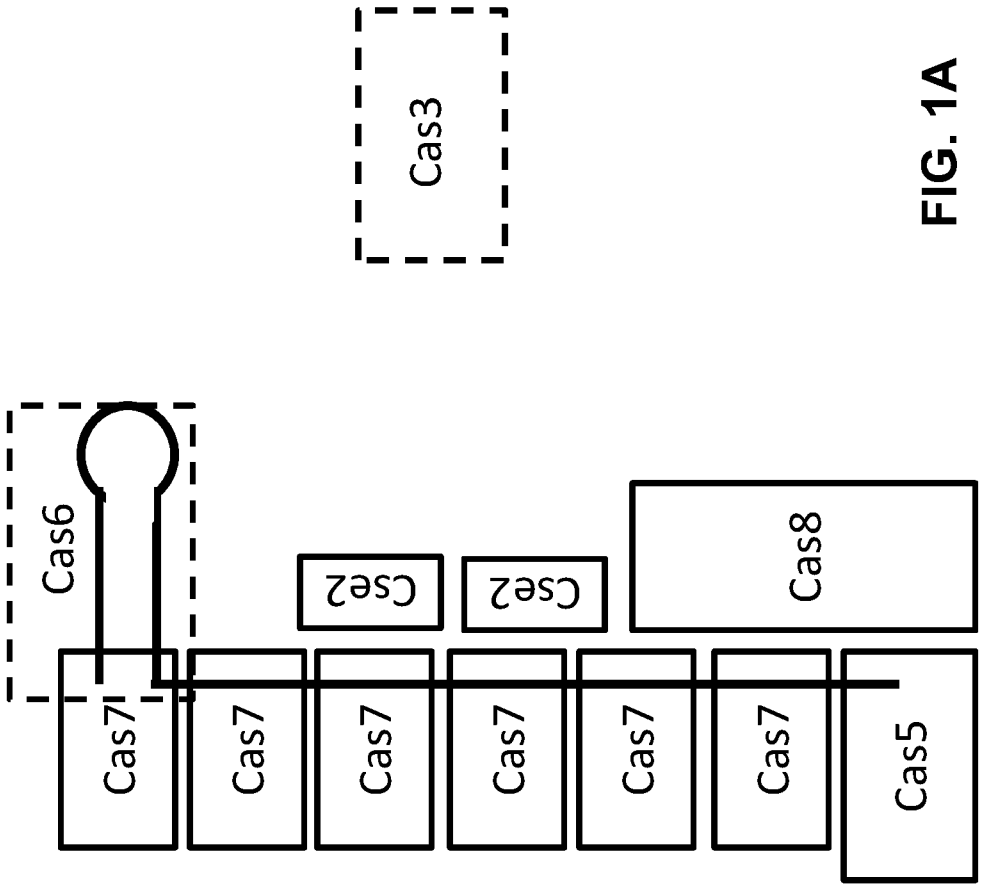


FIG. 1A

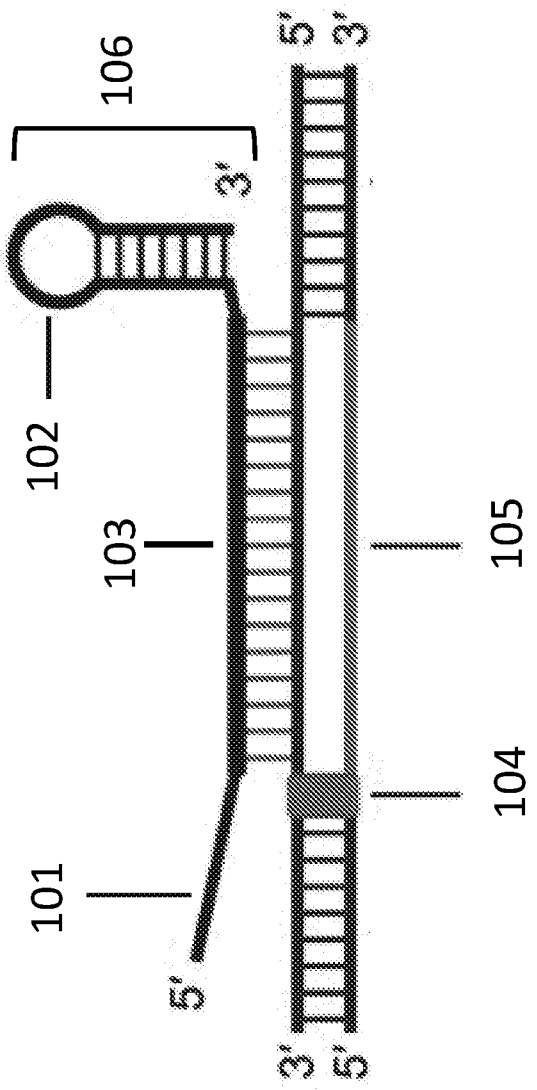


FIG. 1B

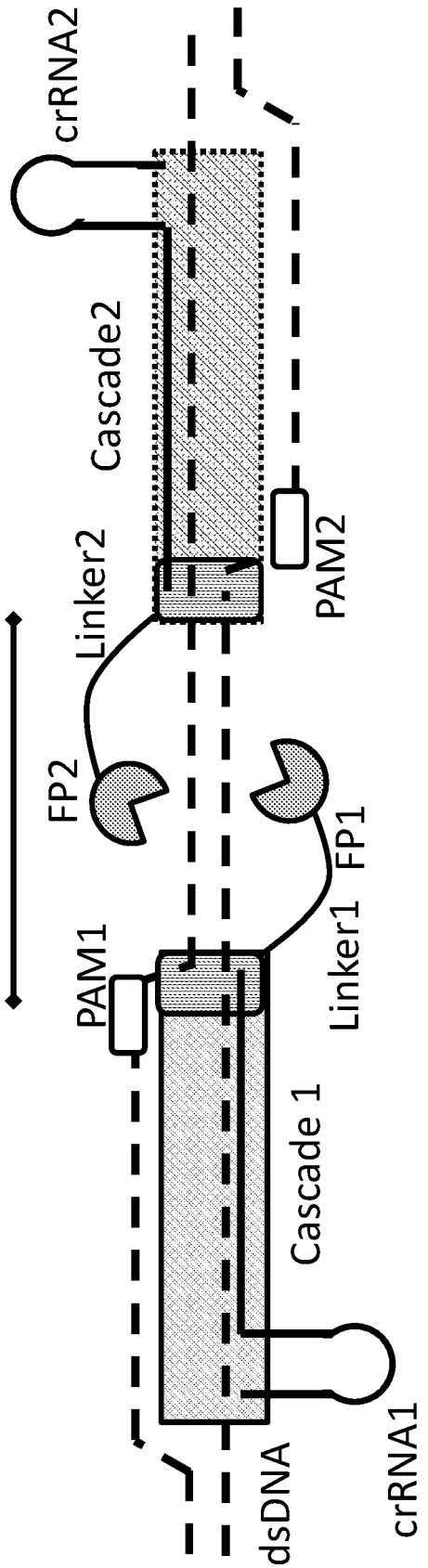

crRNA2
Cascade2
Linker2
PAM2
FP2
FP1
PAM1
Linker1
Cascade 1
dsDNA
crRNA1

FIG. 2A

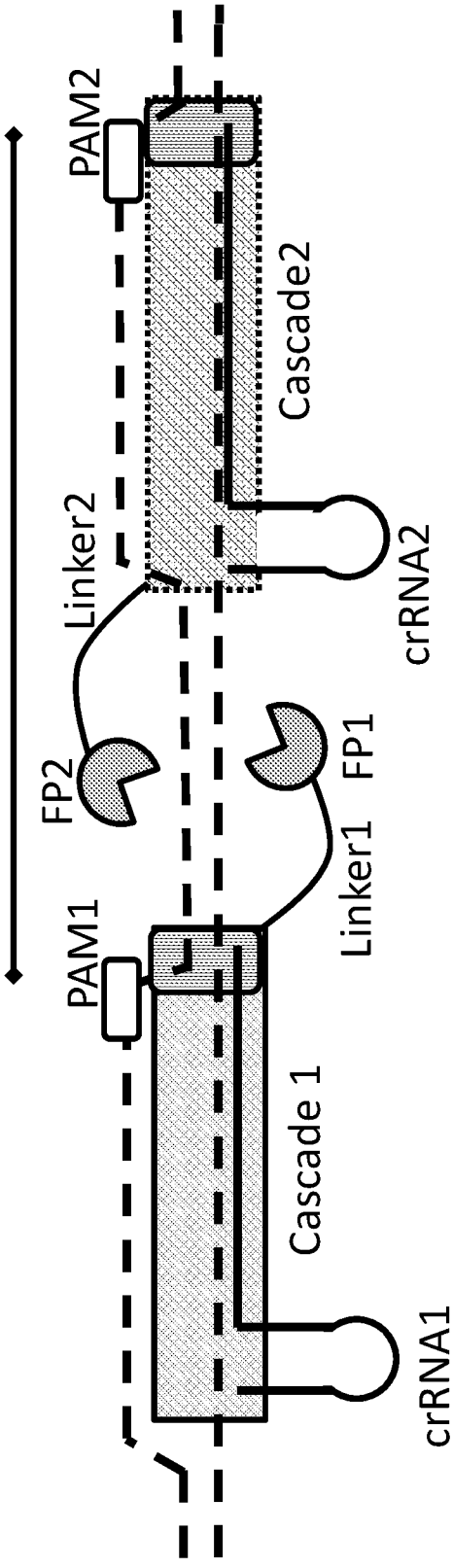

PAM2
Cascade2
Linker2
crRNA2
FP2
FP1
PAM1
Linker1
Cascade 1
crRNA1

FIG. 2B

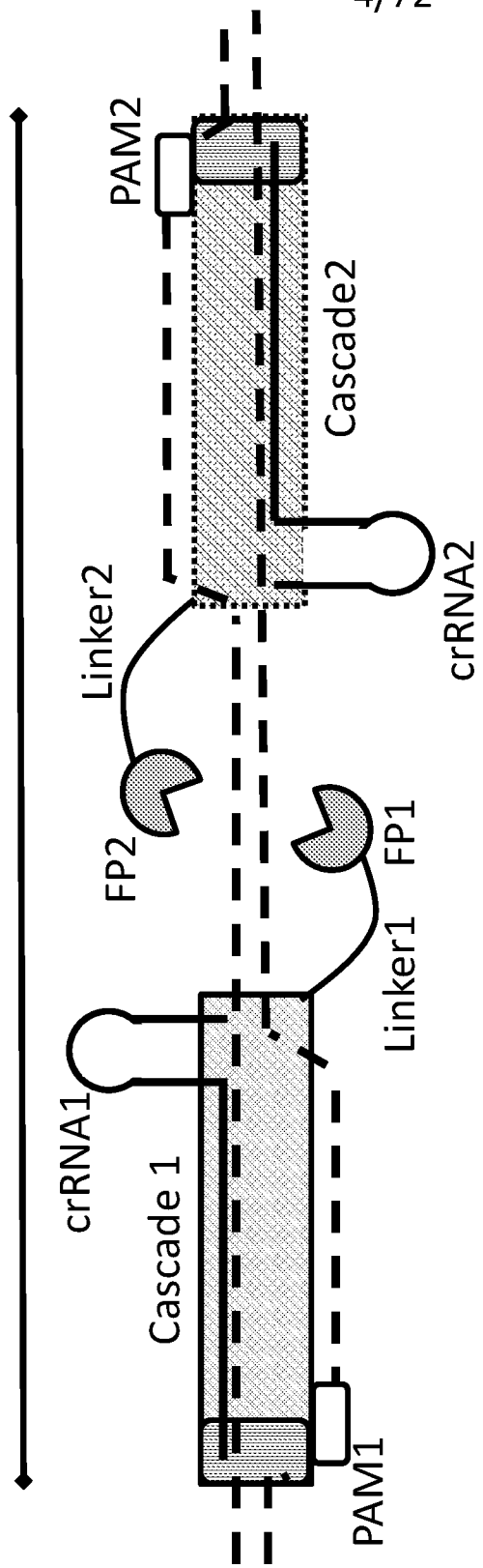

PAM2
Cascade2
Linker2
crRNA2
FP2
FP1
Linker1
crRNA1
Cascade 1
PAM1

FIG. 2C

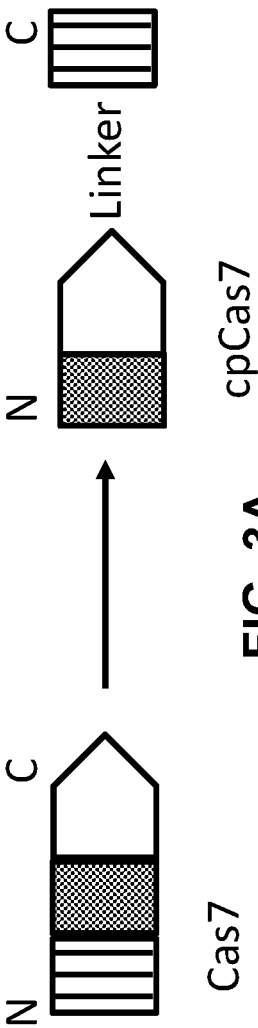


FIG. 3A

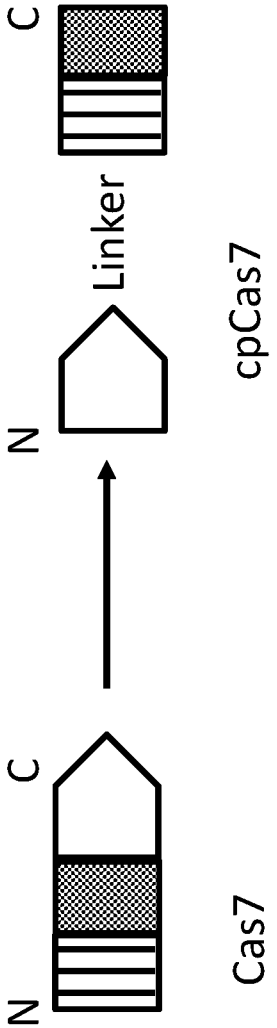


FIG. 3B

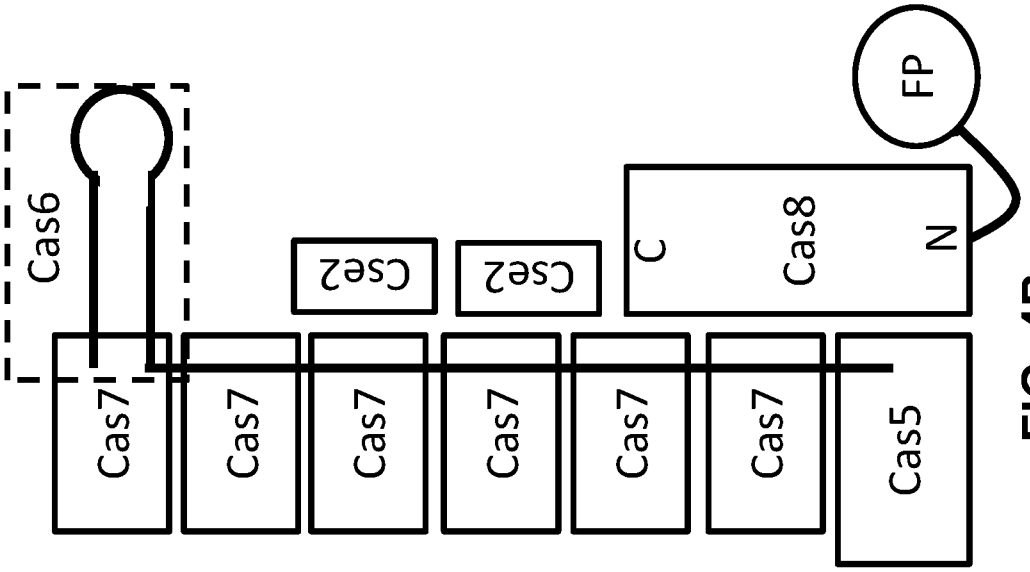

Cas6
Cas7
Cas7
Cas7
Cas7
Cas7
Cas7
Cas5
Cse2
Cse2
C
Cas8
N
FP

FIG. 4B

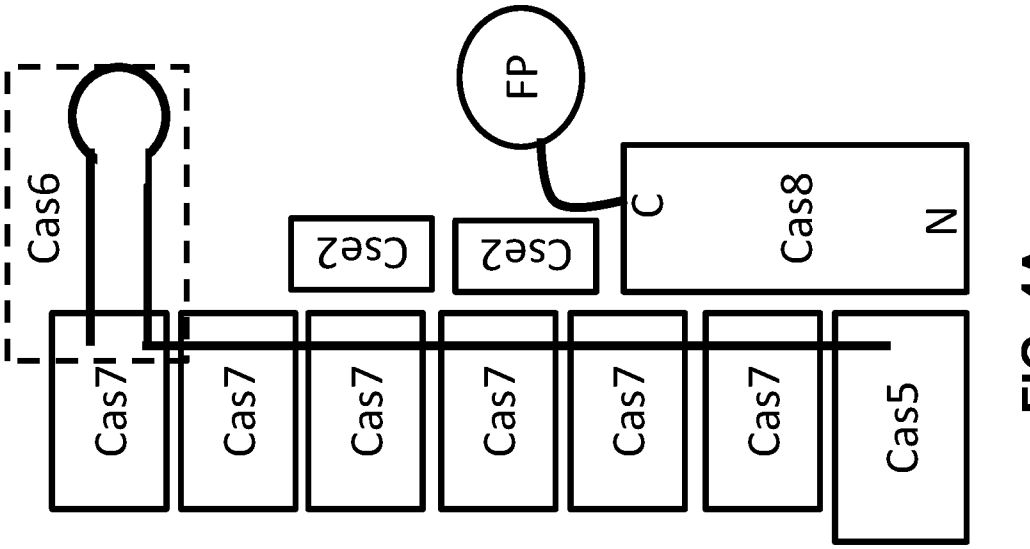

Cas6
Cas7
Cas7
Cas7
Cas7
Cas7
Cas7
Cas5
Cse2
Cse2
C
Cas8
N
FP

FIG. 4A

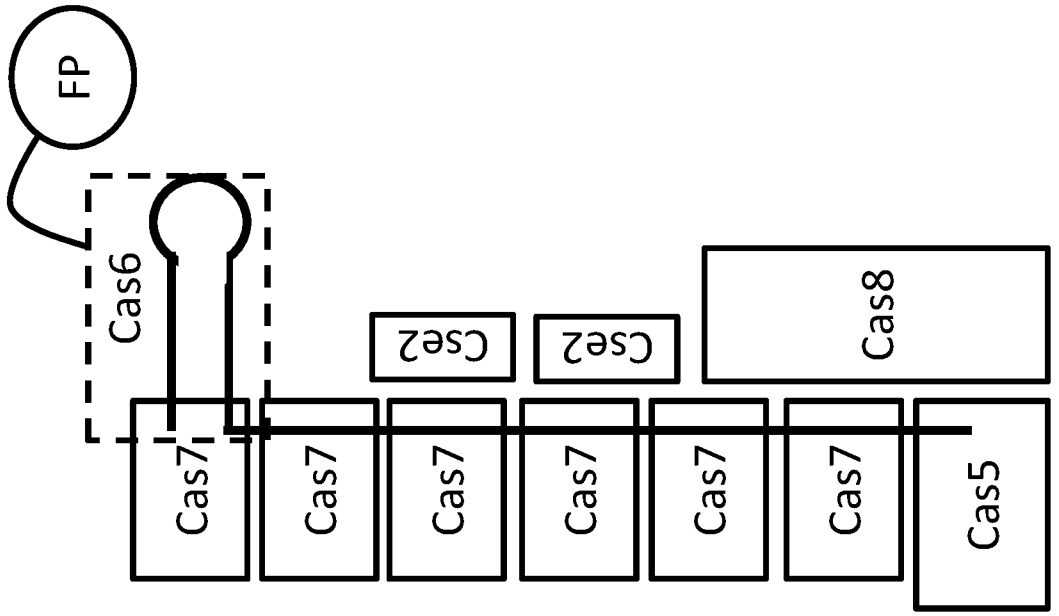

FP
Cas6
Cas7
Cas7
Cas7
Cas7
Cas7
Cas7
Cas5
Cse2
Cse2
Cas8

FIG. 5B

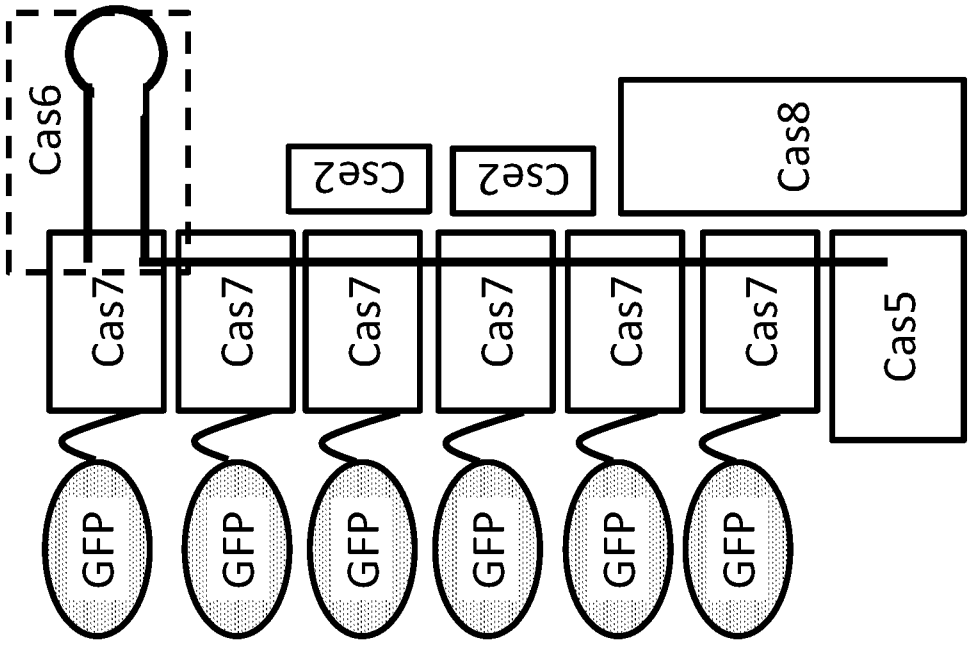

Cas6
Cas7
Cas7
Cas7
Cas7
Cas7
Cas7
Cas5
Cse2
Cse2
Cas8
GFP
GFP
GFP
GFP
GFP
GFP

FIG. 5A

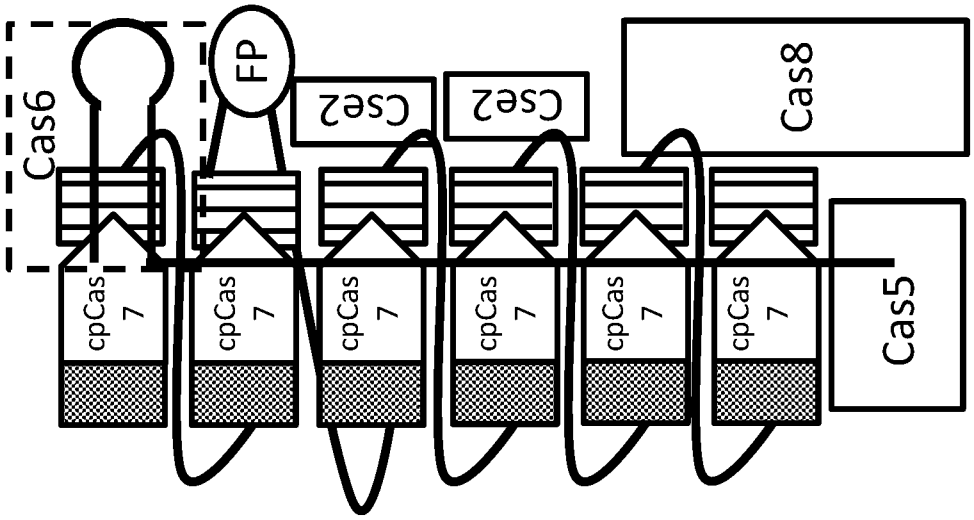


FIG. 6C

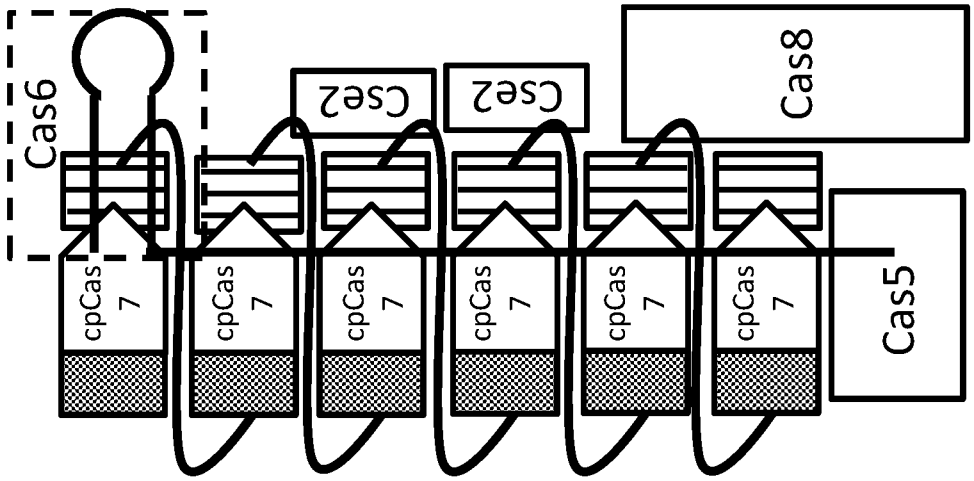


FIG. 6B

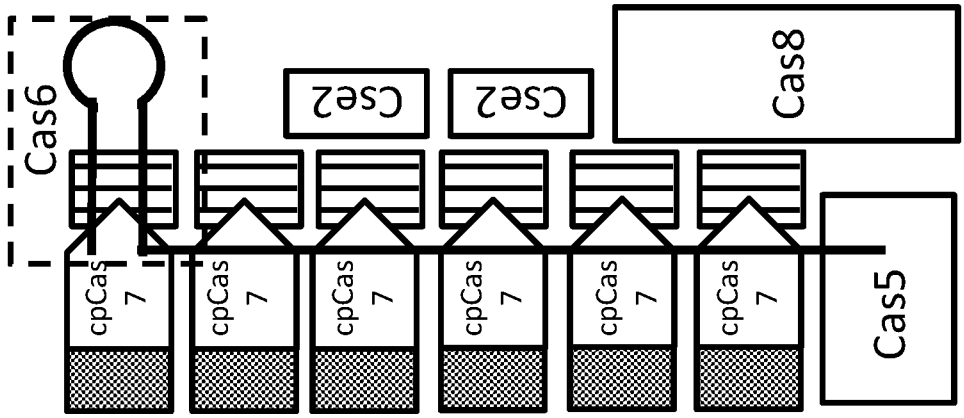


FIG. 6A

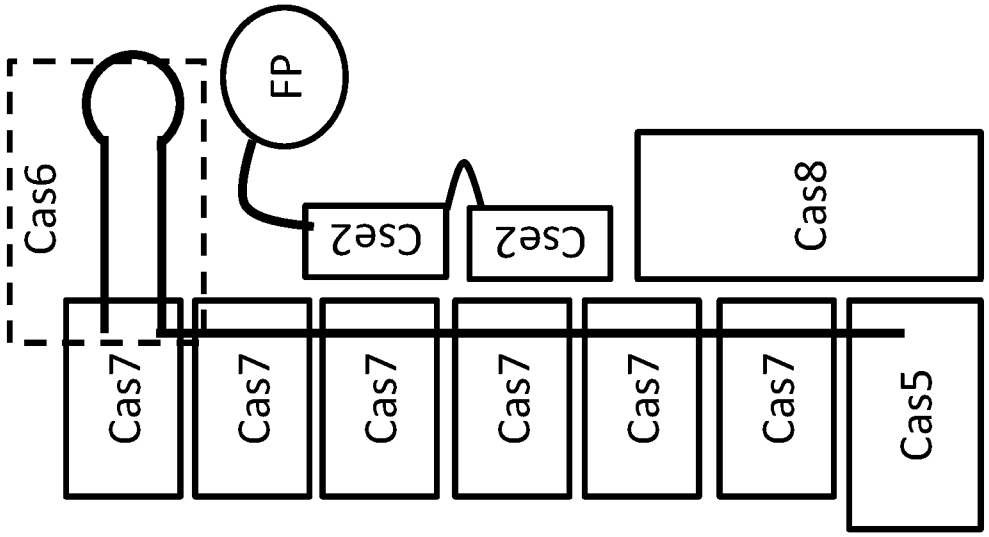

Cas6
FP
Cse2
Cse2
Cas8
Cas7
Cas7
Cas7
Cas7
Cas7
Cas7
Cas5

FIG. 7B

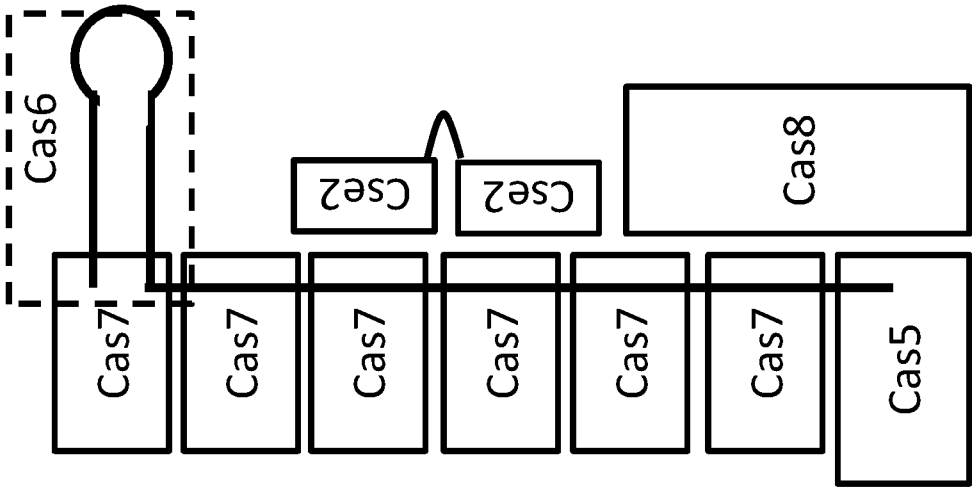

Cas6
Cse2
Cse2
Cas8
Cas7
Cas7
Cas7
Cas7
Cas7
Cas7
Cas5

FIG. 7A

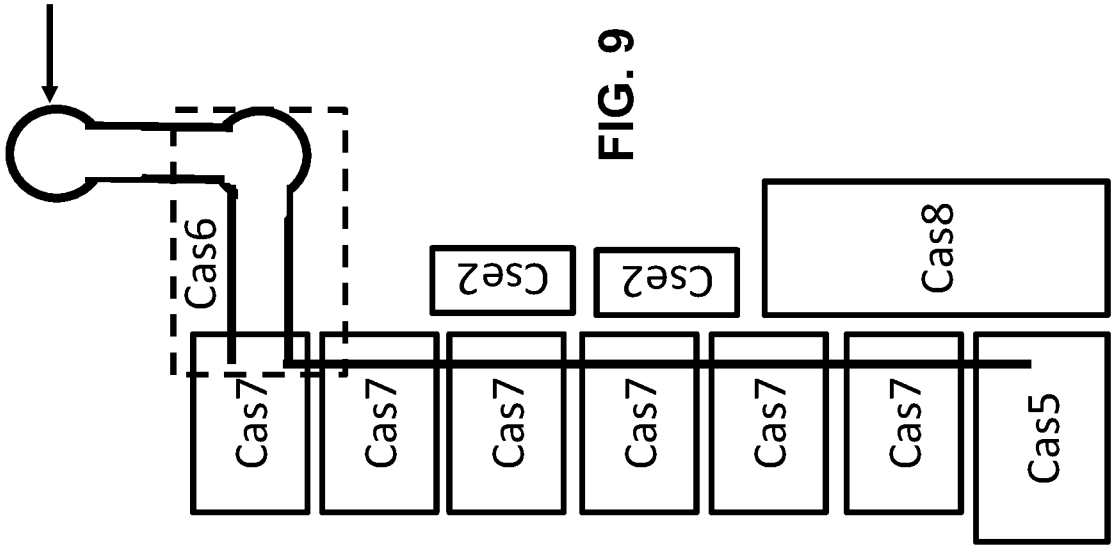

Cas6
Cas7
Cas7
Cas7
Cas7
Cas7
Cas7
Cas5
Cse2
Cse2
Cas8

FIG. 9

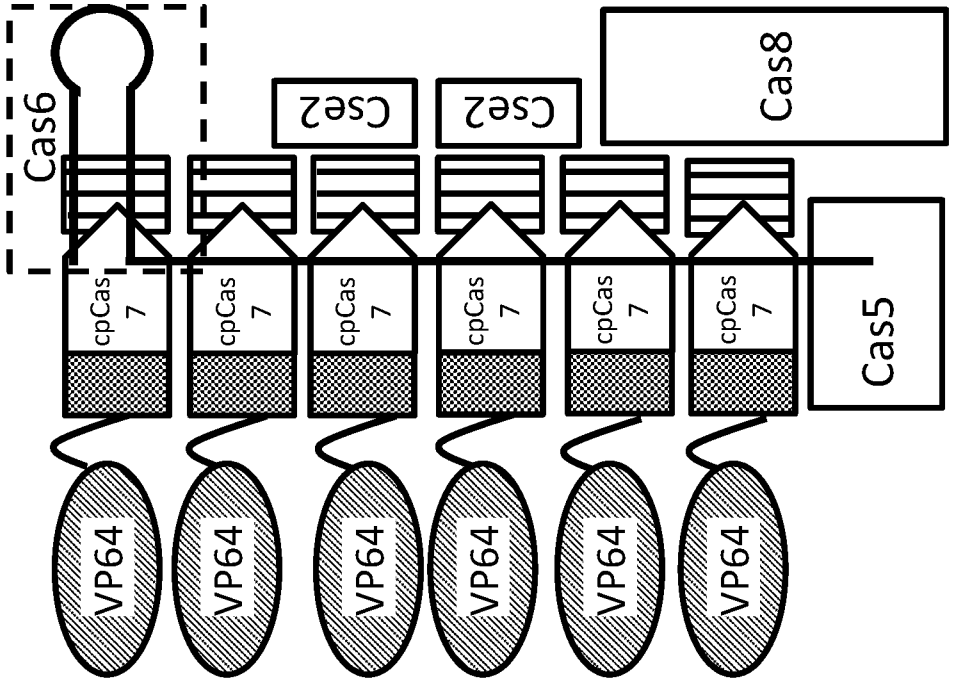

Cas6
cpCas 7
cpCas 7
cpCas 7
cpCas 7
cpCas 7
cpCas 7
Cas5
Cse2
Cse2
Cas8
VP64
VP64
VP64
VP64
VP64
VP64

FIG. 8

FIG. 10B

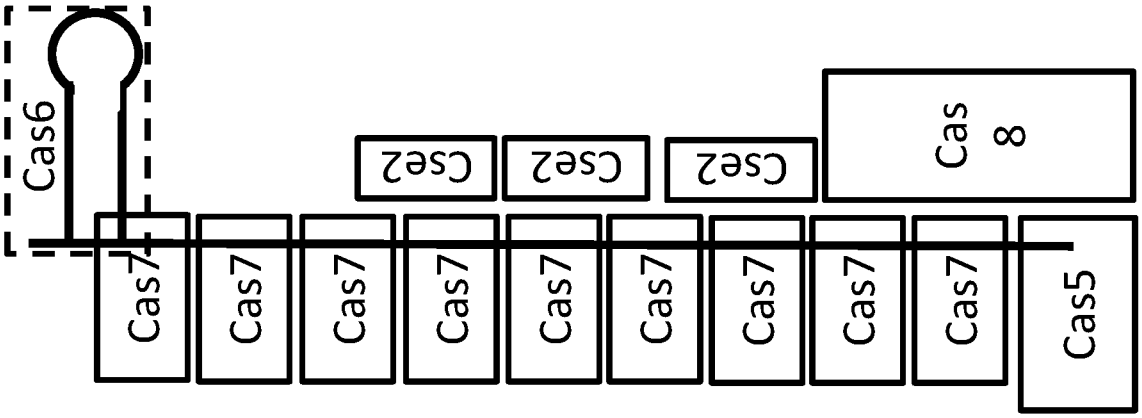
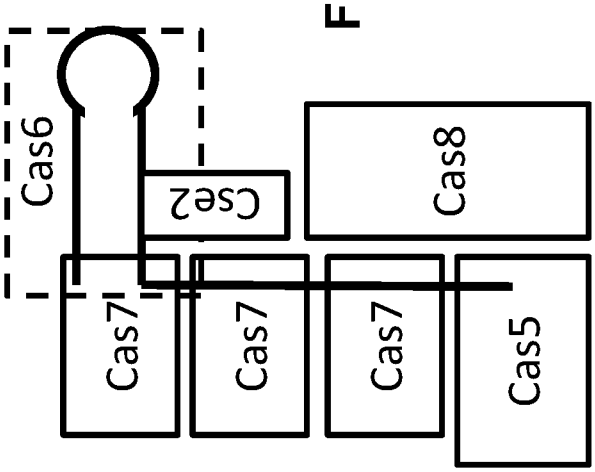


FIG. 10A



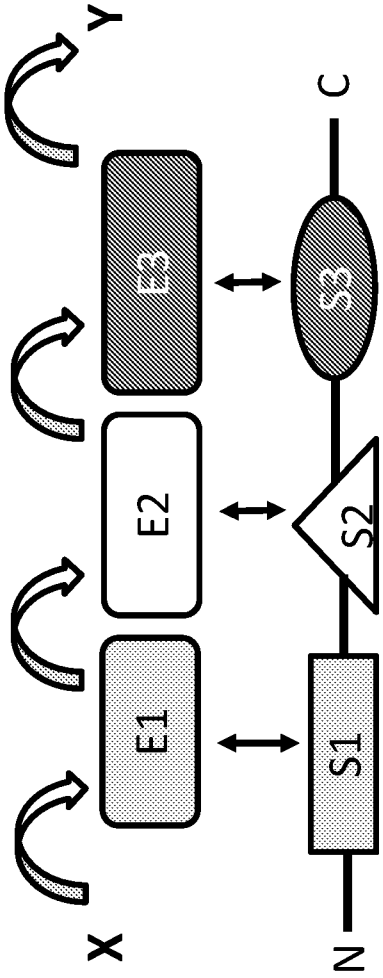


FIG. 11A

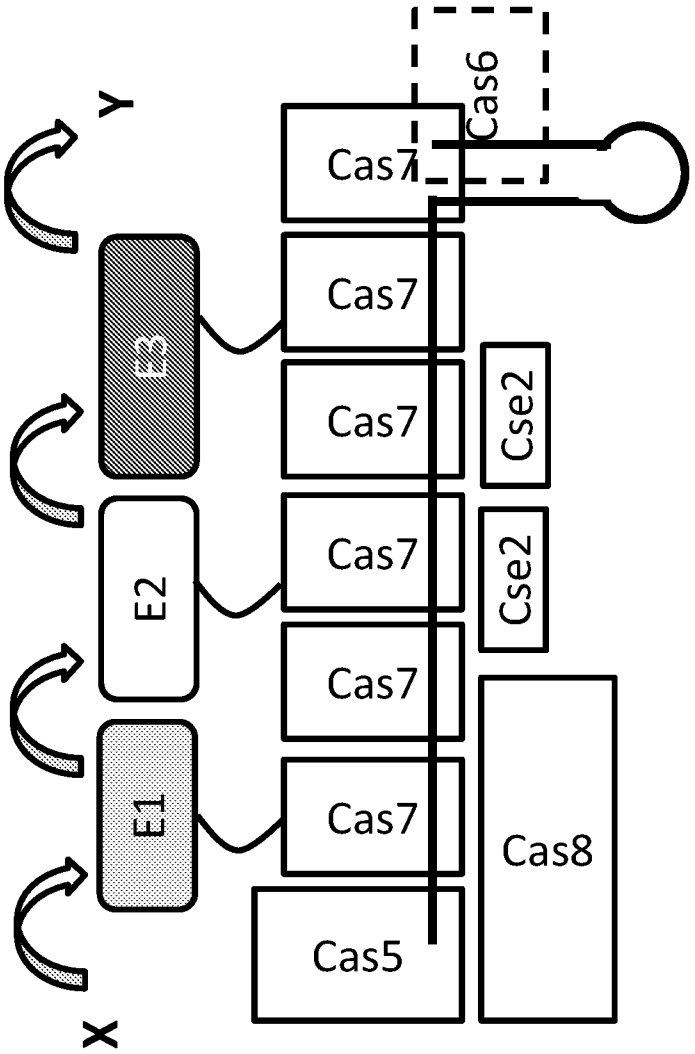


FIG. 11B

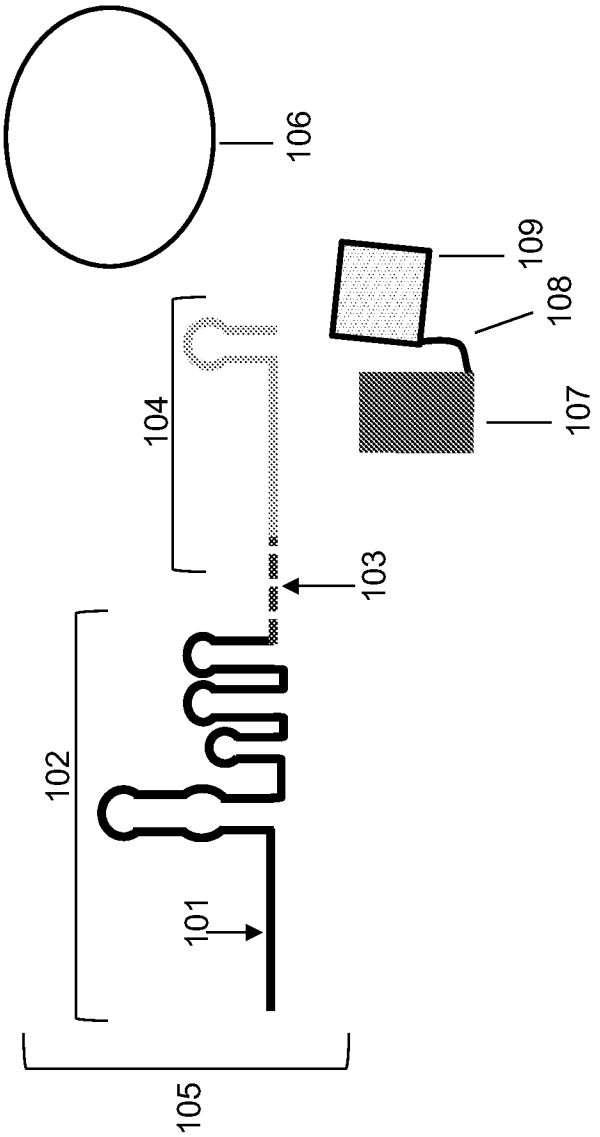


FIG. 12A

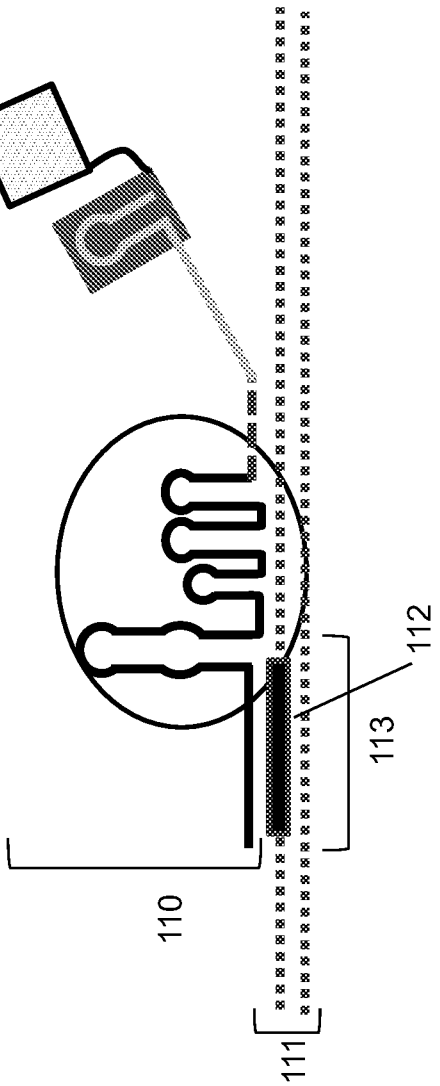


FIG. 12B

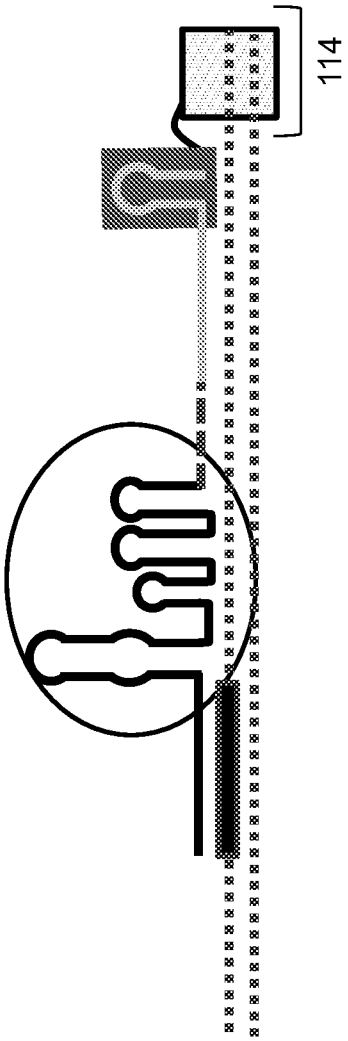


FIG. 12C

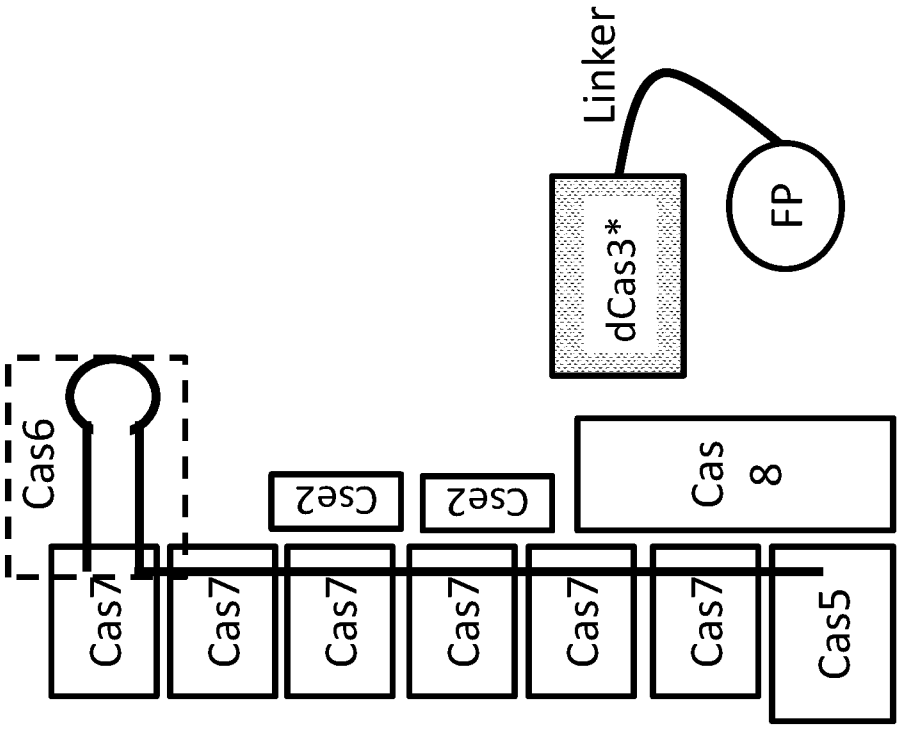


FIG. 13B

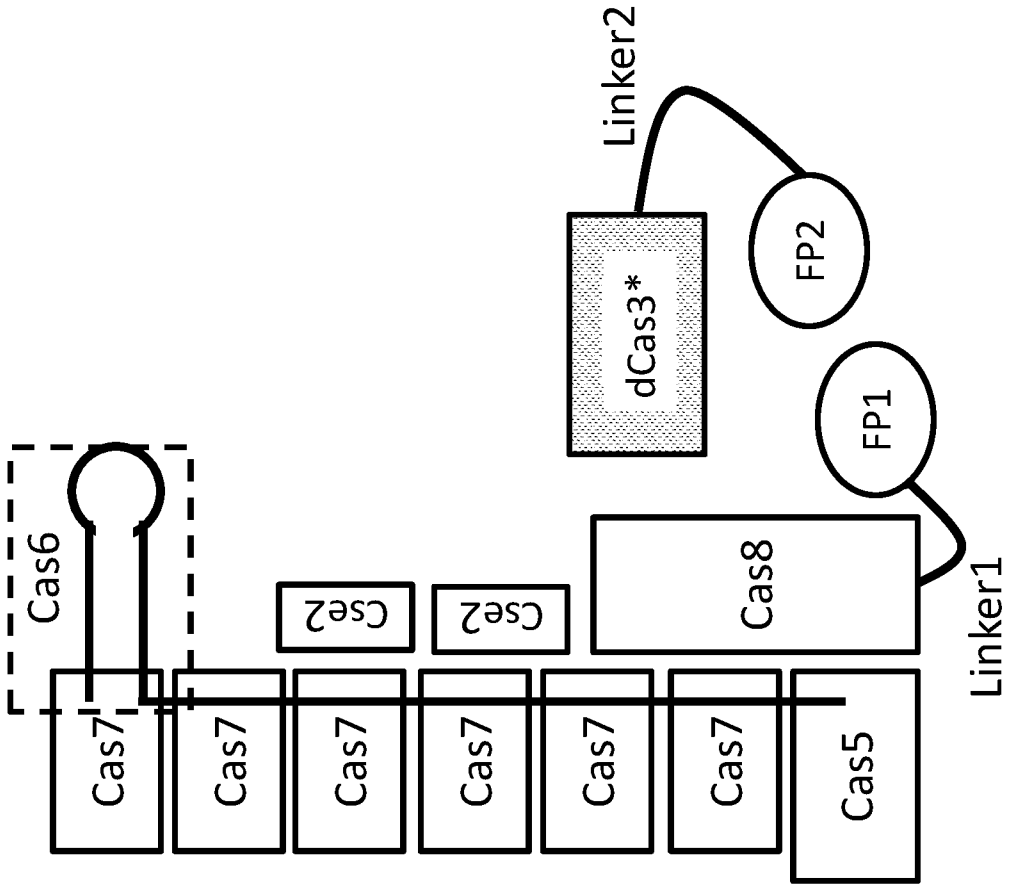
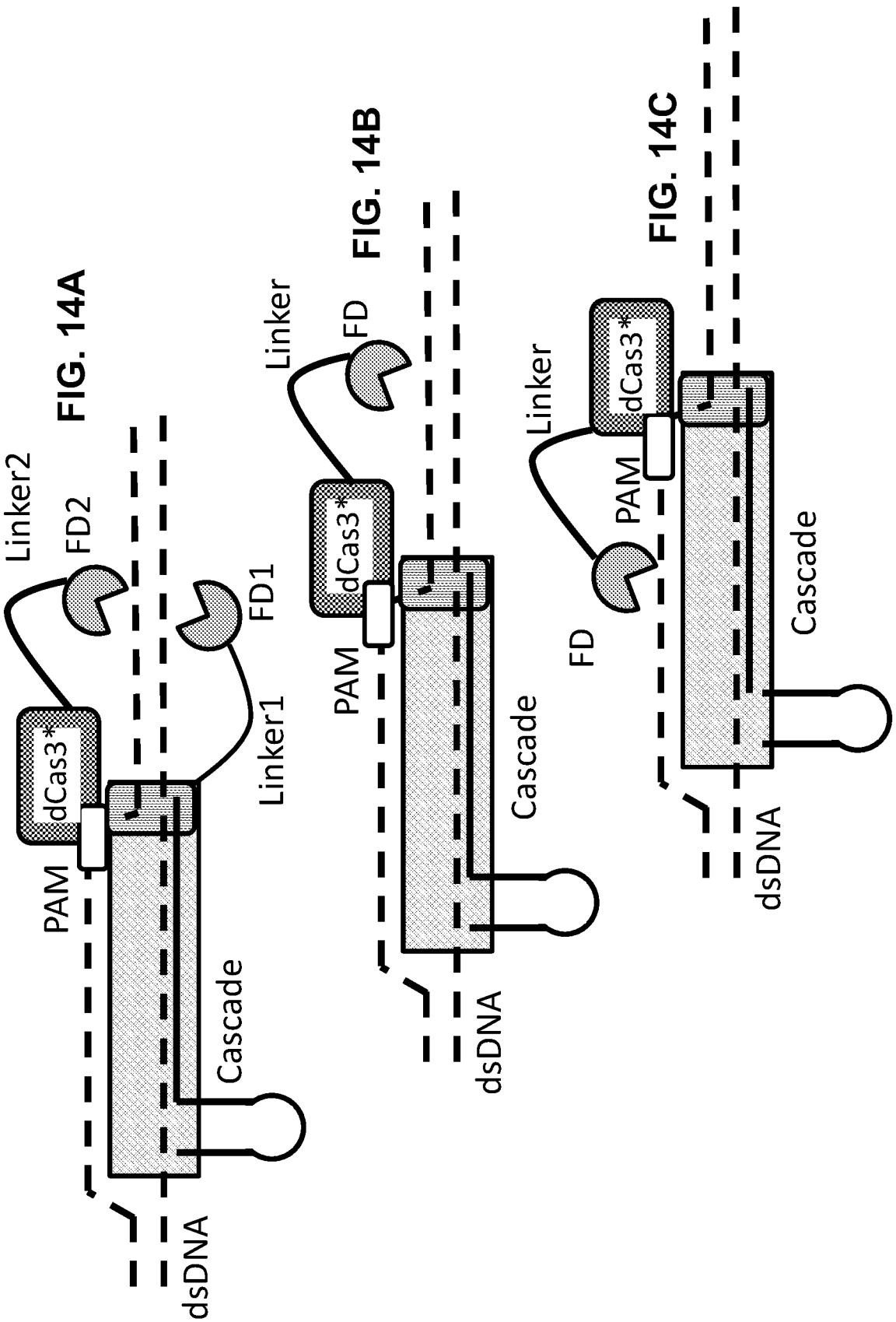


FIG. 13A


FIG. 14A
Linker2
FD2
FD1
Linker1
dCas3*
PAM
Cascade
dsDNA
FIG. 14B
Linker
FD
dCas3*
PAM
Cascade
dsDNA
FIG. 14C
Linker
FD
dCas3*
PAM
Cascade
dsDNA

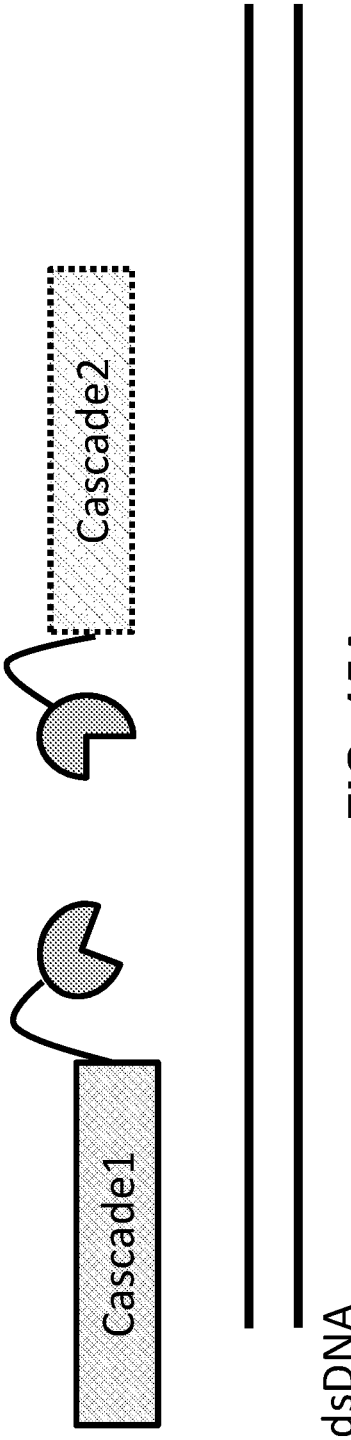


FIG. 15A

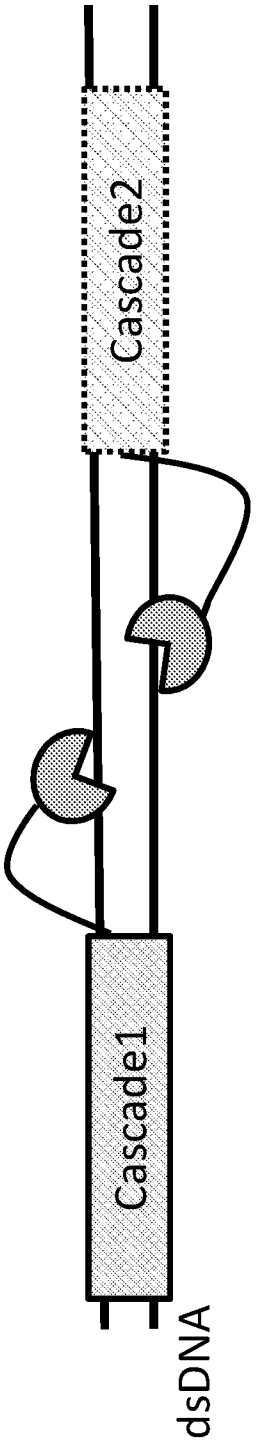


FIG. 15B

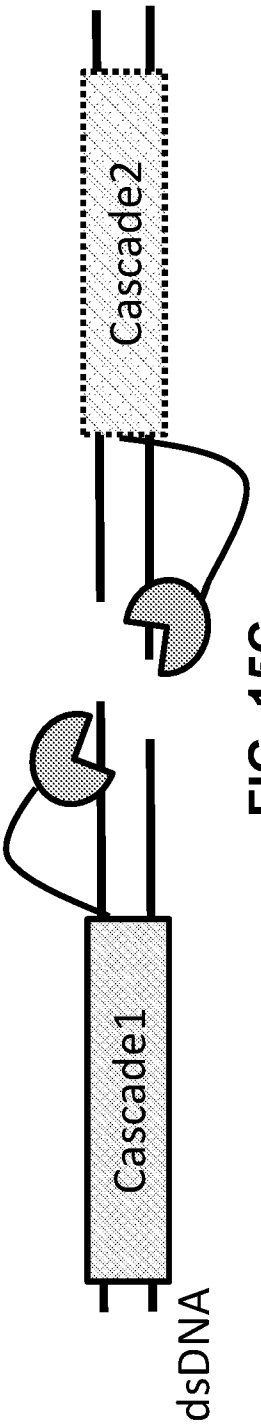


FIG. 15C

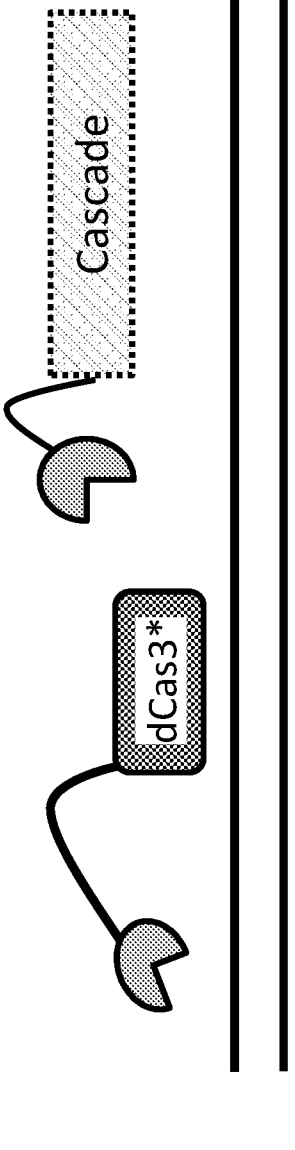


FIG. 16A

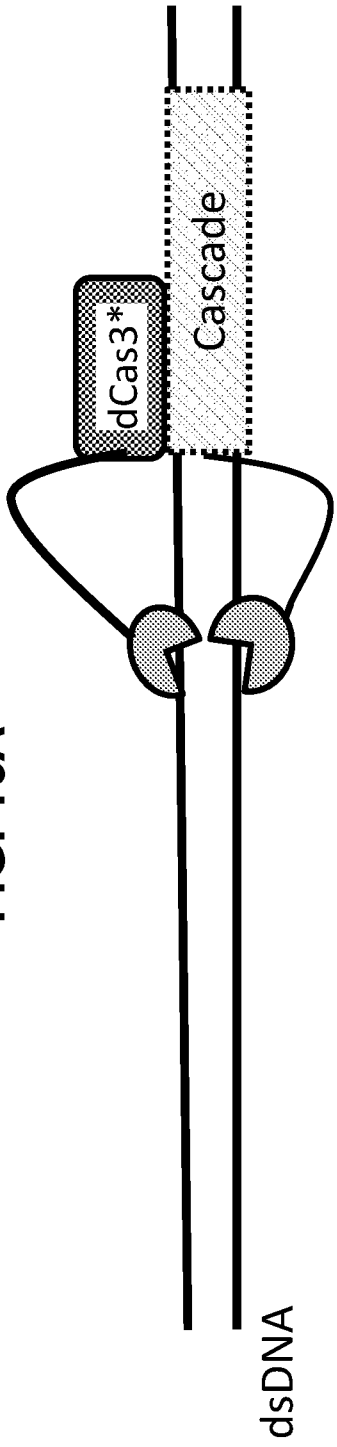


FIG. 16B

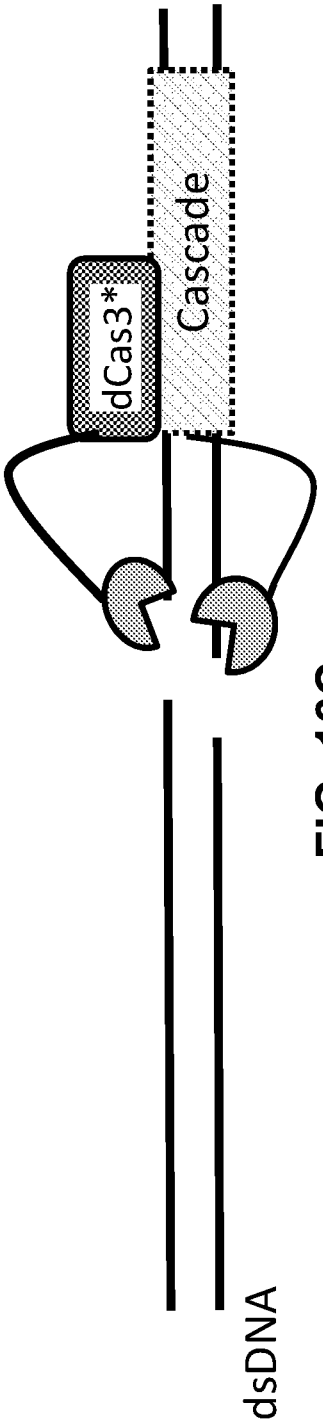


FIG. 16C

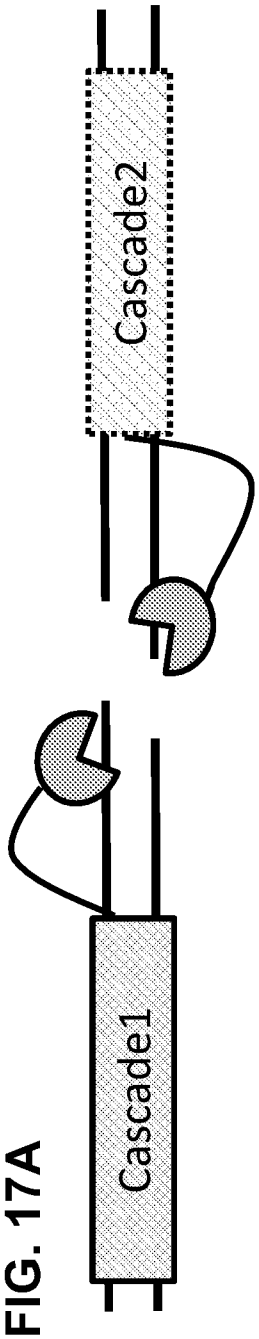


FIG. 17A

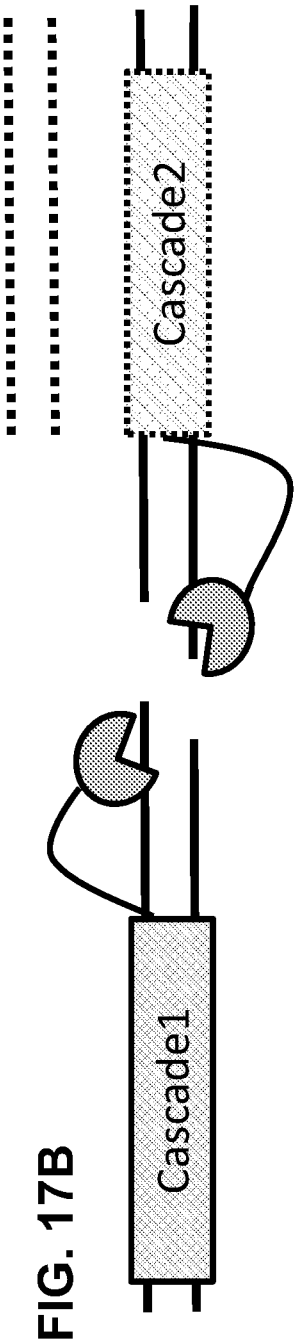


FIG. 17B

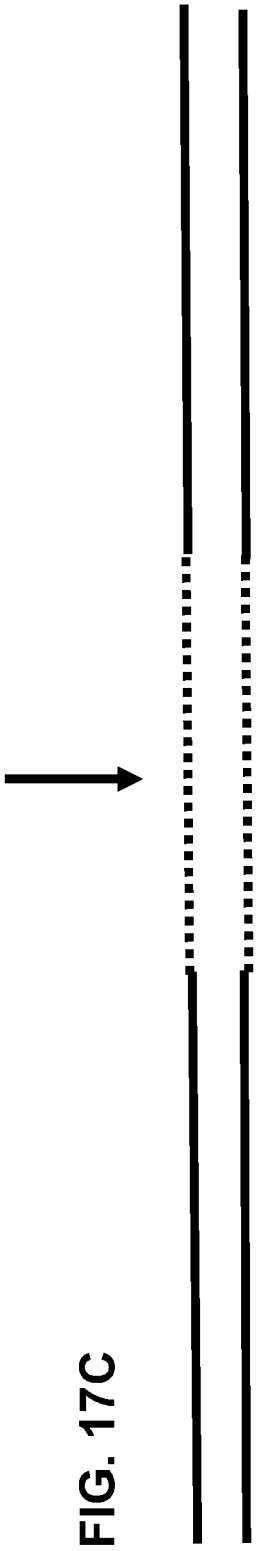
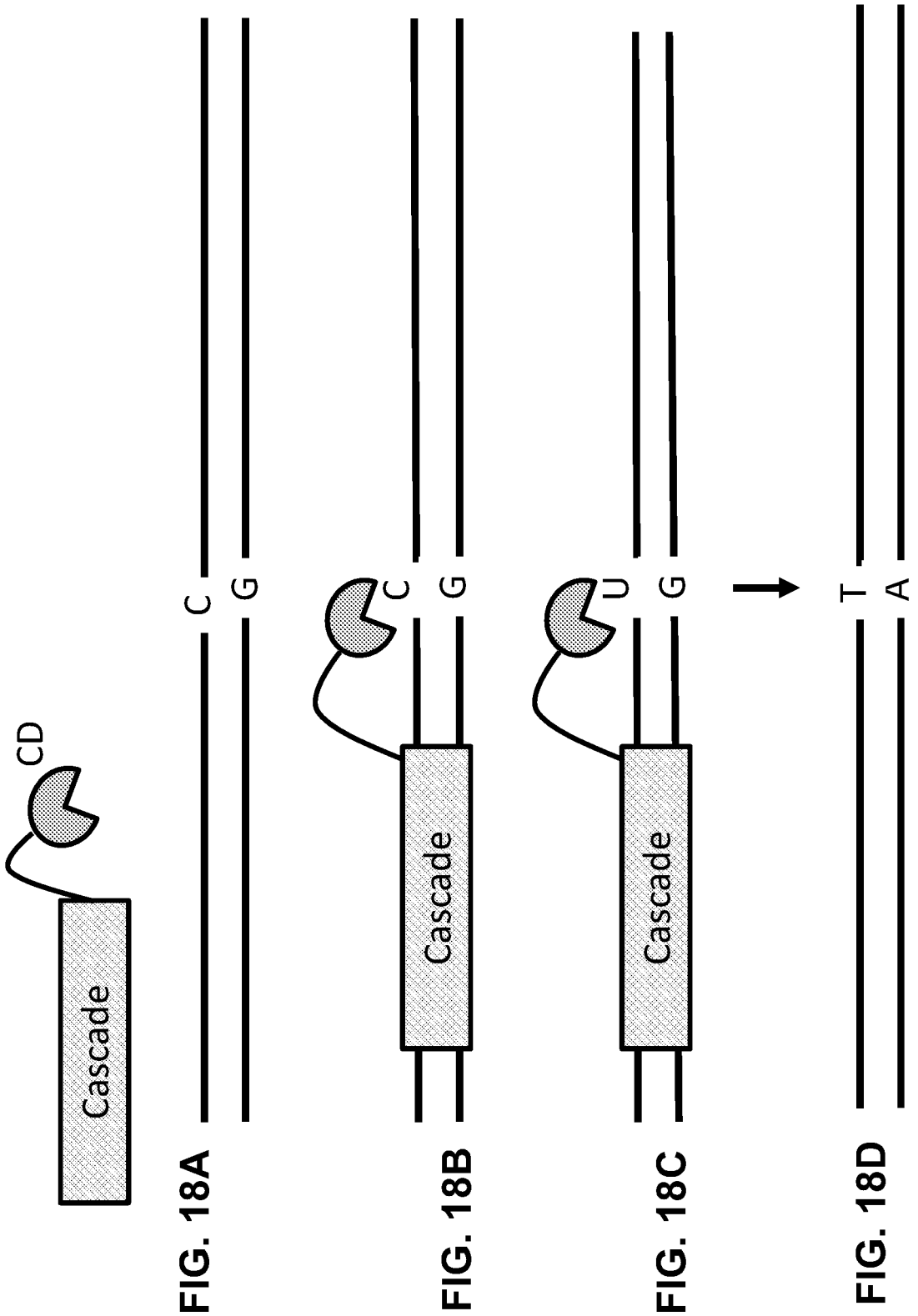


FIG. 17C


CD
Cascade
C
G
FIG. 18A
C
G
Cascade
FIG. 18B
U
G
Cascade
FIG. 18C
T
A
FIG. 18D

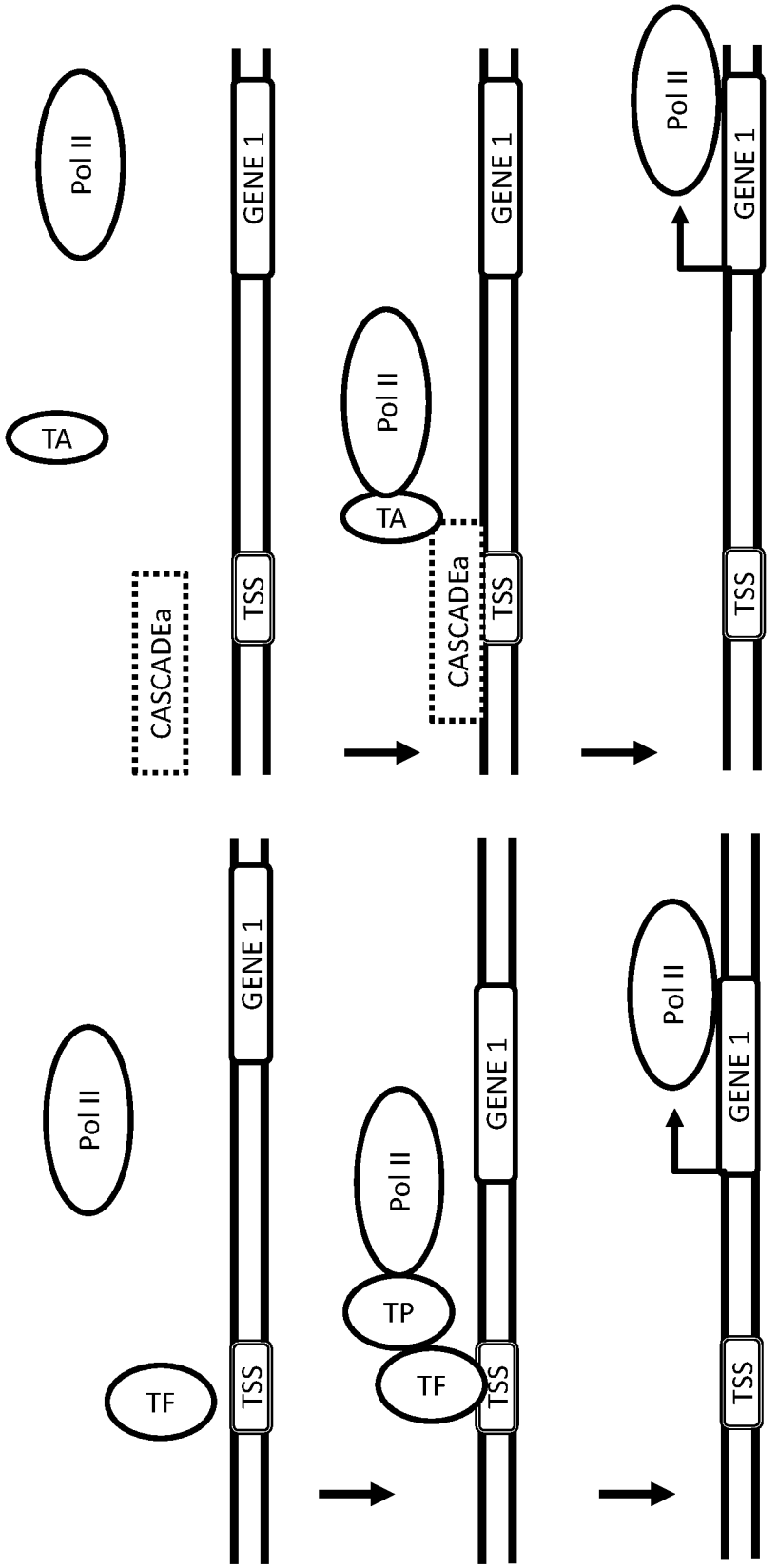


FIG. 19B

FIG. 19A

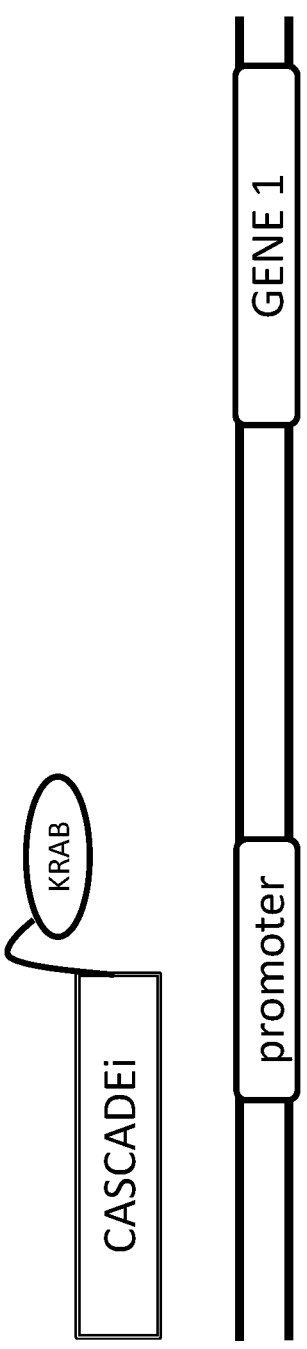


FIG. 20A

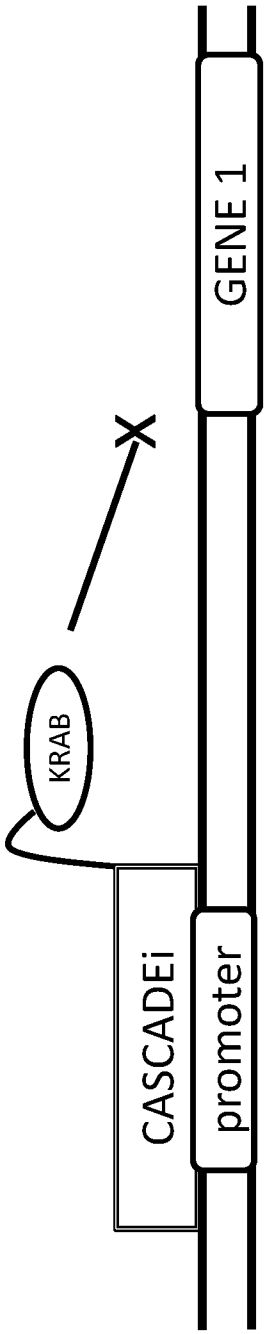


FIG. 20B

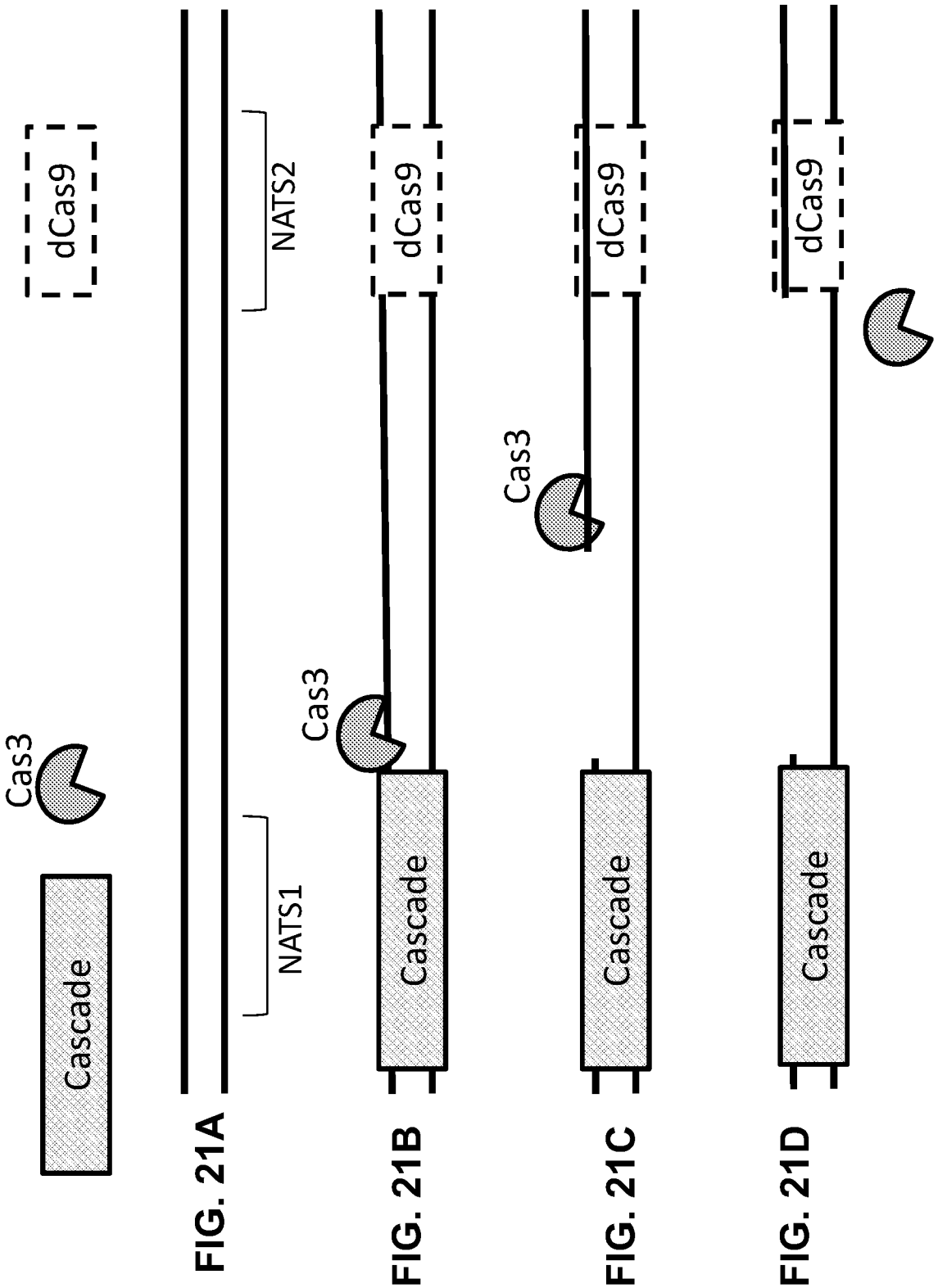


FIG. 21A

FIG. 21B

FIG. 21C

FIG. 21D

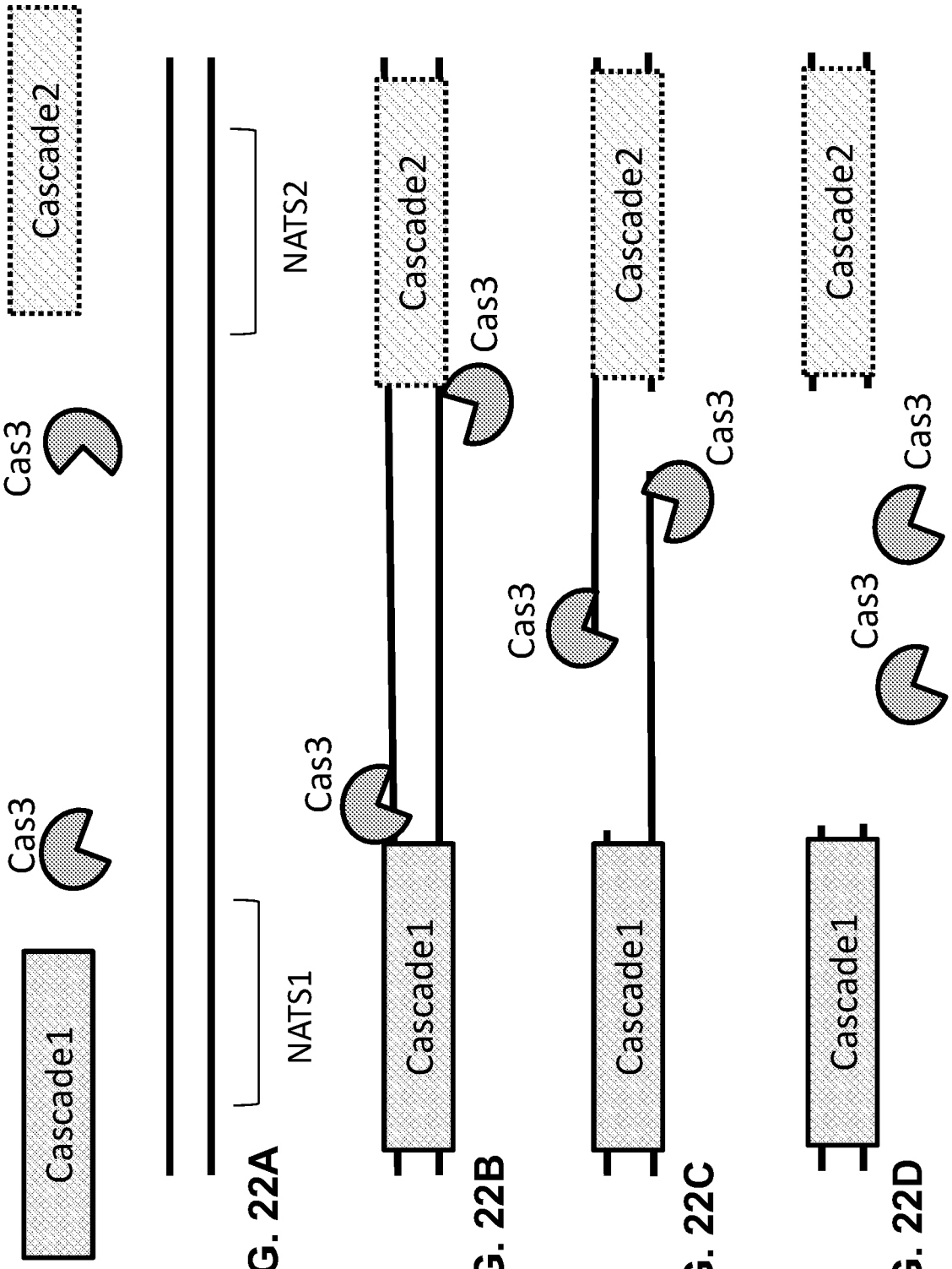


FIG. 22A

FIG. 22B

FIG. 22C

FIG. 22D

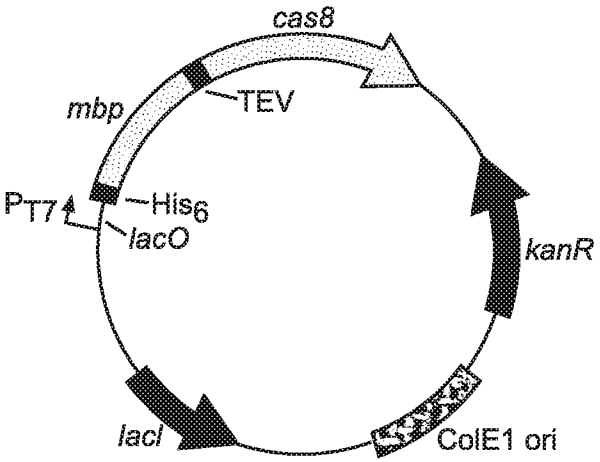


FIG. 23A

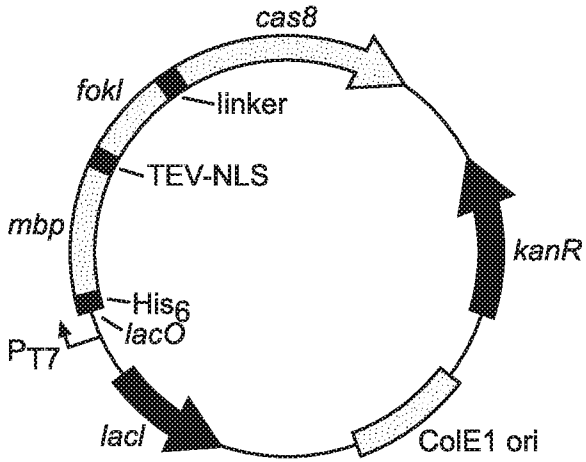


FIG. 23B

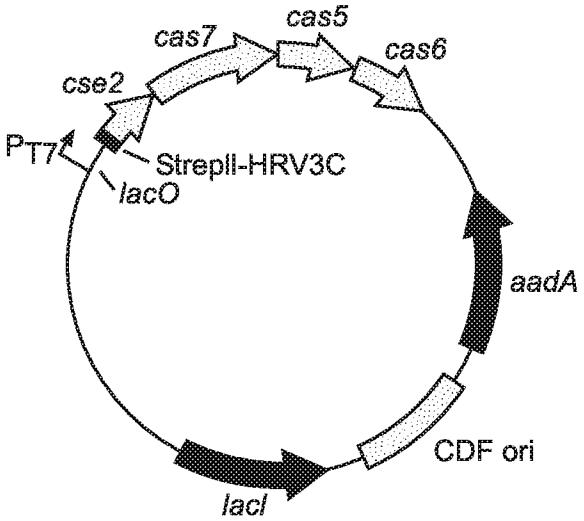


FIG. 23C

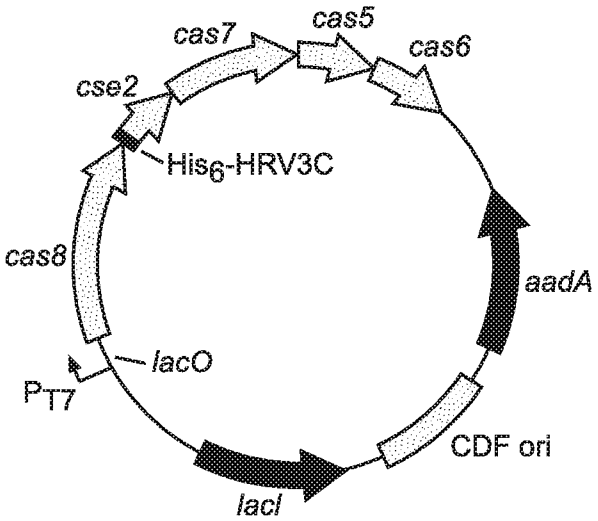


FIG. 23D

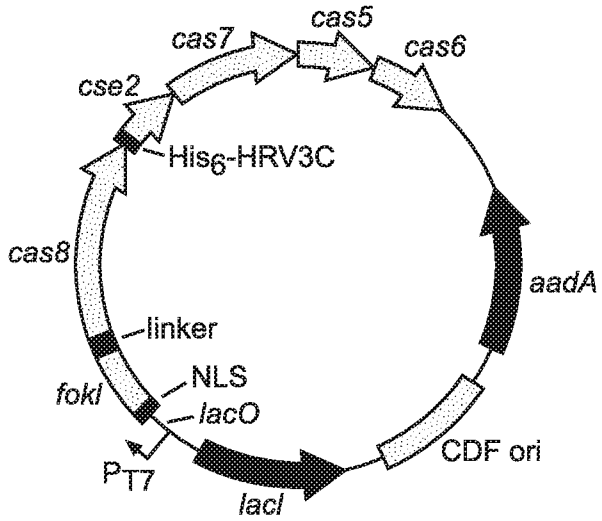


FIG. 23E

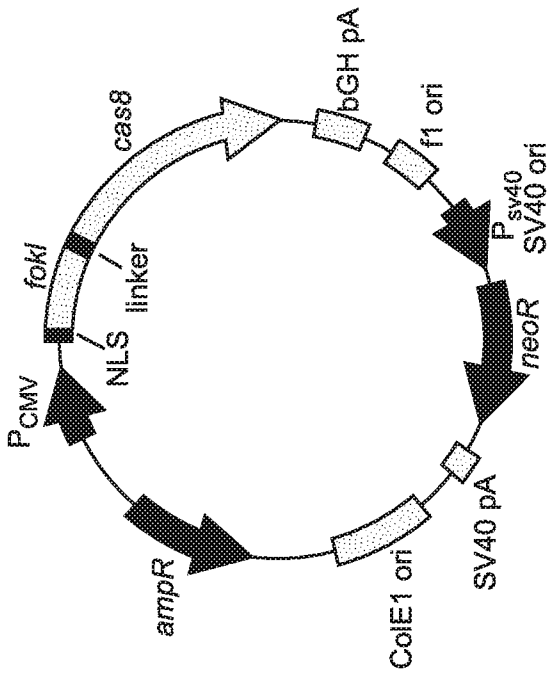


FIG. 25

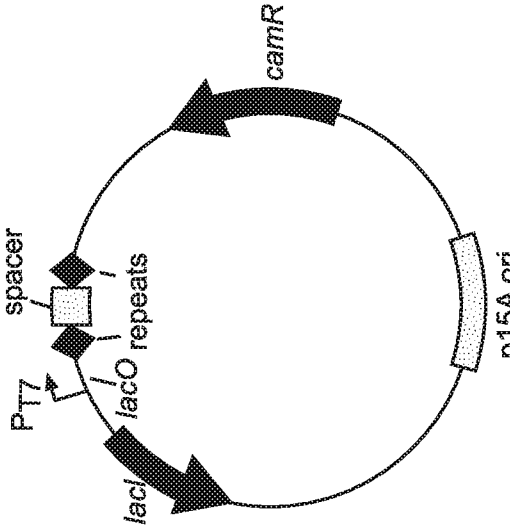


FIG. 24

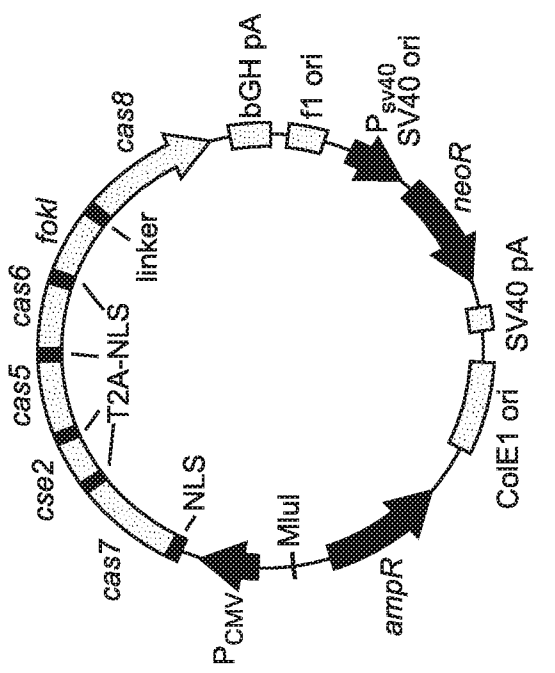


FIG. 27

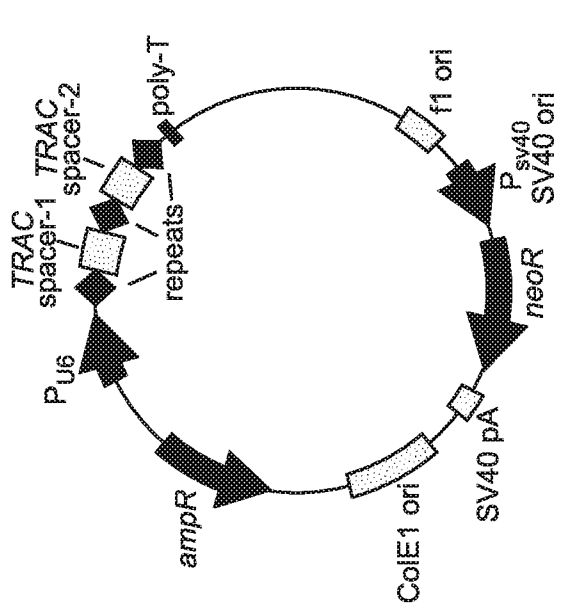


FIG. 26

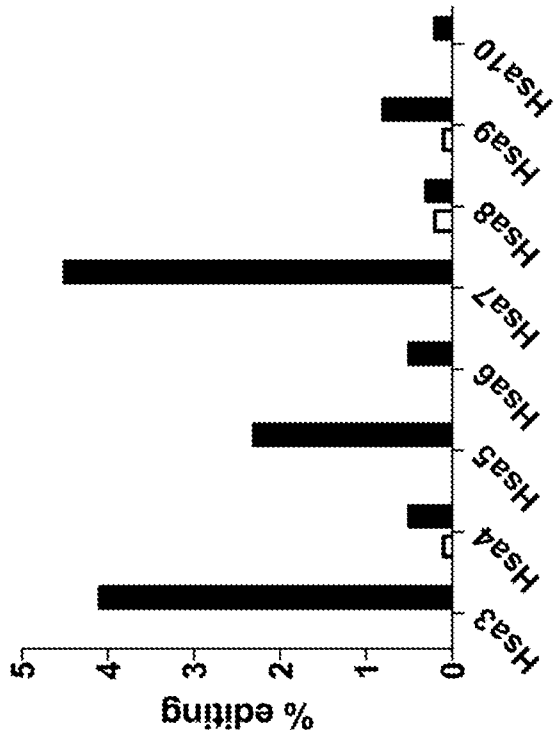

% editing
0
1
2
3
4
5
Hsa3
Hsa4
Hsa5
Hsa6
Hsa7
Hsa8
Hsa9
Hsa10

FIG. 28

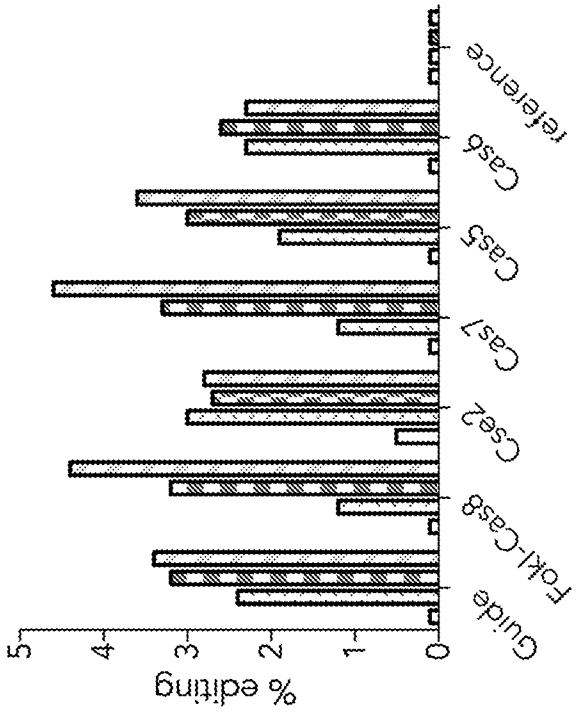

% editing
0
1
2
3
4
5
Guide
FokI-Cas8
Cse2
Cas7
Cas5
Cas6
reference

FIG. 29

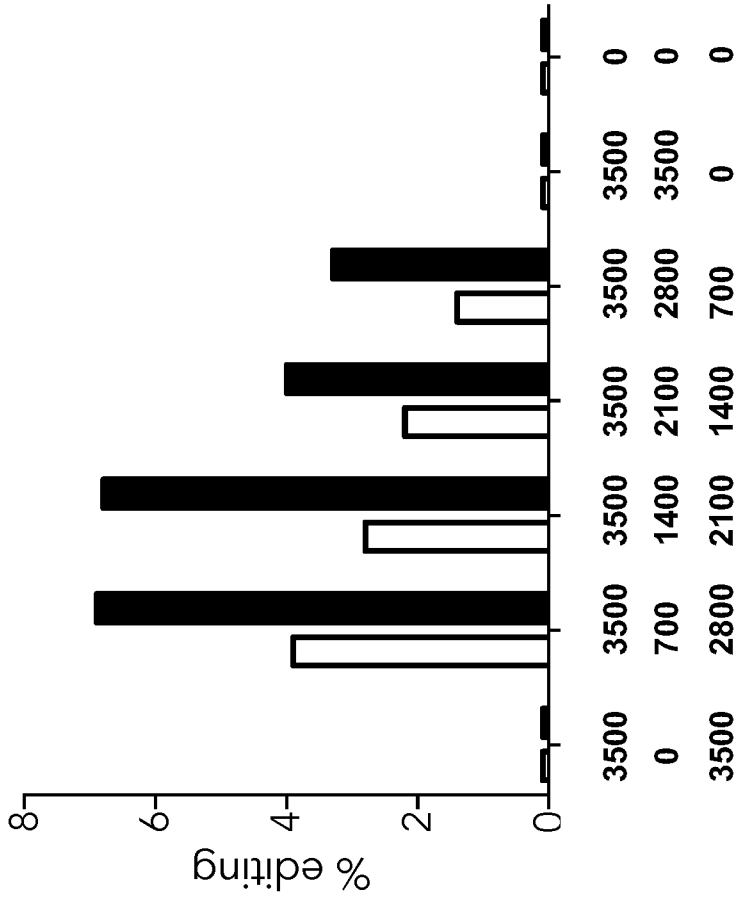

% editing
0
2
4
6
8
3500 0
3500 700
3500 2800
3500 1400
3500 2100
3500 2100
3500 1400
3500 2800
3500 700
3500 0
3500 0
3500 0

FIG. 30

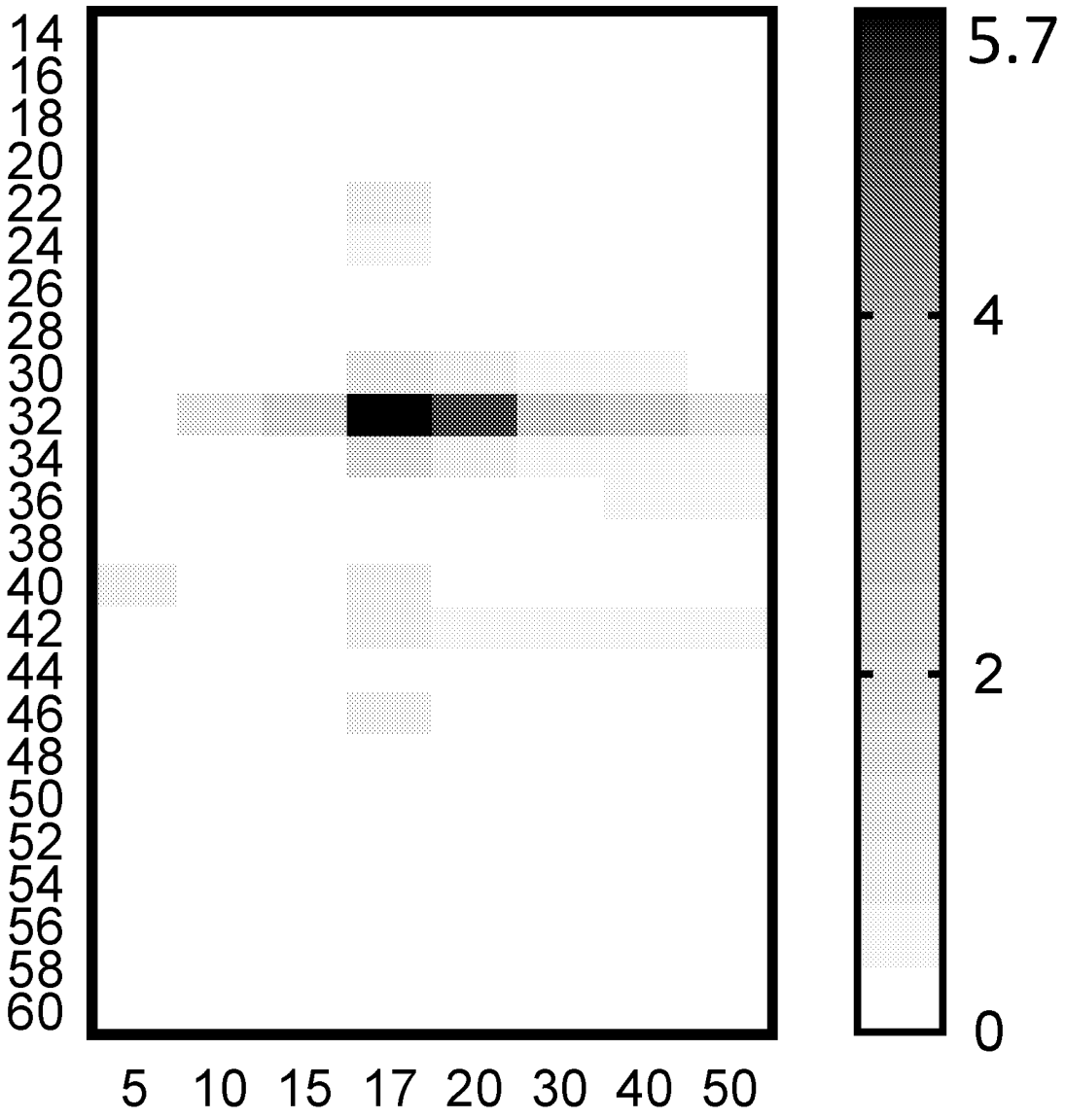
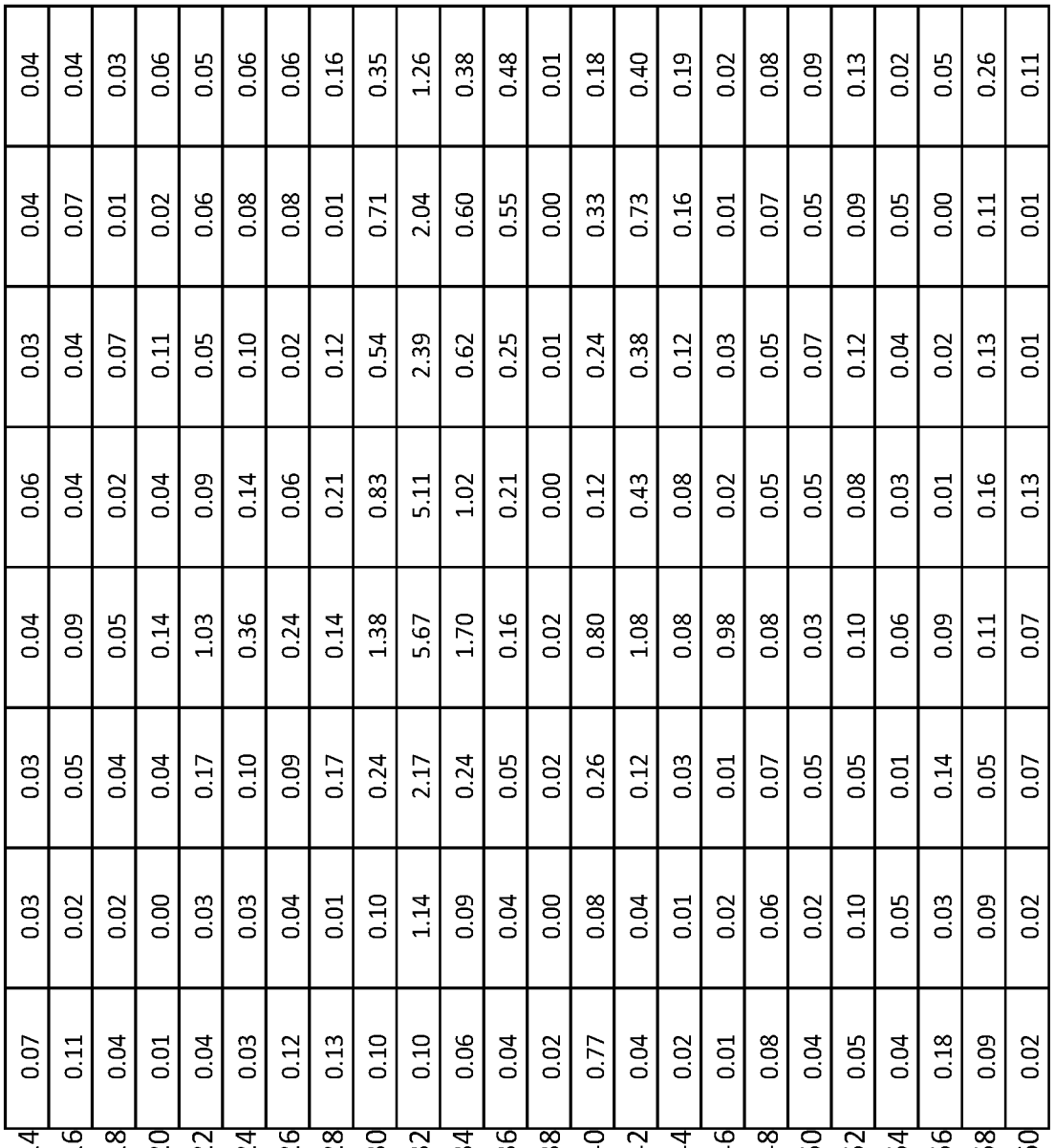

14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
58
60
5
10
15
17
20
30
40
50
5.7
4
2
0

FIG. 31A



14	0.07	0.03	0.03	0.04	0.06	0.03	0.04	0.04		
16	0.11	0.02	0.05	0.09	0.04	0.04	0.07	0.04		
18	0.04	0.02	0.04	0.05	0.02	0.07	0.01	0.03		
20	0.01	0.00	0.04	0.14	0.04	0.11	0.02	0.06		
22	0.04	0.03	0.17	1.03	0.09	0.05	0.06	0.05		
24	0.03	0.03	0.10	0.36	0.14	0.10	0.08	0.06		
26	0.12	0.04	0.09	0.24	0.06	0.02	0.08	0.06		
28	0.13	0.01	0.17	0.14	0.21	0.12	0.01	0.16		
30	0.10	0.10	0.24	1.38	0.83	0.54	0.71	0.35		
32	0.10	1.14	2.17	5.67	5.11	2.39	2.04	1.26		
34	0.06	0.09	0.24	1.70	1.02	0.62	0.60	0.38		
36	0.04	0.04	0.05	0.16	0.21	0.25	0.55	0.48		
38	0.02	0.00	0.02	0.02	0.00	0.01	0.00	0.01		
40	0.77	0.08	0.26	0.80	0.12	0.24	0.33	0.18		
42	0.04	0.04	0.12	1.08	0.43	0.38	0.73	0.40		
44	0.02	0.01	0.03	0.08	0.08	0.12	0.16	0.19		
46	0.01	0.02	0.01	0.98	0.02	0.03	0.01	0.02		
48	0.08	0.06	0.07	0.08	0.05	0.05	0.07	0.08		
50	0.04	0.02	0.05	0.03	0.05	0.07	0.05	0.09		
52	0.05	0.10	0.05	0.10	0.08	0.12	0.09	0.13		
54	0.04	0.05	0.01	0.06	0.03	0.04	0.05	0.02		
56	0.18	0.03	0.14	0.09	0.01	0.02	0.00	0.05		
58	0.09	0.09	0.05	0.11	0.16	0.13	0.11	0.26		
60	0.02	0.02	0.07	0.07	0.13	0.01	0.01	0.11		
	5	10	15	17	20	30	40	50		

FIG. 31B

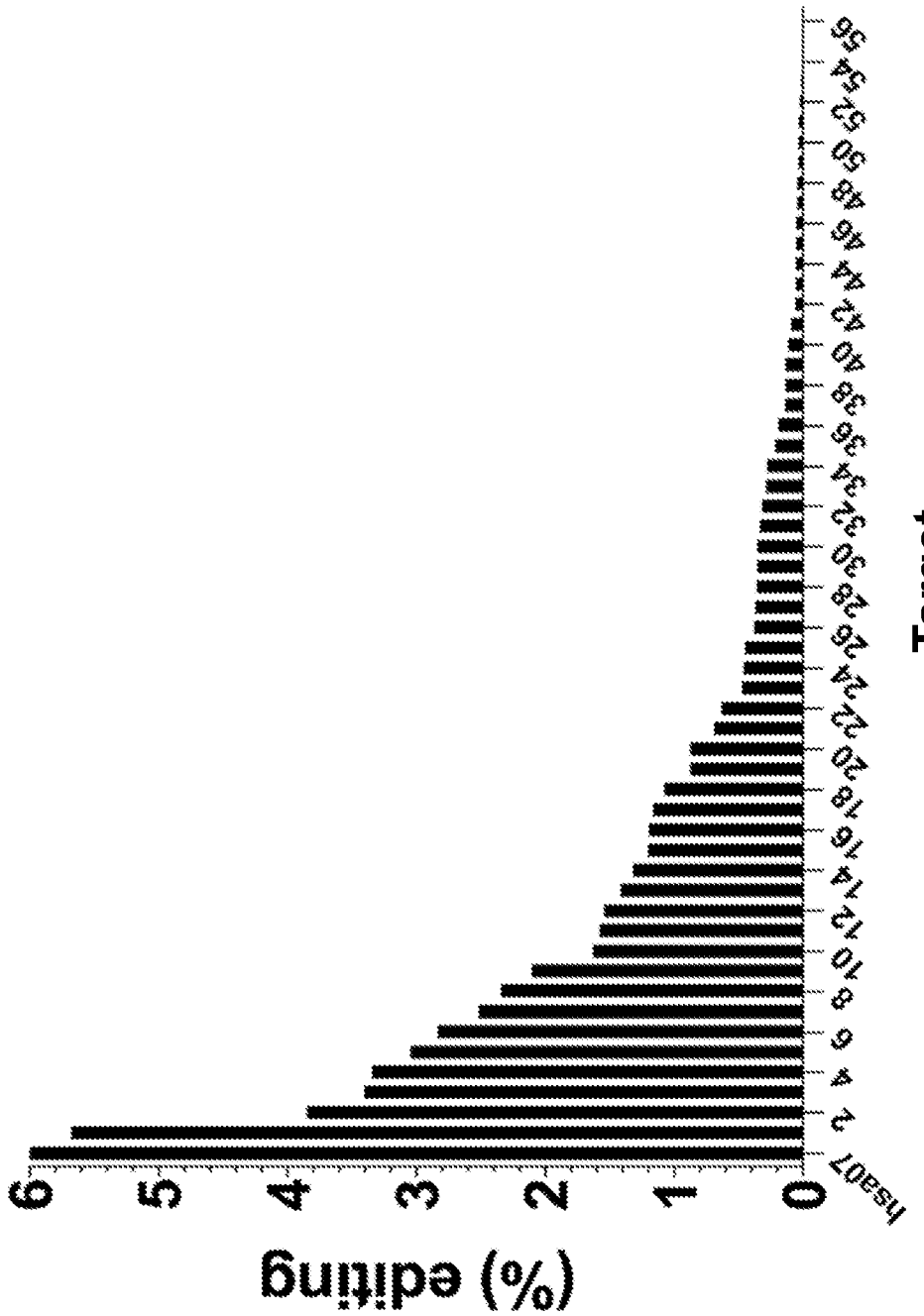

(%) editing
0
1
2
3
4
5
6
hsa07
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
Target

FIG. 32

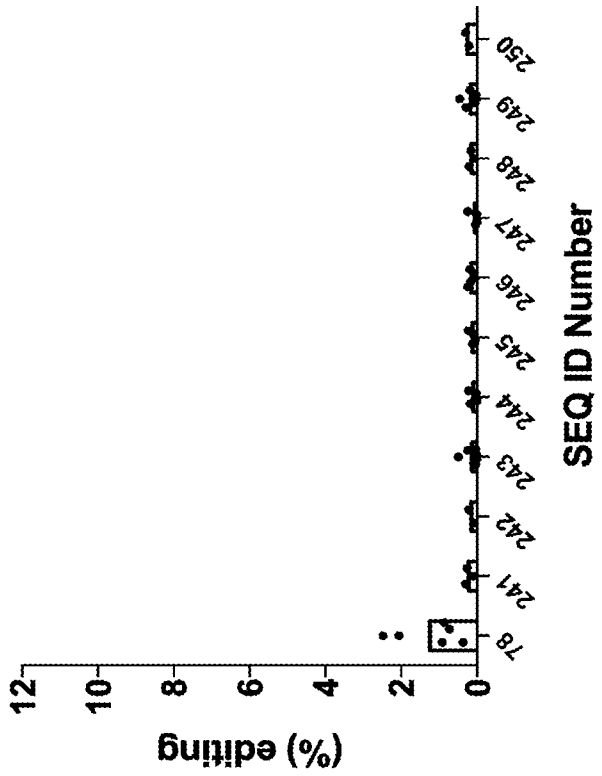


FIG. 33B

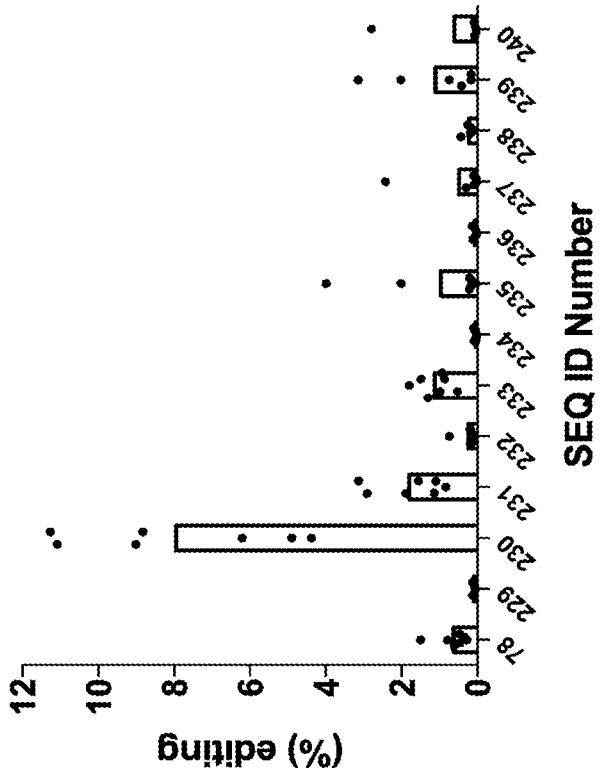


FIG. 33A

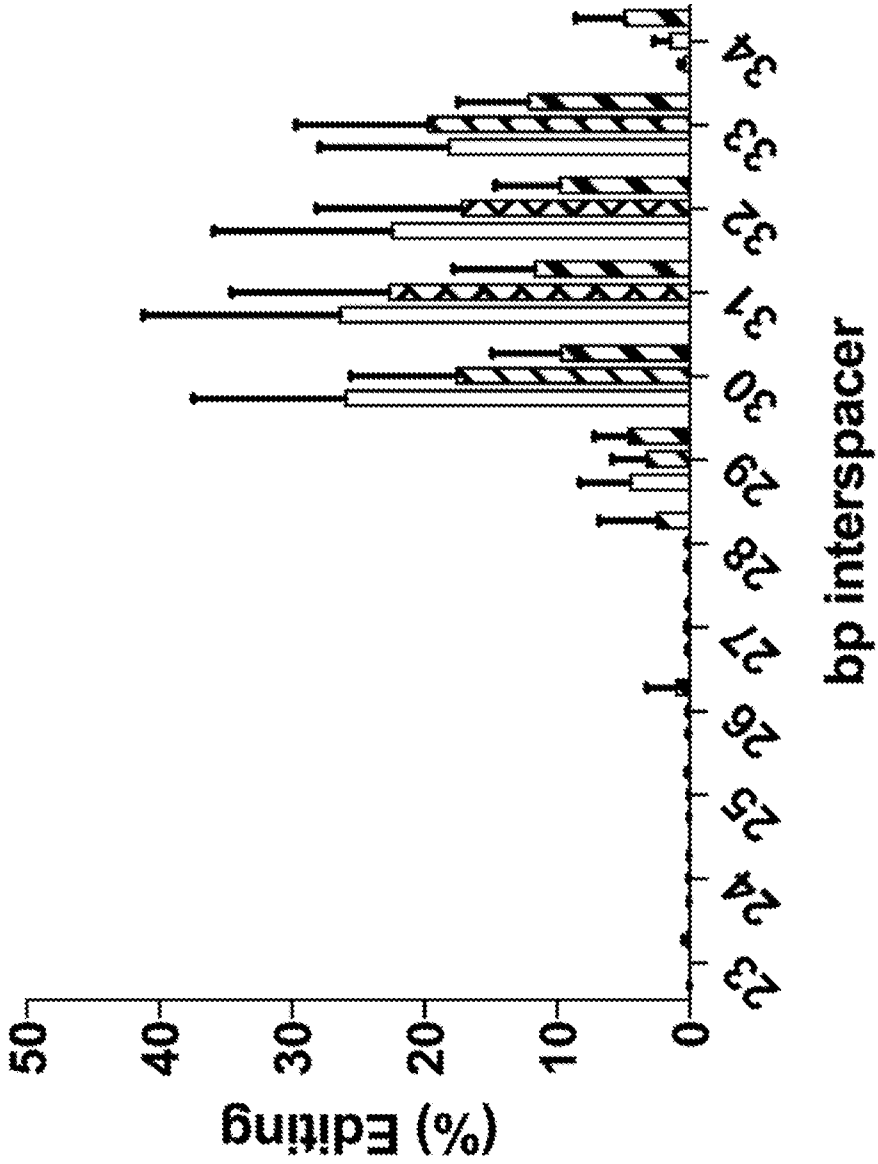

(%) Editing
50
40
30
20
10
0
23
24
25
26
27
28
29
30
31
32
33
34
bp interspacer

FIG. 34

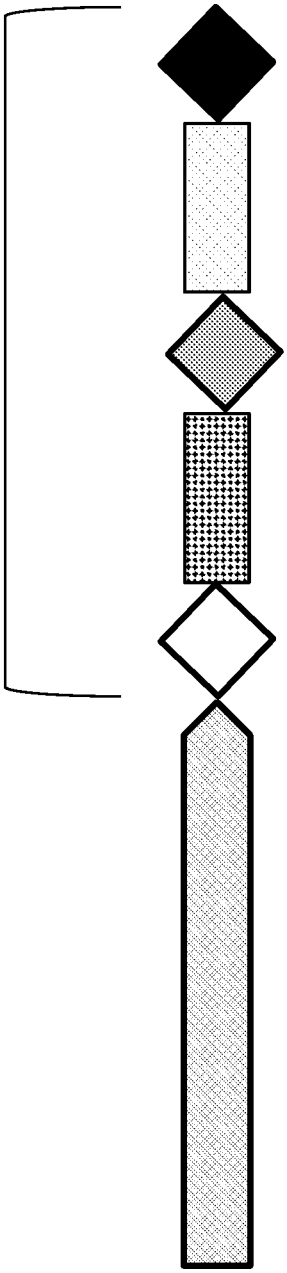


FIG. 35

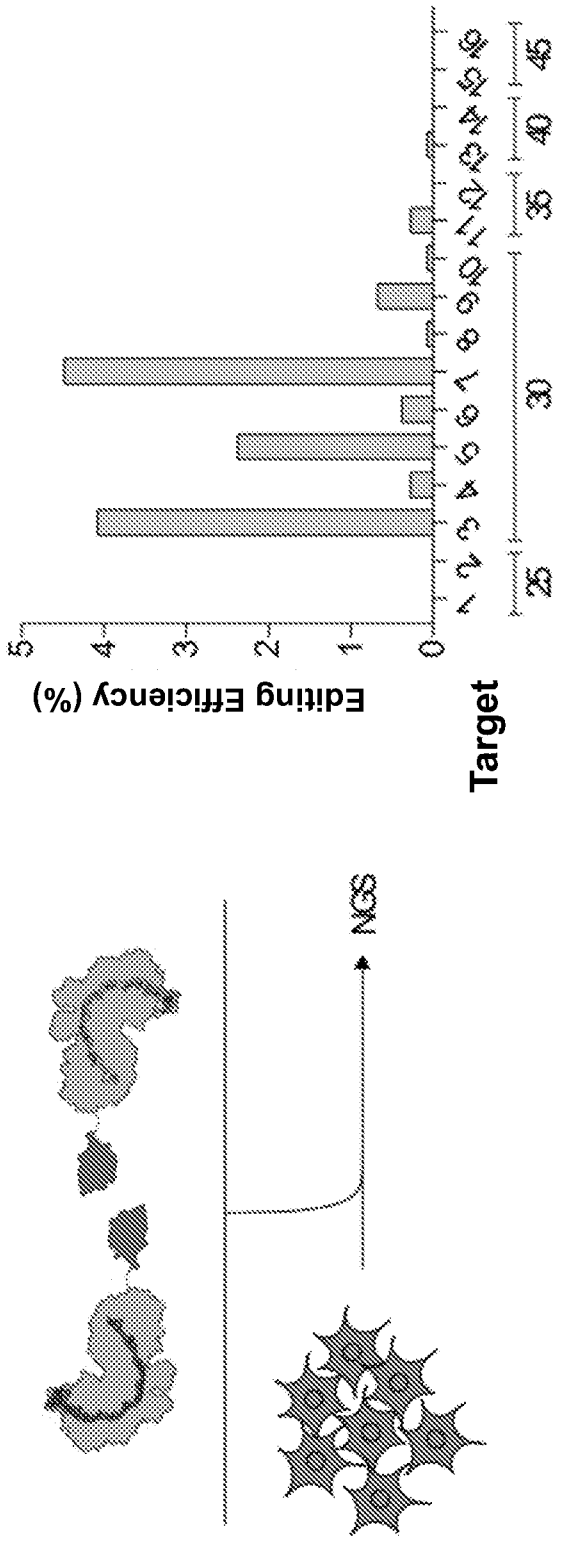

Editing Efficiency (%)
0
1
2
3
4
5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Target
NGS

FIG. 36A

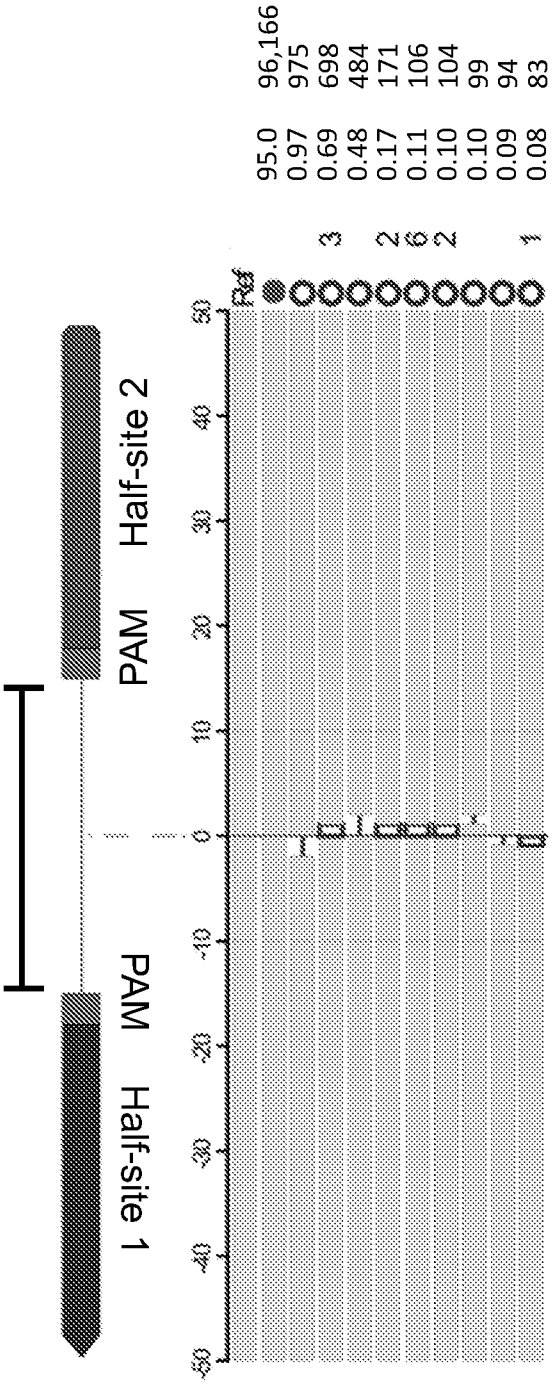
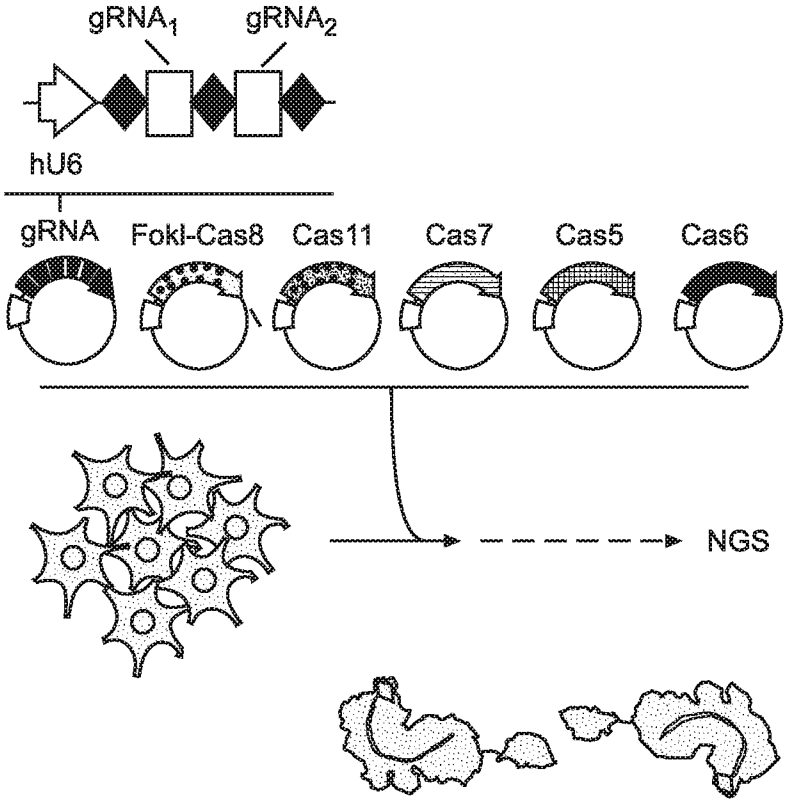

Half-site 1
PAM
Half-site 2
PAM
Ref
95.0
0.97
0.69
0.48
0.17
0.11
0.10
0.10
0.09
0.08
96,166
975
698
484
171
106
104
99
94
83
3
2
6
2
1
-50
-40
-30
-20
-10
0
10
20
30
40
50

FIG. 36B


gRNA1
gRNA2
hU6
gRNA
FokI-Cas8
Cas11
Cas7
Cas5
Cas6
NGS

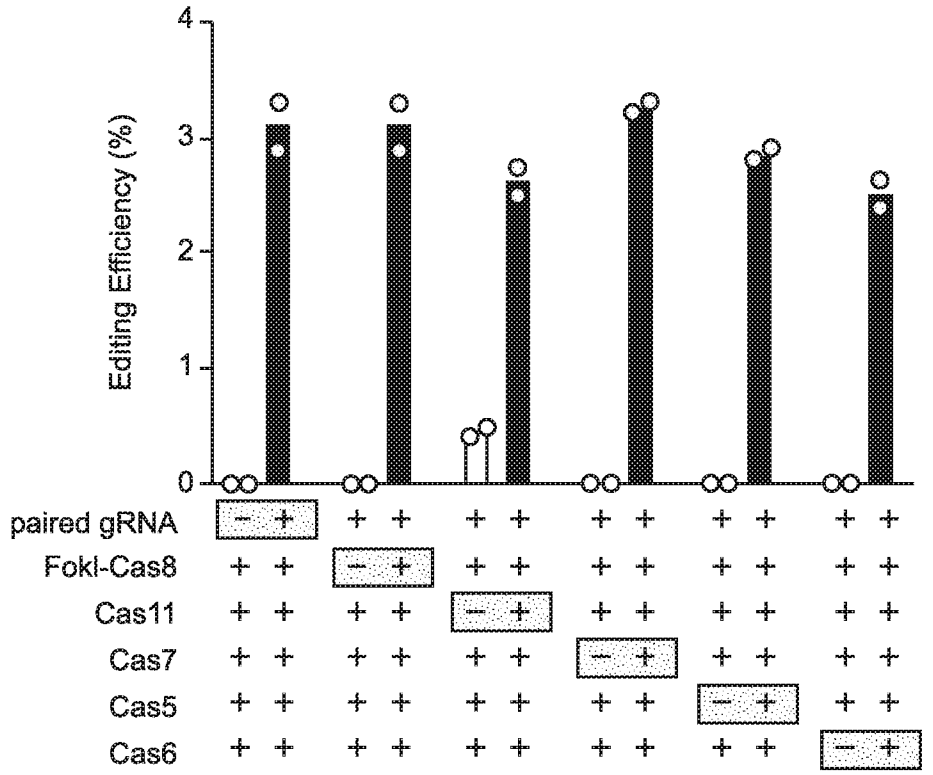

Editing Efficiency (%)
0
1
2
3
4
paired gRNA
FokI-Cas8
Cas11
Cas7
Cas5
Cas6

FIG. 36C

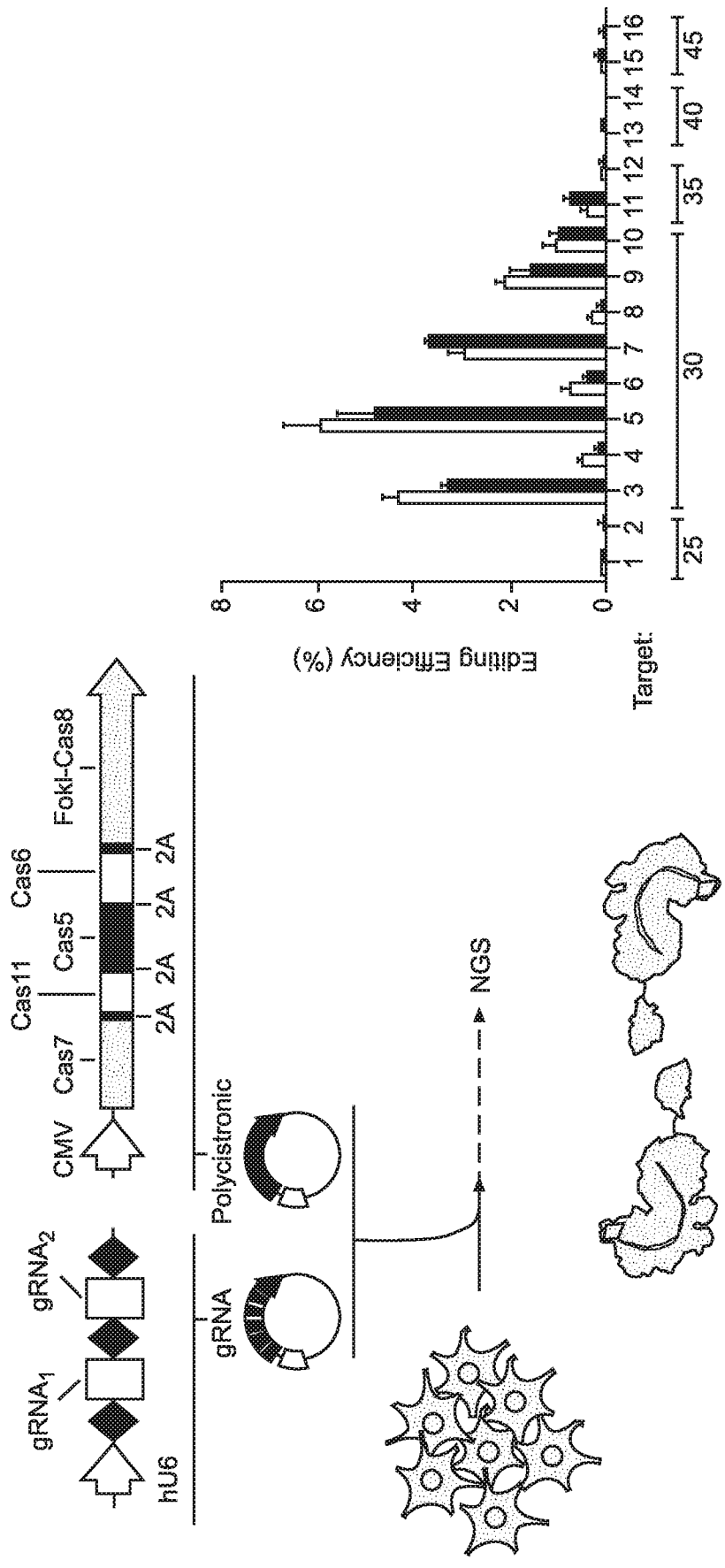

hU6
gRNA1
gRNA2
gRNA
Polycistronic
CMV
Cas7
Cas11
Cas5
Cas6
FokI-Cas8
2A
NGS
Editing Efficiency (%)
0
2
4
6
8
Target:
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
25
30
35
40
45

FIG. 36D

FIG. 37A

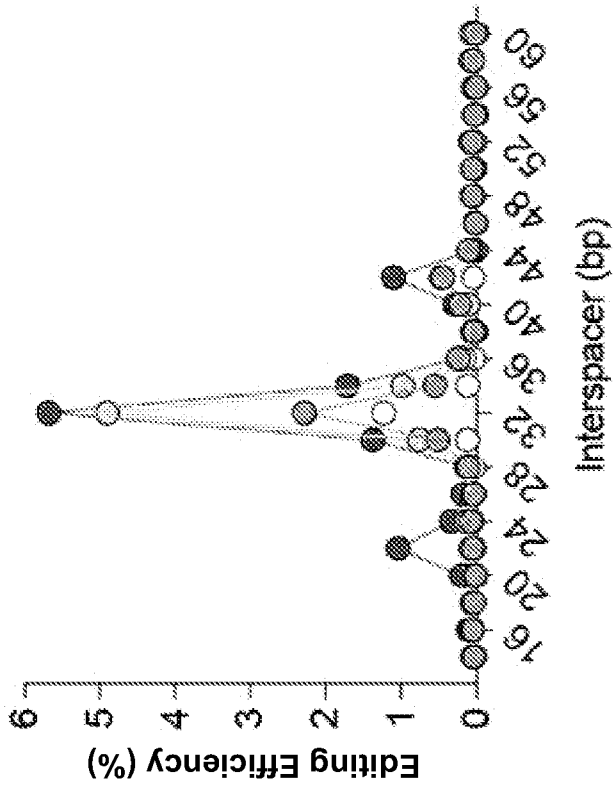
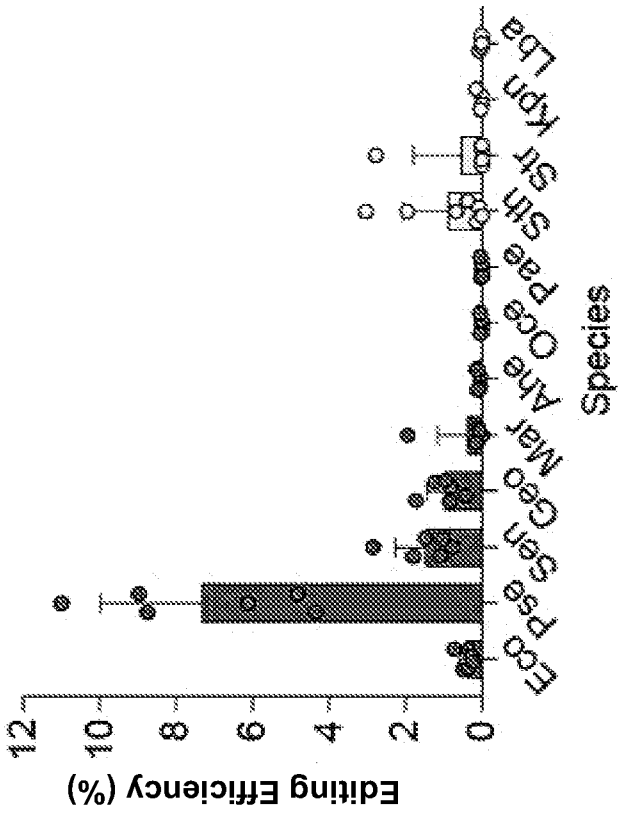


FIG. 37B



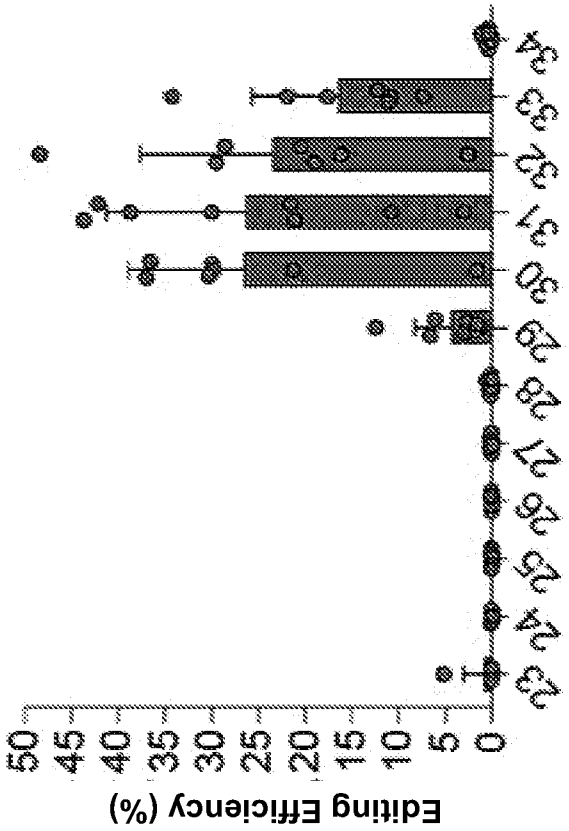


FIG. 37C

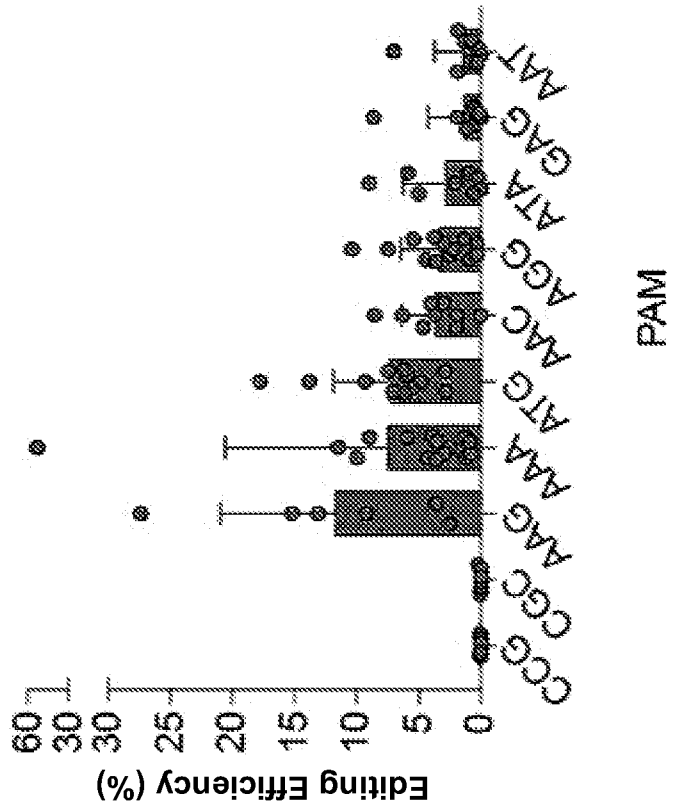


FIG. 37D

FIG. 37F

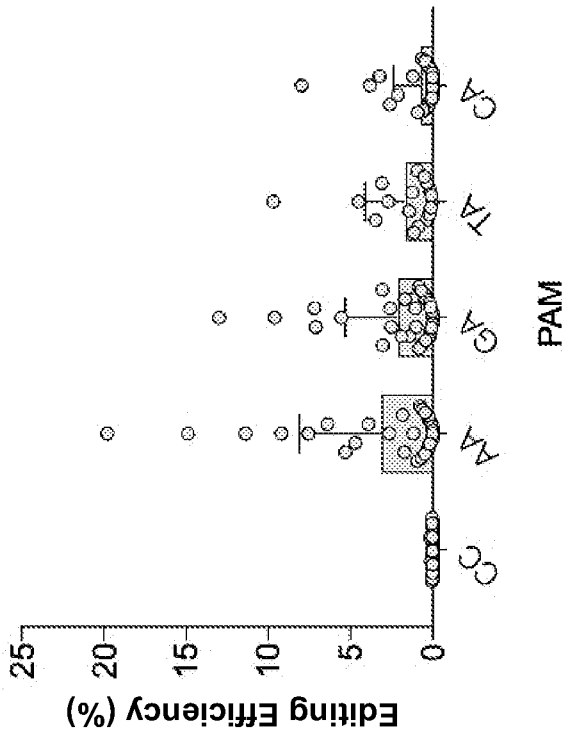
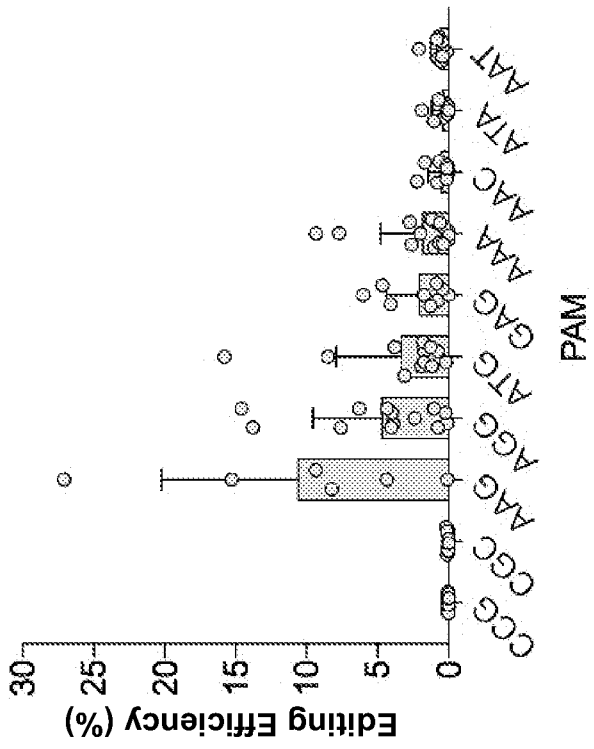


FIG. 37E



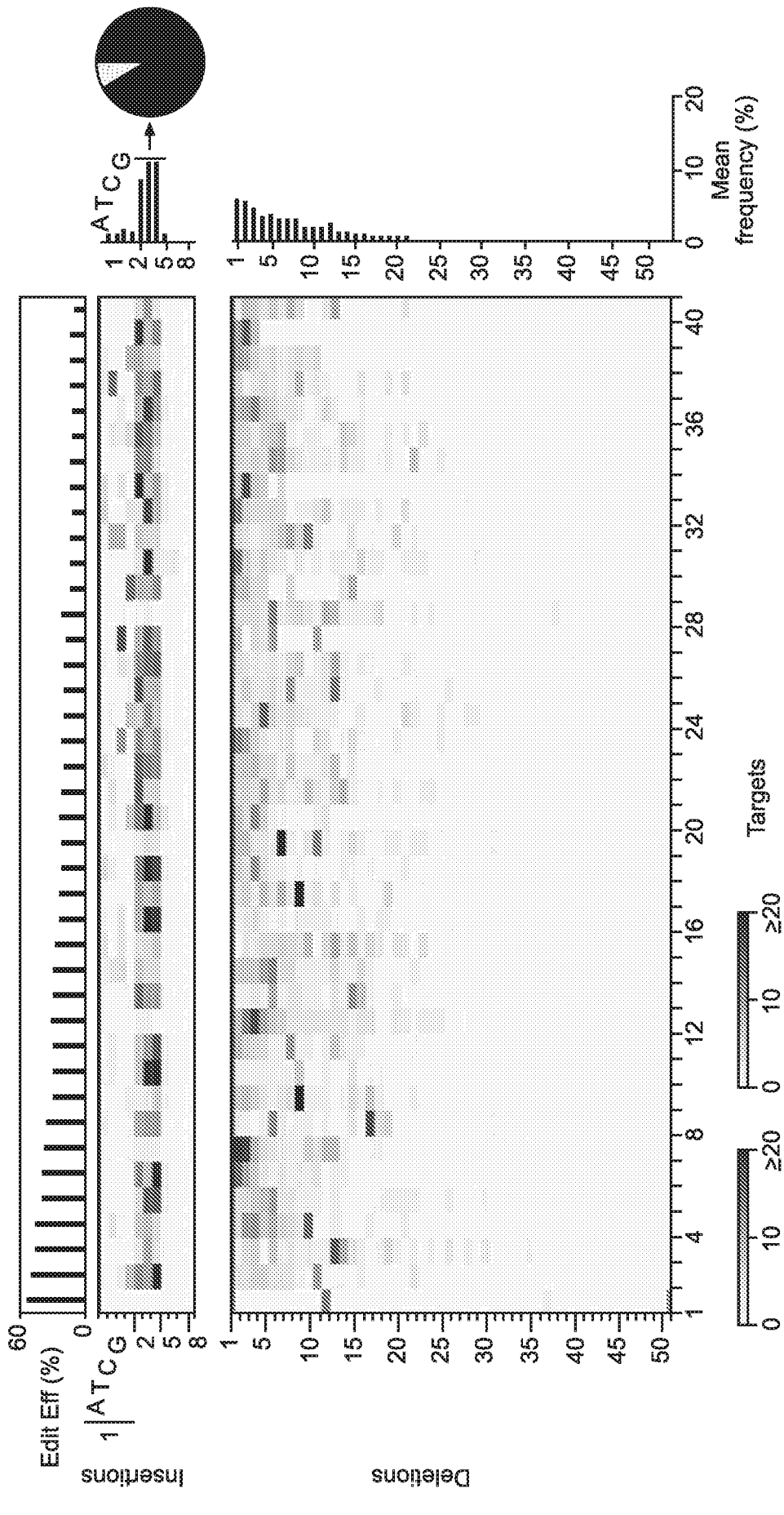

Edit Eff (%)
60
0
Insertions
1
A
T
C
G
2
5
8
Deletions
1
5
10
15
20
25
30
35
40
45
50
1
4
8
12
16
20
24
28
32
36
40
Mean frequency (%)
0
10
20
Ins Freq (%)
0
10
≥20
Del Freq (%)
0
10
≥20
Targets

FIG. 37G

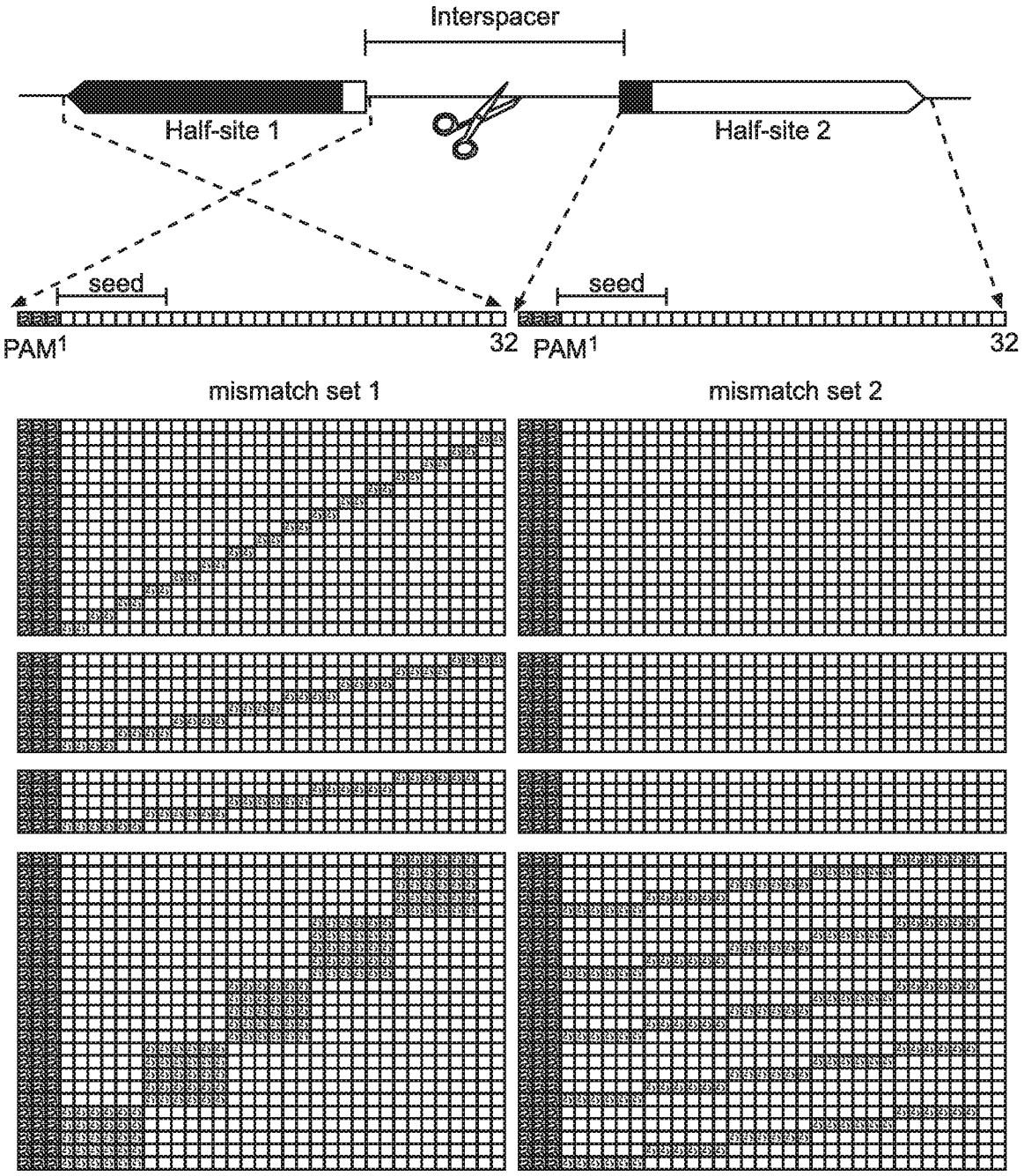

Interspacer
Half-site 1
Half-site 2
seed
seed
PAM1
32
PAM1
32
mismatch set 1
mismatch set 2

FIG. 38A

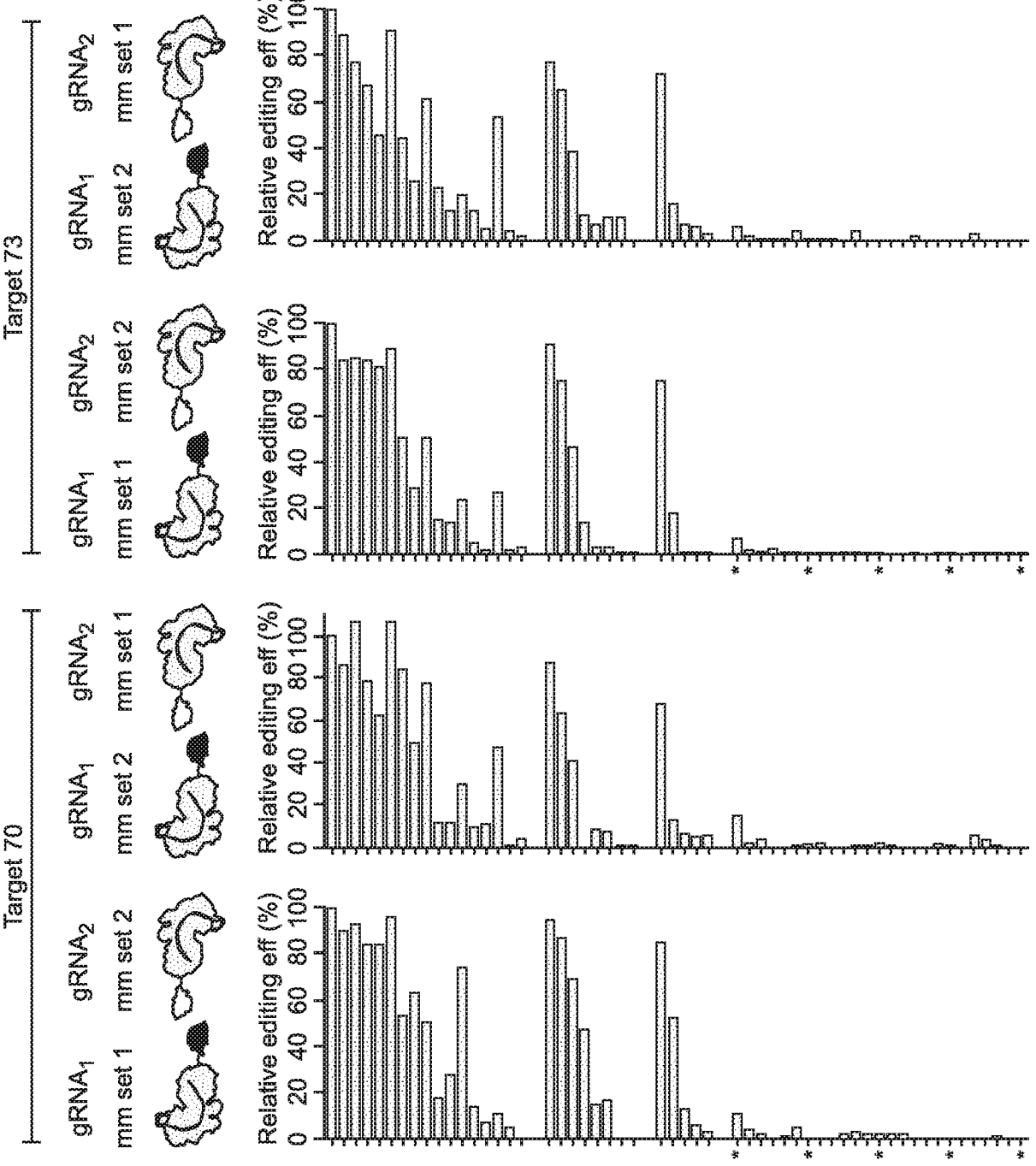


FIG. 38B

FIG. 38C

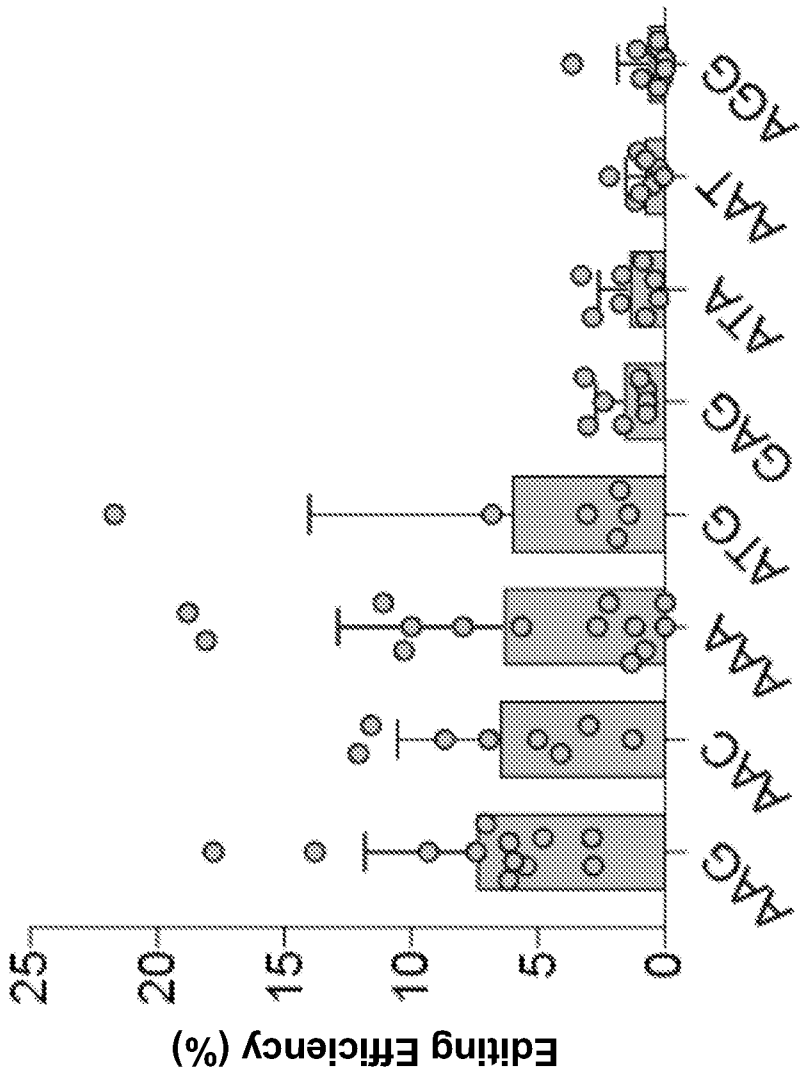

Editing Efficiency (%)
25
20
15
10
5
0
AAG
AAC
AAA
ATG
CAG
ATA
AAT
AGG
PAM

FIG. 39A

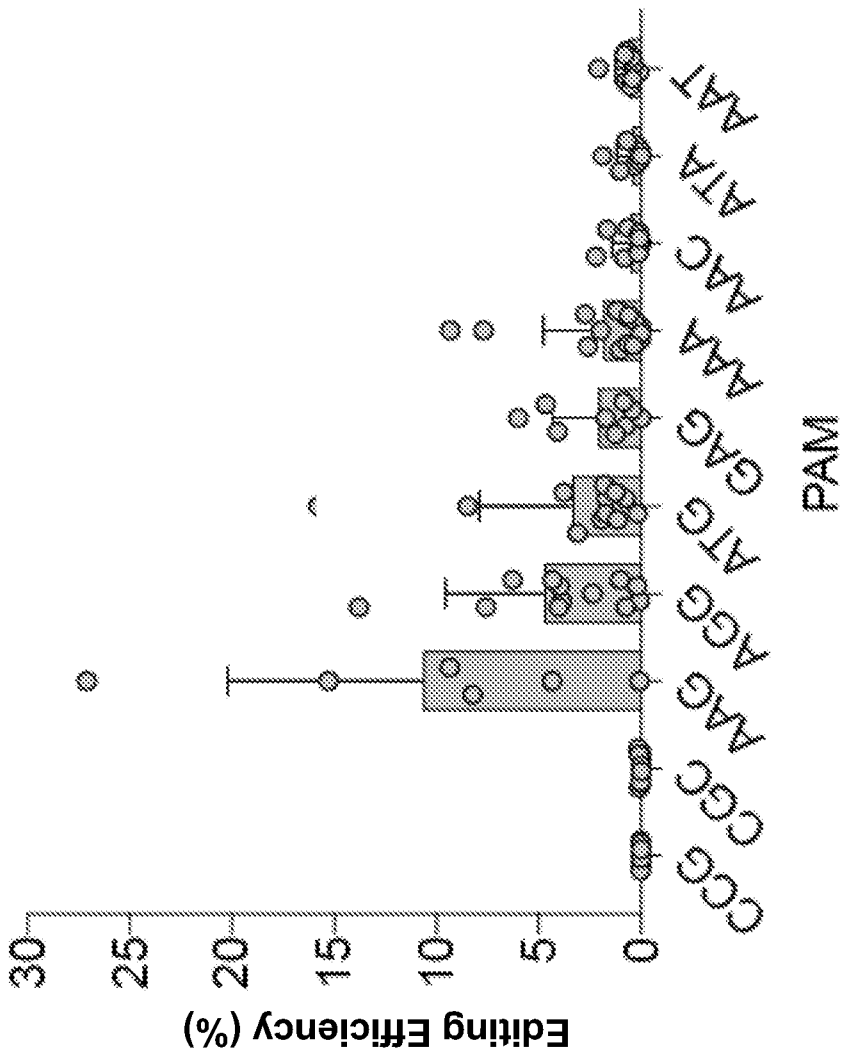

Editing Efficiency (%)
30
25
20
15
10
5
0
CCG
CGG
AAG
AGG
ATG
GAG
AAA
ATC
ATA
AAT
PAM

FIG. 39B

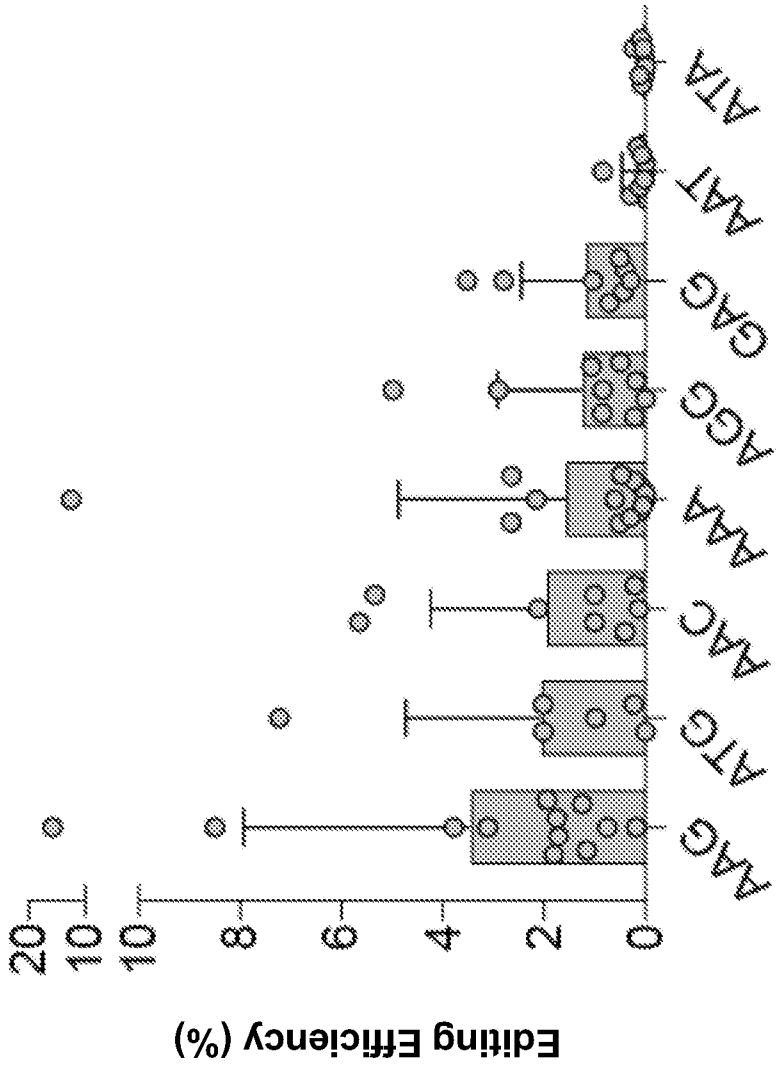

Editing Efficiency (%)
0
2
4
6
8
10
10
20
AAG
ATG
AAC
AAA
AGG
GAG
AAT
ATA
PAM

FIG. 39C

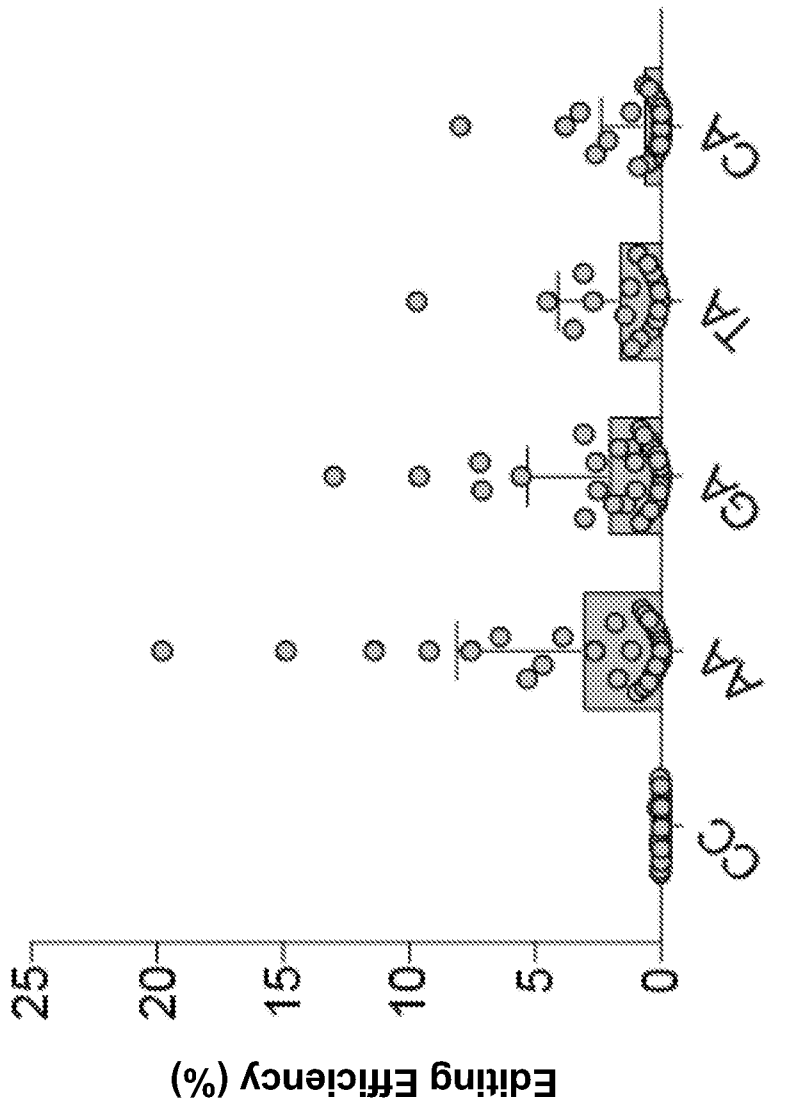


FIG. 39D

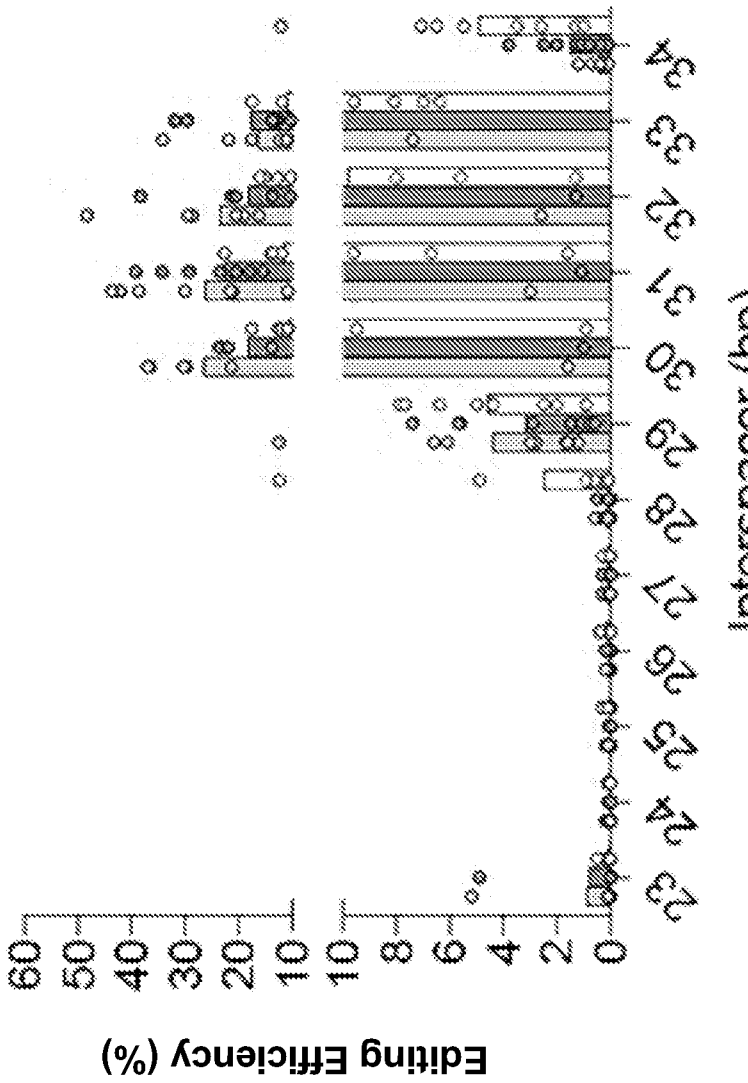

Editing Efficiency (%)
60
50
40
30
20
10
10
8
6
4
2
0
23
24
25
26
27
28
29
30
31
32
33
34
Interspacer (bp)

FIG. 40A

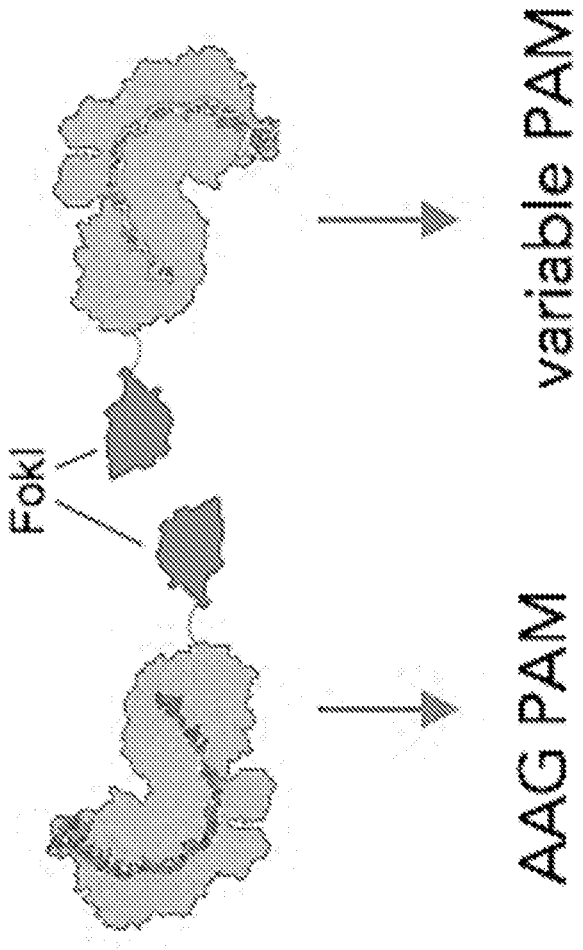


FIG. 40B

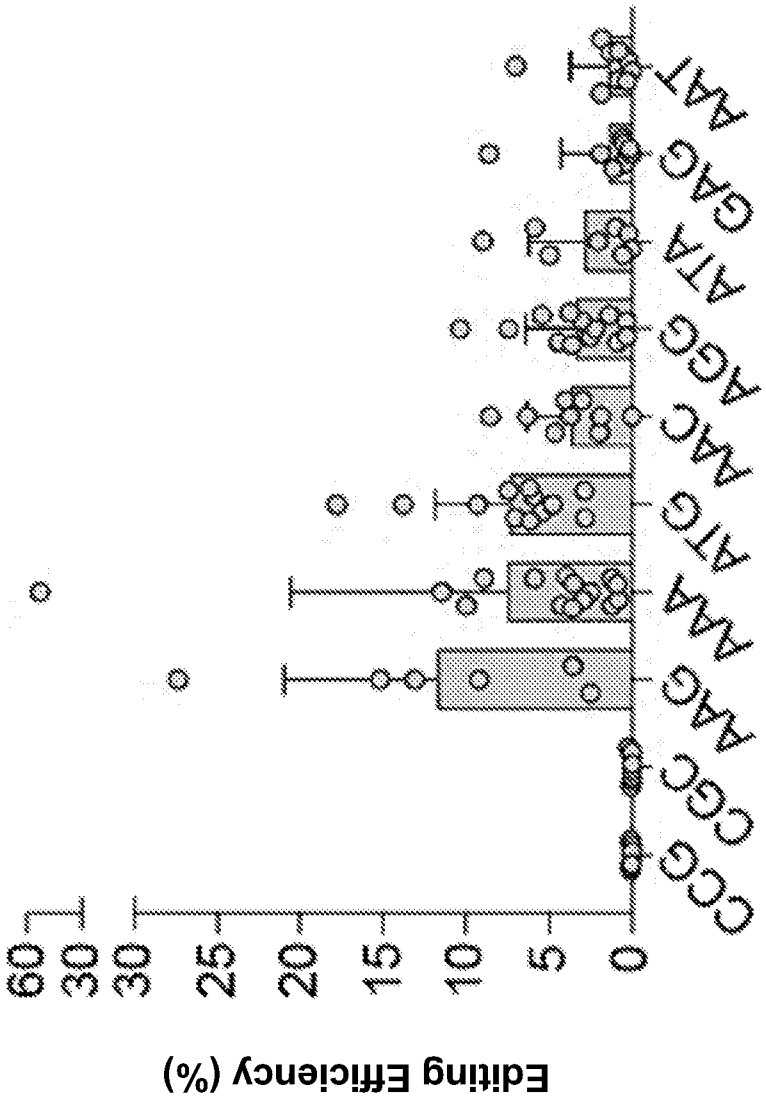

Editing Efficiency (%)
60
30
30
25
20
15
10
5
0
CCG
CGC
AAG
AAA
ATG
AAC
AGG
ATA
GAG
AAT
PAM

FIG. 40C

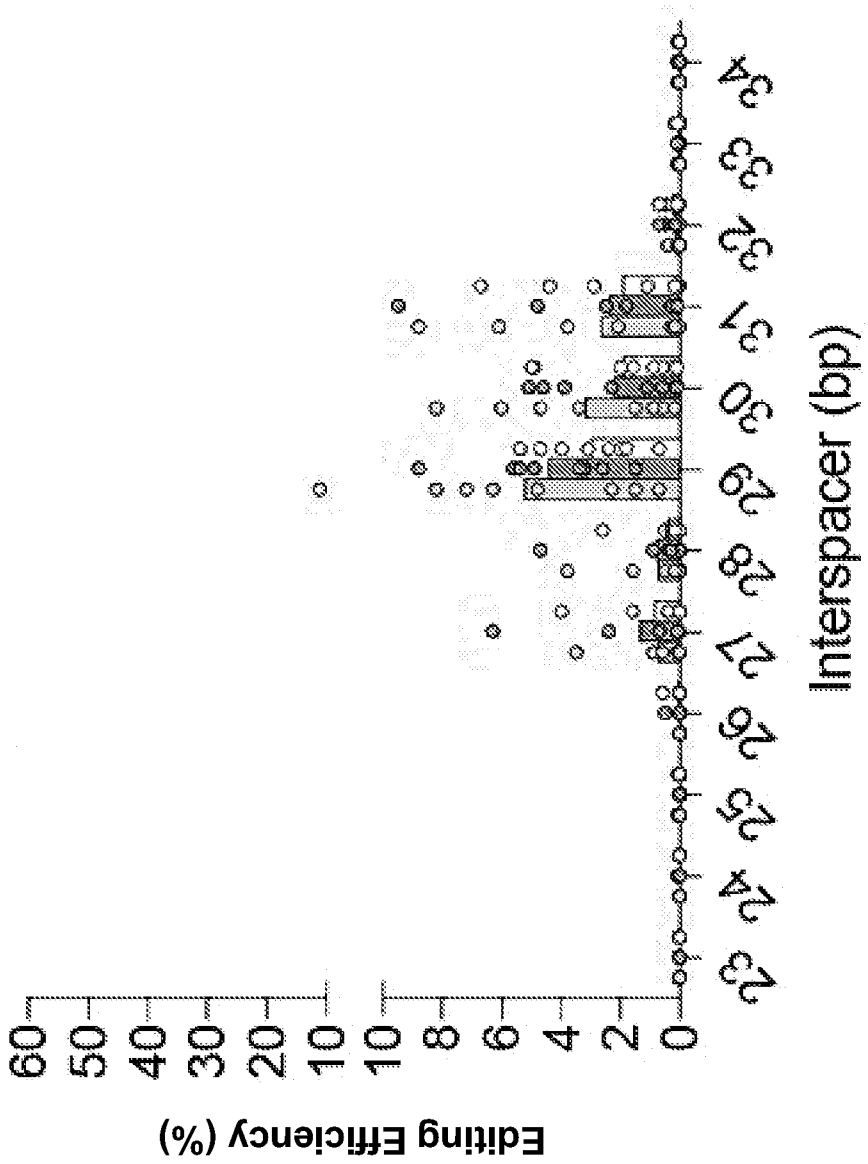

Editing Efficiency (%)
60
50
40
30
20
10
10
8
6
4
2
0
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
Interspacer (bp)

FIG. 40D

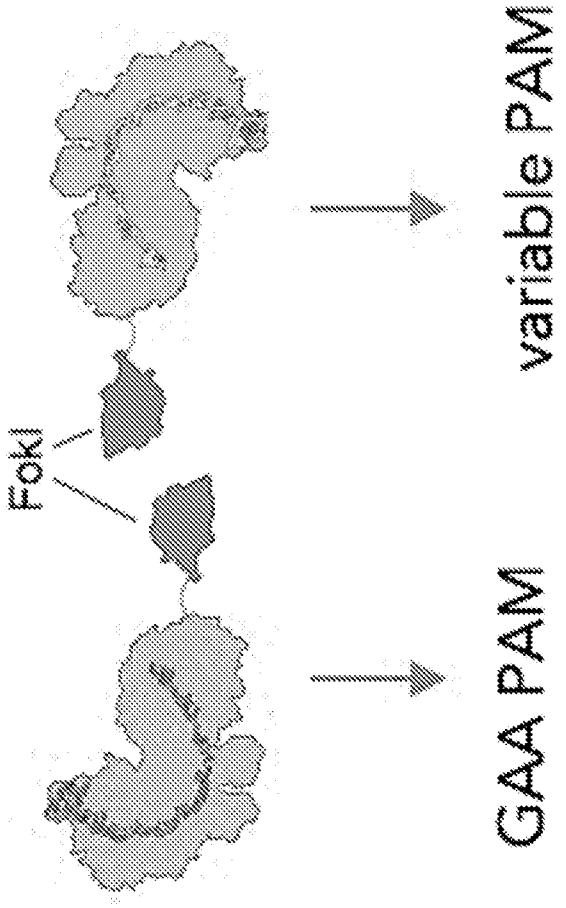


FIG. 40E

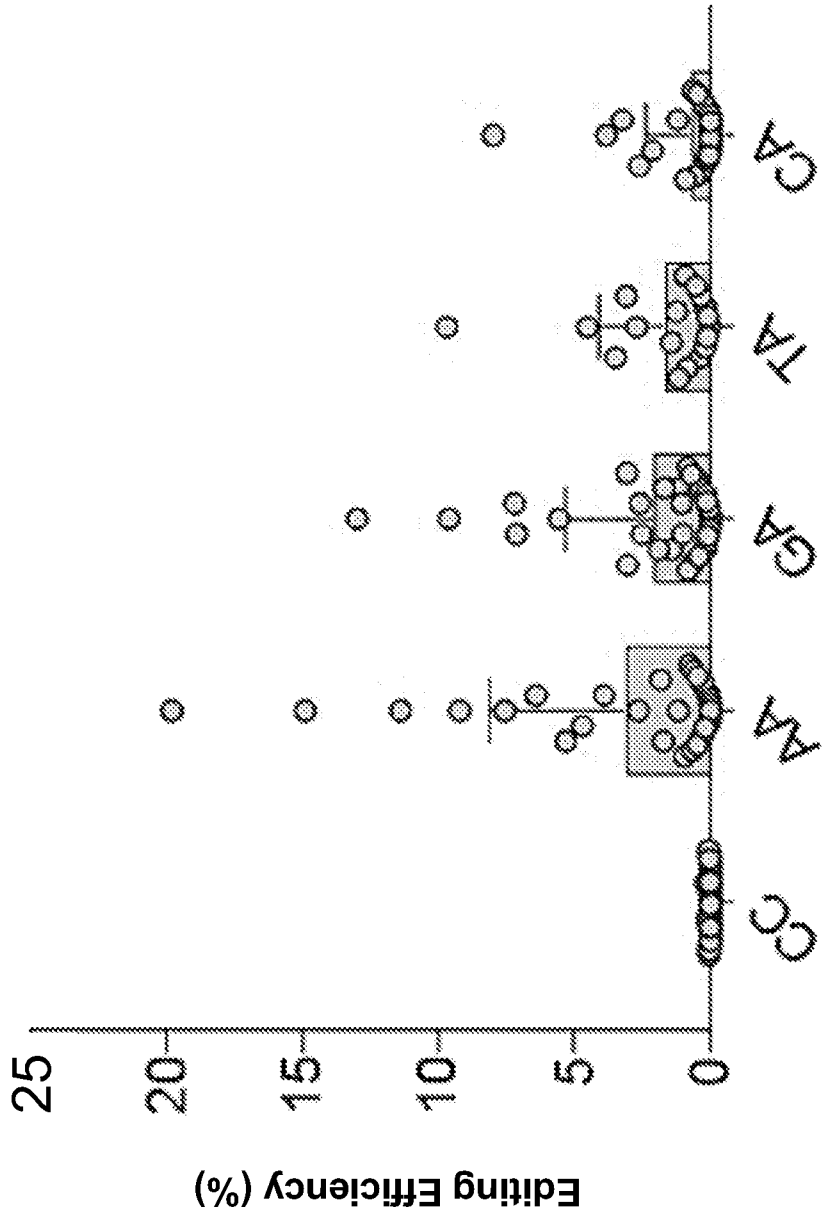


FIG. 40F

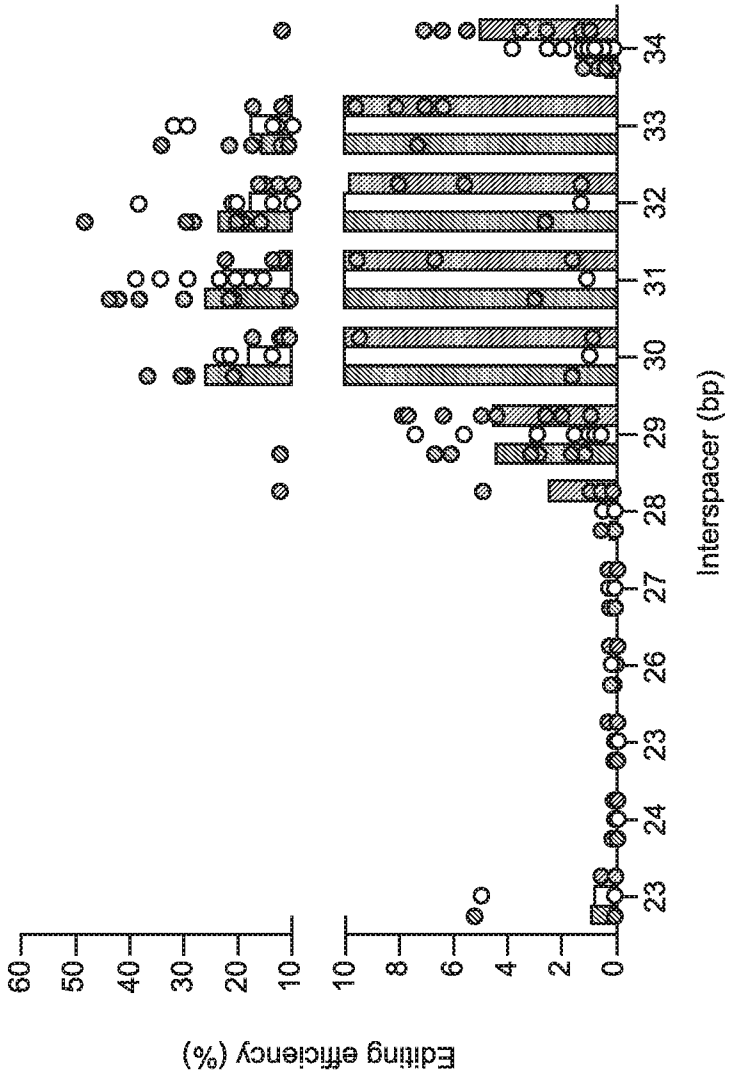

Editing efficiency (%)
60
50
40
30
20
10
10
8
6
4
2
0
23
24
23
26
27
28
29
30
31
32
33
34
Interspacer (bp)

FIG. 41A

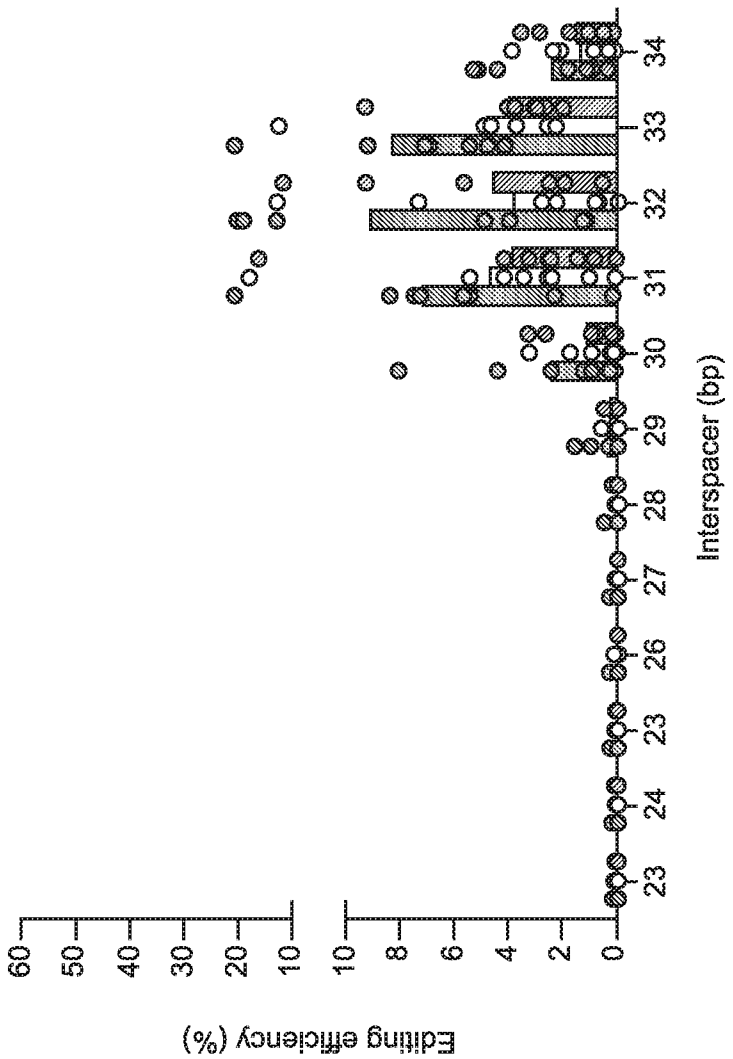

Editing efficiency (%)
60
50
40
30
20
10
10
8
6
4
2
0
23
24
23
26
27
28
29
30
31
32
33
34
Interspacer (bp)

FIG. 41B

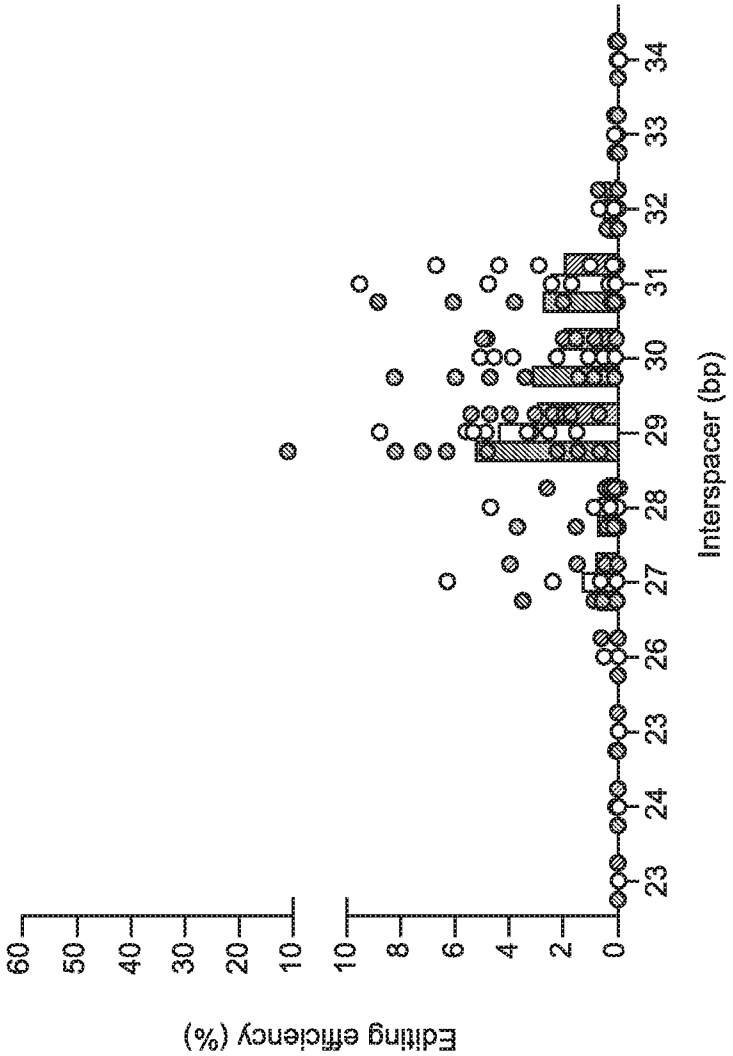

Editing efficiency (%)
60
50
40
30
20
10
10
8
6
4
2
0
23
24
23
26
27
28
29
30
31
32
33
34
Interspacer (bp)

FIG. 41C

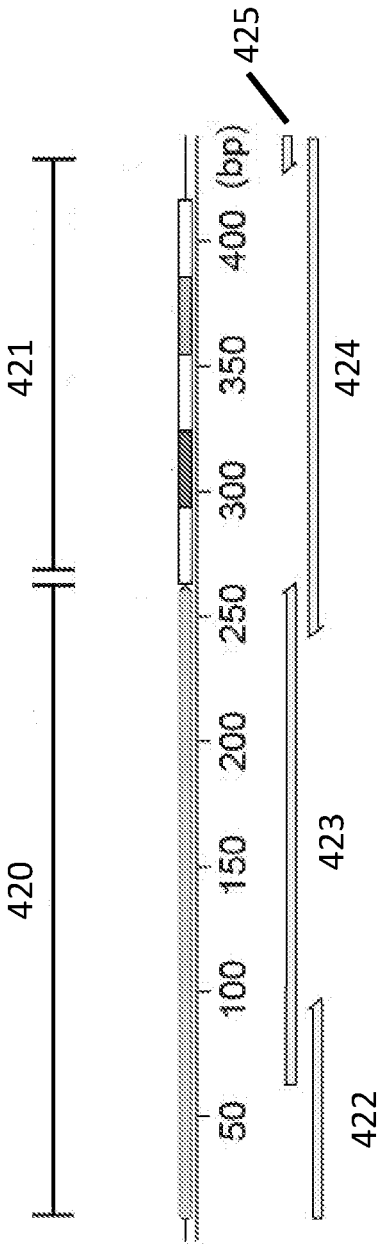


FIG. 42A

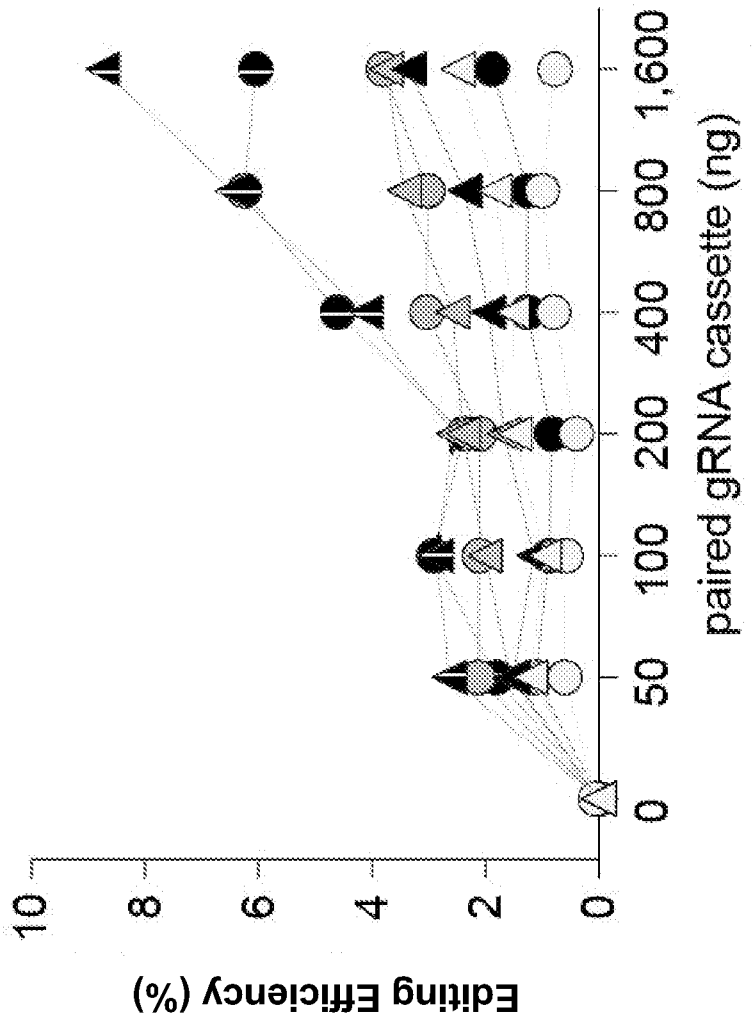

Editing Efficiency (%)
0
2
4
6
8
10
0
50
100
200
400
800
1,600
paired gRNA cassette (ng)

FIG. 42B

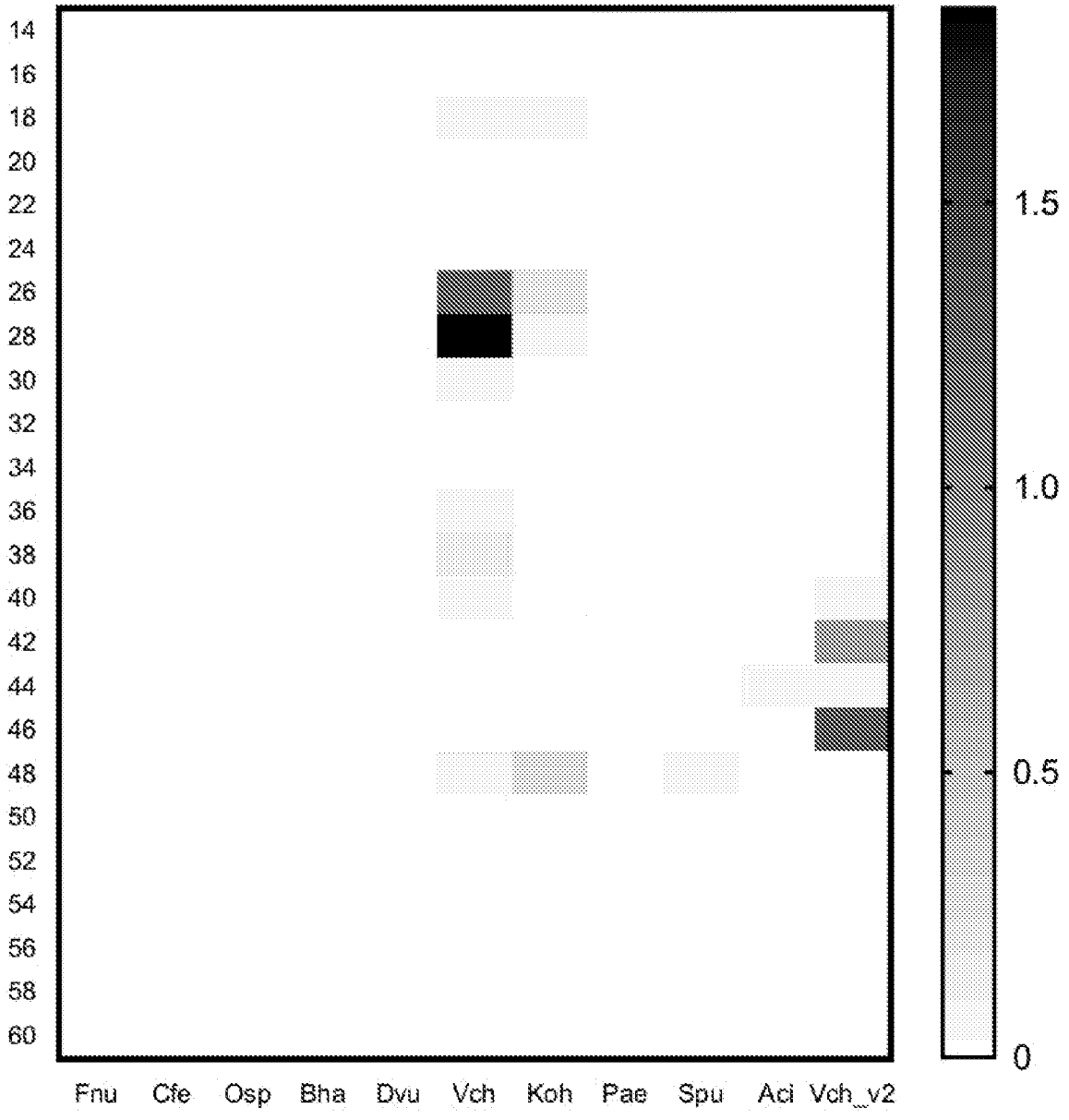

14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
50
52
54
56
58
60
Fnu
Cfe
Osp
Bha
Dvu
Vch
Koh
Pae
Spu
Aci
Vch_v2
1.5
1.0
0.5
0

FIG. 43

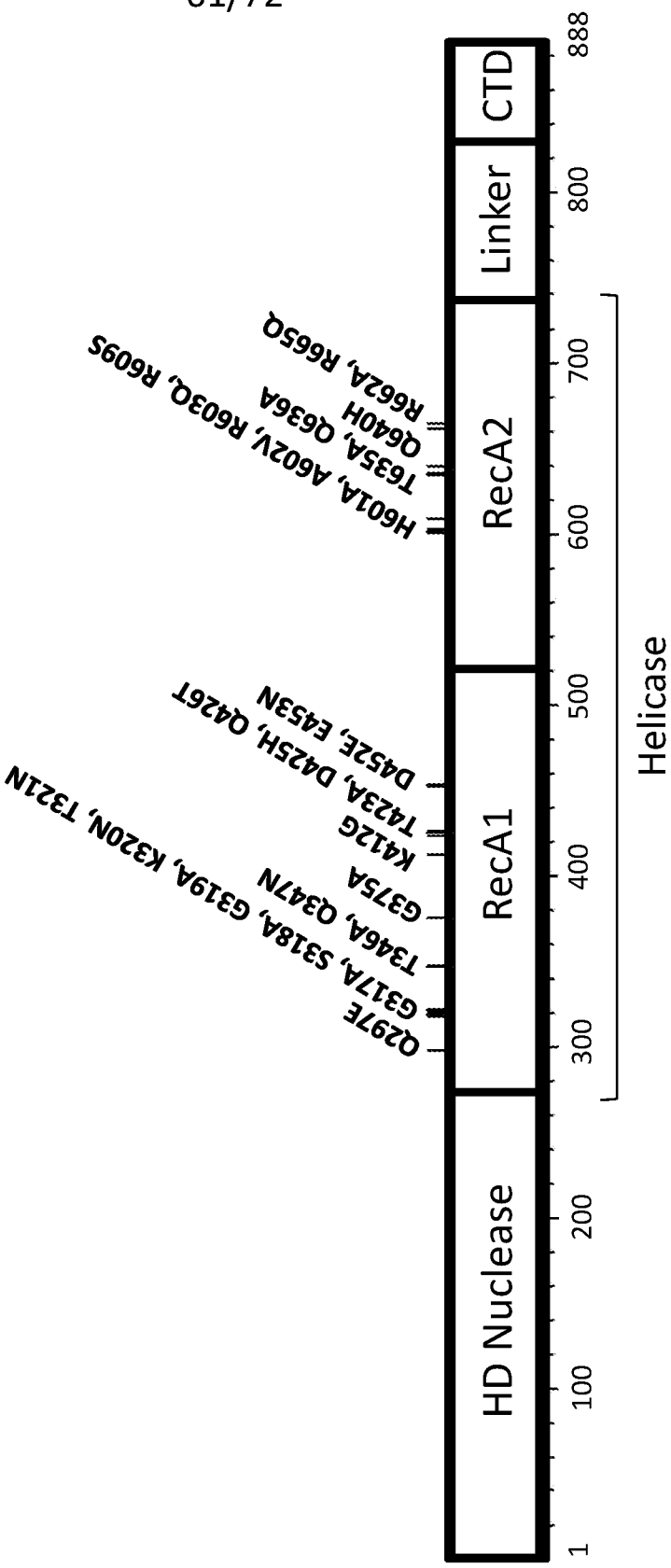

HD Nuclease
RecA1
RecA2
Linker
CTD
Helicase
1
100
200
300
400
500
600
700
800
888
Q297E
G317A, S318A, G319A, K320N, T321N
T346A, Q347N
G375A
K412G
T423A, D425H, Q426T
D452E, E453N
H601A, A602V, R603Q, R609S
T635A, Q636A
Q640H
R662A, R665Q

FIG. 44

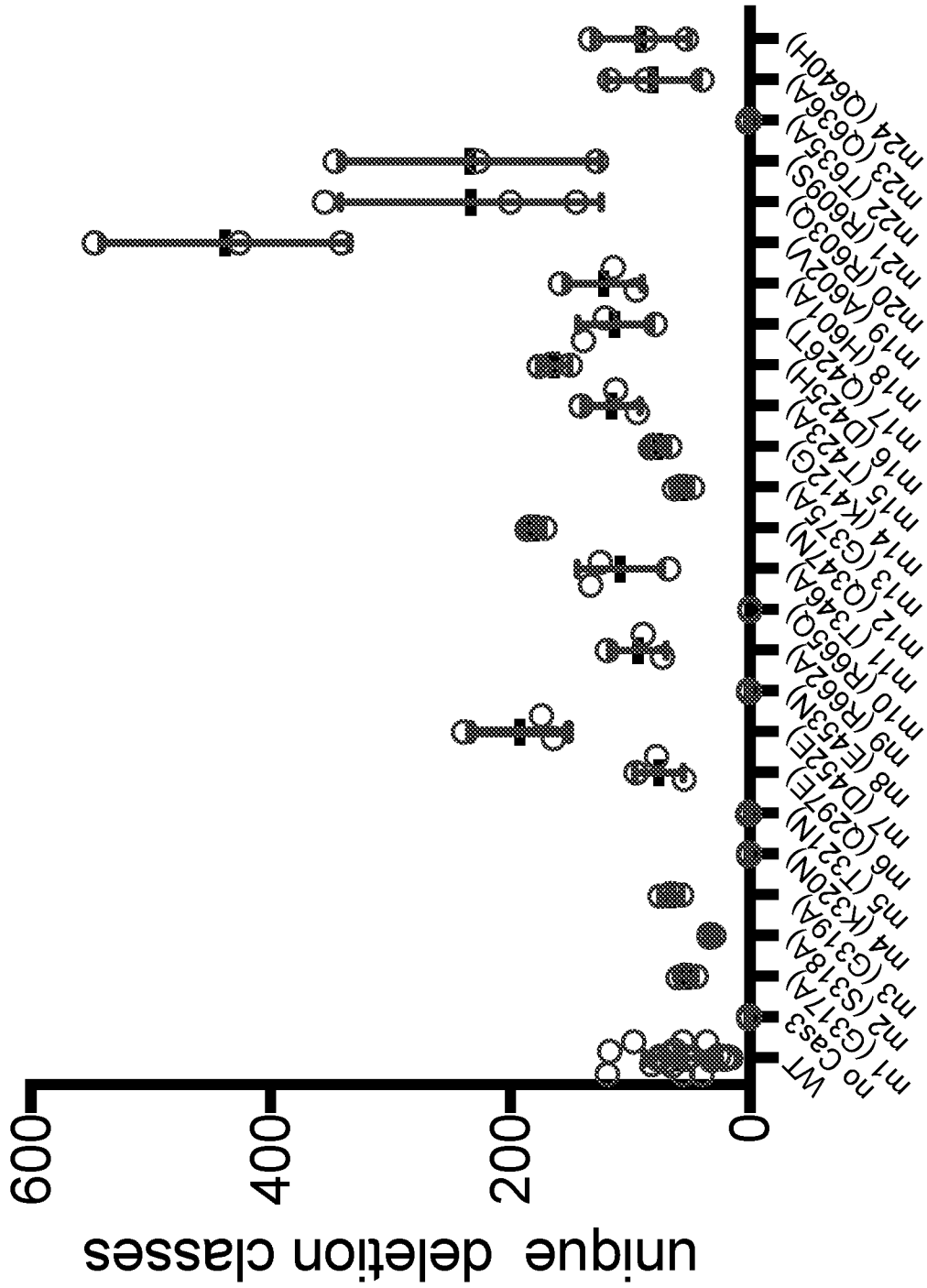

unique deletion classes
600
400
200
0
WT
no Cas3
m1 (G317A)
m2 (S318A)
m3 (G319A)
m4 (K320N)
m5 (T321N)
m6 (Q297E)
m7 (D452E)
m8 (E453N)
m9 (R662A)
m10 (R665Q)
m11 (T346A)
m12 (Q347N)
m13 (G375A)
m14 (K412G)
m15 (T423A)
m16 (D425H)
m17 (Q426T)
m18 (H601A)
m19 (A602V)
m20 (R603Q)
m21 (R609S)
m22 (T635A)
m23 (Q636A)
m24 (Q640H)

FIG. 45A

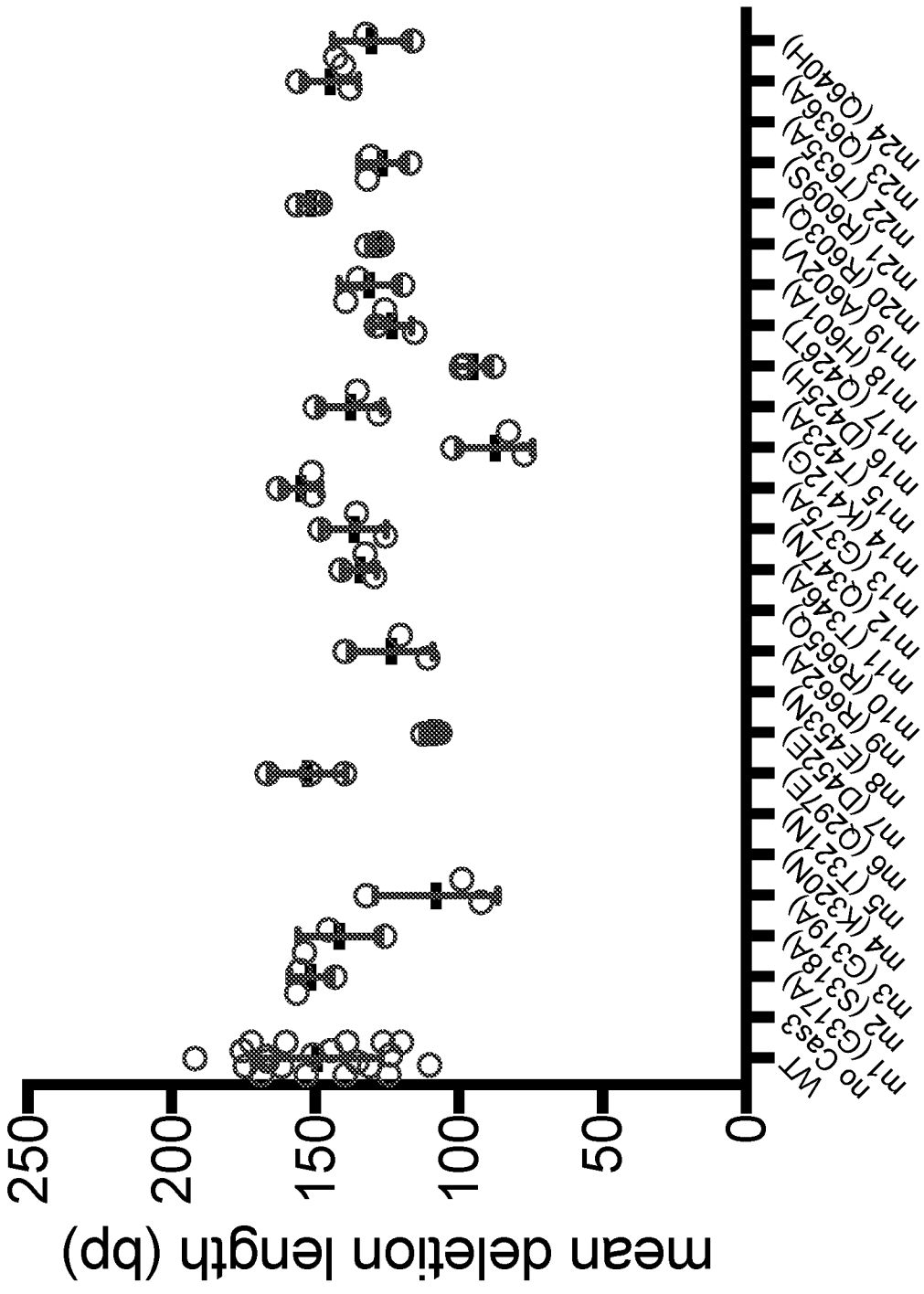

mean deletion length (bp)
250
200
150
100
50
0
WT
no Cas3
m1 (G317A)
m2 (S318A)
m3 (G319A)
m4 (K320N)
m5 (T321N)
m6 (Q297E)
m7 (D452E)
m8 (E453N)
m9 (R662A)
m10 (R665O)
m11 (T346A)
m12 (Q347N)
m13 (G375A)
m14 (K412G)
m15 (T423A)
m16 (D425H)
m17 (Q426T)
m18 (H601A)
m19 (A602V)
m20 (R603O)
m21 (R609S)
m22 (T635A)
m23 (Q636A)
m24 (Q640H)

FIG. 45B

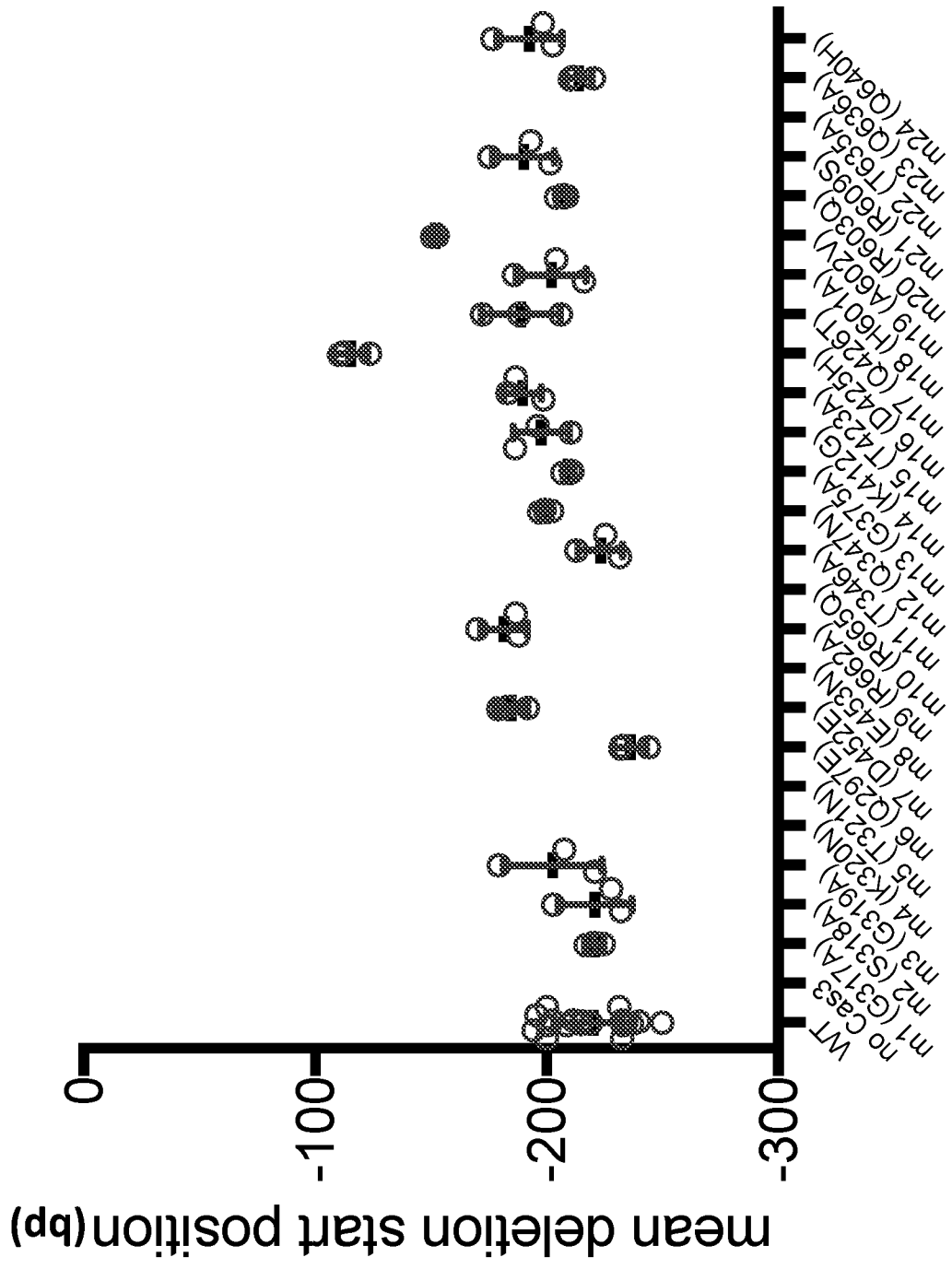

mean deletion start position(bp)
0
-100
-200
-300
WT
no Cas3
m1 (G317A)
m2 (S318A)
m3 (G319A)
m4 (K320N)
m5 (T321N)
m6 (Q229E)
m7 (D452E)
m8 (E453N)
m9 (R662A)
m10 (R665Q)
m11 (T346A)
m12 (Q347N)
m13 (G375A)
m14 (K412G)
m15 (T423A)
m16 (D425H)
m17 (Q426T)
m18 (H601A)
m19 (A602V)
m20 (R603Q)
m21 (R609S)
m22 (T635A)
m23 (Q636A)
m24 (Q640H)

FIG. 45C

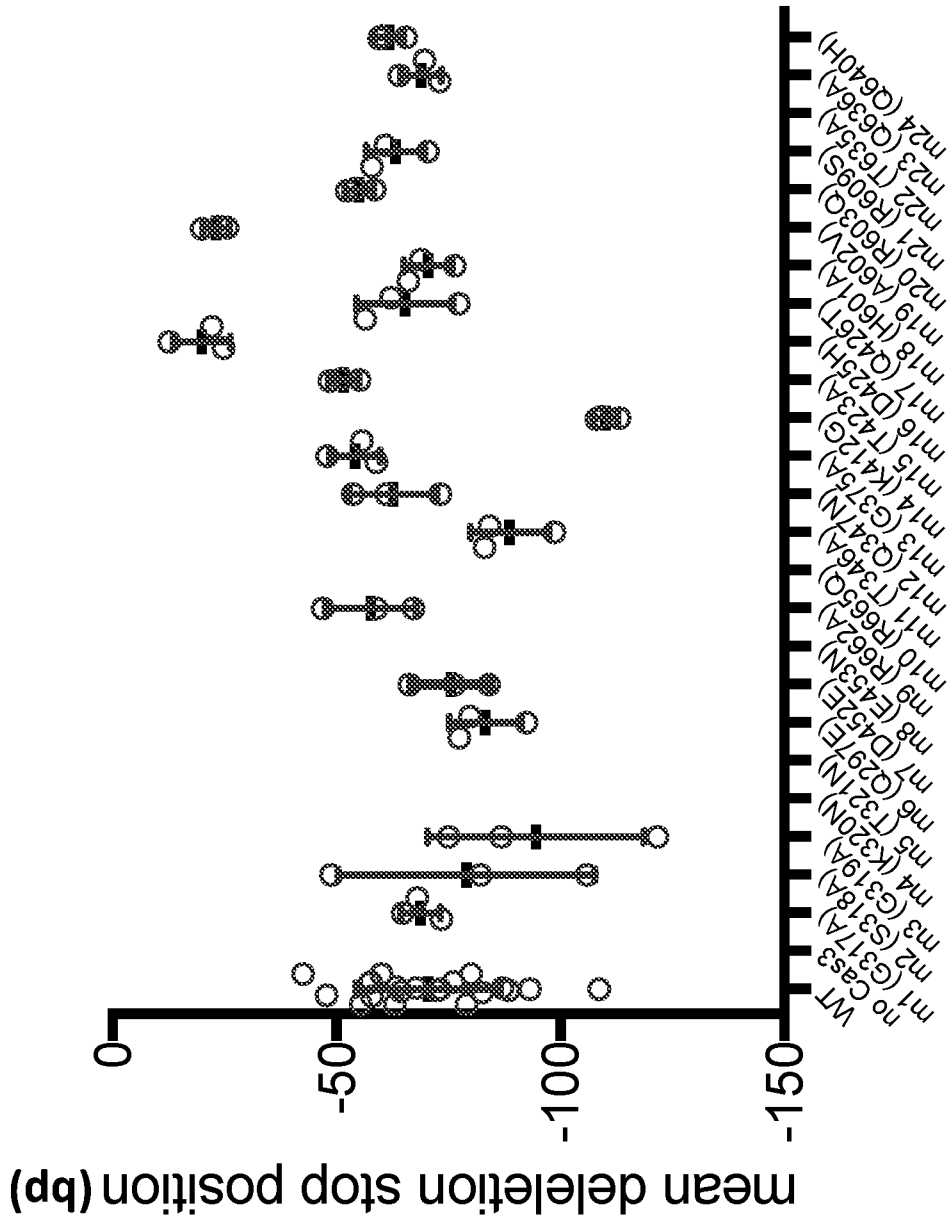

mean deletion stop position (bp)
0
-50
-100
-150
WT
no Cas3
m1 (G317A)
m2 (S318A)
m3 (G319A)
m4 (K320N)
m5 (T321N)
m6 (Q297E)
m7 (D452E)
m8 (E453N)
m9 (R662A)
m10 (R665Q)
m11 (T346Q)
m12 (Q347A)
m13 (G375N)
m14 (K412A)
m15 (T423G)
m16 (D425A)
m17 (Q426H)
m18 (H601T)
m19 (A602A)
m20 (R602V)
m21 (R603Q)
m22 (T609S)
m23 (Q635A)
m24 (Q636A)
(Q640H)

FIG. 45D

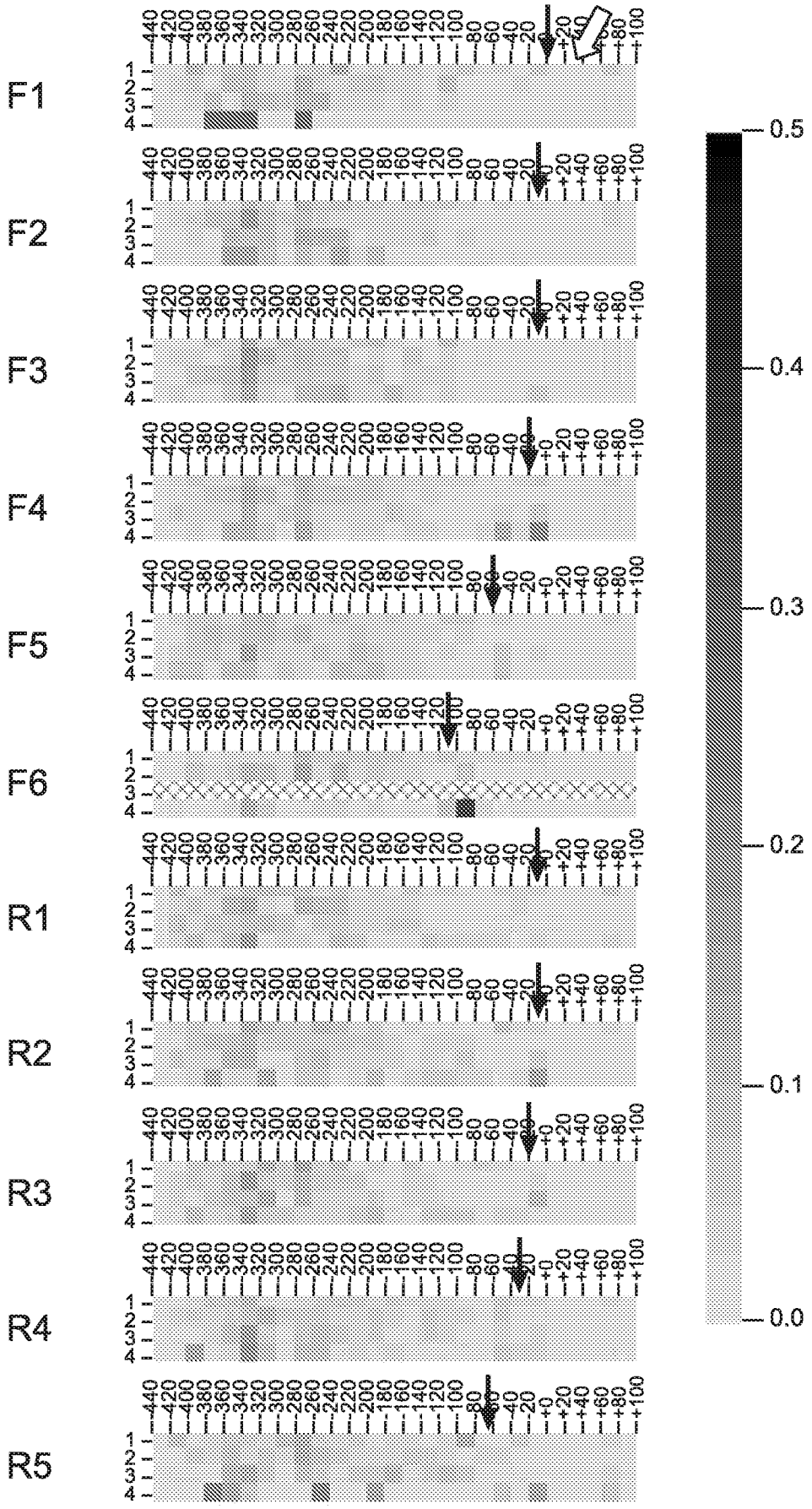


FIG. 46A

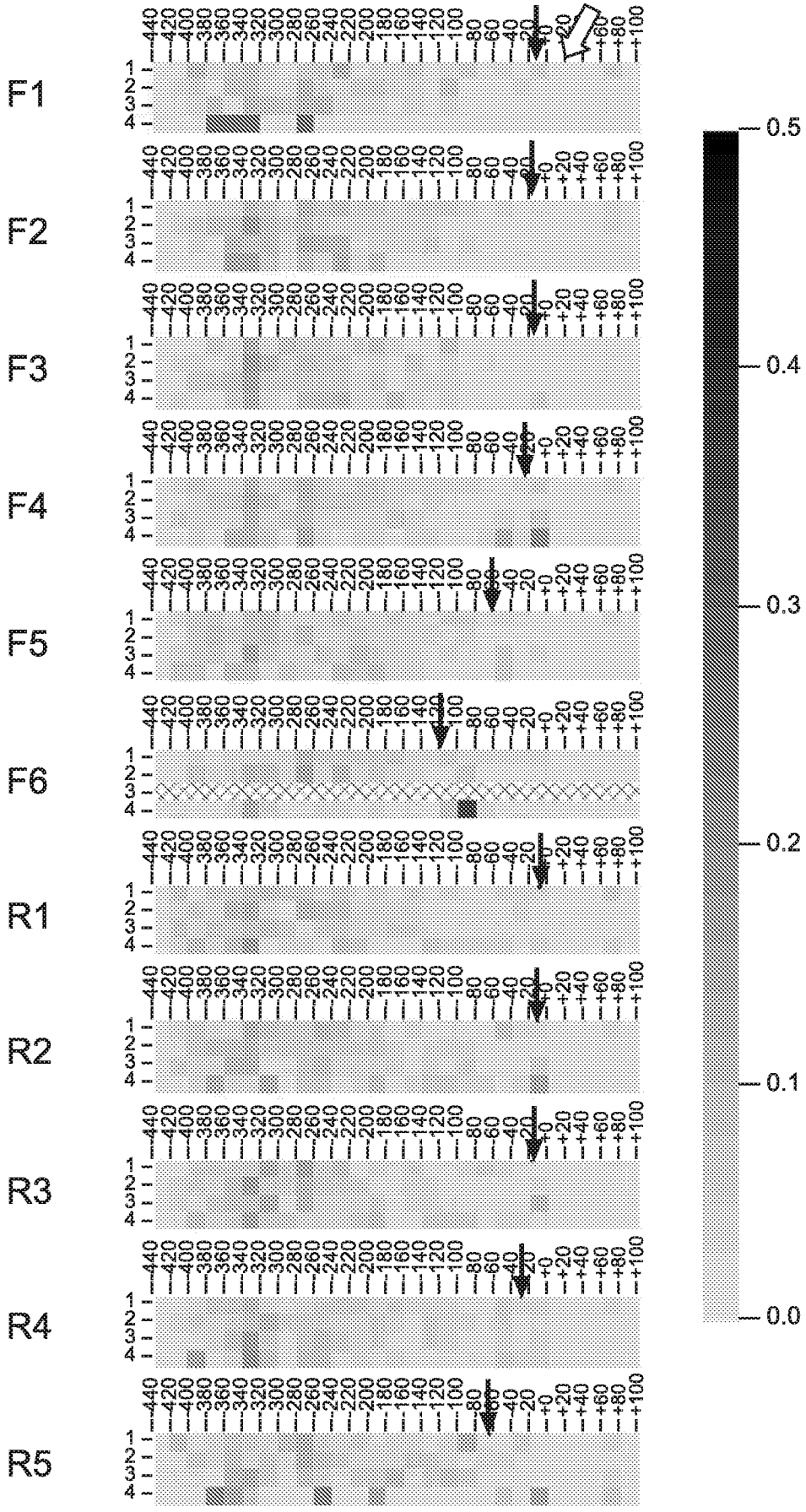


FIG. 46B

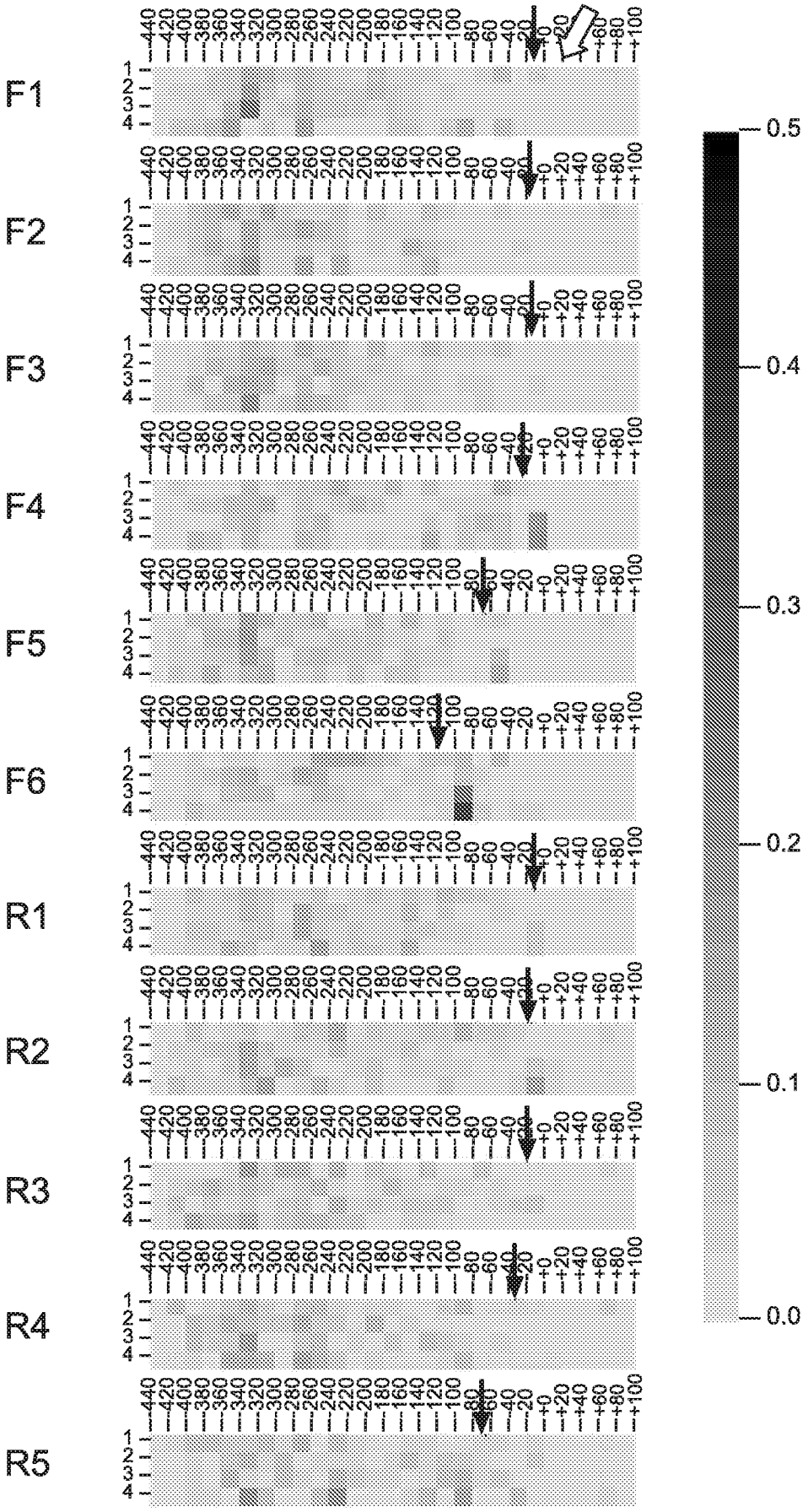


FIG. 46C

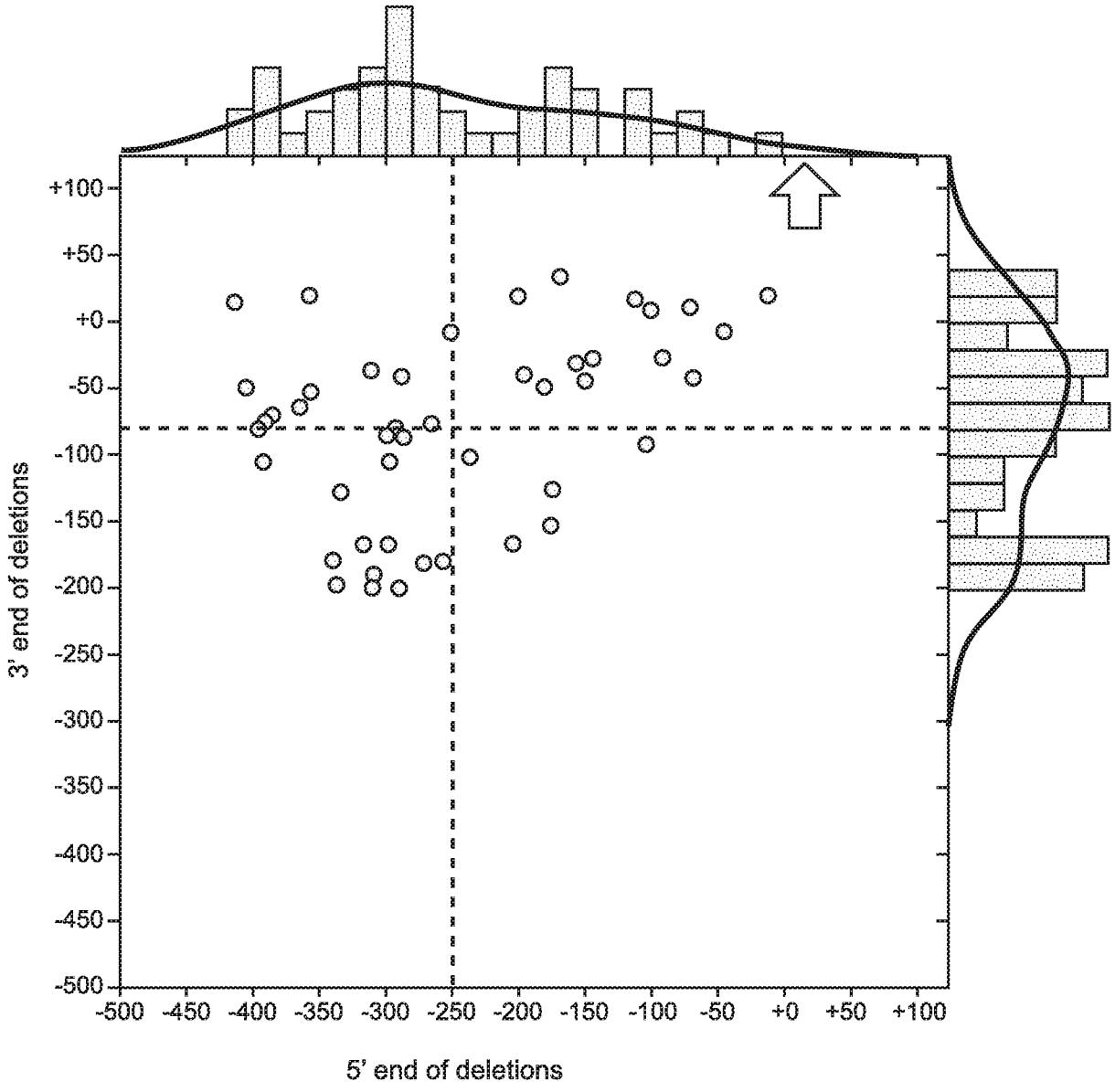

+100
+50
+0
-50
-100
-150
-200
-250
-300
-350
-400
-450
-500
3' end of deletions
-500 -450 -400 -350 -300 -250 -200 -150 -100 -50 +0 +50 +100
5' end of deletions

FIG. 47A

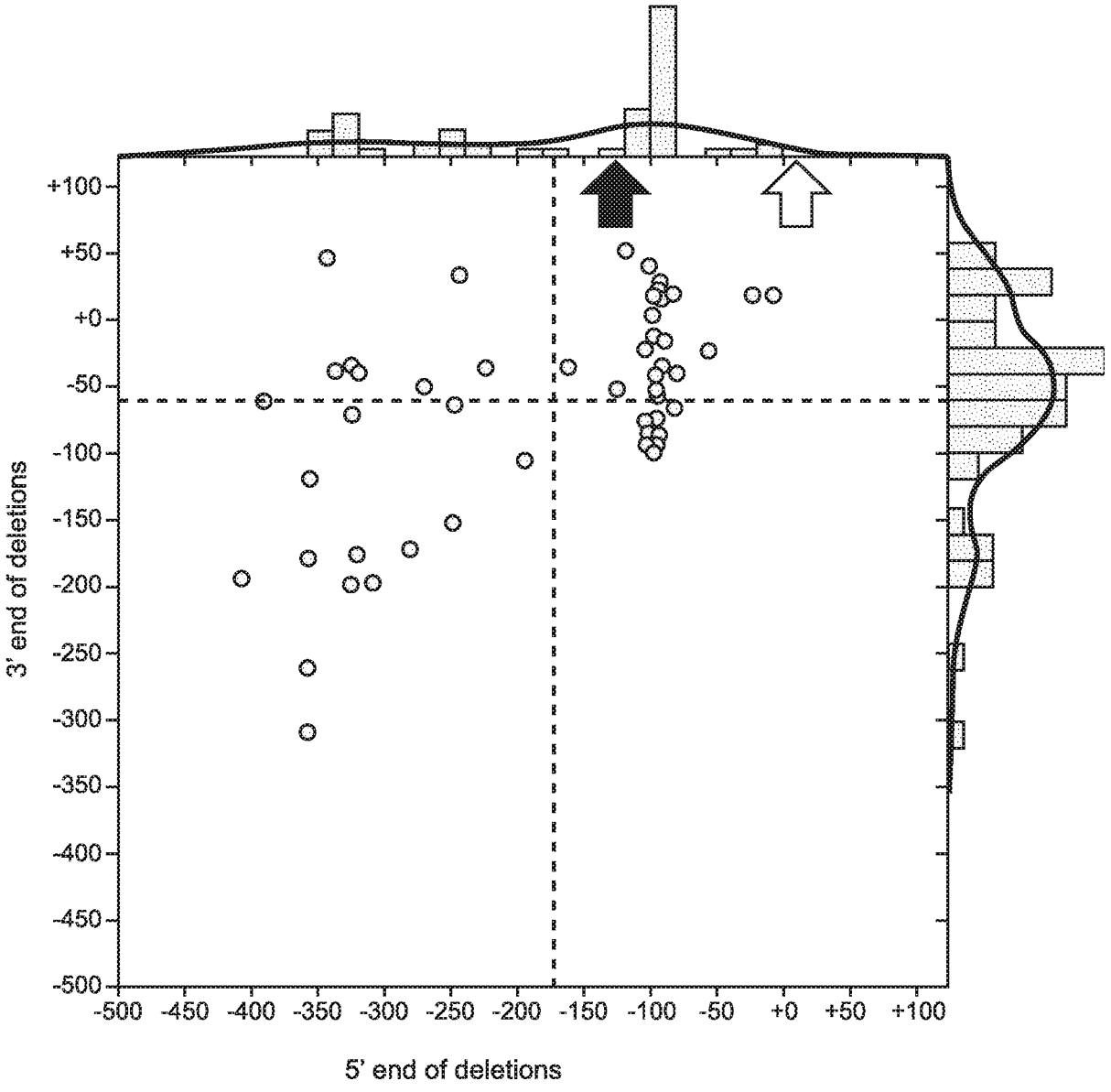

+100
+50
+0
-50
-100
-150
-200
-250
-300
-350
-400
-450
-500
3' end of deletions
-500 -450 -400 -350 -300 -250 -200 -150 -100 -50 +0 +50 +100
5' end of deletions

FIG. 47B

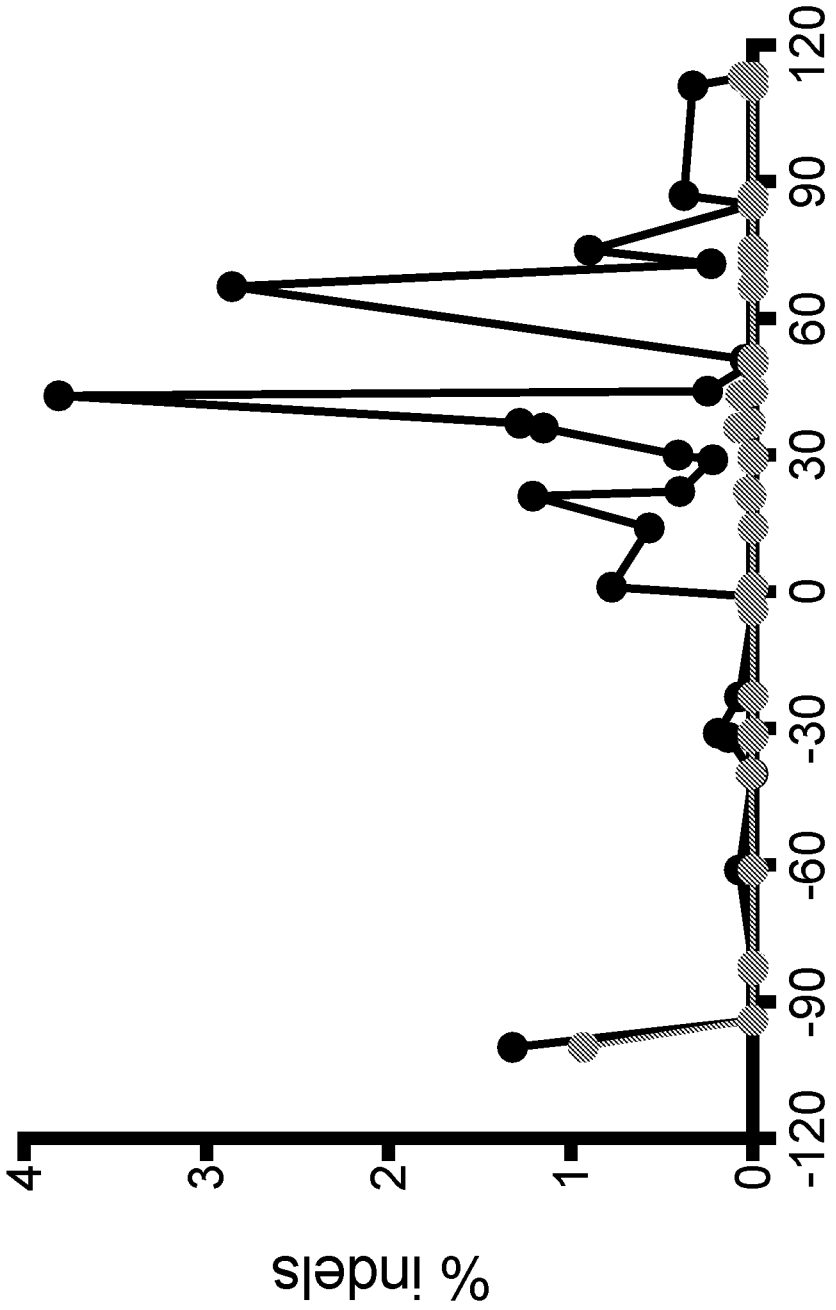


FIG. 48

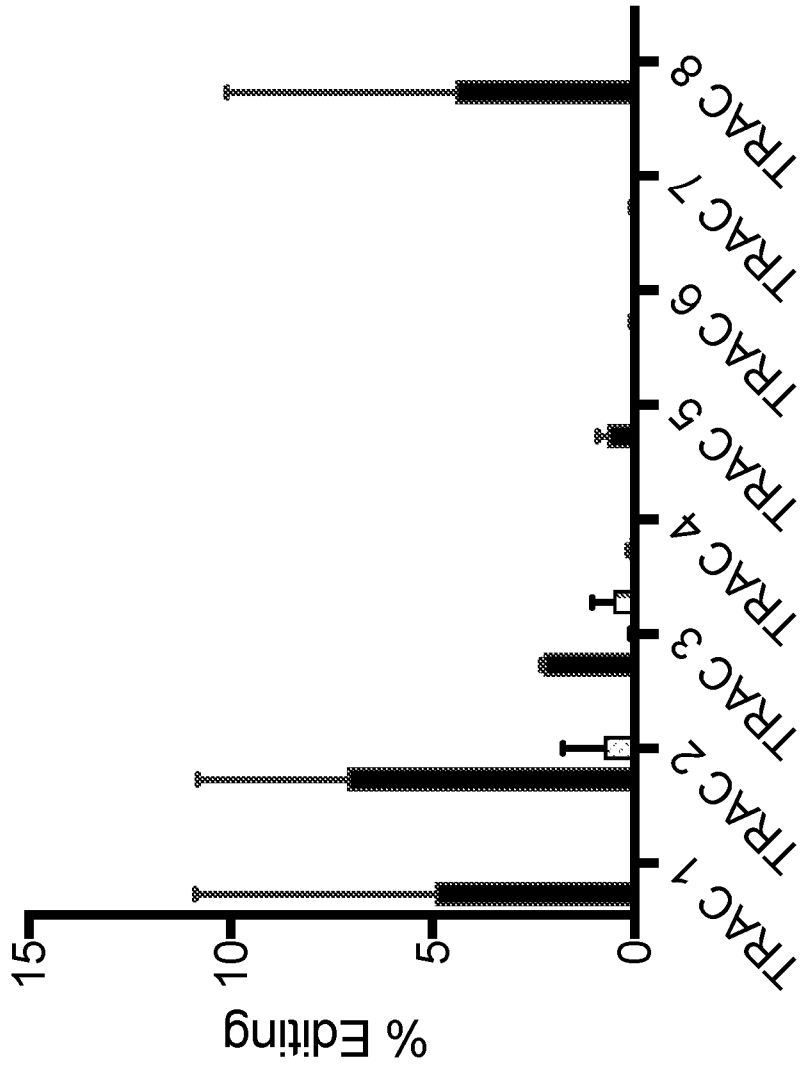

% Editing
15
10
5
0
TRAC 1
TRAC 2
TRAC 3
TRAC 4
TRAC 5
TRAC 6
TRAC 7
TRAC 8

FIG. 49