FIG. 1A

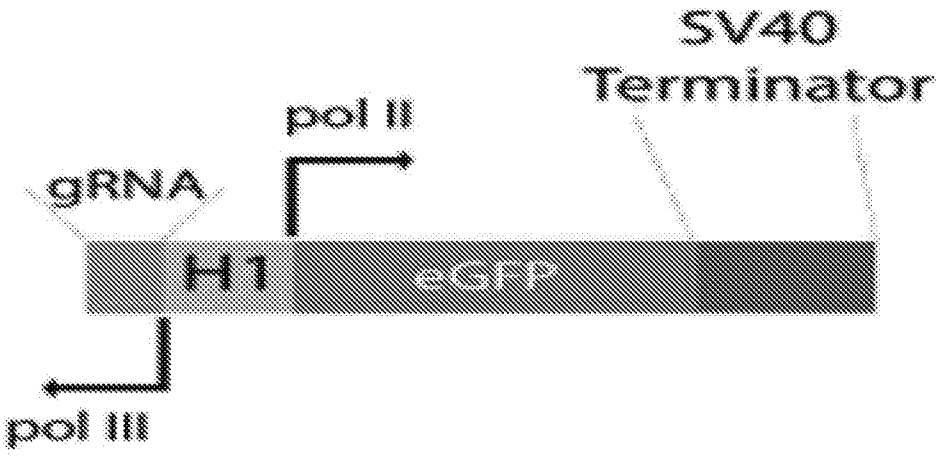


FIG. 1B

5'UTR and Translational Enhancers

Strong Kozak	9bp
Beta-Globin 5'UTR	50bp
6.947[4]	91bp
6.967[4]	75bp

FIG. 1C

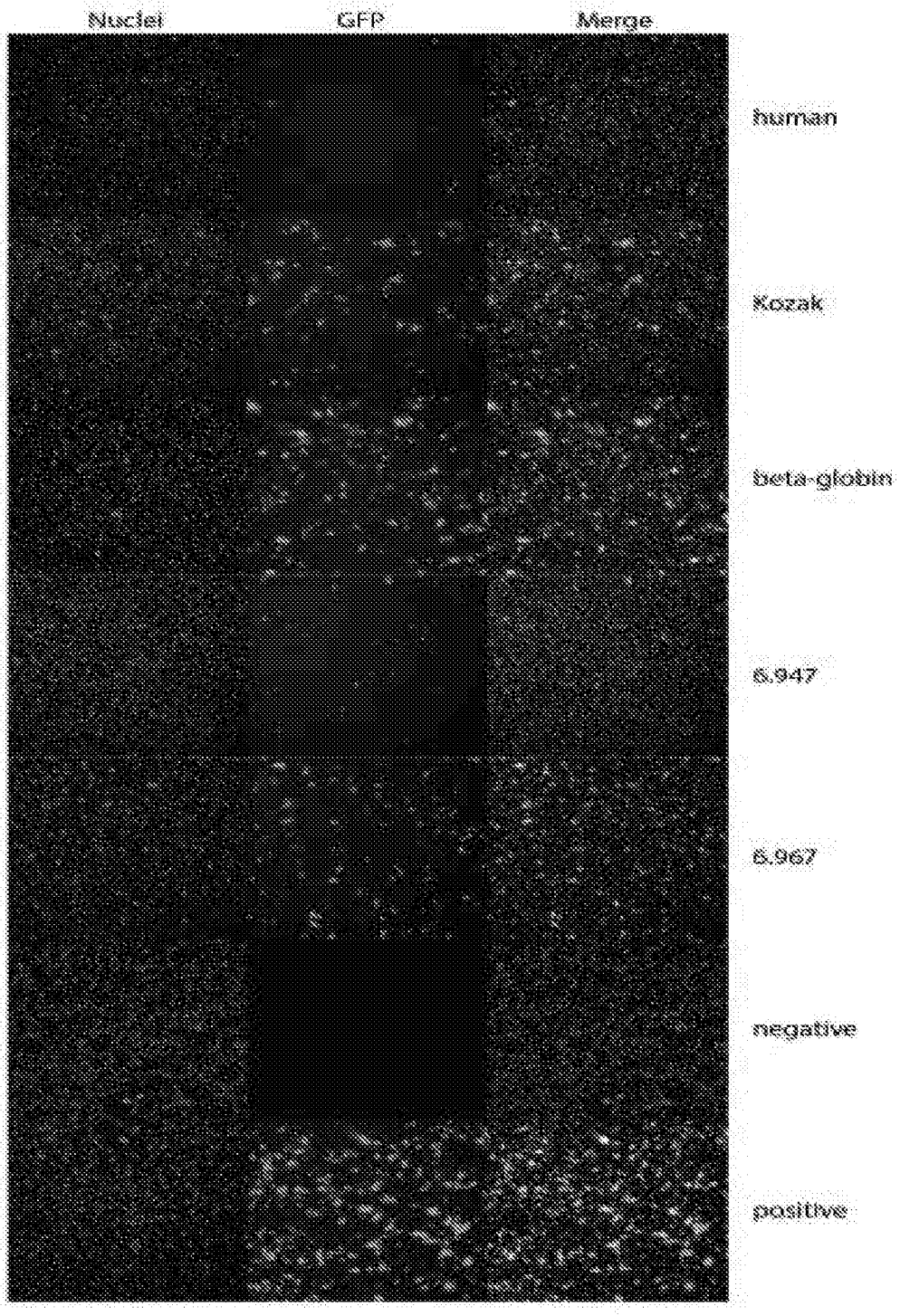


FIG. 1D

9nt Kozak sequence: GCCGCCACC

6nt Kozak sequence: GCCACC

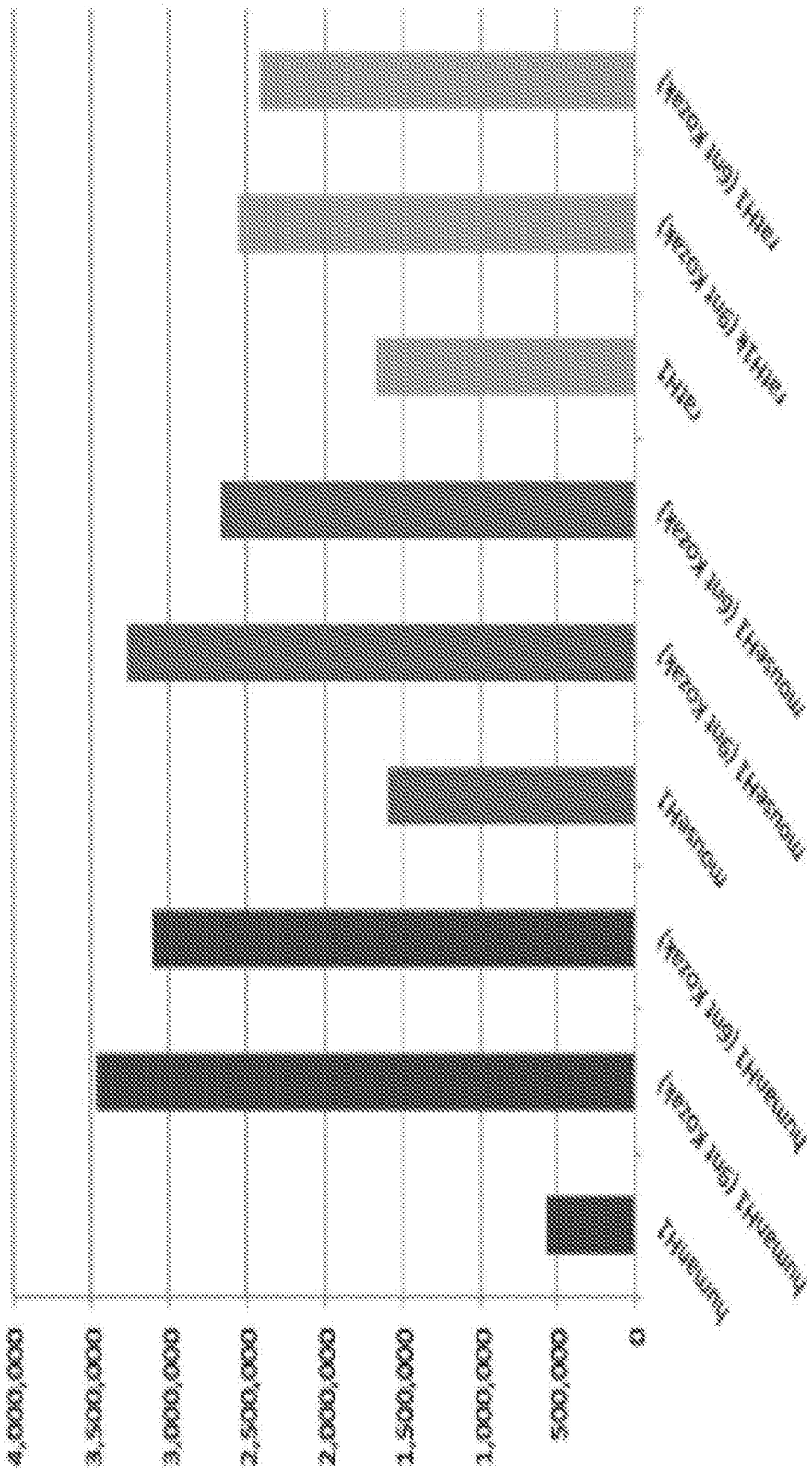

5'UTR Kozak modifications enhance bidirectional expression of pol II gene
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
(hEF1A Int) tHBG
(hEF1A Int) tHBG
tHBG
(hEF1A Int) tHBGmut
(hEF1A Int) tHBGmut
tHBGmut
(hEF1A Int) tHBGmut
(hEF1A Int) tHBGmut
tHBGmut

FIG. 1E

FIG. 1F

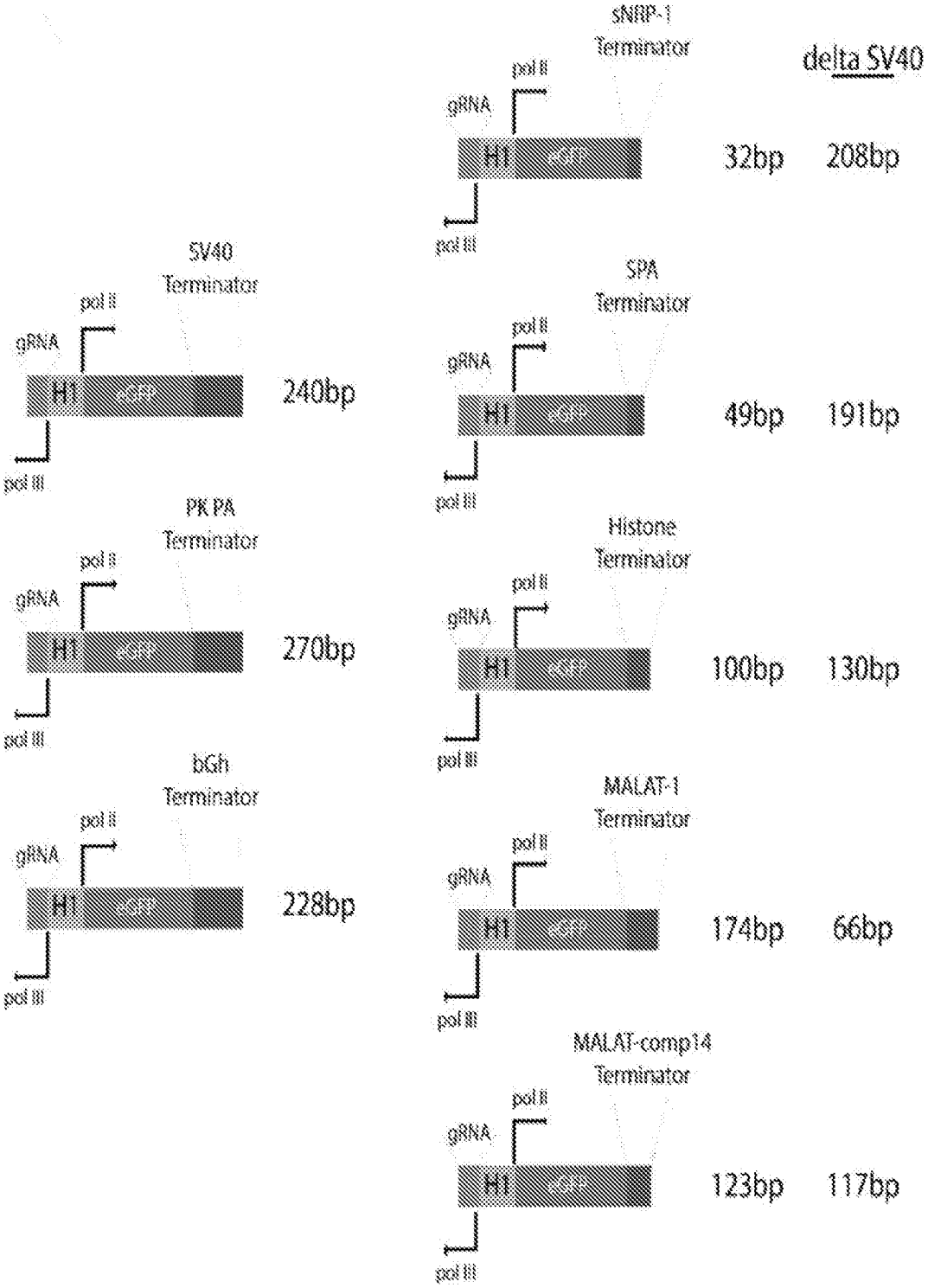
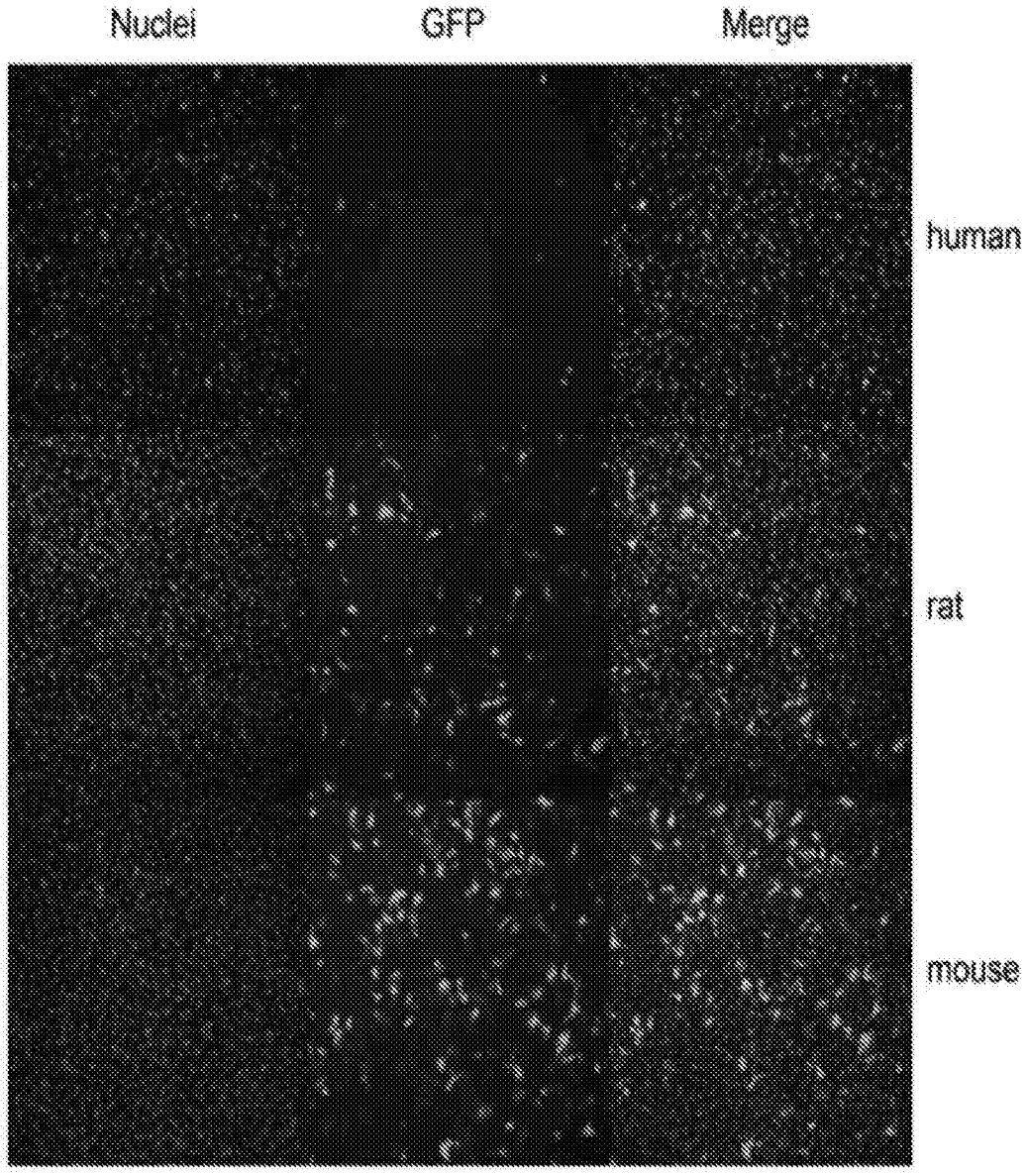

sNRP-1 Terminator
delta SV40
pol II
gRNA
H1
pol III
32bp
208bp
SV40 Terminator
240bp
SPA Terminator
49bp
191bp
PK PA Terminator
270bp
Histone Terminator
100bp
130bp
bGh Terminator
228bp
MALAT-1 Terminator
174bp
66bp
MALAT-comp14 Terminator
123bp
117bp

FIG. 2A

H1 Promoter	Human	230bp
H1 Promoter	Mouse	176bp
H1 Promoter	Rat	207bp

FIG. 2B



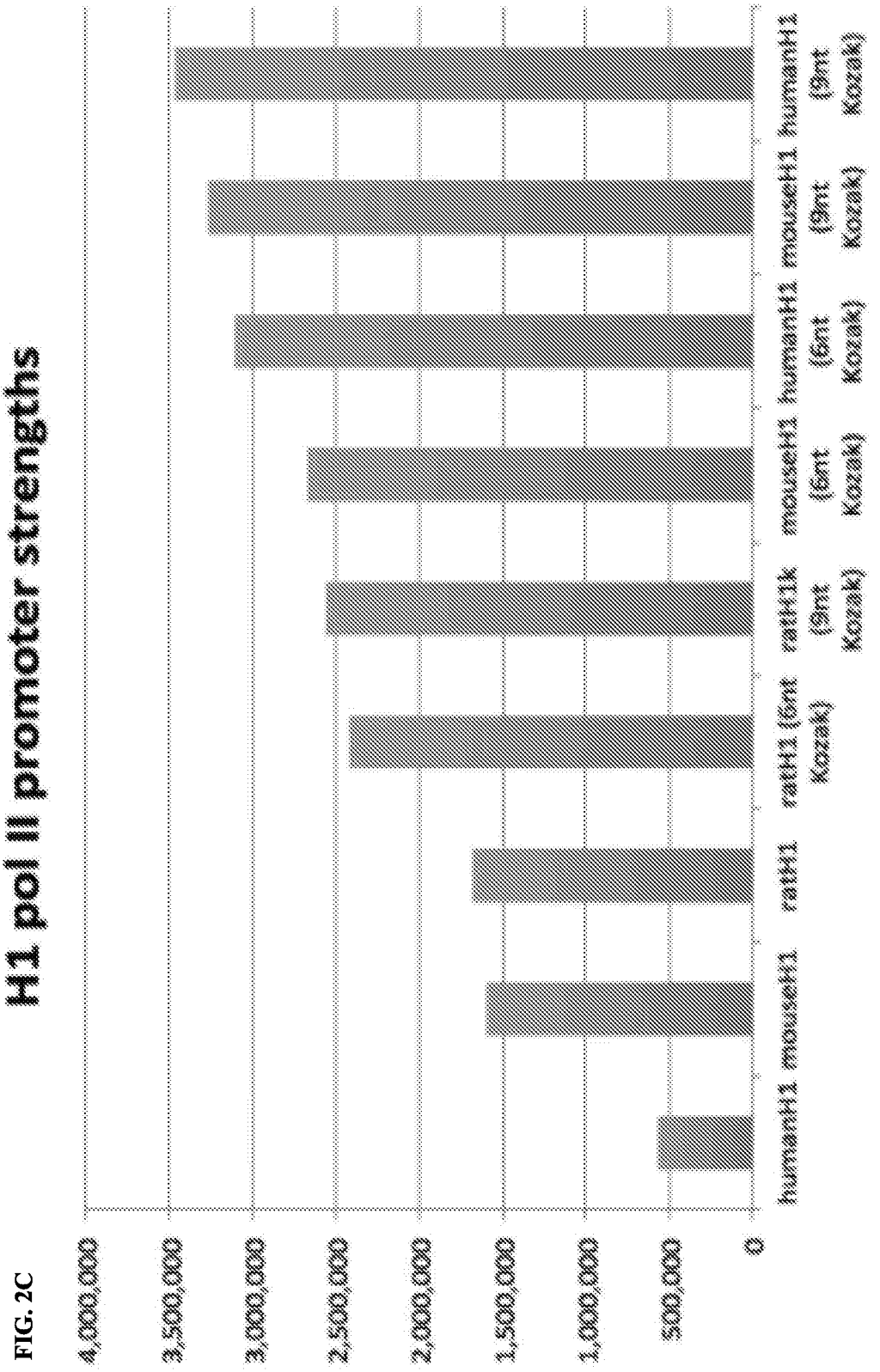
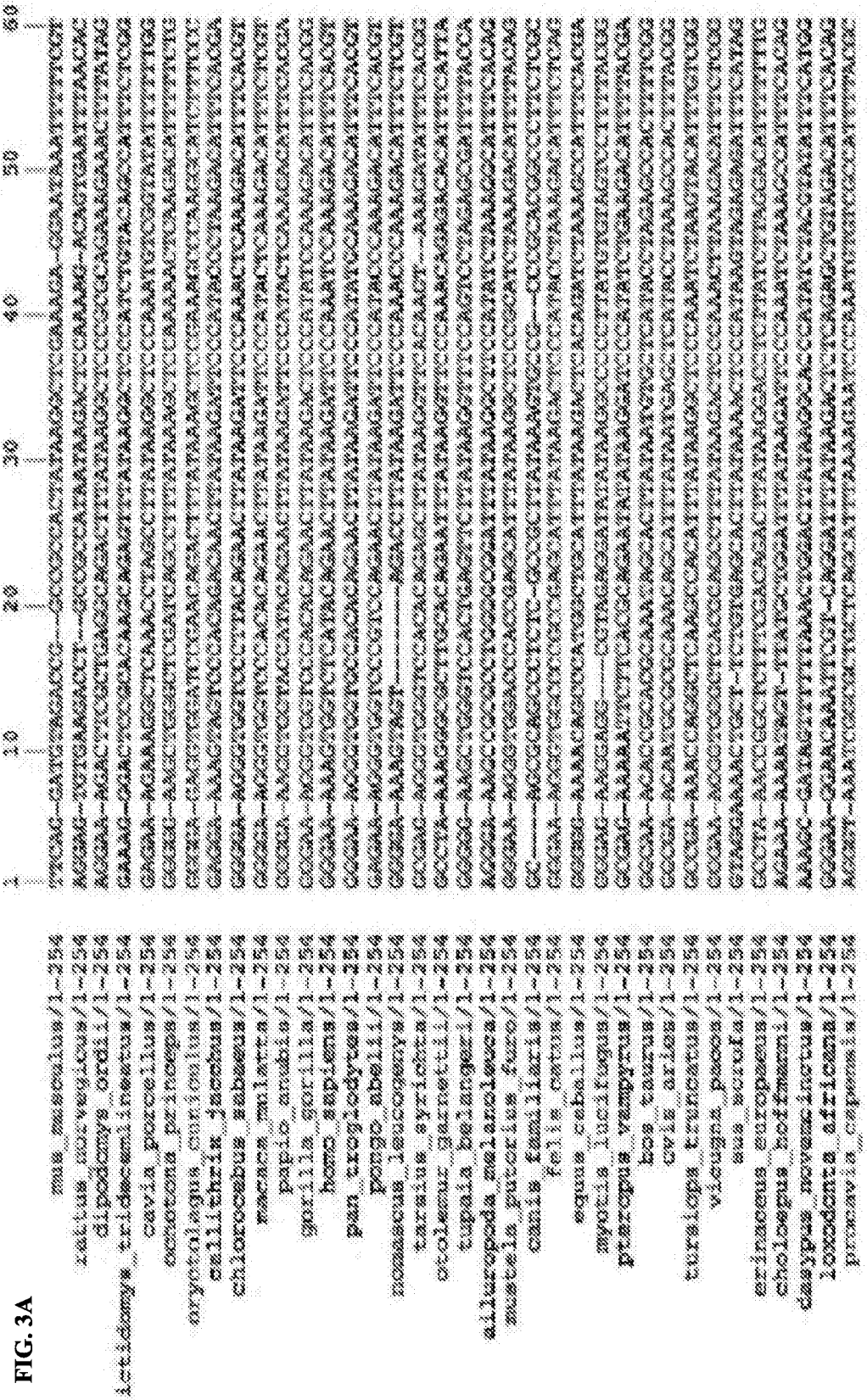

H1 pol II promoter strengths
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
humanH1
mouseH1
ratH1
ratH1 (6nt Kozak)
ratH1k (9nt Kozak)
mouseH1 (6nt Kozak)
humanH1 (6nt Kozak)
mouseH1 (9nt Kozak)
humanH1 (9nt Kozak)

FIG. 2C

FIG. 3A



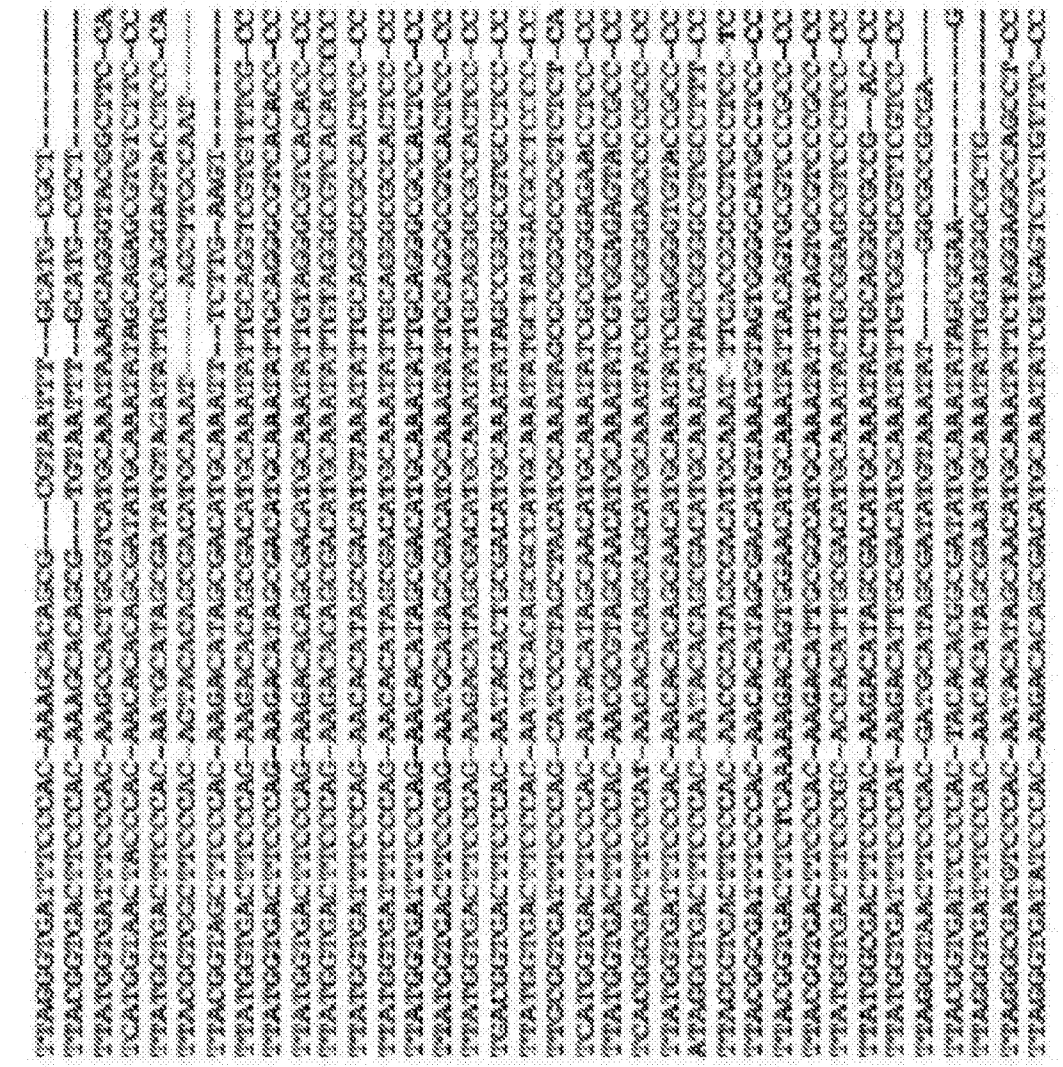


FIG. 3A(cont.)

FIG. 3B

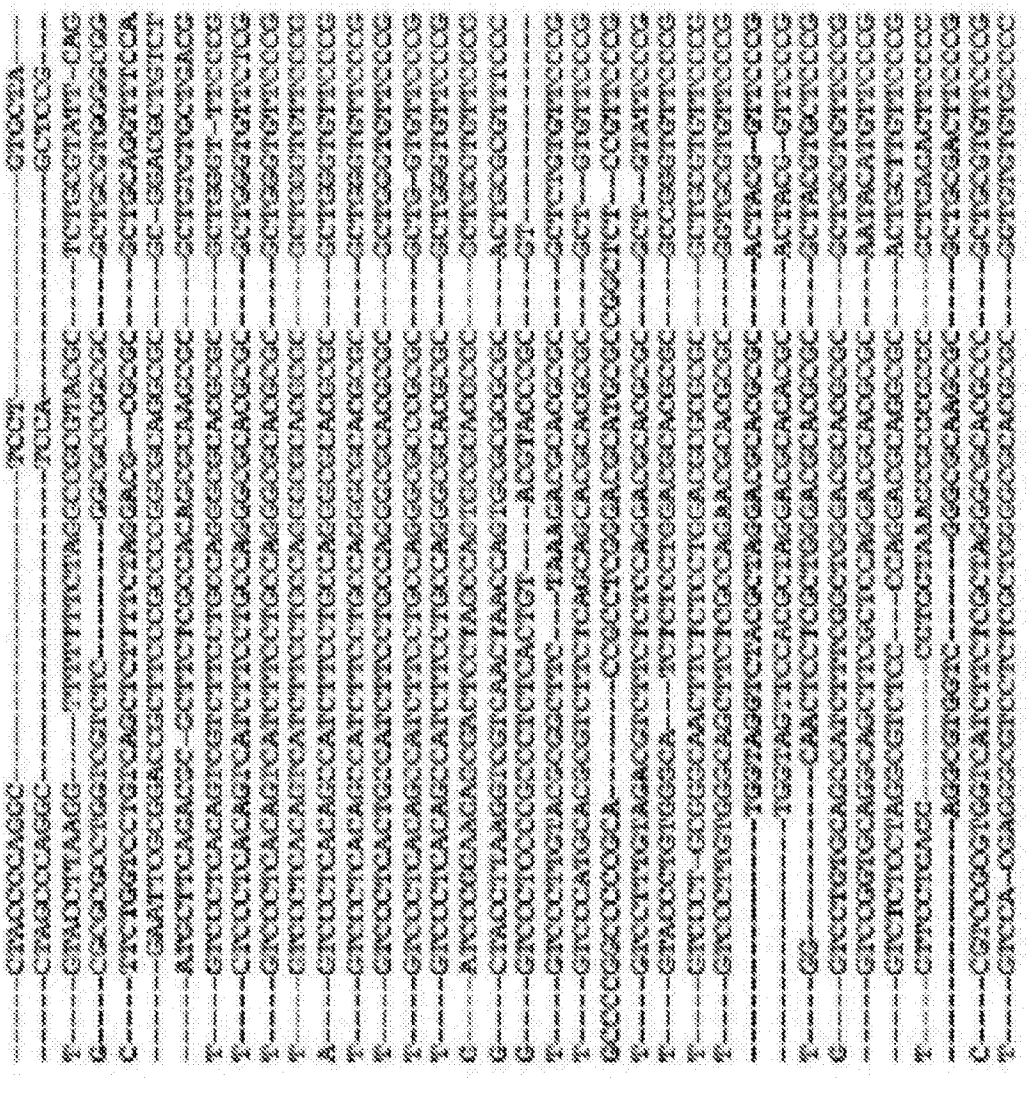
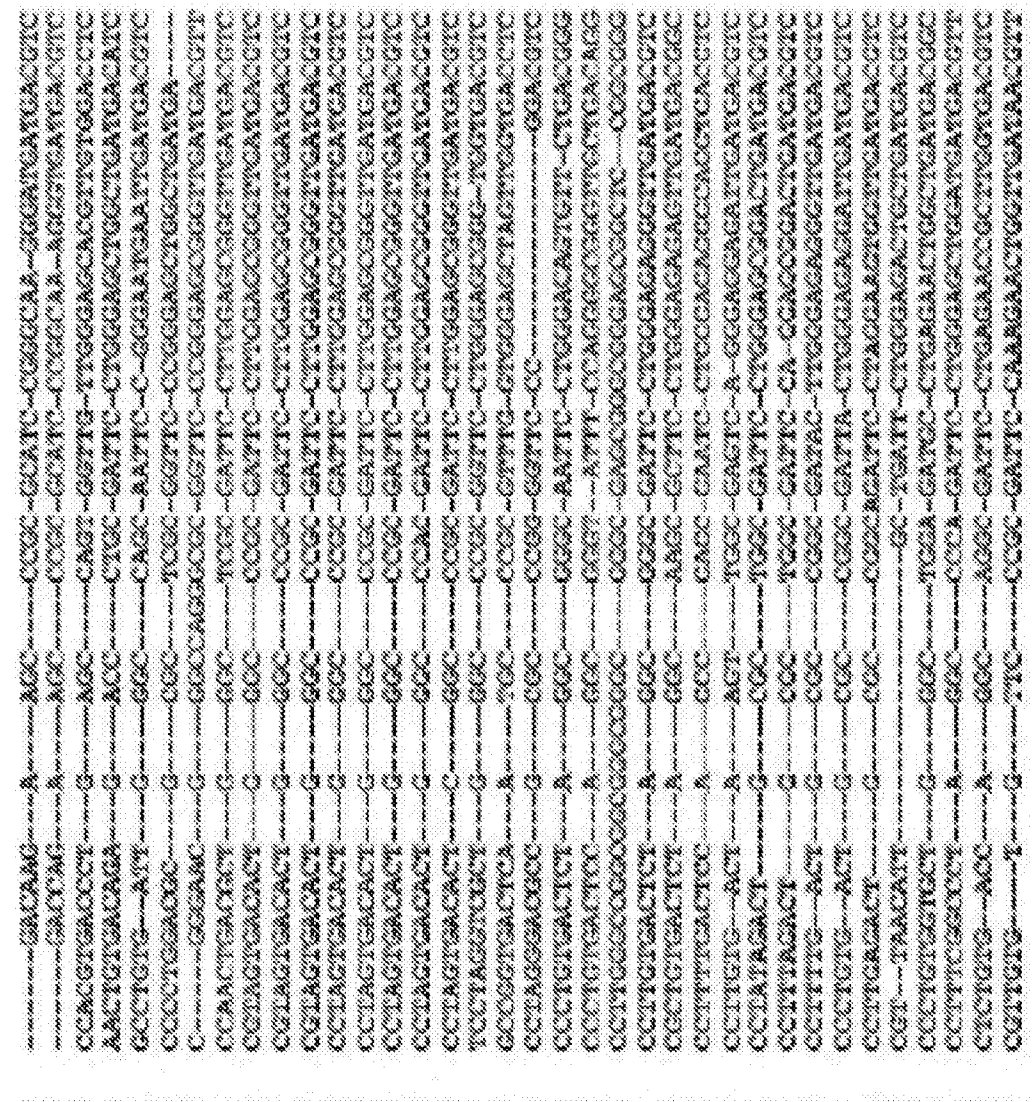


FIG. 3B(cont.)



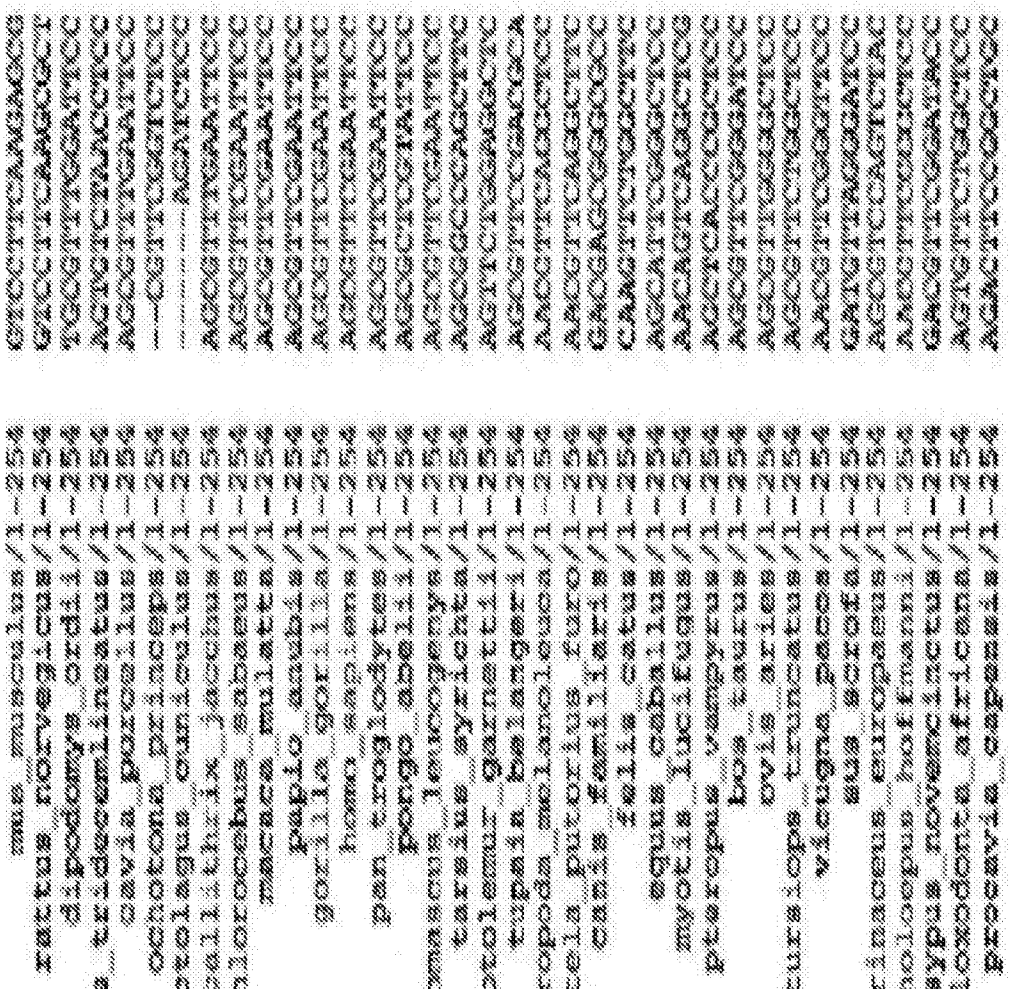
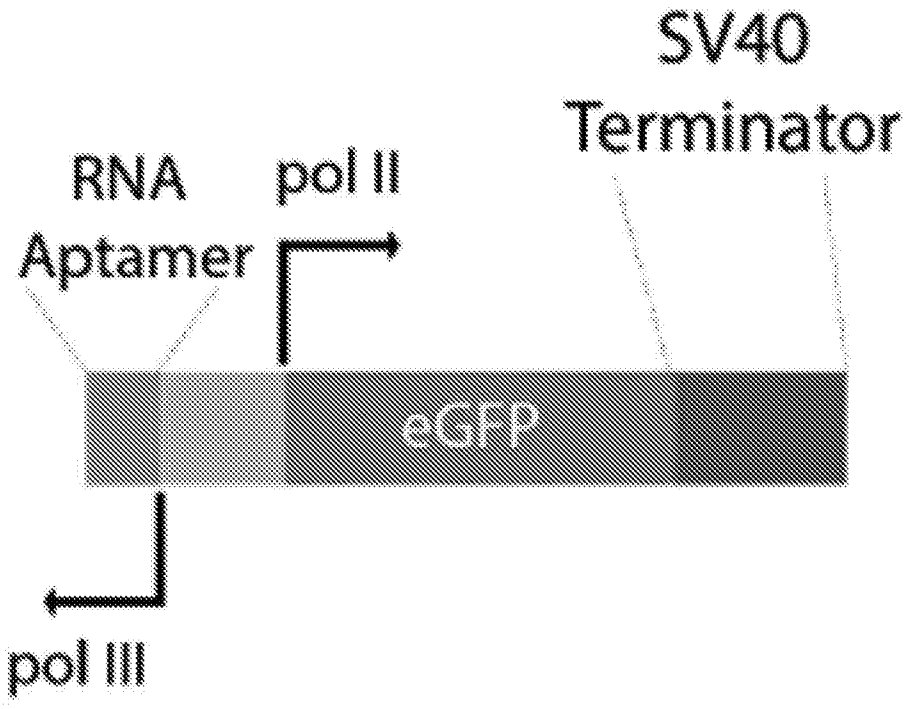

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rattus_norvegicus/1-254 GTCGTTCAAGAGCT
dipodomys_ordii/1-254 AGCGTTCGAATCC
ictidomys_tridecemlineatus/1-254 AGCGTTCTACGCTCC
cavia_porcellus/1-254 ACCGTTCGAATCC
octodon_degus/1-254 ----------AGATCC
oryctolagus_cuniculus/1-254 ACCGTTCGAATCC
callithrix_jacchus/1-254 ACCGTTCGAATCC
chlorocebus_sabaeus/1-254 ACCGTTCGAATCC
macaca_mulatta/1-254 ACCGTTCGAATCC
papio_anubis/1-254 ACCGTTCGAATCC
gorilla_gorilla/1-254 ACCGTTCGAATCC
homo_sapiens/1-254 ACCGTTCGAATCC
pan_troglodytes/1-254 ACCGTTCGAATCC
pongo_abelii/1-254 AGCGCTCGTAATCC
nomascus_leucogenys/1-254 ACCGTTCGAATCC
carlito_syrichta/1-254 AGCGGCTCCAGCTCC
otolemur_garnettii/1-254 AGTTCTCGAAGGCTC
tupaia_belangeri/1-254 ACCGTTCCAAGGCA
ailuropoda_melanoleuca/1-254 AACGTTCACGCTCC
mustela_putorius_furo/1-254 AACGTTCACGCTCC
canis_familiaris/1-254 GACGAGCCGAGGCCC
felis_catus/1-254 CAAGTTCTCGCTTC
equus_caballus/1-254 ACCATTCCAGCTCC
myotis_lucifugus/1-254 AACAGTCCAGCTCG
pteropus_vampyrus/1-254 AGCTCACCCCCTCC
bos_taurus/1-254 AGCGTTCCGAATCC
ovis_aries/1-256 ACCGTTCGAATCC
tursiops_truncatus/1-254 ACCGTTCTCGCTCC
vicugna_pacos/1-254 AACGTTCCCGATCC
sus_scrofa/1-254 GATGTTAGCGATCC
erinaceus_europaeus/1-254 ACCGTTCCAGTCTAC
choloepus_hoffmanni/1-254 ANCGTTCCCGCTCC
dasypus_novemcinctus/1-254 GACGTTCCGGATACC
loxodonta_africana/1-254 AGTGTTCTCGGCTCC
procavia_capensis/1-254 AGACTTCCCGCTCC

FIG. 3B(cont.)

FIG. 4A



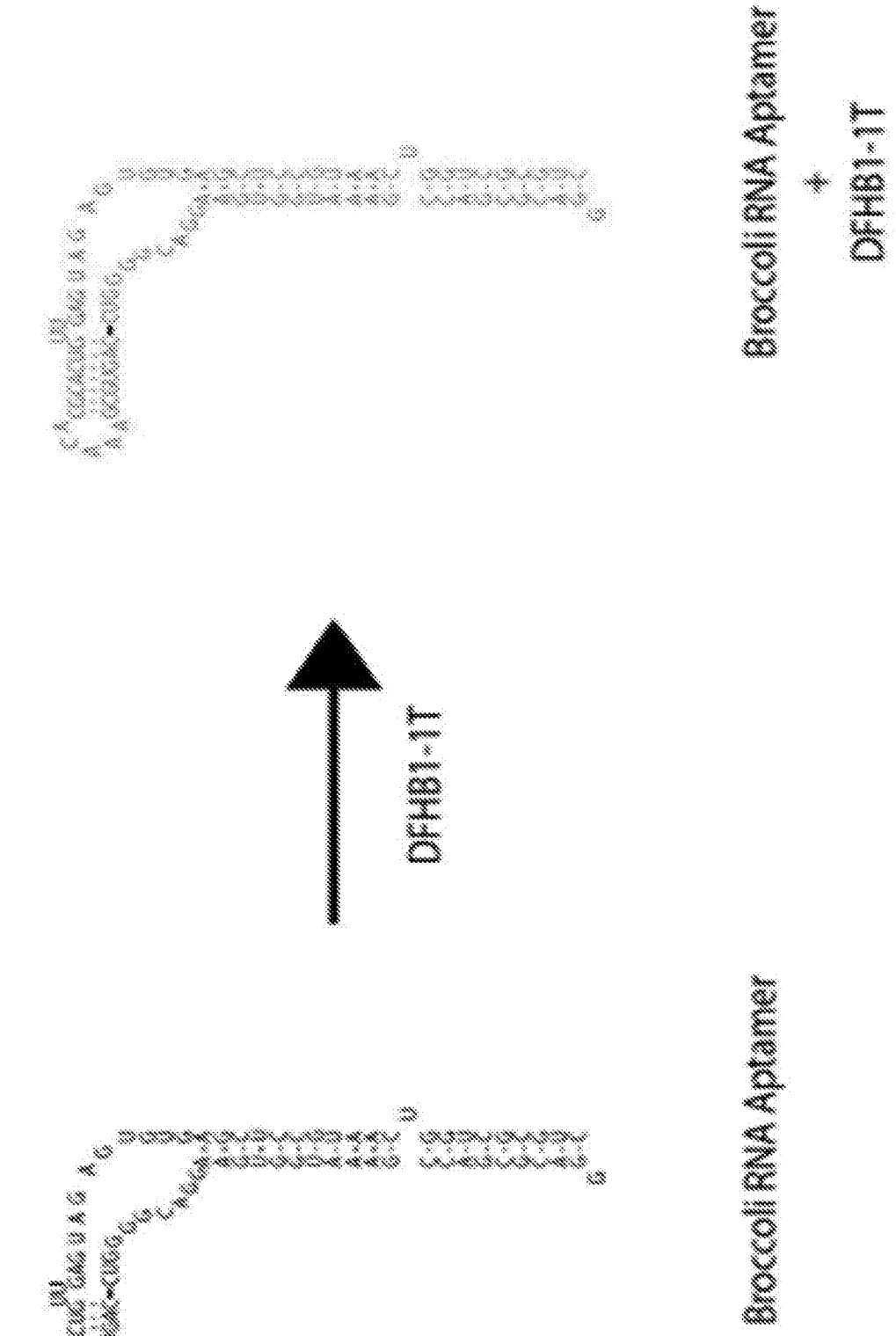

Broccoli RNA Aptamer
+
DFHB1-1T
DFHB1-1T
Broccoli RNA Aptamer

FIG. 4B

FIG. 5

```
>mus_musculus/1-254
TTCAG-CATGTAGACCG--GCCGCCACTATAAGGCTCGAAAGA-GGAATAAATTTTTCGTTTAGGGTGATTTCCCAC-AA
AGCACAGCG----CGTAATTT---GCATG-CGCT----------CTACCCCCAGGC----------------TCCT---
-------------GTGCTA-----------CACAAG-----A-------AGC-----CCGC-GCATC-CGGGCAA-GGGATGATGACGTC
GTCCTTCAAGAGCG
>rattus_norvegicus/1-254
AGGAG-TGTGAAGACCT---GCCGCCATAAATAAGACTCCAAAAG-ACAGTGAATTTAACACTTACGGTGACTTCCCAC-AA
AGCACAGCG-----TGTAATTT---GCATG-CGCT----------CTAGCCCCAGGC----------------TCCA----
-------------GCTCCG-----------GACCAG-----A-------AGC-----CCGC-GCATC-CCGGCAA-AGGGTGATGACGTC
GTCCTTCAAGCGCT
>dipodomys_ordii/1-254
AGGAA-AGACTTCGCTGAGGCAGACTTTATAAGGCTCCCGCGCAGAAAGAAACTTTATAGTTATGGTGATTTCCCAC-AA
GCCACTGCGTCATGCAAATAAAGCAGGGTACGGCTTC-CAT-----GTACCTTAAGG-------TTTTTTTCTAGGCCGCGTAC
GC-----TCTGCGTATT-CAGCCACGTGACCCT-----G-------AGC-----CAGT-GGTTG-TTGGGAGCACGTTGTGGACCTC
TGCGTTTGGATTCC
>ictidomys_tridecemlineatus/1-254
GAAAG-GGACTCCGCACAAGCAGAGTTTATAAGGCTCCCATCTGTACAGCCATTTCTCGGTCATGGTAACTACCCAC-AA
CACACAGCGATATGCAAATATAGCAGAGCGTGTCTTTC-CCG-----CGCCGCCTGGTCGTCTC-----------GGCGCCGGC
GC-----GCTGCGTGGGCGGAACTGTGACAGA----G-------ACC-----CTGC-GATTC-CTGGGAGCTGGCTGATGACATC
AGTGTCTAACCTCC
>cavia_porcellus/1-254
GAGAA-AGAAAGGCTCAAACCTAGCCTTATAAGGCTCCCAAATGTCGGTATATTTTTTGGTTATGGTGACTTCCCAC-AA
TGCATAGCGATATGTAGATATTGCCAGGAGTACCTCC-CAC-----TTCTGGTCCTGTCAGCTCTTTTCTAGGACG---CGC
GC-----GCTGCAGGTTTCCAGCCTGTG---ATT-----G-------GGC-----CAGC-AATTC-C-GGGAATGAATTGATGACGTC
AGCGTTTGAATTCC
>ochotona_princeps/1-254
GGGGG-AAGCTGGGCTCGATCAGCCTTTATAAAAGCTCCAAAAACTCAAGACATTTTTCTGTTACGGTGGCTTCCCAC-AG
TACACAGCGACATGCAAAT-----------AGCTTGCCAAT-----------------GAATTCGCGGACCGCTTCCCCGCCCCGGGCGCAGGC
GC-----GC-GGACGCTGTCTCCCCTGGACGC-----G-------CGC-----TCGC-GGTTC-CCGGGAGCTGGCTGATGA-----
--CGTTCGGTCTCC
>oryctolagus_cuniculus/1-254
GGGGA-GAGGTGGATCCGAACAGACTTTATAAAGCTCCGAAAGCCCAAGGCATCTTTCCCTTACGGTAGCTTCCCAC-AA
GACATAGCGACATGCAAATT---TCTTG-AAGT-----------------ATGCTTCAGACGC-GCTTCTCGCCACAGCGCAAGC
GC-----GCTGTGTGCTGACGC-----GGAAC-----G-------GGCCAGGGCGC-GGTTC-CCGGGAGCGGGTTGATGACGTT
-------AGATCTCC
```

FIG. 5(cont.)

```
>callithrix_jacchus/1-254
GAGGA-AAAGTAGTCCCACACAGACAACTTTATAAGATTCCCATACCCTAAGACATTTCACGATTATGGTGACTTCCCAG-AA
GACACAGCGACATGCAAATATTGCAGGTCGTGTTTCG-CCT-----GTCCCTCACAGTCGTCTTCCTGCCAGGGCGCACGC
GC------GCTGGGT-TTCCCGCCAACTGACGCT----G-----GGC-----TCGC-GATTC-CTTGGAGCGGTTGATGACGTC
AGCGTTTGAATTCC
>chlorocebus_sabaeus/1-254
GGGGA-AGGGTGGTCCCTTACAGAACTTATAAGATTCCCAAACTCAAAGACATTTCACGTTTATGGTGACTTCCCAG-AA
GACATAGCGACATGCAAATATTGCAGGCGTCACACC-CCT-----CTCCCTCACAGTCATCTTCCTGCCAGGGCGCACGC
GC------GCTGGGTGTTCTCGCGTAGTGACACT----G-----GGC-----CCGC-GATTC-CTTGGAGCGGGTTGATGACGTC
AGCGTTCGAATTCC
>macaca_mulatta/1-254
GGGGA-AGGGTGGTCCCACACAGAACTTTATAAGATTCCCATACTCAAAGACATTTCTCGTTTATGGTGACTTCCCAG-AA
GACACAGCGACATGCAAATATTGTAGGGCGTCACACC-CCT-----GTCCCTCACAGTCATCTTCCTGCCAGGGCGCACGC
GC------GCTGGGTGTTCTCCCGCGTAGTGACACT---G-----GGC-----CCGC-GATTC-CTTGGAGCGGGTTGATGACGTC
AGCGTTCGAATTCC
>papio_anubis/1-254
GGGGA-AAGGTGGTACCATACAGAACTTATAAGATTCCCATACTCAAAGACATTTCACGATTATGGTGACTTCCCAG-AA
GACACAGCGACATGCAAATATTGTAGGCGGTCACACCCCCT----GTCCCTCACAGTCATCTTCCTGCCAGGGCGCACGC
GC------GCTGGGTGTTCTCCCGCGTAGTGACACT---G-----GGC-----CCGC-GATTC-CTTGGAGCGGGTTGATGACGTC
AGCGTTCGAATTCC
>gorilla_gorilla/1-254
GGGAA-AGGGTGGTCCCACACAGAACTTTATAAGACTCCCATATCCAAAGACATTTCACGGTTATGGTGATTTCCCAG-AA
CACATAGCGACATGTAAATATTTGCAGGGCGCCACTCC-CCA-----GTCCCTCACAGCCATCTTCCTGCCAGGGCGCACGC
GC------GCTGGGTGTTCCCGCCTAGTGACACT----G-----GGC-----CCGC-GATTC-CTTGGAGCCGGTTGATGACGTC
AGCGTTCGAATTCC
>homo_sapiens/1-254
GGGAA-AAAGTGGTCTCATACAGAACTTATAAGATTCCCAAATCCAAAGACATTTCACGTTTATGGTGATTTCCCAG-AA
CACATAGCGACATGCAAATATTGCAGGGCGCCACTCC-CCT-----GTCCCTCACAGCCATCTTCCTGCCAGGGCGCACGC
GC------GCTGGGTGTTCCCGCCTAGTGACACT----G-----GGC-----CCGC-GATTC-CTTGGAGCGGGTTGATGACGTC
AGCGTTCGAATTCC
>pan_troglodytes/1-254
GGGAA-AGGGTGGTGCCACACAGAACTTTATAAGATTCCCATATGCAAAGACATTTCACGTTTATGGTGATTTCCCAG-AA
CACATAGCGACATGCAAATATTGCAGGGCGCCACTCC-CCT-----GTCCCTCACTGCCATCTTCCTGCCAGGGCGCACGC
GC------GCTGGGTGTTCCCGCCTAGTGACACT----G-----GGC-----CCGC-GATTC-CTTGGAGCGGGTTGATGACGTC
AGCGTTCGAATTCC
```

FIG. 5(cont.)

```
>pongo_abelii/1-254
GAGAA-AGGGTGGTCCCGTCCAGAACTTATAAGATTCCCATACCCAAAGACATTTCACGTTTATGGTGACTTCCCAG-AA
TGCATAGCGACATGCAAATATTGCAGGGCGTCACTCC-CCT-----GTCCCTCACAGCCATCTTCCTGCCAGGGCGCCCGC
GC------GCTG-GTGTTCCCGCCTAGTGACACT-----G------GGC-------CCAC-GATTC-CTTGGAGCGGGTTGATGACGTC
AGCGCTCGTATTCC
>nomascus_leucogenys/1-254
CGCGA-AAAGTAGT----------AGACCTTTATAAGATTCCCAAACCCAAAGACATTTCTCGTTTATGGTGACTTCCCAG-AA
GACATAGCGACATGCAAATATTGCAGGGCGCCACTCC-CCT-----GTCCCTCACAGCCATCTTCCTGCCAGGGCGCACGC
GC------GCTGGGTGTTCCCGCCTAGTGACACT-----C------GGC-------CCGC-GATTC-CTTGGAGCGGGTTGATGACGTC
AGCGTTCGAATTCC
>tarsius_syrichta/1-254
GCGAG-AGGGTGGGTCCACACAGAGCTTATAAGGTTCACAAGT--AAAGATATTTCACGGTGACGGTGACTTCCCAC-AA
TACACTCCGACATGCAAATATAGCCGCGCGTGCCTCC-CCG-----ATCCCCGGAAGAGCGACTCCTAGCCACGTGCGCACGCC
GC------GCTGCGTGTTCGCGTCCTAGGTCGCT-----G------GGC-------CCGC-GGTTC-CTGGGAGCGGG-TGGTGACGTC
AGCGGCCCAGCTTC
>otolemur_garnettii/1-254
GCCTA-AAAGGGCGCTTGCACAGAATTTATAAGGTTCCCAAACAGAGACACATTTCATTATATTATGGTGACTTCCCAC-AA
TGCACAGCGCCATGCAAATATGCTAGGACGCCTCCCCC-CCG-----CTACCTTAAGGTCGTCAACTAACCAGTGCGCGCGC
GC------ACTGCCGCGTTTCCCGCCGGTGACTCA------A------TGC-------CCGC-GTTTG-GTCGCAGCTAGTTGGTGACCTC
AGTTCTGGAGGCTC
>tupaia_belangeri/1-254
GGGGG-AAGCTGGGTCCACTGAGTTCTTATAAGGTTTCCAGTCCTAGAGCGATTTTACCATTGCGGTGATTTCCCAG-CA
TCCGTAGCTACATGCAAATAGCGCGGGGCGCGTCTCT-CAG-----GTCCCTCCCCGCCCTCTCACTGT-------ACGTACCC
GC------GT----------CCTAGGGACGCC-----G------CGC-------CCGG-GGTTC-CC----------GGACGTC
AGCGTTCCGACGCA
>ailuropoda_melanoleuca/1-254
AGGGA-AAGCCGCGCCTGGGGCGGATTTATAAGGCTTCCATATCTAAAGGCATTTCACAGTCATGGTGACTTCCCAC-AA
TACATAGCAACATGCAAATATCGCGGGGAGAACCTCC-CCT-----GTCCCTTGTACGCGGCTTC-----TAAAGACGCACGC
GC------GCTCTGTGTTCCCGCCCTGTGACTCT-----A------GGC-------GCGC-AATTC-CTGGGACAGTGTT-CTGACGGG
AACGTTCAGGCTCC
>mustela_putorius_furo/1-254
GCGAA-AGGGTGGACCCACCGAGCATTTTATAAGGCTCCCGCATCTAAAGACATTTTACAGTTATGGTGACTTCCCAC-AA
CGCGTAGCAACATGCAAATATCGTGGAGAGTACCGCC-CCT-----GTCCCATGCACGCGTCTTCTTCAGCAGCACGCACGCC
GC------GCT--GTGTTCCCGCCCTGTGACTCC---A------GGC-------GGGT--ATTT-CCAGGGGCGGGTTGCTGACAGG
AACGTTCAGGCTTC
```

FIG. 5(cont.)

```
>canis_familiaris/1-254
GC-----AGCGCAGCCCCTCTC-GCCGCTTATAAAGTGCCG--CCCGCACGGCCCTTCTCGCTCACGGCGACTTCCCAT-AA
CACACAGCAGCATGCAAATACCGCGCGGGAGCCCCGCC-CCGCCCCCGGCCCCCGCA---------CCGCCTCGGGACGCATGC
GCCCGCTCT--CCGTTCCCGCCTTGGGCCGGCGGGCGGGGC----GGGC-GAGCGGGCCGGAGCGGCTC--CGGCGGG
GACGAGCGGGGCGCC
>felis_catus/1-254
GGCAA-AGGGTGGCCCCGCCGAGCATTTATAAGACTCCCATACCTAAAGACATTTCTCAGTTATGGTGATTTCCCAC-AA
CACACAGCAACATGCAAATATCGAGCGGTGTACCGCC-CCT-----GTCCTTTGTAGACGTCTTCTCCAGGACGCACCGC
GC-----GCT--GTATTCCCGCCTTGTGACTCT----A-------GGC----GGGC-GATTC-CTGGGAGAGGGTTGATGACGTC
CAAGTTCTGGCTTC
>equus_caballus/1-254
GGGCG-AAAACAGCCCATGCCTGCATTTATAAGACTCACAGATCTAAAGCCATTTCACGAATAGGGTGACTTCCCAC-AA
TACACAGCGACATGCAAACATAGCGGGGCGTGCCTTT-CCT-----GTACCCTGTGTGGGCA----TCTCTCCTGGACGCACGC
GC-----GCCGGGTGTTCCCGCGCTGTGACTCT-----A-------GGC----------AAGC-GCTTC-CTGGGAGAGGTTGATGACGGC
AGCATTCCGGCTCC
>myotis_lucifugus/1-254
CGGAG-AAGGAGC-----CGTAGAGGATATATAAGGCCCCCTTATGTGTAGTCCTTTTACGGTTAGGGTGACTTCCCAC-AA
CGCATAGCGACATGCAAAT-TTGACGGGCGTGCCTCC-TCT-----GTCCCT-GCGGGCAACTTCTCCTCCTGGACGCGCGC
GC-----GCTGCGTGTTCCCGCCTTTTGACTCC----A-------GCCC----GAGC-GAATC-CTGGGAGAGGGCAGGTGACGTC
AACAGTCAGGCTCG
>pteropus_vampyrus/1-254
GCGAG-AAAAATTCTTCACGCAGAATATATAAGGATCCCATATCTGAAGACATTTTACGATTACGGCGATTTCCCAC-AA
CACATAGCGACATGTAAATGTAGTGGGGCATGCCTCC-CCT-----GTCCCTTGTGGGCAGCTTCTCGCCAGAACGCACGC
GC-----GGTGCGTGTTCCCGCCTTTGTG--ACT-----A-------AGT----TGGC-GAGTC-A-GGGAGGAGATTGATGACGTC
AGCTCACCCCGCTCC
>bos_taurus/1-254
GGCAA-ACACCGCACGCAAATAGCACTTATAATGTGCTCATACCTAGAGCCACTTTTCGGTTACGGTGACTTCTCAAAAA
GACAGTGGAACATGCAAATATTACAGTGCGTCCCGCC-CC-----------------TGGTAGGTCTACGCTAGGACGCACGC
GC-----ACTACG-GTTCCCCGCCTATAGACT-------G-------CGC-----TGGC-GATTC-CTGGGAGCCGGACTGATGACGTC
AGCGTTCCGGGATCC
>ovis_aries/1-254
GGCGA-ACAATGCGCGCAAACAGCATTTATAATGAGCTCATACCTAAAGCCACTTTTACGGTTACGGTGACTTCCCAC-AA
GACATTGCGGCATGCAAATATTTTAGTGCGTCCCGCC-CC-----------------TGGTAGTTCCACGCTAGGACGCACAC
GC-----ACTACG-GTTCCCGCCTTTAGACT-------G-------CGC-----TGGC-GATTC-CA-GGAGCGGACTGATGACGTC
AGCGTTGGGGCTCC
>tursiops_truncatus/1-254
GCGCA-AAACCAGGCTCAAGCCACATTTATAAGGCTCCCAAATCTAAGTACATTTGTCGGTTATGGTGACTTCCCGC-AC
CACATTGCGACATGCAAATACTGCGGAGCGTCCCTCC-CCT-----GG-----------------CAACTCCTCGCTGGGACGCACGC
```

FIG. 5(cont.)

```
GC------GCTACGTGCTCCCGCCTTTTG--ACT----G-----CGC-----CGGC-GATAC-TTGGGAGAGGGTTGATGACGTC
AGCGTTCTGGCTCC
>vicugna_pacos/1-254
GGGAA-AGGGTGGGCTCACCGCAGCCTTTATAAGACTCCCAAACTTAAAGACATTTCTCGGTTATGGCGACTTCCCAC-AA
GACATAGCGACATGCAAATACTGCAGGGCGCCG--AC-CCG-----GTCCTGTGCAGCCATCTTTCGGCTGGGACGCACGC
GC------GCTGCGTGTTCCCGCCCTGTG--ACT----G-----CGC-----CGGC-GATTA-CTGGGAGAGGATTGATGACGTC
AACGTTCGGGTTCC
>sus_scrofa/1-254
GTAGGAAAACTGCT-TCTGTGAGCACTTATAAAACTCCCATAAGTAGAGAGATTTCATAGTTATGGTGATTTCCCAT-AA
GACATTGCGACATGCAAATATTGTGGCGCGTTCGTCC-CC-------GTCCGGTGCAGGCAGCTTCGCTCCCAGGACGCACGC
GC------AATACATGTTCCCGCCTTGAGACT-------G-----CGC-----CGGCAGATTC-CTAGGAAGTGGTTGATGACGTC
GATGTTAGGGATCC
>erinaceus_europaeus/1-254
GCCTA-AACCGGCTCTTTCGACAGACTTATAAGGACCTCTTATCTTTAGGACATTTTTTGTTAGGGTAACTTCCCAC-GA
TGCATAGCGATATGTAAAATAT------GGCGCCGCGA-----------GTCTCTCCTAGGCGTCTCC-----CCAGGACGCAGGC
GC------ACTGCTTGTTCCCGCGT--TAACATT-----------------GC-TGATT-CTGGGAGACTGCTGATGACGTC
AGCGTCCAGTCTAC
>choloepus_hoffmanni/1-254
AGAAA-AAAATAGT-TTATGCTGGATTTATAAGATTCCCAAATCTAAAGCCATTTCACAGTTACGGTGATTCCCCAC-TA
CACACGGCGATATGCAAATATAGCGGAA-----------GT-----GTTCCTGAGG-----------CGTGGTAAAGCGCGCGC
GC------GCTGAGAGTTCCCGCCCTGTGGTGCT----G-----GGC-----TGGA-GATGC-CTGAGAACTGGCTGATGACGGC
AACGTTCGGGCTCC
>dasypus_novemcinctus/1-254
AAAGC-GATAGTTTTTTTAAACTGGACTTATAAGGCACCCATATCTACGTATATTTCATGGTTAGGGTGATTTCCCAC-AA
CACATAGCGAAATGCAAATATTGGAGGCGCCTG-----------------------AGGCGTGGTC-----GGGCGCAAGC
GC------GCTGCGACTTCCCGCCTTTCGGCCCT----A-----GGC-----CCCA-GATTC-CTGGGAGCTGGATGATGACGTT
GACGTTCGGATACC
>loxodonta_africana/1-254
GGGAA-GCAACAAATTCGT-CAGGATTTATAAGACTCTCAGAGCTGTAGACATTTCACAGTTAGGGCGATGTCCCAC-AA
TACATAGCAACATGCAAATATTCTAGGAGGCCAGCCT-CCC-----CGTCCGCGGTGGTCATCTTCTCGCTAGGGCGCACGC
CC------GCTGCGTGTTCCCGCTCTGTG--ACC----A-----GGC-----AGGC-GATTC-CTGAGAACCGCTTGGTGACGTC
AGTGTTCTGGCTCC
>procavia_capensis/1-254
AGGGT-AAATCGGCGCTGCTCAGCATTTAAAAGAATCCCAAATGTGTCGCCATTTTACGCTTAGGGTGATATCCCAC-AA
GACACAGCGACATGCAAATATCGTGAGTCTCTGTTTC-CCT-----GTCCA-CGAGGGCGTCCTCTCGCTGTGGGCGCACGC
GC------GGTGTGTGTGCCCCCGTTGTG-----T-----G-----TTC-----CCGC-GATTC-CAAAGAACTGGTTGATAACGTT
AGACTTCCGGCTGC
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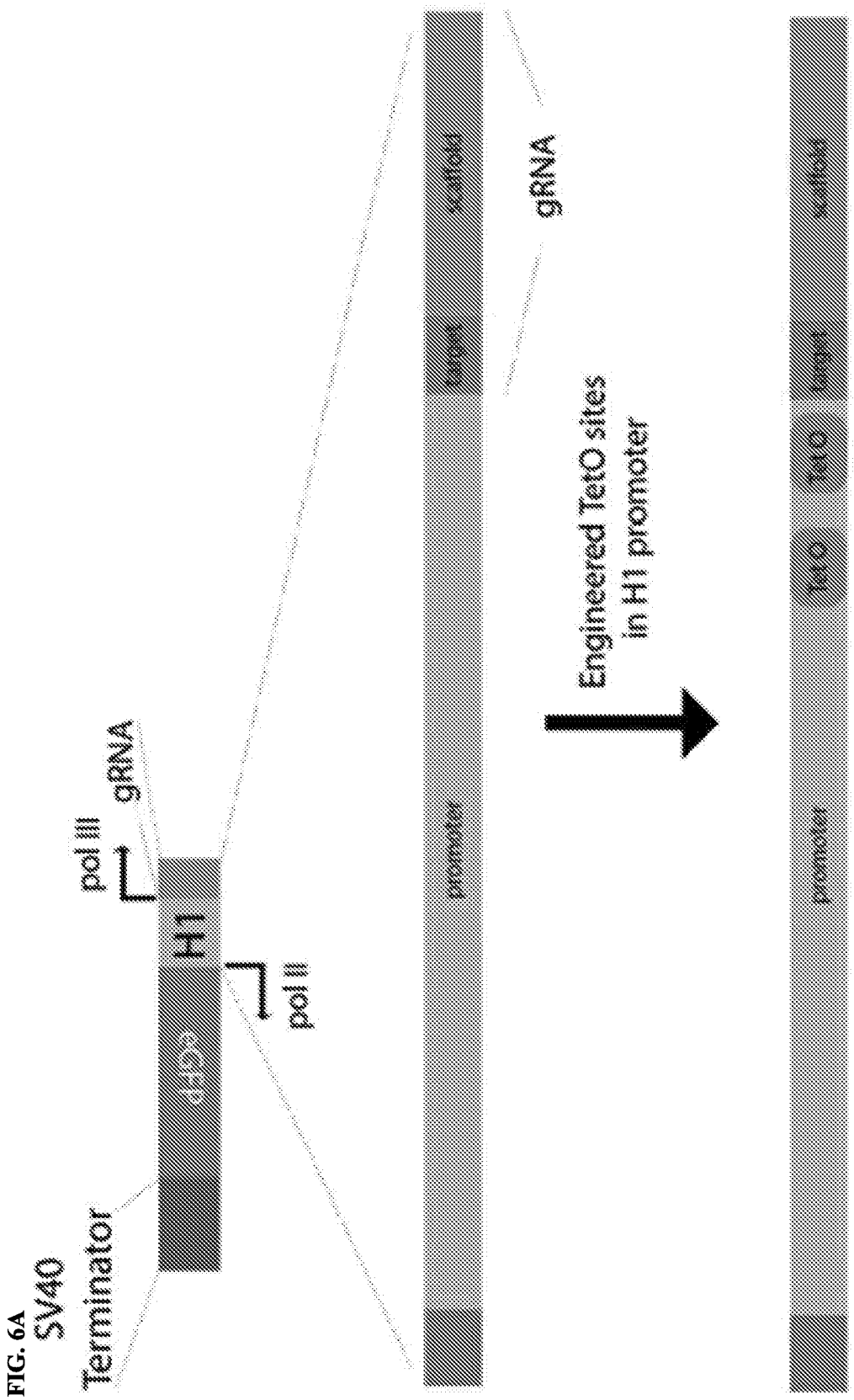

SV40
Terminator
pol III
gRNA
H1
pol II
promoter
Target
gRNA
Engineered TetO sites
in H1 promoter
TetO
TetO
Target
promoter

FIG. 6A

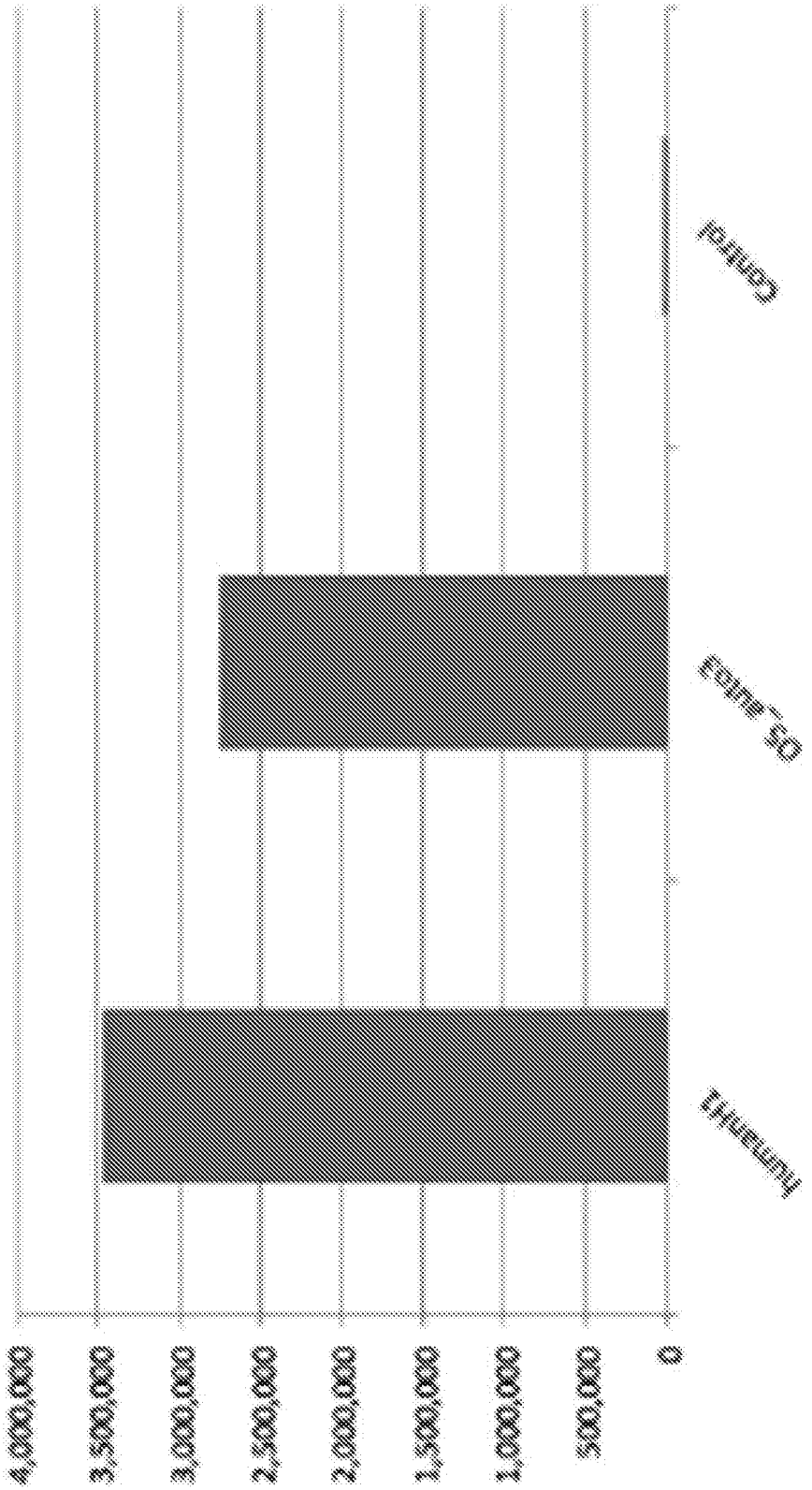
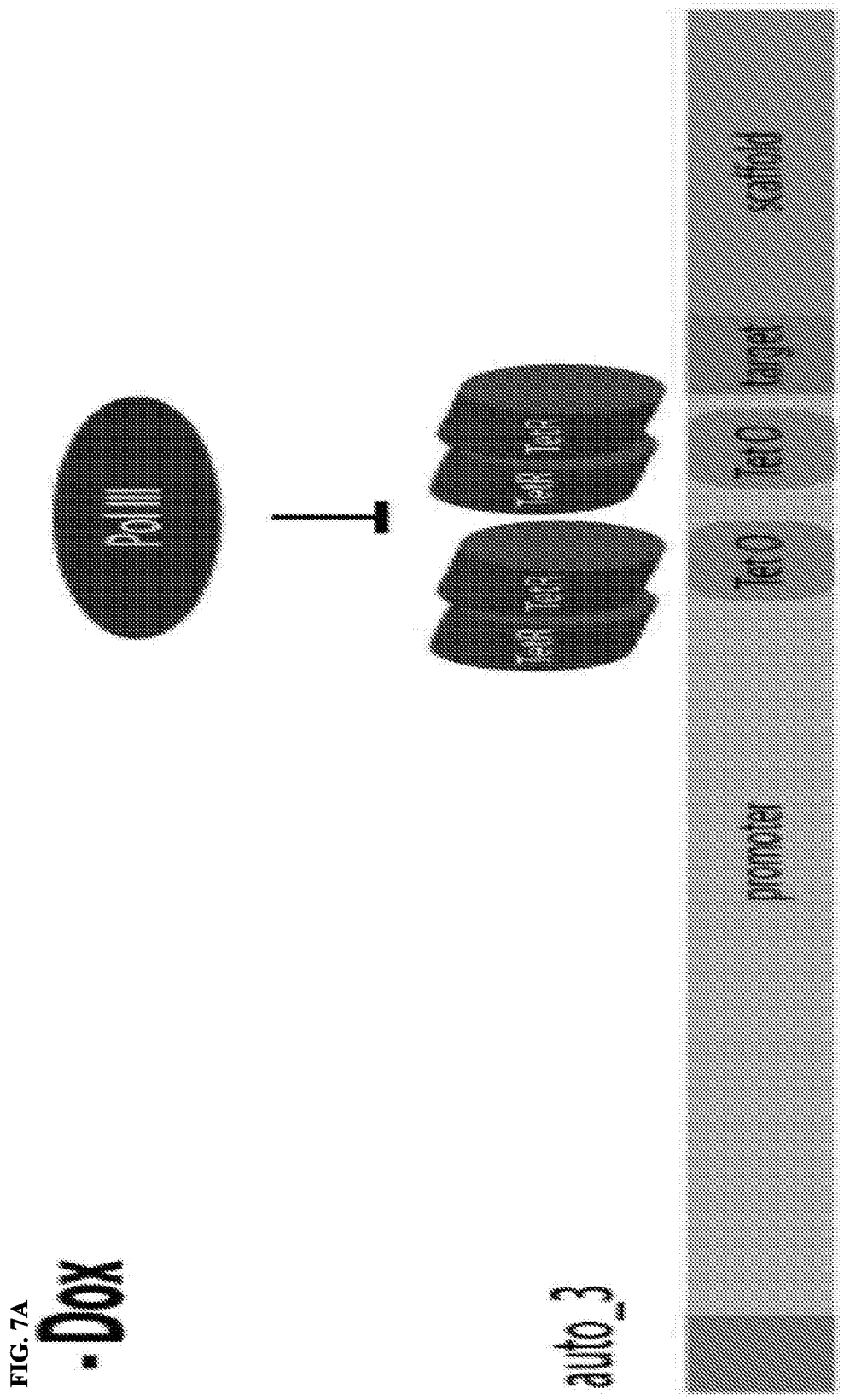

Pol II expression from modified H1 bidirectional promoter
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
Control
Core 50
Human H1

FIG. 6B


-Dox
FIG. 7A
auto.3
promoter
TetO
TetO
target
control
PolIII
Exp. TAR
Exp. TAR

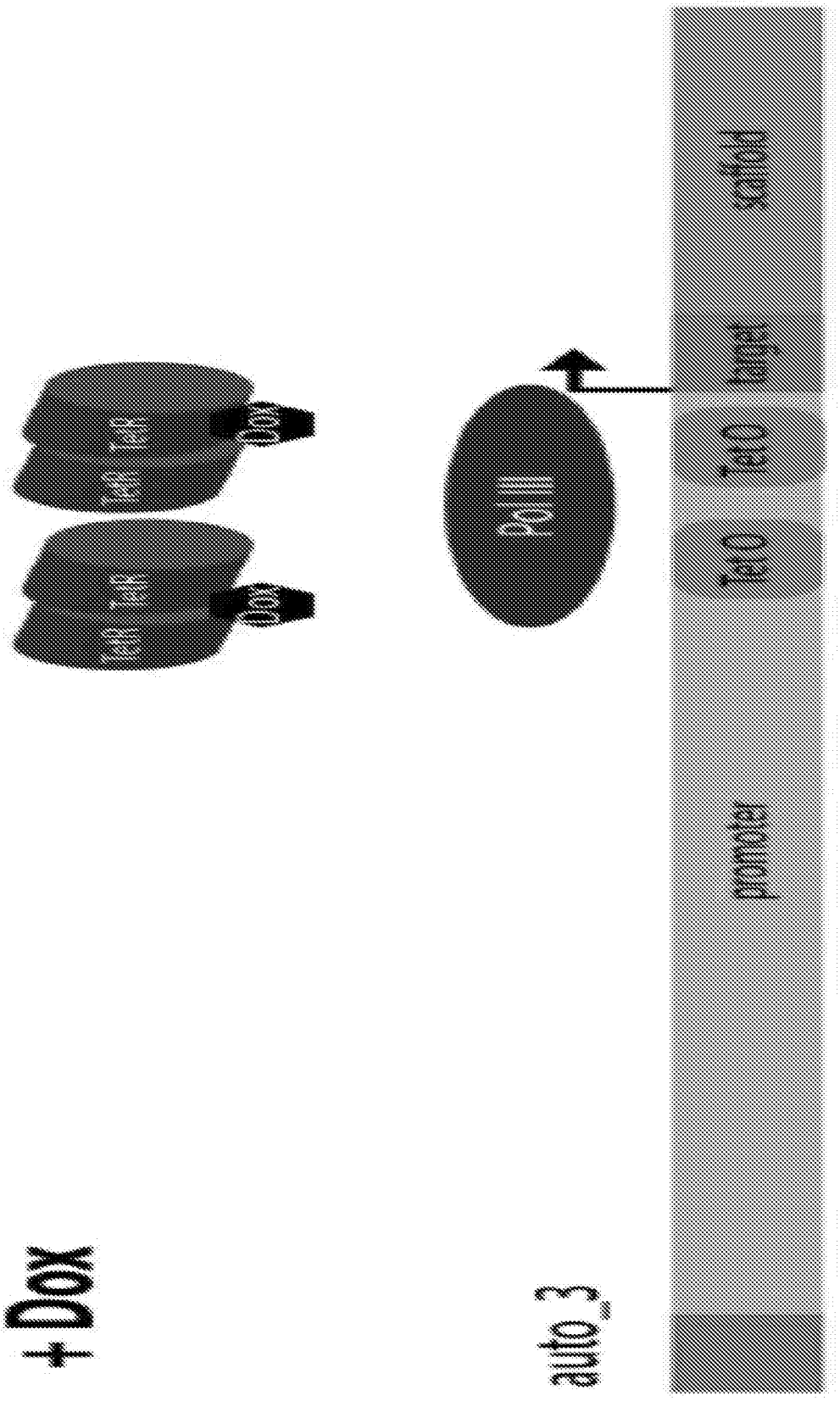

+Dox
auto_3
promoter
TetO
TetO
tagaf
gal4dbd
PolII
rtTA
Dox
rtTA
Dox

FIG. 7B

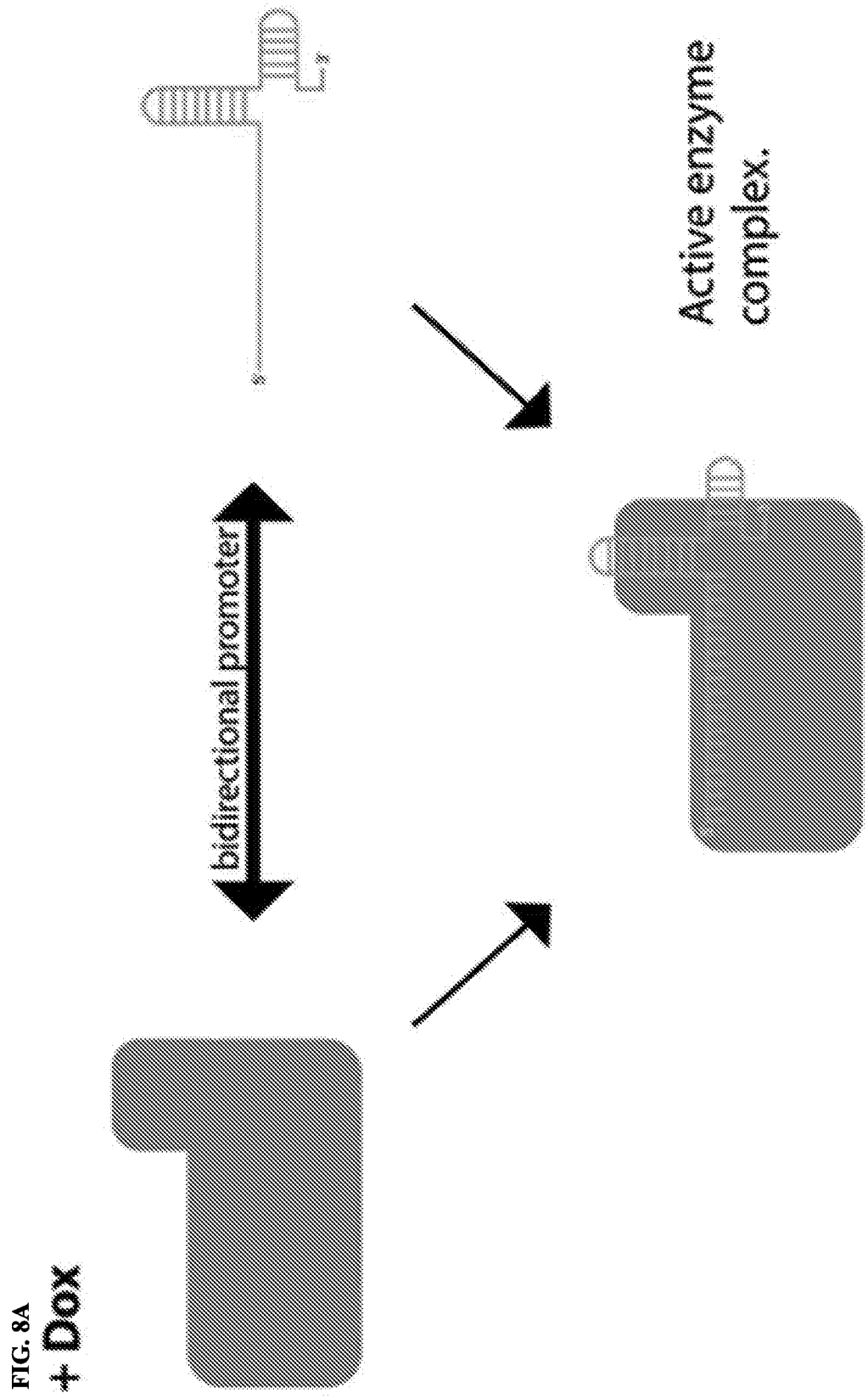


FIG. 8A

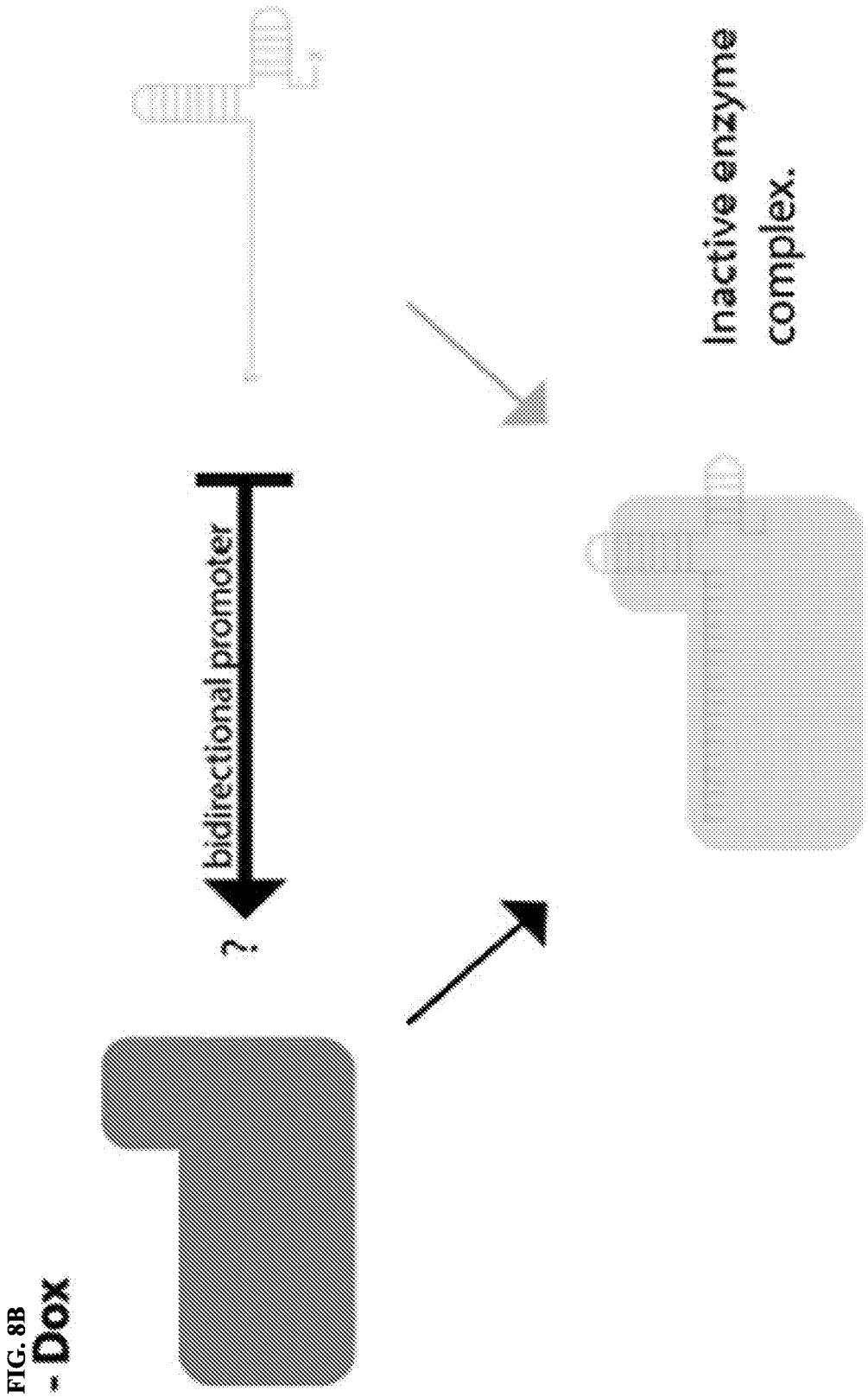


FIG. 8B

FIG. 9A

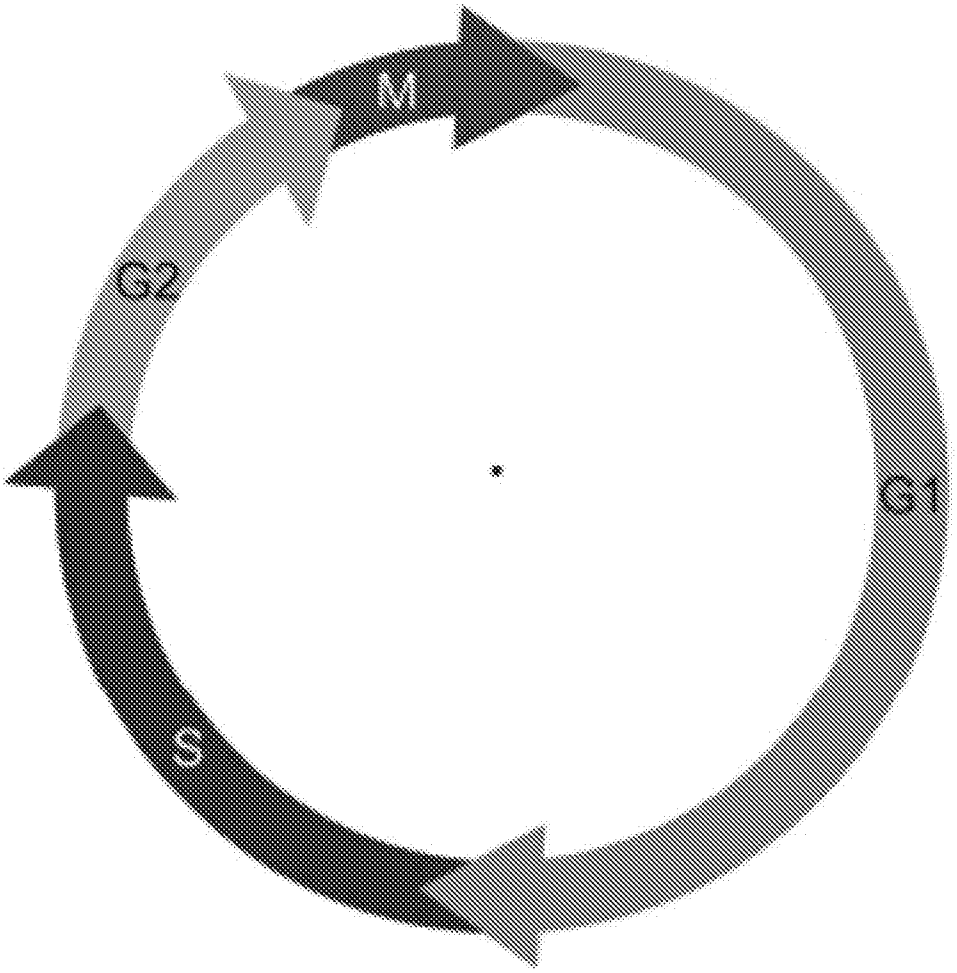
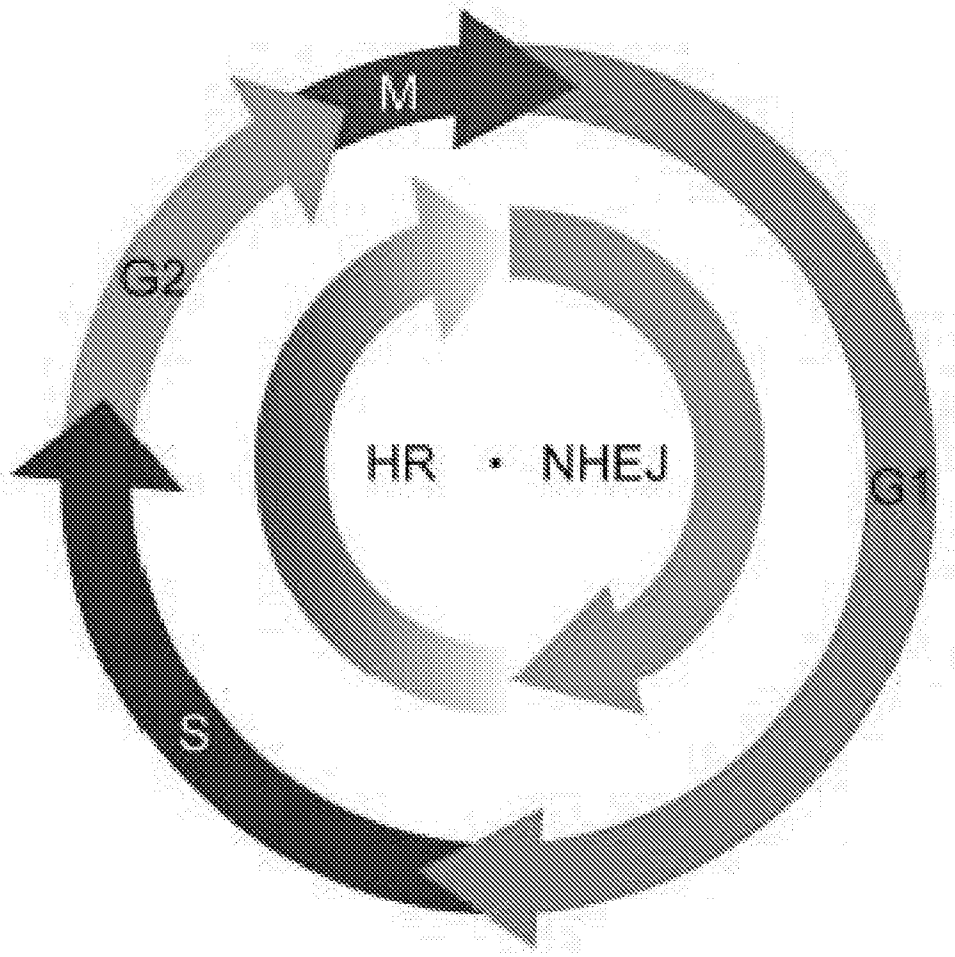


FIG. 9B



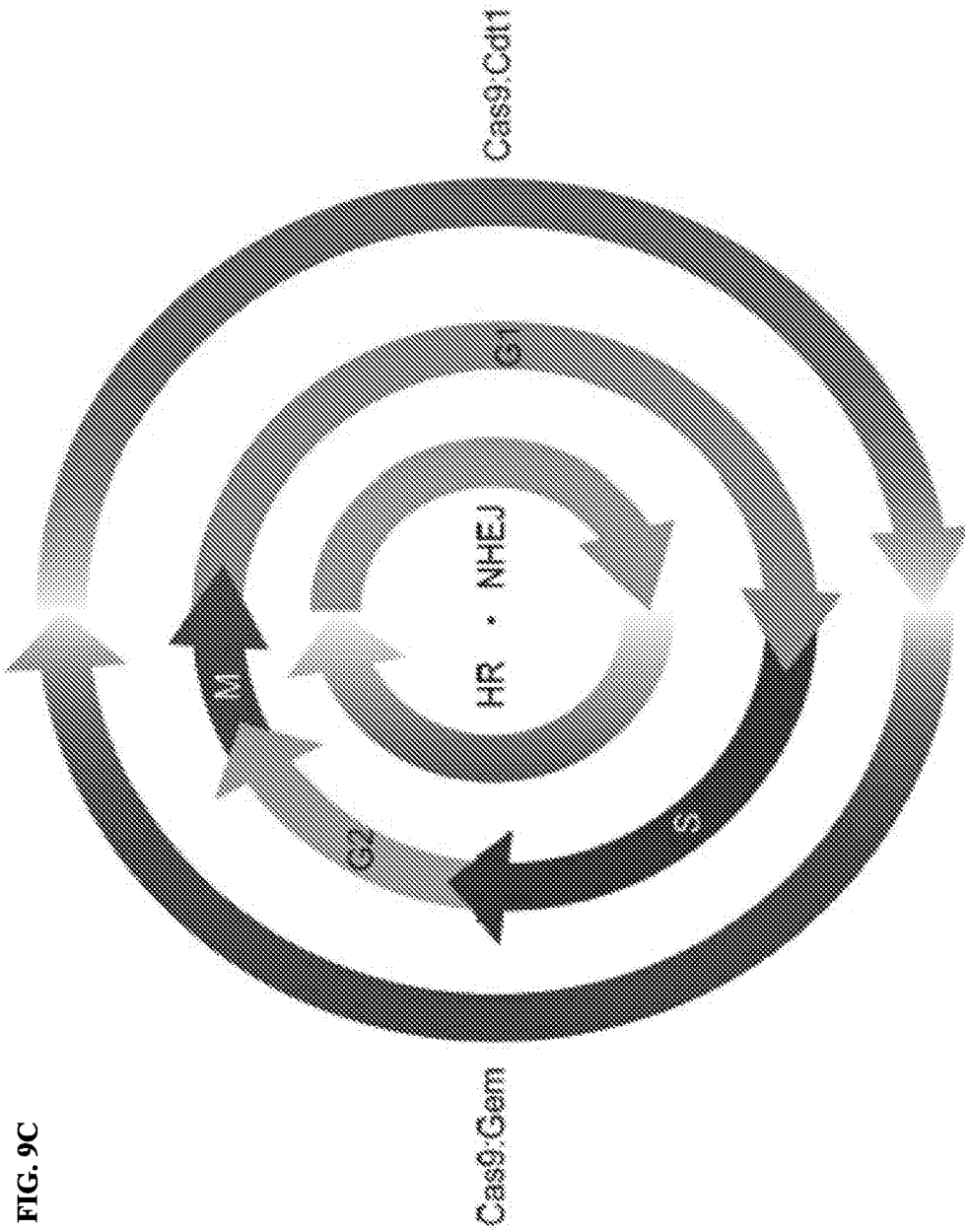


FIG. 9C

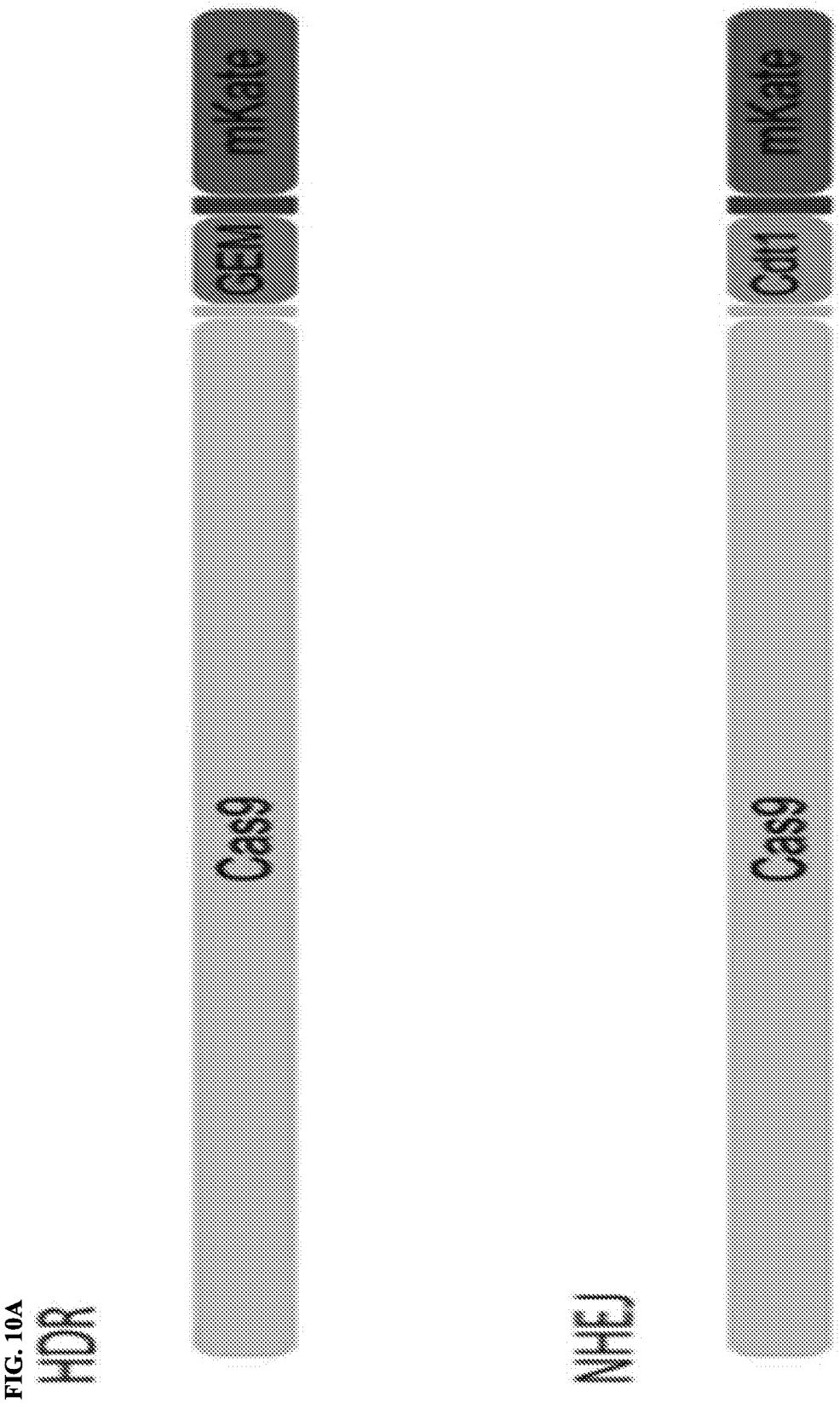


FIG. 10A

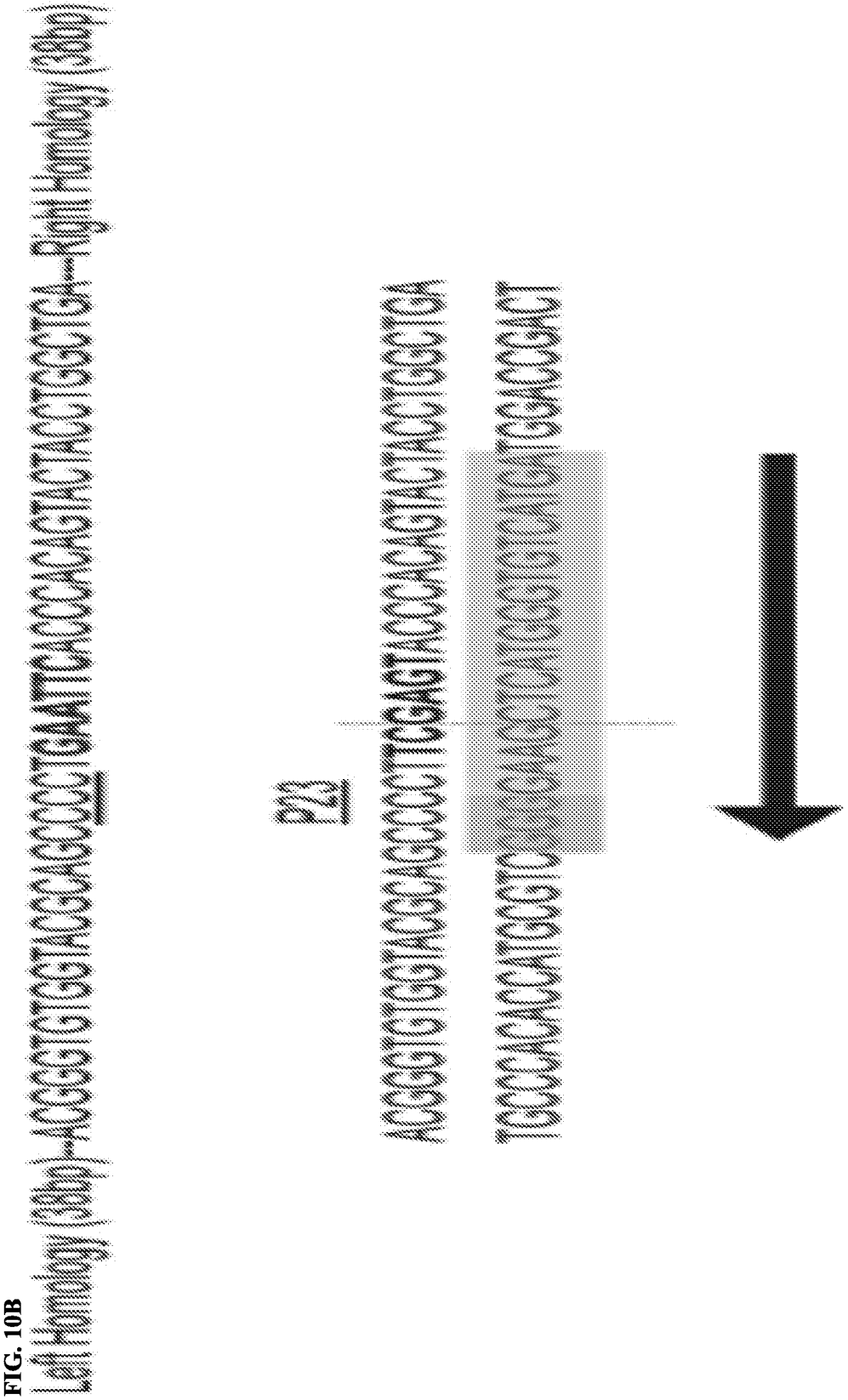

Left Homology (30bp)-ACGGGTGTGGTACGCAGCGCCCTGAATTCACCAAGTACTACCTGGCTGA-Right Homology (30bp)
P23
ACGGGTGTGGTACGCAGCGCCCTTCGAGTACCAAGTACTACCTGGCTGA
TGCCCACACCATGGGTCGAAGCTTAAGTGGTTCATGATGGACCGACT

FIG. 10B

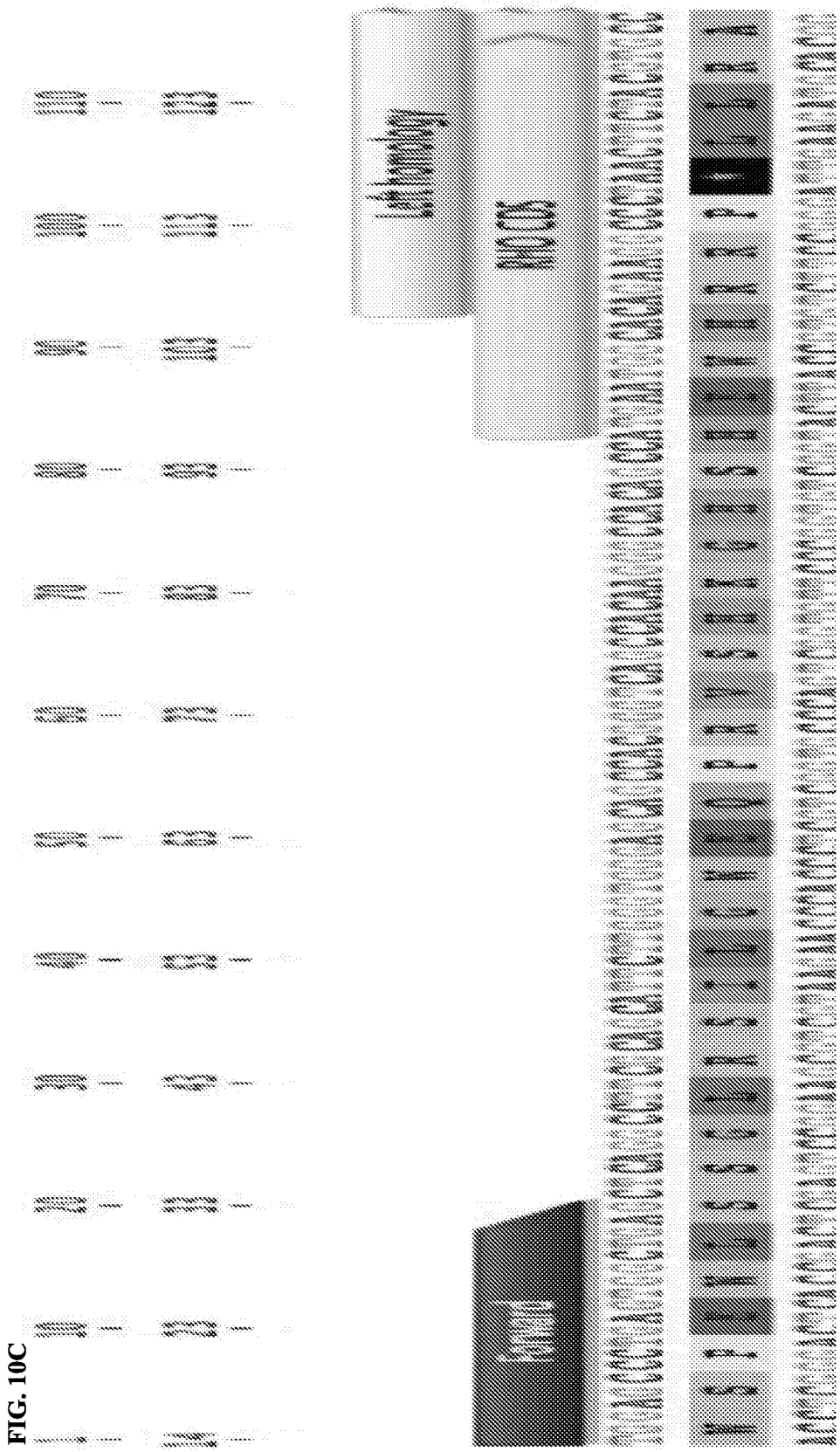


FIG. 10C

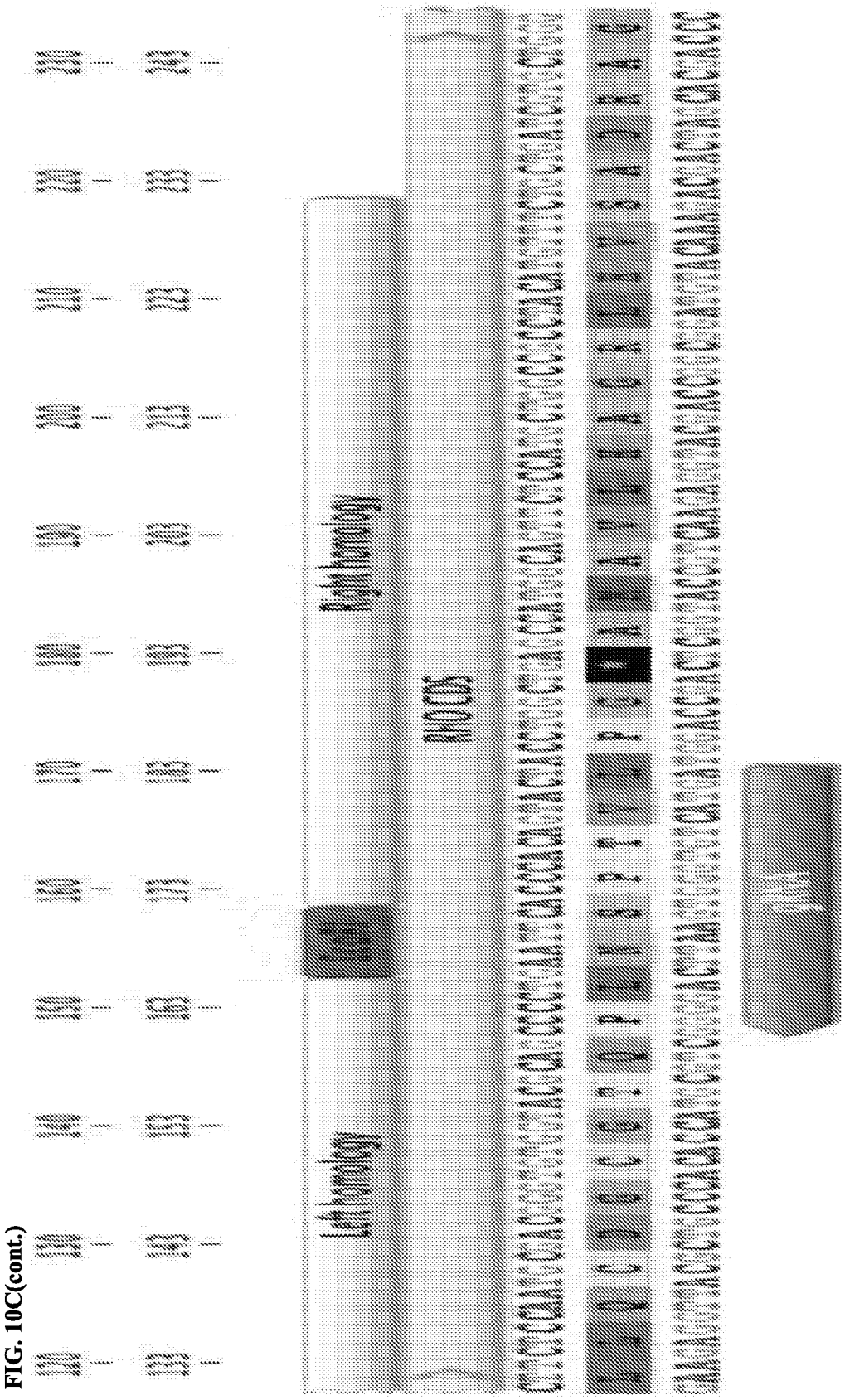


FIG. 10C(cont.)

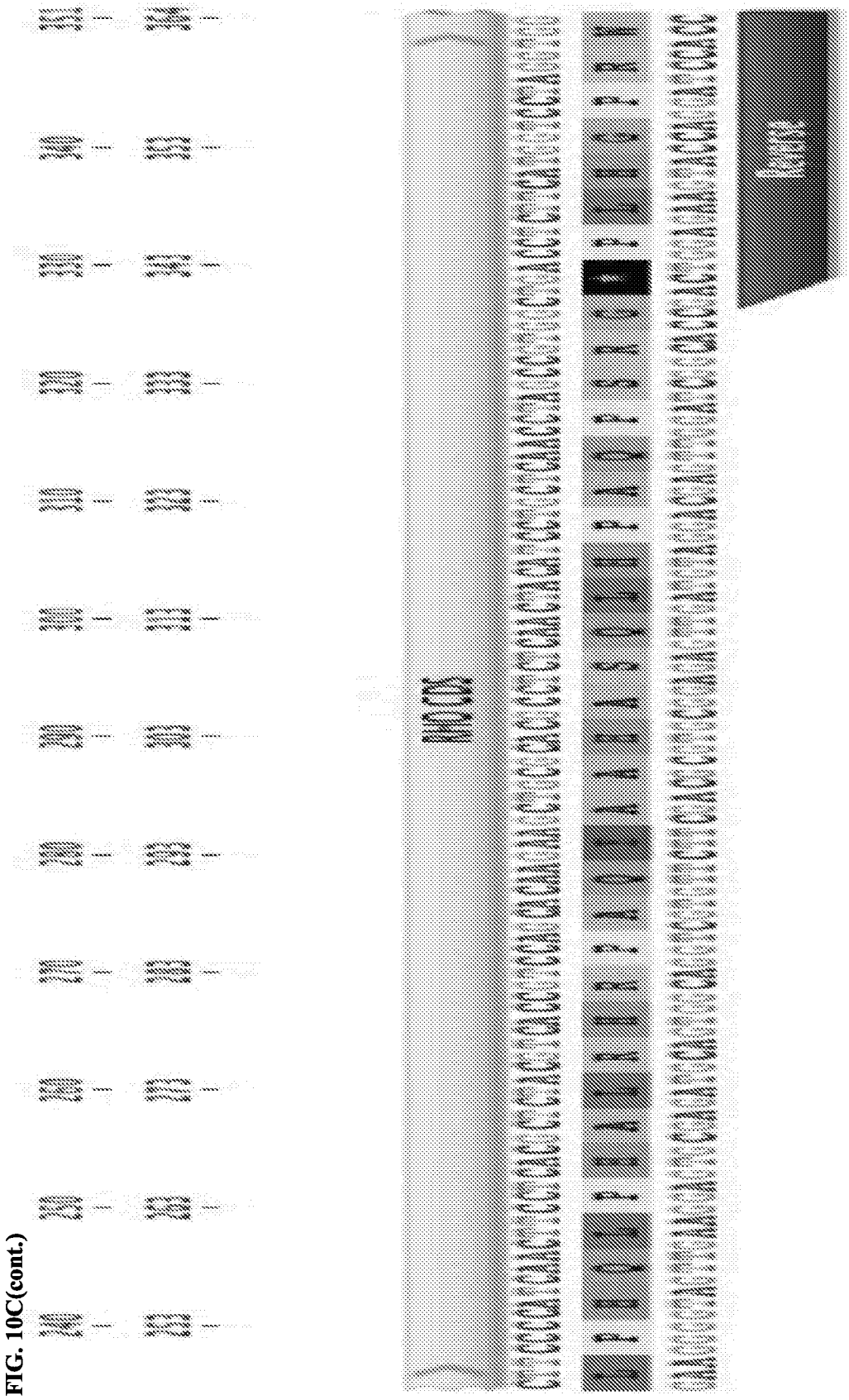


FIG. 10C(cont.)

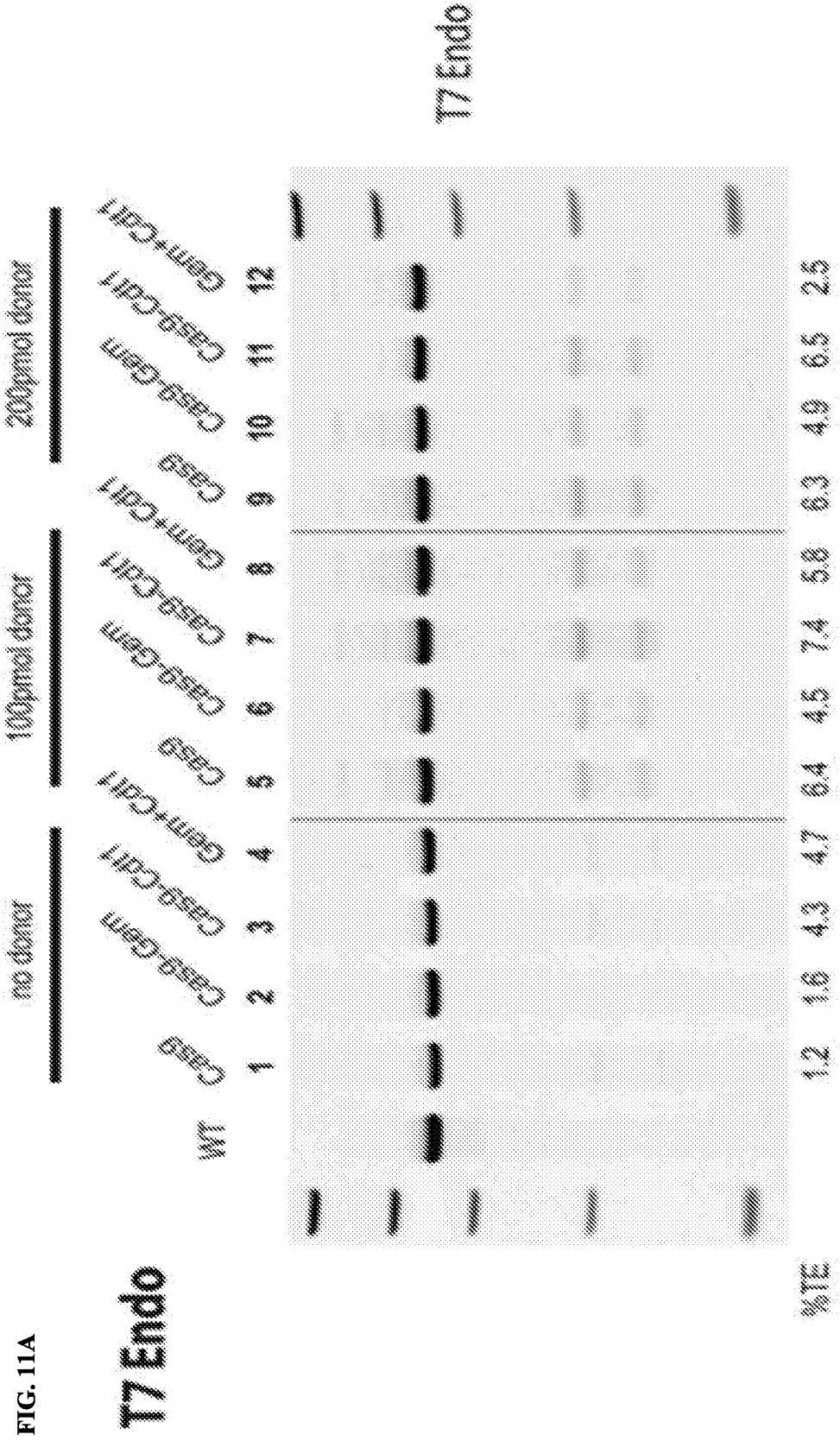

T7 Endo
no donor
100pmol donor
200pmol donor
WT
1
2
3
4
6
6
7
8
6
10
11
12
Cas9
Cas9-Gem
Cas9-Cdt1
Gem+Cdt1
Cas9
Cas9-Gem
Cas9-Cdt1
Gem+Cdt1
Cas9
Cas9-Gem
Cas9-Cdt1
Gem+Cdt1
1.2
1.6
4.3
4.7
6.4
4.5
7.4
5.8
6.3
4.9
6.5
2.5
%TE
T7 Endo

FIG. 11A

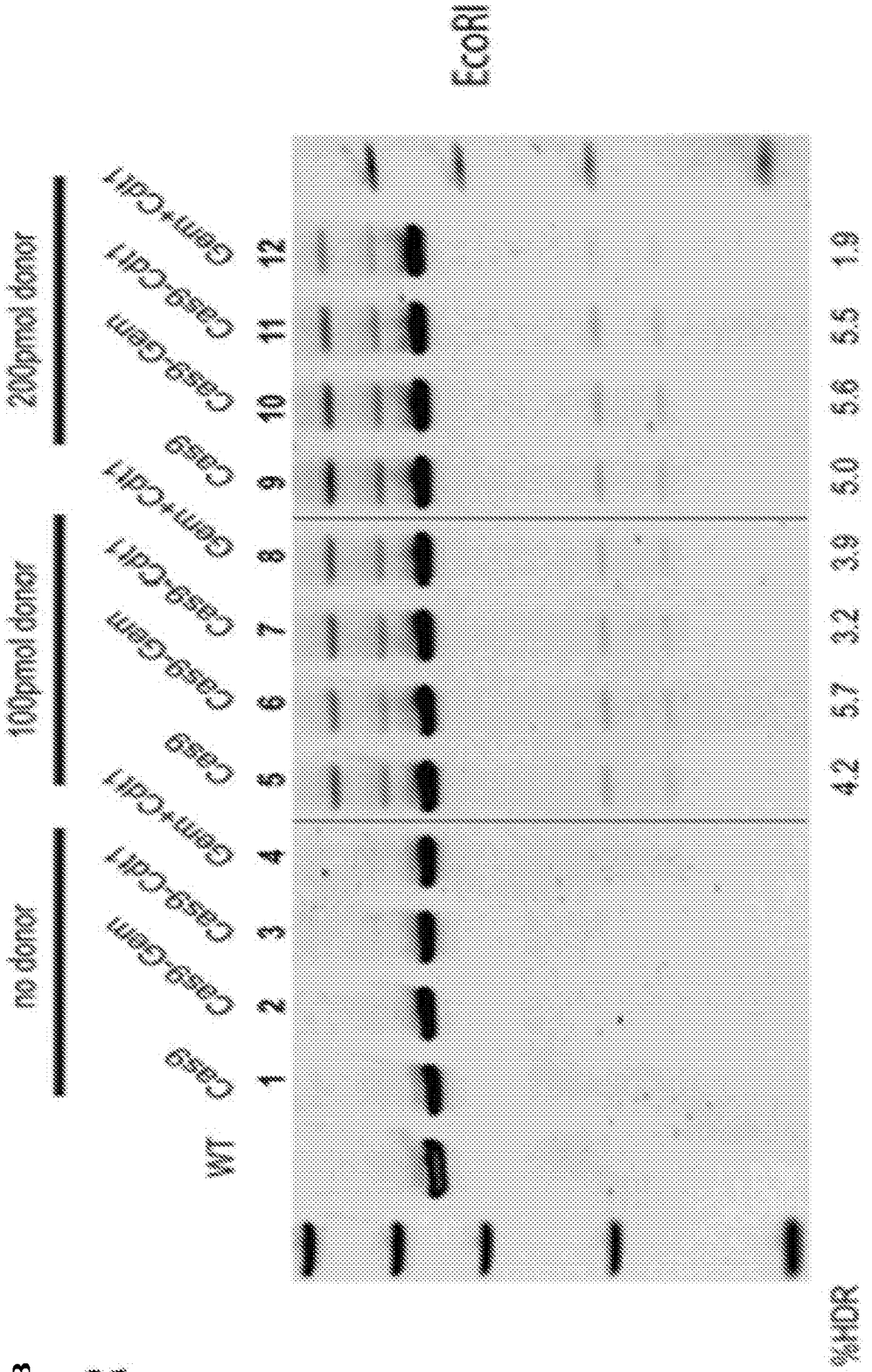

HDR
EcoRI
WT
1
Cas9
2
Cas9-Gem
3
Cas9-Cdt1
4
Gem+Cdt1
5
Cas9
6
Cas9-Gem
7
Cas9-Cdt1
8
Gem+Cdt1
9
Cas9
10
Cas9-Gem
11
Cas9-Cdt1
12
Gem+Cdt1
no donor
100pmol donor
200pmol donor
4.2
5.7
3.2
3.9
5.0
5.6
5.5
1.9
%HDR

FIG. 11B

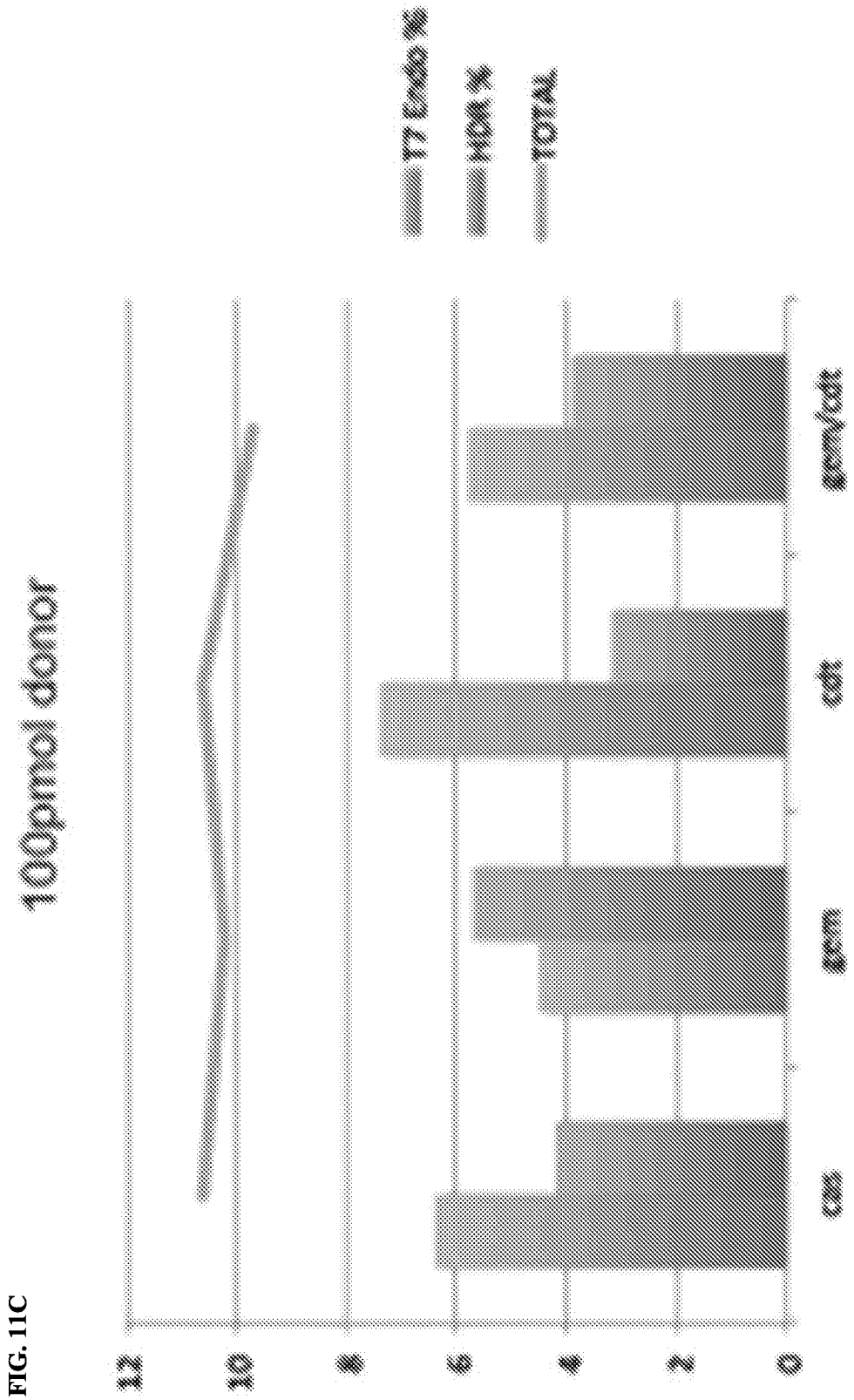

100pmol donor
T7 Endo %
HDR %
TOTAL
12
10
8
6
4
2
0
cas
gcm
crh
gcm/crh

FIG. 11C

FIG. 12A

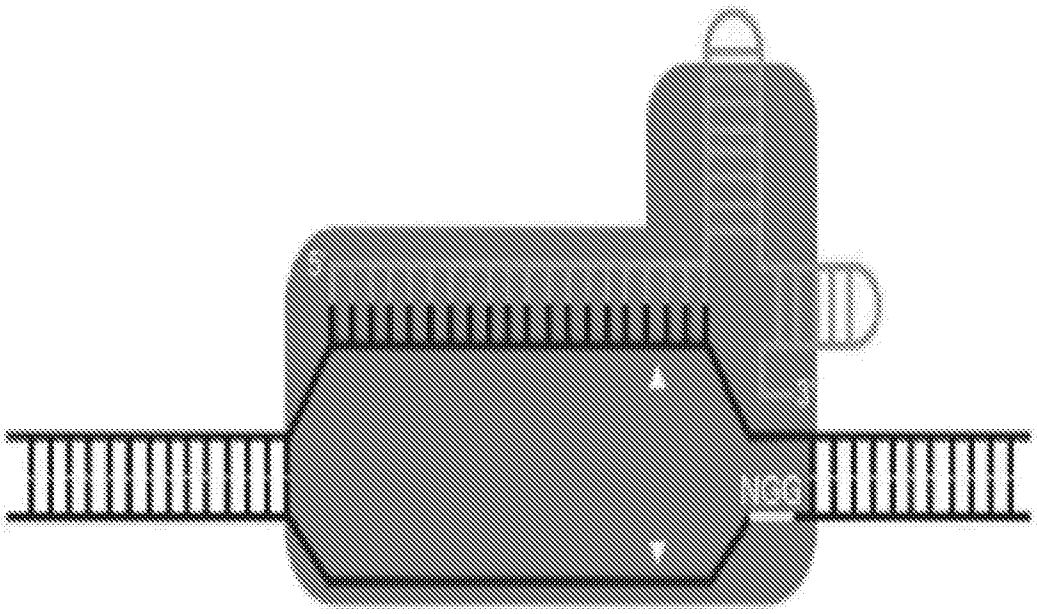


FIG. 12B

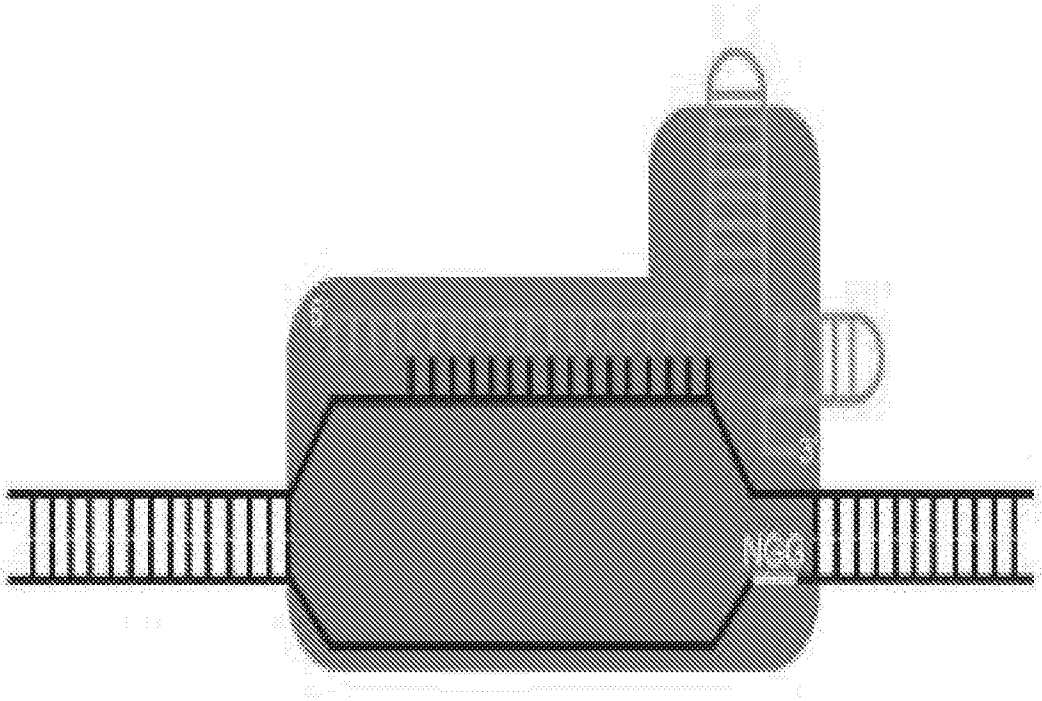


FIG. 13A

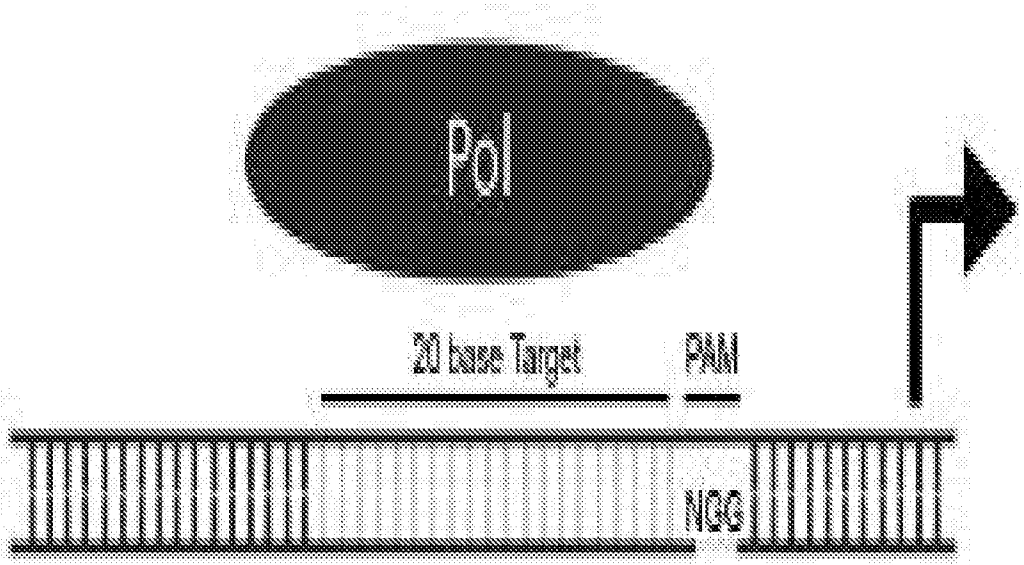


FIG. 13B

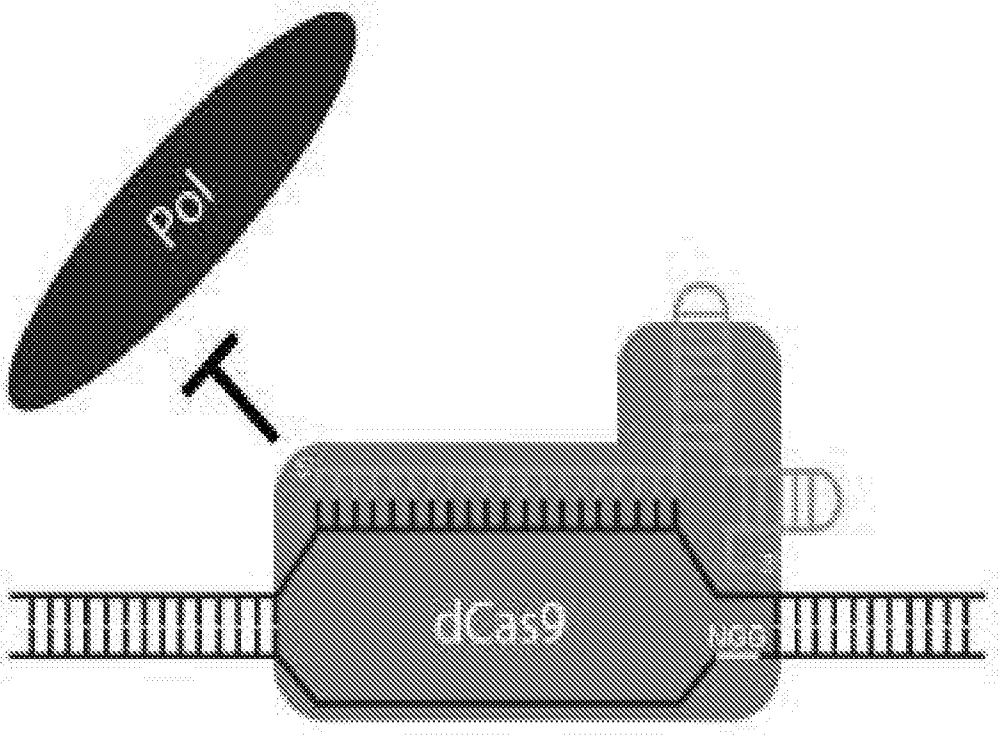
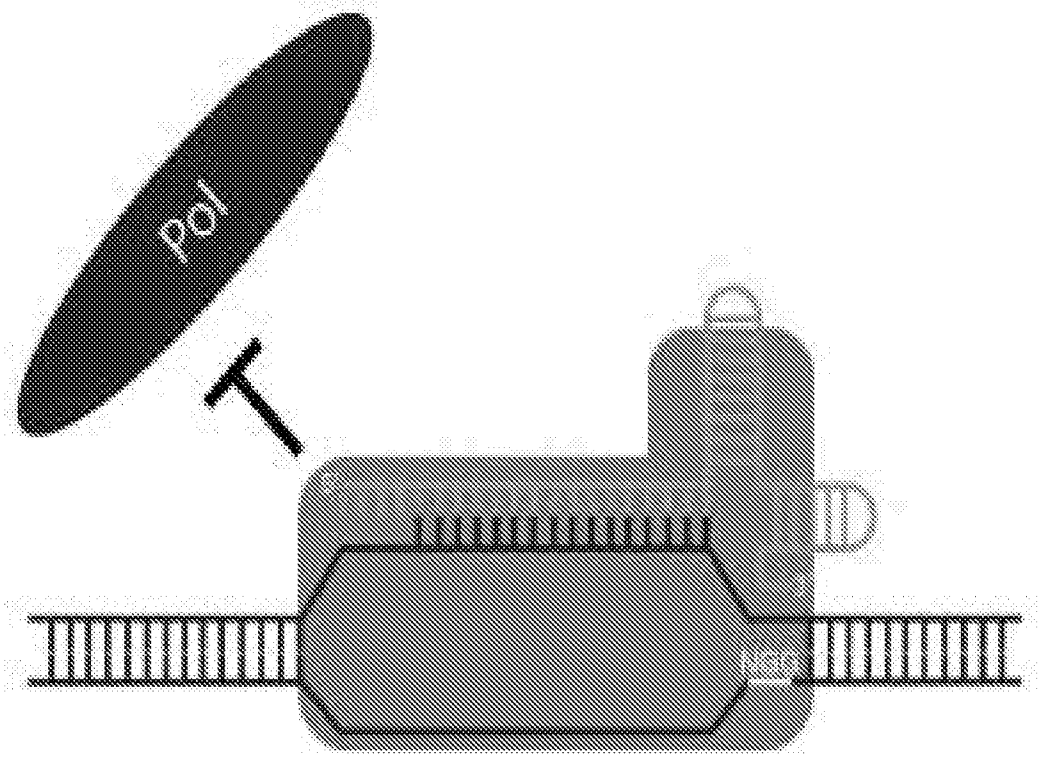


FIG. 13C



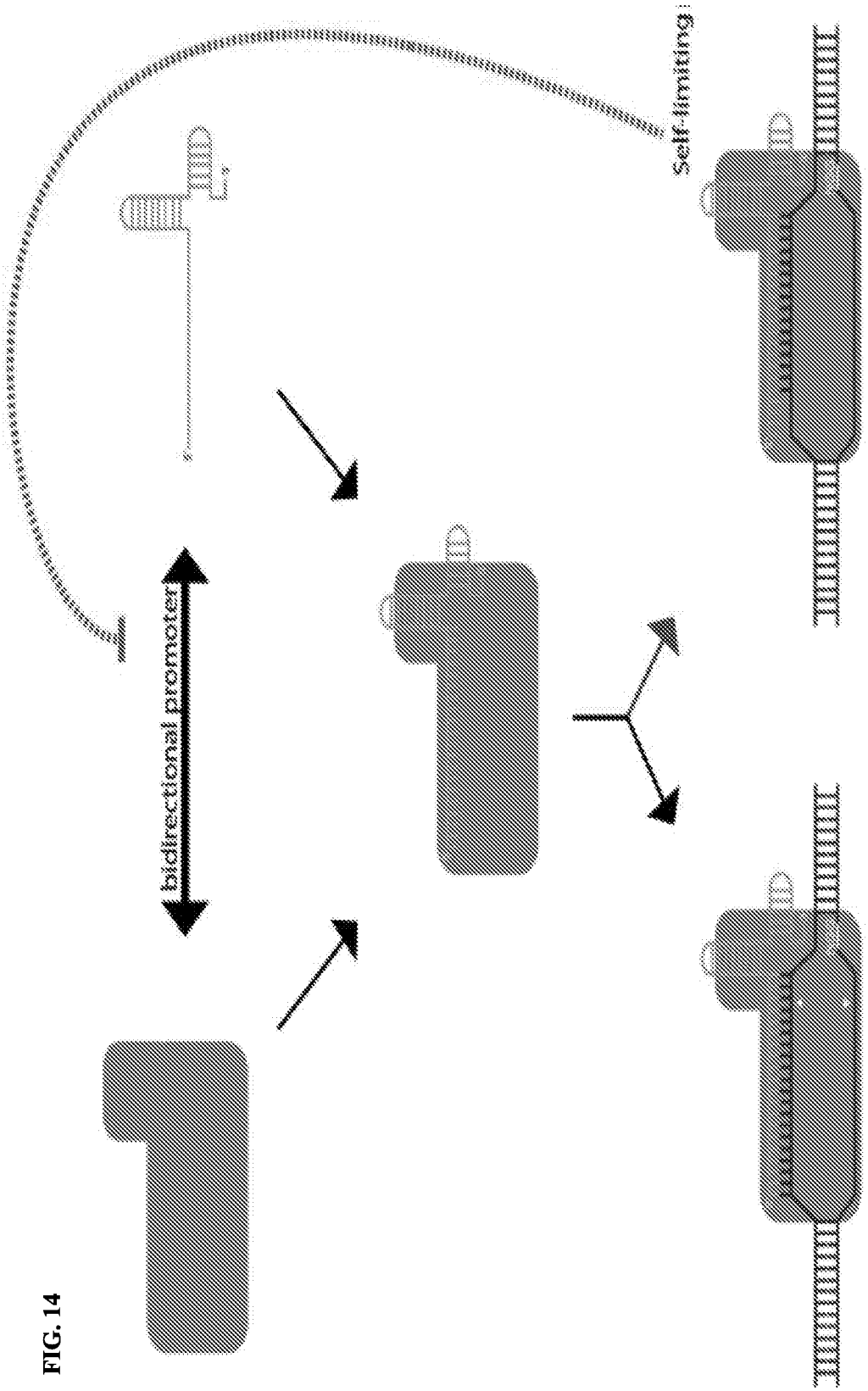


FIG. 14

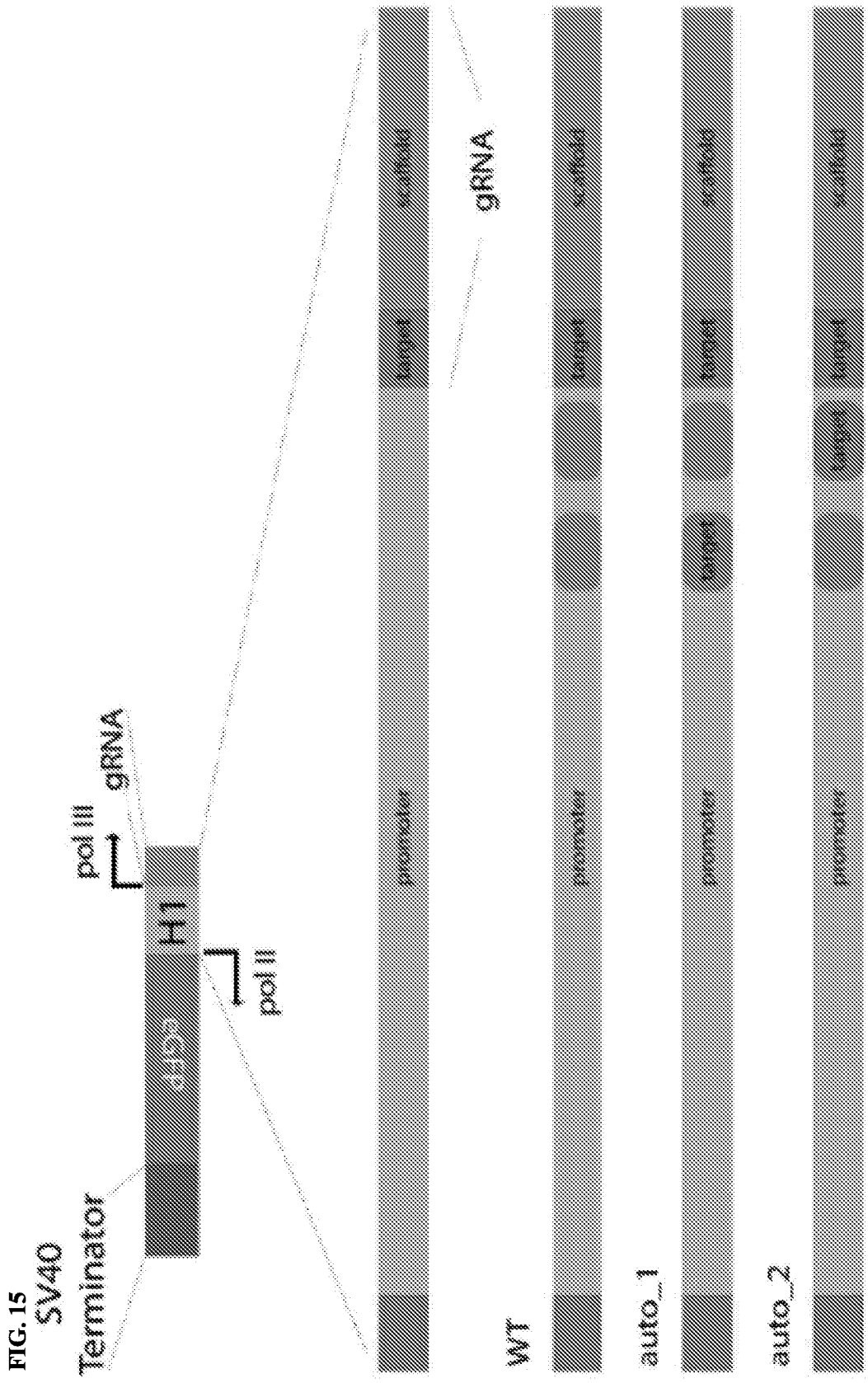
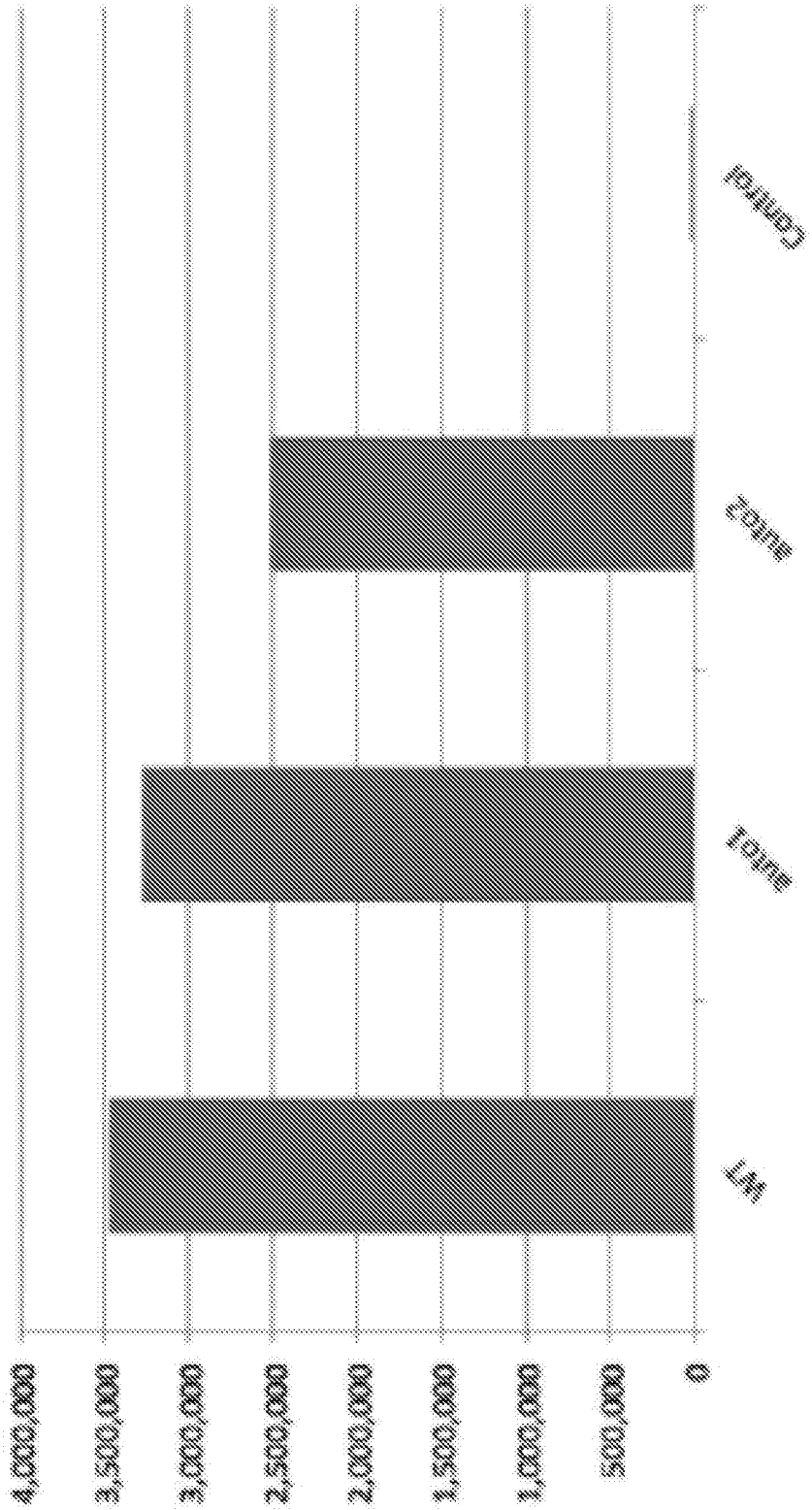

SV40
Terminator
pol III
gRNA
H1
pol II
gRNA
promoter
target
scaffold
WT
auto_1
auto_2

FIG. 15

FIG. 15(cont.)


Pol II expression from modified H1 bidirectional promoter
4,000,000
3,500,000
3,000,000
2,500,000
2,000,000
1,500,000
1,000,000
500,000
0
H1
Bi-H1
Bi-H2
Control

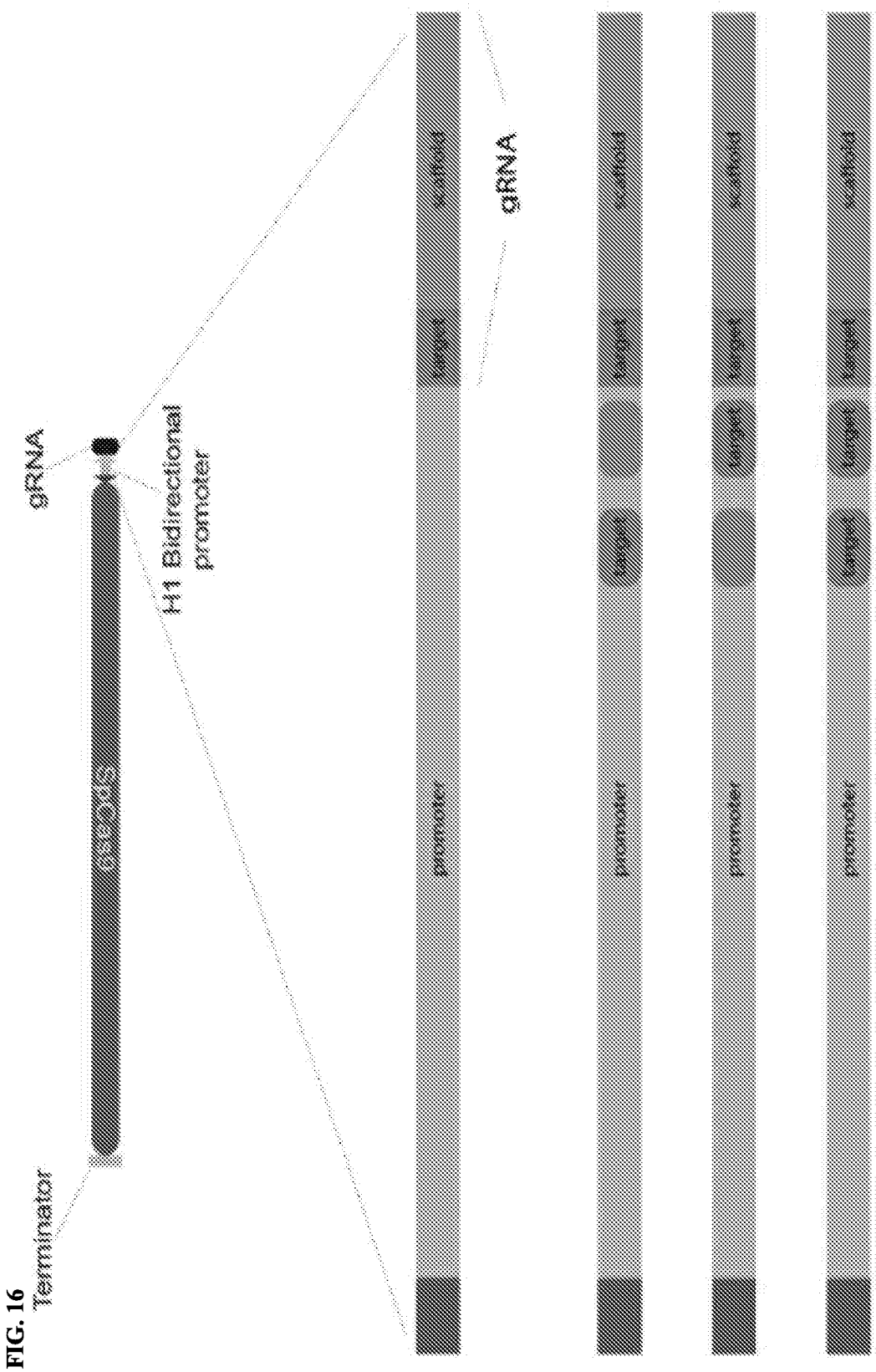

gRNA
H1 Bidirectional promoter
Terminator
gRNA
Target
promoter

FIG. 16

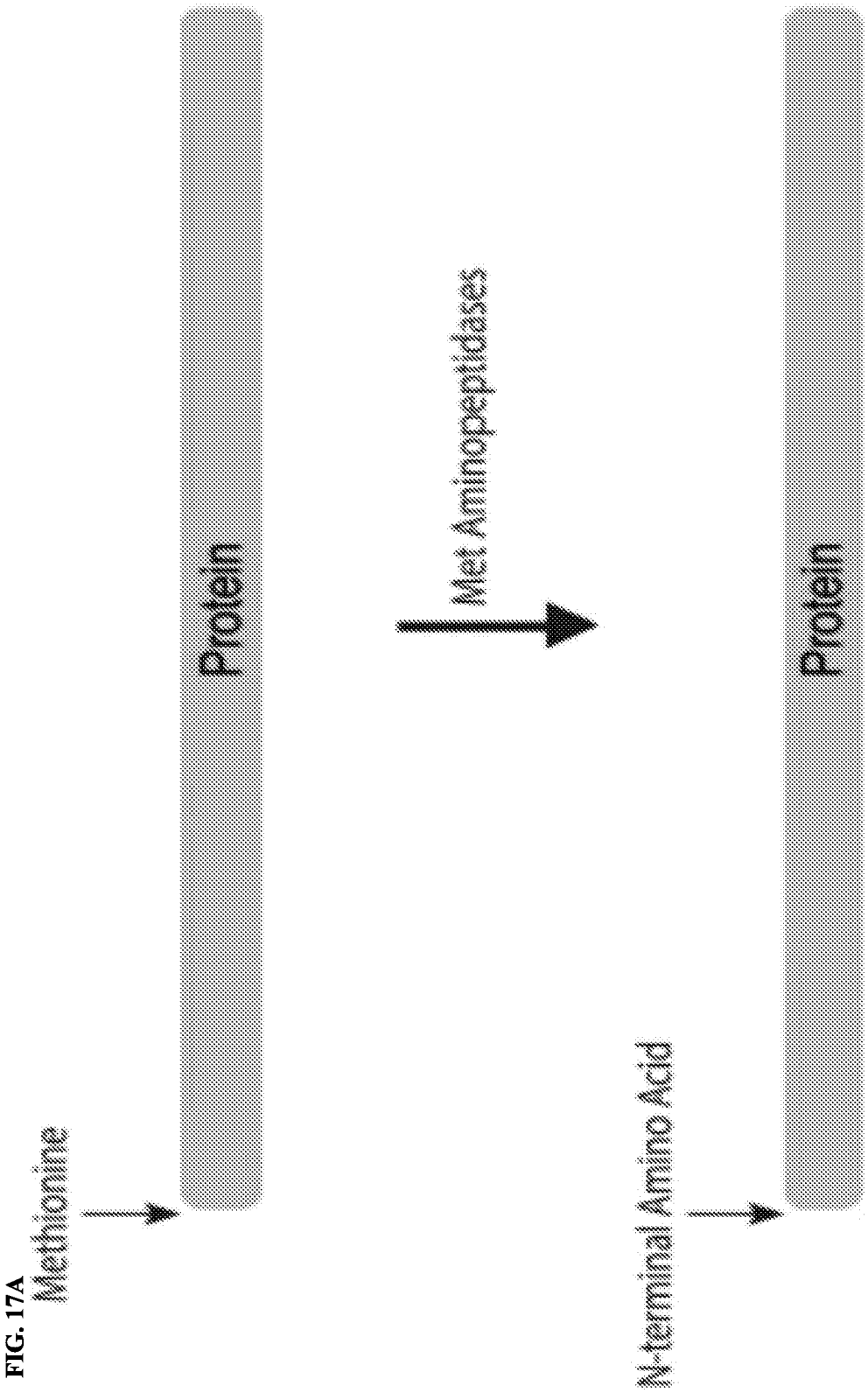

Methionine
Protein
Met Aminopeptidases
N-terminal Amino Acid
Protein

FIG. 17A

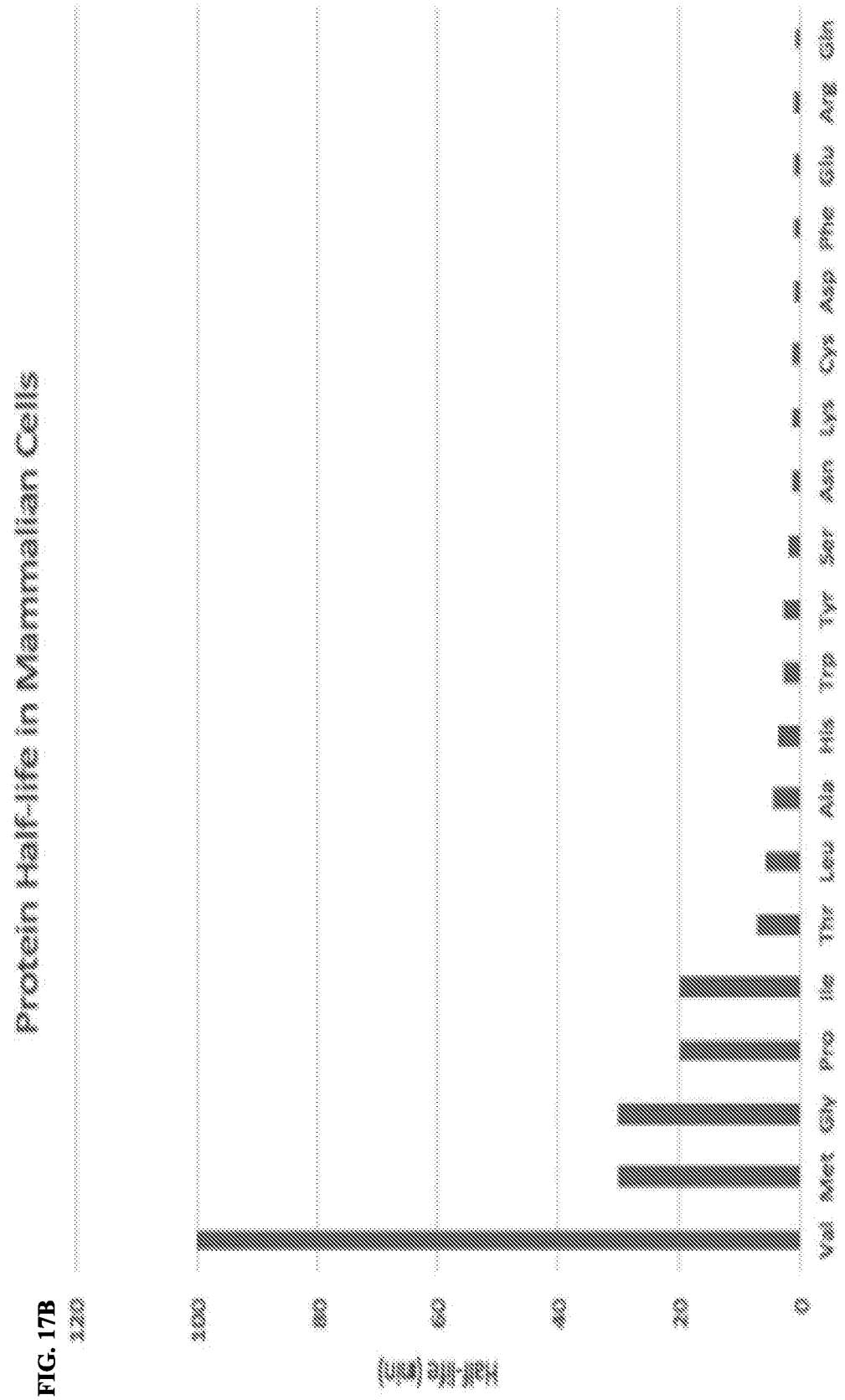

Protein Half-life in Mammalian Cells
Half-life (hrs)
120
100
80
60
40
20
0
Val Met Gly Pro Ile Thr Leu Ala His Trp Tyr Ser Asn Lys Cys Asp Phe Glu Arg Gln
N-terminal Amino Acid

FIG. 17B

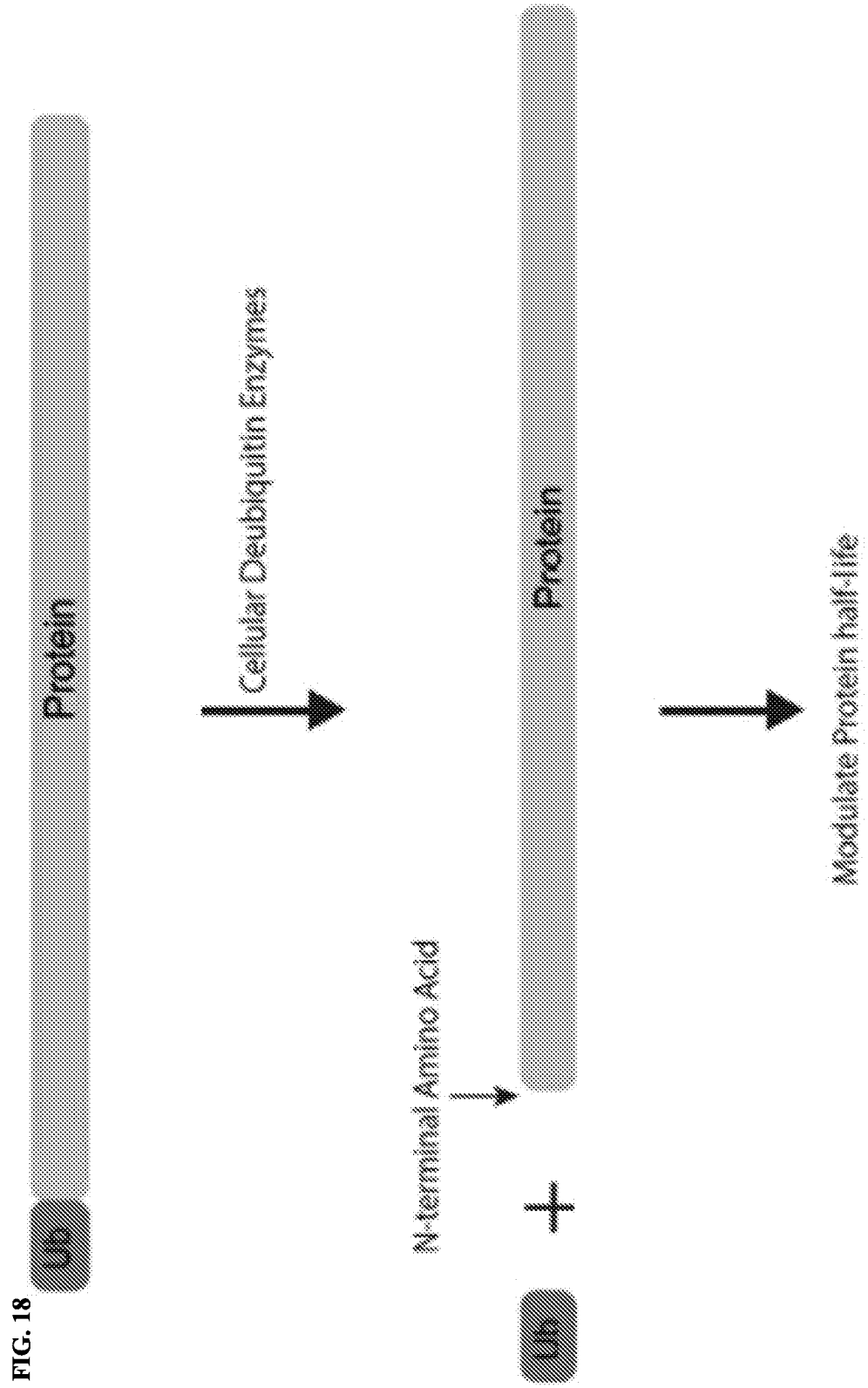

Protein
Ub
Cellular Deubiquitin Enzymes
N-terminal Amino Acid
Protein
Ub
Modulate Protein half-life

FIG. 18

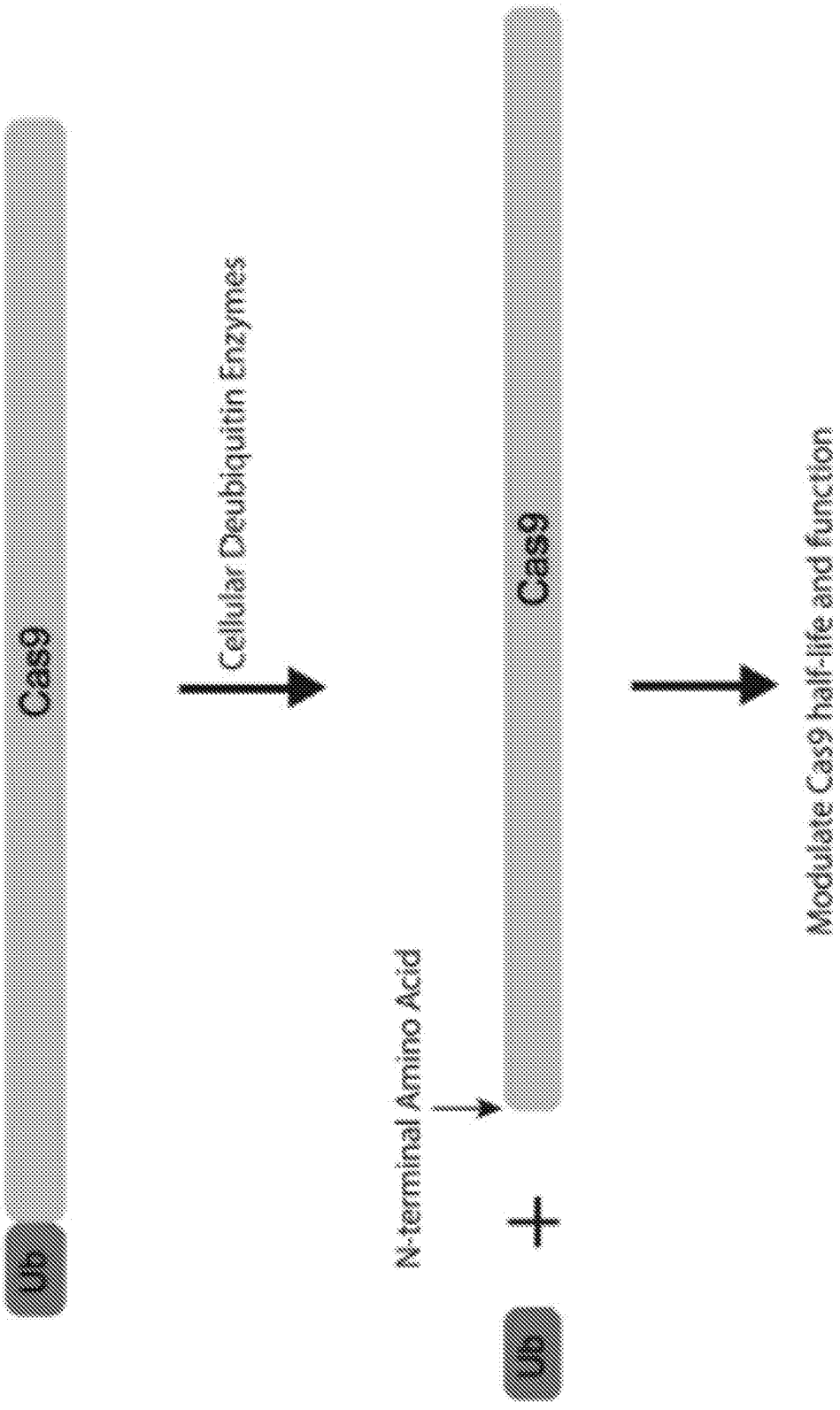

Cas9
Ub
Cellular Deubiquitin Enzymes
N-terminal Amino Acid
Cas9
+
Ub
Modulate Cas9 half-life and function

FIG. 19A

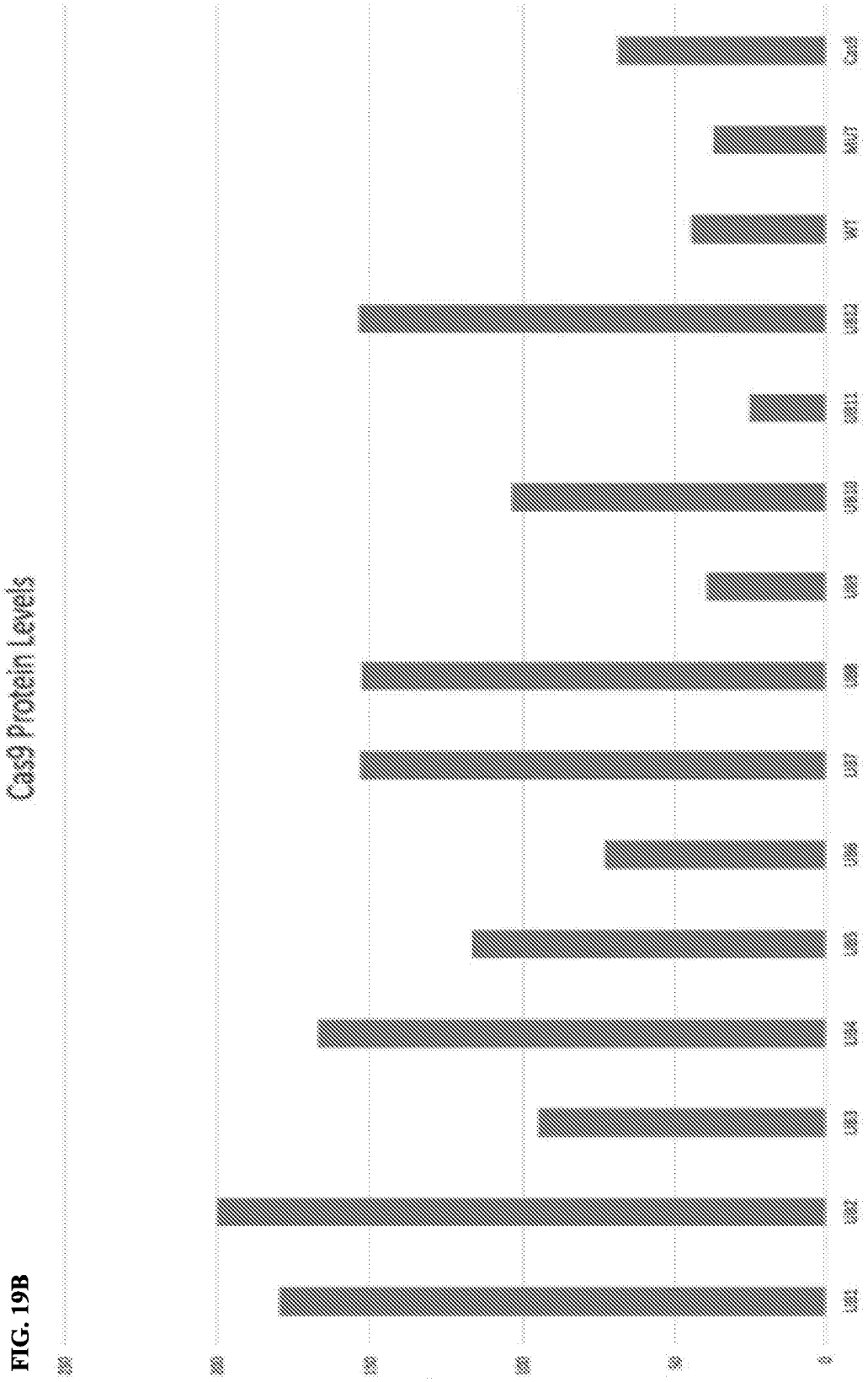


FIG. 19B

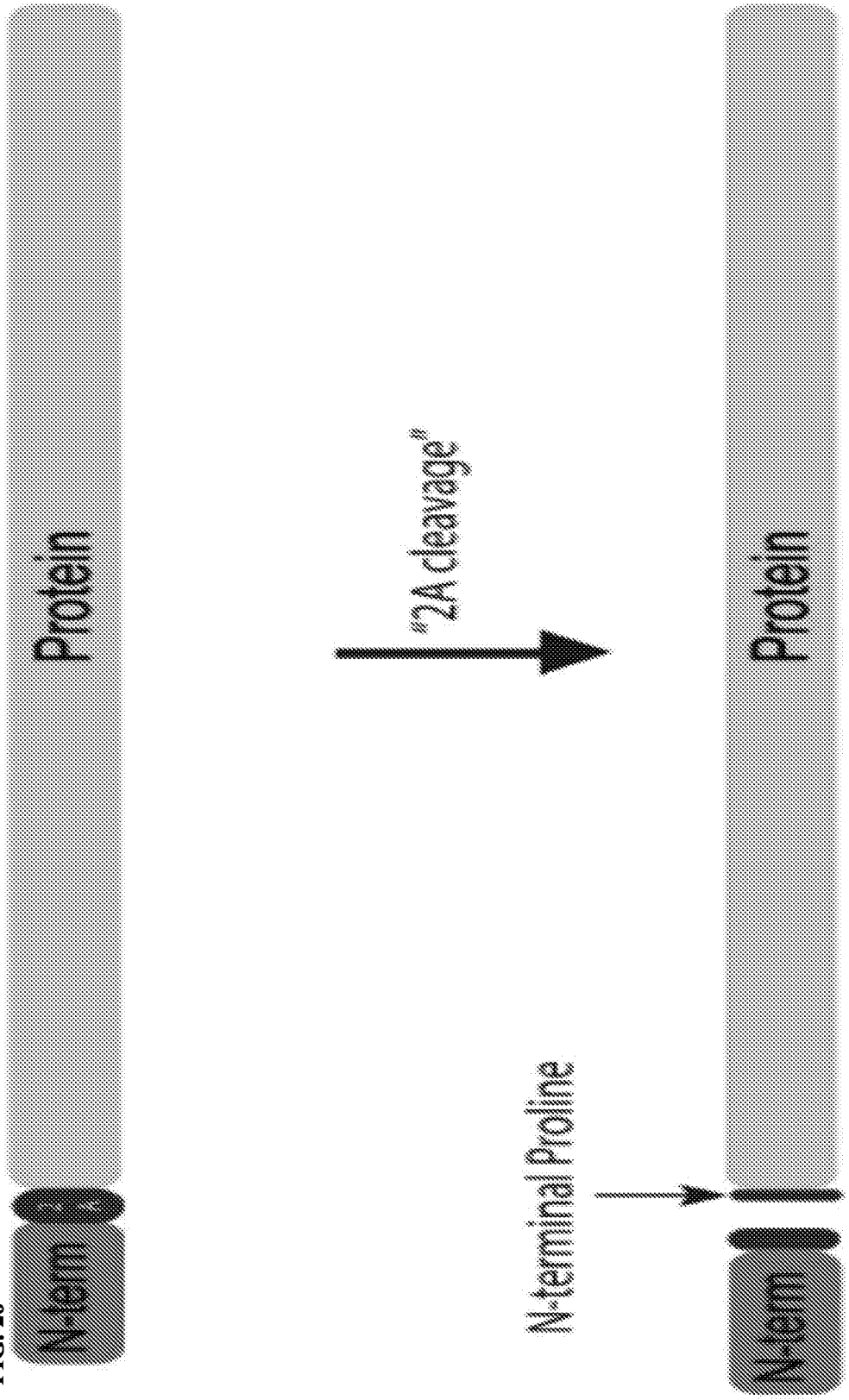
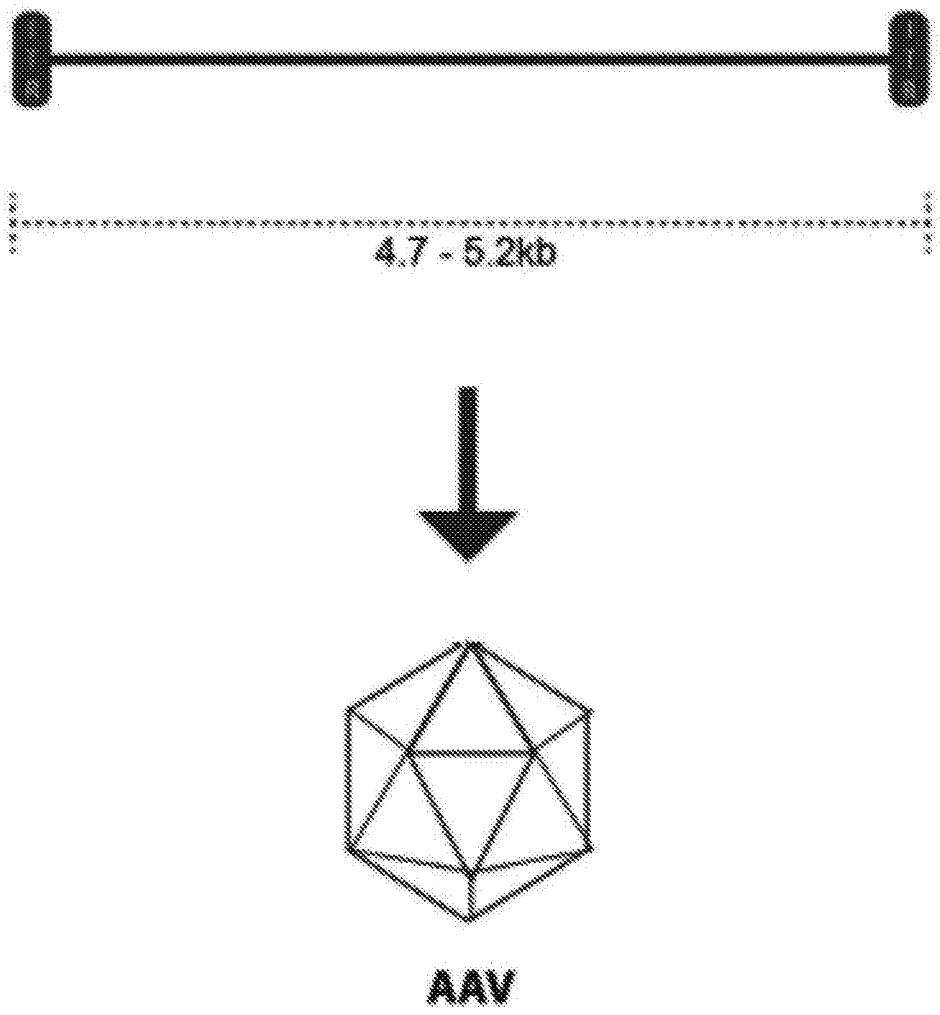

Protein
N-term
"2A cleavage"
N-terminal Proline
Protein
N-term

FIG. 20

FIG. 21A



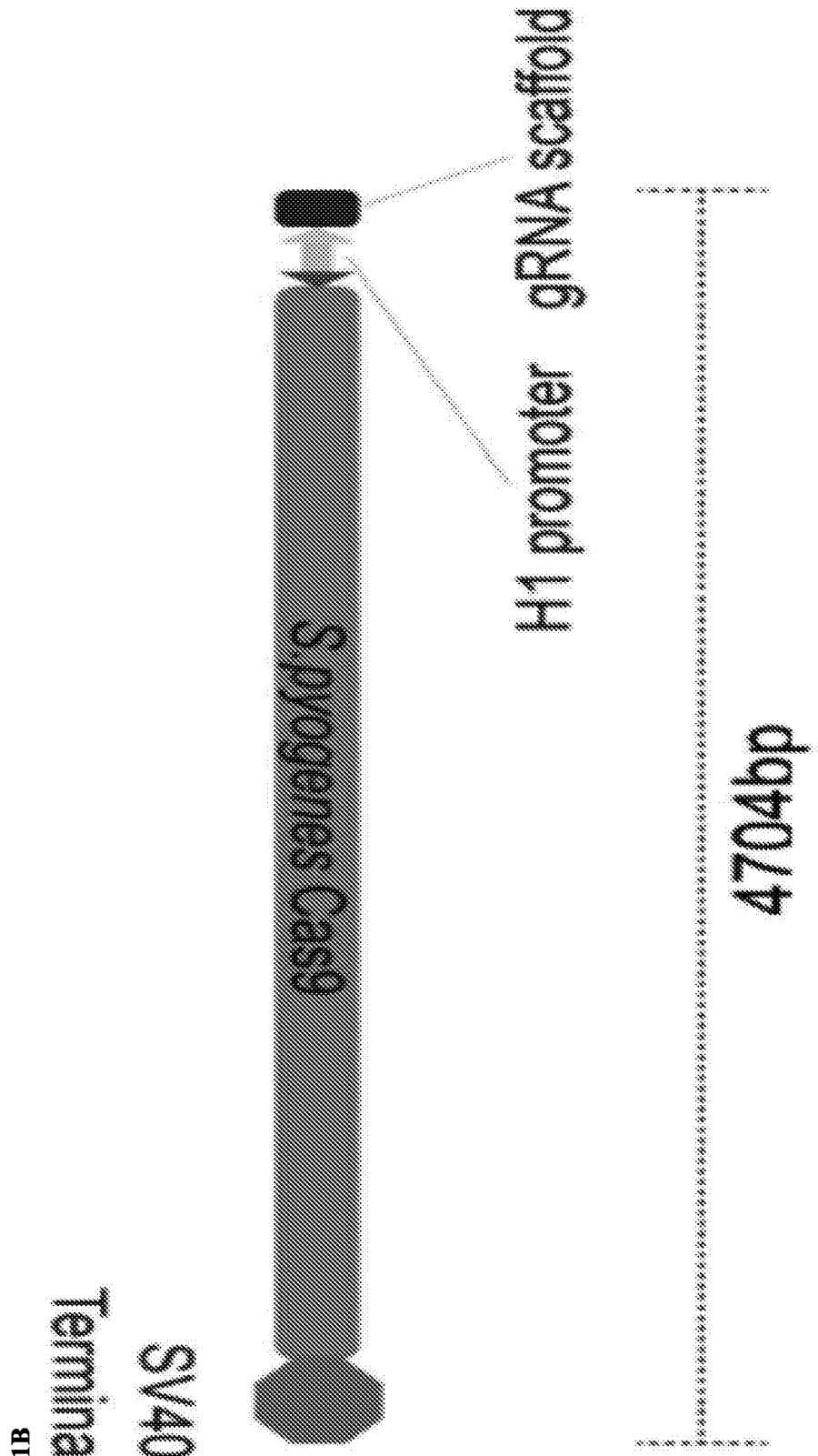

SV40
Terminator
S.pyogenes Cas9
H1 promoter
gRNA scaffold
4704bp

FIG. 21B

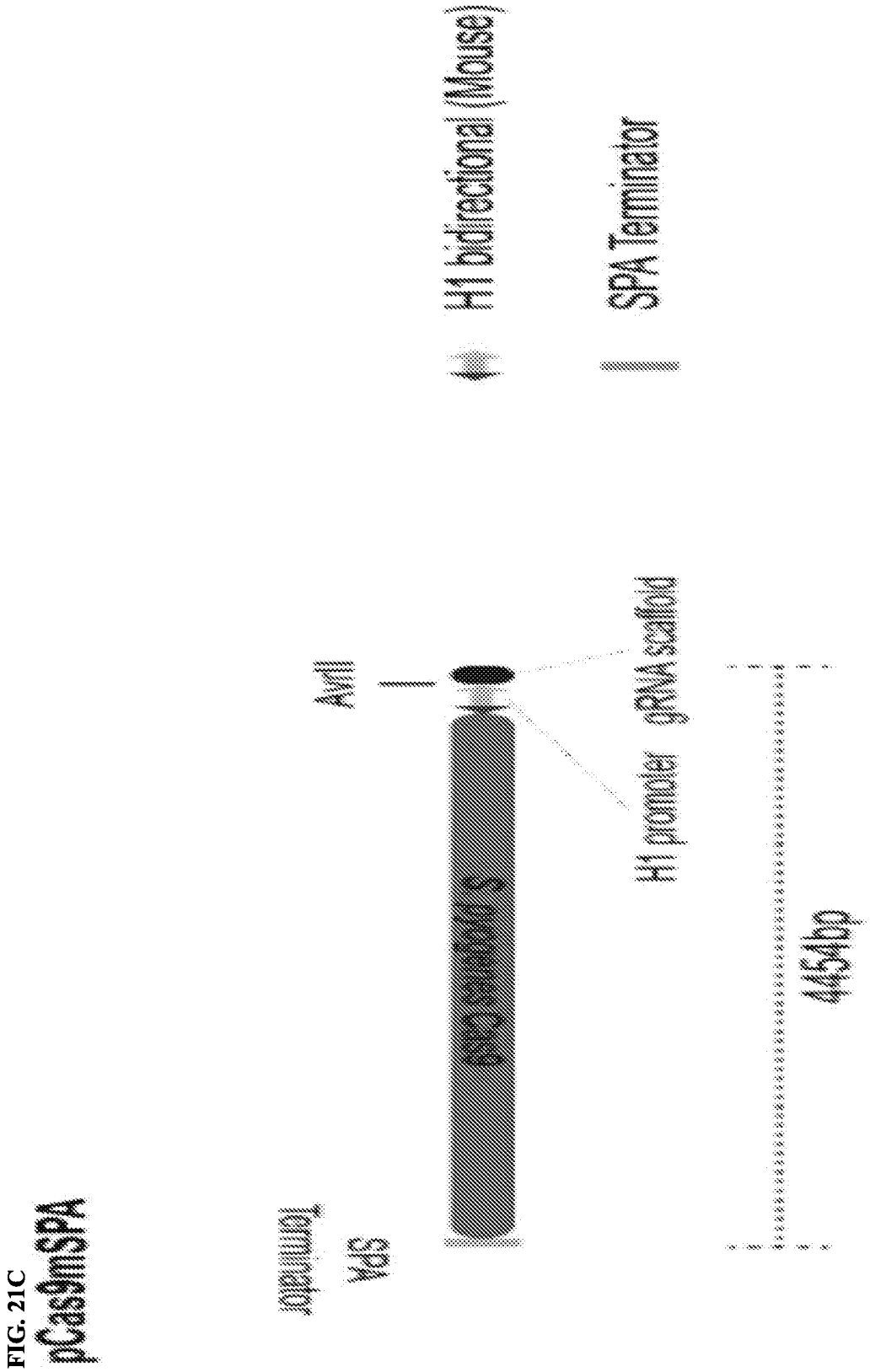


FIG. 21C
pCas9mSPA

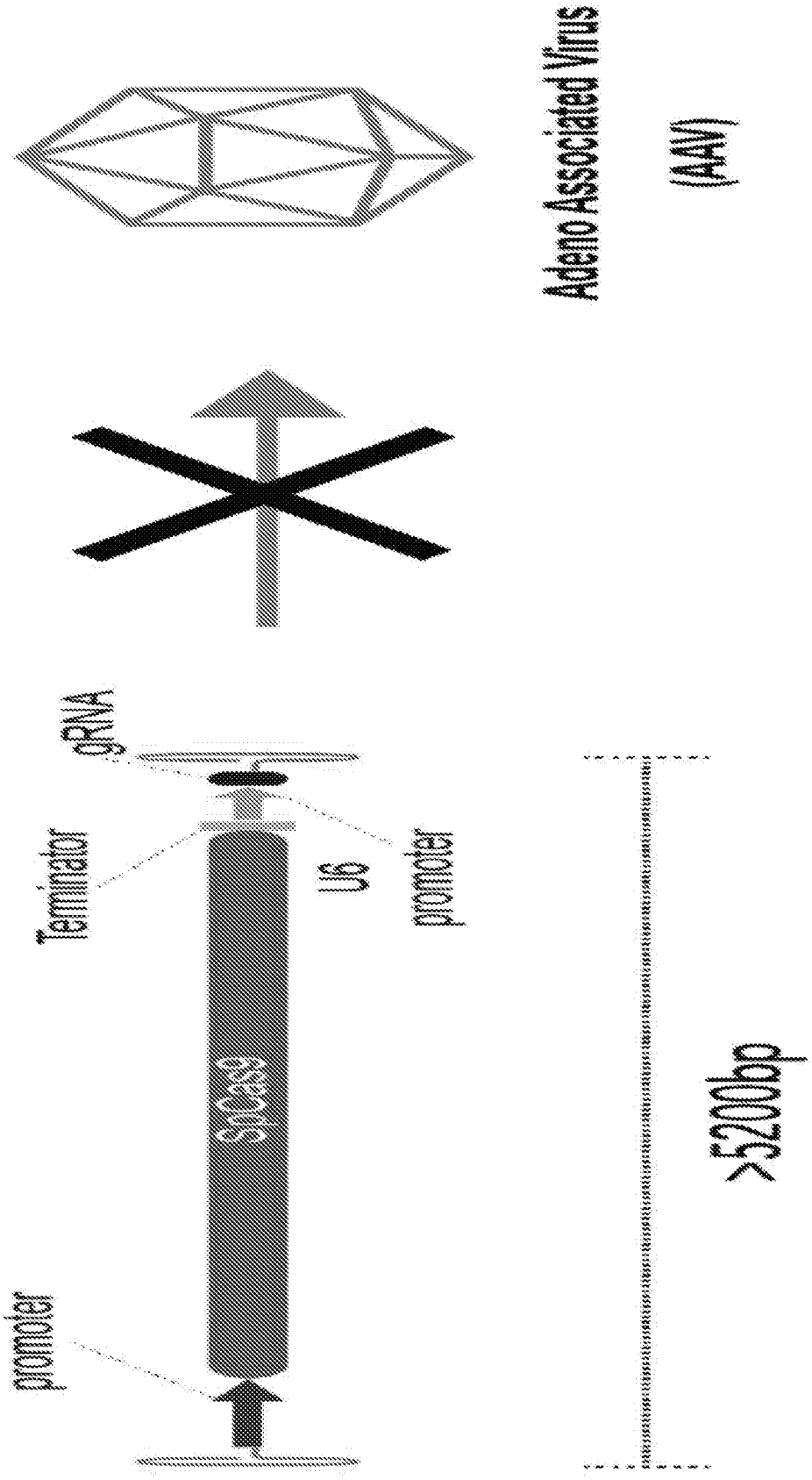

Adeno Associated Virus
(AAV)
CMV
promoter
SaCas9
Terminator
gRNA
U6
promoter
<5200bp

FIG. 22A

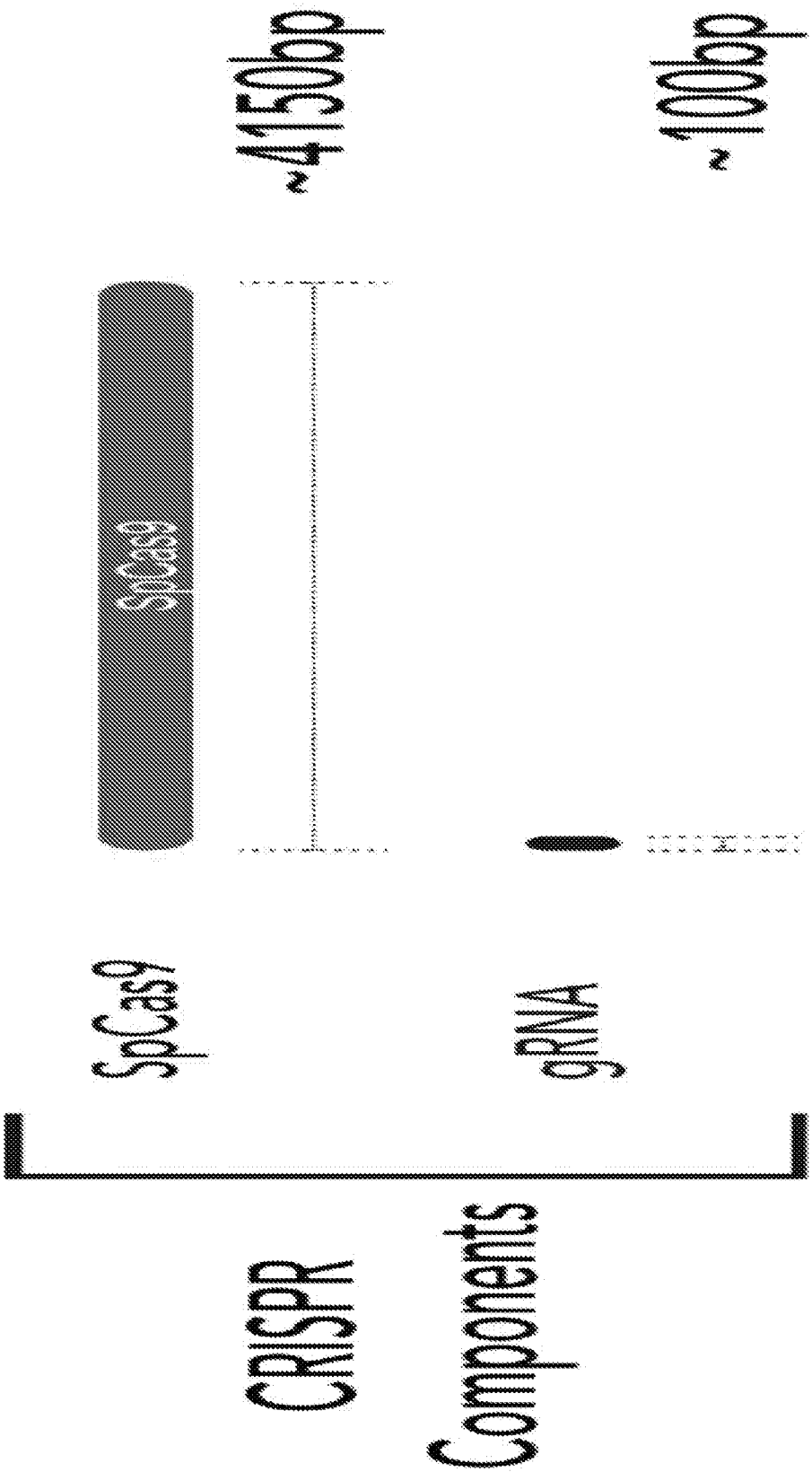


FIG. 22A(cont.)

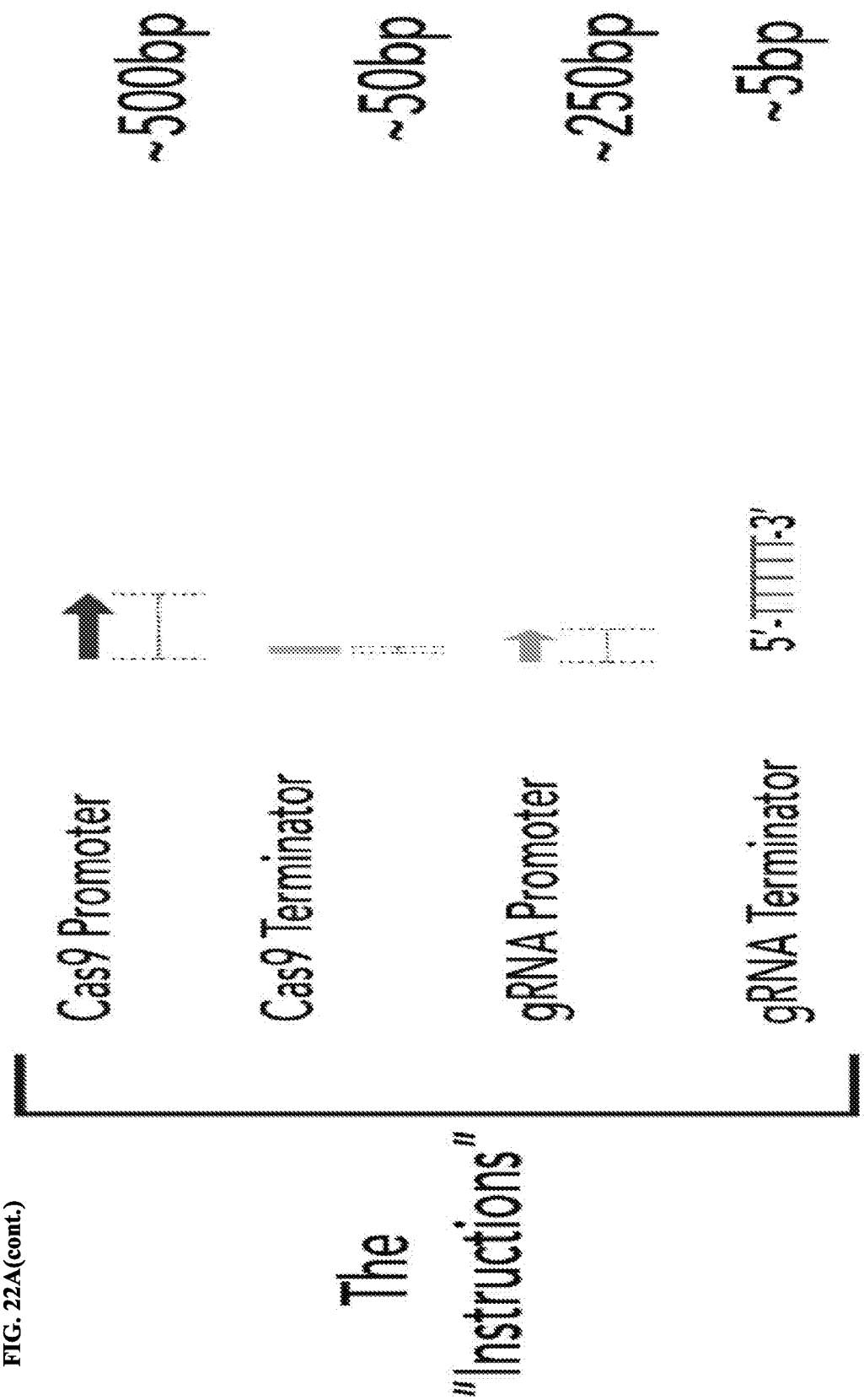

The "Instructions"
Cas9 Promoter
Cas9 Terminator
gRNA Promoter
gRNA Terminator
5'-TTTTT-3'
~500bp
~50bp
~250bp
~5bp

FIG. 22A(cont.)

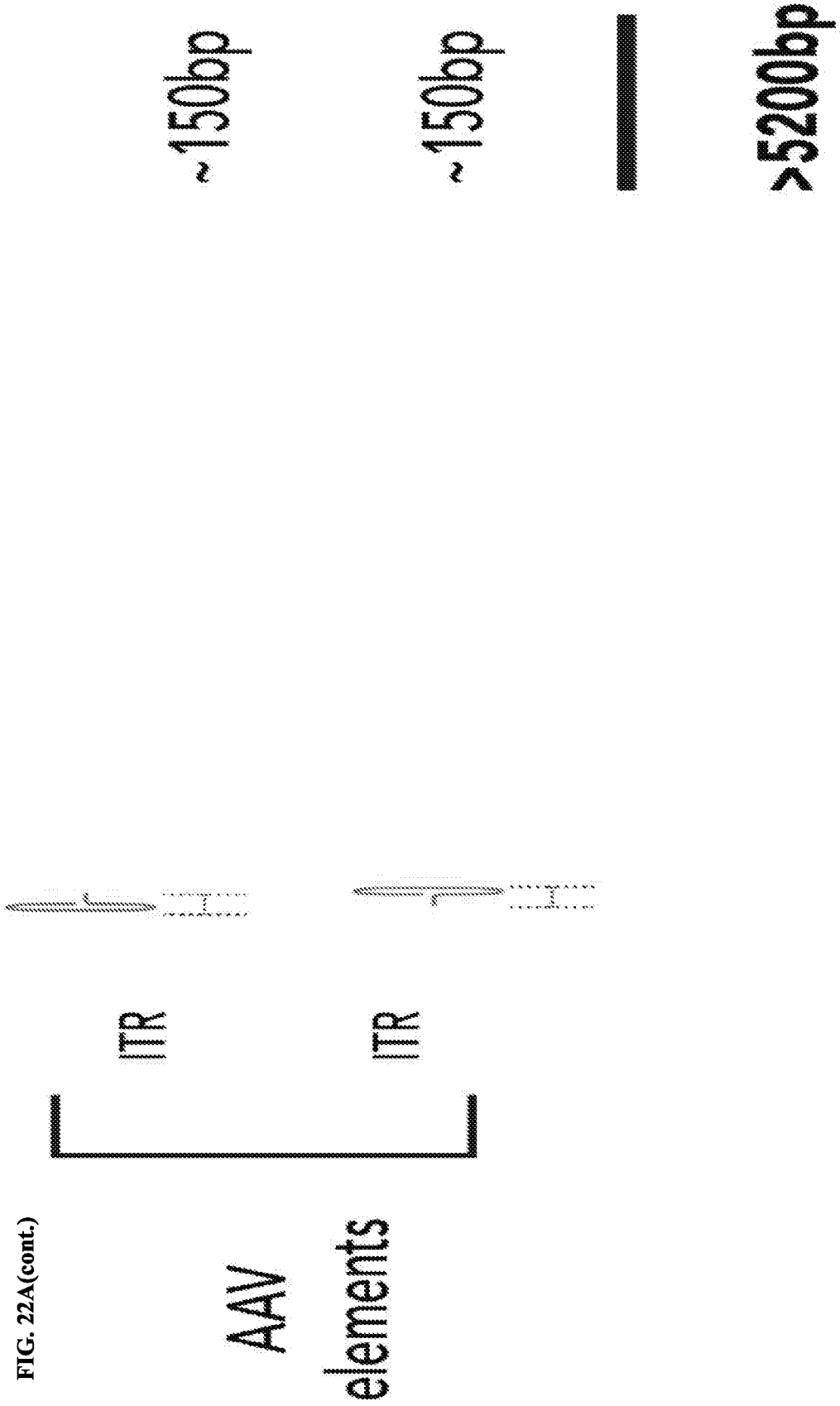


FIG. 22A(cont.)

FIG. 22B

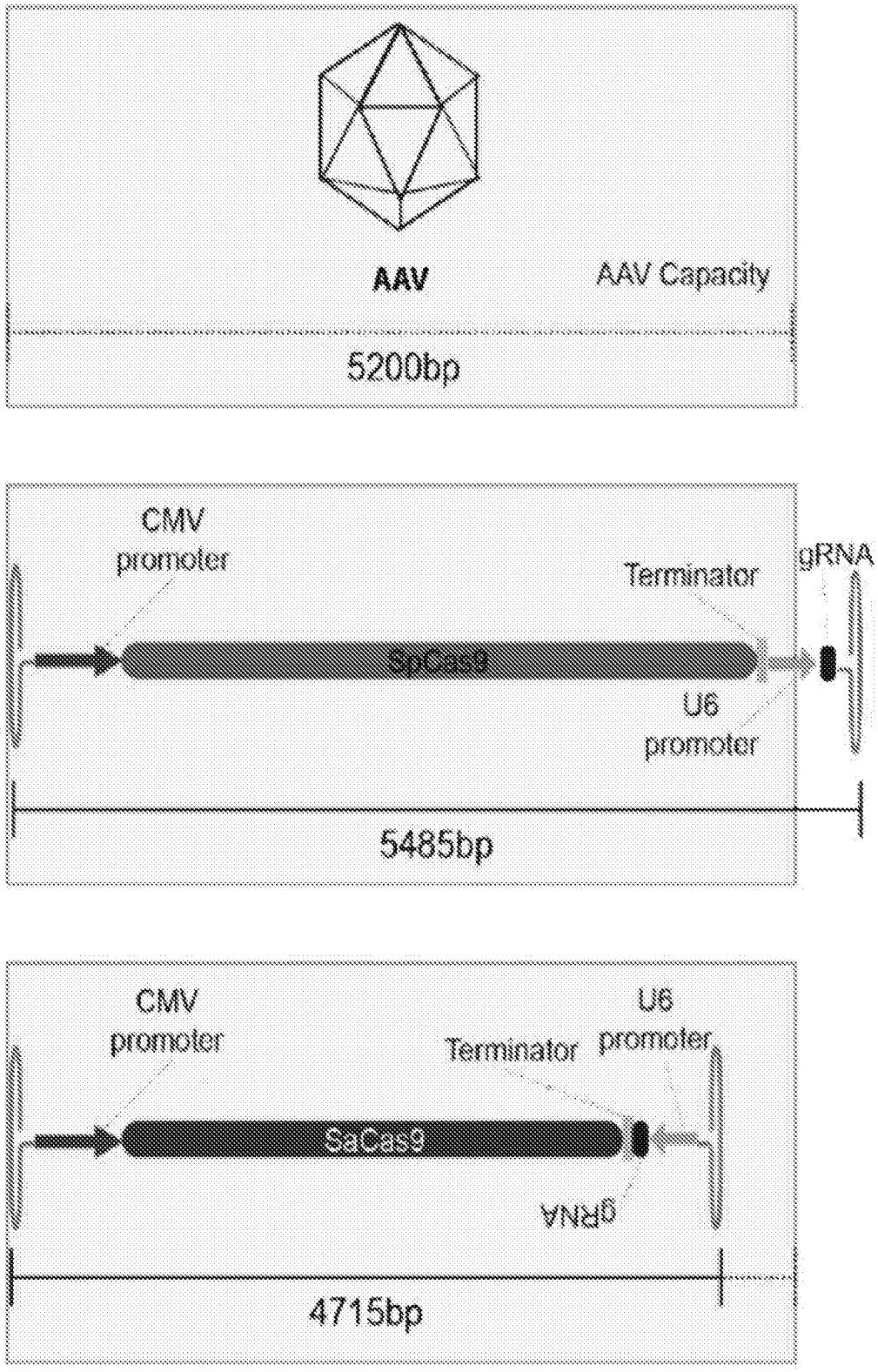
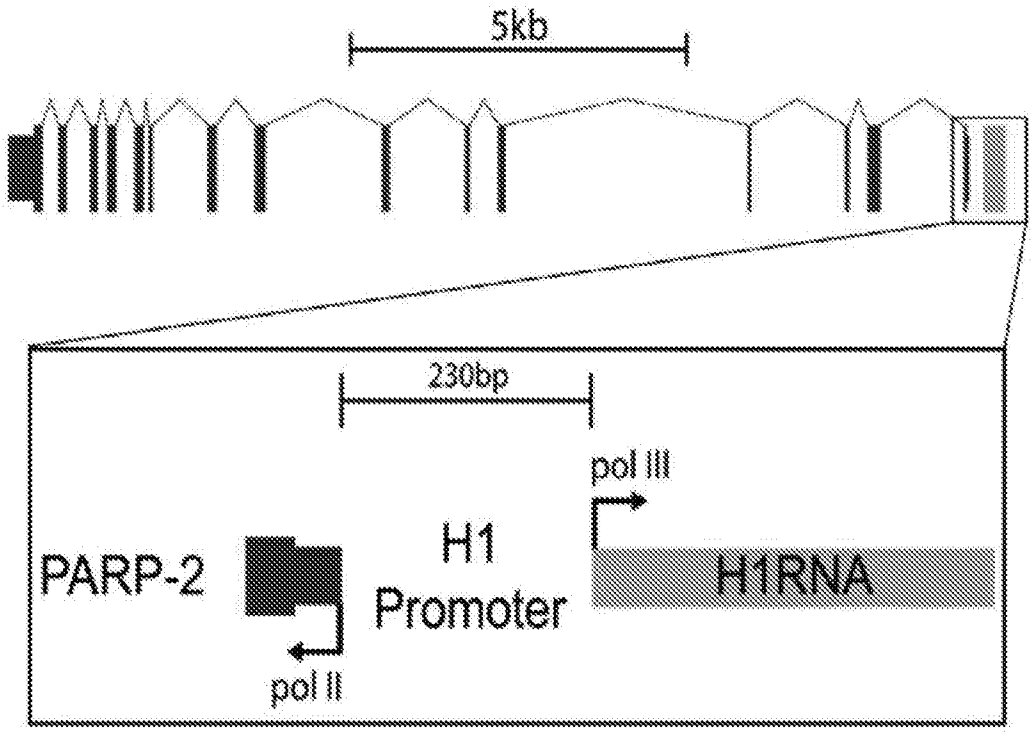

AAV
AAV Capacity
5200bp
CMV promoter
Terminator
gRNA
SpCas9
U6 promoter
5485bp
CMV promoter
U6 promoter
Terminator
SaCas9
gRNA
4715bp

FIG. 22C



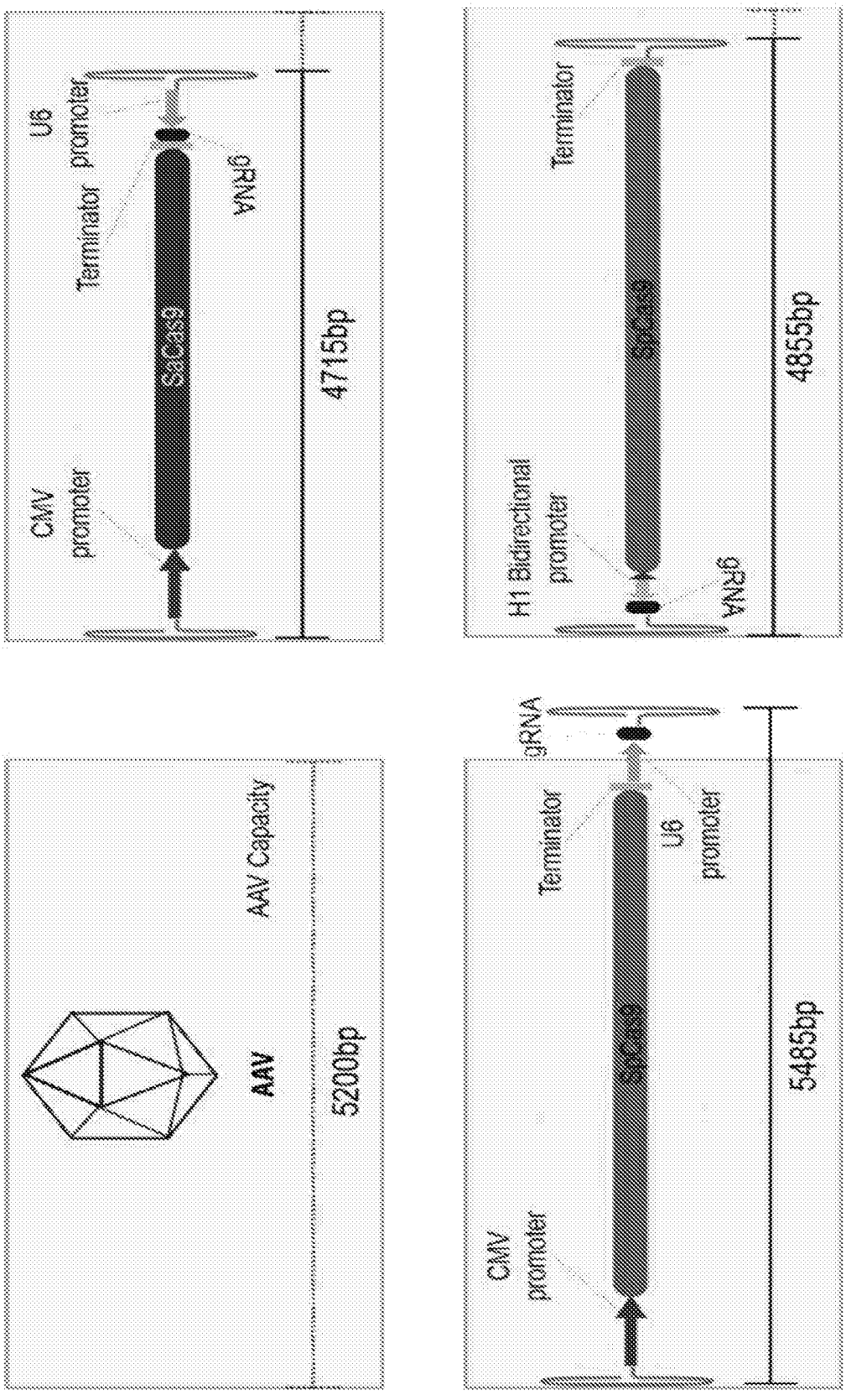

AAV
AAV Capacity
5200bp
CMV promoter
SaCas9
U6 Terminator promoter
gRNA
4715bp
H1 Bidirectional promoter
SaCas9
Terminator
gRNA
4855bp
CMV promoter
SaCas9
Terminator
U6 promoter
gRNA
5485bp

FIG. 22D

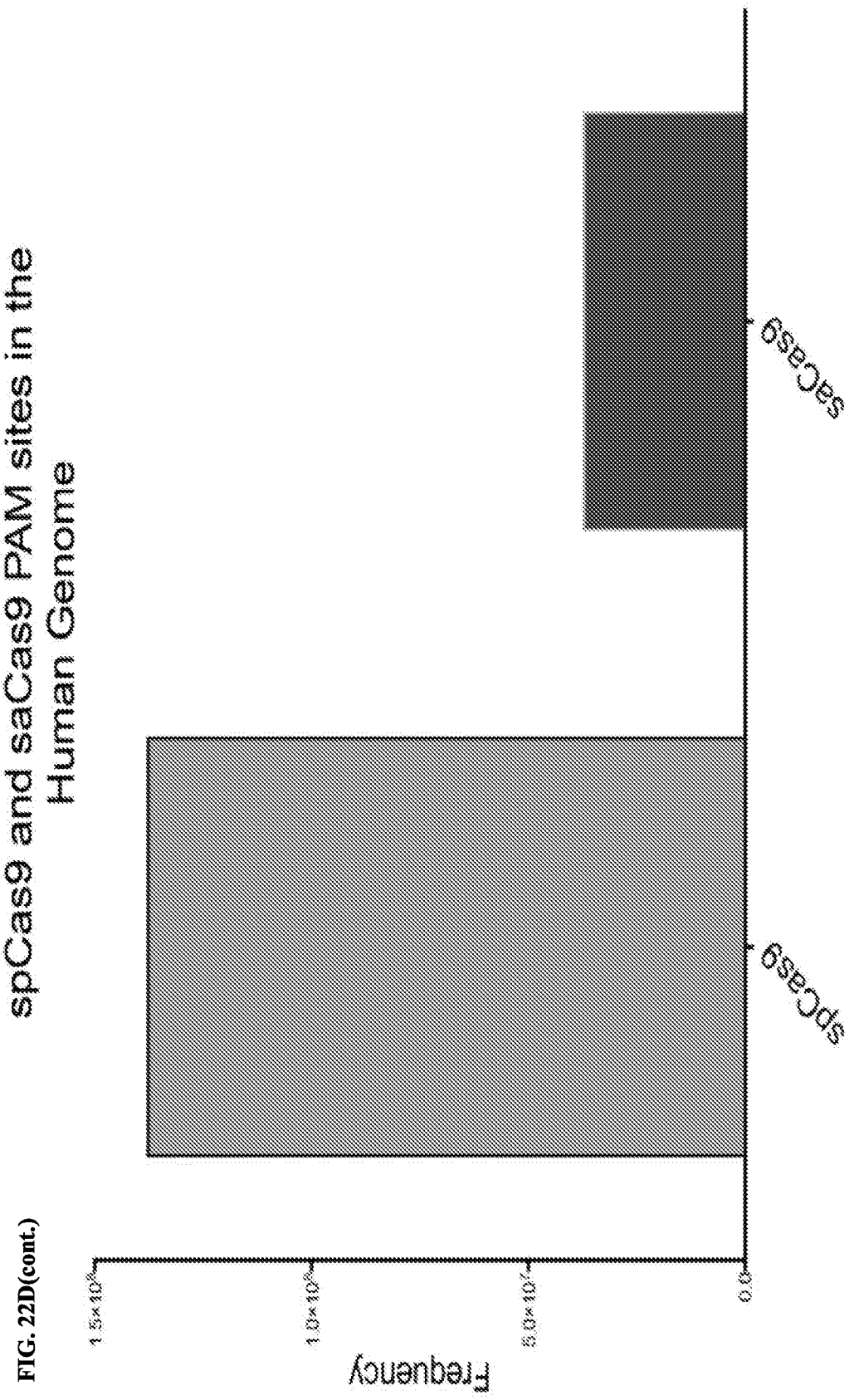

spCas9 and saCas9 PAM sites in the Human Genome
Frequency
1.5×10^8
1.0×10^8
5.0×10^7
0.0
spCas9
saCas9

FIG. 22D(cont.)

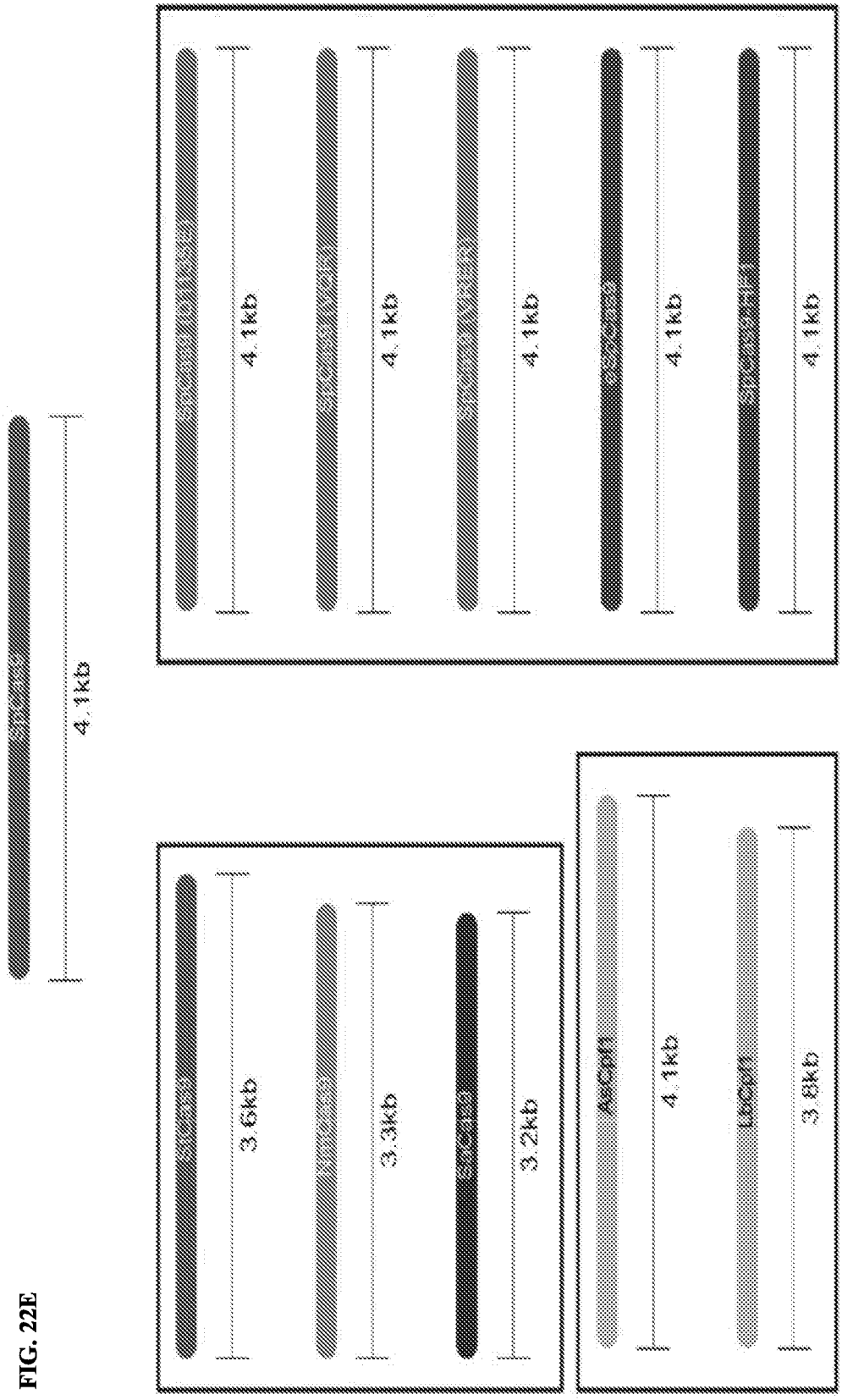
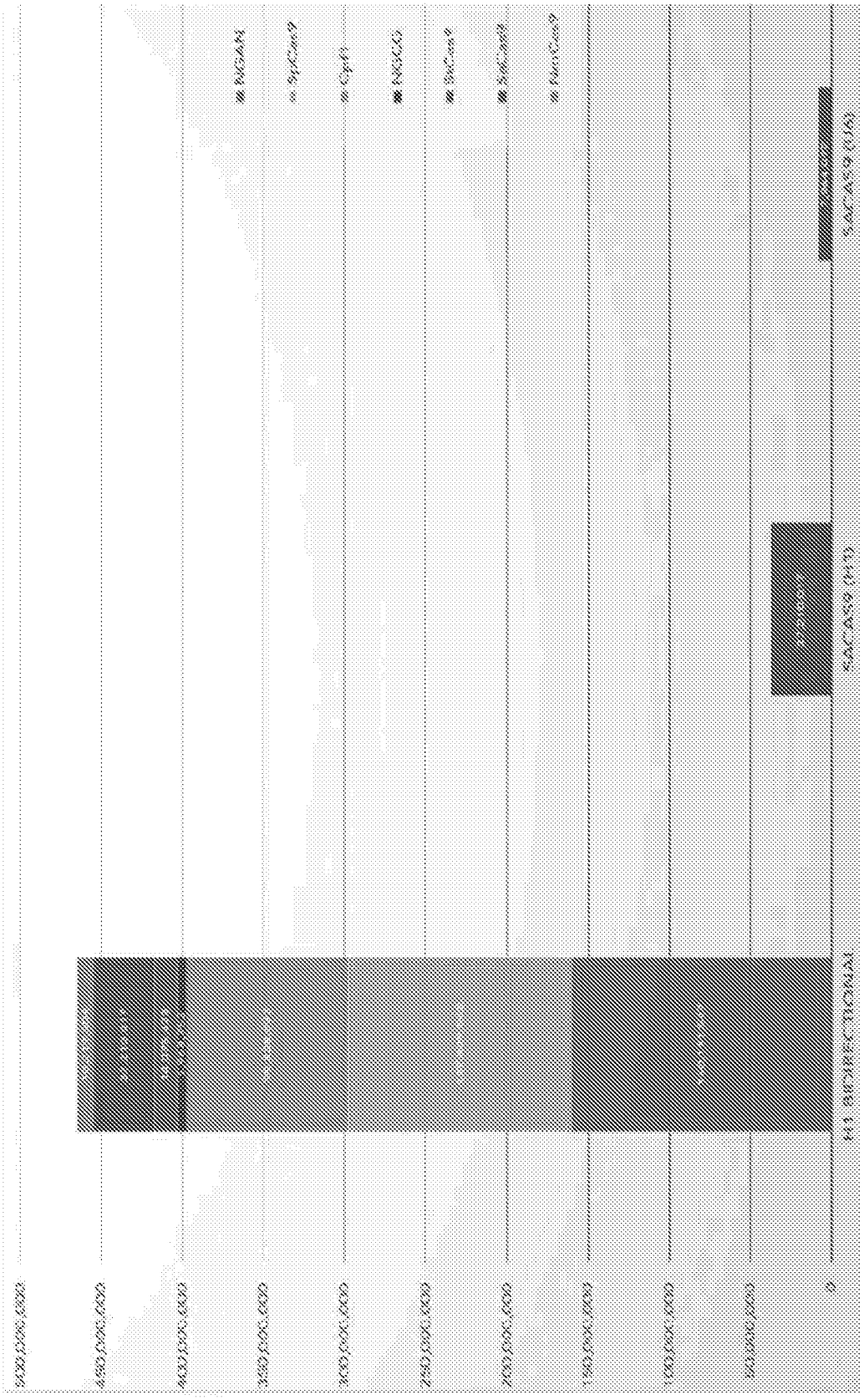


FIG. 22E

FIG. 22F


500,000,000
450,000,000
400,000,000
350,000,000
300,000,000
250,000,000
200,000,000
150,000,000
100,000,000
50,000,000
0
HI-SCRIBE HUMAN
SACAS9 (HI)
SACAS9 (UA)
NGAG
SpCas9
CjeCas9
NGGO
StCas9
SaCas9
NmCas9

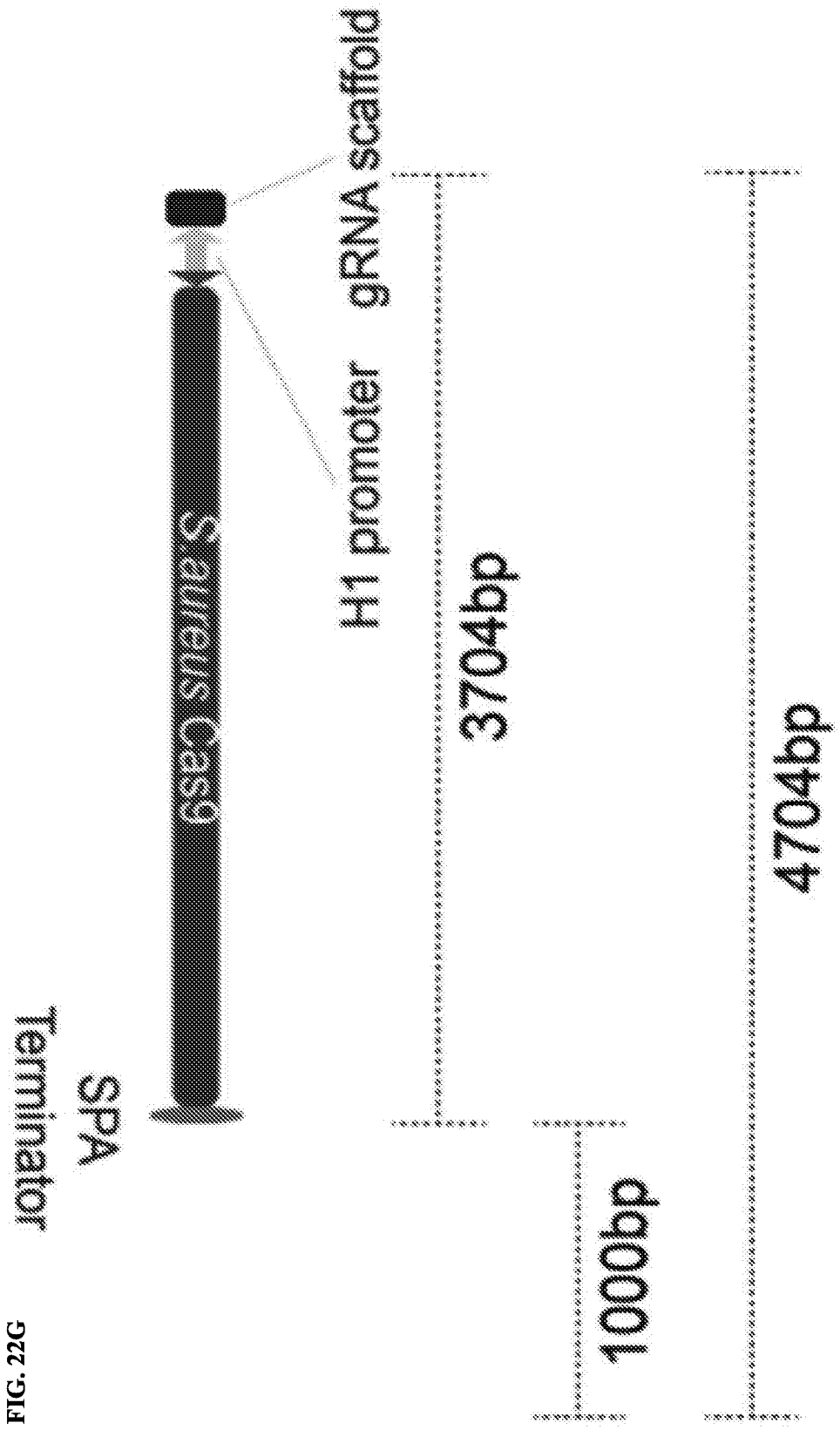

H1 promoter
gRNA scaffold
S. aureus Cas9
SPA Terminator
3704bp
1000bp
4704bp

FIG. 22G

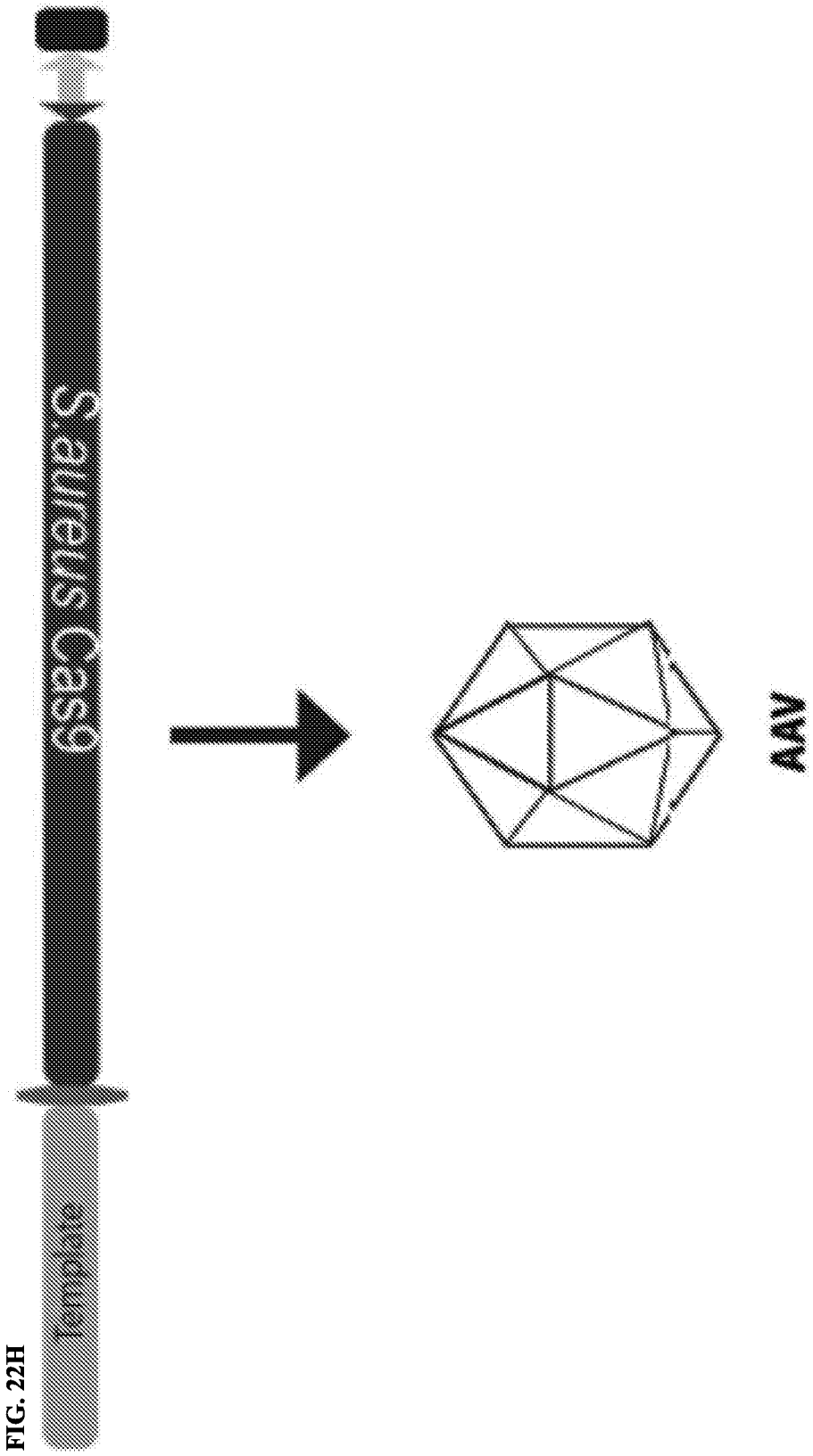


FIG. 22H

FIG. 22I

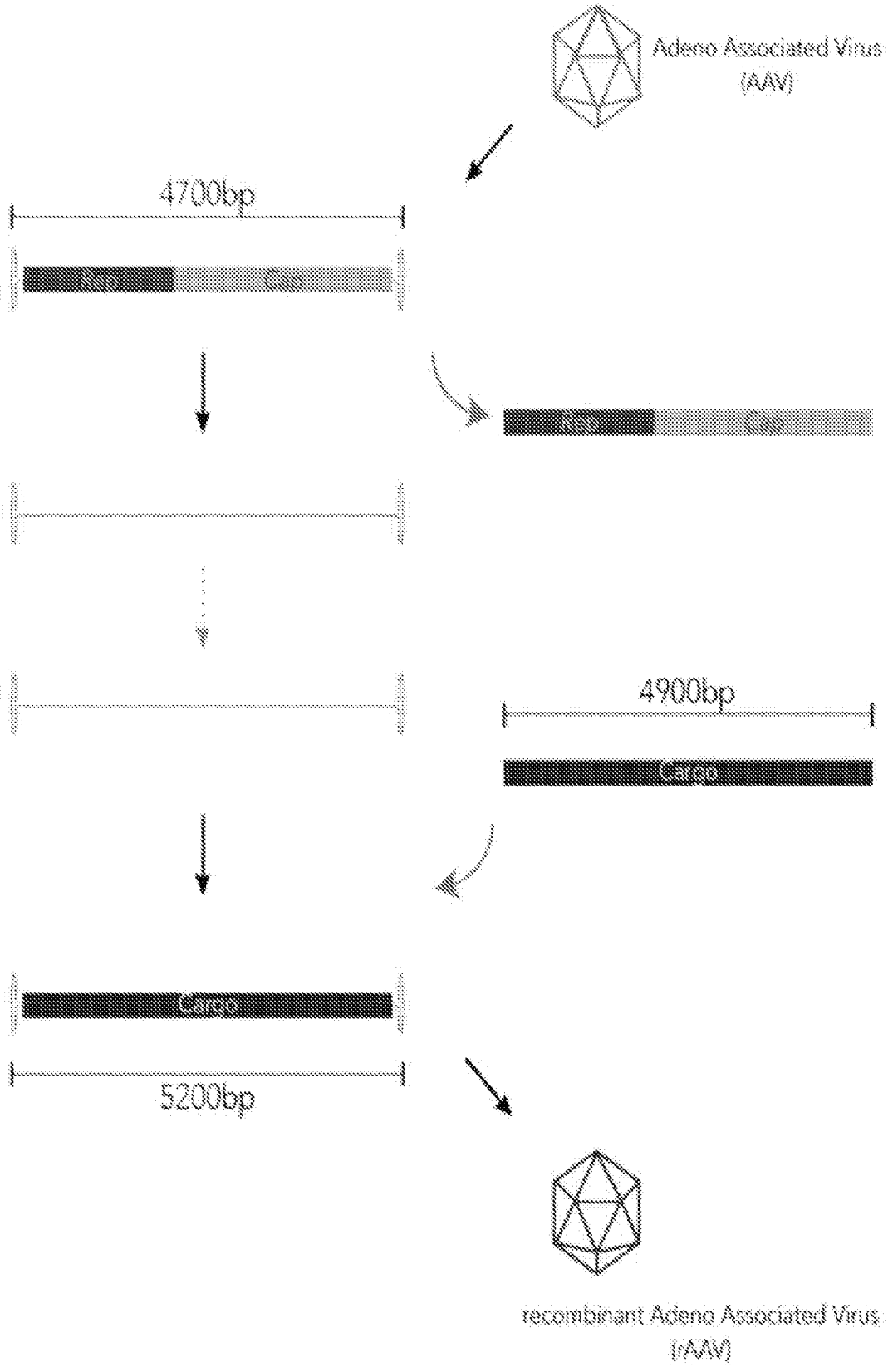
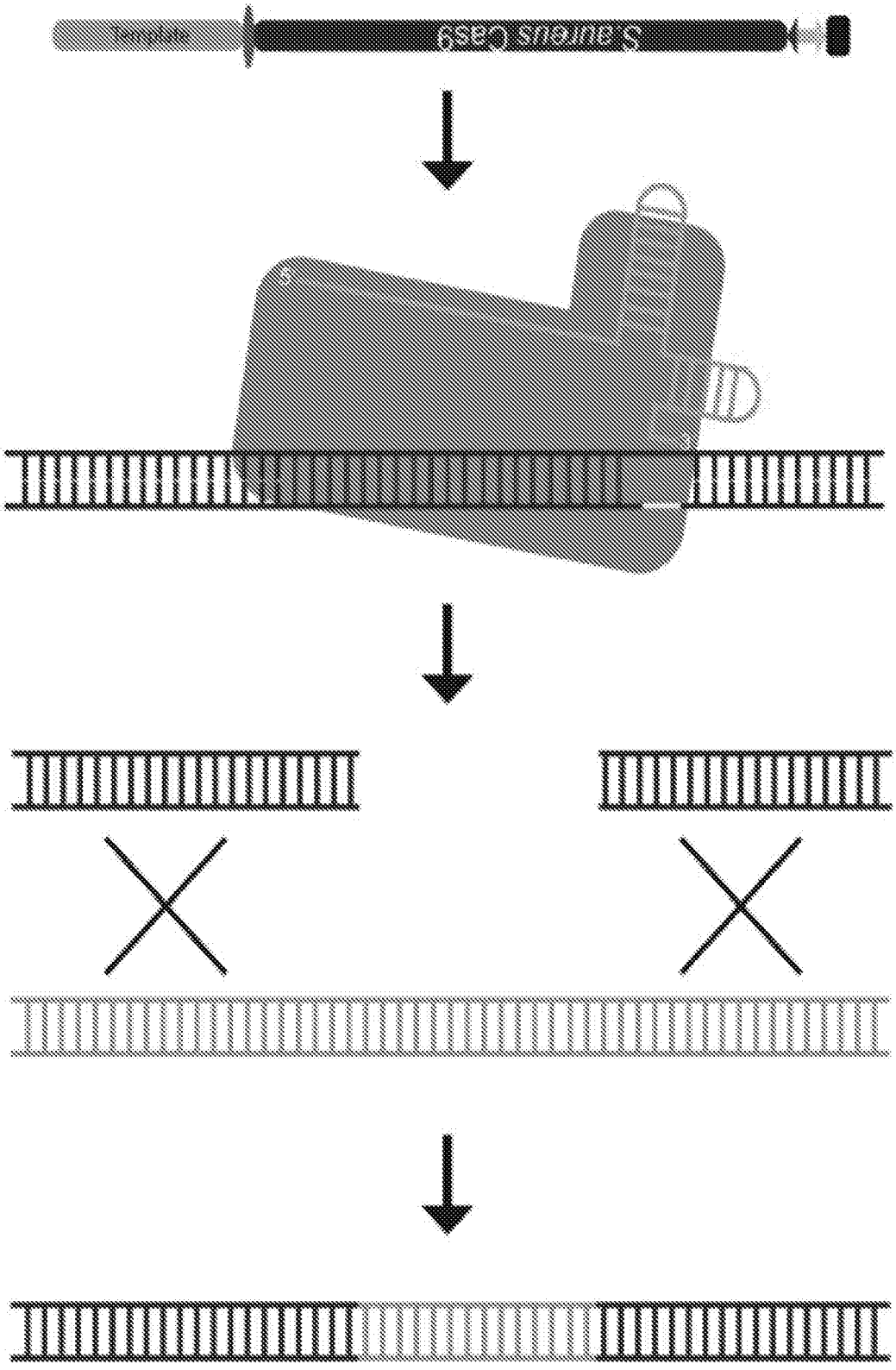

Adeno Associated Virus
(AAV)
4700bp
4900bp
5200bp
recombinant Adeno Associated Virus
(rAAV)

FIG. 23




Some Potential Configurations:
Template
SaCas9
SaCas9
Template

FIG. 24A

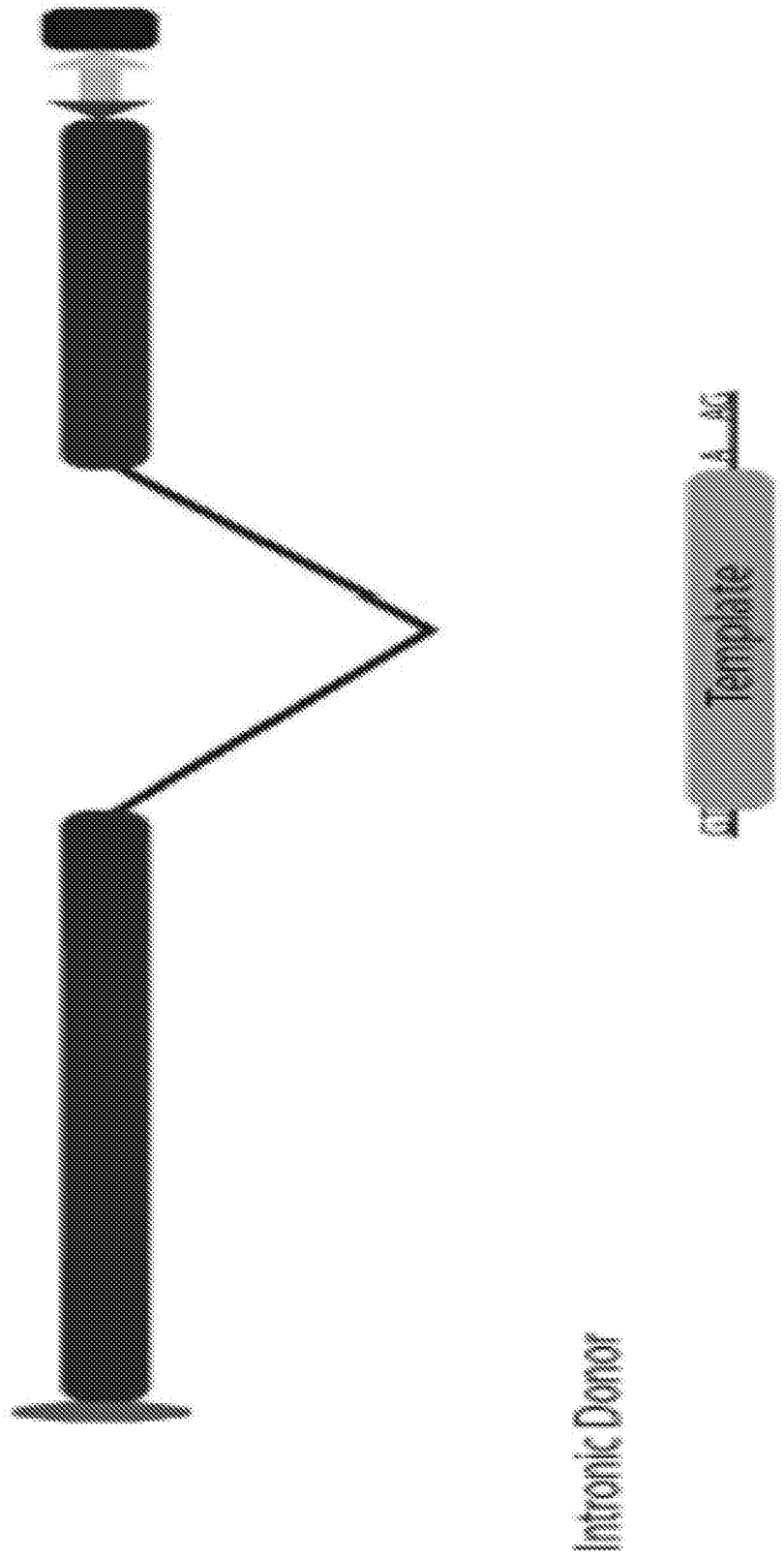


FIG. 24B

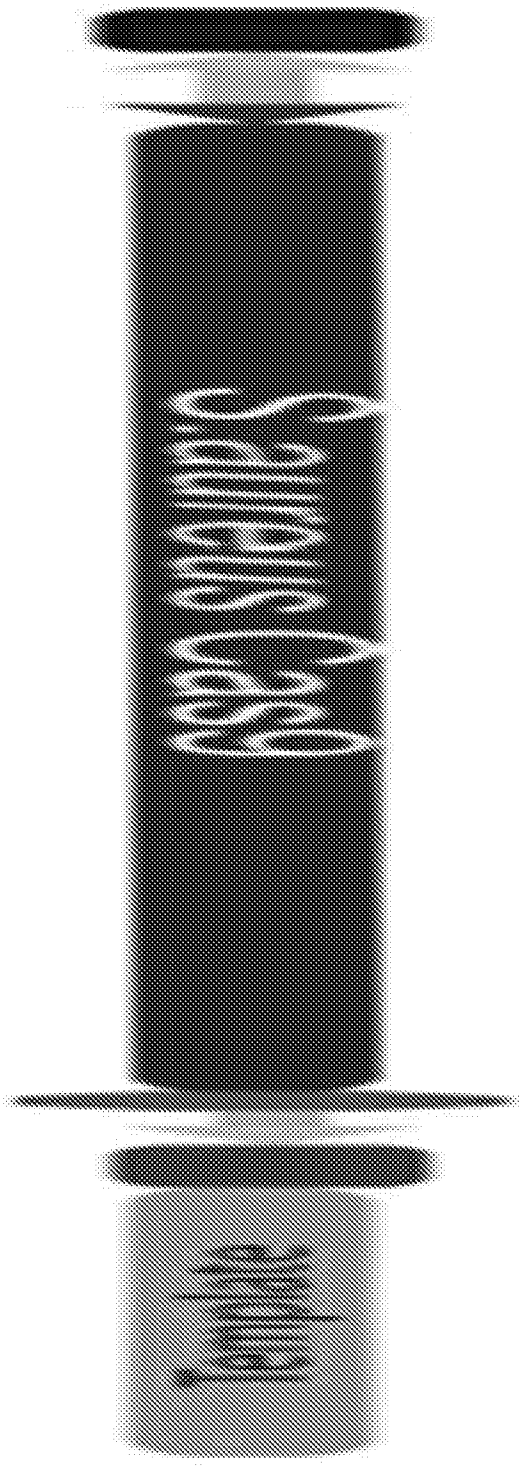
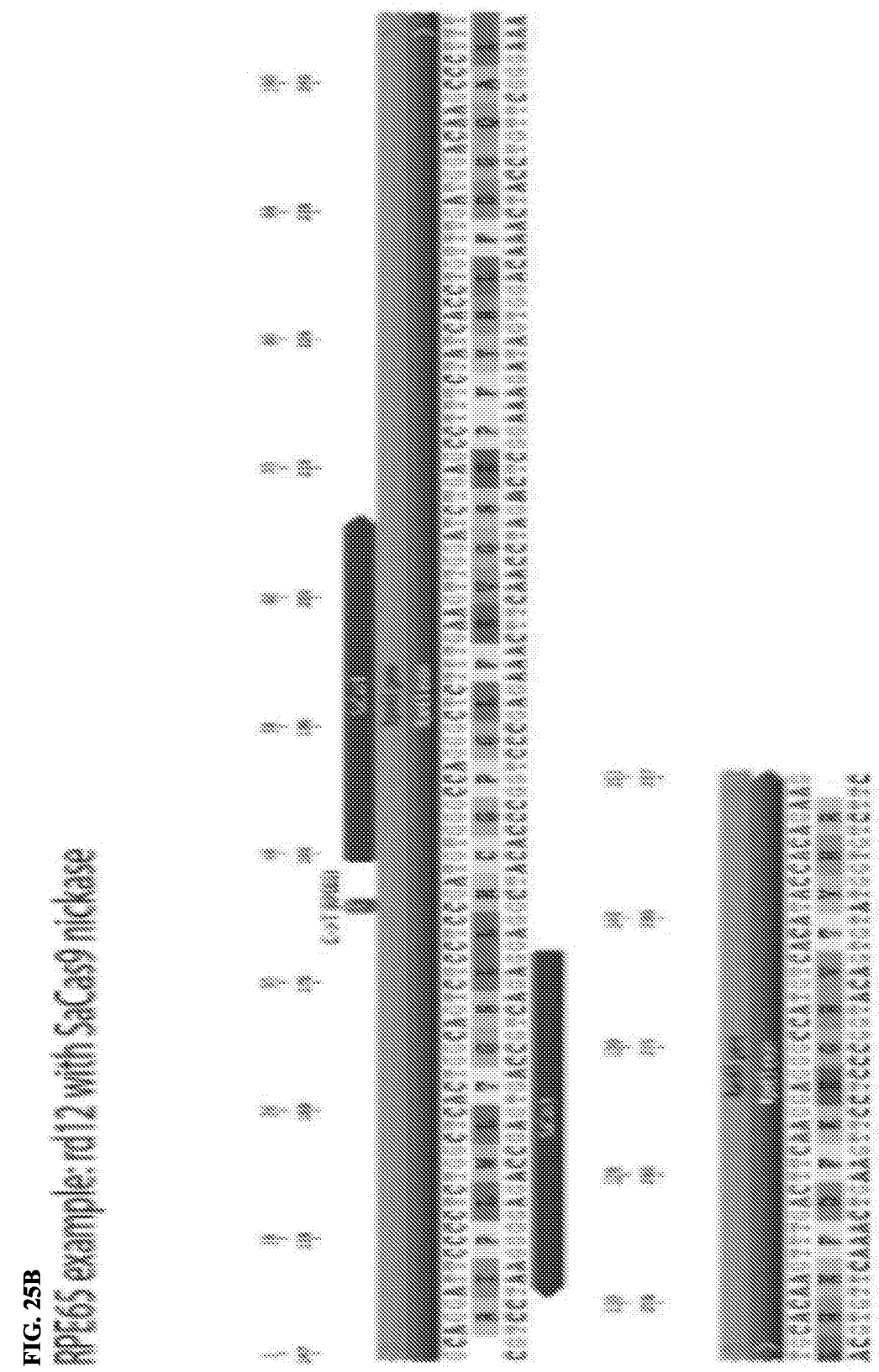


FIG. 25A

FIG. 25B



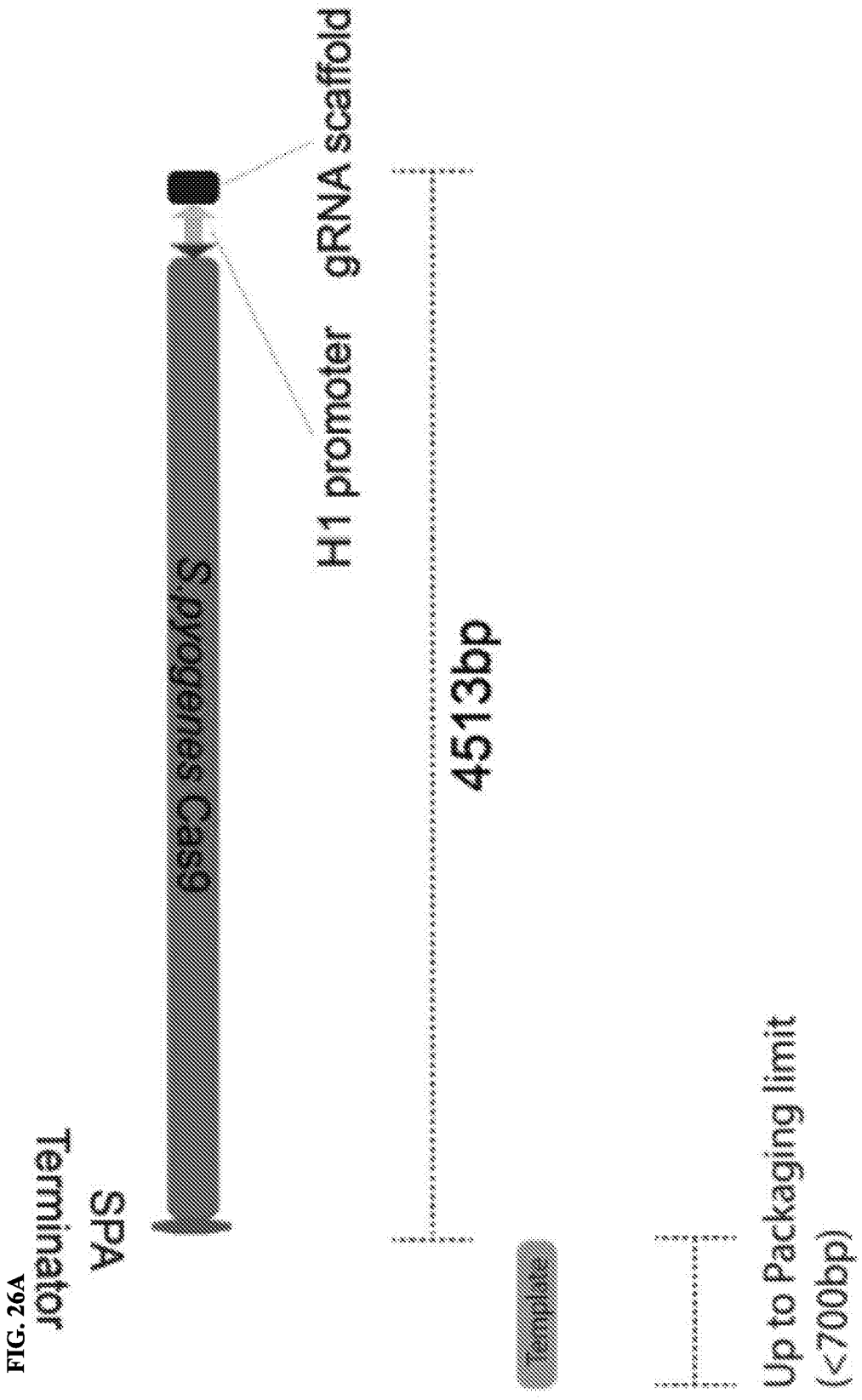

SpCas9
H1 promoter
gRNA scaffold
4513bp
SPA
Terminator
Template
Up to Packaging limit
(<700bp)

FIG. 26A

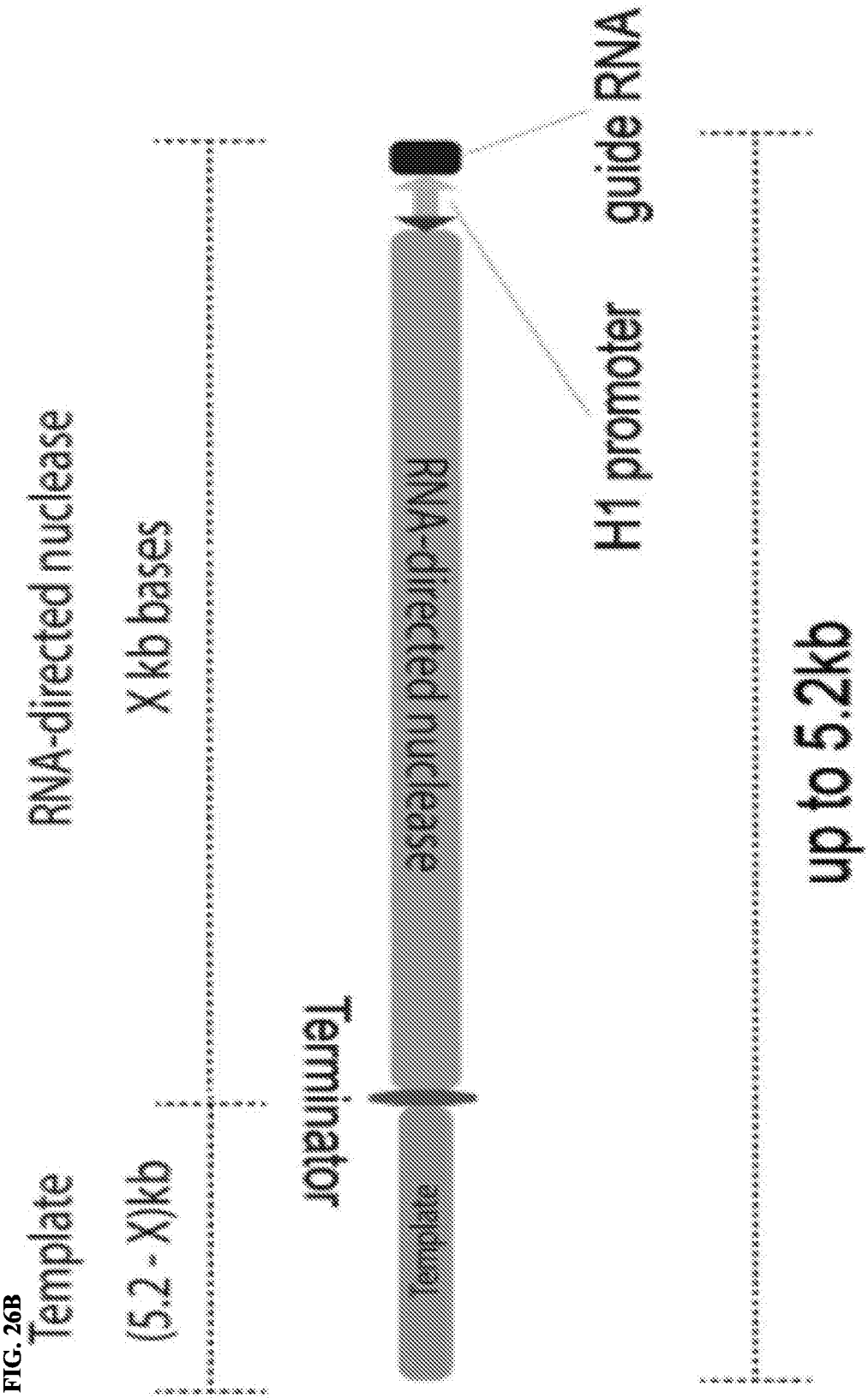

Template
(5.2 - X)kb
Terminator
RNA-directed nuclease
X kb bases
RNA-directed nuclease
H1 promoter
guide RNA
up to 5.2kb

FIG. 26B

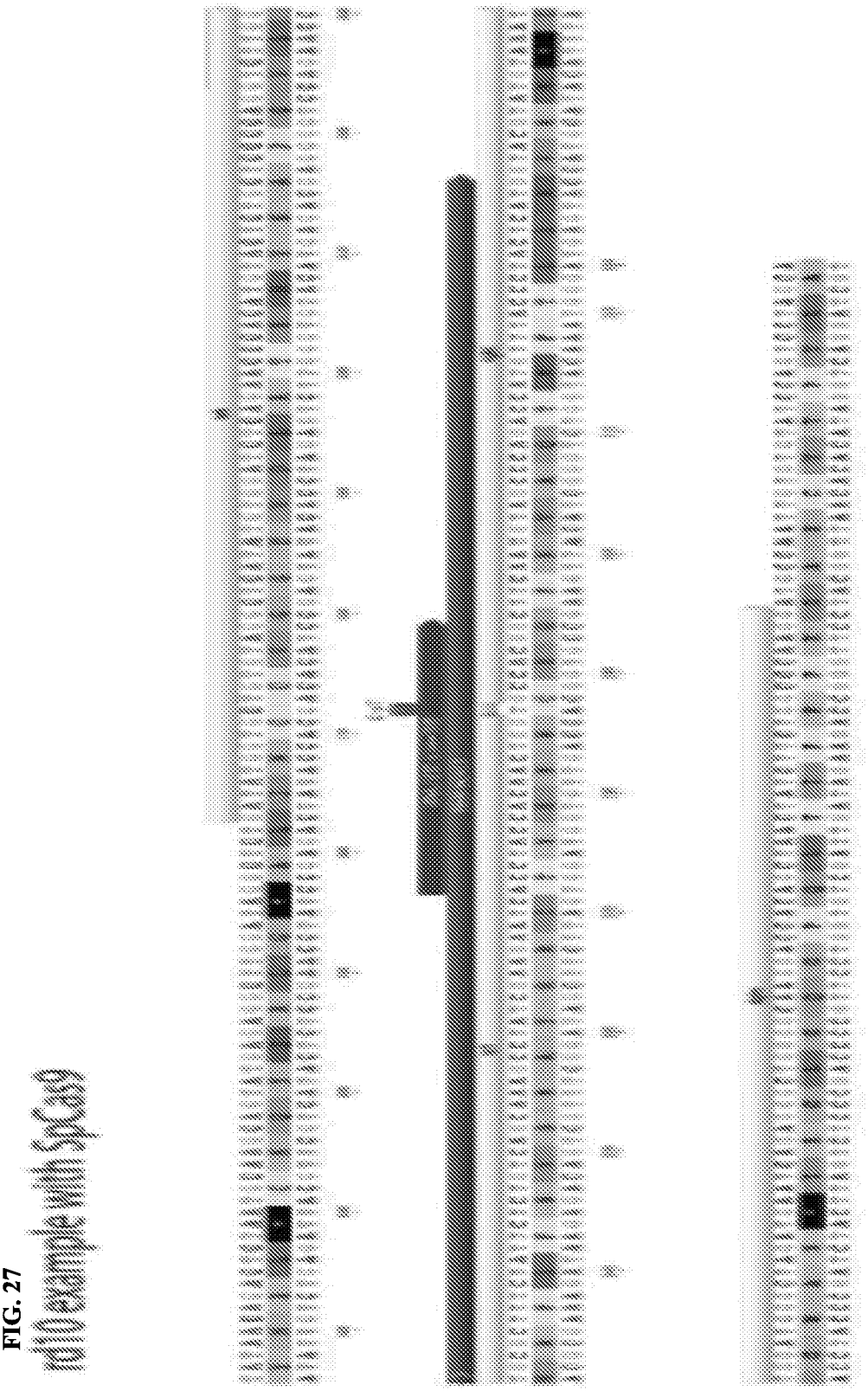


FIG. 27

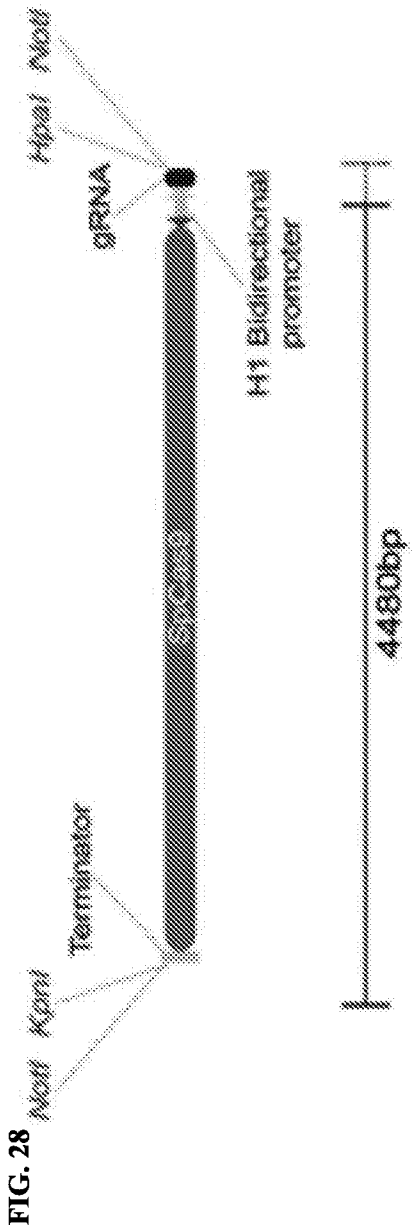


FIG. 28

Gibson Cloning of HDR templates - either KpnI digest (left), or HpaI digest (right)

KpnI (N's = HDR template)

GAGAGTGCACCATAGCGGCCGCGNNNGTACCCACACACAAAAAACCAACACACAG

HpaI (N's = HDR template)

GGCACCGAGTCGGTGCTTTTTTGTTNNNNNNAACGCGGCCGCCTAGAGTCGAC

ncRNA gene	Protein gene	Distance
RPPH1	PARP2	230bp
SRP	RPS29	233bp
7sk1	GSTA4	239bp
SNAR-G1	CGB1	308bp
SNAR	CGB2	308bp
RMRP	CCDC107	361bp
tRNA(Lys)	ALOXE3	376bp
RNU6-9	MED16	412bp
tRNA (Gly)	DPP9	484bp
SNORD13	C8orf41	847bp

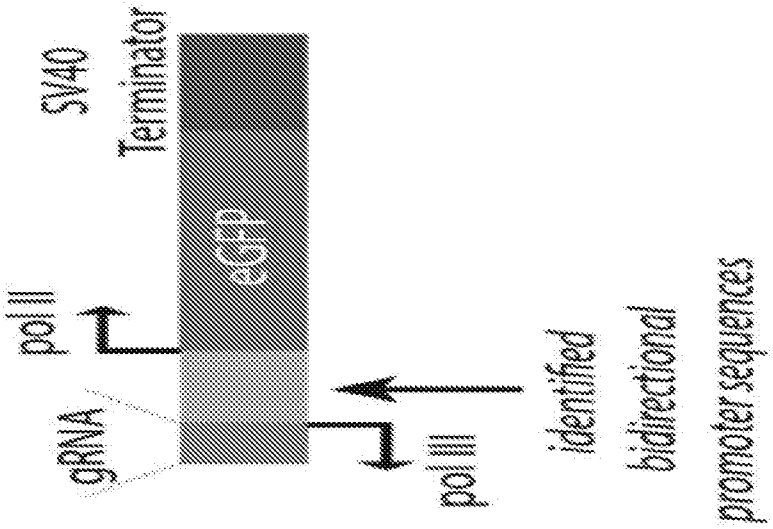


FIG. 29A

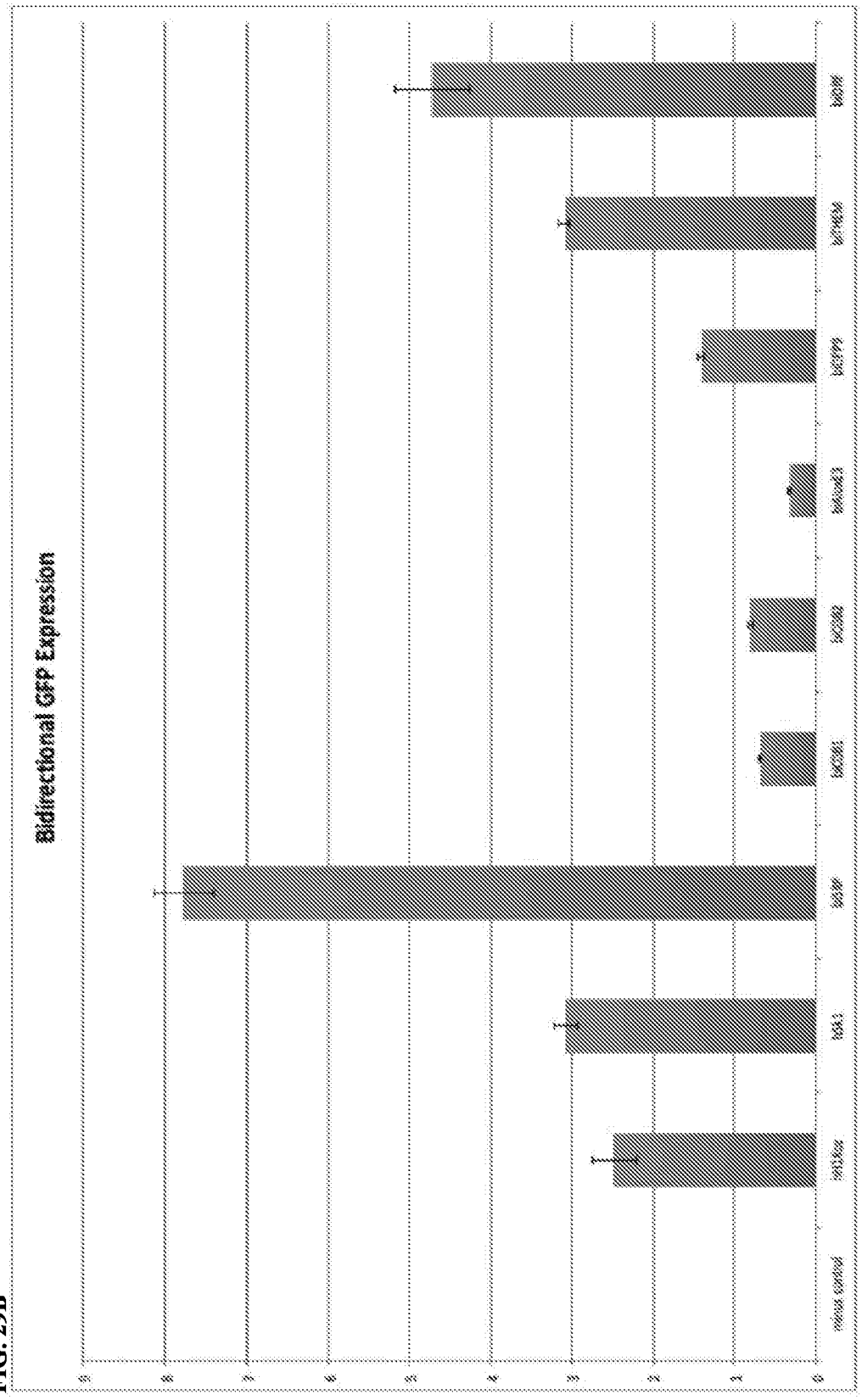


FIG. 29B

ncRNA gene	Protein gene	Distance	Organism
RPPH1	PARP2	230bp	Human
RPPH1	PARP2	172bp	Mouse
RPPH1	PARP2	201bp	Rat

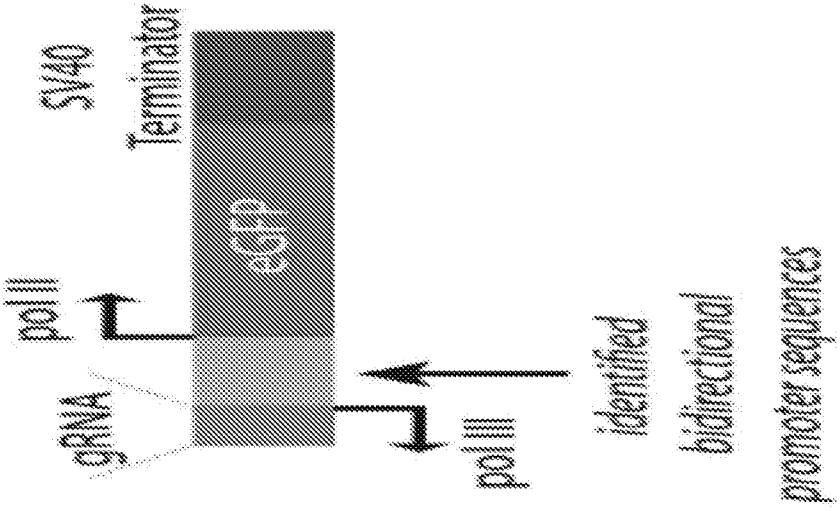


FIG. 30A

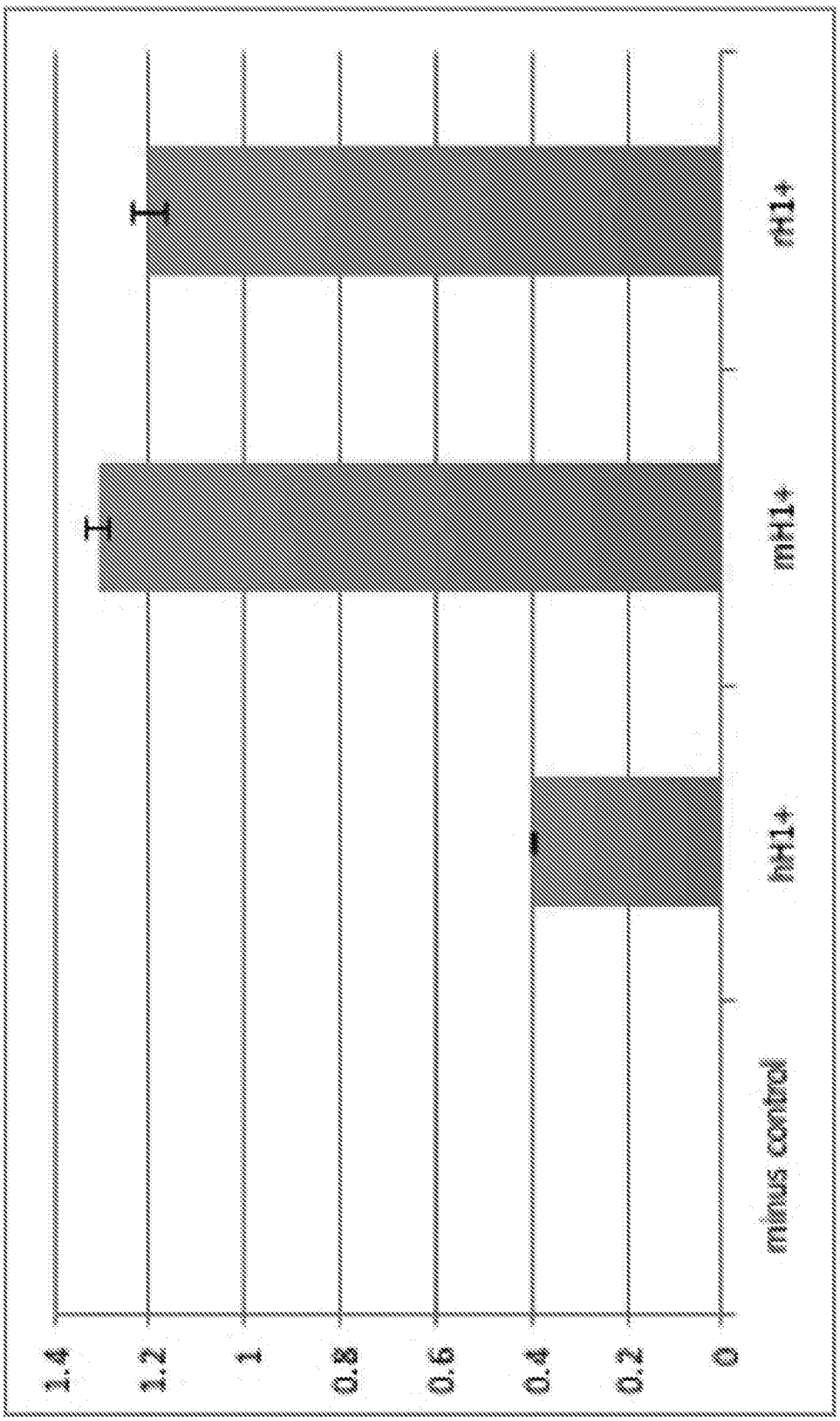


FIG. 30B