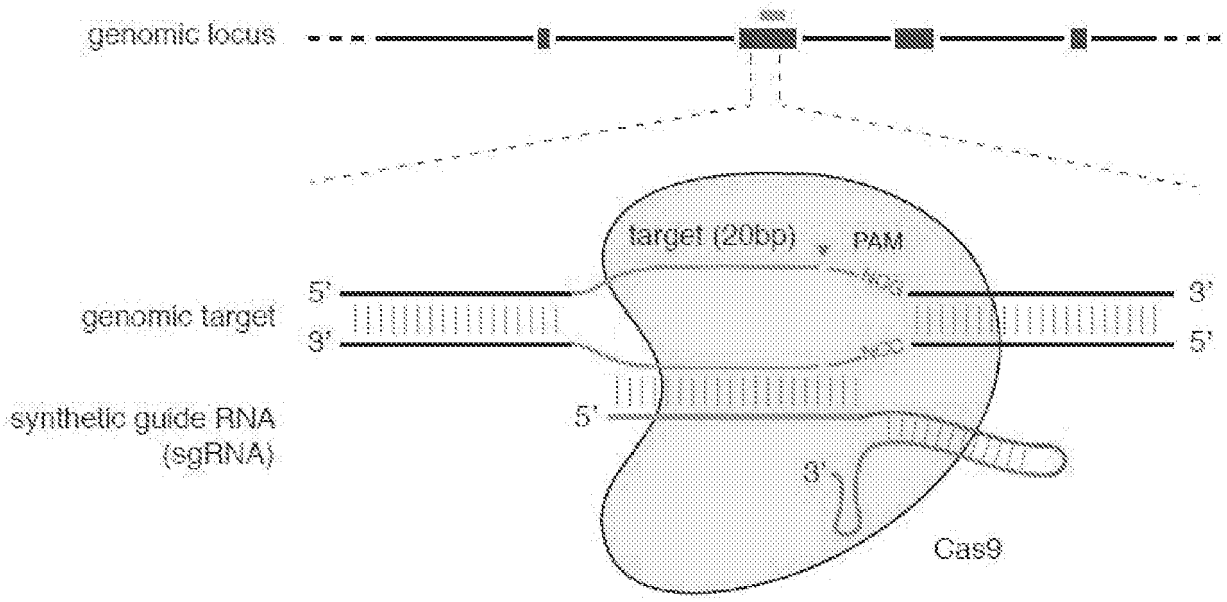

genomic locus
target (20bp)
PAM
5'
3'
genomic target
3'
5'
synthetic guide RNA
(sgRNA)
5'
3'
Cas9

FIG. 1

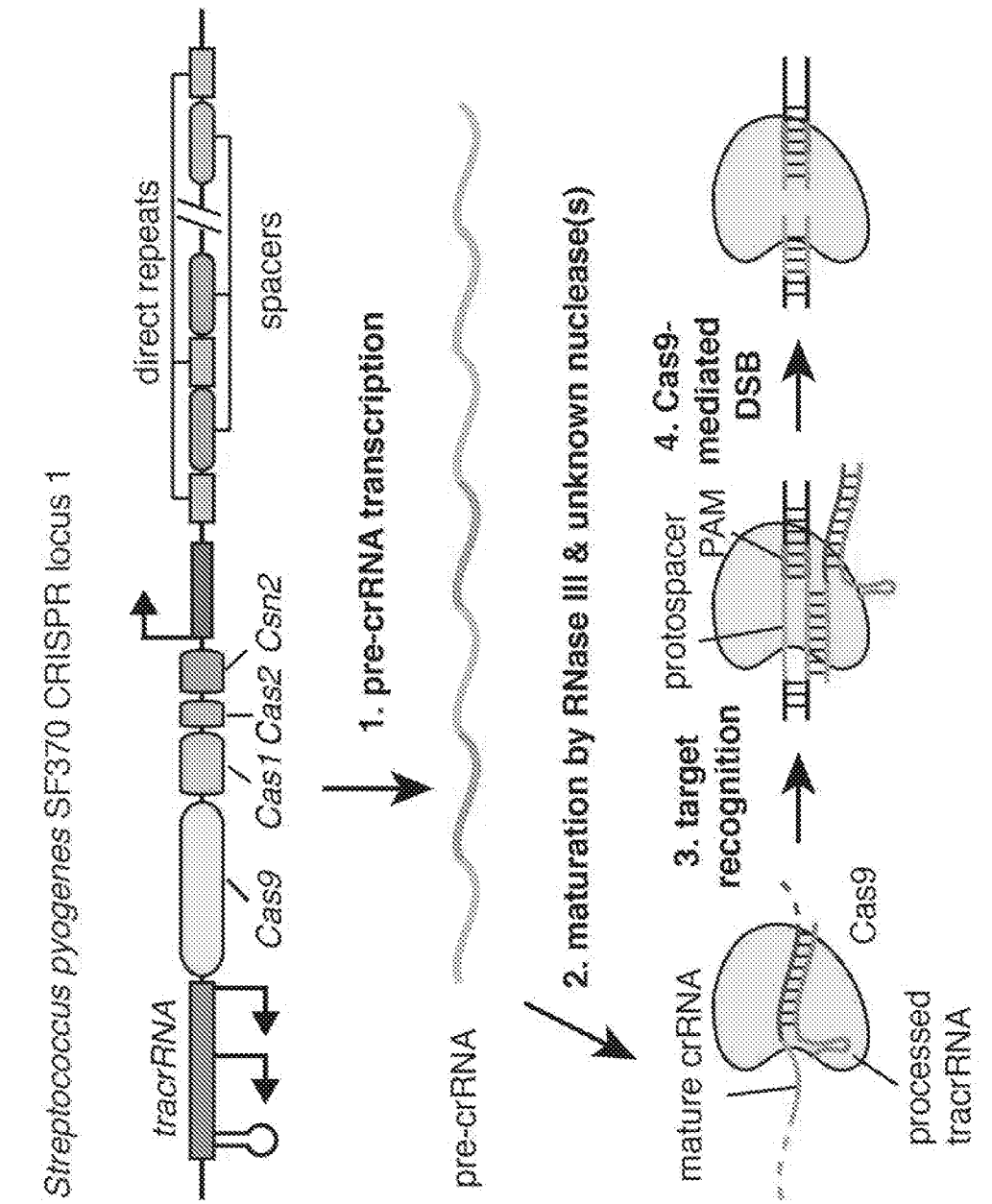

A
Streptococcus pyogenes SF370 CRISPR locus 1
tracrRNA
Cas9
Cas1
Cas2
Csn2
direct repeats
spacers
1. pre-crRNA transcription
pre-crRNA
2. maturation by RNase III & unknown nuclease(s)
mature crRNA
3. target recognition
protospacer
PAM
Cas9
4. Cas9-mediated DSB
processed tracrRNA

FIG. 2A

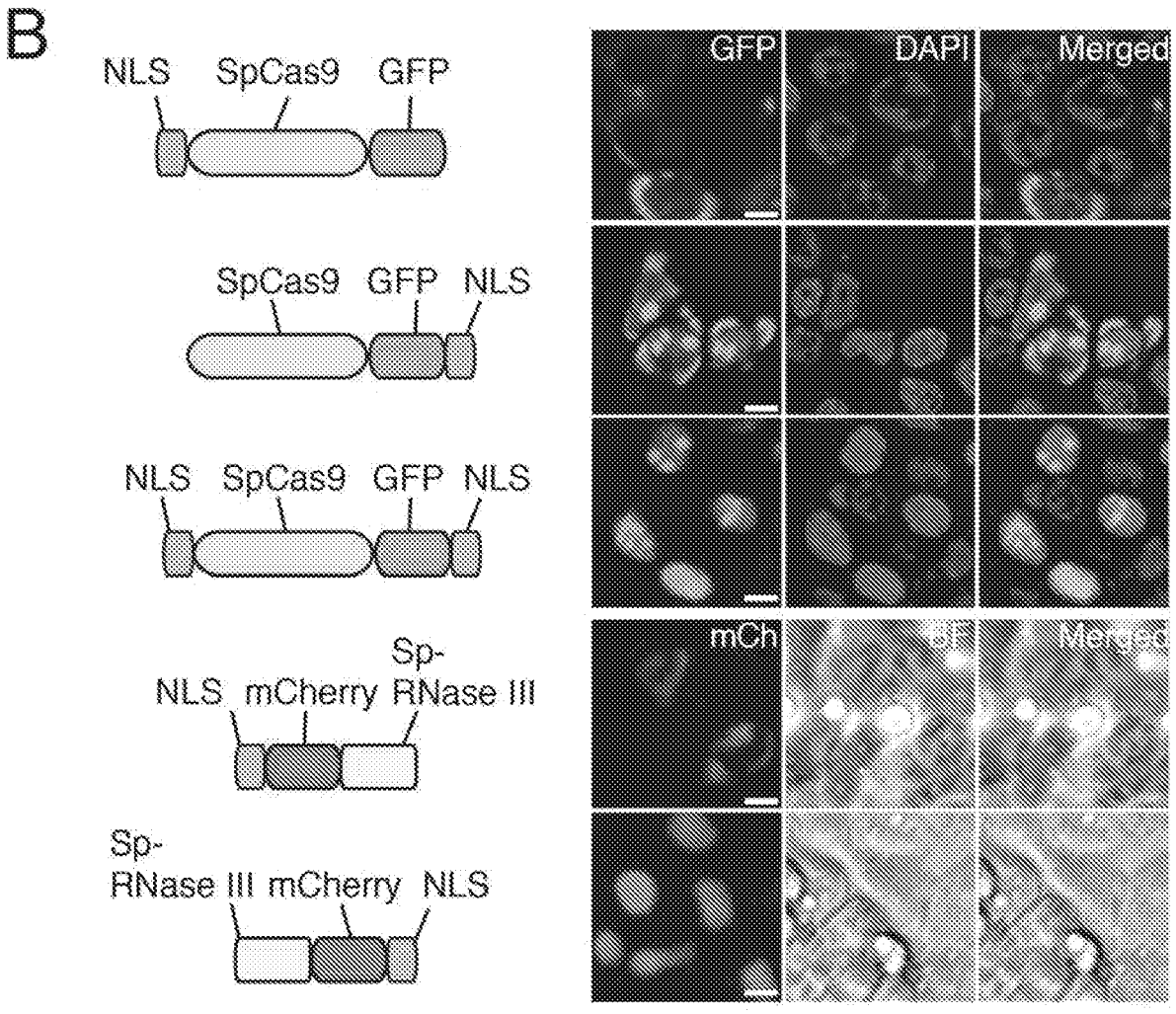

B
NLS SpCas9 GFP
SpCas9 GFP NLS
NLS SpCas9 GFP NLS
Sp-
NLS mCherry RNase III
Sp-
RNase III mCherry NLS
GFP
DAPI
Merged
mCh
BF
Merged

FIG. 2B

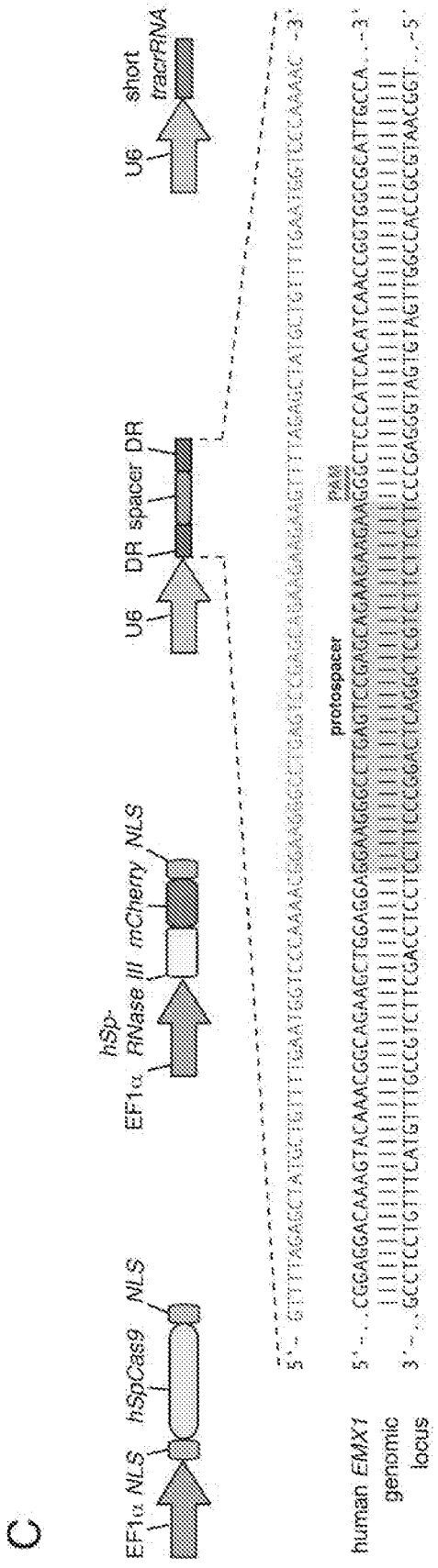

C
EF1α NLS hSpCas9 NLS
hSp-
EF1α RNase III mCherry NLS
U6 DR spacer DR
U6 short tracrRNA
protospacer
PAM
human EMX1 genomic locus
5'- ...CGGAGGACAAAGTACAAACGGCAGAAGCTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCATCACATCAACCGGTGGCGCATTGCCA...-3'
3'-...GCCTCCTGTTTCATGTTTGCCGTCTTCGACCTCCTCCTTCCCGGACTCAGGCTCGTCTTCTTCCCGAGGGTAGTGTAGTTGGCCACCGCGTAACGGT...-5'
5'- GTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAACGGAAGGCCTGAGTCCGAGCAGAAGAAGAAGTTTTAGAGCTATGCTGTTTTGAATGGTCCCAAAAC -3'

FIG. 2C

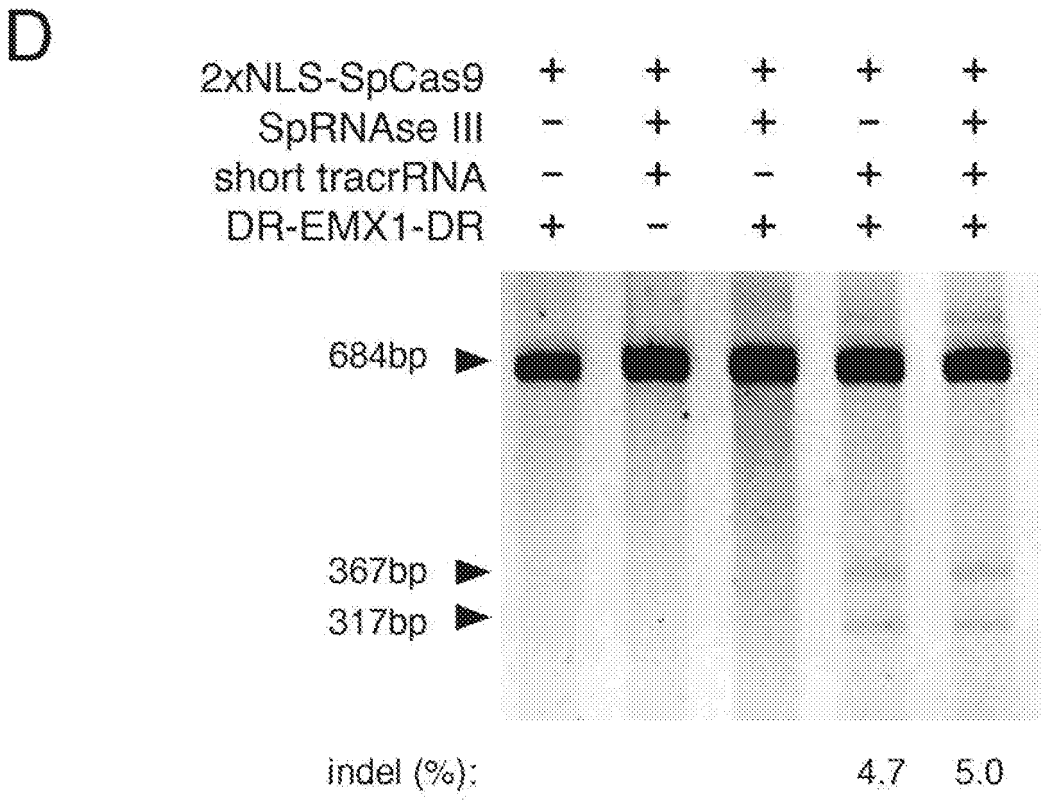

D
2xNLS-SpCas9 + + + + +
SpRNAse III − + + − +
short tracrRNA − + − + +
DR-EMX1-DR + − + + +
684bp
367bp
317bp
indel (%): 4.7 5.0

FIG. 2D

E




F

human *EMX1* protospacer target (mutation in 5 of 43 sequenced clones = 11.6%)

| | |
|---|---|
| WT | 5' - . . CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAG AAGAAGGGCTCCCATCACAT . . -3' |
| Δ1 | CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAG---AGAAGGGCTCCCATCACAT |
| +1 | CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAAGAAGGGCTCCCATCACAT |
| Δ3 | CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAG-----AAGGGCTCCCATCACAT |
| m1, Δ6 | CTGGAGGAGGAAGGGCCTGAGCCCGAGCAGAAG----------GGCTCCCATCACAT |

FIG. 2E-F

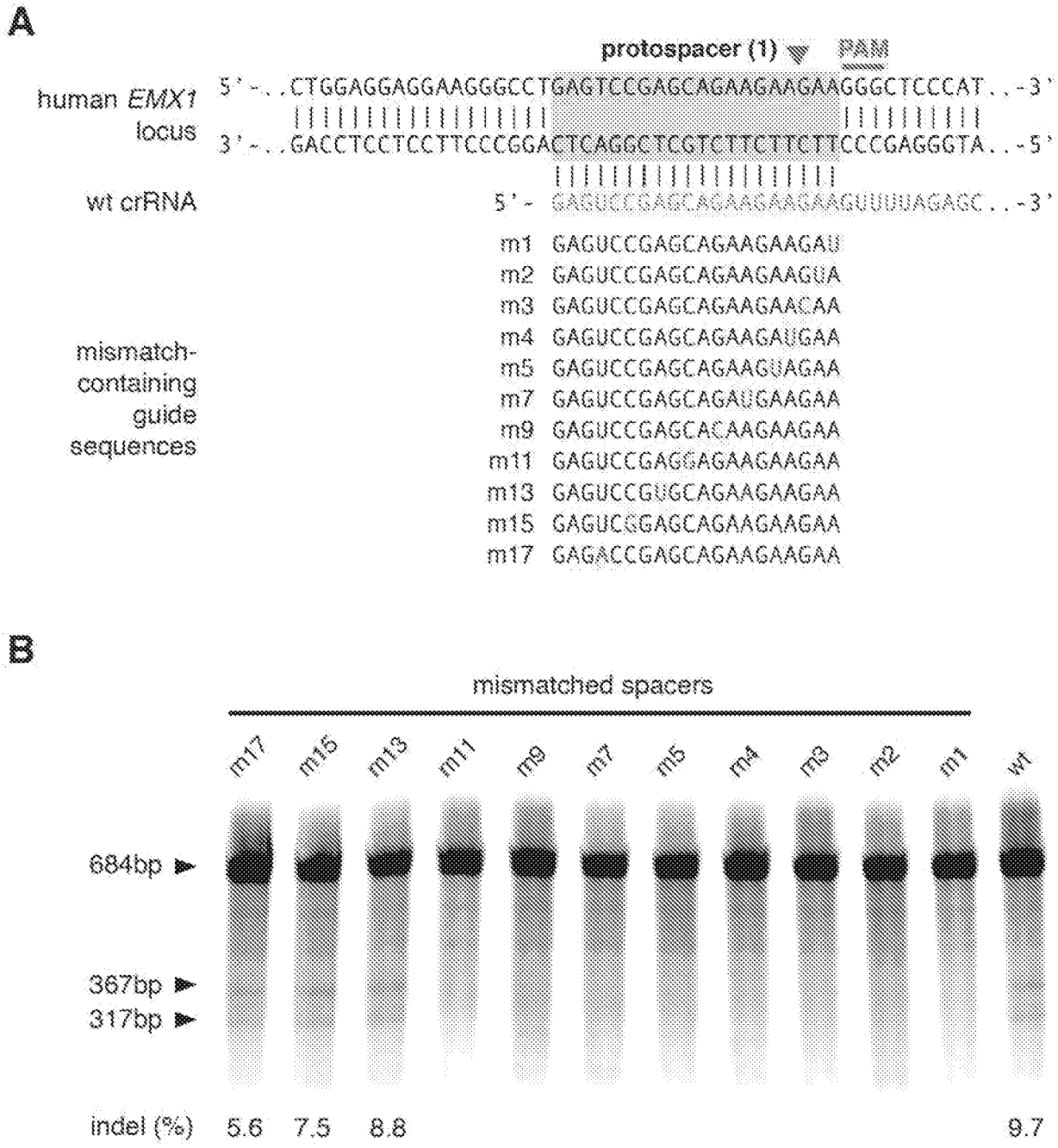

A
protospacer (1)
PAM
human EMX1 locus
5'-..CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCAT..-3'
3'-..GACCTCCTCCTTCCCGGACTCAGGCTCGTCTTCTTCTTCCCGAGGGTA..-5'
wt crRNA
5'- GAGUCCGAGCAGAAGAAGAAGUUUUAGAGC..-3'
mismatch-containing guide sequences
m1 GAGUCCGAGCAGAAGAAGAU
m2 GAGUCCGAGCAGAAGAAGUA
m3 GAGUCCGAGCAGAAGAACAA
m4 GAGUCCGAGCAGAAGAUGAA
m5 GAGUCCGAGCAGAAGUAGAA
m7 GAGUCCGAGCAGAUGAAGAA
m9 GAGUCCGAGCACAAGAAGAA
m11 GAGUCCGAGGAGAAGAAGAA
m13 GAGUCCGUGCAGAAGAAGAA
m15 GAGUCGGAGCAGAAGAAGAA
m17 GAGACCGAGCAGAAGAAGAA
B
mismatched spacers
m17
m15
m13
m11
m9
m7
m5
m4
m3
m2
m1
wt
684bp
367bp
317bp
indel (%)
5.6
7.5
8.8
9.7
C
left TALEN binding site
protospacer (1)
PAM
human EMX1 locus
5'-..CTGGAGGAGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGGGCTCCCAT..-3'
3'-..GACCTCCTCCTTCCCGGACTCAGGCTCGTCTTCTTCTTCCCGAGGGTA..-5'
right TALEN binding site

FIG. 3A-C

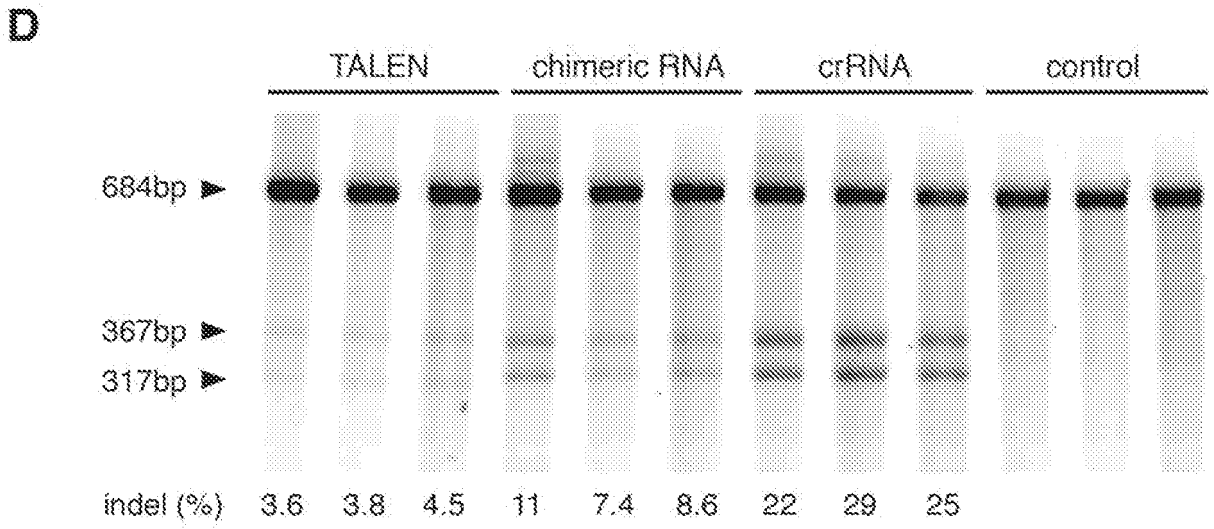

D
TALEN
chimeric RNA
crRNA
control
684bp
367bp
317bp
indel (%)
3.6
3.8
4.5
11
7.4
8.6
22
29
25

FIG. 3D

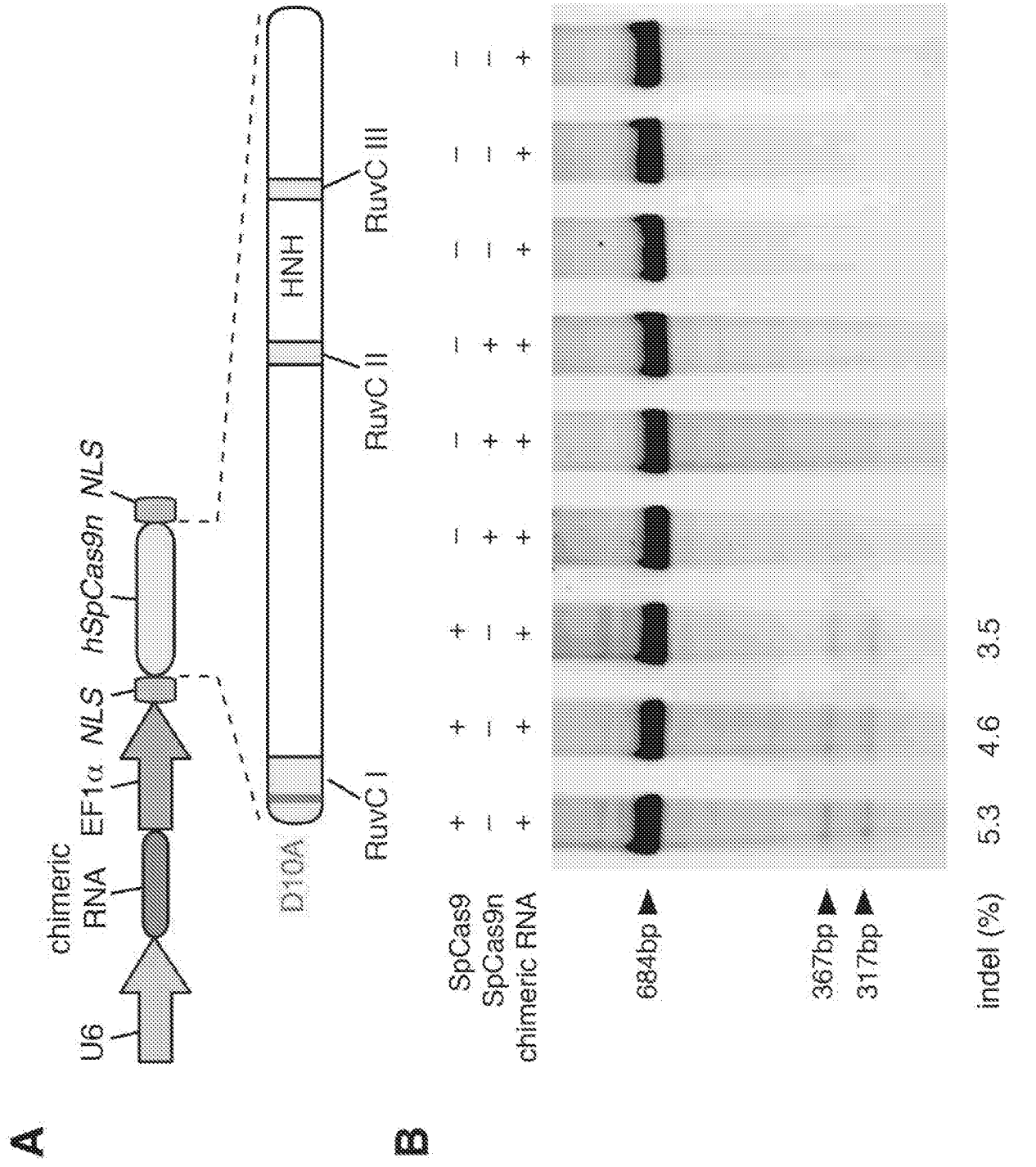

A
U6
chimeric RNA
EF1α
NLS
hSpCas9n
NLS
D10A
RuvC I
RuvC II
HNH
RuvC III
B
SpCas9
SpCas9n
chimeric RNA
684bp
367bp
317bp
indel (%) 5.3 4.6 3.5

FIG. 4A-B

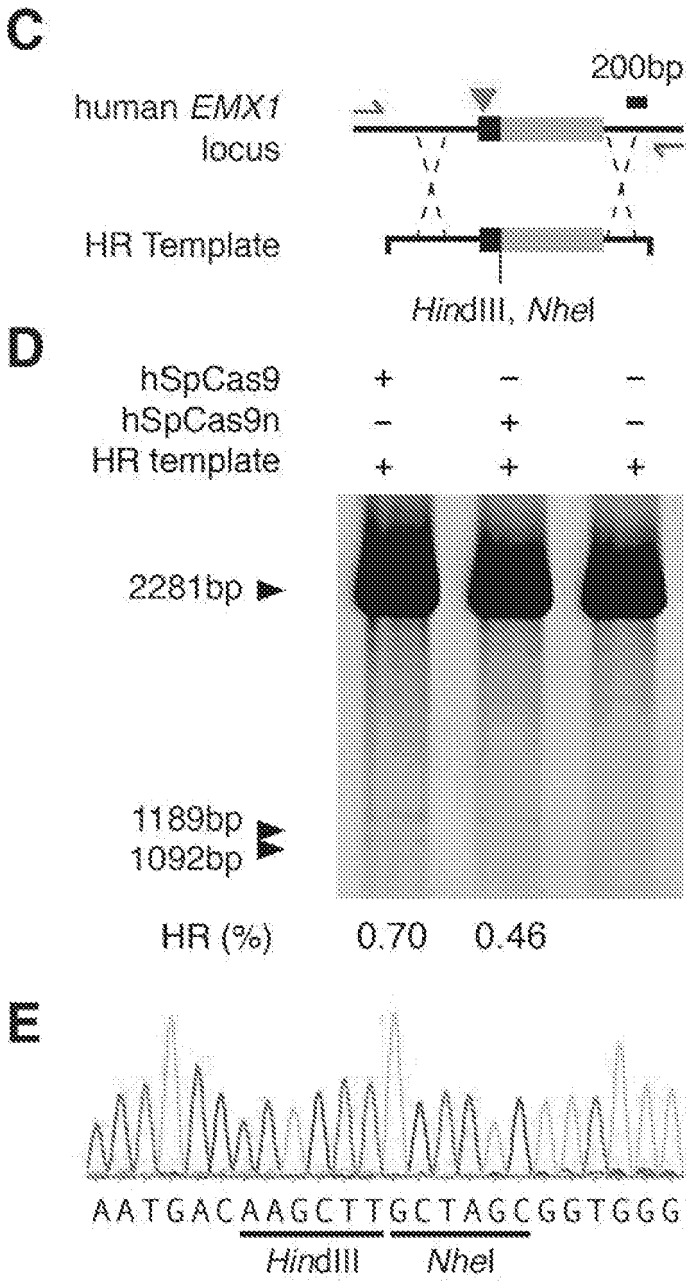

C
200bp
human EMX1 locus
HR Template
HindIII, NheI
D
hSpCas9 + – –
hSpCas9n – + –
HR template + + +
2281bp
1189bp
1092bp
HR (%) 0.70 0.46
E
AATGACAAGCTTGCTAGCGGTGGG
HindIII
NheI

FIG. 4C-E

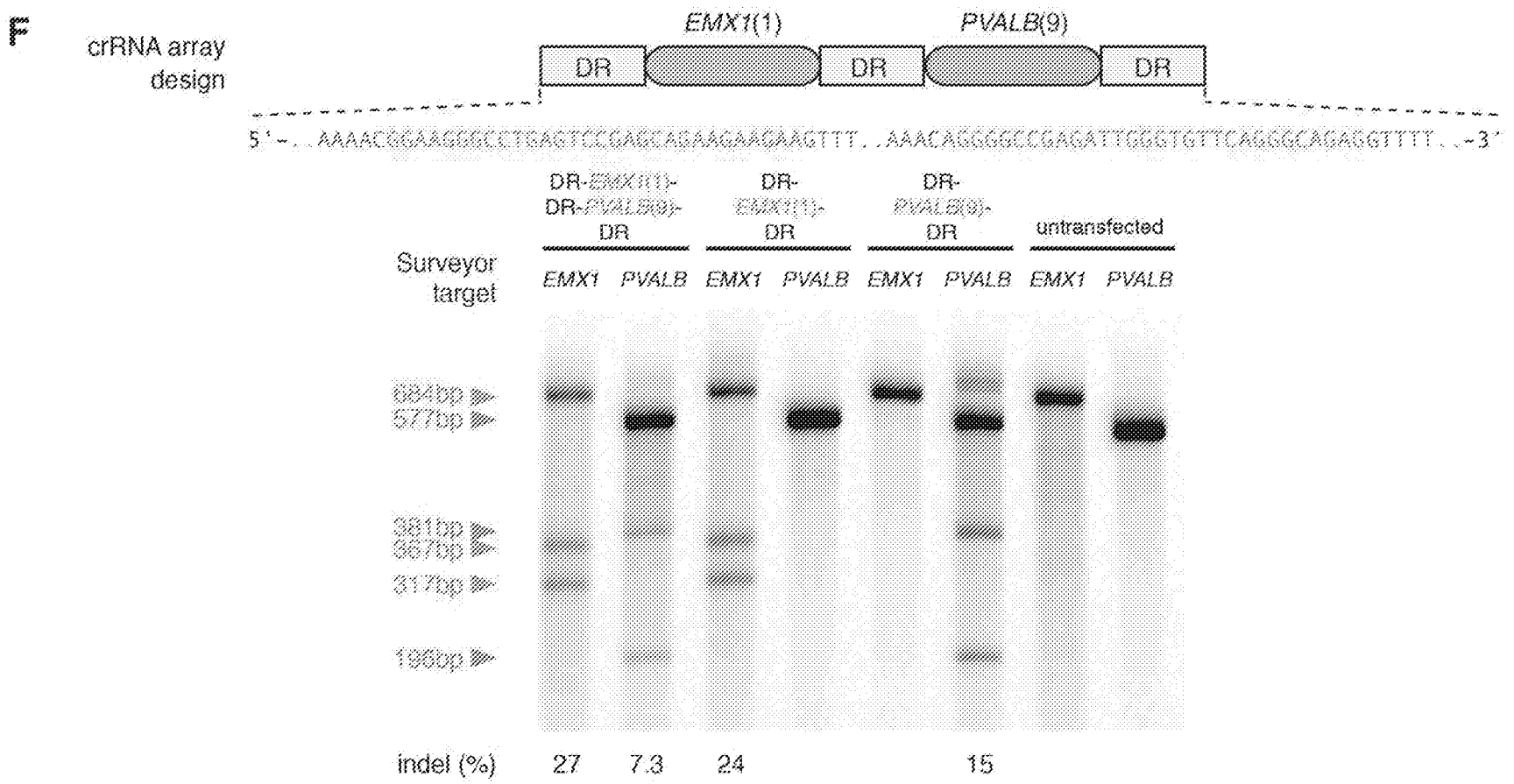

F
crRNA array design
EMX1(1)
PVALB(9)
DR
DR
DR
5'~..AAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTTT..AAACAGGGGCCGAGATTGGGTGTTCAGGGCAGAGGTTTT..~3'
DR-EMX1(1)-DR-PVALB(9)-DR
DR-EMX1(1)-DR
DR-PVALB(9)-DR
untransfected
Surveyor target
EMX1
PVALB
EMX1
PVALB
EMX1
PVALB
EMX1
PVALB
684bp
577bp
381bp
367bp
317bp
196bp
indel (%)
27
7.3
24
15

FIG. 4F

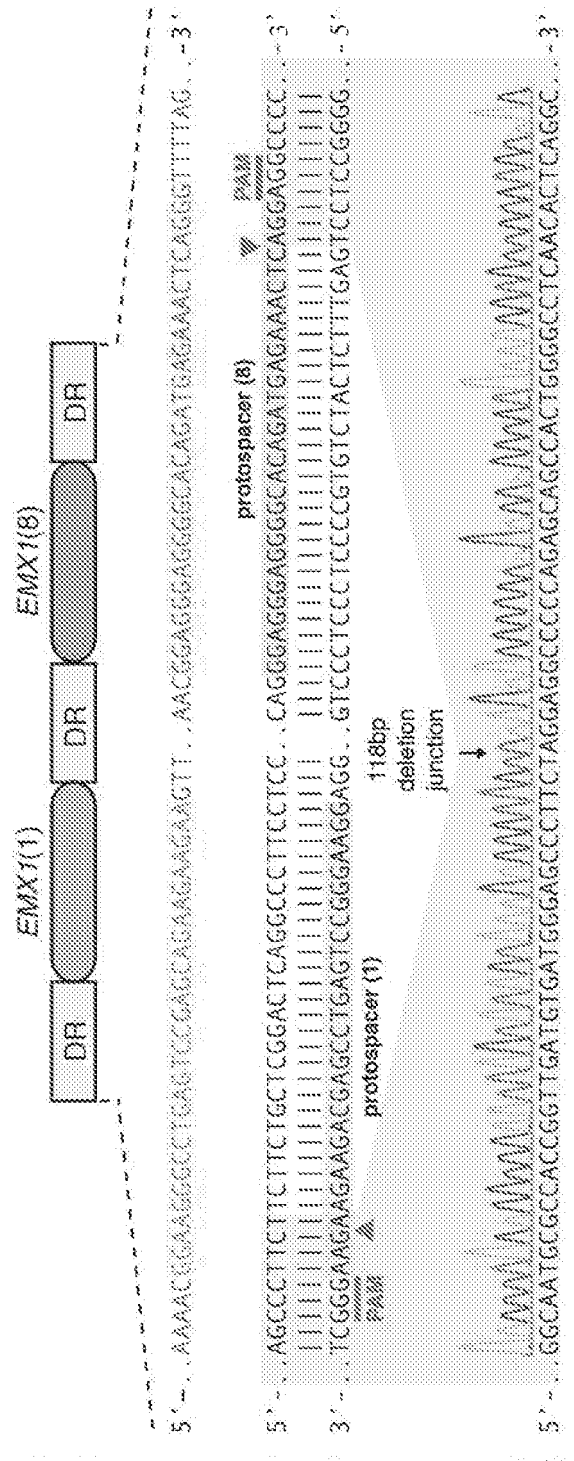
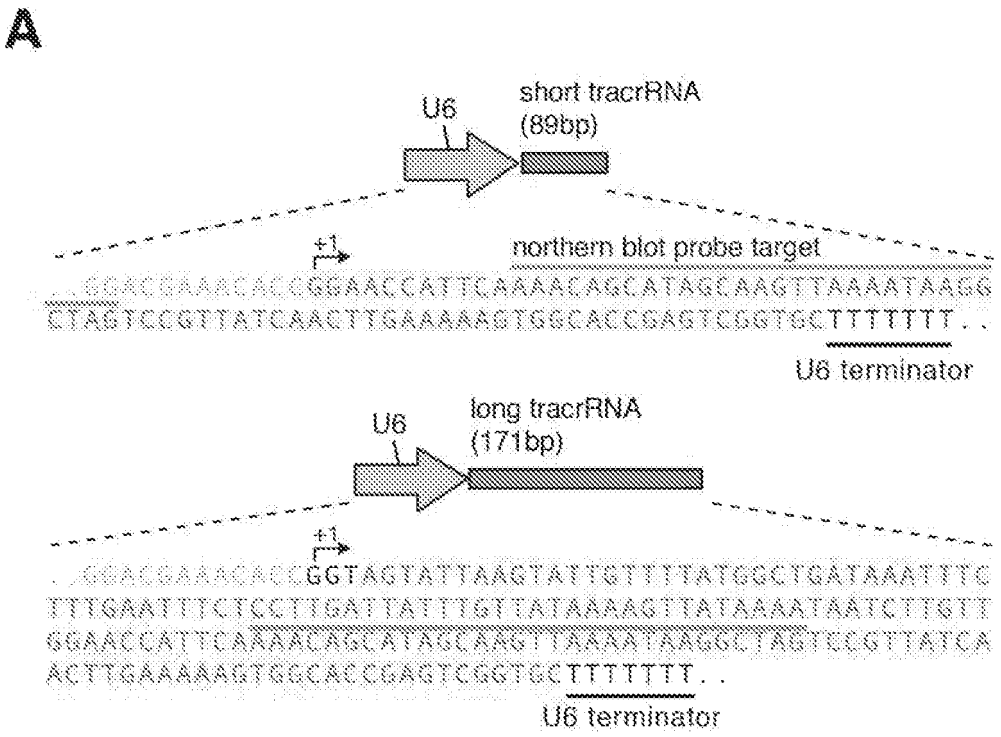

G
crRNA array design
DR
EMX1(1)
DR
EMX1(8)
DR
5'- ..AAAACGGAAGGGGCCCTGAGTCCCAGGCAGAAGAAGAAGTT..AACGGAAGGGAGGGGCACAGATGAAAACTCAGGGTTTTTAG..-3'
human EMX1 locus
5'- ..AGCCCTTCTTCTTGCTCGGACTCAGGCCCTTCCTCC..CAGGGAGGGAGGGGCACAGATGAGAAACTCAGGAGGCCCC..-3'
3'- ..TCGGGAAGAAGACGAGCCTGAGTCCGGGAAGGAGG..GTCCCTCCCTCCCCGTGTCTACTCTTTGAGTCCTCCGGGG..-5'
protospacer (8)
PAM
protospacer (1)
PAM
118bp deletion junction
deletion result
5'- ..GGCAATGCGCCACCGGTTGATGTGATGGGAGCCCTTCTAGGAGGCCCCCAGAGCAGCCACTGGGGCCTCAACACTCAGGC..-3'

FIG. 4G

| Cas9 | target species | gene | protospacer ID | protospacer sequence (5' to 3') | PAM | strand | cell line tested | % indel (pre-crRNA + tracrRNA) | % indel (chimeric RNA) |
|---|---|---|---|---|---|---|---|---|---|
| *S. pyogenes* SF370 type II CRISPR | *Homo sapiens* | *EMX1* | 1 | GGAGGGCCTGAGTCCGAGCAGAAGAA | GGG | + | 293FT | 20 ± 1.8 | 6.7 ± 0.62 |
| | | *EMX1* | 2 | CATTGAAGGTGACATCGATGTCCTCCCCAT | TGG | – | 293FT | 2.1 ± 0.31 | N.D. |
| | | *EMX1* | 3 | GGACATCGATGTCACCTCCAATGACTAGGG | TGG | + | 293FT | 14 ± 1.1 | N.D. |
| | | *EMX1* | 4 | CATCGATGTCCTCCCCATTGGCCTGCTTCG | TGG | – | 293FT | 11 ± 1.7 | N.D. |
| | | *EMX1* | 5 | TTCGTGGCAATGCGCCACCGGTTGATGTGA | TGG | – | 293FT | 4.3 ± 0.46 | 2.1 ± 0.51 |
| | | *EMX1* | 6 | TCGTGCAATGCGCCCACCGGTTGATGTGAT | GGG | – | 293FT | 4.0 ± 0.66 | 0.41 ± 0.25 |
| | | *EMX1* | 7 | TCCAGCTTCTGCCCGTTTGTACTTTGTCCTC | CGG | – | 293FT | 1.5 ± 0.12 | N.D. |
| | | *EMX1* | 8 | GGAGGAGGCCACAGATGAGAAACTCAGG | AGG | – | 293FT | 7.8 ± 0.83 | 2.3 ± 1.2 |
| CRISPR | *Homo sapiens* | *PVALB* | 9 | ACGGGCCCAGATTGGGTGTTCAGGGCAGAG | AGG | + | 293FT | 21 ± 2.6 | 6.5 ± 0.32 |
| | | *PVALB* | 10 | ATGCAGGAGGTGGCTGACAGGGGCCCACAT | TGG | + | 293FT | N.D. | N.D. |
| | | *PVALB* | 11 | GGTGCCGAGAGGGCCGAGATTGGGTGTTC | AGC | + | 293FT | N.D. | N.D. |
| | *Mus musculus* | Th | 12 | CAGCACTGAGTGCCATTAGCTAAATGCAT | AGG | – | Neuro2A | 27 ± 4.3 | 4.1 ± 2.2 |
| | | Th | 13 | AATGCATAGGGTACCACCCACAGGTGCCAG | GGG | – | Neuro2A | 4.6 ± 1.2 | N.D. |
| | | Th | 14 | ACACACATGGGAAAGCCTCTGGGCCAGGAA | AGG | + | Neuro2A | 11.3 ± 1.3 | N.D. |
| *S. thermophilus LMD-9 CRISPR1* | *Homo sapiens* | *EMX1* | 15 | GGAGGAGGTAGTATACAGAAACACAGAGAA | GTAGAAT | – | 293FT | 14 ± 0.88 | N.T. |
| | | *EMX1* | 16 | ACAATGTAGAGGAGTCACAGAAACTCAGCA | CTAGAAA | – | 293FT | 7.8 ± 0.77 | N.T. |

FIG. 5


A
U6
short tracrRNA
(89bp)
+1
northern blot probe target
..GGACGAAACACCGGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGG
CTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT..
U6 terminator
U6
long tracrRNA
(171bp)
+1
..GGACGAAACACCGGTAGTATTAAGTATTGTTTTATGGCTGATAAATTTC
TTTGAATTTCTCCTTGATTATTTGTTATAAAAGTTATAAAATAATCTTGTT
GGAACCATTCAAAACAGCATAGCAAGTTAAAATAAGGCTAGTCCGTTATCA
ACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTT..
U6 terminator

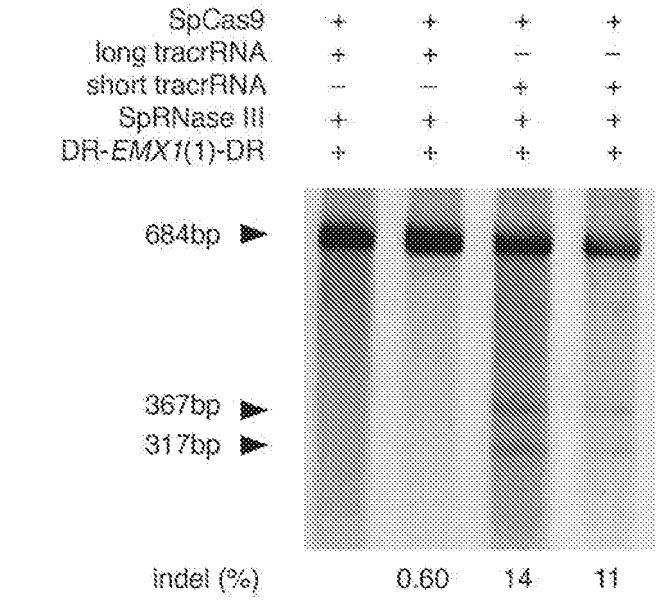

B
SpCas9 + + + +
long tracrRNA + + – –
short tracrRNA – – + +
SpRNase III + + + +
DR-EMX1(1)-DR + + + +
684bp
367bp
317bp
indel (%) 0.60 14 11

FIG. 6A-B

C

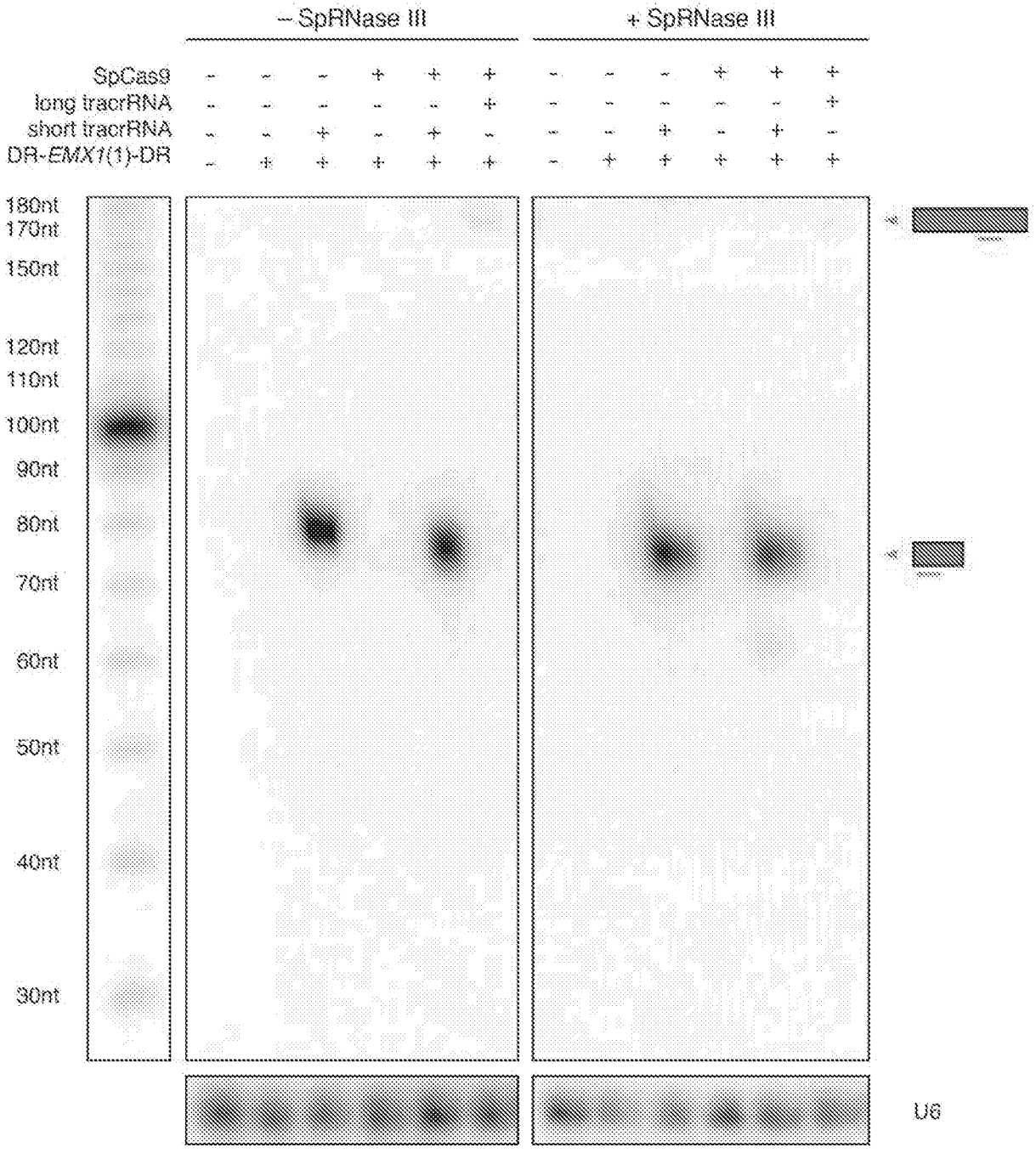

– SpRNase III
+ SpRNase III
SpCas9
long tracrRNA
short tracrRNA
DR-EMX1(1)-DR
180nt
170nt
150nt
120nt
110nt
100nt
90nt
80nt
70nt
60nt
50nt
40nt
30nt
U6

FIG. 6C

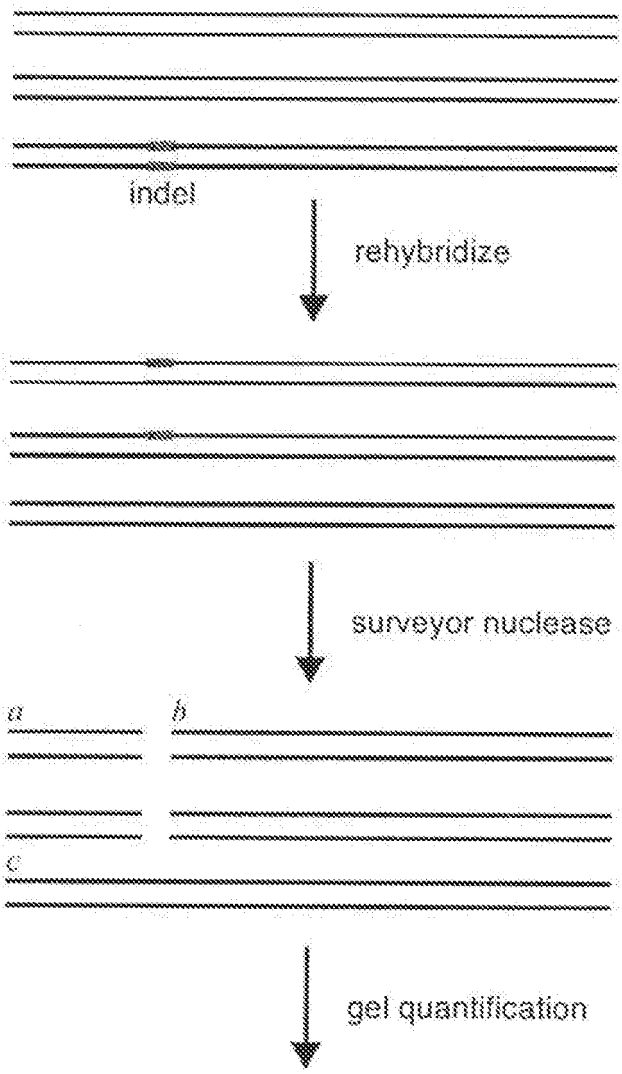

indel
rehybridize
surveyor nuclease
a
b
c
gel quantification
% indel = ( 1 - √1 - (a + b)/(a + b + c) ) *100

FIG. 7

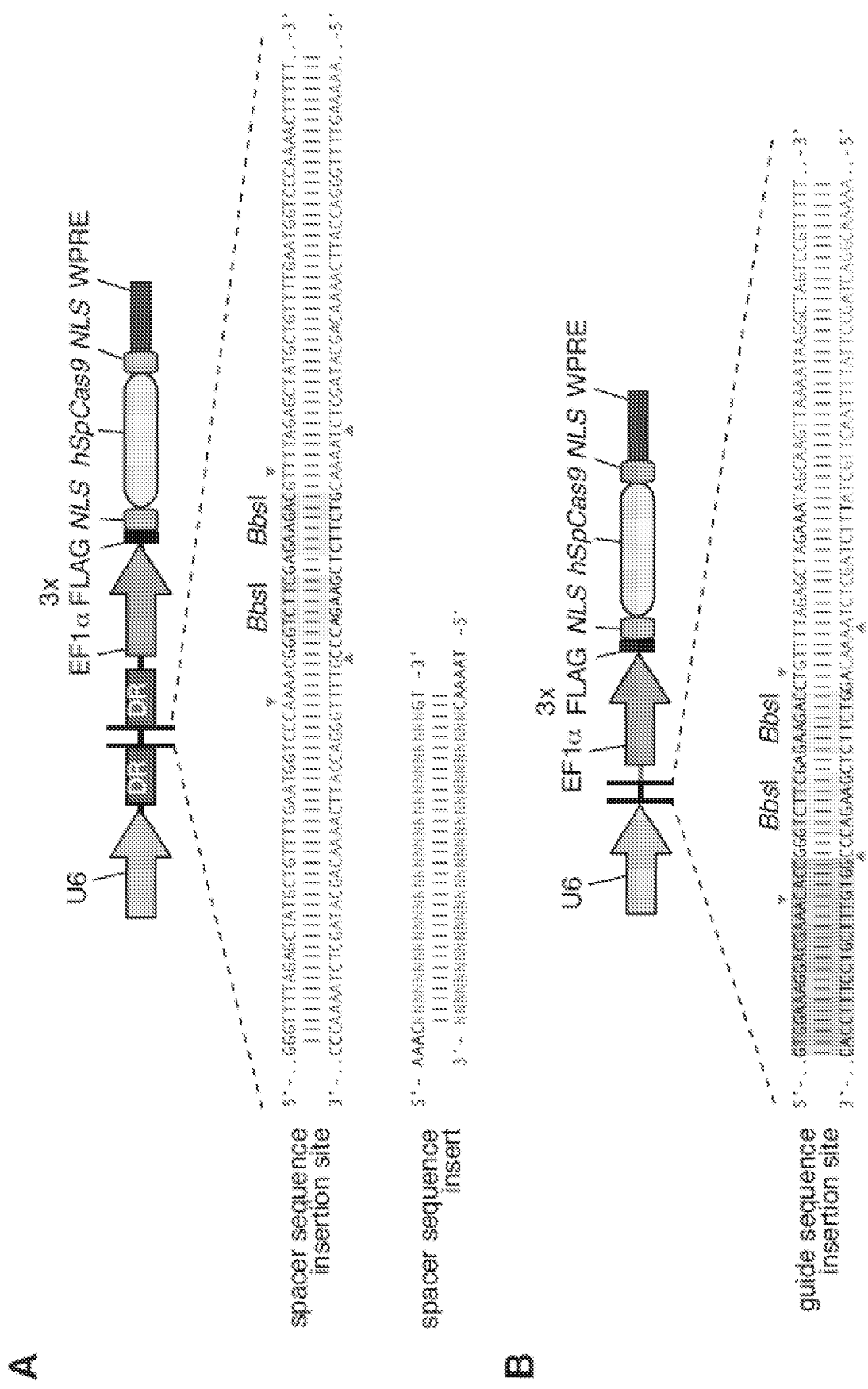
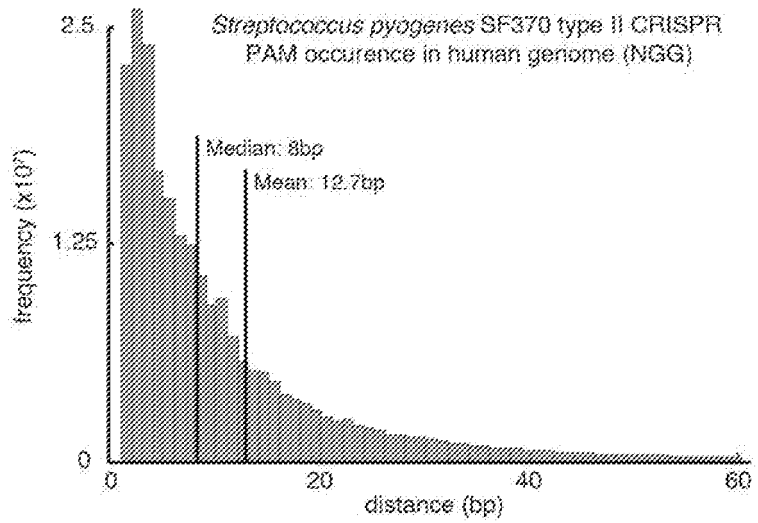
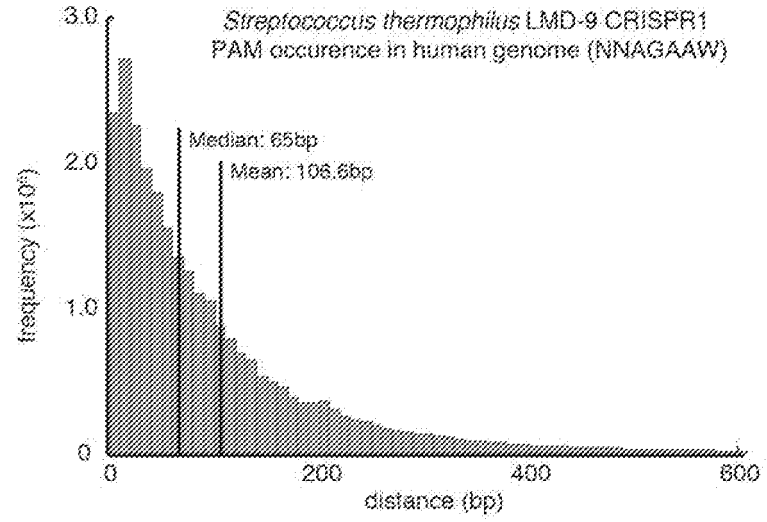

A
U6
3x
EF1α FLAG NLS hSpCas9 NLS WPRE
BbsI
BbsI
spacer sequence insertion site
spacer sequence insert
B
U6
3x
EF1α FLAG NLS hSpCas9 NLS WPRE
BbsI
BbsI
guide sequence insertion site
guide sequence insert

FIG. 8A-B

A




B




C

| | NGG | | NNAGAAW | |
|---|---|---|---|---|
| Chr | median | mean | median | mean |
| 1 | 7 | 12.8 | 67 | 115.8 |
| 2 | 8 | 12.7 | 64 | 100.8 |
| 3 | 8 | 13.0 | 63 | 98.5 |
| 4 | 9 | 14.0 | 61 | 94.5 |
| 5 | 8 | 13.1 | 63 | 97.9 |
| 6 | 8 | 13.1 | 63 | 98.5 |
| 7 | 8 | 12.4 | 64 | 102.9 |
| 8 | 8 | 12.8 | 64 | 100.9 |
| 9 | 7 | 13.9 | 65 | 120.5 |
| 10 | 7 | 12.1 | 66 | 107.0 |
| 11 | 7 | 12.0 | 65 | 105.8 |
| 12 | 8 | 12.4 | 65 | 103.5 |
| 13 | 8 | 13.6 | 62 | 94.6 |
| 14 | 8 | 12.0 | 65 | 101.5 |
| 15 | 7 | 11.5 | 68 | 107.7 |
| 16 | 7 | 11.7 | 74 | 136.8 |
| 17 | 6 | 10.3 | 76 | 127.9 |
| 18 | 8 | 13.4 | 63 | 101.8 |
| 19 | 6 | 9.4 | 82 | 145.4 |
| 20 | 7 | 11.1 | 72 | 121.8 |
| 21 | 7 | 13.4 | 64 | 111.4 |
| 22 | 6 | 9.2 | 85 | 140.3 |
| X | 8 | 13.2 | 63 | 99.0 |
| Y | 8 | 29.2 | 62 | 223.7 |

FIG. 9A-C

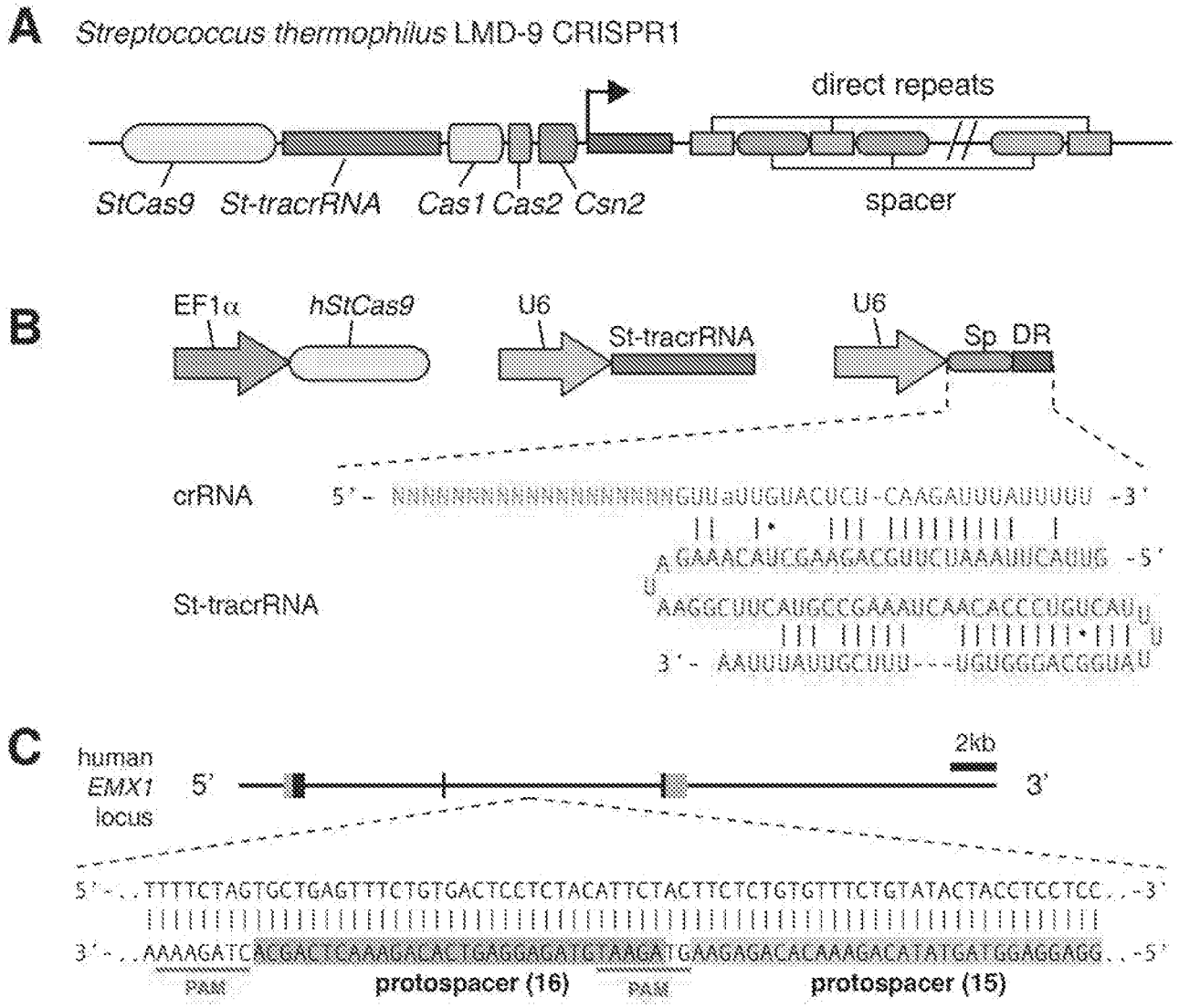

A
Streptococcus thermophilus LMD-9 CRISPR1
direct repeats
StCas9
St-tracrRNA
Cas1
Cas2
Csn2
spacer
B
EF1α
hStCas9
U6
St-tracrRNA
U6
Sp
DR
crRNA
St-tracrRNA
C
human
EMX1
locus
5'
2kb
3'
protospacer (16)
protospacer (15)

FIG. 10A-C

D

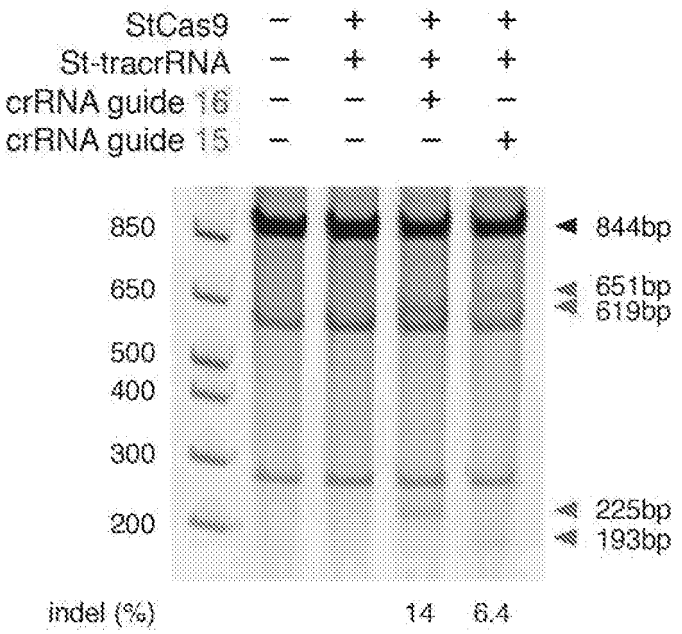

StCas9 − + + +
St-tracrRNA − + + +
crRNA guide 16 − − + −
crRNA guide 15 − − − +
850
650
500
400
300
200
844bp
651bp
619bp
225bp
193bp
indel (%)
14
6.4

FIG. 10D

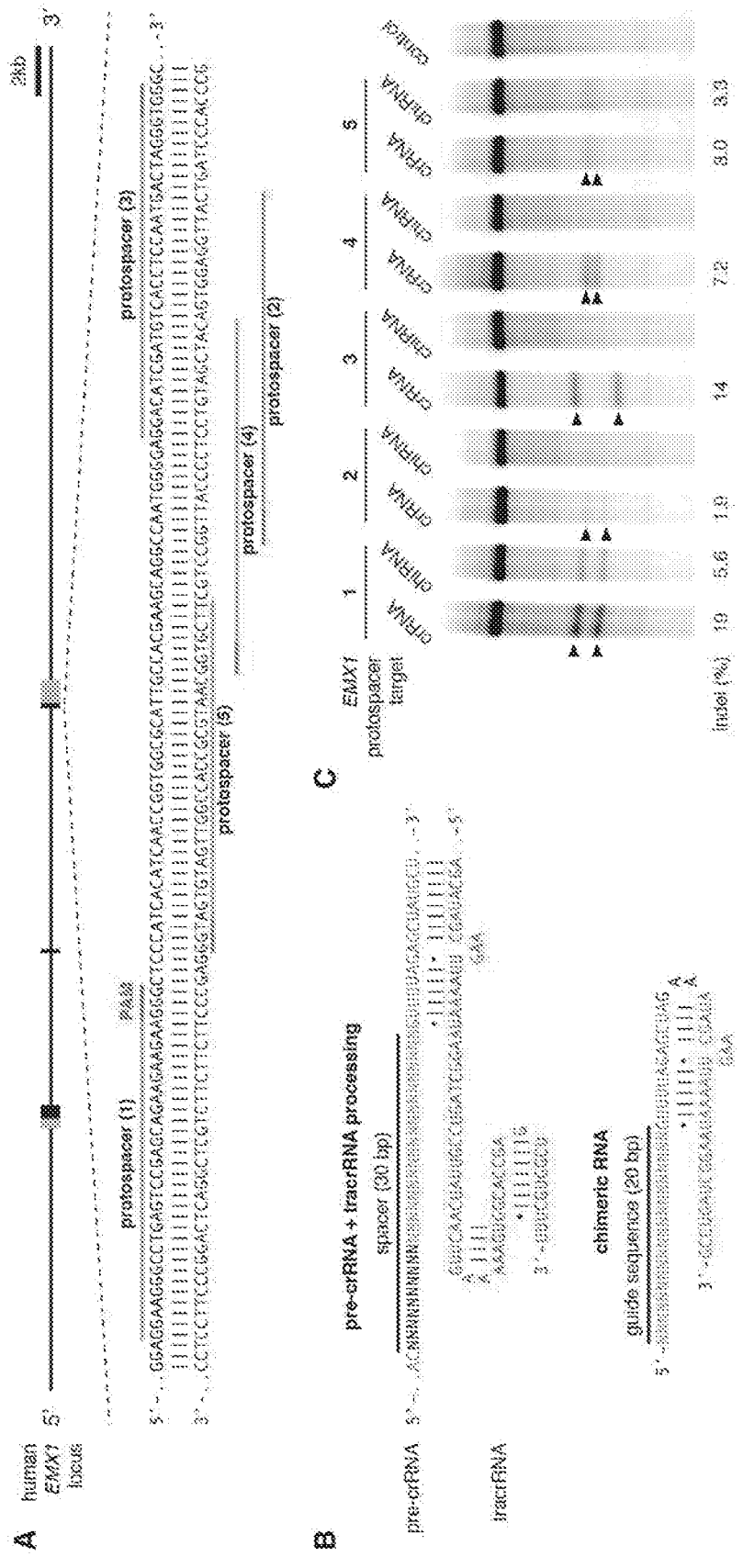

A
human EMX1 locus
5'
3'
2kb
protospacer (1)
PAM
protospacer (3)
protospacer (4)
protospacer (2)
protospacer (5)
B
pre-crRNA + tracrRNA processing
pre-crRNA
tracrRNA
chimeric RNA
spacer (20 bp)
guide sequence (20 bp)
C
EMX1 protospacer target
crRNA
chiRNA
1
2
3
4
5
control
Indel (%)
19
5.6
1.9
14
7.2
9.0
3.3

FIG. 11A-C

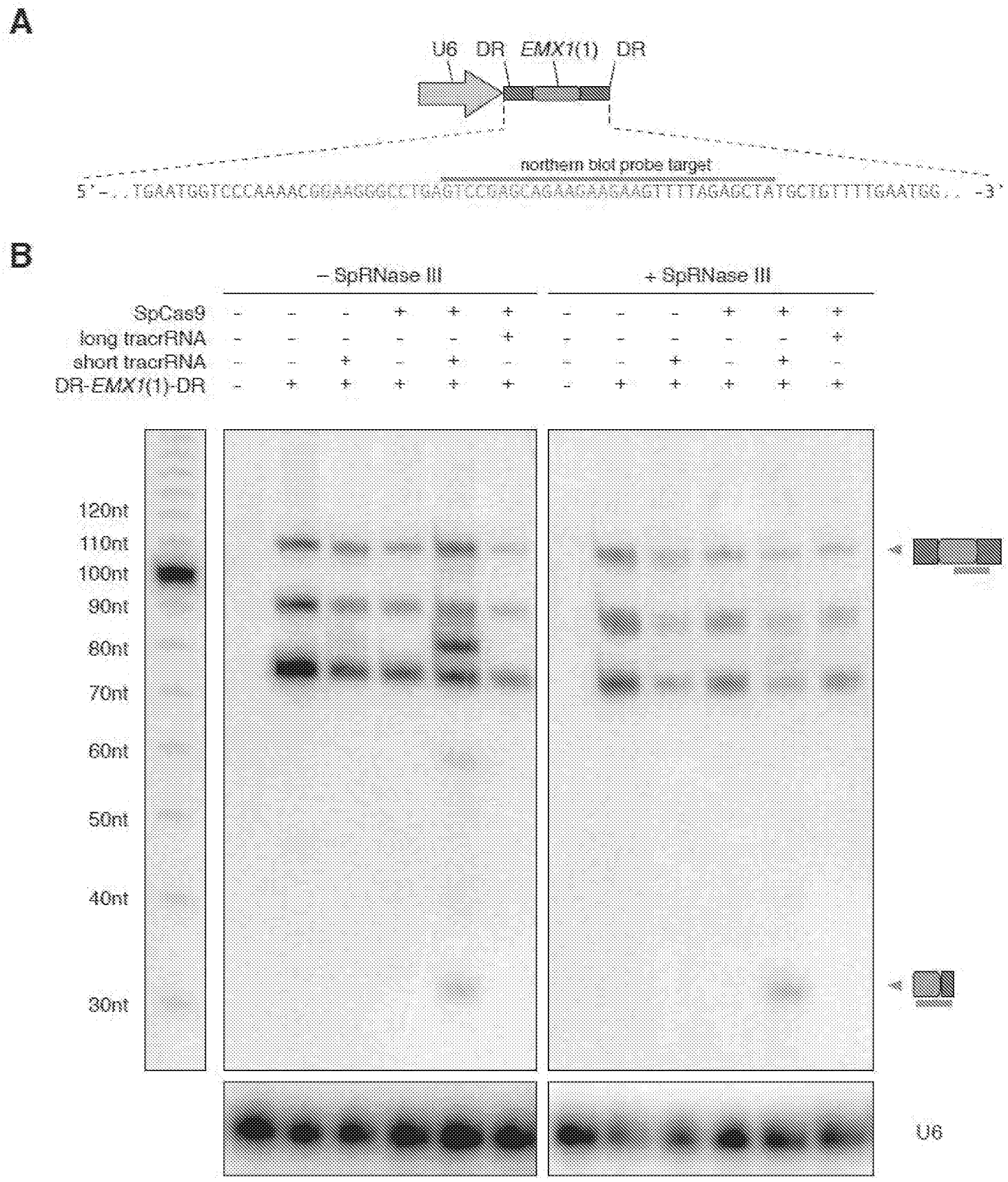

A
U6 DR EMX1(1) DR
northern blot probe target
5'-. . TGAATGGTCCCAAAACGGAAGGGCCTGAGTCCGAGCAGAAGAAGAAGTTTTAGAGCTATGCTGTTTTGAATGG. . -3'
B
– SpRNase III
+ SpRNase III
SpCas9
long tracrRNA
short tracrRNA
DR-EMX1(1)-DR
120nt
110nt
100nt
90nt
80nt
70nt
60nt
50nt
40nt
30nt
U6

FIG. 12A-B

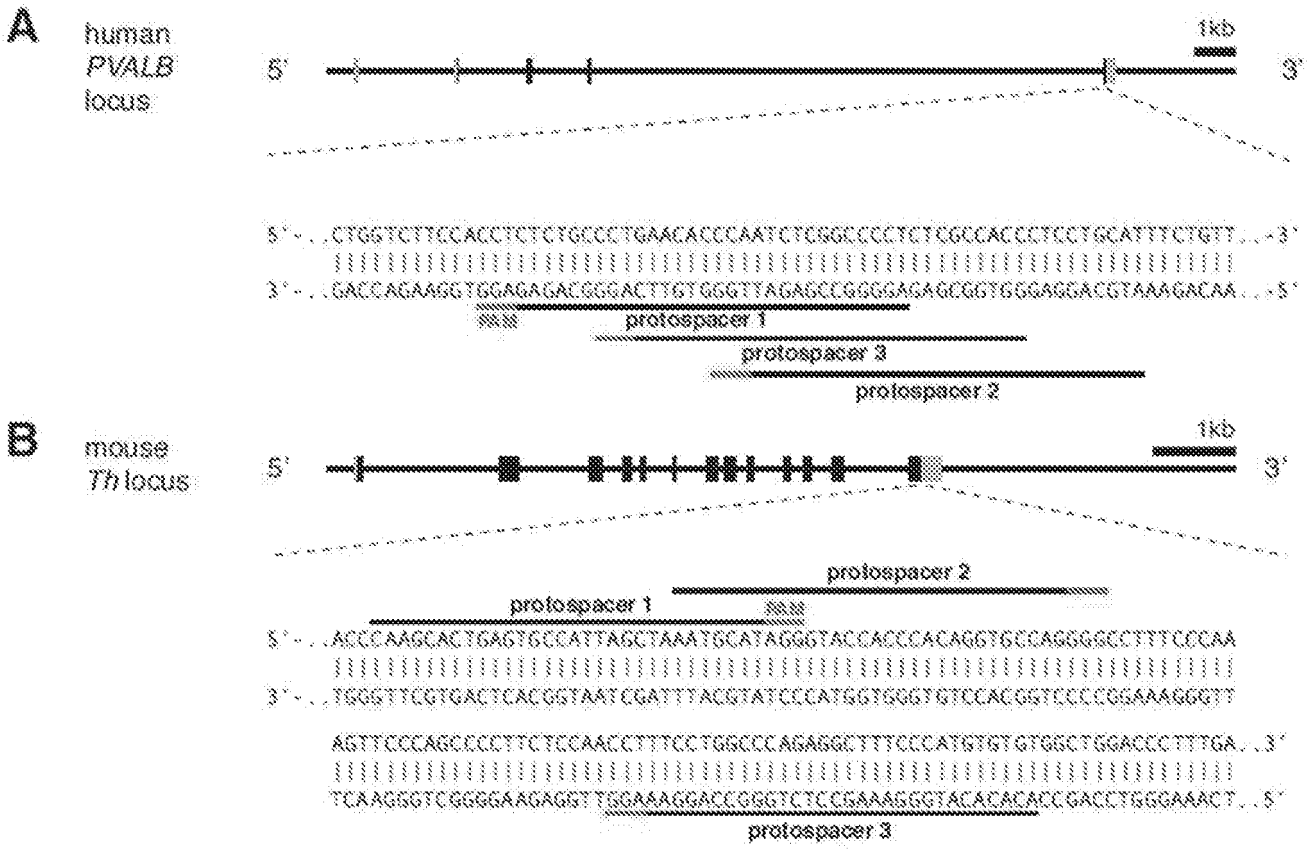

A
human
PVALB
locus
5'
1kb
3'
5'-..CTGGTCTTCCACCTCTCTGCCCTGAACACCCAATCTCGGCCCCTCTCGCCACCCTCCTGCATTTCTGTT..-3'
3'-..GACCAGAAGGTGGAGAGACGGGACTTGTGGGTTAGAGCCGGGGAGAGCGGTGGGAGGACGTAAAGACAA..-5'
protospacer 1
protospacer 3
protospacer 2
B
mouse
Th locus
5'
1kb
3'
protospacer 2
protospacer 1
5'-..ACCCAAGCACTGAGTGCCATTAGCTAAATGCATAGGGTACCACCCACAGGTGCCAGGGGCCTTTCCCAA
3'-..TGGGTTCGTGACTCACGGTAATCGATTTACGTATCCCATGGTGGGTGTCCACGGTCCCCGGAAAGGGTT
AGTTCCCAGCCCCTTCTCCAACCTTTCCTGGCCCAGAGGCTTTCCCATGTGTGTGGCTGGACCCTTTGA..3'
TCAAGGGTCGGGGAAGAGGTTGGAAAGGACCGGGTCTCCGAAAGGGTACACACACCGACCTGGGAAACT..5'
protospacer 3

FIG. 13A-B

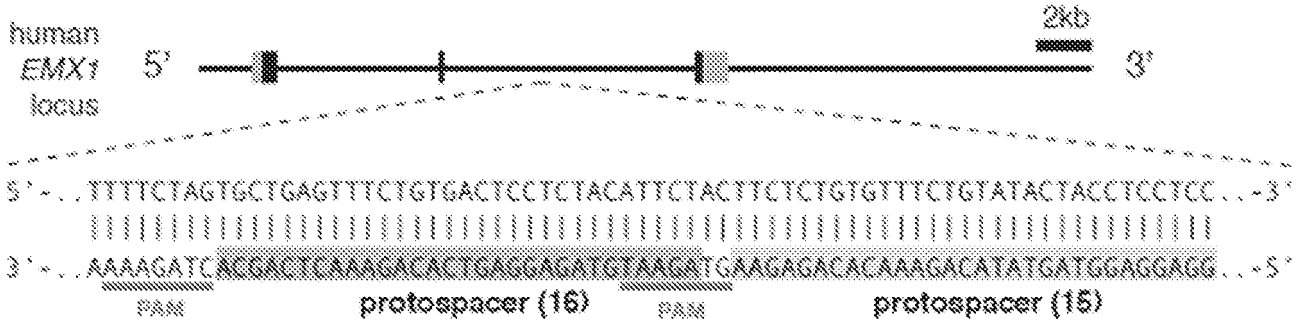

human
EMX1
locus
5'
2kb
3'
5'-..TTTTCTAGTGCTGAGTTTCTGTGACTCCTCTACATTCTACTTCTCTGTGTTTCTGTATACTACCTCCTCC..-3'
3'-..AAAAGATCACGACTCAAAGACACTGAGGAGATGTAAGATGAAGAGACACAAAGACATATGATGGAGGAGG..-5'
PAM
protospacer (16)
PAM
protospacer (15)

FIG. 14

| Primer name | Assay | Genomic Target | Primer sequence |
|---|---|---|---|
| Sp-EMX1-F | SURVEYOR assay, sequencing | *EMX1* | AAAACCACCCTTCTCTCTGGC |
| Sp-EMX1-R | SURVEYOR assay, sequencing | *EMX1* | GGAGATTGGAGACACGGAGAG |
| Sp-PVALB-F | SURVEYOR assay, sequencing | *PVALB* | CTGGAAAGCCAATGCCTGAC |
| Sp-PVALB-R | SURVEYOR assay, sequencing | *PVALB* | GGCAGCAAACTCCTTGTCCT |
| Sp-Th-F | SURVEYOR assay, sequencing | *Th* | GTGCTTTGCAGAGGCCTACC |
| Sp-Th-R | SURVEYOR assay, sequencing | *Th* | CCTGGAGCGCATGCAGTAGT |
| St-EMX1-F | SURVEYOR assay, sequencing | *EMX1* | ACCTTCTGTGTTTCCACCATTC |
| St-EMX1-R | SURVEYOR assay, sequencing | *EMX1* | TTGGGGAGTGCACAGACTTC |
| Sp-EMX1-RFLP-F | RFLP, sequencing | *EMX1* | GGCTCCCTGGGTTCAAAGTA |
| Sp-EMX1-RFLP-R | RFLP, sequencing | *EMX1* | AGAGGGGTCTGGATGTCGTAA |
| Pb_EMX1_sp1 | Northern Blot Probe | Not applicable | TAGCTCTAAAACTTCTTCTTCTGCTCGGAC |
| Pb_tracrRNA | Northern Blot Probe | Not applicable | CTAGCCTTATTTTAACTTGCTATGCTGTTT |

FIG. 15

a

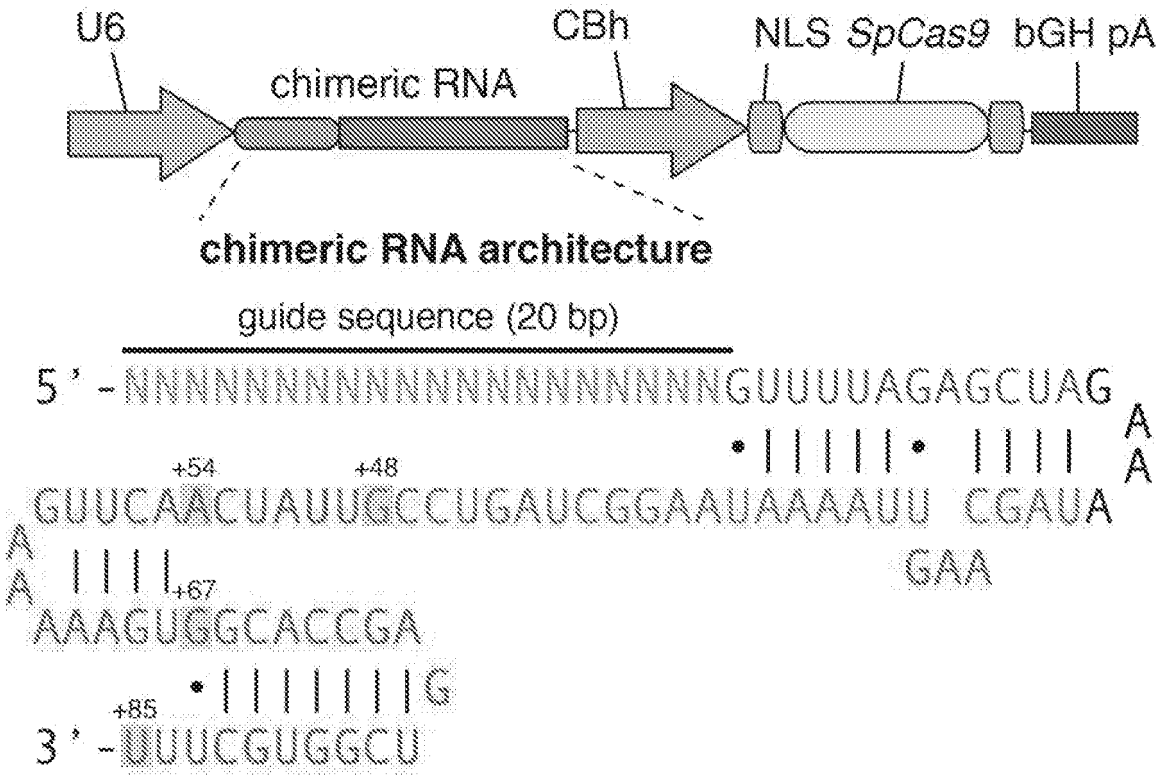

U6
CBh
NLS SpCas9 bGH pA
chimeric RNA
chimeric RNA architecture
guide sequence (20 bp)
5' - NNNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAG
A
A
+54
+48
GUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUA
A
A
GAA
+67
AAAGUGGCACCGA
G
+85
3' - UUUCGUGGCU

FIG. 16A

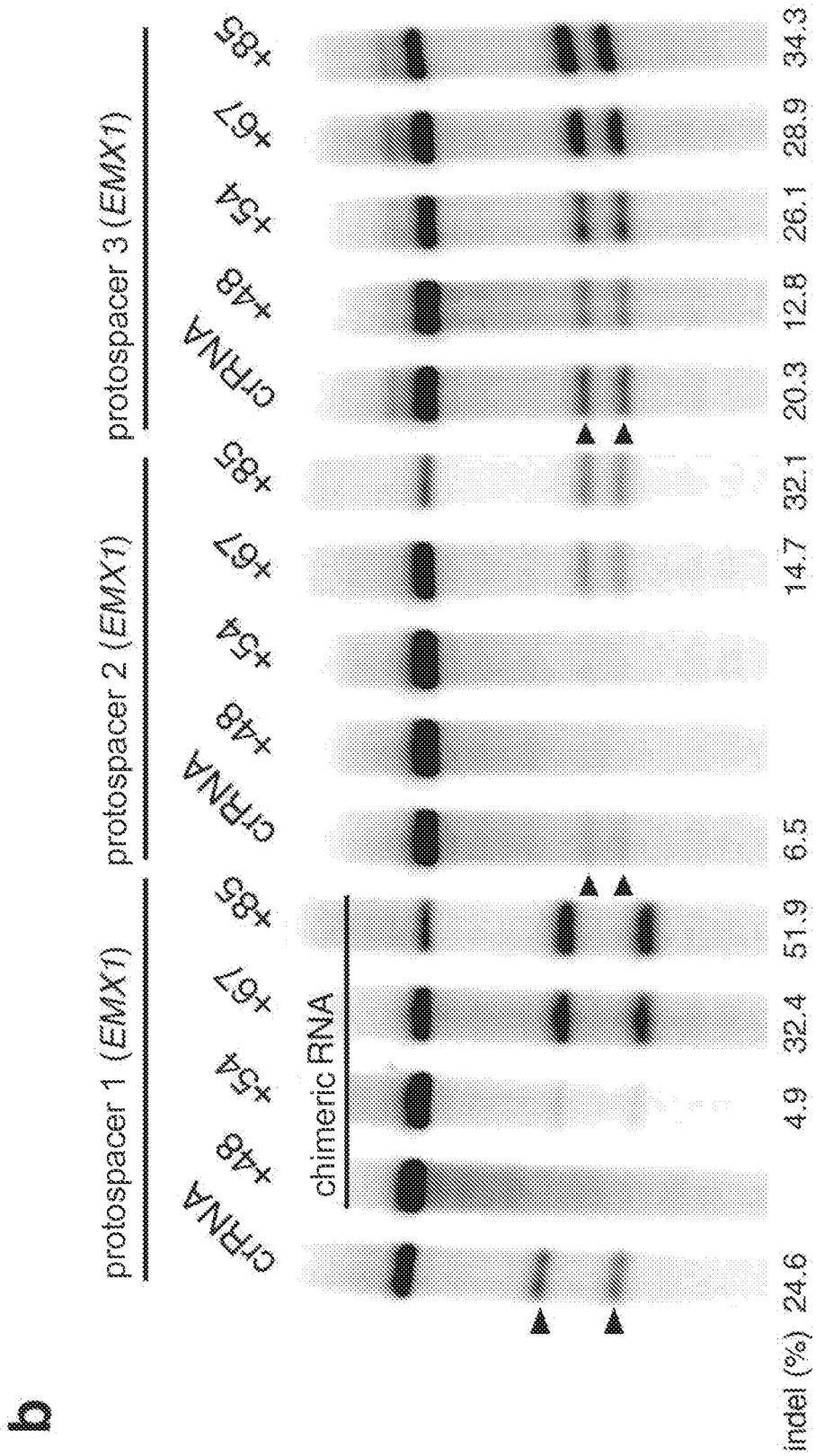

b
protospacer 1 (EMX1)
protospacer 2 (EMX1)
protospacer 3 (EMX1)
chimeric RNA
crRNA
+48
+54
+67
+85
indel (%) 24.6 4.9 32.4 51.9 6.5 14.7 32.1 20.3 12.8 26.1 28.9 34.3

FIG. 16B

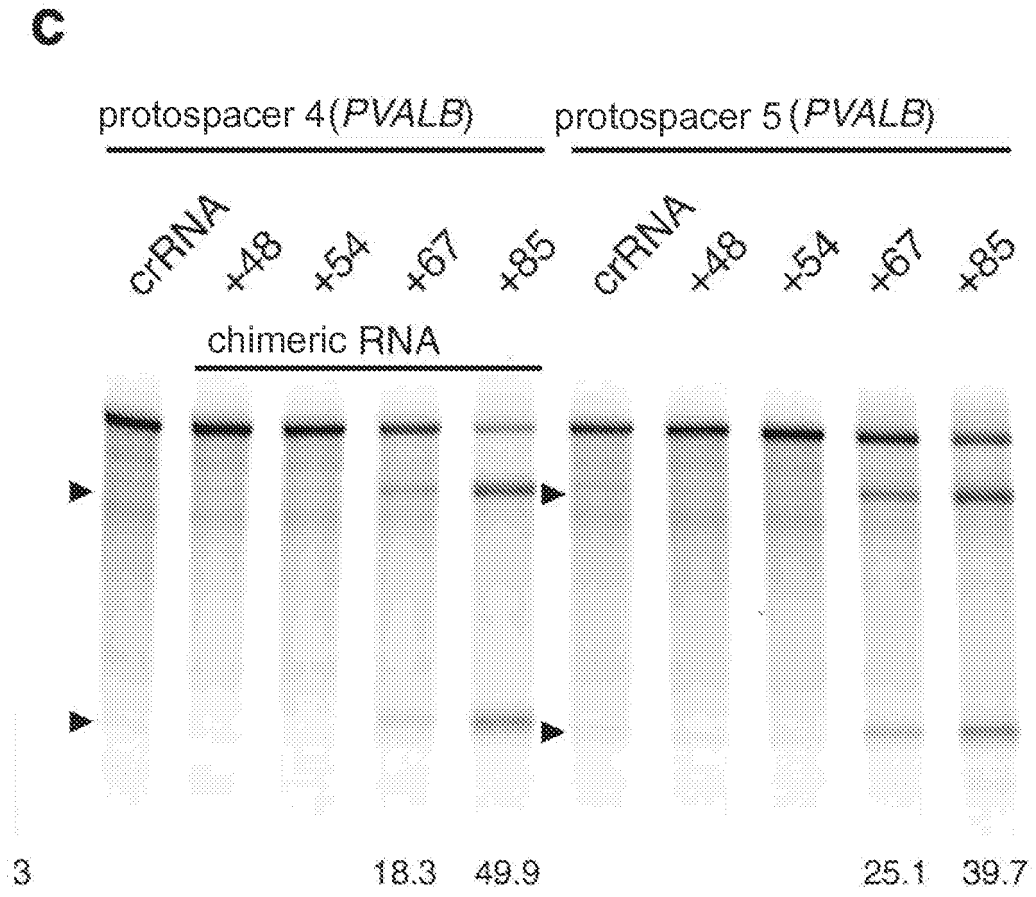
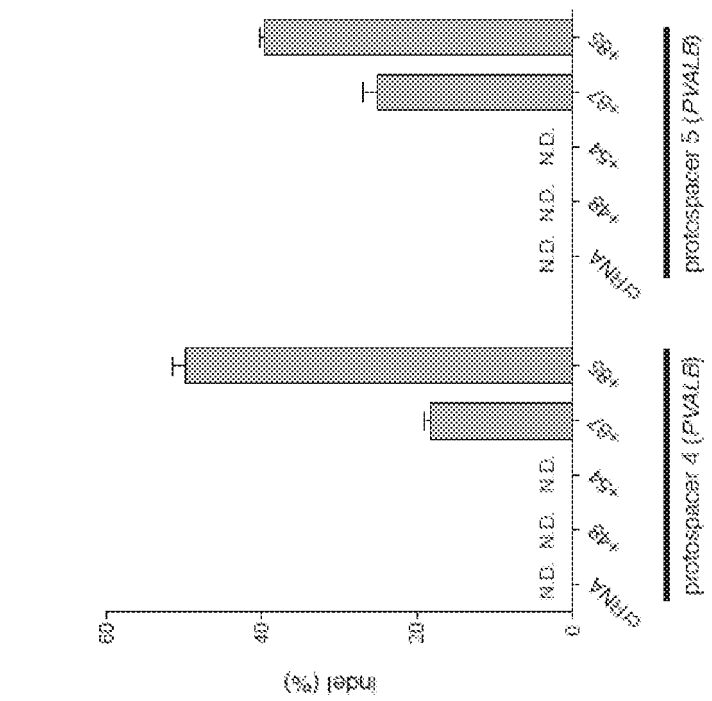

C
protospacer 4 (PVALB)
protospacer 5 (PVALB)
crRNA
+48
+54
+67
+85
crRNA
+48
+54
+67
+85
chimeric RNA
3
18.3
49.9
25.1
39.7

FIG. 16C


b
Indel (%)
0
20
40
60
gRNA
+48
+54
+67
+85
N.D. N.D. N.D.
N.D. N.D. N.D.
protospacer 4 (PVALB)
protospacer 5 (PVALB)

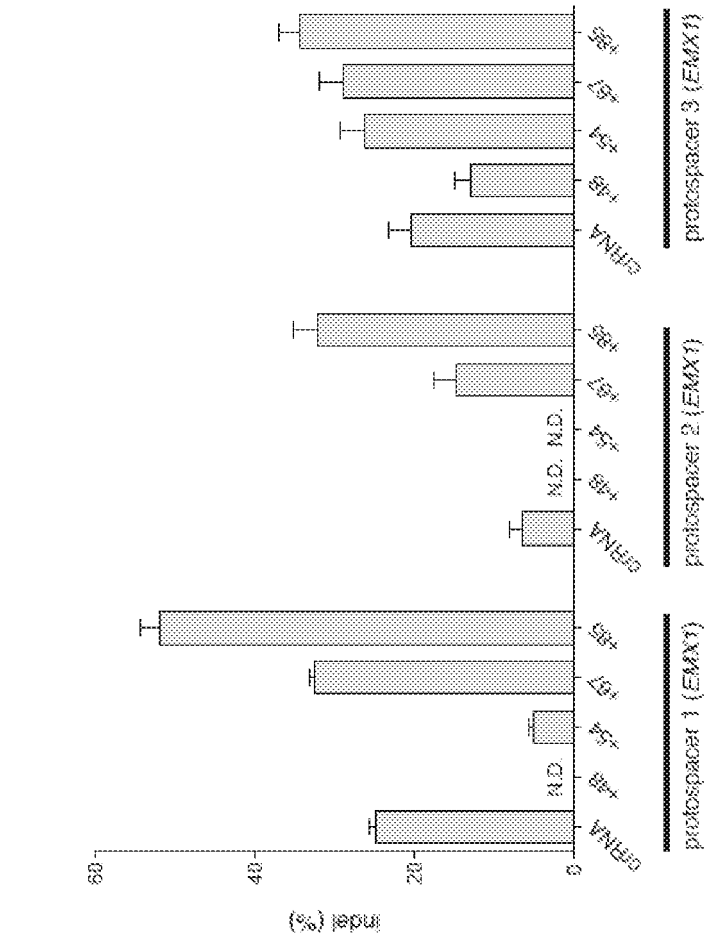

a
Indel (%)
0
20
40
60
gRNA
+48
+54
+67
+85
N.D
N.D. N.D.
protospacer 1 (EMX1)
protospacer 2 (EMX1)
protospacer 3 (EMX1)

FIG. 17A-B

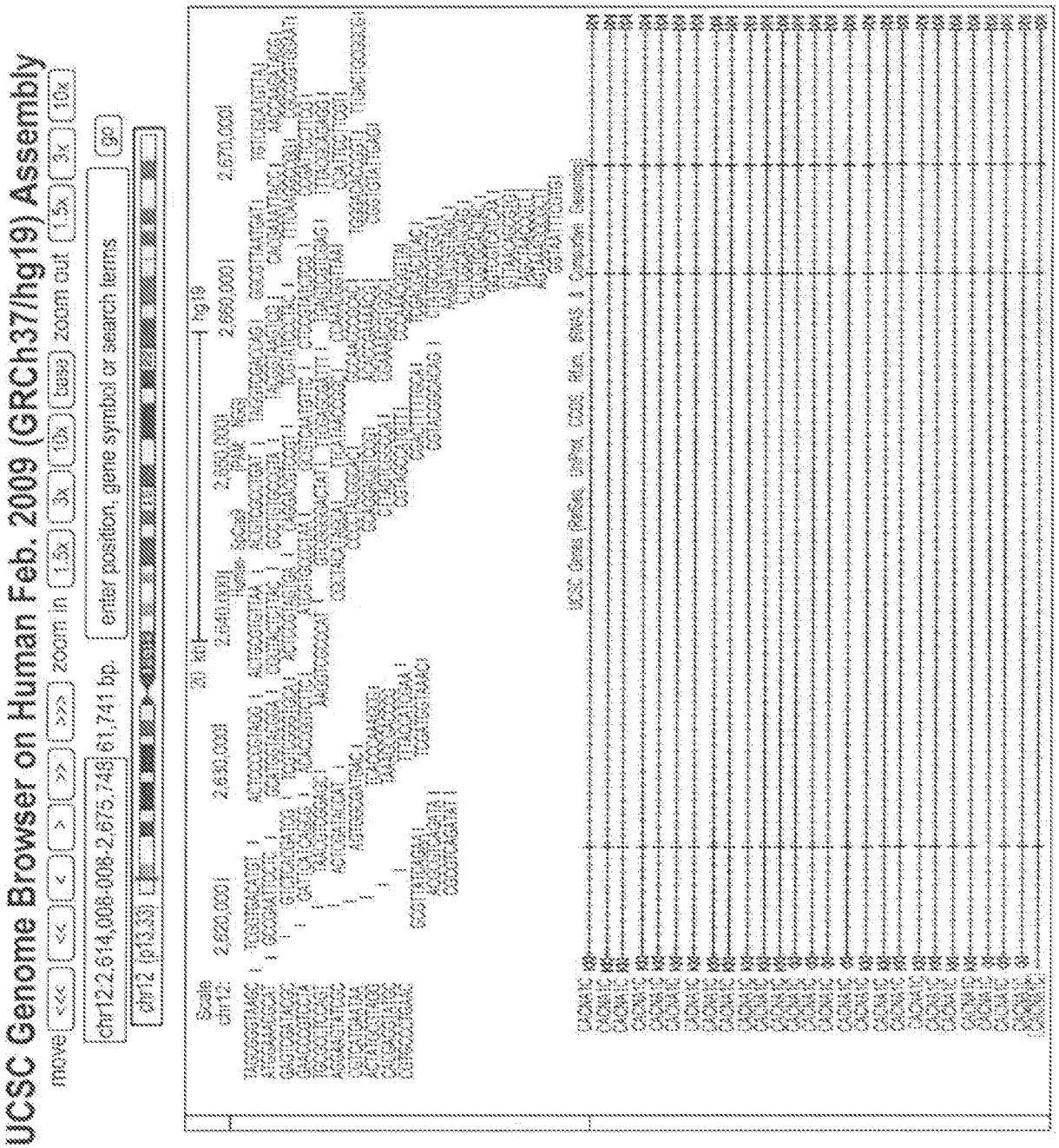

UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly
move
zoom in
zoom out
enter position, gene symbol or search terms
go
chr12:2,614,008-2,675,748
61,741 bp.
chr12 (p13.33)
Scale
chr12
20 kb
hg19
2,620,000
2,630,000
2,640,000
2,650,000
2,660,000
2,670,000
UCSC Genes (RefSeq, UniProt, CCDS, tRNA, RNAi) Composite Sequence

FIG. 18

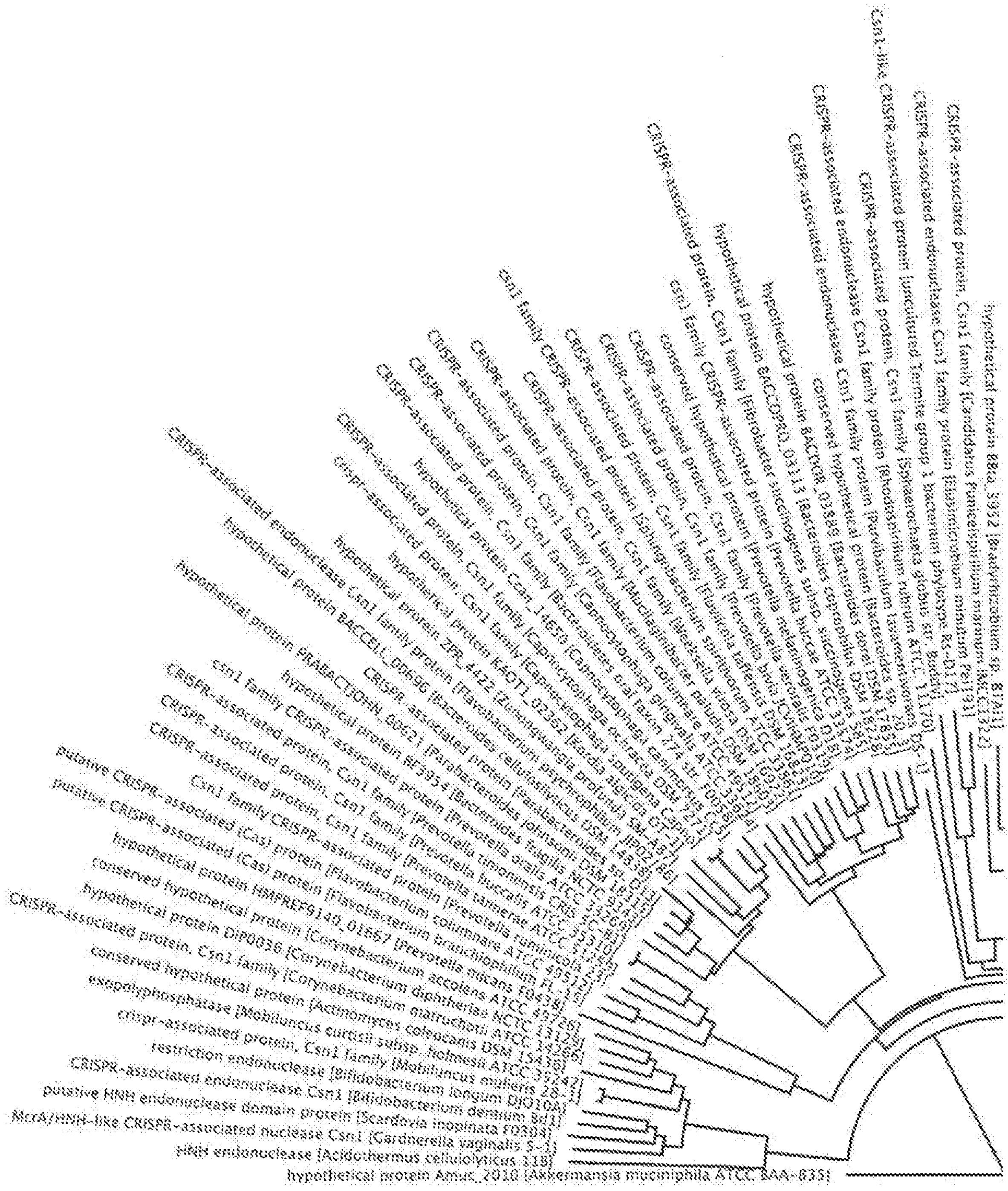


FIG. 19A



FIG. 19B

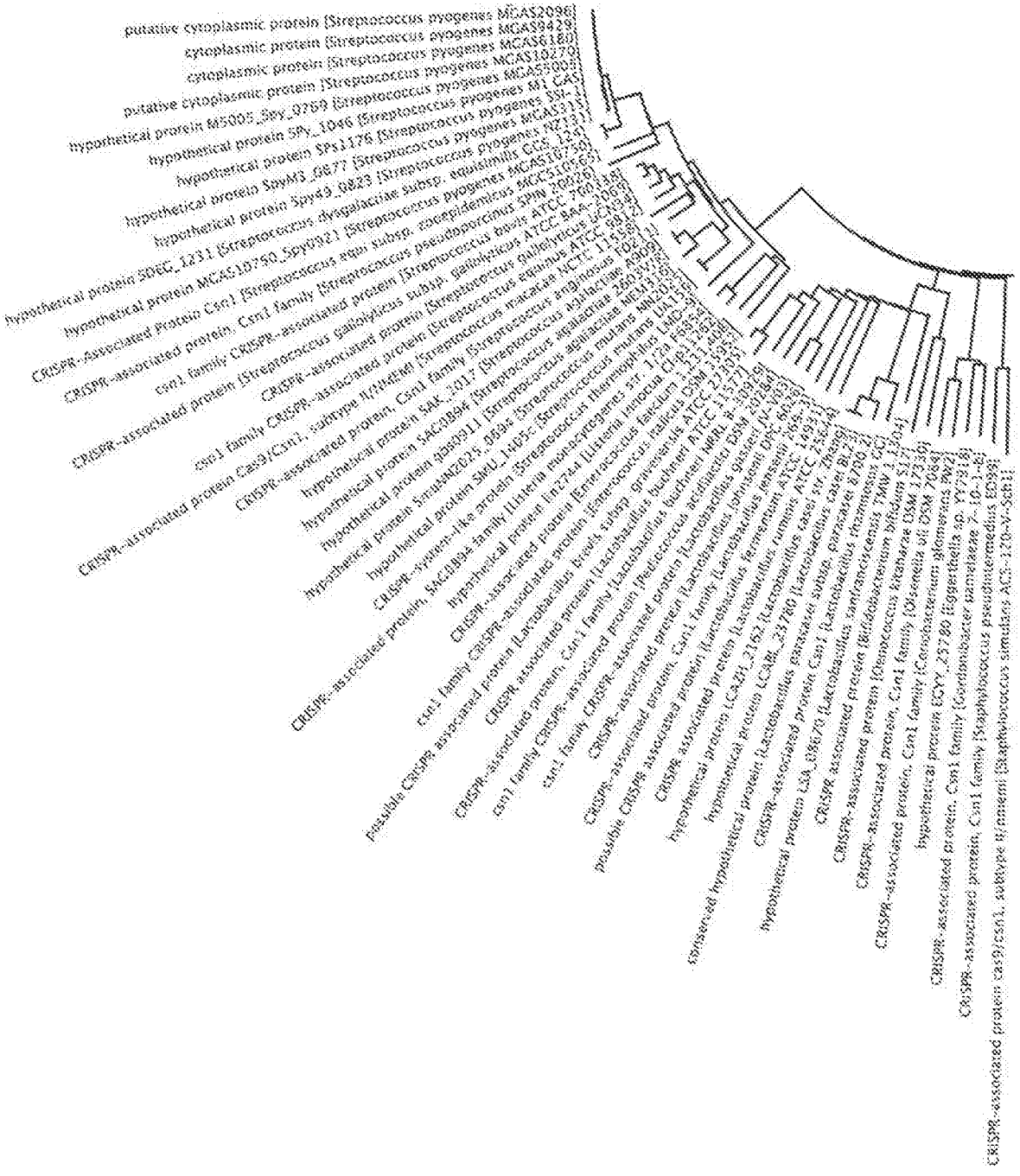

putative cytoplasmic protein [Streptococcus pyogenes MGAS2096]
cytoplasmic protein [Streptococcus pyogenes MGAS9429]
cytoplasmic protein [Streptococcus pyogenes MGAS6180]
putative cytoplasmic protein [Streptococcus pyogenes MGAS10270]
hypothetical protein M5005_Spy_0769 [Streptococcus pyogenes MGAS5005]
hypothetical protein SPy_1046 [Streptococcus pyogenes M1 GAS]
hypothetical protein SPy1176 [Streptococcus pyogenes MGAS315]
hypothetical protein SpyM3_0877 [Streptococcus pyogenes MGAS10750]
hypothetical protein Spy49_0823 [Streptococcus pyogenes MGAS10394]
hypothetical protein SDEG_1231 [Streptococcus dysgalactiae subsp. equisimilis GGS_124]
hypothetical protein MGAS10750_Spy0921 [Streptococcus pyogenes MGAS10750]
CRISPR-associated protein Csn1 [Streptococcus agalactiae 2603V/R]
CRISPR-associated protein, Csn1 family [Streptococcus pseudoporcinus SPIN 20026]
Csn1 family CRISPR-associated protein [Streptococcus gallolyticus UCN34]
CRISPR-associated protein [Streptococcus gallolyticus ATCC BAA-2069]
CRISPR-associated protein [Streptococcus equinus ATCC 700338]
Csn1 family CRISPR-associated protein [Streptococcus agalactiae NCTC 11079]
CRISPR-associated protein Csn3/Csn1, subtype II/NMEM [Streptococcus agalactiae 2603V/R]
hypothetical protein, Csn1 family [Streptococcus agalactiae ATCC 27591]
hypothetical protein SAK_1017 [Streptococcus agalactiae 18RS21]
hypothetical protein SAG3846 [Streptococcus agalactiae NEM316]
CRISPR-associated protein gbs0911 [Streptococcus agalactiae 2603V/R]
hypothetical protein SAN2MGH2025_0896 [Streptococcus agalactiae MGH 2025]
hypothetical protein SAUL_1485c [Streptococcus agalactiae 09mas018883]
CRISPR-system-like protein [Streptococcus agalactiae GD201008-001]
CRISPR-associated protein, SAG1896 family [Streptococcus agalactiae CJB111]
hypothetical protein lin2744 [Listeria monocytogenes str. 1/2a F6854]
CRISPR-associated protein [Streptococcus mutans UA159]
CRISPR-associated protein [Lactobacillus brevis subsp. gravesensis ATCC 27305]
csn1 family CRISPR-associated protein [Lactobacillus buchneri NRRL B-30929]
CRISPR-associated protein, Csn1 family [Pediococcus acidilactici DSM 20284]
csn1 family CRISPR-associated protein [Lactobacillus gasseri JV-V03]
possible CRISPR associated protein [Lactobacillus helveticus DPC 4571]
CRISPR-associated protein, Csn1 family [Lactobacillus rhamnosus ATCC 21052]
CRISPR associated protein [Lactobacillus rhamnosus ATCC 53103]
hypothetical protein LCABL_21610 [Lactobacillus jensenii 269.3]
hypothetical protein LCAS_23780 [Lactobacillus casei str. Zhang]
CRISPR-associated protein [Lactobacillus paracasei subsp. paracasei 8700:2]
hypothetical protein LSA_03670 [Lactobacillus casei BL23]
CRISPR associated protein [Bifidobacterium longum DJO10A]
CRISPR-associated protein [Olsenella uli DSM 7084]
CRISPR-associated protein, Csn1 family [Olsenella uli DSM 7084]
hypothetical protein EGY_25780 [Eggerthella sp. YY7918]
CRISPR-associated protein, Csn1 family [Coriobacterium glomerans PW2]
CRISPR-associated protein, Csn1 family [Gordonibacter pamelaeae 7-10-1-b]
CRISPR-associated protein Csn3/Csn1, subtype II/nmeni [Staphylococcus pseudintermedius ED99]
CRISPR-associated protein Csn3/Csn1, subtype II/nmeni [Staphylococcus simulans ACC-120-V-Sch1]

FIG. 19C

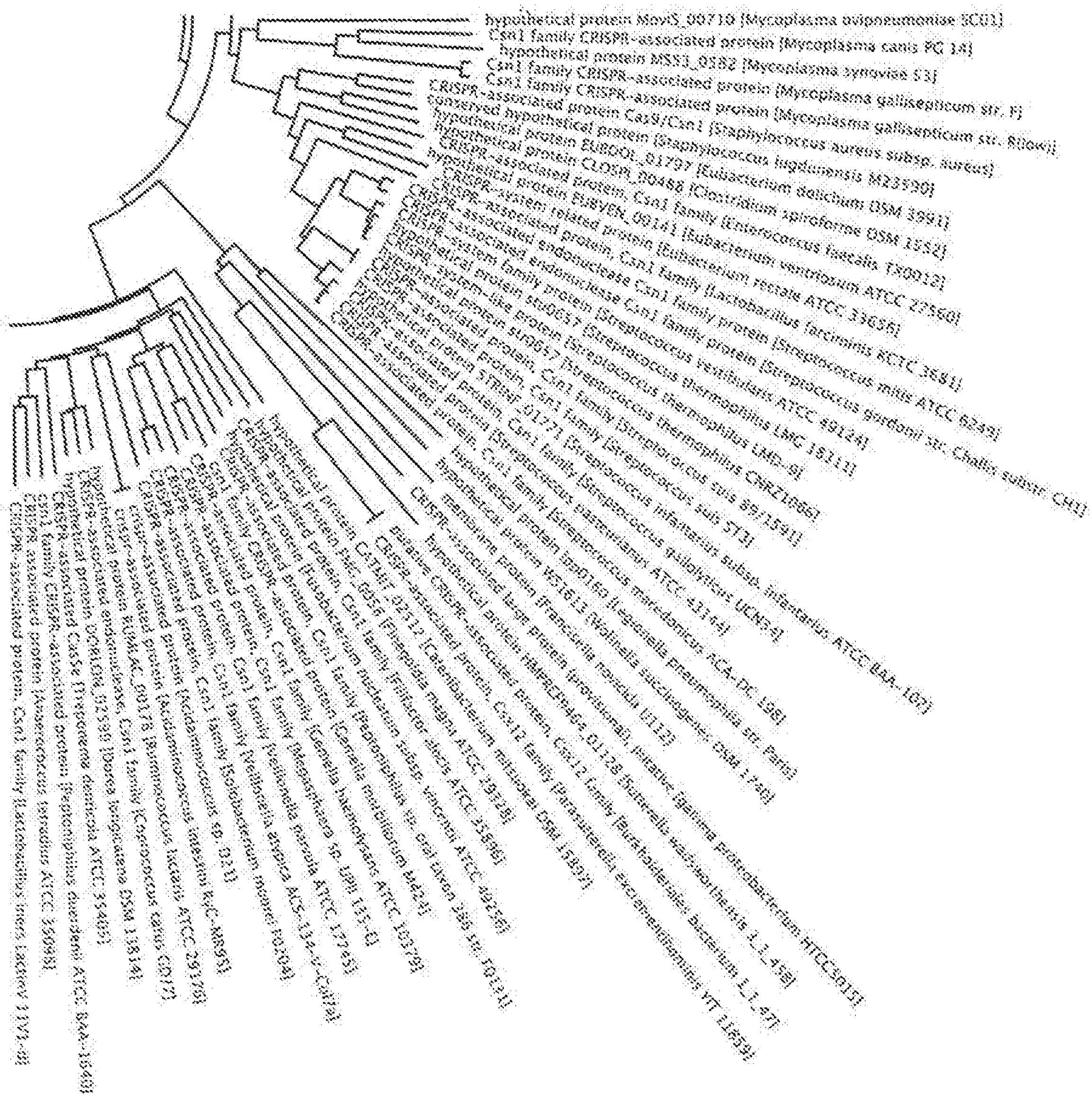

hypothetical protein MoviS_00710 [Mycoplasma ovipneumoniae SC01]
Csn1 family CRISPR-associated protein [Mycoplasma canis PG 14]
hypothetical protein MSS3_0582 [Mycoplasma synoviae 53]
Csn1 family CRISPR-associated protein [Mycoplasma gallisepticum str. F]

FIG. 19D



FIG. 20A



FIG. 20B

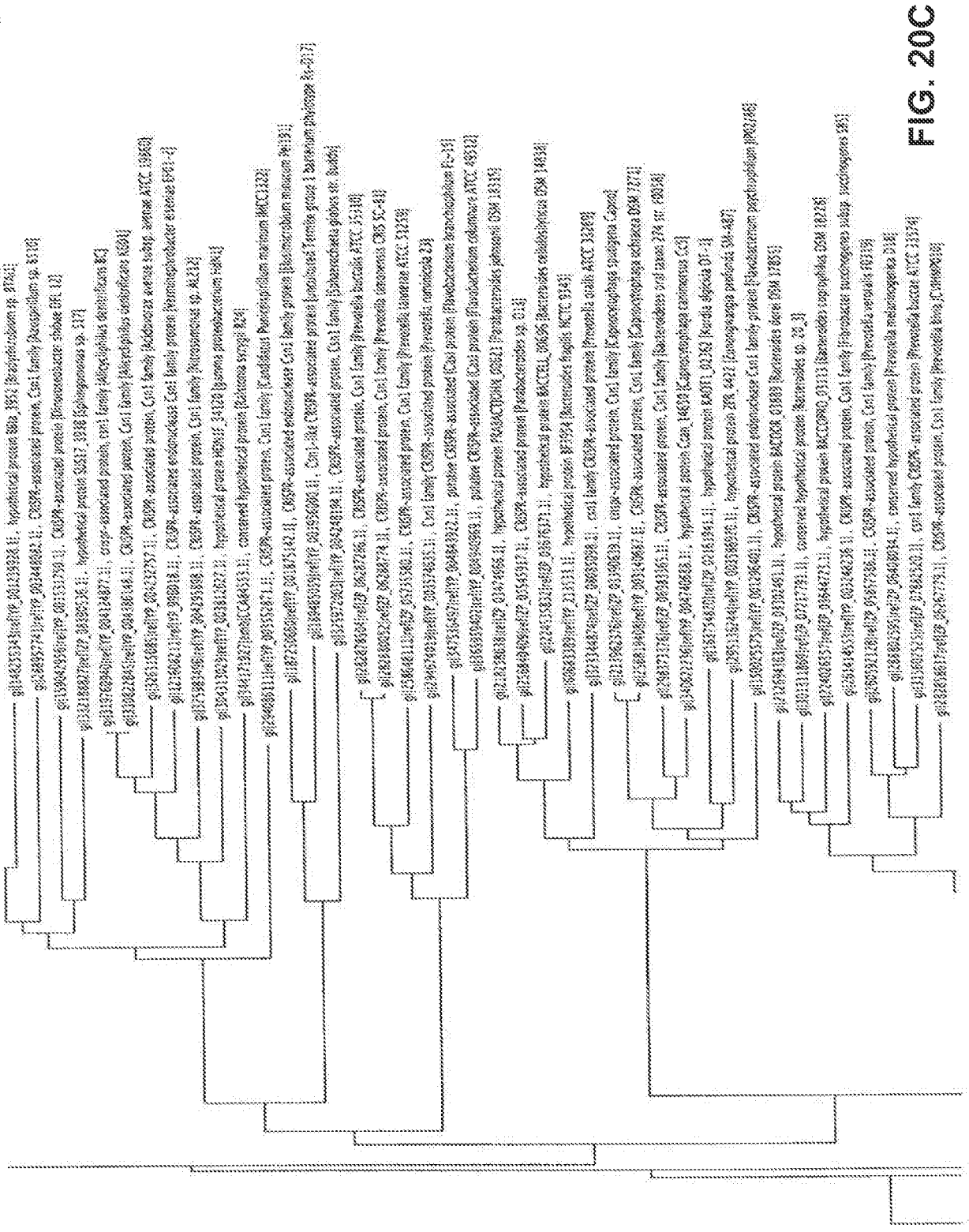


FIG. 20C

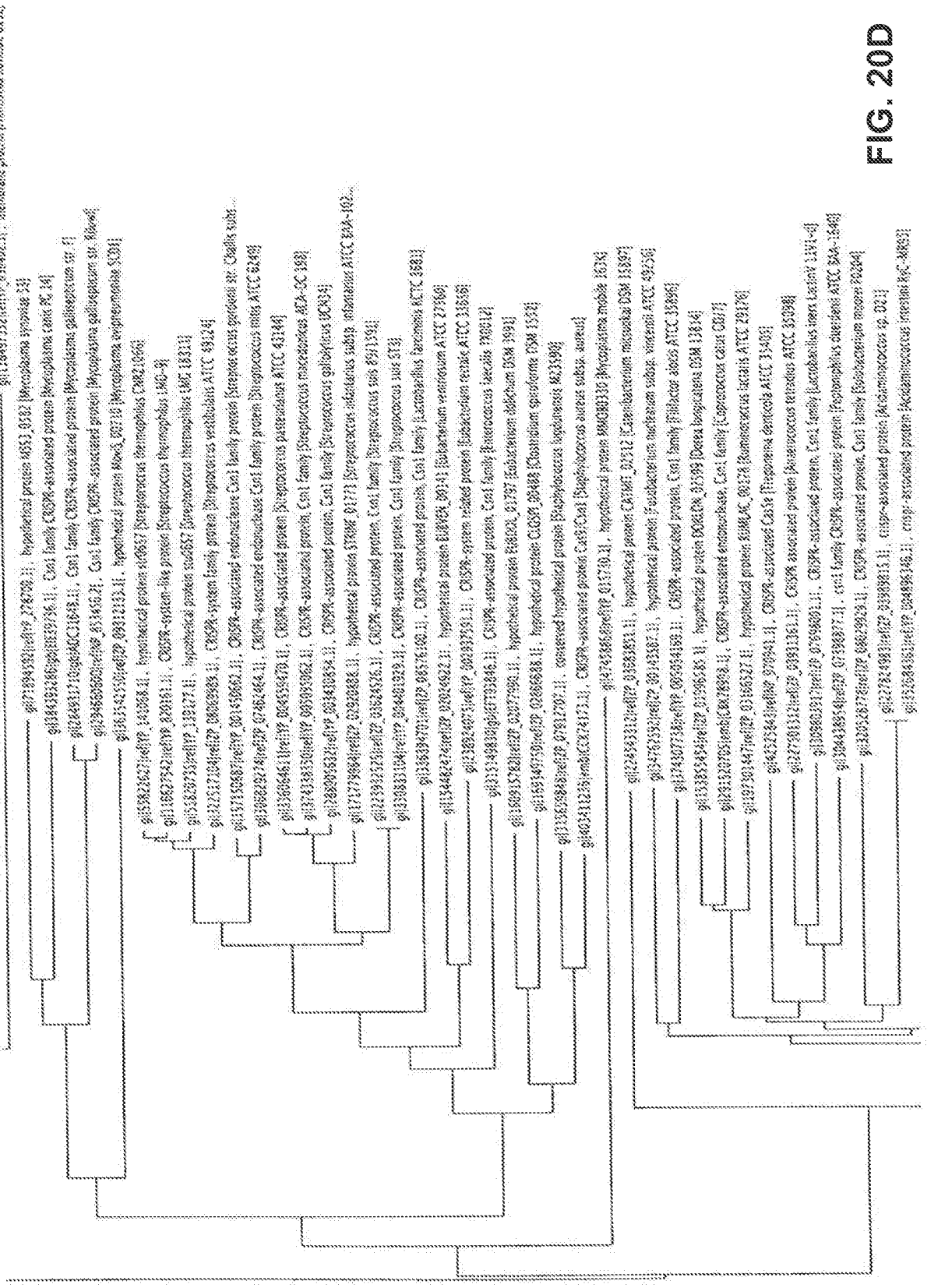


FIG. 20D

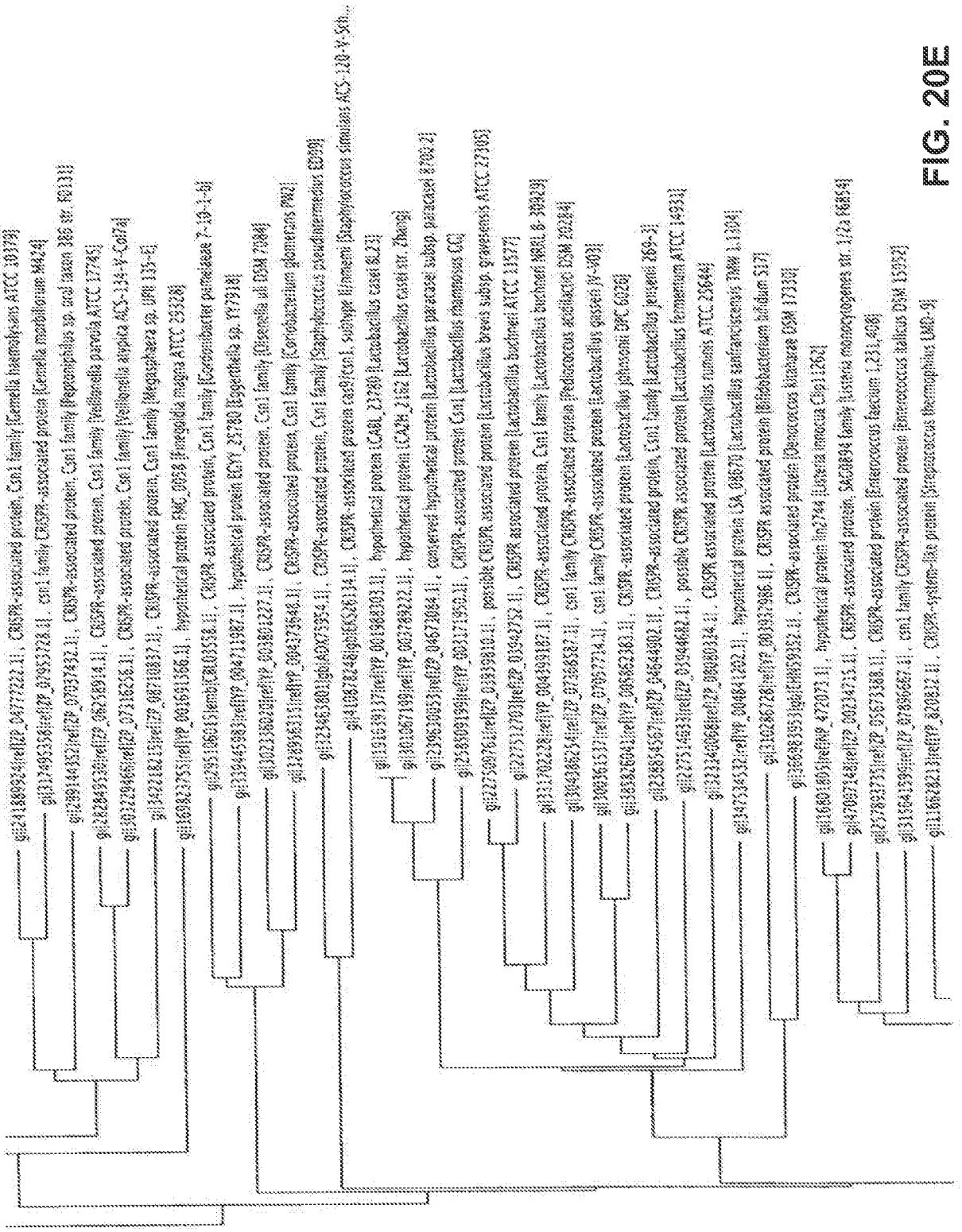

gi|241889934|ref|ZP_04777222.1|, CRISPR-associated protein, Cas1 family [Kemella haemolysans ATCC 10379]
gi|213695356|ref|ZP_03053728.1|, cas1 family CRISPR-associated protein [Kemella moltiformis M424]
gi|239914032|ref|ZP_07037432.1|, CRISPR-associated protein, Cas1 family [Propionibacterium sp. oral taxon 386 str. W5131]
gi|282849338|ref|ZP_06258914.1|, CRISPR-associated protein, Cas1 family [Veillonella parvula ATCC 17745]
gi|302329466|ref|ZP_07256256.1|, CRISPR-associated protein, Cas1 family [Veillonella atypica ACS-134-V-Col7a]
gi|342211812|ref|ZP_08710837.1|, CRISPR-associated protein, Cas1 family [Megasphaera sp. UPII 135-E]
gi|160932375|ref|YP_001561366.1|, hypothetical protein PMC_0038 [Paenibacillus mucilaginosus KNP414]
gi|325118618|emb|CBZ03558.1|, CRISPR-associated protein, Cas1 family [Lactobacillus paracasei 7-10-1-4]
gi|335445983|ref|YP_004711987.1|, hypothetical protein KCYT_21780 [Kingella oralis ATCC 51911]
gi|302213882|ref|YP_003081227.1|, CRISPR-associated protein, Cas1 family [Slackia exigua ATCC 700122]
gi|116095511|ref|YP_809437.1|, CRISPR-associated protein, Cas1 family [Lactobacillus plantarum WCFS1]
gi|323463601|gb|ADX73954.1|, CRISPR-associated protein, Cas1 family [Staphylococcus pseudintermedius ED99]
gi|418787424|gb|AKS03114.1|, CRISPR-associated protein cas1/cse1, subtype I [Staphylococcus simulans ACS-120-V-Sch...
gi|111018117|ref|YP_701088.1|, hypothetical protein rCAS_2228 [Lactobacillus casei BL23]
gi|301067199|ref|YP_3637822.1|, hypothetical protein LCABL_2162 [Lactobacillus casei str. Zhang]
gi|239636033|ref|ZP_04671084.1|, conserved hypothetical protein [Lactobacillus paracasei subsp. paracasei 8700:2]
gi|258509195|ref|YP_003171950.1|, CRISPR-associated protein Cas1 [Lactobacillus rhamnosus GG]
gi|227512760|ref|ZP_03942752.1|, possible CRISPR associated protein [Lactobacillus brevis subsp. gravesensis ATCC 27305]
gi|227512770|ref|ZP_03942752.1|, CRISPR associated protein [Lactobacillus buchneri ATCC 11577]
gi|331702228|ref|YP_004399187.1|, CRISPR-associated protein, Cas1 family [Lactobacillus buchneri NRRL B-30929]
gi|304436254|ref|ZP_07396237.1|, cas1 family CRISPR-associated protein [Peptoniphilus duerdenii ATCC BAA-1640]
gi|300361511|ref|ZP_07057714.1|, cas1 family CRISPR-associated protein [Lactobacillus gasseri JV-V03]
gi|336382604|ref|YP_004952360.1|, CRISPR-associated protein [Lactobacillus johnsonii DPC 6026]
gi|238854567|ref|ZP_04644002.1|, CRISPR-associated protein, Cas1 family [Lactobacillus jensenii 269-3]
gi|227514631|ref|ZP_03944682.1|, possible CRISPR associated protein [Lactobacillus fermentum ATCC 14931]
gi|312144668|ref|ZP_07020814.1|, CRISPR associated protein [Lactobacillus ruminis ATCC 25644]
gi|347734532|ref|YP_004841262.1|, hypothetical protein GA_00670 [Lactobacillus sanfranciscensis TMW 1.1304]
gi|310286728|ref|YP_003937986.1|, CRISPR associated protein [Bifidobacterium bifidum S17]
gi|336393935|gb|AEI33932.1|, CRISPR-associated protein [Oenococcus kitaharae DSM 17331]
gi|116618059|ref|YP_817071.1|, hypothetical protein Insp2244 [Listeria innocua Clip11262]
gi|347627148|ref|ZP_08823735.1|, CRISPR-associated protein, SACOL0894 family [Listeria monocytogenes str. 1/2a 08854]
gi|227891731|ref|ZP_03961388.1|, CRISPR-associated protein [Lactobacillus crispatus 1,231,408]
gi|315644199|ref|ZP_07896687.1|, cas1 family CRISPR-associated protein [Enterococcus italicus DSM 15952]
gi|116622813|ref|YP_820932.1|, CRISPR-system-like protein [Streptococcus thermophilus LMD-9]

FIG. 20E

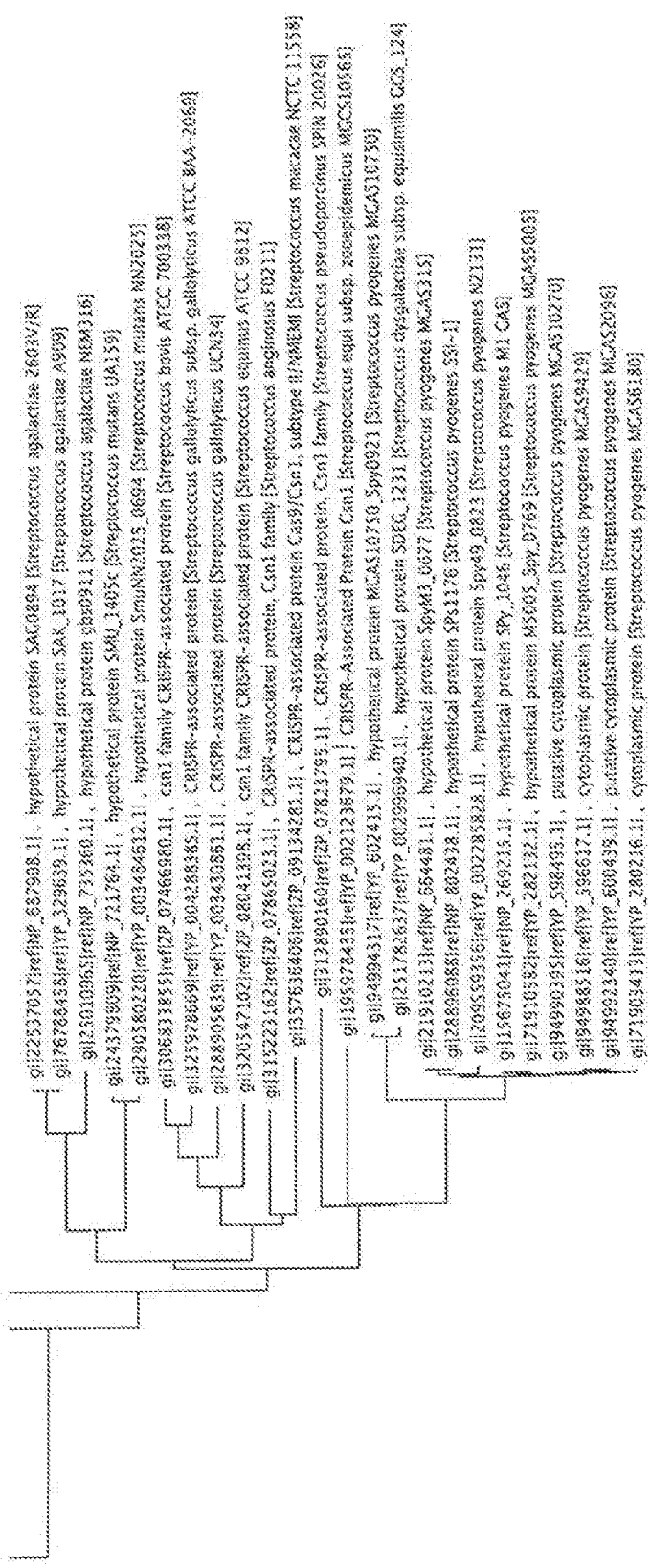

gi|22533705|ref|NP_687938.1| hypothetical protein SAG0894 [Streptococcus agalactiae 2603V/R]
gi|76788438|ref|YP_329639.1| hypothetical protein SAK_1017 [Streptococcus agalactiae A909]
gi|23501085|ref|NP_735360.1| hypothetical protein gbs0911 [Streptococcus agalactiae NEM316]
gi|24378009|ref|NP_731764.1| hypothetical protein SMU_1405c [Streptococcus mutans UA159]
gi|23018021|ref|YP_813484.1| hypothetical protein SmuNN2025_0694 [Streptococcus mutans NN2025]
gi|30681338|ref|ZP_07466980.1| Cas1 family CRISPR-associated protein [Streptococcus bovis ATCC 700338]
gi|32197866|ref|YP_004286385.1| CRISPR-associated protein [Streptococcus gallolyticus subsp. gallolyticus ATCC BAA-2069]
gi|28895638|ref|YP_803430851.1| CRISPR-associated protein [Streptococcus gallolyticus UCN34]
gi|32034702|ref|ZP_08041998.1| cas1 family CRISPR-associated protein [Streptococcus equinus ATCC 9812]
gi|31322162|ref|ZP_07665023.1| CRISPR-associated protein, Cas1 family [Streptococcus anginosus F0211]
gi|13576384|ref|ZP_09134281.1| CRISPR-associated protein Cas9/Csn1, subtype II/NMEN4 [Streptococcus mutacae NCTC 11558]
gi|31389160|ref|ZP_07623795.1| CRISPR-associated protein, Csn1 family [Streptococcus pseudoporcinus SPIK 200261]
gi|19597843|ref|YP_002123879.1| CRISPR-Associated Protein Csn1 [Streptococcus equi subsp. zooepidemicus MGCS10565]
gi|94994317|ref|YP_602415.1| hypothetical protein MGAS10750_Spy0921 [Streptococcus pyogenes MGAS10750]
gi|25178263|ref|YP_002996940.1| hypothetical protein SDEG_1211 [Streptococcus dysgalactiae subsp. equisimilis GGS_124]
gi|21910213|ref|NP_664481.1| hypothetical protein Spy_0677 [Streptococcus pyogenes MGAS315]
gi|22889088|ref|NP_802428.1| hypothetical protein SPs1176 [Streptococcus pyogenes SSI-1]
gi|20955936|ref|YP_002285828.1| hypothetical protein Spy49_0823 [Streptococcus pyogenes NZ131]
gi|15676043|ref|NP_269218.1| hypothetical protein SPy_1048 [Streptococcus pyogenes M1 GAS]
gi|71910582|ref|YP_282182.1| hypothetical protein MS605_Spy_0769 [Streptococcus pyogenes MGAS10003]
gi|94990395|ref|YP_598493.1| putative cytoplasmic protein [Streptococcus pyogenes MGAS10270]
gi|94988516|ref|YP_596617.1| cytoplasmic protein [Streptococcus pyogenes MGAS9429]
gi|94992340|ref|YP_600438.1| putative cytoplasmic protein [Streptococcus pyogenes MGAS2096]
gi|71903413|ref|YP_280216.1| cytoplasmic protein [Streptococcus pyogenes MGAS6180]

FIG. 20F

a

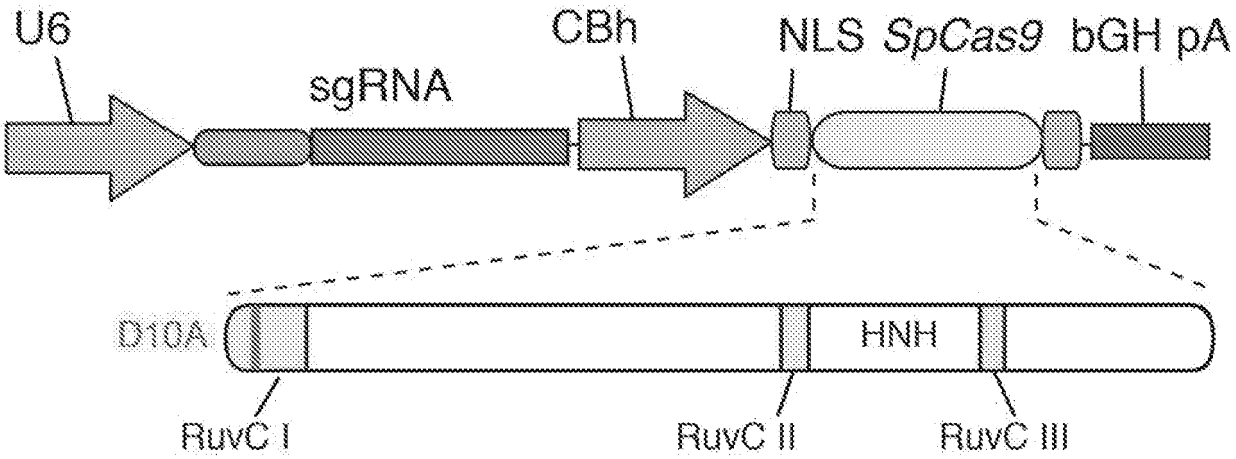



FIG. 21A

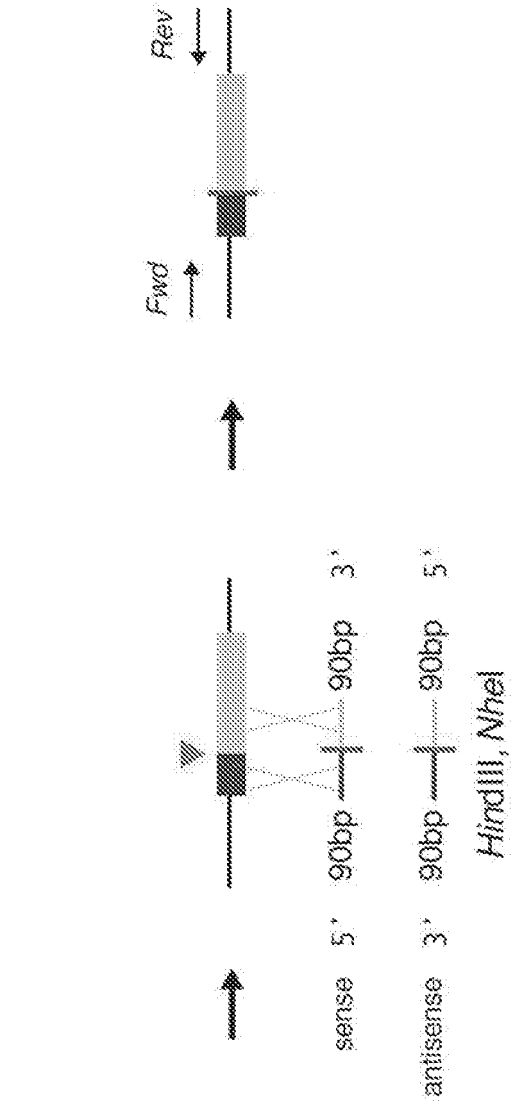

b
hEMX1 locus
ssODN
repair template
Fwd
Rev
sense
5'
90bp
90bp
3'
antisense
3'
90bp
90bp
5'
HindIII, NheI

FIG. 21B

C
hEMX1 locus
genomic target
PAM
5'-...CAGAAGAAGAAGGGC...CCAATGGGGAGGACATCGATGTCACCTCCAATGACTAGGGTGGTGGGCAAC...CTCTGGCCACTCCCT...-3'
3'-...GTCTTCTTCTTCCCG...GGTTACCCCTCCTGTAGCTACAGTGGAGGTTACTGATCCCCACCACCCGTTG...GAGACCGGTGAGGGA...-5'
sense 5'- CAGAAGAAGAAGGGC...ACATCGATGTCACCTCCAATGACAAGCTTGCTAGTGGTGGGCAACCACAAAC...CTCTGGCCACTCCCT -3'
HindIII NheI
antisense 3'- GTCTTCTTCTTCCCG...TGTAGCTACAGTGGAGGTTACTGTTCGAACGATCGGCCACCCGTTGGTGTTTG...GAGACCGGTGAGGGA -5'
HindIII NheI

FIG. 21C

d

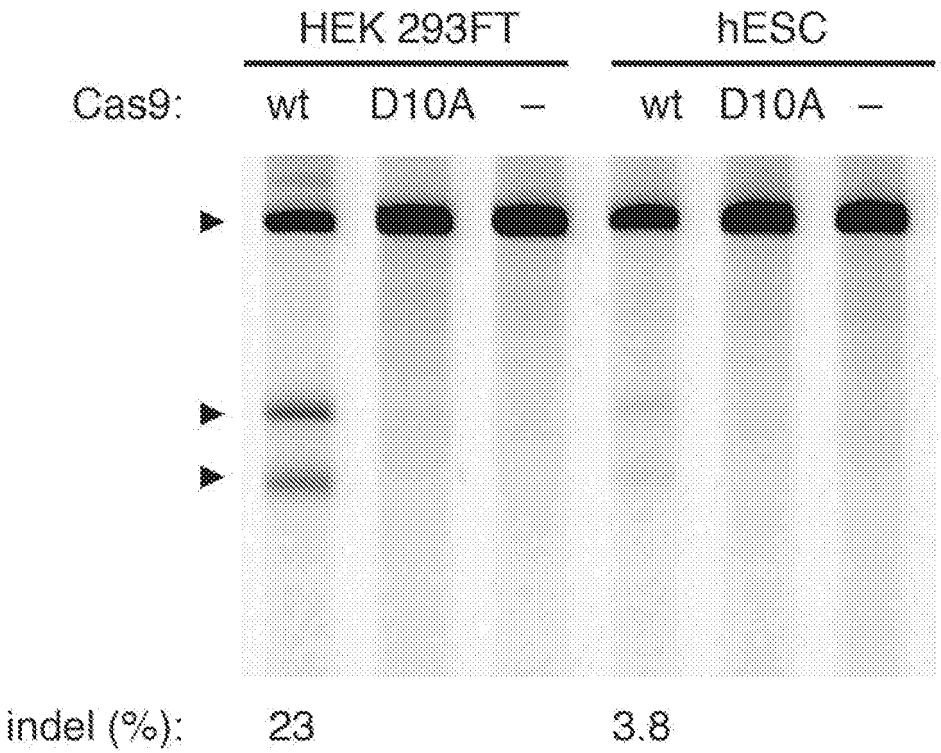



FIG. 21D

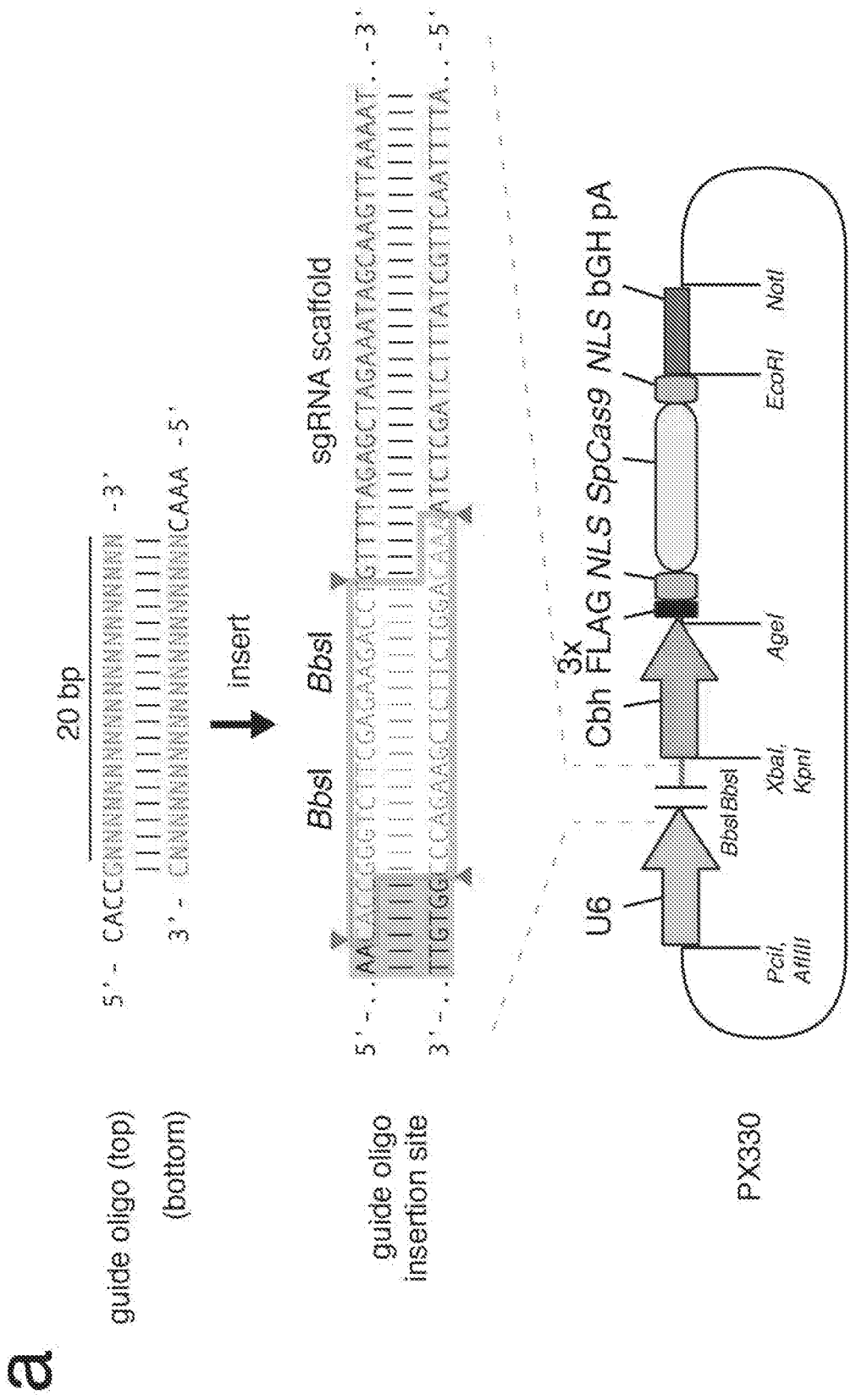

a
guide oligo (top)
(bottom)
20 bp
5' - CACCNNNNNNNNNNNNNNNNNNN - 3'
3' - CNNNNNNNNNNNNNNNNNNNCAAA - 5'
insert
BbsI
BbsI
sgRNA scaffold
guide oligo insertion site
5' - ..AACACCGGTCTTCGAGAAGACC GTTTTAGAGCTAGAAATAGCAAGTTAAAAT..-3'
3' - ..TTGTGGCCAGAAGCTCTTCTGGA CAAAATCTCGATCTTTATCGTTCAATTTTA..-5'
PX330
U6
3x
Cbh FLAG NLS SpCas9 NLS bGH pA
PciI, AflIII
BbsI BbsI
XbaI, KpnI
AgeI
EcoRI
NotI

FIG. 22A

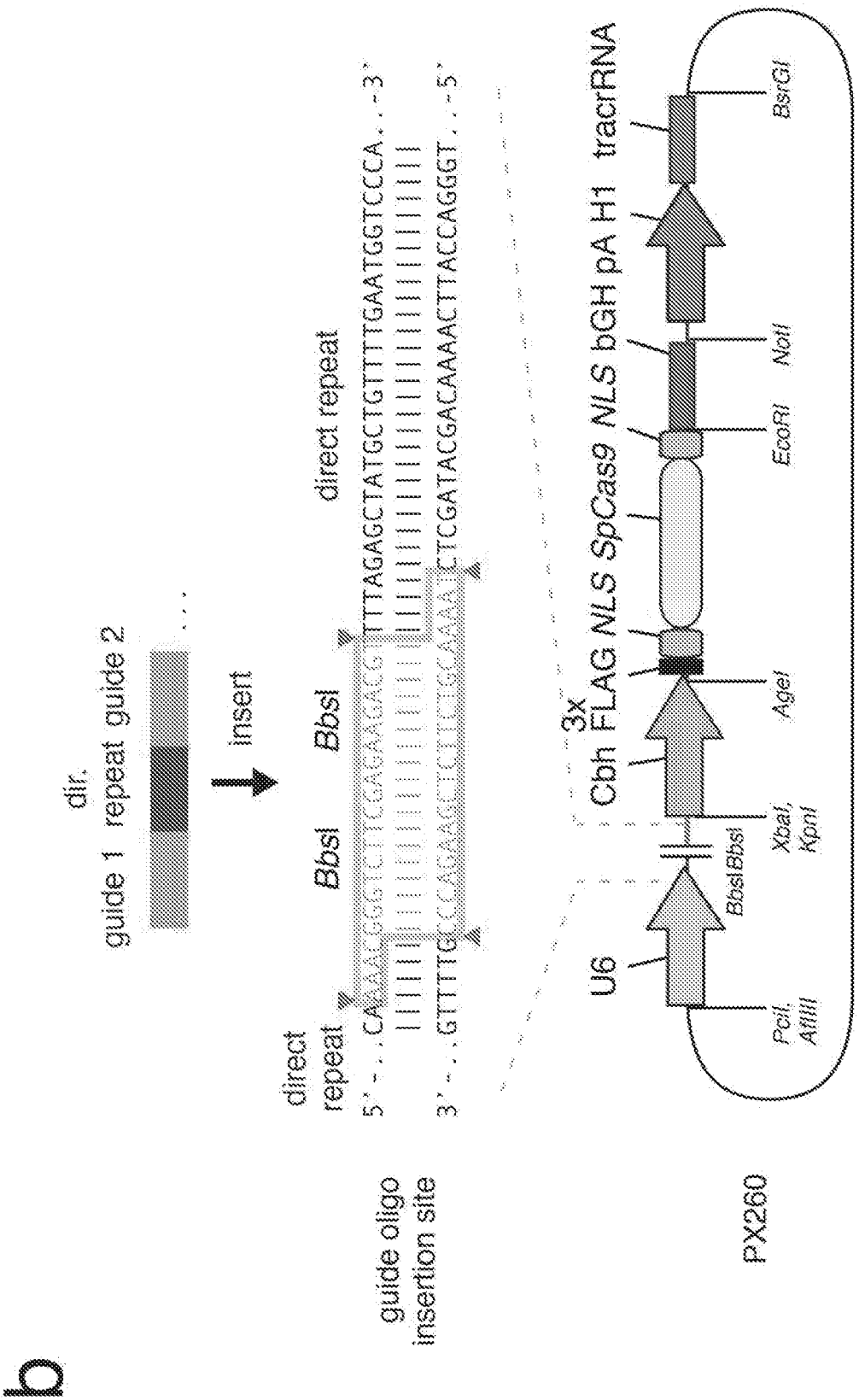

b
dir.
guide 1 repeat guide 2
insert
BbsI
BbsI
direct repeat
direct repeat
5' - . . . CA
TTTAGAGCTATGCTGTTTTGAATGGTCCCA. . . -3'
3' - . . . GTTTTG
CTCGATACGACAAAACTTACCAGGGT. . . -5'
guide oligo insertion site
3x
U6
Cbh FLAG NLS SpCas9 NLS bGH pA H1 tracrRNA
PX260
PciI, AflIII
BbsI BbsI
XbaI, KpnI
AgeI
EcoRI
NotI
BsrGI

FIG. 22B

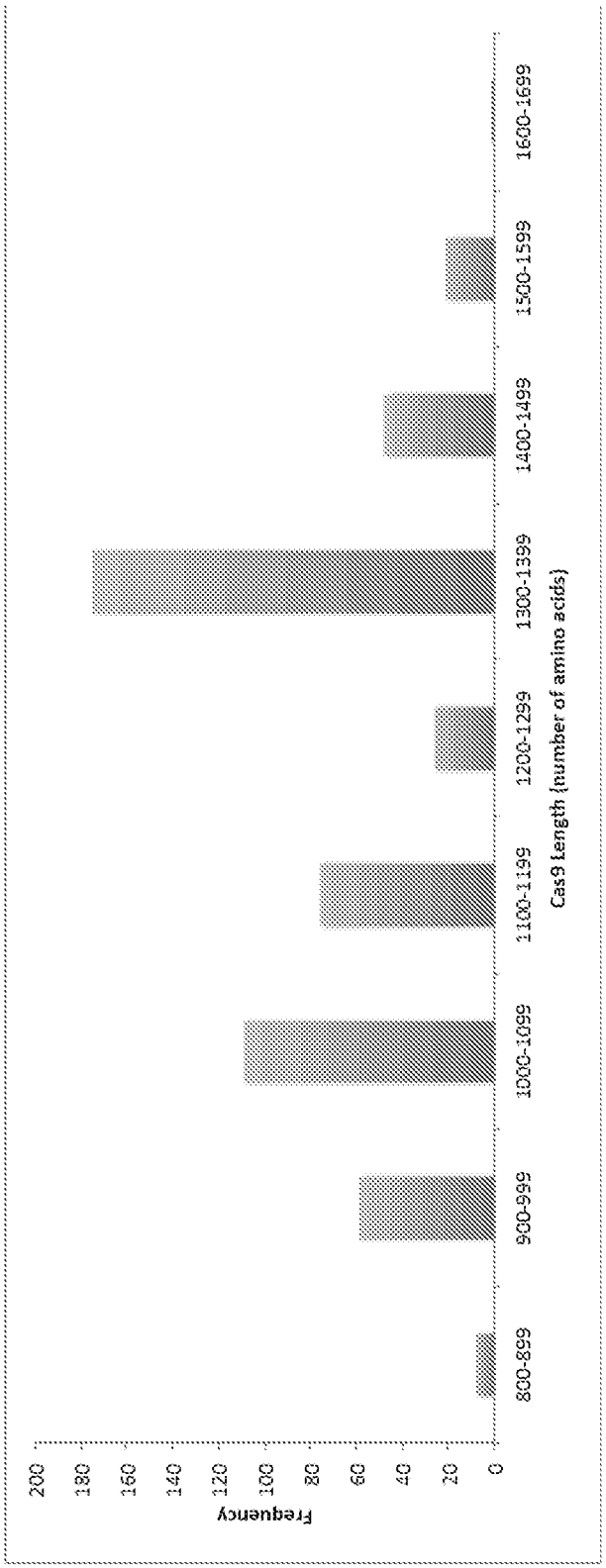

Frequency
0
20
40
60
80
100
120
140
160
180
200
800-899
900-999
1000-1099
1100-1199
1200-1299
1300-1399
1400-1499
1500-1599
1600-1699
Cas9 Length (number of amino acids)

FIG. 23

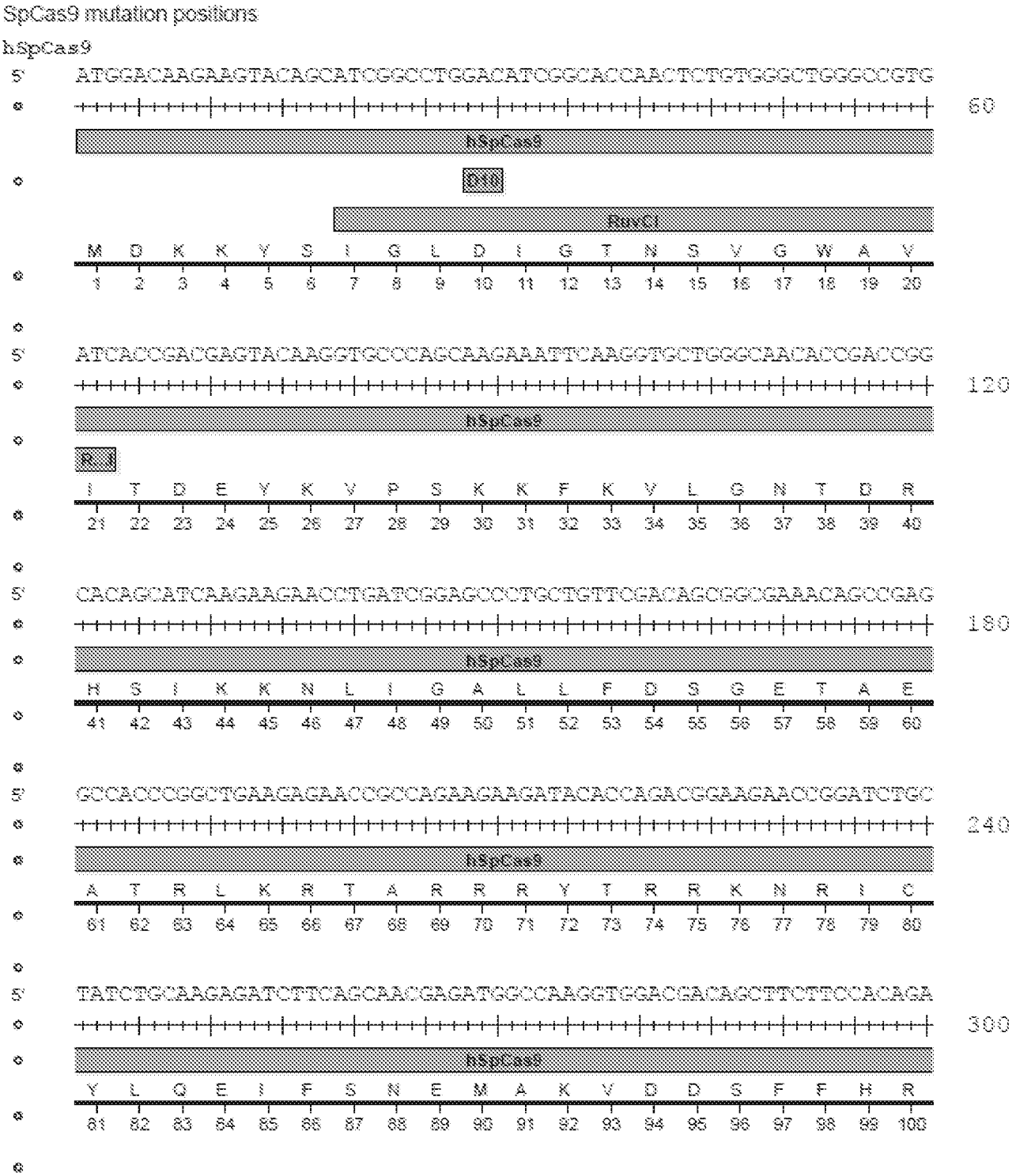

SpCas9 mutation positions
hSpCas9
5' ATGGACAAGAAGTACAGCATCGGCCTGGACATCGGCACCAACTCTGTGGGCTGGGCCGTG
60
hSpCas9
RuvC I
M D K K Y S I G L D I G T N S V G W A V
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
5' ATCACCGACGAGTACAAGGTGCCCAGCAAGAAATTCAAGGTGCTGGGCAACACCGACCGG
120
hSpCas9
R 1
I T D E Y K V P S K K F K V L G N T D R
21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
5' CACAGCATCAAGAAGAACCTGATCGGAGCCCTGCTGTTCGACAGCGGCGAAACAGCCGAG
180
hSpCas9
H S I K K N L I G A L L F D S G E T A E
41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
5' GCCACCCGGCTGAAGAGAACCGCCAGAAGAAGATACACCAGACGGAAGAACCGGATCTGC
240
hSpCas9
A T R L K R T A R R R Y T R R K N R I C
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
5' TATCTGCAAGAGATCTTCAGCAACGAGATGGCCAAGGTGGACGACAGCTTCTTCCACAGA
300
hSpCas9
Y L Q E I F S N E M A K V D D S F F H R
81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100

FIG. 24A

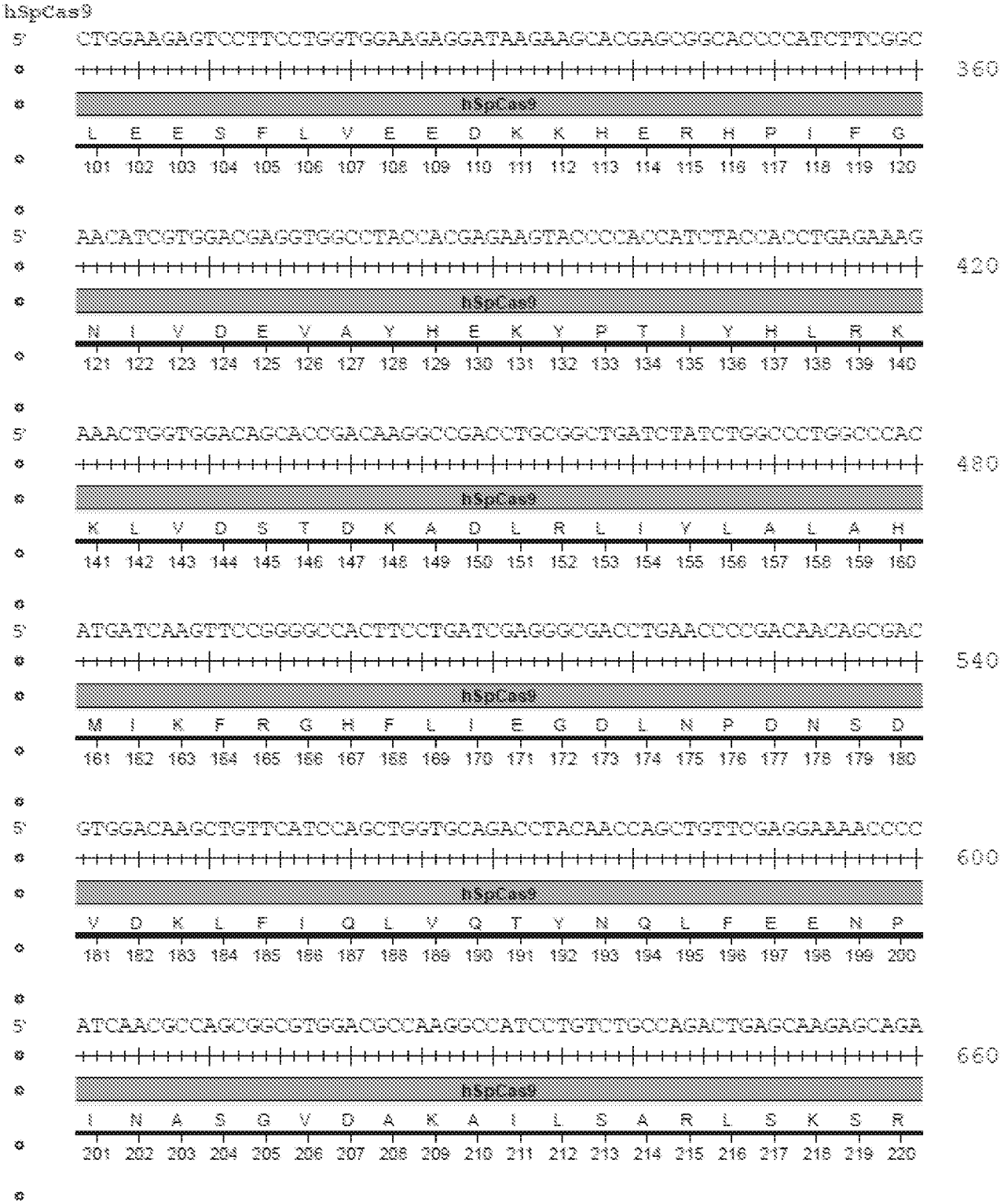

hSpCas9
5' CTGGAAGAGTCCTTCCTGGTGGAAGAGGATAAGAAGCACGAGCGGCACCCCATCTTCGGC
360
hSpCas9
L E E S F L V E E D K K H E R H P I F G
101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
5' AACATCGTGGACGAGGTGGCCTACCACGAGAAGTACCCCACCATCTACCACCTGAGAAAAG
420
hSpCas9
N I V D E V A Y H E K Y P T I Y H L R K
121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140
5' AAACTGGTGGACAGCACCGACAAGGCCGACCTGCGGCTGATCTATCTGGCCCTGGCCCAC
480
hSpCas9
K L V D S T D K A D L R L I Y L A L A H
141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160
5' ATGATCAAGTTCCGGGGCCACTTCCTGATCGAGGGCGACCTGAACCCCGACAACAGCGAC
540
hSpCas9
M I K F R G H F L I E G D L N P D N S D
161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
5' GTGGACAAGCTGTTCATCCAGCTGGTGCAGACCTACAACCAGCTGTTCGAGGAAAACCCC
600
hSpCas9
V D K L F I Q L V Q T Y N Q L F E E N P
181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200
5' ATCAACGCCAGCGGCGTGGACGCCAAGGCCATCCTGTCTGCCAGACTGAGCAAGAGCAGA
660
hSpCas9
I N A S G V D A K A I L S A R L S K S R
201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220

FIG. 24B

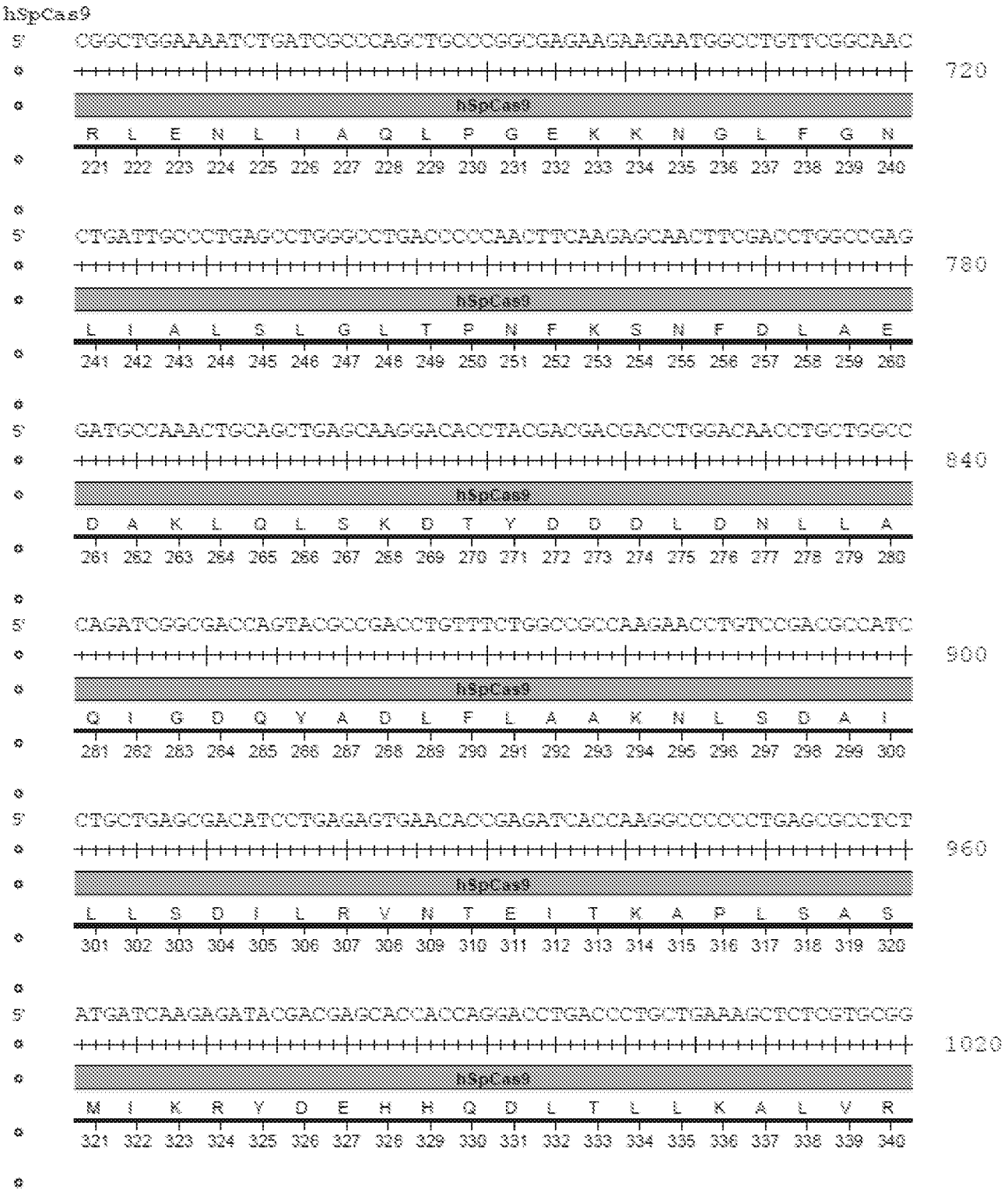

hSpCas9
5' CGGCTGGAAAATCTGATCGCCCAGCTGCCCGGCGAGAAGAAGAATGGCCTGTTCGGCAAC 720
hSpCas9
R L E N L I A Q L P G E K K N G L F G N
221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
5' CTGATTGCCCTGAGCCTGGGCCTGACCCCCAACTTCAAGAGCAACTTCGACCTGGCCGAG 780
hSpCas9
L I A L S L G L T P N F K S N F D L A E
241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260
5' GATGCCAAACTGCAGCTGAGCAAGGACACCTACGACGACGACCTGGACAACCTGCTGGCC 840
hSpCas9
D A K L Q L S K D T Y D D D L D N L L A
261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280
5' CAGATCGGCGACCAGTACGCCGACCTGTTTCTGGCCGCCAAGAACCTGTCCGACGCCATC 900
hSpCas9
Q I G D Q Y A D L F L A A K N L S D A I
281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
5' CTGCTGAGCGACATCCTGAGAGTGAACACCGAGATCACCAAGGCCCCCCTGAGCGCCTCT 960
hSpCas9
L L S D I L R V N T E I T K A P L S A S
301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320
5' ATGATCAAGAGATACGACGAGCACCACCAGGACCTGACCCTGCTGAAAGCTCTCGTGCGG 1020
hSpCas9
M I K R Y D E H H Q D L T L L K A L V R
321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340

FIG. 24C

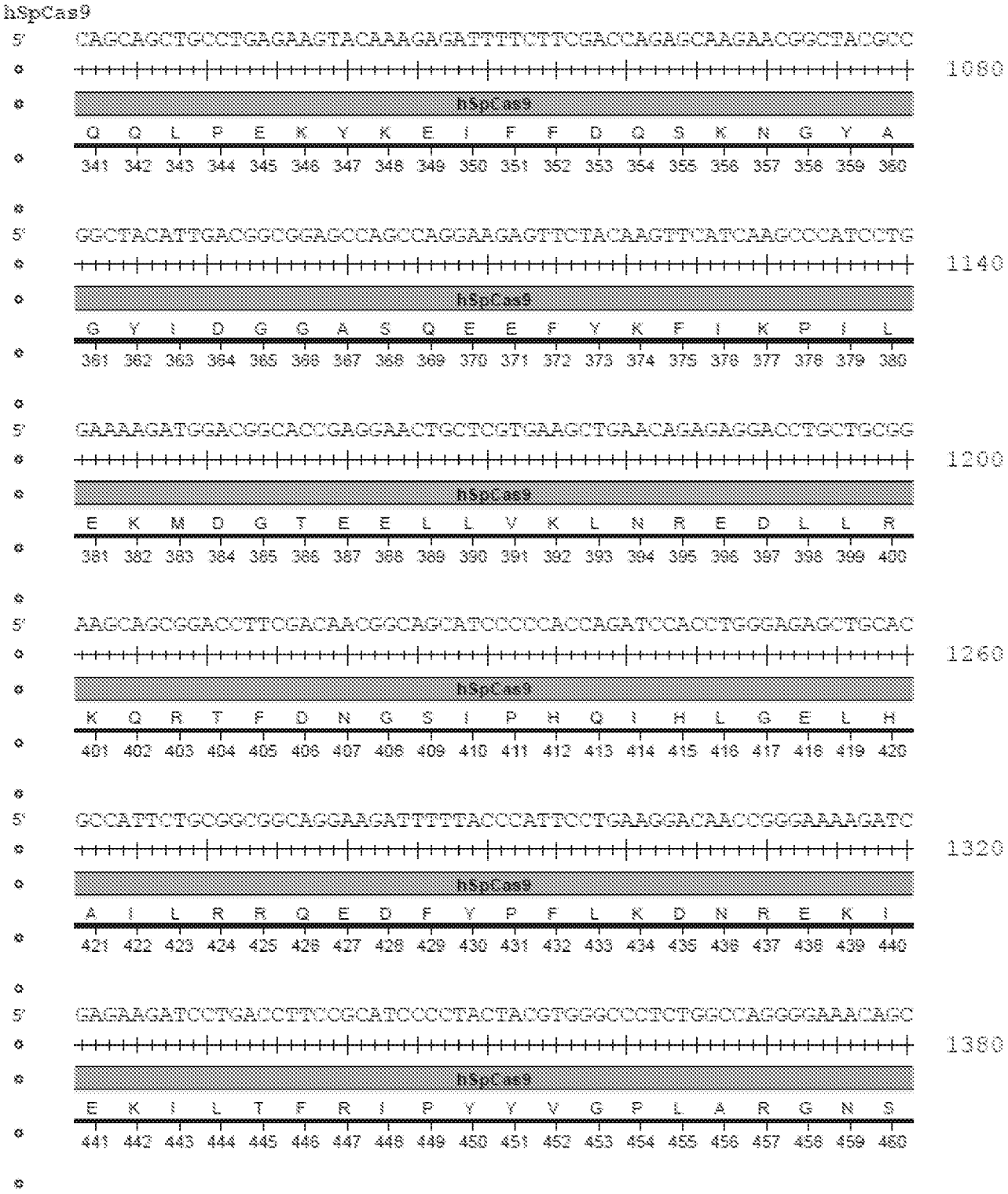

hSpCas9
5' CAGCAGCTGCCTGAGAAGTACAAAGAGATTTTCTTCGACCAGAGCAAGAACGGCTACGCC
1080
hSpCas9
Q Q L P E K Y K E I F F D Q S K N G Y A
341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
5' GGCTACATTGACGGCGGAGCCAGCCAGGAAGAGTTCTACAAGTTCATCAAGCCCATCCTG
1140
hSpCas9
G Y I D G G A S Q E E F Y K F I K P I L
361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380
5' GAAAAGATGGACGGCACCGAGGAACTGCTCGTGAAGCTGAACAGAGAGGACCTGCTGCGG
1200
hSpCas9
E K M D G T E E L L V K L N R E D L L R
381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400
5' AAGCAGCGGACCTTCGACAACGGCAGCATCCCCCACCAGATCCACCTGGGAGAGCTGCAC
1260
hSpCas9
K Q R T F D N G S I P H Q I H L G E L H
401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
5' GCCATTCTGCGGCGGCAGGAAGATTTTTACCCATTCCTGAAGGACAACCGGGAAAAGATC
1320
hSpCas9
A I L R R Q E D F Y P F L K D N R E K I
421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440
5' GAGAAGATCCTGACCTTCCGCATCCCCTACTACGTGGGCCCTCTGGCCAGGGGAAACAGC
1380
hSpCas9
E K I L T F R I P Y Y V G P L A R G N S
441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460

FIG. 24D

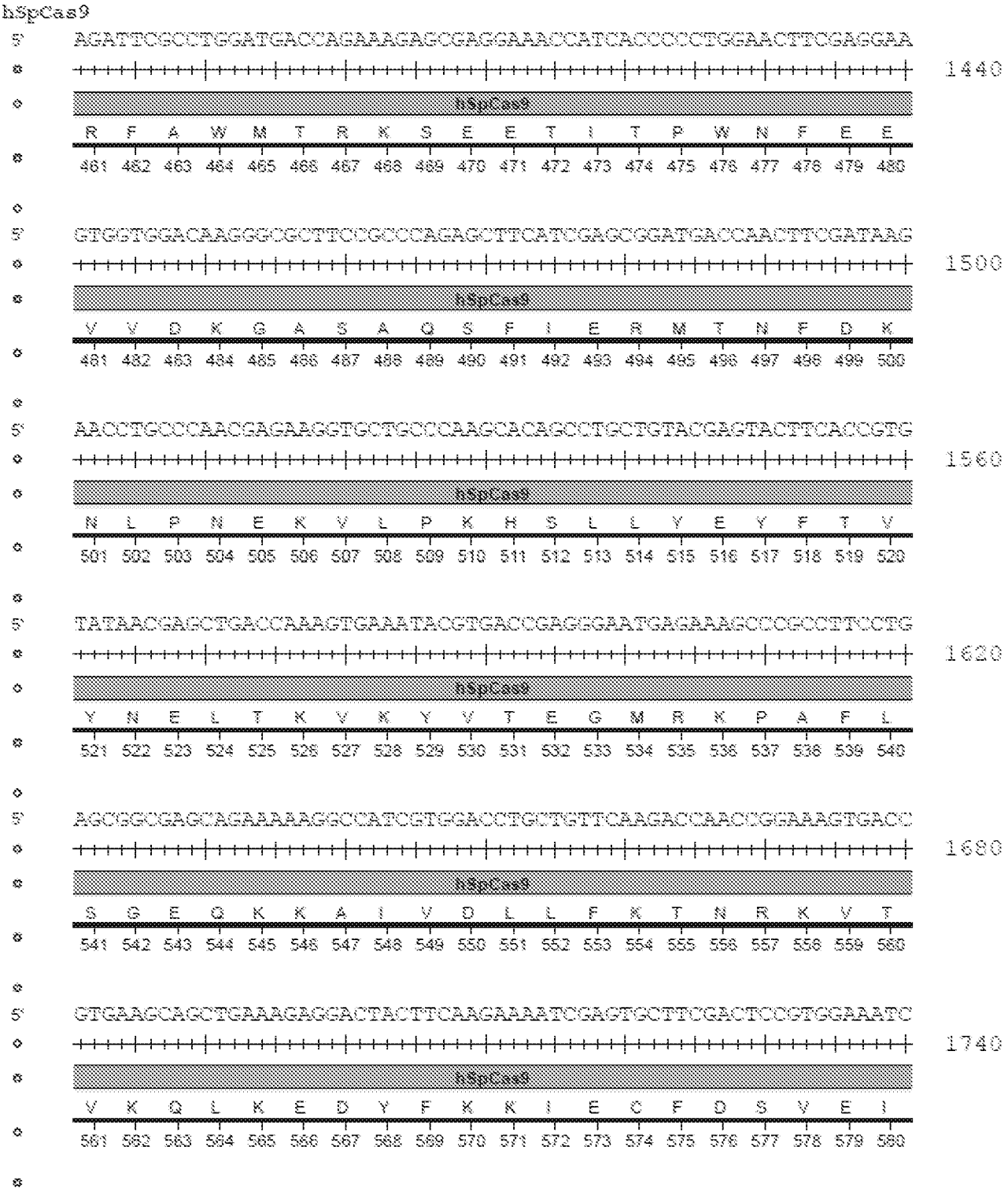

hSpCas9
5' AGATTCGCCTGGATGACCAGAAAGAGCGAGGAAACCATCACCCCCTGGAACTTCGAGGAA 1440
hSpCas9
R F A W M T R K S E E T I T P W N F E E
461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480
5' GTGGTGGACAAGGGCGCTTCCGCCCAGAGCTTCATCGAGCGGATGACCAACTTCGATAAG 1500
hSpCas9
V V D K G A S A Q S F I E R M T N F D K
481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500
5' AACCTGCCCAACGAGAAGGTGCTGCCCAAGCACAGCCTGCTGTACGAGTACTTCACCGTG 1560
hSpCas9
N L P N E K V L P K H S L L Y E Y F T V
501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520
5' TATAACGAGCTGACCAAAGTGAAATACGTGACCGAGGGAATGAGAAAGCCCGCCTTCCTG 1620
hSpCas9
Y N E L T K V K Y V T E G M R K P A F L
521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540
5' AGCGGCGAGCAGAAAAAGGCCATCGTGGACCTGCTGTTCAAGACCAACCGGAAAGTGACC 1680
hSpCas9
S G E Q K K A I V D L L F K T N R K V T
541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560
5' GTGAAGCAGCTGAAAGAGGACTACTTCAAGAAAATCGAGTGCTTCGACTCCGTGGAAATC 1740
hSpCas9
V K Q L K E D Y F K K I E C F D S V E I
561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580

FIG. 24E

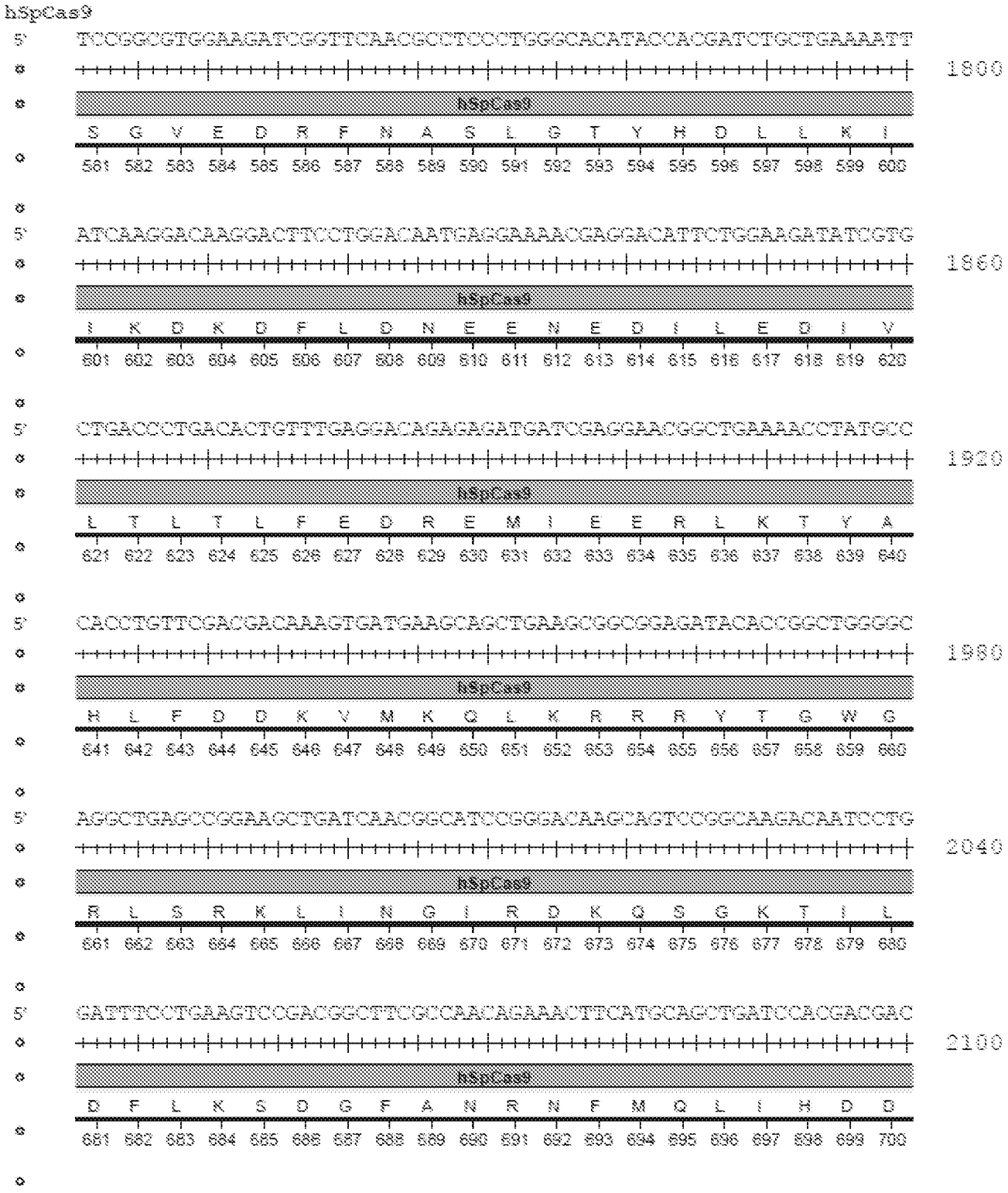

hSpCas9
5' TCCGGCGTGGAAGATCGGTTCAACGCCTCCCTGGGCACATACCACGATCTGCTGAAAATT
1800
hSpCas9
S G V E D R F N A S L G T Y H D L L K I
561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580
5' ATCAAGGACAAGGACTTCCTGGACAATGAGGAAAACGAGGACATTCTGGAAGATATCGTG
1860
hSpCas9
I K D K D F L D N E E N E D I L E D I V
581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600
5' CTGACCCTGACACTGTTTGAGGACAGAGAGATGATCGAGGAACGGCTGAAAACCTATGCC
1920
hSpCas9
L T L T L F E D R E M I E E R L K T Y A
601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620
5' CACCTGTTCGACGACAAAGTGATGAAGCAGCTGAAGCGGCGGAGATACACCGGCTGGGGC
1980
hSpCas9
H L F D D K V M K Q L K R R R Y T G W G
621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640
5' AGGCTGAGCCGGAAGCTGATCAACGGCATCCGGGACAAGCAGTCCGGCAAGACAATCCTG
2040
hSpCas9
R L S R K L I N G I R D K Q S G K T I L
641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660
5' GATTTCCTGAAGTCCGACGGCTTCGCCAACAGAAACTTCATGCAGCTGATCCACGACGAC
2100
hSpCas9
D F L K S D G F A N R N F M Q L I H D D
661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680

FIG. 24F

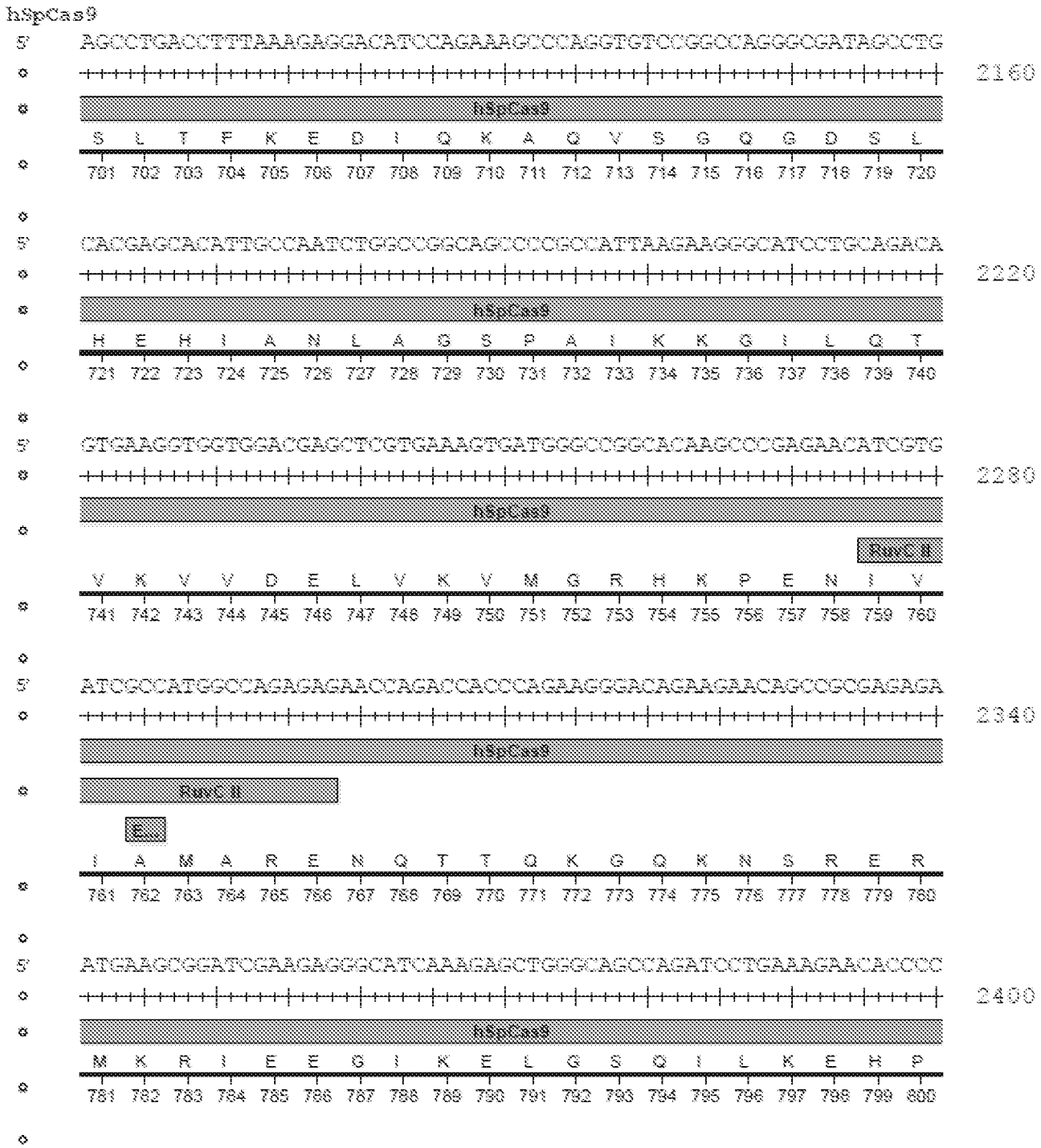

hSpCas9
5' AGCCTGACCTTTAAAGAGGACATCCAGAAAGCCCAGGTGTCCGGCCAGGGCGATAGCCTG
2160
hSpCas9
S L T F K E D I Q K A Q V S G Q G D S L
791 792 793 794 795 796 797 798 799 800
5' CACGAGCACATTGCCAATCTGGCCGGCAGCCCCGCCATTAAGAAGGGCATCCTGCAGACA
2220
hSpCas9
H E H I A N L A G S P A I K K G I L Q T
721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740
5' GTGAAGGTGGTGGACGAGCTCGTGAAAGTGATGGGCCGGCACAAGCCCGAGAACATCGTG
2280
hSpCas9
RuvC II
V K V V D E L V K V M G R H K P E N I V
741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760
5' ATCGCCATGGCCAGAGAGAACCAGACCACCCAGAAGGGACAGAAGAACAGCCGCGAGAGA
2340
hSpCas9
RuvC II
E
I A M A R E N Q T T Q K G Q K N S R E R
761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780
5' ATGAAGCGGATCGAAGAGGGCATCAAAGAGCTGGGCAGCCAGATCCTGAAAGAACACCCC
2400
hSpCas9
M K R I E E G I K E L G S Q I L K E H P
781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800

FIG. 24G

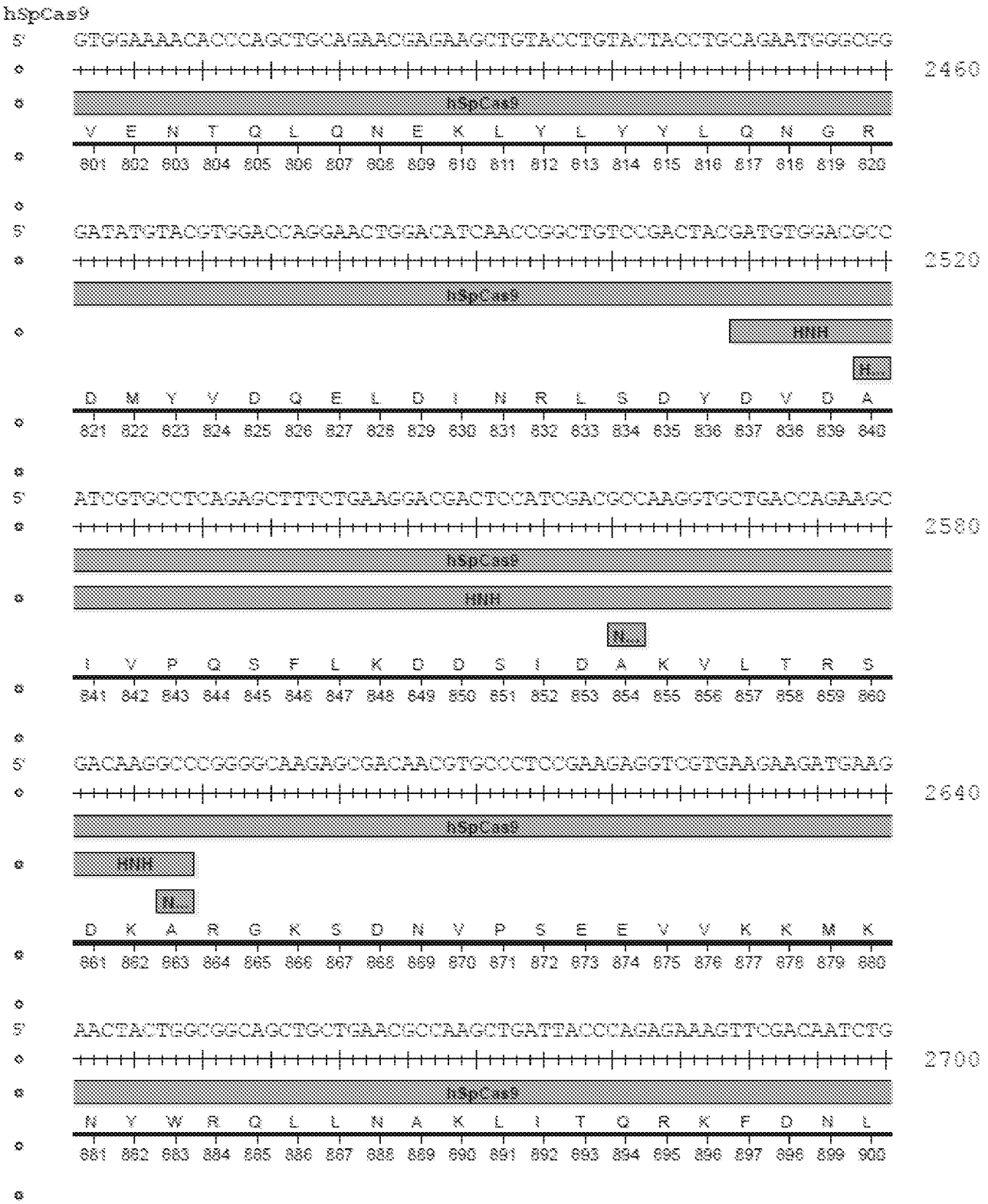

hSpCas9
5' GTGGAAAACACCCAGCTGCAGAACGAGAAGCTGTACCTGTACTACCTGCAGAATGGGCGG
2460
hSpCas9
V E N T Q L Q N E K L Y L Y Y L Q N G R
801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820
5' GATATGTACGTGGACCAGGAACTGGACATCAACCGGCTGTCCGACTACGATGTGGACGCC
2520
hSpCas9
HNH
N..
D M Y V D Q E L D I N R L S D Y D V D A
821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840
5' ATCGTGCCTCAGAGCTTTCTGAAGGACGACTCCATCGACGCCAAGGTGCTGACCAGAAGC
2580
hSpCas9
HNH
N..
I V P Q S F L K D D S I D A K V L T R S
841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860
5' GACAAGGCCCGGGGCAAGAGCGACAACGTGCCCTCCGAAGAGGTCGTGAAGAAGATGAAG
2640
hSpCas9
HNH
N..
D K A R G K S D N V P S E E V V K K M K
861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880
5' AACTACTGGCGGCAGCTGCTGAACGCCAAGCTGATTACCCAGAGAAAGTTCGACAATCTG
2700
hSpCas9
N Y W R Q L L N A K L I T Q R K F D N L
881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900

FIG. 24H

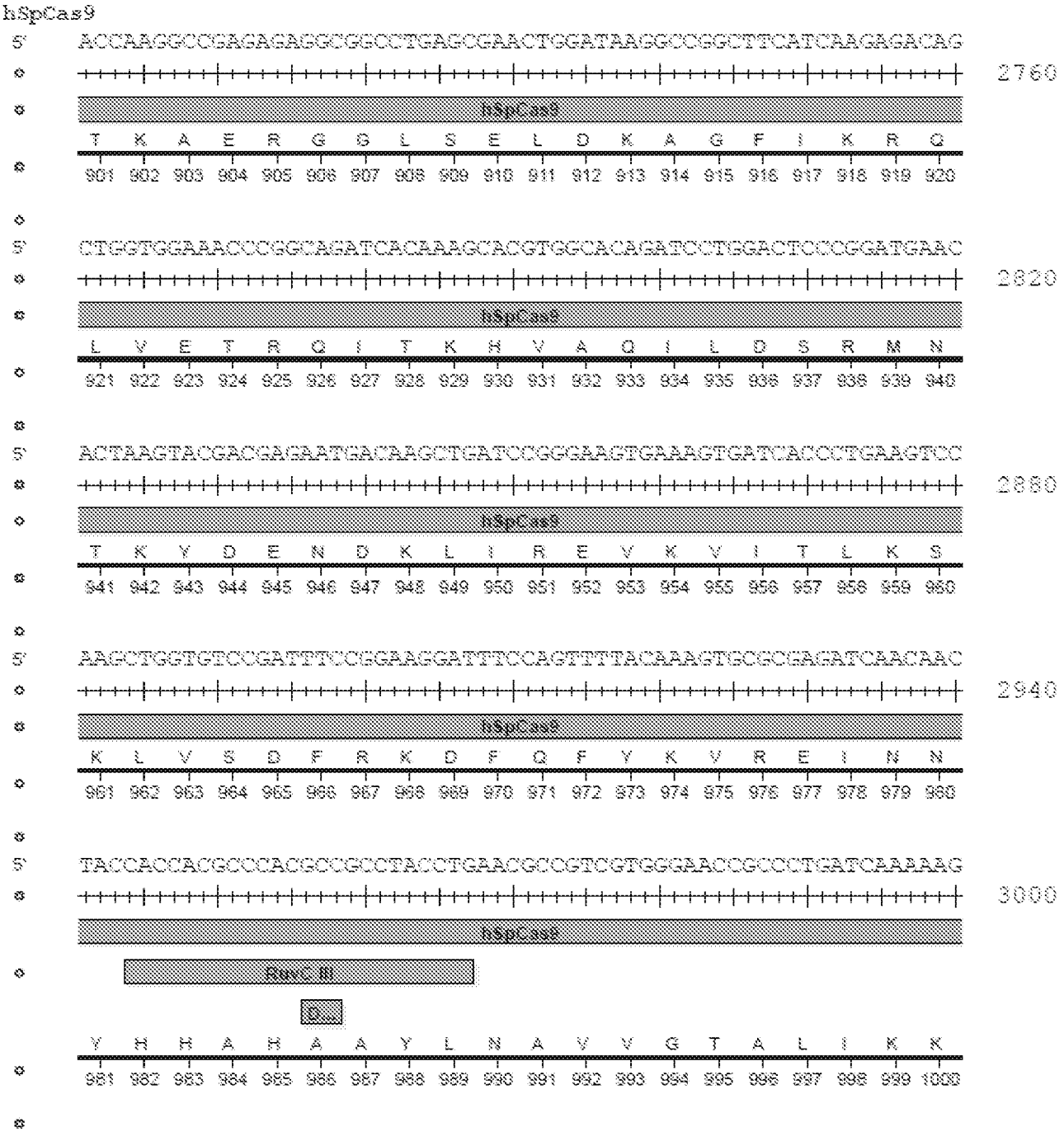

hSpCas9
5' ACCAAGGCCGAGAGAGGCGGCCTGAGCGAACTGGATAAGGCCGGCTTCATCAAGAGACAG
2760
hSpCas9
T K A E R G G L S E L D K A G F I K R Q
901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920
5' CTGGTGGAAACCCGGCAGATCACAAAGCACGTGGCACAGATCCTGGACTCCCGGATGAAC
2820
hSpCas9
L V E T R Q I T K H V A Q I L D S R M N
921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940
5' ACTAAGTACGACGAGAATGACAAGCTGATCCGGGAAGTGAAAGTGATCACCCTGAAGTCC
2880
hSpCas9
T K Y D E N D K L I R E V K V I T L K S
941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960
5' AAGCTGGTGTCCGATTTCCGGAAGGATTTCCAGTTTTACAAAGTGCGCGAGATCAACAAC
2940
hSpCas9
K L V S D F R K D F Q F Y K V R E I N N
961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980
5' TACCACCACGCCCACGCCGCCTACCTGAACGCCGTCGTGGGAACCGCCCTGATCAAAAAG
3000
hSpCas9
RuvC III
S...
Y H H A H A A Y L N A V V G T A L I K K
981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000

FIG. 24I

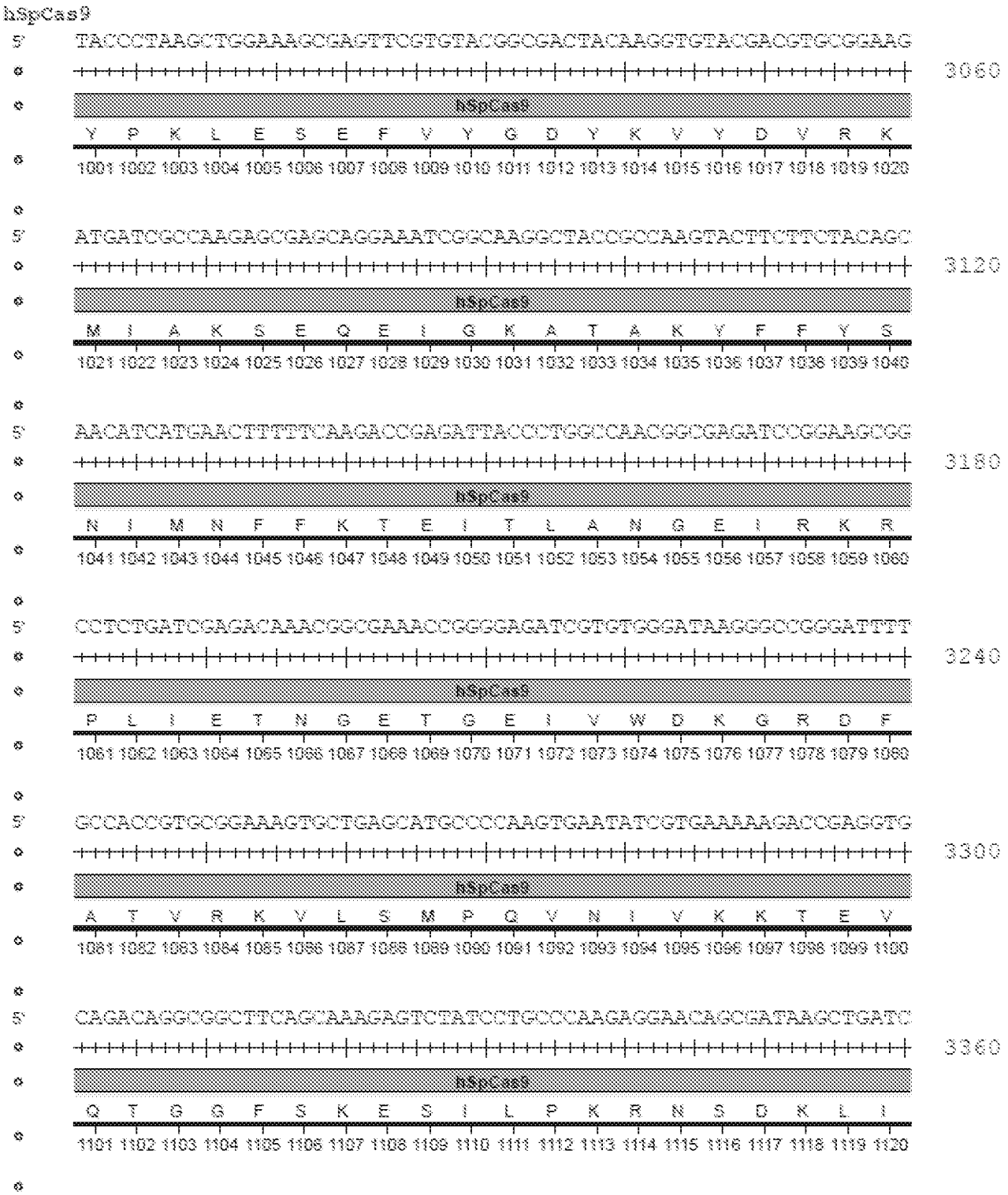

hSpCas9
5' TACCCTAAGCTGGAAAGCGAGTTCGTGTACGGCGACTACAAGGTGTACGACGTGCGGAAG
3060
hSpCas9
Y P K L E S E F V Y G D Y K V Y D V R K
1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020
5' ATGATCGCCAAGAGCGAGCAGGAAATCGGCAAGGCTACCGCCAAGTACTTCTTCTACAGC
3120
hSpCas9
M I A K S E Q E I G K A T A K Y F F Y S
1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040
5' AACATCATGAACTTTTTCAAGACCGAGATTACCCTGGCCAACGGCGAGATCCGGAAGCGG
3180
hSpCas9
N I M N F F K T E I T L A N G E I R K R
1041 1042 1043 1044 1045 1046 1047 1048 1049 1050 1051 1052 1053 1054 1055 1056 1057 1058 1059 1060
5' CCTCTGATCGAGACAAACGGCGAAACCGGGGAGATCGTGTGGGATAAGGGCCGGGATTTT
3240
hSpCas9
P L I E T N G E T G E I V W D K G R D F
1061 1062 1063 1064 1065 1066 1067 1068 1069 1070 1071 1072 1073 1074 1075 1076 1077 1078 1079 1080
5' GCCACCGTGCGGAAAGTGCTGAGCATGCCCCAAGTGAATATCGTGAAAAAGACCGAGGTG
3300
hSpCas9
A T V R K V L S M P Q V N I V K K T E V
1081 1082 1083 1084 1085 1086 1087 1088 1089 1090 1091 1092 1093 1094 1095 1096 1097 1098 1099 1100
5' CAGACAGGCGGCTTCAGCAAAGAGTCTATCCTGCCCAAGAGGAACAGCGATAAGCTGATC
3360
hSpCas9
Q T G G F S K E S I L P K R N S D K L I
1101 1102 1103 1104 1105 1106 1107 1108 1109 1110 1111 1112 1113 1114 1115 1116 1117 1118 1119 1120

FIG. 24J

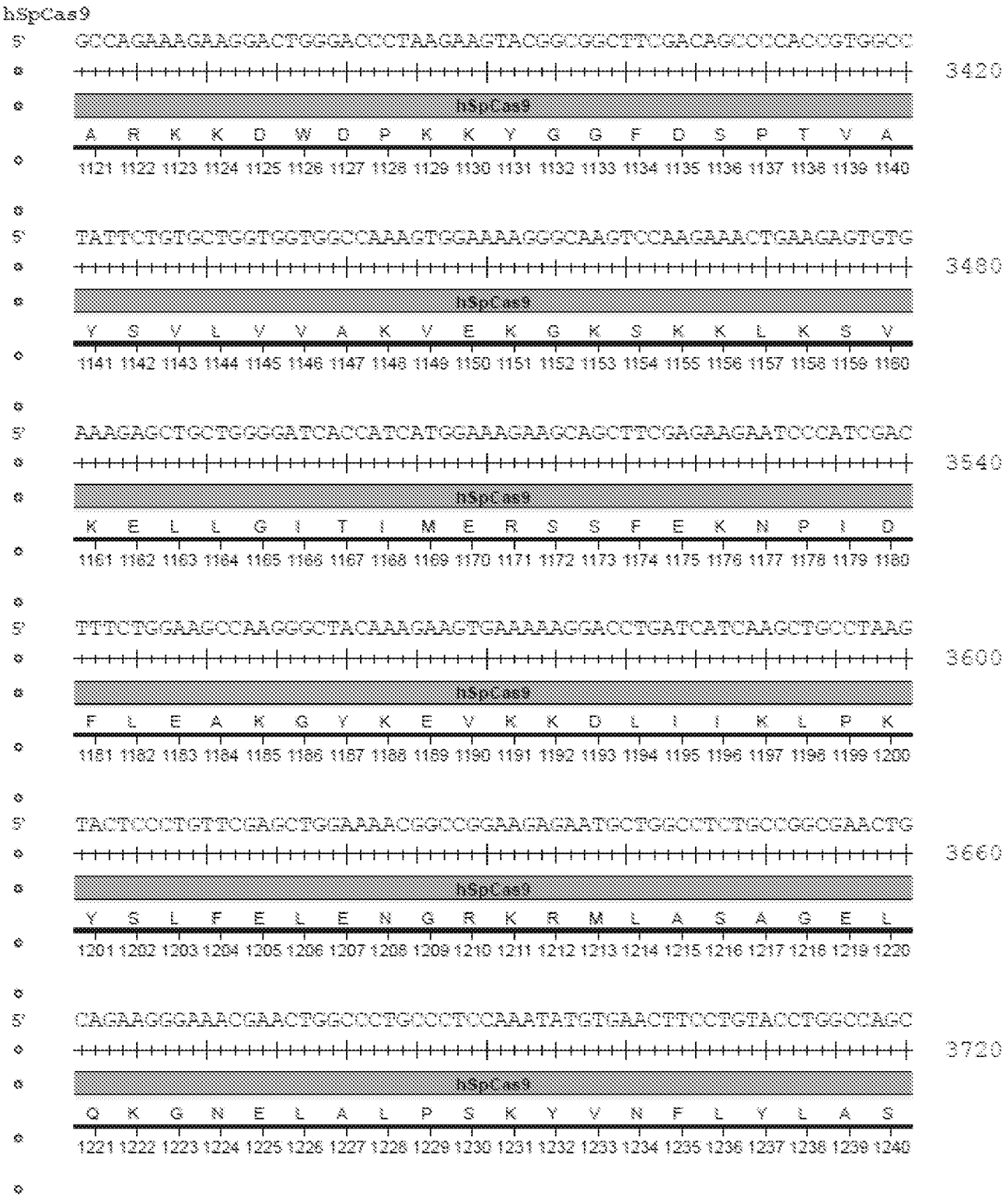

hSpCas9
5' GCCAGAAAGAAGGACTGGGACCCTAAGAAGTACGGCGGCTTCGACAGCCCCACCGTGGCC
3420
hSpCas9
A R K K D W D P K K Y G G F D S P T V A
1121 1122 1123 1124 1125 1126 1127 1128 1129 1130 1131 1132 1133 1134 1135 1136 1137 1138 1139 1140
5' TATTCTGTGCTGGTGGTGGCCAAAGTGGAAAAGGGCAAGTCCAAGAAACTGAAGAGTGTG
3480
hSpCas9
Y S V L V V A K V E K G K S K K L K S V
1141 1142 1143 1144 1145 1146 1147 1148 1149 1150 1151 1152 1153 1154 1155 1156 1157 1158 1159 1160
5' AAAGAGCTGCTGGGGATCACCATCATGGAAAGAAGCAGCTTCGAGAAGAATCCCATCGAC
3540
hSpCas9
K E L L G I T I M E R S S F E K N P I D
1161 1162 1163 1164 1165 1166 1167 1168 1169 1170 1171 1172 1173 1174 1175 1176 1177 1178 1179 1180
5' TTTCTGGAAGCCAAGGGCTACAAAGAAGTGAAAAAGGACCTGATCATCAAGCTGCCTAAG
3600
hSpCas9
F L E A K G Y K E V K K D L I I K L P K
1181 1182 1183 1184 1185 1186 1187 1188 1189 1190 1191 1192 1193 1194 1195 1196 1197 1198 1199 1200
5' TACTCCCTGTTCGAGCTGGAAAACGGCCGGAAGAGAATGCTGGCCTCTGCCGGCGAACTG
3660
hSpCas9
Y S L F E L E N G R K R M L A S A G E L
1201 1202 1203 1204 1205 1206 1207 1208 1209 1210 1211 1212 1213 1214 1215 1216 1217 1218 1219 1220
5' CAGAAGGGAAACGAACTGGCCCTGCCCTCCAAATATGTGAACTTCCTGTACCTGGCCAGC
3720
hSpCas9
Q K G N E L A L P S K Y V N F L Y L A S
1221 1222 1223 1224 1225 1226 1227 1228 1229 1230 1231 1232 1233 1234 1235 1236 1237 1238 1239 1240

FIG. 24K

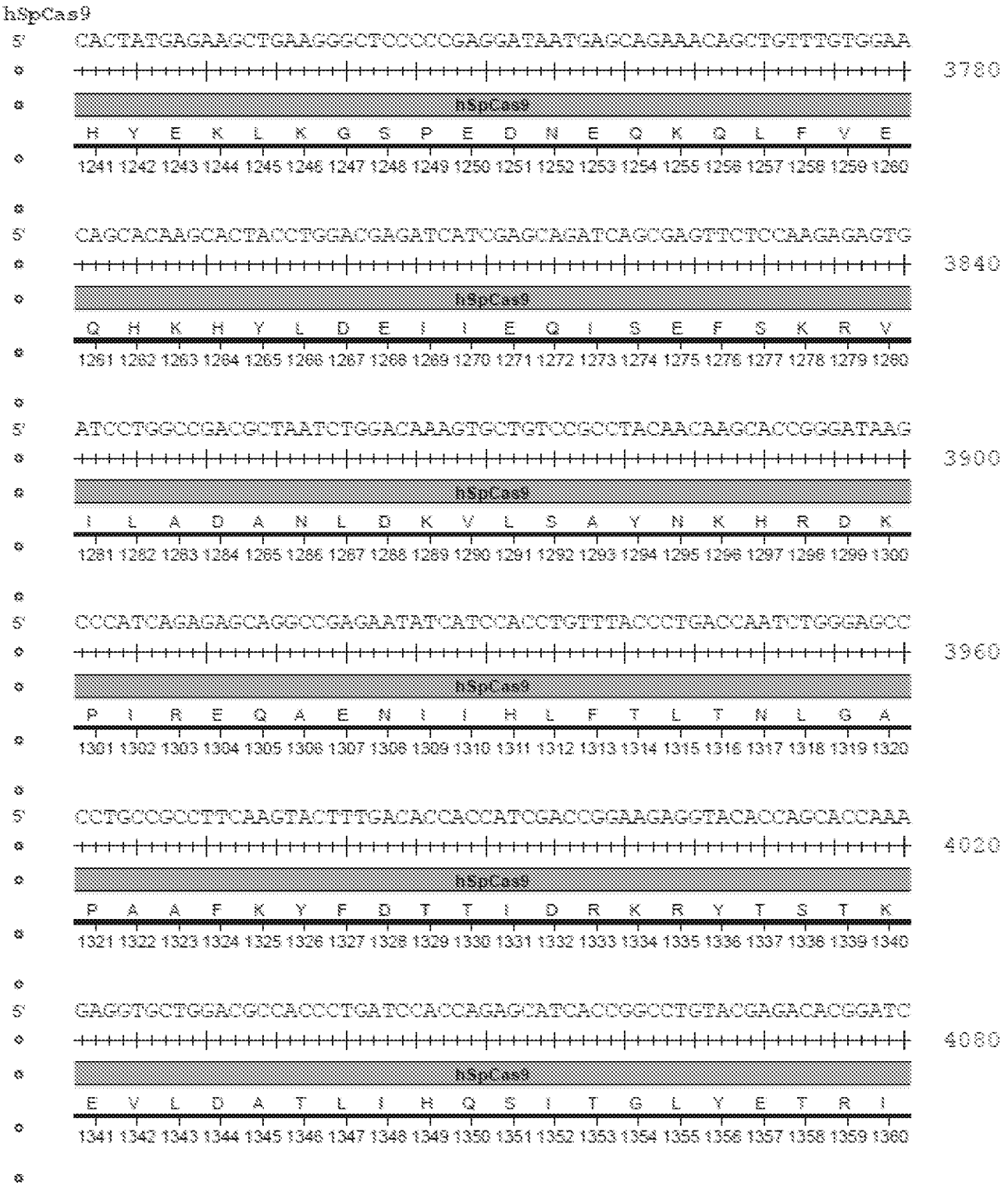

hSpCas9
5' CACTATGAGAAGCTGAAGGGCTCCCCCGAGGATAATGAGCAGAAACAGCTGTTTGTGGAA 3780
H Y E K L K G S P E D N E Q K Q L F V E
1241 1242 1243 1244 1245 1246 1247 1248 1249 1250 1251 1252 1253 1254 1255 1256 1257 1258 1259 1260
5' CAGCACAAGCACTACCTGGACGAGATCATCGAGCAGATCAGCGAGTTCTCCAAGAGAGTG 3840
Q H K H Y L D E I I E Q I S E F S K R V
1261 1262 1263 1264 1265 1266 1267 1268 1269 1270 1271 1272 1273 1274 1275 1276 1277 1278 1279 1280
5' ATCCTGGCCGACGCTAATCTGGACAAAGTGCTGTCCGCCTACAACAAGCACCGGGATAAG 3900
I L A D A N L D K V L S A Y N K H R D K
1281 1282 1283 1284 1285 1286 1287 1288 1289 1290 1291 1292 1293 1294 1295 1296 1297 1298 1299 1300
5' CCCATCAGAGAGCAGGCCGAGAATATCATCCACCTGTTTACCCTGACCAATCTGGGAGCC 3960
P I R E Q A E N I I H L F T L T N L G A
1301 1302 1303 1304 1305 1306 1307 1308 1309 1310 1311 1312 1313 1314 1315 1316 1317 1318 1319 1320
5' CCTGCCGCCTTCAAGTACTTTGACACCACCATCGACCGGAAGAGGTACACCAGCACCAAA 4020
P A A F K Y F D T T I D R K R Y T S T K
1321 1322 1323 1324 1325 1326 1327 1328 1329 1330 1331 1332 1333 1334 1335 1336 1337 1338 1339 1340
5' GAGGTGCTGGACGCCACCCTGATCCACCAGAGCATCACCGGCCTGTACGAGACACGGATC 4080
E V L D A T L I H Q S I T G L Y E T R I
1341 1342 1343 1344 1345 1346 1347 1348 1349 1350 1351 1352 1353 1354 1355 1356 1357 1358 1359 1360

FIG. 24L

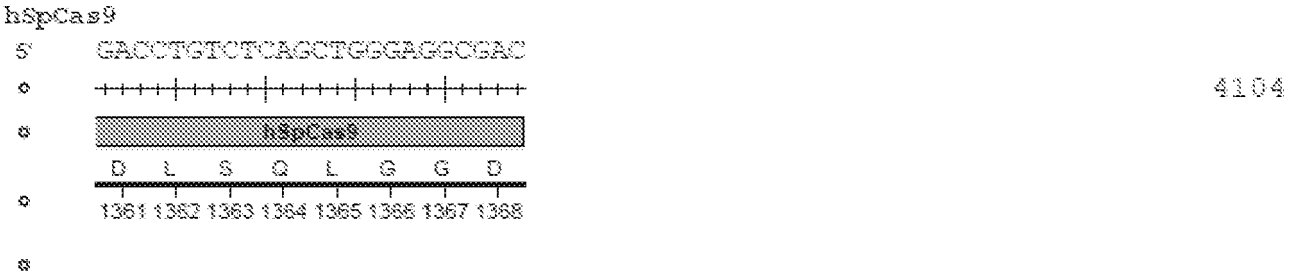

hSpCas9
5'
GACCTGTCTCAGCTGGGAGGCGAC
4104
hSpCas9
D L S Q L G G D
1361 1362 1363 1364 1365 1366 1367 1368

FIG. 24M

Conditional Cas9, Rosa26 targeting vector map

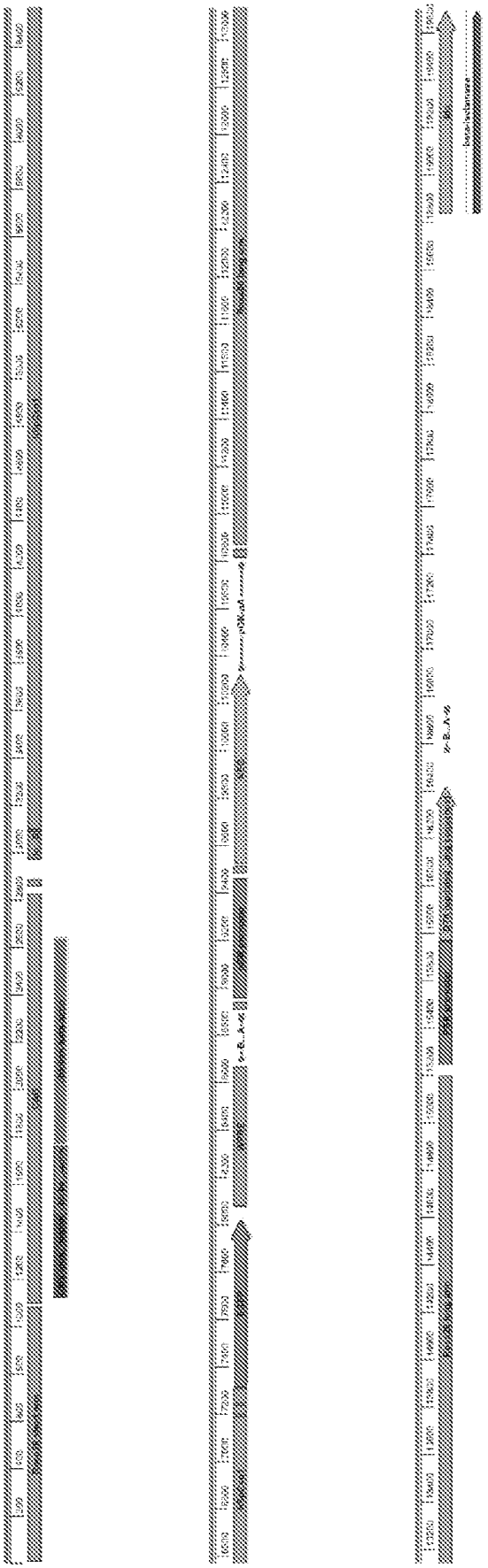


FIG. 25A

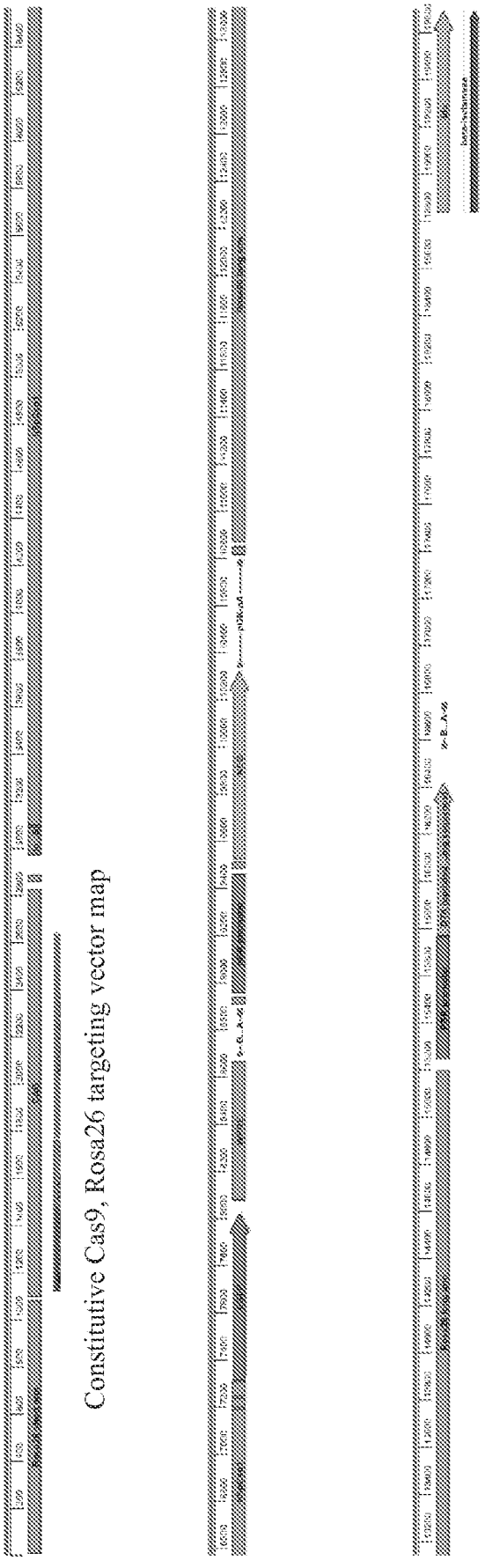



FIG. 25B

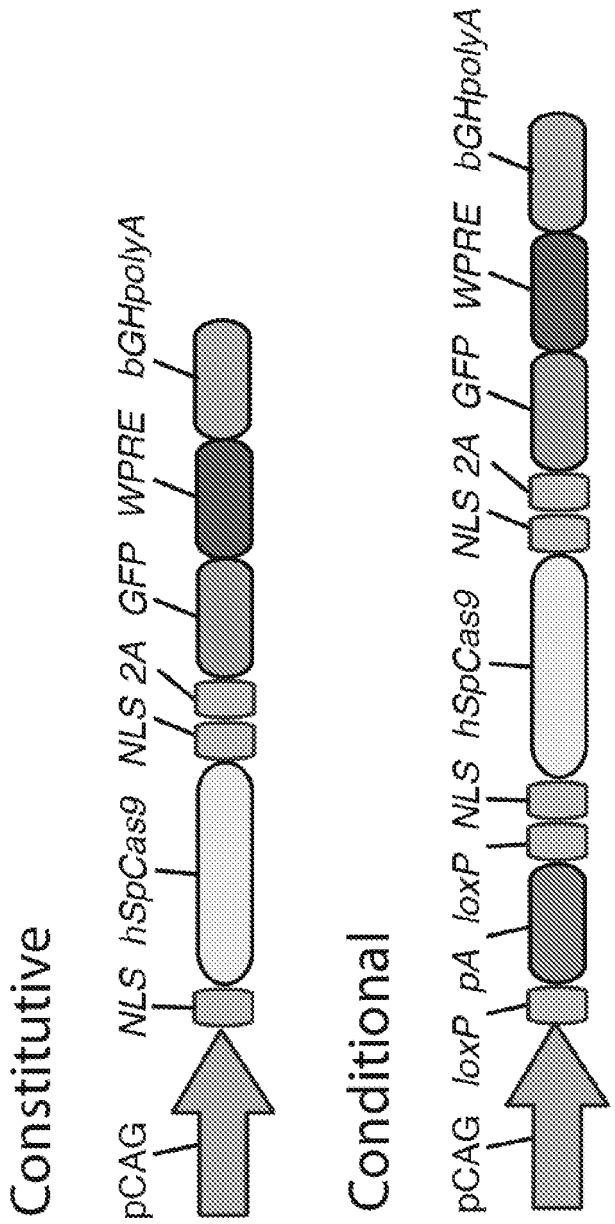
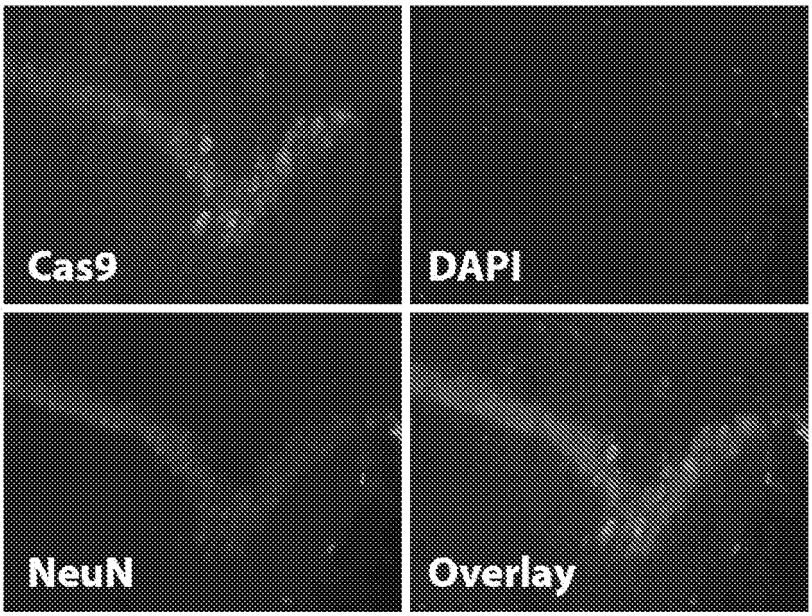

Constitutive
pCAG
NLS
hSpCas9
NLS
2A
GFP
WPRE
bGHpolyA
Conditional
pCAG
loxP
pA
loxP
NLS
hSpCas9
NLS
2A
GFP
WPRE
bGHpolyA

FIG. 26

Cas9 Expression in Mouse Hippocampus (AAV)




Cas9 Expression in Mouse Cortex (AAV)

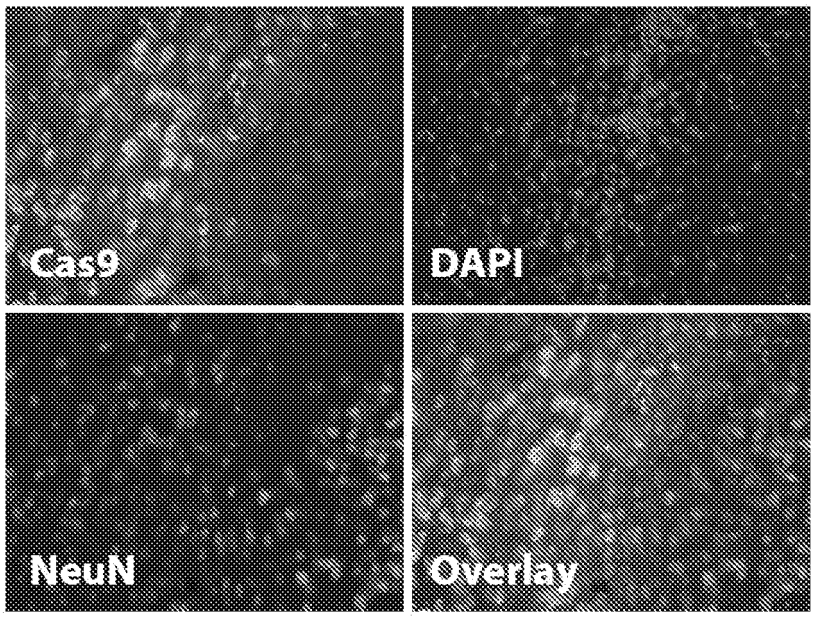



FIG. 27

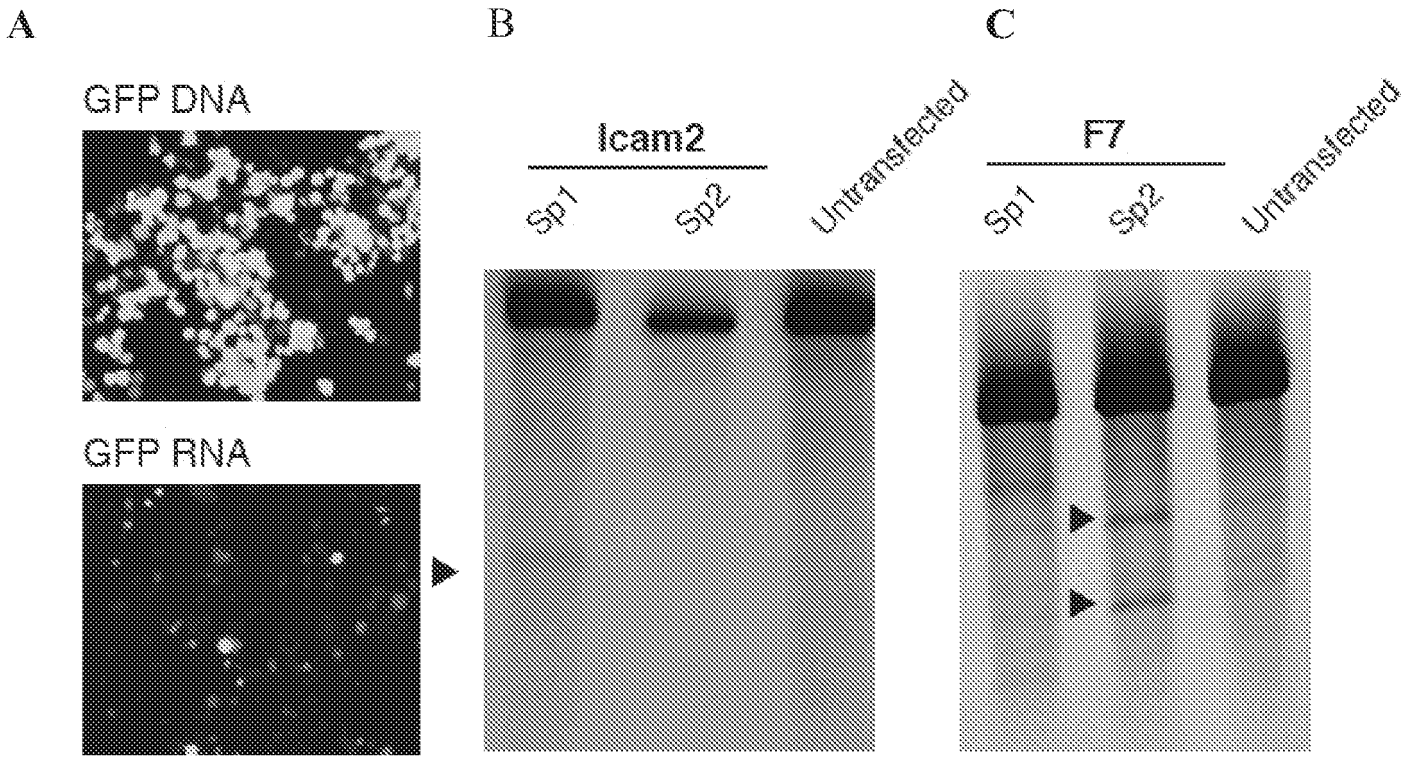

A
B
C
GFP DNA
GFP RNA
Icam2
Sp1
Sp2
Untransfected
F7
Sp1
Sp2
Untransfected

FIG. 28

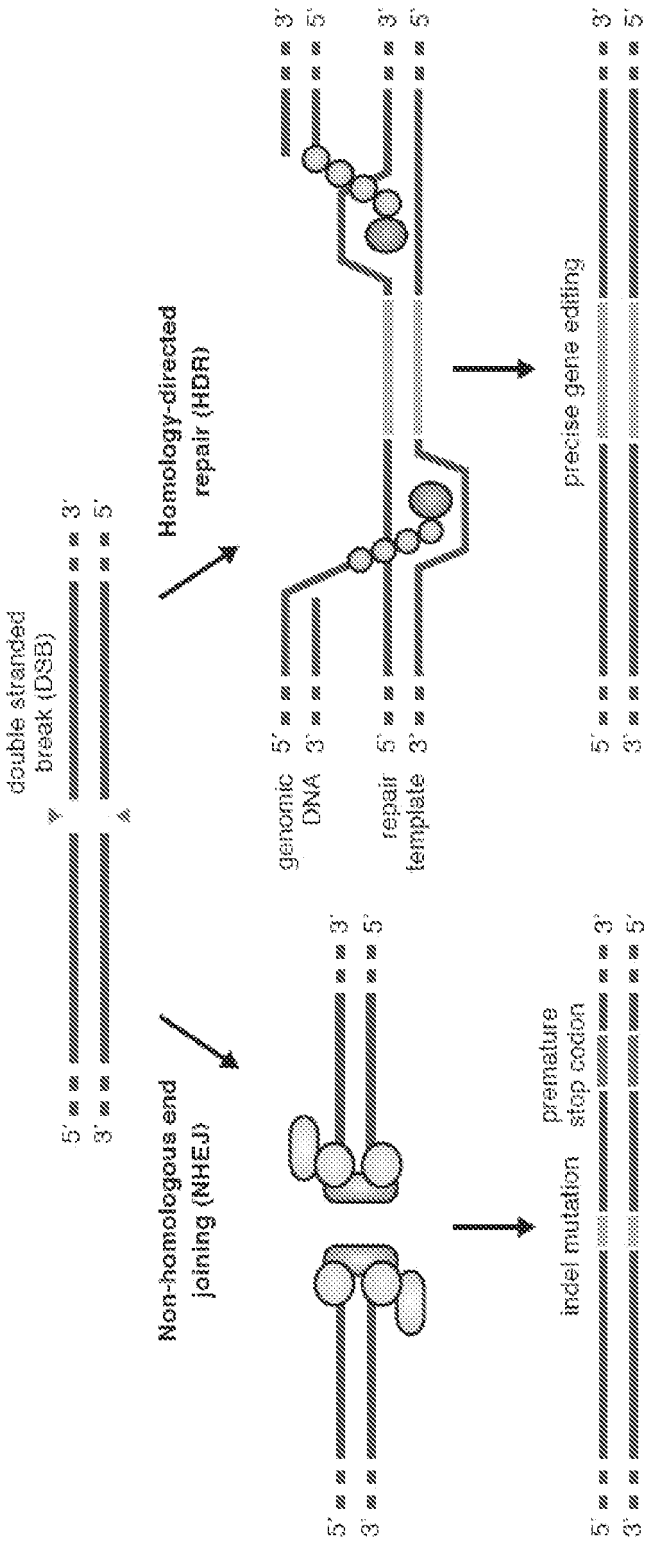

double stranded break (DSB)
5'
3'
Homology-directed repair (HDR)
genomic DNA
repair template
precise gene editing
Non-homologous end joining (NHEJ)
indel mutation
premature stop codon

FIG. 29

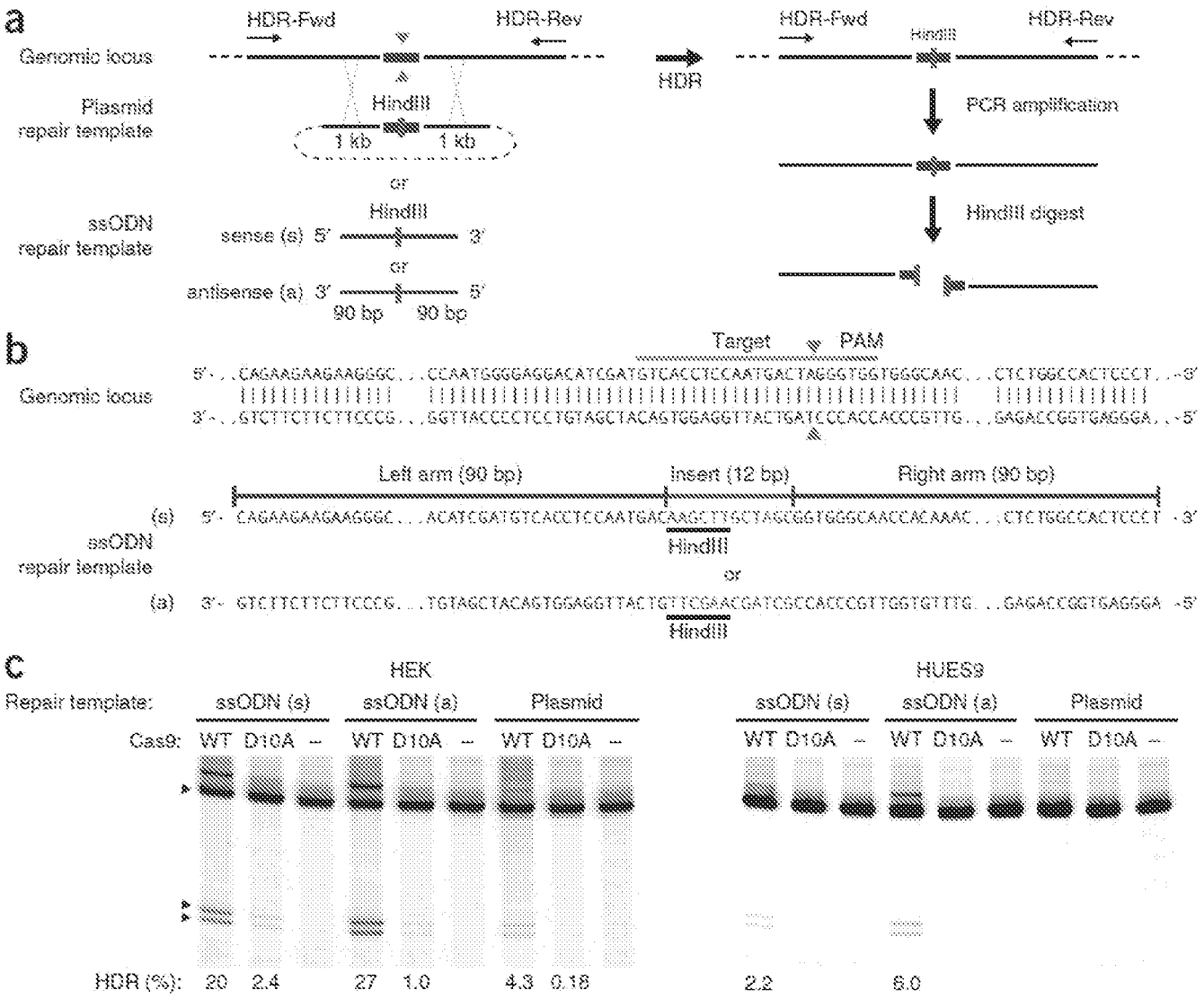

a
HDR-Fwd
HDR-Rev
Genomic locus
Plasmid repair template
HindIII
1 kb
1 kb
or
ssODN repair template
sense (s) 5′
3′
antisense (a) 3′
5′
90 bp
90 bp
HDR
HDR-Fwd
HindIII
HDR-Rev
PCR amplification
HindIII digest
b
Target
PAM
Genomic locus
Left arm (90 bp)
Insert (12 bp)
Right arm (90 bp)
(s)
ssODN repair template
HindIII
or
(a)
HindIII
c
HEK
HUES9
Repair template:
ssODN (s)
ssODN (a)
Plasmid
Cas9:
WT D10A –
HDR (%):
20 2.4 27 1.0 4.3 0.18 2.2 8.0

FIG. 30A-C

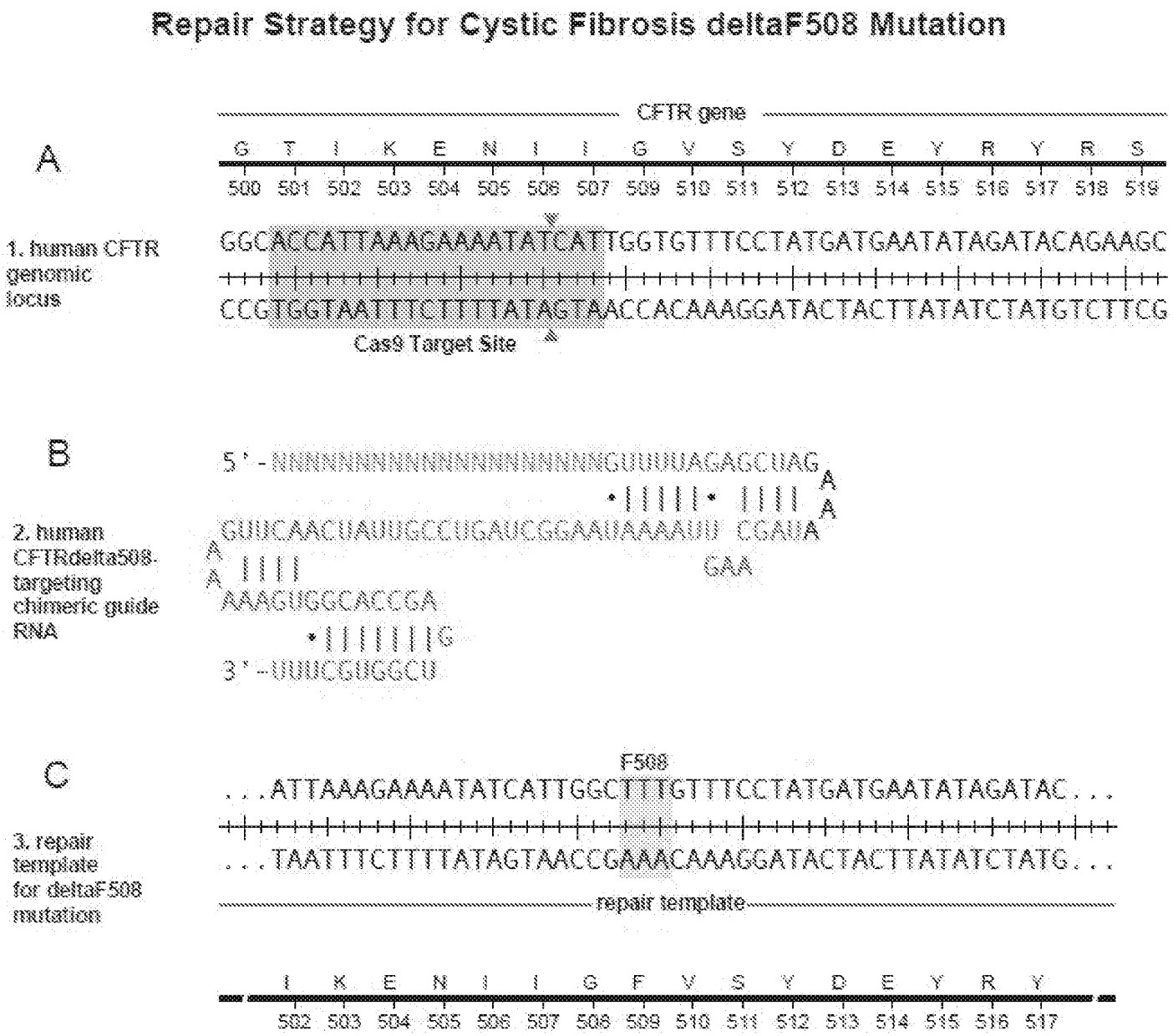
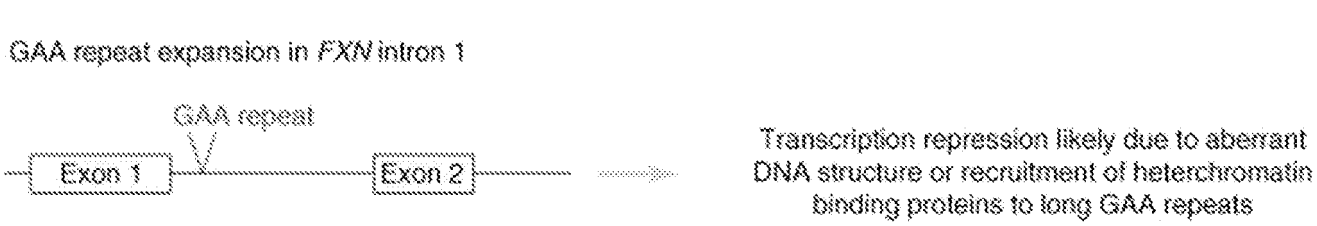

Repair Strategy for Cystic Fibrosis deltaF508 Mutation
A
CFTR gene
G T I K E N I I G V S Y D E Y R Y R S
500 501 502 503 504 505 506 507 509 510 511 512 513 514 515 516 517 518 519
1. human CFTR genomic locus
GGCACCATTAAAGAAAATATCATTGGTGTTTCCTATGATGAATATAGATACAGAAGC
CCGTGGTAATTTCTTTTATAGTAACCACAAAGGATACTACTTATATCTATGTCTTCG
Cas9 Target Site
B
5'-NNNNNNNNNNNNNNNNNNNGUUUUAGAGCUAG
GUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAUA
AAAGUGGCACCGA
3'-UUUCGUGGCU
2. human CFTRdelta508-targeting chimeric guide RNA
C
F508
...ATTAAAGAAAATATCATTGGCTTTGTTTCCTATGATGAATATAGATAC...
...TAATTTCTTTTATAGTAACCGAAACAAAGGATACTACTTATATCTATG...
repair template
I K E N I I G F V S Y D E Y R Y
502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517
3. repair template for deltaF508 mutation

FIG. 31

A


GAA repeat expansion in *FXN* intron 1
GAA repeat
Exon 1
Exon 2
Transcription repression likely due to aberrant DNA structure or recruitment of heterochromatin binding proteins to long GAA repeats

B

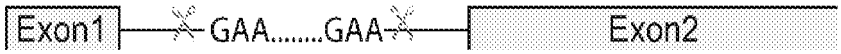

Exon1
GAA.....GAA
Exon2

FIG. 32

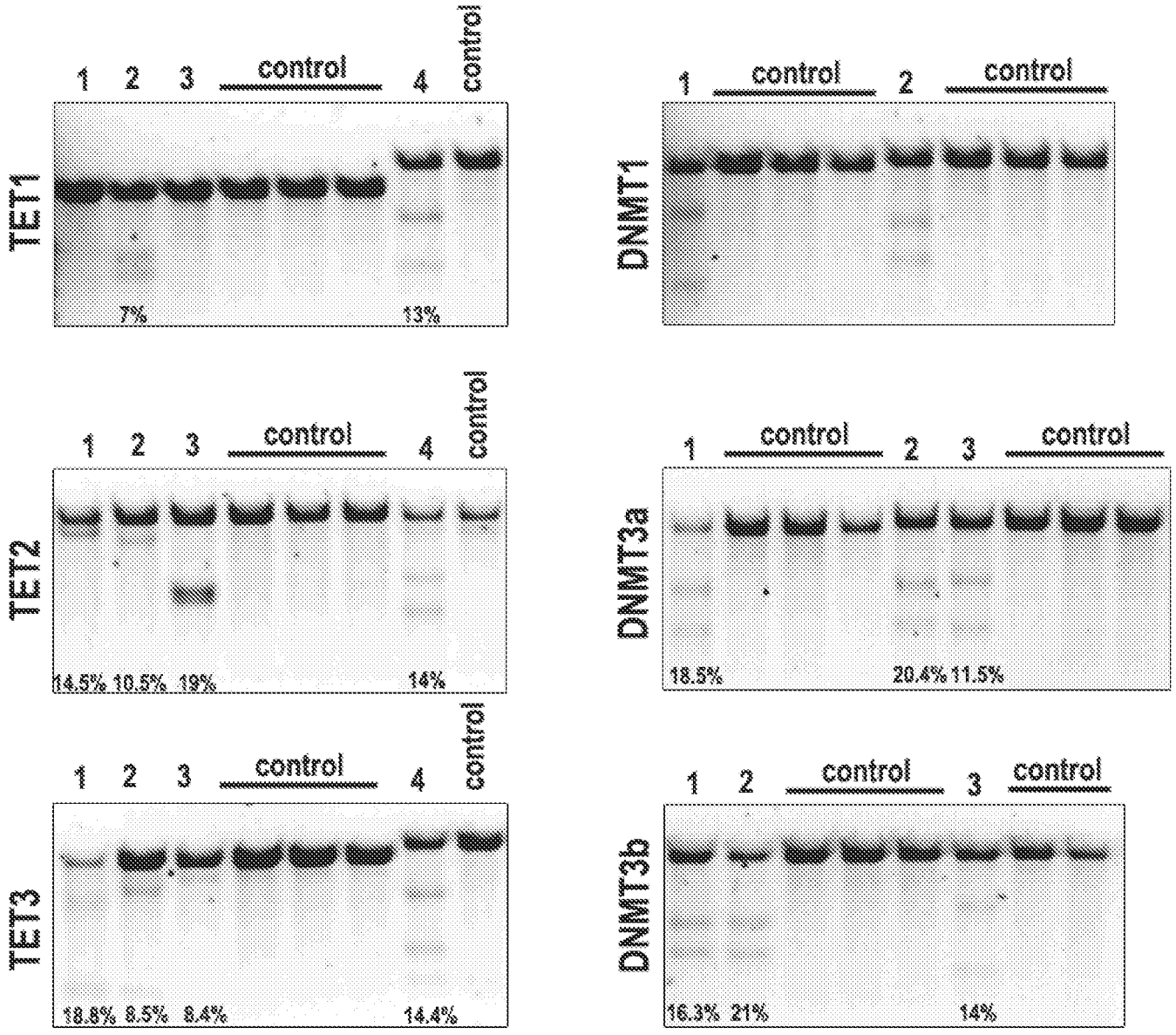

TET1
1 2 3 control 4 control
7% 13%
DNMT1
1 control 2 control
TET2
1 2 3 control 4 control
14.5% 10.5% 19% 14%
DNMT3a
1 control 2 3 control
18.5% 20.4% 11.5%
TET3
1 2 3 control 4 control
18.8% 8.5% 8.4% 14.4%
DNMT3b
1 2 control 3 control
16.3% 21% 14%

FIG. 33

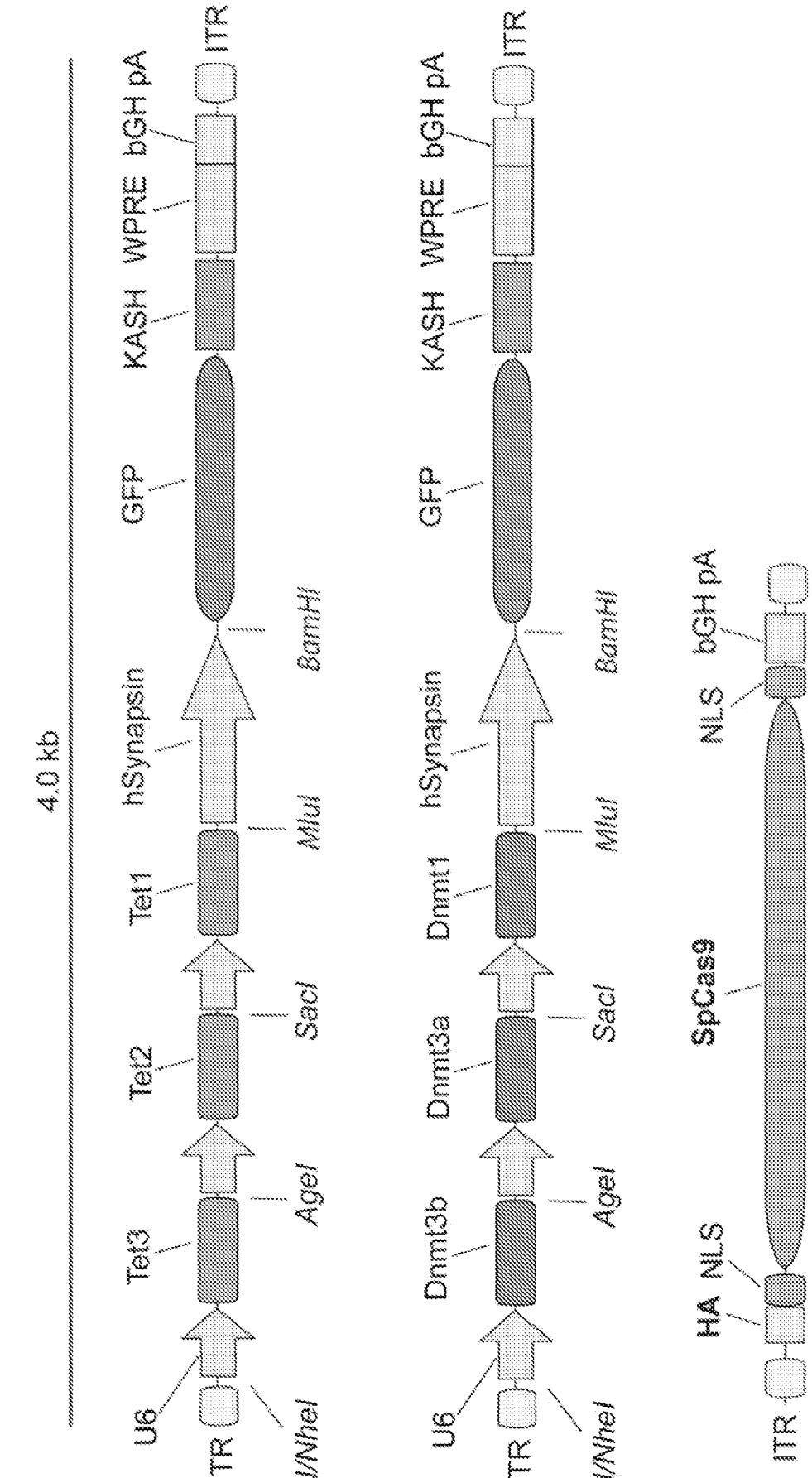

a
4.0 kb
ITR
U6
NotI/NheI
Tet3
AgeI
Tet2
SacI
Tet1
MluI
hSynapsin
GFP
KASH
WPRE
bGH pA
ITR
BamHI
ITR
U6
NotI/NheI
Dnmt3b
AgeI
Dnmt3a
SacI
Dnmt1
MluI
hSynapsin
GFP
KASH
WPRE
bGH pA
ITR
BamHI
ITR
HA
NLS
SpCas9
NLS
bGH pA

FIG. 34

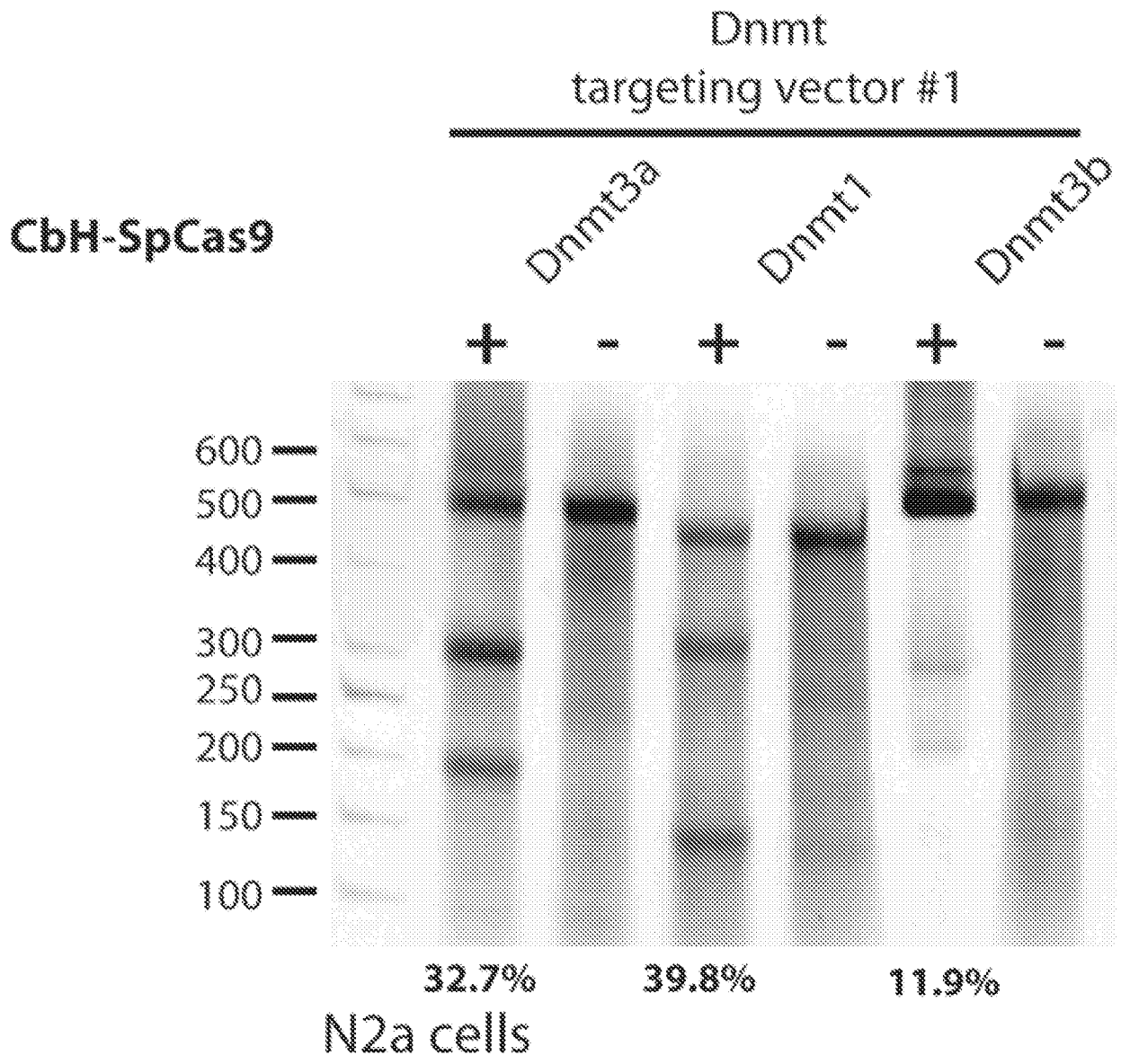

Dnmt
targeting vector #1
CbH-SpCas9
Dnmt3a
Dnmt1
Dnmt3b
+
-
+
-
+
-
600
500
400
300
250
200
150
100
32.7%
39.8%
11.9%
N2a cells

FIG. 35

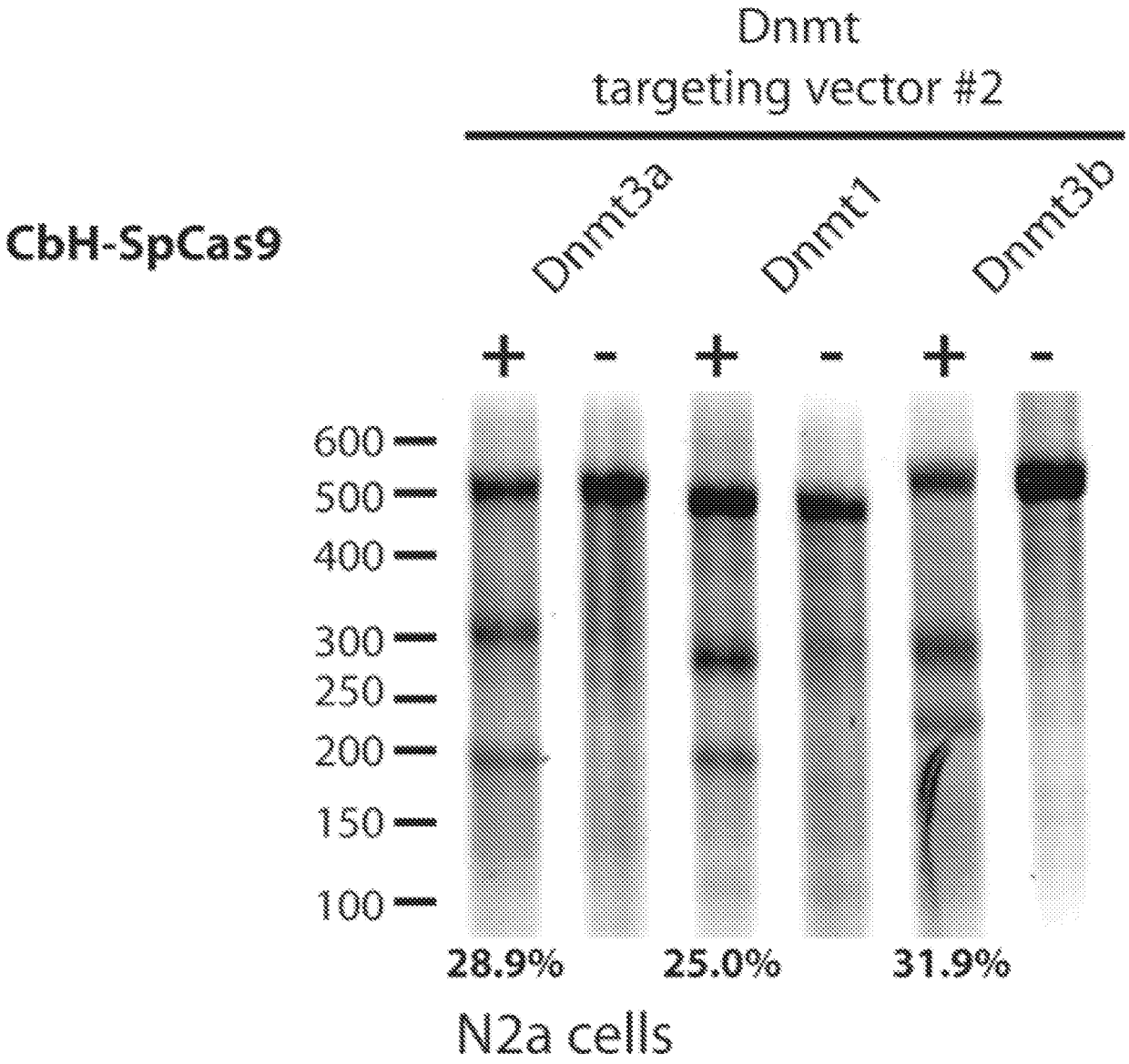

Dnmt
targeting vector #2
CbH-SpCas9
Dnmt3a
Dnmt1
Dnmt3b
+ - + - + -
600
500
400
300
250
200
150
100
28.9%
25.0%
31.9%
N2a cells

FIG. 36

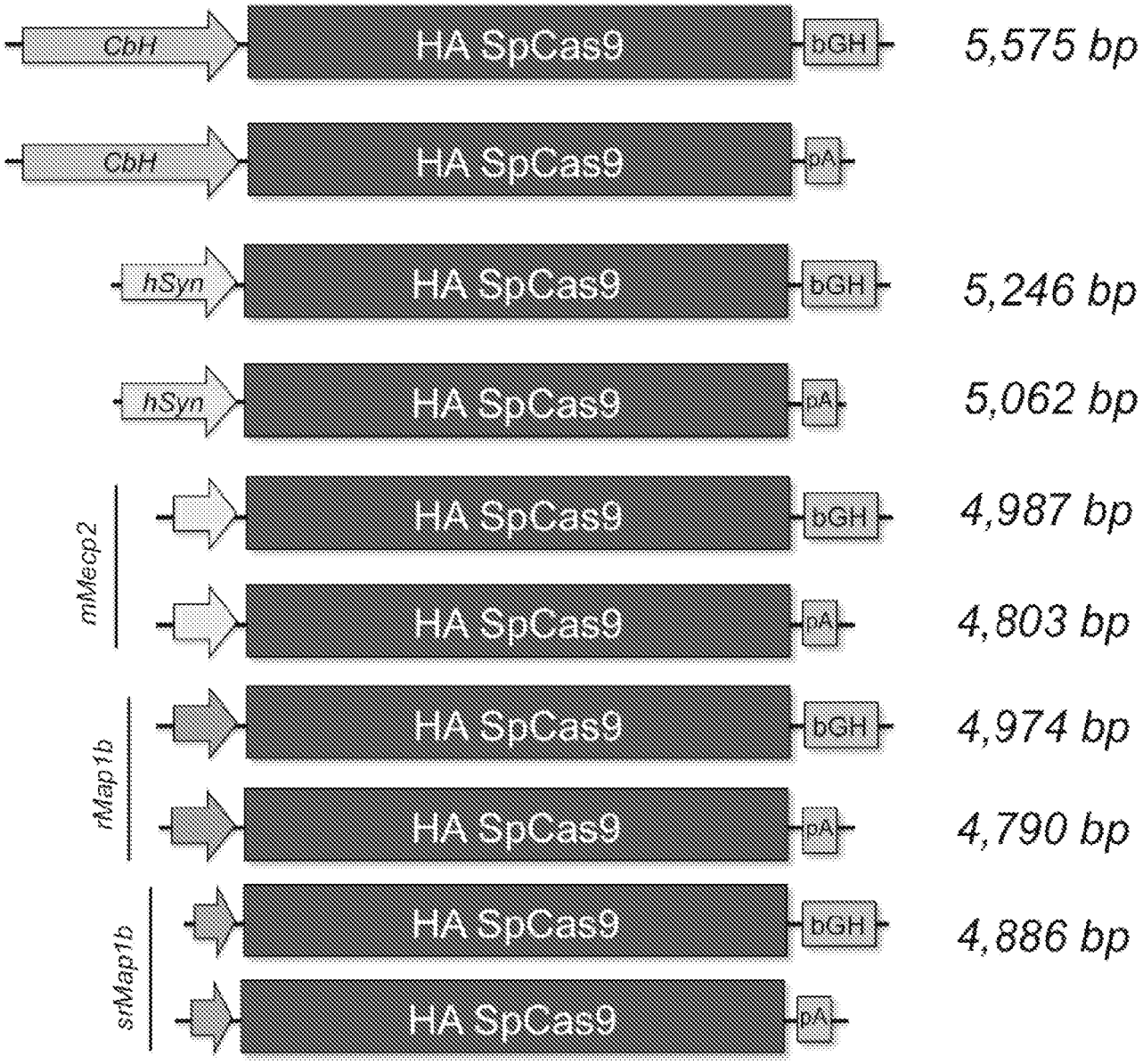

CbH
HA SpCas9
bGH
5,575 bp
CbH
HA SpCas9
pA
hSyn
HA SpCas9
bGH
5,246 bp
hSyn
HA SpCas9
pA
5,062 bp
mMecp2
HA SpCas9
bGH
4,987 bp
HA SpCas9
pA
4,803 bp
rMap1b
HA SpCas9
bGH
4,974 bp
HA SpCas9
pA
4,790 bp
srMap1b
HA SpCas9
bGH
4,886 bp
HA SpCas9
pA

FIG. 37

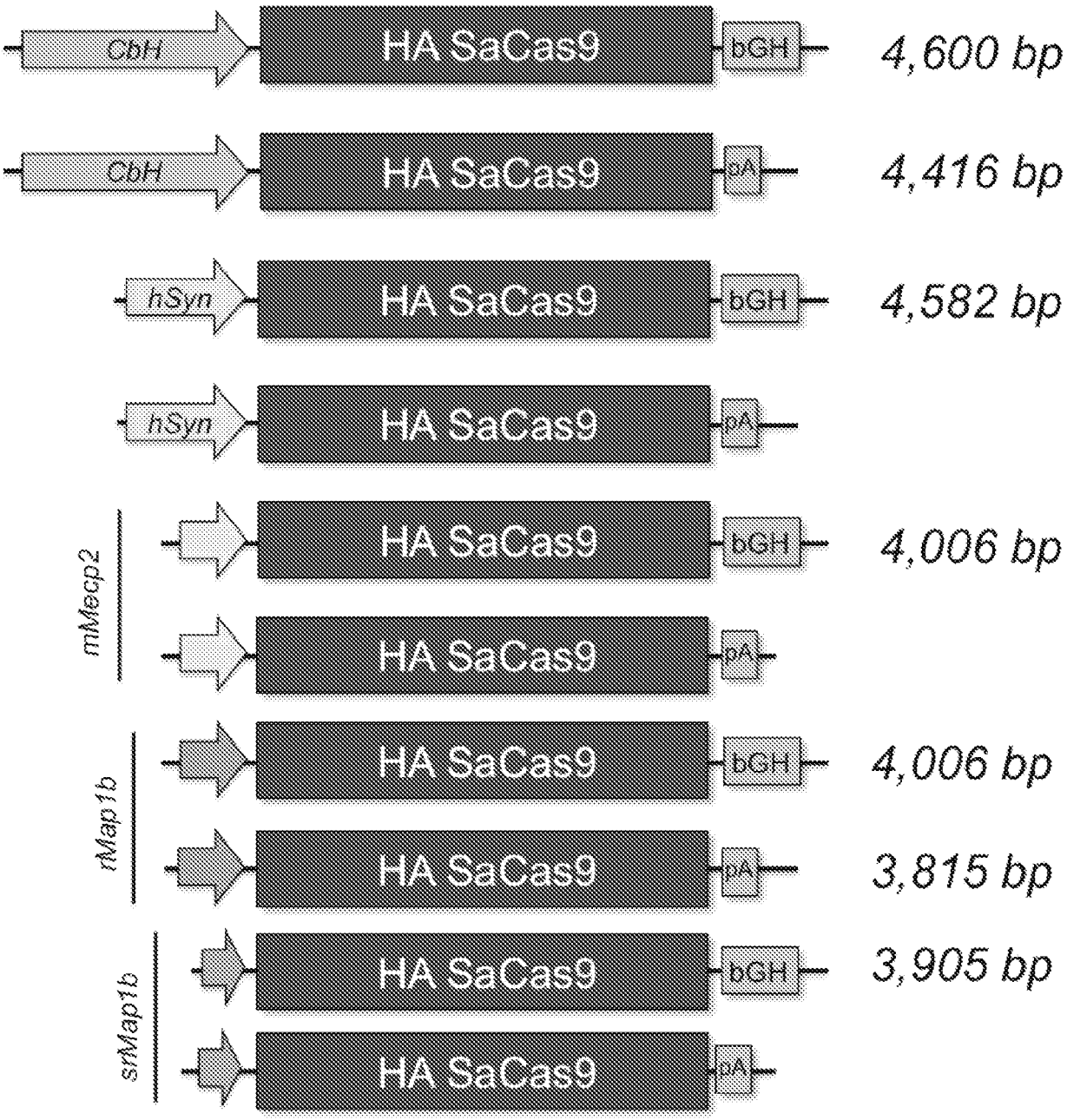

CbH
HA SaCas9
bGH
4,600 bp
CbH
HA SaCas9
pA
4,416 bp
hSyn
HA SaCas9
bGH
4,582 bp
hSyn
HA SaCas9
pA
mMecp2
HA SaCas9
bGH
4,006 bp
HA SaCas9
pA
rMap1b
HA SaCas9
bGH
4,006 bp
HA SaCas9
pA
3,815 bp
srMap1b
HA SaCas9
bGH
3,905 bp
HA SaCas9
pA

FIG. 38

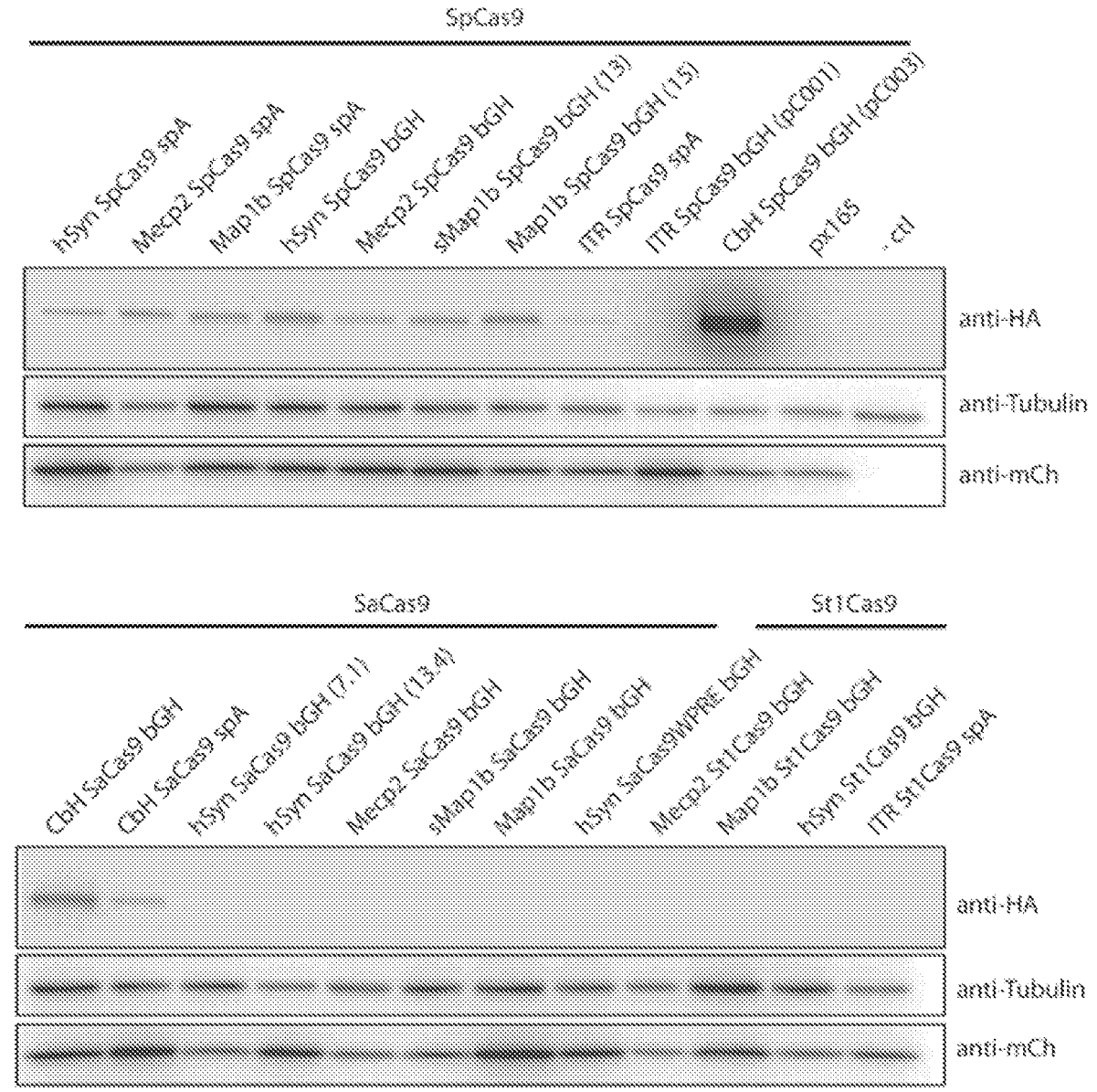

Expression of SpCas9 & SaCas9 in N2a cells
SpCas9
hSyn SpCas9 spA
Mecp2 SpCas9 spA
Map1b SpCas9 spA
hSyn SpCas9 bGH
Mecp2 SpCas9 bGH
sMap1b SpCas9 bGH (13)
Map1b SpCas9 bGH (15)
ITR SpCas9 spA
ITR SpCas9 bGH (pC001)
CbH SpCas9 bGH (pC003)
px165
- ctl
anti-HA
anti-Tubulin
anti-mCh
SaCas9
St1Cas9
CbH SaCas9 bGH
CbH SaCas9 spA
hSyn SaCas9 bGH (7.1)
hSyn SaCas9 bGH (13.4)
Mecp2 SaCas9 bGH
sMap1b SaCas9 bGH
Map1b SaCas9 bGH
hSyn SaCas9WPRE bGH
Mecp2 St1Cas9 bGH
Map1b St1Cas9 bGH
hSyn St1Cas9 bGH
ITR St1Cas9 spA
anti-HA
anti-Tubulin
anti-mCh

FIG. 39

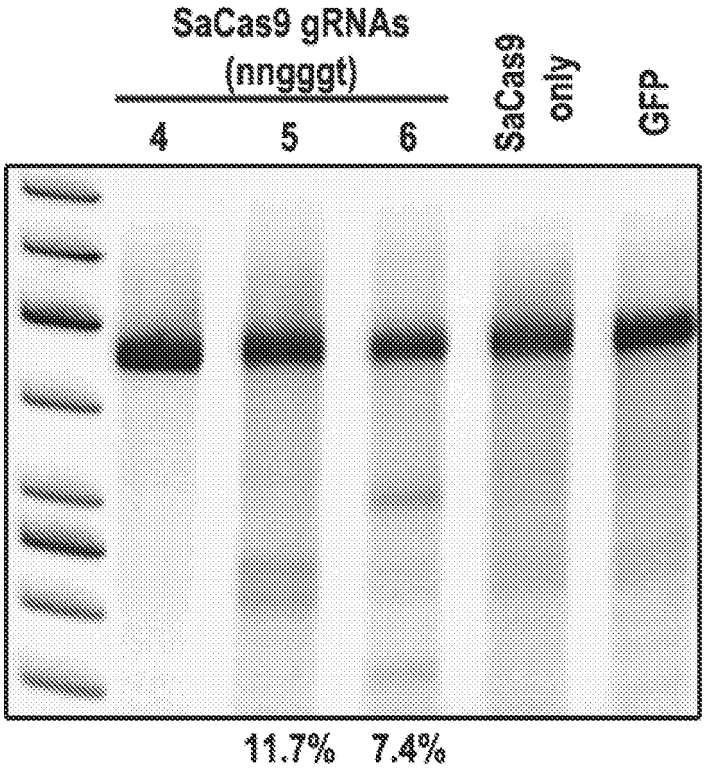



FIG. 40

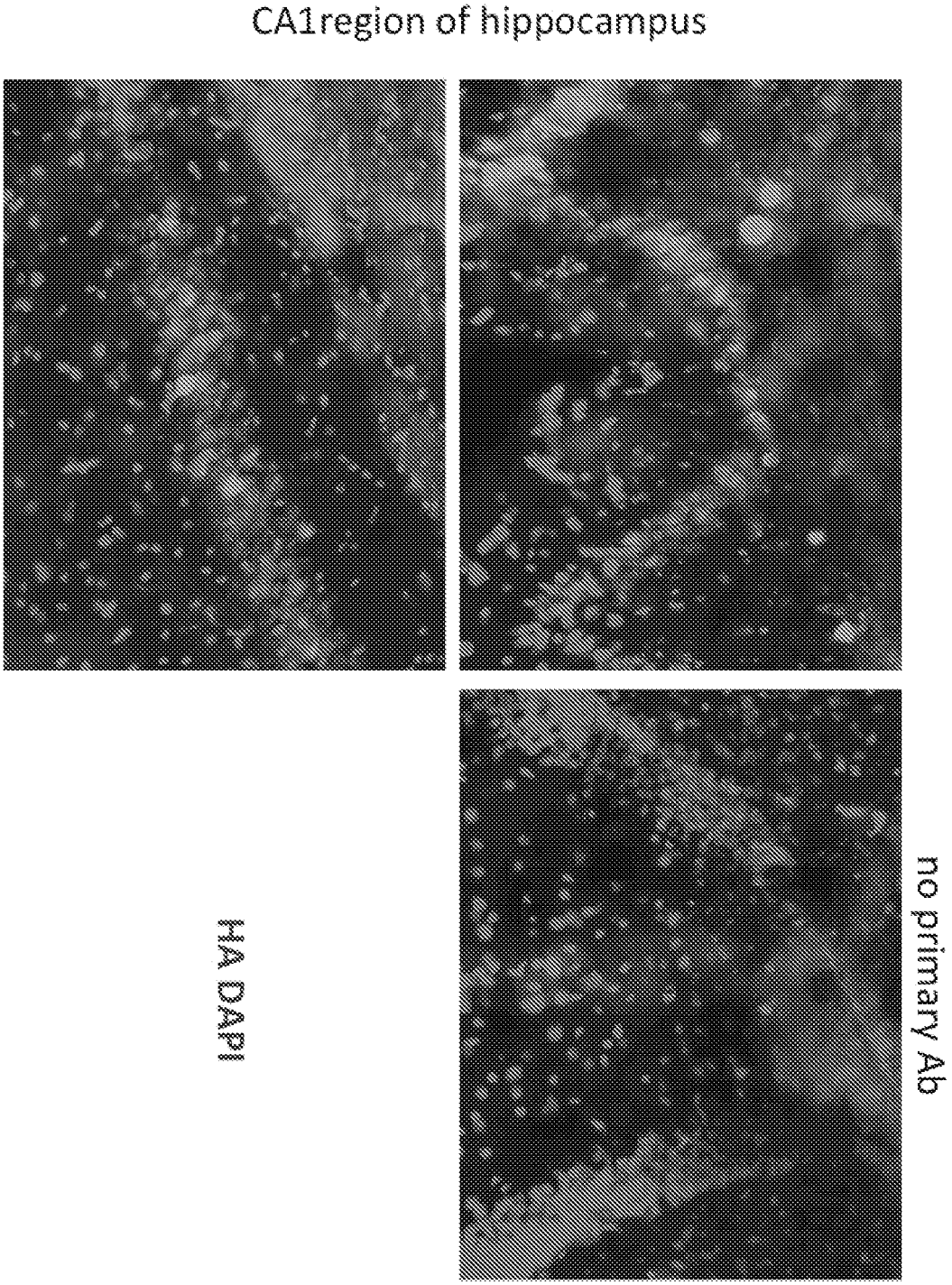



FIG. 41

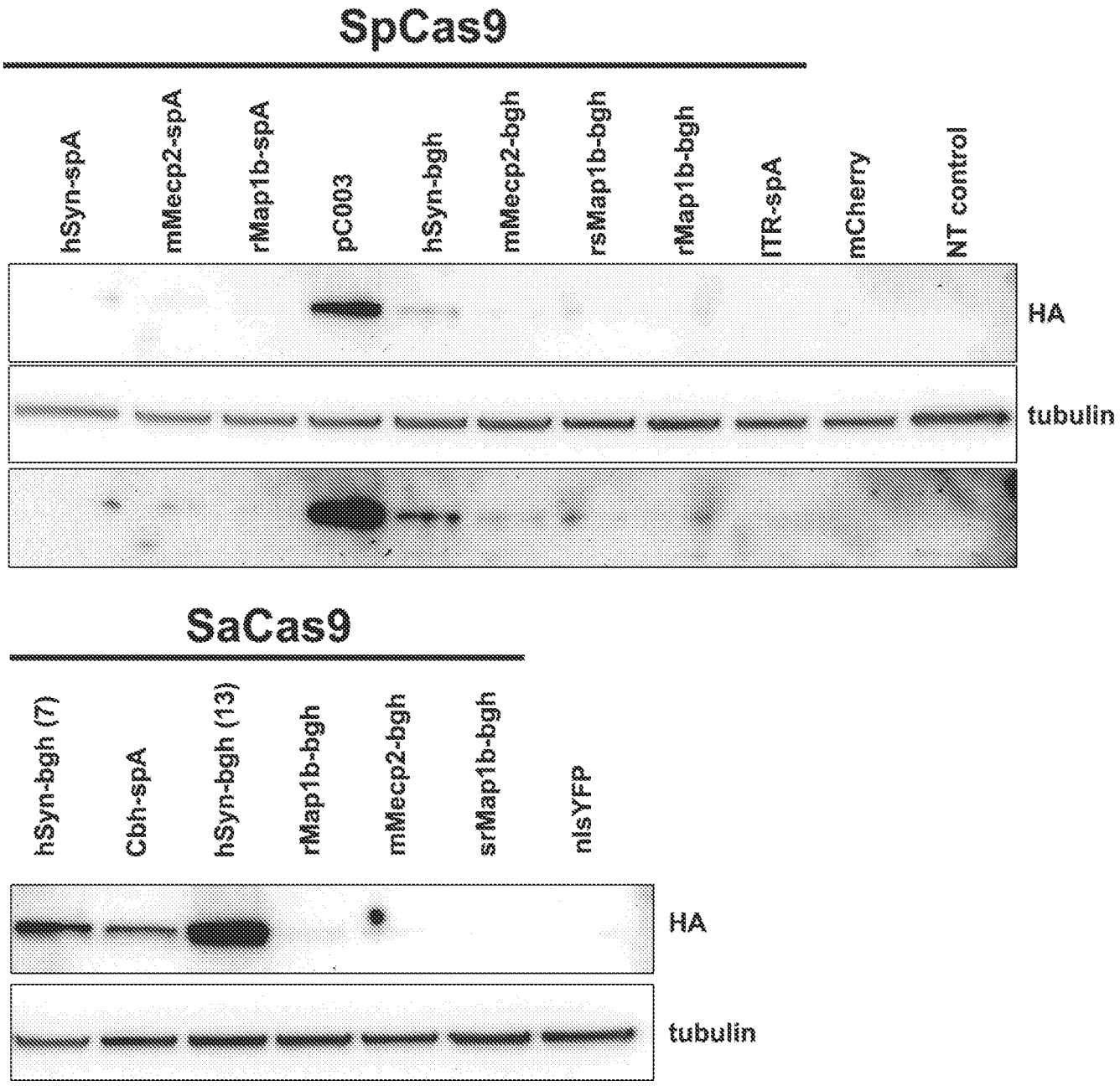

SpCas9
hSyn-spA
mMecp2-spA
rMap1b-spA
pC003
hSyn-bgh
mMecp2-bgh
rsMap1b-bgh
rMap1b-bgh
ITR-spA
mCherry
NT control
HA
tubulin
SaCas9
hSyn-bgh (7)
Cbh-spA
hSyn-bgh (13)
rMap1b-bgh
mMecp2-bgh
srMap1b-bgh
nlsYFP
HA
tubulin

FIG. 42

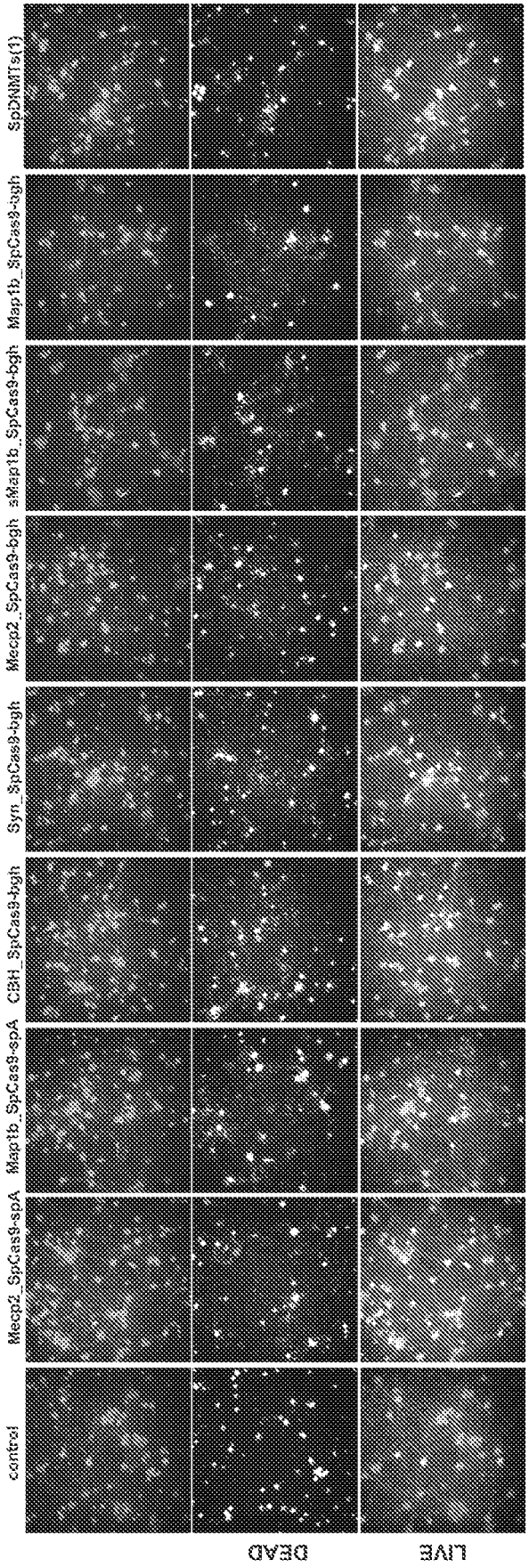

control
Mecp2_SpCas9-spA
Map1b_SpCas9-spA
CBH_SpCas9-bgh
Syn_SpCas9-bgh
Mecp2_SpCas9-bgh
sMap1b_SpCas9-bgh
Map1b_SpCas9-bgh
SpDNMTs(1)
DEAD
LIVE

FIG. 43

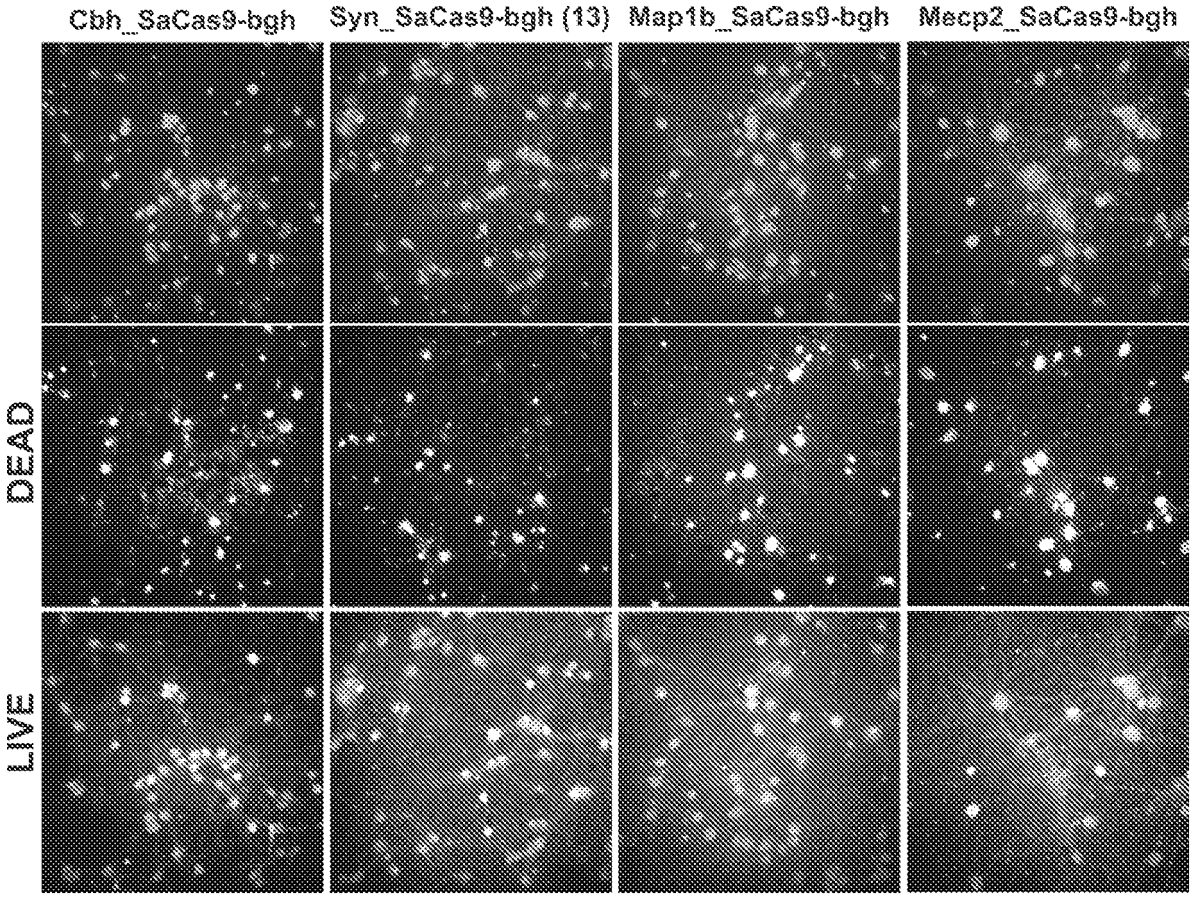

Cbh_SaCas9-bgh
Syn_SaCas9-bgh (13)
Map1b_SaCas9-bgh
Mecp2_SaCas9-bgh
DEAD
LIVE

FIG. 44

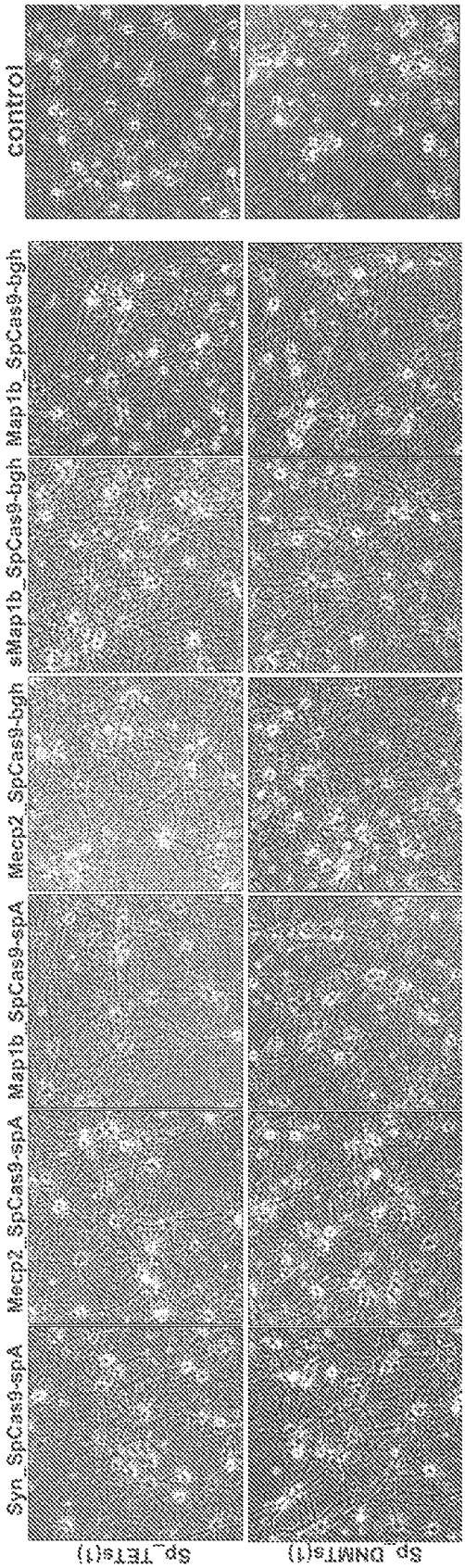

control
Map1b_SpCas9-bgh
sMap1b_SpCas9-bgh
Mecp2_SpCas9-bgh
Map1b_SpCas9-spA
Mecp2_SpCas9-spA
Syn_SpCas9-spA
Sp_DNMTs(1)
Sp_TETs(1)

FIG. 45

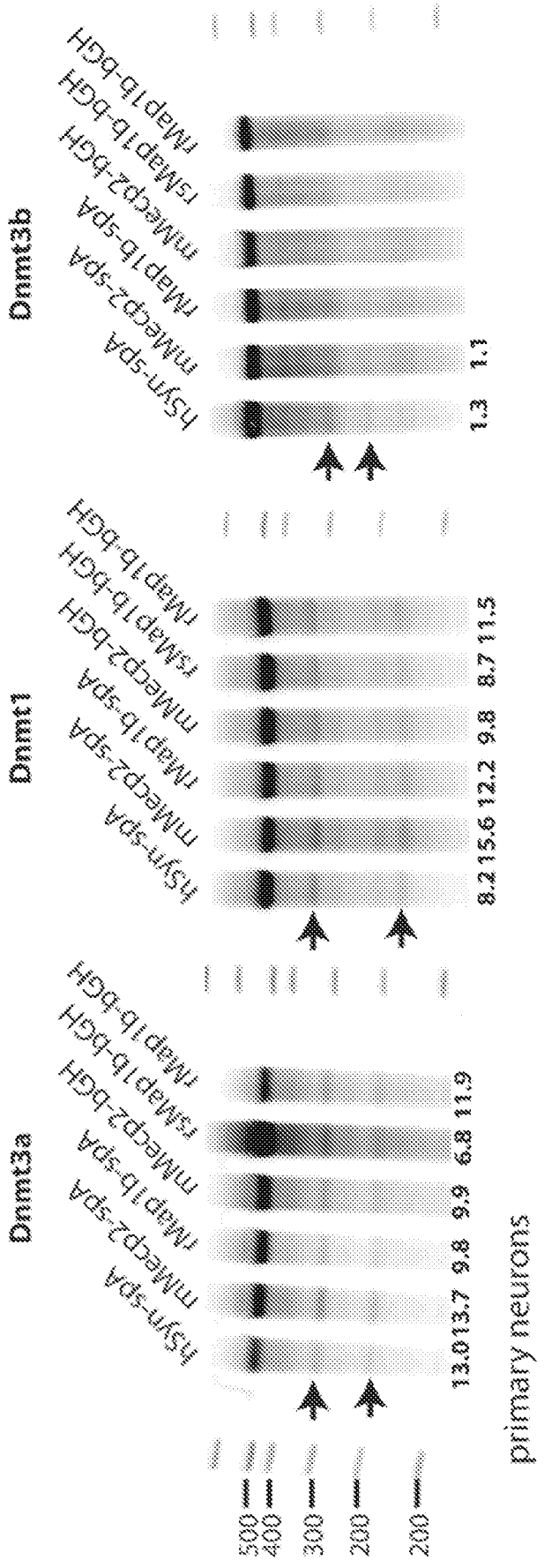

Dnmt3a
Dnmt1
Dnmt3b
hSyn-spA
mMecp2-spA
rMap1b-spA
rMecp2-bGH
rMap1b-bGH
13.0
13.7
9.8
9.9
6.8
11.9
8.2
15.6
12.2
9.8
8.7
11.5
1.3
1.1
500
400
300
200
200
primary neurons

FIG. 46

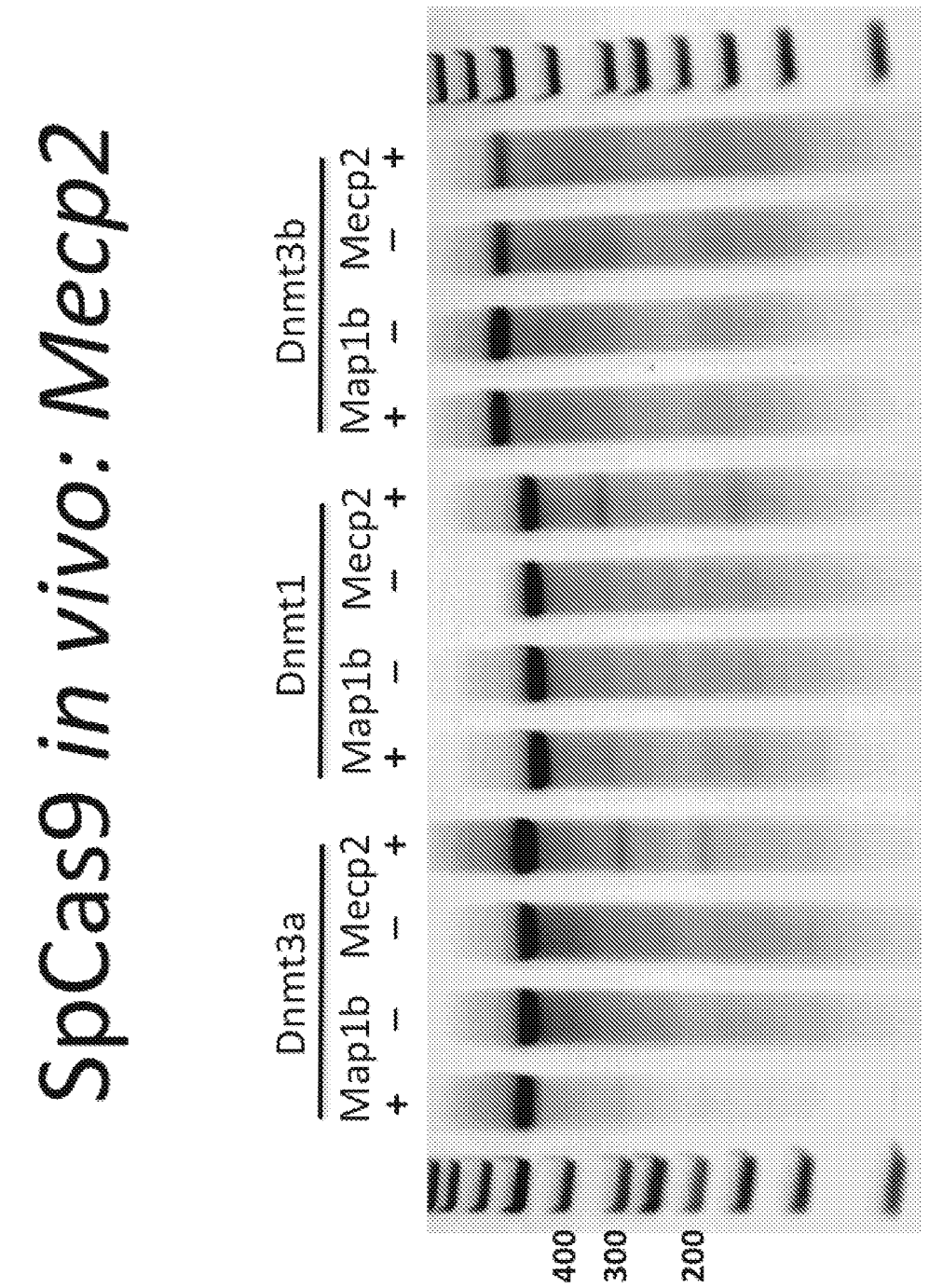

SpCas9 in vivo: Mecp2
Dnmt3a
Map1b + + + +
Mecp2 − − + +
Dnmt1
Map1b + + + +
Mecp2 − − + +
Dnmt3b
Map1b + + + +
Mecp2 − − + +
400
300
200
12.0%
17.0%

FIG. 47

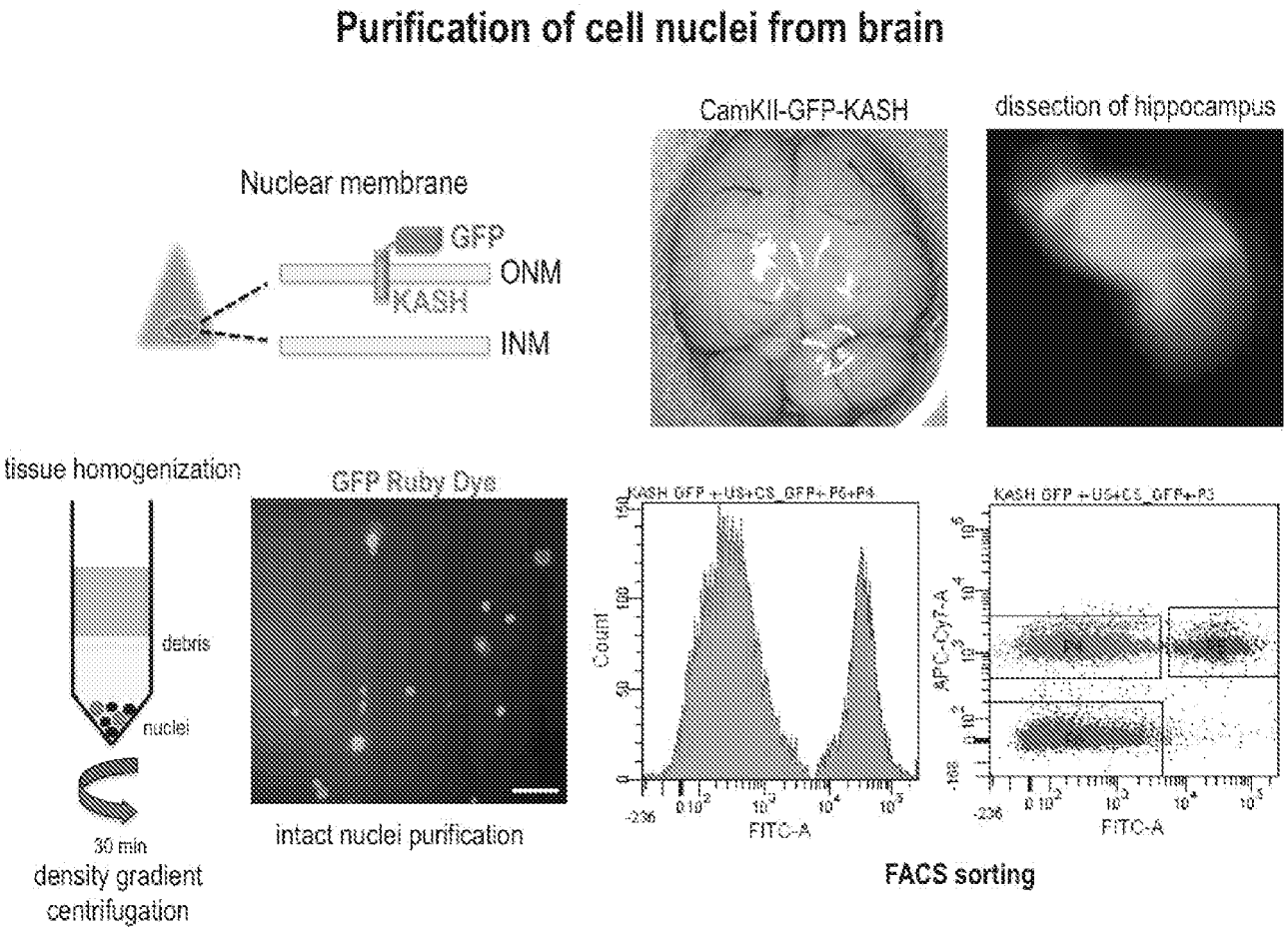

Purification of cell nuclei from brain
Nuclear membrane
GFP
ONM
KASH
INM
CamKII-GFP-KASH
dissection of hippocampus
tissue homogenization
debris
nuclei
30 min
density gradient centrifugation
GFP Ruby Dye
intact nuclei purification
Count
FITC-A
APC-Cy7-A
FACS sorting

FIG. 48

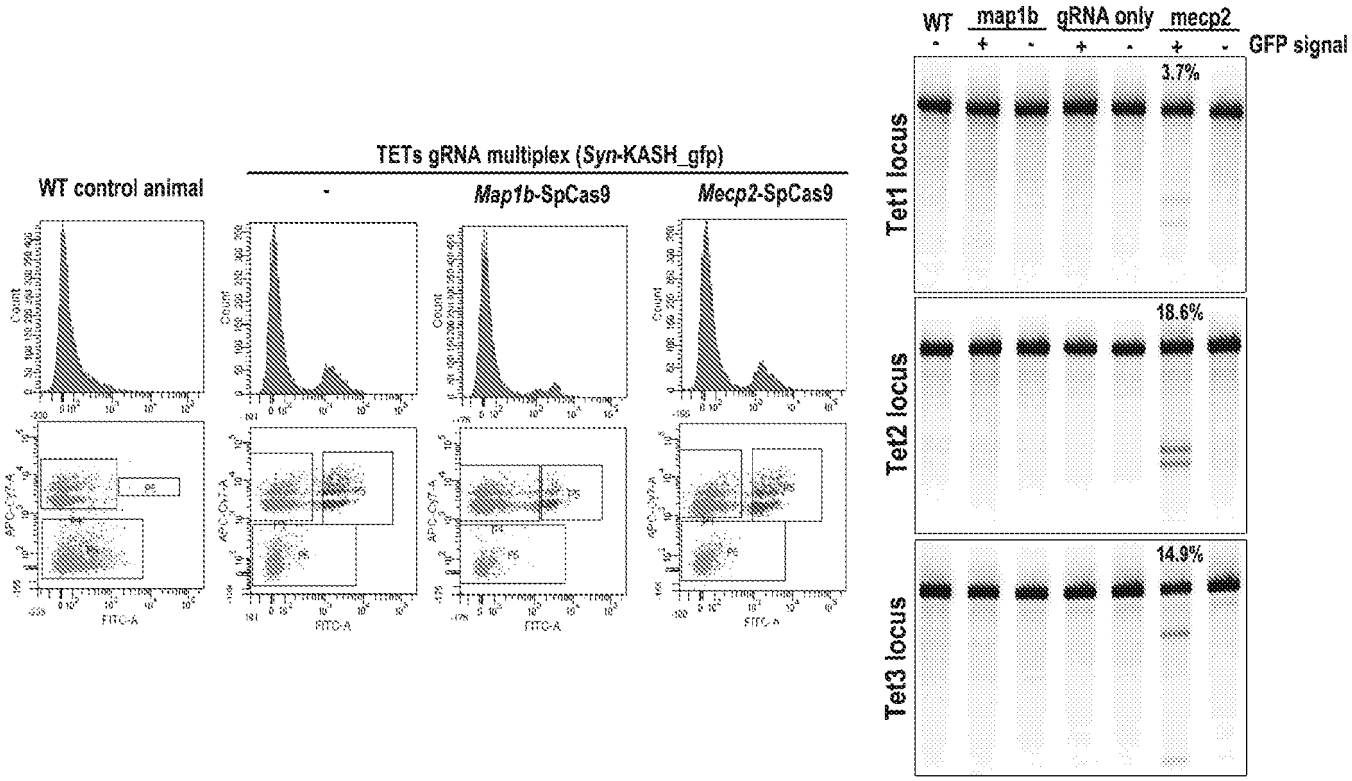

WT control animal
TETs gRNA multiplex (Syn-KASH_gfp)
-
Map1b-SpCas9
Mecp2-SpCas9
Count
APC-Cy7-A
FITC-A
WT
map1b
gRNA only
mecp2
GFP signal
Tet1 locus
Tet2 locus
Tet3 locus
3.7%
18.6%
14.9%

FIG. 49

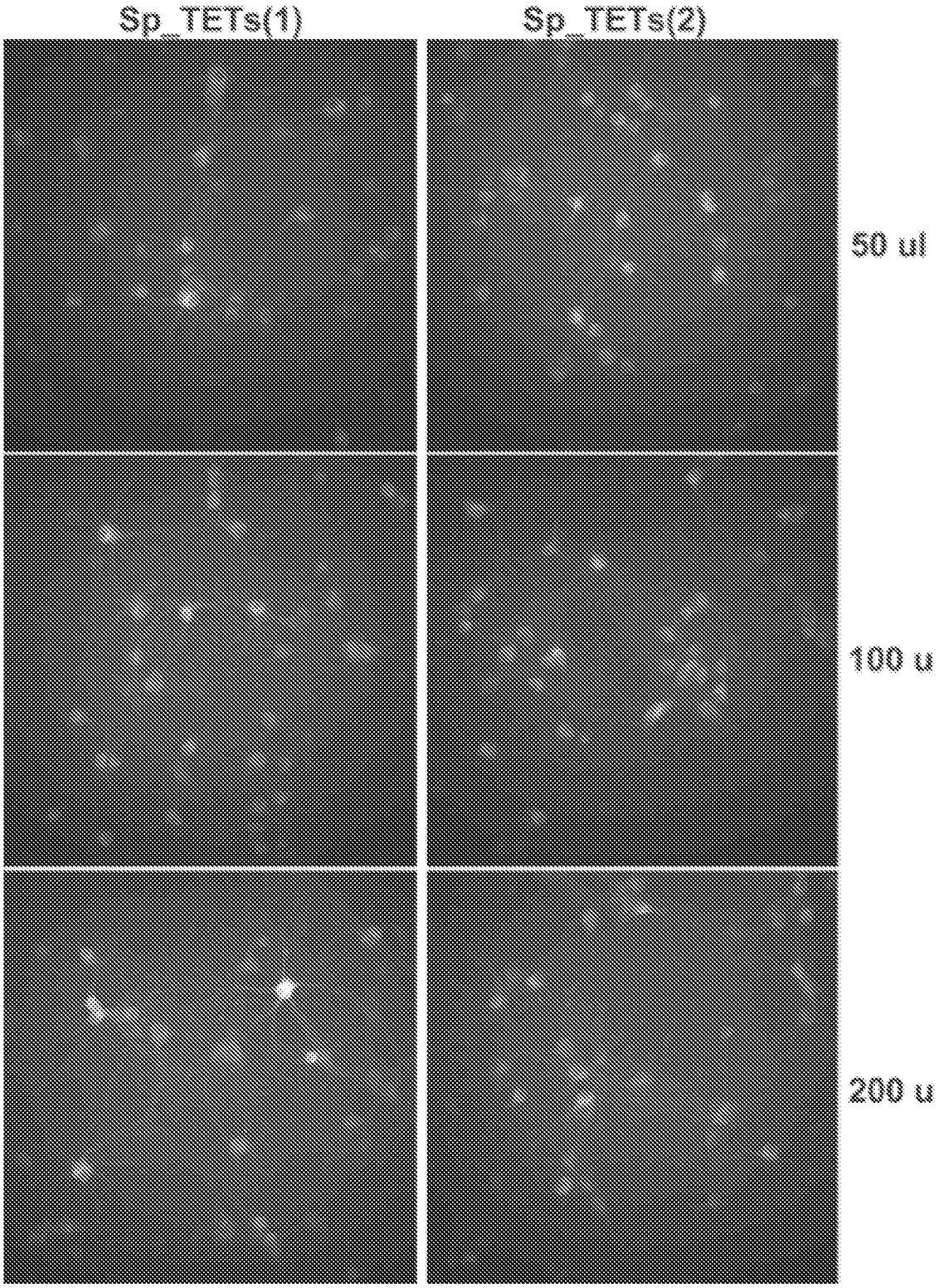
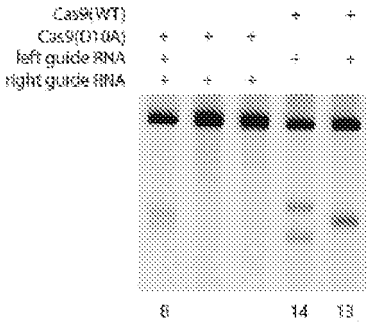
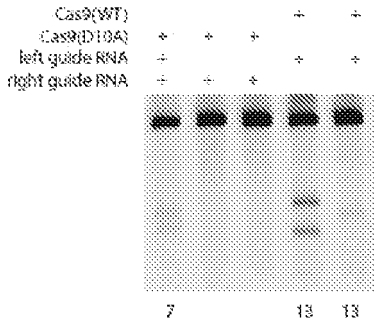



FIG. 50

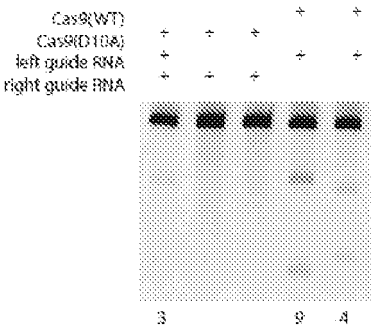
| | pattern | overhang |
|---|---|---|
| 1 | CCnNNNNNNNNNNNNNNNNnnnnnnnnnnnnNNNNNNNNNNNNNNNNnGG | 38 |
| 2 | CCnNNNNNNNNNNNNNNNNnnnnnnnnnNNNNNNNNNNNNNNNNnGG | 34 |
| 3 | CCnNNNNNNNNNNNNNNNNnnnnnNNNNNNNNNNNNNNNNnGG | 30 |
| 4 | CCnNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNnGG | 26 |
| 5 | CCnNNNNNNNNNNNNNNNNNNNNNNNNNNNNNnGG | 22 |
| 6 | CCnNNNNNNNNNNNNNNNNNNNNNNNNNnGG | 18 |
| 7 | CCnNNNNNNNNNNNNNNNNNNNNNNnGG | 14 |
| 8 | CCnNNNNNNNNNNNNNNNNNNnGG | 10 |
| 9 | CCnNNNNNNNNNNNNNNnGG | 6 |
| 10 | CCnNNNNNNNNNNNNnGG | 4 |
| 11 | CCnNNNNNNNNNNnGG | 3 |
| 12 | CCnNNNNNNNNNnGG | 2 |
| 13 | CCnNNNNNNNNnGG | 1 |
| 14 | CCnNNNNNNNnGG | 0 |
| 15 | CCnNNNNNNnGG | 1 |
| 16 | CCnNNNNNnGG | 2 |
| 17 | CCnNNNNnGG | 3 |
| 18 | CCnnnGG | 4 |
| 19 | CCGG | 8 |
| 20 | GGCC | 10 |
| 21 | GGNNCC | 12 |
| 22 | GGNNNCC | 14 |
| 23 | GGNNNNCC | 16 |
| 24 | GGNNNNNNNCC | 20 |



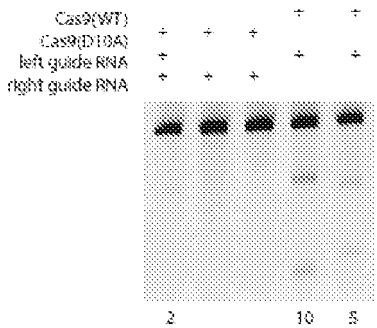

Pattern 1




Pattern 2




Pattern 3




Pattern 4

FIG. 51

Reverse PCR primers for generating U6-guide RNA expression Cassette (pair with U6 forward primer)

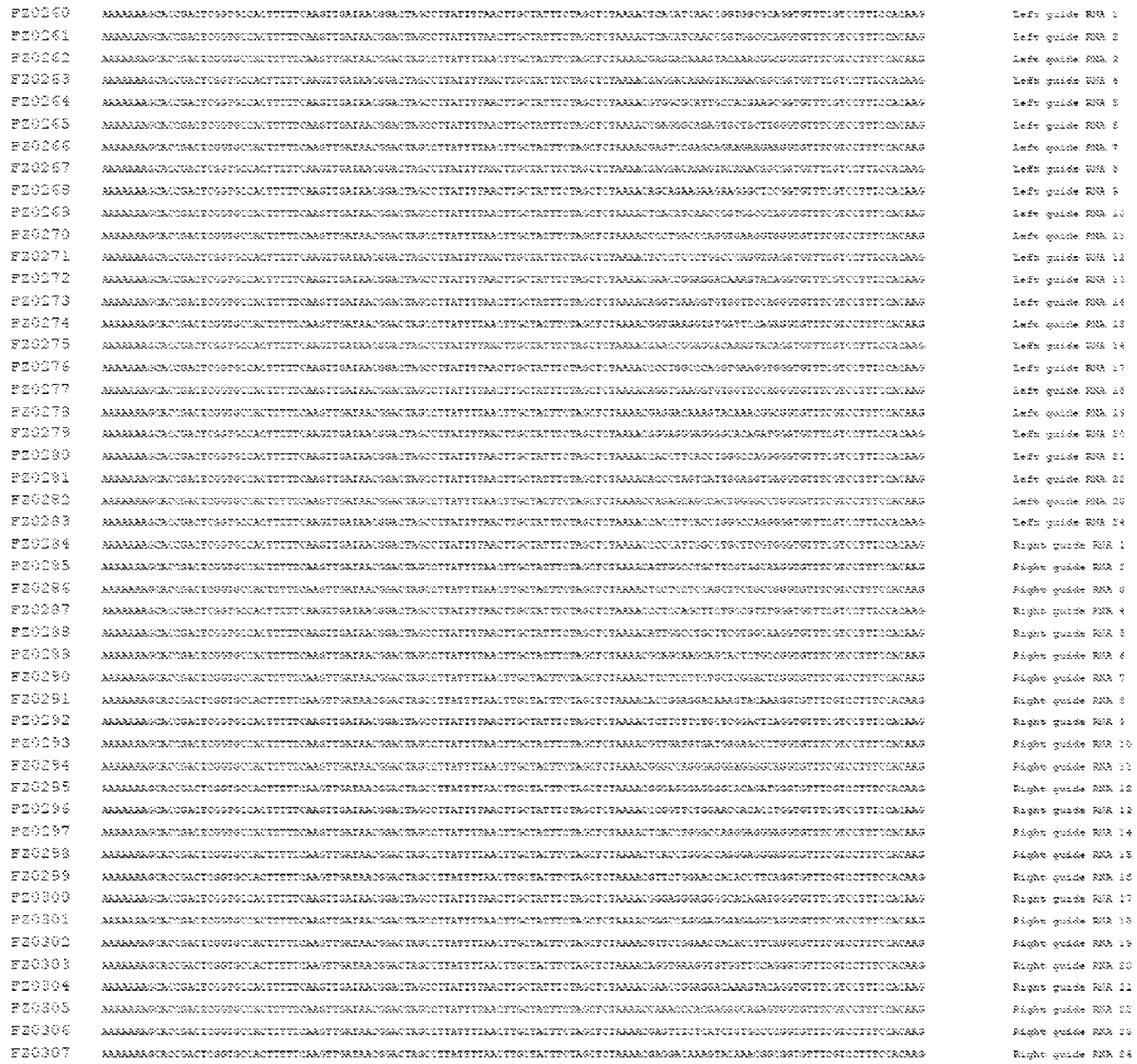


FIG. 52

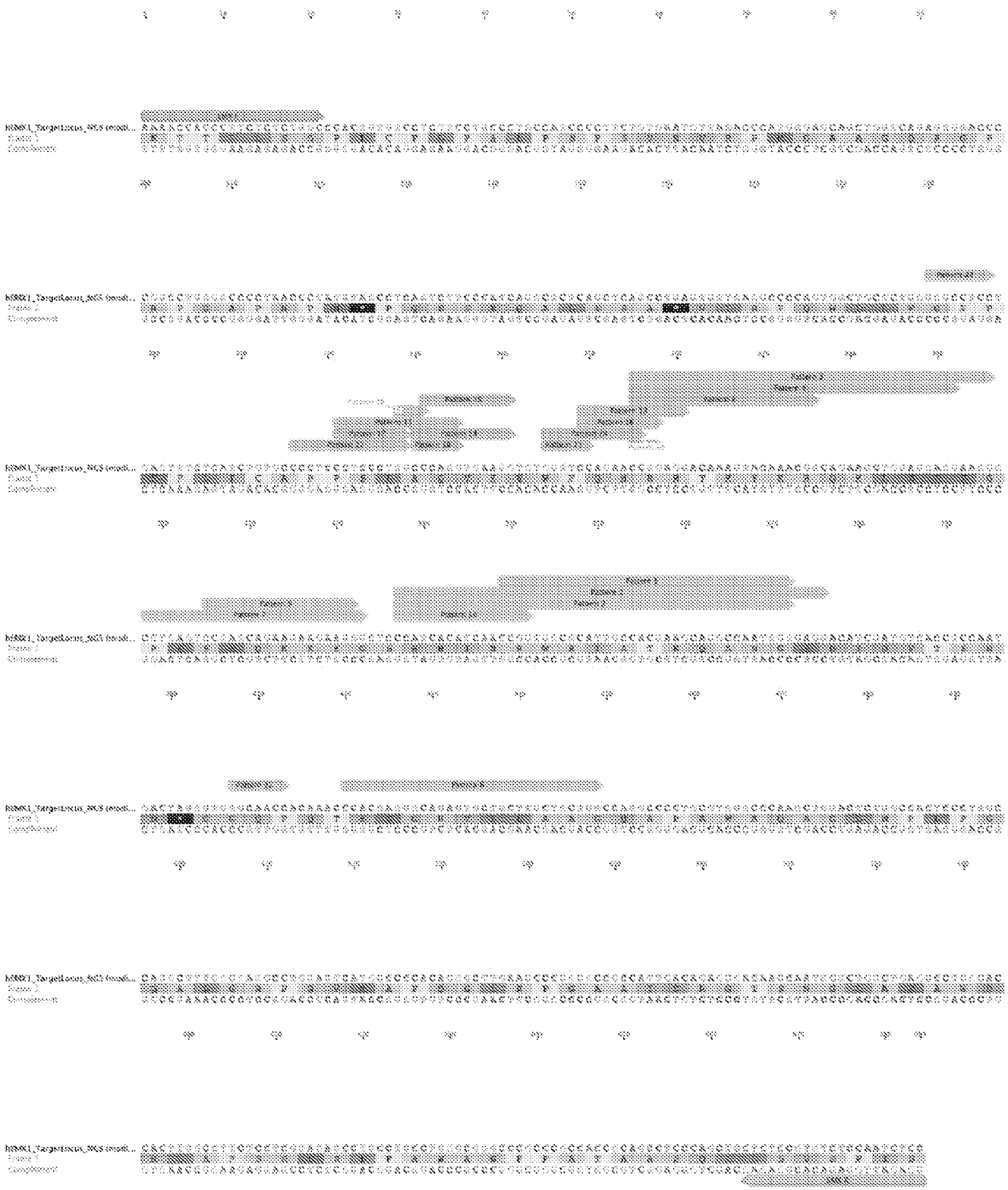


FIG. 53

| lane # | Cas9 to use | Left gRNA | Right gRNA | Overhang (D10A) - number of bases protruding from 5' |
|---|---|---|---|---|
| 1 | D10A | left 23 | left 12 | -36 |
| 2 | D10A | right 4 | left 9 | -25 |
| 3 | D10A | left 23 | right 23 | -16 |
| 4 | D10A | right 7 | left 10 | -15 |
| 5 | D10A | right 16 | left 3 | -8 |
| 6 | D10A | right 22 | right 6 | 26 |
| 7 | D10A | left 12 | right 16 | 31 |
| 8 | D10A | left 12 | right 13 | 34 |
| 9 | D10A | left 10 | right 1 | 38 |
| 10 | D10A | right 23 | right 16 | 51 |
| 11 | D10A | right 23 | right 13 | 54 |
| 12 | D10A | left 3 | right 7 | 57 |
| 13 | D10A | left 12 | right 4 | 65 |
| 14 | D10A | left 12 | right 3 | 69 |
| 15 | D10A | left 3 | right 10 | 76 |
| 16 | D10A | right 23 | right 4 | 85 |
| 17 | D10A | left 12 | right 9 | 95 |
| 18 | D10A | left 12 | right 10 | 115 |
| 19 | D10A | right 23 | right 10 | 135 |
| 20 | D10A | left 12 | right 2 | 145 |
| 21 | D10A | left 12 | left 22 | 181 |
| 22 | D10A | right 23 | left 22 | 201 |
| 23 | D10A | left 12 | right 6 | 222 |
| 24 | D10A | right 23 | right 6 | 242 |

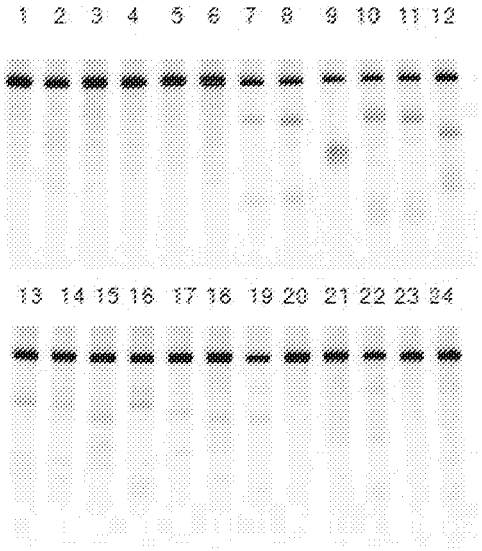



FIG. 54

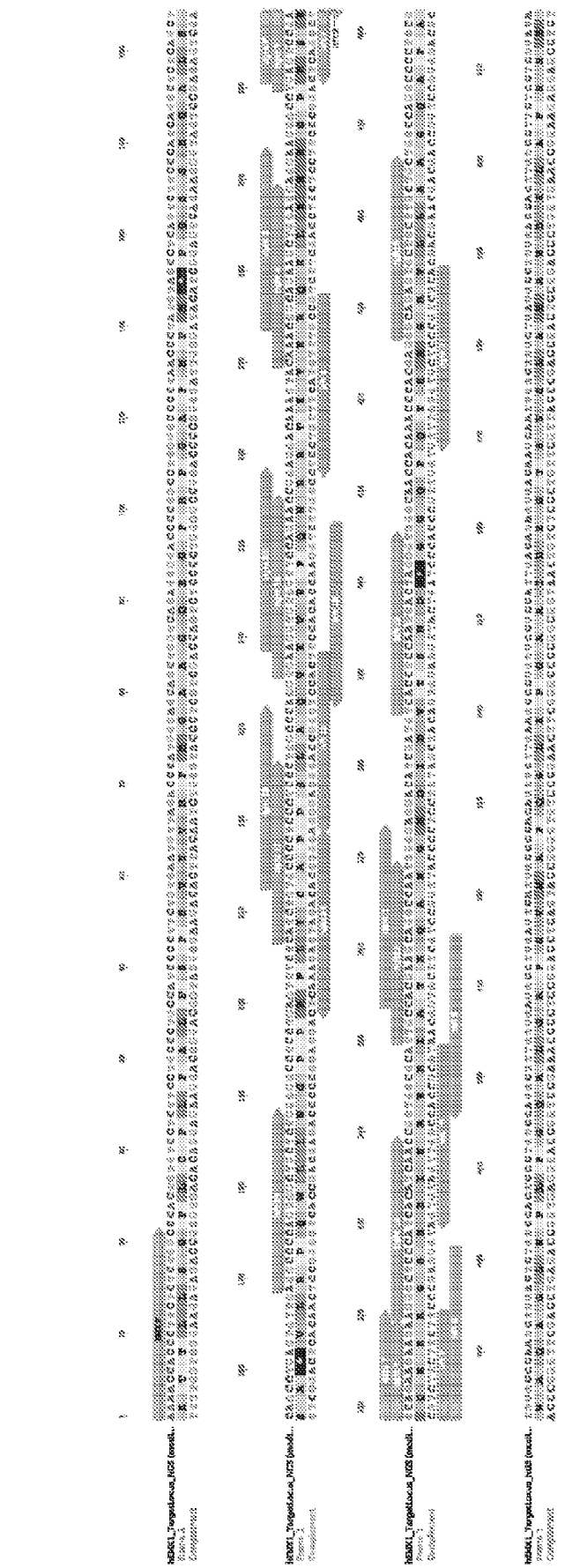


FIG. 55

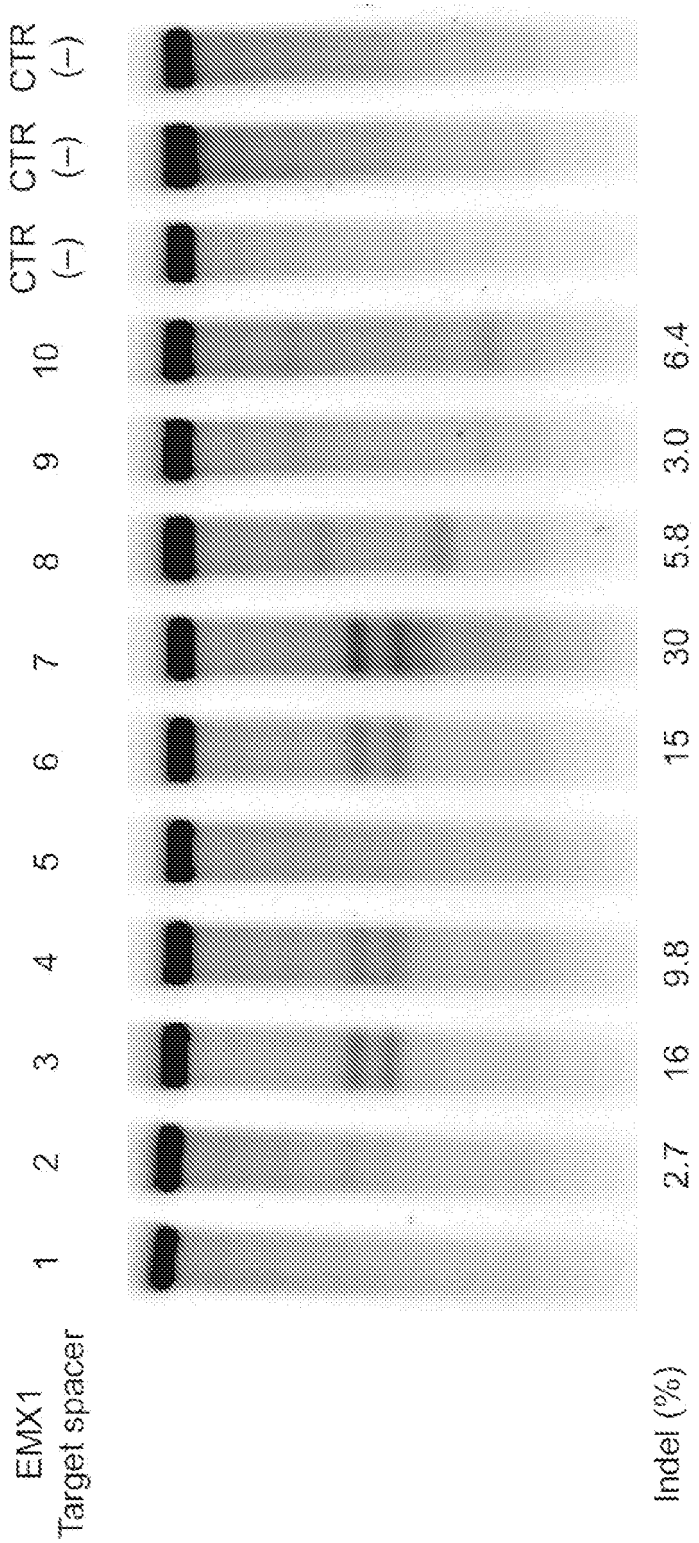

EMX1
Target spacer
1
2
3
4
5
6
7
8
9
10
CTR (–)
CTR (–)
CTR (–)
Indel (%)
2.7
16
9.8
15
30
5.8
3.0
6.4

FIG. 56

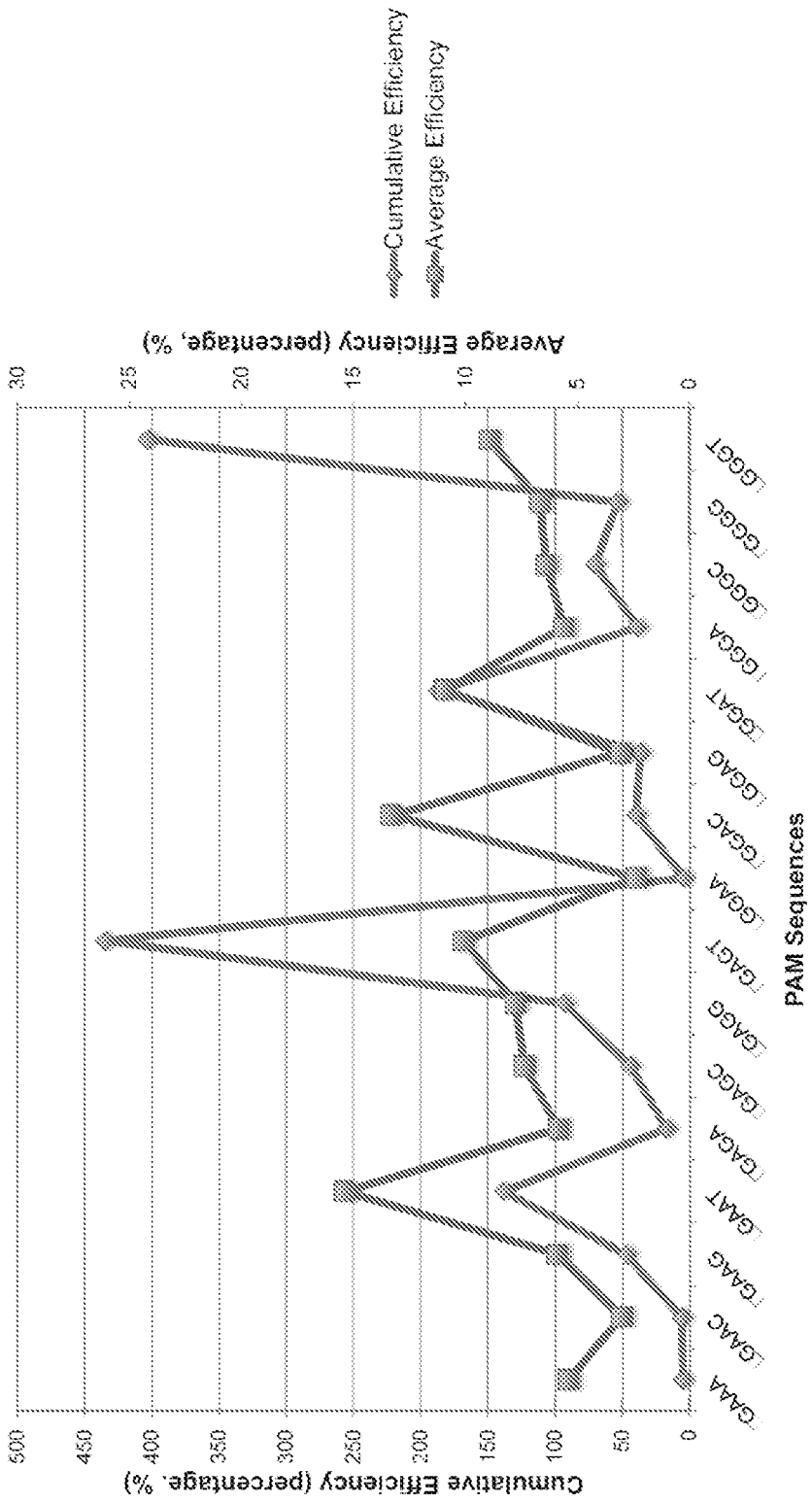

Cumulative Efficiency
Average Efficiency
Average Efficiency (percentage, %)
30
25
20
15
10
5
0
Cumulative Efficiency (percentage, %)
500
450
400
350
300
250
200
150
100
50
0
GAAA
GAAC
GAAG
GAAT
GACA
GACC
GACG
GACT
GAGA
GAGC
GAGG
GAGT
GATA
GATC
GATG
GATT
GCAA
GCAC
GCAG
GCAT
GCCA
GCCC
GCCG
GCCT
PAM Sequences

FIG. 57

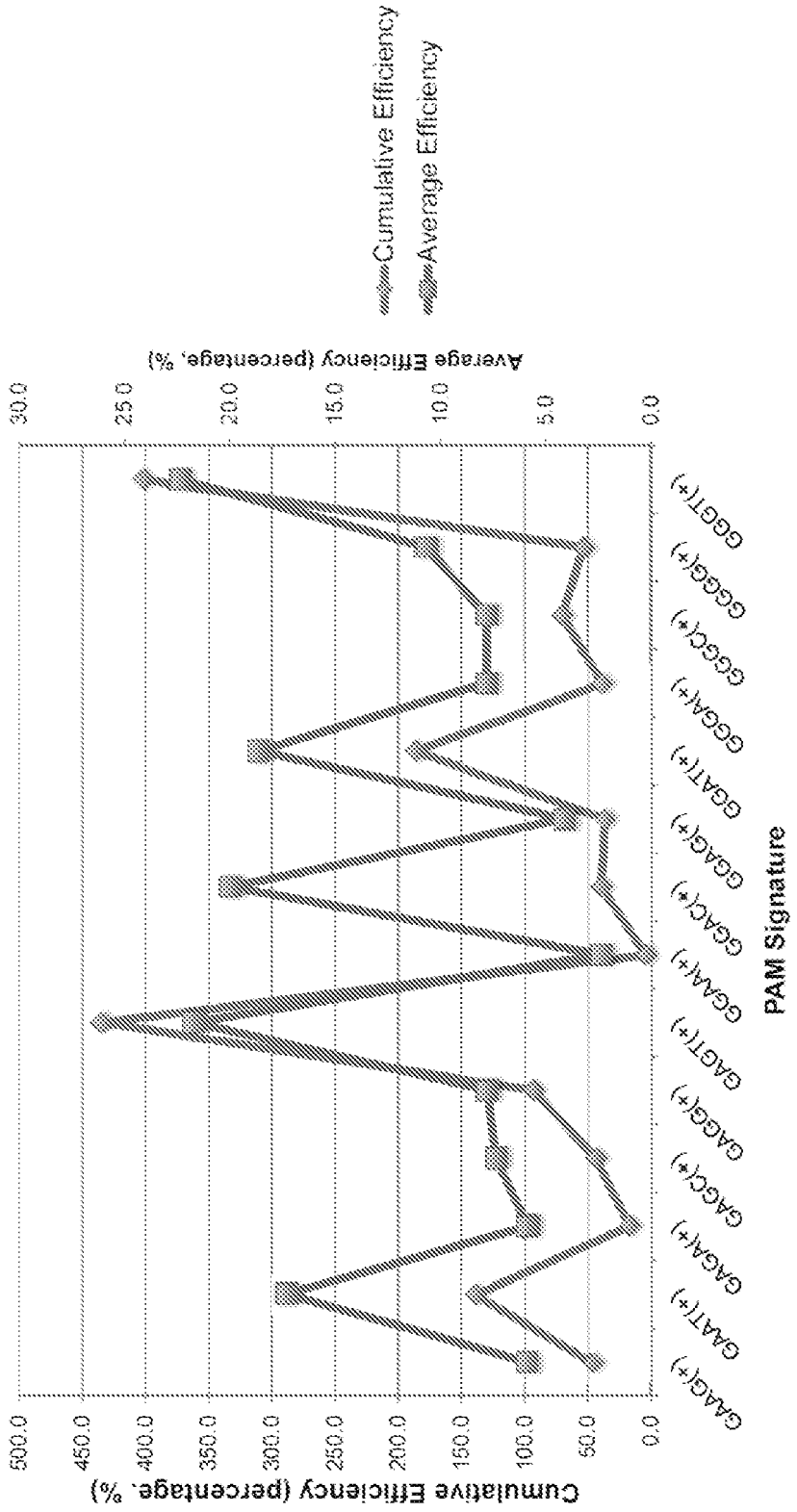

Cumulative Efficiency
Average Efficiency
Average Efficiency (percentage, %)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Cumulative Efficiency (percentage, %)
500.0
450.0
400.0
350.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0
GAAG(+)
GAAT(+)
GACA(+)
GACC(+)
GAGG(+)
GAGT(+)
GAAA(+)
GACA(+)
GAAG(+)
GAAT(+)
GGCA(+)
GGCC(+)
GGGA(+)
GGGT(+)
PAM Signature

FIG. 58

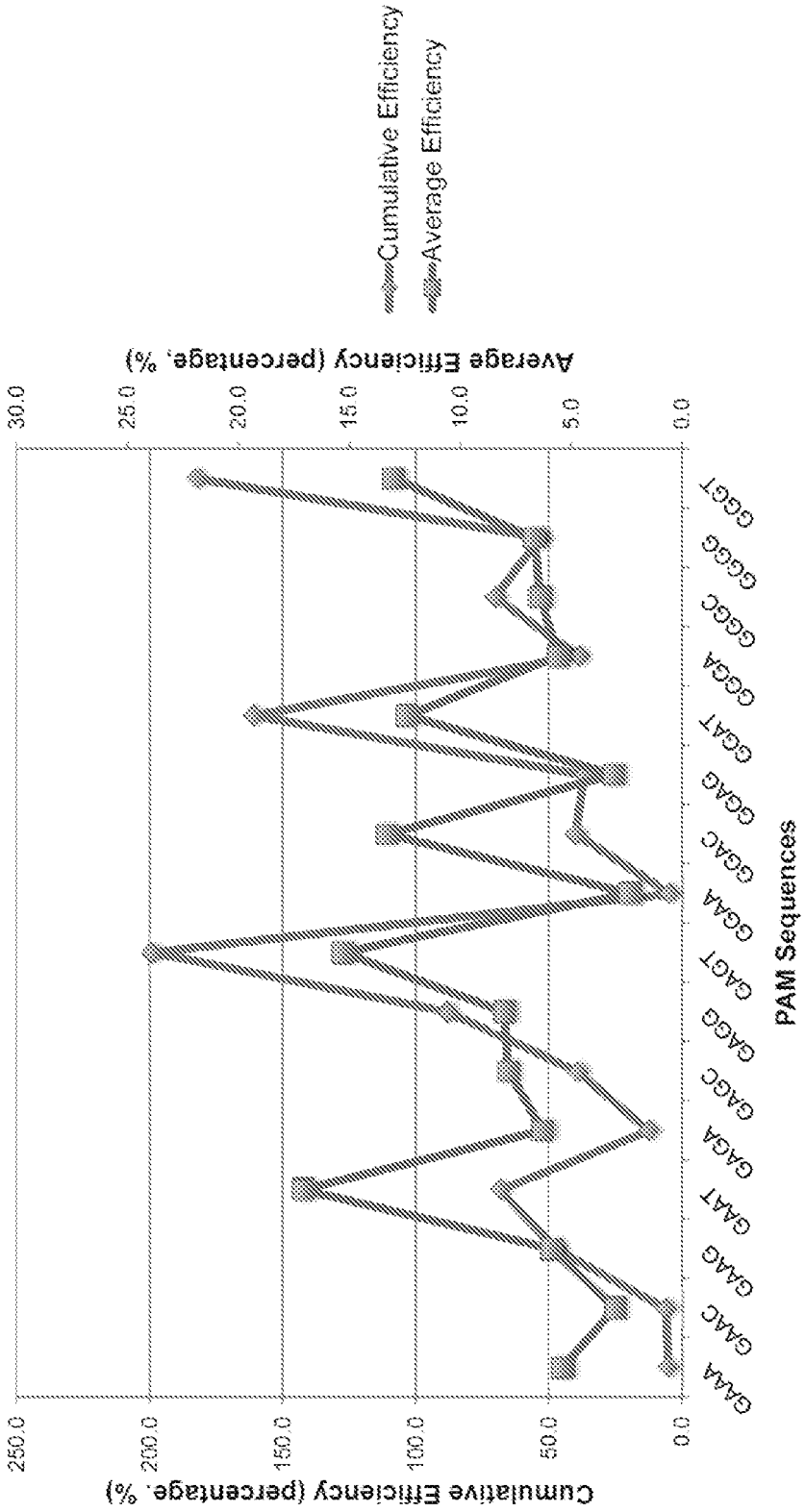

Cumulative Efficiency
Average Efficiency
Average Efficiency (percentage, %)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Cumulative Efficiency (percentage, %)
250.0
200.0
150.0
100.0
50.0
0.0
PAM Sequences
GAAA
GAAC
GAAG
GAAT
GACA
GACC
GACG
GACT
GAGA
GAGC
GAGG
GAGT
GCAA
GCAC
GCAG
GCAT
GCGA
GCGC
GCGG
GCGT

FIG. 59

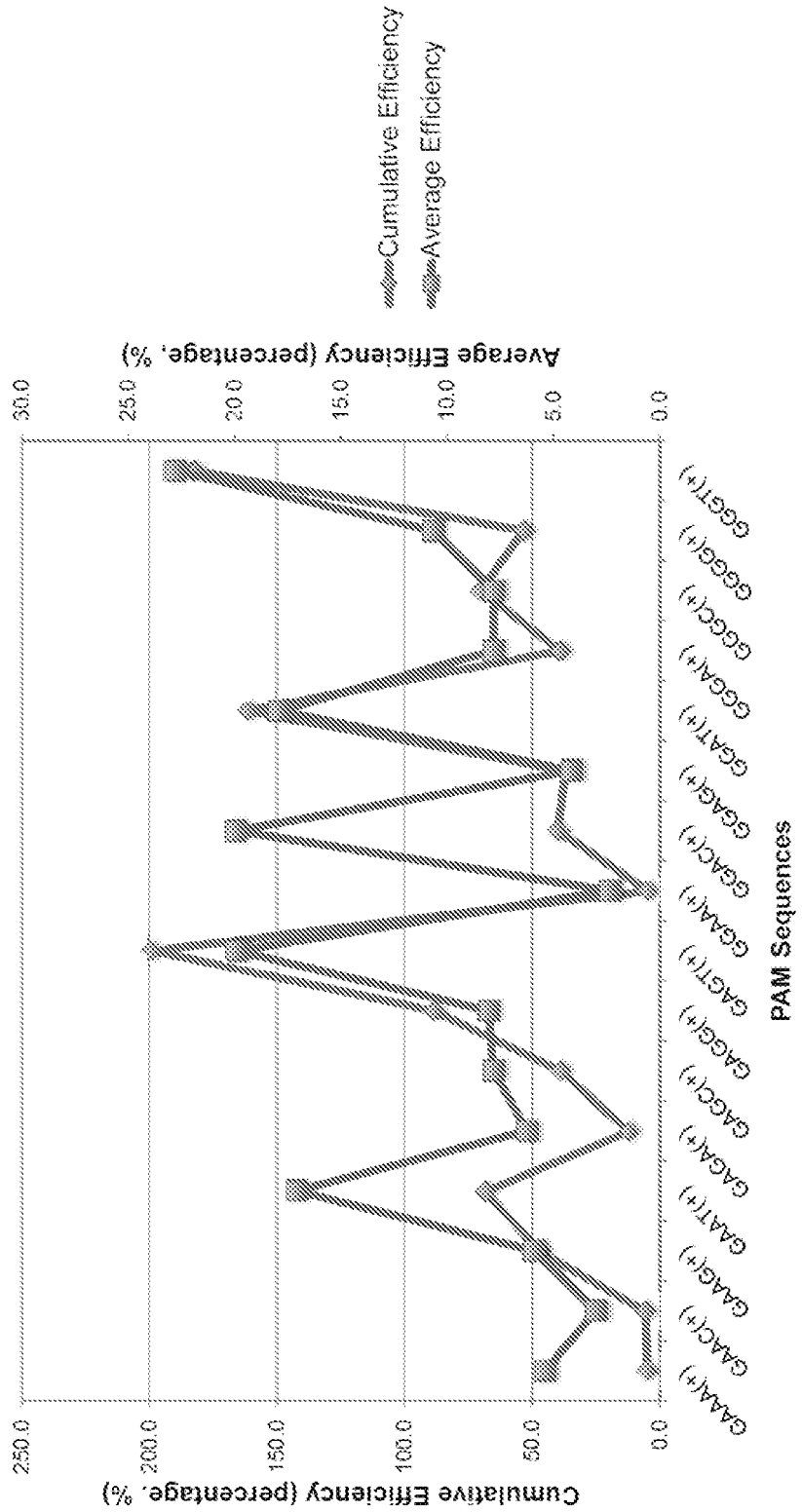

Cumulative Efficiency
Average Efficiency
Average Efficiency (percentage, %)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Cumulative Efficiency (percentage, %)
250.0
200.0
150.0
100.0
50.0
0.0
PAM Sequences
GAAA(+)
GAAC(+)
GAAG(+)
GAAT(+)
GACA(+)
GACC(+)
GACG(+)
GACT(+)
GAGA(+)
GAGC(+)
GAGG(+)
GAGT(+)
GATA(+)
GATC(+)
GATG(+)
GATT(+)
GCAA(+)
GCAC(+)
GCAG(+)
GCAT(+)
GCCA(+)
GCCC(+)
GCCG(+)
GCCT(+)
GGAA(+)
GGAC(+)
GGAG(+)
GGAT(+)
GGCA(+)
GGCC(+)
GGCG(+)
GGCT(+)

FIG. 60

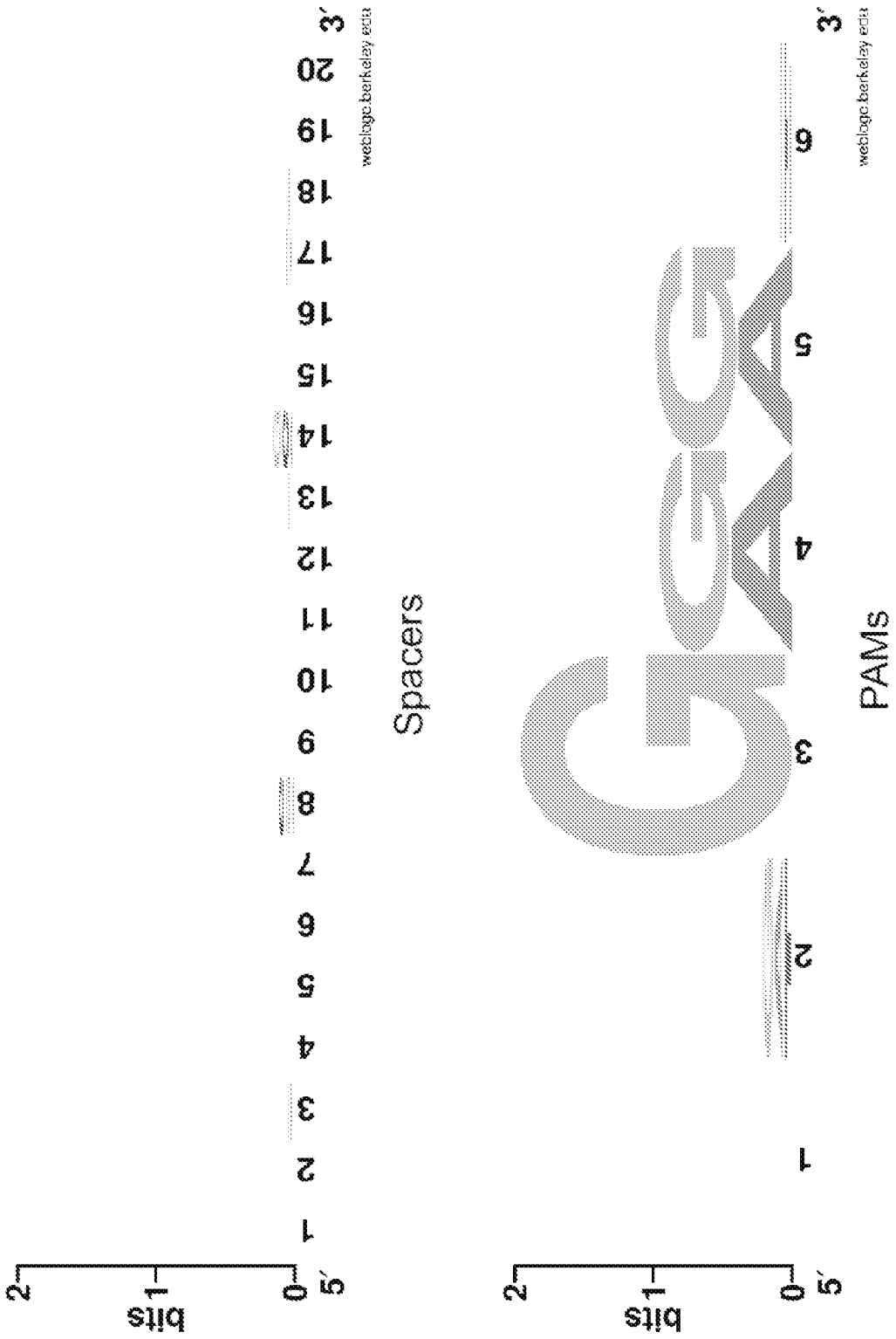

weblogo.berkeley.edu
Spacers
bits
5′
3′
PAMs
weblogo.berkeley.edu
bits
5′
3′

FIG. 61

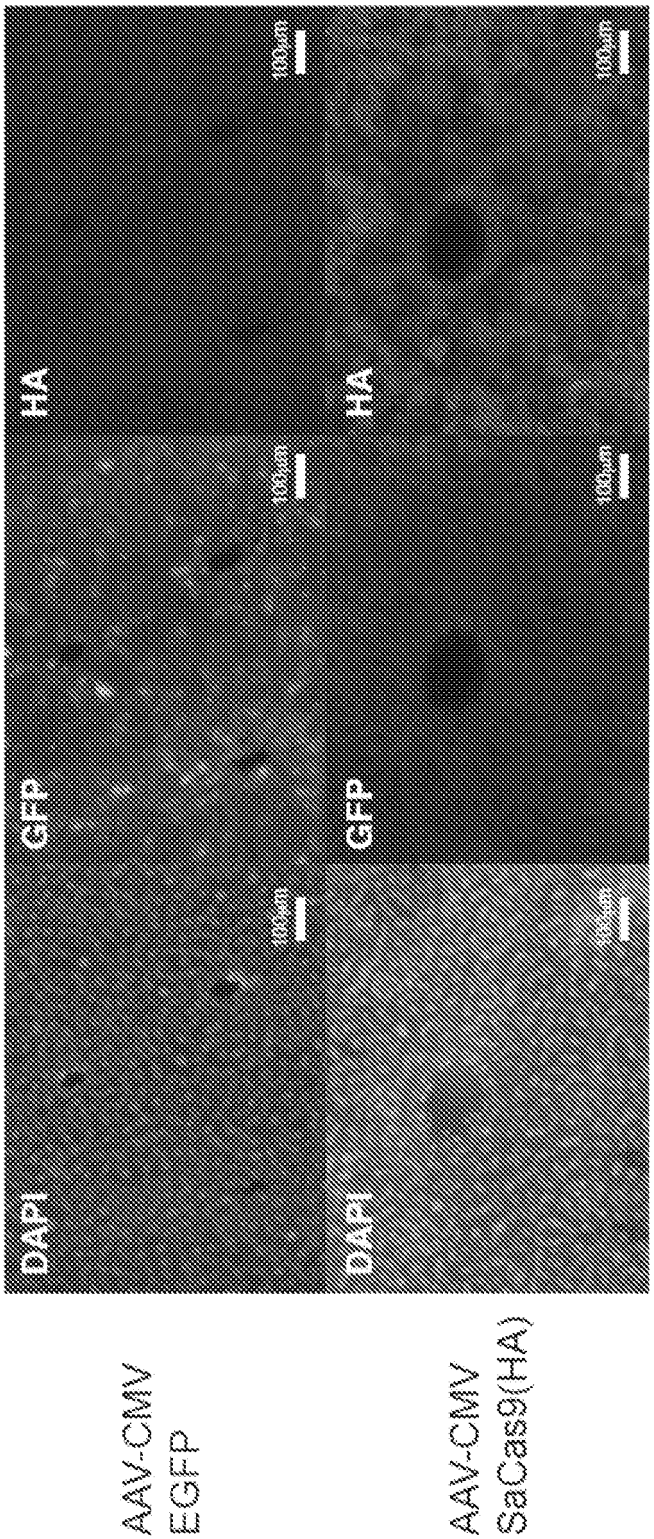



FIG. 62

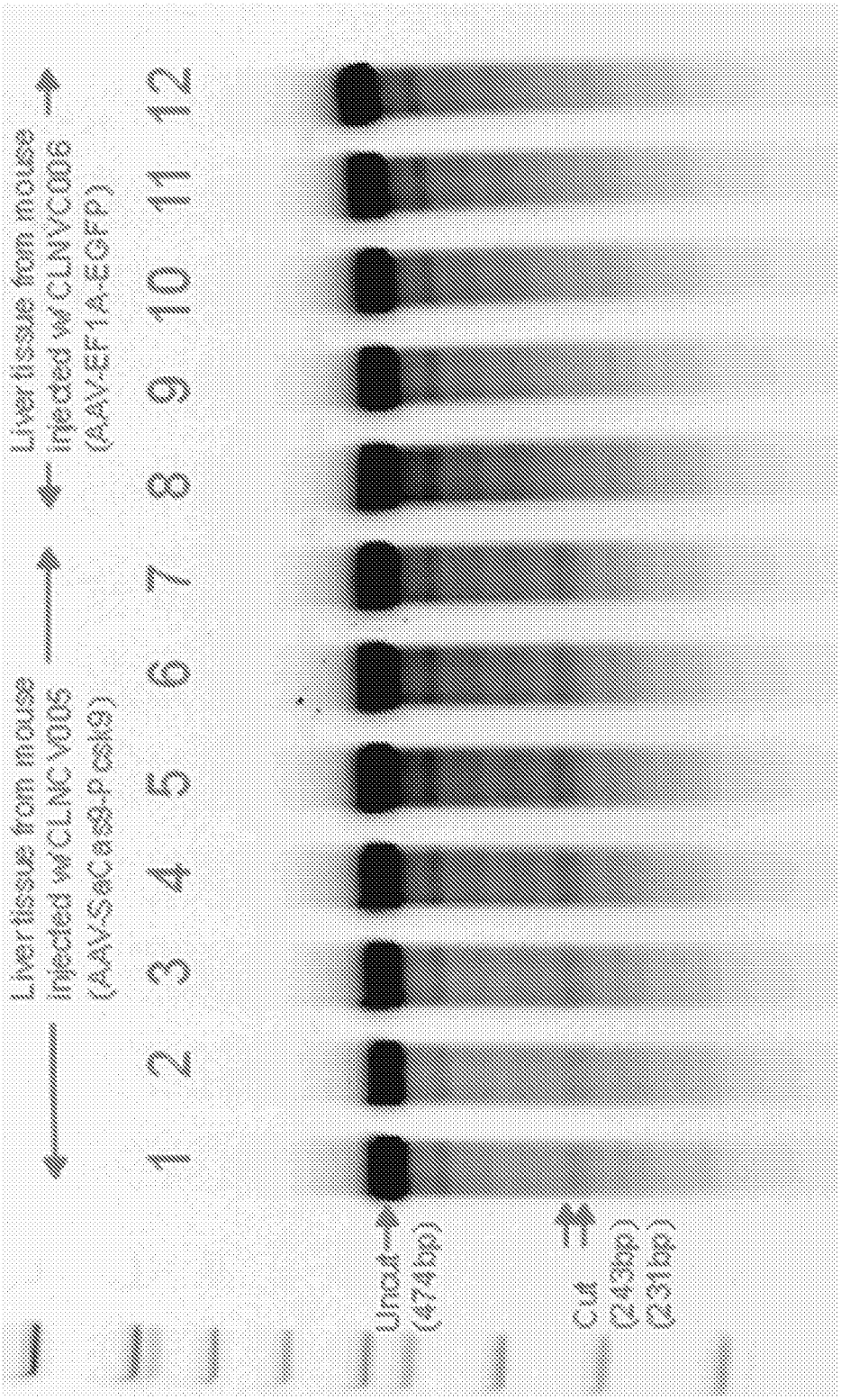

Liver tissue from mouse injected w/ CLNVC005 (AAV-SaCas9-P csl9)
Liver tissue from mouse injected w/ CLNVC006 (AAV-EF1A-EGFP)
1 2 3 4 5 6 7 8 9 10 11 12
Uncut (474bp)
Cut (243bp) (231bp)

FIG. 63

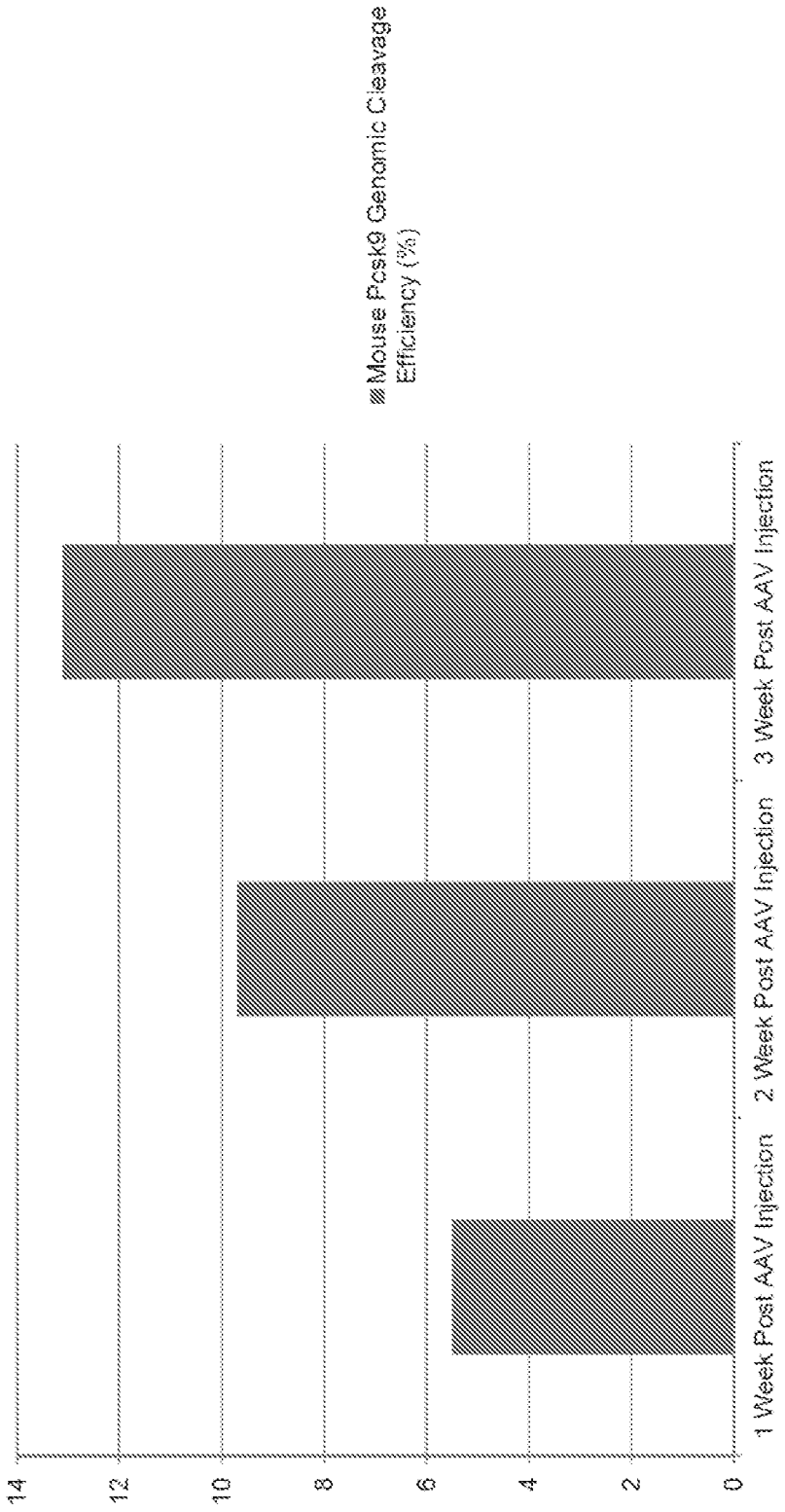

Mouse Pcsk9 Genomic Cleavage Efficiency (%)
14
12
10
8
6
4
2
0
1 Week Post AAV Injection
2 Week Post AAV Injection
3 Week Post AAV Injection

FIG. 64

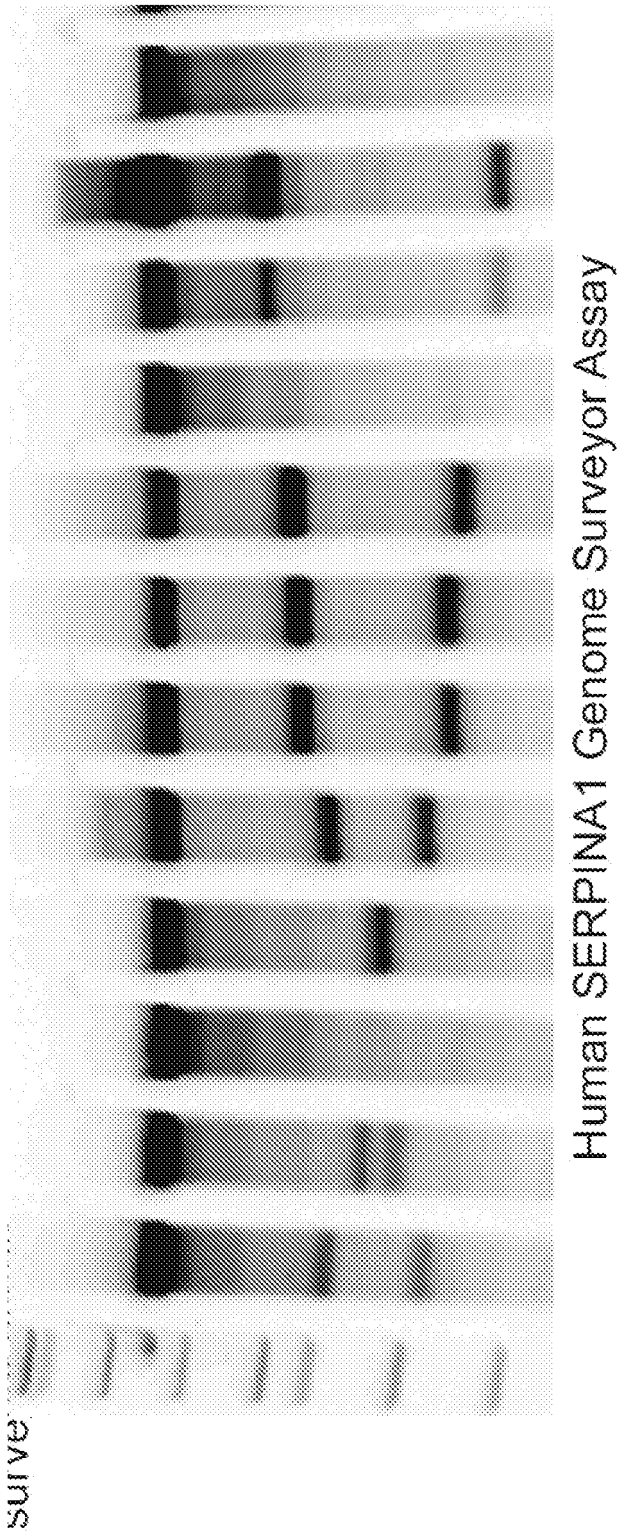



FIG. 65

| Genome Target Sapcers | Genome Cleavage Efficiency (%) |
|---|---|
| MmHmgcr-Spacer 1 | 15.4 |
| MmHmgcr-Spacer 2 | 18.3 |
| MmHmgcr-Spacer 3 | 6.1 |
| MmHmgcr-Spacer 4 | 6.5 |
| MmHmgcr-Spacer 5 | 11.8 |
| MmHmgcr-Spacer 6 | 5.8 |

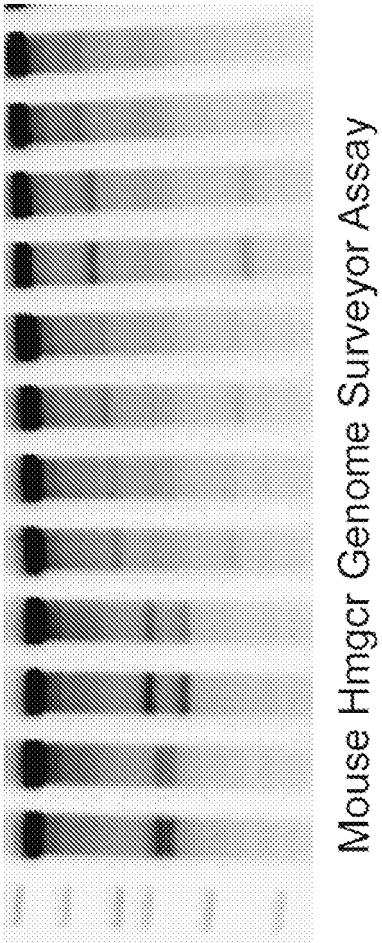



FIG. 66

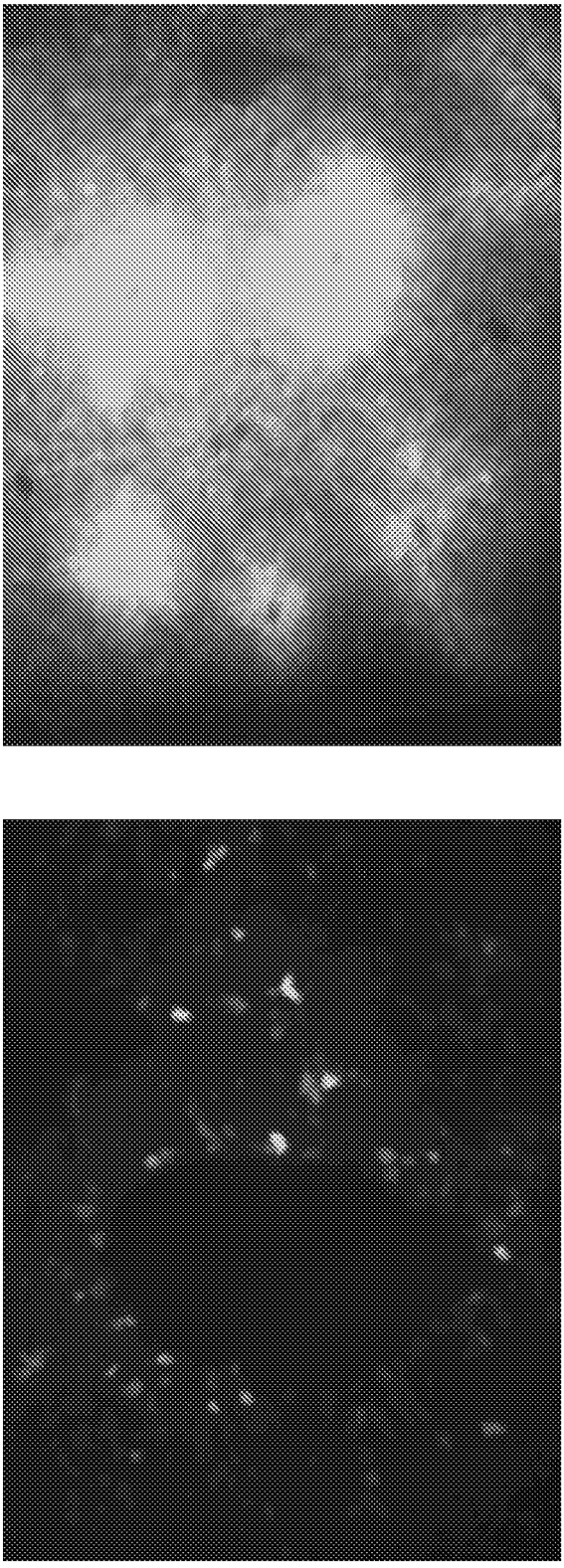
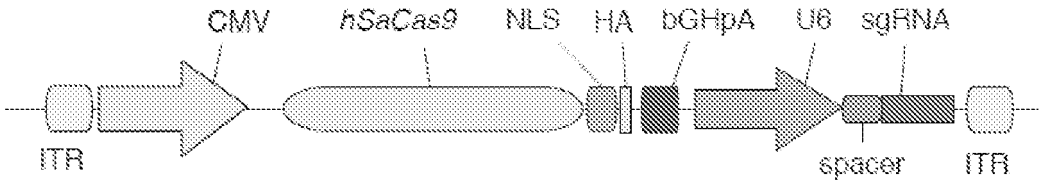



FIG. 67

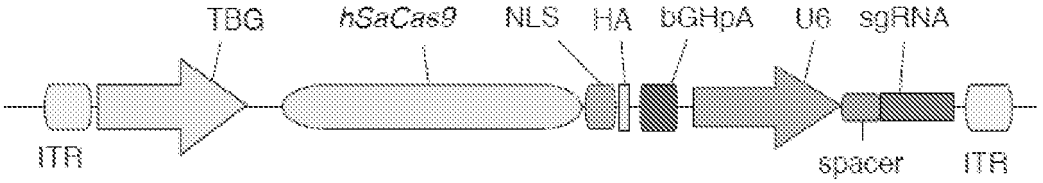
A




pAAV-CMV-SaCas9-U6-sgRNA architecture

(CMV: ubiquitous cytomegalovirus promoter)

B

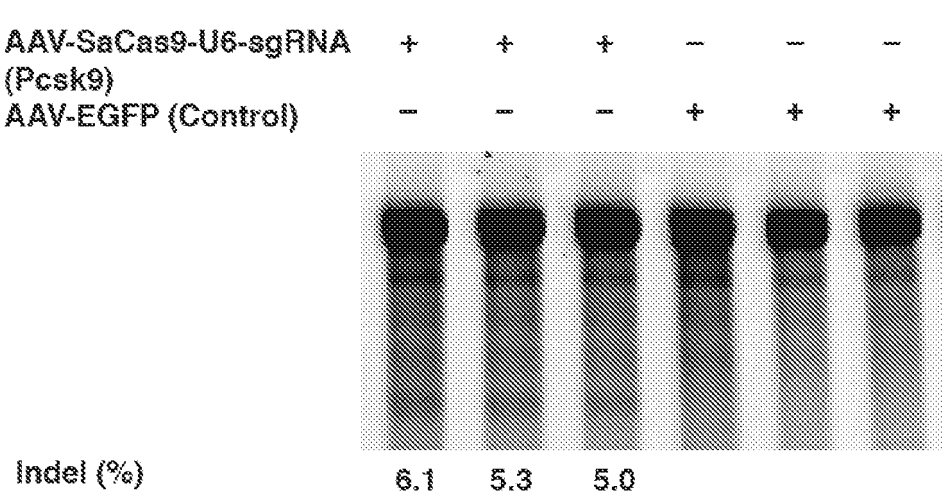



pAAV-TBG-SaCas9-U6-sgRNA architecture

(TBG: Thyroxine-binding globulin liver-specific promoter)

FIG. 68

A


AAV-SaCas9-U6-sgRNA (Pcsk9)
+ + + – – –
AAV-EGFP (Control)
– – – + + +
Indel (%)
6.1 5.3 5.0

B

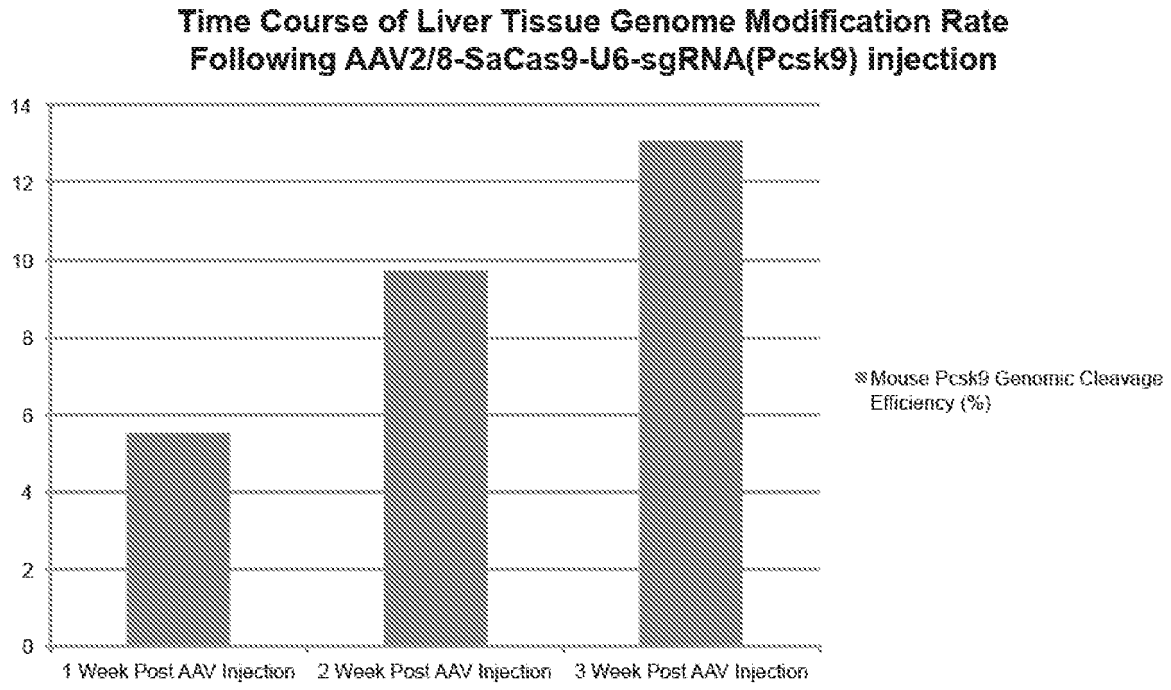

Time Course of Liver Tissue Genome Modification Rate
Following AAV2/8-SaCas9-U6-sgRNA(Pcsk9) injection
14
12
10
8
6
4
2
0
1 Week Post AAV Injection
2 Week Post AAV Injection
3 Week Post AAV Injection
※Mouse Pcsk9 Genomic Cleavage Efficiency (%)

FIG. 69

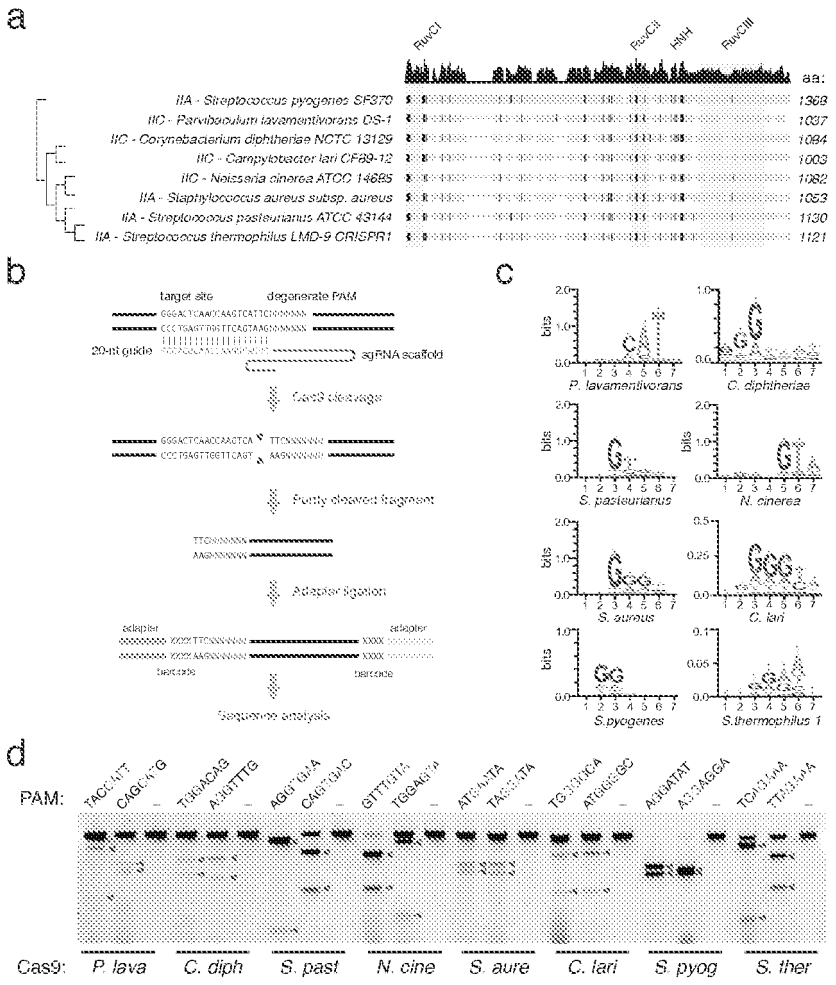

a
RuvCI
RuvCII
HNH
RuvCIII
aa:
IIA - Streptococcus pyogenes SF370 1368
IIC - Parvibaculum lavamentivorans DS-1 1037
IIC - Corynebacterium diphtheriae NCTC 13129 1094
IIC - Campylobacter lari CF89-12 1003
IIC - Neisseria cinerea ATCC 14685 1082
IIA - Staphylococcus aureus subsp. aureus 1053
IIA - Streptococcus pasteurianus ATCC 43144 1130
IIA - Streptococcus thermophilus LMD-9 CRISPR1 1121
b
target site
degenerate PAM
20-nt guide
sgRNA scaffold
Cas9 cleavage
Partly cleaved fragment
Adapter ligation
adapter
barcode
Sequence analysis
c
bits
P. lavamentivorans
C. diphtheriae
S. pasteurianus
N. cinerea
S. aureus
C. lari
S.pyogenes
S.thermophilus 1
d
PAM:
Cas9:
P. lava
C. diph
S. past
N. cine
S. aure
C. lari
S. pyog
S. ther

FIG. 70A-D

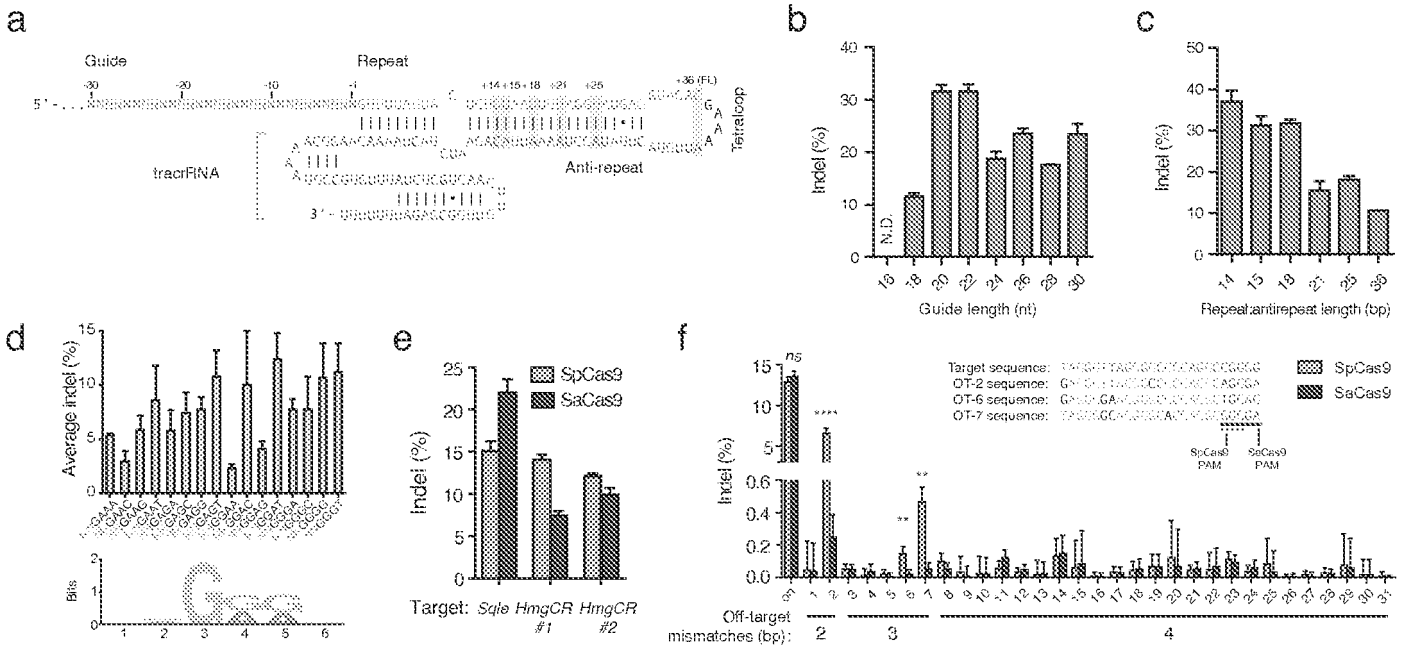

a
Guide
Repeat
tracrRNA
Anti-repeat
Tetraloop
b
Indel (%)
N.D.
Guide length (nt)
c
Indel (%)
Repeat:antirepeat length (bp)
d
Average indel (%)
Bits
e
Indel (%)
SpCas9
SaCas9
Target: Sqle HmgCR #1 HmgCR #2
f
Indel (%)
Target sequence:
OT-2 sequence:
OT-6 sequence:
OT-7 sequence:
SpCas9 PAM
SaCas9 PAM
SpCas9
SaCas9
Off-target mismatches (bp):

FIG. 71A-F

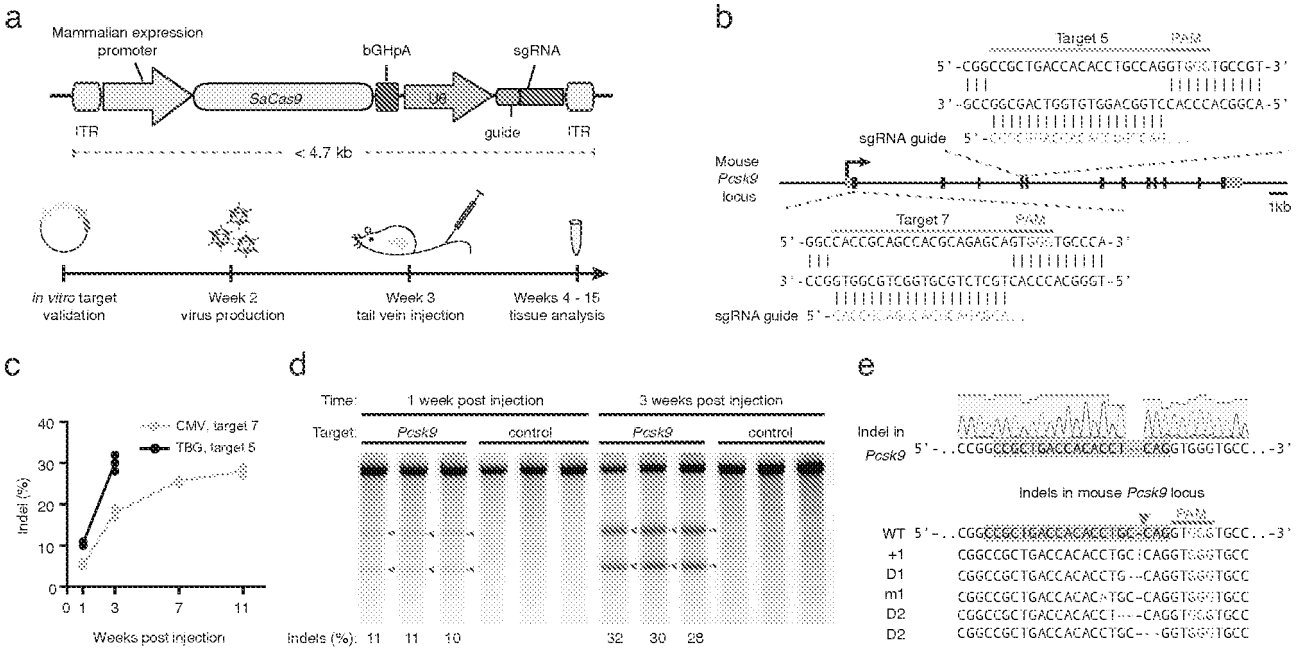

a
Mammalian expression promoter
bGHpA
sgRNA
SaCas9
ITR
guide
ITR
< 4.7 kb
in vitro target validation
Week 2 virus production
Week 3 tail vein injection
Weeks 4 - 15 tissue analysis
b
Target 5
PAM
5'-CGGCCGCTGACCACACCTGCCAGGTGGGTGCCGT-3'
3'-GCCGGCGACTGGTGTGGACGGTCCACCCACGGCA-5'
sgRNA guide
Mouse Pcsk9 locus
1kb
Target 7
PAM
5'-GGCCACCGCAGCCACGCAGAGCAGTGGGTGCCCA-3'
3'-CCGGTGGCGTCGGTGCGTCTCGTCACCCACGGGT-5'
sgRNA guide
c
CMV, target 7
TBG, target 5
Indel (%)
Weeks post injection
d
Time:
1 week post injection
3 weeks post injection
Target:
Pcsk9
control
Pcsk9
control
Indels (%): 11 11 10 32 30 28
e
Indel in Pcsk9
5'-...CCGGCCGCTGACCACACCT--CAGGTGGGTGCC...-3'
Indels in mouse Pcsk9 locus
PAM
WT 5'-...CGGCCGCTGACCACACCTGCCAGGTGGGTGCC...-3'
+1 CGGCCGCTGACCACACCTGCTCAGGTGGGTGCC
D1 CGGCCGCTGACCACACCTG--CAGGTGGGTGCC
m1 CGGCCGCTGACCACACATGC-CAGGTGGGTGCC
D2 CGGCCGCTGACCACACCT----CAGGTGGGTGCC
D2 CGGCCGCTGACCACACCTGC----GGTGGGTGCC

FIG. 72A-E

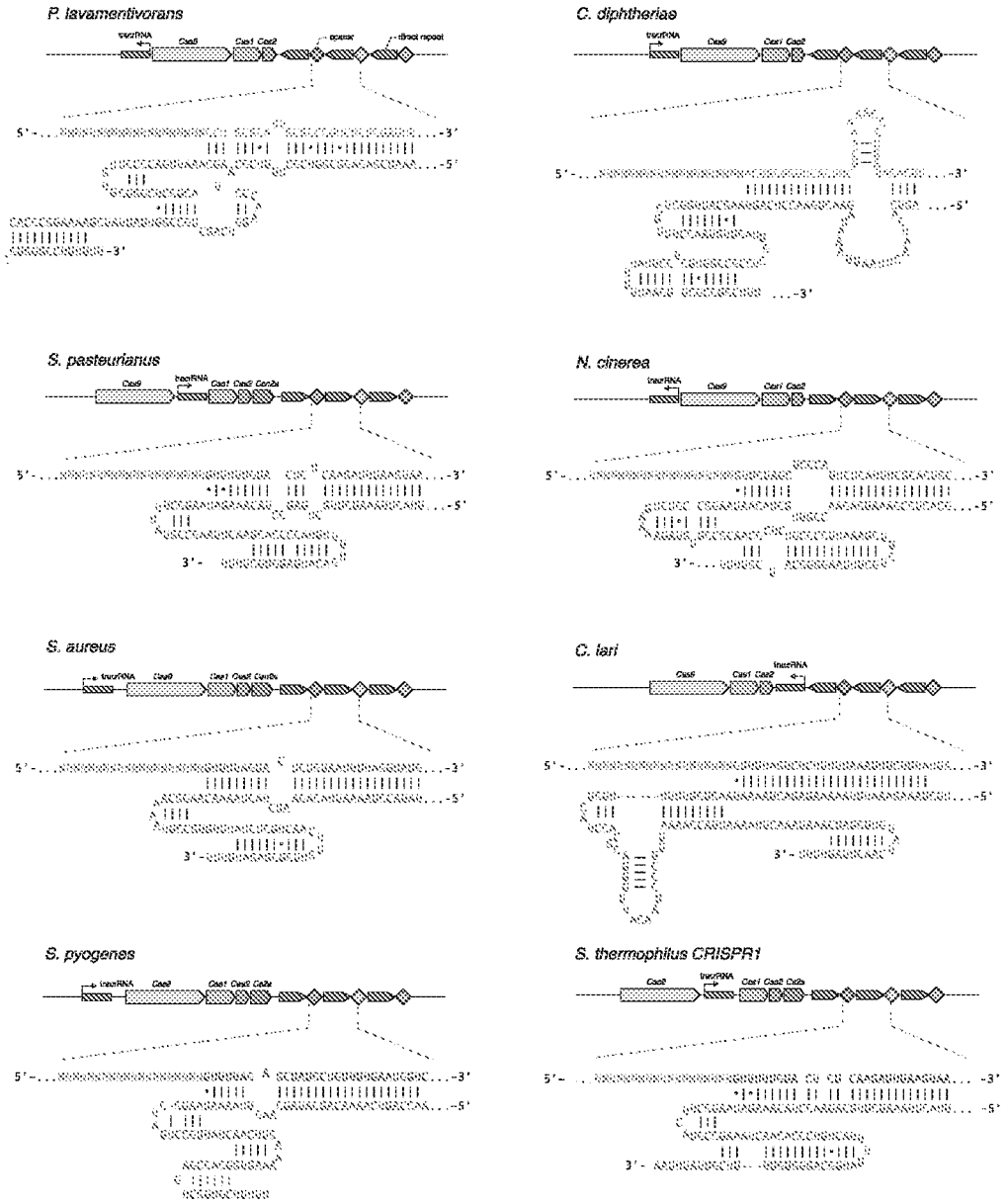

P. lavamentivorans
C. diphtheriae
S. pasteurianus
N. cinerea
S. aureus
C. lari
S. pyogenes
S. thermophilus CRISPR1

FIG. 73

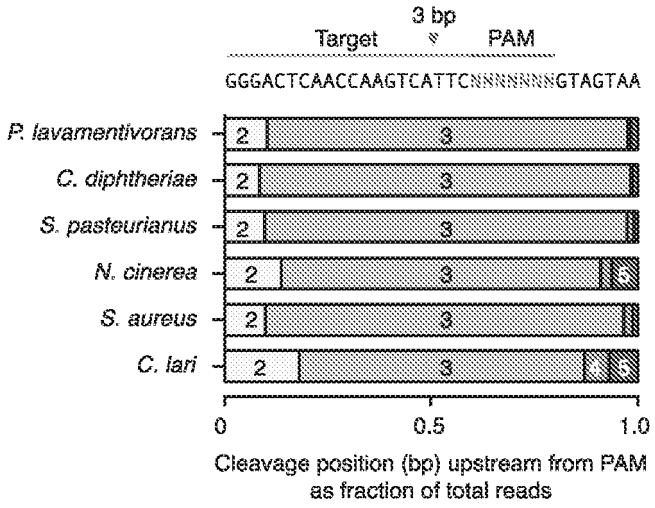

3 bp
Target
PAM
GGGACTCAACCAAGTCATTCNNNNNNNGTAGTAA
P. lavamentivorans
C. diphtheriae
S. pasteurianus
N. cinerea
S. aureus
C. lari
2
3
5
0
0.5
1.0
Cleavage position (bp) upstream from PAM
as fraction of total reads

FIG. 74

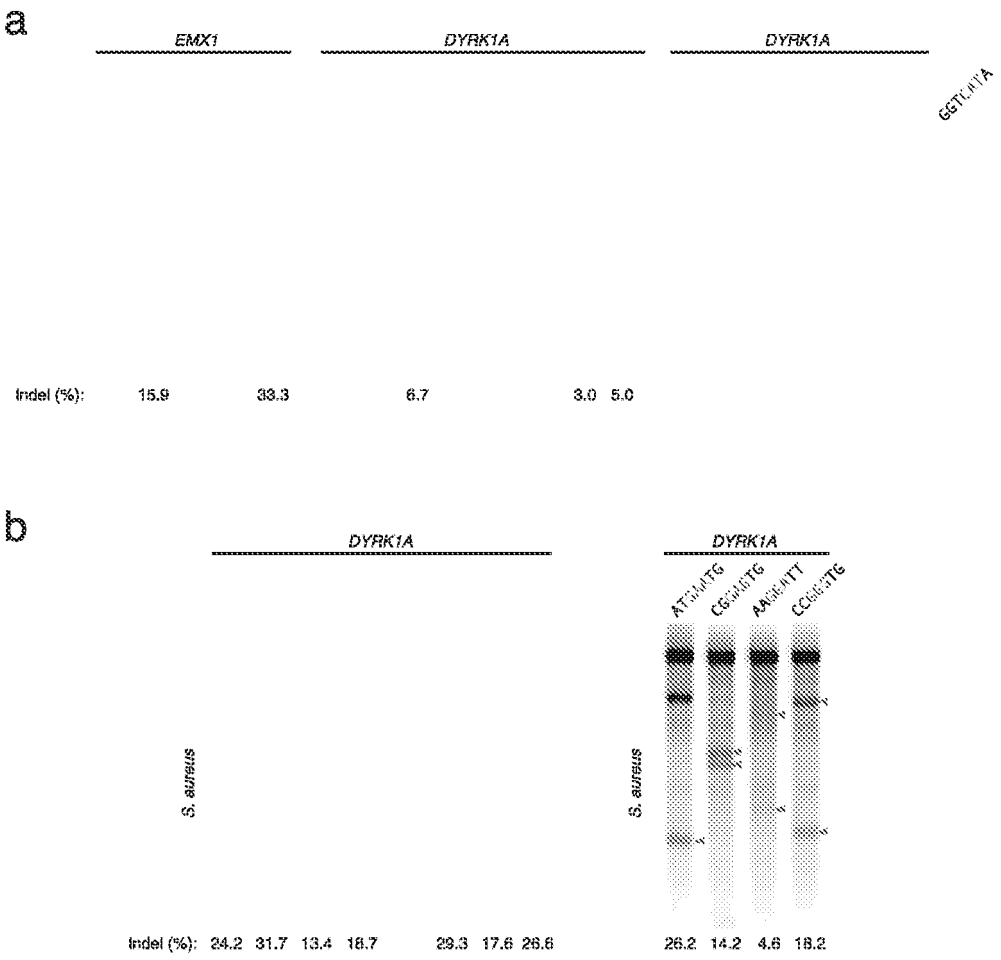
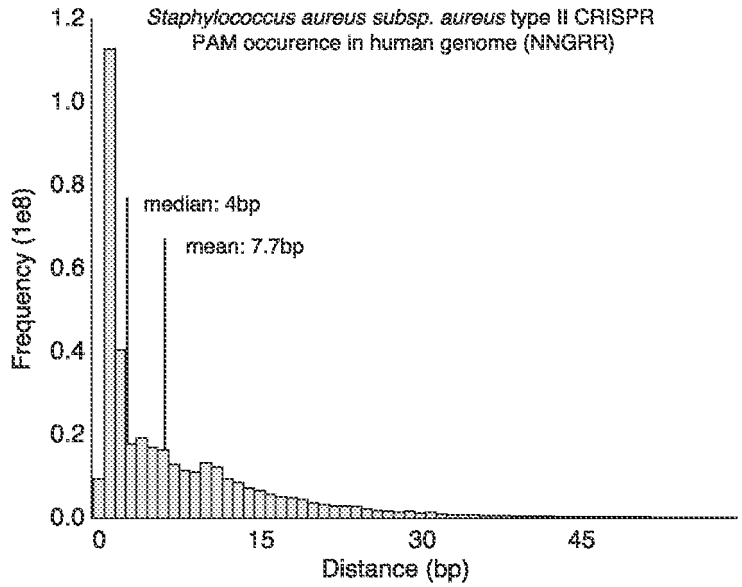

a
EMX1
DYRK1A
DYRK1A
Indel (%): 15.9 33.3 6.7 3.0 5.0
b
DYRK1A
DYRK1A
S. aureus
S. aureus
Indel (%): 24.2 31.7 13.4 18.7 29.3 17.6 26.6 26.2 14.2 4.6 18.2

FIG.75A-B

a

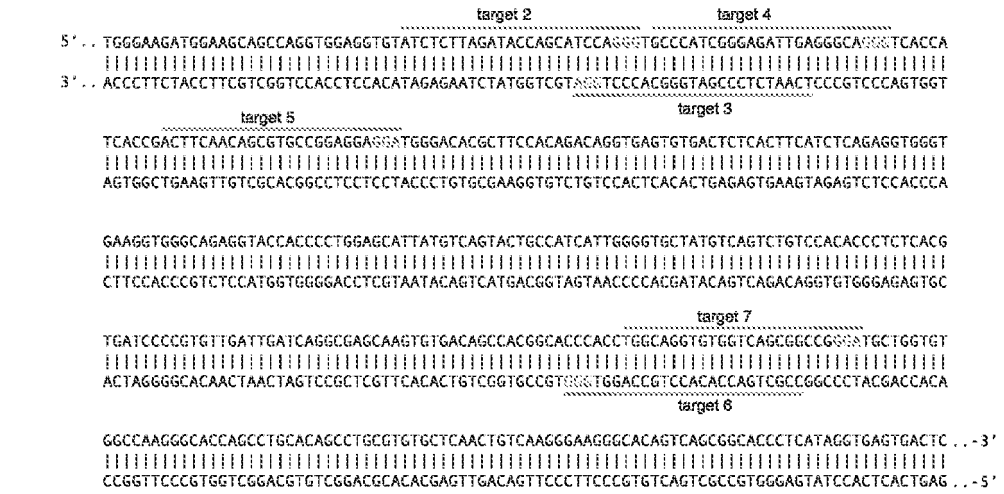



b

| | NNGRR | |
|---|---|---|
| Chr | median | mean |
| 1 | 4 | 7.5 |
| 2 | 4 | 7.9 |
| 3 | 4 | 7.9 |
| 4 | 4 | 8.3 |
| 5 | 4 | 8.0 |
| 6 | 4 | 7.9 |
| 7 | 4 | 7.7 |
| 8 | 4 | 7.8 |
| 9 | 4 | 7.7 |
| 10 | 4 | 7.5 |
| 11 | 4 | 7.5 |
| 12 | 4 | 7.7 |
| 13 | 4 | 8.2 |
| 14 | 4 | 7.7 |
| 15 | 4 | 7.5 |
| 16 | 4 | 7.0 |
| 17 | 4 | 6.8 |
| 18 | 4 | 7.8 |
| 19 | 3 | 6.5 |
| 20 | 4 | 7.1 |
| 21 | 4 | 8.0 |
| 22 | 3 | 6.7 |
| X | 4 | 8.0 |
| Y | 4 | 8.1 |

FIG. 76A-B

a


target 2
target 4
target 3
target 5
target 7
target 6

b

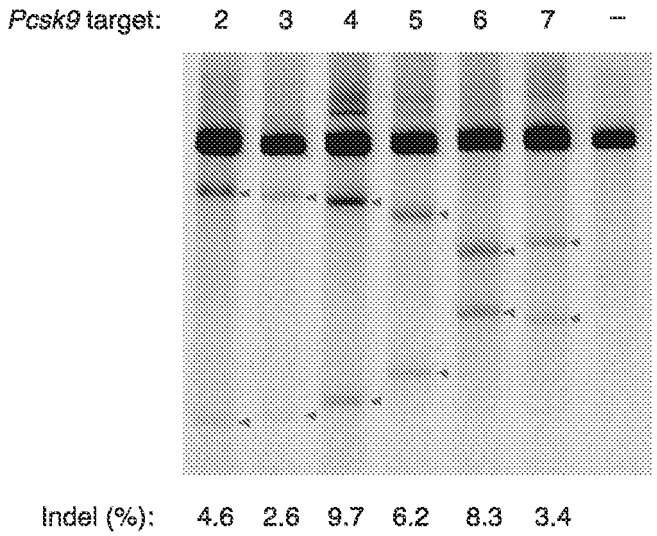
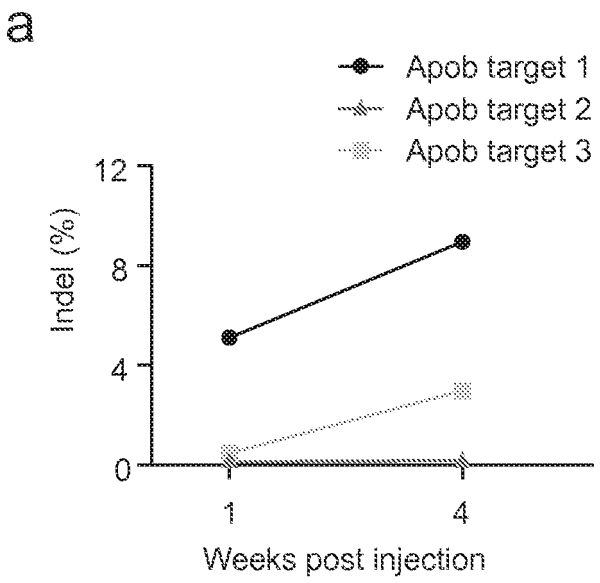
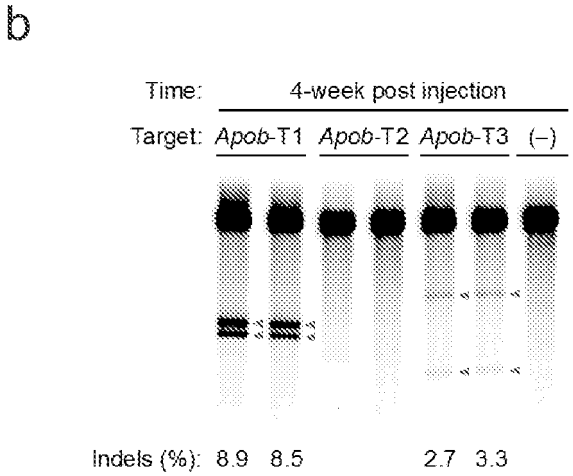

Pcsk9 target: 2 3 4 5 6 7 –
Indel (%): 4.6 2.6 9.7 6.2 8.3 3.4

FIG. 77A-B




ApoB-T1 = Target 1; ApoB-T2 = Target 2, etc.

FIG. 78

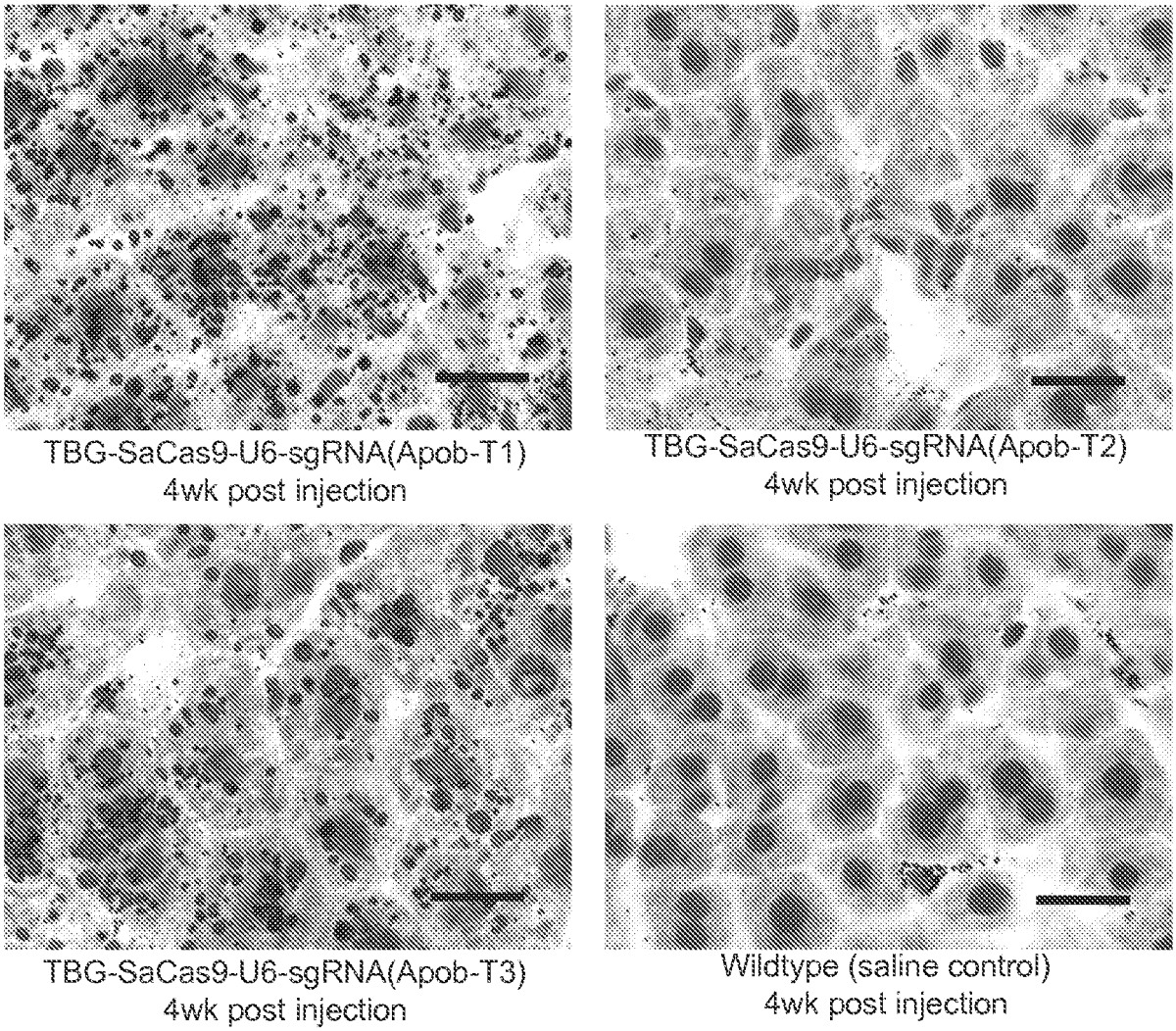

TBG-SaCas9-U6-sgRNA(Apob-T1)
4wk post injection
TBG-SaCas9-U6-sgRNA(Apob-T2)
4wk post injection
TBG-SaCas9-U6-sgRNA(Apob-T3)
4wk post injection
Wildtype (saline control)
4wk post injection

FIG. 79

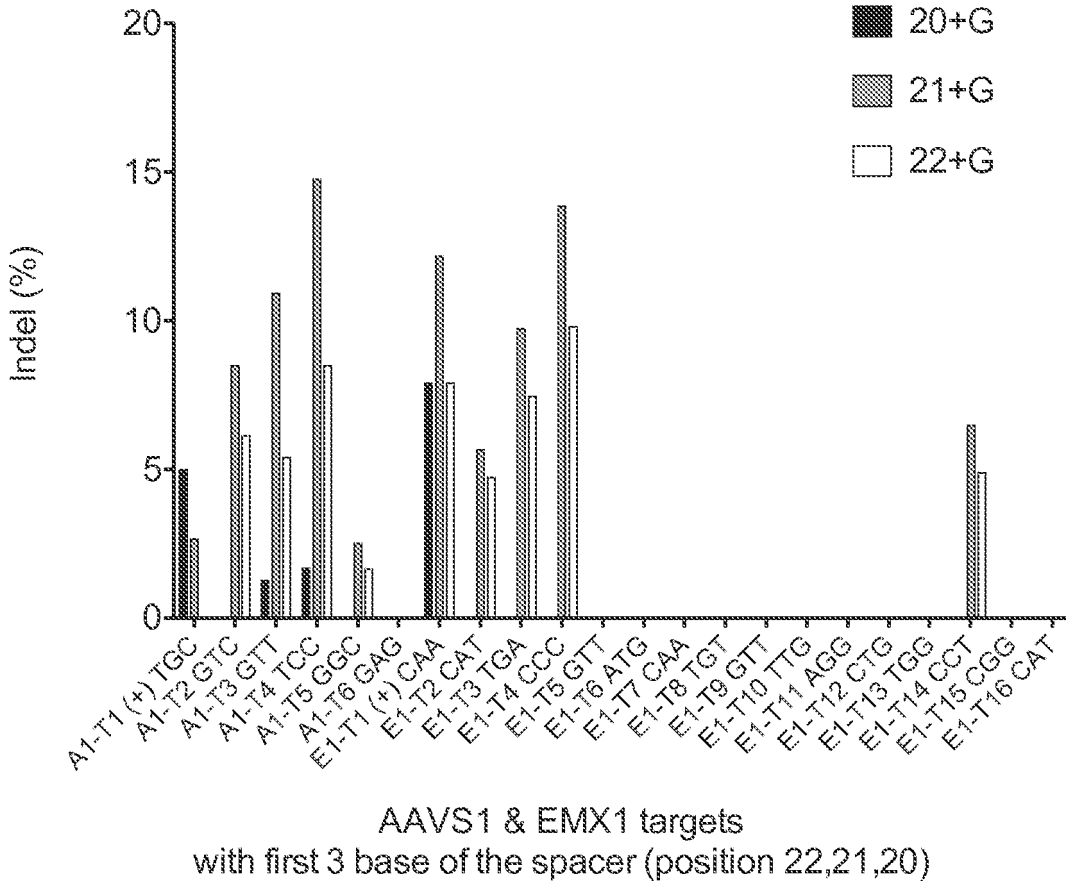

20+G
21+G
22+G
Indel (%)
20
15
10
5
0
A1-T1 (+) TGC
A1-T2 GTC
A1-T3 GTT
A1-T4 TCC
A1-T5 GGC
A1-T6 GAG
E1-T1 (+) CAA
E1-T2 CAT
E1-T3 TGA
E1-T4 CCC
E1-T5 GTT
E1-T6 ATG
E1-T7 CAA
E1-T8 TGT
E1-T9 GTT
E1-T10 TTG
E1-T11 AGG
E1-T12 CTG
E1-T13 TGG
E1-T14 CCT
E1-T15 CGG
E1-T16 CAT
AAVS1 & EMX1 targets
with first 3 base of the spacer (position 22,21,20)

FIG. 80

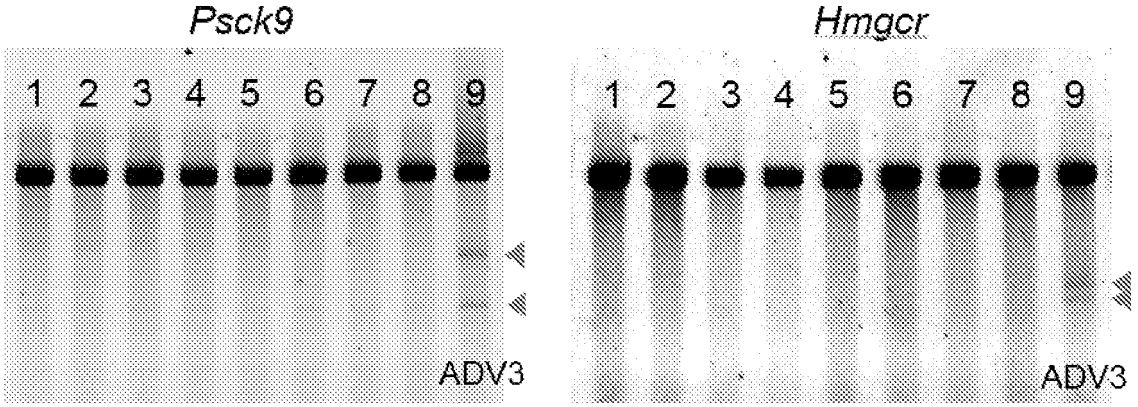



FIG. 81

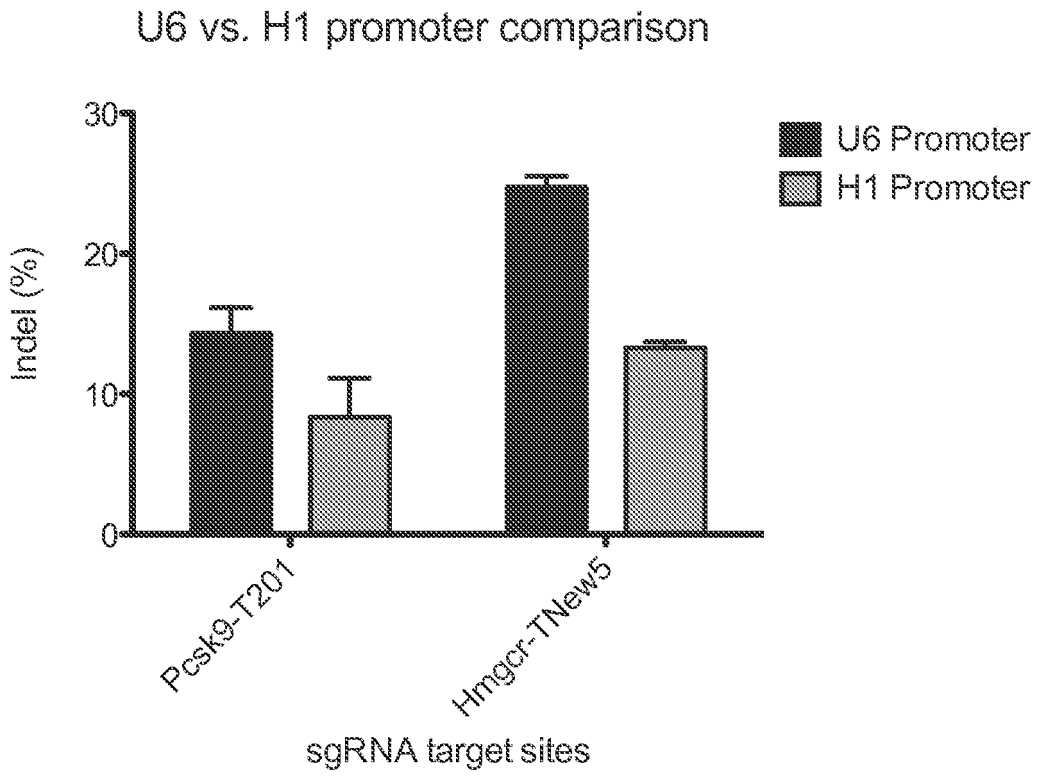

U6 vs. H1 promoter comparison
U6 Promoter
H1 Promoter
Indel (%)
30
20
10
0
Pcsk9-T201
Hmgcr-TNew5
sgRNA target sites

FIG. 82

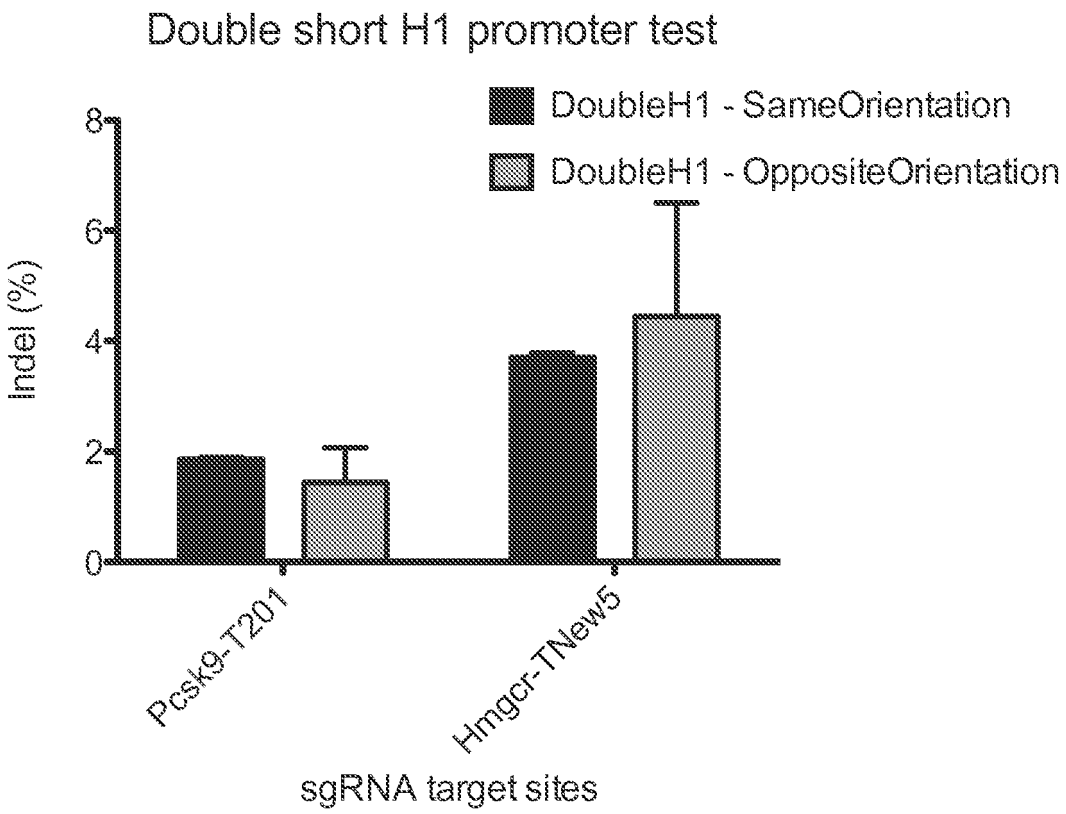

Double short H1 promoter test
DoubleH1 - SameOrientation
DoubleH1 - OppositeOrientation
Indel (%)
0
2
4
6
8
Pcsk9-T201
Hmgcr-TNew5
sgRNA target sites

FIG. 83

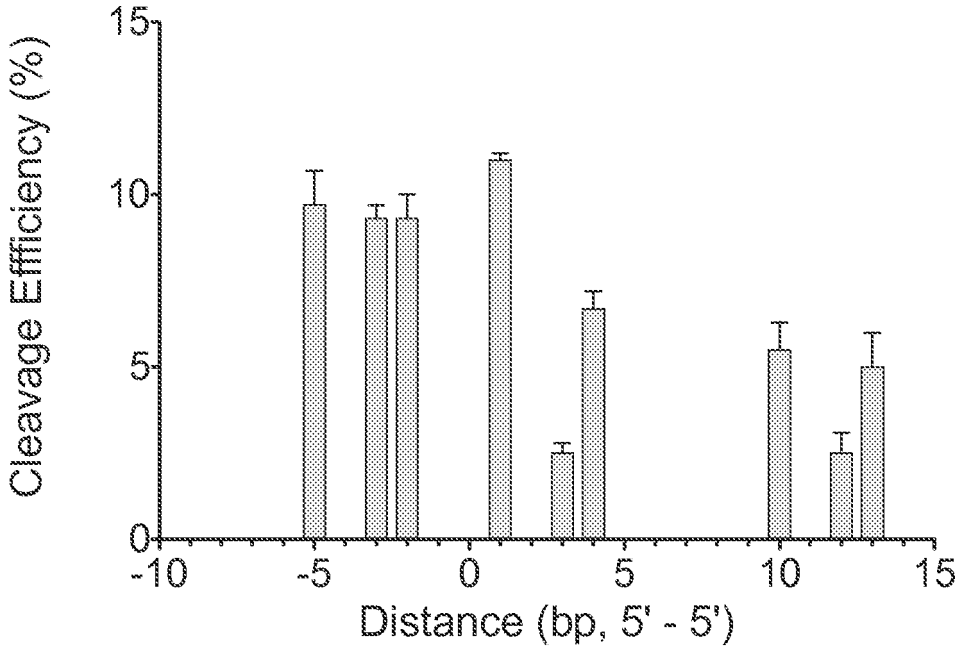



FIG. 84

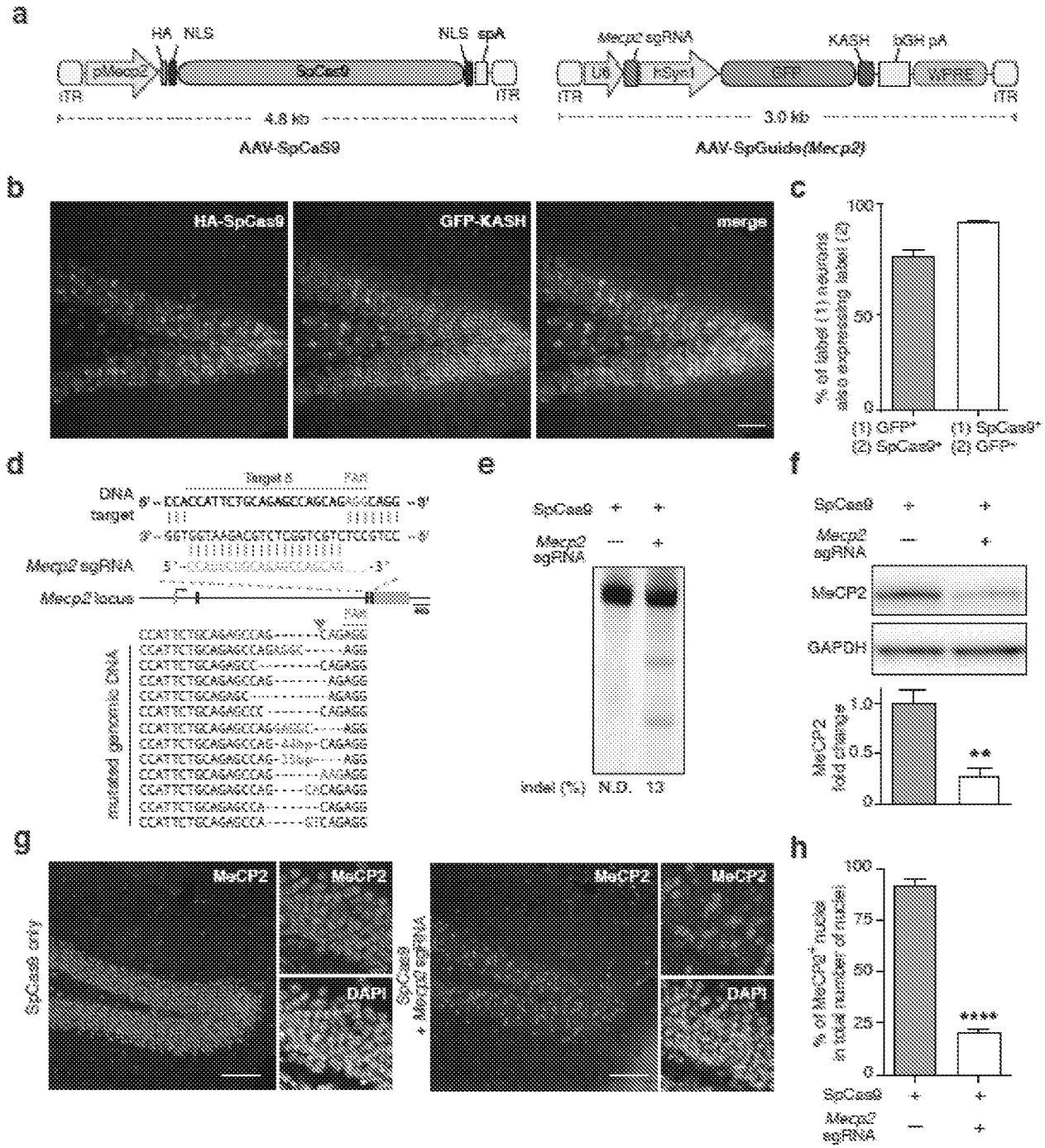

a
HA NLS
NLS spA
pMecp2
SpCas9
ITR
ITR
4.8 kb
AAV-SpCas9
Mecp2 sgRNA
KASH
bGH pA
U6
hSyn1
GFP
WPRE
ITR
ITR
3.0 kb
AAV-SpGuide(Mecp2)
b
HA-SpCas9
GFP-KASH
merge
c
% of labeled (1) neurons also expressing label (2)
100
50
0
(1) GFP+
(2) SpCas9+
(1) SpCas9+
(2) GFP+
d
Target 6
PAM
DNA target
Mecp2 sgRNA
Mecp2 locus
mutated genomic DNA
e
SpCas9 + +
Mecp2 sgRNA — +
indel (%) N.D. 13
f
SpCas9 + +
Mecp2 sgRNA — +
MeCP2
GAPDH
MeCP2 fold change
1.0
0.5
0
**
g
SpCas9 only
SpCas9 + Mecp2 sgRNA
MeCP2
MeCP2
DAPI
MeCP2
MeCP2
DAPI
h
% of MeCP2+ nuclei in total number of nuclei
100
75
50
25
0
****
SpCas9 + +
Mecp2 sgRNA — +

FIG. 85

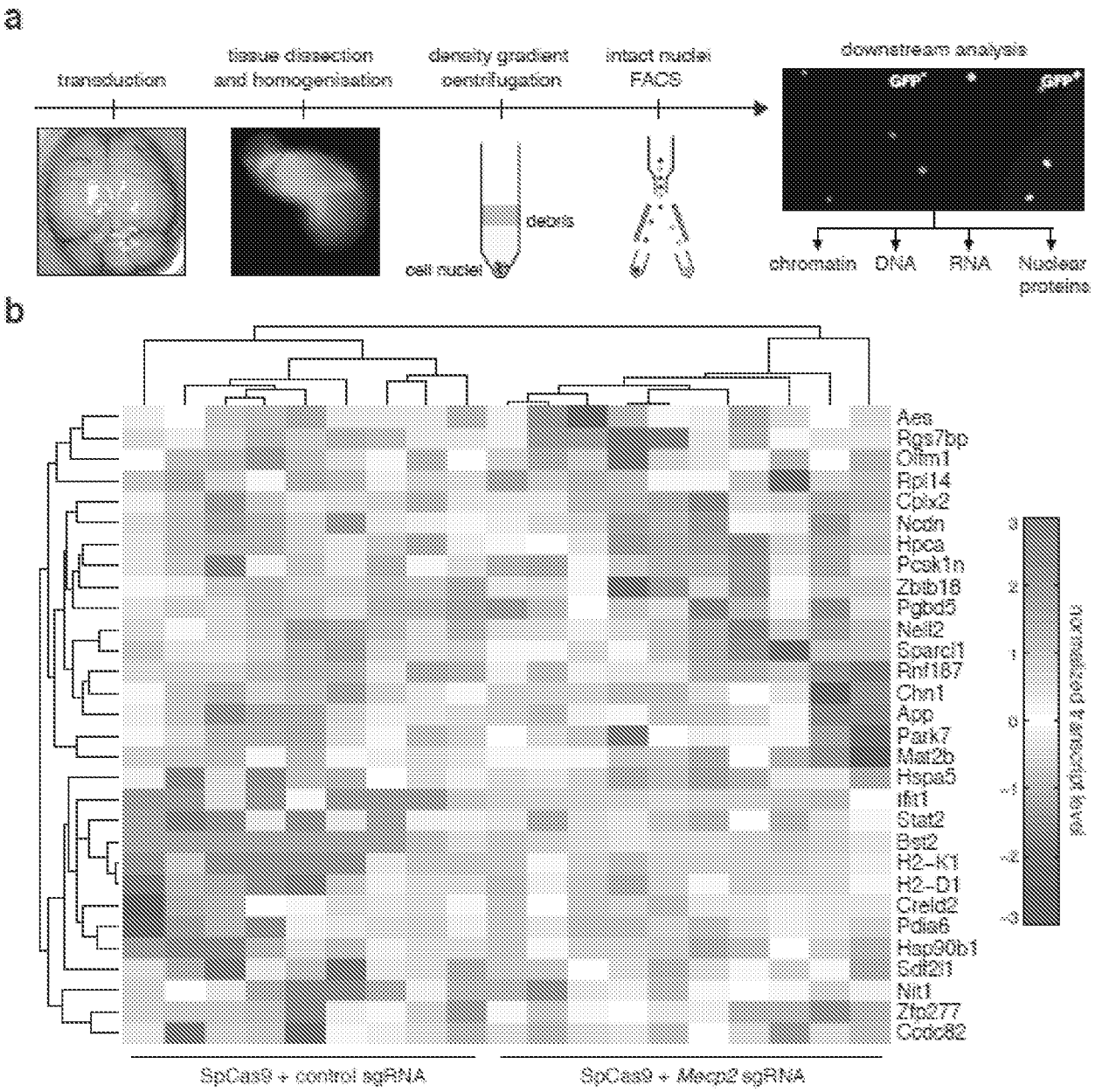

a
transduction
tissue dissection and homogenisation
density gradient centrifugation
intact nuclei FACS
downstream analysis
GFP
debris
cell nuclei
chromatin
DNA
RNA
Nuclear proteins
b
Aes
Rgs7bp
Cfim1
Rbl14
Cdx2
Ncdn
Hpca
Pcsk1n
Zbtb18
Pgbd5
Neil2
Sparcl1
Rnf187
Chn1
Ago
Park7
Mat2b
Hspa5
ifit1
Stat2
Bst2
H2-K1
H2-D1
Creld2
Pdia6
Hsp90b1
Sdf2l1
Nit1
Zfp277
Cosc82
SpCas9 + control sgRNA
SpCas9 + Mecp2 sgRNA
gene expression level

FIG. 86

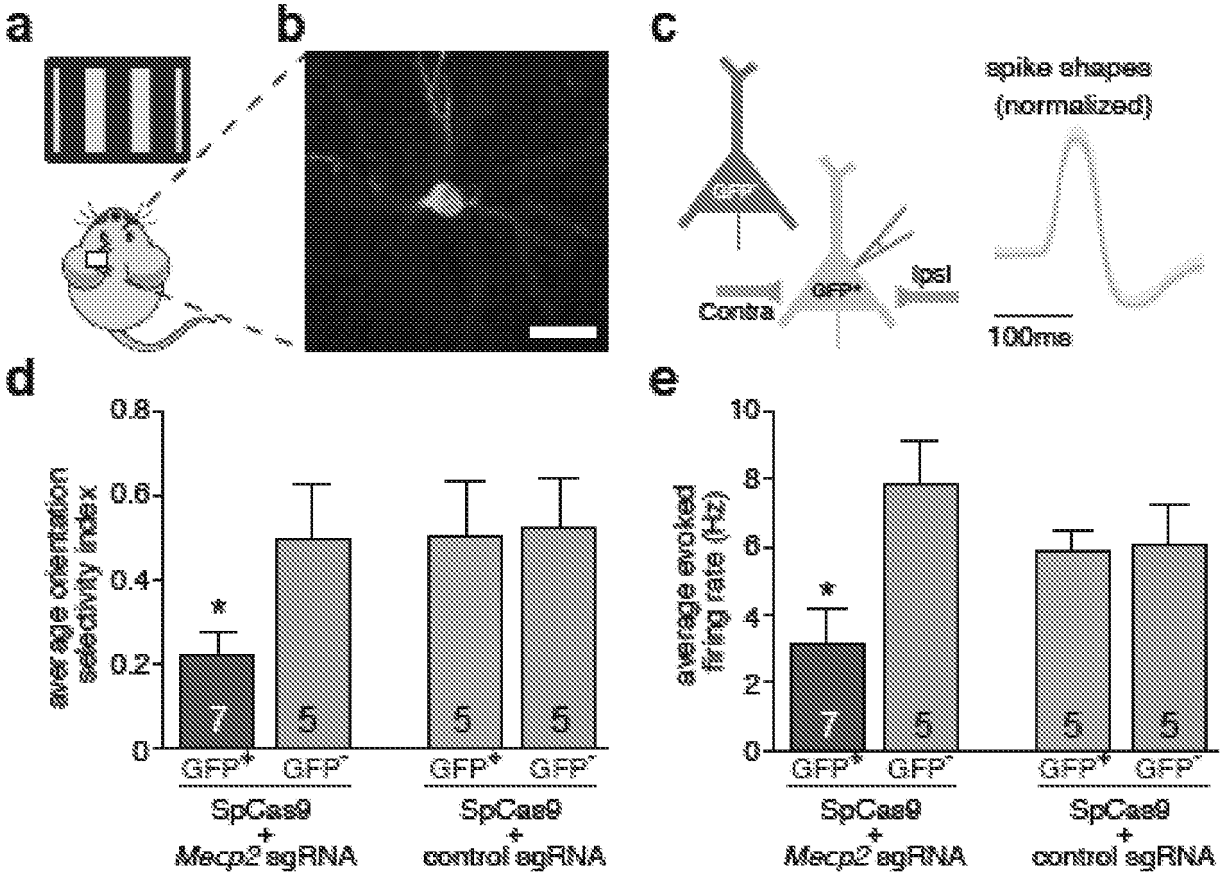
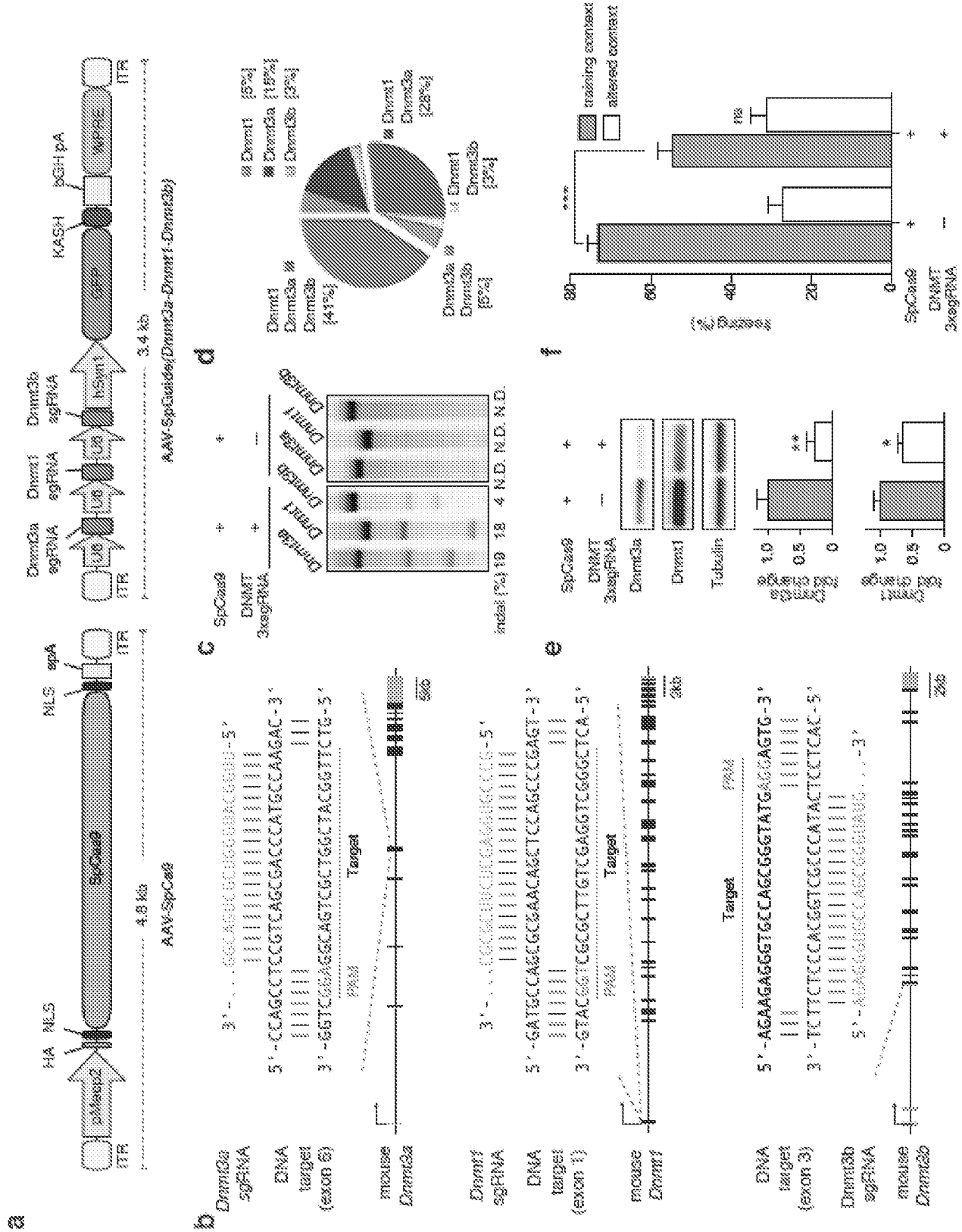

a
b
c
spike shapes
(normalized)
GFP+
GFP-
Contra
Ipsi
100ms
d
e
average orientation selectivity index
average evoked firing rate (Hz)
0.8
0.6
0.4
0.2
0
10
8
6
4
2
0
*
7
5
5
5
GFP+
GFP-
GFP+
GFP-
SpCas9 + Mecp2 sgRNA
SpCas9 + control sgRNA

FIG. 87


a
ITR
pAAV-ssp2
HA
NLS
SpCas9
NLS
spA
ITR
4.8 kb
AAV-SpCas9
Dnmt3a sgRNA
U6
Dnmt1 sgRNA
U6
Dnmt3b sgRNA
hSyn1
KASH
bGH pA
WPRE
ITR
3.4 kb
AAV-SpCas9-Dnmt3a-Dnmt1-Dnmt3b
b
Dnmt3a sgRNA
DNA target (exon 6)
PAM
Target
mouse Dnmt3a
5kb
Dnmt1 sgRNA
DNA target (exon 1)
mouse Dnmt1
Dnmt3b sgRNA
DNA target (exon 3)
mouse Dnmt3b
2kb
c
SpCas9
DNMT 3xsgRNA
indel (%) 19 18 4 N.D. N.D. N.D.
d
Dnmt1 [5%]
Dnmt3a [18%]
Dnmt3b [3%]
Dnmt1 Dnmt3a Dnmt3b [41%]
Dnmt1 Dnmt3a [28%]
Dnmt1 Dnmt3b [3%]
Dnmt3a Dnmt3b [6%]
e
SpCas9
DNMT 3xsgRNA
Dnmt3a
Dnmt1
Tubulin
f
***
ns
training context
altered context
SpCas9
DNMT 3xsgRNA

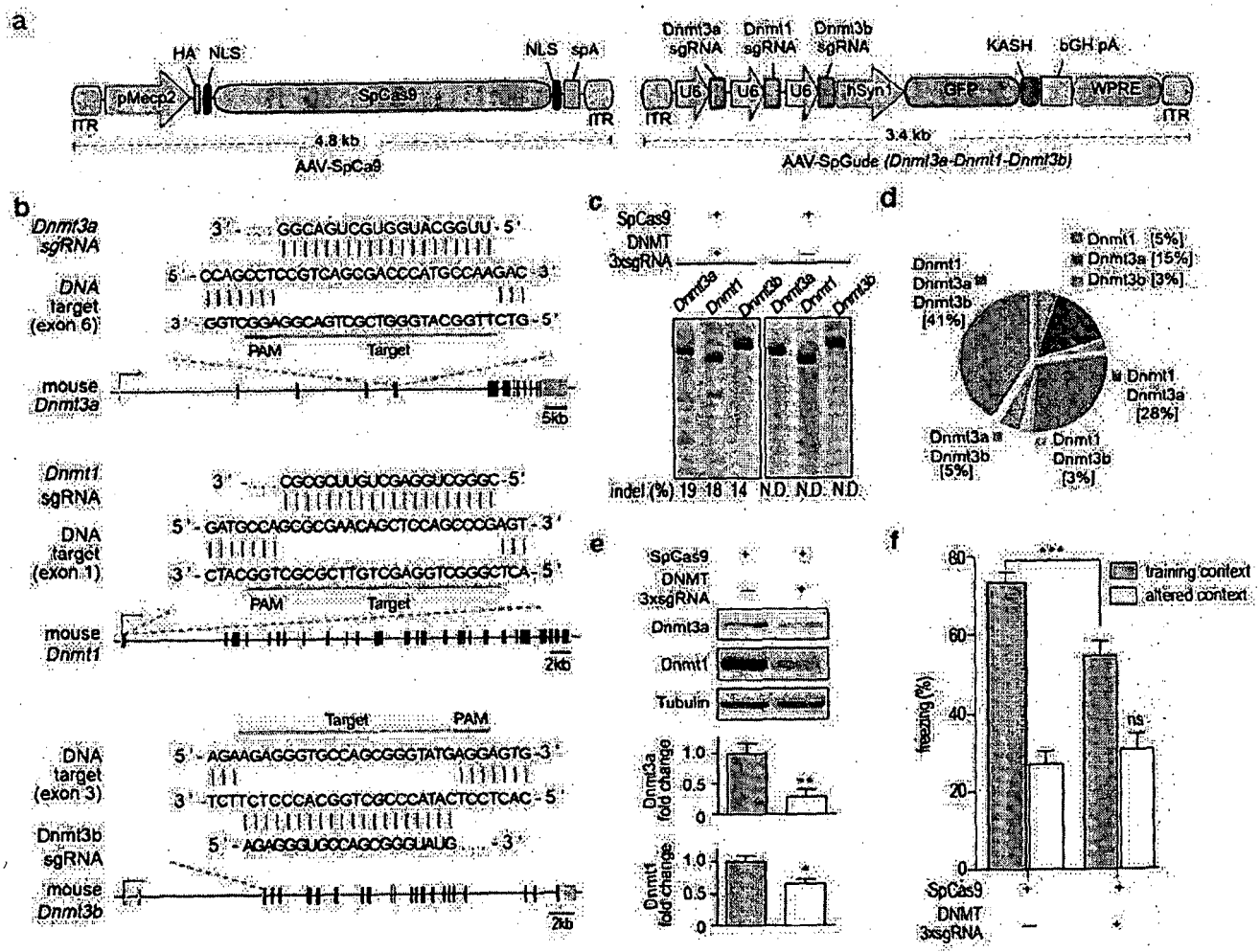

a
HA NLS
NLS spA
pMecp2
SpCas9
ITR
ITR
4.8 kb
AAV-SpCas9
Dnmt3a sgRNA
Dnmt1 sgRNA
Dnmt3b sgRNA
KASH
bGH pA
U6
U6
U6
hSyn1
GFP
WPRE
ITR
ITR
3.4 kb
AAV-SpGuide (Dnmt3a-Dnmt1-Dnmt3b)
b
Dnmt3a sgRNA
3' - GGCAGUCGUGGUACGGUU - 5'
DNA target (exon 6)
5' - CCAGCCTCCGTCAGCGACCCATGCCAAGAC - 3'
3' - GGTCGGAGGCAGTCGCTGGGTACGGTTCTG - 5'
PAM
Target
mouse Dnmt3a
5kb
Dnmt1 sgRNA
3' - CGCGCUUGUCGAGGUCGGGC - 5'
DNA target (exon 1)
5' - GATGCCAGCGCGAACAGCTCCAGCCCGAGT - 3'
3' - CTACGGTCGCGCTTGTCGAGGTCGGGCTCA - 5'
PAM
Target
mouse Dnmt1
2kb
Target
PAM
DNA target (exon 3)
5' - AGAAGAGGGTGCCAGCGGGTATGAGGAGTG - 3'
3' - TCTTCTCCCACGGTCGCCCATACTCCTCAC - 5'
Dnmt3b sgRNA
5' - AGAGGCUGCCAGCGGGUAUG - 3'
mouse Dnmt3b
2kb
c
SpCas9
DNMT 3xsgRNA
Dnmt3a
Dnmt1
Dnmt3b
Dnmt3a
Dnmt1
Dnmt3b
indel (%) 19 18 14 N.D. N.D. N.D.
d
Dnmt1 [5%]
Dnmt3a [15%]
Dnmt3b [3%]
Dnmt1 Dnmt3a [28%]
Dnmt1 Dnmt3b [3%]
Dnmt3a Dnmt3b [5%]
Dnmt3a Dnmt3b [41%]
e
SpCas9
DNMT 3xsgRNA
Dnmt3a
Dnmt1
Tubulin
Dnmt3a fold change
Dnmt1 fold change
1.0
0.5
0
f
training context
altered context
freezing (%)
80
60
40
20
0
ns
SpCas9
DNMT 3xsgRNA

FIG. 88

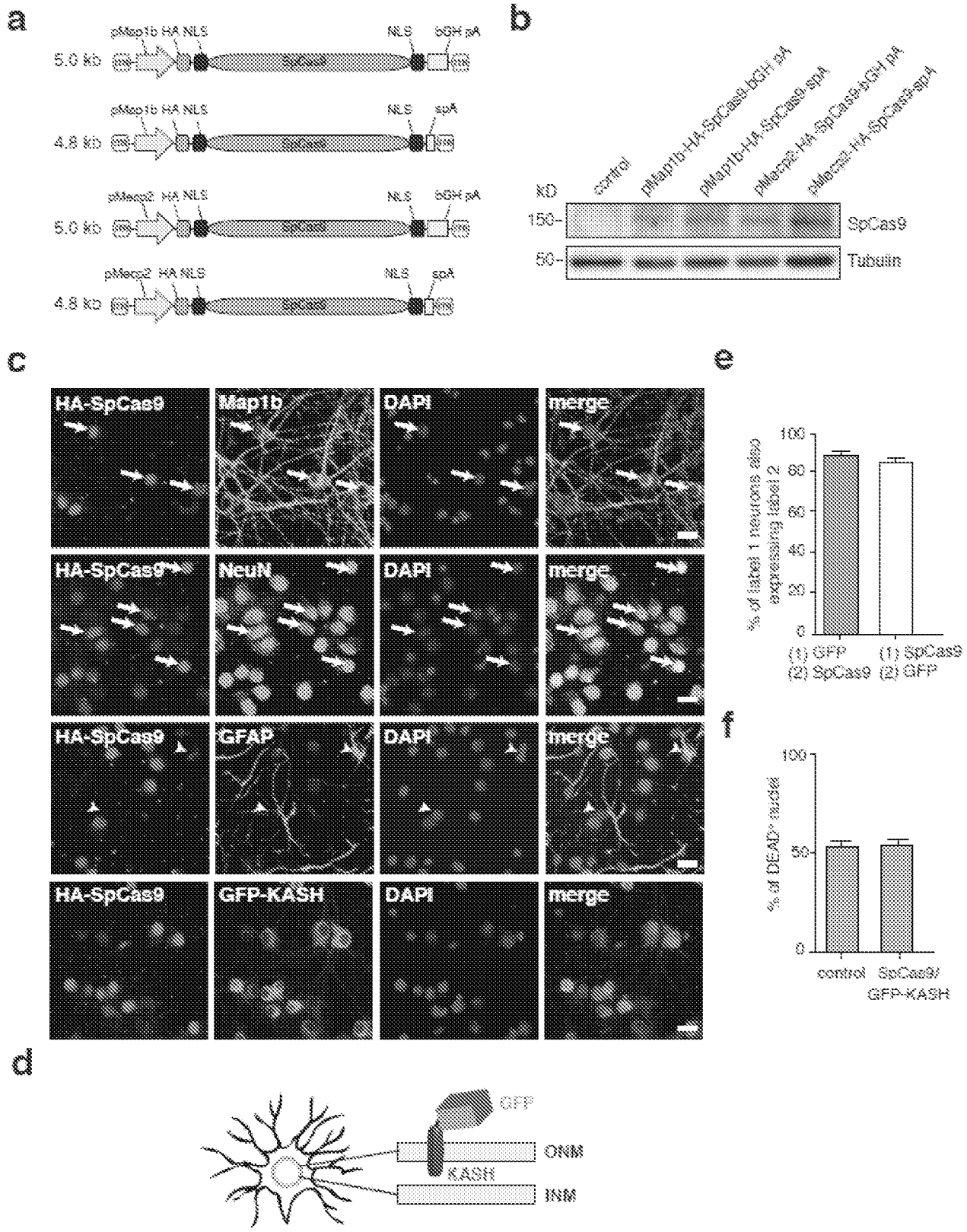

a
pMap1b HA NLS
NLS bGH pA
5.0 kb
SpCas9
pMap1b HA NLS
NLS spA
4.8 kb
SpCas9
pMecp2 HA NLS
NLS bGH pA
5.0 kb
SpCas9
pMecp2 HA NLS
NLS spA
4.8 kb
SpCas9
b
kD
control
pMap1b-HA-SpCas9-bGH pA
pMap1b-HA-SpCas9-spA
pMecp2-HA-SpCas9-bGH pA
pMecp2-HA-SpCas9-spA
150-
SpCas9
50-
Tubulin
c
HA-SpCas9
Map1b
DAPI
merge
HA-SpCas9
NeuN
DAPI
merge
HA-SpCas9
GFAP
DAPI
merge
HA-SpCas9
GFP-KASH
DAPI
merge
e
% of label 1 neurons also expressing label 2
100
80
60
40
20
0
(1) GFP
(2) SpCas9
(1) SpCas9
(2) GFP
f
% of DEAD cells
100
50
0
control
SpCas9/ GFP-KASH
d
GFP
ONM
KASH
INM

FIG. 89

a

| | *Mecp2* target sequence | PAM |
|---|---|---|
| Target 1 | CTGGGAGAGGGAGCCCCTCC | AGG |
| Target 2 | AAAGGTGGGAGACACCTCCT | TGG |
| Target 3 | TCCAACCTTCAGGCAAGGTG | GGG |
| Target 4 | AGGAAGTCTGGCCGATCTGC | TGG |
| Target 5 | CCATTCTGCAGAGCCAGCAG | AGG |
| Target 6 | CTCTGAGGCCCTGGAGATCC | TGG |

b

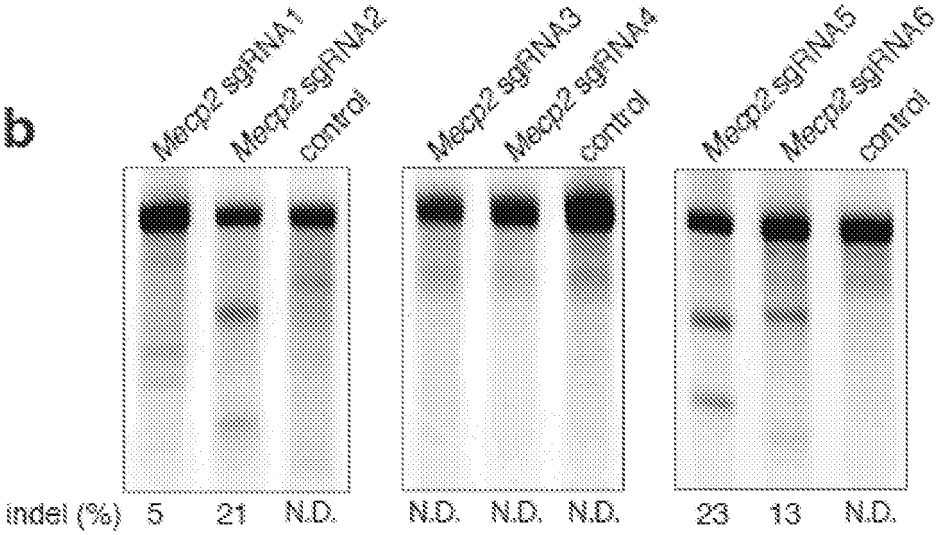



FIG. 90

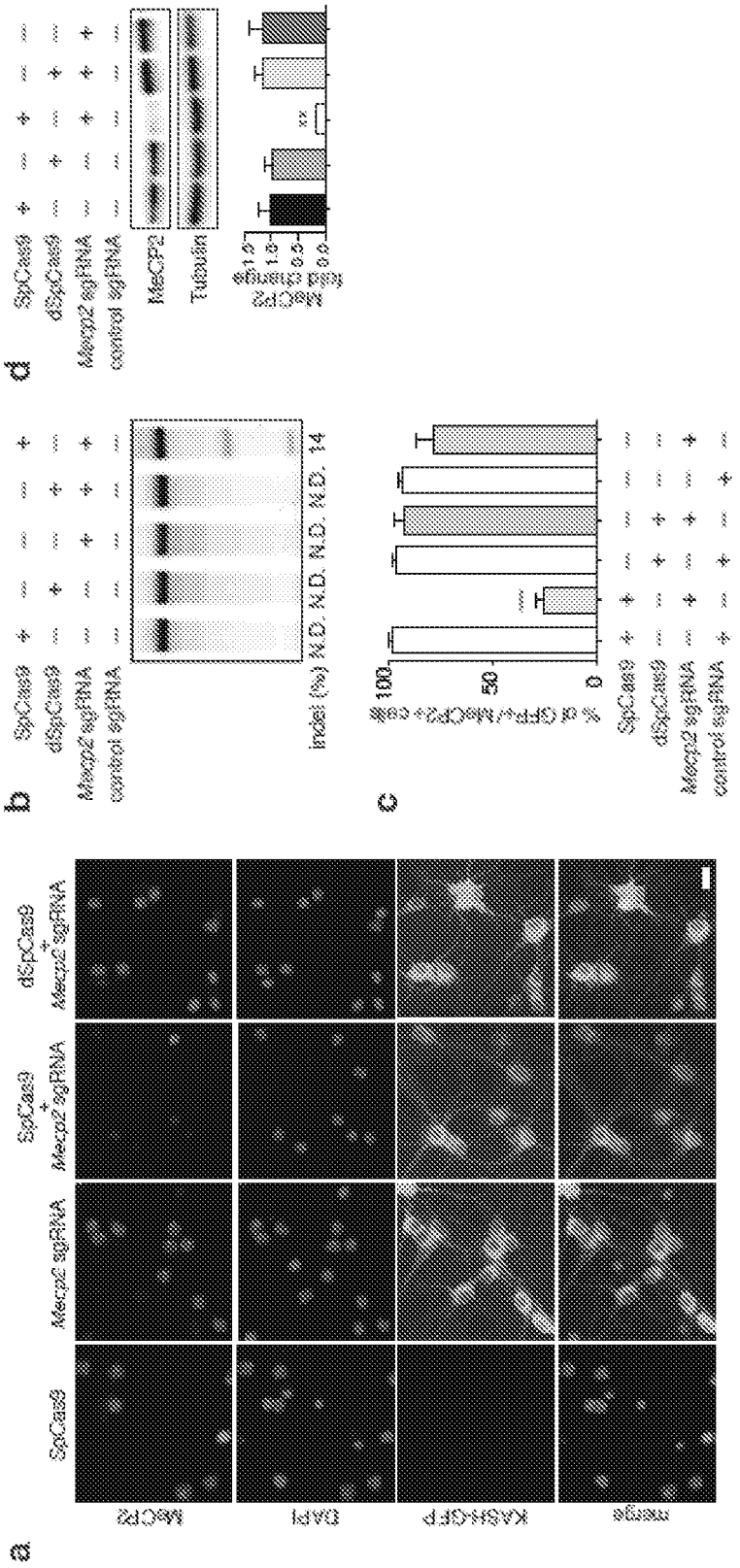

a
SpCas9
Mecp2 sgRNA
SpCas9 + Mecp2 sgRNA
dSpCas9 + Mecp2 sgRNA
MeCP2
DAPI
KASHGFP
merge
b
SpCas9
dSpCas9
Mecp2 sgRNA
control sgRNA
indel (%) N.D. N.D. N.D. N.D. 14
c
% of GFP+/MeCP2+ cells
100
50
0
d
MeCP2
Tubulin
relative MeCP2 expression
1.5
1.0
0.5
0.0

FIG. 91

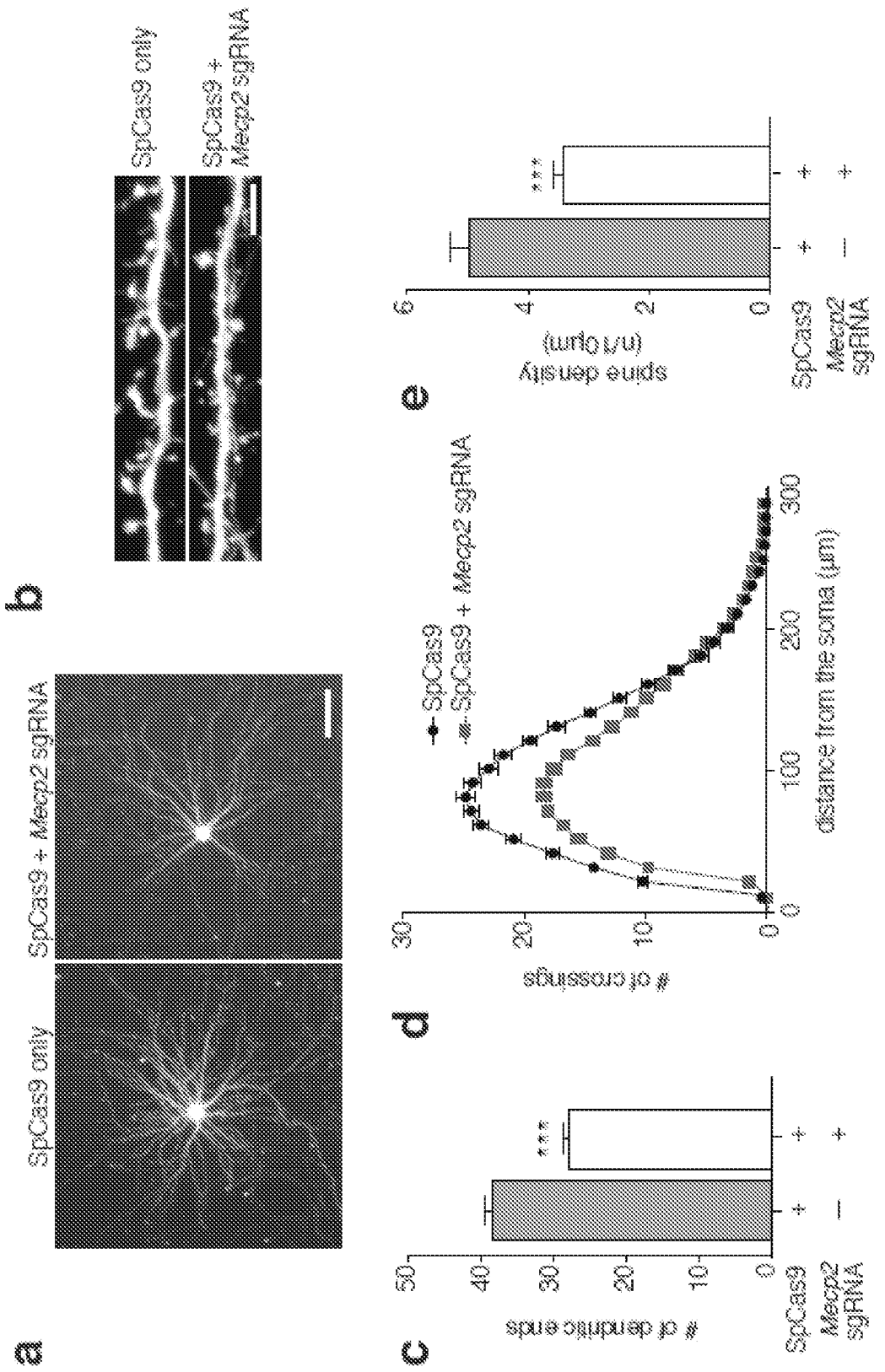

a
SpCas9 only
SpCas9 + Mecp2 sgRNA
b
SpCas9 only
SpCas9 + Mecp2 sgRNA
c
# of dendritic ends
***
SpCas9
Mecp2 sgRNA
d
SpCas9
SpCas9 + Mecp2 sgRNA
# of crossings
distance from the soma (μm)
e
spine density (n/5 0μm)
***
SpCas9
Mecp2 sgRNA

FIG. 92

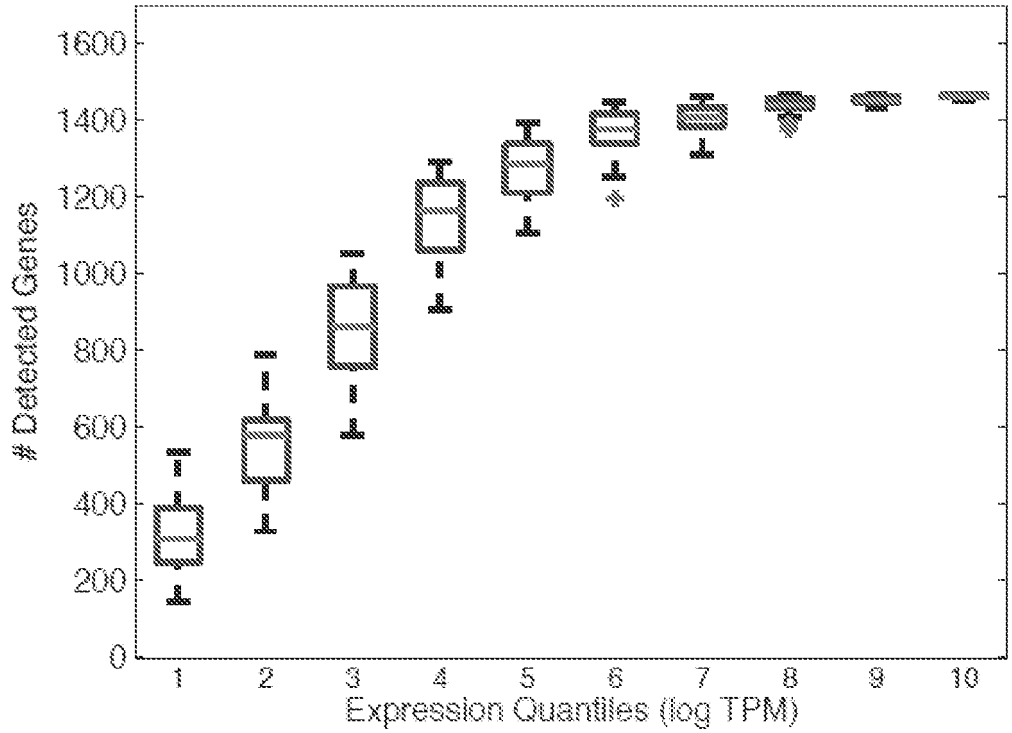

# Detected Genes
0
200
400
600
800
1000
1200
1400
1600
1
2
3
4
5
6
7
8
9
10
Expression Quantiles (log TPM)

FIG. 93

a

| target | target sequence | PAM |
|---|---|---|
| *Dnmt3a* | TTGGCATGGGTCGCTGACGG | AGG |
| *Dnmt1* | CGGGCTGGAGCTGTTCGCGC | TGG |
| *Dnmt3b* | AGAGGGTGCCAGCGGGTATG | AGG |

b

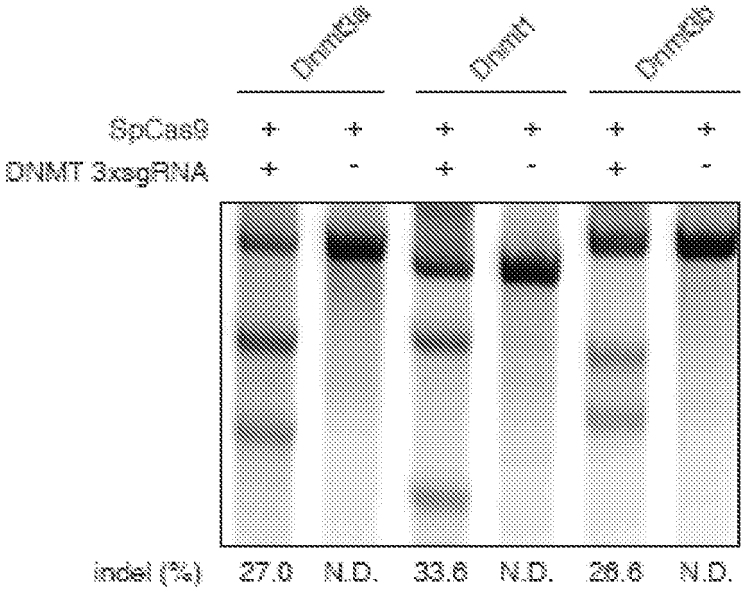



FIG. 94

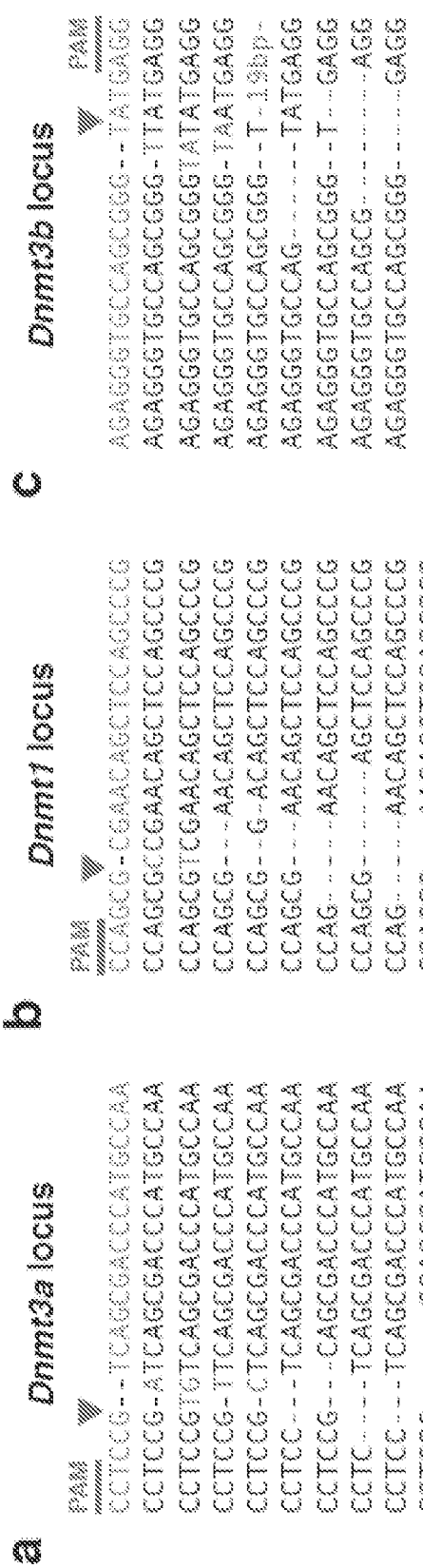

a
Dnmt3a locus
PAM
CCTCCG--TCAGCGACCCATGCCAA
CCTCCG-ATCAGCGACCCATGCCAA
CCTCCGTGTCAGCGACCCATGCCAA
CCTCCG-TTCAGCGACCCATGCCAA
CCTCCG-CTCAGCGACCCATGCCAA
CCTCC---TCAGCGACCCATGCCAA
CCTCCG---CAGCGACCCATGCCAA
CCTC----TCAGCGACCCATGCCAA
CCTCC---TCAGCGACCCATGCCAA
CCTCCG------CGACCCATGCCAA
CCTC------AGCGACCCATGCCAA
C---------TCAGCGACCCATGCCAA
b
Dnmt1 locus
PAM
CCAGCG-CGAACAGCTCCAGCCCG
CCAGCGCCGAACAGCTCCAGCCCG
CCAGCGTCGAACAGCTCCAGCCCG
CCAGCG---AACAGCTCCAGCCCG
CCAGCG--G-ACAGCTCCAGCCCG
CCAGCG---AACAGCTCCAGCCCG
CCAG------AACAGCTCCAGCCCG
CCAGCG------AGCTCCAGCCCG
CCAG------AACAGCTCCAGCCCG
CCAGCG---AACAGCTCCAGCCCG
CCAGCG------ACAGCTCCAGCCCG
CCAGCGC---------TCCAGCCCG
c
Dnmt3b locus
PAM
AGAGGGTGCCAGCGGG--TATGAGG
AGAGGGTGCCAGCGGG-TTATGAGG
AGAGGGTGCCAGCGGGTATATGAGG
AGAGGGTGCCAGCGGG-TAATGAGG
AGAGGGTGCCAGCGGG--T-19bp-
AGAGGGTGCCAG------TATGAGG
AGAGGGTGCCAGCGGG--T---GAGG
AGAGGGTGCCAGCG----------AGG
AGAGGGTGCCAGCGGG-------GAGG

FIG. 95

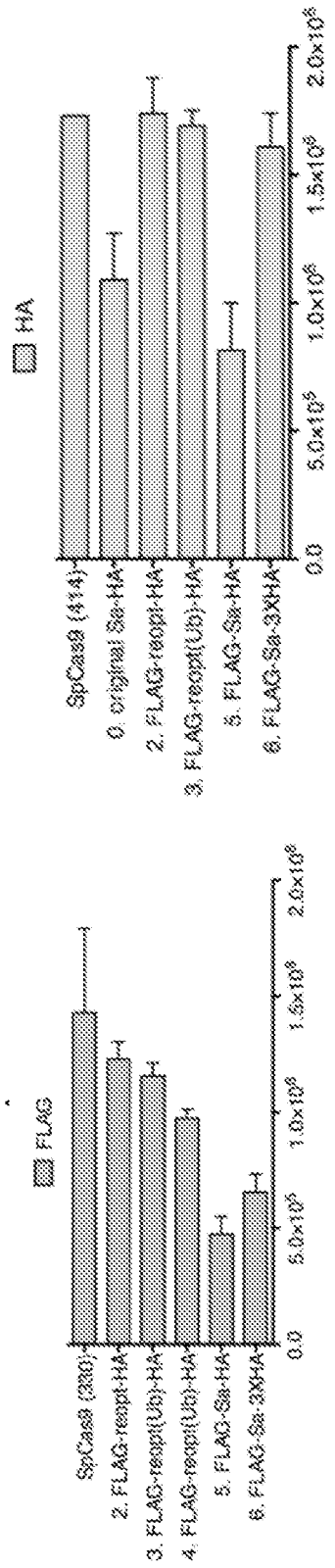

HA
SpCas9 (1414)
0. original Sa-HA
2. FLAG-nespt-HA
3. FLAG-nespt(UD)-HA
5. FLAG-Sa-HA
6. FLAG-Sa-3XHA
0.0
5.0x10^5
1.0x10^6
1.5x10^6
2.0x10^6
FLAG
SpCas9 (320)
2. FLAG-nespt-HA
3. FLAG-nespt(UD)-HA
4. FLAG-nespt(UD)-HA
5. FLAG-Sa-HA
6. FLAG-Sa-3XHA
0.0
5.0x10^5
1.0x10^6
1.5x10^6
2.0x10^6

FIG. 96

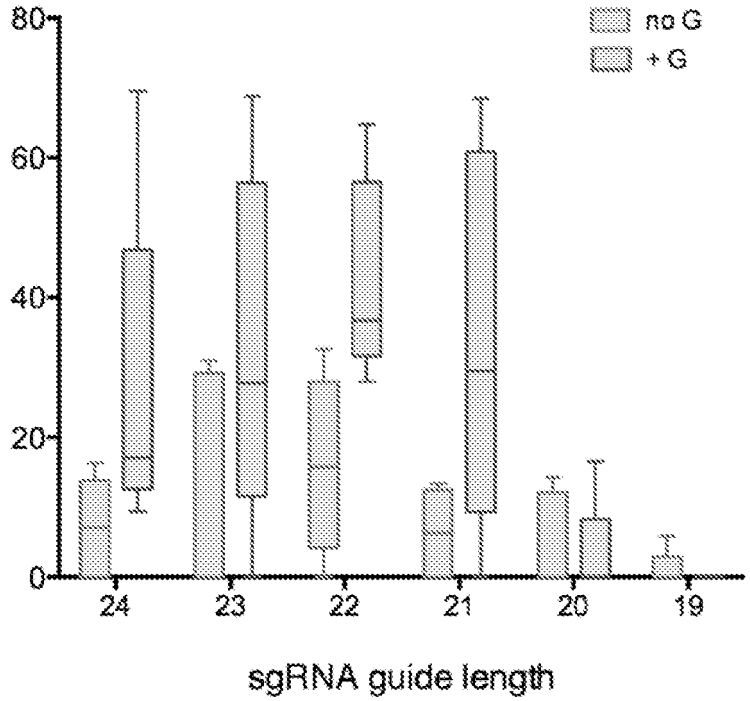



FIG. 97

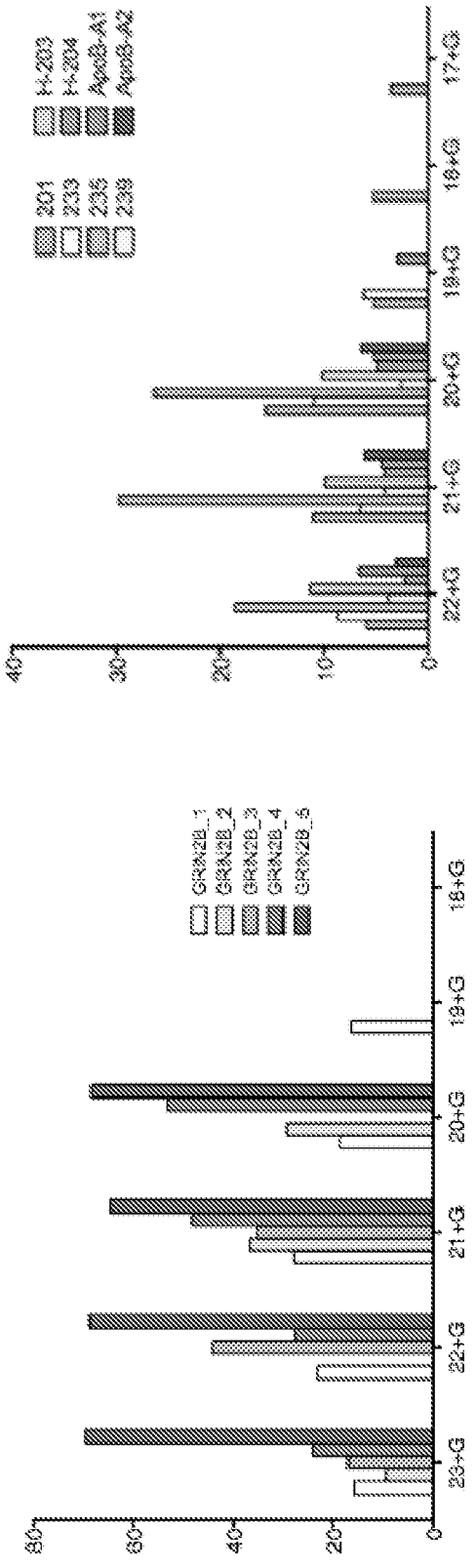

201
233
235
239
I+203
I+204
Apo8-A1
Apo8-A2
40
30
20
10
0
22+G
21+G
20+G
19+G
18+G
17+G
GRIN2B_1
GRIN2B_2
GRIN2B_3
GRIN2B_4
GRIN2B_5
80
60
40
20
0
23+G
22+G
21+G
20+G
19+G
18+G

FIG. 98