| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | G | G | A | A | T | C | C | C | T | T | C |
| APOBEC (BE3) | 0.02% | 0.09% | **99.97%** | **99.96%** | 0.01% | 1.17% | 1.49% | 1.58% | 0.01% | 0.01% | 0.27% | 0.01% |
| A | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | **59.97%** | **65.83%** | **66.67%** | 0.01% | 0.01% | **84.62%** | 0.02% |
| C | **99.96%** | **99.89%** | 0.01% | 0.02% | 0.00% | 0.84% | 0.35% | 0.41% | 0.00% | 0.00% | 0.27% | 0.00% |
| G | 0.02% | 0.02% | 0.01% | 0.02% | **99.98%** | 38.02% | 32.34% | 31.33% | **99.98%** | **99.99%** | 14.83% | **99.97%** |
| T | | | | | | | | | | | | |
| ADAR | | | | | | | | | | | | |
| A | 0.02% | 0.04% | **99.97%** | **99.96%** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | **99.38%** | **99.53%** | **99.66%** | 0.00% | 0.01% | **99.97%** | 0.02% |
| G | **99.97%** | **99.95%** | 0.02% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.02% | 0.01% | 0.02% | **99.98%** | 0.61% | 0.46% | 0.33% | **99.98%** | **99.98%** | 0.03% | **99.96%** |
| ADA | | | | | | | | | | | | |
| A | 0.05% | 0.05% | **99.96%** | **99.97%** | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | **99.67%** | **99.87%** | **99.81%** | 0.01% | 0.01% | **99.96%** | 0.02% |
| G | **99.94%** | **99.93%** | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| T | 0.02% | 0.01% | 0.01% | 0.01% | **99.98%** | 0.32% | 0.12% | 0.18% | **99.98%** | **99.98%** | 0.04% | **99.97%** |
| | | | | | | | | | | | | |
| ADAT | | | | | | | | | | | | |
| A | 0.03% | 0.06% | **99.97%** | **99.97%** | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| C | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | **99.80%** | **99.91%** | **99.94%** | 0.01% | 0.01% | **99.95%** | 0.02% |
| G | **99.96%** | **99.92%** | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.02% | **99.99%** | 0.19% | 0.08% | 0.05% | **99.98%** | **99.98%** | 0.04% | **99.97%** |
| untreated | | | | | | | | | | | | |
| A | 0.03% | 0.06% | **99.97%** | **99.97%** | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| C | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | **99.80%** | **99.91%** | **99.94%** | 0.01% | 0.01% | **99.95%** | 0.02% |
| G | **99.96%** | **99.92%** | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.02% | **99.99%** | 0.19% | 0.08% | 0.05% | **99.98%** | **99.98%** | 0.04% | **99.97%** |

FIGURE 1

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | A | G | C | A | C | C | T | G | G | |
| 0.05% | 0.05% | **99.93%** | 0.05% | 0.00% | **99.97%** | 0.00% | 0.05% | 0.02% | 0.06% | 0.08% | **99.97%** | 0.01% |
| 0.00% | **99.60%** | 0.02% | 0.12% | **99.85%** | 0.00% | **99.97%** | **99.93%** | 0.09% | 0.00% | 0.00% | 0.01% | 0.01% |
| **99.93%** | 0.00% | 0.03% | **99.82%** | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | **99.93%** | **99.91%** | 0.01% | 0.00% |
| 0.02% | 0.34% | 0.01% | 0.01% | 0.15% | 0.01% | 0.03% | 0.02% | **99.89%** | 0.01% | 0.01% | 0.01% | **99.98%** |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| 0.14% | 0.01% | **99.93%** | 0.04% | 0.01% | **99.98%** | 0.00% | 0.01% | 0.01% | 0.11% | 0.06% | **99.97%** | 0.02% |
| 0.00% | **99.80%** | 0.01% | 0.00% | **99.91%** | 0.00% | **99.98%** | **99.87%** | 0.06% | 0.00% | 0.00% | 0.01% | 0.01% |
| **99.84%** | 0.00% | 0.04% | **99.95%** | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | **99.88%** | **99.93%** | 0.01% | 0.00% |
| 0.02% | 0.19% | 0.01% | 0.01% | 0.09% | 0.01% | 0.02% | 0.12% | **99.92%** | 0.01% | 0.01% | 0.01% | **99.97%** |
| | | | | | | | | | | | | |
| 0.04% | 0.00% | **99.94%** | 0.04% | 0.00% | **99.97%** | 0.00% | 0.01% | 0.01% | 0.07% | 0.07% | **99.97%** | 0.01% |
| 0.00% | **99.73%** | 0.02% | 0.00% | **99.98%** | 0.00% | **99.98%** | **99.94%** | 0.13% | 0.00% | 0.00% | 0.01% | 0.01% |
| **99.94%** | 0.00% | 0.03% | **99.93%** | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | **99.91%** | **99.92%** | 0.01% | 0.00% |
| 0.02% | 0.26% | 0.01% | 0.03% | 0.01% | 0.02% | 0.02% | 0.05% | **99.85%** | 0.01% | 0.01% | 0.01% | **99.98%** |
| | | | | | | | | | | | | |
| | | | | | | | | | | | | |
| 0.06% | 0.01% | **99.95%** | 0.06% | 0.00% | **99.97%** | 0.00% | 0.02% | 0.01% | 0.03% | 0.09% | **99.97%** | 0.02% |
| 0.00% | **99.80%** | 0.01% | 0.00% | **99.98%** | 0.00% | **99.96%** | **99.94%** | 0.06% | 0.00% | 0.00% | 0.00% | 0.01% |
| **99.92%** | 0.00% | 0.02% | **99.93%** | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | **99.96%** | **99.90%** | 0.01% | 0.00% |
| 0.01% | 0.19% | 0.01% | 0.01% | 0.02% | 0.02% | 0.04% | 0.04% | **99.91%** | 0.01% | 0.01% | 0.01% | **99.97%** |
| | | | | | | | | | | | | |
| 0.06% | 0.01% | **99.95%** | 0.06% | 0.00% | **99.97%** | 0.00% | 0.02% | 0.01% | 0.03% | 0.09% | **99.97%** | 0.02% |
| 0.00% | **99.80%** | 0.01% | 0.00% | **99.98%** | 0.00% | **99.96%** | **99.94%** | 0.06% | 0.00% | 0.00% | 0.00% | 0.01% |
| **99.92%** | 0.00% | 0.02% | **99.93%** | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | **99.96%** | **99.90%** | 0.01% | 0.00% |
| 0.01% | 0.19% | 0.01% | 0.01% | 0.02% | 0.02% | 0.04% | 0.04% | **99.91%** | 0.01% | 0.01% | 0.01% | **99.97%** |

FIGURE 1 (Continued)

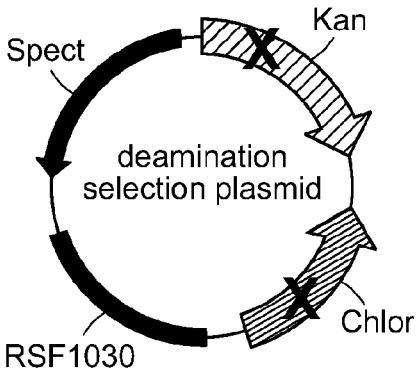



FIGURE 2

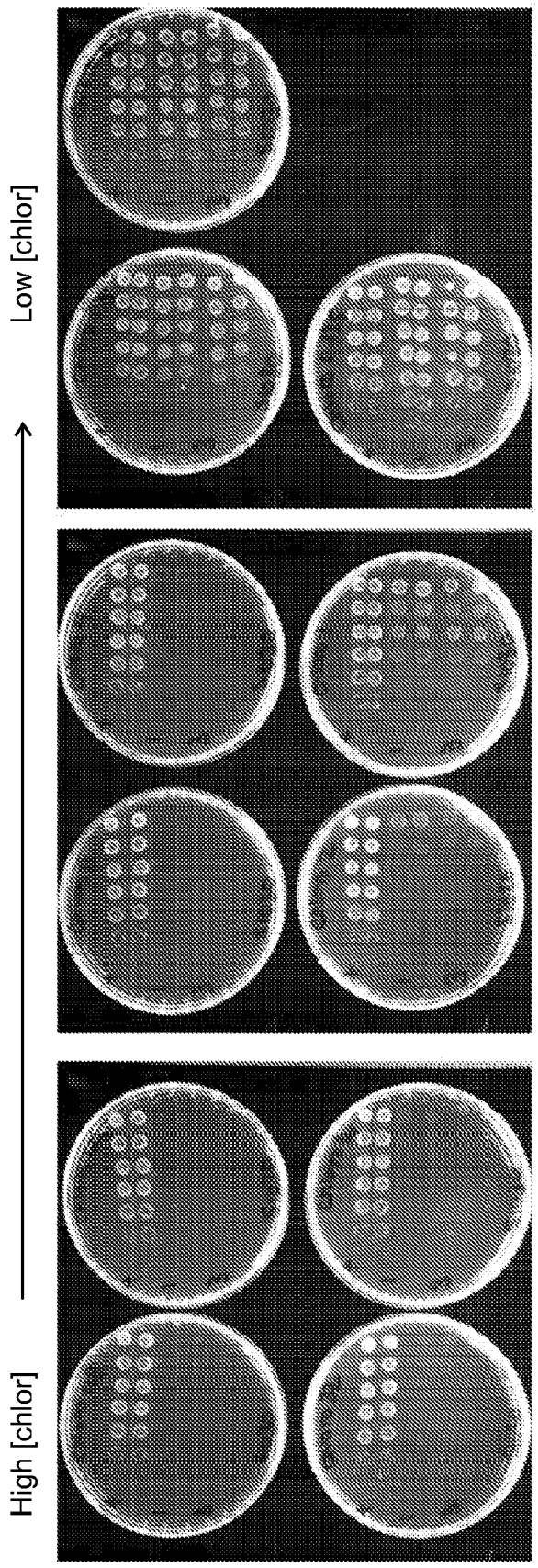



FIGURE 3

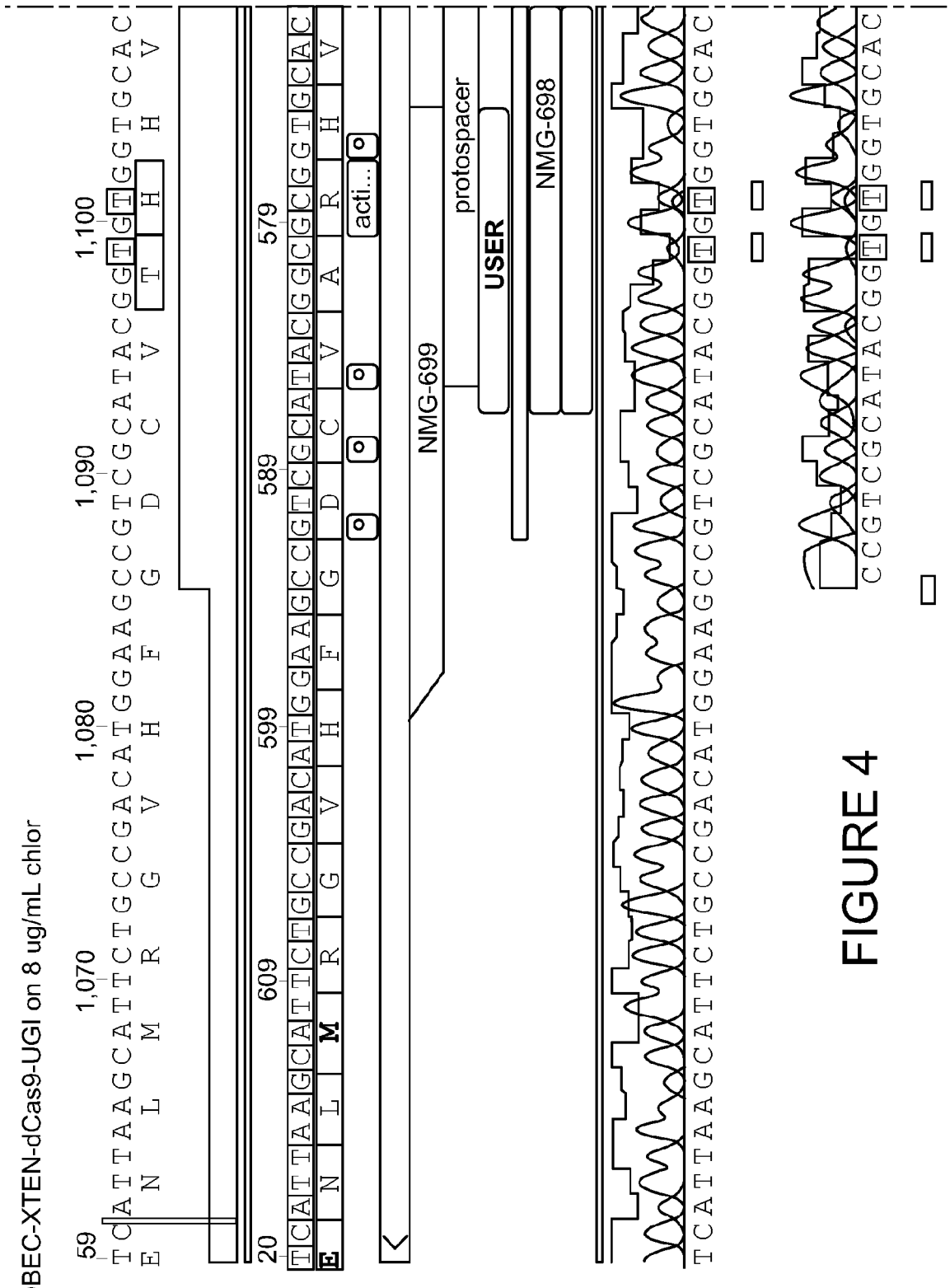

rAPOBEC-XTEN-dCas9-UGI on 8 ug/mL chlor
protospacer
USER
NMG-699
NMG-698

FIGURE 4

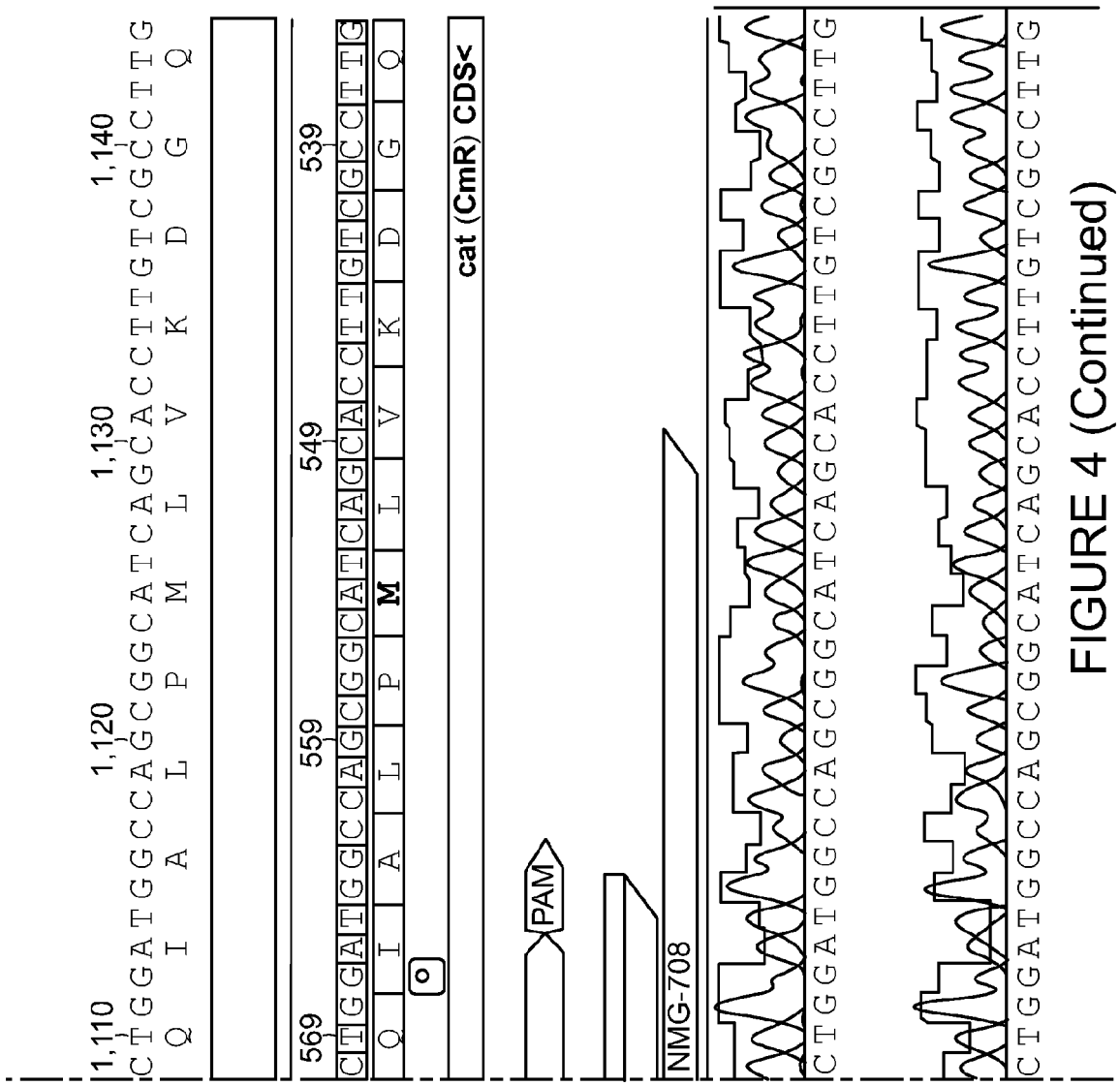



FIGURE 4 (Continued)

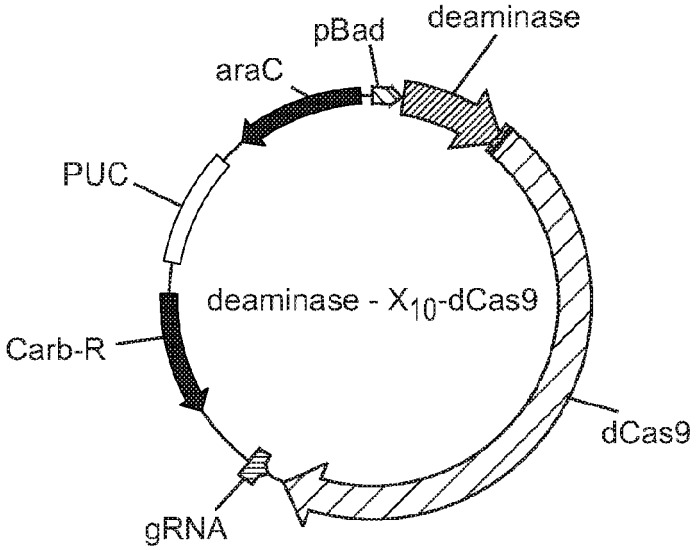

pBad
deaminase
araC
PUC
deaminase - X10-dCas9
Carb-R
dCas9
gRNA

FIGURE 5

FIGURE 6

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 359 | 458 SNP | G | K | 0.40% | G.GGT @ 100 -> X.KGT | CDS | CDS with | 227 | 0 | |
| 361 | 460 SNP | T | C | 0.90% | G.GGT @ 100 -> G.GGc | CDS | CDS with | 227 | 0 | |
| 363 | 462 SNP | G | K | 0.40% | R.GGC @ 101 -> X.GJC | CDS | CDS with | 227 | 0 | |
| 366 | 465 SNP | T | K | 1.80% | V.GTG @ 102 -> X.GKG | CDS | CDS with | 227 | 0 | |
| 367 | 466 SNP | G | A | 2.60% | V.GTG @ 102 -> V.GTa | CDS | CDS with | 227 | 5 | |
| 368 | 467 SNP | G | K | 0.40% | V.GTC @ 103 -> X.KTC | CDS | CDS with | 227 | 0 | |
| 369 | 468 SNP | T | A | 6.20% | V.GTC @ 103 -> D.GaC | CDS | CDS with | 227 | 10 | |
| 371 | 470 SNP | T | A | 4.40% | F.TTT @ 104 -> I.aTT | CDS | CDS with | 227 | 7 | |
| 373 | 472 SNP | T | A | 2.60% | F.TTT @ 104 -> L.TTa | CDS | CDS with | 227 | 4 | |
| 374 | 473 SNP | G | T | 1.30% | G.GGT @ 105 -> C.tGT | CDS | CDS with | 227 | 0 | |
| 376 | 475 SNP | T | K | 1.80% | G.GGT @ 105 -> G.GGk | CDS | CDS with | 227 | 2 | |
| 378 | 477 SNP | C | T | 7.00% | A.GCG @ 106 -> V.GTG | CDS | CDS with | 227 | 0 | |
| 379 | 478 SNP | G | A | 1.80% | A.GCG @ 106 -> A.GCa | CDS | CDS with | 227 | 2 | |
| 380 | 479 SNP | C | T | 14.10% | R.CGT @ 107 -> C.tGT | CDS | CDS with | 227 | 0 | |
| 381 | 480 SNP | G | C | 2.20% | R.CGT @ 107 -> P.CGT | CDS | CDS with | 227 | 2 | |
| 383 | 482 SNP | G | A | 3.10% | D.GAC @ 108 -> N.aAC | CDS | CDS with | 227 | 6 | |
| 384 | 483 SNP | A | G | 6.60% | D.GAC @ 108 -> G.GgC | CDS | CDS with | 227 | | |
| 385 | 484 SNP | C | G | 1.80% | D.GAC @ 108 -> E.GAg | CDS | CDS with | 227 | 0 | |
| 388 | 487 SNP | G | R | 0.90% | A.GCG @ 109 -> A.GCr | CDS | CDS with | 227 | 0 | |
| 391 | 490 SNP | A | R | 0.40% | K.AAA @ 110 -> K.AAr | CDS | CDS with | 227 | | |
| 392 | 491 SNP | A | R | 0.40% | T.ACT @ 111 -> X.rCT | CDS | CDS with | 227 | | |
| 393 | 492 SNP | C | M | 0.90% | T.ACT @ 111 -> X.AmT | CDS | CDS with | 227 | 0 | |
| 394 | 493 SNP | T | W | 2.60% | T.ACT @ 111 -> T.ACw | CDS | CDS with | 227 | 2 | |
| 395 | 494 SNP | G | R | 1.30% | G.GGC @ 112 -> X.rGC | CDS | CDS with | 227 | 0 | |
| 396 | 495 SNP | G | R | 1.80% | G.GGC @ 112 -> X.GrC | CDS | CDS with | 227 | 0 | |
| 398 | 497 SNP | G | S | 0.40% | A.GCT @ 113 -> X.SCT | CDS | CDS with | 227 | 0 | |
| 400 | 499 SNP | T | Y | 0.40% | A.GCT @ 113 -> A.GCy | CDS | CDS with | 227 | 0 | |
| 404 | 503 SNP | G | C | 3.50% | G.GGA @ 115 -> R.cGA | CDS | CDS with | 227 | 0 | |
| 405 | 505 SNP | A | W | 0.90% | G.GGA @ 115 -> G.GGw | CDS | CDS with | 227 | | |

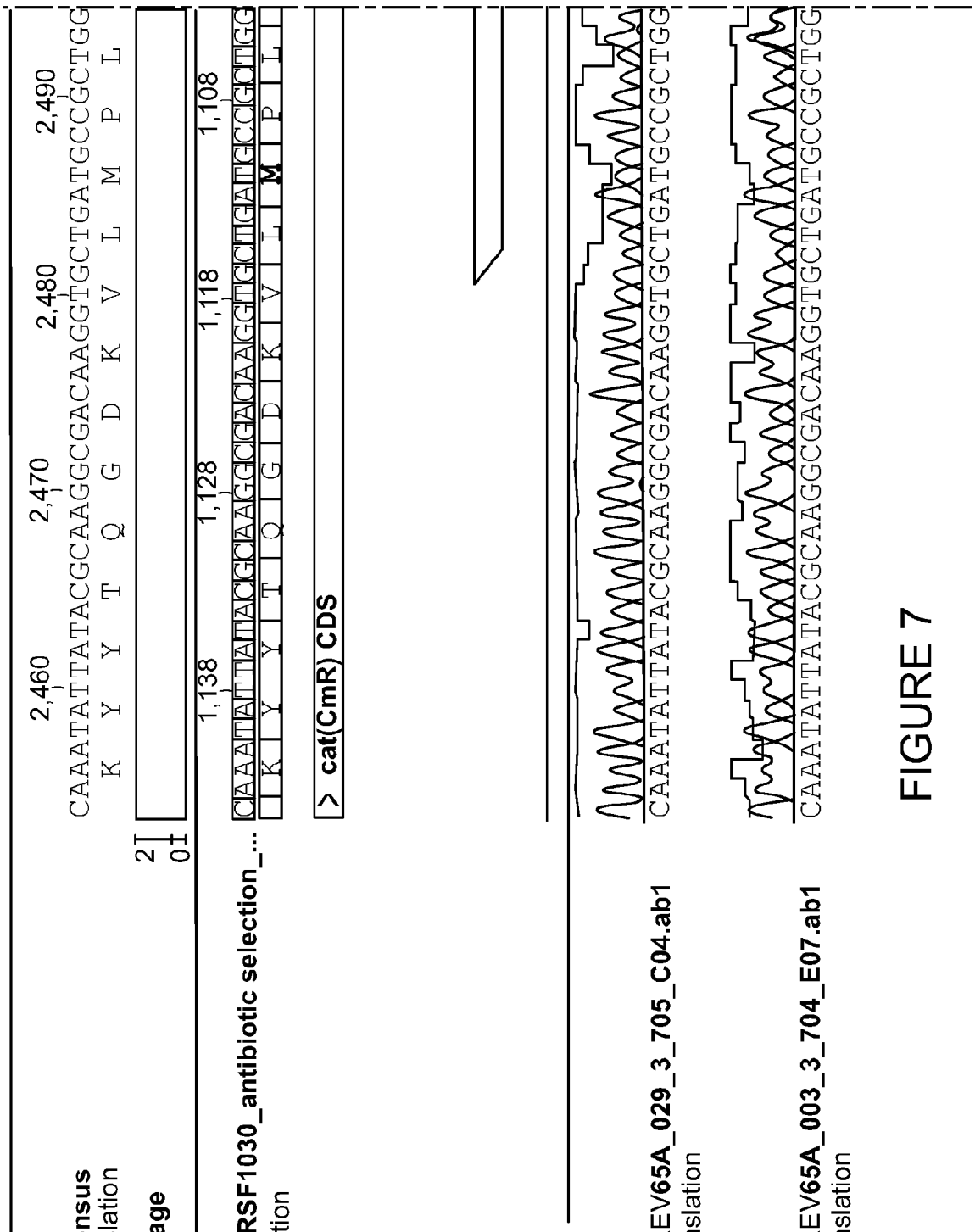

Consensus
Translation
Coverage
D◆REVRSF1030_antibiotic selection_...
Translation
> cat(CmR) CDS
D◆REV65A_029_3_705_C04.ab1
Translation
D◆REV65A_003_3_704_E07.ab1
Translation

FIGURE 7

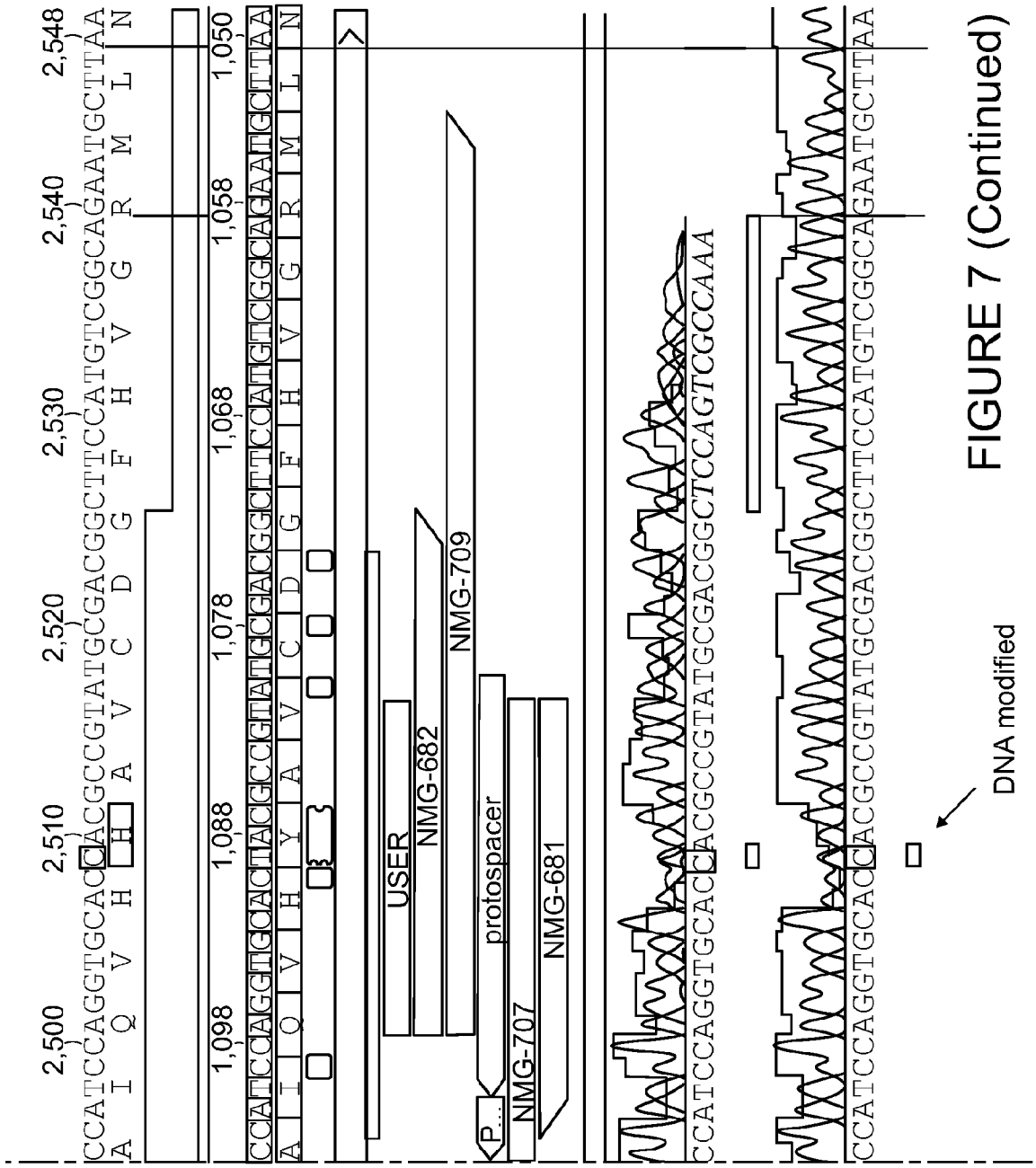



FIGURE 7 (Continued)

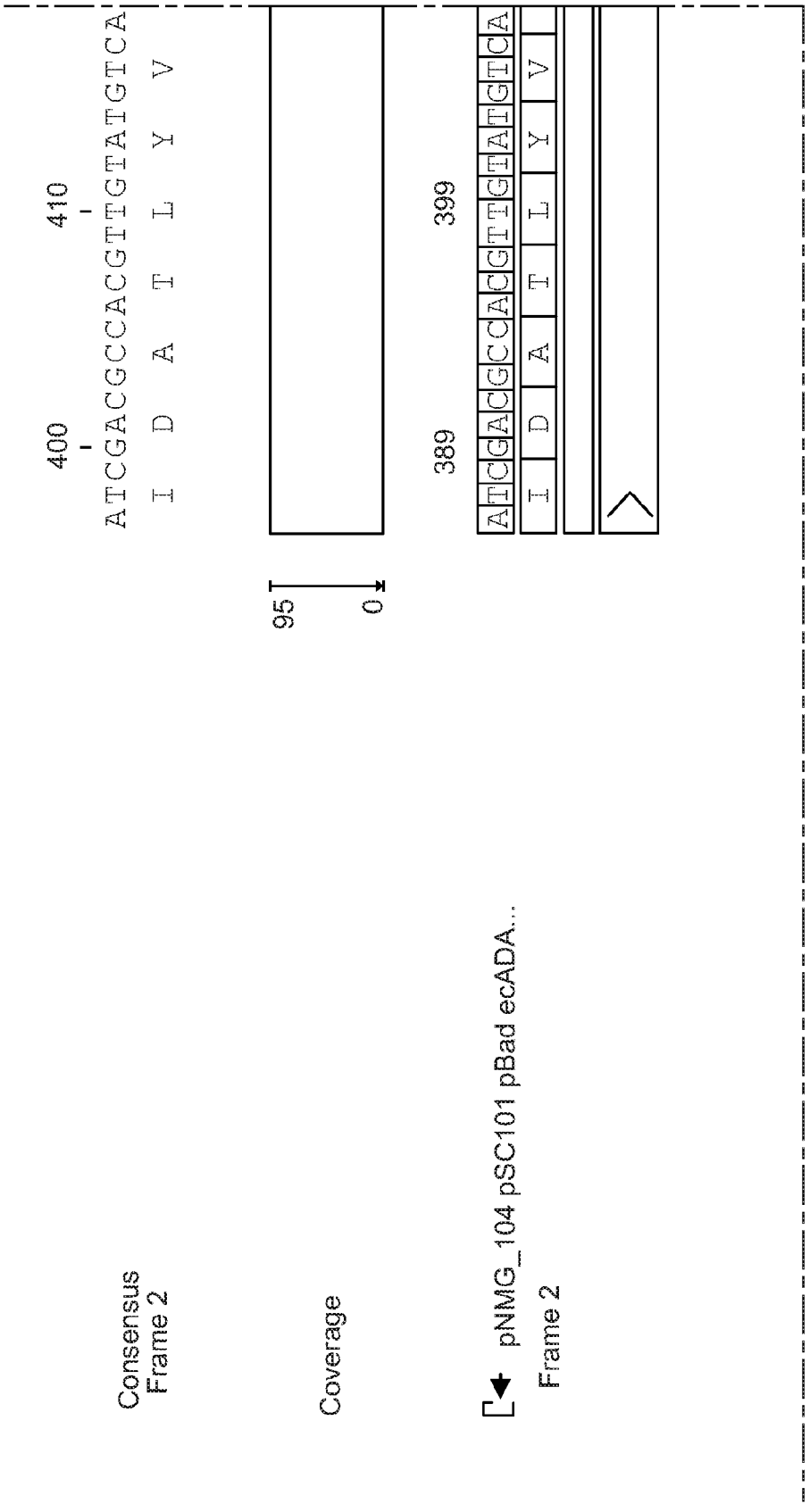

400
410
ATCGACGCCACGTTGTATGTCA
I D A T L Y V
Consensus
Frame 2
Coverage
95
0
389
399
ATCGACGCCACGTTGTATGTCA
I D A T L Y V
pNMG_104 pSC101 pBad ecADA...
Frame 2

FIGURE 8

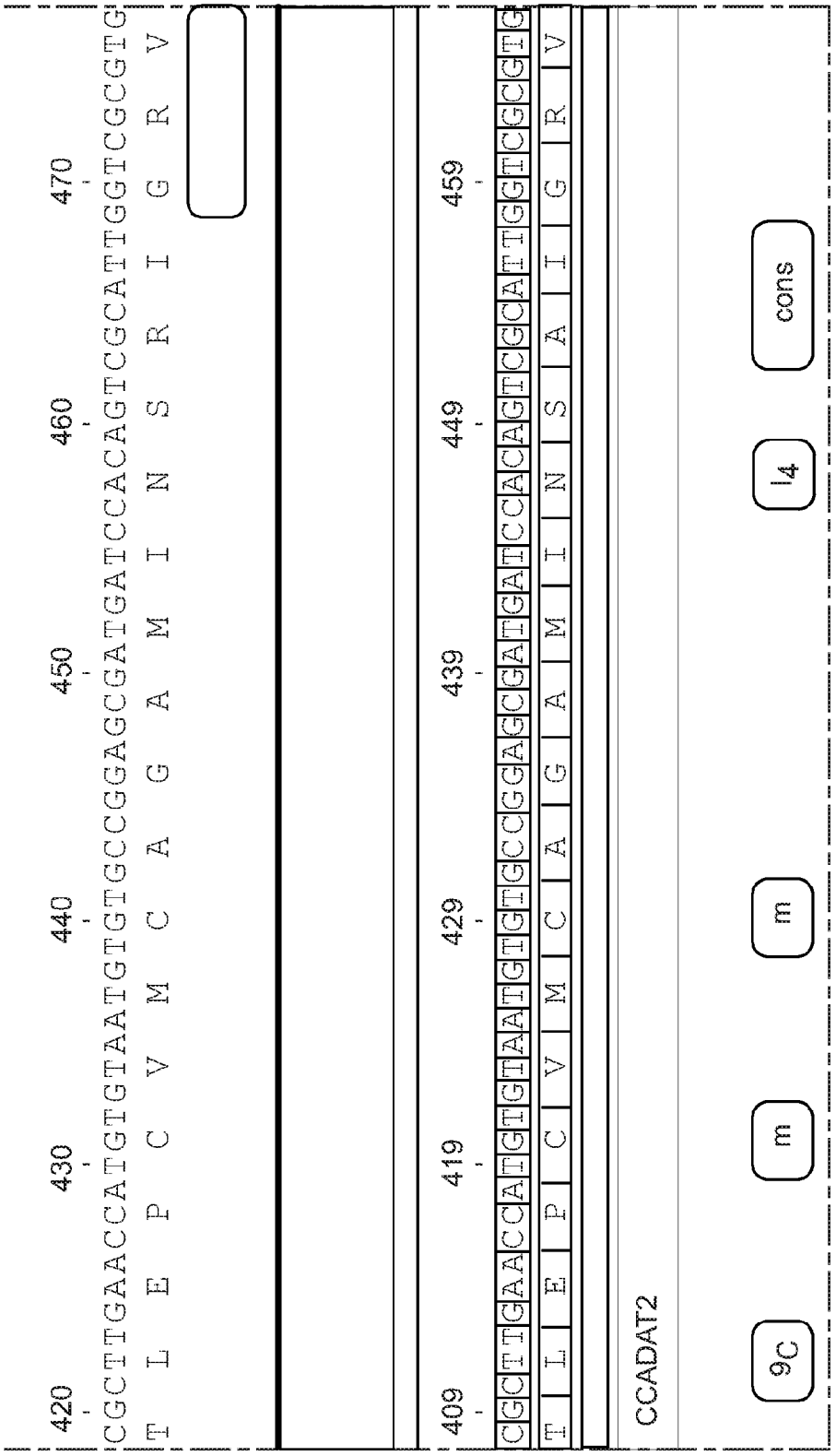



FIGURE 8 (Continued)

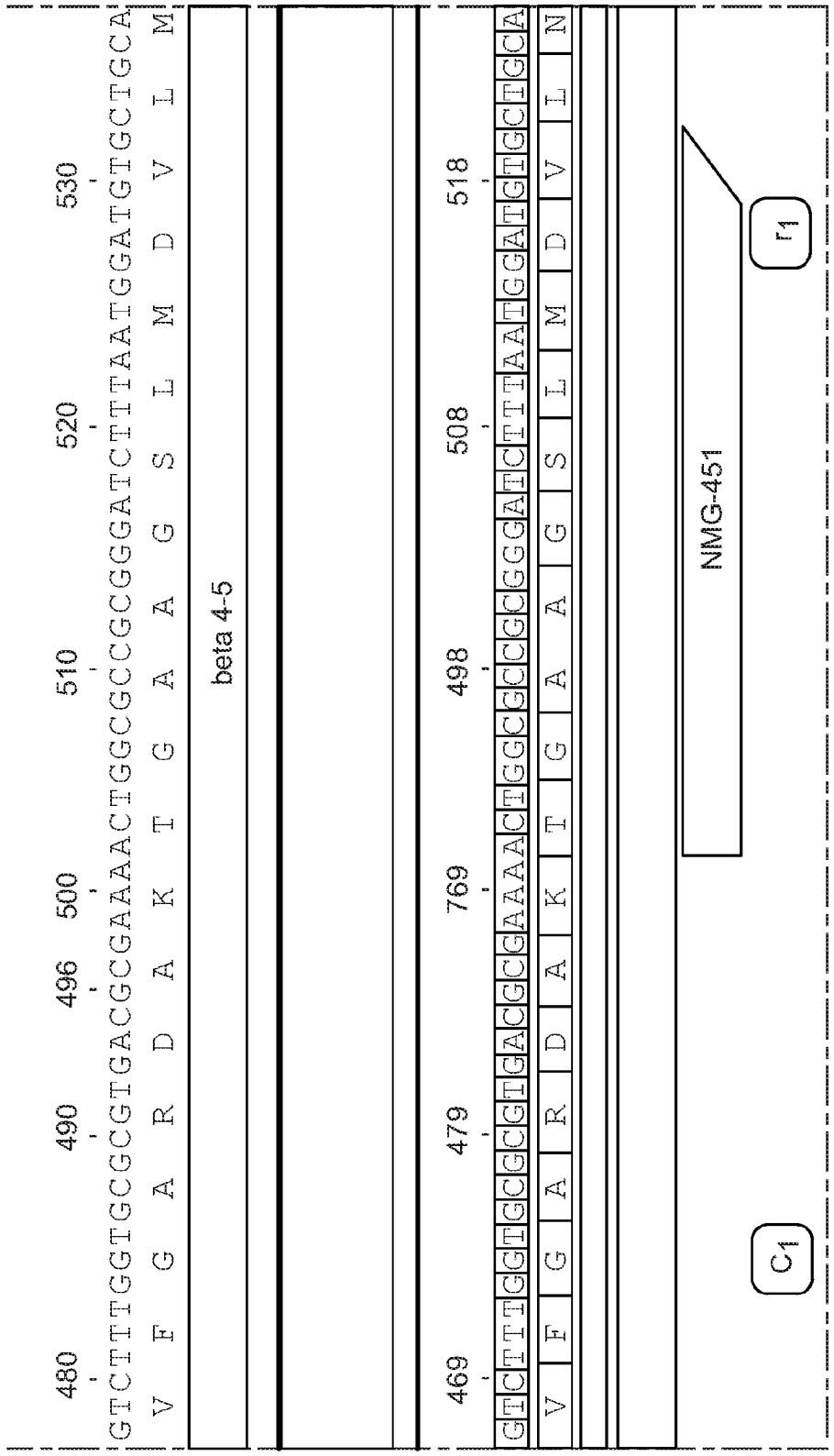



FIGURE 8 (Continued)

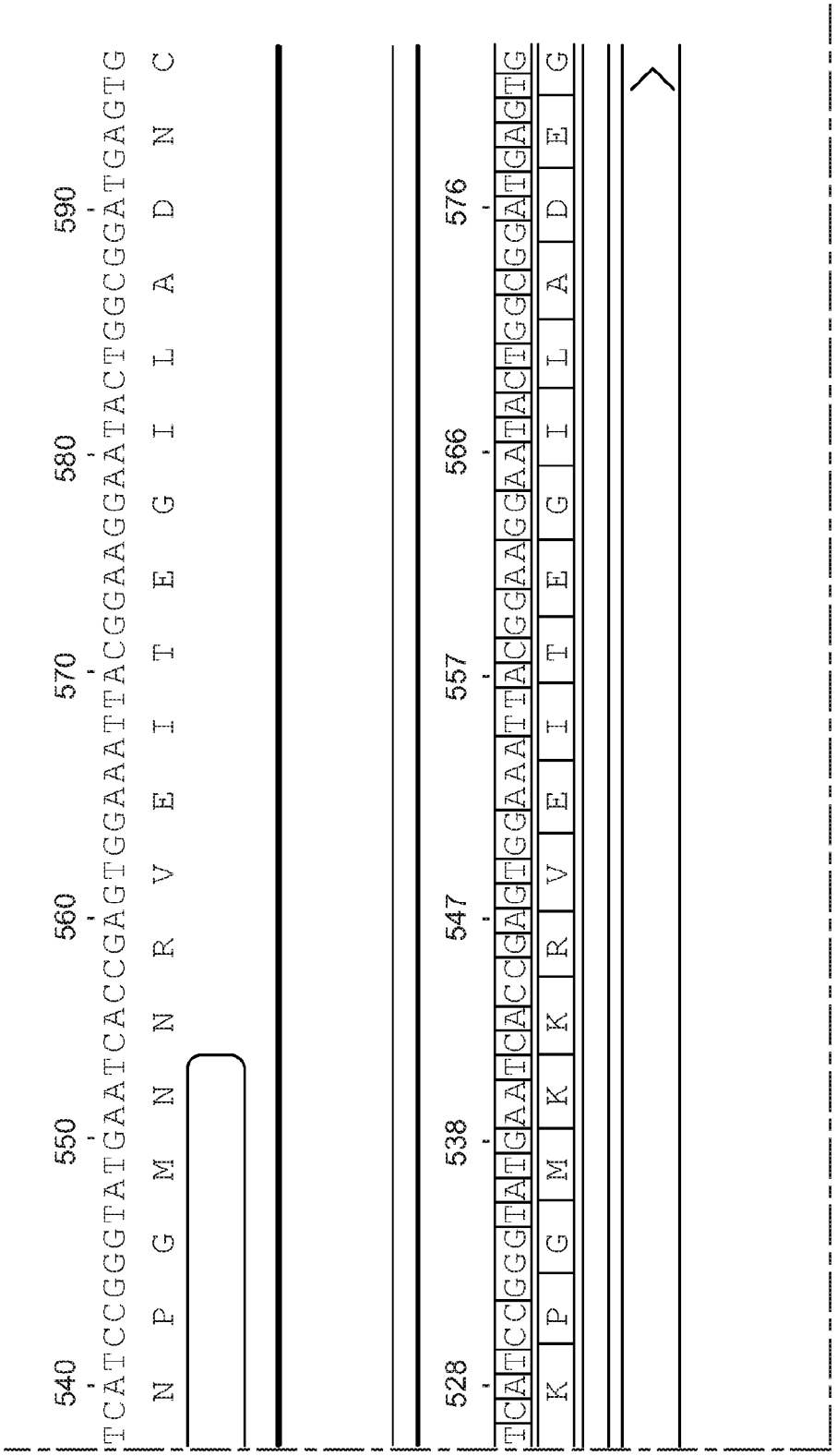



FIGURE 8 (Continued)

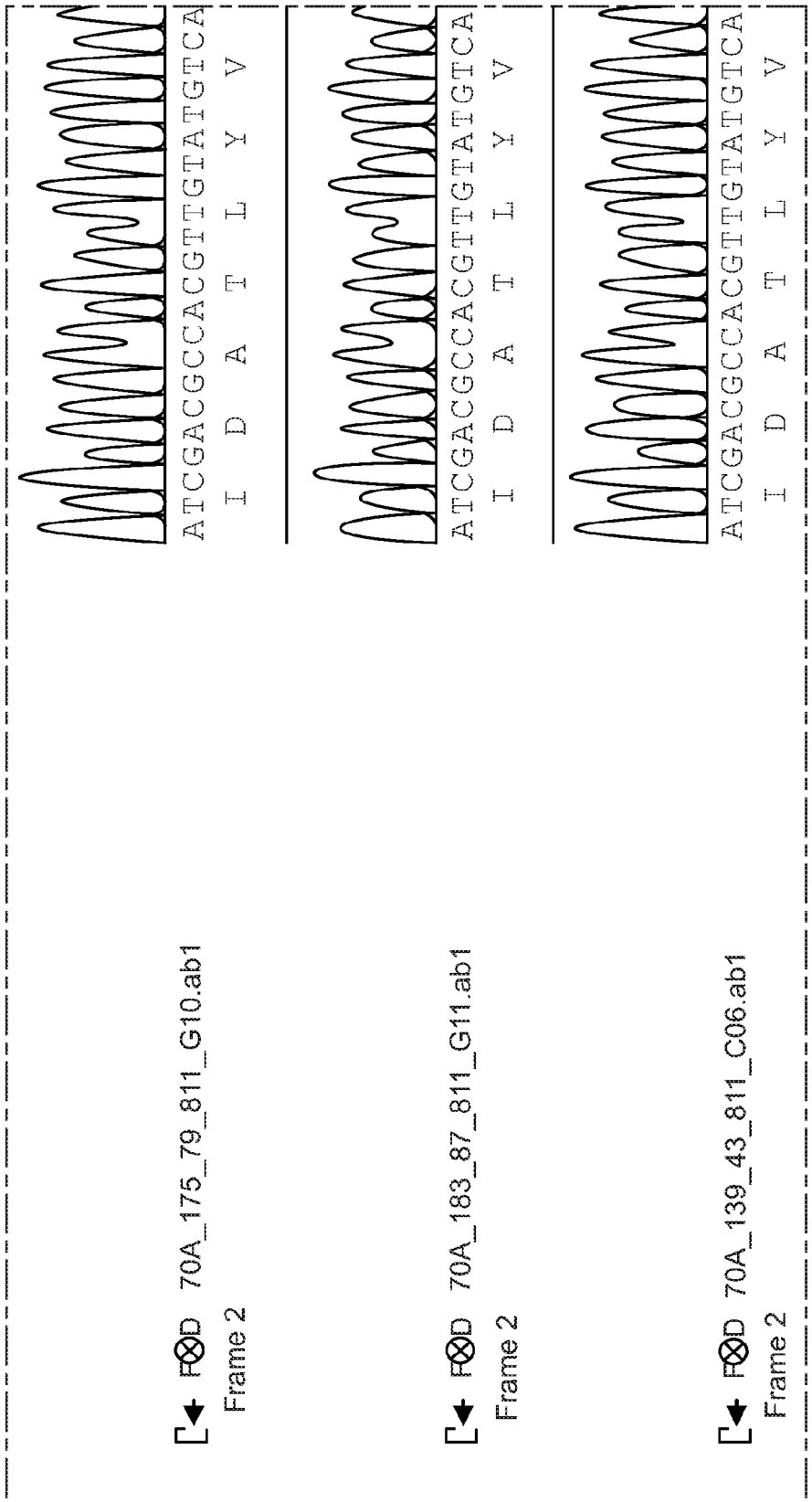



FIGURE 8 (Continued)

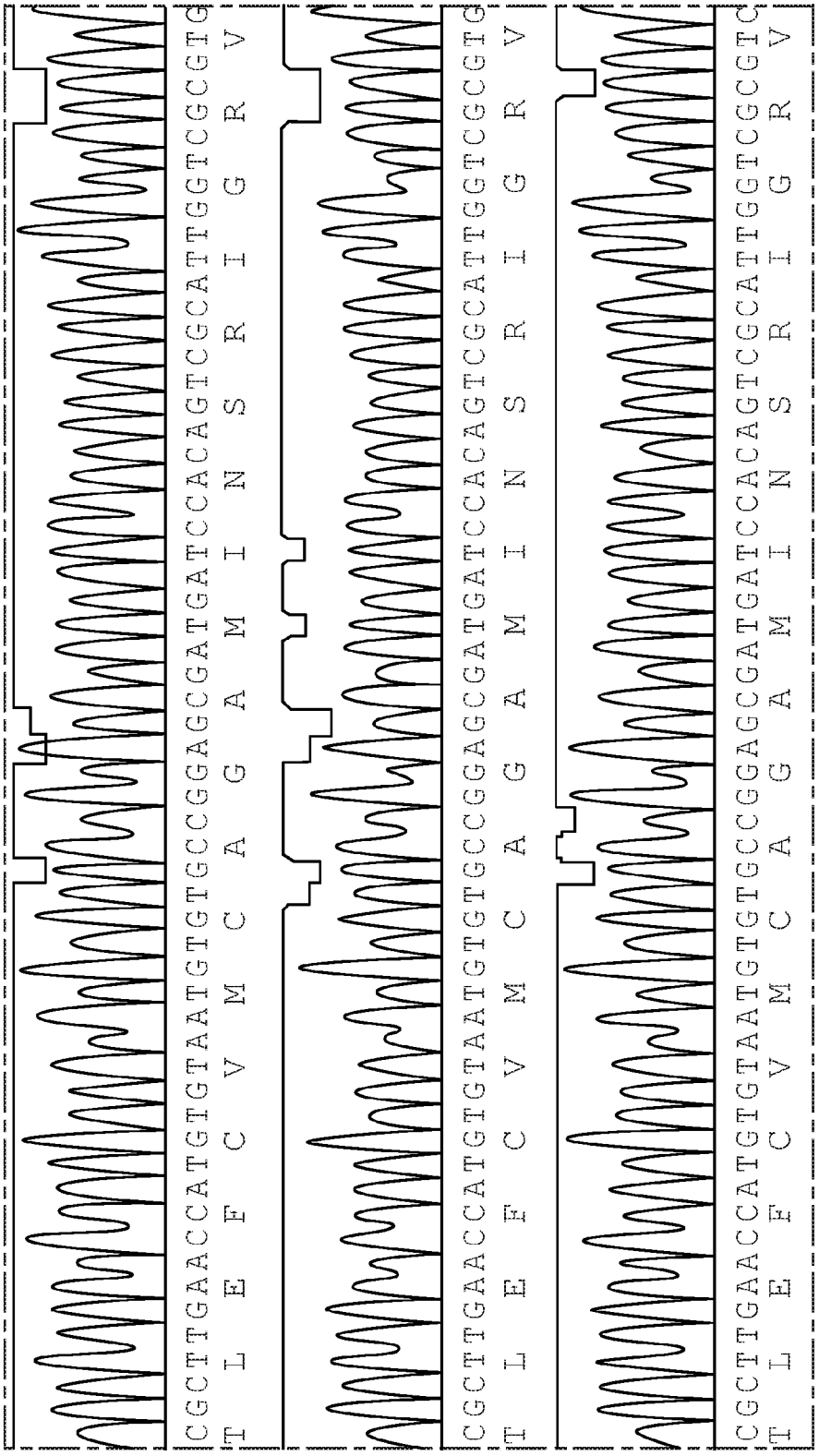



FIGURE 8 (Continued)

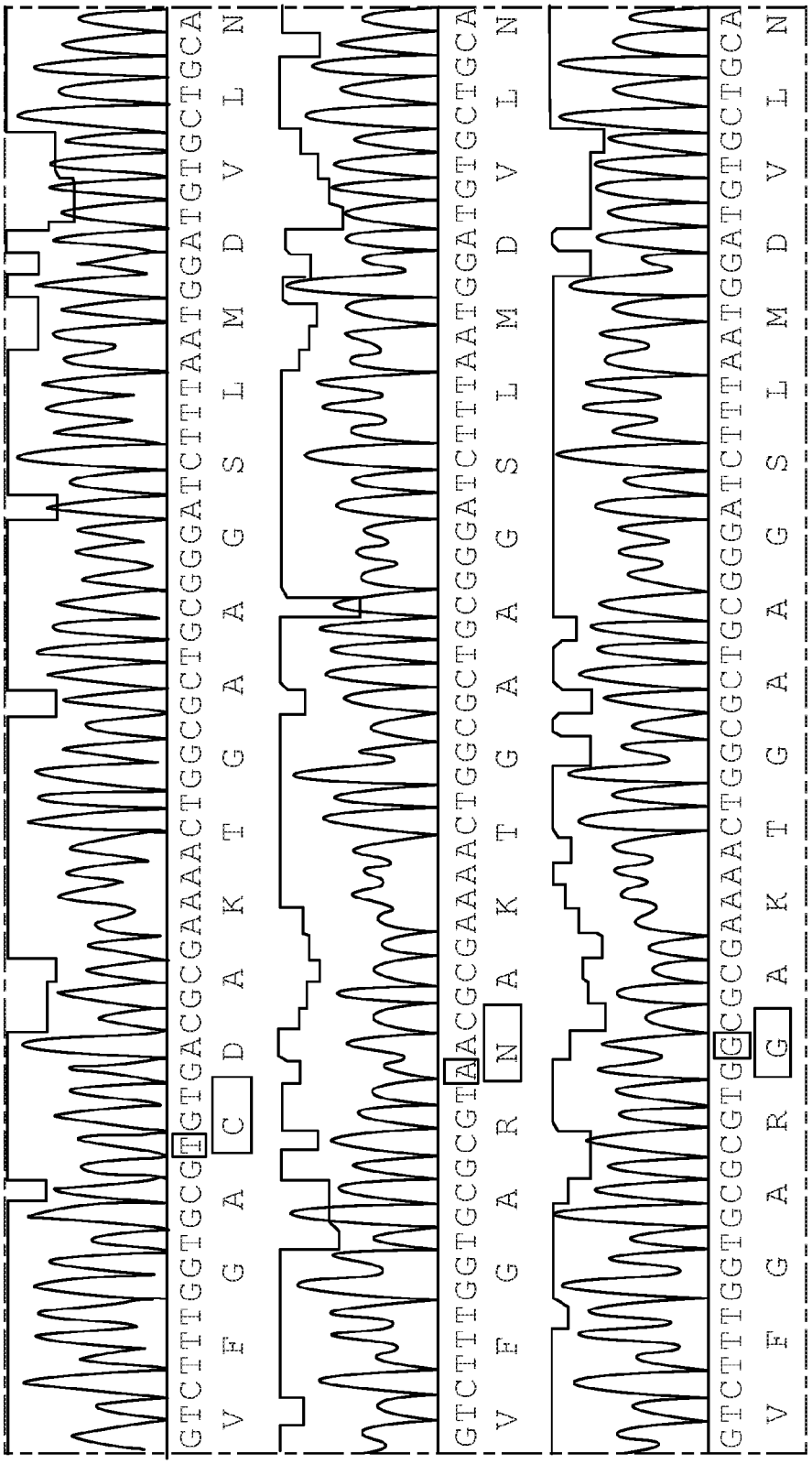



FIGURE 8 (Continued)

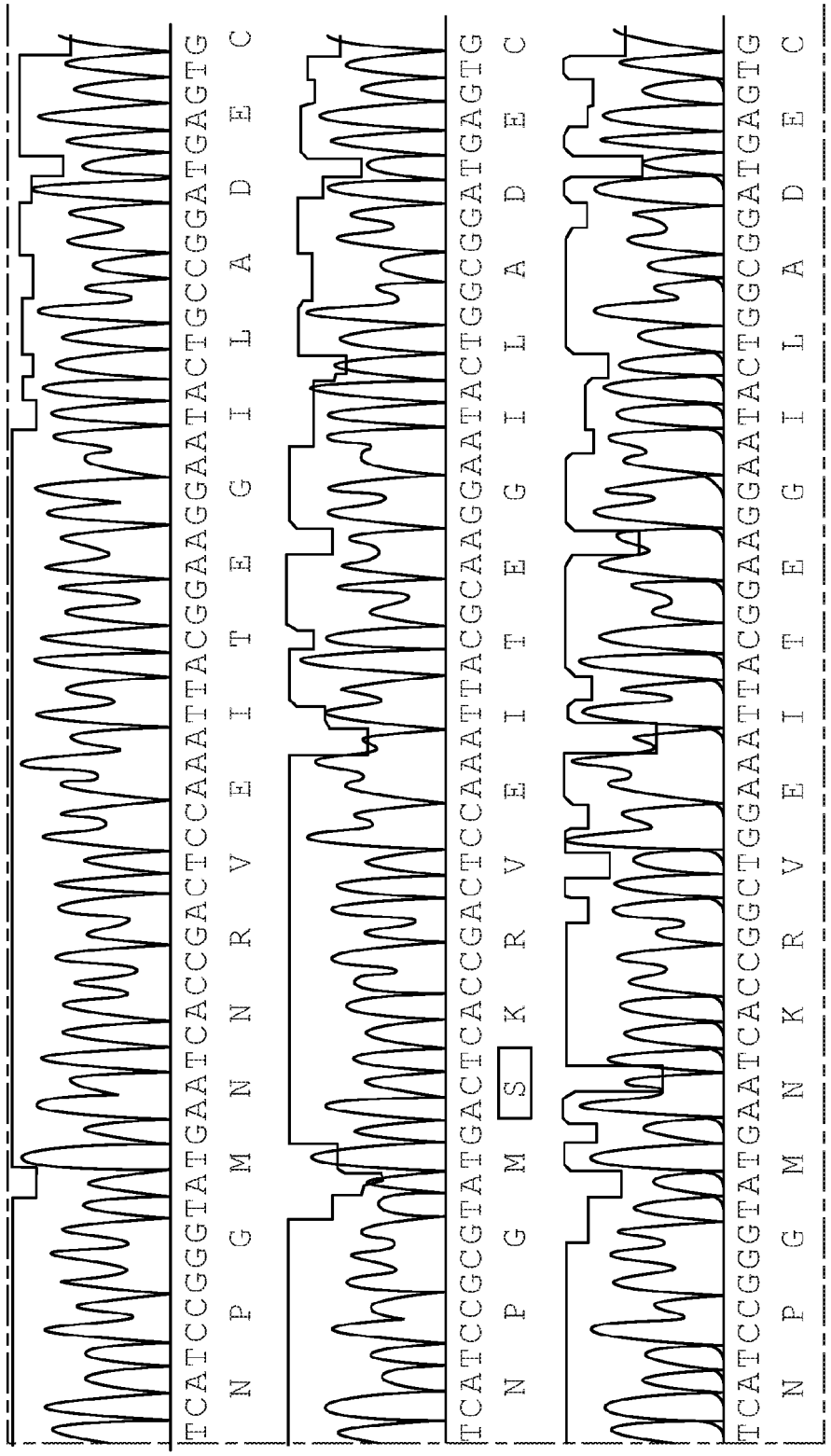



FIGURE 8 (Continued)

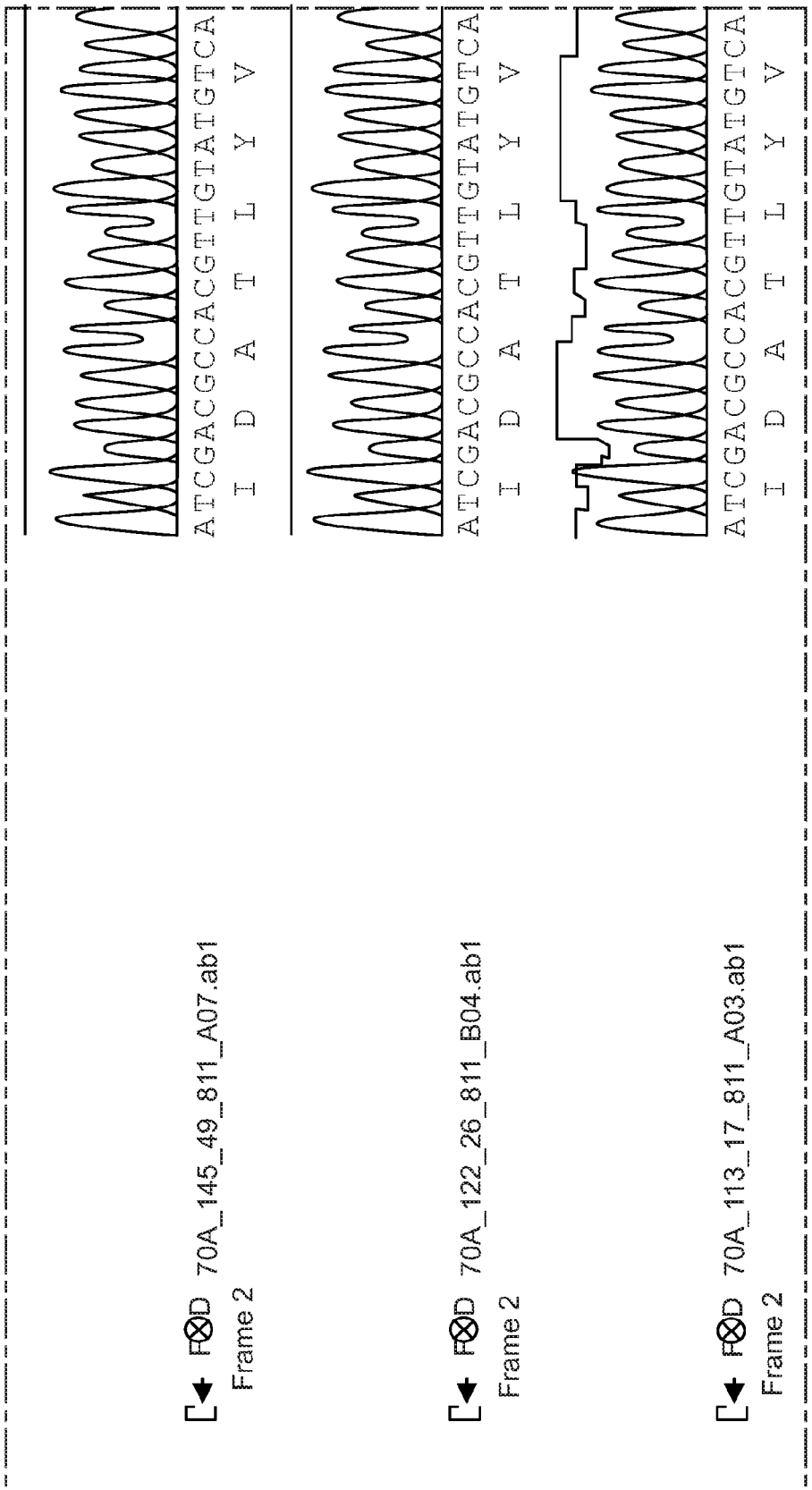



FIGURE 8 (Continued)

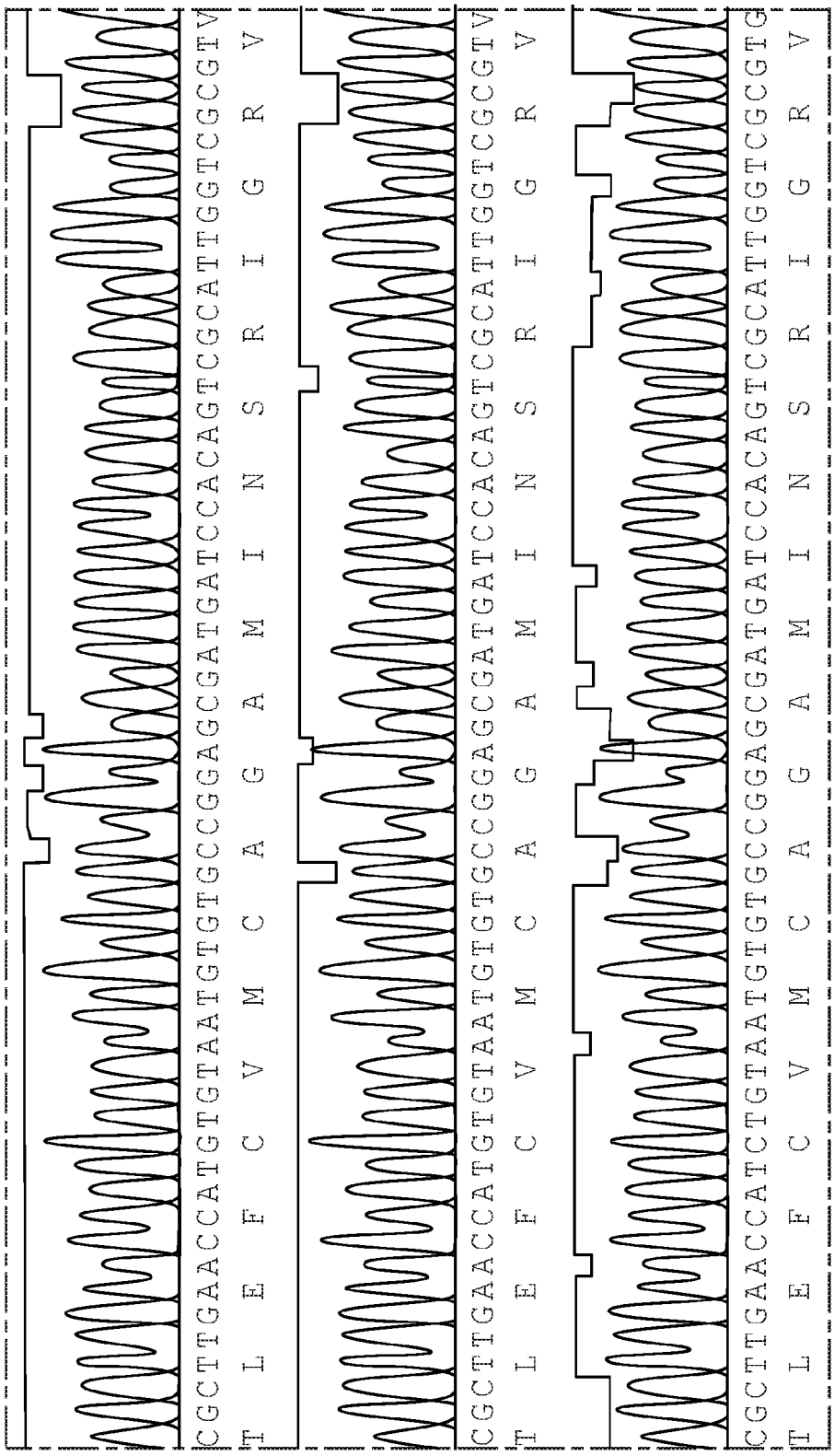



FIGURE 8 (Continued)

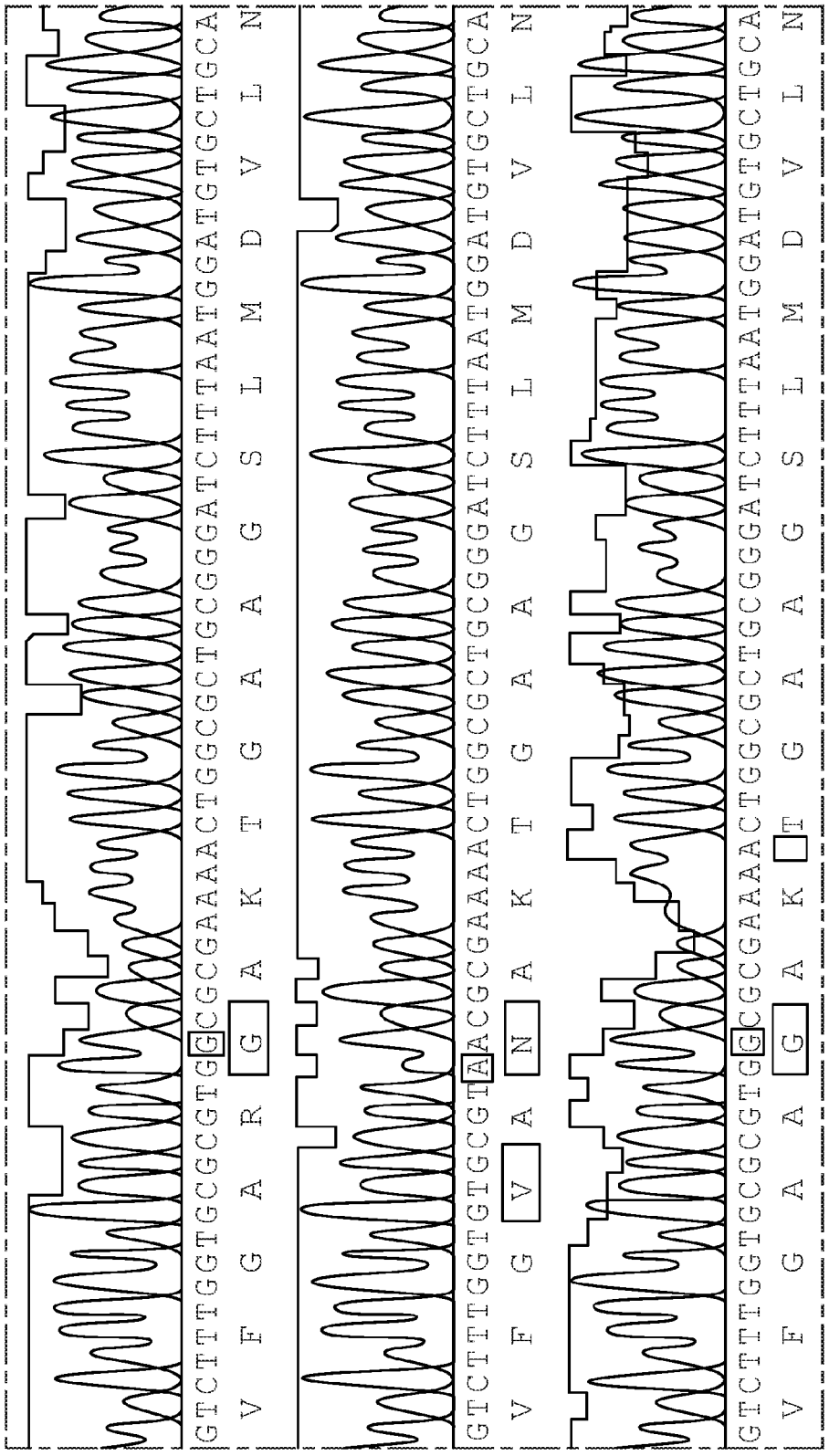



FIGURE 8 (Continued)

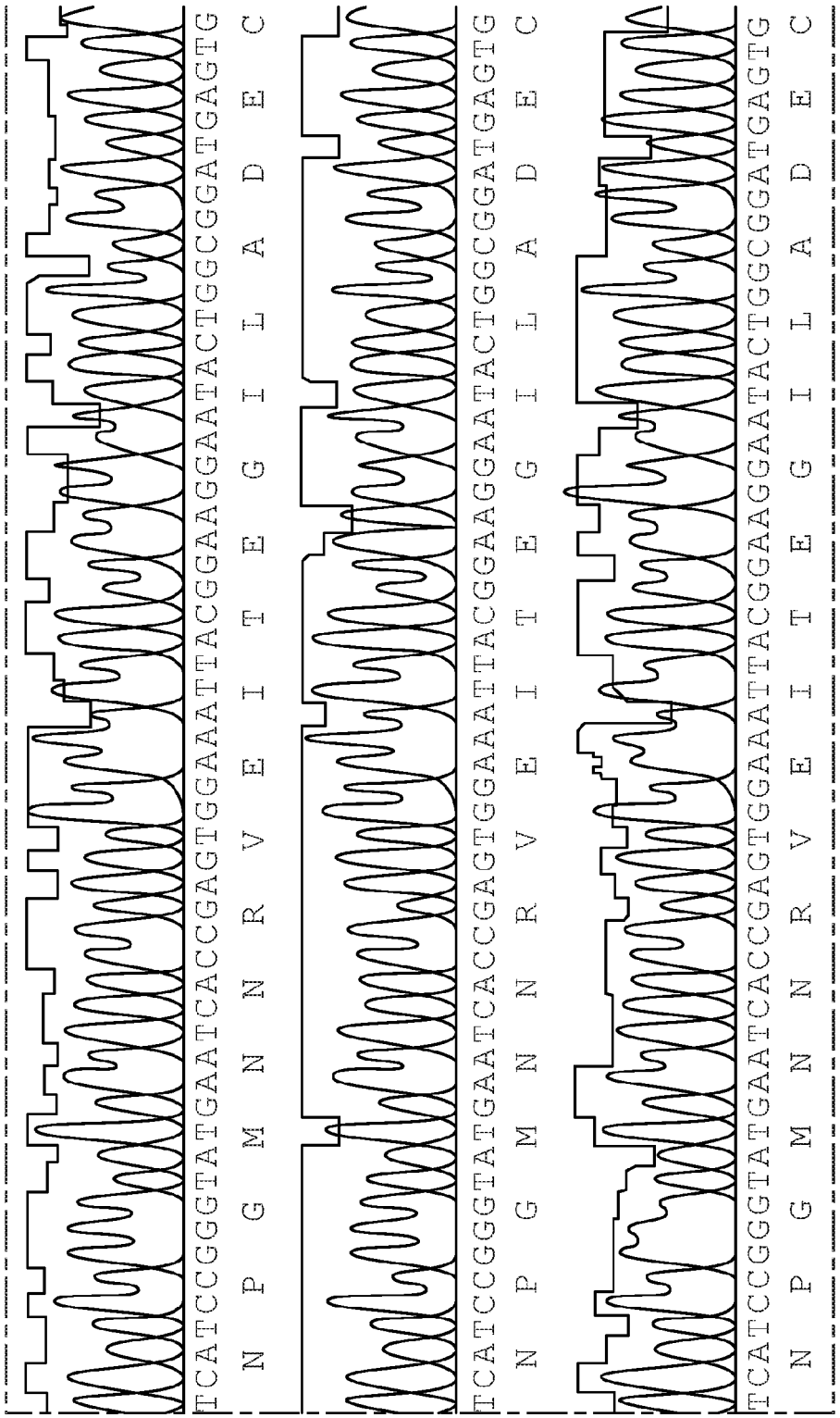



FIGURE 8 (Continued)

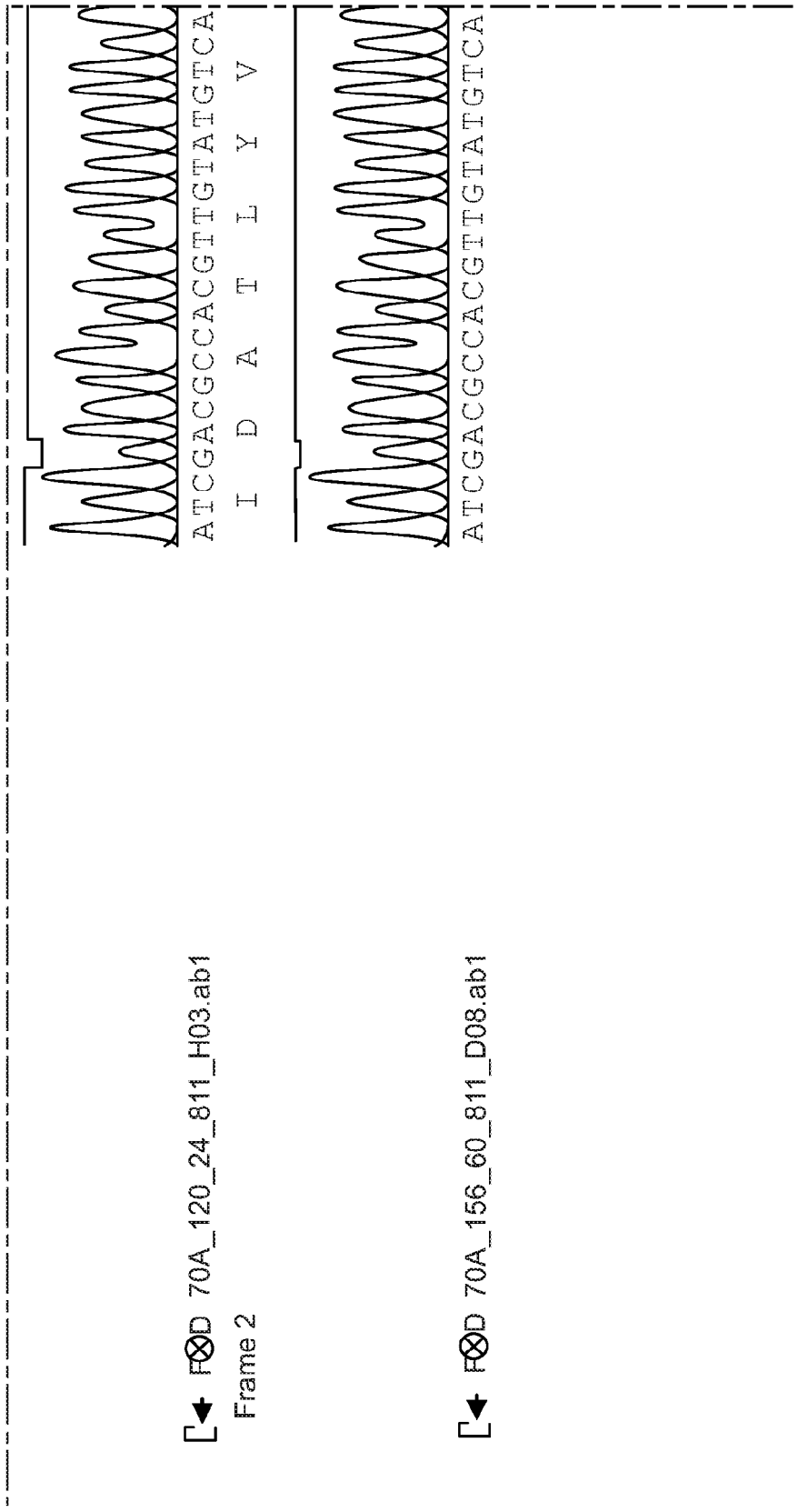



FIGURE 8 (Continued)

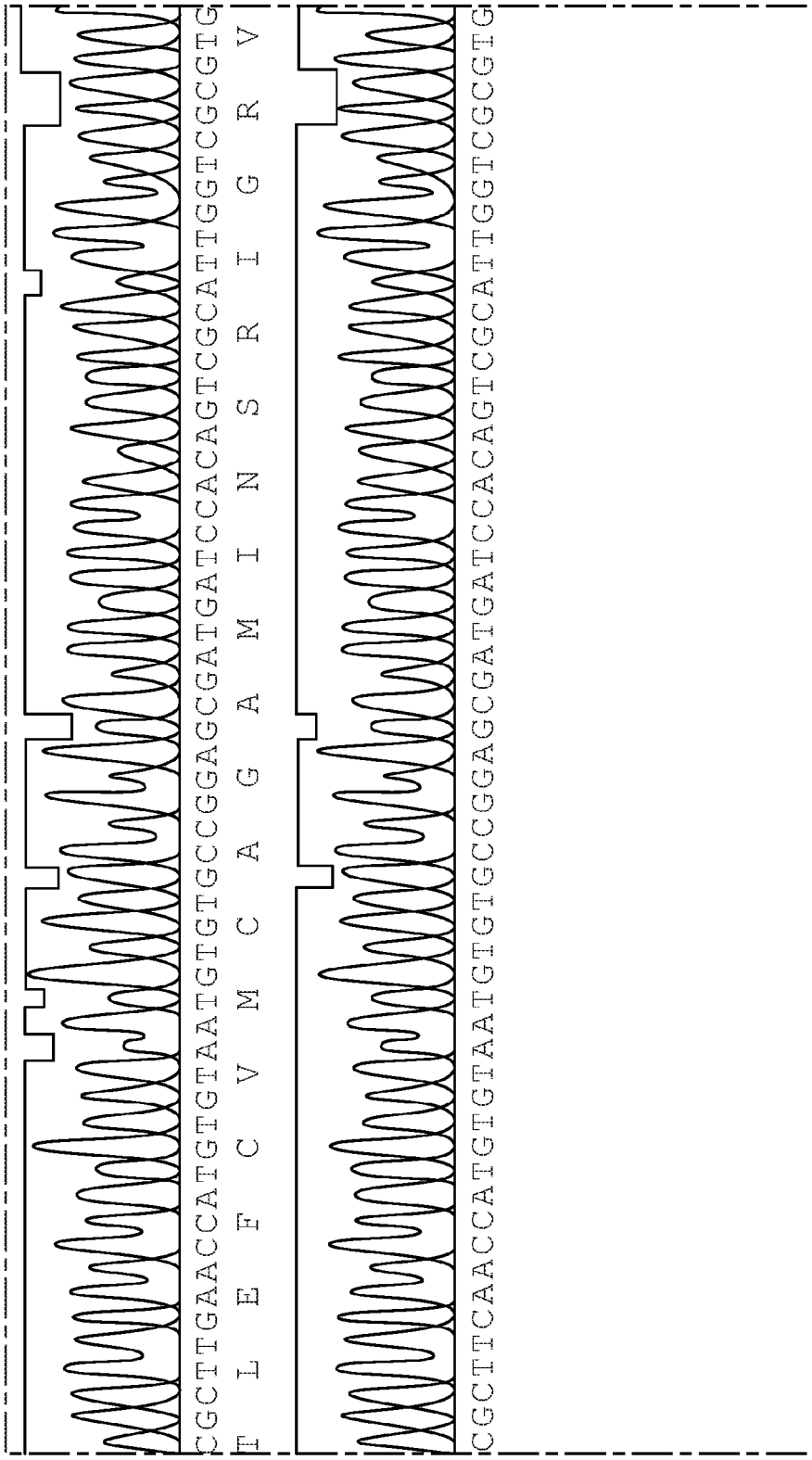



FIGURE 8 (Continued)

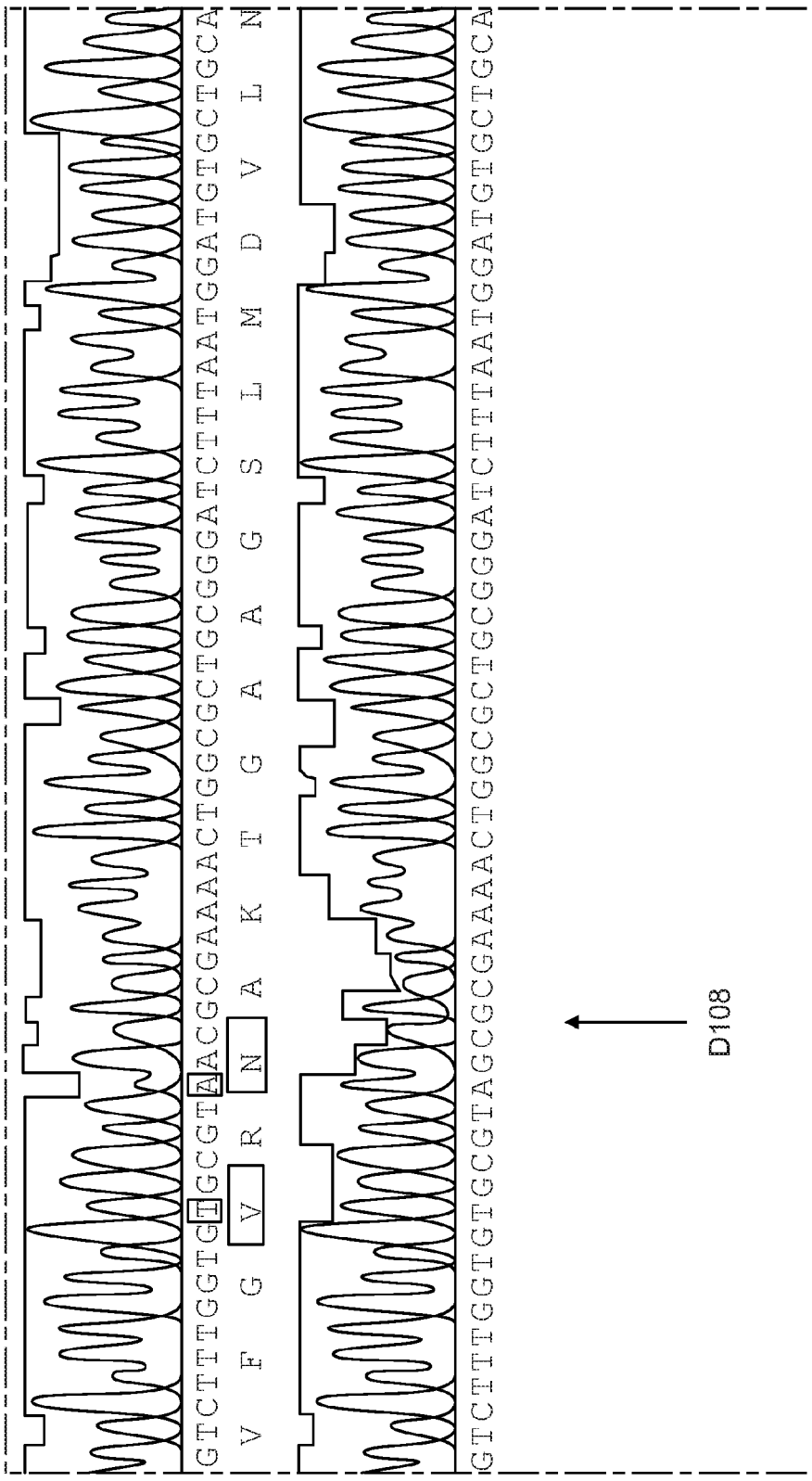



FIGURE 8 (Continued)

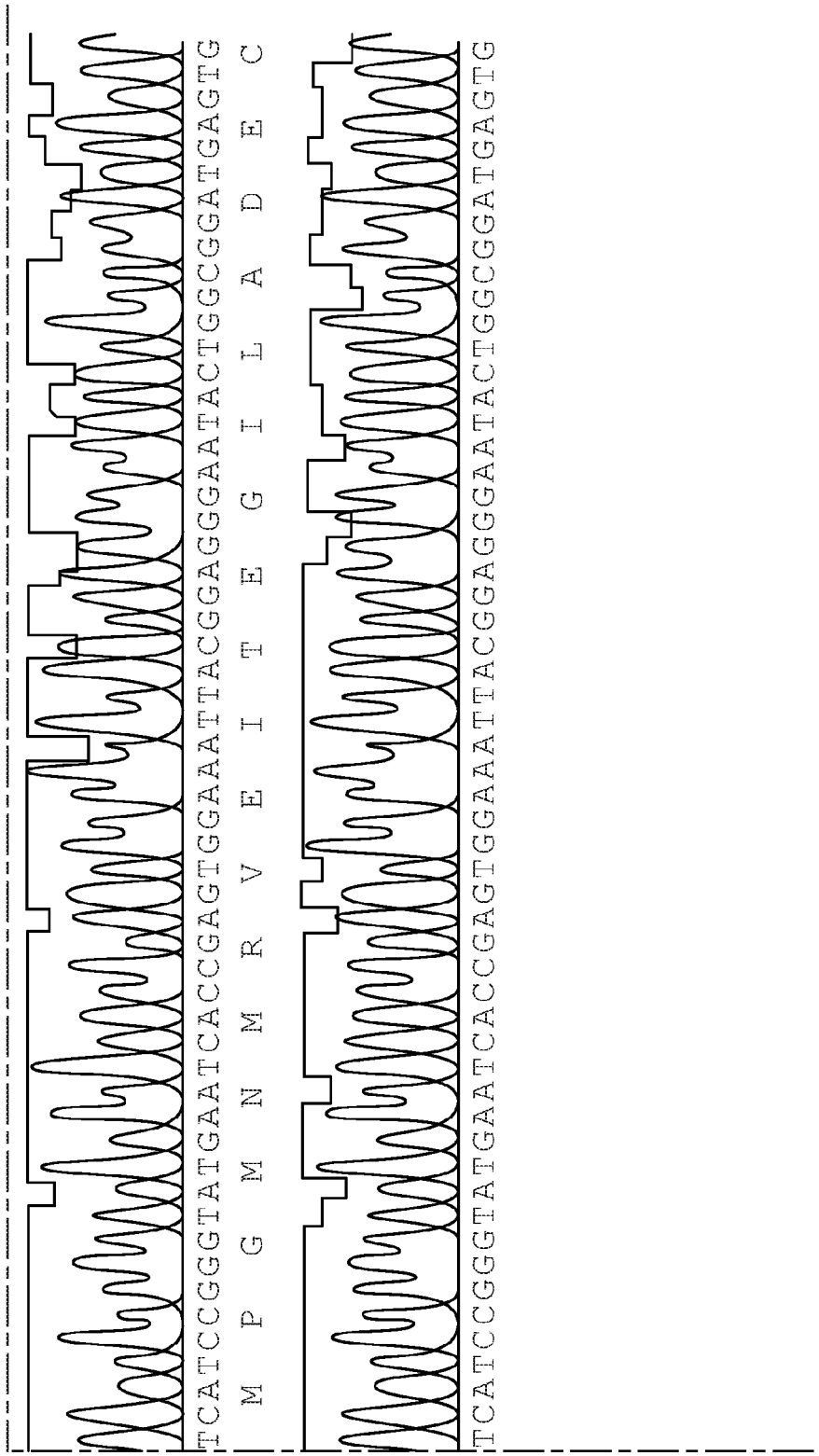



FIGURE 8 (Continued)

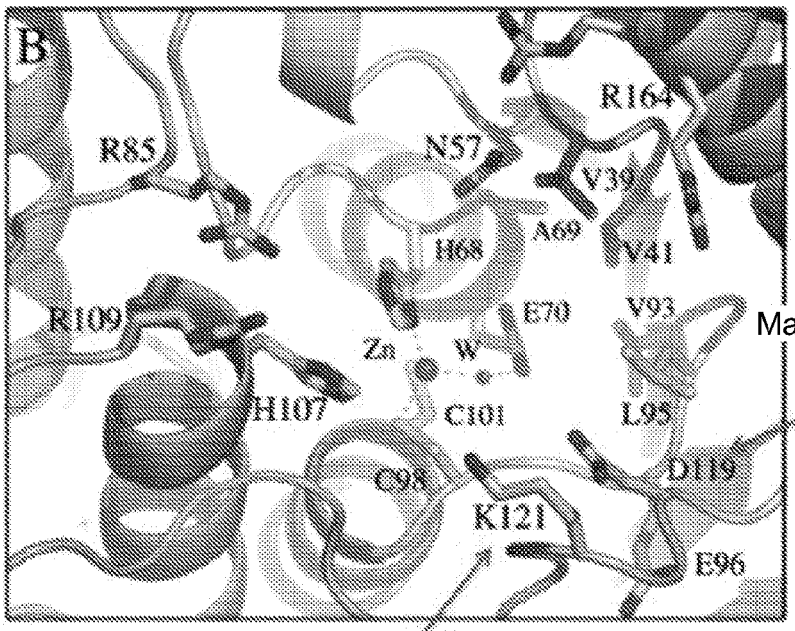

B
R164
R85
N57
V39
A69
H68
V41
R109
E70
V93
Zn
W
H107
C101
L95
C98
D119
K121
E96
Many mutations at this site
See mutations at this site

FIGURE 9

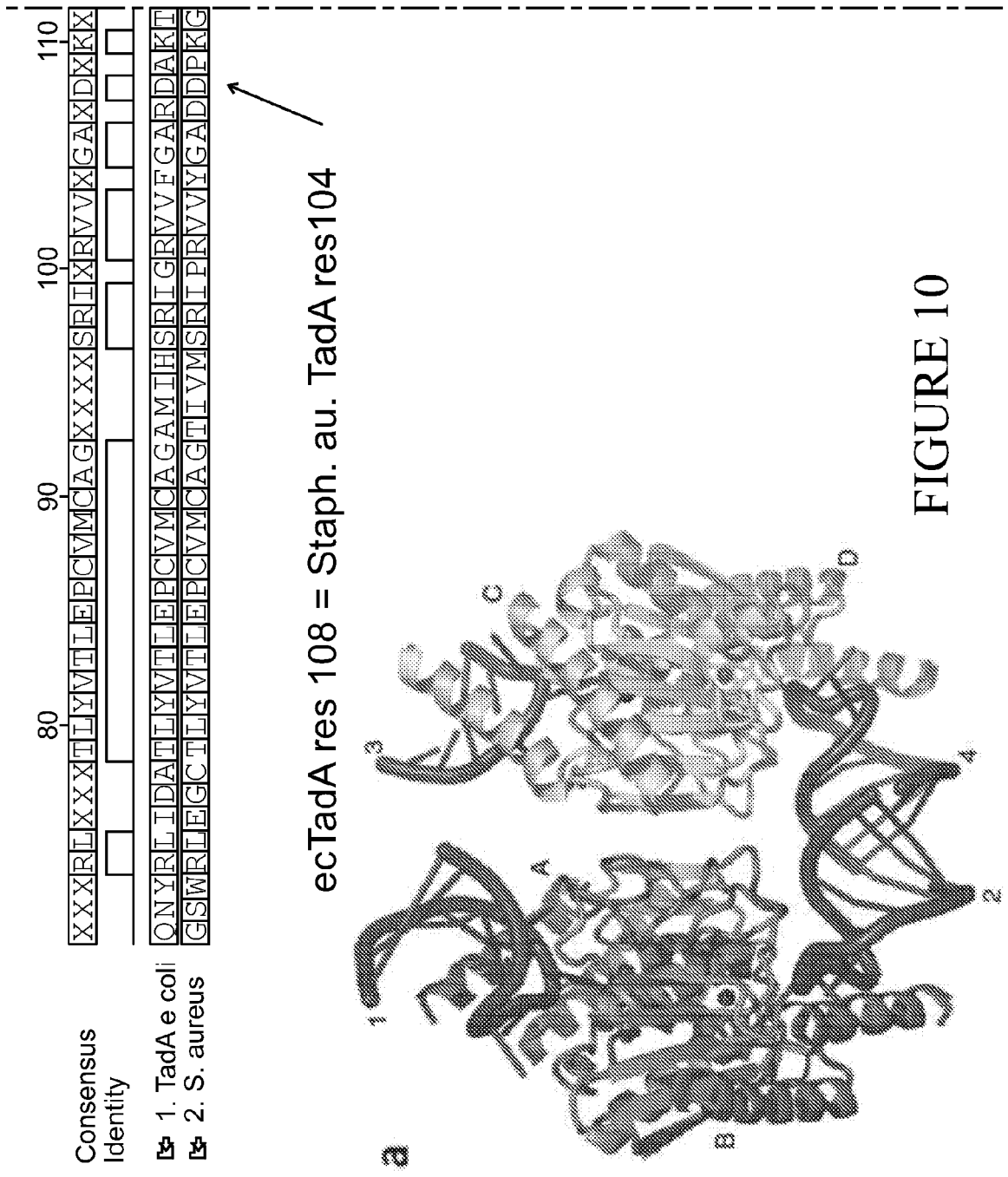

80
90
100
110
Consensus
Identity
1. TadA e coli
2. S. aureus
QNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKT
GSWRLEGCTLYVTLEPCVMCAGTIVMSRIPRVVYGADDPKG
ecTadA res 108 = Staph. au. TadA res104
a

FIGURE 10

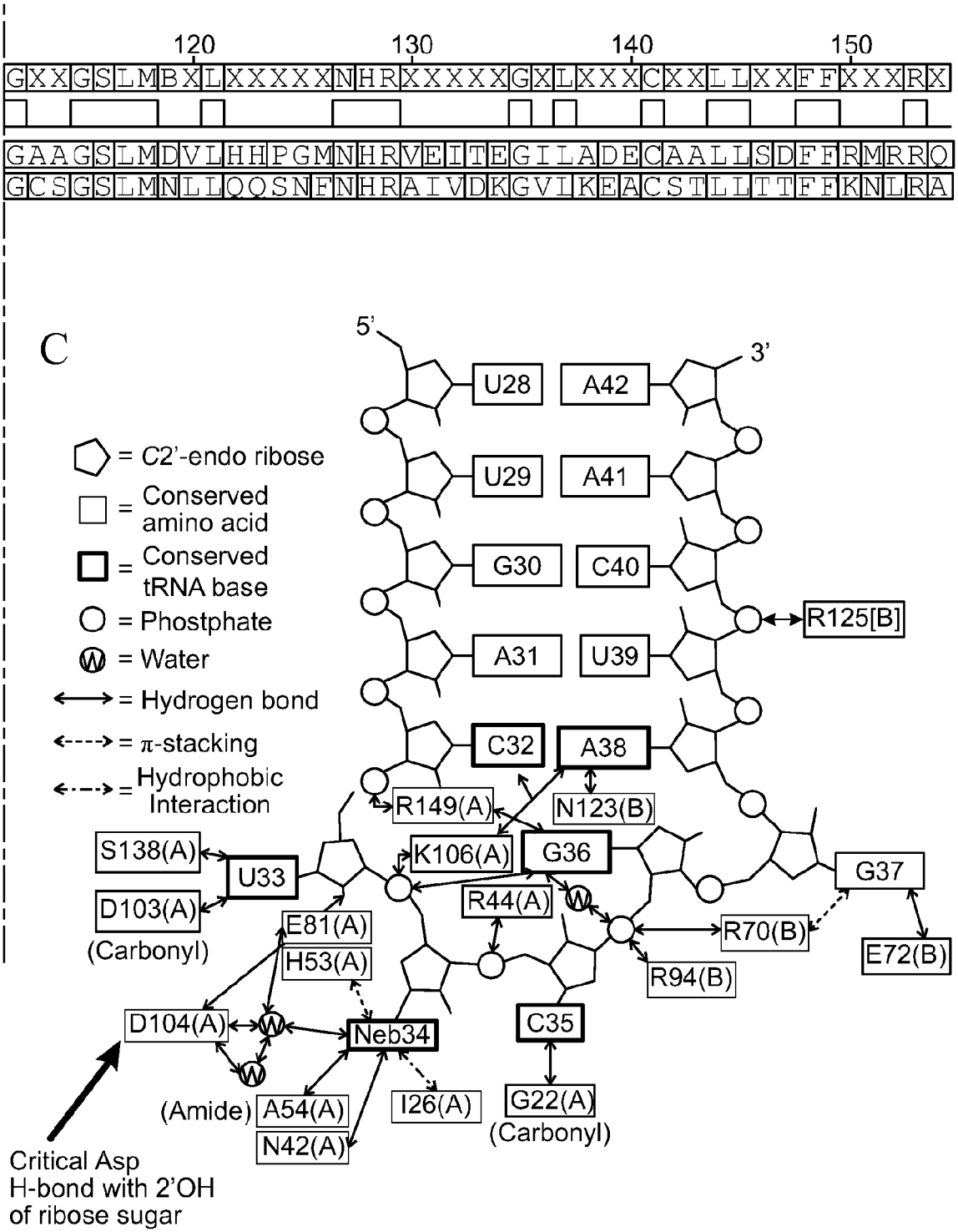



FIGURE 10 (Continued)

| construct | clone | position: | 8 | 17 | 18 | 23 | 34 | 45 | 51 | 56 | 59 | 85 | 94 | 95 | 102 | 104 | 106 | 107 | 108 | 110 | 118 | 127 | 138 | 149 | 151 | 153 | 154 | 156 | 157 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | wt res: | His | Thr | Leu | Trp | Leu | Trp | Arg | Ala | Glu | Glu | Met | Ile | Val | Phe | Ala | Arg | Asp | Lys | Met | Asn | Ala | Phe | Met | Arg | Gln | Ile | Lys |
| 1 | 2 | | | | | | | | | | | | | | | | | | Gly | | | | | | | | | | |
| 2 | 4 | | | | | | | | | | | | | | | | | Cys | | | | | | | | | | | |
| 3 | 5 | | Tyr | | | | | | | | | | | | | | | | Asn | | | Ser | | | | | | | |
| 4 | 7 | | | | | | | | | | | | | | | | Val | | Asn | | | | | Tyr | | | | | |
| 5 | 10 | | | | | | | | | Glu | | | | | | | | Cys | | | | | | | | | | | Arg |
| 6 | 11 | | | | | | | Leu | | | | | | | | | | His | Gly | | | | | | | | | | |
| 7 | 13 | | | | | | | | | | | Lys | | | | | | | | | | | | | | | | Asp | |
| 8 | 16 | | | | | | | | | | | Gly | | | | Leu | | | Asn | | | | | Tyr | | | | | |
| 9 | 18 | | | | | | | | | | | | | | | | | | Val | | | | | | | | | | |
| 10 | 19 | | | Ser | | | Ser | | | | Gly | | | | | | | | | Ile | | | | | | | | | |
| 11 | 21 | | | | | | | | | | | | | | | | | | Asn | | | | Val | | Val | Cys | | | |
| 12 | 23 | | | | | | | | | | | Lys | | | | | | | | | | | | | | | | Asp | |
| 13 | 25 | | | | | Leu | | | | | | | | Ile | | | | Pro | | | Lys | | | | | | | | |
| 14 | 28 | | | | | | | | His | Ser | | | | | Ala | | | | Ala | | | | | | | | | | |
| 15 | 29 | | | | Glu | | | | | | | | Leu | | | | | | Tyr | | | | | | | | Leu | | |
| 16 | 32 | | | | | | | | | | | | | | | | | Cys | Asn | | | | | | | | | | |

FIGURE 11

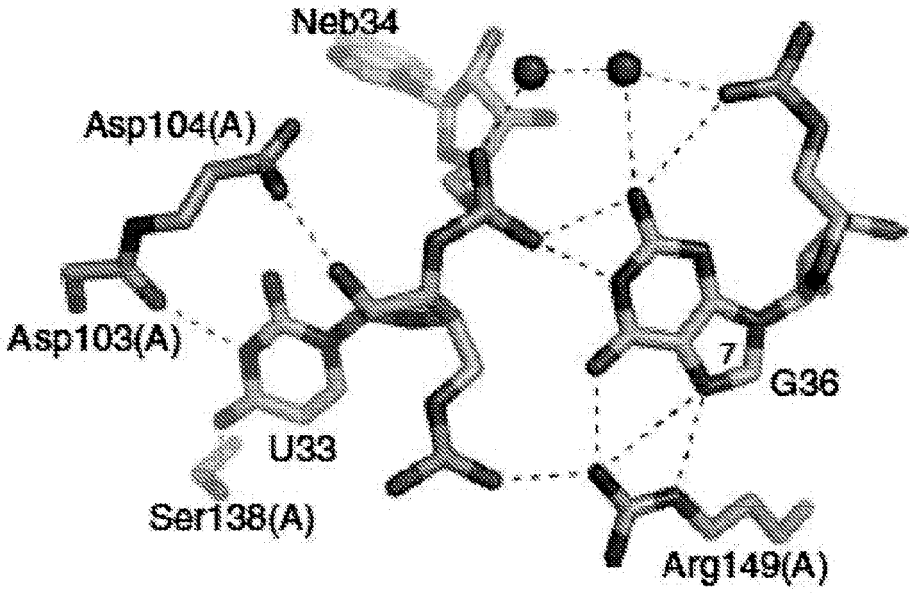



FIGURE 11 (Continued)

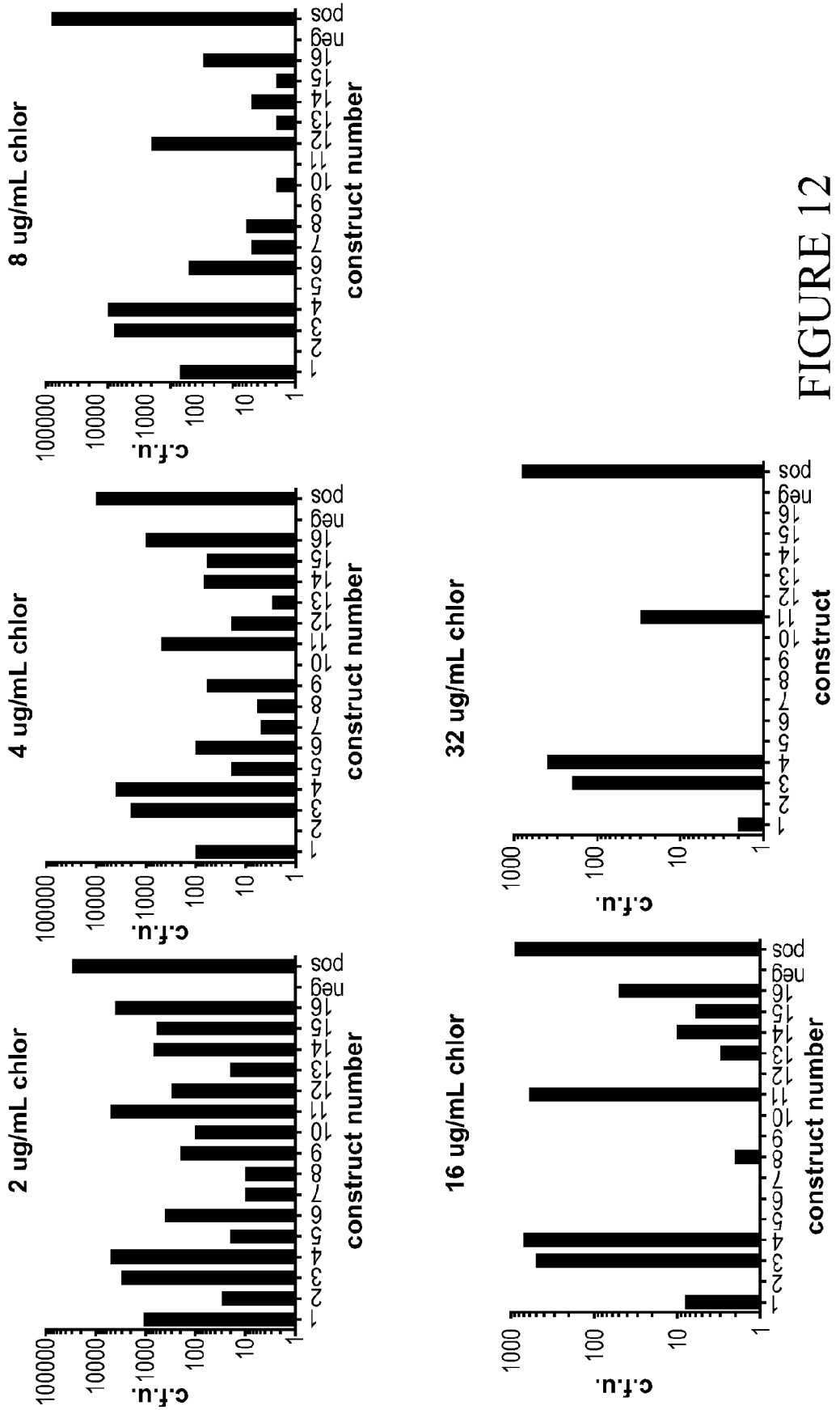

2 ug/mL chlor
4 ug/mL chlor
8 ug/mL chlor
16 ug/mL chlor
32 ug/mL chlor
c.f.u.
construct number
construct
pos
neg

FIGURE 12

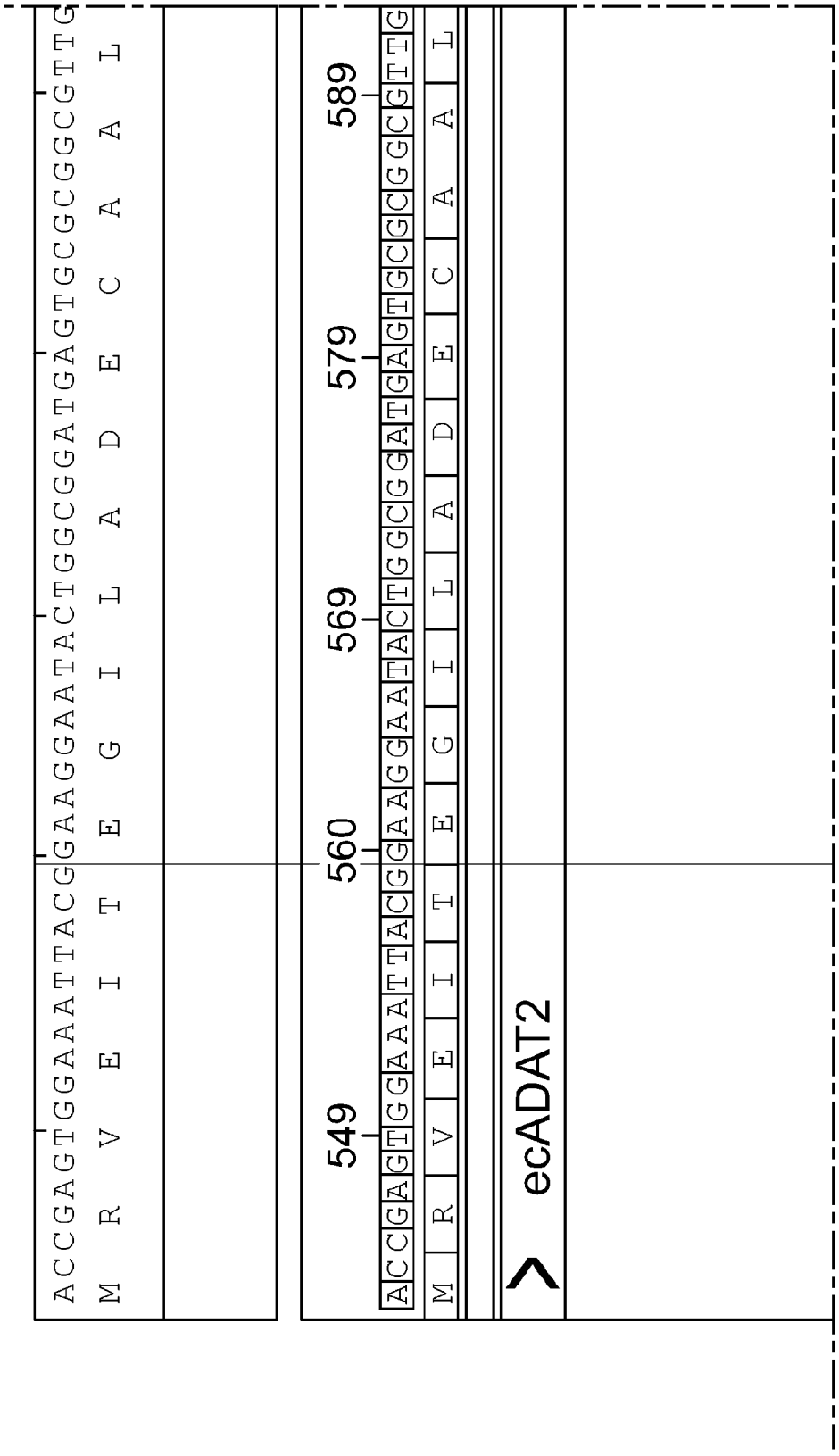



FIGURE 13

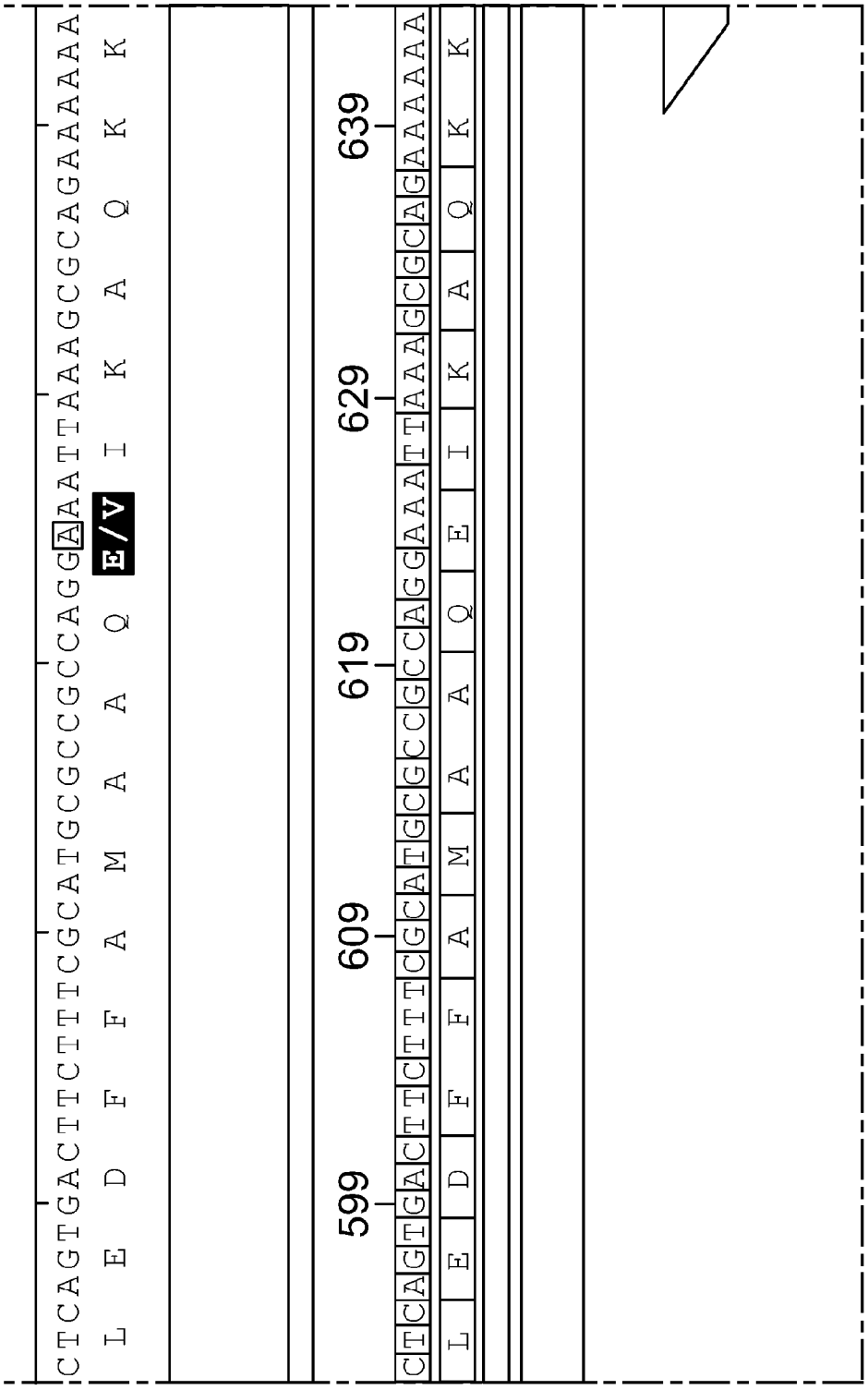



FIGURE 13 (Continued)

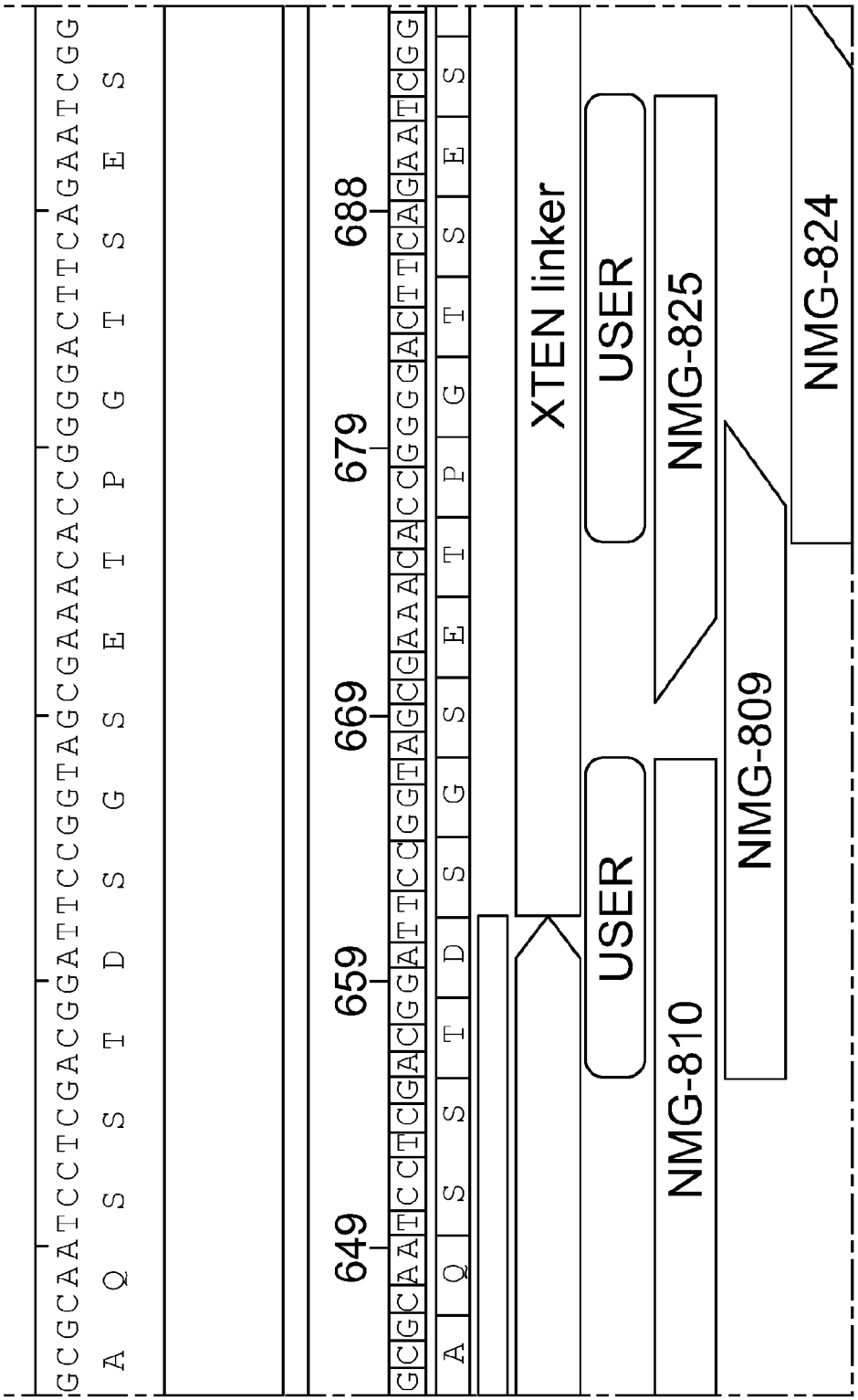



FIGURE 13 (Continued)

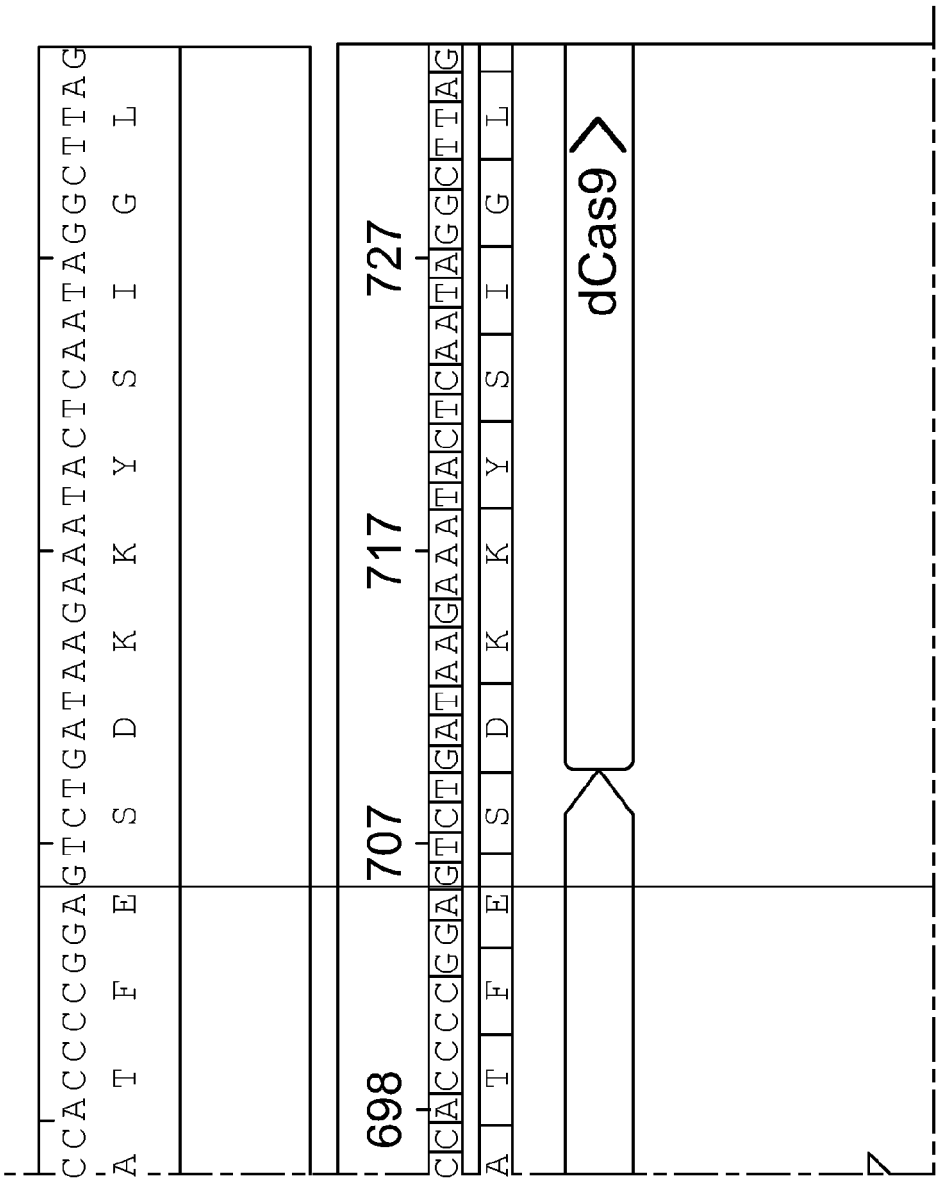



FIGURE 13 (Continued)

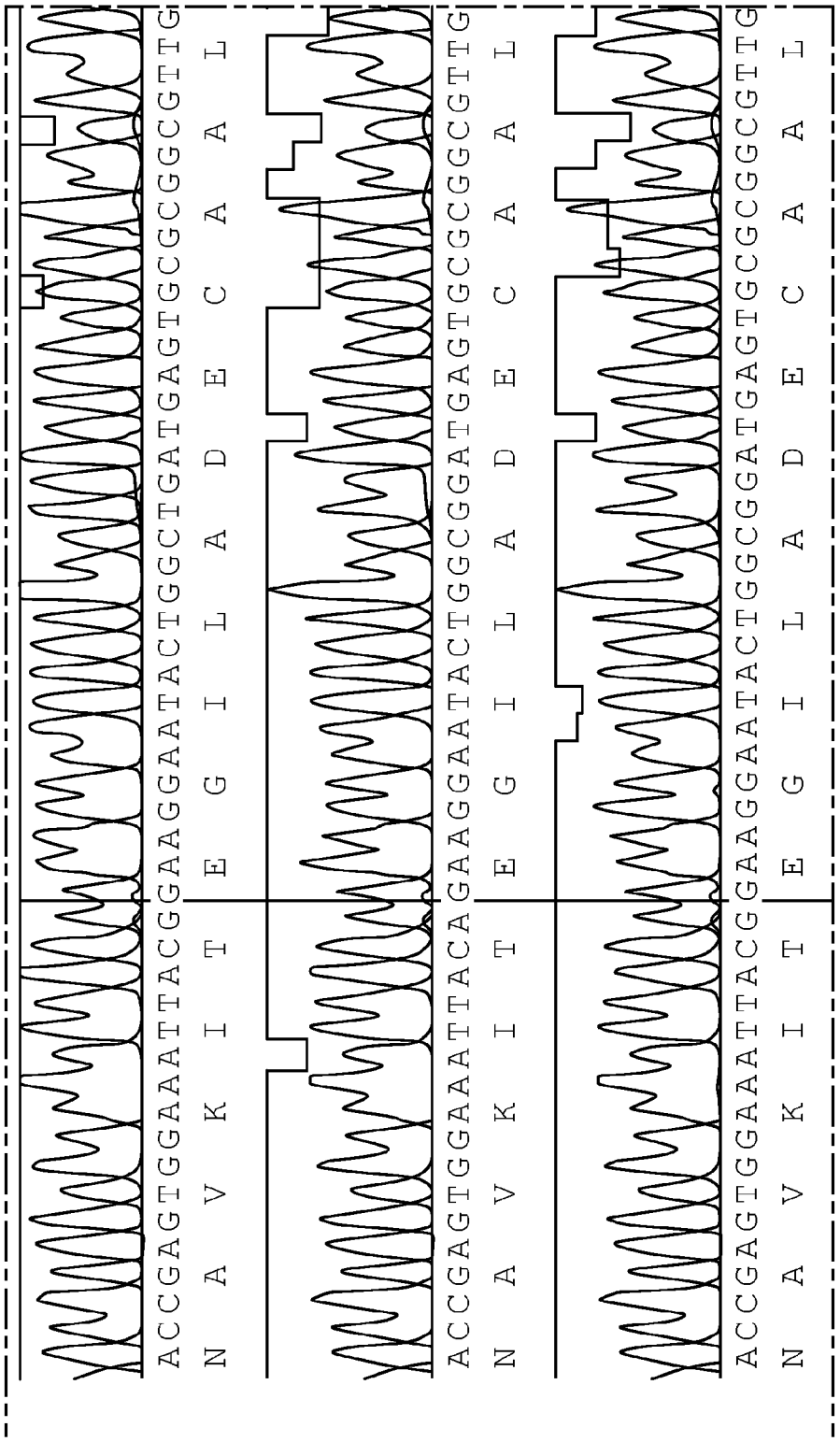



FIGURE 13 (Continued)

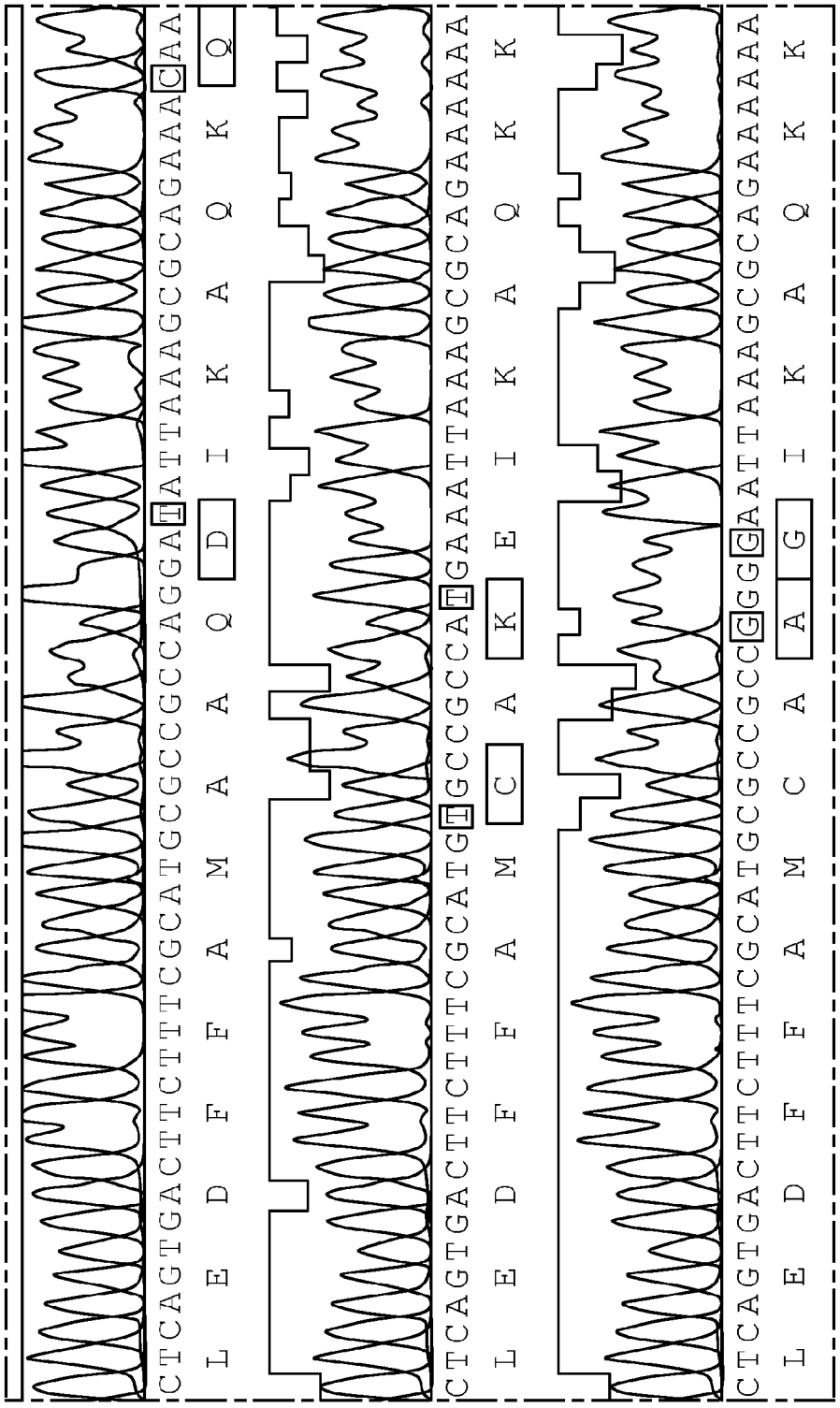



FIGURE 13 (Continued)

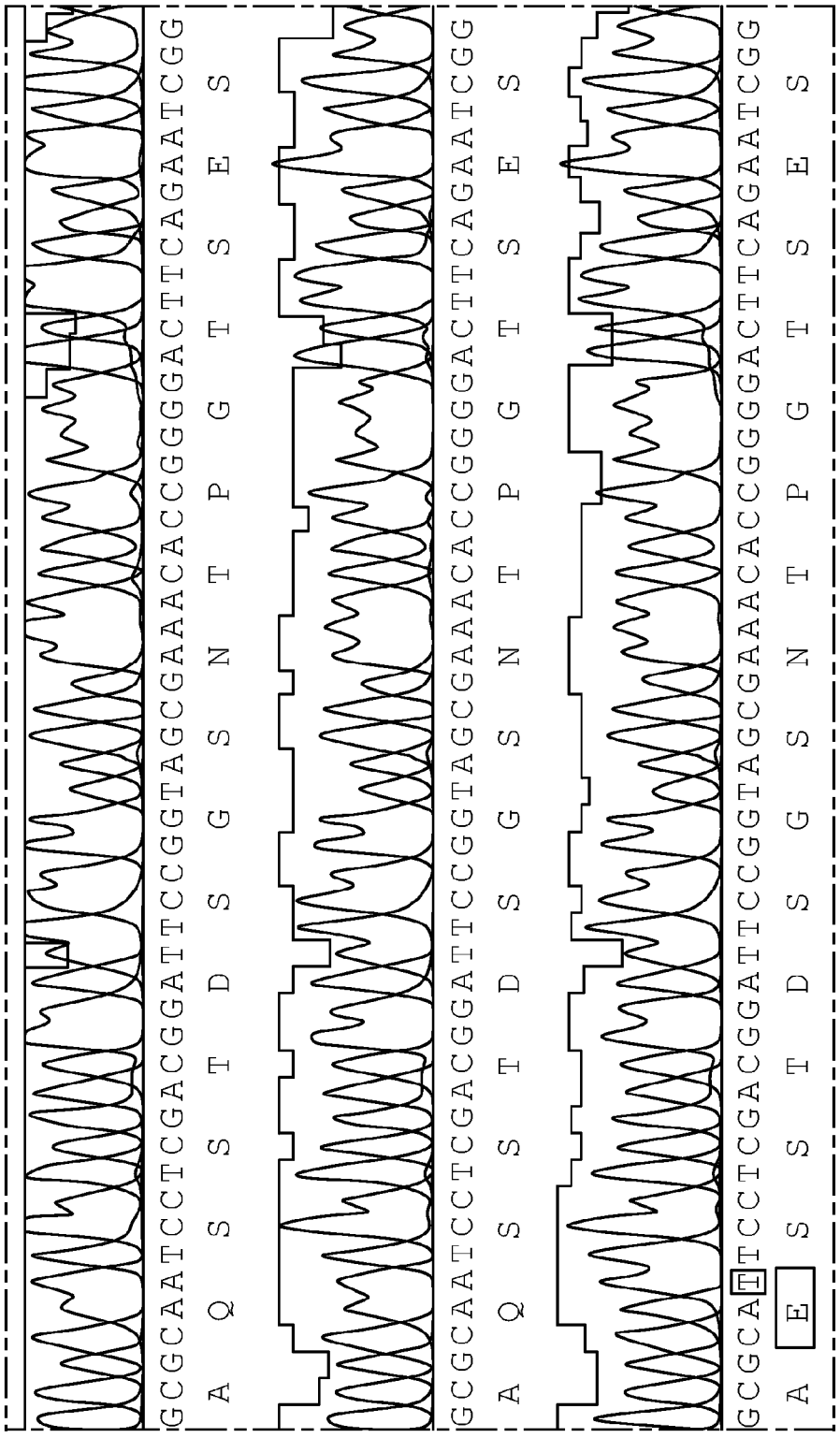



FIGURE 13 (Continued)

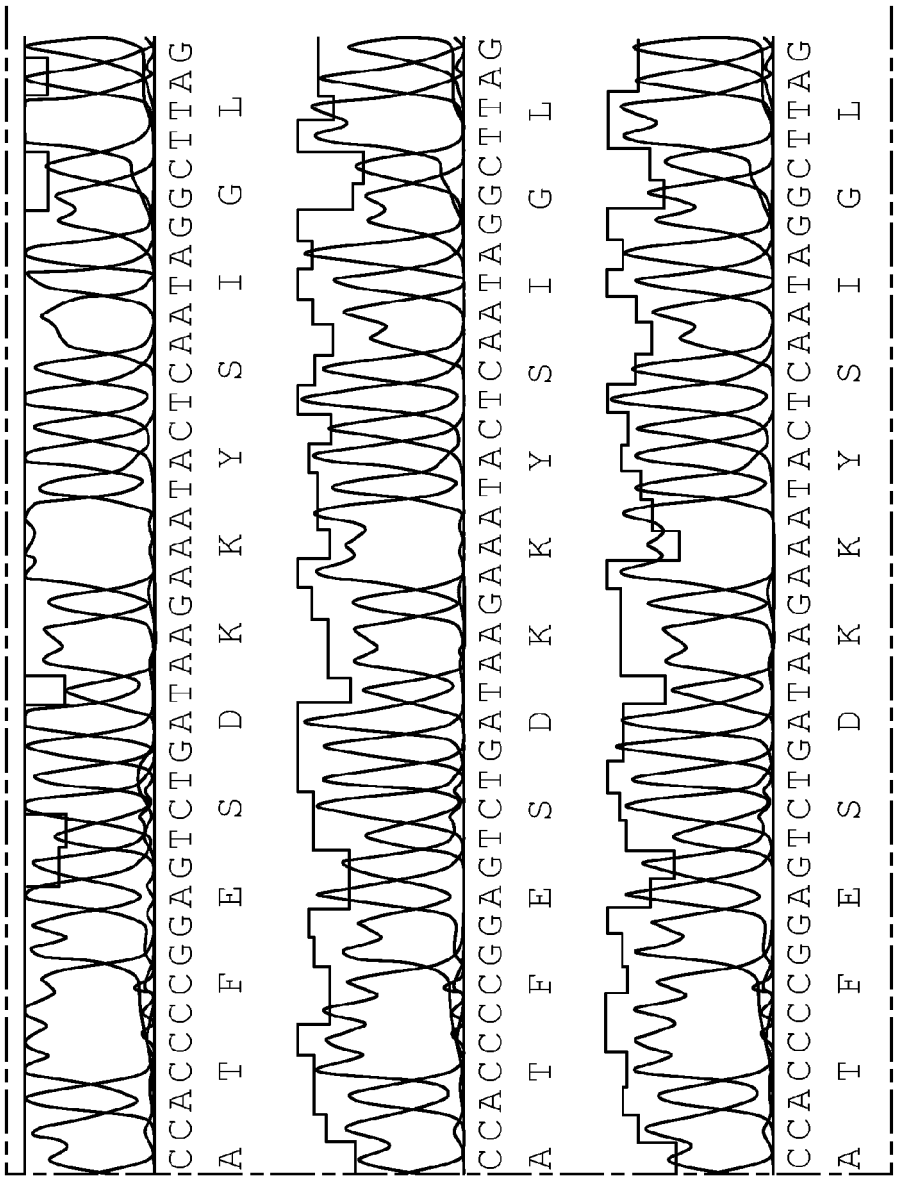



FIGURE 13 (Continued)

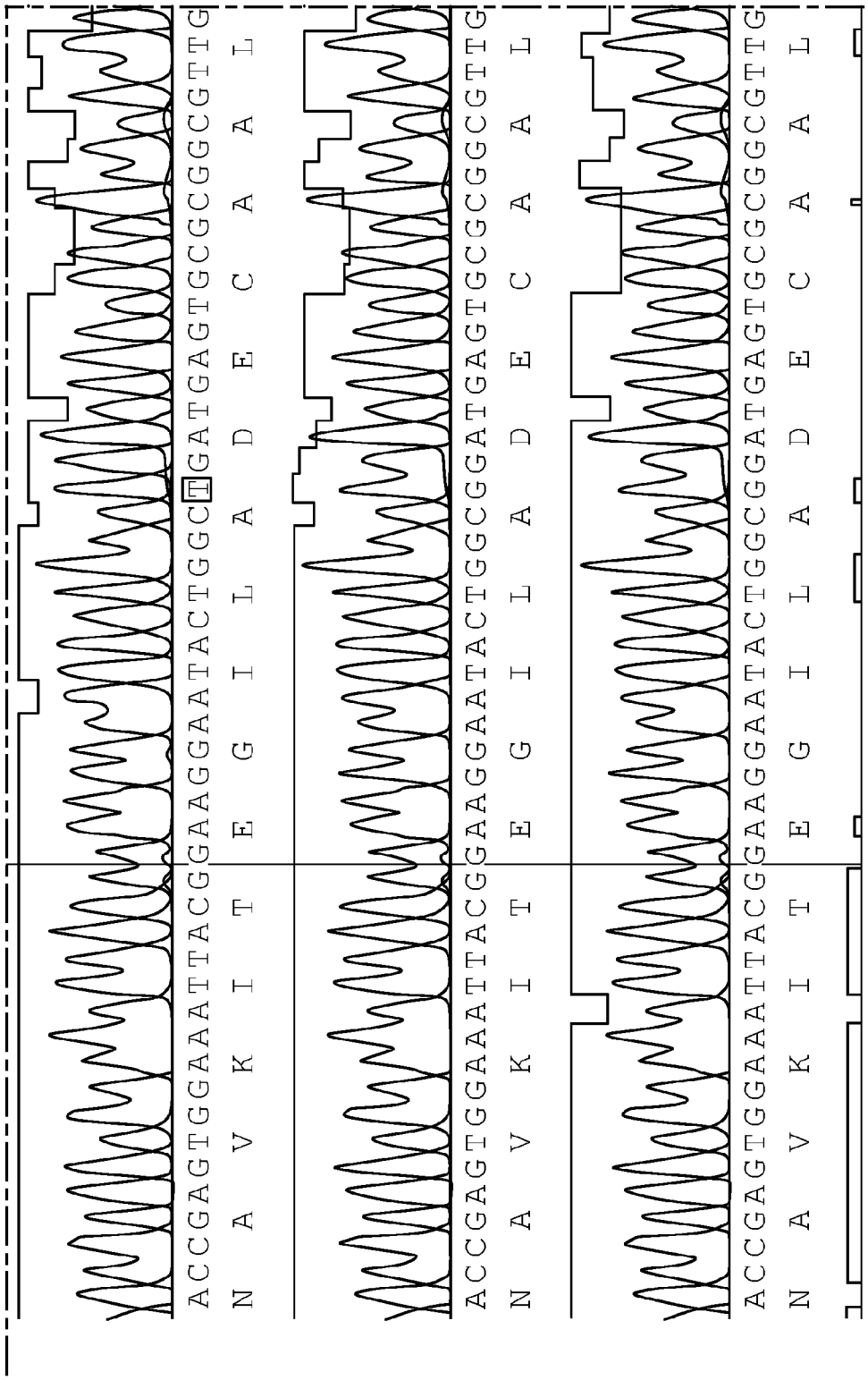



FIGURE 13 (Continued)

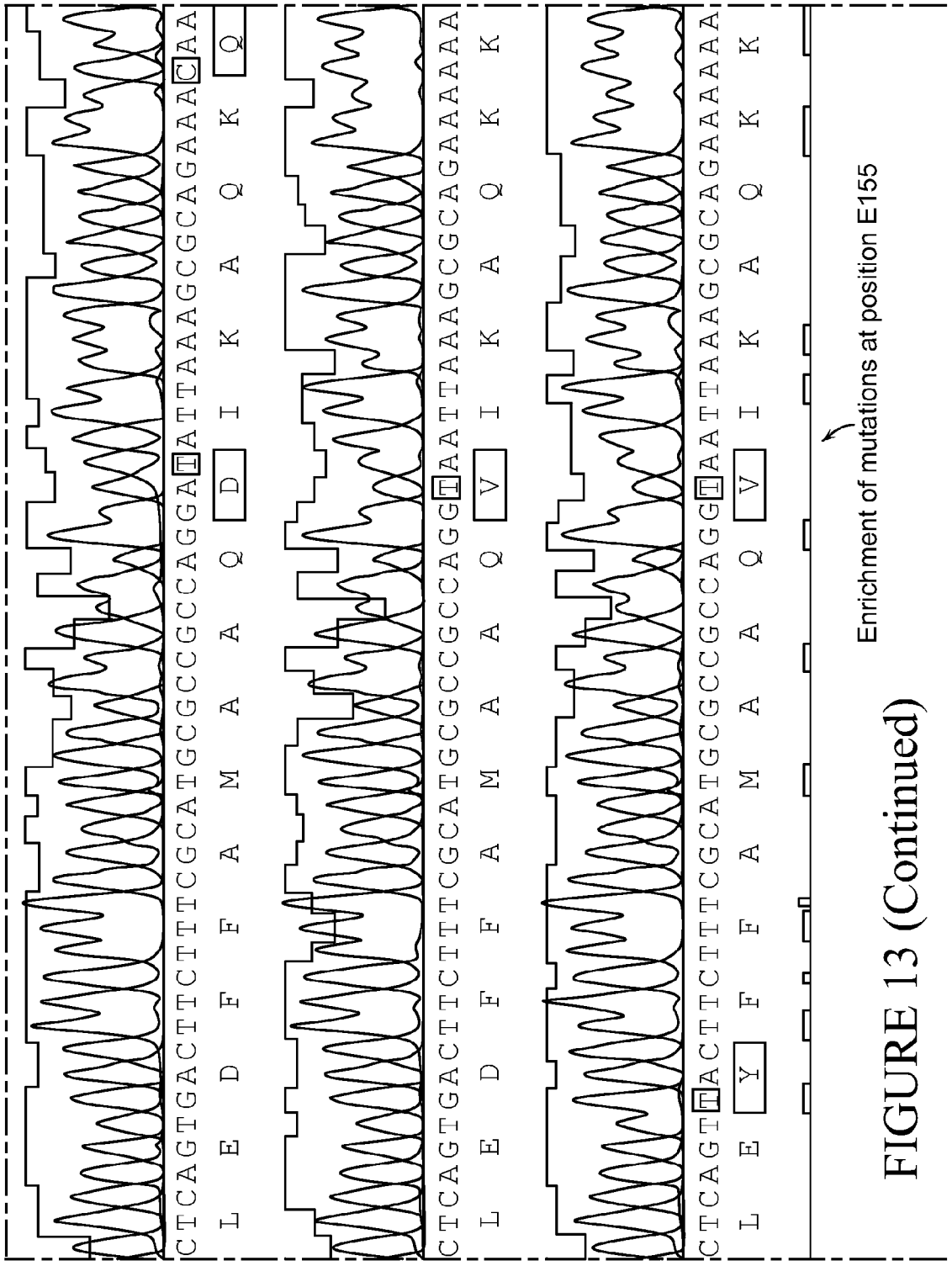



FIGURE 13 (Continued)

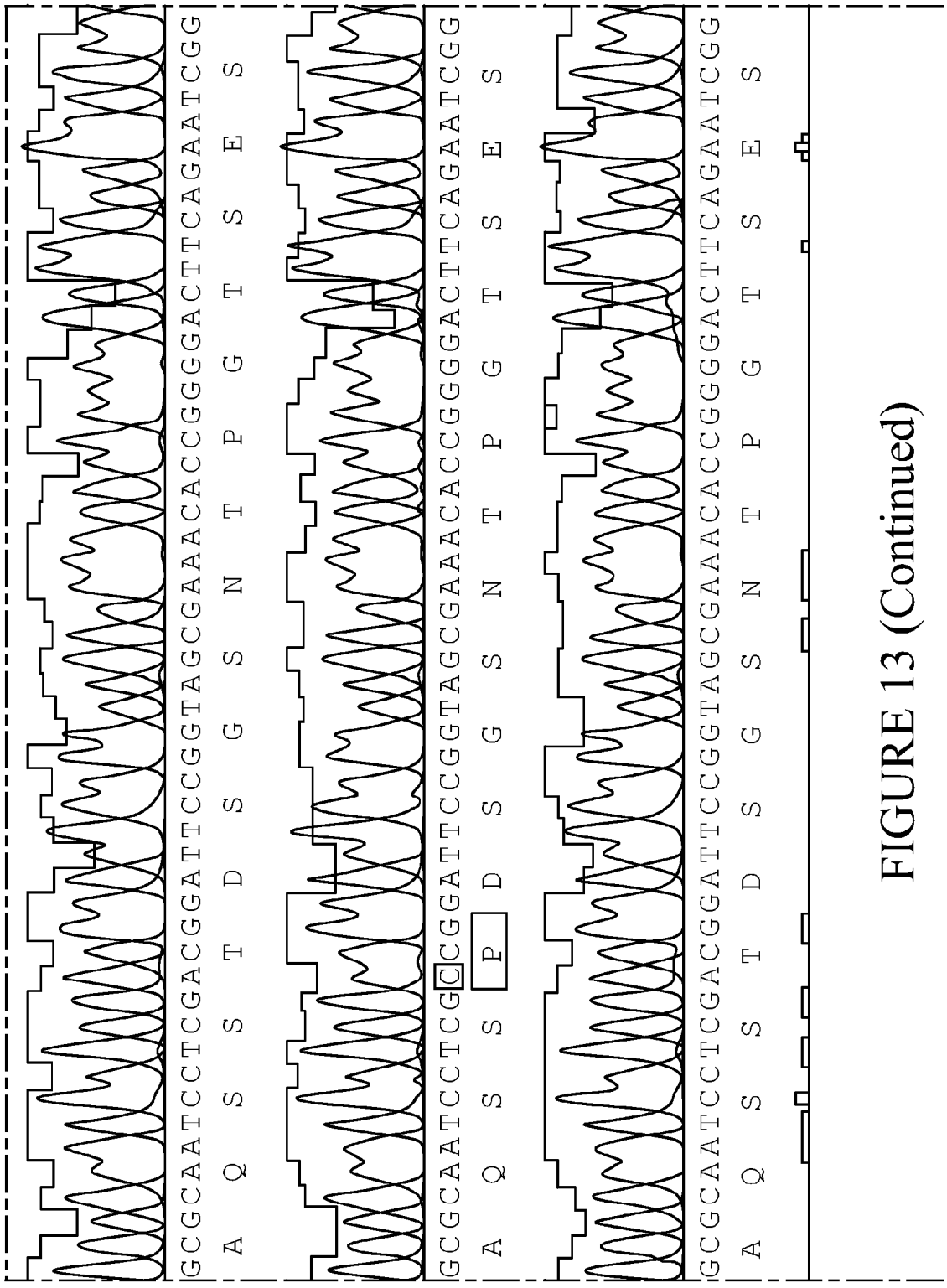



FIGURE 13 (Continued)

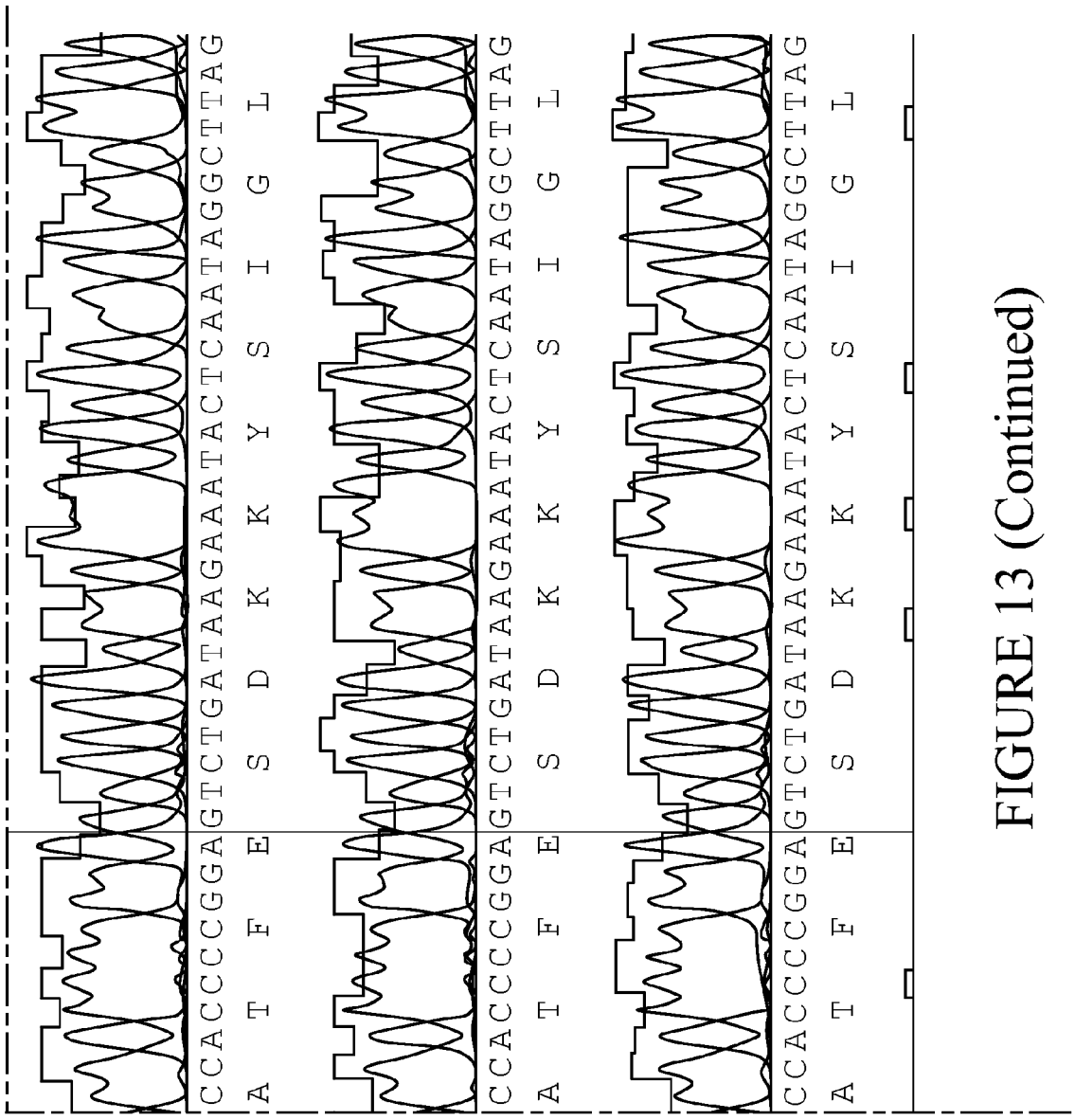



FIGURE 13 (Continued)

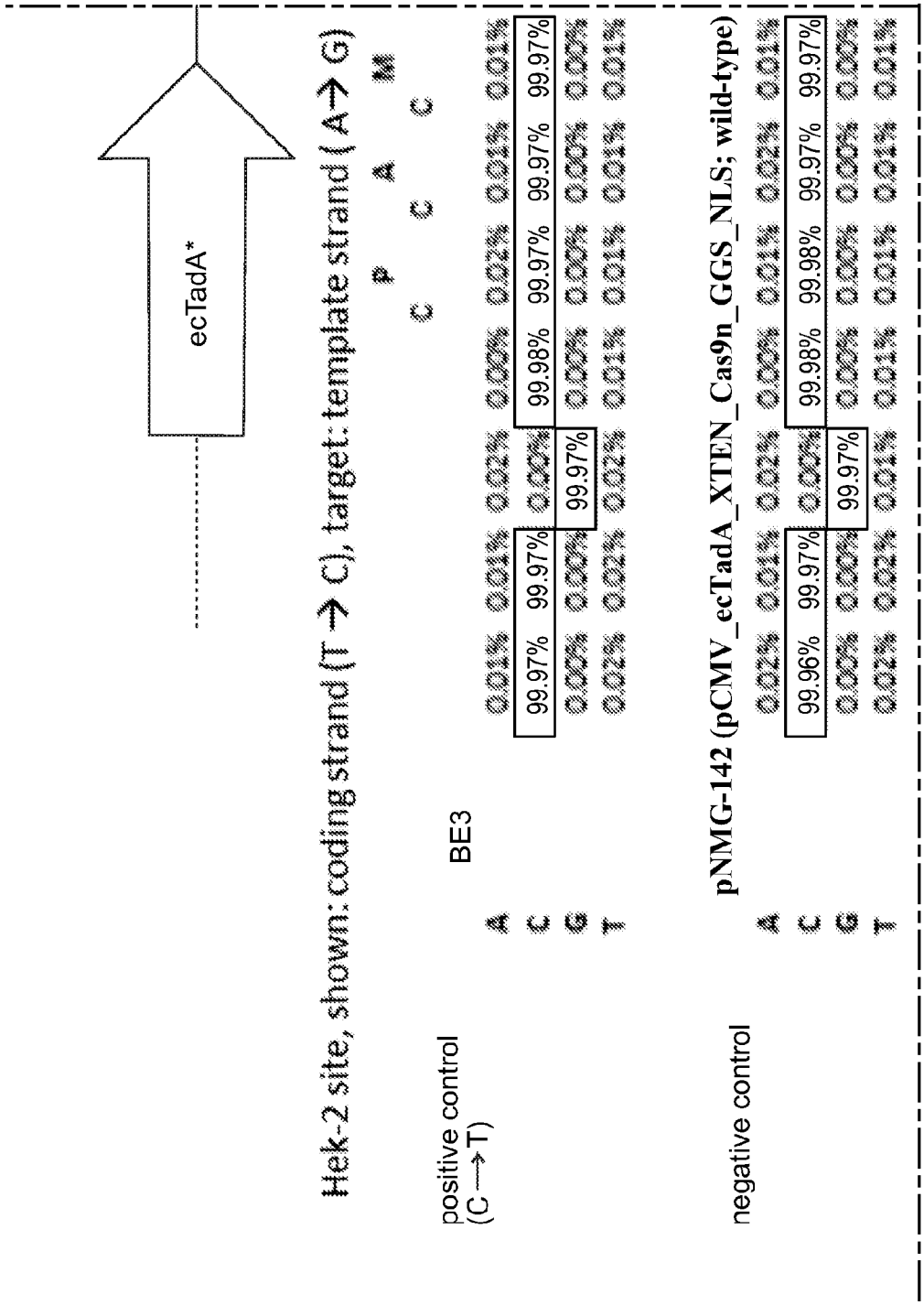

ecTadA*
Hek-2 site, shown: coding strand (T → C), target: template strand (A→ G)
P A M
C C C
positive control (C ⟶ T)
BE3
A 0.01% 0.01% 0.02% 0.00% 0.02% 0.01% 0.01%
C 99.97% 99.97% 0.00% 99.98% 99.97% 99.97% 99.97%
G 0.00% 0.00% 99.97% 0.00% 0.00% 0.00% 0.00%
T 0.02% 0.02% 0.02% 0.01% 0.01% 0.01% 0.01%
negative control
pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)
A 0.02% 0.01% 0.02% 0.00% 0.01% 0.02% 0.01%
C 99.96% 99.97% 0.00% 99.98% 99.98% 99.97% 99.97%
G 0.00% 0.00% 99.97% 0.00% 0.00% 0.00% 0.00%
T 0.02% 0.02% 0.01% 0.01% 0.01% 0.01% 0.01%

FIGURE 14

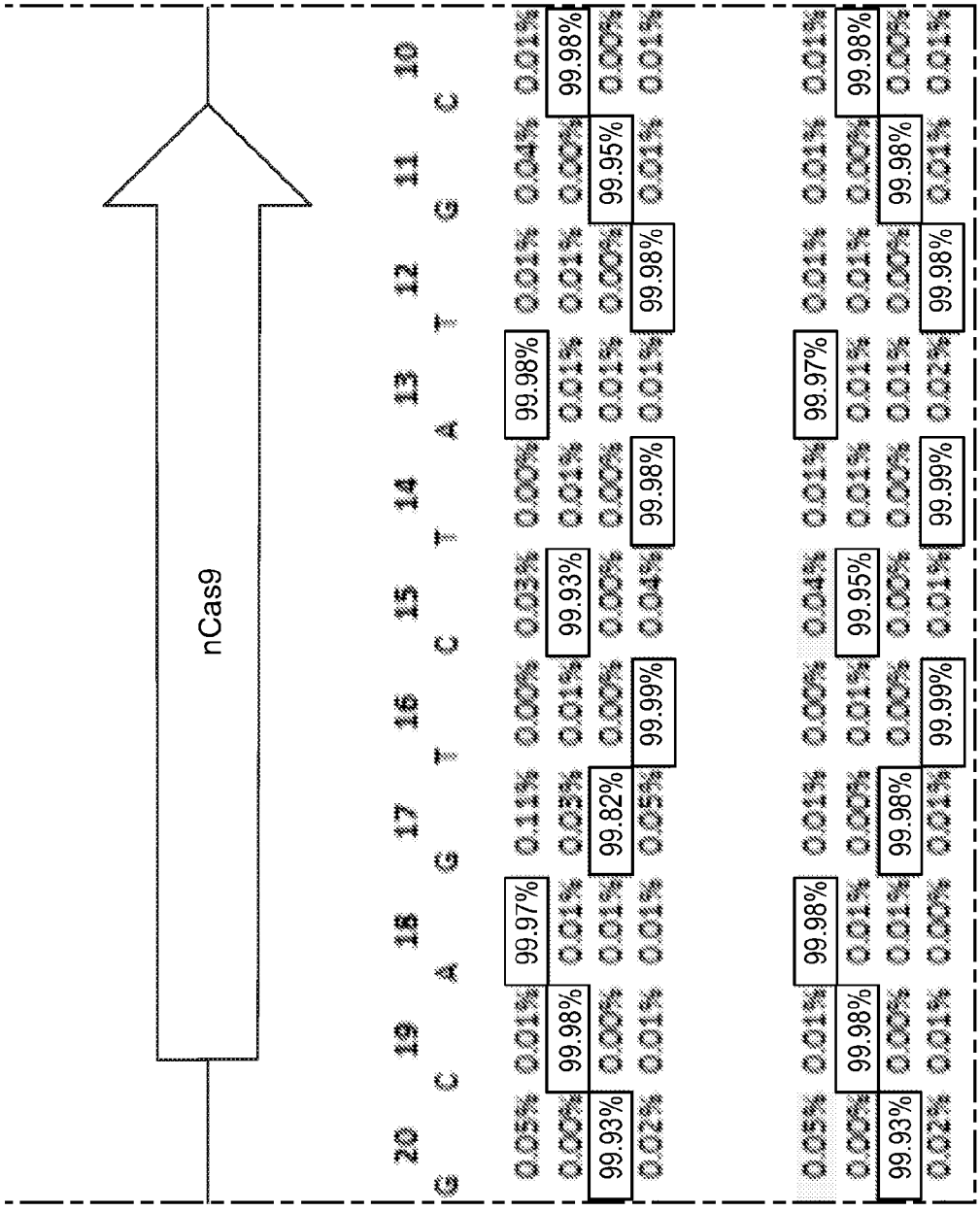



FIGURE 14 (Continued)

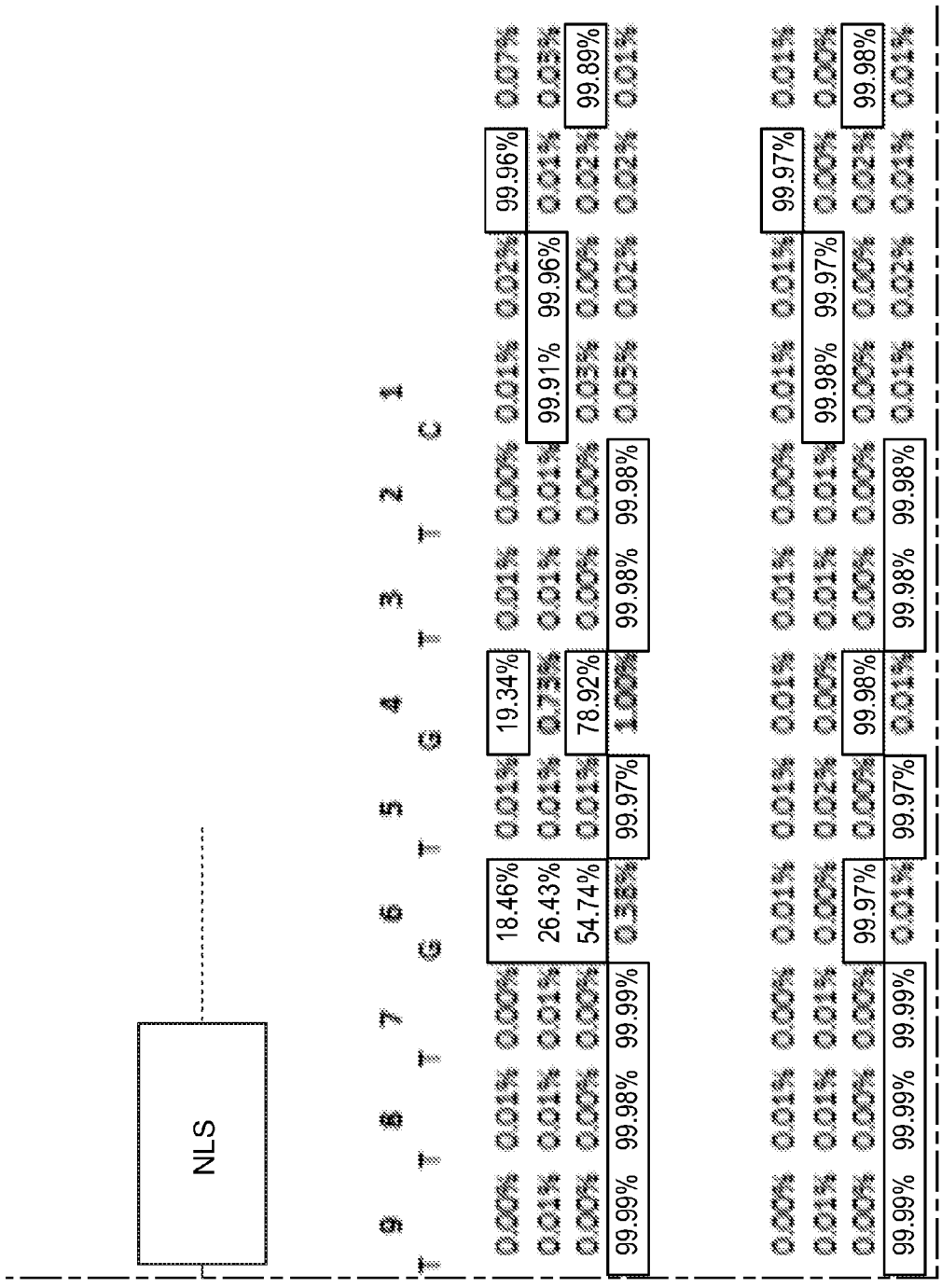



FIGURE 14 (Continued)

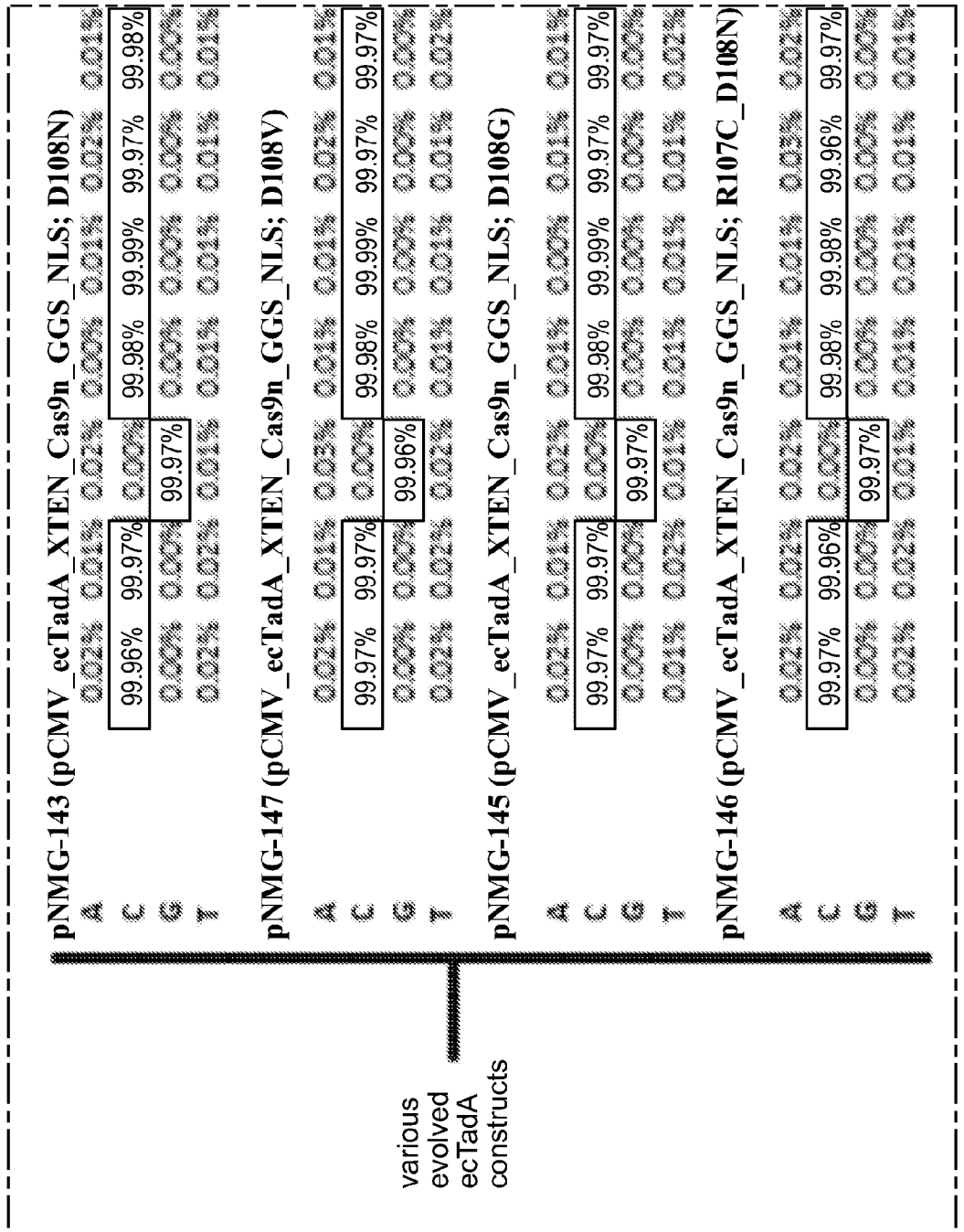



FIGURE 14 (Continued)



FIGURE 14 (Continued)

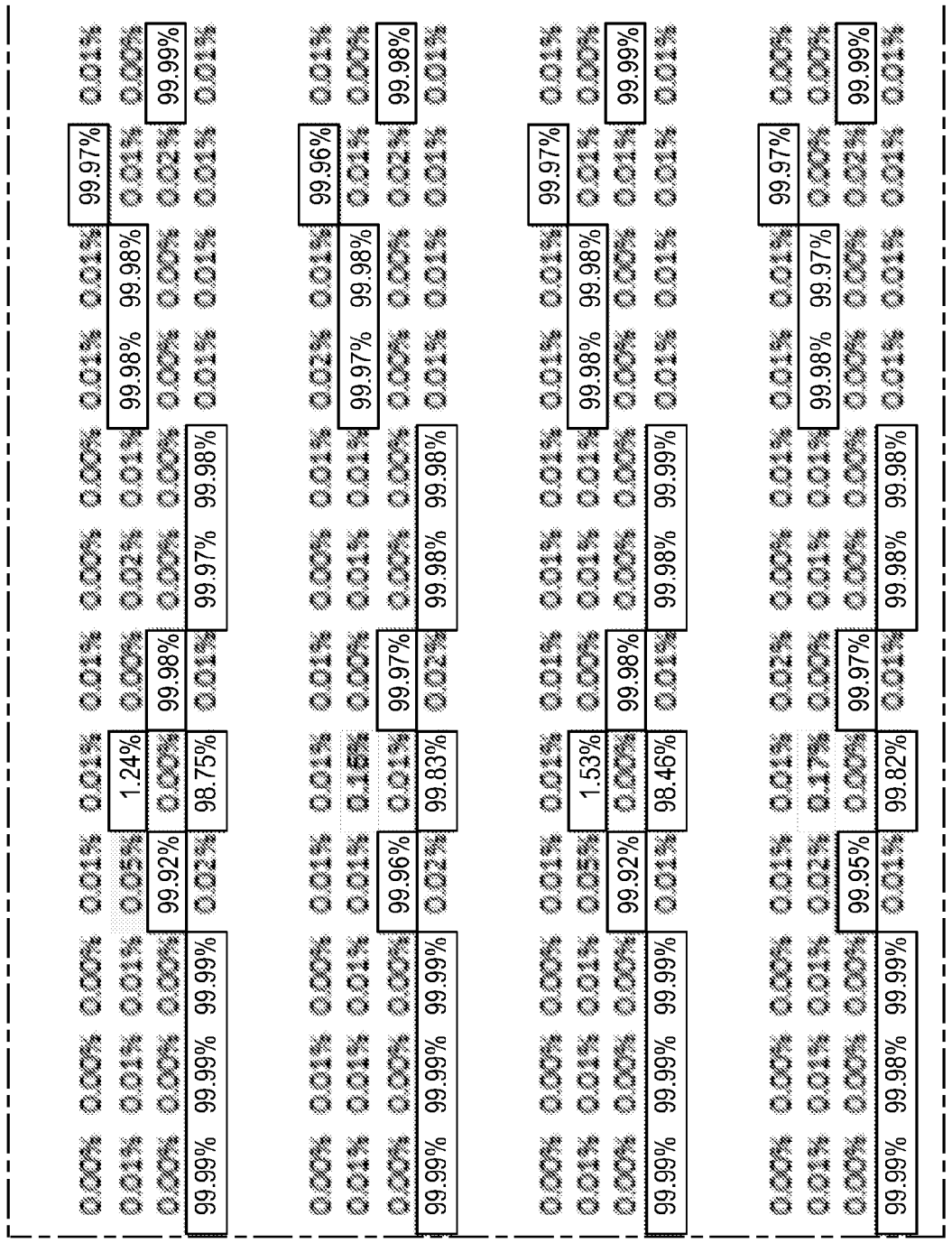


FIGURE 14 (Continued)

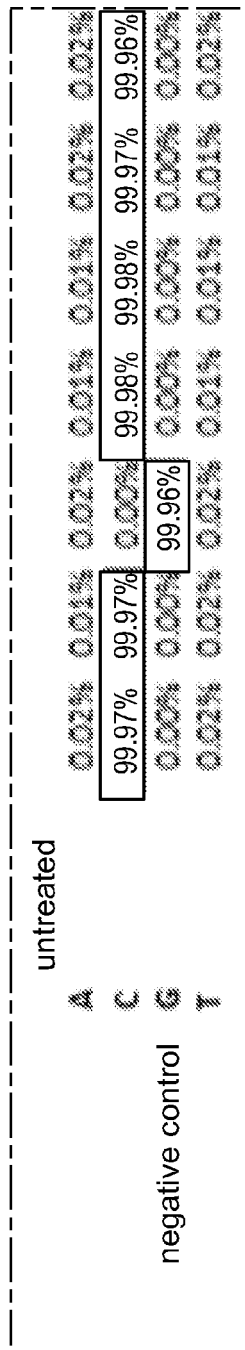



FIGURE 14 (Continued)

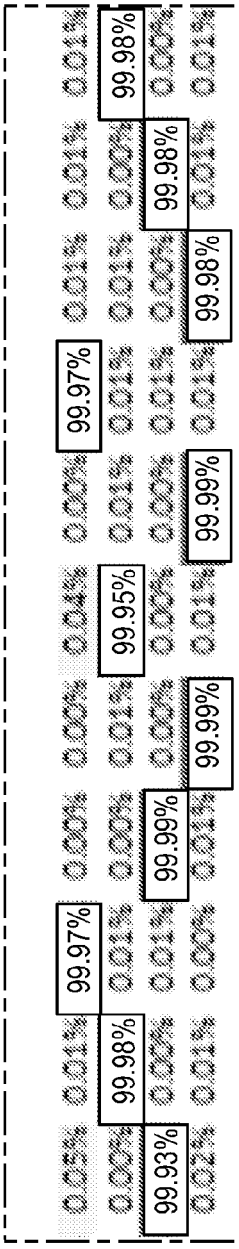



FIGURE 14 (Continued)

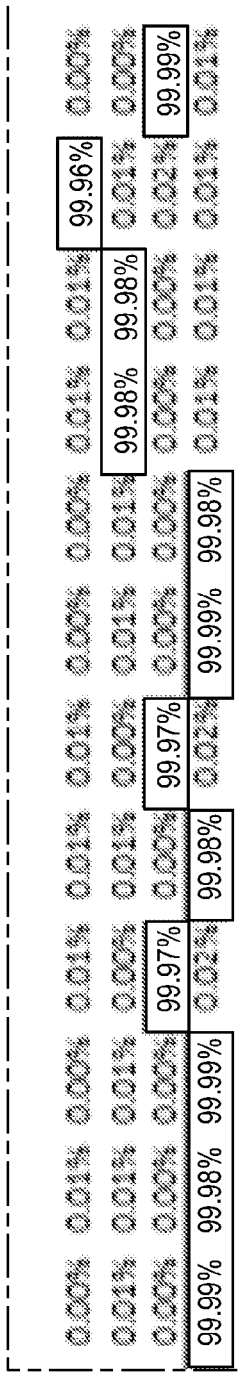



FIGURE 14 (Continued)

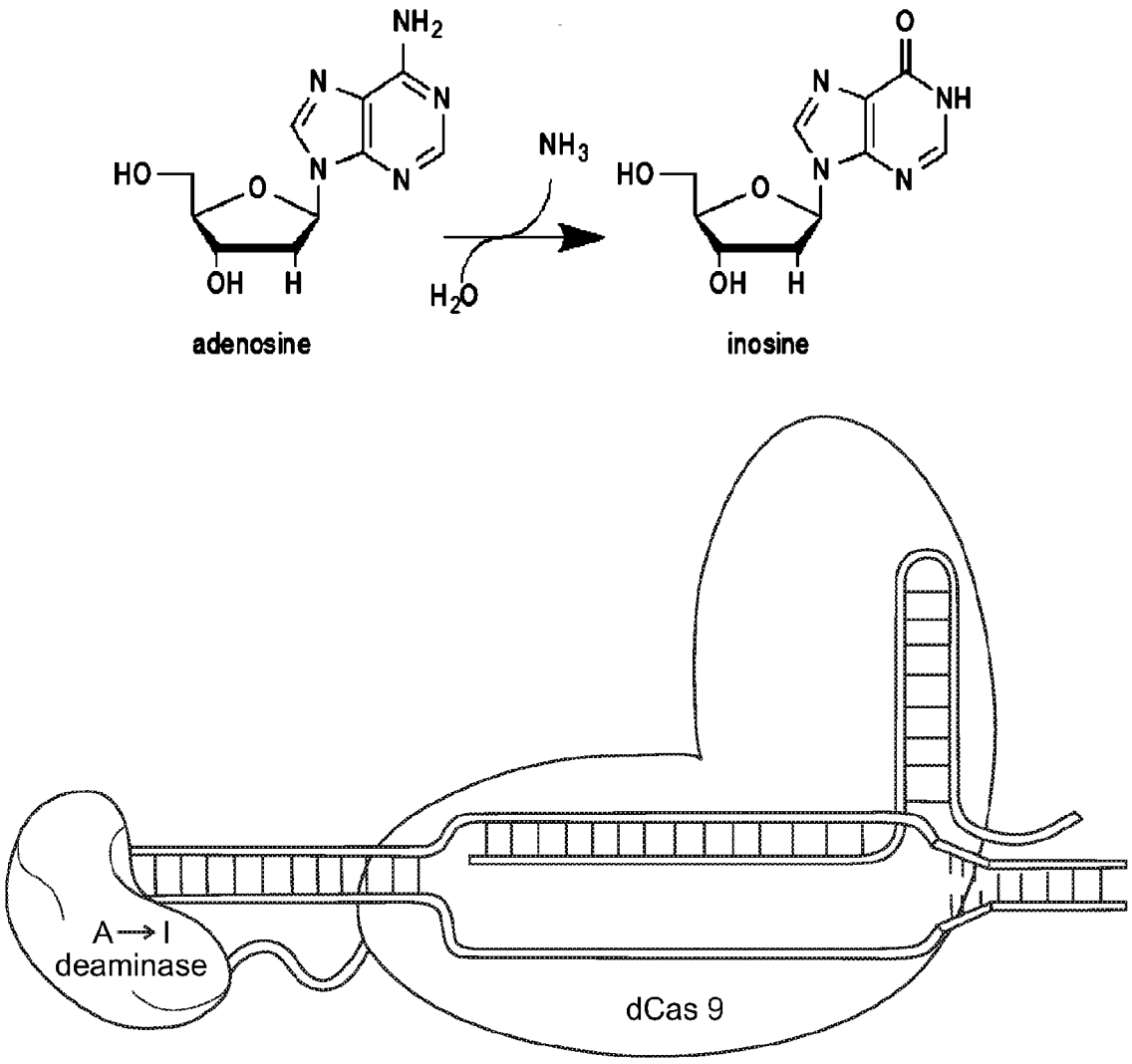
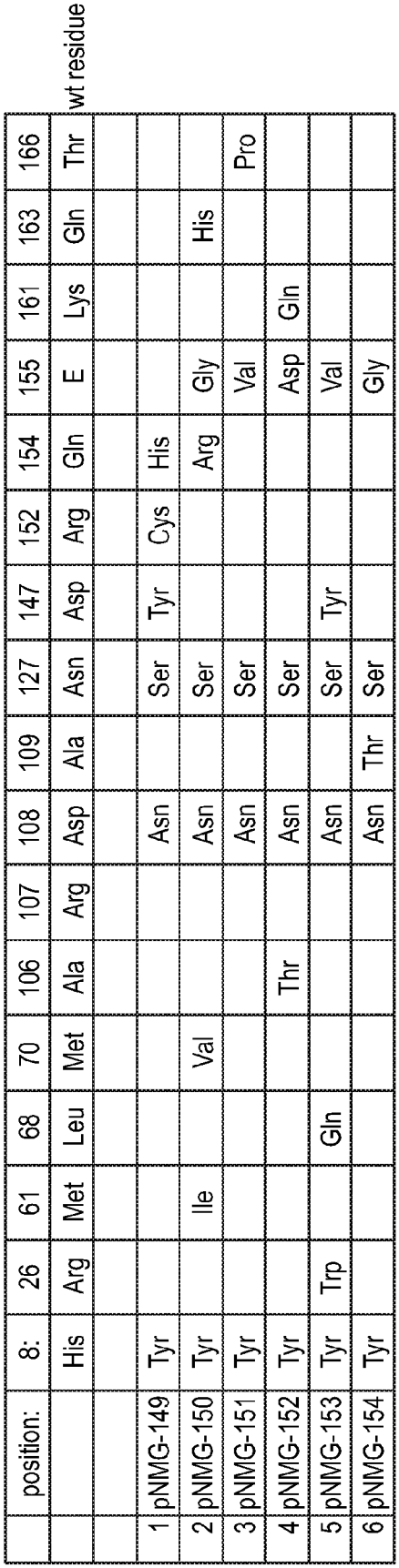

NH2
N
N
N
N
HO
O
OH
H
adenosine
NH3
H2O
O
NH
N
N
N
N
HO
O
OH
H
inosine
A→I
deaminase
dCas 9

FIGURE 15



| | position: | 8: | 26 | 61 | 68 | 70 | 106 | 107 | 108 | 109 | 127 | 147 | 152 | 154 | 155 | 161 | 163 | 166 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | His | Arg | Met | Leu | Met | Ala | Arg | Asp | Ala | Asn | Asp | Arg | Gln | E | Lys | Gln | Thr |
| | | | | | | | | | | | | | | | | | | wt residue |
| 1 | pNMG-149 | Tyr | | | | | | | Asn | | Ser | Tyr | Cys | His | | | | |
| 2 | pNMG-150 | Tyr | | Ile | | Val | | | Asn | | Ser | | | Arg | Gly | | His | |
| 3 | pNMG-151 | Tyr | | | | | | | Asn | | Ser | | | | Val | | | Pro |
| 4 | pNMG-152 | Tyr | | | | | Thr | | Asn | | Ser | | | | Asp | Gln | | |
| 5 | pNMG-153 | Tyr | Trp | | Gln | | | | Asn | | Ser | Tyr | | | Val | | | |
| 6 | pNMG-154 | Tyr | | | | | | | Asn | Thr | Ser | | | | Gly | | | |

FIGURE 16

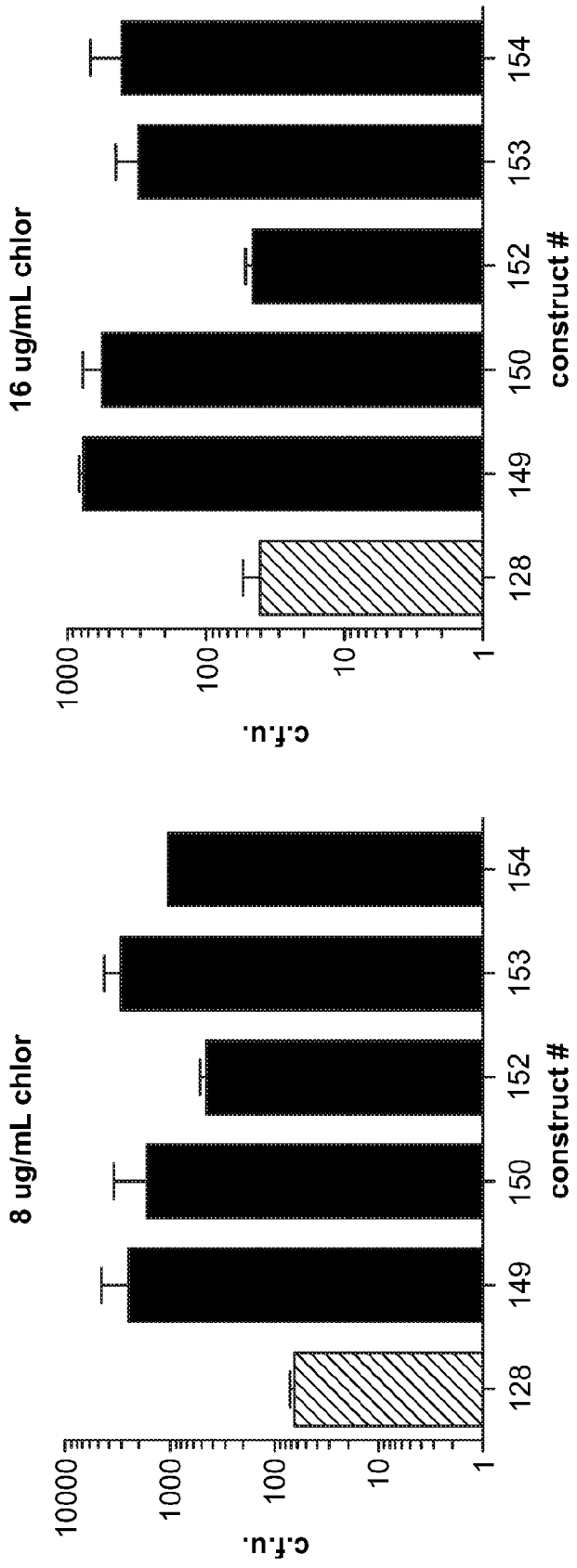

16 ug/mL chlor
c.f.u.
1000
100
10
1
128
149
150
152
153
154
construct #
8 ug/mL chlor
c.f.u.
10000
1000
100
10
1
128
149
150
152
153
154
construct #

FIGURE 17

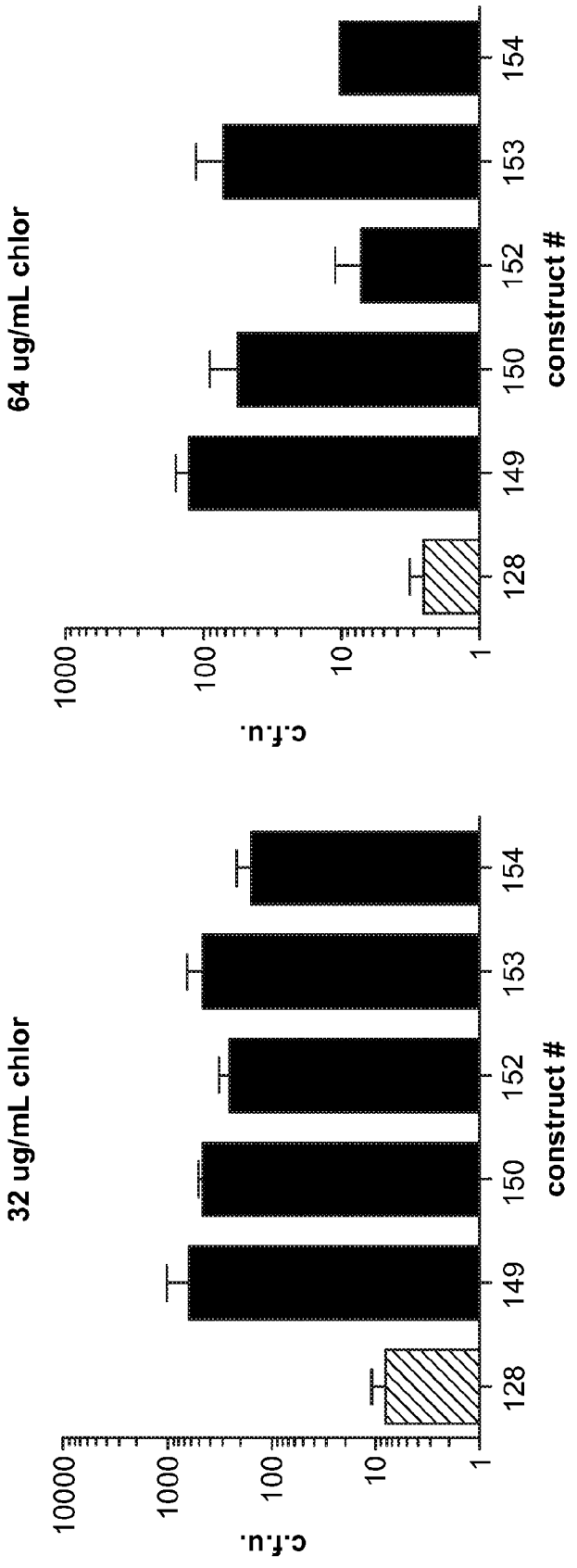



FIGURE 17 (Continued)

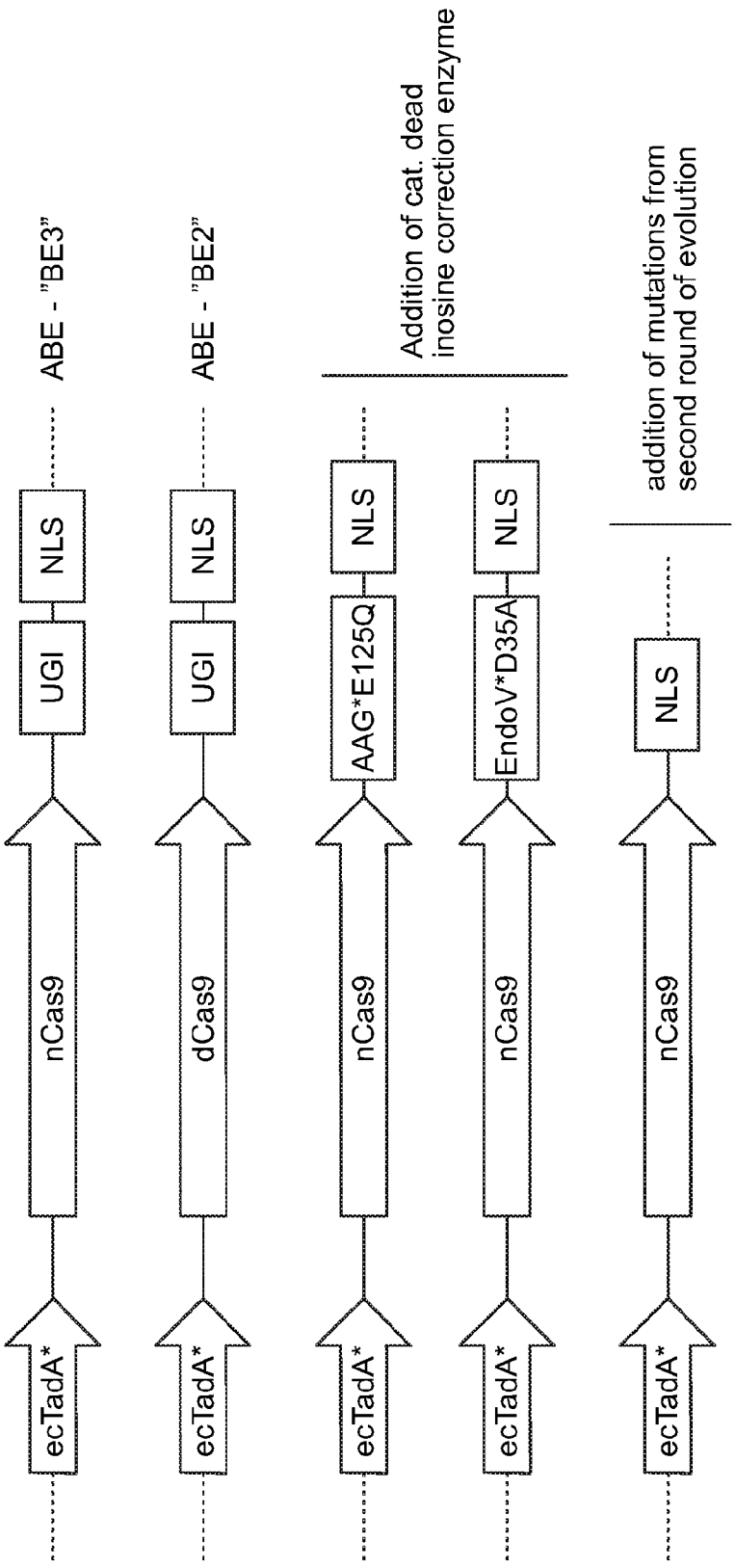

ABE - "BE3"
ABE - "BE2"
Addition of cat. dead inosine correction enzyme
addition of mutations from second round of evolution
ecTadA*
nCas9
UGI
NLS
dCas9
AAG*E125Q
EndoV*D35A

FIGURE 18

FIGURE 19

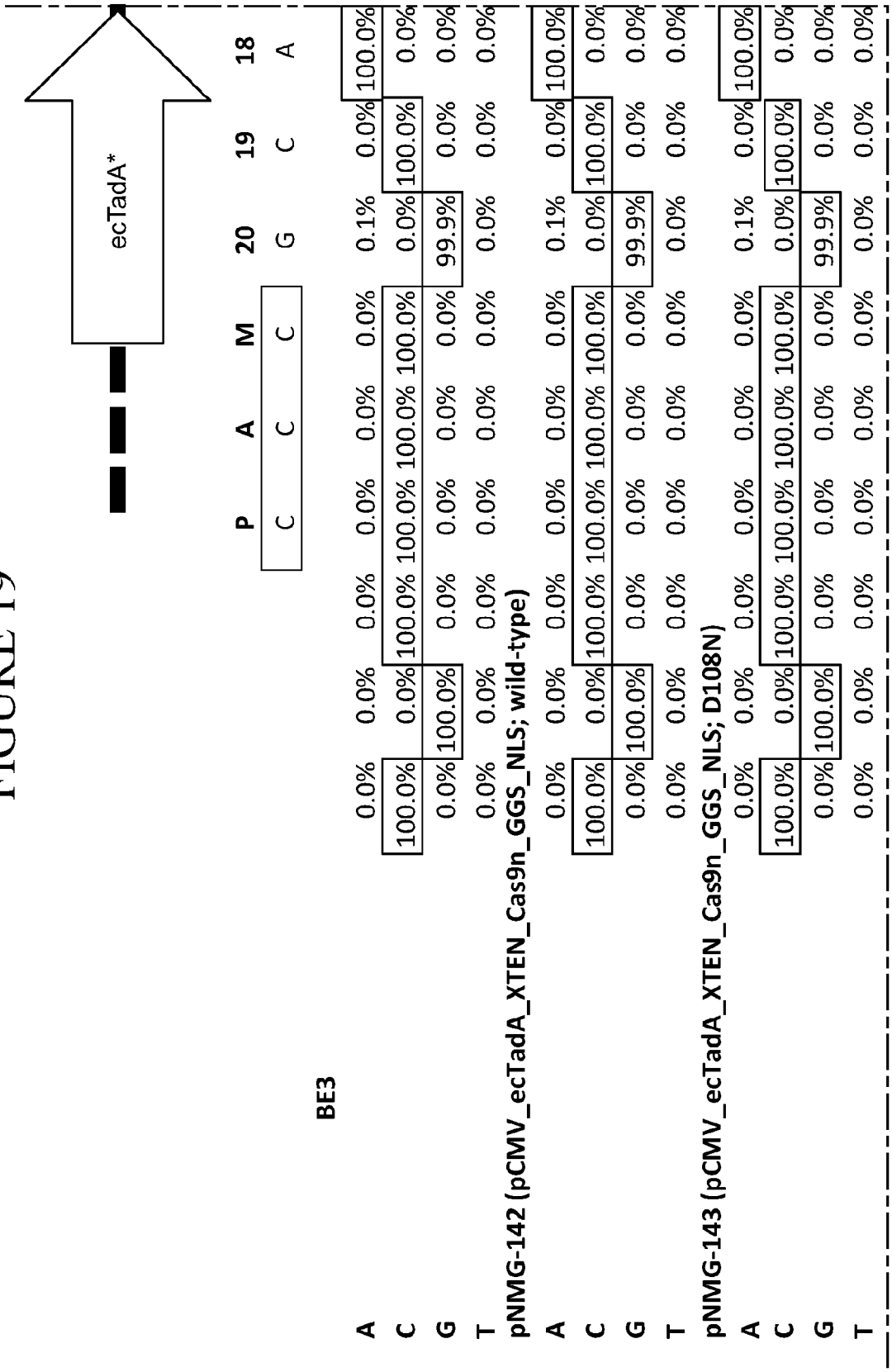

ecTadA*
P A M 20 19 18
C C C G C A
BE3
A 0.0% 0.0% 0.0% 0.0% 0.0% 0.1% 0.0% 100.0%
C 100.0% 0.0% 100.0% 100.0% 100.0% 0.0% 100.0% 0.0%
G 0.0% 100.0% 0.0% 0.0% 0.0% 99.9% 0.0% 0.0%
T 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%
pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)
A 0.0% 0.0% 0.0% 0.0% 0.0% 0.1% 0.0% 100.0%
C 100.0% 0.0% 100.0% 100.0% 100.0% 0.0% 100.0% 0.0%
G 0.0% 100.0% 0.0% 0.0% 0.0% 99.9% 0.0% 0.0%
T 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%
pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)
A 0.0% 0.0% 0.0% 0.0% 0.0% 0.1% 0.0% 100.0%
C 100.0% 0.0% 100.0% 100.0% 100.0% 0.0% 100.0% 0.0%
G 0.0% 100.0% 0.0% 0.0% 0.0% 99.9% 0.0% 0.0%
T 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%

FIGURE 19 (Continued)

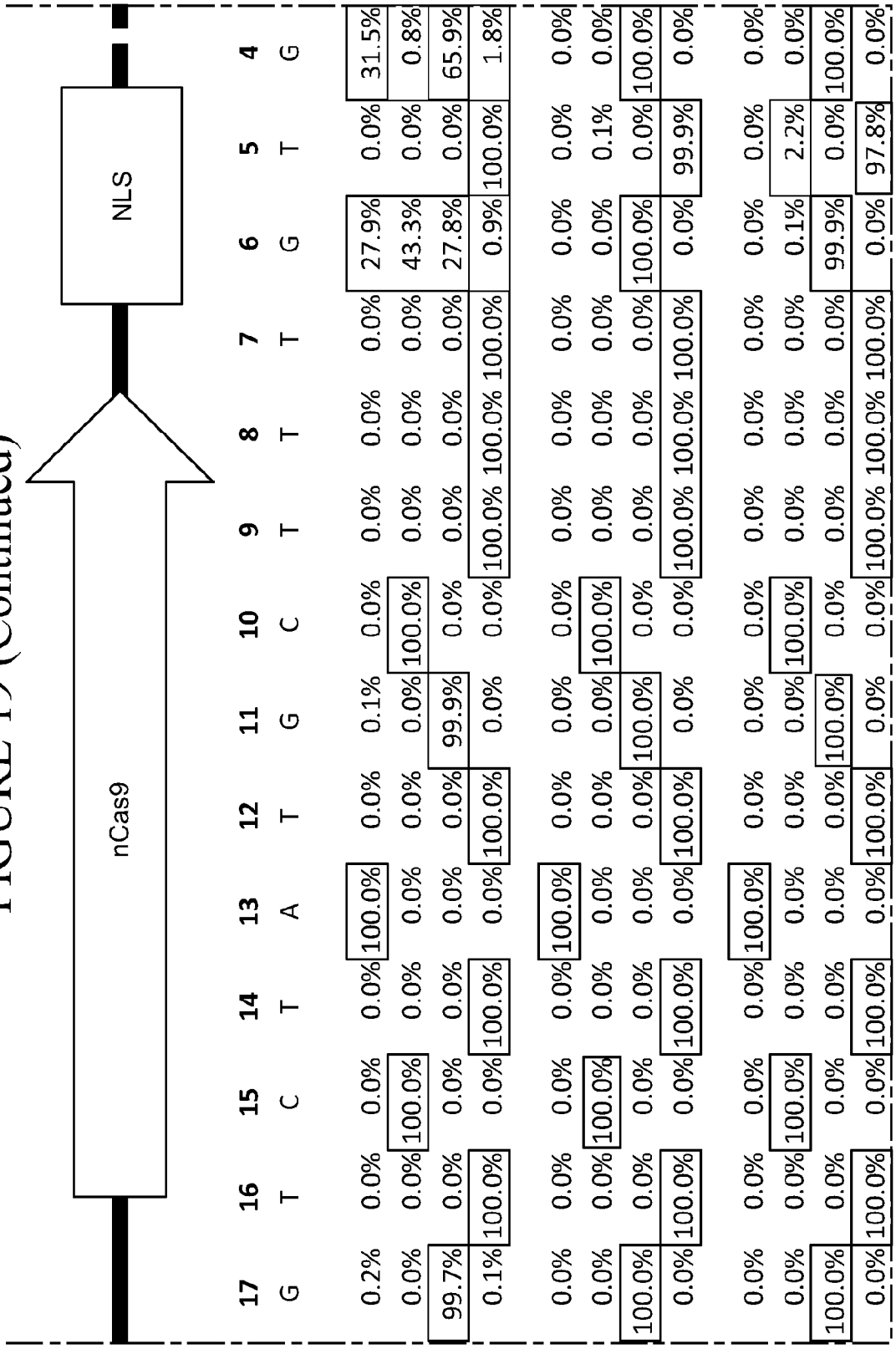


FIGURE 19 (Continued)

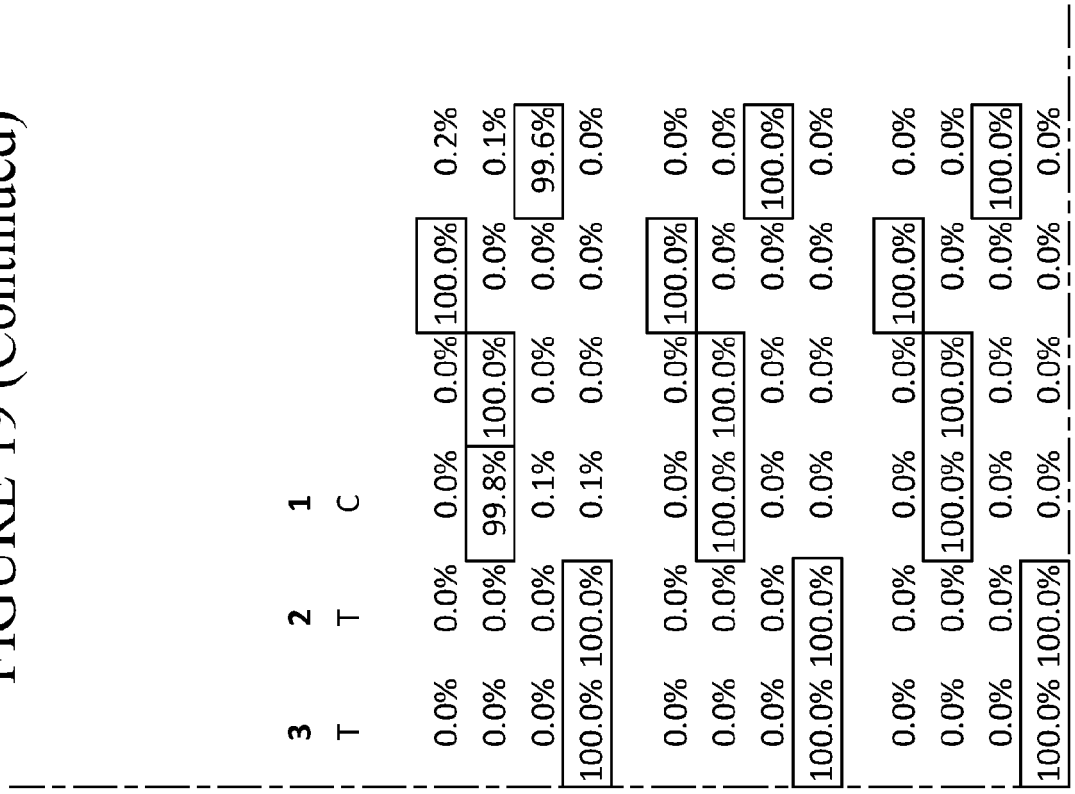


FIGURE 19 (Continued)

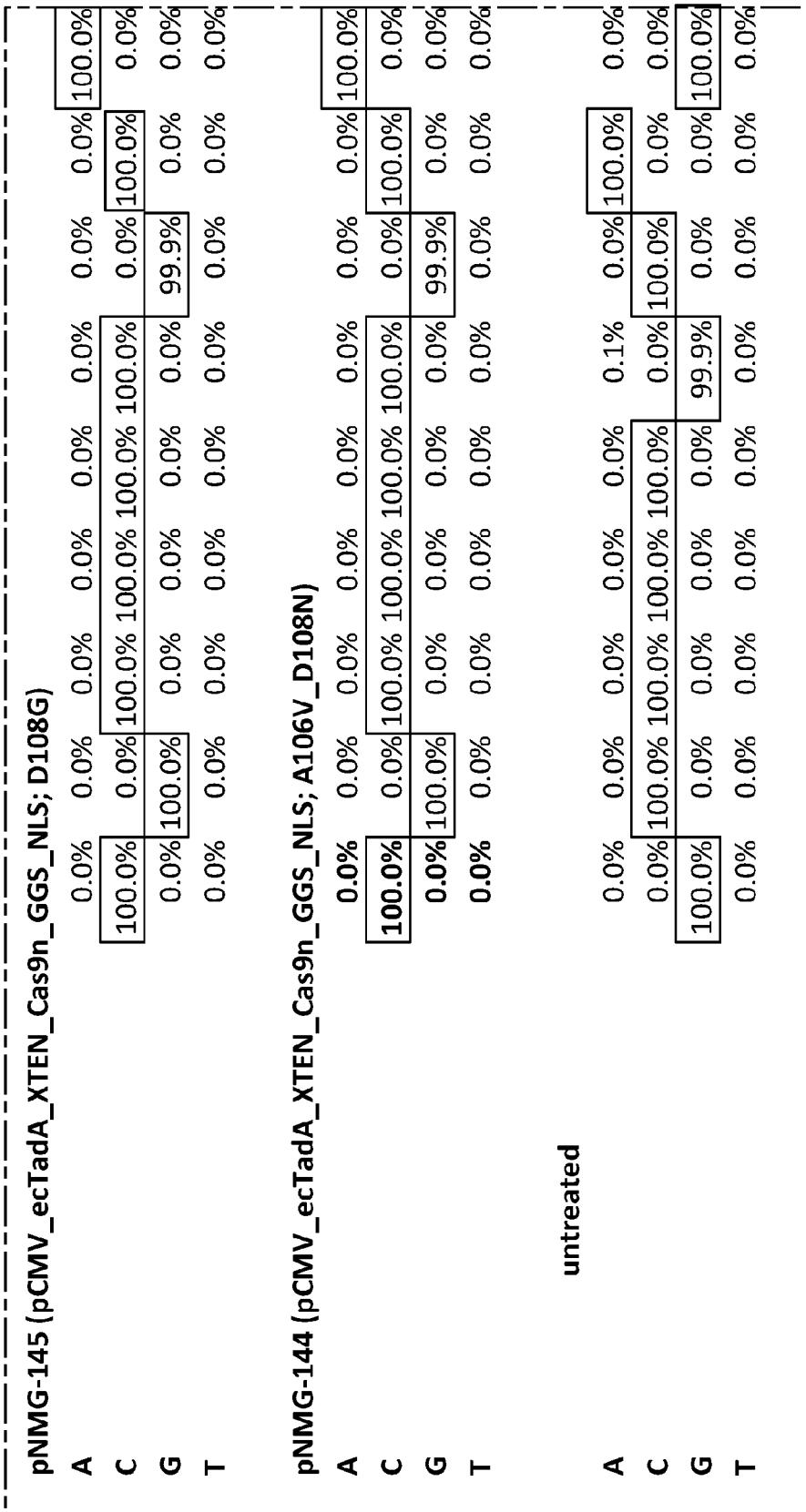


FIGURE 19 (Continued)

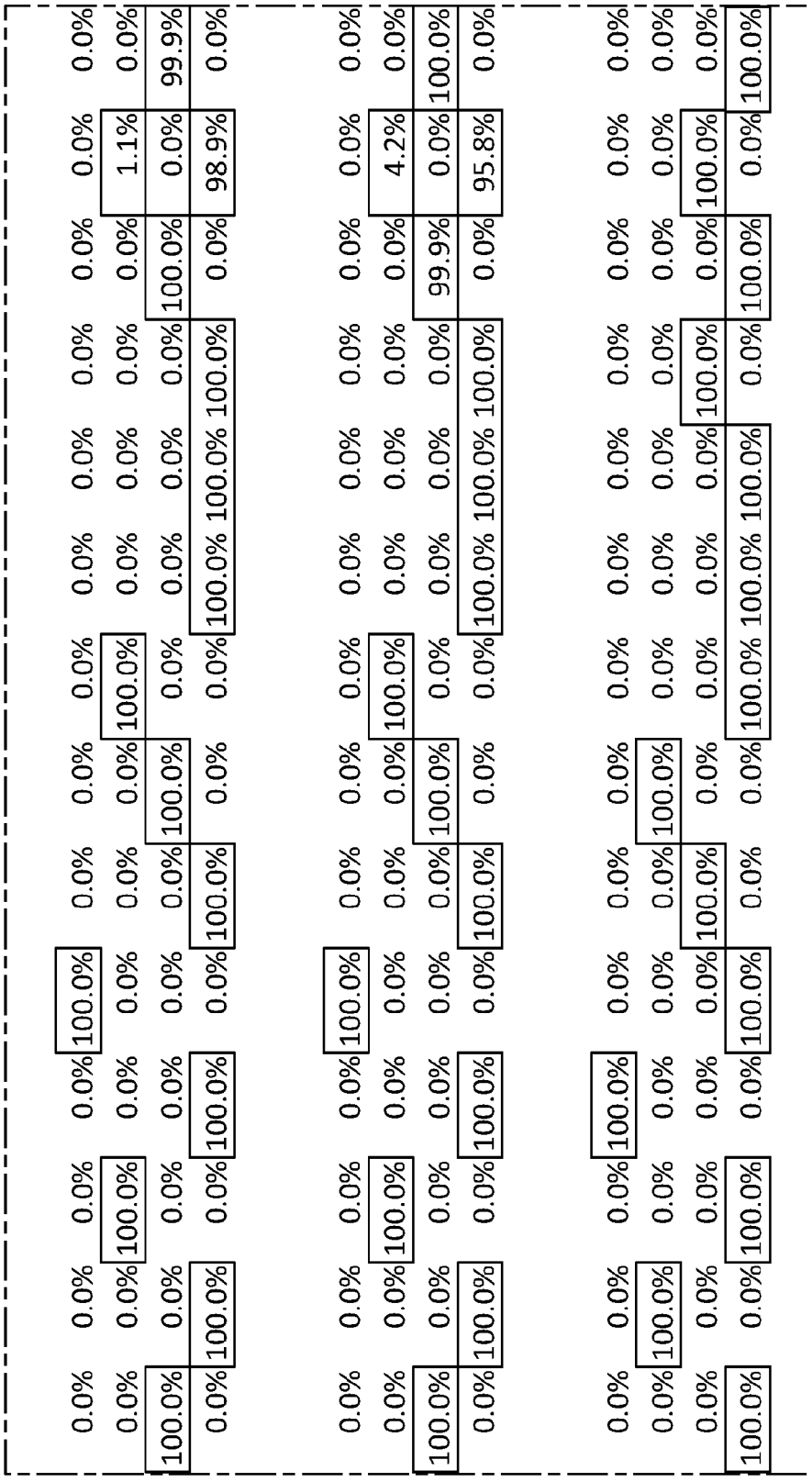
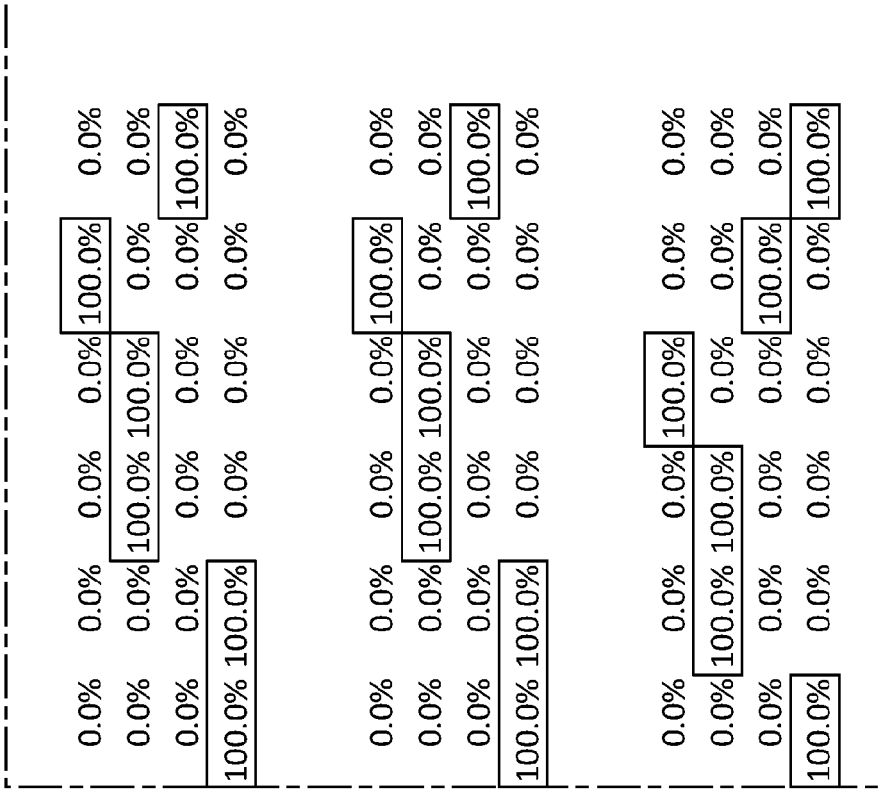


FIGURE 19 (Continued)



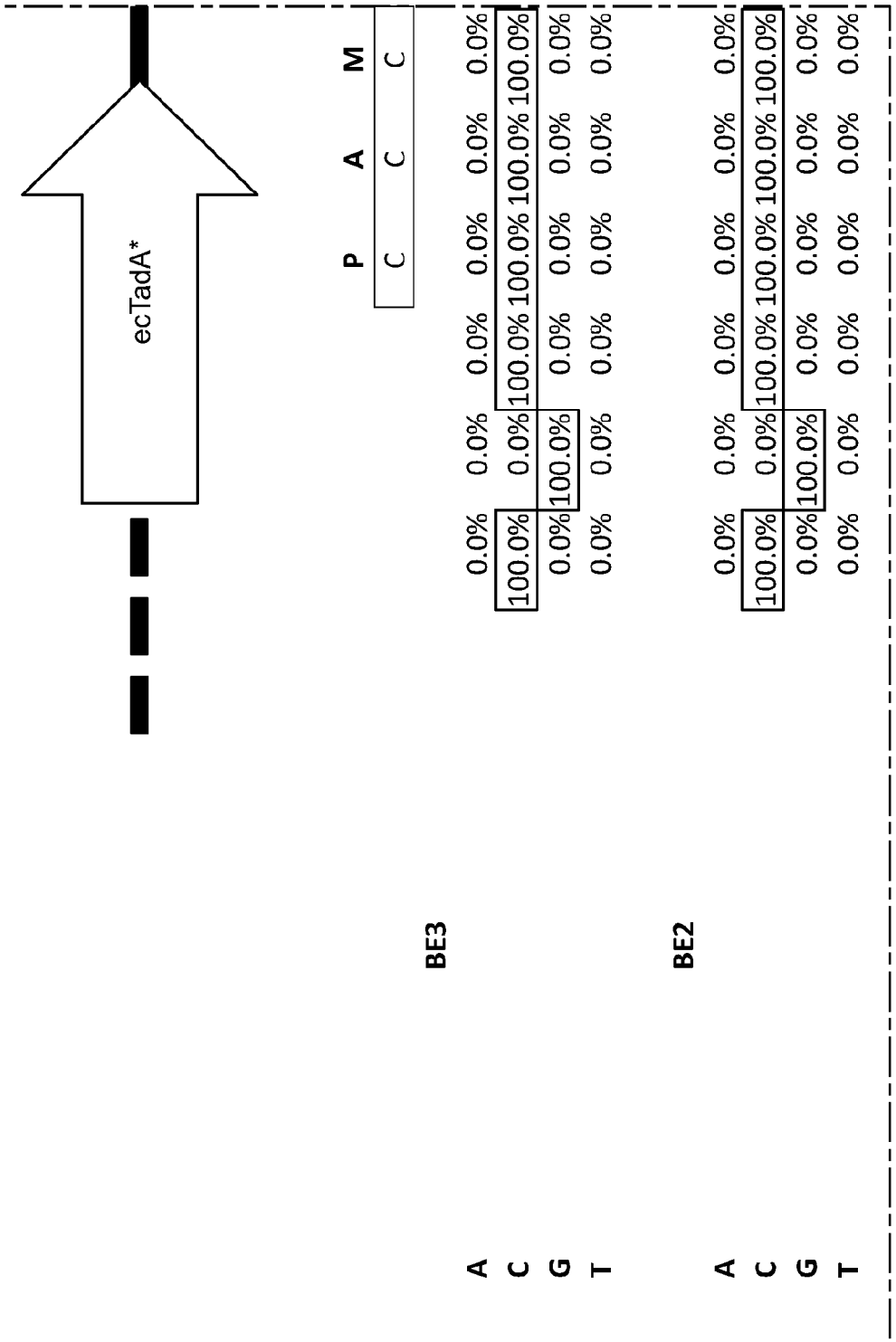

ecTadA*
P A M
C C C
BE3
A 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%
C 100.0% 0.0% 100.0% 100.0% 100.0% 100.0% 100.0% 100.0%
G 0.0% 100.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%
T 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%
BE2
A 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%
C 100.0% 0.0% 100.0% 100.0% 100.0% 100.0% 100.0% 100.0%
G 0.0% 100.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%
T 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0% 0.0%

FIGURE 20

FIGURE 20 (Continued)

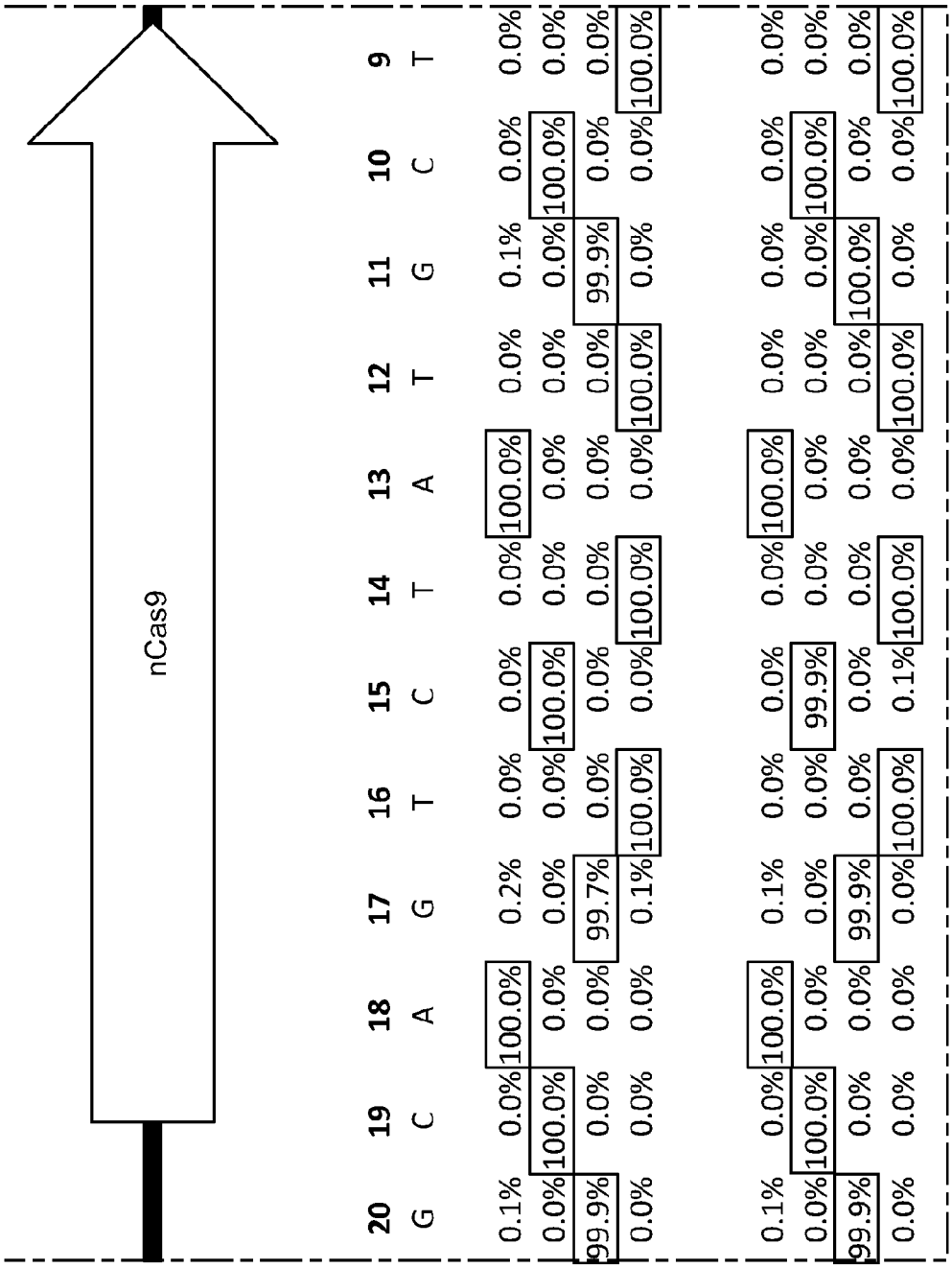


FIGURE 20 (Continued)

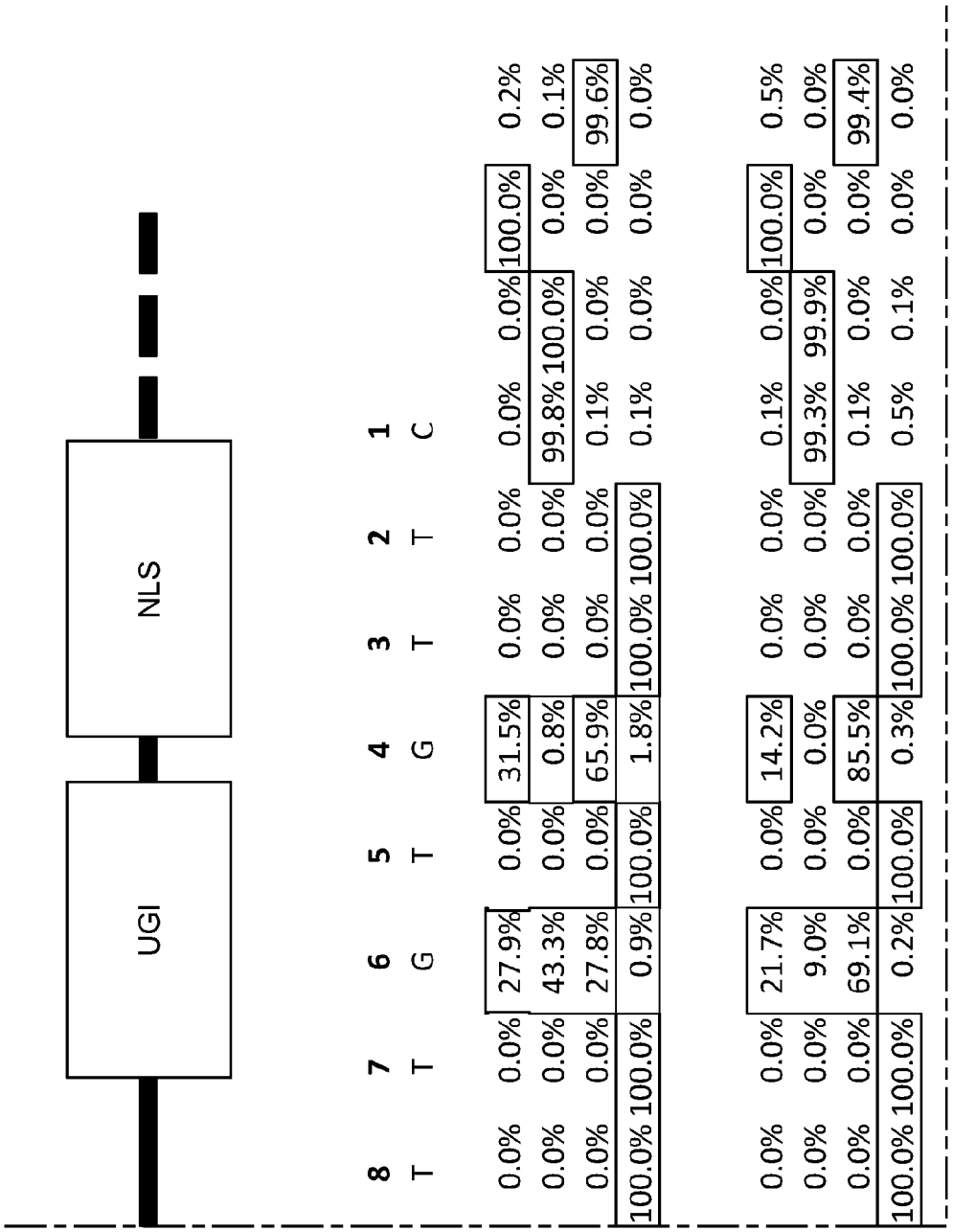
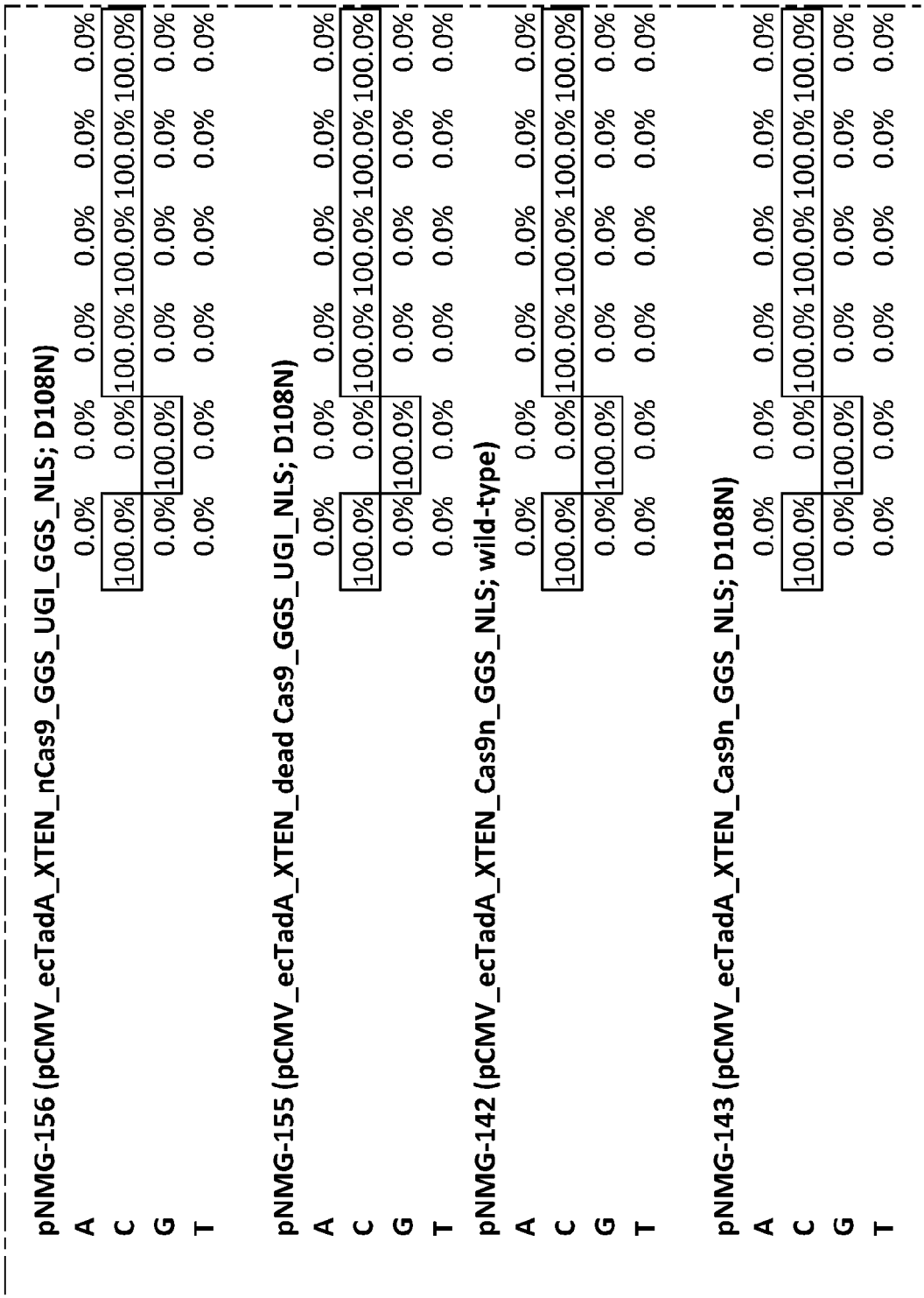


FIGURE 20 (Continued)



| pNMG-156 (pCMV_ecTadA_XTEN_nCas9_GGS_UGI_GGS_NLS; D108N) | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| C | 100.0% | 0.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| G | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| T | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

| pNMG-155 (pCMV_ecTadA_XTEN_dead Cas9_GGS_UGI_NLS; D108N) | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| C | 100.0% | 0.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| G | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| T | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

| pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| C | 100.0% | 0.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| G | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| T | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

| pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N) | | | | | | | |
|---|---|---|---|---|---|---|---|
| A | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| C | 100.0% | 0.0% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| G | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| T | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

FIGURE 20 (Continued)

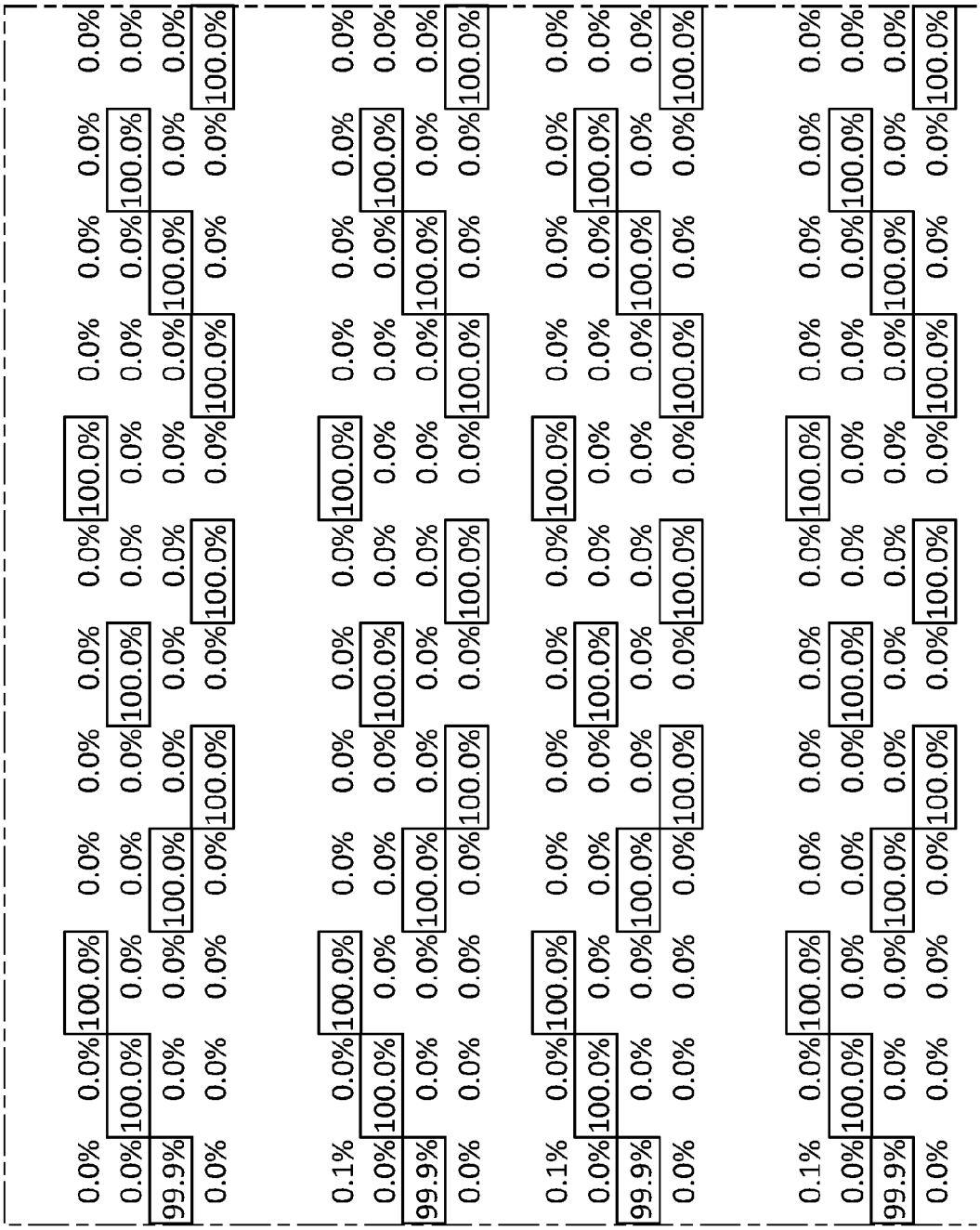


FIGURE 20 (Continued)

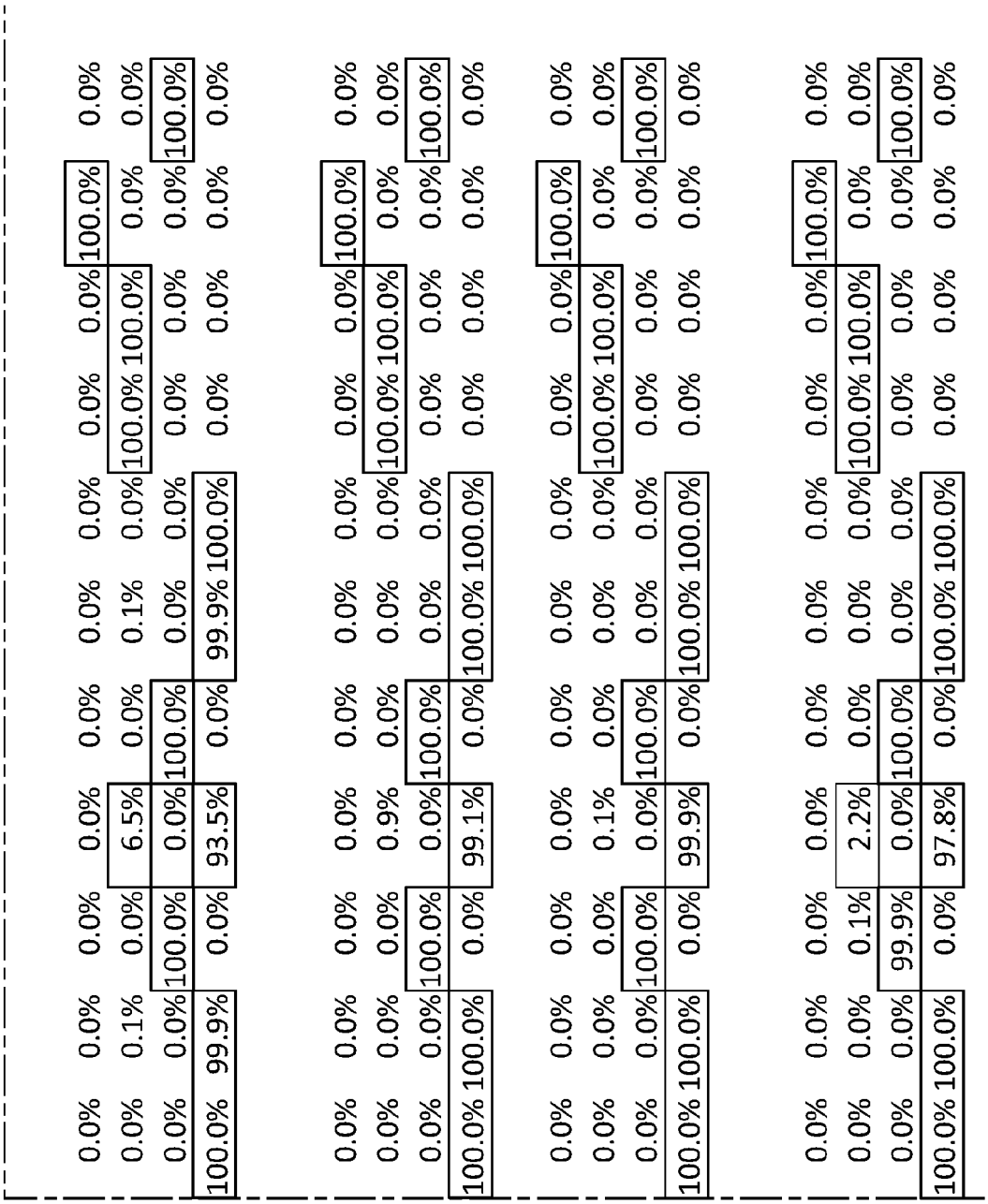


FIGURE 21

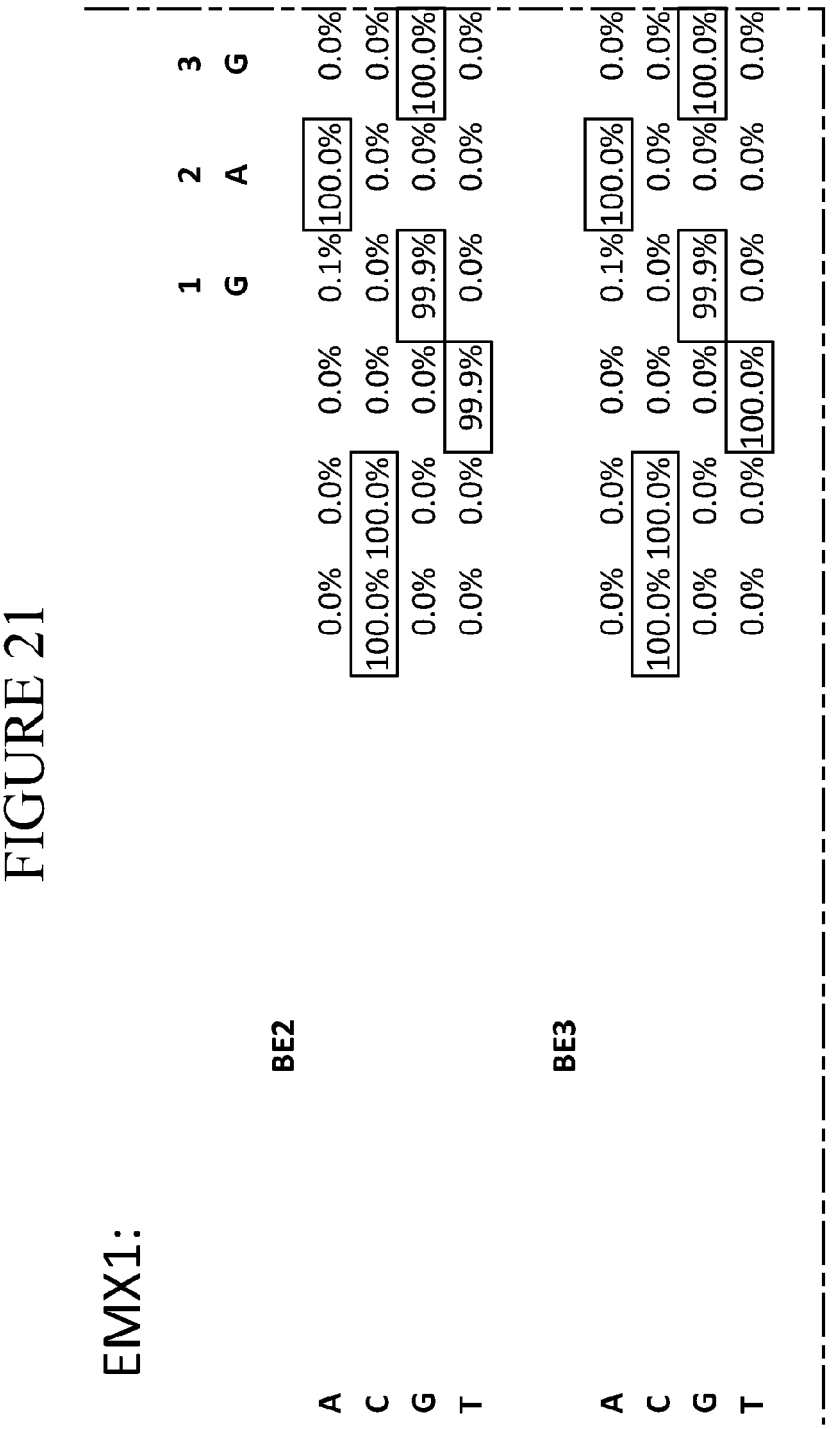
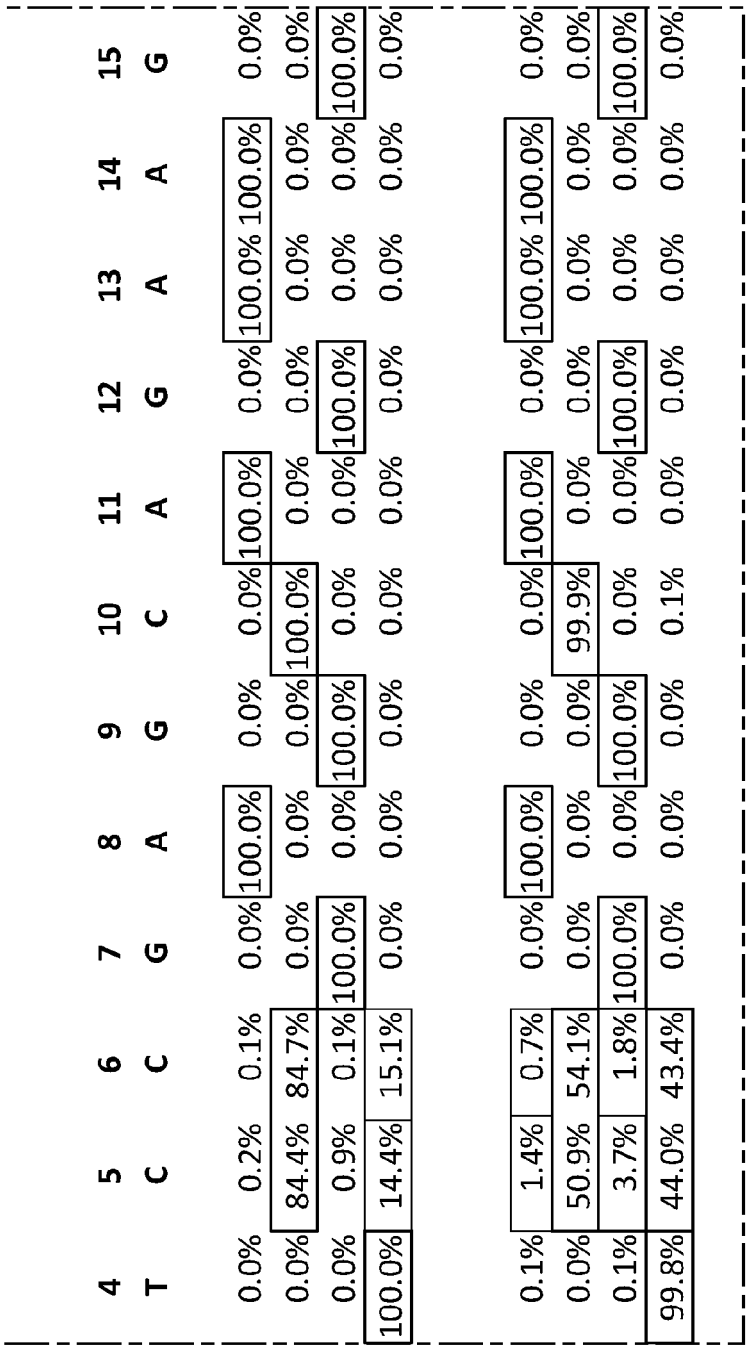

EMX1:
BE2
BE3
1 2 3
G A G
A C G T
0.0% 0.0% 0.0% 0.1% 100.0% 0.0%
100.0% 100.0% 0.0% 0.0% 0.0% 0.0%
0.0% 0.0% 0.0% 99.9% 0.0% 100.0%
0.0% 0.0% 99.9% 0.0% 0.0% 0.0%
0.0% 0.0% 0.0% 0.1% 100.0% 0.0%
100.0% 100.0% 0.0% 0.0% 0.0% 0.0%
0.0% 0.0% 0.0% 99.9% 0.0% 100.0%
0.0% 0.0% 100.0% 0.0% 0.0% 0.0%

FIGURE 21 (Continued)



| 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | C | C | G | A | G | C | A | G | A | A | G |
| 0.0% | 0.2% | 0.1% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% | 0.0% |
| 0.0% | 84.4% | 84.7% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.9% | 0.1% | 100.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% |
| 100.0% | 14.4% | 15.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.1% | 1.4% | 0.7% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% | 0.0% |
| 0.0% | 50.9% | 54.1% | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.1% | 3.7% | 1.8% | 100.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% |
| 99.8% | 44.0% | 43.4% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

FIGURE 21 (Continued)

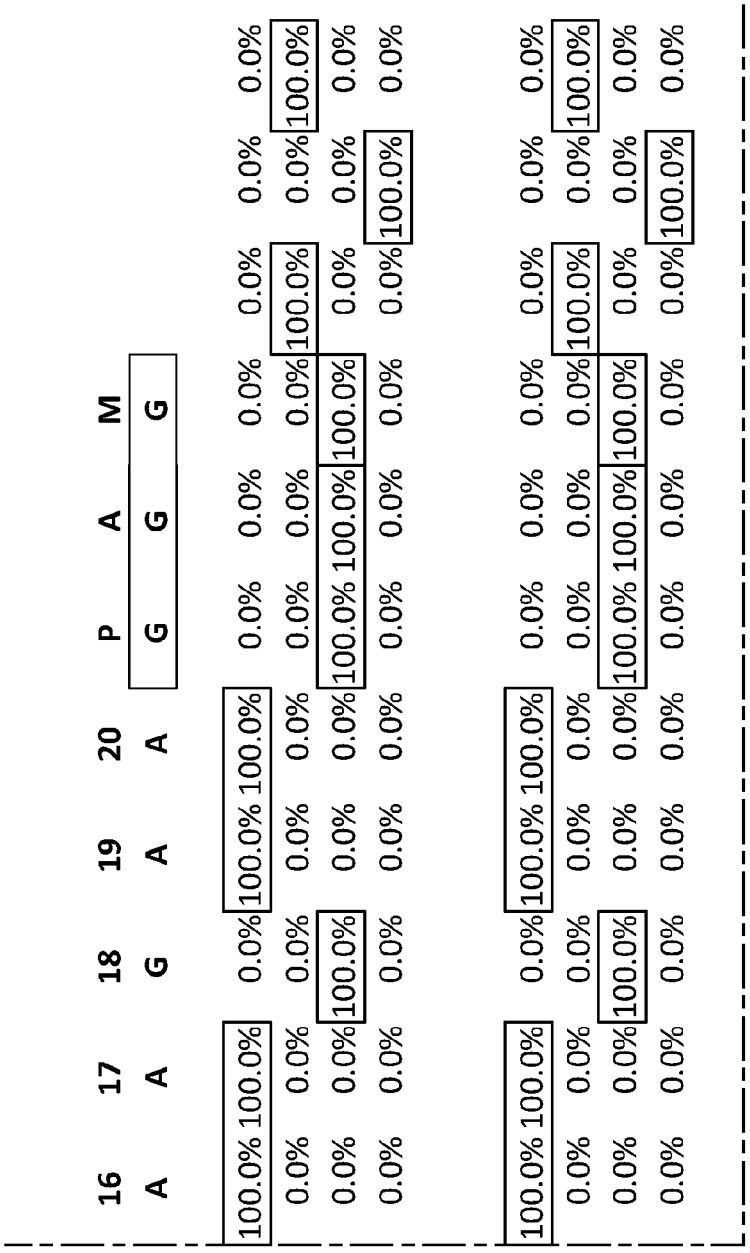


FIGURE 21 (Continued)

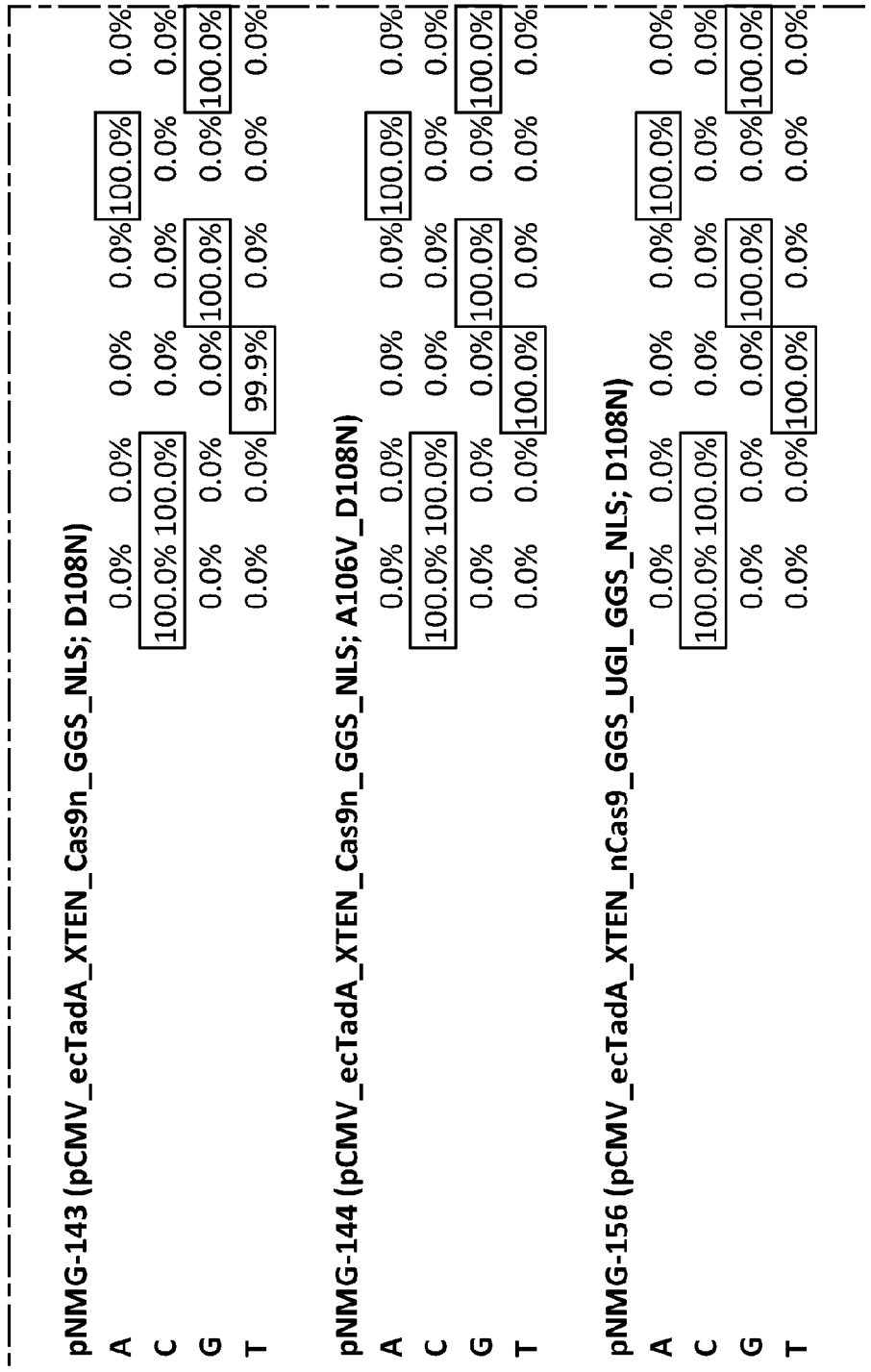


FIGURE 21 (Continued)

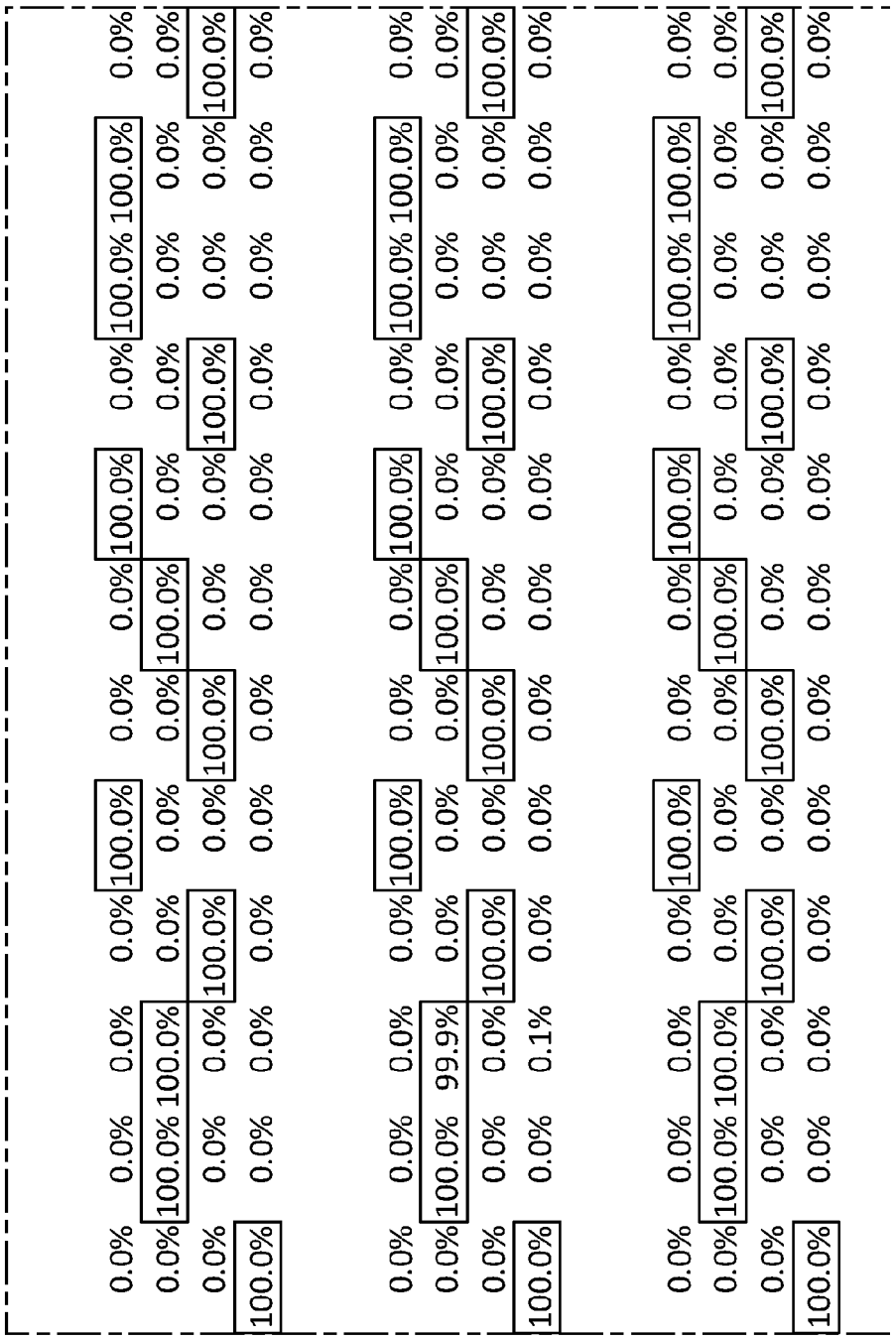


FIGURE 21 (Continued)

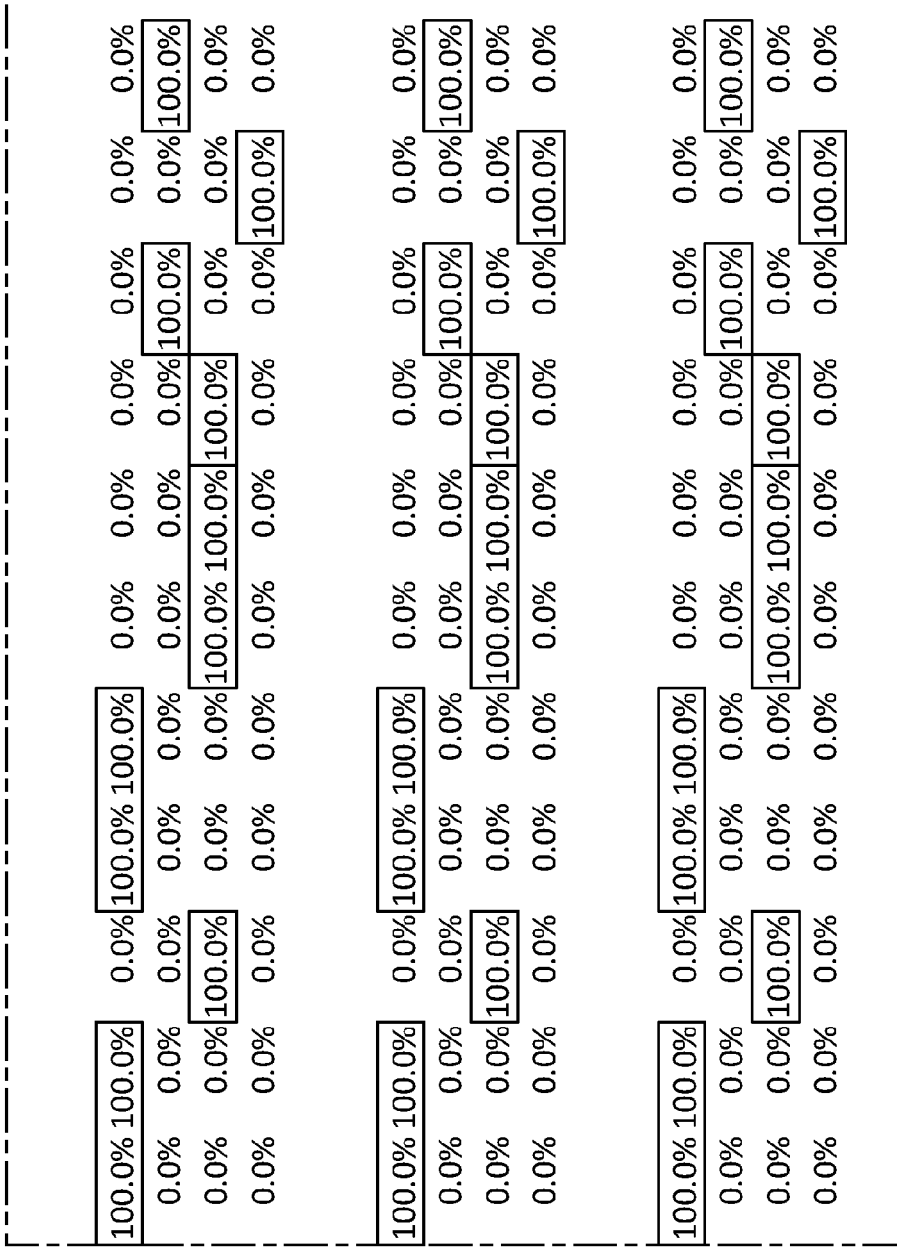


FIGURE 22

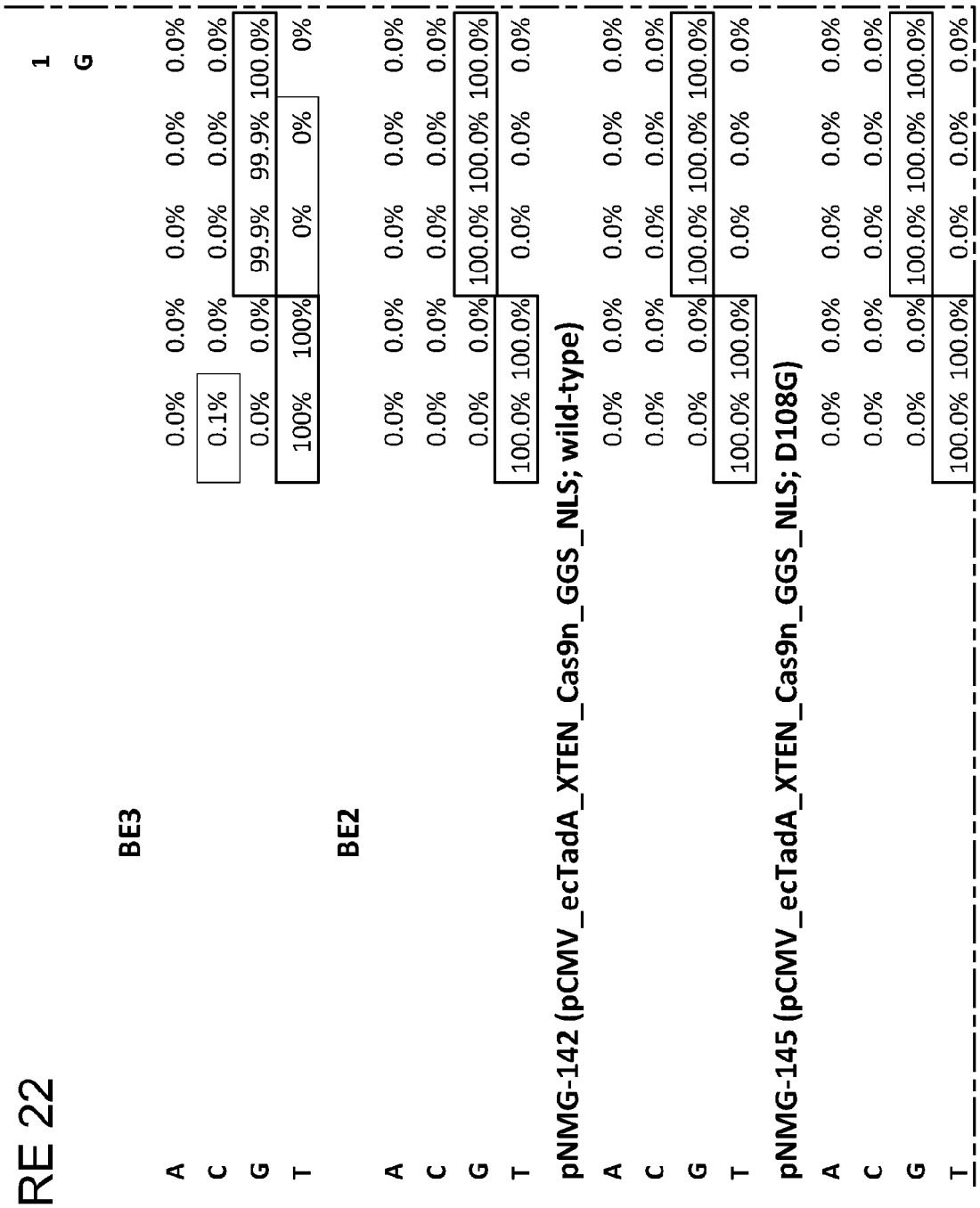

1
G
BE3
A 0.0% 0.0% 0.0% 0.0%
C 0.1% 0.0% 0.0% 0.0%
G 0.0% 0.0% 99.9% 99.9% 100.0%
T 100% 100% 0% 0% 0%
BE2
A 0.0% 0.0% 0.0% 0.0% 0.0%
C 0.0% 0.0% 0.0% 0.0% 0.0%
G 0.0% 0.0% 100.0% 100.0% 100.0%
T 100.0% 100.0% 0.0% 0.0% 0.0%
pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)
A 0.0% 0.0% 0.0% 0.0% 0.0%
C 0.0% 0.0% 0.0% 0.0% 0.0%
G 0.0% 0.0% 100.0% 100.0% 100.0%
T 100.0% 100.0% 0.0% 0.0% 0.0%
pNMG-145 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108G)
A 0.0% 0.0% 0.0% 0.0% 0.0%
C 0.0% 0.0% 0.0% 0.0% 0.0%
G 0.0% 0.0% 100.0% 100.0% 100.0%
T 100.0% 100.0% 0.0% 0.0% 0.0%

FIGURE 22 (Continued)

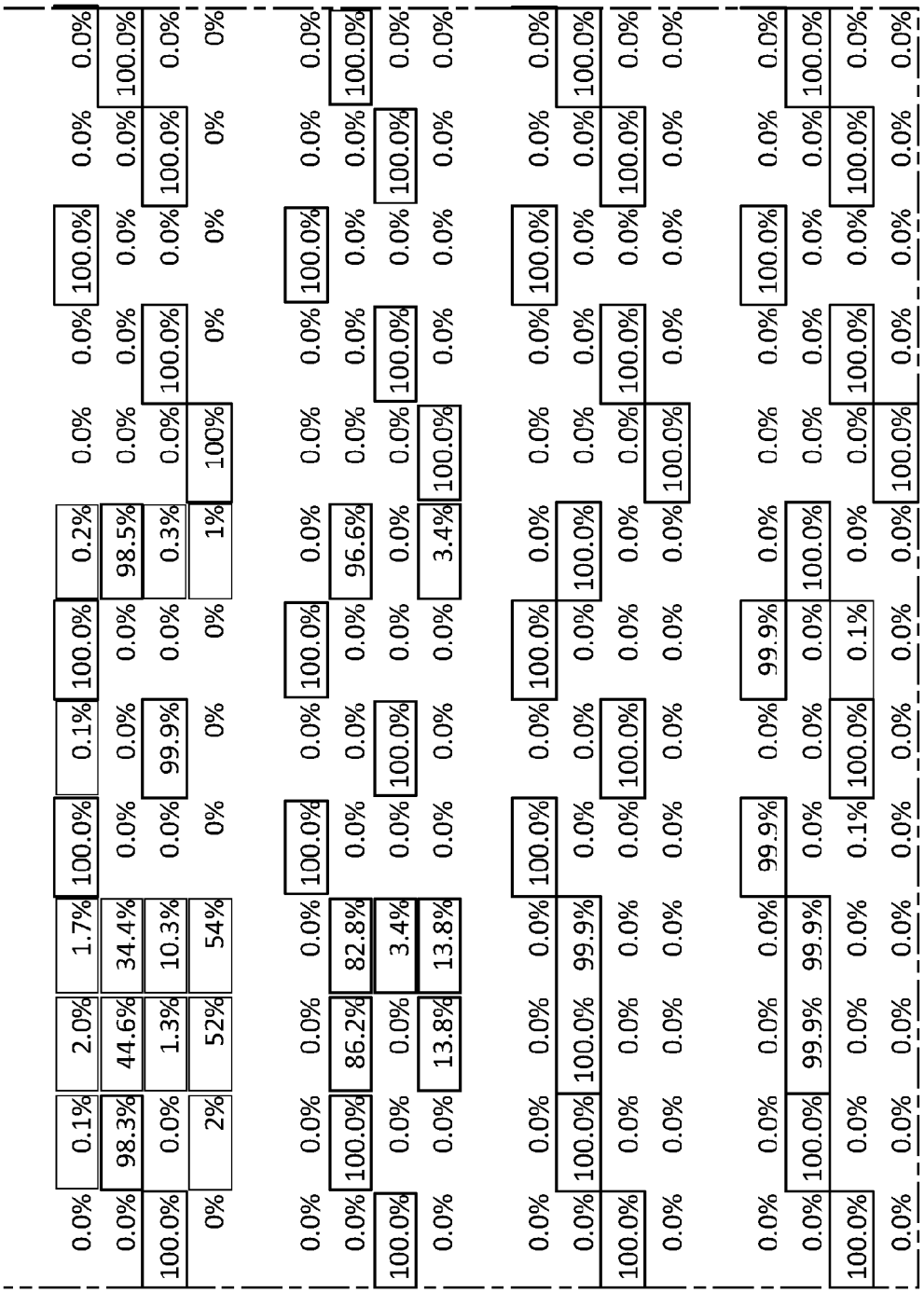
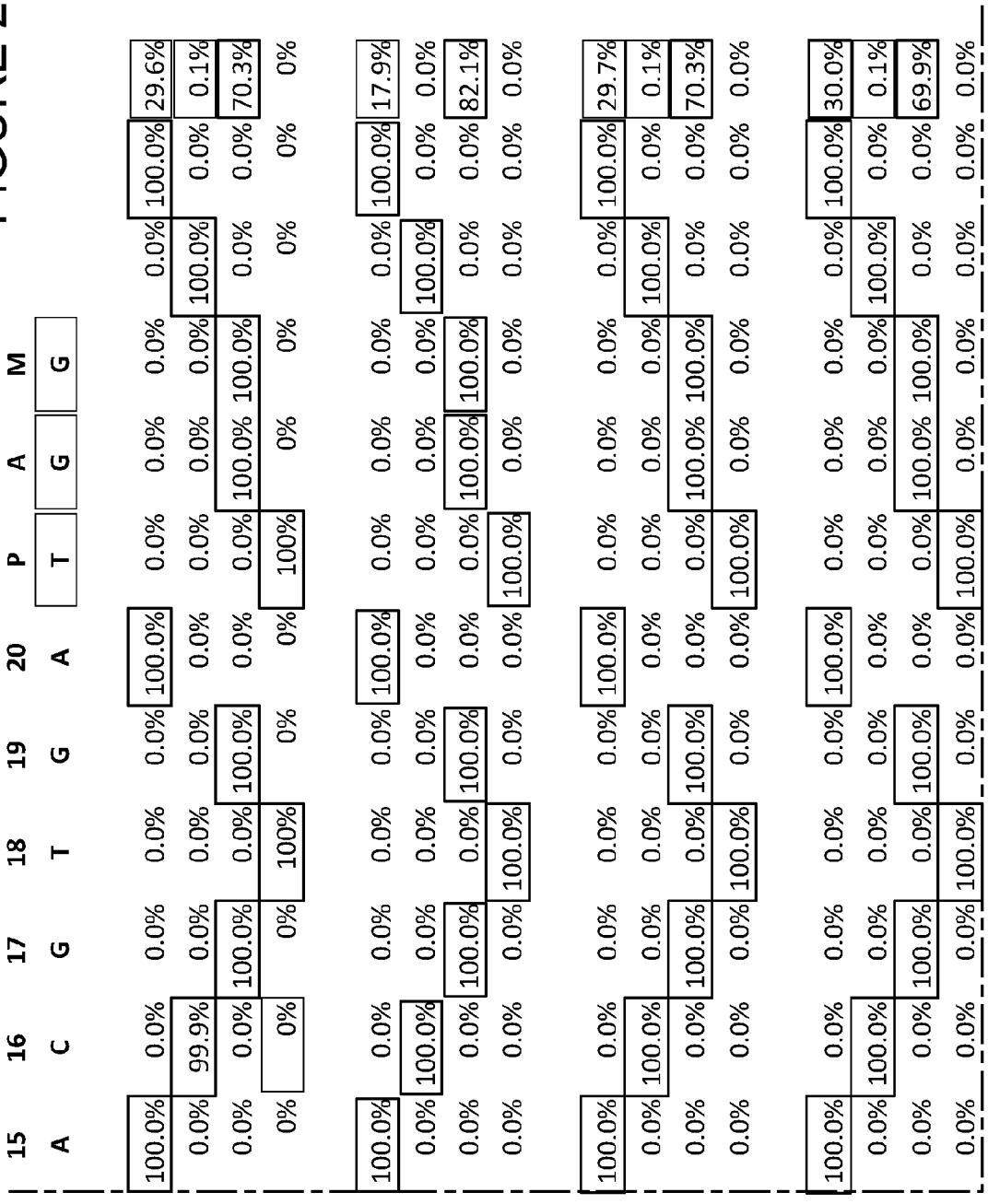


FIGURE 22 (Continued)



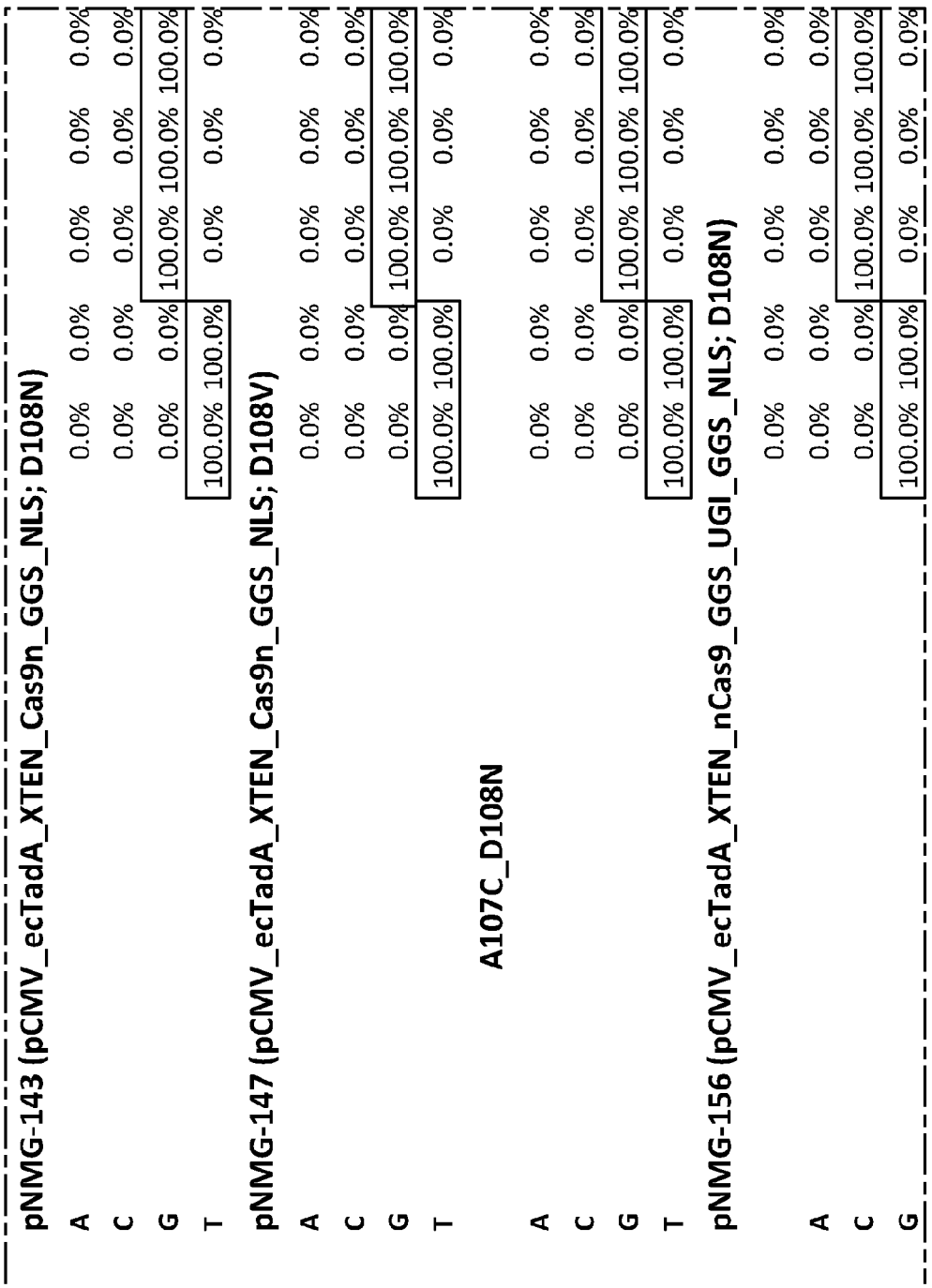



FIGURE 22 (Continued)

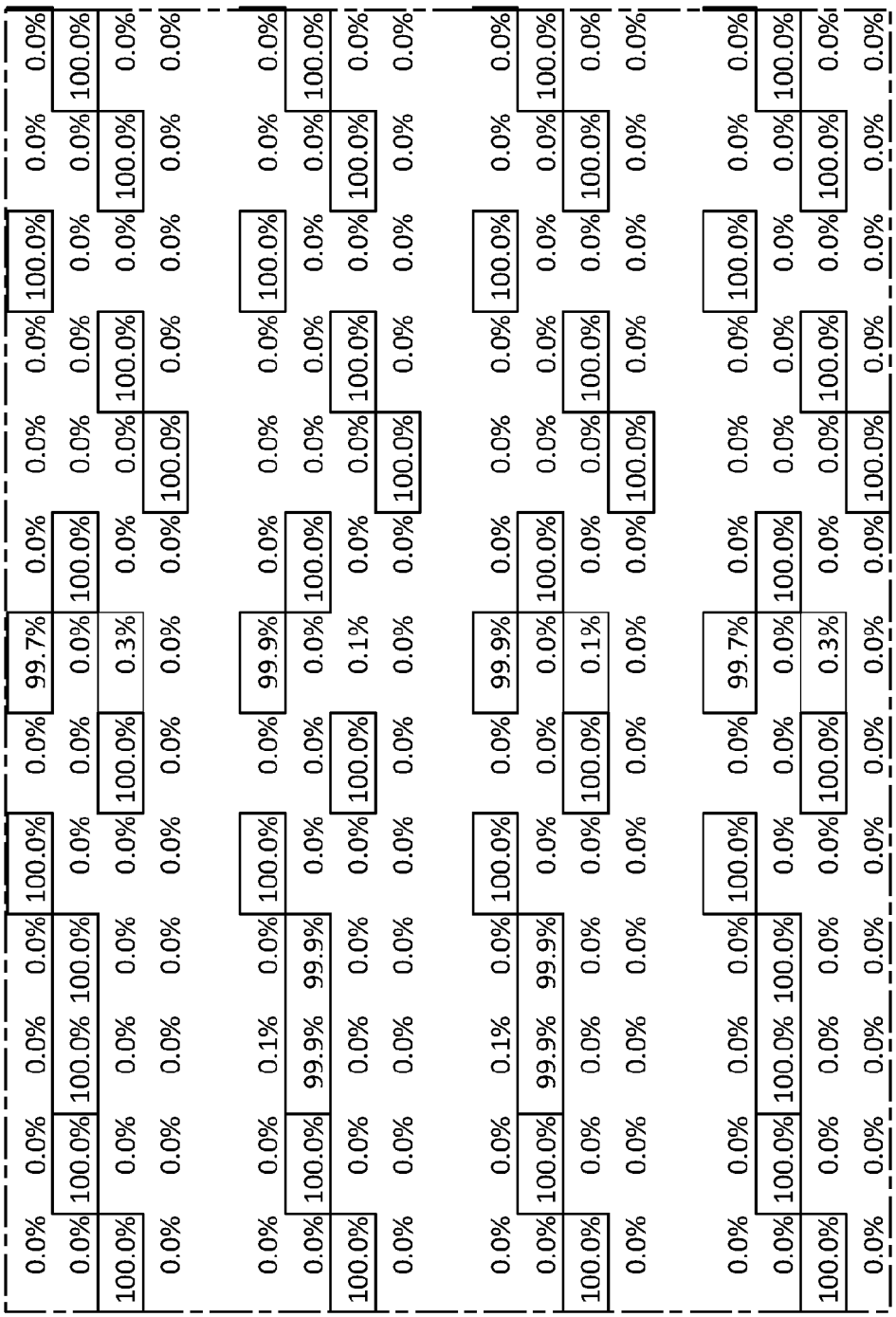



FIGURE 22 (Continued)

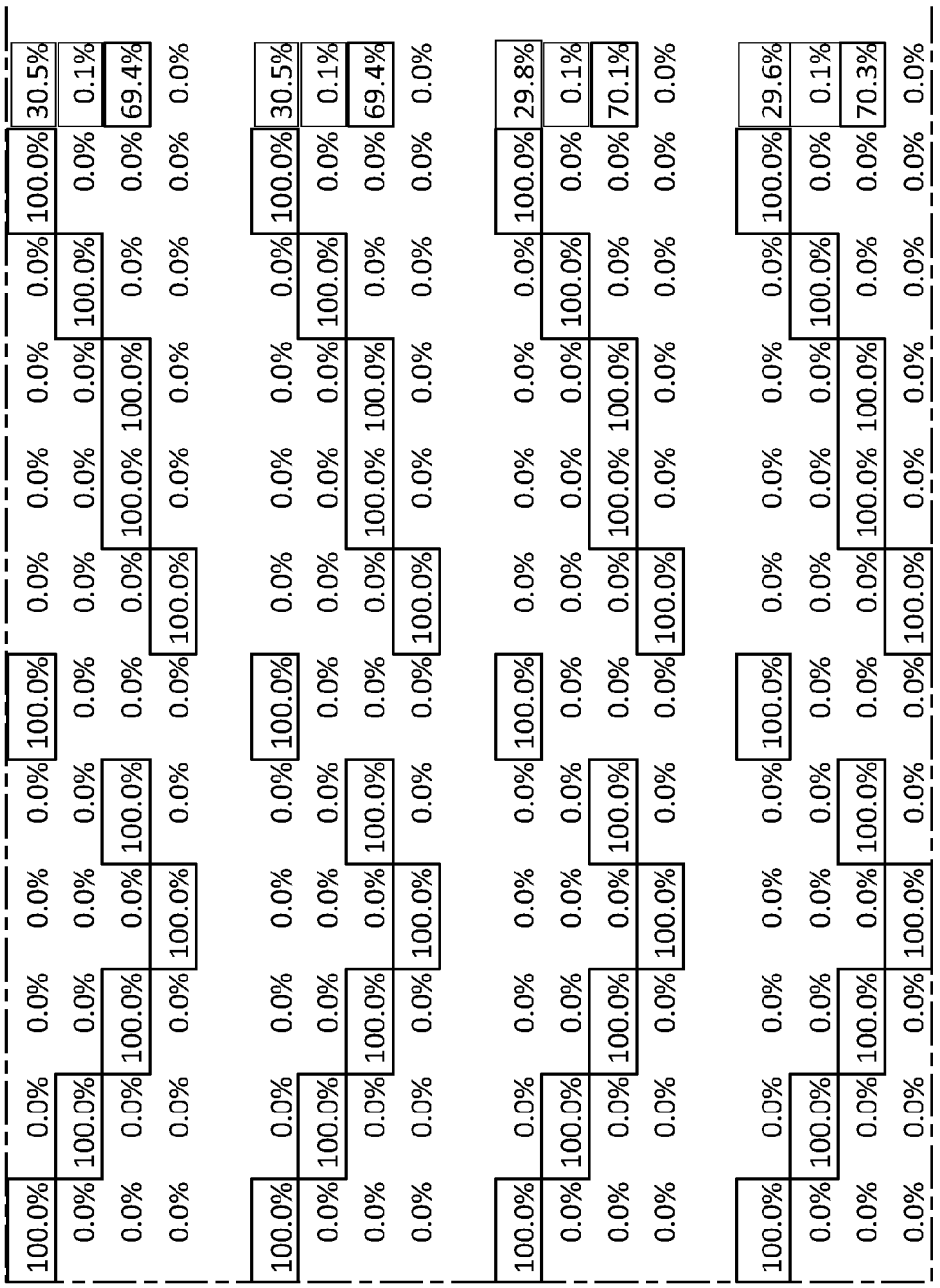



FIGURE 22 (Continued)

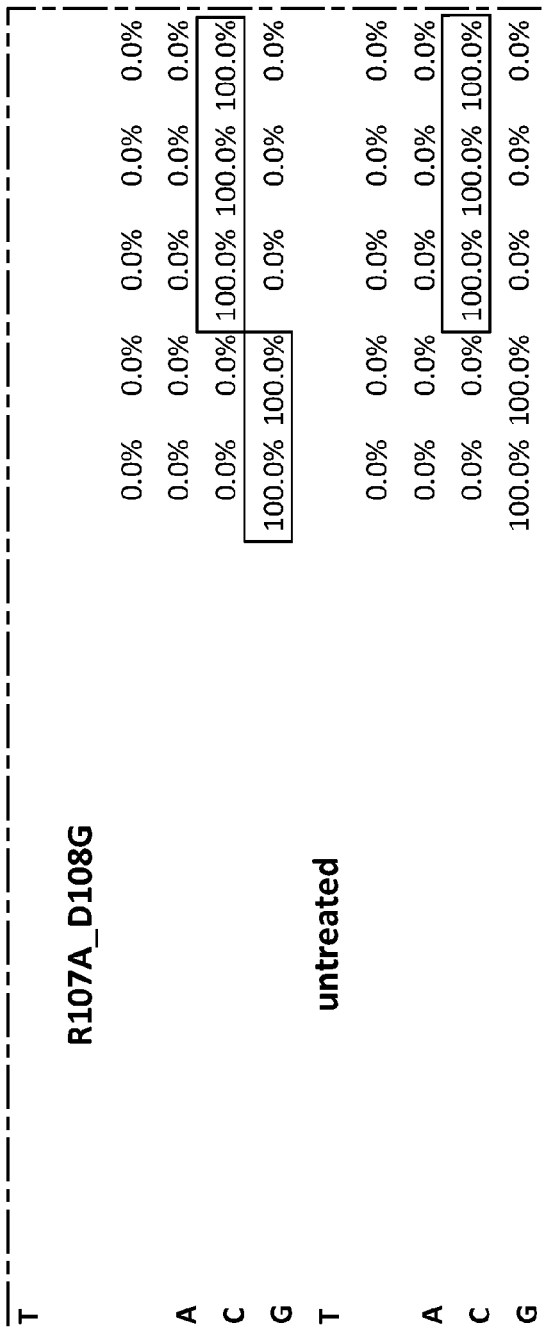



FIGURE 22 (Continued)

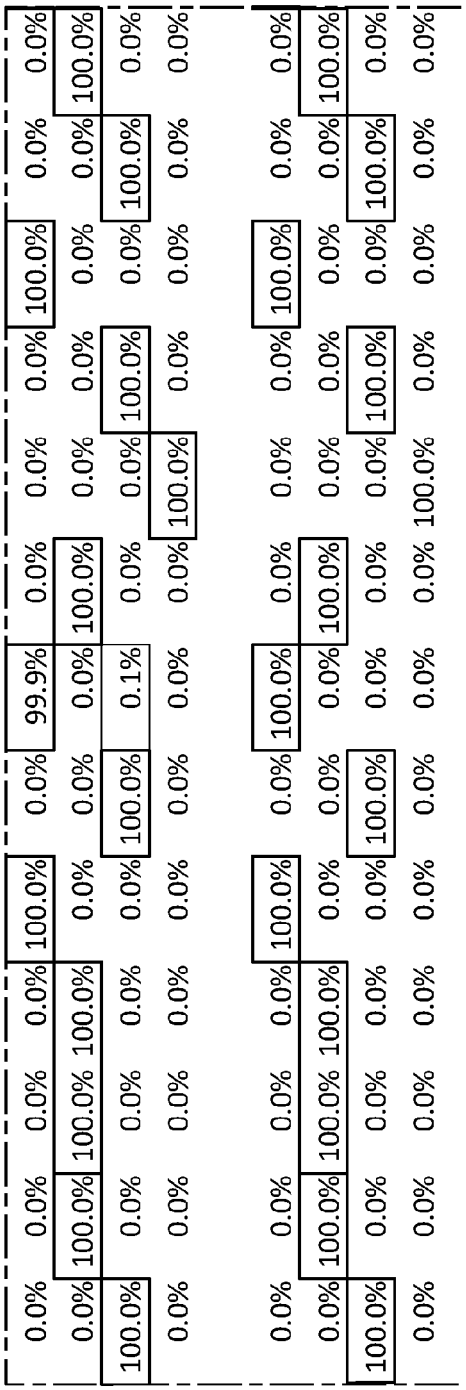



FIGURE 22 (Continued)

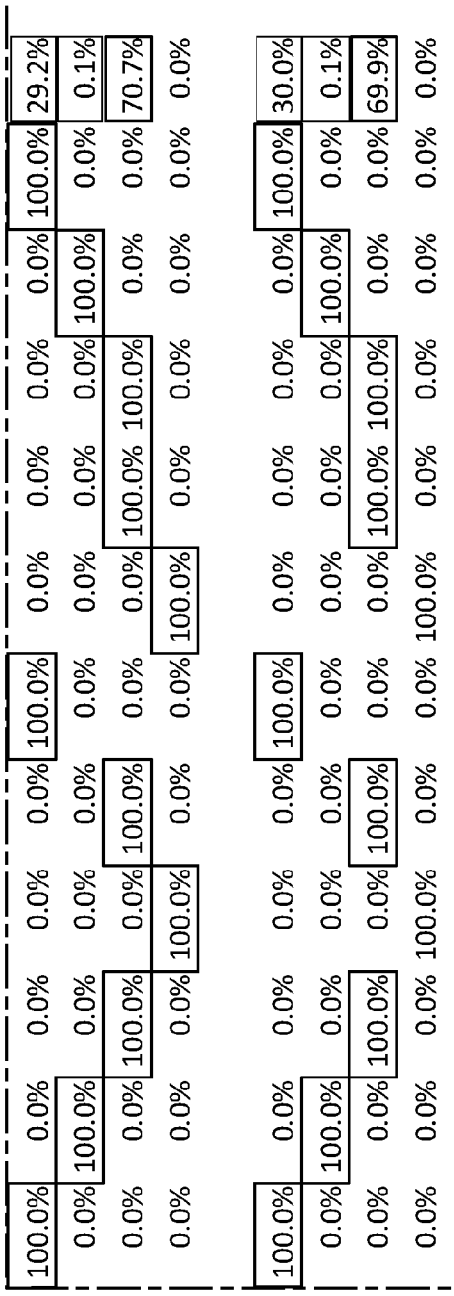



FIGURE 22 (Continued)

# FIGURE 23

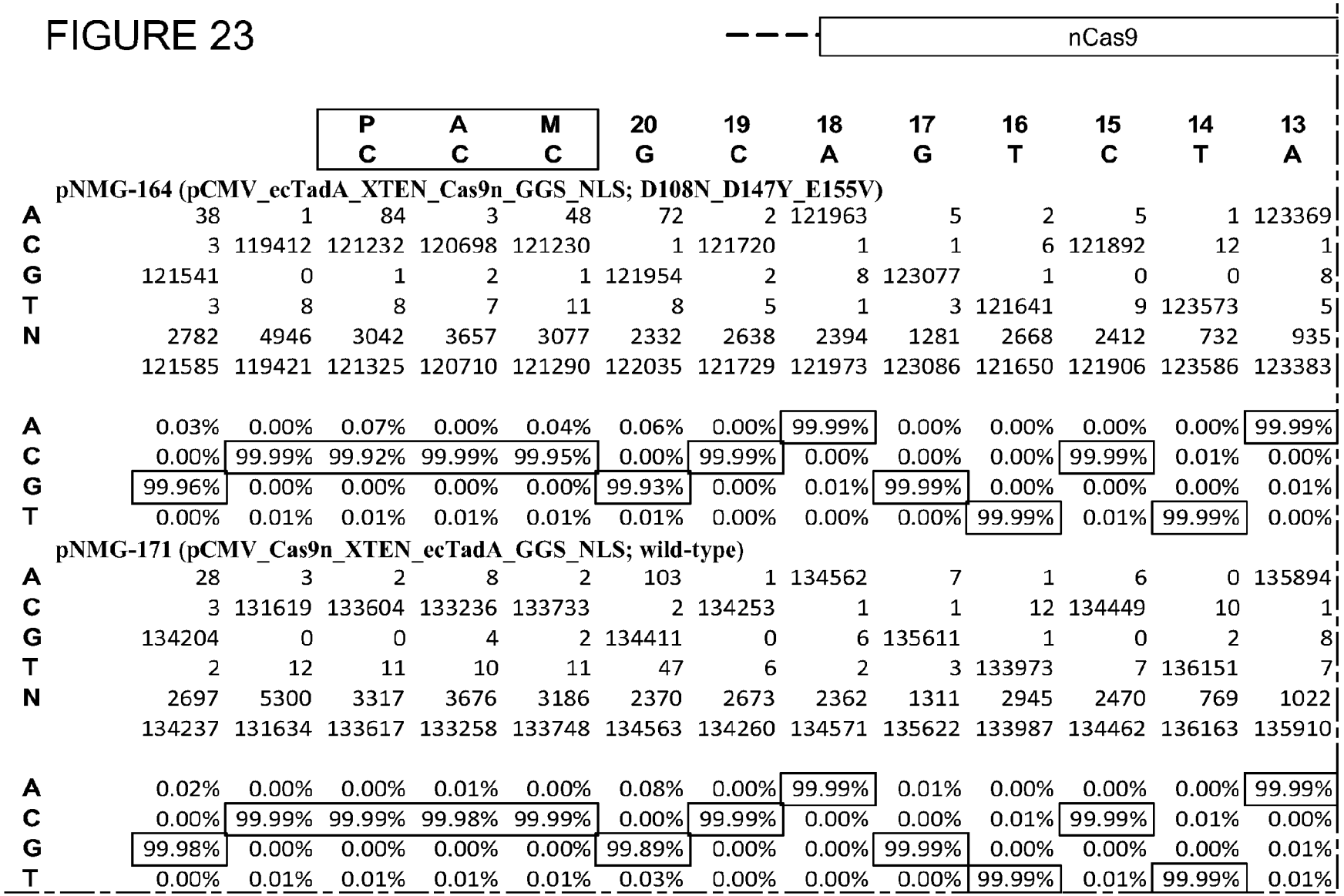

nCas9
P A M 20 19 18 17 16 15 14 13
C C C G C A G T C T A
pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)
A 38 1 84 3 48 72 2 121963 5 2 5 1 123369
C 3 119412 121232 120698 121230 1 121720 1 1 6 121892 12 1
G 121541 0 1 2 1 121954 2 8 123077 1 0 0 8
T 3 8 8 7 11 8 5 1 3 121641 9 123573 5
N 2782 4946 3042 3657 3077 2332 2638 2394 1281 2668 2412 732 935
121585 119421 121325 120710 121290 122035 121729 121973 123086 121650 121906 123586 123383
A 0.03% 0.00% 0.07% 0.00% 0.04% 0.06% 0.00% 99.99% 0.00% 0.00% 0.00% 0.00% 99.99%
C 0.00% 99.99% 99.92% 99.99% 99.95% 0.00% 99.99% 0.00% 0.00% 0.00% 99.99% 0.01% 0.00%
G 99.96% 0.00% 0.00% 0.00% 0.00% 99.93% 0.00% 0.01% 99.99% 0.00% 0.00% 0.00% 0.01%
T 0.00% 0.01% 0.01% 0.01% 0.01% 0.01% 0.00% 0.00% 0.00% 99.99% 0.01% 99.99% 0.00%
pNMG-171 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; wild-type)
A 28 3 2 8 2 103 1 134562 7 1 6 0 135894
C 3 131619 133604 133236 133733 2 134253 1 1 12 134449 10 1
G 134204 0 0 4 2 134411 0 6 135611 1 0 2 8
T 2 12 11 10 11 47 6 2 3 133973 7 136151 7
N 2697 5300 3317 3676 3186 2370 2673 2362 1311 2945 2470 769 1022
134237 131634 133617 133258 133748 134563 134260 134571 135622 133987 134462 136163 135910
A 0.02% 0.00% 0.00% 0.01% 0.00% 0.08% 0.00% 99.99% 0.01% 0.00% 0.00% 0.00% 99.99%
C 0.00% 99.99% 99.99% 99.98% 99.99% 0.00% 99.99% 0.00% 0.00% 0.01% 99.99% 0.01% 0.00%
G 99.98% 0.00% 0.00% 0.00% 0.00% 99.89% 0.00% 0.00% 99.99% 0.00% 0.00% 0.00% 0.01%
T 0.00% 0.01% 0.01% 0.01% 0.01% 0.03% 0.00% 0.00% 0.00% 99.99% 0.01% 99.99% 0.01%

FIGURE 23 (Continued)




| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C | | | |
| 4 | 7 | 0 | 1 | 3 | 2 | 681 | 4 | 483 | 1 | 1 | 3 | 5 | 12281 | 4 |
| 14 | 1 | 122088 | 14 | 4 | 22 | 418 | 22793 | 1 | 276 | 60 | 123533 | 123272 | | 2 |
| 0 | 123011 | 0 | 4 | 0 | 2 | 122428 | 1 | 121990 | 3 | 1 | 1 | 0 | 1 | 5 |
| 123328 | 3 | 7 | 123732 | 123882 | 123443 | 3 | 99925 | 2 | 122991 | 123813 | 10 | 6 | | 7 |
| 972 | 1296 | 2223 | 567 | 429 | 849 | 788 | 1595 | 1842 | 1047 | 442 | 770 | 1034 | 147 | 9 |
| 123346 | 123022 | 122095 | 123751 | 123889 | 123469 | 123530 | 122723 | 122476 | 123271 | 123875 | 123547 | 123283 | 12283 | 8 |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.55% | 0.00% | 0.39% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98 | % |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.02% | 0.34% | 18.57% | 0.00% | 0.22% | 0.05% | 99.99% | 99.99% | 0.00 | % |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.11% | 0.00% | 99.60% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01 | % |
| 99.99% | 0.00% | 0.01% | 99.98% | 99.99% | 99.98% | 0.00% | 81.42% | 0.00% | 99.77% | 99.95% | 0.01% | 0.00% | 0.01 | % |
| 7 | 12 | 2 | 2 | 8 | 0 | 1386 | 3 | 888 | 6 | 1 | 9 | 5 | 13527 | 0 |
| 9 | 2 | 134514 | 6 | 6 | 16 | 789 | 394 | 0 | 21 | 8 | 136054 | 135699 | | 3 |
| 0 | 135522 | 1 | 0 | 2 | 3 | 133879 | 0 | 134317 | 2 | 0 | 4 | 1 | 1 | 6 |
| 135869 | 1 | 11 | 136276 | 136497 | 135993 | 4 | 134832 | 10 | 135950 | 136566 | 17 | 13 | | 8 |
| 1047 | 1395 | 2404 | 648 | 419 | 920 | 874 | 1703 | 1717 | 952 | 356 | 847 | 1213 | 163 | 4 |
| 135885 | 135537 | 134528 | 136284 | 136513 | 136012 | 136058 | 135229 | 135215 | 135979 | 136575 | 136084 | 135718 | 13529 | 7 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 1.02% | 0.00% | 0.66% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98 | % |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.58% | 0.29% | 0.00% | 0.02% | 0.01% | 99.98% | 99.99% | 0.00 | % |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 98.40% | 0.00% | 99.34% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01 | % |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.00% | 99.71% | 0.01% | 99.98% | 99.99% | 0.01% | 0.01% | 0.01 | % |

**pNMG-172 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N)**

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 17 | 0 | 0 | 4 | 9 | 57 | 58 | 119654 | 7 | 1 | 7 | 3 | 120929 | | |
| C | 1 | 117211 | 118953 | 118582 | 119038 | 1 | 119393 | 1 | 1 | 8 | 119588 | 8 | 2 | | |
| G | 119497 | 0 | 0 | 0 | 0 | 119606 | 1 | 13 | 120687 | 1 | 1 | 0 | 6 | | |
| T | 5 | 9 | 8 | 5 | 8 | 53 | 3 | 1 | 1 | 119310 | 6 | 121178 | 6 | | |
| N | 2346 | 4646 | 2904 | 3274 | 2810 | 2148 | 2410 | 2196 | 1169 | 2545 | 2263 | 676 | 922 | | |
| | 119520 | 117220 | 118961 | 118591 | 119055 | 119717 | 119455 | 119669 | 120696 | 119320 | 119602 | 121189 | 120943 | | |
| A | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.05% | 0.05% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | | |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.99% | 0.00% | 99.95% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | | |
| G | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.91% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.04% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | | |

**pNMG-173 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 29 | 1 | 5 | 7 | 8 | 127 | 0 | 190886 | 53 | 3 | 8 | 4 | 192759 | | |
| C | 1 | 186991 | 189769 | 189139 | 189740 | 4 | 190483 | 7 | 1 | 9 | 190805 | 17 | 0 | | |
| G | 190363 | 2 | 0 | 2 | 1 | 190703 | 0 | 12 | 192402 | 0 | 0 | 0 | 14 | | |
| T | 9 | 12 | 10 | 10 | 17 | 34 | 15 | 0 | 3 | 190239 | 16 | 193241 | 10 | | |
| N | 3847 | 7243 | 4461 | 5087 | 4479 | 3377 | 3747 | 3400 | 1846 | 4054 | 3476 | 1043 | 1463 | | |
| | 190402 | 187006 | 189784 | 189158 | 189766 | 190868 | 190498 | 190905 | 192459 | 190251 | 190829 | 193262 | 192783 | | |
| A | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.07% | 0.00% | 99.99% | 0.03% | 0.00% | 0.00% | 0.00% | 99.99% | | |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | | |
| G | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.91% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% | 0.00% | 0.01% | | |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% | | |

FIGURE 23 (Continued)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 4 | 2 | 2 | 5 | 3 | 2997 | 8 | 1855 | 6 | 3 | 1 | 5 | 120400 |
| 15 | 0 | 119788 | 9 | 6 | 7 | 2486 | 763 | 84 | 11 | 21 | 121129 | 120831 | 1 |
| 1 | 120672 | 1 | 2 | 0 | 1 | 115421 | 0 | 118302 | 3 | 0 | 3 | 1 | 20 |
| 120972 | 1 | 4 | 121319 | 121518 | 121048 | 71 | 119601 | 62 | 120995 | 121508 | 13 | 9 | 3 |
| 869 | 1188 | 2070 | 533 | 336 | 806 | 890 | 1493 | 1562 | 850 | 332 | 718 | 1018 | 1440 |
| 120996 | 120677 | 119795 | 121332 | 121529 | 121059 | 120975 | 120372 | 120303 | 121015 | 121532 | 121146 | 120846 | 120424 |
| | | | | | | | | | | | | | |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 2.48% | 0.01% | 1.54% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 2.05% | 0.63% | 0.07% | 0.01% | 0.02% | 99.99% | 99.99% | 0.00% |
| 0.00% | ###### | 0.00% | 0.00% | 0.00% | 0.00% | 95.41% | 0.00% | 98.34% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% |
| 99.98% | 0.00% | 0.00% | 99.99% | 99.99% | 99.99% | 0.06% | 99.36% | 0.05% | 99.98% | 99.98% | 0.01% | 0.01% | 0.00% |
| | | | | | | | | | | | | | |
| 10 | 10 | 5 | 1 | 7 | 4 | 2498 | 14 | 1832 | 8 | 5 | 4 | 6 | 191897 |
| 20 | 2 | 190923 | 10 | 15 | 10 | 1898 | 702 | 1 | 36 | 23 | 192927 | 192631 | 4 |
| 1 | 192222 | 1 | 0 | 3 | 0 | 188656 | 0 | 189940 | 3 | 0 | 1 | 1 | 48 |
| 192766 | 3 | 15 | 193330 | 193631 | 192952 | 9 | 191216 | 95 | 192844 | 193717 | 117 | 17 | 12 |
| 1449 | 2009 | 3302 | 905 | 590 | 1280 | 1185 | 2314 | 2378 | 1355 | 501 | 1197 | 1591 | 2285 |
| 192797 | 192237 | 190944 | 193341 | 193656 | 192966 | 193061 | 191932 | 191868 | 192891 | 193745 | 193049 | 192655 | 191961 |
| | | | | | | | | | | | | | |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 1.29% | 0.01% | 0.95% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.98% | 0.37% | 0.00% | 0.02% | 0.01% | 99.94% | 99.99% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 97.72% | 0.00% | 99.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% |
| 99.98% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.00% | 99.63% | 0.05% | 99.98% | 99.99% | 0.06% | 0.01% | 0.01% |

FIGURE 23 (Continued)

# FIGURE 24

| | P | A | M | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | C | C | G | C | A | G | T | C | T | A | T |
| **pNMG-174 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V)** | | | | | | | | | | | | |
| **A** | 34 | 29 | 11 | 13 | 56 | 135 | 40 | 198571 | 8 | 4 | 6 | 6 | 200496 | 6 |
| **C** | 5 | 194278 | 197293 | 196565 | 197221 | 3 | 197946 | 1 | 1 | 17 | 198396 | 12 | 3 | 11 |
| **G** | 198028 | 2 | 3 | 2 | 2 | 198374 | 1 | 36 | 200229 | 22 | 2 | 1 | 18 | 1 |
| **T** | 16 | 20 | 8 | 15 | 16 | 7 | 7 | 3 | 8 | 197713 | 12 | 200893 | 9 | 200532 |
| **N** | 4035 | 7789 | 4803 | 5523 | 4823 | 3598 | 4123 | 3506 | 1871 | 4294 | 3634 | 1138 | 1502 | 1478 |
| | 198083 | 194329 | 197315 | 196595 | 197295 | 198519 | 197994 | 198611 | 200246 | 197756 | 198416 | 200912 | 200526 | 200550 |
| **A** | 0.02% | 0.01% | 0.01% | 0.01% | 0.03% | 0.07% | 0.02% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **C** | 0.00% | 99.97% | 99.99% | 99.98% | 99.96% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.01% |
| **G** | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.02% | 99.99% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| **T** | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 0.00% | 99.99% |
| **pNMG-175 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | | |
| **A** | 56 | 53 | 2 | 4 | 4 | 133 | 4 | 216224 | 7 | 5 | 10 | 6 | 218978 | 15 |
| **C** | 5 | 211711 | 215382 | 214607 | 215400 | 6 | 216221 | 0 | 3 | 12 | 216218 | 14 | 1 | 13 |
| **G** | 215974 | 3 | 0 | 1 | 1 | 216344 | 0 | 17 | 218187 | 2 | 0 | 3 | 15 | 0 |
| **T** | 7 | 33 | 8 | 20 | 28 | 14 | 13 | 2 | 12 | 214966 | 17 | 219351 | 7 | 218891 |
| **N** | 4541 | 8783 | 5191 | 5951 | 5150 | 4086 | 4345 | 4340 | 2374 | 5598 | 4338 | 1209 | 1582 | 1664 |
| | 216042 | 211800 | 215392 | 214632 | 215433 | 216497 | 216238 | 216243 | 218209 | 214985 | 216245 | 219374 | 219001 | 218919 |
| **A** | 0.03% | 0.03% | 0.00% | 0.00% | 0.00% | 0.06% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| **C** | 0.00% | 99.96% | ###### | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.01% |
| **G** | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| **T** | 0 00% | 0 02% | 0 00% | 0 01% | 0 01% | 0 01% | 0 01% | 0 00% | 0 01% | 99 99% | 0 01% | 99 99% | 0 00% | 99 99% |

FIGURE 24 (Continued)

| 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | C | T | T | T | G | T | G | T | T | C | | |
| 15 | 1 | 0 | 3 | 3 | 5325 | 10 | 4115 | 6 | 6 | 17 | 2 | 199496 |
| 1 | 198558 | 13 | 12 | 15 | 3158 | 679 | 94 | 14 | 28 | 200564 | 200238 | 2 |
| 199893 | 2 | 1 | 0 | 1 | 191773 | 1 | 195041 | 4 | 0 | 97 | 4 | 32 |
| 29 | 18 | 201149 | 201414 | 200686 | 170 | 198971 | 252 | 200575 | 201420 | 113 | 16 | 7 |
| 2089 | 3448 | 864 | 598 | 1321 | 1600 | 2365 | 2524 | 1427 | 569 | 1232 | 1763 | 2486 |
| 199938 | 198579 | 201163 | 201429 | 200705 | 200426 | 199661 | 199502 | 200599 | 201454 | 200791 | 200260 | 199537 |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 2.66% | 0.01% | 2.06% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% |
| 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 1.58% | 0.34% | 0.05% | 0.01% | 0.01% | 99.89% | 99.99% | 0.00% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 95.68% | 0.00% | 97.76% | 0.00% | 0.00% | 0.05% | 0.00% | 0.02% |
| 0.01% | 0.01% | 99.99% | 99.99% | 99.99% | 0.08% | 99.65% | 0.13% | 99.99% | 99.98% | 0.06% | 0.01% | 0.00% |
| 8 | 50 | 3 | 9 | 4 | 122 | 15 | 70 | 5 | 6 | 3 | 27 | 217793 |
| 3 | 216695 | 15 | 13 | 14 | 132 | 721 | 5 | 24 | 18 | 219294 | 218658 | 1 |
| 218010 | 2 | 1 | 61 | 0 | 218890 | 0 | 217812 | 3 | 1 | 3 | 3 | 31 |
| 2 | 19 | 219582 | 219837 | 219102 | 9 | 217267 | 3 | 218907 | 219881 | 21 | 21 | 15 |
| 2560 | 3817 | 979 | 660 | 1460 | 1365 | 2515 | 2628 | 1579 | 611 | 1196 | 1807 | 2675 |
| 218023 | 216766 | 219601 | 219920 | 219120 | 219153 | 218003 | 217890 | 218939 | 219906 | 219321 | 218709 | 217840 |
| 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.06% | 0.01% | 0.03% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% |
| 0.00% | 99.97% | 0.01% | 0.01% | 0.01% | 0.06% | 0.33% | 0.00% | 0.01% | 0.01% | 99.99% | 99.98% | 0.00% |
| 99.99% | 0.00% | 0.00% | 0.03% | 0.00% | 99.88% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.01% | 99.99% | 99.96% | 99.99% | 0.00% | 99.66% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% |

**pNMG-176 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S)**

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 97 | 3 | 4 | 31 | 9 | 313 | 7 | 232628 | 46 | 3 | 5 | 7 | 235537 | 15 | |
| C | 2 | 227886 | 231587 | 230837 | 231630 | 1 | 232510 | 2 | 2 | 15 | 232455 | 18 | 0 | 13 | |
| G | 232253 | 1 | 1 | 1 | 0 | 232607 | 0 | 16 | 234670 | 0 | 0 | 1 | 14 | 0 | |
| T | 6 | 15 | 13 | 22 | 20 | 14 | 15 | 24 | 4 | 231110 | 19 | 235920 | 10 | 235521 | |
| N | 4910 | 9363 | 5663 | 6377 | 5609 | 4333 | 4736 | 4598 | 2546 | 6140 | 4789 | 1322 | 1707 | 1719 | |
| | 232358 | 227905 | 231605 | 230891 | 231659 | 232935 | 232532 | 232670 | 234722 | 231128 | 232479 | 235946 | 235561 | 235549 | |
| A | 0.04% | 0.00% | 0.00% | 0.01% | 0.00% | 0.13% | 0.00% | 99.98% | 0.02% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | |
| C | 0.00% | 99.99% | 99.99% | 99.98% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.01% | |
| G | 99.95% | 0.00% | 0.00% | 0.00% | 0.00% | 99.86% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 99.99% | |

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 29 | 15 | 1 | 3 | 3 | 84 | 7 | 169056 | 1 | 2 | 9 | 1 | 171447 | 18 | |
| C | 0 | 165720 | 168533 | 167759 | 168505 | 2 | 169201 | 1 | 2 | 13 | 169168 | 4 | 5 | 15 | |
| G | 169032 | 0 | 0 | 0 | 0 | 169211 | 1 | 11 | 170886 | 0 | 1 | 1 | 4 | 0 | |
| T | 12 | 7 | 10 | 11 | 16 | 14 | 24 | 2 | 9 | 168099 | 9 | 171681 | 3 | 171368 | |
| N | 3732 | 7063 | 4261 | 5031 | 4280 | 3493 | 3571 | 3734 | 1906 | 4647 | 3574 | 1074 | 1302 | 1360 | |
| | 169073 | 165742 | 168544 | 167773 | 168524 | 169311 | 169233 | 169070 | 170898 | 168114 | 169187 | 171687 | 171459 | 171401 | |

FIGURE 24 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 3 | 2 | 7 | 5 | 125 | 16 | 332 | 8 | 10 | 3 | 8 | 234458 |
| 1 | 233162 | 23 | 20 | 15 | 465 | 1275 | 0 | 24 | 21 | 235812 | 235191 | 6 |
| 234548 | 4 | 4 | 1 | 13 | 235158 | 4 | 234047 | 3 | 1 | 6 | 4 | 29 |
| 10 | 16 | 236149 | 236478 | 235598 | 45 | 233399 | 5 | 235543 | 236582 | 17 | 25 | 5 |
| 2694 | 4082 | 1089 | 761 | 1636 | 1474 | 2573 | 2883 | 1688 | 649 | 1425 | 2035 | 2765 |
| 234573 | 233185 | 236178 | 236506 | 235631 | 235793 | 234694 | 234384 | 235578 | 236614 | 235838 | 235228 | 234498 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.01% | 0.14% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% |
| 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.20% | 0.54% | 0.00% | 0.01% | 0.01% | 99.99% | 99.98% | 0.00% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 99.73% | 0.00% | 99.86% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.02% | 99.45% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 3 | 0 | 7 | 1 | 108 | 6 | 128 | 4 | 4 | 8 | 3 | 170561 |
| 0 | 169660 | 15 | 75 | 89 | 523 | 45487 | 35 | 702 | 43 | 171671 | 171322 | 1 |
| 170628 | 0 | 3 | 1 | 1 | 170858 | 2 | 169823 | 0 | 0 | 6 | 0 | 16 |
| 9 | 10 | 171982 | 172118 | 171382 | 9 | 125059 | 8 | 170433 | 172038 | 17 | 11 | 3 |
| 2109 | 3088 | 761 | 560 | 1288 | 1263 | 2207 | 2766 | 1621 | 675 | 1058 | 1424 | 2179 |
| 170652 | 169673 | 172000 | 172201 | 171473 | 171498 | 170554 | 169994 | 171139 | 172085 | 171702 | 171336 | 170581 |

FIGURE 24 (Continued)

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FIGURE 25

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T |
|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)** | | | | | | | | | | |
| A | 13 | 3 | 2 | 4 | 68 | 0 | 133554 | 9 | 3 | 0 |
| C | 1 | 130804 | 132806 | 132355 | 132759 | 3 | 133258 | 4 | 1 | 16 | 133495 | 13 |
| G | 133210 | 2 | 0 | 0 | 1 | 133560 | 2 | 11 | 134712 | 0 | 5 | 1 |
| T | 2 | 7 | 7 | 6 | 14 | 5 | 10 | 1 | 2 | 133163 | 15 | 135264 |
| N | 2805 | 5215 | 3213 | 3666 | 3251 | 2393 | 2759 | 2459 | 1304 | 2846 | 2510 | 750 |
| | 133226 | 130816 | 132816 | 132363 | 132778 | 133636 | 133270 | 133570 | 134724 | 133182 | 133518 | 135278 |
| A | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% |
| G | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% |
| **pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)** | | | | | | | | | | |
| A | 15 | 21 | 2 | 2 | 5 | 53 | 5 | 135773 | 4 | 2 | 2 | 0 |
| C | 2 | 132869 | 134987 | 134476 | 134879 | 0 | 135486 | 6 | 1 | 14 | 135650 | 7 |
| G | 135423 | 0 | 2 | 0 | 1 | 135779 | 2 | 9 | 137014 | 0 | 2 | 0 |
| T | 5 | 12 | 7 | 8 | 14 | 10 | 9 | 3 | 4 | 135314 | 9 | 137594 |
| N | 3011 | 5554 | 3458 | 3970 | 3557 | 2614 | 2954 | 2665 | 1433 | 3126 | 2793 | 855 |
| | 135445 | 132902 | 134998 | 134486 | 134899 | 135842 | 135502 | 135791 | 137023 | 135330 | 135663 | 137601 |
| A | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 99.98% | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% |
| G | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% |

FIGURE 25 (Continued)

| 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | T | G | C | T | T | T | G | T | G | T | T | C | |
| 135039 | 7 | 9 | 2 | 3 | 4 | 2 | 243 | 9 | 121 | 4 | 6 | 6 | 3 134343 |
| 1 | 10 | 0 | 133703 | 10 | 44 | 12 | 23 | 3460 | 2 | 13 | 12 | 135206 | 134827 11 |
| 9 | 0 | 134678 | 3 | 6 | 4 | 0 | 135108 | 1 | 134297 | 0 | 3 | 4 | 1 20 |
| 1 | 135040 | 1 | 19 | 135402 | 135556 | 135141 | 4 | 130879 | 2 | 135073 | 135652 | 11 | 12 7 |
| 978 | 971 | 1340 | 2301 | 607 | 420 | 873 | 650 | 1679 | 1606 | 938 | 355 | 801 | 1185 1647 |
| 135050 | 135057 | 134688 | 133727 | 135421 | 135608 | 135155 | 135378 | 134349 | 134422 | 135090 | 135673 | 135227 | 134843 134381 |
| 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.18% | 0.01% | 0.09% | 0.00% | 0.00% | 0.00% | 0.00% 99.97% |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.03% | 0.01% | 0.02% | 2.58% | 0.00% | 0.01% | 0.01% | 99.98% | 99.99% 0.01% |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.80% | 0.00% | 99.91% | 0.00% | 0.00% | 0.00% | 0.00% 0.01% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 99.96% | 99.99% | 0.00% | 97.42% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% 0.01% |
| 137273 | 6 | 5 | 2 | 1 | 8 | 2 | 11 | 10 | 9 | 1 | 1 | 36 | 3 136612 |
| 2 | 10 | 1 | 135935 | 13 | 19 | 15 | 92 | 6920 | 0 | 74 | 11 | 137502 | 137191 4 |
| 7 | 0 | 136932 | 1 | 2 | 1 | 0 | 137570 | 3 | 136573 | 2 | 0 | 1 | 1 22 |
| 9 | 137315 | 6 | 8 | 137783 | 137987 | 137432 | 7 | 129757 | 5 | 137295 | 138038 | 12 | 20 6 |
| 1165 | 1125 | 1512 | 2510 | 657 | 441 | 1007 | 776 | 1766 | 1869 | 1084 | 406 | 905 | 1241 1812 |
| 137291 | 137331 | 136944 | 135946 | 137799 | 138015 | 137449 | 137680 | 136690 | 136587 | 137372 | 138050 | 137551 | 137215 136644 |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.03% | 0.00% 99.98% |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.07% | 5.06% | 0.00% | 0.05% | 0.01% | 99.96% | 99.98% 0.00% |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.92% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% 0.02% |
| 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 99.98% | 99.99% | 0.01% | 94.93% | 0.00% | 99.94% | 99.99% | 0.01% | 0.01% 0.00% |

**D108N_D147Y_E155V**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 38 | 1 | 84 | 3 | 48 | 72 | 2 | 121963 | 5 | 2 | 5 | 1 |
| C | 3 | 119412 | 121232 | 120698 | 121230 | 1 | 121720 | 1 | 1 | 6 | 121892 | 12 |
| G | 121541 | 0 | 1 | 2 | 1 | 121954 | 2 | 8 | 123077 | 1 | 0 | 0 |
| T | 3 | 8 | 8 | 7 | 11 | 8 | 5 | 1 | 3 | 121641 | 9 | 123573 |
| N | 2782 | 4946 | 3042 | 3657 | 3077 | 2332 | 2638 | 2394 | 1281 | 2668 | 2412 | 732 |
| | 121585 | 119421 | 121325 | 120710 | 121290 | 122035 | 121729 | 121973 | 123086 | 121650 | 121906 | 123586 |
| | | | | | | | | | | | | |
| A | 0.03% | 0.00% | 0.07% | 0.00% | 0.04% | 0.06% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 99.99% | 99.92% | 99.99% | 99.95% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| G | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% |

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 29 | 15 | 1 | 3 | 3 | 84 | 7 | 169056 | 1 | 2 | 9 | 1 |
| C | 0 | 165720 | 168533 | 167759 | 168505 | 2 | 169201 | 1 | 2 | 13 | 169168 | 4 |
| G | 169032 | 0 | 0 | 0 | 0 | 169211 | 1 | 11 | 170886 | 0 | 1 | 1 |
| T | 12 | 7 | 10 | 11 | 16 | 14 | 24 | 2 | 9 | 168099 | 9 | 171681 |
| N | 3732 | 7063 | 4261 | 5031 | 4280 | 3493 | 3571 | 3734 | 1906 | 4647 | 3574 | 1074 |
| | 169073 | 165742 | 168544 | 167773 | 168524 | 169311 | 169233 | 169070 | 170898 | 168114 | 169187 | 171687 |
| | | | | | | | | | | | | |
| A | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.99% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| G | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | ###### |

FIGURE 25 (Continued)

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 123369 | 4 | 7 | 0 | 1 | 3 | 2 | 681 | 4 | 483 | 1 | 1 | 3 | 5 | 122814 | | | | | | | | | | |
| 1 | 14 | 1 | 122088 | 14 | 4 | 22 | 418 | 22793 | 1 | 276 | 60 | 123533 | 123272 | 2 | | | | | | | | | | |
| 8 | 0 | 123011 | 0 | 4 | 0 | 2 | 122428 | 1 | 121990 | 3 | 1 | 1 | 0 | 15 | | | | | | | | | | |
| 5 | 123328 | 3 | 7 | 123732 | 123882 | 123443 | 3 | 99925 | 2 | 122991 | 123813 | 10 | 6 | 7 | | | | | | | | | | |
| 935 | 972 | 1296 | 2223 | 567 | 429 | 849 | 788 | 1595 | 1842 | 1047 | 442 | 770 | 1034 | 1479 | | | | | | | | | | |
| 123383 | 123346 | 123022 | 122095 | 123751 | 123889 | 123469 | 123530 | 122723 | 122476 | 123271 | 123875 | 123547 | 123283 | 122838 | | | | | | | | | | |
| 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.55% | 0.00% | 0.39% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | | | | | | | | | | |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.02% | 0.34% | 18.57% | 0.00% | 0.22% | 0.05% | 99.99% | 99.99% | 0.00% | | | | | | | | | | |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.11% | 0.00% | 99.60% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | | | | | | | | | | |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.98% | 99.99% | 99.98% | 0.00% | 81.42% | 0.00% | 99.77% | 99.95% | 0.01% | 0.00% | 0.01% | | | | | | | | | | |
| 171447 | 18 | 15 | 3 | 0 | 7 | 1 | 108 | 6 | 128 | 4 | 4 | 8 | 3 | 170561 | | | | | | | | | | |
| 5 | 15 | 0 | 169660 | 15 | 75 | 89 | 523 | 45487 | 35 | 702 | 43 | 171671 | 171322 | 1 | | | | | | | | | | |
| 4 | 0 | 170628 | 0 | 3 | 1 | 1 | 170858 | 2 | 169823 | 0 | 0 | 6 | 0 | 16 | | | | | | | | | | |
| 3 | 171368 | 9 | 10 | 171982 | 172118 | 171382 | 9 | 125059 | 8 | 170433 | 172038 | 17 | 11 | 3 | | | | | | | | | | |
| 1302 | 1360 | 2109 | 3088 | 761 | 560 | 1288 | 1263 | 2207 | 2766 | 1621 | 675 | 1058 | 1424 | 2179 | | | | | | | | | | |
| 171459 | 171401 | 170652 | 169673 | 172000 | 172201 | 171473 | 171498 | 170554 | 169994 | 171139 | 172085 | 171702 | 171336 | 170581 | | | | | | | | | | |
| 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.06% | 0.00% | 0.08% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | | | | | | | | | | |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.04% | 0.05% | 0.30% | 26.67% | 0.02% | 0.41% | 0.02% | 99.98% | 99.99% | 0.00% | | | | | | | | | | |
| 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.63% | 0.00% | 99.90% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | | | | | | | | | | |
| 0.00% | 99.98% | 0.01% | 0.01% | 99.99% | 99.95% | 99.95% | 0.01% | 73.33% | 0.00% | 99.59% | 99.97% | 0.01% | 0.01% | 0.00% | | | | | | | | | | |

FIGURE 25 (Continued)

# FIGURE 26

| | | | | P C | A C | M C | 20 G | 19 C | 18 A |
|---|---|---|---|---|---|---|---|---|---|
| | D108N_D147Y_E155V | | | | | | | | |
| A | | 38 | 1 | 84 | 3 | 48 | 72 | 2 | 121963 |
| C | | 3 | 119412 | 121232 | 120698 | 121230 | 1 | 121720 | 1 |
| G | | 121541 | 0 | 1 | 2 | 1 | 121954 | 2 | 8 |
| T | | 3 | 8 | 8 | 7 | 11 | 8 | 5 | 1 |
| N | | 2782 | 4946 | 3042 | 3657 | 3077 | 2332 | 2638 | 2394 |
| | | 121585 | 119421 | 121325 | 120710 | 121290 | 122035 | 121729 | 121973 |
| A | | 0.03% | 0.00% | 0.07% | 0.00% | 0.04% | 0.06% | 0.00% | 99.99% |
| C | | 0.00% | 99.99% | 99.92% | 99.99% | 99.95% | 0.00% | 99.99% | 0.00% |
| G | | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% |
| T | | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| | pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | |
| A | | 29 | 15 | 1 | 3 | 3 | 84 | 7 | 169056 |
| C | | 0 | 165720 | 168533 | 167759 | 168505 | 2 | 169201 | 1 |
| G | | 169032 | 0 | 0 | 0 | 0 | 169211 | 1 | 11 |
| T | | 12 | 7 | 10 | 11 | 16 | 14 | 24 | 2 |
| N | | 3732 | 7063 | 4261 | 5031 | 4280 | 3493 | 3571 | 3734 |
| | | 169073 | 165742 | 168544 | 167773 | 168524 | 169311 | 169233 | 169070 |

FIGURE 26 (Continued)

| 17 | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9 | 8 | 7 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | T | C | T | A | T | G | C | T | T | T | G |
| 5 | 2 | 5 | 1 | 123369 | 4 | 7 | 0 | 1 | 3 | 2 | 681 |
| 1 | 6 | 121892 | 12 | 1 | 14 | 1 | 122088 | 14 | 4 | 22 | 418 |
| 123077 | 1 | 0 | 0 | 8 | 0 | 123011 | 0 | 4 | 0 | 2 | 122428 |
| 3 | 121641 | 9 | 123573 | 5 | 123328 | 3 | 7 | 123732 | 123882 | 123443 | 3 |
| 1281 | 2668 | 2412 | 732 | 935 | 972 | 1296 | 2223 | 567 | 429 | 849 | 788 |
| 123086 | 121650 | 121906 | 123586 | 123383 | 123346 | 123022 | 122095 | 123751 | 123889 | 123469 | 123530 |
| 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.55% |
| 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.02% | 0.34% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.11% |
| 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 99.99% | 0.00% | 0.01% | 99.98% | 99.99% | 99.98% | 0.00% |
| 1 | 2 | 9 | 1 | 171447 | 18 | 15 | 3 | 0 | 7 | 1 | 108 |
| 2 | 13 | 169168 | 4 | 5 | 15 | 0 | 169660 | 15 | 75 | 89 | 523 |
| 170886 | 0 | 1 | 1 | 4 | 0 | 170628 | 0 | 3 | 1 | 1 | 170858 |
| 9 | 168099 | 9 | 171681 | 3 | 171368 | 9 | 10 | 171982 | 172118 | 171382 | 9 |
| 1906 | 4647 | 3574 | 1074 | 1302 | 1360 | 2109 | 3088 | 761 | 560 | 1288 | 1263 |
| 170898 | 168114 | 169187 | 171687 | 171459 | 171401 | 170652 | 169673 | 172000 | 172201 | 171473 | 171498 |

FIGURE 26 (Continued)

| 5 | 4 | 3 | 2 | 1 | | |
|---|---|---|---|---|---|---|
| T | G | T | T | C | | |
| 4 | 483 | 1 | 1 | 3 | 5 | 122814 |
| 22793 | 1 | 276 | 60 | 123533 | 123272 | 2 |
| 1 | 121990 | 3 | 1 | 1 | 0 | 15 |
| 99925 | 2 | 122991 | 123813 | 10 | 6 | 7 |
| 1595 | 1842 | 1047 | 442 | 770 | 1034 | 1479 |
| 122723 | 122476 | 123271 | 123875 | 123547 | 123283 | 122838 |
| 0.00% | 0.39% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% |
| 18.57% | 0.00% | 0.22% | 0.05% | 99.99% | 99.99% | 0.00% |
| 0.00% | 99.60% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 81.42% | 0.00% | 99.77% | 99.95% | 0.01% | 0.00% | 0.01% |
| 6 | 128 | 4 | 4 | 8 | 3 | 170561 |
| 45487 | 35 | 702 | 43 | 171671 | 171322 | 1 |
| 2 | 169823 | 0 | 0 | 6 | 0 | 16 |
| 125059 | 8 | 170433 | 172038 | 17 | 11 | 3 |
| 2207 | 2766 | 1621 | 675 | 1058 | 1424 | 2179 |
| 170554 | 169994 | 171139 | 172085 | 171702 | 171336 | 170581 |

# FIGURE 26 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.99% |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.99% | 0.00% | 99.98% | 0.00% |
| G | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% |

**pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 50 | 2 | 5 | 4 | 9 | 85 | 56 | 159313 |
| C | 1 | 156274 | 158813 | 158107 | 158665 | 2 | 159293 | 0 |
| G | 159222 | 1 | 0 | 2 | 0 | 159466 | 0 | 6 |
| T | 5 | 13 | 7 | 16 | 15 | 10 | 32 | 6 |
| N | 3411 | 6399 | 3864 | 4560 | 4000 | 3126 | 3308 | 3364 |
| | 159278 | 156290 | 158825 | 158129 | 158689 | 159563 | 159381 | 159325 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 0.03% | 0.00% | 0.00% | 0.00% | 0.01% | 0.05% | 0.04% | 99.99% |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.98% | 0.00% | 99.94% | 0.00% |
| G | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% |

**pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 41 | 24 | 2 | 6 | 32 | 121 | 5 | 227568 |
| C | 5 | 223134 | 226899 | 226051 | 226787 | 5 | 227805 | 3 |
| G | 227574 | 0 | 3 | 3 | 0 | 227877 | 3 | 35 |
| T | 16 | 9 | 7 | 11 | 23 | 19 | 11 | 4 |
| N | 4761 | 9230 | 5485 | 6325 | 5554 | 4374 | 4572 | 4786 |
| | 227636 | 223167 | 226911 | 226071 | 226842 | 228022 | 227824 | 227610 |

# FIGURE 26 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.06% |
| 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.04% | 0.05% | 0.30% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.63% |
| 0.01% | 99.99% | 0.01% | ###### | 0.00% | 99.98% | 0.01% | 0.01% | 99.99% | 99.95% | 99.95% | 0.01% |
| | | | | | | | | | | | |
| 14 | 2 | 4 | 4 | 161431 | 5 | 14 | 3 | 6 | 4 | 0 | 166 |
| 3 | 18 | 159281 | 14 | 1 | 13 | 0 | 159860 | 9 | 122 | 28 | 20 |
| 160880 | 0 | 0 | 0 | 2 | 0 | 160769 | 5 | 1 | 0 | 0 | 161347 |
| 3 | 158308 | 5 | 161678 | 9 | 161452 | 8 | 11 | 161918 | 162030 | 161429 | 8 |
| 1789 | 4361 | 3399 | 993 | 1246 | 1219 | 1898 | 2810 | 755 | 533 | 1232 | 1148 |
| 160900 | 158328 | 159290 | 161696 | 161443 | 161470 | 160791 | 159879 | 161934 | 162156 | 161457 | 161541 |
| | | | | | | | | | | | |
| 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.10% |
| 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.08% | 0.02% | 0.01% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.88% |
| 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 99.92% | 99.98% | 0.00% |
| | | | | | | | | | | | |
| 11 | 4 | 4 | 1 | 230640 | 6 | 16 | 6 | 2 | 7 | 3 | 92 |
| 1 | 21 | 227683 | 23 | 3 | 17 | 3 | 228340 | 87 | 144 | 311 | 430 |
| 229915 | 64 | 0 | 0 | 10 | 2 | 229581 | 3 | 4 | 0 | 3 | 230189 |
| 14 | 226136 | 8 | 231062 | 13 | 230583 | 49 | 12 | 231226 | 231368 | 230268 | 11 |
| 2455 | 6166 | 4696 | 1305 | 1659 | 1717 | 2676 | 3964 | 1006 | 806 | 1740 | 1603 |
| 229941 | 226225 | 227695 | 231086 | 230666 | 230608 | 229649 | 228361 | 231319 | 231519 | 230585 | 230722 |

FIGURE 26 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 0.00% | 0.08% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | |
| 26.67% | 0.02% | 0.41% | 0.02% | 99.98% | 99.99% | 0.00% | | |
| 0.00% | 99.90% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | | |
| 73.33% | 0.00% | 99.59% | 99.97% | 0.01% | 0.01% | 0.00% | | |
| 8 | 12 | 10 | 5 | 2 | 3 | 160644 | | |
| 30430 | 2 | 652 | 16 | 161722 | 161261 | 4 | | |
| 1 | 160258 | 4 | 1 | 1 | 2 | 23 | | |
| 130371 | 7 | 160606 | 162052 | 10 | 12 | 10 | | |
| 1879 | 2410 | 1417 | 613 | 952 | 1409 | 2006 | | |
| 160810 | 160279 | 161272 | 162074 | 161735 | 161278 | 160681 | | |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | | |
| 18.92% | 0.00% | 0.40% | 0.01% | 99.99% | 99.99% | 0.00% | | |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | | |
| 81.07% | 0.00% | 99.59% | 99.99% | 0.01% | 0.01% | 0.01% | | |
| 11 | 101 | 5 | 8 | 2 | 6 | 229530 | | |
| 60360 | 2 | 1177 | 173 | 230905 | 230420 | 1 | | |
| 4 | 228497 | 1 | 0 | 3 | 4 | 26 | | |
| 169108 | 8 | 228953 | 231213 | 16 | 15 | 8 | | |
| 2842 | 3717 | 2189 | 931 | 1399 | 1880 | 2760 | | |
| 229483 | 228608 | 230136 | 231394 | 230926 | 230445 | 229565 | | |

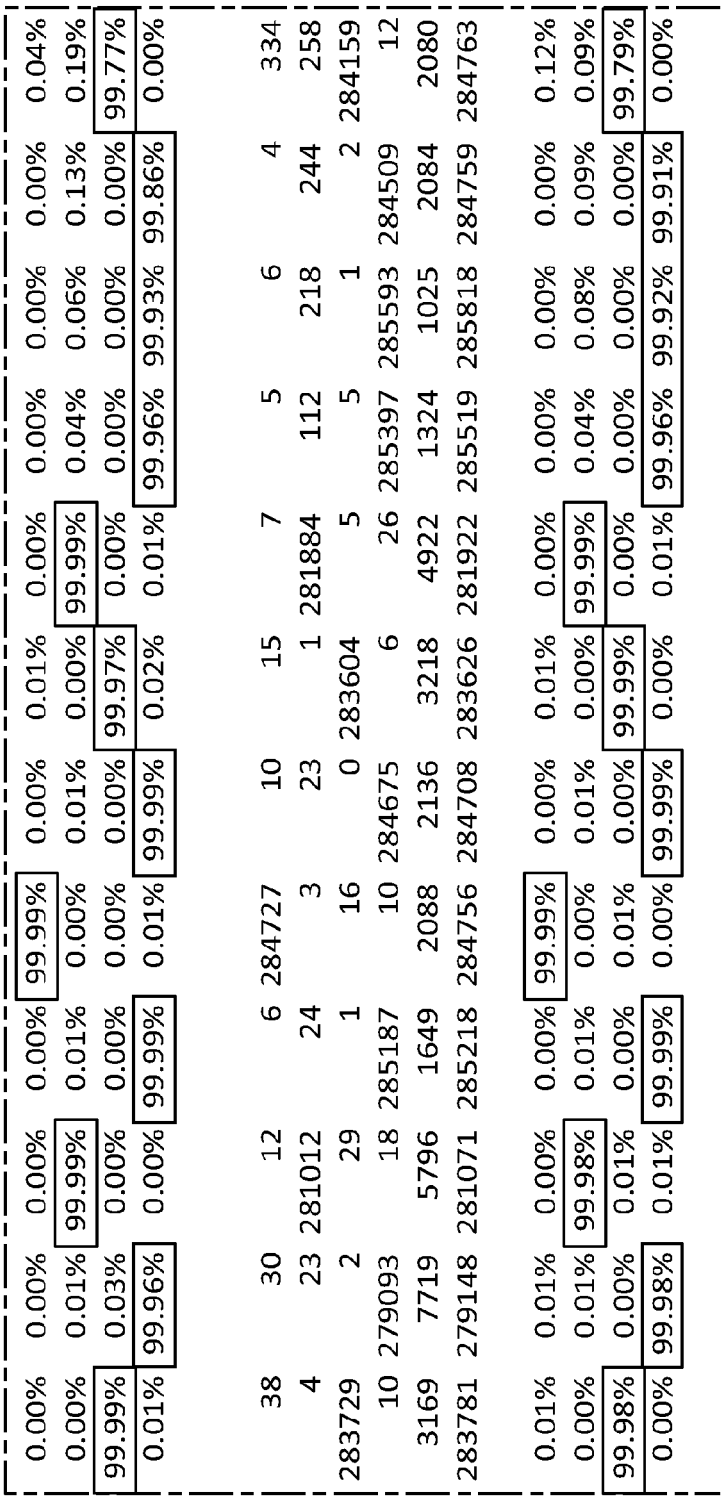
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | 0.05% | 0.00% | 99.98% |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% |
| G | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.02% |
| T | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |

**pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 54 | 6 | 9 | 1 | 9 | 149 | 2 | 280856 |
| C | 5 | 275526 | 280032 | 278910 | 280089 | 3 | 281231 | 3 |
| G | 281030 | 2 | 4 | 1 | 0 | 281422 | 2 | 28 |
| T | 9 | 12 | 10 | 20 | 24 | 35 | 13 | 40 |
| N | 5855 | 11407 | 6895 | 8018 | 6828 | 5341 | 5703 | 6023 |
| | 281098 | 275546 | 280055 | 278932 | 280122 | 281609 | 281248 | 280927 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.97% |
| C | 0.00% | 99.99% | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |

FIGURE 26 (Continued)



| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% |
| 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.04% | 0.06% | 0.13% | 0.00% | 0.19% | | | | | | |
| 99.99% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.77% | | | | | | |
| 0.01% | 99.96% | 0.00% | 99.99% | 0.01% | 99.99% | 0.02% | 0.01% | 99.96% | 99.93% | 99.86% | 0.00% | | | | | | | | |
| 38 | 30 | 12 | 6 | 284727 | 10 | 15 | 7 | 5 | 6 | 4 | 334 | | | | | | | | |
| 4 | 23 | 281012 | 24 | 3 | 23 | 1 | 281884 | 112 | 218 | 244 | 258 | | | | | | | | |
| 283729 | 2 | 29 | 1 | 16 | 0 | 283604 | 5 | 5 | 1 | 2 | 284159 | | | | | | | | |
| 10 | 279093 | 18 | 285187 | 10 | 284675 | 6 | 26 | 285397 | 285593 | 284509 | 12 | | | | | | | | |
| 3169 | 7719 | 5796 | 1649 | 2088 | 2136 | 3218 | 4922 | 1324 | 1025 | 2084 | 2080 | | | | | | | | |
| 283781 | 279148 | 281071 | 285218 | 284756 | 284708 | 283626 | 281922 | 285519 | 285818 | 284759 | 284763 | | | | | | | | |
| 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.12% | | | | | | | | |
| 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.04% | 0.08% | 0.09% | 0.09% | | | | | | | | |
| 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.79% | | | | | | | | |
| 0.00% | 99.98% | 0.01% | 99.99% | 0.00% | 99.99% | 0.00% | 0.01% | 99.96% | 99.92% | 99.91% | 0.00% | | | | | | | | |

FIGURE 26 (Continued)

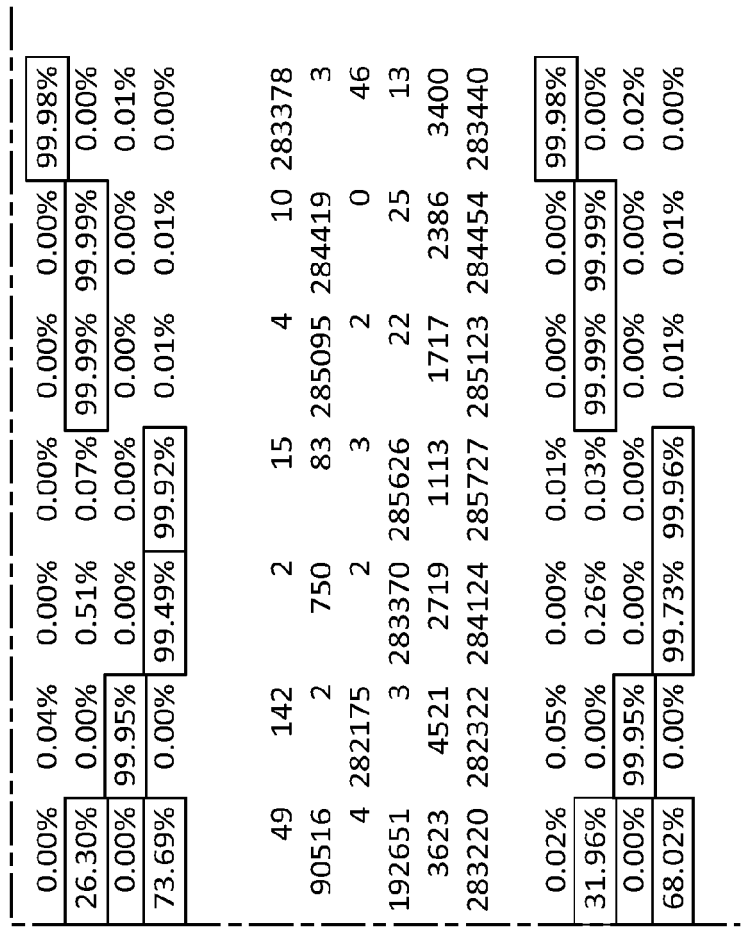



FIGURE 26 (Continued)

# FIGURE 27

| | pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N) | | | 1 G | 2 G | 3 C | 4 C | 5 C | 6 A | 7 G |
|---|---|---|---|---|---|---|---|---|---|---|
| A | | 10 | 9 | 8 | 16 | 2 | 15 | 4 | 139719 | 11 |
| C | | 2 | 4 | 2 | 1 | 141127 | 140716 | 140961 | 3 | 2 |
| G | | 143299 | 141169 | 141433 | 139071 | 3 | 1 | 2 | 50 | 143675 |
| T | | 18 | 9 | 3 | 4 | 10 | 9 | 15 | 3 | 6 |
| N | | 2161 | 4299 | 4044 | 6398 | 4348 | 4749 | 4508 | 5715 | 1796 |
| | | 143329 | 141191 | 141446 | 139092 | 141142 | 140741 | 140982 | 139775 | 143694 |
| A | | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.96% | 0.01% |
| C | | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.98% | 99.99% | 0.00% | 0.00% |
| G | | 99.98% | 99.98% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.04% | 99.99% |
| T | | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |

| | pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 9 | 9 | 13 | 5 | 17 | 3 | 101939 | 4 |
| C | 2 | 1 | 4 | 2 | 102993 | 102770 | 103058 | 1 | 1 |
| G | 104478 | 102367 | 102445 | 100859 | 0 | 0 | 66 | 342 | 105003 |
| T | 4 | 32 | 3 | 6 | 16 | 140 | 135 | 0 | 4 |
| N | 1900 | 3987 | 3935 | 5516 | 3382 | 3469 | 3134 | 4114 | 1384 |
| | 104496 | 102409 | 102461 | 100880 | 103014 | 102927 | 103262 | 102282 | 105012 |
| A | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% | 99.66% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.85% | 99.80% | 0.00% | 0.00% |
| G | 99.98% | 99.96% | 99.98% | 99.98% | 0.00% | 0.00% | 0.06% | 0.33% | 99.99% |
| T | 0.00% | 0.03% | 0.00% | 0.01% | 0.02% | 0.14% | 0.13% | 0.00% | 0.00% |

FIGURE 27 (Continued)

| 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | C | T | G | A | G | C | A | C | G | T | G | A |
| 143159 | 1 | 1 | 13 | 137864 | 12 | 1 | 142750 | 26 | 15 | 2 | 5 | 139440 |
| 1 | 143009 | 25 | 4 | 2 | 2 | 141422 | 3 | 141386 | 1 | 10 | 2 | 2 |
| 17 | 2 | 1 | 143822 | 34 | 142028 | 1 | 5 | 0 | 140547 | 2 | 141902 | 13 |
| 6 | 12 | 143841 | 6 | 3 | 5 | 36 | 10 | 13 | 5 | 141567 | 3 | 6 |
| 2307 | 2466 | 1622 | 1645 | 7587 | 3443 | 4030 | 2722 | 4065 | 4922 | 3882 | 3546 | 5997 |
| 143183 | 143024 | 143868 | 143845 | 137903 | 142047 | 141460 | 142768 | 141425 | 140568 | 141581 | 141912 | 139461 |
| 99.98% | 0.00% | 0.00% | 0.01% | 99.97% | 0.01% | 0.00% | 99.99% | 0.02% | 0.01% | 0.00% | 0.00% | 99.98% |
| 0.00% | 99.99% | 0.02% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 99.98% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% |
| 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.03% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% |
| 104100 | 2 | 1 | 14 | 101157 | 13 | 10 | 104422 | 1 | 13 | 3 | 3 | 102159 |
| 0 | 104619 | 16 | 2 | 0 | 1 | 103686 | 10 | 103538 | 0 | 11 | 0 | 1 |
| 653 | 1 | 2 | 105218 | 24 | 104092 | 1 | 7 | 1 | 102800 | 5 | 103812 | 15 |
| 9 | 9 | 105257 | 5 | 0 | 2 | 20 | 5 | 28 | 10 | 103500 | 1 | 16 |
| 1634 | 1765 | 1120 | 1157 | 5215 | 2288 | 2679 | 1952 | 2828 | 3573 | 2877 | 2580 | 4205 |
| 104762 | 104631 | 105276 | 105239 | 101181 | 104108 | 103717 | 104444 | 103568 | 102823 | 103519 | 103816 | 102191 |
| 99.37% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 99.97% |
| 0.00% | 99.99% | 0.02% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.62% | 0.00% | 0.00% | 99.98% | 0.02% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 100.00% | 0.01% |
| 0.01% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.03% | 0.01% | 99.98% | 0.00% | 0.02% |

FIGURE 27 (Continued)

| PTG | AG | MG | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | 16 | 37 | | 34 | 142595 | | |
| 11 | 5 | 5 | 141742 | | 0 | | |
| 0 | 142689 | 142890 | | 5 | 10 | | |
| 142177 | 4 | 24 | | 13 | 15 | | |
| 3268 | 2744 | 2502 | 3664 | | 2838 | | |
| 142190 | 142714 | 142956 | 141794 | | 142620 | | |
| 0.00% | 0.01% | 0.03% | 0.02% | | 99.98% | | |
| 0.01% | 0.00% | 0.00% | 99.96% | | 0.00% | | |
| 0.00% | 99.98% | 99.95% | 0.00% | | 0.01% | | |
| 99.99% | 0.00% | 0.02% | 0.01% | | 0.01% | | |
| | | | | | | | |
| 1 | 5 | 26 | | 1 | 104286 | | |
| 4 | 1 | 2 | 103701 | | 0 | | |
| 0 | 104429 | 104639 | | 0 | 7 | | |
| 104047 | 3 | 3 | | 10 | 10 | | |
| 2344 | 1958 | 1726 | 2683 | | 2092 | | |
| 104052 | 104438 | 104670 | 103712 | | 104303 | | |
| 0.00% | 0.00% | 0.02% | 0.00% | | 99.98% | | |
| 0.00% | 0.00% | 0.00% | 99.99% | | 0.00% | | |
| 0.00% | 99.99% | 99.97% | 0.00% | | 0.01% | | |
| 100.00% | 0.00% | 0.00% | 0.01% | | 0.01% | | |

# FIGURE 27 (Continued)

**pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 11 | 8 | 15 | 18 | 19 | 3 | 1 | 109369 | 7 |
| C | 0 | 1 | 0 | 1 | 110430 | 110279 | 110601 | 2 | 1 |
| G | 111991 | 109742 | 109817 | 107986 | 0 | 1 | 0 | 215 | 112671 |
| T | 17 | 5 | 4 | 1 | 15 | 27 | 23 | 4 | 4 |
| N | 2155 | 4418 | 4338 | 6168 | 3710 | 3864 | 3549 | 4584 | 1491 |
| | 112019 | 109756 | 109836 | 108006 | 110464 | 110310 | 110625 | 109590 | 112683 |
| | | | | | | | | | |
| A | 0.01% | 0.01% | 0.01% | 0.02% | 0.02% | 0.00% | 0.00% | 99.80% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 99.97% | 99.98% | 0.00% | 0.00% |
| G | 99.98% | 99.99% | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 0.20% | 99.99% |
| T | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 0.02% | 0.00% | 0.00% |
| T | | | | | | | | | |

**pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 3 | 6 | 18 | 0 | 7 | 8 | 121491 | 6 |
| C | 1 | 2 | 1 | 2 | 122985 | 122703 | 122915 | 8 | 0 |
| G | 124439 | 121968 | 122006 | 120090 | 0 | 2 | 3 | 402 | 125206 |
| T | 4 | 5 | 9 | 13 | 22 | 49 | 46 | 4 | 3 |
| N | 2461 | 4939 | 4895 | 6794 | 3910 | 4156 | 3945 | 5012 | 1702 |
| | 124456 | 121978 | 122022 | 120123 | 123007 | 122761 | 122972 | 121905 | 125215 |
| | | | | | | | | | |
| A | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.66% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.95% | 99.95% | 0.01% | 0.00% |
| G | 99.99% | 99.99% | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 0.33% | 99.99% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.02% | 0.04% | 0.04% | 0.00% | 0.00% |

# FIGURE 27 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 111446 | 0 | 2 | 7 | 108443 | 9 | 0 | 112074 | 25 | 10 | 3 | 3 | 109656 |
| 0 | 112200 | 12 | 1 | 2 | 0 | 111107 | 2 | 111120 | 0 | 13 | 2 | 2 |
| 858 | 0 | 0 | 112926 | 19 | 111578 | 12 | 10 | 2 | 110323 | 0 | 111279 | 8 |
| 1 | 8 | 112947 | 4 | 1 | 2 | 11 | 4 | 20 | 27 | 111062 | 7 | 2 |
| 1869 | 1966 | 1213 | 1236 | 5709 | 2585 | 3044 | 2084 | 3032 | 3839 | 3121 | 2908 | 4531 |
| 112305 | 112208 | 112961 | 112938 | 108465 | 111589 | 111130 | 112090 | 111167 | 110360 | 111078 | 111291 | 109668 |
| | | | | | | | | | | | | |
| 99.24% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 99.99% | 0.02% | 0.01% | 0.00% | 0.00% | 99.99% |
| 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.96% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.76% | 0.00% | 0.00% | 99.99% | 0.02% | 99.99% | 0.01% | 0.01% | 0.00% | 99.97% | 0.00% | 99.99% | 0.01% |
| 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.02% | 99.99% | 0.01% | 0.00% |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 124484 | 4 | 2 | 11 | 120721 | 7 | 2 | 124564 | 5 | 12 | 3 | 8 | 121886 |
| 0 | 124697 | 13 | 3 | 2 | 2 | 123533 | 2 | 123498 | 0 | 13 | 1 | 1 |
| 453 | 0 | 0 | 125518 | 17 | 124069 | 1 | 4 | 1 | 122617 | 0 | 123826 | 6 |
| 8 | 12 | 125526 | 2 | 1 | 1 | 11 | 8 | 14 | 2 | 123500 | 3 | 5 |
| 1972 | 2204 | 1376 | 1383 | 6176 | 2838 | 3370 | 2339 | 3399 | 4286 | 3398 | 3076 | 5016 |
| 124945 | 124713 | 125541 | 125534 | 120741 | 124079 | 123547 | 124578 | 123518 | 122631 | 123516 | 123838 | 121898 |
| | | | | | | | | | | | | |
| 99.63% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.99% |
| 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.36% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% |
| 0.01% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% |

FIGURE 27 (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | 8 | 35 | 6 | 111887 | | |
| 2 | 1 | 5 | 111189 | 0 | | |
| 0 | 111970 | 112237 | 0 | 8 | | |
| 111534 | 2 | 2 | 11 | 7 | | |
| 2661 | 2218 | 1920 | 2993 | 2297 | | |
| 111538 | 111981 | 112279 | 111206 | 111902 | | |
| | | | | | | |
| 0.00% | 0.01% | 0.03% | 0.01% | 99.99% | | |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | | |
| 0.00% | 99.99% | 99.96% | 0.00% | 0.01% | | |
| 100.00% | 0.00% | 0.00% | 0.01% | 0.01% | | |
| | | | | | | |
| | | | | | | |
| 3 | 13 | 24 | 6 | 124442 | | |
| 8 | 2 | 6 | 123646 | 3 | | |
| 2 | 124468 | 124685 | 4 | 10 | | |
| 124090 | 2 | 4 | 12 | 9 | | |
| 2811 | 2429 | 2195 | 3246 | 2450 | | |
| 124103 | 124485 | 124719 | 123668 | 124464 | | |
| | | | | | | |
| 0.00% | 0.01% | 0.02% | 0.00% | 99.98% | | |
| 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | | |
| 0.00% | 99.99% | 99.97% | 0.00% | 0.01% | | |
| 99.99% | 0.00% | 0.00% | 0.01% | 0.01% | | |

FIGURE 27 (Continued)

**pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 13 | 11 | 23 | 11 | 20 | 0 | 144076 | 10 | | | |
| C | 2 | 0 | 2 | 3 | 145947 | 145620 | 145835 | 0 | 2 | | | |
| G | 147586 | 144630 | 144868 | 142700 | 11 | 2 | 1 | 600 | 148388 | | | |
| T | 4 | 8 | 12 | 11 | 13 | 17 | 27 | 6 | 18 | | | |
| N | 2772 | 5725 | 5483 | 7639 | 4394 | 4717 | 4513 | 5694 | 1958 | | | |
| | 147604 | 144651 | 144893 | 142737 | 145982 | 145659 | 145863 | 144682 | 148418 | | | |
| | | | | | | | | | | | | |
| A | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 99.58% | 0.01% | | | |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.97% | 99.98% | 0.00% | 0.00% | | | |
| G | 99.99% | 99.99% | 99.98% | 99.97% | 0.01% | 0.00% | 0.00% | 0.41% | 99.98% | | | |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 0.01% | | | |

FIGURE 27 (Continued)

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 146655 | 3 | 1 | 21 | 143031 | 14 | 11 | 147672 | 7 | 18 | 12 | 7 | 144333 | | |
| 1 | 147844 | 14 | 4 | 2 | 3 | 146303 | 1 | 146103 | 2 | 12 | 0 | 2 | | |
| 1431 | 12 | 0 | 148643 | 24 | 147029 | 20 | 22 | 2 | 145188 | 1 | 146546 | 11 | | |
| 13 | 10 | 148732 | 29 | 4 | 8 | 20 | 5 | 16 | 4 | 146327 | 19 | 11 | | |
| 2276 | 2507 | 1629 | 1679 | 7315 | 3322 | 4022 | 2676 | 4247 | 5168 | 4026 | 3806 | 6021 | | |
| 148100 | 147869 | 148747 | 148697 | 143061 | 147054 | 146354 | 147700 | 146128 | 145212 | 146352 | 146572 | 144357 | | |
| 99.02% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | | |
| 0.00% | 99.98% | 0.01% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | | |
| 0.97% | 0.01% | 0.00% | 99.96% | 0.02% | 99.98% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 99.98% | 0.01% | | |
| 0.01% | 0.01% | 99.99% | 0.02% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | | |

FIGURE 27 (Continued)

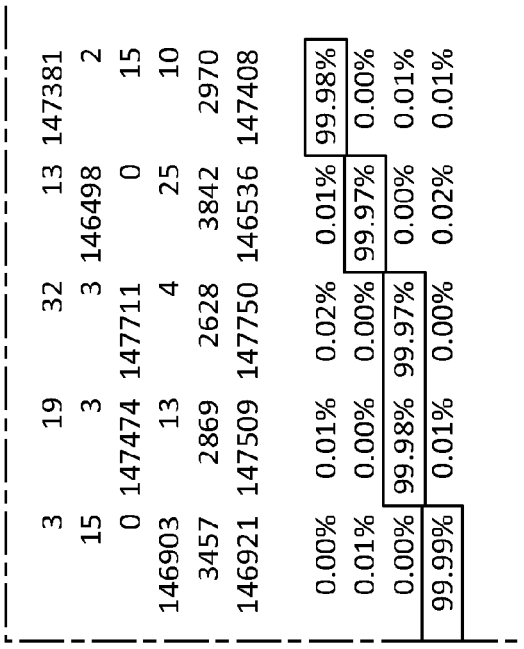


FIGURE 28

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C |
|---|---|---|---|---|---|---|---|---|---|
| **BE3** | | | | | | | | | |
| **A** | 23 | 13 | 30 | 178 | 21 | 357266 | 1103 | 7 | 11 |
| **C** | 356675 | 357330 | 355119 | 12 | 357616 | 13 | 107 | 19 | 359840 |
| **G** | 3 | 7 | 9 | 359941 | 2 | 48 | 358652 | 8 | 22 |
| **T** | 25 | 38 | 54 | 24 | 21 | 15 | 520 | 355970 | 75 |
| **N** | 11090 | 10428 | 12604 | 7661 | 10148 | 10466 | 7409 | 11756 | 7812 |
| | 356726 | 357388 | 355212 | 360155 | 357660 | 357342 | 360382 | 356004 | 359948 |
| **A** | 0.01% | 0.00% | 0.01% | 0.05% | 0.01% | 99.98% | 0.31% | 0.00% | 0.00% |
| **C** | 99.99% | 99.98% | 99.97% | 0.00% | 99.99% | 0.00% | 0.03% | 0.01% | 99.97% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.52% | 0.00% | 0.01% |
| **T** | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 0.14% | 99.99% | 0.02% |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | |
| **A** | 15 | 6 | 18 | 186 | 20 | 320597 | 26 | 4 | 15 |
| **C** | 320337 | 320839 | 319054 | 11 | 321518 | 11 | 2 | 19 | 322606 |
| **G** | 2 | 2 | 0 | 323378 | 3 | 33 | 323947 | 3 | 2 |
| **T** | 18 | 24 | 37 | 24 | 22 | 39 | 22 | 318627 | 20 |
| **N** | 10279 | 9780 | 11542 | 7052 | 9088 | 9970 | 6641 | 11945 | 7955 |
| | 320372 | 320871 | 319109 | 323599 | 321563 | 320680 | 323997 | 318653 | 322643 |
| **A** | 0.00% | 0.00% | 0.01% | 0.06% | 0.01% | 99.97% | 0.01% | 0.00% | 0.00% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% |
| **G** | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 99.99% | 0.01% |

# FIGURE 28 (Continued)

| **14** | **13** | **12** | **11** | **10** | **9** | **8** | **7** | **6** | **5** |
|---|---|---|---|---|---|---|---|---|---|
| **T** | **A** | **T** | **G** | **C** | **T** | **T** | **T** | **G** | **T** |
| 7 | 363880 | 30 | 356 | 5 | 7 | 17 | 14 | 95013 | 82 |
| 19 | 12 | 32 | 29 | 361405 | 24 | 19 | 21 | 212296 | 46 |
| 1 | 39 | 3 | 362189 | 3 | 2 | 32 | 1 | 49905 | 5 |
| 365209 | 33 | 364807 | 48 | 33 | 365136 | 365669 | 363580 | 3722 | 363913 |
| 2544 | 3800 | 2882 | 5134 | 6310 | 2588 | 2020 | 4141 | 6775 | 3665 |
| 365236 | 363964 | 364872 | 362622 | 361446 | 365169 | 365737 | 363616 | 360936 | 364046 |
| 0.00% | 99.98% | 0.01% | 0.10% | 0.00% | 0.00% | 0.00% | 0.00% | 26.32% | 0.02% |
| 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 0.01% | 58.82% | 0.01% |
| 0.00% | 0.01% | 0.00% | 99.88% | 0.00% | 0.00% | 0.01% | 0.00% | 13.83% | 0.00% |
| 99.99% | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% | 99.98% | 99.99% | 1.03% | 99.96% |
| 4 | 326406 | 18 | 23 | 8 | 8 | 15 | 4 | 35 | 26 |
| 25 | 16 | 41 | 11 | 324244 | 30 | 28 | 28 | 24 | 182 |
| 0 | 27 | 1 | 325330 | 3 | 2 | 11 | 5 | 327578 | 16 |
| 327999 | 20 | 327296 | 18 | 14 | 327482 | 328291 | 325838 | 28 | 322223 |
| 2570 | 4129 | 3242 | 5216 | 6325 | 3069 | 2246 | 4716 | 2921 | 8139 |
| 328028 | 326469 | 327356 | 325382 | 324269 | 327522 | 328345 | 325875 | 327665 | 322447 |
| 0.00% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.01% | 0.06% |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% |
| 99.99% | 0.01% | 99.98% | 0.01% | 0.00% | 99.99% | 99.98% | 99.99% | 0.01% | 99.93% |

FIGURE 28 (Continued)

| 4 | 3 | 2 | 1 |
|---|---|---|---|
| G | T | T | C |
| 118603 | 14 | 16 | 39 |
| 5357 | 48 | 60 | 364261 |
| 219943 | 2 | 12 | 80 |
| 9206 | 365064 | 365855 | 472 |
| 14602 | 2582 | 1767 | 2858 |
| 353109 | 365128 | 365943 | 364852 |
| 33.59% | 0.00% | 0.00% | 0.01% |
| 1.52% | 0.01% | 0.02% | 99.84% |
| 62.29% | 0.00% | 0.00% | 0.02% |
| 2.61% | 99.98% | 99.98% | 0.13% |
| 29 | 17 | 13 | 16 |
| 3 | 50 | 44 | 327597 |
| 325012 | 1 | 1 | 8 |
| 24 | 327317 | 328826 | 27 |
| 5518 | 3201 | 1702 | 2938 |
| 325068 | 327385 | 328884 | 327648 |
| 0.01% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.02% | 0.01% | 99.98% |
| 99.98% | 0.00% | 0.00% | 0.00% |
| 0.01% | 99.98% | 99.98% | 0.01% |

FIGURE 28 (Continued)

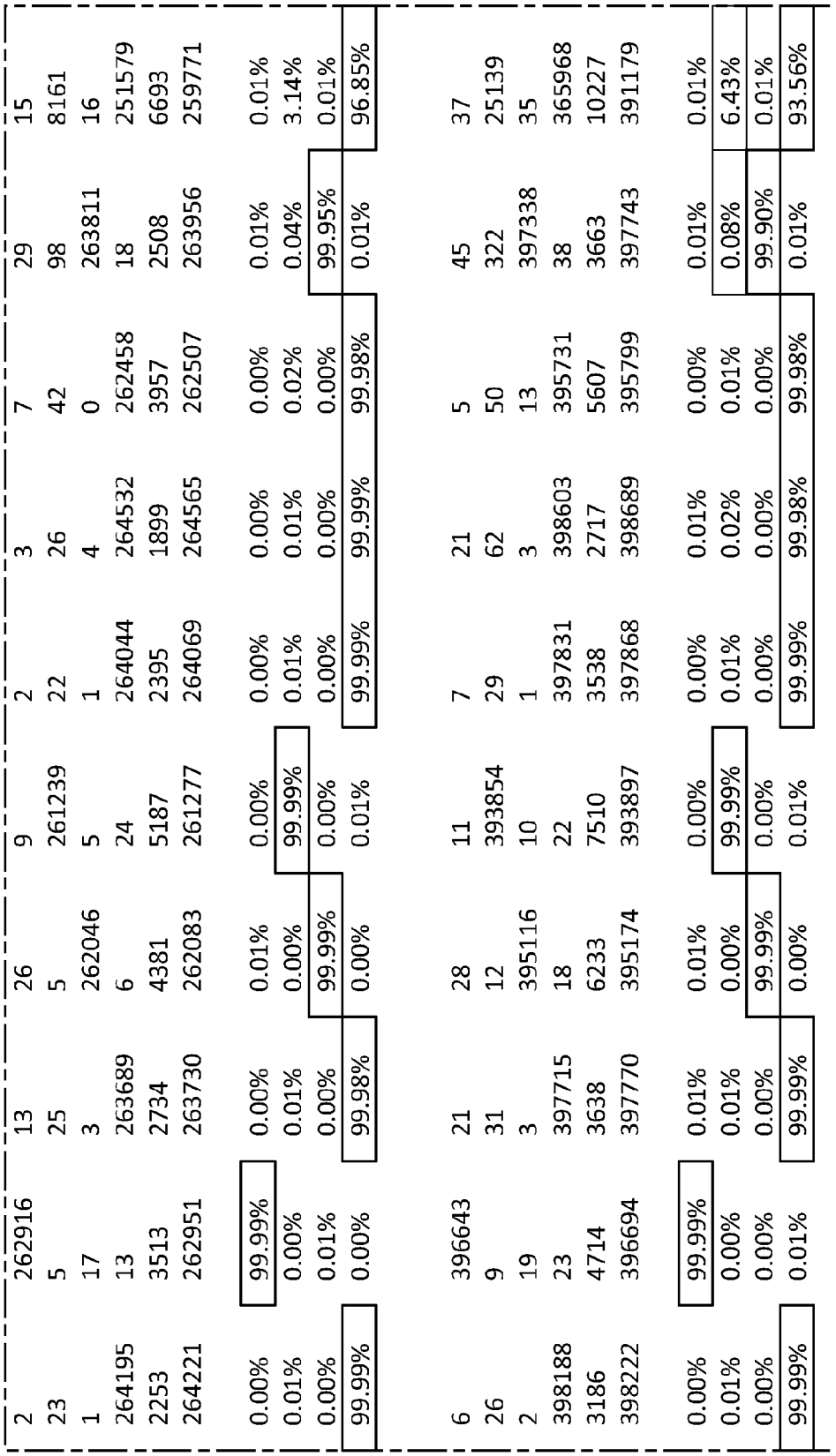
**pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 3 | 13 | 14 | 125 | 23 | 257984 | 15 | 1 | 12 | |
| **C** | 258079 | 258170 | 256876 | 5 | 258943 | 6 | 2 | 18 | 259681 | |
| **G** | 4 | 1 | 1 | 260236 | 3 | 27 | 260928 | 11 | 5 | |
| **T** | 9 | 19 | 24 | 15 | 5 | 16 | 15 | 256634 | 11 | |
| **N** | 8399 | 8291 | 9579 | 6113 | 7520 | 8461 | 5534 | 9810 | 6765 | |
| | 258095 | 258203 | 256915 | 260381 | 258974 | 258033 | 260960 | 256664 | 259709 | |
| | | | | | | | | | | |
| **A** | 0.00% | 0.01% | 0.01% | 0.05% | 0.01% | 99.98% | 0.01% | 0.00% | 0.00% | |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.00% | |

**pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 7 | 19 | 17 | 179 | 24 | 389467 | 24 | 13 | 33 | |
| **C** | 389058 | 389655 | 387623 | 12 | 390637 | 9 | 3 | 31 | 391710 | |
| **G** | 2 | 4 | 4 | 392436 | 1 | 35 | 393368 | 5 | 2 | |
| **T** | 11 | 23 | 39 | 27 | 28 | 32 | 23 | 387033 | 30 | |
| **N** | 12358 | 11735 | 13753 | 8782 | 10722 | 11869 | 8016 | 14322 | 9629 | |
| | 389078 | 389701 | 387683 | 392654 | 390690 | 389543 | 393418 | 387082 | 391775 | |
| | | | | | | | | | | |
| **A** | 0.00% | 0.00% | 0.00% | 0.05% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% | |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 99.99% | 0.01% | |

FIGURE 28 (Continued)



| 2 | 262916 | 13 | 26 | 9 | 2 | 3 | 7 | 29 | 15 |
|---|---|---|---|---|---|---|---|---|---|
| 23 | 5 | 25 | 5 | 261239 | 22 | 26 | 42 | 98 | 8161 |
| 1 | 17 | 3 | 262046 | 5 | 1 | 4 | 0 | 263811 | 16 |
| 264195 | 13 | 263689 | 6 | 24 | 264044 | 264532 | 262458 | 18 | 251579 |
| 2253 | 3513 | 2734 | 4381 | 5187 | 2395 | 1899 | 3957 | 2508 | 6693 |
| 264221 | 262951 | 263730 | 262083 | 261277 | 264069 | 264565 | 262507 | 263956 | 259771 |
| 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.02% | 0.04% | 3.14% |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.95% | 0.01% |
| 99.99% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 99.99% | 99.98% | 0.01% | 96.85% |
| 6 | 396643 | 21 | 28 | 11 | 7 | 21 | 5 | 45 | 37 |
| 26 | 9 | 31 | 12 | 393854 | 29 | 62 | 50 | 322 | 25139 |
| 2 | 19 | 3 | 395116 | 10 | 1 | 3 | 13 | 397338 | 35 |
| 398188 | 23 | 397715 | 18 | 22 | 397831 | 398603 | 395731 | 38 | 365968 |
| 3186 | 4714 | 3638 | 6233 | 7510 | 3538 | 2717 | 5607 | 3663 | 10227 |
| 398222 | 396694 | 397770 | 395174 | 393897 | 397868 | 398689 | 395799 | 397743 | 391179 |
| 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.02% | 0.01% | 0.08% | 6.43% |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.90% | 0.01% |
| 99.99% | 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 99.98% | 99.98% | 0.01% | 93.56% |

FIGURE 28 (Continued)

| | | | |
|---|---|---|---|
| 32 | 7 | 8 | 11 |
| 0 | 40 | 36 | 263993 |
| 261725 | 0 | 0 | 1 |
| 18 | 263731 | 264840 | 18 |
| 4689 | 2686 | 1580 | 2441 |
| 261775 | 263778 | 264884 | 264023 |
| | | | |
| 0.01% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.02% | 0.01% | 99.99% |
| 99.98% | 0.00% | 0.00% | 0.00% |
| 0.01% | 99.98% | 99.98% | 0.01% |
| | | | |
| 42 | 11 | 18 | 13 |
| 4 | 160 | 51 | 398657 |
| 394321 | 2 | 1 | 5 |
| 23 | 397333 | 399161 | 29 |
| 7016 | 3900 | 2175 | 2702 |
| 394390 | 397506 | 399231 | 398704 |
| | | | |
| 0.01% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.04% | 0.01% | 99.99% |
| 99.98% | 0.00% | 0.00% | 0.00% |
| 0.01% | 99.96% | 99.98% | 0.01% |

# FIGURE 28 (Continued)

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-156 (pCMV_ecTadA_XTEN_nCas9_GGS_UGI_GGS_NLS ; D108N)** | | | | | | | | | | | |
| **A** | 5 | 10 | 87 | 199 | 27 | 297532 | 19 | 4 | 17 | 8 | 302895 |
| **C** | 297243 | 297782 | 295952 | 28 | 298305 | 5 | 3 | 25 | 299207 | 31 | 5 |
| **G** | 3 | 3 | 27 | 299704 | 2 | 20 | 300531 | 15 | 5 | 1 | 29 |
| **T** | 19 | 32 | 36 | 30 | 10 | 6 | 25 | 295854 | 27 | 304137 | 22 |
| **N** | 9330 | 8774 | 10499 | 6641 | 8260 | 9041 | 6026 | 10681 | 7323 | 2402 | 3611 |
| | 297270 | 297827 | 296102 | 299961 | 298344 | 297563 | 300578 | 295898 | 299256 | 304177 | 302951 |
| **A** | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% |
| **C** | 100.0% | 100.0% | 99.9% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| **G** | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 0.0% |
| **pNMG-160 (pCMV_ecTadA_XTEN_nCas9_GGS_AAG*(E125Q)_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 3 | 17 | 12 | 170 | 36 | 298411 | 17 | 6 | 7 | 6 | 304129 |
| **C** | 298023 | 298621 | 297168 | 6 | 299302 | 6 | 4 | 21 | 300325 | 18 | 7 |
| **G** | 2 | 3 | 0 | 300860 | 1 | 26 | 301593 | 1 | 1 | 0 | 15 |
| **T** | 10 | 26 | 34 | 11 | 18 | 3 | 16 | 296829 | 23 | 305280 | 13 |
| **N** | 9638 | 9009 | 10462 | 6629 | 8319 | 9230 | 6046 | 10803 | 7304 | 2356 | 3496 |
| | 298038 | 298667 | 297214 | 301047 | 299357 | 298446 | 301630 | 296857 | 300356 | 305304 | 304164 |
| **A** | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% |
| **C** | 100.0% | 100.0% | 100.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| **G** | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 0.0% |

FIGURE 28 (Continued)

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C |
| 18 | 20 | 9 | 2 | 17 | 4 | 63 | 24 | 42 | 6 | 14 | 15 |
| 23 | 2 | 300843 | 30 | 58 | 96 | 90 | 19008 | 8 | 141 | 29 | 303828 |
| 2 | 301704 | 6 | 3 | 15 | 3 | 303582 | 9 | 301174 | 2 | 2 | 5 |
| 303584 | 10 | 25 | 303817 | 304374 | 302098 | 13 | 279747 | 38 | 303334 | 304748 | 23 |
| 2935 | 4826 | 5679 | 2710 | 2098 | 4361 | 2801 | 7761 | 5287 | 3066 | 1756 | 2678 |
| 303627 | 301736 | 300883 | 303852 | 304464 | 302201 | 303748 | 298788 | 301262 | 303483 | 304793 | 303871 |
| 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 6.4% | 0.0% | 0.0% | 0.0% | 100.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 100.0% | 0.0% | 0.0% | 100.0% | 100.0% | 100.0% | 0.0% | 93.6% | 0.0% | 100.0% | 100.0% | 0.0% |
| 11 | 24 | 9 | 2 | 17 | 5 | 61 | 23 | 53 | 11 | 12 | 14 |
| 22 | 10 | 301961 | 27 | 25 | 63 | 115 | 20015 | 1 | 142 | 34 | 304899 |
| 1 | 303015 | 1 | 2 | 2 | 1 | 304680 | 21 | 302358 | 3 | 0 | 4 |
| 304876 | 13 | 21 | 304868 | 305538 | 303328 | 19 | 279698 | 22 | 304396 | 305842 | 20 |
| 2750 | 4599 | 5669 | 2762 | 2079 | 4264 | 2787 | 7906 | 5229 | 3111 | 1775 | 2726 |
| 304910 | 303062 | 301992 | 304899 | 305582 | 303397 | 304875 | 299757 | 302434 | 304552 | 305888 | 304937 |
| 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 6.7% | 0.0% | 0.0% | 0.0% | 100.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 100.0% | 0.0% | 0.0% | 100.0% | 100.0% | 100.0% | 0.0% | 93.3% | 0.0% | 99.9% | 100.0% | 0.0% |

# FIGURE 28 (Continued)

**pNMG-161 (pCMV_ecTadA_XTEN_Cas9n_GGS_EndoV*(D35A)_NLS; D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 5 | 7 | 19 | 173 | 15 | 292247 | 15 | 5 | 8 | 8 | 297503 |
| **C** | 292192 | 292620 | 290929 | 5 | 293102 | 8 | 4 | 17 | 294057 | 28 | 9 |
| **G** | 2 | 1 | 0 | 294613 | 0 | 37 | 295234 | 12 | 11 | 1 | 20 |
| **T** | 11 | 24 | 29 | 27 | 14 | 3 | 22 | 290799 | 17 | 298797 | 26 |
| **N** | 8906 | 8464 | 10139 | 6298 | 7985 | 8821 | 5841 | 10283 | 7023 | 2282 | 3546 |
| | 292210 | 292652 | 290977 | 294818 | 293131 | 292295 | 295275 | 290833 | 294093 | 298834 | 297558 |
| **A** | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% |
| **C** | 100.0% | 100.0% | 100.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| **G** | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 0.0% |

**pNMG-162 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 14 | 12 | 14 | 212 | 34 | 352234 | 17 | 11 | 8 | 5 | 358876 |
| **C** | 351978 | 352512 | 350616 | 9 | 353382 | 11 | 3 | 28 | 354566 | 36 | 12 |
| **G** | 3 | 19 | 12 | 355188 | 0 | 70 | 356072 | 34 | 4 | 3 | 24 |
| **T** | 21 | 17 | 43 | 27 | 24 | 44 | 21 | 350681 | 27 | 360275 | 21 |
| **N** | 11063 | 10519 | 12394 | 7643 | 9639 | 10720 | 6966 | 12286 | 8435 | 2721 | 4072 |
| | 352016 | 352560 | 350685 | 355436 | 353440 | 352359 | 356113 | 350754 | 354605 | 360319 | 358933 |
| **A** | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% |
| **C** | 100.0% | 100.0% | 100.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| **G** | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 0.0% |

# FIGURE 28 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21 | 20 | 5 | 4 | 16 | 1 | 42 | 18 | 49 | 10 | 12 | 12 |
| 29 | 11 | 295609 | 22 | 21 | 34 | 139 | 9174 | 3 | 61 | 47 | 298458 |
| 1 | 296393 | 1 | 2 | 1 | 3 | 298266 | 15 | 296240 | 1 | 2 | 5 |
| 298301 | 24 | 27 | 298493 | 299037 | 296928 | 18 | 284878 | 20 | 298117 | 299423 | 19 |
| 2752 | 4656 | 5462 | 2574 | 2020 | 4129 | 2630 | 7011 | 4784 | 2925 | 1630 | 2620 |
| 298352 | 296448 | 295642 | 298521 | 299075 | 296966 | 298465 | 294085 | 296312 | 298189 | 299484 | 298494 |
| 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 3.1% | 0.0% | 0.0% | 0.0% | 100.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 1000% | 0.0% | 0.0% | 0.0% |
| 100.0% | 0.0% | 0.0% | 100.0% | 100.0% | 100.0% | 0.0% | 96.9% | 0.0% | 100.0% | 100.0% | 0.0% |
| 26 | 51 | 18 | 4 | 14 | 6 | 39 | 21 | 32 | 15 | 9 | 11 |
| 18 | 4 | 356210 | 34 | 96 | 152 | 275 | 37594 | 4 | 386 | 39 | 359847 |
| 1 | 357502 | 2 | 1 | 3 | 1 | 359381 | 21 | 356544 | 5 | 1 | 6 |
| 359743 | 24 | 23 | 359878 | 360503 | 357780 | 18 | 316119 | 22 | 358766 | 360859 | 18 |
| 3217 | 5424 | 6752 | 3088 | 2389 | 5067 | 3293 | 9251 | 6404 | 3834 | 2098 | 3124 |
| 359788 | 357581 | 356253 | 359917 | 360616 | 357939 | 359713 | 353755 | 356602 | 359172 | 360908 | 359882 |
| 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.1% | 10.6% | 0.0% | 0.1% | 0.0% | 100.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.9% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 100.0% | 0.0% | 0.0% | 100.0% | 100.0% | 100.0% | 0.0% | 89.4% | 0.0% | 99.9% | 100.0% | 0.0% |

# FIGURE 28 (Continued)

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-163 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 7 | 9 | 12 | 188 | 24 | 283012 | 33 | 3 | 18 | 3 | 288684 |
| **C** | 282240 | 282151 | 281249 | 4 | 283300 | 13 | 5 | 18 | 284916 | 21 | 4 |
| **G** | 4 | 0 | 1 | 285173 | 1 | 80 | 285905 | 64 | 4 | 0 | 13 |
| **T** | 16 | 26 | 30 | 20 | 19 | 33 | 13 | 282155 | 17 | 289357 | 18 |
| **N** | 9486 | 9567 | 10461 | 6368 | 8409 | 8615 | 5797 | 9447 | 6732 | 2306 | 2903 |
| | 282267 | 282186 | 281292 | 285385 | 283344 | 283138 | 285956 | 282240 | 284955 | 289381 | 288719 |
| **A** | 0.00% | 0.00% | 0.00% | 0.07% | 0.01% | 99.96% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.03% | 99.98% | 0.02% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 99.97% | 0.01% | 99.99% | 0.01% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 9 | 10 | 7 | 111 | 22 | 232096 | 14 | 6 | 12 | 6 | 236540 |
| **C** | 231275 | 231309 | 230163 | 8 | 232017 | 6 | 7 | 13 | 233473 | 12 | 5 |
| **G** | 4 | 3 | 0 | 233713 | 4 | 18 | 234316 | 7 | 1 | 0 | 13 |
| **T** | 16 | 13 | 28 | 15 | 12 | 13 | 10 | 230805 | 17 | 237219 | 20 |
| **N** | 7906 | 7875 | 9012 | 5363 | 7155 | 7075 | 4861 | 8349 | 5677 | 1943 | 2598 |
| | 231304 | 231335 | 230198 | 233847 | 232055 | 232133 | 234347 | 230831 | 233503 | 237237 | 236578 |
| **A** | 0.00% | 0.00% | 0.00% | 0.05% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |



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# FIGURE 28 (Continued)

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C |
| 15 | 21 | 6 | 5 | 8 | 8 | 33 | 14 | 26 | 7 | 14 | 10 |
| 18 | 2 | 286152 | 64 | 78 | 69 | 159 | 53522 | 1 | 999 | 52 | 289017 |
| 3 | 287552 | 2 | 5 | 1 | 1 | 288752 | 6 | 28621 | 3 | 4 | 1 |
| 289093 | 7 | 10 | 289031 | 289597 | 287481 | 14 | 229992 | 13 | 287379 | 289644 | 31 |
| 2493 | 4039 | 5451 | 2515 | 1936 | 4046 | 2648 | 8072 | 5352 | 3218 | 1892 | 2547 |
| 289129 | 287582 | 286170 | 289105 | 289684 | 287559 | 288958 | 283534 | 286254 | 288388 | 289714 | 289059 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.02% | 0.03% | 0.02% | 0.06% | 18.88% | 0.00% | 0.35% | 0.02% | 99.99% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.00% | 99.97% | 99.97% | 99.97% | 0.00% | 81.12% | 0.00% | 99.65% | 99.98% | 0.01% |
| 16 | 20 | 11 | 8 | 5 | 1 | 25 | 16 | 9 | 7 | 11 | 17 |
| 22 | 2 | 234553 | 14 | 21 | 35 | 150 | 8254 | 1 | 61 | 37 | 237148 |
| 2 | 235691 | 4 | 2 | 3 | 1 | 236982 | 12 | 235218 | 1 | 0 | 3 |
| 236982 | 7 | 12 | 237126 | 237564 | 235765 | 15 | 224752 | 8 | 236768 | 237776 | 19 |
| 2154 | 3457 | 4597 | 2027 | 1584 | 3375 | 2005 | 6143 | 3941 | 2340 | 1353 | 1990 |
| 237022 | 235720 | 234580 | 237150 | 237593 | 235802 | 237172 | 233034 | 235236 | 236837 | 237824 | 237187 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.06% | 3.54% | 0.00% | 0.03% | 0.02% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.92% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.98% | 0.00% | 0.01% | 99.99% | 99.99% | 99.98% | 0.01% | 96.45% | 0.00% | 99.97% | 99.98% | 0.01% |

# FIGURE 28 (Continued)

**pNMG-165 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 9 | 10 | 7 | 111 | 22 | 232096 | 14 | 6 | 12 | 6 | 236540 |
| **C** | 231275 | 231309 | 230163 | 8 | 232017 | 6 | 7 | 13 | 233473 | 12 | 5 |
| **G** | 4 | 3 | 0 | 233713 | 4 | 18 | 234316 | 7 | 1 | 0 | 13 |
| **T** | 16 | 13 | 28 | 15 | 12 | 13 | 10 | 230805 | 17 | 237219 | 20 |
| **N** | 7906 | 7875 | 9012 | 5363 | 7155 | 7075 | 4861 | 8349 | 5677 | 1943 | 2598 |
| | 231304 | 231335 | 230198 | 233847 | 232055 | 232133 | 234347 | 230831 | 233503 | 237237 | 236578 |
| **A** | 0.00% | 0.00% | 0.00% | 0.05% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |

**untreated cells**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 10 | 16 | 21 | 173 | 47 | 359094 | 12 | 3 | 6 | 15 | 365895 |
| **C** | 357418 | 357872 | 356168 | 9 | 358865 | 14 | 5 | 27 | 361121 | 22 | 6 |
| **G** | 5 | 0 | 1 | 361456 | 2 | 25 | 362233 | 0 | 1 | 3 | 22 |
| **T** | 16 | 35 | 40 | 36 | 28 | 6 | 23 | 356984 | 20 | 366706 | 15 |
| **N** | 12085 | 11611 | 13304 | 7860 | 10592 | 10395 | 7261 | 12520 | 8386 | 2788 | 3596 |
| | 357449 | 357923 | 356230 | 361674 | 358942 | 359139 | 362273 | 357014 | 361148 | 366746 | 365938 |
| **A** | 0.00% | 0.00% | 0.01% | 0.05% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 99.99% | 0.00% |

FIGURE 28 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 20 | 11 | 8 | 5 | 1 | 25 | 16 | 9 | 7 | 11 | 17 | |
| 22 | 2 | 234553 | 14 | 21 | 35 | 150 | 8254 | 1 | 61 | 37 | 237148 | |
| 2 | 235691 | 4 | 2 | 3 | 1 | 236982 | 12 | 235218 | 1 | 0 | 3 | |
| 236982 | 7 | 12 | 237126 | 237564 | 235765 | 15 | 224752 | 8 | 236768 | 237776 | 19 | |
| 2154 | 3457 | 4597 | 2027 | 1584 | 3375 | 2005 | 6143 | 3941 | 2340 | 1353 | 1990 | |
| 237022 | 235720 | 234580 | 237150 | 237593 | 235802 | 237172 | 233034 | 235236 | 236837 | 237824 | 237187 | |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.06% | 3.54% | 0.00% | 0.03% | 0.02% | 99.98% | |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.92% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | |
| 99.98% | 0.00% | 0.01% | 99.99% | 99.99% | 99.98% | 0.01% | 96.45% | 0.00% | 99.97% | 99.98% | 0.01% | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | 27 | 9 | 1 | 12 | 5 | 27 | 17 | 16 | 15 | 12 | 11 | |
| 24 | 4 | 362487 | 26 | 24 | 34 | 6 | 36 | 4 | 43 | 43 | 366483 | |
| 2 | 364579 | 3 | 3 | 3 | 3 | 366439 | 15 | 363816 | 2 | 5 | 4 | |
| 366451 | 27 | 23 | 366339 | 367181 | 364538 | 23 | 360093 | 28 | 366220 | 367639 | 24 | |
| 3039 | 4897 | 7012 | 3165 | 2315 | 4955 | 3040 | 9374 | 5671 | 3255 | 1836 | 3014 | |
| 366495 | 364637 | 362522 | 366369 | 367220 | 364580 | 366495 | 360161 | 363864 | 366280 | 367699 | 366522 | |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | |
| 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% | 0.01% | 99.98% | 0.01% | 99.98% | 99.98% | 0.01% | |

# FIGURE 29

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BE3** | | | | | | | | | | | |
| **A** | 42 | 58 | 165 | 3773 | 4054 | 149031 | 88 | 154281 | 708 | 3 | 19 |
| **C** | 10 | 23 | 150495 | 52124 | 35749 | 4 | 33 | 36 | 151983 | 22 | 17 |
| **G** | 154158 | 151462 | 5 | 3267 | 22965 | 27 | 154927 | 23 | 709 | 8 | 157023 |
| **T** | 12 | 10 | 2581 | 87941 | 90308 | 16 | 30 | 47 | 1774 | 156586 | 9 |
| **N** | 4176 | 6846 | 5153 | 11292 | 5321 | 9419 | 3418 | 4109 | 3325 | 1885 | 1436 |
| | 154222 | 151553 | 153246 | 147105 | 153076 | 149078 | 155078 | 154387 | 155174 | 156619 | 157068 |
| **A** | 0.03% | 0.04% | 0.11% | 2.56% | 2.65% | 99.97% | 0.06% | 99.93% | 0.46% | 0.00% | 0.01% |
| **C** | 0.01% | 0.02% | 98.20% | 35.43% | 23.35% | 0.00% | 0.02% | 0.02% | 97.94% | 0.01% | 0.01% |
| **G** | 99.96% | 99.94% | 0.00% | 2.22% | 15.00% | 0.02% | 99.90% | 0.01% | 0.46% | 0.01% | 99.97% |
| **T** | 0.01% | 0.01% | 1.68% | 59.78% | 59.00% | 0.01% | 0.02% | 0.03% | 1.14% | 99.98% | 0.01% |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 12 | 21 | 1 | 12 | 8 | 153740 | 11 | 160435 | 7 | 7 | 16 |
| **C** | 1 | 3 | 158349 | 157506 | 157743 | 10 | 1 | 12 | 160802 | 11 | 7 |
| **G** | 156042 | 153877 | 9 | 4 | 4 | 19 | 160902 | 21 | 0 | 2 | 162575 |
| **T** | 9 | 9 | 16 | 28 | 31 | 6 | 4 | 7 | 17 | 162119 | 3 |
| **N** | 8104 | 10258 | 5794 | 6619 | 6383 | 10394 | 3251 | 3694 | 3343 | 2030 | 1568 |
| | 156064 | 153910 | 158375 | 157550 | 157786 | 153775 | 160918 | 160475 | 160826 | 162139 | 162601 |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.97% | 99.97% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% |
| **T** | 0.01% | 0.01% | 0.01% | 0.02% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |



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FIGURE 29 (Continued)

| 12 A | 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 141813 | 8 | 7 | 155006 | 35 | 27 | 7 | 27 | 150598 | 2 | 19 | 25 |
| 8 | 8 | 153438 | 6 | 152786 | 3 | 22 | 2 | 7 | 10 | 2 | 3 |
| 34 | 154088 | 8 | 17 | 7 | 151649 | 2 | 154322 | 11 | 2 | 155004 | 155647 |
| 1 | 13 | 35 | 11 | 230 | 25 | 153529 | 13 | 8 | 154180 | 15 | 15 |
| 16653 | 4446 | 5025 | 3472 | 5463 | 6819 | 4965 | 4160 | 7900 | 4329 | 3481 | 2831 |
| 141856 | 154067 | 153488 | 155040 | 153058 | 151704 | 153560 | 154364 | 150624 | 154194 | 155040 | 155690 |
| 99.97% | 0.01% | 0.00% | 99.98% | 0.02% | 0.02% | 0.00% | 0.02% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.01% | 99.97% | 0.00% | 99.82% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.98% | 0.01% | 0.01% | 0.00% | 99.96% | 0.00% | 99.97% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.02% | 0.01% | 0.15% | 0.02% | 99.98% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% |
| 145407 | 11 | 2 | 160325 | 3 | 19 | 9 | 12 | 155372 | 6 | 12 | 38 |
| 10 | 4 | 158785 | 10 | 158275 | 2 | 22 | 1 | 8 | 11 | 4 | 3 |
| 32 | 159315 | 3 | 6 | 3 | 156702 | 4 | 159296 | 24 | 0 | 160086 | 161033 |
| 5 | 2 | 15 | 5 | 28 | 10 | 158313 | 9 | 8 | 159188 | 10 | 14 |
| 18715 | 4837 | 5364 | 3823 | 5861 | 7437 | 5819 | 4849 | 8755 | 4962 | 4055 | 3079 |
| 145454 | 159332 | 158805 | 160346 | 158309 | 156733 | 158348 | 159318 | 155412 | 159205 | 160112 | 161088 |
| 99.97% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.99% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.02% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% |

# FIGURE 29 (Continued)

**pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 7 | 20 | 0 | 5 | 6 | 104317 | 10 | 108305 | 0 | 3 | 11 |
| **C** | 4 | 4 | 107141 | 106709 | 107157 | 7 | 2 | 5 | 108942 | 13 | 2 |
| **G** | 105732 | 104318 | 1 | 0 | 2 | 48 | 109113 | 522 | 4 | 1 | 110131 |
| **T** | 5 | 6 | 13 | 9 | 10 | 6 | 2 | 6 | 9 | 109854 | 6 |
| **N** | 5384 | 6784 | 3977 | 4409 | 3957 | 6754 | 2005 | 2294 | 2177 | 1261 | 982 |
| | 105748 | 104348 | 107155 | 106723 | 107175 | 104378 | 109127 | 108838 | 108955 | 109871 | 110150 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 99.94% | 0.01% | 99.51% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 99.99% | 99.98% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.05% | 99.99% | 0.48% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% |

**pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 16 | 26 | 0 | 11 | 10 | 199002 | 7 | 206665 | 3 | 6 | 23 |
| **C** | 1 | 5 | 204535 | 203621 | 203879 | 11 | 0 | 12 | 207598 | 15 | 6 |
| **G** | 201070 | 198768 | 5 | 1 | 8 | 149 | 207768 | 616 | 1 | 1 | 209722 |
| **T** | 5 | 6 | 20 | 13 | 19 | 11 | 9 | 16 | 8 | 209186 | 4 |
| **N** | 10724 | 13011 | 7256 | 8170 | 7900 | 12643 | 4032 | 4507 | 4206 | 2608 | 2061 |
| | 201092 | 198805 | 204560 | 203646 | 203916 | 199173 | 207784 | 207309 | 207610 | 209208 | 209755 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.91% | 0.00% | 99.69% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 99.99% | 99.98% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.07% | 99.99% | 0.30% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |

# FIGURE 29 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 98882 | 6 | 0 | 108625 | 3 | 11 | 9 | 6 | 105302 | 0 | 13 | 19 |
| 12 | 0 | 107712 | 8 | 107347 | 0 | 13 | 1 | 6 | 14 | 0 | 2 |
| 19 | 107995 | 2 | 7 | 4 | 106243 | 1 | 108084 | 10 | 0 | 108442 | 109087 |
| 2 | 7 | 17 | 5 | 19 | 7 | 107434 | 6 | 16 | 107820 | 6 | 16 |
| 12217 | 3124 | 3401 | 2487 | 3760 | 4880 | 3680 | 3040 | 5803 | 3303 | 2676 | 2013 |
| 98915 | 108008 | 107731 | 108645 | 107373 | 106261 | 107457 | 108097 | 105334 | 107834 | 108461 | 109124 |
| 99.97% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.02% | 0.00% | 0.02% | 0.01% | 99.98% | 0.01% | 0.02% | 99.99% | 0.01% | 0.01% |
| 188079 | 9 | 4 | 206764 | 0 | 30 | 7 | 9 | 200416 | 10 | 24 | 62 |
| 8 | 4 | 204826 | 19 | 204100 | 6 | 18 | 5 | 8 | 20 | 0 | 4 |
| 32 | 205529 | 5 | 10 | 1 | 202089 | 1 | 205636 | 16 | 0 | 206584 | 207649 |
| 7 | 11 | 35 | 11 | 37 | 15 | 204584 | 10 | 14 | 205495 | 11 | 17 |
| 23690 | 6263 | 6946 | 5012 | 7678 | 9676 | 7218 | 6168 | 11374 | 6303 | 5209 | 4096 |
| 188126 | 205553 | 204870 | 206804 | 204138 | 202140 | 204610 | 205660 | 200454 | 205525 | 206619 | 207732 |
| 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.96% |
| 0.00% | 0.01% | 0.02% | 0.01% | 0.02% | 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% |

# FIGURE 29 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | C | C | C | A | G | A | C | T | G |
| **pNMG-156 (pCMV_ecTadA_XTEN_nCas9_GGS_UGI_GGS_NLS ; D108N)** | | | | | | | | | | | |
| A | 6 | 26 | 0 | 9 | 5 | 126256 | 15 | 130649 | 0 | 3 | 12 |
| C | 1 | 5 | 130060 | 129295 | 129535 | 14 | 0 | 12 | 131909 | 12 | 1 |
| G | 127733 | 126228 | 2 | 0 | 5 | 75 | 132007 | 1035 | 6 | 1 | 133265 |
| T | 20 | 7 | 21 | 46 | 61 | 7 | 5 | 4 | 17 | 132944 | 7 |
| N | 6834 | 8328 | 4511 | 5245 | 4989 | 8243 | 2568 | 2895 | 2663 | 1635 | 1310 |
| | 127760 | 126266 | 130083 | 129350 | 129606 | 126352 | 132027 | 131700 | 131932 | 132960 | 133285 |
| A | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% | 99.92% | 0.01% | 99.20% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.96% | 99.95% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.06% | 99.98% | 0.79% | 0.00% | 0.00% | 99.98% |
| T | 0.02% | 0.01% | 0.02% | 0.04% | 0.05% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% |
| **pNMG-160 (pCMV_ecTadA_XTEN_nCas9_GGS_AAG*(E125Q)_GGS_NLS; D108N)** | | | | | | | | | | | |
| A | 12 | 19 | 0 | 7 | 8 | 154500 | 10 | 160210 | 2 | 1 | 15 |
| C | 1 | 4 | 159163 | 158236 | 158541 | 14 | 4 | 15 | 161291 | 15 | 5 |
| G | 156639 | 154770 | 2 | 1 | 22 | 129 | 161524 | 864 | 4 | 2 | 163057 |
| T | 10 | 8 | 28 | 61 | 54 | 9 | 6 | 7 | 21 | 162605 | 3 |
| N | 7946 | 9809 | 5417 | 6305 | 5985 | 9959 | 3067 | 3515 | 3294 | 1989 | 1532 |
| | 156662 | 154801 | 159193 | 158305 | 158625 | 154652 | 161544 | 161096 | 161318 | 162623 | 163080 |
| A | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.90% | 0.01% | 99.45% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.96% | 99.95% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.01% | 0.08% | 99.99% | 0.54% | 0.00% | 0.00% | 99.99% |
| T | 0.01% | 0.01% | 0.02% | 0.04% | 0.03% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |



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# FIGURE 29 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | G | C | A | C | G | T | G | A | T | G | G |
| 120023 | 4 | 4 | 131575 | 2 | 13 | 8 | 8 | 127618 | 4 | 12 | 30 |
| 9 | 1 | 130248 | 12 | 129933 | 1 | 12 | 3 | 9 | 11 | 1 | 0 |
| 15 | 130696 | 1 | 8 | 0 | 128580 | 1 | 130833 | 14 | 1 | 131372 | 131970 |
| 3 | 6 | 23 | 5 | 19 | 10 | 130031 | 10 | 10 | 130648 | 9 | 11 |
| 14545 | 3888 | 4319 | 2995 | 4641 | 5991 | 4537 | 3735 | 6938 | 3925 | 3195 | 2578 |
| 120050 | 130707 | 130276 | 131600 | 129954 | 128604 | 130052 | 130854 | 127651 | 130664 | 131394 | 132011 |
| 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.98% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% |
| 146588 | 12 | 2 | 160678 | 2 | 19 | 7 | 10 | 155897 | 2 | 10 | 35 |
| 12 | 2 | 159247 | 11 | 158715 | 0 | 21 | 1 | 13 | 18 | 0 | 5 |
| 30 | 159788 | 3 | 13 | 0 | 157287 | 0 | 159930 | 13 | 0 | 160568 | 161462 |
| 3 | 10 | 10 | 11 | 17 | 5 | 159071 | 11 | 11 | 159691 | 6 | 9 |
| 17979 | 4800 | 5350 | 3899 | 5878 | 7301 | 5513 | 4660 | 8678 | 4901 | 4028 | 3101 |
| 146633 | 159812 | 159262 | 160713 | 158734 | 157311 | 159099 | 159952 | 155934 | 159711 | 160584 | 161511 |
| 99.97% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 29 (Continued)

**pNMG-161 (pCMV_ecTadA_XTEN_Cas9n_GGS_EndoV*(D35A)_NLS; D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 17 | 32 | 2 | 8 | 9 | 158241 | 9 | 164433 | 3 | 9 | 17 |
| **C** | 3 | 5 | 162990 | 162006 | 162379 | 10 | 1 | 7 | 165378 | 18 | 5 |
| **G** | 160378 | 158292 | 2 | 2 | 5 | 119 | 165476 | 705 | 1 | 0 | 167249 |
| **T** | 8 | 12 | 24 | 27 | 26 | 13 | 5 | 3 | 10 | 166674 | 8 |
| **N** | 8376 | 10441 | 5764 | 6739 | 6363 | 10399 | 3291 | 3634 | 3390 | 2081 | 1503 |
| | 160406 | 158341 | 163018 | 162043 | 162419 | 158383 | 165491 | 165148 | 165392 | 166701 | 167279 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 99.91% | 0.01% | 99.57% | 0.00% | 0.01% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.98% | 99.98% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.08% | 99.99% | 0.43% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.01% | 0.01% | 0.02% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |

**pNMG-162 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 17 | 17 | 0 | 9 | 5 | 152273 | 8 | 158291 | 4 | 3 | 11 |
| **C** | 2 | 3 | 156961 | 156112 | 156342 | 9 | 3 | 15 | 159243 | 19 | 2 |
| **G** | 154511 | 152462 | 0 | 2 | 2 | 170 | 159281 | 605 | 3 | 0 | 160870 |
| **T** | 2 | 11 | 21 | 13 | 26 | 13 | 7 | 7 | 10 | 160496 | 5 |
| **N** | 7981 | 10020 | 5531 | 6377 | 6138 | 10048 | 3214 | 3595 | 3253 | 1995 | 1625 |
| | 154532 | 152493 | 156982 | 156136 | 156375 | 152465 | 159299 | 158918 | 159260 | 160518 | 160888 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.87% | 0.01% | 99.61% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 99.98% | 99.98% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.11% | 99.99% | 0.38% | 0.00% | 0.00% | 99.99% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |

# FIGURE 29 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 150091 | 10 | 1 | 164894 | 4 | 20 | 2 | 9 | 160130 | 2 | 13 | 35 |
| 8 | 2 | 163486 | 11 | 162941 | 1 | 18 | 0 | 9 | 16 | 2 | 3 |
| 28 | 163944 | 1 | 17 | 0 | 161223 | 0 | 164027 | 16 | 0 | 164725 | 165716 |
| 7 | 9 | 9 | 5 | 26 | 7 | 163031 | 7 | 22 | 163960 | 11 | 9 |
| 18648 | 4817 | 5285 | 3855 | 5811 | 7531 | 5750 | 4758 | 8624 | 4823 | 4050 | 3038 |
| 150134 | 163965 | 163497 | 164927 | 162971 | 161251 | 163051 | 164043 | 160177 | 163978 | 164751 | 165763 |
| 99.97% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.99% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% |
| 144335 | 9 | 1 | 158686 | 2 | 22 | 3 | 9 | 153897 | 1 | 13 | 44 |
| 18 | 0 | 157216 | 29 | 156670 | 13 | 13 | 1 | 16 | 15 | 2 | 4 |
| 33 | 157626 | 1 | 15 | 0 | 155222 | 4 | 157902 | 21 | 0 | 158499 | 159458 |
| 1 | 14 | 7 | 5 | 34 | 9 | 156999 | 11 | 10 | 157742 | 5 | 6 |
| 18126 | 4864 | 5288 | 3778 | 5807 | 7247 | 5525 | 4621 | 8600 | 4786 | 4025 | 3032 |
| 144387 | 157649 | 157225 | 158735 | 156706 | 155266 | 157019 | 157923 | 153944 | 157758 | 158519 | 159512 |
| 99.96% | 0.01% | 0.00% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 0.01% | 0.00% | 99.99% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.02% | 99.99% | 0.00% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 100.0% |
| 0.00% | 0.01% | 0.00% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |

FIGURE 29 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | C | C | C | A | G | A | C | T | G |
| **pNMG-163 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 9 | 15 | 1 | 7 | 3 | 135653 | 5 | 141570 | 4 | 5 | 7 |
| **C** | 4 | 3 | 139415 | 138716 | 139206 | 4 | 1 | 5 | 141850 | 11 | 4 |
| **G** | 136789 | 135816 | 3 | 1 | 3 | 94 | 141888 | 294 | 3 | 2 | 143196 |
| **T** | 8 | 10 | 20 | 9 | 7 | 8 | 7 | 5 | 5 | 143038 | 11 |
| **N** | 7902 | 8868 | 5273 | 5979 | 5493 | 8953 | 2811 | 2838 | 2850 | 1656 | 1494 |
| | 136810 | 135844 | 139439 | 138733 | 139219 | 135759 | 141901 | 141874 | 141862 | 143056 | 143218 |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.92% | 0.00% | 99.79% | 0.00% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.98% | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 0.07% | 99.99% | 0.21% | 0.00% | 0.00% | 99.98% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 16 | 15 | 1 | 1 | 4 | 99736 | 5 | 103620 | 1 | 3 | 10 |
| **C** | 2 | 3 | 102591 | 101921 | 102396 | 8 | 1 | 8 | 104205 | 13 | 3 |
| **G** | 100759 | 100018 | 1 | 1 | 1 | 96 | 104305 | 596 | 36 | 2 | 105350 |
| **T** | 3 | 3 | 14 | 28 | 23 | 24 | 2 | 7 | 5 | 105084 | 6 |
| **N** | 5549 | 6290 | 3722 | 4378 | 3905 | 6465 | 2016 | 2098 | 2082 | 1227 | 960 |
| | 100780 | 100039 | 102607 | 101951 | 102424 | 99864 | 104313 | 104231 | 104247 | 105102 | 105369 |
| **A** | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 99.87% | 0.00% | 99.41% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.97% | 99.97% | 0.01% | 0.00% | 0.01% | 99.96% | 0.01% | 0.00% |
| **G** | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 0.10% | 99.99% | 0.57% | 0.03% | 0.00% | 99.98% |
| **T** | 0.00% | 0.00% | 0.01% | 0.03% | 0.02% | 0.02% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% |

FIGURE 29 (Continued)

| 12 A | 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 130444 | 8 | 1 | 141437 | 3 | 13 | 6 | 12 | 136865 | 11 | 17 | 34 |
| 7 | 3 | 140122 | 12 | 139746 | 4 | 14 | 0 | 7 | 16 | 5 | 5 |
| 31 | 140735 | 2 | 9 | 3 | 138317 | 1 | 140680 | 8 | 0 | 141269 | 142058 |
| 2 | 12 | 16 | 7 | 16 | 9 | 139813 | 8 | 6 | 140537 | 11 | 10 |
| 14228 | 3954 | 4571 | 3247 | 4944 | 6369 | 4881 | 4015 | 7835 | 4157 | 3419 | 2614 |
| 130484 | 140758 | 140141 | 141465 | 139768 | 138343 | 139834 | 140700 | 136886 | 140564 | 141302 | 142107 |
| 99.97% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.99% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% |
| 95630 | 4 | 3 | 103914 | 0 | 17 | 1 | 1 | 100468 | 2 | 8 | 17 |
| 9 | 0 | 102996 | 11 | 102593 | 0 | 10 | 0 | 8 | 9 | 1 | 1 |
| 14 | 103491 | 2 | 7 | 1 | 101655 | 1 | 103337 | 5 | 1 | 103867 | 104361 |
| 0 | 1 | 9 | 7 | 13 | 4 | 102804 | 4 | 11 | 103301 | 2 | 4 |
| 10676 | 2833 | 3319 | 2390 | 3723 | 4654 | 3514 | 2988 | 5838 | 3017 | 2452 | 1947 |
| 95653 | 103496 | 103010 | 103939 | 102607 | 101676 | 102816 | 103342 | 100492 | 103313 | 103878 | 104383 |
| 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.01% | 100.00 % | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 100.00 % | 0.00% | 0.00% | 99.99% | 99.98% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |

# FIGURE 29 (Continued)

**pNMG-165 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 10 | 21 | 2 | 12 | 3 | 143438 | 1 | 149552 | 1 | 3 | 10 |
| **C** | 1 | 4 | 147538 | 146734 | 147101 | 10 | 2 | 11 | 150176 | 9 | 4 |
| **G** | 144847 | 143773 | 3 | 0 | 3 | 90 | 150224 | 581 | 7 | 1 | 151668 |
| **T** | 11 | 14 | 17 | 21 | 18 | 7 | 8 | 9 | 15 | 151376 | 7 |
| **N** | 8269 | 9326 | 5578 | 6371 | 6013 | 9593 | 2903 | 2985 | 2939 | 1749 | 1449 |
| | 144869 | 143812 | 147560 | 146767 | 147125 | 143545 | 150235 | 150153 | 150199 | 151389 | 151689 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.93% | 0.00% | 99.60% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 99.98% | 99.98% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| **G** | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.06% | 99.99% | 0.39% | 0.00% | 0.00% | 99.99% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.00% |

**untreated cells**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8 | 19 | 3 | 9 | 6 | 152229 | 9 | 159092 | 0 | 2 | 13 |
| **A** | 2 | 1 | 156567 | 155502 | 156164 | 10 | 0 | 9 | 159118 | 16 | 4 |
| **C** | 153828 | 152588 | 7 | 0 | 0 | 20 | 159220 | 13 | 2 | 2 | 160752 |
| **G** | 7 | 11 | 18 | 13 | 15 | 6 | 9 | 7 | 16 | 160404 | 6 |
| **T** | 8530 | 9756 | 5780 | 6851 | 6190 | 10110 | 3137 | 3254 | 3239 | 1951 | 1600 |
| **N** | 153845 | 152619 | 156595 | 155524 | 156185 | 152265 | 159238 | 159121 | 159136 | 160424 | 160775 |
| | | | | | | | | | | | |
| | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% |
| **A** | 0.00% | 0.00% | 99.98% | 99.99% | 99.99% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **C** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 99.99% |
| **G** | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 99.99% | 0.00% |

# FIGURE 29 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 138037 | 7 | 2 | 149641 | 6 | 16 | 2 | 9 | 144829 | 4 | 18 | 35 |
| 10 | 1 | 148340 | 10 | 147964 | 1 | 20 | 1 | 6 | 19 | 1 | 2 |
| 22 | 148994 | 3 | 20 | 1 | 146450 | 0 | 148808 | 16 | 0 | 149578 | 150297 |
| 2 | 7 | 23 | 14 | 29 | 13 | 147810 | 12 | 10 | 148717 | 6 | 16 |
| 15072 | 4134 | 4775 | 3458 | 5143 | 6663 | 5313 | 4315 | 8284 | 4405 | 3542 | 2795 |
| 138071 | 149009 | 148368 | 149685 | 148000 | 146480 | 147832 | 148830 | 144861 | 148740 | 149603 | 150350 |
| 99.98% | 0.00% | 0.00% | 99.97% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.01% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.96% |
| 0.00% | 0.00% | 0.02% | 0.01% | 0.02% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% |
| 145920 | 8 | 0 | 158764 | 2 | 17 | 1 | 10 | 153386 | 5 | 15 | 43 |
| 8 | 1 | 157298 | 9 | 156663 | 0 | 8 | 3 | 9 | 16 | 7 | 2 |
| 28 | 157910 | 2 | 15 | 1 | 155056 | 1 | 157589 | 14 | 1 | 158476 | 159225 |
| 3 | 3 | 5 | 9 | 24 | 2 | 156754 | 9 | 9 | 157635 | 6 | 9 |
| 16416 | 4453 | 5070 | 3578 | 5685 | 7300 | 5611 | 4764 | 8957 | 4718 | 3871 | 3096 |
| 145959 | 157922 | 157305 | 158797 | 156690 | 155075 | 156764 | 157611 | 153418 | 157657 | 158504 | 159279 |
| 99.97% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.03% |
| 0.01% | 0.00% | 100.00 % | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% |

# FIGURE 30

| | 1<br>G | 2<br>G | 3<br>C | 4<br>A | 5<br>C | 6<br>T | 7<br>G | 8<br>C | 9<br>G | 10<br>G | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BE3** | | | | | | | | | | | |
| **A** | 3 | 80 | 134 | 120406 | 11164 | 112 | 14 | 99 | 9 | 9 | 0 |
| **C** | 0 | 1 | 126967 | 3 | 43385 | 17 | 1 | 133377 | 2 | 8 | 110953 |
| **G** | 101366 | 122971 | 117 | 9 | 35879 | 1 | 137227 | 42 | 133449 | 123239 | 0 |
| **T** | 112 | 2 | 349 | 9 | 39588 | 129364 | 4 | 2064 | 6 | 4 | 58 |
| **N** | 41409 | 19836 | 15323 | 22463 | 12874 | 13396 | 5644 | 7308 | 9423 | 19629 | 31876 |
| | 101481 | 123054 | 127567 | 120427 | 130016 | 129494 | 137246 | 135582 | 133466 | 123260 | 111011 |
| **A** | 0.00% | 0.07% | 0.11% | 99.98% | 8.59% | 0.09% | 0.01% | 0.07% | 0.01% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 99.53% | 0.00% | 33.37% | 0.01% | 0.00% | 98.37% | 0.00% | 0.01% | 99.95% |
| **G** | 99.89% | 99.93% | 0.09% | 0.01% | 27.60% | 0.00% | 99.99% | 0.03% | 99.99% | 99.98% | 0.00% |
| **T** | 0.11% | 0.00% | 0.27% | 0.01% | 30.45% | 99.90% | 0.00% | 1.52% | 0.00% | 0.00% | 0.05% |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | | | |
| **A** | 5 | 14 | 0 | 129390 | 3 | 9 | 5 | 1 | 5 | 9 | 0 |
| **C** | 0 | 6 | 132740 | 0 | 131508 | 13 | 0 | 138647 | 2 | 0 | 112980 |
| **G** | 104719 | 125294 | 1 | 7 | 1 | 0 | 140688 | 2 | 140637 | 125082 | 2 |
| **T** | 12 | 3 | 7 | 10 | 18 | 137423 | 1 | 71 | 3 | 10 | 7 |
| **N** | 40526 | 19946 | 12515 | 15856 | 13733 | 7818 | 4569 | 6542 | 4616 | 20162 | 32274 |
| | 104736 | 125317 | 132748 | 129407 | 131530 | 137445 | 140694 | 138721 | 140647 | 125101 | 112989 |
| **A** | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.01% | 0.00% | 99.95% | 0.00% | 0.00% | 99.99% |
| **G** | 99.98% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 100.00% | 0.00% | 99.99% | 99.98% | 0.00% |
| **T** | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% | 0.05% | 0.00% | 0.01% | 0.01% |

FIGURE 30 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 38 | 16 | 111613 | 5 | 3 | 0 | 1 | 1 | 3 | 2 | 4 |
| 12 | 0 | 13 | 2 | 0 | 0 | 6 | 3 | 1 | 1 | 2 | 0 |
| 0 | 114701 | 138732 | 14 | 128941 | 135537 | 52 | 98432 | 129460 | 122064 | 124414 | 111364 |
| 106515 | 4 | 36 | 1 | 5 | 6 | 22469 | 9 | 6 | 6 | 3 | 13 |
| 36360 | 28144 | 4090 | 31258 | 13937 | 7342 | 120344 | 44425 | 13402 | 20850 | 18503 | 31543 |
| 106527 | 114743 | 138797 | 111630 | 128951 | 135546 | 22527 | 98445 | 129468 | 122074 | 124421 | 111381 |
| 0.00% | 0.03% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.96% | 99.95% | 0.01% | 99.99% | 99.99% | 0.23% | 99.99% | 99.99% | 99.99% | 99.99% | 99.98% |
| 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 99.74% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0 | 1 | 2 | 112612 | 2 | 5 | 0 | 6 | 1 | 1 | 2 | 4 |
| 5 | 5 | 0 | 4 | 85 | 84 | 10 | 2 | 1 | 0 | 3 | 1 |
| 0 | 115578 | 140824 | 18 | 130675 | 137642 | 51 | 99118 | 131254 | 124137 | 126639 | 113337 |
| 106847 | 15 | 16 | 0 | 7 | 6 | 23267 | 14 | 12 | 11 | 7 | 10 |
| 38411 | 29664 | 4507 | 32715 | 14580 | 7612 | 122021 | 46209 | 14081 | 21200 | 18698 | 31997 |
| 106852 | 115599 | 140842 | 112634 | 130769 | 137737 | 23328 | 99140 | 131268 | 124149 | 126651 | 113352 |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.07% | 0.06% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.98% | 99.99% | 0.02% | 99.93% | 99.93% | 0.22% | 99.98% | 99.99% | 99.99% | 99.99% | 99.99% |
| 100.00 % | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.74% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |

FIGURE 30 (Continued)

| pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 25 | 2 | 97141 | 1 | 5 | 5 | 2 | 10 | 4 | 0 | |
| C | 1 | 4 | 99343 | 10 | 98640 | 13 | 0 | 103657 | 0 | 0 | 84805 | |
| G | 78626 | 93457 | 0 | 135 | 24 | 0 | 105054 | 2 | 104939 | 93332 | 1 | |
| T | 7 | 1 | 4 | 1 | 47 | 102923 | 0 | 53 | 24 | 11 | 3 | |
| N | 29750 | 14899 | 9037 | 11099 | 9674 | 5445 | 3327 | 4672 | 3413 | 15039 | 23577 | |
| | 78636 | 93487 | 99349 | 97287 | 98712 | 102941 | 105059 | 103714 | 104973 | 93347 | 84809 | |
| A | 0.00% | 0.03% | 0.00% | 99.85% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | |
| C | 0.00% | 0.00% | 99.99% | 0.01% | 99.93% | 0.01% | 0.00% | 99.95% | 0.00% | 0.00% | 100.00 % | |
| G | 99.99% | 99.97% | 0.00% | 0.14% | 0.02% | 0.00% | 100.00% | 0.00% | 99.97% | 99.98% | 0.00% | |
| T | 0.01% | 0.00% | 0.00% | 0.00% | 0.05% | 99.98% | 0.00% | 0.05% | 0.02% | 0.01% | 0.00% | |
| pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N) | | | | | | | | | | | | |
| A | 8 | 39 | 1 | 130536 | 6 | 4 | 10 | 1 | 12 | 8 | 0 | |
| C | 1 | 3 | 133797 | 2 | 132664 | 27 | 1 | 139992 | 1 | 2 | 115034 | |
| G | 106779 | 125901 | 1 | 231 | 15 | 0 | 141973 | 3 | 141732 | 126481 | 0 | |
| T | 3 | 1 | 7 | 5 | 42 | 138597 | 3 | 45 | 6 | 10 | 6 | |
| N | 39839 | 20686 | 12824 | 15856 | 13903 | 8002 | 4643 | 6589 | 4879 | 20129 | 31590 | |
| | 106791 | 125944 | 133806 | 130774 | 132727 | 138628 | 141987 | 140041 | 141751 | 126501 | 115040 | |
| A | 0.01% | 0.03% | 0.00% | 99.82% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 99.95% | 0.02% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | |
| G | 99.99% | 99.97% | 0.00% | 0.18% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.03% | 99.98% | 0.00% | 0.03% | 0.00% | 0.01% | 0.01% | |

FIGURE 30 (Continued)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 8 | 2 | 85310 | 2 | 2 | 0 | 0 | 0 | 0 | 4 | 4 | | |
| 6 | 1 | 0 | 1 | 0 | 0 | 4 | 1 | 0 | 1 | 1 | 0 | | |
| 1 | 87034 | 105108 | 16 | 97863 | 102929 | 23 | 74098 | 97966 | 92429 | 94393 | 84662 | | |
| 81038 | 3 | 2 | 1 | 2 | 2 | 17465 | 2 | 4 | 3 | 2 | 5 | | |
| 27341 | 21340 | 3274 | 23058 | 10519 | 5453 | 90894 | 34285 | 10416 | 15953 | 13986 | 23715 | | |
| 81045 | 87046 | 105112 | 85328 | 97867 | 102933 | 17492 | 74101 | 97970 | 92433 | 94400 | 84671 | | |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| 0.00% | 99.99% | 100.00% | 0.02% | 100.00% | 100.00% | 0.13% | 100.00% | 100.00% | 100.00% | 99.99% | 99.99% | | |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.85% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | | |
| 0 | 5 | 4 | 114498 | 1 | 0 | 0 | 2 | 0 | 2 | 5 | 8 | | |
| 9 | 1 | 0 | 3 | 1 | 2 | 7 | 1 | 1 | 0 | 1 | 1 | | |
| 1 | 117443 | 142112 | 18 | 132309 | 138786 | 41 | 101937 | 132840 | 125412 | 127980 | 114330 | | |
| 109408 | 8 | 7 | 0 | 13 | 8 | 24078 | 7 | 7 | 7 | 5 | 8 | | |
| 37212 | 29173 | 4507 | 32111 | 14306 | 7834 | 122504 | 44683 | 13782 | 21209 | 18639 | 32283 | | |
| 109418 | 117457 | 142123 | 114519 | 132321 | 138796 | 24126 | 101947 | 132848 | 125421 | 127991 | 114347 | | |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | | |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| 0.00% | 99.99% | 99.99% | 0.02% | 99.99% | 99.99% | 0.17% | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | | |
| 99.99% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 99.80% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | | |

# Figure 30 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>A | 5<br>C | 6<br>T | 7<br>G | 8<br>C | 9<br>G | 10<br>G | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-156 (pCMV_ecTadA_XTEN_nCas9_GGS_UGI_GGS_NLS ; D108N)** | | | | | | | | | | | |
| **A** | 3 | 31 | 1 | 113058 | 2 | 0 | 4 | 7 | 8 | 7 | 0 |
| **C** | 7 | 2 | 115613 | 7 | 115125 | 8 | 1 | 121117 | 0 | 7 | 99077 |
| **G** | 92071 | 108555 | 0 | 319 | 1 | 1 | 122787 | 1 | 122623 | 108838 | 3 |
| **T** | 5 | 7 | 2 | 11 | 28 | 120100 | 3 | 72 | 8 | 6 | 6 |
| **N** | 34686 | 18177 | 11157 | 13378 | 11617 | 6664 | 3978 | 5576 | 4134 | 17915 | 27687 |
| | 92086 | 108595 | 115616 | 113395 | 115156 | 120109 | 122795 | 121197 | 122639 | 108858 | 99086 |
| **A** | 0.00% | 0.03% | 0.00% | 99.70% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |
| **C** | 0.01% | 0.00% | 100.00 % | 0.01% | 99.97% | 0.01% | 0.00% | 99.93% | 0.00% | 0.01% | 99.99% |
| **G** | 99.98% | 99.96% | 0.00% | 0.28% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% |
| **T** | 0.01% | 0.01% | 0.00% | 0.01% | 0.02% | 99.99% | 0.00% | 0.06% | 0.01% | 0.01% | 0.01% |
| **pNMG-160 (pCMV_ecTadA_XTEN_nCas9_GGS_AAG*(E125Q)_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 7 | 29 | 0 | 129170 | 5 | 3 | 15 | 1 | 9 | 4 | 0 |
| **C** | 1 | 2 | 132221 | 9 | 131319 | 20 | 0 | 138324 | 0 | 7 | 113666 |
| **G** | 105853 | 124439 | 0 | 130 | 2 | 0 | 140125 | 2 | 139875 | 124715 | 0 |
| **T** | 2 | 5 | 6 | 10 | 13 | 137066 | 3 | 47 | 10 | 2 | 6 |
| **N** | 38739 | 20127 | 12375 | 15283 | 13263 | 7513 | 4459 | 6228 | 4708 | 19874 | 30930 |
| | 105863 | 124475 | 132227 | 129319 | 131339 | 137089 | 140143 | 138374 | 139894 | 124728 | 113672 |
| **A** | 0.01% | 0.02% | 0.00% | 99.88% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 100.00 % | 0.01% | 99.98% | 0.01% | 0.00% | 99.96% | 0.00% | 0.01% | 99.99% |
| **G** | 99.99% | 99.97% | 0.00% | 0.10% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| **T** | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% | 0.03% | 0.01% | 0.00% | 0.01% |

Figure 30 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 6 | 1 | 99081 | 2 | 3 | 1 | 3 | 0 | 1 | 3 | 2 |
| 4 | 2 | 2 | 3 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 1 |
| 2 | 101520 | 122793 | 22 | 114222 | 120197 | 35 | 88005 | 115018 | 108129 | 110195 | 98556 |
| 94640 | 11 | 6 | 1 | 7 | 10 | 20842 | 9 | 8 | 4 | 4 | 8 |
| 32120 | 25227 | 3964 | 27659 | 12535 | 6556 | 105885 | 38748 | 11740 | 18632 | 16564 | 28199 |
| 94646 | 101539 | 122802 | 99107 | 114231 | 120210 | 20881 | 88018 | 115026 | 108134 | 110202 | 98567 |
| 0.00% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.98% | 99.99% | 0.02% | 99.99% | 99.99% | 0.17% | 99.99% | 99.99% | 100.00% | 99.99% | 99.99% |
| 99.99% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 99.81% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| 1 | 4 | 6 | 112971 | 3 | 10 | 1 | 3 | 1 | 6 | 5 | 6 |
| 8 | 0 | 1 | 4 | 1 | 1 | 2 | 1 | 0 | 1 | 1 | 0 |
| 0 | 116205 | 140283 | 13 | 130753 | 137115 | 46 | 101038 | 131589 | 123696 | 126146 | 112728 |
| 108346 | 7 | 4 | 4 | 5 | 4 | 24310 | 8 | 5 | 3 | 4 | 7 |
| 36247 | 28386 | 4308 | 31610 | 13840 | 7472 | 120243 | 43552 | 13007 | 20897 | 18447 | 31862 |
| 108355 | 116216 | 140294 | 112992 | 130762 | 137130 | 24359 | 101050 | 131595 | 123706 | 126156 | 112741 |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.00% | 99.99% | 99.99% | 0.01% | 99.99% | 100.0% | 0.2% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.0% | 99.8% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

# Figure 30 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-161 (pCMV_ecTadA_XTEN_Cas9n_GGS_EndoV*(D35A)_NLS; D108N)** | | | | | | | | | | | |
| **A** | 6 | 25 | 1 | 130174 | 11 | 2 | 1 | 4 | 11 | 3 | 0 |
| **C** | 0 | 1 | 132708 | 5 | 132066 | 12 | 0 | 138811 | 0 | 4 | 113881 |
| **G** | 106066 | 124624 | 0 | 108 | 13 | 1 | 140493 | 5 | 140390 | 125055 | 1 |
| **T** | 3 | 2 | 5 | 9 | 10 | 137564 | 3 | 57 | 7 | 12 | 7 |
| **N** | 38942 | 20365 | 12303 | 14721 | 12917 | 7438 | 4520 | 6140 | 4609 | 19943 | 31128 |
| | 106075 | 124652 | 132714 | 130296 | 132100 | 137579 | 140497 | 138877 | 140408 | 125074 | 113889 |
| **A** | 0.01% | 0.02% | 0.00% | 99.91% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 100.00 % | 0.00% | 99.97% | 0.01% | 0.00% | 99.95% | 0.00% | 0.00% | 99.99% |
| **G** | 99.99% | 99.98% | 0.00% | 0.08% | 0.01% | 0.00% | 100.00% | 0.00% | 99.99% | 99.98% | 0.00% |
| **T** | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.00% | 0.04% | 0.00% | 0.01% | 0.01% |
| **pNMG-162 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)** | | | | | | | | | | | |
| **A** | 3 | 32 | 1 | 125425 | 1 | 3 | 4 | 3 | 10 | 5 | 0 |
| **C** | 4 | 1 | 128650 | 10 | 127801 | 18 | 1 | 134456 | 1 | 0 | 110134 |
| **G** | 102793 | 120856 | 1 | 445 | 9 | 0 | 136227 | 2 | 136069 | 121363 | 2 |
| **T** | 1 | 2 | 4 | 2 | 19 | 133251 | 4 | 35 | 6 | 6 | 2 |
| **N** | 37866 | 19776 | 12011 | 14785 | 12837 | 7395 | 4431 | 6171 | 4581 | 19293 | 30529 |
| | 102801 | 120891 | 128656 | 125882 | 127830 | 133272 | 136236 | 134496 | 136086 | 121374 | 110138 |
| **A** | 0.00% | 0.03% | 0.00% | 99.64% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 100.00 % | 0.01% | 99.98% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 100.00 % |
| **G** | 99.99% | 99.97% | 0.00% | 0.35% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| **T** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% |

# Figure 30 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 1 | 113795 | 1 | 3 | 0 | 0 | 0 | 5 | 2 | 5 |
| 5 | 3 | 1 | 2 | 2 | 1 | 8 | 1 | 1 | 1 | 1 | 3 |
| 0 | 116682 | 140651 | 13 | 130910 | 137615 | 41 | 100722 | 131526 | 123991 | 126490 | 112965 |
| 108888 | 3 | 3 | 1 | 11 | 4 | 23833 | 7 | 12 | 7 | 3 | 15 |
| 36123 | 28324 | 4361 | 31206 | 14093 | 7394 | 121135 | 44287 | 13478 | 21013 | 18522 | 32030 |
| 108894 | 116693 | 140656 | 113811 | 130924 | 137623 | 23882 | 100730 | 131539 | 124004 | 126496 | 112988 |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.00% | 99.99% | 100.00% | 0.01% | 100.0% | 100.0% | 0.2% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.0% | 0.0% | 99.8% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 2 | 8 | 3 | 110188 | 4 | 7 | 0 | 6 | 1 | 3 | 1 | 6 |
| 12 | 1 | 0 | 2 | 2 | 0 | 7 | 1 | 0 | 0 | 0 | 1 |
| 2 | 112800 | 136378 | 15 | 126993 | 133360 | 42 | 97079 | 127631 | 120452 | 122864 | 109858 |
| 105123 | 5 | 2 | 0 | 9 | 4 | 22751 | 12 | 5 | 4 | 3 | 8 |
| 35528 | 27853 | 4284 | 30462 | 13659 | 7296 | 117865 | 43567 | 13028 | 20206 | 17797 | 30792 |
| 105139 | 112814 | 136383 | 110205 | 127008 | 133371 | 22800 | 97098 | 127637 | 120459 | 122868 | 109873 |
| 0.00% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.01% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.00% | 100.0% | 100.0% | 0.0% | 100.0% | 100.0% | 0.2% | 100.0% | 100.0% | 100.0% | 100.0% | 100.0% |
| 99.98% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.8% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |



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# Figure 30 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>A | 5<br>C | 6<br>T | 7<br>G | 8<br>C | 9<br>G | 10<br>G | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-163 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 9 | 51 | 3 | 169700 | 5 | 7 | 14 | 0 | 6 | 11 | 0 |
| **C** | 1 | 2 | 171503 | 5 | 171428 | 13 | 0 | 179723 | 0 | 2 | 146052 |
| **G** | 137663 | 160221 | 0 | 359 | 4 | 1 | 182139 | 2 | 182592 | 161927 | 7 |
| **T** | 15 | 3 | 4 | 6 | 26 | 178279 | 6 | 56 | 4 | 5 | 7 |
| **N** | 50404 | 27815 | 16582 | 18022 | 16629 | 9792 | 5933 | 8311 | 5490 | 26147 | 42026 |
| | 137688 | 160277 | 171510 | 170070 | 171463 | 178300 | 182159 | 179781 | 182602 | 161945 | 146066 |
| **A** | 0.01% | 0.03% | 0.00% | 99.78% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 100.00 % | 0.00% | 99.98% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |
| **G** | 99.98% | 99.97% | 0.00% | 0.21% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| **T** | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 5 | 23 | 2 | 150106 | 2 | 3 | 7 | 2 | 19 | 6 | 0 |
| **C** | 0 | 2 | 153049 | 7 | 152396 | 18 | 0 | 159995 | 0 | 0 | 129931 |
| **G** | 121665 | 143205 | 2 | 721 | 4 | 4 | 162290 | 3 | 162671 | 144918 | 2 |
| **T** | 1 | 6 | 8 | 11 | 10 | 158661 | 3 | 85 | 6 | 7 | 7 |
| **N** | 45748 | 24183 | 14358 | 16574 | 15007 | 8733 | 5119 | 7334 | 4723 | 22488 | 37479 |
| | 121671 | 143236 | 153061 | 150845 | 152412 | 158686 | 162300 | 160085 | 162696 | 144931 | 129940 |
| **A** | 0.00% | 0.02% | 0.00% | 99.51% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.94% | 0.00% | 0.00% | 99.99% |
| **G** | 100.00 % | 99.98% | 0.00% | 0.48% | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 99.99% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% | 0.05% | 0.00% | 0.00% | 0.01% |

Figure 30 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 8 | 2 | 147047 | 6 | 0 | 2 | 3 | 8 | 5 | 2 | 4 |
| 10 | 1 | 0 | 4 | 2 | 1 | 8 | 1 | 2 | 1 | 1 | 3 |
| 3 | 152484 | 182850 | 20 | 170007 | 178706 | 59 | 130800 | 171260 | 160350 | 163415 | 146653 |
| 142769 | 3 | 4 | 0 | 6 | 6 | 30168 | 8 | 9 | 8 | 6 | 14 |
| 45310 | 35596 | 5236 | 41021 | 18071 | 9379 | 157855 | 57280 | 16813 | 27728 | 24668 | 41418 |
| 142782 | 152496 | 182856 | 147071 | 170021 | 178713 | 30237 | 130812 | 171279 | 160364 | 163424 | 146674 |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 100.00 % | 0.01% | 99.99% | 100.00 % | 0.20% | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.77% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| 1 | 4 | 3 | 130017 | 2 | 6 | 0 | 8 | 1 | 12 | 3 | 4 |
| 7 | 0 | 1 | 1 | 1 | 0 | 8 | 2 | 0 | 1 | 2 | 1 |
| 2 | 135691 | 162750 | 17 | 151286 | 159172 | 55 | 115251 | 152002 | 143478 | 146302 | 130749 |
| 126186 | 6 | 4 | 0 | 5 | 6 | 26122 | 8 | 10 | 7 | 9 | 14 |
| 41223 | 31718 | 4661 | 37384 | 16125 | 8235 | 141234 | 52150 | 15406 | 23921 | 21103 | 36651 |
| 126196 | 135701 | 162758 | 130035 | 151294 | 159184 | 26185 | 115269 | 152013 | 143498 | 146316 | 130768 |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 100.00 % | 0.01% | 99.99% | 99.99% | 0.21% | 99.98% | 99.99% | 99.99% | 99.99% | 99.99% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.76% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |

# Figure 30 (Continued)

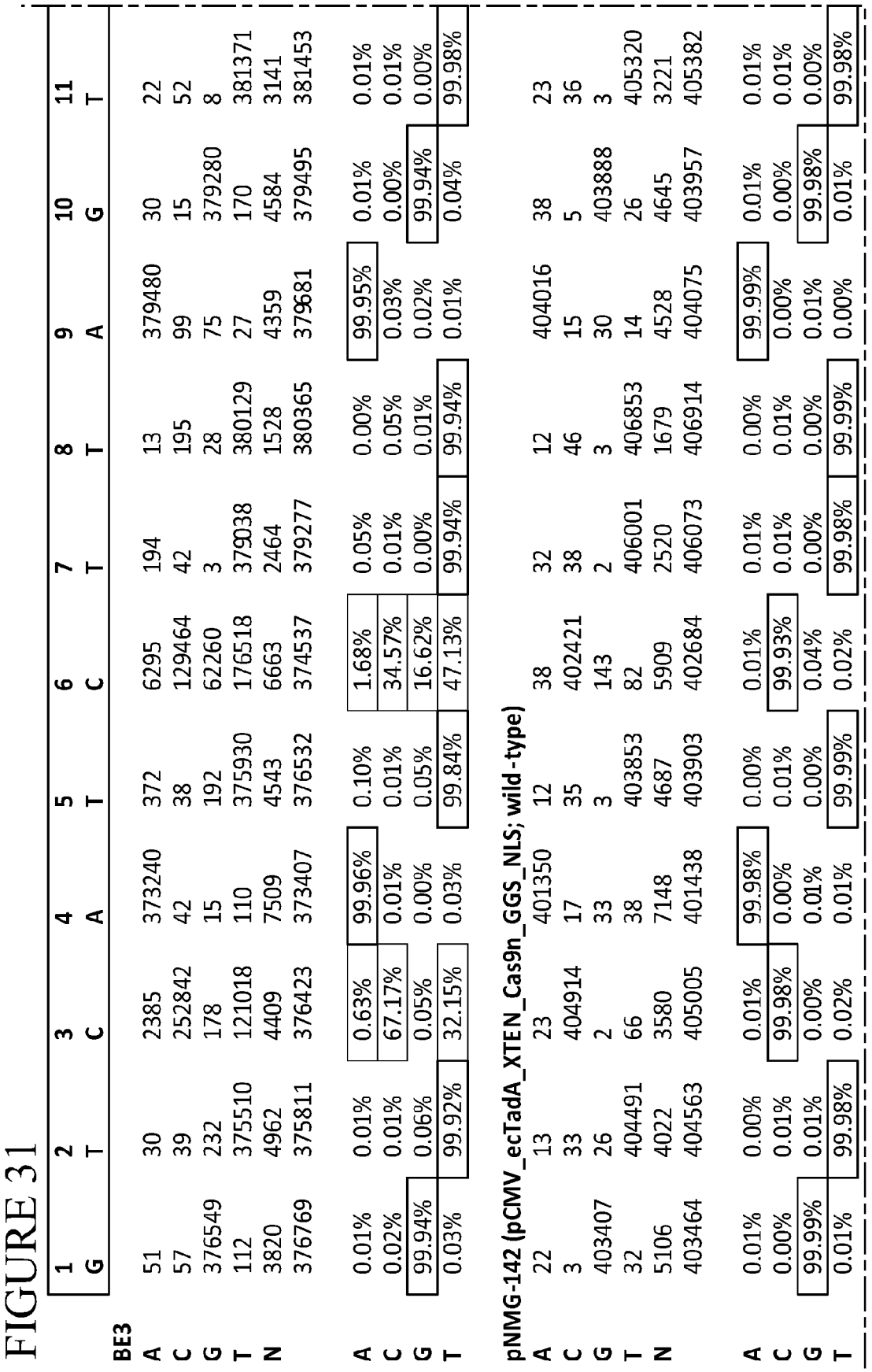
| pNMG-165 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 25 | 2 | 111420 | 2 | 3 | 4 | 2 | 8 | 9 | 0 |
| C | 0 | 2 | 112681 | 8 | 112444 | 13 | 0 | 117613 | 3 | 0 | 95658 |
| G | 89964 | 105113 | 1 | 150 | 2 | 4 | 119149 | 4 | 119504 | 105984 | 1 |
| T | 2 | 0 | 4 | 3 | 6 | 116810 | 2 | 50 | 3 | 10 | 6 |
| N | 33077 | 17908 | 10360 | 11467 | 10594 | 6218 | 3893 | 5379 | 3530 | 17045 | 27383 |
| | 89971 | 105140 | 112688 | 111581 | 112454 | 116830 | 119155 | 117669 | 119518 | 106003 | 95665 |
| A | 0.01% | 0.02% | 0.00% | 99.86% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% | 0.00% | 99.95% | 0.00% | 0.00% | 99.99% |
| G | 99.99% | 99.97% | 0.00% | 0.13% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% | 0.04% | 0.00% | 0.01% | 0.01% |
| **untreated cells** | | | | | | | | | | | |
| A | 9 | 40 | 0 | 153826 | 2 | 2 | 10 | 3 | 14 | 9 | 1 |
| C | 2 | 1 | 155682 | 9 | 155439 | 16 | 0 | 163147 | 0 | 1 | 133477 |
| G | 125582 | 145357 | 2 | 10 | 2 | 1 | 165413 | 3 | 165629 | 147811 | 3 |
| T | 4 | 3 | 3 | 5 | 11 | 161730 | 1 | 50 | 6 | 9 | 5 |
| N | 45024 | 25220 | 14934 | 16770 | 15166 | 8871 | 5196 | 7417 | 4971 | 22790 | 37134 |
| | 125597 | 145401 | 155687 | 153850 | 155454 | 161749 | 165424 | 163203 | 165649 | 147830 | 133486 |
| A | 0.01% | 0.03% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 100.00 % | 0.01% | 99.99% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |
| G | 99.99% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% | 0.03% | 0.00% | 0.01% | 0.00% |

# Figure 30 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 4 | 1 | 97077 | 2 | 4 | 0 | 1 | 1 | 2 | 3 | 8 |
| 3 | 2 | 1 | 2 | 0 | 1 | 6 | 0 | 0 | 0 | 1 | 0 |
| 2 | 99770 | 119594 | 22 | 111397 | 117104 | 38 | 84413 | 111618 | 105045 | 107102 | 96205 |
| 93248 | 6 | 9 | 0 | 7 | 4 | 19266 | 24 | 10 | 11 | 11 | 3 |
| 29795 | 23266 | 3443 | 25947 | 11642 | 5935 | 103738 | 38610 | 11419 | 17990 | 15931 | 26832 |
| 93253 | 99782 | 119605 | 97101 | 111406 | 117113 | 19310 | 84438 | 111629 | 105058 | 107117 | 96216 |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 99.99% | 0.02% | 99.99% | 99.99% | 0.20% | 99.97% | 99.99% | 99.99% | 99.99% | 99.99% |
| 99.99% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.77% | 0.03% | 0.01% | 0.01% | 0.01% | 0.00% |
| 2 | 11 | 1 | 133263 | 5 | 3 | 0 | 3 | 2 | 6 | 7 | 6 |
| 6 | 4 | 2 | 0 | 0 | 0 | 5 | 1 | 1 | 3 | 1 | 0 |
| 0 | 139113 | 165876 | 15 | 154468 | 162054 | 52 | 119299 | 155490 | 146422 | 149179 | 133529 |
| 130128 | 11 | 10 | 0 | 17 | 3 | 27542 | 30 | 19 | 16 | 5 | 8 |
| 40484 | 31481 | 4731 | 37342 | 16130 | 8560 | 143021 | 51287 | 15108 | 24173 | 21428 | 37077 |
| 130136 | 139139 | 165889 | 133278 | 154490 | 162060 | 27599 | 119333 | 155512 | 146447 | 149192 | 133543 |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.98% | 99.99% | 0.01% | 99.99% | 100.00% | 0.19% | 99.97% | 99.99% | 99.98% | 99.99% | 99.99% |
| 99.99% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.79% | 0.03% | 0.01% | 0.01% | 0.00% | 0.01% |

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FIGURE 31



| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T | 9 A | 10 G | 11 T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BE3** | | | | | | | | | | | |
| **A** | 51 | 30 | 2385 | 373240 | 372 | 6295 | 194 | 13 | 379480 | 30 | 22 |
| **C** | 57 | 39 | 252842 | 42 | 38 | 129464 | 42 | 195 | 99 | 15 | 52 |
| **G** | 376549 | 232 | 178 | 15 | 192 | 62260 | 3 | 28 | 75 | 379280 | 8 |
| **T** | 112 | 375510 | 121018 | 110 | 375930 | 176518 | 379038 | 380129 | 27 | 170 | 381371 |
| **N** | 3820 | 4962 | 4409 | 7509 | 4543 | 6663 | 2464 | 1528 | 4359 | 4584 | 3141 |
| | 376769 | 375811 | 376423 | 373407 | 376532 | 374537 | 379277 | 380365 | 379681 | 379495 | 381453 |
| **A** | 0.01% | 0.01% | 0.63% | 99.96% | 0.10% | 1.68% | 0.05% | 0.00% | 99.95% | 0.01% | 0.01% |
| **C** | 0.02% | 0.01% | 67.17% | 0.01% | 0.01% | 34.57% | 0.01% | 0.05% | 0.03% | 0.00% | 0.01% |
| **G** | 99.94% | 0.06% | 0.05% | 0.00% | 0.05% | 16.62% | 0.00% | 0.01% | 0.02% | 99.94% | 0.00% |
| **T** | 0.03% | 99.92% | 32.15% | 0.03% | 99.84% | 47.13% | 99.94% | 99.94% | 0.01% | 0.04% | 99.98% |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | | | |
| **A** | 22 | 13 | 23 | 401350 | 12 | 38 | 32 | 12 | 404016 | 38 | 23 |
| **C** | 3 | 33 | 404914 | 17 | 35 | 402421 | 38 | 46 | 15 | 5 | 36 |
| **G** | 403407 | 26 | 2 | 33 | 3 | 143 | 2 | 3 | 30 | 403888 | 3 |
| **T** | 32 | 404491 | 66 | 38 | 403853 | 82 | 406001 | 406853 | 14 | 26 | 405320 |
| **N** | 5106 | 4022 | 3580 | 7148 | 4687 | 5909 | 2520 | 1679 | 4528 | 4645 | 3221 |
| | 403464 | 404563 | 405005 | 401438 | 403903 | 402684 | 406073 | 406914 | 404075 | 403957 | 405382 |
| **A** | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% |
| **C** | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 99.93% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| **G** | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 0.04% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| **T** | 0.01% | 99.98% | 0.02% | 0.01% | 99.99% | 0.02% | 99.98% | 99.99% | 0.00% | 0.01% | 99.98% |

# FIGURE 31 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | A | G | G | A | C | C | T | G | A | G | G |
| 517 | 378994 | 16 | 15 | 376537 | 125 | 37 | 10 | 59 | 374950 | 10 | 40 |
| 360303 | 25 | 31 | 617 | 31 | 380263 | 377976 | 73 | 27 | 44 | 4 | 4 |
| 850 | 27 | 7 | 11 | 27 | 16 | 3 | 9 | 381999 | 25 | 379506 | 380089 |
| 19575 | 99 | 383093 | 382782 | 88 | 465 | 70 | 380638 | 40 | 46 | 69 | 40 |
| 3379 | 5479 | 1510 | 1253 | 8066 | 3883 | 6683 | 4069 | 2745 | 9847 | 5328 | 4802 |
| 381245 | 379145 | 383147 | 383425 | 376683 | 380869 | 378086 | 380730 | 382125 | 375065 | 379589 | 380173 |
| 0.14% | 99.96% | 0.00% | 0.00% | 99.96% | 0.03% | 0.01% | 0.00% | 0.02% | 99.97% | 0.00% | 0.01% |
| 94.51% | 0.01% | 0.01% | 0.16% | 0.01% | 99.84% | 99.97% | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.22% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.98% | 99.98% |
| 5.13% | 0.03% | 99.99% | 99.83% | 0.02% | 0.12% | 0.02% | 99.98% | 0.01% | 0.01% | 0.02% | 0.01% |
| 24 | 402887 | 8 | 5 | 400381 | 7 | 19 | 10 | 29 | 398498 | 8 | 10 |
| 405246 | 20 | 39 | 52 | 24 | 404431 | 401526 | 59 | 7 | 42 | 5 | 4 |
| 4 | 26 | 3 | 2 | 26 | 2 | 2 | 4 | 405978 | 39 | 403409 | 403726 |
| 62 | 17 | 406954 | 407216 | 24 | 25 | 30 | 404112 | 26 | 35 | 31 | 29 |
| 3267 | 5653 | 1599 | 1328 | 8148 | 4140 | 7027 | 4419 | 2566 | 9992 | 5153 | 4837 |
| 405336 | 402950 | 407004 | 407275 | 400455 | 404465 | 401577 | 404185 | 406040 | 398614 | 403453 | 403769 |
| 0.01% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% |
| 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% |
| 0.02% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.01% |



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# FIGURE 31 (Continued)

| pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 19 | 14 | 9 | 326053 | 10 | 29 | 14 | 8 | 328525 | 13 | 16 |
| C | 4 | 20 | 329171 | 31 | 21 | 327300 | 23 | 39 | 29 | 6 | 25 |
| G | 327902 | 21 | 3 | 67 | 0 | 96 | 5 | 1 | 36 | 328317 | 5 |
| T | 22 | 328813 | 45 | 22 | 328550 | 55 | 330090 | 330826 | 12 | 37 | 329625 |
| N | 4317 | 3413 | 3055 | 6110 | 3702 | 4803 | 2151 | 1409 | 3701 | 3930 | 2632 |
| | 327947 | 328868 | 329228 | 326173 | 328581 | 327480 | 330132 | 330874 | 328602 | 328373 | 329671 |
| | | | | | | | | | | | |
| A | 0.01% | 0.00% | 0.00% | 99.96% | 0.00% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% |
| C | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 99.95% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| G | 99.99% | 0.01% | 0.00% | 0.02% | 0.00% | 0.03% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| T | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% | 0.02% | 99.99% | 99.99% | 0.00% | 0.01% | 99.99% |

| pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 25 | 11 | 13 | 380155 | 11 | 22 | 37 | 7 | 382736 | 20 | 26 |
| C | 5 | 27 | 383655 | 20 | 27 | 381188 | 35 | 34 | 16 | 4 | 40 |
| G | 382210 | 20 | 2 | 127 | 5 | 198 | 6 | 1 | 49 | 382705 | 1 |
| T | 34 | 383309 | 62 | 32 | 382546 | 81 | 384431 | 385377 | 20 | 38 | 384182 |
| N | 4728 | 3652 | 3291 | 6690 | 4441 | 5549 | 2529 | 1620 | 4227 | 4281 | 2802 |
| | 382274 | 383367 | 383732 | 380334 | 382589 | 381489 | 384509 | 385419 | 382821 | 382767 | 384249 |
| | | | | | | | | | | | |
| A | 0.01% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% |
| C | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 99.92% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| G | 99.98% | 0.01% | 0.00% | 0.03% | 0.00% | 0.05% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| T | 0.01% | 99.98% | 0.02% | 0.01% | 99.99% | 0.02% | 99.98% | 99.99% | 0.01% | 0.01% | 99.98% |

# FIGURE 31 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 327487 | 16 | 9 | 325244 | 8 | 12 | 6 | 37 | 324702 | 4 | 9 |
| 329265 | 27 | 25 | 35 | 26 | 328860 | 326515 | 64 | 7 | 35 | 11 | 3 |
| 6 | 28 | 2 | 0 | 24 | 11 | 2 | 5 | 330033 | 20 | 327937 | 328118 |
| 62 | 21 | 330931 | 331112 | 12 | 25 | 31 | 328455 | 42 | 24 | 26 | 27 |
| 2957 | 4740 | 1336 | 1154 | 7005 | 3407 | 5750 | 3781 | 2192 | 7532 | 4335 | 4156 |
| 329346 | 327563 | 330974 | 331156 | 325306 | 328904 | 326560 | 328530 | 330119 | 324781 | 327978 | 328157 |
| | | | | | | | | | | | |
| 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% |
| 99.98% | 0.01% | 0.01% | 0.01% | 0.01% | 99.99% | 99.99% | 0.02% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.99% | 99.99% |
| 0.02% | 0.01% | 99.99% | 99.99% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.01% |
| | | | | | | | | | | | |
| 20 | 381587 | 10 | 17 | 379139 | 9 | 29 | 26 | 34 | 377686 | 7 | 13 |
| 383989 | 19 | 23 | 32 | 24 | 383216 | 380293 | 65 | 7 | 29 | 6 | 7 |
| 12 | 21 | 3 | 2 | 29 | 1 | 2 | 3 | 384448 | 31 | 381891 | 382299 |
| 72 | 27 | 385594 | 385672 | 22 | 37 | 32 | 382836 | 45 | 30 | 32 | 21 |
| 2959 | 5398 | 1422 | 1329 | 7840 | 3791 | 6698 | 4124 | 2520 | 9278 | 5118 | 4714 |
| 384093 | 381654 | 385630 | 385723 | 379214 | 383263 | 380356 | 382930 | 384534 | 377776 | 381936 | 382340 |
| | | | | | | | | | | | |
| 0.01% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% | 0.00% |
| 99.97% | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 99.98% | 0.02% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% |
| 0.02% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.01% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 31 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | T | C | A | T | C | T | T | A | G | T |
| **pNMG-156 (pCMV_ecTadA_XTEN_nCas9_GGS_UGI_GGS_NLS ; D108N)** | | | | | | | | | | | |
| **A** | 28 | 15 | 20 | 329861 | 9 | 17 | 15 | 11 | 332253 | 24 | 18 |
| **C** | 8 | 30 | 333028 | 21 | 25 | 331479 | 28 | 41 | 19 | 9 | 36 |
| **G** | 331561 | 15 | 5 | 83 | 1 | 21 | 6 | 4 | 59 | 332016 | 3 |
| **T** | 25 | 332617 | 59 | 27 | 332045 | 182 | 333663 | 334500 | 17 | 29 | 333258 |
| **N** | 4273 | 3233 | 2801 | 5921 | 3833 | 4219 | 2206 | 1362 | 3613 | 3885 | 2648 |
| | 331622 | 332677 | 333112 | 329992 | 332080 | 331699 | 333712 | 334556 | 332348 | 332078 | 333315 |
| **A** | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| **C** | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 99.9% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **G** | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% |
| **T** | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.1% | 100.0% | 100.0% | 0.0% | 0.0% | 100.0% |
| **pNMG-160 (pCMV_ecTadA_XTEN_nCas9_GGS_AAG*(E125Q)_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 23 | 11 | 13 | 364613 | 6 | 28 | 20 | 4 | 367143 | 18 | 27 |
| **C** | 8 | 36 | 368006 | 19 | 25 | 365576 | 27 | 36 | 15 | 5 | 33 |
| **G** | 366465 | 16 | 2 | 92 | 6 | 76 | 6 | 0 | 30 | 366910 | 1 |
| **T** | 32 | 367547 | 69 | 29 | 367040 | 61 | 368713 | 369665 | 16 | 37 | 368478 |
| **N** | 4711 | 3638 | 3159 | 6496 | 4172 | 5508 | 2486 | 1547 | 4053 | 4288 | 2724 |
| | 366528 | 367610 | 368090 | 364753 | 367077 | 365741 | 368766 | 369705 | 367204 | 366970 | 368539 |
| **A** | 0.01% | 0.00% | 0.00% | 99.96% | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% |
| **C** | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 99.95% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| **G** | 99.98% | 0.00% | 0.00% | 0.03% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| **T** | 0.01% | 99.98% | 0.02% | 0.01% | 99.99% | 0.02% | 99.99% | 99.99% | 0.00% | 0.01% | 99.98% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 31 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | A | G | G | A | C | C | T | G | A | G | G |
| 36 | 331414 | 9 | 11 | 329119 | 12 | 23 | 17 | 46 | 327929 | 13 | 4 |
| 333140 | 22 | 25 | 52 | 25 | 332630 | 330047 | 55 | 5 | 34 | 4 | 1 |
| 4 | 25 | 3 | 3 | 21 | 2 | 3 | 5 | 333730 | 28 | 331688 | 331961 |
| 69 | 17 | 334696 | 334823 | 25 | 43 | 30 | 332312 | 31 | 32 | 24 | 24 |
| 2714 | 4485 | 1235 | 1079 | 6788 | 3292 | 5875 | 3595 | 2180 | 7973 | 4267 | 4006 |
| 333249 | 331478 | 334733 | 334889 | 329190 | 332687 | 330103 | 332389 | 333812 | 328023 | 331729 | 331990 |
| 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% |
| 0.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 19 | 366009 | 9 | 12 | 363740 | 14 | 22 | 15 | 39 | 362059 | 13 | 14 |
| 368256 | 21 | 22 | 35 | 26 | 367497 | 364865 | 67 | 9 | 37 | 9 | 5 |
| 2 | 18 | 2 | 2 | 21 | 2 | 3 | 12 | 368841 | 30 | 366336 | 366847 |
| 73 | 34 | 369822 | 369964 | 29 | 25 | 18 | 367293 | 39 | 43 | 32 | 23 |
| 2914 | 5182 | 1409 | 1251 | 7448 | 3726 | 6355 | 3876 | 2335 | 9096 | 4875 | 4376 |
| 368350 | 366082 | 369855 | 370013 | 363816 | 367538 | 364908 | 367387 | 368928 | 362169 | 366390 | 366889 |
| 0.01% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 100.0% | 0.0% | 0.0% |
| 99.97% | 0.01% | 0.01% | 0.01% | 0.01% | 99.99% | 99.99% | 0.02% | 0.00% | 0.0% | 0.0% | 0.0% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.0% | 100.0% | 100.0% |
| 0.02% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | 99.97% | 0.01% | 0.0% | 0.0% | 0.0% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 31 (Continued)

**pNMG-161 (pCMV_ecTadA_XTEN_Cas9n_GGS_EndoV*(D35A)_NLS; D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 17 | 15 | 15 | 374628 | 13 | 14 | 18 | 13 | 377147 | 19 | 26 |
| **C** | 5 | 44 | 378052 | 27 | 30 | 375859 | 28 | 28 | 20 | 12 | 35 |
| **G** | 376676 | 20 | 2 | 40 | 3 | 43 | 12 | 1 | 41 | 376944 | 1 |
| **T** | 33 | 377455 | 61 | 26 | 377066 | 53 | 378884 | 379845 | 13 | 35 | 378560 |
| **N** | 4610 | 3820 | 3225 | 6634 | 4244 | 5387 | 2415 | 1473 | 4145 | 4357 | 2747 |
| | 376731 | 377534 | 378130 | 374721 | 377112 | 375969 | 378942 | 379887 | 377221 | 377010 | 378622 |
| | | | | | | | | | | | |
| **A** | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.01% |
| **C** | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| **G** | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| **T** | 0.01% | 99.98% | 0.02% | 0.01% | 99.99% | 0.01% | 99.98% | 99.99% | 0.00% | 0.01% | 99.98% |

**pNMG-162 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 23 | 18 | 9 | 394479 | 8 | 30 | 27 | 12 | 397296 | 29 | 33 |
| **C** | 5 | 32 | 398416 | 24 | 40 | 395742 | 25 | 35 | 19 | 6 | 38 |
| **G** | 396870 | 15 | 1 | 282 | 1 | 187 | 4 | 2 | 128 | 397368 | 2 |
| **T** | 18 | 398023 | 63 | 38 | 397411 | 112 | 399311 | 400272 | 17 | 37 | 398970 |
| **N** | 5027 | 3871 | 3470 | 7136 | 4507 | 5897 | 2601 | 1649 | 4528 | 4548 | 2949 |
| | 396916 | 398088 | 398489 | 394823 | 397460 | 396071 | 399367 | 400321 | 397460 | 397440 | 399043 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.00% | 0.00% | 99.91% | 0.00% | 0.01% | 0.01% | 0.00% | 99.96% | 0.01% | 0.01% |
| **C** | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 99.92% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| **G** | 99.99% | 0.00% | 0.00% | 0.07% | 0.00% | 0.05% | 0.00% | 0.00% | 0.03% | 99.98% | 0.00% |
| **T** | 0.00% | 99.98% | 0.02% | 0.01% | 99.99% | 0.03% | 99.99% | 99.99% | 0.00% | 0.01% | 99.98% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 31 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 376187 | 10 | 17 | 373797 | 15 | 15 | 11 | 37 | 372601 | 13 | 10 |
| 378392 | 24 | 31 | 39 | 26 | 377648 | 374848 | 76 | 6 | 37 | 14 | 7 |
| 2 | 14 | 6 | 6 | 35 | 1 | 4 | 4 | 378834 | 23 | 376483 | 376948 |
| 66 | 24 | 379955 | 380058 | 27 | 35 | 26 | 377354 | 32 | 30 | 25 | 25 |
| 2898 | 5120 | 1368 | 1251 | 7488 | 3674 | 6478 | 3926 | 2462 | 8680 | 4836 | 4381 |
| 378471 | 376249 | 380002 | 380120 | 373885 | 377699 | 374893 | 377445 | 378909 | 372691 | 376535 | 376990 |
| 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| 99.98% | 0.01% | 0.01% | 0.01% | 0.01% | 99.99% | 99.99% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% |
| 0.02% | 0.01% | 99.99% | 99.98% | 0.01% | 0.01% | 0.01% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 14 | 396398 | 14 | 11 | 393617 | 18 | 21 | 19 | 49 | 392028 | 13 | 8 |
| 398814 | 19 | 24 | 41 | 27 | 397812 | 394879 | 78 | 5 | 29 | 5 | 4 |
| 6 | 21 | 1 | 3 | 27 | 4 | 2 | 2 | 399284 | 33 | 396706 | 397281 |
| 73 | 24 | 400436 | 400636 | 26 | 46 | 15 | 397618 | 28 | 44 | 34 | 16 |
| 3091 | 5536 | 1526 | 1310 | 8304 | 4123 | 7087 | 4291 | 2643 | 9875 | 5251 | 4700 |
| 398907 | 396462 | 400475 | 400691 | 393697 | 397880 | 394917 | 397717 | 399366 | 392134 | 396758 | 397309 |
| 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% |
| 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 99.99% | 0.02% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% |
| 0.02% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 0.01% | 0.00% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 32

| | 1<br>G | 2<br>G | 3<br>A | 4<br>A | 5<br>T | 6<br>C | 7<br>C | 8<br>C | 9<br>T | 10<br>T | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **BE3** | | | | | | | | | | | |
| **A** | 55 | 92 | 287505 | 286425 | 105 | 5170 | 6416 | 5871 | 75 | 86 | 1711 |
| **C** | 4 | 66 | 19 | 97 | 30 | 145084 | 163065 | 166647 | 46 | 16 | 231537 |
| **G** | 289846 | 291486 | 25 | 31 | 16 | 3045 | 2617 | 3132 | 17 | 4 | 805 |
| **T** | 25 | 36 | 37 | 37 | 293424 | 138058 | 117557 | 114867 | 289274 | 292628 | 56252 |
| **N** | 6419 | 4690 | 8778 | 9774 | 2798 | 5000 | 6701 | 5828 | 6936 | 3622 | 6041 |
| | 289930 | 291680 | 287586 | 286590 | 293575 | 291357 | 289655 | 290517 | 289412 | 292734 | 290305 |
| **A** | 0.02% | 0.03% | 99.97% | 99.94% | 0.04% | 1.77% | 2.22% | 2.02% | 0.03% | 0.03% | 0.59% |
| **C** | 0.00% | 0.02% | 0.01% | 0.03% | 0.01% | 49.80% | 56.30% | 57.36% | 0.02% | 0.01% | 79.76% |
| **G** | 99.97% | 99.93% | 0.01% | 0.01% | 0.01% | 1.05% | 0.90% | 1.08% | 0.01% | 0.00% | 0.28% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 99.95% | 47.38% | 40.59% | 39.54% | 99.95% | 99.96% | 19.38% |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | | | |
| **A** | 35 | 15 | 357734 | 356419 | 9 | 56 | 12 | 18 | 13 | 20 | 10 |
| **C** | 5 | 1 | 6 | 19 | 38 | 357564 | 354727 | 360113 | 17 | 20 | 360704 |
| **G** | 356934 | 358972 | 37 | 30 | 2 | 112 | 6 | 12 | 1 | 0 | 8 |
| **T** | 14 | 20 | 25 | 21 | 363565 | 76 | 39 | 27 | 357736 | 362759 | 33 |
| **N** | 9918 | 7898 | 9106 | 10419 | 3298 | 9104 | 12128 | 6742 | 9142 | 4110 | 6154 |
| | 356988 | 359008 | 357802 | 356489 | 363614 | 357808 | 354784 | 360170 | 357767 | 362799 | 360755 |
| **A** | 0.01% | 0.00% | 99.98% | 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.93% | 99.98% | 99.98% | 0.00% | 0.01% | 99.99% |
| **G** | 99.98% | 99.99% | 0.01% | 0.01% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.02% | 0.01% | 0.01% | 99.99% | 99.99% | 0.01% |

# FIGURE 32 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | A | G | C | A | C | C | T | G | G |
| 21 | 27 | 24 | 292794 | 22 | 18 | 290011 | 10 | 17 | 15 | 36 | 43 |
| 47 | 30 | 292652 | 40 | 3 | 291757 | 20 | 290468 | 290302 | 67 | 2 | 8 |
| 80 | 290093 | 1 | 27 | 292964 | 1 | 26 | 4 | 0 | 7 | 290974 | 290142 |
| 292432 | 102 | 301 | 26 | 15 | 43 | 26 | 49 | 13 | 292472 | 12 | 7 |
| 3776 | 6102 | 3383 | 3465 | 3342 | 4527 | 6262 | 5798 | 5981 | 3751 | 5282 | 6106 |
| 292580 | 290252 | 292978 | 292887 | 293004 | 291819 | 290083 | 290531 | 290332 | 292561 | 291024 | 290200 |
| 0.01% | 0.01% | 0.01% | 99.97% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| 0.02% | 0.01% | 99.89% | 0.01% | 0.00% | 99.98% | 0.01% | 99.98% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.03% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 99.98% |
| 99.95% | 0.04% | 0.10% | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 99.97% | 0.00% | 0.00% |
| 8 | 13 | 2 | 362889 | 22 | 3 | 358932 | 0 | 36 | 15 | 26 | 19 |
| 47 | 2 | 362930 | 24 | 6 | 361655 | 22 | 359974 | 359659 | 80 | 5 | 9 |
| 9 | 360551 | 6 | 28 | 363060 | 1 | 19 | 3 | 2 | 4 | 360520 | 359317 |
| 362186 | 21 | 63 | 4 | 9 | 22 | 21 | 25 | 22 | 362388 | 20 | 12 |
| 4659 | 6328 | 3914 | 3961 | 3820 | 5235 | 7926 | 6917 | 7207 | 4439 | 6355 | 7566 |
| 362250 | 360587 | 363001 | 362945 | 363097 | 361681 | 358994 | 360002 | 359719 | 362487 | 360571 | 359357 |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 99.98% | 0.02% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 99.97% | 0.01% | 0.00% |



SUBSTITUTE SHEET (RULE 26)

FIGURE 32 (Continued)

| pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 34 | 14 | 285275 | 284019 | 7 | 44 | 26 | 9 | 15 | 12 | 5 | |
| C | 4 | 3 | 9 | 16 | 29 | 284580 | 282585 | 286899 | 17 | 17 | 287604 | |
| G | 284298 | 286180 | 41 | 65 | 0 | 372 | 23 | 12 | 1 | 7 | 19 | |
| T | 10 | 12 | 12 | 14 | 289926 | 204 | 69 | 56 | 285096 | 289146 | 31 | |
| N | 8371 | 6508 | 7382 | 8605 | 2755 | 7517 | 10014 | 5741 | 7588 | 3531 | 5054 | |
| | 284346 | 286209 | 285337 | 284114 | 289962 | 285200 | 282703 | 286976 | 285129 | 289182 | 287659 | |
| A | 0.01% | 0.00% | 99.98% | 99.97% | 0.00% | 0.02% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.78% | 99.96% | 99.97% | 0.01% | 0.01% | 99.98% | |
| G | 99.98% | 99.99% | 0.01% | 0.02% | 0.00% | 0.13% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.07% | 0.02% | 0.02% | 99.99% | 99.99% | 0.01% | |
| pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N) | | | | | | | | | | | | |
| A | 40 | 37 | 351183 | 350036 | 13 | 121 | 55 | 23 | 12 | 5 | 18 | |
| C | 3 | 19 | 12 | 26 | 27 | 350780 | 348646 | 353888 | 25 | 18 | 354622 | |
| G | 350792 | 352780 | 70 | 96 | 0 | 514 | 3 | 90 | 1 | 0 | 23 | |
| T | 13 | 20 | 19 | 14 | 357315 | 323 | 163 | 134 | 351653 | 356678 | 68 | |
| N | 9852 | 7844 | 9416 | 10529 | 3345 | 8962 | 11833 | 6565 | 9009 | 3999 | 5969 | |
| | 350848 | 352856 | 351284 | 350172 | 357355 | 351738 | 348867 | 354135 | 351691 | 356701 | 354731 | |
| A | 0.01% | 0.01% | 99.97% | 99.96% | 0.00% | 0.03% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | |
| C | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.73% | 99.94% | 99.93% | 0.01% | 0.01% | 99.97% | |
| G | 99.98% | 99.98% | 0.02% | 0.03% | 0.00% | 0.15% | 0.00% | 0.03% | 0.00% | 0.00% | 0.01% | |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 99.99% | 0.09% | 0.05% | 0.04% | 99.99% | 99.99% | 0.02% | |

FIGURE 32 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 17 | 7 | 289405 | 24 | 1 | 286302 | 1 | 32 | 12 | 13 | 22 | |
| 32 | 2 | 289469 | 20 | 5 | 288367 | 13 | 287084 | 286724 | 58 | 4 | 6 | |
| 2 | 287564 | 3 | 25 | 289529 | 2 | 16 | 0 | 0 | 4 | 287315 | 286360 | |
| 288883 | 10 | 72 | 5 | 15 | 14 | 15 | 16 | 11 | 289014 | 10 | 11 | |
| 3787 | 5120 | 3162 | 3258 | 3140 | 4329 | 6367 | 5608 | 5942 | 3621 | 5366 | 6308 | |
| 288926 | 287593 | 289551 | 289455 | 289573 | 288384 | 286346 | 287101 | 286767 | 289088 | 287342 | 286399 | |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | |
| 0.01% | 0.00% | 99.97% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% | |
| 0.00% | 99.99% | 0.00% | 0.02% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% | |
| 99.99% | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | |
| 16 | 32 | 47 | 356757 | 46 | 2 | 353081 | 5 | 26 | 48 | 22 | 25 | |
| 44 | 33 | 356825 | 28 | 3 | 355600 | 17 | 353743 | 353563 | 79 | 7 | 8 | |
| 1 | 354333 | 10 | 32 | 356752 | 28 | 21 | 0 | 1 | 2 | 354411 | 352972 | |
| 356123 | 36 | 59 | 8 | 17 | 21 | 19 | 17 | 25 | 356026 | 44 | 35 | |
| 4516 | 6266 | 3756 | 3872 | 3879 | 5046 | 7558 | 6915 | 7091 | 4550 | 6207 | 7645 | |
| 356184 | 354434 | 356941 | 356825 | 356818 | 355651 | 353138 | 353765 | 353615 | 356155 | 354484 | 353040 | |
| 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | |
| 0.01% | 0.01% | 99.97% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% | |
| 0.00% | 99.97% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 99.98% | |
| 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 99.96% | 0.01% | 0.01% | |

# Figure 32 (Continued)

| | 1<br>G | 2<br>G | 3<br>A | 4<br>A | 5<br>T | 6<br>C | 7<br>C | 8<br>C | 9<br>T | 10<br>T | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-156 (pCMV_ecTadA_XTEN_nCas9_GGS_UGI_GGS_NLS ; D108N)** | | | | | | | | | | | |
| **A** | 43 | 13 | 299252 | 297920 | 7 | 81 | 11 | 24 | 10 | 17 | 29 |
| **C** | 4 | 5 | 6 | 18 | 26 | 298168 | 296547 | 301020 | 22 | 18 | 301720 |
| **G** | 298626 | 300371 | 71 | 173 | 0 | 266 | 3 | 3 | 2 | 0 | 1 |
| **T** | 16 | 15 | 14 | 25 | 304037 | 777 | 179 | 109 | 299141 | 303434 | 50 |
| **N** | 8229 | 6514 | 7577 | 8783 | 2849 | 7627 | 10179 | 5763 | 7744 | 3450 | 5118 |
| | 298689 | 300404 | 299343 | 298136 | 304070 | 299292 | 296740 | 301156 | 299175 | 303469 | 301800 |
| **A** | 0.0% | 0.0% | 100.0% | 99.9% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **C** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.6% | 99.9% | 100.0% | 0.0% | 0.0% | 100.0% |
| **G** | 100.0% | 100.0% | 0.0% | 0.1% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.3% | 0.1% | 0.0% | 100.0% | 100.0% | 0.0% |
| **pNMG-160 (pCMV_ecTadA_XTEN_nCas9_GGS_AAG*(E125Q)_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 27 | 16 | 309745 | 308518 | 25 | 60 | 10 | 30 | 9 | 21 | 8 |
| **C** | 2 | 5 | 7 | 23 | 30 | 309303 | 307210 | 311732 | 21 | 15 | 312437 |
| **G** | 309129 | 310940 | 29 | 105 | 0 | 187 | 5 | 4 | 3 | 0 | 2 |
| **T** | 13 | 22 | 28 | 11 | 314880 | 157 | 53 | 45 | 309727 | 314144 | 37 |
| **N** | 8522 | 6710 | 7886 | 9038 | 2760 | 7988 | 10417 | 5884 | 7935 | 3515 | 5211 |
| | 309171 | 310983 | 309809 | 308657 | 314935 | 309707 | 307278 | 311811 | 309760 | 314180 | 312484 |
| **A** | 0.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **C** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.9% | 100.0% | 100.0% | 0.0% | 0.0% | 100.0% |
| **G** | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.1% | 0.0% | 0.0% | 100.0% | 100.0% | 0.0% |

Figure 32 (Continued)

| 12 T | 13 G | 14 C | 15 A | 16 G | 17 C | 18 A | 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 19 | 4 | 303540 | 14 | 1 | 300325 | 0 | 48 | 14 | 18 | 17 |
| 30 | 4 | 303621 | 26 | 6 | 302498 | 19 | 300949 | 300637 | 67 | 3 | 5 |
| 3 | 301427 | 13 | 27 | 303620 | 1 | 25 | 0 | 1 | 5 | 301403 | 300251 |
| 302962 | 9 | 67 | 10 | 6 | 14 | 20 | 31 | 13 | 302935 | 10 | 7 |
| 3911 | 5459 | 3213 | 3315 | 3272 | 4404 | 6520 | 5929 | 6210 | 3888 | 5474 | 6627 |
| 303007 | 301459 | 303705 | 303603 | 303646 | 302514 | 300389 | 300980 | 300699 | 303021 | 301434 | 300280 |
| 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 100.0% |
| 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| 8 | 18 | 1 | 314350 | 21 | 6 | 311032 | 0 | 28 | 17 | 20 | 20 |
| 33 | 7 | 314464 | 28 | 3 | 313214 | 19 | 311572 | 311420 | 57 | 4 | 7 |
| 4 | 312266 | 8 | 30 | 314313 | 1 | 15 | 4 | 1 | 4 | 312333 | 311031 |
| 313601 | 15 | 50 | 4 | 10 | 14 | 28 | 12 | 10 | 313784 | 14 | 9 |
| 4049 | 5389 | 3172 | 3283 | 3348 | 4460 | 6601 | 6107 | 6236 | 3822 | 5311 | 6613 |
| 313646 | 312306 | 314523 | 314412 | 314347 | 313235 | 311094 | 311588 | 311459 | 313862 | 312371 | 311067 |
| 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 100.0% |
| 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |

# Figure 32 (Continued)

**pNMG-161 (pCMV_ecTadA_XTEN_Cas9n_GGS_EndoV*(D35A)_NLS; D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 30 | 26 | 337530 | 336244 | 5 | 92 | 51 | 25 | 17 | 15 | 8 |
| **C** | 7 | 5 | 6 | 30 | 35 | 337155 | 334855 | 339695 | 23 | 11 | 340349 |
| **G** | 336802 | 338645 | 51 | 85 | 1 | 214 | 28 | 28 | 4 | 2 | 2 |
| **T** | 18 | 39 | 15 | 10 | 342845 | 267 | 92 | 96 | 337519 | 342118 | 37 |
| **N** | 9192 | 7334 | 8447 | 9680 | 3162 | 8320 | 11022 | 6204 | 8485 | 3902 | 5652 |
| | 336857 | 338715 | 337602 | 336369 | 342886 | 337728 | 335026 | 339844 | 337563 | 342146 | 340396 |
| **A** | 0.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **C** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.8% | 99.9% | 100.0% | 0.0% | 0.0% | 100.0% |
| **G** | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.1% | 0.0% | 0.0% | 100.0% | 100.0% | 0.0% |

**pNMG-162 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 44 | 20 | 355918 | 354215 | 16 | 199 | 30 | 12 | 21 | 16 | 15 |
| **C** | 10 | 7 | 11 | 28 | 29 | 354942 | 353108 | 358292 | 29 | 15 | 359039 |
| **G** | 355397 | 357450 | 140 | 328 | 2 | 632 | 7 | 41 | 6 | 1 | 2 |
| **T** | 17 | 23 | 23 | 12 | 361861 | 345 | 157 | 132 | 356059 | 361029 | 51 |
| **N** | 9710 | 7678 | 9086 | 10595 | 3266 | 9056 | 11872 | 6697 | 9059 | 4113 | 6067 |
| | 355468 | 357500 | 356092 | 354583 | 361908 | 356118 | 353302 | 358477 | 356115 | 361061 | 359107 |
| **A** | 0.0% | 0.0% | 100.0% | 99.9% | 0.0% | 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **C** | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 99.7% | 99.9% | 99.9% | 0.0% | 0.0% | 100.0% |
| **G** | 100.0% | 100.0% | 0.0% | 0.1% | 0.0% | 0.2% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| **T** | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.1% | 0.0% | 0.0% | 100.0% | 100.0% | 0.0% |

**pNMG-163 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 37 | 23 | 332065 | 331652 | 14 | 139 | 42 | 3 | 20 | 10 | 19 |

# Figure 32 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 8 | 25 | 2 | 342228 | 22 | 1 | 338855 | 7 | 19 | 14 | 18 | 14 |
| 40 | 6 | 342443 | 34 | 5 | 341194 | 27 | 339566 | 339295 | 63 | 4 | 7 |
| 3 | 340169 | 7 | 34 | 342352 | 0 | 17 | 2 | 1 | 9 | 340040 | 338855 |
| 341709 | 13 | 55 | 8 | 10 | 11 | 22 | 19 | 30 | 341860 | 14 | 15 |
| 4288 | 5835 | 3542 | 3745 | 3661 | 4844 | 7129 | 6456 | 6716 | 4115 | 5984 | 7166 |
| 341760 | 340213 | 342507 | 342304 | 342389 | 341206 | 338921 | 339594 | 339345 | 341946 | 340076 | 338891 |
| 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 100.0% |
| 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| 8 | 29 | 5 | 361095 | 22 | 6 | 357397 | 4 | 29 | 19 | 35 | 33 |
| 52 | 8 | 361287 | 29 | 5 | 359995 | 20 | 358246 | 357878 | 78 | 3 | 6 |
| 0 | 358713 | 6 | 47 | 361298 | 1 | 14 | 0 | 3 | 7 | 358914 | 357551 |
| 360576 | 24 | 80 | 7 | 19 | 14 | 20 | 17 | 35 | 360522 | 17 | 18 |
| 4538 | 6401 | 3795 | 3995 | 3829 | 5157 | 7722 | 6906 | 7227 | 4546 | 6203 | 7563 |
| 360636 | 358774 | 361378 | 361178 | 361344 | 360016 | 357451 | 358267 | 357945 | 360626 | 358969 | 357608 |
| 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 100.0% |
| 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |
| 7 | 20 | 7 | 338032 | 16 | 19 | 335012 | 1 | 20 | 18 | 19 | 43 |

Figure 32 (Continued)

| | 1 G | 2 G | 3 A | 4 A | 5 T | 6 C | 7 C | 8 C | 9 T | 10 T | 11 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 1 | 4 | 6 | 10 | 21 | 331885 | 329195 | 334440 | 23 | 19 | 334995 |
| G | 332202 | 333841 | 37 | 133 | 1 | 165 | 5 | 0 | 5 | 1 | 33 |
| T | 13 | 17 | 12 | 7 | 337903 | 128 | 60 | 32 | 331760 | 336613 | 33 |
| N | 8654 | 7022 | 8788 | 9106 | 2968 | 8590 | 11605 | 6432 | 9082 | 4247 | 5810 |
| | 332253 | 333885 | 332120 | 331802 | 337939 | 332317 | 329302 | 334475 | 331808 | 336643 | 335080 |
| A | 0.01% | 0.01% | 99.98% | 99.95% | 0.00% | 0.04% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.87% | 99.97% | 99.99% | 0.01% | 0.01% | 99.97% |
| G | 99.98% | 99.99% | 0.01% | 0.04% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| T | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.04% | 0.02% | 0.01% | 99.99% | 99.99% | 0.01% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 44 | 15 | 309428 | 309041 | 12 | 45 | 5 | 37 | 19 | 14 | 3 |
| C | 3 | 4 | 10 | 23 | 20 | 309038 | 306799 | 311702 | 32 | 16 | 312771 |
| G | 310020 | 311674 | 64 | 401 | 0 | 453 | 3 | 45 | 1 | 2 | 4 |
| T | 20 | 26 | 22 | 15 | 315134 | 369 | 173 | 137 | 309458 | 313932 | 3 |
| N | 7851 | 6219 | 8414 | 8458 | 2771 | 8032 | 10957 | 6016 | 8427 | 3973 | 5156 |
| | 310087 | 311719 | 309524 | 309480 | 315166 | 309905 | 306980 | 311921 | 309510 | 313964 | 312781 |
| A | 0.01% | 0.00% | 99.97% | 99.86% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.72% | 99.94% | 99.93% | 0.01% | 0.01% | 100.00 % |
| G | 99.98% | 99.99% | 0.02% | 0.13% | 0.00% | 0.15% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.12% | 0.06% | 0.04% | 99.98% | 99.99% | 0.00% |

Figure 32 (Continued)

| 12 T | 13 G | 14 C | 15 A | 16 G | 17 C | 18 A | 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 9 | 337578 | 14 | 2 | 336084 | 12 | 334611 | 333414 | 64 | 2 | 5 |
| 0 | 335123 | 4 | 33 | 336617 | 2 | 14 | 2 | 3 | 6 | 333632 | 331332 |
| 336360 | 11 | 48 | 3 | 9 | 18 | 15 | 16 | 20 | 336203 | 5 | 10 |
| 4490 | 5727 | 3253 | 2807 | 4245 | 4766 | 5836 | 6259 | 7432 | 4598 | 7231 | 9495 |
| 336400 | 335163 | 337637 | 338082 | 336644 | 336123 | 335053 | 334630 | 333457 | 336291 | 333658 | 331390 |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.98% |
| 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% |
| 13 | 15 | 6 | 315241 | 23 | 20 | 312625 | 9 | 33 | 16 | 22 | 20 |
| 28 | 9 | 314933 | 23 | 2 | 313576 | 16 | 312103 | 311220 | 80 | 5 | 7 |
| 3 | 312717 | 2 | 51 | 313962 | 2 | 26 | 1 | 2 | 1 | 311304 | 309078 |
| 313763 | 58 | 47 | 7 | 6 | 10 | 19 | 14 | 8 | 313699 | 6 | 18 |
| 4131 | 5139 | 2950 | 2616 | 3945 | 4328 | 5250 | 5809 | 6673 | 4140 | 6597 | 8804 |
| 313807 | 312799 | 314988 | 315322 | 313993 | 313608 | 312686 | 312127 | 311263 | 313796 | 311337 | 309123 |
| 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 99.99% | 0.03% | 0.00% | 0.00% |
| 0.00% | 99.97% | 0.00% | 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 99.99% | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 99.97% | 0.00% | 0.01% |

# Figure 32 (Continued)

**pNMG-165 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 49 | 27 | 388084 | 387748 | 16 | 150 | 51 | 39 | 23 | 26 | 6 |
| **C** | 7 | 4 | 3 | 15 | 24 | 387353 | 384940 | 390836 | 22 | 26 | 391721 |
| **G** | 388227 | 390304 | 93 | 87 | 1 | 652 | 12 | 21 | 2 | 1 | 12 |
| **T** | 17 | 29 | 30 | 13 | 394936 | 310 | 44 | 48 | 387855 | 393289 | 17 |
| **N** | 10039 | 7975 | 10131 | 10478 | 3364 | 9875 | 13293 | 7392 | 10434 | 4994 | 6580 |
| | 388300 | 390364 | 388210 | 387863 | 394977 | 388465 | 385047 | 390944 | 387902 | 393342 | 391756 |
| **A** | 0.01% | 0.01% | 99.97% | 99.97% | 0.00% | 0.04% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.71% | 99.97% | 99.97% | 0.01% | 0.01% | 99.99% |
| **G** | 99.98% | 99.98% | 0.02% | 0.02% | 0.00% | 0.17% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.01% | 0.01% | 0.00% | 99.99% | 0.08% | 0.01% | 0.01% | 99.99% | 99.99% | 0.00% |

**untreated cells**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 46 | 26 | 385153 | 384786 | 17 | 8 | 22 | 37 | 18 | 21 | 5 |
| **C** | 3 | 5 | 6 | 16 | 41 | 385538 | 381974 | 387854 | 25 | 23 | 389029 |
| **G** | 385378 | 387417 | 37 | 44 | 0 | 11 | 7 | 7 | 2 | 0 | 1 |
| **T** | 8 | 12 | 21 | 17 | 391982 | 46 | 36 | 24 | 384993 | 390444 | 8 |
| **N** | 10040 | 8015 | 10258 | 10612 | 3433 | 9870 | 13434 | 7551 | 10435 | 4985 | 6430 |
| | 385435 | 387460 | 385217 | 384863 | 392040 | 385603 | 382039 | 387922 | 385038 | 390488 | 389043 |
| **A** | 0.01% | 0.01% | 99.98% | 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.98% | 99.98% | 0.01% | 0.01% | 100.00% |
| **G** | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 99.99% | 99.99% | 0.00% |

Figure 32 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24 | 27 | 5 | 394828 | 16 | 9 | 391834 | 7 | 29 | 25 | 35 | 28 | |
| 40 | 5 | 394425 | 19 | 3 | 392915 | 18 | 391128 | 389950 | 92 | 10 | 13 | |
| 3 | 391842 | 11 | 42 | 393441 | 1 | 18 | 10 | 4 | 1 | 389775 | 387438 | |
| 393250 | 17 | 67 | 6 | 12 | 27 | 18 | 28 | 28 | 393005 | 28 | 12 | |
| 5019 | 6469 | 3852 | 3463 | 4886 | 5406 | 6470 | 7185 | 8352 | 5238 | 8513 | 10869 | |
| 393317 | 391891 | 394508 | 394895 | 393472 | 392952 | 391888 | 391173 | 390011 | 393123 | 389848 | 387491 | |
| 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | |
| 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.98% | 0.02% | 0.00% | 0.00% | |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% | |
| 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.97% | 0.01% | 0.00% | |
| 18 | 24 | 15 | 392023 | 22 | 8 | 388815 | 1 | 39 | 27 | 31 | 30 | |
| 46 | 1 | 391518 | 21 | 0 | 389879 | 14 | 388256 | 387007 | 84 | 4 | 6 | |
| 2 | 388912 | 6 | 42 | 390481 | 8 | 21 | 1 | 1 | 6 | 386894 | 384419 | |
| 390388 | 11 | 66 | 5 | 8 | 14 | 16 | 16 | 11 | 390034 | 32 | 7 | |
| 5019 | 6525 | 3868 | 3382 | 4962 | 5564 | 6607 | 7198 | 8414 | 5321 | 8511 | 11006 | |
| 390454 | 388948 | 391605 | 392091 | 390511 | 389909 | 388866 | 388274 | 387058 | 390151 | 386961 | 384462 | |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 100.00 % | 99.99% | 0.02% | 0.00% | 0.00% | |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% | |
| 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 0.00% | |

FIGURE 33

**BE3**

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 112 | 159022 | 23 | 28 | 3825 | 1510 | 47 | 150753 | 28 | 2 | 169262 |
| C | 2 | 6 | 21 | 11 | 77206 | 86813 | 3 | 2 | 17 | 170270 | 4 |
| G | 181825 | 26 | 178307 | 107 | 9040 | 4939 | 155968 | 47 | 170669 | 1 | 21 |
| T | 20 | 19 | 40 | 160683 | 84197 | 77888 | 10 | 6 | 7 | 102 | 12 |
| N | 5245 | 28134 | 8816 | 26375 | 12940 | 16050 | 31173 | 36393 | 16481 | 16827 | 17903 |
| | 181959 | 159073 | 178391 | 160829 | 174268 | 171150 | 156028 | 150808 | 170721 | 170375 | 169299 |
| A | 0.06% | 99.97% | 0.01% | 0.02% | 2.19% | 0.88% | 0.03% | 99.96% | 0.02% | 0.00% | 99.98% |
| C | 0.00% | 0.00% | 0.01% | 0.01% | 44.30% | 50.72% | 0.00% | 0.00% | 0.01% | 99.94% | 0.00% |
| G | 99.93% | 0.02% | 99.95% | 0.07% | 5.19% | 2.89% | 99.96% | 0.03% | 99.97% | 0.00% | 0.01% |
| T | 0.01% | 0.01% | 0.02% | 99.91% | 48.31% | 45.51% | 0.01% | 0.00% | 0.00% | 0.06% | 0.01% |

**pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 18 | 175183 | 8 | 11 | 3 | 7 | 21 | 153203 | 21 | 1 | 173492 |
| C | 1 | 8 | 1 | 23 | 184431 | 187125 | 5 | 9 | 3 | 174635 | 12 |
| G | 192808 | 32 | 189463 | 0 | 6 | 13 | 162130 | 17 | 174151 | 5 | 15 |
| T | 3 | 8 | 4 | 173116 | 123 | 163 | 12 | 9 | 5 | 12 | 6 |
| N | 5899 | 23499 | 9254 | 25580 | 14170 | 11425 | 36566 | 45496 | 24554 | 24081 | 25209 |
| | 192830 | 175231 | 189476 | 173150 | 184563 | 187308 | 162168 | 153238 | 174180 | 174653 | 173525 |
| A | 0.01% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.93% | 99.90% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% |
| G | 99.99% | 0.02% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.07% | 0.09% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |

FIGURE 33 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | 177122 | 179522 | 13 | 171310 | 175488 | 4 | 179341 | 175682 | 11 | 9 | 8 |
| 0 | 5 | 4 | 19 | 2 | 7 | 0 | 6 | 6 | 2 | 2 | 1 |
| 179167 | 13 | 18 | 181316 | 21 | 15 | 180703 | 22 | 14 | 180502 | 181657 | 175935 |
| 3 | 3 | 7 | 2 | 3 | 7 | 3 | 6 | 6 | 9 | 8 | 5 |
| 8018 | 10059 | 7651 | 5852 | 15866 | 11685 | 6492 | 7827 | 11494 | 6678 | 5526 | 11253 |
| 179184 | 177143 | 179551 | 181350 | 171336 | 175517 | 180710 | 179375 | 175708 | 180524 | 181676 | 175949 |
| 0.01% | 99.99% | 99.98% | 0.01% | 99.98% | 99.98% | 0.00% | 99.98% | 99.99% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 5 | 187437 | 190333 | 2 | 181468 | 185893 | 0 | 190196 | 186321 | 8 | 14 | 11 |
| 0 | 10 | 15 | 1 | 11 | 11 | 2 | 12 | 12 | 0 | 0 | 1 |
| 189831 | 13 | 19 | 192351 | 12 | 8 | 191551 | 16 | 14 | 191361 | 192414 | 186973 |
| 6 | 5 | 9 | 3 | 3 | 9 | 3 | 9 | 17 | 15 | 6 | 2 |
| 8892 | 11269 | 8358 | 6377 | 17240 | 12813 | 7178 | 8501 | 12370 | 7350 | 6300 | 11747 |
| 189842 | 187465 | 190376 | 192357 | 181494 | 185921 | 191556 | 190233 | 186364 | 191384 | 192434 | 186987 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.98% | 0.00% | 0.01% | 0.01% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 100.00% | 0.01% | 0.00% | 100.00% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |

FIGURE 33 (Continued)

**pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 12 | 122969 | 9 | 8 | 2 | 1 | 22 | 108246 | 13 | 0 | 121818 | |
| **C** | 1 | 1 | 0 | 14 | 129754 | 131679 | 1 | 3 | 1 | 122871 | 7 | |
| **G** | 135613 | 20 | 133207 | 1 | 2 | 1 | 114562 | 19 | 122437 | 1 | 18 | |
| **T** | 7 | 3 | 6 | 121325 | 88 | 118 | 8 | 5 | 4 | 16 | 6 | |
| **N** | 4117 | 16760 | 6531 | 18405 | 9911 | 7959 | 25165 | 31485 | 17303 | 16870 | 17909 | |
| | 135633 | 122993 | 133222 | 121348 | 129846 | 131799 | 114593 | 108273 | 122455 | 122888 | 121849 | |
| | | | | | | | | | | | | |
| **A** | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.02% | 99.98% | 0.01% | 0.00% | 99.97% | |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.93% | 99.91% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | |
| **G** | 99.99% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.97% | 0.02% | 99.99% | 0.00% | 0.01% | |
| **T** | 0.01% | 0.00% | 0.00% | 99.98% | 0.07% | 0.09% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | |

**pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 17 | 178992 | 8 | 10 | 23 | 4 | 26 | 155699 | 30 | 0 | 176493 | |
| **C** | 0 | 6 | 0 | 19 | 187594 | 190062 | 3 | 6 | 4 | 177222 | 7 | |
| **G** | 195560 | 45 | 192466 | 0 | 2 | 0 | 165150 | 17 | 177022 | 0 | 17 | |
| **T** | 8 | 9 | 10 | 176436 | 67 | 220 | 12 | 7 | 9 | 16 | 4 | |
| **N** | 6082 | 22622 | 9190 | 25209 | 13990 | 11392 | 36490 | 45952 | 24616 | 24443 | 25161 | |
| | 195585 | 179052 | 192484 | 176465 | 187686 | 190286 | 165191 | 155729 | 177065 | 177238 | 176521 | |
| | | | | | | | | | | | | |
| **A** | 0.01% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | 0.02% | 99.98% | 0.02% | 0.00% | 99.98% | |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.95% | 99.88% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | |
| **G** | 99.99% | 0.03% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | |
| **T** | 0.00% | 0.01% | 0.01% | 99.98% | 0.04% | 0.12% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | |

FIGURE 33 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 131801 | 134001 | 4 | 127507 | 130927 | 4 | 133929 | 130896 | 11 | 5 | 8 |
| 0 | 7 | 7 | 0 | 7 | 11 | 1 | 8 | 9 | 0 | 1 | 1 |
| 133533 | 9 | 11 | 135310 | 6 | 15 | 134709 | 9 | 11 | 134415 | 135284 | 131572 |
| 9 | 2 | 4 | 5 | 6 | 8 | 5 | 8 | 10 | 13 | 3 | 4 |
| 6210 | 7939 | 5735 | 4439 | 12232 | 8797 | 5039 | 5804 | 8832 | 5319 | 4465 | 8173 |
| 133548 | 131819 | 134023 | 135319 | 127526 | 130961 | 134719 | 133954 | 130926 | 134439 | 135293 | 131585 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.97% | 0.00% | 99.98% | 99.98% | 0.01% | 0.00% | 0.01% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| 5 | 190419 | 193197 | 6 | 184225 | 189032 | 5 | 193142 | 189493 | 9 | 13 | 15 |
| 4 | 7 | 7 | 1 | 5 | 6 | 1 | 6 | 10 | 1 | 1 | 2 |
| 192848 | 9 | 16 | 195265 | 13 | 20 | 194392 | 12 | 25 | 194309 | 195191 | 189747 |
| 7 | 2 | 9 | 7 | 4 | 11 | 7 | 16 | 13 | 17 | 5 | 4 |
| 8818 | 11245 | 8453 | 6403 | 17435 | 12613 | 7277 | 8506 | 12141 | 7346 | 6472 | 11914 |
| 192864 | 190437 | 193229 | 195279 | 184247 | 189069 | 194405 | 193176 | 189541 | 194336 | 195210 | 189768 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.97% | 0.00% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |

# FIGURE 33 (Continued)

| | 1<br>G | 2<br>A | 3<br>G | 4<br>T | 5<br>C | 6<br>C | 7<br>G | 8<br>A | 9<br>G | 10<br>C | 11<br>A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-156 (pCMV_ecTadA_XTEN_nCas9_GGS_UGI_GGS_NLS ; D108N)** | | | | | | | | | | | |
| **A** | 10 | 130267 | 6 | 7 | 0 | 20 | 23 | 114294 | 17 | 2 | 128364 |
| **C** | 1 | 5 | 0 | 9 | 136093 | 138053 | 0 | 4 | 1 | 129115 | 5 |
| **G** | 142326 | 29 | 139929 | 0 | 1 | 2 | 120411 | 6 | 129116 | 0 | 12 |
| **T** | 2 | 3 | 4 | 128214 | 236 | 446 | 9 | 8 | 3 | 10 | 5 |
| **N** | 4210 | 16246 | 6611 | 18320 | 10222 | 8033 | 26111 | 32242 | 17417 | 17428 | 18169 |
| | 142339 | 130304 | 139939 | 128230 | 136330 | 138521 | 120443 | 114312 | 129137 | 129127 | 128386 |
| **A** | 0.01% | 99.97% | 0.00% | 0.01% | 0.00% | 0.01% | 0.02% | 99.98% | 0.01% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.83% | 99.66% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.99% | 0.17% | 0.32% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |
| **pNMG-160 (pCMV_ecTadA_XTEN_nCas9_GGS_AAG*(E125Q)_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 24 | 161702 | 9 | 16 | 0 | 4 | 21 | 140665 | 29 | 0 | 158789 |
| **C** | 1 | 10 | 1 | 20 | 169241 | 171414 | 2 | 5 | 4 | 159895 | 16 |
| **G** | 176486 | 21 | 173742 | 0 | 25 | 36 | 148883 | 22 | 159775 | 0 | 15 |
| **T** | 2 | 8 | 7 | 159614 | 126 | 218 | 15 | 6 | 11 | 14 | 6 |
| **N** | 5398 | 20171 | 8153 | 22262 | 12526 | 10249 | 33002 | 41227 | 22106 | 22016 | 23099 |
| | 176513 | 161741 | 173759 | 159650 | 169392 | 171672 | 148921 | 140698 | 159819 | 159909 | 158826 |
| **A** | 0.0% | 100.0% | 0.0% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.02% | 0.00% | 99.98% |
| **C** | 0.0% | 0.0% | 0.0% | 0.01% | 99.91% | 99.85% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| **G** | 100.0% | 0.0% | 100.0% | 0.00% | 0.01% | 0.02% | 99.97% | 0.02% | 99.97% | 0.00% | 0.01% |
| **T** | 0.0% | 0.0% | 0.0% | 99.98% | 0.07% | 0.13% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% |



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FIGURE 33 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 138576 | 140563 | 4 | 134207 | 137420 | 5 | 140509 | 137820 | 8 | 6 | 10 |
| 0 | 5 | 7 | 1 | 8 | 5 | 0 | 6 | 9 | 1 | 2 | 1 |
| 140116 | 3 | 22 | 142075 | 8 | 15 | 141466 | 13 | 20 | 141190 | 141959 | 137993 |
| 3 | 3 | 7 | 1 | 2 | 5 | 2 | 13 | 7 | 19 | 3 | 2 |
| 6430 | 7968 | 5956 | 4474 | 12330 | 9110 | 5082 | 6014 | 8699 | 5337 | 4585 | 8549 |
| 140125 | 138587 | 140599 | 142081 | 134225 | 137445 | 141473 | 140541 | 137856 | 141218 | 141970 | 138006 |
| 0.00% | 99.99% | 99.97% | 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.97% | 0.01% | 0.00% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.02% | 100.00% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| 3 | 171720 | 174379 | 6 | 166380 | 170723 | 4 | 174360 | 171007 | 10 | 13 | 8 |
| 2 | 16 | 13 | 3 | 9 | 16 | 1 | 13 | 15 | 2 | 0 | 2 |
| 173911 | 5 | 9 | 176157 | 14 | 10 | 175558 | 5 | 21 | 175334 | 176187 | 171262 |
| 9 | 3 | 8 | 11 | 7 | 4 | 7 | 7 | 17 | 16 | 11 | 3 |
| 8000 | 10181 | 7516 | 5748 | 15515 | 11172 | 6355 | 7540 | 10865 | 6563 | 5714 | 10650 |
| 173925 | 171744 | 174409 | 176177 | 166410 | 170753 | 175570 | 174385 | 171060 | 175362 | 176211 | 171275 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.98% | 0.00% | 99.99% | 99.97% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |

# FIGURE 33 (Continued)

**pNMG-161 (pCMV_ecTadA_XTEN_Cas9n_GGS_EndoV*(D35A)_NLS; D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 12 | 148682 | 7 | 5 | 12 | 2 | 25 | 130511 | 18 | 0 | 147061 |
| **C** | 2 | 11 | 0 | 27 | 156162 | 158398 | 1 | 3 | 3 | 148110 | 3 |
| **G** | 163014 | 16 | 160282 | 0 | 2 | 32 | 137457 | 31 | 147680 | 1 | 11 |
| **T** | 5 | 2 | 3 | 146487 | 170 | 171 | 8 | 7 | 6 | 20 | 10 |
| **N** | 4802 | 19127 | 7546 | 21319 | 11494 | 9238 | 30352 | 37291 | 20136 | 19712 | 20758 |
| | 163033 | 148711 | 160292 | 146519 | 156346 | 158603 | 137491 | 130552 | 147707 | 148131 | 147085 |
| | | | | | | | | | | | |
| **A** | 0.0% | 100.0% | 0.0% | 0.00% | 0.01% | 0.00% | 0.02% | 99.97% | 0.01% | 0.00% | 99.98% |
| **C** | 0.0% | 0.0% | 0.0% | 0.02% | 99.88% | 99.87% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 100.0% | 0.0% | 100.0% | 0.00% | 0.00% | 0.02% | 99.98% | 0.02% | 99.98% | 0.00% | 0.01% |
| **T** | 0.0% | 0.0% | 0.0% | 99.98% | 0.11% | 0.11% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |

**pNMG-162 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 21 | 144119 | 8 | 13 | 0 | 2 | 31 | 126391 | 19 | 2 | 142410 |
| **C** | 2 | 10 | 3 | 18 | 151325 | 153554 | 1 | 1 | 0 | 143276 | 3 |
| **G** | 158249 | 15 | 155549 | 0 | 39 | 8 | 133306 | 60 | 143050 | 1 | 13 |
| **T** | 3 | 2 | 5 | 142107 | 253 | 272 | 9 | 5 | 4 | 9 | 9 |
| **N** | 4843 | 18972 | 7553 | 20980 | 11501 | 9282 | 29771 | 36661 | 20045 | 19830 | 20683 |
| | 158275 | 144146 | 155565 | 142138 | 151617 | 153836 | 133347 | 126457 | 143073 | 143288 | 142435 |
| | | | | | | | | | | | |
| **A** | 0.0% | 100.0% | 0.0% | 0.01% | 0.00% | 0.00% | 0.02% | 99.95% | 0.01% | 0.00% | 99.98% |
| **C** | 0.0% | 0.0% | 0.0% | 0.01% | 99.81% | 99.82% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 100.0% | 0.0% | 100.0% | 0.00% | 0.03% | 0.01% | 99.97% | 0.05% | 99.98% | 0.00% | 0.01% |
| **T** | 0.0% | 0.0% | 0.0% | 99.98% | 0.17% | 0.18% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% |



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# FIGURE 33 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 158695 | 161184 | 4 | 153601 | 157375 | 1 | 160855 | 157667 | 7 | 8 | 13 |
| 0 | 8 | 5 | 3 | 4 | 6 | 0 | 8 | 8 | 0 | 2 | 0 |
| 160680 | 10 | 12 | 162687 | 11 | 18 | 161893 | 8 | 18 | 161687 | 162718 | 158182 |
| 4 | 2 | 5 | 1 | 3 | 8 | 3 | 6 | 13 | 28 | 5 | 3 |
| 7157 | 9128 | 6637 | 5148 | 14224 | 10436 | 5946 | 6966 | 10137 | 6121 | 5110 | 9645 |
| 160686 | 158715 | 161206 | 162695 | 153619 | 157407 | 161897 | 160877 | 157706 | 161722 | 162733 | 158198 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 100.00% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 100.00% | 0.00% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.02% | 0.00% | 0.00% |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 153897 | 156373 | 1 | 148973 | 152990 | 5 | 156315 | 153135 | 9 | 13 | 12 |
| 0 | 9 | 4 | 2 | 1 | 3 | 0 | 3 | 4 | 2 | 0 | 0 |
| 155926 | 13 | 14 | 157997 | 15 | 12 | 157435 | 13 | 14 | 157060 | 157912 | 153581 |
| 5 | 2 | 7 | 4 | 4 | 6 | 4 | 11 | 11 | 20 | 1 | 0 |
| 7183 | 9197 | 6720 | 5114 | 14125 | 10107 | 5674 | 6776 | 9954 | 6067 | 5232 | 9565 |
| 155935 | 153921 | 156398 | 158004 | 148993 | 153011 | 157444 | 156342 | 153164 | 157091 | 157926 | 153593 |
| 0.00% | 99.98% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.98% | 0.01% | 0.01% | 0.01% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 33 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | A | G | T | C | C | G | A | G | C | A |
| **pNMG-163 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 11 | 141230 | 4 | 16 | 44 | 5 | 30 | 122599 | 16 | 1 | 138697 |
| **C** | 2 | 4 | 0 | 24 | 147818 | 150444 | 2 | 7 | 6 | 139752 | 4 |
| **G** | 155892 | 23 | 152993 | 1 | 63 | 1 | 130154 | 17 | 139056 | 0 | 18 |
| **T** | 2 | 6 | 5 | 138757 | 537 | 686 | 13 | 6 | 8 | 7 | 6 |
| **N** | 5466 | 20112 | 8373 | 22577 | 12914 | 10242 | 31180 | 38751 | 22294 | 21620 | 22655 |
| | 155907 | 141263 | 153002 | 138798 | 148462 | 151136 | 130199 | 122629 | 139086 | 139760 | 138725 |
| **A** | 0.01% | 99.98% | 0.00% | 0.01% | 0.03% | 0.00% | 0.02% | 99.98% | 0.01% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.02% | 99.57% | 99.54% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.02% | 99.99% | 0.00% | 0.04% | 0.00% | 99.97% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.97% | 0.36% | 0.45% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 17 | 179847 | 13 | 17 | 6 | 3 | 31 | 155609 | 30 | 2 | 176863 |
| **C** | 2 | 7 | 0 | 13 | 189183 | 192342 | 3 | 4 | 1 | 178297 | 12 |
| **G** | 199087 | 94 | 195307 | 1 | 1 | 22 | 165748 | 99 | 177348 | 3 | 24 |
| **T** | 4 | 4 | 1 | 176337 | 164 | 323 | 11 | 7 | 13 | 14 | 12 |
| **N** | 7138 | 26296 | 10927 | 29880 | 16896 | 13561 | 40459 | 50533 | 28860 | 27936 | 29341 |
| | 199110 | 179952 | 195321 | 176368 | 189354 | 192690 | 165793 | 155719 | 177392 | 178316 | 176911 |
| **A** | 0.01% | 99.94% | 0.01% | 0.01% | 0.00% | 0.00% | 0.02% | 99.93% | 0.02% | 0.00% | 99.97% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.91% | 99.82% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| **G** | 99.99% | 0.05% | 99.99% | 0.00% | 0.00% | 0.01% | 99.97% | 0.06% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.09% | 0.17% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% |



SUBSTITUTE SHEET (RULE 26)

FIGURE 33 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 151325 | 153802 | 7 | 146621 | 150506 | 7 | 154588 | 151010 | 6 | 13 | 3 |
| 0 | 7 | 7 | 0 | 4 | 8 | 2 | 3 | 8 | 2 | 3 | 4 |
| 153181 | 6 | 19 | 155490 | 6 | 7 | 154820 | 18 | 24 | 154233 | 154289 | 150209 |
| 4 | 0 | 2 | 5 | 4 | 5 | 6 | 4 | 47 | 9 | 4 | 1 |
| 8190 | 10042 | 7550 | 5878 | 14745 | 10854 | 6545 | 6767 | 10291 | 7130 | 7071 | 11163 |
| 153190 | 151338 | 153830 | 155502 | 146635 | 150526 | 154835 | 154613 | 151089 | 154250 | 154309 | 150217 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.95% | 0.00% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 99.99% | 0.01% | 0.02% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.01% | 0.00% | 0.00% |
| 3 | 193493 | 196342 | 3 | 187067 | 191976 | 1 | 197311 | 192647 | 6 | 11 | 6 |
| 1 | 17 | 10 | 1 | 6 | 8 | 1 | 8 | 12 | 1 | 2 | 2 |
| 195501 | 13 | 13 | 198564 | 20 | 13 | 197639 | 18 | 28 | 196881 | 197149 | 192364 |
| 5 | 2 | 6 | 2 | 2 | 3 | 2 | 7 | 12 | 22 | 2 | 10 |
| 10742 | 12727 | 9881 | 7682 | 19157 | 14252 | 8608 | 8907 | 13552 | 9341 | 9100 | 13882 |
| 195510 | 193525 | 196371 | 198570 | 187095 | 192000 | 197643 | 197344 | 192699 | 196910 | 197164 | 192382 |
| 0.00% | 99.98% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.97% | 0.00% | 0.01% | 0.00% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 100.00% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% |

FIGURE 33 (Continued)

**pNMG-165 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; H8Y_D108N_S127S)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 11 | 84334 | 3 | 6 | 0 | 2 | 13 | 73609 | 19 | 1 | 83398 |
| **C** | 0 | 2 | 1 | 10 | 89271 | 90746 | 1 | 3 | 1 | 84191 | 9 |
| **G** | 93876 | 14 | 92037 | 0 | 3 | 2 | 78299 | 13 | 83629 | 0 | 6 |
| **T** | 2 | 4 | 4 | 83186 | 14 | 37 | 4 | 2 | 0 | 8 | 8 |
| **N** | 3201 | 12738 | 5047 | 13890 | 7805 | 6306 | 18776 | 23467 | 13445 | 12894 | 13673 |
| | 93889 | 84354 | 92045 | 83202 | 89288 | 90787 | 78317 | 73627 | 83649 | 84200 | 83421 |
| | | | | | | | | | | | |
| **A** | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 99.98% | 0.02% | 0.00% | 99.97% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.95% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| **G** | 99.99% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% | 0.04% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% |
| | | | | | | | | | | | |
| **untreated cells** | | | | | | | | | | | |
| **A** | 25 | 199101 | 17 | 12 | 2 | 10 | 36 | 171256 | 22 | 2 | 194764 |
| **C** | 5 | 8 | 2 | 20 | 208783 | 212252 | 1 | 8 | 6 | 196157 | 9 |
| **G** | 219047 | 24 | 215226 | 3 | 4 | 1 | 182971 | 23 | 195391 | 0 | 17 |
| **T** | 3 | 5 | 6 | 195499 | 19 | 30 | 22 | 4 | 5 | 9 | 6 |
| **N** | 7919 | 27862 | 11749 | 31466 | 18193 | 14708 | 43971 | 55712 | 31579 | 30835 | 32207 |
| | 219080 | 199138 | 215251 | 195534 | 208808 | 212293 | 183030 | 171291 | 195424 | 196168 | 194796 |
| | | | | | | | | | | | |
| **A** | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.02% | 99.98% | 0.01% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 33 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 91256 | 92595 | 1 | 88208 | 90412 | 3 | 93035 | 90929 | 5 | 6 | 4 | |
| 0 | 7 | 9 | 1 | 5 | 9 | 1 | 10 | 11 | 0 | 0 | 0 | |
| 92228 | 3 | 5 | 93579 | 11 | 12 | 93138 | 6 | 15 | 92761 | 92876 | 90492 | |
| 2 | 1 | 2 | 3 | 3 | 1 | 0 | 1 | 4 | 6 | 1 | 0 | |
| 4862 | 5827 | 4483 | 3510 | 8867 | 6661 | 3953 | 4043 | 6136 | 4323 | 4212 | 6599 | |
| 92232 | 91267 | 92611 | 93584 | 88227 | 90434 | 93142 | 93052 | 90959 | 92772 | 92883 | 90496 | |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.98% | 0.00% | 99.98% | 99.97% | 0.01% | 0.01% | 0.00% | |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | |
| 100.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 100.00% | 0.01% | 0.02% | 99.99% | 99.99% | 100.00% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | |
| 10 | 212755 | 216153 | 2 | 206396 | 211706 | 7 | 217386 | 212626 | 9 | 16 | 4 | |
| 4 | 11 | 9 | 1 | 5 | 9 | 2 | 10 | 11 | 2 | 1 | 2 | |
| 215451 | 16 | 17 | 218592 | 14 | 18 | 217732 | 10 | 16 | 217166 | 217318 | 211398 | |
| 6 | 3 | 4 | 7 | 0 | 5 | 3 | 7 | 14 | 8 | 5 | 2 | |
| 11532 | 14218 | 10820 | 8401 | 20588 | 15265 | 9259 | 9590 | 14336 | 9818 | 9663 | 15597 | |
| 215471 | 212785 | 216183 | 218602 | 206415 | 211738 | 217744 | 217413 | 212667 | 217185 | 217340 | 211406 | |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 0.01% | 0.00% | |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | |
| 99.99% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 99.99% | 100.00% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | |

FIGURE 34

| | P | A | M | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | C | C | G | C | A | G | T | C | T | A |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 7 | 6 | 5 | 95 | 15 | 186688 | 4 | 6 | 11 | 1 | 188598 |
| **C** | 185572 | 185009 | 185631 | 3 | 186401 | 4 | 0 | 12 | 186512 | 13 | 1 |
| **G** | 3 | 2 | 1 | 186658 | 0 | 19 | 188313 | 0 | 1 | 1 | 13 |
| **T** | 13 | 8 | 24 | 17 | 11 | 77 | 4 | 185923 | 1 | 188998 | 11 |
| **N** | 4574 | 5144 | 4508 | 3396 | 3742 | 3381 | 1848 | 4075 | 3491 | 1003 | 1393 |
| | 185595 | 185025 | 185661 | 186773 | 186427 | 186788 | 188321 | 185941 | 186525 | 189013 | 188623 |
| **A** | 0.00% | 0.00% | 0.00% | 0.05% | 0.01% | 99.95% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 100.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.04% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% |
| **pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 3 | 2 | 4 | 68 | 0 | 133554 | 9 | 3 | 3 | 0 | 135039 |
| **C** | 132806 | 132355 | 132759 | 3 | 133258 | 4 | 1 | 16 | 133495 | 13 | 1 |
| **G** | 0 | 0 | 1 | 133560 | 2 | 11 | 134712 | 0 | 5 | 1 | 9 |
| **T** | 7 | 6 | 14 | 5 | 10 | 1 | 2 | 133163 | 15 | 135264 | 1 |
| **N** | 3213 | 3666 | 3251 | 2393 | 2759 | 2459 | 1304 | 2846 | 2510 | 750 | 978 |
| | 132816 | 132363 | 132778 | 133636 | 133270 | 133570 | 134724 | 133182 | 133518 | 135278 | 135050 |
| **A** | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% |

FIGURE 34 (Continued)

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C |
| 4 | 9 | 1 | 2 | 6 | 5 | 5 | 14 | 85 | 7 | 4 | 7 |
| 17 | 0 | 186680 | 20 | 13 | 15 | 58 | 370 | 49 | 18 | 21 | 188802 |
| 0 | 188051 | 1 | 1 | 1 | 1 | 189000 | 2 | 187637 | 1 | 0 | 2 |
| 188620 | 8 | 14 | 189114 | 189438 | 188757 | 6 | 187276 | 5 | 188588 | 189538 | 24 |
| 1375 | 1948 | 3320 | 879 | 558 | 1238 | 947 | 2354 | 2240 | 1402 | 450 | 1178 |
| 188641 | 188068 | 186696 | 189137 | 189458 | 188778 | 189069 | 187662 | 187776 | 188614 | 189563 | 188835 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.05% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.03% | 0.20% | 0.03% | 0.01% | 0.01% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.96% | 0.00% | 99.93% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.00% | 99.79% | 0.00% | 99.99% | 99.99% | 0.01% |
| 7 | 9 | 2 | 3 | 4 | 2 | 243 | 9 | 121 | 4 | 6 | 6 |
| 10 | 0 | 133703 | 10 | 44 | 12 | 23 | 3460 | 2 | 13 | 12 | 135206 |
| 0 | 134678 | 3 | 6 | 4 | 0 | 135108 | 1 | 134297 | 0 | 3 | 4 |
| 135040 | 1 | 19 | 135402 | 135556 | 135141 | 4 | 130879 | 2 | 135073 | 135652 | 11 |
| 971 | 1340 | 2301 | 607 | 420 | 873 | 650 | 1679 | 1606 | 938 | 355 | 801 |
| 135057 | 134688 | 133727 | 135421 | 135608 | 135155 | 135378 | 134349 | 134422 | 135090 | 135673 | 135227 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.18% | 0.01% | 0.09% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.03% | 0.01% | 0.02% | 2.58% | 0.00% | 0.01% | 0.01% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.80% | 0.00% | 99.91% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.96% | 99.99% | 0.00% | 97.42% | 0.00% | 99.99% | 99.98% | 0.01% |

# FIGURE 34 (Continued)

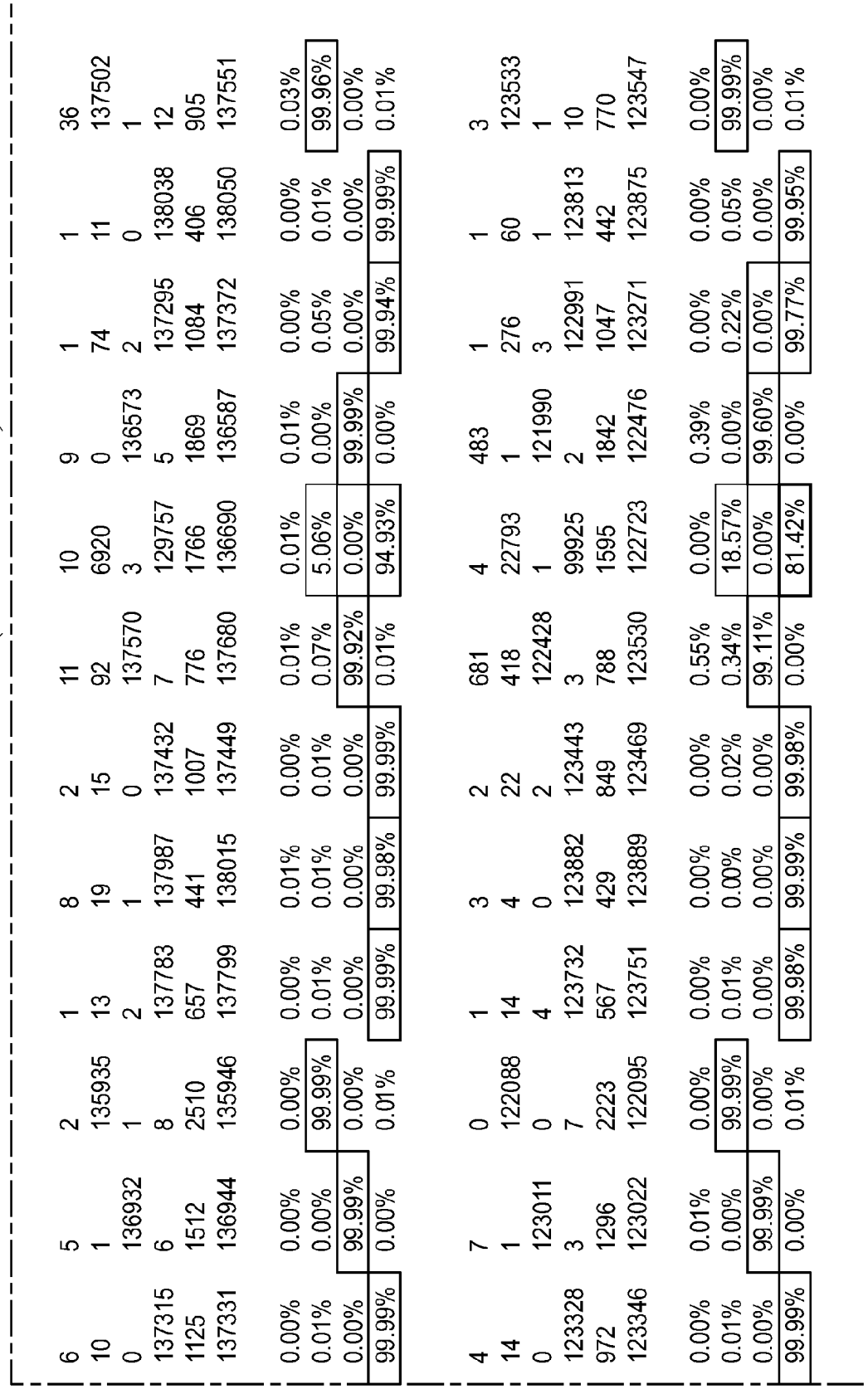
**pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 2 | 2 | 5 | 53 | 5 | 135773 | 4 | 2 | 2 | 0 | 137273 |
| **C** | 134987 | 134476 | 134879 | 0 | 135486 | 6 | 1 | 14 | 135650 | 7 | 2 |
| **G** | 2 | 0 | 1 | 135779 | 2 | 9 | 137014 | 0 | 2 | 0 | 7 |
| **T** | 7 | 8 | 14 | 10 | 9 | 3 | 4 | 135314 | 9 | 137594 | 9 |
| **N** | 3458 | 3970 | 3557 | 2614 | 2954 | 2665 | 1433 | 3126 | 2793 | 855 | 1165 |
| | 134998 | 134486 | 134899 | 135842 | 135502 | 135791 | 137023 | 135330 | 135663 | 137601 | 137291 |
| | | | | | | | | | | | |
| **A** | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |

**pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 84 | 3 | 48 | 72 | 2 | 121963 | 5 | 2 | 5 | 1 | 123369 |
| **C** | 121232 | 120698 | 121230 | 1 | 121720 | 1 | 1 | 6 | 121892 | 12 | 1 |
| **G** | 1 | 2 | 1 | 121954 | 2 | 8 | 123077 | 1 | 0 | 0 | 8 |
| **T** | 8 | 7 | 11 | 8 | 5 | 1 | 3 | 121641 | 9 | 123573 | 5 |
| **N** | 3042 | 3657 | 3077 | 2332 | 2638 | 2394 | 1281 | 2668 | 2412 | 732 | 935 |
| | 121325 | 120710 | 121290 | 122035 | 121729 | 121973 | 123086 | 121650 | 121906 | 123586 | 123383 |
| | | | | | | | | | | | |
| **A** | 0.07% | 0.00% | 0.04% | 0.06% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% |
| **C** | 99.92% | 99.99% | 99.95% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% |

FIGURE 34 (Continued)



| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 5 | 2 | 1 | 8 | 2 | 11 | 10 | 9 | 1 | 1 | 1 | 36 |
| 10 | 1 | 135935 | 13 | 19 | 15 | 92 | 6920 | 0 | 74 | 11 | 11 | 137502 |
| 0 | 136932 | 1 | 2 | 1 | 0 | 137570 | 3 | 136573 | 2 | 0 | 0 | 1 |
| 137315 | 6 | 8 | 137783 | 137987 | 137432 | 7 | 129757 | 5 | 137295 | 138038 | 138038 | 12 |
| 1125 | 1512 | 2510 | 657 | 441 | 1007 | 776 | 1766 | 1869 | 1084 | 406 | 406 | 905 |
| 137331 | 136944 | 135946 | 137799 | 138015 | 137449 | 137680 | 136690 | 136587 | 137372 | 138050 | 138050 | 137551 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.03% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.07% | 5.06% | 0.00% | 0.05% | 0.01% | 0.01% | 99.96% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.92% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.98% | 99.99% | 0.01% | 94.93% | 0.00% | 99.94% | 99.99% | 99.99% | 0.01% |
| 4 | 7 | 0 | 1 | 3 | 2 | 681 | 4 | 483 | 1 | 1 | 1 | 3 |
| 14 | 1 | 122088 | 14 | 4 | 22 | 418 | 22793 | 1 | 276 | 60 | 60 | 123533 |
| 0 | 123011 | 0 | 4 | 0 | 2 | 122428 | 1 | 121990 | 3 | 1 | 1 | 1 |
| 123328 | 3 | 7 | 123732 | 123882 | 123443 | 3 | 99925 | 2 | 122991 | 123813 | 123813 | 10 |
| 972 | 1296 | 2223 | 567 | 429 | 849 | 788 | 1595 | 1842 | 1047 | 442 | 442 | 770 |
| 123346 | 123022 | 122095 | 123751 | 123889 | 123469 | 123530 | 122723 | 122476 | 123271 | 123875 | 123875 | 123547 |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.55% | 0.00% | 0.39% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.02% | 0.34% | 18.57% | 0.00% | 0.22% | 0.05% | 0.05% | 99.99% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.11% | 0.00% | 99.60% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.98% | 99.99% | 99.98% | 0.00% | 81.42% | 0.00% | 99.77% | 99.95% | 99.95% | 0.01% |

# FIGURE 34 (Continued)

| | PC | AC | MC | 20G | 19C | 18A | 17G | 16T | 15C | 14T | 13A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-171 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 2 | 8 | 2 | 103 | 1 | 134562 | 7 | 1 | 6 | 0 | 135894 |
| **C** | 133604 | 133236 | 133733 | 2 | 134253 | 1 | 1 | 12 | 134449 | 10 | 1 |
| **G** | 0 | 4 | 2 | 134411 | 0 | 6 | 135611 | 1 | 0 | 2 | 8 |
| **T** | 11 | 10 | 11 | 47 | 6 | 2 | 3 | 133973 | 7 | 136151 | 7 |
| **N** | 3317 | 3676 | 3186 | 2370 | 2673 | 2362 | 1311 | 2945 | 2470 | 769 | 1022 |
| | 133617 | 133258 | 133748 | 134563 | 134260 | 134571 | 135622 | 133987 | 134462 | 136163 | 135910 |
| **A** | 0.00% | 0.01% | 0.00% | 0.08% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% |
| **C** | 99.99% | 99.98% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.89% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.01% | 0.01% | 0.03% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |
| **pNMG-172 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 0 | 4 | 9 | 57 | 58 | 119654 | 7 | 1 | 7 | 3 | 120929 |
| **C** | 118953 | 118582 | 119038 | 1 | 119393 | 1 | 1 | 8 | 119588 | 8 | 2 |
| **G** | 0 | 0 | 0 | 119606 | 1 | 13 | 120687 | 1 | 1 | 0 | 6 |
| **T** | 8 | 5 | 8 | 53 | 3 | 1 | 1 | 119310 | 6 | 121178 | 6 |
| **N** | 2904 | 3274 | 2810 | 2148 | 2410 | 2196 | 1169 | 2545 | 2263 | 676 | 922 |
| | 118961 | 118591 | 119055 | 119717 | 119455 | 119669 | 120696 | 119320 | 119602 | 121189 | 120943 |
| **A** | 0.00% | 0.00% | 0.01% | 0.05% | 0.05% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.99% | 0.00% | 99.95% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.91% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.00% | 0.01% | 0.04% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% |

# FIGURE 34 (Continued)

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C |
| 7 | 12 | 2 | 2 | 8 | 0 | 1386 | 3 | 888 | 6 | 1 | 9 |
| 9 | 2 | 134514 | 6 | 6 | 16 | 789 | 394 | 0 | 21 | 8 | 136054 |
| 0 | 135522 | 1 | 0 | 2 | 3 | 133879 | 0 | 134317 | 2 | 0 | 4 |
| 135869 | 1 | 11 | 136276 | 136497 | 135993 | 4 | 134832 | 10 | 135950 | 136566 | 17 |
| 1047 | 1395 | 2404 | 648 | 419 | 920 | 874 | 1703 | 1717 | 952 | 356 | 847 |
| 135885 | 135537 | 134528 | 136284 | 136513 | 136012 | 136058 | 135229 | 135215 | 135979 | 136575 | 136084 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 1.02% | 0.00% | 0.66% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.58% | 0.29% | 0.00% | 0.02% | 0.01% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 98.40% | 0.00% | 99.34% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.00% | 99.71% | 0.01% | 99.98% | 99.99% | 0.01% |
| 8 | 4 | 2 | 2 | 5 | 3 | 2997 | 8 | 1855 | 6 | 3 | 1 |
| 15 | 0 | 119788 | 9 | 6 | 7 | 2486 | 763 | 84 | 11 | 21 | 121129 |
| 1 | 120672 | 1 | 2 | 0 | 1 | 115421 | 0 | 118302 | 3 | 0 | 3 |
| 120972 | 1 | 4 | 121319 | 121518 | 121048 | 71 | 119601 | 62 | 120995 | 121508 | 13 |
| 869 | 1188 | 2070 | 533 | 336 | 806 | 890 | 1493 | 1562 | 850 | 332 | 718 |
| 120996 | 120677 | 119795 | 121332 | 121529 | 121059 | 120975 | 120372 | 120303 | 121015 | 121532 | 121146 |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 2.48% | 0.01% | 1.54% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 2.05% | 0.63% | 0.07% | 0.01% | 0.02% | 99.99% |
| 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 95.41% | 0.00% | 98.34% | 0.00% | 0.00% | 0.00% |
| 99.98% | 0.00% | 0.00% | 99.99% | 99.99% | 99.99% | 0.06% | 99.36% | 0.05% | 99.98% | 99.98% | 0.01% |

# FIGURE 34 (Continued)

**pNMG-173 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 7 | 8 | 127 | 0 | 190886 | 53 | 3 | 8 | 4 | 192759 |
| C | 189769 | 189139 | 189740 | 4 | 190483 | 7 | 1 | 9 | 190805 | 17 | 0 |
| G | 0 | 2 | 1 | 190703 | 0 | 12 | 192402 | 0 | 0 | 0 | 14 |
| T | 10 | 10 | 17 | 34 | 15 | 0 | 3 | 190239 | 16 | 193241 | 10 |
| N | 4461 | 5087 | 4479 | 3377 | 3747 | 3400 | 1846 | 4054 | 3476 | 1043 | 1463 |
| | 189784 | 189158 | 189766 | 190868 | 190498 | 190905 | 192459 | 190251 | 190829 | 193262 | 192783 |
| | | | | | | | | | | | |
| A | 0.00% | 0.00% | 0.00% | 0.07% | 0.00% | 99.99% | 0.03% | 0.00% | 0.00% | 0.00% | 99.99% |
| C | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.91% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% | 0.00% | 0.01% |
| T | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |

**pNMG-174 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 11 | 13 | 56 | 135 | 40 | 198571 | 8 | 4 | 6 | 6 | 200496 |
| C | 197293 | 196565 | 197221 | 3 | 197946 | 1 | 1 | 17 | 198396 | 12 | 3 |
| G | 3 | 2 | 2 | 198374 | 1 | 36 | 200229 | 22 | 2 | 1 | 18 |
| T | 8 | 15 | 16 | 7 | 7 | 3 | 8 | 197713 | 12 | 200893 | 9 |
| N | 4803 | 5523 | 4823 | 3598 | 4123 | 3506 | 1871 | 4294 | 3634 | 1138 | 1502 |
| | 197315 | 196595 | 197295 | 198519 | 197994 | 198611 | 200246 | 197756 | 198416 | 200912 | 200526 |
| | | | | | | | | | | | |
| A | 0.01% | 0.01% | 0.03% | 0.07% | 0.02% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% |
| C | 99.99% | 99.98% | 99.96% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.02% | 99.99% | 0.01% | 0.00% | 0.00% | 0.01% |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 0.00% |

**pNMG-175 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 4 | 4 | 133 | 4 | 216224 | 7 | 5 | 10 | 6 | 218978 |

# FIGURE 34 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 10 | 5 | 1 | 7 | 4 | 2498 | 14 | 1832 | 8 | 5 | 4 |
| 20 | 2 | 190923 | 10 | 15 | 10 | 1898 | 702 | 1 | 36 | 23 | 192927 |
| 1 | 192222 | 1 | 0 | 3 | 0 | 188656 | 0 | 189940 | 3 | 0 | 1 |
| 192766 | 3 | 15 | 193330 | 193631 | 192952 | 9 | 191216 | 95 | 192844 | 193717 | 117 |
| 1449 | 2009 | 3302 | 905 | 590 | 1280 | 1185 | 2314 | 2378 | 1355 | 501 | 1197 |
| 192797 | 192237 | 190944 | 193341 | 193656 | 192966 | 193061 | 191932 | 191868 | 192891 | 193745 | 193049 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 1.29% | 0.01% | 0.95% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.98% | 0.37% | 0.00% | 0.02% | 0.01% | 99.94% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 97.72% | 0.00% | 99.00% | 0.00% | 0.00% | 0.00% |
| 99.98% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.00% | 99.63% | 0.05% | 99.98% | 99.99% | 0.06% |
| 6 | 15 | 1 | 0 | 3 | 3 | 5325 | 10 | 4115 | 6 | 6 | 17 |
| 11 | 1 | 198558 | 13 | 12 | 15 | 3158 | 679 | 94 | 14 | 28 | 200564 |
| 1 | 199893 | 2 | 1 | 0 | 1 | 191773 | 1 | 195041 | 4 | 0 | 97 |
| 200532 | 29 | 18 | 201149 | 201414 | 200686 | 170 | 198971 | 252 | 200575 | 201420 | 113 |
| 1478 | 2089 | 3448 | 864 | 598 | 1321 | 1600 | 2365 | 2524 | 1427 | 569 | 1232 |
| 200550 | 199938 | 198579 | 201163 | 201429 | 200705 | 200426 | 199661 | 199502 | 200599 | 201454 | 200791 |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 2.66% | 0.01% | 2.06% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 1.58% | 0.34% | 0.05% | 0.01% | 0.01% | 99.89% |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 95.68% | 0.00% | 97.76% | 0.00% | 0.00% | 0.05% |
| 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% | 0.08% | 99.65% | 0.13% | 99.99% | 99.98% | 0.06% |
| 15 | 8 | 50 | 3 | 9 | 4 | 122 | 15 | 70 | 5 | 6 | 3 |



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FIGURE 34 (Continued)

| | | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | | 215382 | 214607 | 215400 | 6 | 216221 | 0 | 3 | 12 | 216218 | 14 | 1 |
| G | | 0 | 1 | 1 | 216344 | 0 | 17 | 218187 | 2 | 0 | 3 | 15 |
| T | | 8 | 20 | 28 | 14 | 13 | 2 | 12 | 214966 | 17 | 219351 | 7 |
| N | | 5191 | 5951 | 5150 | 4086 | 4345 | 4340 | 2374 | 5598 | 4338 | 1209 | 1582 |
| | | 215392 | 214632 | 215433 | 216497 | 216238 | 216243 | 218209 | 214985 | 216245 | 219374 | 219001 |
| A | | 0.00% | 0.00% | 0.00% | 0.06% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% |
| C | | 100.00% | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| G | | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| T | | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 99.99% | 0.00% |
| **pNMG-176 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S)** | | | | | | | | | | | | |
| A | | 4 | 31 | 9 | 313 | 7 | 232628 | 46 | 3 | 5 | 7 | 235537 |
| C | | 231587 | 230837 | 231630 | 1 | 232510 | 2 | 2 | 15 | 232455 | 18 | 0 |
| G | | 1 | 1 | 0 | 232607 | 0 | 16 | 234670 | 0 | 0 | 1 | 14 |
| T | | 13 | 22 | 20 | 14 | 15 | 24 | 4 | 231110 | 19 | 235920 | 10 |
| N | | 5663 | 6377 | 5609 | 4333 | 4736 | 4598 | 2546 | 6140 | 4789 | 1322 | 1707 |
| | | 231605 | 230891 | 231659 | 232935 | 232532 | 232670 | 234722 | 231128 | 232479 | 235946 | 235561 |
| A | | 0.00% | 0.01% | 0.00% | 0.13% | 0.00% | 99.98% | 0.02% | 0.00% | 0.00% | 0.00% | 99.99% |
| C | | 99.99% | 99.98% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| G | | 0.00% | 0.00% | 0.00% | 99.86% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% |
| T | | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% |

FIGURE 34 (Continued)

| 12 T | 11 G | 10 C | 9 T | 8 T | 7 T | 6 G | 5 T | 4 G | 3 T | 2 T | 1 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 3 | 216695 | 15 | 13 | 14 | 132 | 721 | 5 | 24 | 18 | 219294 |
| 0 | 218010 | 2 | 1 | 61 | 0 | 218890 | 0 | 217812 | 3 | 1 | 3 |
| 218891 | 2 | 19 | 219582 | 219837 | 219102 | 9 | 217267 | 3 | 218907 | 219881 | 21 |
| 1664 | 2560 | 3817 | 979 | 660 | 1460 | 1365 | 2515 | 2628 | 1579 | 611 | 1196 |
| 218919 | 218023 | 216766 | 219601 | 219920 | 219120 | 219153 | 218003 | 217890 | 218939 | 219906 | 219321 |
| 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.06% | 0.01% | 0.03% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.97% | 0.01% | 0.01% | 0.01% | 0.06% | 0.33% | 0.00% | 0.01% | 0.01% | 99.99% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.03% | 0.00% | 99.88% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.96% | 99.99% | 0.00% | 99.66% | 0.00% | 99.99% | 99.99% | 0.01% |
| 15 | 14 | 3 | 2 | 7 | 5 | 125 | 16 | 332 | 8 | 10 | 3 |
| 13 | 1 | 233162 | 23 | 20 | 15 | 465 | 1275 | 0 | 24 | 21 | 235812 |
| 0 | 234548 | 4 | 4 | 1 | 13 | 235158 | 4 | 234047 | 3 | 1 | 6 |
| 235521 | 10 | 16 | 236149 | 236478 | 235598 | 45 | 233399 | 5 | 235543 | 236582 | 17 |
| 1719 | 2694 | 4082 | 1089 | 761 | 1636 | 1474 | 2573 | 2883 | 1688 | 649 | 1425 |
| 235549 | 234573 | 233185 | 236178 | 236506 | 235631 | 235793 | 234694 | 234384 | 235578 | 236614 | 235838 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.01% | 0.14% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.20% | 0.54% | 0.00% | 0.01% | 0.01% | 99.99% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 99.73% | 0.00% | 99.86% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.02% | 99.45% | 0.00% | 99.99% | 99.99% | 0.01% |

FIGURE 34 (Continued)

| pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 1 | 3 | 84 | 7 | 169056 | 1 | 2 | 9 | 1 | 171447 |
| C | 168533 | 167759 | 168505 | 2 | 169201 | 1 | 2 | 13 | 169168 | 4 | 5 |
| G | 0 | 0 | 0 | 169211 | 1 | 11 | 170886 | 0 | 1 | 1 | 4 |
| T | 10 | 11 | 16 | 14 | 24 | 2 | 9 | 168099 | 9 | 171681 | 3 |
| N | 4261 | 5031 | 4280 | 3493 | 3571 | 3734 | 1906 | 4647 | 3574 | 1074 | 1302 |
| | 168544 | 167773 | 168524 | 169311 | 169233 | 169070 | 170898 | 168114 | 169187 | 171687 | 171459 |
| | | | | | | | | | | | |
| A | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% |
| C | 99.99% | 99.99% | 99.99% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 100.00% | 0.00% |
| | | | | | | | | | | | |
| pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | | |
| A | 5 | 4 | 9 | 85 | 56 | 159313 | 14 | 2 | 4 | 4 | 161431 |
| C | 158813 | 158107 | 158665 | 2 | 159293 | 0 | 3 | 18 | 159281 | 14 | 1 |
| G | 0 | 2 | 0 | 159466 | 0 | 6 | 160880 | 0 | 0 | 0 | 2 |
| T | 7 | 16 | 15 | 10 | 32 | 6 | 3 | 158308 | 5 | 161678 | 9 |
| N | 3864 | 4560 | 4000 | 3126 | 3308 | 3364 | 1789 | 4361 | 3399 | 993 | 1246 |
| | 158825 | 158129 | 158689 | 159563 | 159381 | 159325 | 160900 | 158328 | 159290 | 161696 | 161443 |
| | | | | | | | | | | | |
| A | 0.00% | 0.00% | 0.01% | 0.05% | 0.04% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% |
| C | 99.99% | 99.99% | 99.98% | 0.00% | 99.94% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% |

# FIGURE 34 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | 15 | 3 | 0 | 7 | 1 | 108 | 6 | 128 | 4 | 4 | 8 |
| 15 | 0 | 169660 | 15 | 75 | 89 | 523 | 45487 | 35 | 702 | 43 | 171671 |
| 0 | 170628 | 0 | 3 | 1 | 1 | 170858 | 2 | 169823 | 0 | 0 | 6 |
| 171368 | 9 | 10 | 171982 | 172118 | 171382 | 9 | 125059 | 8 | 170433 | 172038 | 17 |
| 1360 | 2109 | 3088 | 761 | 560 | 1288 | 1263 | 2207 | 2766 | 1621 | 675 | 1058 |
| 171401 | 170652 | 169673 | 172000 | 172201 | 171473 | 171498 | 170554 | 169994 | 171139 | 172085 | 171702 |
| | | | | | | | | | | | |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.06% | 0.00% | 0.08% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.04% | 0.05% | 0.30% | 26.67% | 0.02% | 0.41% | 0.02% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.63% | 0.00% | 99.90% | 0.00% | 0.00% | 0.00% |
| 99.98% | 0.01% | 0.01% | 99.99% | 99.95% | 99.95% | 0.01% | 73.33% | 0.00% | 99.59% | 99.97% | 0.01% |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 14 | 3 | 6 | 4 | 0 | 166 | 8 | 12 | 10 | 5 | 2 |
| 13 | 0 | 159860 | 9 | 122 | 28 | 20 | 30430 | 2 | 652 | 16 | 161722 |
| 0 | 160769 | 5 | 1 | 0 | 0 | 161347 | 1 | 160258 | 4 | 1 | 1 |
| 161452 | 8 | 11 | 161918 | 162030 | 161429 | 8 | 130371 | 7 | 160606 | 162052 | 10 |
| 1219 | 1898 | 2810 | 755 | 533 | 1232 | 1148 | 1879 | 2410 | 1417 | 613 | 952 |
| 161470 | 160791 | 159879 | 161934 | 162156 | 161457 | 161541 | 160810 | 160279 | 161272 | 162074 | 161735 |
| | | | | | | | | | | | |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.10% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.08% | 0.02% | 0.01% | 18.92% | 0.00% | 0.40% | 0.01% | 99.99% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.88% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.92% | 99.98% | 0.00% | 81.07% | 0.00% | 99.59% | 99.99% | 0.01% |



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FIGURE 34 (Continued)

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 2 | 6 | 32 | 121 | 5 | 227568 | 11 | 4 | 4 | 1 | 230640 |
| **C** | 226899 | 226051 | 226787 | 5 | 227805 | 3 | 1 | 21 | 227683 | 23 | 3 |
| **G** | 3 | 3 | 0 | 227877 | 3 | 35 | 229915 | 64 | 0 | 0 | 10 |
| **T** | 7 | 11 | 23 | 19 | 11 | 4 | 14 | 226136 | 8 | 231062 | 13 |
| **N** | 5485 | 6325 | 5554 | 4374 | 4572 | 4786 | 2455 | 6166 | 4696 | 1305 | 1659 |
| | 226911 | 226071 | 226842 | 228022 | 227824 | 227610 | 229941 | 226225 | 227695 | 231086 | 230666 |
| **A** | 0.00% | 0.00% | 0.01% | 0.05% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.02% | 99.99% | 0.03% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.96% | 0.00% | 99.99% | 0.01% |
| **pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 9 | 1 | 9 | 149 | 2 | 280856 | 38 | 30 | 12 | 6 | 284727 |
| **C** | 280032 | 278910 | 280089 | 3 | 281231 | 3 | 4 | 23 | 281012 | 24 | 3 |
| **G** | 4 | 1 | 0 | 281422 | 2 | 28 | 283729 | 2 | 29 | 1 | 16 |
| **T** | 10 | 20 | 24 | 35 | 13 | 40 | 10 | 279093 | 18 | 285187 | 10 |
| **N** | 6895 | 8018 | 6828 | 5341 | 5703 | 6023 | 3169 | 7719 | 5796 | 1649 | 2088 |
| | 280055 | 278932 | 280122 | 281609 | 281248 | 280927 | 283781 | 279148 | 281071 | 285218 | 284756 |

FIGURE 34 (Continued)

| 12 T | 11 G | 10 C | 9 T | 8 T | 7 T | 6 G | 5 T | 4 G | 3 T | 2 T | 1 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 16 | 6 | 2 | 7 | 3 | 92 | 11 | 101 | 5 | 8 | 2 |
| 17 | 3 | 228340 | 87 | 144 | 311 | 430 | 60360 | 2 | 1177 | 173 | 230905 |
| 2 | 229581 | 3 | 4 | 0 | 3 | 230189 | 4 | 228497 | 1 | 0 | 3 |
| 230583 | 49 | 12 | 231226 | 231368 | 230268 | 11 | 169108 | 8 | 228953 | 231213 | 16 |
| 1717 | 2676 | 3964 | 1006 | 806 | 1740 | 1603 | 2842 | 3717 | 2189 | 931 | 1399 |
| 230608 | 229649 | 228361 | 231319 | 231519 | 230585 | 230722 | 229483 | 228608 | 230136 | 231394 | 230926 |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.04% | 0.06% | 0.13% | 0.19% | 26.30% | 0.00% | 0.51% | 0.07% | 99.99% |
| 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 99.77% | 0.00% | 99.95% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.02% | 0.01% | 99.96% | 99.93% | 99.86% | 0.00% | 73.69% | 0.00% | 99.49% | 99.92% | 0.01% |
| 10 | 15 | 7 | 5 | 6 | 4 | 334 | 49 | 142 | 2 | 15 | 4 |
| 23 | 1 | 281884 | 112 | 218 | 244 | 258 | 90516 | 2 | 750 | 83 | 285095 |
| 0 | 283604 | 5 | 5 | 1 | 2 | 284159 | 4 | 282175 | 2 | 3 | 2 |
| 284675 | 6 | 26 | 285397 | 285593 | 284509 | 12 | 192651 | 3 | 283370 | 285626 | 22 |
| 2136 | 3218 | 4922 | 1324 | 1025 | 2084 | 2080 | 3623 | 4521 | 2719 | 1113 | 1717 |
| 284708 | 283626 | 281922 | 285519 | 285818 | 284759 | 284763 | 283220 | 282322 | 284124 | 285727 | 285123 |

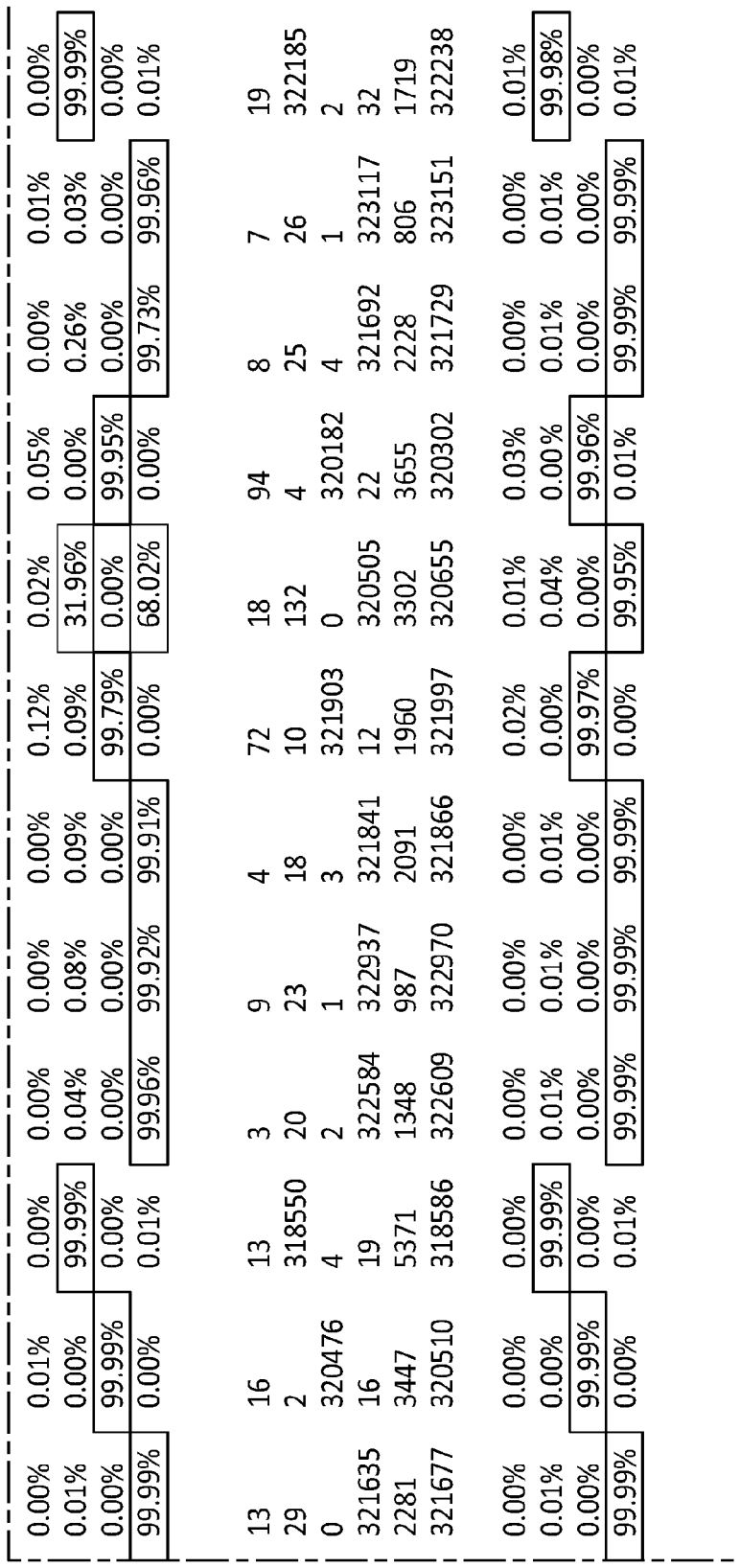
FIGURE 34 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.97% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% |
| C | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 99.99% | 0.00% |

**untreated cells**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 7 | 14 | 6 | 162 | 10 | 318101 | 21 | 2 | 17 | 1 | 321616 |
| C | 316695 | 315568 | 316693 | 2 | 317976 | 0 | 2 | 30 | 317803 | 16 | 4 |
| G | 1 | 2 | 1 | 318196 | 0 | 26 | 320694 | 2 | 4 | 0 | 24 |
| T | 23 | 28 | 37 | 27 | 29 | 4 | 14 | 316128 | 19 | 322324 | 19 |
| N | 7231 | 8345 | 7220 | 5570 | 5942 | 5826 | 3226 | 7795 | 6114 | 1617 | 2295 |
| | 316726 | 315612 | 316737 | 318387 | 318015 | 318131 | 320731 | 316162 | 317843 | 322341 | 321663 |
| A | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% |
| C | 99.99% | 99.99% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |

FIGURE 34 (Continued)



# FIGURE 35

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 8 | 16 | 2 | 15 | 4 | 139719 | 11 | 143159 | 1 | 1 | 13 |
| **C** | 2 | 1 | 141127 | 140716 | 140961 | 3 | 2 | 1 | 143009 | 25 | 4 |
| **G** | 141433 | 139071 | 3 | 1 | 2 | 50 | 143675 | 17 | 2 | 1 | 143822 |
| **T** | 3 | 4 | 10 | 9 | 15 | 3 | 6 | 6 | 12 | 143841 | 6 |
| **N** | 4044 | 6398 | 4348 | 4749 | 4508 | 5715 | 1796 | 2307 | 2466 | 1622 | 1645 |
| | 141446 | 139092 | 141142 | 140741 | 140982 | 139775 | 143694 | 143183 | 143024 | 143868 | 143845 |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.96% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 99.98% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.02% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.04% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| **pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 8 | 17 | 1 | 8 | 3 | 125072 | 2 | 128040 | 2 | 2 | 12 |
| **C** | 2 | 0 | 126380 | 126043 | 126354 | 5 | 1 | 1 | 128147 | 16 | 0 |
| **G** | 126924 | 124669 | 0 | 1 | 0 | 110 | 128733 | 319 | 1 | 0 | 128929 |
| **T** | 9 | 2 | 15 | 4 | 13 | 17 | 11 | 6 | 6 | 128976 | 6 |
| **N** | 3450 | 5705 | 3997 | 4337 | 4023 | 5189 | 1646 | 2027 | 2237 | 1399 | 1446 |
| | 126943 | 124688 | 126396 | 126056 | 126370 | 125204 | 128747 | 128366 | 128156 | 128994 | 128947 |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.89% | 0.00% | 99.75% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.09% | 99.99% | 0.25% | 0.00% | 0.00% | 99.99% |
| **T** | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% |

| 12 A | 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 137864 | 12 | 1 | 142750 | 26 | 15 | 2 | 5 | 139440 | 2 | 16 | 37 |
| 2 | 2 | 141422 | 3 | 141386 | 1 | 10 | 2 | 2 | 11 | 5 | 5 |
| 34 | 142028 | 1 | 5 | 0 | 140547 | 2 | 141902 | 13 | 0 | 142689 | 142890 |
| 3 | 5 | 36 | 10 | 13 | 5 | 141567 | 3 | 6 | 142177 | 4 | 24 |
| 7587 | 3443 | 4030 | 2722 | 4065 | 4922 | 3882 | 3546 | 5997 | 3268 | 2744 | 2502 |
| 137903 | 142047 | 141460 | 142768 | 141425 | 140568 | 141581 | 141912 | 139461 | 142190 | 142714 | 142956 |
| 99.97% | 0.01% | 0.00% | 99.99% | 0.02% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.97% | 0.00% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.95% |
| 0.00% | 0.00% | 0.03% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.02% |
| 123672 | 11 | 5 | 128041 | 2 | 9 | 7 | 13 | 125164 | 1 | 9 | 42 |
| 2 | 3 | 126923 | 2 | 126804 | 0 | 8 | 2 | 2 | 12 | 5 | 4 |
| 18 | 127508 | 3 | 6 | 1 | 125938 | 0 | 127167 | 12 | 0 | 127961 | 128136 |
| 4 | 4 | 19 | 3 | 18 | 1 | 126877 | 3 | 10 | 127419 | 2 | 5 |
| 6697 | 2867 | 3443 | 2341 | 3568 | 4445 | 3493 | 3200 | 5197 | 2953 | 2408 | 2198 |
| 123696 | 127526 | 126950 | 128052 | 126825 | 125948 | 126892 | 127185 | 125188 | 127432 | 127977 | 128187 |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 99.96% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |

# FIGURE 35 (Continued)

**pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 6 | 11 | 1 | 6 | 4 | 93441 | 5 | 95503 | 0 | 2 | 12 |
| **C** | 0 | 4 | 94168 | 93997 | 94249 | 0 | 0 | 0 | 95492 | 2 | 5 |
| **G** | 94583 | 92906 | 0 | 0 | 3 | 9 | 95921 | 157 | 2 | 2 | 96104 |
| **T** | 1 | 3 | 13 | 5 | 5 | 6 | 2 | 3 | 8 | 96137 | 1 |
| **N** | 2559 | 4225 | 2967 | 3141 | 2888 | 3693 | 1221 | 1486 | 1647 | 1006 | 1027 |
| | 94590 | 92924 | 94182 | 94008 | 94261 | 93456 | 95928 | 95663 | 95502 | 96143 | 96122 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 99.83% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.16% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |

**pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 5 | 20 | 1 | 5 | 0 | 99433 | 6 | 101928 | 1 | 1 | 10 |
| **C** | 2 | 0 | 100438 | 100291 | 100645 | 1 | 2 | 2 | 102080 | 6 | 2 |
| **G** | 101004 | 99221 | 2 | 3 | 0 | 196 | 102523 | 237 | 0 | 0 | 102762 |
| **T** | 4 | 1 | 15 | 9 | 2 | 7 | 3 | 5 | 6 | 102771 | 4 |
| **N** | 2929 | 4702 | 3488 | 3636 | 3297 | 4307 | 1410 | 1772 | 1857 | 1166 | 1166 |
| | 101015 | 99242 | 100456 | 100308 | 100647 | 99637 | 102534 | 102172 | 102087 | 102778 | 102778 |
| | | | | | | | | | | | |
| **A** | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 99.80% | 0.01% | 99.76% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.98% | 100.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.20% | 99.99% | 0.23% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |

FIGURE 35 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 92209 | 5 | 3 | 95376 | 1 | 6 | 3 | 3 | 93314 | 0 | 13 | 50 | |
| 0 | 4 | 94706 | 0 | 94676 | 2 | 10 | 3 | 0 | 8 | 0 | 1 | |
| 10 | 94963 | 2 | 10 | 0 | 93943 | 0 | 94845 | 7 | 0 | 95422 | 95523 | |
| 3 | 2 | 10 | 2 | 9 | 2 | 94563 | 1 | 8 | 95075 | 2 | 2 | |
| 4927 | 2175 | 2428 | 1761 | 2463 | 3196 | 2573 | 2297 | 3820 | 2066 | 1712 | 1573 | |
| 92222 | 94974 | 94721 | 95388 | 94686 | 93953 | 94576 | 94852 | 93329 | 95083 | 95437 | 95576 | |
| 99.99% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.05% | |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.94% | |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | |
| 98543 | 7 | 0 | 101975 | 7 | 17 | 4 | 3 | 99773 | 0 | 16 | 35 | |
| 2 | 1 | 101113 | 2 | 101195 | 1 | 18 | 1 | 1 | 11 | 1 | 3 | |
| 11 | 101560 | 0 | 9 | 0 | 100384 | 0 | 101409 | 5 | 1 | 102035 | 102198 | |
| 26 | 4 | 15 | 9 | 14 | 0 | 101124 | 5 | 9 | 101589 | 5 | 2 | |
| 5362 | 2372 | 2816 | 1949 | 2728 | 3542 | 2798 | 2526 | 4156 | 2343 | 1887 | 1706 | |
| 98582 | 101572 | 101128 | 101995 | 101216 | 100402 | 101146 | 101418 | 99788 | 101601 | 102057 | 102238 | |
| 99.96% | 0.01% | 0.00% | 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 99.98% | 0.00% | 0.02% | 0.03% | |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.96% | |
| 0.03% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | |

# FIGURE 35 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | C | C | C | A | G | A | C | T | G |
| **pNMG-171 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 8 | 11 | 1 | 3 | 3 | 110009 | 8 | 112489 | 0 | 2 | 7 |
| **C** | 1 | 4 | 110870 | 110617 | 110883 | 1 | 2 | 1 | 112385 | 9 | 3 |
| **G** | 111154 | 109235 | 2 | 0 | 0 | 12 | 112861 | 10 | 1 | 0 | 113054 |
| **T** | 10 | 3 | 9 | 2 | 12 | 4 | 6 | 5 | 7 | 113106 | 6 |
| **N** | 3160 | 5080 | 3451 | 3711 | 3435 | 4307 | 1456 | 1828 | 1940 | 1216 | 1263 |
| | 111173 | 109253 | 110882 | 110622 | 110898 | 110026 | 112877 | 112505 | 112393 | 113117 | 113070 |
| **A** | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 100.00% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 99.99% |
| **T** | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% |
| **pNMG-172 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 9 | 10 | 3 | 6 | 2 | 98686 | 4 | 101066 | 1 | 0 | 3 |
| **C** | 2 | 1 | 99653 | 99383 | 99594 | 2 | 1 | 0 | 100949 | 71 | 2 |
| **G** | 99910 | 98268 | 0 | 0 | 1 | 9 | 101389 | 2 | 1 | 0 | 101590 |
| **T** | 25 | 1 | 18 | 8 | 11 | 7 | 0 | 9 | 8 | 101511 | 3 |
| **N** | 2713 | 4379 | 2985 | 3262 | 3051 | 3955 | 1265 | 1582 | 1700 | 1077 | 1061 |
| | 99946 | 98280 | 99674 | 99397 | 99608 | 98704 | 101394 | 101077 | 100959 | 101582 | 101598 |
| **A** | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.98% | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.07% | 0.00% |
| **G** | 99.96% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 100.00% | 0.00% | 0.00% | 0.00% | 99.99% |
| **T** | 0.03% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.93% | 0.00% |

# FIGURE 35 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | G | C | A | C | G | T | G | A | T | G | G |
| 108533 | 6 | 3 | 112249 | 2 | 13 | 3 | 15 | 109677 | 15 | 9 | 30 |
| 0 | 0 | 111259 | 1 | 111271 | 2 | 13 | 13 | 0 | 8 | 0 | 12 |
| 20 | 111712 | 1 | 8 | 13 | 110481 | 1 | 111584 | 12 | 0 | 112206 | 112326 |
| 1 | 2 | 16 | 2 | 15 | 5 | 111316 | 4 | 5 | 111739 | 6 | 2 |
| 5779 | 2613 | 3054 | 2073 | 3032 | 3832 | 3000 | 2716 | 4638 | 2570 | 2110 | 1961 |
| 108554 | 111720 | 111279 | 112260 | 111301 | 110501 | 111333 | 111616 | 109694 | 111762 | 112221 | 112370 |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% | 99.97% | 0.01% | 0.00% | 99.99% | 99.96% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| 97465 | 8 | 3 | 100707 | 0 | 11 | 1 | 6 | 98573 | 4 | 7 | 16 |
| 2 | 2 | 99877 | 1 | 99808 | 0 | 10 | 2 | 1 | 8 | 2 | 2 |
| 14 | 100380 | 3 | 7 | 0 | 99032 | 1 | 100170 | 9 | 0 | 100751 | 100864 |
| 4 | 0 | 14 | 3 | 23 | 2 | 99929 | 1 | 10 | 100373 | 0 | 1 |
| 5174 | 2269 | 2762 | 1941 | 2828 | 3614 | 2718 | 2480 | 4066 | 2274 | 1899 | 1776 |
| 97485 | 100390 | 99897 | 100718 | 99831 | 99045 | 99941 | 100179 | 98593 | 100385 | 100760 | 100883 |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 99.98% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |



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# FIGURE 35 (Continued)

**pNMG-173 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 8 | 20 | 29 | 9 | 4 | 108814 | 7 | 111451 | 0 | 0 | 12 |
| **C** | 3 | 1 | 109796 | 109583 | 109885 | 3 | 3 | 2 | 111368 | 9 | 2 |
| **G** | 110053 | 108261 | 2 | 0 | 0 | 42 | 111773 | 82 | 0 | 1 | 111994 |
| **T** | 1 | 28 | 14 | 36 | 10 | 3 | 3 | 2 | 13 | 112085 | 22 |
| **N** | 3257 | 5012 | 3481 | 3694 | 3423 | 4460 | 1536 | 1785 | 1941 | 1227 | 1292 |
| | 110065 | 108310 | 109841 | 109628 | 109899 | 108862 | 111786 | 111537 | 111381 | 112095 | 112030 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.02% | 0.03% | 0.01% | 0.00% | 99.96% | 0.01% | 99.92% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.96% | 99.96% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.95% | 0.00% | 0.00% | 0.00% | 0.04% | 99.99% | 0.07% | 0.00% | 0.00% | 99.97% |
| **T** | 0.00% | 0.03% | 0.01% | 0.03% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.02% |

**pNMG-174 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V )**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 12 | 23 | 4 | 30 | 25 | 154252 | 5 | 158042 | 1 | 5 | 17 |
| **C** | 0 | 0 | 155788 | 154879 | 155181 | 6 | 3 | 0 | 157880 | 5 | 1 |
| **G** | 156313 | 153748 | 3 | 72 | 64 | 17 | 158548 | 72 | 2 | 3 | 158844 |
| **T** | 7 | 28 | 25 | 480 | 450 | 9 | 23 | 5 | 4 | 158897 | 33 |
| **N** | 4312 | 6845 | 4824 | 5183 | 4924 | 6360 | 2065 | 2525 | 2757 | 1734 | 1749 |
| | 156332 | 153799 | 155820 | 155461 | 155720 | 154284 | 158579 | 158119 | 157887 | 158910 | 158895 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.01% | 0.00% | 0.02% | 0.02% | 99.98% | 0.00% | 99.95% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.63% | 99.65% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% |
| **G** | 99.99% | 99.97% | 0.00% | 0.05% | 0.04% | 0.01% | 99.98% | 0.05% | 0.00% | 0.00% | 99.97% |
| **T** | 0.00% | 0.02% | 0.02% | 0.31% | 0.29% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.02% |



SUBSTITUTE SHEET (RULE 26)

FIGURE 35 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 107471 | 4 | 51 | 111232 | 1 | 18 | 1 | 9 | 108695 | 3 | 12 | 35 |
| 0 | 1 | 110126 | 1 | 110211 | 1 | 62 | 0 | 2 | 8 | 1 | 3 |
| 13 | 110737 | 0 | 7 | 1 | 109406 | 0 | 110529 | 10 | 0 | 111261 | 111308 |
| 1 | 3 | 9 | 6 | 11 | 1 | 110197 | 27 | 64 | 110807 | 1 | 4 |
| 5837 | 2577 | 3136 | 2076 | 3098 | 3896 | 3062 | 2757 | 4551 | 2504 | 2047 | 1972 |
| 107485 | 110745 | 110186 | 111246 | 110224 | 109426 | 110260 | 110565 | 108771 | 110818 | 111275 | 111350 |
| 99.99% | 0.00% | 0.05% | 99.99% | 0.00% | 0.02% | 0.00% | 0.01% | 99.93% | 0.00% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.95% | 0.00% | 99.99% | 0.00% | 0.06% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.97% | 0.01% | 0.00% | 99.99% | 99.96% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.94% | 0.02% | 0.06% | 99.99% | 0.00% | 0.00% |
| 152360 | 14 | 14 | 157664 | 31 | 35 | 5 | 11 | 154254 | 3 | 20 | 45 |
| 5 | 0 | 156223 | 2 | 156167 | 1 | 13 | 1 | 0 | 14 | 3 | 3 |
| 32 | 156972 | 2 | 16 | 1 | 155122 | 22 | 156685 | 5 | 1 | 157621 | 157837 |
| 39 | 11 | 23 | 6 | 28 | 0 | 156239 | 35 | 10 | 157034 | 2 | 11 |
| 8208 | 3647 | 4382 | 2956 | 4417 | 5486 | 4364 | 3911 | 6374 | 3591 | 2997 | 2747 |
| 152436 | 156997 | 156262 | 157688 | 156227 | 155158 | 156279 | 156732 | 154269 | 157052 | 157646 | 157896 |
| 99.95% | 0.01% | 0.01% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% |
| 0.00% | 0.00% | 99.98% | 0.0% | 100.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| 0.02% | 99.98% | 0.00% | 0.0% | 0.0% | 100.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 100.0% |
| 0.03% | 0.01% | 0.01% | 0.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% | 100.0% | 0.0% | 0.0% |

# FIGURE 35 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | C | C | C | A | G | A | C | T | G |
| **pNMG-175 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 8 | 11 | 3 | 20 | 5 | 117906 | 6 | 120830 | 0 | 1 | 14 |
| **C** | 2 | 3 | 118998 | 118715 | 118948 | 0 | 0 | 0 | 120641 | 5 | 1 |
| **G** | 117977 | 116191 | 0 | 0 | 1 | 14 | 121147 | 11 | 1 | 0 | 121328 |
| **T** | 6 | 4 | 17 | 10 | 8 | 8 | 8 | 4 | 11 | 121450 | 7 |
| **N** | 4819 | 6603 | 3794 | 4067 | 3850 | 4884 | 1651 | 1967 | 2159 | 1356 | 1462 |
| | 117993 | 116209 | 119018 | 118745 | 118962 | 117928 | 121161 | 120845 | 120653 | 121456 | 121350 |
| **A** | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.97% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% |
| **T** | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 100.00% | 0.01% |
| **pNMG-176 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S)** | | | | | | | | | | | |
| **A** | 3 | 9 | 1 | 7 | 1 | 110570 | 8 | 113378 | 1 | 1 | 12 |
| **C** | 0 | 5 | 111730 | 111278 | 111463 | 1 | 0 | 2 | 113233 | 8 | 3 |
| **G** | 110923 | 109112 | 3 | 0 | 1 | 19 | 113689 | 18 | 1 | 1 | 113920 |
| **T** | 1 | 3 | 15 | 86 | 158 | 14 | 3 | 3 | 31 | 113923 | 6 |
| **N** | 4300 | 6098 | 3478 | 3856 | 3604 | 4623 | 1527 | 1826 | 1961 | 1294 | 1286 |
| | 110927 | 109129 | 111749 | 111371 | 111623 | 110604 | 113700 | 113401 | 113266 | 113933 | 113941 |
| **A** | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.97% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.92% | 99.86% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 0.00% |
| **G** | 100.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.02% | 99.99% | 0.02% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.00% | 0.01% | 0.08% | 0.14% | 0.01% | 0.00% | 0.00% | 0.03% | 99.99% | 0.01% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 35 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | G | C | A | C | G | T | G | A | T | G | G |
| 116594 | 5 | 2 | 120476 | 2 | 12 | 5 | 2 | 117857 | 2 | 11 | 34 |
| 1 | 3 | 119472 | 0 | 119416 | 1 | 8 | 0 | 0 | 8 | 1 | 3 |
| 18 | 119903 | 0 | 5 | 0 | 118527 | 1 | 119715 | 8 | 0 | 120416 | 120626 |
| 2 | 6 | 18 | 7 | 11 | 5 | 119508 | 8 | 9 | 119969 | 8 | 9 |
| 6197 | 2895 | 3320 | 2324 | 3383 | 4267 | 3290 | 3087 | 4938 | 2833 | 2376 | 2140 |
| 116615 | 119917 | 119492 | 120488 | 119429 | 118545 | 119522 | 119725 | 117874 | 119979 | 120436 | 120672 |
| 99.98% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.96% |
| 0.00% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% |
| 109468 | 7 | 1 | 113080 | 0 | 14 | 5 | 9 | 110564 | 3 | 20 | 28 |
| 0 | 0 | 112197 | 0 | 112099 | 1 | 11 | 0 | 0 | 14 | 1 | 3 |
| 21 | 112595 | 0 | 10 | 1 | 111319 | 1 | 112276 | 12 | 0 | 112979 | 113217 |
| 7 | 2 | 11 | 4 | 15 | 2 | 112022 | 3 | 6 | 112540 | 1 | 3 |
| 5731 | 2623 | 3018 | 2133 | 3112 | 3891 | 3188 | 2939 | 4645 | 2670 | 2226 | 1976 |
| 109496 | 112604 | 112209 | 113094 | 112115 | 111336 | 112039 | 112288 | 110582 | 112557 | 113001 | 113251 |
| 99.97% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.02% | 0.02% |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 35 (Continued)

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 9 | 13 | 5 | 17 | 3 | 101939 | 4 | 104100 | 2 | 1 | 14 |
| **C** | 4 | 2 | 102993 | 102770 | 103058 | 1 | 1 | 0 | 104619 | 16 | 2 |
| **G** | 102445 | 100859 | 0 | 0 | 66 | 342 | 105003 | 653 | 1 | 2 | 105218 |
| **T** | 3 | 6 | 16 | 140 | 135 | 0 | 4 | 9 | 9 | 105257 | 5 |
| **N** | 3935 | 5516 | 3382 | 3469 | 3134 | 4114 | 1384 | 1634 | 1765 | 1120 | 1157 |
| | 102461 | 100880 | 103014 | 102927 | 103262 | 102282 | 105012 | 104762 | 104631 | 105276 | 105239 |
| **A** | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% | 99.66% | 0.00% | 99.37% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.85% | 99.80% | 0.00% | 0.00% | 0.00% | 99.99% | 0.02% | 0.00% |
| **G** | 99.98% | 99.98% | 0.00% | 0.00% | 0.06% | 0.33% | 99.99% | 0.62% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.01% | 0.02% | 0.14% | 0.13% | 0.00% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% |

**pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 15 | 18 | 19 | 3 | 1 | 109369 | 7 | 111446 | 0 | 2 | 7 |
| **C** | 0 | 1 | 110430 | 110279 | 110601 | 2 | 1 | 0 | 112200 | 12 | 1 |
| **G** | 109817 | 107986 | 0 | 1 | 0 | 215 | 112671 | 858 | 0 | 0 | 112926 |
| **T** | 4 | 1 | 15 | 27 | 23 | 4 | 4 | 1 | 8 | 112947 | 4 |
| **N** | 4338 | 6168 | 3710 | 3864 | 3549 | 4584 | 1491 | 1869 | 1966 | 1213 | 1236 |
| | 109836 | 108006 | 110464 | 110310 | 110625 | 109590 | 112683 | 112305 | 112208 | 112961 | 112938 |
| **A** | 0.01% | 0.02% | 0.02% | 0.00% | 0.00% | 99.80% | 0.01% | 99.24% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.97% | 99.97% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 0.20% | 99.99% | 0.76% | 0.00% | 0.00% | 99.99% |
| **T** | 0.00% | 0.00% | 0.01% | 0.02% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| **T** | | | | | | | | | | | |

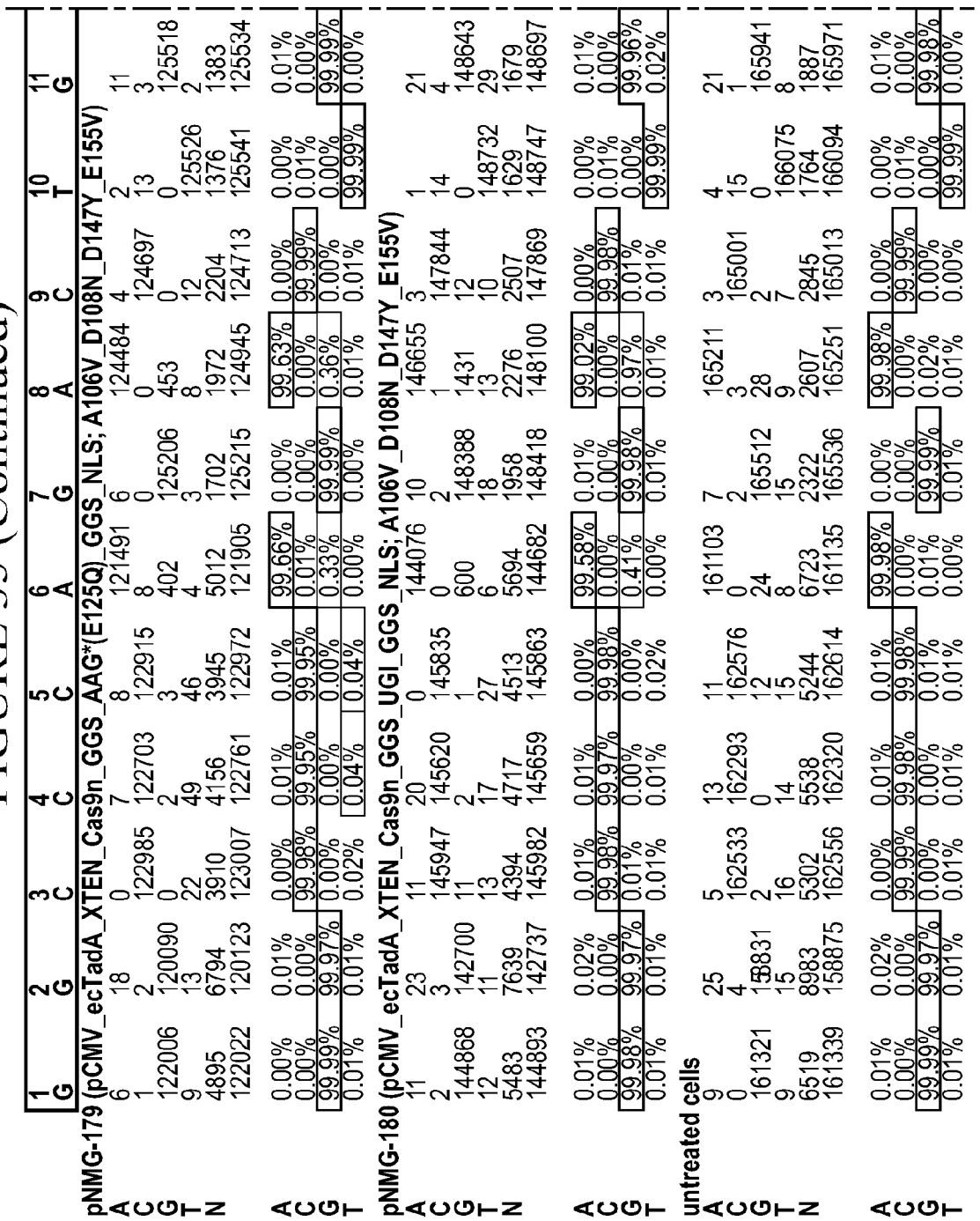


SUBSTITUTE SHEET (RULE 26)

FIGURE 35 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101157 | 13 | 10 | 104422 | 1 | 13 | 3 | 3 | 102159 | 1 | 5 | 26 | |
| 0 | 1 | 103686 | 10 | 103538 | 0 | 11 | 0 | 1 | 4 | 1 | 2 | |
| 24 | 104092 | 1 | 7 | 1 | 102800 | 5 | 103812 | 15 | 0 | 104429 | 104639 | |
| 0 | 2 | 20 | 5 | 28 | 10 | 103500 | 1 | 16 | 104047 | 3 | 3 | |
| 5215 | 2288 | 2679 | 1952 | 2828 | 3573 | 2877 | 2580 | 4205 | 2344 | 1958 | 1726 | |
| 101181 | 104108 | 103717 | 104444 | 103568 | 102823 | 103519 | 103816 | 102191 | 104052 | 104438 | 104670 | |
| 99.98% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 99.97% | 0.00% | 0.00% | 0.02% | |
| 0.00% | 0.00% | 99.97% | 0.01% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.02% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 100.00% | 0.01% | 0.00% | 99.99% | 99.97% | |
| 0.00% | 0.00% | 0.02% | 0.00% | 0.03% | 0.01% | 99.98% | 0.00% | 0.02% | 100.00% | 0.00% | 0.00% | |
| 108443 | 9 | 0 | 112074 | 25 | 10 | 3 | 3 | 109656 | 2 | 8 | 35 | |
| 2 | 0 | 111107 | 2 | 111120 | 0 | 13 | 2 | 2 | 2 | 1 | 5 | |
| 19 | 111578 | 12 | 10 | 2 | 110323 | 0 | 111279 | 8 | 0 | 111970 | 112237 | |
| 1 | 2 | 11 | 4 | 20 | 27 | 111062 | 7 | 2 | 111534 | 2 | 2 | |
| 5709 | 2585 | 3044 | 2084 | 3032 | 3839 | 3121 | 2908 | 4531 | 2661 | 2218 | 1920 | |
| 108465 | 111589 | 111130 | 112090 | 111167 | 110360 | 111078 | 111291 | 109668 | 111538 | 111981 | 112279 | |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.02% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 0.03% | |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.96% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.02% | 99.99% | 0.01% | 0.01% | 0.00% | 99.97% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 99.96% | |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.02% | 99.99% | 0.01% | 0.00% | 100.00% | 0.00% | 0.00% | |

FIGURE 35 (Continued)



| | 1 G | 2 G | 3 C | 4 C | 5 C | 6 A | 7 G | 8 A | 9 C | 10 T | 11 G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 6 | 18 | 0 | 7 | 8 | 121491 | 6 | 124484 | 4 | 2 | 11 |
| C | 1 | 2 | 122985 | 122703 | 122915 | 8 | 0 | 0 | 124697 | 13 | 3 |
| G | 122006 | 120090 | 0 | 2 | 3 | 402 | 125206 | 453 | 0 | 0 | 125518 |
| T | 9 | 13 | 22 | 49 | 46 | 4 | 3 | 8 | 12 | 125526 | 2 |
| N | 4895 | 6794 | 3910 | 4156 | 3945 | 5012 | 1702 | 1972 | 2204 | 1376 | 1383 |
| | 122022 | 120123 | 123007 | 122761 | 122972 | 121905 | 125215 | 124945 | 124713 | 125541 | 125534 |
| A | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.66% | 0.00% | 99.63% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.95% | 99.95% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 0.33% | 99.99% | 0.36% | 0.00% | 0.00% | 99.99% |
| T | 0.01% | 0.01% | 0.02% | 0.04% | 0.04% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.00% |
| **pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 11 | 23 | 11 | 20 | 0 | 144076 | 10 | 146655 | 3 | 1 | 21 |
| C | 2 | 3 | 145947 | 145620 | 145835 | 0 | 2 | 1 | 147844 | 14 | 4 |
| G | 144868 | 142700 | 11 | 2 | 1 | 600 | 148388 | 1431 | 12 | 0 | 148643 |
| T | 12 | 11 | 13 | 17 | 27 | 6 | 18 | 13 | 10 | 148732 | 29 |
| N | 5483 | 7639 | 4394 | 4717 | 4513 | 5694 | 1958 | 2276 | 2507 | 1629 | 1679 |
| | 144893 | 142737 | 145982 | 145659 | 145863 | 144682 | 148418 | 148100 | 147869 | 148747 | 148697 |
| A | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 99.58% | 0.01% | 99.02% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.97% | 99.98% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| G | 99.98% | 99.97% | 0.01% | 0.00% | 0.00% | 0.41% | 99.98% | 0.97% | 0.01% | 0.00% | 99.96% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.02% |
| **untreated cells** | | | | | | | | | | | |
| A | 9 | 25 | 5 | 13 | 11 | 161103 | 7 | 165211 | 3 | 4 | 21 |
| C | 0 | 4 | 162533 | 162293 | 162576 | 0 | 2 | 3 | 165001 | 15 | 1 |
| G | 161321 | 158831 | 2 | 0 | 12 | 24 | 165512 | 28 | 2 | 0 | 165941 |
| T | 9 | 15 | 16 | 14 | 15 | 8 | 15 | 9 | 7 | 166075 | 8 |
| N | 6519 | 8983 | 5302 | 5538 | 5244 | 6723 | 2322 | 2607 | 2845 | 1764 | 1887 |
| | 161339 | 158875 | 162556 | 162320 | 162614 | 161135 | 165536 | 165251 | 165013 | 166094 | 165971 |
| A | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.99% | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.97% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.02% | 0.00% | 0.00% | 99.98% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% |

FIGURE 35 (Continued)

| 12 A | 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 120721 | 7 | 2 | 124564 | 5 | 12 | 3 | 8 | 121886 | 3 | 13 | 24 |
| 2 | 2 | 123533 | 2 | 123498 | 0 | 13 | 1 | 1 | 8 | 2 | 6 |
| 17 | 124069 | 1 | 4 | 1 | 122617 | 0 | 123826 | 6 | 2 | 124468 | 124685 |
| 1 | 1 | 11 | 8 | 14 | 2 | 123500 | 3 | 5 | 124090 | 2 | 4 |
| 6176 | 2838 | 3370 | 2339 | 3399 | 4286 | 3398 | 3076 | 5016 | 2811 | 2429 | 2195 |
| 120741 | 124079 | 123547 | 124578 | 123518 | 122631 | 123516 | 123838 | 121898 | 124103 | 124485 | 124719 |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 99.97% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% |
| 143031 | 14 | 11 | 147672 | 7 | 18 | 12 | 7 | 144333 | 3 | 19 | 32 |
| 2 | 3 | 146303 | 1 | 146103 | 2 | 12 | 0 | 2 | 15 | 3 | 3 |
| 24 | 147029 | 20 | 22 | 2 | 145188 | 1 | 146546 | 11 | 0 | 147474 | 147711 |
| 4 | 8 | 20 | 5 | 16 | 4 | 146327 | 19 | 11 | 146903 | 13 | 4 |
| 7315 | 3322 | 4022 | 2676 | 4247 | 5168 | 4026 | 3806 | 6021 | 3457 | 2869 | 2628 |
| 143061 | 147054 | 146354 | 147700 | 146128 | 145212 | 146352 | 146572 | 144357 | 146921 | 147509 | 147750 |
| 99.98% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.97% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.98% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 99.98% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% |
| 159624 | 11 | 16 | 164706 | 4 | 14 | 2 | 10 | 161199 | 4 | 15 | 36 |
| 1 | 3 | 163407 | 2 | 163259 | 1 | 18 | 2 | 3 | 11 | 3 | 5 |
| 19 | 164025 | 2 | 18 | 0 | 162164 | 0 | 163776 | 19 | 0 | 164606 | 164833 |
| 4 | 9 | 15 | 5 | 16 | 7 | 163209 | 9 | 10 | 163970 | 4 | 7 |
| 8210 | 3810 | 4418 | 3127 | 4579 | 5672 | 4629 | 4061 | 6627 | 3873 | 3230 | 2977 |
| 159648 | 164048 | 163440 | 164731 | 163279 | 162186 | 163229 | 163797 | 161231 | 163985 | 164628 | 164881 |
| 99.98% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.00% |

FIGURE 36

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | C | A | C | T | G | C | G | G | C |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 1 | 92 | 2 | 75062 | 45 | 1 | 2 | 1 | 5 | 37 | 33 |
| **C** | 0 | 0 | 73348 | 1 | 75478 | 14 | 0 | 78095 | 1 | 5 | 62249 |
| **G** | 60630 | 69087 | 0 | 4 | 78 | 1 | 78780 | 3 | 78622 | 67346 | 2 |
| **T** | 27 | 0 | 10 | 3 | 114 | 77698 | 1 | 126 | 4 | 0 | 6 |
| **N** | 20250 | 11729 | 7548 | 5838 | 5193 | 3194 | 2125 | 2683 | 2276 | 13520 | 18618 |
| | 60658 | 69179 | 73360 | 75070 | 75715 | 77714 | 78783 | 78225 | 78632 | 67388 | 62290 |
| **A** | 0.00% | 0.13% | 0.00% | 99.99% | 0.06% | 0.00% | 0.00% | 0.00% | 0.01% | 0.05% | 0.05% |
| **C** | 0.00% | 0.00% | 99.98% | 0.00% | 99.69% | 0.02% | 0.00% | 99.83% | 0.00% | 0.01% | 99.93% |
| **G** | 99.95% | 99.87% | 0.00% | 0.01% | 0.10% | 0.00% | 100.00% | 0.00% | 99.99% | 99.94% | 0.00% |
| **T** | 0.04% | 0.00% | 0.01% | 0.00% | 0.15% | 99.98% | 0.00% | 0.16% | 0.01% | 0.00% | 0.01% |
| **pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 1 | 66 | 1 | 57502 | 0 | 0 | 4 | 0 | 7 | 6 | 1 |
| **C** | 0 | 1 | 56500 | 1 | 57689 | 6 | 0 | 60067 | 0 | 0 | 48510 |
| **G** | 46879 | 53258 | 1 | 60 | 225 | 0 | 60622 | 2 | 60356 | 52582 | 0 |
| **T** | 1 | 0 | 4 | 3 | 225 | 59653 | 1 | 91 | 2 | 1 | 9 |
| **N** | 15415 | 8971 | 5790 | 4730 | 4157 | 2637 | 1669 | 2136 | 1931 | 9707 | 13776 |
| | 46881 | 53325 | 56506 | 57566 | 58139 | 59659 | 60627 | 60160 | 60365 | 52589 | 48520 |
| **A** | 0.00% | 0.12% | 0.00% | 99.89% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 99.99% | 0.00% | 99.23% | 0.01% | 0.00% | 99.85% | 0.00% | 0.00% | 99.98% |
| **G** | 100.00% | 99.87% | 0.00% | 0.10% | 0.39% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.39% | 99.99% | 0.00% | 0.15% | 0.00% | 0.00% | 0.02% |

FIGURE 36 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 4 | 1 | 62446 | 2 | 3 | 0 | 3 | 3 | 1 | 2 | 6 |
| 7 | 2 | 0 | 1 | 0 | 0 | 2 | 1 | 0 | 0 | 1 | 0 |
| 0 | 63948 | 78726 | 15 | 75651 | 78044 | 23 | 60327 | 75472 | 67534 | 68646 | 56922 |
| 61446 | 2 | 20 | 0 | 2 | 1 | 14607 | 4 | 2 | 1 | 0 | 6 |
| 19455 | 16952 | 2161 | 18446 | 5253 | 2860 | 66276 | 20573 | 5431 | 13372 | 12259 | 23974 |
| 61453 | 63956 | 78747 | 62462 | 75655 | 78048 | 14632 | 60335 | 75477 | 67536 | 68649 | 56934 |
| 0.00% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 99.97% | 0.02% | 99.99% | 99.99% | 0.16% | 99.99% | 99.99% | 100.00 % | 100.00 % | 99.98% |
| 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 99.83% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| 1 | 4 | 2 | 47635 | 1 | 2 | 0 | 1 | 0 | 2 | 4 | 2 |
| 4 | 0 | 1 | 0 | 2 | 0 | 2 | 1 | 2 | 0 | 0 | 1 |
| 0 | 49638 | 60596 | 12 | 58094 | 59974 | 20 | 46069 | 57976 | 52679 | 53547 | 44059 |
| 47418 | 0 | 0 | 1 | 1 | 0 | 11103 | 2 | 3 | 0 | 0 | 7 |
| 14873 | 12654 | 1697 | 14648 | 4198 | 2320 | 51171 | 16223 | 4315 | 9615 | 8745 | 18227 |
| 47423 | 49642 | 60599 | 47648 | 58098 | 59976 | 11125 | 46073 | 57981 | 52681 | 53551 | 44069 |
| 0.00% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 100.00 % | 0.03% | 99.99% | 100.00 % | 0.18% | 99.99% | 99.99% | 100.00 % | 99.99% | 99.98% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.80% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% |

FIGURE 36 (Continued)

**pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 5 | 108 | 0 | 54865 | 35 | 0 | 7 | 29 | 7 | 20 | 0 |
| **C** | 1 | 0 | 53649 | 0 | 54978 | 9 | 0 | 56916 | 0 | 0 | 45642 |
| **G** | 44310 | 50392 | 1 | 14 | 160 | 1 | 57538 | 2 | 57289 | 49370 | 2 |
| **T** | 0 | 2 | 12 | 4 | 147 | 56743 | 0 | 136 | 3 | 2 | 8 |
| **N** | 14716 | 8530 | 5370 | 4147 | 3710 | 2277 | 1485 | 1947 | 1731 | 9638 | 13378 |
| | 44316 | 50502 | 53662 | 54883 | 55320 | 56753 | 57545 | 57083 | 57299 | 49392 | 45652 |
| | | | | | | | | | | | |
| **A** | 0.01% | 0.21% | 0.00% | 99.97% | 0.06% | 0.00% | 0.01% | 0.05% | 0.01% | 0.04% | 0.00% |
| **C** | 0.00% | 0.00% | 99.98% | 0.00% | 99.38% | 0.02% | 0.00% | 99.71% | 0.00% | 0.00% | 99.98% |
| **G** | 99.99% | 99.78% | 0.00% | 0.03% | 0.29% | 0.00% | 99.99% | 0.00% | 99.98% | 99.96% | 0.00% |
| **T** | 0.00% | 0.00% | 0.02% | 0.01% | 0.27% | 99.98% | 0.00% | 0.24% | 0.01% | 0.00% | 0.02% |

**pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 2 | 201 | 0 | 69114 | 0 | 1 | 15 | 0 | 2 | 21 | 0 |
| **C** | 1 | 1 | 67566 | 0 | 69536 | 8 | 0 | 71619 | 0 | 0 | 57360 |
| **G** | 55760 | 63433 | 1 | 26 | 65 | 1 | 72400 | 2 | 72254 | 61979 | 0 |
| **T** | 1 | 15 | 15 | 3 | 132 | 71506 | 7 | 193 | 4 | 60 | 43 |
| **N** | 18608 | 10722 | 6790 | 5229 | 4639 | 2856 | 1950 | 2558 | 2112 | 12312 | 16969 |
| | 55764 | 63650 | 67582 | 69143 | 69733 | 71516 | 72422 | 71814 | 72260 | 62060 | 57403 |
| | | | | | | | | | | | |
| **A** | 0.00% | 0.32% | 0.00% | 99.96% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.03% | 0.00% |
| **C** | 0.00% | 0.00% | 99.98% | 0.00% | 99.72% | 0.01% | 0.00% | 99.73% | 0.00% | 0.00% | 99.93% |
| **G** | 99.99% | 99.66% | 0.00% | 0.04% | 0.09% | 0.00% | 99.97% | 0.00% | 99.99% | 99.87% | 0.00% |
| **T** | 0.00% | 0.02% | 0.02% | 0.00% | 0.19% | 99.99% | 0.01% | 0.27% | 0.01% | 0.10% | 0.07% |

FIGURE 36 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 4 | 3 | 45958 | 2 | 0 | 0 | 3 | 1 | 2 | 4 | 5 |
| 4 | 0 | 0 | 1 | 0 | 0 | 7 | 1 | 1 | 0 | 0 | 2 |
| 0 | 46914 | 57395 | 13 | 55121 | 56922 | 20 | 43640 | 54897 | 49481 | 50307 | 41922 |
| 44904 | 0 | 1 | 1 | 1 | 0 | 10742 | 2 | 2 | 1 | 1 | 5 |
| 14118 | 12112 | 1631 | 13057 | 3906 | 2109 | 48262 | 15385 | 4130 | 9547 | 8719 | 17097 |
| 44912 | 46918 | 57399 | 45973 | 55124 | 56922 | 10769 | 43646 | 54901 | 49484 | 50312 | 41934 |
| 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.07% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 99.99% | 0.03% | 99.99% | 100.00% | 0.19% | 99.99% | 99.99% | 99.99% | 99.99% | 99.97% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.75% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0 | 4 | 1 | 57447 | 4 | 1 | 0 | 2 | 0 | 2 | 5 | 8 |
| 57 | 0 | 0 | 0 | 1 | 0 | 4 | 3 | 1 | 0 | 2 | 0 |
| 1 | 58858 | 72311 | 26 | 69344 | 71704 | 20 | 55020 | 69178 | 62009 | 63130 | 52702 |
| 56207 | 2 | 0 | 0 | 31 | 0 | 13522 | 143 | 13 | 4 | 0 | 7 |
| 18107 | 15508 | 2060 | 16899 | 4992 | 2667 | 60826 | 19204 | 5180 | 12357 | 11235 | 21655 |
| 56265 | 58864 | 72312 | 57473 | 69380 | 71705 | 13546 | 55168 | 69192 | 62015 | 63137 | 52717 |
| 0.00% | 0.01% | 0.00% | 99.95% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% |
| 0.10% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 100.00% | 0.05% | 99.95% | 100.00% | 0.15% | 99.73% | 99.98% | 99.99% | 99.99% | 99.97% |
| 99.90% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 99.82% | 0.26% | 0.02% | 0.01% | 0.00% | 0.01% |

FIGURE 36 (Continued)

| | 1 G | 2 G | 3 C | 4 A | 5 C | 6 T | 7 G | 8 C | 9 G | 10 G | 11 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-171 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 5 | 219 | 1 | 66603 | 67 | 2 | 23 | 28 | 9 | 8 | 0 |
| **C** | 0 | 1 | 65034 | 0 | 66950 | 17 | 1 | 69200 | 18 | 0 | 55868 |
| **G** | 54209 | 61131 | 0 | 3 | 18 | 5 | 69984 | 2 | 69740 | 60357 | 1 |
| **T** | 2 | 28 | 12 | 4 | 168 | 68995 | 3 | 215 | 4 | 0 | 22 |
| **N** | 17599 | 10436 | 6768 | 5205 | 4612 | 2796 | 1804 | 2370 | 2044 | 11450 | 15924 |
| | 54216 | 61379 | 65047 | 66610 | 67203 | 69019 | 70011 | 69445 | 69771 | 60365 | 55891 |
| **A** | 0.01% | 0.36% | 0.00% | 99.99% | 0.10% | 0.00% | 0.03% | 0.04% | 0.01% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 99.98% | 0.00% | 99.62% | 0.02% | 0.00% | 99.65% | 0.03% | 0.00% | 99.96% |
| **G** | 99.99% | 99.60% | 0.00% | 0.00% | 0.03% | 0.01% | 99.96% | 0.00% | 99.96% | 99.99% | 0.00% |
| **T** | 0.00% | 0.05% | 0.02% | 0.01% | 0.25% | 99.97% | 0.00% | 0.31% | 0.01% | 0.00% | 0.04% |
| **pNMG-172 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 7 | 199 | 15 | 68570 | 123 | 0 | 9 | 1 | 4 | 24 | 35 |
| **C** | 0 | 2 | 67159 | 0 | 68857 | 27 | 0 | 71311 | 0 | 1 | 57628 |
| **G** | 56008 | 63245 | 0 | 39 | 61 | 0 | 72113 | 2 | 71873 | 62133 | 2 |
| **T** | 3 | 1 | 28 | 3 | 253 | 71081 | 28 | 259 | 3 | 1 | 14 |
| **N** | 17989 | 10560 | 6805 | 5394 | 4712 | 2898 | 1856 | 2433 | 2126 | 11847 | 16327 |
| | 56018 | 63447 | 67202 | 68612 | 69294 | 71108 | 72150 | 71573 | 71880 | 62159 | 57679 |
| **A** | 0.01% | 0.31% | 0.02% | 99.94% | 0.18% | 0.00% | 0.01% | 0.00% | 0.01% | 0.04% | 0.06% |
| **C** | 0.00% | 0.00% | 99.94% | 0.00% | 99.37% | 0.04% | 0.00% | 99.63% | 0.00% | 0.00% | 99.91% |
| **G** | 99.98% | 99.68% | 0.00% | 0.06% | 0.09% | 0.00% | 99.95% | 0.00% | 99.99% | 99.96% | 0.00% |
| **T** | 0.01% | 0.00% | 0.04% | 0.00% | 0.37% | 99.96% | 0.04% | 0.36% | 0.00% | 0.00% | 0.02% |

FIGURE 36 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 55764 | 3 | 2 | 0 | 4 | 0 | 3 | 3 | 7 |
| 10 | 0 | 0 | 0 | 0 | 0 | 9 | 2 | 0 | 0 | 2 | 1 |
| 1 | 57240 | 69840 | 25 | 67053 | 69233 | 21 | 53401 | 67054 | 60518 | 61413 | 50937 |
| 54838 | 9 | 10 | 0 | 35 | 2 | 13048 | 0 | 1 | 3 | 2 | 3 |
| 16966 | 14565 | 1963 | 16026 | 4724 | 2578 | 58737 | 18408 | 4760 | 11291 | 10395 | 20867 |
| 54849 | 57250 | 69852 | 55789 | 67091 | 69237 | 13078 | 53407 | 67055 | 60524 | 61420 | 50948 |
| 0.00% | 0.00% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.07% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.98% | 99.98% | 0.04% | 99.94% | 99.99% | 0.16% | 99.99% | 100.00 % | 99.99% | 99.99% | 99.98% |
| 99.98% | 0.02% | 0.01% | 0.00% | 0.05% | 0.00% | 99.77% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0 | 1 | 0 | 57575 | 1 | 1 | 1 | 2 | 5 | 6 | 8 | 5 |
| 6 | 1 | 1 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 2 |
| 0 | 59047 | 72098 | 24 | 69389 | 71479 | 15 | 55793 | 69278 | 62374 | 63293 | 52738 |
| 56751 | 7 | 0 | 0 | 2 | 0 | 13951 | 89 | 3 | 0 | 0 | 6 |
| 17249 | 14950 | 1907 | 16406 | 4614 | 2526 | 60036 | 18122 | 4720 | 11626 | 10705 | 21255 |
| 56757 | 59056 | 72099 | 57600 | 69392 | 71480 | 13970 | 55884 | 69286 | 62380 | 63301 | 52751 |
| 0.00% | 0.00% | 0.00% | 99.96% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.98% | 100.00 % | 0.04% | 100.00 % | 100.00 % | 0.11% | 99.84% | 99.99% | 99.99% | 99.99% | 99.98% |
| 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 99.86% | 0.16% | 0.00% | 0.00% | 0.00% | 0.01% |

# FIGURE 36 (Continued)

**pNMG-173 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 215 | 1 | 80227 | 0 | 4 | 11 | 2 | 9 | 23 | 12 |
| C | 1 | 0 | 78443 | 0 | 80612 | 21 | 1 | 83456 | 0 | 0 | 67680 |
| G | 65751 | 73604 | 2 | 7 | 173 | 0 | 84292 | 1 | 84116 | 72975 | 1 |
| T | 5 | 19 | 30 | 5 | 288 | 83111 | 19 | 235 | 6 | 1 | 31 |
| N | 20753 | 12677 | 8039 | 6275 | 5441 | 3378 | 2191 | 2820 | 2383 | 13515 | 18790 |
| | 65762 | 73838 | 78476 | 80239 | 81073 | 83136 | 84323 | 83694 | 84131 | 72999 | 67724 |
| | | | | | | | | | | | |
| A | 0.01% | 0.29% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.03% | 0.02% |
| C | 0.00% | 0.00% | 99.96% | 0.00% | 99.43% | 0.03% | 0.00% | 99.72% | 0.00% | 0.00% | 99.94% |
| G | 99.98% | 99.68% | 0.00% | 0.01% | 0.21% | 0.00% | 99.96% | 0.00% | 99.98% | 99.97% | 0.00% |
| T | 0.01% | 0.03% | 0.04% | 0.01% | 0.36% | 99.97% | 0.02% | 0.28% | 0.01% | 0.00% | 0.05% |

**pNMG-174 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V )**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 225 | 25 | 86388 | 30 | 4 | 7 | 14 | 6 | 37 | 1 |
| C | 0 | 2 | 84425 | 1 | 86908 | 19 | 0 | 89844 | 22 | 0 | 72441 |
| G | 70158 | 79440 | 2 | 30 | 35 | 2 | 90740 | 0 | 90477 | 78387 | 1 |
| T | 25 | 1 | 19 | 3 | 208 | 89445 | 1 | 254 | 32 | 13 | 23 |
| N | 23034 | 13551 | 8748 | 6797 | 6038 | 3749 | 2471 | 3107 | 2682 | 14782 | 20753 |
| | 70185 | 79668 | 84471 | 86422 | 87181 | 89470 | 90748 | 90112 | 90537 | 78437 | 72466 |
| | | | | | | | | | | | |
| A | 0.00% | 0.28% | 0.03% | 99.96% | 0.03% | 0.00% | 0.01% | 0.02% | 0.01% | 0.05% | 0.00% |
| C | 0.00% | 0.00% | 99.95% | 0.00% | 99.69% | 0.02% | 0.00% | 99.70% | 0.02% | 0.00% | 99.97% |
| G | 99.96% | 99.71% | 0.00% | 0.03% | 0.04% | 0.00% | 99.99% | 0.00% | 99.93% | 99.94% | 0.00% |
| T | 0.04% | 0.00% | 0.02% | 0.00% | 0.24% | 99.97% | 0.00% | 0.28% | 0.04% | 0.02% | 0.03% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 36 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 4 | 2 | 66892 | 4 | 4 | 0 | 2 | 3 | 12 | 4 | 18 |
| 10 | 1 | 0 | 1 | 2 | 0 | 4 | 0 | 1 | 0 | 1 | 1 |
| 0 | 69359 | 84198 | 31 | 80857 | 83476 | 25 | 64503 | 80604 | 73192 | 74344 | 61496 |
| 66430 | 20 | 6 | 1 | 21 | 3 | 15812 | 3 | 5 | 1 | 1 | 10 |
| 20074 | 17130 | 2308 | 19589 | 5630 | 3031 | 70675 | 22008 | 5903 | 13311 | 12166 | 24991 |
| 66440 | 69384 | 84206 | 66925 | 80884 | 83483 | 15841 | 64508 | 80613 | 73205 | 74350 | 61525 |
| 0.00% | 0.01% | 0.00% | 99.95% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.01% | 0.03% |
| 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.96% | 99.99% | 0.05% | 99.97% | 99.99% | 0.16% | 99.99% | 99.99% | 99.98% | 99.99% | 99.95% |
| 99.98% | 0.03% | 0.01% | 0.00% | 0.03% | 0.00% | 99.82% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% |
| 4 | 5 | 0 | 71786 | 1 | 3 | 1 | 5 | 0 | 2 | 5 | 14 |
| 18 | 2 | 0 | 0 | 0 | 0 | 10 | 1 | 1 | 0 | 0 | 3 |
| 1 | 74092 | 90595 | 19 | 87006 | 89809 | 35 | 69250 | 86988 | 78547 | 79744 | 66239 |
| 71149 | 24 | 18 | 0 | 53 | 1 | 17061 | 27 | 3 | 6 | 5 | 9 |
| 22047 | 19096 | 2606 | 21414 | 6159 | 3407 | 76113 | 23937 | 6228 | 14665 | 13466 | 26955 |
| 71172 | 74123 | 90613 | 71805 | 87060 | 89813 | 17107 | 69283 | 86992 | 78555 | 79754 | 66265 |
| 0.01% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.02% |
| 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.06% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.96% | 99.98% | 0.03% | 99.94% | 100.00 % | 0.20% | 99.95% | 100.00 % | 99.99% | 99.99% | 99.96% |
| 99.97% | 0.03% | 0.02% | 0.00% | 0.06% | 0.00% | 99.73% | 0.04% | 0.00% | 0.01% | 0.01% | 0.01% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 36 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>A | 5<br>C | 6<br>T | 7<br>G | 8<br>C | 9<br>G | 10<br>G | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-175 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 9 | 202 | 14 | 71205 | 1 | 3 | 9 | 31 | 9 | 19 | 1 |
| **C** | 1 | 1 | 69574 | 0 | 71400 | 10 | 0 | 73854 | 0 | 1 | 59444 |
| **G** | 57514 | 65370 | 1 | 11 | 134 | 3 | 74692 | 2 | 74504 | 63818 | 2 |
| **T** | 4 | 0 | 18 | 4 | 291 | 73640 | 2 | 270 | 5 | 47 | 8 |
| **N** | 19102 | 11057 | 7023 | 5410 | 4804 | 2974 | 1927 | 2473 | 2112 | 12745 | 17175 |
| | 57528 | 65573 | 69607 | 71220 | 71826 | 73656 | 74703 | 74157 | 74518 | 63885 | 59455 |
| **A** | 0.02% | 0.31% | 0.02% | 99.98% | 0.00% | 0.00% | 0.01% | 0.04% | 0.01% | 0.03% | 0.00% |
| **C** | 0.00% | 0.00% | 99.95% | 0.00% | 99.41% | 0.01% | 0.00% | 99.59% | 0.00% | 0.00% | 99.98% |
| **G** | 99.98% | 99.69% | 0.00% | 0.02% | 0.19% | 0.00% | 99.99% | 0.00% | 99.98% | 99.90% | 0.00% |
| **T** | 0.01% | 0.00% | 0.03% | 0.01% | 0.41% | 99.98% | 0.00% | 0.36% | 0.01% | 0.07% | 0.01% |
| **pNMG-176 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S)** | | | | | | | | | | | |
| **A** | 7 | 145 | 29 | 69155 | 117 | 2 | 3 | 0 | 5 | 12 | 0 |
| **C** | 0 | 0 | 67673 | 3 | 68926 | 16 | 0 | 71946 | 0 | 1 | 58207 |
| **G** | 56157 | 63541 | 0 | 35 | 396 | 2 | 72644 | 1 | 72530 | 62997 | 2 |
| **T** | 0 | 0 | 15 | 1 | 367 | 71509 | 12 | 196 | 4 | 1 | 7 |
| **N** | 18395 | 10873 | 6842 | 5365 | 4753 | 3030 | 1900 | 2416 | 2020 | 11548 | 16343 |
| | 56164 | 63686 | 67717 | 69194 | 69806 | 71529 | 72659 | 72143 | 72539 | 63011 | 58216 |
| **A** | 0.01% | 0.23% | 0.04% | 99.94% | 0.17% | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 0.00% |
| **C** | 0.00% | 0.00% | 99.94% | 0.00% | 98.74% | 0.02% | 0.00% | 99.73% | 0.00% | 0.00% | 99.98% |
| **G** | 99.99% | 99.77% | 0.00% | 0.05% | 0.57% | 0.00% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% |
| **T** | 0.00% | 0.00% | 0.02% | 0.00% | 0.53% | 99.97% | 0.02% | 0.27% | 0.01% | 0.00% | 0.01% |

FIGURE 36 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 3 | 0 | 60061 | 2 | 6 | 0 | 17 | 5 | 3 | 7 | 14 |
| 10 | 0 | 37 | 0 | 0 | 1 | 8 | 0 | 1 | 0 | 1 | 1 |
| 0 | 61636 | 74716 | 12 | 71916 | 74114 | 26 | 57944 | 71905 | 64284 | 65211 | 54324 |
| 59736 | 4 | 3 | 0 | 5 | 3 | 14307 | 14 | 4 | 12 | 4 | 8 |
| 16884 | 14987 | 1874 | 16557 | 4707 | 2506 | 62289 | 18655 | 4715 | 12331 | 11407 | 22283 |
| 59746 | 61643 | 74756 | 60073 | 71923 | 74124 | 14341 | 57975 | 71915 | 64299 | 65223 | 54347 |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.03% | 0.01% | 0.00% | 0.01% | 0.03% |
| 0.02% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.06% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 99.95% | 0.02% | 99.99% | 99.99% | 0.18% | 99.95% | 99.99% | 99.98% | 99.98% | 99.96% |
| 99.98% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 99.76% | 0.02% | 0.01% | 0.02% | 0.01% | 0.01% |
| 0 | 2 | 2 | 57891 | 2 | 5 | 2 | 0 | 1 | 2 | 3 | 8 |
| 10 | 0 | 0 | 1 | 0 | 1 | 7 | 1 | 0 | 0 | 0 | 1 |
| 3 | 60450 | 72709 | 14 | 69823 | 72016 | 27 | 56052 | 69755 | 63212 | 64105 | 53116 |
| 58362 | 1 | 1 | 1 | 34 | 1 | 13681 | 3 | 1 | 1 | 12 | 8 |
| 16184 | 14106 | 1847 | 16652 | 4700 | 2536 | 60842 | 18503 | 4802 | 11344 | 10439 | 21426 |
| 58375 | 60453 | 72712 | 57907 | 69859 | 72023 | 13717 | 56056 | 69757 | 63215 | 64120 | 53133 |
| 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% |
| 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.01% | 100.00 % | 100.00 % | 0.02% | 99.95% | 99.99% | 0.20% | 99.99% | 100.00 % | 100.00 % | 99.98% | 99.97% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 99.74% | 0.01% | 0.00% | 0.00% | 0.02% | 0.02% |

FIGURE 36 (Continued)

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 6 | 156 | 1 | 63372 | 21 | 0 | 5 | 0 | 5 | 18 | 1 | |
| **C** | 0 | 0 | 62154 | 1 | 63665 | 10 | 0 | 65886 | 0 | 0 | 52951 | |
| **G** | 51193 | 58187 | 0 | 160 | 186 | 0 | 66554 | 1 | 66375 | 57031 | 0 | |
| **T** | 5 | 1 | 13 | 4 | 160 | 65570 | 3 | 165 | 11 | 44 | 15 | |
| **N** | 17122 | 9982 | 6158 | 4789 | 4294 | 2746 | 1764 | 2274 | 1935 | 11233 | 15359 | |
| | 51204 | 58344 | 62168 | 63537 | 64032 | 65580 | 66562 | 66052 | 66391 | 57093 | 52967 | |
| | | | | | | | | | | | | |
| **A** | 0.01% | 0.27% | 0.00% | 99.74% | 0.03% | 0.00% | 0.01% | 0.00% | 0.01% | 0.03% | 0.00% | |
| **C** | 0.00% | 0.00% | 99.98% | 0.00% | 99.43% | 0.02% | 0.00% | 99.75% | 0.00% | 0.00% | 99.97% | |
| **G** | 99.98% | 99.73% | 0.00% | 0.25% | 0.29% | 0.00% | 99.99% | 0.00% | 99.98% | 99.89% | 0.00% | |
| **T** | 0.01% | 0.00% | 0.02% | 0.01% | 0.25% | 99.98% | 0.00% | 0.25% | 0.02% | 0.08% | 0.03% | |

**pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 4 | 154 | 3 | 60752 | 32 | 3 | 9 | 0 | 5 | 13 | 1 | |
| **C** | 0 | 1 | 59678 | 4 | 61270 | 9 | 0 | 63353 | 0 | 0 | 50805 | |
| **G** | 49370 | 55872 | 0 | 419 | 186 | 1 | 63928 | 0 | 63875 | 54772 | 2 | |
| **T** | 0 | 1 | 19 | 2 | 99 | 63010 | 1 | 168 | 1 | 1 | 8 | |
| **N** | 16278 | 9624 | 5952 | 4475 | 4065 | 2629 | 1714 | 2131 | 1770 | 10865 | 14835 | |
| | 49374 | 56028 | 59700 | 61177 | 61587 | 63023 | 63938 | 63521 | 63881 | 54786 | 50816 | |
| | | | | | | | | | | | | |
| **A** | 0.01% | 0.27% | 0.01% | 99.31% | 0.05% | 0.00% | 0.01% | 0.00% | 0.01% | 0.02% | 0.00% | |
| **C** | 0.00% | 0.00% | 99.96% | 0.01% | 99.49% | 0.01% | 0.00% | 99.74% | 0.00% | 0.00% | 99.98% | |
| **G** | 99.99% | 99.72% | 0.00% | 0.68% | 0.30% | 0.00% | 99.98% | 0.00% | 99.99% | 99.97% | 0.00% | |
| **T** | 0.00% | 0.00% | 0.03% | 0.00% | 0.16% | 99.98% | 0.00% | 0.26% | 0.00% | 0.00% | 0.02% | |

FIGURE 36 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 19 | 2 | 53782 | 3 | 3 | 1 | 3 | 2 | 5 | 3 | 5 | |
| 4 | 1 | 0 | 0 | 0 | 0 | 6 | 1 | 0 | 0 | 10 | 0 | |
| 0 | 55064 | 66611 | 11 | 64079 | 66018 | 19 | 51243 | 63857 | 57399 | 58254 | 48532 | |
| 53251 | 5 | 5 | 0 | 4 | 4 | 12618 | 5 | 12 | 5 | 5 | 9 | |
| 15070 | 13237 | 1708 | 14533 | 4240 | 2301 | 55682 | 17074 | 4455 | 10917 | 10054 | 19780 | |
| 53256 | 55089 | 66618 | 53793 | 64086 | 66025 | 12644 | 51252 | 63871 | 57409 | 58272 | 48546 | |
| 0.00% | 0.03% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | |
| 0.00% | 99.95% | 99.99% | 0.02% | 99.99% | 99.99% | 0.15% | 99.98% | 99.98% | 99.98% | 99.97% | 99.97% | |
| 99.99% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.79% | 0.01% | 0.02% | 0.01% | 0.01% | 0.02% | |
| 0 | 8 | 1 | 51396 | 2 | 3 | 0 | 1 | 1 | 4 | 6 | 8 | |
| 6 | 0 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 1 | |
| 0 | 52708 | 64070 | 20 | 61563 | 63482 | 18 | 49528 | 61517 | 54941 | 55816 | 46829 | |
| 51108 | 1 | 0 | 1 | 1 | 0 | 12290 | 3 | 2 | 0 | 0 | 7 | |
| 14537 | 12934 | 1579 | 14234 | 4085 | 2166 | 53339 | 16119 | 4131 | 10706 | 9829 | 18806 | |
| 51114 | 52717 | 64072 | 51417 | 61566 | 63485 | 12312 | 49532 | 61520 | 54945 | 55822 | 46845 | |
| 0.00% | 0.02% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.02% | |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 99.98% | 100.00% | 0.04% | 100.00% | 100.00% | 0.15% | 99.99% | 100.00% | 99.99% | 99.99% | 99.97% | |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.82% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | |

FIGURE 36 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | C | A | C | T | G | C | G | G | C | T |
| **pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | | |
| **A** | 3 | 154 | 2 | 71809 | 0 | 2 | 7 | 3 | 7 | 15 | 0 | 1 |
| **C** | 2 | 0 | 70252 | 1 | 72364 | 24 | 0 | 74670 | 0 | 0 | 60470 | 7 |
| **G** | 58500 | 65995 | 0 | 121 | 134 | 2 | 75414 | 0 | 75201 | 64970 | 2 | 0 |
| **T** | 1 | 0 | 21 | 1 | 148 | 74333 | 1 | 144 | 3 | 0 | 10 | 60812 |
| **N** | 18917 | 11274 | 7148 | 5491 | 4777 | 3062 | 2001 | 2606 | 2212 | 12438 | 16941 | 16603 |
| | 58506 | 66149 | 70275 | 71932 | 72646 | 74361 | 75422 | 74817 | 75211 | 64985 | 60482 | 60820 |
| **A** | 0.01% | 0.23% | 0.00% | 99.83% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.02% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.97% | 0.00% | 99.61% | 0.03% | 0.00% | 99.80% | 0.00% | 0.00% | 99.98% | 0.01% |
| **G** | 99.99% | 99.77% | 0.00% | 0.17% | 0.18% | 0.00% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.03% | 0.00% | 0.20% | 99.96% | 0.00% | 0.19% | 0.00% | 0.00% | 0.02% | 99.99% |

FIGURE 36 (Continued)

| 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 60779 | 1 | 5 | 2 | 2 | 1 | 4 | 7 | 12 |
| 1 | 1 | 0 | 1 | 1 | 6 | 0 | 0 | 1 | 0 | 1 |
| 62691 | 75475 | 19 | 72621 | 74747 | 19 | 58300 | 72476 | 65254 | 66271 | 55138 |
| 1 | 21 | 0 | 2 | 1 | 14351 | 1 | 5 | 1 | 1 | 3 |
| 14729 | 1924 | 16625 | 4798 | 2669 | 63045 | 19120 | 4941 | 12163 | 11144 | 22269 |
| 62694 | 75499 | 60798 | 72625 | 74754 | 14378 | 58303 | 72482 | 65260 | 66279 | 55154 |
| 0.00% | 0.00% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.02% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 99.97% | 0.03% | 99.99% | 99.99% | 0.13% | 99.99% | 99.99% | 99.99% | 99.99% | 99.97% |
| 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 99.81% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |

FIGURE 36 (Continued)

**pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 2 | 274 | 1 | 94337 | 68 | 7 | 16 | 2 | 6 | 38 | 0 | 1 |
| **C** | 2 | 1 | 93216 | 0 | 95374 | 13 | 0 | 98770 | 0 | 2 | 80149 | 8 |
| **G** | 77551 | 87659 | 0 | 688 | 222 | 2 | 99659 | 2 | 99530 | 85960 | 2 | 0 |
| **T** | 4 | 1 | 29 | 4 | 220 | 98222 | 3 | 267 | 6 | 2 | 17 | 80515 |
| **N** | 24864 | 14488 | 9177 | 7393 | 6538 | 4178 | 2744 | 3381 | 2880 | 16420 | 22254 | 21898 |
| | 77559 | 87935 | 93246 | 95029 | 95884 | 98244 | 99678 | 99041 | 99542 | 86002 | 80168 | 80524 |
| **A** | 0.00% | 0.31% | 0.00% | 99.27% | 0.07% | 0.01% | 0.02% | 0.00% | 0.01% | 0.04% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.97% | 0.00% | 99.47% | 0.01% | 0.00% | 99.73% | 0.00% | 0.00% | 99.98% | 0.01% |
| **G** | 99.99% | 99.69% | 0.00% | 0.72% | 0.23% | 0.00% | 99.98% | 0.00% | 99.99% | 99.95% | 0.00% | 0.00% |
| **T** | 0.01% | 0.00% | 0.03% | 0.00% | 0.23% | 99.98% | 0.00% | 0.27% | 0.01% | 0.00% | 0.02% | 99.99% |

**untreated cells**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 5 | 140 | 2 | 85519 | 2 | 4 | 4 | 4 | 13 | 22 | 0 | 1 |
| **C** | 2 | 0 | 83615 | 1 | 86140 | 17 | 0 | 88688 | 0 | 2 | 71985 | 7 |
| **G** | 69897 | 78238 | 0 | 8 | 1 | 0 | 89423 | 3 | 89338 | 77631 | 0 | 2 |
| **T** | 0 | 2 | 8 | 3 | 12 | 8252 | 0 | 127 | 0 | 1 | 15 | 72438 |
| **N** | 21878 | 13402 | 8157 | 6251 | 5627 | 3509 | 2355 | 2960 | 2431 | 14126 | 19782 | 19334 |
| | 69904 | 78380 | 83625 | 85531 | 86155 | 88273 | 89427 | 88822 | 89351 | 77656 | 72000 | 72448 |
| **A** | 0.01% | 0.18% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.03% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.02% | 0.00% | 99.85% | 0.00% | 0.00% | 99.98% | 0.01% |
| **G** | 99.99% | 99.82% | 0.00% | 0.01% | 0.00% | 0.00% | 100.00 % | 0.00% | 99.99% | 99.97% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.14% | 0.00% | 0.00% | 0.02% | 99.99% |

FIGURE 36 (Continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 2 | 80250 | 2 | 5 | 1 | 1 | 3 | 7 | 8 | 16 |
| 2 | 0 | 0 | 1 | 2 | 10 | 3 | 0 | 0 | 1 | 6 |
| 82978 | 99896 | 21 | 96190 | 99118 | 30 | 78046 | 96104 | 86348 | 87670 | 73038 |
| 9 | 8 | 1 | 2 | 7 | 19229 | 9 | 4 | 3 | 2 | 12 |
| 19423 | 2516 | 22150 | 6229 | 3292 | 83154 | 24365 | 6313 | 16066 | 14743 | 29352 |
| 82999 | 99906 | 80272 | 96195 | 99132 | 19270 | 78059 | 96111 | 86358 | 87681 | 73072 |
| 0.01% | 0.00% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.02% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 99.97% | 99.99% | 0.03% | 99.99% | 99.99% | 0.16% | 99.98% | 99.99% | 99.99% | 99.99% | 99.95% |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.79% | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% |
| 5 | 3 | 72312 | 1 | 4 | 0 | 2 | 4 | 5 | 7 | 20 |
| 2 | 0 | 0 | 2 | 2 | 2 | 1 | 0 | 0 | 2 | 0 |
| 74838 | 89552 | 9 | 86255 | 88821 | 27 | 69548 | 86000 | 77880 | 79024 | 65745 |
| 3 | 0 | 0 | 6 | 1 | 17217 | 6 | 3 | 6 | 1 | 7 |
| 16934 | 2227 | 19461 | 5518 | 2954 | 74536 | 22225 | 5775 | 13891 | 12748 | 26010 |
| 74848 | 89555 | 72321 | 86264 | 88828 | 17246 | 69557 | 86007 | 77891 | 79034 | 65772 |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.03% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 100.00 % | 0.01% | 99.99% | 99.99% | 0.16% | 99.99% | 99.99% | 99.99% | 99.99% | 99.96% |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 99.83% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |

FIGURE 37

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild type)** | | | | | | | | | | | |
| A | 27 | 177257 | 7 | 13 | 0 | 31 | 32 | 175928 | 36 | 2 | 173560 |
| C | 2 | 3 | 1 | 25 | 178579 | 179947 | 2 | 2 | 3 | 172305 | 2 |
| G | 182303 | 25 | 182291 | 1 | 13 | 31 | 180770 | 16 | 175637 | 0 | 18 |
| T | 2 | 1 | 6 | 175103 | 492 | 383 | 9 | 8 | 7 | 17 | 9 |
| N | 4999 | 10047 | 5028 | 12191 | 8249 | 6941 | 6520 | 11379 | 11650 | 15009 | 13744 |
| | 182334 | 177286 | 182305 | 175142 | 179084 | 180392 | 180813 | 175954 | 175683 | 172324 | 173589 |
| A | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.02% | 0.02% | 99.99% | 0.02% | 0.00% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.72% | 99.75% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.01% | 0.02% | 99.98% | 0.01% | 99.97% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.27% | 0.21% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| **pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)** | | | | | | | | | | | |
| A | 23 | 165552 | 8 | 11 | 2 | 2 | 27 | 164096 | 26 | 2 | 162114 |
| C | 2 | 3 | 1 | 15 | 166684 | 167957 | 2 | 1 | 5 | 160986 | 2 |
| G | 170201 | 20 | 170171 | 3 | 2 | 1 | 168878 | 87 | 163894 | 0 | 15 |
| T | 3 | 1 | 5 | 163295 | 304 | 384 | 6 | 6 | 1 | 10 | 5 |
| N | 4680 | 9333 | 4724 | 11585 | 7917 | 6565 | 5996 | 10719 | 10983 | 13911 | 12773 |
| | 170229 | 165576 | 170185 | 163324 | 166992 | 168344 | 168913 | 164190 | 163926 | 160998 | 162136 |
| A | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 99.94% | 0.02% | 0.00% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.82% | 99.77% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.05% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.18% | 0.23% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |

FIGURE 37 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 180942 | 182413 | 6 | 179213 | 181006 | 3 | 181673 | 178739 | 15 | 8 | 10 |
| 1 | 3 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 1 |
| 180647 | 10 | 15 | 182989 | 13 | 9 | 182119 | 73 | 25 | 181403 | 181803 | 179743 |
| 2 | 4 | 12 | 5 | 2 | 9 | 6 | 9 | 10 | 19 | 3 | 6 |
| 6680 | 6374 | 4893 | 4332 | 8105 | 6309 | 5204 | 5578 | 8620 | 5957 | 5581 | 7578 |
| 180653 | 180959 | 182440 | 183001 | 179228 | 181024 | 182129 | 181755 | 178775 | 181438 | 181814 | 179760 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.95% | 99.98% | 0.01% | 0.00% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 99.99% | 0.04% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 4 | 168976 | 170344 | 2 | 167102 | 168945 | 2 | 169608 | 166748 | 12 | 16 | 6 |
| 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| 168796 | 8 | 12 | 170881 | 13 | 19 | 169976 | 15 | 22 | 169264 | 169625 | 167877 |
| 3 | 3 | 6 | 4 | 0 | 5 | 1 | 7 | 8 | 5 | 4 | 2 |
| 6105 | 5921 | 4546 | 4022 | 7794 | 5939 | 4930 | 5279 | 8130 | 5627 | 5264 | 7023 |
| 168804 | 168988 | 170363 | 170887 | 167115 | 168970 | 169979 | 169630 | 166779 | 169282 | 169645 | 167886 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.00% | 0.01% | 100.00 % | 0.01% | 0.01% | 100.00 % | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

# FIGURE 37 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)** | | | | | | | | | | | |
| **A** | 18 | 132530 | 4 | 17 | 3 | 0 | 26 | 131454 | 28 | 1 | 129824 |
| **C** | 2 | 1 | 0 | 15 | 133533 | 134545 | 2 | 1 | 2 | 128923 | 1 |
| **G** | 136418 | 17 | 136364 | 1 | 32 | 1 | 135195 | 29 | 131320 | 5 | 21 |
| **T** | 4 | 5 | 3 | 130697 | 143 | 338 | 5 | 6 | 8 | 8 | 6 |
| **N** | 3800 | 7689 | 3871 | 9512 | 6531 | 5358 | 5014 | 8752 | 8884 | 11305 | 10390 |
| | 136442 | 132553 | 136371 | 130730 | 133711 | 134884 | 135228 | 131490 | 131358 | 128937 | 129852 |
| **A** | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 99.97% | 0.02% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.87% | 99.75% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.01% | 99.99% | 0.00% | 0.02% | 0.00% | 99.98% | 0.02% | 99.97% | 0.00% | 0.02% |
| **T** | 0.00% | 0.00% | 0.00% | 99.97% | 0.11% | 0.25% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 16 | 140343 | 4 | 6 | 45 | 27 | 20 | 139333 | 23 | 0 | 137715 |
| **C** | 4 | 1 | 1 | 28 | 141300 | 142378 | 12 | 0 | 5 | 136613 | 0 |
| **G** | 144507 | 30 | 144348 | 1 | 1 | 1 | 143215 | 12 | 139151 | 1 | 10 |
| **T** | 6 | 3 | 3 | 138477 | 374 | 430 | 24 | 5 | 5 | 13 | 7 |
| **N** | 3836 | 7992 | 4013 | 9857 | 6649 | 5533 | 5098 | 9019 | 9185 | 11742 | 10637 |
| | 144533 | 140377 | 144356 | 138512 | 141720 | 142836 | 143271 | 139350 | 139184 | 136627 | 137732 |
| **A** | 0.01% | 99.98% | 0.00% | 0.00% | 0.03% | 0.02% | 0.01% | 99.99% | 0.02% | 0.00% | 99.99% |
| **C** | 0.00% | 0.00% | 0.00% | 0.02% | 99.70% | 99.68% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.96% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.97% | 0.26% | 0.30% | 0.02% | 0.00% | 0.00% | 0.01% | 0.01% |

FIGURE 37 (Continued)

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 135313 | 136513 | 6 | 133946 | 135472 | 1 | 135898 | 133559 | 7 | 9 | 2 | | | | | |
| 1 | 2 | 1 | 2 | 1 | 2 | 0 | 0 | 1 | 1 | 2 | 1 | | | | | |
| 135229 | 10 | 12 | 136896 | 6 | 11 | 136352 | 13 | 13 | 135615 | 135921 | 134623 | | | | | |
| 4 | 0 | 2 | 4 | 5 | 4 | 6 | 6 | 6 | 9 | 4 | 2 | | | | | |
| 5004 | 4916 | 3713 | 3333 | 6283 | 4752 | 3882 | 4324 | 6662 | 4609 | 4305 | 5613 | | | | | |
| 135237 | 135325 | 136528 | 136908 | 133958 | 135489 | 136359 | 135917 | 133579 | 135632 | 135936 | 134628 | | | | | |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | | | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | | | |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 100.00 % | | | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | | | | | |
| 2 | 143439 | 144560 | 3 | 141906 | 143270 | 2 | 143931 | 141356 | 8 | 14 | 4 | | | | | |
| 2 | 6 | 1 | 1 | 0 | 2 | 4 | 0 | 4 | 0 | 1 | 0 | | | | | |
| 143207 | 13 | 16 | 144997 | 8 | 11 | 144263 | 8 | 20 | 143532 | 143875 | 142444 | | | | | |
| 4 | 2 | 5 | 3 | 2 | 2 | 0 | 6 | 5 | 13 | 1 | 14 | | | | | |
| 5154 | 4909 | 3787 | 3365 | 6453 | 5084 | 4099 | 4423 | 6983 | 4815 | 4477 | 5906 | | | | | |
| 143215 | 143460 | 144582 | 145004 | 141916 | 143285 | 144269 | 143945 | 141385 | 143553 | 143891 | 142462 | | | | | |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% | | | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | | | |
| 99.99% | 0.01% | 0.01% | 100.00 % | 0.01% | 0.01% | 100.00 % | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% | | | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | | | | | |

FIGURE 37 (Continued)

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-171 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 19 | 166701 | 5 | 8 | 3 | 5 | 20 | 165275 | 22 | 4 | 163291 |
| **C** | 2 | 1 | 2 | 20 | 167760 | 169043 | 2 | 0 | 1 | 162104 | 0 |
| **G** | 171323 | 22 | 171263 | 1 | 1 | 58 | 169870 | 13 | 164938 | 1 | 13 |
| **T** | 3 | 4 | 5 | 164459 | 426 | 429 | 6 | 7 | 6 | 5 | 9 |
| **N** | 4689 | 9308 | 4761 | 11548 | 7846 | 6501 | 6138 | 10741 | 11069 | 13922 | 12723 |
| | 171347 | 166728 | 171275 | 164488 | 168190 | 169535 | 169898 | 165295 | 164967 | 162114 | 163313 |
| **A** | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 99.99% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.74% | 99.71% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% | 0.03% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.25% | 0.25% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| **pNMG-172 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 24 | 164766 | 11 | 5 | 25 | 2 | 32 | 163069 | 30 | 2 | 161174 |
| **C** | 0 | 0 | 1 | 19 | 165737 | 166680 | 1 | 1 | 5 | 160075 | 1 |
| **G** | 168897 | 17 | 169008 | 0 | 2 | 2 | 167734 | 13 | 162993 | 4 | 13 |
| **T** | 6 | 2 | 4 | 162593 | 345 | 483 | 6 | 5 | 2 | 8 | 7 |
| **N** | 4577 | 8719 | 4480 | 10887 | 7395 | 6337 | 5731 | 10416 | 10474 | 13415 | 12309 |
| | 168927 | 164785 | 169024 | 162617 | 166109 | 167167 | 167773 | 163088 | 163030 | 160089 | 161195 |
| **A** | 0.01% | 99.99% | 0.01% | 0.00% | 0.02% | 0.00% | 0.02% | 99.99% | 0.02% | 0.00% | 99.99% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.78% | 99.71% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.99% | 0.21% | 0.29% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 37 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 170020 | 171494 | 7 | 168343 | 170122 | 7 | 170812 | 168075 | 11 | 8 | 6 |
| 1 | 3 | 0 | 1 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 0 |
| 169864 | 10 | 24 | 171915 | 16 | 13 | 171117 | 13 | 16 | 170433 | 170711 | 168974 |
| 3 | 1 | 6 | 3 | 2 | 7 | 3 | 8 | 11 | 9 | 4 | 1 |
| 6167 | 6002 | 4512 | 4110 | 7675 | 5893 | 4907 | 5202 | 7933 | 5582 | 5312 | 7055 |
| 169869 | 170034 | 171524 | 171926 | 168361 | 170143 | 171129 | 170834 | 168103 | 170454 | 170724 | 168981 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 100.00 % |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 6 | 167606 | 169033 | 8 | 166048 | 167856 | 1 | 168515 | 165771 | 11 | 15 | 6 |
| 0 | 5 | 1 | 2 | 0 | 2 | 1 | 0 | 2 | 1 | 2 | 3 |
| 167705 | 6 | 8 | 169610 | 8 | 15 | 168810 | 14 | 17 | 168185 | 168512 | 166751 |
| 5 | 3 | 6 | 7 | 4 | 4 | 5 | 6 | 9 | 12 | 4 | 3 |
| 5788 | 5884 | 4456 | 3877 | 7444 | 5627 | 4687 | 4969 | 7704 | 5294 | 4970 | 6740 |
| 167716 | 167620 | 169048 | 169627 | 166060 | 167877 | 168817 | 168535 | 165799 | 168209 | 168533 | 166763 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 100.00 % | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |

# FIGURE 37 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-173 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)** | | | | | | | | | | | |
| **A** | 18 | 180485 | 11 | 13 | 4 | 64 | 68 | 179317 | 31 | 3 | 177270 |
| **C** | 3 | 1 | 1 | 17 | 182001 | 183428 | 2 | 1 | 5 | 176016 | 0 |
| **G** | 185644 | 22 | 185624 | 6 | 28 | 31 | 184136 | 21 | 179061 | 4 | 9 |
| **T** | 5 | 3 | 0 | 178375 | 306 | 248 | 7 | 4 | 4 | 15 | 13 |
| **N** | 4920 | 10079 | 4954 | 12179 | 8251 | 6819 | 6378 | 11248 | 11490 | 14553 | 13299 |
| | 185670 | 180511 | 185636 | 178411 | 182339 | 183771 | 184213 | 179343 | 179101 | 176038 | 177292 |
| **A** | 0.01% | 99.99% | 0.01% | 0.01% | 0.00% | 0.03% | 0.04% | 99.99% | 0.02% | 0.00% | 99.99% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.81% | 99.81% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.01% | 99.99% | 0.00% | 0.02% | 0.02% | 99.96% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.17% | 0.13% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| **pNMG-174 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V )** | | | | | | | | | | | |
| **A** | 17 | 177789 | 8 | 17 | 3 | 27 | 26 | 176217 | 27 | 0 | 174196 |
| **C** | 3 | 0 | 1 | 14 | 178955 | 180390 | 0 | 5 | 0 | 172914 | 0 |
| **G** | 182597 | 22 | 182663 | 3 | 4 | 32 | 181118 | 22 | 176039 | 2 | 17 |
| **T** | 5 | 3 | 3 | 175272 | 362 | 296 | 5 | 8 | 3 | 14 | 2 |
| **N** | 5107 | 9915 | 5054 | 12423 | 8405 | 6984 | 6580 | 11477 | 11660 | 14799 | 13514 |
| | 182622 | 177814 | 182675 | 175306 | 179324 | 180745 | 181149 | 176252 | 176069 | 172930 | 174215 |
| **A** | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.02% | 0.00% | 99.99% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.79% | 99.80% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% | 0.02% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.20% | 0.16% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |

FIGURE 37 (Continued)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 184286 | 185889 | 6 | 182538 | 184422 | 2 | 184921 | 181999 | 6 | 5 | 6 | | |
| 1 | 3 | 1 | 3 | 1 | 1 | 1 | 1 | 2 | 2 | 1 | 0 | | |
| 184117 | 10 | 30 | 186220 | 13 | 14 | 185542 | 15 | 19 | 184716 | 184900 | 183259 | | |
| 2 | 1 | 3 | 4 | 4 | 5 | 4 | 8 | 15 | 11 | 6 | 2 | | |
| 6466 | 6290 | 4667 | 4357 | 8034 | 6148 | 5040 | 5644 | 8554 | 5854 | 5677 | 7322 | | |
| 184124 | 184300 | 185923 | 186233 | 182556 | 184442 | 185549 | 184945 | 182035 | 184735 | 184912 | 183267 | | |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| 100.00 | | | | | | 100.00 | | | | | 100.00 | | |
| % | 0.01% | 0.02% | 99.99% | 0.01% | 0.01% | % | 0.01% | 0.01% | 99.99% | 99.99% | % | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | | |
| 5 | 181220 | 182782 | 3 | 179566 | 181463 | 8 | 182114 | 179176 | 9 | 15 | 8 | | |
| 1 | 2 | 3 | 1 | 0 | 1 | 0 | 0 | 3 | 0 | 4 | 0 | | |
| 181136 | 14 | 13 | 183260 | 27 | 29 | 182505 | 14 | 24 | 181737 | 182031 | 180249 | | |
| 7 | 0 | 6 | 2 | 1 | 15 | 1 | 8 | 8 | 8 | 2 | 1 | | |
| 6580 | 6493 | 4939 | 4477 | 8149 | 6235 | 5229 | 5607 | 8532 | 5989 | 5691 | 7485 | | |
| 181149 | 181236 | 182804 | 183266 | 179594 | 181508 | 182514 | 182136 | 179211 | 181754 | 182052 | 180258 | | |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 0.01% | 0.00% | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| | | | 100.00 | | | 100.00 | | | | | 100.00 | | |
| 99.99% | 0.01% | 0.01% | % | 0.02% | 0.02% | % | 0.01% | 0.01% | 99.99% | 99.99% | % | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |

FIGURE 37 (Continued)

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-175 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 15 | 162319 | 4 | 9 | 24 | 4 | 46 | 161280 | 23 | 3 | 159335 |
| C | 2 | 4 | 1 | 9 | 163645 | 164798 | 3 | 0 | 1 | 158063 | 0 |
| G | 167004 | 17 | 166843 | 0 | 1 | 28 | 165587 | 17 | 161246 | 2 | 20 |
| T | 2 | 6 | 6 | 160467 | 328 | 322 | 4 | 7 | 3 | 5 | 13 |
| N | 4471 | 9148 | 4640 | 11009 | 7496 | 6342 | 5854 | 10190 | 10221 | 13421 | 12126 |
| | 167023 | 162346 | 166854 | 160485 | 163998 | 165152 | 165640 | 161304 | 161273 | 158073 | 159368 |
| A | 0.01% | 99.98% | 0.00% | 0.01% | 0.01% | 0.00% | 0.03% | 99.99% | 0.01% | 0.00% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.78% | 99.79% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% | 0.02% | 99.97% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.99% | 0.20% | 0.19% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| **pNMG-176 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S)** | | | | | | | | | | | |
| A | 44 | 143425 | 3 | 11 | 26 | 27 | 69 | 142422 | 26 | 2 | 140814 |
| C | 2 | 0 | 3 | 16 | 144217 | 145263 | 0 | 3 | 3 | 139859 | 1 |
| G | 147453 | 22 | 147467 | 4 | 0 | 41 | 146180 | 22 | 142440 | 1 | 13 |
| T | 2 | 3 | 2 | 141470 | 503 | 541 | 2 | 10 | 3 | 15 | 5 |
| N | 3943 | 7994 | 3969 | 9943 | 6698 | 5572 | 5193 | 8987 | 8972 | 11567 | 10611 |
| | 147501 | 143450 | 147475 | 141501 | 144746 | 145872 | 146251 | 142457 | 142472 | 139877 | 140833 |
| A | 0.03% | 99.98% | 0.00% | 0.01% | 0.02% | 0.02% | 0.05% | 99.98% | 0.02% | 0.00% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.63% | 99.58% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.97% | 0.02% | 99.99% | 0.00% | 0.00% | 0.03% | 99.95% | 0.02% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.35% | 0.37% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |

# FIGURE 37 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 165558 | 167192 | 1 | 163980 | 165816 | 1 | 166499 | 163799 | 7 | 13 | 12 |
| 4 | 6 | 3 | 1 | 0 | 0 | 1 | 1 | 3 | 0 | 1 | 2 |
| 165656 | 10 | 21 | 167607 | 41 | 11 | 166725 | 8 | 17 | 166149 | 166283 | 164714 |
| 3 | 3 | 3 | 3 | 4 | 33 | 1 | 11 | 14 | 14 | 4 | 1 |
| 5830 | 5917 | 4311 | 3918 | 7505 | 5670 | 4802 | 5011 | 7697 | 5360 | 5229 | 6800 |
| 165664 | 165577 | 167219 | 167612 | 164025 | 165860 | 166728 | 166519 | 163833 | 166170 | 166301 | 164729 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.97% | 99.97% | 0.00% | 99.99% | 99.98% | 0.00% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.01% | 0.01% | 100.00 % | 0.02% | 0.01% | 100.00 % | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| 5 | 146333 | 147581 | 2 | 144795 | 146388 | 6 | 146991 | 144522 | 8 | 7 | 12 |
| 1 | 6 | 2 | 1 | 0 | 0 | 3 | 0 | 3 | 3 | 0 | 0 |
| 146335 | 4 | 14 | 148004 | 16 | 9 | 147263 | 15 | 16 | 146680 | 146878 | 145512 |
| 2 | 1 | 3 | 4 | 1 | 3 | 2 | 7 | 5 | 14 | 2 | 2 |
| 5101 | 5100 | 3844 | 3432 | 6631 | 5043 | 4169 | 4430 | 6897 | 4738 | 4556 | 5917 |
| 146343 | 146344 | 147600 | 148011 | 144812 | 146400 | 147274 | 147013 | 144546 | 146705 | 146887 | 145526 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.00% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 100.00 % | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

FIGURE 37 (Continued)

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 17 | 132826 | 28 | 2 | 3 | 44 | 53 | 132030 | 26 | 3 | 130658 | |
| **C** | 3 | 1 | 0 | 21 | 133804 | 134677 | 1 | 1 | 3 | 129589 | 1 | |
| **G** | 136607 | 43 | 136603 | 3 | 30 | 1 | 135495 | 19 | 132060 | 2 | 8 | |
| **T** | 2 | 0 | 2 | 131076 | 368 | 436 | 2 | 9 | 0 | 13 | 10 | |
| **N** | 3729 | 7488 | 3725 | 9256 | 6153 | 5200 | 4807 | 8299 | 8269 | 10751 | 9681 | |
| | 136629 | 132870 | 136633 | 131102 | 134205 | 135158 | 135551 | 132059 | 132089 | 129607 | 130677 | |
| **A** | 0.01% | 99.97% | 0.02% | 0.00% | 0.00% | 0.03% | 0.04% | 99.98% | 0.02% | 0.00% | 99.99% | |
| **C** | 0.00% | 0.00% | 0.00% | 0.02% | 99.70% | 99.64% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | |
| **G** | 99.98% | 0.03% | 99.98% | 0.00% | 0.02% | 0.00% | 99.96% | 0.01% | 99.98% | 0.00% | 0.01% | |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.27% | 0.32% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | |

**pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 10 | 133061 | 9 | 2 | 3 | 41 | 29 | 132500 | 30 | 1 | 131014 | |
| **C** | 2 | 1 | 0 | 17 | 133900 | 135007 | 0 | 1 | 0 | 130047 | 2 | |
| **G** | 137012 | 20 | 136932 | 1 | 27 | 75 | 135879 | 30 | 132680 | 3 | 10 | |
| **T** | 4 | 6 | 0 | 131338 | 631 | 545 | 8 | 6 | 2 | 5 | 7 | |
| **N** | 3647 | 7587 | 3734 | 9317 | 6114 | 5007 | 4759 | 8138 | 7963 | 10619 | 9642 | |
| | 137028 | 133088 | 136941 | 131358 | 134561 | 135668 | 135916 | 132537 | 132712 | 130056 | 131033 | |
| **A** | 0.01% | 99.98% | 0.01% | 0.00% | 0.00% | 0.03% | 0.02% | 99.97% | 0.02% | 0.00% | 99.99% | |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.51% | 99.51% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | |
| **G** | 99.99% | 0.02% | 99.99% | 0.00% | 0.02% | 0.06% | 99.97% | 0.02% | 99.98% | 0.00% | 0.01% | |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.47% | 0.40% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | |

# FIGURE 37 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 135658 | 136769 | 2 | 134092 | 135602 | 3 | 136158 | 133763 | 6 | 9 | 7 |
| 0 | 2 | 4 | 1 | 0 | 2 | 1 | 3 | 7 | 1 | 0 | 0 |
| 135770 | 14 | 8 | 137117 | 10 | 11 | 136474 | 6 | 17 | 135814 | 136031 | 134765 |
| 3 | 2 | 2 | 2 | 6 | 8 | 25 | 6 | 7 | 5 | 3 | 3 |
| 4584 | 4682 | 3575 | 3236 | 6250 | 4735 | 3855 | 4185 | 6564 | 4532 | 4315 | 5583 |
| 135774 | 135676 | 136783 | 137122 | 134108 | 135623 | 136503 | 136173 | 133794 | 135826 | 136043 | 134775 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 100.00% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 136066 | 137147 | 0 | 134511 | 135875 | 1 | 136598 | 134136 | 9 | 13 | 5 |
| 1 | 0 | 0 | 2 | 0 | 1 | 3 | 1 | 8 | 3 | 2 | 2 |
| 136005 | 10 | 28 | 137467 | 6 | 9 | 136820 | 11 | 15 | 136226 | 136487 | 135247 |
| 1 | 3 | 3 | 3 | 2 | 6 | 7 | 5 | 7 | 8 | 4 | 2 |
| 4662 | 4595 | 3496 | 3202 | 6155 | 4783 | 3843 | 4059 | 6508 | 4428 | 4168 | 5418 |
| 136012 | 136079 | 137178 | 137472 | 134519 | 135891 | 136831 | 136615 | 134166 | 136246 | 136506 | 135256 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.02% | 100.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |

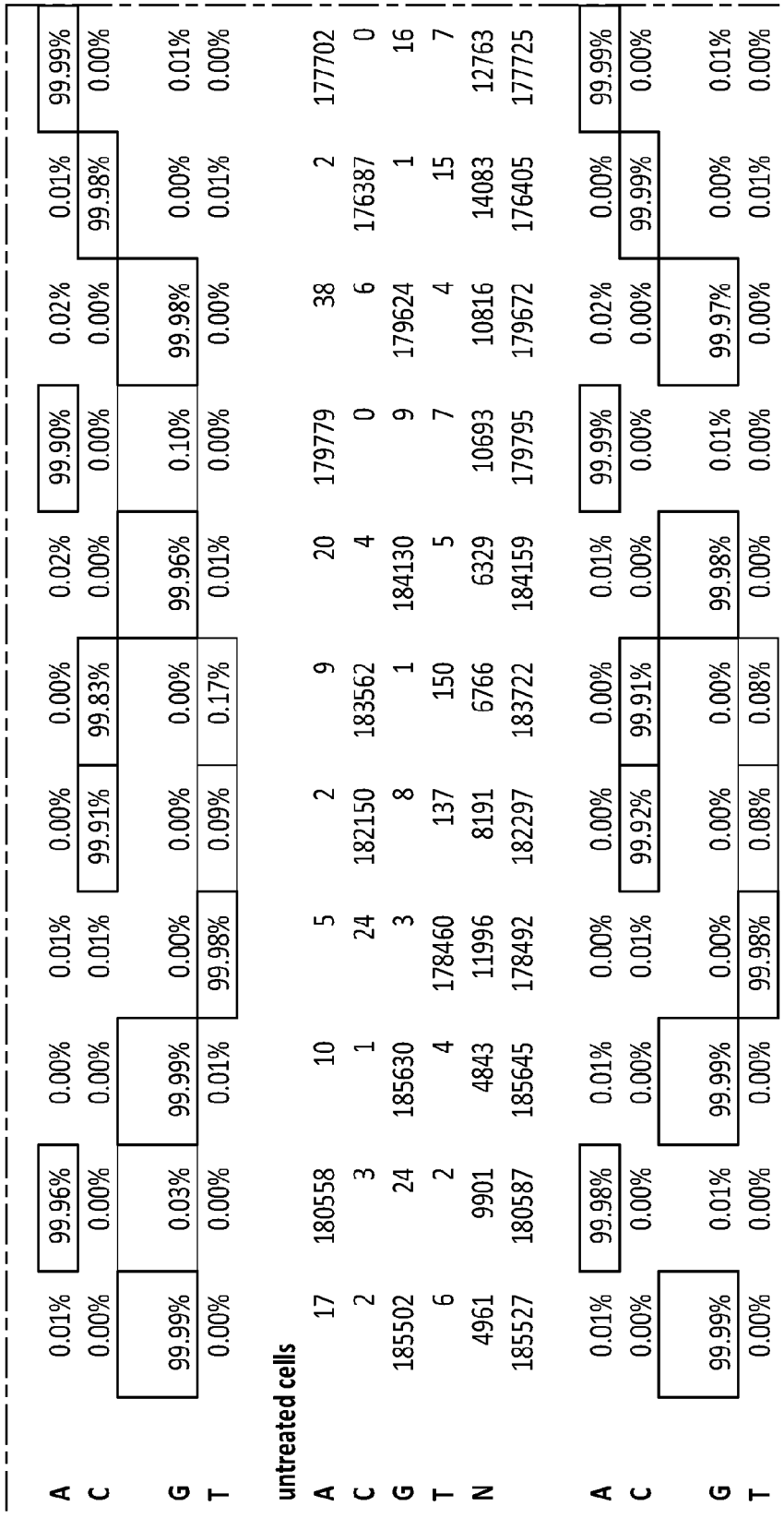
FIGURE 37 (Continued)

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 15 | 153754 | 11 | 10 | 3 | 3 | 54 | 152636 | 32 | 0 | 150823 |
| **C** | 4 | 2 | 1 | 20 | 154806 | 155946 | 2 | 0 | 3 | 149734 | 2 |
| **G** | 157822 | 23 | 157966 | 1 | 12 | 3 | 156582 | 52 | 152752 | 1 | 13 |
| **T** | 5 | 2 | 2 | 151635 | 177 | 243 | 4 | 4 | 2 | 8 | 9 |
| **N** | 4266 | 8331 | 4132 | 10446 | 7114 | 5917 | 5469 | 9419 | 9322 | 12368 | 11264 |
| | 157846 | 153781 | 157980 | 151666 | 154998 | 156195 | 156642 | 152692 | 152789 | 149743 | 150847 |
| | | | | | | | | | | | |
| **A** | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.03% | 99.96% | 0.02% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.88% | 99.84% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.96% | 0.03% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.11% | 0.16% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| **pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 14 | 176300 | 6 | 11 | 8 | 4 | 41 | 174568 | 29 | 9 | 172852 |
| **C** | 2 | 2 | 0 | 20 | 177834 | 178766 | 2 | 1 | 4 | 171403 | 1 |
| **G** | 180971 | 60 | 180985 | 1 | 2 | 3 | 179584 | 168 | 174979 | 4 | 15 |
| **T** | 4 | 7 | 14 | 174370 | 157 | 303 | 26 | 7 | 4 | 15 | 8 |
| **N** | 4835 | 9457 | 4821 | 11424 | 7825 | 6750 | 6173 | 11082 | 10810 | 14395 | 12950 |
| | 180991 | 176369 | 181005 | 174402 | 178001 | 179076 | 179653 | 174744 | 175016 | 171431 | 172876 |

FIGURE 37 (Continued)

| 12 GG | 13 AA | 14 AA | 15 GG | 16 AA | 17 AA | 18 GG | 19 AA | 20 AA | P GG | A GG | M GG |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 156801 | 158076 | 1 | 155190 | 156801 | 4 | 157455 | 154961 | 10 | 11 | 13 |
| 1 | 1 | 1 | 7 | 0 | 2 | 0 | 1 | 6 | 1 | 0 | 2 |
| 156751 | 11 | 17 | 158412 | 9 | 13 | 157785 | 12 | 12 | 157046 | 157278 | 155697 |
| 1 | 5 | 8 | 2 | 2 | 6 | 4 | 4 | 10 | 5 | 3 | 4 |
| 5356 | 5293 | 4009 | 3688 | 6909 | 5288 | 4304 | 4625 | 7108 | 5035 | 4805 | 6381 |
| 156755 | 156818 | 158102 | 158422 | 155201 | 156822 | 157793 | 157472 | 154989 | 157062 | 157292 | 155716 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 6 | 179495 | 181083 | 1 | 177817 | 179813 | 5 | 180498 | 177655 | 6 | 10 | 16 |
| 1 | 3 | 2 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 1 |
| 179558 | 13 | 10 | 181687 | 27 | 17 | 180839 | 14 | 10 | 180193 | 180351 | 178494 |
| 3 | 2 | 2 | 2 | 6 | 18 | 1 | 3 | 10 | 17 | 3 | 5 |
| 6258 | 6313 | 4746 | 4151 | 7993 | 5994 | 4997 | 5327 | 8167 | 5627 | 5479 | 7327 |
| 179568 | 179513 | 181097 | 181692 | 177850 | 179849 | 180846 | 180516 | 177676 | 180216 | 180364 | 178516 |

FIGURE 37 (Continued)



| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 99.96% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 99.90% | 0.02% | 0.01% | 99.99% | | | | | | | | |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.91% | 99.83% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | | | | | | | | |
| G | 99.99% | 0.03% | 99.99% | 0.00% | 0.00% | 0.00% | 99.96% | 0.10% | 99.98% | 0.00% | 0.01% | | | | | | | | |
| T | 0.00% | 0.00% | 0.01% | 99.98% | 0.09% | 0.17% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | | | | | | | | |
| **untreated cells** | | | | | | | | | | | | | | | | | | | |
| A | 17 | 180558 | 10 | 5 | 2 | 9 | 20 | 179779 | 38 | 2 | 177702 | | | | | | | | |
| C | 2 | 3 | 1 | 24 | 182150 | 183562 | 4 | 0 | 6 | 176387 | 0 | | | | | | | | |
| G | 185502 | 24 | 185630 | 3 | 8 | 1 | 184130 | 9 | 179624 | 1 | 16 | | | | | | | | |
| T | 6 | 2 | 4 | 178460 | 137 | 150 | 5 | 7 | 4 | 15 | 7 | | | | | | | | |
| N | 4961 | 9901 | 4843 | 11996 | 8191 | 6766 | 6329 | 10693 | 10816 | 14083 | 12763 | | | | | | | | |
| | 185527 | 180587 | 185645 | 178492 | 182297 | 183722 | 184159 | 179795 | 179672 | 176405 | 177725 | | | | | | | | |
| A | 0.01% | 99.98% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.02% | 0.00% | 99.99% | | | | | | | | |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.92% | 99.91% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | | | | | | | | |
| G | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.97% | 0.00% | 0.01% | | | | | | | | |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.08% | 0.08% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | | | | | | | | |

FIGURE 37 (Continued)

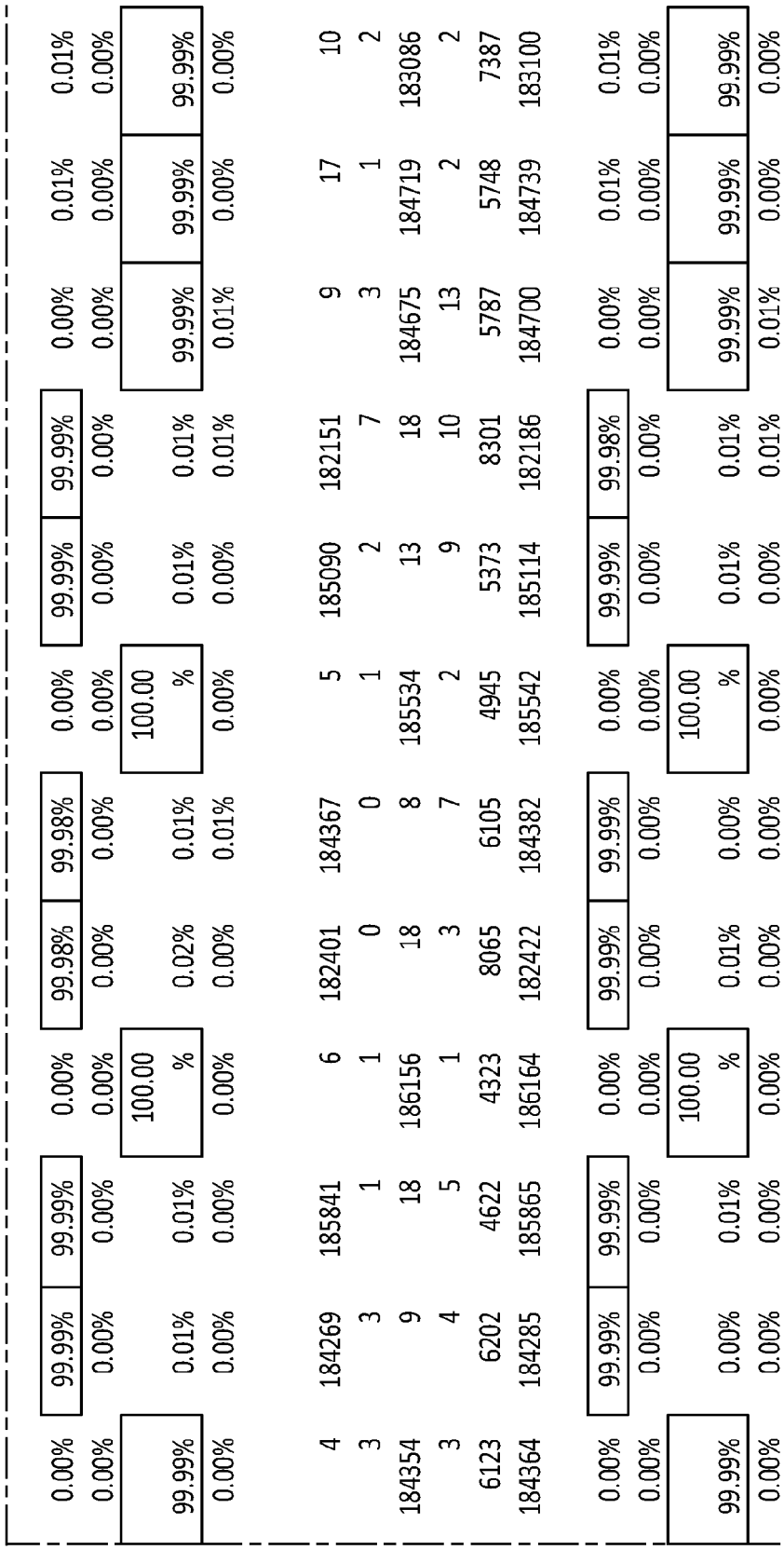


FIGURE 38

| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T | 9 A | 10 G | 11 T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 13 | 15 | 65 | 260044 | 10 | 6 | 11 | 3 | 261614 | 21 | 24 |
| **C** | 2 | 15 | 260820 | 58 | 23 | 261261 | 14 | 18 | 9 | 4 | 26 |
| **G** | 261608 | 5 | 3 | 16 | 0 | 211 | 1 | 1 | 25 | 261723 | 3 |
| **T** | 17 | 261439 | 24 | 21 | 261613 | 31 | 262463 | 262901 | 24 | 21 | 262969 |
| **N** | 2002 | 2189 | 2752 | 3525 | 2018 | 2155 | 1175 | 742 | 1995 | 1898 | 645 |
| | 261640 | 261474 | 260912 | 260139 | 261646 | 261509 | 262489 | 262923 | 261672 | 261769 | 263022 |
| **A** | 0.00% | 0.01% | 0.02% | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.01% |
| **C** | 0.00% | 0.01% | 99.96% | 0.02% | 0.01% | 99.91% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| **G** | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.08% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| **T** | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 99.98% |
| **pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)** | | | | | | | | | | | |
| **A** | 16 | 9 | 5 | 233859 | 7 | 4 | 17 | 4 | 235266 | 12 | 26 |
| **C** | 3 | 4 | 234720 | 55 | 13 | 235092 | 20 | 17 | 9 | 3 | 16 |
| **G** | 235303 | 2 | 1 | 91 | 0 | 2 | 1 | 0 | 13 | 235465 | 3 |
| **T** | 13 | 235124 | 19 | 13 | 235254 | 71 | 236055 | 236397 | 18 | 13 | 236375 |
| **N** | 1696 | 1912 | 2306 | 3034 | 1778 | 1884 | 960 | 635 | 1748 | 1561 | 634 |
| | 235335 | 235139 | 234745 | 234018 | 235274 | 235169 | 236093 | 236418 | 235306 | 235493 | 236420 |
| **A** | 0.01% | 0.00% | 0.00% | 99.93% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% |
| **C** | 0.00% | 0.00% | 99.99% | 0.02% | 0.01% | 99.97% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| **G** | 99.99% | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| **T** | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.03% | 99.98% | 99.99% | 0.01% | 0.01% | 99.98% |

FIGURE 38 (Continued)

| 12 C | 13 A | 14 G | 15 G | 16 A | 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 261211 | 13 | 7 | 260984 | 9 | 10 | 14 | 36 | 261083 | 6 | 8 |
| 262352 | 60 | 20 | 25 | 8 | 261819 | 260535 | 43 | 4 | 58 | 5 | 3 |
| 8 | 19 | 3 | 0 | 16 | 2 | 3 | 3 | 262744 | 27 | 262160 | 261387 |
| 39 | 11 | 263056 | 263039 | 14 | 14 | 16 | 261533 | 34 | 29 | 34 | 24 |
| 1245 | 2366 | 575 | 596 | 2645 | 1823 | 3103 | 2073 | 848 | 2470 | 1462 | 2245 |
| 262422 | 261301 | 263092 | 263071 | 261022 | 261844 | 260564 | 261593 | 262818 | 261197 | 262205 | 261422 |
| 0.01% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.01% | 99.96% | 0.00% | 0.00% |
| 99.97% | 0.02% | 0.01% | 0.01% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.98% | 99.99% |
| 0.01% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.01% |
| 14 | 234908 | 6 | 3 | 234746 | 3 | 6 | 7 | 20 | 234854 | 9 | 6 |
| 235954 | 46 | 13 | 20 | 12 | 235332 | 234224 | 37 | 5 | 45 | 5 | 1 |
| 2 | 17 | 2 | 0 | 14 | 4 | 4 | 2 | 236281 | 17 | 235795 | 235086 |
| 31 | 11 | 236546 | 236507 | 19 | 20 | 91 | 235302 | 34 | 16 | 39 | 23 |
| 1053 | 2072 | 487 | 524 | 2263 | 1695 | 2729 | 1706 | 714 | 2122 | 1206 | 1938 |
| 236001 | 234982 | 236567 | 236530 | 234791 | 235359 | 234325 | 235348 | 236340 | 234932 | 235848 | 235116 |
| 0.01% | 99.97% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% |
| 99.98% | 0.02% | 0.01% | 0.01% | 0.01% | 99.99% | 99.96% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 99.99% |
| 0.01% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.04% | 99.98% | 0.01% | 0.01% | 0.02% | 0.01% |

FIGURE 38 (Continued)

| pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4 | 1 | 5 | 112312 | 3 | 2 | 3 | 3 | 113016 | 10 | 9 | | | | | | | | | |
| C | 1 | 9 | 112701 | 20 | 6 | 112936 | 5 | 8 | 1 | 0 | 11 | | | | | | | | | |
| G | 113119 | 0 | 1 | 25 | 2 | 41 | 1 | 0 | 50 | 113060 | 0 | | | | | | | | | |
| T | 3 | 112980 | 8 | 6 | 113040 | 5 | 113434 | 113597 | 11 | 12 | 113618 | | | | | | | | | |
| N | 793 | 939 | 1214 | 1566 | 879 | 947 | 488 | 324 | 854 | 850 | 318 | | | | | | | | | |
| | 113127 | 112990 | 112715 | 112363 | 113051 | 112984 | 113443 | 113608 | 113078 | 113082 | 113638 | | | | | | | | | |
| A | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.00% | 0.00% | 0.00% | 99.95% | 0.01% | 0.01% | | | | | | | | | |
| C | 0.00% | 0.01% | 99.99% | 0.02% | 0.01% | 99.96% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | | | | | | | | | |
| G | 99.99% | 0.00% | 0.00% | 0.02% | 0.00% | 0.04% | 0.00% | 0.00% | 0.04% | 99.98% | 0.00% | | | | | | | | | |
| T | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 99.98% | | | | | | | | | |
| pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | | | | | | | | | | | |
| A | 9 | 7 | 4 | 188683 | 7 | 4 | 6 | 0 | 190287 | 8 | 6 | | | | | | | | | |
| C | 2 | 15 | 189720 | 43 | 18 | 190213 | 12 | 11 | 9 | 6 | 20 | | | | | | | | | |
| G | 190390 | 4 | 1 | 301 | 3 | 49 | 4 | 2 | 113 | 190454 | 2 | | | | | | | | | |
| T | 14 | 190156 | 42 | 17 | 190337 | 9 | 191012 | 191298 | 18 | 10 | 191318 | | | | | | | | | |
| N | 1368 | 1619 | 2034 | 2757 | 1436 | 1527 | 768 | 491 | 1393 | 1342 | 474 | | | | | | | | | |
| | 190415 | 190182 | 189767 | 189044 | 190365 | 190275 | 191034 | 191311 | 190427 | 190478 | 191346 | | | | | | | | | |
| A | 0.00% | 0.00% | 0.00% | 99.81% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.00% | | | | | | | | | |
| C | 0.00% | 0.01% | 99.98% | 0.02% | 0.01% | 99.97% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | | | | | | | | | |
| G | 99.99% | 0.00% | 0.00% | 0.16% | 0.00% | 0.03% | 0.00% | 0.00% | 0.06% | 99.99% | 0.00% | | | | | | | | | |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 99.99% | | | | | | | | | |
| pNMG-171 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; wild type) | | | | | | | | | | | | | | | | | | | | |
| A | 13 | 11 | 4 | 222824 | 8 | 26 | 14 | 2 | 224059 | 16 | 19 | | | | | | | | | |
| C | 1 | 9 | 223471 | 53 | 6 | 223941 | 20 | 18 | 28 | 26 | 39 | | | | | | | | | |
| G | 223993 | 2 | 4 | 6 | 0 | 26 | 2 | 25 | 15 | 224204 | 4 | | | | | | | | | |
| T | 31 | 223866 | 28 | 12 | 224027 | 38 | 224766 | 225111 | 18 | 13 | 225135 | | | | | | | | | |

# FIGURE 38 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 112888 | 6 | 1 | 112850 | 1 | 5 | 4 | 13 | 112902 | 2 | 4 |
| 113363 | 20 | 3 | 6 | 2 | 113126 | 112588 | 20 | 1 | 22 | 2 | 0 |
| 3 | 4 | 0 | 0 | 5 | 1 | 2 | 1 | 113551 | 11 | 113308 | 112987 |
| 15 | 8 | 113717 | 113692 | 7 | 11 | 16 | 113060 | 11 | 12 | 10 | 9 |
| 571 | 1036 | 230 | 257 | 1092 | 817 | 1345 | 871 | 380 | 1009 | 634 | 973 |
| 113385 | 112920 | 113726 | 113699 | 112864 | 113139 | 112611 | 113085 | 113576 | 112947 | 113322 | 113000 |
| 0.00% | 99.97% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 0.00% | 0.00% |
| 99.98% | 0.02% | 0.00% | 0.01% | 0.00% | 99.99% | 99.98% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% |
| 0.01% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.01% |
| 12 | 190067 | 13 | 1 | 189948 | 3 | 2 | 7 | 12 | 190007 | 3 | 5 |
| 190818 | 41 | 9 | 13 | 8 | 190438 | 189450 | 26 | 0 | 39 | 0 | 0 |
| 0 | 15 | 0 | 0 | 16 | 2 | 1 | 3 | 191174 | 12 | 190844 | 190198 |
| 24 | 7 | 191352 | 191396 | 14 | 19 | 48 | 190257 | 34 | 17 | 32 | 25 |
| 966 | 1690 | 446 | 410 | 1834 | 1358 | 2319 | 1527 | 600 | 1745 | 941 | 1592 |
| 190854 | 190130 | 191374 | 191410 | 189986 | 190462 | 189501 | 190293 | 191220 | 190075 | 190879 | 190228 |
| 0.01% | 99.97% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 0.00% | 0.00% |
| 99.98% | 0.02% | 0.00% | 0.01% | 0.00% | 99.99% | 99.97% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 99.98% |
| 0.01% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.03% | 99.98% | 0.02% | 0.01% | 0.02% | 0.01% |
| 6 | 223767 | 7 | 9 | 223602 | 29 | 1 | 7 | 19 | 223622 | 3 | 15 |
| 224688 | 37 | 36 | 14 | 5 | 224185 | 223114 | 59 | 2 | 36 | 2 | 1 |
| 2 | 21 | 0 | 25 | 17 | 6 | 3 | 4 | 225084 | 21 | 224567 | 223949 |
| 28 | 8 | 225282 | 225282 | 16 | 17 | 15 | 224009 | 31 | 22 | 25 | 18 |

FIGURE 38 (Continued)

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | G | T | C | A | T | C | T | T | A | G | T |
| N | | 1685 | 1877 | 2258 | 2870 | 1724 | 1734 | 963 | 609 | 1645 | 1506 | 568 |
| | | 224038 | 223888 | 223507 | 222895 | 224041 | 224031 | 224802 | 225156 | 224120 | 224259 | 225197 |
| A | | 0.01% | 0.00% | 0.00% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | 99.97% | 0.01% | 0.01% |
| C | | 0.00% | 0.00% | 99.98% | 0.02% | 0.00% | 99.96% | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% |
| G | | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% |
| T | | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.02% | 99.98% | 99.98% | 0.01% | 0.01% | 99.97% |
| **pNMG-172 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N)** | | | | | | | | | | | | |
| A | | 17 | 10 | 5 | 262192 | 4 | 7 | 7 | 2 | 263843 | 11 | 23 |
| C | | 1 | 17 | 263077 | 64 | 14 | 263512 | 19 | 53 | 6 | 3 | 28 |
| G | | 263811 | 4 | 5 | 13 | 3 | 8 | 2 | 0 | 22 | 263910 | 2 |
| T | | 13 | 263653 | 23 | 13 | 263624 | 16 | 264663 | 265063 | 25 | 47 | 265187 |
| N | | 1993 | 2171 | 2745 | 3573 | 2210 | 2313 | 1196 | 770 | 2018 | 1943 | 675 |
| | | 263842 | 263684 | 263110 | 262282 | 263645 | 263543 | 264691 | 265118 | 263896 | 263971 | 265240 |
| A | | 0.01% | 0.00% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% |
| C | | 0.00% | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% |
| G | | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| T | | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 99.98% | 0.01% | 0.02% | 99.98% |
| **pNMG-173 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)** | | | | | | | | | | | | |
| A | | 0 | 0 | 0 | 186 | 0 | 0 | 0 | 0 | 189 | 0 | 0 |
| C | | 0 | 0 | 184 | 0 | 0 | 190 | 0 | 0 | 0 | 0 | 0 |
| G | | 188 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 191 | 0 |
| T | | 0 | 185 | 0 | 0 | 183 | 0 | 189 | 192 | 0 | 0 | 191 |
| N | | 4 | 7 | 9 | 7 | 10 | 3 | 4 | 1 | 4 | 2 | 2 |
| | | 188 | 185 | 184 | 186 | 183 | 190 | 189 | 192 | 189 | 191 | 191 |

# FIGURE 38 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | A | G | G | A | C | C | T | G | A | G | G |
| 1041 | 1932 | 466 | 461 | 2151 | 1556 | 2660 | 1714 | 657 | 2112 | 1216 | 1830 |
| 224724 | 223833 | 225325 | 225330 | 223640 | 224237 | 223133 | 224079 | 225136 | 223701 | 224597 | 223983 |
| 0.00% | 99.97% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% | 0.00% | 0.01% | 99.96% | 0.00% | 0.01% |
| 99.98% | 0.02% | 0.02% | 0.01% | 0.00% | 99.98% | 99.99% | 0.03% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.98% |
| 0.01% | 0.00% | 99.98% | 99.98% | 0.01% | 0.01% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.01% |
| 10 | 263350 | 7 | 7 | 263275 | 6 | 6 | 15 | 33 | 263197 | 11 | 16 |
| 264585 | 53 | 16 | 94 | 12 | 264038 | 262665 | 53 | 2 | 46 | 6 | 2 |
| 3 | 17 | 1 | 2 | 15 | 7 | 6 | 1 | 265034 | 16 | 264416 | 263611 |
| 65 | 15 | 265321 | 265231 | 23 | 16 | 38 | 263866 | 35 | 28 | 33 | 18 |
| 1252 | 2480 | 570 | 581 | 2590 | 1848 | 3200 | 1980 | 811 | 2628 | 1449 | 2268 |
| 264663 | 263435 | 265345 | 265334 | 263325 | 264067 | 262715 | 263935 | 265104 | 263287 | 264466 | 263647 |
| 0.00% | 99.97% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 99.97% | 0.00% | 0.01% |
| 99.97% | 0.02% | 0.01% | 0.04% | 0.00% | 99.99% | 99.98% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.98% | 99.99% |
| 0.02% | 0.01% | 99.99% | 99.96% | 0.01% | 0.01% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.01% |
| 0 | 187 | 0 | 0 | 189 | 0 | 0 | 0 | 0 | 188 | 1 | 0 |
| 189 | 0 | 1 | 0 | 0 | 189 | 182 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 192 | 0 | 191 | 188 |
| 0 | 0 | 190 | 190 | 0 | 0 | 0 | 188 | 0 | 0 | 0 | 0 |
| 4 | 6 | 2 | 3 | 4 | 4 | 11 | 5 | 1 | 5 | 1 | 5 |
| 189 | 187 | 191 | 190 | 189 | 189 | 182 | 188 | 192 | 188 | 192 | 188 |

# FIGURE 38 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% |
| T | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% | 100.00 % | 100.00 % | 0.00% | 0.00% | 100.00 % |

**pNMG-174 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V )**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 19 | 18 | 5 | 272021 | 10 | 5 | 16 | 5 | 273725 | 14 | 21 |
| C | 4 | 23 | 273090 | 73 | 17 | 273441 | 22 | 24 | 13 | 4 | 25 |
| G | 273846 | 0 | 2 | 24 | 1 | 68 | 4 | 1 | 30 | 273905 | 1 |
| T | 14 | 273616 | 24 | 9 | 273712 | 14 | 274652 | 275080 | 23 | 21 | 275194 |
| N | 2040 | 2295 | 2832 | 3827 | 2214 | 2427 | 1261 | 846 | 2165 | 2012 | 735 |
| | 273883 | 273657 | 273121 | 272127 | 273740 | 273528 | 274694 | 275110 | 273791 | 273944 | 275241 |
| A | 0.01% | 0.01% | 0.00% | 99.96% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% |
| C | 0.00% | 0.01% | 99.99% | 0.03% | 0.01% | 99.97% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| G | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| T | 0.01% | 99.99% | 0.01% | 0.00% | 99.99% | 0.01% | 99.98% | 99.99% | 0.01% | 0.01% | 99.98% |

**pNMG-175 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 17 | 8 | 2 | 237171 | 3 | 6 | 8 | 2 | 238165 | 9 | 18 |
| C | 2 | 26 | 237432 | 64 | 16 | 237852 | 17 | 30 | 16 | 7 | 24 |
| G | 237491 | 1 | 1 | 9 | 1 | 45 | 0 | 1 | 22 | 237650 | 1 |
| T | 14 | 237857 | 33 | 21 | 237972 | 9 | 238266 | 238625 | 24 | 18 | 238862 |
| N | 1898 | 1561 | 1985 | 2188 | 1461 | 1541 | 1162 | 795 | 1244 | 1787 | 566 |

FIGURE 38 (Continued)

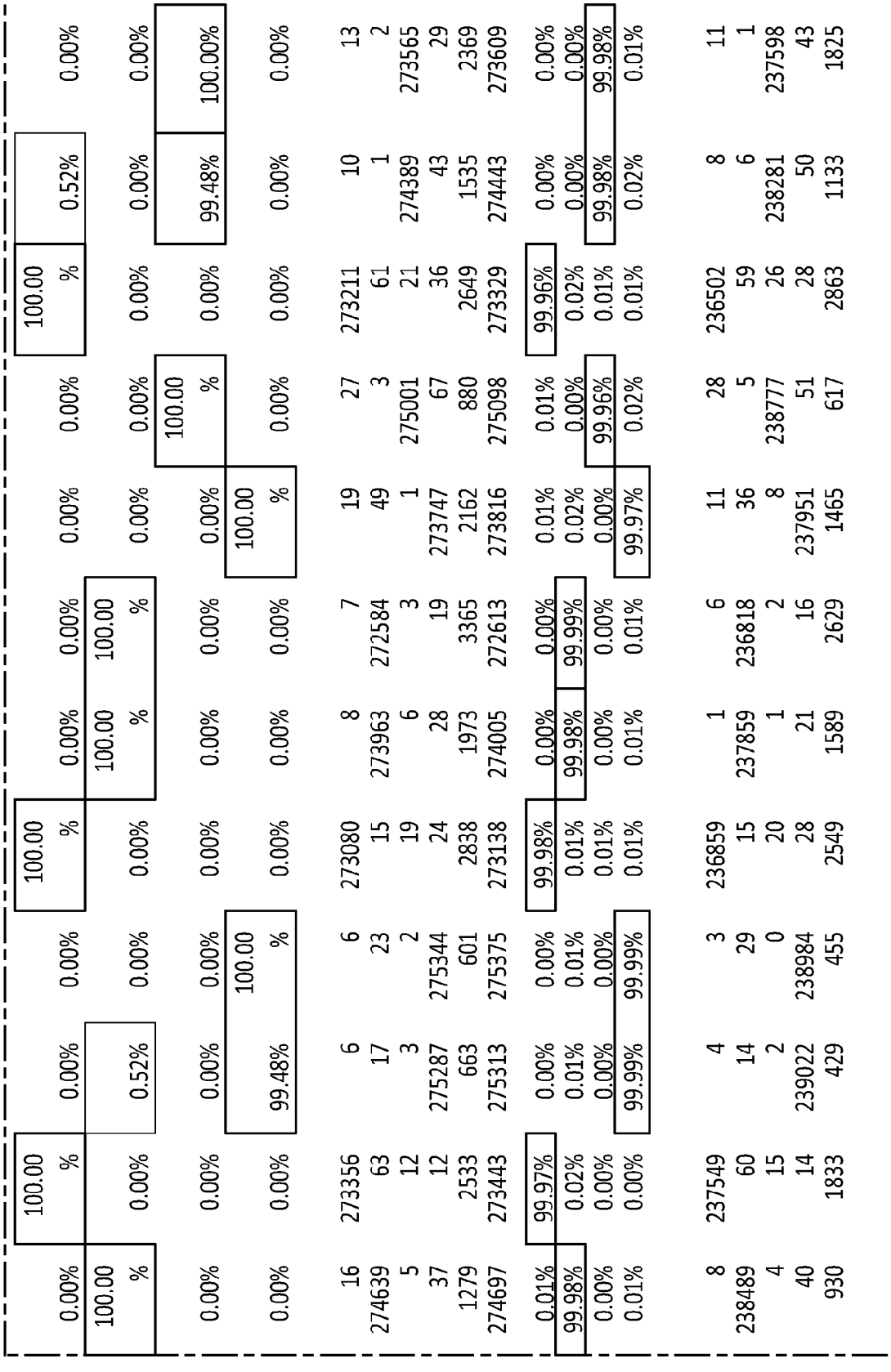


FIGURE 38 (Continued)

| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T | 9 A | 10 G | 11 T |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 237524 | 237892 | 237468 | 237265 | 237992 | 237912 | 238291 | 238658 | 238227 | 237684 | 238905 |
| A | 0.01% | 0.00% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 0.01% |
| C | 0.00% | 0.01% | 99.98% | 0.03% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| G | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| T | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 99.98% |
| **pNMG-176 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S)** | | | | | | | | | | | |
| A | 12 | 6 | 10 | 209833 | 3 | 5 | 2 | 1 | 210501 | 21 | 19 |
| C | 1 | 14 | 209843 | 43 | 19 | 210262 | 15 | 19 | 11 | 4 | 30 |
| G | 209873 | 3 | 5 | 13 | 0 | 27 | 2 | 0 | 14 | 210099 | 1 |
| T | 11 | 210244 | 21 | 23 | 210371 | 11 | 210527 | 210913 | 28 | 18 | 211042 |
| N | 1729 | 1378 | 1766 | 1734 | 1253 | 1342 | 1101 | 714 | 1093 | 1505 | 555 |
| | 209897 | 210267 | 209879 | 209912 | 210393 | 210305 | 210546 | 210933 | 210554 | 210142 | 211092 |
| A | 0.01% | 0.00% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 0.01% |
| C | 0.00% | 0.01% | 99.98% | 0.02% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| G | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% |
| T | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 99.98% |
| **pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 15 | 11 | 5 | 205333 | 6 | 7 | 8 | 4 | 206417 | 11 | 6 |
| C | 0 | 12 | 205779 | 54 | 21 | 206227 | 16 | 21 | 11 | 4 | 13 |
| G | 205786 | 1 | 2 | 310 | 9 | 34 | 3 | 0 | 135 | 206005 | 5 |
| T | 15 | 206247 | 21 | 13 | 206326 | 25 | 206535 | 206905 | 14 | 20 | 207085 |
| N | 1795 | 1375 | 1839 | 1937 | 1285 | 1355 | 1087 | 719 | 1072 | 1609 | 549 |
| | 205816 | 206271 | 205807 | 205710 | 206362 | 206293 | 206562 | 206930 | 206577 | 206040 | 207109 |

# FIGURE 38 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | A | G | G | A | C | C | T | G | A | G | G |
| 238541 | 237638 | 239042 | 239016 | 236922 | 237882 | 236842 | 238006 | 238861 | 236615 | 238345 | 237653 |
| 0.00% | 99.96% | 0.00% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 0.01% | 99.95% | 0.00% | 0.00% |
| 99.98% | 0.03% | 0.01% | 0.01% | 0.01% | 99.99% | 99.99% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.96% | 0.01% | 99.97% | 99.98% |
| 0.02% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.02% | 0.01% | 0.02% | 0.02% |
| 14 | 209912 | 5 | 1 | 209419 | 9 | 7 | 3 | 20 | 209054 | 7 | 7 |
| 210771 | 43 | 13 | 18 | 9 | 210257 | 209258 | 40 | 1 | 44 | 3 | 1 |
| 1 | 11 | 3 | 2 | 18 | 7 | 1 | 1 | 211029 | 19 | 210666 | 210083 |
| 25 | 11 | 211189 | 211231 | 20 | 21 | 12 | 210274 | 30 | 28 | 24 | 19 |
| 836 | 1670 | 437 | 395 | 2181 | 1353 | 2369 | 1329 | 567 | 2502 | 947 | 1537 |
| 210811 | 209977 | 211210 | 211252 | 209466 | 210294 | 209278 | 210318 | 211080 | 209145 | 210700 | 210110 |
| 0.01% | 99.97% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 0.00% | 0.00% |
| 99.98% | 0.02% | 0.01% | 0.01% | 0.00% | 99.98% | 99.99% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 99.99% |
| 0.01% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.01% |
| 12 | 205909 | 4 | 2 | 205410 | 8 | 6 | 12 | 21 | 205101 | 2 | 7 |
| 206739 | 45 | 18 | 17 | 7 | 206257 | 205325 | 29 | 4 | 47 | 1 | 2 |
| 1 | 10 | 1 | 2 | 13 | 1 | 4 | 0 | 207026 | 14 | 206651 | 206023 |
| 28 | 9 | 207196 | 207266 | 23 | 18 | 16 | 206226 | 47 | 29 | 51 | 31 |
| 878 | 1685 | 439 | 371 | 2205 | 1374 | 2307 | 1391 | 560 | 2467 | 953 | 1606 |
| 206780 | 205973 | 207219 | 207287 | 205453 | 206284 | 205351 | 206267 | 207098 | 205191 | 206705 | 206063 |



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# FIGURE 38 (Continued)

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 0.01% | 0.00% | 99.82% | 0.00% | 0.00% | 0.00% | 0.00% | 99.92% | 0.01% | 0.00% | | | |
| C | 0.00% | 0.01% | 99.99% | 0.03% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | | | |
| G | 99.99% | 0.00% | 0.00% | 0.15% | 0.00% | 0.02% | 0.00% | 0.00% | 0.07% | 99.98% | 0.00% | | | |
| T | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 99.99% | | | |
| **pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | | | | |
| A | 13 | 8 | 4 | 235817 | 4 | 5 | 9 | 3 | 236803 | 12 | 15 | | | |
| C | 2 | 18 | 236093 | 58 | 22 | 236381 | 23 | 11 | 12 | 5 | 24 | | | |
| G | 236016 | 3 | 5 | 150 | 2 | 10 | 5 | 1 | 103 | 236386 | 2 | | | |
| T | 14 | 236487 | 44 | 32 | 236758 | 224 | 236951 | 237309 | 30 | 20 | 237487 | | | |
| N | 2003 | 1558 | 1928 | 2019 | 1290 | 1456 | 1088 | 752 | 1161 | 1687 | 595 | | | |
| | 236045 | 236516 | 236146 | 236057 | 236786 | 236620 | 236988 | 237324 | 236948 | 236423 | 237528 | | | |
| A | 0.01% | 0.00% | 0.00% | 99.90% | 0.00% | 0.00% | 0.00% | 0.00% | 99.94% | 0.01% | 0.01% | | | |
| C | 0.00% | 0.01% | 99.98% | 0.02% | 0.01% | 99.90% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | | | |
| G | 99.99% | 0.00% | 0.00% | 0.06% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 99.98% | 0.00% | | | |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.09% | 99.98% | 99.99% | 0.01% | 0.01% | 99.98% | | | |
| **pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | | | | |
| A | 16 | 16 | 6 | 264566 | 7 | 9 | 5 | 4 | 265730 | 10 | 13 | | | |
| C | 7 | 15 | 265022 | 75 | 21 | 265379 | 22 | 29 | 9 | 5 | 29 | | | |
| G | 264815 | 2 | 3 | 200 | 0 | 90 | 3 | 3 | 53 | 265342 | 3 | | | |
| T | 22 | 265342 | 28 | 29 | 265475 | 45 | 265776 | 266212 | 24 | 28 | 266409 | | | |
| N | 2242 | 1747 | 2063 | 2252 | 1619 | 1599 | 1316 | 874 | 1312 | 1743 | 676 | | | |
| | 264860 | 265375 | 265059 | 264870 | 265503 | 265523 | 265806 | 266248 | 265816 | 265385 | 266454 | | | |

FIGURE 38 (Continued)

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.01% | 99.97% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 99.96% | 0.00% | 0.00% | | | | | | | | | | | | | |
| 99.98% | 0.02% | 0.01% | 0.01% | 0.00% | 99.99% | 99.99% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | | | | | | | | | | | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.97% | 99.98% | | | | | | | | | | | | | |
| 0.01% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.02% | 0.01% | 0.02% | 0.02% | | | | | | | | | | | | | |
| 14 | 236243 | 7 | 1 | 235623 | 10 | 6 | 4 | 17 | 235424 | 6 | 16 | | | | | | | | | | | | | |
| 237099 | 55 | 13 | 21 | 11 | 236514 | 235410 | 45 | 11 | 53 | 4 | 4 | | | | | | | | | | | | | |
| 7 | 19 | 0 | 1 | 16 | 4 | 6 | 4 | 237487 | 19 | 237105 | 236402 | | | | | | | | | | | | | |
| 67 | 15 | 237647 | 237679 | 23 | 23 | 17 | 236578 | 42 | 40 | 48 | 19 | | | | | | | | | | | | | |
| 936 | 1791 | 456 | 421 | 2450 | 1573 | 2695 | 1503 | 578 | 2599 | 972 | 1694 | | | | | | | | | | | | | |
| 237187 | 236332 | 237667 | 237702 | 235673 | 236551 | 235439 | 236631 | 237557 | 235536 | 237163 | 236441 | | | | | | | | | | | | | |
| 0.01% | 99.96% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.95% | 0.00% | 0.01% | | | | | | | | | | | | | |
| 99.96% | 0.02% | 0.01% | 0.01% | 0.00% | 99.98% | 99.99% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% | | | | | | | | | | | | | |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.98% | 99.98% | | | | | | | | | | | | | |
| 0.03% | 0.01% | 99.99% | 99.99% | 0.01% | 0.01% | 0.01% | 99.98% | 0.02% | 0.02% | 0.02% | 0.01% | | | | | | | | | | | | | |
| 11 | 264948 | 4 | 4 | 264356 | 9 | 11 | 4 | 30 | 263979 | 6 | 13 | | | | | | | | | | | | | |
| 266022 | 69 | 14 | 56 | 14 | 265259 | 264020 | 44 | 5 | 67 | 2 | 0 | | | | | | | | | | | | | |
| 1 | 15 | 2 | 0 | 29 | 1 | 2 | 6 | 266389 | 22 | 265901 | 265097 | | | | | | | | | | | | | |
| 39 | 15 | 266569 | 266615 | 31 | 36 | 22 | 265340 | 42 | 29 | 45 | 23 | | | | | | | | | | | | | |
| 1057 | 2083 | 541 | 455 | 2700 | 1825 | 3075 | 1736 | 665 | 3034 | 1177 | 1998 | | | | | | | | | | | | | |
| 266073 | 265047 | 266589 | 266675 | 264430 | 265305 | 264055 | 265394 | 266466 | 264097 | 265954 | 265133 | | | | | | | | | | | | | |

| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T |
|---|---|---|---|---|---|---|---|
| A | 0.01% | 0.01% | 0.00% | 99.89% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.01% | 99.99% | 0.03% | 0.01% | 99.95% | 0.01% |
| G | 99.98% | 0.00% | 0.00% | 0.08% | 0.00% | 0.03% | 0.00% |
| T | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.02% | 99.99% |
| **pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | |
| A | 21 | 11 | 7 | 278816 | 2 | 6 | 9 |
| C | 3 | 18 | 279252 | 52 | 25 | 279591 | 23 |
| G | 279336 | 3 | 1 | 285 | 1 | 23 | 11 |
| T | 25 | 279882 | 43 | 29 | 279914 | 224 | 280164 |
| N | 2242 | 1741 | 2353 | 2474 | 1714 | 1814 | 1455 |
| | 279385 | 279914 | 279303 | 279182 | 279942 | 279844 | 280207 |
| A | 0.01% | 0.00% | 0.00% | 99.87% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.01% | 99.98% | 0.02% | 0.01% | 99.91% | 0.01% |
| G | 99.98% | 0.00% | 0.00% | 0.10% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.08% | 99.98% |
| **untreated cells** | | | | | | | |
| A | 14 | 10 | 10 | 281734 | 5 | 8 | 10 |
| C | 1 | 20 | 281864 | 59 | 24 | 282320 | 23 |
| G | 281824 | 2 | 2 | 12 | 1 | 3 | 8 |
| T | 20 | 282327 | 34 | 15 | 282459 | 12 | 282768 |
| N | 2374 | 1887 | 2339 | 2429 | 1760 | 1911 | 1445 |
| | 281859 | 282359 | 281910 | 281820 | 282489 | 282343 | 282809 |
| A | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.01% | 99.98% | 0.02% | 0.01% | 99.99% | 0.01% |
| G | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 99.99% |

FIGURE 38
(Continued)

FIGURE 38
(Continued)

| 8 T | 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G |
|---|---|---|---|---|---|---|---|
| 0.00% | 99.97% | 0.00% | 0.00% | 0.00% | 99.96% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.03% | 0.01% | 0.02% |
| 0.00% | 0.02% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% | 99.98% |
| 2 | 279922 | 17 | 15 | 12 | 279371 | 8 | 6 |
| 38 | 15 | 6 | 24 | 280603 | 54 | 17 | 38 |
| 2 | 282 | 279647 | 5 | 12 | 18 | 4 | 1 |
| 280652 | 34 | 39 | 281016 | 48 | 24 | 281129 | 281168 |
| 968 | 1450 | 1994 | 650 | 1035 | 2243 | 564 | 509 |
| 280694 | 280253 | 279709 | 281060 | 280675 | 279467 | 281158 | 281213 |
| 0.00% | 99.88% | 0.01% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% |
| 0.01% | 0.01% | 0.00% | 0.01% | 99.97% | 0.02% | 0.01% | 0.01% |
| 0.00% | 0.10% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.98% | 0.02% | 0.01% | 99.99% | 99.98% |
| 4 | 282782 | 13 | 18 | 20 | 281885 | 9 | 7 |
| 36 | 8 | 3 | 31 | 283072 | 62 | 16 | 26 |
| 3 | 25 | 282176 | 1 | 4 | 16 | 0 | 0 |
| 283253 | 26 | 25 | 283489 | 39 | 19 | 283729 | 283667 |
| 958 | 1414 | 2038 | 717 | 1121 | 2274 | 502 | 556 |
| 283296 | 282841 | 282217 | 283539 | 283135 | 281982 | 283754 | 283700 |
| 0.00% | 99.98% | 0.00% | 0.01% | 0.01% | 99.97% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.02% | 0.01% | 0.01% |
| 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 99.98% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% | 99.99% |

# FIGURE 39

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | A | A | T | C | C | C | T | T | C |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild type)** | | | | | | | | | | | |
| A | 18 | 10 | 210454 | 212180 | 10 | 1 | 2 | 4 | 11 | 11 | 4 |
| C | 2 | 3 | 4 | 2 | 17 | 212977 | 210945 | 214564 | 12 | 20 | 215157 |
| G | 214306 | 214030 | 19 | 14 | 0 | 97 | 3 | 2 | 0 | 0 | 1 |
| T | 8 | 12 | 6 | 11 | 215980 | 89 | 56 | 81 | 214635 | 216459 | 56 |
| N | 3779 | 4058 | 7630 | 5907 | 2107 | 4950 | 7108 | 3463 | 3456 | 1624 | 2896 |
| | 214334 | 214055 | 210483 | 212207 | 216007 | 213164 | 211006 | 214651 | 214658 | 216490 | 215218 |
| A | 0.01% | 0.00% | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.91% | 99.97% | 99.96% | 0.01% | 0.01% | 99.97% |
| G | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.00% | 0.01% | 99.99% | 0.04% | 0.03% | 0.04% | 99.99% | 99.99% | 0.03% |
| **pNMG-143 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N)** | | | | | | | | | | | |
| A | 10 | 6 | 161482 | 162734 | 5 | 1 | 6 | 1 | 11 | 5 | 3 |
| C | 2 | 4 | 7 | 0 | 13 | 163350 | 161823 | 164757 | 10 | 10 | 165189 |
| G | 164467 | 164291 | 33 | 17 | 1 | 1 | 5 | 3 | 3 | 1 | 46 |
| T | 12 | 3 | 5 | 41 | 165892 | 124 | 82 | 30 | 164777 | 166231 | 5 |
| N | 3067 | 3254 | 6031 | 4766 | 1647 | 4082 | 5641 | 2766 | 2756 | 1310 | 2314 |
| | 164491 | 164304 | 161527 | 162792 | 165911 | 163476 | 161916 | 164791 | 164801 | 166247 | 165243 |
| A | 0.01% | 0.00% | 99.97% | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.92% | 99.94% | 99.98% | 0.01% | 0.01% | 99.97% |
| G | 99.99% | 99.99% | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% |
| T | 0.01% | 0.00% | 0.00% | 0.03% | 99.99% | 0.08% | 0.05% | 0.02% | 99.99% | 99.99% | 0.00% |

# FIGURE 39 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | A | G | C | A | C | C | T | G | G |
| 4 | 19 | 3 | 217050 | 9 | 2 | 214992 | 1 | 4 | 11 | 23 | 14 |
| 16 | 4 | 216351 | 13 | 1 | 215721 | 7 | 213699 | 214333 | 46 | 2 | 4 |
| 0 | 213912 | 7 | 17 | 216480 | 3 | 14 | 0 | 1 | 5 | 214582 | 213252 |
| 215719 | 8 | 40 | 3 | 6 | 5 | 13 | 8 | 7 | 215508 | 6 | 8 |
| 2375 | 4171 | 1713 | 1031 | 1618 | 2383 | 3088 | 4405 | 3768 | 2511 | 3468 | 4802 |
| 215739 | 213943 | 216401 | 217083 | 216496 | 215731 | 215026 | 213708 | 214345 | 215570 | 214613 | 213278 |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 100.00% | 0.00% | 100.00% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 99.99% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 99.97% | 0.00% | 0.00% |
| 4 | 17 | 1 | 166783 | 7 | 6 | 165043 | 0 | 1 | 11 | 9 | 17 |
| 27 | 0 | 166093 | 0 | 0 | 165612 | 9 | 164245 | 164661 | 30 | 3 | 8 |
| 0 | 164239 | 4 | 11 | 166268 | 0 | 7 | 3 | 1 | 3 | 164903 | 163847 |
| 165700 | 6 | 34 | 3 | 3 | 7 | 8 | 8 | 12 | 165475 | 5 | 3 |
| 1826 | 3295 | 1425 | 760 | 1279 | 1932 | 2489 | 3300 | 2881 | 2037 | 2636 | 3680 |
| 165731 | 164262 | 166132 | 166797 | 166278 | 165625 | 165067 | 164256 | 164675 | 165519 | 164920 | 163875 |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| 0.02% | 0.00% | 99.98% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.98% |
| 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% |

FIGURE 39 (Continued)

**pNMG-144 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 10 | 2 | 115692 | 116529 | 2 | 47 | 1 | 2 | 3 | 1 | 3 | |
| C | 6 | 0 | 1 | 0 | 7 | 117026 | 115985 | 118013 | 8 | 4 | 118343 | |
| G | 117876 | 117749 | 22 | 63 | 1 | 42 | 1 | 1 | 10 | 0 | 16 | |
| T | 10 | 11 | 5 | 6 | 118852 | 34 | 5 | 6 | 118004 | 119096 | 31 | |
| N | 2143 | 2283 | 4324 | 3446 | 1182 | 2895 | 4052 | 2023 | 2020 | 944 | 1652 | |
| | 117902 | 117762 | 115720 | 116598 | 118862 | 117149 | 115992 | 118022 | 118025 | 119101 | 118393 | |
| | | | | | | | | | | | | |
| A | 0.01% | 0.00% | 99.98% | 99.94% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.90% | 99.99% | 99.99% | 0.01% | 0.00% | 99.96% | |
| G | 99.98% | 99.99% | 0.02% | 0.05% | 0.00% | 0.04% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | |
| T | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.03% | 0.00% | 0.01% | 99.98% | 100.00% | 0.03% | |

**pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 15 | 13 | 151693 | 152749 | 4 | 3 | 2 | 7 | 4 | 6 | 37 | |
| C | 3 | 5 | 2 | 2 | 18 | 153357 | 152124 | 154645 | 8 | 12 | 155108 | |
| G | 154538 | 154353 | 11 | 219 | 0 | 154 | 0 | 1 | 1 | 2 | 3 | |
| T | 3 | 2 | 11 | 11 | 155742 | 224 | 100 | 113 | 154741 | 156122 | 75 | |
| N | 2791 | 2977 | 5633 | 4369 | 1586 | 3612 | 5124 | 2584 | 2596 | 1208 | 2127 | |
| | 154559 | 154373 | 151717 | 152981 | 155764 | 153738 | 152226 | 154766 | 154754 | 156142 | 155223 | |
| | | | | | | | | | | | | |
| A | 0.01% | 0.01% | 99.98% | 99.85% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.75% | 99.93% | 99.92% | 0.01% | 0.01% | 99.93% | |
| G | 99.99% | 99.99% | 0.01% | 0.14% | 0.00% | 0.10% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |

# FIGURE 39 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 6 | 3 | 119412 | 7 | 4 | 118296 | 1 | 2 | 3 | 9 | 10 |
| 14 | 0 | 119006 | 2 | 1 | 118630 | 2 | 117532 | 117975 | 13 | 2 | 5 |
| 1 | 117680 | 1 | 10 | 119089 | 1 | 3 | 0 | 2 | 1 | 118183 | 117409 |
| 118745 | 5 | 12 | 2 | 1 | 20 | 7 | 40 | 17 | 118660 | 2 | 5 |
| 1279 | 2354 | 1023 | 619 | 947 | 1390 | 1737 | 2472 | 2049 | 1368 | 1848 | 2615 |
| 118766 | 117691 | 119022 | 119426 | 119098 | 118655 | 118308 | 117573 | 117996 | 118677 | 118196 | 117429 |
| 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 99.98% | 0.00% | 99.97% | 99.98% | 0.01% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.98% |
| 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 0.01% | 0.03% | 0.01% | 99.99% | 0.00% | 0.00% |
| 7 | 18 | 18 | 156520 | 8 | 0 | 155088 | 1 | 3 | 8 | 12 | 18 |
| 19 | 1 | 156015 | 11 | 4 | 155568 | 5 | 154185 | 154629 | 24 | 2 | 5 |
| 1 | 154328 | 3 | 9 | 156164 | 2 | 8 | 1 | 0 | 2 | 154899 | 153937 |
| 155718 | 2 | 29 | 0 | 3 | 8 | 11 | 17 | 4 | 155575 | 6 | 5 |
| 1605 | 3001 | 1286 | 811 | 1172 | 1773 | 2239 | 3147 | 2715 | 1760 | 2450 | 3402 |
| 155745 | 154349 | 156065 | 156540 | 156179 | 155578 | 155112 | 154204 | 154636 | 155609 | 154919 | 153965 |
| 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.97% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 100.00 % | 0.02% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.98% |

# FIGURE 39 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | A | A | T | C | C | C | T | T | C |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.15% | 0.07% | 0.07% | 99.99% | 99.99% | 0.05% |
| **pNMG-171 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; wild type)** | | | | | | | | | | | |
| A | 15 | 15 | 171779 | 173217 | 6 | 2 | 3 | 4 | 5 | 7 | 3 |
| C | 2 | 4 | 2 | 1 | 15 | 173801 | 172172 | 175212 | 17 | 9 | 175573 |
| G | 174922 | 174674 | 14 | 20 | 1 | 70 | 16 | 12 | 0 | 12 | 3 |
| T | 25 | 4 | 7 | 5 | 176299 | 84 | 32 | 54 | 175148 | 176718 | 11 |
| N | 3134 | 3401 | 6296 | 4855 | 1754 | 4118 | 5852 | 2793 | 2905 | 1329 | 2485 |
| | 174964 | 174697 | 171802 | 173243 | 176321 | 173957 | 172223 | 175282 | 175170 | 176746 | 175590 |
| A | 0.01% | 0.01% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.91% | 99.97% | 99.96% | 0.01% | 0.01% | 99.99% |
| G | 99.98% | 99.99% | 0.01% | 0.01% | 0.00% | 0.04% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.05% | 0.02% | 0.03% | 99.99% | 99.98% | 0.01% |
| **pNMG-172 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N)** | | | | | | | | | | | |
| A | 9 | 7 | 180709 | 182106 | 7 | 63 | 5 | 36 | 5 | 12 | 25 |
| C | 2 | 2 | 2 | 1 | 11 | 182054 | 180648 | 183567 | 7 | 10 | 184551 |
| G | 184083 | 183785 | 19 | 19 | 3 | 83 | 2 | 32 | 2 | 1 | 3 |
| T | 4 | 3 | 7 | 8 | 185449 | 747 | 553 | 573 | 184236 | 185894 | 289 |
| N | 3182 | 3483 | 6543 | 5146 | 1810 | 4333 | 6072 | 3072 | 3030 | 1363 | 2413 |
| | 184098 | 183797 | 180737 | 182134 | 185470 | 182947 | 181208 | 184208 | 184250 | 185917 | 184868 |
| A | 0.00% | 0.00% | 99.98% | 99.98% | 0.00% | 0.03% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.51% | 99.69% | 99.65% | 0.00% | 0.01% | 99.83% |

# FIGURE 39 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | A | G | C | A | C | C | T | G | G |
| 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% |
| 5 | 14 | 3 | 177184 | 10 | 4 | 175472 | 0 | 25 | 17 | 23 | 18 |
| 20 | 0 | 176554 | 5 | 4 | 176153 | 4 | 174531 | 174923 | 71 | 3 | 4 |
| 1 | 174562 | 5 | 17 | 176731 | 0 | 17 | 1 | 0 | 5 | 175347 | 174167 |
| 176172 | 6 | 69 | 4 | 2 | 13 | 32 | 10 | 8 | 175947 | 3 | 19 |
| 1876 | 3492 | 1443 | 864 | 1327 | 1904 | 2549 | 3532 | 3118 | 2034 | 2698 | 3844 |
| 176198 | 174582 | 176631 | 177210 | 176747 | 176170 | 175525 | 174542 | 174956 | 176040 | 175376 | 174208 |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 99.97% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.96% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.98% | 0.04% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 99.98% |
| 99.99% | 0.00% | 0.04% | 0.00% | 0.00% | 0.01% | 0.02% | 0.01% | 0.00% | 99.95% | 0.00% | 0.01% |
| 7 | 18 | 5 | 186320 | 6 | 0 | 184614 | 0 | 5 | 5 | 11 | 12 |
| 21 | 2 | 185717 | 5 | 2 | 185210 | 0 | 183544 | 184073 | 36 | 3 | 7 |
| 2 | 183685 | 6 | 22 | 185874 | 0 | 8 | 2 | 0 | 4 | 184465 | 183231 |
| 185280 | 4 | 79 | 6 | 2 | 5 | 21 | 10 | 6 | 185123 | 1 | 3 |
| 1971 | 3572 | 1474 | 928 | 1397 | 2066 | 2638 | 3725 | 3197 | 2113 | 2800 | 4026 |
| 185310 | 183709 | 185807 | 186353 | 185884 | 185215 | 184643 | 183556 | 184084 | 185168 | 184480 | 183253 |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.95% | 0.00% | 0.00% | 100.00% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |

# FIGURE 39 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | 0.05% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.41% | 0.31% | 0.31% | 99.99% | 99.99% | 0.16% |

**pNMG-173 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S_D147Y_Q154H)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 20 | 11 | 176047 | 177440 | 4 | 2 | 7 | 4 | 12 | 11 | 55 |
| C | 6 | 1 | 2 | 2 | 9 | 178118 | 176417 | 179353 | 11 | 9 | 179804 |
| G | 179035 | 178783 | 22 | 12 | 0 | 1 | 1 | 0 | 0 | 0 | 4 |
| T | 30 | 1 | 10 | 10 | 180519 | 13 | 11 | 9 | 179325 | 180806 | 6 |
| N | 3124 | 3419 | 6134 | 4751 | 1683 | 4081 | 5779 | 2849 | 2867 | 1389 | 2346 |
| | 179091 | 178796 | 176081 | 177464 | 180532 | 178134 | 176436 | 179366 | 179348 | 180826 | 179869 |
| | | | | | | | | | | | |
| A | 0.01% | 0.01% | 99.98% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.03% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% | 99.99% | 0.01% | 0.00% | 99.96% |
| G | 99.97% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.02% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 0.01% | 99.99% | 99.99% | 0.00% |

**pNMG-174 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_R24W_D108N_N127S_D147Y_E155V )**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 14 | 13 | 124305 | 125318 | 4 | 18 | 0 | 4 | 4 | 2 | 6 |
| C | 0 | 3 | 2 | 0 | 12 | 125927 | 124575 | 126835 | 4 | 3 | 127203 |
| G | 126713 | 126458 | 17 | 7 | 0 | 0 | 3 | 13 | 0 | 0 | 3 |
| T | 9 | 6 | 8 | 11 | 127679 | 26 | 28 | 16 | 126764 | 128012 | 3 |
| N | 2229 | 2485 | 4632 | 3628 | 1269 | 2993 | 4358 | 2096 | 2192 | 947 | 1749 |
| | 126736 | 126480 | 124332 | 125336 | 127695 | 125971 | 124606 | 126868 | 126772 | 128017 | 127215 |
| | | | | | | | | | | | |
| A | 0.01% | 0.01% | 99.98% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 99.98% | 99.97% | 0.00% | 0.00% | 99.99% |



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FIGURE 39 (Continued)

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% | | | | | | | |
| 99.98% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | | | | | | | |
| 6 | 7 | 4 | 181283 | 6 | 4 | 179655 | 0 | 3 | 8 | 17 | 9 | | | | | | | | |
| 19 | 0 | 180688 | 7 | 5 | 180165 | 5 | 178767 | 179309 | 26 | 5 | 1 | | | | | | | | |
| 0 | 178763 | 5 | 12 | 180752 | 1 | 10 | 0 | 3 | 2 | 179384 | 178424 | | | | | | | | |
| 180393 | 8 | 42 | 4 | 81 | 10 | 8 | 11 | 2 | 180228 | 3 | 1 | | | | | | | | |
| 1797 | 3437 | 1476 | 909 | 1371 | 2035 | 2537 | 3437 | 2898 | 1951 | 2805 | 3777 | | | | | | | | |
| 180418 | 178778 | 180739 | 181306 | 180844 | 180180 | 179678 | 178778 | 179317 | 180264 | 179409 | 178435 | | | | | | | | |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | | | | | | | | |
| 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 100.00 % | 0.01% | 0.00% | 0.00% | | | | | | | | |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.95% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% | | | | | | | | |
| 99.99% | 0.00% | 0.02% | 0.00% | 0.04% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | | | | | | | | |
| 9 | 15 | 20 | 128386 | 10 | 1 | 127093 | 0 | 2 | 7 | 8 | 5 | | | | | | | | |
| 11 | 2 | 127905 | 2 | 1 | 127512 | 1 | 126329 | 126725 | 21 | 1 | 3 | | | | | | | | |
| 0 | 126383 | 0 | 6 | 128021 | 2 | 4 | 1 | 0 | 1 | 126956 | 126124 | | | | | | | | |
| 127546 | 1 | 22 | 2 | 1 | 11 | 12 | 10 | 21 | 127424 | 2 | 8 | | | | | | | | |
| 1399 | 2564 | 1025 | 576 | 939 | 1441 | 1857 | 2627 | 2219 | 1514 | 2000 | 2826 | | | | | | | | |
| 127566 | 126401 | 127947 | 128396 | 128033 | 127526 | 127110 | 126340 | 126748 | 127453 | 126967 | 126140 | | | | | | | | |
| 0.01% | 0.01% | 0.02% | 99.99% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | | | | | | | | |
| 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.98% | 0.02% | 0.00% | 0.00% | | | | | | | | |

FIGURE 39 (Continued)

| | 1 G | 2 G | 3 A | 4 A | 5 T | 6 C | 7 C | 8 C | 9 T | 10 T | 11 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 99.98% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.02% | 0.02% | 0.01% | 99.99% | 100.00% | 0.00% |

**pNMG-175 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 13 | 12 | 264383 | 260043 | 9 | 25 | 36 | 29 | 7 | 8 | 41 |
| C | 2 | 7 | 2 | 1 | 29 | 263371 | 261236 | 265394 | 16 | 8 | 265961 |
| G | 265023 | 264362 | 14 | 16 | 0 | 39 | 5 | 46 | 1 | 0 | 44 |
| T | 5 | 36 | 12 | 10 | 266923 | 205 | 197 | 128 | 265398 | 267552 | 103 |
| N | 4303 | 4929 | 4935 | 9275 | 2384 | 5705 | 7871 | 3748 | 3923 | 1778 | 3197 |
| | 265043 | 264417 | 264411 | 260070 | 266961 | 263640 | 261474 | 265597 | 265422 | 267568 | 266149 |
| A | 0.00% | 0.00% | 99.99% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.02% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.90% | 99.91% | 99.92% | 0.01% | 0.00% | 99.93% |
| G | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | 0.02% |
| T | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.08% | 0.08% | 0.05% | 99.99% | 99.99% | 0.04% |

**pNMG-176 (pCMV_Cas9n_XTEN_ecTadA_GGS_NLS; H8Y_D108N_S127S)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 32 | 16 | 233265 | 229058 | 6 | 33 | 26 | 44 | 12 | 5 | 33 |
| C | 7 | 3 | 0 | 2 | 33 | 232272 | 230536 | 233985 | 7 | 12 | 234699 |
| G | 233909 | 233330 | 21 | 15 | 0 | 2 | 18 | 1 | 1 | 3 | 1 |
| T | 5 | 6 | 16 | 14 | 235488 | 409 | 323 | 328 | 234194 | 236078 | 144 |
| N | 3728 | 4326 | 4379 | 8592 | 2153 | 4964 | 6777 | 3322 | 3466 | 1582 | 2803 |
| | 233953 | 233355 | 233302 | 229089 | 235527 | 232716 | 230903 | 234358 | 234214 | 236098 | 234877 |

FIGURE 39 (Continued)

| 12 T | 13 G | 14 C | 15 A | 16 G | 17 C | 18 A | 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 99.98% | 0.00% | 0.01% |
| 12 | 22 | 0 | 268106 | 12 | 2 | 265629 | 0 | 6 | 12 | 25 | 19 |
| 30 | 4 | 267258 | 4 | 4 | 266756 | 2 | 264892 | 265660 | 79 | 2 | 3 |
| 1 | 265487 | 3 | 29 | 267593 | 1 | 17 | 47 | 1 | 4 | 266055 | 264968 |
| 267077 | 11 | 48 | 5 | 5 | 6 | 18 | 9 | 9 | 266736 | 7 | 23 |
| 2228 | 3823 | 2039 | 1204 | 1733 | 2582 | 3727 | 4444 | 3716 | 2561 | 3301 | 4374 |
| 267120 | 265524 | 267309 | 268144 | 267614 | 266765 | 265666 | 264948 | 265676 | 266831 | 266089 | 265013 |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 100.00% | 0.00% | 99.98% | 99.99% | 0.03% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 0.02% | 0.00% | 0.00% | 99.99% | 99.98% |
| 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 99.96% | 0.00% | 0.01% |
| 12 | 16 | 2 | 236576 | 33 | 3 | 234428 | 2 | 4 | 1 | 13 | 13 |
| 21 | 2 | 235762 | 3 | 1 | 235447 | 2 | 233890 | 234425 | 45 | 1 | 6 |
| 0 | 234125 | 2 | 19 | 236125 | 0 | 16 | 1 | 1 | 1 | 234797 | 233914 |
| 235600 | 33 | 34 | 2 | 6 | 10 | 19 | 13 | 12 | 235380 | 5 | 4 |
| 2047 | 3504 | 1881 | 1081 | 1516 | 2221 | 3216 | 3775 | 3239 | 2254 | 2865 | 3742 |
| 235633 | 234176 | 235800 | 236600 | 236165 | 235460 | 234465 | 233906 | 234442 | 235427 | 234816 | 233937 |

# FIGURE 39 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 0.01% | 99.98% | 99.99% | 0.00% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.81% | 99.84% | 99.84% | 0.00% | 0.01% | 99.92% |
| G | 99.98% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 99.98% | 0.18% | 0.14% | 0.14% | 99.99% | 99.99% | 0.06% |

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 16 | 7 | 187731 | 183920 | 2 | 124 | 20 | 25 | 39 | 12 | 4 |
| C | 2 | 3 | 1 | 1 | 14 | 186175 | 185476 | 188307 | 13 | 10 | 188958 |
| G | 188245 | 187861 | 76 | 387 | 0 | 429 | 21 | 0 | 1 | 1 | 1 |
| T | 9 | 12 | 13 | 14 | 189565 | 520 | 252 | 328 | 188607 | 190111 | 75 |
| N | 3215 | 3604 | 3666 | 7165 | 1905 | 4238 | 5717 | 2826 | 2826 | 1352 | 2448 |
| | 188272 | 187883 | 187821 | 184322 | 189581 | 187248 | 185769 | 188660 | 188660 | 190134 | 189038 |
| A | 0.01% | 0.00% | 99.95% | 99.78% | 0.00% | 0.07% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.43% | 99.84% | 99.81% | 0.01% | 0.01% | 99.96% |
| G | 99.99% | 99.99% | 0.04% | 0.21% | 0.00% | 0.23% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.28% | 0.14% | 0.17% | 99.97% | 99.99% | 0.04% |

**pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 20 | 203081 | 199340 | 4 | 4 | 22 | 36 | 7 | 7 | 9 |
| C | 3 | 3 | 2 | 0 | 20 | 201972 | 200738 | 203816 | 14 | 14 | 204304 |
| G | 203511 | 203061 | 54 | 75 | 0 | 133 | 1 | 0 | 0 | 0 | 46 |
| T | 5 | 3 | 12 | 9 | 204954 | 556 | 167 | 107 | 203917 | 205502 | 49 |
| N | 3433 | 3877 | 3815 | 7540 | 1986 | 4299 | 6036 | 3005 | 3026 | 1441 | 2556 |
| | 203531 | 203087 | 203149 | 199424 | 204978 | 202665 | 200928 | 203959 | 203938 | 205523 | 204408 |
| A | 0.01% | 0.01% | 99.97% | 99.96% | 0.00% | 0.00% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.66% | 99.91% | 99.93% | 0.01% | 0.01% | 99.95% |

FIGURE 39 (Continued)

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | | | | | | | | |
| 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% | 0.00% | | | | | | | | |
| 0.00% | 99.98% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% | 99.99% | | | | | | | | |
| 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | | | | | | | | |
| 9 | 21 | 4 | 190533 | 15 | 5 | 188753 | 20 | 6 | 7 | 9 | 6 | | | | | | | | | |
| 31 | 3 | 189834 | 5 | 3 | 189552 | 2 | 188168 | 188678 | 23 | 2 | 9 | | | | | | | | | |
| 1 | 188533 | 0 | 20 | 190127 | 1 | 9 | 1 | 0 | 1 | 188984 | 188269 | | | | | | | | | |
| 189752 | 9 | 57 | 1 | 7 | 6 | 15 | 15 | 17 | 189585 | 8 | 6 | | | | | | | | | |
| 1693 | 2920 | 1591 | 927 | 1334 | 1922 | 2697 | 3273 | 2794 | 1879 | 2492 | 3201 | | | | | | | | | |
| 189793 | 188566 | 189895 | 190559 | 190152 | 189564 | 188779 | 188204 | 188701 | 189616 | 189003 | 188290 | | | | | | | | | |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | |
| 0.02% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 99.99% | 0.01% | 0.00% | 0.00% | | | | | | | | | |
| 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% | | | | | | | | | |
| 99.98% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% | 0.00% | | | | | | | | | |
| 7 | 13 | 5 | 206029 | 12 | 5 | 204063 | 0 | 8 | 9 | 12 | 15 | | | | | | | | | |
| 24 | 1 | 205229 | 5 | 2 | 204985 | 1 | 203456 | 204017 | 40 | 5 | 7 | | | | | | | | | |
| 3 | 203958 | 3 | 14 | 205624 | 2 | 11 | 1 | 0 | 2 | 204267 | 203646 | | | | | | | | | |
| 205242 | 4 | 30 | 2 | 21 | 53 | 11 | 5 | 9 | 205010 | 22 | 4 | | | | | | | | | |
| 1688 | 2988 | 1697 | 914 | 1305 | 1919 | 2876 | 3499 | 2927 | 1900 | 2655 | 3288 | | | | | | | | | |
| 205276 | 203976 | 205267 | 206050 | 205659 | 205045 | 204086 | 203462 | 204034 | 205061 | 204306 | 203672 | | | | | | | | | |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | | | | | | | | | |
| 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.97% | 0.00% | 100.00% | 99.99% | 0.02% | 0.00% | 0.00% | | | | | | | | | |

FIGURE 39 (Continued)

| | 1 G | 2 G | 3 A | 4 A | 5 T | 6 C | 7 C | 8 C | 9 T | 10 T | 11 C | 12 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 99.99% | 99.99% | 0.03% | 0.04% | 0.00% | 0.07% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.27% | 0.08% | 0.05% | 99.99% | 99.99% | 0.02% | 99.98% |

**pNMG-179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 10 | 11 | 239242 | 234734 | 5 | 15 | 27 | 9 | 9 | 3 | 5 | 7 |
| C | 1 | 4 | 0 | 0 | 21 | 238265 | 236592 | 240167 | 10 | 14 | 240781 | 27 |
| G | 239812 | 239270 | 57 | 313 | 1 | 286 | 1 | 29 | 0 | 0 | 3 | 3 |
| T | 12 | 14 | 8 | 8 | 241420 | 126 | 41 | 51 | 240181 | 242047 | 40 | 241658 |
| N | 3834 | 4370 | 4362 | 8614 | 2221 | 4976 | 7007 | 3412 | 3468 | 1604 | 2839 | 1973 |
| | 239835 | 239299 | 239307 | 235055 | 241447 | 238692 | 236661 | 240256 | 240200 | 242064 | 240829 | 241695 |
| A | 0.00% | 0.00% | 99.97% | 99.86% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.82% | 99.97% | 99.96% | 0.00% | 0.01% | 99.98% | 0.01% |
| G | 99.99% | 99.99% | 0.02% | 0.13% | 0.00% | 0.12% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.05% | 0.02% | 0.02% | 99.99% | 99.99% | 0.02% | 99.98% |

FIGURE 39 (Continued)

| 13 G | 14 C | 15 A | 16 G | 17 C | 18 A | 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|
| 99.99% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% |
| 0.00% | 0.01% | 0.00% | 0.01% | 0.03% | 0.01% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| 14 | 5 | 242612 | 15 | 0 | 240352 | 2 | 6 | 15 | 20 | 17 |
| 5 | 241760 | 4 | 2 | 241378 | 0 | 239805 | 240409 | 70 | 3 | 3 |
| 240168 | 4 | 21 | 242062 | 2 | 10 | 0 | 2 | 3 | 240754 | 239817 |
| 11 | 34 | 4 | 12 | 9 | 13 | 9 | 7 | 241321 | 10 | 7 |
| 3470 | 1866 | 1028 | 1578 | 2280 | 3294 | 3852 | 3243 | 2258 | 2880 | 3819 |
| 240198 | 241803 | 242641 | 242091 | 241389 | 240375 | 239816 | 240424 | 241409 | 240787 | 239844 |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| 0.00% | 99.98% | 0.00% | 0.00% | 100.00% | 0.00% | 100.00% | 99.99% | 0.03% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 99.96% | 0.00% | 0.00% |

FIGURE 39 (Continued)

**pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 21 | 14 | 302284 | 296270 | 10 | 45 | 14 | 12 | 20 | 14 | 5 | 10 |
| **C** | 4 | 3 | 0 | 2 | 27 | 299878 | 298590 | 303422 | 20 | 26 | 304313 | 40 |
| **G** | 302939 | 302353 | 73 | 601 | 0 | 318 | 2 | 29 | 2 | 0 | 3 | 1 |
| **T** | 16 | 17 | 13 | 9 | 304980 | 1200 | 281 | 178 | 303465 | 305842 | 62 | 305285 |
| **N** | 4816 | 5409 | 5426 | 10914 | 2779 | 6355 | 8909 | 4155 | 4289 | 1914 | 3413 | 2460 |
| | 302980 | 302387 | 302370 | 296882 | 305017 | 301441 | 298887 | 303641 | 303507 | 305882 | 304383 | 305336 |
| **A** | 0.01% | 0.00% | 99.97% | 99.79% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.48% | 99.90% | 99.93% | 0.01% | 0.01% | 99.98% | 0.01% |
| **G** | 99.99% | 99.99% | 0.02% | 0.20% | 0.00% | 0.11% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.40% | 0.09% | 0.06% | 99.99% | 99.99% | 0.02% | 99.98% |
| **untreated cells** | | | | | | | | | | | | |
| **A** | 23 | 21 | 332577 | 326833 | 8 | 10 | 28 | 47 | 12 | 16 | 14 | 10 |
| **C** | 4 | 5 | 5 | 1 | 32 | 331592 | 328887 | 333898 | 20 | 18 | 334688 | 46 |
| **G** | 333134 | 332412 | 27 | 18 | 0 | 4 | 4 | 0 | 4 | 0 | 3 | 2 |
| **T** | 16 | 12 | 18 | 10 | 335444 | 70 | 57 | 64 | 333687 | 336319 | 25 | 335815 |
| **N** | 5393 | 6120 | 5943 | 11708 | 3085 | 6893 | 9593 | 4560 | 4846 | 2216 | 3839 | 269670 |
| | 333177 | 332450 | 332627 | 326862 | 335484 | 331676 | 328976 | 334009 | 333723 | 336353 | 334730 | 335873 |
| **A** | 0.01% | 0.01% | 99.98% | 99.99% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 99.97% | 99.97% | 0.01% | 0.01% | 99.99% | 0.01% |
| **G** | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.02% | 0.02% | 0.02% | 99.99% | 99.99% | 0.01% | 99.98% |

# FIGURE 39 (Continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 23 | 2 | 306503 | 34 | 3 | 303706 | 2 | 9 | 12 | 34 | 17 |
| 2 | 305466 | 2 | 2 | 304989 | 3 | 302884 | 303725 | 63 | 5 | 6 |
| 303605 | 1 | 26 | 305871 | 3 | 12 | 4 | 4 | 6 | 304172 | 303060 |
| 10 | 64 | 7 | 6 | 47 | 22 | 11 | 25 | 305004 | 11 | 13 |
| 4156 | 2263 | 1258 | 1883 | 2754 | 4053 | 4895 | 4033 | 2711 | 3573 | 4697 |
| 303640 | 305533 | 306538 | 305913 | 305042 | 303743 | 302901 | 303763 | 305085 | 304222 | 303096 |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% |
| 0.00% | 0.02% | 0.00% | 0.00% | 0.02% | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.00% |
| 29 | 4 | 337124 | 17 | 13 | 334016 | 3 | 22 | 15 | 28 | 14 |
| 4 | 335944 | 3 | 4 | 335479 | 6 | 333201 | 334043 | 41 | 3 | 7 |
| 333712 | 4 | 38 | 336462 | 1 | 13 | 3 | 2 | 2 | 334524 | 333315 |
| 9 | 55 | 10 | 15 | 16 | 12 | 22 | 21 | 335503 | 21 | 13 |
| 4815 | 2562 | 1394 | 2071 | 3060 | 4522 | 5340 | 4481 | 3006 | 3990 | 5215 |
| 333754 | 336007 | 337175 | 336498 | 335509 | 334047 | 333229 | 334088 | 335561 | 334576 | 333349 |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| 0.00% | 99.98% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.01% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% |
| 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% |

FIGURE 40

| | P | A | M | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | C | C | G | C | A | G | T | C | T | A | T |
| **Cas9** | | | | | | | | | | | | |
| **A** | 43 | 24 | 73 | 85 | 37 | 62131 | 54 | 19 | 6 | 13 | 63114 | 6 |
| **C** | 61372 | 61194 | 61521 | 4 | 61944 | 49 | 47 | 25 | 62609 | 13 | 1 | 10 |
| **G** | 1 | 40 | 23 | 62064 | 50 | 106 | 62784 | 83 | 11 | 6 | 14 | 3 |
| **T** | 66 | 66 | 74 | 64 | 64 | 202 | 236 | 62108 | 44 | 63380 | 15 | 63198 |
| **N** | 1570 | 1742 | 1435 | 1171 | 1358 | 1163 | 811 | 1601 | 1196 | 453 | 668 | 620 |
| | 61482 | 61324 | 61691 | 62217 | 62095 | 62488 | 63121 | 62235 | 62670 | 63412 | 63144 | 63217 |
| **A** | 0.07% | 0.04% | 0.12% | 0.14% | 0.06% | 99.43% | 0.09% | 0.03% | 0.01% | 0.02% | 99.95% | 0.01% |
| **C** | 99.82% | 99.79% | 99.72% | 0.01% | 99.76% | 0.08% | 0.07% | 0.04% | 99.90% | 0.02% | 0.00% | 0.02% |
| **G** | 0.00% | 0.07% | 0.04% | 99.75% | 0.08% | 0.17% | 99.47% | 0.13% | 0.02% | 0.01% | 0.02% | 0.00% |
| **T** | 0.11% | 0.11% | 0.12% | 0.10% | 0.10% | 0.32% | 0.37% | 99.80% | 0.07% | 99.95% | 0.02% | 99.97% |
| **pNMG-247 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; wild type)** | | | | | | | | | | | | |
| **A** | 1 | 10 | 14 | 45 | 28 | 49157 | 12 | 26 | 24 | 16 | 50206 | 9 |
| **C** | 48877 | 48708 | 48904 | 2 | 49181 | 90 | 64 | 27 | 49733 | 17 | 8 | 14 |
| **G** | 1 | 5 | 10 | 49318 | 13 | 85 | 49913 | 102 | 15 | 7 | 19 | 0 |
| **T** | 20 | 33 | 62 | 17 | 45 | 185 | 136 | 49147 | 20 | 50421 | 22 | 50259 |
| **N** | 1147 | 1330 | 1126 | 896 | 1115 | 922 | 630 | 1300 | 996 | 343 | 478 | 458 |
| | 48899 | 48756 | 48990 | 49382 | 49267 | 49517 | 50125 | 49302 | 49792 | 50461 | 50255 | 50282 |
| **A** | 0.00% | 0.02% | 0.03% | 0.09% | 0.06% | 99.27% | 0.02% | 0.05% | 0.05% | 0.03% | 99.90% | 0.02% |
| **C** | 99.96% | 99.90% | 99.82% | 0.00% | 99.83% | 0.18% | 0.13% | 0.05% | 99.88% | 0.03% | 0.02% | 0.03% |
| **G** | 0.00% | 0.01% | 0.02% | 99.87% | 0.03% | 0.17% | 99.58% | 0.21% | 0.03% | 0.01% | 0.04% | 0.00% |
| **T** | 0.04% | 0.07% | 0.13% | 0.03% | 0.09% | 0.37% | 0.27% | 99.69% | 0.04% | 99.92% | 0.04% | 99.95% |

FIGURE 40 (Continued)

| 11 G | 10 C | 9 T | 8 T | 7 T | 6 G | 5 T | 4 G | 3 T | 2 T | 1 C |
|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 10 | 2 | 5 | 1 | 7 | 4 | 6 | 0 | 47 | 0 |
| 2 | 62622 | 7 | 4 | 7 | 49 | 14 | 0 | 48 | 8 | 63453 |
| 63012 | 2 | 2 | 1 | 0 | 63461 | 2 | 62980 | 1 | 0 | 0 |
| 3 | 5 | 63537 | 63632 | 63403 | 4 | 62910 | 4 | 63352 | 63500 | 6 |
| 816 | 1205 | 305 | 267 | 498 | 389 | 980 | 925 | 514 | 361 | 457 |
| 63026 | 62639 | 63548 | 63642 | 63411 | 63521 | 62930 | 62990 | 63401 | 63555 | 63459 |
| 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.07% | 0.00% |
| 0.00% | 99.97% | 0.01% | 0.01% | 0.01% | 0.08% | 0.02% | 0.00% | 0.08% | 0.01% | 99.99% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.91% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.01% | 99.98% | 99.98% | 99.99% | 0.01% | 99.97% | 0.01% | 99.92% | 99.91% | 0.01% |
| 14 | 4 | 2 | 1 | 1 | 8 | 6 | 6 | 5 | 127 | 2 |
| 13 | 49749 | 3 | 3 | 7 | 126 | 13 | 0 | 133 | 7 | 50562 |
| 50148 | 0 | 1 | 12 | 1 | 50459 | 0 | 50176 | 2 | 0 | 0 |
| 0 | 14 | 50533 | 50713 | 50472 | 5 | 50090 | 6 | 50359 | 50522 | 12 |
| 597 | 1005 | 231 | 190 | 438 | 314 | 803 | 724 | 413 | 256 | 336 |
| 50175 | 49767 | 50539 | 50729 | 50481 | 50598 | 50109 | 50188 | 50499 | 50656 | 50576 |
| 0.03% | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.25% | 0.00% |
| 0.03% | 99.96% | 0.01% | 0.01% | 0.01% | 0.25% | 0.03% | 0.00% | 0.26% | 0.01% | 99.97% |
| 99.95% | 0.00% | 0.00% | 0.02% | 0.00% | 99.73% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.03% | 99.99% | 99.97% | 99.98% | 0.01% | 99.96% | 0.01% | 99.72% | 99.74% | 0.02% |

FIGURE 40 (Continued)

| pNMG-248 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 7 | 18 | 8 | 40 | 13 | 50733 | 71 | 43 | 18 | 32 | 51920 | 34 |
| C | 50367 | 50126 | 50447 | 12 | 50613 | 88 | 38 | 29 | 51428 | 6 | 12 | 24 |
| G | 5 | 7 | 27 | 50679 | 34 | 81 | 51498 | 34 | 1 | 0 | 11 | 0 |
| T | 8 | 6 | 21 | 41 | 63 | 140 | 146 | 50899 | 5 | 52079 | 5 | 51862 |
| N | 1229 | 1457 | 1146 | 1020 | 1174 | 975 | 672 | 1388 | 1009 | 362 | 508 | 536 |
| | 50387 | 50157 | 50503 | 50772 | 50723 | 51042 | 51753 | 51005 | 51452 | 52117 | 51948 | 51920 |
| A | 0.01% | 0.04% | 0.02% | 0.08% | 0.03% | 99.39% | 0.14% | 0.08% | 0.03% | 0.06% | 99.95% | 0.07% |
| C | 99.96% | 99.94% | 99.89% | 0.02% | 99.78% | 0.17% | 0.07% | 0.06% | 99.95% | 0.01% | 0.02% | 0.05% |
| G | 0.01% | 0.01% | 0.05% | 99.82% | 0.07% | 0.16% | 99.51% | 0.07% | 0.00% | 0.00% | 0.02% | 0.00% |
| T | 0.02% | 0.01% | 0.04% | 0.08% | 0.12% | 0.27% | 0.28% | 99.79% | 0.01% | 99.93% | 0.01% | 99.89% |
| pNMG-249 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | | | | |
| A | 16 | 26 | 30 | 63 | 27 | 60982 | 54 | 44 | 20 | 2 | 62025 | 6 |
| C | 60537 | 60247 | 60609 | 5 | 61001 | 100 | 65 | 41 | 61433 | 30 | 1 | 5 |
| G | 3 | 12 | 28 | 61027 | 36 | 136 | 61815 | 79 | 14 | 0 | 25 | 3 |
| T | 32 | 26 | 22 | 45 | 42 | 141 | 53 | 60937 | 5 | 62288 | 7 | 62054 |
| N | 1533 | 1816 | 1488 | 1244 | 1394 | 1220 | 812 | 1624 | 1264 | 439 | 639 | 630 |
| | 60588 | 60311 | 60689 | 61140 | 61106 | 61359 | 61987 | 61101 | 61472 | 62320 | 62058 | 62068 |
| A | 0.03% | 0.04% | 0.05% | 0.10% | 0.04% | 99.39% | 0.09% | 0.07% | 0.03% | 0.00% | 99.95% | 0.01% |
| C | 99.92% | 99.89% | 99.87% | 0.01% | 99.83% | 0.16% | 0.10% | 0.07% | 99.94% | 0.05% | 0.00% | 0.01% |
| G | 0.00% | 0.02% | 0.05% | 99.82% | 0.06% | 0.22% | 99.72% | 0.13% | 0.02% | 0.00% | 0.04% | 0.00% |
| T | 0.05% | 0.04% | 0.04% | 0.07% | 0.07% | 0.23% | 0.09% | 99.73% | 0.01% | 99.95% | 0.01% | 99.98% |

FIGURE 40 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | 1 | 2 | 3 | 0 | 1 | 2 | 7 | 2 | 37 | 2 | |
| 0 | 51467 | 5 | 4 | 1 | 53 | 48 | 1 | 43 | 5 | 52161 | |
| 51735 | 1 | 2 | 2 | 0 | 52172 | 0 | 51709 | 1 | 0 | 1 | |
| 5 | 3 | 52208 | 52292 | 52095 | 2 | 51669 | 3 | 52077 | 52216 | 1 | |
| 705 | 992 | 247 | 199 | 414 | 282 | 798 | 797 | 394 | 259 | 352 | |
| 51758 | 51472 | 52217 | 52301 | 52096 | 52228 | 51719 | 51720 | 52123 | 52258 | 52165 | |
| 0.03% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.07% | 0.00% | |
| 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 0.10% | 0.09% | 0.00% | 0.08% | 0.01% | 99.99% | |
| 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 99.89% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | |
| 0.01% | 0.01% | 99.98% | 99.98% | 100.00 % | 0.00% | 99.90% | 0.01% | 99.91% | 99.92% | 0.00% | |
| 5 | 2 | 1 | 3 | 4 | 2 | 7 | 1 | 4 | 60 | 5 | |
| 5 | 61400 | 3 | 7 | 6 | 61 | 93 | 5 | 65 | 10 | 62321 | |
| 61834 | 2 | 0 | 12 | 0 | 62356 | 1 | 61811 | 0 | 6 | 3 | |
| 11 | 5 | 62415 | 62539 | 62245 | 6 | 61723 | 3 | 62211 | 62384 | 5 | |
| 851 | 1297 | 288 | 235 | 541 | 358 | 965 | 969 | 509 | 329 | 455 | |
| 61855 | 61409 | 62419 | 62561 | 62255 | 62425 | 61824 | 61820 | 62280 | 62460 | 62334 | |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.10% | 0.01% | |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.01% | 0.10% | 0.15% | 0.01% | 0.10% | 0.02% | 99.98% | |
| 99.97% | 0.00% | 0.00% | 0.02% | 0.00% | 99.89% | 0.00% | 99.99 | 0.00% | 0.01% | 0.00% | |
| 0.02% | 0.01% | 99.99% | 99.96% | 99.98% | 0.01% | 99.84% | 0.00% | 99.89% | 99.88% | 0.01% | |

# FIGURE 40 (Continued)

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A | 12 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-250 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | | |
| **A** | 4 | 9 | 34 | 84 | 34 | 70666 | 41 | 61 | 60 | 3 | 71808 | 7 |
| **C** | 70179 | 69757 | 70247 | 21 | 70466 | 92 | 84 | 68 | 71149 | 37 | 3 | 59 |
| **G** | 3 | 32 | 35 | 70676 | 10 | 81 | 71467 | 83 | 23 | 8 | 72 | 1 |
| **T** | 18 | 26 | 53 | 29 | 47 | 147 | 208 | 70466 | 22 | 72193 | 19 | 71895 |
| **N** | 1807 | 2222 | 1720 | 1478 | 1740 | 1454 | 859 | 1912 | 1443 | 486 | 767 | 719 |
| | 70204 | 69824 | 70369 | 70810 | 70557 | 70986 | 71800 | 70678 | 71254 | 72241 | 71902 | 71962 |
| **A** | 0.01% | 0.01% | 0.05% | 0.12% | 0.05% | 99.55% | 0.06% | 0.09% | 0.08% | 0.00% | 99.87% | 0.01% |
| **C** | 99.96% | 99.90% | 99.83% | 0.03% | 99.87% | 0.13% | 0.12% | 0.10% | 99.85% | 0.05% | 0.00% | 0.08% |
| **G** | 0.00% | 0.05% | 0.05% | 99.81% | 0.01% | 0.11% | 99.54% | 0.12% | 0.03% | 0.01% | 0.10% | 0.00% |
| **T** | 0.03% | 0.04% | 0.08% | 0.04% | 0.07% | 0.21% | 0.29% | 99.70% | 0.03% | 99.93% | 0.03% | 99.91% |
| **pNMG-251 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | | |
| **A** | 10 | 10 | 32 | 71 | 45 | 95737 | 35 | 10 | 19 | 0 | 97259 | 3 |
| **C** | 95117 | 94772 | 95163 | 5 | 95388 | 83 | 8 | 10 | 96193 | 12 | 0 | 38 |
| **G** | 3 | 8 | 15 | 95699 | 22 | 72 | 96863 | 37 | 4 | 0 | 41 | 0 |
| **T** | 16 | 12 | 15 | 34 | 39 | 87 | 72 | 95542 | 10 | 97621 | 6 | 97201 |
| **N** | 2461 | 2850 | 2446 | 1933 | 2341 | 1975 | 1284 | 2646 | 2048 | 672 | 987 | 1052 |
| | 95146 | 94802 | 95225 | 95809 | 95494 | 95979 | 96978 | 95599 | 96226 | 97633 | 97306 | 97242 |
| **A** | 0.01% | 0.01% | 0.03% | 0.07% | 0.05% | 99.75% | 0.04% | 0.01% | 0.02% | 0.00% | 99.95% | 0.00% |
| **C** | 99.97% | 99.97% | 99.93% | 0.01% | 99.89% | 0.09% | 0.01% | 0.01% | 99.97% | 0.01% | 0.00% | 0.04% |
| **G** | 0.00% | 0.01% | 0.02% | 99.89% | 0.02% | 0.08% | 99.88% | 0.04% | 0.00% | 0.00% | 0.04% | 0.00% |
| **T** | 0.02% | 0.01% | 0.02% | 0.04% | 0.04% | 0.09% | 0.07% | 99.94% | 0.01% | 99.99% | 0.01% | 99.96% |

# FIGURE 40 (Continued)

| 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|
| G | C | T | T | T | G | T | G | T | T | C |
| 51 | 1 | 1 | 5 | 1 | 2 | 4 | 5 | 4 | 20 | 5 |
| 7 | 71221 | 6 | 19 | 18 | 20 | 32 | 1 | 29 | 10 | 72239 |
| 71619 | 2 | 4 | 18 | 4 | 72276 | 0 | 71576 | 1 | 0 | 37 |
| 4 | 9 | 72354 | 72413 | 72154 | 3 | 71554 | 6 | 72084 | 72387 | 5 |
| 1004 | 1454 | 324 | 272 | 550 | 408 | 1119 | 1122 | 592 | 337 | 480 |
| 71681 | 71233 | 72365 | 72455 | 72177 | 72301 | 71590 | 71588 | 72118 | 72417 | 72286 |
| 0.07% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.03% | 0.01% |
| 0.01% | 99.98% | 0.01% | 0.03% | 0.02% | 0.03% | 0.04% | 0.00% | 0.04% | 0.01% | 99.93% |
| 99.91% | 0.00% | 0.01% | 0.02% | 0.01% | 99.97% | 0.00% | 99.98% | 0.00% | 0.00% | 0.05% |
| 0.01% | 0.01% | 99.98% | 99.94% | 99.97% | 0.00% | 99.95% | 0.01% | 99.95% | 99.96% | 0.01% |
| 39 | 5 | 3 | 2 | 1 | 16 | 7 | 13 | 3 | 8 | 9 |
| 6 | 96282 | 12 | 4 | 10 | 0 | 205 | 1 | 15 | 9 | 97586 |
| 96882 | 0 | 2 | 0 | 1 | 97669 | 2 | 96791 | 2 | 0 | 1 |
| 6 | 4 | 97832 | 97897 | 97476 | 7 | 96503 | 5 | 97485 | 97757 | 12 |
| 1376 | 2018 | 455 | 401 | 816 | 612 | 1587 | 1494 | 799 | 530 | 696 |
| 96933 | 96291 | 97849 | 97903 | 97488 | 97692 | 96717 | 96810 | 97505 | 97774 | 97608 |
| 0.04% | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |
| 0.01% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 0.21% | 0.00% | 0.02% | 0.01% | 99.98% |
| 99.95% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.98% | 99.99% | 99.99% | 0.01% | 99.78% | 0.01% | 99.98% | 99.98% | 0.01% |

FIGURE 40 (Continued)

**pNMG-181(pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 8 | 9 | 7 | 131 | 6 | 158423 | 8 | 5 | 9 | 6 | 160073 | 4 |
| **C** | 157684 | 156805 | 157592 | 2 | 157919 | 8 | 3 | 19 | 158420 | 17 | 3 | 14 |
| **G** | 2 | 0 | 0 | 158451 | 1 | 10 | 159599 | 1 | 8 | 0 | 13 | 0 |
| **T** | 7 | 14 | 23 | 14 | 7 | 2 | 15 | 157456 | 16 | 160717 | 20 | 160183 |
| **N** | 4145 | 5018 | 4224 | 3248 | 3913 | 3403 | 2221 | 4365 | 3393 | 1106 | 1737 | 1645 |
| | 157701 | 156828 | 157622 | 158598 | 157933 | 158443 | 159625 | 157481 | 158453 | 160740 | 160109 | 160201 |
| | | | | | | | | | | | | |
| **A** | 0.01% | 0.01% | 0.00% | 0.08% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% |
| **G** | 0.00% | 0.00% | 0.00% | 99.91% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 99.99% | 0.01% | 99.99% |

**pNMG-182 (pCMV_ecTadA_GGS_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 10 | 9 | 6 | 90 | 12 | 151204 | 10 | 4 | 26 | 5 | 152664 | 11 |
| **C** | 150380 | 149557 | 150556 | 1 | 150697 | 4 | 7 | 21 | 151148 | 22 | 2 | 11 |
| **G** | 2 | 3 | 0 | 151137 | 1 | 17 | 152253 | 3 | 0 | 0 | 15 | 0 |
| **T** | 8 | 14 | 15 | 14 | 5 | 1 | 10 | 150254 | 9 | 153153 | 13 | 152620 |
| **N** | 3743 | 4560 | 3566 | 2901 | 3428 | 2917 | 1863 | 3851 | 2950 | 953 | 1438 | 1490 |
| | 150400 | 149583 | 150577 | 151242 | 150715 | 151226 | 152280 | 150282 | 151183 | 153180 | 152694 | 152642 |
| | | | | | | | | | | | | |
| **A** | 0.01% | 0.01% | 0.00% | 0.06% | 0.01% | 99.99% | 0.01% | 0.00% | 0.02% | 0.00% | 99.98% | 0.01% |
| **C** | 99.99% | 99.98% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% |
| **G** | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 99.98% | 0.01% | 99.99% |

**pNMG-183 (pCMV_ecTadA_(GGS)2-XTEN-(GGS)2_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

# FIGURE 40 (Continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 7 | 3 | 9 | 6 | 24 | 11 | 24 | 7 | 5 | 12 |
| 8 | 158570 | 12 | 42 | 33 | 74 | 15442 | 1 | 316 | 19 | 160587 |
| 159510 | 0 | 1 | 0 | 3 | 160771 | 4 | 159043 | 2 | 1 | 0 |
| 5 | 14 | 161101 | 161121 | 160410 | 11 | 143891 | 7 | 159996 | 160909 | 19 |
| 2303 | 3255 | 720 | 665 | 1385 | 957 | 2489 | 2762 | 1516 | 903 | 1219 |
| 159543 | 158591 | 161117 | 161172 | 160452 | 160880 | 159348 | 159075 | 160321 | 160934 | 160618 |
| | | | | | | | | | | |
| 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% |
| 0.01% | 99.99% | 0.01% | 0.03% | 0.02% | 0.05% | 9.69% | 0.00% | 0.20% | 0.01% | 99.98% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.01% | 99.99% | 99.97% | 99.97% | 0.01% | 90.30% | 0.00% | 99.80% | 99.98% | 0.01% |
| | | | | | | | | | | |
| 11 | 12 | 7 | 7 | 13 | 18 | 10 | 17 | 8 | 3 | 2 |
| 2 | 151115 | 14 | 38 | 54 | 76 | 18664 | 3 | 172 | 37 | 153032 |
| 152149 | 0 | 1 | 5 | 3 | 153167 | 1 | 151612 | 1 | 1 | 2 |
| 11 | 13 | 153443 | 153529 | 152847 | 12 | 133131 | 5 | 152599 | 153265 | 20 |
| 1959 | 2992 | 666 | 552 | 1214 | 854 | 2321 | 2490 | 1347 | 821 | 1071 |
| 152173 | 151140 | 153465 | 153579 | 152917 | 153273 | 151806 | 151637 | 152780 | 153306 | 153056 |
| | | | | | | | | | | |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.00% | 99.98% | 0.01% | 0.02% | 0.04% | 0.05% | 12.29% | 0.00% | 0.11% | 0.02% | 99.98% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.01% | 99.99% | 99.97% | 99.95% | 0.01% | 87.70% | 0.00% | 99.88% | 99.97% | 0.01% |

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# FIGURE 40 (Continued)

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A | 12 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3 | 3 | 7 | 42 | 5 | 108748 | 3 | 4 | 2 | 2 | 109877 | 9 |
| C | 107855 | 107219 | 108006 | 3 | 108194 | 7 | 1 | 3 | 108755 | 9 | 2 | 7 |
| G | 2 | 0 | 0 | 108642 | 1 | 37 | 109441 | 26 | 2 | 1 | 4 | 1 |
| T | 5 | 23 | 8 | 11 | 5 | 35 | 4 | 108158 | 14 | 110079 | 10 | 109756 |
| N | 3009 | 3629 | 2853 | 2176 | 2663 | 2041 | 1419 | 2643 | 2061 | 743 | 916 | 1036 |
| | 107865 | 107245 | 108021 | 108698 | 108205 | 108827 | 109449 | 108191 | 108773 | 110091 | 109893 | 109773 |
| A | 0.00% | 0.00% | 0.01% | 0.04% | 0.00% | 99.93% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| C | 99.99% | 99.98% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% | 0.01% |
| G | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.03% | 99.99% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.02% | 0.01% | 0.01% | 0.00% | 0.03% | 0.00% | 99.97% | 0.01% | 99.99% | 0.01% | 99.98% |
| **pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | | |
| A | 6 | 1 | 15 | 64 | 7 | 127598 | 8 | 0 | 12 | 0 | 128944 | 2 |
| C | 126591 | 125744 | 126752 | 2 | 126977 | 7 | 1 | 7 | 127570 | 8 | 0 | 12 |
| G | 1 | 0 | 1 | 127476 | 2 | 12 | 128471 | 6 | 0 | 1 | 7 | 0 |
| T | 6 | 12 | 20 | 9 | 12 | 12 | 5 | 126893 | 13 | 129135 | 11 | 128824 |
| N | 3442 | 4289 | 3258 | 2495 | 3048 | 2417 | 1561 | 3130 | 2441 | 892 | 1068 | 1182 |
| | 126604 | 125757 | 126788 | 127551 | 126998 | 127629 | 128485 | 126906 | 127595 | 129144 | 128962 | 128838 |
| A | 0.00% | 0.00% | 0.01% | 0.05% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |
| C | 99.99% | 99.99% | 99.97% | 0.00% | 99.98% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| T | 0.00% | 0.01% | 0.02% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% | 99.99% |

# FIGURE 40 (Continued)

| 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|
| G | C | T | T | T | G | T | G | T | T | C |
| 9 | 4 | 3 | 6 | 2 | 356 | 4 | 200 | 4 | 4 | 4 |
| 4 | 108581 | 33 | 24 | 29 | 665 | 24395 | 3 | 155 | 36 | 110009 |
| 109460 | 0 | 0 | 2 | 0 | 109088 | 2 | 108614 | 1 | 0 | 3 |
| 4 | 5 | 110301 | 110353 | 109846 | 11 | 84606 | 65 | 109627 | 110128 | 15 |
| 1332 | 2219 | 471 | 423 | 931 | 688 | 1801 | 1926 | 1021 | 640 | 777 |
| 109477 | 108590 | 110337 | 110385 | 109877 | 110120 | 109007 | 108882 | 109787 | 110168 | 110031 |
| 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.32% | 0.00% | 0.18% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.03% | 0.02% | 0.03% | 0.60% | 22.38% | 0.00% | 0.14% | 0.03% | 99.98% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.06% | 0.00% | 99.75% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 99.97% | 99.97% | 99.97% | 0.01% | 77.62% | 0.06% | 99.85% | 99.96% | 0.01% |
| 13 | 8 | 2 | 5 | 3 | 81 | 11 | 15 | 4 | 5 | 8 |
| 2 | 127355 | 12 | 36 | 43 | 135 | 16449 | 0 | 264 | 12 | 129164 |
| 128460 | 0 | 1 | 2 | 2 | 129124 | 24 | 127869 | 1 | 0 | 4 |
| 5 | 10 | 129446 | 129485 | 128976 | 12 | 111411 | 25 | 128655 | 129310 | 15 |
| 1540 | 2647 | 559 | 492 | 996 | 668 | 2125 | 2111 | 1096 | 693 | 829 |
| 128480 | 127373 | 129461 | 129528 | 129024 | 129352 | 127895 | 127909 | 128924 | 129327 | 129191 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.06% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.00% | 99.99% | 0.01% | 0.03% | 0.03% | 0.10% | 12.86% | 0.00% | 0.20% | 0.01% | 99.98% |
| 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.82% | 0.02% | 99.97% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.01% | 99.99% | 99.97% | 99.96% | 0.01% | 87.11% | 0.02% | 99.79% | 99.99% | 0.01% |



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# FIGURE 40 (Continued)

**pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 4 | 7 | 59 | 7 | 125089 | 3 | 0 | 10 | 4 | 126393 | 12 |
| C | 124236 | 123476 | 124332 | 2 | 124475 | 8 | 2 | 6 | 125070 | 13 | 2 | 16 |
| G | 1 | 3 | 0 | 124921 | 0 | 9 | 125929 | 0 | 0 | 0 | 7 | 1 |
| T | 7 | 20 | 17 | 14 | 10 | 2 | 14 | 124384 | 15 | 126623 | 10 | 126282 |
| N | 3182 | 3929 | 3076 | 2436 | 2940 | 2324 | 1484 | 3042 | 2337 | 792 | 1020 | 1121 |
| | 124250 | 123503 | 124356 | 124996 | 124492 | 125108 | 125948 | 124390 | 125095 | 126640 | 126412 | 126311 |
| A | 0.00% | 0.00% | 0.01% | 0.05% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% |
| C | 99.99% | 99.98% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% | 0.01% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 0.02% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 100.00 % | 0.01% | 99.99% | 0.01% | 99.98% |

**pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 6 | 7 | 57 | 10 | 123389 | 3 | 2 | 12 | 5 | 124609 | 4 |
| C | 122417 | 121705 | 122503 | 1 | 122673 | 6 | 1 | 10 | 123369 | 9 | 1 | 11 |
| G | 2 | 0 | 6 | 123268 | 1 | 6 | 124242 | 1 | 0 | 0 | 5 | 0 |
| T | 4 | 11 | 19 | 10 | 7 | 2 | 6 | 122643 | 7 | 124931 | 6 | 124581 |
| N | 3312 | 4018 | 3205 | 2404 | 3040 | 2328 | 1479 | 3075 | 2343 | 785 | 1109 | 1134 |
| | 122428 | 121722 | 122535 | 123336 | 122691 | 123403 | 124252 | 122656 | 123388 | 124945 | 124621 | 124596 |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 40 (Continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 7 | 3 | 6 | 4 | 6 | 6 | 5 | 3 | 4 | 5 |
| 1 | 124969 | 16 | 54 | 13 | 130 | 15162 | 0 | 151 | 10 | 126613 |
| 125953 | 4 | 1 | 9 | 1 | 126659 | 1 | 125491 | 2 | 1 | 3 |
| 10 | 7 | 126873 | 126932 | 126429 | 13 | 110279 | 18 | 126227 | 126776 | 10 |
| 1452 | 2445 | 539 | 431 | 985 | 615 | 1975 | 1909 | 1040 | 632 | 792 |
| 125980 | 124987 | 126893 | 127001 | 126447 | 126808 | 125448 | 125514 | 126383 | 126791 | 126631 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.01% | 0.04% | 0.01% | 0.10% | 12.09% | 0.00% | 0.12% | 0.01% | 99.99% |
| 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.88% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.01% | 99.98% | 99.95% | 99.99% | 0.01% | 87.91% | 0.01% | 99.88% | 99.99% | 0.01% |
| 10 | 6 | 2 | 7 | 1 | 38 | 9 | 36 | 6 | 6 | 6 |
| 0 | 123243 | 8 | 10 | 9 | 34 | 47 | 2 | 15 | 8 | 124875 |
| 124245 | 1 | 1 | 0 | 0 | 125030 | 4 | 123870 | 3 | 0 | 2 |
| 5 | 7 | 125144 | 125236 | 124719 | 10 | 123725 | 6 | 124740 | 125164 | 10 |
| 1470 | 2473 | 575 | 477 | 1001 | 618 | 1945 | 1816 | 966 | 552 | 837 |
| 124260 | 123257 | 125155 | 125253 | 124729 | 125112 | 123785 | 123914 | 124764 | 125178 | 124893 |



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FIGURE 40 (Continued)

| | P | A | M | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | C | C | G | C | A | G | T | C | T | A | T |
| A | 0.00% | 0.00% | 0.01% | 0.05% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |
| C | 99.99% | 99.99% | 99.97% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 99.99% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | | |
| A | 7 | 6 | 5 | 65 | 5 | 138080 | 6 | 0 | 13 | 4 | 139556 | 10 |
| C | 137052 | 136188 | 137174 | 1 | 137371 | 10 | 0 | 12 | 137939 | 8 | 0 | 24 |
| G | 2 | 1 | 0 | 137815 | 1 | 17 | 138993 | 5 | 1 | 0 | 19 | 0 |
| T | 5 | 7 | 21 | 19 | 12 | 2 | 12 | 137303 | 7 | 139795 | 7 | 139429 |
| N | 3720 | 4584 | 3586 | 2886 | 3397 | 2687 | 1785 | 3476 | 2836 | 989 | 1210 | 1329 |
| | 137066 | 136202 | 137200 | 137900 | 137389 | 138109 | 139011 | 137320 | 137960 | 139807 | 139582 | 139463 |
| A | 0.01% | 0.00% | 0.00% | 0.05% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% |
| C | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.02% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| T | 0.00% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 99.99% | 0.01% | 99.98% |

FIGURE 40 (Continued)

| 11 G | 10 C | 9 T | 8 T | 7 T | 6 G | 5 T | 4 G | 3 T | 2 T | 1 C |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.03% | 0.01% | 0.03% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.03% | 0.04% | 0.00% | 0.01% | 0.01% | 99.99% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.01% | 99.95% | 0.00% | 99.98% | 99.99% | 0.01% |
| 18 | 6 | 4 | 6 | 1 | 4 | 6 | 13 | 3 | 4 | 6 |
| 3 | 137863 | 10 | 45 | 16 | 56 | 13064 | 4 | 237 | 27 | 13982 |
| 139067 | 2 | 1 | 11 | 14 | 139961 | 0 | 138564 | 1 | 2 | 2 |
| 8 | 6 | 140184 | 140198 | 139603 | 26 | 125494 | 1 | 139326 | 139981 | 12 |
| 1696 | 2915 | 592 | 531 | 1157 | 734 | 2217 | 2199 | 1214 | 767 | 933 |
| 139096 | 137877 | 140199 | 140260 | 139634 | 140047 | 138564 | 138582 | 139567 | 140014 | 13984 |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.01% | 0.03% | 0.01% | 0.04% | 9.43% | 0.00% | 0.17% | 0.02% | 99.99% |
| 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 99.94% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 99.96% | 99.98% | 0.02% | 90.57% | 0.00% | 99.83% | 99.98% | 0.01% |

# FIGURE 40 (Continued)

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 4 | 9 | 7 | 79 | 8 | 145291 | 20 | 2 | 17 | 6 | 146886 | 10 |
| C | 144147 | 143271 | 144336 | 1 | 144546 | 13 | 0 | 12 | 145273 | 8 | 2 | 13 |
| G | 1 | 0 | 0 | 145090 | 1 | 22 | 146322 | 12 | 3 | 2 | 7 | 0 |
| T | 8 | 9 | 17 | 22 | 11 | 1 | 3 | 144449 | 16 | 147205 | 10 | 146753 |
| N | 4094 | 4965 | 3894 | 3062 | 3688 | 2927 | 1909 | 3779 | 2945 | 1033 | 1337 | 1466 |
| | 144160 | 143289 | 144360 | 145192 | 144566 | 145327 | 146345 | 144475 | 145309 | 147221 | 146905 | 146776 |
| A | 0.00% | 0.01% | 0.00% | 0.05% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% |
| C | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% |
| G | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.02% | 99.98% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 0.01% | 99.98% |

**pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 8 | 10 | 19 | 79 | 3 | 165789 | 12 | 1 | 10 | 6 | 167640 | 6 |
| C | 164469 | 163202 | 164507 | 3 | 164857 | 8 | 0 | 12 | 165767 | 13 | 2 | 15 |
| G | 3 | 2 | 1 | 165491 | 2 | 26 | 166912 | 13 | 2 | 1 | 12 | 1 |
| T | 7 | 17 | 22 | 13 | 13 | 6 | 13 | 164840 | 17 | 168030 | 9 | 167553 |
| N | 4725 | 5981 | 4663 | 3626 | 4337 | 3383 | 2276 | 4347 | 3417 | 1163 | 1538 | 1626 |
| | 164487 | 163231 | 164549 | 165586 | 164875 | 165829 | 166937 | 164866 | 165796 | 168050 | 167663 | 167575 |
| A | 0.00% | 0.01% | 0.01% | 0.05% | 0.00% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |
| C | 99.99% | 99.98% | 99.97% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.02% | 99.99% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 99.99% | 0.01% | 99.99% |

FIGURE 40 (Continued)

| 9 | 10 | 0 | 13 | 5 | 82 | 7 | 98 | 3 | 5 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 145073 | 14 | 17 | 59 | 65 | 15274 | 2 | 173 | 21 | 14718 |
| 146362 | 3 | 1 | 1 | 4 | 147290 | 2 | 145735 | 1 | 2 | 3 |
| 6 | 11 | 147580 | 147631 | 146968 | 8 | 130470 | 6 | 146829 | 147449 | 8 |
| 1864 | 3145 | 647 | 580 | 1206 | 797 | 2489 | 2401 | 1236 | 765 | 1037 |
| 146378 | 145097 | 147595 | 147662 | 147036 | 147445 | 145753 | 145841 | 147006 | 147477 | 14720 |
| 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.06% | 0.00% | 0.07% | 0.00% | 0.00% | 0.01% |
| 0.00% | 99.98% | 0.01% | 0.01% | 0.04% | 0.04% | 10.48% | 0.00% | 0.12% | 0.01% | 99.99% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.89% | 0.00% | 99.93% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.01% | 99.99% | 99.98% | 99.95% | 0.01% | 89.51% | 0.00% | 99.88% | 99.98% | 0.01% |
| 12 | 12 | 2 | 7 | 4 | 25 | 20 | 32 | 5 | 4 | 12 |
| 3 | 165506 | 42 | 87 | 167 | 53 | 30594 | 1 | 315 | 27 | 16795 |
| 167029 | 1 | 3 | 3 | 3 | 168116 | 1 | 166120 | 6 | 0 | 2 |
| 7 | 11 | 168368 | 168448 | 167577 | 14 | 135711 | 11 | 167242 | 168175 | 18 |
| 2150 | 3671 | 786 | 656 | 1450 | 993 | 2875 | 3037 | 1633 | 995 | 1215 |
| 167051 | 165530 | 168415 | 168545 | 167751 | 168208 | 166326 | 166164 | 167568 | 168206 | 16798 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% |
| 0.00% | 99.99% | 0.02% | 0.05% | 0.10% | 0.03% | 18.39% | 0.00% | 0.19% | 0.02% | 99.98% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.01% | 99.97% | 99.94% | 99.90% | 0.01% | 81.59% | 0.01% | 99.81% | 99.98% | 0.01% |

# FIGURE 40 (Continued)

| | P<br>C | A<br>C | M<br>C | 20<br>G | 19<br>C | 18<br>A | 17<br>G | 16<br>T | 15<br>C | 14<br>T | 13<br>A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **untreated cells** | | | | | | | | | | | |
| **A** | 5 | 8 | 8 | 46 | 8 | 126539 | 5 | 0 | 14 | 3 | 127809 |
| **C** | 125520 | 124786 | 125615 | 3 | 125782 | 5 | 1 | 9 | 126407 | 12 | 0 |
| **G** | 1 | 0 | 1 | 126309 | 0 | 12 | 127437 | 0 | 1 | 0 | 9 |
| **T** | 6 | 16 | 16 | 8 | 19 | 2 | 3 | 125700 | 16 | 128134 | 11 |
| **N** | 3437 | 4159 | 3329 | 2604 | 3161 | 2412 | 1524 | 3261 | 2532 | 821 | 1141 |
| | 125532 | 124810 | 125640 | 126366 | 125809 | 126558 | 127446 | 125709 | 126438 | 128149 | 127829 |
| **A** | 0.00% | 0.01% | 0.01% | 0.04% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% |
| **C** | 99.99% | 99.98% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |
| **pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)** | | | | | | | | | | | |
| **A** | 4 | 7 | 9 | 66 | 6 | 127824 | 8 | 2 | 6 | 3 | 129157 |
| **C** | 126843 | 126230 | 127009 | 5 | 127181 | 7 | 0 | 11 | 128004 | 4 | 1 |
| **G** | 1 | 2 | 0 | 127581 | 0 | 11 | 128741 | 1 | 1 | 0 | 8 |
| **T** | 5 | 4 | 19 | 8 | 3 | 2 | 5 | 127095 | 4 | 129542 | 9 |
| **N** | 3474 | 4084 | 3290 | 2667 | 3137 | 2483 | 1573 | 3218 | 2312 | 778 | 1152 |
| | 126853 | 126243 | 127037 | 127660 | 127190 | 127844 | 128754 | 127109 | 128015 | 129549 | 129175 |
| **A** | 0.00% | 0.01% | 0.01% | 0.05% | 0.00% | 99.98% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% |

# FIGURE 40 (Continued)

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C |
| 11 | 8 | 5 | 2 | 4 | 2 | 11 | 8 | 10 | 1 | 6 | 10 |
| 15 | 1 | 126344 | 7 | 11 | 11 | 0 | 21 | 1 | 16 | 8 | 128120 |
| 0 | 127408 | 0 | 3 | 1 | 1 | 128298 | 0 | 127118 | 3 | 2 | 0 |
| 127771 | 6 | 7 | 128356 | 128490 | 127956 | 5 | 126892 | 7 | 127990 | 128397 | 15 |
| 1173 | 1547 | 2614 | 602 | 464 | 1000 | 656 | 2049 | 1834 | 960 | 557 | 825 |
| 127797 | 127423 | 126356 | 128368 | 128506 | 127970 | 128314 | 126921 | 127136 | 128010 | 128413 | 128145 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.98% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.00% | 99.98% | 0.01% | 99.98% | 99.99% | 0.01% |
| 13 | 7 | 9 | 1 | 8 | 1 | 13 | 11 | 10 | 3 | 6 | 7 |
| 14 | 2 | 127717 | 10 | 9 | 10 | 14 | 26 | 0 | 14 | 15 | 129453 |
| 0 | 128746 | 2 | 2 | 1 | 0 | 129597 | 0 | 128432 | 2 | 2 | 1 |
| 129132 | 10 | 3 | 129753 | 129825 | 129267 | 8 | 128228 | 7 | 129310 | 129685 | 9 |
| 1168 | 1562 | 2596 | 561 | 484 | 1049 | 695 | 2062 | 1878 | 998 | 619 | 857 |
| 129159 | 128765 | 127731 | 129766 | 129843 | 129278 | 129632 | 128265 | 128449 | 129329 | 129708 | 129470 |
| 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.99% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.98% | 0.01% | 0.00% | 99.99% | 99.99% | 99.99% | 0.01% | 99.97% | 0.01% | 99.99% | 99.98% | 0.01% |

# FIGURE 40 (Continued)

**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/**
**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 0 | 0 | 0 | 2 | 0 | 1118 | 0 | 0 | 0 | 0 | 1127 |
| **C** | 1112 | 1098 | 1108 | 0 | 1114 | 0 | 0 | 0 | 1118 | 0 | 0 |
| **G** | 0 | 0 | 0 | 1121 | 0 | 1 | 1121 | 0 | 0 | 0 | 0 |
| **T** | 1 | 1 | 2 | 0 | 1 | 0 | 1 | 1112 | 0 | 1131 | 0 |
| **N** | 26 | 40 | 29 | 16 | 24 | 20 | 17 | 27 | 21 | 8 | 12 |
| | 1113 | 1099 | 1110 | 1123 | 1115 | 1119 | 1122 | 1112 | 1118 | 1131 | 1127 |
| **A** | 0.00% | 0.00% | 0.00% | 0.18% | 0.00% | 99.91% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % |
| **C** | 99.91% | 99.91% | 99.82% | 0.00% | 99.91% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.82% | 0.00% | 0.09% | 99.91% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.09% | 0.09% | 0.18% | 0.00% | 0.09% | 0.00% | 0.09% | 100.00 % | 0.00% | 100.00 % | 0.00% |

**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/**
**pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 4 | 9 | 4 | 64 | 6 | 116171 | 3 | 1 | 5 | 6 | 117306 |
| **C** | 115481 | 114884 | 115574 | 1 | 115776 | 7 | 2 | 11 | 116336 | 7 | 0 |
| **G** | 0 | 0 | 1 | 116114 | 0 | 32 | 117016 | 16 | 1 | 0 | 6 |
| **T** | 4 | 11 | 13 | 12 | 7 | 11 | 6 | 115554 | 14 | 117701 | 13 |



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FIGURE 40 (Continued)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1112 | 1 | 2 | 1 | 3 | 417 | 0 | 37 | 4 | 1129 | 8 | 16 |
| 0 | 1122 | 0 | 0 | 0 | 0 | 1128 | 0 | 1116 | 0 | 0 | 0 | 0 | 0 |
| 1128 | 1 | 1 | 1131 | 1130 | 1126 | 1 | 701 | 0 | 1083 | 1123 | 1 | 1 | 1 |
| 10 | 15 | 25 | 6 | 6 | 11 | 6 | 20 | 22 | 18 | 11 | 8 | 8 | 3 |
| 1128 | 1123 | 1113 | 1132 | 1132 | 1127 | 1132 | 1118 | 1116 | 1120 | 1127 | 1130 | 1 | 117075 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 117394 | 6 |
| 0.00% | 0.00% | 99.91% | 0.09% | 0.18% | 0.09% | 0.27% | 37.30% | 0.00% | 3.30% | 0.35% | 99.91% | 3 | 117901 |
| 0.00% | 99.91% | 0.00% | 0.00% | 0.00% | 0.00% | 99.65% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 117991 | 117502 |
| 100.00% | 0.09% | 0.09% | 99.91% | 99.82% | 99.91% | 0.09% | 62.70% | 0.00% | 96.70% | 99.65% | 0.09% | 117901 | 117502 |

# FIGURE 40 (Continued)

| | P | A | M | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | C | C | G | C | A | G | T | C | T | A |
| N | 2948 | 3533 | 2845 | 2246 | 2648 | 2215 | 1410 | 2855 | 2081 | 723 | 1097 |
| | 115489 | 114904 | 115592 | 116191 | 115789 | 116221 | 117027 | 115582 | 116356 | 117714 | 117325 |
| A | 0.00% | 0.01% | 0.00% | 0.06% | 0.01% | 99.96% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% |
| C | 99.99% | 99.98% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.03% | 99.99% | 0.01% | 0.00% | 0.00% | 0.01% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 99.99% | 0.01% |

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 7 | 6 | 12 | 58 | 11 | 131354 | 5 | 2 | 8 | 6 | 132681 |
| C | 130391 | 129686 | 130471 | 2 | 130778 | 6 | 3 | 7 | 131498 | 12 | 0 |
| G | 0 | 3 | 0 | 131221 | 1 | 13 | 132288 | 0 | 1 | 1 | 5 |
| T | 6 | 13 | 22 | 16 | 16 | 3 | 5 | 130597 | 11 | 133041 | 8 |
| N | 3505 | 4201 | 3404 | 2612 | 3103 | 2533 | 1608 | 3302 | 2390 | 848 | 1214 |
| | 130404 | 129708 | 130505 | 131297 | 130806 | 131376 | 132301 | 130606 | 131518 | 133060 | 132694 |
| A | 0.01% | 0.00% | 0.01% | 0.04% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% |
| C | 99.99% | 99.98% | 99.97% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |

FIGURE 40 (Continued)

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C |
| 1011 | 1322 | 2278 | 504 | 414 | 910 | 616 | 1852 | 1713 | 864 | 522 | 804 |
| 117411 | 117100 | 116144 | 117918 | 118008 | 117512 | 117806 | 116570 | 116709 | 117558 | 117900 | 117618 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 0.02% | 1.06% | 0.00% | 0.01% | 0.01% | 99.98% |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.00% | 99.99% | 99.99% | 99.99% | 0.00% | 98.93% | 0.01% | 99.98% | 99.99% | 0.01% |
| 12 | 8 | 10 | 3 | 7 | 7 | 6 | 5 | 17 | 6 | 6 | 6 |
| 11 | 3 | 131183 | 13 | 13 | 6 | 0 | 94 | 2 | 16 | 18 | 132920 |
| 1 | 132293 | 3 | 2 | 2 | 1 | 133228 | 0 | 132019 | 2 | 0 | 2 |
| 132677 | 5 | 5 | 133306 | 133401 | 132839 | 7 | 131661 | 7 | 132874 | 133288 | 29 |
| 1207 | 1599 | 2707 | 584 | 485 | 1055 | 667 | 2148 | 1863 | 1010 | 596 | 951 |
| 132701 | 132309 | 131201 | 133324 | 133423 | 132853 | 133241 | 131760 | 132045 | 132898 | 133312 | 132957 |
| 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.07% | 0.00% | 0.01% | 0.01% | 99.97% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 99.98% | 0.00% | 0.00% | 99.99% | 99.98% | 99.99% | 0.01% | 99.92% | 0.01% | 99.98% | 99.98% | 0.02% |

FIGURE 40 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 8 | 3 | 5 | 57 | 9 | 139236 | 6 | 0 | 15 | 5 | 141073 |
| **C** | 138447 | 137168 | 138603 | 3 | 138749 | 15 | 3 | 10 | 139569 | 11 | 1 |
| **G** | 0 | 3 | 0 | 139177 | 14 | 12 | 140472 | 0 | 1 | 1 | 15 |
| **T** | 8 | 12 | 16 | 14 | 9 | 4 | 6 | 138735 | 10 | 141375 | 6 |
| **N** | 3847 | 5124 | 3686 | 3059 | 3529 | 3043 | 1823 | 3565 | 2715 | 918 | 1215 |
| | 138463 | 137186 | 138624 | 139251 | 138781 | 139267 | 140487 | 138745 | 139595 | 141392 | 141095 |
| **A** | 0.01% | 0.00% | 0.00% | 0.04% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% |
| **C** | 99.99% | 99.99% | 99.98% | 0.00% | 99.98% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.95% | 0.01% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% |
| **pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | | | |
| **A** | 5 | 9 | 14 | 55 | 7 | 120051 | 6 | 2 | 22 | 3 | 121701 |
| **C** | 119344 | 118249 | 119536 | 1 | 119788 | 2 | 2 | 8 | 120380 | 30 | 1 |
| **G** | 1 | 0 | 1 | 120055 | 2 | 14 | 121144 | 0 | 0 | 0 | 4 |
| **T** | 6 | 9 | 18 | 6 | 10 | 2 | 6 | 119772 | 7 | 121926 | 5 |
| **N** | 3438 | 4527 | 3225 | 2677 | 2987 | 2725 | 1636 | 3012 | 2385 | 835 | 1083 |
| | 119356 | 118267 | 119569 | 120117 | 119807 | 120069 | 121158 | 119782 | 120409 | 121959 | 121711 |
| **A** | 0.00% | 0.01% | 0.01% | 0.05% | 0.01% | 99.99% | 0.00% | 0.00% | 0.02% | 0.00% | 99.99% |
| **C** | 99.99% | 99.98% | 99.97% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.98% | 0.02% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.97% | 0.00% |
| **pNMG-276 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (wild-type) + (A106V_D108N_D147Y_E155V))** | | | | | | | | | | | |
| **A** | 10 | 11 | 10 | 63 | 11 | 151143 | 7 | 0 | 19 | 10 | 152789 |
| **C** | 150035 | 149220 | 150235 | 1 | 150381 | 9 | 1 | 9 | 151198 | 10 | 3 |
| **G** | 0 | 2 | 1 | 151112 | 0 | 16 | 152330 | 7 | 0 | 0 | 10 |
| **T** | 8 | 12 | 21 | 10 | 7 | 6 | 11 | 150179 | 16 | 153318 | 11 |

# FIGURE 40 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | 10 | 7 | 2 | 10 | 3 | 26 | 14 | 38 | 3 | 6 | 11 |
| 27 | 1 | 139475 | 58 | 194 | 225 | 78 | 64710 | 2 | 2813 | 188 | 141176 |
| 2 | 140498 | 1 | 0 | 2 | 0 | 141284 | 2 | 139316 | 1 | 1 | 5 |
| 140936 | 9 | 7 | 141649 | 141574 | 140967 | 15 | 75111 | 7 | 137749 | 141096 | 12 |
| 1325 | 1792 | 2820 | 601 | 530 | 1115 | 907 | 2473 | 2947 | 1744 | 1019 | 1106 |
| 140985 | 140518 | 139490 | 141709 | 141780 | 141195 | 141403 | 139837 | 139363 | 140566 | 141291 | 141204 |
| 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.01% | 0.03% | 0.00% | 0.00% | 0.01% |
| 0.02% | 0.00% | 99.99% | 0.04% | 0.14% | 0.16% | 0.06% | 46.28% | 0.00% | 2.00% | 0.13% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.92% | 0.00% | 99.97% | 0.00% | 0.00% | 0.00% |
| 99.97% | 0.01% | 0.01% | 99.96% | 99.85% | 99.84% | 0.01% | 53.71% | 0.01% | 98.00% | 99.86% | 0.01% |
| | | | | | | | | | | | |
| 6 | 9 | 9 | 1 | 4 | 7 | 29 | 11 | 40 | 6 | 7 | 9 |
| 12 | 1 | 120284 | 12 | 8 | 7 | 35 | 66422 | 0 | 60 | 10 | 121801 |
| 0 | 121223 | 1 | 3 | 0 | 1 | 121881 | 1 | 120089 | 2 | 0 | 3 |
| 121557 | 8 | 16 | 122290 | 122306 | 121767 | 13 | 54203 | 8 | 121289 | 121880 | 15 |
| 1219 | 1553 | 2484 | 488 | 476 | 1012 | 836 | 2157 | 2657 | 1437 | 897 | 966 |
| 121575 | 121241 | 120310 | 122306 | 122318 | 121782 | 121958 | 120637 | 120137 | 121357 | 121897 | 121828 |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.02% | 0.01% | 0.03% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 0.01% | 0.03% | 55.06% | 0.00% | 0.05% | 0.01% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% | 0.01% | 44.93% | 0.01% | 99.94% | 99.99% | 0.01% |
| | | | | | | | | | | | |
| 15 | 11 | 6 | 7 | 9 | 6 | 13 | 7 | 16 | 7 | 10 | 5 |
| 24 | 1 | 151116 | 12 | 11 | 11 | 54 | 482 | 0 | 29 | 17 | 153222 |
| 1 | 152369 | 5 | 1 | 1 | 1 | 153420 | 2 | 151946 | 1 | 0 | 2 |
| 152909 | 5 | 12 | 153655 | 153746 | 153082 | 9 | 151373 | 9 | 153083 | 153580 | 11 |

# FIGURE 40 (Continued)

| | P | A | M | 20 | 19 | 18 | 17 | 16 | 15 | 14 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | C | C | G | C | A | G | T | C | T | A |
| N | 4344 | 5151 | 4129 | 3210 | 3995 | 3220 | 2045 | 4179 | 3141 | 1036 | 1561 |
| | 150053 | 149245 | 150267 | 151186 | 150399 | 151174 | 152349 | 150195 | 151233 | 153338 | 152813 |
| A | 0.01% | 0.01% | 0.01% | 0.04% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.01% | 99.98% |
| C | 99.99% | 99.98% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 99.99% | 0.01% |
| **pNMG-277 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (A106V_D108N_D147Y_E155V) + (A106V_D108N_D147Y_E155V))** | | | | | | | | | | | |
| A | 12 | 7 | 12 | 54 | 7 | 141057 | 12 | 3 | 12 | 1 | 142849 |
| C | 140187 | 138830 | 140355 | 3 | 140753 | 6 | 1 | 8 | 141320 | 12 | 1 |
| G | 0 | 1 | 1 | 141067 | 0 | 11 | 142220 | 3 | 1 | 0 | 9 |
| T | 5 | 11 | 11 | 17 | 15 | 1 | 6 | 140472 | 10 | 143177 | 5 |
| N | 3915 | 5270 | 3740 | 2978 | 3344 | 3024 | 1860 | 3613 | 2756 | 909 | 1235 |
| | 140204 | 138849 | 140379 | 141141 | 140775 | 141075 | 142239 | 140486 | 141343 | 143190 | 142864 |
| A | 0.01% | 0.01% | 0.01% | 0.04% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% |
| C | 99.99% | 99.99% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% |

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FIGURE 40 (Continued)

| 12 T | 11 G | 10 C | 9 T | 8 T | 7 T | 6 G | 5 T | 4 G | 3 T | 2 T | 1 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1425 | 1987 | 3234 | 697 | 605 | 1272 | 876 | 2508 | 2401 | 1252 | 765 | 1132 |
| 152949 | 152386 | 151139 | 153675 | 153767 | 153100 | 153496 | 151864 | 151971 | 153120 | 153607 | 153240 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| 0.02% | 0.00% | 99.98% | 0.01% | 0.01% | 0.01% | 0.04% | 0.32% | 0.00% | 0.02% | 0.01% | 99.99% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 99.97% | 0.00% | 0.01% | 99.99% | 99.99% | 99.99% | 0.01% | 99.68% | 0.01% | 99.98% | 99.98% | 0.01% |
| 10 | 13 | 7 | 3 | 8 | 1 | 15 | 17 | 29 | 9 | 7 | 11 |
| 10 | 1 | 141250 | 208 | 366 | 310 | 82 | 73997 | 0 | 4984 | 267 | 143054 |
| 0 | 142328 | 5 | 2 | 1 | 0 | 143023 | 7 | 140924 | 0 | 2 | 0 |
| 142720 | 9 | 5 | 143277 | 143166 | 142613 | 10 | 67549 | 32 | 137217 | 142744 | 12 |
| 1359 | 1748 | 2832 | 608 | 559 | 1176 | 970 | 2530 | 3115 | 1890 | 1080 | 1023 |
| 142740 | 142351 | 141267 | 143490 | 143541 | 142924 | 143130 | 141570 | 140985 | 142210 | 143020 | 143077 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.14% | 0.25% | 0.22% | 0.06% | 52.27% | 0.00% | 3.50% | 0.19% | 99.98% |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 99.96% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.00% | 99.85% | 99.74% | 99.78% | 0.01% | 47.71% | 0.02% | 96.49% | 99.81% | 0.01% |

# FIGURE 40 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-278 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108Q_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 5 | 5 | 7 | 41 | 6 | 103643 | 4 | 0 | 10 | 3 | 104806 |
| **C** | 102703 | 102054 | 102882 | 0 | 103003 | 9 | 4 | 13 | 103650 | 9 | 2 |
| **G** | 2 | 0 | 0 | 103431 | 0 | 6 | 104371 | 0 | 2 | 1 | 4 |
| **T** | 10 | 7 | 8 | 11 | 8 | 2 | 6 | 102952 | 14 | 104939 | 10 |
| **N** | 2950 | 3604 | 2773 | 2187 | 2653 | 2010 | 1285 | 2701 | 1990 | 714 | 844 |
| | 102720 | 102066 | 102897 | 103483 | 103017 | 103660 | 104385 | 102965 | 103676 | 104952 | 104822 |
| **A** | 0.00% | 0.00% | 0.01% | 0.04% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% |
| **C** | 99.98% | 99.99% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 99.99% | 0.01% |
| **pNMG-279 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108M_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 5 | 12 | 10 | 56 | 10 | 114864 | 11 | 4 | 6 | 1 | 116205 |
| **C** | 113907 | 113012 | 113978 | 1 | 114275 | 12 | 2 | 6 | 114960 | 6 | 2 |
| **G** | 0 | 0 | 0 | 114741 | 0 | 9 | 115687 | 0 | 0 | 0 | 4 |
| **T** | 11 | 10 | 22 | 15 | 22 | 12 | 8 | 114262 | 9 | 116379 | 9 |
| **N** | 3249 | 4138 | 3162 | 2359 | 2865 | 2275 | 1456 | 2885 | 2182 | 771 | 937 |
| | 113923 | 113034 | 114010 | 114813 | 114307 | 114897 | 115708 | 114272 | 114975 | 116386 | 116220 |
| **A** | 0.00% | 0.01% | 0.01% | 0.05% | 0.01% | 99.97% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% |
| **C** | 99.99% | 99.98% | 99.97% | 0.00% | 99.97% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |
| **G** | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.01% | 0.02% | 0.01% | 0.02% | 0.01% | 0.01% | 99.99% | 0.01% | 99.99% | 0.01% |
| **pNMG-280 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108L_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 4 | 10 | 13 | 41 | 7 | 105927 | 5 | 3 | 6 | 2 | 107018 |
| **C** | 105021 | 104373 | 105175 | 0 | 105340 | 11 | 1 | 5 | 105918 | 12 | 6 |

FIGURE 40 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 12 | 7 | 3 | 7 | 0 | 9 | 5 | 6 | 1 | 1 | 11 |
| 5 | 0 | 103320 | 9 | 19 | 9 | 38 | 2959 | 2 | 37 | 12 | 104884 |
| 1 | 104288 | 1 | 0 | 0 | 1 | 105101 | 1 | 104240 | 1 | 1 | 1 |
| 104661 | 7 | 6 | 105135 | 105220 | 104777 | 10 | 100898 | 5 | 104758 | 105089 | 7 |
| 992 | 1359 | 2332 | 519 | 420 | 880 | 509 | 1804 | 1414 | 870 | 564 | 764 |
| 104674 | 104307 | 103334 | 105147 | 105246 | 104787 | 105158 | 103863 | 104253 | 104797 | 105103 | 104903 |
| 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.00% | 99.99% | 0.01% | 0.02% | 0.01% | 0.04% | 2.85% | 0.00% | 0.04% | 0.01% | 99.98% |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 99.98% | 99.99% | 0.01% | 97.15% | 0.00% | 99.96% | 99.99% | 0.01% |
| 4 | 8 | 7 | 2 | 4 | 4 | 9 | 17 | 17 | 4 | 3 | 7 |
| 4 | 1 | 114516 | 19 | 14 | 115 | 22 | 21535 | 1 | 231 | 25 | 11625 |
| 0 | 115667 | 0 | 2 | 2 | 0 | 116522 | 9 | 115309 | 0 | 0 | 9 |
| 116044 | 7 | 10 | 116563 | 116668 | 116088 | 8 | 93452 | 8 | 115811 | 116472 | 0 |
| 1105 | 1474 | 2624 | 571 | 469 | 950 | 596 | 2144 | 1822 | 1111 | 657 | 16 |
| 116052 | 115683 | 114533 | 116586 | 116688 | 116207 | 116561 | 115013 | 115335 | 116046 | 116500 | 875 |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 11628 |
| 0.00% | 0.00% | 99.99% | 0.02% | 0.01% | 0.10% | 0.02% | 18.72% | 0.00% | 0.20% | 0.02% | 2 |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% |
| 99.99% | 0.01% | 0.01% | 99.98% | 99.98% | 99.90% | 0.01% | 81.25% | 0.01% | 99.80% | 99.98% | 99.98 % |
| 7 | 10 | 13 | 2 | 7 | 1 | 6 | 7 | 12 | 5 | 2 | 0.00% |
| 7 | 1 | 105536 | 10 | 7 | 11 | 40 | 3414 | 2 | 85 | 11 | 3 |
| | | | | | | | | | | | 10714 |

FIGURE 40 (Continued)

| | P C | A C | M C | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 3 | 2 | 0 | 105808 | 1 | 7 | 106646 | 2 | 0 | 0 | 4 |
| T | 4 | 7 | 7 | 16 | 6 | 2 | 6 | 105194 | 12 | 107222 | 10 |
| N | 2916 | 3556 | 2753 | 2083 | 2594 | 1999 | 1288 | 2732 | 2000 | 700 | 895 |
| | 105032 | 104392 | 105195 | 105865 | 105354 | 105947 | 106658 | 105204 | 105936 | 107236 | 107038 |
| A | 0.00% | 0.01% | 0.01% | 0.04% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% |
| C | 99.99% | 99.98% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% | 0.01% | 0.01% |
| G | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 99.99% | 0.01% |
| **pNMG-281 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108K_D147Y_E155V)** | | | | | | | | | | | |
| A | 2 | 10 | 8 | 38 | 4 | 103231 | 6 | 1 | 10 | 3 | 104465 |
| C | 102387 | 101783 | 102441 | 5 | 102669 | 8 | 0 | 4 | 103400 | 7 | 4 |
| G | 2 | 1 | 0 | 103160 | 1 | 11 | 103964 | 3 | 0 | 1 | 5 |
| T | 8 | 5 | 18 | 18 | 7 | 2 | 6 | 102646 | 6 | 104637 | 7 |
| N | 2920 | 3520 | 2852 | 2098 | 2638 | 2067 | 1343 | 2665 | 1903 | 671 | 834 |
| | 102399 | 101799 | 102467 | 103221 | 102681 | 103252 | 103976 | 102654 | 103416 | 104648 | 104481 |
| A | 0.00% | 0.01% | 0.01% | 0.04% | 0.00% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% |
| C | 99.99% | 99.98% | 99.97% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.00% | 0.02% | 0.02% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 99.99% | 0.01% |

FIGURE 40 (Continued)

| 12 T | 11 G | 10 C | 9 T | 8 T | 7 T | 6 G | 5 T | 4 G | 3 T | 2 T | 1 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | 8 |
| 0 | 106553 | 2 | 1 | 4 | 1 | 107371 | 0 | 106458 | 2 | 0 | 0 |
| 106938 | 5 | 7 | 107405 | 107507 | 107071 | 12 | 102708 | 8 | 106999 | 107404 | 19 |
| 982 | 1365 | 2376 | 516 | 409 | 850 | 501 | 1801 | 1450 | 840 | 514 | 761 |
| 106952 | 106569 | 105558 | 107418 | 107525 | 107084 | 107429 | 106129 | 106480 | 107091 | 107417 | 10717 |
| 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0 0.00% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 0.01% | 0.04% | 3.22% | 0.00% | 0.08% | 0.01% | 99.98 % |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.98% | 99.99% | 0.01% | 96.78% | 0.01% | 99.91% | 99.99% | 0.02% |
| 4 | 7 | 6 | 1 | 1 | 1 | 25 | 8 | 9 | 5 | 3 | 2 |
| 6 | 1 | 102959 | 18 | 21 | 98 | 104 | 2954 | 0 | 91 | 29 | 10448 9 |
| 0 | 103979 | 5 | 0 | 0 | 2 | 104663 | 2 | 103900 | 0 | 0 | 4 |
| 104376 | 3 | 5 | 104729 | 104865 | 104380 | 6 | 100568 | 3 | 104381 | 104774 | 11 |
| 929 | 1325 | 2340 | 567 | 428 | 834 | 517 | 1783 | 1403 | 838 | 509 | 809 |
| 104386 | 103990 | 102975 | 104748 | 104887 | 104481 | 104798 | 103532 | 103912 | 104477 | 104806 | 10450 6 |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.98% | 0.02% | 0.02% | 0.09% | 0.10% | 2.85% | 0.00% | 0.09% | 0.03% | 99.98 % |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.87% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.00% | 99.98% | 99.98% | 9990% | 0.01% | 97.14% | 0.00% | 99.91% | 99.97% | 0.01% |

# FIGURE 40 (Continued)

**pNMG-282 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108I_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 8 | 7 | 20 | 89 | 4 | 106622 | 9 | 2 | 5 | 3 | 107863 |
| C | 105754 | 105024 | 105778 | 0 | 105973 | 5 | 2 | 5 | 106573 | 6 | 2 |
| G | 1 | 1 | 0 | 106449 | 2 | 17 | 107419 | 11 | 0 | 1 | 9 |
| T | 5 | 16 | 15 | 8 | 9 | 1 | 8 | 105995 | 14 | 107988 | 12 |
| N | 3024 | 3744 | 2979 | 2246 | 2804 | 2152 | 1359 | 2784 | 2205 | 799 | 900 |
| | 105768 | 105048 | 105813 | 106546 | 105988 | 106645 | 107438 | 106013 | 106592 | 107998 | 107886 |
| A | 0.01% | 0.01% | 0.02% | 0.08% | 0.00% | 99.98% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% |
| C | 99.99% | 99.98% | 99.97% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.91% | 0.00% | 0.02% | 99.98% | 0.01% | 0.00% | 0.00% | 0.01% |
| T | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 99.99% | 0.01% |

**pNMG-283 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108F_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 4 | 5 | 60 | 6 | 114772 | 4 | 2 | 11 | 0 | 116108 |
| C | 113825 | 112921 | 113809 | 2 | 113973 | 5 | 2 | 7 | 114780 | 8 | 1 |
| G | 2 | 2 | 1 | 114611 | 2 | 11 | 115546 | 2 | 0 | 0 | 4 |
| T | 6 | 13 | 12 | 10 | 2 | 11 | 8 | 113969 | 10 | 116243 | 6 |
| N | 3323 | 4221 | 3334 | 2478 | 3178 | 2362 | 1601 | 3157 | 2336 | 886 | 1017 |
| | 113838 | 112940 | 113827 | 114683 | 113983 | 114799 | 115560 | 113980 | 114801 | 116251 | 116119 |

# FIGURE 40 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 13 | 6 | 2 | 7 | 2 | 3 | 9 | 11 | 6 | 0 | 6 |
| 10 | 3 | 106372 | 6 | 7 | 14 | 30 | 777 | 1 | 30 | 18 | 107923 |
| 0 | 107410 | 0 | 2 | 1 | 1 | 108185 | 0 | 107375 | 5 | 1 | 0 |
| 107736 | 4 | 9 | 108270 | 108321 | 107847 | 11 | 106237 | 4 | 107869 | 108226 | 11 |
| 1035 | 1356 | 2399 | 506 | 450 | 922 | 557 | 1763 | 1395 | 876 | 541 | 846 |
| 107751 | 107430 | 106387 | 108280 | 108336 | 107864 | 108229 | 107023 | 107391 | 107910 | 108245 | 107940 |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.03% | 0.73% | 0.00% | 0.03% | 0.02% | 99.98% |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.96% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 99.99% | 99.98% | 0.01% | 99.27% | 0.00% | 99.96% | 99.98% | 0.01% |
| 3 | 8 | 9 | 1 | 5 | 2 | 6 | 3 | 14 | 4 | 6 | 4 |
| 10 | 3 | 114266 | 11 | 12 | 20 | 22 | 2181 | 0 | 50 | 5 | 116235 |
| 1 | 115528 | 1 | 1 | 0 | 3 | 116456 | 0 | 115465 | 1 | 1 | 1 |
| 115944 | 8 | 12 | 116496 | 116602 | 116070 | 13 | 112955 | 12 | 116050 | 116542 | 9 |
| 1177 | 1588 | 2847 | 626 | 516 | 1040 | 638 | 1996 | 1644 | 1030 | 581 | 886 |
| 115958 | 115547 | 114288 | 116509 | 116619 | 116095 | 116497 | 115139 | 115491 | 116105 | 116554 | 116249 |

# FIGURE 40 (Continued)

| | PC | AC | MC | 20 G | 19 C | 18 A | 17 G | 16 T | 15 C | 14 T | 13 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.00% | 0.00% | 0.05% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% |
| C | 99.99% | 99.98% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.94% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 99.99% | 0.01% |
| **untreated cells** | | | | | | | | | | | |
| A | 6 | 8 | 6 | 40 | 6 | 111900 | 6 | 2 | 8 | 1 | 113201 |
| C | 110856 | 110092 | 110895 | 5 | 111193 | 8 | 1 | 7 | 111911 | 7 | 1 |
| G | 0 | 0 | 1 | 111745 | 0 | 12 | 112609 | 0 | 2 | 2 | 5 |
| T | 4 | 10 | 17 | 13 | 7 | 3 | 5 | 111199 | 13 | 113414 | 9 |
| N | 3383 | 4139 | 3330 | 2446 | 3043 | 2326 | 1628 | 3041 | 2315 | 825 | 1033 |
| | 110866 | 110110 | 110919 | 111803 | 111206 | 111923 | 112621 | 111208 | 111934 | 113424 | 113216 |
| A | 0.01% | 0.01% | 0.01% | 0.04% | 0.01% | 99.98% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% |
| C | 99.99% | 99.98% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.95% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.01% |

# FIGURE 40 (Continued)

| 12 | 11 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | T | T | T | G | T | G | T | T | C |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 0.02% | 0.02% | 1.89% | 0.00% | 0.04% | 0.00% | 99.99% |
| 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.96% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.98% | 0.01% | 98.10% | 0.01% | 99.95% | 99.99% | 0.01% |
| 9 | 9 | 5 | 2 | 3 | 2 | 5 | 5 | 3 | 4 | 2 | 4 |
| 5 | 0 | 111576 | 6 | 9 | 8 | 1 | 38 | 3 | 10 | 16 | 113252 |
| 0 | 112633 | 1 | 0 | 0 | 1 | 113633 | 1 | 112600 | 0 | 0 | 1 |
| 113117 | 7 | 10 | 113638 | 113722 | 113224 | 10 | 112219 | 6 | 113221 | 113620 | 17 |
| 1118 | 1600 | 2657 | 603 | 515 | 1014 | 600 | 1986 | 1637 | 1014 | 611 | 975 |
| 113131 | 112649 | 111592 | 113646 | 113734 | 113235 | 113649 | 112263 | 112612 | 113235 | 113638 | 113274 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 99.99% | 0.01% | 0.01% | 0.01% | 0.00% | 0.03% | 0.00% | 0.01% | 0.01% | 99.98% |
| 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% | 0.01% | 99.96% | 0.01% | 99.99% | 99.98% | 0.02% |

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FIGURE 40 (Continued)

| NTC | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 1 | 0 | 5 | 1 | 5382 | 0 | 0 | 0 | 0 | 5426 |
| C | 5336 | 5301 | 5326 | 1 | 5348 | 0 | 0 | 0 | 5377 | 0 | 0 |
| G | 0 | 0 | 0 | 5376 | 0 | 1 | 5420 | 0 | 0 | 0 | 0 |
| T | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 5337 | 1 | 5444 | 1 |
| N | 147 | 180 | 157 | 100 | 134 | 100 | 63 | 146 | 105 | 39 | 56 |
| | 5336 | 5303 | 5326 | 5383 | 5349 | 5383 | 5420 | 5337 | 5378 | 5444 | 5427 |
| A | 0.00% | 0.02% | 0.00% | 0.09% | 0.02% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% |
| C | 100.00 % | 99.96% | 100.00 % | 0.02% | 99.98% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 99.87% | 0.00% | 0.02% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 100.00% | 0.02% | 100.00 % | 0.02% |
| NTC | | | | | | | | | | | |
| A | 0 | 0 | 0 | 0 | 36 | 452 | 0 | 0 | 0 | 0 | 458 |
| C | 454 | 455 | 454 | 0 | 414 | 0 | 0 | 0 | 460 | 0 | 0 |
| G | 0 | 0 | 0 | 455 | 0 | 0 | 459 | 0 | 0 | 0 | 0 |
| T | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 451 | 0 | 465 | 0 |
| N | 13 | 12 | 13 | 12 | 17 | 15 | 8 | 16 | 7 | 2 | 9 |
| | 454 | 455 | 454 | 455 | 450 | 452 | 459 | 451 | 460 | 465 | 458 |
| A | 0.00% | 0.00% | 0.00% | 0.00% | 8.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % |
| C | 100.00 % | 100.00 % | 100.00 % | 0.00% | 92.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% |
| G | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 100.00 % | 0.00% |

FIGURE 40 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 1 | 0 | 119 | 1 | 0 | 0 | 0 | 1 | |
| 0 | 0 | 5364 | 1 | 1 | 1 | 1 | 164 | 0 | 2 | 0 | 5449 | |
| 0 | 5262 | 0 | 0 | 0 | 0 | 5326 | 0 | 5415 | 0 | 0 | 0 | |
| 5430 | 147 | 0 | 5448 | 5457 | 5440 | 0 | 5223 | 0 | 5441 | 5455 | 1 | |
| 52 | 74 | 119 | 34 | 24 | 42 | 37 | 95 | 68 | 40 | 28 | 32 | |
| 5431 | 5409 | 5364 | 5449 | 5459 | 5441 | 5446 | 5388 | 5415 | 5443 | 5455 | 5451 | |
| 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 2.19% | 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 99.96 % |
| 0.00% | 0.00% | 100.00% | 0.02% | 0.02% | 0.02% | 0.02% | 3.04% | 0.00% | 0.04% | 0.00% | 0.00% | |
| 0.00% | 97.28% | 0.00% | 0.00% | 0.00% | 0.00% | 97.80% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | |
| 99.98% | 2.72% | 0.00% | 99.98% | 99.96% | 99.98% | 0.00% | 96.94% | 0.00% | 99.96% | 100.00 % | 0.02% | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 1 | 0 | 458 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 460 | |
| 0 | 461 | 0 | 0 | 0 | 0 | 461 | 0 | 457 | 0 | 0 | 1 | |
| 464 | 0 | 0 | 465 | 463 | 463 | 0 | 455 | 0 | 462 | 462 | 0 | |
| 2 | 6 | 9 | 2 | 4 | 4 | 6 | 8 | 10 | 5 | 5 | 6 | |
| 465 | 461 | 458 | 465 | 463 | 463 | 461 | 459 | 457 | 462 | 462 | 461 | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.22% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.87% | 0.00% | 0.00% | 0.00% | 99.78 % | |
| 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 100.00 % | 0.00% | 0.00% | 0.22% | |
| 99.78% | 0.00% | 0.00% | 100.00 % | 100.00 % | 100.00% | 0.00% | 99.13% | 0.00% | 100.00 % | 100.00 % | 0.00% | |

# FIGURE 41

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cas9** | | | | | | | | | | | |
| **A** | 1 | 0 | 1 | 0 | 2 | 19984 | 0 | 20453 | 3 | 0 | 7 |
| **C** | 1 | 0 | 19949 | 20011 | 20111 | 0 | 0 | 0 | 20467 | 2 | 3 |
| **G** | 20400 | 19881 | 0 | 0 | 0 | 4 | 20590 | 0 | 1 | 0 | 20578 |
| **T** | 0 | 1 | 2 | 2 | 1 | 1 | 0 | 4 | 0 | 20597 | 0 |
| **N** | 509 | 1028 | 957 | 893 | 790 | 918 | 317 | 450 | 436 | 308 | 320 |
| | 20402 | 19882 | 19952 | 20013 | 20114 | 19989 | 20590 | 20457 | 20471 | 20599 | 20588 |
| **A** | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 99.98% | 0.01% | 0.00% | 0.03% |
| **C** | 0.00% | 0.00% | 99.98% | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.01% |
| **G** | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 0.02% | 100.00% | 0.00% | 0.00% | 0.00% | 99.95% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 99.99% | 0.00% |
| **pNMG-247 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 0 | 1 | 0 | 1 | 0 | 9858 | 2 | 10090 | 0 | 1 | 3 |
| **C** | 0 | 0 | 9930 | 9947 | 9939 | 0 | 0 | 0 | 10096 | 2 | 0 |
| **G** | 10079 | 9850 | 0 | 0 | 0 | 0 | 10159 | 1 | 0 | 0 | 10175 |
| **T** | 2 | 2 | 0 | 0 | 2 | 0 | 2 | 2 | 1 | 10182 | 0 |
| **N** | 269 | 497 | 419 | 395 | 402 | 485 | 180 | 250 | 246 | 154 | 160 |
| | 10081 | 9853 | 9930 | 9948 | 9941 | 9858 | 10163 | 10093 | 10097 | 10185 | 10178 |
| **A** | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 100.00% | 0.02% | 99.97% | 0.00% | 0.01% | 0.03% |
| **C** | 0.00% | 0.00% | 100.00% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.02% | 0.00% |
| **G** | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% | 99.96% | 0.01% | 0.00% | 0.00% | 99.97% |
| **T** | 0.02% | 0.02% | 0.00% | 0.00% | 0.02% | 0.00% | 0.02% | 0.02% | 0.01% | 99.97% | 0.00% |

FIGURE 41 (Continued)

| 12 A | 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 19900 | 2 | 2 | 20325 | 13 | 49 | 38 | 28 | 19550 | 17 | 33 | 12 |
| 4 | 1 | 20239 | 35 | 20010 | 43 | 34 | 30 | 26 | 17 | 5 | 3 |
| 7 | 20346 | 6 | 7 | 58 | 19759 | 80 | 19959 | 8 | 14 | 19961 | 20089 |
| 6 | 4 | 13 | 3 | 46 | 30 | 19798 | 12 | 13 | 19994 | 5 | 7 |
| 996 | 560 | 651 | 539 | 776 | 1019 | 798 | 702 | 1085 | 619 | 624 | 503 |
| 19917 | 20353 | 20260 | 20370 | 20127 | 19881 | 19950 | 20029 | 19597 | 20042 | 20004 | 20111 |
| 99.91% | 0.01% | 0.01% | 99.78% | 0.06% | 0.25% | 0.19% | 0.14% | 99.76% | 0.08% | 0.16% | 0.06% |
| 0.02% | 0.00% | 99.90% | 0.17% | 99.42% | 0.22% | 0.17% | 0.15% | 0.13% | 0.08% | 0.02% | 0.01% |
| 0.04% | 99.97% | 0.03% | 0.03% | 0.29% | 99.39% | 0.40% | 99.65% | 0.04% | 0.07% | 99.79% | 99.89% |
| 0.03% | 0.02% | 0.06% | 0.01% | 0.23% | 0.15% | 99.24% | 0.06% | 0.07% | 99.76% | 0.02% | 0.03% |
| 9822 | 2 | 6 | 10083 | 31 | 41 | 44 | 5 | 9398 | 13 | 16 | 10 |
| 0 | 0 | 9985 | 7 | 9819 | 53 | 29 | 27 | 22 | 22 | 7 | 7 |
| 1 | 10058 | 0 | 1 | 60 | 9674 | 92 | 9668 | 7 | 13 | 9615 | 9670 |
| 0 | 2 | 10 | 6 | 25 | 49 | 9547 | 17 | 27 | 9623 | 5 | 9 |
| 515 | 281 | 352 | 255 | 408 | 514 | 413 | 354 | 571 | 324 | 310 | 251 |
| 9823 | 10062 | 10001 | 10097 | 9935 | 9817 | 9712 | 9717 | 9454 | 9671 | 9643 | 9696 |
| 99.99% | 0.02% | 0.06% | 99.86% | 0.31% | 0.42% | 0.45% | 0.05% | 99.41% | 0.13% | 0.17% | 0.10% |
| 0.00% | 0.00% | 99.84% | 0.07% | 98.83% | 0.54% | 0.30% | 0.28% | 0.23% | 0.23% | 0.07% | 0.07% |
| 0.01% | 99.96% | 0.00% | 0.01% | 0.60% | 98.54% | 0.95% | 99.50% | 0.07% | 0.13% | 99.71% | 99.73% |
| 0.00% | 0.02% | 0.10% | 0.06% | 0.25% | 0.50% | 98.30% | 0.17% | 0.29% | 99.50% | 0.05% | 0.09% |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-248 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 0 | 2 | 0 | 2 | 0 | 16831 | 0 | 17248 | 1 | 3 | 6 |
| **C** | 2 | 0 | 16891 | 16955 | 16995 | 0 | 0 | 0 | 17254 | 2 | 1 |
| **G** | 17143 | 16757 | 1 | 0 | 0 | 3 | 17331 | 3 | 0 | 0 | 17316 |
| **T** | 0 | 3 | 2 | 2 | 3 | 1 | 1 | 4 | 1 | 17366 | 0 |
| **N** | 472 | 854 | 719 | 653 | 614 | 776 | 279 | 356 | 355 | 240 | 288 |
| | 17145 | 16762 | 16894 | 16959 | 16998 | 16835 | 17332 | 17255 | 17256 | 17371 | 17323 |
| **A** | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.96% | 0.01% | 0.02% | 0.03% |
| **C** | 0.01% | 0.00% | 99.98% | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.01% |
| **G** | 99.99% | 99.97% | 0.01% | 0.00% | 0.00% | 0.02% | 99.99% | 0.02% | 0.00% | 0.00% | 99.96% |
| **T** | 0.00% | 0.02% | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% | 0.02% | 0.01% | 99.97% | 0.00% |
| **pNMG-249 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 2 | 4 | 1 | 2 | 0 | 15815 | 2 | 16187 | 0 | 0 | 3 |
| **C** | 2 | 0 | 15832 | 15893 | 15904 | 0 | 0 | 8 | 16206 | 2 | 0 |
| **G** | 16107 | 15738 | 0 | 2 | 1 | 0 | 16275 | 0 | 0 | 2 | 16300 |
| **T** | 1 | 0 | 2 | 1 | 1 | 3 | 1 | 0 | 0 | 16312 | 0 |
| **N** | 434 | 804 | 711 | 648 | 640 | 734 | 274 | 357 | 346 | 234 | 247 |
| | 16112 | 15742 | 15835 | 15898 | 15906 | 15818 | 16278 | 16195 | 16206 | 16316 | 16303 |
| **A** | 0.01% | 0.03% | 0.01% | 0.01% | 0.00% | 99.98% | 0.01% | 99.95% | 0.00% | 0.00% | 0.02% |
| **C** | 0.01% | 0.00% | 99.98% | 99.97% | 99.99% | 0.00% | 0.00% | 0.05% | 100.00% | 0.01% | 0.00% |



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# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 16769 | 1 | 0 | 17155 | 14 | 46 | 64 | 13 | 16480 | 10 | 15 | 2 |
| 6 | 0 | 17077 | 20 | 16881 | 50 | 28 | 18 | 19 | 11 | 5 | 8 |
| 5 | 17134 | 1 | 8 | 41 | 16674 | 76 | 16831 | 12 | 14 | 16786 | 16876 |
| 0 | 1 | 6 | 17 | 38 | 21 | 16636 | 25 | 14 | 16803 | 5 | 6 |
| 834 | 478 | 530 | 410 | 627 | 813 | 637 | 535 | 842 | 496 | 488 | 403 |
| 16780 | 17136 | 17084 | 17200 | 16974 | 16791 | 16804 | 16887 | 16525 | 16838 | 16811 | 16892 |
| 99.93% | 0.01% | 0.00% | 99.74% | 0.08% | 0.27% | 0.38% | 0.08% | 99.73% | 0.06% | 0.09% | 0.01% |
| 0.04% | 0.00% | 99.96% | 0.12% | 99.45% | 0.30% | 0.17% | 0.11% | 0.11% | 0.07% | 0.03% | 0.05% |
| 0.03% | 99.99% | 0.01% | 0.05% | 0.24% | 99.30% | 0.45% | 99.67% | 0.07% | 0.08% | 99.85% | 99.91% |
| 0.00% | 0.01% | 0.04% | 0.10% | 0.22% | 0.13% | 99.00% | 0.15% | 0.08% | 99.79% | 0.03% | 0.04% |
| 15743 | 0 | 0 | 16098 | 23 | 71 | 54 | 21 | 15396 | 6 | 60 | 3 |
| 5 | 5 | 16024 | 25 | 15855 | 34 | 36 | 32 | 42 | 60 | 12 | 0 |
| 5 | 16110 | 2 | 9 | 62 | 15612 | 96 | 15793 | 18 | 4 | 15670 | 15837 |
| 0 | 5 | 2 | 16 | 36 | 46 | 15606 | 17 | 9 | 15765 | 6 | 6 |
| 797 | 430 | 520 | 399 | 569 | 777 | 628 | 535 | 861 | 471 | 520 | 406 |
| 15753 | 16120 | 16028 | 16148 | 15976 | 15763 | 15792 | 15863 | 15465 | 15835 | 15748 | 15846 |
| 99.94% | 0.00% | 0.00% | 99.69% | 0.14% | 0.45% | 0.34% | 0.13% | 99.55% | 0.04% | 0.38% | 0.02% |
| 0.03% | 0.03% | 99.98% | 0.15% | 99.24% | 0.22% | 0.23% | 0.20% | 0.27% | 0.38% | 0.08% | 0.00% |

# FIGURE 41 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 99.97% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 99.98% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% |
| **pNMG-250 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 2 | 3 | 0 | 1 | 0 | 21880 | 8 | 22422 | 1 | 11 | 2 |
| C | 0 | 1 | 21832 | 21999 | 22014 | 0 | 1 | 0 | 22461 | 3 | 5 |
| G | 22297 | 21809 | 0 | 0 | 0 | 2 | 22539 | 3 | 8 | 0 | 22569 |
| T | 2 | 3 | 1 | 0 | 4 | 2 | 1 | 0 | 6 | 22606 | 1 |
| N | 626 | 1111 | 1092 | 919 | 908 | 1040 | 375 | 499 | 447 | 303 | 343 |
| | 22301 | 21816 | 21833 | 22000 | 22018 | 21884 | 22549 | 22425 | 22476 | 22620 | 22577 |
| A | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 0.04% | 99.99% | 0.00% | 0.05% | 0.01% |
| C | 0.00% | 0.00% | 100.00% | 100.00% | 99.98% | 0.00% | 0.00% | 0.00% | 99.93% | 0.01% | 0.02% |
| G | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 0.01% | 0.04% | 0.00% | 99.96% |
| T | 0.01% | 0.01% | 0.00% | 0.00% | 0.02% | 0.01% | 0.00% | 0.00% | 0.03% | 99.94% | 0.00% |
| **pNMG-251 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 1 | 6 | 0 | 4 | 5 | 36404 | 2 | 37263 | 0 | 0 | 3 |
| C | 0 | 0 | 36492 | 36642 | 36724 | 0 | 0 | 0 | 37339 | 3 | 1 |
| G | 37121 | 36324 | 1 | 0 | 1 | 5 | 37547 | 4 | 1 | 0 | 37571 |
| T | 1 | 0 | 3 | 3 | 4 | 5 | 2 | 0 | 3 | 37628 | 0 |
| N | 1019 | 1812 | 1646 | 1493 | 1408 | 1728 | 591 | 875 | 798 | 510 | 566 |
| | 37123 | 36330 | 36496 | 36649 | 36734 | 36414 | 37551 | 37267 | 37343 | 37631 | 37575 |
| A | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 99.97% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.99% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 99.99% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.00% |

# FIGURE 41 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | G | C | A | C | G | T | G | A | T | G | G |
| 0.03% | 99.94% | 0.01% | 0.06% | 0.39% | 99.04% | 0.61% | 99.56% | 0.12% | 0.03% | 99.50% | 99.94% |
| 0.00% | 0.03% | 0.01% | 0.10% | 0.23% | 0.29% | 98.82% | 0.11% | 0.06% | 99.56% | 0.04% | 0.04% |
| 21787 | 1 | 1 | 22321 | 25 | 39 | 67 | 21 | 21447 | 37 | 21 | 10 |
| 3 | 0 | 22247 | 41 | 21981 | 60 | 31 | 17 | 35 | 21 | 4 | 2 |
| 12 | 22324 | 0 | 9 | 84 | 21670 | 87 | 21880 | 15 | 3 | 21964 | 22084 |
| 2 | 3 | 12 | 26 | 26 | 43 | 21678 | 26 | 13 | 21978 | 5 | 6 |
| 1122 | 619 | 684 | 537 | 811 | 1119 | 901 | 794 | 1184 | 641 | 646 | 530 |
| 21804 | 22328 | 22260 | 22397 | 22116 | 21812 | 21863 | 21944 | 21510 | 22039 | 21994 | 22102 |
| 99.92% | 0.00% | 0.00% | 99.66% | 0.11% | 0.18% | 0.31% | 0.10% | 99.71% | 0.17% | 0.10% | 0.05% |
| 0.01% | 0.00% | 99.94% | 0.18% | 99.39% | 0.28% | 0.14% | 0.08% | 0.16% | 0.10% | 0.02% | 0.01% |
| 0.06% | 99.98% | 0.00% | 0.04% | 0.38% | 99.35% | 0.40% | 99.71% | 0.07% | 0.01% | 99.86% | 99.92% |
| 0.01% | 0.01% | 0.05% | 0.12% | 0.12% | 0.20% | 99.15% | 0.12% | 0.06% | 99.72% | 0.02% | 0.03% |
| 36283 | 4 | 2 | 37164 | 7 | 72 | 43 | 8 | 35826 | 10 | 22 | 12 |
| 0 | 0 | 36945 | 20 | 36717 | 30 | 36 | 15 | 20 | 26 | 6 | 3 |
| 5 | 37047 | 4 | 15 | 67 | 36192 | 73 | 36563 | 12 | 2 | 36638 | 36838 |
| 1 | 3 | 3 | 8 | 25 | 13 | 36335 | 11 | 14 | 36670 | 6 | 3 |
| 1852 | 1094 | 1193 | 940 | 1329 | 1824 | 1409 | 1283 | 1949 | 1106 | 1115 | 920 |
| 36289 | 37054 | 36954 | 37207 | 36816 | 36307 | 36487 | 36597 | 35872 | 36708 | 36672 | 36856 |
| 99.98% | 0.01% | 0.01% | 99.88% | 0.02% | 0.20% | 0.12% | 0.02% | 99.87% | 0.03% | 0.06% | 0.03% |
| 0.00% | 0.00% | 99.98% | 0.05% | 99.73% | 0.08% | 0.10% | 0.04% | 0.06% | 0.07% | 0.02% | 0.01% |
| 0.01% | 99.98% | 0.01% | 0.04% | 0.18% | 99.68% | 0.20% | 99.91% | 0.03% | 0.01% | 99.91% | 99.95% |
| 0.00% | 0.01% | 0.01% | 0.02% | 0.07% | 0.04% | 99.58% | 0.03% | 0.04% | 99.90% | 0.02% | 0.01% |



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# FIGURE 41 (Continued)

| pNMG-181 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 7 | 9 | 2 | 5 | 5 | 82475 | 5 | 84662 | 1 | 0 | 11 |
| C | 0 | 2 | 82899 | 83345 | 83466 | 0 | 0 | 0 | 85134 | 7 | 0 |
| G | 84626 | 82474 | 0 | 2 | 0 | 184 | 85466 | 267 | 1 | 0 | 85559 |
| T | 4 | 1 | 10 | 6 | 7 | 2 | 2 | 3 | 7 | 85788 | 4 |
| N | 2518 | 4669 | 4244 | 3797 | 3677 | 4494 | 1682 | 2223 | 2012 | 1360 | 1581 |
| | 84637 | 82486 | 82911 | 83358 | 83478 | 82661 | 85473 | 84932 | 85143 | 85795 | 85574 |
| | | | | | | | | | | | |
| A | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.77% | 0.01% | 99.68% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.99% | 99.98% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.99% | 0.00% | 0.00% | 0.00% | 0.22% | 99.99% | 0.31% | 0.00% | 0.00% | 99.98% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| | | | | | | | | | | | |
| pNMG-182 (pCMVecTadA_GGS_Cas9n_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | | |
| A | 5 | 8 | 3 | 3 | 3 | 73311 | 3 | 75152 | 2 | 0 | 3 |
| C | 0 | 3 | 73759 | 74004 | 74114 | 0 | 0 | 0 | 75485 | 5 | 1 |
| G | 75044 | 73314 | 0 | 1 | 1 | 179 | 75741 | 174 | 0 | 0 | 75772 |
| T | 4 | 2 | 6 | 6 | 10 | 6 | 3 | 6 | 7 | 75967 | 5 |
| N | 2043 | 3769 | 3328 | 3082 | 2968 | 3600 | 1349 | 1764 | 1602 | 1124 | 1315 |
| | 75053 | 73327 | 73768 | 74014 | 74128 | 73496 | 75747 | 75332 | 75494 | 75972 | 75781 |
| | | | | | | | | | | | |
| A | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.75% | 0.00% | 99.76% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 99.99% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |



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# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 82372 | 4 | 3 | 84781 | 2 | 14 | 5 | 5 | 82273 | 4 | 7 | 20 |
| 1 | 1 | 84117 | 2 | 83798 | 1 | 12 | 1 | 5 | 5 | 1 | 3 |
| 10 | 84382 | 1 | 8 | 2 | 82616 | 1 | 83990 | 5 | 0 | 84352 | 84806 |
| 1 | 4 | 9 | 0 | 13 | 3 | 83630 | 7 | 6 | 84407 | 3 | 6 |
| 4771 | 2764 | 3025 | 2364 | 3340 | 4521 | 3507 | 3152 | 4866 | 2739 | 2792 | 2320 |
| 82384 | 84391 | 84130 | 84791 | 83815 | 82634 | 83648 | 84003 | 82289 | 84416 | 84363 | 84835 |
| 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.02% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 99.97% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 99.98% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% |
| 73285 | 6 | 2 | 75190 | 4 | 10 | 1 | 2 | 73069 | 2 | 14 | 16 |
| 0 | 3 | 74627 | 0 | 74192 | 0 | 7 | 1 | 0 | 5 | 1 | 0 |
| 14 | 74888 | 1 | 4 | 1 | 73411 | 2 | 74483 | 7 | 0 | 74766 | 75209 |
| 0 | 4 | 9 | 2 | 12 | 2 | 74160 | 6 | 6 | 74827 | 4 | 7 |
| 3797 | 2195 | 2457 | 1900 | 2887 | 3673 | 2926 | 2604 | 4014 | 2262 | 2311 | 1864 |
| 73299 | 74901 | 74639 | 75196 | 74209 | 73423 | 74170 | 74492 | 73082 | 74834 | 74785 | 75232 |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.02% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 41 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.24% | 99.99% | 0.23% | 0.00% | 0.00% | 99.99% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% |
| **pNMG-183 (pCMV_ecTadA_(GGS)2-XTEN-(GGS)2_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 4 | 11 | 1 | 6 | 1 | 56984 | 0 | 58084 | 2 | 1 | 5 |
| C | 0 | 1 | 57332 | 57469 | 57526 | 1 | 0 | 0 | 58616 | 7 | 0 |
| G | 57308 | 56342 | 4 | 0 | 0 | 122 | 58914 | 579 | 0 | 0 | 58963 |
| T | 1 | 2 | 8 | 6 | 21 | 2 | 1 | 3 | 4 | 58999 | 1 |
| N | 2476 | 3433 | 2444 | 2308 | 2241 | 2680 | 874 | 1123 | 1167 | 782 | 820 |
| | 57313 | 56356 | 57345 | 57481 | 57548 | 57109 | 58915 | 58666 | 58622 | 59007 | 58969 |
| A | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | 99.78% | 0.00% | 99.01% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.98% | 99.96% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.98% | 0.01% | 0.00% | 0.00% | 0.21% | 100.00% | 0.99% | 0.00% | 0.00% | 99.99% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.04% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.00% |
| **pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 1 | 9 | 5 | 3 | 2 | 60176 | 2 | 61415 | 2 | 0 | 7 |
| C | 0 | 0 | 60694 | 60722 | 60920 | 0 | 2 | 0 | 61934 | 3 | 2 |
| G | 60793 | 59768 | 0 | 3 | 7 | 163 | 62238 | 509 | 0 | 1 | 62313 |
| T | 1 | 0 | 18 | 13 | 13 | 1 | 1 | 4 | 2 | 62362 | 9 |
| N | 2363 | 3381 | 2441 | 2417 | 2216 | 2818 | 915 | 1230 | 1220 | 792 | 827 |
| | 60795 | 59777 | 60717 | 60741 | 60942 | 60340 | 62243 | 61928 | 61938 | 62366 | 62331 |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 41 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | G | C | A | C | G | T | G | A | T | G | G |
| 0.02% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.97% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% |
| 57075 | 4 | 3 | 58270 | 2 | 3 | 0 | 7 | 56622 | 5 | 6 | 10 |
| 1 | 0 | 57872 | 0 | 57659 | 0 | 3 | 1 | 2 | 5 | 0 | 0 |
| 8 | 58418 | 1 | 4 | 2 | 57068 | 0 | 57810 | 6 | 1 | 58149 | 58392 |
| 1 | 0 | 7 | 2 | 7 | 1 | 57575 | 1 | 1 | 58082 | 1 | 3 |
| 2704 | 1367 | 1906 | 1513 | 2119 | 2717 | 2211 | 1970 | 3158 | 1696 | 1633 | 1384 |
| 57085 | 58422 | 57883 | 58276 | 57670 | 57072 | 57578 | 57819 | 56631 | 58093 | 58156 | 58405 |
| 99.98% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 99.98% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% |
| 60356 | 6 | 4 | 61690 | 3 | 5 | 1 | 4 | 59875 | 1 | 10 | 16 |
| 0 | 1 | 61149 | 5 | 60985 | 4 | 8 | 0 | 3 | 10 | 1 | 2 |
| 8 | 61765 | 3 | 5 | 0 | 60400 | 0 | 61248 | 13 | 1 | 61420 | 61773 |
| 2 | 4 | 3 | 5 | 8 | 4 | 60962 | 3 | 6 | 61363 | 2 | 2 |
| 2792 | 1382 | 1999 | 1453 | 2162 | 2745 | 2187 | 1903 | 3261 | 1783 | 1725 | 1365 |
| 60366 | 61776 | 61159 | 61705 | 60996 | 60413 | 60971 | 61255 | 59897 | 61375 | 61433 | 61793 |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.02% | 0.01% | 0.00% | 0.00% | 99.73% | 0.00% | 99.17% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.96% | 99.97% | 99.96% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% |
| G | 100.00 % | 99.98% | 0.00% | 0.00% | 0.01% | 0.27% | 99.99% | 0.82% | 0.00% | 0.00% | 99.97% |
| T | 0.00% | 0.00% | 0.03% | 0.02% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 5 | 12 | 3 | 3 | 3 | 51890 | 6 | 53113 | 1 | 1 | 4 |
| C | 1 | 2 | 52277 | 52366 | 52443 | 0 | 1 | 0 | 53431 | 3 | 1 |
| G | 52367 | 51475 | 2 | 0 | 0 | 87 | 53680 | 296 | 1 | 0 | 53760 |
| T | 0 | 3 | 4 | 3 | 13 | 1 | 1 | 2 | 4 | 53768 | 2 |
| N | 2102 | 2983 | 2189 | 2103 | 2016 | 2497 | 787 | 1064 | 1038 | 703 | 708 |
| | 52373 | 51492 | 52286 | 52372 | 52459 | 51978 | 53688 | 53411 | 53437 | 53772 | 53767 |
| A | 0.01% | 0.02% | 0.01% | 0.01% | 0.01% | 99.83% | 0.01% | 99.44% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 0.17% | 99.99% | 0.55% | 0.00% | 0.00% | 99.99% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| **pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)/ pNMG-261** | | | | | | | | | | | |
| A | 3 | 11 | 2 | 6 | 2 | 64058 | 2 | 65753 | 1 | 0 | 9 |
| C | 2 | 0 | 64394 | 64527 | 64641 | 1 | 3 | 0 | 65750 | 8 | 0 |
| G | 64502 | 63461 | 3 | 1 | 1 | 5 | 66061 | 10 | 1 | 1 | 66142 |
| T | 1 | 2 | 9 | 5 | 2 | 4 | 3 | 5 | 3 | 66177 | 7 |
| N | 2618 | 3652 | 2718 | 2587 | 2480 | 3058 | 1057 | 1358 | 1371 | 940 | 968 |
| | 64508 | 63474 | 64408 | 64539 | 64646 | 64068 | 66069 | 65768 | 65755 | 66186 | 66158 |
| A | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 99.98% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.96% | 0.00% | 0.02% | 0.03% |
| 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.01% | 0.02% | 0.00% | 0.00% |
| 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.02% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% |
| 52023 | 5 | 1 | 53217 | 2 | 7 | 0 | 5 | 51557 | 2 | 10 | 9 |
| 0 | 0 | 52789 | 0 | 52594 | 1 | 4 | 0 | 3 | 4 | 0 | 1 |
| 13 | 53229 | 0 | 0 | 0 | 51952 | 0 | 52733 | 7 | 0 | 52996 | 53241 |
| 0 | 0 | 4 | 5 | 5 | 2 | 52501 | 2 | 6 | 52863 | 1 | 3 |
| 2439 | 1241 | 1681 | 1253 | 1874 | 2513 | 1970 | 1735 | 2902 | 1606 | 1468 | 1221 |
| 52036 | 53234 | 52794 | 53222 | 52601 | 51962 | 52505 | 52740 | 51573 | 52869 | 53007 | 53254 |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.02% | 0.02% |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.98% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |
| 64047 | 5 | 2 | 65536 | 3 | 9 | 3 | 4 | 63626 | 4 | 7 | 26 |
| 0 | 1 | 64969 | 1 | 64798 | 0 | 10 | 0 | 1 | 6 | 0 | 5 |
| 9 | 65514 | 0 | 6 | 0 | 64105 | 1 | 64953 | 6 | 1 | 65201 | 65598 |
| 0 | 3 | 4 | 4 | 13 | 1 | 64651 | 2 | 3 | 65185 | 2 | 4 |
| 3070 | 1603 | 2151 | 1579 | 2312 | 3011 | 2461 | 2167 | 3490 | 1930 | 1916 | 1493 |
| 64056 | 65523 | 64975 | 65547 | 64814 | 64115 | 64665 | 64959 | 63636 | 65196 | 65210 | 65633 |
| 99.99% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 0.04% |

# FIGURE 41 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **C** | 0.00% | 0.00% | 99.98% | 99.98% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.02% | 0.00% | 0.00% | 99.98% |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% |
| **pNMG -164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)/ pNMG261** | | | | | | | | | | | |
| **A** | 5 | 13 | 3 | 9 | 2 | 74698 | 4 | 76766 | 0 | 0 | 3 |
| **C** | 1 | 0 | 75043 | 75202 | 75487 | 0 | 0 | 0 | 76828 | 7 | 0 |
| **G** | 75283 | 73950 | 0 | 1 | 0 | 3 | 77175 | 7 | 0 | 2 | 77322 |
| **T** | 1 | 2 | 4 | 6 | 4 | 2 | 3 | 7 | 4 | 77376 | 0 |
| **N** | 3109 | 4434 | 3349 | 3181 | 2906 | 3696 | 1217 | 1619 | 1567 | 1014 | 1074 |
| | 75290 | 73965 | 75050 | 75218 | 75493 | 74703 | 77182 | 76780 | 76832 | 77385 | 77325 |
| **A** | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.99% | 99.98% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 100.00 % |
| **T** | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.00% |
| **pNMG -177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)/ pNMG261** | | | | | | | | | | | |
| **A** | 6 | 14 | 0 | 4 | 8 | 69862 | 7 | 71850 | 3 | 1 | 7 |
| **C** | 1 | 4 | 70096 | 70375 | 70470 | 1 | 2 | 0 | 71817 | 8 | 0 |
| **G** | 70329 | 69027 | 0 | 1 | 2 | 10 | 72117 | 6 | 0 | 0 | 72228 |
| **T** | 1 | 3 | 8 | 30 | 34 | 6 | 3 | 3 | 8 | 72286 | 2 |
| **N** | 2947 | 4236 | 3180 | 2874 | 2770 | 3405 | 1155 | 1425 | 1456 | 989 | 1047 |
| | 70337 | 69048 | 70104 | 70410 | 70514 | 69879 | 72129 | 71859 | 71828 | 72295 | 72237 |

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# FIGURE 41 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | G | C | A | C | G | T | G | A | T | G | G |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 99.95% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.02% | 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% |
| 74765 | 4 | 6 | 76522 | 3 | 4 | 2 | 6 | 74259 | 1 | 11 | 14 |
| 0 | 0 | 75866 | 0 | 75677 | 0 | 7 | 0 | 2 | 5 | 1 | 2 |
| 7 | 76562 | 2 | 4 | 0 | 74857 | 0 | 75912 | 5 | 1 | 76201 | 76673 |
| 2 | 2 | 9 | 5 | 8 | 2 | 75596 | 4 | 10 | 76105 | 3 | 1 |
| 3625 | 1831 | 2516 | 1868 | 2711 | 3536 | 2794 | 2477 | 4123 | 2287 | 2183 | 1709 |
| 74774 | 76568 | 75883 | 76531 | 75688 | 74863 | 75605 | 75922 | 74276 | 76112 | 76216 | 76690 |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.98% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.00% |
| 69804 | 6 | 3 | 71458 | 4 | 13 | 1 | 8 | 69428 | 0 | 9 | 26 |
| 0 | 2 | 70929 | 0 | 70647 | 1 | 7 | 0 | 3 | 8 | 1 | 8 |
| 10 | 71549 | 1 | 1 | 1 | 69951 | 1 | 70996 | 9 | 1 | 71178 | 71606 |
| 1 | 2 | 5 | 5 | 14 | 3 | 70634 | 3 | 3 | 71176 | 3 | 5 |
| 3469 | 1725 | 2346 | 1820 | 2618 | 3316 | 2641 | 2277 | 3841 | 2099 | 2093 | 1639 |
| 69815 | 71559 | 70938 | 71464 | 70666 | 69968 | 70643 | 71007 | 69443 | 71185 | 71191 | 71645 |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.01% | 99.99% | 99.95% | 99.94% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| **G** | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.00% | 99.99% |
| **T** | 0.00% | 0.00% | 0.01% | 0.04% | 0.05% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| **pNMG -180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)/ pNMG261** | | | | | | | | | | | |
| **A** | 3 | 15 | 2 | 11 | 1 | 69193 | 6 | 71347 | 1 | 3 | 5 |
| **C** | 2 | 0 | 69534 | 69752 | 69976 | 0 | 1 | 0 | 71415 | 9 | 2 |
| **G** | 69825 | 68485 | 0 | 0 | 0 | 7 | 71730 | 5 | 2 | 1 | 71887 |
| **T** | 1 | 2 | 12 | 8 | 12 | 3 | 5 | 8 | 8 | 71947 | 1 |
| **N** | 3143 | 4472 | 3426 | 3203 | 2985 | 3771 | 1232 | 1614 | 1548 | 1014 | 1079 |
| | 69831 | 68502 | 69548 | 69771 | 69989 | 69203 | 71742 | 71360 | 71426 | 71960 | 71895 |
| **A** | 0.00% | 0.02% | 0.00% | 0.02% | 0.00% | 99.99% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.97% | 99.98% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 0.00% | 99.99% |
| **T** | 0.00% | 0.00% | 0.02% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% |
| **untreated cells** | | | | | | | | | | | |
| **A** | 0 | 3 | 0 | 0 | 0 | 10489 | 1 | 10765 | 0 | 0 | 0 |
| **C** | 0 | 0 | 10544 | 10550 | 10568 | 1 | 0 | 0 | 10761 | 1 | 0 |
| **G** | 10562 | 10385 | 0 | 0 | 2 | 0 | 10798 | 1 | 0 | 0 | 10844 |
| **T** | 0 | 0 | 1 | 1 | 3 | 1 | 1 | 1 | 3 | 10848 | 0 |
| **N** | 427 | 601 | 444 | 438 | 416 | 498 | 189 | 222 | 225 | 140 | 145 |
| | 10562 | 10388 | 10545 | 10551 | 10573 | 10491 | 10800 | 10767 | 10764 | 10849 | 10844 |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 99.98% | 0.01% | 0.00% | 99.99% | 0.01% | 0.02% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.04% |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% |
| 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.01% | 0.00% | 99.98% | 99.95% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.02% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% |
| 69292 | 7 | 1 | 71050 | 3 | 12 | 2 | 5 | 68871 | 2 | 13 | 17 |
| 0 | 2 | 70445 | 1 | 70256 | 0 | 9 | 1 | 2 | 6 | 2 | 3 |
| 8 | 71069 | 3 | 5 | 1 | 69426 | 0 | 70522 | 3 | 0 | 70728 | 71179 |
| 0 | 2 | 10 | 3 | 10 | 1 | 70157 | 2 | 5 | 70703 | 5 | 5 |
| 3674 | 1894 | 2515 | 1915 | 2704 | 3535 | 2806 | 2444 | 4093 | 2263 | 2226 | 1770 |
| 69300 | 71080 | 7045 | 71059 | 70270 | 69439 | 70168 | 70530 | 68881 | 70711 | 70748 | 71204 |
| 99.99% | 0.01% | 0.00% | 99.99% | 0.00% | 0.02% | 0.00% | 0.01% | 99.99% | 0.00% | 0.02% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 99.97% | 99.96% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% |
| 10460 | 0 | 0 | 10691 | 0 | 2 | 0 | 2 | 10399 | 0 | 2 | 4 |
| 1 | 0 | 10640 | 1 | 10593 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 10727 | 0 | 0 | 0 | 10471 | 0 | 10652 | 2 | 0 | 10666 | 10733 |
| 0 | 0 | 1 | 3 | 3 | 0 | 10583 | 0 | 3 | 10675 | 0 | 0 |
| 528 | 262 | 348 | 294 | 393 | 516 | 406 | 335 | 585 | 314 | 321 | 252 |
| 10461 | 10727 | 10641 | 10695 | 10596 | 10473 | 10583 | 10654 | 10404 | 10675 | 10668 | 10737 |

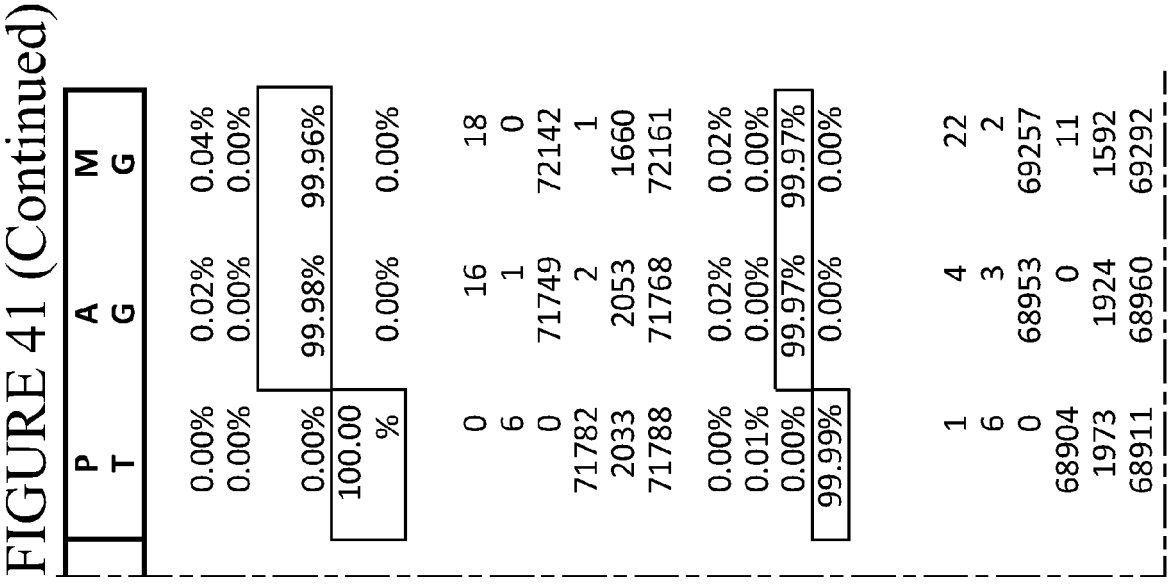
# FIGURE 41 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 99.99% | 99.99% | 99.95% | 0.01% | 0.00% | 0.00% | 99.97% | 0.01% |
| G | 100.00 % | 99.97% | 0.00% | 0.00% | 0.02% | 0.00% | 99.98% | 0.01% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.03% | 0.01% | 0.01% | 0.01% | 0.03% | 99.99% |
| **pNMG274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild type)** | | | | | | | | | | |
| A | 5 | 13 | 5 | 7 | 3 | 70497 | 5 | 72333 | 5 | 0 |
| C | 2 | 2 | 70691 | 71061 | 71156 | 0 | 0 | 0 | 72388 | 14 |
| G | 70966 | 69775 | 3 | 1 | 1 | 14 | 72653 | 6 | 1 | 0 |
| T | 1 | 1 | 8 | 5 | 6 | 1 | 2 | 3 | 8 | 72823 |
| N | 2848 | 4031 | 3115 | 2748 | 2656 | 3309 | 1161 | 1479 | 1419 | 984 |
| | 70974 | 69791 | 70707 | 71074 | 71166 | 70512 | 72660 | 72342 | 72402 | 72837 |
| A | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 99.98% | 0.01% | 99.99% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 99.98% | 99.98% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% |
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.02% | 99.99% | 0.01% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% |
| **pNMG274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild type)/ pNMG177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | |
| A | 8 | 15 | 3 | 3 | 6 | 67071 | 1 | 67771 | 0 | 1 |
| C | 0 | 4 | 67993 | 68295 | 68391 | 1 | 1 | 0 | 69480 | 8 |
| G | 68224 | 67174 | 1 | 8 | 2 | 616 | 69803 | 1637 | 3 | 0 |
| T | 1 | 0 | 7 | 12 | 12 | 3 | 0 | 11 | 1 | 69878 |
| N | 2651 | 3691 | 2880 | 2566 | 2473 | 3193 | 1079 | 1465 | 1400 | 997 |
| | 68233 | 67193 | 68004 | 68318 | 68411 | 67691 | 69805 | 69419 | 69484 | 69887 |

FIGURE 41 (Continued)

| 11 G | 12 A | 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A |
|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 99.99% | 0.00% | 0.00% | 99.96% | 0.00% | 0.02% | 0.00% | 0.02% | 99.95% |
| 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 99.97% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.02% |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.03% | 0.03% | 0.00% | 100.00 % | 0.00% | 0.03% |
| 11 | 70574 | 5 | 4 | 72002 | 3 | 4 | 2 | 4 | 69976 |
| 2 | 1 | 1 | 71445 | 2 | 71280 | 0 | 6 | 1 | 4 |
| 72715 | 12 | 71959 | 0 | 3 | 1 | 70604 | 0 | 71553 | 2 |
| 4 | 5 | 3 | 9 | 2 | 13 | 0 | 71195 | 1 | 6 |
| 1089 | 3229 | 1853 | 2363 | 1812 | 2524 | 3213 | 2618 | 2262 | 3833 |
| 72732 | 70592 | 71968 | 71458 | 72009 | 71297 | 70608 | 71203 | 71559 | 69988 |
| 0.02% | 99.97% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% |
| 99.98% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% |
| 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 99.99% | 0.00% | 0.01% |
| 11 | 67747 | 8 | 5 | 69063 | 6 | 6 | 1 | 0 | 67136 |
| 1 | 2 | 1 | 68593 | 2 | 68335 | 0 | 7 | 0 | 4 |
| 69778 | 10 | 69171 | 2 | 10 | 1 | 67679 | 0 | 68644 | 4 |
| 2 | 2 | 3 | 7 | 4 | 7 | 1 | 68327 | 2 | 7 |
| 1092 | 3123 | 1701 | 2277 | 1805 | 2535 | 3198 | 2549 | 2238 | 3733 |
| 69792 | 67761 | 69183 | 68607 | 69079 | 68349 | 67686 | 68335 | 68646 | 67151 |

FIGURE 41 (Continued)



| PT | AG | MG |
|---|---|---|
| 0.00% | 0.02% | 0.04% |
| 0.00% | 0.00% | 0.00% |
| 0.00% | 99.98% | 99.96% |
| 100.00% | 0.00% | 0.00% |
| 0 | 16 | 18 |
| 6 | 1 | 0 |
| 0 | 71749 | 72142 |
| 71782 | 2 | 1 |
| 2033 | 2053 | 1660 |
| 71788 | 71768 | 72161 |
| 0.00% | 0.02% | 0.02% |
| 0.01% | 0.00% | 0.00% |
| 0.00% | 99.97% | 99.97% |
| 99.99% | 0.00% | 0.00% |
| 1 | 4 | 22 |
| 6 | 3 | 2 |
| 0 | 68953 | 69257 |
| 68904 | 0 | 11 |
| 1973 | 1924 | 1592 |
| 68911 | 68960 | 69292 |

# FIGURE 41 (Continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 99.08% | 0.00% | 97.63% | 0.00% | 0.00% |
| C | 0.00% | 0.01% | 99.98% | 99.97% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| G | 99.99% | 99.97% | 0.00% | 0.01% | 0.00% | 0.91% | 100.00% | 2.36% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.02% | 0.02% | 0.00% | 0.00% | 0.02% | 0.00% | 99.99% |

**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 14 | 2 | 5 | 6 | 69004 | 5 | 70792 | 1 | 3 |
| C | 0 | 2 | 69299 | 69546 | 69715 | 0 | 0 | 0 | 70882 | 8 |
| G | 69617 | 68418 | 1 | 1 | 0 | 19 | 71238 | 11 | 0 | 1 |
| T | 2 | 2 | 9 | 12 | 7 | 5 | 2 | 10 | 3 | 71301 |
| N | 2645 | 3834 | 2959 | 2706 | 2542 | 3242 | 1025 | 1457 | 1384 | 957 |
| | 69625 | 68436 | 69311 | 69564 | 69728 | 69028 | 71245 | 70813 | 70886 | 71313 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.97% | 0.01% | 99.97% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 99.98% | 99.97% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| G | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 0.03% | 99.99% | 0.02% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.98% |

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 10 | 10 | 1 | 3 | 4 | 70781 | 2 | 72733 | 5 | 0 |
| C | 1 | 2 | 71094 | 71530 | 71592 | 2 | 0 | 1 | 72749 | 5 |
| G | 71488 | 70271 | 0 | 0 | 0 | 6 | 73077 | 7 | 0 | 1 |

# FIGURE 41 (Continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.02% | 99.98% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 99.98% |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% |
| 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 100.00 % | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% |
| | | | | | | | | | |
| 13 | 69154 | 7 | 4 | 70667 | 0 | 4 | 8 | 16 | 68572 |
| 1 | 0 | 0 | 70022 | 0 | 69789 | 1 | 9 | 0 | 1 |
| 71156 | 14 | 70527 | 0 | 4 | 0 | 69081 | 8 | 69972 | 6 |
| 3 | 2 | 1 | 7 | 2 | 22 | 2 | 69618 | 1 | 8 |
| 1097 | 3100 | 1735 | 2237 | 1597 | 2459 | 3183 | 2628 | 2282 | 3684 |
| 71173 | 69170 | 70535 | 70033 | 70673 | 69811 | 69088 | 69643 | 69989 | 68587 |
| | | | | | | | | | |
| 0.02% | 99.98% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 0.01% | 0.02% | 99.98% |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% |
| 99.98% | 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 99.98% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.03% | 0.00% | 99.96% | 0.00% | 0.01% |
| | | | | | | | | | |
| 11 | 71006 | 4 | 2 | 72410 | 1 | 12 | 2 | 3 | 70391 |
| 0 | 0 | 2 | 71853 | 0 | 71554 | 0 | 7 | 1 | 1 |
| 73130 | 11 | 72378 | 1 | 4 | 0 | 70902 | 1 | 71936 | 6 |

# FIGURE 41 (Continued)

| | | | |
|---|---|---|---|
| 99.98% | 0.00% | 0.01% | 0.03% |
| 0.01% | 0.01% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.99% | 99.95% |
| 0.01% | 99.99% | 0.00% | 0.02% |

| | | | |
|---|---|---|---|
| 68572 | 10 | 11 | 26 |
| 1 | 8 | 1 | 1 |
| 6 | 0 | 70287 | 70621 |
| 8 | 70239 | 1 | 9 |
| 3684 | 2014 | 1967 | 1610 |
| 68587 | 70257 | 70300 | 70657 |

| | | | |
|---|---|---|---|
| 99.98% | 0.01% | 0.02% | 0.04% |
| 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 0.00% | 99.98% | 99.95% |
| 0.01% | 99.97% | 0.00% | 0.01% |

| | | | |
|---|---|---|---|
| 70391 | 5 | 5 | 10 |
| 1 | 3 | 1 | 0 |
| 6 | 0 | 72214 | 72599 |

FIGURE 41 (Continued)

| | 1 G | 2 G | 3 C | 4 C | 5 C | 6 A | 7 G | 8 A | 9 C | 10 T | 11 G | 12 A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T | 3 | 0 | 5 | 2 | 9 | 5 | 7 | 2 | 4 | 73259 | 5 | 1 |
| N | 2750 | 3969 | 3152 | 2717 | 2647 | 3458 | 1166 | 1509 | 1494 | 987 | 1106 | 3234 |
| | 71502 | 70283 | 71100 | 71535 | 71605 | 70794 | 73086 | 72743 | 72758 | 73265 | 73146 | 71018 |
| A | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 0.02% | 99.98% |
| C | 0.00% | 0.00% | 99.99% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% |
| G | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 99.98% | 0.02% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% |

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 10 | 4 | 3 | 3 | 69616 | 1 | 71039 | 3 | 0 | 6 | 70406 |
| C | 1 | 1 | 70421 | 70940 | 70990 | 1 | 0 | 0 | 72127 | 9 | 0 | 0 |
| G | 70969 | 69802 | 1 | 0 | 11 | 578 | 72478 | 1056 | 0 | 1 | 72474 | 14 |
| T | 2 | 4 | 8 | 8 | 19 | 4 | 2 | 6 | 5 | 72676 | 4 | 0 |
| N | 2695 | 3856 | 3239 | 2722 | 2650 | 3474 | 1192 | 1572 | 1538 | 987 | 1189 | 3253 |
| | 70978 | 69817 | 70434 | 70951 | 71023 | 70199 | 72481 | 72101 | 72135 | 72686 | 72484 | 70420 |
| A | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 99.17% | 0.00% | 98.53% | 0.00% | 0.00% | 0.01% | 99.98% |
| C | 0.00% | 0.00% | 99.98% | 99.98% | 99.95% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% |
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.02% | 0.82% | 100.00% | 1.46% | 0.00% | 0.00% | 99.99% | 0.02% |
| T | 0.00% | 0.01% | 0.01% | 0.01% | 0.03% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% |

# FIGURE 41 (Continued)

| 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 5 | 6 | 9 | 3 | 71592 | 2 | 5 | 72119 | 0 | 3 |
| 1866 | 2391 | 1832 | 2688 | 3335 | 2650 | 2310 | 3849 | 2125 | 2032 | 1640 |
| 72386 | 71861 | 72420 | 71564 | 70917 | 71602 | 71942 | 70403 | 72127 | 72220 | 72612 |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.02% | 0.00% | 0.00% | 99.98% | 0.01% | 0.01% | 0.01% |
| 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.99% | 99.98% |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| 6 | 5 | 71899 | 2 | 8 | 1 | 4 | 69813 | 2 | 4 | 18 |
| 0 | 71286 | 0 | 71141 | 0 | 2 | 0 | 5 | 7 | 1 | 1 |
| 71779 | 0 | 9 | 0 | 70387 | 1 | 71399 | 2 | 1 | 71644 | 72068 |
| 1 | 16 | 3 | 11 | 2 | 71020 | 1 | 6 | 71620 | 5 | 4 |
| 1887 | 2366 | 1762 | 2519 | 3276 | 2649 | 2269 | 3847 | 2043 | 2019 | 1582 |
| 71786 | 71307 | 71911 | 71154 | 70397 | 71024 | 71404 | 69826 | 71630 | 71654 | 72091 |
| 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 99.97% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 99.99% | 99.97% |
| 0.00% | 0.02% | 0.00% | 0.02% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% |

SUBSTITUTE SHEET (RULE 26)

# FIGURE 41 (Continued)

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 7 | 8 | 2 | 6 | 1 | 61968 | 3 | 63886 | 1 | 0 | 6 | 62271 |
| **C** | 0 | 0 | 62366 | 62681 | 62840 | 0 | 1 | 1 | 63960 | 8 | 1 | 0 |
| **G** | 62634 | 61605 | 1 | 0 | 10 | 136 | 64246 | 24 | 0 | 2 | 64237 | 7 |
| **T** | 4 | 2 | 3 | 3 | 10 | 7 | 1 | 6 | 4 | 64397 | 1 | 3 |
| **N** | 2632 | 3662 | 2905 | 2587 | 2416 | 3166 | 1026 | 1360 | 1312 | 870 | 1032 | 2996 |
| | 62645 | 61615 | 62372 | 62690 | 62861 | 62111 | 64251 | 63917 | 63965 | 64407 | 64245 | 62281 |
| | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.77% | 0.00% | 99.95% | 0.00% | 0.00% | 0.01% | 99.98% |
| **C** | 0.00% | 0.00% | 99.99% | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% |
| **G** | 99.98% | 99.98% | 0.00% | 0.00% | 0.02% | 0.22% | 99.99% | 0.04% | 0.00% | 0.00% | 99.99% | 0.01% |
| **T** | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% | 0.00% |

**pNMG-276 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (wild-type) + (A106V_D108N_D147Y_E155V))**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 4 | 10 | 4 | 11 | 5 | 70844 | 2 | 72845 | 6 | 1 | 12 | 71021 |
| **C** | 0 | 2 | 71121 | 71502 | 71755 | 0 | 1 | 0 | 72960 | 8 | 0 | 3 |
| **G** | 71521 | 70222 | 1 | 0 | 2 | 15 | 73378 | 11 | 1 | 1 | 73337 | 14 |
| **T** | 6 | 3 | 6 | 3 | 10 | 4 | 2 | 1 | 4 | 73548 | 2 | 4 |
| **N** | 3040 | 4333 | 3438 | 3054 | 2798 | 3707 | 1187 | 1713 | 1599 | 1012 | 1219 | 3528 |
| | 71531 | 70237 | 71132 | 71516 | 71772 | 70863 | 73383 | 72857 | 72971 | 73558 | 73351 | 71042 |
| **A** | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 99.97% | 0.00% | 99.98% | 0.01% | 0.00% | 0.02% | 99.97% |
| **C** | 0.00% | 0.00% | 99.98% | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% | 0.00% |
| **G** | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.02% | 99.99% | 0.02% | 0.00% | 0.00% | 99.98% | 0.02% |
| **T** | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |

**pNMG-277 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (A106V_D108N_D147Y_E155V) + (A106V_D108N_D147Y_E155V))**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 6 | 8 | 2 | 7 | 6 | 71604 | 6 | 72728 | 2 | 3 | 4 | 72290 |

# FIGURE 41 (Continued)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 1 | 63616 | 2 | 9 | 3 | 4 | 61844 | 1 | 9 | 9 |
| 2 | 63174 | 1 | 62975 | 0 | 7 | 0 | 6 | 6 | 0 | 3 |
| 63590 | 1 | 4 | 1 | 62341 | 0 | 63212 | 5 | 0 | 63400 | 63778 |
| 0 | 7 | 6 | 4 | 3 | 62967 | 1 | 9 | 63413 | 3 | 2 |
| 1682 | 2094 | 1650 | 2295 | 2924 | 2300 | 2060 | 3413 | 1857 | 1865 | 1485 |
| 63595 | 63183 | 63627 | 62982 | 62353 | 62977 | 63217 | 61864 | 63420 | 63412 | 63792 |
| | | | | | | | | | | |
| 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.01% | 0.01% |
| 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.98% |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| | | | | | | | | | | |
| 6 | 2 | 72668 | 3 | 10 | 7 | 6 | 70411 | 1 | 7 | 17 |
| 1 | 72043 | 3 | 71882 | 3 | 8 | 0 | 5 | 7 | 3 | 2 |
| 72567 | 2 | 3 | 2 | 71050 | 0 | 72003 | 2 | 0 | 72322 | 72835 |
| 2 | 7 | 4 | 15 | 3 | 71763 | 5 | 7 | 72341 | 2 | 0 |
| 1994 | 2516 | 1892 | 2668 | 3504 | 2787 | 2551 | 4140 | 2216 | 2231 | 1711 |
| 72576 | 72054 | 72678 | 71902 | 71066 | 71778 | 72014 | 70425 | 72349 | 72334 | 72854 |
| | | | | | | | | | | |
| 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 99.98% | 0.00% | 99.97% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.02% | 0.00% | 99.98% | 0.01% | 0.01% | 99.99% | 0.00% | 0.00% |
| | | | | | | | | | | |
| 6 | 4 | 74020 | 2 | 16 | 4 | 3 | 71872 | 5 | 8 | 13 |

# FIGURE 41 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 0 | 3 | 72572 | 72893 | 73123 | 0 | 0 | 0 | 74295 | 9 | 1 |
| G | 72951 | 71823 | 2 | 1 | 4 | 693 | 74623 | 1457 | 0 | 1 | 74640 |
| T | 4 | 3 | 13 | 15 | 13 | 4 | 8 | 3 | 4 | 74817 | 1 |
| N | 2869 | 3993 | 3241 | 2914 | 2684 | 3529 | 1193 | 1642 | 1529 | 1000 | 1184 |
| | 72961 | 71837 | 72589 | 72916 | 73146 | 72301 | 74637 | 74188 | 74301 | 74830 | 74646 |
| A | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.04% | 0.01% | 98.03% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.97% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.01% | 0.96% | 99.98% | 1.96% | 0.00% | 0.00% | 99.99% |
| T | 0.01% | 0.00% | 0.02% | 0.02% | 0.02% | 0.01% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% |
| **pNMG-278 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108Q_D147Y_E155V)** | | | | | | | | | | | |
| A | 5 | 7 | 0 | 4 | 4 | 61380 | 3 | 62954 | 1 | 1 | 4 |
| C | 1 | 1 | 61815 | 61916 | 62105 | 0 | 3 | 1 | 63236 | 7 | 0 |
| G | 61960 | 60941 | 0 | 0 | 1 | 35 | 63421 | 110 | 0 | 0 | 63533 |
| T | 3 | 4 | 10 | 2 | 5 | 5 | 2 | 3 | 3 | 63591 | 2 |
| N | 2527 | 3543 | 2671 | 2574 | 2381 | 3076 | 1067 | 1428 | 1256 | 897 | 957 |
| | 61969 | 60953 | 61825 | 61922 | 62115 | 61420 | 63429 | 63068 | 63240 | 63599 | 63539 |
| A | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.93% | 0.00% | 99.82% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.06% | 99.99% | 0.17% | 0.00% | 0.00% | 99.99% |
| T | 0.00% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |

FIGURE 41 (Continued)

| 12 A | 13 G | 14 C | 15 A | 16 C | 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 73419 | 0 | 73217 | 0 | 6 | 1 | 3 | 3 | 2 | 3 |
| 11 | 73866 | 0 | 6 | 0 | 72446 | 1 | 73419 | 13 | 0 | 73714 | 74118 |
| 2 | 1 | 8 | 1 | 12 | 3 | 73157 | 3 | 5 | 73700 | 2 | 6 |
| 3526 | 1955 | 2399 | 1803 | 2599 | 3365 | 2662 | 2404 | 3937 | 2122 | 2104 | 1690 |
| 72304 | 73875 | 73431 | 74027 | 73231 | 72465 | 73168 | 73426 | 71893 | 73708 | 73726 | 74140 |
| 99.98% | 0.01% | 0.01% | 99.99% | 0.00% | 0.02% | 0.01% | 0.00% | 99.97% | 0.01% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.97% | 0.00% | 99.99% | 0.02% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |
| 61687 | 3 | 2 | 63015 | 3 | 13 | 4 | 5 | 61051 | 1 | 9 | 16 |
| 0 | 1 | 62589 | 0 | 62260 | 1 | 12 | 0 | 2 | 7 | 0 | 0 |
| 8 | 62904 | 1 | 5 | 0 | 61618 | 1 | 62427 | 4 | 0 | 62669 | 62943 |
| 0 | 4 | 2 | 6 | 10 | 3 | 62118 | 3 | 6 | 62651 | 2 | 0 |
| 2801 | 1584 | 1902 | 1470 | 2222 | 2859 | 2359 | 2059 | 3431 | 1835 | 1815 | 1536 |
| 61695 | 62912 | 62594 | 63026 | 62273 | 61635 | 62135 | 62435 | 61063 | 62659 | 62680 | 62959 |
| 99.99% | 0.00% | 0.00% | 99.98% | 0.00% | 0.02% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.97% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.00% | 0.01% | 0.02% | 0.00% | 99.97% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pNMG-279 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108M_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 5 | 4 | 0 | 3 | 2 | 60658 | 2 | 62063 | 5 | 2 | 8 |
| **C** | 1 | 0 | 61251 | 61335 | 61542 | 0 | 0 | 0 | 62558 | 9 | 3 |
| **G** | 61427 | 60570 | 1 | 0 | 1 | 224 | 62773 | 326 | 1 | 1 | 62928 |
| **T** | 7 | 3 | 9 | 22 | 15 | 2 | 4 | 5 | 0 | 62987 | 3 |
| **N** | 2376 | 3239 | 2555 | 2456 | 2256 | 2932 | 1037 | 1422 | 1252 | 817 | 874 |
| | 61440 | 60577 | 61261 | 61360 | 61560 | 60884 | 62779 | 62394 | 62564 | 62999 | 62942 |
| **A** | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.63% | 0.00% | 99.47% | 0.01% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 99.98% | 99.96% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.98% | 99.99% | 0.00% | 0.00% | 0.00% | 0.37% | 99.99% | 0.52% | 0.00% | 0.00% | 99.98% |
| **T** | 0.01% | 0.00% | 0.01% | 0.04% | 0.02% | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% |
| **pNMG-280 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108L_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 4 | 12 | 2 | 8 | 4 | 66577 | 1 | 68312 | 2 | 0 | 11 |
| **C** | 0 | 3 | 67190 | 67280 | 67442 | 3 | 0 | 0 | 68646 | 4 | 2 |
| **G** | 67295 | 66223 | 0 | 1 | 0 | 30 | 68876 | 76 | 0 | 4 | 69047 |
| **T** | 4 | 2 | 3 | 19 | 7 | 8 | 2 | 4 | 6 | 69041 | 2 |
| **N** | 2695 | 3758 | 2803 | 2690 | 2545 | 3380 | 1119 | 1606 | 1344 | 949 | 936 |
| | 67303 | 66240 | 67195 | 67308 | 67453 | 66618 | 68879 | 68392 | 68654 | 69049 | 69062 |
| **A** | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.94% | 0.00% | 99.88% | 0.00% | 0.00% | 0.02% |
| **C** | 0.00% | 0.00% | 99.99% | 99.96% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| **G** | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 0.05% | 100.00% | 0.11% | 0.00% | 0.01% | 99.98% |
| **T** | 0.01% | 0.00% | 0.00% | 0.03% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.00% |
| **pNMG-281 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108K_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 9 | 16 | 1 | 7 | 5 | 78632 | 5 | 80522 | 13 | 0 | 9 |
| **C** | 1 | 1 | 79295 | 79336 | 79560 | 3 | 3 | 0 | 80987 | 7 | 3 |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 61070 | 8 | 3 | 62343 | 3 | 8 | 2 | 5 | 60572 | 1 | 5 | 11 |
| 0 | 0 | 61950 | 0 | 61653 | 1 | 8 | 0 | 3 | 4 | 1 | 2 |
| 10 | 62256 | 1 | 14 | 0 | 60971 | 0 | 61871 | 5 | 0 | 62078 | 62369 |
| 0 | 5 | 16 | 2 | 8 | 4 | 61580 | 5 | 6 | 62074 | 4 | 3 |
| 2736 | 1547 | 1846 | 1457 | 2152 | 2832 | 2226 | 1935 | 3230 | 1737 | 1727 | 1430 |
| 61080 | 62269 | 61970 | 62359 | 61664 | 60984 | 61590 | 61881 | 60586 | 62079 | 62088 | 62385 |
| 99.98% | 0.01% | 0.00% | 99.97% | 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.97% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.98% | 0.00% | 0.02% | 0.00% | 99.98% | 0.00% | 99.98% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.01% | 0.03% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% |
| 66924 | 7 | 2 | 68476 | 0 | 5 | 4 | 2 | 66386 | 3 | 13 | 24 |
| 0 | 0 | 67959 | 1 | 67692 | 1 | 3 | 0 | 0 | 6 | 3 | 1 |
| 13 | 68356 | 1 | 5 | 0 | 66930 | 0 | 67824 | 6 | 1 | 68091 | 68422 |
| 0 | 3 | 5 | 2 | 8 | 2 | 67533 | 2 | 4 | 68056 | 3 | 3 |
| 3061 | 1632 | 2031 | 1514 | 2298 | 3060 | 2457 | 2169 | 3601 | 1931 | 1887 | 1547 |
| 66937 | 68366 | 67967 | 68484 | 67700 | 66938 | 67540 | 67828 | 66396 | 68066 | 68110 | 68450 |
| 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.02% | 0.04% |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.97% | 99.96% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| 79028 | 13 | 3 | 80776 | 3 | 17 | 5 | 6 | 78396 | 4 | 10 | 23 |
| 0 | 2 | 80202 | 0 | 79842 | 4 | 8 | 6 | 3 | 11 | 0 | 3 |

# FIGURE 41 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>C | 5<br>C | 6<br>A | 7<br>G | 8<br>A | 9<br>C | 10<br>T | 11<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 79453 | 78187 | 2 | 1 | 5 | 42 | 81260 | 212 | 0 | 2 | 81540 |
| T | 4 | 1 | 4 | 3 | 20 | 3 | 1 | 5 | 4 | 81539 | 3 |
| N | 3230 | 4492 | 3394 | 3349 | 3107 | 4017 | 1428 | 1958 | 1693 | 1149 | 1142 |
| | 79467 | 78205 | 79302 | 79347 | 79590 | 78680 | 81269 | 80739 | 81004 | 81548 | 81555 |
| | | | | | | | | | | | |
| A | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.94% | 0.01% | 99.73% | 0.02% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.99% | 99.99% | 99.96% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.00% |
| G | 99.98% | 99.98% | 0.00% | 0.00% | 0.01% | 0.05% | 99.99% | 0.26% | 0.00% | 0.00% | 99.98% |
| T | 0.01% | 0.00% | 0.01% | 0.00% | 0.03% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |
| | | | | | | | | | | | |
| **pNMG-282 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108I_D147Y_E155V)** | | | | | | | | | | | |
| A | 7 | 14 | 0 | 6 | 11 | 74167 | 2 | 76161 | 3 | 1 | 11 |
| C | 0 | 2 | 74686 | 74764 | 75153 | 0 | 1 | 1 | 76527 | 8 | 1 |
| G | 74889 | 73687 | 1 | 0 | 1 | 17 | 76748 | 19 | 1 | 1 | 76917 |
| T | 6 | 4 | 10 | 8 | 12 | 4 | 3 | 5 | 2 | 76977 | 3 |
| N | 3132 | 4327 | 3337 | 3256 | 2857 | 3846 | 1280 | 1848 | 1501 | 1047 | 1102 |
| | 74902 | 73707 | 74697 | 74778 | 75177 | 74188 | 76754 | 76186 | 76533 | 76987 | 76932 |
| | | | | | | | | | | | |
| A | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.97% | 0.00% | 99.97% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.99% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% |
| G | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.02% | 99.99% | 0.02% | 0.00% | 0.00% | 99.98% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 41 (Continued)

| 12<br>A | 13<br>G | 14<br>C | 15<br>A | 16<br>C | 17<br>G | 18<br>T | 19<br>G | 20<br>A | P<br>T | A<br>G | M<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 10 | 80700 | 1 | 9 | 4 | 78978 | 3 | 80141 | 8 | 0 | 80432 | 80782 |
| 1 | 4 | 13 | 2 | 8 | 5 | 79784 | 4 | 10 | 80266 | 3 | 2 |
| 3658 | 1978 | 2478 | 1910 | 2840 | 3693 | 2893 | 2536 | 4276 | 2412 | 2248 | 1883 |
| 79039 | 80719 | 80219 | 80787 | 79857 | 79004 | 79800 | 80157 | 78417 | 80281 | 80445 | 80810 |
| 99.99% | 0.02% | 0.00% | 99.99% | 0.00% | 0.02% | 0.01% | 0.01% | 99.97% | 0.00% | 0.01% | 0.03% |
| 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.98% | 0.00% | 0.01% | 0.01% | 99.97% | 0.00% | 99.98% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% |
| 74586 | 4 | 2 | 76260 | 2 | 13 | 10 | 4 | 73905 | 0 | 10 | 14 |
| 0 | 1 | 75824 | 5 | 75475 | 1 | 8 | 0 | 3 | 4 | 2 | 2 |
| 10 | 76178 | 3 | 6 | 0 | 74583 | 0 | 75605 | 8 | 0 | 75899 | 76322 |
| 2 | 3 | 5 | 3 | 8 | 4 | 75224 | 3 | 7 | 75766 | 2 | 4 |
| 3436 | 1848 | 2200 | 1760 | 2549 | 3433 | 2788 | 2418 | 4107 | 2262 | 2119 | 1690 |
| 74598 | 76186 | 75834 | 76274 | 75485 | 74601 | 75242 | 75612 | 73923 | 75770 | 75913 | 76342 |
| 99.98% | 0.01% | 0.00% | 99.98% | 0.00% | 0.02% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 41 (Continued)

| pNMG-283 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108F_D147Y_E155V) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 6 | 10 | 0 | 2 | 1 | 59948 | 4 | 61704 | 1 | 1 | 5 |
| C | 0 | 1 | 60435 | 60535 | 60855 | 1 | 0 | 0 | 61999 | 3 | 1 |
| G | 60732 | 59603 | 5 | 0 | 3 | 35 | 62189 | 48 | 0 | 0 | 62341 |
| T | 4 | 1 | 5 | 7 | 9 | 4 | 3 | 2 | 4 | 62394 | 9 |
| N | 2495 | 3622 | 2792 | 2693 | 2369 | 3249 | 1041 | 1483 | 1233 | 839 | 881 |
| | 60742 | 59615 | 60445 | 60544 | 60868 | 59988 | 62196 | 61754 | 62004 | 62398 | 62356 |
| A | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 99.93% | 0.01% | 99.92% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.98% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% |
| G | 99.98% | 99.98% | 0.01% | 0.00% | 0.00% | 0.06% | 99.99% | 0.08% | 0.00% | 0.00% | 99.98% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% |
| **untreated cells** | | | | | | | | | | | |
| A | 5 | 13 | 2 | 4 | 8 | 86208 | 4 | 88810 | 2 | 2 | 5 |
| C | 1 | 3 | 86833 | 87055 | 87490 | 0 | 0 | 0 | 89259 | 9 | 1 |
| G | 87259 | 85749 | 2 | 1 | 1 | 8 | 89495 | 9 | 0 | 1 | 89745 |
| T | 3 | 1 | 8 | 3 | 5 | 6 | 3 | 3 | 2 | 89914 | 4 |
| N | 3937 | 5439 | 4360 | 4142 | 3701 | 4983 | 1703 | 2383 | 1942 | 1279 | 1450 |
| | 87268 | 85766 | 86845 | 87063 | 87504 | 86222 | 89502 | 88822 | 89263 | 89926 | 89755 |
| A | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 99.98% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 99.99% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 100.00% | 0.01% | 0.00% |
| G | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 99.99% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **NTC** | | | | | | | | | | | |
| A | 0 | 0 | 0 | 0 | 0 | 973 | 0 | 1001 | 0 | 0 | 0 |
| C | 0 | 0 | 983 | 942 | 943 | 0 | 0 | 0 | 1003 | 0 | 0 |

# FIGURE 41 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 60342 | 2 | 1 | 61839 | 5 | 7 | 1 | 1 | 59944 | 2 | 16 | 23 |
| 0 | 1 | 61433 | 9 | 61179 | 10 | 8 | 0 | 1 | 3 | 1 | 0 |
| 9 | 61699 | 0 | 1 | 1 | 60454 | 1 | 61276 | 18 | 0 | 61489 | 61876 |
| 4 | 2 | 8 | 6 | 18 | 2 | 60983 | 4 | 6 | 61419 | 3 | 3 |
| 2882 | 1533 | 1795 | 1382 | 2034 | 2764 | 2252 | 1964 | 3276 | 1821 | 1736 | 1343 |
| 60355 | 61704 | 61442 | 61855 | 61203 | 60473 | 60993 | 61281 | 59969 | 61424 | 61509 | 61902 |
| 99.98% | 0.00% | 0.00% | 99.97% | 0.01% | 0.01% | 0.00% | 0.00% | 99.96% | 0.00% | 0.03% | 0.04% |
| 0.00% | 0.00% | 99.99% | 0.01% | 99.96% | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 99.99% | 0.03% | 0.00% | 99.97% | 99.96% |
| 0.01% | 0.00% | 0.01% | 0.01% | 0.03% | 0.00% | 99.98% | 0.01% | 0.01% | 99.99% | 0.00% | 0.00% |
| 86721 | 1 | 2 | 88972 | 2 | 8 | 2 | 5 | 86098 | 2 | 11 | 21 |
| 0 | 2 | 88336 | 3 | 88011 | 2 | 6 | 1 | 1 | 8 | 3 | 6 |
| 9 | 88706 | 0 | 12 | 0 | 86930 | 0 | 88137 | 7 | 1 | 88505 | 89022 |
| 3 | 2 | 7 | 4 | 11 | 2 | 87773 | 4 | 7 | 88436 | 2 | 3 |
| 4472 | 2494 | 2860 | 2214 | 3181 | 4263 | 3424 | 3058 | 5092 | 2758 | 2684 | 2153 |
| 86733 | 88711 | 88345 | 88991 | 88024 | 86942 | 87781 | 88147 | 86113 | 88447 | 88521 | 89052 |
| 99.99% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 9998% | 0.00% | 0.01% | 0.02% |
| 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% |
| 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 99.97% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| 975 | 0 | 0 | 1008 | 0 | 0 | 0 | 0 | 975 | 0 | 0 | 0 |
| 0 | 0 | 988 | 0 | 990 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

FIGURE 41 (Continued)

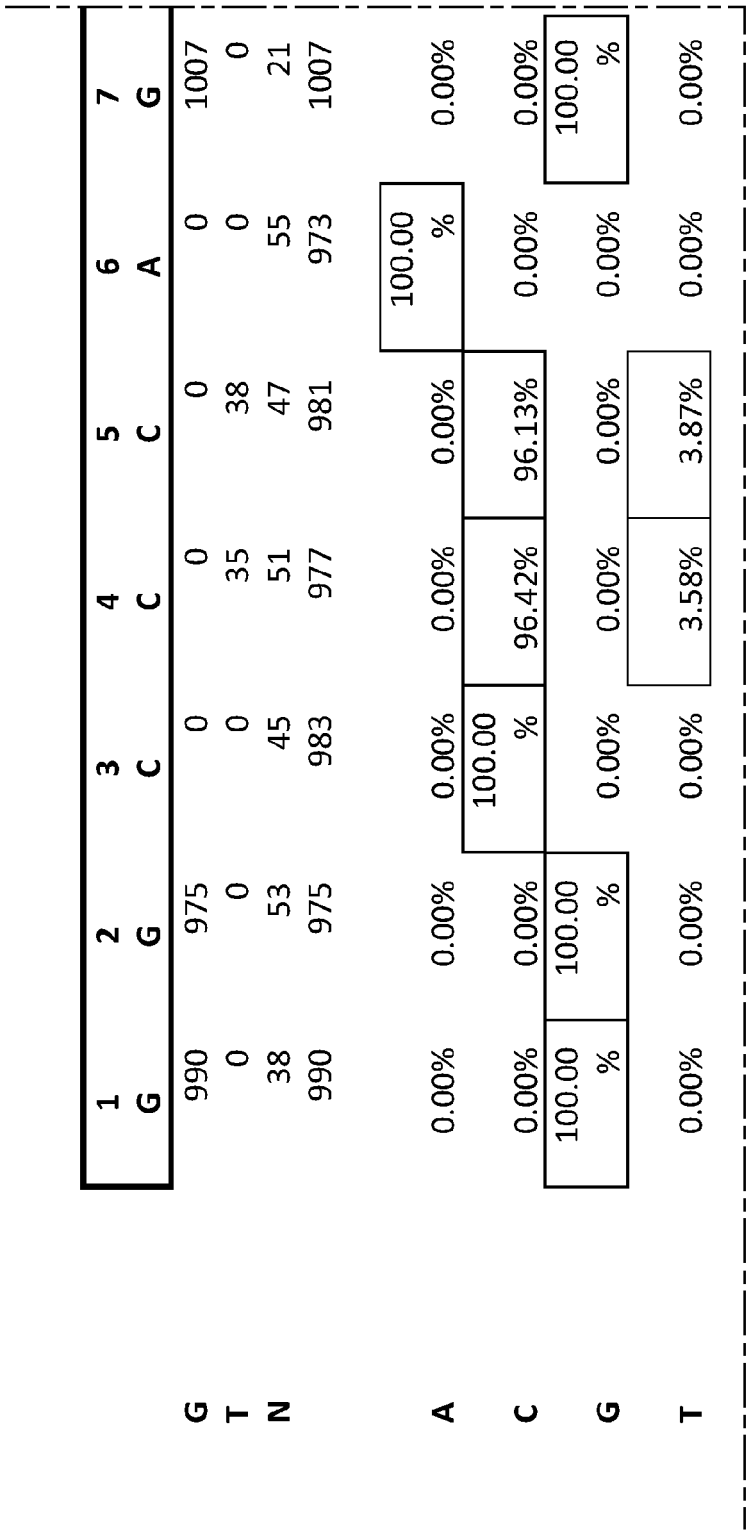
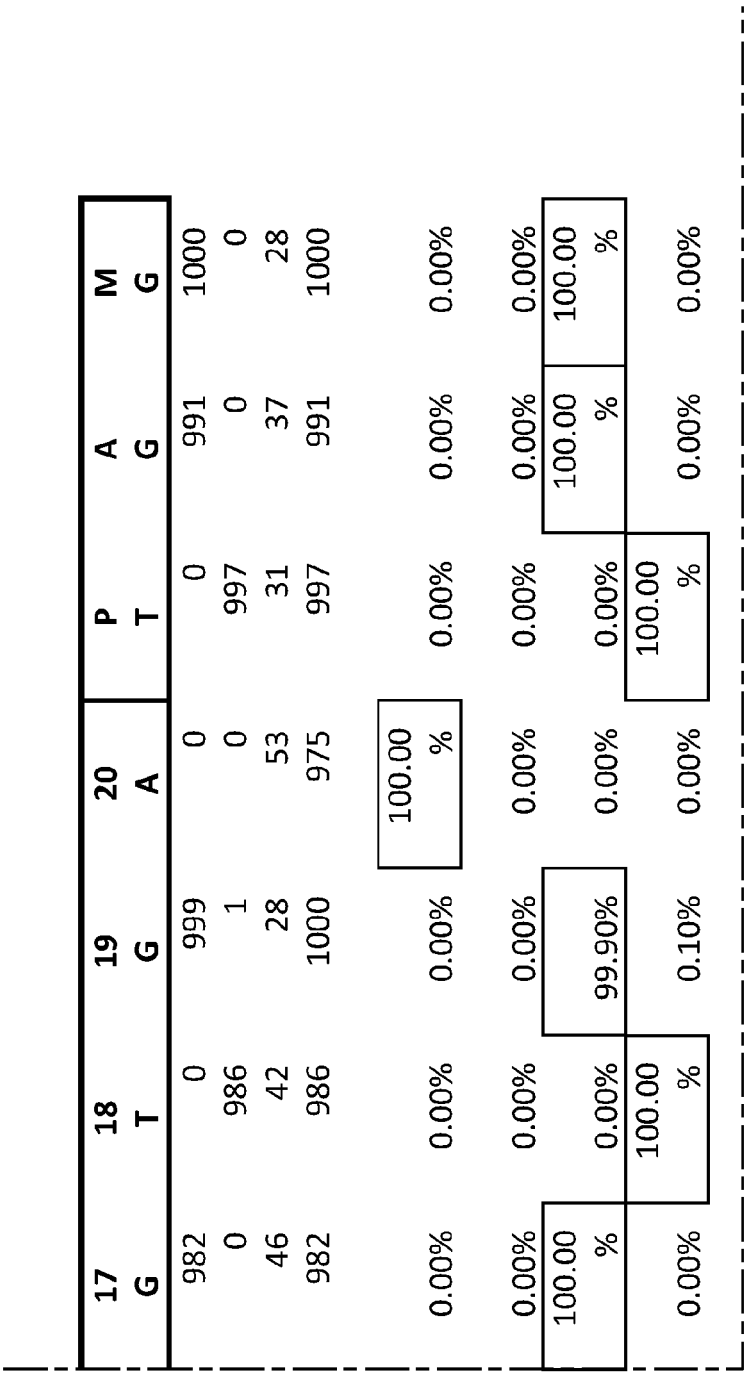


FIGURE 41 (Continued)

| 8 A | 9 C | 10 T | 11 G | 12 A | 13 G | 14 C | 15 A | 16 C |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 1013 | 0 | 1001 | 0 | 0 | 0 |
| 0 | 0 | 1011 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 | 25 | 17 | 15 | 53 | 27 | 40 | 20 | 38 |
| 1001 | 1003 | 1011 | 1013 | 975 | 1001 | 988 | 1008 | 990 |
| 100.00 % | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% |
| 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 100.00 % |
| 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 41 (Continued)



| 17 G | 18 T | 19 G | 20 A | P T | A G | M G |
|---|---|---|---|---|---|---|
| 982 | 0 | 999 | 0 | 0 | 991 | 1000 |
| 0 | 986 | 1 | 0 | 997 | 0 | 0 |
| 46 | 42 | 28 | 53 | 31 | 37 | 28 |
| 982 | 986 | 1000 | 975 | 997 | 991 | 1000 |
| | | | 100.00 % | | | |
| 0.00% | 0.00% | 0.00% | | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | | | | | 100.00 % | 100.00 % |
| | 0.00% | 99.90% | 0.00% | 0.00% | | |
| | 100.00 % | | | 100.00 % | | |
| 0.00% | | 0.10% | 0.00% | | 0.00% | 0.00% |

FIGURE 41 (Continued)

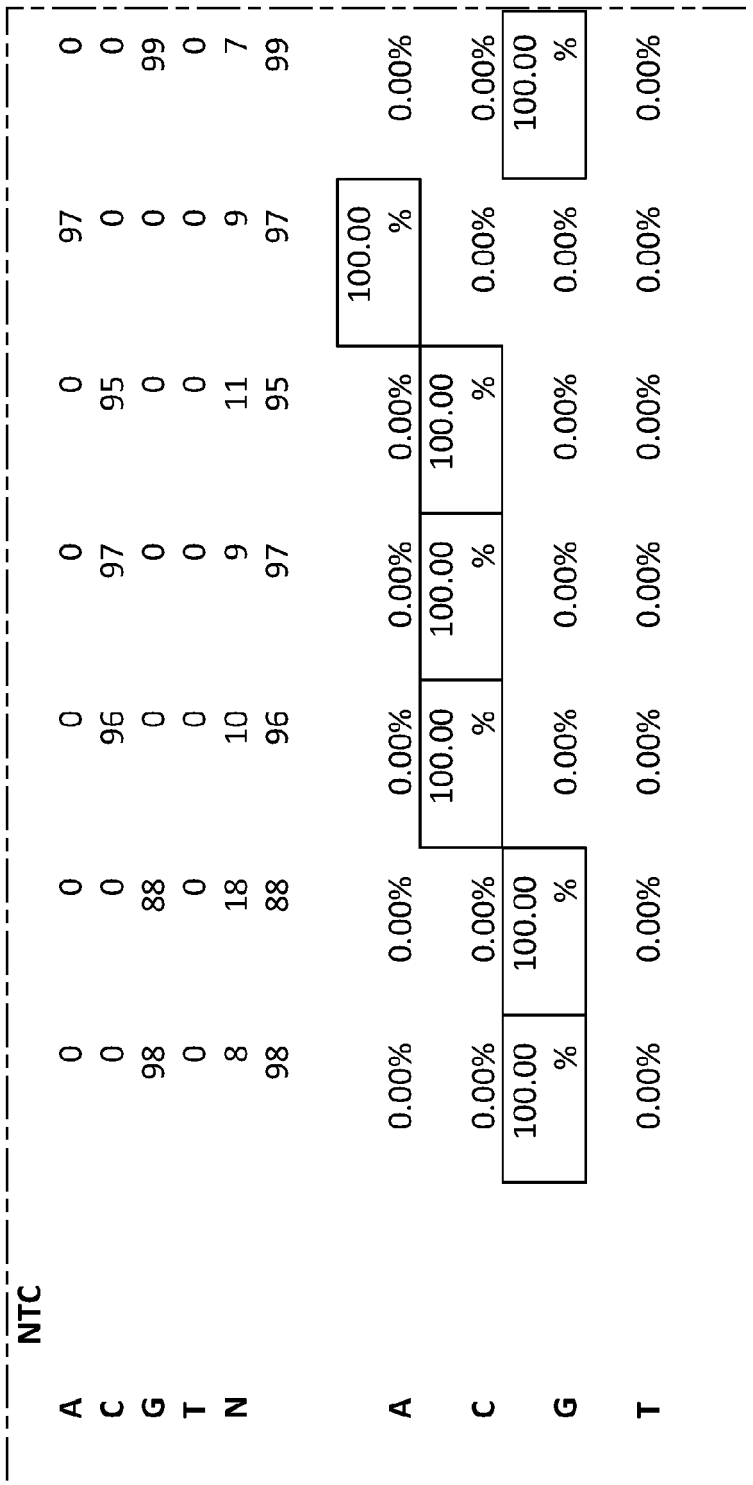


FIGURE 41 (Continued)

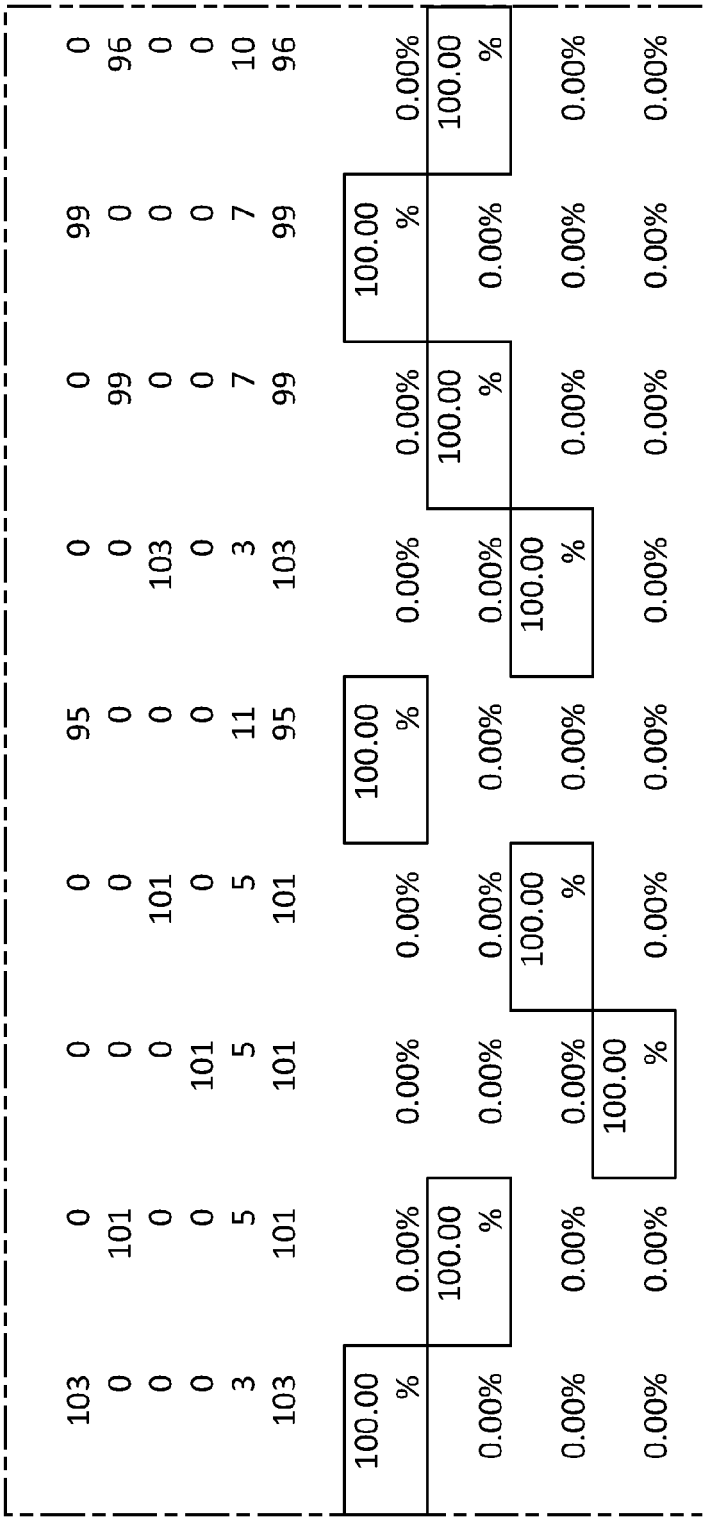


FIGURE 41 (Continued)

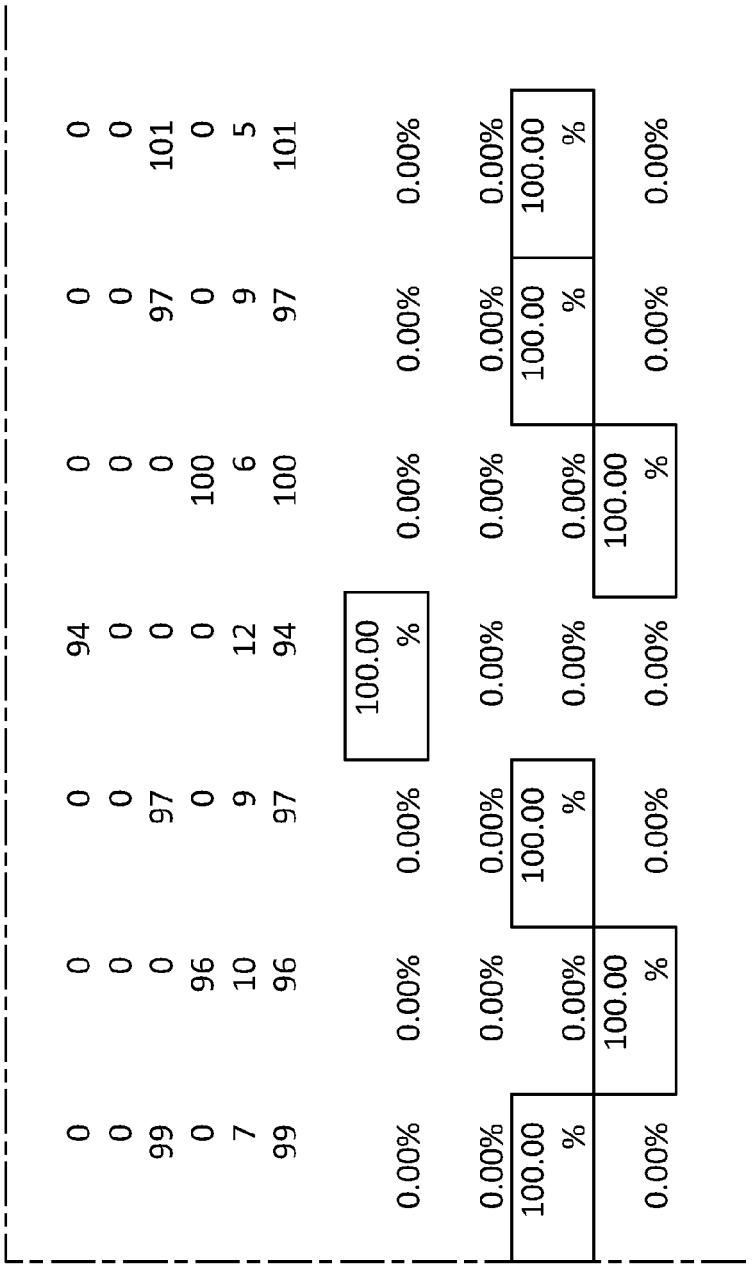
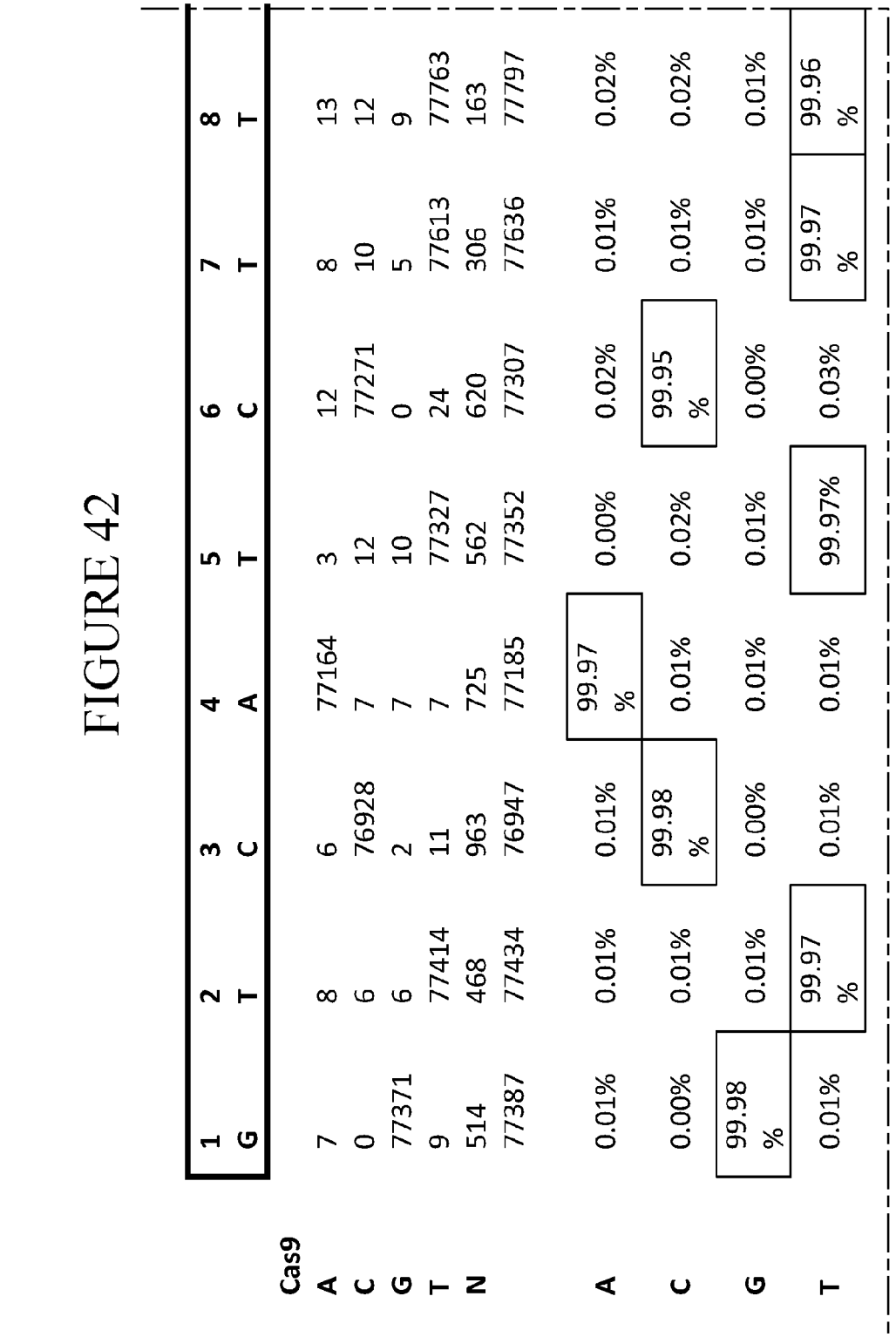
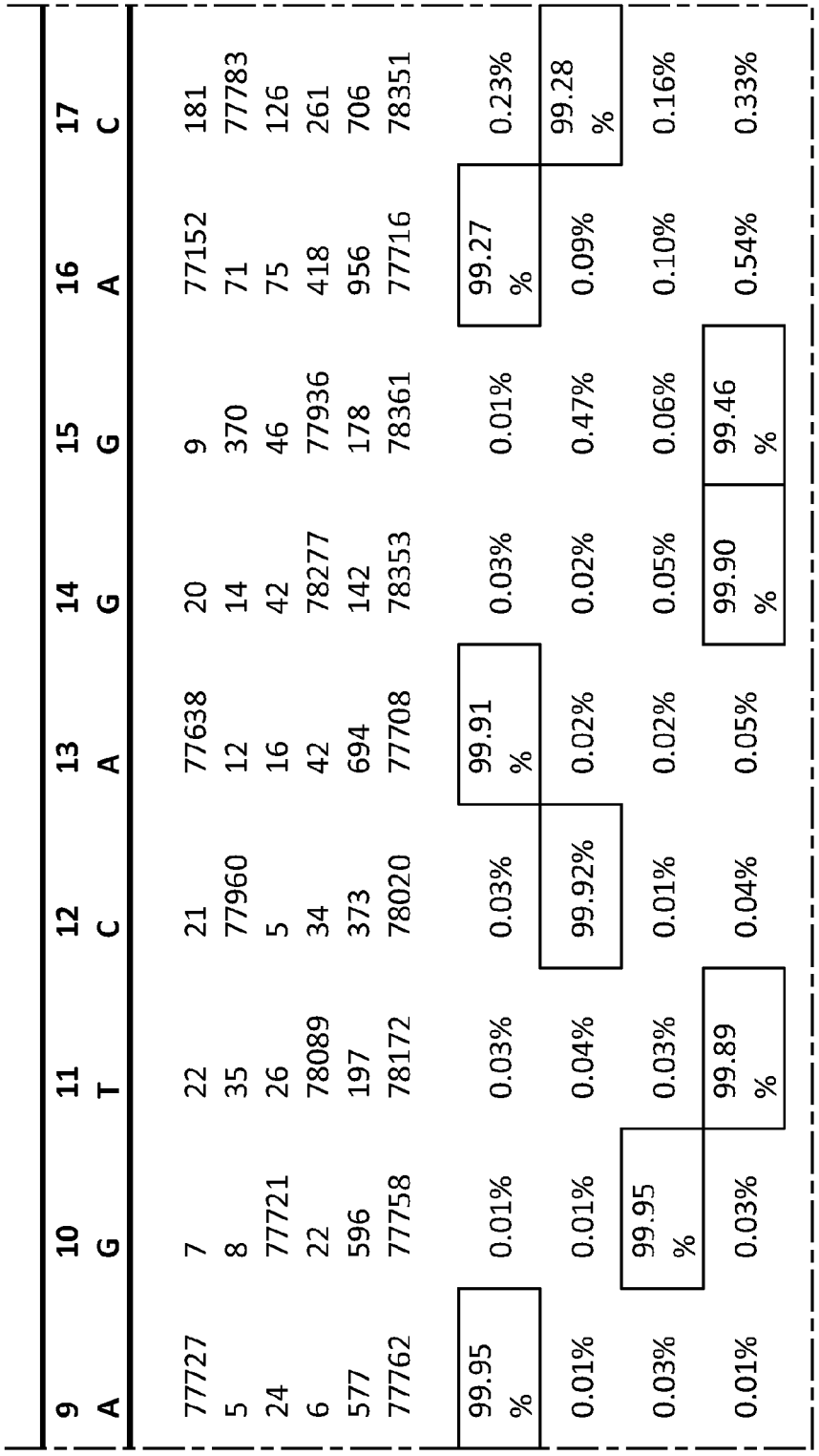


FIGURE 42



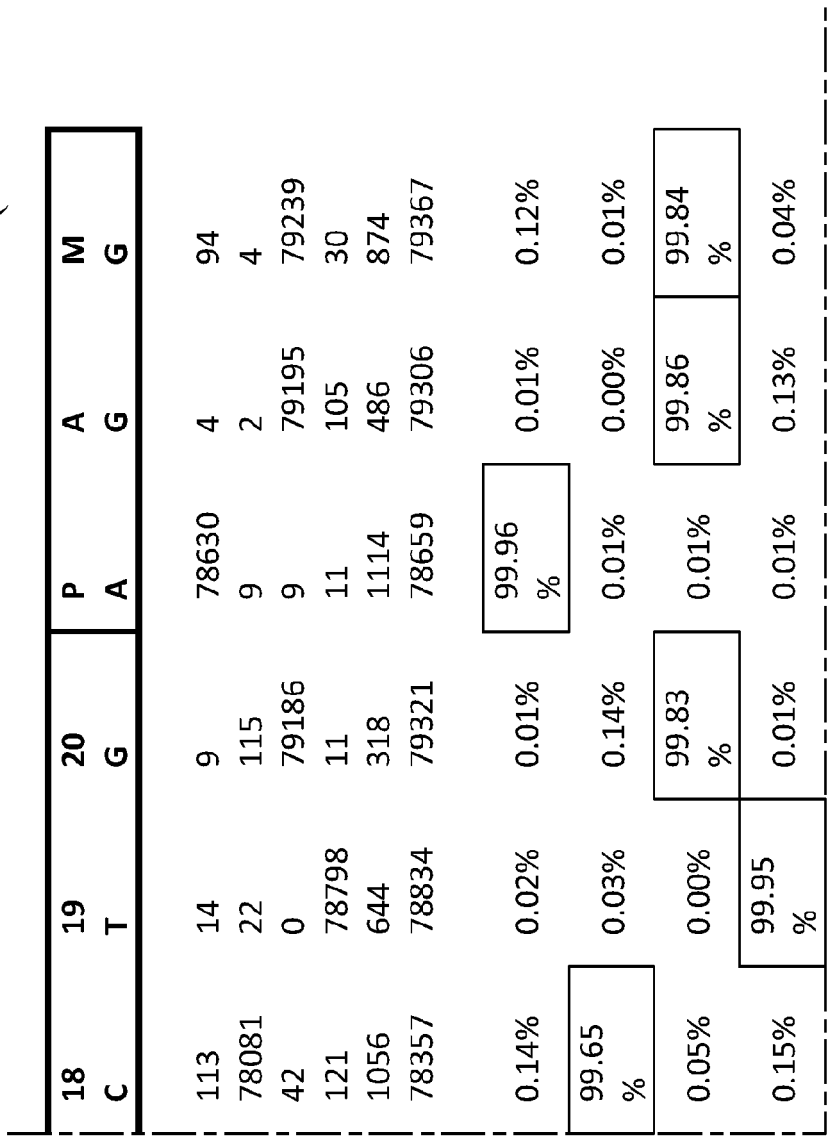
| Cas9 | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T |
|---|---|---|---|---|---|---|---|---|
| A | 7 | 8 | 6 | 77164 | 3 | 12 | 8 | 13 |
| C | 0 | 6 | 76928 | 7 | 12 | 77271 | 10 | 12 |
| G | 77371 | 6 | 2 | 7 | 10 | 0 | 5 | 9 |
| T | 9 | 77414 | 11 | 7 | 77327 | 24 | 77613 | 77763 |
| N | 514 | 468 | 963 | 725 | 562 | 620 | 306 | 163 |
| | 77387 | 77434 | 76947 | 77185 | 77352 | 77307 | 77636 | 77797 |
| A | 0.01% | 0.01% | 0.01% | 99.97 % | 0.00% | 0.02% | 0.01% | 0.02% |
| C | 0.00% | 0.01% | 99.98 % | 0.01% | 0.02% | 99.95 % | 0.01% | 0.02% |
| G | 99.98 % | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |
| T | 0.01% | 99.97 % | 0.01% | 0.01% | 99.97% | 0.03% | 99.97 % | 99.96 % |

FIGURE 42 (Continued)



| 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G | 16 A | 17 C |
|---|---|---|---|---|---|---|---|---|
| 77727 | 7 | 22 | 21 | 77638 | 20 | 9 | 77152 | 181 |
| 5 | 8 | 35 | 77960 | 12 | 14 | 370 | 71 | 77783 |
| 24 | 77721 | 26 | 5 | 16 | 42 | 46 | 75 | 126 |
| 6 | 22 | 78089 | 34 | 42 | 78277 | 77936 | 418 | 261 |
| 577 | 596 | 197 | 373 | 694 | 142 | 178 | 956 | 706 |
| 7762 | 77758 | 78172 | 78020 | 77708 | 78353 | 78361 | 77716 | 78351 |
| 99.95 % | 0.01% | 0.03% | 0.03% | 99.91 % | 0.03% | 0.01% | 99.27 % | 0.23% |
| 0.01% | 0.01% | 0.04% | 99.92% | 0.02% | 0.02% | 0.47% | 0.09% | 99.28 % |
| 0.03% | 99.95 % | 0.03% | 0.01% | 0.02% | 0.05% | 0.06% | 0.10% | 0.16% |
| 0.01% | 0.03% | 99.89 % | 0.04% | 0.05% | 99.90 % | 99.46 % | 0.54% | 0.33% |

FIGURE 42 (Continued)



| 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|
| 113 | 14 | 9 | 78630 | 4 | 94 |
| 78081 | 22 | 115 | 9 | 2 | 4 |
| 42 | 0 | 79186 | 9 | 79195 | 79239 |
| 121 | 78798 | 11 | 11 | 105 | 30 |
| 1056 | 644 | 318 | 1114 | 486 | 874 |
| 78357 | 78834 | 79321 | 78659 | 79306 | 79367 |
| 0.14% | 0.02% | 0.01% | 99.96 % | 0.01% | 0.12% |
| 99.65 % | 0.03% | 0.14% | 0.01% | 0.00% | 0.01% |
| 0.05% | 0.00% | 99.83 % | 0.01% | 99.86 % | 99.84 % |
| 0.15% | 99.95 % | 0.01% | 0.01% | 0.13% | 0.04% |

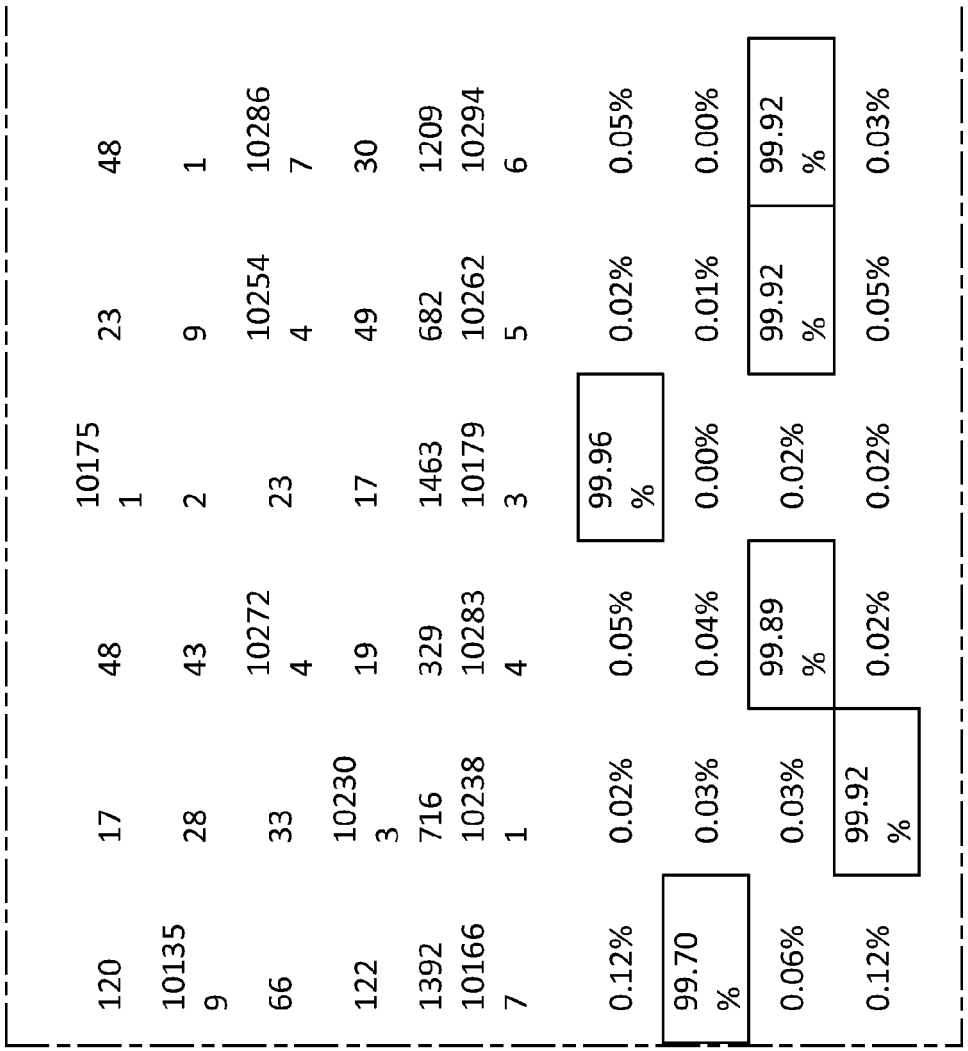
FIGURE 42 (Continued)

| pNMG-247 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; wild type) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 4 | 7 | 10083 | 2 | 14 | 8 | 14 | |
| C | 0 | 2 | 10049 | 18 | 7 | 10087 | 16 | 17 | |
| G | 10120 | 1 | 1 | 3 | 2 | 2 | 2 | 1 | |
| T | 12 | 10119 | 20 | 11 | 10109 | 14 | 10141 | 10162 | |
| N | 637 | 660 | 1334 | 993 | 762 | 973 | 445 | 229 | |
| | 10121 | 10119 | 10052 | 10087 | 10110 | 10090 | 10143 | 10166 | |
| | 8 | 7 | 6 | 1 | 9 | 5 | 8 | 0 | |
| A | 0.00% | 0.00% | 0.01% | 99.97% | 0.00% | 0.01% | 0.01% | 0.01% | |
| C | 0.00% | 0.00% | 99.97% | 0.02% | 0.01% | 99.97% | 0.02% | 0.02% | |
| G | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.01% | 99.97% | 99.97% | |

FIGURE 42 (Continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 10146 2 | 13 | 16 | 7 | 10128 6 | 8 | 9 | 10076 6 | 224 | |
| 17 | 3 | 21 | 101849 | 28 | 30 | 461 | 48 | 10147 0 | |
| 14 | 10145 6 | 2 | 14 | 16 | 28 | 17 | 93 | 62 | |
| 18 | 10 | 10204 1 | 37 | 25 | 10211 6 | 10170 2 | 476 | 217 | |
| 817 | 847 | 267 | 455 | 1028 | 232 | 249 | 1215 | 951 | |
| 10151 | 10148 | 10208 | 101907 | 10135 | 10218 | 10218 | 10138 | 10197 | |
| 1 | 2 | 0 | | 5 | 2 | 9 | 3 | 3 | |
| 99.95 % | 0.01% | 0.02% | 0.01% | 99.93 % | 0.01% | 0.01% | 99.39 % | 0.22% | 99.51 % |
| 0.02% | 0.00% | 0.02% | 99.94% | 0.03% | 0.03% | 0.45% | 0.05% | | |
| 0.01% | 99.97 % | 0.00% | 0.01% | 0.02% | 0.03% | 0.02% | 0.09% | 0.06% | |
| 0.02% | 0.01% | 99.96 % | 0.04% | 0.02% | 99.94 % | 99.52 % | 0.47% | 0.21% | |

FIGURE 42 (Continued)

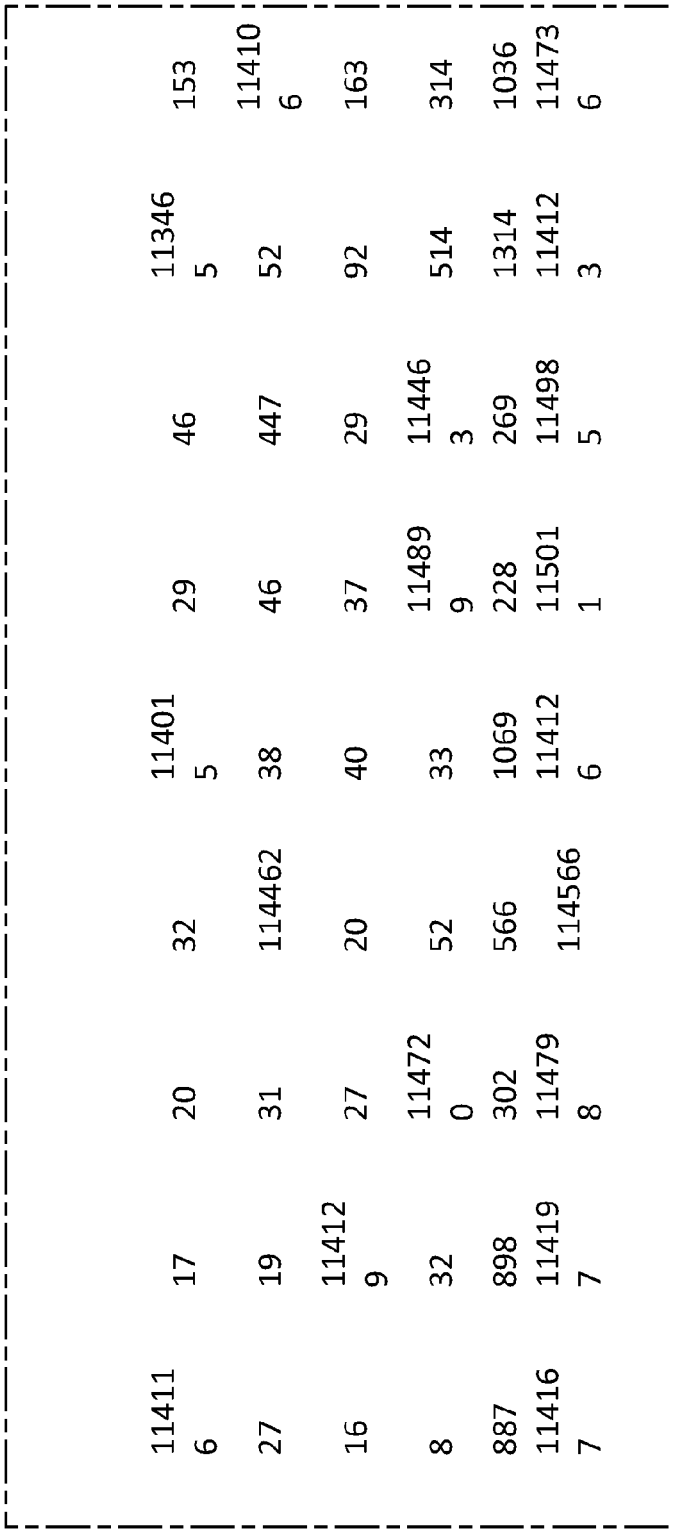


| | | | | | | |
|---|---|---|---|---|---|---|
| 120 | 17 | 48 | 101751 | 23 | 48 | |
| 101359 | 28 | 43 | 2 | 9 | 1 | |
| 66 | 33 | 10272 4 | 23 | 10254 4 | 10286 7 | |
| 122 | 102303 | 19 | 17 | 49 | 30 | |
| 1392 | 716 | 329 | 1463 | 682 | 1209 | |
| 10166 7 | 10238 1 | 10283 4 | 10179 3 | 10262 5 | 10294 6 | |
| 0.12% | 0.02% | 0.05% | 99.96 % | 0.02% | 0.05% | |
| 99.70 % | 0.03% | 0.04% | 0.00% | 0.01% | 0.00% | |
| 0.06% | 0.03% | 99.89 % | 0.02% | 99.92 % | 99.92 % | |
| 0.12% | 99.92 % | 0.02% | 0.02% | 0.05% | 0.03% | |

FIGURE 42 (Continued)

| pNMG-248 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 14 | 3 | 10 | 11346 | 9 | 11 | 20 | 10 | | |
| C | 0 | 16 | 11315 | 21 | 19 | 11350 | 9 | 21 | | |
| G | 11381 | 2 | 1 | 8 | 0 | 1 | 20 | 14 | | |
| T | 7 | 11383 | 22 | 15 | 11378 | 15 | 11407 | 11434 | | |
| N | 763 | 750 | 1426 | 1118 | 834 | 1115 | 526 | 273 | | |
| | 11383 | 11385 | 11318 | 11350 | 11380 | 11353 | 11412 | 11438 | | |
| | 6 | 7 | 5 | 9 | 8 | 1 | 2 | 5 | | |

FIGURE 42 (Continued)



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 11411 6 | 17 | 20 | 32 | 11401 5 | 29 | 46 | 11346 5 | 153 | |
| 27 | 19 | 31 | 114462 | 38 | 46 | 447 | 52 | 11410 6 | |
| 16 | 11412 9 | 27 | 20 | 40 | 37 | 29 | 92 | 163 | |
| 8 | 32 | 11472 0 | 52 | 33 | 11489 9 | 11446 3 | 514 | 314 | |
| 887 | 898 | 302 | 566 | 1069 | 228 | 269 | 1314 | 1036 | |
| 11416 7 | 11419 7 | 11479 8 | 114566 | 11412 6 | 11501 1 | 11498 5 | 11412 3 | 11473 6 | |

FIGURE 42 (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 129 | 45 | 16 | 114649 | 5 | 77 | |
| 114049 | 21 | 75 | 10 | 8 | 2 | |
| 80 | 38 | 115544 | 25 | 115390 | 115688 | |
| 153 | 114998 | 22 | 12 | 84 | 17 | |
| 1515 | 835 | 407 | 1533 | 751 | 1258 | |
| 11441 | 11510 | 11565 | 11469 | 11548 | 11578 | |
| 1 | 2 | 7 | 6 | 7 | 4 | |

FIGURE 42 (Continued)

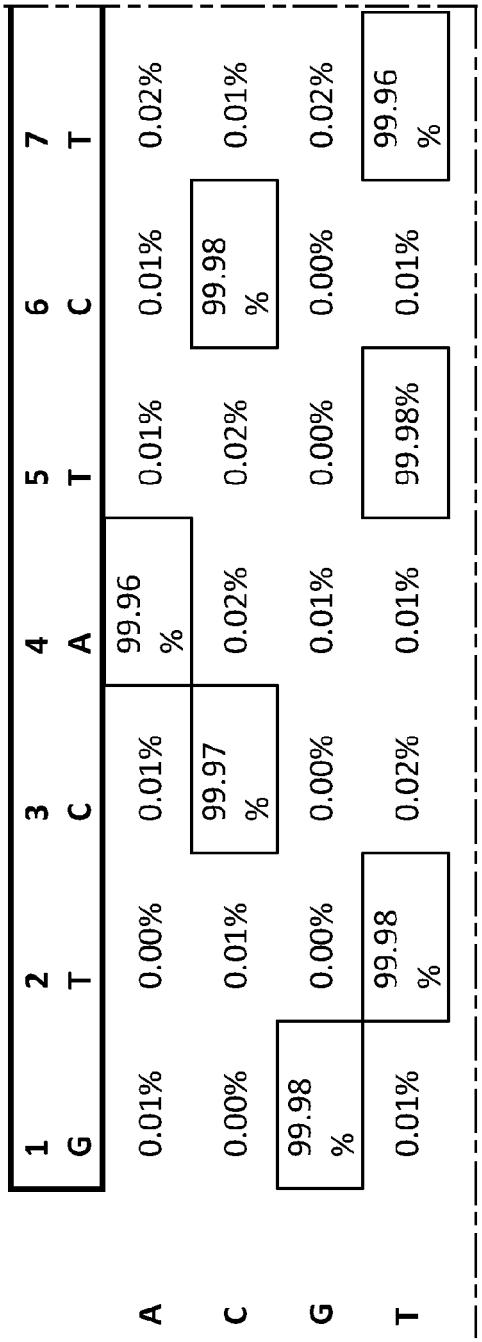
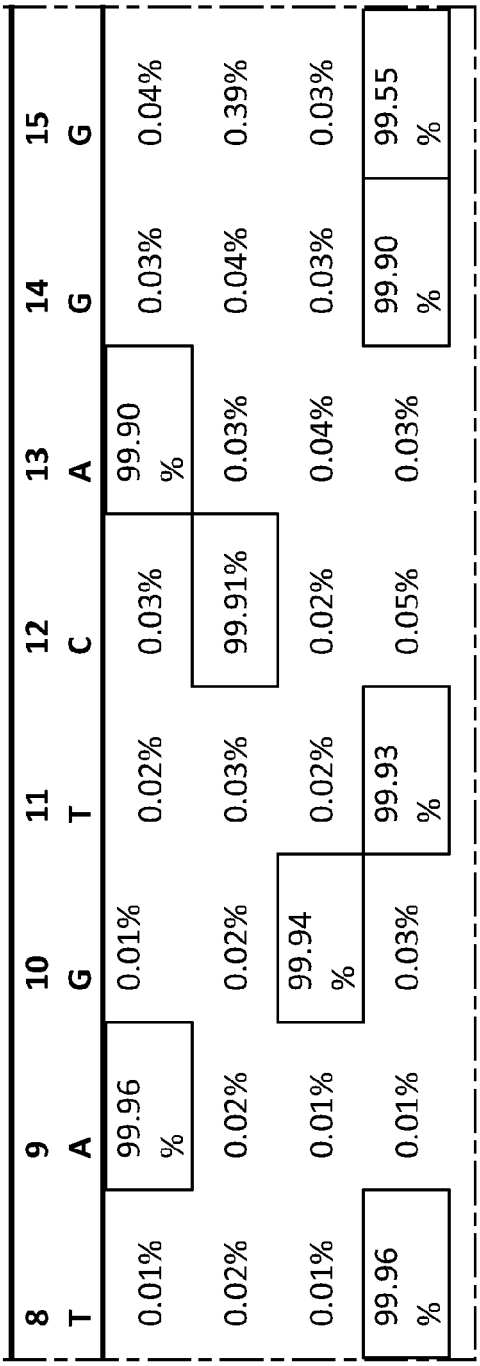


FIGURE 42 (Continued)

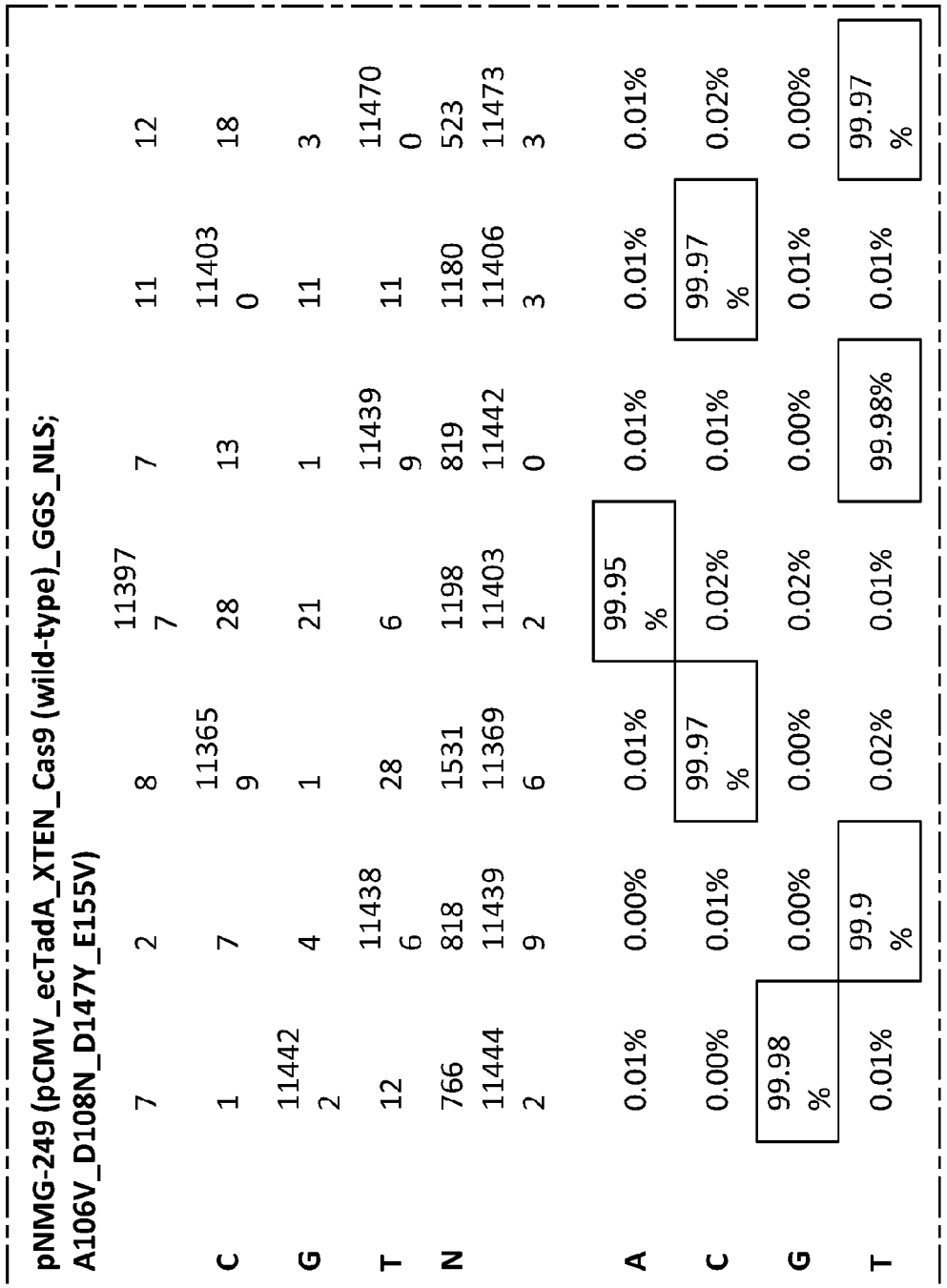


| 8 T | 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G |
|---|---|---|---|---|---|---|---|
| 0.01% | 99.96 % | 0.01% | 0.02% | 0.03% | 99.90 % | 0.03% | 0.04% |
| 0.02% | 0.02% | 0.02% | 0.03% | 99.91% | 0.03% | 0.04% | 0.39% |
| 0.01% | 0.01% | 99.94 % | 0.02% | 0.02% | 0.04% | 0.03% | 0.03% |
| 99.96 % | 0.01% | 0.03% | 99.93 % | 0.05% | 0.03% | 99.90 % | 99.55 % |

FIGURE 42 (Continued)

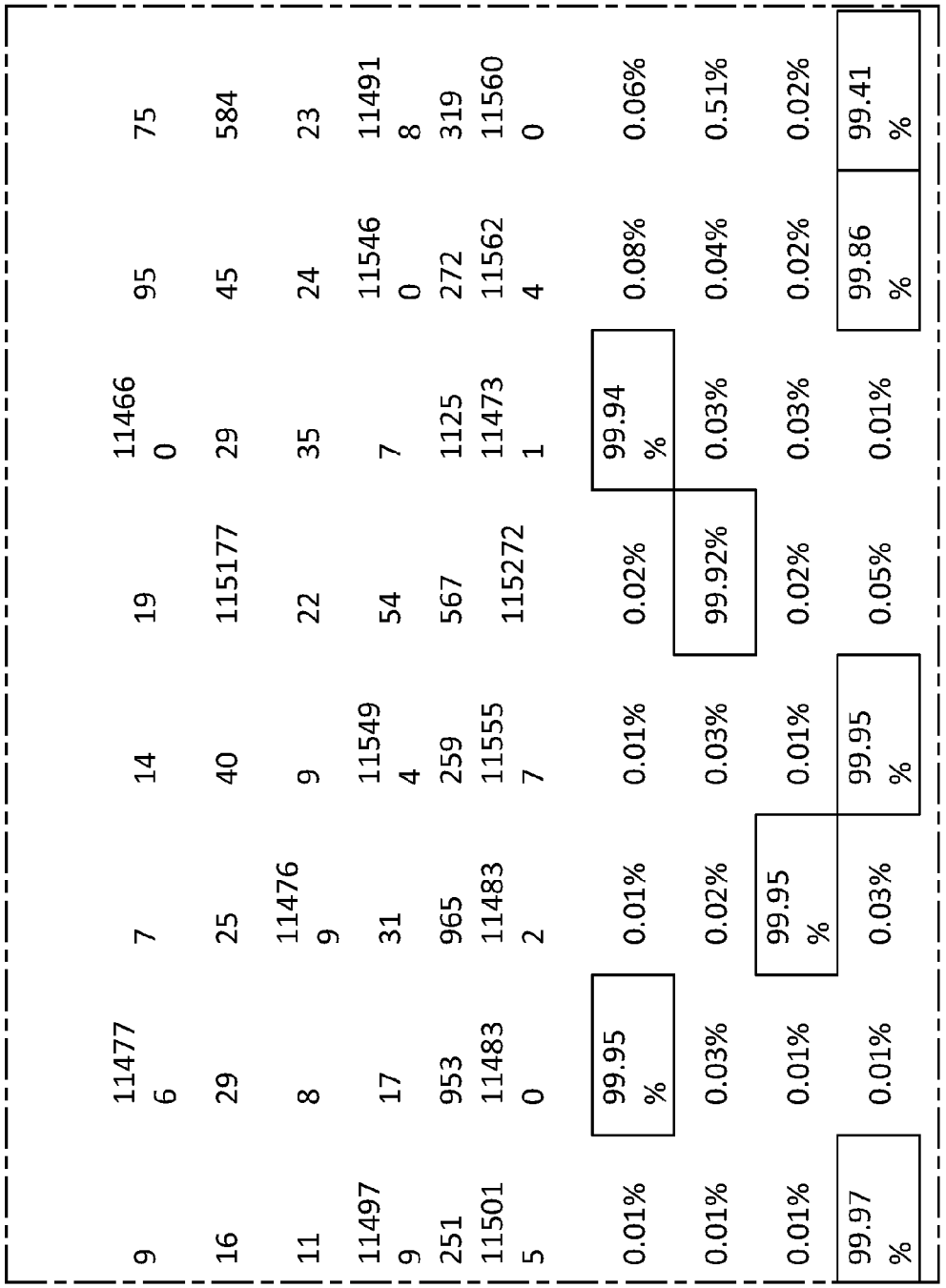
| 16 A | 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|---|
| 99.42 % | 0.13% | 0.11% | 0.04% | 0.01% | 99.96 % | 0.00% | 0.07% |
| 0.05% | 99.45 % | 99.68 % | 0.02% | 0.06% | 0.01% | 0.01% | 0.00% |
| 0.08% | 0.14% | 0.07% | 0.03% | 99.90 % | 0.02% | 99.92 % | 99.92 % |
| 0.45% | 0.27% | 0.13% | 99.91 % | 0.02% | 0.01% | 0.07% | 0.01% |

FIGURE 42 (Continued)



| pNMG-249 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 7 | 2 | 8 | 11397 | 7 | 11 | 12 | | | | |
| C | 1 | 7 | 11365 | 28 | 13 | 11403 | 18 | | | | |
| | 11442 | | | | | | | | | | |
| G | 2 | 4 | 1 | 21 | 1 | 11 | 3 | | | | |
| | 11438 | | | | 11439 | | 11470 | | | | |
| T | 12 | 6 | 28 | 6 | 9 | 11 | 0 | | | | |
| N | 766 | 818 | 1531 | 1198 | 819 | 1180 | 523 | | | | |
| | 11444 | 11439 | 11369 | 11403 | 11442 | 11406 | 11473 | | | | |
| | 2 | 9 | 6 | 2 | 0 | 3 | 3 | | | | |
| A | 0.01% | 0.00% | 0.01% | 99.95 % | 0.01% | 0.01% | 0.01% | | | | |
| C | 0.00% | 0.01% | 99.97 % | 0.02% | 0.01% | 99.97 % | 0.02% | | | | |
| G | 99.98 % | 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% | | | | |
| T | 0.01% | 99.9 % | 0.02% | 0.01% | 99.98% | 0.01% | 99.97 % | | | | |

FIGURE 42 (Continued)



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 9 | 11477 6 | 7 | 14 | 19 | 11466 0 | 95 | 75 | | |
| 16 | 29 | 25 | 40 | 115177 | 29 | 45 | 584 | | |
| 11 | 8 | 11476 9 | 9 | 22 | 35 | 24 | 23 | | |
| 11497 9 | 17 | 31 | 11549 4 | 54 | 7 | 11546 0 | 11491 8 | | |
| 251 | 953 | 965 | 259 | 567 | 1125 | 272 | 319 | | |
| 11501 5 | 11483 0 | 11483 2 | 11555 7 | 115272 | 11473 1 | 11562 4 | 11560 0 | | |
| 0.01% | 99.95 % | 0.01% | 0.01% | 0.02% | 99.94 % | 0.08% | 0.06% | | |
| 0.01% | 0.03% | 0.02% | 0.03% | 99.92% | 0.03% | 0.04% | 0.51% | | |
| 0.01% | 0.01% | 99.95 % | 0.01% | 0.02% | 0.03% | 0.02% | 0.02% | | |
| 99.97 % | 0.01% | 0.03% | 99.95 % | 0.05% | 0.01% | 99.86 % | 99.41 % | | |

FIGURE 42 (Continued)

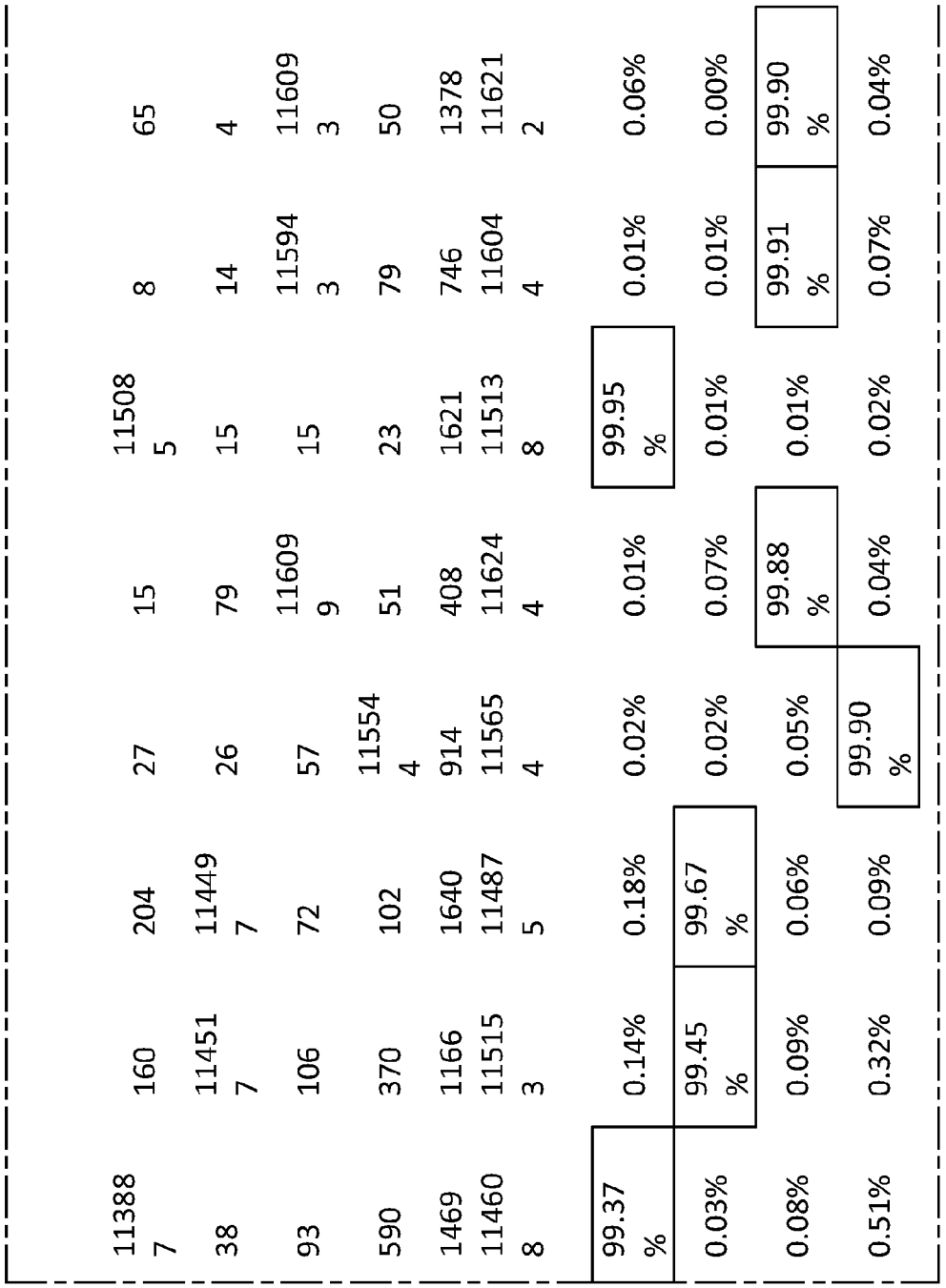
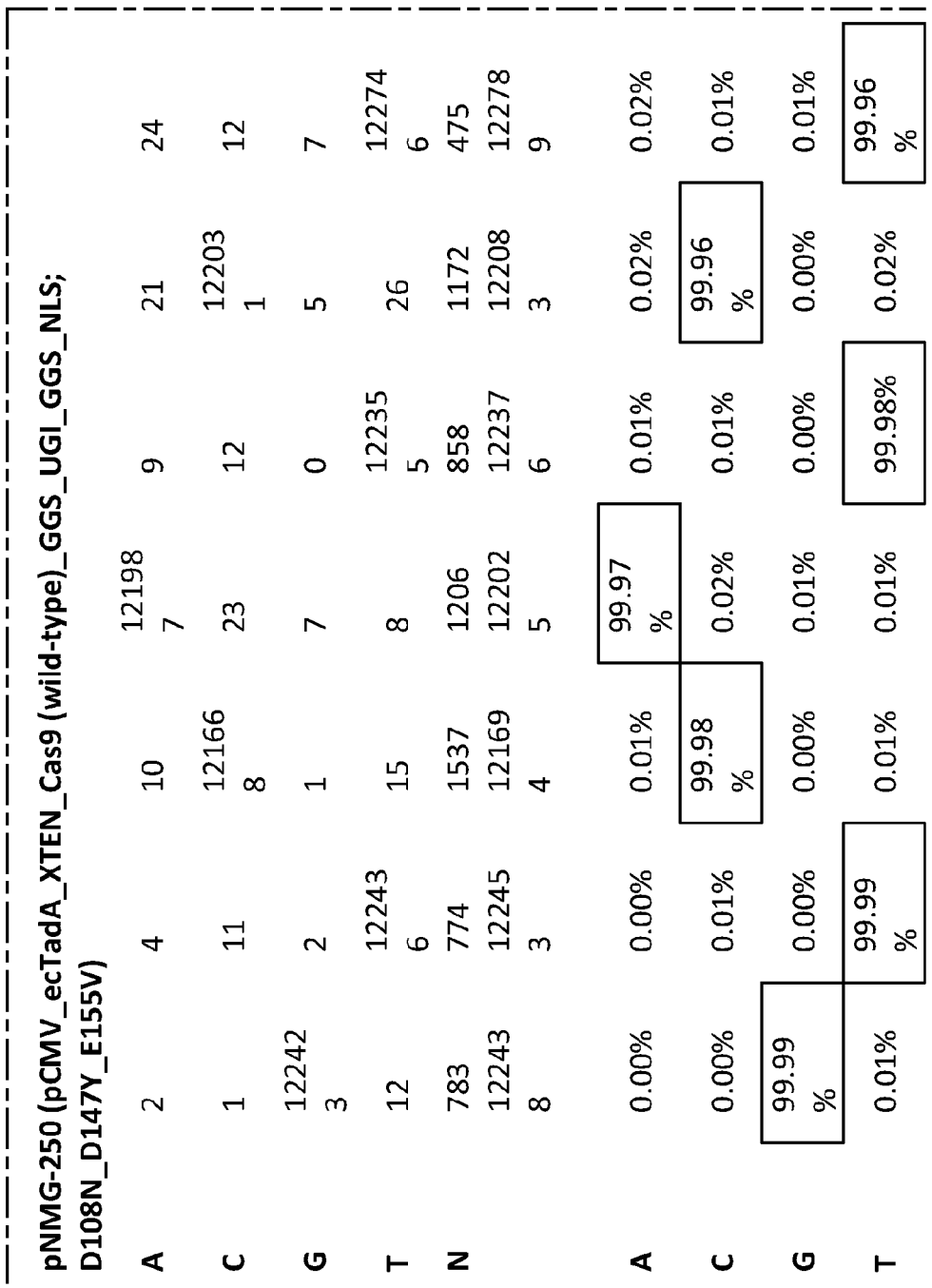
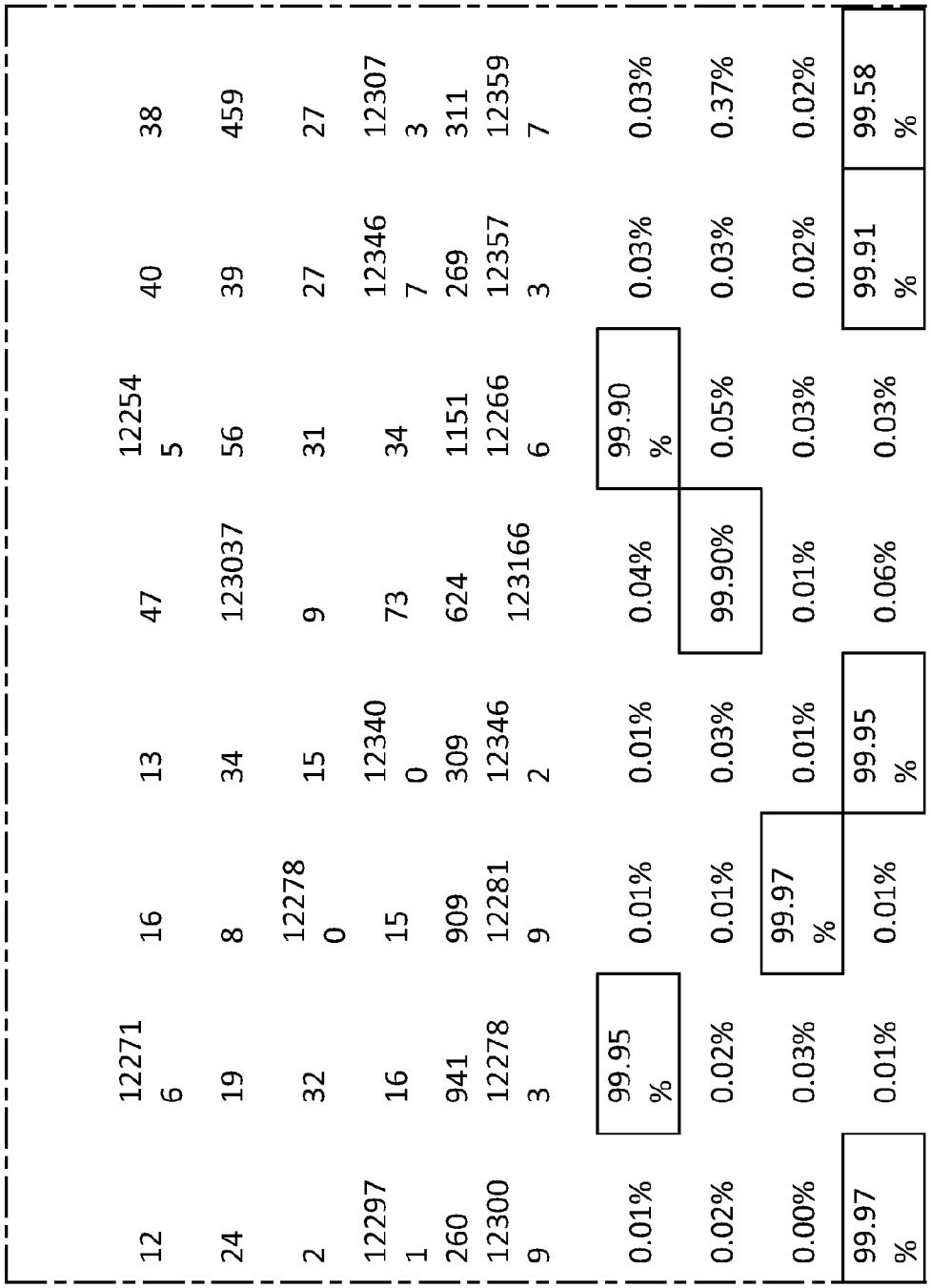


FIGURE 42 (Continued)



| pNMG-250 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_UGI_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 4 | 10 | 12198 7 | 9 | 21 | 24 | | | |
| C | 1 | 11 | 12166 8 | 23 | 12 | 12203 1 | 12 | | | |
| G | 12242 3 | 2 | 1 | 7 | 0 | 5 | 7 | | | |
| T | 12 | 12243 6 | 15 | 8 | 12235 5 | 26 | 12274 6 | | | |
| N | 783 | 774 | 1537 | 1206 | 858 | 1172 | 475 | | | |
| | 12243 8 | 12245 3 | 12169 4 | 12202 5 | 12237 6 | 12208 3 | 12278 9 | | | |
| A | 0.00% | 0.00% | 0.01% | 99.97 % | 0.01% | 0.02% | 0.02% | | | |
| C | 0.00% | 0.01% | 99.98 % | 0.02% | 0.01% | 99.96 % | 0.01% | | | |
| G | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | | | |
| T | 0.01% | 99.99 % | 0.01% | 0.01% | 99.98% | 0.02% | 99.96 % | | | |

FIGURE 42 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 12 | 122716 | 16 | 13 | 47 | 122545 | 40 | 38 | |
| 24 | 19 | 8 | 34 | 123037 | 56 | 39 | 459 | |
| 2 | 32 | 122780 | 15 | 9 | 31 | 27 | 27 | |
| 12297 | 16 | 15 | 123400 | 73 | 34 | 123467 | 123073 | |
| 260 | 941 | 909 | 309 | 624 | 1151 | 269 | 311 | |
| 12300 | 12278 | 12281 | 12346 | 123166 | 12266 | 12357 | 12359 | |
| 9 | 3 | 9 | 2 | | 6 | 3 | 7 | |
| 0.01% | 99.95% | 0.01% | 0.01% | 0.04% | 99.90% | 0.03% | 0.03% | |
| 0.02% | 0.02% | 0.01% | 0.03% | 99.90% | 0.05% | 0.03% | 0.37% | |
| 0.00% | 0.03% | 99.97% | 0.01% | 0.01% | 0.03% | 0.02% | 0.02% | |
| 99.97% | 0.01% | 0.01% | 99.95% | 0.06% | 0.03% | 99.91% | 99.58% | |

FIGURE 42 (Continued)

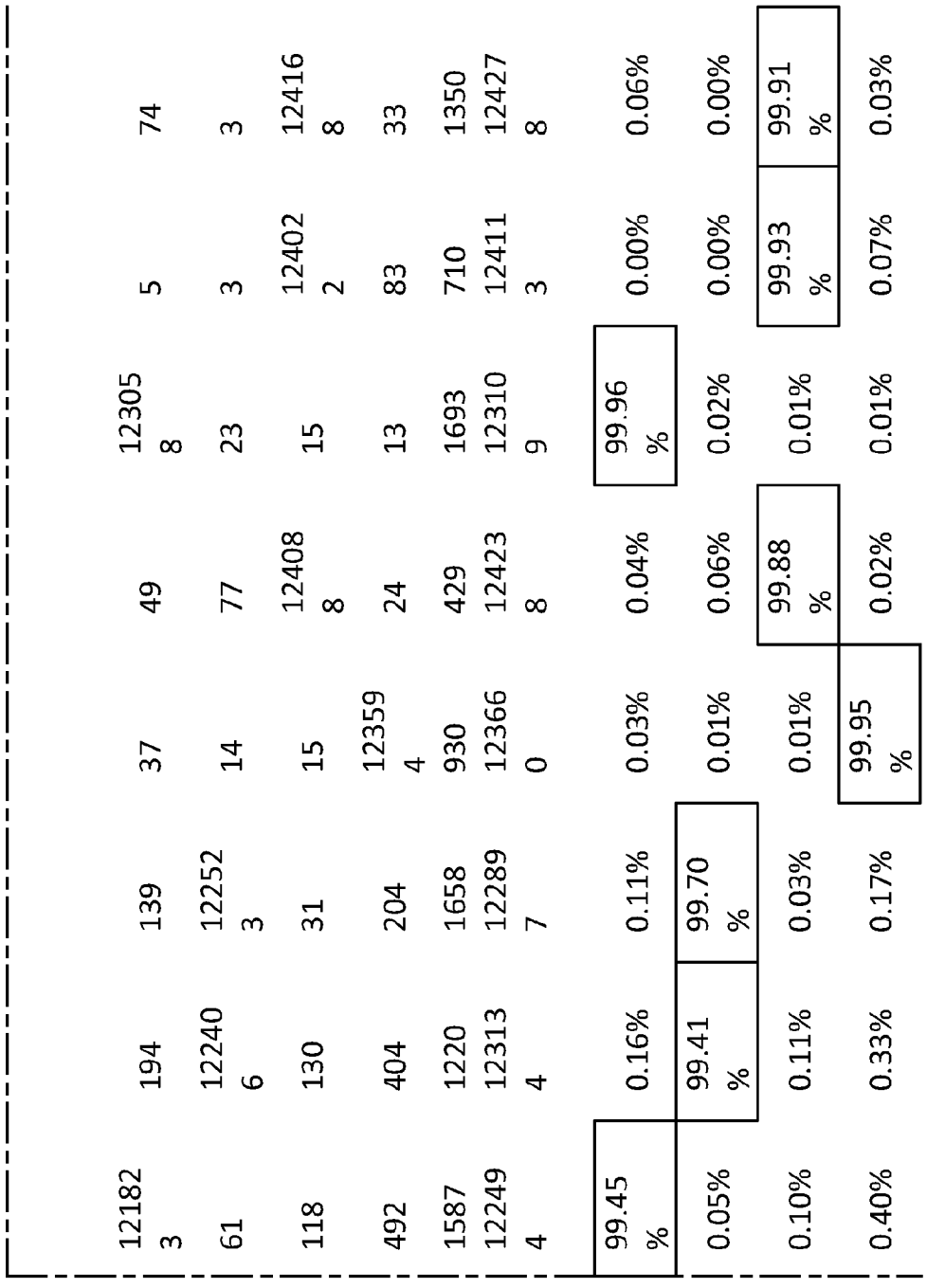
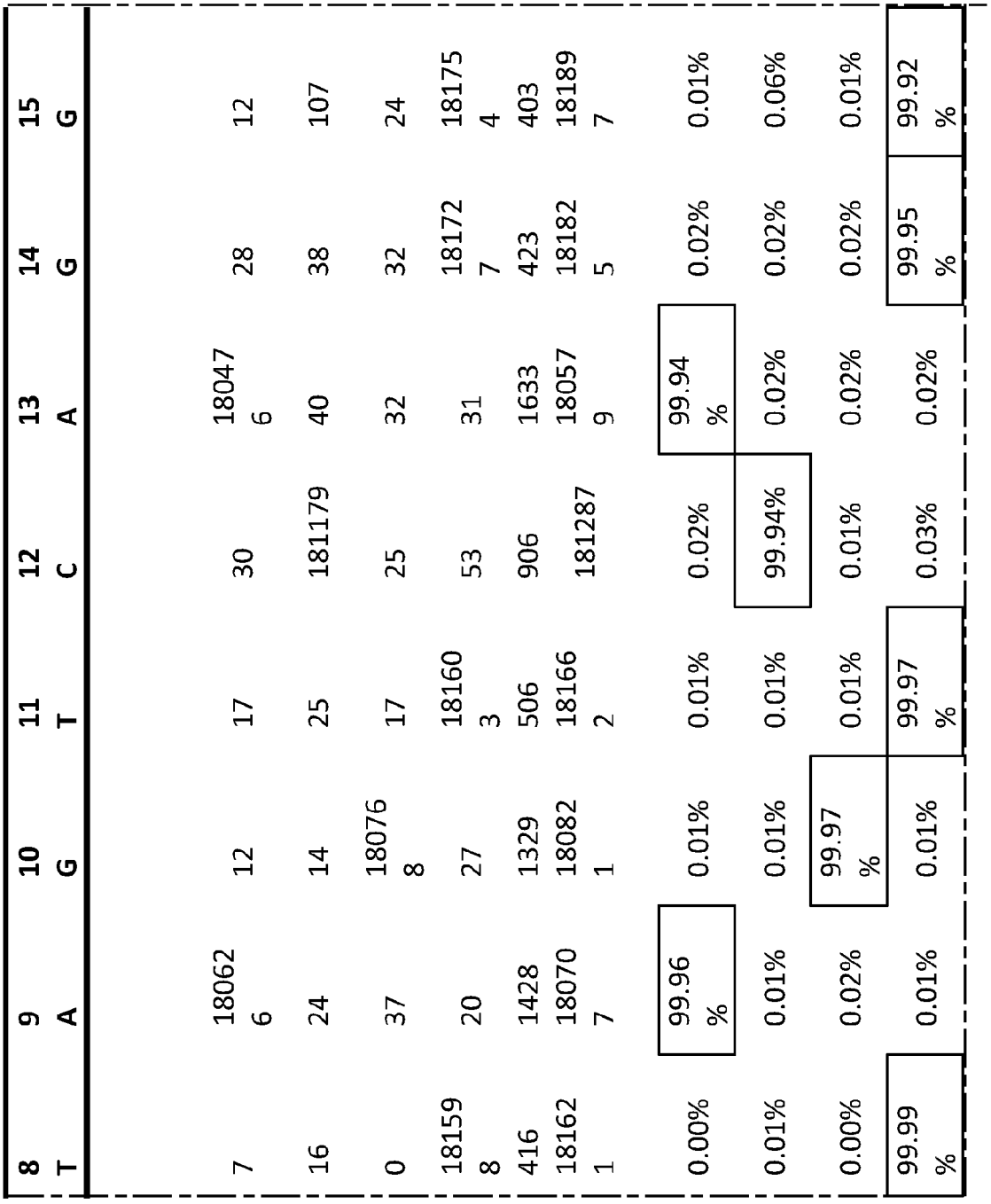


FIGURE 42 (Continued)

pNMG-251 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)

| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T |
|---|---|---|---|---|---|---|---|
| A | 15 | 4 | 16 | 18017 1 | 10 | 20 | 21 |
| C | 0 | 9 | 17958 8 | 33 | 11 | 18031 4 | 24 |
| G | 18088 0 | 6 | 1 | 17 | 7 | 15 | 1 |
| T | 12 | 18079 7 | 40 | 14 | 18071 2 | 19 | 18120 9 |
| N | 1091 | 1185 | 2360 | 1770 | 1286 | 1663 | 781 |
| | 18090 | 18081 | 17964 | 18023 | 18074 | 18036 | 18125 |
| | 7 | 6 | 5 | 5 | 0 | 8 | 5 |
| A | 0.01% | 0.00% | 0.01% | 99.96 % | 0.01% | 0.01% | 0.01% |
| C | 0.00% | 0.00% | 99.97 % | 0.02% | 0.01% | 99.97 % | 0.01% |
| G | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 99.99 % | 0.02% | 0.01% | 99.98% | 0.01% | 99.97 % |

FIGURE 42 (Continued)



| 8 T | 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G |
|---|---|---|---|---|---|---|---|
| 7 | 180626 | 12 | 17 | 30 | 180476 | 28 | 12 |
| 16 | 24 | 14 | 25 | 181179 | 40 | 38 | 107 |
| 0 | 37 | 180768 | 17 | 25 | 32 | 32 | 24 |
| 18159 | 20 | 27 | 18160 | 53 | 31 | 18172 | 18175 |
| 8 | | | 3 | | | 7 | 4 |
| 416 | 1428 | 1329 | 506 | 906 | 1633 | 423 | 403 |
| 18162 | 18070 | 18082 | 18166 | 181287 | 18057 | 18182 | 18189 |
| 1 | 7 | 1 | 2 | | 9 | 5 | 7 |
| 0.00% | 99.96% | 0.01% | 0.01% | 0.02% | 99.94% | 0.02% | 0.01% |
| 0.01% | 0.01% | 0.01% | 0.01% | 99.94% | 0.02% | 0.02% | 0.06% |
| 0.00% | 0.02% | 99.97% | 0.01% | 0.01% | 0.02% | 0.02% | 0.01% |
| 99.99% | 0.01% | 0.01% | 99.97% | 0.03% | 0.02% | 99.95% | 99.92% |

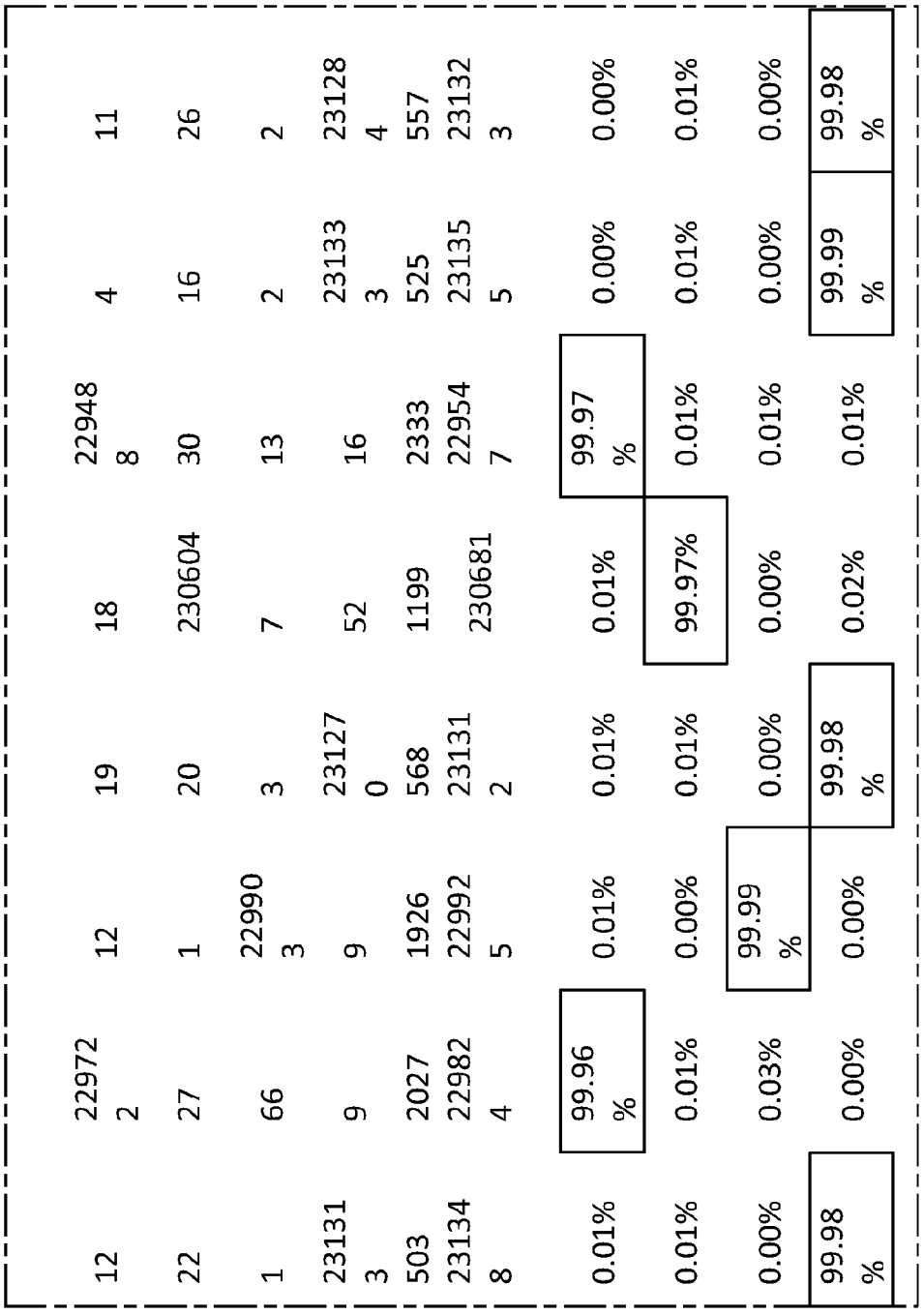
FIGURE 42 (Continued)

| 16 A | 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|---|
| 179977 | 100 | 92 | 7 | 20 | 180444 | 10 | 69 |
| 71 | 180737 | 180299 | 24 | 56 | 2 | 1 | 7 |
| 31 | 28 | 2 | 2 | 182171 | 23 | 18179 | 18119 |
| 127 | 127 | 60 | 181469 | 16 | 24 | 67 | 5 |
| 2215 | 1694 | 2394 | 1372 | 627 | 2433 | 1054 | 1960 |
| 18020 | 18099 | 18045 | 18150 | 18226 | 18049 | 18187 | 18127 |
| 6 | 2 | 3 | 2 | 3 | 3 | 5 | 6 |
| 99.87% | 0.06% | 0.05% | 0.00% | 0.01% | 99.97% | 0.01% | 0.04% |
| 0.04% | 99.86% | 99.91% | 0.01% | 0.03% | 0.00% | 0.00% | 0.00% |
| 0.02% | 0.02% | 0.00% | 0.00% | 99.95% | 0.01% | 99.96% | 99.96% |
| 0.07% | 0.07% | 0.03% | 99.98% | 0.01% | 0.01% | 0.04% | 0.00% |

FIGURE 42 (Continued)

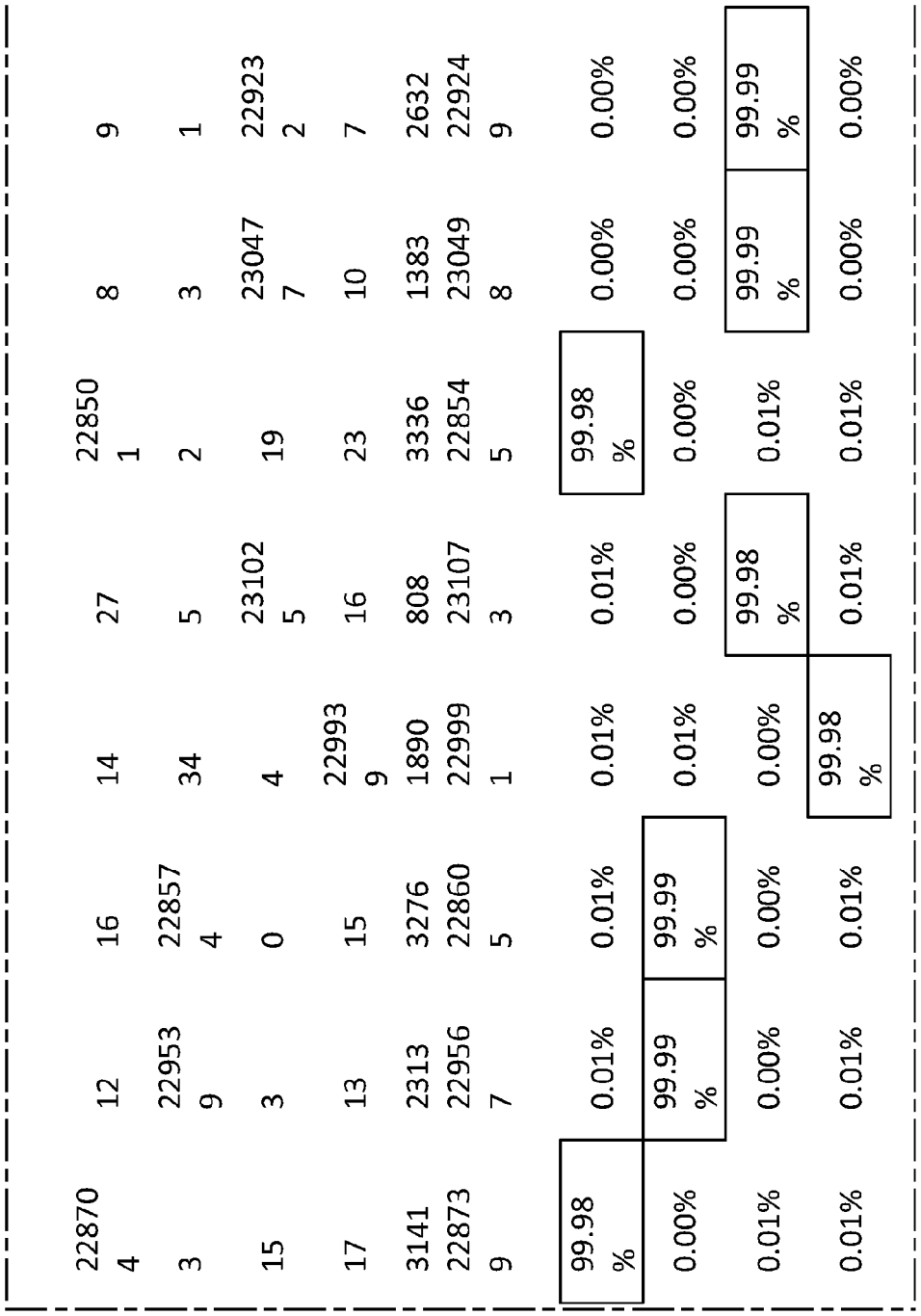
| pNMG -181 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 12 | 19 | 22902 4 | 6 | 10 | 17 | | |
| C | 3 | 11 | 22860 8 | 56 | 11 | 22935 8 | 15 | | |
| G | 23019 2 | 2 | 3 | 113 | 0 | 70 | 10 | | |
| T | 15 | 23018 9 | 56 | 21 | 23007 8 | 70 | 23073 2 | | |
| N | 1625 | 1637 | 3165 | 2637 | 1756 | 2343 | 1077 | | |
| | 23022 | 23021 | 22868 | 22921 | 23009 | 22950 | 23077 | | |
| | 2 | 4 | 6 | 4 | 5 | 8 | 4 | | |
| A | 0.01% | 0.01% | 0.01% | 99.92 % | 0.00% | 0.00% | 0.01% | | |
| C | 0.00% | 0.00% | 99.97 % | 0.02% | 0.00% | 99.93 % | 0.01% | | |
| G | 99.99 % | 0.00% | 0.00% | 0.05% | 0.00% | 0.03% | 0.00% | | |
| T | 0.01% | 99.99 % | 0.02% | 0.01% | 99.99% | 0.03% | 99.98 % | | |

FIGURE 42 (Continued)



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 12 | 22972 | 12 | 19 | 18 | 22948 | 4 | 11 |
| | 2 | | | | 8 | | |
| 22 | 27 | 1 | 20 | 230604 | 30 | 16 | 26 |
| | | 22990 | | | | | |
| 1 | 66 | 3 | 3 | 7 | 13 | 2 | 2 |
| 23131 | | | 23127 | | | 23133 | 23128 |
| 3 | 9 | 9 | 0 | 52 | 16 | 3 | 4 |
| 503 | 2027 | 1926 | 568 | 1199 | 2333 | 525 | 557 |
| 23134 | 22982 | 22992 | 23131 | 230681 | 22954 | 23135 | 23132 |
| 8 | 4 | 5 | 2 | | 7 | 5 | 3 |
| 0.01% | 99.96 % | 0.01% | 0.01% | 0.01% | 99.97 % | 0.00% | 0.00% |
| 0.01% | 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% |
| 0.00% | 0.03% | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 99.98 % | 0.00% | 0.00% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.98 % |

FIGURE 42 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 22870 4 | 12 | 16 | 14 | 27 | 22850 1 | 8 | 9 | |
| 3 | 22953 9 | 22857 4 | 34 | 5 | 2 | 3 | 1 | |
| 15 | 3 | 0 | 4 | 23102 5 | 19 | 23047 7 | 22923 2 | |
| 17 | 13 | 15 | 22993 9 | 16 | 23 | 10 | 7 | |
| 3141 | 2313 | 3276 | 1890 | 808 | 3336 | 1383 | 2632 | |
| 22873 | 22956 | 22860 | 22999 | 23107 | 22854 | 23049 | 22924 | |
| 9 | 7 | 5 | 1 | 3 | 5 | 8 | 9 | |
| 99.98 % | 0.01% | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.00% | |
| 0.00% | 99.99 % | 99.99 % | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.01% | 0.00% | 0.00% | 0.00% | 99.98 % | 0.01% | 99.99 % | 99.99 % | |
| 0.01% | 0.01% | 0.01% | 99.98 % | 0.01% | 0.01% | 0.00% | 0.00% | |

FIGURE 42 (Continued)

| pNMG-182 (pCMV_ecTadA_GGS_Cas9n_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 16 | 10 | 20 | 19621 | 4 | 20 | 13 | | |
| C | 4 | 18 | 19569 | 32 | 12 | 19615 | 21 | | |
| G | 19693 | 4 | 5 | 98 | 0 | 35 | 0 | | |
| T | 20 | 19697 | 49 | 18 | 19684 | 222 | 19742 | | |

FIGURE 42 (Continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9 | 196630 | 14 | 22 | 16 | 196503 | 9 | 9 |
| 17 | 24 | 0 | 13 | 197281 | 22 | 15 | 19 |
| 1 | 12 | 196786 | 0 | 4 | 9 | 0 | 1 |
| 19782 | 14 | 14 | 19773 | 68 | 15 | 19779 | 19776 |

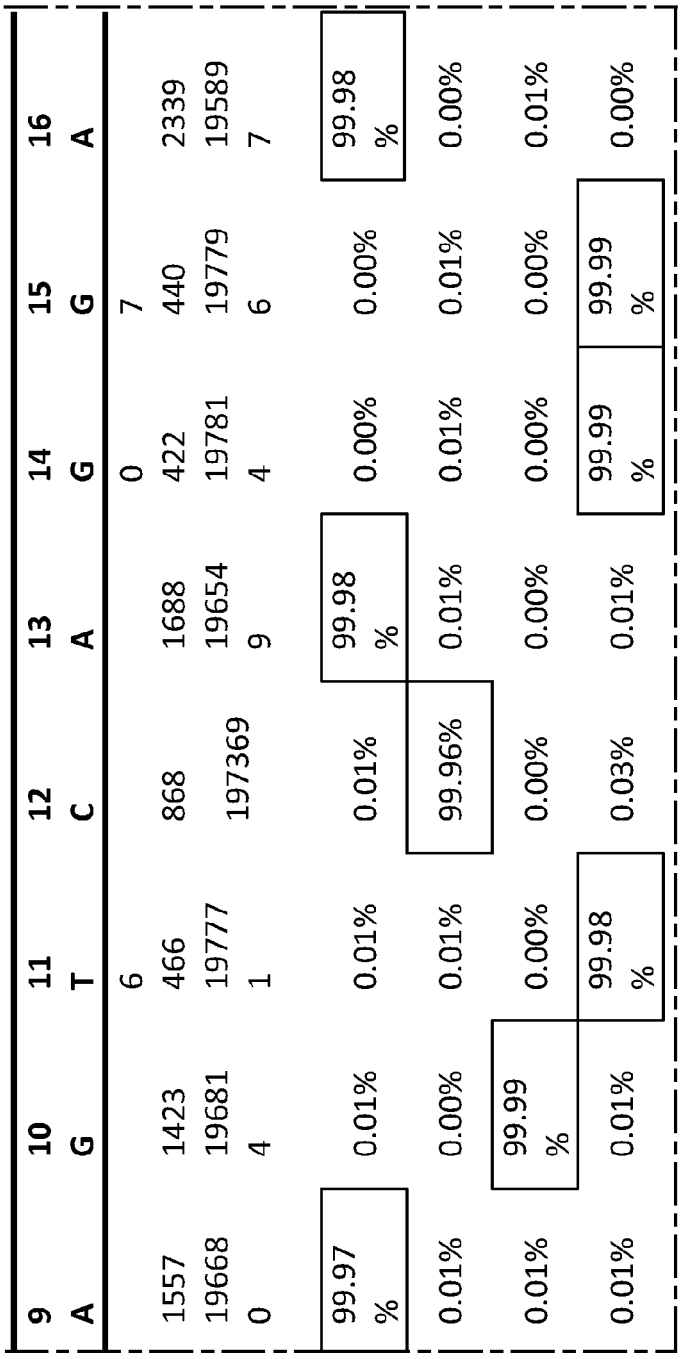
FIGURE 42 (Continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 19586 | 15 | 15 | 8 | 25 | 19566 | 9 | 12 | | |
| 7 | | | | | 0 | | | | |
| 4 | 19651 | 19569 | 43 | 3 | 2 | 3 | 2 | | |
| | 4 | 0 | | | | | | | |
| 17 | 0 | 2 | 3 | 19761 | 11 | 19715 | 19627 | | |
| | | | | 9 | | 1 | 1 | | |
| 9 | 15 | 24 | 19671 | 11 | 20 | 11 | 8 | | |

FIGURE 42 (Continued)

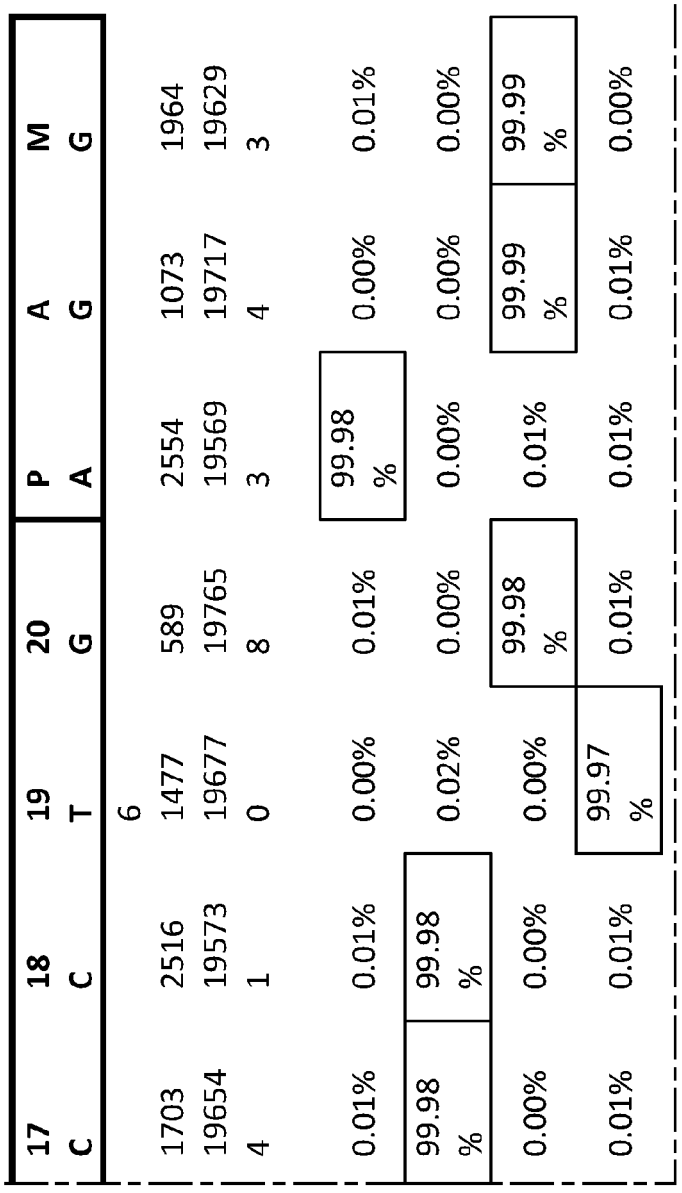
| N | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T |
|---|---|---|---|---|---|---|---|---|
| | 1265 | 1226 | 2469 | 1870 | 1377 | 1806 | 774 | 384 |
| | 19697 | 19701 | 19576 | 19636 | 19685 | 19643 | 19746 | 19785 |
| | 1 | 0 | 7 | 6 | 9 | 0 | 2 | 2 |
| A | 0.01% | 0.01% | 0.01% | 99.92 % | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.01% | 99.96 % | 0.02% | 0.01% | 99.86 % | 0.01% | 0.01% |
| G | 99.98 % | 0.00% | 0.00% | 0.05% | 0.00% | 0.02% | 0.00% | 0.00% |
| T | 0.01% | 99.98 % | 0.03% | 0.01% | 99.99% | 0.11% | 99.98 % | 99.99 % |

FIGURE 42 (Continued)



| 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G | 16 A |
|---|---|---|---|---|---|---|---|
| | | 6 | | | 0 | 7 | |
| 1557 | 1423 | 466 | 868 | 1688 | 422 | 440 | 2339 |
| 19668 | 19681 | 19777 | 197369 | 19654 | 19781 | 19779 | 19589 |
| 0 | 4 | 1 | | 9 | 4 | 6 | 7 |
| 99.97 % | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.00% | 99.98 % |
| 0.01% | 0.00% | 0.01% | 99.96% | 0.01% | 0.01% | 0.01% | 0.00% |
| 0.01% | 99.99 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.01% | 99.98 % | 0.03% | 0.01% | 99.99 % | 99.99 % | 0.00% |

FIGURE 42 (Continued)



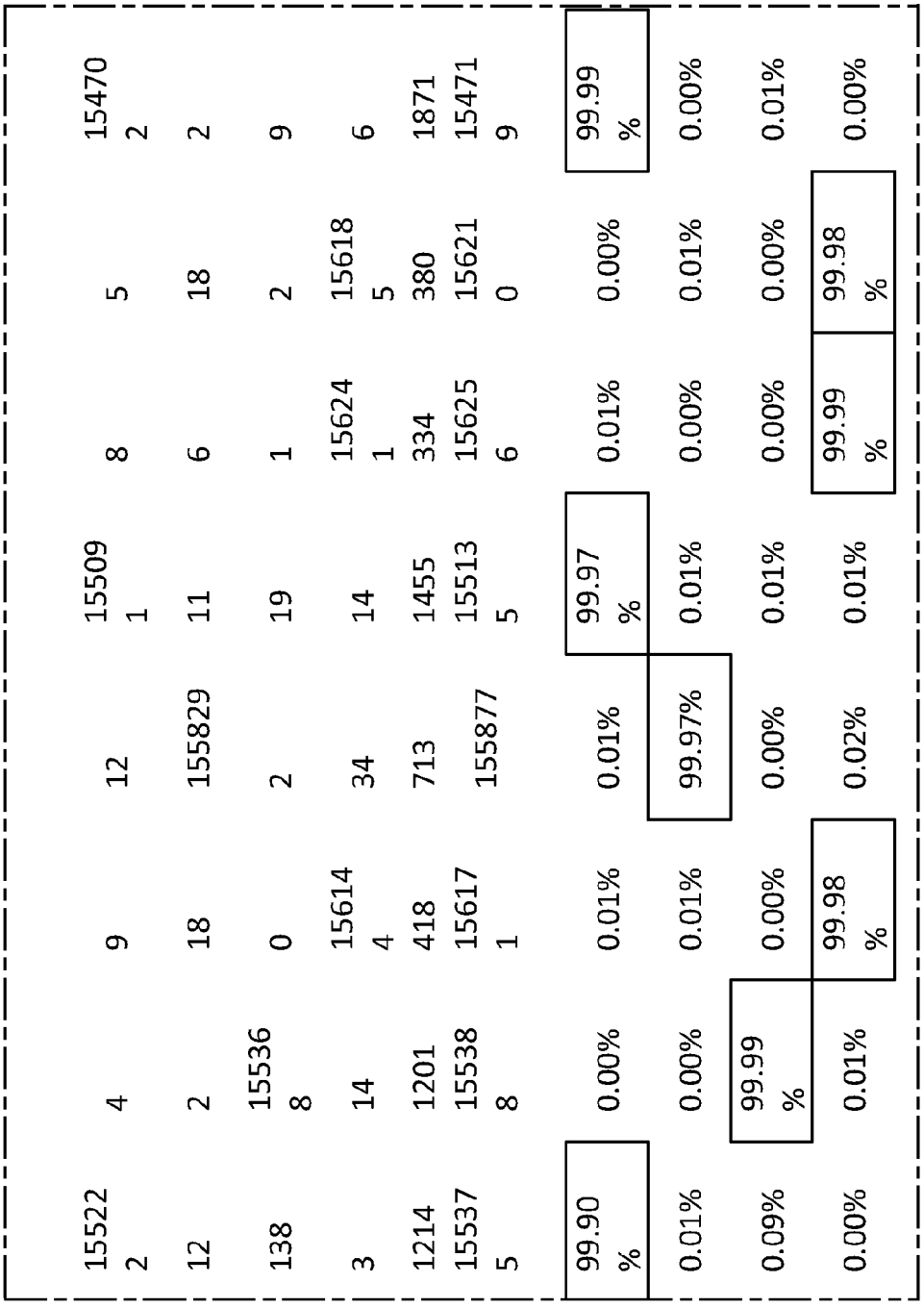
| 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|
| | | 6 | | | | |
| 1703 | 2516 | 1477 | 589 | 2554 | 1073 | 1964 |
| 19654 | 19573 | 19677 | 19765 | 19569 | 19717 | 19629 |
| 4 | 1 | 0 | 8 | 3 | 4 | 3 |
| 0.01% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% |
| 99.98% | 99.98% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% |
| 0.01% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% |

FIGURE 42 (Continued)

pNMG-183 (pCMV_ecTadA_(GGS)2-XTEN-(GGS)2_Cas9n_GGS_NLS; D108N_D147Y_E155V)

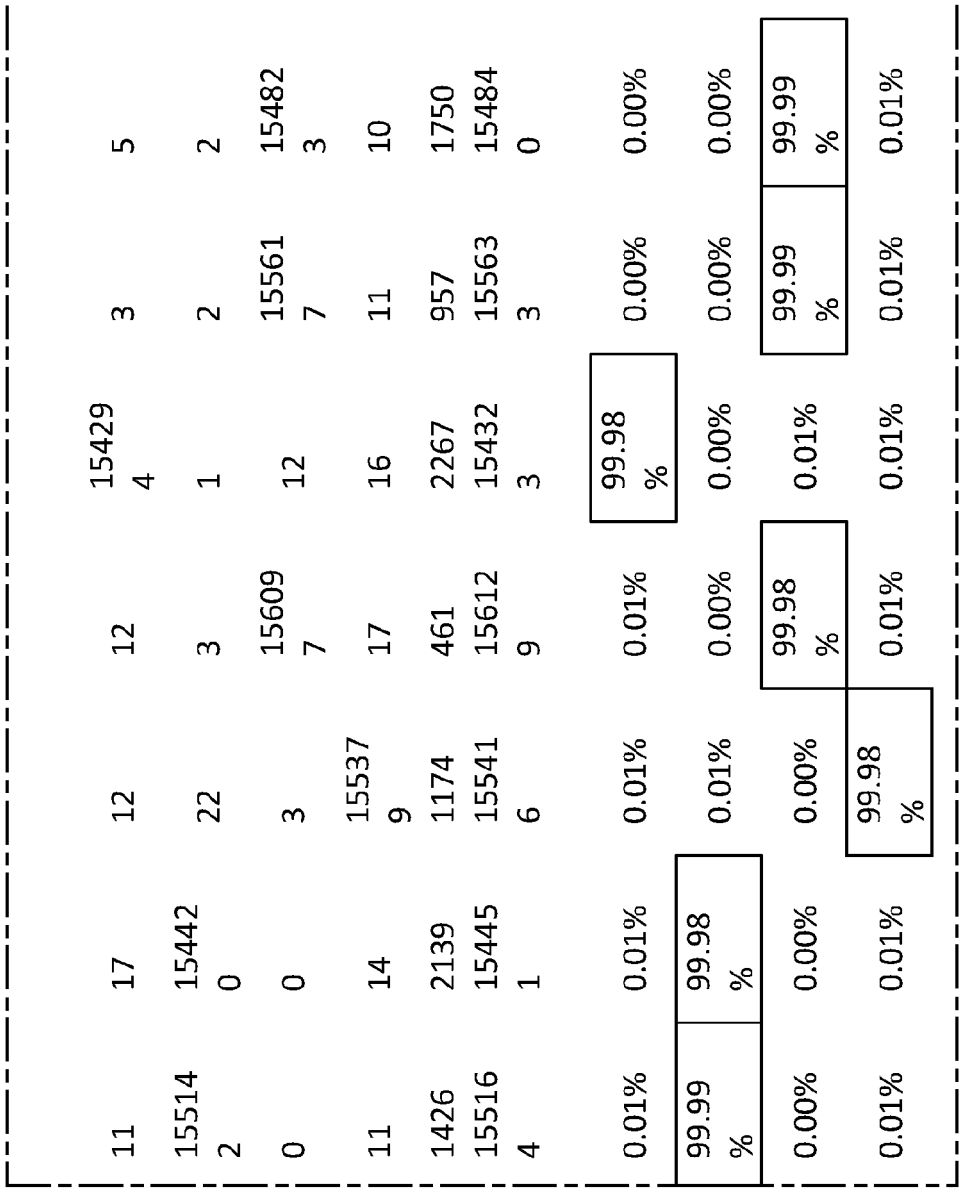
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 6 | 10 | 15496 | 5 | 12 | 8 | 1 | |
| C | 7 | 12 | 15452 | 18 | 14 | 15507 | 10 | 12 | |
| G | 15554 | 3 | 1 | 86 | 0 | 48 | 3 | 2 | |
| T | 15 | 15546 | 33 | 19 | 15542 | 193 | 15587 | 15622 | |
| N | 1013 | 1106 | 2020 | 1501 | 1145 | 1266 | 696 | 353 | |
| | 15557 | 15548 | 15456 | 15508 | 15544 | 15532 | 15589 | 15623 | |
| | 2 | 3 | 9 | 8 | 4 | 3 | 3 | 6 | |
| A | 0.00% | 0.00% | 0.01% | 99.92% | 0.00% | 0.01% | 0.01% | 0.00% | |
| C | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 99.84% | 0.01% | 0.01% | |
| G | 99.98% | 0.00% | 0.00% | 0.06% | 0.00% | 0.03% | 0.00% | 0.00% | |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.12% | 99.99% | 99.99% | |

FIGURE 42 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15522 | 4 | 9 | 12 | 15509 | 8 | 5 | | 15470 |
| 2 | | | | 1 | | | | 2 |
| 12 | 2 | 18 | 155829 | 11 | 6 | 18 | 2 | |
| 138 | 15536 | 0 | 2 | 19 | 1 | 2 | 9 | |
| | 8 | | | | | | | |
| 3 | 14 | 15614 | 34 | 14 | 15624 | 15618 | 6 | |
| | | 4 | | | 1 | 5 | | |
| 1214 | 1201 | 418 | 713 | 1455 | 334 | 380 | 1871 | |
| 15537 | 15538 | 15617 | 155877 | 15513 | 15625 | 15621 | 15471 | |
| 5 | 8 | 1 | | 5 | 6 | 0 | 9 | |
| 99.90 % | 0.00% | 0.01% | 0.01% | 99.97 % | 0.01% | 0.00% | 99.99 % | |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.00% | 0.01% | 0.00% | |
| 0.09% | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | |
| 0.00% | 0.01% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.98 % | 0.00% | |

FIGURE 42 (Continued)



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 11 | 17 | 12 | 12 | 15429 | 3 | 5 | |
| 15514 | 15442 | 22 | 3 | 1 | 2 | 2 | |
| 2 | 0 | | | 4 | | | |
| 0 | 0 | 3 | 15609 | 12 | 15561 | 15482 | |
| | | | 7 | | 7 | 3 | |
| 11 | 14 | 15537 | 17 | 16 | 11 | 10 | |
| | | 9 | | | | | |
| 1426 | 2139 | 1174 | 461 | 2267 | 957 | 1750 | |
| 15516 | 15445 | 15541 | 15612 | 15432 | 15563 | 15484 | |
| 4 | 1 | 6 | 9 | 3 | 3 | 0 | |
| 0.01% | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.00% | |
| 99.99 % | 99.98 % | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 99.98 % | 0.01% | 99.99 % | 99.99 % | |
| 0.01% | 0.01% | 99.98 % | 0.01% | 0.01% | 0.01% | 0.01% | |

FIGURE 42 (Continued)

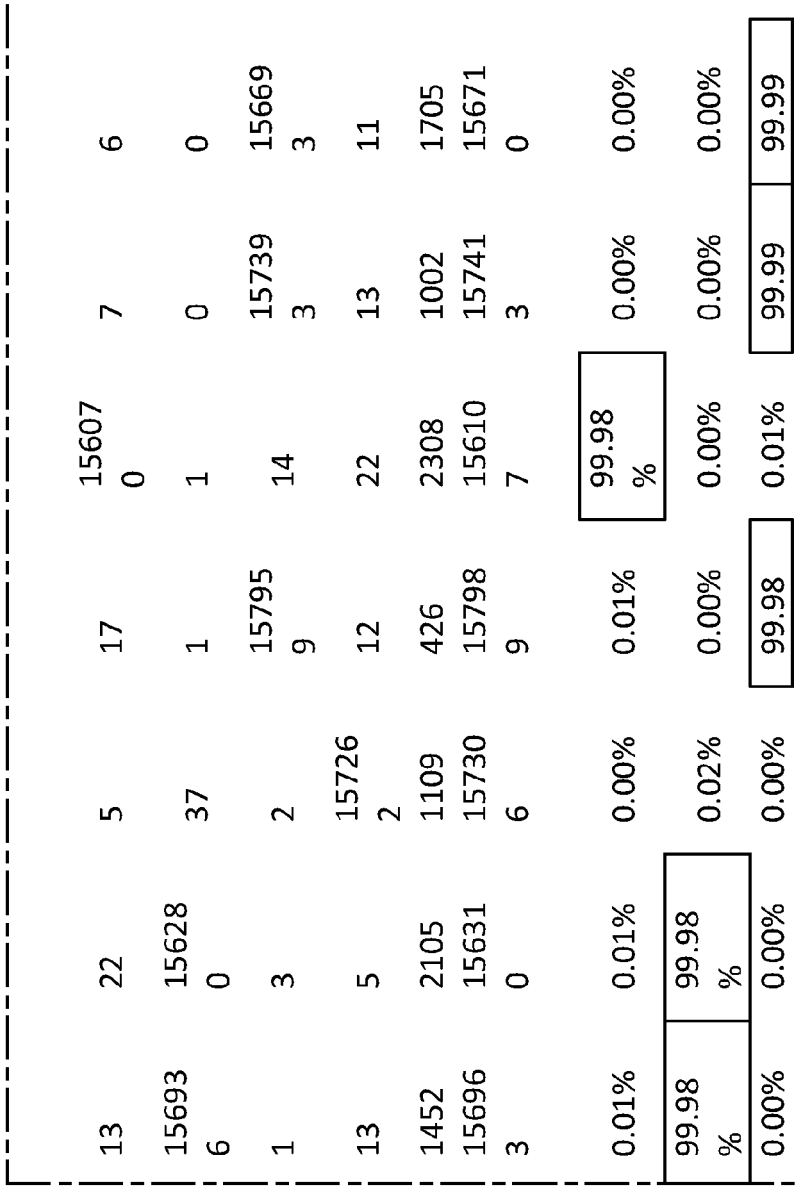
pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 7 | 21 | 15675 | 2 | 17 | 9 | 5 | |
| C | 2 | 14 | 15640 | 43 | 11 | 15656 | 5 | 13 | |
| G | 15733 | 3 | 2 | 77 | 1 | 76 | 4 | 1 | |
| T | 21 | 15722 | 23 | 8 | 15721 | 228 | 15769 | 15805 | |
| N | 1036 | 1154 | 1951 | 1527 | 1176 | 1522 | 702 | 337 | |
| | 15736 | 15725 | 15645 | 15687 | 15722 | 15688 | 15771 | 15807 | |
| | 6 | 1 | 4 | 8 | 9 | 9 | 0 | 7 | |
| A | 0.01% | 0.00% | 0.01% | 99.92% | 0.00% | 0.01% | 0.01% | 0.00% | |
| C | 0.00% | 0.01% | 99.97% | 0.03% | 0.01% | 99.80% | 0.00% | 0.01% | |
| G | 99.98 | 0.00% | 0.00% | 0.05% | 0.00% | 0.05% | 0.00% | 0.00% | |

FIGURE 42 (Continued)

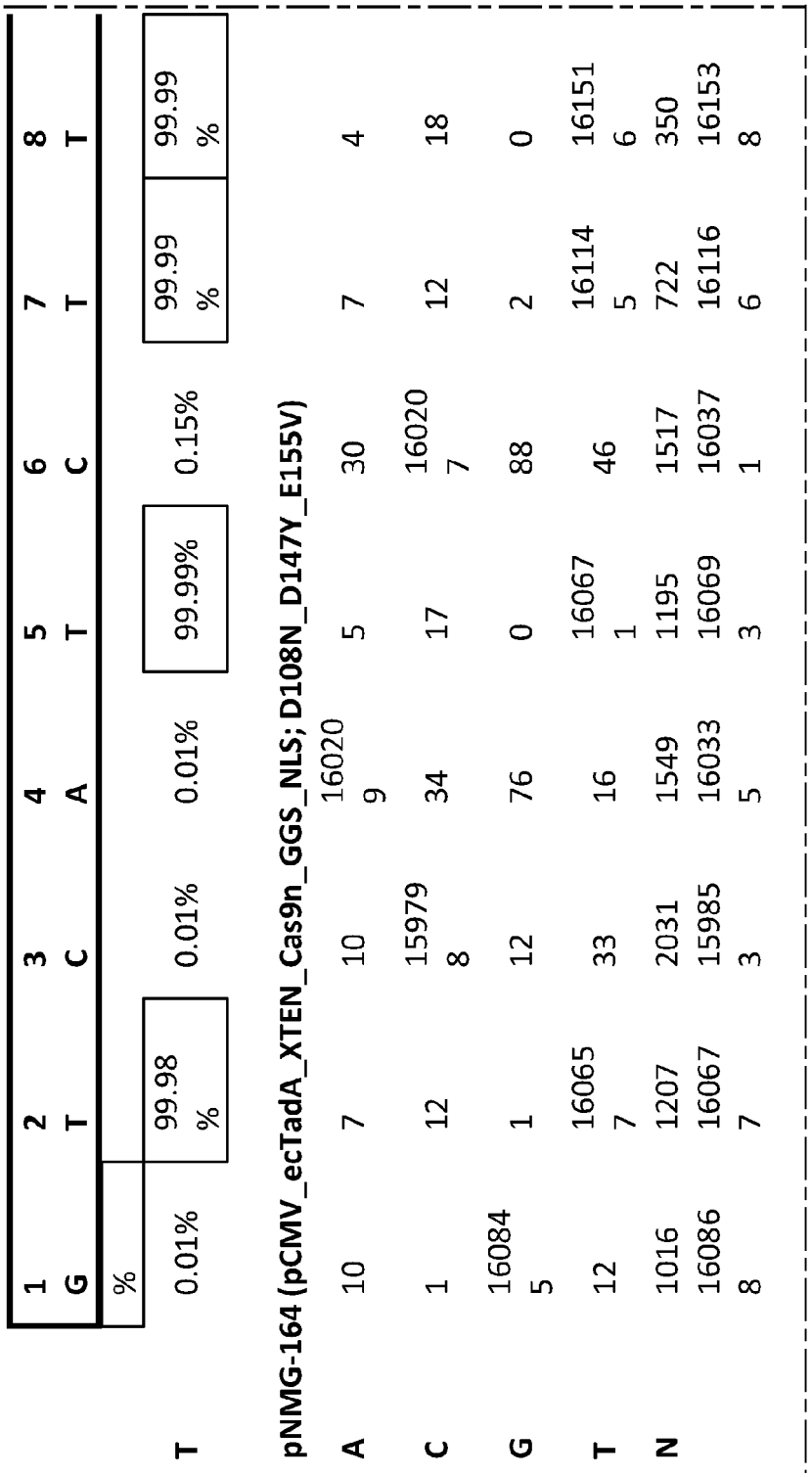
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15707 | 10 | 15 | 12 | 15686 | 5 | 3 | 15645 | |
| 3 | | | | 1 | | | 8 | |
| 22 | 3 | 15 | 157611 | 24 | 5 | 13 | 3 | |
| 62 | 15712 | 0 | 6 | 19 | 0 | 2 | 10 | |
| | 4 | | | | | | | |
| 7 | 11 | 15796 | 52 | 18 | 15809 | 15802 | 8 | |
| | | 9 | | | 8 | 2 | | |
| 1250 | 1266 | 415 | 733 | 1493 | 307 | 375 | 1936 | |
| 15716 | 15714 | 15799 | 157681 | 15692 | 15810 | 15804 | 15647 | |
| 4 | 8 | 9 | | 2 | 8 | 0 | 9 | |
| 99.94 % | 0.01% | 0.01% | 0.01% | 99.96 % | 0.00% | 0.00% | 99.99 % | |
| 0.01% | 0.00% | 0.01% | 99.96% | 0.02% | 0.00% | 0.01% | 0.00% | |
| 0.04% | 99.98 | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | |

FIGURE 42 (Continued)



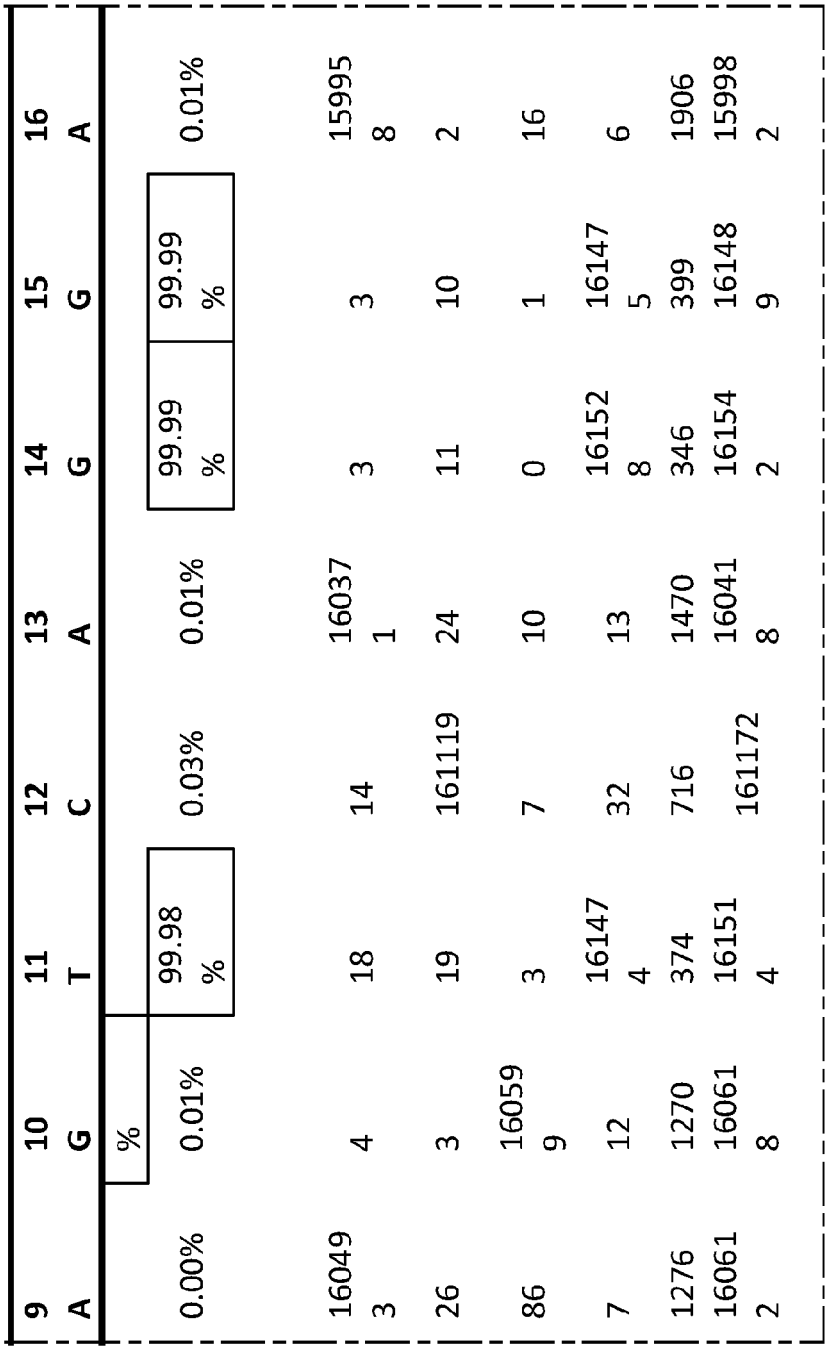
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 13 | 22 | 5 | 17 | 15607 | 7 | 6 | |
| 15693 | 15628 | 37 | 1 | 1 | 0 | 0 | |
| 6 | 0 | | | 0 | | | |
| 1 | 3 | 2 | 15795 | 14 | 15739 | 15669 | |
| | | | 9 | | 3 | 3 | |
| 13 | 5 | 15726 | 12 | 22 | 13 | 11 | |
| | | 2 | | | | | |
| 1452 | 2105 | 1109 | 426 | 2308 | 1002 | 1705 | |
| 15696 | 15631 | 15730 | 15798 | 15610 | 15741 | 15671 | |
| 3 | 0 | 6 | 9 | 7 | 3 | 0 | |
| 0.01% | 0.01% | 0.00% | 0.01% | 99.98 % | 0.00% | 0.00% | |
| 99.98 % | 99.98 % | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 99.98 | 0.01% | 99.99 | 99.99 | |

FIGURE 42 (Continued)



| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T |
|---|---|---|---|---|---|---|---|---|
| | % | | | | | | | |
| T | 0.01% | 99.98 % | 0.01% | 0.01% | 99.99% | 0.15% | 99.99 % | 99.99 % |
| pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | |
| A | 10 | 7 | 10 | 16020 9 | 5 | 30 | 7 | 4 |
| C | 1 | 12 | 15979 8 | 34 | 17 | 16020 7 | 12 | 18 |
| G | 16084 5 | 1 | 12 | 76 | 0 | 88 | 2 | 0 |
| T | 12 | 16065 7 | 33 | 16 | 16067 1 | 46 | 16114 5 | 16151 6 |
| N | 1016 | 1207 | 2031 | 1549 | 1195 | 1517 | 722 | 350 |
| | 16086 8 | 16067 7 | 15985 3 | 16033 5 | 16069 3 | 16037 1 | 16116 6 | 16153 8 |

FIGURE 42 (Continued)



| 9 A | 10 G | % | 11 T | 12 C | 13 A | 14 G | 15 G | 16 A |
|---|---|---|---|---|---|---|---|---|
| 0.00% | 0.01% | | 99.98 % | 0.03% | 0.01% | 99.99 % | 99.99 % | 0.01% |
| 16049 3 | 4 | | 18 | 14 | 16037 1 | 3 | 3 | 15995 8 |
| 26 | 3 | | 19 | 161119 | 24 | 11 | 10 | 2 |
| 86 | 16059 9 | | 3 | 7 | 10 | 0 | 1 | 16 |
| 7 | 12 | | 16147 4 | 32 | 13 | 16152 8 | 16147 5 | 6 |
| 1276 | 1270 | | 374 | 716 | 1470 | 346 | 399 | 1906 |
| 16061 2 | 16061 8 | | 16151 4 | 161172 | 16041 8 | 16154 2 | 16148 9 | 15998 2 |

FIGURE 42 (Continued)

| 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|
| 0.01% | 0.00% | 99.97 % | 0.01% | 0.01% | 0.01% | 0.01% |
| 9 | 10 | 5 | 14 | 15963 9 | 3 | 12 |
| 16039 4 | 15965 3 | 29 | 4 | 5 | 0 | 1 |
| 2 | 2 | 0 | 16138 8 | 16 | 16096 9 | 16012 7 |
| 16 | 5 | 16063 3 | 9 | 22 | 7 | 6 |
| 1467 | 2218 | 1221 | 473 | 2206 | 909 | 1742 |
| 16042 | 15967 | 16066 | 16141 | 15968 | 16097 | 16014 |
| 1 | 0 | 7 | 5 | 2 | 9 | 6 |

FIGURE 42 (Continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 0.00% | 0.01% | 99.92% | 0.00% | 0.02% | 0.00% | 0.00% | |
| C | 0.00% | 0.01% | 99.97% | 0.02% | 0.01% | 99.90% | 0.01% | 0.01% | |
| G | 99.99% | 0.00% | 0.01% | 0.05% | 0.00% | 0.05% | 0.00% | 0.00% | |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.03% | 99.99% | 99.99% | |

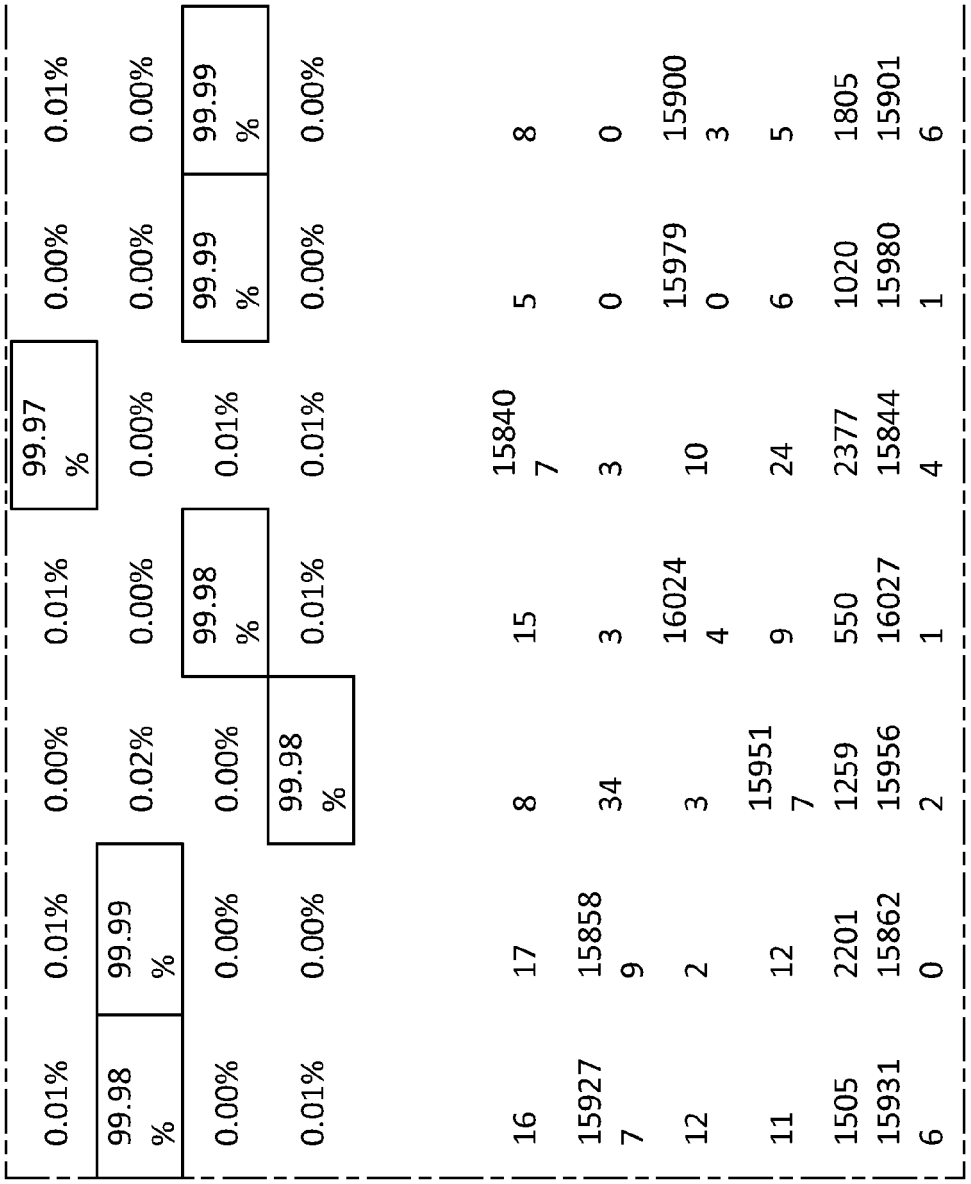
**pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)/ pNMG-260**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 10 | 9 | 12 | 15918 | 2 | 27 | 8 | 9 | |
| C | 3 | 15 | 15873 | 21 | 21 | 15913 | 13 | 13 | |
| G | 15973 | 1 | 1 | 7 | 0 | 16 | 2 | 1 | |
| T | 16 | 15970 | 47 | 12 | 15961 | 55 | 16010 | 16040 | |
| N | 1054 | 1095 | 2024 | 1600 | 1187 | 1590 | 690 | 389 | |
| | 15976 | 15972 | 15879 | 15922 | 15963 | 15923 | 16013 | 16043 | |
| | 5 | 5 | 6 | 1 | 4 | 1 | 1 | 2 | |

FIGURE 42 (Continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 99.93% | 0.00% | 0.01% | 0.01% | 99.97% | 0.00% | 0.00% | 99.98% | | |
| 0.02% | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% | | |
| 0.05% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | | |
| 0.00% | 0.01% | 99.98% | 0.02% | 0.01% | 99.99% | 99.99% | 0.00% | | |
| 159462 | 15 | 12 | 8 | 159225 | 5 | 9 | 15871 | | |
| 15 | 0 | 14 | 160044 | 16 | 10 | 7 | 5 | | |
| 11 | 159489 | 1 | 4 | 11 | 2 | 1 | 15 | | |
| 7 | 7 | 160431 | 26 | 12 | 160476 | 160391 | 13 | | |
| 1326 | 1310 | 363 | 739 | 1557 | 328 | 413 | 2077 | | |
| 15949 | 15951 | 16045 | 160082 | 15926 | 16049 | 16040 | 15874 | | |
| 5 | 1 | 8 | 4 | 4 | 3 | 8 | 4 | | |

FIGURE 42 (Continued)



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.01% | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.01% | | | |
| 99.98% | 99.99% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | | | |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% | | | |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | | | |
| 16 | 17 | 8 | 15 | 158407 | 5 | 8 | | | |
| 159277 | 158589 | 34 | 3 | 3 | 0 | 0 | | | |
| 12 | 2 | 3 | 160244 | 10 | 159790 | 159003 | | | |
| 11 | 12 | 159517 | 9 | 24 | 6 | 5 | | | |
| 1505 | 2201 | 1259 | 550 | 2377 | 1020 | 1805 | | | |
| 15931 | 15862 | 15956 | 16027 | 15844 | 15980 | 15901 | | | |
| 6 | 0 | 2 | 1 | 4 | 1 | 6 | | | |

FIGURE 42 (Continued)

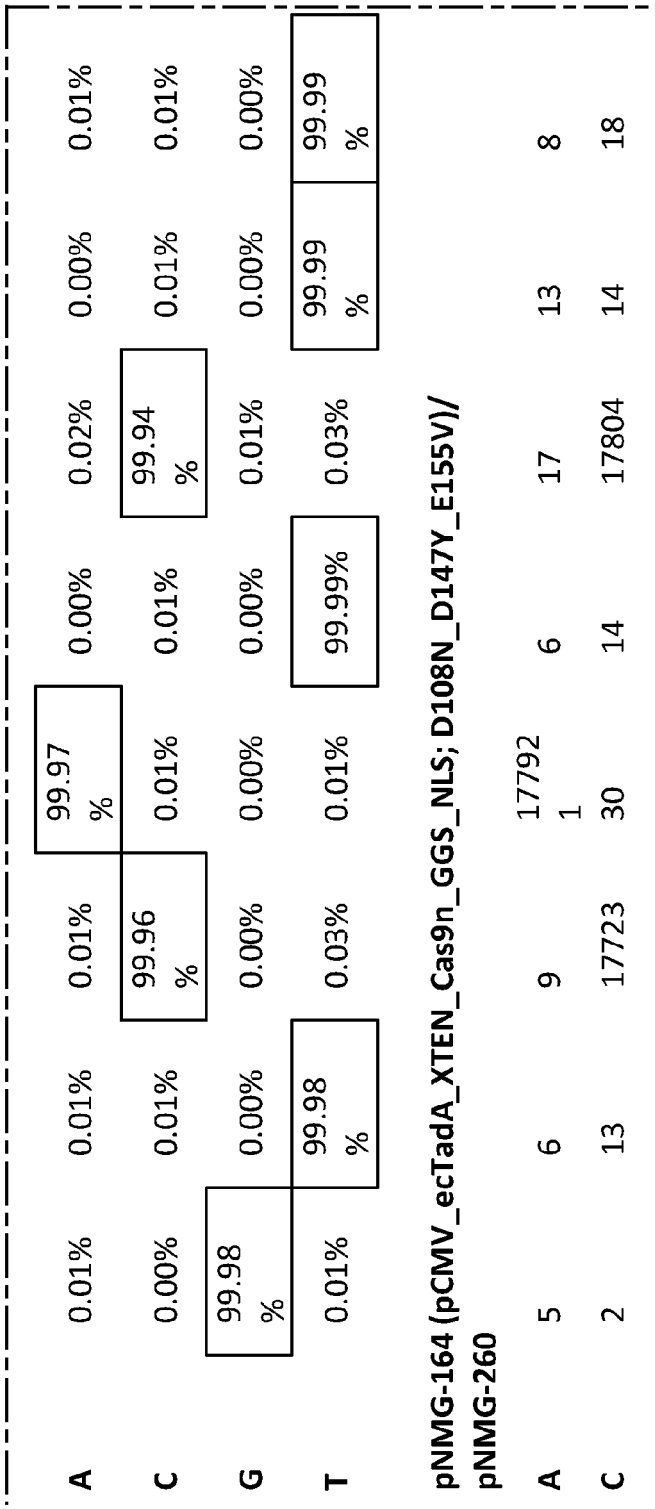


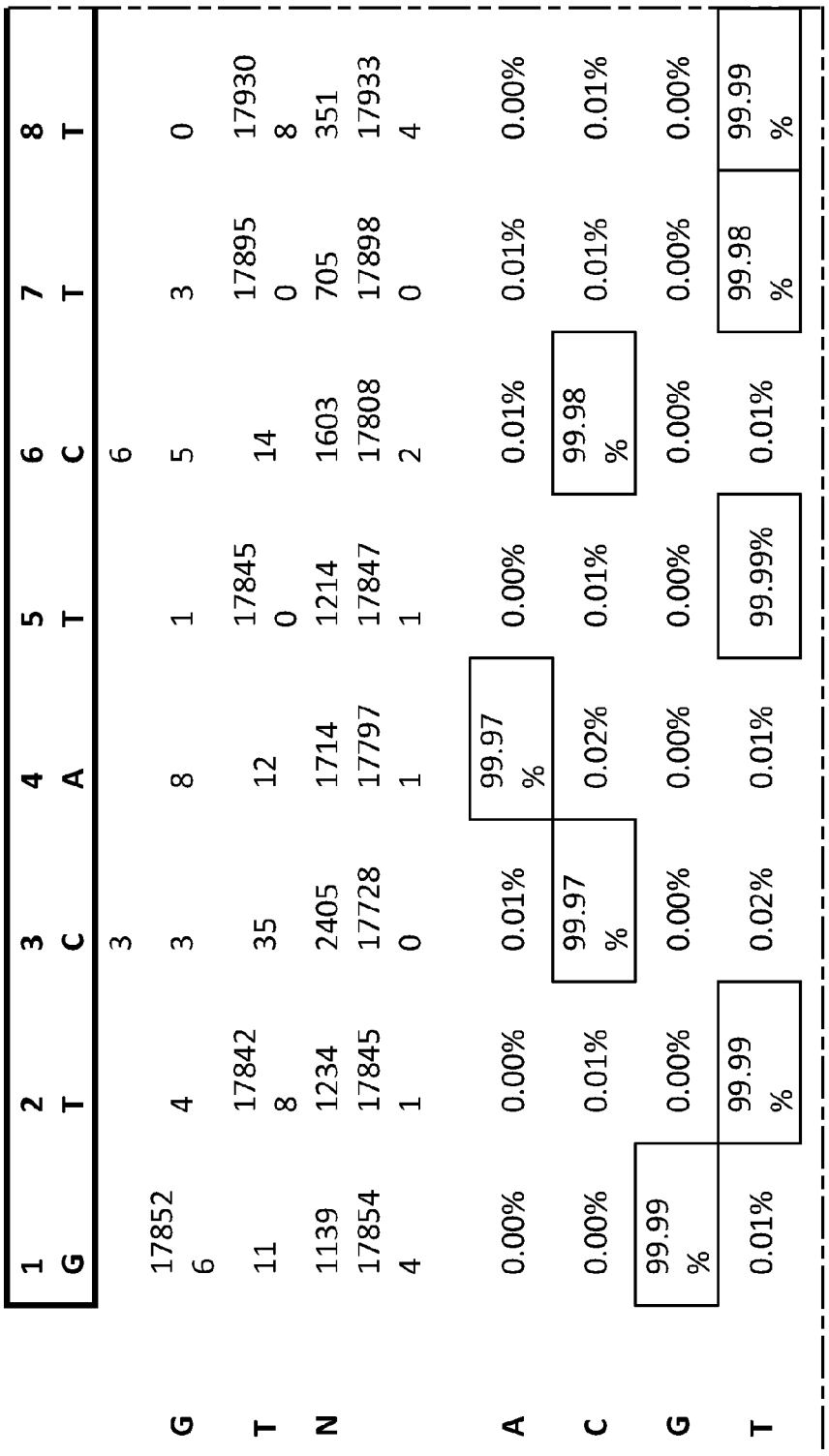
FIGURE 42 (Continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 99.98 % | 0.01% | 0.01% | 0.00% | 99.98 % | 0.00% | 0.01% | 99.98 % |
| 0.01% | 0.00% | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% |
| 0.01% | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.00% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.99 % | 0.01% |
| 178317 | 14 | 17 | 14 | 177953 | 10 | 5 | 177448 |
| 16 | 2 | 12 | 178731 | 19 | 7 | 15 | 4 |

FIGURE 42 (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.01% | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.01% |
| 99.98 % | 99.98 % | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 99.98 % | 0.01% | 99.99 % | 99.99 % |
| 0.01% | 0.01% | 99.97 % | 0.01% | 0.02% | 0.00% | 0.00% |
| 12 | 19 | 12 | 13 | 17719 8 | 5 | 5 |
| 17795 | 17716 | 28 | 9 | 0 | 4 | 0 |

FIGURE 42 (Continued)

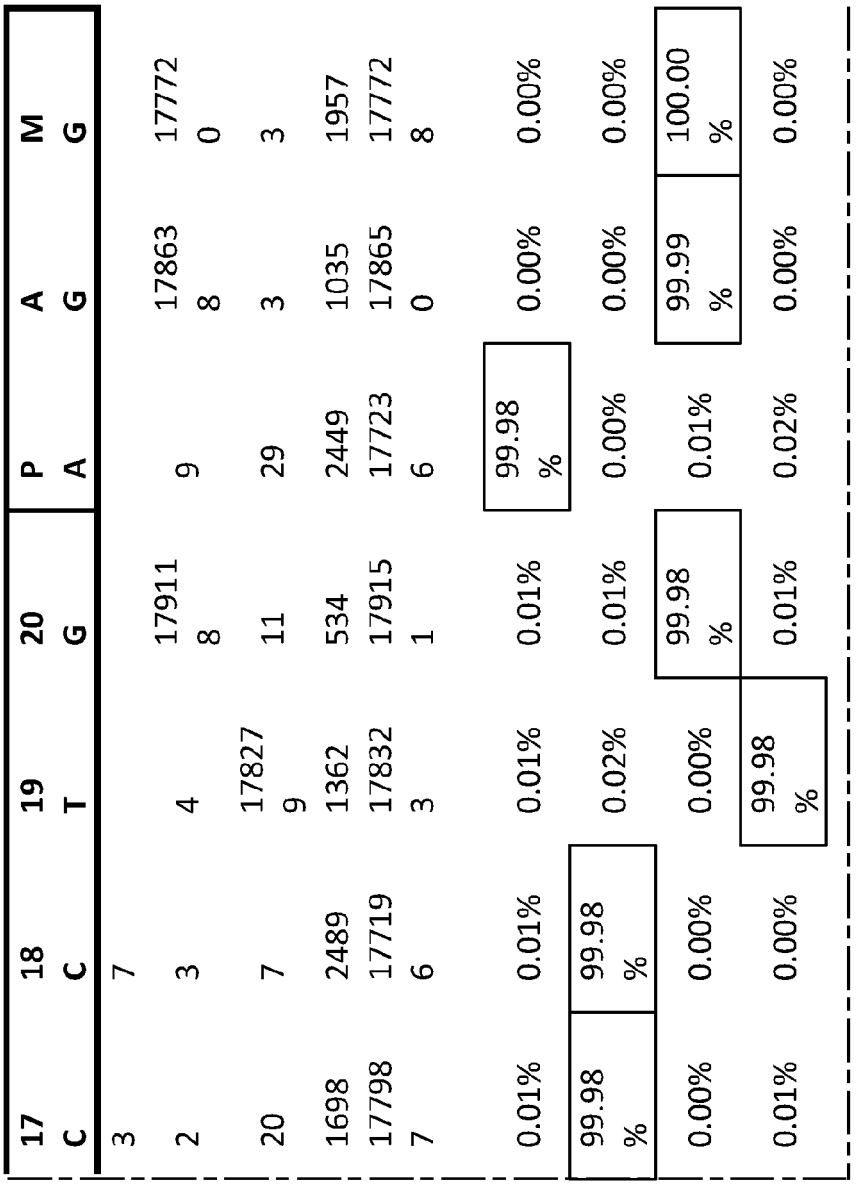


| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T |
|---|---|---|---|---|---|---|---|---|
| | | | 3 | | | 6 | | |
| G | 17852 6 | 4 | 3 | 8 | 1 | 5 | 3 | 0 |
| T | 11 | 17842 8 | 35 | 12 | 17845 0 | 14 | 17895 0 | 17930 8 |
| N | 1139 | 1234 | 2405 | 1714 | 1214 | 1603 | 705 | 351 |
| | 17854 4 | 17845 1 | 17728 0 | 17797 1 | 17847 1 | 17808 2 | 17898 0 | 17933 4 |
| A | 0.00% | 0.00% | 0.01% | 99.97 % | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.01% | 99.97 % | 0.02% | 0.01% | 99.98 % | 0.01% | 0.01% |
| G | 99.99 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 99.99 % | 0.02% | 0.01% | 99.99% | 0.01% | 99.98 % | 99.99 % |

FIGURE 42 (Continued)

| 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G | 16 A |
|---|---|---|---|---|---|---|---|
| 11 | 17825 1 | 0 | 4 | 15 | 0 | 1 | 11 |
| 3 | 13 | 17916 1 | 39 | 12 | 17930 4 | 17921 4 | 16 |
| 1338 | 1405 | 495 | 897 | 1686 | 364 | 450 | 2206 |
| 17834 | 17828 | 17919 | 178788 | 17799 | 17932 | 17923 | 17747 |
| 7 | 0 | 0 | | 9 | 1 | 5 | 9 |
| 99.98 % | 0.01% | 0.01% | 0.01% | 99.97 % | 0.01% | 0.00% | 99.98 % |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.00% | 0.01% | 0.00% |
| 0.01% | 99.98 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.01% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.99 % | 0.01% |

FIGURE 42 (Continued)



| 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|
| 3 | 7 | | | | | |
| 2 | 3 | 4 | 17911 8 | 9 | 17863 8 | 17772 0 |
| 20 | 7 | 17827 9 | 11 | 29 | 3 | 3 |
| 1698 | 2489 | 1362 | 534 | 2449 | 1035 | 1957 |
| 17798 | 17719 | 17832 | 17915 | 17723 | 17865 | 17772 |
| 7 | 6 | 3 | 1 | 6 | 0 | 8 |
| 0.01% | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.00% |
| 99.98 % | 99.98 % | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98 % | 0.01% | 99.99 % | 100.00 % |
| 0.01% | 0.00% | 99.98 % | 0.01% | 0.02% | 0.00% | 0.00% |

FIGURE 42 (Continued)

**pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)/ pNMG-260**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **A** | 10 | 12 | 21 | 17232 | 7 | 19 | 10 | 4 | |
| **C** | 3 | 13 | 17178 | 24 | 19 | 17225 | 22 | 14 | |
| **G** | 17288 | 3 | 2 | 6 | 0 | 64 | 0 | 0 | |
| **T** | 26 | 17277 | 84 | 14 | 17278 | 96 | 17329 | 17365 | |
| **N** | 1149 | 1270 | 2181 | 1707 | 1266 | 1648 | 752 | 403 | |
| | 17292 | 17280 | 17189 | 17237 | 17281 | 17243 | 17332 | 17367 | |
| | 6 | 5 | 5 | 1 | 2 | 0 | 6 | 5 | |
| **A** | 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% | |
| **C** | 0.00% | 0.01% | 99.94% | 0.01% | 0.01% | 99.90% | 0.01% | 0.01% | |
| **G** | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | |
| **T** | 0.02% | 99.98% | 0.05% | 0.01% | 99.98% | 0.06% | 99.98% | 99.99% | |

FIGURE 42 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17264 | 4 | 15 | 21 | 17233 | 12 | 4 | | 17175 |
| 8 | | | | 7 | | | | 5 |
| 15 | 5 | 7 | 173113 | 17 | 14 | 18 | | 2 |
| 12 | 17265 | 2 | 6 | 9 | 2 | 1 | | 16 |
| | 7 | | | | | | | |
| 15 | 7 | 17356 | 50 | 18 | 17369 | 17366 | | 11 |
| | | 8 | | | 9 | 1 | | |
| 1388 | 1405 | 486 | 888 | 1697 | 351 | 394 | | 2294 |
| 17269 | 17267 | 17359 | 173190 | 17238 | 17372 | 17368 | | 17178 |
| 0 | 3 | 2 | | 1 | 7 | 4 | | 4 |
| 99.98 % | 0.00% | 0.01% | 0.01% | 99.97 % | 0.01% | 0.00% | | 99.98 % |
| 0.01% | 0.00% | 0.00% | 99.96% | 0.01% | 0.01% | 0.01% | | 0.00% |
| 0.01% | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | | 0.01% |
| 0.01% | 0.00% | 99.99 % | 0.03% | 0.01% | 99.98 % | 99.99 % | | 0.01% |

FIGURE 42 (Continued)

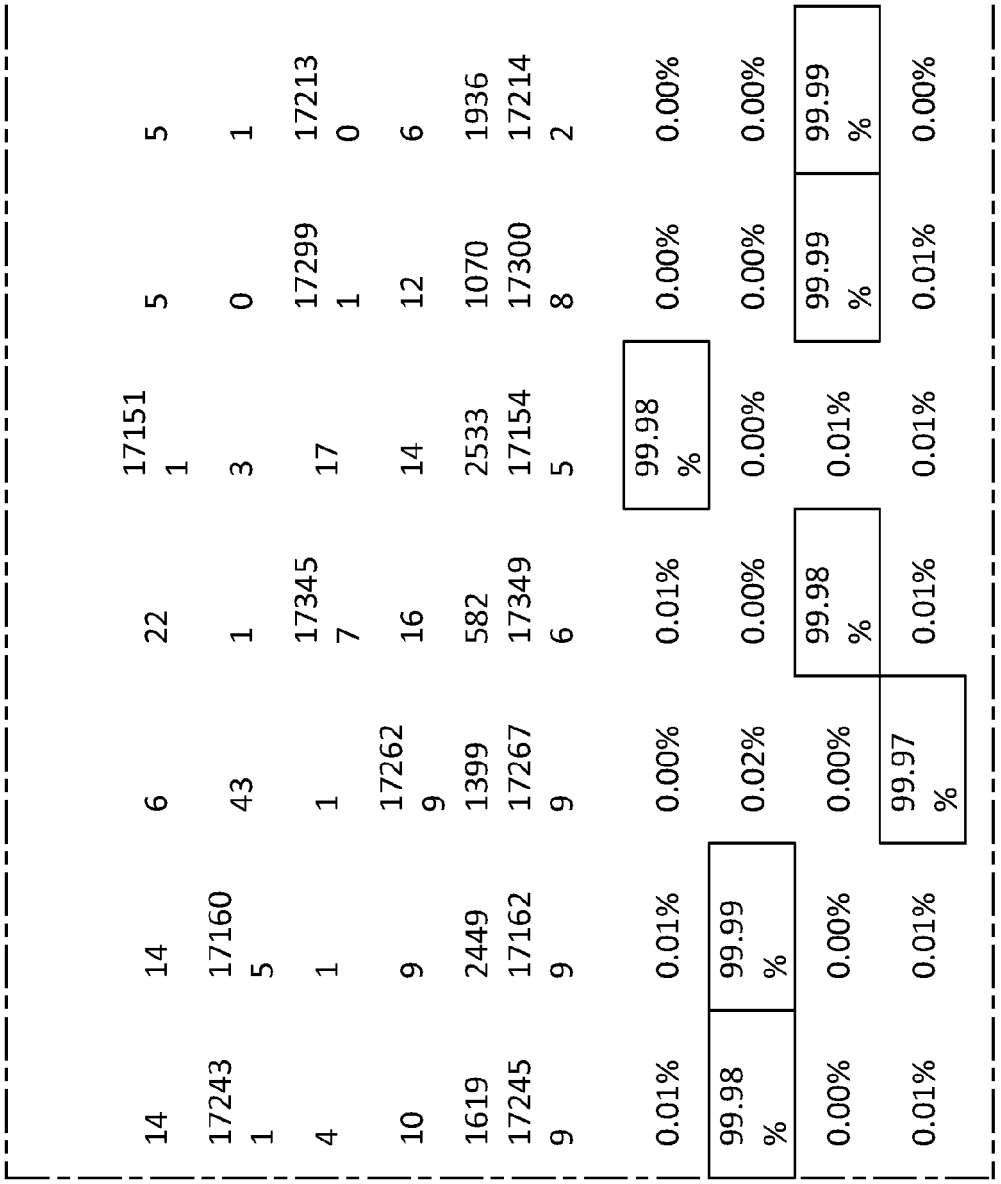


FIGURE 42 (Continued)

| pNMG-180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V)/ pNMG-260 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 10 | 19 | 169899 | 3 | 15 | 11 | 4 | |
| C | 3 | 11 | 169490 | 22 | 13 | 170007 | 12 | 23 | |
| G | 170625 | 2 | 3 | 10 | 1 | 23 | 1 | 0 | |
| T | 16 | 170504 | 37 | 12 | 170541 | 38 | 171106 | 171429 | |
| N | 1232 | 1354 | 2332 | 1938 | 1323 | 1798 | 751 | 425 | |
| | 170649 | 170527 | 169540 | 169943 | 170558 | 170083 | 171136 | 171456 | |

FIGURE 42 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17031 | 11 | 21 | 10 | 17005 | 1 | 10 | 16941 | |
| 0 | | | | 4 | | | 9 | |
| 17 | 1 | 19 | 170933 | 17 | 11 | 15 | 8 | |
| 14 | 17035 | 1 | 2 | 6 | 2 | 1 | 14 | |
| | 5 | | | | | | | |
| 9 | 7 | 17135 | 39 | 11 | 17144 | 17140 | 13 | |
| | | 6 | | | 3 | 1 | | |
| 1531 | 1507 | 484 | 897 | 1793 | 423 | 453 | 2426 | |
| 17035 | 17037 | 17139 | 170984 | 17008 | 17145 | 17142 | 16945 | |
| 0 | 4 | 7 | | 8 | 7 | 7 | 4 | |

FIGURE 42 (Continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9 | 10 | 4 | 25 | 169120 | 9 | 4 | |
| 17006 3 | 169277 | 25 | 5 | 2 | 1 | 3 | |
| 2 | 1 | 2 | 171260 | 16 | 170774 | 16974 7 | |
| 14 | 6 | 170413 | 10 | 17 | 4 | 7 | |
| 1792 | 2586 | 1436 | 580 | 2725 | 1092 | 2119 | |
| 17008 | 16929 | 17044 | 17130 | 16915 | 17078 | 16976 | |
| 8 | 4 | 4 | 0 | 5 | 8 | 1 | |

FIGURE 42 (Continued)

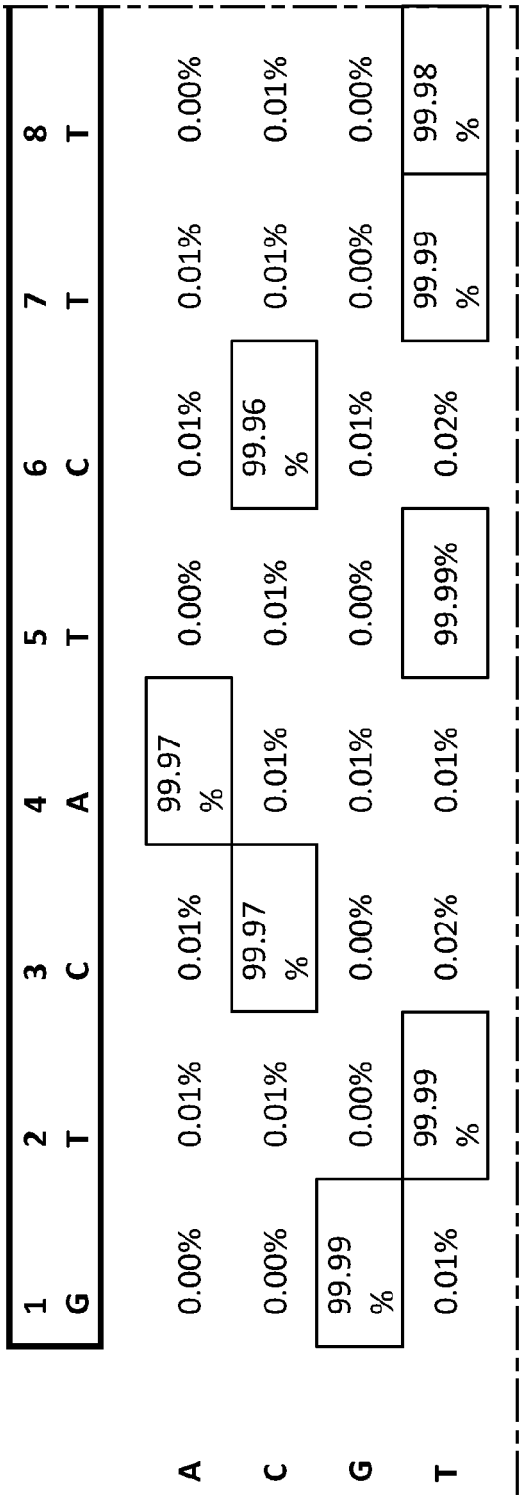


FIGURE 42 (Continued)

| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|
| A | G | T | C | A | G | G | A |
| 99.98 % | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.01% | 99.98 % |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% |
| 0.01% | 99.99 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.98 % | 0.01% |

FIGURE 42 (Continued)

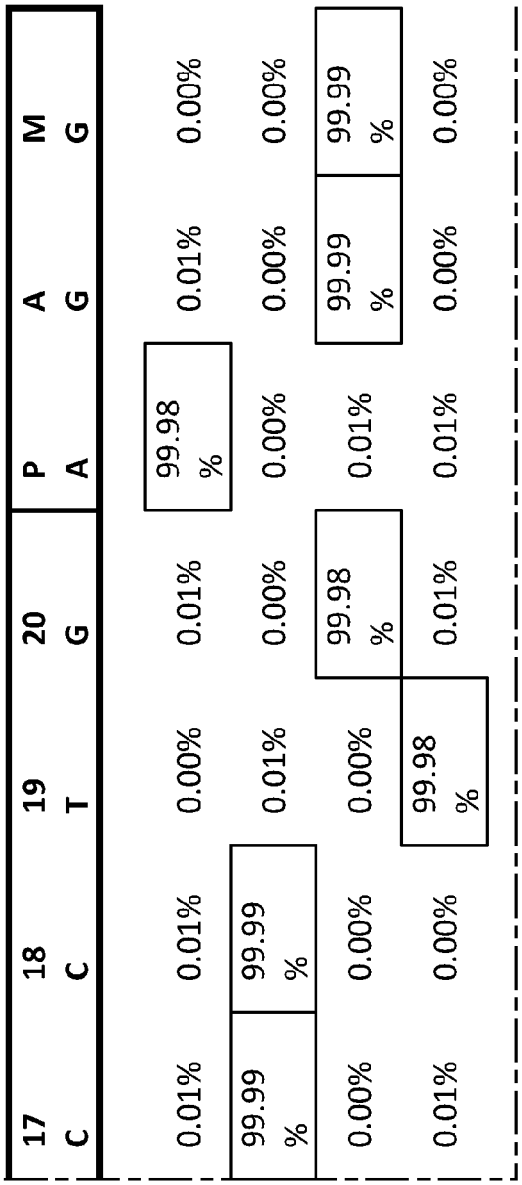
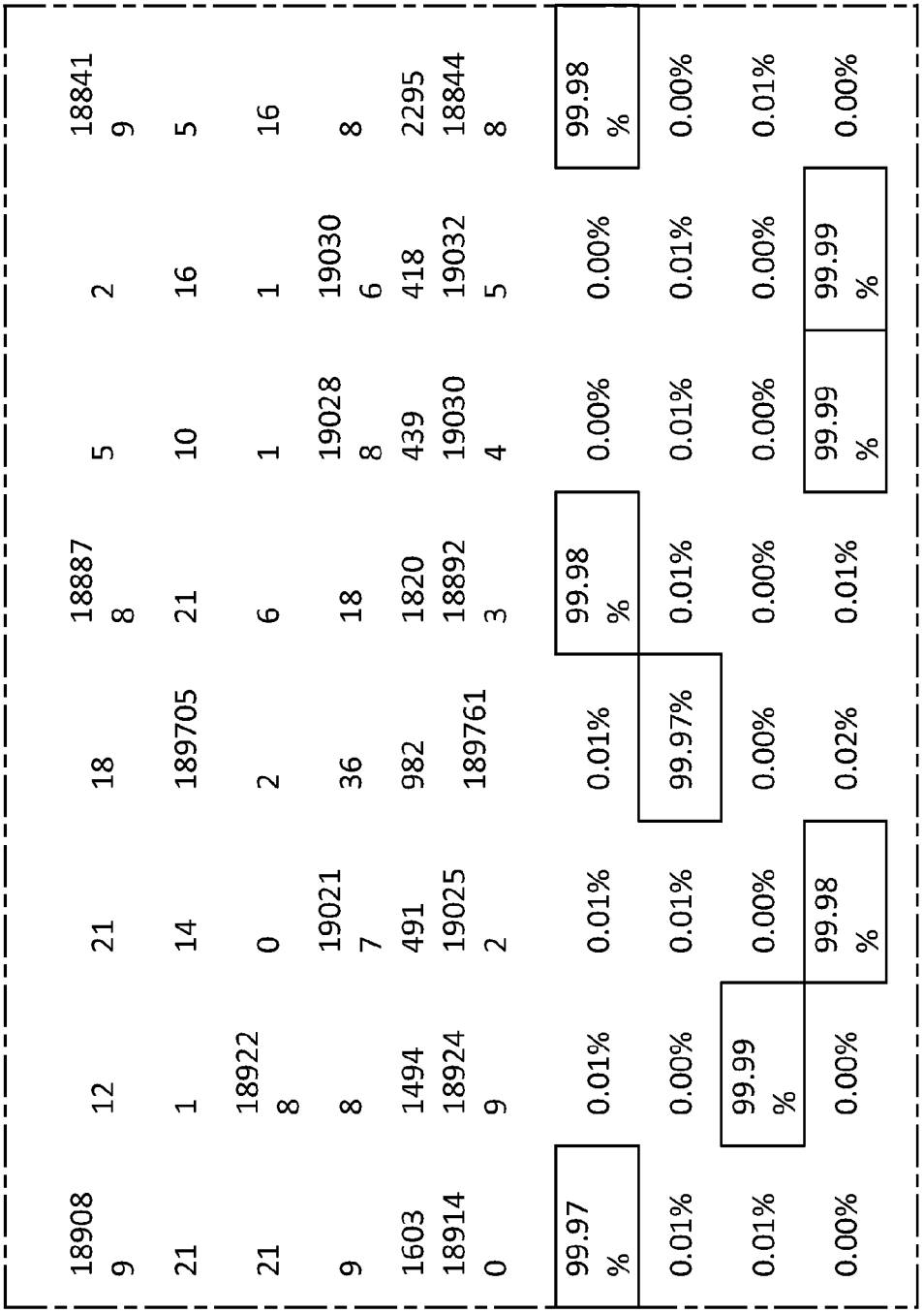


FIGURE 42 (Continued)

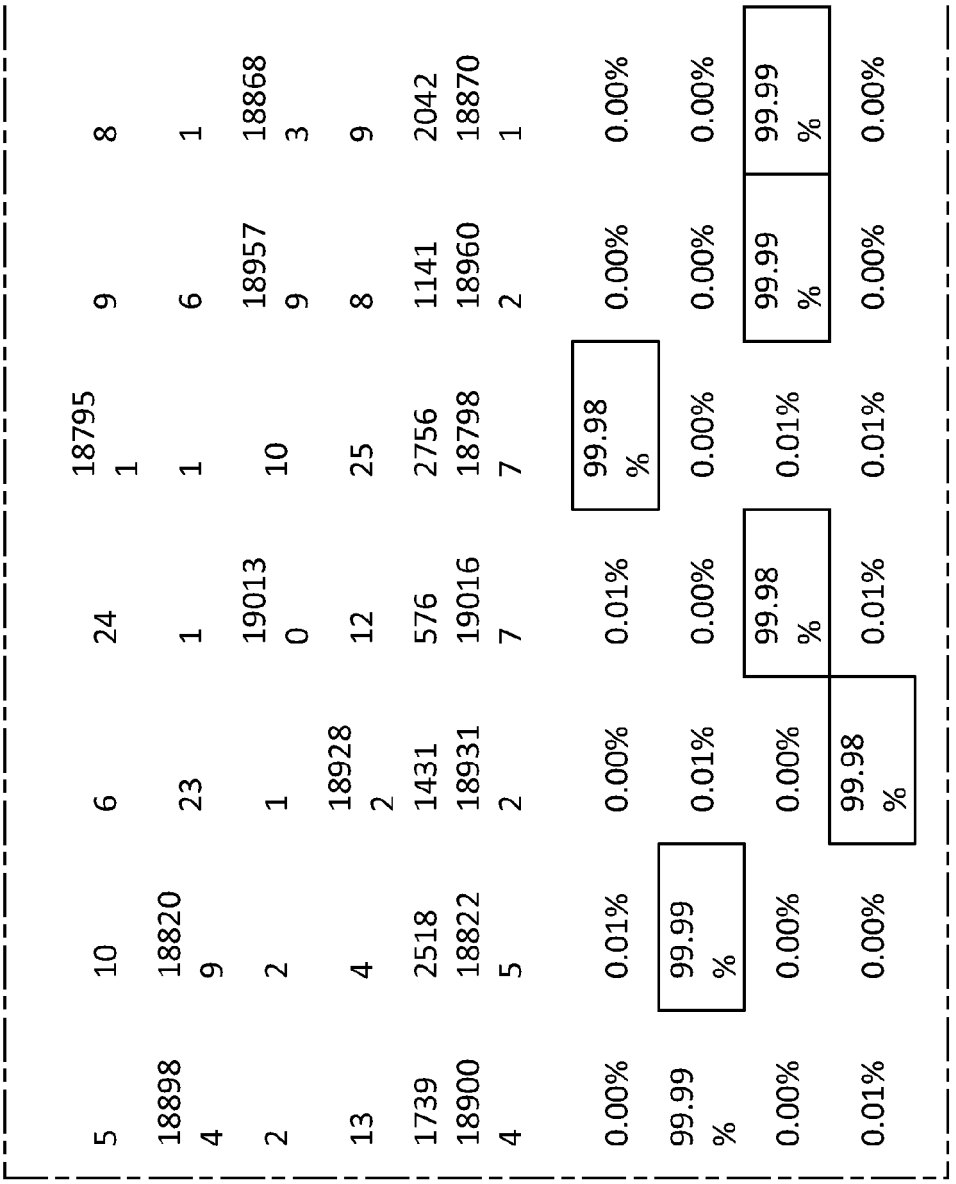
| untreated cells | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 10 | 8 | 13 | 18885 | 2 | 11 | 7 | 11 | | |
| C | 3 | 15 | 18822 | 37 | 21 | 18887 | 24 | 9 | | |
| G | 18949 | 3 | 1 | 5 | 1 | 0 | 1 | 0 | | |
| T | 16 | 18935 | 47 | 21 | 18928 | 23 | 18991 | 19032 | | |
| N | 1222 | 1360 | 2454 | 1822 | 1431 | 1831 | 799 | 401 | | |
| | 18952 | 18938 | 18828 | 18892 | 18931 | 18891 | 18994 | 19034 | | |
| | 1 | 3 | 9 | 1 | 2 | 2 | 4 | 2 | | |
| A | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.01% | 0.00% | 0.01% | | |
| C | 0.00% | 0.01% | 99.97% | 0.02% | 0.01% | 99.98% | 0.01% | 0.00% | | |
| G | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.01% | 99.98% | 99.99% | | |

FIGURE 42 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 18908 | 12 | 21 | 18 | 18887 | 5 | 2 | 18841 | |
| 9 | | | | 8 | | | 9 | |
| 21 | 1 | 14 | 189705 | 21 | 10 | 16 | 5 | |
| 21 | 18922 | 0 | 2 | 6 | 1 | 1 | 16 | |
| | 8 | | | | | | | |
| 9 | 8 | 19021 | 36 | 18 | 19028 | 19030 | 8 | |
| | | 7 | | | 8 | 6 | | |
| 1603 | 1494 | 491 | 982 | 1820 | 439 | 418 | 2295 | |
| 18914 | 18924 | 19025 | 189761 | 18892 | 19030 | 19032 | 18844 | |
| 0 | 9 | 2 | | 3 | 4 | 5 | 8 | |
| 99.97 % | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.00% | 99.98 % | |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% | |
| 0.01% | 99.99 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | |
| 0.00% | 0.00% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.99 % | 0.00% | |

FIGURE 42 (Continued)



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 | 10 | 6 | 24 | 187951 | 9 | 8 | |
| 18898 | 188209 | 23 | 1 | 1 | 6 | 1 | |
| 2 | 2 | 1 | 190130 | 10 | 18957 | 188683 | |
| 13 | 4 | 189282 | 12 | 25 | 8 | 9 | |
| 1739 | 2518 | 1431 | 576 | 2756 | 1141 | 2042 | |
| 18900 | 18822 | 18931 | 19016 | 18798 | 18960 | 18870 | |
| 4 | 5 | 2 | 7 | 7 | 2 | 1 | |
| 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | |
| 99.99% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% | |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | |

FIGURE 42 (Continued)

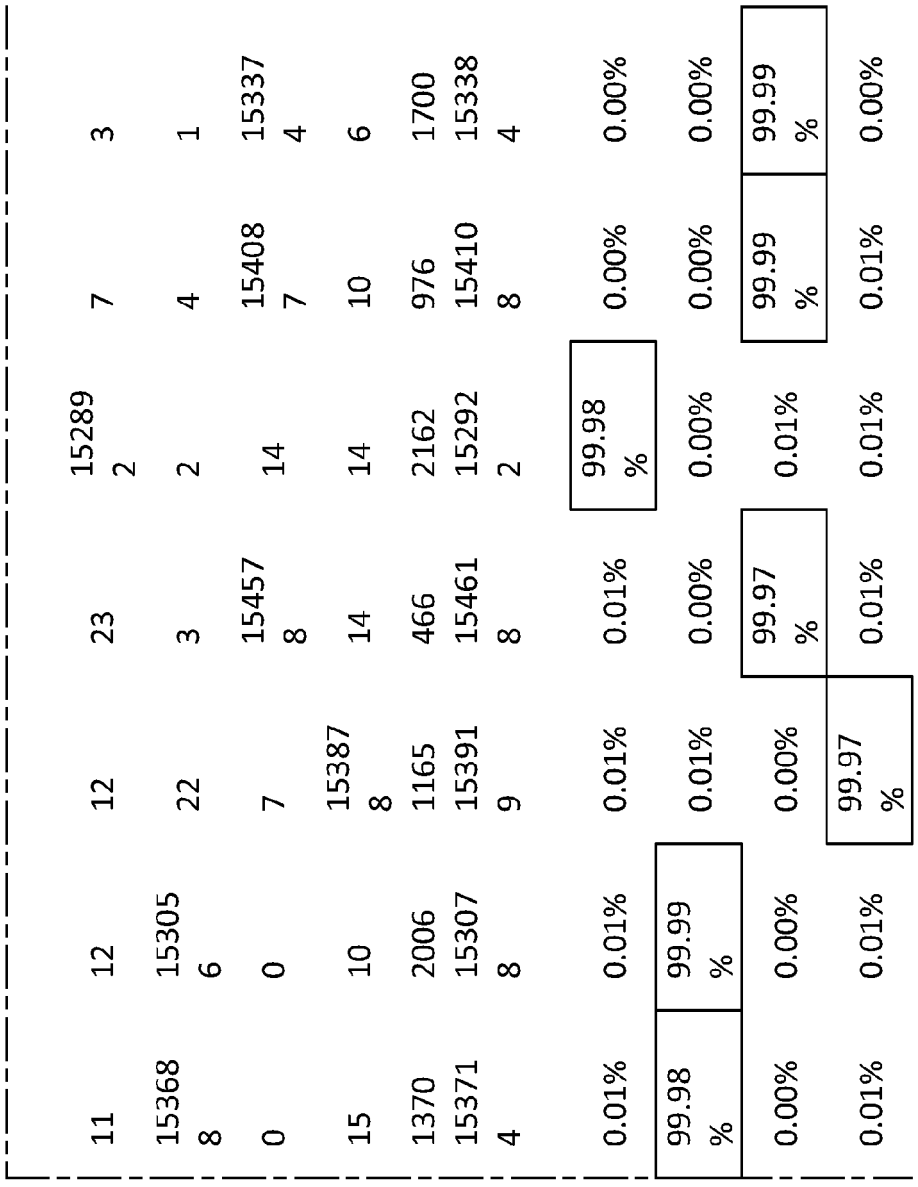
**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 7 | 2 | 13 | 15352 7 | 4 | 17 | 10 | 4 | | |
| **C** | 0 | 15 | 15308 8 | 22 | 6 | 15374 1 | 15 | 9 | | |
| **G** | 15405 7 | 7 | 1 | 10 | 0 | 3 | 5 | 2 | | |
| **T** | 9 | 15417 8 | 40 | 12 | 15390 0 | 21 | 15432 8 | 15466 9 | | |
| **N** | 998 | 872 | 1932 | 1503 | 1164 | 1292 | 716 | 390 | | |
| | 15407 3 | 15420 2 | 15314 2 | 15357 1 | 15391 0 | 15378 2 | 15435 8 | 15468 4 | | |
| **A** | 0.00% | 0.00% | 0.01% | 99.97 % | 0.00% | 0.01% | 0.01% | 0.00% | | |
| **C** | 0.00% | 0.01% | 99.96 % | 0.01% | 0.00% | 99.97 % | 0.01% | 0.01% | | |
| **G** | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| **T** | 0.01% | 99.98 % | 0.03% | 0.01% | 99.99% | 0.01% | 99.98 % | 99.99 % | | |

FIGURE 42 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 15388 | 10 | 11 | 13 | 15362 | 9 | 4 | | 15322 |
| 5 | | | | 6 | | | | 0 |
| 15 | 3 | 17 | 154287 | 15 | 11 | 16 | 2 | |
| 11 | 15385 | 1 | 2 | 11 | 1 | 3 | 14 | |
| | 6 | | | | | | | |
| 7 | 11 | 15465 | 40 | 6 | 15473 | 15469 | 3 | |
| | | 1 | | | 0 | 0 | | |
| 1166 | 1204 | 404 | 742 | 1426 | 333 | 371 | 1845 | |
| 15391 | 15388 | 15468 | 154342 | 15365 | 15475 | 15471 | 15323 | |
| 8 | 0 | 0 | | 8 | 1 | 3 | 9 | |
| 99.98 % | 0.01% | 0.01% | 0.01% | 99.98 % | 0.01% | 0.00% | 99.99 % | 0.00% |
| 0.01% | 0.00% | 0.01% | 99.96% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% |
| 0.01% | 99.98 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| 0.00% | 0.01% | 99.98 % | 0.03% | 0.00% | 99.99 % | 99.99 % | 0.00% | 0.00% |

FIGURE 42 (Continued)



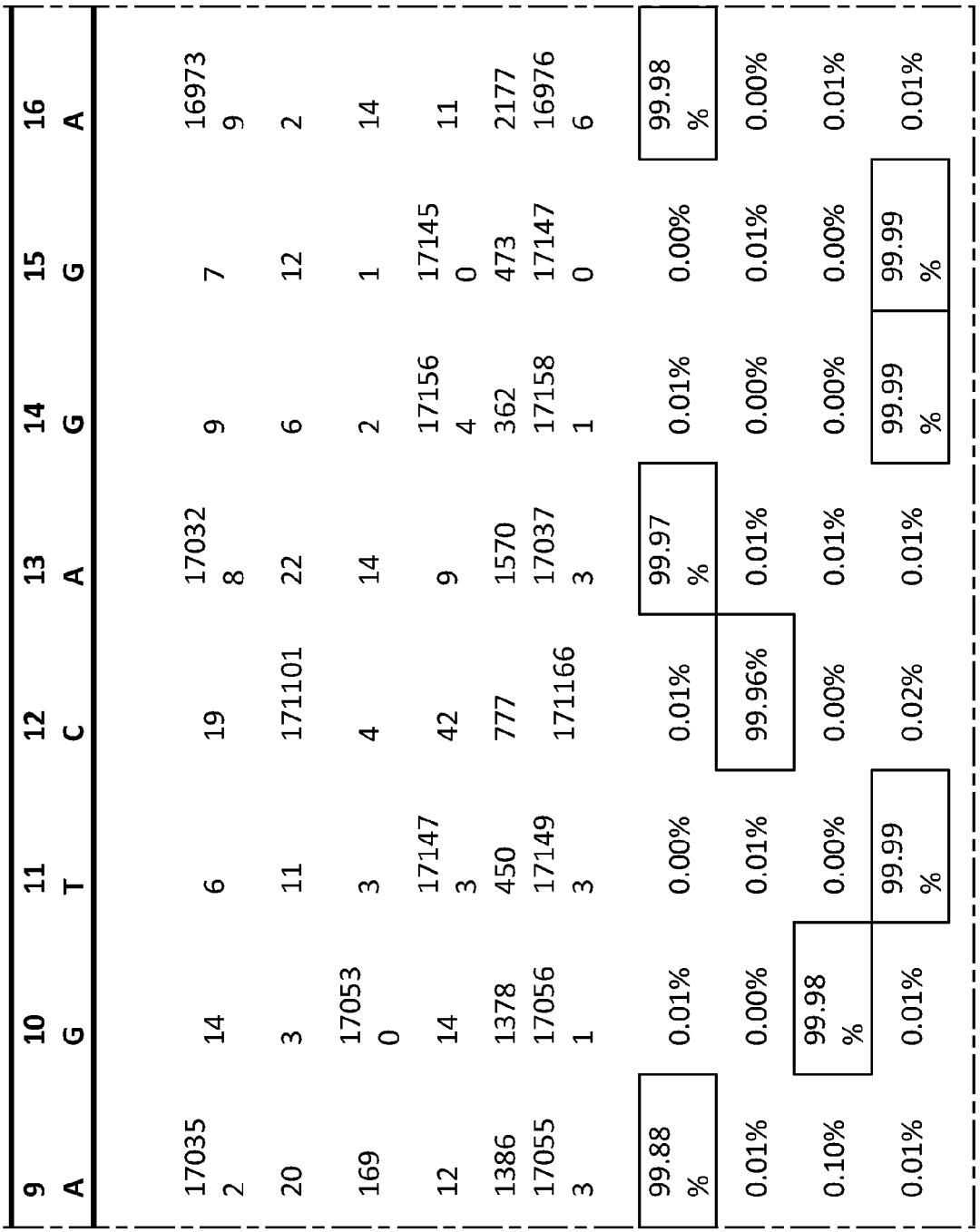
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 11 | 12 | 12 | 23 | 15289 2 | 7 | 3 | |
| 15368 8 | 15305 6 | 22 | 3 | 2 | 4 | 1 | |
| 0 | 0 | 7 | 15457 8 | 14 | 15408 7 | 15337 4 | |
| 15 | 10 | 15387 8 | 14 | 14 | 10 | 6 | |
| 1370 | 2006 | 1165 | 466 | 2162 | 976 | 1700 | |
| 15371 | 15307 | 15391 | 15461 | 15292 | 15410 | 15338 | |
| 4 | 8 | 9 | 8 | 2 | 8 | 4 | |
| 0.01% | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.00% | |
| 99.98 % | 99.99 % | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 99.97 % | 0.01% | 99.99 % | 99.99 % | |
| 0.01% | 0.01% | 99.97 % | 0.01% | 0.01% | 0.01% | 0.00% | |

FIGURE 42 (Continued)

pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/
pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)

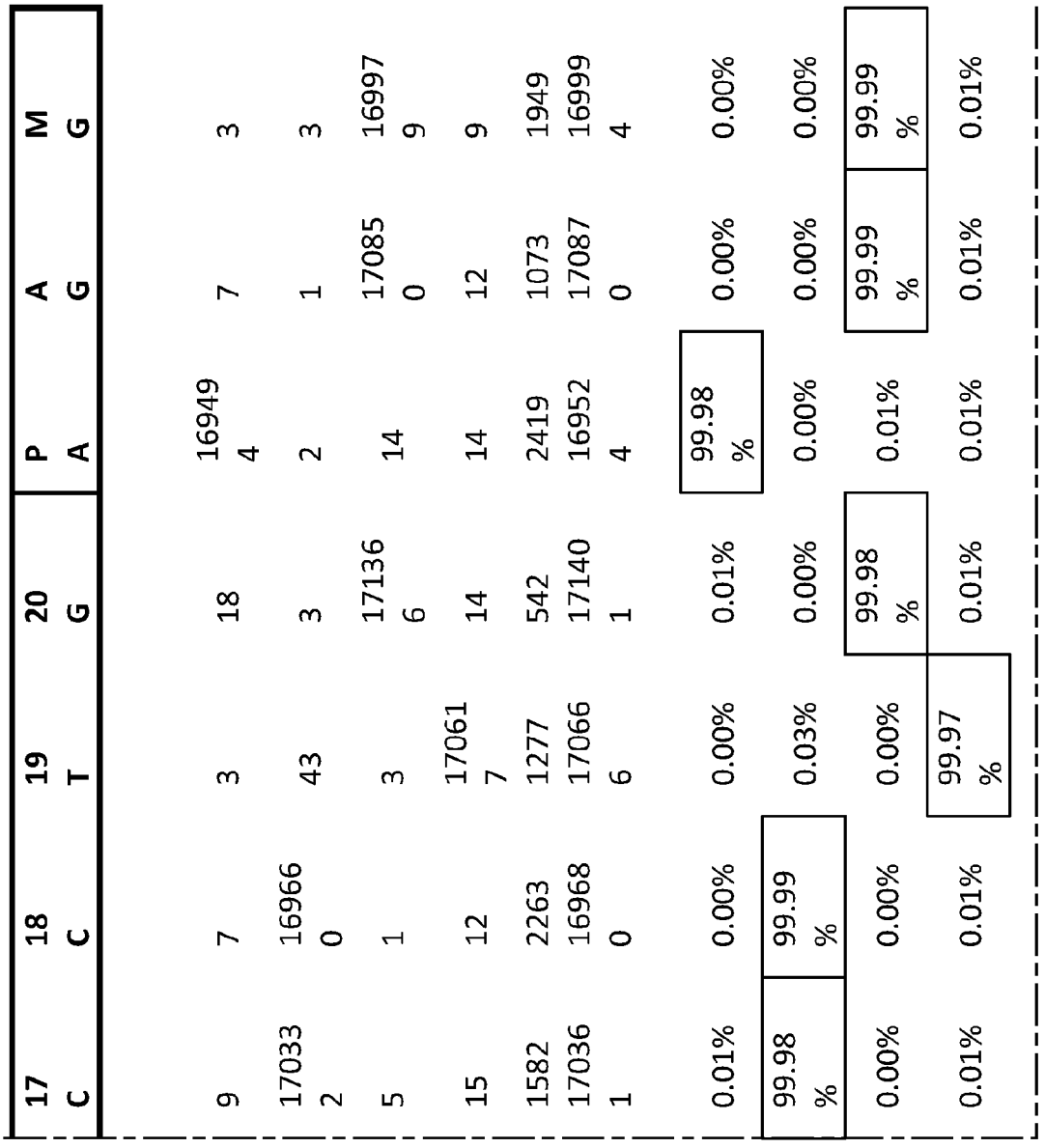
| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T |
|---|---|---|---|---|---|---|---|---|
| A | 11 | 6 | 10 | 16951 | 6 | 24 | 8 | 8 |
| C | 2 | 8 | 16969 | 27 | 20 | 16992 | 14 | 9 |
| G | 17068 | 2 | 5 | 553 | 1 | 207 | 5 | 0 |
| T | 15 | 17099 | 50 | 18 | 17057 | 53 | 17105 | 17153 |
| N | 1223 | 924 | 2179 | 1823 | 1332 | 1731 | 851 | 385 |
| | 17071 | 17101 | 16975 | 17011 | 17060 | 17020 | 17108 | 17155 |
| | 1 | 2 | 7 | 3 | 4 | 5 | 5 | 1 |
| A | 0.01% | 0.00% | 0.01% | 99.65 % | 0.00% | 0.01% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 99.96 % | 0.02% | 0.01% | 99.83 % | 0.01% | 0.01% |
| G | 99.98 % | 0.00% | 0.00% | 0.33% | 0.00% | 0.12% | 0.00% | 0.00% |
| T | 0.01% | 99.99 % | 0.03% | 0.01% | 99.98% | 0.03% | 99.98 % | 99.99 % |

FIGURE 42 (Continued)



| 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G | 16 A |
|---|---|---|---|---|---|---|---|
| 17035 2 | 14 | 6 | 19 | 17032 8 | 9 | 7 | 16973 9 |
| 20 | 3 | 11 | 171101 | 22 | 6 | 12 | 2 |
| 169 | 17053 0 | 3 | 4 | 14 | 2 | 1 | 14 |
| 12 | 14 | 17147 3 | 42 | 9 | 17156 4 | 17145 0 | 11 |
| 1386 | 1378 | 450 | 777 | 1570 | 362 | 473 | 2177 |
| 17055 3 | 17056 1 | 17149 3 | 171166 | 17037 3 | 17158 1 | 17147 0 | 16976 6 |
| 99.88 % | 0.01% | 0.00% | 0.01% | 99.97 % | 0.01% | 0.00% | 99.98 % |
| 0.01% | 0.00% | 0.01% | 99.96% | 0.01% | 0.00% | 0.01% | 0.00% |
| 0.10% | 99.98 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.01% | 99.99 % | 0.02% | 0.01% | 99.99 % | 99.99 % | 0.01% |

FIGURE 42 (Continued)

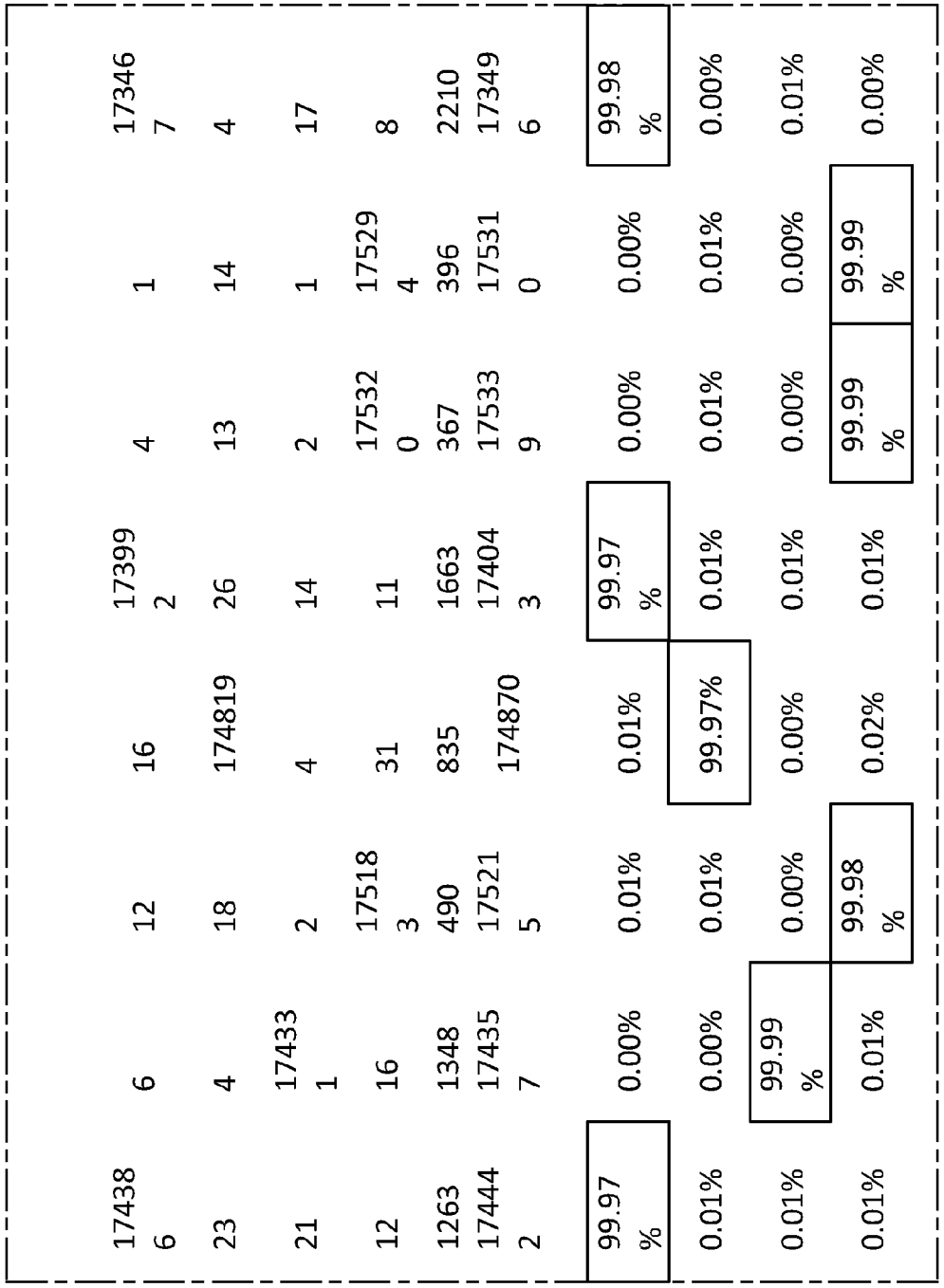


| 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|
| 9 | 7 | 3 | 18 | 16949 4 | 7 | 3 |
| 17033 2 | 16966 0 | 43 | 3 | 2 | 1 | 3 |
| 5 | 1 | 3 | 17136 6 | 14 | 17085 0 | 16997 9 |
| 15 | 12 | 17061 7 | 14 | 14 | 12 | 9 |
| 1582 | 2263 | 1277 | 542 | 2419 | 1073 | 1949 |
| 17036 1 | 16968 0 | 17066 6 | 17140 1 | 16952 4 | 17087 0 | 16999 4 |
| 0.01% | 0.00% | 0.00% | 0.01% | 99.98 % | 0.00% | 0.00% |
| 99.98 % | 99.99 % | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98 % | 0.01% | 99.99 % | 99.99 % |
| 0.01% | 0.01% | 99.97 % | 0.01% | 0.01% | 0.01% | 0.01% |

FIGURE 42 (Continued)

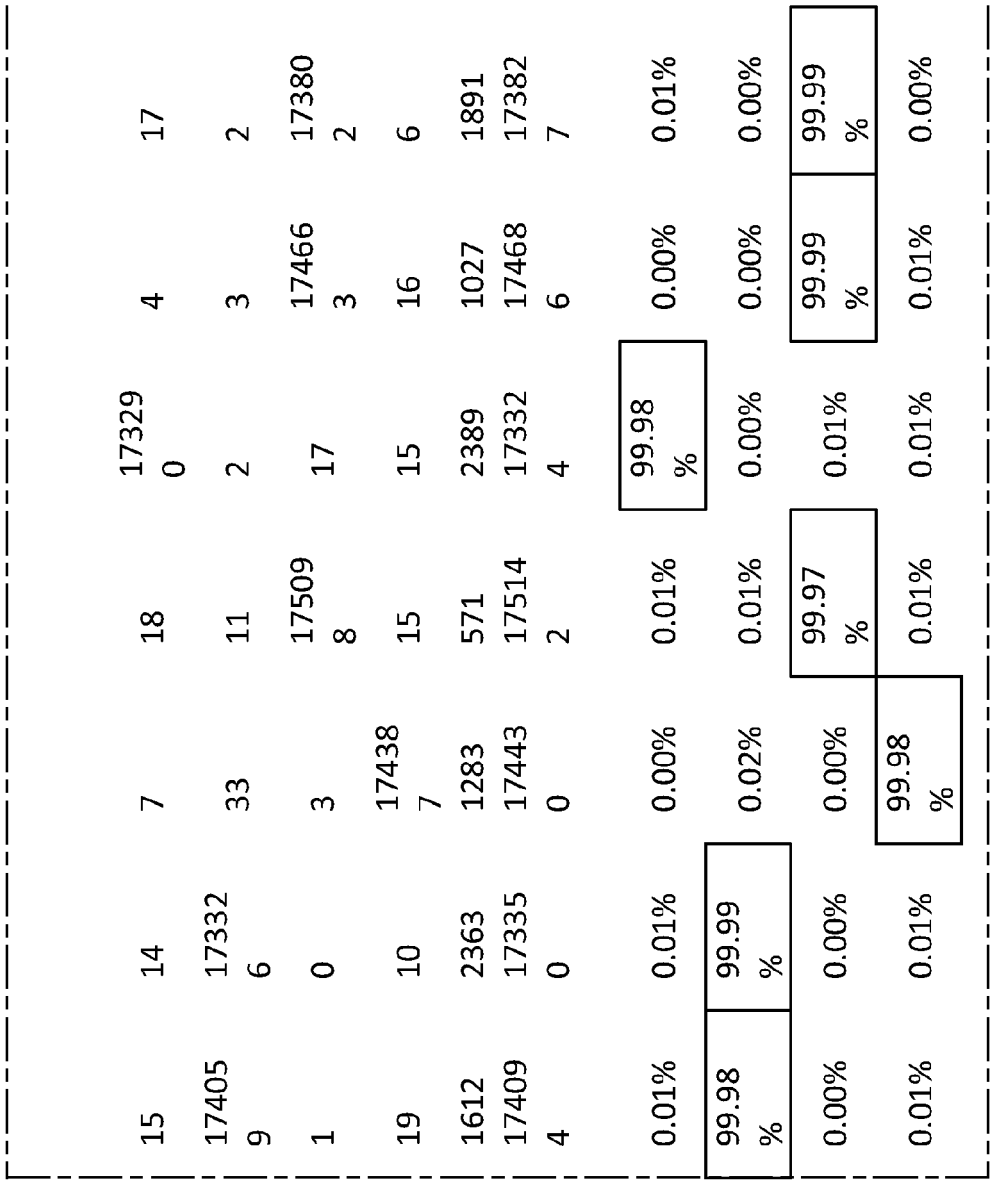
| pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 6 | 11 | 17390 | 10 | 12 | 22 | 6 | | |
| C | 0 | 15 | 17341 | 34 | 11 | 17377 | 6 | 27 | | |
| G | 17448 | 17 | 0 | 13 | 1 | 97 | 4 | 0 | | |
| T | 8 | 17466 | 29 | 15 | 17435 | 77 | 17484 | 17527 | | |
| N | 1189 | 985 | 2242 | 1723 | 1320 | 1739 | 817 | 394 | | |
| | 17450 | 17470 | 17345 | 17396 | 17437 | 17395 | 17488 | 17530 | | |
| | 1 | 7 | 0 | 9 | 2 | 9 | 1 | 4 | | |
| A | 0.00% | 0.00% | 0.01% | 99.96 % | 0.01% | 0.01% | 0.01% | 0.00% | | |
| C | 0.00% | 0.01% | 99.98 % | 0.02% | 0.01% | 99.89 % | 0.00% | 0.02% | | |
| G | 99.99 % | 0.01% | 0.00% | 0.01% | 0.00% | 0.06% | 0.00% | 0.00% | | |
| T | 0.00% | 99.98 % | 0.02% | 0.01% | 99.99% | 0.04% | 99.98 % | 99.98 % | | |

FIGURE 42 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17438 | 6 | 12 | 16 | 17399 | 4 | 1 | 17346 | |
| 6 | | | | 2 | | | 7 | |
| 23 | 4 | 18 | 174819 | 26 | 13 | 14 | 4 | |
| 21 | 17433 | 2 | 4 | 14 | 2 | 1 | 17 | |
| | 1 | | | | | | | |
| 12 | 16 | 17518 | 31 | 11 | 17532 | 17529 | 8 | |
| | | 3 | | | 0 | 4 | | |
| 1263 | 1348 | 490 | 835 | 1663 | 367 | 396 | 2210 | |
| 17444 | 17435 | 17521 | 174870 | 17404 | 17533 | 17531 | 17349 | |
| 2 | 7 | 5 | | 3 | 9 | 0 | 6 | |
| 99.97 % | 0.00% | 0.01% | 0.01% | 99.97 % | 0.00% | 0.00% | 99.98 % | |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% | |
| 0.01% | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | |
| 0.01% | 0.01% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.99 % | 0.00% | |

FIGURE 42 (Continued)



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 15 | 14 | 7 | 18 | 173290 | 4 | 17 | |
| 17405 | 17332 | 33 | 11 | 2 | 3 | 2 | |
| 9 | 6 | | | | | | |
| 1 | 0 | 3 | 175098 | 17 | 17466 | 17380 | |
| | | | | | 3 | 2 | |
| 19 | 10 | 174387 | 15 | 15 | 16 | 6 | |
| 1612 | 2363 | 1283 | 571 | 2389 | 1027 | 1891 | |
| 17409 | 17335 | 17443 | 17514 | 17332 | 17468 | 17382 | |
| 4 | 0 | 0 | 2 | 4 | 6 | 7 | |
| 0.01% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | |
| 99.98% | 99.99% | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 99.99% | 99.99% | |
| 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.00% | |

FIGURE 42 (Continued)

| pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 10 | 8 | 13 | 17039 2 | 2 | 14 | 10 | 0 | |
| C | 1 | 8 | 16997 1 | 19 | 12 | 17052 8 | 16 | 16 | |
| G | 17102 1 | 2 | 0 | 6 | 0 | 1 | 4 | 2 | |
| T | 13 | 17122 | 30 | 18 | 17088 | 7 | 17140 | 17185 | |

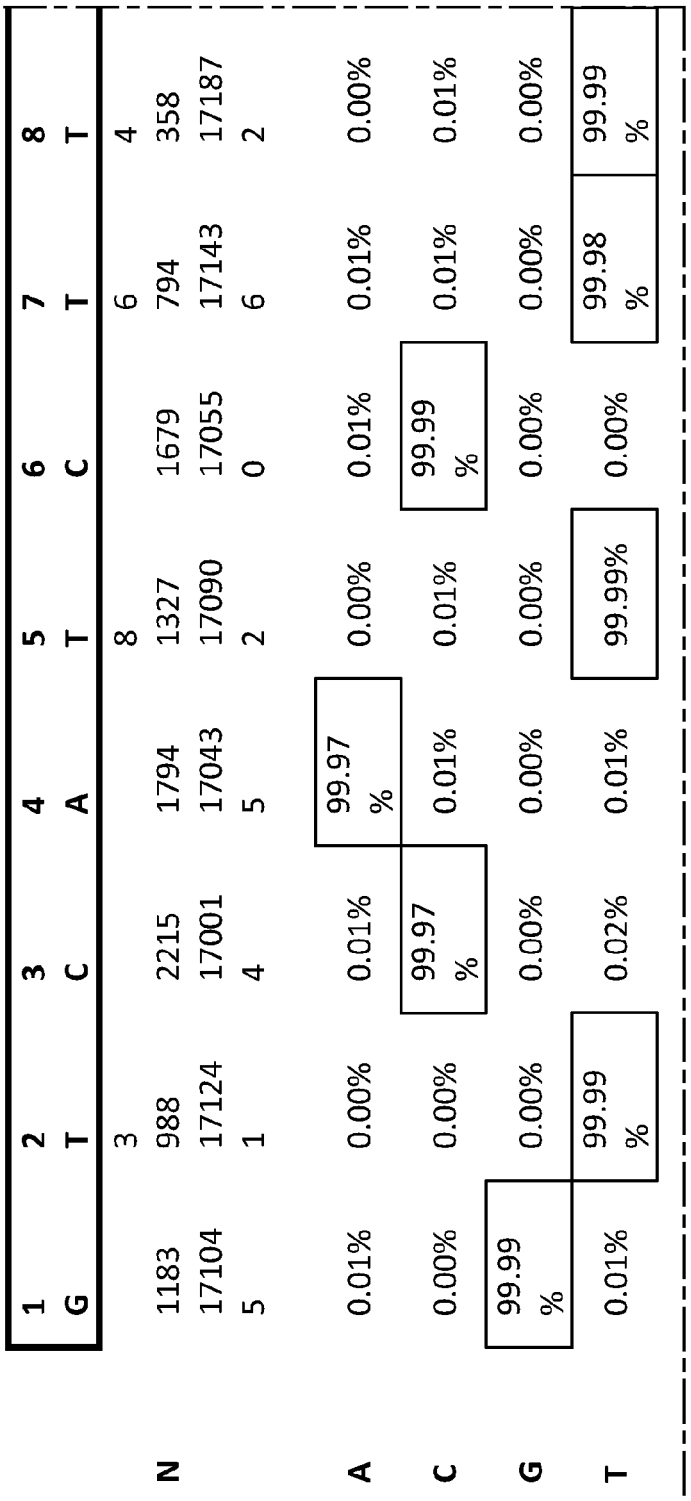
FIGURE 42 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17081 5 | 6 | 6 | 16 | 17055 9 | 10 | 4 | 16999 9 | |
| 18 | 1 | 18 | 171378 | 17 | 12 | 12 | 6 | |
| 12 | 17081 5 | 2 | 4 | 22 | 1 | 0 | 15 | |
| 8 | 12 | 17183 | 35 | 11 | 17182 | 17175 | 11 | |

FIGURE 42 (Continued)

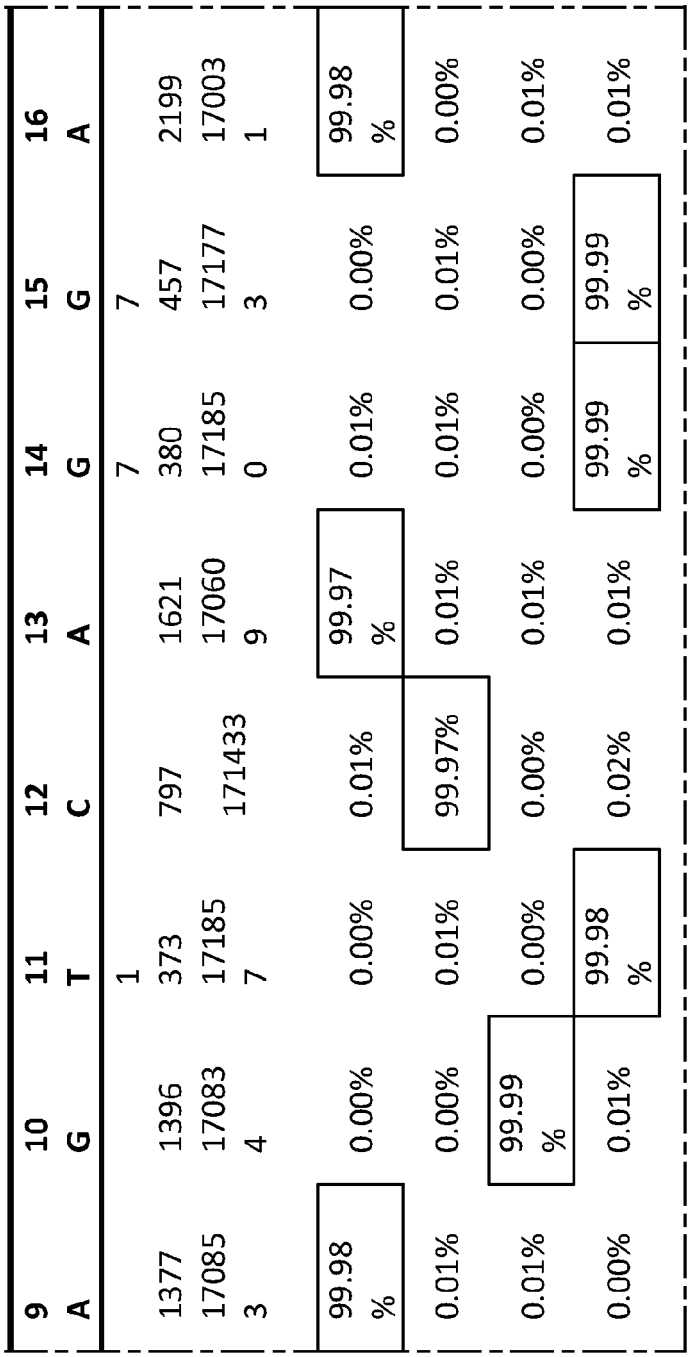
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 11 | 23 | 8 | 26 | 16967 1 | 5 | 5 | |
| 17056 9 | 16988 1 | 33 | 2 | 3 | 3 | 1 | |
| 1 | 2 | 4 | 17162 8 | 11 | 17113 1 | 17037 5 | |
| 21 | 8 | 17084 | 9 | 23 | 16 | 10 | |

FIGURE 42 (Continued)



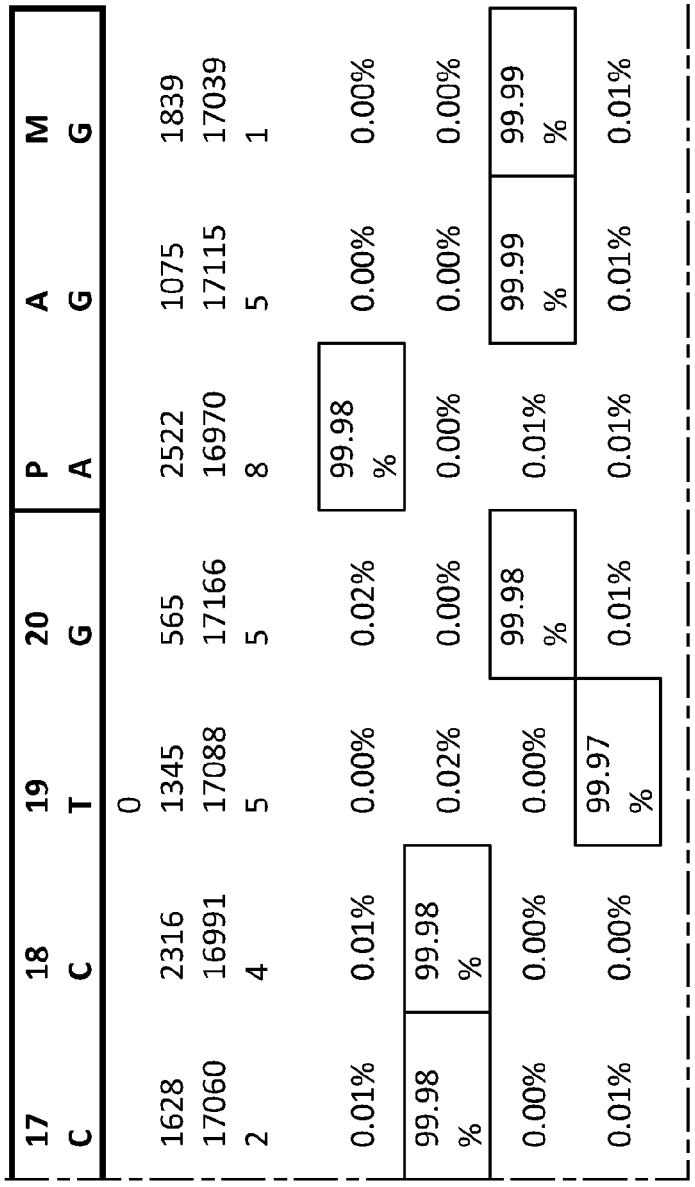
| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T |
|---|---|---|---|---|---|---|---|---|
| N | 1183 | 988 | 2215 | 1794 | 1327 | 1679 | 794 | 358 |
| | 17104 | 17124 | 17001 | 17043 | 17090 | 17055 | 17143 | 17187 |
| | 5 | 1 | 4 | 5 | 2 | 0 | 6 | 2 |
| A | 0.01% | 0.00% | 0.01% | 99.97% | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 99.97% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% |
| G | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.00% | 99.98% | 99.99% |

FIGURE 42 (Continued)



| 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G | 16 A |
|---|---|---|---|---|---|---|---|
| | | 1 | | | 7 | 7 | |
| 1377 | 1396 | 373 | 797 | 1621 | 380 | 457 | 2199 |
| 17085 | 17083 | 17185 | 171433 | 17060 | 17185 | 17177 | 17003 |
| 3 | 4 | 7 | | 9 | 0 | 3 | 1 |
| 99.98 % | 0.00% | 0.00% | 0.01% | 99.97 % | 0.01% | 0.00% | 99.98 % |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% |
| 0.01% | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.01% | 99.98 % | 0.02% | 0.01% | 99.99 % | 99.99 % | 0.01% |

FIGURE 42 (Continued)

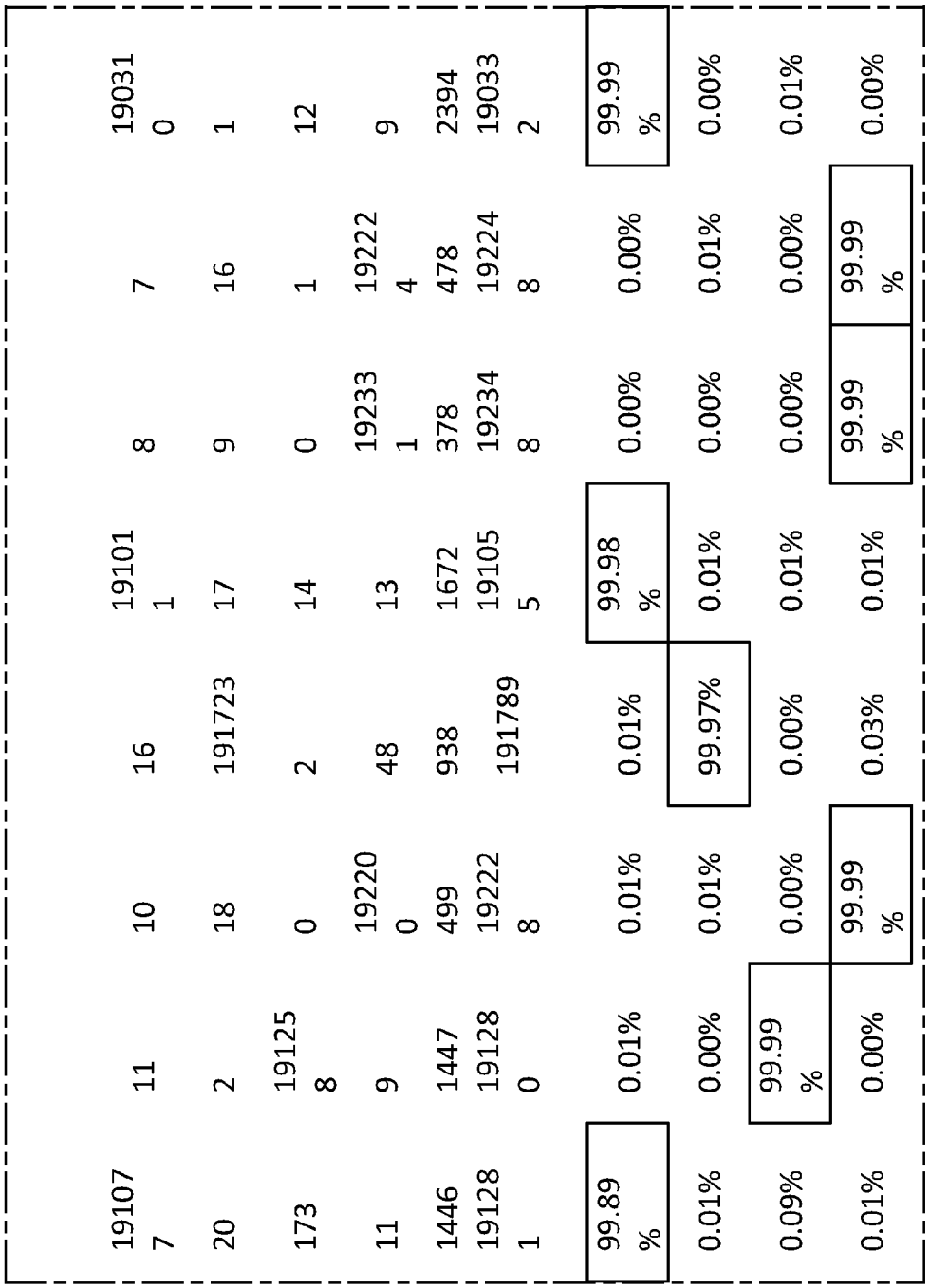


| 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|
| | | 0 | | | | |
| 1628 | 2316 | 1345 | 565 | 2522 | 1075 | 1839 |
| 17060 | 16991 | 17088 | 17166 | 16970 | 17115 | 17039 |
| 2 | 4 | 5 | 5 | 8 | 5 | 1 |
| 0.01% | 0.01% | 0.00% | 0.02% | 99.98 % | 0.00% | 0.00% |
| 99.98 % | 99.98 % | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98 % | 0.01% | 99.99 % | 99.99 % |
| 0.01% | 0.00% | 99.97 % | 0.01% | 0.01% | 0.01% | 0.01% |

FIGURE 42 (Continued)

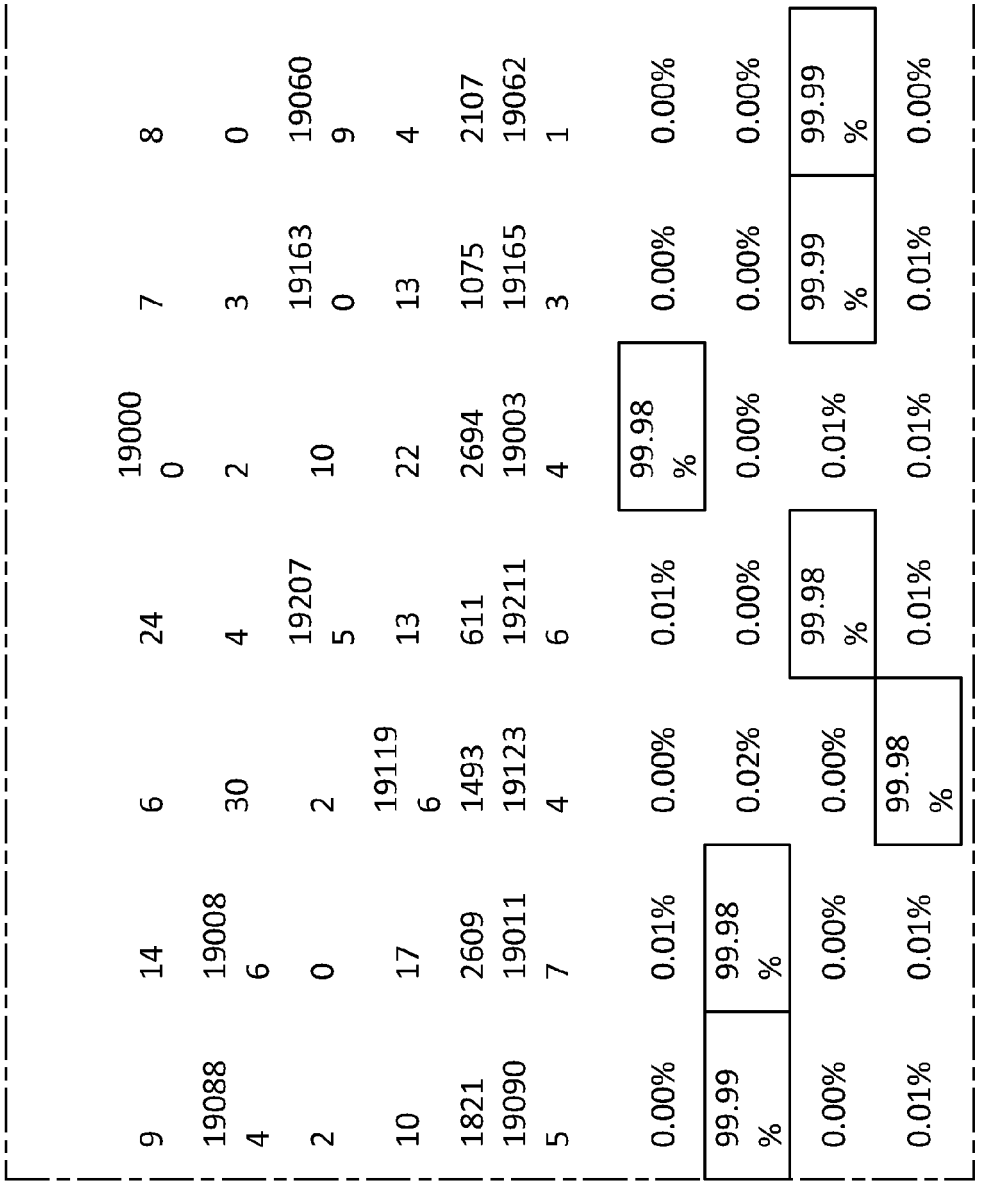
| pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 11 | 5 | 16 | 19021 7 | 6 | 23 | 10 | 4 | |
| C | 1 | 12 | 19013 8 | 27 | 16 | 19068 4 | 15 | 26 | |
| G | 19140 0 | 6 | 0 | 430 | 1 | 81 | 4 | 4 | |
| T | 11 | 19162 5 | 41 | 19 | 19132 4 | 43 | 19187 9 | 19225 2 | |
| N | 1300 | 1075 | 2528 | 2030 | 1376 | 1892 | 815 | 437 | |
| | 19142 | 19164 | 19019 | 19069 | 19134 | 19083 | 19190 | 19228 | |
| | 3 | 8 | 5 | 3 | 7 | 1 | 8 | 6 | |
| A | 0.01% | 0.00% | 0.01% | 99.75 % | 0.00% | 0.01% | 0.01% | 0.00% | |
| C | 0.00% | 0.01% | 99.97 % | 0.01% | 0.01% | 99.92 % | 0.01% | 0.01% | |
| G | 99.99 % | 0.00% | 0.00% | 0.23% | 0.00% | 0.04% | 0.00% | 0.00% | |
| T | 0.01% | 99.99 % | 0.02% | 0.01% | 99.99% | 0.02% | 99.98 % | 99.98 % | |

FIGURE 42 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 19107 | 11 | 10 | 16 | 19101 | 8 | 7 | 19031 | |
| 7 | | | | 1 | | | 0 | |
| 20 | 2 | 18 | 191723 | 17 | 9 | 16 | 1 | |
| | 19125 | | | | | | | |
| 173 | 8 | 0 | 2 | 14 | 0 | 1 | 12 | |
| | | 19220 | | | 19233 | 19222 | | |
| 11 | 9 | 0 | 48 | 13 | 1 | 4 | 9 | |
| 1446 | 1447 | 499 | 938 | 1672 | 378 | 478 | 2394 | |
| 19128 | 19128 | 19222 | 191789 | 19105 | 19234 | 19224 | 19033 | |
| 1 | 0 | 8 | | 5 | 8 | 8 | 2 | |
| 99.89 % | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.00% | 99.99 % | |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.00% | 0.01% | 0.00% | |
| 0.09% | 99.99 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | |
| 0.01% | 0.00% | 99.99 % | 0.03% | 0.01% | 99.99 % | 99.99 % | 0.00% | |

FIGURE 42 (Continued)

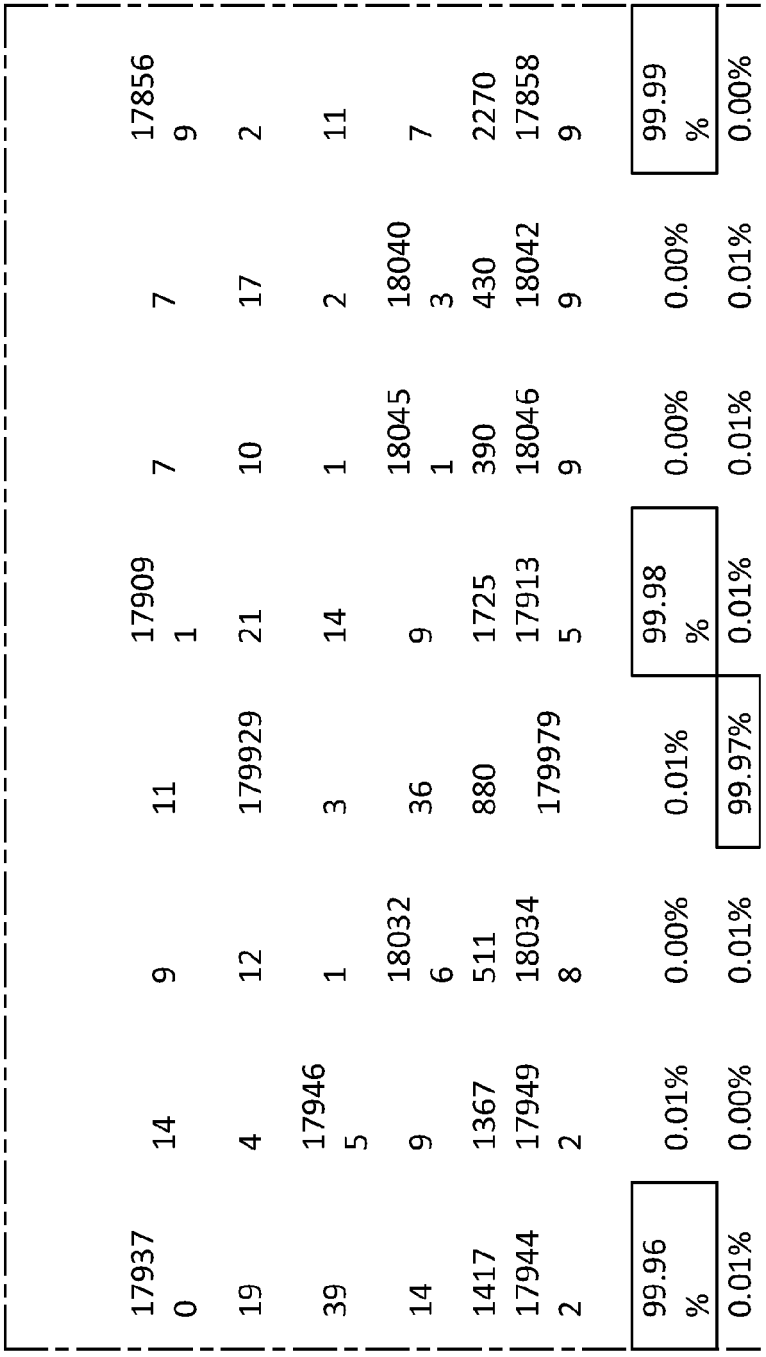


| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9 | 14 | 6 | 24 | 190000 | 7 | 8 | |
| 19088 | 19008 | 30 | 4 | 2 | 3 | 0 | |
| 4 | 6 | | | | | | |
| 2 | 0 | 2 | 192075 | 10 | 191630 | 190609 | |
| 10 | 17 | 191196 | 13 | 22 | 13 | 4 | |
| 1821 | 2609 | 1493 | 611 | 2694 | 1075 | 2107 | |
| 19090 | 19011 | 19123 | 19211 | 19003 | 19165 | 19062 | |
| 5 | 7 | 4 | 6 | 4 | 3 | 1 | |
| 0.00% | 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | |
| 99.99% | 99.98% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.99% | 99.99% | |
| 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.01% | 0.00% | |

FIGURE 42 (Continued)

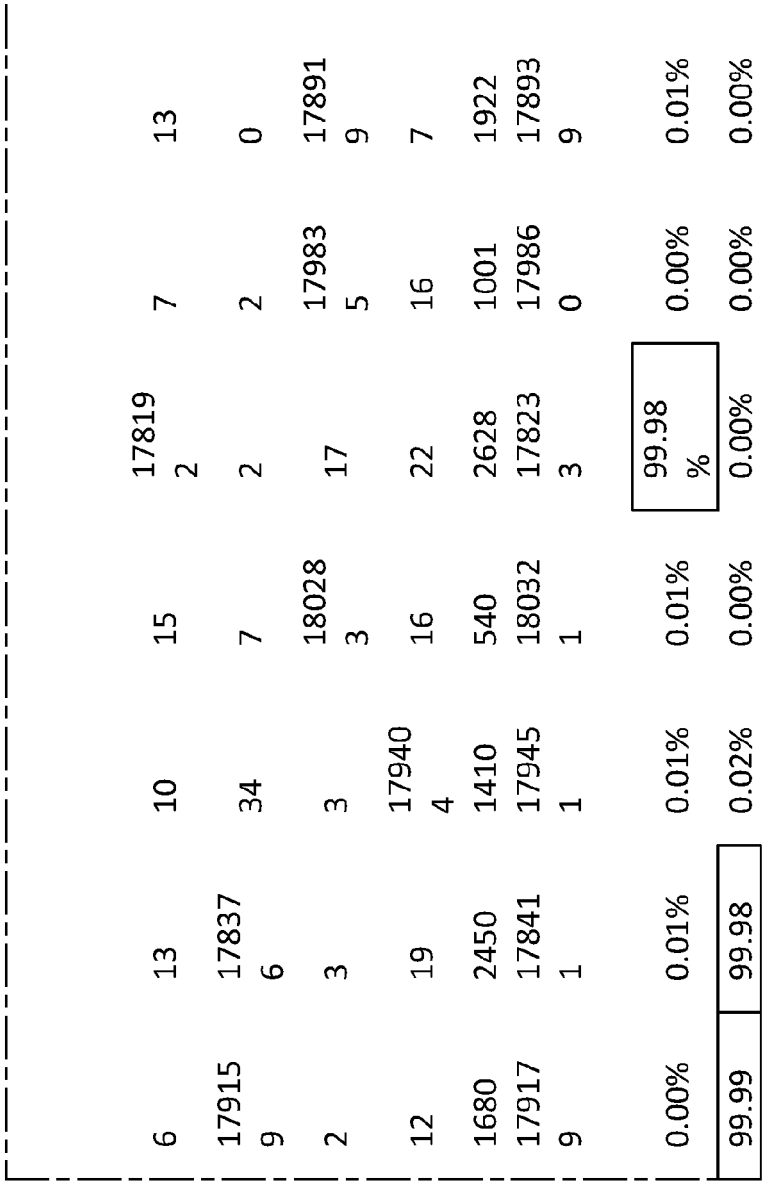
| pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 16 | 5 | 14 | 17862 3 | 4 | 33 | 9 | 7 | |
| C | 4 | 6 | 17851 3 | 29 | 9 | 17899 1 | 16 | 15 | |
| G | 17957 1 | 1 | 1 | 295 | 0 | 17 | 1 | 1 | |
| T | 14 | 17980 1 | 29 | 13 | 17957 4 | 29 | 18006 9 | 18043 4 | |
| N | 1254 | 1046 | 2302 | 1899 | 1272 | 1789 | 764 | 402 | |
| | 17960 5 | 17981 3 | 17855 7 | 17896 0 | 17958 7 | 17907 0 | 18009 5 | 18045 7 | |
| A | 0.01% | 0.00% | 0.01% | 99.81 % | 0.00% | 0.02% | 0.00% | 0.00% | |
| C | 0.00% | 0.00% | 99.98 | 0.02% | 0.01% | 99.96 | 0.01% | 0.01% | |

FIGURE 42 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 17937 | 14 | 9 | 11 | 17909 | 7 | 7 | | 17856 |
| 0 | | | | 1 | | | | 9 |
| 19 | 4 | 12 | 179929 | 21 | 10 | 17 | | 2 |
| 39 | 17946 | 1 | 3 | 14 | 1 | 2 | | 11 |
| | 5 | | | | | | | |
| 14 | 9 | 18032 | 36 | 9 | 18045 | 18040 | | 7 |
| | | 6 | | | 1 | 3 | | |
| 1417 | 1367 | 511 | 880 | 1725 | 390 | 430 | | 2270 |
| 17944 | 17949 | 18034 | 179979 | 17913 | 18046 | 18042 | | 17858 |
| 2 | 2 | 8 | | 5 | 9 | 9 | | 9 |
| 99.96 % | 0.01% | 0.00% | 0.01% | 99.98 % | 0.00% | 0.00% | | 99.99 % |
| 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.01% | 0.01% | | 0.00% |

FIGURE 42 (Continued)



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | 13 | 10 | 15 | 17819 | 7 | 13 | |
| | | | | 2 | | | |
| 17915 | 17837 | 34 | 7 | 2 | 2 | 0 | |
| 9 | 6 | | | | | | |
| 2 | 3 | 3 | 18028 | 17 | 17983 | 17891 | |
| | | | 3 | | 5 | 9 | |
| 12 | 19 | 17940 | 16 | 22 | 16 | 7 | |
| | | 4 | | | | | |
| 1680 | 2450 | 1410 | 540 | 2628 | 1001 | 1922 | |
| 17917 | 17841 | 17945 | 18032 | 17823 | 17986 | 17893 | |
| 9 | 1 | 1 | 1 | 3 | 0 | 9 | |
| 0.00% | 0.01% | 0.01% | 0.01% | 99.98 % | 0.00% | 0.01% | |
| 99.99 | 99.98 | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | |

FIGURE 42 (Continued)

| | 1<br>G | 2<br>T | 3<br>C | 4<br>A | 5<br>T | 6<br>C | 7<br>T | 8<br>T |
|---|---|---|---|---|---|---|---|---|
| G | 99.98<br>% | 0.00% | 0.00% | 0.16% | 0.00% | 0.01% | 0.00% | 0.00% |
| T | 0.01% | 99.99<br>% | 0.02% | 0.01% | 99.99% | 0.02% | 99.99<br>% | 99.99<br>% |

**pNMG-276 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (wild-type) + (A106V_D108N_D147Y_E155V))**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 12 | 4 | 15 | 18245<br>4 | 13 | 21 | 10 | 3 |
| C | 1 | 11 | 18201<br>6 | 20 | 5 | 18243<br>2 | 12 | 19 |
| G | 18327<br>9 | 5 | 2 | 11 | 2 | 162 | 1 | 1 |
| T | 20 | 18339<br>8 | 42 | 13 | 18317<br>1 | 83 | 18374<br>5 | 18416<br>6 |
| N | 1314 | 1209 | 2553 | 2130 | 1437 | 1930 | 860 | 439 |
| | 18331 | 18341 | 18207 | 18249 | 18319 | 18269 | 18376 | 18418 |
| | 2 | 8 | 5 | 8 | 1 | 8 | 8 | 9 |

FIGURE 42 (Continued)

| 9 A | 10 G | 11 T | 12 C | | 13 A | 14 G | 15 G | 16 A | 17 C |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | % |
| 0.02% | 99.98 % | 0.00% | 0.00% | | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| 0.01% | 0.01% | 99.99 % | 0.02% | | 0.01% | 99.99 % | 99.99 % | 0.00% | 0.01% |
| 18299 | 7 | 13 | 9 | | 18271 | 7 | 13 | 18200 | 9 |
| 8 | | | | | 9 | | | 4 | |
| 14 | 2 | 16 | 183608 | | 16 | 13 | 15 | 1 | 18272 |
| | | | | | | | | | 2 |
| 22 | 18303 | 5 | 5 | | 5 | 2 | 0 | 10 | 3 |
| | 3 | | | | | | | | |
| 9 | 19 | 18408 | 56 | | 9 | 18413 | 18412 | 11 | 16 |
| | | 9 | | | | 9 | 9 | | |
| 1589 | 1571 | 509 | 954 | | 1883 | 471 | 475 | 2606 | 1892 |
| 18304 | 18306 | 18412 | 183678 | | 18274 | 18416 | 18415 | 18202 | 18275 |
| 3 | 1 | 3 | | | 9 | 1 | 7 | 6 | 0 |

FIGURE 42 (Continued)

| 18 C | 19 T | 20 G | PA | A G | M G |
|---|---|---|---|---|---|
| % | | | | | |
| 0.00% | 0.00% | 99.98 % | 0.01% | 99.99 % | 99.99 % |
| 0.01% | 99.97 % | 0.01% | 0.01% | 0.01% | 0.00% |
| 15 | 17 | 27 | 18167 0 | 4 | 9 |
| 18182 4 | 31 | 1 | 1 | 3 | 0 |
| 2 | 1 | 18392 6 | 10 | 18344 8 | 18241 8 |
| 10 | 18300 6 | 13 | 25 | 22 | 23 |
| 2791 | 1587 | 675 | 2939 | 1168 | 2195 |
| 18185 | 18305 | 18396 | 18170 | 18347 | 18245 |
| 1 | 5 | 7 | 6 | 7 | 0 |

FIGURE 42 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 0.00% | 0.01% | 99.98 % | 0.01% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.01% | 99.97 % | 0.01% | 0.00% | 99.85 % | 0.01% | 0.01% |
| G | 99.98 % | 0.00% | 0.00% | 0.01% | 0.00% | 0.09% | 0.00% | 0.00% |
| T | 0.01% | 99.99 % | 0.02% | 0.01% | 99.99% | 0.05% | 99.99 % | 99.99 % |

**pNMG-277 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (A106V_D108N_D147Y_E155V) + (A106V_D108N_D147Y_E155V))**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 12 | 7 | 13 | 18835 5 | 6 | 29 | 13 | 6 |
| C | 1 | 15 | 18850 2 | 33 | 17 | 18878 2 | 13 | 25 |
| G | 18970 1 | 5 | 2 | 612 | 1 | 202 | 1 | 0 |
| T | 22 | 18990 7 | 38 | 29 | 18964 9 | 79 | 19015 5 | 19056 2 |
| N | 1275 | 1077 | 2457 | 1983 | 1339 | 1920 | 830 | 419 |
| | 18973 6 | 18993 4 | 18855 5 | 18902 9 | 18967 3 | 18909 2 | 19018 2 | 19059 3 |

# FIGURE 42 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% |
| 0.01% | 0.00% | 0.01% | 99.96% | 0.01% | 0.01% | 0.01% | 0.00% | 99.98% |
| 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| 0.00% | 0.01% | 99.98% | 0.03% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% |
| 189326 | 11 | 13 | 19 | 189199 | 9 | 1 | 188661 | 11 |
| 26 | 1 | 18 | 190073 | 27 | 9 | 23 | 3 | 189267 |
| 239 | 189626 | 2 | 2 | 22 | 0 | 0 | 11 | 2 |
| 12 | 5 | 190490 | 43 | 18 | 190595 | 190568 | 15 | 11 |
| 1420 | 1380 | 502 | 888 | 1759 | 411 | 432 | 2334 | 1733 |
| 189603 | 189643 | 190523 | 190137 | 189266 | 190613 | 190592 | 188690 | 189291 |

FIGURE 42 (Continued)

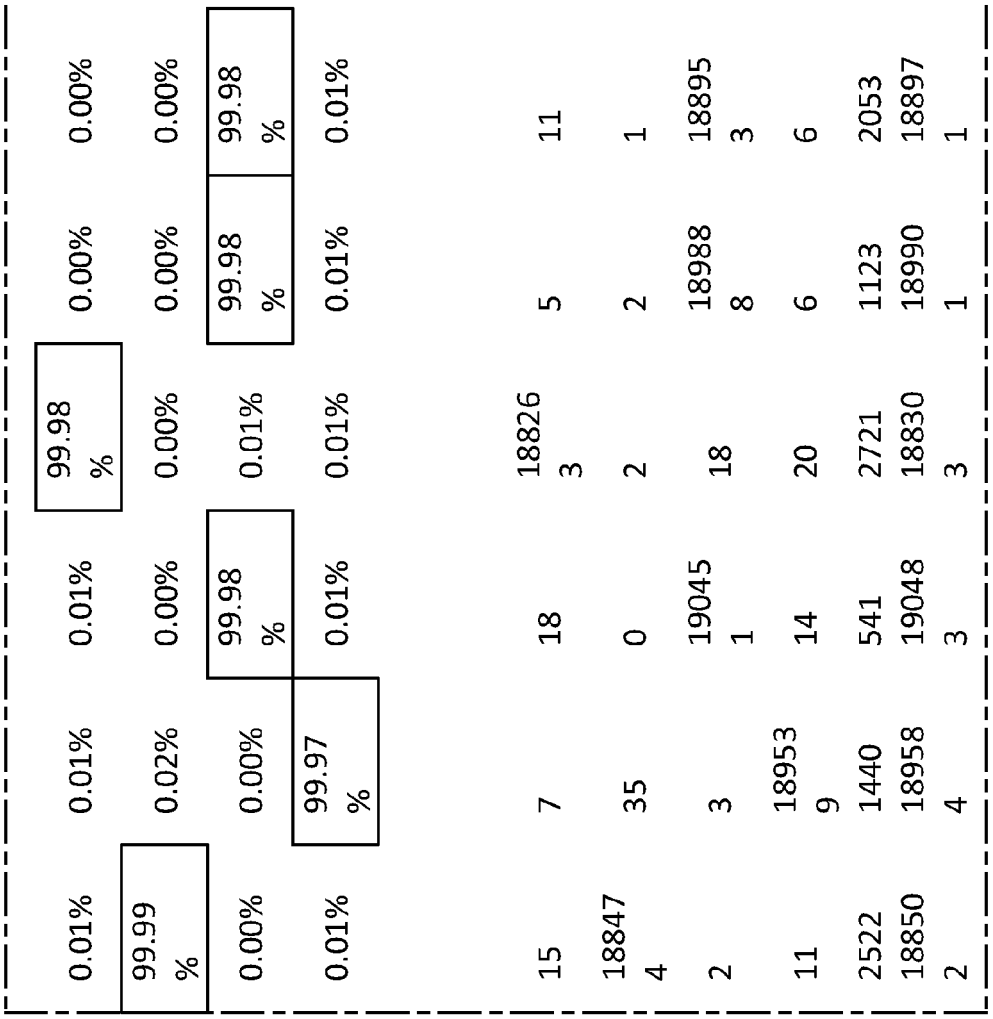


FIGURE 42 (Continued)

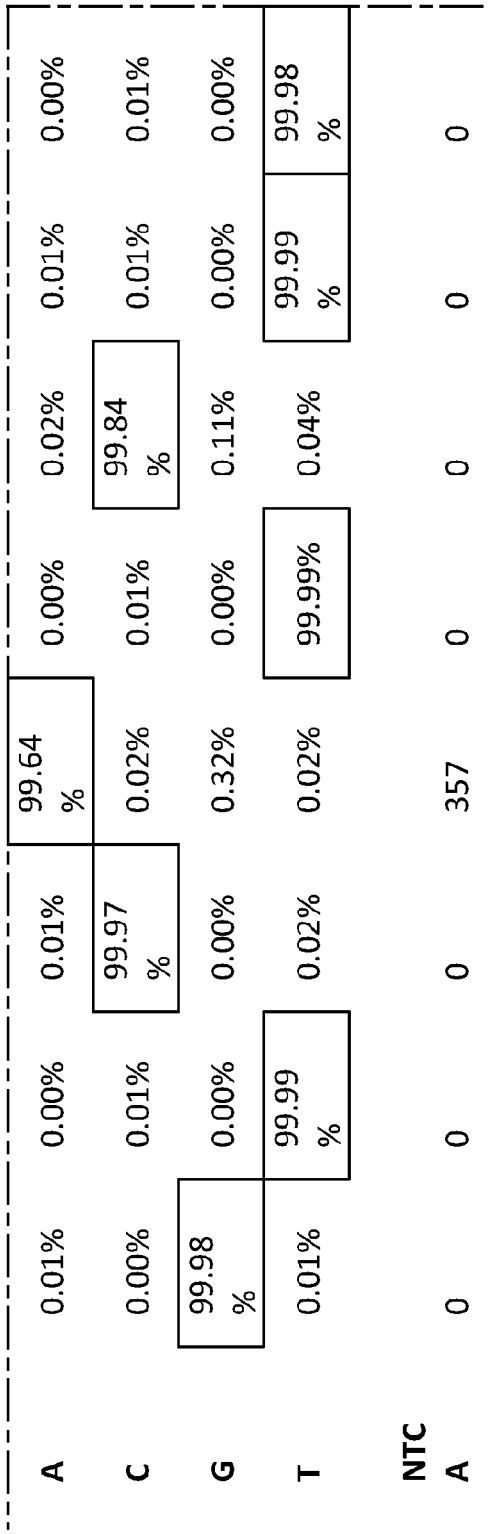


FIGURE 42 (Continued)

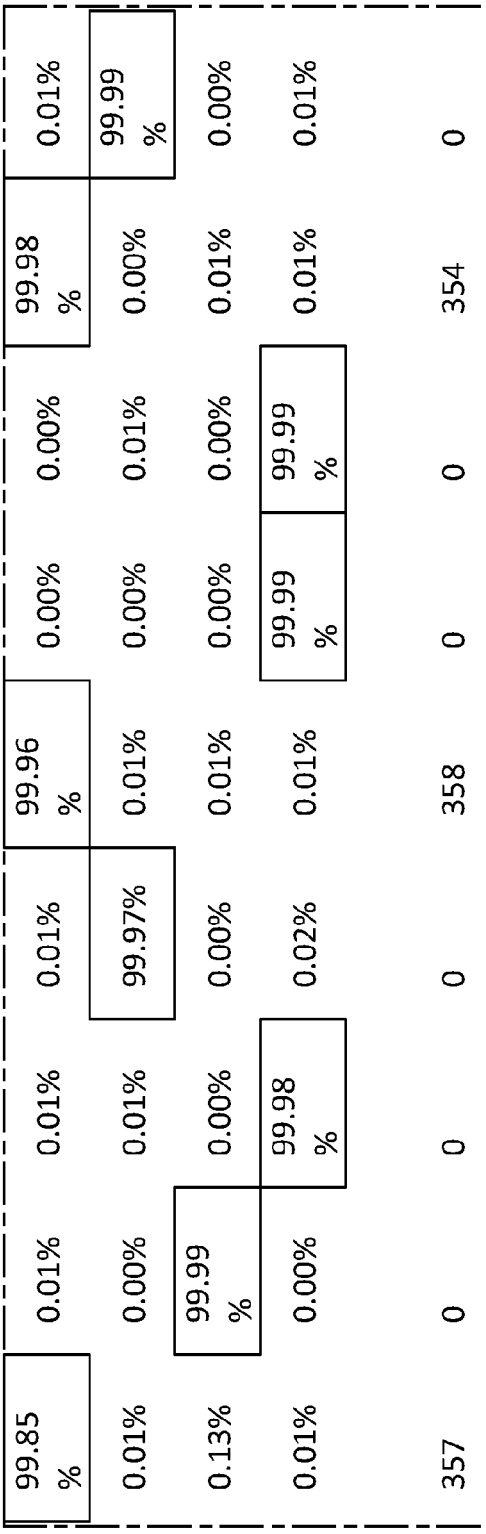


FIGURE 42 (Continued)

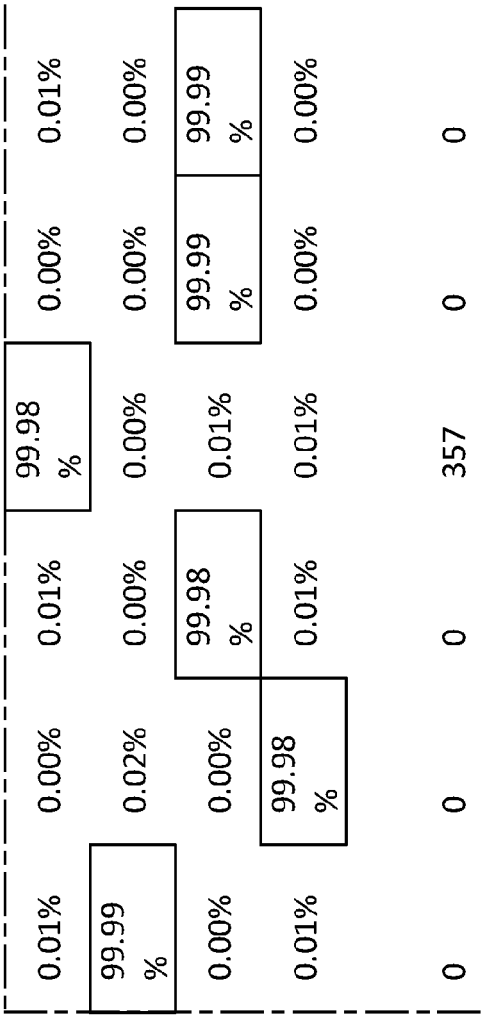


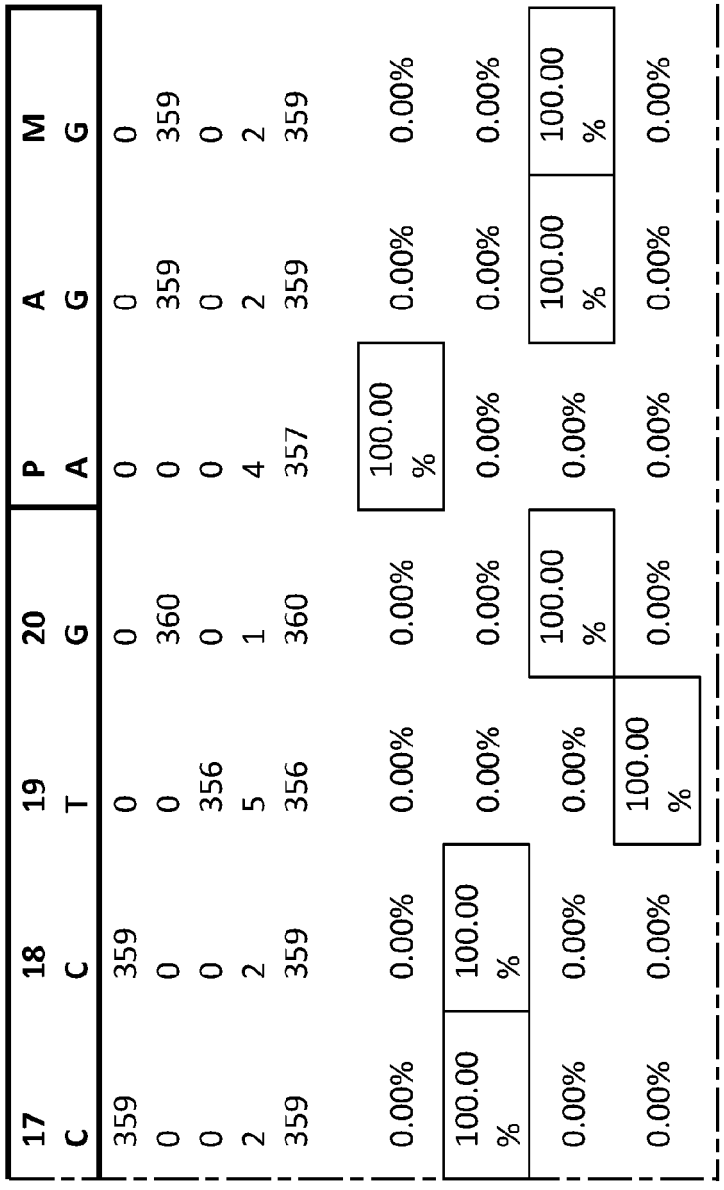
FIGURE 42 (Continued)

| | 1 G | 2 T | 3 C | 4 A | 5 T | 6 C | 7 T | 8 T |
|---|---|---|---|---|---|---|---|---|
| C | 0 | 0 | 358 | 0 | 0 | 358 | 0 | 0 |
| G | 361 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | 359 | 0 | 0 | 358 | 0 | 359 | 361 |
| N | 0 | 2 | 3 | 4 | 3 | 3 | 2 | 0 |
| | 361 | 359 | 358 | 357 | 358 | 358 | 359 | 361 |
| A | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% |
| G | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% | 100.00 % | 100.00 % |

FIGURE 42 (Continued)

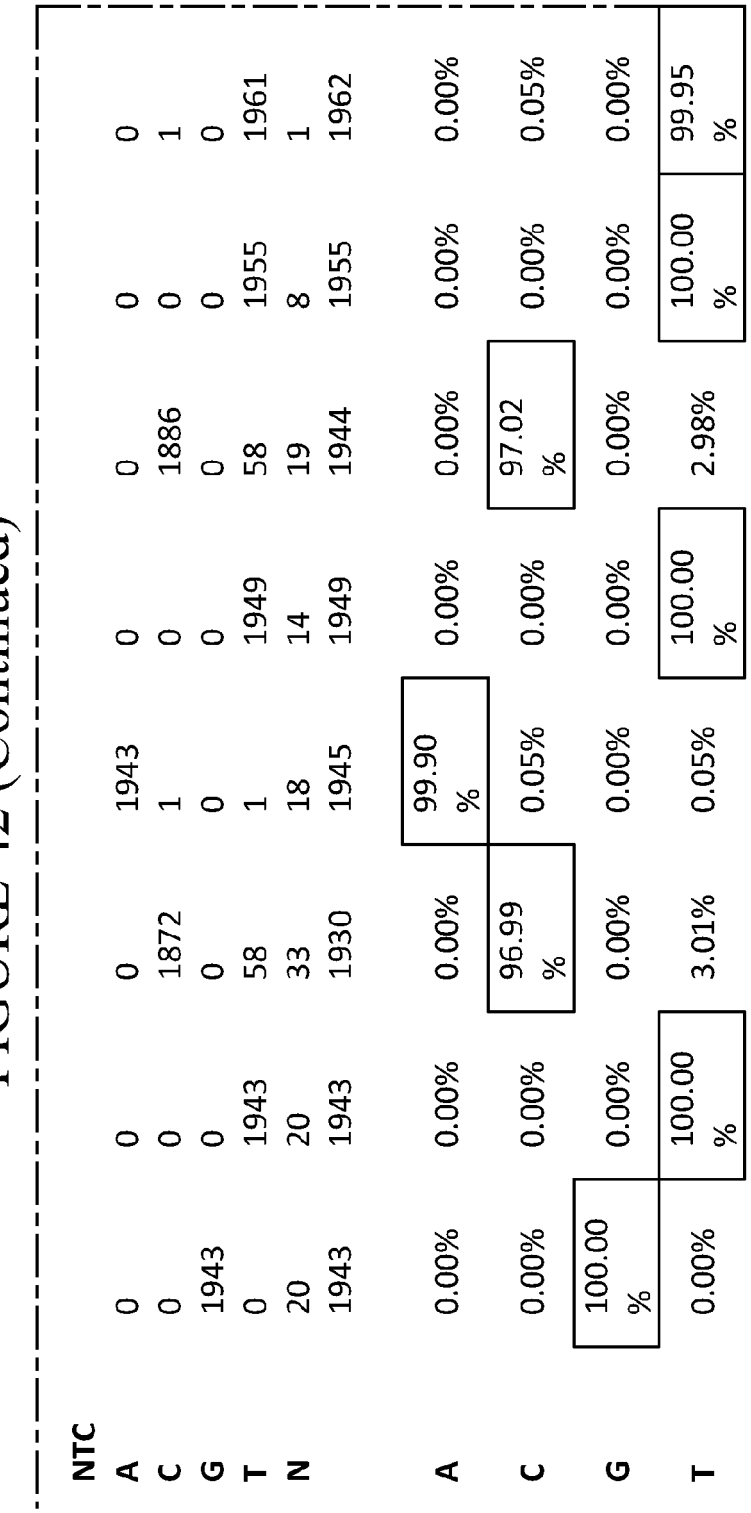
| 9 A | 10 G | 11 T | 12 C | 13 A | 14 G | 15 G | 16 A |
|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 358 | 0 | 0 | 0 | 0 |
| 0 | 359 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 361 | 0 | 0 | 359 | 361 | 0 |
| 4 | 2 | 0 | 3 | 3 | 2 | 0 | 7 |
| 357 | 359 | 361 | 358 | 358 | 359 | 361 | 354 |
| 100.00 % | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % |
| 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 100.00 % | 0.00% |

FIGURE 42 (Continued)



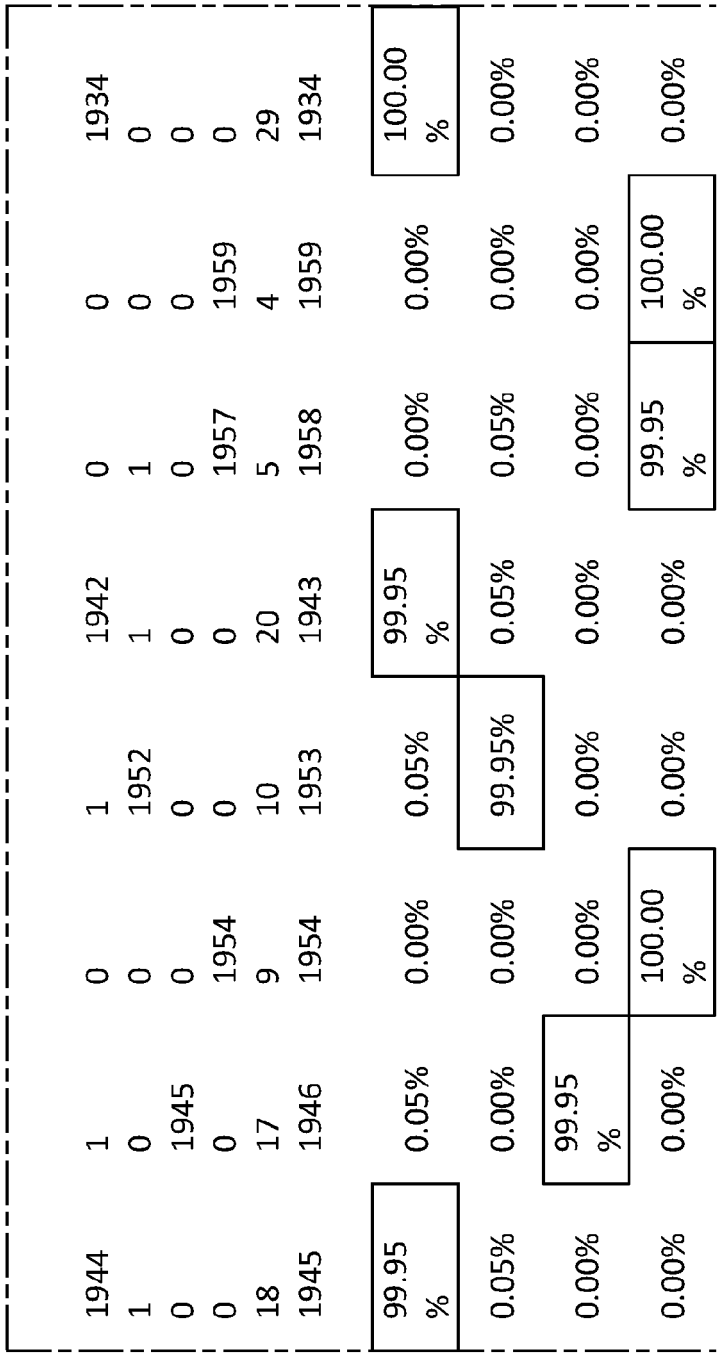
| 17 C | 18 C | 19 T | 20 G | P A | A G | M G |
|---|---|---|---|---|---|---|
| 359 | 359 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 360 | 0 | 359 | 359 |
| 0 | 0 | 356 | 0 | 0 | 0 | 0 |
| 2 | 2 | 5 | 1 | 4 | 2 | 2 |
| 359 | 359 | 356 | 360 | 357 | 359 | 359 |
| 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% |
| 100.00 % | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 100.00 % | 100.00 % |
| 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 42 (Continued)



| NTC | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 0 | 0 | 0 | 1943 | 0 | 0 | 0 | 0 | 0 |
| C | 0 | 0 | 1872 | 1 | 0 | 1886 | 0 | 0 | 1 |
| G | 1943 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | 1943 | 58 | 1 | 1949 | 58 | 1955 | 1961 | |
| N | 20 | 20 | 33 | 18 | 14 | 19 | 8 | 1 | |
| | 1943 | 1943 | 1930 | 1945 | 1949 | 1944 | 1955 | 1962 | |
| A | 0.00% | 0.00% | 0.00% | 99.90 % | 0.00% | 0.00% | 0.00% | 0.00% | |
| C | 0.00% | 0.00% | 96.99 % | 0.05% | 0.00% | 97.02 % | 0.00% | 0.05% | |
| G | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| T | 0.00% | 100.00 % | 3.01% | 0.05% | 100.00 % | 2.98% | 100.00 % | 99.95 % | |

FIGURE 42 (Continued)



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1944 | 1 | 0 | 1 | 1942 | 0 | 0 | 1934 | | |
| 1 | 0 | 0 | 1952 | 1 | 1 | 0 | 0 | | |
| 0 | 1945 | 0 | 0 | 0 | 0 | 0 | 0 | | |
| 0 | 0 | 1954 | 0 | 0 | 1957 | 1959 | 0 | | |
| 18 | 17 | 9 | 10 | 20 | 5 | 4 | 29 | | |
| 1945 | 1946 | 1954 | 1953 | 1943 | 1958 | 1959 | 1934 | | |
| 99.95 % | 0.05% | 0.00% | 0.05% | 99.95 % | 0.00% | 0.00% | 100.00 % | | |
| 0.05% | 0.00% | 0.00% | 99.95% | 0.05% | 0.05% | 0.00% | 0.00% | | |
| 0.00% | 99.95 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 99.95 % | 100.00 % | 0.00% | | |

FIGURE 42 (Continued)

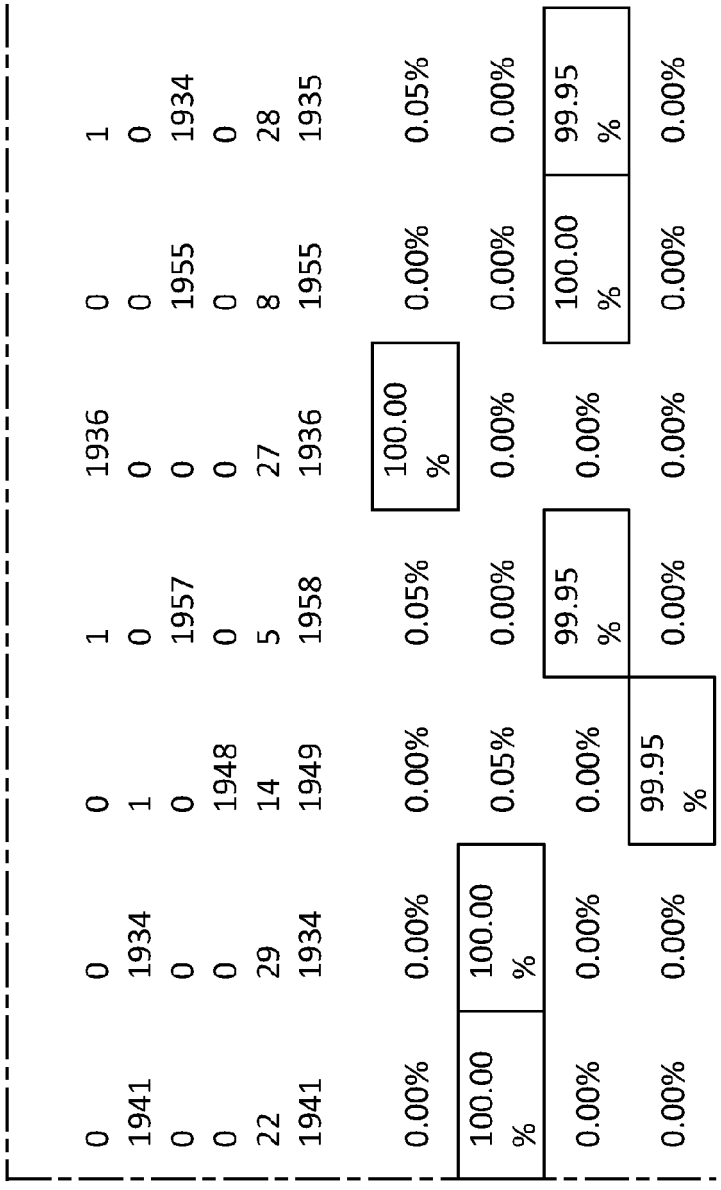


FIGURE 43

| | | 1 G | 2 G | 3 C | 4 A | 5 C | 6 T | 7 G | 8 C | 9 G | 10 G | 11 C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cas9 | | | | | | | | | | | | |
| | A | 1 | 8 | 3 | 32426 | 1 | 1 | 3 | 0 | 1 | 0 | 0 |
| | C | 0 | 0 | 31772 | 0 | 32709 | 4 | 1 | 33848 | 0 | 1 | 28542 |
| | G | 27402 | 29547 | 0 | 3 | 32 | 0 | 34276 | 0 | 34143 | 30841 | 0 |
| | T | 1 | 2 | 0 | 1 | 0 | 33721 | 1 | 11 | 0 | 0 | 2 |
| | N | 7811 | 5658 | 3400 | 2745 | 2433 | 1449 | 894 | 1280 | 995 | 4295 | 6592 |
| | | 27404 | 29557 | 31775 | 32430 | 32742 | 33726 | 34281 | 33859 | 34144 | 30842 | 28544 |
| | A | 0.00% | 0.03% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.00% | 0.00% | 99.99% | 0.00% | 99.90% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |
| | G | 99.99% | 99.97% | 0.00% | 0.01% | 0.10% | 0.00% | 99.99% | 0.00% | 100.00 % | 100.00 % | 0.00% |
| | T | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.01% |
| pNMG-247 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; wild type) | | | | | | | | | | | | |
| | A | 3 | 5 | 0 | 26252 | 1 | 0 | 0 | 5 | 1 | 0 | 23 |
| | C | 1 | 0 | 25761 | 0 | 26432 | 3 | 0 | 27314 | 0 | 1 | 23148 |
| | G | 22205 | 23879 | 0 | 2 | 30 | 27 | 27687 | 2 | 27629 | 24957 | 0 |
| | T | 0 | 1 | 0 | 30 | 3 | 27248 | 0 | 30 | 0 | 1 | 1 |
| | N | 6219 | 4543 | 2644 | 2120 | 1938 | 1126 | 717 | 1053 | 774 | 3445 | 5232 |
| | | 22209 | 23885 | 25761 | 26284 | 26466 | 27278 | 27687 | 27351 | 27630 | 24959 | 23172 |
| | A | 0.01% | 0.02% | 0.00% | 99.88% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.10% |
| | C | 0.00% | 0.00% | 100.00 % | 0.00% | 99.87% | 0.01% | 0.00% | 99.86% | 0.00% | 0.00% | 99.90% |
| | G | 99.98% | 99.97% | 0.00% | 0.01% | 0.11% | 0.10% | 100.00 % | 0.01% | 100.00 % | 99.99% | 0.00% |
| | T | 0.00% | 0.00% | 0.00% | 0.11% | 0.01% | 99.89% | 0.00% | 0.11% | 0.00% | 0.00% | 0.00% |

FIGURE 43 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 2 | 1 | 25822 | 1 | 1 | 16 | 3 | 1 | 0 | 2 | 3 |
| 2 | 1 | 0 | 0 | 0 | 0 | 27 | 1 | 1 | 0 | 1 | 0 |
| 0 | 28530 | 34043 | 5 | 32200 | 33503 | 32 | 25987 | 32772 | 30748 | 31012 | 27230 |
| 27180 | 1 | 0 | 1 | 2 | 0 | 6117 | 1 | 0 | 0 | 0 | 1 |
| 7954 | 6602 | 1092 | 9308 | 2933 | 1632 | 28916 | 9073 | 2269 | 4296 | 4028 | 7809 |
| 27182 | 28534 | 34044 | 25828 | 32203 | 33504 | 6192 | 25992 | 32774 | 30748 | 31015 | 27234 |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.26% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.44% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 100.00 % | 0.02% | 99.99% | 100.00 % | 0.52% | 99.98% | 99.99% | 100.00 % | 99.99% | 99.99% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 98.79% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 29 | 1 | 1 | 20859 | 29 | 1 | 6 | 13 | 19 | 2 | 14 | 1 |
| 1 | 0 | 33 | 1 | 0 | 23 | 22 | 0 | 7 | 13 | 1 | 0 |
| 0 | 23097 | 27470 | 33 | 26065 | 27110 | 157 | 21258 | 26793 | 24881 | 25293 | 22246 |
| 22023 | 0 | 0 | 1 | 2 | 1 | 5111 | 4 | 1 | 123 | 0 | 0 |
| 6351 | 5306 | 894 | 7504 | 2299 | 1260 | 23009 | 7000 | 1709 | 3509 | 3213 | 6274 |
| 22053 | 23098 | 27504 | 20894 | 26096 | 27135 | 5296 | 21275 | 26820 | 25019 | 25308 | 22247 |
| 0.13% | 0.00% | 0.00% | 99.83% | 0.11% | 0.00% | 0.11% | 0.06% | 0.07% | 0.01% | 0.06% | 0.00% |
| 0.00% | 0.00% | 0.12% | 0.00% | 0.00% | 0.08% | 0.42% | 0.00% | 0.03% | 0.05% | 0.00% | 0.00% |
| 0.00% | 100.00 % | 99.88% | 0.16% | 99.88% | 99.91% | 2.96% | 99.92% | 99.90% | 99.45% | 99.94% | 100.00 % |
| 99.86% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 96.51% | 0.02% | 0.00% | 0.49% | 0.00% | 0.00% |

FIGURE 43 (Continued)

| pNMG-248 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3 | 10 | 0 | 29595 | 0 | 0 | 0 | 0 | 2 | 2 | 6 | |
| C | 0 | 0 | 28887 | 0 | 29739 | 3 | 0 | 30756 | 0 | 7 | 26026 | |
| G | 25103 | 26695 | 1 | 1 | 0 | 0 | 31097 | 0 | 31016 | 28055 | 0 | |
| T | 0 | 0 | 1 | 0 | 0 | 30657 | 0 | 16 | 2 | 0 | 2 | |
| N | 6812 | 5213 | 3016 | 2308 | 2157 | 1236 | 799 | 1124 | 876 | 3832 | 5862 | |
| | 25106 | 26705 | 28889 | 29596 | 29739 | 30660 | 31097 | 30772 | 31020 | 28064 | 26034 | |
| | | | | 100.00 | | | | | | | | |
| A | 0.01% | 0.04% | 0.00% | % | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.02% | |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 100.00 % | 0.01% | 0.00% | 99.95% | 0.00% | 0.02% | 99.97% | |
| G | 99.99% | 99.96% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 99.99% | 99.97% | 0.00% | |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.05% | 0.01% | 0.00% | 0.01% | |
| pNMG-249 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | | | | |
| A | 1 | 6 | 0 | 27195 | 0 | 0 | 2 | 1 | 1 | 2 | 0 | |
| C | 0 | 0 | 26675 | 0 | 27351 | 4 | 0 | 28346 | 1 | 0 | 23983 | |
| G | 22984 | 24716 | 0 | 0 | 0 | 0 | 28680 | 0 | 28668 | 25809 | 5 | |
| T | 0 | 0 | 2 | 0 | 30 | 28320 | 1 | 11 | 0 | 1 | 3 | |
| N | 6463 | 4726 | 2771 | 2253 | 2067 | 1124 | 765 | 1090 | 775 | 3633 | 5455 | |
| | 22985 | 24722 | 26677 | 27195 | 27381 | 28324 | 28683 | 28358 | 28670 | 25812 | 23991 | |

FIGURE 43 (Continued)

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 1 | 2 | 23642 | 1 | 3 | 0 | 1 | 0 | 1 | 2 | 0 | | | | | | | |
| 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | | | | | | | |
| 0 | 26161 | 30853 | 4 | 29323 | 30410 | 53 | 23722 | 29812 | 28032 | 28375 | 24893 | | | | | | | |
| 25016 | 0 | 6 | 0 | 0 | 1 | 5586 | 1 | 2 | 2 | 0 | 2 | | | | | | | |
| 6878 | 5734 | 1035 | 8248 | 2566 | 1460 | 26192 | 8107 | 2015 | 3794 | 3559 | 7044 | | | | | | | |
| 25018 | 26162 | 30861 | 23648 | 29324 | 30414 | 5639 | 23724 | 29814 | 28035 | 28378 | 24895 | | | | | | | |
| | | | | | | | | | | | | | | | | | | |
| 0.00% | 0.00% | 0.01% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | | | | | | | |
| | | | | | | | | | | | | | | | | | | |
| 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | |
| | 100.00 | | | 100.00 | | | | | | | | | | | | | | |
| 0.00% | % | 99.97% | 0.02% | % | 99.99% | 0.94% | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | | | | | | | |
| 99.99% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 99.06% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | | | | | | | |
| 0 | 0 | 1 | 21626 | 1 | 0 | 13 | 1 | 1 | 0 | 1 | 0 | | | | | | | |
| 0 | 0 | 1 | 1 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 0 | | | | | | | |
| 0 | 23917 | 28516 | 2 | 26931 | 28091 | 8 | 21754 | 27401 | 25637 | 25924 | 22727 | | | | | | | |
| 22841 | 2 | 0 | 1 | 40 | 0 | 5207 | 1 | 2 | 0 | 0 | 2 | | | | | | | |
| 6605 | 5526 | 927 | 7815 | 2473 | 1354 | 24111 | 7447 | 1794 | 3580 | 3293 | 6485 | | | | | | | |
| 22841 | 23919 | 28518 | 21630 | 26972 | 28091 | 5233 | 21756 | 27404 | 25638 | 25925 | 22729 | | | | | | | |

FIGURE 43 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | G | C | A | C | T | G | C | G | G | C |
| A | 0.00% | 0.02% | 0.00% | 100.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 99.89% | 0.01% | 0.00% | 99.96% | 0.00% | 0.00% | 99.97% |
| G | 100.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.02% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.11% | 99.99% | 0.00% | 0.04% | 0.00% | 0.00% | 0.01% |

**pNMG-250 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 12 | 0 | 27212 | 0 | 2 | 4 | 1 | 4 | 1 | 0 |
| C | 0 | 0 | 26533 | 0 | 27401 | 4 | 1 | 28284 | 0 | 0 | 23842 |
| G | 22902 | 24446 | 0 | 2 | 1 | 1 | 28634 | 1 | 28569 | 25676 | 1 |
| T | 1 | 1 | 4 | 1 | 3 | 28165 | 1 | 16 | 0 | 1 | 4 |
| N | 6466 | 4912 | 2834 | 2156 | 1966 | 1199 | 731 | 1069 | 798 | 3693 | 5524 |
| | 22905 | 24459 | 26537 | 27215 | 27405 | 28172 | 28640 | 28302 | 28573 | 25678 | 23847 |
| A | 0.01% | 0.05% | 0.00% | 99.99% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 0.01% | 0.00% | 99.94% | 0.00% | 0.00% | 99.98% |
| G | 99.99% | 99.95% | 0.00% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% |
| T | 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 99.98% | 0.00% | 0.06% | 0.00% | 0.00% | 0.02% |

FIGURE 43 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | |
| 0.00% | 0.00% | 0.00% | | 0.00% | 0.00% | 0.25% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.10% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | | | 0.00% | | | | | | | | |
| | | | | | 100.00 | | | | | | |
| 0.00% | 99.99% | 99.99% | 0.01% | 99.85% | % | 0.15% | 99.99% | 99.99% | 100.00 % | 100.00 % | 99.99% |
| 100.00 % | 0.01% | 0.00% | 0.00% | 0.15% | 0.00% | 99.50% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 1 | 0 | 1 | 21502 | 0 | 77 | 0 | 0 | 2 | 1 | 1 | 0 |
| 5 | 0 | 46 | 0 | 0 | 39 | 16 | 0 | 0 | 6 | 0 | 1 |
| 0 | 23852 | 28402 | 43 | 27067 | 27994 | 5 | 21803 | 27396 | 25642 | 25843 | 22701 |
| 22701 | 0 | 1 | 1 | 2 | 20 | 5336 | 1 | 25 | 1 | 7 | 2 |
| 6664 | 5519 | 918 | 7931 | 2409 | 1348 | 24027 | 7553 | 1913 | 3680 | 3427 | 6573 |
| 22707 | 23852 | 28450 | 21546 | 27069 | 28130 | 5357 | 21804 | 27423 | 25650 | 25851 | 22704 |
| 0.00% | 0.00% | 0.00% | 99.80% | 0.00% | 0.27% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0.02% | 0.00% | 0.16% | 0.00% | 0.00% | 0.14% | 0.30% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% |
| | 100.00 | | | | | | 100.00 | | | | |
| 0.00% | % | 99.83% | 0.20% | 99.99% | 99.52% | 0.09% | % | 99.90% | 99.97% | 99.97% | 99.99% |
| 99.97% | 0.00% | 0.00% | 0.00% | 0.01% | 0.07% | 99.61% | 0.00% | 0.09% | 0.00% | 0.03% | 0.01% |

FIGURE 43 (Continued)

pNMG-251 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 7 | 2 | 51744 | 1 | 0 | 3 | 0 | 1 | 4 | 0 | |
| C | 0 | 1 | 50422 | 0 | 52059 | 8 | 0 | 53818 | 0 | 0 | 45074 | |
| G | 43110 | 46662 | 0 | 11 | 28 | 0 | 54520 | 1 | 54353 | 48875 | 1 | |
| T | 2 | 0 | 4 | 2 | 5 | 53650 | 1 | 18 | 1 | 1 | 4 | |
| N | 12794 | 9238 | 5480 | 4150 | 3814 | 2249 | 1383 | 2070 | 1552 | 7027 | 10828 | |
| | 43114 | 46670 | 50428 | 51757 | 52093 | 53658 | 54524 | 53837 | 54355 | 48880 | 45079 | |
| A | 0.00% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 99.93% | 0.01% | 0.00% | 99.96% | 0.00% | 0.00% | 99.99% | |
| G | 99.99% | 99.98% | 0.00% | 0.02% | 0.05% | 0.00% | 99.99% | 0.00% | 100.00 % | 99.99% | 0.00% | |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.01% | |

pNMG-181 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; D108N_D147Y_E155V)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 13 | 30 | 63667 | 66 | 0 | 2 | 2 | 5 | 3 | 17 | |
| C | 1 | 0 | 62189 | 0 | 63742 | 2 | 0 | 66486 | 0 | 0 | 55152 | |
| G | 52827 | 57410 | 0 | 125 | 133 | 0 | 67410 | 0 | 67144 | 60177 | 0 | |
| T | 47 | 11 | 6 | 5 | 345 | 66246 | 10 | 40 | 36 | 8 | 2 | |
| N | 16331 | 11777 | 6986 | 5414 | 4925 | 2963 | 1789 | 2683 | 2026 | 9023 | 14040 | |
| | 52880 | 57434 | 62225 | 63797 | 64286 | 66248 | 67422 | 66528 | 67185 | 60188 | 55171 | |
| A | 0.01% | 0.02% | 0.05% | 99.80% | 0.10% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.03% | |
| C | 0.00% | 0.00% | 99.94% | 0.00% | 99.15% | 0.00% | 0.00% | 99.94% | 0.00% | 0.00% | 99.97% | |
| G | 99.90% | 99.96% | 0.00% | 0.20% | 0.21% | 0.00% | 99.98% | 0.00% | 99.94% | 99.98% | 0.00% | |
| T | 0.09% | 0.02% | 0.01% | 0.01% | 0.54% | 100.00 % | 0.01% | 0.06% | 0.05% | 0.01% | 0.00% | |

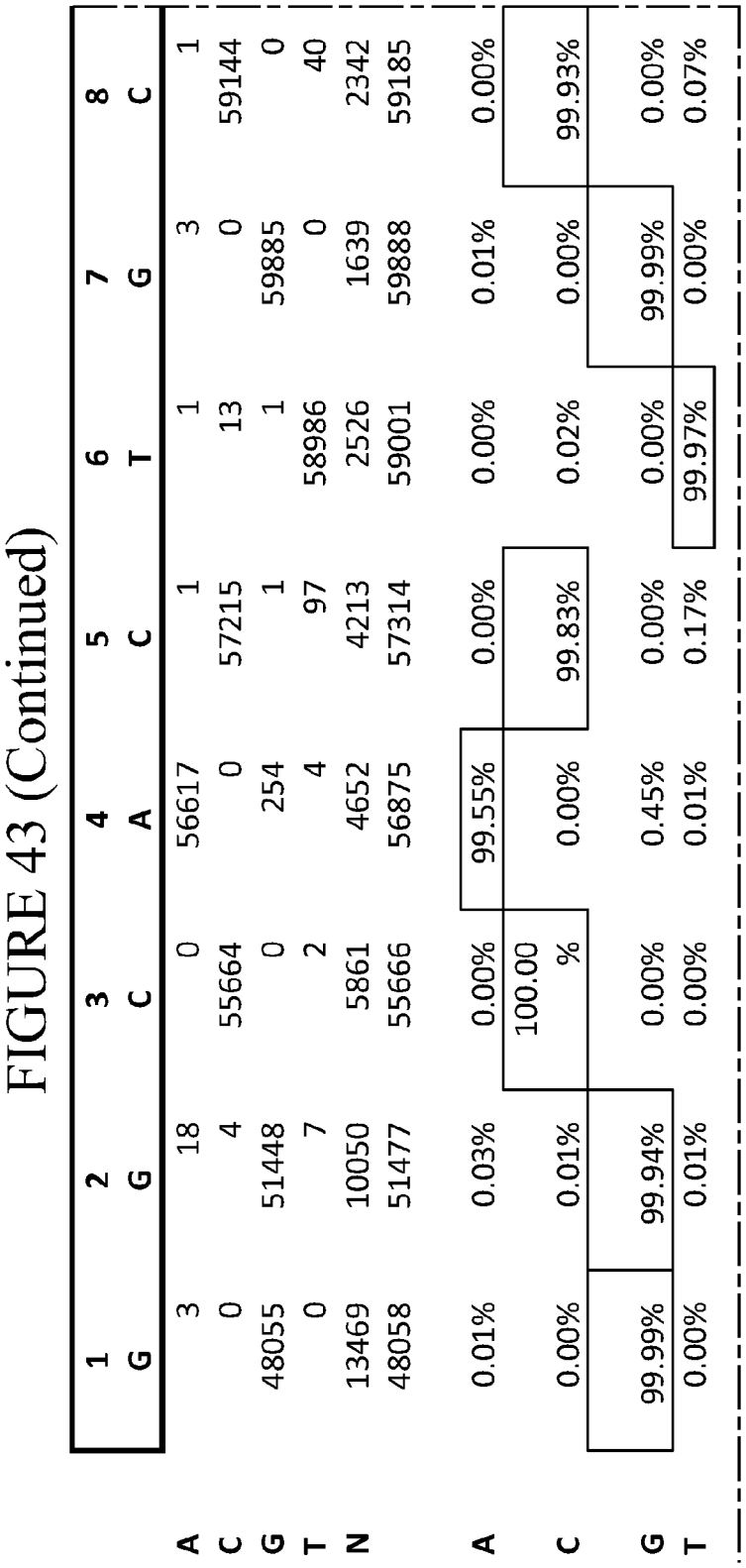
pNMG-182(pCMV_ecTadA_GGS_Cas9n_GGS_NLS; D108N_D147Y_E155V)

# FIGURE 43 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 3 | 2 | 40630 | 0 | 1 | 0 | 0 | 2 | 3 | 1 | 2 |
| 3 | 0 | 1 | 0 | 0 | 0 | 5 | 1 | 0 | 1 | 0 | 3 |
| 0 | 45097 | 54068 | 2 | 51241 | 53308 | 15 | 40823 | 52086 | 48791 | 49240 | 43241 |
| 42899 | 1 | 0 | 0 | 0 | 1 | 9382 | 1 | 2 | 0 | 0 | 2 |
| 13005 | 10806 | 1836 | 15275 | 4666 | 2597 | 46442 | 15005 | 3737 | 7032 | 6586 | 12575 |
| 42902 | 45101 | 54071 | 40632 | 51241 | 53310 | 9402 | 40825 | 52090 | 48795 | 49241 | 43248 |
| 0.00% | 0.01% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.00% | 99.99% | 99.99% | 0.00% | 100.00 % | 100.00 % | 0.16% | 100.00 % | 99.99% | 99.99% | 100.00 % | 99.98% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.79% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

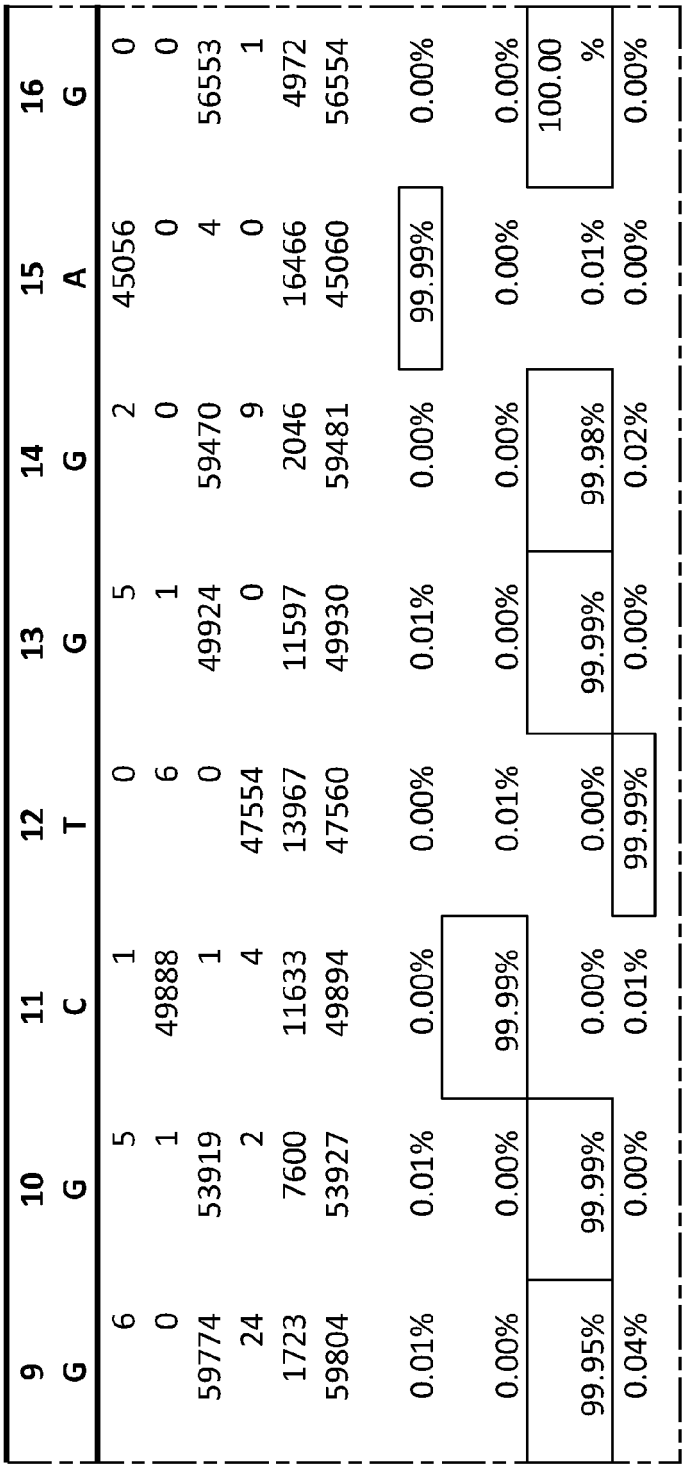
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 2 | 5 | 49585 | 3 | 2 | 1 | 2 | 4 | 1 | 1 | 4 |
| 5 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 2 | 2 | 1 | 0 |
| 0 | 55266 | 66835 | 8 | 62996 | 65842 | 10 | 50036 | 64480 | 60127 | 60678 | 53048 |
| 52436 | 1 | 11 | 0 | 41 | 21 | 11554 | 8 | 5 | 25 | 7 | 8 |
| 16770 | 13940 | 2360 | 19618 | 6169 | 3347 | 57644 | 19165 | 4720 | 9056 | 8524 | 16151 |
| 52441 | 55271 | 66851 | 49593 | 63042 | 65865 | 11567 | 50046 | 64491 | 60155 | 60687 | 53060 |
| 0.00% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 99.98% | 0.02% | 99.93% | 99.97% | 0.09% | 99.98% | 99.98% | 99.95% | 99.99% | 99.98% |
| 99.99% | 0.00% | 0.02% | 0.00% | 0.07% | 0.03% | 99.89% | 0.02% | 0.01% | 0.04% | 0.01% | 0.02% |

FIGURE 43 (Continued)



| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| | | G | G | C | A | C | T | G | C |
| A | | 3 | 18 | 0 | 56617 | 1 | 1 | 3 | 1 |
| C | | 0 | 4 | 55664 | 0 | 57215 | 13 | 0 | 59144 |
| G | | 48055 | 51448 | 0 | 254 | 1 | 1 | 59885 | 0 |
| T | | 0 | 7 | 2 | 4 | 97 | 58986 | 0 | 40 |
| N | | 13469 | 10050 | 5861 | 4652 | 4213 | 2526 | 1639 | 2342 |
| | | 48058 | 51477 | 55666 | 56875 | 57314 | 59001 | 59888 | 59185 |
| | A | 0.01% | 0.03% | 0.00% | 99.55% | 0.00% | 0.00% | 0.01% | 0.00% |
| | C | 0.00% | 0.01% | 100.00 % | 0.00% | 99.83% | 0.02% | 0.00% | 99.93% |
| | G | 99.99% | 99.94% | 0.00% | 0.45% | 0.00% | 0.00% | 99.99% | 0.00% |
| | T | 0.00% | 0.01% | 0.00% | 0.01% | 0.17% | 99.97% | 0.00% | 0.07% |

FIGURE 43 (Continued)

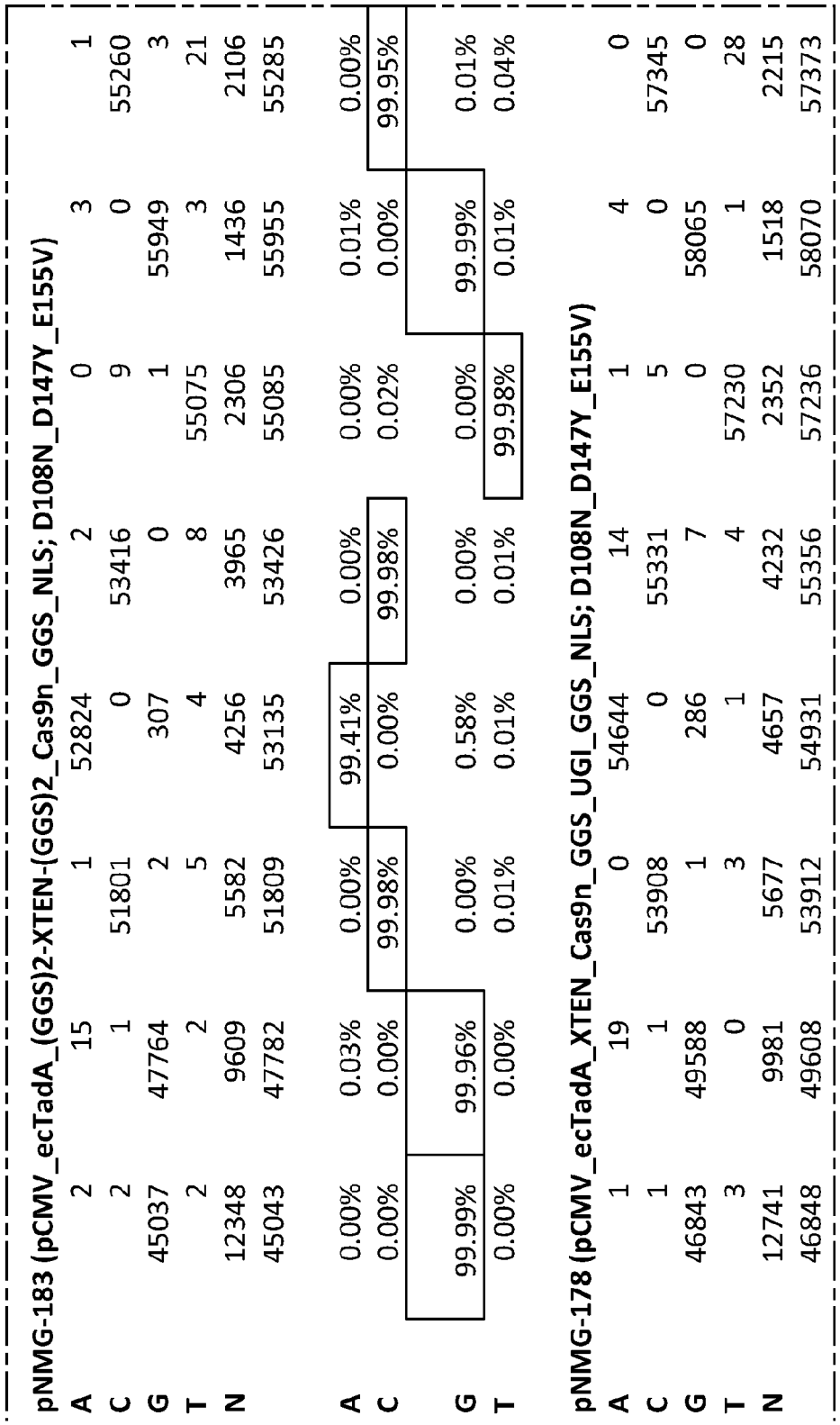


| 9 G | 10 G | 11 C | 12 T | 13 G | 14 G | 15 A | 16 G |
|---|---|---|---|---|---|---|---|
| 6 | 5 | 1 | 0 | 5 | 2 | 45056 | 0 |
| 0 | 1 | 49888 | 6 | 1 | 0 | 0 | 0 |
| 59774 | 53919 | 1 | 0 | 49924 | 59470 | 4 | 56553 |
| 24 | 2 | 4 | 47554 | 0 | 9 | 0 | 1 |
| 1723 | 7600 | 11633 | 13967 | 11597 | 2046 | 16466 | 4972 |
| 59804 | 53927 | 49894 | 47560 | 49930 | 59481 | 45060 | 56554 |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% |
| 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.95% | 99.99% | 0.00% | 0.00% | 99.99% | 99.98% | 0.01% | 100.00 % |
| 0.04% | 0.00% | 0.01% | 99.99% | 0.00% | 0.02% | 0.00% | 0.00% |

FIGURE 43 (Continued)

| 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|
| 2 | 0 | 3 | 2 | 0 | 1 | 2 |
| 0 | 2 | 2 | 0 | 0 | 1 | 0 |
| 58762 | 10 | 45531 | 57669 | 53946 | 54473 | 47595 |
| 0 | 10541 | 1 | 1 | 1 | 0 | 2 |
| 2762 | 50963 | 15979 | 3844 | 7569 | 7041 | 13917 |
| 58764 | 10553 | 45537 | 57672 | 53947 | 54475 | 47599 |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.09% | 99.99% | 99.99% | 100.00 % | 100.00 % | 99.99% |
| 0.00% | 99.89% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 43 (Continued)



| pNMG-183 (pCMV_ecTadA_(GGS)2-XTEN-(GGS)2_Cas9n_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 2 | 15 | 1 | 52824 | 2 | 0 | 3 | 1 | | |
| C | 2 | 1 | 51801 | 0 | 53416 | 9 | 0 | 55260 | | |
| G | 45037 | 47764 | 2 | 307 | 0 | 1 | 55949 | 3 | | |
| T | 2 | 2 | 5 | 4 | 8 | 55075 | 3 | 21 | | |
| N | 12348 | 9609 | 5582 | 4256 | 3965 | 2306 | 1436 | 2106 | | |
| | 45043 | 47782 | 51809 | 53135 | 53426 | 55085 | 55955 | 55285 | | |
| | | | | | | | | | | |
| A | 0.00% | 0.03% | 0.00% | 99.41% | 0.00% | 0.00% | 0.01% | 0.00% | | |
| C | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.02% | 0.00% | 99.95% | | |
| G | 99.99% | 99.96% | 0.00% | 0.58% | 0.00% | 0.00% | 99.99% | 0.01% | | |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.04% | | |
| | | | | | | | | | | |
| pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | |
| A | 1 | 19 | 0 | 54644 | 14 | 1 | 4 | 0 | | |
| C | 1 | 1 | 53908 | 0 | 55331 | 5 | 0 | 57345 | | |
| G | 46843 | 49588 | 1 | 286 | 7 | 0 | 58065 | 0 | | |
| T | 3 | 0 | 3 | 1 | 4 | 57230 | 1 | 28 | | |
| N | 12741 | 9981 | 5677 | 4657 | 4232 | 2352 | 1518 | 2215 | | |
| | 46848 | 49608 | 53912 | 54931 | 55356 | 57236 | 58070 | 57373 | | |

FIGURE 43 (Continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 6 | 6 | 0 | 0 | 3 | 3 | 41825 | 1 |
| 0 | 0 | 46756 | 4 | 0 | 0 | 0 | 0 |
| 55849 | 50395 | 1 | 0 | 46547 | 55565 | 4 | 52683 |
| 1 | 2 | 3 | 44534 | 3 | 2 | 1 | 3 |
| 1535 | 6988 | 10631 | 12853 | 10838 | 1821 | 15561 | 4704 |
| 55856 | 50403 | 46760 | 44538 | 46553 | 55570 | 41830 | 52687 |
| | | | | | | | |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.00% |
| 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 99.98% | 0.00% | 0.00% | 99.99% | 99.99% | 0.01% | 99.99% |
| 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 0.01% |
| | | | | | | | |
| 3 | 3 | 0 | 0 | 1 | 1 | 43273 | 2 |
| 0 | 0 | 48597 | 8 | 1 | 2 | 1 | 1 |
| 57958 | 52365 | 0 | 0 | 48434 | 57719 | 5 | 54836 |
| 1 | 0 | 3 | 46333 | 1 | 0 | 0 | 0 |
| 1626 | 7220 | 10988 | 13247 | 11151 | 1866 | 16309 | 4749 |
| 57962 | 52368 | 48600 | 46341 | 48437 | 57722 | 43279 | 54839 |

# FIGURE 43 (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 5 | 2 | 2 |
| 0 | 4 | 0 | 0 | 0 | 0 | 1 |
| 54835 | 12 | 42948 | 53805 | 50526 | 50967 | 44661 |
| 1 | 10076 | 13 | 5 | 2 | 2 | 3 |
| 2554 | 47299 | 14430 | 3581 | 6858 | 6420 | 12724 |
| 54837 | 10092 | 42961 | 53810 | 50533 | 50971 | 44667 |
| | | | | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.12% | 99.97% | 99.99% | 99.99% | 99.99% | 99.99% |
| 0.00% | 99.84% | 0.03% | 0.01% | 0.00% | 0.00% | 0.01% |
| | | | | | | |
| 1 | 0 | 0 | 1 | 2 | 4 | 5 |
| 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 56943 | 21 | 45091 | 56084 | 52501 | 52976 | 46446 |
| 0 | 10715 | 2 | 1 | 1 | 0 | 2 |
| 2645 | 48852 | 14496 | 3502 | 7085 | 6609 | 13136 |
| 56944 | 10737 | 45093 | 56087 | 52504 | 52980 | 46453 |

FIGURE 43 (Continued)

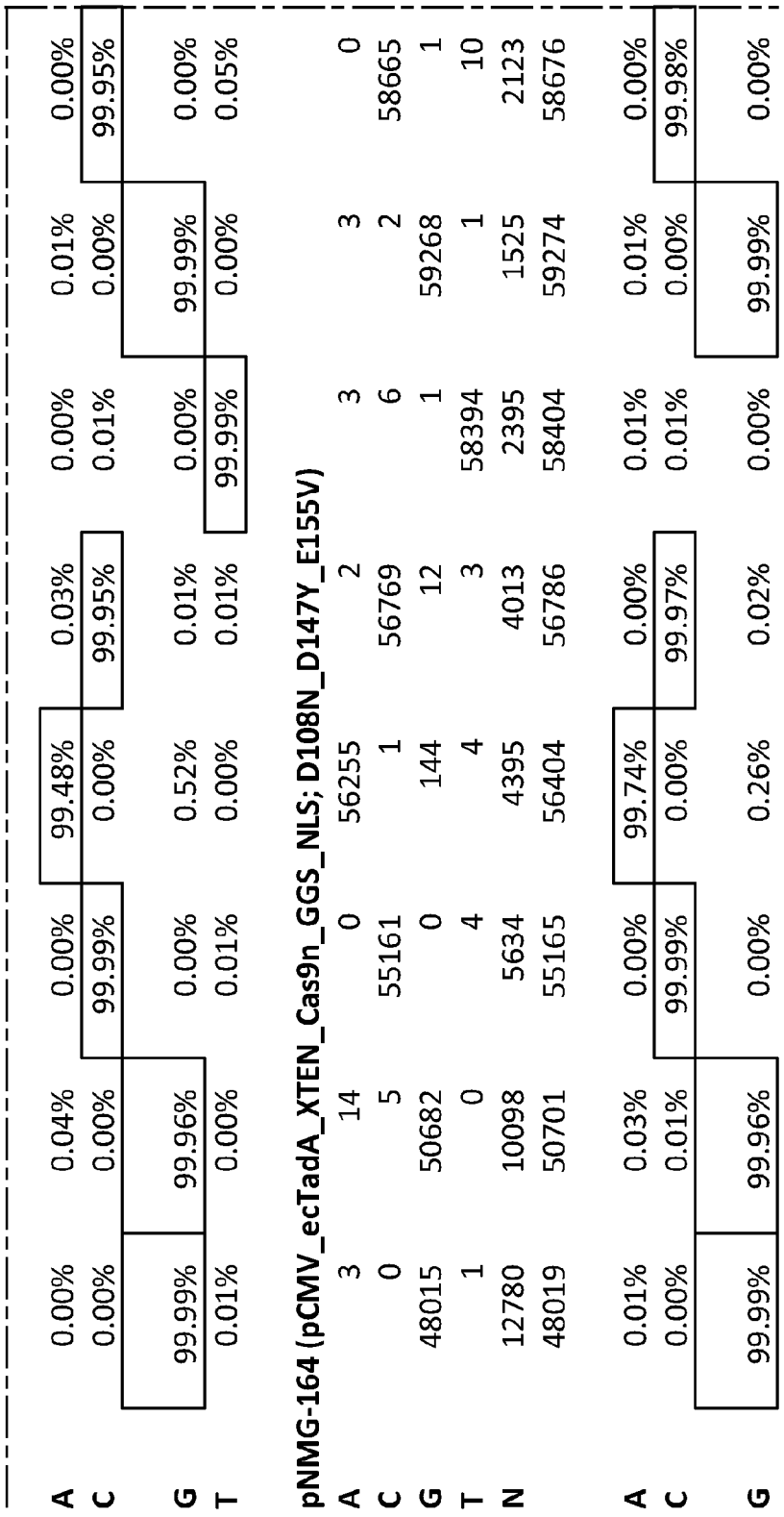
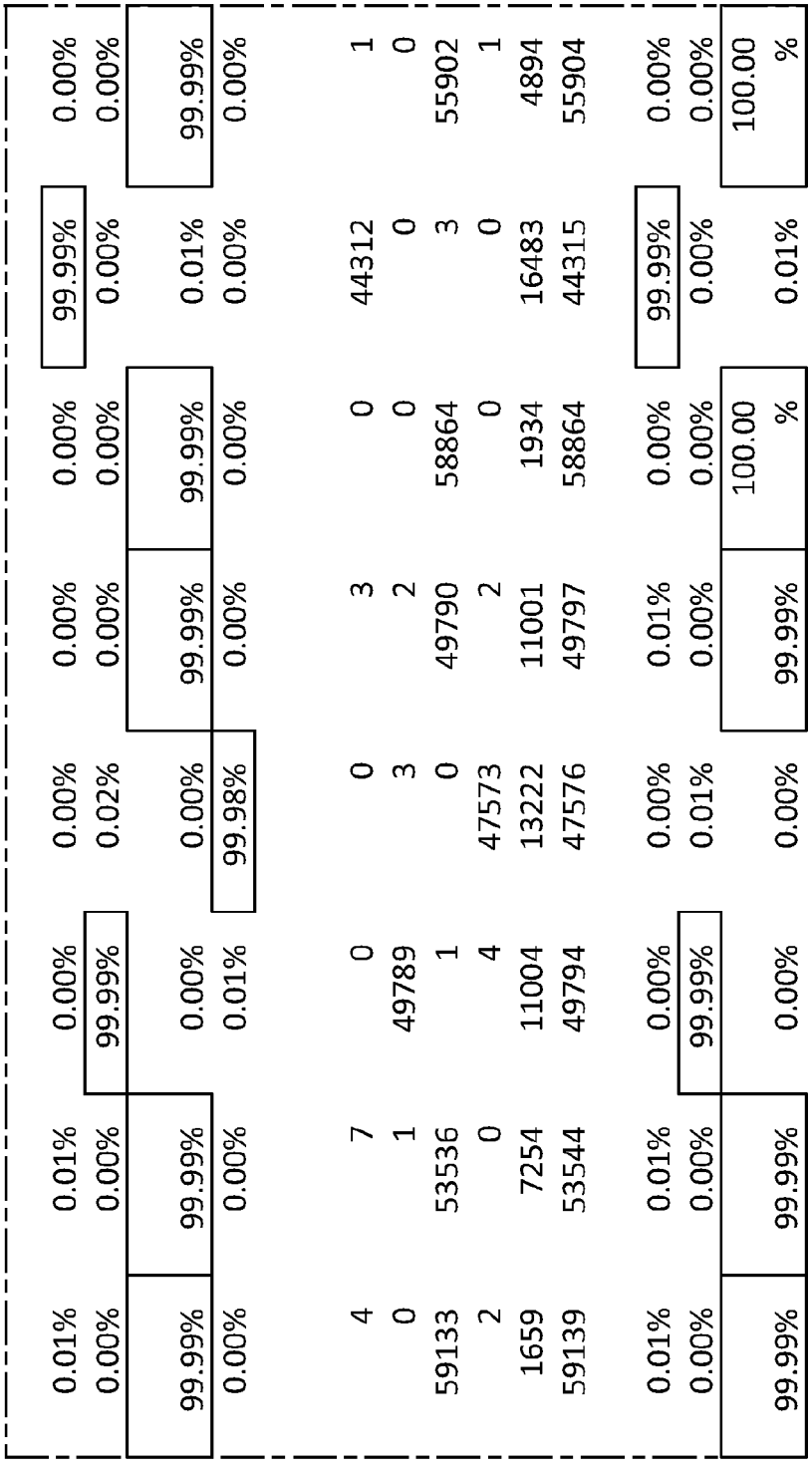


FIGURE 43 (Continued)



| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | | | | | | | | | | | | | | | |
| 0.00% | 0.00% | 99.99% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | | | | |
| 99.99% | 99.99% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 0.01% | 99.99% | | | | | | | | | | | | | | | |
| 0.00% | 0.00% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | | | | |
| 4 | 7 | 0 | 0 | 3 | 0 | 44312 | 1 | | | | | | | | | | | | | | | | |
| 0 | 1 | 49789 | 3 | 2 | 0 | 0 | 0 | | | | | | | | | | | | | | | | |
| 59133 | 53536 | 1 | 0 | 49790 | 58864 | 3 | 55902 | | | | | | | | | | | | | | | | |
| 2 | 0 | 4 | 47573 | 2 | 0 | 0 | 1 | | | | | | | | | | | | | | | | |
| 1659 | 7254 | 11004 | 13222 | 11001 | 1934 | 16483 | 4894 | | | | | | | | | | | | | | | | |
| 59139 | 53544 | 49794 | 47576 | 49797 | 58864 | 44315 | 55904 | | | | | | | | | | | | | | | | |
| 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.00% | | | | | | | | | | | | | | | | |
| 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | | | | | | | | | | | | | | | | |
| 99.99% | 99.99% | 0.00% | 0.00% | 99.99% | 100.00 | 0.01% | 100.00 | | | | | | | | | | | | | | | | |
| | | | | | % | | % | | | | | | | | | | | | | | | | |

# FIGURE 43 (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.20% | 100.00 % | 99.99% | 99.99% | 99.99% | 99.98% |
| 0.00% | 99.80% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 4 | 0 | 2 | 1 | 1 | 2 | 3 |
| 0 | 11 | 0 | 0 | 1 | 2 | 3 |
| 58078 | 6 | 45535 | 57073 | 53637 | 54149 | 47629 |
| 0 | 10767 | 1 | 0 | 1 | 0 | 1 |
| 2716 | 50014 | 15260 | 3724 | 7158 | 6645 | 13162 |
| 58082 | 10784 | 45538 | 57074 | 53640 | 54153 | 47636 |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.10% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 99.99% | 0.06% | 99.99% | 100.00 % | 99.99% | 99.99% | 99.99% |

FIGURE 43 (Continued)

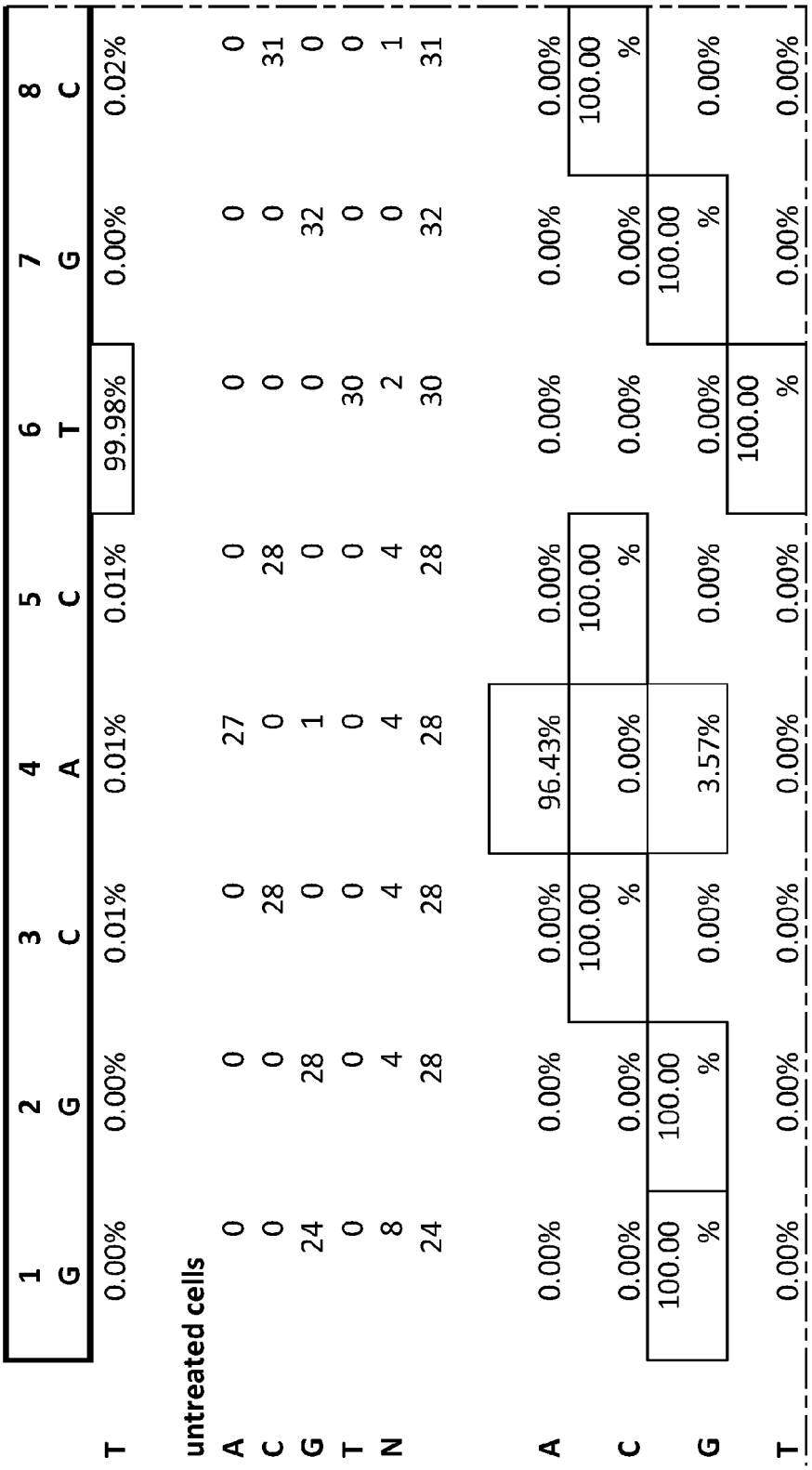
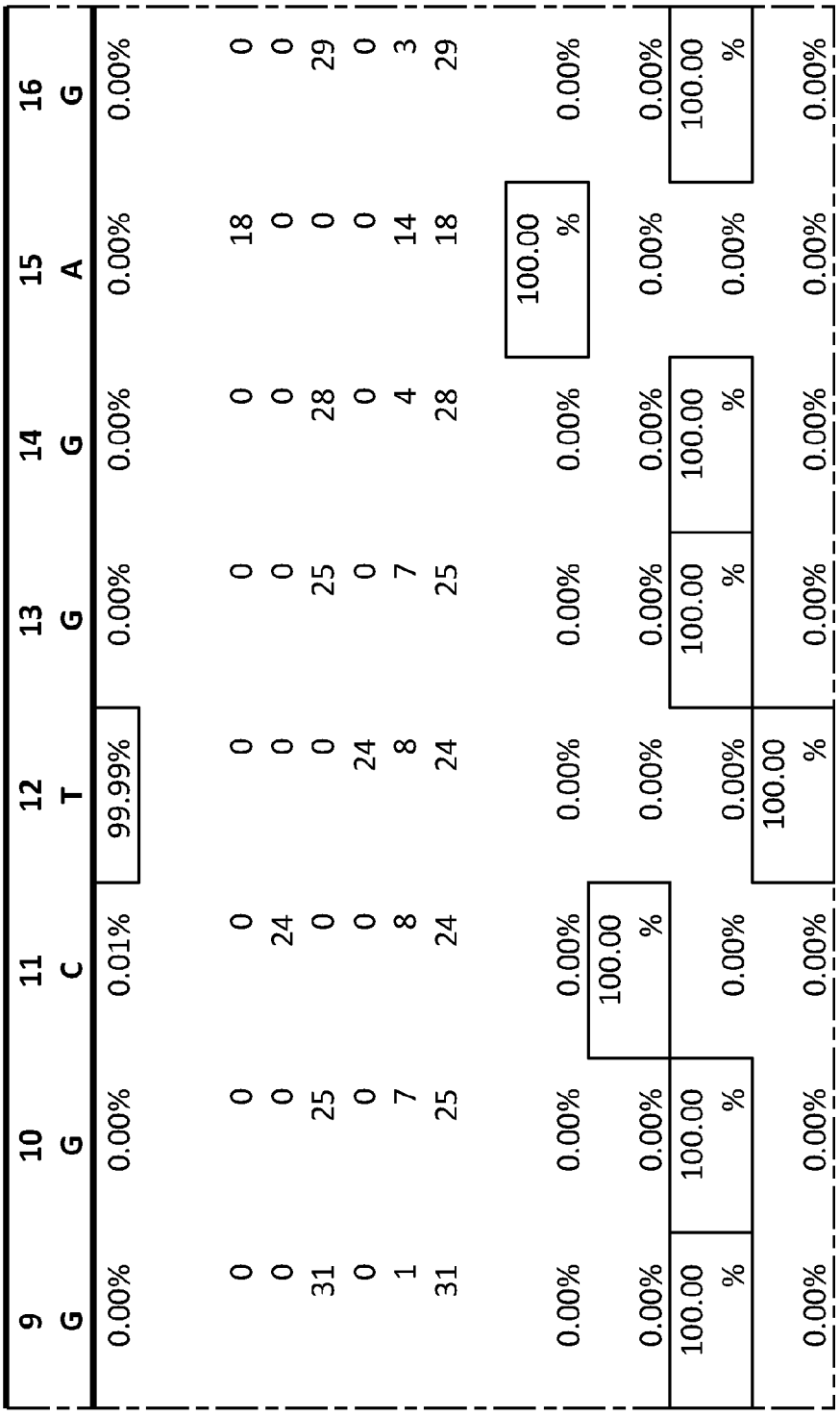
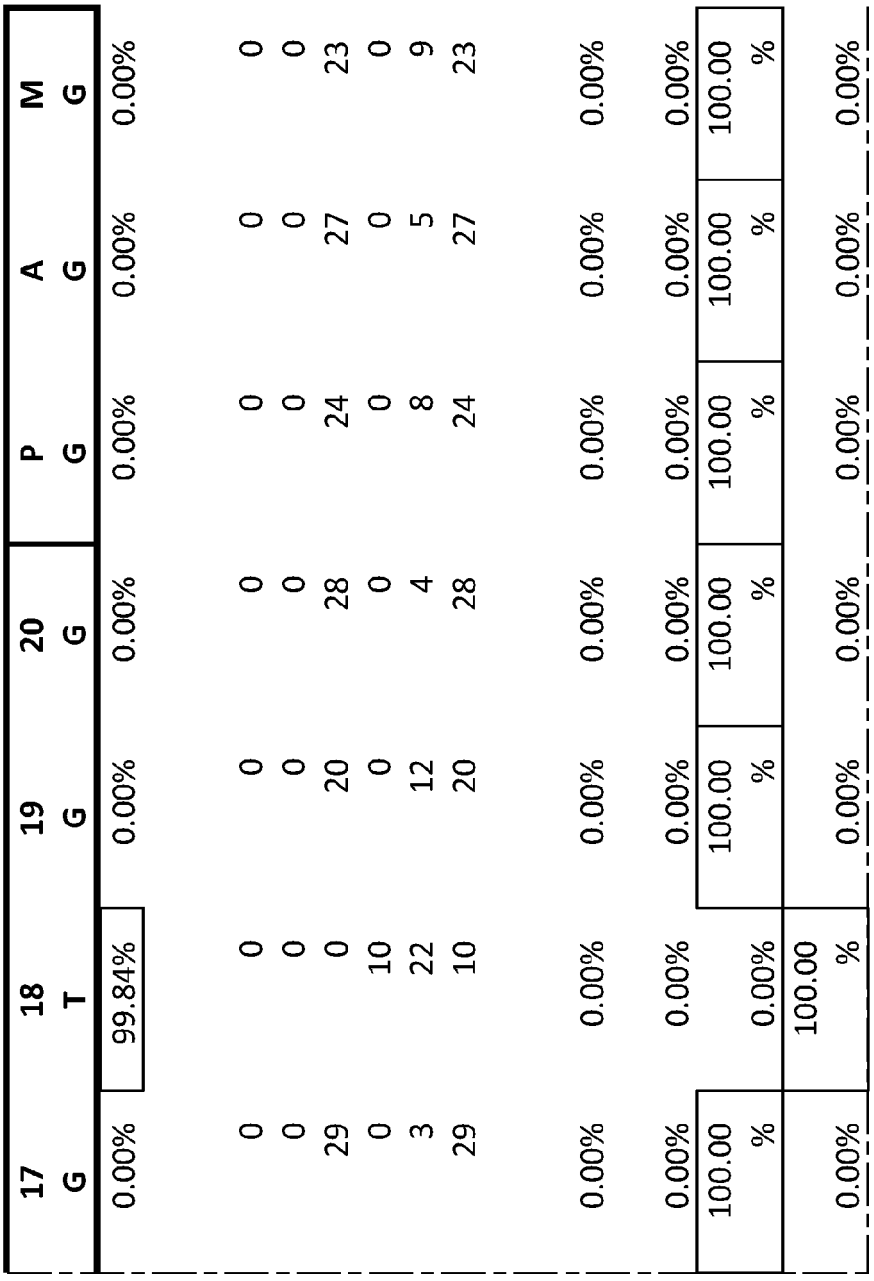


FIGURE 43 (Continued)



| 9 G | 10 G | 11 C | 12 T | 13 G | 14 G | 15 A | 16 G |
|---|---|---|---|---|---|---|---|
| 0.00% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0 | 0 | 0 | 0 | 0 | 0 | 18 | 0 |
| 0 | 0 | 24 | 0 | 0 | 0 | 0 | 0 |
| 31 | 25 | 0 | 0 | 25 | 28 | 0 | 29 |
| 0 | 0 | 0 | 24 | 0 | 0 | 0 | 0 |
| 1 | 7 | 8 | 8 | 7 | 4 | 14 | 3 |
| 31 | 25 | 24 | 24 | 25 | 28 | 18 | 29 |
| | | | | | | 100.00 % | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | 0.00% |
| 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 100.00 % | 0.00% | 0.00% | 100.00 % | 100.00 % | 0.00% | 100.00 % |
| 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 43 (Continued)



| 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|
| 0.00% | 99.84% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | 0 | 20 | 28 | 24 | 27 | 23 |
| 0 | 10 | 0 | 0 | 0 | 0 | 0 |
| 3 | 22 | 12 | 4 | 8 | 5 | 9 |
| 29 | 10 | 20 | 28 | 24 | 27 | 23 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.00% | 100.00 % | 100.00 % | 100.00 % | 100.00 % | 100.00 % |
| | 100.00 % | | | | | |
| 0.00% | | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 43 (Continued)

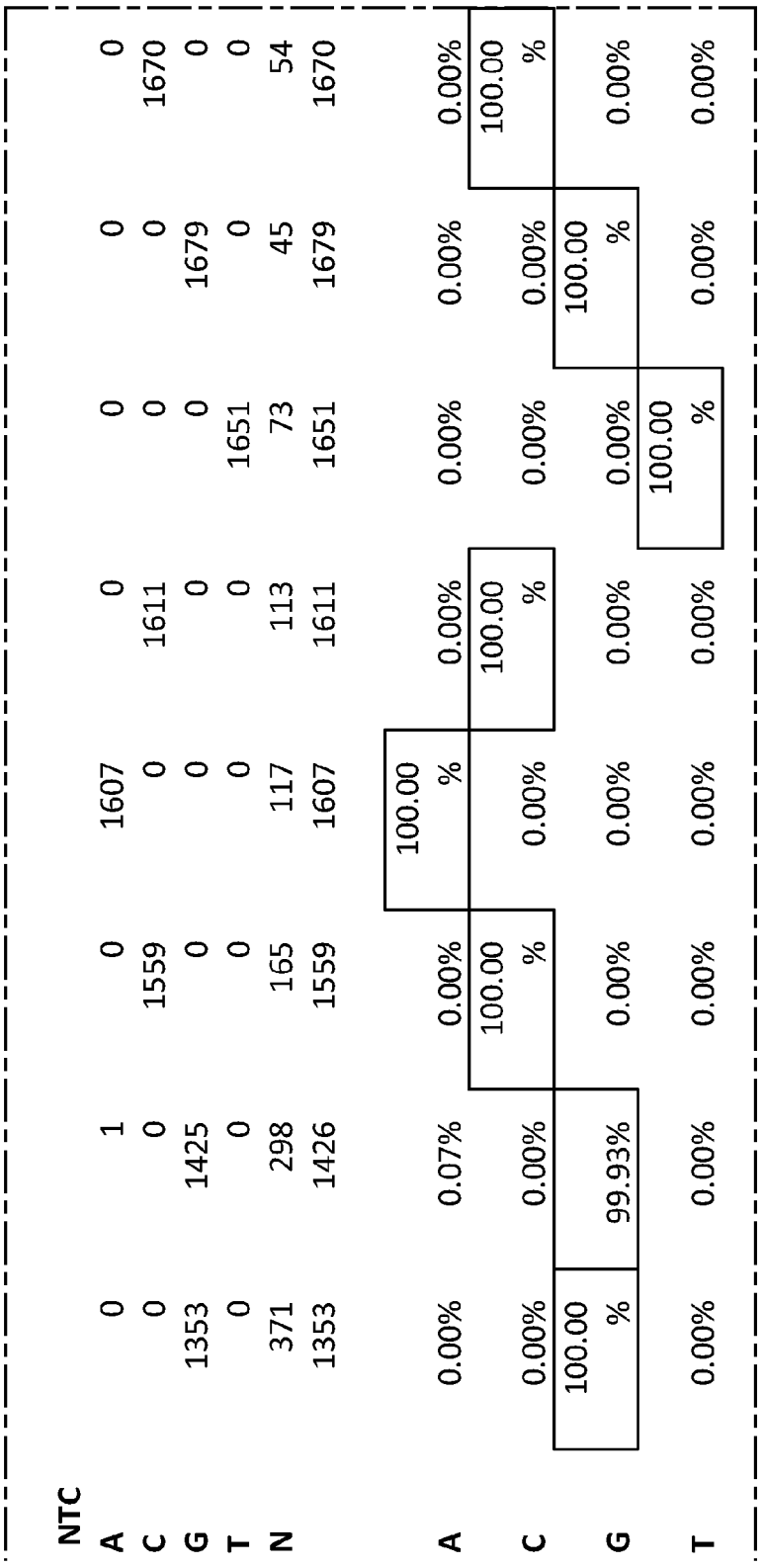


FIGURE 43 (Continued)

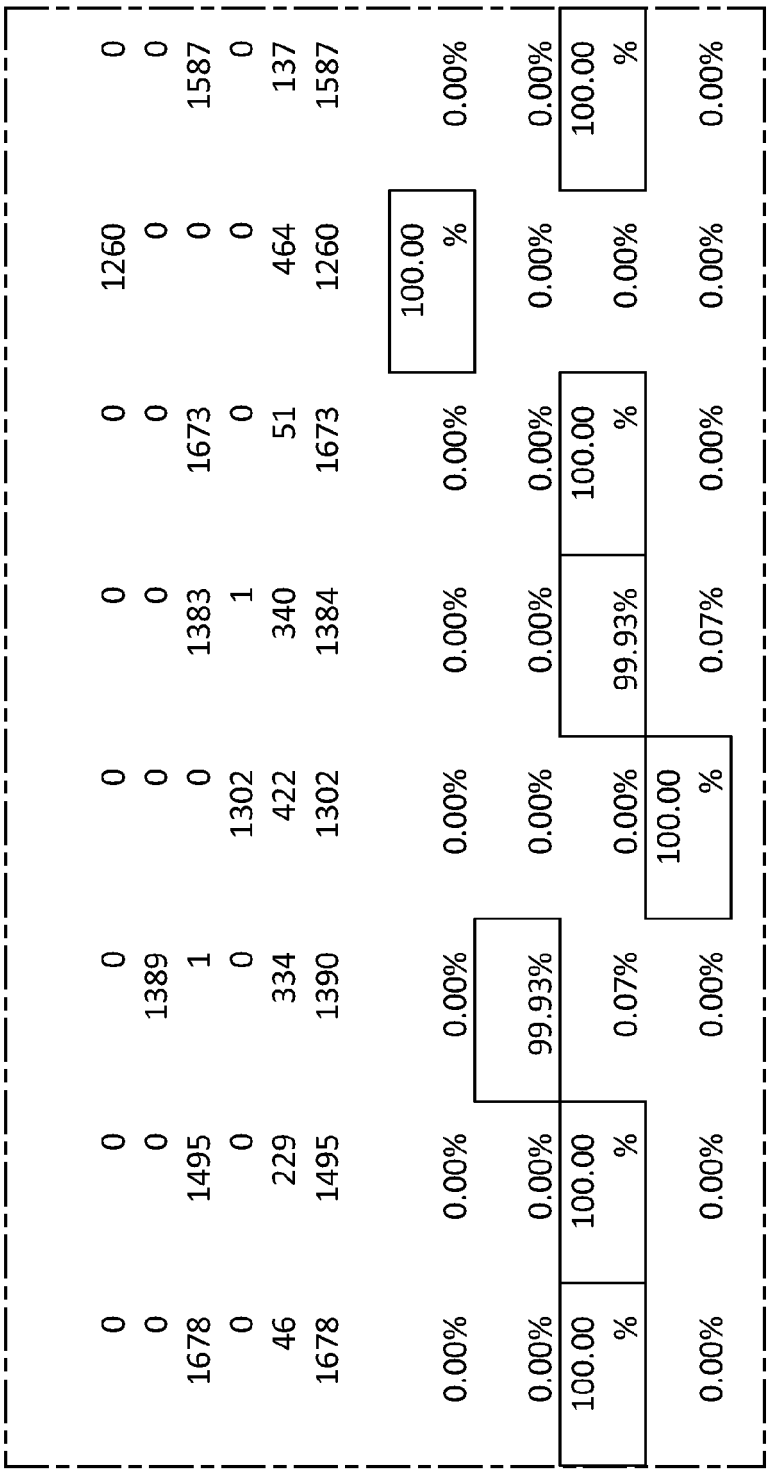
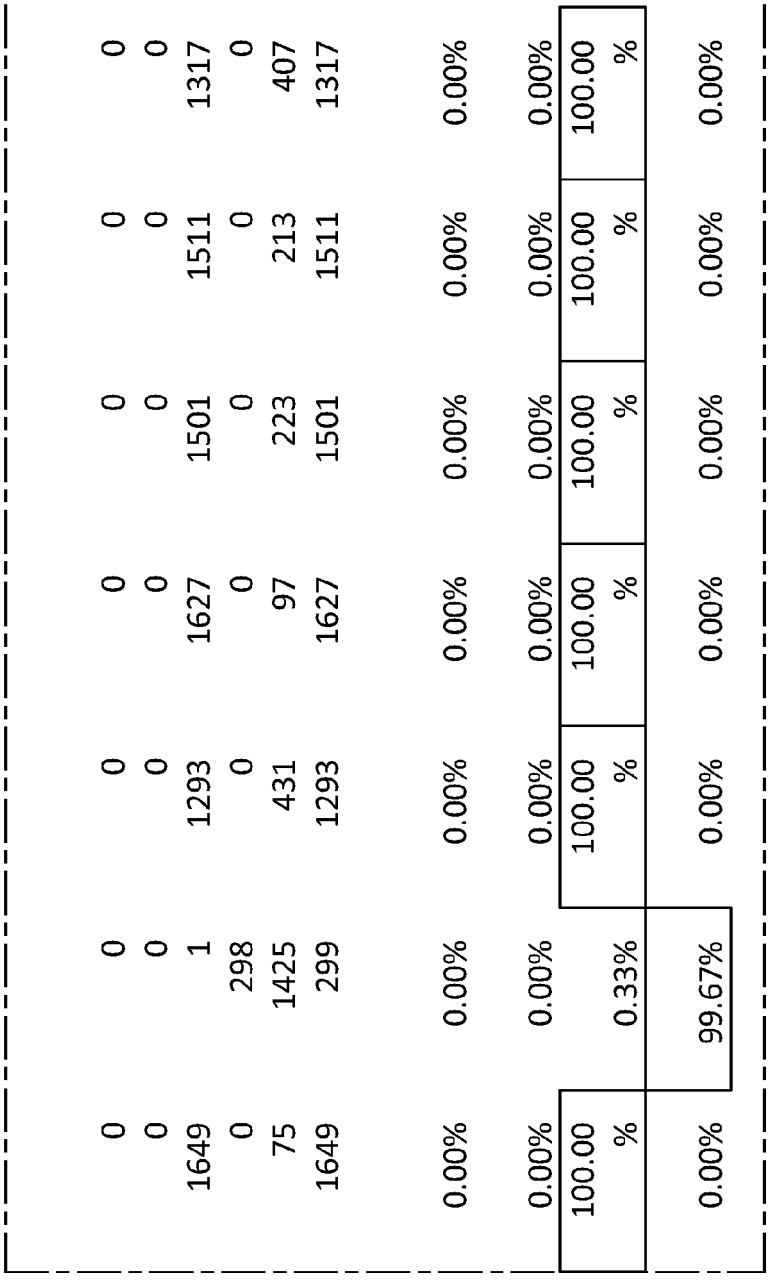
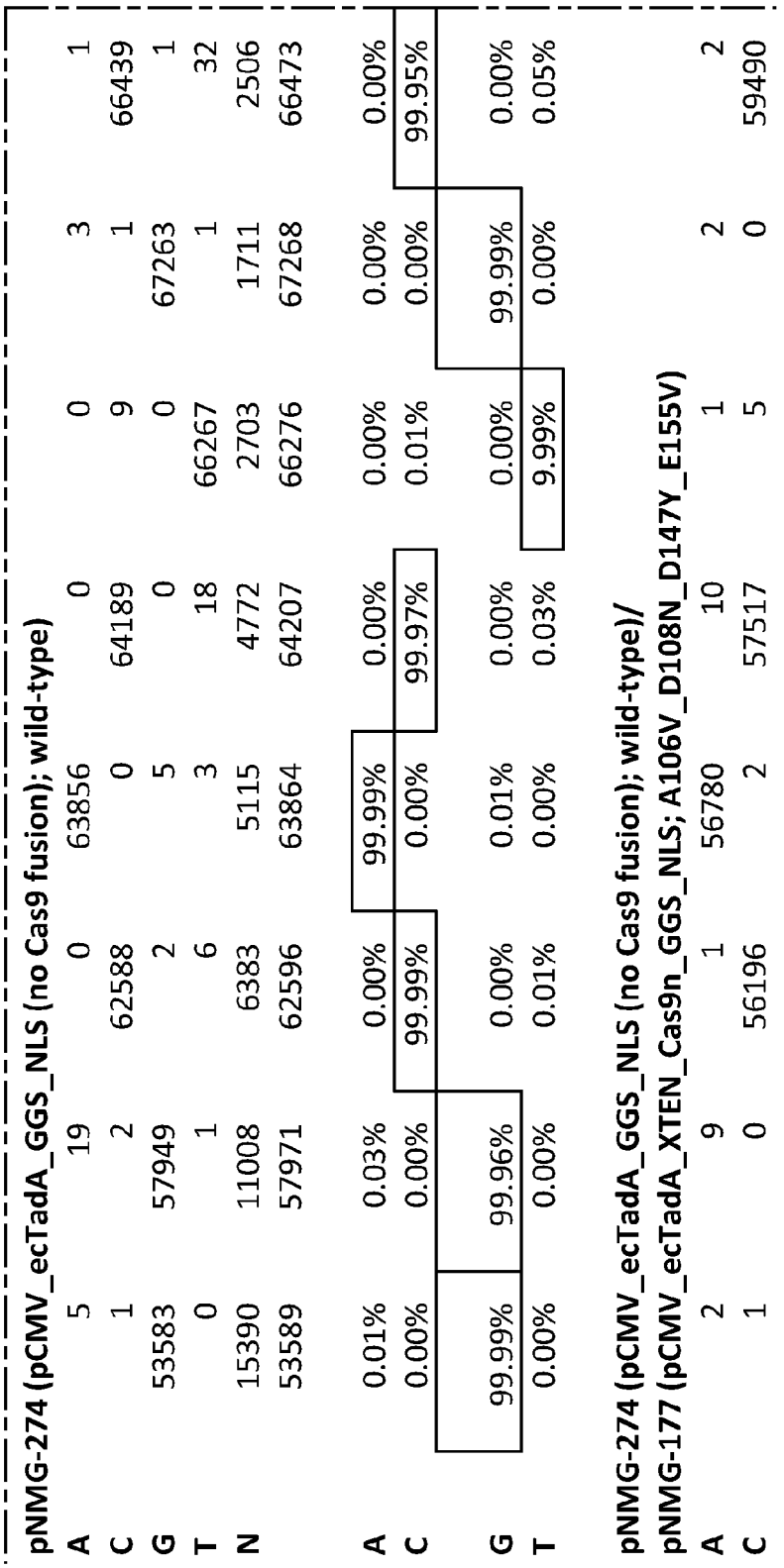


FIGURE 43 (Continued)



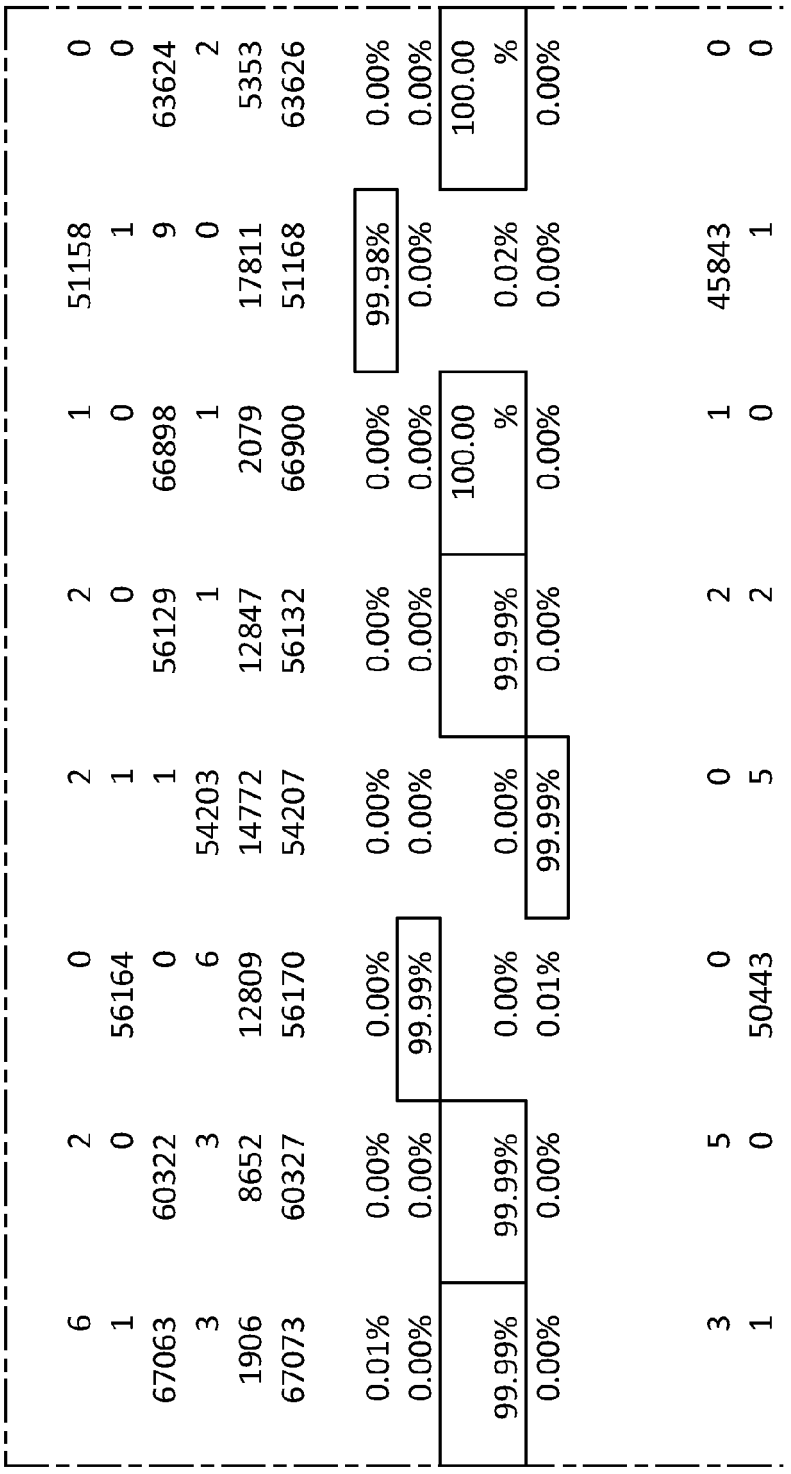
| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1649 | 1 | 1293 | 1627 | 1501 | 223 | 1511 | 213 | 1511 | 407 | 1317 | 1317 | 1649 | 299 | 1293 | 1627 | 1501 | 223 | 1511 | 213 | 1511 | 407 | 1317 | 1317 |
| 0 | 298 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 75 | 1425 | 431 | 97 | 223 | 0 | 213 | 0 | 407 | 0 | 0 | 0 | 75 | 1425 | 431 | 97 | 223 | 0 | 213 | 0 | 407 | 0 | 0 | 0 |
| 1649 | 299 | 1293 | 1627 | 1501 | 223 | 1511 | 213 | 1511 | 407 | 1317 | 1317 | 1649 | 299 | 1293 | 1627 | 1501 | 223 | 1511 | 213 | 1511 | 407 | 1317 | 1317 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.33% | 100.00 % | 100.00 % | 100.00 % | 0.00% | 100.00 % | 0.00% | 100.00 % | 0.00% | 100.00 % | 100.00 % | 0.00% | 99.67% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 43 (Continued)



| pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 19 | 0 | 63856 | 0 | 0 | 3 | | 1 | | |
| C | 1 | 2 | 62588 | 0 | 64189 | 9 | 1 | | 66439 | | |
| G | 53583 | 57949 | 2 | 5 | 0 | 0 | 67263 | | 1 | | |
| T | 0 | 1 | 6 | 3 | 18 | 66267 | 1 | | 32 | | |
| N | 15390 | 11008 | 6383 | 5115 | 4772 | 2703 | 1711 | | 2506 | | |
| | 53589 | 57971 | 62596 | 63864 | 64207 | 66276 | 67268 | | 66473 | | |
| A | 0.01% | 0.03% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | | 0.00% | | |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 99.97% | 0.01% | 0.00% | | 99.95% | | |
| G | 99.99% | 99.96% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | | 0.00% | | |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.03% | 9.99% | 0.00% | | 0.05% | | |
| pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | | | |
| A | 2 | 9 | 1 | 56780 | 10 | 1 | 2 | | 2 | | |
| C | 1 | 0 | 56196 | 2 | 57517 | 5 | 0 | | 59490 | | |

FIGURE 43 (Continued)



# FIGURE 43 (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 0 | 0 | 1 | 0 | 2 | 0 | 2 |
| 0 | 3 | 0 | 0 | 0 | 0 | 1 |
| 66017 | 19 | 52407 | 64841 | 60759 | 61236 | 53755 |
| 2 | 12601 | 1 | 15 | 3 | 12 | 2 |
| 2960 | 56356 | 16570 | 4123 | 8215 | 7731 | 15219 |
| 66019 | 12623 | 52409 | 64856 | 60764 | 61248 | 53760 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00% | 0.15% | 100.00% | 99.98% | 99.99% | 99.98% | 99.99% |
| 0.00% | 99.83% | 0.00% | 0.02% | 0.00% | 0.02% | 0.00% |
| 0 | 1 | 5 | 1 | 2 | 3 | 4 |
| 0 | 5 | 0 | 0 | 1 | 0 | 0 |

# FIGURE 43 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>A | 5<br>C | 6<br>T | 7<br>G | 8<br>C | 9<br>G | 10<br>G | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 48042 | 52111 | 0 | 412 | 22 | 0 | 60223 | 2 | 60034 | 54200 | 0 |
| T | 1 | 1 | 2 | 3 | 16 | 59268 | 0 | 20 | 1 | 1 | 1 |
| N | 13724 | 9649 | 5571 | 4573 | 4205 | 2496 | 1545 | 2256 | 1731 | 7564 | 11326 |
| | 48046 | 52121 | 56199 | 57197 | 57565 | 59274 | 60225 | 59514 | 60039 | 54206 | 50444 |
| A | 0.00% | 0.02% | 0.00% | 99.27% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 99.92% | 0.01% | 0.00% | 99.96% | 0.00% | 0.00% | 100.00% |
| G | 99.99% | 99.98% | 0.00% | 0.72% | 0.04% | 0.00% | 100.00% | 0.00% | 99.99% | 99.99% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.01% | 0.03% | 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% |
| **pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | | | |
| A | 0 | 11 | 0 | 68349 | 0 | 0 | 8 | 3 | 4 | 5 | 1 |
| C | 0 | 2 | 66984 | 0 | 68767 | 18 | 0 | 71081 | 0 | 1 | 60200 |
| G | 57506 | 62099 | 0 | 10 | 0 | 0 | 71882 | 0 | 71692 | 64676 | 1 |
| T | 3 | 2 | 4 | 5 | 1 | 70825 | 2 | 17 | 1 | 3 | 3 |
| N | 16099 | 11494 | 6620 | 5244 | 4840 | 2765 | 1716 | 2507 | 1911 | 8923 | 13403 |
| | 57509 | 62114 | 66988 | 68364 | 68768 | 70843 | 71892 | 71101 | 71697 | 64685 | 60205 |
| A | 0.00% | 0.02% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 100.00% | 0.03% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |
| G | 99.99% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 99.97% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% |

# FIGURE 43 (Continued)

| 12 T | 13 G | 14 G | 15 A | 16 G | 17 G | 18 T | 19 G | 20 G | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 50503 | 59937 | 1 | 57091 | 59170 | 12 | 47528 | 58319 | 54556 | 54975 | 48221 |
| 48818 | 2 | 1 | 1 | 1 | 3 | 11417 | 2 | 3 | 4 | 0 | 4 |
| 12947 | 11261 | 1831 | 15924 | 4678 | 2597 | 50335 | 14235 | 3447 | 7207 | 6792 | 13541 |
| 48823 | 50509 | 59939 | 45846 | 57092 | 59173 | 11435 | 47535 | 58323 | 54563 | 54978 | 48229 |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 100.00 % | 0.00% | 100.00 % | 99.99% | 0.10% | 99.99% | 99.99% | 99.99% | 99.99% | 99.98% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.84% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% |
| 0 | 8 | 1 | 54906 | 0 | 2 | 0 | 2 | 4 | 0 | 3 | 4 |
| 6 | 1 | 0 | 1 | 0 | 0 | 6 | 0 | 0 | 0 | 7 | 0 |
| 1 | 60389 | 71531 | 10 | 68129 | 70556 | 16 | 56105 | 69422 | 65074 | 65633 | 57804 |
| 58237 | 4 | 3 | 1 | 11 | 2 | 13429 | 2 | 1 | 2 | 2 | 5 |
| 15364 | 13206 | 2073 | 18690 | 5468 | 3048 | 60157 | 17499 | 4181 | 8531 | 7962 | 15794 |
| 58244 | 60402 | 71535 | 54918 | 68140 | 70560 | 13451 | 56109 | 69427 | 65076 | 65645 | 57813 |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| 0.00% | 99.98% | 99.99% | 0.02% | 99.98% | 99.99% | 0.12% | 99.99% | 99.99% | 100.00 % | 99.98% | 99.98% |
| 99.99% | 0.01% | 0.00% | 0.00% | 0.02% | 0.00% | 99.84% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 43 (Continued)

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 2 | 16 | 2 | 60767 | 1 | 0 | 5 | 1 | 1 | 7 | 0 |
| **C** | 1 | 3 | 59727 | 0 | 61157 | 10 | 0 | 63304 | 0 | 2 | 53514 |
| **G** | 50984 | 55300 | 0 | 4 | 1 | 1 | 64097 | 1 | 63852 | 57658 | 1 |
| **T** | 2 | 1 | 4 | 3 | 3 | 63110 | 0 | 24 | 1 | 1 | 1 |
| **N** | 14739 | 10408 | 5995 | 4954 | 4566 | 2607 | 1626 | 2398 | 1873 | 8059 | 12211 |
| | 50989 | 55320 | 59733 | 60774 | 61162 | 63121 | 64102 | 63330 | 63854 | 57668 | 53516 |
| **A** | 0.00% | 0.03% | 0.00% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| **C** | 0.00% | 0.01% | 99.99% | 0.00% | 99.99% | 0.02% | 0.00% | 99.96% | 0.00% | 0.00% | 100.00% |
| **G** | 99.99% | 99.96% | 0.00% | 0.01% | 0.00% | 0.00% | 99.99% | 0.00% | 100.00% | 99.98% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% |

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 2 | 13 | 4 | 70957 | 24 | 1 | 2 | 1 | 6 | 3 | 1 |
| **C** | 0 | 2 | 70232 | 0 | 72001 | 11 | 0 | 74543 | 0 | 2 | 62882 |
| **G** | 60118 | 65047 | 2 | 725 | 52 | 0 | 75448 | 1 | 75285 | 67677 | 1 |
| **T** | 0 | 1 | 10 | 4 | 50 | 74207 | 2 | 23 | 0 | 1 | 3 |
| **N** | 17349 | 12406 | 7221 | 5782 | 5341 | 3249 | 2016 | 2900 | 2177 | 9785 | 14581 |
| | 60120 | 65063 | 70248 | 71686 | 72127 | 74219 | 75452 | 74568 | 75291 | 67683 | 62887 |
| **A** | 0.00% | 0.02% | 0.01% | 98.98% | 0.03% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 99.98% | 0.00% | 99.83% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |

# FIGURE 43 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 4 | 2 | 48571 | 1 | 2 | 0 | 2 | 6 | 0 | 2 | 7 |
| 3 | 1 | 0 | 2 | 1 | 1 | 6 | 1 | 1 | 1 | 2 | 2 |
| 0 | 53596 | 63800 | 6 | 60598 | 62892 | 11 | 50120 | 61922 | 58024 | 58475 | 51452 |
| 51833 | 4 | 2 | 0 | 0 | 0 | 11991 | 1 | 3 | 1 | 0 | 0 |
| 13891 | 12122 | 1923 | 17148 | 5127 | 2832 | 53719 | 15603 | 3795 | 7701 | 7248 | 14266 |
| 51836 | 53605 | 63804 | 48579 | 60600 | 62895 | 12008 | 50124 | 61932 | 58026 | 58479 | 51461 |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.98% | 99.99% | 0.01% | 100.00% | 100.00% | 0.09% | 99.99% | 99.98% | 100.00% | 99.99% | 99.98% |
| 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 99.86% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 1 | 57222 | 0 | 0 | 0 | 1 | 2 | 2 | 1 | 4 |
| 2 | 1 | 1 | 2 | 0 | 0 | 6 | 0 | 1 | 1 | 0 | 1 |
| 1 | 62902 | 75123 | 11 | 71409 | 74181 | 23 | 58655 | 72965 | 68130 | 68742 | 60409 |
| 60803 | 1 | 3 | 1 | 1 | 0 | 13863 | 0 | 2 | 0 | 0 | 3 |
| 16661 | 14562 | 2340 | 20232 | 6058 | 3287 | 63576 | 18812 | 4498 | 9335 | 8725 | 17051 |
| 60807 | 62906 | 75128 | 57236 | 71410 | 74181 | 13892 | 58656 | 72970 | 68133 | 68743 | 60417 |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

# FIGURE 43 (Continued)

| | 1<br>G | 2<br>G | 3<br>C | 4<br>A | 5<br>C | 6<br>T | 7<br>G | 8<br>C | 9<br>G | 10<br>G | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 100.00 % | 99.98% | 0.00% | 1.01% | 0.07% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.07% | 99.98% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% |
| **pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | | | |
| A | 2 | 24 | 1 | 68696 | 1 | 2 | 19 | 1 | 7 | 9 | 0 |
| C | 2 | 1 | 67819 | 0 | 69582 | 8 | 0 | 71926 | 0 | 0 | 60598 |
| G | 57793 | 62867 | 1 | 450 | 38 | 1 | 72895 | 2 | 72725 | 65450 | 0 |
| T | 0 | 2 | 5 | 6 | 28 | 71811 | 0 | 36 | 1 | 0 | 1 |
| N | 17076 | 11979 | 7047 | 5721 | 5224 | 3051 | 1959 | 2908 | 2140 | 9414 | 14274 |
| | 57797 | 62894 | 67826 | 69152 | 69649 | 71822 | 72914 | 71965 | 72733 | 65459 | 60599 |
| A | 0.00% | 0.04% | 0.00% | 99.34% | 0.00% | 0.00% | 0.03% | 0.00% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 99.99% | 0.00% | 99.90% | 0.01% | 0.00% | 99.95% | 0.00% | 0.00% | 100.00 % |
| G | 99.99% | 99.96% | 0.00% | 0.65% | 0.05% | 0.00% | 99.97% | 0.00% | 99.99% | 99.99% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 0.04% | 99.98% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% |
| **pNMG-276 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (wild-type) + (A106V_D108N_D147Y_E155V))** | | | | | | | | | | | |
| A | 0 | 0 | 0 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 0 | 0 | 45 | 0 | 48 | 0 | 0 | 54 | 0 | 0 | 30 |
| G | 33 | 42 | 0 | 0 | 0 | 0 | 53 | 0 | 53 | 37 | 0 |
| T | 0 | 0 | 0 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| N | 27 | 18 | 15 | 16 | 12 | 10 | 7 | 6 | 7 | 23 | 30 |
| | 33 | 42 | 45 | 44 | 48 | 50 | 53 | 54 | 53 | 37 | 30 |

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# FIGURE 43 (Continued)

| 12<br>T | 13<br>G | 14<br>G | 15<br>A | 16<br>G | 17<br>G | 18<br>T | 19<br>G | 20<br>G | P<br>G | A<br>G | M<br>G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 99.99% | 99.99% | 0.02% | 100.00 % | 100.00 % | 0.17% | 100.00 % | 99.99% | 100.00 % | 100.00 % | 99.99% |
| 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.79% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 3 | 4 | 0 | 55144 | 1 | 1 | 3 | 3 | 4 | 1 | 3 | 3 |
| 5 | 0 | 0 | 2 | 0 | 1 | 5 | 1 | 0 | 0 | 0 | 0 |
| 1 | 60743 | 72562 | 4 | 68903 | 71658 | 19 | 56164 | 70273 | 65802 | 66406 | 58469 |
| 58500 | 3 | 1 | 3 | 1 | 0 | 13308 | 1 | 1 | 0 | 0 | 3 |
| 16364 | 14123 | 2310 | 19720 | 5968 | 3213 | 61538 | 18704 | 4595 | 9070 | 8464 | 16398 |
| 58509 | 60750 | 72563 | 55153 | 68905 | 71660 | 13335 | 56169 | 70278 | 65803 | 66409 | 58475 |
| 0.01% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 100.00 % | 0.01% | 100.00 % | 100.00 % | 0.14% | 99.99% | 99.99% | 100.00 % | 100.00 % | 99.99% |
| 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 99.80% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0 | 0 | 0 | 34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 34 | 57 | 0 | 44 | 50 | 0 | 36 | 48 | 36 | 39 | 32 |
| 29 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 |
| 31 | 26 | 3 | 26 | 16 | 10 | 52 | 24 | 12 | 24 | 21 | 28 |
| 29 | 34 | 57 | 34 | 44 | 50 | 8 | 36 | 48 | 36 | 39 | 32 |



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# FIGURE 43 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 100.00% | 0.00% | 100.00% | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 100.00% |
| G | 100.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 100.00% | 100.00% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

**pNMG-277 (pCMVecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (A106V_D108N_D147Y_E155V) + (A106V_D108N_D147Y_E155V))**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 3 | 24 | 0 | 69983 | 1 | 1 | 6 | 1 | 8 | 3 | 0 |
| C | 1 | 3 | 69828 | 0 | 71641 | 9 | 0 | 74193 | 1 | 0 | 62401 |
| G | 59632 | 64601 | 0 | 1163 | 55 | 0 | 75023 | 3 | 74770 | 67261 | 0 |
| T | 1 | 0 | 11 | 6 | 8 | 73858 | 1 | 23 | 2 | 1 | 0 |
| N | 17423 | 12432 | 7221 | 5908 | 5355 | 3192 | 2030 | 2840 | 2279 | 9795 | 14659 |
| | 59637 | 64628 | 69839 | 71152 | 71705 | 73868 | 75030 | 74220 | 74781 | 67265 | 62401 |
| A | 0.01% | 0.04% | 0.00% | 98.36% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| C | 0.00% | 0.00% | 99.98% | 0.00% | 99.91% | 0.01% | 0.00% | 99.96% | 0.00% | 0.00% | 100.00% |
| G | 99.99% | 99.96% | 0.00% | 1.63% | 0.08% | 0.00% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% |
| T | 0.00% | 0.00% | 0.02% | 0.01% | 0.01% | 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% |



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# FIGURE 43 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | 100.00% | 100.00% | 0.00% | 100.00% | 100.00% | 0.00% | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% |
| % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0 | 1 | 3 | 56522 | 0 | 1 | 1 | 2 | 2 | 11 | 2 | 1 |
| 5 | 1 | 0 | 1 | 0 | 0 | 4 | 0 | 1 | 0 | 1 | 0 |
| 1 | 62424 | 74764 | 5 | 71076 | 73805 | 15 | 58308 | 72476 | 67691 | 68304 | 59894 |
| 60166 | 1 | 3 | 3 | 3 | 1 | 13721 | 1 | 1 | 1 | 0 | 3 |
| 16887 | 14632 | 2289 | 20528 | 5980 | 3252 | 63318 | 18748 | 4579 | 9356 | 8752 | 17161 |
| 60172 | 62427 | 74770 | 56531 | 71079 | 73807 | 13741 | 58311 | 72480 | 67703 | 68307 | 59898 |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 0.00% | 100.00% | 99.99% | 0.01% | 100.00% | 100.00% | 0.11% | 99.99% | 99.99% | 99.98% | 100.00% | 99.99% |
| 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 99.85% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |

# FIGURE 44

| | 1<br>G | 2<br>A | 3<br>G | 4<br>T | 5<br>C | 6<br>C | 7<br>G | 8<br>A | 9<br>G | 10<br>C | 11<br>A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cas9** | | | | | | | | | | | |
| **A** | 9 | 39506 | 3 | 13 | 1 | 2 | 3 | 39293 | 16 | 4 | 38812 |
| **C** | 1 | 0 | 7 | 5 | 39893 | 40270 | 0 | 6 | 0 | 38662 | 0 |
| **G** | 40974 | 5 | 40798 | 8 | 1 | 0 | 40375 | 3 | 39501 | 1 | 5 |
| **T** | 2 | 0 | 2 | 38909 | 17 | 11 | 4 | 0 | 0 | 3 | 1 |
| **N** | 1179 | 2654 | 1355 | 3230 | 2253 | 1873 | 1774 | 2854 | 2625 | 3481 | 3333 |
| | 40986 | 39511 | 40810 | 38935 | 39912 | 40283 | 40382 | 39302 | 39517 | 38670 | 38818 |
| **A** | 0.02% | 99.99% | 0.01% | 0.03% | 0.00% | 0.00% | 0.01% | 99.98% | 0.04% | 0.01% | 99.98% |
| **C** | 0.00% | 0.00% | 0.02% | 0.01% | 99.95% | 99.97% | 0.00% | 0.02% | 0.00% | 99.98% | 0.00% |
| **G** | 99.97% | 0.01% | 99.97% | 0.02% | 0.00% | 0.00% | 99.98% | 0.01% | 99.96% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.93% | 0.04% | 0.03% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| **pNMG-247 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 7 | 54127 | 4 | 10 | 2 | 4 | 3 | 53712 | 7 | 2 | 53086 |
| **C** | 1 | 0 | 5 | 8 | 54780 | 55119 | 0 | 0 | 0 | 52891 | 0 |
| **G** | 55987 | 6 | 55844 | 0 | 1 | 0 | 55222 | 6 | 54002 | 0 | 3 |
| **T** | 3 | 0 | 4 | 53483 | 8 | 12 | 6 | 3 | 5 | 4 | 3 |
| **N** | 1748 | 3613 | 1889 | 4245 | 2955 | 2611 | 2515 | 4025 | 3725 | 4842 | 4646 |
| | 55998 | 54133 | 55857 | 53501 | 54791 | 55135 | 55231 | 53721 | 54014 | 52897 | 53092 |
| **A** | 0.01% | 99.99% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% | 99.99% |
| **C** | 0.00% | 0.00% | 0.01% | 0.01% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.01% | 0.00% | 0.01% | 99.97% | 0.01% | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |

# FIGURE 44 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | A | A | G | A | A | G | A | A | G | G | G |
| 1 | 40751 | 41159 | 0 | 39983 | 40503 | 8 | 40469 | 39655 | 3 | 13 | 2 |
| 0 | 3 | 12 | 0 | 33 | 31 | 10 | 1 | 20 | 0 | 0 | 4 |
| 40833 | 13 | 1 | 41172 | 19 | 4 | 40802 | 27 | 11 | 40555 | 40637 | 40050 |
| 1 | 2 | 12 | 15 | 11 | 1 | 23 | 17 | 13 | 17 | 1 | 1 |
| 1311 | 1371 | 977 | 974 | 2104 | 1639 | 1230 | 1503 | 2314 | 1442 | 1360 | 1950 |
| 40835 | 40769 | 41184 | 41187 | 40046 | 40539 | 40843 | 40514 | 39699 | 40575 | 40651 | 40057 |
| 0.00% | 99.96% | 99.94% | 0.00% | 99.84% | 99.91% | 0.02% | 99.89% | 99.89% | 0.01% | 0.03% | 0.00% |
| 0.00% | 0.01% | 0.03% | 0.00% | 0.08% | 0.08% | 0.02% | 0.00% | 0.05% | 0.00% | 0.00% | 0.01% |
| 100.00% | 0.03% | 0.00% | 99.96% | 0.05% | 0.01% | 99.90% | 0.07% | 0.03% | 99.95% | 99.97% | 99.98% |
| 0.00% | 0.00% | 0.03% | 0.04% | 0.03% | 0.00% | 0.06% | 0.04% | 0.03% | 0.04% | 0.00% | 0.00% |
| 1 | 55756 | 56362 | 1 | 54879 | 55464 | 1 | 55598 | 54446 | 5 | 4 | 26 |
| 1 | 0 | 1 | 3 | 23 | 5 | 12 | 13 | 6 | 1 | 1 | 7 |
| 55792 | 2 | 7 | 56395 | 21 | 6 | 56061 | 8 | 7 | 55831 | 55960 | 54954 |
| 2 | 0 | 3 | 1 | 2 | 21 | 7 | 9 | 1 | 2 | 4 | 1 |
| 1939 | 1977 | 1374 | 1347 | 2821 | 2266 | 1671 | 2086 | 3255 | 1918 | 1781 | 2756 |
| 55796 | 55758 | 56373 | 56400 | 54925 | 55496 | 56081 | 55628 | 54460 | 55839 | 55969 | 54988 |
| 0.00% | 100.00% | 99.98% | 0.00% | 99.92% | 99.94% | 0.00% | 99.95% | 99.97% | 0.01% | 0.01% | 0.05% |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.04% | 0.01% | 0.02% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.04% | 0.01% | 99.96% | 0.01% | 0.01% | 99.99% | 99.98% | 99.94% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.04% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% |

FIGURE 44 (Continued)

| pNMG-248 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; D108N_D147Y_E155V) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 11 | 52989 | 3 | 3 | 3 | 2 | 10 | 52738 | 12 | 5 | 51999 |
| C | 1 | 0 | 7 | 9 | 53642 | 54012 | 0 | 0 | 0 | 51921 | 0 |
| G | 54891 | 7 | 54726 | 0 | 0 | 1 | 54120 | 2 | 53011 | 0 | 3 |
| T | 1 | 0 | 0 | 52293 | 4 | 12 | 0 | 2 | 0 | 5 | 5 |
| N | 1571 | 3479 | 1739 | 4169 | 2819 | 2439 | 2333 | 3721 | 3438 | 4534 | 4458 |
| | 54904 | 52996 | 54736 | 52305 | 53649 | 54027 | 54130 | 52742 | 53023 | 51931 | 52007 |
| A | 0.02% | 99.99% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% | 99.99% | 0.02% | 0.01% | 99.98% |
| C | 0.00% | 0.00% | 0.01% | 0.02% | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% |
| G | 99.98% | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| pNMG-249 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | | | |
| A | 3 | 48310 | 4 | 6 | 1 | 1 | 3 | 48009 | 5 | 2 | 47362 |
| C | 0 | 1 | 2 | 8 | 48854 | 49274 | 0 | 0 | 0 | 47237 | 7 |
| G | 50000 | 5 | 49969 | 0 | 0 | 0 | 49372 | 6 | 48246 | 2 | 1 |
| T | 3 | 1 | 0 | 47631 | 4 | 12 | 2 | 1 | 2 | 2 | 5 |
| N | 1550 | 3239 | 1580 | 3910 | 2696 | 2268 | 2178 | 3539 | 3301 | 4307 | 4175 |
| | 50006 | 48317 | 49975 | 47645 | 48859 | 49287 | 49377 | 48016 | 48253 | 47243 | 47375 |
| A | 0.01% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.00% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.02% | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% |
| G | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% |

FIGURE 44 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 54594 | 55152 | 2 | 53590 | 54273 | 5 | 54321 | 53260 | 3 | 1 | 5 | |
| 0 | 0 | 1 | 0 | 71 | 2 | 6 | 15 | 4 | 0 | 1 | 20 | |
| 54666 | 6 | 3 | 55168 | 29 | 7 | 54899 | 5 | 7 | 54493 | 54628 | 53718 | |
| 0 | 1 | 12 | 0 | 3 | 18 | 4 | 4 | 8 | 4 | 15 | 3 | |
| 1799 | 1861 | 1311 | 1309 | 2785 | 2178 | 1549 | 2022 | 3096 | 1887 | 1765 | 2660 | |
| 54666 | 54601 | 55168 | 55170 | 53693 | 54300 | 54914 | 54345 | 53279 | 54500 | 54645 | 53746 | |
| 0.00% | 99.99% | 99.97% | 0.00% | 99.81% | 99.95% | 0.01% | 99.96% | 99.96% | 0.01% | 0.00% | 0.01% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.13% | 0.00% | 0.01% | 0.03% | 0.01% | 0.00% | 0.00% | 0.04% | |
| 100.00% | 0.01% | 0.01% | 100.00% | 0.05% | 0.01% | 99.97% | 0.01% | 0.01% | 99.99% | 99.97% | 99.95% | |
| 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 0.03% | 0.01% | 0.01% | 0.02% | 0.01% | 0.03% | 0.01% | |
| 1 | 49809 | 50360 | 7 | 48966 | 49470 | 5 | 49700 | 48666 | 2 | 6 | 16 | |
| 5 | 2 | 4 | 1 | 42 | 0 | 8 | 17 | 7 | 0 | 1 | 16 | |
| 49769 | 7 | 10 | 50352 | 76 | 11 | 50069 | 13 | 1 | 49806 | 49921 | 49065 | |
| 8 | 2 | 3 | 2 | 3 | 69 | 3 | 10 | 4 | 7 | 5 | 2 | |
| 1759 | 1720 | 1226 | 1241 | 2516 | 2078 | 1516 | 1816 | 2890 | 1749 | 1657 | 2490 | |
| 49783 | 49820 | 50377 | 50362 | 49087 | 49550 | 50085 | 49740 | 48678 | 49815 | 49933 | 49099 | |
| 0.00% | 99.98% | 99.97% | 0.01% | 99.75% | 99.84% | 0.01% | 99.92% | 99.98% | 0.00% | 0.01% | 0.03% | |
| 0.01% | 0.00% | 0.01% | 0.00% | 0.09% | 0.00% | 0.02% | 0.03% | 0.01% | 0.00% | 0.00% | 0.03% | |
| 99.97% | 0.01% | 0.02% | 99.98% | 0.15% | 0.02% | 99.97% | 0.03% | 0.00% | 99.98% | 99.98% | 99.93% | |

FIGURE 44 (Continued)

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | 0.01% | 0.00% | 0.00% | 99.97% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| **pNMG-250 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 5 | 51228 | 2 | 1 | 0 | 6 | 6 | 50970 | 8 | 0 | 50427 |
| C | 0 | 1 | 1 | 8 | 51803 | 52277 | 0 | 2 | 0 | 50183 | 1 |
| G | 53227 | 5 | 53048 | 1 | 0 | 0 | 52515 | 3 | 51301 | 0 | 6 |
| T | 3 | 1 | 2 | 50597 | 5 | 11 | 2 | 1 | 0 | 9 | 4 |
| N | 1567 | 3567 | 1749 | 4195 | 2994 | 2508 | 2278 | 3825 | 3480 | 4600 | 4354 |
| | 53235 | 51235 | 53053 | 50607 | 51808 | 52294 | 52523 | 50976 | 51309 | 50192 | 50438 |
| A | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.02% | 0.00% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.02% | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 0.01% |
| **pNMG-251 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 12 | 79659 | 2 | 2 | 1 | 2 | 20 | 79422 | 6 | 2 | 78329 |
| C | 0 | 0 | 4 | 9 | 80520 | 81396 | 0 | 0 | 3 | 77990 | 0 |
| G | 82737 | 13 | 82510 | 0 | 0 | 0 | 81567 | 14 | 79734 | 0 | 7 |
| T | 2 | 4 | 0 | 78310 | 11 | 14 | 1 | 4 | 2 | 5 | 7 |
| N | 2527 | 5602 | 2762 | 6957 | 4746 | 3866 | 3690 | 5838 | 5533 | 7281 | 6935 |
| | 82751 | 79676 | 82516 | 78321 | 80532 | 81412 | 81588 | 79440 | 79745 | 77997 | 78343 |
| A | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 99.98% | 0.01% | 0.00% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.97% | 0.02% | 99.99% | 0.00% | 0.01% |
| T | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |

FIGURE 44 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.02% | 0.00% | 0.01% | 0.00% | 0.01% | 0.14% | 0.01% | 0.02% | 0.01% | 0.01% | 0.01% | 0.00% |
| 1 | 52907 | 53469 | 3 | 51900 | 52473 | 17 | 52676 | 51517 | 1 | 11 | 2 |
| 1 | 1 | 18 | 20 | 55 | 24 | 10 | 18 | 6 | 2 | 6 | 10 |
| 53011 | 24 | 3 | 53443 | 26 | 11 | 53092 | 11 | 9 | 52839 | 52984 | 52144 |
| 1 | 6 | 7 | 3 | 6 | 28 | 7 | 8 | 19 | 2 | 3 | 2 |
| 1772 | 1842 | 1306 | 1340 | 2819 | 2301 | 1681 | 2047 | 3227 | 1940 | 1773 | 2625 |
| 53014 | 52938 | 53497 | 53469 | 51987 | 52536 | 53126 | 52713 | 51551 | 52844 | 53004 | 52158 |
| 0.00% | 99.94% | 99.95% | 0.01% | 99.83% | 99.88% | 0.03% | 99.93% | 99.93% | 0.00% | 0.02% | 0.00% |
| 0.00% | 0.00% | 0.03% | 0.04% | 0.11% | 0.05% | 0.02% | 0.03% | 0.01% | 0.00% | 0.01% | 0.02% |
| 99.99% | 0.05% | 0.01% | 99.95% | 0.05% | 0.02% | 99.94% | 0.02% | 0.02% | 99.99% | 99.96% | 99.97% |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.05% | 0.01% | 0.02% | 0.04% | 0.00% | 0.01% | 0.00% |
| 1 | 82181 | 83172 | 0 | 80922 | 81726 | 2 | 82021 | 80309 | 6 | 8 | 4 |
| 0 | 2 | 0 | 1 | 1 | 3 | 2 | 2 | 6 | 0 | 3 | 1 |
| 82376 | 4 | 8 | 83211 | 6 | 12 | 82631 | 14 | 9 | 82119 | 82358 | 81108 |
| 1 | 3 | 7 | 1 | 2 | 11 | 16 | 5 | 15 | 8 | 6 | 3 |
| 2895 | 3083 | 2086 | 2060 | 4342 | 3517 | 2591 | 3183 | 4877 | 3077 | 2832 | 4091 |
| 82378 | 82190 | 83187 | 83213 | 80931 | 81752 | 82651 | 82042 | 80339 | 82133 | 82375 | 81116 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.97% | 0.00% | 99.97% | 99.96% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.00% | 0.01% | 100.00% | 0.01% | 0.01% | 99.98% | 0.02% | 0.01% | 99.98% | 99.98% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.02% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% |

# FIGURE 44 (Continued)

**pNMG-181 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 9 | 97324 | 2 | 5 | 4 | 1 | 11 | 97074 | 20 | 3 | 95925 |
| **C** | 2 | 1 | 1 | 6 | 98574 | 99616 | 0 | 0 | 3 | 95365 | 1 |
| **G** | 101562 | 20 | 101047 | 0 | 0 | 1 | 99950 | 29 | 97622 | 1 | 16 |
| **T** | 4 | 7 | 4 | 95762 | 12 | 21 | 3 | 6 | 2 | 5 | 11 |
| **N** | 3028 | 7253 | 3551 | 8832 | 6015 | 4966 | 4641 | 7496 | 6958 | 9231 | 8652 |
| | 101577 | 97352 | 101054 | 95773 | 98590 | 99639 | 99964 | 97109 | 97647 | 95374 | 95953 |
| **A** | 0.01% | 99.97% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.96% | 0.02% | 0.00% | 99.97% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.03% | 99.97% | 0.00% | 0.02% |
| **T** | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |

**pNMG-182 (pCMV_ecTadA_GGS_Cas9n_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 16 | 84523 | 3 | 9 | 3 | 2 | 14 | 84141 | 12 | 4 | 83033 |
| **C** | 2 | 1 | 0 | 13 | 85538 | 86146 | 2 | 0 | 2 | 82690 | 0 |
| **G** | 87655 | 10 | 87403 | 0 | 14 | 1 | 86557 | 12 | 84600 | 1 | 7 |
| **T** | 3 | 0 | 5 | 83248 | 29 | 64 | 5 | 2 | 3 | 4 | 9 |
| **N** | 2573 | 5715 | 2838 | 6979 | 4665 | 4036 | 3671 | 6094 | 5632 | 7550 | 7200 |
| | 87676 | 84534 | 87411 | 83270 | 85584 | 86213 | 86578 | 84155 | 84617 | 82699 | 83049 |
| **A** | 0.02% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 99.98% | 0.01% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.02% | 99.95% | 99.92% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |

FIGURE 44 (Continued)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 100951 | 102012 | 1 | 99263 | 100252 | 1 | 100489 | 98160 | 9 | 7 | 2 | | |
| 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | | |
| 101024 | 6 | 7 | 101957 | 3 | 12 | 101307 | 5 | 9 | 100583 | 100872 | 99341 | | |
| 3 | 0 | 2 | 6 | 0 | 6 | 3 | 5 | 9 | 7 | 1 | 0 | | |
| 3572 | 3648 | 2583 | 2640 | 5339 | 4335 | 3294 | 4106 | 6426 | 4005 | 3725 | 5261 | | |
| 101033 | 100957 | 102022 | 101965 | 99266 | 100270 | 101311 | 100499 | 98179 | 100600 | 100880 | 99344 | | |
| 0.00% | 99.99% | 99.99% | 0.00% | 100.00 % | 99.98% | 0.00% | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.00% | 0.01% | 100.00 % | 0.00% | 0.01% | 99.98% | 99.99% | 100.00 % | | |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | | |
| 1 | 87191 | 88061 | 2 | 85708 | 86666 | 4 | 86983 | 85147 | 7 | 12 | 7 | | |
| 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 1 | | |
| 87358 | 5 | 8 | 88078 | 6 | 2 | 87648 | 11 | 9 | 87084 | 87412 | 86027 | | |
| 3 | 1 | 5 | 3 | 2 | 8 | 2 | 4 | 9 | 8 | 3 | 2 | | |
| 2887 | 3050 | 2173 | 2164 | 4532 | 3572 | 2594 | 3249 | 5081 | 3148 | 2820 | 4209 | | |
| 87362 | 87198 | 88075 | 88084 | 85716 | 86676 | 87654 | 86999 | 85167 | 87100 | 87428 | 86037 | | |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.98% | 0.01% | 0.01% | 0.01% | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | |

# FIGURE 44 (Continued)

| | 1<br>G | 2<br>A | 3<br>G | 4<br>T | 5<br>C | 6<br>C | 7<br>G | 8<br>A | 9<br>G | 10<br>C | 11<br>A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.02% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.01% | 99.97% | 0.03% | 0.07% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| **pNMG-183 (pCMV_ecTadA_(GGS)2-XTEN-(GGS)2_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 9 | 76347 | 1 | 5 | 7 | 3 | 13 | 75867 | 16 | 1 | 75035 |
| C | 1 | 0 | 0 | 8 | 77116 | 77734 | 0 | 0 | 3 | 74819 | 0 |
| G | 79023 | 10 | 78786 | 0 | 1 | 1 | 78046 | 137 | 76218 | 0 | 14 |
| T | 2 | 0 | 4 | 75362 | 21 | 40 | 5 | 5 | 2 | 1 | 5 |
| N | 2257 | 4935 | 2501 | 5917 | 4147 | 3514 | 3228 | 5283 | 5053 | 6471 | 6238 |
| | 79035 | 76357 | 78791 | 75375 | 77145 | 77778 | 78064 | 76009 | 76239 | 74821 | 75054 |
| A | 0.01% | 99.99% | 0.00% | 0.01% | 0.01% | 0.00% | 0.02% | 99.81% | 0.02% | 0.00% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 99.94% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.18% | 99.97% | 0.00% | 0.02% |
| T | 0.00% | 0.00% | 0.01% | 99.98% | 0.03% | 0.05% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| **pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 9 | 87842 | 5 | 5 | 1 | 5 | 16 | 87371 | 13 | 5 | 86297 |
| C | 0 | 3 | 0 | 8 | 88804 | 89321 | 2 | 0 | 2 | 86017 | 0 |
| G | 90896 | 13 | 90633 | 1 | 8 | 0 | 89704 | 49 | 87759 | 0 | 13 |
| T | 6 | 3 | 6 | 86876 | 36 | 62 | 5 | 6 | 3 | 6 | 6 |
| N | 2460 | 5510 | 2727 | 6481 | 4522 | 3983 | 3644 | 5945 | 5594 | 7343 | 7055 |
| | 90911 | 87861 | 90644 | 86890 | 88849 | 89388 | 89727 | 87426 | 87777 | 86028 | 86316 |

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FIGURE 44 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 100.00 % | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.98% | 99.98% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 4 | 78678 | 79414 | 3 | 77488 | 78234 | 4 | 78589 | 76804 | 7 | 6 | 1 |
| 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 1 |
| 78752 | 5 | 5 | 79460 | 6 | 6 | 79029 | 9 | 5 | 78588 | 78802 | 77505 |
| 4 | 0 | 7 | 2 | 1 | 1 | 3 | 4 | 5 | 5 | 3 | 3 |
| 2532 | 2608 | 1865 | 1827 | 3797 | 3050 | 2256 | 2690 | 4483 | 2697 | 2487 | 3788 |
| 78760 | 78684 | 79427 | 79465 | 77495 | 78242 | 79036 | 78602 | 76815 | 78601 | 78811 | 77510 |
| 0.01% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.01% | 99.98% | 99.99% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| 4 | 90336 | 91308 | 2 | 89008 | 90009 | 3 | 90215 | 88429 | 9 | 3 | 1 |
| 0 | 2 | 1 | 0 | 0 | 0 | 1 | 2 | 2 | 1 | 1 | 0 |
| 90468 | 4 | 10 | 91358 | 5 | 5 | 90872 | 7 | 7 | 90433 | 90613 | 89123 |
| 4 | 1 | 6 | 8 | 2 | 3 | 1 | 4 | 7 | 7 | 1 | 0 |
| 2895 | 3028 | 2046 | 2003 | 4356 | 3357 | 2497 | 3146 | 4929 | 2924 | 2756 | 4250 |
| 90476 | 90343 | 91325 | 91368 | 89015 | 90017 | 90877 | 90228 | 88445 | 90450 | 90618 | 89124 |

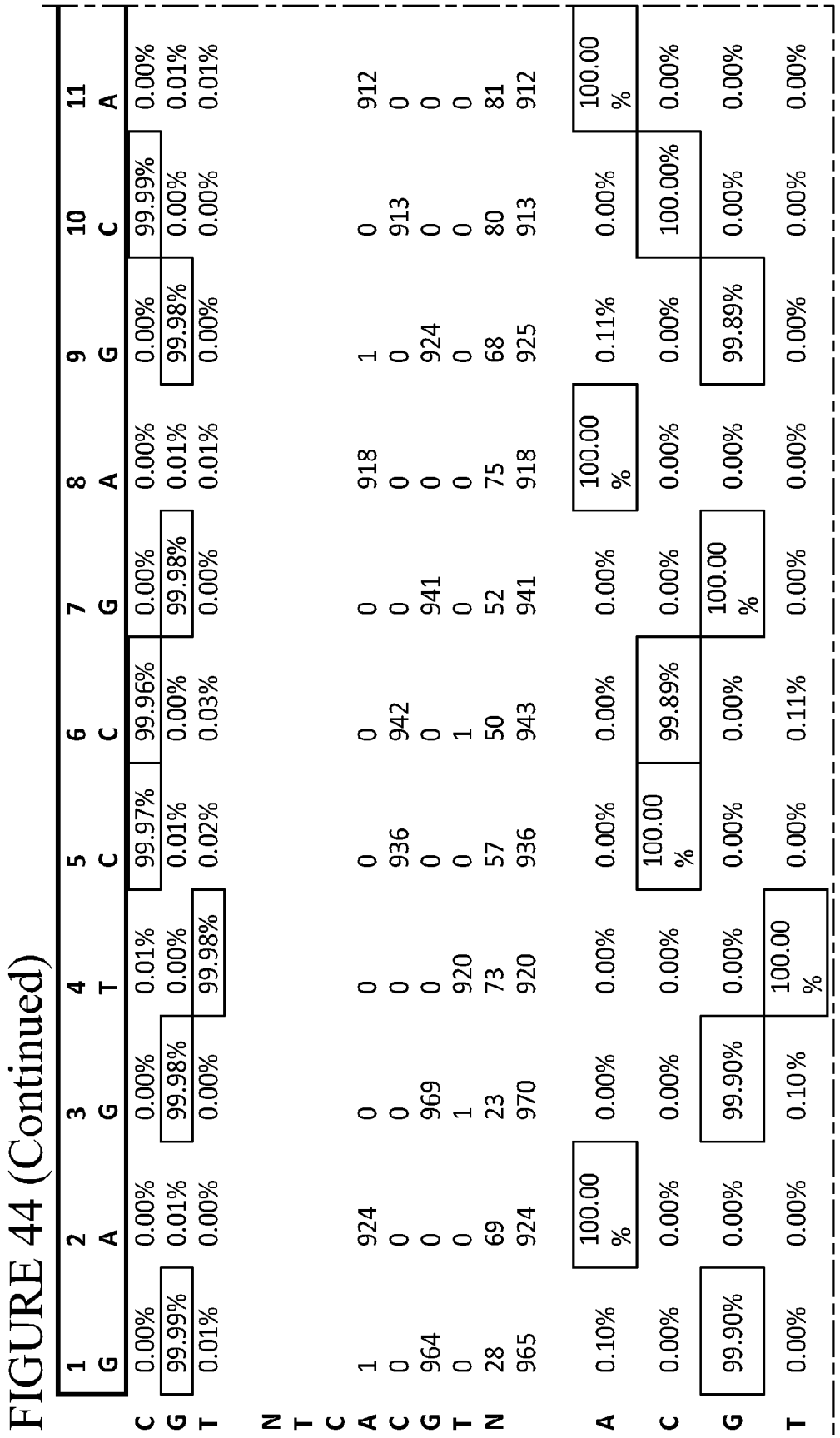
# FIGURE 44 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.01% | 0.02% | 99.94% | 0.01% | 0.01% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.95% | 99.93% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.97% | 0.06% | 99.98% | 0.00% | 0.02% |
| T | 0.01% | 0.00% | 0.01% | 99.98% | 0.04% | 0.07% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 11 | 95615 | 3 | 4 | 3 | 1 | 11 | 95391 | 12 | 4 | 94123 |
| C | 3 | 2 | 0 | 7 | 96731 | 97438 | 0 | 0 | 5 | 93810 | 0 |
| G | 98857 | 13 | 98574 | 1 | 3 | 0 | 97748 | 20 | 95591 | 1 | 9 |
| T | 1 | 3 | 4 | 94516 | 17 | 55 | 2 | 7 | 4 | 7 | 2 |
| N | 2659 | 5898 | 2950 | 7003 | 4777 | 4037 | 3770 | 6113 | 5919 | 7709 | 7397 |
| | 98872 | 95633 | 98581 | 94528 | 96754 | 97494 | 97761 | 95418 | 95612 | 93822 | 94134 |
| A | 0.01% | 99.98% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 0.01% | 0.00% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.94% | 0.00% | 0.00% | 0.01% | 99.99% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.99% | 0.02% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.99% | 0.02% | 0.06% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| **untreated cells** | | | | | | | | | | | |
| A | 6 | 95824 | 8 | 10 | 2 | 5 | 14 | 95481 | 10 | 3 | 94220 |
| C | 0 | 1 | 3 | 8 | 96889 | 97556 | 0 | 1 | 2 | 93970 | 1 |
| G | 99134 | 11 | 98872 | 0 | 5 | 2 | 97900 | 7 | 95756 | 1 | 6 |
| T | 5 | 2 | 4 | 94644 | 20 | 29 | 3 | 6 | 3 | 4 | 9 |
| N | 2750 | 6057 | 3008 | 7233 | 4979 | 4303 | 3978 | 6400 | 6124 | 7917 | 7659 |
| | 99145 | 95838 | 98887 | 94662 | 96916 | 97592 | 97917 | 95495 | 95771 | 93978 | 94236 |
| A | 0.01% | 99.99% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 99.98% |

FIGURE 44 (Continued)

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.01% | 0.00% | 0.00% | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 99.99% | 100.00 % | | | |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | | | |
| 2 | 98432 | 99332 | 1 | 96949 | 97868 | 2 | 98148 | 96186 | 2 | 8 | 3 | | | |
| 2 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 1 | 1 | | | |
| 98499 | 2 | 5 | 99269 | 5 | 7 | 98797 | 10 | 8 | 98338 | 98599 | 97080 | | | |
| 4 | 2 | 10 | 0 | 3 | 2 | 1 | 3 | 8 | 8 | 1 | 0 | | | |
| 3024 | 3093 | 2184 | 2261 | 4574 | 3654 | 2730 | 3370 | 5327 | 3183 | 2922 | 4447 | | | |
| 98507 | 98438 | 99347 | 99270 | 96957 | 97877 | 98801 | 98161 | 96204 | 98348 | 98609 | 97084 | | | |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 0.01% | 0.00% | | | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | |
| 99.99% | 0.00% | 0.01% | 100.00% | 0.01% | 0.01% | 100.00 % | 0.01% | 0.01% | 99.99% | 99.99% | 100.00 % | | | |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | | | |
| 2 | 98652 | 99650 | 1 | 97187 | 98206 | 3 | 98353 | 96468 | 2 | 8 | 5 | | | |
| 2 | 3 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 1 | | | |
| 98818 | 3 | 9 | 99639 | 9 | 9 | 99103 | 6 | 13 | 98615 | 98770 | 97155 | | | |
| 2 | 1 | 4 | 0 | 0 | 12 | 3 | 7 | 1 | 8 | 1 | 1 | | | |
| 3071 | 3236 | 2232 | 2251 | 4698 | 3667 | 2785 | 3527 | 5412 | 3268 | 3113 | 4732 | | | |
| 98824 | 98659 | 99663 | 99643 | 97196 | 98227 | 99109 | 98367 | 96482 | 98626 | 98781 | 97162 | | | |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 0.01% | 0.01% | | | |

FIGURE 44 (Continued)



| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 99.96% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.99% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 99.98% | 0.02% | 0.03% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| N | | | | | | | | | | | |
| T | | | | | | | | | | | |
| C | | | | | | | | | | | |
| A | 1 | 924 | 0 | 0 | 0 | 0 | 0 | 918 | 1 | 0 | 912 |
| C | 0 | 0 | 0 | 0 | 936 | 942 | 0 | 0 | 0 | 913 | 0 |
| G | 964 | 0 | 969 | 0 | 0 | 0 | 941 | 0 | 924 | 0 | 0 |
| T | 0 | 0 | 1 | 920 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| N | 28 | 69 | 23 | 73 | 57 | 50 | 52 | 75 | 68 | 80 | 81 |
| | 965 | 924 | 970 | 920 | 936 | 943 | 941 | 918 | 925 | 913 | 912 |
| A | 0.10% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00% | 0.11% | 0.00% | 100.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 100.00% | 99.89% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% |
| G | 99.90% | 0.00% | 99.90% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 99.89% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.10% | 100.00% | 0.00% | 0.11% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 44 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 100.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| 0 | 952 | 960 | 0 | 950 | 955 | 0 | 963 | 939 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 960 | 0 | 0 | 963 | 0 | 0 | 962 | 0 | 0 | 962 | 971 | 945 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33 | 41 | 33 | 30 | 43 | 38 | 31 | 30 | 54 | 31 | 22 | 48 |
| 960 | 952 | 960 | 963 | 950 | 955 | 962 | 963 | 939 | 962 | 971 | 945 |
| 0.00% | 100.00 % | 100.00 % | 0.00% | 100.00 % | 100.00 % | 0.00% | 100.00 % | 100.00 % | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 100.00 % | 100.00 % |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 44 (Continued)

**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 12 | 85503 | 1 | 6 | 2 | 5 | 10 | 85029 | 13 | 3 | 84108 |
| **C** | 1 | 2 | 0 | 11 | 86223 | 86869 | 1 | 1 | 1 | 83725 | 1 |
| **G** | 88215 | 9 | 88001 | 0 | 0 | 2 | 87132 | 11 | 85287 | 1 | 10 |
| **T** | 6 | 1 | 1 | 84239 | 4 | 11 | 1 | 8 | 3 | 7 | 4 |
| **N** | 2384 | 5103 | 2615 | 6362 | 4389 | 3731 | 3474 | 5569 | 5314 | 6882 | 6495 |
| | 88234 | 85515 | 88003 | 84256 | 86229 | 86887 | 87144 | 85049 | 85304 | 83736 | 84123 |
| **A** | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.02% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.01% | 100.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 99.98% | 0.00% | 0.01% |
| **T** | 0.01% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |

**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 11 | 96254 | 8 | 4 | 3 | 2 | 12 | 95338 | 24 | 0 | 94446 |
| **C** | 1 | 1 | 1 | 10 | 97327 | 97899 | 1 | 0 | 2 | 94146 | 0 |
| **G** | 99474 | 167 | 99298 | 0 | 2 | 0 | 98226 | 309 | 96056 | 0 | 34 |
| **T** | 4 | 1 | 3 | 95103 | 37 | 97 | 4 | 2 | 3 | 5 | 6 |
| **N** | 2679 | 5746 | 2859 | 7052 | 4800 | 4171 | 3926 | 6520 | 6084 | 8018 | 7683 |
| | 99490 | 96423 | 99310 | 95117 | 97369 | 97998 | 98243 | 95649 | 96085 | 94151 | 94486 |
| **A** | 0.01% | 99.82% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.67% | 0.02% | 0.00% | 99.96% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 99.90% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.17% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.32% | 99.97% | 0.00% | 0.04% |
| **T** | 0.00% | 0.00% | 0.00% | 99.99% | 0.04% | 0.10% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |

**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 11 | 97089 | 1 | 9 | 0 | 13 | 14 | 96835 | 11 | 2 | 95593 |
| **C** | 1 | 1 | 0 | 7 | 98106 | 98825 | 1 | 0 | 2 | 95111 | 0 |

FIGURE 44 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 87769 | 88599 | 2 | 86459 | 87340 | 4 | 87925 | 86385 | 2 | 6 | 2 | |
| 2 | 3 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | |
| 87913 | 2 | 7 | 88516 | 9 | 4 | 88152 | 6 | 11 | 87739 | 87339 | 86190 | |
| 1 | 1 | 5 | 1 | 3 | 11 | 2 | 4 | 10 | 5 | 1 | 1 | |
| 2702 | 2843 | 2007 | 2099 | 4146 | 3261 | 2459 | 2683 | 4211 | 2871 | 3271 | 4425 | |
| 87916 | 87775 | 88611 | 88519 | 86472 | 87357 | 88159 | 87935 | 86407 | 87747 | 87347 | 86193 | |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.98% | 0.00% | 99.99% | 99.97% | 0.00% | 0.01% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 100.00% | 0.00% | 0.01% | 100.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 100.00% | |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 98886 | 99901 | 0 | 97523 | 98625 | 3 | 99121 | 97273 | 8 | 9 | 2 | |
| 0 | 2 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 3 | |
| 99171 | 10 | 5 | 99908 | 13 | 6 | 99414 | 5 | 4 | 98924 | 98657 | 97175 | |
| 1 | 2 | 5 | 0 | 3 | 8 | 2 | 5 | 5 | 4 | 3 | 1 | |
| 2993 | 3269 | 2257 | 2261 | 4629 | 3530 | 2749 | 3038 | 4886 | 3233 | 3499 | 4988 | |
| 99176 | 98900 | 99912 | 99908 | 97540 | 98639 | 99420 | 99131 | 97283 | 98936 | 98670 | 97181 | |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.99% | 0.00% | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 99.99% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 99.99% | 99.99% | 99.99% | |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 99885 | 100874 | 5 | 98407 | 99407 | 1 | 99974 | 98148 | 7 | 8 | 4 |
| 1 | 1 | 1 | 3 | 0 | 1 | 0 | 1 | 4 | 1 | 2 | 0 |

FIGURE 44 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | A | G | T | C | C | G | A | G | C | A |
| G | 100366 | 14 | 100044 | 1 | 2 | 0 | 99136 | 12 | 97150 | 0 | 7 |
| T | 2 | 3 | 3 | 95865 | 22 | 18 | 4 | 3 | 6 | 10 | 9 |
| N | 2592 | 5865 | 2924 | 7090 | 4842 | 4116 | 3817 | 6122 | 5803 | 7849 | 7363 |
| | 100380 | 97107 | 100048 | 95882 | 98130 | 98856 | 99155 | 96850 | 97169 | 95123 | 95609 |
| A | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.99% | 0.01% | 100.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% | 0.02% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| **pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| A | 11 | 104607 | 5 | 4 | 6 | 5 | 23 | 104186 | 25 | 4 | 102871 |
| C | 1 | 2 | 1 | 9 | 105669 | 106510 | 1 | 1 | 3 | 102327 | 1 |
| G | 108291 | 11 | 107982 | 0 | 12 | 0 | 106882 | 7 | 104608 | 1 | 5 |
| T | 3 | 1 | 1 | 103264 | 28 | 56 | 10 | 6 | 4 | 7 | 6 |
| N | 2907 | 6592 | 3224 | 7936 | 5498 | 4642 | 4297 | 7013 | 6573 | 8874 | 8330 |
| | 108306 | 104621 | 107989 | 103277 | 105715 | 106571 | 106916 | 104200 | 104640 | 102339 | 102883 |
| A | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 99.99% | 0.02% | 0.00% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 99.94% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.99% | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 99.97% | 0.01% | 99.97% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 99.99% | 0.03% | 0.05% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |

FIGURE 44 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 99981 | 4 | 7 | 100819 | 5 | 6 | 100245 | 2 | 15 | 99759 | 99516 | 98059 |
| 1 | 0 | 9 | 1 | 14 | 9 | 2 | 4 | 6 | 8 | 7 | 0 |
| 2989 | 3082 | 2081 | 2144 | 4546 | 3559 | 2734 | 3001 | 4809 | 3207 | 3449 | 4919 |
| 99983 | 99890 | 100891 | 100828 | 98426 | 99423 | 100248 | 99981 | 98173 | 99775 | 99533 | 98063 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.98% | 0.00% | 99.99% | 99.97% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.00% | 0.01% | 99.99% | 0.01% | 0.01% | 100.00 % | 0.00% | 0.02% | 99.98% | 99.98% | 100.00 % |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |
| 1 | 107647 | 108785 | 2 | 106016 | 107113 | 1 | 107868 | 105744 | 4 | 10 | 7 |
| 3 | 1 | 3 | 1 | 0 | 1 | 1 | 0 | 3 | 1 | 0 | 1 |
| 107817 | 6 | 8 | 108710 | 9 | 5 | 108152 | 9 | 16 | 107648 | 107286 | 105773 |
| 5 | 3 | 5 | 2 | 3 | 3 | 5 | 6 | 11 | 7 | 3 | 2 |
| 3387 | 3556 | 2412 | 2498 | 5185 | 4091 | 3054 | 3330 | 5439 | 3553 | 3914 | 5430 |
| 107826 | 107657 | 108801 | 108715 | 106028 | 107122 | 108159 | 107883 | 105774 | 107660 | 107299 | 105783 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.97% | 0.00% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 100.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.02% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |

FIGURE 44 (Continued)

| pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 9 | 96988 | 4 | 5 | 2 | 6 | 11 | 96516 | 14 | 1 | 95659 |
| **C** | 0 | 2 | 0 | 10 | 98262 | 98931 | 1 | 1 | 1 | 95288 | 0 |
| **G** | 100538 | 101 | 100250 | 1 | 3 | 6 | 99257 | 241 | 97271 | 1 | 14 |
| **T** | 2 | 3 | 2 | 95904 | 36 | 111 | 3 | 8 | 5 | 9 | 10 |
| **N** | 2749 | 6204 | 3042 | 7378 | 4995 | 4244 | 4026 | 6532 | 6007 | 7999 | 7615 |
| | 100549 | 97094 | 100256 | 95920 | 98303 | 99054 | 99272 | 96766 | 97291 | 95299 | 95683 |
| **A** | 0.01% | 99.89% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.74% | 0.01% | 0.00% | 99.97% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 99.88% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.10% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.25% | 99.98% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.04% | 0.11% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| **pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)** | | | | | | | | | | | |
| **A** | 15 | 102909 | 8 | 5 | 4 | 0 | 14 | 102467 | 21 | 2 | 101174 |
| **C** | 1 | 3 | 1 | 10 | 103881 | 104868 | 1 | 0 | 3 | 100889 | 0 |
| **G** | 106573 | 8 | 106262 | 0 | 0 | 4 | 105132 | 20 | 102841 | 0 | 9 |
| **T** | 5 | 2 | 3 | 101236 | 32 | 29 | 3 | 5 | 2 | 6 | 4 |
| **N** | 2973 | 6645 | 3293 | 8316 | 5650 | 4666 | 4417 | 7075 | 6700 | 8670 | 8380 |
| | 106594 | 102922 | 106274 | 101251 | 103917 | 104901 | 105150 | 102492 | 102867 | 100897 | 101187 |
| **A** | 0.01% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 0.02% | 0.00% | 99.99% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.97% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% | 99.97% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.99% | 0.03% | 0.03% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| **pNMG-276 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (wild-type) + (A106V_D108N_D147Y_E155V))** | | | | | | | | | | | |
| **A** | 19 | 106316 | 8 | 9 | 1 | 3 | 15 | 106235 | 21 | 3 | 104862 |
| **C** | 1 | 1 | 1 | 14 | 107532 | 108644 | 1 | 1 | 0 | 104468 | 0 |
| **G** | 110516 | 14 | 109989 | 0 | 0 | 0 | 109063 | 8 | 106642 | 0 | 8 |

FIGURE 44 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 100143 | 101103 | 3 | 98510 | 99463 | 4 | 100161 | 98253 | 3 | 10 | 2 | |
| 0 | 5 | 0 | 1 | 0 | 1 | 0 | 0 | 3 | 0 | 1 | 0 | |
| 100306 | 7 | 7 | 101004 | 12 | 10 | 100460 | 11 | 15 | 99895 | 99679 | 98365 | |
| 1 | 3 | 6 | 5 | 2 | 2 | 2 | 6 | 5 | 9 | 2 | 2 | |
| 2986 | 3139 | 2181 | 2284 | 4773 | 3821 | 2831 | 3119 | 5021 | 3390 | 3605 | 4928 | |
| 100312 | 100158 | 101116 | 101013 | 98524 | 99476 | 100466 | 100178 | 98276 | 99907 | 99692 | 98369 | |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.98% | 0.00% | 0.01% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.02% | 99.99% | 99.99% | 100.00 % | |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | |
| 5 | 106016 | 107123 | 5 | 104439 | 105503 | 1 | 106165 | 104208 | 5 | 7 | 7 | |
| 1 | 1 | 3 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | |
| 106181 | 7 | 10 | 107136 | 10 | 4 | 106413 | 10 | 14 | 105862 | 105505 | 104145 | |
| 1 | 3 | 5 | 3 | 2 | 5 | 4 | 10 | 8 | 7 | 4 | 0 | |
| 3379 | 3540 | 2426 | 2422 | 5115 | 4055 | 3147 | 3382 | 5337 | 3692 | 4051 | 5415 | |
| 106188 | 106027 | 107141 | 107145 | 104452 | 105512 | 106420 | 106185 | 104230 | 105875 | 105516 | 104152 | |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.99% | 0.00% | 99.98% | 99.98% | 0.00% | 0.01% | 0.01% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | |
| 4 | 109931 | 111082 | 3 | 108131 | 109256 | 4 | 110101 | 107824 | 2 | 7 | 8 | |
| 1 | 0 | 1 | 1 | 2 | 0 | 1 | 3 | 0 | 1 | 3 | 1 | |
| 110060 | 3 | 11 | 111036 | 26 | 13 | 110314 | 9 | 8 | 109663 | 109220 | 107721 | |

FIGURE 44 (Continued)

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | 4 | 0 | 3 | 104589 | 10 | 23 | 2 | 4 | 3 | 8 | 7 |
| N | 3119 | 7328 | 3658 | 9047 | 6116 | 4989 | 4578 | 7411 | 6993 | 9180 | 8782 |
| | 110540 | 106331 | 110001 | 104612 | 107543 | 108670 | 109081 | 106248 | 106666 | 104479 | 104877 |
| A | 0.02% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.02% | 0.00% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| **pNMG-277 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (A106V_D108N_D147Y_E155V) + (A106V_D108N_D147Y_E155V))** | | | | | | | | | | | |
| A | 12 | 93237 | 3 | 3 | 4 | 1 | 13 | 92716 | 31 | 3 | 91851 |
| C | 3 | 0 | 1 | 8 | 94493 | 95028 | 1 | 1 | 1 | 91544 | 0 |
| G | 96591 | 153 | 96304 | 0 | 5 | 0 | 95346 | 245 | 93334 | 0 | 55 |
| T | 2 | 0 | 6 | 92172 | 9 | 23 | 8 | 3 | 3 | 8 | 3 |
| N | 2628 | 5846 | 2922 | 7053 | 4725 | 4184 | 3868 | 6271 | 5867 | 7683 | 7329 |
| | 96608 | 93390 | 96314 | 92183 | 94511 | 95052 | 95368 | 92965 | 93369 | 91555 | 91909 |
| A | 0.01% | 99.84% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.73% | 0.03% | 0.00% | 99.94% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.16% | 99.99% | 0.00% | 0.01% | 0.00% | 99.98% | 0.26% | 99.96% | 0.00% | 0.06% |
| T | 0.00% | 0.00% | 0.01% | 99.99% | 0.01% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |

# FIGURE 44 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| G | A | A | G | A | A | G | A | A | G | G | G |
| 5 | 3 | 6 | 6 | 1 | 25 | 0 | 8 | 11 | 4 | 2 | 1 |
| 3589 | 3722 | 2575 | 2629 | 5515 | 4381 | 3356 | 3554 | 5832 | 4005 | 4443 | 5939 |
| 110070 | 109937 | 111100 | 111046 | 108160 | 109294 | 110319 | 110121 | 107843 | 109670 | 109232 | 107731 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.97% | 99.97% | 0.00% | 99.98% | 99.98% | 0.00% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 99.99% | 0.02% | 0.01% | 100.00 % | 0.01% | 0.01% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| 1 | 96107 | 97086 | 4 | 94700 | 95648 | 1 | 96285 | 94484 | 6 | 5 | 6 |
| 3 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 2 | 1 |
| 96272 | 10 | 12 | 97054 | 8 | 12 | 96458 | 13 | 9 | 96021 | 95772 | 94464 |
| 3 | 1 | 8 | 3 | 1 | 9 | 2 | 5 | 7 | 8 | 2 | 0 |
| 2959 | 3119 | 2133 | 2179 | 4531 | 3571 | 2778 | 2936 | 4738 | 3204 | 3459 | 4769 |
| 96279 | 96119 | 97107 | 97061 | 94709 | 95669 | 96462 | 96304 | 94502 | 96036 | 95781 | 94471 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.98% | 0.01% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 100.00 % | 0.01% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |

FIGURE 44 (Continued)

**pNMG-278 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108Q_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 8 | 83830 | 3 | 6 | 0 | 2 | 11 | 83439 | 19 | 3 | 82307 |
| **C** | 2 | 0 | 2 | 1 | 84664 | 85398 | 0 | 1 | 1 | 81994 | 2 |
| **G** | 86690 | 5 | 86479 | 1 | 4 | 0 | 85608 | 13 | 83601 | 1 | 9 |
| **T** | 1 | 2 | 2 | 82780 | 14 | 28 | 2 | 3 | 1 | 6 | 4 |
| **N** | 2371 | 5235 | 2586 | 6284 | 4390 | 3644 | 3451 | 5616 | 5450 | 7068 | 6750 |
| | 86701 | 83837 | 86486 | 82788 | 84682 | 85428 | 85621 | 83456 | 83622 | 82004 | 82322 |
| **A** | 0.01% | 99.99% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.02% | 0.00% | 99.98% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.96% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| **G** | 99.99% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% | 99.97% | 0.00% | 0.01% |
| **T** | 0.00% | 0.00% | 0.00% | 99.99% | 0.02% | 0.03% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |

**pNMG-279 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108M_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 11 | 86842 | 6 | 6 | 0 | 4 | 11 | 86206 | 16 | 6 | 85096 |
| **C** | 1 | 1 | 1 | 10 | 87745 | 88272 | 1 | 2 | 1 | 84874 | 1 |
| **G** | 89777 | 16 | 89447 | 0 | 2 | 2 | 88576 | 30 | 86573 | 0 | 4 |
| **T** | 1 | 2 | 3 | 85819 | 15 | 18 | 6 | 5 | 2 | 8 | 4 |
| **N** | 2449 | 5378 | 2782 | 6404 | 4477 | 3943 | 3645 | 5996 | 5647 | 7361 | 7144 |
| | 89790 | 86861 | 89457 | 85835 | 87762 | 88296 | 88594 | 86243 | 86592 | 84888 | 85105 |
| **A** | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.96% | 0.02% | 0.01% | 99.99% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% |
| **G** | 99.99% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.03% | 99.98% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% | 0.02% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |

**pNMG-280 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108L_D147Y_E155V)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 14 | 79682 | 6 | 12 | 1 | 1 | 10 | 79444 | 14 | 2 | 78438 |
| **C** | 1 | 1 | 0 | 11 | 80614 | 81133 | 2 | 1 | 1 | 78189 | 8 |
| **G** | 82389 | 10 | 82096 | 1 | 0 | 10 | 81410 | 5 | 79736 | 1 | 9 |

FIGURE 44 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 86286 | 87102 | 1 | 85032 | 85751 | 1 | 86158 | 84288 | 4 | 4 | 4 | 10 |
| 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 2 | 1 | 0 | 0 | 0 |
| 86340 | 6 | 9 | 87121 | 5 | 8 | 86692 | 9 | 7 | 86175 | 86362 | 86362 | 85004 |
| 4 | 5 | 8 | 3 | 1 | 7 | 1 | 1 | 9 | 4 | 0 | 0 | 1 |
| 2724 | 2775 | 1952 | 1947 | 4034 | 3305 | 2377 | 2903 | 4765 | 2887 | 2705 | 2705 | 4056 |
| 86348 | 86297 | 87120 | 87125 | 85038 | 85767 | 86695 | 86168 | 84306 | 86184 | 86366 | 86366 | 85015 |
| 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 100.00% | 0.01% | 0.01% | 99.99% | 100.00% | 100.00% | 99.99% |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| 2 | 89195 | 90097 | 3 | 87939 | 88832 | 2 | 88970 | 87305 | 3 | 2 | 4 | |
| 0 | 1 | 3 | 0 | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | |
| 89320 | 13 | 10 | 90189 | 3 | 1 | 89658 | 18 | 13 | 89282 | 89447 | 87886 | |
| 1 | 2 | 19 | 1 | 2 | 4 | 2 | 9 | 13 | 4 | 0 | 1 | |
| 2926 | 3038 | 2120 | 2056 | 4305 | 3411 | 2587 | 3252 | 4915 | 2965 | 2805 | 4363 | |
| 89323 | 89211 | 90129 | 90193 | 87944 | 88838 | 89662 | 88997 | 87334 | 89289 | 89449 | 87891 | |
| 0.00% | 99.98% | 99.96% | 0.00% | 99.99% | 99.99% | 0.00% | 99.97% | 99.97% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| 100.00% | 0.01% | 0.01% | 100.00% | 0.00% | 0.00% | 100.00% | 0.02% | 0.01% | 99.99% | 100.00% | 99.99% | |
| 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | |
| 3 | 81982 | 82729 | 1 | 80771 | 81559 | 3 | 81836 | 80164 | 5 | 7 | 5 | |
| 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 2 | |
| 82144 | 9 | 5 | 82782 | 13 | 8 | 82334 | 10 | 13 | 81951 | 82133 | 80915 | |

FIGURE 44 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | G | A | G | T | C | C | G | A | G | C | A |
| T | 3 | 1 | 3 | 78750 | 7 | 12 | 6 | 3 | 7 | 14 | 5 |
| N | 2127 | 4840 | 2429 | 5760 | 3912 | 3378 | 3106 | 5081 | 4776 | 6328 | 6074 |
| | 82407 | 79694 | 82105 | 78774 | 80622 | 81156 | 81428 | 79453 | 79758 | 78206 | 78460 |
| A | 0.02% | 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 99.99% | 0.02% | 0.00% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 99.97% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 99.97% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.97% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.02% | 0.01% |
| **pNMG-281 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108K_D147Y_E155V)** | | | | | | | | | | | |
| A | 12 | 95030 | 3 | 10 | 3 | 5 | 9 | 94617 | 12 | 3 | 93295 |
| C | 0 | 1 | 1 | 12 | 96045 | 96660 | 0 | 1 | 1 | 92980 | 0 |
| G | 98200 | 17 | 97975 | 0 | 1 | 6 | 97052 | 31 | 94959 | 0 | 5 |
| T | 4 | 2 | 3 | 93791 | 47 | 56 | 7 | 7 | 1 | 6 | 1 |
| N | 2744 | 5910 | 2978 | 7147 | 4864 | 4233 | 3892 | 6304 | 5987 | 7971 | 7659 |
| | 98216 | 95050 | 97982 | 93813 | 96096 | 96727 | 97068 | 94656 | 94973 | 92989 | 93301 |
| A | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.96% | 0.01% | 0.00% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.95% | 99.93% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.02% | 99.99% | 0.00% | 0.00% | 0.01% | 99.98% | 0.03% | 99.99% | 0.00% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.05% | 0.06% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |
| **pNMG-282 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108I_D147Y_E155V)** | | | | | | | | | | | |
| A | 13 | 96997 | 3 | 5 | 2 | 3 | 9 | 96617 | 13 | 1 | 95462 |
| C | 3 | 0 | 1 | 12 | 98094 | 98846 | 3 | 0 | 3 | 95075 | 1 |
| G | 100541 | 13 | 100062 | 1 | 3 | 0 | 99158 | 11 | 96877 | 0 | 12 |

FIGURE 44 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 4 | 3 | 3 | 0 | 4 | 1 | 4 | 5 | 5 | 4 | 1 |
| 2382 | 2539 | 1797 | 1747 | 3750 | 2963 | 2195 | 2684 | 4351 | 2572 | 2390 | 3611 |
| 82152 | 81995 | 82737 | 82787 | 80784 | 81571 | 82339 | 81850 | 80183 | 81962 | 82144 | 80923 |
| 0.00% | 99.98% | 99.99% | 0.00% | 99.98% | 99.99% | 0.00% | 99.98% | 99.98% | 0.01% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.02% | 0.01% | 99.99% | 0.01% | 0.02% | 99.99% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 1 | 97740 | 98673 | 1 | 96293 | 97256 | 3 | 97447 | 95472 | 1 | 6 | 3 |
| 2 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 0 |
| 97834 | 5 | 6 | 98734 | 3 | 8 | 98148 | 4 | 10 | 97668 | 97902 | 96275 |
| 4 | 5 | 4 | 3 | 2 | 4 | 4 | 3 | 6 | 7 | 0 | 0 |
| 3119 | 3210 | 2277 | 2222 | 4662 | 3691 | 2805 | 3505 | 5471 | 3283 | 3052 | 4682 |
| 97841 | 97750 | 98683 | 98738 | 96298 | 97269 | 98155 | 97455 | 95489 | 97677 | 97908 | 96278 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 100.00% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% | 99.99% | 99.99% | 100.00 % |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| 4 | 99934 | 100888 | 2 | 98284 | 99311 | 5 | 99554 | 97487 | 3 | 4 | 1 |
| 2 | 2 | 0 | 0 | 0 | 2 | 0 | 1 | 3 | 1 | 2 | 0 |
| 100049 | 12 | 10 | 100856 | 8 | 11 | 100356 | 13 | 14 | 99790 | 100021 | 98510 |

FIGURE 44 (Continued)

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 99.98% | 0.01% | 0.00% | 99.98% | | | | | | | | | |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | | | | | | | | | |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | | | | | | | | | |
| T | 0.00% | 0.00% | 0.00% | 99.98% | 0.02% | 0.02% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | | | | | | | | | |
| **pNMG-283 (pCMV_ecTadA_XTEN_nCas9_GGS_NLS; D108F_D147Y_E155V)** | | | | | | | | | | | | | | | | | | | | |
| A | 12 | 90536 | 5 | 12 | 3 | 3 | 12 | 90387 | 14 | 2 | 89293 | | | | | | | | | |
| C | 2 | 0 | 1 | 13 | 91705 | 92454 | 0 | 0 | 0 | 88864 | 0 | | | | | | | | | |
| G | 93968 | 20 | 93657 | 2 | 1 | 1 | 92665 | 5 | 90502 | 0 | 7 | | | | | | | | | |
| T | 5 | 2 | 2 | 89293 | 13 | 25 | 8 | 3 | 1 | 6 | 14 | | | | | | | | | |
| N | 2640 | 6069 | 2962 | 7307 | 4905 | 4144 | 3942 | 6232 | 6110 | 7755 | 7313 | | | | | | | | | |
| | 93987 | 90558 | 93665 | 89320 | 91722 | 92483 | 92685 | 90395 | 90517 | 88872 | 89314 | | | | | | | | | |
| A | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.02% | 0.00% | 99.98% | | | | | | | | | |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | | | | | | | | | |
| G | 99.98% | 0.02% | 99.99% | 0.00% | 0.00% | 0.00% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% | | | | | | | | | |
| T | 0.01% | 0.00% | 0.00% | 99.97% | 0.01% | 0.03% | 0.01% | 0.00% | 0.00% | 0.01% | 0.02% | | | | | | | | | |
| **untreated cells** | | | | | | | | | | | | | | | | | | | | |
| A | 9 | 82687 | 4 | 6 | 11 | 7 | 15 | 82405 | 15 | 1 | 81425 | | | | | | | | | |
| C | 1 | 1 | 1 | 8 | 83569 | 84545 | 1 | 0 | 1 | 81102 | 0 | | | | | | | | | |
| G | 86078 | 8 | 85764 | 1 | 8 | 11 | 84813 | 6 | 82754 | 4 | 6 | | | | | | | | | |
| T | 5 | 3 | 1 | 81488 | 90 | 91 | 2 | 6 | 4 | 4 | 6 | | | | | | | | | |
| N | 2445 | 5839 | 2768 | 7035 | 4860 | 3884 | 3707 | 6121 | 5764 | 7427 | 7101 | | | | | | | | | |
| | 86093 | 82699 | 85770 | 81503 | 83678 | 84654 | 84831 | 82417 | 82774 | 81111 | 81437 | | | | | | | | | |
| T | 2 | 3 | 2 | 95669 | 18 | 23 | 4 | 8 | 2 | 10 | 7 | | | | | | | | | |
| N | 2677 | 6223 | 3168 | 7549 | 5119 | 4364 | 4062 | 6600 | 6341 | 8154 | 7758 | | | | | | | | | |
| | 100559 | 97013 | 100068 | 95687 | 98117 | 98872 | 99174 | 96636 | 96895 | 95086 | 95482 | | | | | | | | | |

# FIGURE 44 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 99.98% | 99.98% | 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% | 99.99% | 100.00 % |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| 2 | 93479 | 94442 | 0 | 92073 | 92826 | 3 | 93271 | 91294 | 9 | 11 | 8 |
| 0 | 2 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 1 | 2 |
| 93607 | 3 | 3 | 94416 | 9 | 12 | 93876 | 8 | 5 | 93378 | 93598 | 92065 |
| 4 | 0 | 5 | 4 | 0 | 7 | 2 | 7 | 9 | 7 | 1 | 1 |
| 3014 | 3143 | 2177 | 2207 | 4545 | 3782 | 2743 | 3341 | 5318 | 3233 | 3016 | 4551 |
| 93613 | 93484 | 94450 | 94420 | 92082 | 92845 | 93884 | 93286 | 91309 | 93394 | 93611 | 92076 |
| 0.00% | 99.99% | 99.99% | 0.00% | 99.99% | 99.98% | 0.00% | 99.98% | 99.98% | 0.01% | 0.01% | 0.01% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.00% | 100.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 99.99% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| 3 | 85690 | 86538 | 1 | 84168 | 85051 | 1 | 85254 | 83476 | 6 | 6 | 3 |
| 0 | 2 | 2 | 0 | 0 | 1 | 1 | 0 | 7 | 0 | 0 | 0 |
| 85664 | 1 | 8 | 86433 | 2 | 3 | 86008 | 9 | 11 | 85278 | 85583 | 84313 |
| 2 | 4 | 7 | 0 | 1 | 7 | 2 | 9 | 5 | 4 | 1 | 0 |
| 2869 | 2841 | 1983 | 2104 | 4367 | 3476 | 2526 | 3266 | 5039 | 3250 | 2948 | 4222 |
| 85669 | 85697 | 86555 | 86434 | 84171 | 85062 | 86012 | 85272 | 83499 | 85288 | 85590 | 84316 |
| 4 | 3 | 14 | 4 | 0 | 5 | 4 | 9 | 11 | 5 | 5 | 0 |
| 3181 | 3289 | 2328 | 2378 | 4948 | 3911 | 2875 | 3663 | 5725 | 3441 | 3208 | 4729 |
| 100059 | 99951 | 100912 | 100862 | 98292 | 99329 | 100365 | 99577 | 97515 | 99799 | 100032 | 98511 |

FIGURE 44 (Continued)

| | 1 G | 2 A | 3 G | 4 T | 5 C | 6 C | 7 G | 8 A | 9 G | 10 C | 11 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 0.01% | 99.99% | 0.00% | 0.01% | 0.01% | 0.01% | 0.02% | 99.99% | 0.02% | 0.00% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 99.87% | 99.87% | 0.00% | 0.00% | 0.00% | 99.99% | 0.00% |
| G | 99.98% | 0.01% | 99.99% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 99.98% | 0.00% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 99.98% | 0.11% | 0.11% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| N | | | | | | | | | | | |
| T | | | | | | | | | | | |
| C | | | | | | | | | | | |
| A | 0 | 1440 | 0 | 1 | 0 | 0 | 0 | 1414 | 0 | 0 | 1412 |
| C | 0 | 0 | 0 | 0 | 1439 | 1453 | 0 | 0 | 0 | 1393 | 0 |
| G | 1478 | 0 | 1472 | 0 | 0 | 0 | 1459 | 0 | 1430 | 0 | 0 |
| T | 0 | 0 | 0 | 1400 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N | 43 | 81 | 49 | 120 | 82 | 68 | 62 | 107 | 91 | 128 | 109 |
| | 1478 | 1440 | 1472 | 1401 | 1439 | 1453 | 1459 | 1414 | 1430 | 1393 | 1412 |
| A | 0.00% | 100.00% | 0.00% | 0.07% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 100.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 100.00% | 100.00% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% |
| G | 100.00% | 0.00% | 100.00% | 0.00% | 0.00% | 0.00% | 100.00% | 0.00% | 100.00% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 99.93% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 44 (Continued)

| 12 G | 13 A | 14 A | 15 G | 16 A | 17 A | 18 G | 19 A | 20 A | P G | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 99.99% | 99.98% | 0.00% | 100.00 % | 99.99% | 0.00% | 99.98% | 99.97% | 0.01% | 0.01% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| 99.99% | 0.00% | 0.01% | 100.00% | 0.00% | 0.00% | 100.00 % | 0.01% | 0.01% | 99.99% | 99.99% | 100.00 % |
| 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0 | 1468 | 1491 | 0 | 1450 | 1456 | 1 | 1458 | 1426 | 0 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1469 | 0 | 0 | 1482 | 0 | 0 | 1471 | 1 | 2 | 1457 | 1470 | 1446 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 52 | 53 | 30 | 39 | 71 | 65 | 49 | 62 | 93 | 64 | 50 | 75 |
| 1469 | 1468 | 1491 | 1482 | 1450 | 1456 | 1472 | 1459 | 1428 | 1457 | 1471 | 1446 |
| 0.00% | 100.00 % | 100.00 % | 0.00% | 100.00 % | 100.00 % | 0.07% | 99.93% | 99.86% | 0.00% | 0.07% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00 % | 0.00% | 0.00% | 100.00% | 0.00% | 0.00% | 99.93% | 0.07% | 0.14% | 100.00 % | 99.93% | 100.00 % |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 45

| | 1 G | 2 G | 3 A | 4 A | 5 T | 6 C | 7 C | 8 C | 9 T | 10 T | 11 C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cas9** | | | | | | | | | | | |
| **A** | 7 | 4 | 70228 | 69855 | 2 | 3 | 2 | 11 | 7 | 2 | 5 |
| **C** | 0 | 3 | 13 | 7 | 2 | 70410 | 69701 | 71064 | 6 | 5 | 71328 |
| **G** | 70702 | 70557 | 3 | 5 | 10 | 9 | 3 | 8 | 8 | 3 | 0 |
| **T** | 15 | 12 | 0 | 11 | 71570 | 21 | 19 | 17 | 70935 | 71637 | 10 |
| **N** | 1590 | 1738 | 2069 | 2432 | 726 | 1867 | 2584 | 1209 | 1329 | 638 | 930 |
| | 70724 | 70576 | 70244 | 69878 | 71584 | 70443 | 69725 | 71100 | 70956 | 71647 | 71343 |
| **A** | 0.01% | 0.01% | 99.98% | 99.97% | 0.00% | 0.00% | 0.00% | 0.02% | 0.01% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 0.02% | 0.01% | 0.00% | 99.95% | 99.97% | 99.95% | 0.01% | 0.01% | 99.98% |
| **G** | 99.97% | 99.97% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| **T** | 0.02% | 0.02% | 0.00% | 0.02% | 99.98% | 0.03% | 0.03% | 0.02% | 99.97% | 99.99% | 0.01% |
| **pNMG-247 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; wild type)** | | | | | | | | | | | |
| **A** | 11 | 8 | 88799 | 88480 | 1 | 20 | 10 | 12 | 11 | 51 | 3 |
| **C** | 1 | 19 | 0 | 20 | 6 | 89169 | 88205 | 90048 | 10 | 9 | 90283 |
| **G** | 89495 | 89323 | 10 | 12 | 1 | 1 | 2 | 2 | 0 | 1 | 0 |
| **T** | 6 | 6 | 13 | 6 | 90598 | 20 | 23 | 18 | 89785 | 90567 | 9 |
| **N** | 2020 | 2181 | 2714 | 3017 | 929 | 2325 | 3295 | 1455 | 1689 | 853 | 1186 |
| | 89513 | 89356 | 88822 | 88518 | 90606 | 89210 | 88240 | 90080 | 89806 | 90628 | 90295 |
| **A** | 0.01% | 0.01% | 99.97% | 99.96% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.06% | 0.00% |
| **C** | 0.00% | 0.02% | 0.00% | 0.02% | 0.01% | 99.95% | 99.96% | 99.96% | 0.01% | 0.01% | 99.99% |
| **G** | 99.98% | 99.96% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.01% | 0.01% | 0.01% | 99.99% | 0.02% | 0.03% | 0.02% | 99.98% | 99.93% | 0.01% |

FIGURE 45 (Continued)

| 12 T | 13 G | 14 C | 15 A | 16 G | 17 C | 18 A | 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 12 | 8 | 12 | 71743 | 46 | 110 | 70078 | 22 | 54 | 30 | 21 | 27 |
| 12 | 18 | 71618 | 1 | 56 | 71049 | 42 | 69865 | 69952 | 38 | 30 | 3 |
| 0 | 71010 | 1 | 15 | 71458 | 46 | 124 | 74 | 42 | 18 | 70155 | 69621 |
| 71406 | 14 | 13 | 15 | 18 | 124 | 176 | 112 | 67 | 70502 | 16 | 17 |
| 853 | 1233 | 644 | 423 | 610 | 861 | 1375 | 1591 | 1392 | 879 | 1187 | 1727 |
| 71430 | 71050 | 71644 | 71774 | 71578 | 71329 | 70420 | 70073 | 70115 | 70588 | 70222 | 69668 |
| 0.02% | 0.01% | 0.02% | 99.96% | 0.06% | 0.15% | 99.51% | 0.03% | 0.08% | 0.04% | 0.03% | 0.04% |
| 0.02% | 0.03% | 99.96% | 0.00% | 0.08% | 99.61% | 0.06% | 99.70% | 99.77% | 0.05% | 0.04% | 0.00% |
| 0.00% | 99.94% | 0.00% | 0.02% | 99.83% | 0.06% | 0.18% | 0.11% | 0.06% | 0.03% | 99.90% | 99.93% |
| 99.97% | 0.02% | 0.02% | 0.02% | 0.03% | 0.17% | 0.25% | 0.16% | 0.10% | 99.88% | 0.02% | 0.02% |
| 50 | 17 | 20 | 90798 | 45 | 176 | 89113 | 15 | 55 | 34 | 39 | 16 |
| 11 | 15 | 90583 | 1 | 44 | 89873 | 31 | 88780 | 88986 | 39 | 18 | 6 |
| 63 | 89838 | 19 | 30 | 90448 | 62 | 72 | 64 | 60 | 30 | 89302 | 88606 |
| 90249 | 67 | 18 | 11 | 7 | 115 | 216 | 132 | 21 | 89653 | 18 | 10 |
| 1095 | 1531 | 824 | 520 | 808 | 1112 | 1671 | 2030 | 1780 | 1128 | 1446 | 2179 |
| 90373 | 89937 | 90640 | 90840 | 90544 | 90226 | 89432 | 88991 | 89122 | 89756 | 89377 | 88638 |
| 0.06% | 0.02% | 0.02% | 99.95% | 0.05% | 0.20% | 99.64% | 0.02% | 0.06% | 0.04% | 0.04% | 0.02% |
| 0.01% | 0.02% | 99.94% | 0.00% | 0.05% | 99.61% | 0.03% | 99.76% | 99.85% | 0.04% | 0.02% | 0.01% |
| 0.07% | 99.89% | 0.02% | 0.03% | 99.89% | 0.07% | 0.08% | 0.07% | 0.07% | 0.03% | 99.92% | 99.96% |
| 99.86% | 0.07% | 0.02% | 0.01% | 0.01% | 0.13% | 0.24% | 0.15% | 0.02% | 99.89% | 0.02% | 0.01% |

FIGURE 45 (Continued)

**pNMG-248 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 6 | 4 | 84256 | 83979 | 3 | 7 | 3 | 6 | 6 | 11 | 3 | |
| **C** | 1 | 1 | 0 | 4 | 6 | 84556 | 83708 | 85374 | 3 | 1 | 85537 | |
| **G** | 84778 | 84678 | 6 | 4 | 0 | 6 | 1 | 0 | 2 | 0 | 0 | |
| **T** | 3 | 6 | 1 | 7 | 85856 | 22 | 21 | 19 | 85015 | 85826 | 4 | |
| **N** | 1980 | 2079 | 2505 | 2774 | 903 | 2177 | 3035 | 1369 | 1679 | 867 | 1151 | |
| | 84788 | 84689 | 84263 | 83994 | 85865 | 84591 | 83733 | 85399 | 85026 | 85838 | 85544 | |
| | | | | | | | | | | | | |
| **A** | 0.01% | 0.00% | 99.99% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.96% | 99.97% | 99.97% | 0.00% | 0.00% | 99.99% | |
| **G** | 99.99% | 99.99% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | |
| **T** | 0.00% | 0.01% | 0.00% | 0.01% | 99.99% | 0.03% | 0.03% | 0.02% | 99.99% | 99.99% | 0.00% | |

**pNMG-249 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_NLS; A106V_D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 4 | 5 | 91853 | 91409 | 1 | 30 | 2 | 5 | 6 | 53 | 25 | |
| **C** | 1 | 12 | 1 | 18 | 9 | 92097 | 91130 | 93028 | 8 | 16 | 93184 | |
| **G** | 92421 | 92283 | 12 | 8 | 14 | 16 | 8 | 10 | 13 | 1 | 0 | |
| **T** | 7 | 2 | 4 | 17 | 93630 | 8 | 12 | 12 | 92708 | 93551 | 3 | |
| **N** | 2158 | 2289 | 2720 | 3138 | 933 | 2436 | 3435 | 1532 | 1777 | 891 | 1277 | |
| | 92433 | 92302 | 91870 | 91452 | 93654 | 92151 | 91152 | 93055 | 92735 | 93621 | 93212 | |
| | | | | | | | | | | | | |
| **A** | 0.00% | 0.01% | 99.98% | 99.95% | 0.00% | 0.03% | 0.00% | 0.01% | 0.01% | 0.06% | 0.03% | |
| **C** | 0.00% | 0.01% | 0.00% | 0.02% | 0.01% | 99.94% | 99.98% | 99.97% | 0.01% | 0.02% | 99.97% | |
| **G** | 99.99% | 99.98% | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | |
| **T** | 0.01% | 0.00% | 0.00% | 0.02% | 99.97% | 0.01% | 0.01% | 0.01% | 99.97% | 99.93% | 0.00% | |

**pNMG-250 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)**

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 7 | 4 | 91827 | 91416 | 4 | 13 | 2 | 6 | 8 | 43 | 17 | |
| **C** | 1 | 12 | 0 | 14 | 10 | 92101 | 91120 | 92940 | 2 | 25 | 93208 | |
| **G** | 92465 | 92319 | 8 | 7 | 0 | 6 | 3 | 6 | 8 | 0 | 2 | |
| **T** | 6 | 3 | 4 | 6 | 93601 | 29 | 20 | 21 | 92630 | 93524 | 28 | |

# FIGURE 45 (Continued)

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | 12 | 25 | 86137 | 98 | 195 | 84580 | 22 | 17 | 59 | 26 | 19 |
| 7 | 31 | 85902 | 2 | 77 | 85288 | 46 | 84287 | 84489 | 37 | 16 | 14 |
| 30 | 85193 | 2 | 22 | 85748 | 57 | 77 | 81 | 12 | 4 | 84634 | 83983 |
| 85547 | 17 | 16 | 9 | 26 | 104 | 185 | 68 | 17 | 84903 | 6 | 17 |
| 1085 | 1442 | 736 | 476 | 698 | 995 | 1509 | 1865 | 1664 | 1142 | 1378 | 2009 |
| 85610 | 85253 | 85945 | 86170 | 85949 | 85644 | 84888 | 84458 | 84535 | 85003 | 84682 | 84033 |
| 0.03% | 0.01% | 0.03% | 99.96% | 0.11% | 0.23% | 99.64% | 0.03% | 0.02% | 0.07% | 0.03% | 0.02% |
| 0.01% | 0.04% | 99.95% | 0.00% | 0.09% | 99.58% | 0.05% | 99.80% | 99.95% | 0.04% | 0.02% | 0.02% |
| 0.04% | 99.93% | 0.00% | 0.03% | 99.77% | 0.07% | 0.09% | 0.10% | 0.01% | 0.00% | 99.94% | 99.94% |
| 99.93% | 0.02% | 0.02% | 0.01% | 0.03% | 0.12% | 0.22% | 0.08% | 0.02% | 99.88% | 0.01% | 0.02% |
| 42 | 16 | 18 | 93732 | 36 | 165 | 91979 | 30 | 12 | 16 | 13 | 11 |
| 11 | 12 | 93560 | 4 | 48 | 92870 | 35 | 91638 | 91929 | 35 | 6 | 11 |
| 79 | 92742 | 12 | 8 | 93377 | 39 | 47 | 49 | 29 | 5 | 92244 | 91550 |
| 93157 | 84 | 42 | 11 | 24 | 107 | 205 | 117 | 52 | 92569 | 13 | 9 |
| 1196 | 1631 | 840 | 568 | 820 | 1098 | 1744 | 2109 | 1823 | 1196 | 1501 | 2196 |
| 93289 | 92854 | 93632 | 93755 | 93485 | 93181 | 92266 | 91834 | 92022 | 92625 | 92276 | 91581 |
| 0.05% | 0.02% | 0.02% | 99.98% | 0.04% | 0.18% | 99.69% | 0.03% | 0.01% | 0.02% | 0.01% | 0.01% |
| 0.01% | 0.01% | 99.92% | 0.00% | 0.05% | 99.67% | 0.04% | 99.79% | 99.90% | 0.04% | 0.01% | 0.01% |
| 0.08% | 99.88% | 0.01% | 0.01% | 99.88% | 0.04% | 0.05% | 0.05% | 0.03% | 0.01% | 99.97% | 99.97% |
| 99.86% | 0.09% | 0.04% | 0.01% | 0.03% | 0.11% | 0.22% | 0.13% | 0.06% | 99.94% | 0.01% | 0.01% |
| 34 | 25 | 41 | 93840 | 39 | 137 | 92123 | 50 | 57 | 33 | 31 | 18 |
| 12 | 34 | 93557 | 1 | 124 | 92976 | 63 | 91680 | 91772 | 40 | 11 | 14 |
| 39 | 92800 | 1 | 15 | 93441 | 9 | 72 | 72 | 28 | 11 | 91964 | 91293 |
| 93155 | 47 | 19 | 9 | 22 | 144 | 136 | 59 | 32 | 92461 | 13 | 12 |

# FIGURE 45 (Continued)

| | 1<br>G | 2<br>G | 3<br>A | 4<br>A | 5<br>T | 6<br>C | 7<br>C | 8<br>C | 9<br>T | 10<br>T | 11<br>C |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **N** | 2071 | 2212 | 2701 | 3097 | 925 | 2391 | 3395 | 1557 | 1853 | 908 | 1242 |
| | 92479 | 92338 | 91839 | 91443 | 93615 | 92149 | 91145 | 92973 | 92648 | 93592 | 93255 |
| **A** | 0.01% | 0.00% | 99.99% | 99.97% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.05% | 0.02% |
| **C** | 0.00% | 0.01% | 0.00% | 0.02% | 0.01% | 99.95% | 99.97% | 99.96% | 0.00% | 0.03% | 99.95% |
| **G** | 99.98% | 99.98% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| **T** | 0.01% | 0.00% | 0.00% | 0.01% | 99.99% | 0.03% | 0.02% | 0.02% | 99.98% | 99.93% | 0.03% |
| **pNMG-251 (pCMV_ecTadA_XTEN_Cas9 (wild-type)_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 9 | 11 | 128645 | 128041 | 1 | 6 | 6 | 10 | 8 | 4 | 8 |
| **C** | 2 | 4 | 2 | 8 | 6 | 128991 | 127660 | 130240 | 14 | 7 | 130645 |
| **G** | 129439 | 129205 | 10 | 10 | 0 | 5 | 2 | 0 | 4 | 0 | 4 |
| **T** | 6 | 9 | 11 | 7 | 131008 | 11 | 4 | 11 | 129897 | 131011 | 5 |
| **N** | 2751 | 2978 | 3539 | 4141 | 1192 | 3194 | 4535 | 1946 | 2273 | 1147 | 1496 |
| | 129456 | 129229 | 128668 | 128066 | 131015 | 129013 | 127672 | 130261 | 129923 | 131022 | 130662 |
| **A** | 0.01% | 0.01% | 99.98% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% | 99.99% | 99.98% | 0.01% | 0.01% | 99.99% |
| **G** | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 0.01% | 99.98% | 99.99% | 0.00% |
| **pNMG-181 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | |
| **A** | 15 | 11 | 164329 | 163299 | 3 | 59 | 25 | 26 | 9 | 12 | 12 |
| **C** | 2 | 3 | 0 | 6 | 17 | 164618 | 163058 | 166449 | 8 | 20 | 167016 |
| **G** | 165378 | 165048 | 24 | 128 | 0 | 158 | 1 | 4 | 0 | 0 | 1 |
| **T** | 7 | 11 | 14 | 8 | 167514 | 122 | 22 | 27 | 166022 | 167528 | 22 |
| **N** | 3841 | 4170 | 4876 | 5802 | 1709 | 4286 | 6137 | 2737 | 3204 | 1683 | 2192 |
| | 165402 | 165073 | 164367 | 163441 | 167534 | 164957 | 163106 | 166506 | 166039 | 167560 | 167051 |

FIGURE 45 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T | G | C | A | G | C | A | C | C | T | G | G |
| 1240 | 1554 | 853 | 548 | 786 | 1142 | 1633 | 2035 | 1843 | 1146 | 1518 | 2183 |
| 93240 | 92906 | 93618 | 93865 | 93626 | 93266 | 92394 | 91861 | 91889 | 92545 | 92019 | 91337 |
| 0.04% | 0.03% | 0.04% | 99.97% | 0.04% | 0.15% | 99.71% | 0.05% | 0.06% | 0.04% | 0.03% | 0.02% |
| 0.01% | 0.04% | 99.93% | 0.00% | 0.13% | 99.69% | 0.07% | 99.80% | 99.87% | 0.04% | 0.01% | 0.02% |
| 0.04% | 99.89% | 0.00% | 0.02% | 99.80% | 0.01% | 0.08% | 0.08% | 0.03% | 0.01% | 99.94% | 99.95% |
| 99.91% | 0.05% | 0.02% | 0.01% | 0.02% | 0.15% | 0.15% | 0.06% | 0.03% | 99.91% | 0.01% | 0.01% |
| 8 | 15 | 19 | 131332 | 21 | 51 | 129574 | 14 | 37 | 36 | 15 | 8 |
| 17 | 0 | 130990 | 4 | 32 | 130538 | 8 | 129156 | 129474 | 31 | 6 | 11 |
| 0 | 130179 | 7 | 13 | 131029 | 18 | 36 | 36 | 21 | 6 | 129842 | 129024 |
| 130627 | 2 | 29 | 25 | 1 | 41 | 99 | 46 | 4 | 130254 | 11 | 11 |
| 1506 | 1962 | 1101 | 738 | 1025 | 1455 | 2216 | 2640 | 2325 | 1517 | 1917 | 2733 |
| 130652 | 130196 | 131045 | 131374 | 131083 | 130648 | 129717 | 129252 | 129536 | 130327 | 129874 | 129054 |
| 0.01% | 0.01% | 0.01% | 99.97% | 0.02% | 0.04% | 99.89% | 0.01% | 0.03% | 0.03% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.96% | 0.00% | 0.02% | 99.92% | 0.01% | 99.93% | 99.95% | 0.02% | 0.00% | 0.01% |
| 0.00% | 99.99% | 0.01% | 0.01% | 99.96% | 0.01% | 0.03% | 0.03% | 0.02% | 0.00% | 99.98% | 99.98% |
| 99.98% | 0.00% | 0.02% | 0.02% | 0.00% | 0.03% | 0.08% | 0.04% | 0.00% | 99.94% | 0.01% | 0.01% |
| 8 | 9 | 14 | 168220 | 13 | 8 | 166022 | 10 | 9 | 6 | 18 | 15 |
| 19 | 0 | 167699 | 8 | 0 | 167183 | 4 | 165457 | 165883 | 22 | 2 | 5 |
| 3 | 166388 | 3 | 18 | 167787 | 3 | 4 | 0 | 0 | 4 | 166478 | 165284 |
| 167057 | 6 | 34 | 2 | 9 | 9 | 15 | 22 | 10 | 167128 | 11 | 3 |
| 2163 | 2847 | 1500 | 1002 | 1441 | 2047 | 3205 | 3761 | 3347 | 2089 | 2739 | 3941 |
| 167087 | 166403 | 167750 | 168248 | 167809 | 167203 | 166045 | 165489 | 165902 | 167160 | 166509 | 165307 |

FIGURE 45 (Continued)

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 0.01% | 0.01% | 99.98% | 99.91% | 0.00% | 0.04% | 0.02% | 0.02% | 0.01% | 0.01% | 0.01% | | | |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.79% | 99.97% | 99.97% | 0.00% | 0.01% | 99.98% | | | |
| **G** | 99.99% | 99.98% | 0.01% | 0.08% | 0.00% | 0.10% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | | | |
| **T** | 0.00% | 0.01% | 0.01% | 0.00% | 99.99% | 0.07% | 0.01% | 0.02% | 99.99% | 99.98% | 0.01% | | | |
| **pNMG-182 (pCMV_ecTadA_GGS_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | | | | |
| **A** | 12 | 2 | 151143 | 150219 | 16 | 140 | 9 | 15 | 9 | 11 | 18 | | | |
| **C** | 0 | 2 | 3 | 7 | 12 | 150403 | 149987 | 152969 | 10 | 15 | 153494 | | | |
| **G** | 152081 | 151862 | 31 | 241 | 1 | 358 | 1 | 18 | 3 | 1 | 0 | | | |
| **T** | 14 | 9 | 6 | 13 | 153898 | 819 | 38 | 31 | 152608 | 154008 | 20 | | | |
| **N** | 3335 | 3567 | 4259 | 4962 | 1515 | 3722 | 5407 | 2409 | 2812 | 1407 | 1910 | | | |
| | 152107 | 151875 | 151183 | 150480 | 153927 | 151720 | 150035 | 153033 | 152630 | 154035 | 153532 | | | |
| **A** | 0.01% | 0.00% | 99.97% | 99.83% | 0.01% | 0.09% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | | | |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.13% | 99.97% | 99.96% | 0.01% | 0.01% | 99.98% | | | |
| **G** | 99.98% | 99.99% | 0.02% | 0.16% | 0.00% | 0.24% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | | | |
| **T** | 0.01% | 0.01% | 0.00% | 0.01% | 99.98% | 0.54% | 0.03% | 0.02% | 99.99% | 99.98% | 0.01% | | | |
| **pNMG-183 (pCMV_ecTadA_(GGS)2-XTEN-(GGS)2_Cas9n_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | | | | | | |
| **A** | 10 | 12 | 145434 | 145694 | 6 | 35 | 20 | 9 | 15 | 13 | 3 | | | |
| **C** | 4 | 5 | 2 | 7 | 13 | 145072 | 144136 | 147363 | 6 | 5 | 147768 | | | |
| **G** | 146883 | 146528 | 42 | 175 | 0 | 295 | 2 | 0 | 1 | 2 | 2 | | | |
| **T** | 9 | 6 | 15 | 10 | 148624 | 540 | 54 | 56 | 147267 | 148551 | 21 | | | |
| **N** | 2910 | 3274 | 4332 | 3939 | 1182 | 3883 | 5613 | 2397 | 2536 | 1254 | 2031 | | | |
| | 146906 | 146551 | 145493 | 145886 | 148643 | 145942 | 144212 | 147428 | 147289 | 148571 | 147794 | | | |
| **A** | 0.01% | 0.01% | 99.96% | 99.87% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | | | |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.40% | 99.95% | 99.96% | 0.00% | 0.00% | 99.98% | | | |

FIGURE 45 (Continued)

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 99.99% | 0.01% | 0.00% | 0.00% | 0.00% |
| 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% | |
| 99.98% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% | |
| 7 | 11 | 12 | 154540 | 10 | 10 | 152712 | 8 | 11 | 6 | 14 | 9 | |
| 25 | 6 | 154103 | 1 | 1 | 153674 | 3 | 152151 | 152604 | 29 | 3 | 3 | |
| 0 | 152994 | 1 | 13 | 154191 | 0 | 10 | 1 | 0 | 0 | 153067 | 152096 | |
| 153567 | 10 | 33 | 2 | 14 | 12 | 19 | 17 | 13 | 153617 | 9 | 10 | |
| 1843 | 2421 | 1298 | 891 | 1231 | 1751 | 2703 | 3270 | 2819 | 1795 | 2354 | 3329 | |
| 153599 | 153021 | 154149 | 154556 | 154216 | 153696 | 152744 | 152177 | 152628 | 153652 | 153093 | 152118 | |
| 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | |
| 0.02% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% | 99.98% | 99.98% | 0.02% | 0.00% | 0.00% | |
| 0.00% | 99.98% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% | |
| 99.98% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | |
| 10 | 14 | 6 | 148953 | 13 | 4 | 147350 | 2 | 1 | 7 | 11 | 9 | |
| 12 | 1 | 148700 | 2 | 2 | 148131 | 3 | 146358 | 146682 | 34 | 2 | 2 | |
| 0 | 147782 | 1 | 25 | 148333 | 1 | 11 | 2 | 1 | 1 | 147075 | 145652 | |
| 148071 | 11 | 33 | 2 | 8 | 20 | 8 | 7 | 6 | 147866 | 9 | 4 | |
| 1732 | 2017 | 1095 | 853 | 1479 | 1679 | 2463 | 3466 | 3145 | 1927 | 2738 | 4167 | |
| 148093 | 147808 | 148740 | 148982 | 148356 | 148156 | 147372 | 146369 | 146690 | 147908 | 147097 | 145667 | |
| 0.01% | 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.99% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | |
| 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.98% | 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% | |

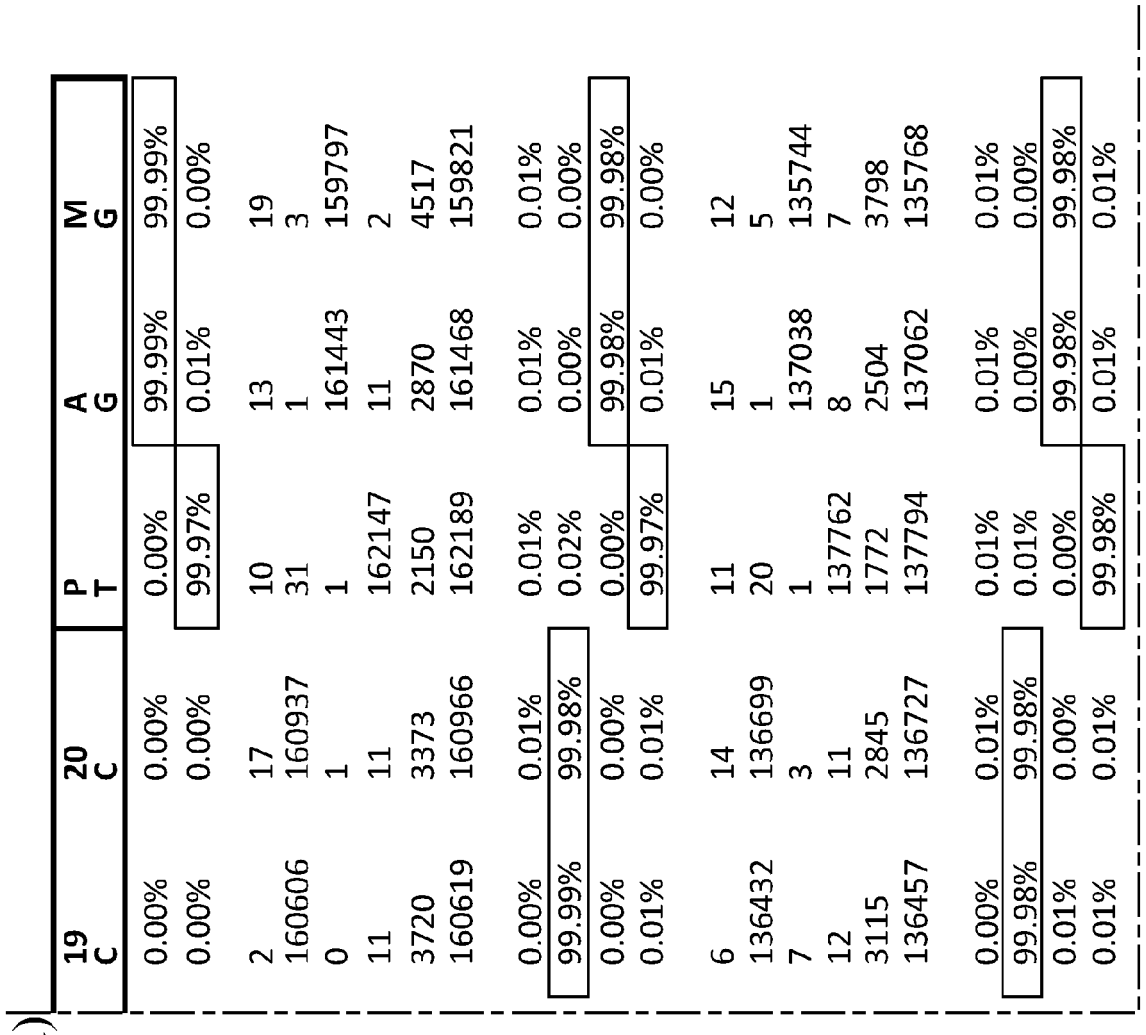
FIGURE 45 (Continued)

| | 1<br>G | 2<br>G | 3<br>A | 4<br>A | 5<br>T | 6<br>C | 7<br>C | 8<br>C | 9<br>T |
|---|---|---|---|---|---|---|---|---|---|
| G | 99.98% | 99.98% | 0.03% | 0.12% | 0.00% | 0.20% | 0.00% | 0.00% | 0.00% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.37% | 0.04% | 0.04% | 99.99% |
| **pNMG-178 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; D108N_D147Y_E155V)** | | | | | | | | | |
| A | 22 | 8 | 159239 | 159769 | 6 | 39 | 10 | 12 | 17 |
| C | 0 | 3 | 2 | 7 | 13 | 159408 | 157977 | 161539 | 11 |
| G | 161097 | 160745 | 59 | 144 | 1 | 96 | 1 | 23 | 4 |
| T | 10 | 10 | 7 | 8 | 162959 | 541 | 21 | 20 | 161421 |
| N | 3210 | 3573 | 5032 | 4411 | 1360 | 4255 | 6330 | 2745 | 2886 |
| | 161129 | 160766 | 159307 | 159928 | 162979 | 160084 | 158009 | 161594 | 161453 |
| A | 0.01% | 0.00% | 99.96% | 99.90% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.58% | 99.98% | 99.97% | 0.01% |
| G | 99.98% | 99.99% | 0.04% | 0.09% | 0.00% | 0.06% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 0.01% | 0.00% | 0.01% | 99.99% | 0.34% | 0.01% | 0.01% | 99.98% |
| **pNMG-164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NS; D108N_D147Y_E155V)** | | | | | | | | | |
| A | 21 | 10 | 135460 | 135755 | 1 | 14 | 14 | 35 | 7 |
| C | 0 | 0 | 0 | 7 | 9 | 135620 | 134231 | 137165 | 7 |
| G | 136871 | 136513 | 30 | 55 | 0 | 220 | 4 | 20 | 2 |
| T | 6 | 4 | 6 | 5 | 138459 | 95 | 17 | 17 | 137123 |
| N | 2677 | 3048 | 4079 | 3753 | 1106 | 3626 | 5309 | 2338 | 2436 |
| | 136898 | 136527 | 135496 | 135822 | 138469 | 135949 | 134266 | 137237 | 137139 |
| A | 0.02% | 0.01% | 99.97% | 99.95% | 0.00% | 0.01% | 0.01% | 0.03% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.76% | 99.97% | 99.95% | 0.01% |
| G | 99.98% | 99.99% | 0.02% | 0.04% | 0.00% | 0.16% | 0.00% | 0.01% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 99.99% | 0.07% | 0.01% | 0.01% | 99.99% |

FIGURE 45 (Continued)

| 10 T | 11 C | 12 T | 13 G | 14 C | 15 A | 16 G | 17 C | 18 A |
|---|---|---|---|---|---|---|---|---|
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.02% | 99.98% | 0.00% | 0.01% |
| 99.99% | 0.01% | 99.99% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% |
| 8 | 9 | 10 | 9 | 12 | 163353 | 9 | 9 | 161513 |
| 7 | 162048 | 22 | 3 | 163094 | 7 | 2 | 162453 | 1 |
| 0 | 2 | 3 | 162103 | 7 | 18 | 162709 | 1 | 10 |
| 162959 | 12 | 162342 | 10 | 30 | 5 | 5 | 6 | 13 |
| 1365 | 2268 | 1962 | 2214 | 1196 | 956 | 1614 | 1870 | 2802 |
| 162974 | 162071 | 162377 | 162125 | 163143 | 163383 | 162725 | 162469 | 161537 |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.01% | 99.99% |
| 0.00% | 99.99% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |
| 99.99% | 0.01% | 99.98% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% |
| 11 | 8 | 5 | 13 | 2 | 138809 | 8 | 10 | 137213 |
| 9 | 137692 | 16 | 1 | 138528 | 1 | 2 | 137869 | 2 |
| 1 | 8 | 0 | 137732 | 3 | 11 | 138187 | 1 | 8 |
| 138376 | 17 | 137950 | 12 | 27 | 0 | 8 | 12 | 6 |
| 1176 | 1848 | 1601 | 1814 | 1012 | 751 | 1367 | 1680 | 2343 |
| 138397 | 137725 | 137971 | 137758 | 138560 | 138821 | 138205 | 137892 | 137229 |
| 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.99% |
| 0.01% | 99.98% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 99.98% | 0.00% |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |
| 99.98% | 0.01% | 99.98% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 0.00% |

FIGURE 45 (Continued)



| 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|
| 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 0.00% | 0.00% | 99.97% | 0.01% | 0.00% |
| 2 | 17 | 10 | 13 | 19 |
| 160606 | 160937 | 31 | 1 | 3 |
| 0 | 1 | 1 | 161443 | 159797 |
| 11 | 11 | 162147 | 11 | 2 |
| 3720 | 3373 | 2150 | 2870 | 4517 |
| 160619 | 160966 | 162189 | 161468 | 159821 |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| 99.99% | 99.98% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98% | 99.98% |
| 0.01% | 0.01% | 99.97% | 0.01% | 0.00% |
| 6 | 14 | 11 | 15 | 12 |
| 136432 | 136699 | 20 | 1 | 5 |
| 7 | 3 | 1 | 137038 | 135744 |
| 12 | 11 | 137762 | 8 | 7 |
| 3115 | 2845 | 1772 | 2504 | 3798 |
| 136457 | 136727 | 137794 | 137062 | 135768 |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| 99.98% | 99.98% | 0.01% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 99.98% | 99.98% |
| 0.01% | 0.01% | 99.98% | 0.01% | 0.01% |

# FIGURE 45 (Continued)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **untreated cells** | | | | | | | | | |
| **A** | 20 | 7 | 160902 | 161306 | 10 | 4 | 6 | 10 | 9 |
| **C** | 0 | 4 | 2 | 6 | 12 | 161564 | 159532 | 163107 | 10 |
| **G** | 162525 | 162224 | 14 | 12 | 0 | 2 | 5 | 0 | 2 |
| **T** | 6 | 3 | 11 | 8 | 164523 | 12 | 18 | 6 | 163036 |
| **N** | 3320 | 3633 | 4942 | 4539 | 1326 | 4289 | 6310 | 2748 | 2814 |
| | 162551 | 162238 | 160929 | 161332 | 164545 | 161582 | 159561 | 163123 | 163057 |
| **A** | 0.01% | 0.00% | 99.98% | 99.98% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.99% | 99.98% | 99.99% | 0.01% |
| **G** | 99.98% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 99.99% |
| **NTC** | | | | | | | | | |
| **A** | 1 | 0 | 2947 | 2952 | 0 | 0 | 0 | 0 | 0 |
| **C** | 0 | 0 | 0 | 0 | 0 | 2838 | 2825 | 2867 | 1 |
| **G** | 2975 | 2981 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **T** | 0 | 0 | 2 | 0 | 3025 | 122 | 89 | 122 | 2992 |
| **N** | 64 | 59 | 91 | 88 | 15 | 80 | 126 | 51 | 47 |
| | 2976 | 2981 | 2949 | 2952 | 3025 | 2960 | 2914 | 2989 | 2993 |
| **A** | 0.03% | 0.00% | 99.93% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 95.88% | 96.95% | 95.92% | 0.03% |
| **G** | 99.97% | 100.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.07% | 0.00% | 100.00 | 4.12% | 3.05% | 4.08% | 99.97% |

FIGURE 45 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 5 | 10 | 7 | 7 | 5 | 164919 | 12 | 2 | 163033 |
| 7 | 163643 | 18 | 1 | 164590 | 1 | 2 | 163930 | 1 |
| 0 | 1 | 1 | 163598 | 3 | 16 | 164169 | 3 | 14 |
| 164518 | 5 | 163936 | 7 | 18 | 4 | 6 | 2 | 15 |
| 1341 | 2212 | 1909 | 2258 | 1256 | 932 | 1683 | 1935 | 2809 |
| 164530 | 163659 | 163962 | 163613 | 164616 | 164940 | 164189 | 163937 | 163063 |
| 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 0.01% | 0.00% | 99.98% |
| 0.00% | 99.99% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 100.00 % | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |
| 99.99% | 0.00% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| 0 | 45 | 0 | 1 | 0 | 3020 | 0 | 0 | 2992 |
| 0 | 2945 | 3 | 0 | 3007 | 0 | 0 | 2999 | 0 |
| 0 | 0 | 0 | 2992 | 0 | 0 | 3003 | 0 | 0 |
| 3015 | 0 | 3004 | 0 | 0 | 0 | 0 | 0 | 1 |
| 25 | 50 | 33 | 47 | 33 | 20 | 37 | 41 | 47 |
| 3015 | 2990 | 3007 | 2993 | 3007 | 3020 | 3003 | 2999 | 2993 |
| 0.00% | 1.51% | 0.00% | 0.03% | 0.00% | 100.00 % | 0.00% | 0.00% | 99.97% |
| 0.00% | 98.49% | 0.10% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.97% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% |
| 100.00 | 0.00% | 99.90% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% |

FIGURE 45 (Continued)

| | | | | | |
|---|---|---|---|---|---|
| 3 | 13 | 11 | 8 | 13 | |
| 162074 | 162358 | 34 | 1 | 1 | |
| 0 | 1 | 4 | 162842 | 161345 | |
| 18 | 5 | 163594 | 10 | 4 | |
| 3777 | 3495 | 2229 | 3011 | 4509 | |
| 162095 | 162377 | 163643 | 162861 | 161363 | |
| 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | |
| 99.99% | 99.99% | 0.02% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 99.99% | 99.99% | |
| 0.01% | 0.00% | 99.97% | 0.01% | 0.00% | |
| 0 | 0 | 0 | 0 | 2 | |
| 2962 | 2976 | 0 | 0 | 0 | |
| 0 | 0 | 0 | 2982 | 2960 | |
| 0 | 0 | 3009 | 0 | 0 | |
| 78 | 64 | 31 | 58 | 78 | |
| 2962 | 2976 | 3009 | 2982 | 2962 | |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.07% | |
| 100.00% | 100.00% | 0.00% | 0.00% | 0.00% | |
| 0.00% | 0.00% | 0.00% | 100.00% | 99.93% | |
| 0.00% | 0.00% | 100.00 | 0.00% | 0.00% | |

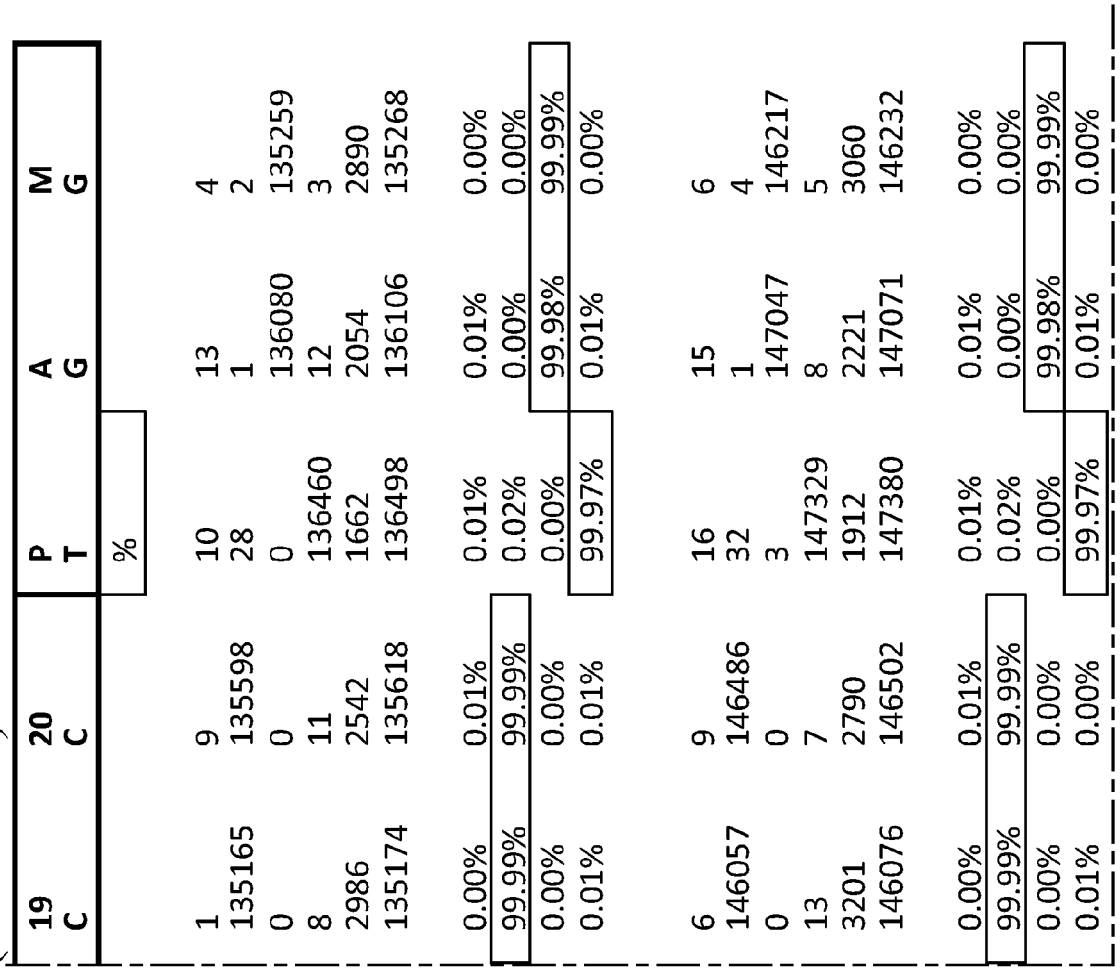
FIGURE 45 (Continued)

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| | G | G | A | A | T | C | C | C | T |
| | | | | | % | | | | |
| **pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)** | | | | | | | | | |
| **A** | 16 | 10 | 134439 | 134285 | 5 | 7 | 4 | 13 | 5 |
| **C** | 2 | 2 | 1 | 6 | 13 | 134694 | 133286 | 135996 | 7 |
| **G** | 135238 | 135052 | 14 | 7 | 0 | 2 | 1 | 0 | 2 |
| **T** | 8 | 5 | 13 | 11 | 136922 | 12 | 5 | 7 | 135988 |
| **N** | 2896 | 3091 | 3693 | 3851 | 1220 | 3445 | 4864 | 2144 | 2158 |
| | 135264 | 135069 | 134467 | 134309 | 136940 | 134715 | 133296 | 136016 | 136002 |
| **A** | 0.01% | 0.01% | 99.98% | 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.98% | 99.99% | 99.99% | 0.01% |
| **G** | 99.98% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| **T** | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 0.01% | 99.99% |
| **pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)** | | | | | | | | | |
| **A** | 11 | 11 | 145029 | 144697 | 0 | 83 | 18 | 7 | 7 |
| **C** | 1 | 2 | 0 | 3 | 11 | 144294 | 143805 | 146843 | 11 |
| **G** | 146159 | 145913 | 69 | 327 | 1 | 795 | 20 | 6 | 3 |
| **T** | 4 | 7 | 9 | 5 | 147990 | 267 | 44 | 27 | 146863 |
| **N** | 3105 | 3347 | 4173 | 4248 | 1278 | 3841 | 5393 | 2396 | 2394 |
| | 146175 | 145933 | 145107 | 145032 | 148002 | 145439 | 143887 | 146883 | 146884 |
| **A** | 0.01% | 0.01% | 99.95% | 99.77% | 0.00% | 0.06% | 0.01% | 0.00% | 0.00% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.21% | 99.94% | 99.97% | 0.01% |
| **G** | 99.99% | 99.99% | 0.05% | 0.23% | 0.00% | 0.55% | 0.01% | 0.00% | 0.00% |
| **T** | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.18% | 0.03% | 0.02% | 99.99% |

FIGURE 45 (Continued)

| 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|
| T | C | T | G | C | A | G | C | A |
| % | | | | | | | | |
| 8 | 8 | 12 | 10 | 8 | 137188 | 8 | 7 | 135529 |
| 7 | 136345 | 13 | 1 | 136982 | 2 | 1 | 136534 | 2 |
| 0 | 1 | 1 | 136168 | 0 | 13 | 136984 | 2 | 6 |
| 137196 | 7 | 136636 | 4 | 38 | 3 | 8 | 6 | 11 |
| 949 | 1799 | 1498 | 1977 | 1132 | 954 | 1159 | 1611 | 2612 |
| 137211 | 136361 | 136662 | 136183 | 137028 | 137206 | 137001 | 136549 | 135548 |
| 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 99.99% |
| 0.01% | 99.99% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.00% |
| 99.99% | 0.01% | 99.98% | 0.00% | 0.03% | 0.00% | 0.01% | 0.00% | 0.01% |
| 10 | 7 | 3 | 13 | 10 | 148249 | 10 | 3 | 146486 |
| 13 | 147360 | 20 | 4 | 148091 | 3 | 0 | 147571 | 4 |
| 0 | 0 | 1 | 147188 | 4 | 12 | 148038 | 2 | 8 |
| 148223 | 7 | 147571 | 7 | 25 | 2 | 8 | 13 | 9 |
| 1032 | 1904 | 1683 | 2066 | 1148 | 1012 | 1222 | 1689 | 2770 |
| 148246 | 147374 | 147595 | 147212 | 148130 | 148266 | 148056 | 147589 | 146507 |
| 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% | 99.99% |
| 0.01% | 99.99% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |
| 99.98% | 0.00% | 99.98% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% |

FIGURE 45 (Continued)



| 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|
| | | % | | |
| 1 | 9 | 10 | 13 | 4 |
| 135165 | 135598 | 28 | 1 | 2 |
| 0 | 0 | 0 | 136080 | 135259 |
| 8 | 11 | 136460 | 12 | 3 |
| 2986 | 2542 | 1662 | 2054 | 2890 |
| 135174 | 135618 | 136498 | 136106 | 135268 |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |
| 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98% | 99.99% |
| 0.01% | 0.01% | 99.97% | 0.01% | 0.00% |
| 6 | 9 | 16 | 15 | 6 |
| 146057 | 146486 | 32 | 1 | 4 |
| 0 | 0 | 3 | 147047 | 146217 |
| 13 | 7 | 147329 | 8 | 5 |
| 3201 | 2790 | 1912 | 2221 | 3060 |
| 146076 | 146502 | 147380 | 147071 | 146232 |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |
| 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.98% | 99.99% |
| 0.01% | 0.00% | 99.97% | 0.01% | 0.00% |

FIGURE 45 (Continued)

**pNMG-274 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); wild-type)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

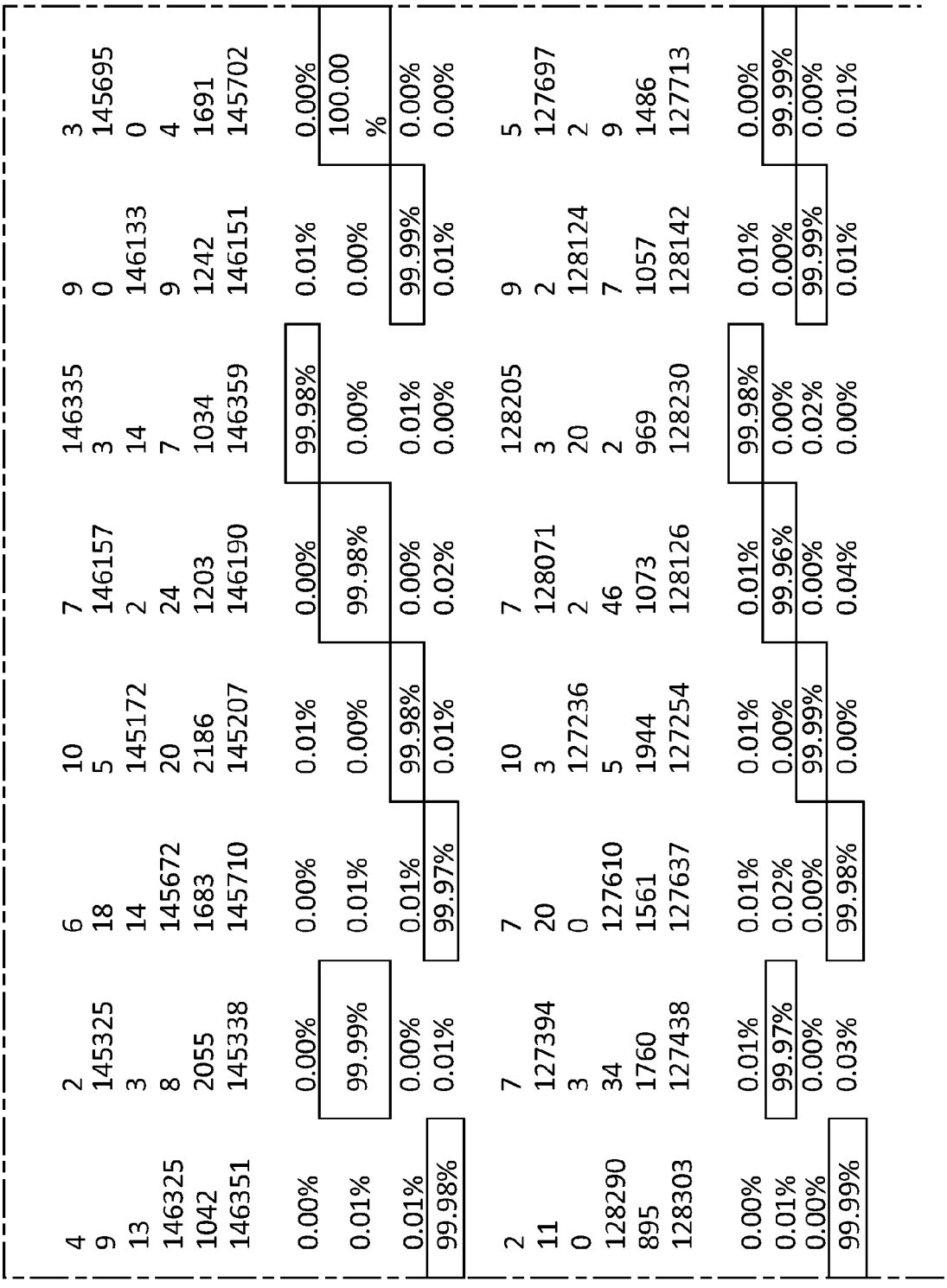
| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 16 | 8 | 143194 | 143263 | 3 | 43 | 14 | 17 | 6 |
| C | 2 | 0 | 1 | 9 | 17 | 143354 | 141952 | 144902 | 18 |
| G | 144269 | 143961 | 16 | 7 | 12 | 155 | 0 | 33 | 4 |
| T | 8 | 9 | 11 | 16 | 146007 | 64 | 24 | 32 | 145005 |
| N | 3098 | 3415 | 4171 | 4098 | 1354 | 3777 | 5403 | 2409 | 2360 |
| | 144295 | 143978 | 143222 | 143295 | 146039 | 143616 | 141990 | 144984 | 145033 |
| | | | | | | | | | |
| A | 0.01% | 0.01% | 99.98% | 99.98% | 0.00% | 0.03% | 0.01% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.82% | 99.97% | 99.94% | 0.01% |
| G | 99.98% | 99.99% | 0.01% | 0.00% | 0.01% | 0.11% | 0.00% | 0.02% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 99.98% | 0.04% | 0.02% | 0.02% | 99.98% |

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 5 | 13 | 125522 | 125373 | 4 | 6 | 6 | 11 | 5 |
| C | 1 | 4 | 0 | 7 | 6 | 125815 | 124408 | 126977 | 11 |
| G | 126417 | 126135 | 9 | 13 | 0 | 2 | 4 | 1 | 0 |
| T | 4 | 8 | 8 | 6 | 128092 | 56 | 61 | 56 | 127071 |
| N | 2771 | 3038 | 3659 | 3799 | 1096 | 3319 | 4719 | 2153 | 2111 |
| | 126427 | 126160 | 125539 | 125399 | 128102 | 125879 | 124479 | 127045 | 127087 |
| | | | | | | | | | |
| A | 0.00% | 0.01% | 99.99% | 99.98% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 99.95% | 99.94% | 99.95% | 0.01% |
| G | 99.99% | 99.98% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 99.99% | 0.04% | 0.05% | 0.04% | 99.99% |

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)**

FIGURE 45 (Continued)



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 4 | 2 | 6 | 10 | 7 | 146335 | 9 | 3 | |
| 9 | 145325 | 18 | 5 | 146157 | 3 | 0 | 145695 | |
| 13 | 3 | 14 | 145172 | 2 | 14 | 146133 | 0 | |
| 146325 | 8 | 145672 | 20 | 24 | 7 | 9 | 4 | |
| 1042 | 2055 | 1683 | 2186 | 1203 | 1034 | 1242 | 1691 | |
| 146351 | 145338 | 145710 | 145207 | 146190 | 146359 | 146151 | 145702 | |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | |
| 0.01% | 99.99% | 0.01% | 0.00% | 99.98% | 0.00% | 0.00% | 100.00% | |
| 0.01% | 0.00% | 0.01% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% | |
| 99.98% | 0.01% | 99.97% | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% | |
| 2 | 7 | 7 | 10 | 7 | 128205 | 9 | 5 | |
| 11 | 127394 | 20 | 3 | 128071 | 3 | 2 | 127697 | |
| 0 | 3 | 0 | 127236 | 2 | 20 | 128124 | 2 | |
| 128290 | 34 | 127610 | 5 | 46 | 2 | 7 | 9 | |
| 895 | 1760 | 1561 | 1944 | 1073 | 969 | 1057 | 1486 | |
| 128303 | 127438 | 127637 | 127254 | 128126 | 128230 | 128142 | 127713 | |
| 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% | |
| 0.01% | 99.97% | 0.02% | 0.00% | 99.96% | 0.00% | 0.00% | 99.99% | |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.02% | 99.99% | 0.00% | |
| 99.99% | 0.03% | 99.98% | 0.00% | 0.04% | 0.00% | 0.01% | 0.01% | |

FIGURE 45 (Continued)

| | | | | | |
|---|---|---|---|---|---|
| 144560 | 2 | 12 | 7 | 10 | 19 |
| 1 | 144150 | 144647 | 30 | 1 | 3 |
| 16 | 1 | 3 | 3 | 145158 | 144311 |
| 11 | 26 | 10 | 145488 | 5 | 7 |
| 2805 | 3214 | 2721 | 1865 | 2219 | 3052 |
| 144588 | 144179 | 144672 | 145528 | 145174 | 144340 |
| 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |
| 0.00% | 99.98% | 99.98% | 0.02% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.98% |
| 0.01% | 0.02% | 0.01% | 99.97% | 0.00% | 0.00% |
| 126732 | 6 | 9 | 6 | 10 | 12 |
| 2 | 126376 | 126671 | 22 | 2 | 3 |
| 6 | 2 | 1 | 2 | 127238 | 126421 |
| 6 | 12 | 5 | 127567 | 8 | 7 |
| 2453 | 2803 | 2513 | 1601 | 1940 | 2754 |
| 126746 | 126396 | 126686 | 127597 | 127258 | 126443 |
| 99.99% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |
| 0.00% | 99.98% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 99.98% |
| 0.00% | 0.01% | 0.00% | 99.98% | 0.01% | 0.01% |

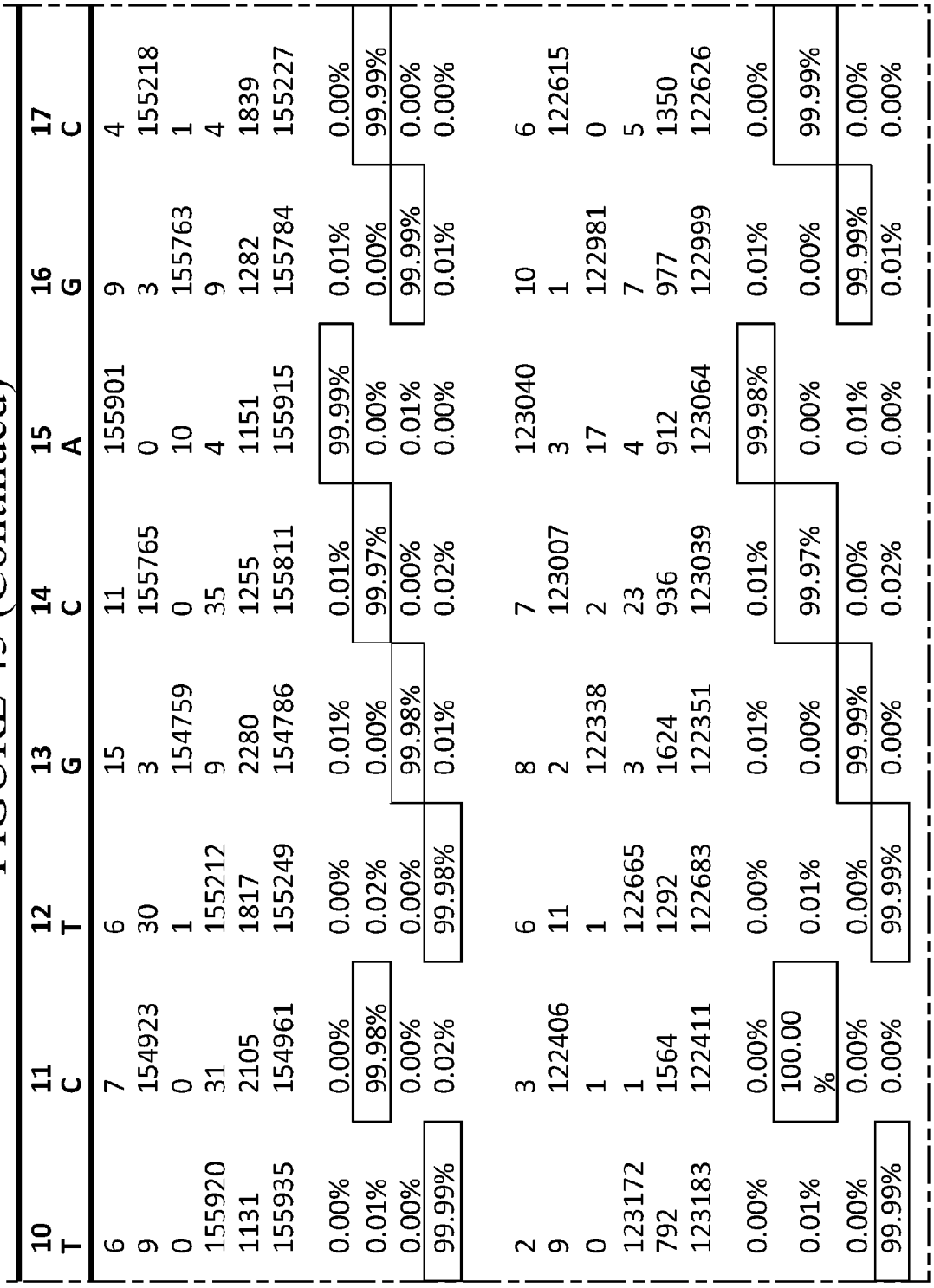
# FIGURE 45 (Continued)

| | 1<br>G | 2<br>G | 3<br>A | 4<br>A | 5<br>T | 6<br>C | 7<br>C | 8<br>C | 9<br>T |
|---|---|---|---|---|---|---|---|---|---|
| A | 12 | 8 | 152613 | 152304 | 4 | 52 | 14 | 15 | 17 |
| C | 2 | 2 | 1 | 3 | 10 | 152850 | 151443 | 154428 | 13 |
| G | 153665 | 153425 | 130 | 363 | 9 | 130 | 1 | 29 | 1 |
| T | 11 | 6 | 11 | 14 | 155689 | 93 | 18 | 34 | 154488 |
| N | 3376 | 3625 | 4311 | 4382 | 1354 | 3941 | 5590 | 2560 | 2547 |
| | 153690 | 153441 | 152755 | 152684 | 155712 | 153125 | 151476 | 154506 | 154519 |
| A | 0.01% | 0.01% | 99.91% | 99.75% | 0.00% | 0.03% | 0.01% | 0.01% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.82% | 99.98% | 99.95% | 0.01% |
| G | 99.98% | 99.99% | 0.09% | 0.24% | 0.01% | 0.08% | 0.00% | 0.02% | 0.00% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.06% | 0.01% | 0.02% | 99.98% |

**pNMG-275 (pCMV_ecTadA_GGS_NLS (no Cas9 fusion); A106V_D108N_D147Y_E155V)/ pNMG-142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 9 | 8 | 120729 | 120331 | 4 | 5 | 3 | 12 | 1 |
| C | 1 | 1 | 4 | 7 | 8 | 120981 | 119708 | 122065 | 7 |
| G | 121432 | 121186 | 8 | 256 | 0 | 35 | 4 | 0 | 2 |
| T | 6 | 4 | 10 | 4 | 122968 | 7 | 7 | 4 | 122081 |
| N | 2527 | 2776 | 3224 | 3377 | 995 | 2947 | 4253 | 1894 | 1884 |
| | 121448 | 121199 | 120751 | 120598 | 122980 | 121028 | 119722 | 122081 | 122091 |
| A | 0.01% | 0.01% | 99.98% | 99.78% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.96% | 99.99% | 99.99% | 0.01% |
| G | 99.99% | 99.99% | 0.01% | 0.21% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 0.00% | 99.99% |

FIGURE 45 (Continued)



| 10 T | 11 C | 12 T | 13 G | 14 C | 15 A | 16 G | 17 C |
|---|---|---|---|---|---|---|---|
| 6 | 7 | 6 | 15 | 11 | 155901 | 9 | 4 |
| 9 | 154923 | 30 | 3 | 155765 | 0 | 3 | 155218 |
| 0 | 0 | 1 | 154759 | 0 | 10 | 155763 | 1 |
| 155920 | 31 | 155212 | 9 | 35 | 4 | 9 | 4 |
| 1131 | 2105 | 1817 | 2280 | 1255 | 1151 | 1282 | 1839 |
| 155935 | 154961 | 155249 | 154786 | 155811 | 155915 | 155784 | 155227 |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% |
| 0.01% | 99.98% | 0.02% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |
| 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 99.99% | 0.00% |
| 99.99% | 0.02% | 99.98% | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% |
| 2 | 3 | 6 | 8 | 7 | 123040 | 10 | 6 |
| 9 | 122406 | 11 | 2 | 123007 | 3 | 1 | 122615 |
| 0 | 1 | 1 | 122338 | 2 | 17 | 122981 | 0 |
| 123172 | 1 | 122665 | 3 | 23 | 4 | 7 | 5 |
| 792 | 1564 | 1292 | 1624 | 936 | 912 | 977 | 1350 |
| 123183 | 122411 | 122683 | 122351 | 123039 | 123064 | 122999 | 122626 |
| 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 99.98% | 0.01% | 0.00% |
| 0.01% | 100.00 % | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% |
| 99.99% | 0.00% | 99.99% | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% |

FIGURE 45 (Continued)

| 18 A | 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|---|
| 154133 | 4 | 10 | 6 | 12 | 12 |
| 4 | 153689 | 154073 | 37 | 2 | 2 |
| 10 | 1 | 1 | 2 | 154742 | 153854 |
| 14 | 9 | 6 | 155110 | 6 | 8 |
| 2905 | 3363 | 2976 | 1911 | 2304 | 3189 |
| 154161 | 153703 | 154090 | 155155 | 154762 | 153876 |
| 99.98% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |
| 0.00% | 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 0.01% | 0.01% | 0.00% | 99.97% | 0.00% | 0.01% |
| 121748 | 5 | 8 | 8 | 12 | 4 |
| 1 | 121391 | 121823 | 27 | 2 | 3 |
| 7 | 0 | 0 | 2 | 122296 | 121676 |
| 14 | 14 | 10 | 122537 | 4 | 6 |
| 2206 | 2566 | 2150 | 1417 | 1677 | 2301 |
| 121770 | 121410 | 121841 | 122574 | 122314 | 121689 |
| 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |
| 0.00% | 99.98% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.99% |
| 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.00% |

# FIGURE 45 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **pNMG-276 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (wild-type) + (A106V_D108N_D147Y_E155V))** | | | | | | | | |
| **A** | 22 | 12 | 163894 | 163602 | 3 | 48 | 9 | 23 |
| **C** | 2 | 0 | 3 | 4 | 13 | 163868 | 162116 | 165529 |
| **G** | 164842 | 164627 | 11 | 11 | 0 | 143 | 23 | 24 |
| **T** | 7 | 10 | 18 | 14 | 167081 | 257 | 173 | 176 |
| **N** | 3691 | 3915 | 4638 | 4933 | 1467 | 4248 | 6243 | 2810 |
| | 164873 | 164649 | 163926 | 163631 | 167097 | 164316 | 162321 | 165752 |
| | | | | | | | | |
| **A** | 0.01% | 0.01% | 99.98% | 99.98% | 0.00% | 0.03% | 0.01% | 0.01% |
| **C** | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 99.73% | 99.87% | 99.87% |
| **G** | 99.98% | 99.99% | 0.01% | 0.01% | 0.00% | 0.09% | 0.01% | 0.01% |
| **T** | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.16% | 0.11% | 0.11% |
| **pNMG-277 (pCMV_ecTadA-(GGS)2-XTEN-(GGS)2_ecTadA_XTEN_nCas9_GGS_NLS; (A106V_D108N_D147Y_E155V) + (A106V_D108N_D147Y_E155V))** | | | | | | | | |
| **A** | 11 | 7 | 150677 | 150090 | 3 | 143 | 6 | 8 |
| **C** | 0 | 4 | 3 | 10 | 24 | 150472 | 149374 | 152535 |
| **G** | 151823 | 151585 | 120 | 640 | 0 | 441 | 2 | 9 |
| **T** | 7 | 11 | 12 | 5 | 153705 | 161 | 46 | 37 |
| **N** | 3277 | 3511 | 4306 | 4373 | 1386 | 3901 | 5690 | 2527 |
| | 151841 | 151607 | 150812 | 150745 | 153732 | 151217 | 149428 | 152589 |
| | | | | | | | | |
| **A** | 0.01% | 0.00% | 99.91% | 99.57% | 0.00% | 0.09% | 0.00% | 0.01% |
| **C** | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 99.51% | 99.96% | 99.96% |
| **G** | 99.99% | 99.99% | 0.08% | 0.42% | 0.00% | 0.29% | 0.00% | 0.01% |
| **T** | 0.00% | 0.01% | 0.01% | 0.00% | 99.98% | 0.11% | 0.03% | 0.02% |
| **untreated cells** | | | | | | | | |
| **A** | 11 | 7 | 134526 | 107403 | 1 | 14 | 5 | 6 |
| **C** | 4 | 2 | 4 | 8 | 7 | 146613 | 147145 | 152872 |

FIGURE 45 (Continued)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 10 | 7 | 16 | 8 | 13 | 13 | 167207 | 13 | 8 |
| 6 | 7 | 166079 | 24 | 2 | 167035 | 5 | 3 | 166517 |
| 0 | 0 | 10 | 2 | 166134 | 12 | 14 | 167124 | 1 |
| 165848 | 167317 | 77 | 166532 | 9 | 40 | 4 | 16 | 10 |
| 2698 | 1224 | 2373 | 1989 | 2397 | 1455 | 1325 | 1399 | 2019 |
| 165864 | 167331 | 166182 | 166566 | 166158 | 167100 | 167230 | 167156 | 166536 |
| 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.00% |
| 0.00% | 0.00% | 99.94% | 0.01% | 0.00% | 99.96% | 0.00% | 0.00% | 99.99% |
| 0.00% | 0.00% | 0.01% | 0.00% | 99.99% | 0.01% | 0.01% | 99.98% | 0.00% |
| 99.99% | 99.99% | 0.05% | 99.98% | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% |
| 10 | 6 | 7 | 8 | 12 | 11 | 153952 | 23 | 10 |
| 12 | 6 | 153006 | 18 | 3 | 153832 | 5 | 1 | 153273 |
| 7 | 0 | 0 | 2 | 152831 | 0 | 11 | 153788 | 0 |
| 152606 | 154015 | 20 | 153303 | 8 | 34 | 5 | 11 | 7 |
| 2481 | 1089 | 2083 | 1786 | 2263 | 1240 | 1144 | 1294 | 1827 |
| 152635 | 154027 | 153033 | 153331 | 152854 | 153877 | 153973 | 153823 | 153290 |
| 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% |
| 0.01% | 0.00% | 99.98% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% |
| 0.00% | 0.00% | 0.00% | 0.00% | 99.98% | 0.00% | 0.01% | 99.98% | 0.00% |
| 99.98% | 99.99% | 0.01% | 99.98% | 0.01% | 0.02% | 0.00% | 0.01% | 0.00% |
| 10 | 12 | 11 | 8 | 11 | 6 | 155150 | 7 | 10 |
| 8 | 7 | 153951 | 13 | 1 | 154809 | 2 | 0 | 154281 |

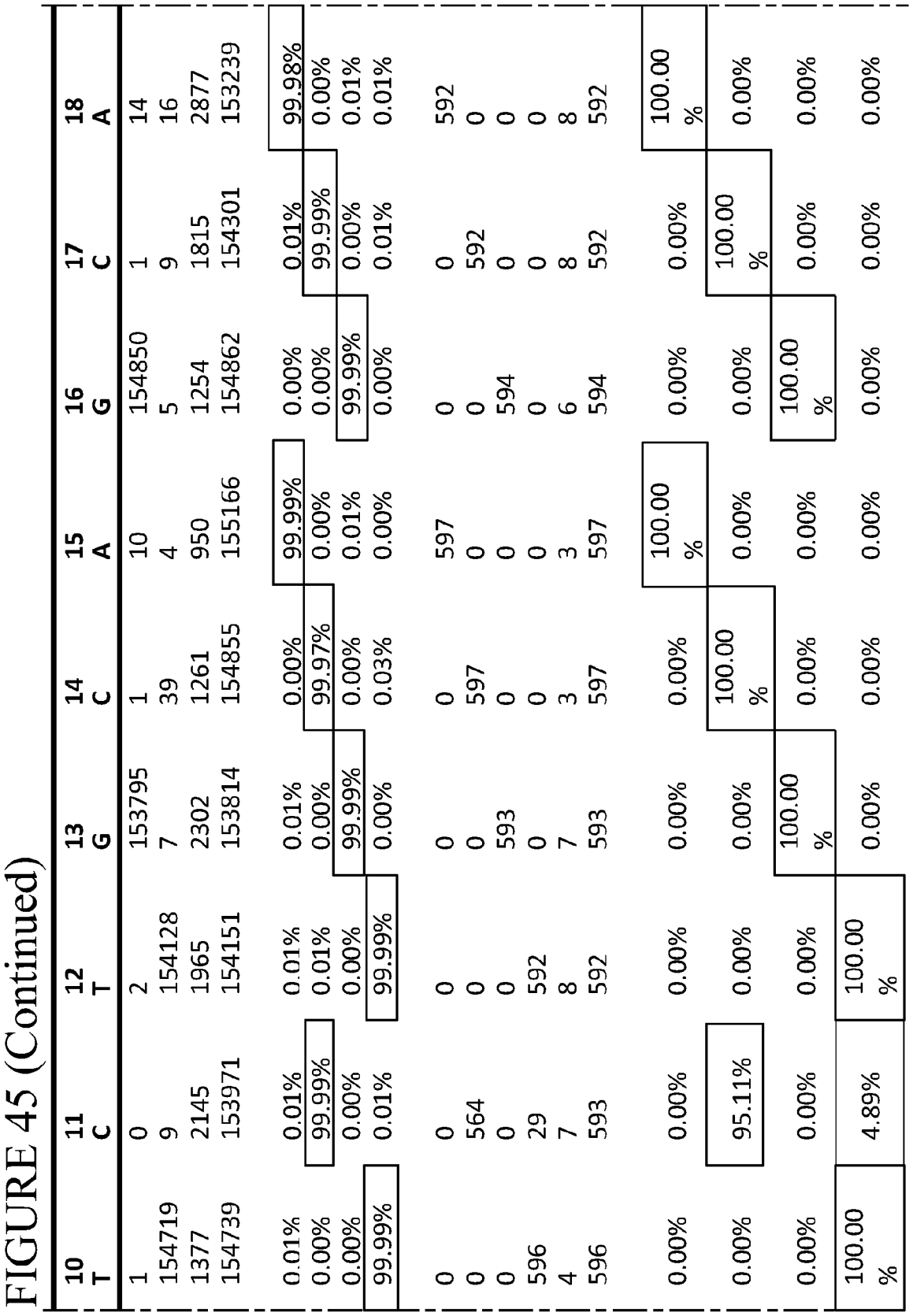
FIGURE 45 (Continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| 165242 | 3 | 17 | 11 | 13 | 18 | |
| 5 | 164757 | 165271 | 37 | 3 | 7 | |
| 10 | 1 | 1 | 2 | 165978 | 165079 | |
| 21 | 22 | 10 | 166427 | 8 | 4 | |
| 3277 | 3772 | 3256 | 2077 | 2551 | 3445 | |
| 165278 | 164783 | 165299 | 166477 | 166002 | 165108 | |
| 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | |
| 0.00% | 99.98% | 99.98% | 0.02% | 0.00% | 0.00% | |
| 0.01% | 0.00% | 0.00% | 0.00% | 99.99% | 99.98% | |
| 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.00% | |
| 152221 | 6 | 13 | 13 | 17 | 9 | |
| 4 | 151823 | 152154 | 28 | 4 | 0 | |
| 14 | 0 | 2 | 2 | 152812 | 151949 | |
| 8 | 13 | 18 | 153222 | 6 | 5 | |
| 2870 | 3275 | 2930 | 1852 | 2278 | 3154 | |
| 152247 | 151842 | 152187 | 153265 | 152839 | 151963 | |
| 99.98% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | |
| 0.00% | 99.99% | 99.98% | 0.02% | 0.00% | 0.00% | |
| 0.01% | 0.00% | 0.00% | 0.00% | 99.98% | 99.99% | |
| 0.01% | 0.01% | 0.01% | 99.97% | 0.00% | 0.00% | |
| 153206 | 5 | 6 | 12 | 11 | 18 | |
| 3 | 152824 | 153186 | 29 | 3 | 2 | |

# FIGURE 45 (Continued)

| | 1<br>G | 2<br>G | 3<br>A | 4<br>A | 5<br>T | 6<br>C | 7<br>C | 8<br>C | 9<br>T |
|---|---|---|---|---|---|---|---|---|---|
| G | 152582 | 152466 | 8 | 7 | 1 | 2 | 1 | 0 | 1 |
| T | 7 | 2 | 15 | 6 | 146251 | 16 | 13 | 13 | 153223 |
| N | 3513 | 3640 | 21564 | 48693 | 9857 | 9472 | 8953 | 3226 | 2874 |
| | 152604 | 152477 | 134553 | 107424 | 146260 | 146645 | 147164 | 152891 | 153242 |
| A | 0.01% | 0.00% | 99.98% | 99.98% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 99.98% | 99.99% | 99.99% | 0.01% |
| G | 99.99% | 99.99% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.01% | 99.99% | 0.01% | 0.01% | 0.01% | 99.99% |
| **NTC** | | | | | | | | | |
| A | 0 | 0 | 534 | 434 | 0 | 0 | 0 | 29 | 0 |
| C | 0 | 0 | 0 | 0 | 0 | 540 | 573 | 561 | 0 |
| G | 587 | 588 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T | 0 | 0 | 0 | 0 | 569 | 30 | 0 | 0 | 593 |
| N | 13 | 12 | 66 | 166 | 31 | 30 | 27 | 10 | 7 |
| | 587 | 588 | 534 | 434 | 569 | 570 | 573 | 590 | 593 |
| A | 0.00% | 0.00% | 100.00 % | 100.00% | 0.00% | 0.00% | 0.00% | 4.92% | 0.00% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 94.74% | 100.00 % | 95.08% | 0.00% |
| G | 100.00 % | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 5.26% | 0.00% | 0.00% | 100.00 % |

FIGURE 45 (Continued)



| 10 T | 11 C | 12 T | 13 G | 14 C | 15 A | 16 G | 17 C | 18 A |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 2 | 153795 | 1 | 10 | 154850 | 1 | 14 |
| 154719 | 9 | 154128 | 7 | 39 | 4 | 5 | 9 | 16 |
| 1377 | 2145 | 1965 | 2302 | 1261 | 950 | 1254 | 1815 | 2877 |
| 154739 | 153971 | 154151 | 153814 | 154855 | 155166 | 154862 | 154301 | 153239 |
| 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 99.99% | 0.00% | 0.01% | 99.98% |
| 0.00% | 99.99% | 0.01% | 0.00% | 99.97% | 0.00% | 0.00% | 99.99% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.99% | 0.00% | 0.01% | 99.99% | 0.00% | 0.01% |
| 99.99% | 0.01% | 99.99% | 0.00% | 0.03% | 0.00% | 0.00% | 0.01% | 0.01% |
| 0 | 0 | 0 | 0 | 0 | 597 | 0 | 0 | 592 |
| 0 | 564 | 0 | 0 | 597 | 0 | 0 | 592 | 0 |
| 0 | 0 | 0 | 593 | 0 | 0 | 594 | 0 | 0 |
| 596 | 29 | 592 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | 7 | 8 | 7 | 3 | 3 | 6 | 8 | 8 |
| 596 | 593 | 592 | 593 | 597 | 597 | 594 | 592 | 592 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % |
| 0.00% | 95.11% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% |
| 0.00% | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% | 100.00 % | 0.00% | 0.00% |
| 100.00 % | 4.89% | 100.00 % | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

FIGURE 45 (Continued)

| 19 C | 20 C | P T | A G | M G |
|---|---|---|---|---|
| 1 | 1 | 1 | 153592 | 152537 |
| 11 | 3 | 154127 | 8 | 5 |
| 3275 | 2920 | 1947 | 2502 | 3553 |
| 152841 | 153196 | 154169 | 153614 | 152562 |
| 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| 99.99% | 99.99% | 0.02% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 99.99% | 99.98% |
| 0.01% | 0.00% | 99.97% | 0.01% | 0.00% |
| 0 | 0 | 0 | 0 | 0 |
| 586 | 587 | 1 | 0 | 0 |
| 0 | 0 | 0 | 590 | 590 |
| 0 | 0 | 590 | 0 | 0 |
| 14 | 13 | 9 | 10 | 10 |
| 586 | 587 | 591 | 590 | 590 |
| 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| 100.00% | 100.00% | 0.17% | 0.00% | 0.00% |
| 0.00% | 0.00% | 0.00% | 100.00% | 100.00% |
| 0.00% | 0.00% | 99.83% | 0.00% | 0.00% |

FIGURE 46

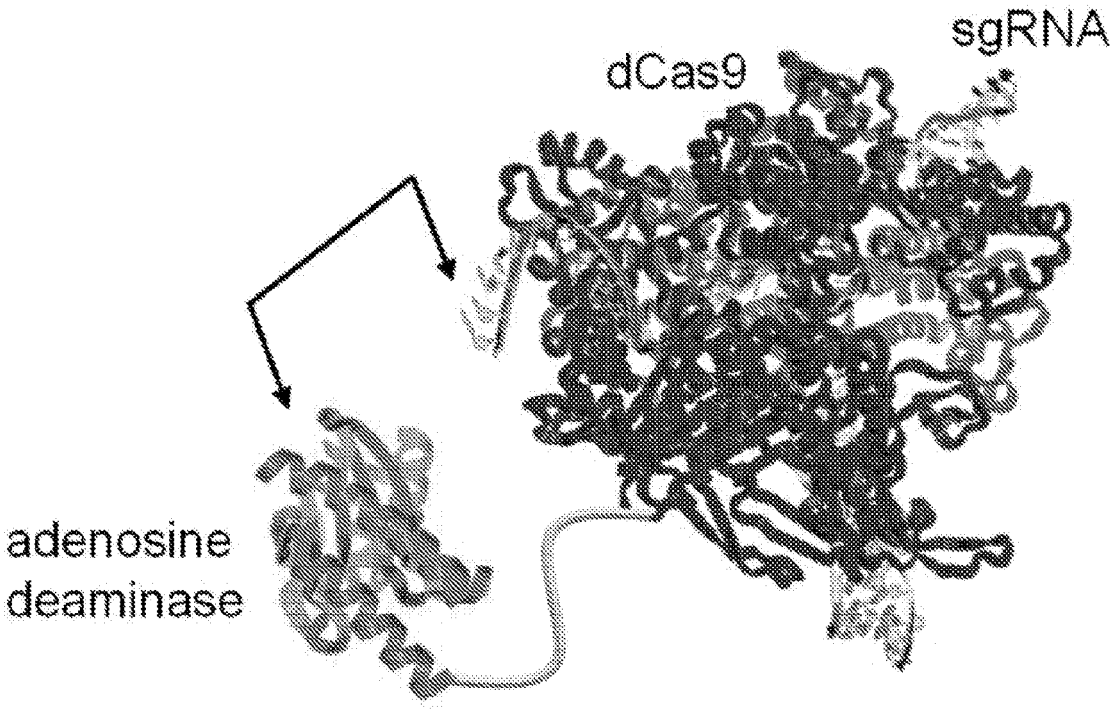



FIGURE 47

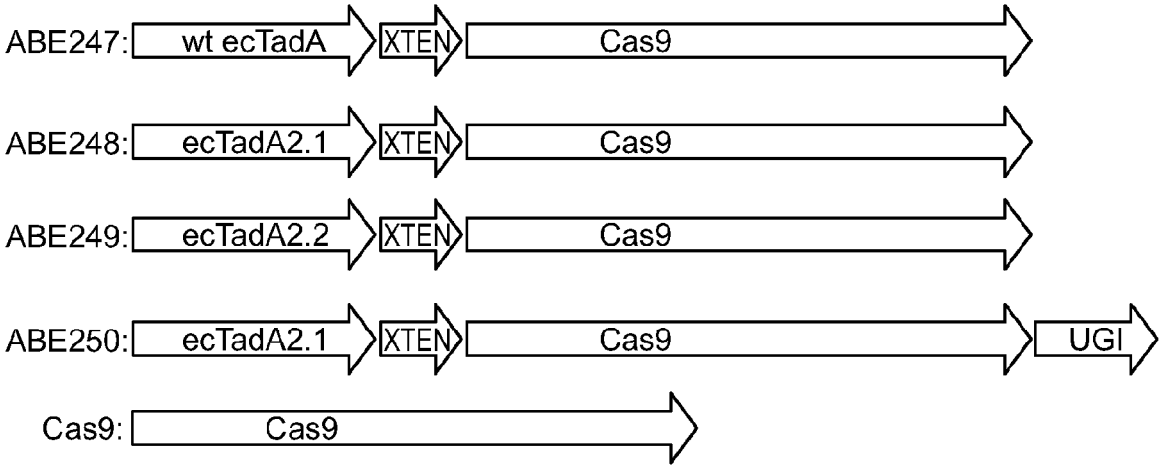


FIGURE 48

| | EMX1 | FANCF | HEK2 | HEK3 | HEK4 | RNF2 |
|---|---|---|---|---|---|---|
| wtCas9 | 35.4% | 37.0% | 32.9% | 56.8% | 23.8% | 33.8% |
| ABE247 | 25.5% | 30.2% | 45.7% | 76.3% | 36.5% | 26.8% |
| ABE248 | 24.8% | 26.6% | 39.8% | 64.2% | 35.1% | 26.5% |
| ABE249 | 30.2% | 28.6% | 42.0% | 66.7% | 40.0% | 27.1% |
| ABE250 | 25.0% | 25.2% | 31.3% | 56.3% | 36.4% | 25.2% |

FIGURE 49

protospacer PAM

EMX1: $GA_2GTCCGA_8GCAGAAGAAGAGGG$

FANCF: $GGA_3A_4TCCCTTCTGCAGCACCTGG$

HEK293 site 2: $GA_2A_3CA_5CA_7A_8A_9GCATAGACTGCGGG$

HEK293 site 3: $GGCCCA_6GA_8CTGAGCACGTGATGG$

HEK293 site 4: $GGCA_4CTGCGGCTGGAGGTCCGGG$

RNF2: $GTCA_4TCTTA_9GTCATTACCTGAGG$

FIGURE 50

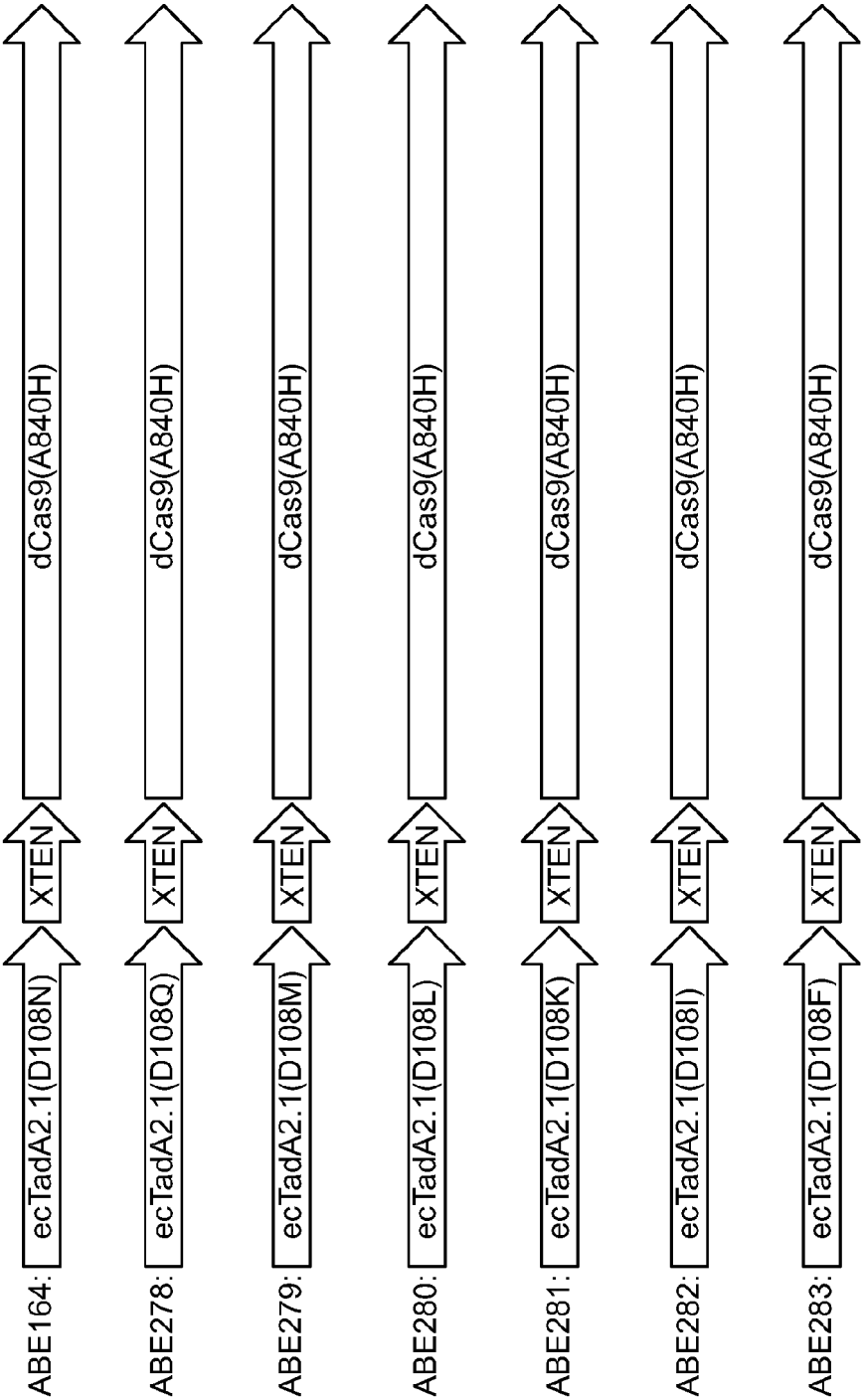

ABE164:
ecTadA2.1(D108N)
XTEN
dCas9(A840H)
ABE278:
ecTadA2.1(D108Q)
XTEN
dCas9(A840H)
ABE279:
ecTadA2.1(D108M)
XTEN
dCas9(A840H)
ABE280:
ecTadA2.1(D108L)
XTEN
dCas9(A840H)
ABE281:
ecTadA2.1(D108K)
XTEN
dCas9(A840H)
ABE282:
ecTadA2.1(D108I)
XTEN
dCas9(A840H)
ABE283:
ecTadA2.1(D108F)
XTEN
dCas9(A840H)

FIGURE 51

HEK293 site 2: $GA_2A_3CA_5CA_7A_8A_9GCATAGACTGCGGG$ (see high editing at A-5)

EMX1: $GA_2GTCCGA_8GCAGAAGAAGAAGGG$ (see no editing)

HEK293 site 3: $GGCCCA_6GA_8CTGAGCACGTGATGG$ (see low editing)

# FIGURE 52

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| untreated | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D108N (pNMG-164; pCMV_ecTadA_XTEN_Cas9n_GGS_NLS) | A | 100.0 | 99.8 | 90.6 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | 9.4 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D108Q (pNMG-278; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 97.1 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 2.8 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 99.8 | 81.3 | 99.9 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | 18.7 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 52 (Continued)

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| D108L (pNMG-280; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 99.9 | 96.8 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 3.2 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| D108K (pNMG-281; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 99.9 | 97.1 | 99.9 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 2.9 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| D108I (pNMG-282; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 99.3 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.7 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| D108F (pNMG-283; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 98.1 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 1.9 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 53

| | | EMX1 | | HEK3 | |
|---|---|---|---|---|---|
| | | $A_2$ | $A_8$ | $A_6$ | $A_8$ |
| untreated | A | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| D108N (pNMG-164; pCMV_ecTadA_XTEN_Cas9n_GGS_NLS) | A | 100.0 | 100.0 | 99.8 | 99.4 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.2 | 0.6 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| D108Q (pNMG-278; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 99.9 | 99.8 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.1 | 0.2 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 99.6 | 99.5 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.4 | 0.5 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 53 (Continued)

| | | EMX1 | | HEK3 | |
|---|---|---|---|---|---|
| | | $A_2$ | $A_8$ | $A_6$ | $A_8$ |
| D108L (pNMG-280; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 99.9 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| D108K (pNMG-281; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 99.9 | 99.7 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.1 | 0.3 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| D108I (pNMG-282; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| D108F (pNMG-283; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 100.0 | 99.9 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.1 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 54

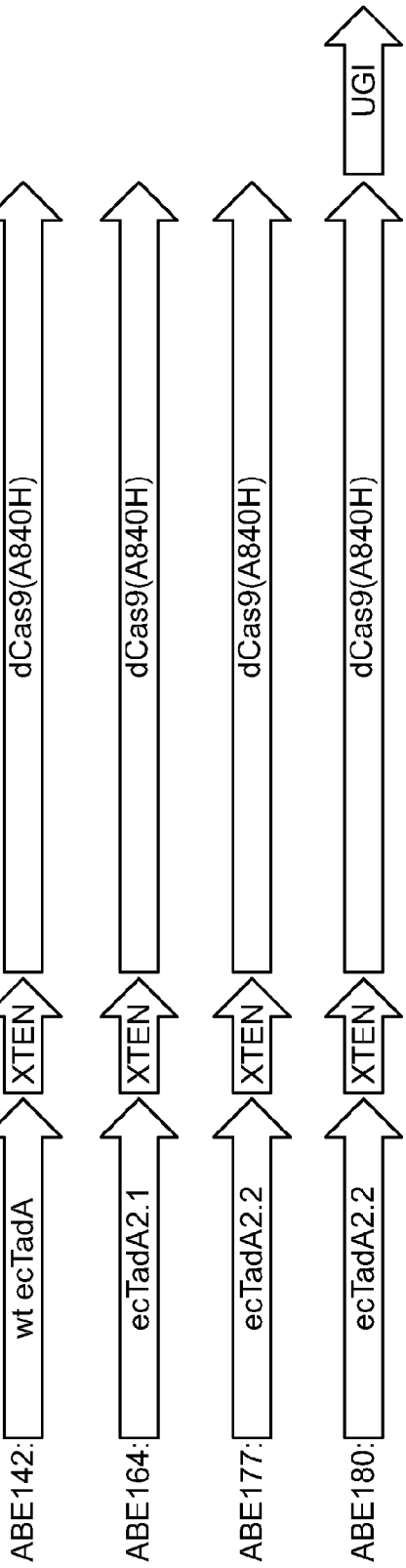


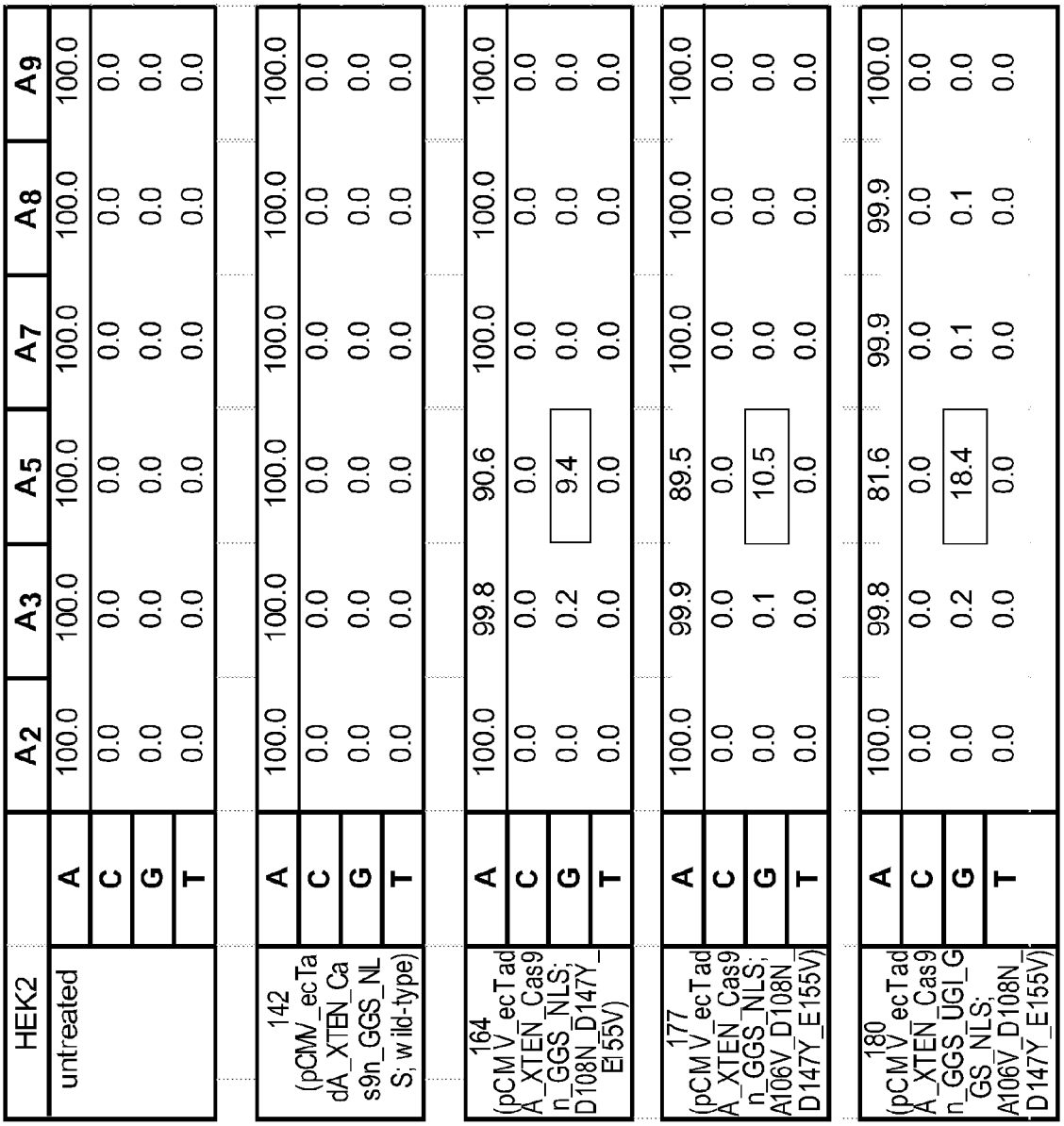

FIGURE 55

**HEK293 site 2:** $GA_2A_3CA_5CA_7A_8A_9GCATAGACTGCGGG$

**RNF2 multi-A:** $A_1GA_3A_4A_5A_6A_7CA_9A_{10}TTTTAGTATTTGG$

**HEK3 multi-A:** $GCA_3GA_5A_6A_7TA_9GA_{11}CTAATTGCATGG$

FIGURE 56



| HEK2 | | A2 | A3 | A5 | A7 | A8 | A9 |
|---|---|---|---|---|---|---|---|
| untreated | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V) | A | 100.0 | 99.8 | 90.6 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | 9.4 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.9 | 89.5 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 10.5 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.8 | 81.6 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | 18.4 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 57

| HEK3 | | A3 | A5 | A6 | A7 | A9 | A11 |
|---|---|---|---|---|---|---|---|
| untreated | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| 142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| 164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | 99.9 | 98.4 | 98.9 | 99.9 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 1.6 | 1.1 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | 100.0 | 98.4 | 98.8 | 99.8 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 1.6 | 1.2 | 0.2 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | A | 100.0 | 98.1 | 98.4 | 99.7 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 1.9 | 1.6 | 0.3 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 57
(Continued)

| RNF2 | | A1 | A3 | A4 | A5 | A6 | A7 | A9 | A10 |
|---|---|---|---|---|---|---|---|---|---|
| untreated | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 164 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

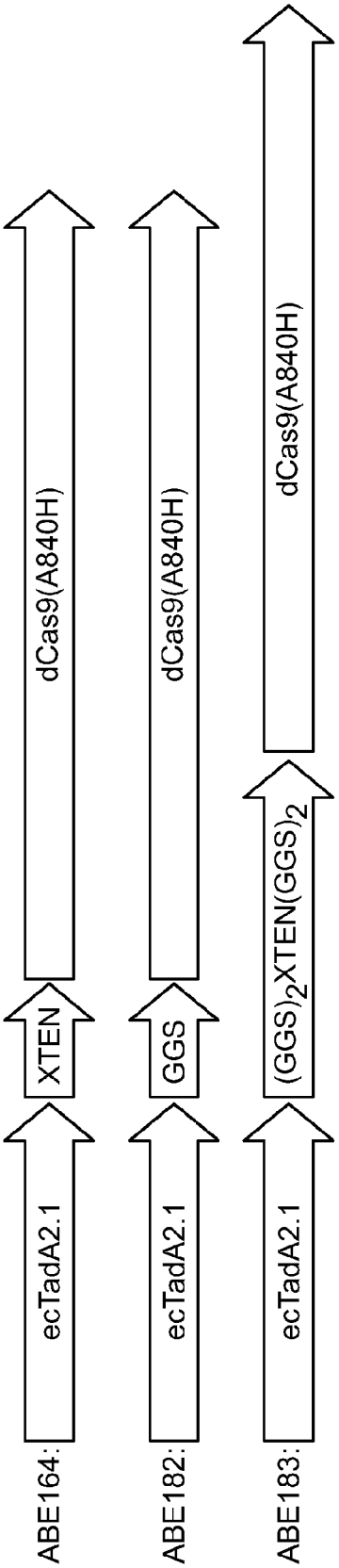

ABE164:
ecTadA2.1
XTEN
dCas9(A840H)
ABE182:
ecTadA2.1
GGS
dCas9(A840H)
ABE183:
ecTadA2.1
(GGS)2XTEN(GGS)2
dCas9(A840H)

FIGURE 58

# FIGURE 59

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| untreated | A | 100 | 100 | 100 | 100 | 100 | 100 |
| | C | 0 | 0 | 0 | 0 | 0 | 0 |
| | G | 0 | 0 | 0 | 0 | 0 | 0 |
| | T | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | |
| XTEN | A | 100 | 99.8 | 90.6 | 100 | 100 | 100 |
| | C | 0 | 0 | 0 | 0 | 0 | 0 |
| | G | 0 | 0.2 | 9.4 | 0 | 0 | 0 |
| | T | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 0 | 0 | 0 | 0 | 0 | 0 |
| GGS | A | 100 | 99.9 | 87.7 | 100 | 100 | 100 |
| | C | 0 | 0 | 0 | 0 | 0 | 0 |
| | G | 0 | 0.1 | 12.3 | 0 | 0 | 0 |
| | T | 0 | 0 | 0 | 0 | 0 | 0 |
| | | | | | | | |
| $(GGC)_2$XTEN $(GGS)_2$ | A | 100 | 99.9 | 77.6 | 100 | 100 | 100 |
| | C | 0 | 0 | 0 | 0 | 0 | 0 |
| | G | 0 | 0.1 | 22.4 | 0 | 0 | 0 |
| | T | 0 | 0 | 0 | 0 | 0 | 0 |

FIGURE 60

| | EMX1 | A2 | A8 | FANCF | A3 | A4 | HEK3 | A6 | A8 | HEK4 | A4 | RNF2 | A4 | A9 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| untreated | A | 100.0 | 100.0 | | 100.0 | 100.0 | | 100.0 | 100.0 | | 100 | | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0 | | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0 | | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0 | | 0.0 | 0.0 |
| XTEN (pNMG-164; pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; D108N_D147Y_E155V) | A | 100.0 | 100.0 | | 100.0 | 100.0 | | 99.8 | 99.4 | | 99.7 | | 99.9 | 99.9 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.2 | 0.6 | | 0.3 | | 0.0 | 0.1 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | | 0.0 | 0.0 |
| GGS (pNMG-182; pCMV_ecTadA_GGS_Cas9n_GGS_NLS; D108N_D147Y_E155V) | A | 100.0 | 100.0 | | 100.0 | 99.8 | | 99.7 | 99.8 | | 99.5 | | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | | 0.0 | 0.2 | | 0.2 | 0.2 | | 0.4 | | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | | 0.0 | 0.0 |
| $(GGS)_2$XTEN(GGS)2 (pNMG-183;pCMV_ecTadA_(GGS)2-XTEN-(GGS)2_Cas9n_GGS_NLS; D108N_D147Y_E155V) | A | 100.0 | 99.8 | | 100.0 | 99.9 | | 99.8 | 99.0 | | 99.4 | | 99.9 | 99.9 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | | 0.0 | 0.1 | | 0.2 | 1.0 | | 0.6 | | 0.1 | 0.1 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | 0.0 | | 0.0 | | 0.0 | 0.0 |

# FIGURE 61

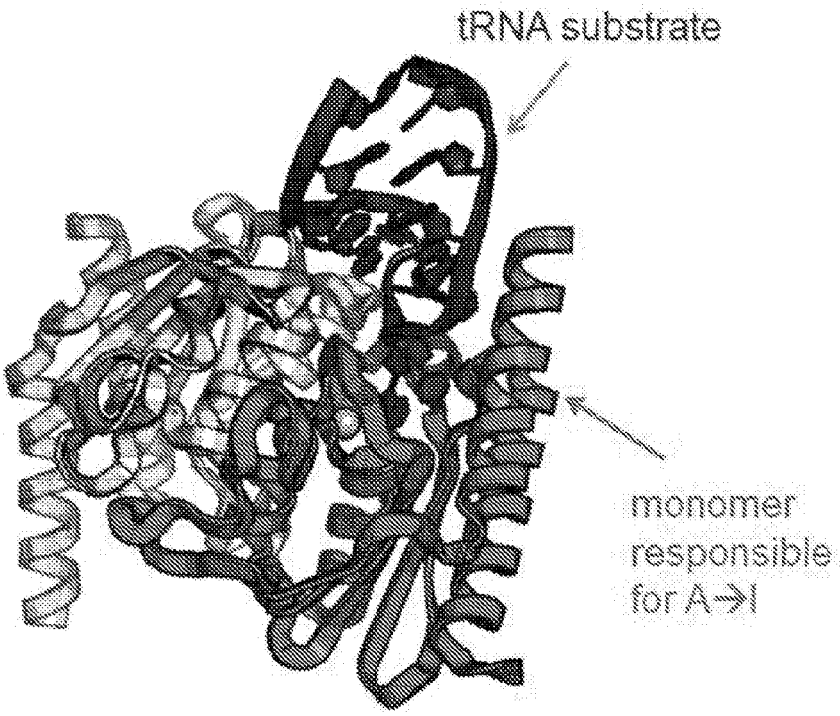


FIGURE 62

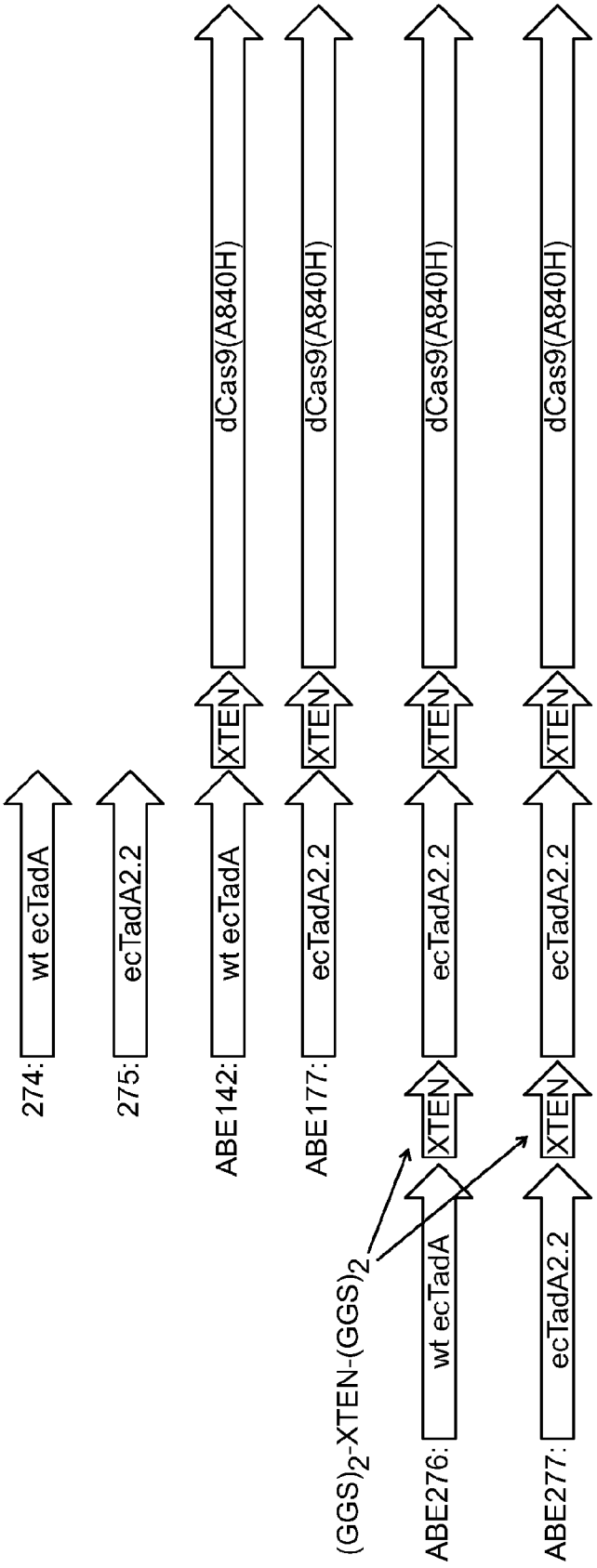
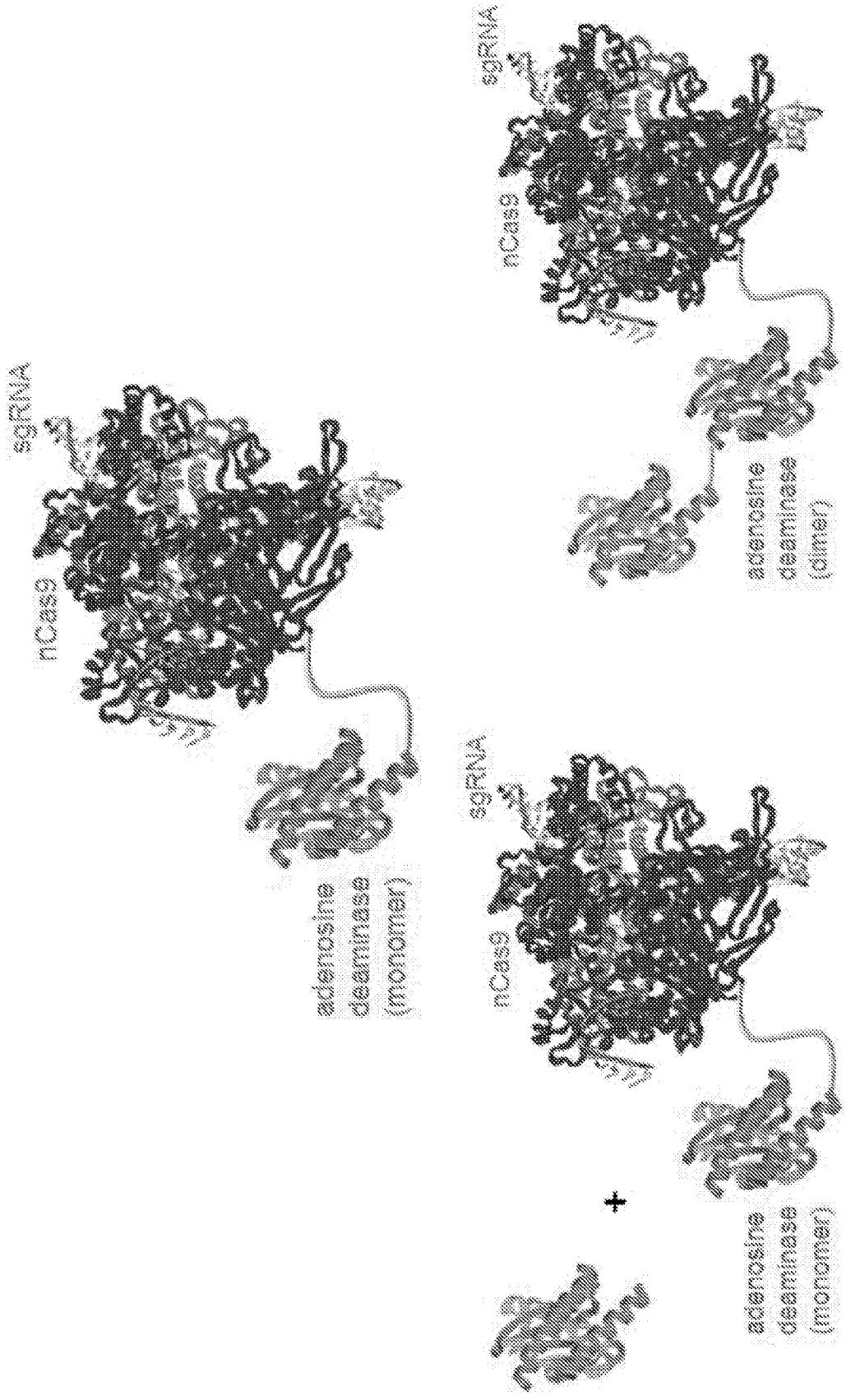

274:
wt ecTadA
275:
ecTadA2.2
ABE142:
wt ecTadA
XTEN
dCas9(A840H)
ABE177:
ecTadA2.2
XTEN
dCas9(A840H)
(GGS)2-XTEN-(GGS)2
ABE276:
wt ecTadA
XTEN
ecTadA2.2
XTEN
dCas9(A840H)
ABE277:
ecTadA2.2
XTEN
ecTadA2.2
XTEN
dCas9(A840H)

FIGURE 63


sgRNA
nCas9
adenosine deaminase (monomer)
sgRNA
nCas9
adenosine deaminase (dimer)
sgRNA
nCas9
adenosine deaminase (monomer)
+

# FIGURE 64

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| wtTadA-Cas9n (142) | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-Cas9n (177) | A | 100.0 | 99.9 | 89.5 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 10.5 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA + wtTadA-Cas9n (274 + 142) | A | 100.0 | 100.0 | 98.9 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 1.1 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA + mutTadA-Cas9n (274+177) | A | 99.6 | 96.7 | 62.7 | 99.9 | 99.8 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.4 | 3.3 | 37.3 | 0.1 | 0.2 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 64 (Continued)

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| mutTadA + wtTadA-Cas9n (275 + 142) | A | 100.0 | 99.9 | 44.9 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 55.1 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA + mutTadA-Cas9n (275 + 177) | A | 99.9 | 98.0 | 53.7 | 99.8 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 2.0 | 46.3 | 0.2 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA-mutTadA-Cas9n (276) | A | 100.0 | 100.0 | 99.7 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.3 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-mutTadA-Cas9n (277) | A | 99.8 | 96.5 | 47.7 | 99.8 | 99.7 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.2 | 3.5 | 52.3 | 0.2 | 0.3 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 65

| | | EMX1 | | | FANCF | |
|---|---|---|---|---|---|---|
| | | $A_2$ | $A_8$ | | $A_3$ | $A_4$ |
| wtTadA-Cas9n (142) | A | 100.0 | 100.0 | | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 |
| mutTadA-Cas9n (177) | A | 100.0 | 100.0 | | 100.0 | 99.8 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | | 0.0 | 0.2 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 |
| wtTadA + wtTadA-Cas9n (274+142) | A | 100.0 | 100.0 | | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 |
| wtTadA + mutTadA-Cas9n (274+177) | A | 99.8 | 99.7 | | 99.9 | 99.8 |
| | C | 0.0 | 0.0 | | 0.0 | 0.0 |
| | G | 0.2 | 0.3 | | 0.0 | 0.2 |
| | T | 0.0 | 0.0 | | 0.0 | 0.0 |

# FIGURE 65 (Continued)

| | | EMX1 | | FANCF | |
|---|---|---|---|---|---|
| | | $A_2$ | $A_8$ | $A_3$ | $A_4$ |
| mutTadA + wtTadA-Cas9n (275 +142) | A | 100.0 | 100.0 | 100.0 | 99.8 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.2 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA + mutTadA-Cas9n (275+177) | A | 99.9 | 99.7 | 99.9 | 99.8 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 0.2 | 0.1 | 0.2 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA-mutTadA-Cas9n (276) | A | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-mutTadA-Cas9n (277) | A | 99.8 | 99.7 | 99.9 | 99.6 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.2 | 0.3 | 0.1 | 0.4 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 66

| | | HEK3 | | HEK4 | RNF2 | |
|---|---|---|---|---|---|---|
| | | $A_6$ | $A_8$ | $A_4$ | $A_4$ | $A_9$ |
| wtTadA-Cas9n | **A** | **100.0** | **100.0** | **100.0** | **100.0** | **100.0** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-Cas9n | **A** | **99.7** | **99.4** | **99.7** | **99.8** | **99.8** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | 0.3 | 0.6 | 0.3 | 0.2 | 0.2 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA + wtTadA-Cas9n | **A** | **100.0** | **100.0** | **100.0** | **100.0** | **100.0** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA + mutTadA-Cas9n | **A** | **99.1** | **97.6** | **99.3** | **99.6** | **99.6** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | **0.9** | **2.4** | **0.7** | 0.3 | 0.3 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 66 (Continued)

| | | HEK3 | | HEK4 | RNF2 | |
|---|---|---|---|---|---|---|
| | | $A_6$ | $A_8$ | $A_4$ | $A_4$ | $A_9$ |
| mutTadA + wtTadA- Cas9n | **A** | **99.8** | **100.0** | **99.3** | **99.8** | **99.8** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | 0.2 | 0.0 | 0.7 | 0.2 | 0.2 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA + mutTadA- Cas9n | **A** | **99.2** | **98.5** | **99.0** | **99.8** | **99.8** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | 0.8 | 1.5 | 1.0 | 0.2 | 0.2 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA- mutTadA- Cas9n | **A** | **100.0** | **100.0** | 0.0 | **100.0** | **100.0** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA- mutTadA- Cas9n | **A** | **99.0** | **98.0** | **98.4** | **99.6** | **99.6** |
| | **C** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | **G** | **1.0** | **2.0** | **1.6** | 0.3 | 0.3 |
| | **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 67

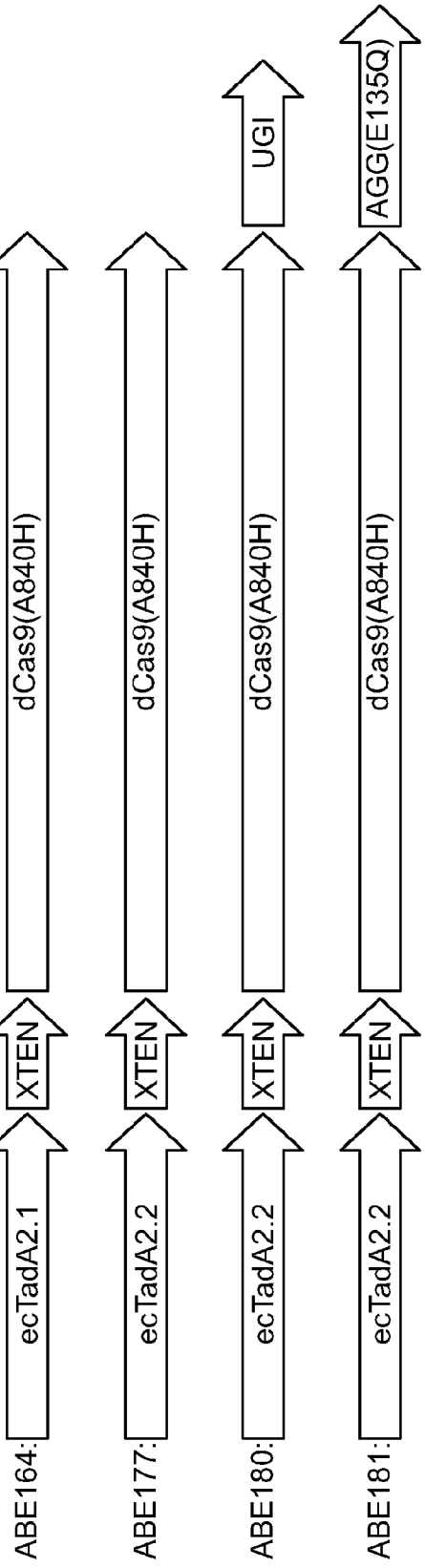

ABE164:
ecTadA2.1
XTEN
dCas9(A840H)
ABE177:
ecTadA2.2
XTEN
dCas9(A840H)
ABE180:
ecTadA2.2
XTEN
dCas9(A840H)
UGI
ABE181:
ecTadA2.2
XTEN
dCas9(A840H)
AGG(E135Q)

FIGURE 68

| | modification | protospacer | position of target A | strand modification | origin | MIC (S1030) Kan |
|---|---|---|---|---|---|---|
| pNMG-208 | stop in Kan gene, W15 | 5'-GCTTAGGTGGAGCGCCTATT-3' | 5 | coding | RSF1030 | 32 ug/mL |
| pNMG-209 | stop in Kan gene, R18 | 5'-AGTCACTCCACCCAAGCGGC-3' | 5 | template | RSF1030 | 256 ug/mL |
| pNMG-210 | stop in Kan gene, R 44 | 5'-GTCACCCCTGCGCTGACAGC-3' | 4 | template | RSF1030 | 128 ug/mL |
| pNMG-211 | stop in Kan gene, Q4 | 5'-ATCTTATTCGATCATGCGAA-3' | 6 | template | RSF1030 | 16 ug/mL |
| pNMG-212 | wt Kan gene | control plasmid | n/a | n/a | RSF1030 | >1056 ug/mL |
| pNMG-213 | pNMG-208 w/ SD8 RBS | 5'-GCTTAGGTGGAGCGCCTATT-3' | 5 | template | RSF1030 | 528 ug/mL |
| pNMG-214 | pNMG-208 w/ SD3 RBS | 5'-GCTTAGGTGGAGCGCCTATT-3' | 5 | template | RSF1030 | 128 ug/mL |
| pNMG-215 | pNMG-208 w/ SD2 RBS | 5'-GCTTAGGTGGAGCGCCTATT-3' | 5 | template | RSF1030 | unknown |
| pNMG-216 | 2 stop, Q4 + R18 | 5'-ATCTTATTCGATCATGCGAA-3', 5'-AGTCACTCCACCCAAGCGGC-3' | 6 + 5 | template | RSF1030 | 8 ug/mL |
| pNMG-217 | 2 stop, W15 + R44 | 5'-GCTTAGGTGGAGCGCCTATT-3', 5'-GTCACCCCTGCGCTGACAGC-3' | 5 + 4 | both | RSF1030 | 8 ug/mL |
| pNMG-221 | 2 stop, W15 + R44 | 5'-GCTTAGGTGGAGCGCCTATT-3', 5'-GTCACCCCTGCGCTGACAGC-3' | 5 + 4 | both | ClaDF3 | 4 ug/mL |

FIGURE 68 (Continued)

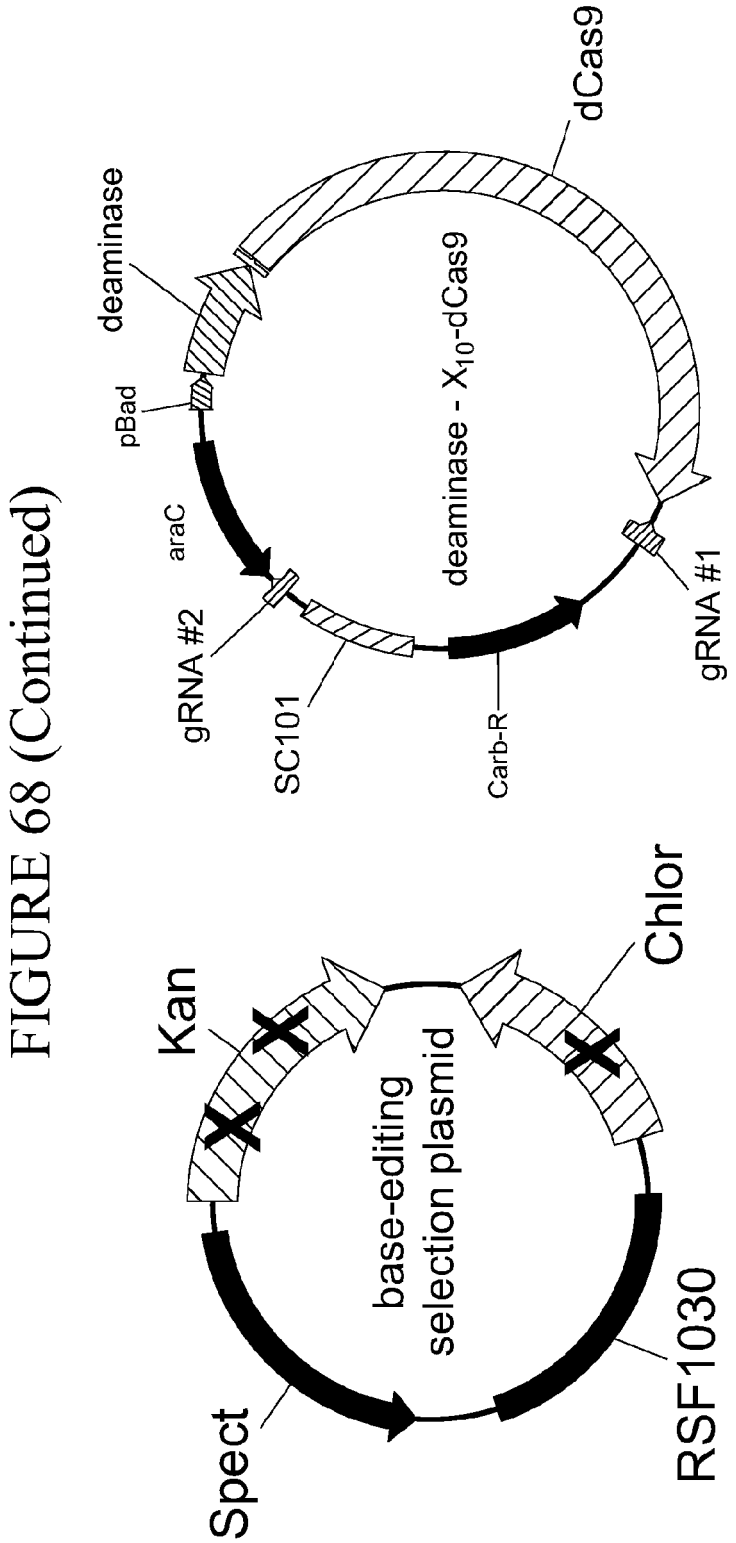


FIGURE 69

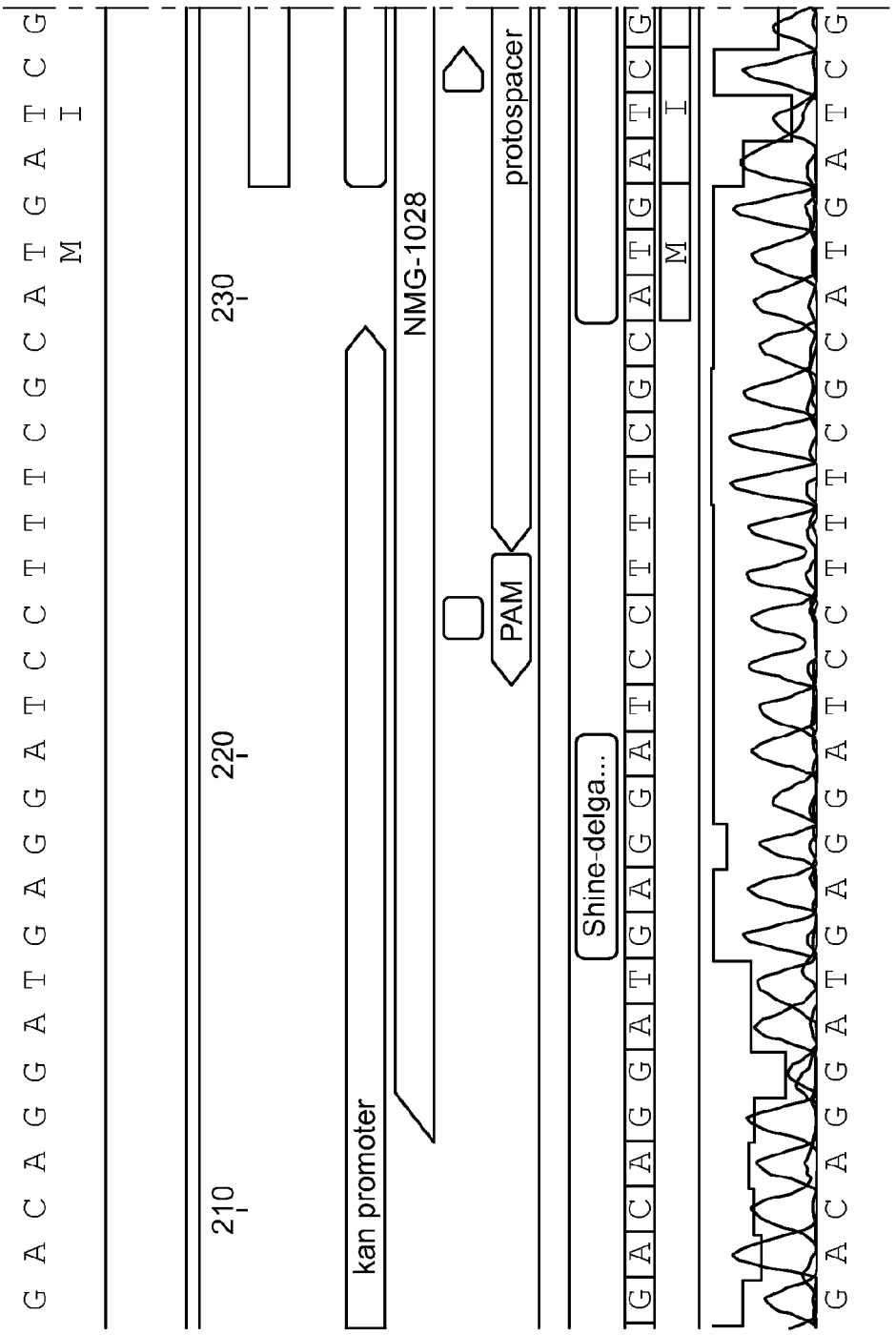

G A C A G G G A T G A G G A T C C C T T T C G G C A T G A T C G
M I
210
220
230
kan promoter
NMG-1028
PAM
protospacer
Shine-delga...
G A C A G G G A T G A G G A T C C C T T T C G G C A T G A T C G
M I

FIGURE 69 (Continued)

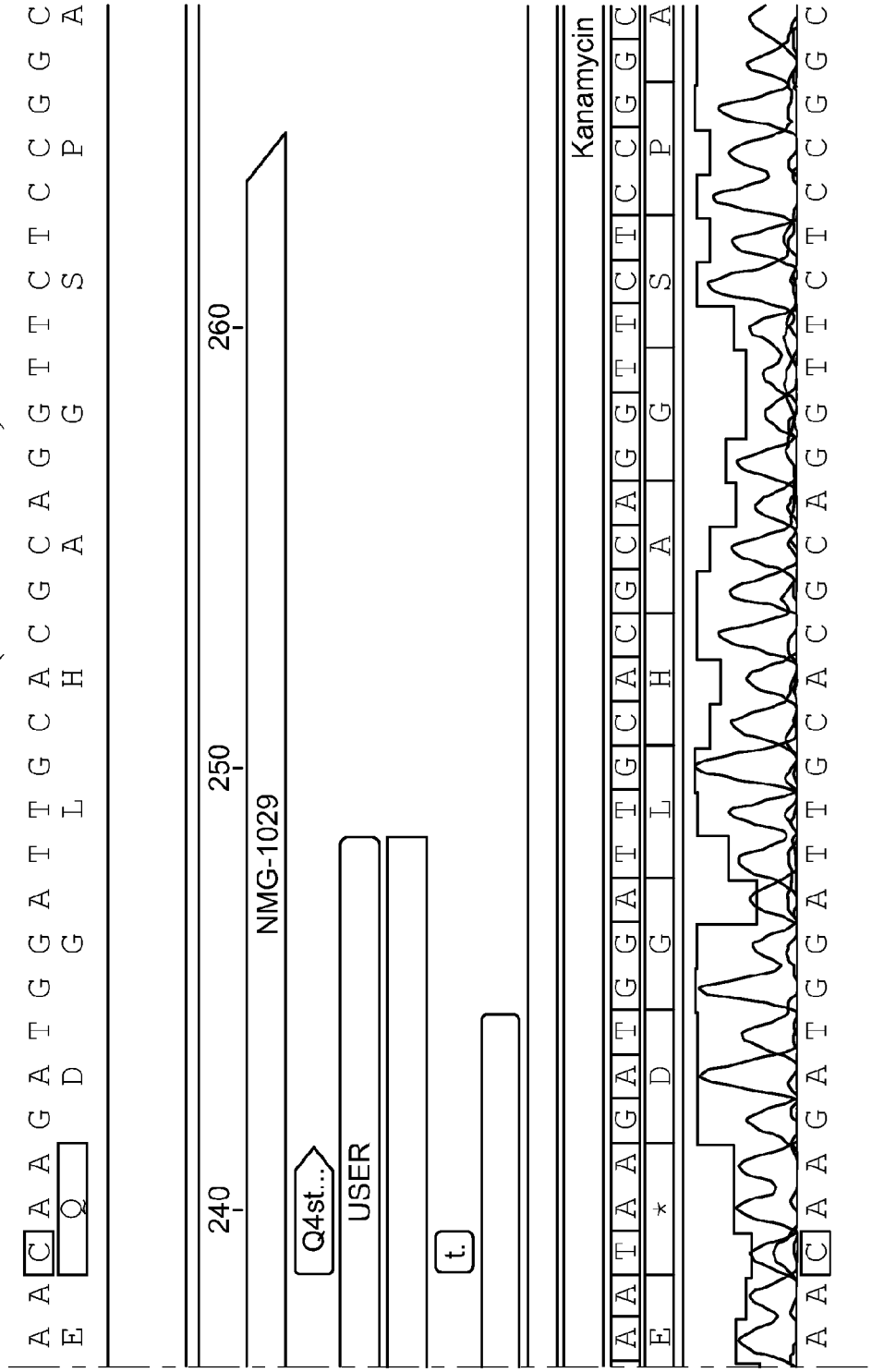


FIGURE 70

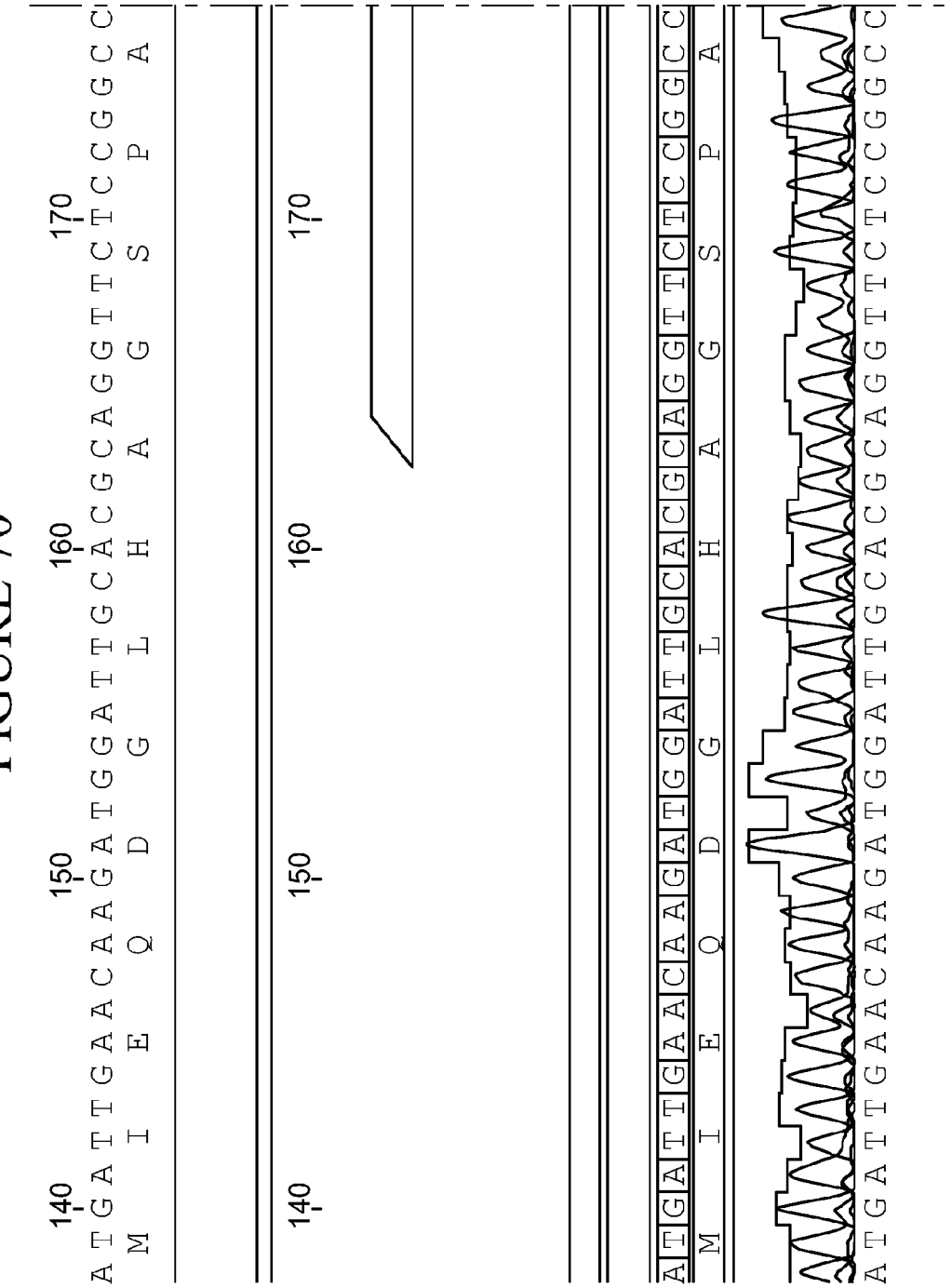

140
150
160
170
ATGATTGAACAAGATGGATTGCACGGCAGGTTCTCCGGCC
M I E Q D G L H A G S P A
ATGATTGAACAAGATGGATTGCACGGCAGGTTCTCCGGCC
M I E Q D G L H A G S P A

FIGURE 70 (Continued)

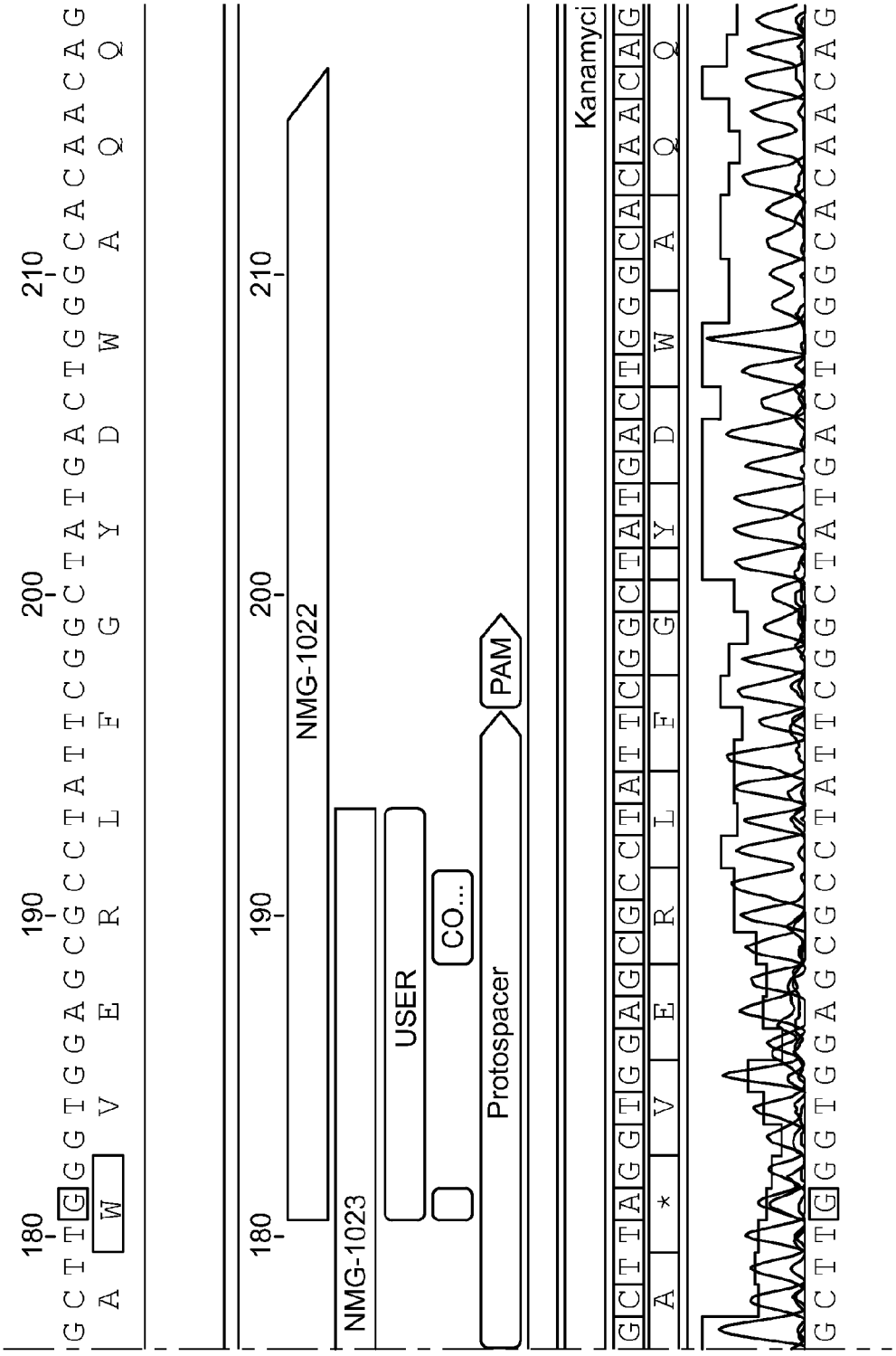


FIGURE 71

HEK293 site 2: $GA_2A_3CA_5CA_7A_8A_9GCATAGACTGCGGG$
(showing as T to C)

FIGURE 72

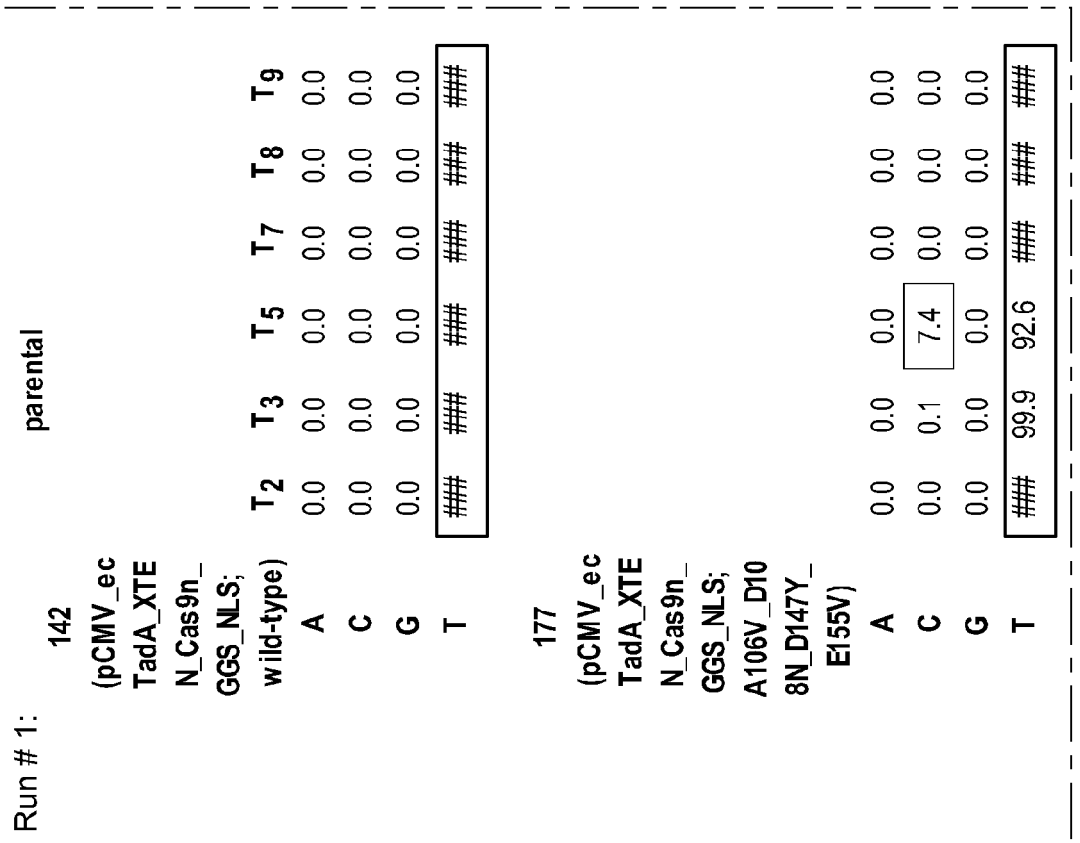

Run # 1:
142
(pCMV_ec
TadA_XTE
N_Cas9n_
GGS_NLS;
wild-type)
A 0.0 0.0 0.0 0.0 0.0 0.0
C 0.0 0.0 0.0 0.0 0.0 0.0
G 0.0 0.0 0.0 0.0 0.0 0.0
T ### ### ### ### ### ###
177
(pCMV_ec
TadA_XTE
N_Cas9n_
GGS_NLS;
A106V_D10
8N_D147Y_
E155V)
A 0.0 0.0 0.0 0.0 0.0 0.0
C 0.0 0.1 7.4 0.0 0.0 0.0
G 0.0 0.0 0.0 0.0 0.0 0.0
T ### 99.9 92.6 ### ### ###

FIGURE 72 (Continued)

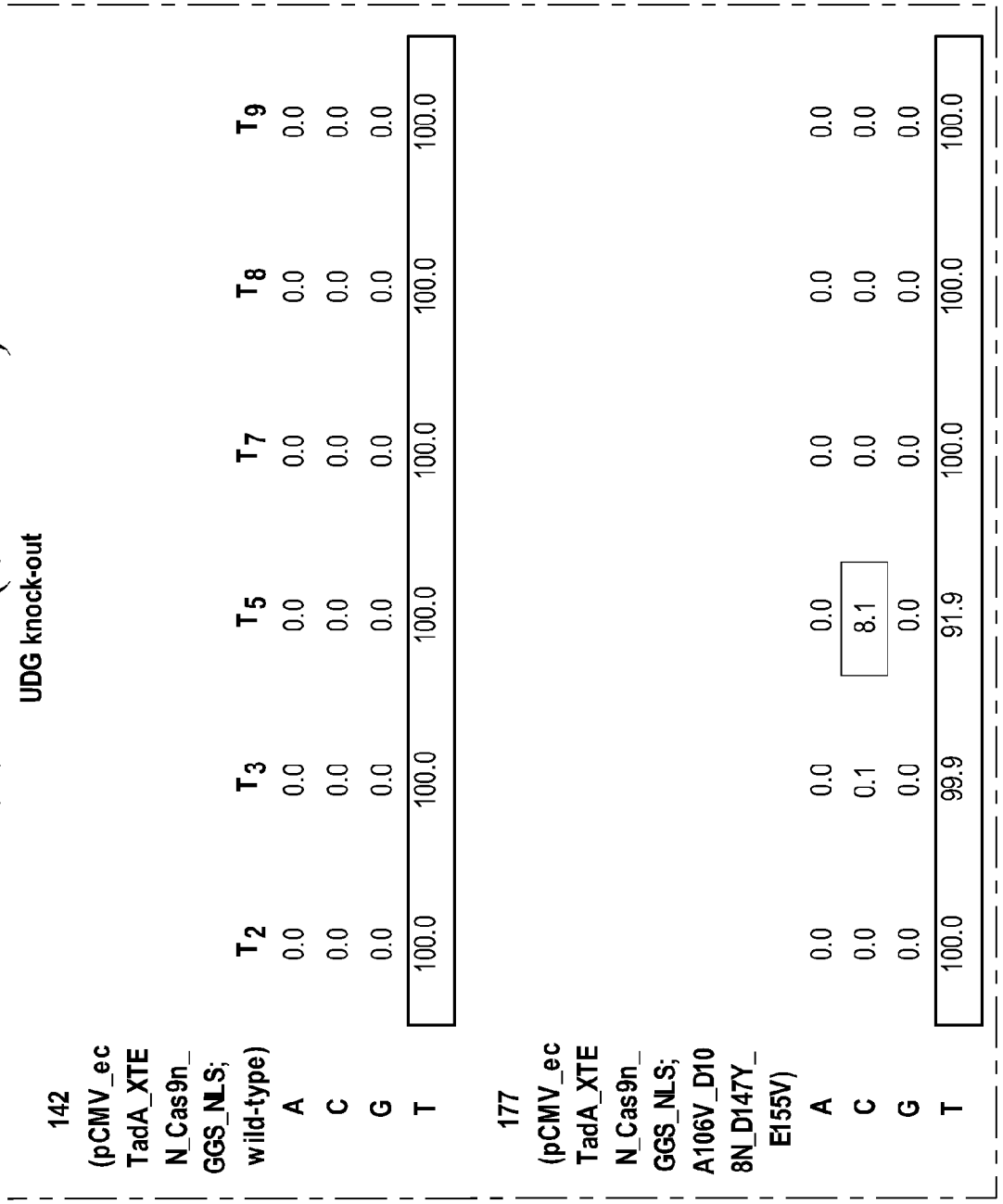
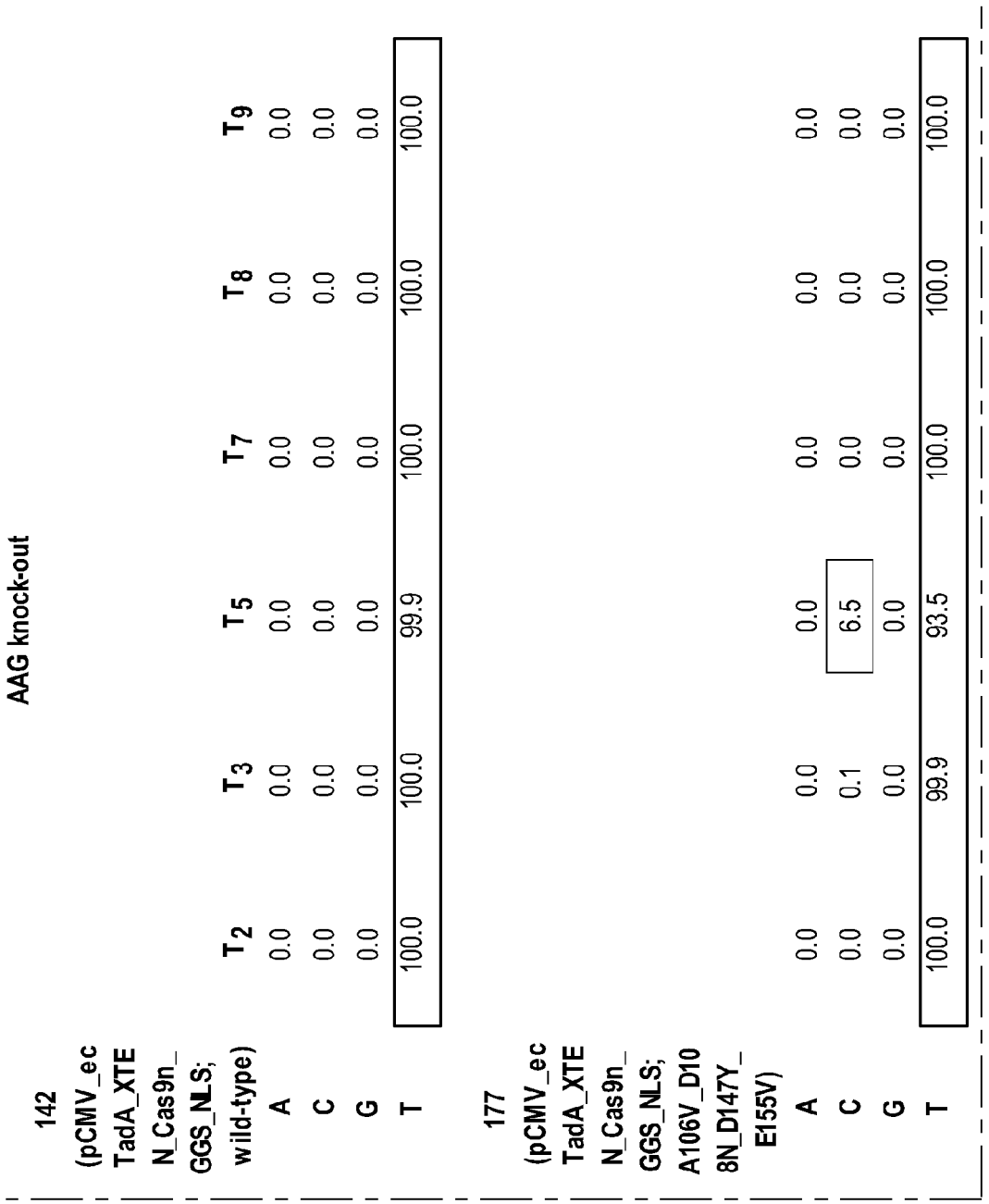


FIGURE 72 (Continued)



142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) AAG knock-out

| | $T_2$ | $T_3$ | $T_5$ | $T_7$ | $T_8$ | $T_9$ |
|---|---|---|---|---|---|---|
| A | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| T | 100.0 | 100.0 | 99.9 | 100.0 | 100.0 | 100.0 |

177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)

| | $T_2$ | $T_3$ | $T_5$ | $T_7$ | $T_8$ | $T_9$ |
|---|---|---|---|---|---|---|
| A | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| C | 0.0 | 0.1 | 6.5 | 0.0 | 0.0 | 0.0 |
| G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| T | 100.0 | 99.9 | 93.5 | 100.0 | 100.0 | 100.0 |

FIGURE 72 (Continued)

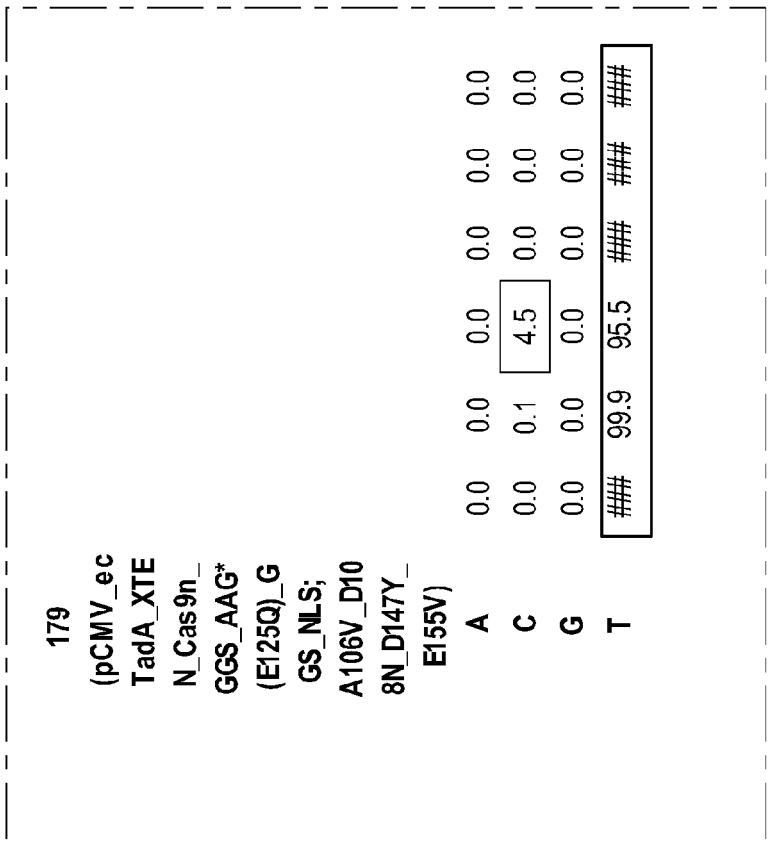


FIGURE 72 (Continued)

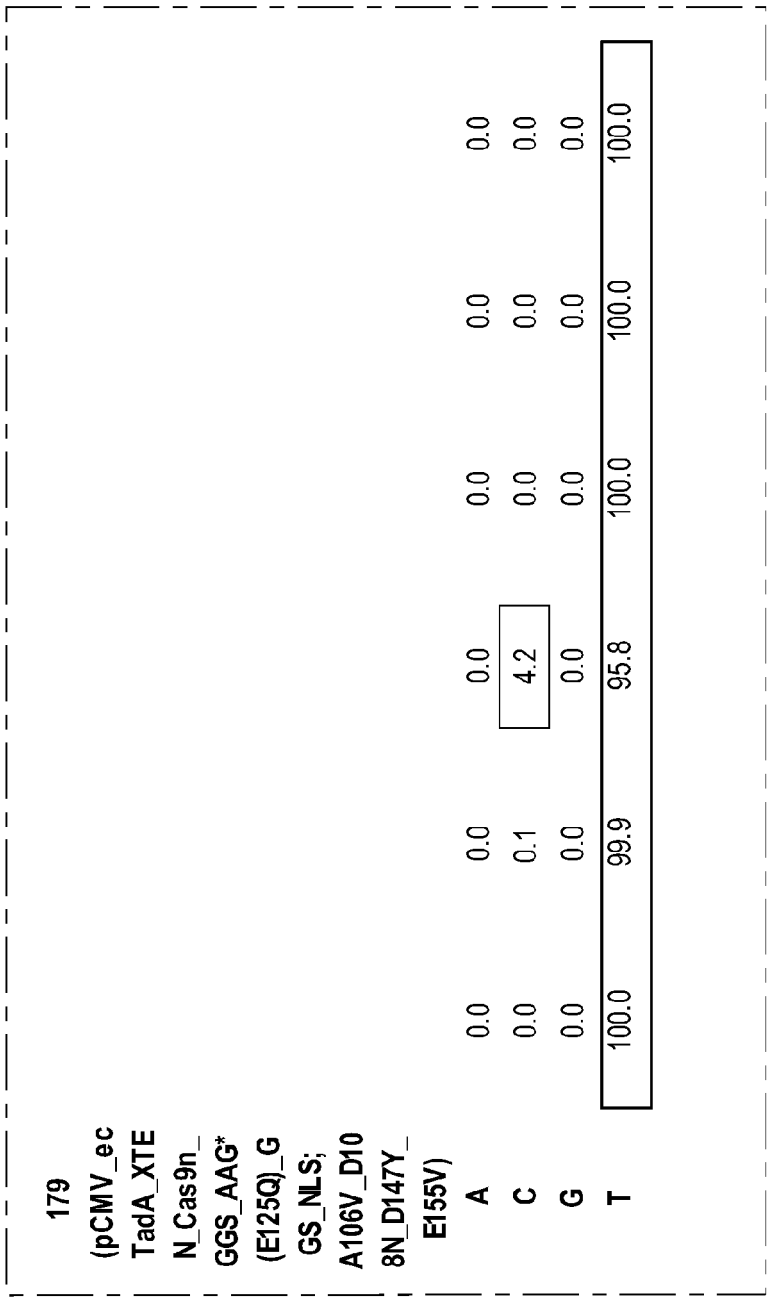


FIGURE 72 (Continued)

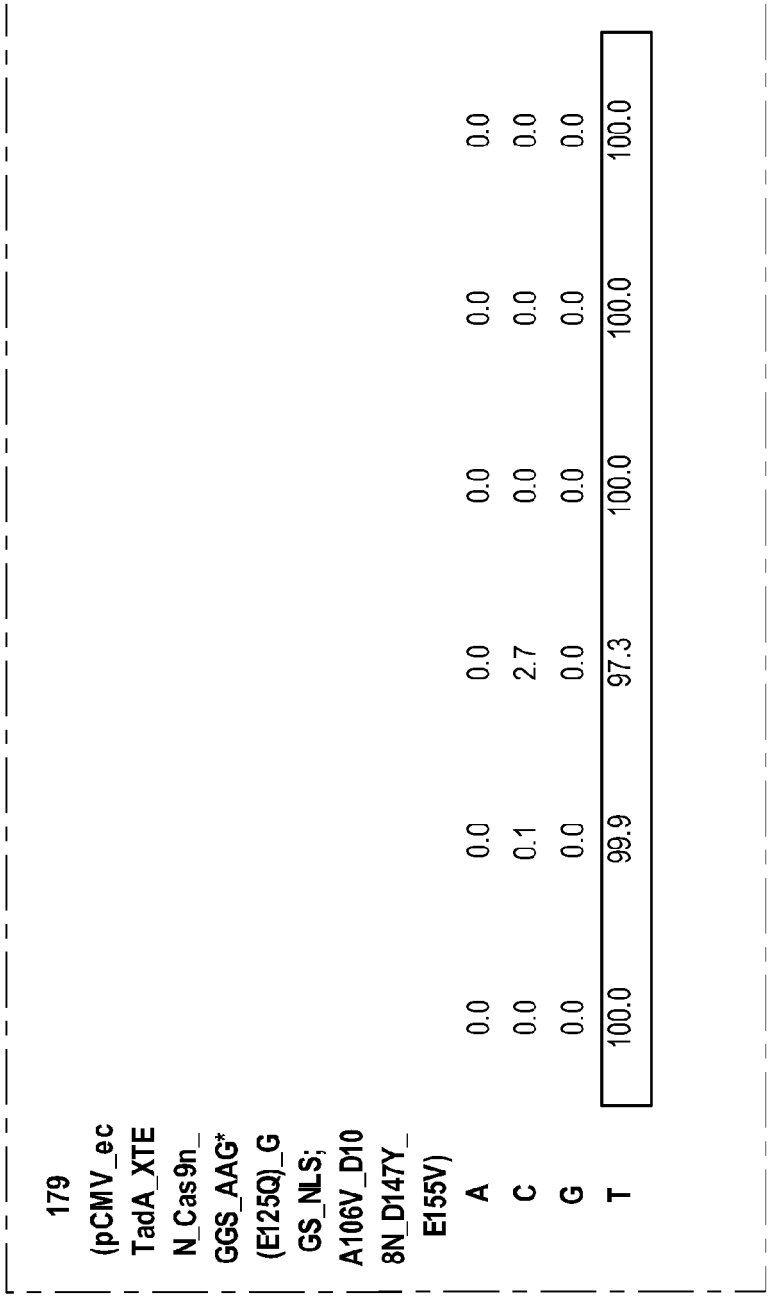


FIGURE 72 (Continued)

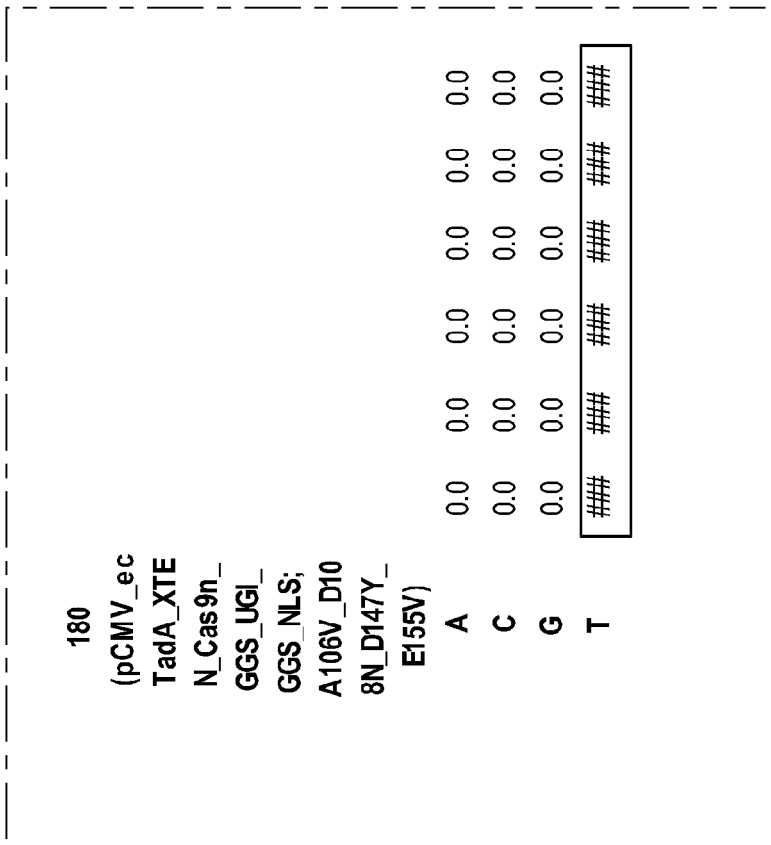


FIGURE 72 (Continued)

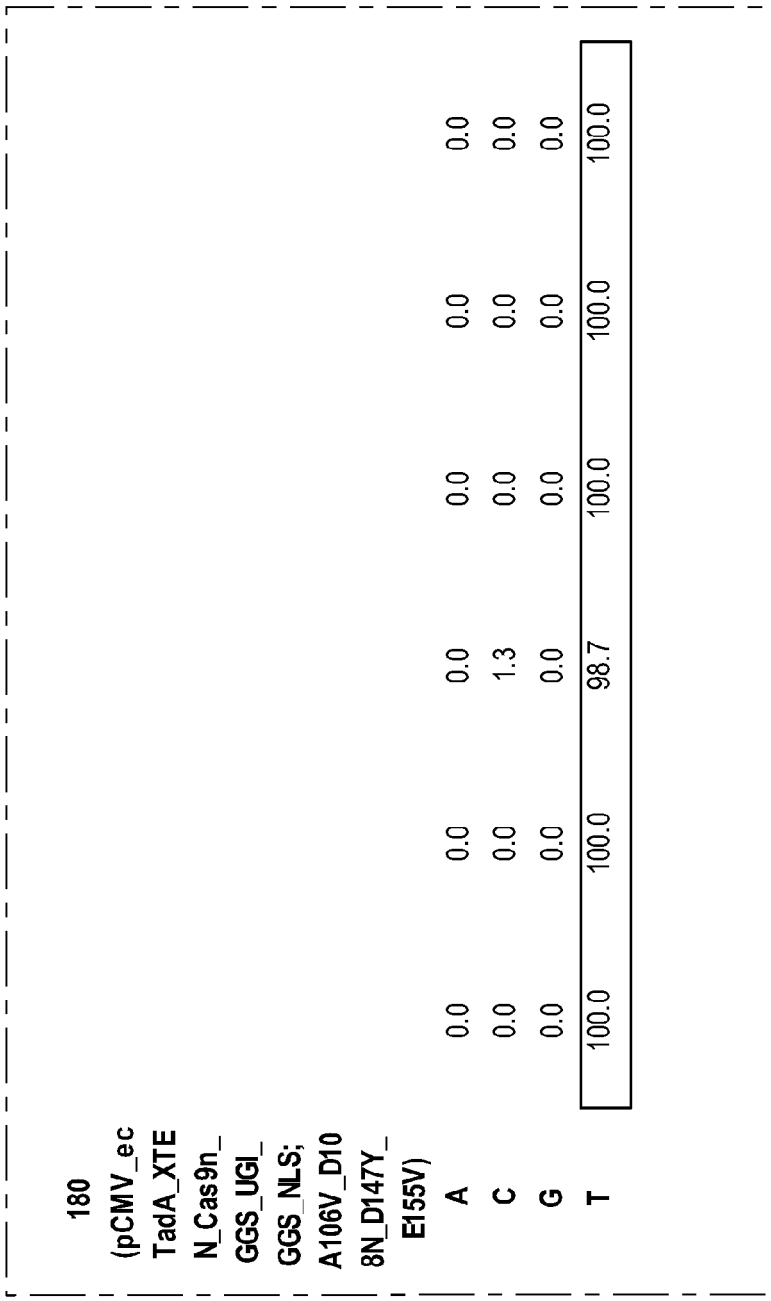


FIGURE 72 (Continued)

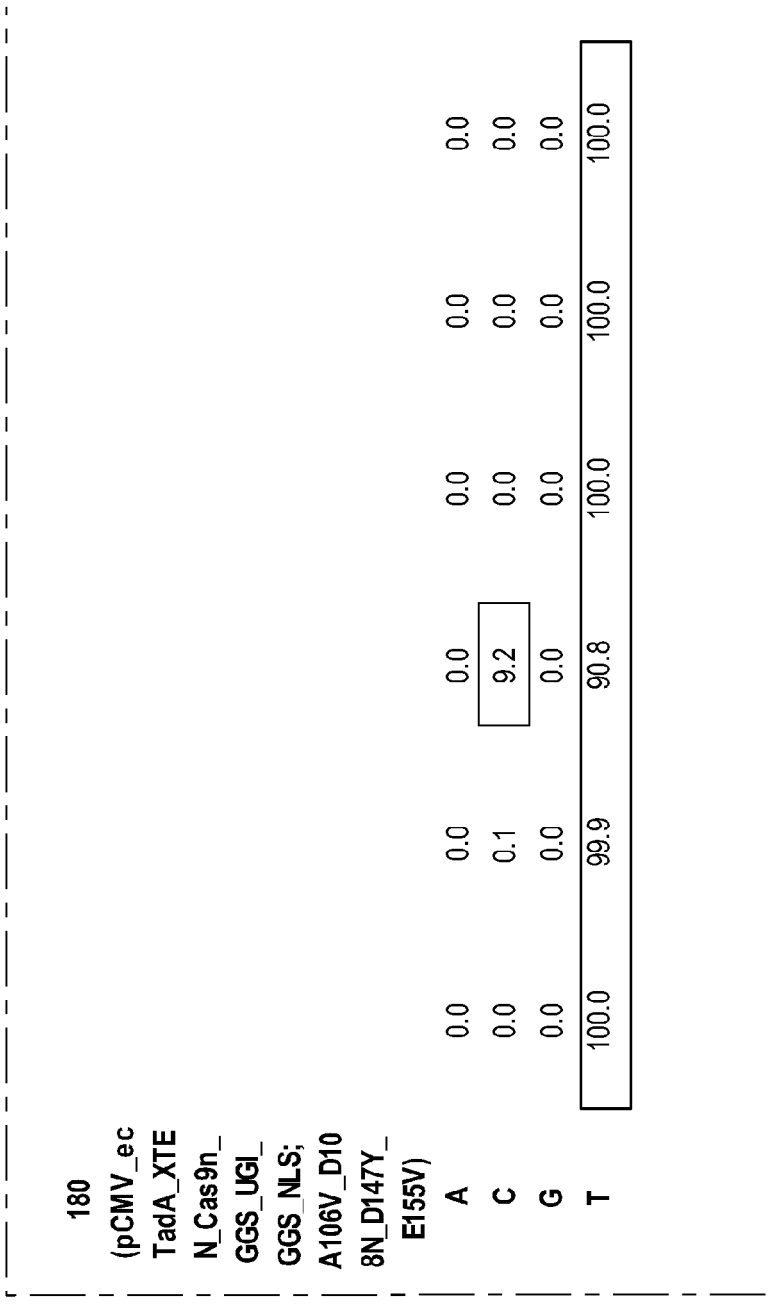


FIGURE 73

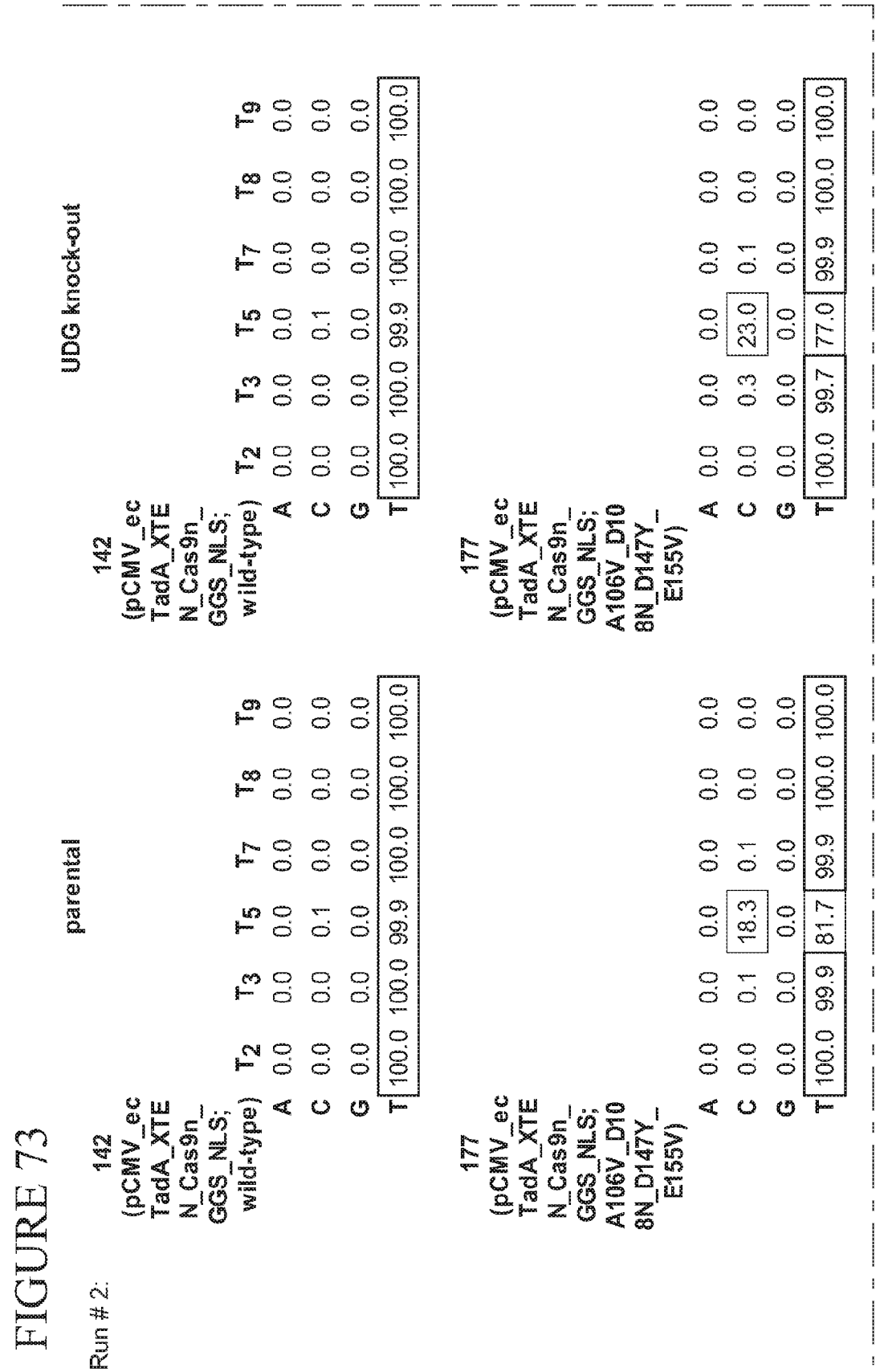

Run # 2:
parental
142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)
A 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
C 0.0 0.0 0.0 0.1 0.0 0.0 0.0 0.0
G 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
T 100.0 100.0 99.9 100.0 100.0 100.0 100.0
177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)
A 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
C 0.0 0.1 18.3 0.1 0.0 0.0 0.0
G 0.0 0.0 0.0 0.0 0.0 0.0 0.0
T 100.0 99.9 81.7 99.9 100.0 100.0 100.0
UDG knock-out
142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type)
A 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
C 0.0 0.0 0.0 0.1 0.0 0.0 0.0 0.0
G 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
T 100.0 100.0 99.9 100.0 100.0 100.0 100.0
177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V)
A 0.0 0.0 0.0 0.0 0.0 0.0 0.0
C 0.0 0.3 23.0 0.1 0.0 0.0
G 0.0 0.0 0.0 0.0 0.0 0.0
T 100.0 99.7 77.0 99.9 100.0 100.0

FIGURE 73 (Continued)

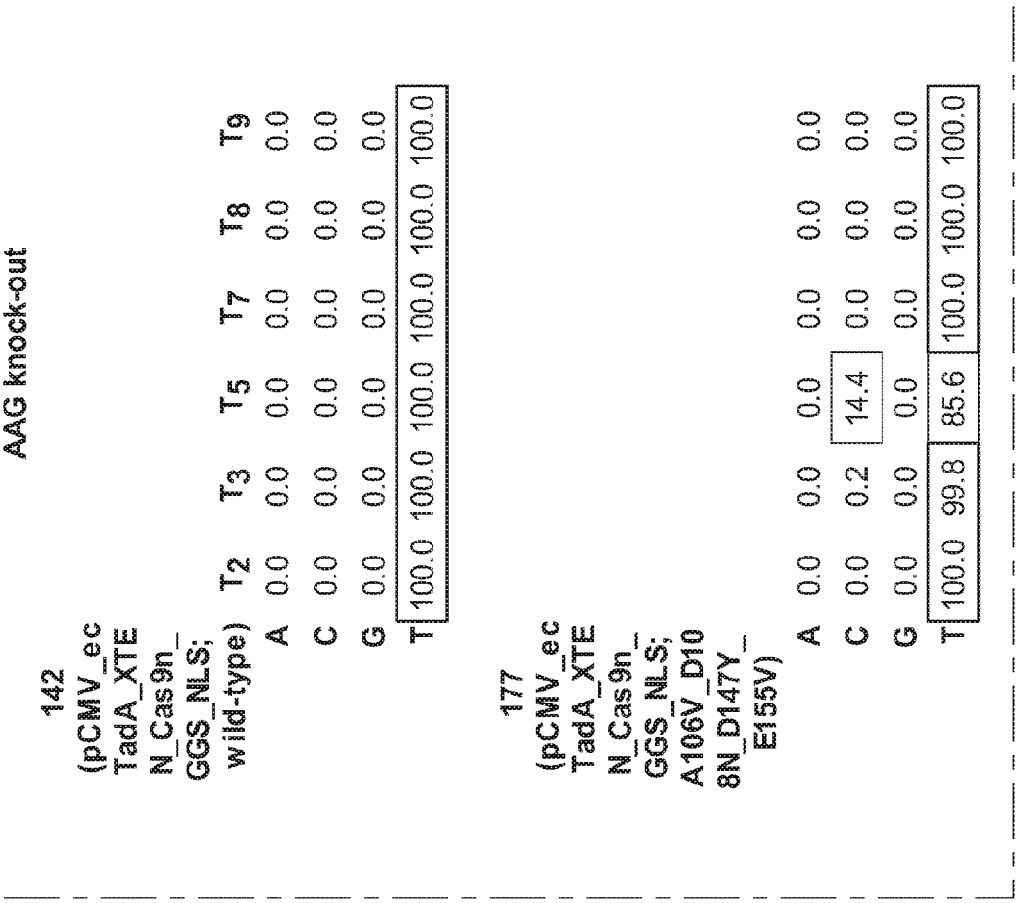


FIGURE 73 (Continued)

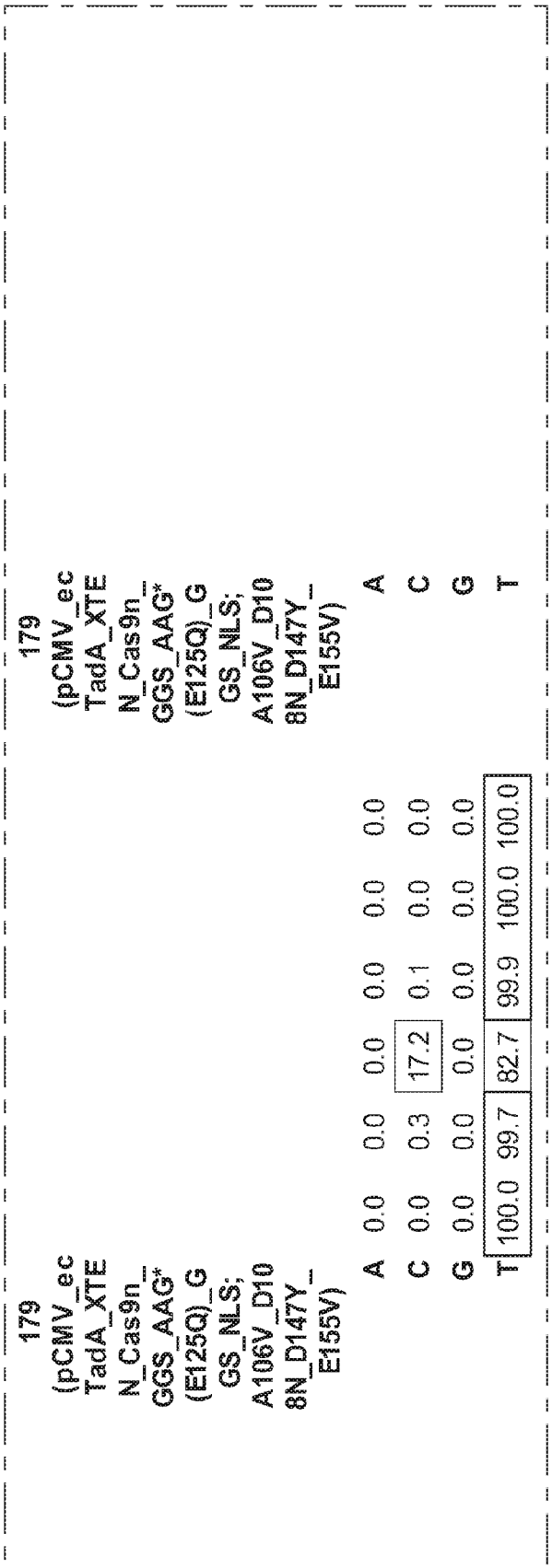


FIGURE 73 (Continued)

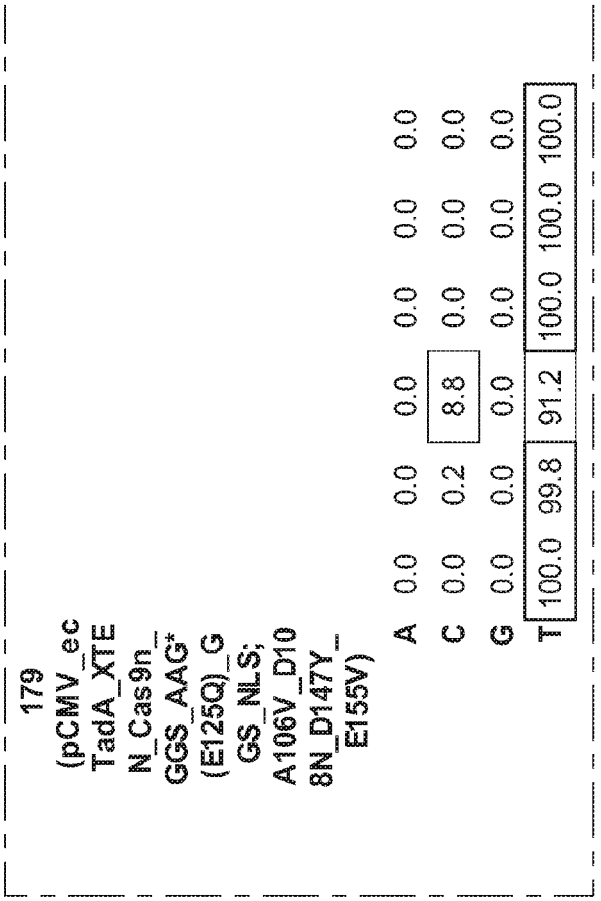


FIGURE 73 (Continued)

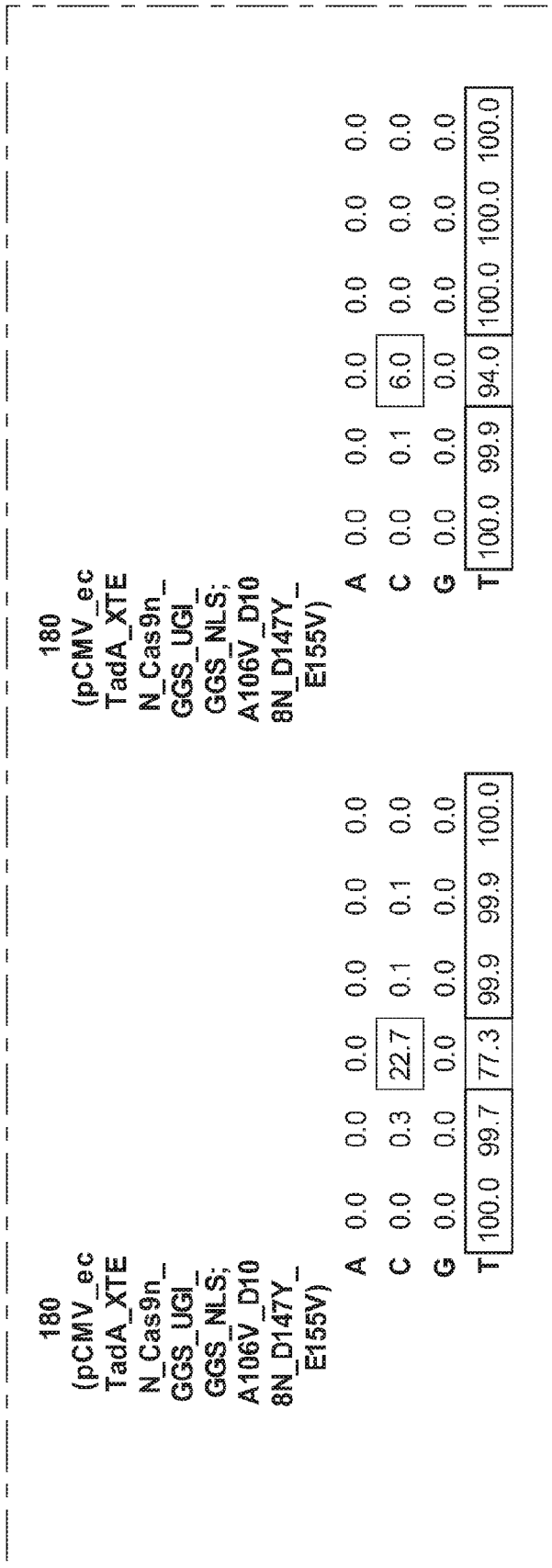


FIGURE 73 (Continued)

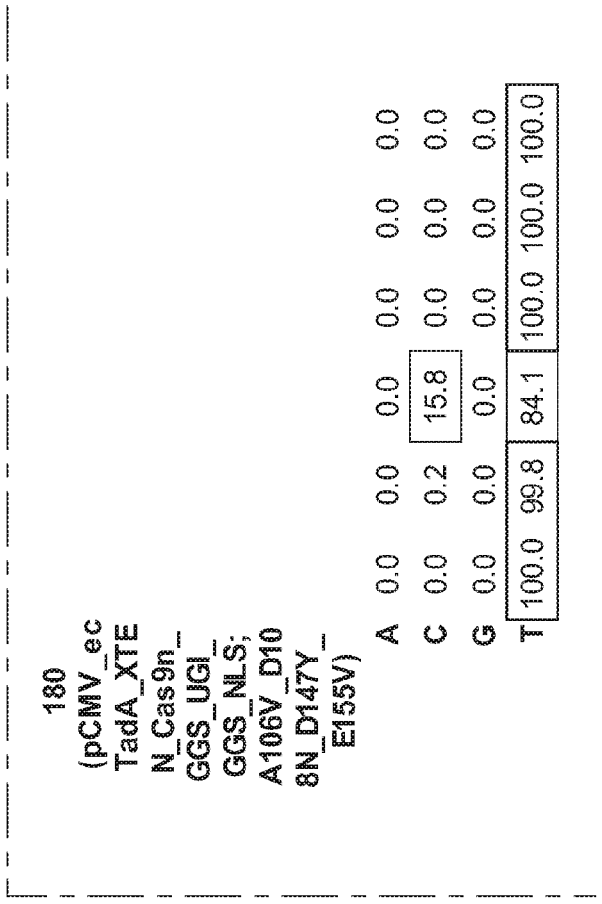


FIGURE 74

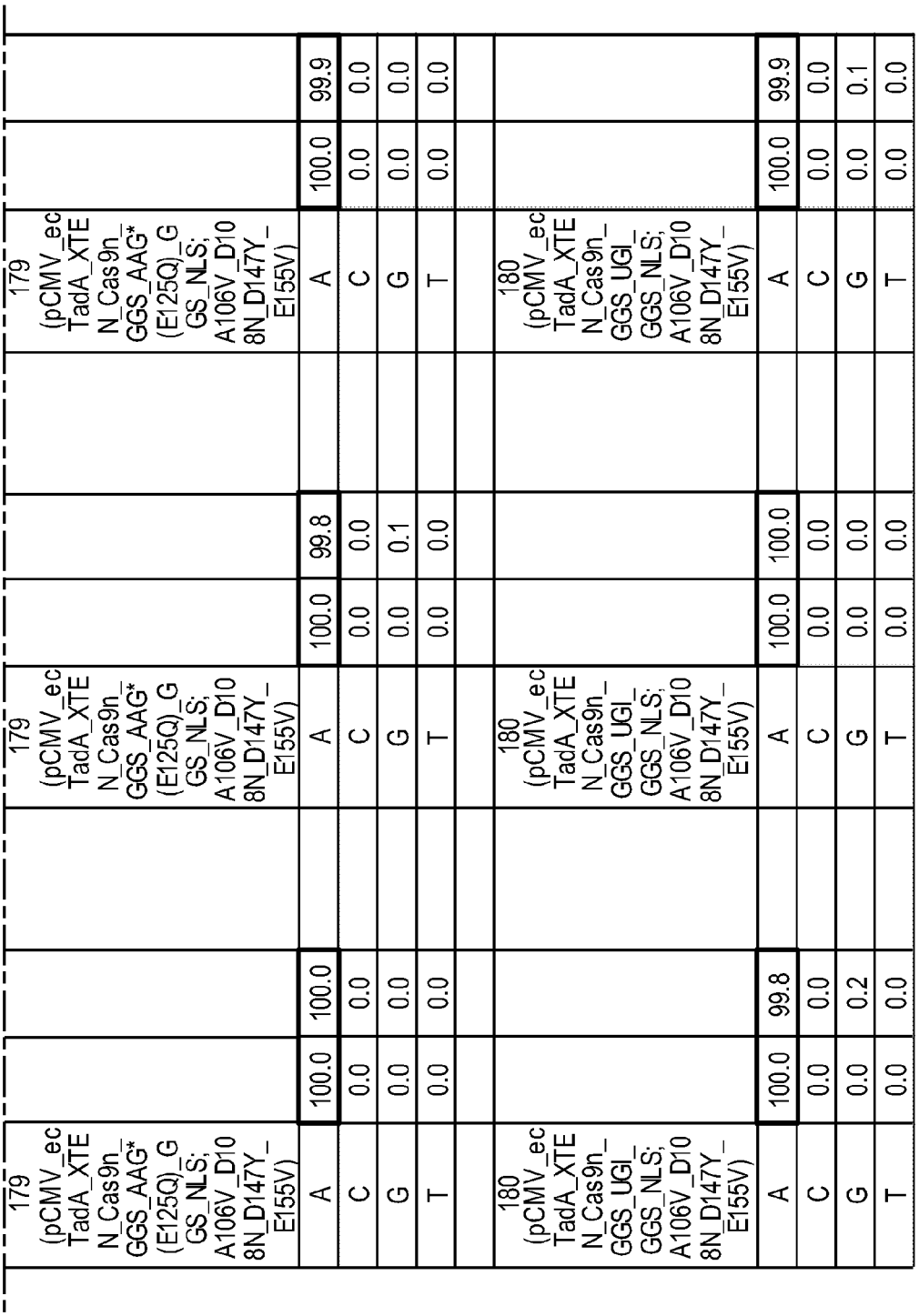
FANCF: $GGA_3A_4TCCCTTCTGCAGCACCTGG$

FIGURE 75

Run # 2:

| | parental | | | | out | | | | out | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | $A_3$ | $A_4$ | | 142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | $A_3$ | $A_4$ | | 142 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; wild-type) | $A_3$ | $A_4$ |
| A | 100.0 | 100.0 | | A | 100.0 | 100.0 | | A | 100.0 | 100.0 |
| C | 0.0 | 0.0 | | C | 0.0 | 0.0 | | C | 0.0 | 0.0 |
| G | 0.0 | 0.0 | | G | 0.0 | 0.0 | | G | 0.0 | 0.0 |
| T | 0.0 | 0.0 | | T | 0.0 | 0.0 | | T | 0.0 | 0.0 |
| | | | | | | | | | | |
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | | |
| A | 100.0 | 99.7 | | A | 100.0 | 99.8 | | A | 99.9 | 99.8 |
| C | 0.0 | 0.0 | | C | 0.0 | 0.0 | | C | 0.0 | 0.0 |
| G | 0.0 | 0.3 | | G | 0.0 | 0.2 | | G | 0.1 | 0.2 |
| T | 0.0 | 0.0 | | T | 0.0 | 0.0 | | T | 0.0 | 0.0 |
| | | | | | | | | | | |

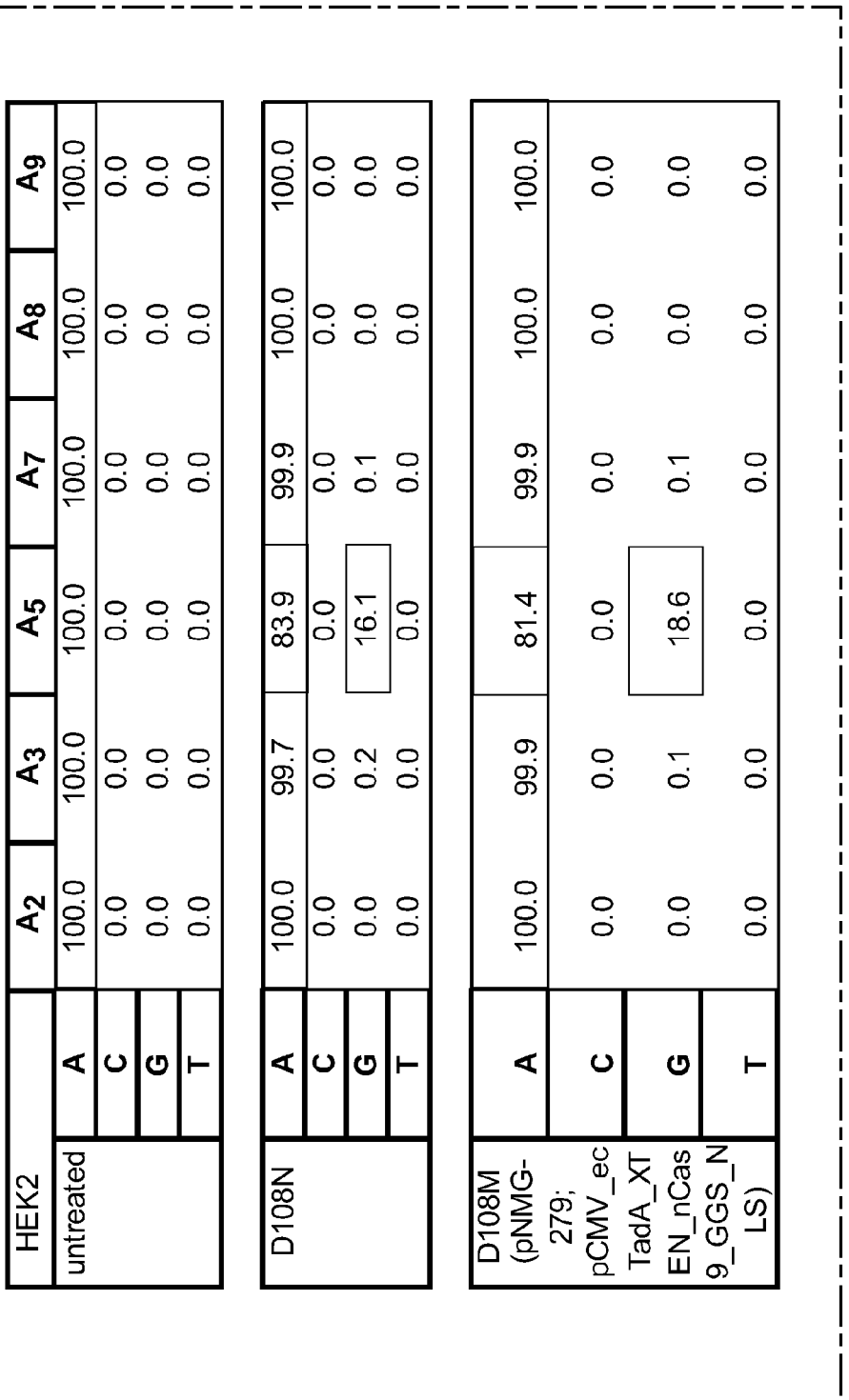
FIGURE 75 (Continued)



| 179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG* (E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | 179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG* (E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 100.0 | 100.0 | | | | | | | A | 100.0 | 99.8 | | | | | | |
| C | 0.0 | 0.0 | | | | | | | C | 0.0 | 0.0 | | | | | | |
| G | 0.0 | 0.0 | | | | | | | G | 0.0 | 0.1 | | | | | | |
| T | 0.0 | 0.0 | | | | | | | T | 0.0 | 0.0 | | | | | | |
| | | | | | | | | | | | | | | | | | |
| 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | |
| A | 100.0 | 99.8 | | | | | | | A | 100.0 | 100.0 | | | | | | |
| C | 0.0 | 0.0 | | | | | | | C | 0.0 | 0.0 | | | | | | |
| G | 0.0 | 0.2 | | | | | | | G | 0.0 | 0.0 | | | | | | |
| T | 0.0 | 0.0 | | | | | | | T | 0.0 | 0.0 | | | | | | |

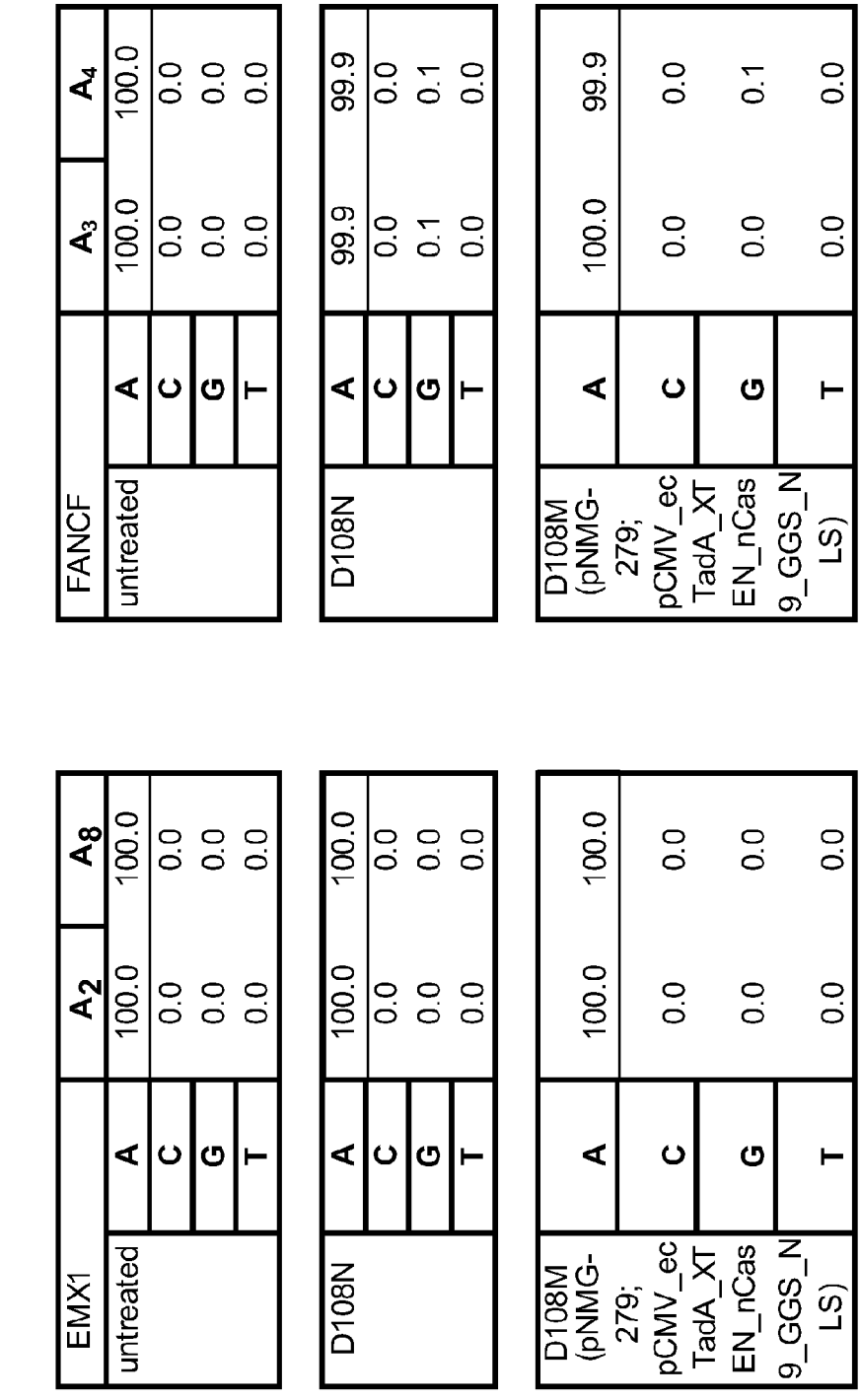
| 179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG* (E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | 100.0 | 99.9 | | | | | | | |
| C | 0.0 | 0.0 | | | | | | | |
| G | 0.0 | 0.0 | | | | | | | |
| T | 0.0 | 0.0 | | | | | | | |
| | | | | | | | | | |
| 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | | | | | | | | | |
| A | 100.0 | 99.9 | | | | | | | |
| C | 0.0 | 0.0 | | | | | | | |
| G | 0.0 | 0.1 | | | | | | | |
| T | 0.0 | 0.0 | | | | | | | |

FIGURE 76



| HEK2 | | A2 | A3 | A5 | A7 | A8 | A9 |
|---|---|---|---|---|---|---|---|
| untreated | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| D108N | A | 100.0 | 99.7 | 83.9 | 99.9 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | 16.1 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | A | 100.0 | 99.9 | 81.4 | 99.9 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 18.6 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 76 (Continued)



| EMX1 | | $A_2$ | $A_8$ |
|---|---|---|---|
| untreated | A | 100.0 | 100.0 |
| | C | 0.0 | 0.0 |
| | G | 0.0 | 0.0 |
| | T | 0.0 | 0.0 |

| D108N | | | |
|---|---|---|---|
| | A | 100.0 | 100.0 |
| | C | 0.0 | 0.0 |
| | G | 0.0 | 0.0 |
| | T | 0.0 | 0.0 |

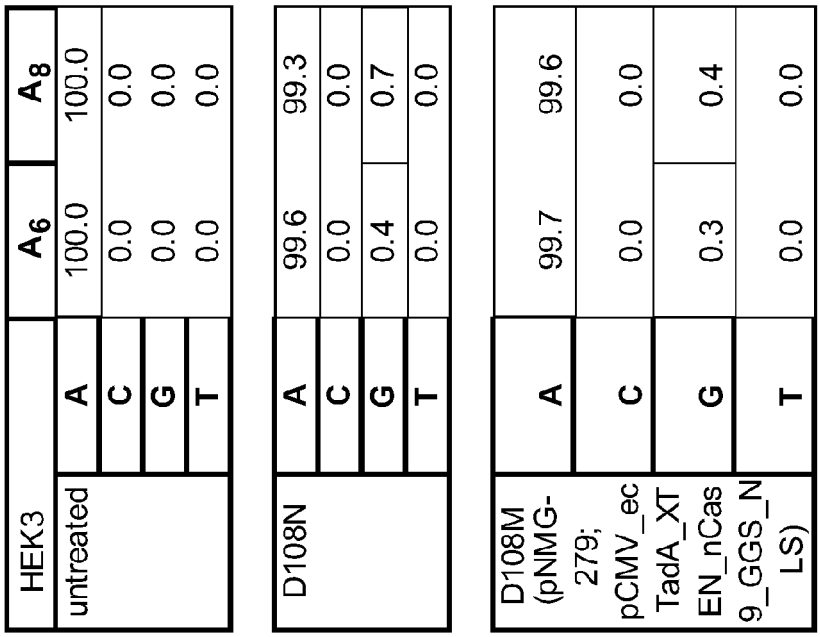
| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | | | |
|---|---|---|---|
| | A | 100.0 | 100.0 |
| | C | 0.0 | 0.0 |
| | G | 0.0 | 0.0 |
| | T | 0.0 | 0.0 |

| FANCF | | $A_3$ | $A_4$ |
|---|---|---|---|
| untreated | A | 100.0 | 100.0 |
| | C | 0.0 | 0.0 |
| | G | 0.0 | 0.0 |
| | T | 0.0 | 0.0 |

| D108N | | | |
|---|---|---|---|
| | A | 99.9 | 99.9 |
| | C | 0.0 | 0.0 |
| | G | 0.1 | 0.1 |
| | T | 0.0 | 0.0 |

| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | | | |
|---|---|---|---|
| | A | 100.0 | 99.9 |
| | C | 0.0 | 0.0 |
| | G | 0.0 | 0.1 |
| | T | 0.0 | 0.0 |

FIGURE 76 (Continued)

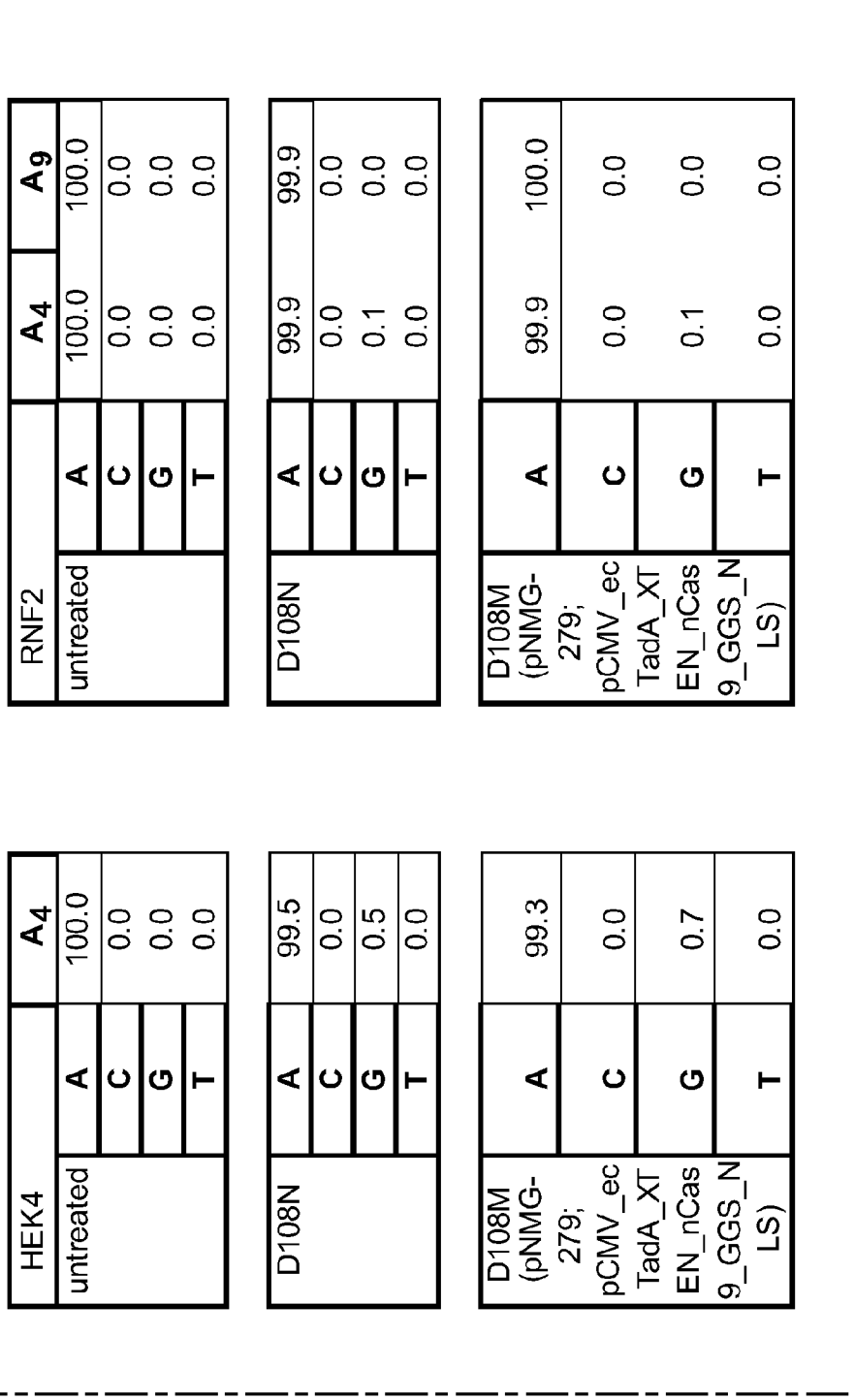


| HEK3 | | $A_6$ | $A_8$ |
|---|---|---|---|
| untreated | A | 100.0 | 100.0 |
| | C | 0.0 | 0.0 |
| | G | 0.0 | 0.0 |
| | T | 0.0 | 0.0 |

| D108N | | $A_6$ | $A_8$ |
|---|---|---|---|
| | A | 99.6 | 99.3 |
| | C | 0.0 | 0.0 |
| | G | 0.4 | 0.7 |
| | T | 0.0 | 0.0 |

| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | | $A_6$ | $A_8$ |
|---|---|---|---|
| | A | 99.7 | 99.6 |
| | C | 0.0 | 0.0 |
| | G | 0.3 | 0.4 |
| | T | 0.0 | 0.0 |

FIGURE 76 (Continued)



| HEK4 | | $A_4$ |
|---|---|---|
| untreated | A | 100.0 |
| | C | 0.0 |
| | G | 0.0 |
| | T | 0.0 |

| D108N | | |
|---|---|---|
| | A | 99.5 |
| | C | 0.0 |
| | G | 0.5 |
| | T | 0.0 |

| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | | |
|---|---|---|
| | A | 99.3 |
| | C | 0.0 |
| | G | 0.7 |
| | T | 0.0 |

| RNF2 | | $A_4$ | $A_9$ |
|---|---|---|---|
| untreated | A | 100.0 | 100.0 |
| | C | 0.0 | 0.0 |
| | G | 0.0 | 0.0 |
| | T | 0.0 | 0.0 |

| D108N | | | |
|---|---|---|---|
| | A | 99.9 | 99.9 |
| | C | 0.0 | 0.0 |
| | G | 0.1 | 0.0 |
| | T | 0.0 | 0.0 |

| D108M (pNMG-279; pCMV_ecTadA_XTEN_nCas9_GGS_NLS) | | | |
|---|---|---|---|
| | A | 99.9 | 100.0 |
| | C | 0.0 | 0.0 |
| | G | 0.1 | 0.0 |
| | T | 0.0 | 0.0 |

# FIGURE 77

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| wtTadA + wtTadA- Cas9n | A | 100.0 | 100.0 | 98.9 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 1.1 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA + mutTadA- Cas9n | A | 99.6 | 96.7 | 62.7 | 99.9 | 99.8 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.4 | 3.3 | 37.3 | 0.1 | 0.2 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA + wtTadA- Cas9n | A | 100.0 | 99.9 | 44.9 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 55.1 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA + mutTadA- Cas9n | A | 99.9 | 98.0 | 53.7 | 99.8 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 2.0 | 46.3 | 0.2 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 77 (Continued)

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| wtTadA-Cas9n | A | 100.0 | 100.0 | 99.9 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-Cas9n | A | 100.0 | 99.8 | 80.1 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | 19.9 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA-[short]-wtTadA-Cas9n | A | 100.0 | 100.0 | 99.8 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-[short]-mutTadA-Cas9n | A | 99.8 | 97.0 | 52.0 | 99.7 | 99.7 | 99.8 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 3.0 | 47.9 | 0.3 | 0.3 | 0.2 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA-[long]-mutTadA-Cas9n | A | 99.9 | 98.4 | 72.6 | 99.8 | 99.8 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 1.6 | 27.4 | 0.2 | 0.2 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-[long]-mutTadA-Cas9n | A | 99.9 | 98.0 | 61.9 | 99.8 | 99.6 | 99.8 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 2.0 | 38.1 | 0.2 | 0.4 | 0.2 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 78

| | | EMX1 | | FANCF | HEK3 | | HEK4 | RNF2 | |
|---|---|---|---|---|---|---|---|---|---|
| | | $A_2$ | $A_8$ | $A_3$ | $A_4$ | $A_6$ | $A_8$ | $A_4$ | $A_4$ | $A_9$ |
| wtTadA-Cas9n | A | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-Cas9n | A | 100.0 | 100.0 | 99.9 | 99.7 | 99.7 | 99.4 | 99.8 | 99.8 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.2 | 0.3 | 0.5 | 0.2 | 0.2 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA-[short]-wtTadA-Cas9n | A | 100.0 | 100.0 | | | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-[short]-mutTadA-Cas9n | A | 99.9 | 99.8 | 99.9 | 99.6 | 99.3 | 98.3 | 99.1 | 99.5 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 0.2 | 0.1 | 0.4 | 0.7 | 1.6 | 0.9 | 0.4 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| wtTadA-[long]-mutTadA-Cas9n | A | 99.9 | 99.8 | 100.0 | 99.9 | 99.6 | 98.8 | 99.7 | 99.8 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 0.2 | 0.0 | 0.1 | 0.4 | 1.2 | 0.2 | 0.2 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| mutTadA-[long]-mutTadA-Cas9n | A | 99.9 | 99.8 | 99.9 | 99.7 | 99.4 | 98.4 | 99.4 | 99.7 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 0.2 | 0.1 | 0.3 | 0.6 | 1.6 | 0.5 | 0.3 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 79

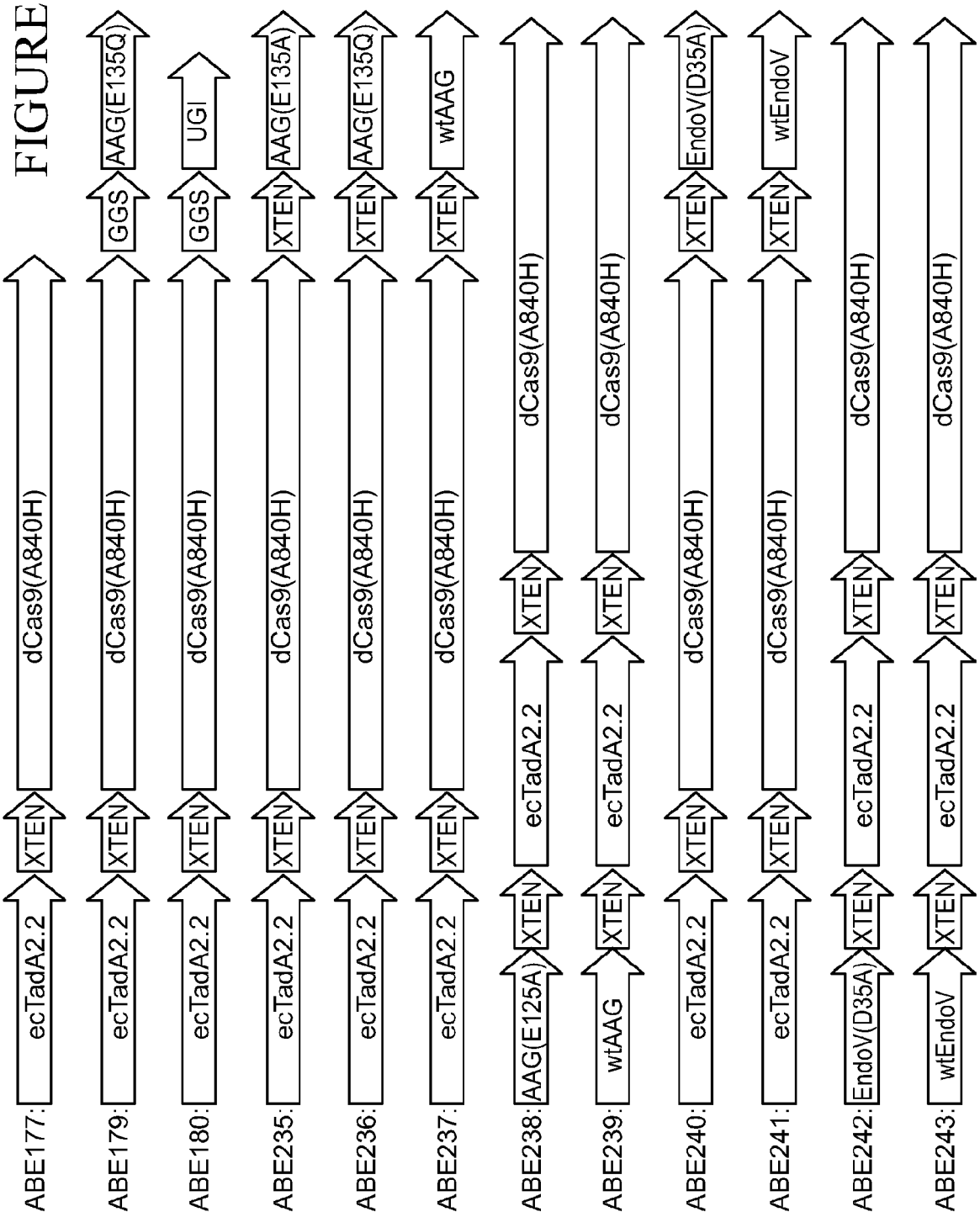

ABE177:
ecTadA2.2
XTEN
dCas9(A840H)
ABE179:
ecTadA2.2
XTEN
dCas9(A840H)
GGS
AAG(E135Q)
ABE180:
ecTadA2.2
XTEN
dCas9(A840H)
GGS
UGI
ABE235:
ecTadA2.2
XTEN
dCas9(A840H)
XTEN
AAG(E135A)
ABE236:
ecTadA2.2
XTEN
dCas9(A840H)
XTEN
AAG(E135Q)
ABE237:
ecTadA2.2
XTEN
dCas9(A840H)
XTEN
wtAAG
ABE238:
AAG(E125A)
XTEN
ecTadA2.2
XTEN
dCas9(A840H)
ABE239:
wtAAG
XTEN
ecTadA2.2
XTEN
dCas9(A840H)
ABE240:
ecTadA2.2
XTEN
dCas9(A840H)
XTEN
EndoV(D35A)
ABE241:
ecTadA2.2
XTEN
dCas9(A840H)
XTEN
wtEndoV
ABE242:
EndoV(D35A)
XTEN
ecTadA2.2
XTEN
dCas9(A840H)
ABE243:
wtEndoV
XTEN
ecTadA2.2
XTEN
dCas9(A840H)

FIGURE 80

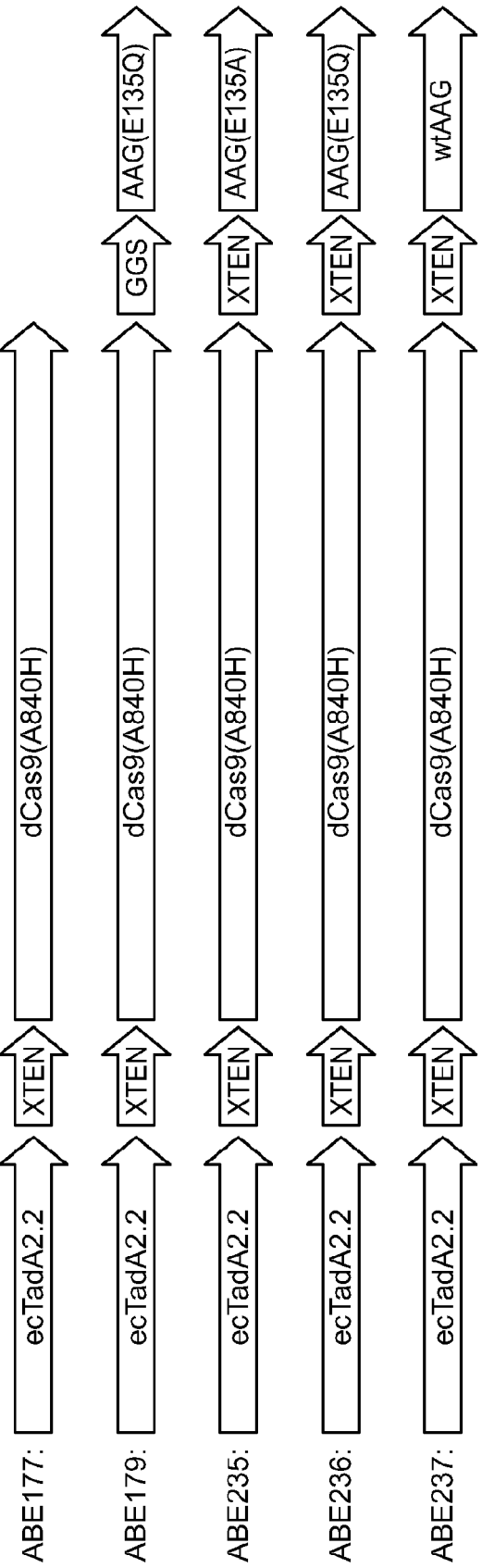



FIGURE 81



# FIGURE 82

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.7 | 74.1 | 100.0 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.3 | 25.9 | 0.0 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.6 | 69.8 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.4 | 30.2 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 235 (pCMV_ecTadA_XTEN_Cas9n_XTEN_AAG*(E125A)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.7 | 77.4 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.3 | 22.6 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 82 (Continued)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 236 (pCMV_ecTadA_XTEN_Cas9n_XTEN_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.9 | 94.4 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 5.6 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 237 (pCMV_ecTadA_XTEN_Cas9n_XTEN_AAG*(wt)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.5 | 62.8 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.5 | 37.1 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 83

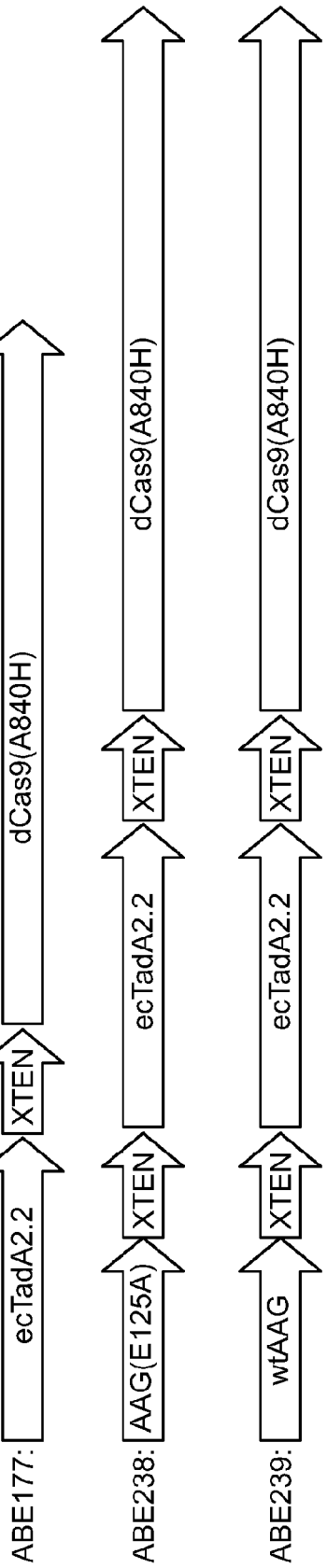
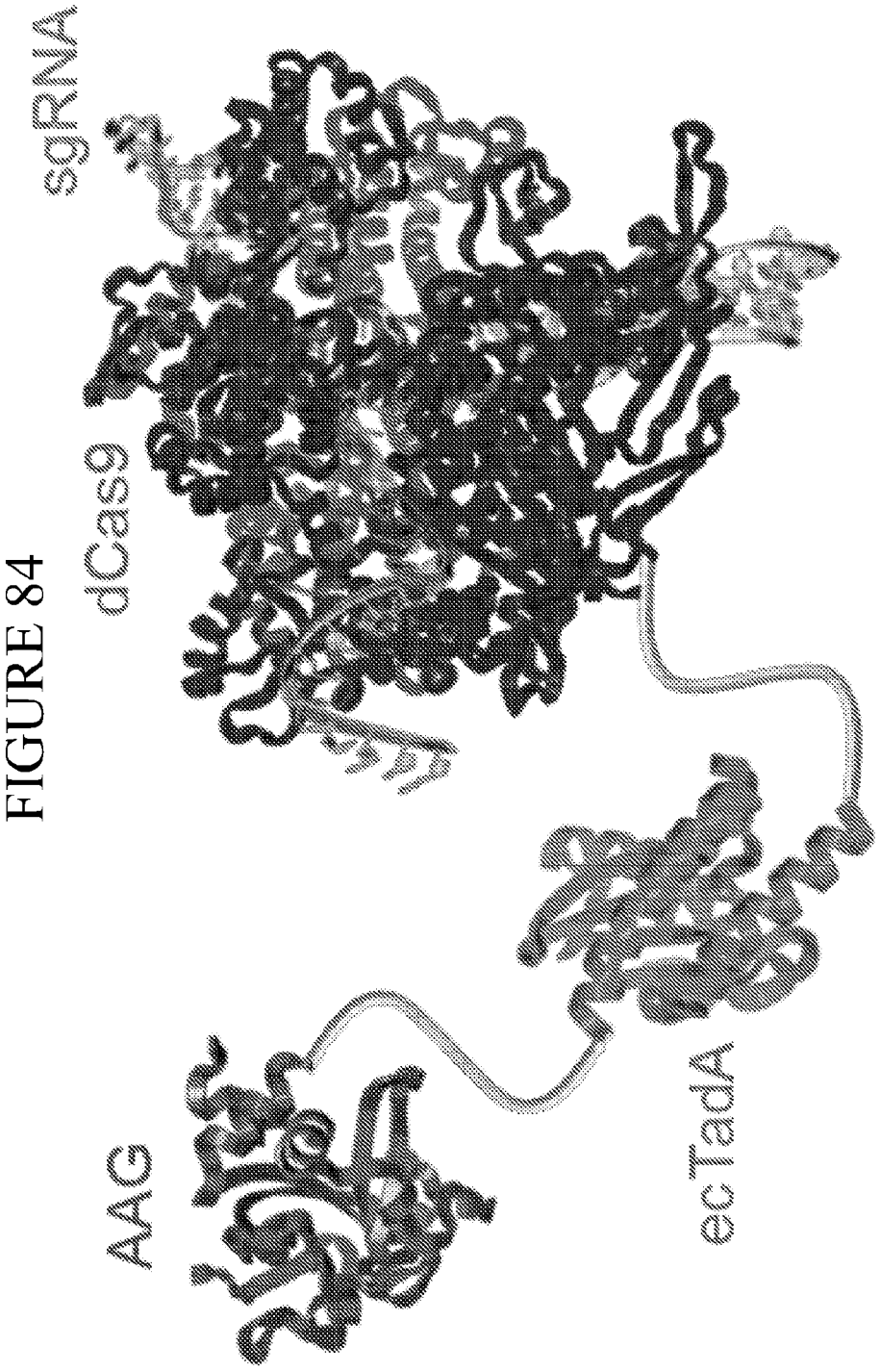

ABE177:
ecTadA2.2
XTEN
dCas9(A840H)
ABE238:
AAG(E125A)
XTEN
ecTadA2.2
XTEN
dCas9(A840H)
ABE239:
wtAAG
XTEN
ecTadA2.2
XTEN
dCas9(A840H)

FIGURE 84



# FIGURE 85

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.7 | 74.1 | 100.0 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.3 | 25.9 | 0.0 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 238 (pCMV_AAG*(E125A)_XTEN_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E15 | A | 100.0 | 99.9 | 74.8 | 99.9 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 25.2 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 239 (pCMV_AAG*(wt)_XTEN_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E15 | A | 100.0 | 99.8 | 69.0 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.2 | 30.9 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 86

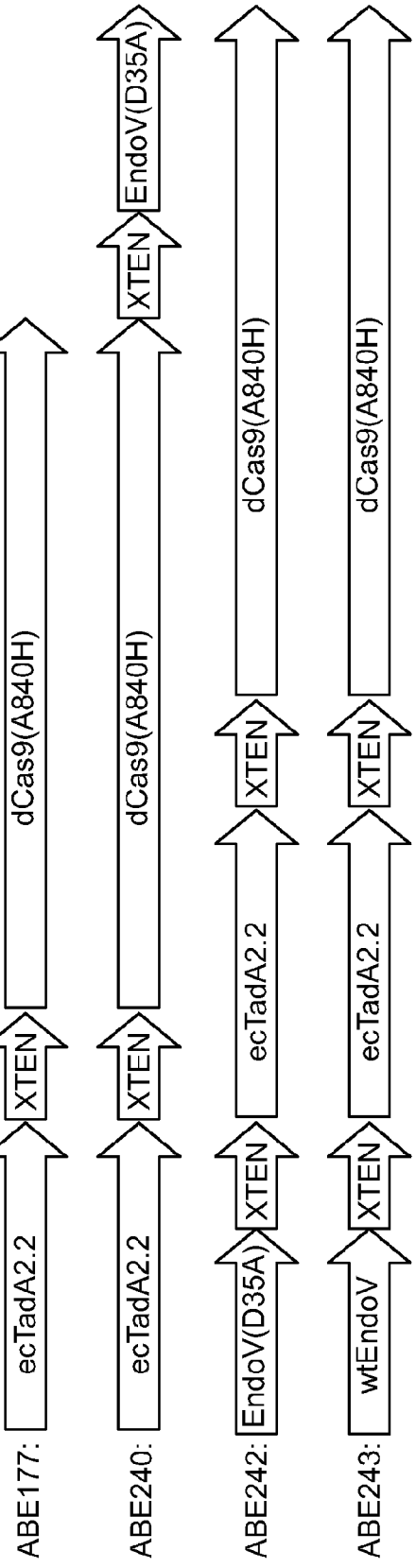

ABE177:
ecTadA2.2
XTEN
dCas9(A840H)
ABE240:
ecTadA2.2
XTEN
dCas9(A840H)
XTEN
EndoV(D35A)
ABE242:
EndoV(D35A)
XTEN
ecTadA2.2
XTEN
dCas9(A840H)
ABE243:
wtEndoV
XTEN
ecTadA2.2
XTEN
dCas9(A840H)

FIGURE 87



FIGURE 88

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.7 | 74.1 | 100.0 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.3 | 25.9 | 0.0 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 240 (pCMV_ecTadA_XTEN_Cas9n_XTEN_EndoV*(D35A)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.7 | 64.8 | 99.9 | 99.9 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.3 | 35.2 | 0.1 | 0.1 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 88 (Continued)

| 242 (pCMV_EndoV*(D35A)_XTEN_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.8 | 53.8 | 100.0 | 99.9 | 100.0 |
|---|---|---|---|---|---|---|---|
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 46.2 | 0.0 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| 243 (pCMV_EndoV*(wt)_XTEN_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.9 | 67.9 | 99.9 | 99.9 | 100.0 |
|---|---|---|---|---|---|---|---|
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| | G | 0.0 | 0.1 | 32.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 89


ABE177:
ecTadA2.2
XTEN
dCas9(A840H)
ABE179:
ecTadA2.2
XTEN
dCas9(A840H)
GGS
AAG(E135Q)
ABE180:
ecTadA2.2
XTEN
dCas9(A840H)
GGS
UGI

# FIGURE 90

Run # 1:

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.6 | 73.3 | 99.9 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.4 | 26.7 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 99.9 | 99.5 | 73.7 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 0.5 | 26.3 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.7 | 68.0 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.3 | 32.0 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

# FIGURE 90 (Continued)

Run # 2:

| HEK2 | | $A_2$ | $A_3$ | $A_5$ | $A_7$ | $A_8$ | $A_9$ |
|---|---|---|---|---|---|---|---|
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.7 | 74.1 | 100.0 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.3 | 25.9 | 0.0 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG*(E125Q)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.6 | 69.8 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.4 | 30.2 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 180 (pCMV_ecTadA_XTEN_Cas9n_GGS_UGI_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 99.9 | 99.6 | 63.9 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.1 | 0.4 | 36.1 | 0.1 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 91

| | | EMX1 | | FANCF | | HEK3 | | HEK4 | RNF2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $A_1$ | $A_0$ | $A_0$ | $A_1$ | $A_0$ | $A_0$ | $A_1$ | $A_1$ | $A_0$ |
| 177 (pCMV_ecTadA_XTEN_Cas9n_GGS_NLS. A106V_D108N_D147Y_E155V) | A | 100.0 | 99.9 | 99.9 | 99.7 | 99.5 | 99.1 | 99.4 | 99.8 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 0.0 | 0.3 | 0.4 | 0.9 | 0.6 | 0.2 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 179 (pCMV_ecTadA_XTEN_Cas9n_GGS_AAG-(E125Q)_GGS_NLS : A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 99.9 | 99.6 | 99.2 | 99.8 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.8 | 0.2 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 91 (Continued)

| | | EMX1 | | FANCF | | HEK3 | | HEK4 | RNF2 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $A_1$ | $A_0$ | $A_0$ | $A_1$ | $A_0$ | $A_0$ | $A_1$ | $A_1$ | $A_0$ |
| 238 (pCMV_AAG-(E125A)_XTEN_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 99.9 | 99.6 | 99.6 | 99.9 | 99.9 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.3 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 239 (pCMV_AAG-(_wt_XTEN_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 99.8 | 99.3 | 99.1 | 99.7 | 99.8 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.2 | 0.6 | 0.9 | 0.3 | 0.2 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 91 (Continued)

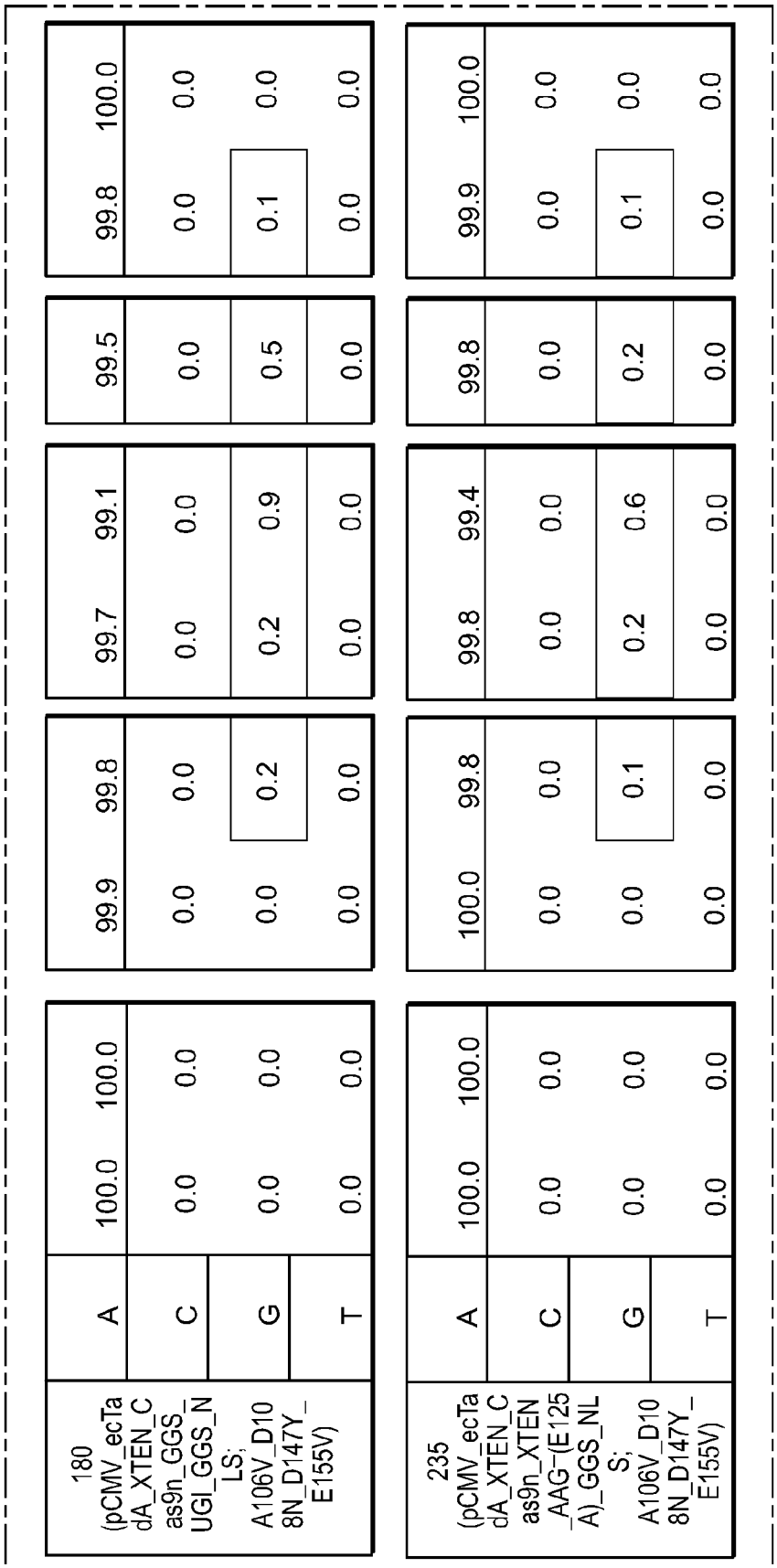


FIGURE 91 (Continued)

| 240 (pCMV_ecTadA_XTEN_Cas9n_XTEN_EndoV-(D35A)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 99.8 | 99.5 | 99.3 | 99.5 | 99.8 | 99.9 |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.2 | 0.5 | 0.7 | 0.5 | 0.2 | 0.1 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| 242 (pCMV_EndoV-(D35A)_XTEN_ecTadA_XTEN_Cas9n GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 99.7 | 99.5 | 99.1 | 99.7 | 99.8 | 100.0 |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.3 | 0.5 | 0.9 | 0.3 | 0.1 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

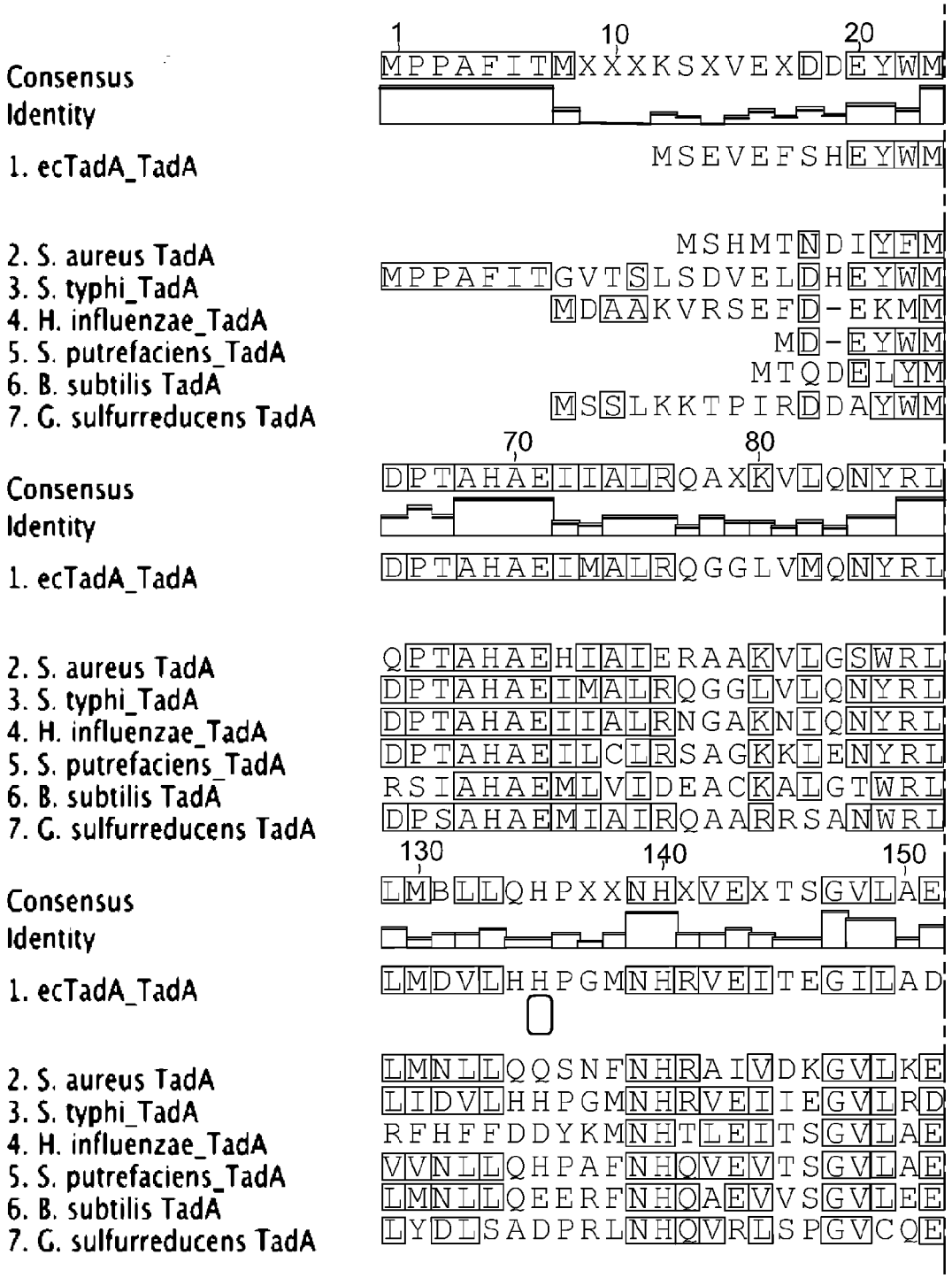
FIGURE 91 (Continued)

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 236 (pCMV_ecTadA_XTEN_Cas9n_XTEN_AAG_(E125Q_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 100.0 | 100.0 | 100.0 | 99.9 | 99.9 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 237 (pCMV_ecTadA_XTEN_Cas9n_XTEN_AAG−(wt)_GGS_NLS; A106V_D108N_D147Y_E155V) | A | 100.0 | 99.9 | 99.9 | 99.6 | 99.3 | 99.0 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.7 | 99.9 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | G | 0.0 | 0.1 | 0.1 | 0.4 | 0.6 | 1.0 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

FIGURE 91 (Continued)

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 243 (pCMV_EndoV_(wt)_XTEN_ecTadA_XTEN_Cas9n_GGS_NLS; A106V_D108N_D147Y_E1055V) | A | 100.0 | 100.0 | 100.0 | 100.0 | 99.8 | 99.8 | 99.9 | 99.9 | 99.9 | 99.9 | | | | | | | | |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | |
| | G | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.2 | 0.1 | 0.1 | 0.1 | 0.0 | | | | | | | | |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | | | | | | | |

# FIGURE 92


1
10
20
Consensus
Identity
1. ecTadA_TadA
2. S. aureus TadA
3. S. typhi_TadA
4. H. influenzae_TadA
5. S. putrefaciens_TadA
6. B. subtilis TadA
7. G. sulfurreducens TadA
70
80
130
140
150

# FIGURE 92 (Continued)



FIGURE 93

| | ecTadA_T... | S. aureus ... | S. typhi_T... | H. influenz... | S. putrefac... | B. subtilis ... | G. sulfurre... |
|---|---|---|---|---|---|---|---|
| ecTadA_TadA | | 41% | 88% | 56% | 58% | 42% | 45% |
| S. aureus TadA | 41% | | 43% | 42% | 49% | 61% | 48% |
| S. typhi_TadA | 88% | 43% | | 53% | 59% | 42% | 44% |
| H. influenzae_TadA | 56% | 42% | 53% | | 55% | 42% | 42% |
| S. putrefaciens_TadA | 58% | 49% | 59% | 55% | | 50% | 44% |
| B. subtilis TadA G. | 42% | 61% | 42% | 42% | 50% | | 52% |
| sulfurreducens TadA | 45% | 48% | 44% | 42% | 44% | 52% | |

FIGURE 94

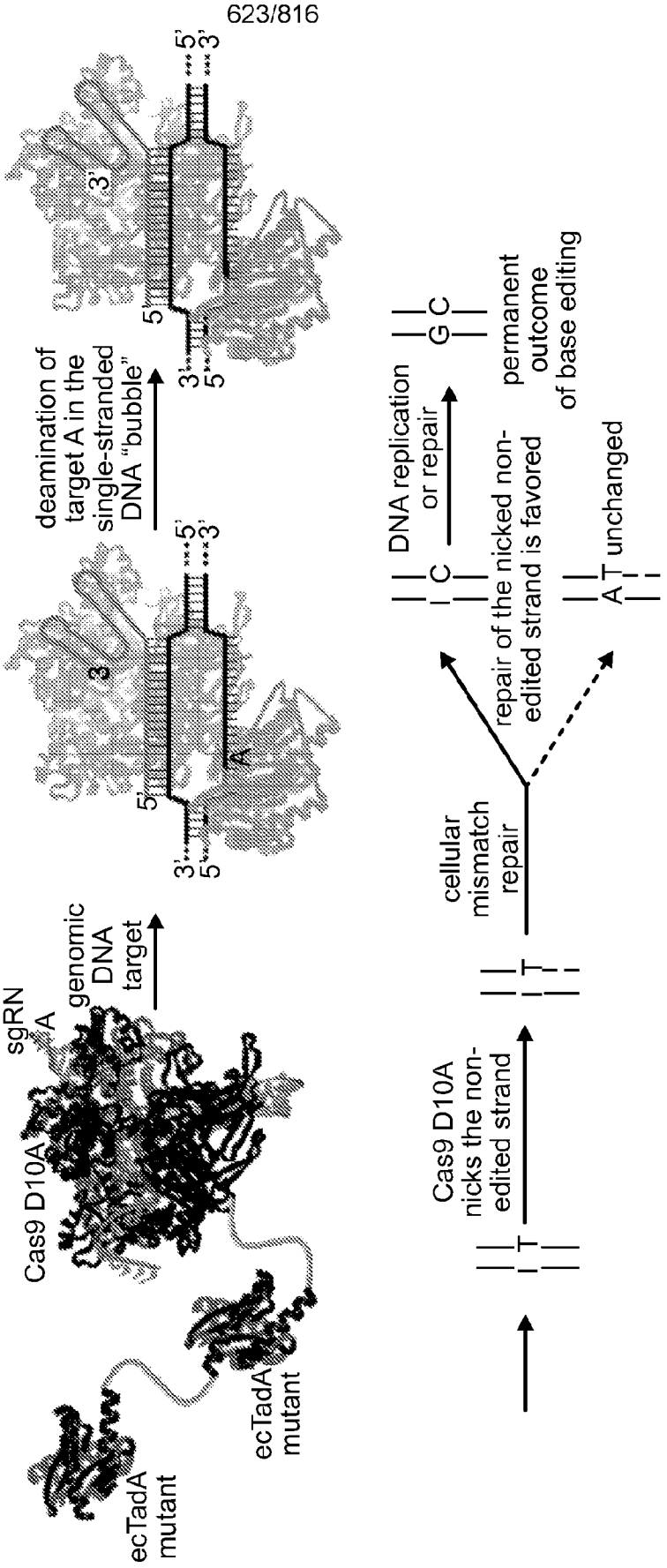

ecTadA mutant
ecTadA mutant
Cas9 D10A
sgRNA
genomic DNA target
deamination of target A in the single-stranded DNA "bubble"
Cas9 D10A nicks the non-edited strand
cellular mismatch repair
repair of the nicked non-edited strand is favored
DNA replication or repair
permanent outcome of base editing
unchanged

FIGURE 95

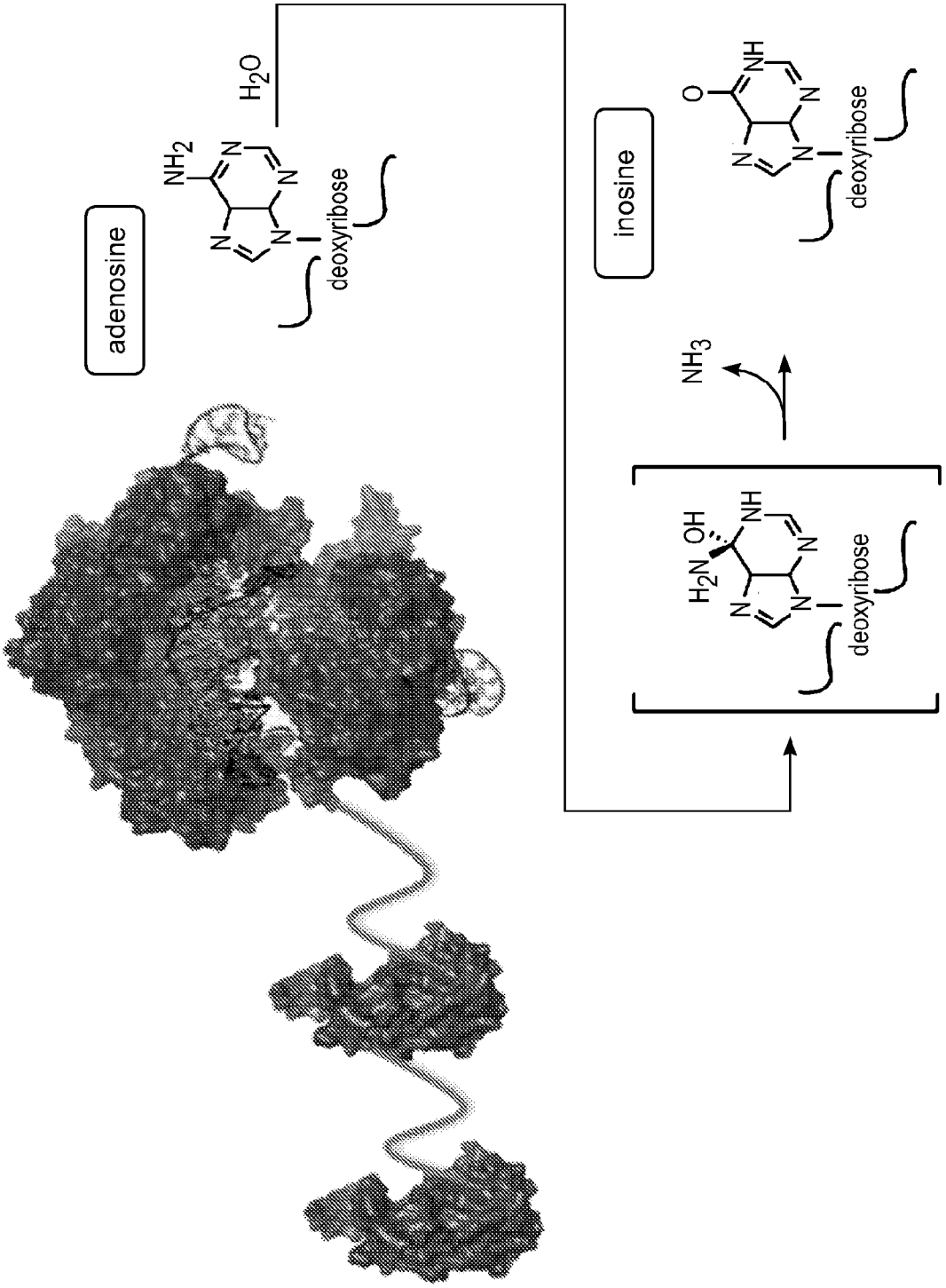

adenosine
$H_2O$
$NH_2$
deoxyribose
inosine
O
NH
deoxyribose
$NH_3$
$H_2N$
OH
NH
deoxyribose

FIGURE 96

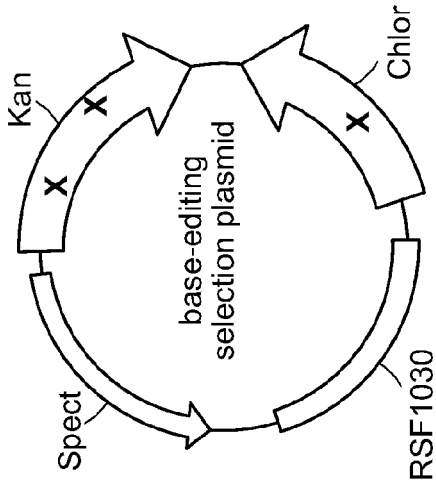



FIGURE 97

| wt res | S2 | H8 | I49 | L84 | A106 | D108 | H123 | N127 | D147 | E155 | I156 | K160 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| clone 1 | | | | Phe | Val | Arg | Tyr | | Tyr | Val | Phe | |
| clone 2 | Ala | | Phe | | Val | Arg | | | Tyr | Val | | |
| clone 3 | | Tyr | | | Thr | Arg | | Ser | Asp | Glu | | Ser |

FIGURE 98

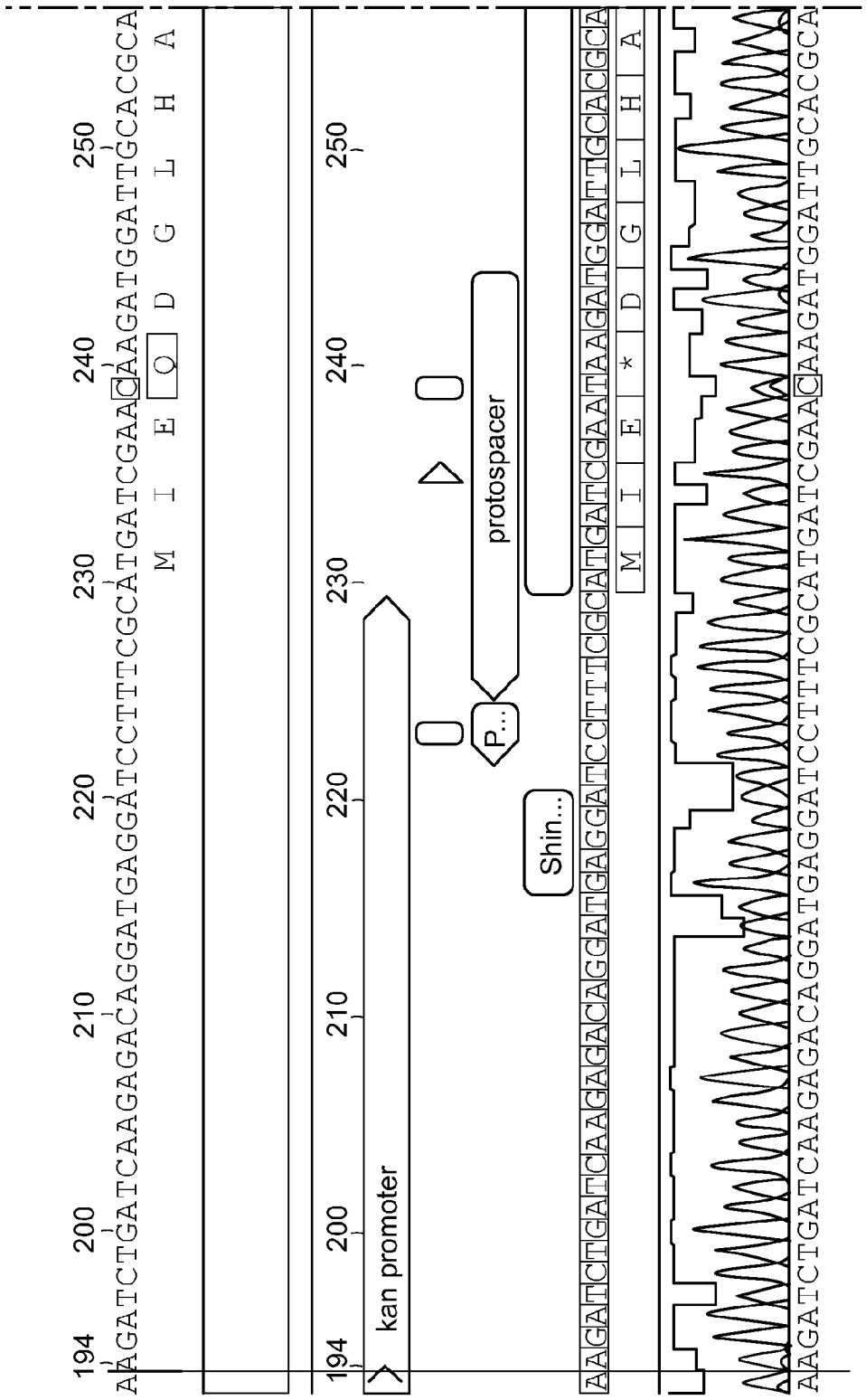

194
200
210
220
230
240
250
kan promoter
P...
protospacer
Shin...
M I E Q D G L H A
M I E * D G L H A

FIGURE 98 (Continued)

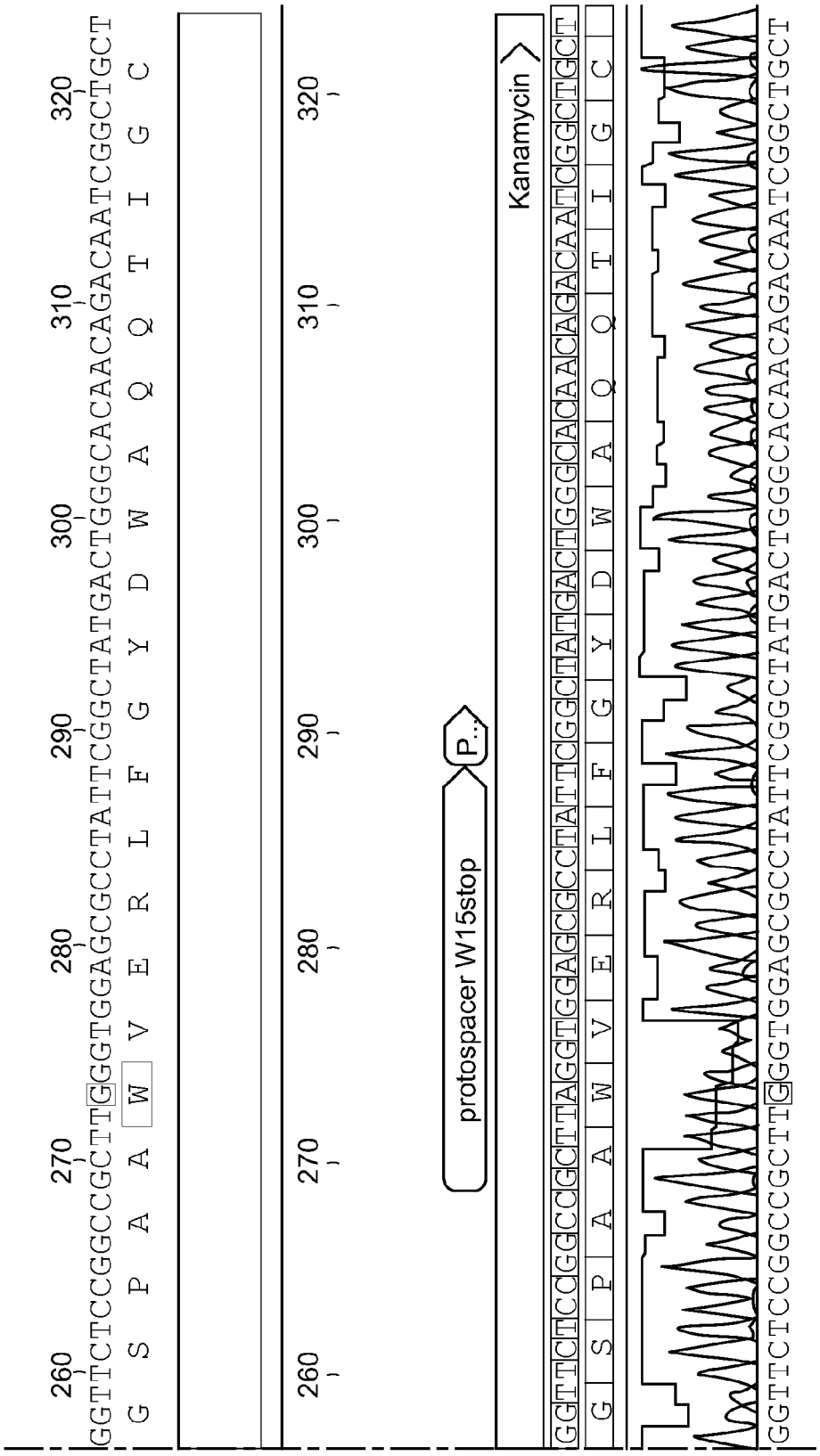
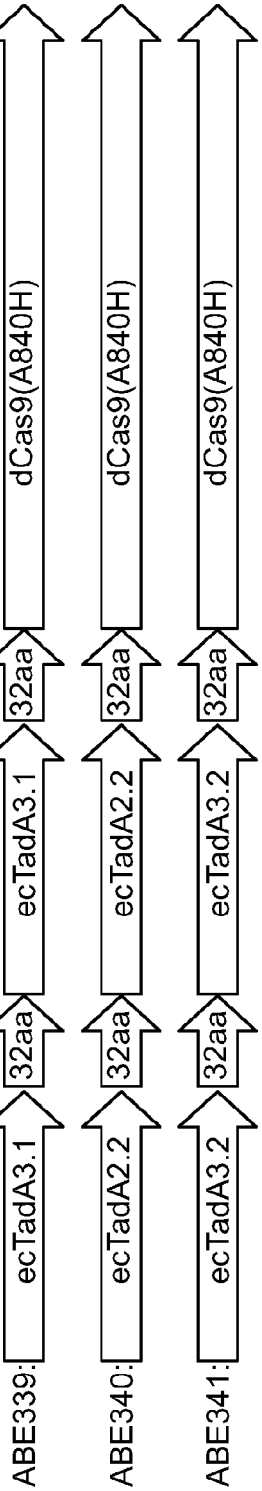


FIGURE 99


ABE339:
ecTadA3.1
32aa
ecTadA3.1
32aa
dCas9(A840H)
ABE340:
ecTadA2.2
32aa
ecTadA2.2
32aa
dCas9(A840H)
ABE341:
ecTadA3.2
32aa
ecTadA3.2
32aa
dCas9(A840H)

# FIGURE 100

| HEK2 | G | $A_2$ | $A_3$ | C | $A_5$ | C | $A_7$ | $A_8$ | $A_9$ | G |
|---|---|---|---|---|---|---|---|---|---|---|
| 339 | | 0.1% | 5.0% | | 58.6% | | 1.6% | 1.9% | 0.9% | |
| 340 | | 0.1% | 2.6% | | 61.2% | | 0.5% | 1.1% | 0.6% | |
| 341 | | 0.1% | 6.0% | | 67.2% | | 1.7% | 1.6% | 1.1% | |

| HEK2 | G | G | $A_3$ | $A_4$ | C | $A_6$ | C | $A_8$ | $A_9$ | $A_{10}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| 339 | | | 2.2% | 56.7% | | 59.0% | | 6.6% | 23.3% | 2.1% |
| 340 | | | 1.4% | 31.0% | | 36.0% | | 2.6% | 19.6% | 1.5% |
| 341 | | | 1.9% | 57.1% | | 61.2% | | 5.7% | 18.1% | 2.0% |

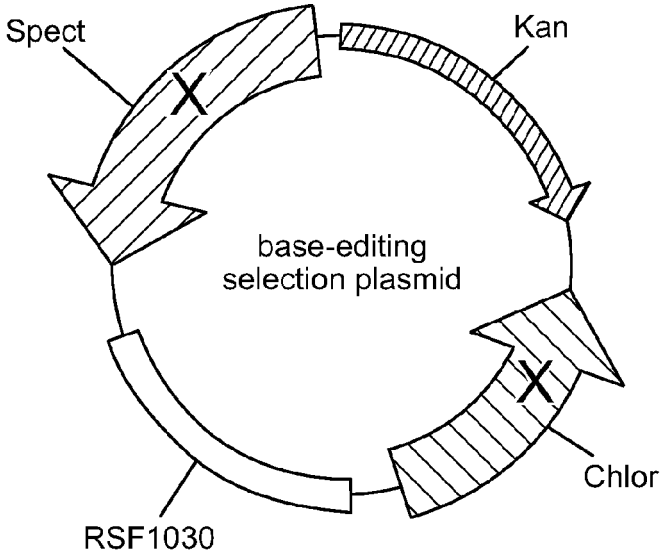
| HEK2-3 | G | T | A | $A_4$ | $A_5$ | C | $A_7$ | $A_8$ | $A_9$ | G |
|---|---|---|---|---|---|---|---|---|---|---|
| 339 | | | 0.5% | 1.0% | 19.3% | | 0.6% | 1.5% | 0.7% | |
| 340 | | | 0.2% | 0.4% | 17.2% | | 0.2% | 0.7% | 0.3% | |
| 341 | | | 0.5% | 1.0% | 19.5% | | 0.6% | 1.3% | 0.5% | |

| HEK2-6 | G | $A_2$ | $A_3$ | G | $A_5$ | C | C | $A_8$ | $A_9$ | G |
|---|---|---|---|---|---|---|---|---|---|---|
| 339 | | 0.0% | 0.1% | | 7.2% | | | 0.4% | 0.3% | |
| 340 | | 0.0% | 0.0% | | 6.1% | | | 0.2% | 0.1% | |
| 341 | | 0.0% | 0.0% | | 6.6% | | | 0.4% | 0.3% | |

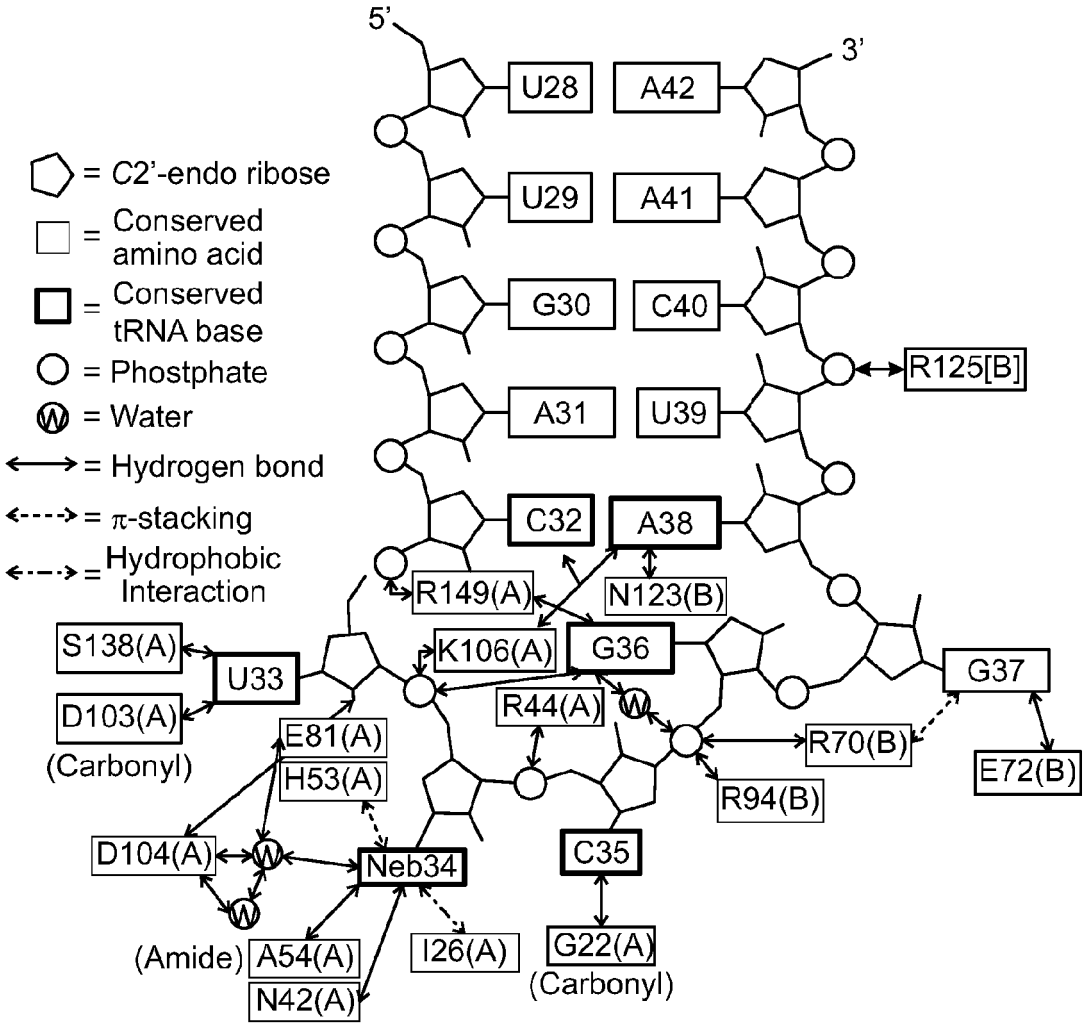
| HEK2-7 | G | $A_2$ | $A_3$ | $A_4$ | $A_5$ | C | $A_7$ | $A_8$ | $A_9$ | T |
|---|---|---|---|---|---|---|---|---|---|---|
| 339 | | 0.0% | 0.0% | 0.0% | 0.6% | | 0.0% | 0.0% | 0.0% | |
| 340 | | 0.0% | 0.0% | 0.0% | 0.3% | | 0.0% | 0.0% | 0.0% | |
| 341 | | 0.0% | 0.0% | 0.0% | 0.4% | | 0.1% | 0.0% | 0.0% | |

| HEK2-8 | G | A | T | C | $A_5$ | C | $A_7$ | $A_8$ | $A_9$ | G |
|---|---|---|---|---|---|---|---|---|---|---|
| 339 | | 0.2% | | | 23.9% | | 0.5% | 0.5% | 0.3% | |
| 340 | | 0.1% | | | 26.0% | | 0.2% | 0.2% | 0.1% | |
| 341 | | 0.2% | | | 27.5% | | 0.5% | 0.4% | 0.3% | |

# FIGURE 101



# FIGURE 102


5'
3'
U28
A42
U29
A41
G30
C40
A31
U39
C32
A38
R125[B]
= C2'-endo ribose
= Conserved amino acid
= Conserved tRNA base
= Phostphate
= Water
= Hydrogen bond
= π-stacking
= Hydrophobic Interaction
R149(A)
N123(B)
S138(A)
U33
K106(A)
G36
G37
D103(A)
(Carbonyl)
E81(A)
H53(A)
R44(A)
R70(B)
E72(B)
R94(B)
D104(A)
Neb34
C35
(Amide)
A54(A)
N42(A)
I26(A)
G22(A)
(Carbonyl)

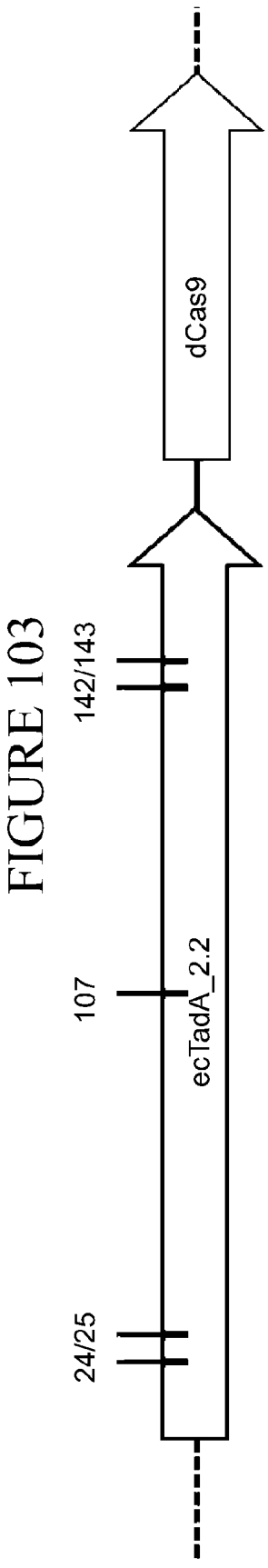



FIGURE 103

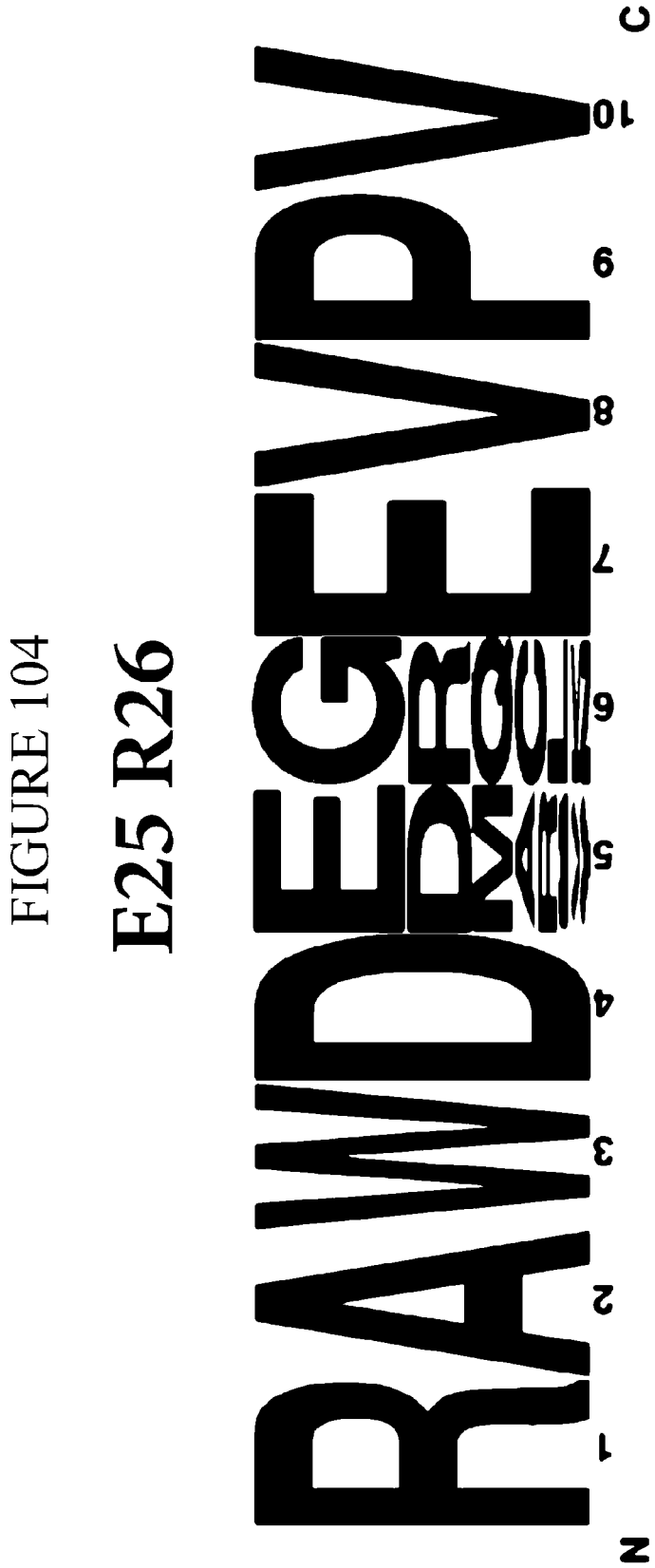



FIGURE 104

E25 R26

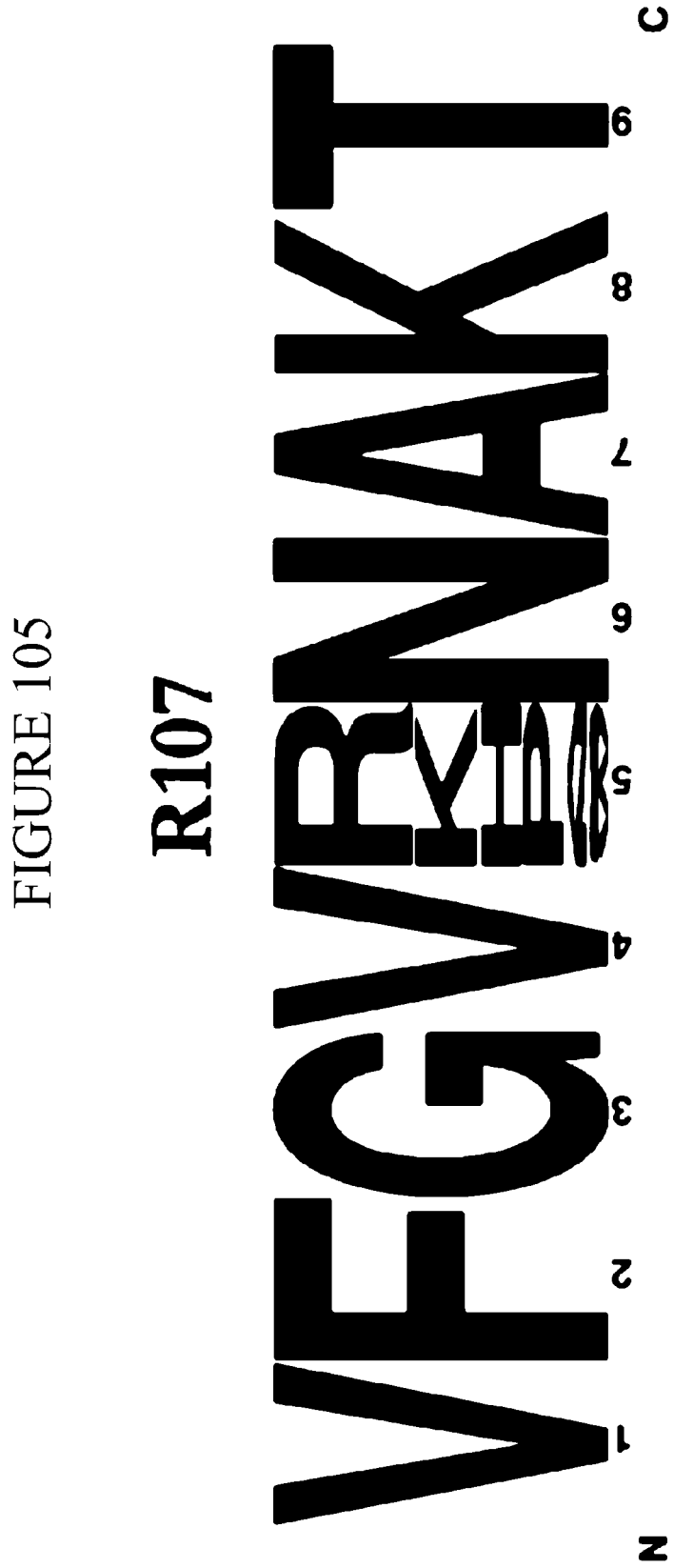



FIGURE 105




FIGURE 106

FIGURE 107

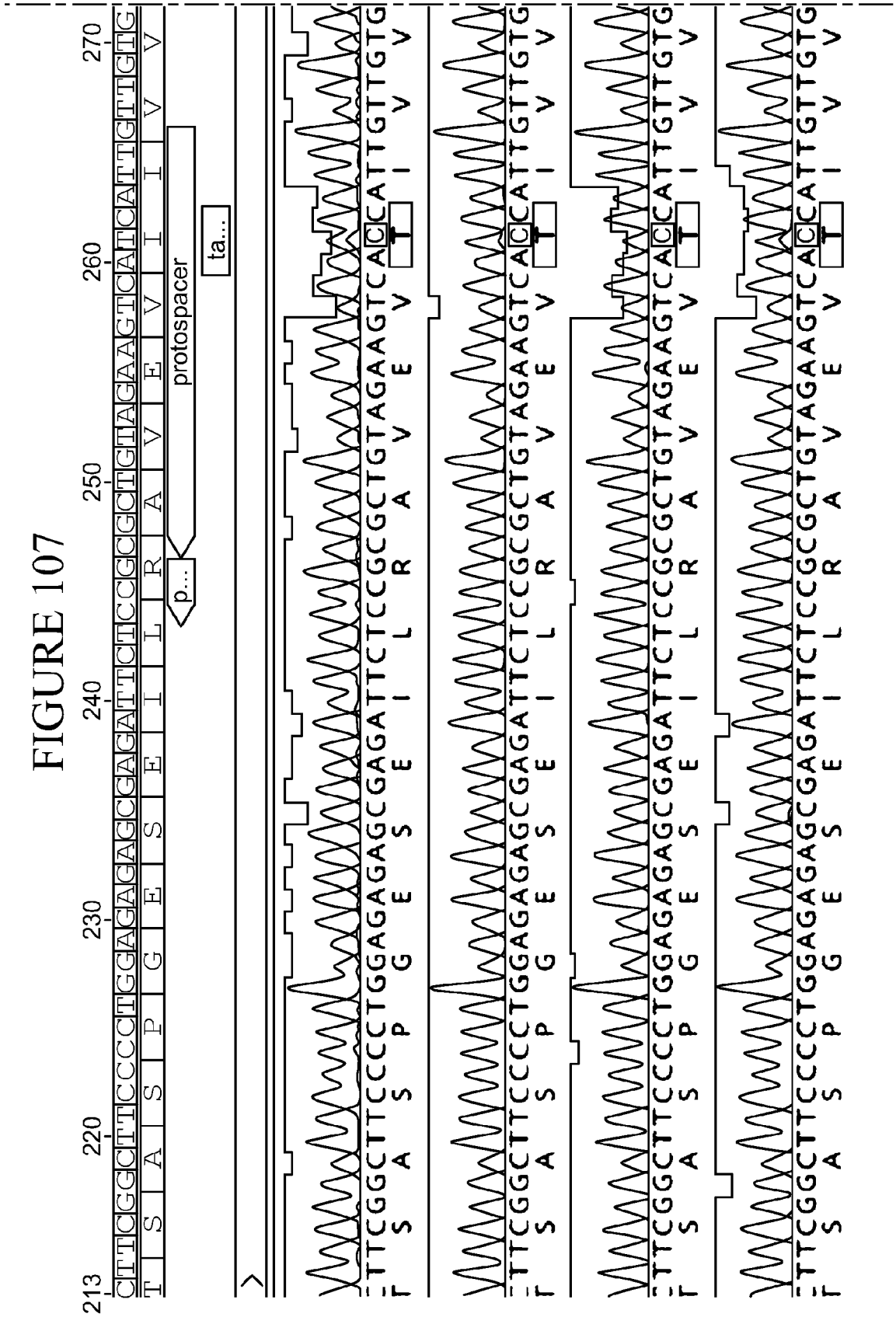
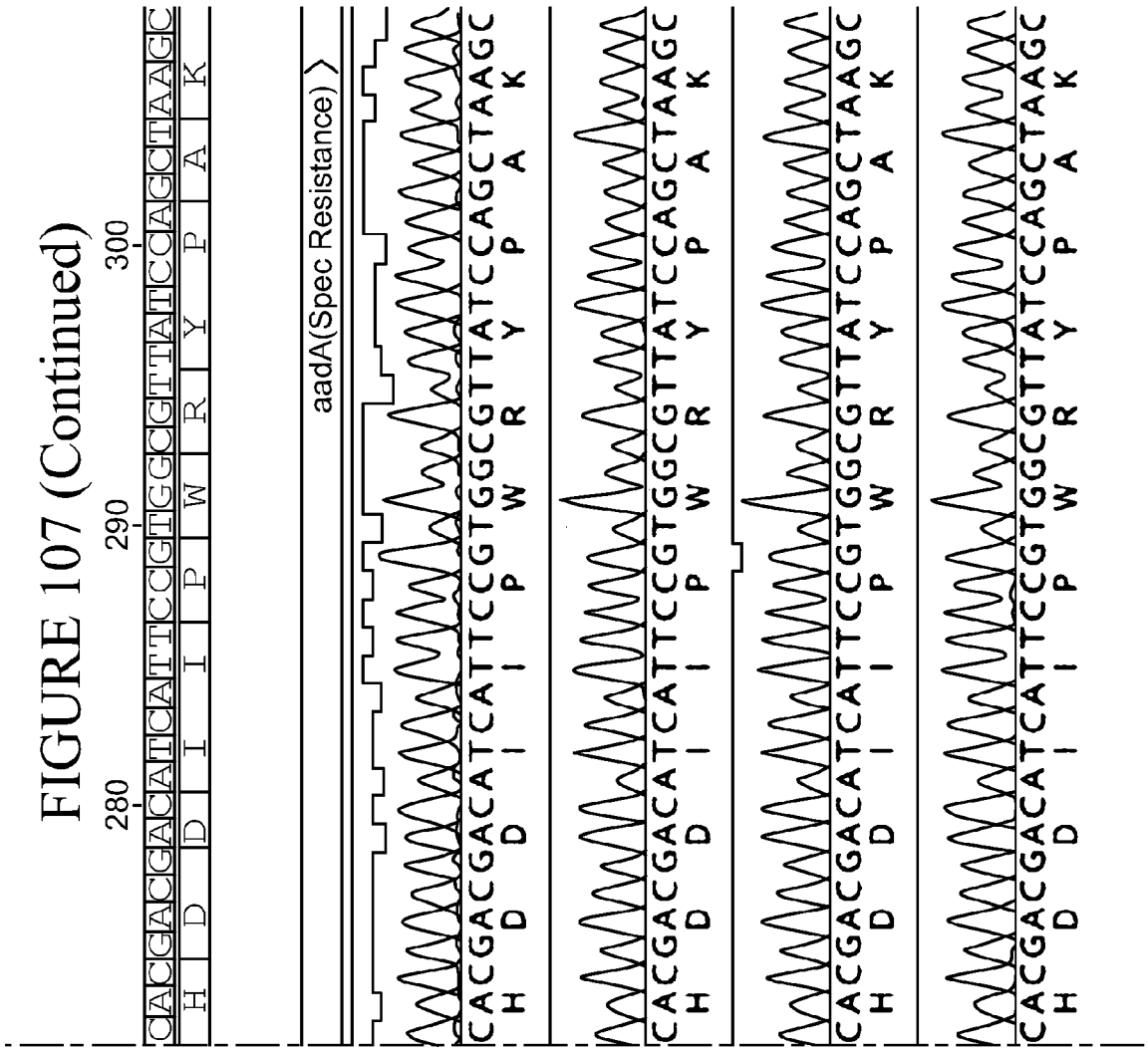


FIGURE 107 (Continued)



# FIGURE 108

Hek-2 site: 5'-GA$_2$A$_3$CA$_5$CA$_7$A$_8$A$_9$GCA$_{12}$TA$_{14}$GA$_{16}$CTGCGGG-3'

| HEK2 pNMG-369 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #3 | A | 99.93% | 97.44% | 60.06% | 99.03% | 99.17% | 99.59% | 99.96% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.07% | 2.56% | 39.91% | 0.96% | 0.83% | 0.41% | 0.03% | 0.02% | 0.0% |
| | T | 0.00% | 0.01% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.0% |

| HEK2 pNMG-370 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #2 | A | 99.94% | 98.55% | 57.73% | 99.71% | 99.40% | 99.80% | 99.96% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.05% | 1.44% | 42.25% | 0.29% | 0.59% | 0.20% | 0.03% | 0.02% | 0.0% |
| | T | 0.01% | 0.01% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.0% |

| HEK2 pNMG-371 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #3 | A | 99.91% | 96.97% | 54.22% | 99.11% | 99.06% | 99.41% | 99.96% | 99.98% | 100.0% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.09% | 3.02% | 45.76% | 0.89% | 0.93% | 0.59% | 0.03% | 0.01% | 0.0% |
| | T | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.0% |

FIGURE 108 (Continued)

| HEK2 pNMG-360 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.92% | 98.69% | 72.23% | 99.68% | 99.71% | 99.92% | 99.95% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.07% | 1.30% | 27.75% | 0.32% | 0.28% | 0.07% | 0.04% | 0.02% | 0.0% |
| | T | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.0% |

| HEK2 pNMG-361 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.94% | 98.96% | 89.63% | 99.81% | 99.68% | 99.92% | 99.98% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.05% | 1.04% | 10.36% | 0.18% | 0.31% | 0.08% | 0.01% | 0.02% | 0.0% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |

| HEK2 pNMG-362 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.96% | 99.14% | 67.24% | 99.78% | 99.79% | 99.86% | 99.91% | 99.98% | 100.0% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.04% | 0.86% | 32.75% | 0.21% | 0.20% | 0.14% | 0.08% | 0.01% | 0.0% |
| | T | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.0% |

# FIGURE 108 (Continued)

Hek - 2 site: 5'-$GA_2A_3CA_5CA_7A_8A_9GCA_{12}TA_{14}GA_{16}$ CTGCGGG-3'

| HEK2 pNMG-363 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.92% | 98.69% | 72.23% | 99.68% | 99.71% | 99.92% | 99.95% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.07% | 1.30% | 27.75% | 0.32% | 0.28% | 0.07% | 0.04% | 0.02% | 0.0% |
| | T | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.0% |

| HEK2 pNMG-364 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.97% | 99.01% | 65.38% | 99.68% | 99.78% | 99.90% | 99.95% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.03% | 0.98% | 34.60% | 0.32% | 0.21% | 0.10% | 0.04% | 0.02% | 0.0% |
| | T | 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.0% |

| HEK2 pNMG-365 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.97% | 99.08% | 72.04% | 99.81% | 99.78% | 99.90% | 99.95% | 99.98% | 100.0% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.03% | 0.91% | 27.94% | 0.18% | 0.21% | 0.10% | 0.04% | 0.01% | 0.0% |
| | T | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.0% |

FIGURE 108 (Continued)

| HEK2 pNMG-366 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.90% | 98.23% | 71.10% | 99.59% | 99.73% | 99.85% | 99.93% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.10% | 1.76% | 28.89% | 0.41% | 0.26% | 0.15% | 0.06% | 0.02% | 0.0% |
| | T | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.0% |

| HEK2 pNMG-367 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.92% | 98.73% | 66.04% | 99.68% | 99.78% | 99.90% | 99.95% | 99.97% | 100.0% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.08% | 1.26% | 33.94% | 0.32% | 0.21% | 0.10% | 0.04% | 0.02% | 0.0% |
| | T | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.0% |

| HEK2 pNMG-368 | | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| Evo #4 | A | 99.97% | 99.72% | 99.32% | 99.95% | 99.92% | 99.96% | 99.97% | 99.98% | 100.0% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.0% |
| | G | 0.02% | 0.27% | 0.67% | 0.05% | 0.08% | 0.03% | 0.02% | 0.01% | 0.0% |
| | T | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.0% |

# FIGURE 109

HEK2-3 site: 5'-GT$A_3A_4A_5CA_7A_8A_9GCA_{12}TA_{14}GA_{16}$ CTGAGGG-3'

| HEK2-3 pNMG-369 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.73% | 99.58% | 93.69% | 99.55% | 99.15% | 99.93% | 99.96% | 99.98% | 100.00% |
| C | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| G | 0.25% | 0.41% | 6.30% | 0.44% | 0.85% | 0.07% | 0.02% | 0.01% | 0.00% |
| T | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK2-3 pNMG-370 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.93% | 99.89% | 94.96% | 99.92% | 99.74% | 99.98% | 99.99% | 99.99% | 99.99% |
| C | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.06% | 0.11% | 5.03% | 0.07% | 0.25% | 0.02% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |

| HEK2-3 pNMG-371 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.69% | 99.59% | 93.55% | 99.75% | 99.46% | 99.94% | 99.96% | 99.98% | 99.99% |
| C | 0.01% | 0.01% | 0.01% | 0.02% | 0.02% | 0.01% | 0.02% | 0.01% | 0.01% |
| G | 0.30% | 0.40% | 6.45% | 0.23% | 0.52% | 0.05% | 0.01% | 0.01% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

FIGURE 109 (Continued)

| HEK2-3 pNMG-360 | | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| | A | 99.82% | 99.89% | 98.74% | 99.90% | 99.92% | 99.98% | 99.98% | 99.99% | 99.99% |
| | C | 0.00% | 0.00% | 0.01% | 0.02% | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.18% | 0.10% | 1.25% | 0.08% | 0.06% | 0.01% | 0.01% | 0.01% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK2-3 pNMG-361 | | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| | A | 99.97% | 99.98% | 99.54% | 99.97% | 99.98% | 99.99% | 99.98% | 99.99% | 99.99% |
| | C | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.03% | 0.02% | 0.44% | 0.02% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |

| HEK2-3 pNMG-362 | | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| | A | 99.91% | 99.94% | 98.06% | 99.93% | 99.89% | 99.96% | 99.98% | 99.98% | 99.99% |
| | C | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.09% | 0.06% | 1.93% | 0.06% | 0.10% | 0.04% | 0.02% | 0.02% | 0.00% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |

# FIGURE 109 (Continued)

HEK2-3 site: 5'-GT$A_3A_4A_5CA_7A_8A_9GCA_{12}TA_{14}GA_{16}$ CTGAGGG-3'

| HEK2-3 pNMG-363 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.80% | 99.91% | 97.59% | 99.90% | 99.89% | 99.97% | 99.96% | 99.99% | 99.99% |
| C | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| G | 0.19% | 0.09% | 2.40% | 0.09% | 0.10% | 0.03% | 0.02% | 0.01% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% |

| HEK2-3 pNMG-364 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.96% | 99.97% | 99.47% | 99.96% | 99.98% | 100.00% | 99.99% | 99.98% | 100.00% |
| C | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.03% | 0.02% | 0.51% | 0.04% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |

| HEK2-3 pNMG-365 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.91% | 99.97% | 98.49% | 99.95% | 99.92% | 99.97% | 99.92% | 99.98% | 100.00% |
| C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.09% | 0.02% | 1.51% | 0.04% | 0.07% | 0.02% | 0.07% | 0.01% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

FIGURE 109 (Continued)

| HEK2-3 pNMG-366 | | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| | A | 99.70% | 99.86% | 97.73% | 99.68% | 99.77% | 99.97% | 99.98% | 99.99% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.29% | 0.14% | 2.27% | 0.30% | 0.22% | 0.02% | 0.02% | 0.00% | 0.00% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |

| HEK2-3 pNMG-367 | | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| | A | 99.87% | 99.89% | 97.80% | 99.87% | 99.91% | 99.97% | 99.92% | 99.98% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | G | 0.12% | 0.10% | 2.19% | 0.11% | 0.08% | 0.02% | 0.05% | 0.02% | 0.01% |
| | T | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |

| HEK2-3 pNMG-368 | | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A |
| | A | 99.97% | 100.00% | 100.00% | 99.99% | 99.98% | 100.00% | 99.98% | 99.98% | 100.00% |
| | C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| | G | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |
| | T | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |

# FIGURE 110

HEK2-6: 5'-G$A_2A_3G A_5CCA_8A_9GGA_{12}TA_{14}$GACTGCTGG-3'

| HEK2-6 pNMG-369 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.80% | 99.94% | 95.27% | 99.70% | 99.74% | 99.94% | 99.94% |
| | C | 0.07% | 0.06% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.13% | 0.00% | 4.67% | 0.30% | 0.26% | 0.06% | 0.06% |
| | T | 0.00% | 0.00% | 0.06% | 0.00% | 0.00% | 0.00% | 0.00% |

| HEK2-6 pNMG-370 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.97% | 99.98% | 96.45% | 99.77% | 99.92% | 99.98% | 99.98% |
| | C | 0.03% | 0.02% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% |
| | G | 0.00% | 0.00% | 3.50% | 0.23% | 0.03% | 0.02% | 0.02% |
| | T | 0.00% | 0.00% | 0.05% | 0.00% | 0.03% | 0.00% | 0.00% |

| HEK2-6 pNMG-371 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.97% | 99.94% | 96.33% | 99.80% | 99.78% | 100.00% | 100.00% |
| | C | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.03% | 0.00% | 3.67% | 0.20% | 0.19% | 0.00% | 0.00% |
| | T | 0.00% | 0.03% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% |

FIGURE 110 (Continued)

| HEK2-6 pNMG-360 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 100.00% | 99.96% | 99.03% | 99.96% | 99.91% | 99.96% | 100.00% |
| | C | 0.00% | 0.04% | 0.00% | 0.00% | 0.09% | 0.00% | 0.00% |
| | G | 0.00% | 0.00% | 0.97% | 0.04% | 0.00% | 0.04% | 0.00% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

| HEK2-6 361 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.97% | 99.97% | 99.67% | 100.00% | 99.97% | 99.97% | 100.00% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.03% | 0.03% | 0.30% | 0.00% | 0.00% | 0.03% | 0.00% |
| | T | 0.00% | 0.00% | 0.03% | 0.00% | 0.03% | 0.00% | 0.00% |

| HEK2-6 362 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.96% | 99.92% | 98.65% | 99.94% | 99.98% | 99.94% | 99.98% |
| | C | 0.02% | 0.08% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.02% | 0.00% | 1.35% | 0.04% | 0.02% | 0.04% | 0.02% |
| | T | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.02% | 0.00% |

# FIGURE 110 (Continued)

HEK2-6: 5'-$GA_2A_3GA_5CCA_8A_9GGA_{12}TA_{14}$GACTGCTGG-3'

| HEK2-6 363 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.97% | 99.97% | 95.64% | 99.93% | 99.99% | 99.99% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.03% | 0.03% | 4.36% | 0.05% | 0.01% | 0.00% | 0.00% |
| | T | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |

| HEK2-6 364 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.98% | 99.98% | 99.59% | 99.98% | 99.90% | 99.98% | 100.00% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% |
| | G | 0.00% | 0.02% | 0.39% | 0.02% | 0.06% | 0.02% | 0.00% |
| | T | 0.02% | 0.00% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |

| HEK2-6 365 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A |
| | A | 99.95% | 100.00% | 97.12% | 99.95% | 99.82% | 99.85% | 100.00% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% |
| | G | 0.03% | 0.00% | 2.86% | 0.02% | 0.18% | 0.10% | 0.00% |
| | T | 0.03% | 0.00% | 0.02% | 0.02% | 0.00% | 0.03% | 0.00% |

FIGURE 110 (Continued)

| HEK2-6 366 | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A |
| A | 99.90% | 99.96% | 97.54% | 99.82% | 99.98% | 99.88% | 99.98% |
| C | 0.04% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% |
| G | 0.02% | 0.04% | 2.46% | 0.15% | 0.00% | 0.08% | 0.02% |
| T | 0.04% | 0.00% | 0.00% | 0.04% | 0.00% | 0.04% | 0.00% |

| HEK2-6 367 | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A |
| A | 99.89% | 99.99% | 97.00% | 99.93% | 99.94% | 99.96% | 99.99% |
| C | 0.03% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% |
| G | 0.06% | 0.01% | 2.97% | 0.04% | 0.03% | 0.00% | 0.00% |
| T | 0.02% | 0.00% | 0.01% | 0.01% | 0.01% | 0.03% | 0.01% |

| HEK2-6 368 | 2 | 3 | 5 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A |
| A | 99.92% | 99.96% | 99.96% | 99.99% | 99.99% | 99.98% | 100.00% |
| C | 0.02% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| G | 0.04% | 0.02% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |
| T | 0.02% | 0.00% | 0.03% | 0.00% | 0.00% | 0.01% | 0.00% |

# FIGURE 111

HEK2-7: 3'-CCTGCAGT$_{16}$CAAT$_{12}$GAT$_9$T$_8$T$_7$GT$_5$T$_4$T$_3$T$_2$G-5'

| HEK2-7 pNMG-369 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.08% | 0.09% | 0.05% | 0.83% | 0.06% | 0.03% | 0.02% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.91% | 99.91% | 99.95% | 99.16% | 99.93% | 99.96% | 99.98% |

| HEK2-7 pNMG-370 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.24% | 0.01% | 0.01% | 0.01% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% |
| | T | 99.99% | 99.99% | 99.99% | 99.99% | 99.98% | 99.75% | 99.98% | 99.98% | 99.98% |

| HEK2-7 pNMG-371 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | C | 0.00% | 0.01% | 0.06% | 0.04% | 0.10% | 0.35% | 0.02% | 0.01% | 0.00% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% |
| | T | 100.00% | 99.99% | 99.94% | 99.96% | 99.90% | 99.63% | 99.98% | 99.99% | 99.97% |

FIGURE 111 (Continued)

| HEK2-7 pNMG-360 | | 16 | 12 | 9 | 8 | 7 | 5 | 4 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| | C | 0.01% | 0.00% | 0.01% | 0.03% | 0.00% | 0.09% | 0.03% | 0.02% | 0.02% |
| | G | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | T | 99.99% | 100.00% | 99.98% | 99.95% | 99.99% | 99.89% | 99.97% | 99.98% | 99.95% |

| HEK2-7 pNMG-361 | | 16 | 12 | 9 | 8 | 7 | 5 | 4 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T |
| | A | 0.02% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.02% | 0.04% | 0.00% | 0.00% | 0.02% | 0.06% | 0.02% | 0.00% | 0.02% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.96% | 99.94% | 100.00% | 100.00% | 99.98% | 99.94% | 99.98% | 100.00% | 99.98% |

| HEK2-7 pNMG-362 | | 16 | 12 | 9 | 8 | 7 | 5 | 4 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T |
| | A | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% |
| | C | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.10% | 0.00% | 0.05% | 0.02% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 100.00% | 100.00% | 99.95% | 100.00% | 99.90% | 100.00% | 99.95% | 99.95% |

# FIGURE 111 (Continued)

HEK2-7: 3'-CCTGCAGT$_{16}$CAAT$_{12}$GAT$_9$T$_8$T$_7$GT$_5$T$_4$T$_3$T$_2$G-5'

| HEK2-7 pNMG-363 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.16% | 0.00% | 0.00% | 0.00% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 100.00% | 99.97% | 100.00% | 99.97% | 100.00% | 99.84% | 100.00% | 100.00% | 100.00% |

| HEK2-7 pNMG-364 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.02% | 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 0.02% | 0.02% | 0.04% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 100.00% | 99.98% | 100.00% | 99.98% | 99.98% | 99.98% | 99.96% |

| HEK2-7 pNMG-365 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | C | 0.01% | 0.01% | 0.04% | 0.01% | 0.01% | 0.07% | 0.01% | 0.01% | 0.00% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | T | 99.98% | 99.99% | 99.95% | 99.98% | 99.99% | 99.93% | 99.99% | 99.99% | 99.97% |



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# FIGURE 111 (Continued)

| HEK2-7 pNMG-366 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| | C | 0.02% | 0.02% | 0.02% | 0.04% | 0.03% | 0.07% | 0.00% | 0.02% | 0.01% |
| | G | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.97% | 99.96% | 99.97% | 99.92% | 100.00% | 99.98% | 99.99% |

| HEK2-7 pNMG-367 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.03% | 0.01% | 0.01% | 0.00% | 0.07% | 0.01% | 0.02% | 0.00% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.96% | 99.99% | 99.99% | 100.00% | 99.92% | 99.98% | 99.98% | 99.99% |

| HEK2-7 pNMG-368 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| | C | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.02% | 0.01% |
| | G | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 100.00% | 99.99% | 99.97% | 99.98% | 99.97% | 100.00% | 99.97% | 99.98% |



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# FIGURE 112

HEK2-10: 3'-CCATCAT$_{17}$T$_{16}$C T$_{14}$AT$_{12}$T$_{11}$CT$_9$T$_8$T$_7$AT$_5$GT$_3$T$_2$C-5'

| HEK2-10 pNMG-369 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |
| | C | 0.02% | 0.01% | 0.01% | 0.14% | 0.38% | 0.31% | 0.88% | 1.20% | 7.43% | 1.02% | 0.27% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.96% | 99.98% | 99.86% | 99.62% | 99.68% | 99.11% | 98.79% | 92.56% | 98.97% | 99.72% |

| HEK2-10 pNMG-370 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.05% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% |
| | C | 0.01% | 0.01% | 0.02% | 0.29% | 0.10% | 0.08% | 0.60% | 0.43% | 3.51% | 0.30% | 0.30% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.94% | 99.97% | 99.70% | 99.89% | 99.91% | 99.39% | 99.57% | 96.48% | 99.70% | 99.69% |

| HEK2-10 pNMG-371 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.10% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.47% | 0.20% | 0.30% | 1.12% | 1.35% | 7.27% | 0.70% | 0.54% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.89% | 99.98% | 99.52% | 99.79% | 99.70% | 98.88% | 98.64% | 92.72% | 99.29% | 99.46% |

# FIGURE 112 (Continued)

| HEK2-10 pNMG-360 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.01% | 0.09% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| | C | 0.01% | 0.01% | 0.03% | 0.60% | 0.24% | 0.03% | 0.66% | 0.14% | 1.20% | 0.17% | 0.62% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.96% | 99.91% | 99.97% | 99.40% | 99.74% | 99.96% | 99.33% | 99.84% | 98.80% | 99.83% | 99.38% |

| HEK2-10 pNMG-361 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.16% | 0.00% | 0.00% | 0.04% | 0.03% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |
| | C | 0.01% | 0.00% | 0.06% | 0.52% | 0.12% | 0.01% | 0.58% | 0.11% | 0.21% | 0.04% | 0.42% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.02% | 0.03% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.84% | 99.93% | 99.47% | 99.83% | 99.96% | 99.40% | 99.85% | 99.78% | 99.95% | 99.57% |

| HEK2-10 pNMG-362 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.07% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% |
| | C | 0.01% | 0.01% | 0.01% | 0.26% | 1.06% | 0.11% | 0.40% | 0.26% | 1.37% | 0.17% | 0.28% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.92% | 99.98% | 99.73% | 98.93% | 99.88% | 99.59% | 99.73% | 98.62% | 99.81% | 99.71% |



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# FIGURE 112 (Continued)

HEK2-10: 3'-CCATCAT$_{17}$T$_{16}$C T$_{14}$AT$_{12}$T$_{11}$CT$_{9}$T$_{8}$T$_{7}$AT$_{5}$GT$_{3}$T$_{2}$C-5'

| HEK2-10 pNMG-363 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.02% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% |
| | C | 0.02% | 0.01% | 0.01% | 0.15% | 1.24% | 0.13% | 0.30% | 0.35% | 3.03% | 0.41% | 0.23% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.97% | 99.98% | 99.85% | 98.74% | 99.87% | 99.69% | 99.64% | 96.96% | 99.58% | 99.76% |

| HEK2-10 pNMG-364 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.07% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.02% | 0.01% | 0.01% | 0.38% | 0.04% | 0.01% | 0.39% | 0.04% | 0.49% | 0.14% | 0.36% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.92% | 99.98% | 99.61% | 99.95% | 99.99% | 99.60% | 99.95% | 99.51% | 99.85% | 99.64% |

| HEK2-10 pNMG-365 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.12% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| | C | 0.01% | 0.01% | 0.02% | 0.40% | 1.68% | 0.06% | 0.48% | 0.13% | 1.51% | 0.24% | 0.40% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.87% | 99.97% | 99.60% | 98.31% | 99.94% | 99.51% | 99.86% | 98.49% | 99.75% | 99.59% |

# FIGURE 112 (Continued)

| HEK2-10 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pNMG-366 | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.08% | 0.28% | 0.15% | 0.35% | 0.66% | 2.82% | 0.61% | 0.31% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.97% | 99.98% | 99.91% | 99.71% | 99.85% | 99.64% | 99.33% | 97.17% | 99.38% | 99.68% |

| HEK2-10 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pNMG-367 | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.07% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| | C | 0.01% | 0.01% | 0.01% | 0.38% | 0.84% | 0.11% | 0.52% | 0.26% | 2.27% | 0.27% | 0.51% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.92% | 99.98% | 99.61% | 99.15% | 99.89% | 99.47% | 99.74% | 97.73% | 99.72% | 99.48% |

| HEK2-10 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pNMG-368 | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.05% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% |
| | C | 0.02% | 0.01% | 0.01% | 0.22% | 0.01% | 0.01% | 0.21% | 0.01% | 0.02% | 0.01% | 0.19% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.94% | 99.98% | 99.78% | 99.98% | 99.98% | 99.78% | 99.98% | 99.97% | 99.99% | 99.79% |



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# FIGURE 113

Hek3- 5'-GGCCCA$_6$GA$_8$CTGA$_{12}$GCA$_{15}$CGTGATGG-3'

| HEK3 pNMG-369 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | **A** | 98.95% | 97.14% | 99.96% | 99.98% |
| | **C** | 0.00% | 0.00% | 0.00% | 0.00% |
| | **G** | 1.04% | 2.85% | 0.03% | 0.01% |
| | **T** | 0.00% | 0.01% | 0.00% | 0.01% |

| HEK3 pNMG-370 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | **A** | 99.44% | 98.20% | 99.97% | 99.98% |
| | **C** | 0.00% | 0.01% | 0.00% | 0.01% |
| | **G** | 0.56% | 1.79% | 0.03% | 0.01% |
| | **T** | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK3 pNMG-371 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | **A** | 98.86% | 97.60% | 99.96% | 99.98% |
| | **C** | 0.00% | 0.00% | 0.01% | 0.00% |
| | **G** | 1.12% | 2.39% | 0.03% | 0.01% |
| | **T** | 0.01% | 0.01% | 0.00% | 0.01% |

# FIGURE 113 (Continued)

| HEK3 pNMG-360 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.79% | 99.69% | 99.89% | 99.98% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% |
| | G | 0.18% | 0.29% | 0.05% | 0.01% |
| | T | 0.02% | 0.01% | 0.04% | 0.01% |

| HEK3 pNMG-361 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.87% | 99.44% | 99.97% | 99.98% |
| | C | 0.01% | 0.00% | 0.00% | 0.01% |
| | G | 0.11% | 0.55% | 0.02% | 0.00% |
| | T | 0.02% | 0.01% | 0.00% | 0.01% |

| HEK3 pNMG-362 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.53% | 97.91% | 99.97% | 99.98% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% |
| | G | 0.47% | 2.08% | 0.02% | 0.00% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% |

| HEK3 pNMG-363 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.54% | 96.79% | 99.97% | 99.97% |
| | C | 0.00% | 0.00% | 0.00% | 0.02% |
| | G | 0.44% | 3.20% | 0.03% | 0.00% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

# FIGURE 113 (Continued)

| HEK3 pNMG-364 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.87% | 99.33% | 99.95% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.13% | 0.67% | 0.05% | 0.00% |
| | T | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK3 pNMG-365 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.53% | 98.14% | 99.95% | 99.99% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.44% | 1.86% | 0.05% | 0.01% |
| | T | 0.02% | 0.00% | 0.00% | 0.00% |

| HEK3 366 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.16% | 97.43% | 99.97% | 99.99% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% |
| | G | 0.84% | 2.56% | 0.02% | 0.00% |
| | T | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK3 367 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.39% | 97.22% | 99.93% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.59% | 2.76% | 0.06% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% |

# FIGURE 114

FANCF- 5'-GG$A_3A_4$TCCCTTCTGC$A_{15}$GC$A_{18}$CCTGG-3'

| FanCF pNMG-369 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.91% | 99.33% | 99.97% | 99.98% |
| | C | 0.01% | 0.01% | 0.01% | 0.00% |
| | G | 0.08% | 0.66% | 0.02% | 0.01% |
| | T | 0.01% | 0.00% | 0.00% | 0.01% |

| FanCF pNMG-370 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.96% | 99.70% | 99.98% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.04% | 0.29% | 0.01% | 0.00% |
| | T | 0.00% | 0.00% | 0.00% | 0.01% |

| FanCF pNMG-371 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.88% | 99.24% | 99.98% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.01% |
| | G | 0.11% | 0.75% | 0.01% | 0.00% |
| | T | 0.00% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-360 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.97% | 99.85% | 99.97% | 99.99% |
| | C | 0.01% | 0.01% | 0.01% | 0.00% |
| | G | 0.01% | 0.13% | 0.01% | 0.00% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% |

# FIGURE 114 (Continued)

| FanCF pNMG-361 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.94% | 99.51% | 99.99% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.03% | 0.48% | 0.01% | 0.00% |
| | T | 0.03% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-362 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.87% | 99.53% | 99.98% | 99.98% |
| | C | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.12% | 0.46% | 0.01% | 0.01% |
| | T | 0.01% | 0.00% | 0.00% | 0.01% |

| FanCF pNMG-363 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.81% | 99.63% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.19% | 0.37% | 0.02% | 0.01% |
| | T | 0.00% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-364 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.96% | 99.83% | 99.98% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.03% | 0.16% | 0.01% | 0.01% |
| | T | 0.01% | 0.00% | 0.00% | 0.01% |

# FIGURE 114 (Continued)

| FanCF pNMG-365 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.88% | 99.72% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.01% | 0.00% |
| | G | 0.11% | 0.26% | 0.01% | 0.01% |
| | T | 0.00% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-366 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.79% | 99.55% | 99.97% | 99.98% |
| | C | 0.01% | 0.00% | 0.01% | 0.00% |
| | G | 0.20% | 0.44% | 0.02% | 0.01% |
| | T | 0.00% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-367 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.86% | 99.60% | 99.98% | 99.99% |
| | C | 0.01% | 0.00% | 0.00% | 0.01% |
| | G | 0.13% | 0.39% | 0.01% | 0.00% |
| | T | 0.01% | 0.01% | 0.00% | 0.00% |

| FanCF pNMG-368 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.97% | 99.98% | 99.98% | 99.99% |
| | C | 0.01% | 0.01% | 0.01% | 0.01% |
| | G | 0.01% | 0.01% | 0.01% | 0.00% |
| | T | 0.00% | 0.01% | 0.00% | 0.00% |

# FIGURE 115

Hek-2 site: 3'-CCCGCAGT$_{16}$CT$_{14}$AT$_{12}$GCT$_9$T$_8$T$_7$GT$_5$GT$_3$T$_2$C-5'

| HEK2 pNMG-370/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% | 0.04% | 0.01% | 0.00% |
| | C | 0.01% | 0.03% | 0.01% | 0.34% | 0.67% | 0.44% | 54.03% | 1.20% | 0.10% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% |
| | T | 99.98% | 99.96% | 99.97% | 99.65% | 99.32% | 99.56% | 45.90% | 98.79% | 99.89% |

| HEK2 pNMG-371/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% |
| | C | 0.01% | 0.02% | 0.05% | 0.73% | 1.26% | 1.46% | 65.41% | 3.91% | 0.11% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.98% | 99.97% | 99.94% | 99.26% | 98.73% | 98.53% | 34.57% | 96.07% | 99.89% |

| HEK2 pNMG-382/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.03% | 0.02% | 0.23% | 0.31% | 48.37% | 0.55% | 0.06% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% |
| | T | 99.97% | 99.98% | 99.96% | 99.98% | 99.76% | 99.69% | 51.61% | 99.45% | 99.93% |



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FIGURE 115 (Continued)

| HEK2 pNMG-383/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.01% | 0.00% |
| | C | 0.01% | 0.02% | 0.02% | 0.10% | 0.07% | 0.13% | 52.86% | 0.29% | 0.05% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.97% | 99.89% | 99.91% | 99.87% | 47.11% | 99.69% | 99.94% |

| HEK2 pNMG-384/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |
| | C | 0.02% | 0.03% | 0.03% | 0.04% | 0.05% | 0.11% | 44.62% | 0.12% | 0.03% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.97% | 99.97% | 99.95% | 99.94% | 99.88% | 55.36% | 99.88% | 99.97% |

| HEK2 pNMG-385/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.02% | 0.02% | 0.01% | 0.06% | 0.09% | 40.31% | 0.12% | 0.05% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.98% | 99.99% | 99.92% | 99.91% | 59.67% | 99.88% | 99.95% |

| HEK2 pNMG-386/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.02% | 0.01% | 0.07% | 0.44% | 0.13% | 40.97% | 0.80% | 0.04% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.97% | 99.98% | 99.98% | 99.93% | 99.54% | 99.87% | 59.01% | 99.20% | 99.96% |

# FIGURE 115 (Continued)

Hek-2 site: 3'-CCCGCAGT$_{16}$CT$_{14}$AT$_{12}$GCT$_9$T$_8$T$_7$GT$_5$GT$_3$T$_2$C-5'

| HEK2 pNMG-387/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.02% | 0.02% | 0.05% | 0.18% | 0.13% | 44.77% | 0.63% | 0.02% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.98% | 99.97% | 99.97% | 99.94% | 99.79% | 99.86% | 55.21% | 99.37% | 99.98% |

| HEK2 pNMG-388/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.02% | 0.01% | 0.04% | 0.18% | 0.13% | 43.62% | 0.55% | 0.07% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.99% | 99.97% | 99.98% | 99.95% | 99.81% | 99.87% | 56.37% | 99.44% | 99.93% |

| HEK2 pNMG-389/299 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| | C | 0.02% | 0.01% | 0.01% | 0.09% | 0.19% | 0.22% | 40.60% | 0.40% | 0.07% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.99% | 99.89% | 99.79% | 99.77% | 59.37% | 99.60% | 99.92% |

FIGURE 115 (Continued)

| HEK2 pNMG-370/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.03% | 0.01% | 0.01% |
| | C | 0.01% | 0.02% | 0.01% | 0.90% | 10.56% | 1.26% | 25.05% | 19.42% | 0.73% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.97% | 99.98% | 99.09% | 89.38% | 98.73% | 74.92% | 80.57% | 99.26% |

| HEK2 pNMG-371/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.05% | 0.02% | 0.02% | 0.03% | 0.00% |
| | C | 0.01% | 0.02% | 0.04% | 1.52% | 13.26% | 4.44% | 61.17% | 54.59% | 1.66% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.03% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.95% | 98.48% | 86.67% | 95.54% | 38.78% | 45.38% | 98.33% |

| HEK2 pNMG-382/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.02% | 0.13% | 6.00% | 0.73% | 18.75% | 12.15% | 0.80% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.05% | 0.00% | 0.00% | 0.03% | 0.00% |
| | T | 99.98% | 99.98% | 99.97% | 99.87% | 93.94% | 99.27% | 81.24% | 87.82% | 99.20% |

| HEK2 pNMG-383/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% |
| | C | 0.01% | 0.02% | 0.01% | 0.51% | 3.02% | 0.62% | 23.79% | 10.65% | 0.30% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.98% | 99.49% | 96.97% | 99.37% | 76.20% | 89.34% | 99.69% |

# FIGURE 115 (Continued)

Hek-2 site: 3'-CCCGCAGT$_{16}$CT$_{14}$AT$_{12}$GCT$_9$T$_8$T$_7$GT$_5$GT$_3$T$_2$C-5'

| HEK2 pNMG-384/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.02% | 0.01% | 0.42% | 2.72% | 0.18% | 13.40% | 6.88% | 0.12% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.97% | 99.58% | 97.25% | 99.82% | 86.59% | 93.11% | 99.87% |

| HEK2 pNMG-385/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% |
| | C | 0.00% | 0.02% | 0.01% | 0.06% | 0.43% | 0.06% | 8.73% | 2.84% | 0.11% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.98% | 99.93% | 99.56% | 99.94% | 91.27% | 97.16% | 99.88% |

| HEK2 pNMG-386/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.02% | 0.01% | 0.64% | 2.94% | 0.28% | 15.51% | 7.73% | 0.23% |
| | G | 0.05% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% |
| | T | 99.94% | 99.98% | 99.98% | 99.35% | 97.04% | 99.72% | 84.48% | 92.26% | 99.76% |

FIGURE 115 (Continued)

| HEK2 pNMG-387/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.04% | 0.13% | 3.90% | 0.44% | 15.02% | 10.22% | 0.57% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.95% | 99.86% | 96.08% | 99.56% | 84.96% | 89.78% | 99.43% |

| HEK2 pNMG-388/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |
| | C | 0.00% | 0.01% | 0.02% | 0.12% | 4.98% | 0.61% | 20.09% | 12.33% | 0.70% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.97% | 99.88% | 95.00% | 99.39% | 79.90% | 87.67% | 99.29% |

| HEK2 pNMG-389/301 | | 16 T | 14 T | 12 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% |
| | C | 0.01% | 0.02% | 0.01% | 0.27% | 9.95% | 0.60% | 17.48% | 9.82% | 0.86% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.98% | 99.73% | 90.02% | 99.39% | 82.50% | 90.17% | 99.13% |

# FIGURE 116

Hek2-2 site: 5'- $GA_2A_3TA_5CTA_8A_9GCA_{12}TA_{14}GA_{16}$ CTCCAGG-3'

| HEK2-2 pNMG-370 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| | A | 99.81% | 99.80% | 70.89% | 99.53% | 99.93% | 99.97% | 99.99% | 99.99% |
| | C | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% |
| | G | 0.14% | 0.19% | 29.09% | 0.46% | 0.06% | 0.01% | 0.00% | 0.01% |
| | T | 0.02% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |

| HEK2-2 pNMG-371 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| | A | 99.47% | 99.28% | 49.87% | 99.33% | 99.65% | 99.98% | 99.99% | 100.00% |
| | C | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.50% | 0.71% | 50.12% | 0.66% | 0.35% | 0.01% | 0.01% | 0.00% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK2-2 pNMG-382 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| | A | 99.91% | 99.88% | 75.50% | 99.85% | 99.97% | 99.97% | 99.95% | 99.97% |
| | C | 0.03% | 0.01% | 0.01% | 0.03% | 0.02% | 0.02% | 0.04% | 0.01% |
| | G | 0.05% | 0.10% | 24.48% | 0.12% | 0.01% | 0.01% | 0.01% | 0.00% |
| | T | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% |

FIGURE 116 (Continued)

| HEK2-2 pNMG-383 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| | A | 99.91% | 99.97% | 76.15% | 99.93% | 99.95% | 99.98% | 99.99% | 99.98% |
| | C | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.07% | 0.03% | 23.84% | 0.07% | 0.04% | 0.01% | 0.01% | 0.01% |
| | T | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |

| HEK2-2 pNMG-384 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| | A | 99.92% | 99.99% | 78.15% | 99.96% | 99.95% | 99.97% | 99.97% | 99.99% |
| | C | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |
| | G | 0.05% | 0.00% | 21.83% | 0.03% | 0.04% | 0.01% | 0.01% | 0.00% |
| | T | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

# FIGURE 116 (Continued)

Hek2-2 site: 5'-$GA_2A_3TA_5CTA_8A_9GCA_{12}TA_{14}GA_{16}$CTCCAGG-3'

| HEK2-2 pNMG-385 | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A |
| A | 99.95% | 99.99% | 88.71% | 99.96% | 99.99% | 99.99% | 99.99% | 99.99% |
| C | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.04% | 0.01% | 11.29% | 0.03% | 0.01% | 0.00% | 0.01% | 0.00% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |

| HEK2-2 pNMG-386 | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A |
| A | 99.92% | 99.95% | 79.42% | 99.88% | 99.96% | 99.95% | 99.97% | 99.98% |
| C | 0.04% | 0.02% | 0.02% | 0.04% | 0.03% | 0.04% | 0.02% | 0.02% |
| G | 0.03% | 0.02% | 20.54% | 0.08% | 0.00% | 0.00% | 0.01% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK2-2 pNMG-387 | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A |
| A | 99.86% | 99.93% | 78.43% | 99.82% | 99.94% | 99.98% | 99.99% | 99.99% |
| C | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.11% | 0.06% | 21.56% | 0.17% | 0.05% | 0.01% | 0.01% | 0.01% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% |

FIGURE 116 (Continued)

| HEK2-2 pNMG-388 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.87% | 99.88% | 76.34% | 99.90% | 99.98% | 99.98% | 99.98% | 99.98% |
| | C | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% |
| | G | 0.12% | 0.12% | 23.64% | 0.09% | 0.01% | 0.01% | 0.00% | 0.00% |
| | T | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.02% |

| HEK2-2 pNMG-389 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.82% | 99.95% | 76.62% | 99.90% | 99.97% | 99.99% | 99.98% | 99.99% |
| | C | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.16% | 0.04% | 23.37% | 0.10% | 0.02% | 0.01% | 0.01% | 0.00% |
| | T | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% |

# FIGURE 117

Hek 2-3 site: 5'-GT$A_3$ $A_4$ $A_5$ $CA_7$ $A_8$ $A_9$ $GCA_{12}$ $TA_{14}$ $GA_{16}$ CTGAGGG -3'

| HEK2-3 pNMG-370 | 3 A | 4 A | 5 A | 7 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| A | 99.86% | 99.81% | 89.59% | 99.89% | 99.50% | 99.67% | 99.97% | 99.97% | 99.99% |
| C | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.14% | 0.01% | 0.02% | 0.00% |
| G | 0.12% | 0.17% | 10.39% | 0.10% | 0.48% | 0.19% | 0.02% | 0.01% | 0.00% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |

| HEK2-3 pNMG-371 | 3 A | 4 A | 5 A | 7 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| A | 99.61% | 98.90% | 79.92% | 99.00% | 98.38% | 99.18% | 99.94% | 99.97% | 99.99% |
| C | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.13% | 0.00% | 0.01% | 0.00% |
| G | 0.39% | 1.08% | 20.07% | 1.00% | 1.60% | 0.69% | 0.04% | 0.02% | 0.00% |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.01% |

| HEK2-3 pNMG-382 | 3 A | 4 A | 5 A | 7 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| C | 0.01% | 0.01% | 0.03% | 0.02% | 0.23% | 0.31% | 48.37% | 0.55% | 0.06% |
| G | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% |
| T | 99.97% | 99.98% | 99.96% | 99.98% | 99.76% | 99.69% | 51.61% | 99.45% | 99.93% |

| HEK2-3 pNMG-383 | 3 A | 4 A | 5 A | 7 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|
| A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.01% | 0.00% |
| C | 0.01% | 0.02% | 0.02% | 0.10% | 0.07% | 0.13% | 52.86% | 0.29% | 0.05% |
| G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| T | 99.98% | 99.98% | 99.97% | 99.89% | 99.91% | 99.87% | 47.11% | 99.69% | 99.94% |

FIGURE 117 (Continued)

| HEK2-3 pNMG-384 | | 3 A | 4 A | 5 A | 7 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% |
| | C | 0.02% | 0.03% | 0.03% | 0.04% | 0.05% | 0.11% | 44.62% | 0.12% | 0.03% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.97% | 99.97% | 99.95% | 99.94% | 99.88% | 55.36% | 99.88% | 99.97% |

| HEK2-3 pNMG-385 | | 3 A | 4 A | 5 A | 7 A | 8 A | 9 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.02% | 0.02% | 0.01% | 0.06% | 0.09% | 40.31% | 0.12% | 0.05% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.98% | 99.99% | 99.92% | 99.91% | 59.67% | 99.88% | 99.95% |

# FIGURE 117 (Continued)

Hek 2-3 site: 5'-GT$A_3A_4A_5CA_7A_8A_9GCA_{12}TA_{14}GA_{16}$CTGAGGG -3'

| HEK2-3 pNMG-386 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| C | 0.01% | 0.02% | 0.01% | 0.07% | 0.44% | 0.13% | 40.97% | 0.80% | 0.04% |
| G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| T | 99.97% | 99.98% | 99.98% | 99.93% | 99.54% | 99.87% | 59.01% | 99.20% | 99.96% |

| HEK2-3 pNMG-387 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 0.00% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.01% | 0.00% | 0.00% |
| C | 0.01% | 0.02% | 0.02% | 0.05% | 0.18% | 0.13% | 44.77% | 0.63% | 0.02% |
| G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| T | 99.98% | 99.97% | 99.97% | 99.94% | 99.79% | 99.86% | 55.21% | 99.37% | 99.98% |

| HEK2-3 pNMG-388 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| C | 0.01% | 0.02% | 0.01% | 0.04% | 0.18% | 0.13% | 43.62% | 0.55% | 0.07% |
| G | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| T | 99.99% | 99.97% | 99.98% | 99.95% | 99.81% | 99.87% | 56.37% | 99.44% | 99.93% |

| HEK2-3 pNMG-389 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 0.00% | 0.02% | 0.00% | 0.00% |
| C | 0.02% | 0.01% | 0.01% | 0.09% | 0.19% | 0.22% | 40.60% | 0.40% | 0.07% |
| G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| T | 99.98% | 99.98% | 99.99% | 99.89% | 99.79% | 99.77% | 59.37% | 99.60% | 99.92% |

# FIGURE 118

HEK2-6- 5'-$GA_2A_3GA_5CCA_8A_9GGA_{12}TA_{14}GA_{16}$CTGCTGG-3'

| HEK2-6 pNMG-370 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.96% | 99.94% | 95.58% | 99.77% | 99.84% | 99.96% | 99.98% | 99.97% |
| | C | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% |
| | G | 0.03% | 0.04% | 4.40% | 0.21% | 0.14% | 0.02% | 0.02% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% | 0.01% |

| HEK2-6 pNMG-371 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.95% | 99.91% | 92.37% | 99.60% | 99.63% | 99.98% | 99.99% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | G | 0.04% | 0.08% | 7.61% | 0.39% | 0.35% | 0.01% | 0.01% | 0.00% |
| | T | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |

| HEK2-6 pNMG-382 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.97% | 99.97% | 95.39% | 99.88% | 99.90% | 99.99% | 99.99% | 99.96% |
| | C | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| | G | 0.03% | 0.01% | 4.59% | 0.10% | 0.08% | 0.01% | 0.00% | 0.02% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |

FIGURE 118 (Continued)

| HEK2-6 pNMG-383 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.97% | 99.97% | 94.48% | 99.97% | 99.90% | 99.94% | 99.97% | 99.98% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |
| | G | 0.02% | 0.01% | 5.50% | 0.03% | 0.07% | 0.04% | 0.01% | 0.00% |
| | T | 0.00% | 0.01% | 0.02% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% |

| HEK2-6 pNMG-384 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.96% | 99.97% | 97.20% | 99.98% | 99.94% | 99.97% | 99.98% | 99.97% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% |
| | G | 0.03% | 0.01% | 2.79% | 0.01% | 0.04% | 0.01% | 0.01% | 0.01% |
| | T | 0.00% | 0.01% | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% | 0.00% |

# FIGURE 118 (Continued)

HEK2-6- 5'-$GA_2A_3GA_5CCA_8A_9GGA_{12}TA_{14}GA_{16}CTGCTGG$-3'

| HEK2-6 pNMG-385 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.96% | 99.97% | 98.54% | 99.98% | 99.97% | 99.97% | 99.95% | 99.98% |
| | C | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| | G | 0.02% | 0.01% | 1.45% | 0.01% | 0.02% | 0.01% | 0.03% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% |

| HEK2-6 pNMG-386 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.98% | 99.97% | 97.56% | 99.95% | 99.93% | 99.95% | 99.96% | 99.97% |
| | C | 0.01% | 0.01% | 0.01% | 0.02% | 0.02% | 0.01% | 0.01% | 0.02% |
| | G | 0.01% | 0.01% | 2.41% | 0.02% | 0.04% | 0.03% | 0.02% | 0.00% |
| | T | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% | 0.00% |

| HEK2-6 pNMG-387 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.93% | 99.97% | 97.46% | 99.94% | 99.97% | 99.98% | 99.97% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| | G | 0.05% | 0.01% | 2.53% | 0.05% | 0.02% | 0.01% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |

FIGURE 118 (Continued)

| HEK2-6 pNMG-388 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.97% | 99.98% | 96.81% | 99.94% | 99.90% | 99.94% | 99.99% | 99.99% |
| | C | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.02% | 0.01% | 3.18% | 0.06% | 0.08% | 0.06% | 0.00% | 0.00% |
| | T | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% |

| HEK2-6 pNMG-389 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.97% | 99.96% | 97.46% | 99.97% | 99.85% | 99.89% | 99.97% | 99.97% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.02% |
| | G | 0.02% | 0.02% | 2.51% | 0.03% | 0.13% | 0.10% | 0.01% | 0.01% |
| | T | 0.01% | 0.02% | 0.03% | 0.00% | 0.01% | 0.01% | 0.02% | 0.00% |

# FIGURE 119

HEK2-7- 3'-CCTGCAGT$_{16}$CAAT$_{12}$GAT$_9$T$_8$T$_7$GT$_5$T$_4$T$_3$T$_2$G-5'

| HEK2-7 pNMG-370 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% |
| | C | 0.02% | 0.01% | 0.04% | 0.02% | 0.01% | 0.30% | 0.01% | 0.01% | 0.01% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.99% | 99.96% | 99.98% | 99.99% | 99.69% | 99.99% | 99.99% | 99.99% |

| HEK2-7 pNMG-371 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.02% | 0.01% | 0.07% | 0.05% | 0.05% | 0.75% | 0.04% | 0.02% | 0.01% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.92% | 99.95% | 99.94% | 99.24% | 99.96% | 99.98% | 99.99% |

| HEK2-7 pNMG-382 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.26% | 0.01% | 0.01% | 0.01% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.99% | 99.99% | 99.99% | 99.74% | 99.99% | 99.99% | 99.98% |

FIGURE 119 (Continued)

| HEK2-7 pNMG-383 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.23% | 0.01% | 0.01% | 0.00% |
| | G | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.99% | 99.98% | 99.99% | 99.76% | 99.98% | 99.99% | 99.99% |

| HEK2-7 pNMG-384 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.02% | 0.00% | 0.01% | 0.10% | 0.01% | 0.02% | 0.01% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.98% | 99.98% | 99.99% | 99.99% | 99.89% | 99.99% | 99.98% | 99.99% |

# FIGURE 119 (Continued)

HEK2-7- 3'-CCTGCAGT$_{16}$CAAT$_{12}$GAT$_{9}$T$_{8}$T$_{7}$GT$_{5}$T$_{4}$T$_{3}$T$_{2}$G-5'

| HEK2-7 pNMG-385 | | 16 | 12 | 9 | 8 | 7 | 5 | 4 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.02% | 0.00% | 0.09% | 0.02% | 0.01% | 0.01% |
| | G | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.99% | 99.97% | 99.99% | 99.97% | 100.00% | 99.91% | 99.98% | 99.99% | 99.98% |

| HEK2-7 pNMG-386 | | 16 | 12 | 9 | 8 | 7 | 5 | 4 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% | 0.14% | 0.01% | 0.01% | 0.00% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.99% | 99.98% | 99.99% | 99.98% | 99.85% | 99.99% | 99.99% | 99.99% |

| HEK2-7 pNMG-387 | | 16 | 12 | 9 | 8 | 7 | 5 | 4 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T |
| | A | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.02% | 0.02% | 0.01% | 0.14% | 0.01% | 0.02% | 0.01% |
| | G | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.98% | 99.98% | 99.98% | 99.85% | 99.98% | 99.97% | 99.99% |

FIGURE 119 (Continued)

| HEK2-7 pNMG-388 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | C | 0.02% | 0.02% | 0.00% | 0.02% | 0.02% | 0.38% | 0.05% | 0.00% | 0.03% |
| | G | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% |
| | T | 99.98% | 99.97% | 99.99% | 99.98% | 99.98% | 99.62% | 99.95% | 99.99% | 99.95% |

| HEK2-7 pNMG-389 | | 16 T | 12 T | 9 T | 8 T | 7 T | 5 T | 4 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | C | 0.02% | 0.01% | 0.02% | 0.02% | 0.01% | 0.27% | 0.02% | 0.01% | 0.01% |
| | G | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.98% | 99.98% | 99.98% | 99.97% | 99.98% | 99.73% | 99.98% | 99.99% | 99.99% |

# FIGURE 120

Hek2-10 site: 3'-CCATCAT$_{17}$T$_{16}$CT$_{14}$AT$_{12}$T$_{11}$CT$_{9}$T$_{8}$T$_{7}$AT$_{5}$GT$_{3}$T$_{2}$C-5'

| HEK2-10 pNMG-370 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.05% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% |
| | C | 0.01% | 0.01% | 0.02% | 0.19% | 0.22% | 0.19% | 0.57% | 0.94% | 6.27% | 0.53% | 0.26% |
| | G | 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.94% | 99.98% | 99.80% | 99.76% | 99.80% | 99.43% | 99.05% | 93.72% | 99.46% | 99.74% |

| HEK2-10 pNMG-371 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.09% | 0.69% | 0.73% | 2.06% | 4.06% | 21.40% | 2.52% | 0.53% |
| | G | 0.02% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |
| | T | 99.97% | 99.98% | 99.98% | 99.91% | 99.29% | 99.26% | 97.94% | 95.94% | 78.59% | 97.47% | 99.46% |

| HEK2-10 pNMG-382 | | 17 | 16 | 14 | 12 | 11 | 9 | 8 | 7 | 5 | 3 | 2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | T | T | T | T | T | T | T | T | T | T | T |
| | A | 0.00% | 0.07% | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.23% | 0.18% | 0.03% | 0.33% | 0.23% | 3.62% | 0.29% | 0.31% |
| | G | 0.02% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.93% | 99.98% | 99.76% | 99.79% | 99.97% | 99.66% | 99.76% | 96.38% | 99.70% | 99.69% |

FIGURE 120 (Continued)

| HEK2-10 pNMG-383 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |
| | C | 0.01% | 0.01% | 0.01% | 0.05% | 0.75% | 0.08% | 0.15% | 0.20% | 3.71% | 0.19% | 0.12% |
| | G | 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.98% | 99.98% | 99.95% | 99.23% | 99.91% | 99.85% | 99.80% | 96.28% | 99.80% | 99.87% |

| HEK2-10 pNMG-384 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| | C | 0.01% | 0.01% | 0.01% | 0.11% | 0.83% | 0.04% | 0.13% | 0.11% | 3.14% | 0.12% | 0.14% |
| | G | 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.97% | 99.98% | 99.89% | 99.14% | 99.96% | 99.86% | 99.89% | 96.85% | 99.87% | 99.85% |

# FIGURE 120 (Continued)

Hek2-10 site: 3'-CCATCAT$_{17}$T$_{16}$CT$_{14}$AT$_{12}$T$_{11}$CT$_{9}$T$_{8}$T$_{7}$AT$_{5}$GT$_{3}$T$_{2}$C-5'

| HEK2-10 pNMG-385 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.06% | 0.28% | 0.03% | 0.09% | 0.10% | 1.52% | 0.05% | 0.11% |
| | G | 0.02% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.97% | 99.98% | 99.93% | 99.69% | 99.97% | 99.91% | 99.89% | 98.47% | 99.94% | 99.88% |

| HEK2-10 pNMG-386 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.07% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% |
| | C | 0.01% | 0.01% | 0.01% | 0.24% | 0.70% | 0.05% | 0.27% | 0.10% | 2.78% | 0.11% | 0.23% |
| | G | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.92% | 99.97% | 99.76% | 99.28% | 99.94% | 99.72% | 99.90% | 97.21% | 99.88% | 99.76% |

| HEK2-10 pNMG-387 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.01% | 0.05% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |
| | C | 0.01% | 0.01% | 0.01% | 0.26% | 0.18% | 0.08% | 0.38% | 0.19% | 3.15% | 0.21% | 0.39% |
| | G | 0.01% | 0.00% | 0.01% | 0.00% | 0.02% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.95% | 99.98% | 99.74% | 99.80% | 99.92% | 99.62% | 99.80% | 96.83% | 99.79% | 99.61% |



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FIGURE 120 (Continued)

| HEK2-10 pNMG-388 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.03% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |
| | C | 0.01% | 0.01% | 0.01% | 0.13% | 0.16% | 0.05% | 0.25% | 0.24% | 4.14% | 0.22% | 0.22% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.96% | 99.98% | 99.87% | 99.83% | 99.94% | 99.75% | 99.75% | 95.85% | 99.77% | 99.77% |

| HEK2-10 pNMG-389 | | 17 T | 16 T | 14 T | 12 T | 11 T | 9 T | 8 T | 7 T | 5 T | 3 T | 2 T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 0.00% | 0.02% | 0.01% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | C | 0.01% | 0.01% | 0.01% | 0.09% | 0.23% | 0.06% | 0.18% | 0.23% | 3.10% | 0.15% | 0.21% |
| | G | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | T | 99.97% | 99.97% | 99.98% | 99.91% | 99.76% | 99.93% | 99.82% | 99.77% | 96.89% | 99.84% | 99.78% |

# FIGURE 121

Hek3- 5'-GGCCCA$_6$GA$_8$CTGA$_{12}$GCA$_{15}$CGTGATGG-3'

| HEK3 pNMG-370 | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|
| A | 98.94% | 97.53% | 99.94% | 99.97% |
| C | 0.01% | 0.01% | 0.01% | 0.02% |
| G | 1.04% | 2.46% | 0.04% | 0.01% |
| T | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK3 pNMG-371 | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|
| A | 98.05% | 95.74% | 99.97% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 1.95% | 4.25% | 0.03% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 0.00% |

| HEK3 pNMG-382 | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|
| A | 99.39% | 97.01% | 99.93% | 99.98% |
| C | 0.01% | 0.01% | 0.01% | 0.01% |
| G | 0.60% | 2.98% | 0.05% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.00% |

| HEK3 pNMG-385 | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|
| A | 99.75% | 98.93% | 99.98% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.25% | 1.06% | 0.02% | 0.01% |
| T | 0.01% | 0.01% | 0.00% | 0.00% |

| HEK3 pNMG-386 | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|
| A | 99.55% | 97.94% | 99.94% | 99.96% |
| C | 0.03% | 0.02% | 0.03% | 0.03% |
| G | 0.41% | 2.04% | 0.03% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 0.01% |

| HEK3 pNMG-387 | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|
| A | 99.64% | 97.14% | 99.95% | 99.98% |
| C | 0.00% | 0.00% | 0.01% | 0.00% |
| G | 0.36% | 2.86% | 0.04% | 0.01% |
| T | 0.00% | 0.01% | 0.00% | 0.01% |

FIGURE 121 (Continued)

| HEK3 pNMG-383 | 6 | 8 | 12 | 15 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.49% | 97.31% | 99.95% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.50% | 2.68% | 0.04% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 0.00% |

| HEK3 pNMG-384 | 6 | 8 | 12 | 15 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.70% | 98.15% | 99.96% | 99.98% |
| C | 0.01% | 0.01% | 0.01% | 0.01% |
| G | 0.28% | 1.83% | 0.03% | 0.01% |
| T | 0.01% | 0.01% | 0.00% | 0.00% |

| HEK3 pNMG-385 | 6 | 8 | 12 | 15 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.75% | 98.93% | 99.98% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.25% | 1.06% | 0.02% | 0.01% |
| T | 0.01% | 0.01% | 0.00% | 0.00% |

| HEK3 pNMG-388 | 6 | 8 | 12 | 15 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.39% | 97.54% | 99.94% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.59% | 2.45% | 0.05% | 0.01% |
| T | 0.01% | 0.01% | 0.00% | 0.01% |

| HEK3 pNMG-389 | 6 | 8 | 12 | 15 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.53% | 97.37% | 99.96% | 99.98% |
| C | 0.01% | 0.00% | 0.00% | 0.00% |
| G | 0.46% | 2.63% | 0.03% | 0.01% |
| T | 0.01% | 0.01% | 0.00% | 0.01% |

# FIGURE 122

FANCF- 5'-GGA$_3$A$_4$TCCCTTCTGCA$_{15}$GCA$_{18}$CCTGG-3'

| FANCF pNMG-370 | 3 | 4 | 15 | 18 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.93% | 99.40% | 99.98% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.06% | 0.60% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 0.00% |

| FANCF pNMG-371 | 3 | 4 | 15 | 18 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.67% | 97.88% | 99.98% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.32% | 2.11% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 0.01% |

| FANCF pNMG-382 | 3 | 4 | 15 | 18 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.93% | 99.69% | 99.99% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.06% | 0.30% | 0.01% | 0.00% |
| T | 0.00% | 0.01% | 0.00% | 0.01% |

| FANCF pNMG-385 | 3 | 4 | 15 | 18 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.94% | 99.82% | 99.99% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.04% | 0.17% | 0.00% | 0.00% |
| T | 0.02% | 0.01% | 0.00% | 0.01% |

| FANCF pNMG-386 | 3 | 4 | 15 | 18 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.94% | 99.82% | 99.98% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.05% | 0.17% | 0.01% | 0.00% |
| T | 0.01% | 0.01% | 0.00% | 0.01% |

| FANCF pNMG-387 | 3 | 4 | 15 | 18 |
|---|---|---|---|---|
| | A | A | A | A |
| A | 99.94% | 99.74% | 99.98% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.05% | 0.25% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 0.00% |

FIGURE 122 (Continued)

| FANCF pNMG-383 | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|
| A | 99.94% | 99.76% | 99.98% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.05% | 0.23% | 0.02% | 0.01% |
| T | 0.00% | 0.00% | 0.00% | 0.01% |

| FANCF pNMG-384 | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|
| A | 99.92% | 99.83% | 99.98% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.06% | 0.16% | 0.01% | 0.00% |
| T | 0.01% | 0.01% | 0.00% | 0.01% |

| FANCF pNMG-388 | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|
| A | 99.94% | 99.69% | 99.98% | 99.98% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.05% | 0.31% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.00% | 0.01% |

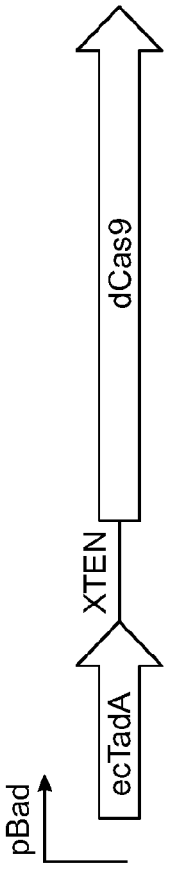
| FANCF pNMG-389 | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|
| A | 99.92% | 99.85% | 99.99% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.08% | 0.14% | 0.01% | 0.00% |
| T | 0.00% | 0.00% | 0.00% | 0.01% |

FIGURE 123

| Site | Protospacer and PAM sequence | pNMG370 | pNMG371 | pNMG382-389 | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HEK2 | GAACACAAAGCATAGACTGCT**GG** | 54.0 | 65.4 | 48.4 | 52.9 | 44.6 | 40.3 | 41.0 | 44.8 | 43.6 | 40.6 | |
| HEK2-2 | GAA*TA*CT*A*AGCATAGACT*CC***AGG** | 29.1 | 50.1 | 24.5 | 23.8 | 21.8 | 11.3 | 20.5 | 21.6 | 23.6 | 23.4 | |
| HEK2-3 | G*TAA***A**CAAAGCATAGACTG*A***GGG** | 10.4 | 20.1 | 8.2 | 11.0 | 4.7 | 3.5 | 8.0 | 4.2 | 8.5 | 6.2 | |
| HEK2-6 | GAAG***A***C*C*AAGGATAGACTGCT**GG** | 4.4 | 7.6 | 4.6 | 5.5 | 2.8 | 1.4 | 2.4 | 2.5 | 3.2 | 2.5 | |
| HEK2-7 | GAA*A***A**CAAA*T*GAT*T*GACTGC**AGG** | 0.3 | 0.7 | 0.3 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.4 | 0.3 | |
| HEK2-10 | GAAC***A***T*A*AAGAATAGAATG*A***TGG** | 6.3 | 21.4 | 3.6 | 6.3 | 4.6 | 2.0 | 4.2 | 4.1 | 5.5 | 4.0 | |

382 389

FIGURE 124



# FIGURE 125

| | position: | 11 | 35 | 36 | 37 | 47 | 48 | 49 | 51 | 69 | 70 | 72 | 77 | 84 | 88 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | Trp | Val | His | Asn | Arg | Pro | Ile | Arg | Val | Met | Asn | Asp | Leu | Val |
| 1 | | | | | | | | | Leu | | | | | Phe | |
| 2 | | | | | Ser | | | | His | | | | Gly | Phe | |
| 3 | | | | | | | | | | | Thr | | | Phe | |
| 4 | | | | Leu | | | Leu | | | | | | | Phe | |
| 5 | | | | | Ser | | | | | | | | | Phe | |
| 6 | | | | | | | | | | | | Ser | | Phe | |
| 7 | | | | | Thr | | Thr | Val | | | Leu | | | Phe | |
| 8 | | | | | | | | | | Ala | | | | Phe | |
| 9 | | | | Leu | | | | | | | | | | Phe | |
| 10 | | | Glu | | | | | | | | | | | Phe | |
| 11 | | | | | | | | | | | | | | Phe | |
| 12 | | | | | | | | | | | | | | Phe | |
| 13 | | | | | | | | | | | | | | Phe | |
| 14 | | | | | | | | | | | | | | Phe | |
| 15 | | | | Leu | | | Leu | | | | | | | Phe | |
| 16 | | | | | | | | | | | | | | Phe | |
| 17 | | | | | | | | | | | | | Gly | Phe | |
| 18 | | | | | Ser | Ser | | | | | | | | Phe | |
| 19 | | Arg | | | | | | | | Ala | | | | Phe | |
| 20 | | | | | Ser | | | | His | | | Ser | | Phe | |
| 21 | | | | Leu | | | Leu | | | | | | | Phe | |
| 22 | | | | | | | | | | | | | | Phe | |
| 23 | | | | | | | | | Leu | | | | | Phe | |
| 24 | | | | Leu | | | | | | | | | | Phe | |
| 25 | | | | Leu | | | Leu | | | | | | | Phe | |
| 26 | | | | | Thr | | Thr | Val | | | Leu | | | Phe | |
| 27 | | | | | | | | | | | | | | Phe | |

evolution #1

evolution #2

evolution #3

# FIGURE 125 (Continued)

| 106 | 107 | 108 | 111 | 123 | 134 | 137 | 146 | 147 | 154 | 155 | 156 | 157 | 158 | 160 | 161 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ala | Arg | Asp | Thr | His | Glu | Leu | Ser | Asp | Gln | Glu | Ile | Lys | Ala | Lys | Lys |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | His | Val | Phe | | | | |
| Val | | Asn | | Tyr | Gly | | | Tyr | | Val | Phe | | Ser | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Ala | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | Ile | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | Asn | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | Thr |
| Val | | Asn | | Tyr | Gly | | | Cys | | Val | Phe | | Ser | | |
| Val | | Asn | | Tyr | | | Cys | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Ala | Phe | | | | |
| Val | | Asn | | Tyr | | | Arg | Tyr | | Val | Phe | | | Ile | |
| Val | Cys | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | Thr |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Ala | Phe | | | | |
| Val | | Asn | | Tyr | Gly | | | Tyr | His | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | Cys | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | Asn | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | Asn | | | |
| Val | | Asn | | Tyr | Gly | | | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | Arg | Tyr | | Val | Phe | | | | |
| Val | | Asn | | Tyr | | | | Tyr | | Val | Phe | | | | |

FIGURE 126A

**384 ug/mL spectinomycin (5h):**

| | position: | 36 | 37 | 47 | 48 | 49 | 51 | 69 | 70 | 72 | 77 | 84 | 106 | 108 | 123 | 134 | 146 | 147 | 154 | 155 | 156 | 157 | 159 | 160 | 161 | 163 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | His | Asn | Arg | Pro | Ile | Arg | Val | Met | Asn | Asp | Leu | Ala | Asp | His | Glu | Ser | Asp | Gln | Glu | Ile | Lys | Ala | Lys | Lys | Gln |
| 1 | | | Thr | | Thr | Val | | | Leu | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 2 | | Leu | | | | | | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | Asn | | | | |
| 3 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | Asn | | | | |
| 4 | | | | | | | Leu | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | Asn | | | | |
| 5 | | | Ser | | | | His | | | | Gly | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 6 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | Cys | Tyr | | Val | Phe | | | | Thr | |
| 7 | | Leu | | | | | | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 8 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | Arg | Tyr | | Val | Phe | | | | Thr | |
| 8 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |

evolution #1
evolution #2
evolution #3

# FIGURE 126B

**384 ug/mL spectinomycin (7h):**

| | position: | 36 | 37 | 47 | 48 | 49 | 51 | 69 | 70 | 72 | 77 | 84 | 106 | 108 | 123 | 134 | 146 | 147 | 154 | 155 | 156 | 157 | 159 | 160 | 161 | 163 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | His | Asn | Arg | Pro | Ile | Arg | Val | Met | Asn | Asp | Leu | Ala | Asp | His | Glu | Ser | Asp | Gln | Glu | Ile | Lys | Ala | Lys | Lys | Gln |
| 1 | | | Ser | | | | Leu | | | | | Phe | Val | Asn | Tyr | | Cys | Tyr | | Val | Phe | | | | | |
| 2 | | | | | | | Leu | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 4 | | | | | | | | | | Ser | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | Ile | | |
| 5 | | | Ser | | | | His | | | | | Phe | Val | Asn | Tyr | | | Tyr | | | Phe | | | | | |
| 6 | | | | | | | Leu | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | Asn | | | | |
| 7 | | | Ser | | | | His | | | | Gly | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 8 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |

evolution #1
evolution #2
evolution #3

# FIGURE 126C

**128 ug/mL chloramphenicol (7h):**

| | position: | 36 | 37 | 47 | 48 | 49 | 51 | 69 | 70 | 72 | 77 | 84 | 106 | 108 | 123 | 134 | 146 | 147 | 154 | 155 | 156 | 157 | 159 | 160 | 161 | 163 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | His | Asn | Arg | Pro | Ile | Arg | Val | Met | Asn | Asp | Leu | Ala | Asp | His | Glu | Ser | Asp | Gln | Glu | Ile | Lys | Ala | Lys | Lys | Gln |
| 1 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | Arg | Tyr | | Val | Phe | | | | | |
| 2 | | | | | | | Leu | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 3 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | Arg | Tyr | | Val | Phe | | | | Thr | |
| 4 | | | | | Thr | Val | | | Leu | | | Phe | Val | Asn | Tyr | | Cys | Tyr | | Val | Phe | | | | | |
| 5 | | | Thr | | Thr | Val | | | Leu | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 2 | | | | | | | Leu | | | | | Phe | Val | Asn | Tyr | | Arg | Tyr | | Val | Phe | | | | | |
| 3 | | | | | | | | | | | Gly | Phe | Val | Asn | Tyr | | | Tyr | His | Val | Phe | | | | | |

evolution #1

evolution #2

evolution #3

# FIGURE 126D

**128 ug/mL chloramphenicol + 128 ug/mL spectinomycin (overnight):**

| | position: | 36 | 37 | 47 | 48 | 49 | 51 | 69 | 70 | 72 | 77 | 84 | 106 | 108 | 123 | 134 | 146 | 147 | 154 | 155 | 156 | 157 | 159 | 160 | 161 | 163 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | His | Asn | Arg | Pro | Ile | Arg | Val | Met | Asn | Asp | Leu | Ala | Asp | His | Glu | Ser | Asp | Gln | Glu | Ile | Lys | Ala | Lys | Lys | Gln |
| 1 | | | | | | | | | Thr | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 2 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |

evolution #1

evolution #2

evolution #3

FIGURE 126E

**128 ug/mL chloramphenicol + 256 ug/mL spectinomycin (overnight):**

| | position: | 36 | 37 | 47 | 48 | 49 | 51 | 69 | 70 | 72 | 77 | 84 | 106 | 108 | 123 | 134 | 146 | 147 | 154 | 155 | 156 | 157 | 159 | 160 | 161 | 163 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | His | Asn | Arg | Pro | Ile | Arg | Val | Met | Asn | Asp | Leu | Ala | Asp | His | Glu | Ser | Asp | Gln | Glu | Ile | Lys | Ala | Lys | Lys | Gln |
| 1 | | | | | | | | | | | | Phe | Val | Asn | Tyr | Gly | | Tyr | | Val | Phe | | | | Thr | |
| 2 | | | Ser | | | | | | | Ser | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |
| 3 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | Cys | Tyr | | Val | Phe | | | | | |
| 4 | | | | | | | | | | | | Phe | Val | Asn | Tyr | | | Tyr | | Val | Phe | | | | | |

evolution #1
evolution #2
evolution #3

FIGURE 127

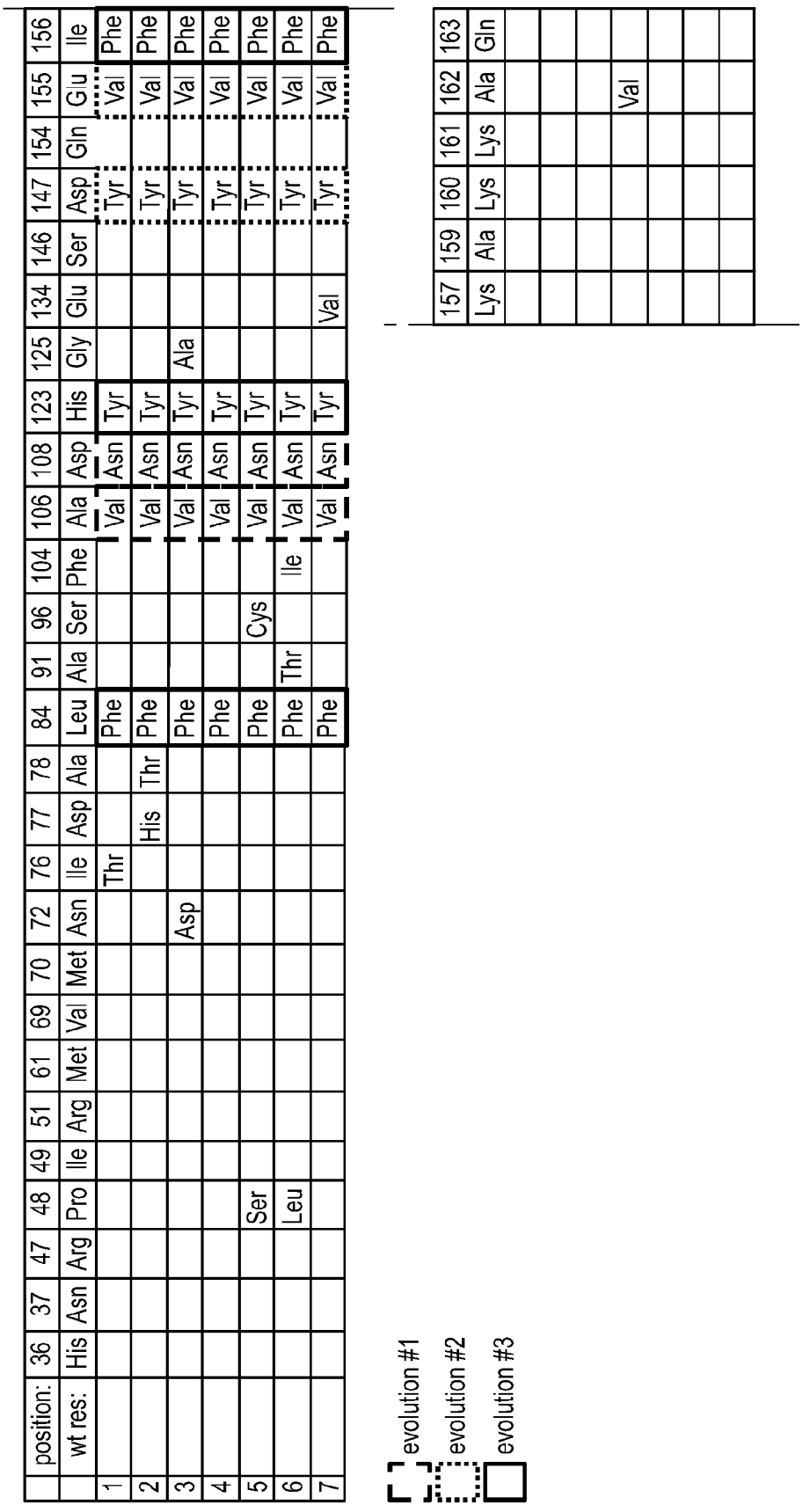

position: 36 37 47 48 49 51 61 69 70 72 76 77 78 84 91 96 104 106 108 123 125 134 146 147 154 155 156
wt res: His Asn Arg Pro Ile Arg Met Val Met Asn Ile Asp Ala Leu Ala Ser Phe Ala Asp His Gly Glu Ser Asp Gln Glu Ile
1 Thr Phe Val Asn Tyr Tyr Val Phe
2 His Thr Phe Val Asn Tyr Tyr Val Phe
3 Asp Phe Val Asn Tyr Ala Tyr Val Phe
4 Phe Val Asn Tyr Tyr Val Phe
5 Ser Cys Phe Val Asn Tyr Tyr Val Phe
6 Leu Phe Thr Ile Val Asn Tyr Tyr Val Phe
7 Phe Val Asn Tyr Val Tyr Val Phe
157 159 160 161 162 163
Lys Ala Lys Lys Ala Gln
Val
evolution #1
evolution #2
evolution #3

FIGURE 128

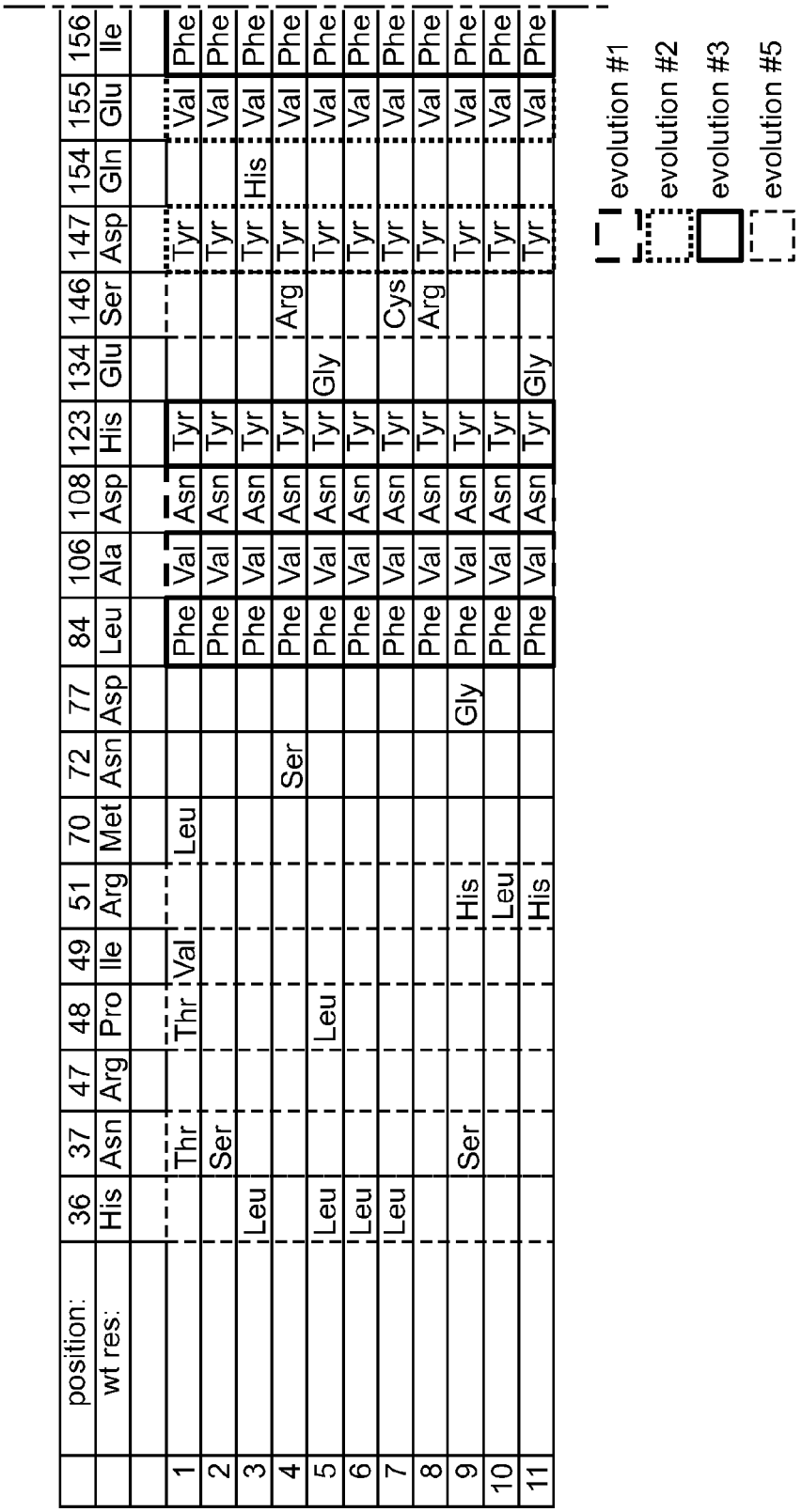

position: 36 37 47 48 49 51 70 72 77 84 106 108 123 134 146 147 154 155 156
wt res: His Asn Arg Pro Ile Arg Met Asn Asp Leu Ala Asp His Glu Ser Asp Gln Glu Ile
1 Thr Val Leu Phe Val Asn Tyr Tyr Val Phe
2 Ser Phe Val Asn Tyr Tyr Val Phe
3 Leu Phe Val Asn Tyr Tyr His Val Phe
4 Ser Phe Val Asn Tyr Arg Tyr Val Phe
5 Leu Leu Phe Val Asn Tyr Gly Tyr Val Phe
6 Leu Phe Val Asn Tyr Tyr Val Phe
7 Leu Phe Val Asn Tyr Cys Tyr Val Phe
8 Phe Val Asn Tyr Arg Tyr Val Phe
9 Ser His Gly Phe Val Asn Tyr Tyr Val Phe
10 Leu Phe Val Asn Tyr Tyr Val Phe
11 His Phe Val Asn Tyr Gly Tyr Val Phe
evolution #1
evolution #2
evolution #3
evolution #5

FIGURE 128 (Continued)

| 157 | 161 | | | | | |
|---|---|---|---|---|---|---|
| Lys | Lys | position | # | mammalian vector | | bacterial vector |
| | | plate1, E11 | 85 | pNMG-392 | | pNMG-413 |
| | Thr | plate1, B11 | 82 | pNMG-393 | | pNMG-414 |
| | | plate1, H5 | 40 | pNMG-394 | | pNMG-415 |
| | | plate1, G10 | 79 | pNMG-395 | | pNMG-416 |
| | | plate2, H11 | 88 | pNMG-396 | | n/a |
| Asn | | plate1, G1 | 7 | pNMG-397 | | pNMG-417 |
| | | plate1, H3 | 24 | pNMG-398 | | pNMG-418 |
| | Thr | plate1, G3 | 23 | pNMG-399 | | pNMG-419 |
| | | plate1, D2 | 12 | pNMG-400 | | pNMG-420 |
| Asn | | plate1, F3 | 22 | pNMG-401 | | pNMG-421 |
| Asn | | plate2, G10 | 79 | n/a | | pNMG-422 |

FIGURE 129

Hek-2 site: 5'-$GA_2A_3CA_5CA_7A_8A_9$ $GCA_{12}TA_{14}GA_{16}$CTGCGGG-3'

| HEK2 pNMG-339 | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.8% | 95.9% | 44.1% | 98.4% | 98.5% | 99.3% | 99.9% | 100.0% | 100.0% |
| C | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| G | 0.2% | 4.1% | 55.9% | 1.6% | 1.5% | 0.7% | 0.0% | 0.0% | 0.0% |
| T | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |

| HEK2 pNMG-340 | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.89% | 97.88% | 34.81% | 99.43% | 98.98% | 99.54% | 99.98% | 99.99% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.10% | 2.12% | 65.18% | 0.56% | 1.02% | 0.45% | 0.01% | 0.01% | 0.01% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |

| HEK2 pNMG-341 | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.80% | 94.79% | 29.76% | 98.16% | 98.08% | 99.02% | 99.92% | 99.98% | 99.99% |
| C | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.19% | 5.19% | 70.23% | 1.84% | 1.91% | 0.98% | 0.07% | 0.02% | 0.01% |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |

| HEK2 pNMG-346 | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.97% | 99.89% | 93.08% | 99.94% | 99.98% | 99.89% | 99.99% | 99.98% | 99.99% |
| C | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.02% | 0.10% | 6.91% | 0.04% | 0.01% | 0.10% | 0.01% | 0.01% | 0.01% |
| T | 0.00% | 0.00% | 0.01% | 0.02% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% |

FIGURE 129 (Continued)

| HEK2 pNMG-347 | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.97% | 99.68% | 87.50% | 99.89% | 99.86% | 99.74% | 99.99% | 99.99% | 99.99% |
| C | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.03% | 0.31% | 12.49% | 0.11% | 0.13% | 0.26% | 0.01% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% |

| HEK2 pNMG-348 | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.98% | 99.72% | 84.66% | 99.93% | 99.93% | 99.90% | 99.92% | 99.98% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.01% | 0.27% | 15.34% | 0.06% | 0.06% | 0.10% | 0.07% | 0.02% | 0.01% |
| T | 0.00% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% |

| HEK2 pNMG-349 | 2 | 3 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.98% | 99.71% | 85.59% | 99.94% | 99.84% | 99.92% | 99.97% | 99.97% | 99.99% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.02% | 0.28% | 14.40% | 0.05% | 0.15% | 0.07% | 0.02% | 0.02% | 0.01% |
| T | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% |

FIGURE 130

Hek 2-1 site: 5'-G$A_2$$A_3$$A_4$$A_5$$A_6$$A_7$$A_8$$A_9$GC$A_{12}$G$A_{14}$GACTGCTGG-3'

| HEK2-1 pNMG-339 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.99% | 99.99% | 99.97% | 99.90% | 99.97% | 99.98% | 99.98% | 99.97% | 99.97% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.01% | 0.03% | 0.10% | 0.03% | 0.01% | 0.01% | 0.02% | 0.02% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |

| HEK2-1 pNMG-340 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.99% | 99.99% | 99.98% | 99.88% | 99.98% | 99.99% | 99.99% | 99.97% | 99.98% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.01% | 0.01% | 0.11% | 0.02% | 0.01% | 0.01% | 0.03% | 0.01% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |

| HEK2-1 pNMG-341 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.98% | 99.99% | 99.94% | 99.80% | 99.93% | 99.98% | 99.98% | 99.98% | 99.98% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.01% | 0.05% | 0.20% | 0.07% | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% |
| | T | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |

| HEK2-1 pNMG-346 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | 99.97% | 99.98% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |

FIGURE 130 (Continued)

| HEK2-1 pNMG-347 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.99% | 99.99% | 99.99% | 99.98% | 99.99% | 99.99% | 99.99% | 99.98% | 99.97% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.00% | 0.01% | 0.02% | 0.01% | 0.00% | 0.01% | 0.02% | 0.02% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |

| HEK2-1 pNMG-348 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | 99.99% | 99.98% | 99.98% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.00% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.02% | 0.01% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |

| HEK2-1 pNMG-349 | | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.99% | 99.99% | 100.00% | 99.99% | 99.99% | 99.99% | 99.99% | 99.98% | 99.97% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.02% | 0.02% | 0.01% |
| | T | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |

FIGURE 131

Hek 2-2 site: 5'-$GA_2A_3TA_5CTA_8A_9GCA_{12}TA_{14}GA_{16}$CTCCAGG-3'

| HEK2-2 pNMG-339 | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A |
| A | 99.63% | 99.44% | 69.55% | 99.54% | 99.65% | 99.97% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.36% | 0.55% | 30.44% | 0.45% | 0.35% | 0.01% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% |

| HEK2-2 pNMG-340 | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A |
| A | 99.84% | 99.83% | 66.30% | 99.59% | 99.91% | 99.98% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.15% | 0.16% | 33.68% | 0.40% | 0.09% | 0.01% | 0.01% | 0.01% |
| T | 0.00% | 0.01% | 0.02% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |

| HEK2-2 pNMG-341 | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A |
| A | 99.58% | 99.29% | 52.66% | 99.35% | 99.60% | 99.95% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.42% | 0.70% | 47.32% | 0.64% | 0.40% | 0.03% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% |

| HEK2-2 pNMG-346 | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A |
| A | 99.99% | 99.98% | 90.58% | 99.94% | 99.98% | 99.98% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.00% | 0.02% | 9.40% | 0.05% | 0.02% | 0.01% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |

FIGURE 131 (Continued)

| HEK2-2 pNMG-347 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.99% | 99.96% | 84.82% | 99.93% | 99.97% | 99.97% | 99.97% | 99.97% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.03% | 15.16% | 0.06% | 0.03% | 0.01% | 0.01% | 0.01% |
| | T | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.02% | 0.01% | 0.01% |

| HEK2-2 pNMG-348 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.98% | 99.93% | 85.38% | 99.91% | 99.98% | 99.97% | 99.97% | 99.97% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.07% | 14.61% | 0.08% | 0.02% | 0.01% | 0.01% | 0.01% |
| | T | 0.01% | 0.00% | 0.02% | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% |

| HEK2-2 pNMG-349 | | 2 | 3 | 5 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A |
| | A | 99.98% | 99.95% | 86.93% | 99.92% | 99.99% | 99.97% | 99.97% | 99.97% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.05% | 13.06% | 0.07% | 0.01% | 0.01% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% |

FIGURE 132

Hek 2-3 site: 5'-GT$A_3A_4A_5CA_7A_8A_9GCA_{12}TA_{14}GA_{16}$CTGAGGG-3'

| HEK2-3 pNMG-339 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.98% | 99.98% | 99.43% | 99.98% | 99.97% | 99.98% | 99.97% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.01% | 0.02% | 0.56% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |
| T | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |

| HEK2-3 pNMG-340 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.97% | 99.96% | 99.02% | 99.96% | 99.97% | 99.76% | 99.96% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.19% | 0.00% | 0.00% | 0.00% |
| G | 0.03% | 0.03% | 0.97% | 0.03% | 0.03% | 0.05% | 0.02% | 0.01% | 0.01% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.02% | 0.01% | 0.01% |

| HEK2-3 pNMG-341 | 3 | 4 | 5 | 7 | 8 | 9 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A |
| A | 99.97% | 99.95% | 98.58% | 99.97% | 99.97% | 99.79% | 99.96% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.19% | 0.00% | 0.00% | 0.00% |
| G | 0.02% | 0.05% | 1.42% | 0.01% | 0.02% | 0.02% | 0.02% | 0.01% | 0.01% |
| T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% |

# FIGURE 133

Hek 2-4 site: 5'-GG$A_3$C$A_5$C$A_7A_8$A9GCTT$A_{14}$G$A_{16}$CTCCAGG-3'

| HEK2-4 pNMG-339 | | 3 A | 5 A | 7 A | 8 A | 9 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|
| | A | 95.12% | 75.77% | 99.50% | 99.35% | 99.67% | 99.98% | 99.98% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 4.87% | 24.21% | 0.49% | 0.64% | 0.32% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% |

| HEK2-4 pNMG-340 | | 3 A | 5 A | 7 A | 8 A | 9 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|
| | A | 97.41% | 74.87% | 99.76% | 99.67% | 99.82% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 2.58% | 25.12% | 0.23% | 0.32% | 0.17% | 0.02% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |

| HEK2-4 pNMG-341 | | 3 A | 5 A | 7 A | 8 A | 9 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|
| | A | 93.73% | 64.91% | 99.34% | 99.22% | 99.69% | 99.98% | 99.98% |
| | C | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 6.26% | 35.06% | 0.65% | 0.78% | 0.30% | 0.02% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% |



SUBSTITUTE SHEET (RULE 26)

# FIGURE 134

Hek2-6 similar: 5'G$A_2$ $A_3$G$A_5$CC$A_8$ $A_9$GGATAGACTGCTGG-3'

| HEK2-6 pNMG-339 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A |
|---|---|---|---|---|---|---|---|---|
| | A | 99.94% | 99.86% | 95.56% | 99.74% | 99.90% | 99.97% | 99.98% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.04% | 0.12% | 4.43% | 0.24% | 0.08% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.02% | 0.00% |

| HEK2-6 pNMG-340 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A |
|---|---|---|---|---|---|---|---|---|
| | A | 99.97% | 99.97% | 95.74% | 99.81% | 99.90% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.02% | 0.02% | 4.25% | 0.17% | 0.09% | 0.01% | 0.01% |
| | T | 0.01% | 0.00% | 0.02% | 0.01% | 0.01% | 0.01% | 0.00% |

| HEK2-6 pNMG-341 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A |
|---|---|---|---|---|---|---|---|---|
| | A | 99.94% | 99.86% | 95.56% | 99.74% | 99.90% | 99.97% | 99.98% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.04% | 0.12% | 4.43% | 0.24% | 0.08% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.02% | 0.01% | 0.02% | 0.00% |

| HEK2-6 pNMG-346 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A |
|---|---|---|---|---|---|---|---|---|
| | A | 99.98% | 99.99% | 99.76% | 99.97% | 99.98% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.00% | 0.23% | 0.02% | 0.01% | 0.00% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% |

# FIGURE 134 (Continued)

| HEK2-6 pNMG-347 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A |
|---|---|---|---|---|---|---|---|---|
| | A | 99.95% | 99.97% | 99.62% | 99.97% | 99.95% | 99.97% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.03% | 0.01% | 0.35% | 0.02% | 0.03% | 0.01% | 0.01% |
| | T | 0.02% | 0.01% | 0.02% | 0.00% | 0.02% | 0.02% | 0.00% |

| HEK2-6 pNMG-348 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A |
|---|---|---|---|---|---|---|---|---|
| | A | 99.97% | 99.98% | 99.68% | 99.97% | 99.97% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.02% | 0.02% | 0.30% | 0.01% | 0.01% | 0.00% | 0.01% |
| | T | 0.01% | 0.01% | 0.02% | 0.01% | 0.02% | 0.02% | 0.01% |

| HEK2-6 pNMG-349 | | 2 A | 3 A | 5 A | 8 A | 9 A | 12 A | 14 A |
|---|---|---|---|---|---|---|---|---|
| | A | 99.97% | 99.97% | 99.74% | 99.97% | 99.97% | 99.97% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.02% | 0.01% | 0.25% | 0.01% | 0.02% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% |

FIGURE 135 Hek2-9 site: 5'-$GA_2A_3A_4A_5CA_7A_8A_9A_{10}CA_{12}TA_{14}$GAGTGCTGG-3'

| HEK2-9 pNMG-339 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A | A |
| A | 99.95% | 99.90% | 99.69% | 96.43% | 99.74% | 99.76% | 99.54% | 98.67% | 99.97% | 99.97% |
| C | 0.00% | 0.00% | 0.03% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| G | 0.04% | 0.10% | 0.28% | 3.56% | 0.24% | 0.24% | 0.46% | 1.32% | 0.01% | 0.02% |
| T | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |

| HEK2-9 pNMG-340 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A | A |
| A | 99.96% | 99.97% | 99.80% | 96.26% | 99.94% | 99.92% | 99.76% | 99.33% | 99.98% | 99.97% |
| C | 0.00% | 0.00% | 0.03% | 0.01% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% |
| G | 0.03% | 0.03% | 0.17% | 3.73% | 0.05% | 0.07% | 0.24% | 0.66% | 0.01% | 0.02% |
| T | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% |

| HEK2-9 pNMG-341 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A | A |
| A | 99.90% | 99.83% | 99.47% | 94.53% | 99.62% | 99.65% | 99.33% | 98.64% | 99.92% | 99.97% |
| C | 0.00% | 0.00% | 0.02% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| G | 0.10% | 0.17% | 0.51% | 5.46% | 0.37% | 0.35% | 0.66% | 1.35% | 0.06% | 0.02% |
| T | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.02% | 0.01% |

| HEK2-9 pNMG-346 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | A | A | A | A | A | A | A | A | A |
| A | 99.98% | 99.99% | 99.96% | 99.90% | 99.99% | 99.98% | 99.99% | 99.95% | 99.98% | 99.98% |
| C | 0.00% | 0.00% | 0.03% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| G | 0.01% | 0.01% | 0.01% | 0.08% | 0.01% | 0.02% | 0.01% | 0.04% | 0.01% | 0.01% |
| T | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |

FIGURE 135 (Continued)

| HEK2-9 pNMG-347 | | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 100.00% | 99.98% | 99.94% | 99.76% | 99.98% | 99.97% | 99.97% | 99.93% | 99.98% | 99.97% |
| | C | 0.00% | 0.00% | 0.04% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | G | 0.00% | 0.01% | 0.02% | 0.23% | 0.01% | 0.02% | 0.03% | 0.06% | 0.01% | 0.02% |
| | T | 0.00% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% |

| HEK2-9 pNMG-348 | | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.97% | 99.98% | 99.96% | 99.85% | 99.99% | 99.99% | 99.99% | 99.96% | 99.98% | 99.94% |
| | C | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | G | 0.01% | 0.02% | 0.02% | 0.14% | 0.01% | 0.01% | 0.01% | 0.03% | 0.00% | 0.04% |
| | T | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% |

| HEK2-9 pNMG-349 | | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 12 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.98% | 99.98% | 99.62% | 99.66% | 99.97% | 99.99% | 99.99% | 99.63% | 99.98% | 99.96% |
| | C | 0.00% | 0.00% | 0.35% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% |
| | G | 0.01% | 0.01% | 0.03% | 0.34% | 0.02% | 0.01% | 0.01% | 0.37% | 0.01% | 0.03% |
| | T | 0.01% | 0.01% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.01% | 0.00% |

# FIGURE 136

Hek2-10 site: 5'-G$A_2$$A_3$C$A_5$T$A_7$$A_8$$A_9$G$A_{11}$$A_{12}$T$A_{14}$G$A_{16}$ATGATGG-3'

| HEK2-10 pNMG-339 | | 2 A | 3 A | 5 A | 7 A | 8 A | 9 A | 11 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 99.71% | 98.28% | 89.09% | 98.35% | 98.78% | 99.52% | 99.46% | 99.98% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.28% | 1.71% | 10.90% | 1.64% | 1.21% | 0.47% | 0.52% | 0.02% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |

| HEK2-10 pNMG-340 | | 2 A | 3 A | 5 A | 7 A | 8 A | 9 A | 11 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 99.87% | 99.47% | 93.87% | 99.11% | 99.59% | 99.79% | 99.75% | 99.98% | 99.97% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.12% | 0.52% | 6.12% | 0.89% | 0.40% | 0.20% | 0.22% | 0.02% | 0.02% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |

| HEK2-10 pNMG-341 | | 2 A | 3 A | 5 A | 7 A | 8 A | 9 A | 11 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 99.61% | 97.99% | 84.59% | 97.09% | 98.45% | 99.40% | 99.47% | 99.97% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.00% | 0.01% | 0.00% | 0.00% | 0.00% |
| | G | 0.39% | 2.00% | 15.40% | 2.90% | 1.53% | 0.59% | 0.50% | 0.02% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.00% | 0.01% | 0.01% |

| HEK2-10 pNMG-346 | | 2 A | 3 A | 5 A | 7 A | 8 A | 9 A | 11 A | 12 A | 14 A | 16 A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | 99.98% | 99.96% | 99.67% | 99.71% | 99.96% | 99.95% | 99.97% | 99.99% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% |
| | G | 0.02% | 0.03% | 0.32% | 0.29% | 0.03% | 0.04% | 0.01% | 0.01% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |



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FIGURE 136 (Continued)

| HEK2-10 pNMG-347 | | 2 | 3 | 5 | 7 | 8 | 9 | 11 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.98% | 99.93% | 99.06% | 99.56% | 99.93% | 99.95% | 99.97% | 99.99% | 99.97% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.06% | 0.93% | 0.43% | 0.06% | 0.04% | 0.01% | 0.01% | 0.02% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% | 0.01% |

| HEK2-10 pNMG-348 | | 2 | 3 | 5 | 7 | 8 | 9 | 11 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.96% | 99.94% | 98.39% | 99.50% | 99.92% | 99.97% | 99.97% | 99.99% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.02% | 0.00% | 0.00% | 0.00% |
| | G | 0.03% | 0.05% | 1.59% | 0.49% | 0.08% | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.00% | 0.01% | 0.01% | 0.01% | 0.01% |

| HEK2-10 pNMG-349 | | 2 | 3 | 5 | 7 | 8 | 9 | 11 | 12 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | A | A | A | A | A | A | A | A | A |
| | A | 99.98% | 99.94% | 98.75% | 99.54% | 99.93% | 99.96% | 99.96% | 99.99% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.01% | 0.03% | 0.00% | 0.00% | 0.00% |
| | G | 0.01% | 0.04% | 1.24% | 0.46% | 0.06% | 0.02% | 0.01% | 0.01% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% | 0.00% | 0.01% | 0.01% |

# FIGURE 137

Hek3- 5'-GGCCCA$_6$GA$_8$CTGA$_{12}$GCA$_{15}$CGTGATGG-3'

| HEK3 pNMG-339 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 97.97% | 94.85% | 99.95% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 2.02% | 5.15% | 0.04% | 0.01% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

| HEK3 pNMG-340 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 98.96% | 96.57% | 99.95% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 1.03% | 3.43% | 0.04% | 0.01% |
| | T | 0.01% | 0.00% | 0.01% | 0.01% |

| HEK3 pNMG-341 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 97.73% | 94.69% | 99.96% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 2.25% | 5.30% | 0.03% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% |

# FIGURE 137 (Continued)

| HEK3 pNMG-346 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.86% | 99.76% | 99.95% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.12% | 0.23% | 0.05% | 0.01% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

| HEK3 pNMG-347 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.73% | 99.43% | 99.96% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.26% | 0.56% | 0.03% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% |

| HEK3 pNMG-348 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.76% | 99.62% | 99.95% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.22% | 0.37% | 0.04% | 0.00% |
| | T | 0.02% | 0.01% | 0.00% | 0.01% |

| HEK3 pNMG-349 | | 6 A | 8 A | 12 A | 15 A |
|---|---|---|---|---|---|
| | A | 99.85% | 99.46% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.14% | 0.53% | 0.02% | 0.00% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

# FIGURE 138

RNF2- 5'-GTCA$_4$TCTTA$_9$GTCA$_{13}$TTA$_{16}$CCTGAGG-3'

| RNF2 pNMG-339 | | 4 A | 9 A | 13 A | 16 A |
|---|---|---|---|---|---|
| | **A** | 99.21% | 98.98% | 99.98% | 99.97% |
| | **C** | 0.00% | 0.02% | 0.00% | 0.00% |
| | **G** | 0.78% | 0.99% | 0.01% | 0.02% |
| | **T** | 0.01% | 0.01% | 0.01% | 0.01% |

| RNF2 pNMG-340 | | 4 A | 9 A | 13 A | 16 A |
|---|---|---|---|---|---|
| | **A** | 99.75% | 99.69% | 99.99% | 99.98% |
| | **C** | 0.00% | 0.01% | 0.00% | 0.00% |
| | **G** | 0.24% | 0.29% | 0.01% | 0.01% |
| | **T** | 0.01% | 0.01% | 0.01% | 0.01% |

| RNF2 pNMG-341 | | 4 A | 9 A | 13 A | 16 A |
|---|---|---|---|---|---|
| | **A** | 98.86% | 98.94% | 99.97% | 99.97% |
| | **C** | 0.00% | 0.02% | 0.00% | 0.00% |
| | **G** | 1.12% | 1.03% | 0.01% | 0.02% |
| | **T** | 0.02% | 0.01% | 0.01% | 0.01% |

# FIGURE 138 (Continued)

| RNF2 pNMG-346 | | 4 A | 9 A | 13 A | 16 A |
|---|---|---|---|---|---|
| | A | 99.97% | 99.94% | 99.98% | 99.98% |
| | C | 0.00% | 0.02% | 0.00% | 0.00% |
| | G | 0.02% | 0.04% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% |

| RNF2 pNMG-347 | | 4 A | 9 A | 13 A | 16 A |
|---|---|---|---|---|---|
| | A | 99.95% | 99.90% | 99.98% | 99.98% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% |
| | G | 0.04% | 0.08% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.01% | 0.01% |

| RNF2 pNMG-348 | | 4 A | 9 A | 13 A | 16 A |
|---|---|---|---|---|---|
| | A | 99.93% | 99.96% | 99.97% | 99.97% |
| | C | 0.00% | 0.01% | 0.00% | 0.00% |
| | G | 0.06% | 0.02% | 0.01% | 0.02% |
| | T | 0.01% | 0.00% | 0.01% | 0.01% |

| RNF2 pNMG-349 | | 4 A | 9 A | 13 A | 16 A |
|---|---|---|---|---|---|
| | A | 99.94% | 99.95% | 99.98% | 99.98% |
| | C | 0.00% | 0.02% | 0.00% | 0.00% |
| | G | 0.05% | 0.02% | 0.01% | 0.01% |
| | T | 0.01% | 0.00% | 0.01% | 0.01% |

# FIGURE 139

FANCF- 5'-GG$A_3A_4$TCCCTTCTGC$A_{15}$GC$A_{18}$CCTGG-3'

| FanCF pNMG-339 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.72% | 98.46% | 99.98% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.26% | 1.52% | 0.02% | 0.01% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-340 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.95% | 99.50% | 99.98% | 99.99% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.04% | 0.48% | 0.01% | 0.00% |
| | T | 0.01% | 0.00% | 0.00% | 0.01% |

| FanCF pNMG-341 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.69% | 98.46% | 99.98% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.29% | 1.52% | 0.02% | 0.01% |
| | T | 0.01% | 0.00% | 0.00% | 0.01% |

# FIGURE 139 (Continued)

| FanCF pNMG-346 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.97% | 99.85% | 99.98% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.01% | 0.13% | 0.02% | 0.01% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-347 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.95% | 99.65% | 99.98% | 99.99% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.04% | 0.34% | 0.02% | 0.00% |
| | T | 0.01% | 0.00% | 0.01% | 0.01% |

| FanCF pNMG-348 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.94% | 99.08% | 99.98% | 99.98% |
| | C | 0.00% | 0.00% | 0.00% | 0.00% |
| | G | 0.05% | 0.90% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

| FanCF pNMG-349 | | 3 A | 4 A | 15 A | 18 A |
|---|---|---|---|---|---|
| | A | 99.93% | 99.33% | 99.98% | 99.98% |
| | C | 0.01% | 0.01% | 0.00% | 0.00% |
| | G | 0.05% | 0.65% | 0.01% | 0.01% |
| | T | 0.01% | 0.01% | 0.00% | 0.01% |

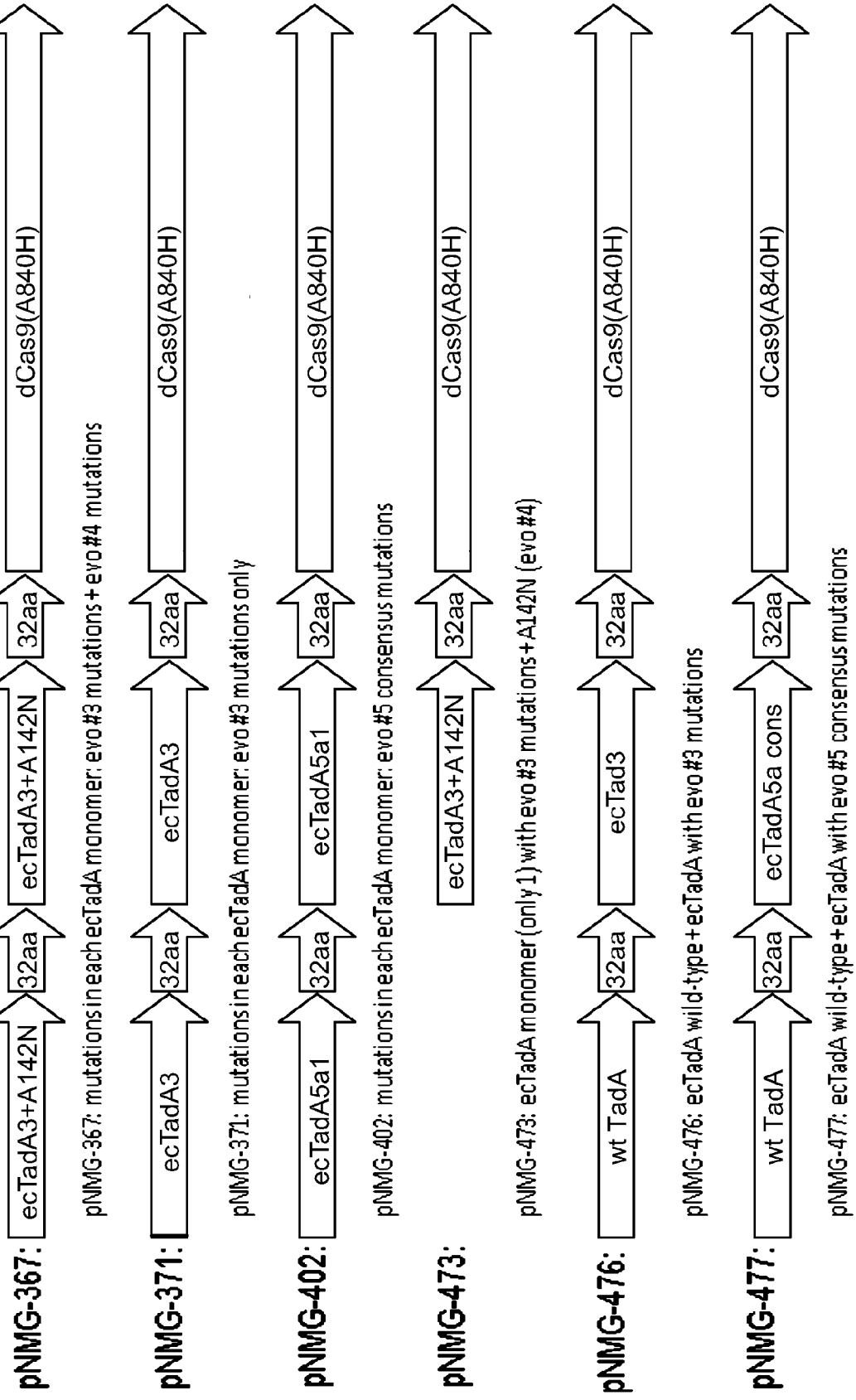

pNMG-367:
ecTadA3+A142N
32aa
ecTadA3+A142N
32aa
dCas9(A840H)
pNMG-367: mutations in each ecTadA monomer: evo #3 mutations + evo #4 mutations
pNMG-371:
ecTadA3
32aa
ecTadA3
32aa
dCas9(A840H)
pNMG-371: mutations in each ecTadA monomer: evo #3 mutations only
pNMG-402:
ecTadA5a1
32aa
ecTadA5a1
32aa
dCas9(A840H)
pNMG-402: mutations in each ecTadA monomer: evo #5 consensus mutations
pNMG-473:
ecTadA3+A142N
32aa
dCas9(A840H)
pNMG-473: ecTadA monomer (only 1) with evo #3 mutations + A142N (evo #4)
pNMG-476:
wt TadA
32aa
ecTad3
32aa
dCas9(A840H)
pNMG-476: ecTadA wild-type + ecTadA with evo #3 mutations
pNMG-477:
wt TadA
32aa
ecTadA5a cons
32aa
dCas9(A840H)
pNMG-477: ecTadA wild-type + ecTadA with evo #5 consensus mutations

Figure 140

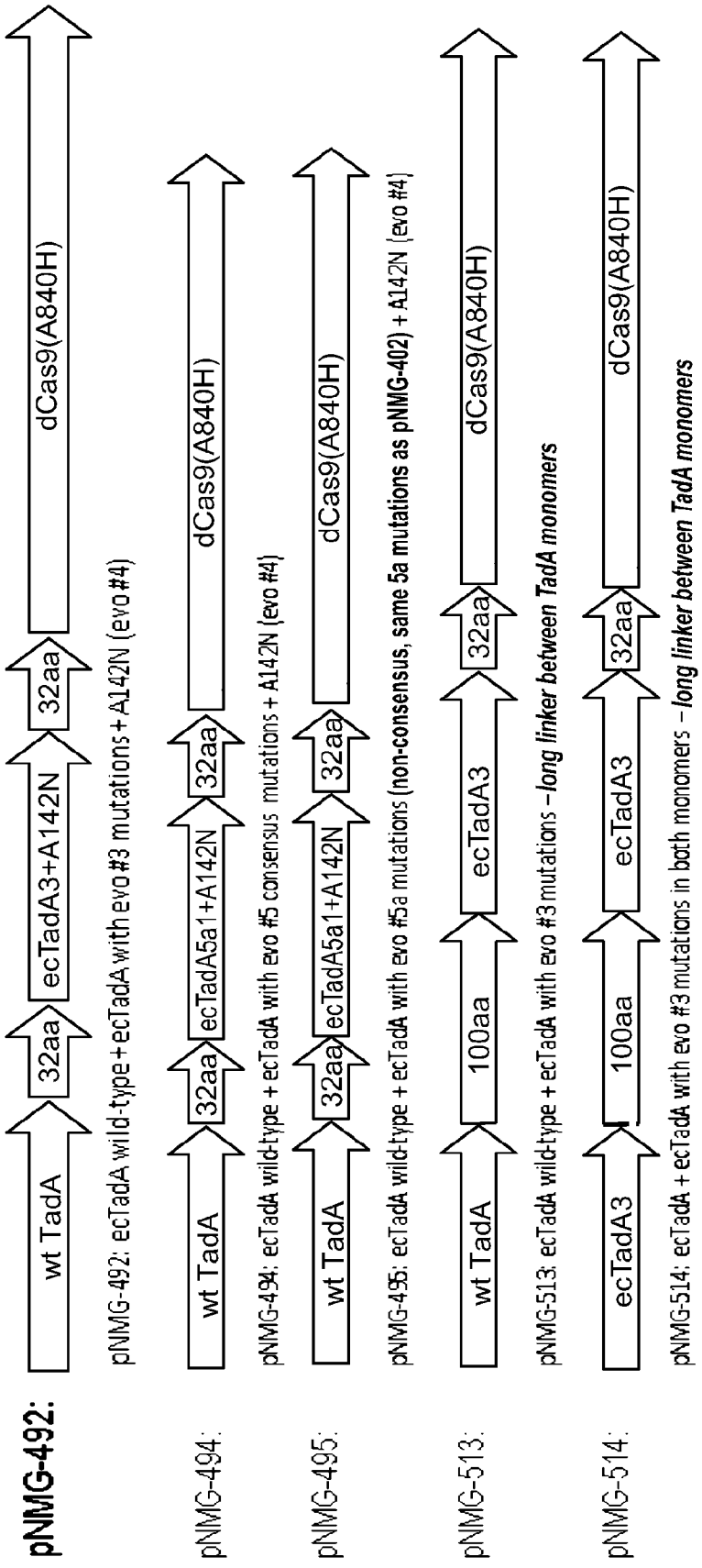



Figure 140 (Continued)

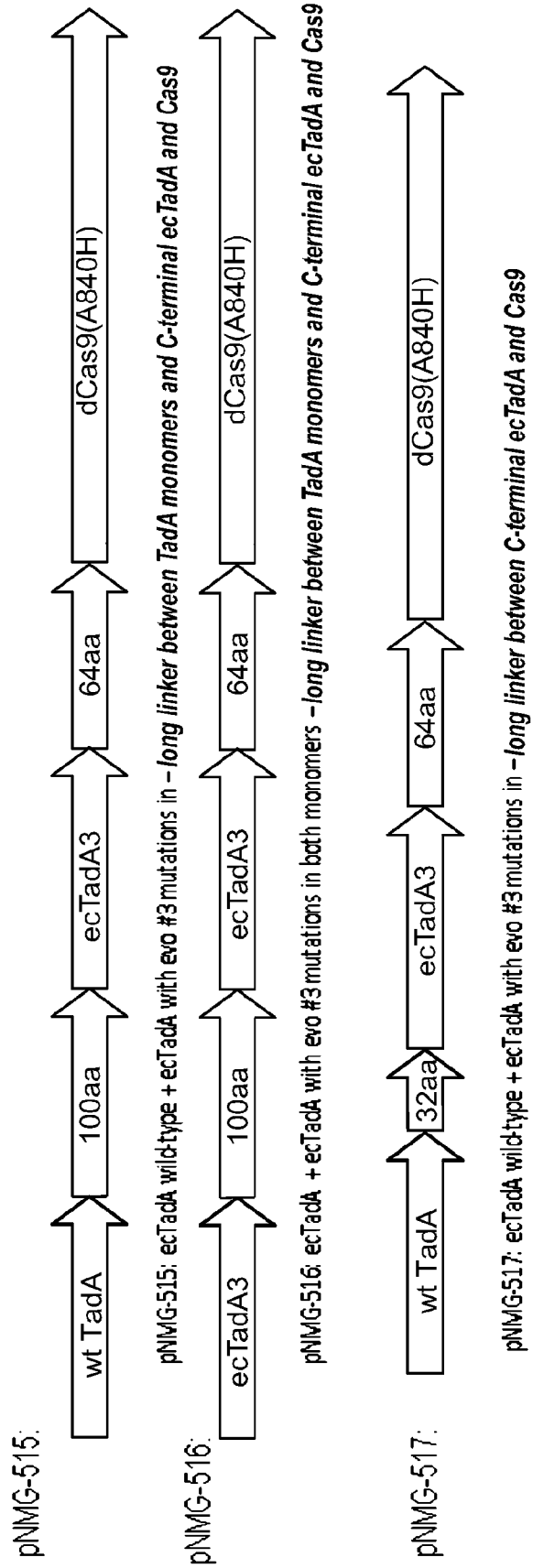



Figure 140 (Continued)

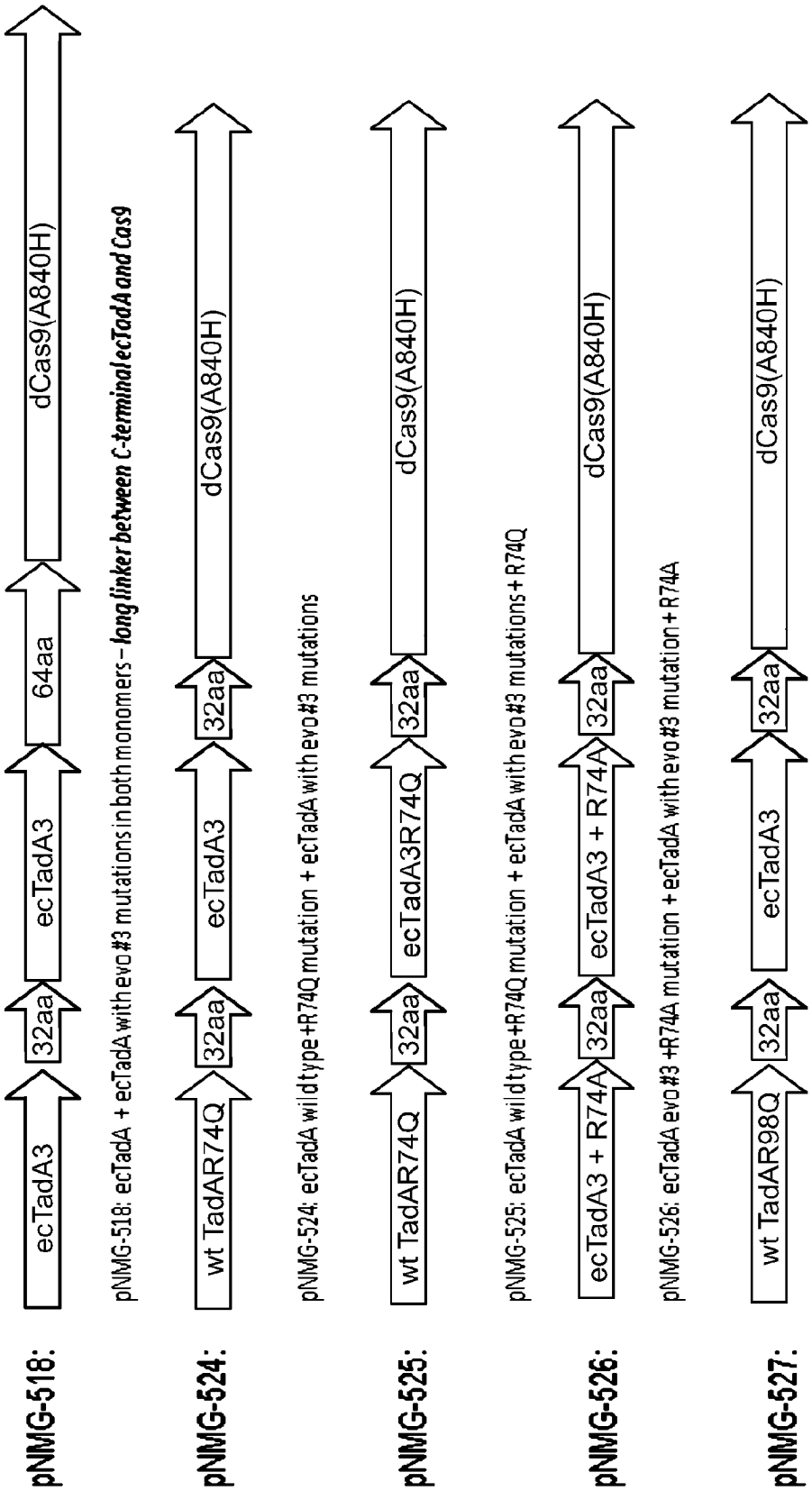



Figure 140 (Continued)

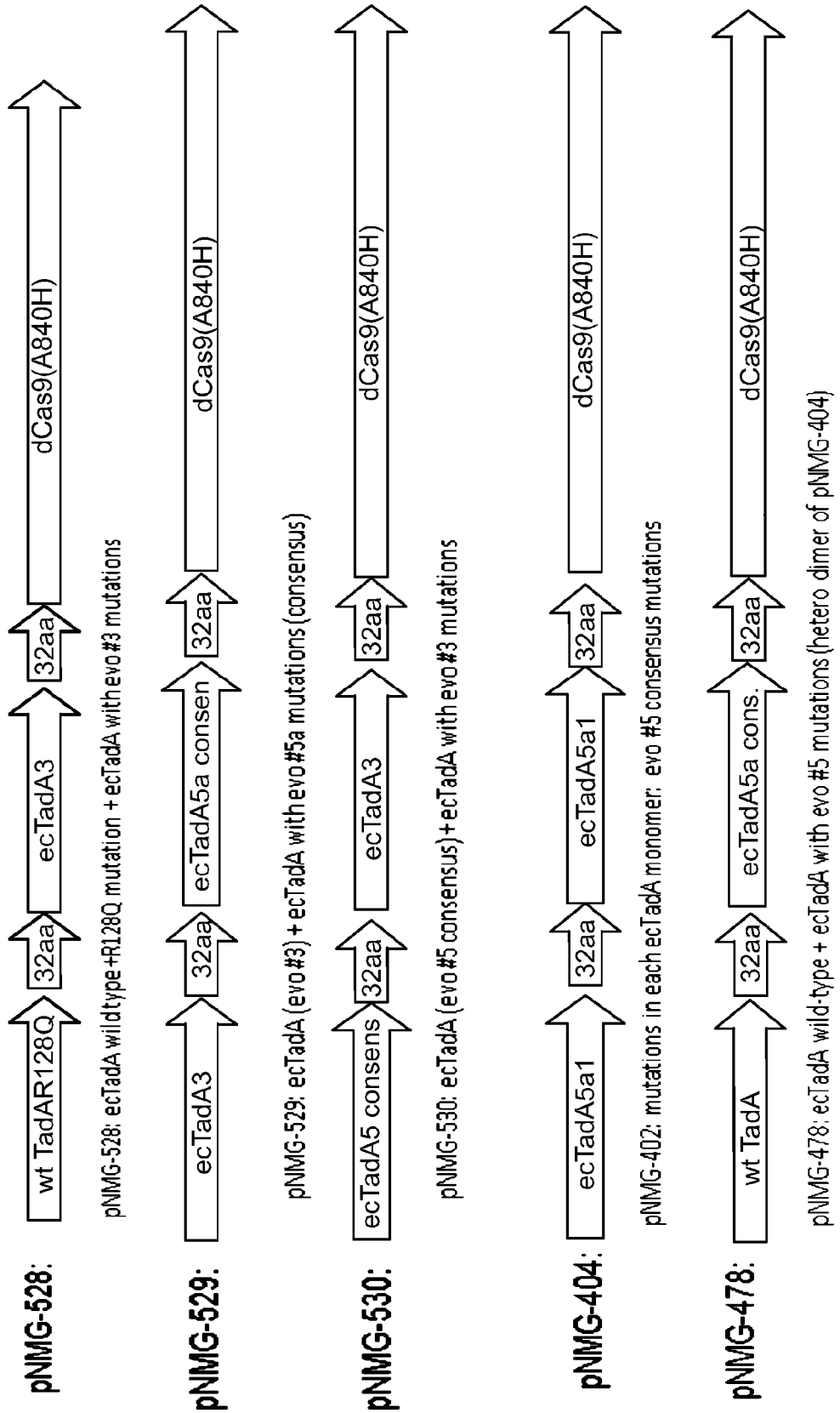



Figure 140 (Continued)

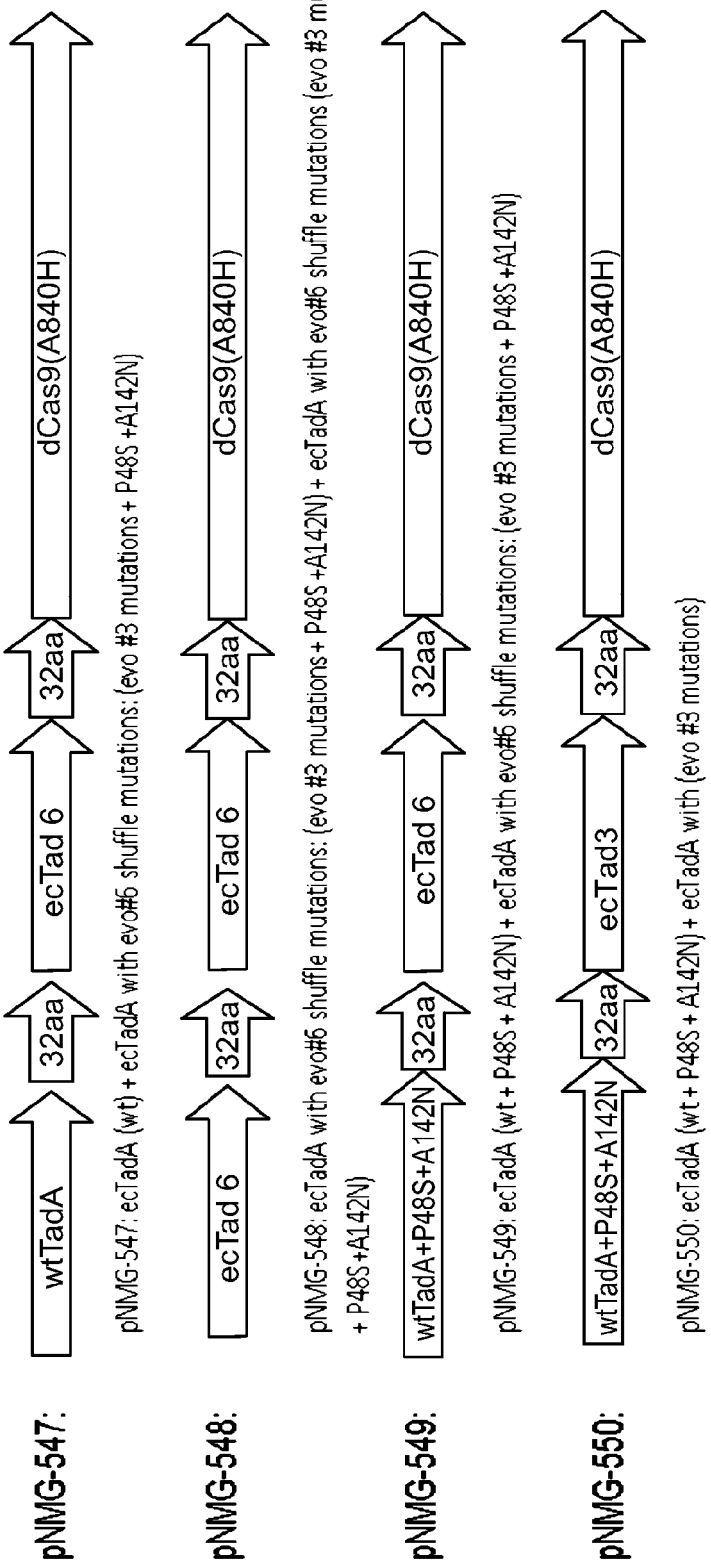



Figure 140 (Continued)

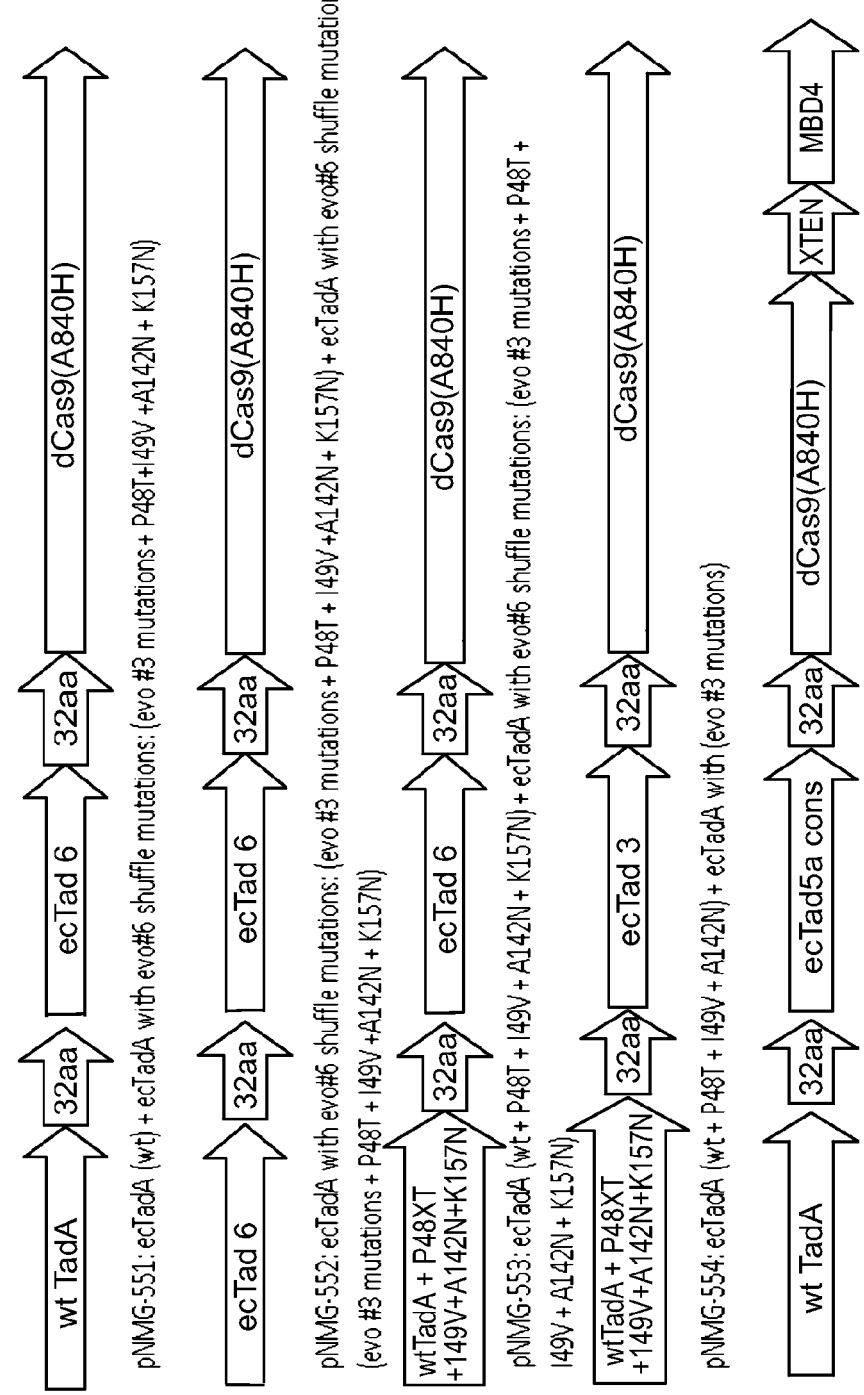



Figure 140 (Continued)

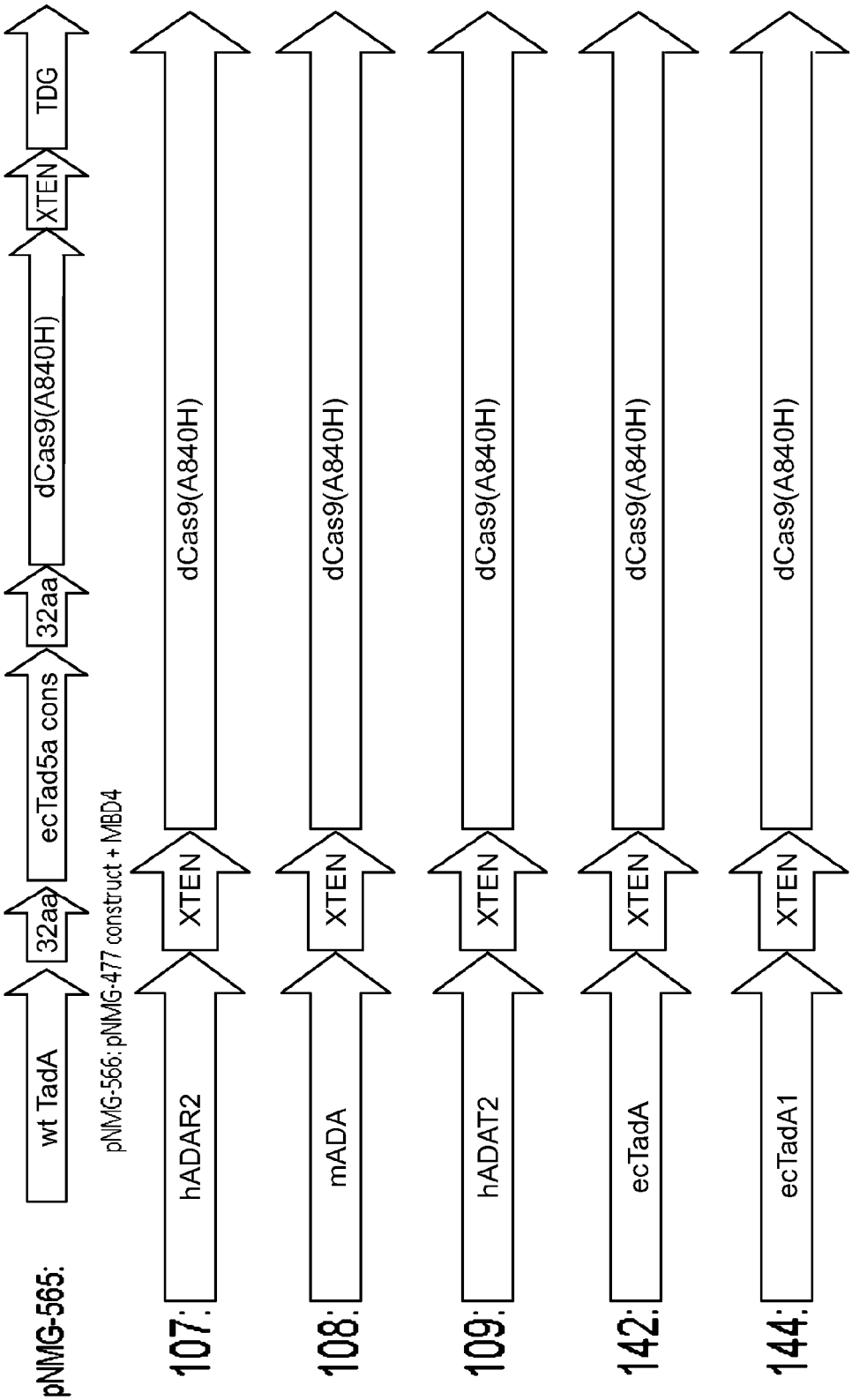



Figure 140 (Continued)

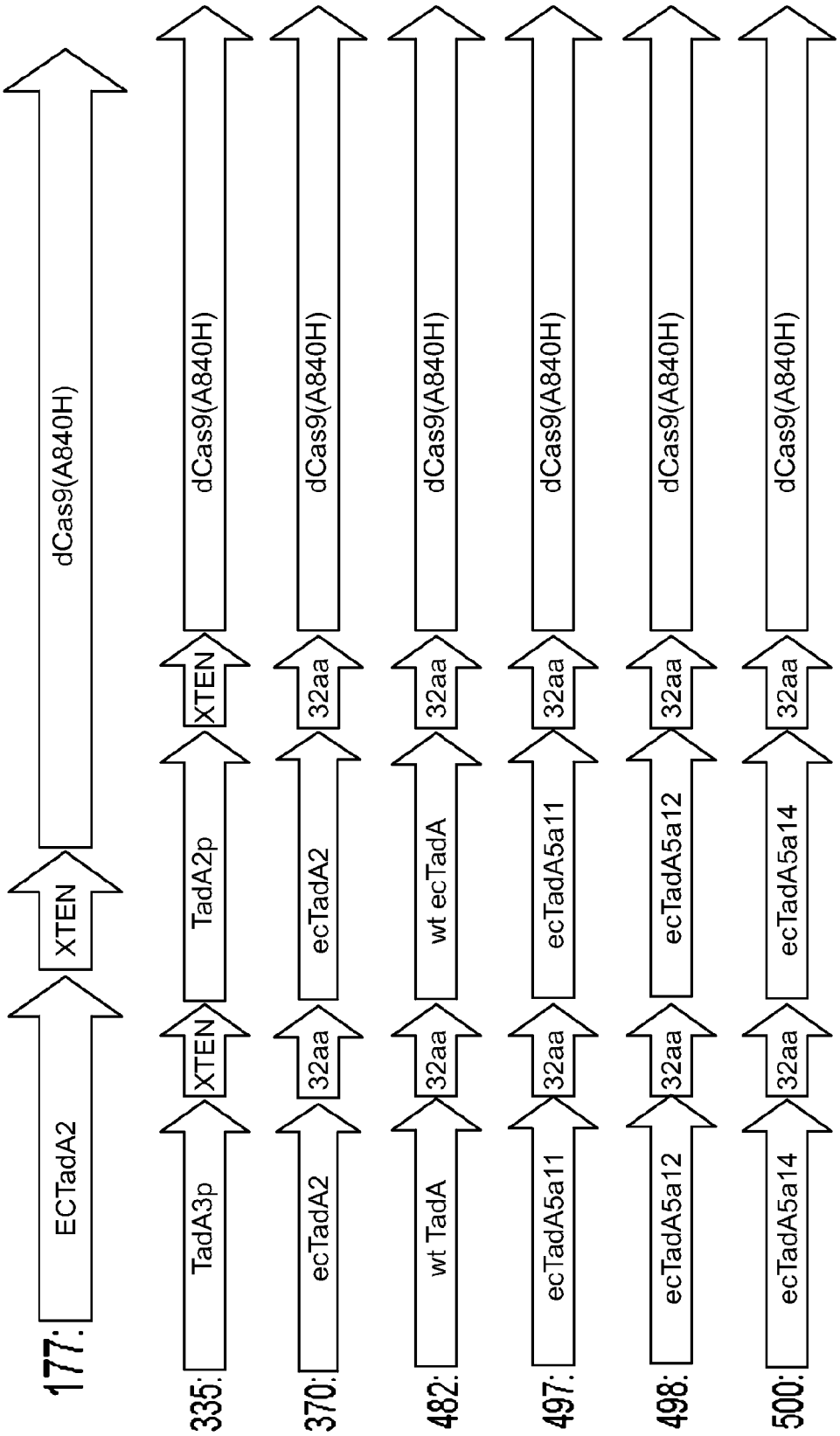



Figure 140 (Continued)

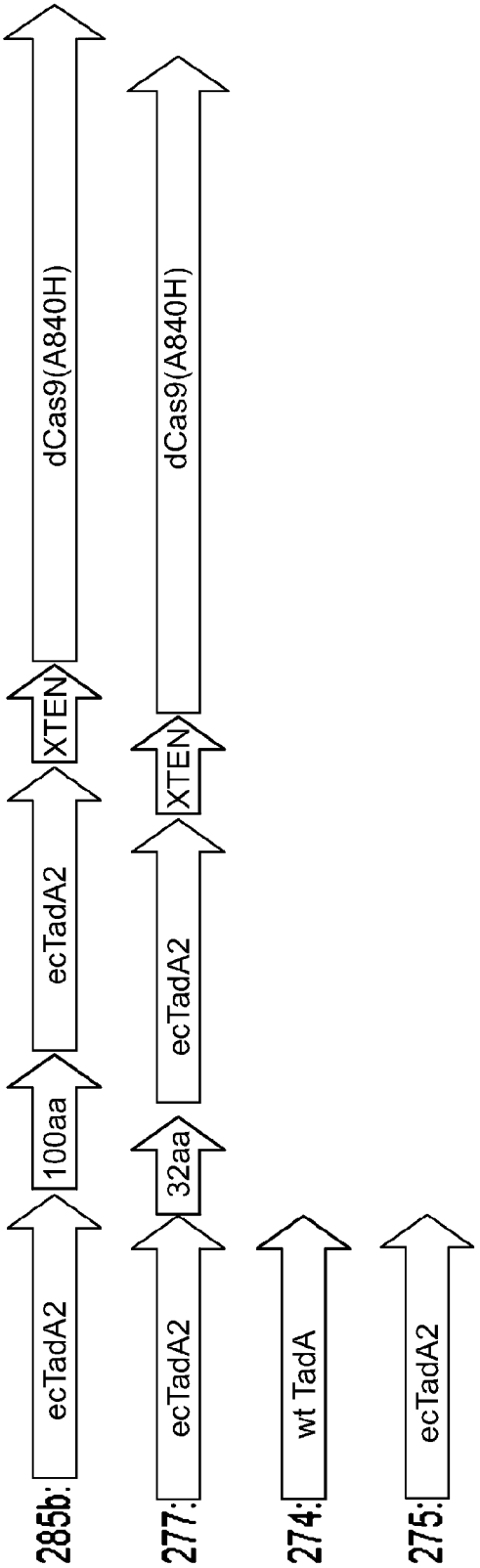



Figure 140 (Continued)

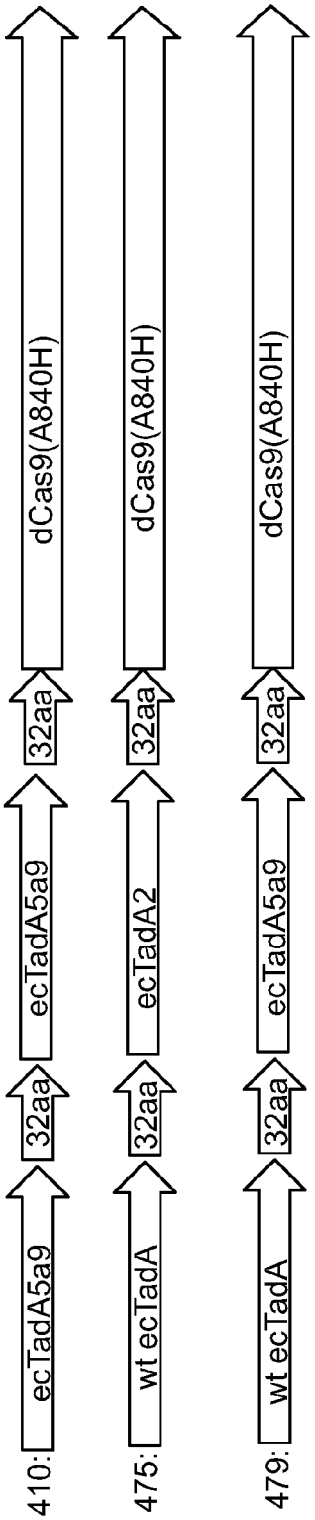



Figure 140 (Continued)

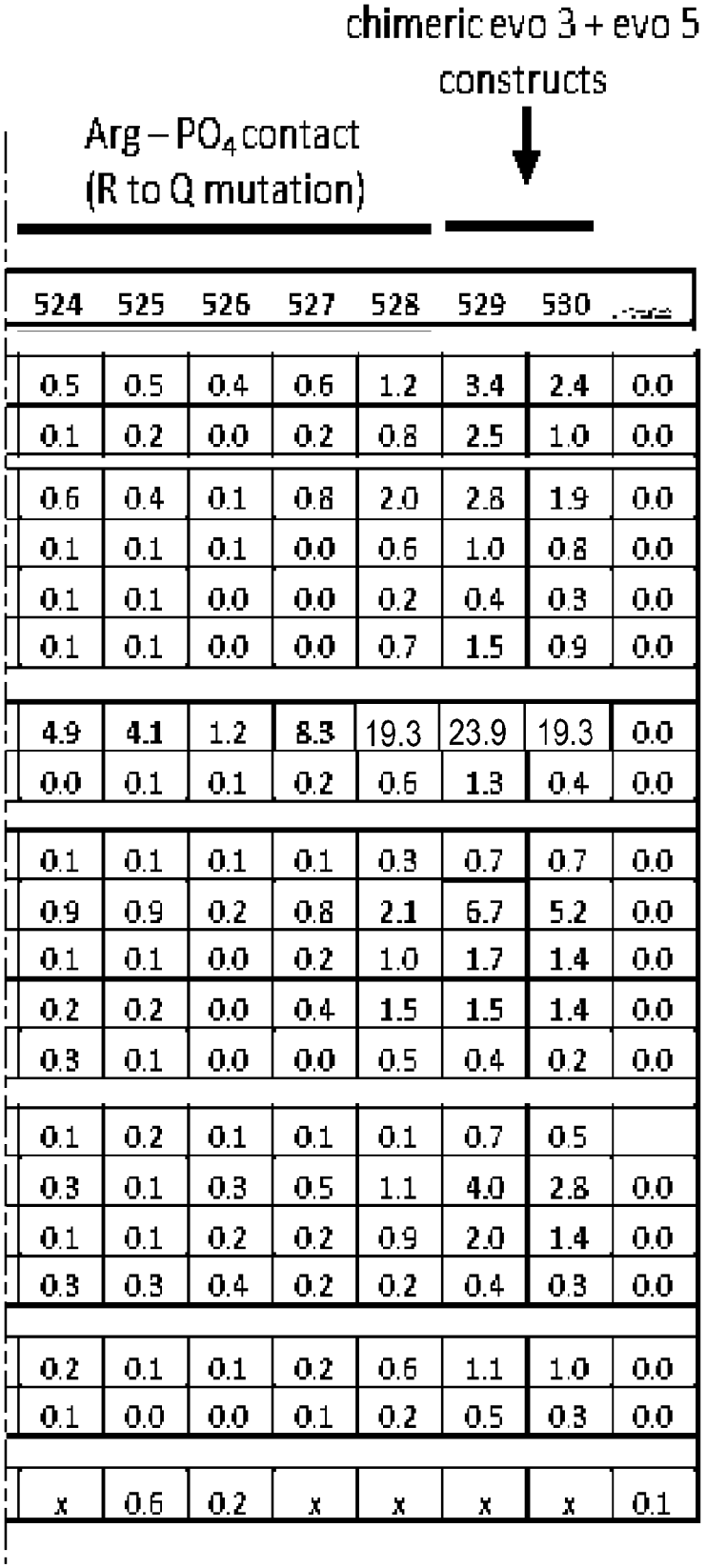
| | ABE plasmid # | 367 | 371 | 402 |
|---|---|---|---|---|
| sgRNA plasmid # | | | | |
| pNMG-466 | GAAGA(5)TAGAGAATAGACTGCTGG | 1.5 | 2.6 | 2.4 |
| | GAAGATA(7)GAGAATAGACTGCTGG | 0.1 | 1.0 | 2.0 |
| pNMG-467 | GGCTA(5)AAGACCATAGACTGTGGG | 0.1 | 2.3 | 2.7 |
| | GGCTAA(6)AGACCATAGACTGTGGG | 0.2 | 1.1 | 0.9 |
| | GGCTAAA(7)GACCATAGACTGTGGG | 0.0 | 0.4 | 0.3 |
| | GGCTAAAGA(9)CCATAGACTGTGGG | 0.6 | 1.3 | 1.4 |
| pNMG-469 | GAGTA(5)TGAGGCATAGACTGCAGG | 7.2 | 17.1 | 22.2 |
| | GAGTATGA(8)GGCATAGACTGCAGG | 1.0 | 0.5 | 1.0 |
| pNMG-470 | GTCA(4)AGAAAGCAGAGACTGCCGG | 0.3 | 0.7 | 1.2 |
| | GTCAA(5)GAAAGCAGAGACTGCCGG | 2.7 | 5.4 | 6.4 |
| | GTCAAGA(7)AAGCAGAGACTGCCGG | 2.5 | 1.1 | 1.5 |
| | GTCAAGAA(8)AGCAGAGACTGCCGG | 1.3 | 1.2 | 1.0 |
| | GTCAAGAAA(9)GCAGAGACTGCCGG | 0.4 | 0.2 | 0.3 |
| pNMG-501 | GACA(4)AAGAGGAAGAGAGACGGGG | 0.1 | 0.4 | 0.4 |
| | GACAA(5)AGAGGAAGAGAGACGGGG | 0.9 | 2.7 | 3.5 |
| | GACAAA(6)GAGGAAGAGAGACGGGG | 0.3 | 1.3 | 1.9 |
| | GACAAAGA(8)GGAAGAGAGACGGGG | 0.4 | 0.3 | 0.3 |
| pNMG-509 | GCCTA(5)GGCAGTGGGGGTGCAGGG | 0.2 | 0.8 | 1.1 |
| | GCCTAGGCA(9)GTGGGGGTGCAGGG | 0.2 | 0.2 | 0.5 |
| pNMG-502 | GGGGA(5)CGCGCTGGCTTCCCGGGG | x | x | x |

FIGURE 141

longer linkers between ecTadA monomers and/or C-terminal ecTadA and C9

| 473 | 476 | 477 | 492 | 494 | 495 | 513 | 514 | 515 | 516 | 517 | 518 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.9 | 5.9 | 8.1 | 0.8 | 1.9 | 0.8 | 1.0 | 1.0 | 0.2 | 0.2 | 0.5 | 0.5 |
| 0.0 | 1.2 | 4.1 | 0.2 | 0.4 | 0.2 | 0.3 | 0.6 | 0.1 | 0.1 | 0.2 | 0.1 |
| 0.1 | 4.0 | 4.7 | 0.4 | 0.7 | 0.2 | 1.7 | 1.4 | 0.1 | 0.0 | 0.4 | 0.2 |
| 0.1 | 0.9 | 0.9 | 0.2 | 0.4 | 0.1 | 0.7 | 0.8 | 0.0 | 0.0 | 0.1 | 0.1 |
| 0.0 | 0.3 | 0.3 | 0.1 | 0.1 | 0.0 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.1 | 1.1 | 1.5 | 0.6 | 0.9 | 0.2 | 0.5 | 0.6 | 0.0 | 0.0 | 0.2 | 0.2 |
| 1.2 | 31.3 | 33.1 | 5.8 | 14.3 | 3.2 | 23.8 | 15.6 | n/a | 2.2 | 7.4 | 3.2 |
| 0.1 | 0.6 | 1.3 | 0.8 | 1.7 | 0.5 | 0.4 | 0.4 | n/a | 0.1 | 0.2 | 0.1 |
| 0.1 | 0.3 | 0.7 | 0.3 | 0.6 | 0.4 | 0.2 | 0.6 | 0.0 | 0.1 | 0.1 | 0.1 |
| 0.3 | 7.3 | 8.9 | 4.0 | 6.5 | 1.9 | 6.9 | 5.0 | 1.2 | 1.6 | 2.4 | 1.9 |
| 1.4 | 1.0 | 1.4 | 2.0 | 2.8 | 2.8 | 0.8 | 1.4 | 0.3 | 0.7 | 0.8 | 0.3 |
| 0.0 | 1.1 | 1.4 | 1.2 | 1.3 | 1.2 | 0.7 | 2.7 | 0.7 | 0.5 | 1.9 | 0.5 |
| 0.2 | 0.4 | 0.5 | 0.5 | 0.6 | 0.0 | 0.2 | 0.6 | 0.1 | 0.3 | 0.0 | 0.2 |
| 0.0 | 0.4 | 0.5 | 0.1 | 0.3 | 0.1 | 0.2 | 0.3 | 0.1 | 0.1 | 0.1 | 0.3 |
| 0.3 | 1.4 | 2.0 | 0.4 | 0.6 | 0.4 | 2.0 | 1.4 | 0.2 | 0.8 | 0.2 | 2.0 |
| 0.0 | 0.9 | 1.5 | 0.6 | 0.4 | 1.6 | 0.9 | 0.3 | 0.4 | 0.2 | 0.7 | 0.2 |
| 0.1 | 0.2 | 0.4 | 0.3 | 0.5 | 0.2 | 0.4 | 0.4 | 0.2 | 0.1 | 0.4 | 0.2 |
| 0.1 | 2.3 | 4.9 | 0.4 | 0.8 | 0.1 | 0.8 | 0.6 | 0.6 | 0.3 | 0.7 | 0.4 |
| 0.0 | 0.2 | 0.5 | 0.2 | 0.5 | 0.2 | 0.3 | 0.3 | 0.2 | 0.5 | 0.3 | 0.3 |
| x | x | x | x | x | x | x | 2.9 | 0.9 | x | 1.3 | x |

FIGURE 141 (Continued)




| 524 | 525 | 526 | 527 | 528 | 529 | 530 | |
|---|---|---|---|---|---|---|---|
| 0.5 | 0.5 | 0.4 | 0.6 | 1.2 | 3.4 | 2.4 | 0.0 |
| 0.1 | 0.2 | 0.0 | 0.2 | 0.8 | 2.5 | 1.0 | 0.0 |
| 0.6 | 0.4 | 0.1 | 0.8 | 2.0 | 2.8 | 1.9 | 0.0 |
| 0.1 | 0.1 | 0.1 | 0.0 | 0.6 | 1.0 | 0.8 | 0.0 |
| 0.1 | 0.1 | 0.0 | 0.0 | 0.2 | 0.4 | 0.3 | 0.0 |
| 0.1 | 0.1 | 0.0 | 0.0 | 0.7 | 1.5 | 0.9 | 0.0 |
| 4.9 | 4.1 | 1.2 | 8.3 | 19.3 | 23.9 | 19.3 | 0.0 |
| 0.0 | 0.1 | 0.1 | 0.2 | 0.6 | 1.3 | 0.4 | 0.0 |
| 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.7 | 0.7 | 0.0 |
| 0.9 | 0.9 | 0.2 | 0.8 | 2.1 | 6.7 | 5.2 | 0.0 |
| 0.1 | 0.1 | 0.0 | 0.2 | 1.0 | 1.7 | 1.4 | 0.0 |
| 0.2 | 0.2 | 0.0 | 0.4 | 1.5 | 1.5 | 1.4 | 0.0 |
| 0.3 | 0.1 | 0.0 | 0.0 | 0.5 | 0.4 | 0.2 | 0.0 |
| 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.7 | 0.5 | |
| 0.3 | 0.1 | 0.3 | 0.5 | 1.1 | 4.0 | 2.8 | 0.0 |
| 0.1 | 0.1 | 0.2 | 0.2 | 0.9 | 2.0 | 1.4 | 0.0 |
| 0.3 | 0.3 | 0.4 | 0.2 | 0.2 | 0.4 | 0.3 | 0.0 |
| 0.2 | 0.1 | 0.1 | 0.2 | 0.6 | 1.1 | 1.0 | 0.0 |
| 0.1 | 0.0 | 0.0 | 0.1 | 0.2 | 0.5 | 0.3 | 0.0 |
| x | 0.6 | 0.2 | x | x | x | x | 0.1 |

FIGURE 141 (Continued)

| sgRNA | site | 107 | 108 | 109 | 142 | 144 | 177 | 335 | 370 |
|---|---|---|---|---|---|---|---|---|---|
| 299 | GAACA$_5$CAAAGCATAGACTGC | 0.2 | 0.2 | 1.0 | 0.3 | 2.9 | 14.8 | 0.4 | 52.5 |
| 299 | GAACAC$_6$AAAGCATAGACTGC | | | | | | | | |
| 502 | GGGGA$_5$CGCGCTGGCTTCCCG | 0.1 | 0.0 | 0.0 | 0.1 | 0.3 | 1.2 | 0.0 | 2.0 |
| 504 | GCCA$_4$CTTCTAAGCCCTTGAT | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 1.3 | 0.0 | 4.3 |
| 504 | GCCACTTC$_8$TAAGCCCTTGAT | | | | | | | | |
| 508 | GATGA$_5$GATAATGATGAGTCA | 0.0 | 0.0 | 0.0 | 0.1 | 0.2 | 2.7 | 0.0 | 2.5 |
| 508 | GATGAGA$_7$TAATGATGAGTCA | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 | 0.9 | 0.0 | 1.5 |
| 509 | GCCTA$_5$GGCAGTGGGGGTGCA | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.0 | 0.7 |
| 509 | GCC$_3$TAGGCAGTGGGGGTGCA | | | | | | | | |
| 469 | GAGTA$_5$TGAGGCATAGACTGC | 0.2 | 0.2 | 0.1 | 0.3 | 0.8 | 5.7 | 0.0 | 7.4 |

FIGURE 142

| 371 | 402 | 404 | 476 | 477 | 478 | 482 | 494 | 492 | 497 | 498 | 500 | BE3 | BE3B | Cas9 (indel %) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 57.7 | 54.0 | 55.8 | 62.9 | 64.0 | 61.0 | 0.7 | 39.3 | 34.1 | 45.6 | 56.5 | 29.5 | | | 28.92 |
| | | | | | | | | | | | | 38.9 | 45.8 | |
| 3.6 | 4.0 | 3.1 | 6.1 | 5.1 | 4.9 | 0.1 | 2.7 | | | | | | | |
| 21.3 | 19.2 | 15.8 | 7.2 | 5.8 | 6.0 | 0.0 | 3.6 | 4.6 | 3.3 | 4.4 | 1.3 | | | 31.98 |
| | | | | | | | | | | | | 32.5 | 34.8 | |
| 3.6 | 4.2 | 3.6 | 12.9 | 13.9 | 12.1 | 0.0 | 5.4 | 8.9 | 10.1 | 12.5 | 2.6 | | | 39.19 |
| 4.1 | 4.3 | 5.0 | 5.0 | 4.9 | 4.2 | 0.0 | 5.2 | 7.8 | 3.4 | 4.5 | 1.5 | | | |
| 1.4 | 1.5 | 1.0 | 3.8 | 5.2 | 3.2 | 0.0 | 0.3 | 1.0 | 4.0 | 3.9 | 0.9 | | | 17.16 |
| | | | | | | | | | | | | 9.1 | 0.3 | |
| 26.7 | 28.8 | 20.3 | 37.7 | 42.3 | 0.3 | 0.3 | 5.9 | 13.0 | 30.4 | 32.7 | 11.2 | | | |

FIGURE 142 (Continued)

| construct | HEK2 | Site 2 | site 4 | site 8 | site 9 |
|---|---|---|---|---|---|
| **107** | 0.00 | 0.01 | 0.01 | 0.06 | 0.06 |
| **108** | 0.53 | 0.18 | 0.02 | 0.02 | 0.10 |
| **109** | 0.01 | 0.03 | 0.04 | 0.05 | 0.00 |
| **142** | 0.05 | 0.01 | 0.03 | 0.13 | 0.10 |
| **144** | 0.00 | 0.07 | 0.01 | 0.02 | 0.02 |
| **177** | 0.12 | 0.12 | 0.08 | 0.03 | 0.04 |
| **335** | 0.04 | 0.03 | 0.04 | 0.00 | 0.04 |
| **370** | 0.32 | 0.09 | 0.09 | 0.01 | 0.03 |
| **371** | 0.36 | 0.17 | 0.23 | 0.09 | 0.03 |
| **402** | 0.26 | 0.16 | 0.33 | 0.00 | 0.01 |
| **404** | 0.13 | 0.17 | 0.12 | 0.11 | 0.02 |
| **476** | 0.01 | 0.14 | 0.05 | 0.00 | 0.10 |
| **477** | 0.05 | 0.10 | 0.07 | 0.09 | 0.06 |
| **478** | 0.03 | 0.20 | 0.07 | 0.03 | 0.02 |
| **482** | 0.03 | 0.16 | 0.02 | 0.09 | 0.16 |
| **494** | 0.00 | 0.11 | 0.11 | 0.08 | 0.01 |
| **492** | 0.43 | | 0.07 | 0.02 | 0.03 |
| **497** | 0.05 | | 0.05 | 0.07 | 0.12 |
| **498** | 0.13 | | 0.08 | 0.05 | 0.14 |
| **500** | 0.01 | | 0.03 | 0.05 | 0.01 |
| **BE3** | 1.05 | | 1.69 | 0.13 | 0.20 |
| **BE3B** | 6.34 | | 6.90 | 0.13 | 0.18 |
| **Cas9** | 28.92 | | 31.98 | 39.19 | 17.16 |

Figure 143

| sgRNA | site | 482 | 476 | 476+274 | 476+275 | 477 | 477+274 | 477+275 | 285b | 285b+274 | 285b+275 | 277 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 299 | GAACA$_5$CAAAGCATAGACTGC | 1.9 | 67.5 | 59.6 | 51.2 | 72.9 | 63.9 | 60.1 | 30.8 | 31.2 | 27.0 | 45.2 |
| 301 | GGAA$_4$CACAAAGCATAGACTG | 0.3 | 29.5 | 23.3 | 18.5 | 37.1 | 24.6 | 32.0 | 16.9 | 14.5 | 12.5 | 19.9 |
| 301 | GGAACA$_6$CAAAGCATAGACTG | 2.8 | 53.0 | 38.3 | 33.7 | 61.0 | 44.3 | 47.6 | 19.7 | 17.1 | 15.4 | 24.4 |
| 502 | GGGGA$_5$CGCGCTGGCTTCCCG | 0.0 | 4.1 | 4.7 | 3.6 | 3.5 | 4.1 | 3.1 | 2.7 | 2.0 | 1.3 | 1.0 |
| 505 | GGGA$_4$AAGACCCAGCATCCGT | 0.0 | 0.1 | 0.2 | 0.2 | 0.2 | 0.1 | 0.8 | 0.6 | 0.0 | 0.0 | 0.0 |
| 505 | GGGAA$_5$AGACCCAGCATCCGT | 0.0 | 0.6 | 0.6 | 0.1 | 1.1 | 0.2 | 0.2 | 1.2 | 0.1 | 0.1 | 0.5 |
| 505 | GGGAAA$_6$GACCCAGCATCCGT | 0.0 | 1.4 | 0.1 | 0.3 | 0.3 | 1.2 | 0.8 | 0.6 | 0.6 | 0.4 | 0.0 |
| 505 | GGGAAAGA$_8$CCCAGCATCCGT | 0.7 | 3.1 | 1.3 | 1.2 | 3.2 | 1.4 | 1.9 | 0.5 | 0.1 | 0.2 | 0.6 |
| 507 | GAAA$_4$CTGGTCCCGTTTACAG | 0.0 | 0.5 | 0.2 | 0.1 | 0.5 | 0.5 | 0.3 |  | 0.5 | 0.2 | 0.3 |
| 509 | GCCTA$_5$GGCAGTGGGGGTGCA | 0.0 | 7.7 | 2.0 | 2.0 | 5.8 | 1.7 | 2.0 | 1.3 | 0.1 | 0.1 | 0.7 |

Figure 144

| Site | Protospacer and PAM sequence | 371 | 402 | 404 | 410 | 476 | 477 | 478 | 479 | 475 |
|---|---|---|---|---|---|---|---|---|---|---|
| CAC (Hek2) | GAACACAAAGCATAGACTGCTGG | 45.4 | 48.1 | 41.0 | 46.2 | 51.8 | 39.6 | 45.9 | 35.2 | |
| AAA | GAAAAAAAAGCAGAGACTGCTGG | 0.1 | 0.1 | 0.0 | 0.1 | 0.2 | 0.3 | 0.2 | 0.0 | |
| TAC | GAATACTAAGCATAGACTCCAGG | 37.7 | 39.9 | 38.5 | 43.4 | 45.1 | 41.4 | 38.3 | 25.7 | |
| AAC | GTAAACAAAGCATAGACTGAGGG | 17.8 | 21.8 | 16.9 | 19.5 | 14.1 | 14.8 | 14.2 | 9.7 | |
| GAC | GAAGACCAAGGATAGACTGCTGG | 7.7 | 6.5 | 4.7 | 11.4 | 7.6 | 9.3 | 7.4 | 2.3 | |
| CAT | GAACATAAAGAATAGAATGATGG | 16.4 | 20.8 | 16.0 | 21.7 | 16.7 | 22.3 | 21.3 | 12.9 | |
| CAG | GGACAGGCAGCATAGACTGTGGG | 9.6 | 16.9 | 9.4 | 13.7 | 24.9 | 22.7 | 29.0 | 26.7 | |
| GAA | GTAGAAAAAGTATAGACTGCAGG | 2.9 | 2.8 | 2.5 | 4.8 | 8.7 | 6.4 | 6.0 | 3.7 | 11.1 |
| GAG | GGAGAGAGAGCATAGACTGCTGG | 7.6 | 10.6 | 5.6 | 10.4 | 16.5 | 26.0 | 14.1 | 9.2 | |
| GAT | GAAGATAGAGAAATAGACTGCTGG | 2.6 | 4.1 | 2.2 | 6.1 | 7.1 | 7.3 | 5.6 | 3.2 | 0.5 |
| TAA | GGCTAAAGACCATAGACTGTGGG | 2.3 | 3.7 | 1.8 | 2.8 | 4.2 | 5.6 | 4.1 | 2.2 | 9.8 |
| TAG | GTCTAGAAAGCTTAGACTGCTGG | 10.1 | 14.9 | 8.1 | 9.1 | 24.3 | 28.3 | 20.3 | 13.6 | 31.4 |
| TAT | GAGTATGAGGCATAGACTGCAGG | 21.0 | 38.1 | 18.3 | 32.3 | 37.0 | 43.3 | 40.1 | 28.4 | 11.7 |
| AAG | GTCAAGAAAGCAGAGACTGCCGG | 6.1 | 6.5 | 5.6 | 10.7 | 11.9 | 12.6 | 9.8 | 7.8 | 18.7 |
| AAT | GGGAATAAATCATAGAATCCTGG | 5.9 | 11.2 | 6.4 | 16.7 | 20.1 | 15.3 | 16.0 | 11.1 | |
| CAA | GAGCAAAGAGAAATAGACTGTAGG | 2.5 | 5.4 | 2.8 | 3.2 | 7.4 | 13.3 | 6.9 | 6.2 | 0.3 |

Figure 145

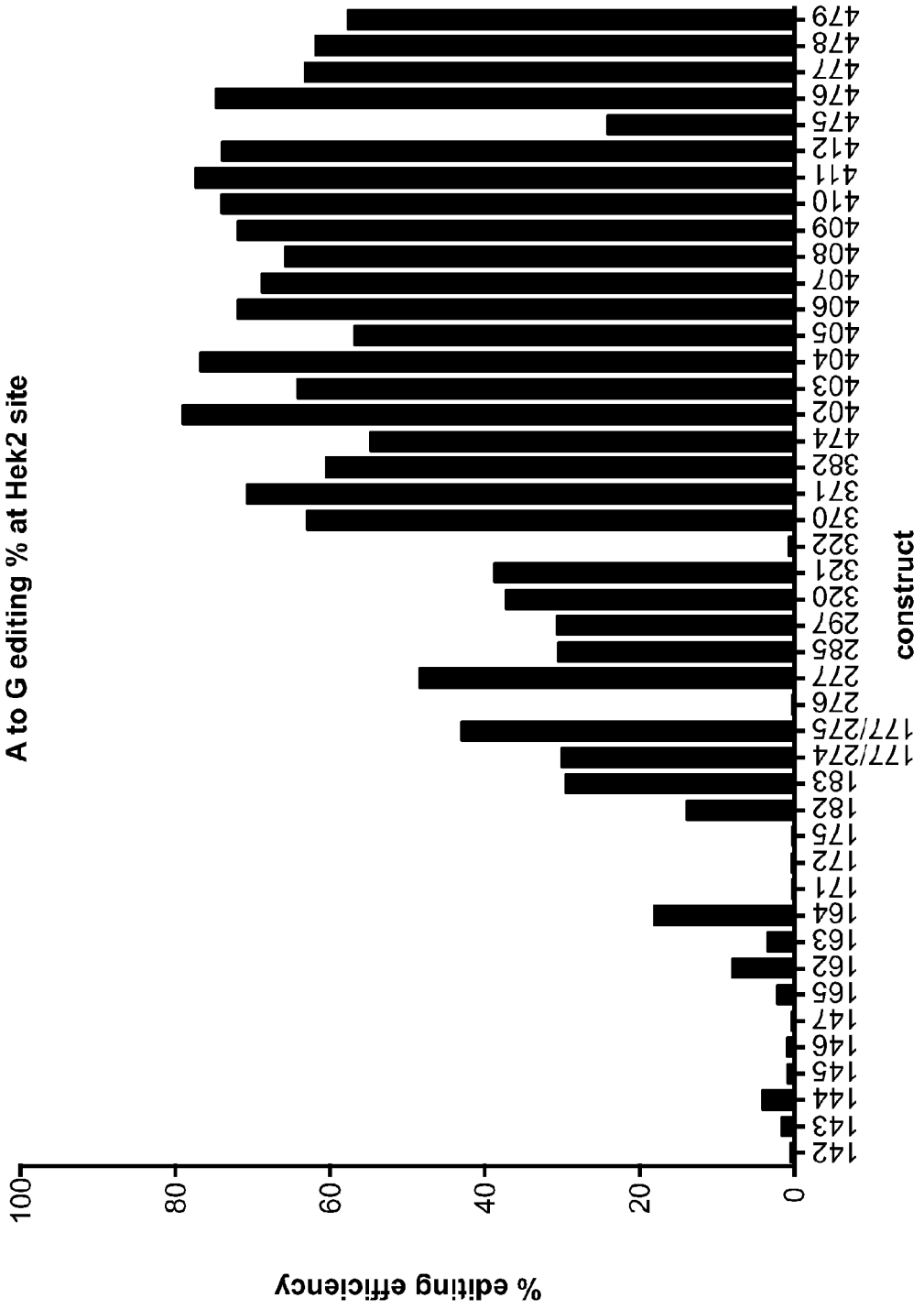

A to G editing % at Hek2 site
% editing efficiency
100
80
60
40
20
0
construct
142
143
144
145
146
147
165
162
163
164
171
172
175
182
183
177/274
177/275
276
277
285
297
320
321
322
370
371
382
474
402
403
404
405
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407
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409
410
411
412
475
476
477
478
479

Figure 146

FIGURE 147

| Site | Protospacer and PAM sequence | Cas9 (indel %) | L84F A106V D108N H123Y D147Y E155V I156F = 3rd round | 3rd round + N37S K161T | 3rd round + D24G Q71R H96L K160E | 3rd round + H36L G67V S146T | 3rd round + Q71L L137M A143E | 3rd round + E25G Q159L | 3rd round + A91T F104I | 3rd round + N72D G125A | 3rd round + P48S S97C | 3rd round + A142N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HEK2 | GAACACAAAGCATAGACTGCTGG | 76.9 | 78.3 | 77.4 | 43.6 | 2.9 | 62.6 | 16.3 | 10.7 | 71.4 | 66.5 | 61.2 |
| HEK2-1 | GAAAAAAAGCAGAGACTGCTGG | 17.9 | 0.6 | 0.4 | 1.2 | 0.0 | 0.2 | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 |
| HEK2-2 | GAATACTAAGCATAGACTCCAGG | 33.0 | 57.0 | 56.3 | 16.2 | 1.1 | 34.7 | 6.9 | 4.0 | 49.2 | 39.7 | 31.3 |
| HEK2-3 | GTAAACAAAGCATAGACTGAGGG | 43.0 | 29.5 | 31.1 | 14.8 | 0.4 | 8.7 | 1.5 | 4.2 | 17.3 | 14.1 | 13.3 |
| HEK2-6 | GAAGACCAAGGATAGACTGCTGG | 23.5 | 8.4 | | 2.6 | 0.2 | 2.1 | 1.0 | 0.7 | 3.0 | 2.8 | 5.1 |
| HEK2-10 | GAACATAAAGAATAGAATGATGG | 20.9 | 21.0 | 16.9 | 7.9 | 0.4 | 6.7 | 2.0 | 1.5 | 8.4 | 16.7 | 8.2 |

| sgRNA | site | evo 3 | synthetic 5a | evo 5a | evo 4 | best 5b | synthetic 4 | synthetic 3 | synthetic 5a | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 476 | 477 | 478 | 479 | 492 | 493 | 494 | 495 | 496 | 497 | 498 | 499 | 500 |
| 299 | GAACA$_5$CAAAGCATAGACTGC | 52.2 | 50.0 | 52.0 | 54.1 | 47.5 | 50.5 | 51.3 | 40.1 | 58.8 | 61.1 | 50.1 | 27.6 | 28.4 |
| 463 | GGACA$_5$GGCAGCATAGACTGT | 32.5 | 27.2 | 21.4 | 18.2 | 9.3 | 19.6 | 11.2 | 6.3 | 21.9 | 25.8 | 18.5 | 3.9 | 5.1 |
| 466 | GAAGA$_5$TAGAGAATAGACTGC | 5.0 | 3.8 | 2.0 | 1.0 | 1.2 | 2.6 | 1.5 | 0.8 | 2.6 | 2.2 | 1.5 | 0.4 | 0.4 |
| 509 | GCCTA$_5$GGCAGTGGGGGTGCA | 5.4 | 2.1 | 1.9 | 0.5 | 1.0 | 0.9 | 0.5 | 0.1 | 1.7 | 1.5 | 2.1 | 0.3 | 0.3 |
| 501 | GACAA$_5$AGAGGAAGAGAGACG | 1.7 | 1.5 | 1.0 | 1.1 | 0.7 | 0.9 | 0.4 | 0.1 | 1.9 | 1.2 | 0.6 | 0.1 | 0.2 |
| 465 | GGAGA$_5$GAGAGCATAGACTGC | 18.2 | 18.6 | 10.1 | 6.0 | 5.7 | 6.7 | 13.5 | 5.2 | 12.4 | 16.0 | 11.2 | 2.6 | 6.9 |
| 467 | GGCTA$_5$AAGACCATAGACTGT | 3.7 | 3.4 | 1.6 | 1.4 | 0.3 | 1.1 | 0.6 | 0.3 | 1.9 | 2.2 | 1.7 | 0.3 | 0.4 |
| 469 | GAGTA$_5$TGAGGCATAGACTGC | 45.3 | 40.3 | 36.9 | 22.9 | 6.6 | 29.1 | 14.5 | 7.5 | 34.2 | 37.0 | 27.3 | 10.8 | 12.2 |




FIGURE 148

| sgRNA | site | ABE2 | ABE3 | ABE4 | ABE5-1 | ABE5-2 | ABE5-3 |
|---|---|---|---|---|---|---|---|
| 299 | GAACA$_5$CAAAGCATAGACTGC | 13.6 | 58.5 | **54.4** | **77.6** | **69.5** | **57.3** |
| 502 | GGGGA$_5$CGCGCTGGCTTCCCG | 0.9 | 5.6 | 3.0 | 5.8 | 3.0 | 3.3 |
| 504 | GCCA$_4$CTTCTAAGCCCTTGAT | 1.0 | 7.4 | 4.2 | 7.6 | 5.1 | 5.4 |
| 505 | GGGA$_4$AAGACCCAGCATCCGT | 0.1 | 0.2 | 0.7 | 0.3 | 0.1 | 0.3 |
| 505 | GGGAA$_5$AGACCCAGCATCCGT | 0.1 | 0.4 | 0.5 | 0.5 | 0.2 | 1.0 |
| 505 | GGGAAA$_6$GACCCAGCATCCGT | 0.3 | 0.6 | 0.4 | 0.2 | 0.2 | 0.5 |
| 505 | GGGAAAGA$_8$CCCAGCATCCGT | 0.6 | 1.5 | 1.5 | 3.0 | 1.3 | 3.6 |
| 507 | GAAA$_4$CTGGTCCCGTTTACAG | 0.1 | 0.6 | 0.3 | 0.9 | 0.4 | 0.6 |
| 508 | GATGA$_5$GATAATGATGAGTCA | 1.7 | 11.5 | 0.4 | 15.6 | 8.8 | 6.1 |
| 508 | GATGAGA$_7$TAATGATGAGTCA | 1.4 | 5.1 | 0.1 | 6.0 | 3.5 | 4.7 |
| 509 | GCCTA$_5$GGCAGTGGGGGTGCA | 0.2 | 3.1 | 0.6 | 5.9 | 2.3 | 1.3 |

*

FIGURE 149

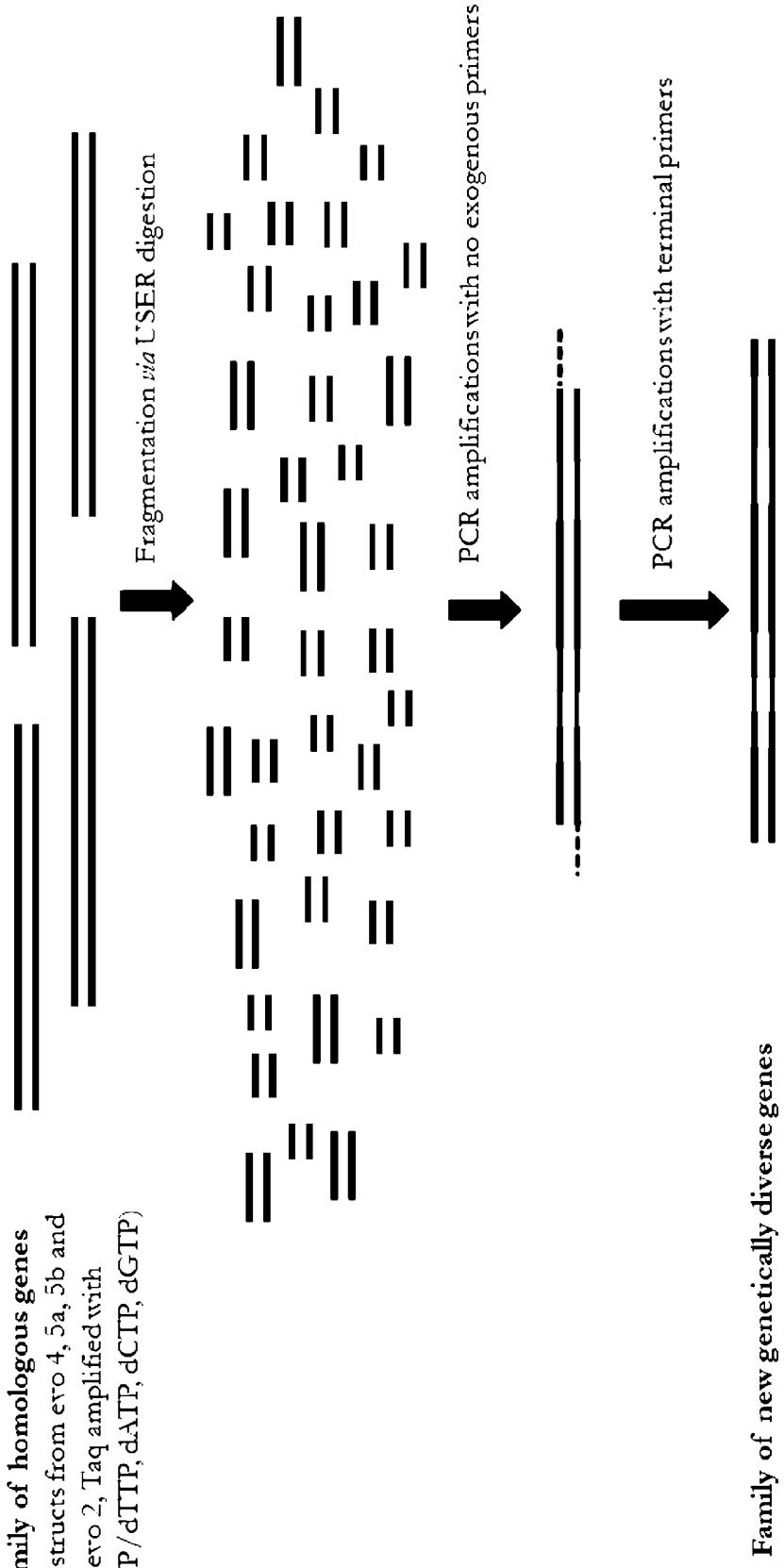

Family of homologous genes
(constructs from evo 4, 5a, 5b and evo 2, Taq amplified with dUTP/dTTP, dATP, dCTP, dGTP)
Fragmentation *via* USER digestion
PCR amplifications with no exogenous primers
PCR amplifications with terminal primers
Family of new genetically diverse genes

FIGURE 150

# FIGURE 151 DNA Shuffle (NeXT)

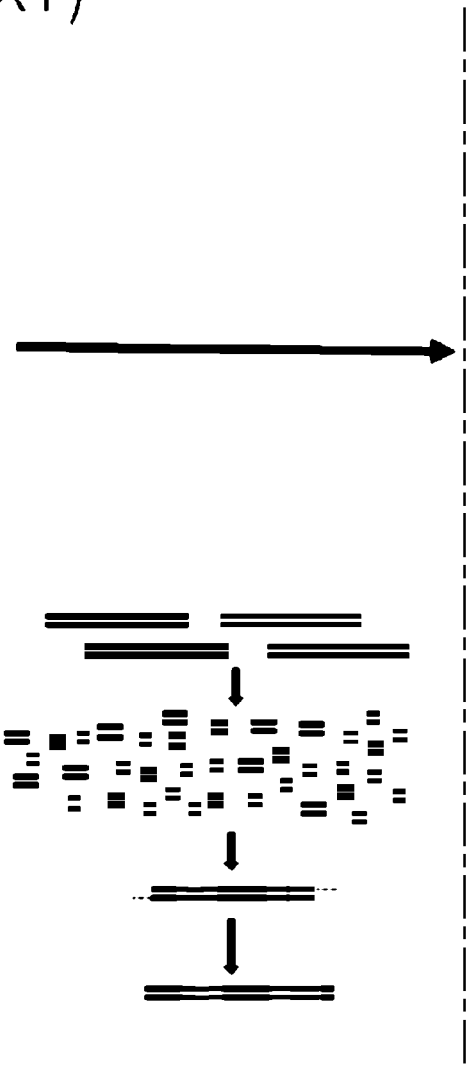
1. generated shuffled library including constructs from evo #4, 5a, 5b and evo #2

2. transformed library into S1030 + pNMG-333 selection plasmid

3. induce ABE expression for 7h and plated on selection conditions:

   a. 128 ug/mL chlor
   b. 192 ug/mL chlor
   c. 384 ug/mL spect
   d. 256 ug/mL spect, 64 ug/mL chlor

4. selection plate incubated at 37$^0$C, 48h → surviving colonies sequenced



Spect target: 5'-CAATGATGACTTCTACAGCG-3'

Chlor target: 5'-TACGGCGTAGTGCACCTGGA-3'

FIGURE 151 (Continued)

**outcome:**

- >95% of clones surviving on chlor and chlor + spect plates (200 colonies sequenced total) contained Evo #3 mutations only
- clones sequenced from spect only selection condition, however, had high frequency of mutation at A142 and A143, also high frequency of mutations in *C*-terminal portion of ecTadA (K157, Q159, K160 and K161) – see chart on next slide
- clones sequenced from spect only selection condition had a low relative frequency of evo #3 mutations only (<10% of total constructs sequenced) – very different outcome than colonies sequenced from chlor only plates.

FIGURE 152

| | position: | 25 | 36 | 37 | 48 | 49 | 51 | 52 | 62 | 70 | 71 | 76 | 77 | 84 | 96 | 99 | 100 | 106 | 107 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | Glu | His | Asn | Pro | Ile | Arg | His | Ala | Met | Gln | Ile | Asp | Leu | His | Gly | Val | Ala | Arg |
| 1 | | | | | | | | | | | | | | Phe | | | | Val | |
| 2 | | | | | | | | | | | | | | Phe | | | | Val | |
| 3 | | | | | | | His | | | | | | | Phe | | | | Val | |
| 4 | | | | Thr | Thr | Val | | | | Leu | | | | Phe | | | | Val | |
| 5 | | | | | | | | | | | | | | Phe | | Ser | | Val | |
| 6 | | | | | | | | | | | | | | Phe | | | | Val | |
| 7 | | | | | | | | | | | | Val | | Phe | | | | Val | |
| 8 | | | | | | | | | | | | | | Phe | | | | Val | |
| 9 | | | | | Thr | Val | | | | | | | | Phe | | | | Val | |
| 10 | | | | | | | | | | | | | | Phe | | | | Val | |
| 11 | | | Leu | | Ala | | | | | | | | | Phe | | | | Val | |
| 12 | | | | | | | | | | | Arg | | | Phe | Pro | | Ala | Val | |
| 13 | | | | | | | | | | | | | | Phe | | | | Val | |
| 14 | | | | | Thr | Val | | | | Leu | | | | Phe | | | | Val | His |
| 15 | | | Leu | | Thr | Val | | | | | | | | Phe | | | | Val | |
| 16 | | | Leu | | | | | | | | | | | Phe | Leu | | | Val | |
| 17 | | | | | | | | | | Leu | | | | | | | | Val | |
| 18 | | | | | | | | | | | | | | Phe | | | | Val | |
| 19 | | Leu | | | | | | | | | | | | Phe | | | | Val | |
| 20 | | | | | | | | | Val | | | | | | Leu | | | Val | |
| 21 | | | Leu | | | | His | | | | | | | Phe | Leu | | | Val | |

FIGURE 152 (Continued)

| 108 | 122 | 123 | 134 | 137 | 142 | 143 | 146 | 147 | 151 | 153 | 154 | 155 | 156 | 157 | 159 | 160 | 161 | 162 | 167 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Asp | His | His | Glu | Leu | Ala | Ala | Ser | Asp | Met | Arg | Gln | Glu | Ile | Lys | Gln | Lys | Lys | Ala | Asp |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | | Thr | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | Asn | | | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | Met | | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | Glu | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | Asn | | | Thr | | |
| Asn | | | | | Asn | | | Tyr | | | | Val | | | | | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | Leu | | | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | Glu | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | Gly | | | | | Tyr | | | | Val | Phe | | Leu | | | | |
| Asn | | | | | Asn | | | Tyr | | | | Val | Phe | | | | Thr | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | Asn | | | | | |
| Asn | Arg | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | | | Tyr | | | His | Val | Phe | | | | | | |
| Asn | | | | | Asn | | | Tyr | | | | Val | Phe | | Leu | | | | |
| Asn | | Tyr | | Met | | Glu | | Tyr | | | | Val | Phe | | | | Thr | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | Asn | | | | | |
| Asn | | Tyr | | | | | Arg | Tyr | | | | Val | Phe | | | | | | |

FIGURE 152 (Continued)

| | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 22 | | | | | | | | | | | | | | | | | | | | | | Phe | | Val |
| 23 | | | | Ser | | | | | Leu | | | | | | | | | | | | | Phe | | Val |
| 24 | | | | | | | | | | | | | | | | | | | | | | | | Val |
| 25 | | | | | | | | | | | | | | | | | | | | | | | | Val |
| 26 | | | | | | | | | | Leu | | | | | | | | | | | | | Arg | Val |
| 27 | | | | Ser | Thr | Val | | | Leu | | | | | | | | | | | | | Phe | | Val |
| 28 | | | Leu | | | | | | | | | | | | | | | | | | | Phe | | Val |
| 29 | | | | | | | | | | | | | | | | | | | | | | Phe | Leu | Val |
| 30 | | | Leu | | | | | | | | | | | | | | | | | | | Phe | | Val |
| 31 | | | | | | | | | | | | | | | | | | | | | | Phe | | Val |
| 32 | | | | | Thr | Val | | | | | | | | | | | | | | | | Phe | | Val |
| 33 | | | Leu | | | | Leu | | | | | | | | | | | | | | | Phe | | Val |
| 34 | | | | | | | | | | | | | | | | | | | | | | Phe | | Val |
| 35 | | | | | | | | | | | | | | | | | | | | | | Phe | | Val |
| 36 | | | | | | | | | | | | | | | | | | | | | Gly | Phe | | Val |
| 37 | | | | | | | | Arg | | | | | | | | | | | | | | Phe | Leu | Val |
| 38 | | | | | | | Leu | | | | | | | | | | | | | | | Phe | Leu | Val |
| 39 | | | | | | | | | | | | | | | | | | | | | | Phe | | Val |
| 40 | | | | | | | His | | | | | | | | | | | | | | | Phe | | Val |
| 41 | | | | | Thr | Val | | | | Arg | | | | | | | | | | | | Phe | | Val |
| 42 | | | | | | | Leu | | | | | | | | | | | | | | | Phe | | Val |
| 43 | | | | | | | | | Leu | | | | | | | | | | | | | Phe | | Val |
| 44 | | | | | | | | | | | | | | | | | | | | | | Phe | | Val |

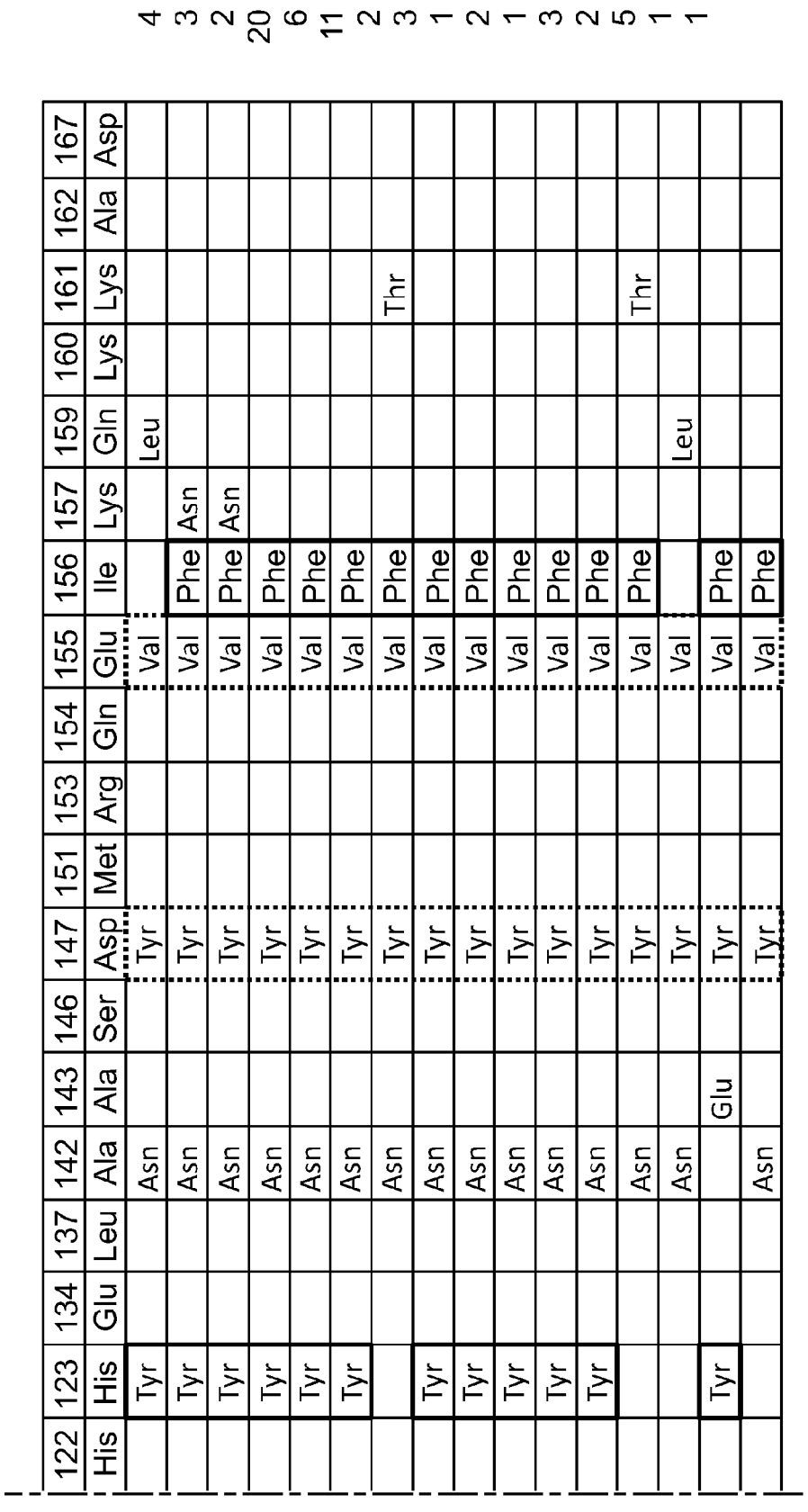
# FIGURE 152 (Continued)

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Asn | | Tyr | Gly | | | | | Tyr | | | | Val | Phe | | | Glu | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | | | | Asn | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | | | | Asn | | | Tyr | | | | Val | Phe | | | | Thr | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | Glu | | | |
| Asn | | Tyr | | | | | | Tyr | | Cys | | Val | Phe | | Leu | | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | Glu | | | |
| Asn | | | | | | | | Tyr | Val | | | Val | Phe | | | Glu | | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | Leu | | | | Gly |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | | Thr | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | | | Leu | | | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | Glu | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | Glu | | Tyr | | | | Val | Phe | Asn | | | | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | Met | | | | Tyr | | | | Val | Phe | | | Glu | | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | | | | | |
| Asn | | Tyr | | | | Glu | | Tyr | | | | Val | Phe | | Leu | | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | | | | Glu | | | |
| Asn | | Tyr | | | Asn | | | Tyr | | | | Val | Phe | | Leu | | | | |
| Asn | | Tyr | | | | | | Tyr | | | | Val | Phe | | | | Thr | | |
| Asn | | Tyr | | Met | | Glu | | Tyr | | | | Val | Phe | Asn | | | | | |
| Asn | | | | | Asn | | Cys | Tyr | | | | Val | Phe | | | | | | |

FIGURE 153

| | position: | 25 | 36 | 37 | 48 | 49 | 51 | 52 | 62 | 70 | 71 | 76 | 77 | 84 | 96 | 99 | 100 | 106 | 107 | 108 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | wt res: | Glu | His | Asn | Pro | Ile | Arg | His | Ala | Met | Gln | Ile | Asp | Leu | His | Gly | Val | Ala | Arg | Asp |
| | | | | | Thr | Val | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | Thr | Val | | | | | | | | Phe | | | | Val | | Asn |
| | | | Leu | | Thr | Val | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | Ser | | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | Ser | | | | | Leu | | | | Phe | | | | Val | | Asn |
| | | | Leu | | Ala | | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | | | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | | | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | | | | | | | | | Gly | Phe | | | | Val | | Asn |
| | | | Leu | | Ser | | | | | Leu | | | | Phe | | | | Val | | Asn |
| | | | | | Ala | | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | | | Leu | | | | | | | Phe | | | | Val | | Asn |
| | | | | | | | Leu | | | | | | | Phe | Leu | | | Val | | Asn |
| | | | | | | | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | Thr | Val | | | | | | | | Phe | | | | Val | | Asn |
| | | | | | Ser | | | | | Leu | | | | Phe | | | | Val | | Asn |
| | | | | | | | | | | | Arg | | | Phe | Leu | | | Val | | Asn |

FIGURE 153 (Continued)



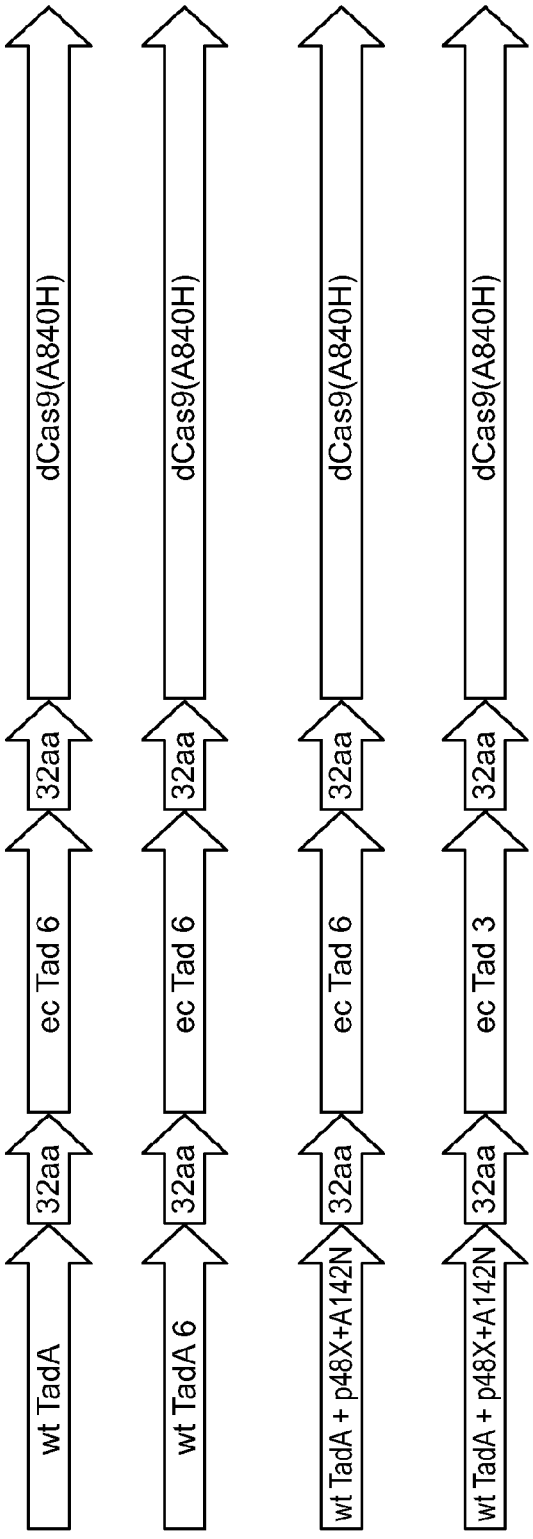

wt TadA
32aa
ec Tad 6
32aa
dCas9(A840H)
wt TadA 6
32aa
ec Tad 6
32aa
dCas9(A840H)
wt TadA + p48X+A142N
32aa
ec Tad 6
32aa
dCas9(A840H)
wt TadA + p48X+A142N
32aa
ec Tad 3
32aa
dCas9(A840H)

FIGURE 154

FIGURE 155

| | pNMG | | new ABE name |
|---|---|---|---|
| 1 | 107 | hADAR2 | |
| 2 | 108 | mADA | |
| 3 | 109 | hADAT2 | |
| 4 | 142 | ecTadA wt | |
| 5 | 143 | round #1 | ABE 1a |
| 6 | 144 | round #1 | ABE 1b |
| 7 | 177 | round #2 | ABE 2a |
| 8 | 235 | round #2 + AAG | ABE 2b |
| 9 | 240 | round #2 +dEndoV | ABE 2c |
| 10 | 175b | C terminal version of 177 | ABE 2d |
| 11 | 182b | GGS of 177 | ABE 2e |
| 12 | 183b | 32aa of 177 | ABE 2f |
| 13 | 177 +274 | dimerization + wt | ABE 2g |
| 14 | 177 +275 | dimerization + mutation | ABE 2h |
| 15 | 276 | wt + wt dimer of TadA | ABE 2i |
| 16 | 277 | ecTadA (evo 2) + ecTadA (evo2) | ABE 2j |
| 17 | 320 | hetero dimer- wt + ecTadA (evo 2) | ABE 2k |
| 18 | 321 | ecTadA E59A + ecTadA (evo2) | ABE 2l |
| 19 | 322 | ecTadA (evo 2)---32---ecTadA (mut)--XTEN---Cas9 | ABE 2m |
| 20 | 371 | ecTadA (evo 3)---32---ecTadA (evo 3)--32---Cas9 | ABE 3a |
| 21 | 476 | evo 3 hetero dimer | ABE 3b |
| 22 | 513 | wt ---100 ---ecTadA evo (3)--32 Cas9 | ABE 3c |
| 23 | 514 | ecTadA evo (3)---100 ---ecTadA evo (3)--32---Cas9 | ABE 3d |
| 24 | 515 | wt ---100 ---ecTadA evo (3)--64 Cas9 | ABE 3e |

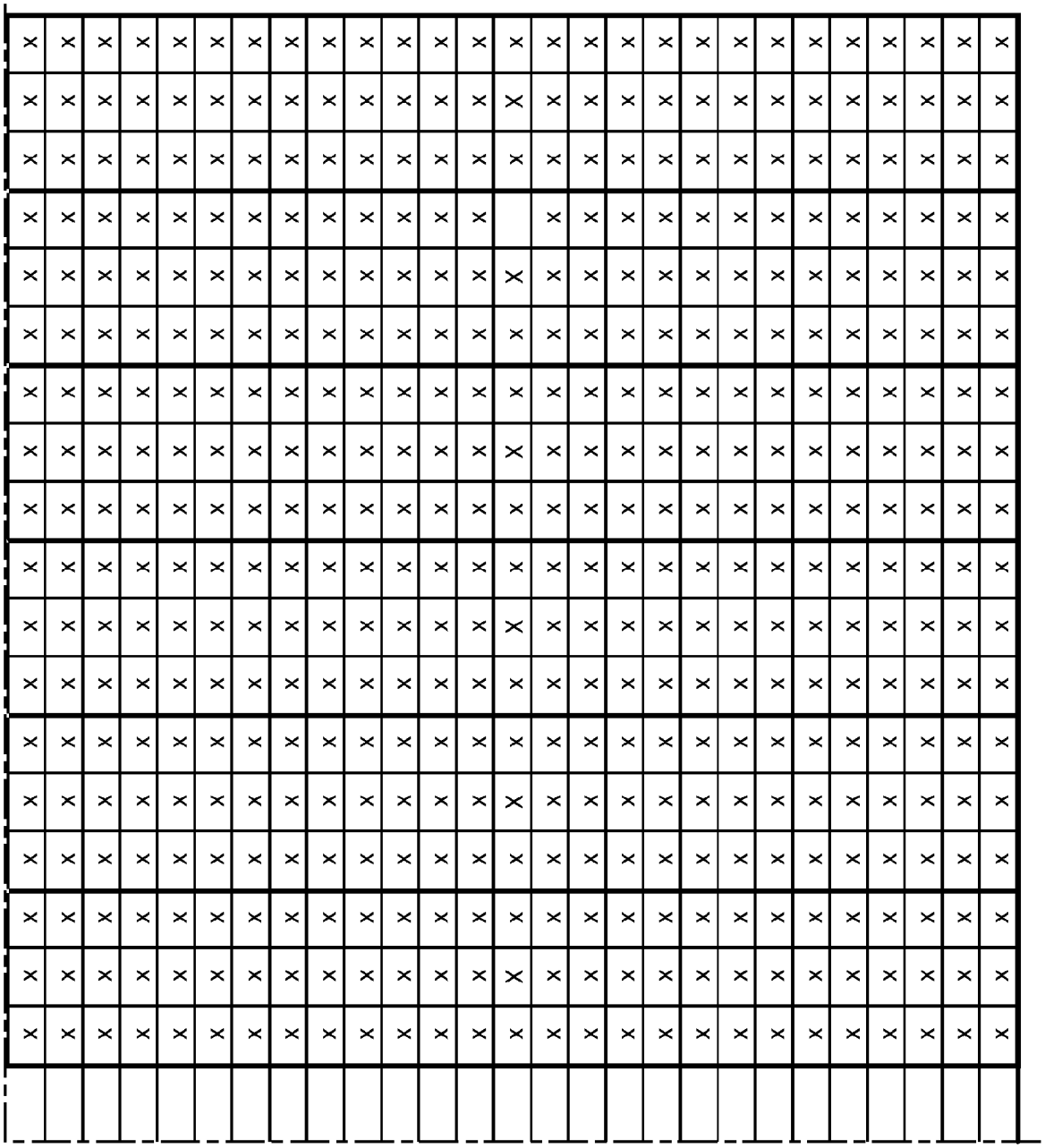
FIGURE 155
(Continued)

| | sgRNA # | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 469 | | | 470 | | | 472 | | | 508 | | | 536 | | | 299 | |
| | n=1 | n=2 | n=3 | n=1 | n=2 | n=3 | n=1 | n=2 | n=3 | n=1 | n=2 | n=3 | n=1 | n=2 | n=3 | n=1 | n=2 | n=3 |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |

FIGURE 155 (Continued)

| | | | |
|---|---|---|---|
| 25 | 516 | ecTadA evo (3)--100 ---ecTadA evo (3)--64---- Cas9 | ABE 3f |
| 26 | 517 | wt ---32 ---ecTadA evo (3)--64 Cas9 | ABE 3g |
| 27 | 518 | ecTadA evo (3)--32 ---ecTadA evo (3)--64---- Cas9 | ABE 3h |
| 28 | 382 | evo 4 construct (A142N only) - homodimer | ABE 4a |
| 29 | 383 | evo 4 construct (R26G +A142N) - homodimer | ABE 4b |
| 30 | 474 | evo (3 + A 142N) - 32aa - evo 3 + A142N - 32 aa-Cas9 | ABE 4c |
| 31 | 402 | evo 5 consensus homodimer | ABE 5a |
| 32 | 404 | evo 5 highest frequency homodimer | ABE 5b |
| 33 | 477 | evo 5 (402) heterodimer | ABE 5c |
| 34 | 478 | evo 5 (404) heterodimer | ABE 5d |
| 35 | 555 | wt---24 ---ecTadA (evo 5)----24----Cas9 | ABE 5e |
| 36 | 556 | wt---24 ---ecTadA (evo 5)----32----Cas9 | ABE 5f |
| 37 | 557 | wt---24 ---ecTadA (evo 5)----40----Cas9 | ABE 5g |
| 38 | 558 | wt---32 ---ecTadA (evo 5)----24----Cas9 | ABE 5h |
| 39 | 559 | wt---32 ---ecTadA (evo 5)----40----Cas9 | ABE 5i |
| 40 | 560 | wt---40 ---ecTadA (evo 5)----24----Cas9 | ABE 5j |
| 41 | 561 | wt---40 ---ecTadA (evo 5)----32----Cas9 | ABE 5k |
| 42 | 562 | wt---40 ---ecTadA (evo 5)----40----Cas9 | ABE 5l |
| 43 | 454 | evo 6 homodimer (5b) | ABE 6a |
| 44 | 455 | evo 6 homodimer (5b) - contains P48S | ABE 6b |
| 45 | 547 | wt - 32 - ecTadA 7 (shuffle) - 32 Cas9 P48S + A142 (evo 3) | ABE 7a |
| 46 | 548 | ecTadA 7 (shuffle) - 32 ecTadA 7 (shuffle)-Cas9 P48S + A142 (evo 3) | ABE 7b |
| 47 | 551 | wt - 32 - ecTadA 7 (shuffle) - 32 Cas9 | ABE 7c |
| 48 | 576 | wt --32--ecTadA (evo 5 + P48S) --32--Cas9 | ABE 7d |
| 49 | 577 | wt --32--ecTadA (evo 5 + P48S + A142 N) --32--Cas9 | ABE 7e |
| 50 | 578 | wt --32--ecTadA (evo 5 + P48T) --32--Cas9 | ABE 7f |
| 51 | 579 | wt --32--ecTadA (evo 5 + P48T + A142N) --32--Cas9 | ABE 7g |

FIGURE 155
(Continued)



| genetic locus | sequence | position of target A | target sequence |
|---|---|---|---|
| pNMG-469 | TAT | 5 | GAGTATGAGGCATAGACTGC |
| pNMG-470 | AAG | 5 | GTCAAGAAAGCAGAGACTGC |
| pNMG-472 | CAA | 5 | GGGAATAAATCATAGAATCC |
| pNMG-508 | GAG | 5 | GATGAGATAATGATGAGTCA |
| pNMG-536 | GAC | 7 | GGATTGACCCAGGCCAGGGC |
| pNMG-299 | CAC | 5 | GAACACAAAGCATAGACTGC |

FIGURE 155 (Continued)

FIGURE 156

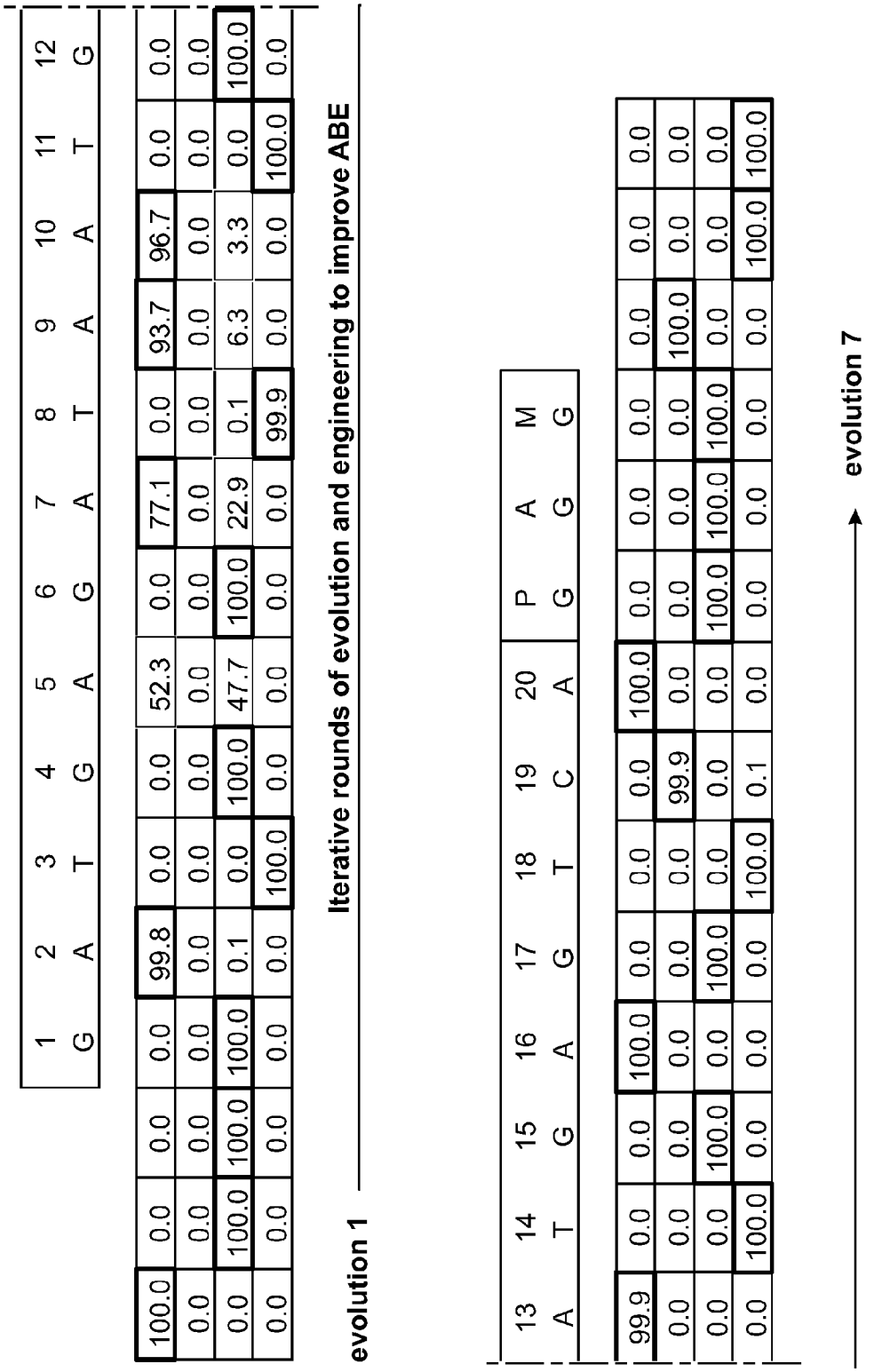
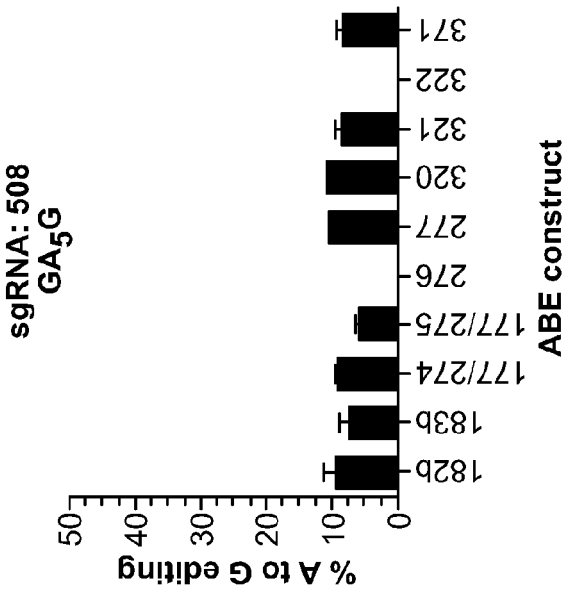
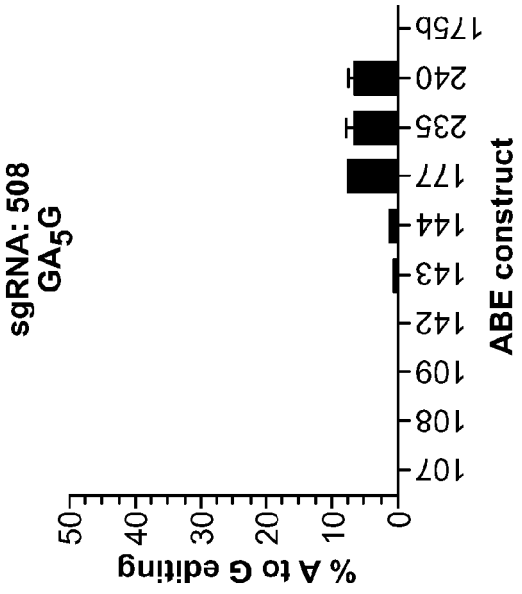

1 2 3 4 5 6 7 8 9 10 11 12
G A T G A G A T A A T G
A 100.0 0.0 0.0 0.0 99.8 0.0 0.0 52.3 0.0 77.1 0.0 93.7 96.7 0.0 0.0
C 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
G 0.0 100.0 100.0 100.0 0.1 0.0 100.0 47.7 100.0 22.9 0.1 6.3 3.3 0.0 100.0
T 0.0 0.0 0.0 0.0 0.0 100.0 0.0 0.0 0.0 0.0 99.9 0.0 0.0 100.0 0.0
Iterative rounds of evolution and engineering to improve ABE
evolution 1
13 14 15 16 17 18 19 20
A T G A G T C A
P A M
G G G
99.9 0.0 0.0 100.0 0.0 0.0 0.0 100.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
0.0 0.0 0.0 0.0 0.0 0.0 99.9 0.0 0.0 0.0 0.0 100.0 0.0 0.0 0.0
0.0 0.0 100.0 0.0 100.0 0.0 0.0 0.0 100.0 100.0 100.0 0.0 0.0 0.0 0.0
0.0 100.0 0.0 0.0 0.0 100.0 0.1 0.0 0.0 0.0 0.0 0.0 100.0 100.0
evolution 7

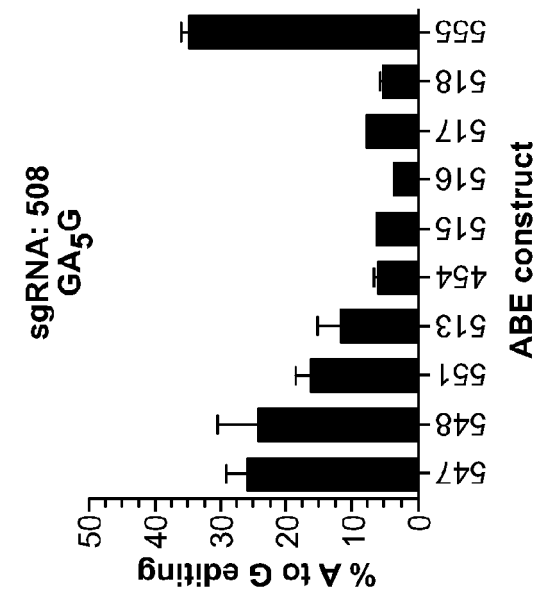
FIGURE 156 (Continued)

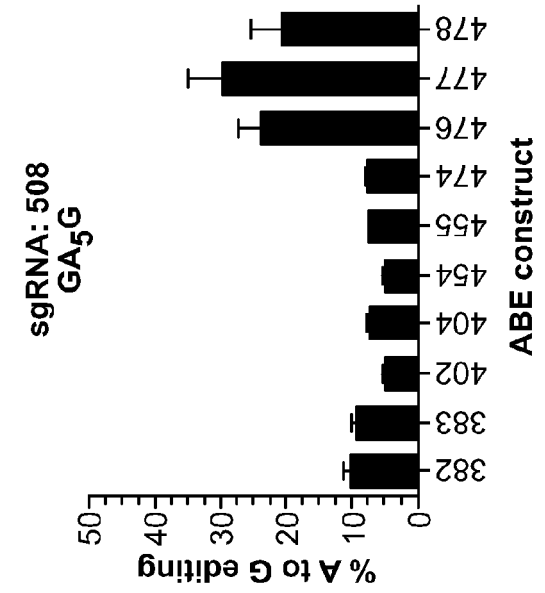



Target sequence for "508" genomic site

**5′ –GATGAGATAATGATGAGTCA– 3′**



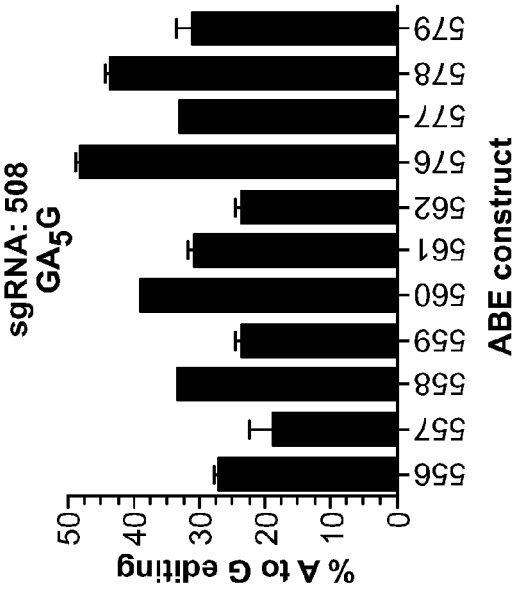
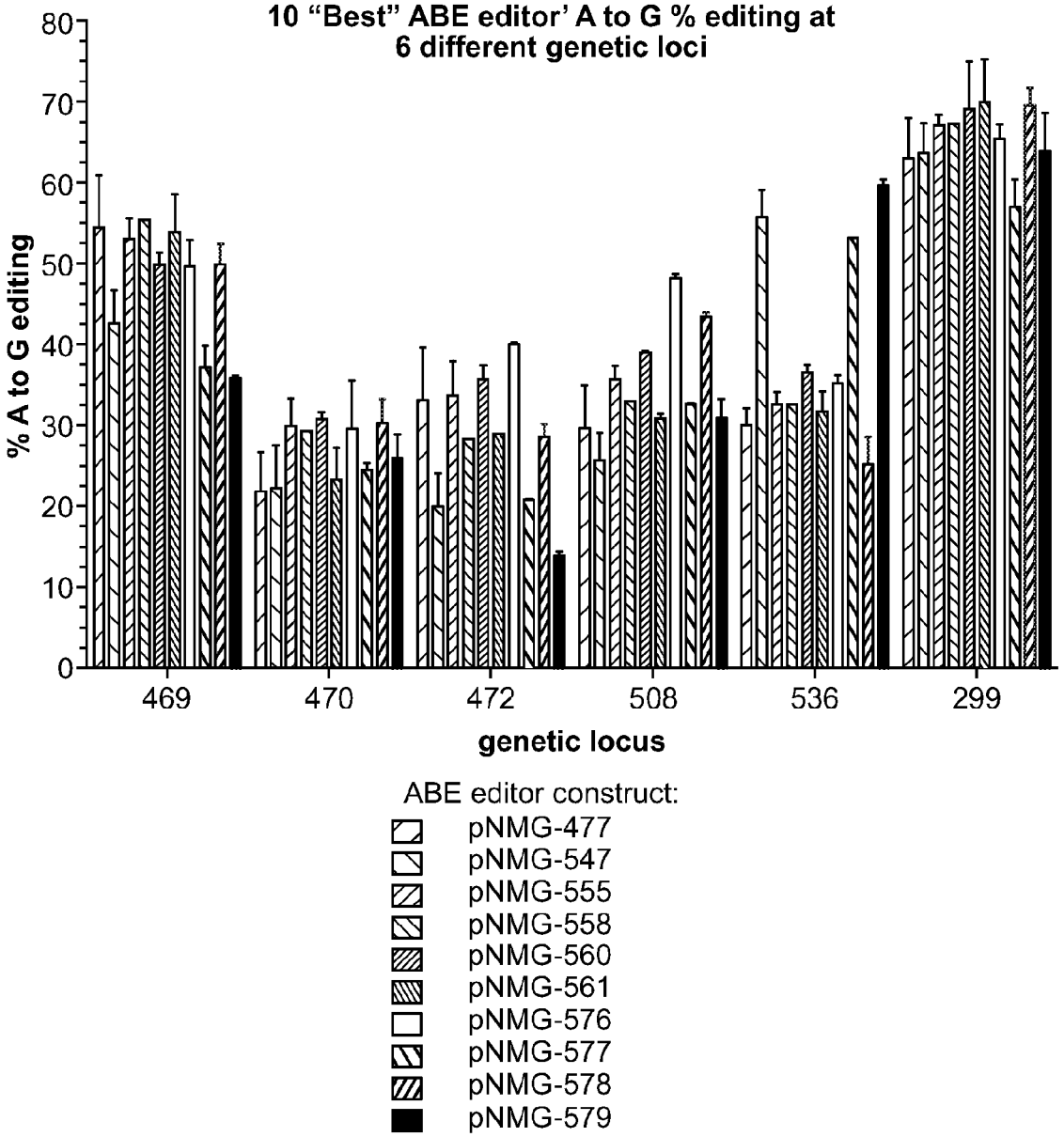




FIGURE 156
(Continued)

# FIGURE 157

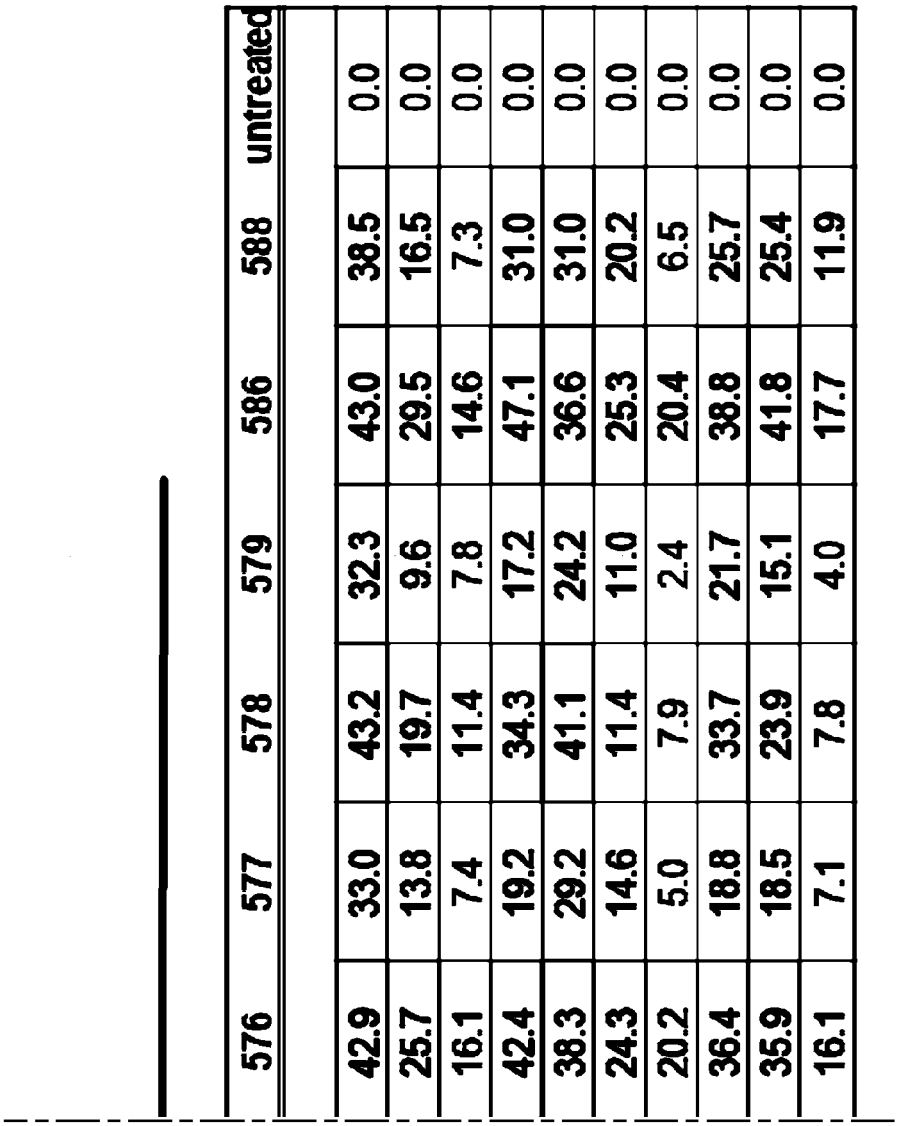



| genetic locus | sequence | position of target A | target site |
|---|---|---|---|
| pNMG-469 | TAT | 5 | GAGTATGAGGCATAGACTGC |
| pNMG-470 | AAG | 5 | GTCAAGAAAGCAGAGACTGC |
| pNMG-472 | CAA | 5 | GGGAATAAATCATAGAATCC |
| pNMG-508 | GAG | 5 | GATGAGATAATGATGAGTCA |
| pNMG-536 | GAC | 7 | GGATTGACCCAGGCCAGGGC |
| pNMG-299 | CAC | 5 | GAACACAAAGCATAGACTGC |

top 10 ABEs

| sgRNA # | NAN | target sequence | 477 | 547 | 555 | 558 | 560 | 561 |
|---|---|---|---|---|---|---|---|---|
| 310 | TAC | GAATACTAAGCATAGACTCC | 33.0 | 27.7 | 47.7 | 47.8 | 46.8 | 44.1 |
| 311 | AAC | GTAAACAAAGCATAGACTGA | 15.7 | 12.1 | 27.9 | 29.5 | 28.0 | 20.0 |
| 314 | ACC | GAAGACCAAGGATAGACTGC | 10.4 | 6.4 | 16.0 | 18.1 | 19.3 | 12.4 |
| 318 | CAT | GAACATAAAGAATAGAATGA | 20.7 | 20.2 | 40.3 | 43.1 | 37.1 | 33.2 |
| 463 | CAG | GGACAGGCAGCATAGACTGT | 31.2 | 35.2 | 41.7 | 45.8 | 38.4 | 39.6 |
| 464 | GAA | GTAGAAAAAGTATAGACTGC | 9.3 | 15.2 | 16.7 | 19.0 | 15.5 | 9.5 |
| 467 | TAA | GGCTAAAGACCATAGACTGT | 7.6 | 4.0 | 14.2 | 14.9 | 12.5 | 9.5 |
| 468 | TAG | GTCTAGAAAGCTTAGACTGC | 20.1 | 15.6 | 30.8 | 28.7 | 26.0 | 28.8 |
| 471 | AAT | GGGAATAAATCATAGAATCC | 20.0 | 22.1 | 30.6 | 31.7 | 29.1 | 20.9 |
| 501 | AAA | GACAAAGAGGAAGAGAGACG | 8.1 | 7.2 | 9.4 | 10.4 | 8.8 | 7.1 |

Figure 158



| 576 | 577 | 578 | 579 | 586 | 588 | untreated |
|---|---|---|---|---|---|---|
| 42.9 | 33.0 | 43.2 | 32.3 | 43.0 | 38.5 | 0.0 |
| 25.7 | 13.8 | 19.7 | 9.6 | 29.5 | 16.5 | 0.0 |
| 16.1 | 7.4 | 11.4 | 7.8 | 14.6 | 7.3 | 0.0 |
| 42.4 | 19.2 | 34.3 | 17.2 | 47.1 | 31.0 | 0.0 |
| 38.3 | 29.2 | 41.1 | 24.2 | 36.6 | 31.0 | 0.0 |
| 24.3 | 14.6 | 11.4 | 11.0 | 25.3 | 20.2 | 0.0 |
| 20.2 | 5.0 | 7.9 | 2.4 | 20.4 | 6.5 | 0.0 |
| 36.4 | 18.8 | 33.7 | 21.7 | 38.8 | 25.7 | 0.0 |
| 35.9 | 18.5 | 23.9 | 15.1 | 41.8 | 25.4 | 0.0 |
| 16.1 | 7.1 | 7.8 | 4.0 | 17.7 | 11.9 | 0.0 |

Figure 158 (Continued)

# Figure 159

| | | | | | alternating AS | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | 1 | 2 | 3 | 4 | 5 |
| | | | | | A | C | A | C | A |
| 477 | | | | | | | | | |
| **A** | 100.0 | 0.0 | 99.6 | 0.0 | 99.3 | 0.0 | 99.6 | 0.0 | 87.4 |
| **C** | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| **G** | 0.0 | 100.0 | 0.4 | 0.0 | 0.7 | 0.0 | 3.4 | 0.0 | 12.6 |
| **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **total reads** | 48139 | 48072 | 48293 | 48527 | 48538 | 48614 | 48810 | 48010 | 47431 |
| 586 | | | | | | | | | |
| **A** | 99.9 | 0.0 | 99.7 | 0.0 | 99.1 | 0.0 | 99.6 | 0.0 | 86.5 |
| **C** | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| **G** | 0.0 | 100.0 | 0.3 | 0.0 | 0.9 | 0.0 | 3.4 | 0.0 | 13.5 |
| **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **total reads** | 51282 | 51319 | 51503 | 51717 | 51891 | 51794 | 52073 | 51114 | 50764 |
| 588 | | | | | | | | | |
| **A** | 100.0 | 0.0 | 99.9 | 0.0 | 99.4 | 0.0 | 97.6 | 0.0 | 91.8 |
| **C** | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| **G** | 0.0 | 100.0 | 0.1 | 0.0 | 0.6 | 0.0 | 2.4 | 0.0 | 8.1 |
| **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **total reads** | 34512 | 34483 | 34579 | 34858 | 34831 | 34907 | 35009 | 34450 | 34114 |
| BE3 | | | | | | | | | |
| **A** | 100.0 | 0.5 | 100.0 | 0.0 | 100.0 | 0.1 | 100.0 | 1.3 | 100.0 |
| **C** | 0.0 | 0.1 | 0.0 | 99.5 | 0.0 | 96.8 | 0.0 | 87.3 | 0.0 |
| **G** | 0.0 | 99.4 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.3 | 0.0 |
| **T** | 0.0 | 0.0 | 0.0 | 0.5 | 0.0 | 3.0 | 0.0 | 11.0 | 0.0 |
| **total reads** | 74023 | 73919 | 74176 | 74558 | 74638 | 74700 | 74995 | 73842 | 73816 |
| **untreated** | | | | | | | | | |
| **A** | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 99.9 |
| **C** | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| **G** | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 |
| **T** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| **total reads** | 43330 | 43221 | 43407 | 43639 | 43746 | 43716 | 43924 | 43215 | 43308 |

# Figure 159 (Continued)

| 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|
| C | A | C | A | C | T | T | A | G | A | A |
| 0.0 | 90.7 | 0.0 | 85.0 | 0.0 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 | 100.0 |
| 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 9.3 | 0.0 | 15.0 | 0.0 | 0.0 | 0.0 | 0.1 | 100.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 48067 | 47341 | 48271 | 47402 | 47697 | 48589 | 48863 | 48515 | 48251 | 46740 | 46930 |
| 0.0 | 88.0 | 0.0 | 75.1 | 0.0 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 | 100.0 |
| 100.0 | 0.0 | 99.9 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 12.0 | 0.0 | 24.9 | 0.0 | 0.0 | 0.0 | 0.1 | 100.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 51188 | 50530 | 51436 | 50217 | 50635 | 51683 | 52069 | 51280 | 51383 | 49132 | 49579 |
| 0.0 | 90.6 | 0.0 | 76.7 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 100.0 |
| 100.0 | 0.0 | 99.9 | 0.0 | 99.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 9.3 | 0.0 | 23.3 | 0.1 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 34563 | 33966 | 34588 | 33859 | 34096 | 34848 | 35107 | 34650 | 34586 | 32795 | 33204 |
| 2.9 | 100.0 | 0.6 | 100.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 100.0 |
| 66.7 | 0.0 | 88.4 | 0.0 | 99.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 10.3 | 0.0 | 0.4 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 20.1 | 0.0 | 10.7 | 0.0 | 0.3 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 73867 | 73518 | 74476 | 73833 | 73925 | 75011 | 75338 | 74473 | 74081 | 70538 | 71142 |
| 0.0 | 100.0 | 0.1 | 99.8 | 0.0 | 0.1 | 0.0 | 99.8 | 0.1 | 99.7 | 99.4 |
| 100.0 | 0.0 | 99.8 | 0.0 | 99.9 | 0.0 | 0.1 | 0.1 | 0.2 | 0.1 | 0.2 |
| 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 99.7 | 0.0 | 0.3 |
| 0.0 | 0.0 | 0.1 | 0.1 | 0.0 | 99.9 | 99.8 | 0.1 | 0.0 | 0.2 | 0.0 |
| 43391 | 43036 | 43538 | 42920 | 42989 | 43687 | 43744 | 43381 | 43128 | 40812 | 41266 |

# Figure 159 (Continued)

| 17 | 18 | 19 | 20 | P | A | M |
|---|---|---|---|---|---|---|
| T | C | T | C | T | G | G |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 100.0 | 0.0 | 100.0 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 48590 | 48198 | 48888 | 48506 | 47281 | 48464 | 48071 | 48082 | 47369 | 48069 | 46263 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 100.0 | 0.0 | 100.0 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 51463 | 51202 | 51936 | 51629 | 50320 | 51539 | 51265 | 51252 | 50030 | 51191 | 49049 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 34681 | 34510 | 34925 | 34766 | 33854 | 34745 | 34486 | 34499 | 33892 | 34428 | 33140 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 99.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 99.9 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 100.0 | 0.1 | 100.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 74313 | 73944 | 75064 | 74518 | 72819 | 74548 | 73960 | 74011 | 72919 | 73903 | 71061 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.3 | 0.1 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 99.8 | 0.1 | 0.0 |
| 0.0 | 99.2 | 0.0 | 0.2 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.1 |
| 0.1 | 0.5 | 0.0 | 99.7 | 0.0 | 99.9 | 99.7 | 99.9 | 0.0 | 99.7 | 0.0 |
| 99.7 | 0.2 | 100.0 | 0.1 | 99.8 | 0.0 | 0.1 | 0.0 | 0.1 | 0.2 | 99.9 |
| 43143 | 42922 | 43548 | 43194 | 42107 | 43192 | 42682 | 42741 | 42082 | 42871 | 41221 |

# Figure 160

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | 1 C | 2 A | 3 C |
| 477 | | | | | | | |
| A | 0.0 | 99.6 | 0.0 | 99.3 | 0.0 | 95.4 | 0.0 |
| C | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| G | 100.0 | 0.4 | 0.0 | 0.7 | 0.0 | 4.6 | 0.0 |
| T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total reads | 112439 | 112834 | 113528 | 113538 | 113681 | 113985 | 112176 |
| 586 | | | | | | | |
| A | 0.0 | 99.7 | 0.0 | 99.5 | 0.0 | 95.3 | 0.0 |
| C | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 99.9 |
| G | 100.0 | 0.3 | 0.0 | 0.5 | 0.0 | 4.7 | 0.0 |
| T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total reads | 132178 | 132477 | 133286 | 133509 | 133494 | 133925 | 131908 |
| 588 | | | | | | | |
| A | 0.0 | 99.8 | 0.0 | 99.5 | 0.0 | 96.8 | 0.0 |
| C | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| G | 100.0 | 0.2 | 0.0 | 0.4 | 0.0 | 3.2 | 0.0 |
| T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| total reads | 103850 | 104287 | 105033 | 105072 | 105289 | 105486 | 103955 |
| BE3 | | | | | | | |
| A | 0.6 | 100.0 | 0.0 | 100.0 | 0.1 | 100.0 | 0.7 |
| C | 0.0 | 0.0 | 99.4 | 0.0 | 97.3 | 0.0 | 89.0 |
| G | 99.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 |
| T | 0.0 | 0.0 | 0.6 | 0.0 | 2.6 | 0.0 | 10.1 |
| total reads | 28505 | 28558 | 28709 | 28672 | 28742 | 28860 | 28443 |
| untreated | | | | | | | |
| A | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 99.9 | 0.0 |
| C | 0.0 | 0.0 | 99.9 | 0.0 | 99.9 | 0.0 | 99.9 |
| G | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| total reads | 70330 | 70714 | 71213 | 71252 | 71212 | 71514 | 70350 |

# Figure 160 (Continued)

alternating As

| 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|
| A | C | A | C | A | C | T | T |
| 87.7 | 0.0 | 88.7 | 0.0 | 82.1 | 0.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 12.3 | 0.0 | 11.3 | 0.0 | 17.8 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 100.0 |
| 110957 | 112329 | 110610 | 112793 | 110457 | 111399 | 113626 | 114317 |
| 88.7 | 0.0 | 87.6 | 0.0 | 72.5 | 0.0 | 0.0 | 0.0 |
| 0.0 | 99.9 | 0.0 | 99.9 | 0.0 | 99.5 | 0.0 | 0.0 |
| 11.3 | 0.0 | 12.4 | 0.0 | 27.5 | 0.3 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 100.0 | 100.0 |
| 130947 | 132047 | 130240 | 132612 | 129061 | 130139 | 132919 | 134000 |
| 92.4 | 0.0 | 92.2 | 0.0 | 75.6 | 0.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 99.4 | 0.0 | 0.0 |
| 7.6 | 0.0 | 7.8 | 0.0 | 24.4 | 0.4 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 100.0 | 100.0 |
| 103035 | 104083 | 102666 | 104460 | 102199 | 102791 | 104894 | 105715 |
| 100.0 | 4.2 | 100.0 | 1.5 | 100.0 | 0.1 | 0.0 | 0.0 |
| 0.0 | 69.5 | 0.0 | 80.2 | 0.0 | 98.9 | 0.0 | 0.0 |
| 0.0 | 6.3 | 0.0 | 1.7 | 0.0 | 0.1 | 0.0 | 0.0 |
| 0.0 | 20.0 | 0.0 | 16.6 | 0.0 | 1.0 | 100.0 | 100.0 |
| 28420 | 28451 | 28279 | 28612 | 28393 | 28463 | 28935 | 29020 |
| 99.9 | 0.0 | 99.9 | 0.2 | 99.7 | 0.1 | 0.0 | 0.0 |
| 0.0 | 99.9 | 0.0 | 99.7 | 0.0 | 99.8 | 0.1 | 0.1 |
| 0.1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.1 | 0.0 | 0.2 | 0.0 | 99.9 | 99.9 |
| 70576 | 70682 | 69963 | 70778 | 69863 | 69870 | 70972 | 70778 |

# Figure 160 (Continued)

| 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|
| A | G | A | A | T | C | T | G |
| 99.8 | 0.0 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.2 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 113263 | 112615 | 109228 | 109821 | 113539 | 112723 | 114158 | 113310 |
| 99.5 | 0.0 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.5 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 132129 | 132354 | 127031 | 127734 | 132630 | 131981 | 133789 | 132992 |
| 99.9 | 0.0 | 100.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.1 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 104341 | 104298 | 99151 | 100343 | 104469 | 103959 | 105327 | 104630 |
| 100.0 | 0.0 | 100.0 | 100.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 99.5 | 0.0 | 0.0 |
| 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.3 | 100.0 | 0.0 |
| 28673 | 28514 | 27128 | 27359 | 28617 | 28492 | 28885 | 28682 |
| 99.9 | 0.0 | 99.8 | 99.7 | 0.2 | 0.1 | 0.1 | 0.0 |
| 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 99.3 | 0.1 | 0.1 |
| 0.0 | 99.8 | 0.0 | 0.3 | 0.0 | 0.4 | 0.1 | 99.8 |
| 0.1 | 0.0 | 0.2 | 0.0 | 99.8 | 0.1 | 99.8 | 0.1 |
| 70480 | 69804 | 66108 | 66873 | 69970 | 69771 | 70540 | 70015 |

# Figure 160 (Continued)

| 20 T | P G | A G | M G |
|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 110668 | 113316 | 112402 | 112481 | 110476 | 112258 | 108248 | 112056 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 129636 | 132937 | 131938 | 132156 | 129283 | 131875 | 126433 | 131758 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 102118 | 104786 | 103982 | 103997 | 102166 | 103831 | 100042 | 103242 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 100.0 | 100.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 28048 | 28743 | 28513 | 28490 | 28001 | 28434 | 27354 | 28398 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 0.1 | 0.0 | 0.0 | 0.1 | 99.9 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 |
| 0.0 | 99.9 | 99.8 | 99.9 | 0.0 | 99.9 | 0.0 | 99.9 |
| 99.9 | 0.0 | 0.1 | 0.0 | 0.1 | 0.1 | 99.9 | 0.0 |
| 68618 | 69894 | 68462 | 68560 | 67892 | 68649 | 66444 | 68392 |

Figure 161

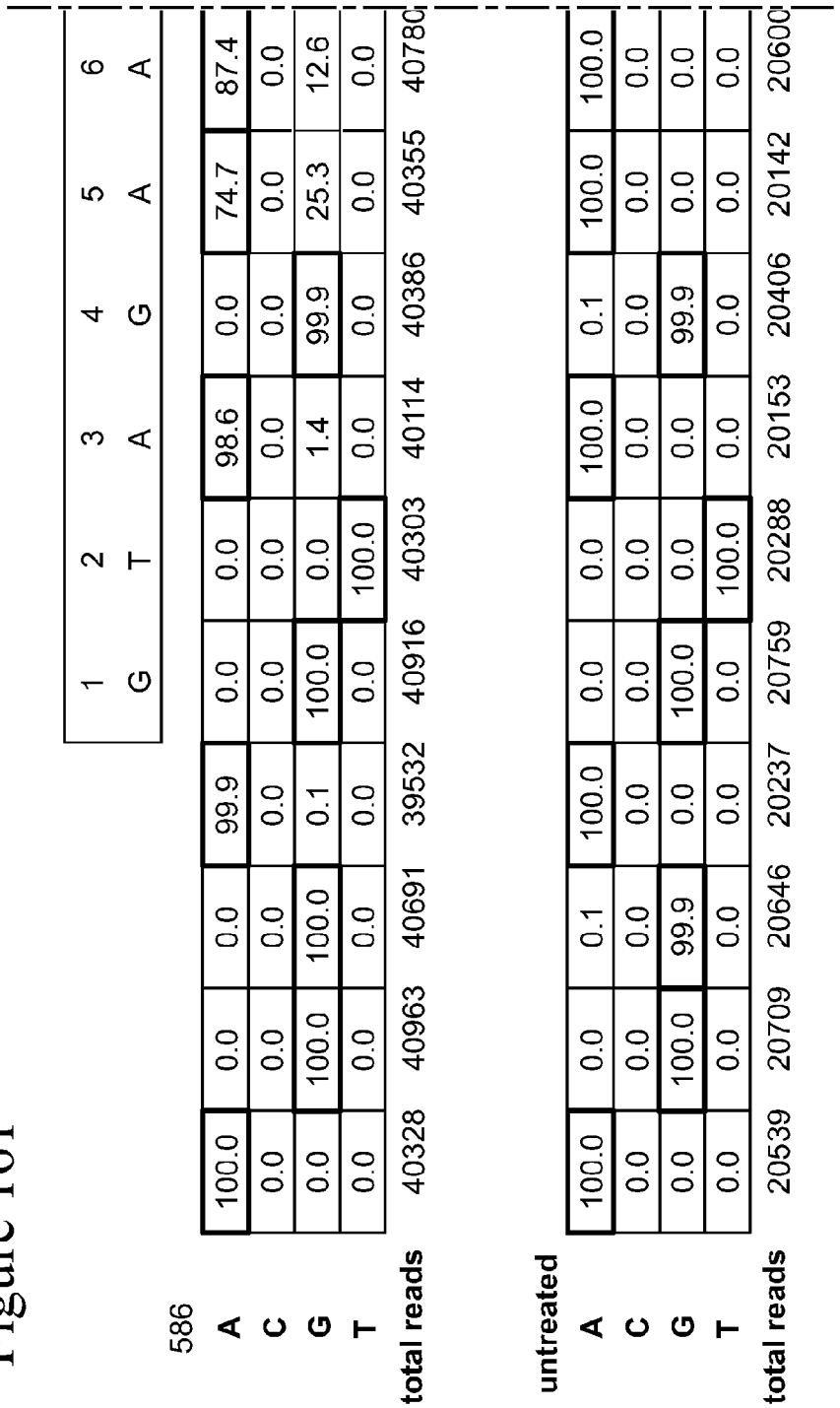

586
1 2 3 4 5 6
G T A G A A
A 100.0 0.0 0.0 99.9 0.0 0.0 98.6 0.0 74.7 87.4
C 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
G 0.0 100.0 100.0 0.1 100.0 0.0 1.4 99.9 25.3 12.6
T 0.0 0.0 0.0 0.0 0.0 100.0 0.0 0.0 0.0 0.0
total reads 40328 40963 40691 39532 40916 40303 40114 40386 40355 40780
untreated
A 100.0 0.0 0.1 100.0 0.0 0.0 100.0 0.1 100.0 100.0
C 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
G 0.0 100.0 99.9 0.0 100.0 0.0 0.0 99.9 0.0 0.0
T 0.0 0.0 0.0 0.0 0.0 100.0 0.0 0.0 0.0 0.0
total reads 20539 20709 20646 20237 20759 20288 20153 20406 20142 20600

Figure 161 (Continued)

| 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|
| A | A | A | G | T | A | T | A | G | A | C |
| 94.4 | 96.6 | 96.6 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 | 0.1 | 100.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 5.6 | 3.4 | 3.4 | 100.0 | 0.0 | 0.1 | 0.0 | 0.0 | 99.9 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 40398 | 40607 | 40231 | 40175 | 37223 | 40133 | 40728 | 41151 | 39326 | 40711 | 40733 |
| 100.0 | 100.0 | 100.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 20371 | 20608 | 20300 | 20273 | 18016 | 20111 | 20684 | 20630 | 20157 | 20574 | 20592 |

Figure 161 (Continued)

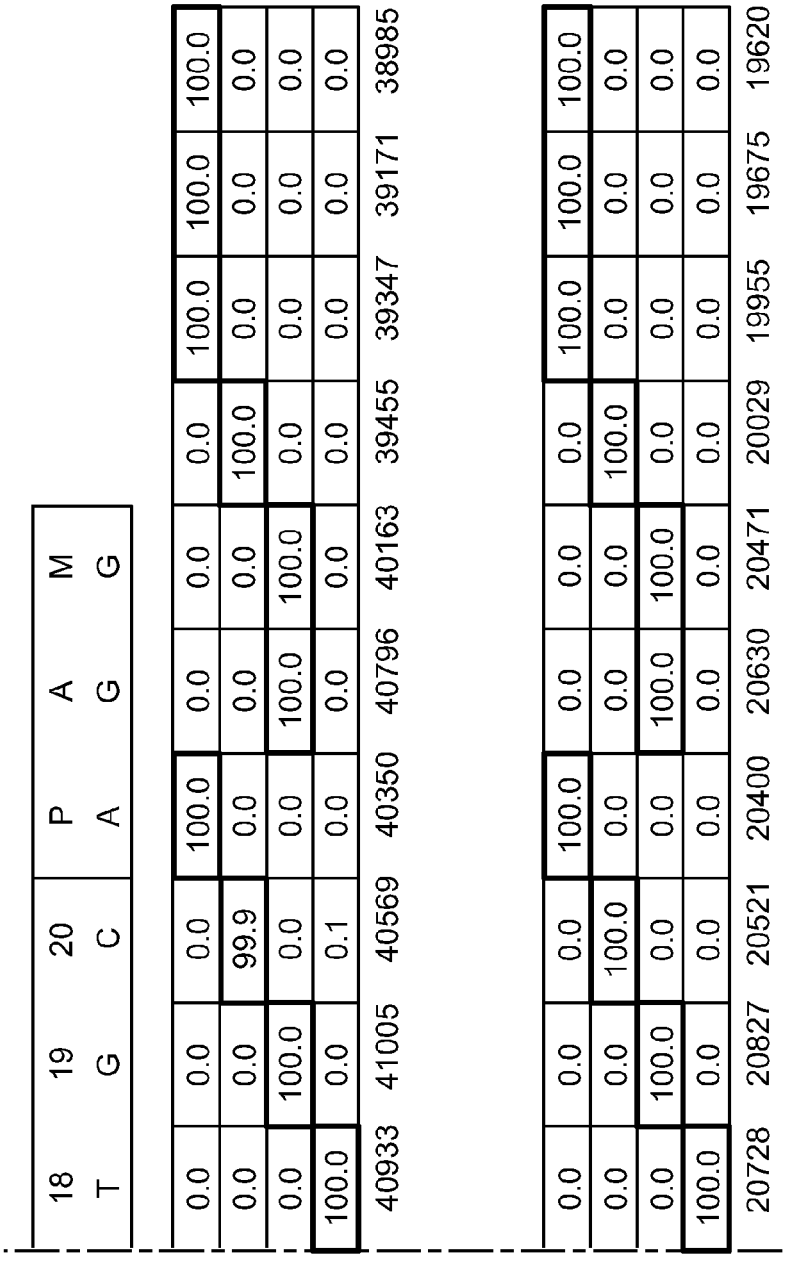


Figure 161 (Continued)

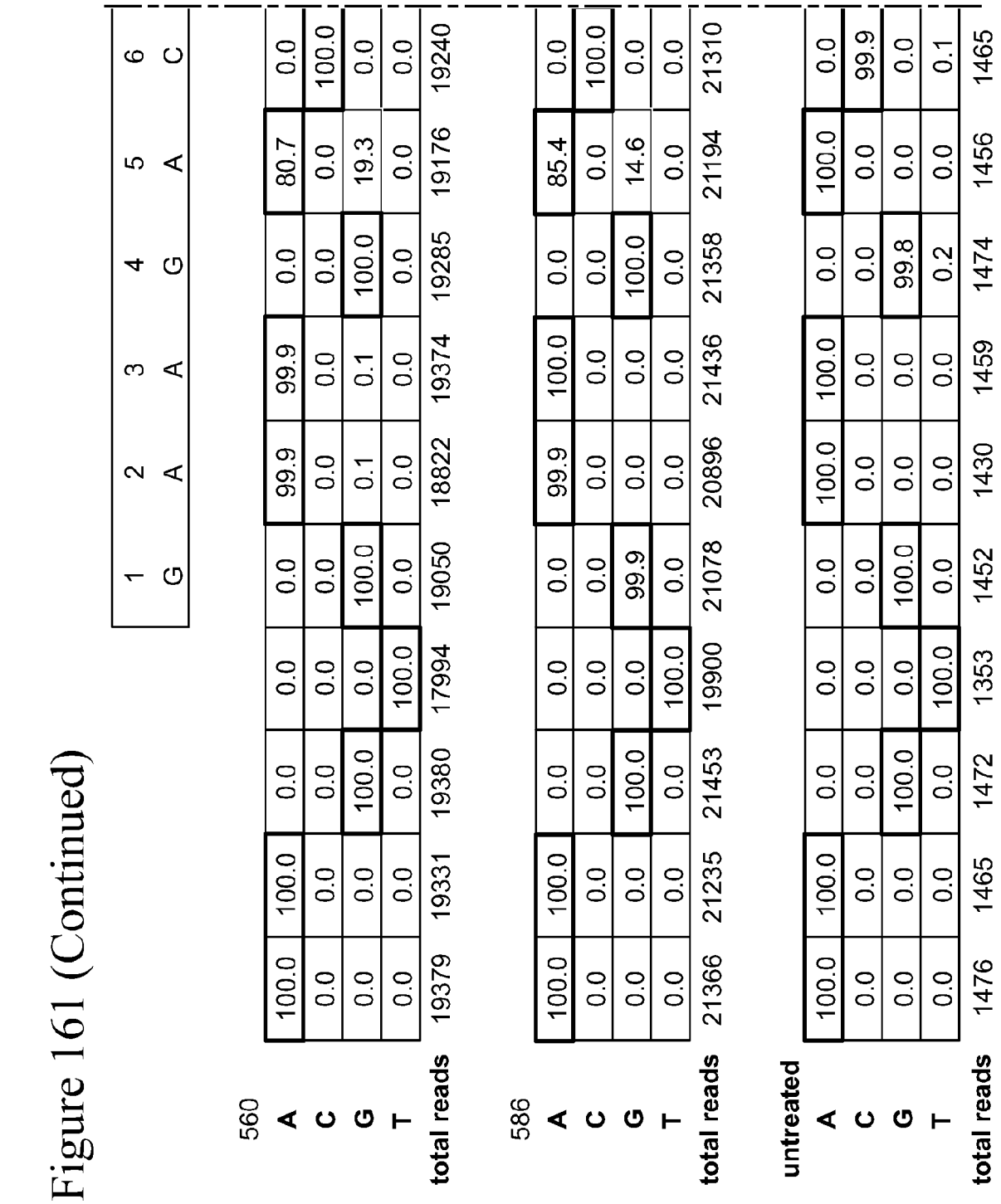
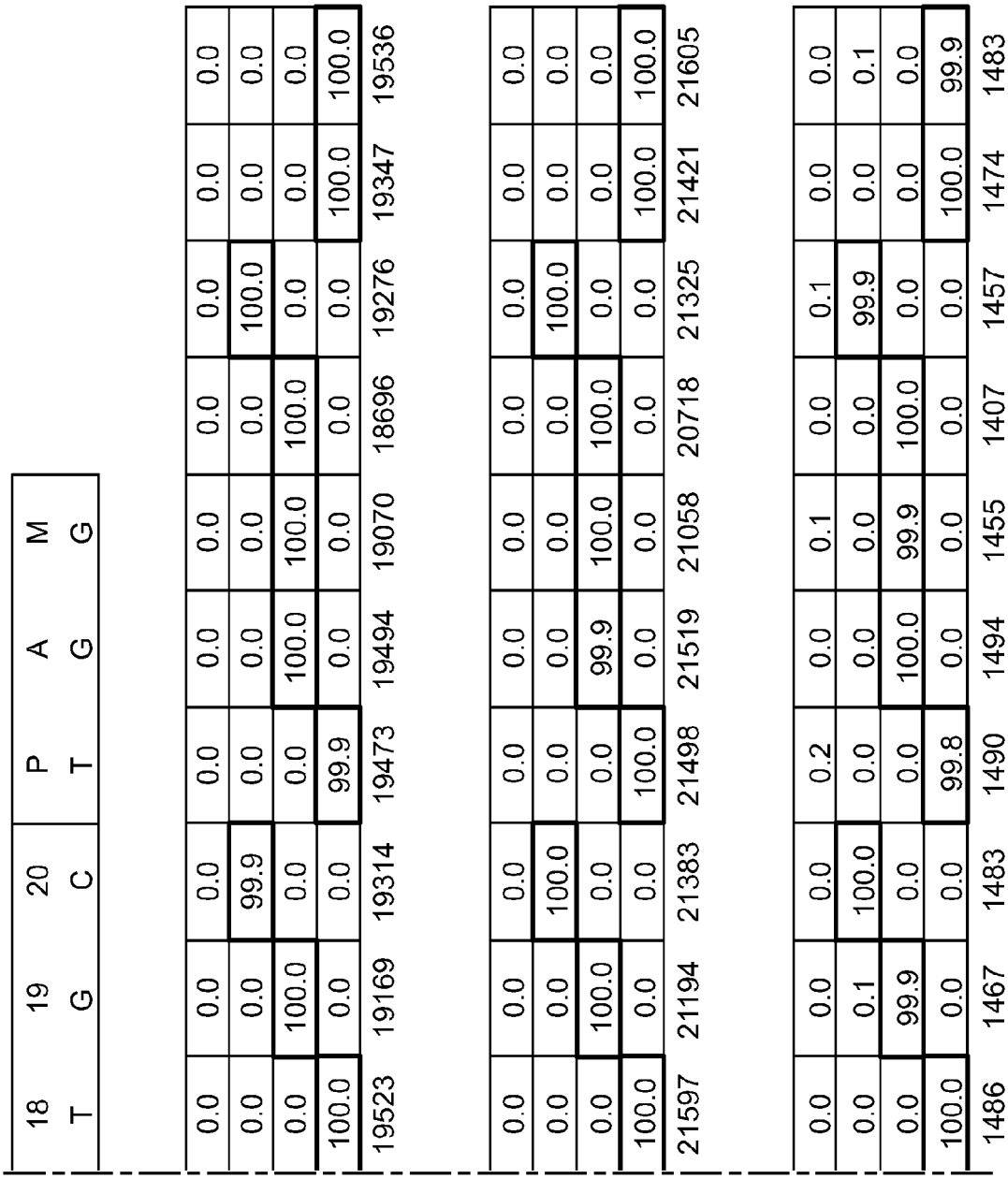


Figure 161 (Continued)

| 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|
| C | A | A | G | G | A | T | A | G | A | C |
| 0.0 | 98.9 | 99.4 | 0.0 | 0.1 | 99.9 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 1.1 | 0.6 | 100.0 | 99.9 | 0.1 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 19289 | 19406 | 18396 | 19111 | 18728 | 18639 | 19205 | 19347 | 19233 | 19203 | 19496 |
| 0.0 | 98.9 | 99.1 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 1.1 | 0.9 | 100.0 | 99.9 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 21294 | 21446 | 20355 | 21196 | 20790 | 20615 | 21205 | 21459 | 21333 | 21282 | 21537 |
| 0.0 | 100.0 | 99.9 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 | 100.0 | 0.0 |
| 99.8 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| 0.0 | 0.0 | 0.1 | 100.0 | 99.8 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 |
| 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 1464 | 1479 | 1401 | 1452 | 1427 | 1416 | 1460 | 1472 | 1464 | 1462 | 1481 |

Figure 161 (Continued)



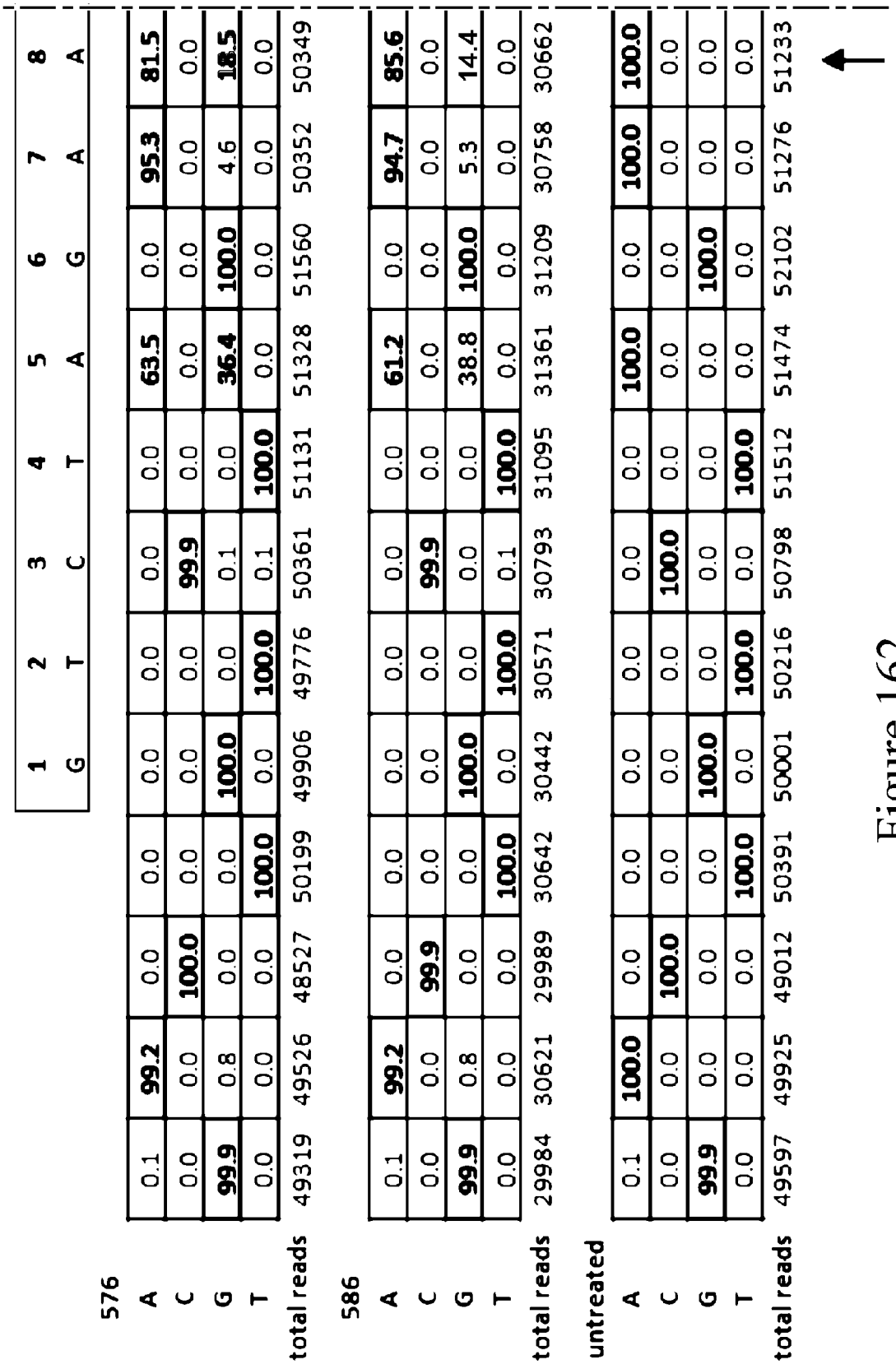
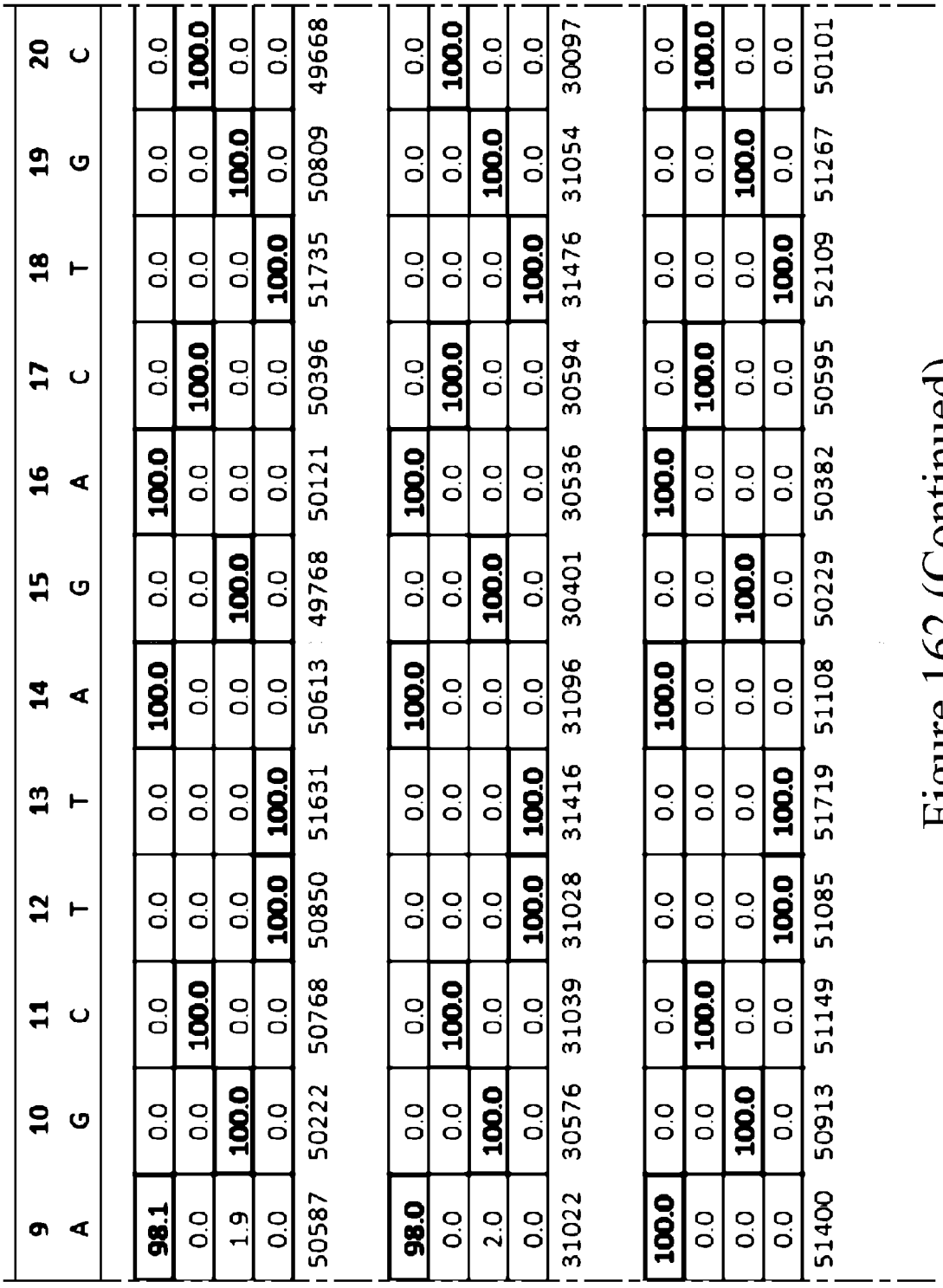

1 2 3 4 5 6 7 8
G T C T A G A A
576
A 0.1 99.2 0.0 0.0 0.0 0.0 63.5 0.0 95.3 81.5
C 0.0 0.0 100.0 0.0 0.0 99.9 0.0 0.0 0.0 0.0
G 99.9 0.8 0.0 0.0 100.0 0.1 36.4 100.0 4.6 18.5
T 0.0 0.0 0.0 100.0 0.0 100.0 0.0 0.0 0.0 0.0
total reads 49319 49526 48527 50199 49906 49776 50361 51131 51328 51560 50352 50349
586
A 0.1 99.2 0.0 0.0 0.0 0.0 0.0 0.0 61.2 0.0 94.7 85.6
C 0.0 0.0 99.9 0.0 0.0 0.0 99.9 0.0 0.0 0.0 0.0 0.0
G 99.9 0.8 0.0 0.0 100.0 0.0 0.0 0.0 38.8 100.0 5.3 14.4
T 0.0 0.0 0.0 100.0 0.0 100.0 0.1 100.0 0.0 0.0 0.0 0.0
total reads 29984 30621 29989 30642 30442 30571 30793 31095 31361 31209 30758 30662
untreated
A 0.1 100.0 0.0 0.0 0.0 0.0 0.0 0.0 100.0 0.0 100.0 100.0
C 0.0 0.0 100.0 0.0 0.0 0.0 100.0 0.0 0.0 0.0 0.0 0.0
G 99.9 0.0 0.0 0.0 100.0 0.0 0.0 0.0 0.0 100.0 0.0 0.0
T 0.0 0.0 0.0 100.0 0.0 100.0 0.0 100.0 0.0 0.0 0.0 0.0
total reads 49597 49925 49012 50391 50001 50216 50798 51512 51474 52102 51276 51233

Figure 162



| 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A | G | C | T | T | A | G | A | C | T | G | C |
| **98.1** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | **100.0** |
| 1.9 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 |
| 0.0 | 0.0 | 0.0 | **100.0** | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 |
| 50587 | 50222 | 50768 | 50850 | 51631 | 50613 | 49768 | 50121 | 50396 | 51735 | 50809 | 49668 |
| **98.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | **100.0** |
| 2.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 |
| 0.0 | 0.0 | 0.0 | **100.0** | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 |
| 31022 | 30576 | 31039 | 31028 | 31416 | 31096 | 30401 | 30536 | 30594 | 31476 | 31054 | 30097 |
| **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | **100.0** |
| 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 |
| 0.0 | 0.0 | 0.0 | **100.0** | **100.0** | 0.0 | 0.0 | 0.0 | 0.0 | **100.0** | 0.0 | 0.0 |
| 51400 | 50913 | 51149 | 51085 | 51719 | 51108 | 50229 | 50382 | 50595 | 52109 | 51267 | 50101 |

Figure 162 (Continued)

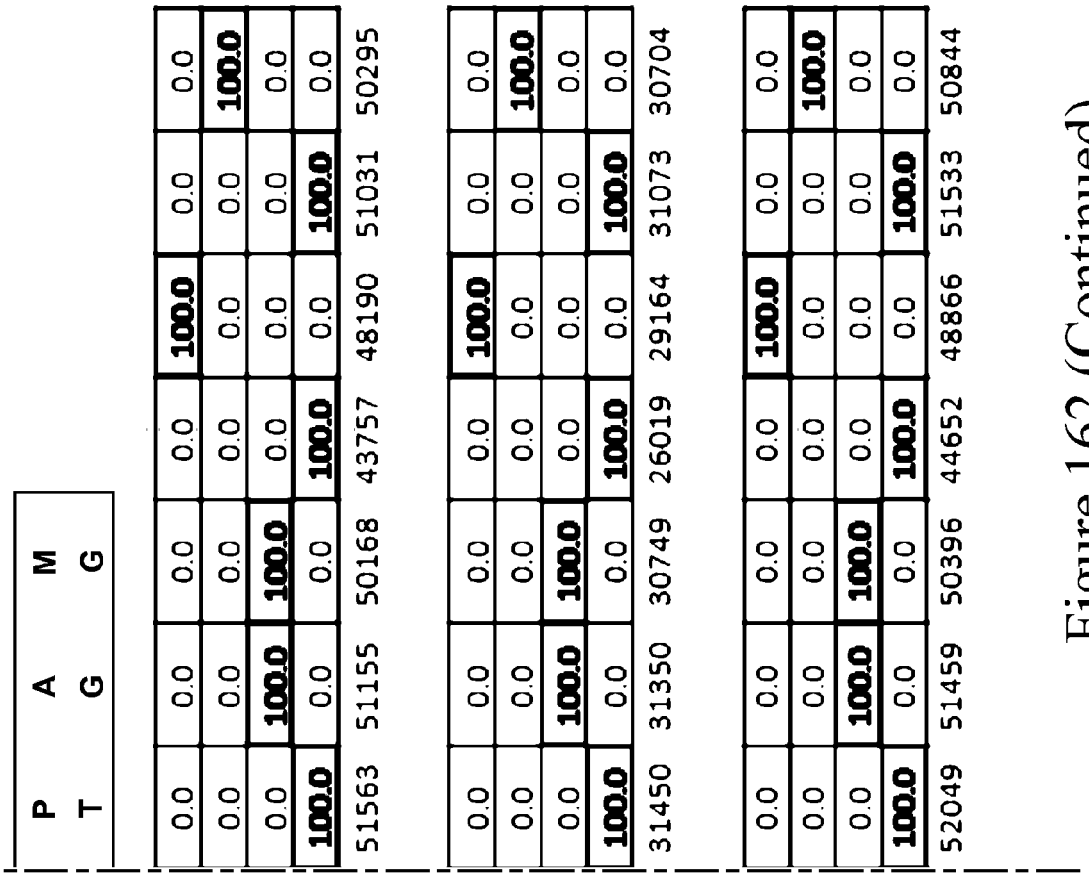



Figure 162 (Continued)

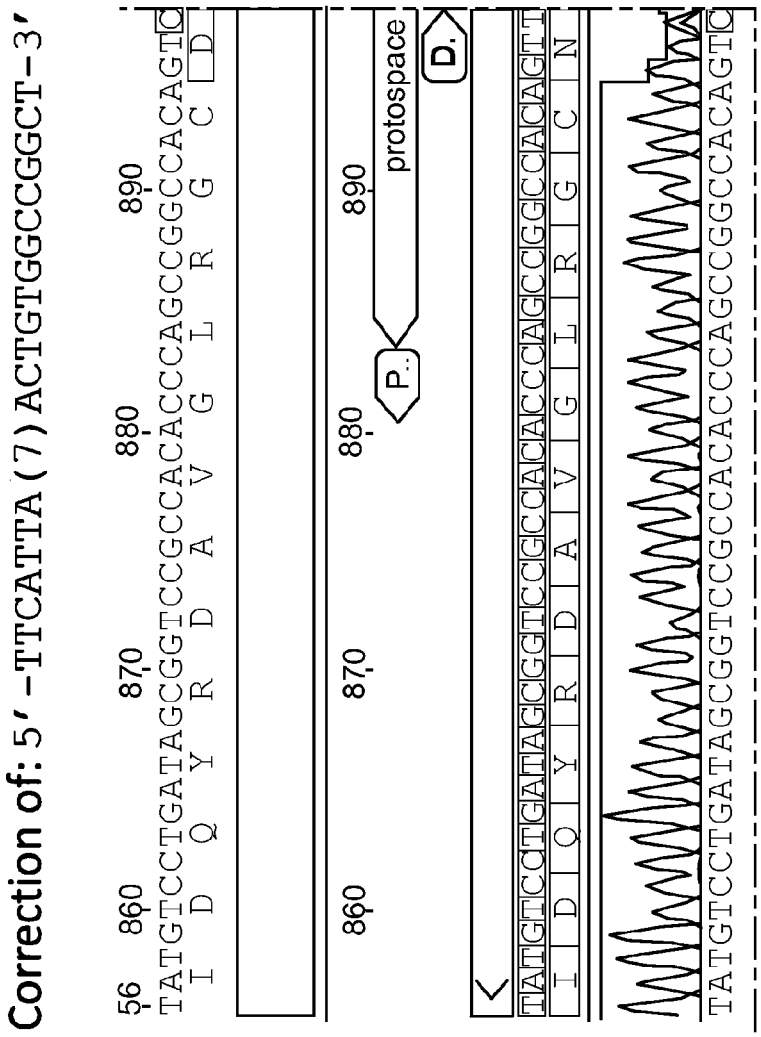

Correction of: 5′ –TTCATTA ( 7 ) ACTGTGGCCGGCT–3′
56
860
870
880
890
TATGTCCTGATAGCGGTCCGCCACACCCAGCCGGCCACAGTC
I D Q Y R D A V G L R G C D
protospace
P..
D.

Figure 163

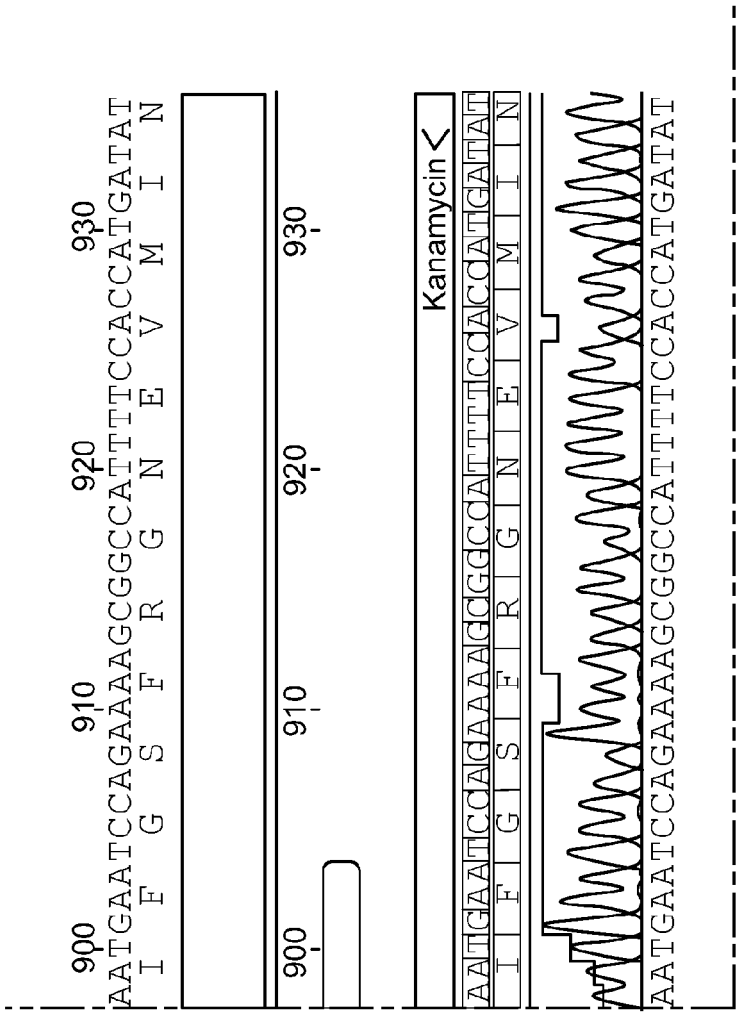



Figure 163 (Continued)

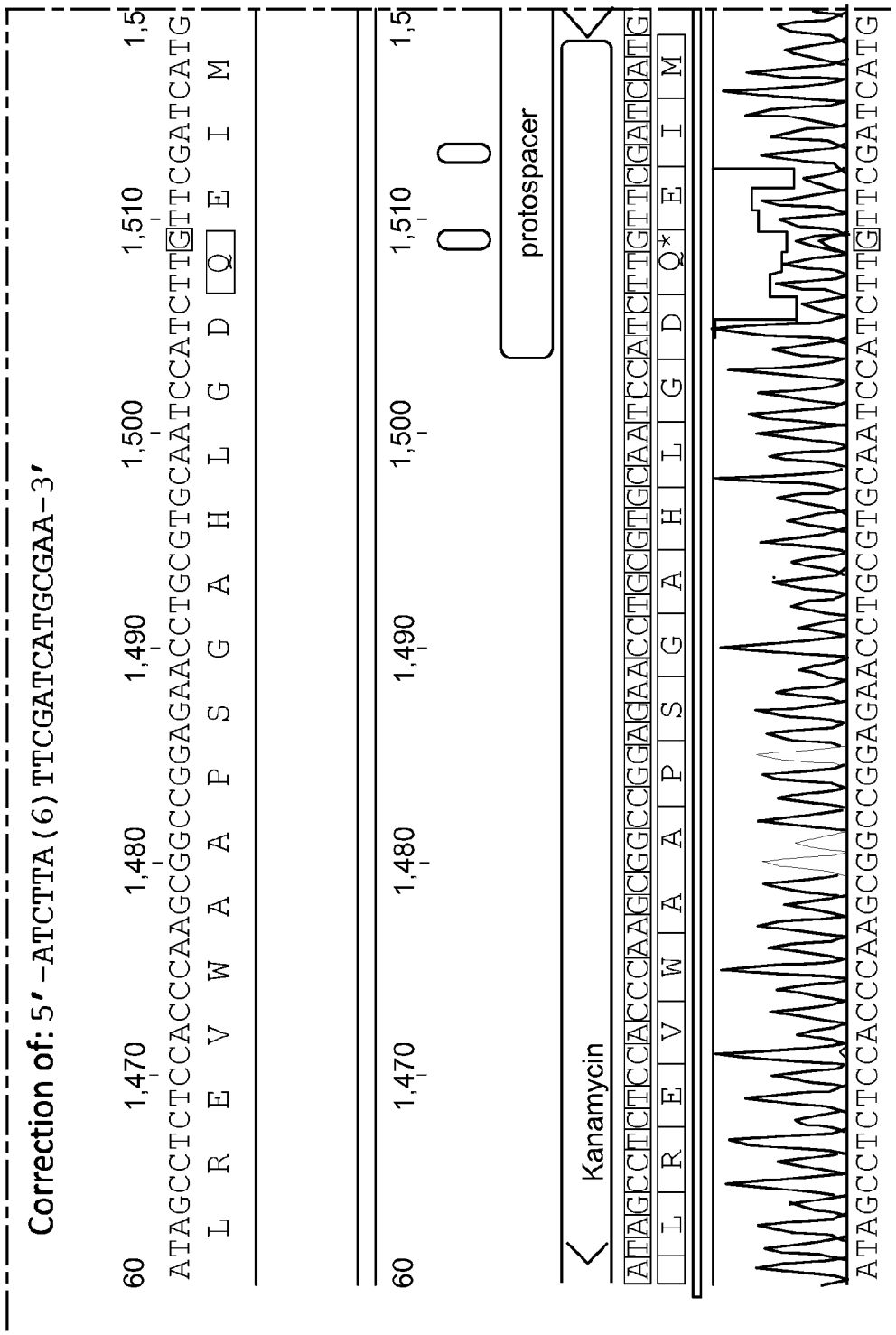



Figure 163 (Continued)

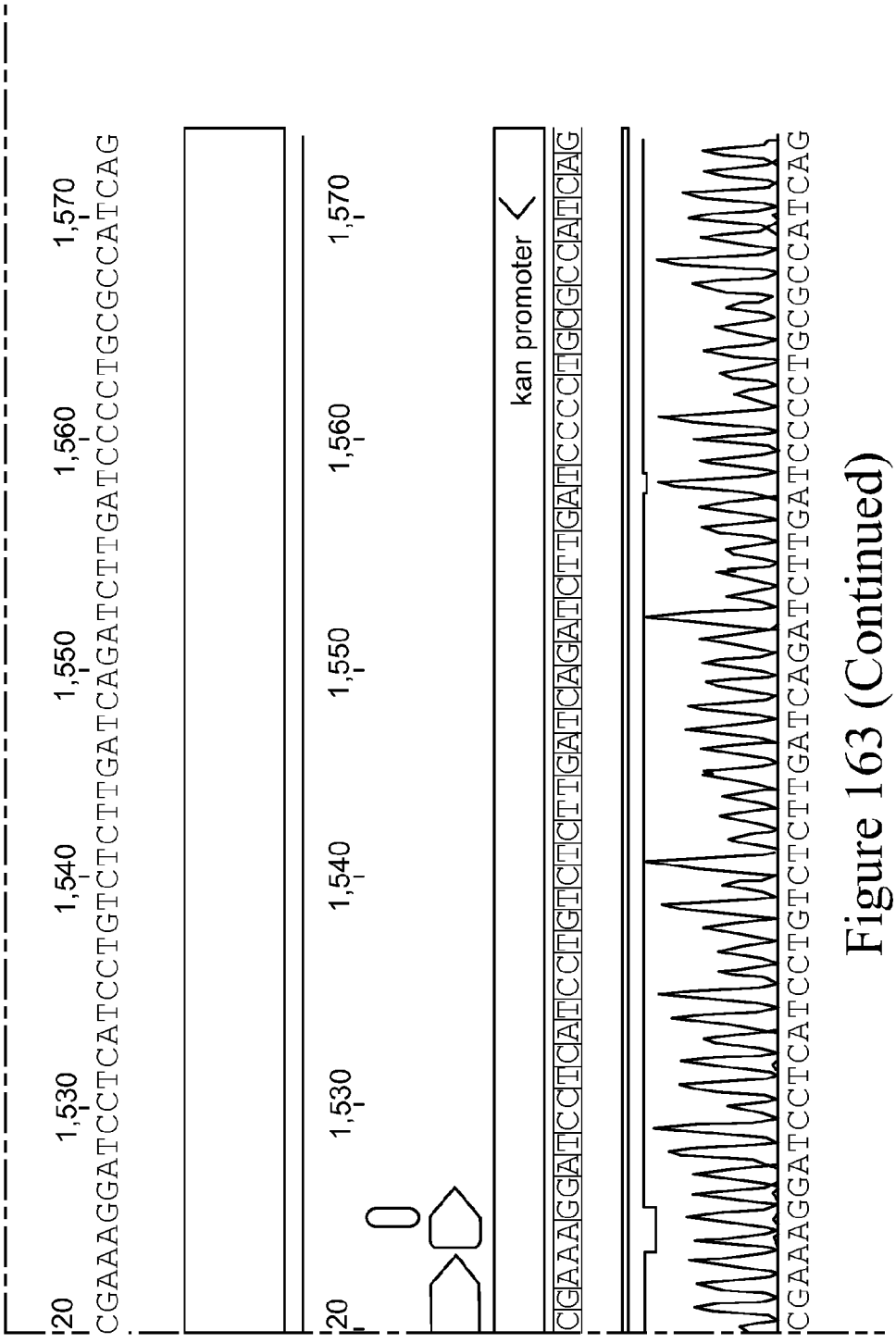
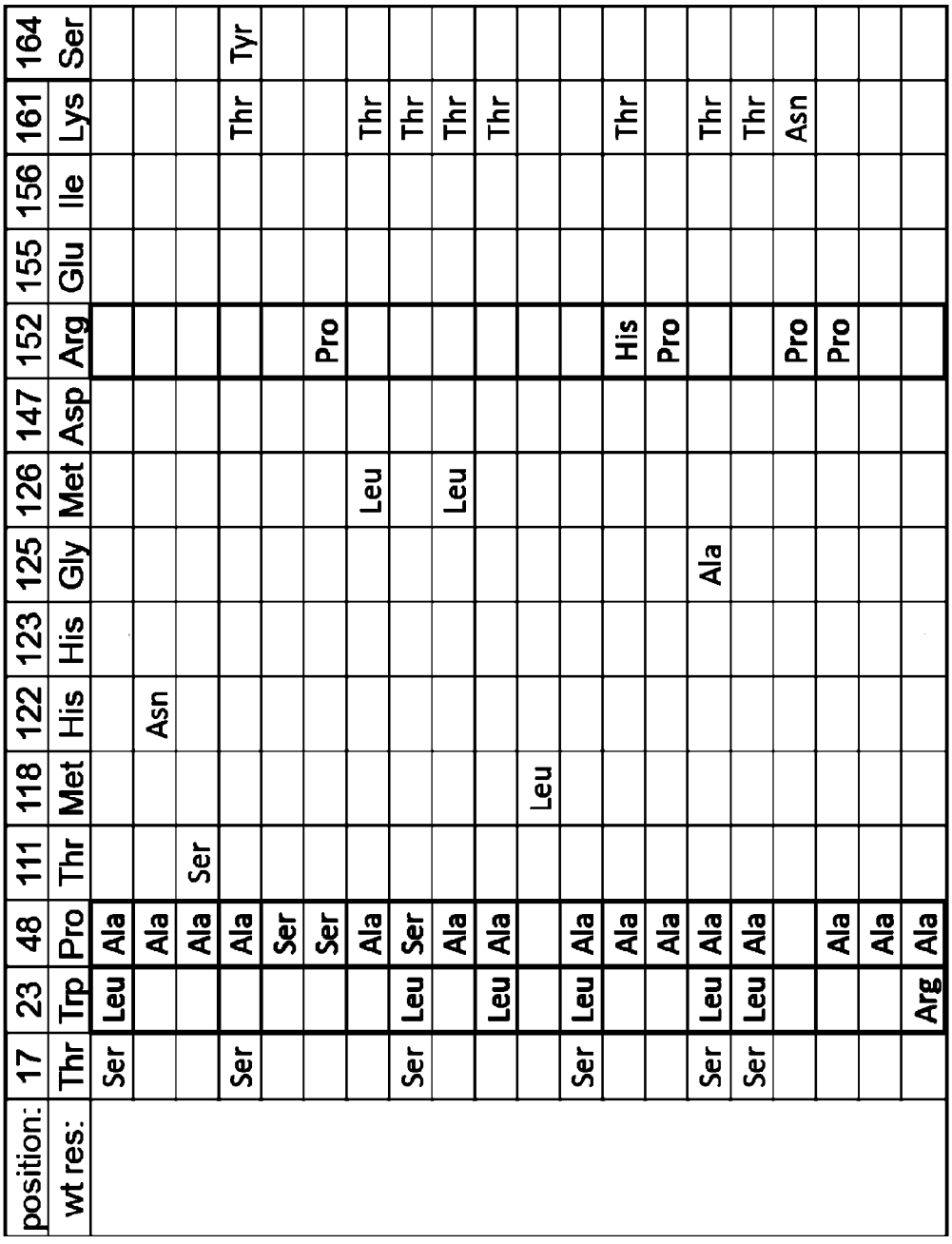



Figure 163 (Continued)



| position: | 17 | 23 | 48 | 111 | 118 | 122 | 123 | 125 | 126 | 147 | 152 | 155 | 156 | 161 | 164 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| wt res: | Thr | Trp | Pro | Thr | Met | His | His | Gly | Met | Asp | Arg | Glu | Ile | Lys | Ser |
| | Ser | Leu | Ala | | | | | | | | | | | | |
| | | | Ala | | | Asn | | | | | | | | | |
| | | | Ala | Ser | | | | | | | | | | | |
| | Ser | | Ala | | | | | | | | | | | Thr | Tyr |
| | | | Ser | | | | | | | | | | | | |
| | | | Ser | | | | | | | | Pro | | | | |
| | | | Ala | | | | | | Leu | | | | | Thr | |
| | Ser | Leu | Ser | | | | | | | | | | | Thr | |
| | | | Ala | | | | | | Leu | | | | | Thr | |
| | | Leu | Ala | | | | | | | | | | | Thr | |
| | | | | | Leu | | | | | | | | | | |
| | Ser | Leu | Ala | | | | | | | | | | | | |
| | | | Ala | | | | | | | | His | | | Thr | |
| | | | Ala | | | | | | | | Pro | | | | |
| | Ser | Leu | Ala | | | | | Ala | | | | | | Thr | |
| | Ser | Leu | Ala | | | | | | | | | | | Thr | |
| | | | | | | | | | | | Pro | | | Asn | |
| | | | Ala | | | | | | | | Pro | | | | |
| | | | Ala | | | | | | | | | | | | |
| | | Arg | Ala | | | | | | | | | | | | |

Figure 164

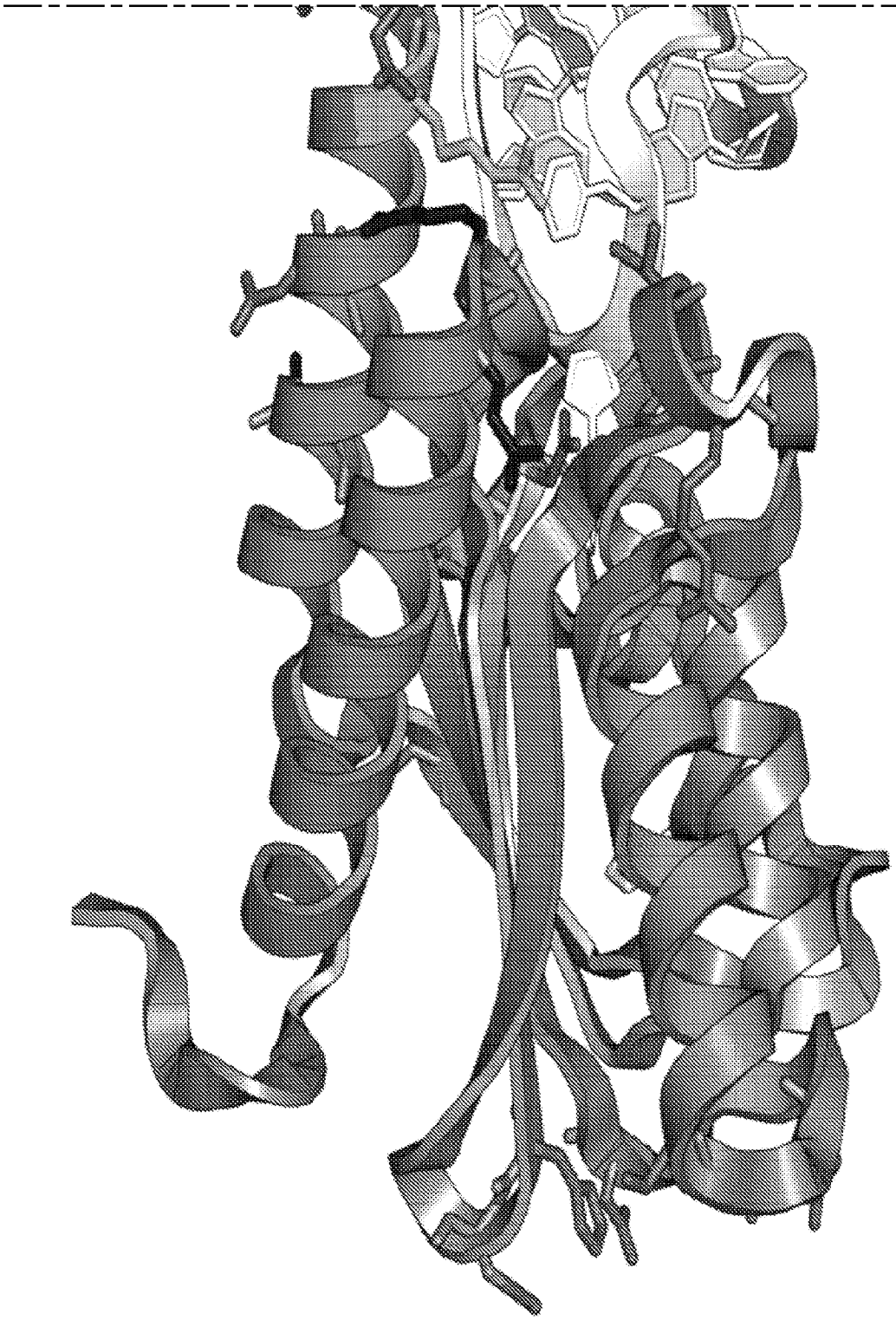


Figure 164 (Continued)

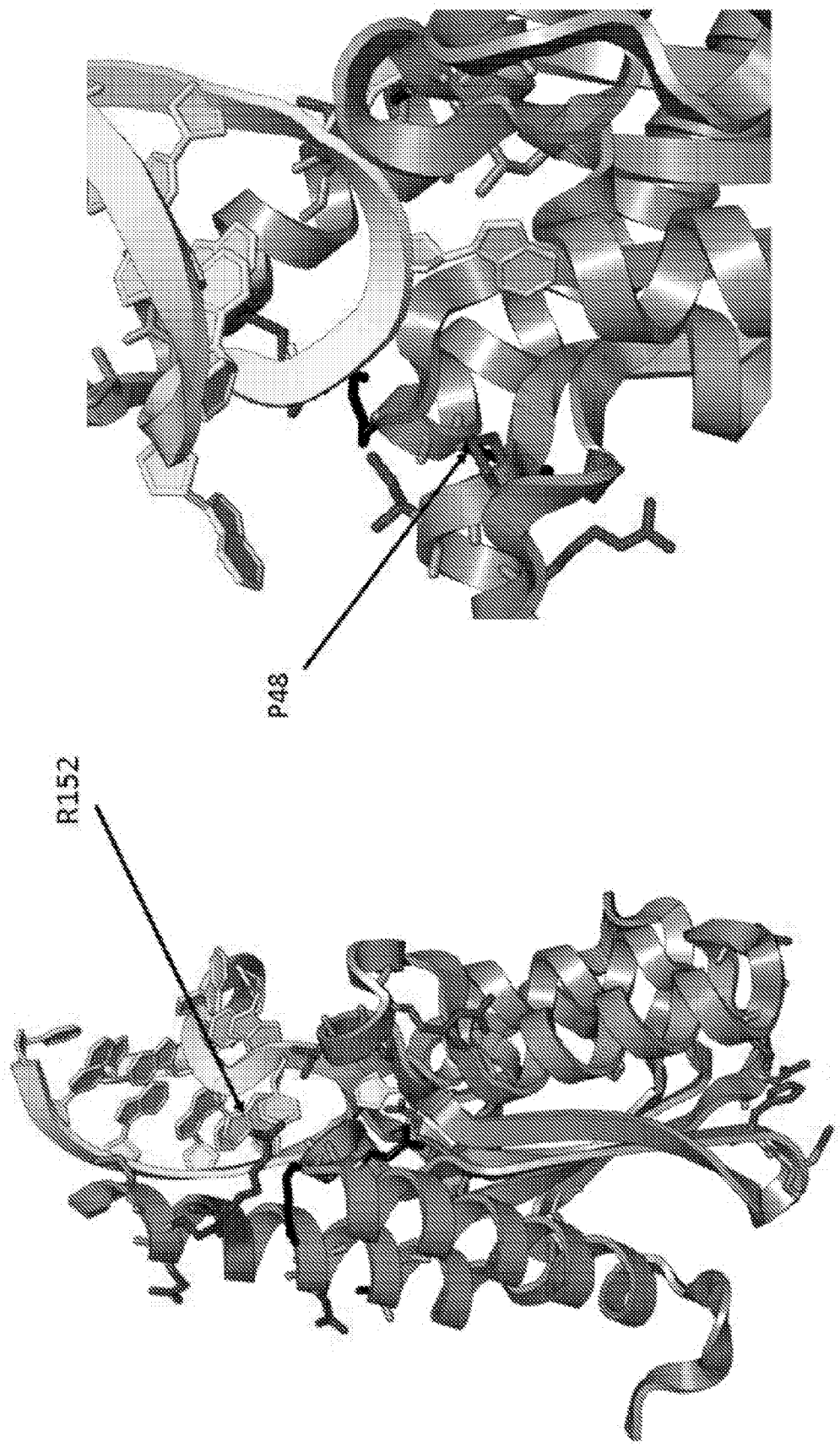



Figure 165

| sgRNA plasmid | protospacer | %editing | ABE | cell line |
|---|---|---|---|---|
| pNMG-510 | GACTC**A**GATAAGATGCTGAGG | <0.15% | pNMG-478 | R196* TP53 (Calu-6) |
| pNMG-511 | GCAT**A**TGTAACAGTTCCTGCA | <0.80% | pNMG-402 | M237I TP53 (T98G) |
| pNMG-512 | GTGC**A**TGTTTGTGCCTGTCC | <0.13% | pNMG-477 | R273H TP53 (NCI-H1975) |

Figure 166

Figure 167

**A>G editing at position 5**

| sgRNA plasmid # | ABE plasmid # | 371 | 402 | 404 | 429 | 430 | 476 | 477 |
|---|---|---|---|---|---|---|---|---|
| pNMG-466 | GAAG**A**(5)TAGAGAATAGACTGCTGG | 8.0% | 5.4% | 6.5% | 7.3% | 7.1% | 13.4% | 14.5% |
| pNMG-467 | GGCT**A**(5)AAGACCATAGACTGTGGG | 5.8% | 3.9% | 4.3% | 6.0% | 5.0% | 9.1% | 11.0% |
| pNMG-469 | GAGT**A**(5)TGAGGCATAGACTGCAGG | 46.8% | 40.6% | 43.6% | 42.7% | 39.7% | 52.4% | 52.5% |
| pNMG-472 | GAGC**A**(5)AAGAGAATAGACTGTAGG | 16.2% | 16.2% | 14.9% | 14.7% | 14.7% | 24.2% | 34.4% |
| pNMG-502 | GGGG**A**(5)CGCGCTGGCTTCCCGGGG | 5.7% | 5.3% | 4.5% | 7.8% | 5.8% | 8.5% | 8.6% |
| pNMG-509 | GCCT**A**(5)GGCAGTGGGGGTGCAGGG | 3.2% | 3.0% | 2.9% | 4.2% | 2.6% | 7.8% | 9.7% |

GAT TAA TAT CAA GAC TAG

Figure 167 (Continued)

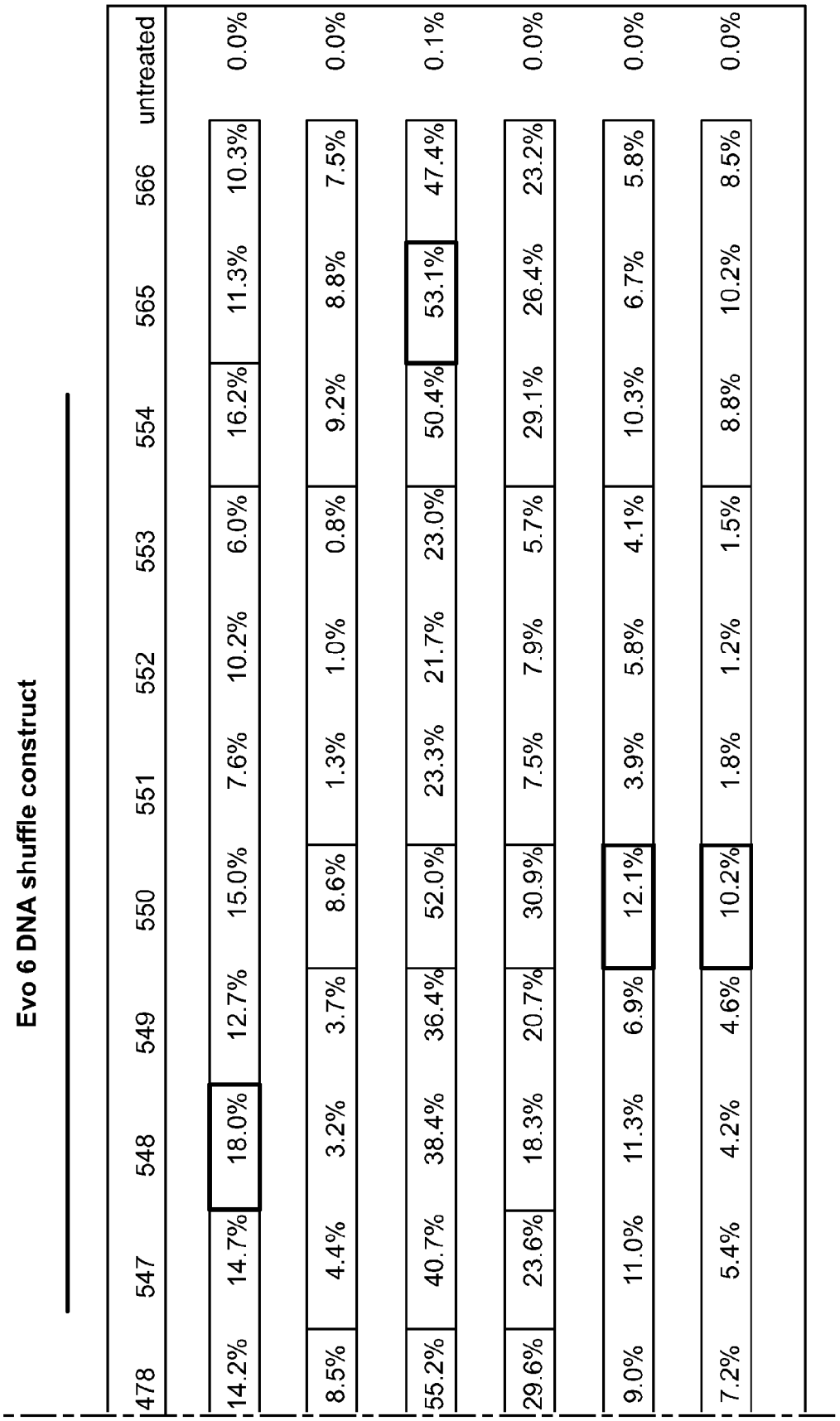
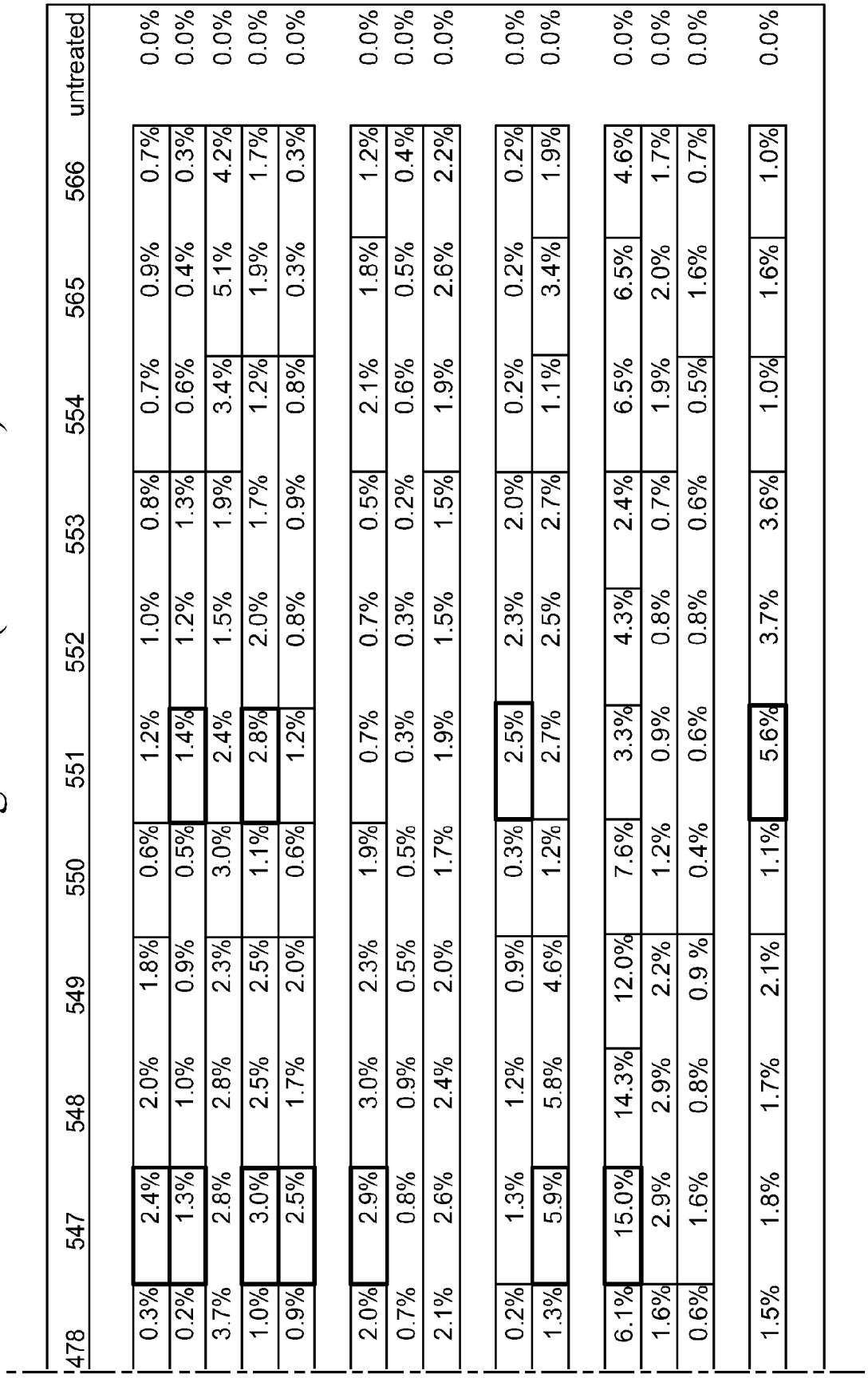


Figure 167 (Continued)

**A>G editing at all other positions**

| | ABE plasmid # | 371 | 402 | 404 | 429 | 430 | 476 | 477 |
|---|---|---|---|---|---|---|---|---|
| sgRNA plasmid # | | | | | | | | |
| pNMG-466 | G**A**(2)AGATAGAGAATAGACTGCTGG | 0.6% | 0.3% | 0.3% | 0.7% | 0.6% | 0.5% | 0.9% |
| | GA**A**(3)GATAGAGAATAGACTGCTGG | 0.6% | 0.2% | 0.3% | 0.5% | 0.5% | 0.4% | 0.3% |
| | GAAGAT**A**(7)GAGAATAGACTGCTGG | 3.0% | 2.5% | 3.1% | 4.8% | 2.8% | 2.9% | 6.7% |
| | GAAGATAG**A**(9)GAATAGACTGCTGG | 0.7% | 0.6% | 0.7% | 1.6% | 1.0% | 0.9% | 2.1% |
| | GAAGATAGAG**A**(11)ATAGACTGCTGG | 0.4% | 0.1% | 0.7% | 0.2% | 0.5% | 0.5% | 0.3% |
| pNMG-467 | GGCTA**A**(6)AGACCATAGACTGTGGG | 2.5% | 1.4% | 1.9% | 2.0% | 2.0% | 1.7% | 1.9% |
| | GGCTAA**A**(7)GACCATAGACTGTGGG | 0.8% | 0.4% | 0.6% | 0.6% | 0.5% | 0.6% | 0.6% |
| | GGCTAAAG**A**(9)CCATAGACTGTGGG | 2.3% | 1.0% | 1.7% | 2.9% | 1.6% | 1.8% | 2.7% |
| pNMG-469 | G**A**(2)GTATGAGGCATAGACTGCAGG | 0.3% | 0.1% | 0.4% | 0.3% | 0.3% | 0.3% | 0.3% |
| | GAGTATG**A**(8)GGCATAGACTGCAGG | 1.4% | 1.8% | 1.5% | 2.5% | 0.9% | 1.1% | 2.4% |
| pNMG-472 | GAGCA**A**(6)AGAGAATAGACTGTAGG | 9.3% | 6.5% | 8.1% | 7.5% | 9.3% | 5.3% | 8.5% |
| | GAGCAA**A**(7)GAGAATAGACTGTAGG | 1.4% | 1.2% | 1.8% | 2.0% | 2.0% | 1.4% | 3.2% |
| | GAGCAAAG**A**(9)GAATAGACTGTAGG | 0.3% | 0.6% | 0.7% | 1.4% | 0.4% | 0.6% | 2.1% |
| pNMG-509 | GCCTAGGC**A**(9)GTGGGGGTGCAGGG | 1.2% | 0.8% | 1.5% | 1.9% | 1.0% | 1.1% | 1.4% |

Figure 167 (Continued)



| 478 | 547 | 548 | 549 | 550 | 551 | 552 | 553 | 554 | 565 | 566 | untreated |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.3% | 2.4% | 2.0% | 1.8% | 0.6% | 1.2% | 1.0% | 0.8% | 0.7% | 0.9% | 0.7% | 0.0% |
| 0.2% | 1.3% | 1.0% | 0.9% | 0.5% | 1.4% | 1.2% | 1.3% | 0.6% | 0.4% | 0.3% | 0.0% |
| 3.7% | 2.8% | 2.8% | 2.3% | 3.0% | 2.4% | 1.5% | 1.9% | 3.4% | 5.1% | 4.2% | 0.0% |
| 1.0% | 3.0% | 2.5% | 2.5% | 1.1% | 2.8% | 2.0% | 1.7% | 1.2% | 1.9% | 1.7% | 0.0% |
| 0.9% | 2.5% | 1.7% | 2.0% | 0.6% | 1.2% | 0.8% | 0.9% | 0.8% | 0.3% | 0.3% | 0.0% |
| 2.0% | 2.9% | 3.0% | 2.3% | 1.9% | 0.7% | 0.7% | 0.5% | 2.1% | 1.8% | 1.2% | 0.0% |
| 0.7% | 0.8% | 0.9% | 0.5% | 0.5% | 0.3% | 0.3% | 0.2% | 0.6% | 0.5% | 0.4% | 0.0% |
| 2.1% | 2.6% | 2.4% | 2.0% | 1.7% | 1.9% | 1.5% | 1.5% | 1.9% | 2.6% | 2.2% | 0.0% |
| 0.2% | 1.3% | 1.2% | 0.9% | 0.3% | 2.5% | 2.3% | 2.0% | 0.2% | 0.2% | 0.2% | 0.0% |
| 1.3% | 5.9% | 5.8% | 4.6% | 1.2% | 2.7% | 2.5% | 2.7% | 1.1% | 3.4% | 1.9% | 0.0% |
| 6.1% | 15.0% | 14.3% | 12.0% | 7.6% | 3.3% | 4.3% | 2.4% | 6.5% | 6.5% | 4.6% | 0.0% |
| 1.6% | 2.9% | 2.9% | 2.2% | 1.2% | 0.9% | 0.8% | 0.7% | 1.9% | 2.0% | 1.7% | 0.0% |
| 0.6% | 1.6% | 0.8% | 0.9 % | 0.4% | 0.6% | 0.8% | 0.6% | 0.5% | 1.6% | 0.7% | 0.0% |
| 1.5% | 1.8% | 1.7% | 2.1% | 1.1% | 5.6% | 3.7% | 3.6% | 1.0% | 1.6% | 1.0% | 0.0% |

# Figure 168

| | | Round 3 | A = 32<br>B = 32 | A = 24<br>B = 24 | A = 24<br>B = 32 |
|---|---|---|---|---|---|
| | ABE plasmid # | 476 | 477 | 555 | 556 |
| sgRNA plasmid # | | | | | |
| pNMG-466 | GAAGA (5) TAGAGAATAGACTGCTGG | 14.0% | 14.5% | 14.9% | 9.2% |
| | GAAGATA (7) GAGAATAGACTGCTGG | 3.1% | 6.7% | 6.2% | 4.2% |
| | GAAGATAGA (9) GAATAGACTGCTGG | 1.0% | 2.1% | 1.0% | 0.8% |
| pNMG-468 | GTCTA (5) GAAAGCTTAGACTGCTGG | 34.8% | 38.2% | 33.5% | 31.8% |
| | GTCTAGA (7) AAGCTTAGACTGCTGG | 2.6% | 3.7% | 2.2% | 3.4% |
| | GTCTAGAA (8) AGCTTAGACTGCTGG | 27.1% | 21.6% | 11.9% | 18.1% |
| | GTCTAGAAA (9) GCTTAGACTGCTGG | 0.7% | 0.9% | 0.0% | 0.6% |
| pNMG-469 | GAGTA (5) TGAGGCATAGACTGCAGG | 38.2% | 44.8% | 42.1% | 33.4% |
| | GAGTATGA (8) GGCATAGACTGCAGG | 0.9% | 1.7% | 1.8% | 1.4% |
| pNMG-472 | GAGCA (5) AAGAGAATAGACTGTAGG | 25.8% | 24.3% | 25.0% | 25.6% |
| | GAGCAA (6) AGAGAATAGACTGTAGG | 7.1% | 6.1% | 7.8% | 6.2% |
| | GAGCAAA (7) GAGAATAGACTGTAGG | 1.8% | 1.8% | 2.1% | 1.2% |
| | GAGCAAAGA (9) GAATAGACTGTAGG | 0.5% | 1.5% | 0.3% | 0.5% |
| pNMG-509 | GCCTA (5) GGCAGTGGGGGTGCAGGG | 10.7% | 10.9% | 20.7% | 11.7% |
| | GCCTAGGCA (9) GTGGGGGTGCAGGG | 1.5% | 2.3% | 0.6% | 0.9% |
| pNMG-539 | GGGGA (5) CTTTGGGAGGTGATCAGG | 13.8% | 14.2% | 12.5% | 9.7% |

*pNMG-558 transfection needs to be repeated

Figure 168 (Continued)

new linker ABE

| A = 24 B = 40 | A = 32 B = 24 | A = 32 B = 40 | A = 40 B = 24 | A = 40 B = 32 | | |
|---|---|---|---|---|---|---|
| 557 | 558 | 559 | 560 | 561 | 562 | untreated |
| 9.1% | 0.7% | 11.2% | 16.7% | 12.7% | 10.0% | 0.0% |
| 4.9% | 0.3% | 5.7% | 7.1% | 6.0% | 5.6% | 0.0% |
| 1.7% | 0.0% | 2.2% | 1.2% | 1.7% | 1.7% | 0.0% |
| 24.0% | 1.0% | 26.1% | 29.5% | 40.1% | 31.4% | 0.0% |
| 2.8% | 0.0% | 2.8% | 1.2% | 0.8% | 3.3% | 0.0% |
| 14.6% | 0.5% | 16.7% | 12.8% | 27.8% | 18.6% | 0.0% |
| 0.7% | 0.0% | 0.8% | 0.0% | 0.9% | 1.1% | 0.0% |
| 39.5% | 3.7% | 38.5% | 41.8% | 41.6% | 40.0% | 0.1% |
| 2.3% | 0.1% | 1.8% | 1.7% | 2.0% | 2.6% | 0.0% |
| 22.5% | 2.1% | 22.1% | 36.1% | 26.9% | 22.7% | 0.0% |
| 4.7% | 0.4% | 4.5% | 8.6% | 5.5% | 5.9% | 0.0% |
| 1.4% | 0.1% | 1.4% | 1.9% | 1.0% | 2.0% | 0.0% |
| 1.1% | 0.1% | 1.1% | 0.1% | 1.3% | 1.2% | 0.0% |
| 9.1% | 1.4% | 10.5% | 16.5% | 12.5% | 8.9% | 0.6% |
| 1.5% | 0.2% | 2.7% | 1.2% | 1.3% | 1.5% | 0.0% |
| 10.8% | 0.5% | 13.5% | 20.8% | 23.6% | 7.6% | 0.6% |

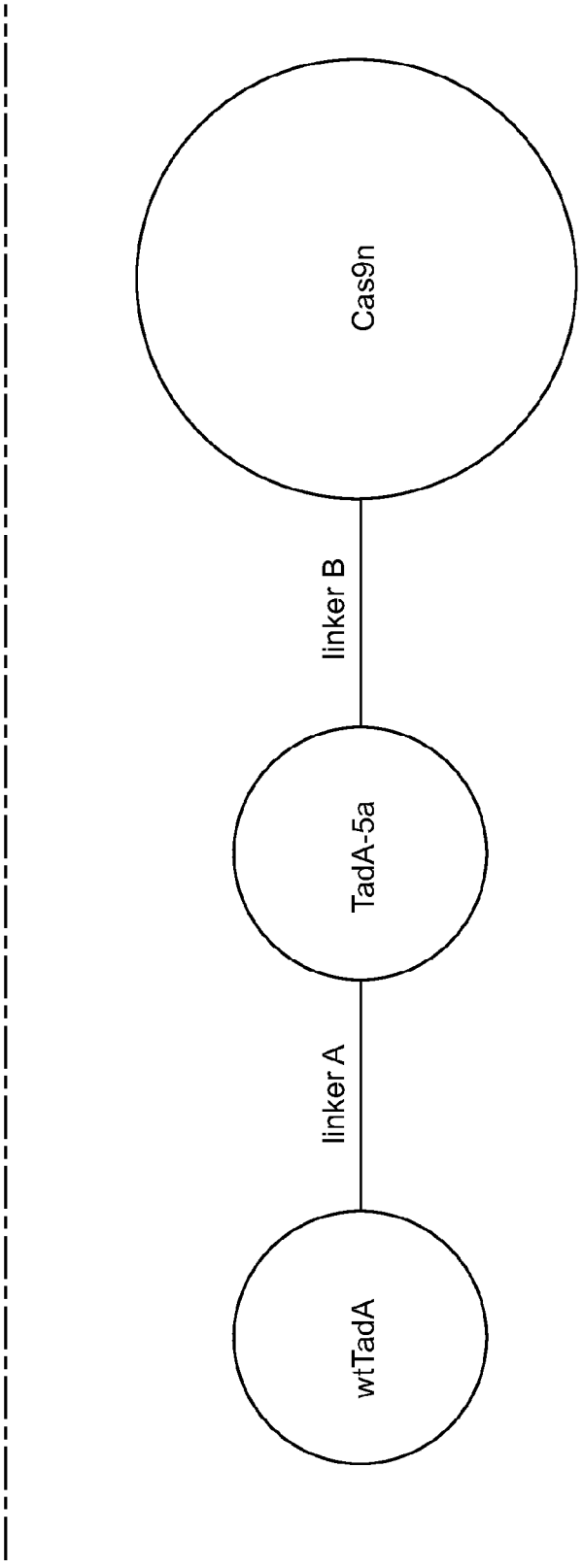



Figure 168 (Continued)

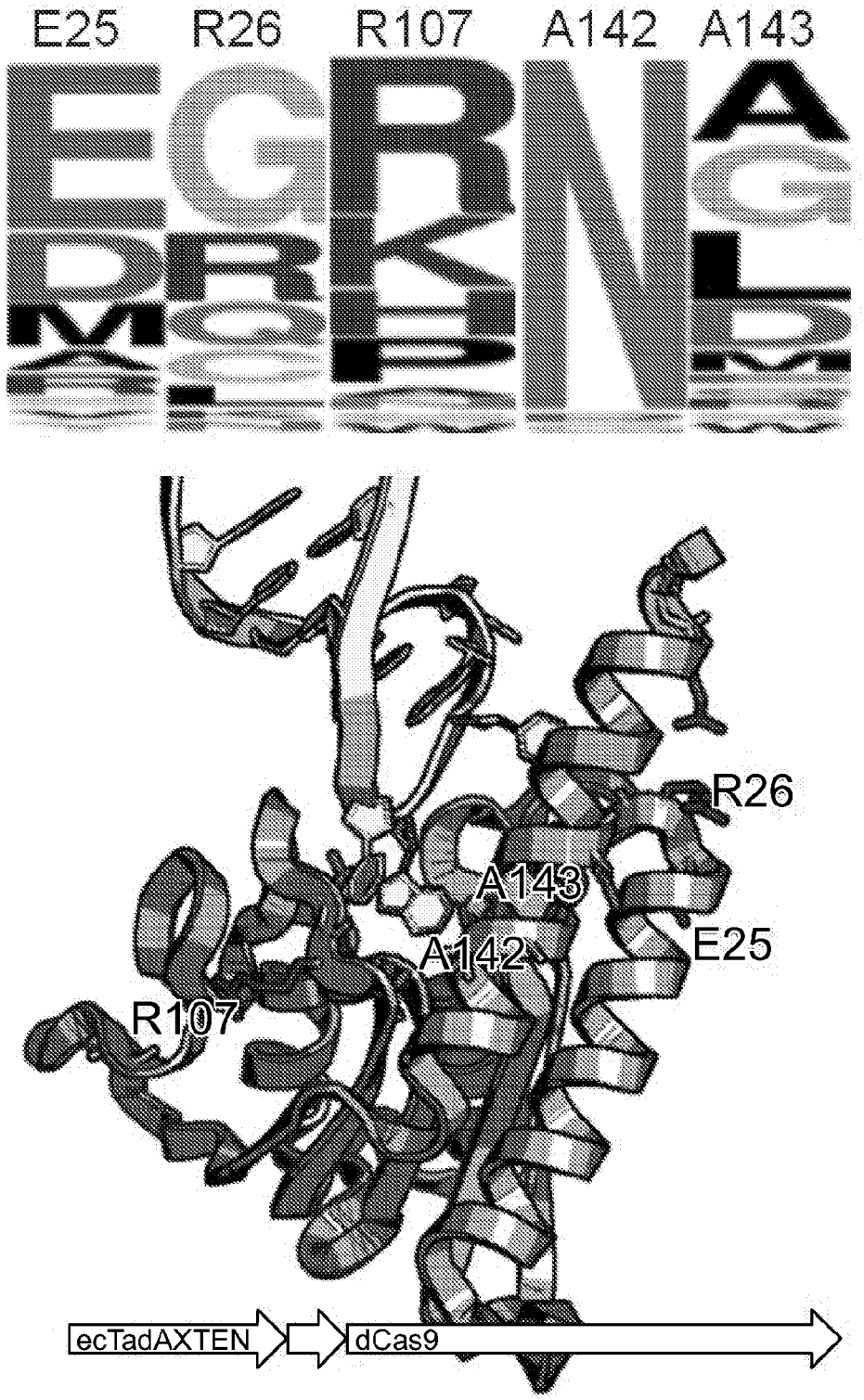

E25
R26
R107
A142
A143
R26
A143
A142
E25
R107
ecTadAXTEN
dCas9

Figure 169

| Site | Protospacer and PAM sequence | Evo Round #2 (*in cis* homodimer) | Evo Round #3 (*in cis* homodimer) | Evo Round #4 A142N (*in cis* homodimer) | Evo Round #4 R25G +A142N (*in cis* homodimer) | Evo Round #4 R25G + R107H +A142N +A143D (*in cis*homodimer) | Evo Round #4 A142N (*in trans* heterodimer) | Evo Round #4 R25G +A142N (*in trans* heterodimer) | Evo Round #4 R25G + R107H +A142N +A143D (*in trans*heterodimer) |
|---|---|---|---|---|---|---|---|---|---|
| HEK2 | GAACACAAAGCATAGACTGCTGG | 54.0 | 65.4 | 48.4 | 52.9 | 40.3 | 23.9 | 23.4 | 17.7 |
| HEK2-2 | GAATACTAAGCATAGACTCCAGG | 29.1 | 50.1 | 24.5 | 23.8 | 11.3 | 10.5 | 13.9 | 8.3 |
| HEK2-3 | GTAAACAAAGCATAGACTGAGGG | 10.4 | 20.1 | 8.2 | 11.0 | 3.5 | 3.1 | 3.9 | 2.2 |
| HEK2-6 | GAAGACCAAGGATAGACTGCTGG | 4.4 | 7.6 | 4.6 | 5.5 | 1.4 | 1.2 | 1.3 | 0.6 |
| HEK2-7 | GAAAACAAATCATTGACTGCAGG | 0.3 | 0.7 | 0.3 | 0.2 | 0.1 | 0.1 | 0.1 | 0.0 |
| HEK2-10 | GAACATAAAGAATAGAATGATGG | 6.3 | 21.4 | 3.6 | 6.3 | 2.0 | 1.5 | 1.1 | 0.7 |

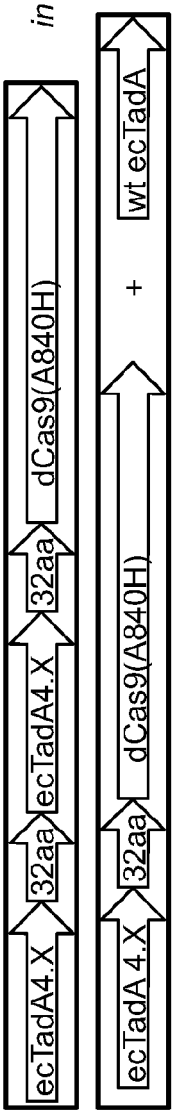
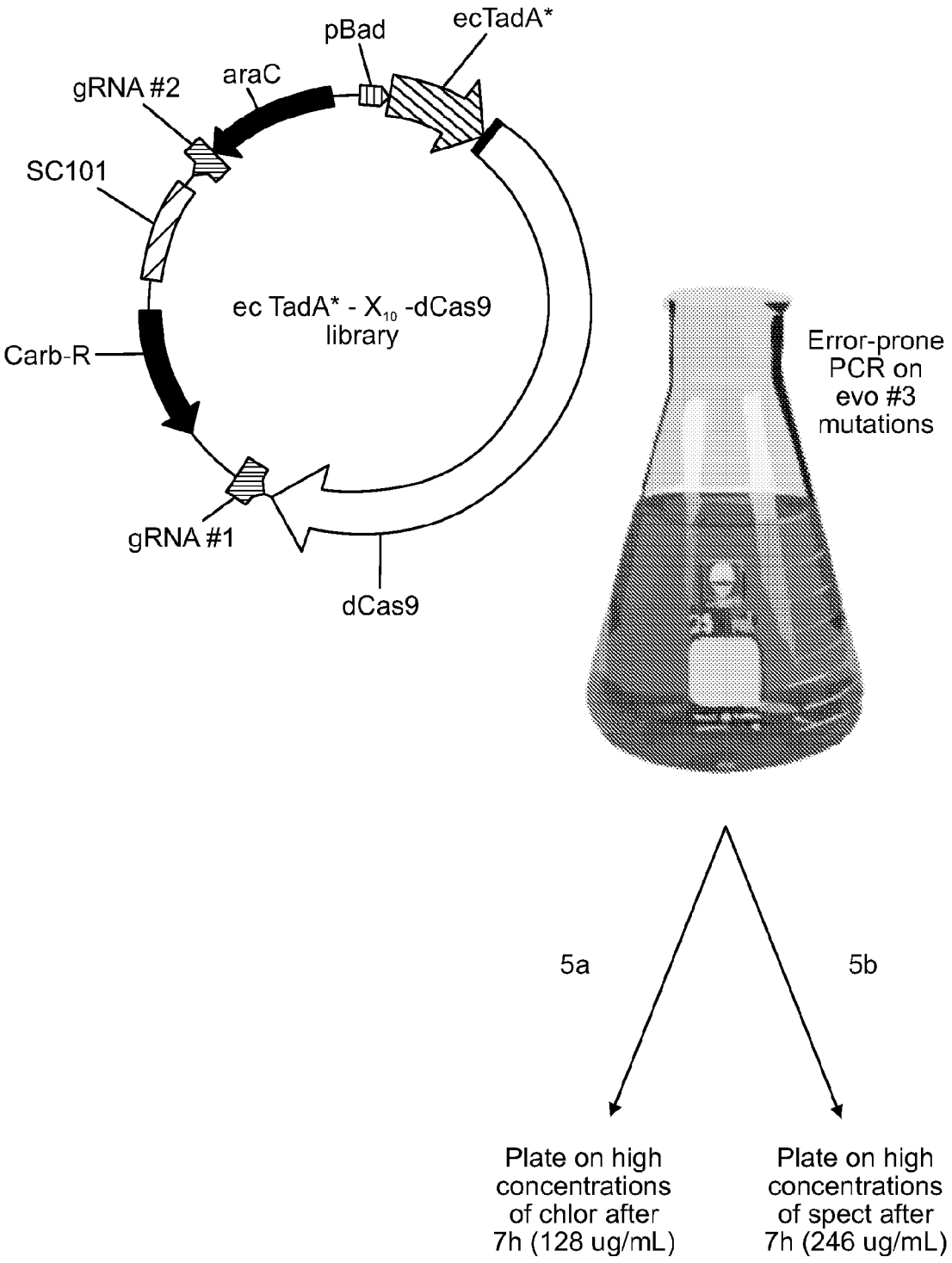



Figure 170

# Figure 171


pBad
ecTadA*
araC
gRNA #2
SC101
ec TadA* - X10 -dCas9
library
Carb-R
gRNA #1
dCas9
Error-prone
PCR on
evo #3
mutations
5a
5b
Plate on high
concentrations
of chlor after
7h (128 ug/mL)
Plate on high
concentrations
of spect after
7h (246 ug/mL)

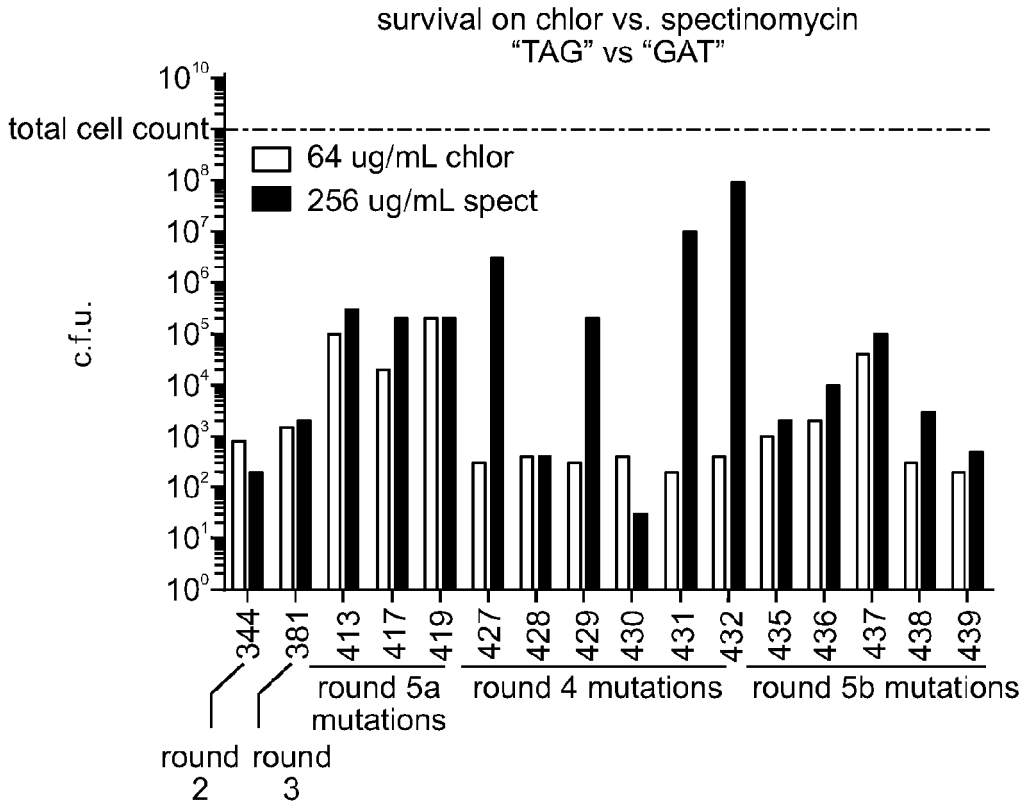



Figure 171 (Continued)

Figure 172

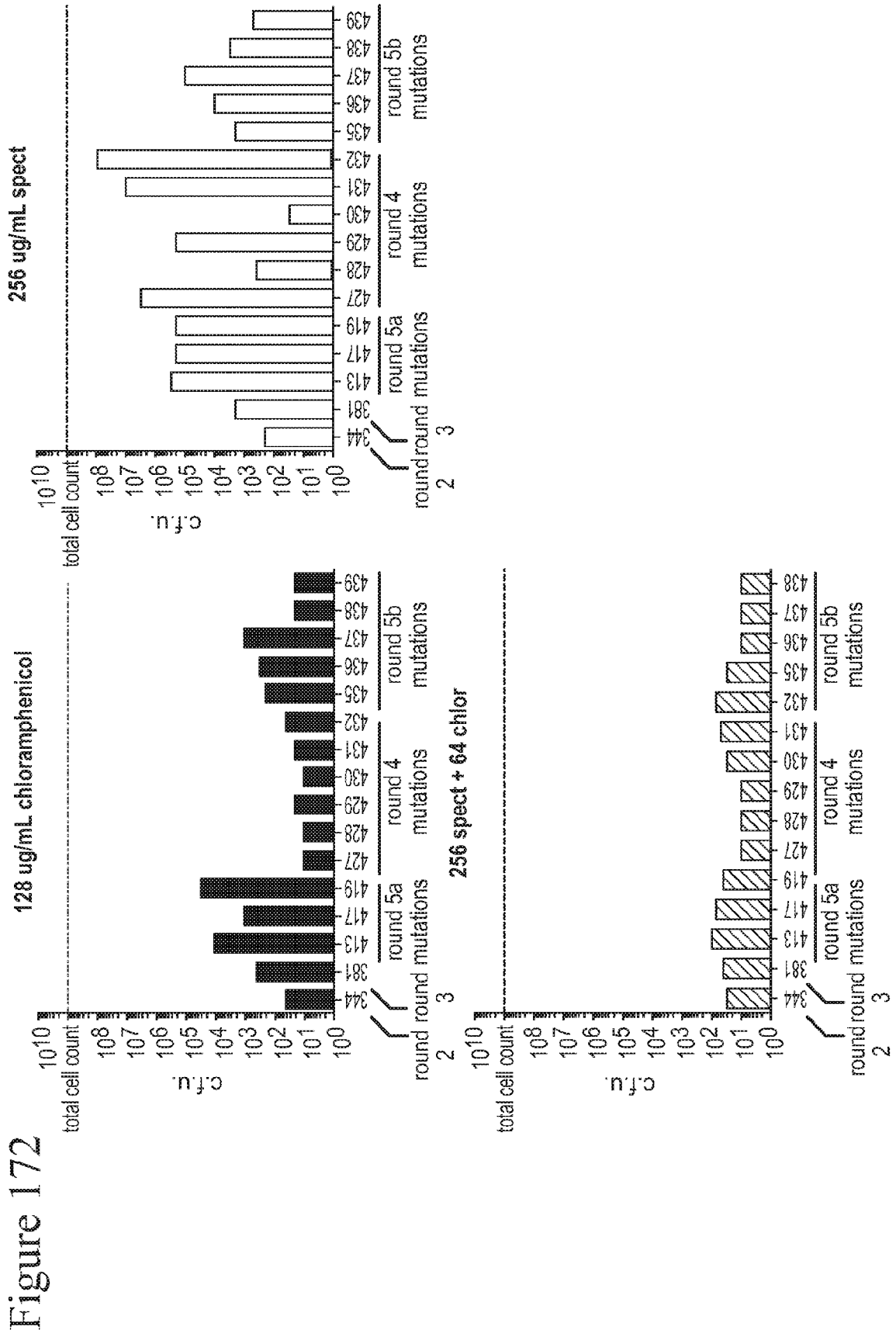

256 ug/mL spect
128 ug/mL chloramphenicol
256 spect + 64 chlor
total cell count
c.f.u.
round 2
round 3
round 5a mutations
round 4 mutations
round 5b mutations

Figure 173

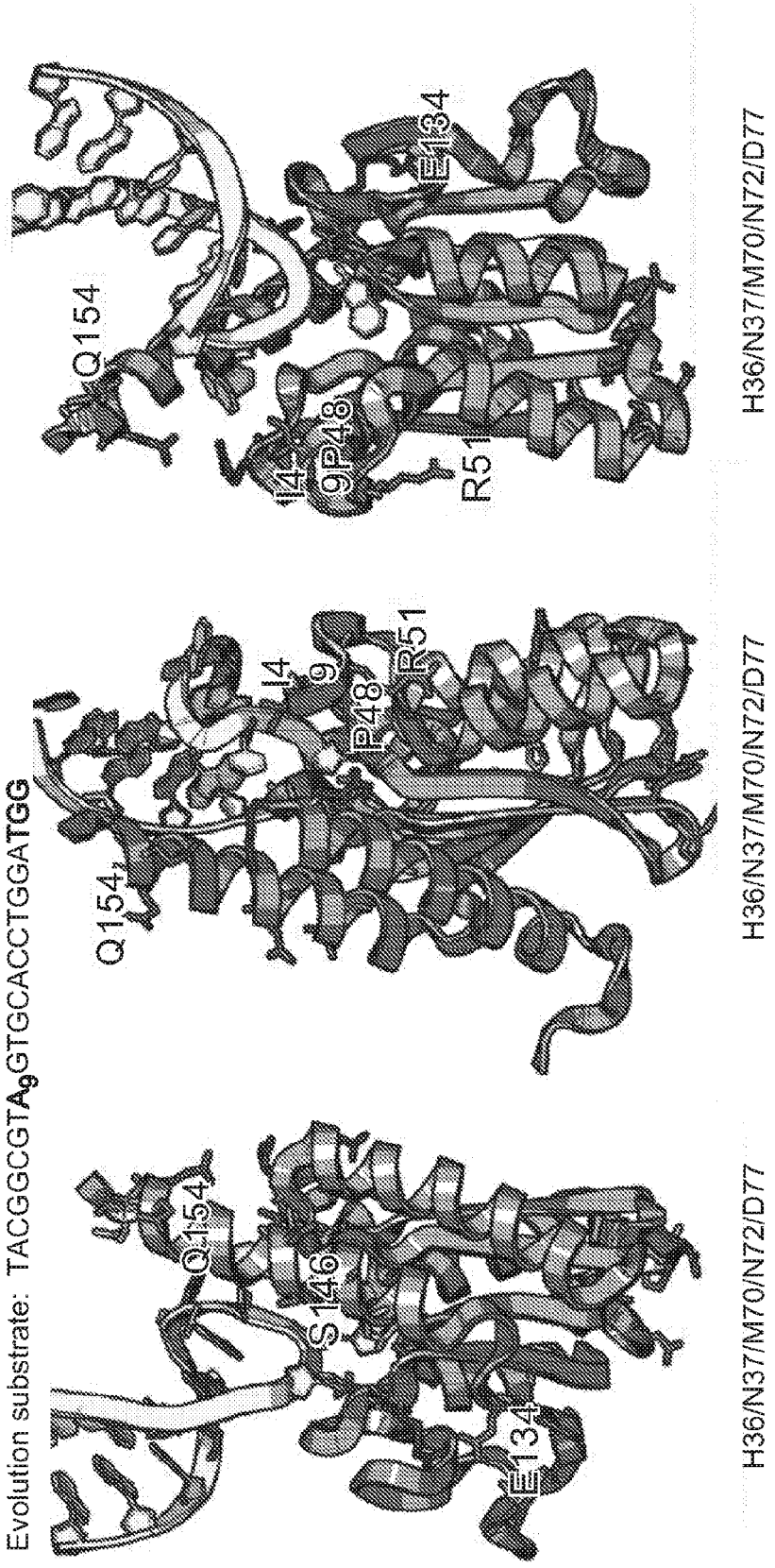

Evolution substrate: TACGGCGTA9GTGCACCTGGATGG
Q154
S146
E134
H36/N37/M70/N72/D77
Q154
I4
9
P48
R51
H36/N37/M70/N72/D77
Q154
I4
9P48
R51
E134
H36/N37/M70/N72/D77

Figure 174

| Site | Protospacer and PAM sequence | L84F **A106V D108N** H123Y **D147Y E155V** I156F = 3rd round | 3rd round + **H36L R51L S146C K157N** | 3rd round + **N37T P48T I49V M70L** | 3rd round + **H36L P48L E134G** | 3rd round + **R51L K157N** |
|---|---|---|---|---|---|---|
| HEK2 | GAA**C**A**C**AAAGCATAGACTGCT**G**G | 62.7 | 62.6 | 56.0 | 63.4 | 66.7 |
| HEK2-2 | GAA**TAC**TAAGCATAGACT**C**CA**G**G | 27.7 | 40.6 | 35.3 | 31.9 | 45.8 |
| HEK2-3 | GT**AA**ACAAAGCATAGACTGA**G**GG | 14.5 | 20.2 | 14.6 | 20.6 | 19.6 |
| HEK2-6 | GAA**G**AC**C**AAG**G**ATAGACTGCT**G**G | 3.5 | 7.3 | 6.9 | 6.5 | 7.4 |
| HEK2-7 | GAAA**C**AAA**T**CATTGACTGCA**G**G | 0.4 | 0.9 | 0.6 | 1.0 | 0.8 |
| HEK2-10 | GAAC**AT**AAAGA**A**TAGAATGA**T**GG | 11.2 | 24.8 | 22.1 | 22.0 | 22.5 |

# Figure 175

| Site | Protospacer and PAM sequence | Evo Round #3 (*in cis* homodimer) | Round #5a constructs | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 402 | 403 | 404 | 405 | 406 | 407 | 408 | 409 | 410 | 411 | 412 |
| HEK2 | GAAC**A**CAAAGCATAGACTGC**TGG** | 54.7 | 64.9 | 56.3 | 72.4 | 66.9 | 58.9 | 64.0 | 49.3 | 52.5 | 54.5 | 58.9 | 59.4 |
| HEK2 GSQ | GAAC**A**CAA**T**GCATAGA**T**TGC**TGG** | 18.6 | 36.9 | 24.1 | 33.9 | 29.5 | 27.6 | 28.0 | 15.5 | 16.2 | 28.2 | 28.2 | 32.1 |
| HEK2-1 | GAA**AAA**AAAGCA**G**AGACTGC**TGG** | 0.2 | 0.3 | 0.2 | 0.4 | 0.4 | 0.4 | 0.9 | 0.2 | 0.1 | 0.2 | 0.4 | 0.5 |
| HEK2-2 | GAA**TA**C**T**AAGCATAGACT**C**C**AGG** | 29.0 | 44.2 | 30.2 | 40.7 | 38.4 | 31.7 | 35.9 | 28.5 | 26.4 | 35.8 | 41.5 | |
| HEK2-3 | GT**AA**ACAAAGCATAGACTG**AGGG** | 14.7 | 15.8 | 10.8 | 14.1 | 14.3 | 11.6 | 16.3 | 14.4 | 8.9 | 14.2 | 16.3 | 18.3 |
| HEK2-4 | G**G**AC**A**CAAAGC**T**TAGACT**C**C**AGG** | 32.7 | 41.7 | 33.6 | 41.5 | 39.8 | 33.1 | 31.2 | 35.7 | 29.6 | 34.8 | 38.0 | 37.0 |
| HEK2-6 | GAA**GA**C**C**AAG**G**ATAGACTGC**TGG** | 4.3 | 6.0 | 5.5 | 4.4 | | 7.5 | 7.9 | 3.5 | 4.6 | 7.0 | 6.5 | 3.8 |
| HEK2-7 | GAA**AA**CAAA**T**CA**TT**GACTGC**AGG** | 0.6 | | 1.0 | 1.3 | 1.3 | 1.0 | 1.8 | 0.7 | 0.9 | 0.9 | 1.5 | 1.0 |
| HEK2-8 | GA**T**C**A**CAAAGCAT**G**GACTG**AAGG** | 25.9 | 37.7 | 25.3 | 36.8 | 32.5 | 28.3 | 29.3 | 25.0 | 28.7 | 36.4 | 38.8 | 32.9 |
| HEK2-10 | GAAC**AT**AAAG**A**ATAGA**A**TG**ATGG** | 11.2 | 14.6 | 14.3 | 11.9 | 11.3 | 11.1 | 20.5 | 9.2 | 9.1 | 14.4 | 16.2 | 13.9 |



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Figure 176

| HEK2 | | G | G | $A_3$ | $A_4$ | C | $A_6$ | C | $A_8$ | $A_9$ | $A_{10}$ | G | C | A | T | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| L84F A106V D108N H123Y D147Y E155V I156F =3rd round 402 | A | 0.0 | 0.0 | 99.0 | 63.6 | 0.1 | 57.9 | 0.0 | 96.9 | 89.9 | 98.9 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.9 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 1.0 | 36.4 | 0.0 | 42.0 | 0.0 | 3.1 | 10.1 | 1.1 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + H36L R51L S146C K157N 402 | A | 0.0 | 0.1 | 98.6 | 57.5 | 0.0 | 39.0 | 0.0 | 91.7 | 90.3 | 98.4 | 0.0 | 0.8 | 99.8 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.8 | 0.0 | 99.1 | 0.0 | 0.0 | 0.0 | 0.0 | 99.2 | 0.0 | 0.1 | 0.0 |
| | G | 100.0 | 99.9 | 1.4 | 42.5 | 0.1 | 61.0 | 0.0 | 8.2 | 9.7 | 1.6 | 100.0 | 0.0 | 0.2 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.9 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 99.9 | 0.0 |
| 3rd round + N37T P48T I49V M70L 403 | A | 0.0 | 0.0 | 98.4 | 56.0 | 1.4 | 52.3 | 0.0 | 94.1 | 83.6 | 95.1 | 0.1 | 0.0 | 100.0 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 95.1 | 0.0 | 99.4 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 1.6 | 44.0 | 1.0 | 47.6 | 0.5 | 5.9 | 16.3 | 4.9 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 2.4 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + N37S K161T 404 | A | 0.0 | 0.0 | 99.4 | 63.0 | 0.1 | 47.6 | 0.0 | 95.6 | 89.4 | 98.7 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.8 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 0.6 | 37.0 | 0.0 | 52.4 | 0.0 | 4.4 | 10.6 | 1.3 | 100.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + H36L Q154H 405 | A | 0.0 | 0.0 | 97.9 | 40.9 | 0.1 | 37.6 | 0.0 | 93.3 | 81.7 | 98.0 | 0.0 | 0.0 | 99.6 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.8 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 2.1 | 59.0 | 0.1 | 62.4 | 0.0 | 6.7 | 18.2 | 2.0 | 100.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + N72S S146R 406 | A | 0.0 | 0.0 | 96.4 | 31.6 | 0.1 | 40.9 | 0.0 | 92.3 | 85.6 | 97.8 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.7 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 3.6 | 68.3 | 0.0 | 59.1 | 0.0 | 7.7 | 14.4 | 2.2 | 100.0 | 0.0 | 0.1 | 100.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |

Figure 177

| HEK2 | | G | G | $A_3$ | $A_4$ | C | $A_6$ | C | $A_8$ | $A_9$ | $A_{10}$ | G | C | A | T | A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3rd round + H36L P48L E134G 407 | A | 0.0 | 0.0 | 96.2 | 45.7 | 0.4 | 55.6 | 0.0 | 93.8 | 83.7 | 96.4 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 98.3 | 0.0 | 99.8 | 0.0 | 0.0 | 0.0 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 3.8 | 54.2 | 0.3 | 44.3 | 0.1 | 6.2 | 16.2 | 3.6 | 99.9 | 0.0 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 1.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + H36L K157N 408 | A | 0.0 | 0.0 | 99.3 | 71.5 | 0.1 | 61.7 | 0.0 | 97.6 | 93.9 | 99.3 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.8 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 0.7 | 28.5 | 0.1 | 38.3 | 0.0 | 2.4 | 6.0 | 0.7 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + H36L S146C 409 | A | 0.0 | 0.0 | 98.7 | 58.2 | 0.0 | 55.0 | 0.0 | 93.6 | 87.5 | 98.5 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.8 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 1.3 | 41.8 | 0.0 | 45.0 | 0.0 | 6.4 | 12.5 | 1.5 | 100.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + S146R K161T 410 | A | 0.0 | 0.0 | 97.7 | 40.5 | 0.1 | 41.8 | 0.0 | 91.3 | 88.3 | 98.3 | 0.0 | 0.0 | 99.9 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.7 | 0.0 | 99.9 | 0.0 | 0.1 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 2.3 | 59.5 | 0.1 | 58.2 | 0.1 | 8.7 | 11.7 | 1.7 | 100.0 | 0.0 | 0.1 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + N37S R51H D77G 411 | A | 0.0 | 0.0 | 95.6 | 48.0 | 0.1 | 35.7 | 0.0 | 94.4 | 90.1 | 98.8 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.8 | 0.0 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 4.4 | 52.0 | 0.0 | 64.3 | 0.1 | 5.5 | 9.9 | 1.2 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| 3rd round + R51L K157N 412 | A | 0.0 | 0.0 | 98.9 | 55.0 | 0.1 | 39.2 | 0.0 | 94.9 | 90.1 | 99.0 | 0.0 | 0.0 | 100.0 | 0.0 | 100.0 |
| | C | 0.0 | 0.0 | 0.0 | 0.0 | 99.7 | 0.1 | 99.9 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 | 0.0 | 0.0 |
| | G | 100.0 | 100.0 | 1.1 | 45.0 | 0.1 | 60.7 | 0.1 | 5.1 | 9.9 | 1.0 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | T | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 | 0.0 |

HEK2 :GAACACAAAGCATAGACTGCGGG
AAA: GAAAAAAAAGCAGAGACTGCTGG
TAC: GAATACTAAGCATAGACTCCAGG
AAC: GTAAACAAAGCATAGACTGAGGG
GAC: GAAGACCAAGGATAGACTGCTGG
CAT: GAACATAAAGAATAGAATGATGG

GAG: GGAGAGAGAGCATAGACTGCTGG
GAT: GAAGATAGAGAATAGACTGCTGG
GAA: GTAGAAAAAGTATAGACTGCAGG
AAG: GTCAAGAAAGCAGAGACTGCCGG
TAT: GAGTATGAGGCATAGACTGCAGG
TAG: GTCTAGAAAGCTTAGACTGCTGG
CAG: GGACAGGCAGCATAGACTGTGGG
CAA: GAGCAAAGAGAATAGACTGTAGG
TAA: GGCTAAAGACCATAGACTGTGGG
AAT: GGGAATAAATCATAGAATCCTGG

Figure 178

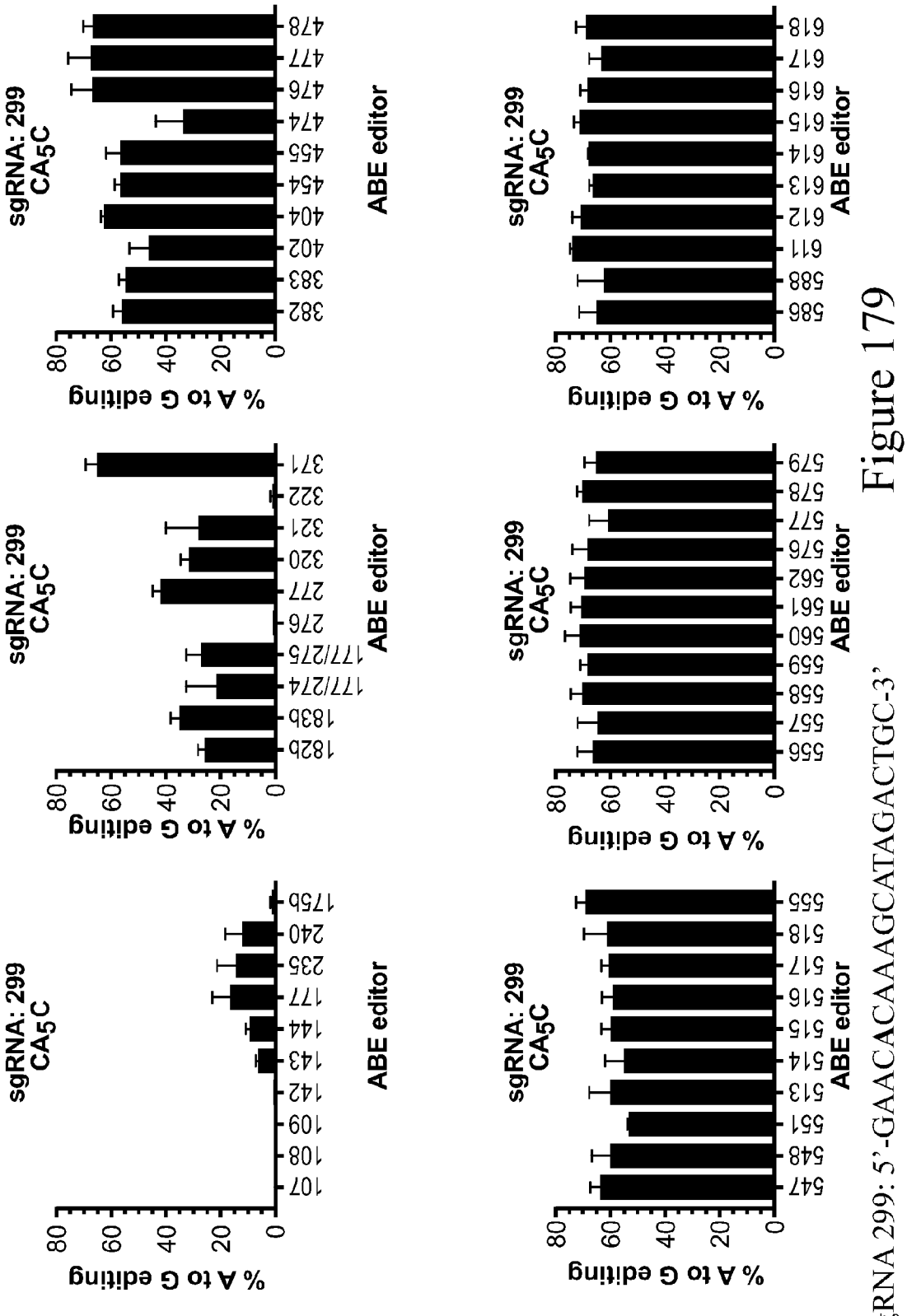



Figure 179

sgRNA 299: 5'-GAACACAAAGCATAGACTGC-3'

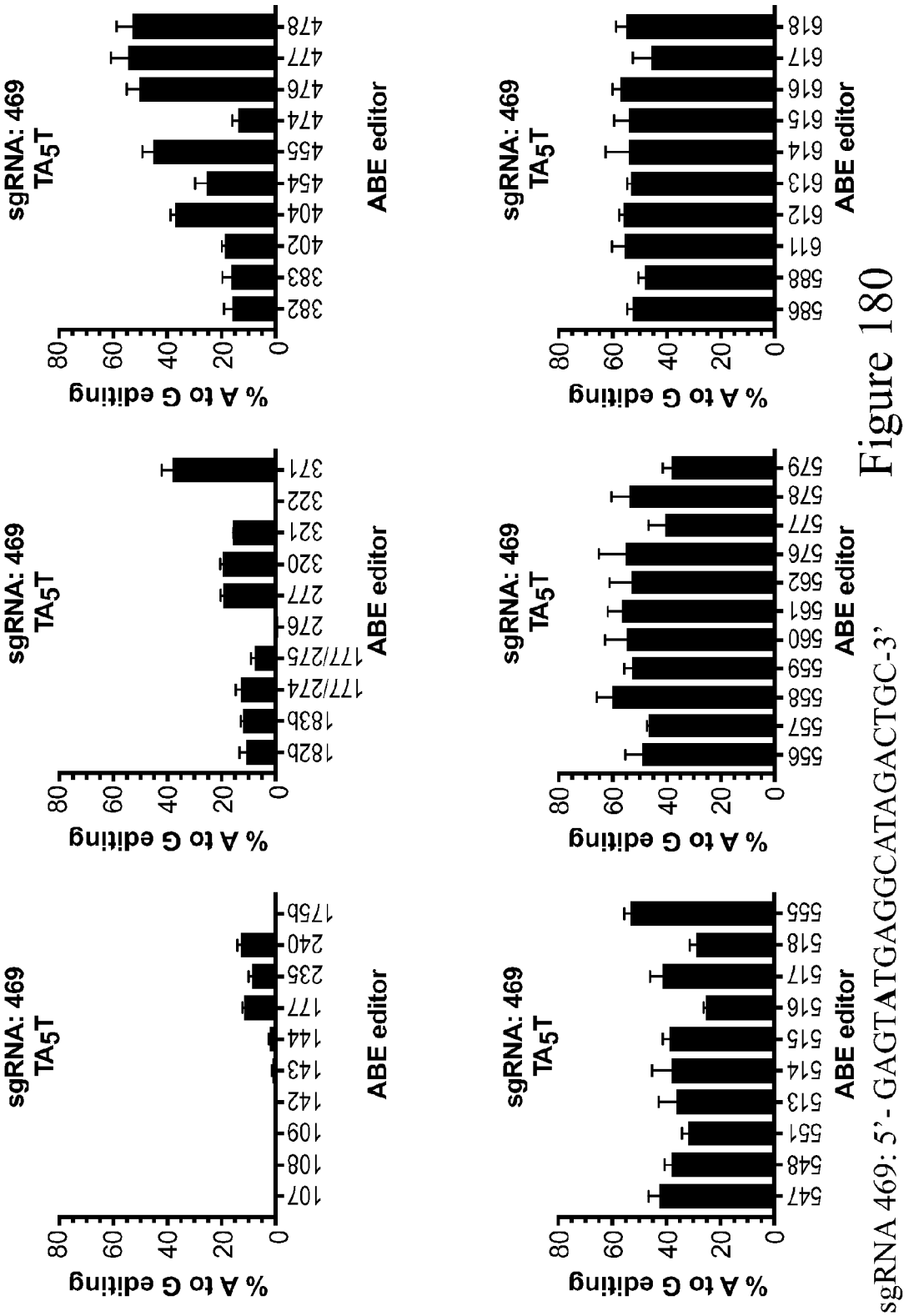



Figure 180

sgRNA 469: 5'- GAGTATGAGGCATAGACTGC-3'

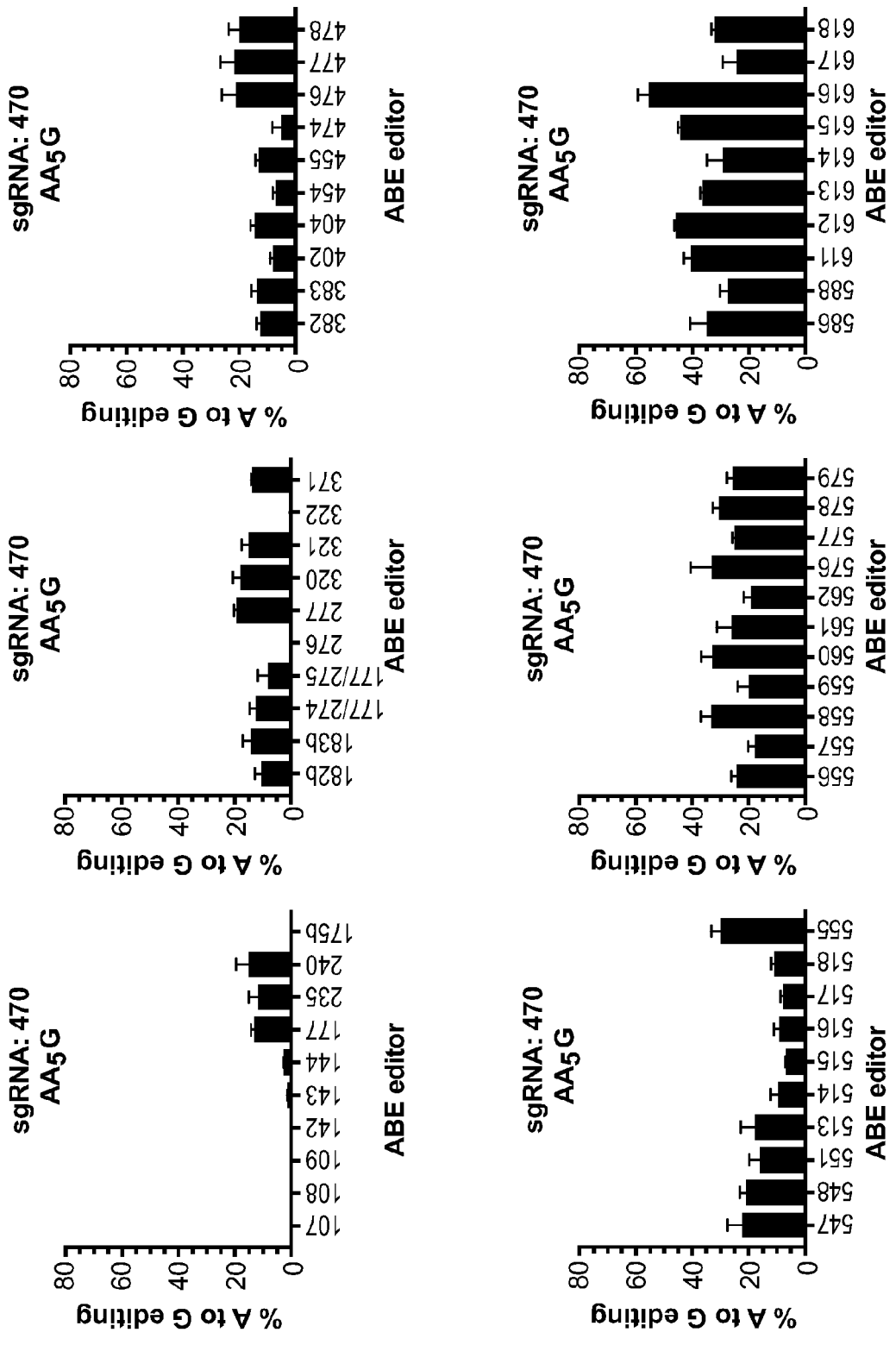



Figure 181

sgRNA 470: 5',-GTCAAGAAAGCAGAGACTGC-3',

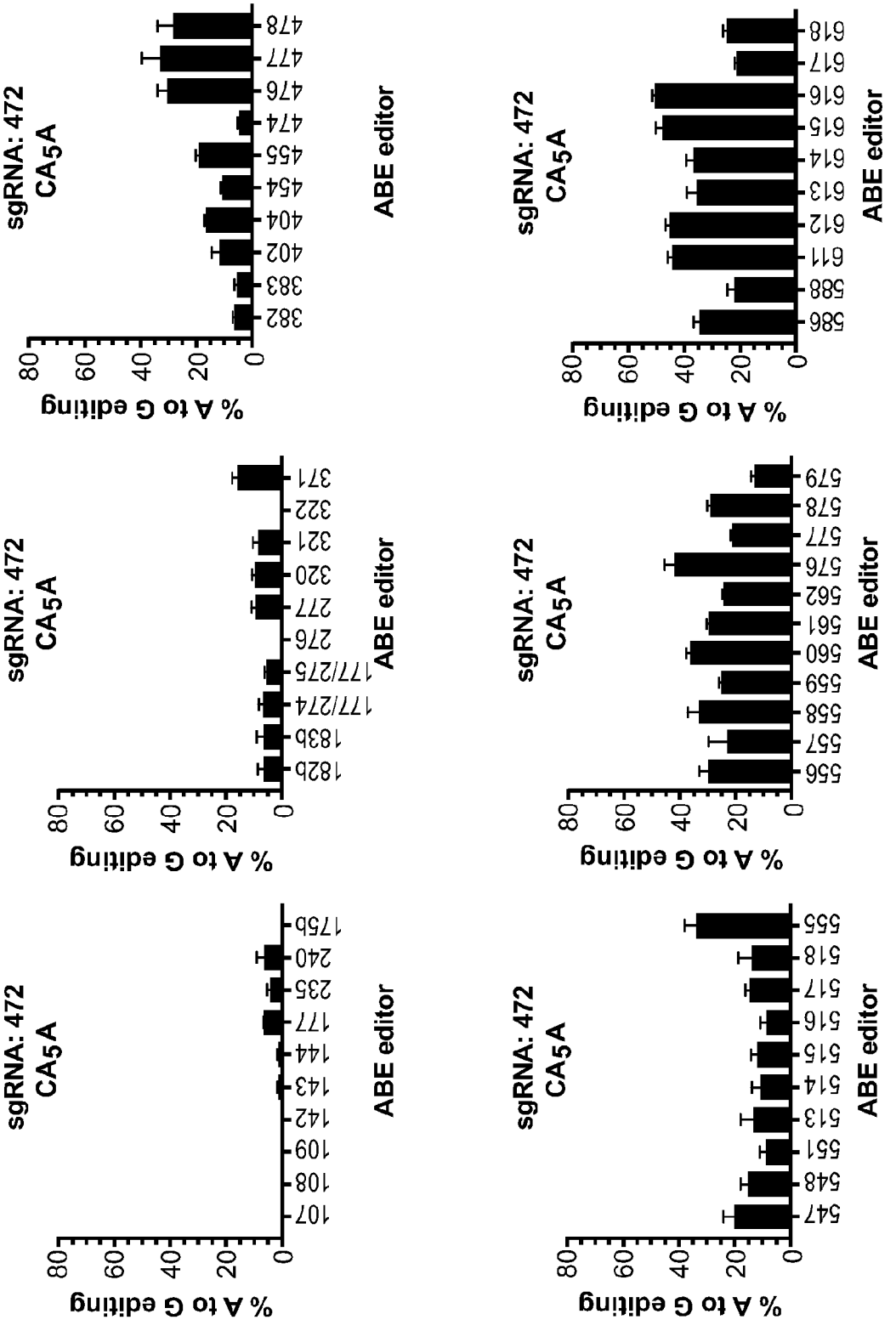



Figure 182

sgRNA 472: 5',-GAGCAAAGACAATACACTGT-3',

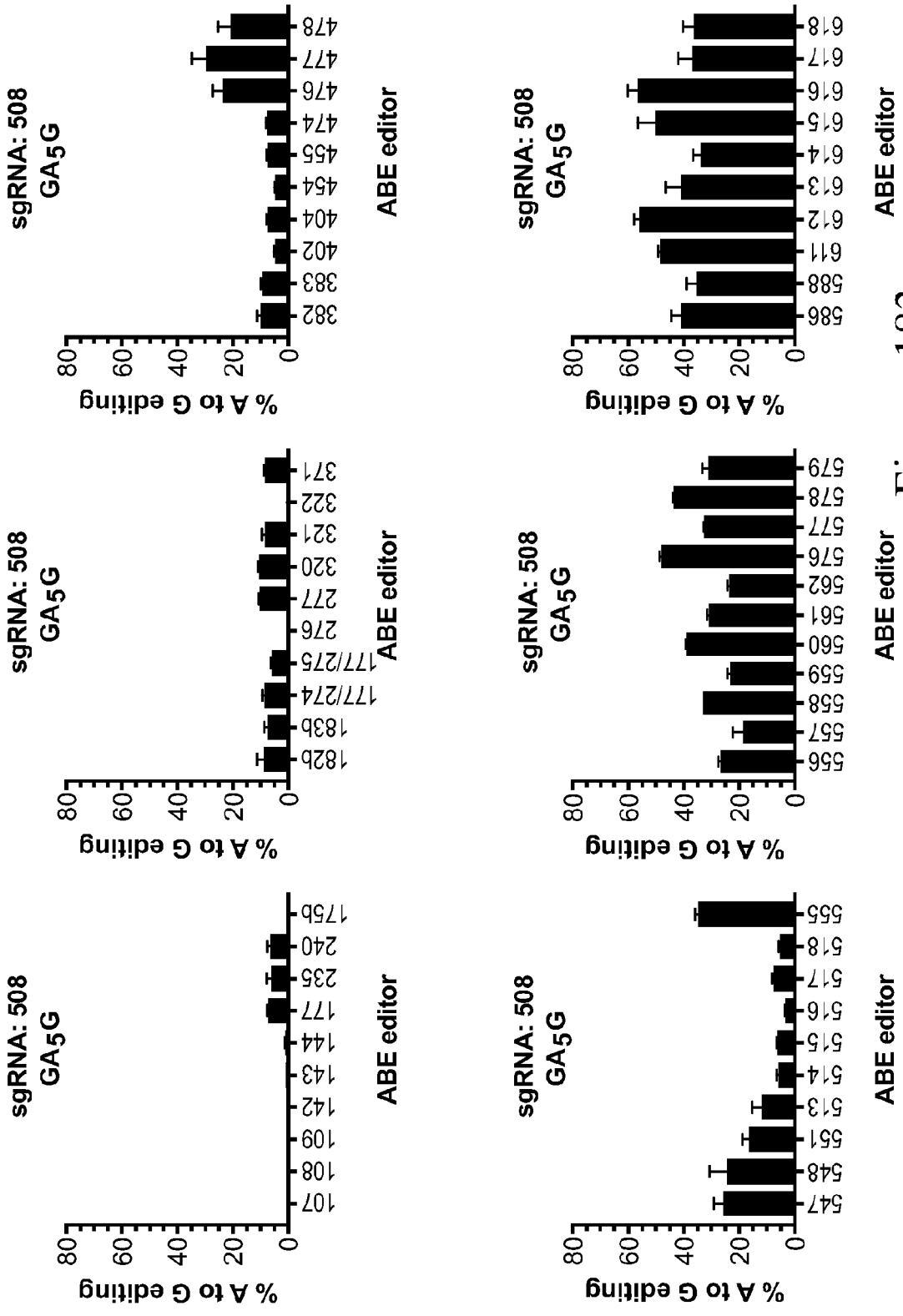



Figure 183

sgRNA 508: 5'-GATGAGATAATGATGAGTCA-3'

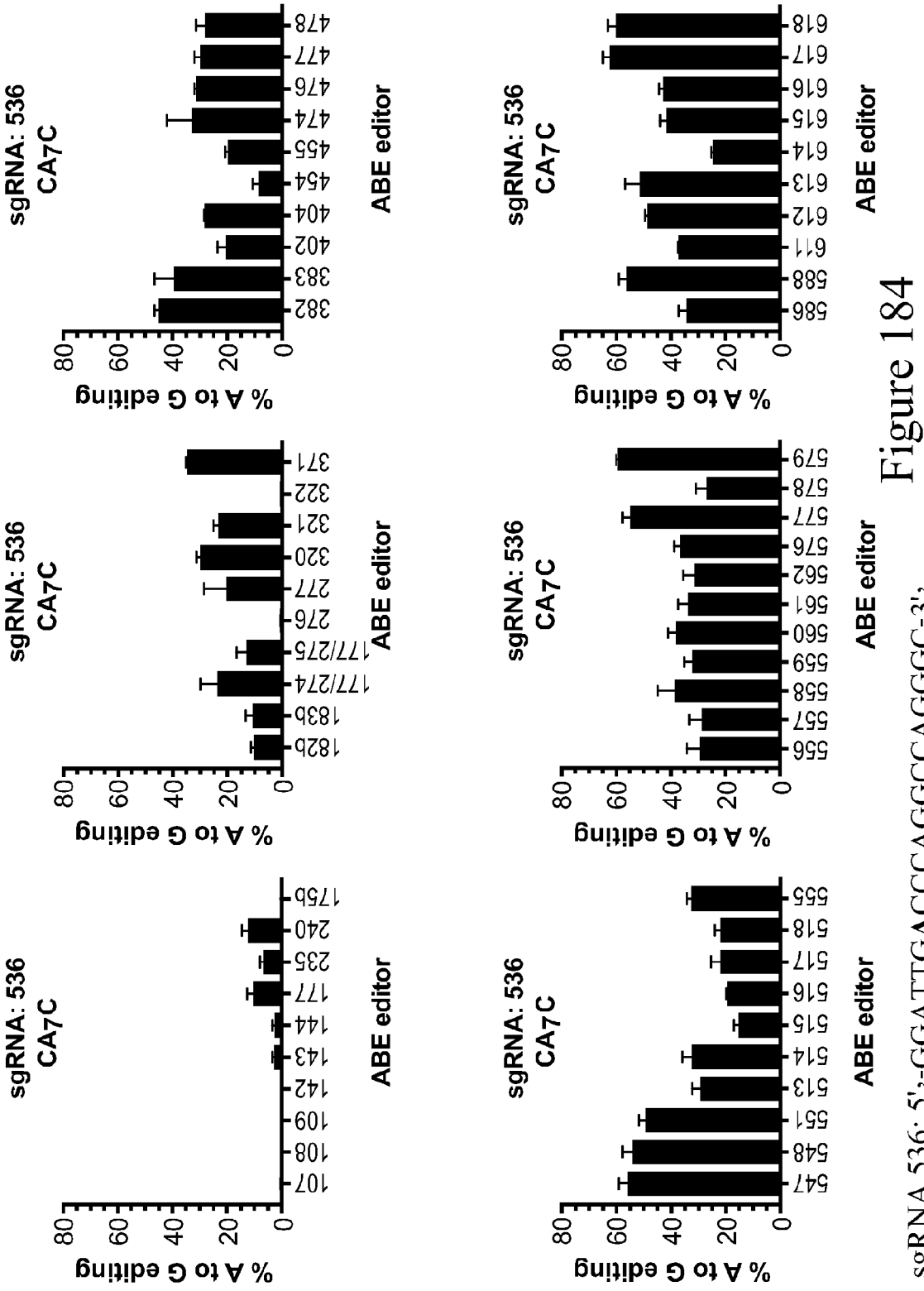



Figure 184

sgRNA 536: 5'-GGATTGACCCAGGCCAGGGC-3',

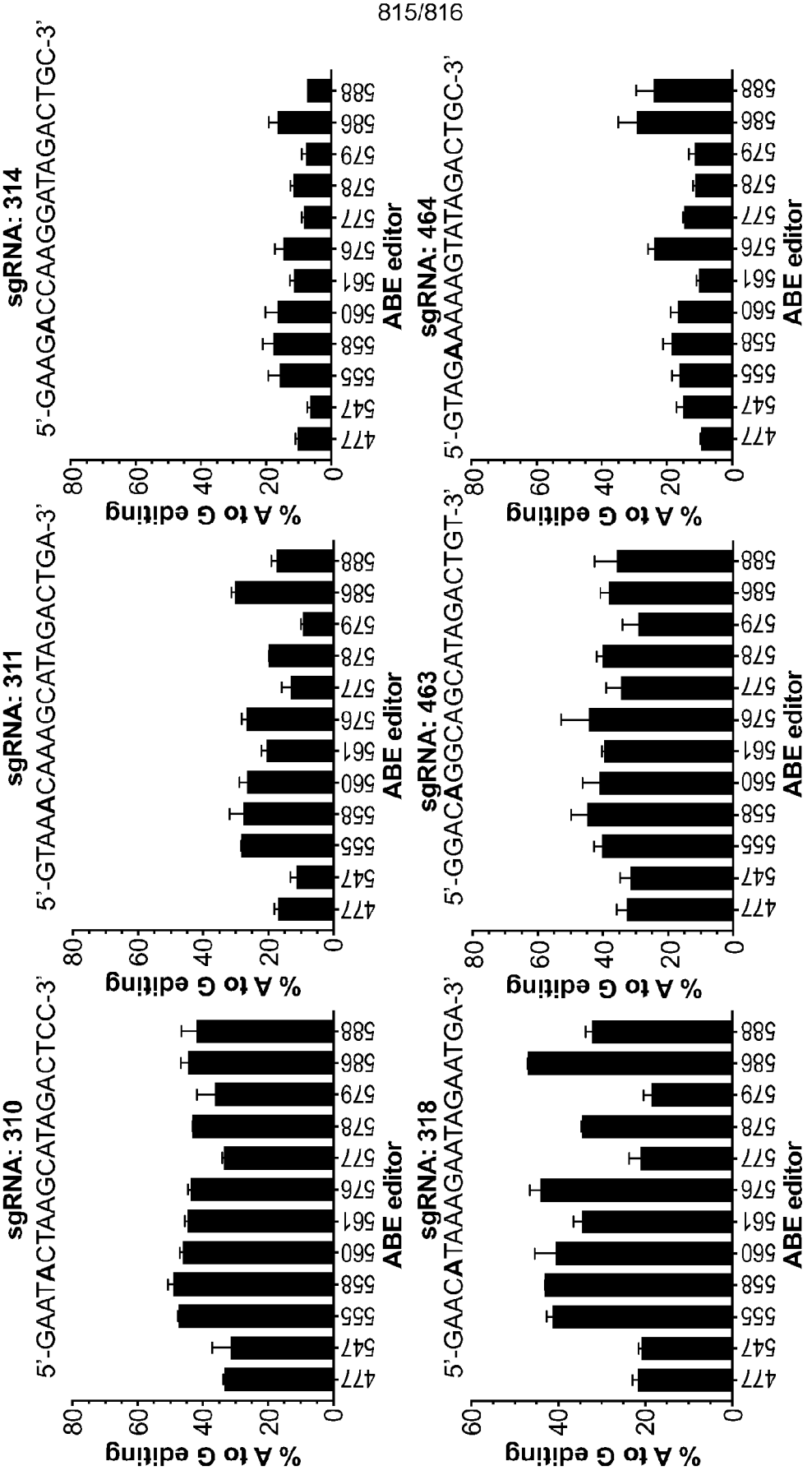

sgRNA: 310
5'-GAATACTAAGCATAGACTCC-3'
sgRNA: 311
5'-GTAAACAAAGCATAGACTGA-3'
sgRNA: 314
5'-GAAGACCAAGGATAGACTGC-3'
sgRNA: 318
5'-GAACATAAAGAATAGAATGA-3'
sgRNA: 463
5'-GGACAGGCAGCATAGACTGT-3'
sgRNA: 464
5'-GTAGAAAAAGTATAGACTGC-3'
% A to G editing
ABE editor
0
20
40
60
80
477
547
555
558
560
561
576
577
578
579
586
588

Figure 185

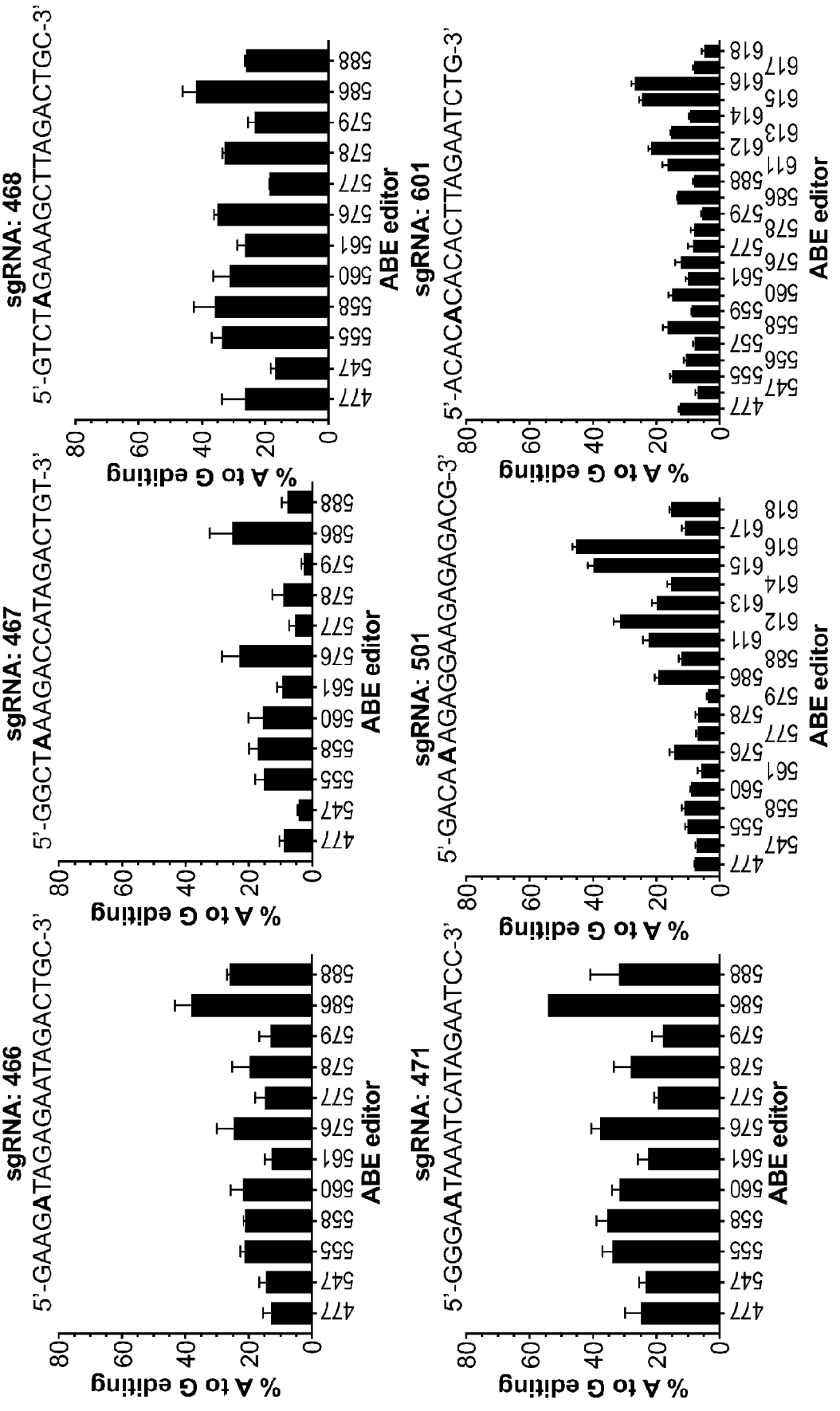

sgRNA: 466
5'-GAAGATAGAGAATAGACTGC-3'
sgRNA: 467
5'-GGCTAAAGACCATAGACTGT-3'
sgRNA: 468
5'-GTCTAGAAAGCTTAGACTGC-3'
sgRNA: 471
5'-GGGAATAAATCATAGAATCC-3'
sgRNA: 501
5'-GACAAAGAGGAAGAGAGACG-3'
sgRNA: 601
5'-ACACACACACTTAGAATCTG-3'
% A to G editing
ABE editor

Figure 186