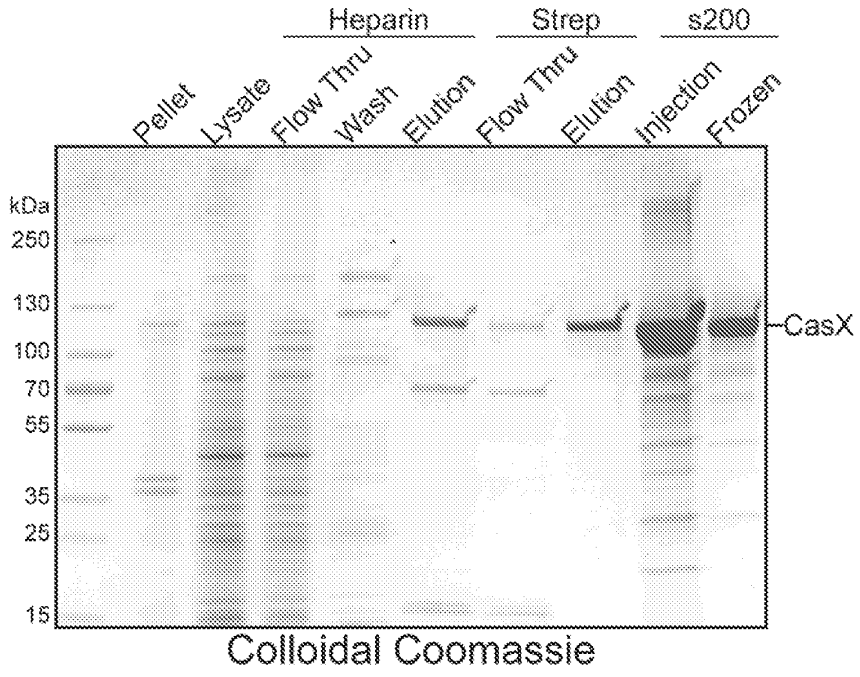
Heparin
Strep
s200
Pellet
Lysate
Flow Thru
Wash
Elution
Flow Thru
Elution
Injection
Frozen
kDa
250
130
100
70
55
35
25
15
CasX
Colloidal Coomassie

FIG. 1

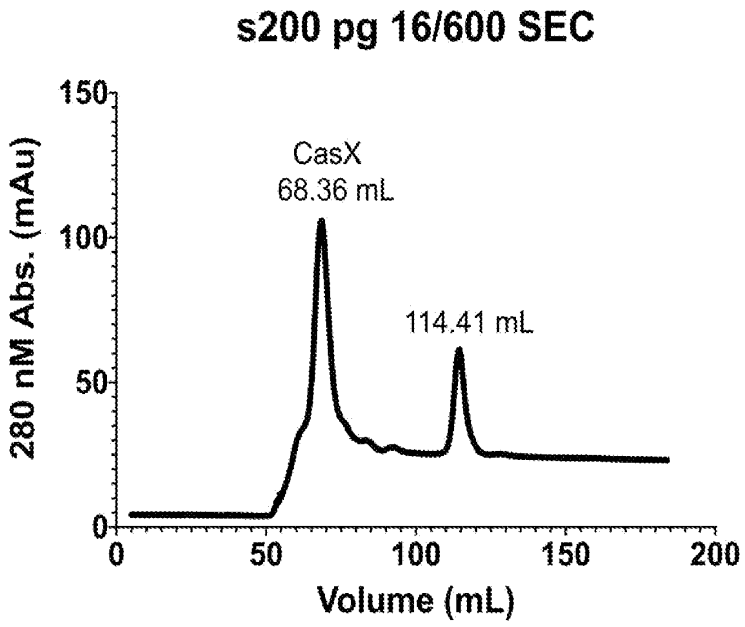
s200 pg 16/600 SEC
280 nM Abs. (mAu)
150
100
50
0
CasX
68.36 mL
114.41 mL
0
50
100
150
200
Volume (mL)

FIG. 2

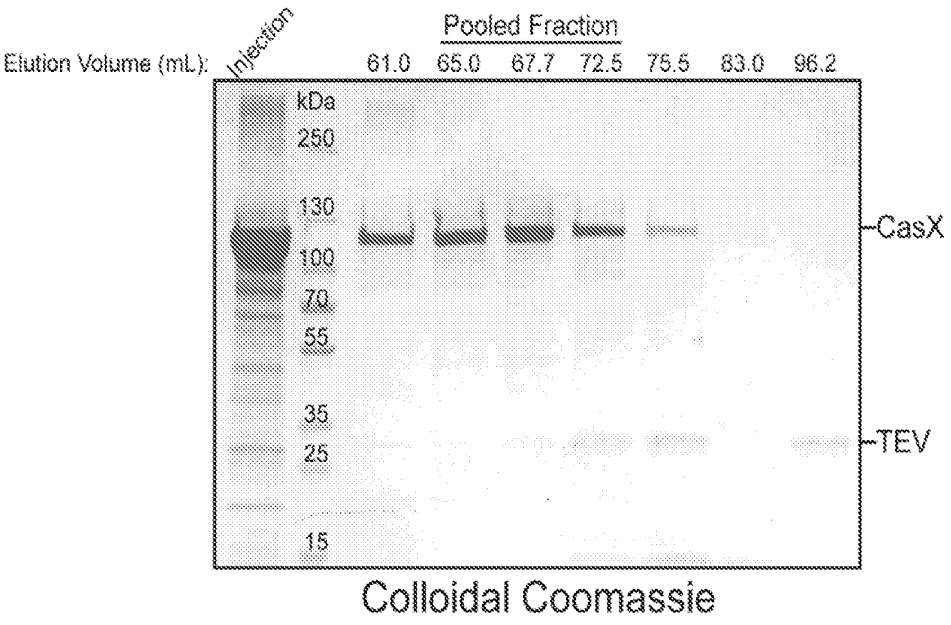
Elution Volume (mL):
Injection
Pooled Fraction
61.0
65.0
67.7
72.5
75.5
83.0
96.2
kDa
250
130
100
70
55
35
25
15
CasX
TEV
Colloidal Coomassie

FIG. 3

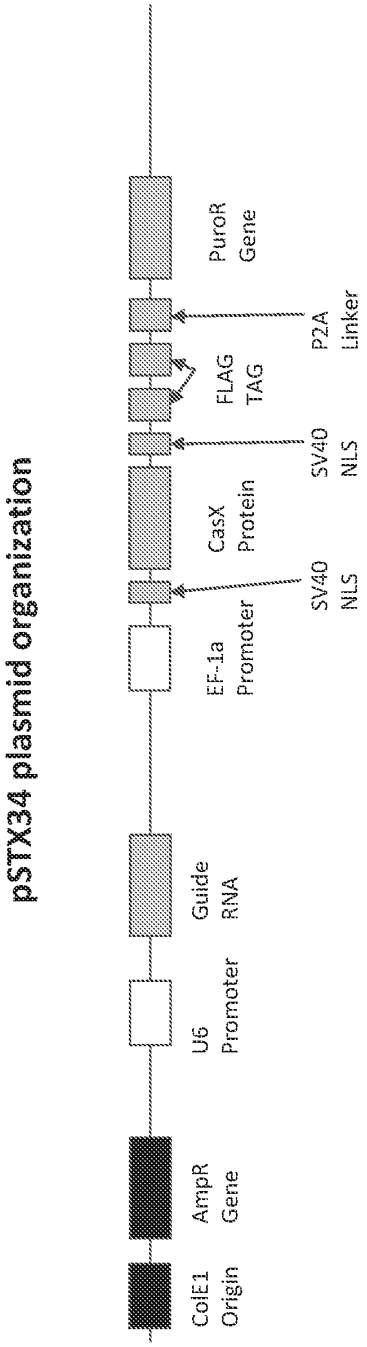
pSTX34 plasmid organization
ColE1 Origin
AmpR Gene
U6 Promoter
Guide RNA
EF-1a Promoter
SV40 NLS
CasX Protein
SV40 NLS
FLAG TAG
P2A Linker
PuroR Gene

FIG. 4

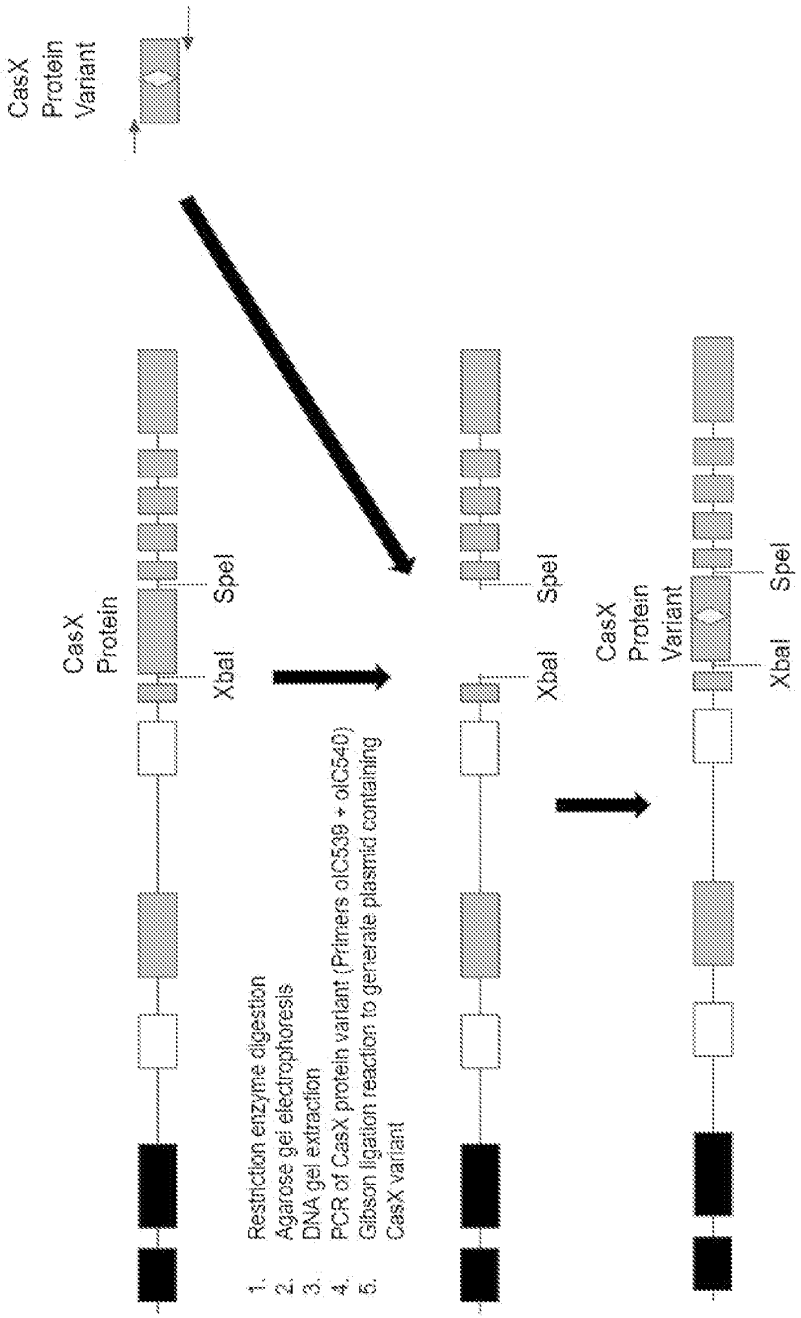
CasX Protein Variant
CasX Protein
XbaI
SpeI
1. Restriction enzyme digestion
2. Agarose gel electrophoresis
3. DNA gel extraction
4. PCR of CasX protein variant (Primers oIC539 + oIC540)
5. Gibson ligation reaction to generate plasmid containing CasX variant
XbaI
SpeI
CasX Protein Variant
XbaI
SpeI

FIG. 5

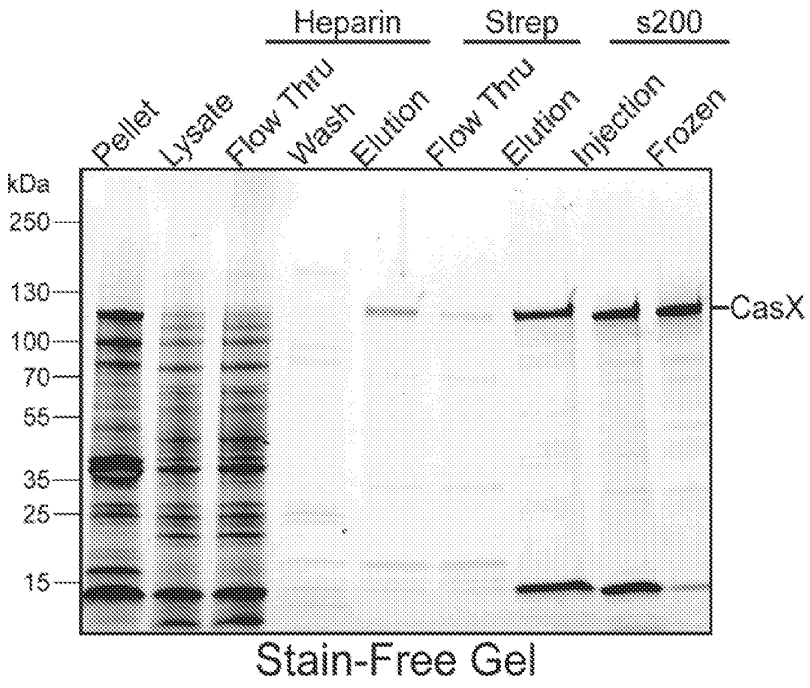
Heparin
Strep
s200
Pellet
Lysate
Flow Thru
Wash
Elution
Flow Thru
Elution
Injection
Frozen
kDa
250
130
100
70
55
35
25
15
CasX
Stain-Free Gel

FIG. 6

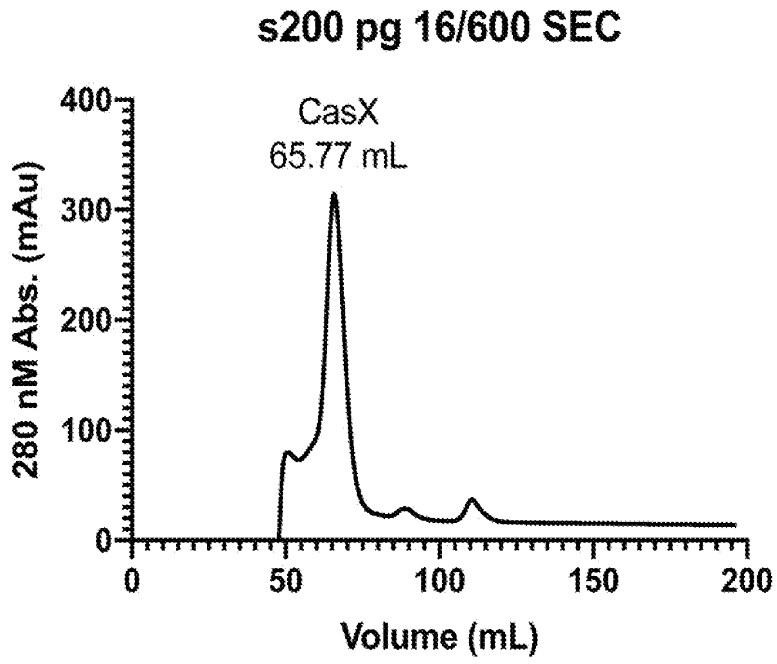
s200 pg 16/600 SEC
CasX
65.77 mL
280 nM Abs. (mAu)
400
300
200
100
0
0
50
100
150
200
Volume (mL)

FIG. 7

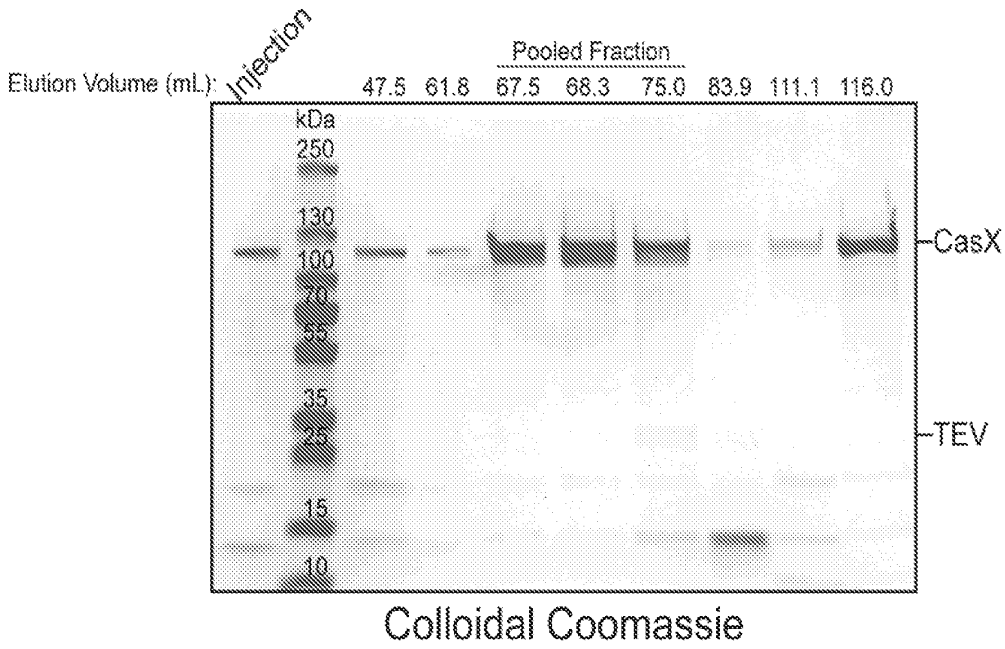
Elution Volume (mL):
Injection
Pooled Fraction
47.5
61.8
67.5
68.3
75.0
83.9
111.1
116.0
kDa
250
130
100
70
55
35
25
15
10
CasX
TEV
Colloidal Coomassie

FIG. 8

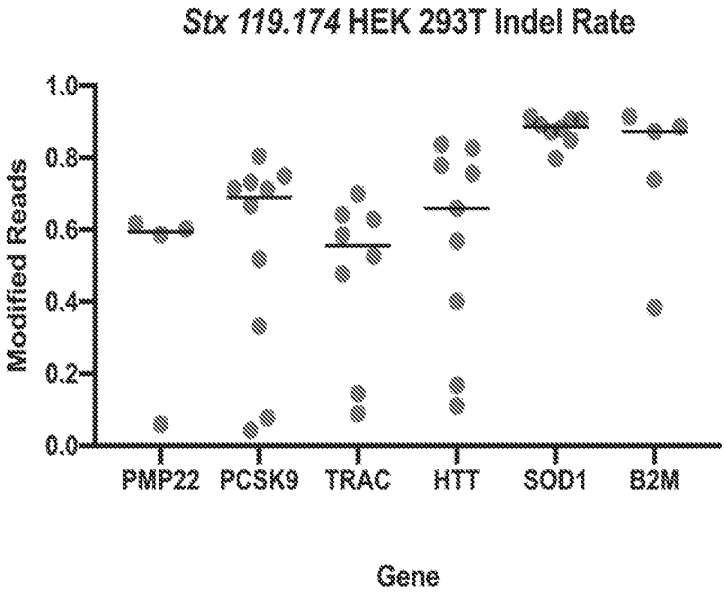
Stx 119.174 HEK 293T Indel Rate
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
PMP22
PCSK9
TRAC
HTT
SOD1
B2M
Gene

FIG. 9

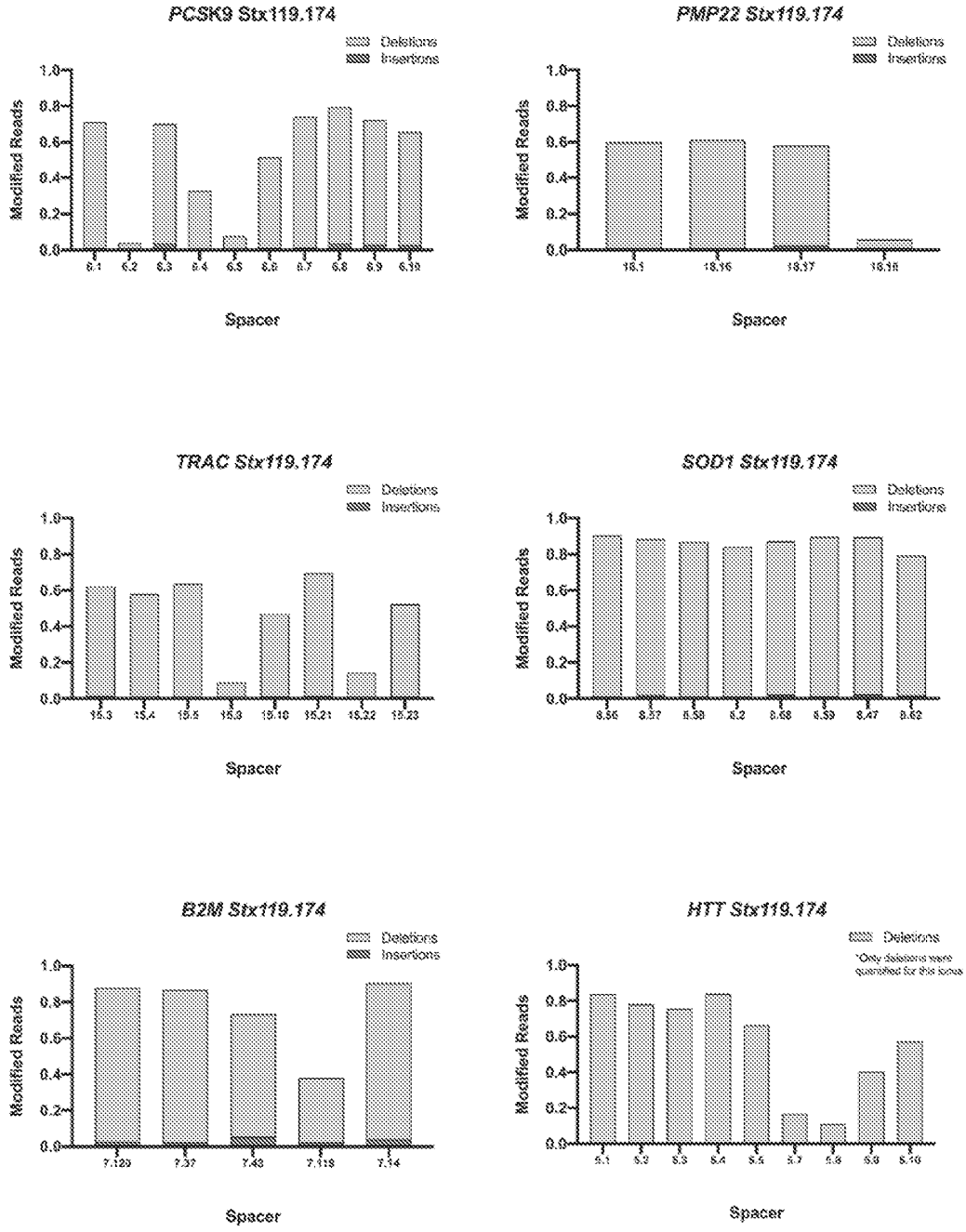
PCSK9 Stx119.174
Deletions
Insertions
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
6.1
6.2
6.3
6.4
6.5
6.6
6.7
6.8
6.9
6.10
Spacer
PMP22 Stx119.174
Deletions
Insertions
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
18.1
18.16
18.17
18.18
Spacer
TRAC Stx119.174
Deletions
Insertions
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
15.3
15.4
15.5
15.9
15.10
15.21
15.22
15.23
Spacer
SOD1 Stx119.174
Deletions
Insertions
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
8.56
8.57
8.58
8.2
8.68
8.59
8.47
8.62
Spacer
B2M Stx119.174
Deletions
Insertions
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
7.129
7.37
7.43
7.119
7.14
Spacer
HTT Stx119.174
Deletions
*Only deletions were quantified for this locus
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
5.1
5.2
5.3
5.4
5.5
5.7
5.8
5.9
5.10
Spacer

FIG. 10

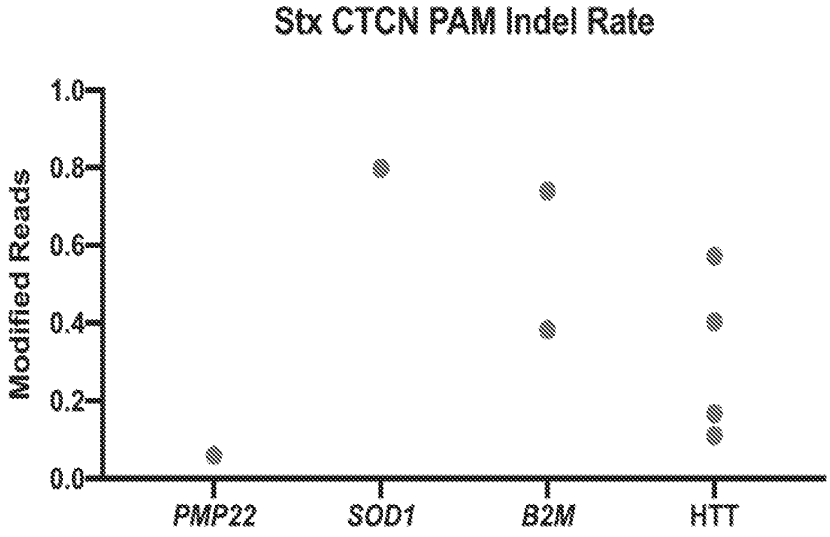
Stx CTCN PAM Indel Rate
Modified Reads
1.0
0.8
0.6
0.4
0.2
0.0
PMP22
SOD1
B2M
HTT

FIG. 11

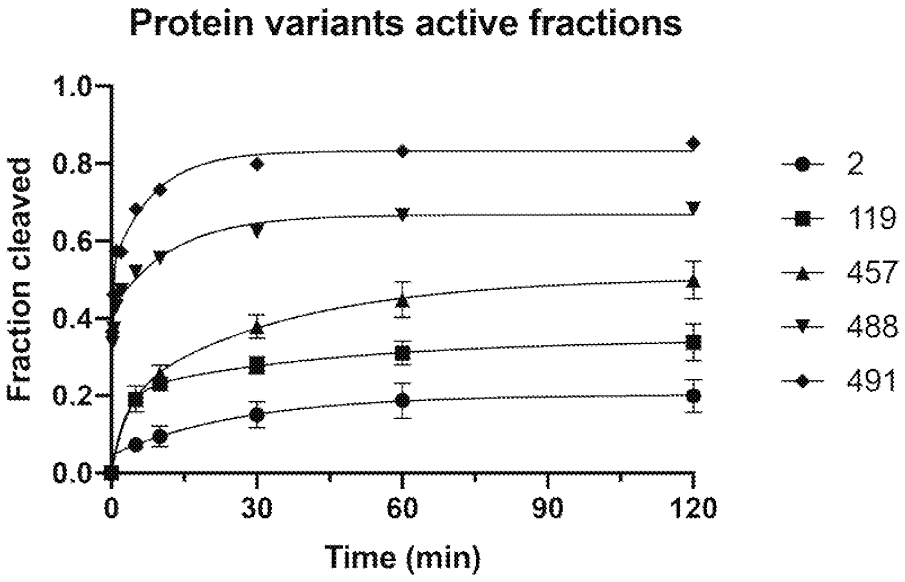
Protein variants active fractions
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
30
60
90
120
Time (min)
2
119
457
488
491

FIG. 12

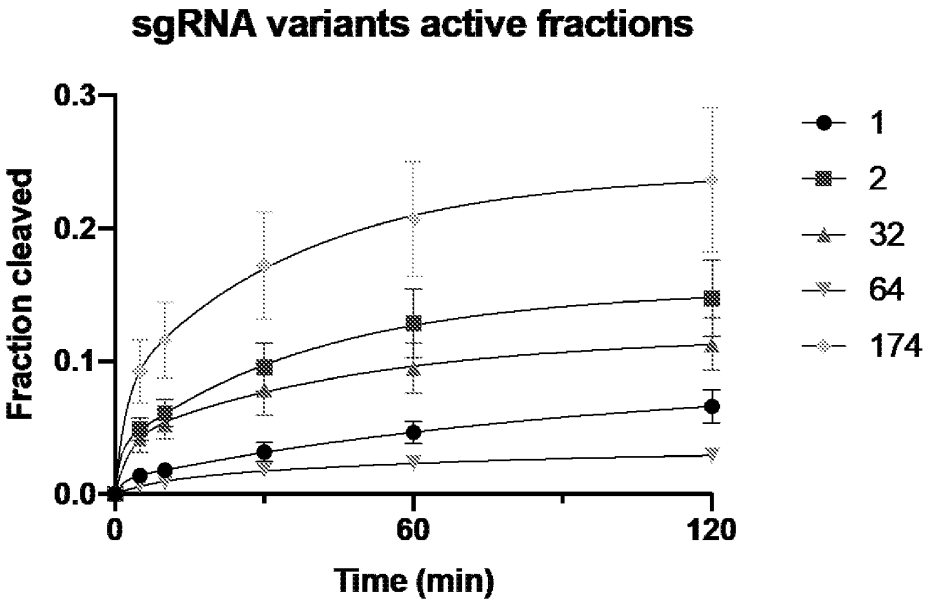
sgRNA variants active fractions
Fraction cleaved
0.3
0.2
0.1
0.0
0
60
120
Time (min)
1
2
32
64
174

FIG. 13

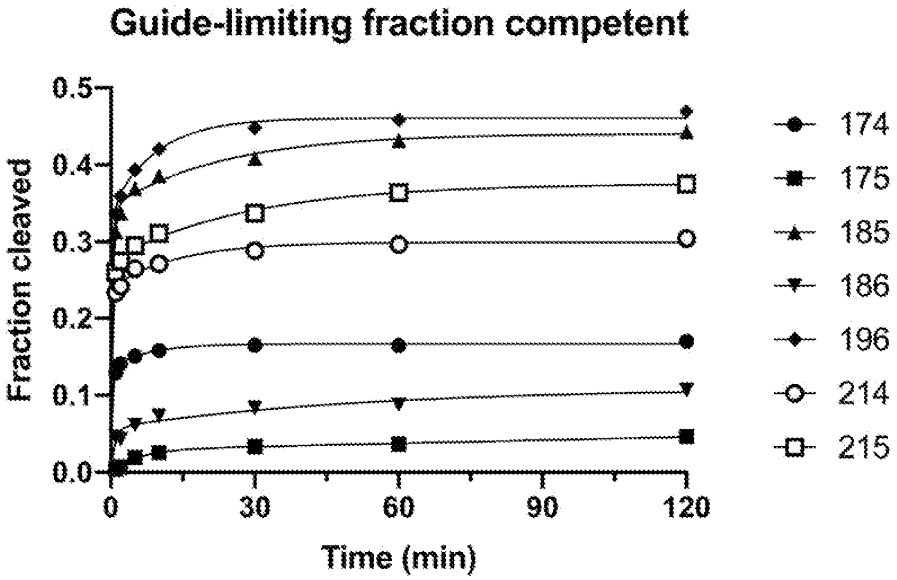
Guide-limiting fraction competent
Fraction cleaved
0.5
0.4
0.3
0.2
0.1
0.0
0
30
60
90
120
Time (min)
174
175
185
186
196
214
215

FIG. 14

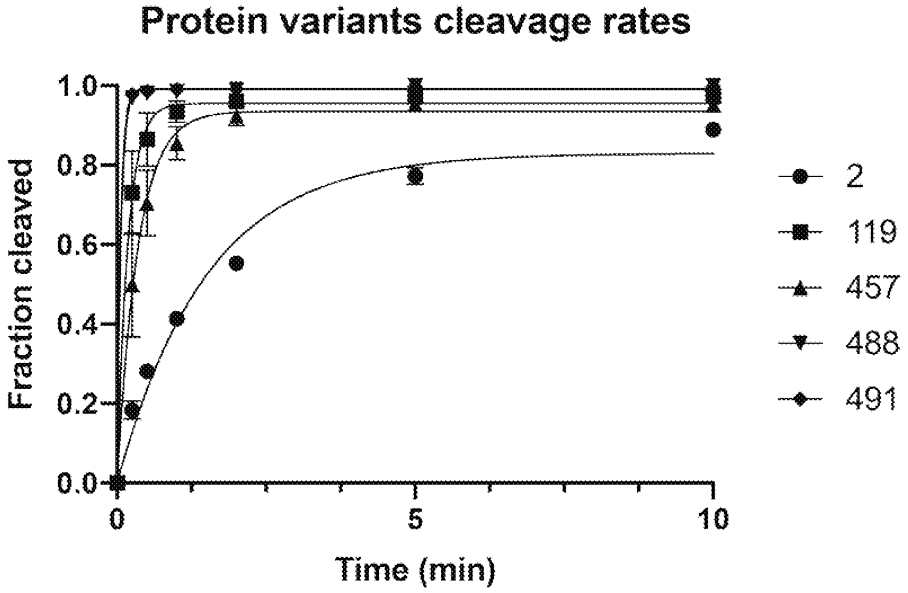
Protein variants cleavage rates
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
Time (min)
2
119
457
488
491

FIG. 15

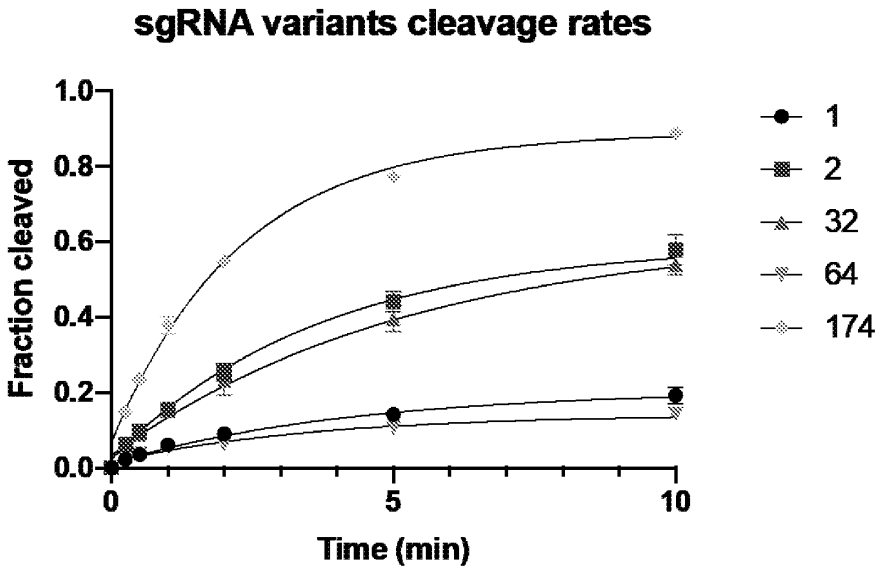
sgRNA variants cleavage rates
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
Time (min)
1
2
32
64
174

FIG. 16

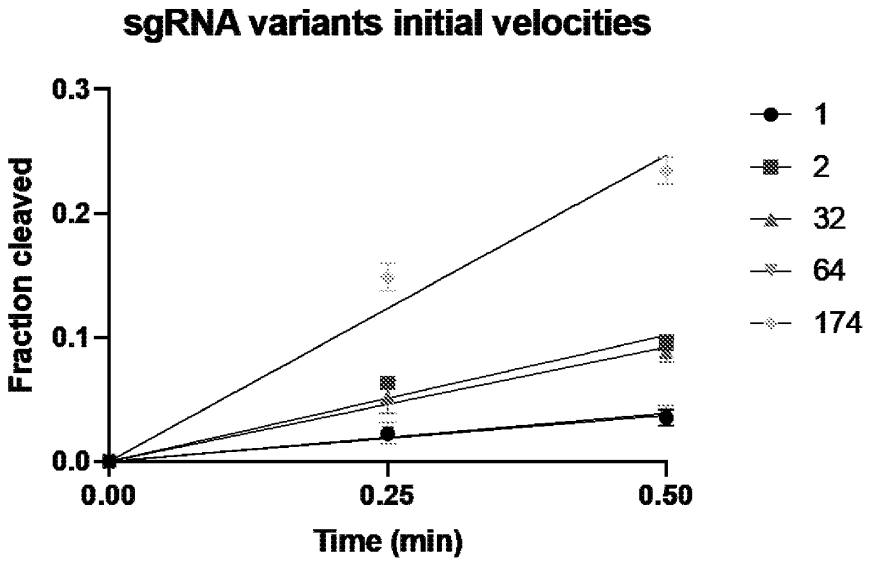
sgRNA variants initial velocities
Fraction cleaved
0.3
0.2
0.1
0.0
0.00
0.25
0.50
Time (min)
1
2
32
64
174

FIG. 17

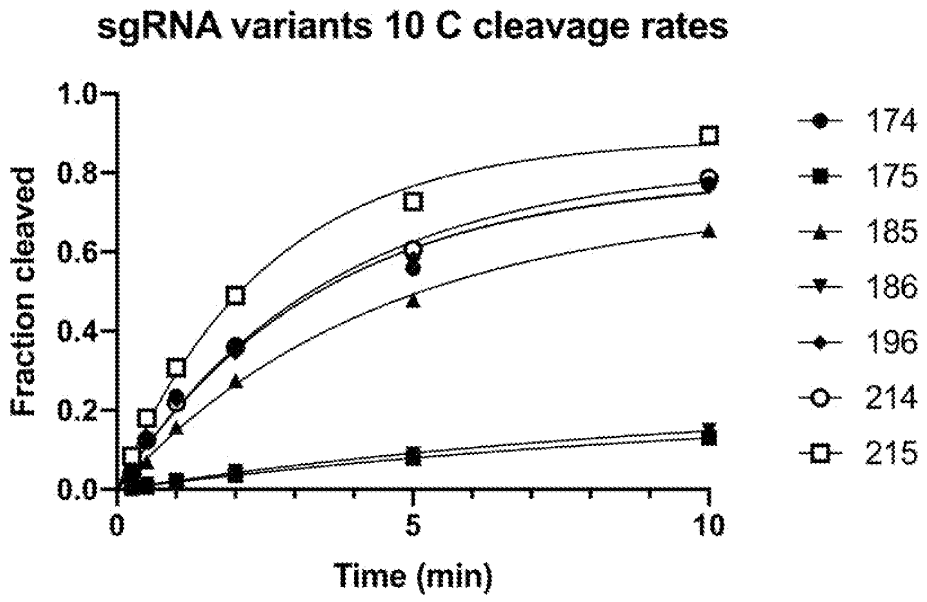
sgRNA variants 10 C cleavage rates
Fraction cleaved
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
Time (min)
174
175
185
186
196
214
215

FIG. 18

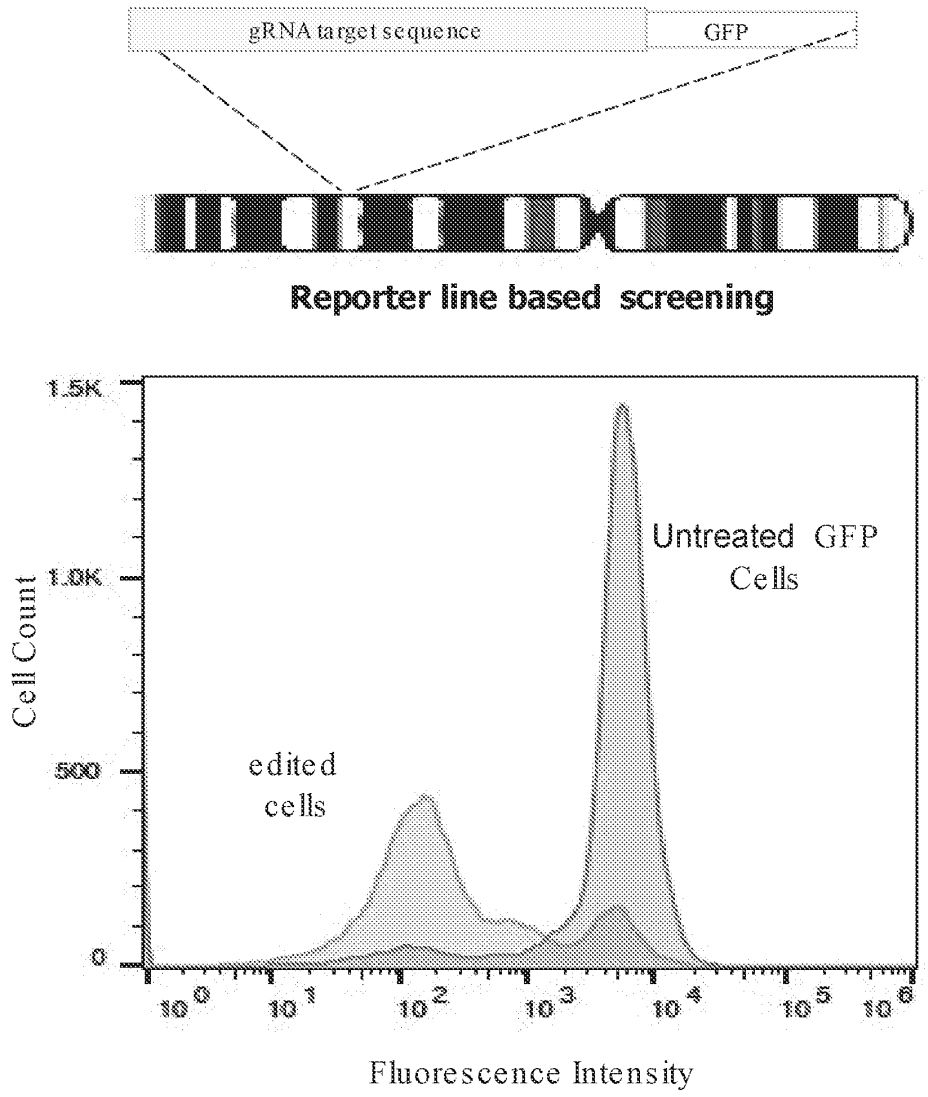
gRNA target sequence
GFP
Reporter line based screening
1.5K
1.0K
500
0
Cell Count
Untreated GFP Cells
edited cells
10 0
10 1
10 2
10 3
10 4
10 5
10 6
Fluorescence Intensity

FIG. 19

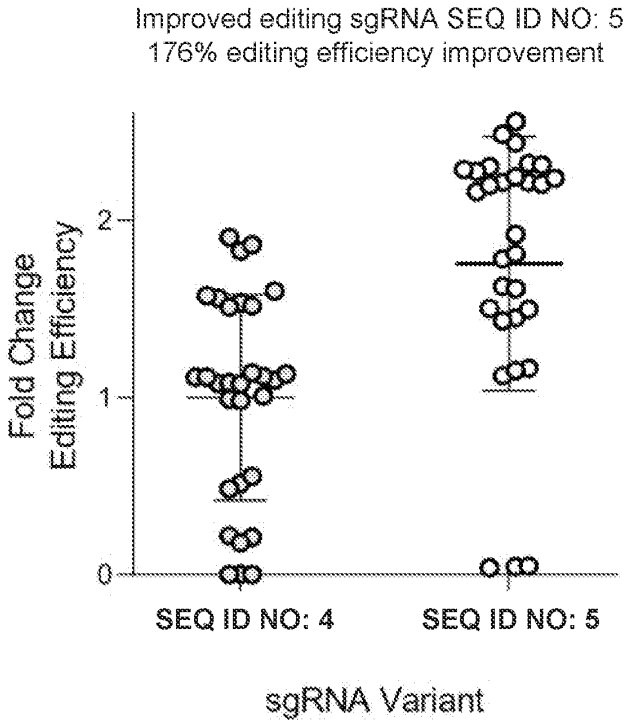
Improved editing sgRNA SEQ ID NO: 5
176% editing efficiency improvement
Fold Change
Editing Efficiency
2
1
0
SEQ ID NO: 4
SEQ ID NO: 5
sgRNA Variant

FIG. 20

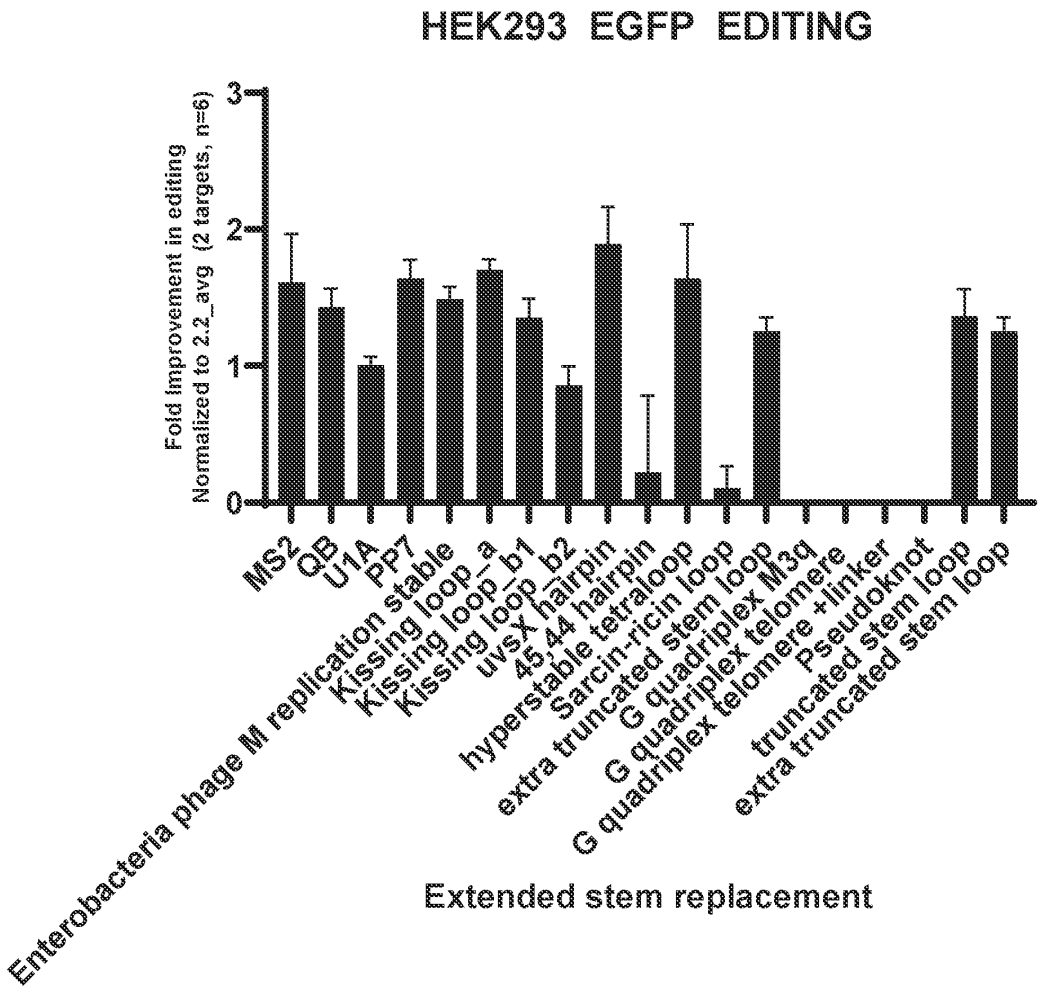
HEK293 EGFP EDITING
Fold improvement in editing
Normalized to 2.2_avg (2 targets, n=6)
0
1
2
3
MS2
QB
U1A
PP7
Kissing stable
Kissing loop_a
Kissing loop_b1
Kissing loop_b2
uvsX hairpin
45,44 hairpin
hyperstable tetraloop
Sarcin-ricin loop
extra truncated stem loop
G quadriplex M3q
G quadriplex telomere
G quadriplex telomere +linker
Pseudoknot
truncated stem loop
extra truncated stem loop
Enterobacteria phage M replication
Extended stem replacement

FIG. 21

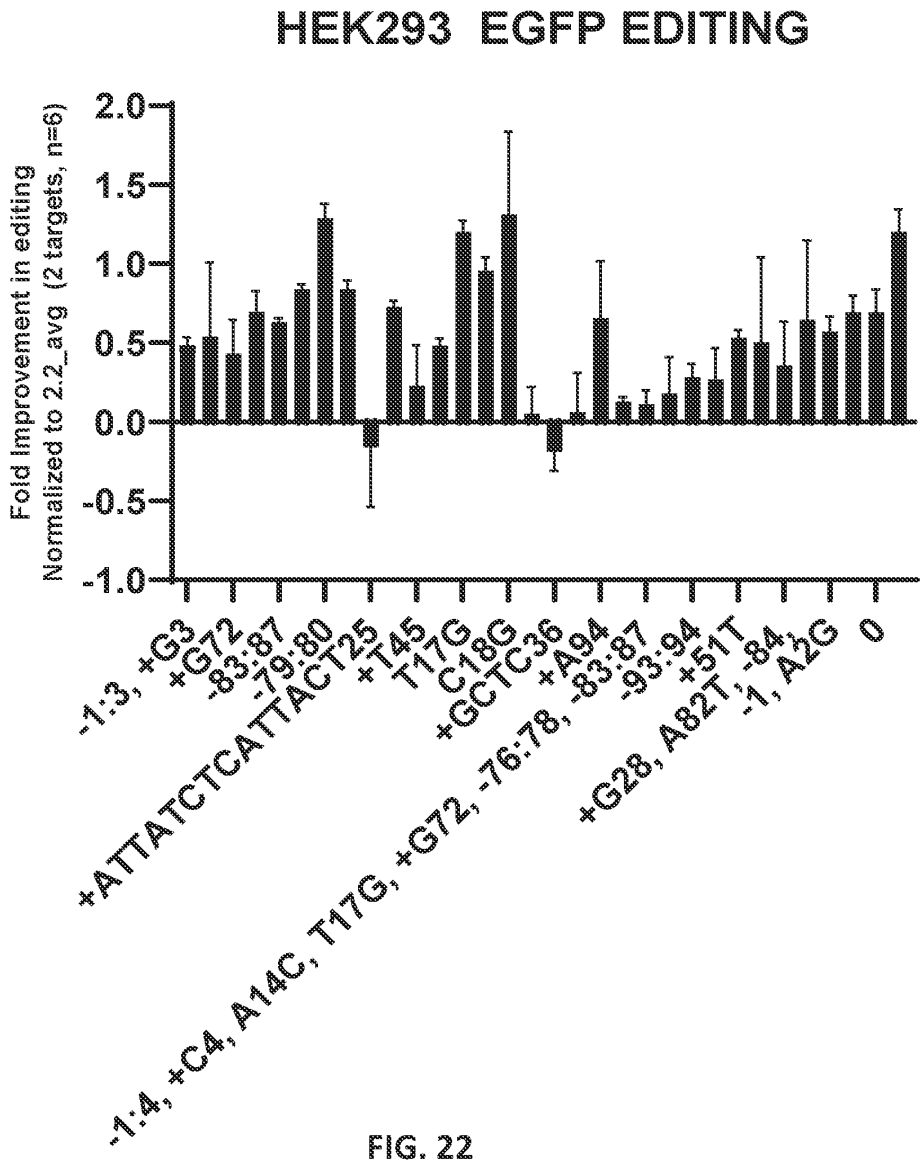
HEK293 EGFP EDITING
Fold Improvement in editing
Normalized to 2.2_avg (2 targets, n=6)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1:3, +G3
+G72
-83:87
-79:80
+ATTATCTCATTACT25
+T45
T17G
C18G
+GCTC36
+A94
-93:94
+51T
-1, A2G
0
-1:4, +C4, A14C, T17G, +G72, -76:78, -83:87
+G28, A82T, -84,

FIG. 22

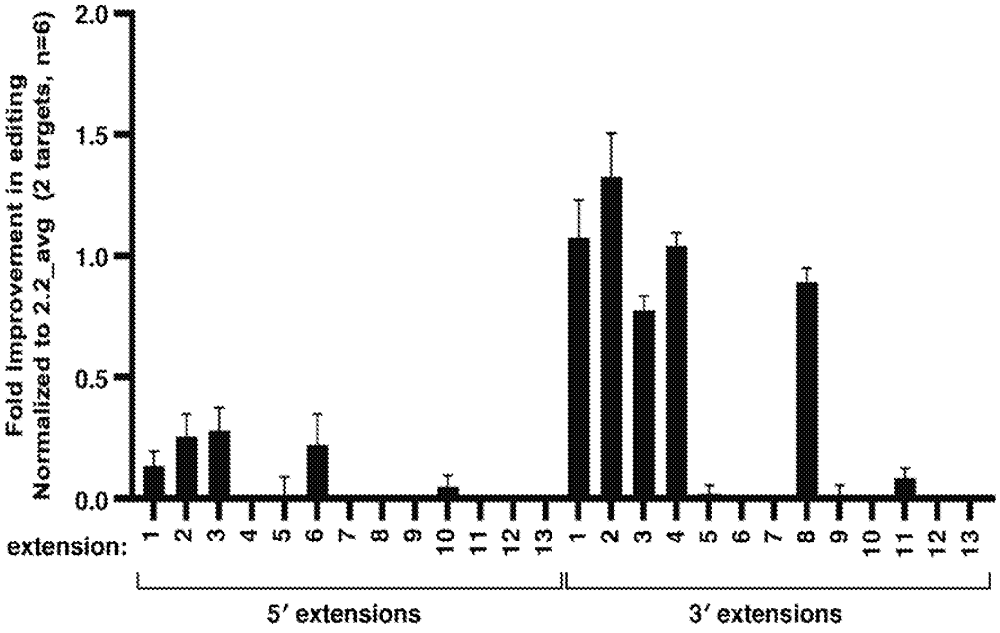
Fold Improvement in editing
Normalized to 2.2_avg (2 targets, n=6)
2.0
1.5
1.0
0.5
0.0
extension:
1 2 3 4 5 6 7 8 9 10 11 12 13
1 2 3 4 5 6 7 8 9 10 11 12 13
5′ extensions
3′ extensions

FIG. 23

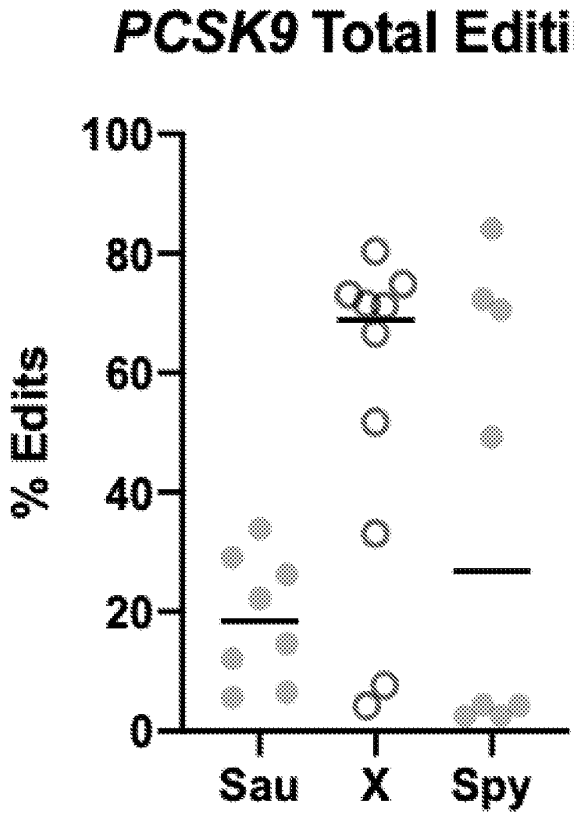

FIG. 24

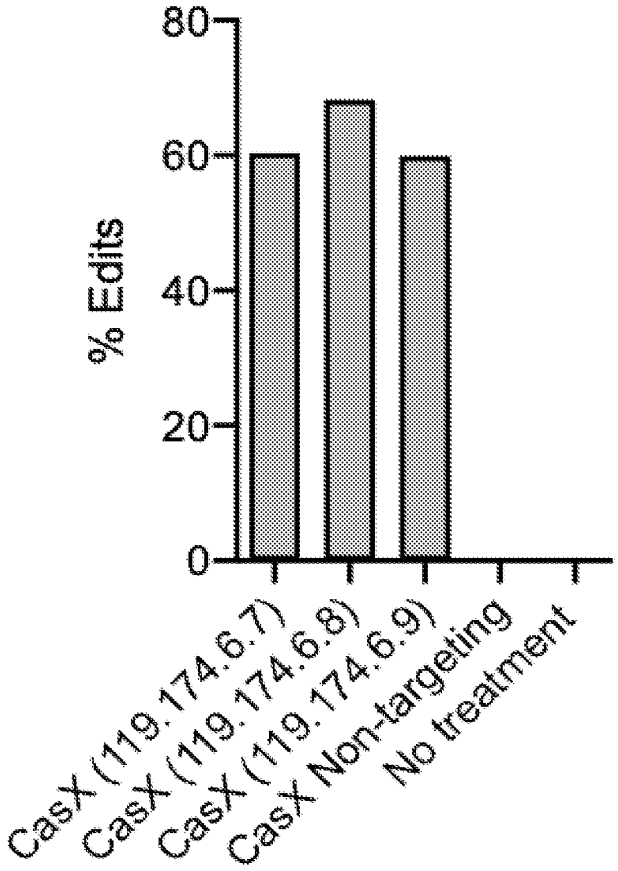
80
60
40
20
0
% Edits
CasX (119.174.6.7)
CasX (119.174.6.8)
CasX (119.174.6.9)
CasX Non-targeting
No treatment

FIG. 25

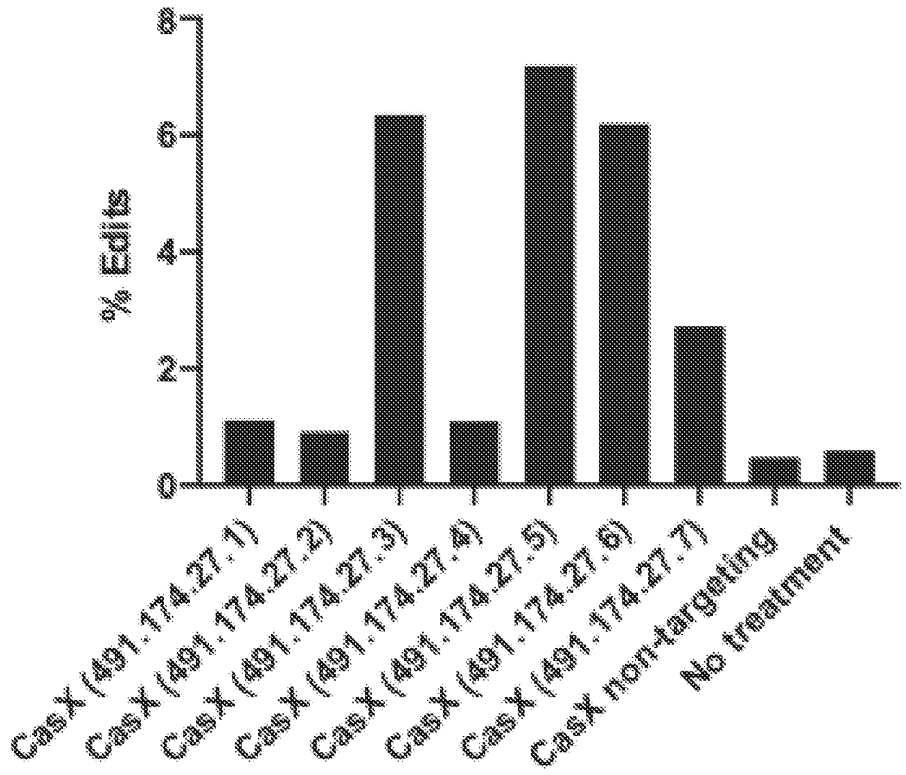
% Edits
0
2
4
6
8
CasX (491.174.27.1)
CasX (491.174.27.2)
CasX (491.174.27.3)
CasX (491.174.27.4)
CasX (491.174.27.5)
CasX (491.174.27.6)
CasX (491.174.27.7)
CasX non-targeting
No treatment

FIG. 26