FIG. 1A

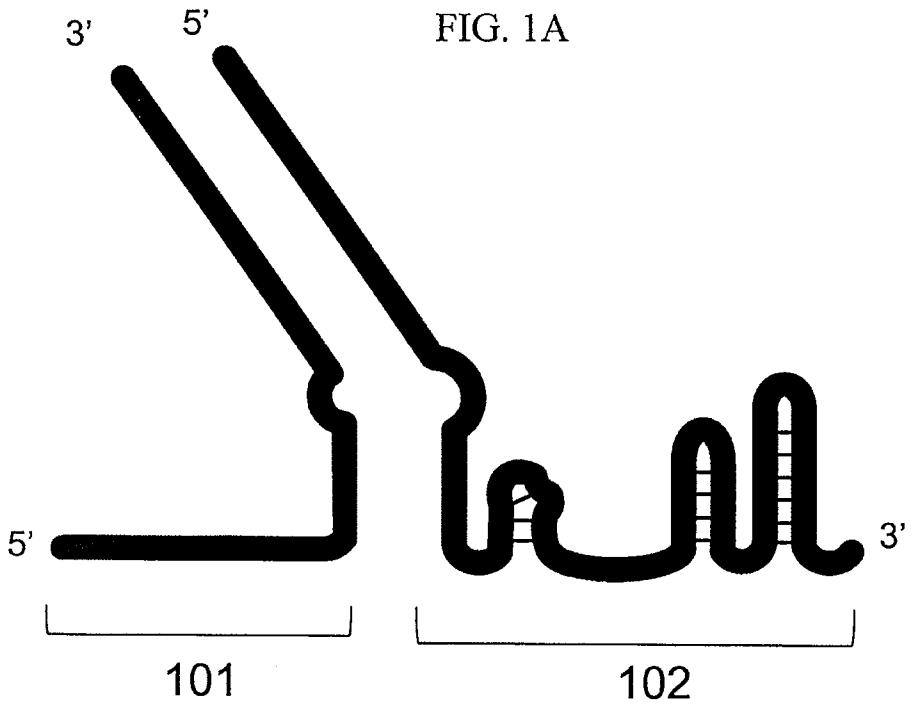
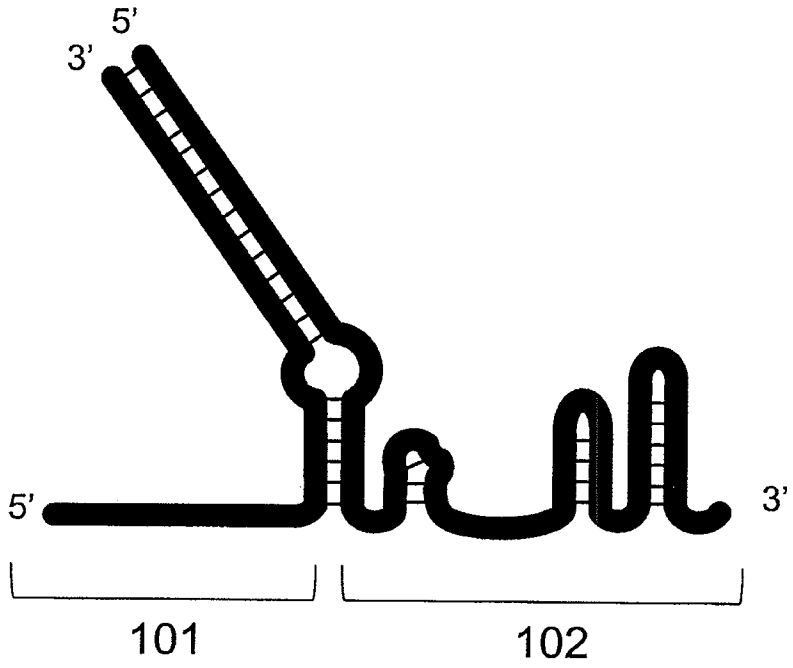


FIG. 1B



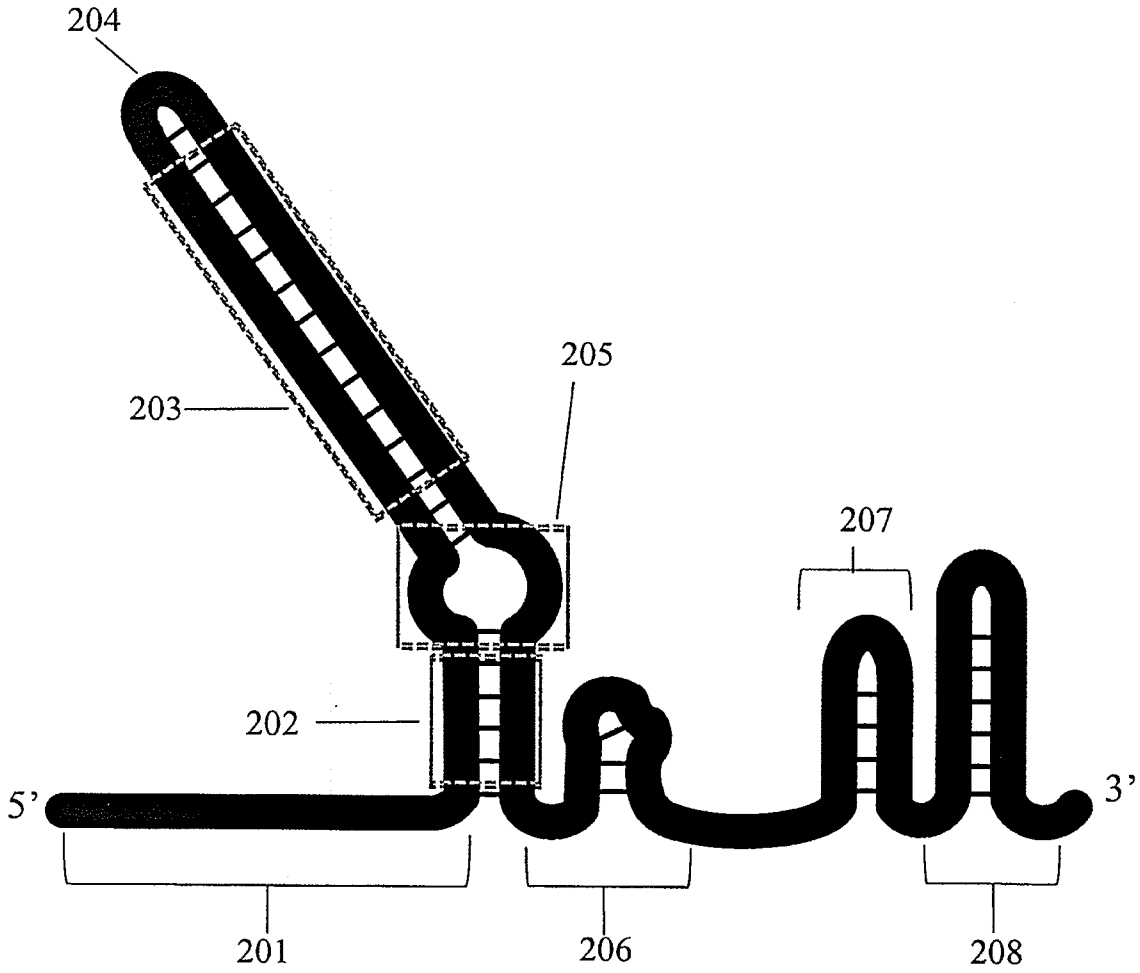
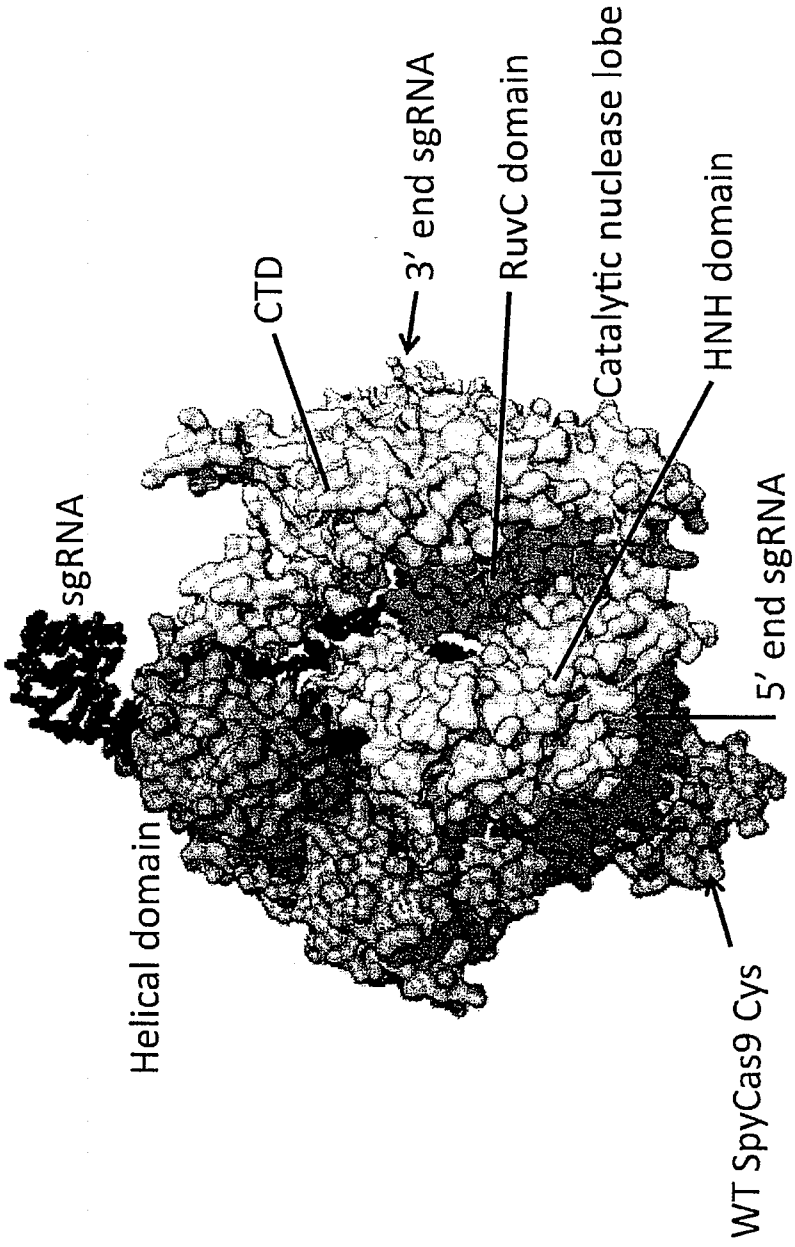


FIG. 2

FIG. 3A


sgRNA
Helical domain
CTD
3' end sgRNA
RuvC domain
Catalytic nuclease lobe
HNH domain
5' end sgRNA
WT SpyCas9 Cys

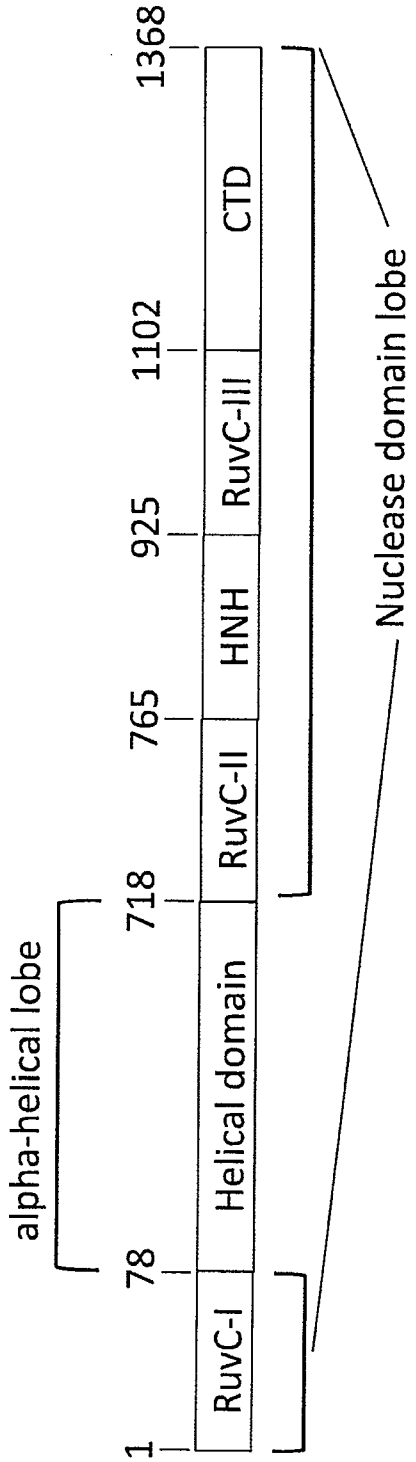

1
78
718
765
925
1102
1368
RuvC-I
Helical domain
RuvC-II
HNH
RuvC-III
CTD
alpha-helical lobe
Nuclease domain lobe

FIG. 3B

FIG. 4

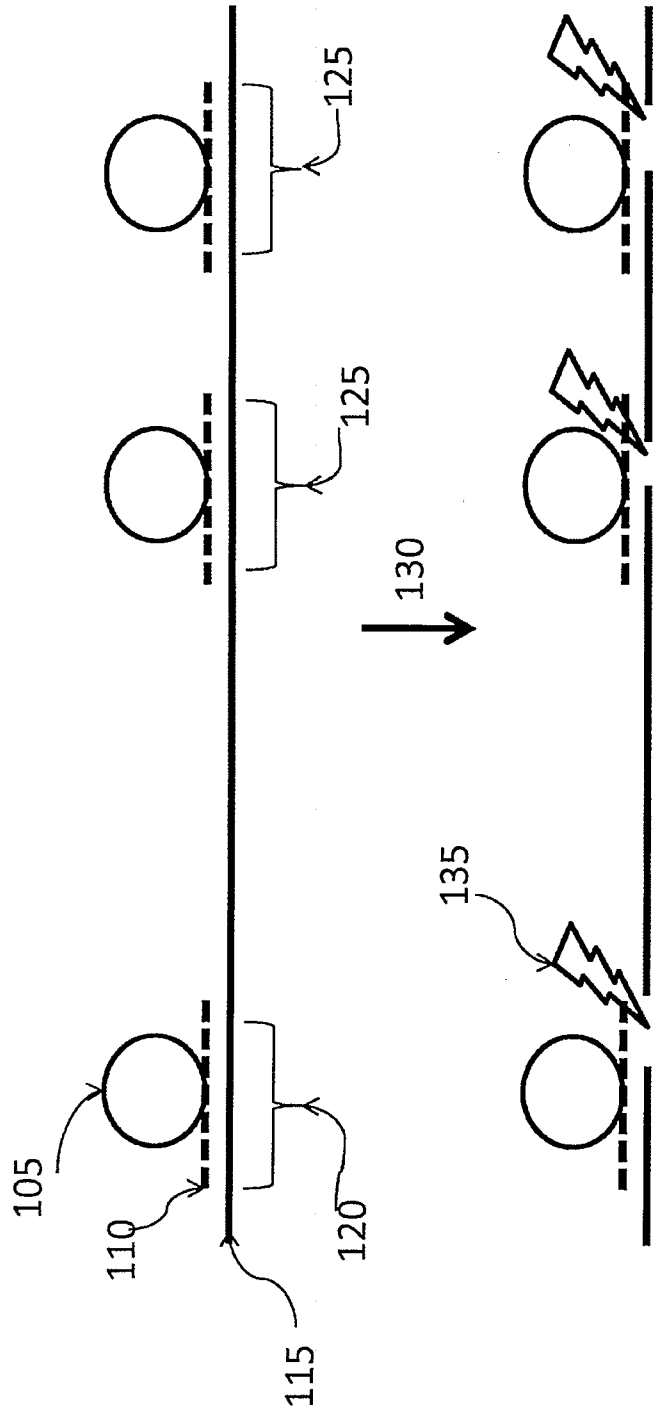
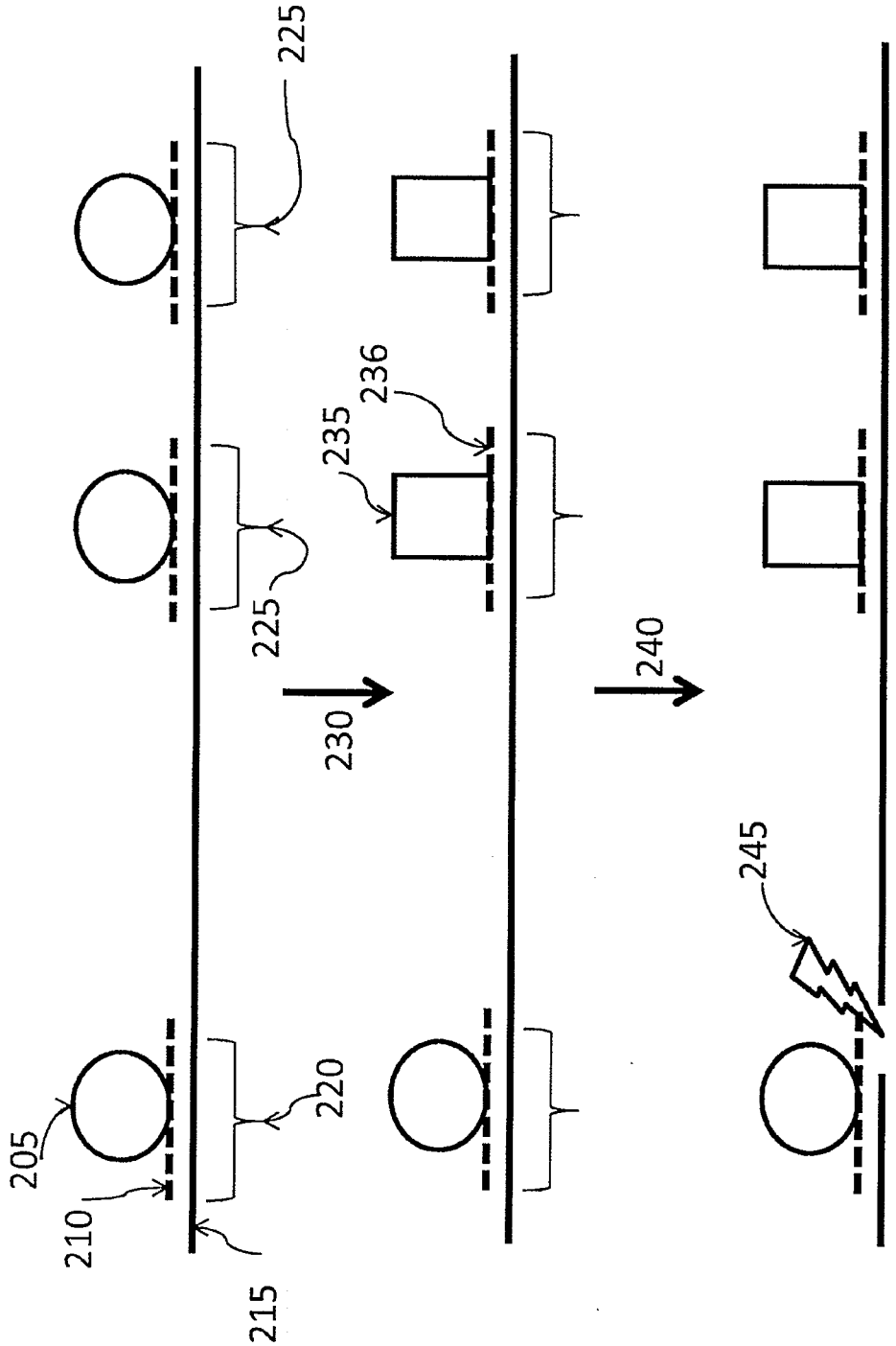


FIG. 5



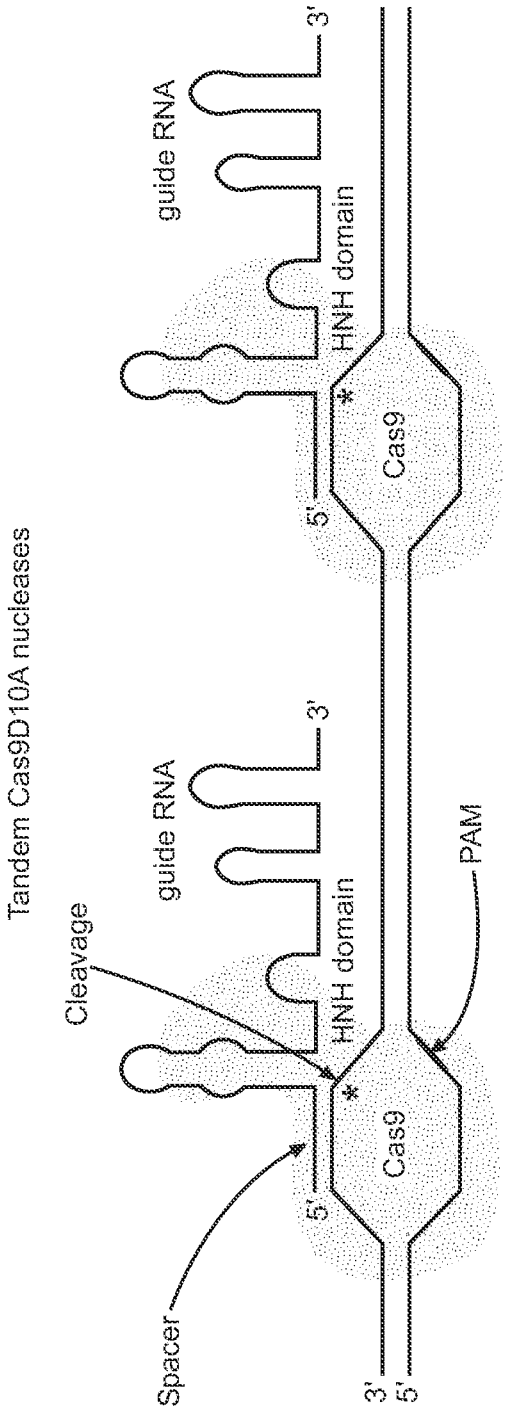


FIG. 6A



FIG. 6B

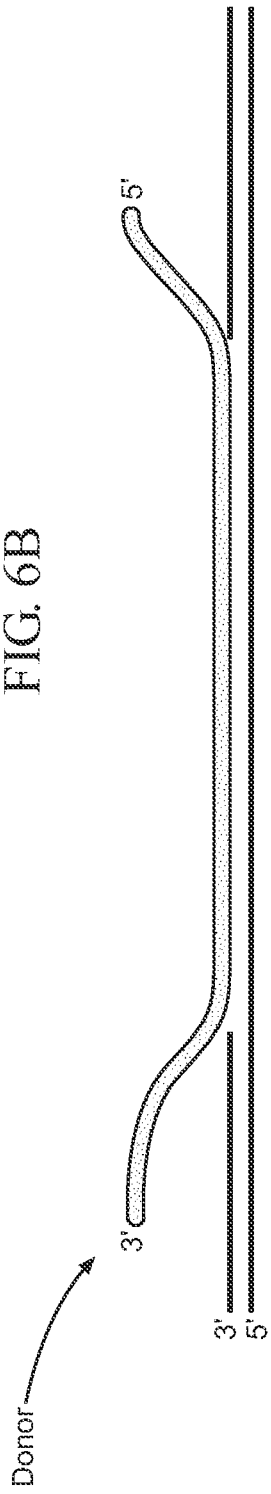


FIG. 6C

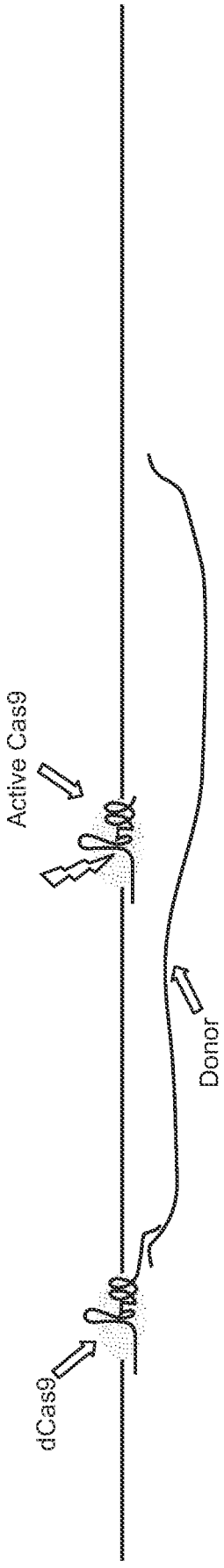


FIG. 7A

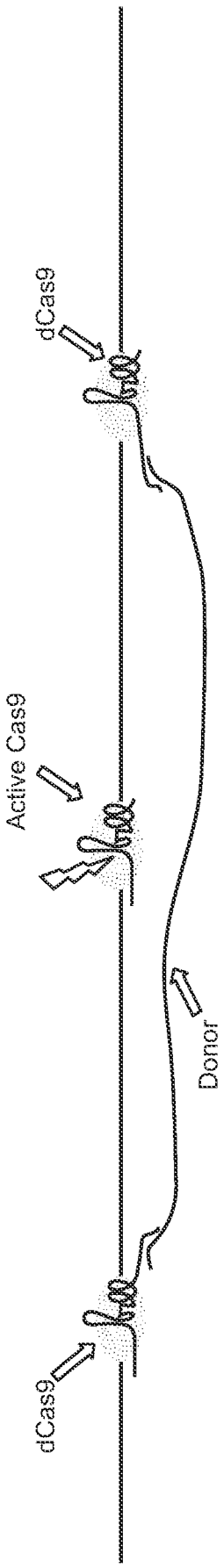


FIG. 7B

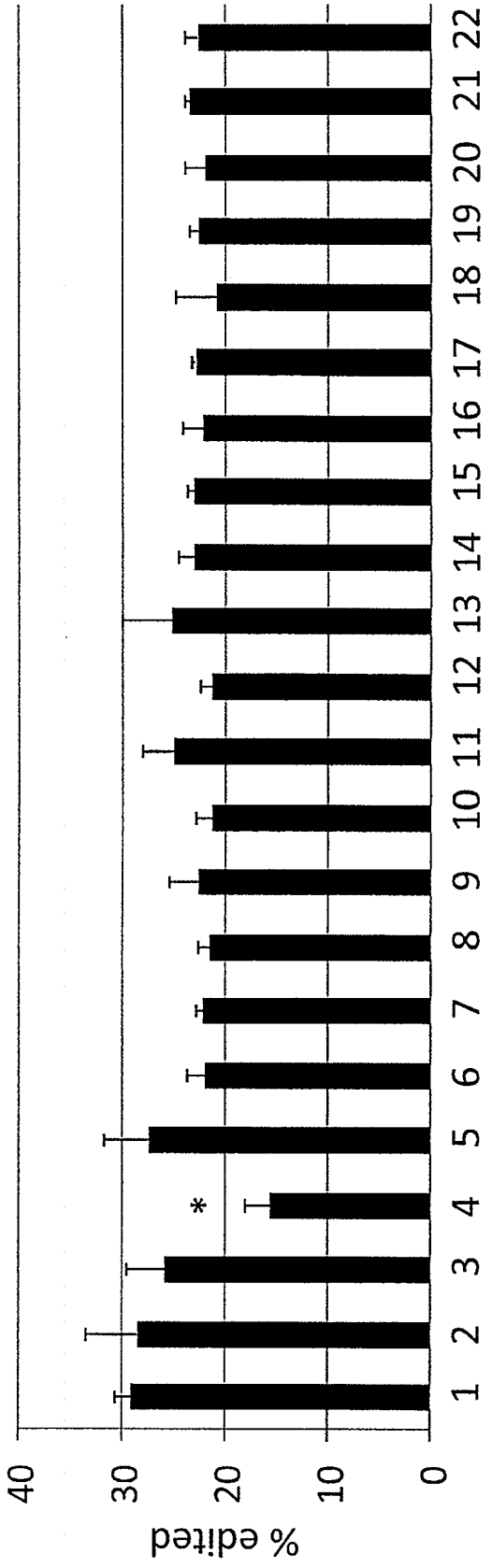

% edited
0
10
20
30
40
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
*

FIG. 8

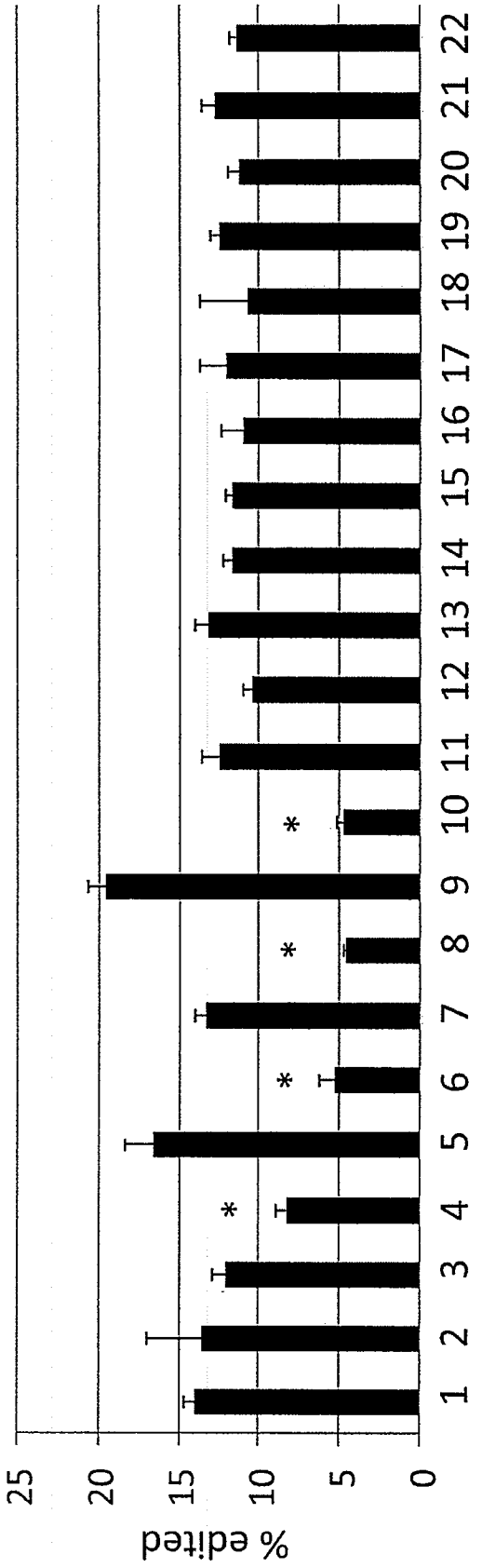

% edited
25
20
15
10
5
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
*
*
*
*
*

FIG. 9

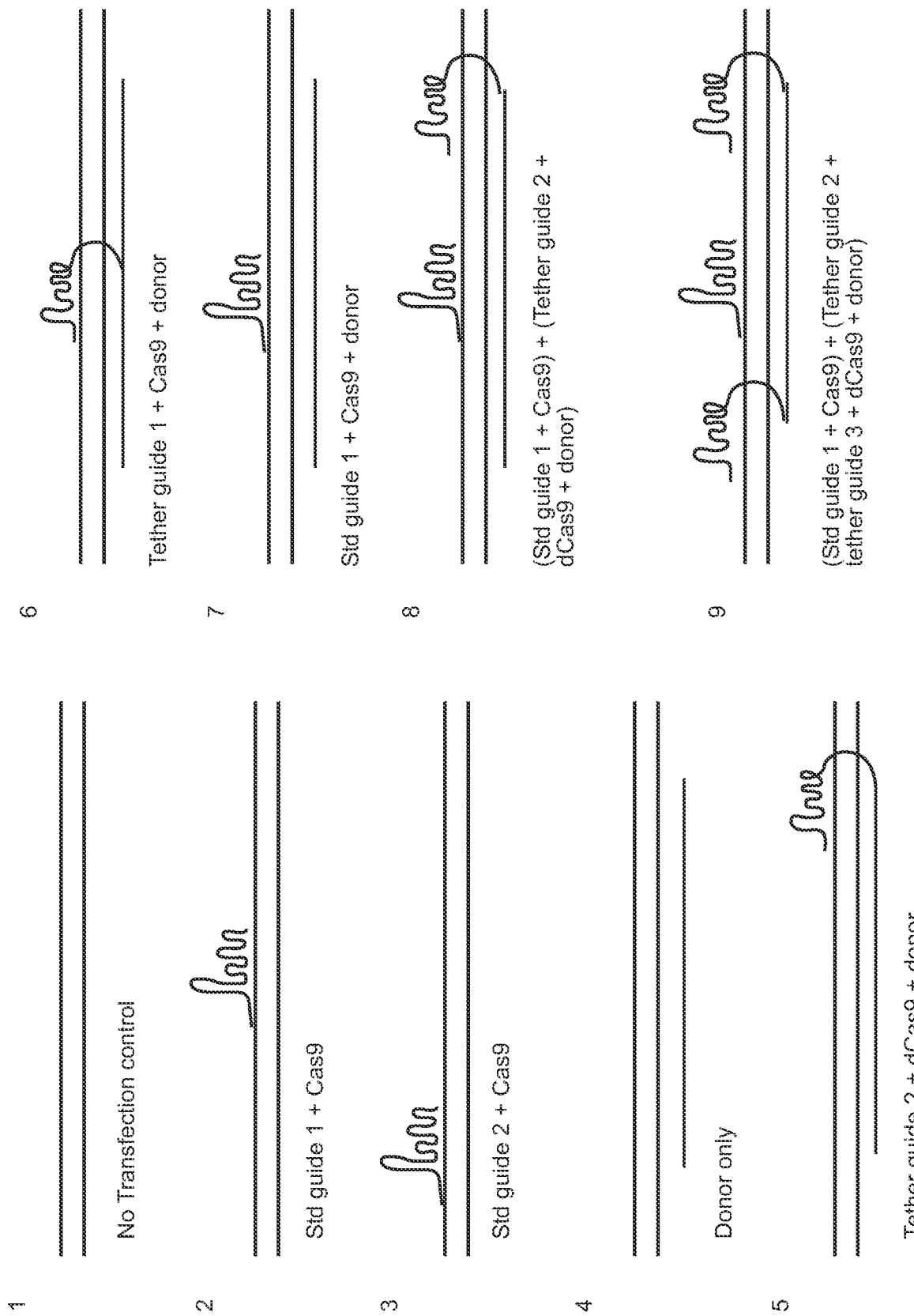

1
No Transfection control
2
Std guide 1 + Cas9
3
Std guide 2 + Cas9
4
Donor only
5
Tether guide 2 + dCas9 + donor
6
Tether guide 1 + Cas9 + donor
7
Std guide 1 + Cas9 + donor
8
(Std guide 1 + Cas9) + (Tether guide 2 + dCas9 + donor)
9
(Std guide 1 + Cas9) + (Tether guide 2 + tether guide 3 + dCas9 + donor)

FIG. 10

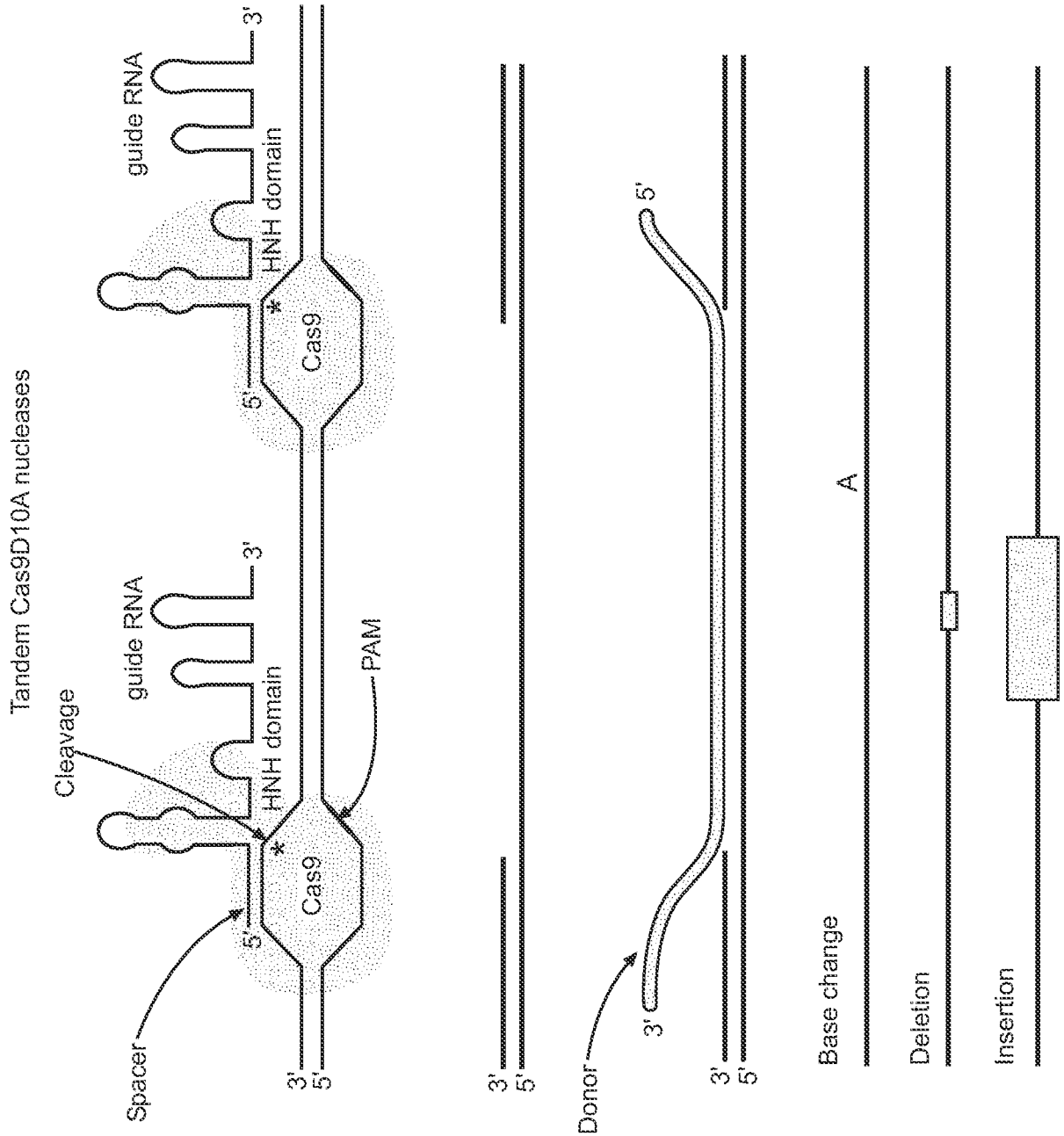

Tandem Cas9D10A nucleases
Cleavage
guide RNA
3'
HNH domain
Cas9
5'
PAM
Spacer
guide RNA
3'
HNH domain
Cas9
5'
3'
5'
3'
5'
Donor
5'
3'
3'
5'
A
Base change
Deletion
Insertion

FIG. 11

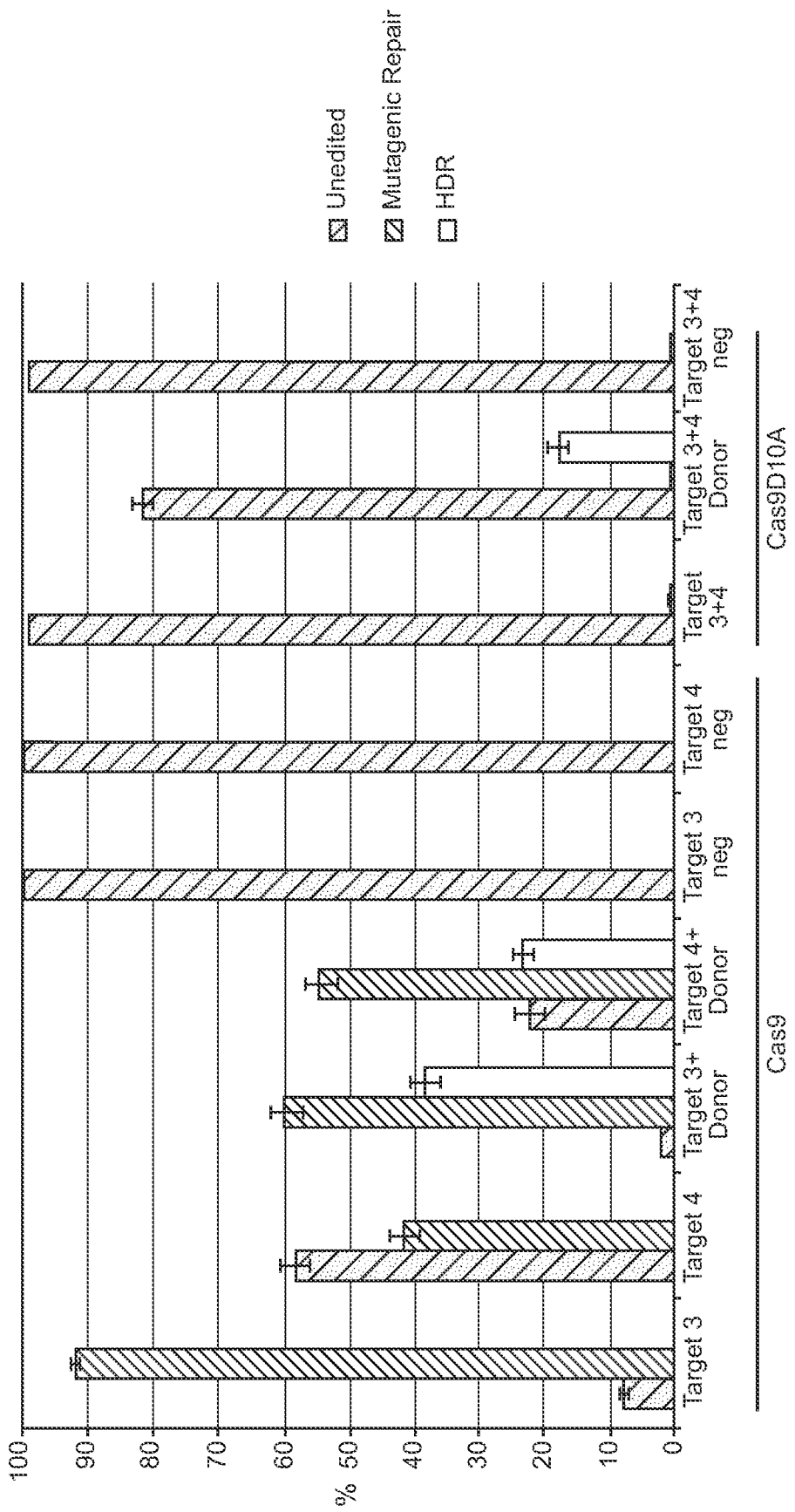

Unedited
Mutagenic Repair
HDR
100
90
80
70
60
50
40
30
20
10
0
%
Target 3
Target 4
Target 3+ Donor
Target 4+ Donor
Target 3 neg
Target 4 neg
Target 3+4
Target 3+4 Donor
Target 3+4 neg
Cas9
Cas9D10A

FIG. 12