

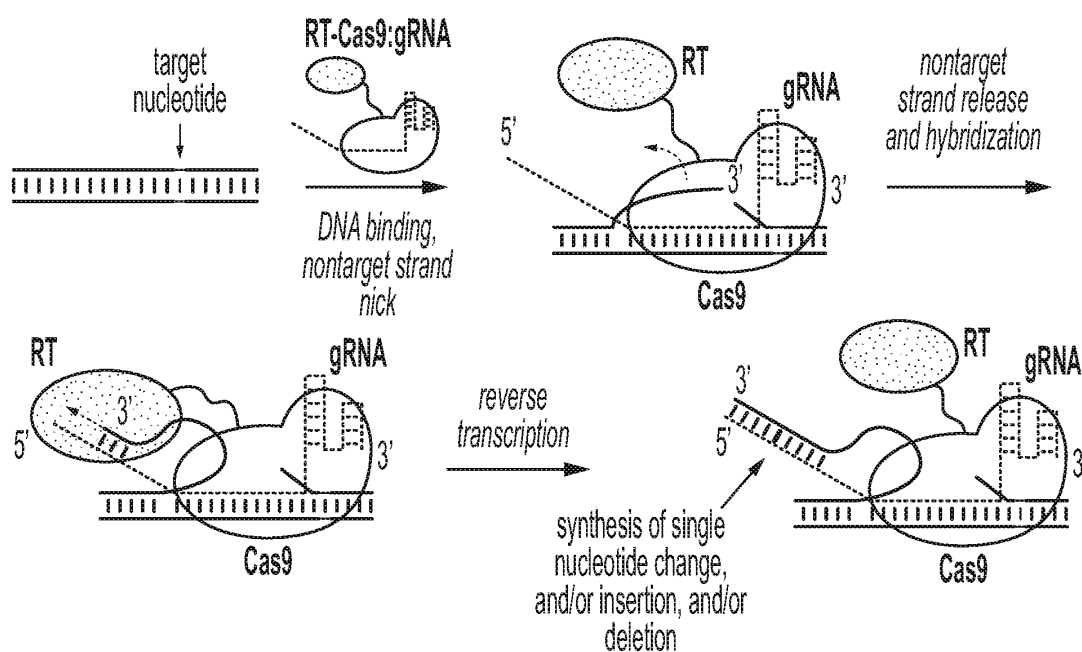


FIG. 1A

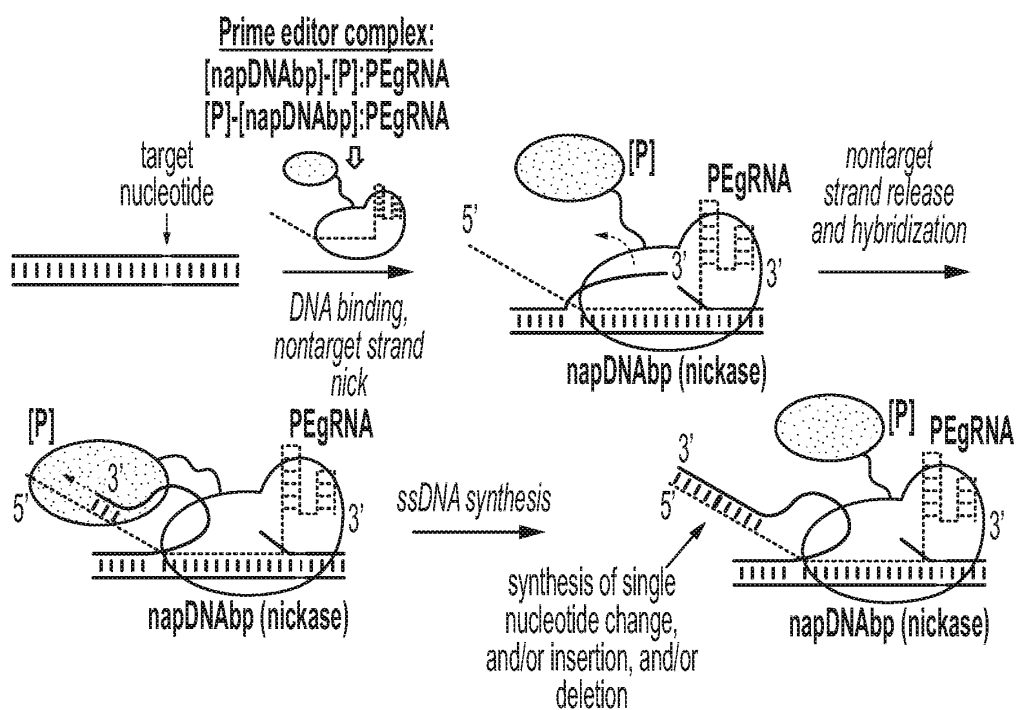


FIG. 1B

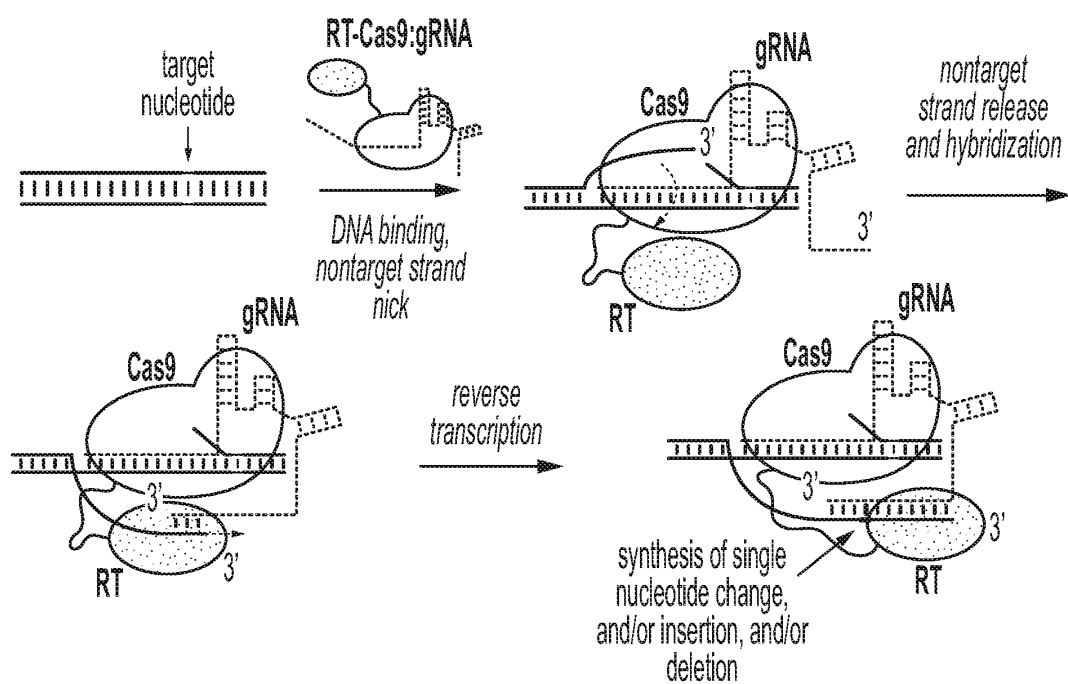


FIG. 1C

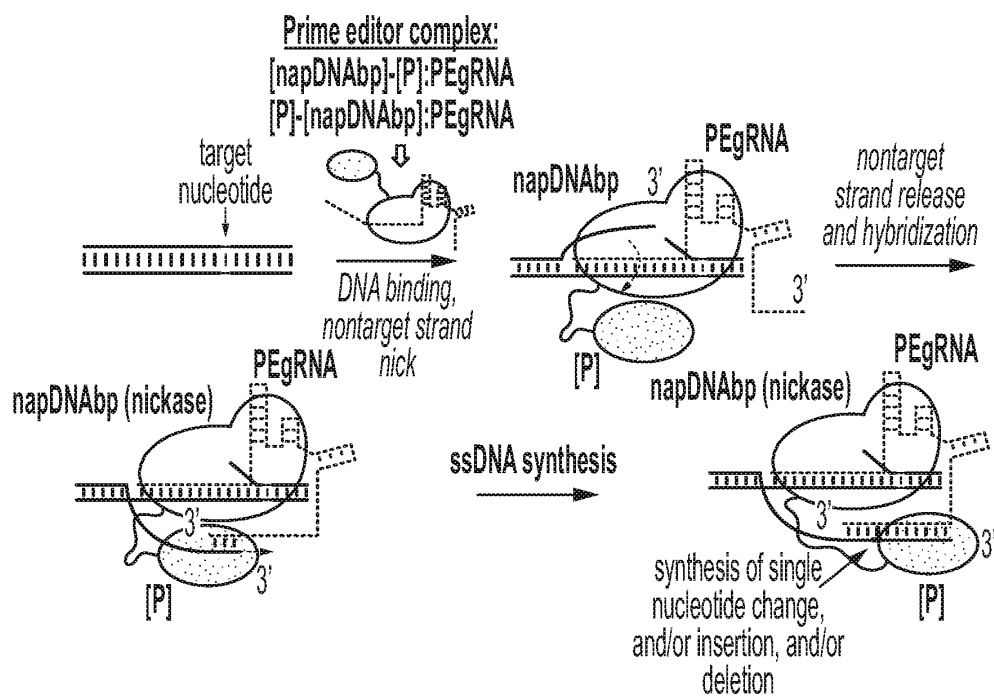


FIG. 1D

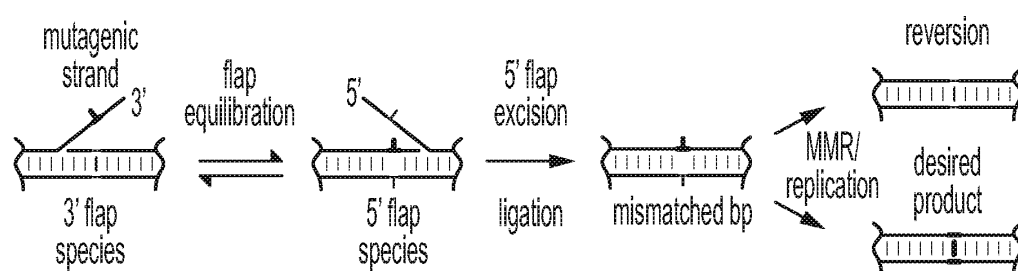


FIG. 1E

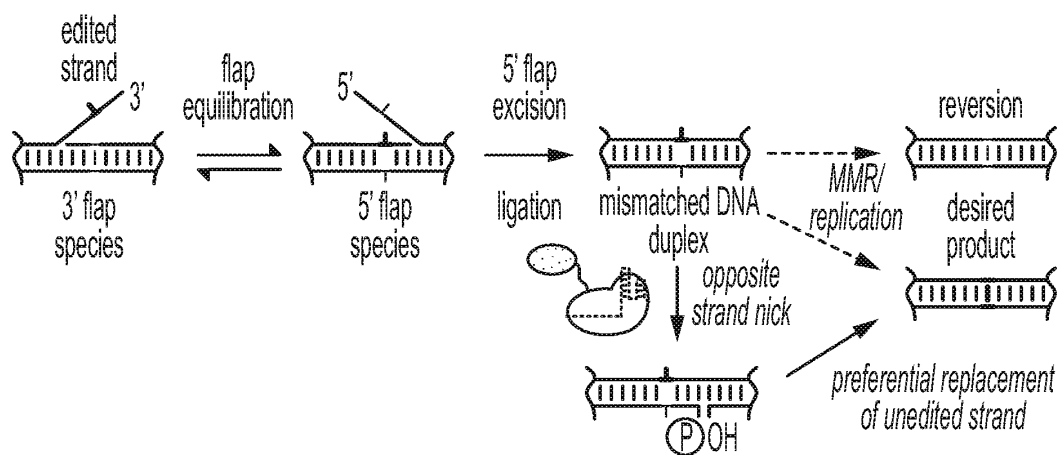


FIG. 1F

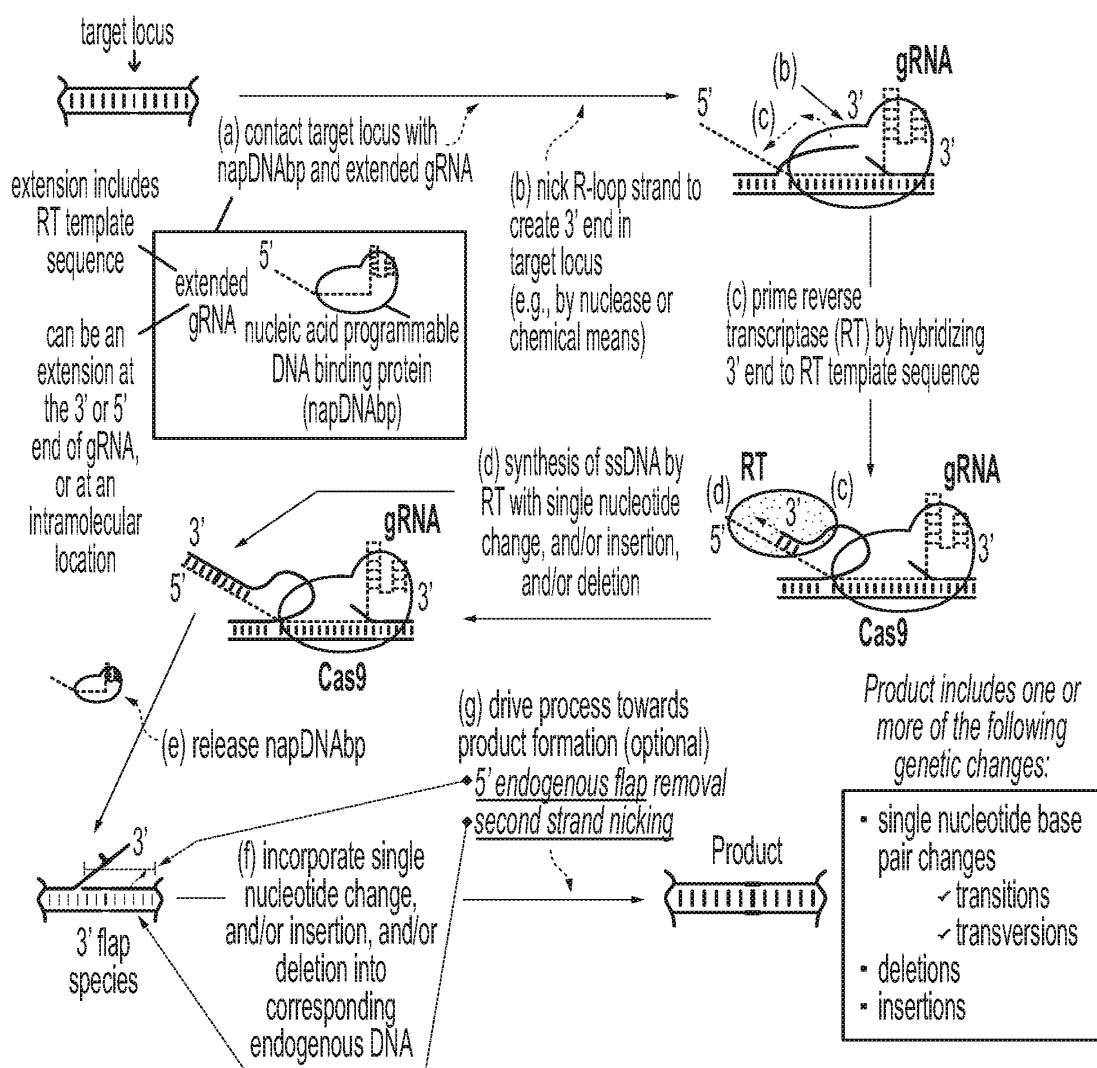


FIG. 1G

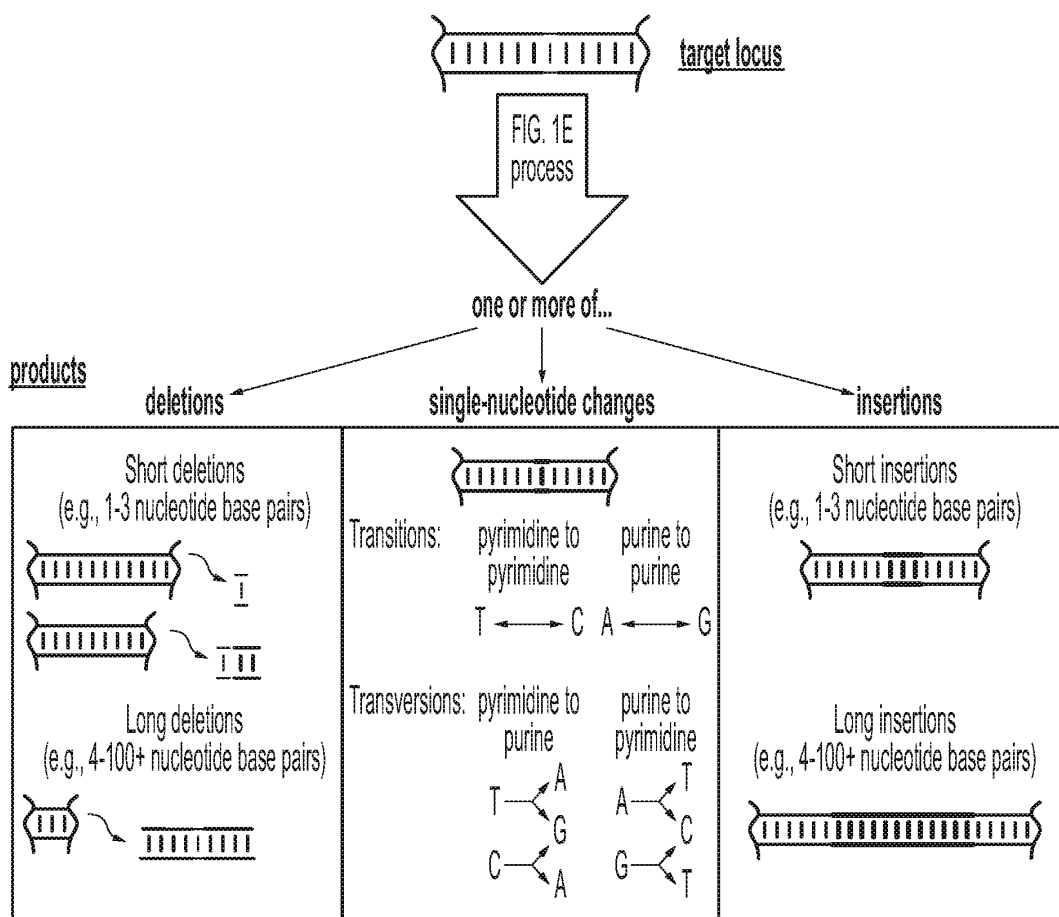


FIG. 1H

*PE3b temporal nicking.*

**PE3b editing system: edit-specific nicked of the complementary (unedited) DNA strand**

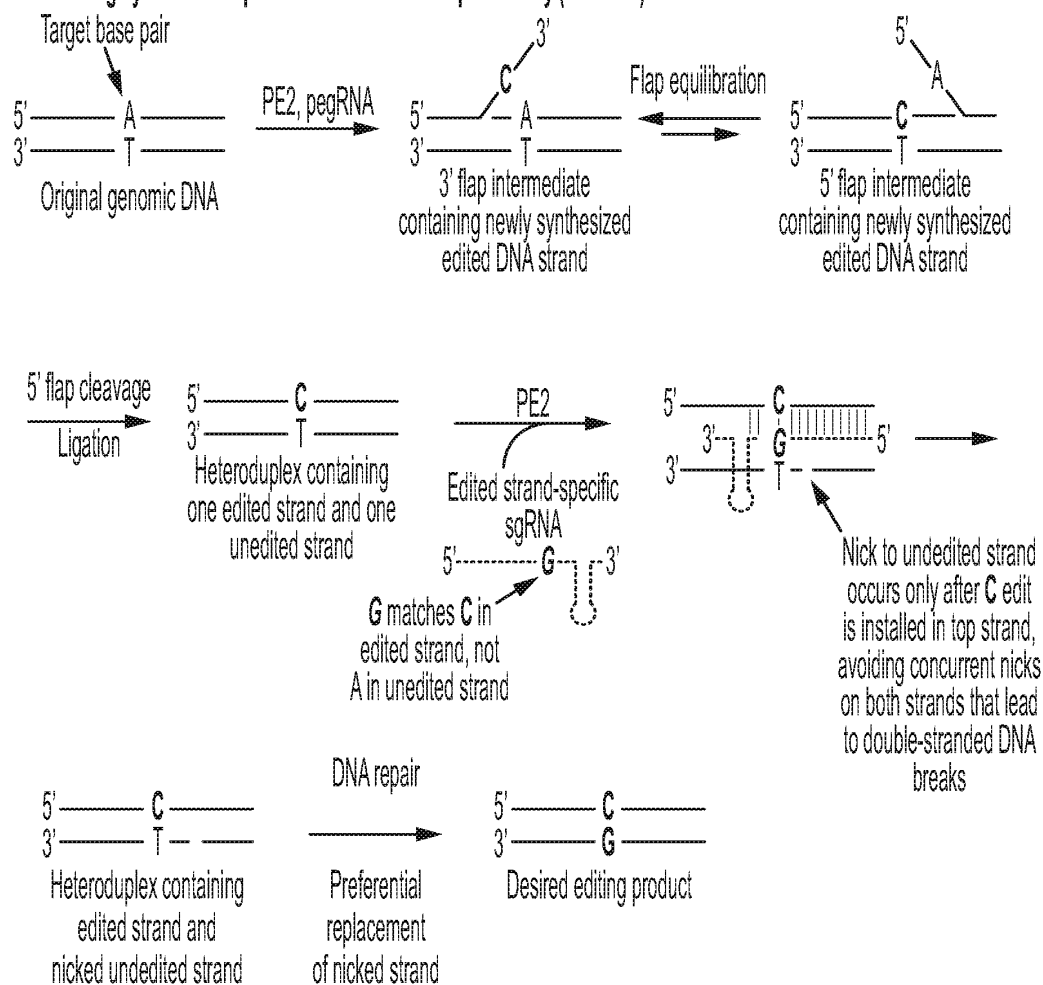


FIG. 1I

Prime editing with any programmable nuclease

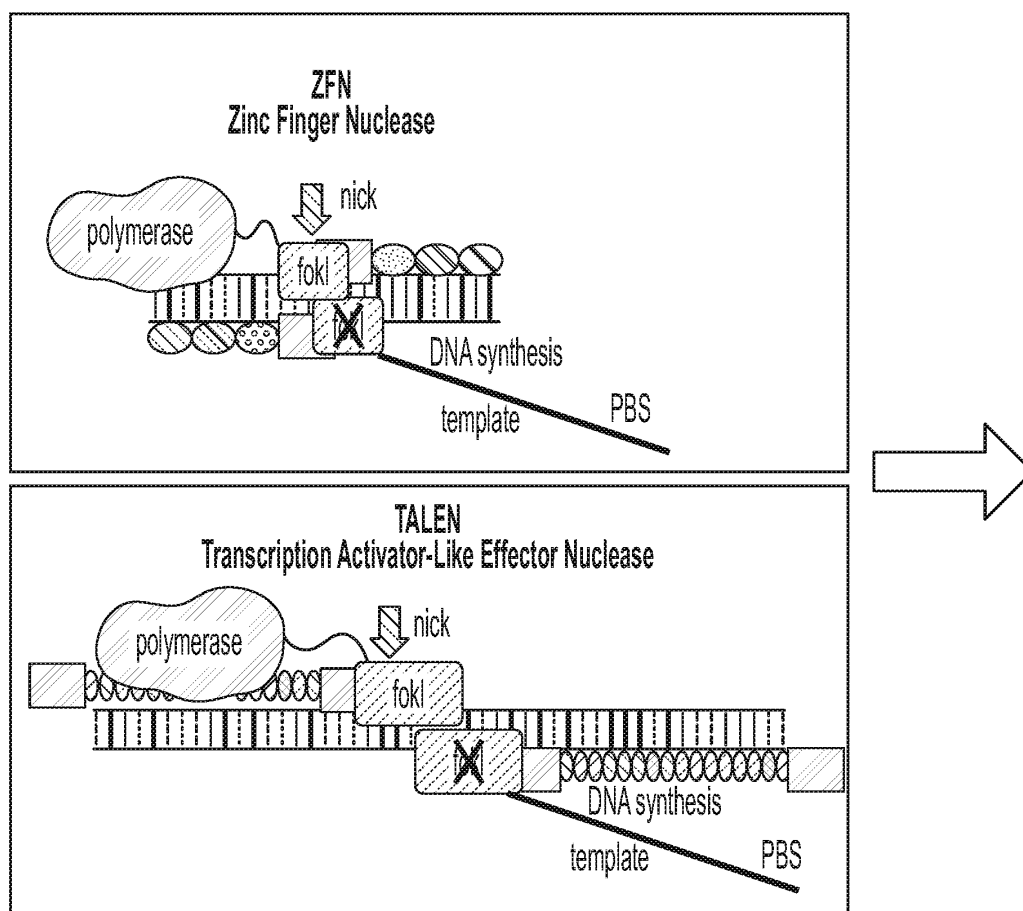


FIG. 1J

Polymerase-dependent synthesis of a replacement strand encoded by the polymerase against the DNA synthesis template and which contains a desired edit. The mechanism is envisioned to operate similarly to prime editing. Additional factors or effectors may be added *in trans* or as fusions to facilitate the reaction as a whole (e.g., (a) a helicase to unwind the DNA at the cut site to make the cut strand with the 3' end available as a primer, (b) a FEN1 to help remove the endogenous strand on the cut strand to drive the reaction towards replacement of the endogenous strand with the synthesized strand, or (c) a nCas9:gRNA complex to create a second site nick on the opposite strand, which may help drive the integration of the synthesized repair through favored cellular repair of the non-edited strand)

FIG. 1K

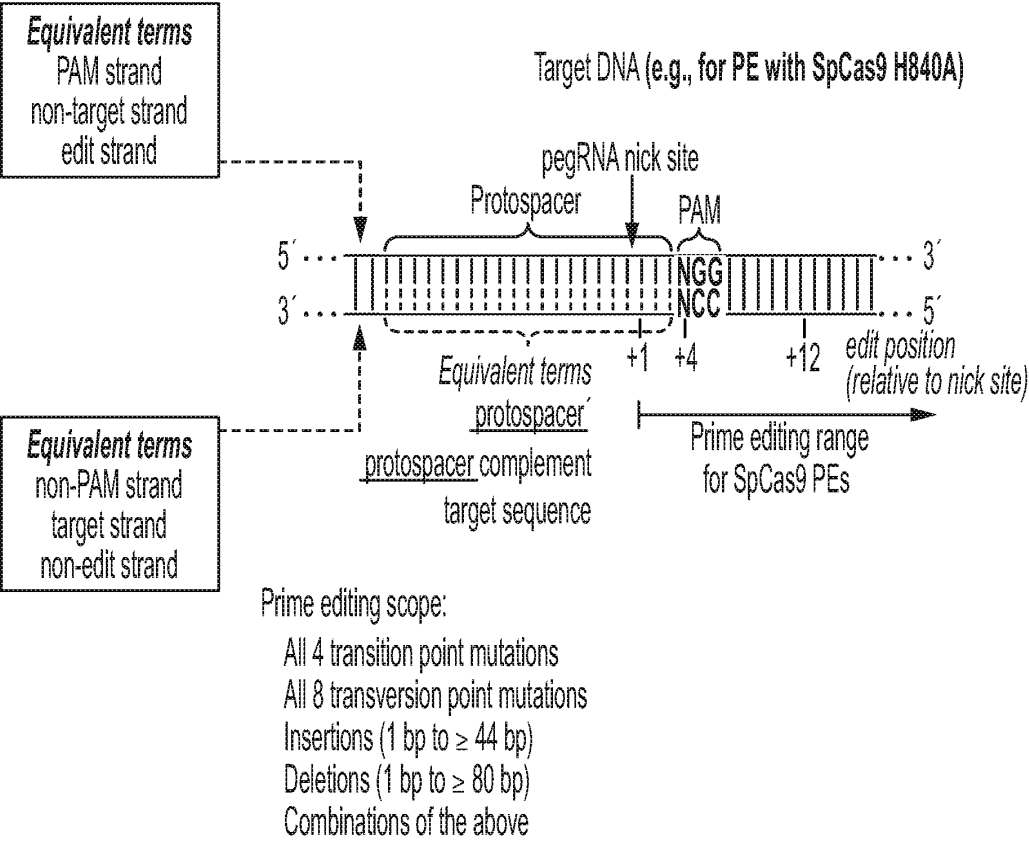


FIG. 1L

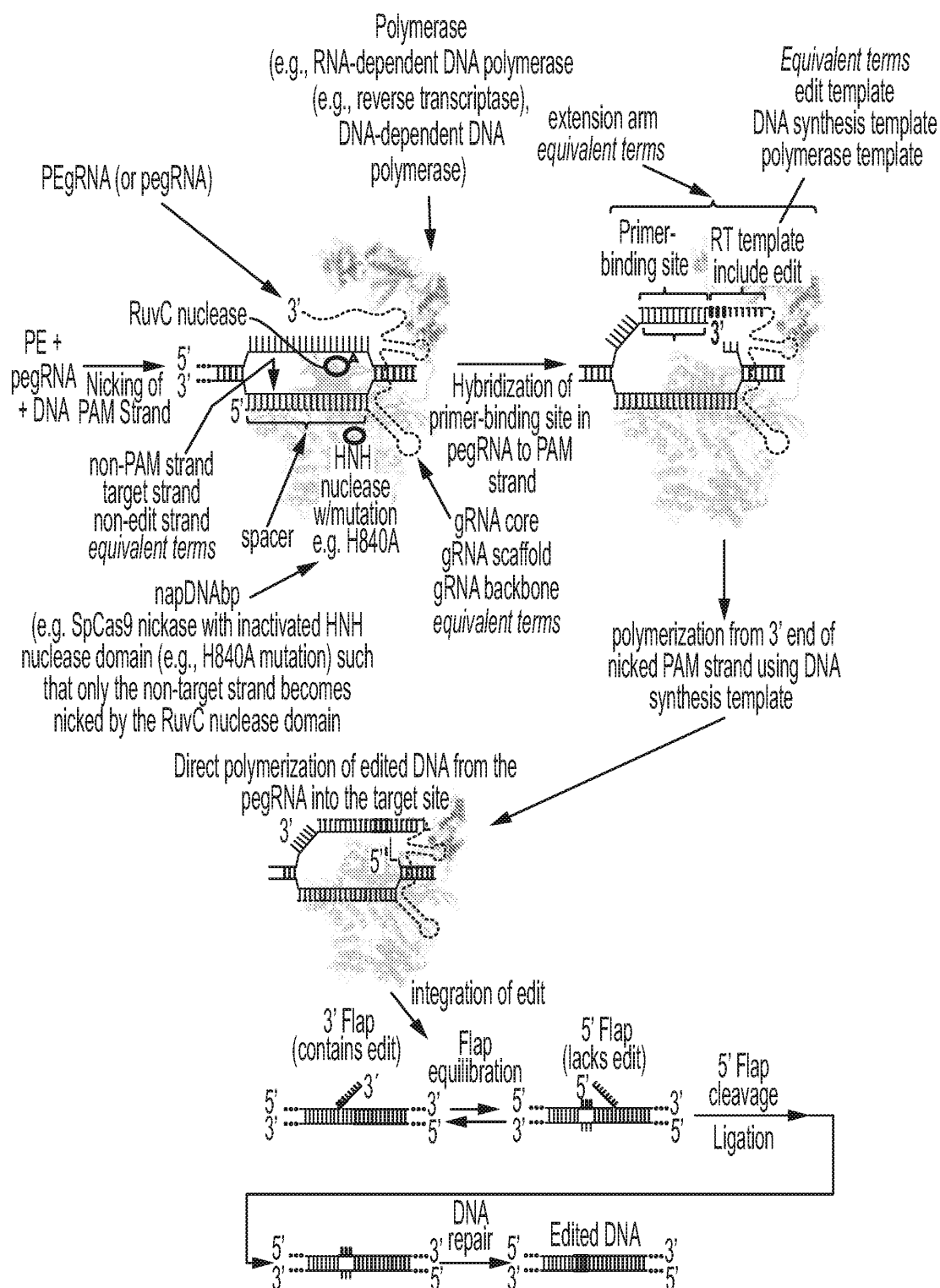


FIG. 1M

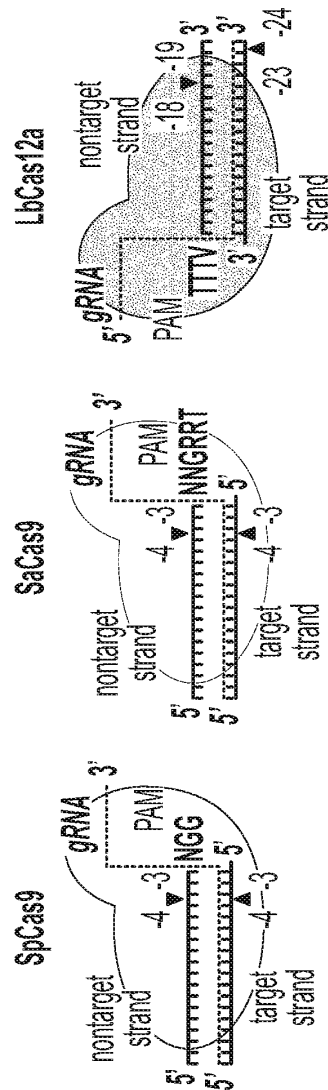


FIG. 2

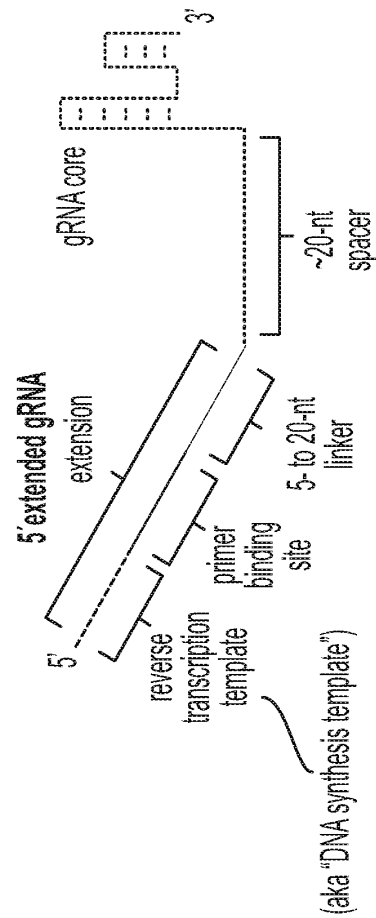


FIG. 3A

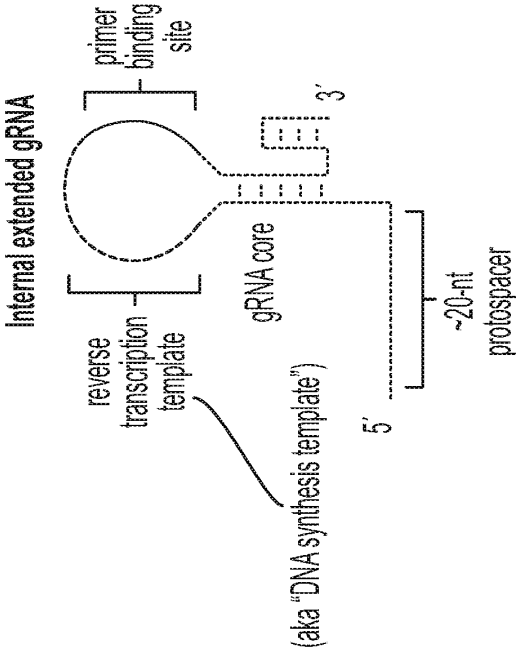


FIG. 3C

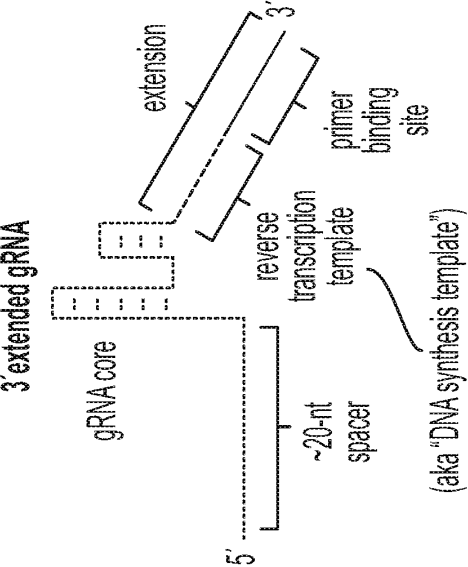
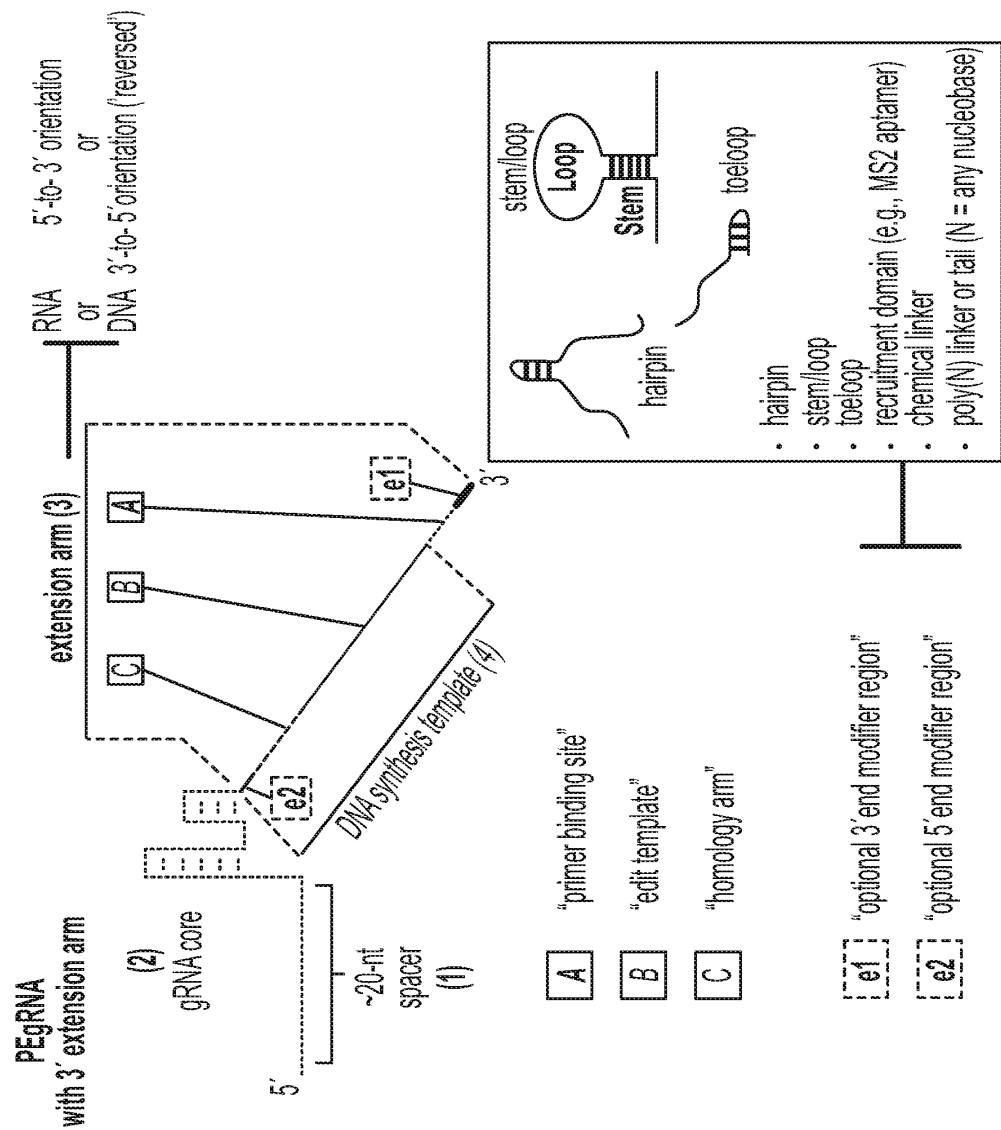


FIG. 3B



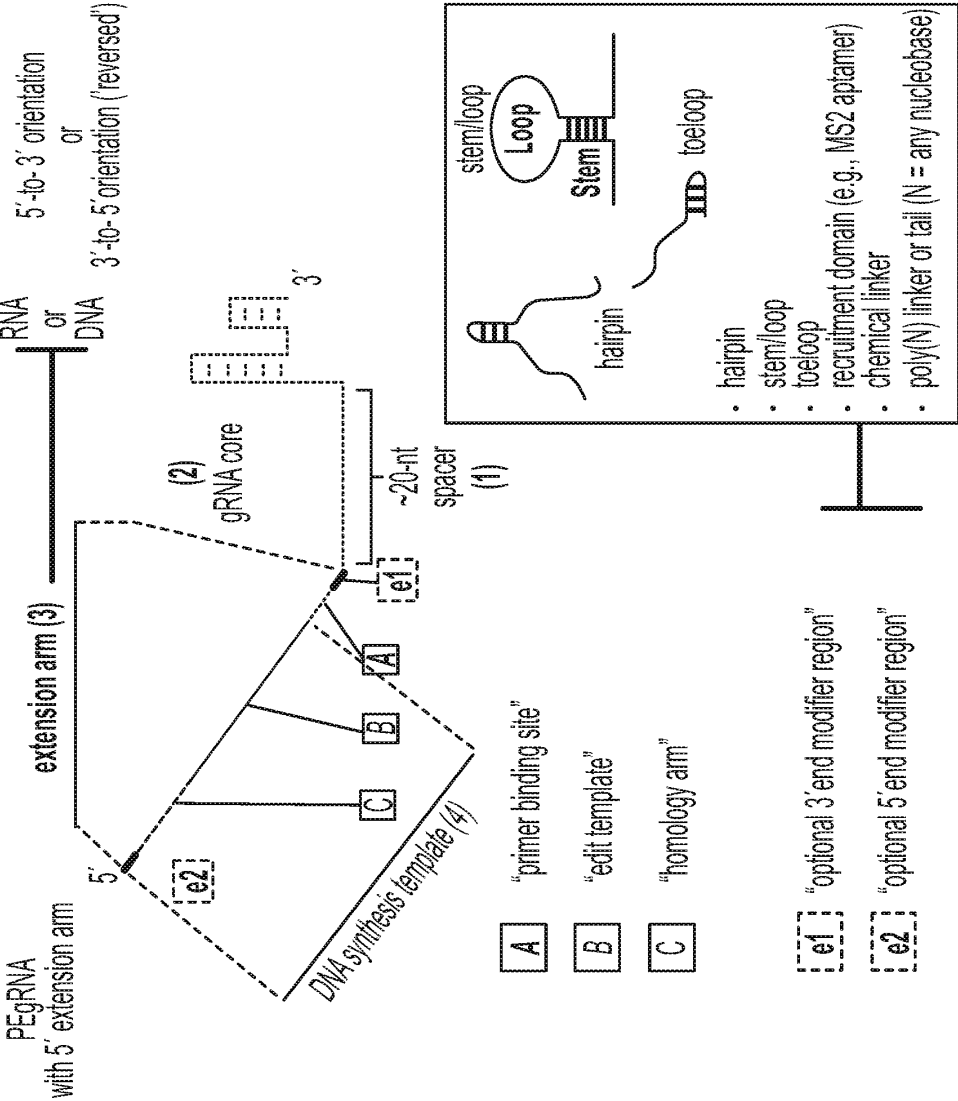


FIG. 3E

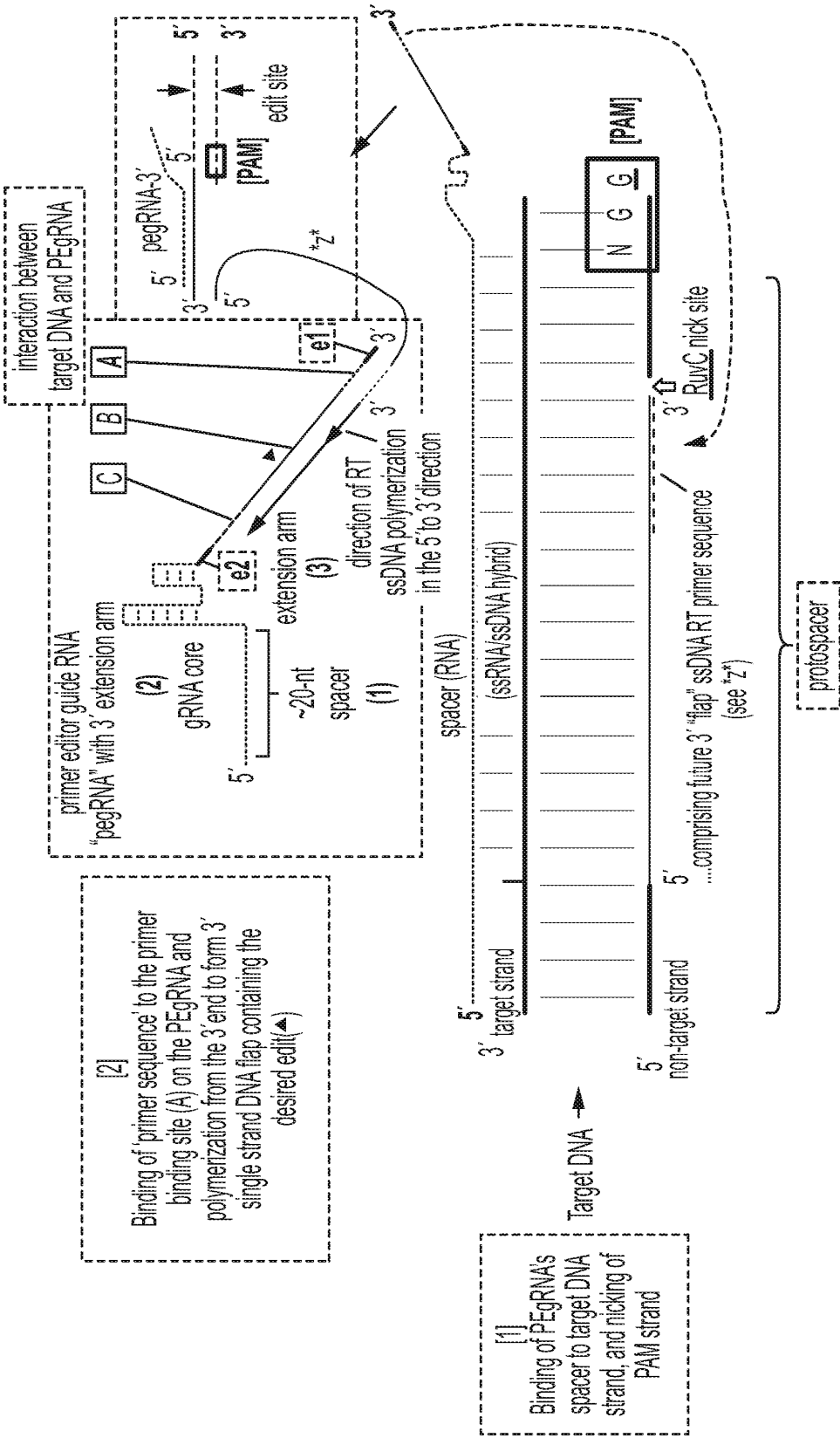
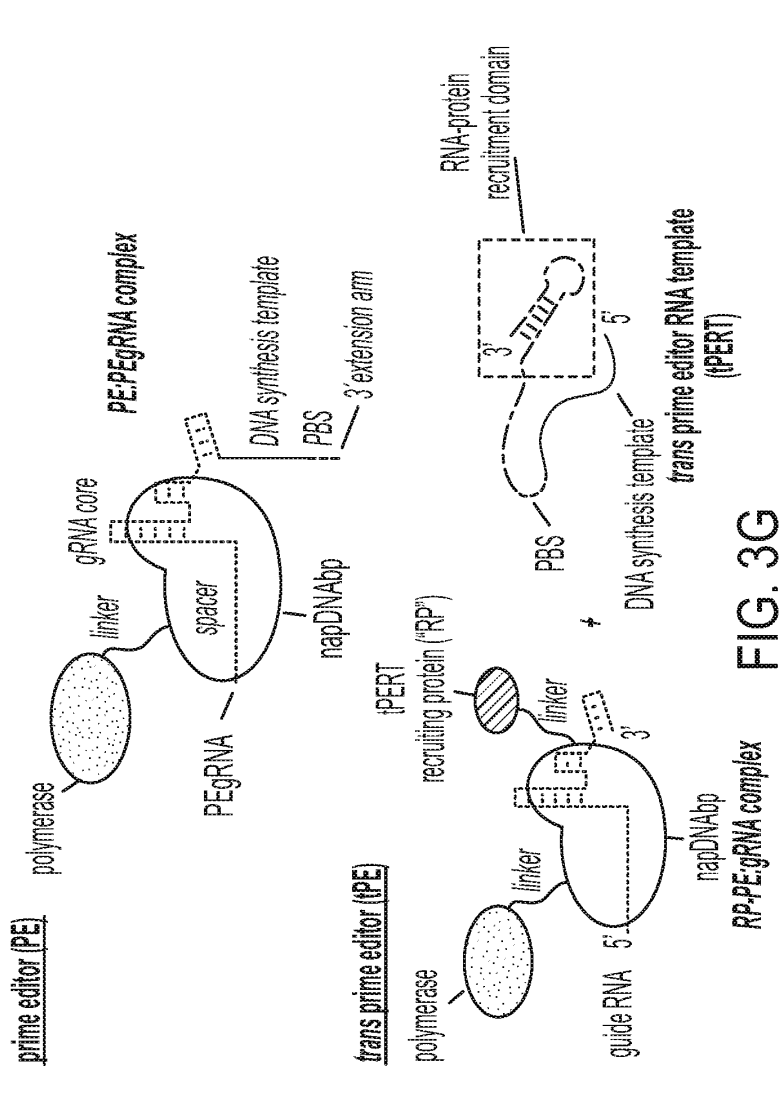
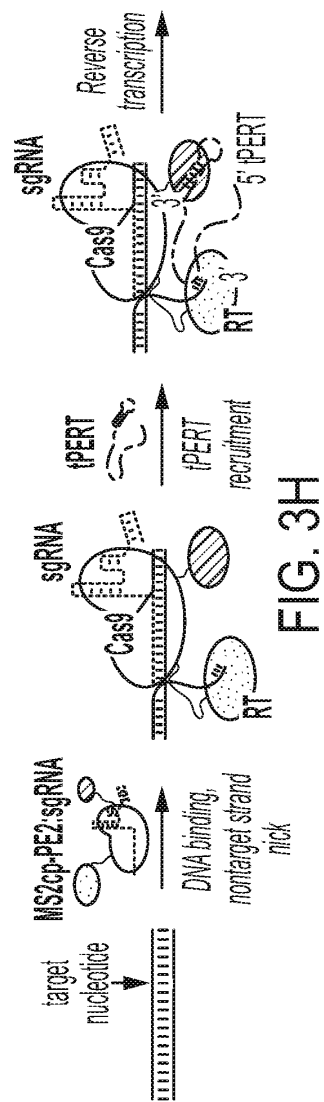


FIG. 3F



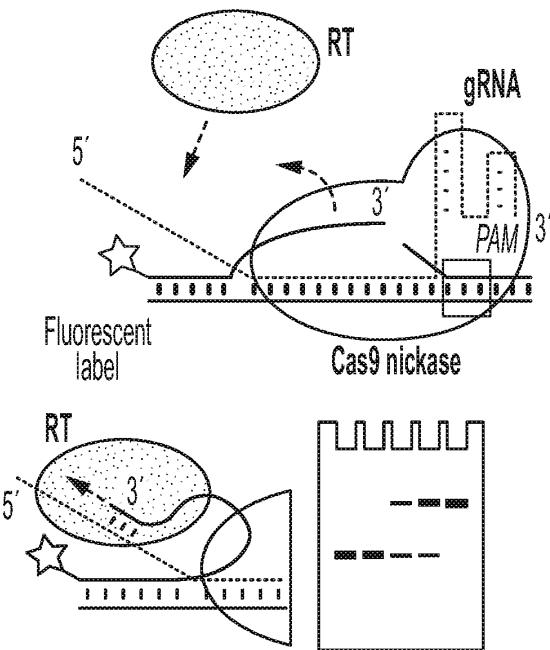


FIG. 4A

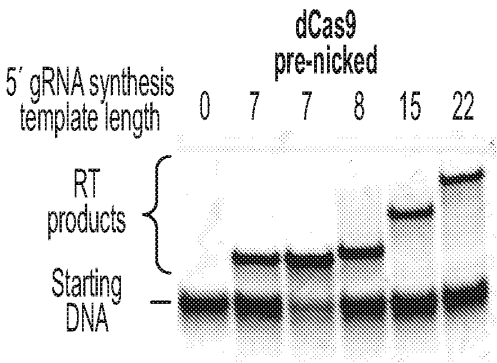


FIG. 4B

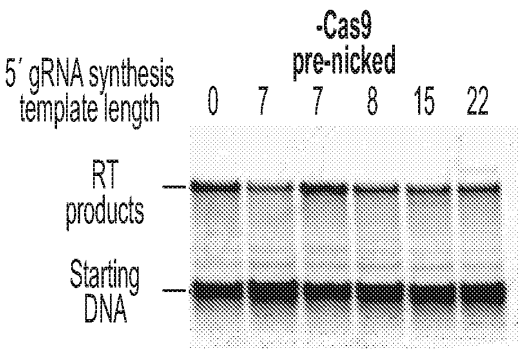


FIG. 4C

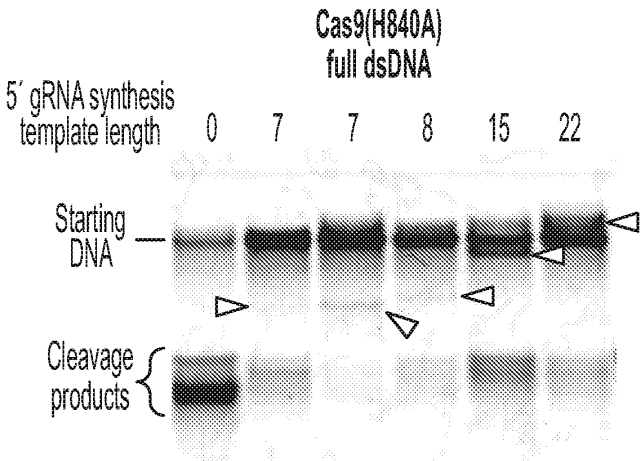


FIG. 4D

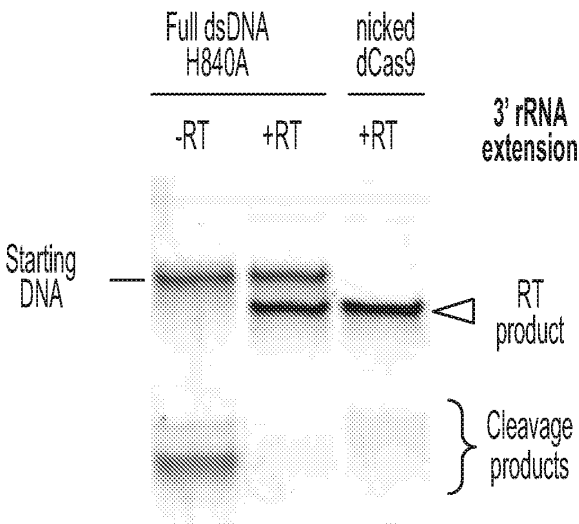
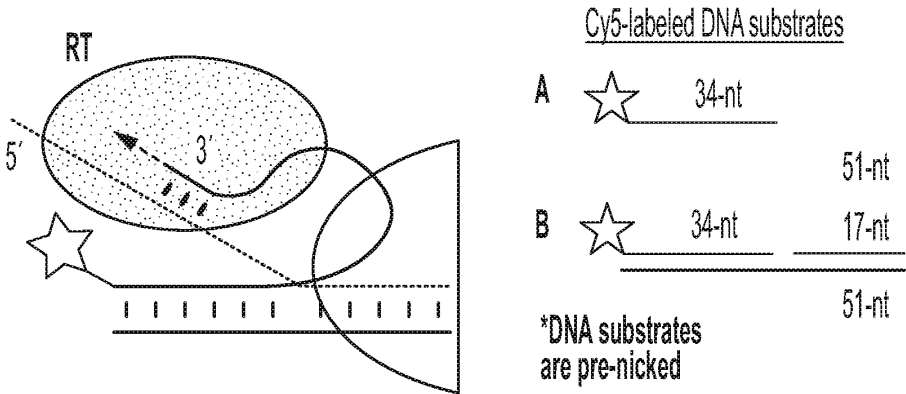


FIG. 4E



gRNA\_0: no extension  
gRNA\_1: 7 bp template  
gRNA\_2: 7 bp template  
gRNA\_3: 8 bp template  
gRNA\_4: 15 bp template  
gRNA\_5: 22 bp template

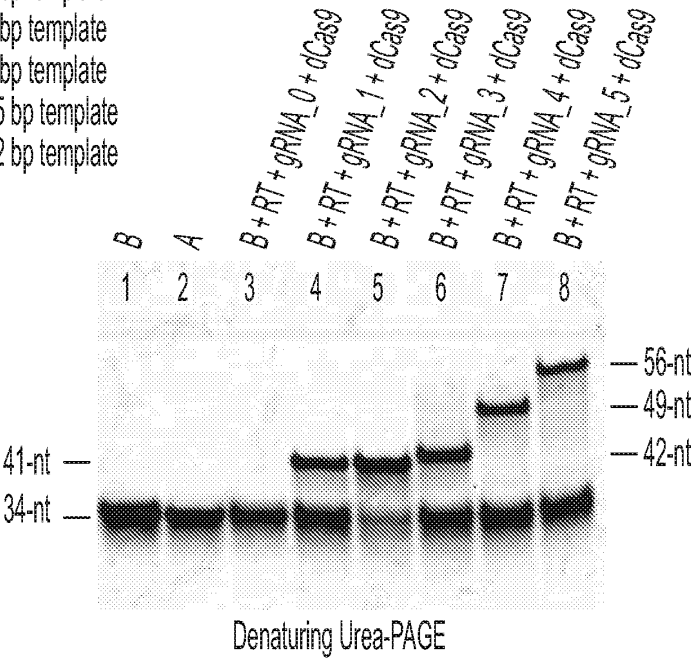
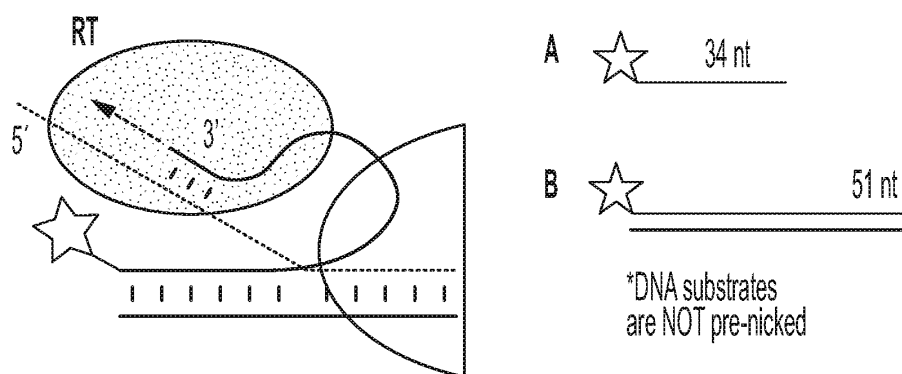


FIG. 5



gRNA\_0: no extension  
 gRNA\_1: 7 bp template  
 gRNA\_2: 7 bp template  
 gRNA\_3: 8 bp template  
 gRNA\_4: 15 bp template  
 gRNA\_5: 22 bp template

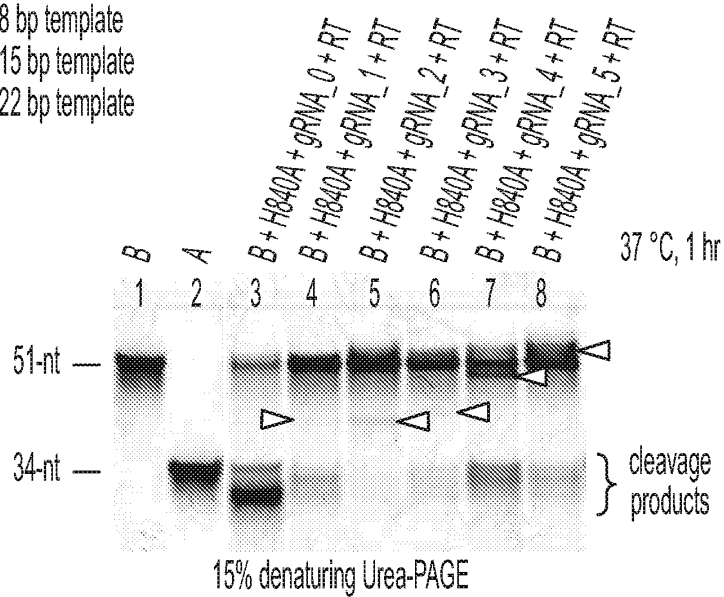
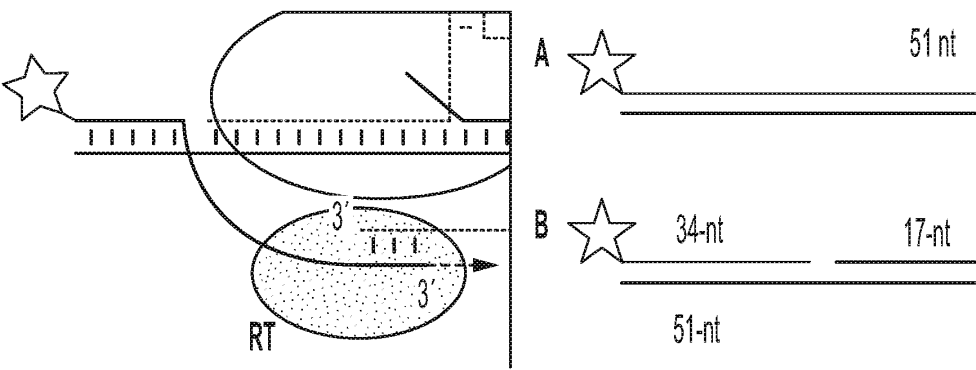


FIG. 6



gRNA\_0: no extension  
gRNA\_1: 15 bp template

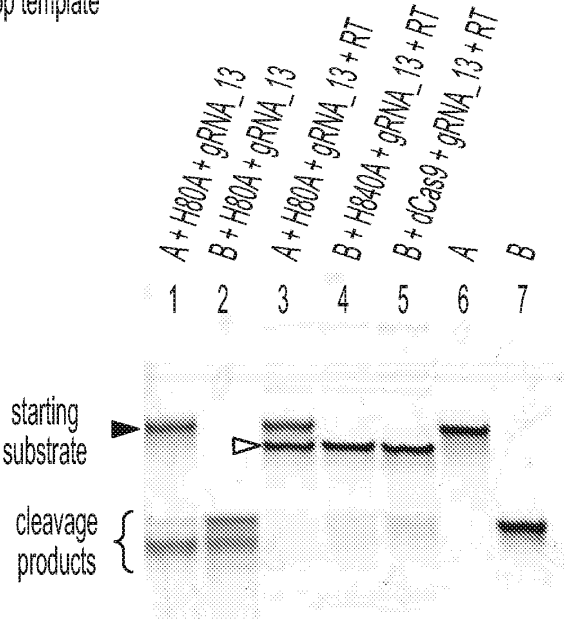


FIG. 7

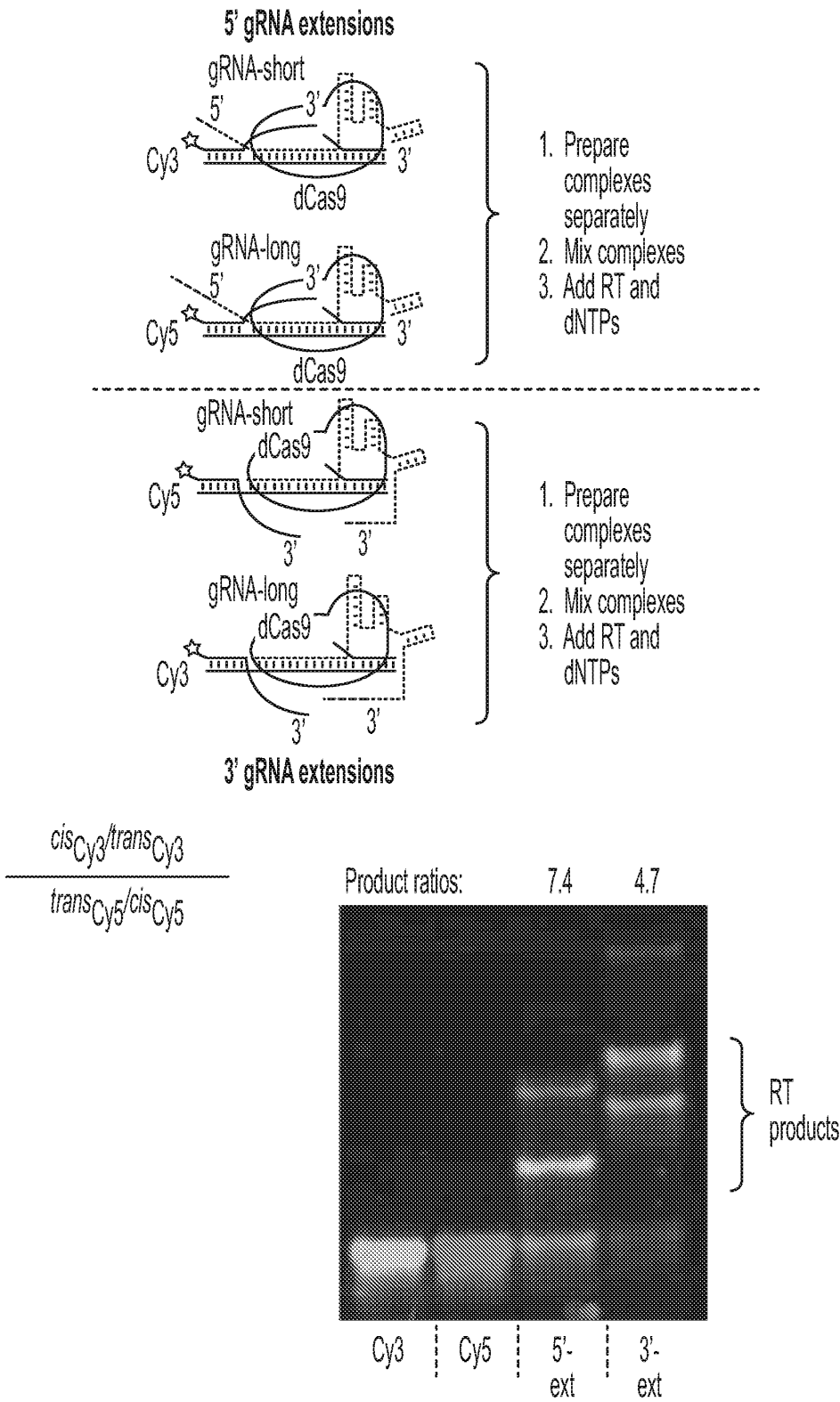


FIG. 8

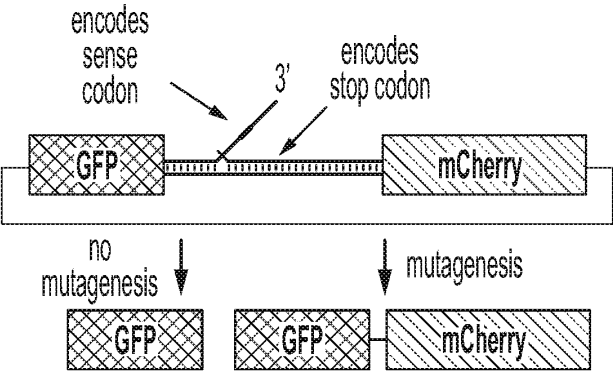


FIG. 9A

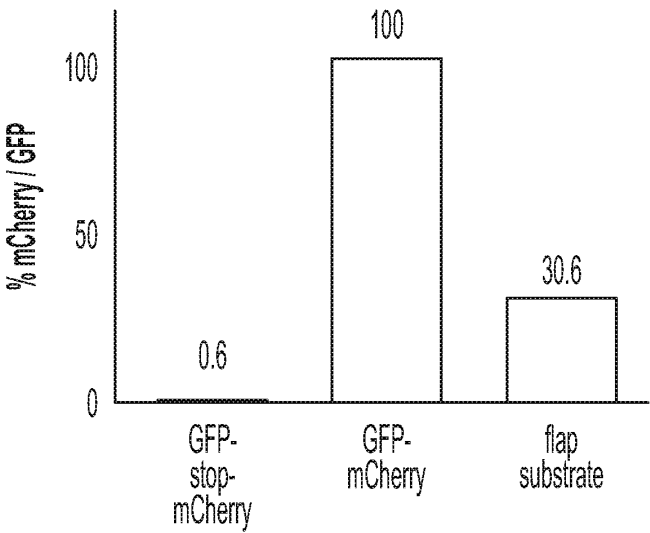


FIG. 9B

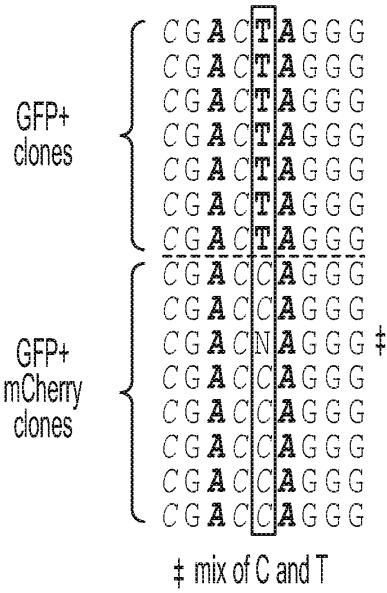


FIG. 9C

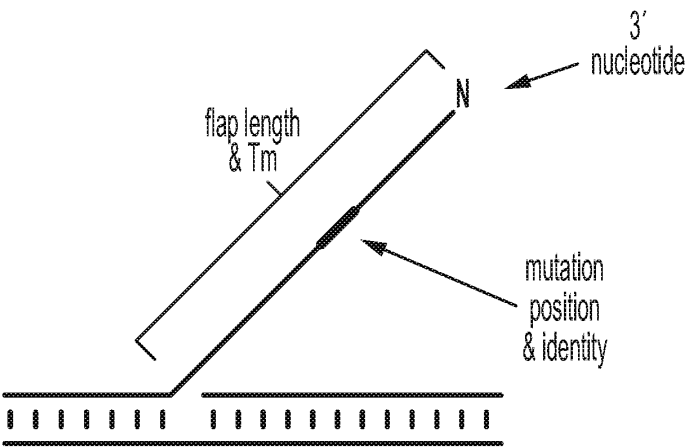


FIG. 9D

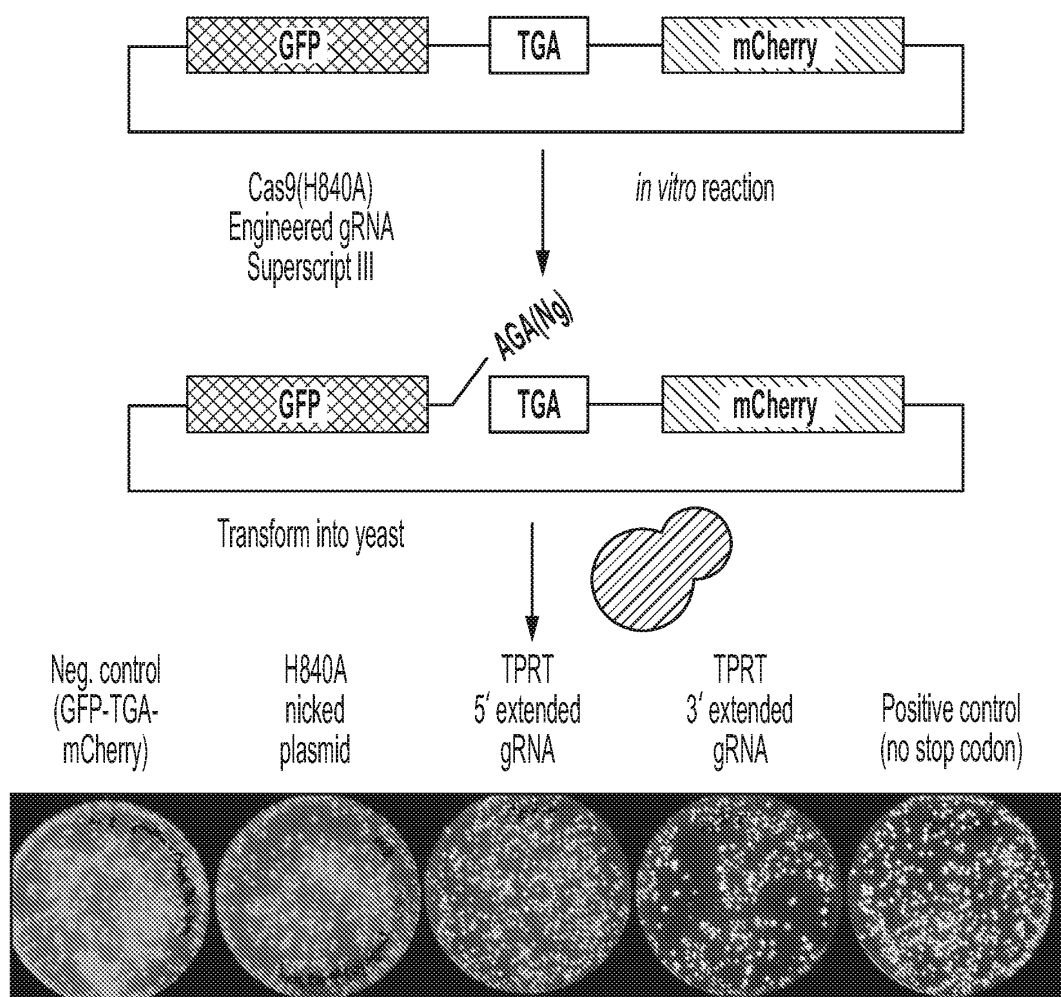


FIG. 10

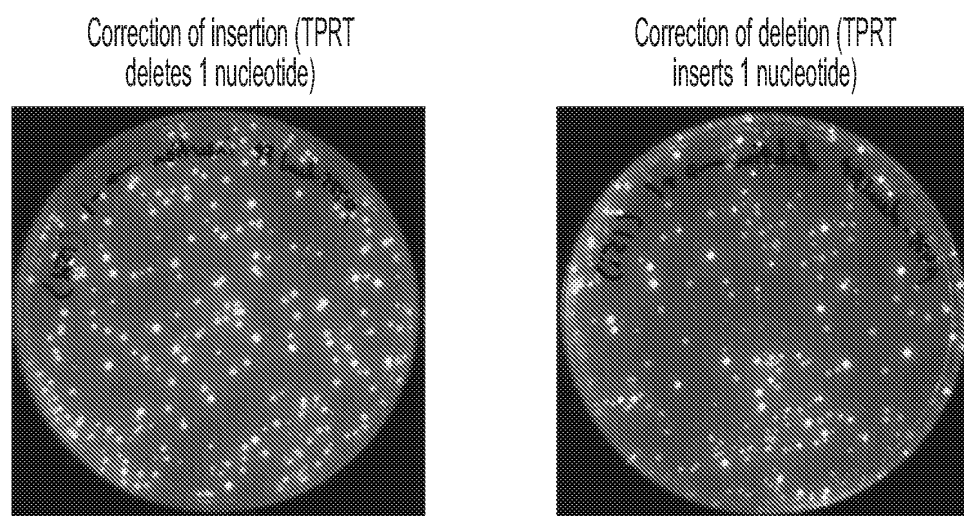


FIG. 11

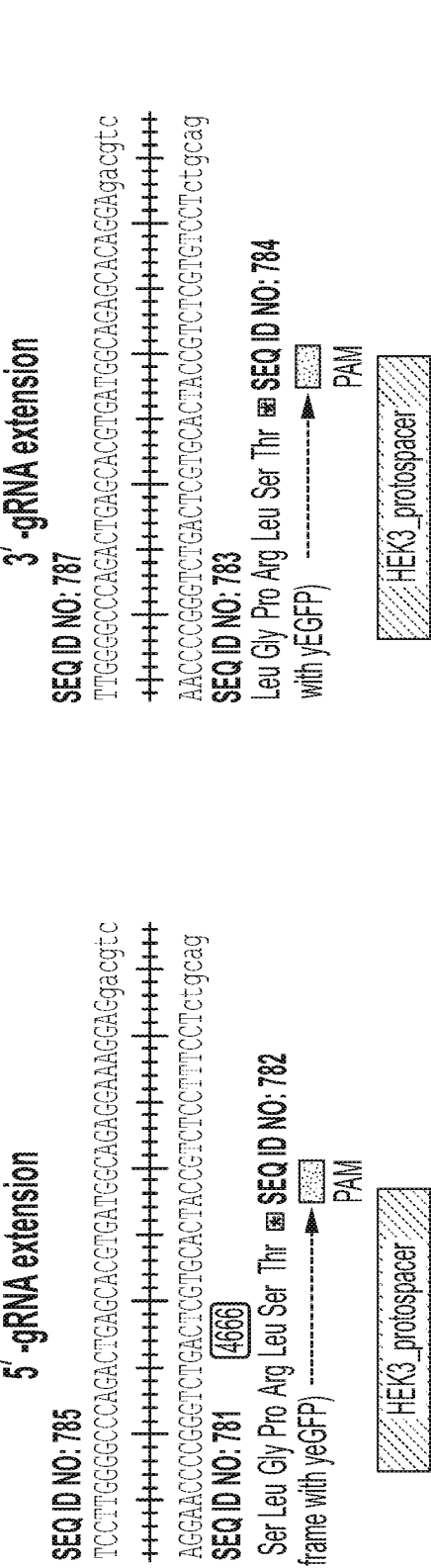
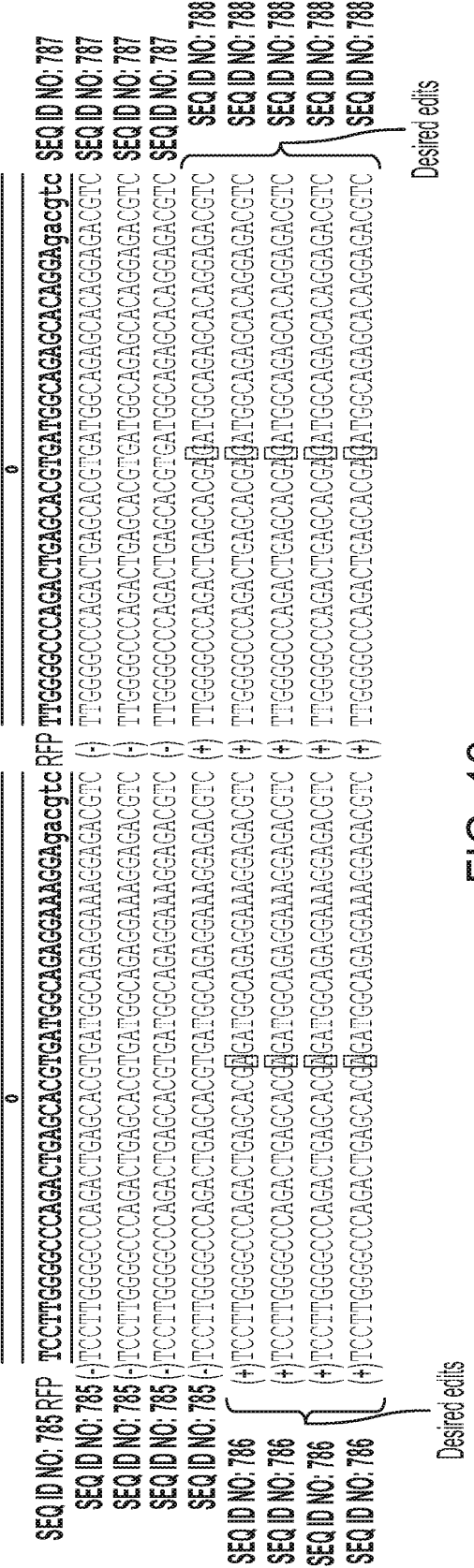


FIG. 12

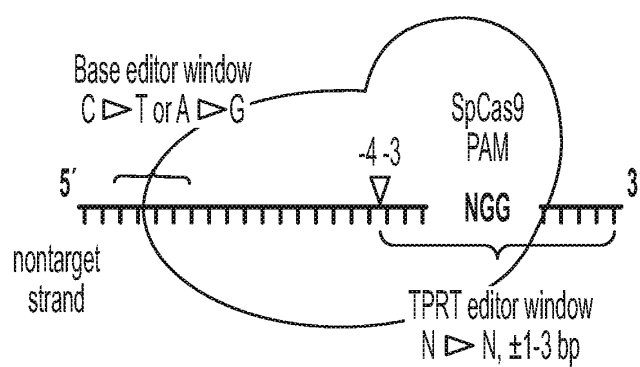


FIG. 13

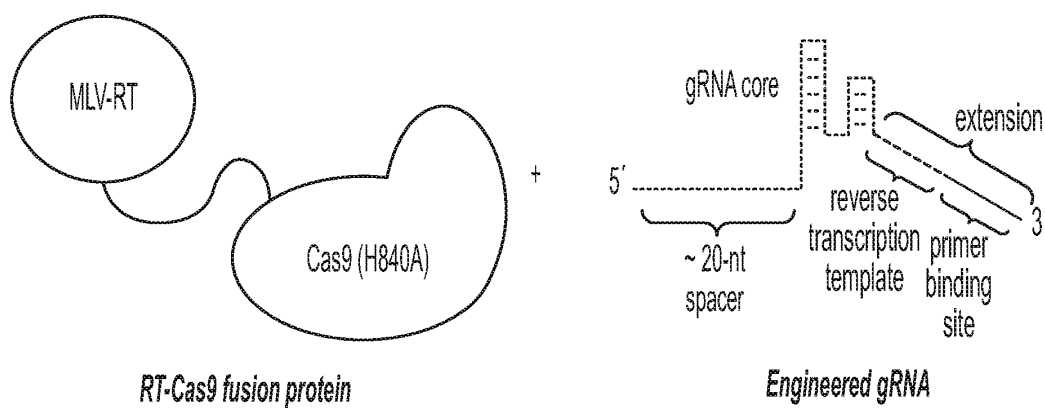


FIG. 14

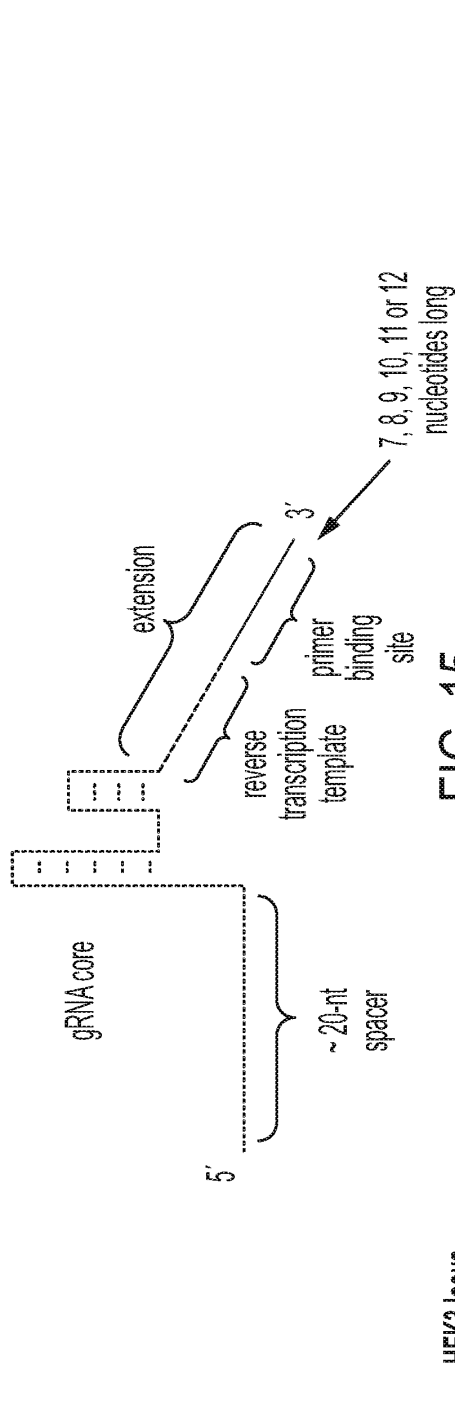


FIG. 15

HEK3 locus

SEQ ID NO: 789

5' ATGTGGGTCCTAGAAAGGCATGGATGAGAGAGCTGGACAGGGATCCAGGGAAACGCCCATGCAATTAGTCTATTCTGTGCAAGTAAGCATG  
3' TACACCGACGGATCTTTCCGTACTACTCTCTTCGGACCTGTCTCCTAGGTCCCTTTGGGGTACGTTATCAGATAAAGACGACGTTCAATTCGTAC

SEQ ID NO: 790

CATTGTAGGCTTGATGCTTTTCTGCTTCTCCAGCCCTGGCCTGGGTCAATCCTTGGGGCCAGACTGACACGTGTATGGCAGGAAGGAAGCCC  
GTAAACATCCGAACCTACGAAAAAAGACGAGAGGTGCGGACCGGACCCAGTATAGGAACCCCGGGTCTCACTCGTGCACTACCGTCTCCTTCCCTTCGGG

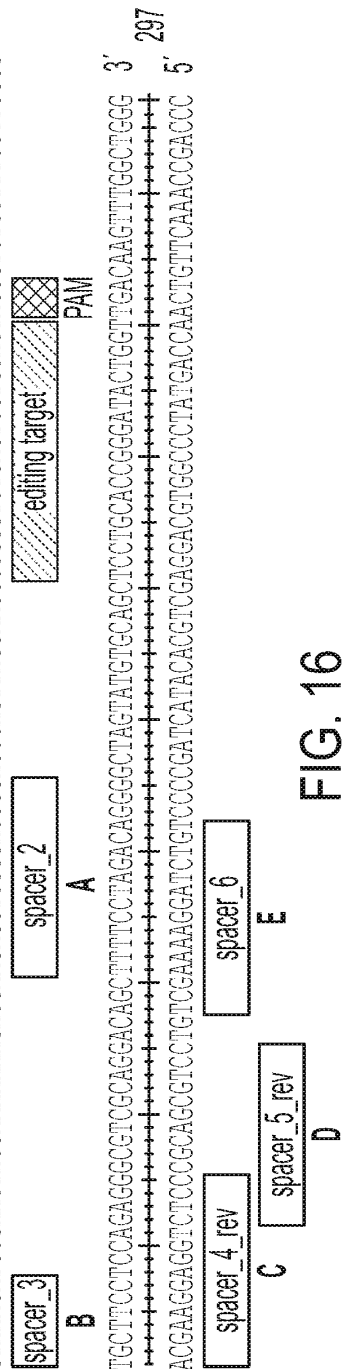


FIG. 16

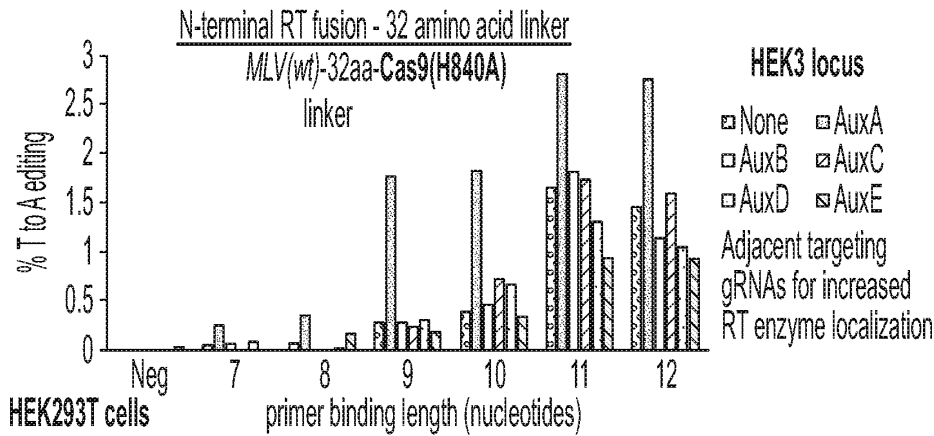


FIG. 17A

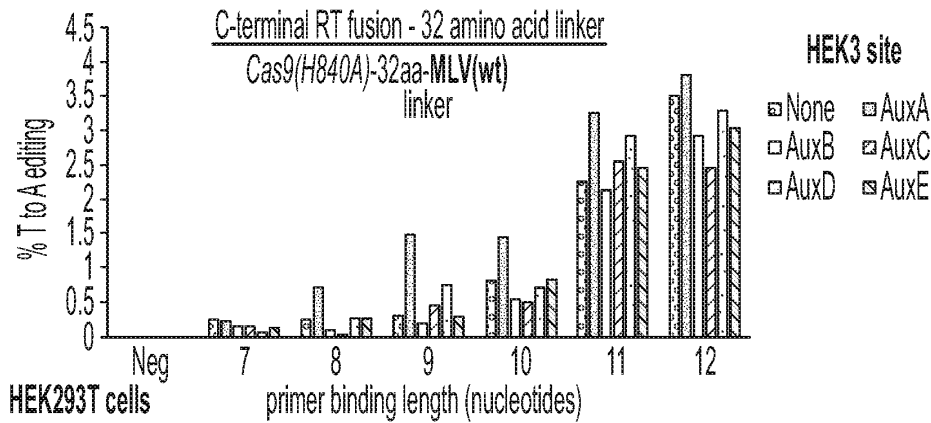


FIG. 17B

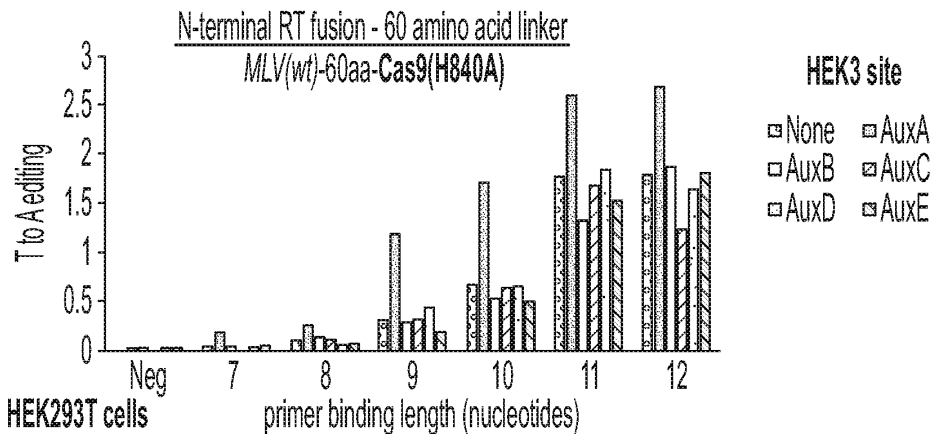


FIG. 17C

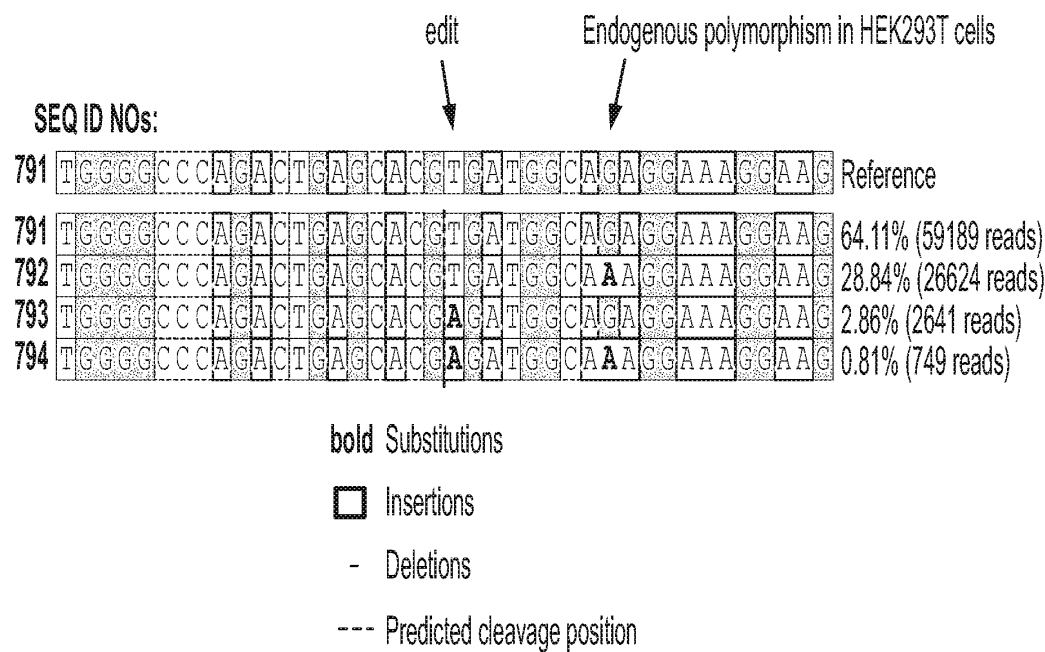


FIG. 18

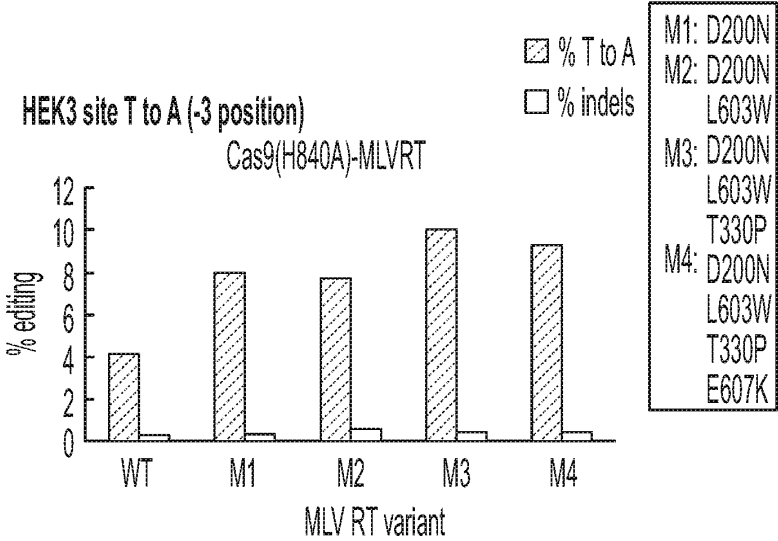


FIG. 19

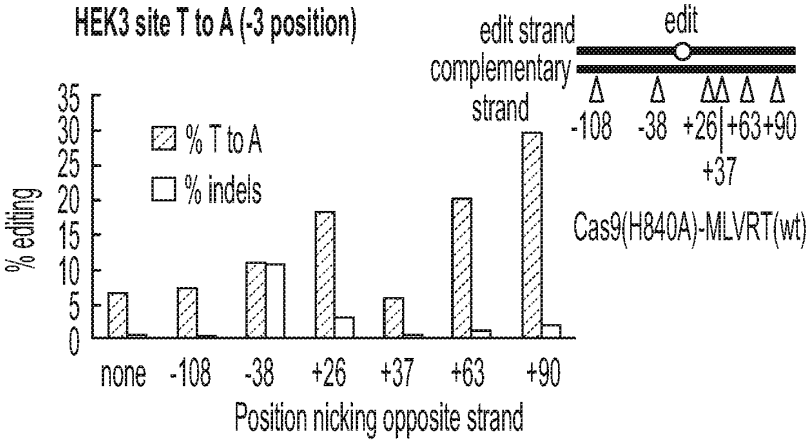


FIG. 20

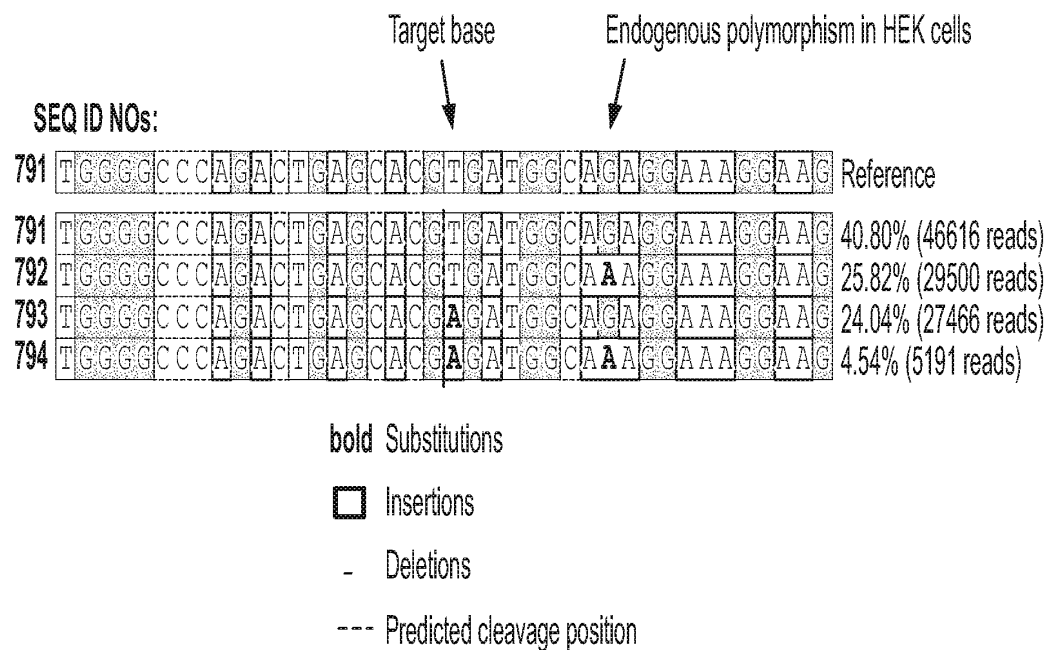


FIG. 21

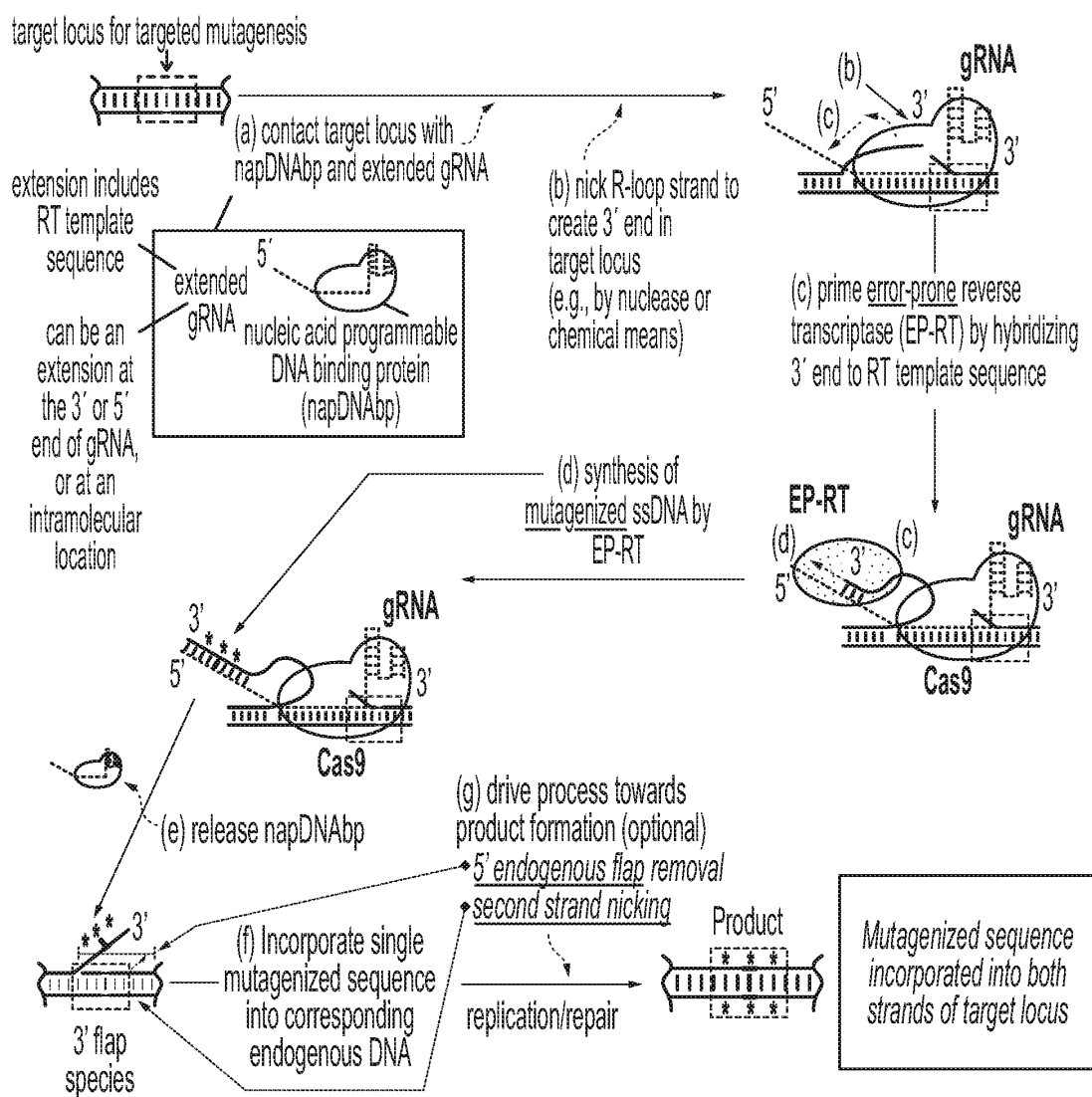
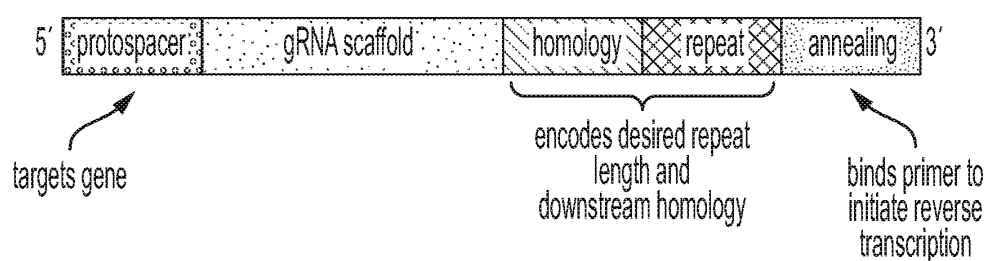


FIG. 22

### gRNA design for contracting trinucleotide repeat sequences



### Trinucleotide repeat contraction with TPRT genome editing

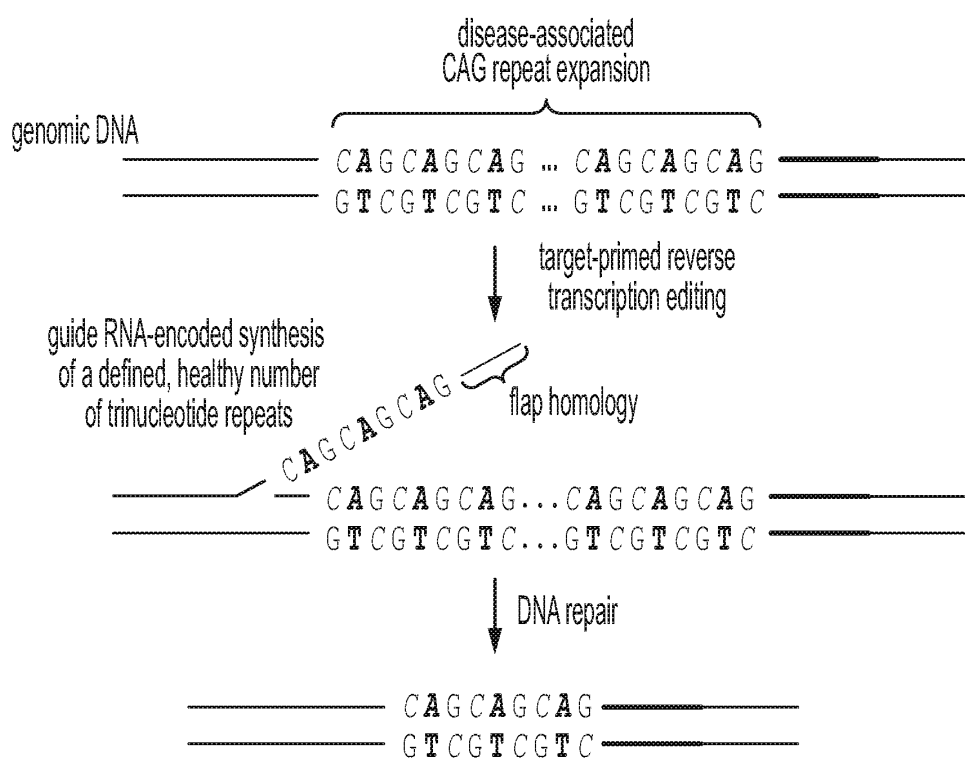


FIG. 23

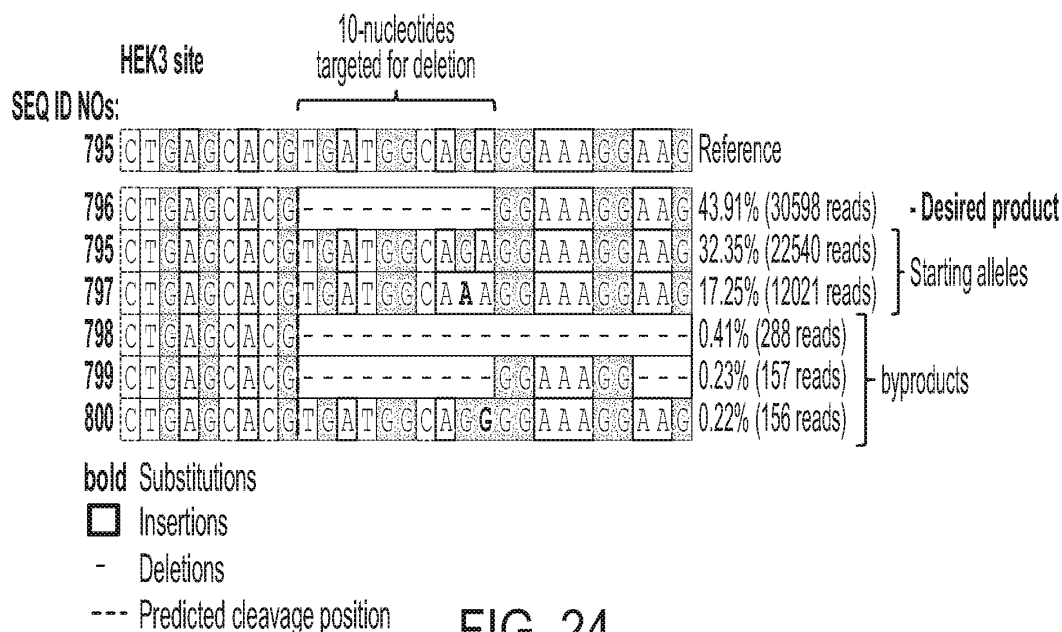


FIG. 24

## gRNA design for peptide tagging genes at endogenous genomic loci

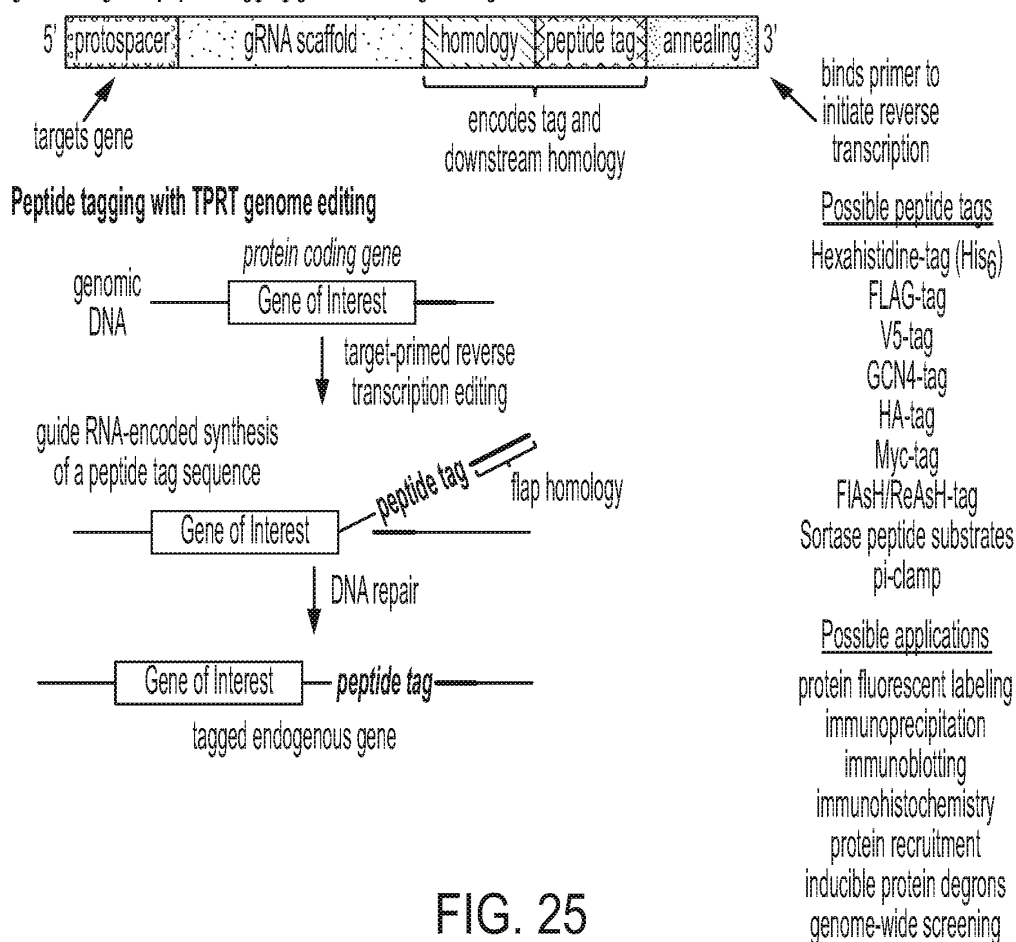


FIG. 25

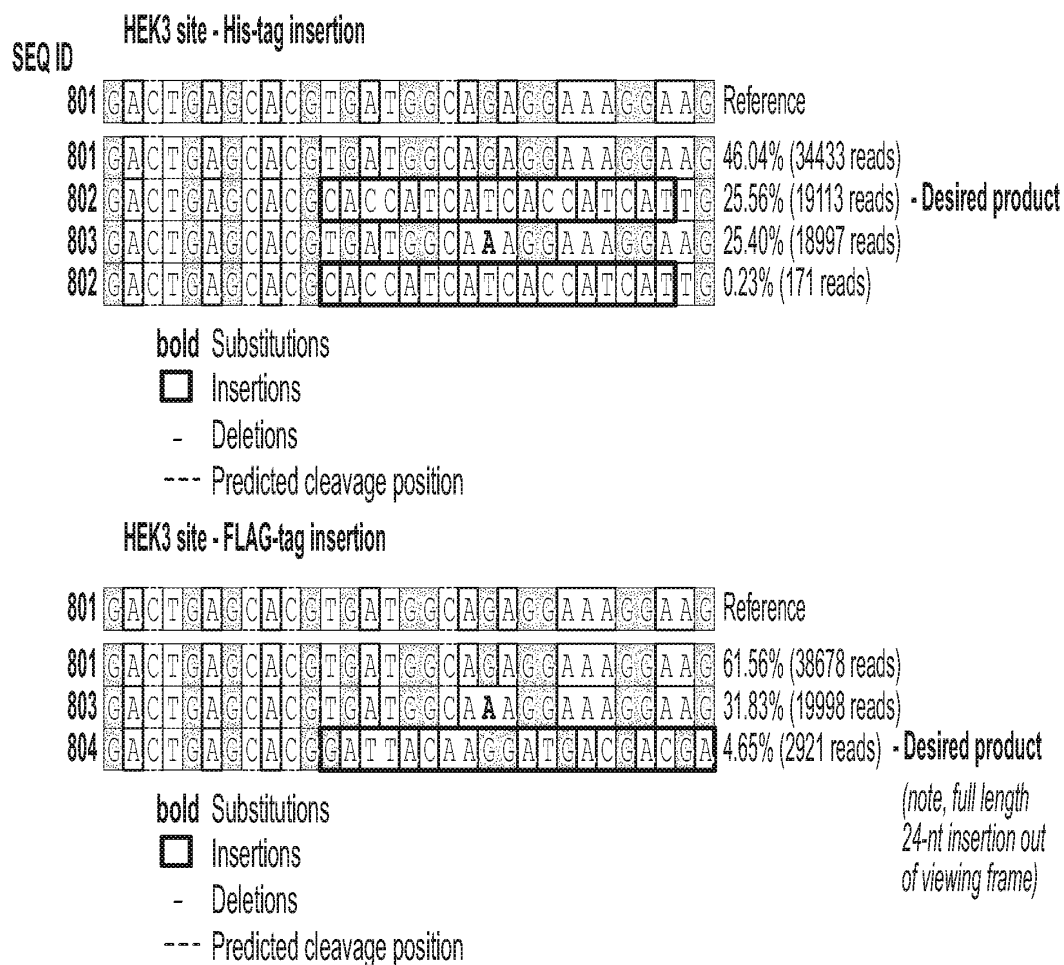


FIG. 26A

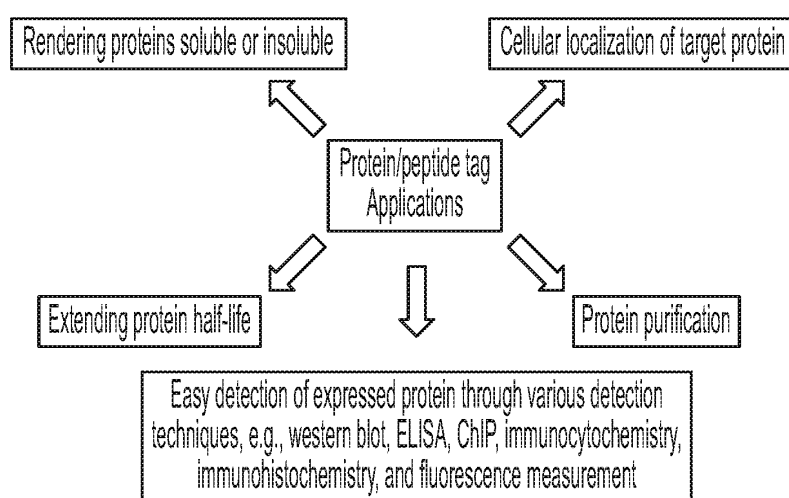


FIG. 26B

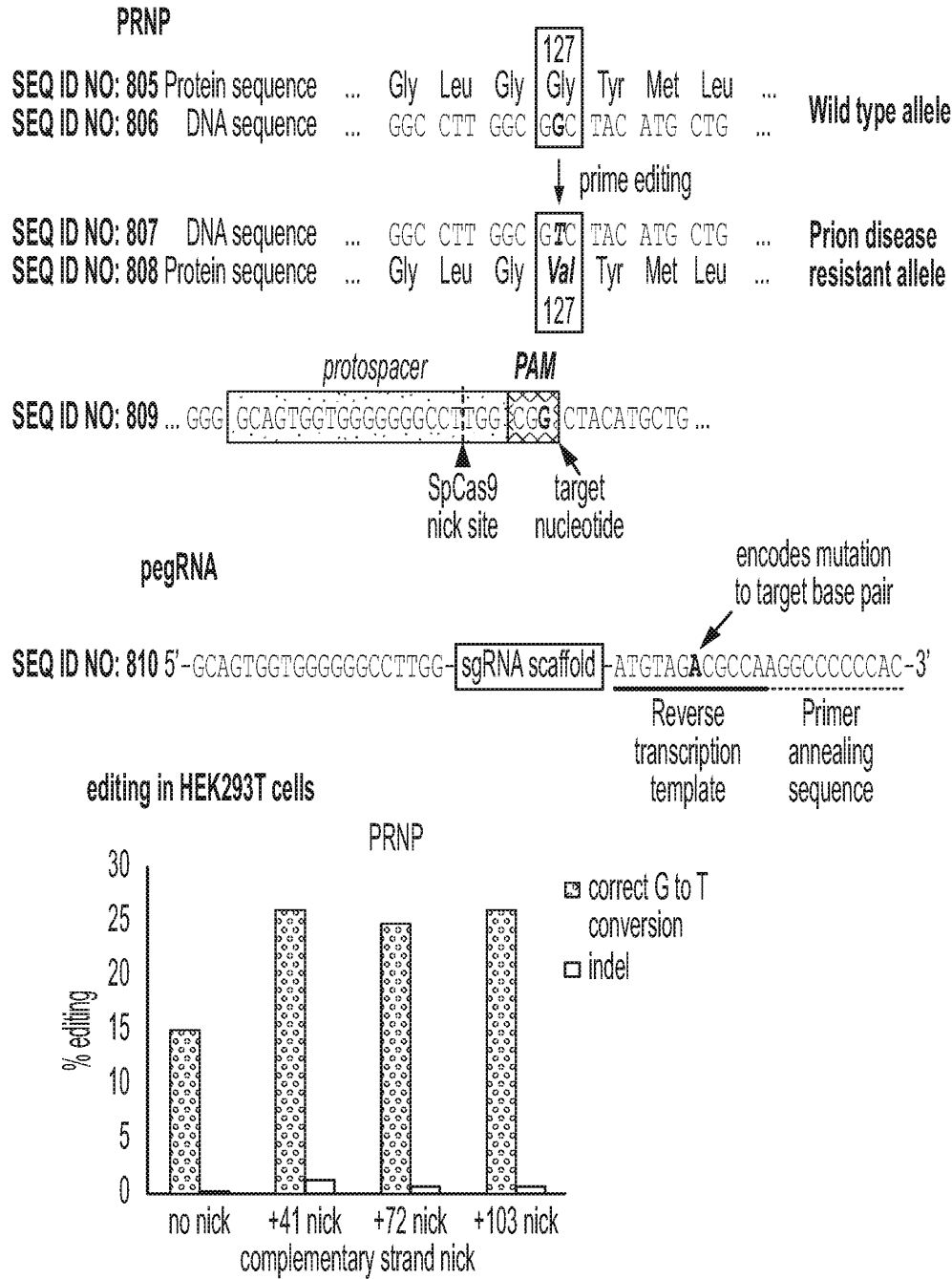


FIG. 27

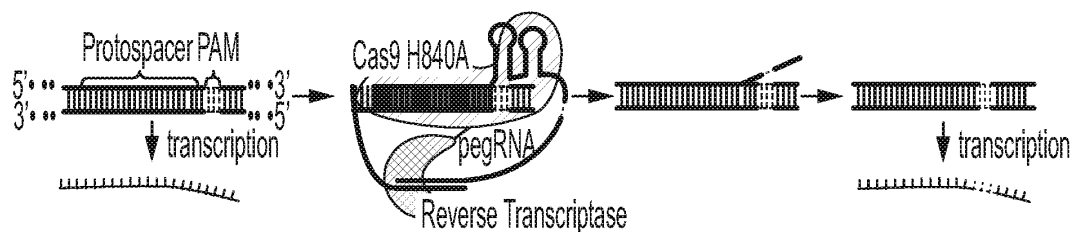


FIG. 28A

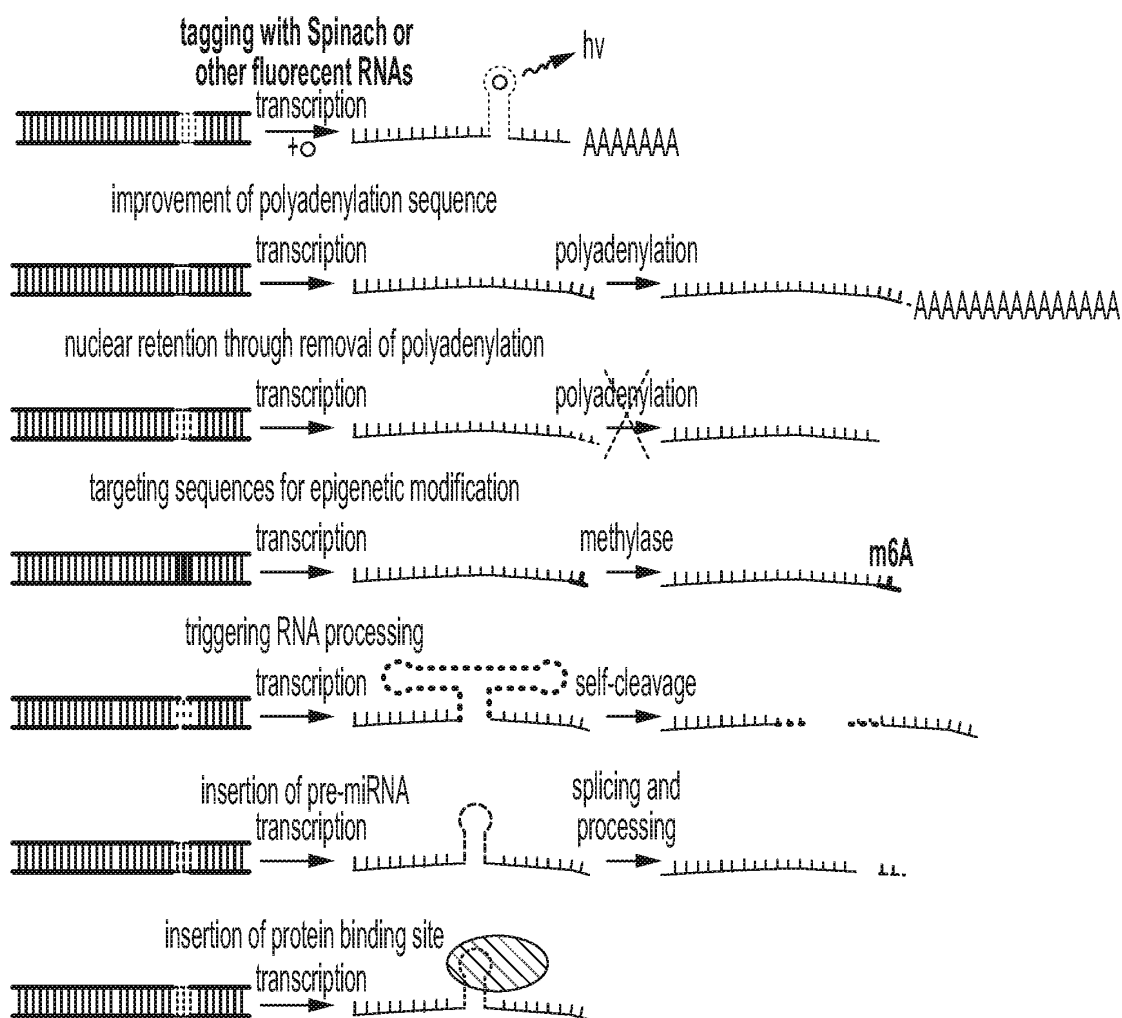


FIG. 28B

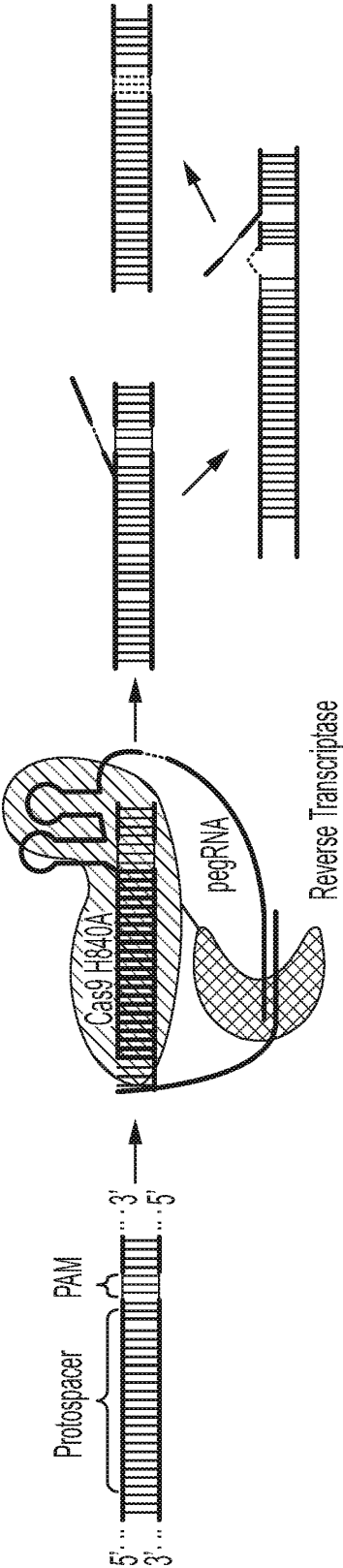


FIG. 29A

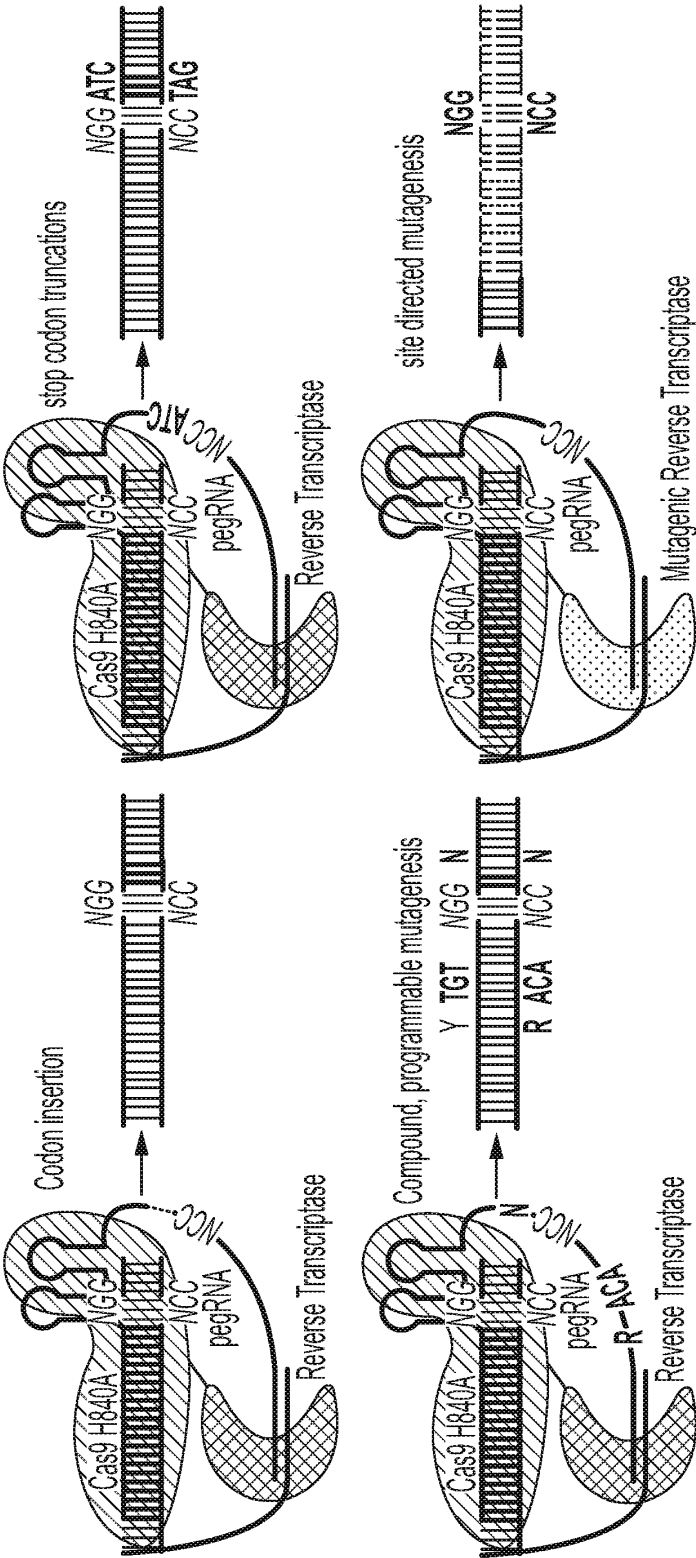


FIG. 29B

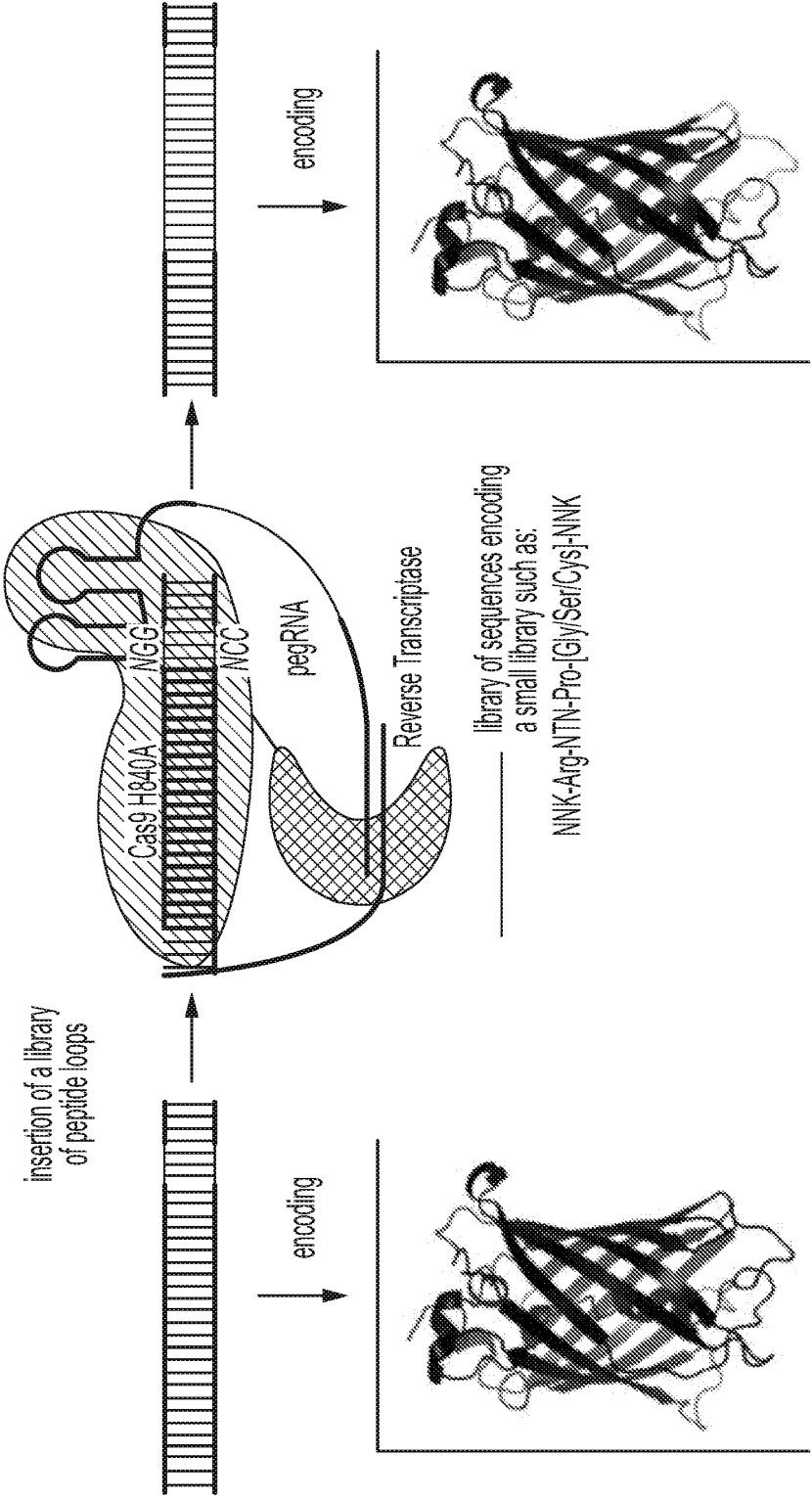


FIG. 29C

| programmable codon deletion and truncations |            |                           |
|---------------------------------------------|------------|---------------------------|
| SEQ ID NO: 811                              | <b>GFP</b> | NFKIRHNIEDGSVQLADHYQQNTPI |
| SEQ ID NO: 812                              | del5       | NFKI-HNIEDGSVQLADHYQQNTPI |
| SEQ ID NO: 813                              | del6       | NFKIR-NIEDGSVQLADHYQQNTPI |
| SEQ ID NO: 814                              | del8       | NFKIRHN-EDGSVQLADHYQQNTPI |
| SEQ ID NO: 815                              | del 8-13   | NFKIRHN-----QLADHYQQNTPI  |
| SEQ ID NO: 816                              | del 1-5    | -----HNIEDGSVQLADHYQQNTPI |
| SEQ ID NO: 817                              | del 15-25  | NFKIRHNIEDGSVQ-----       |

FIG. 29D

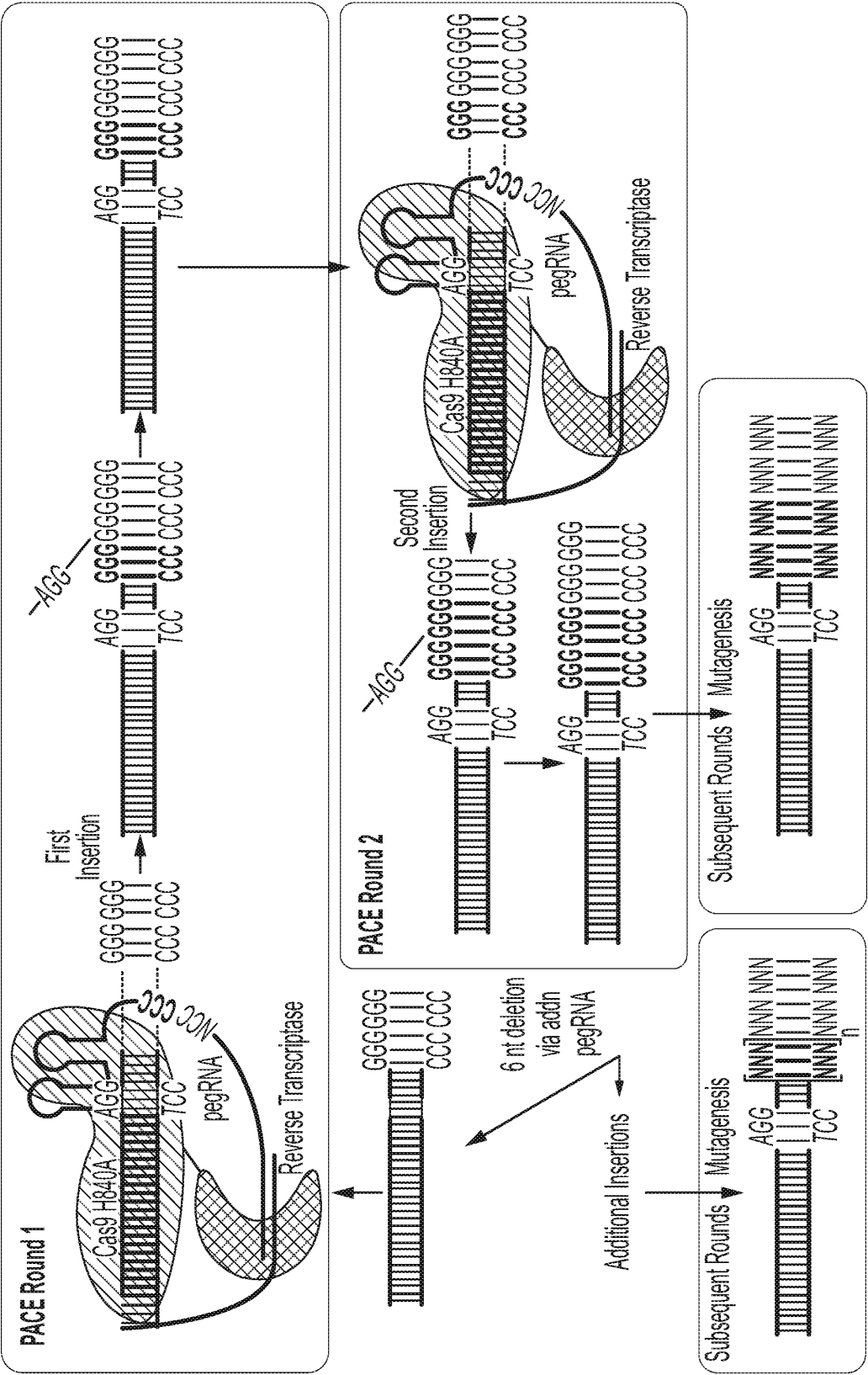


FIG. 30

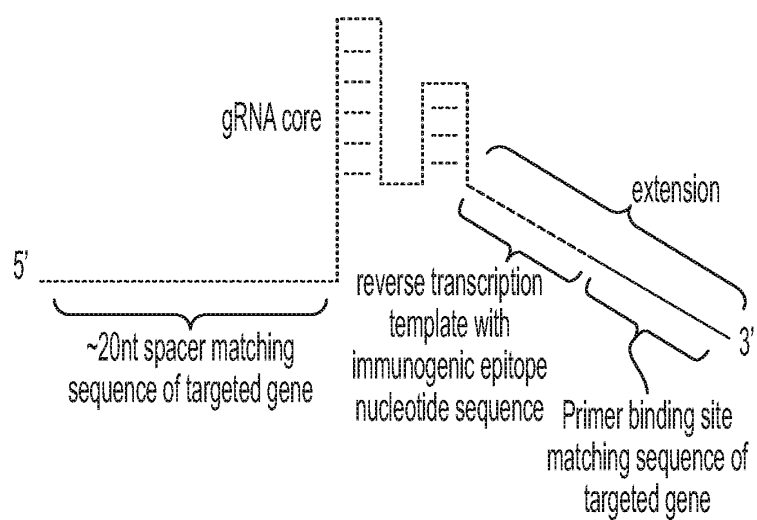


FIG. 31

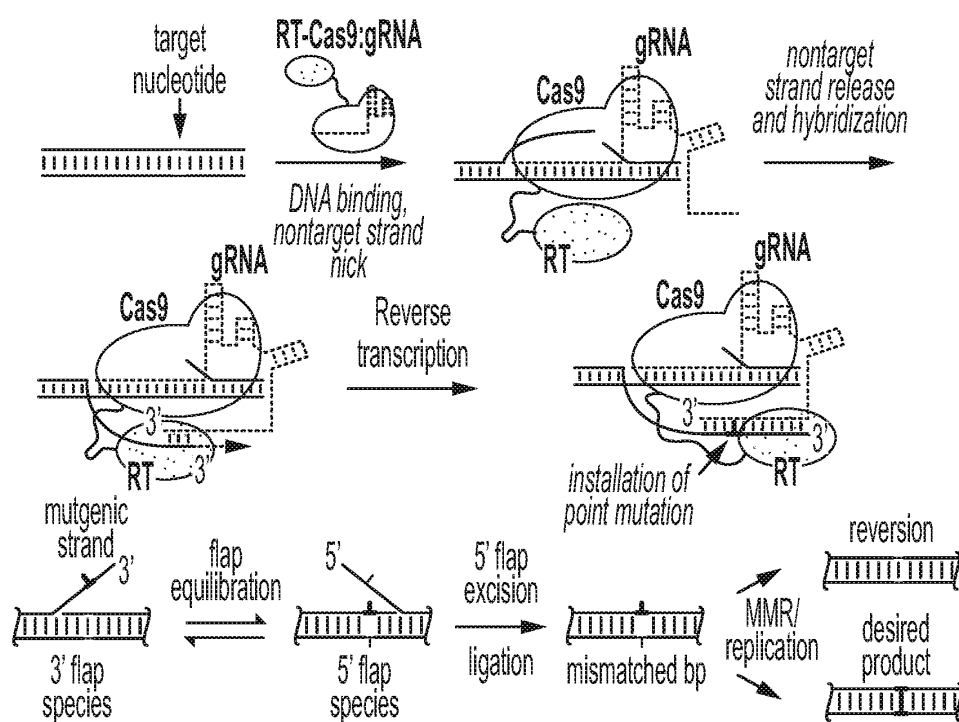
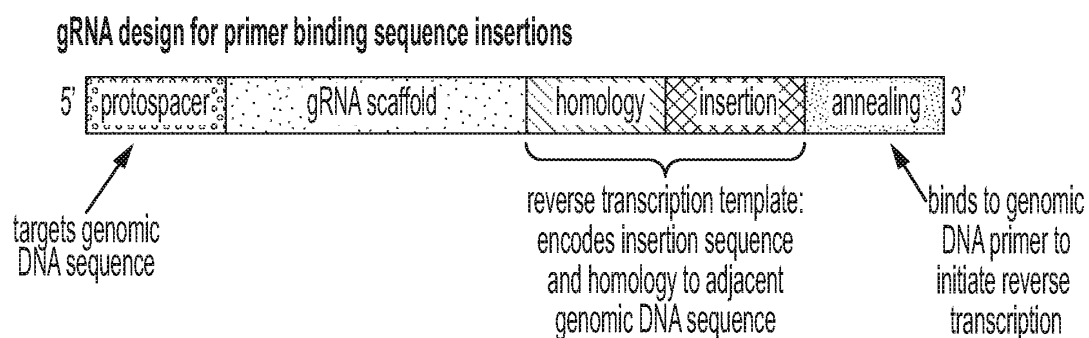


FIG. 32



**Primer binding sequence insertion into genomic DNA using prime editing**

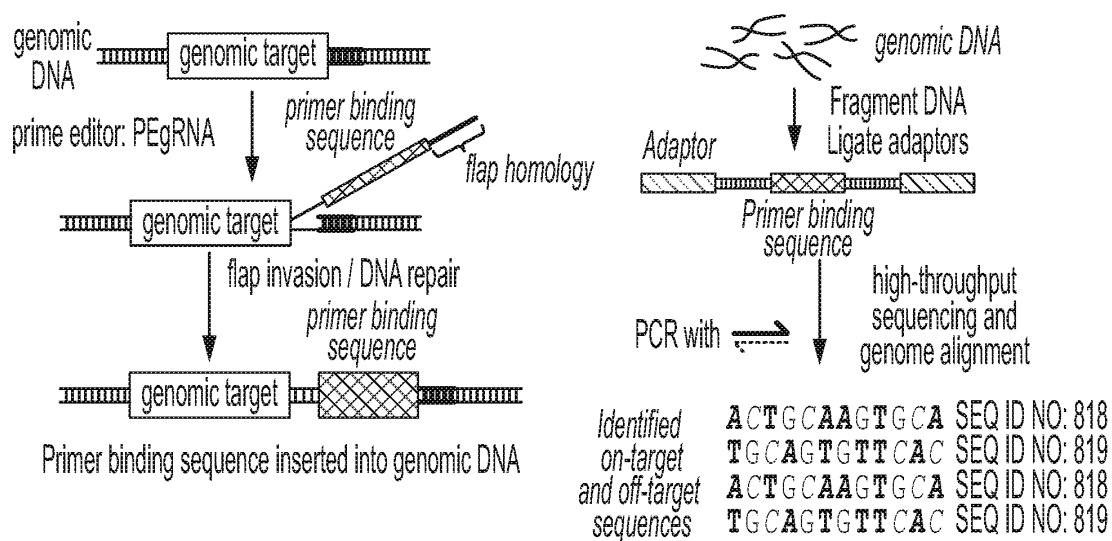


**FIG. 33**

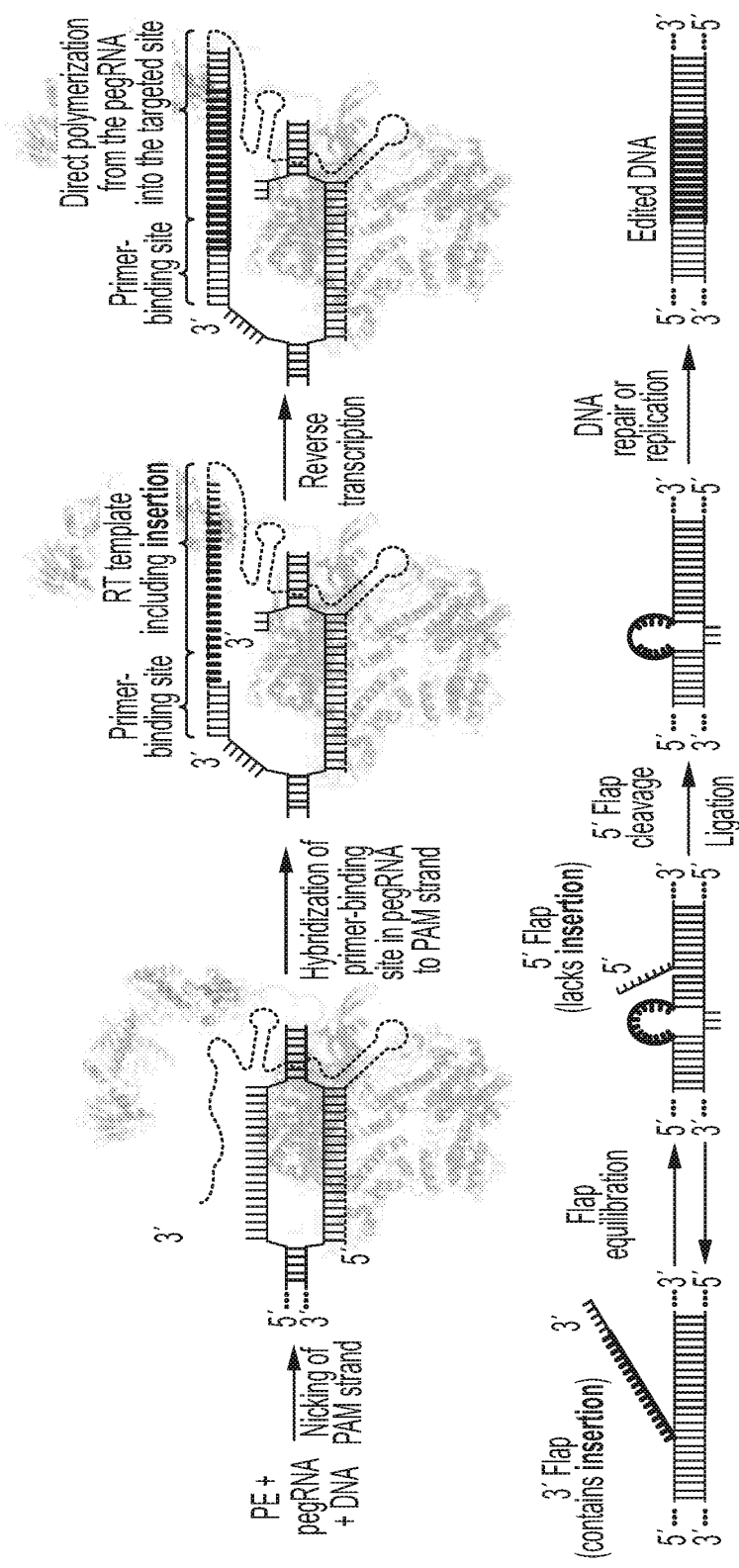


FIG. 34

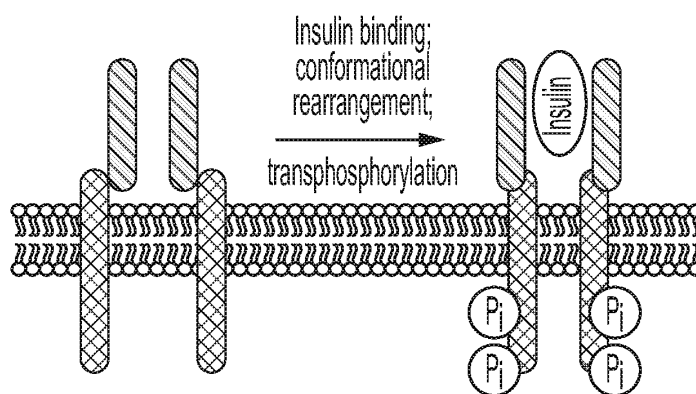


FIG. 35A

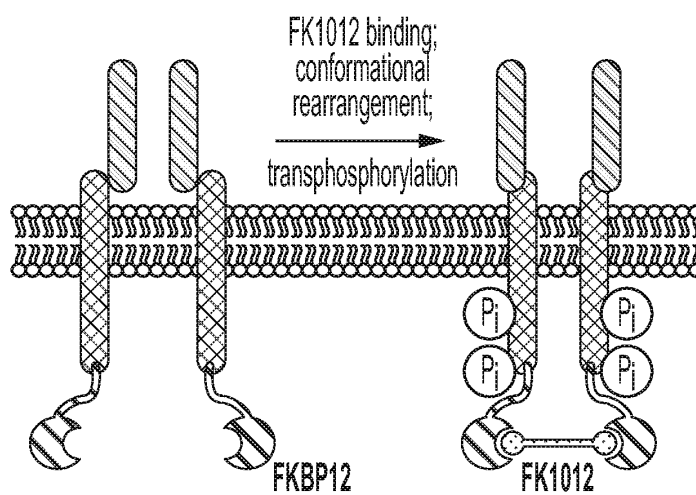


FIG. 35B

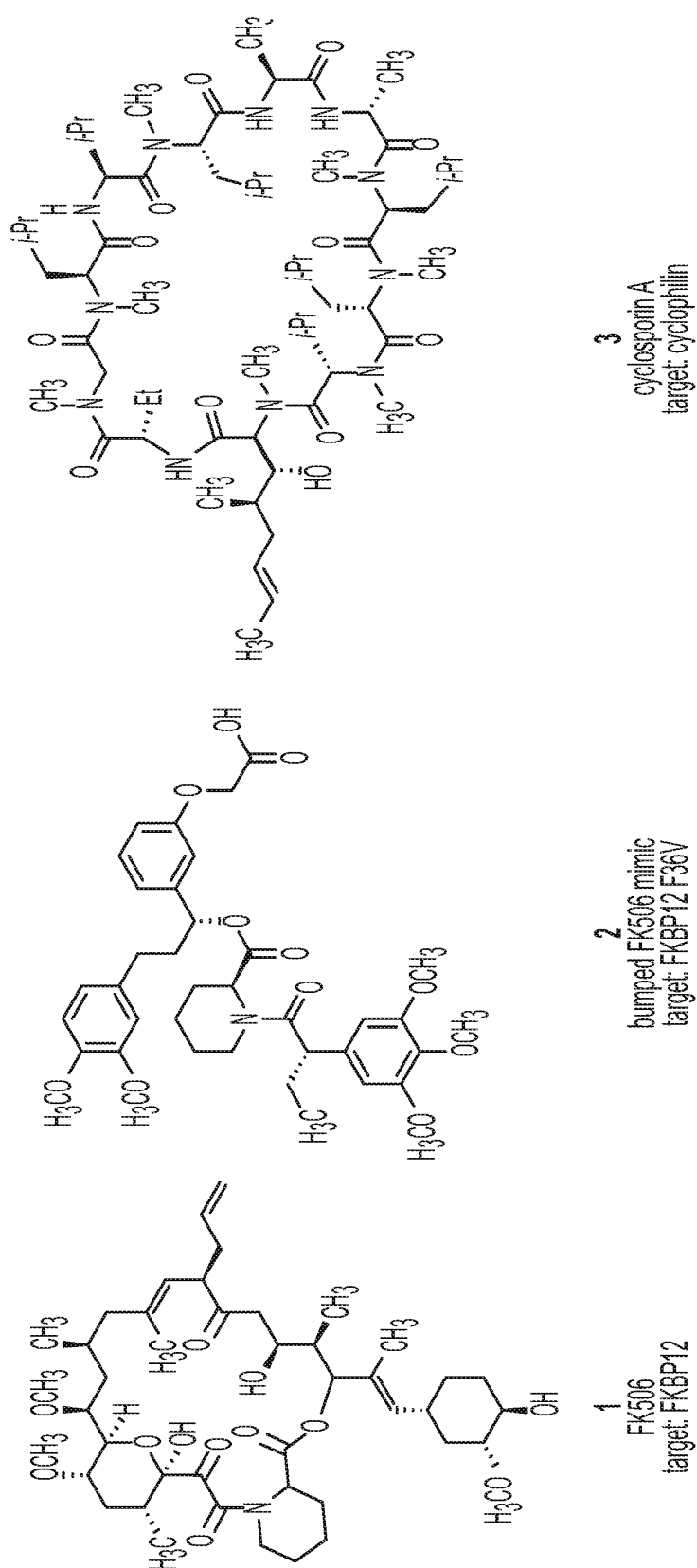


FIG. 36

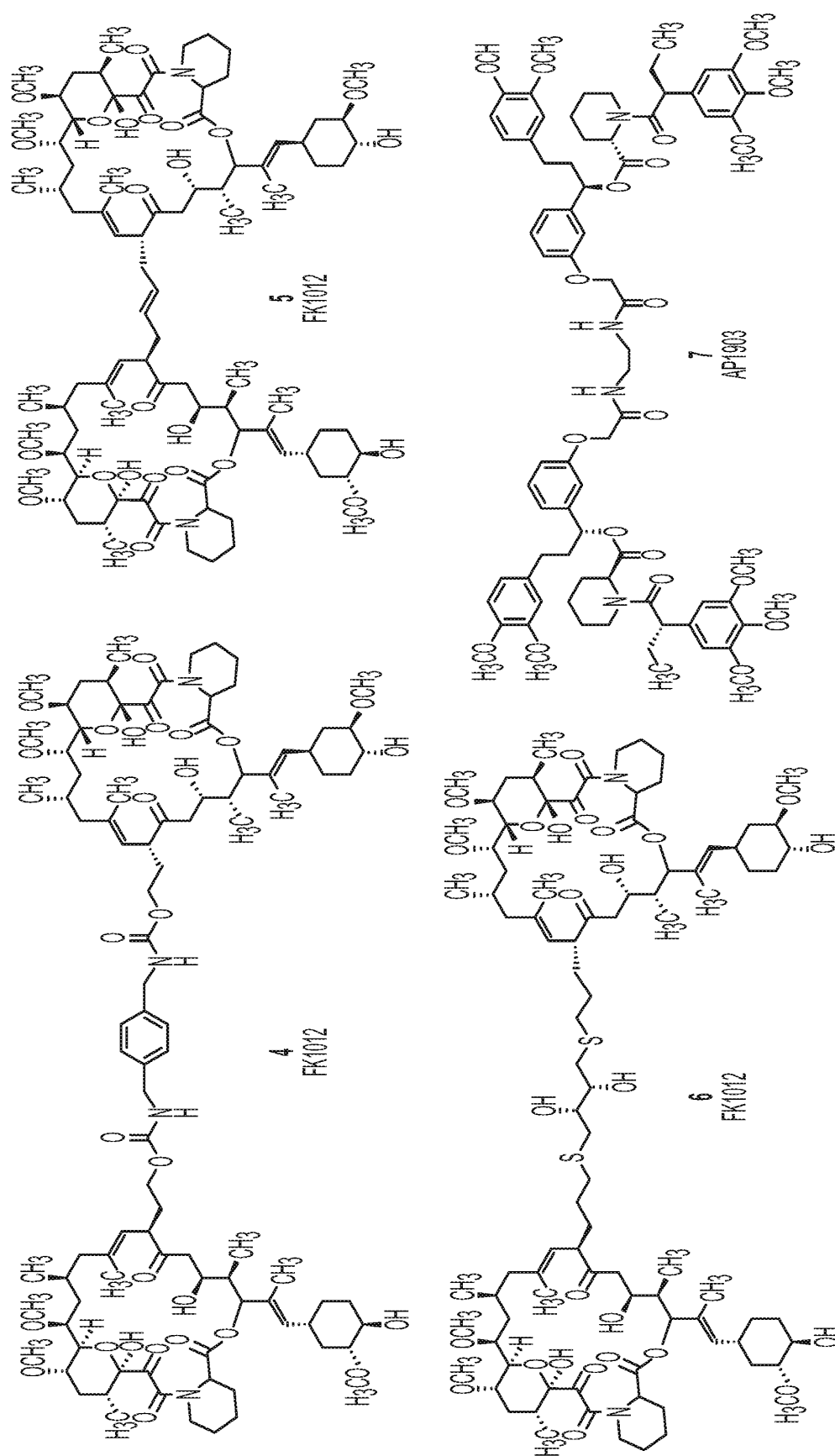


FIG. 37A

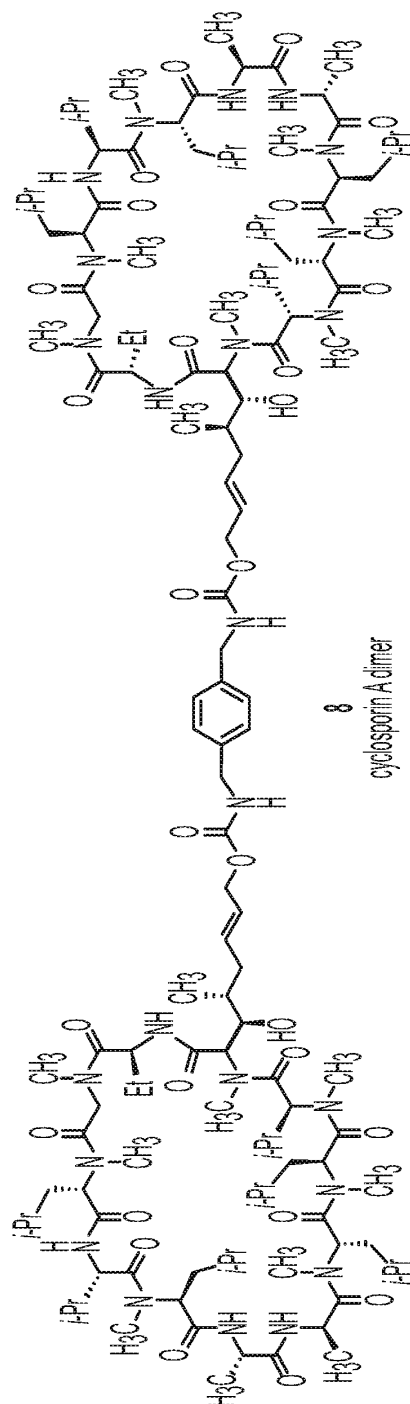
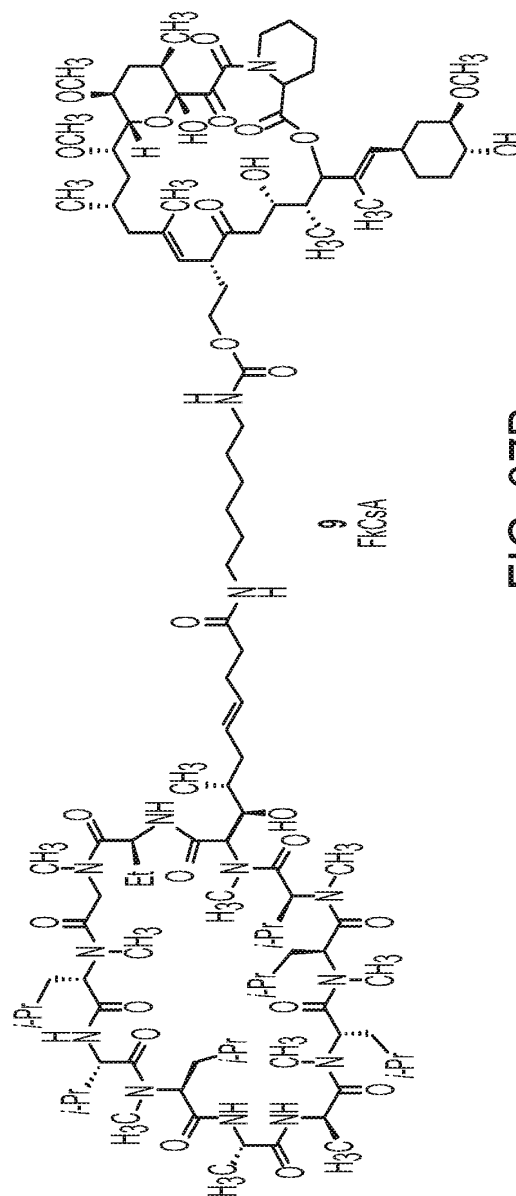


FIG. 37B

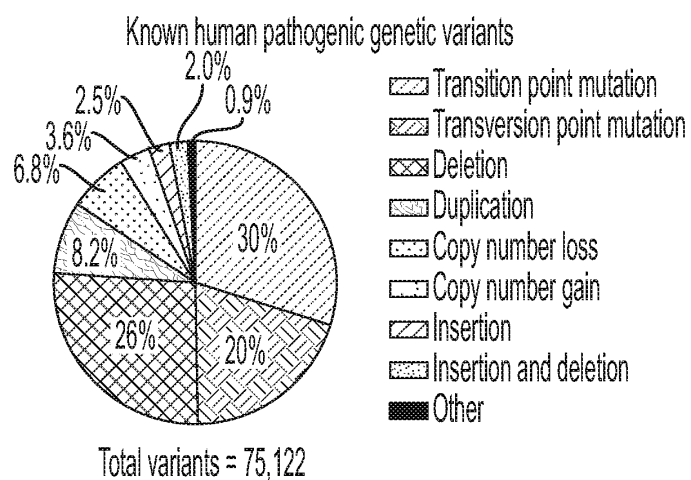


FIG. 38A

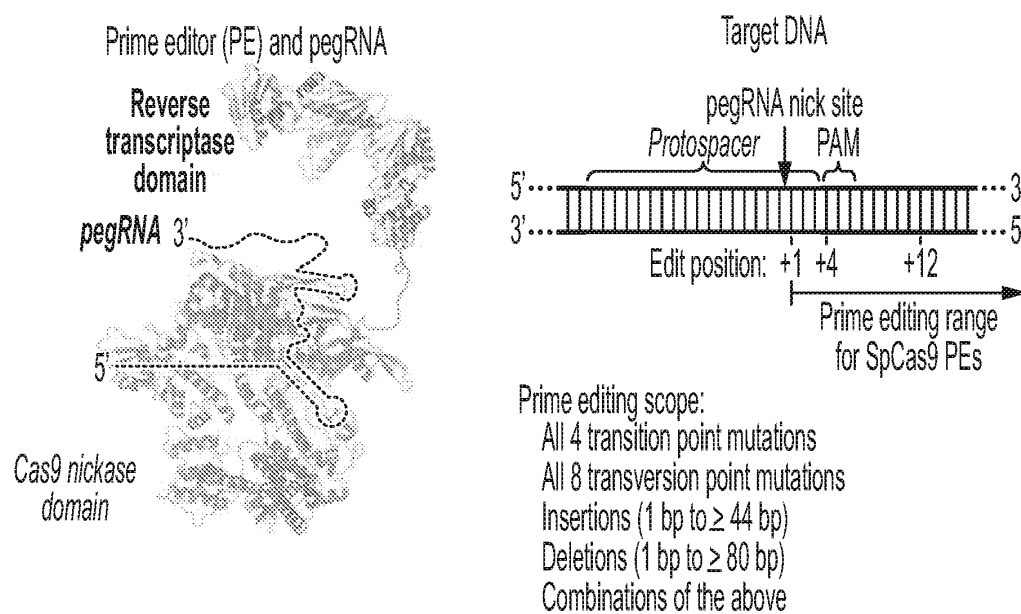


FIG. 38B

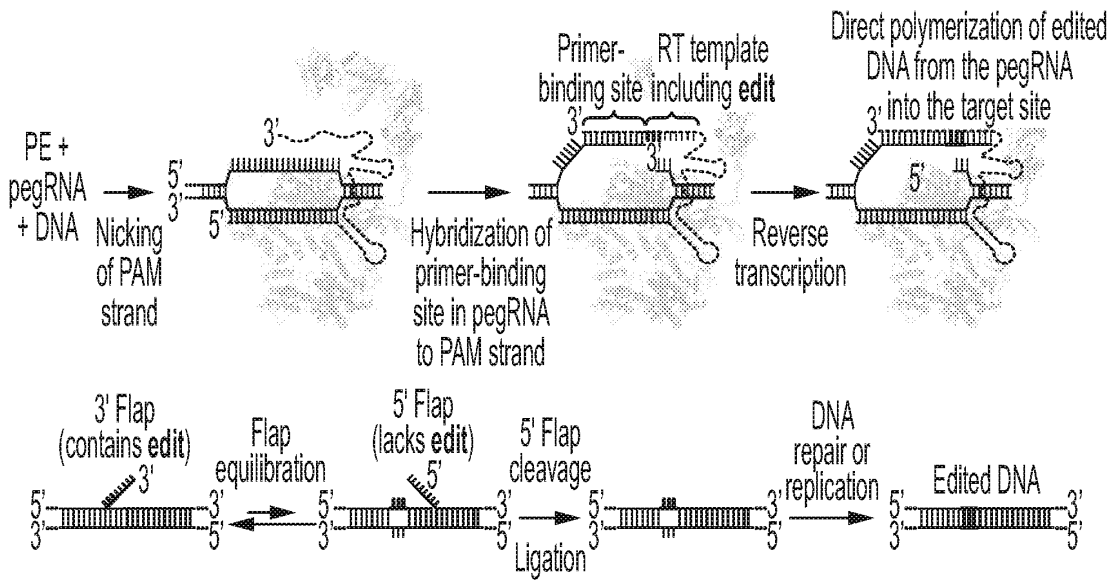


FIG. 38C

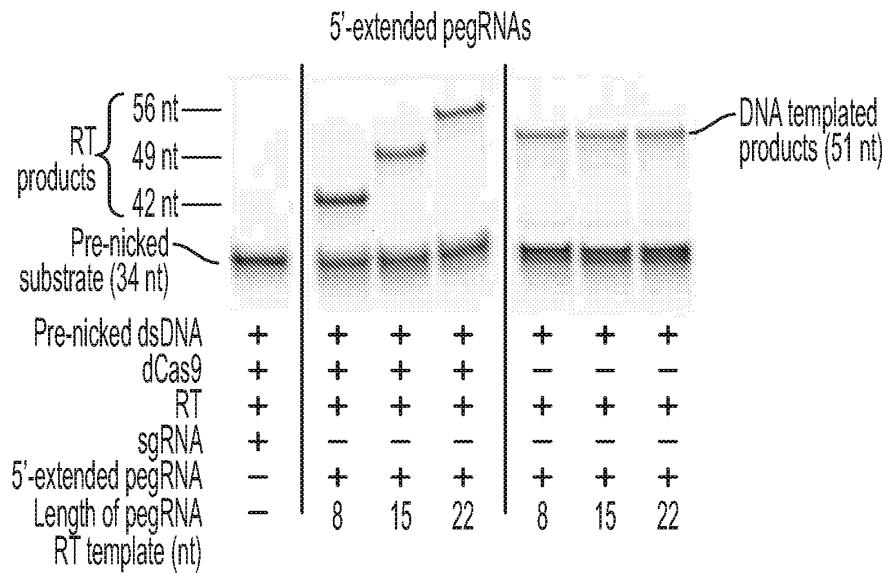


FIG. 38D

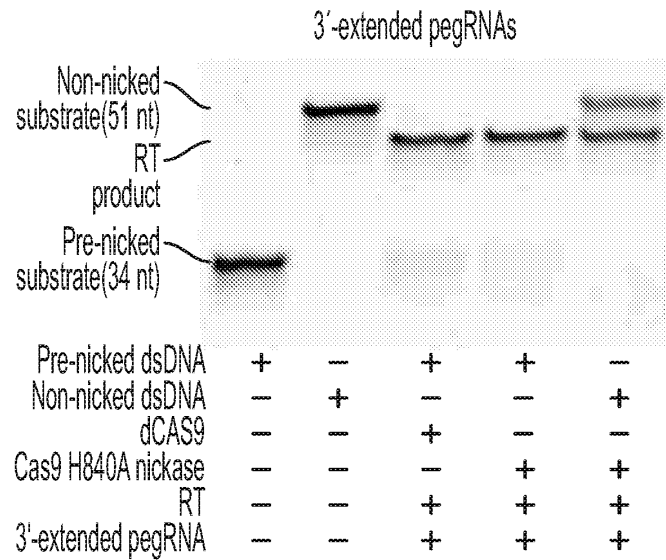


FIG. 38E

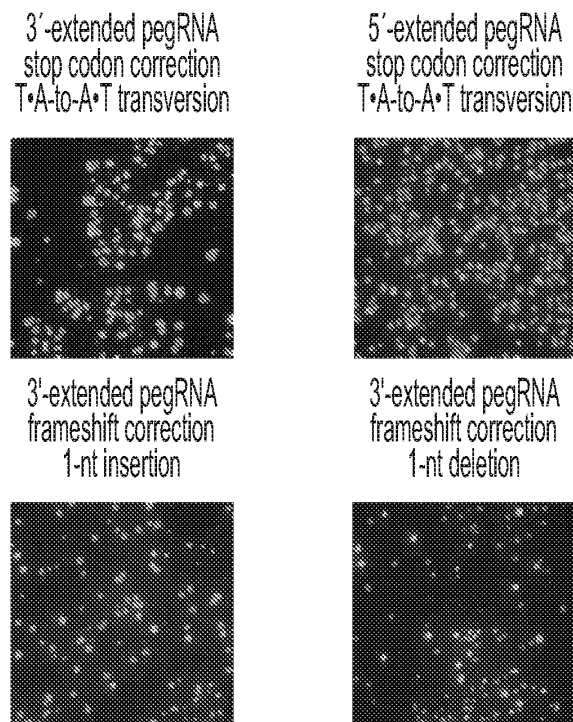


FIG. 38F

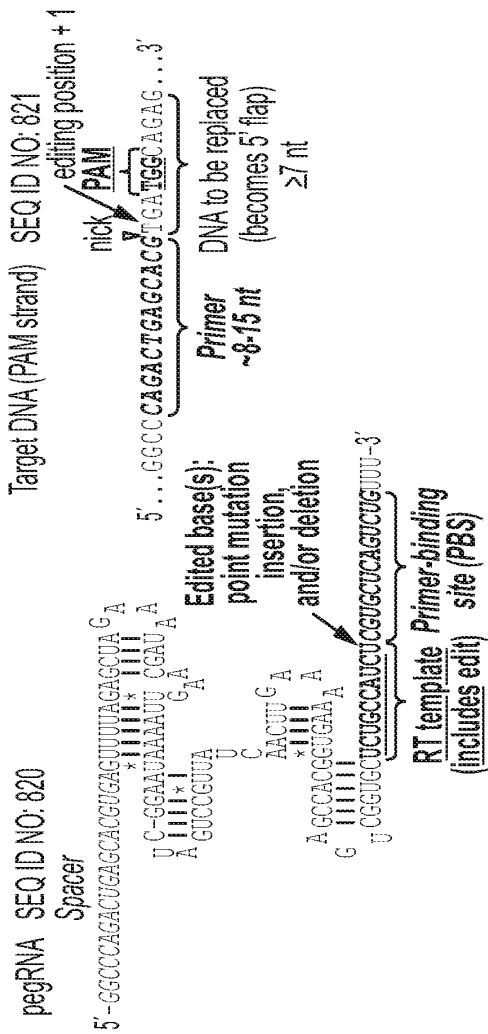


FIG. 39A

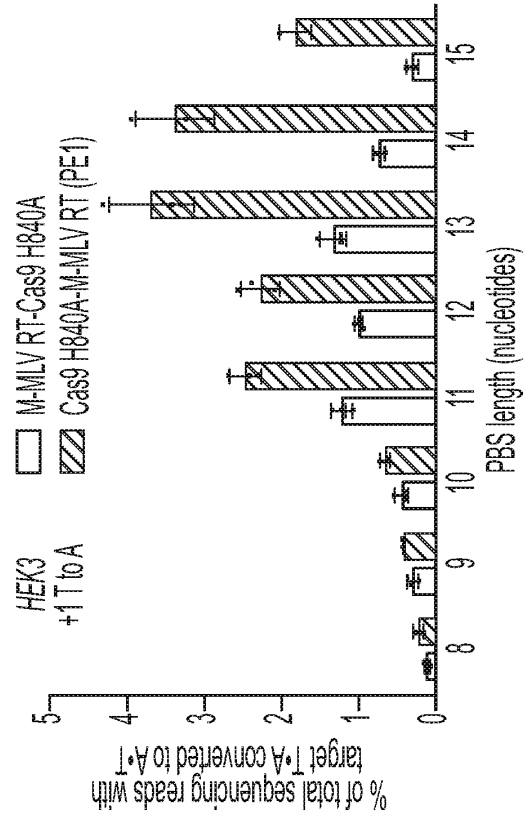
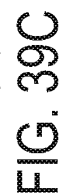


FIG. 39B



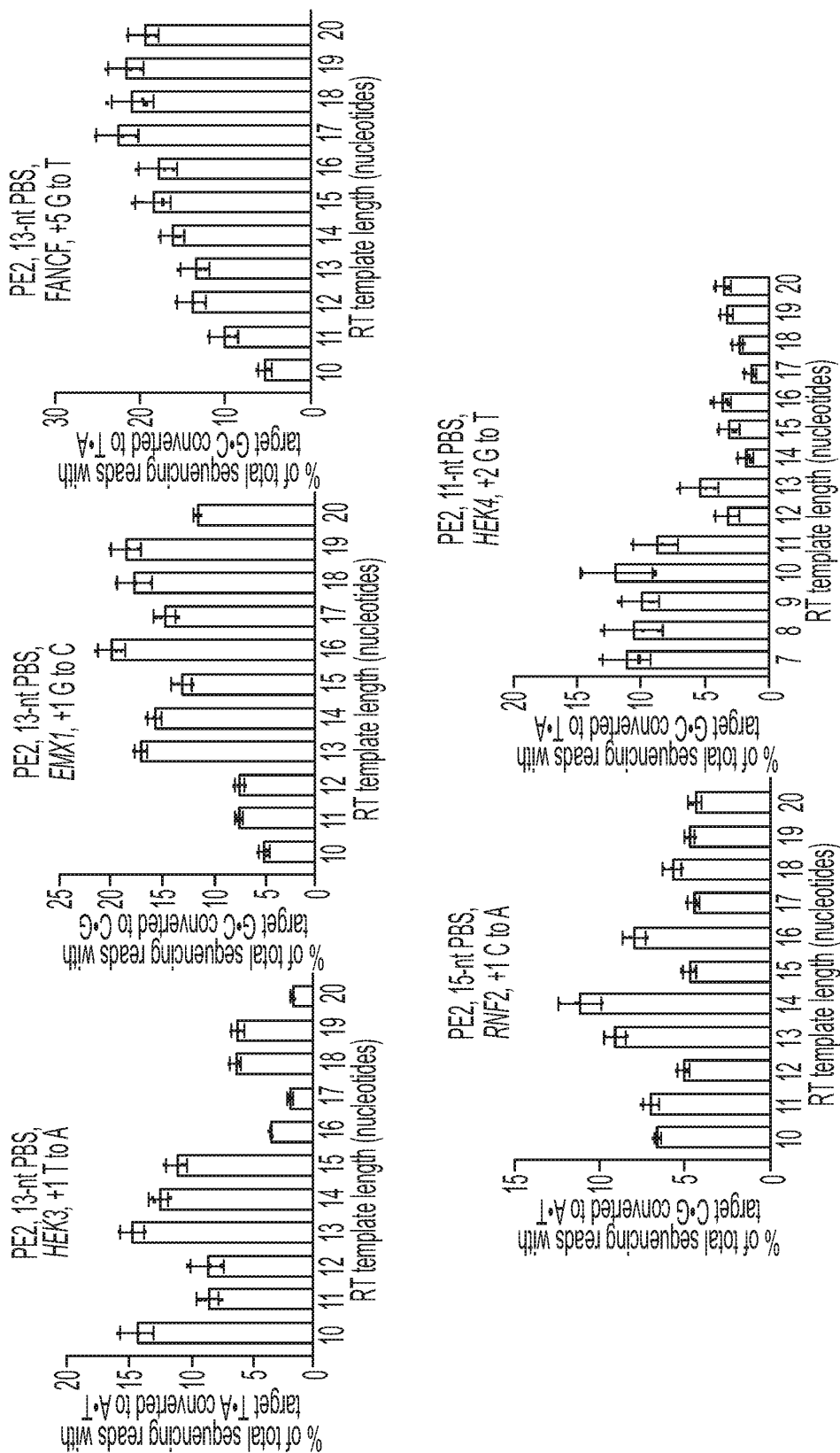


FIG. 39D

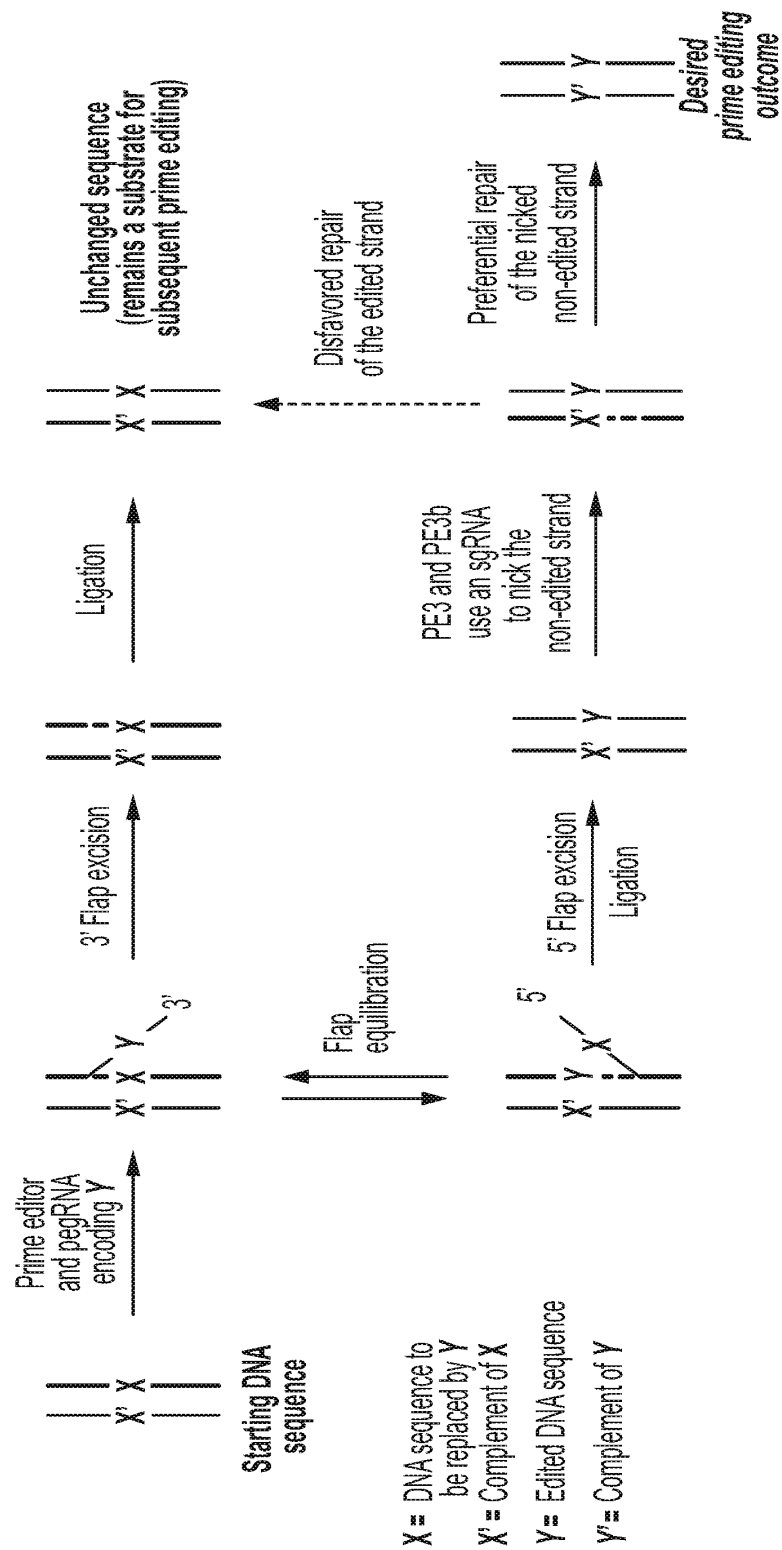


FIG. 40A

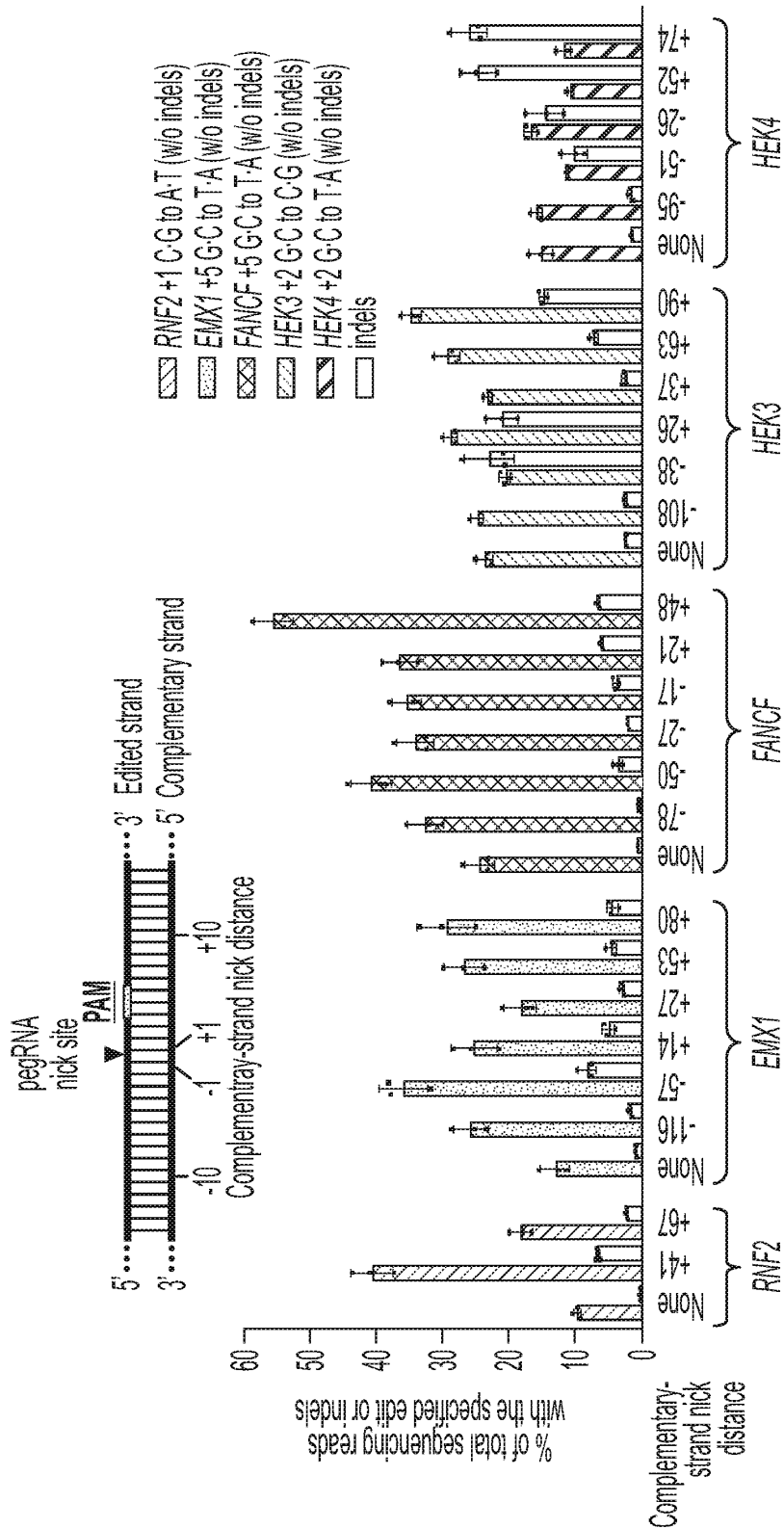


FIG. 40B

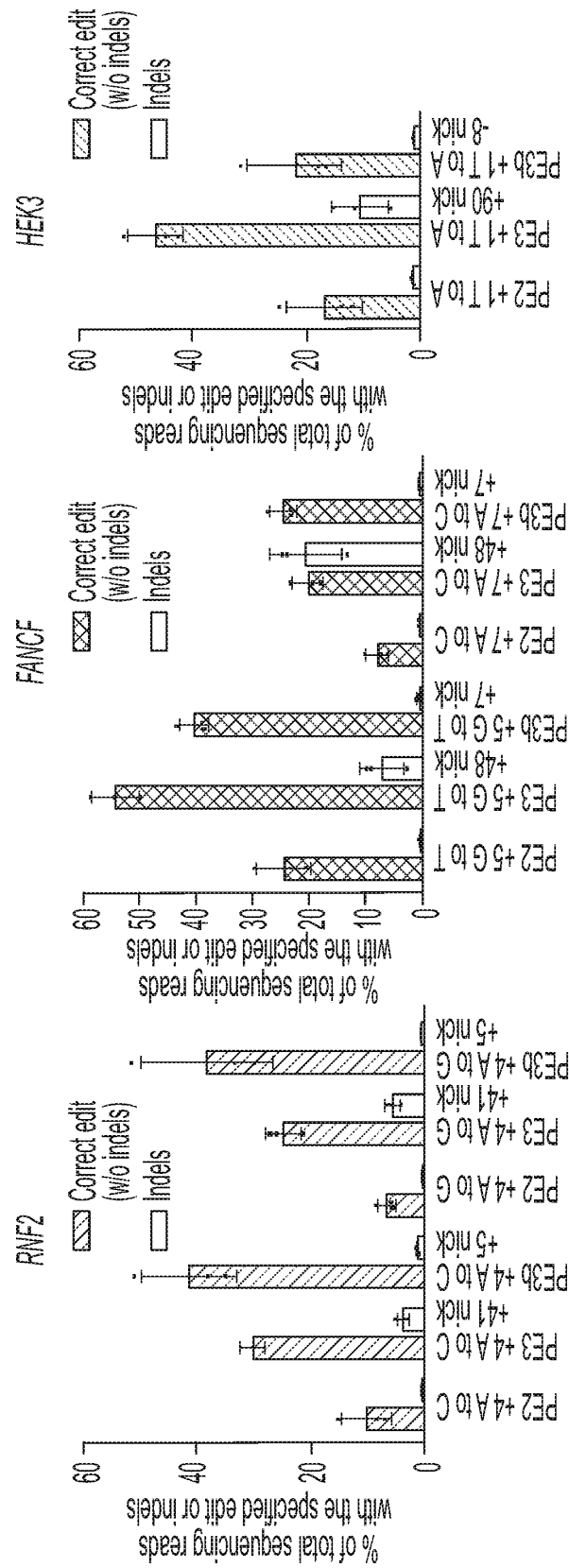


FIG. 40C

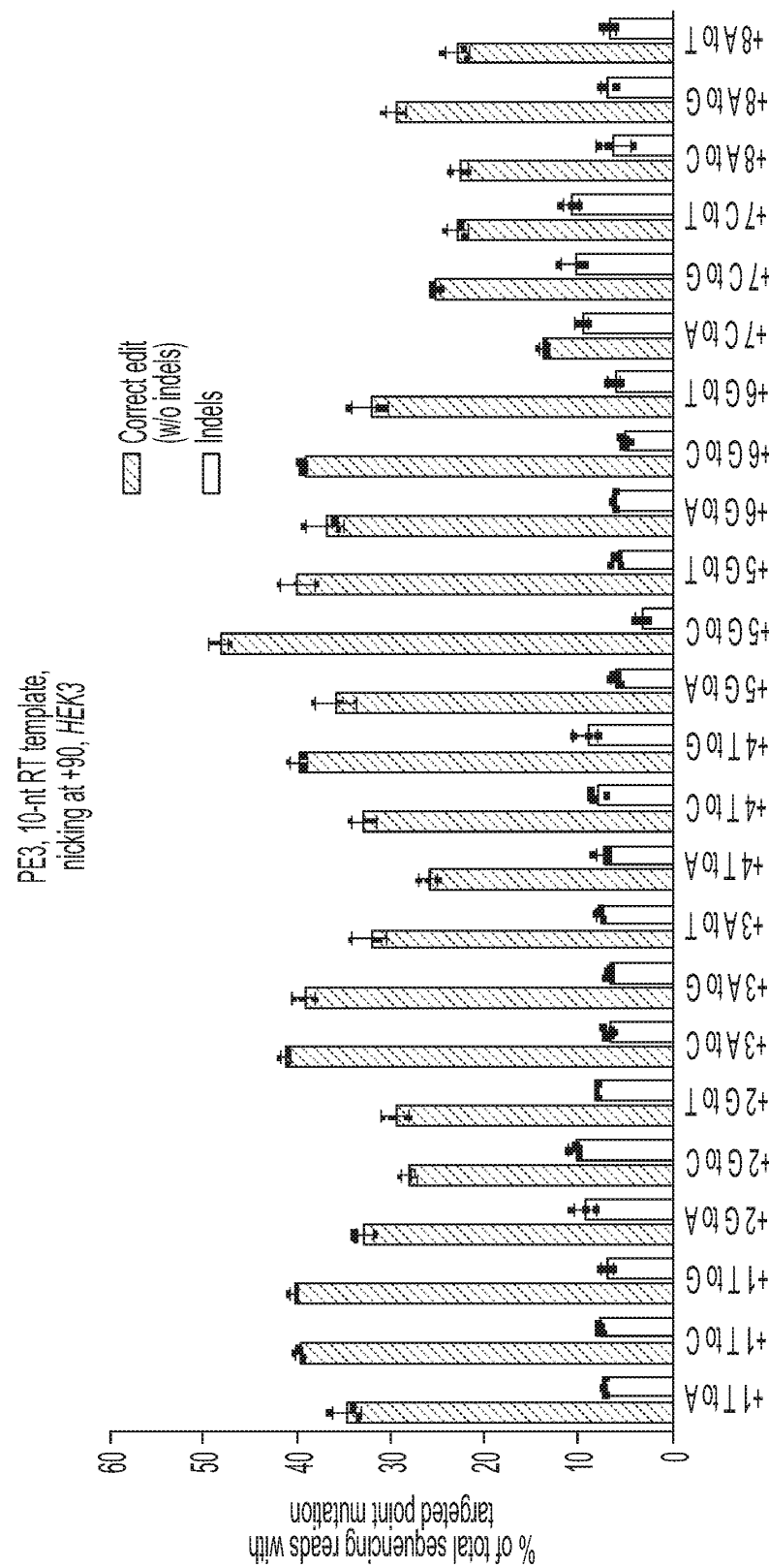


FIG. 41A

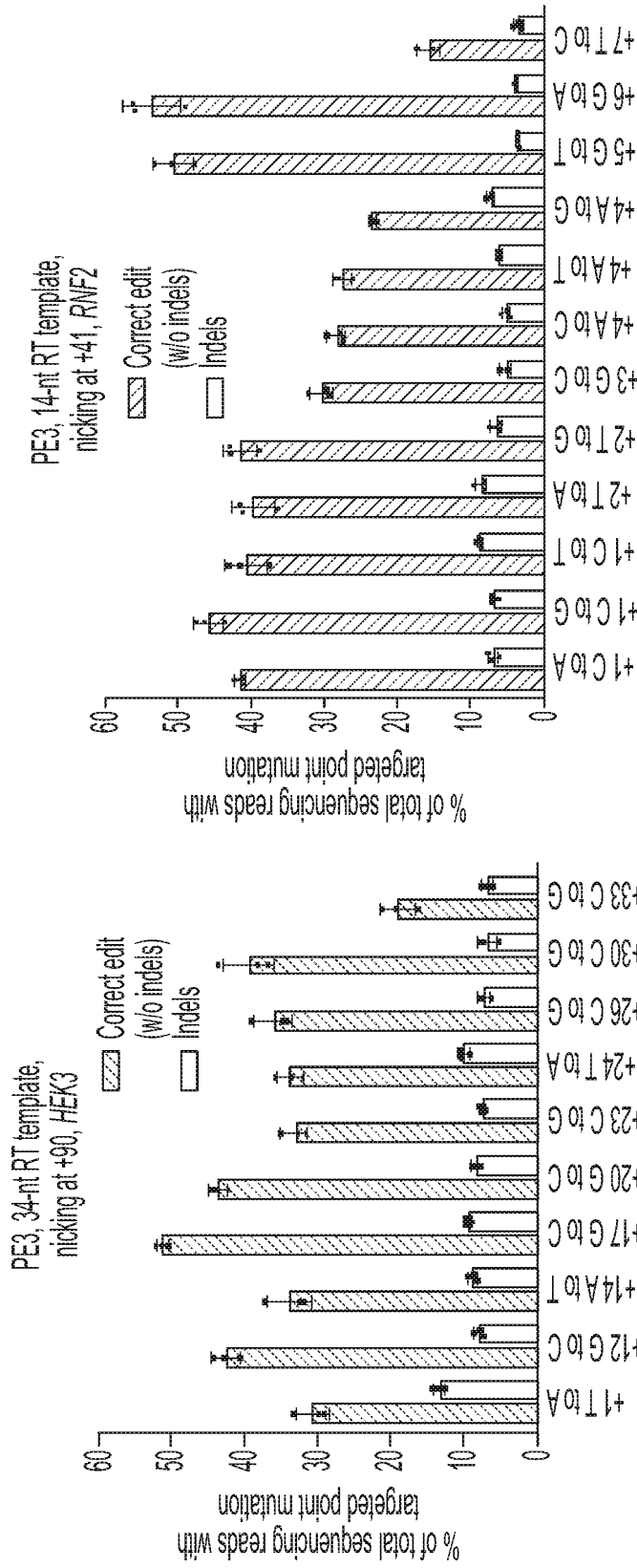


FIG. 41C

FIG. 41B

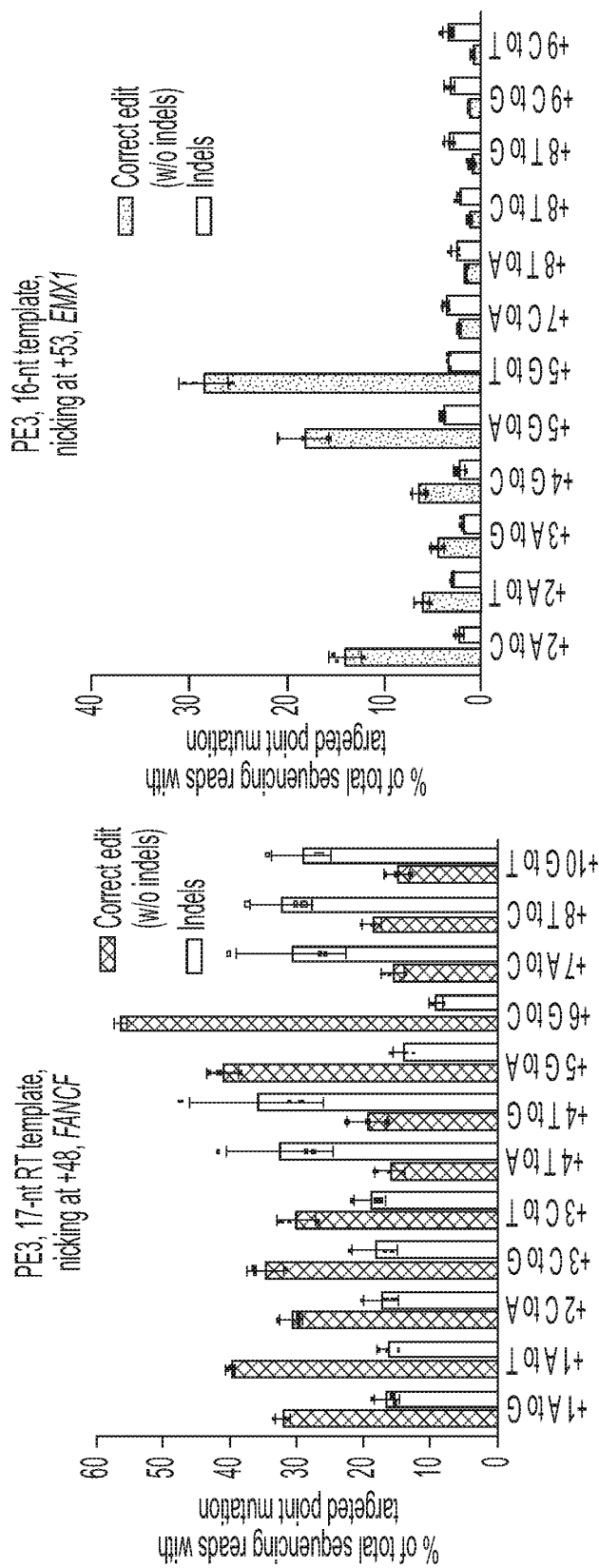


FIG. 41E

FIG. 41D

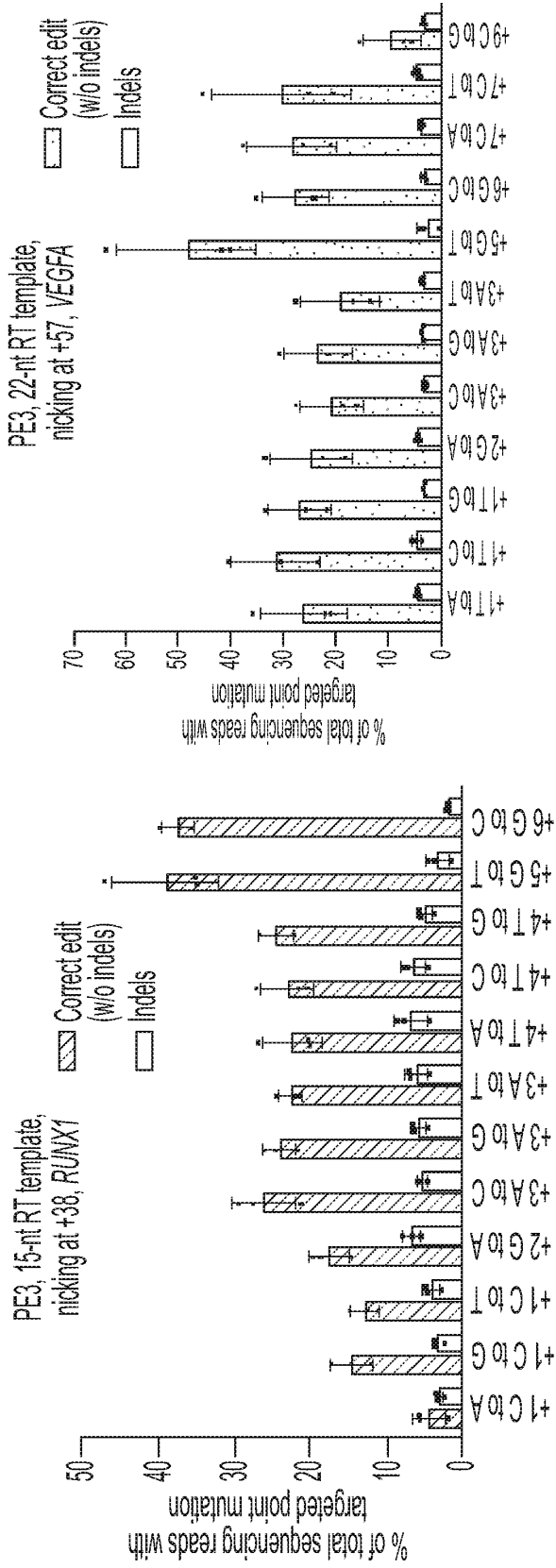


FIG. 41G

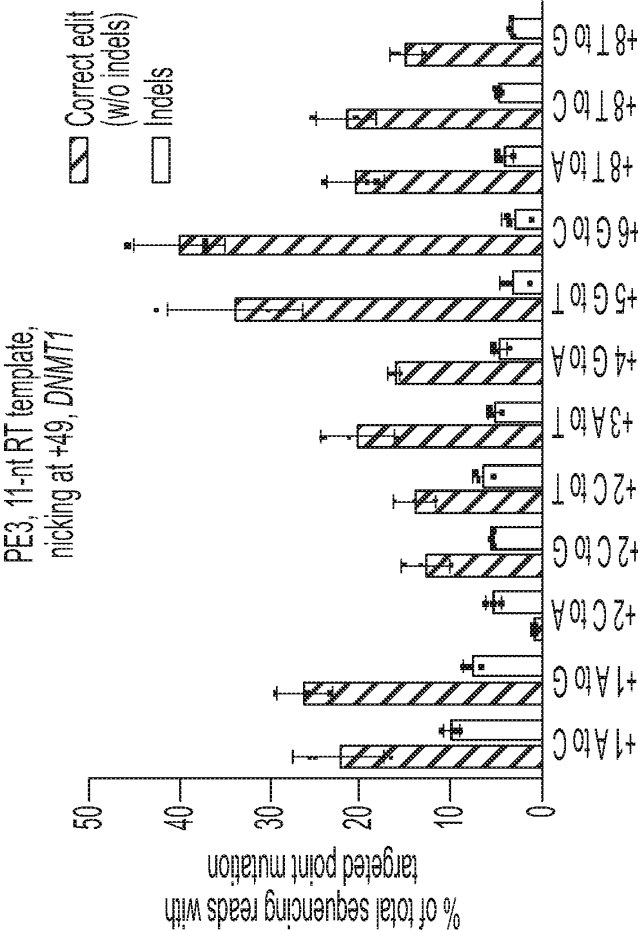


FIG. 41H

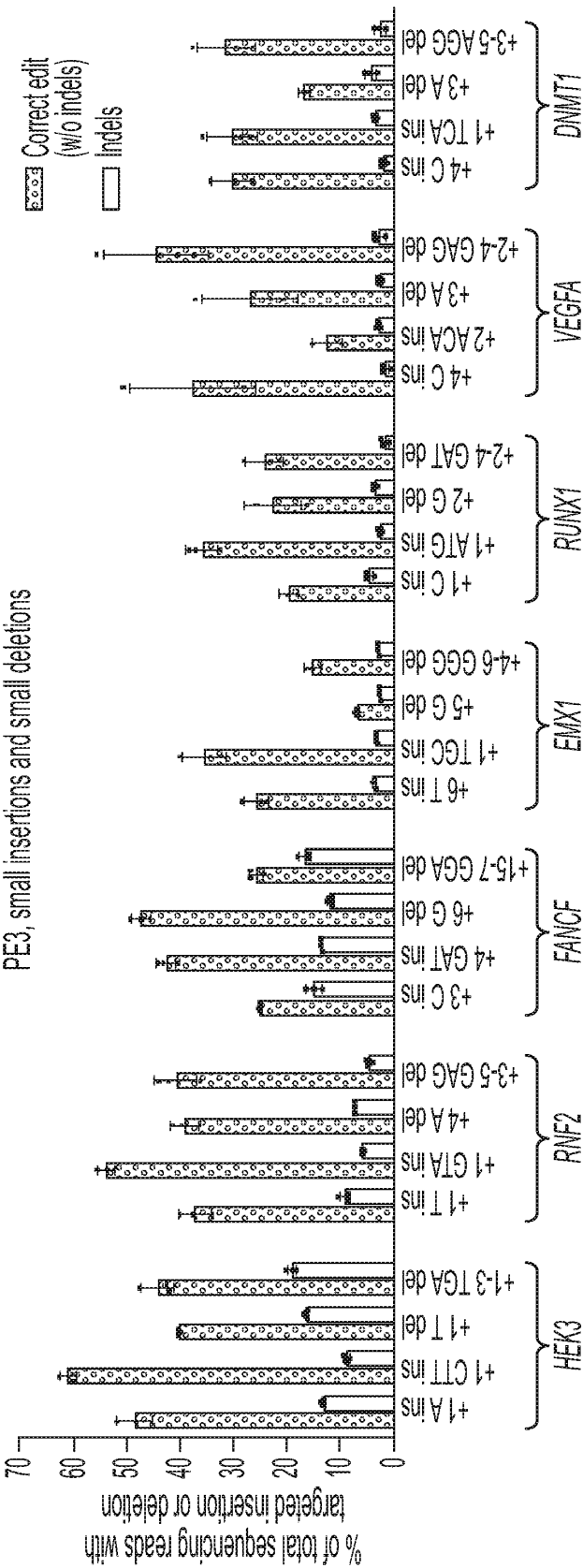


FIG. 41I

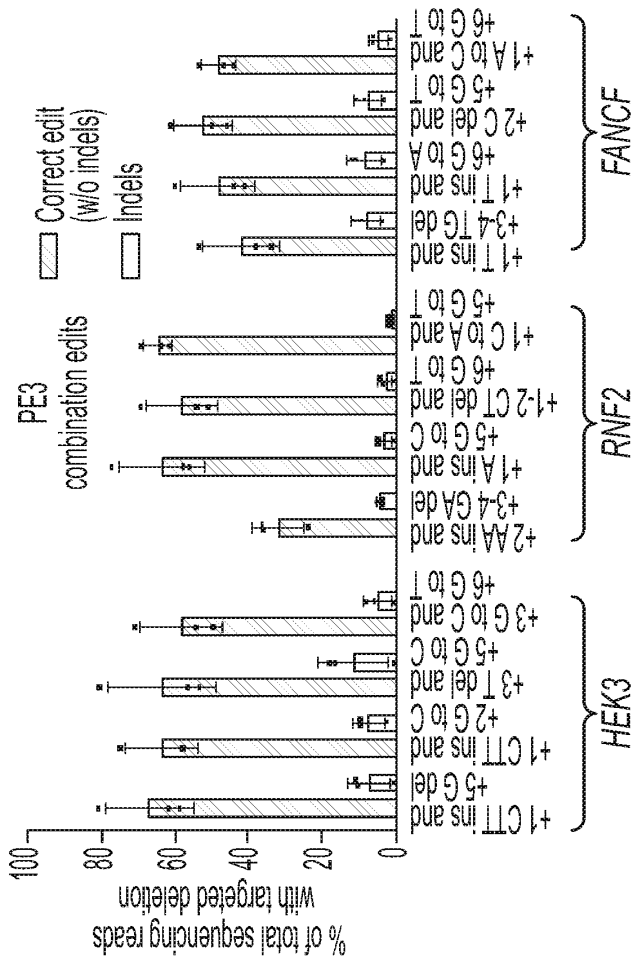


FIG. 41K

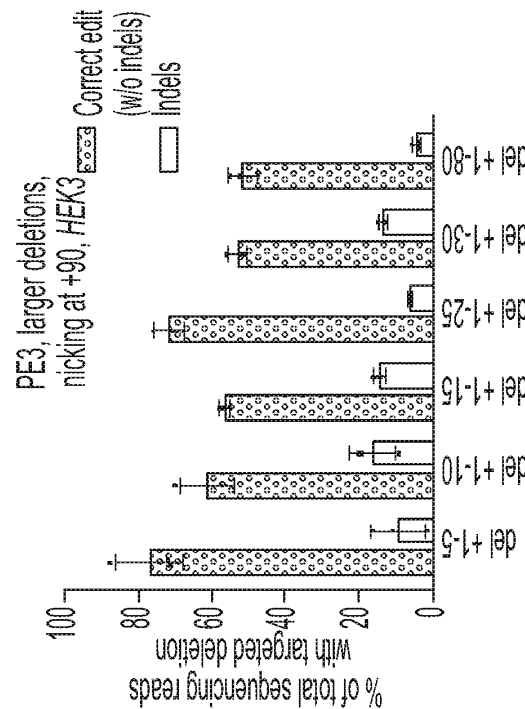


FIG. 41J

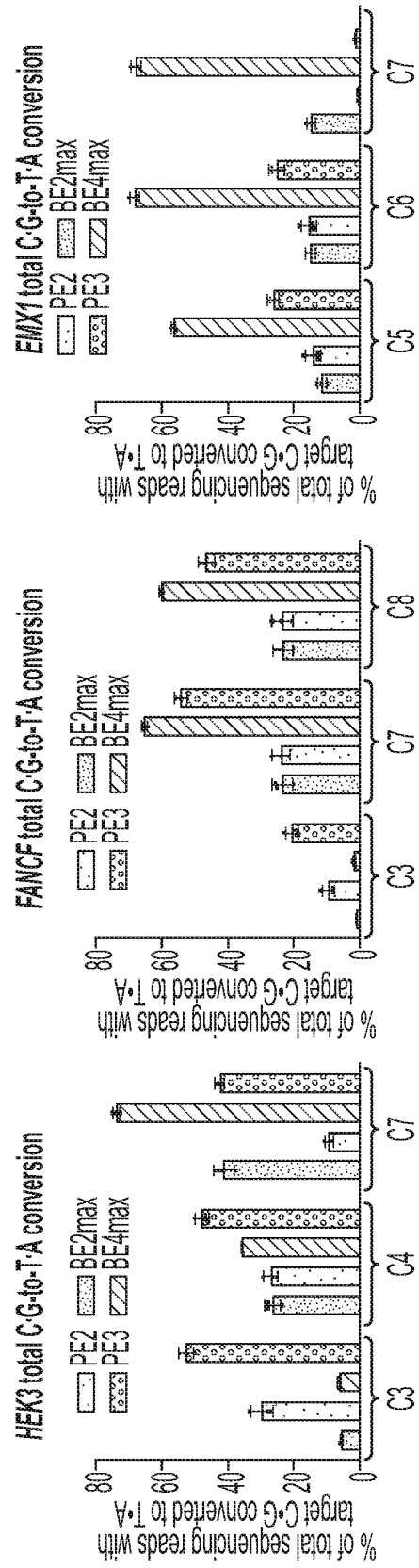


FIG. 42A

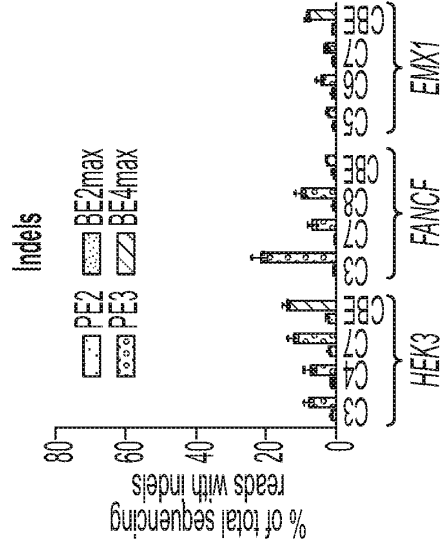


FIG. 42B

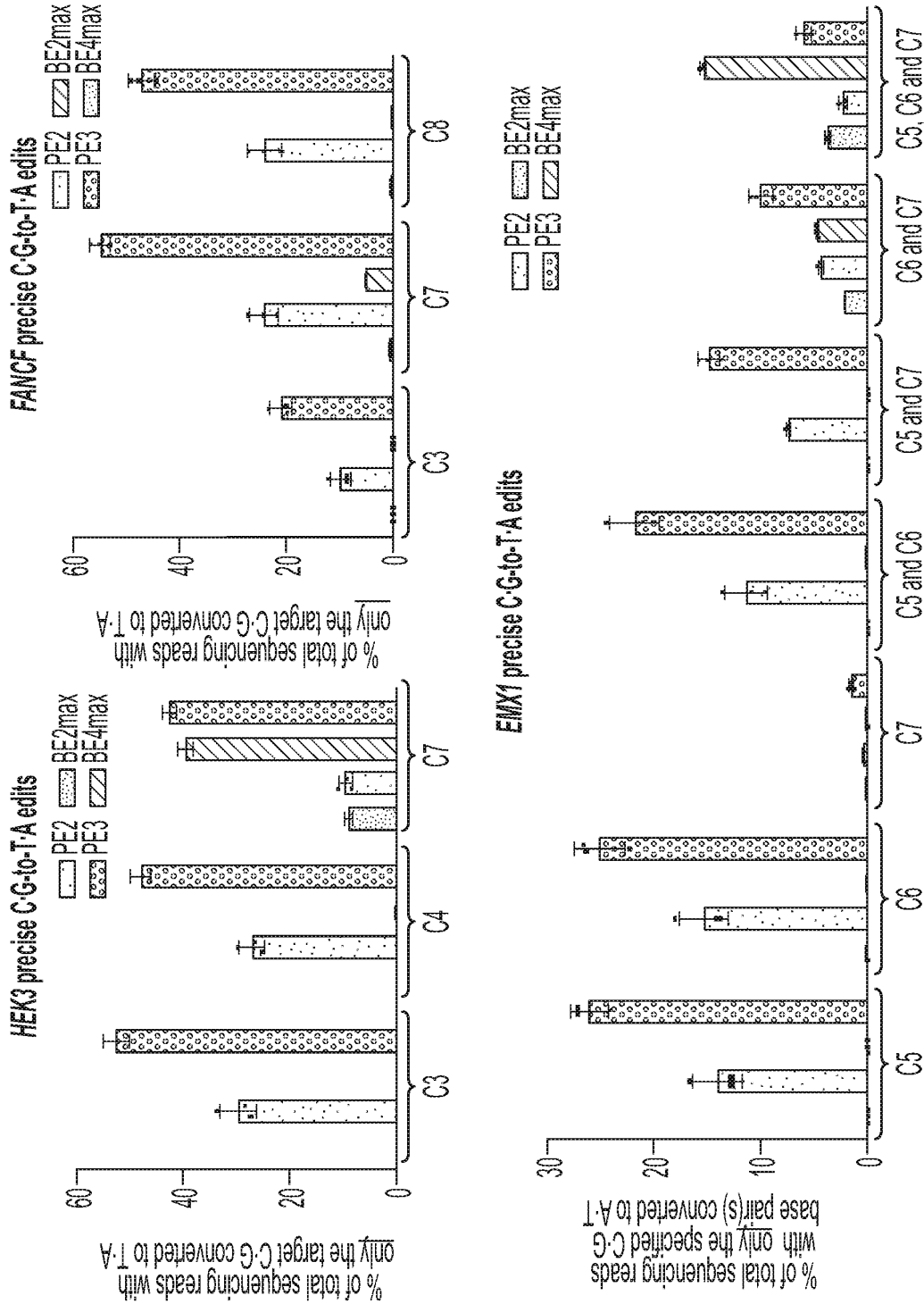


FIG. 42C

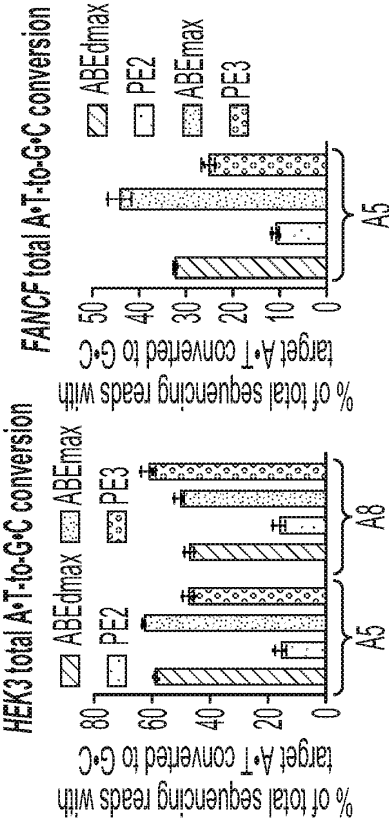


FIG. 42D

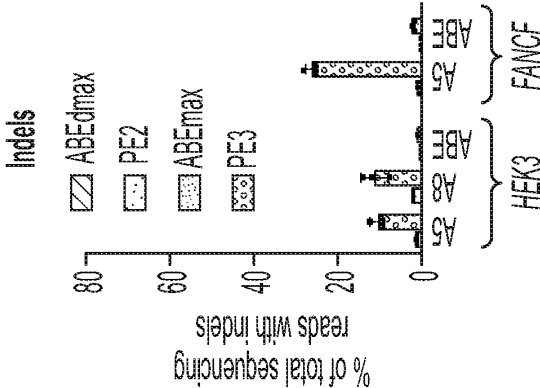


FIG. 42E

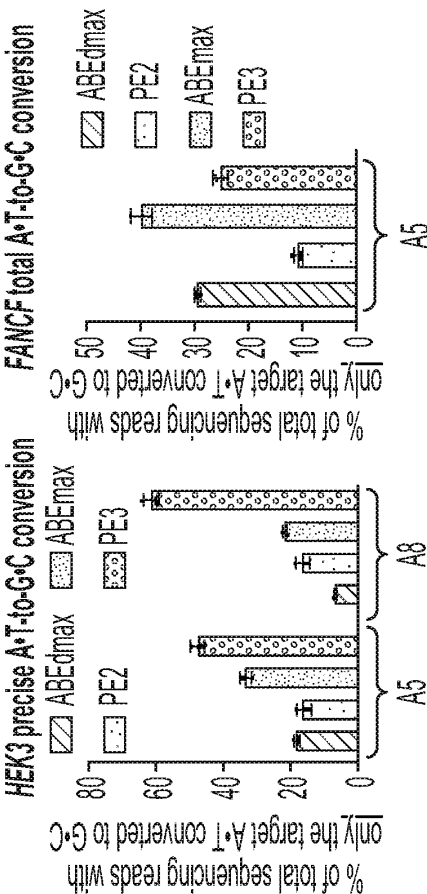


FIG. 42F

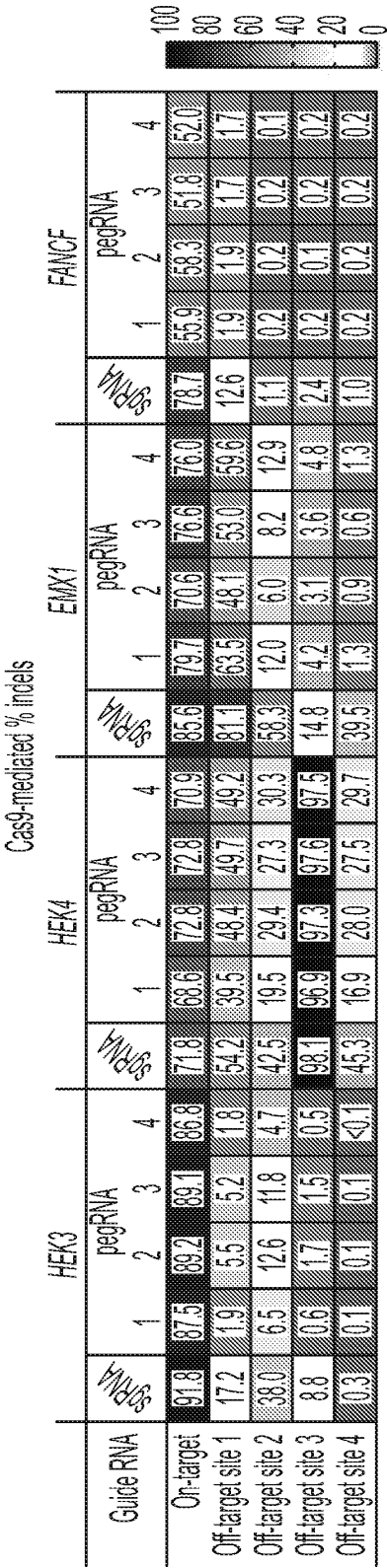


FIG. 42G

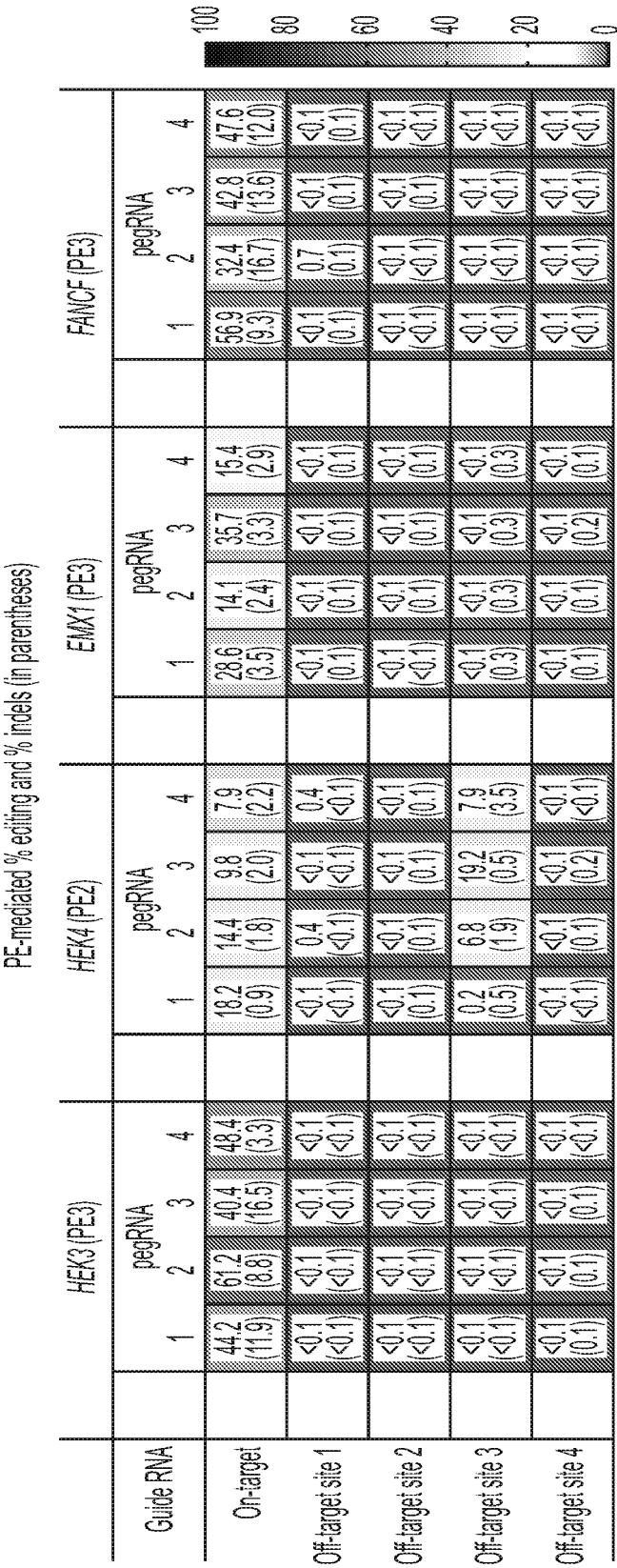


FIG. 42H

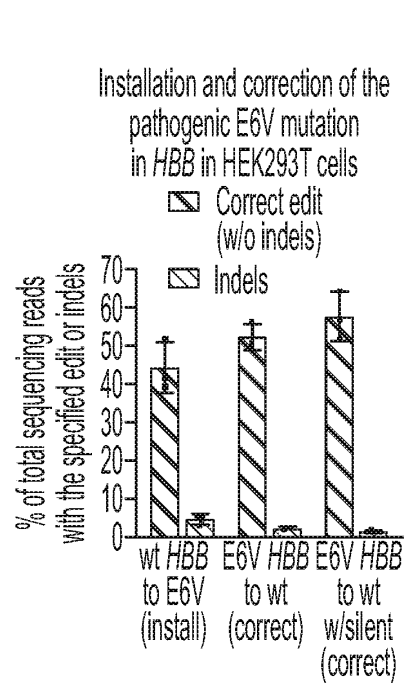


FIG. 43A

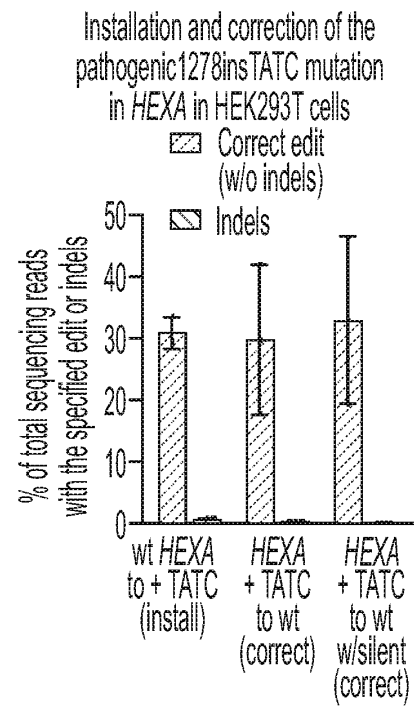


FIG. 43B

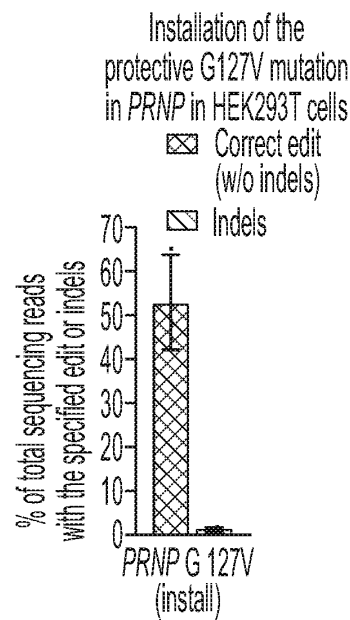


FIG. 43C

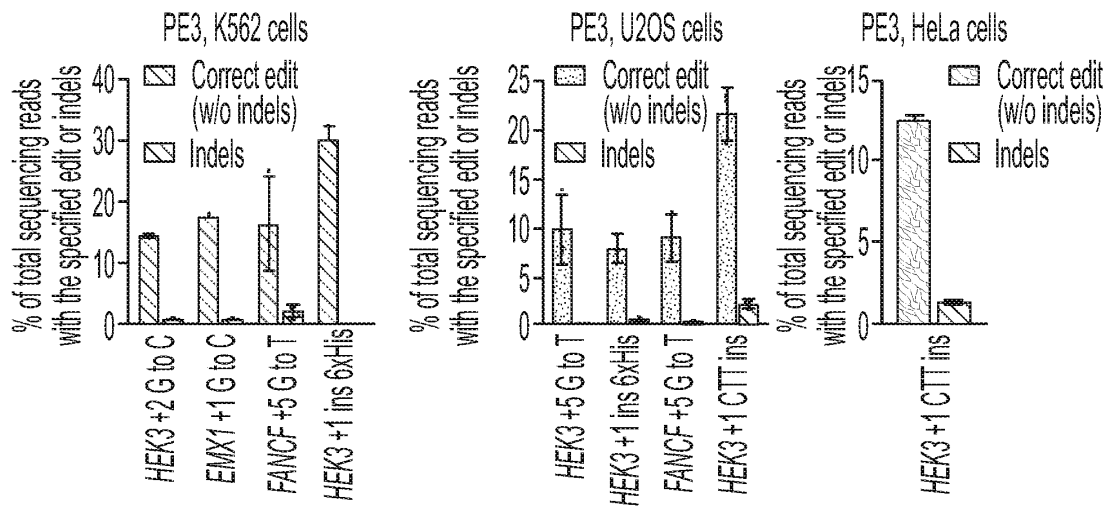


FIG. 43D

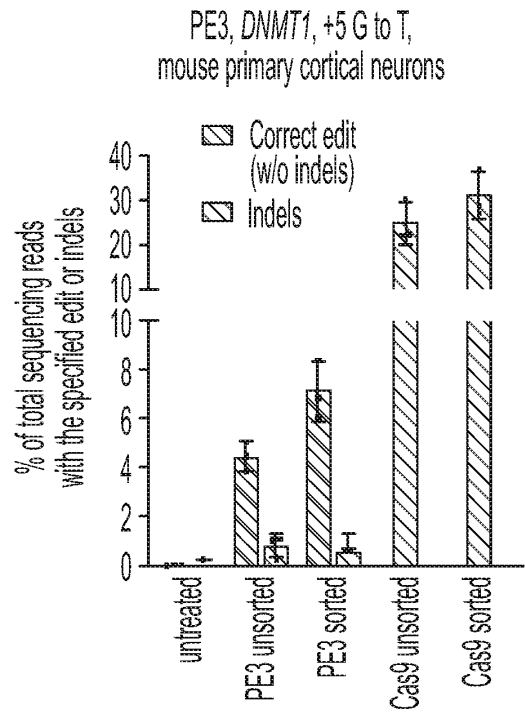


FIG. 43E

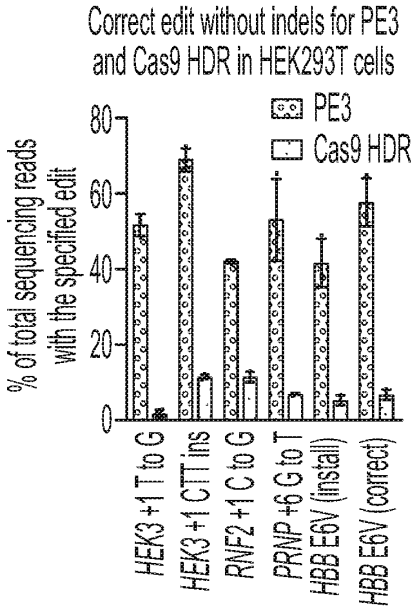


FIG. 43F

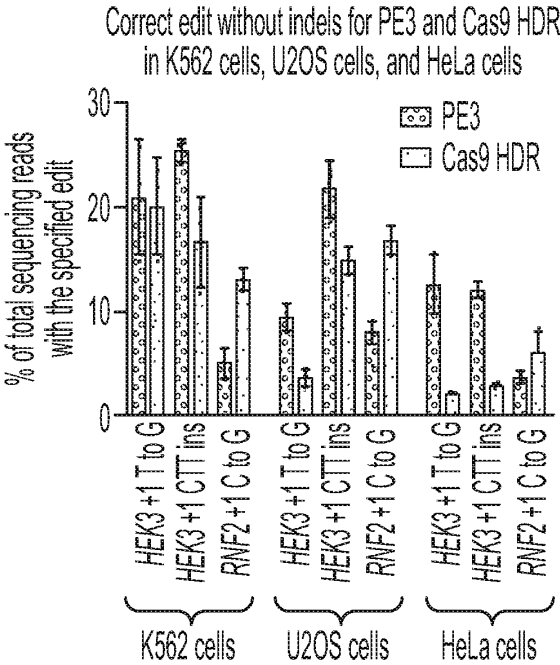


FIG. 43G

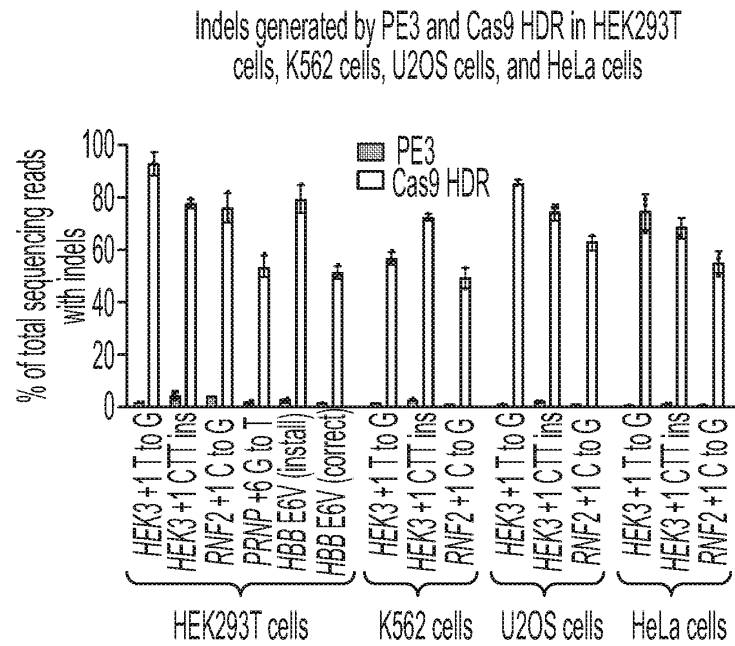


FIG. 43H

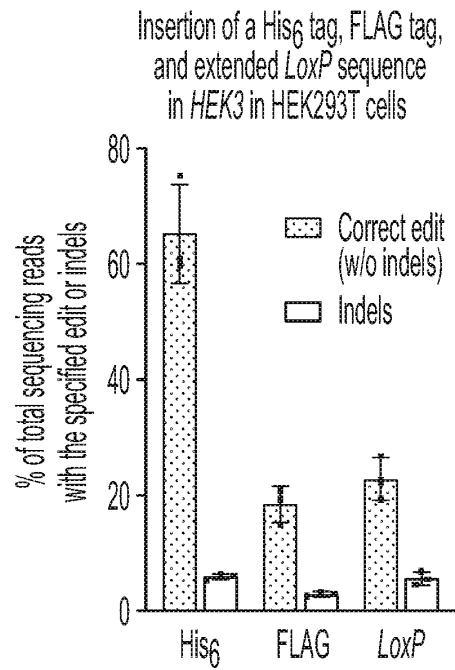


FIG. 43I

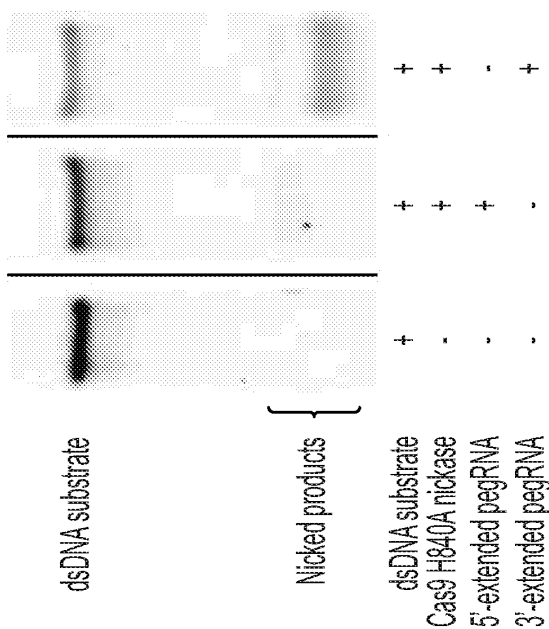


FIG. 44B

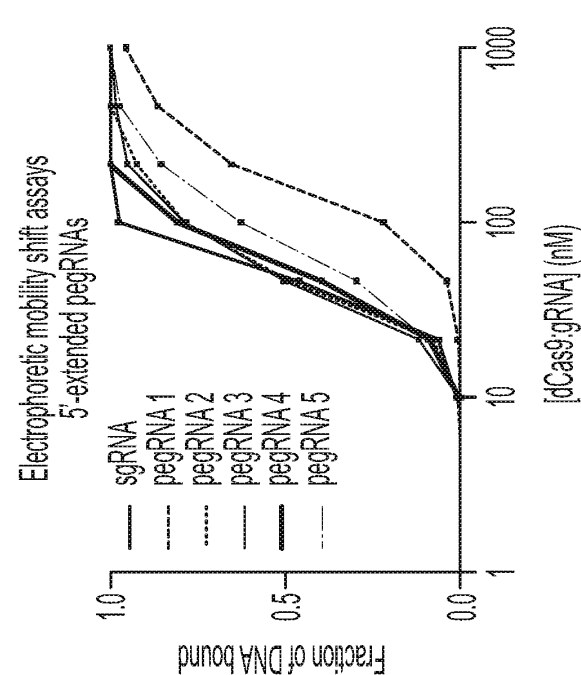


FIG. 44A

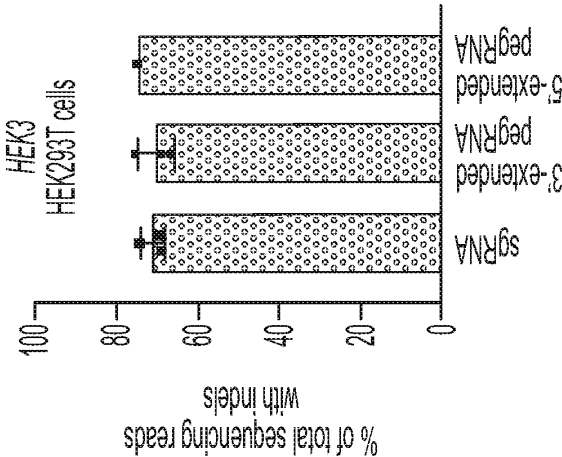


FIG. 44C

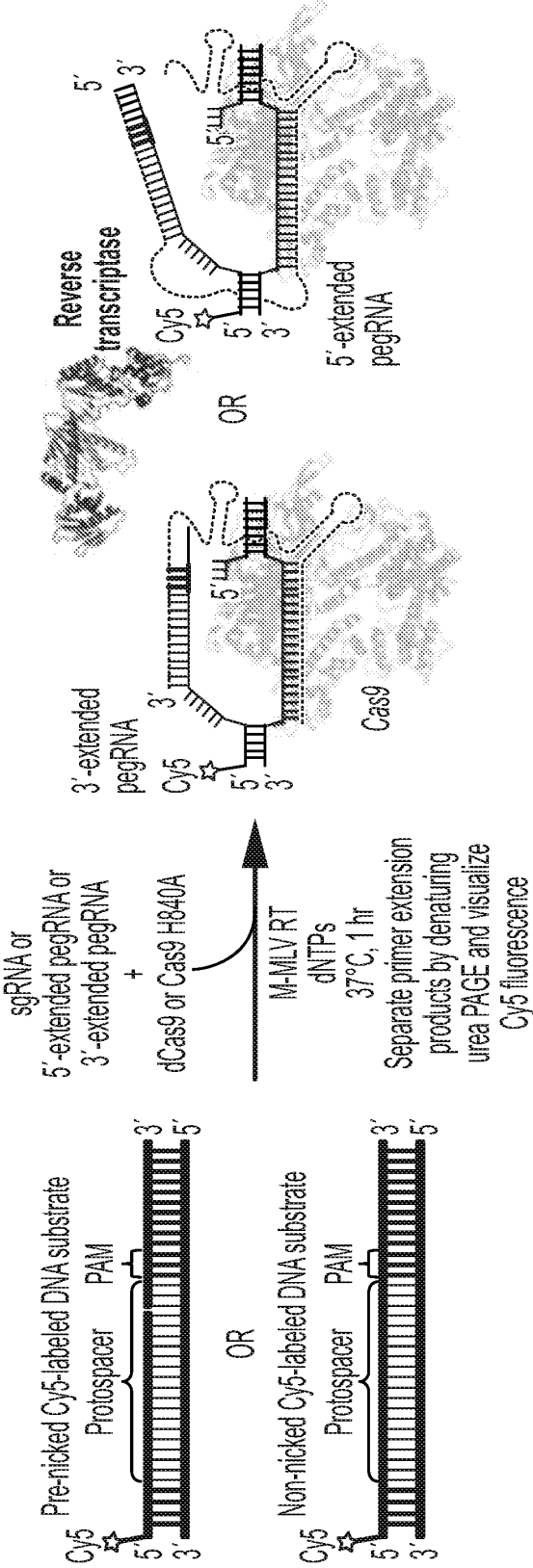


FIG. 44D

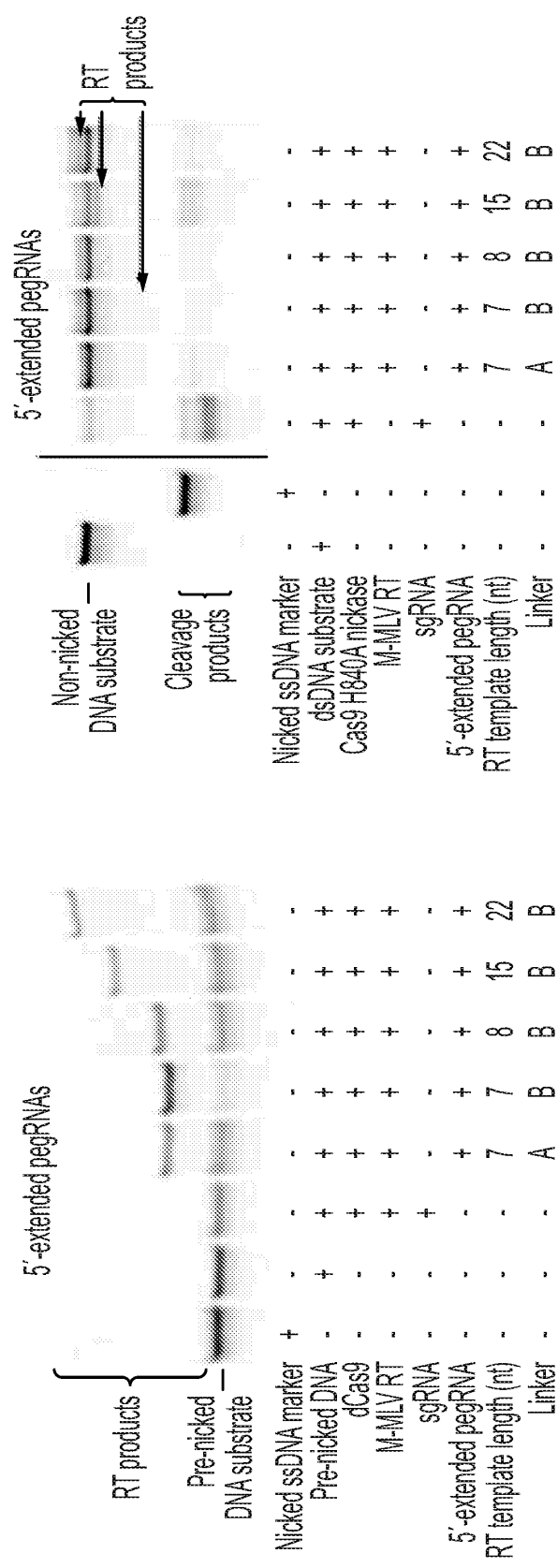


FIG. 44E

FIG. 44F

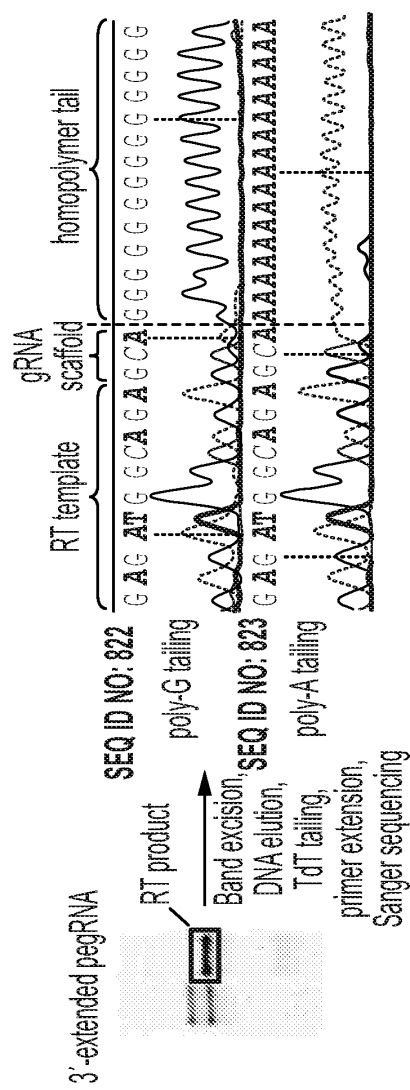


FIG. 44G

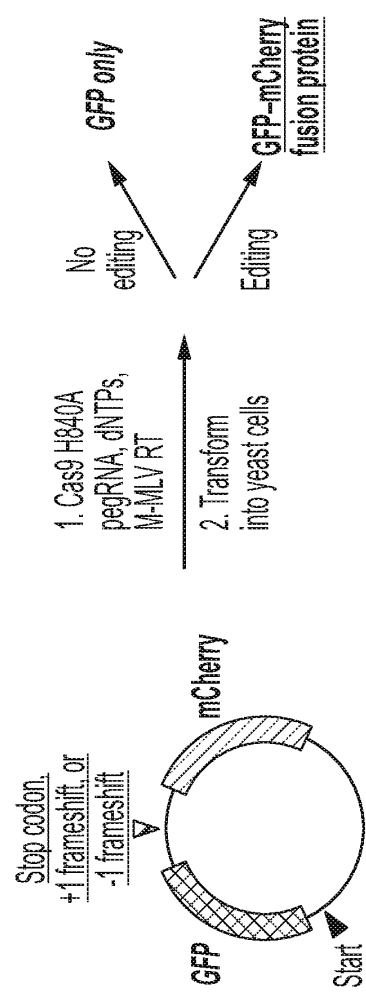


FIG. 45A

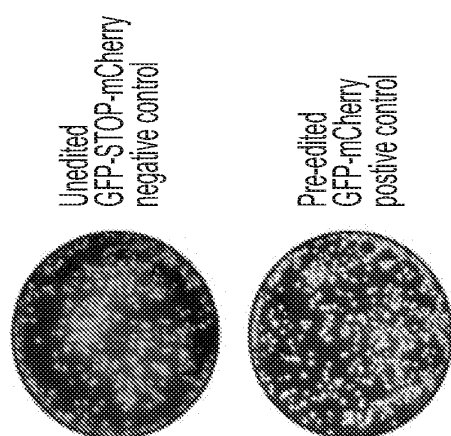


FIG. 45B

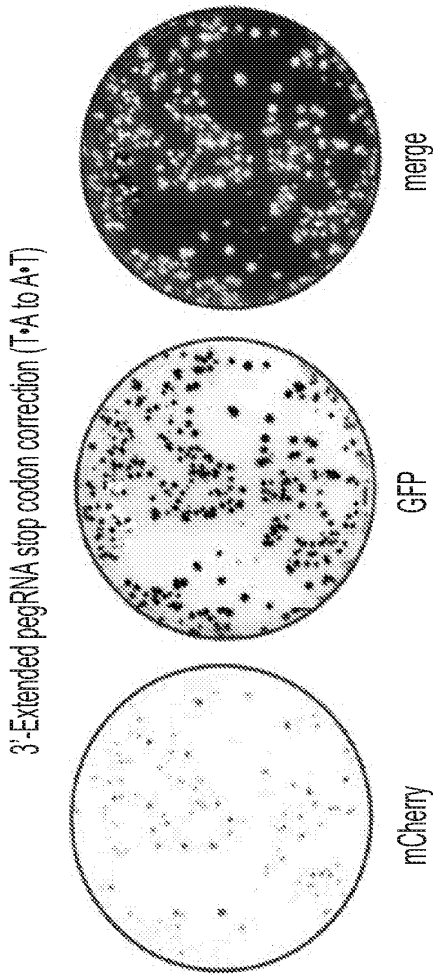


FIG. 45C

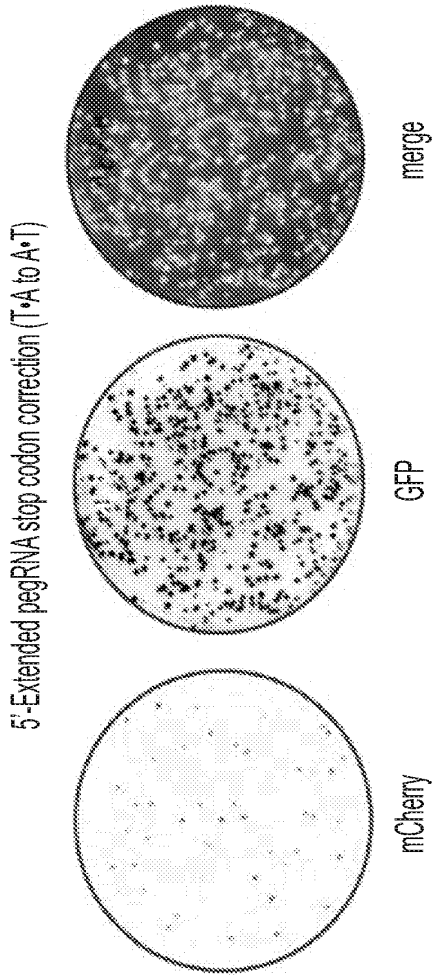


FIG. 45D

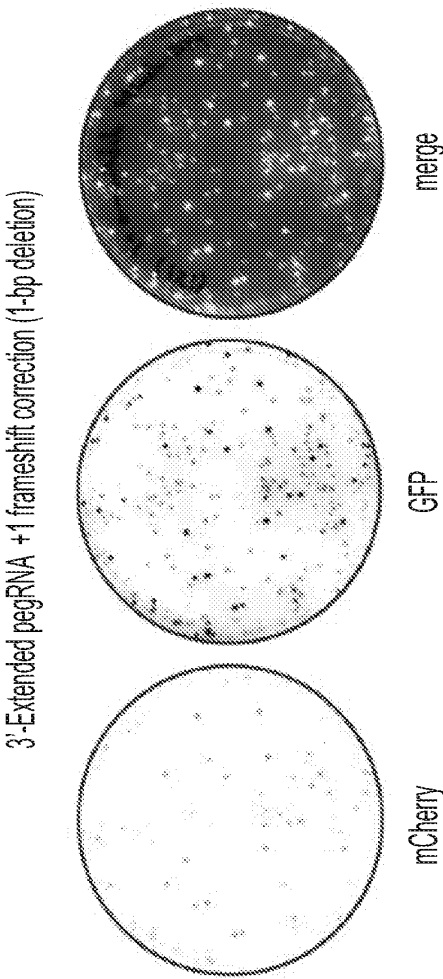


FIG. 45E

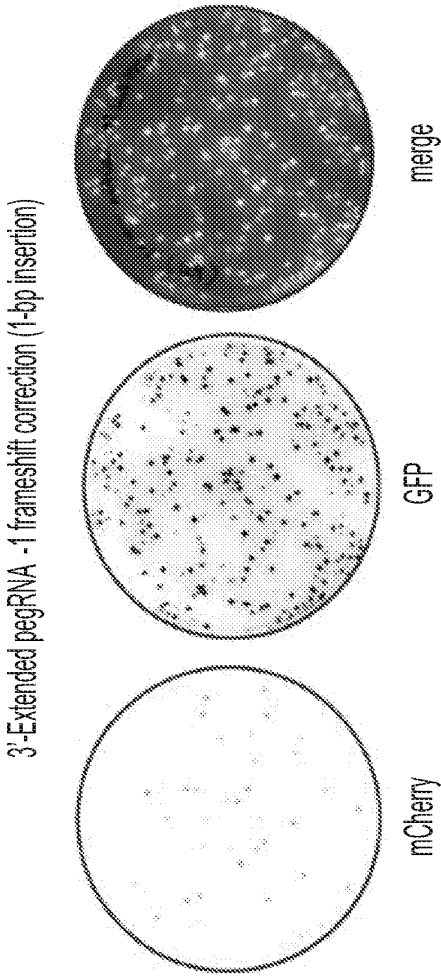


FIG. 45F

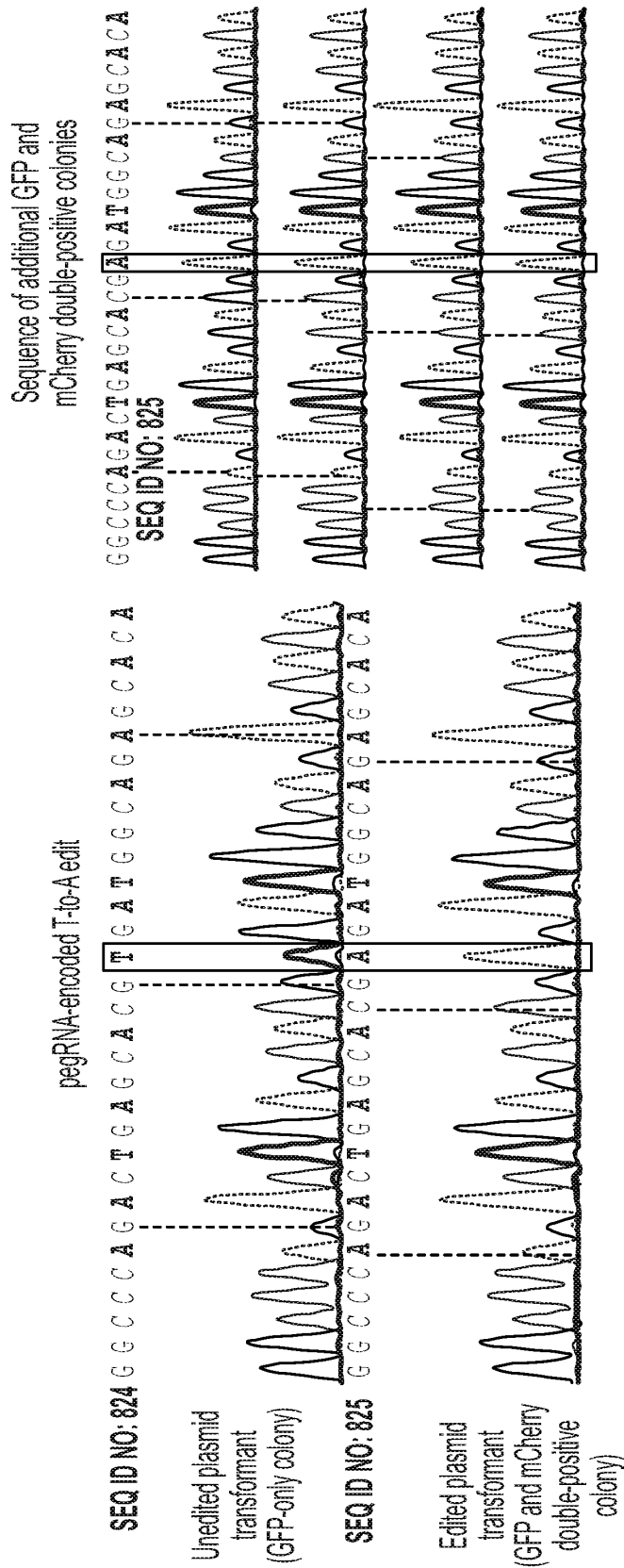


FIG. 45G

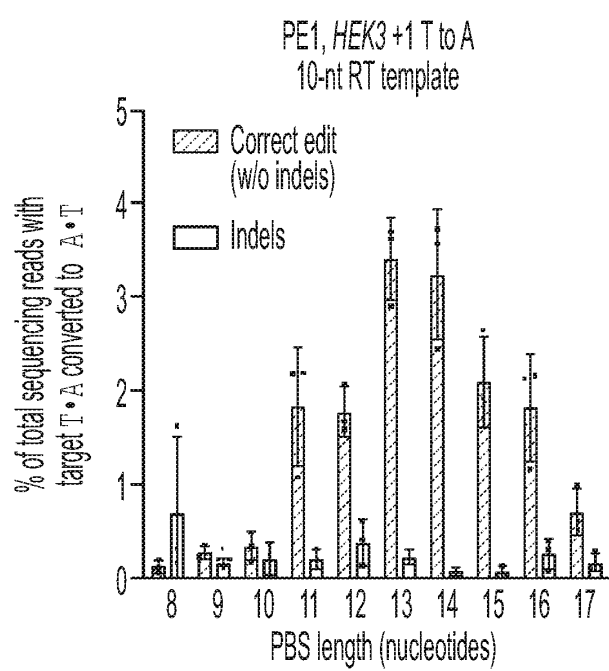


FIG. 46A

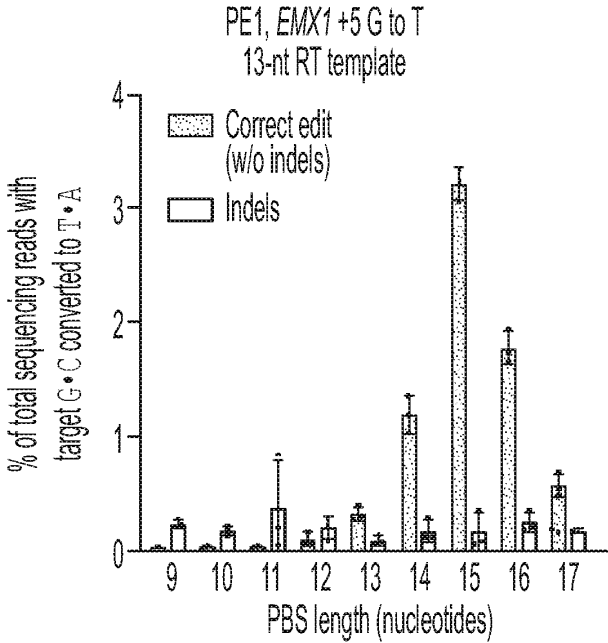


FIG. 46B

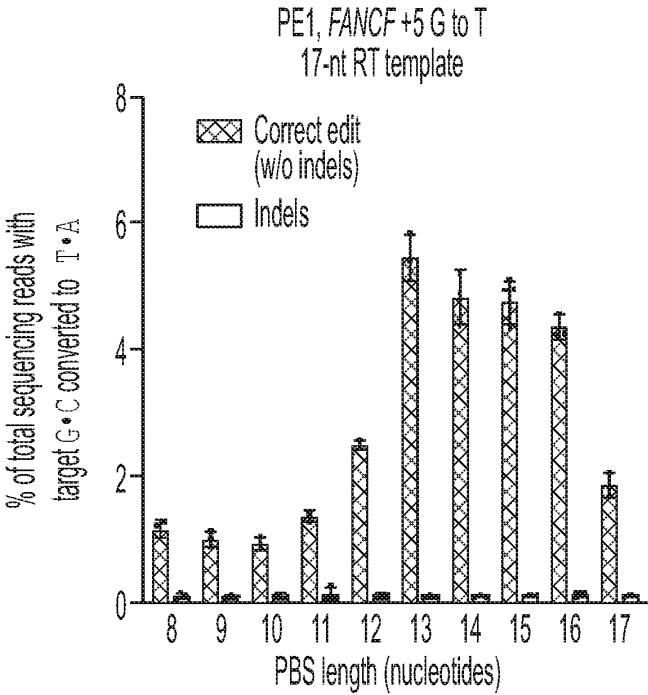


FIG. 46C

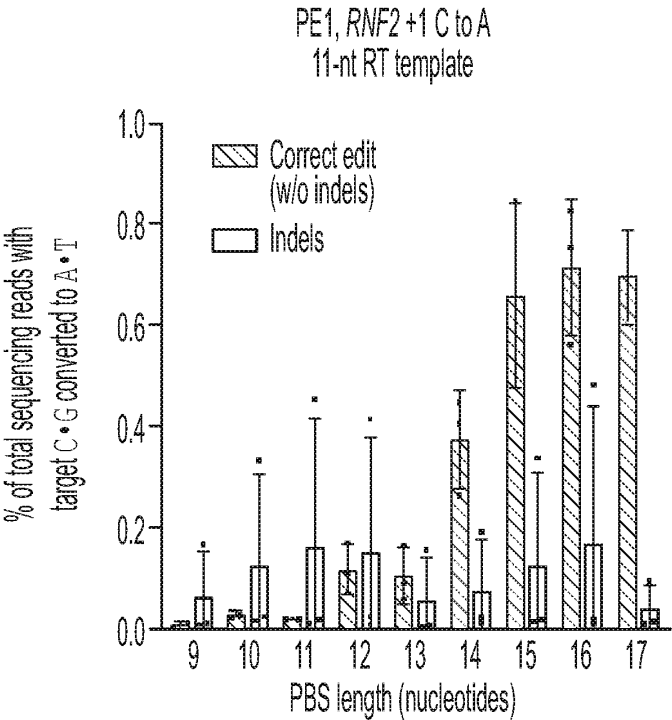


FIG. 46D

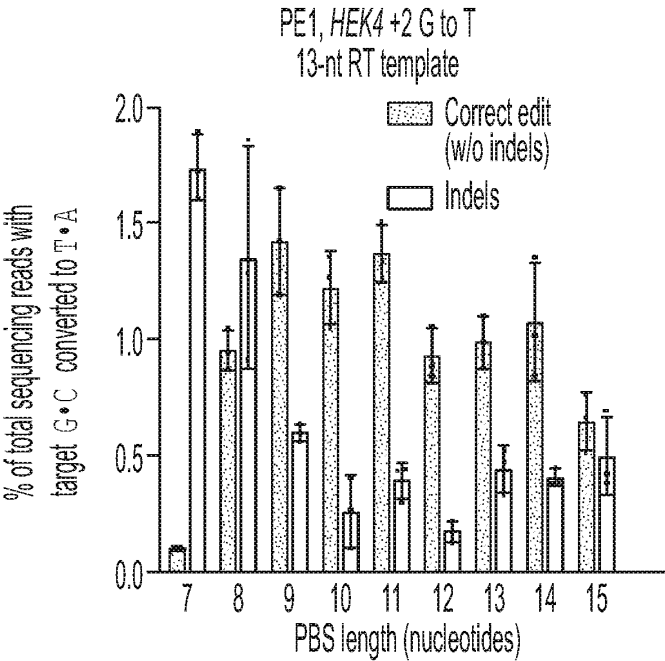


FIG. 46E

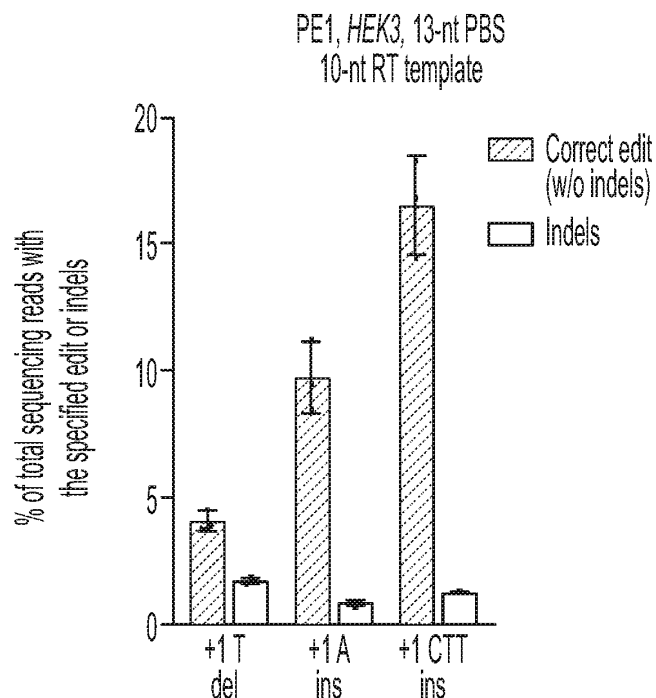


FIG. 46F

|           |                                                                                                               |
|-----------|---------------------------------------------------------------------------------------------------------------|
| PE1       | Cas9 H840A-M-MLV RT (wt)                                                                                      |
| PE1-M1    | Cas9 H840A-M-MLV RT (D200N)                                                                                   |
| PE1-M2    | Cas9 H840A-M-MLV RT (D200N+L603W)                                                                             |
| PE1-M3    | Cas9 H840A-M-MLV RT (D200N+L603W+T330P)                                                                       |
| PE1-M6    | Cas9 H840A-M-MLV RT (D200N+L603W+T330P+D524G+E562Q+D583N)                                                     |
| PE1-M15   | Cas9 H840A-M-MLV RT (P51L+S67R+E67K+T197A+H204R+E302K+F309N+W1331F+T330P+L345G+N454K+D524G+D583N+H594Q+D653N) |
| PE1-M3inv | M-MLV RT (D200N+L603W+T330P)-Cas9 H840A                                                                       |
| PE2       | Cas9 H840A-M-MLV RT (D200N+L603W+T330P+T306K+W313F)                                                           |

FIG. 47A

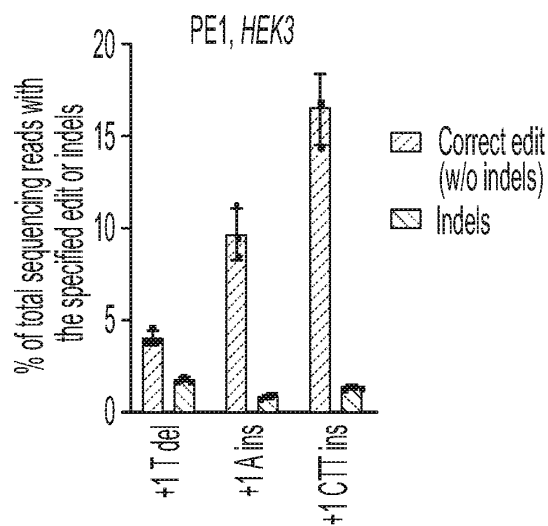


FIG. 47B

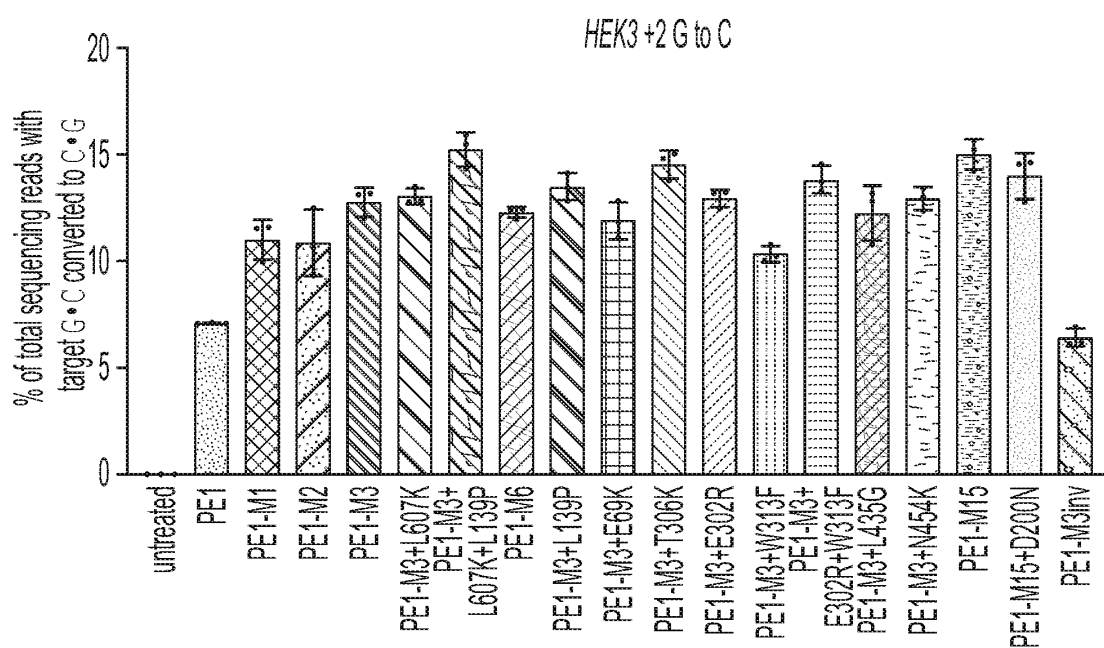


FIG. 47C

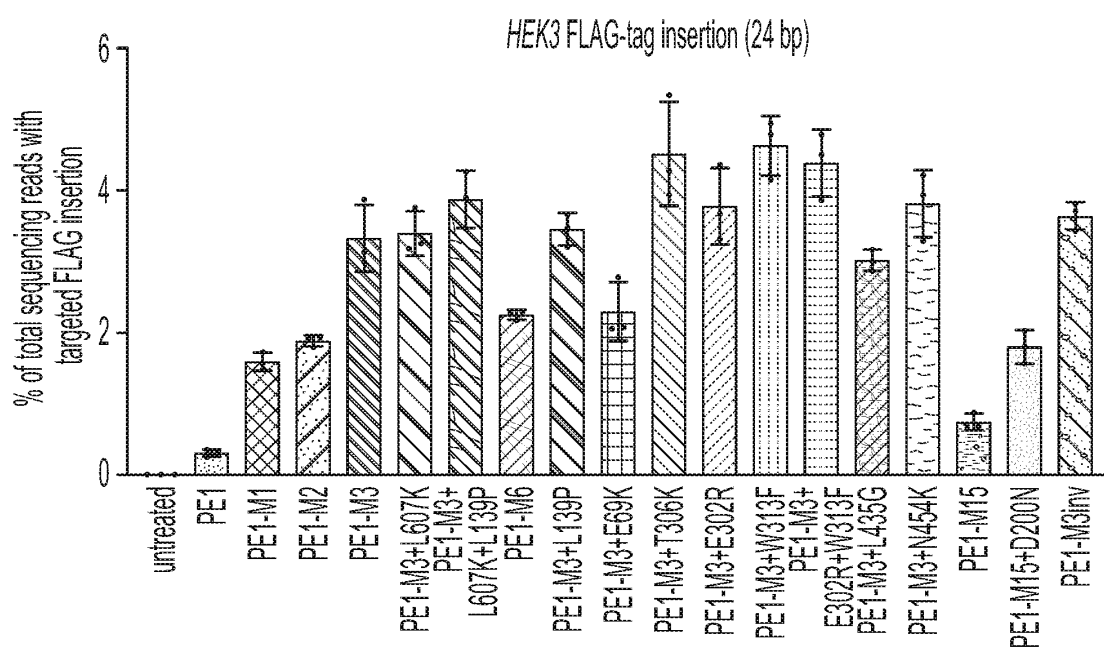


FIG. 47D

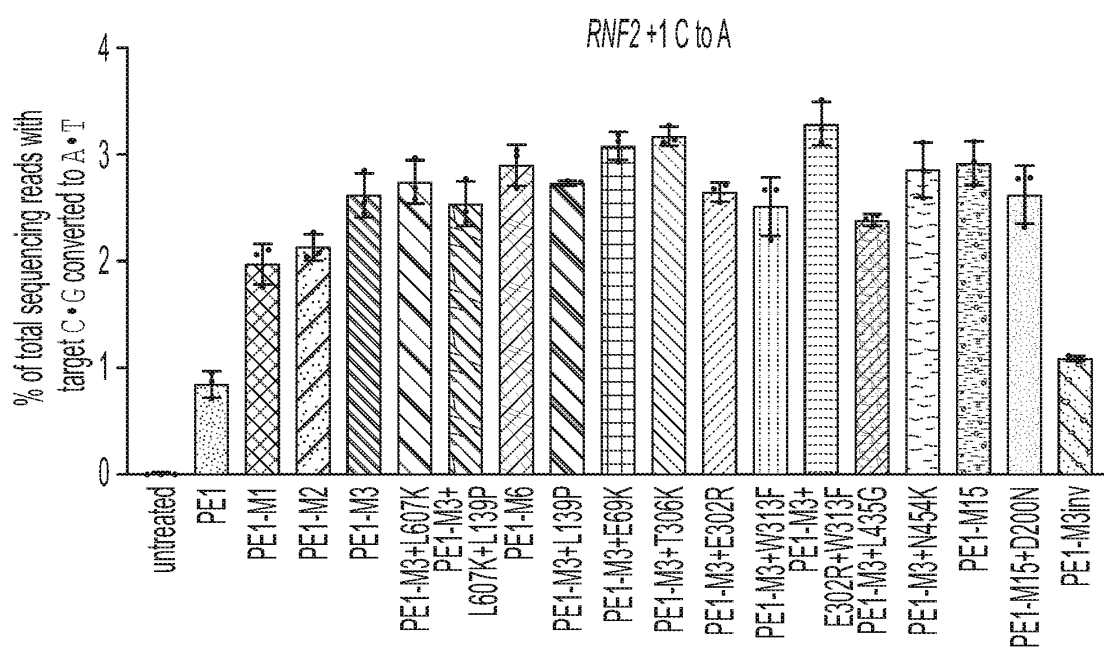


FIG. 47E

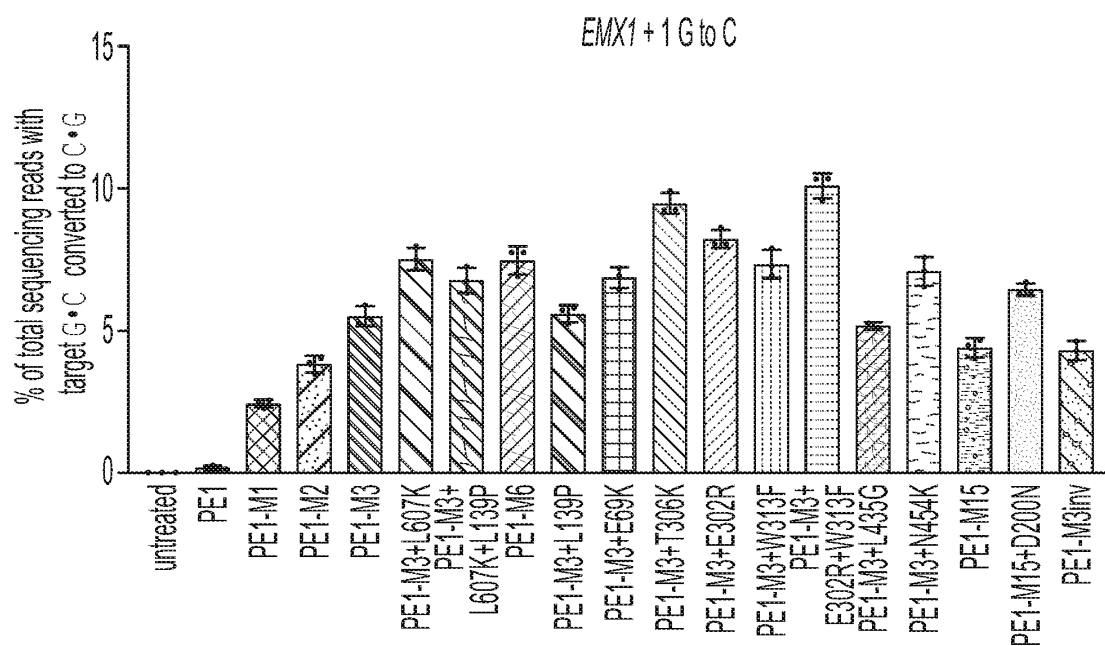


FIG. 47F

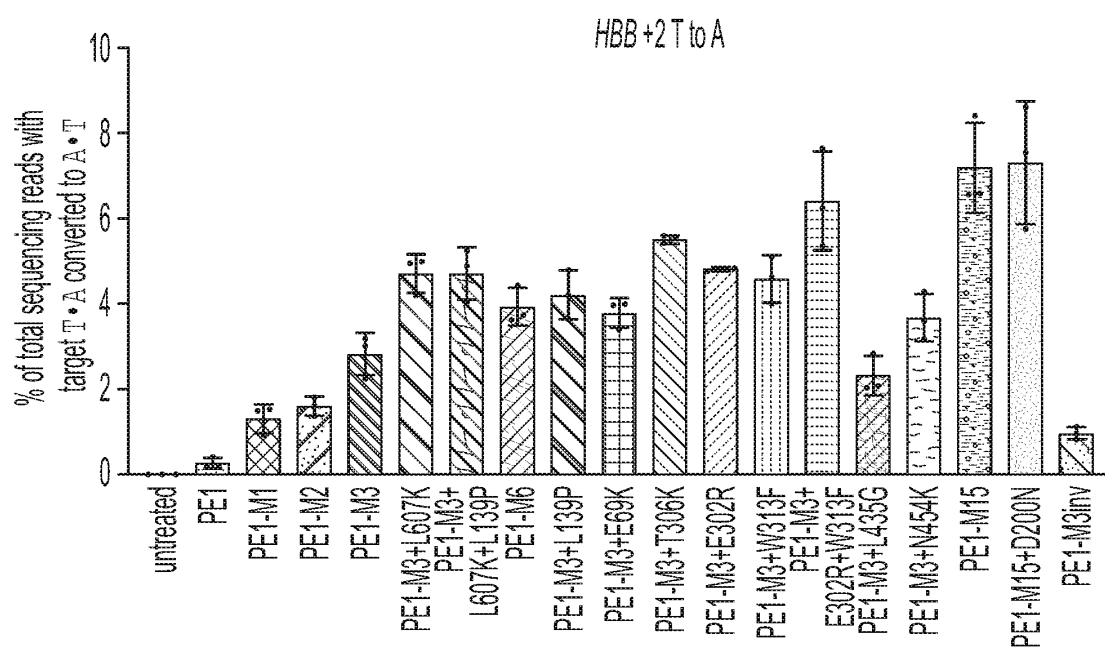


FIG. 47G

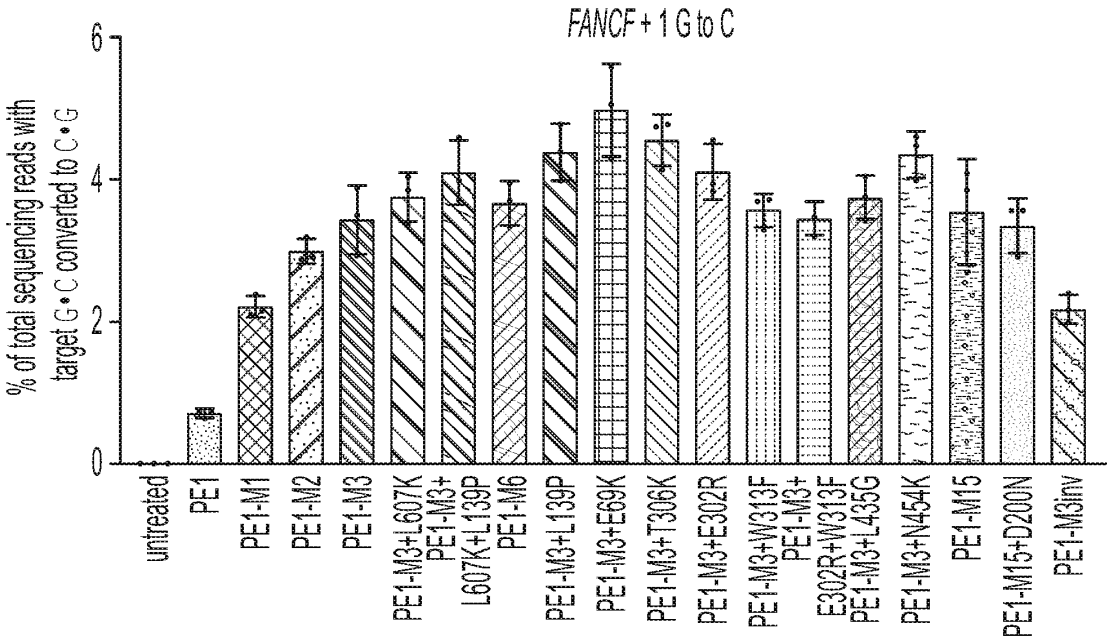


FIG. 47H

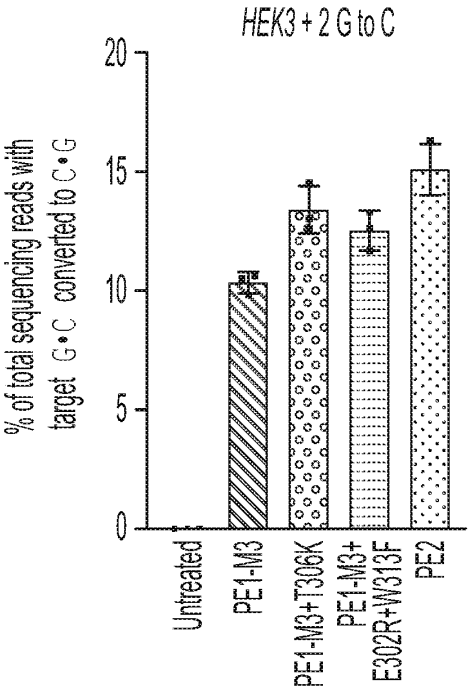


FIG. 47I

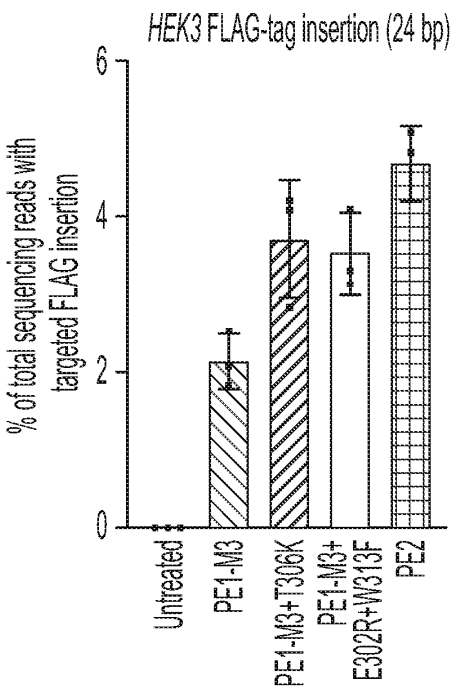


FIG. 47J

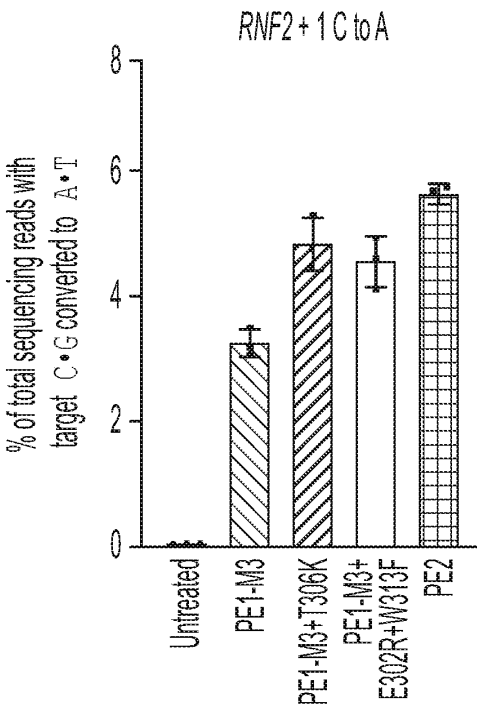


FIG. 47K

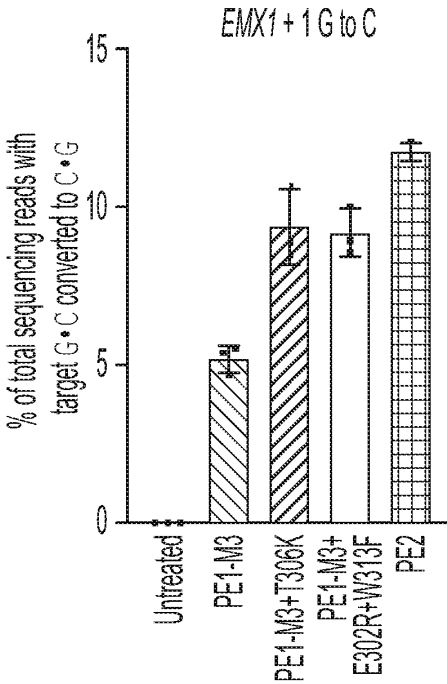


FIG. 47L

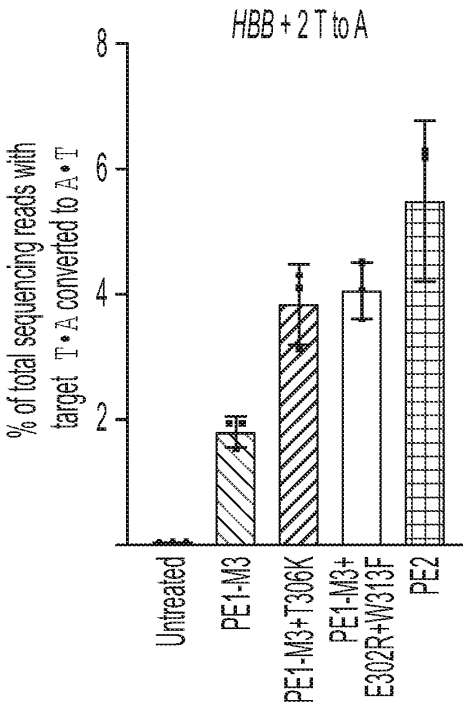


FIG. 47M

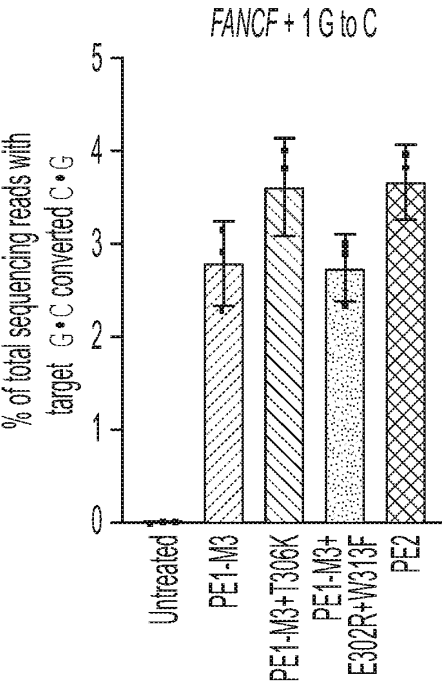


FIG. 47N

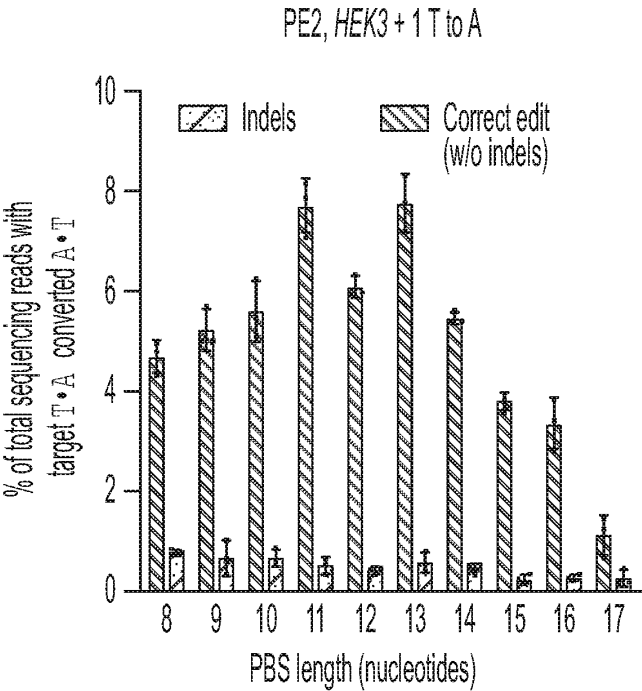


FIG. 47O

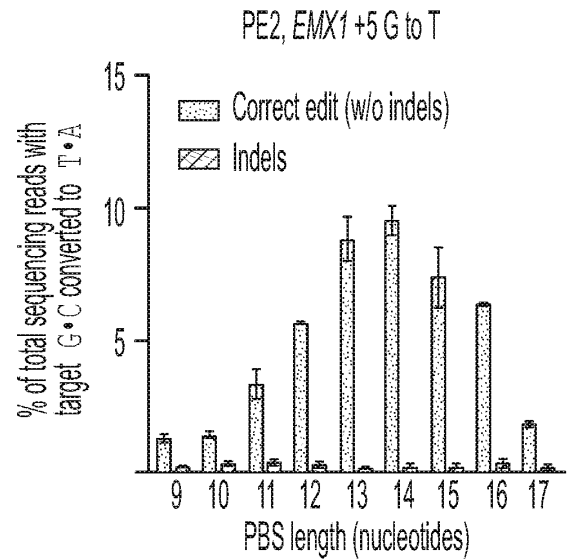


FIG. 47P

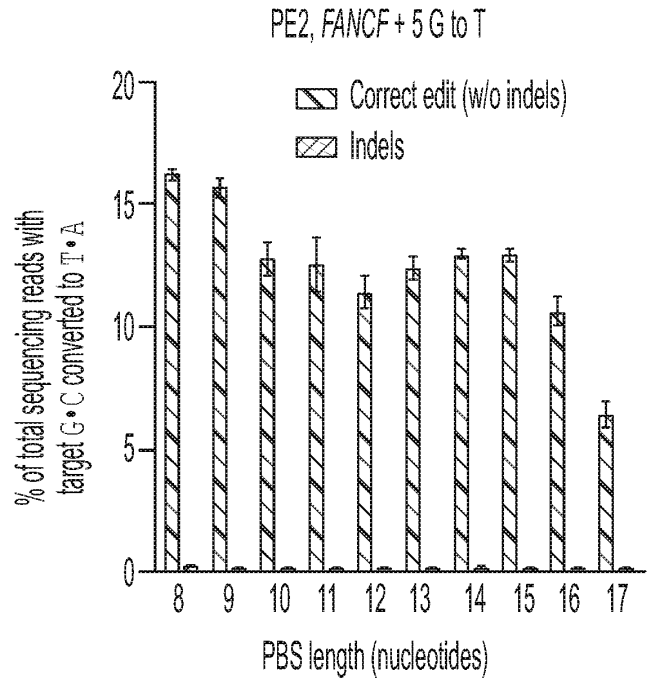


FIG. 47Q

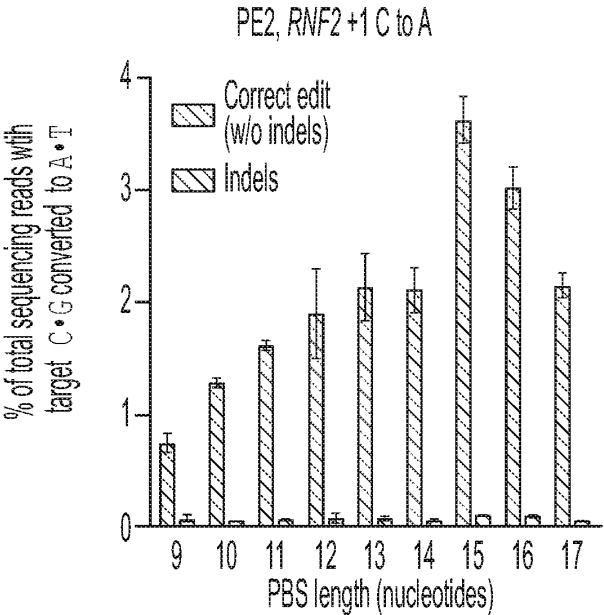


FIG. 47R

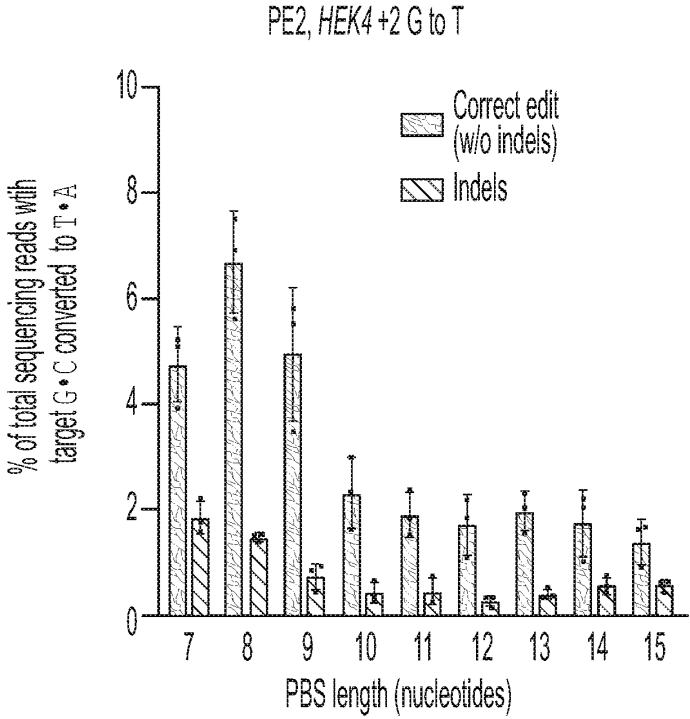


FIG. 47S

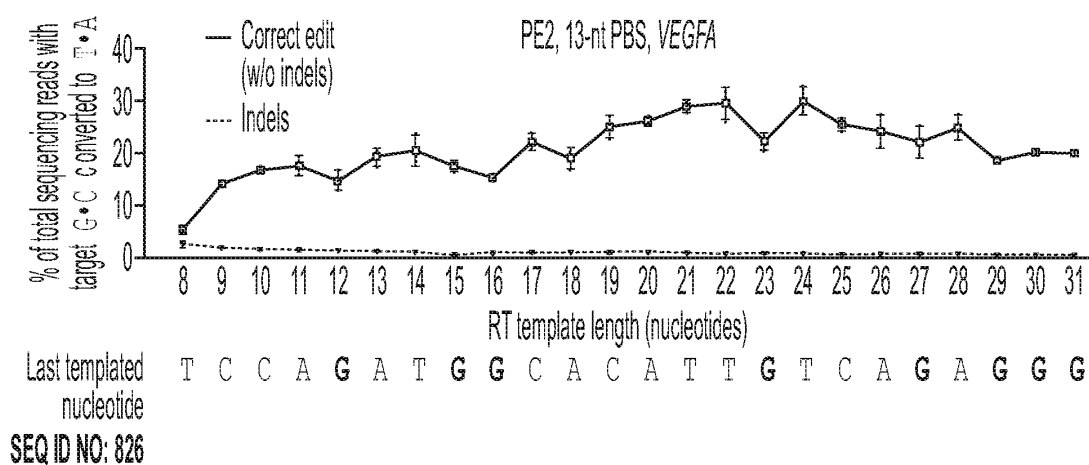


FIG. 48A

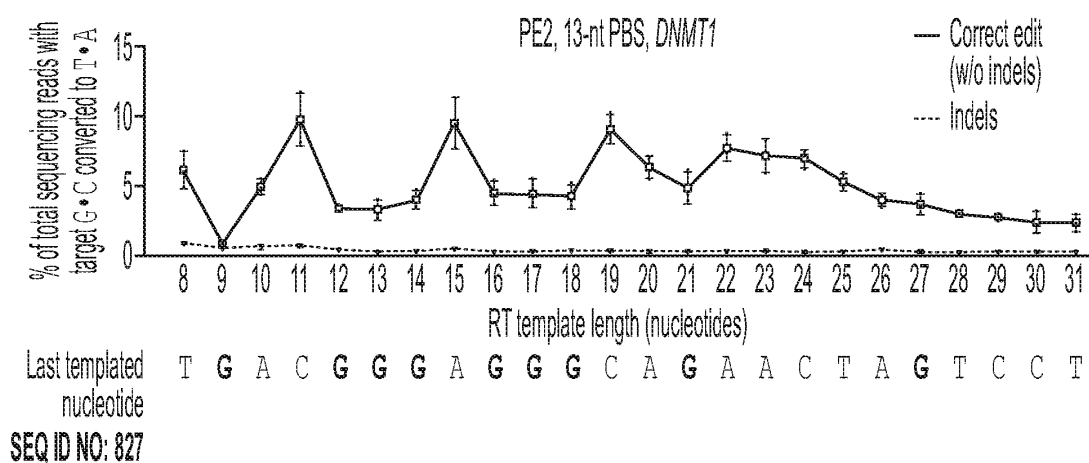


FIG. 48B

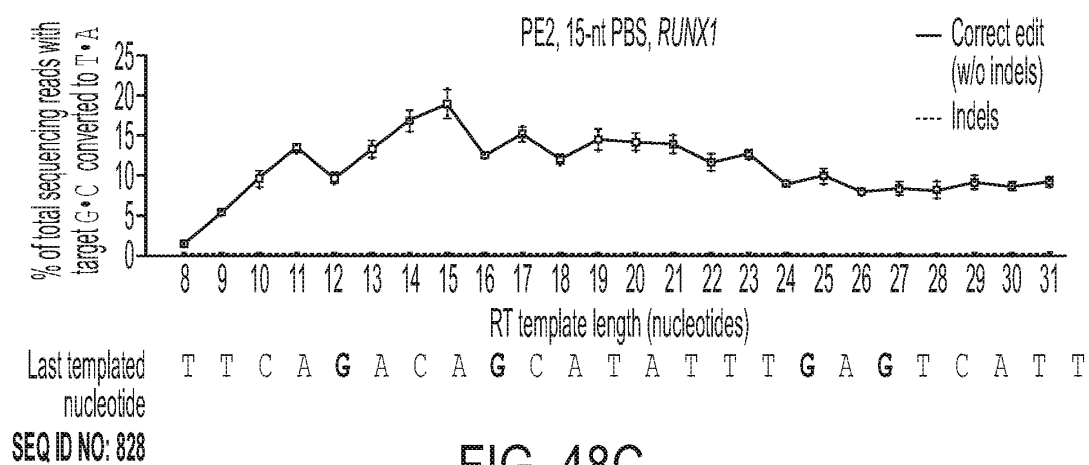


FIG. 48C

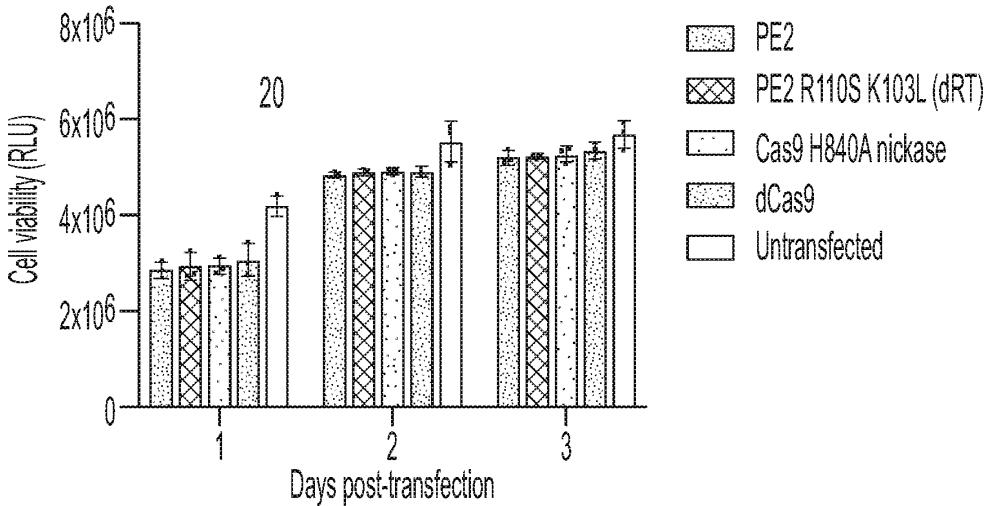


FIG. 49A

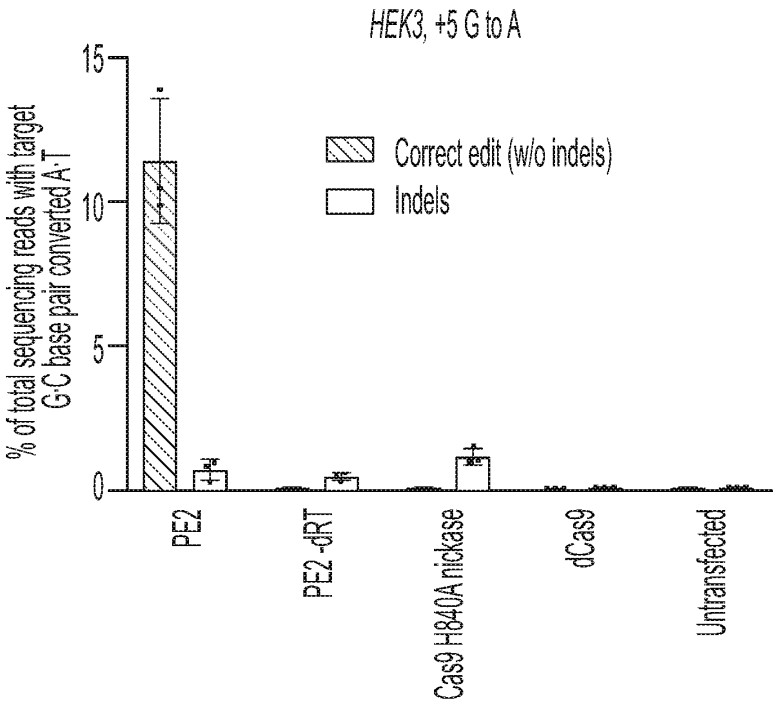


FIG. 49B

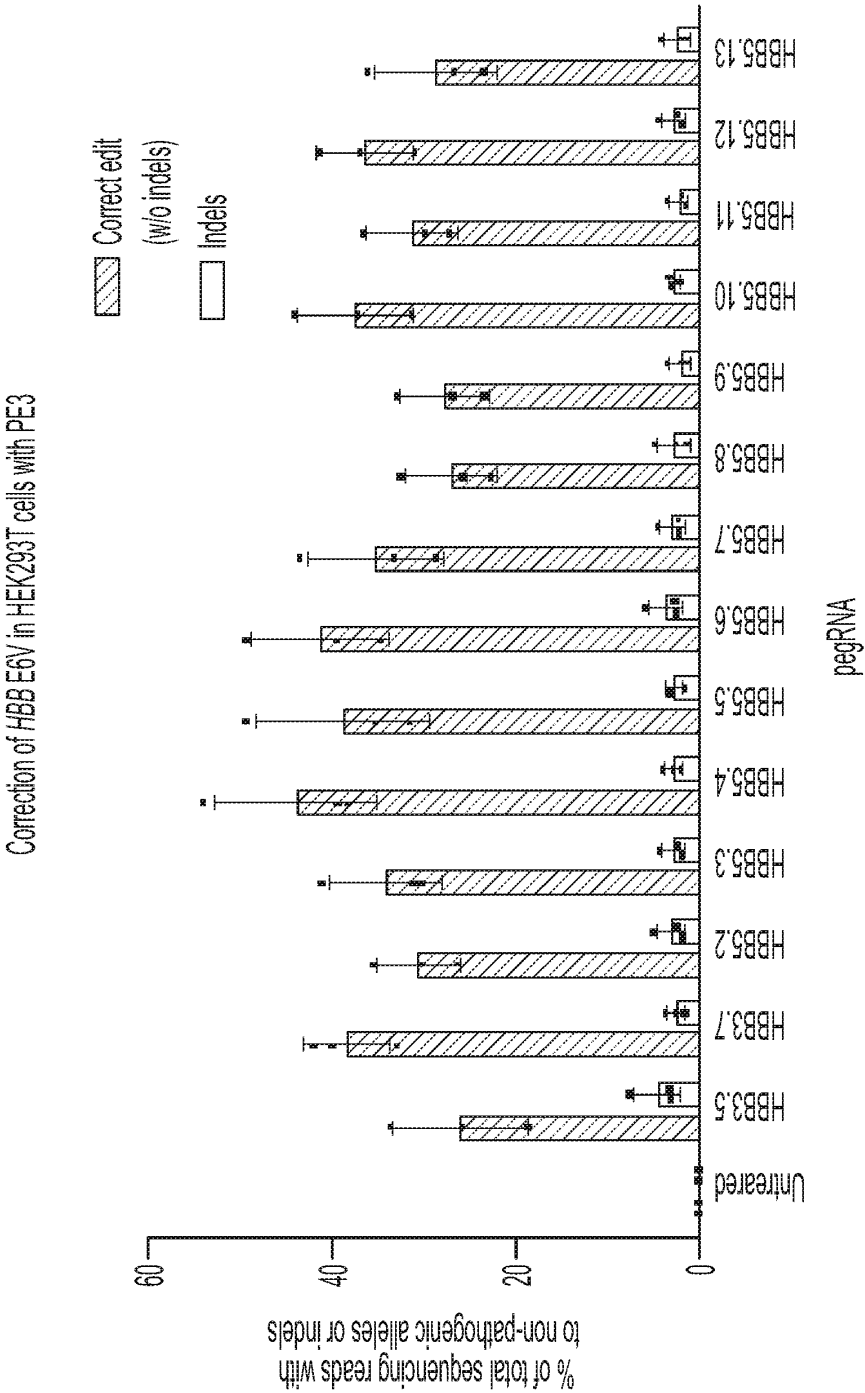


FIG. 50A

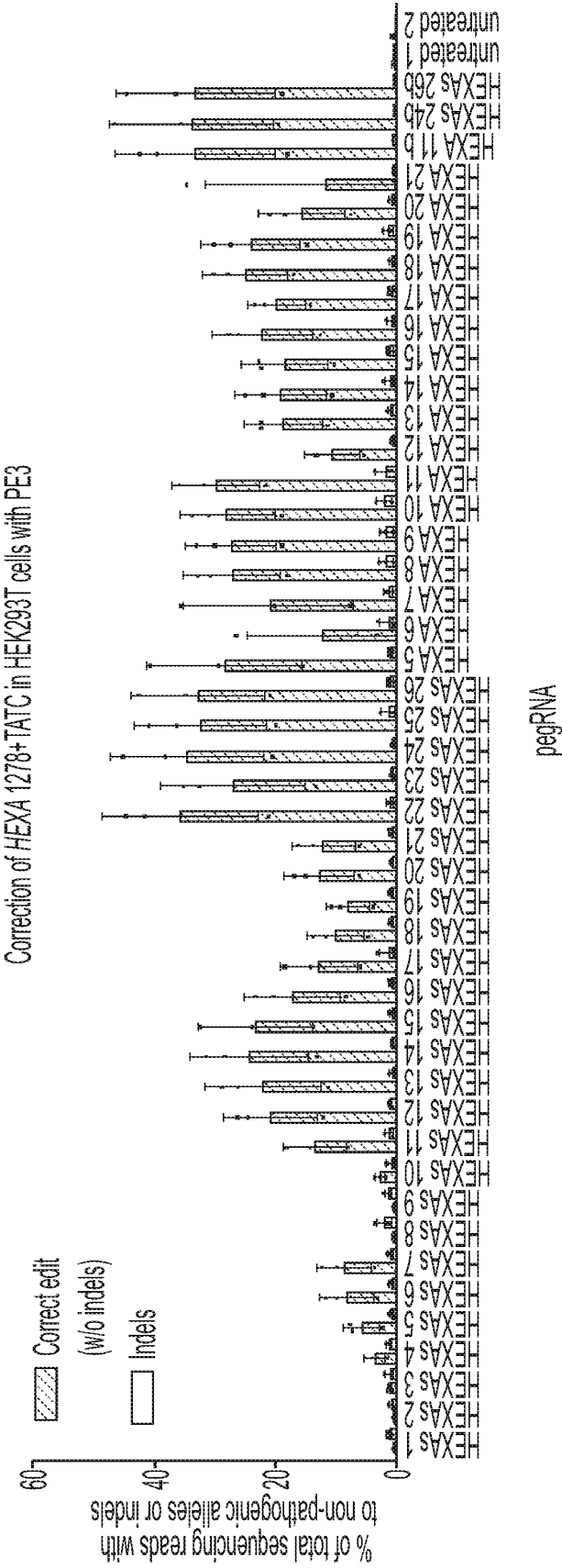


FIG. 50B

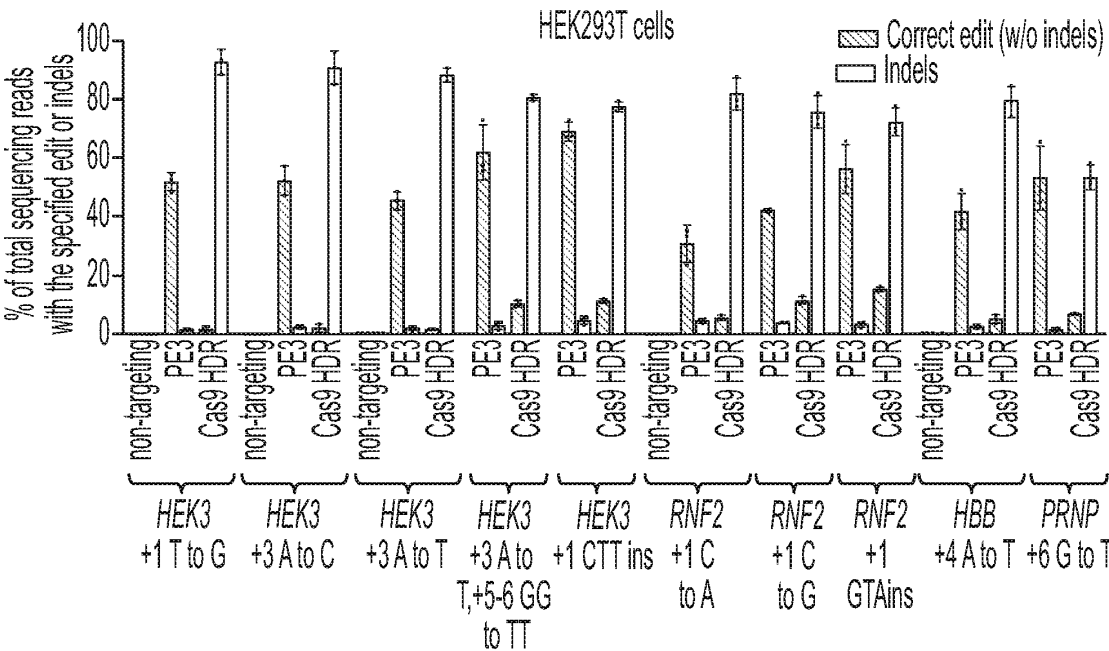


FIG. 51A

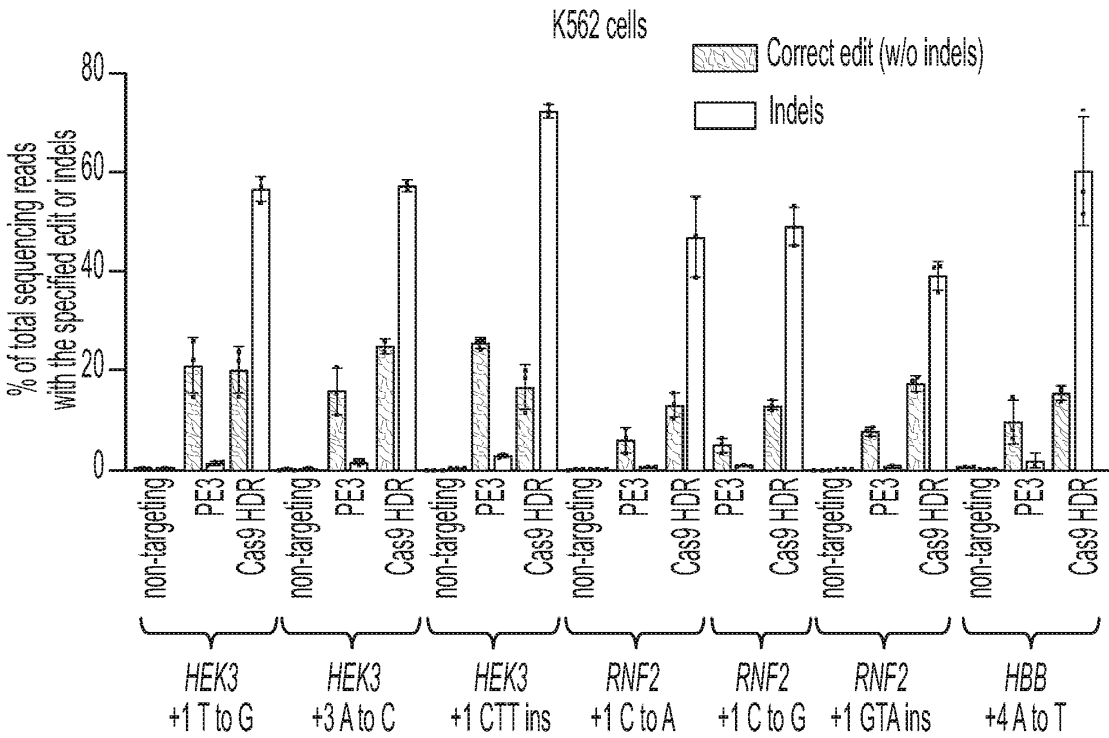


FIG. 51B

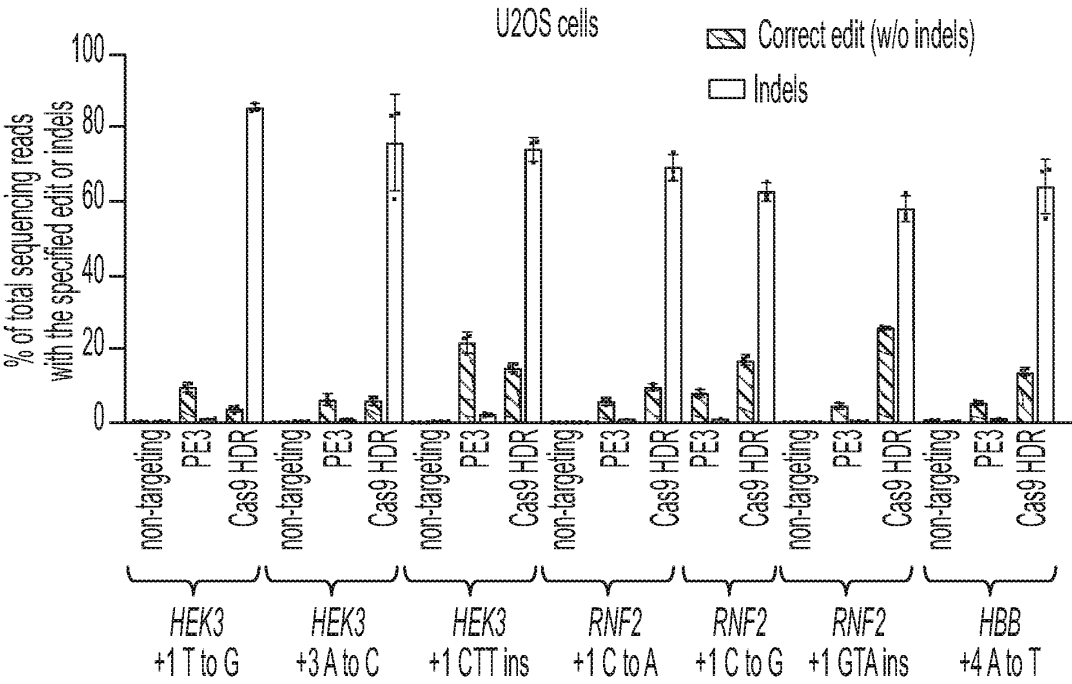


FIG. 51C

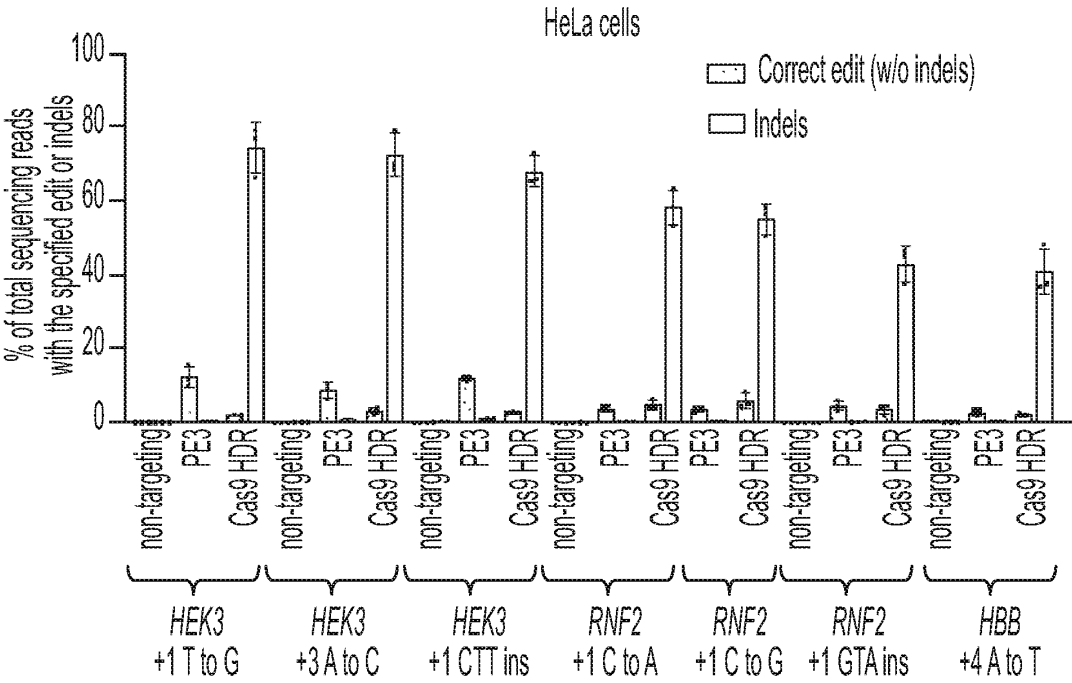


FIG. 51D

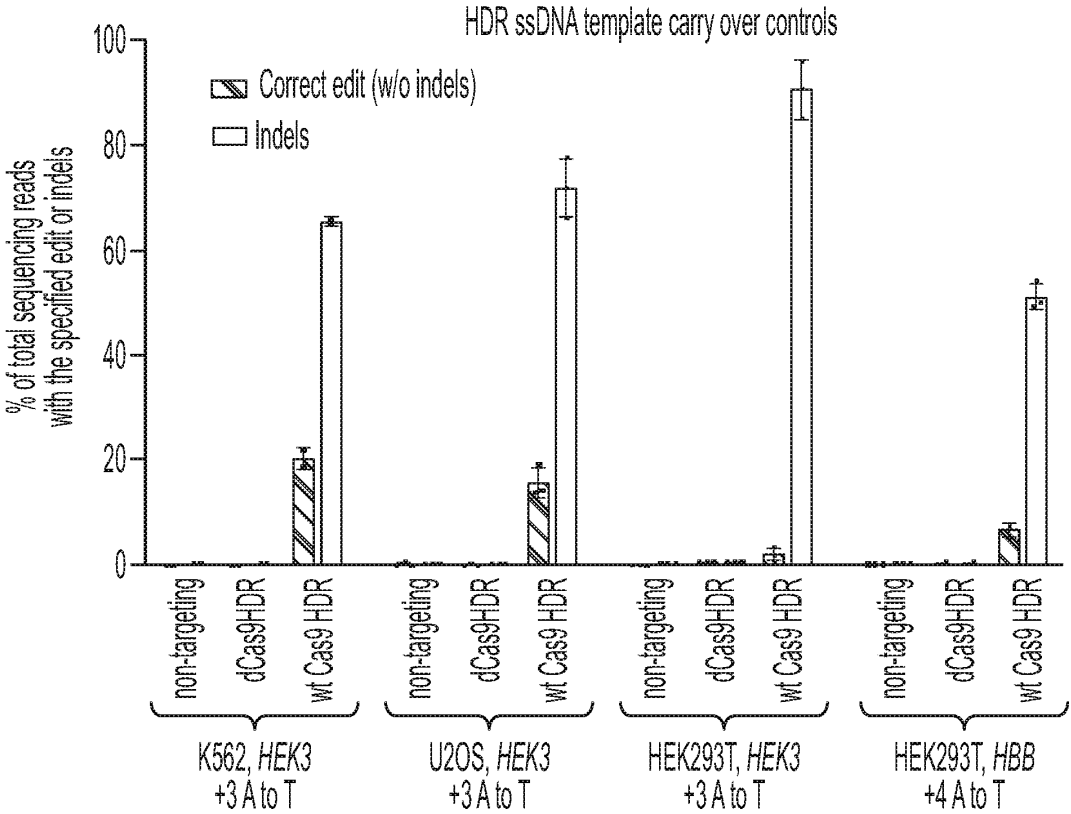


FIG. 51E

SEQ ID NO:

|                                                   |                                           |                       |
|---------------------------------------------------|-------------------------------------------|-----------------------|
| 791                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAAG | -Reference            |
| K562 HEK3 negative control (non-targeting pegRNA) |                                           |                       |
| 791                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAAG | -93.54% (47190 reads) |
| 791                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAAG | -0.47% (238 reads)    |
| 829                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGCGGAAAGGAAAG | -0.21% (108 reads)    |
| K562 HEK3 PE3+1 CTT insertion                     |                                           |                       |
| 791                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAAG | -69.66% (39061 reads) |
| 830                                               | TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGG  | -22.80% (12784 reads) |
| 791                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAAG | -0.32% (179 reads)    |
| 830                                               | TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGG  | -0.21% (120 reads)    |
| 831                                               | TGGGGCCCAGACTGAGCACGCTTTTATGGCAGAGGAAAGG  | -0.21% (116 reads)    |
| K562 HEK3 Cas9-initiated HDR +1 CTT insertion     |                                           |                       |
| 832                                               | TGGGGCCCAGACTGAGCAC-TGATGGCAGAGGAAAGGAAAG | -17.15% (7256 reads)  |
| 791                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAAG | -13.86% (5864 reads)  |
| 830                                               | TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGG  | -10.55% (4461 reads)  |
| 833                                               | TGGGGCCCAGACTGAGCA--TGATGGCAGAGGAAAGGAAAG | -9.72% (4111 reads)   |
| 834                                               | TGGGGCCCAGACTGAGCACG--ATGGCAGAGGAAAGGAAAG | -5.14% (2174 reads)   |
| 835                                               | TGGGGCCCAGACTGAGCACGCTGATGGCAGAGGAAAGGAA  | -4.77% (2016 reads)   |
| 836                                               | TGGGGCCCAGACTGAGCACG-GATGGCAGAGGAAAGGAAAG | -3.82% (1616 reads)   |
| 837                                               | TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAA   | -3.17% (1342 reads)   |
| 838                                               | TGGGGCCCAGACTGA-----TGATGGCAGAGGAAAGGAAAG | -1.92% (814 reads)    |
| 839                                               | TGGGGCCCAGACTGAGCACGATGATGGCAGAGGAAAGGAA  | -1.63% (689 reads)    |
| 840                                               | TGGGGCCCAGACTGAGCACG-----CAGAGGAAAGGAAAG  | -1.26% (535 reads)    |
| 841                                               | TGGGGC-----TGATGGCAGAGGAAAGGAAAG          | -0.78% (329 reads)    |
| 842                                               | TGGGGCCCAGACTGAGCA-----GAGGAAAGGAAAG      | -0.61% (257 reads)    |
| 843                                               | TGGGGCCCAGACTGAGCACGCTGATGGCAGAGGAAAGGAA  | -0.59% (250 reads)    |
| 844                                               | TGGGGCC-----TGATGGCAGAGGAAAGGAAAG         | -0.56% (237 reads)    |

FIG. 51F

|     |          |                              |               |                    |                    |
|-----|----------|------------------------------|---------------|--------------------|--------------------|
| 845 | TGGGG    | -----                        | TGATGGCAGAGG  | AAAGGAAG           | -0.52% (219 reads) |
| 846 | TGGGG    | CCCAGACTGAGCACG              | -----         | AGGAAAGGAAG        | -0.47% (197 reads) |
| 847 | TGGGG    | CCCAGACTGAGCACG              | -----         | AGAGGAAAGGAAG      | -0.43 (181 reads)  |
| 848 | TGGGG    | CCCAGACTGAGCACG              | -----         | GGCAGAGGAAAGGAAG   | -0.41 (173 reads)  |
| 849 | TGGGG    | CCCAGACTGAG                  | -----         | GAAAGGAAG          | -0.40% (169 reads) |
| 850 | TGGGG    | C-----                       | -----         | AGAGGAAAGGAAG      | -0.38% (159 reads) |
| 851 | TGGGG    | CCCAGACTGAGCACG              | -----         | GAAAGGAAG          | -0.37% (156 reads) |
| 852 | TGGGG    | CCCAGA-----                  | -----         | TGGCAGAGGAAAGGAAG  | -0.35% (148 reads) |
| 853 | TGGGG    | CCCAGACTGAGCACG              | -----         | AAAGGAAG           | -0.35% (146 reads) |
| 854 | TG-----  | -----                        | -----         | ATGGCAGAGGAAAGGAAG | -0.31% (132 reads) |
| 855 | TGGGG    | CCCAGACTGAGC <b>TT</b> ----- | TGATGGCAGAGG  | AAAGGAAG           | -0.30% (125 reads) |
| 856 | TGGGG    | CCCAGACTGAGCACG              | -----         | AAAG               | -0.30% (125 reads) |
| 857 | TGGGG    | CCCAGACTGAGCACG              | -----         | GAGGAAAGGAAG       | -0.29% (122 reads) |
| 858 | TGGGG    | CCCAGACTGAGCACG              | -----         | GAAAG              | -0.29% (121 reads) |
| 859 | TGGGG    | CCCAGA-----                  | -----         | GGAAAGGAAG         | -0.27% (114 reads) |
| 860 | TGGGG    | CCCAG-----                   | TGATGGCAGAGG  | AAAGGAAG           | -0.26% (112 reads) |
| 861 | TGG----- | -----                        | TGATGGCAGAGG  | AAAGGAAG           | -0.26% (112 reads) |
| 862 | TGGGG    | CCCAGACTGAGCACG              | <b>TCT</b> GA | TGGCAGAGGAAAGGA    | -0.26% (110 reads) |
| 863 | -----    | -----                        | -----         | ATGGCAGAGGAAAGGAAG | -0.24% (100 reads) |
| 864 | TGGGG    | CCCAGACTG-----               | TGATGGCAGAGG  | AAAGGAAG           | -0.23% (96 reads)  |
| 865 | TGGGG    | CCCAGACTGAG-----             | TGATGGCAGAGG  | AAAGGAAG           | -0.23% (96 reads)  |
| 866 | TGGGG    | CCC-----                     | TGATGGCAGAGG  | AAAGGAAG           | -0.22% (93 reads)  |
| 867 | TGGGG    | CCCAGACTGAGCACG              | -----         | GCAGAGGAAAGGAAG    | -0.21% (87 reads)  |

**bold** Substitutions

**□** Insertions

- Deletions

---- Predicted

cleavage position

FIG. 51G

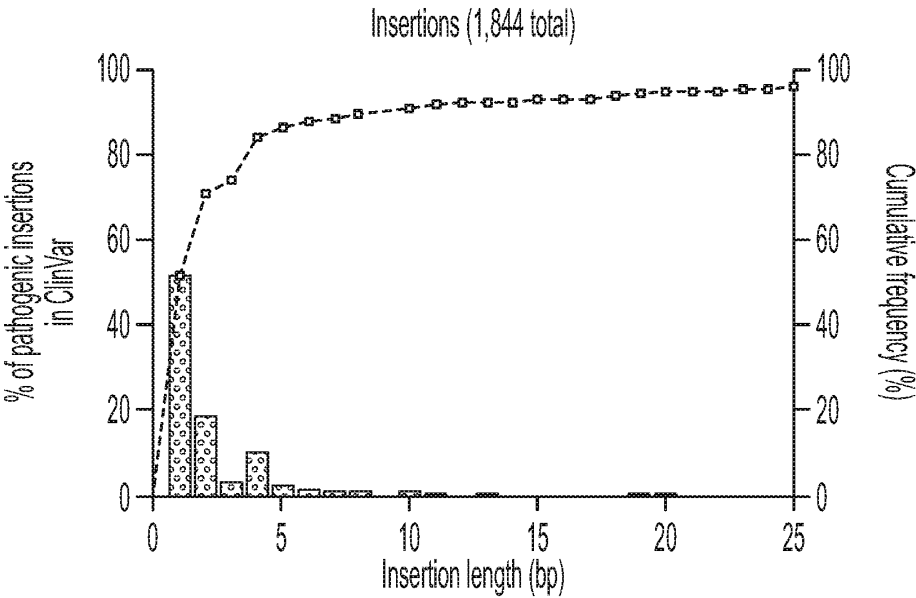


FIG. 52A

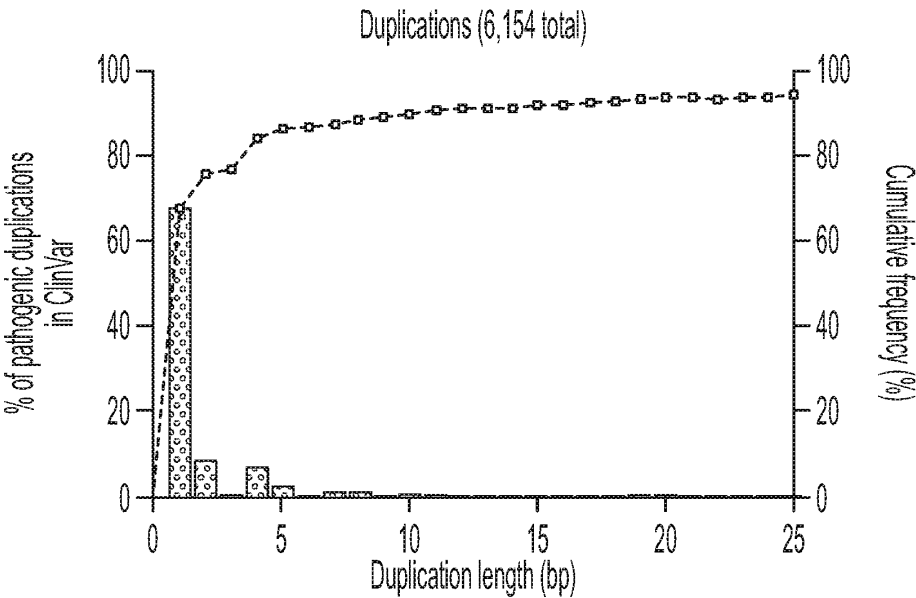


FIG. 52B

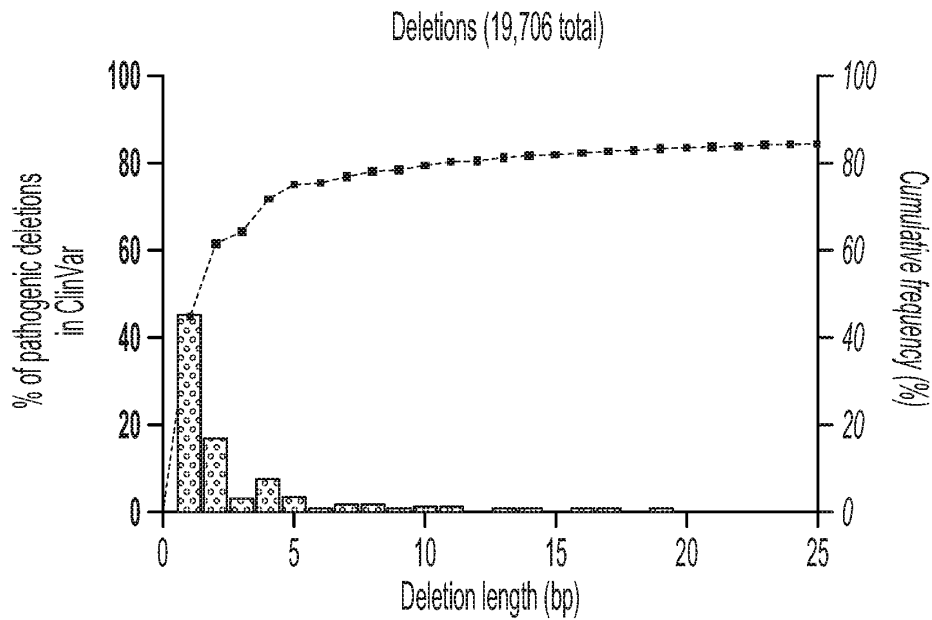


FIG. 52C

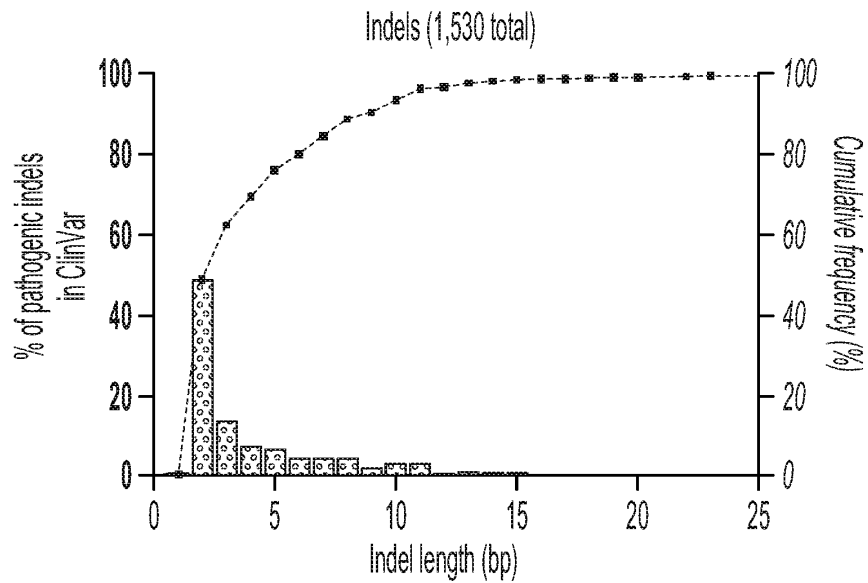
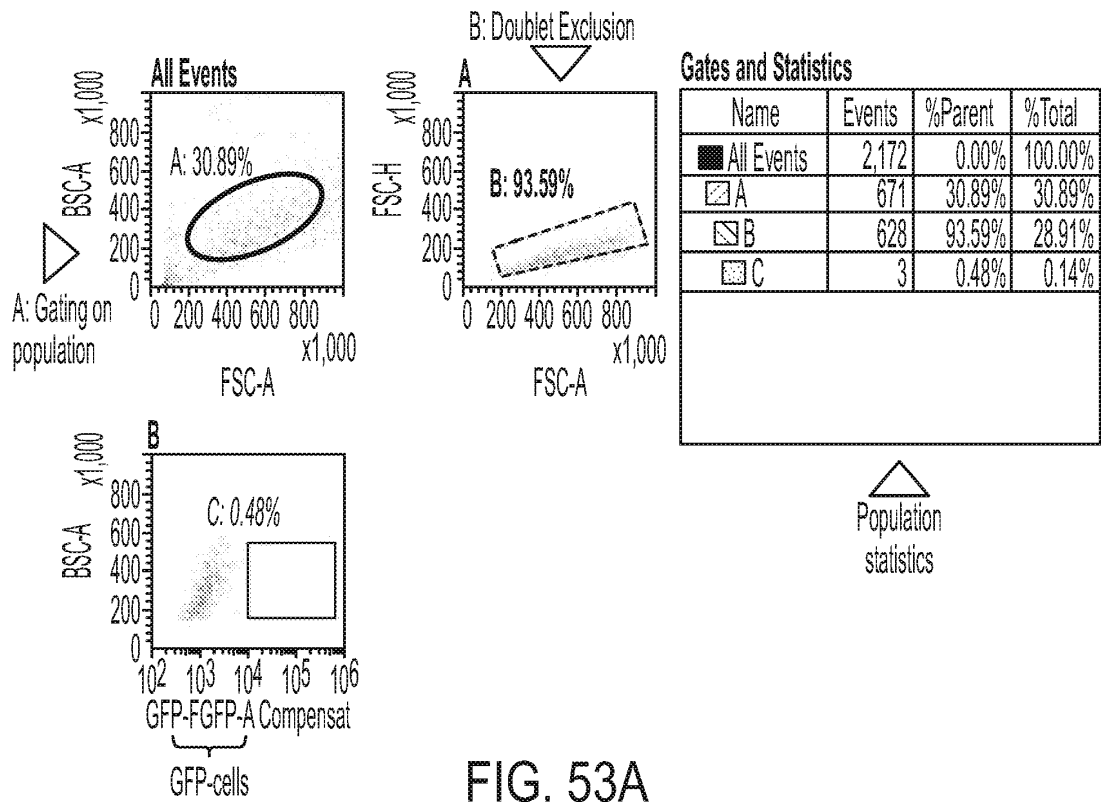
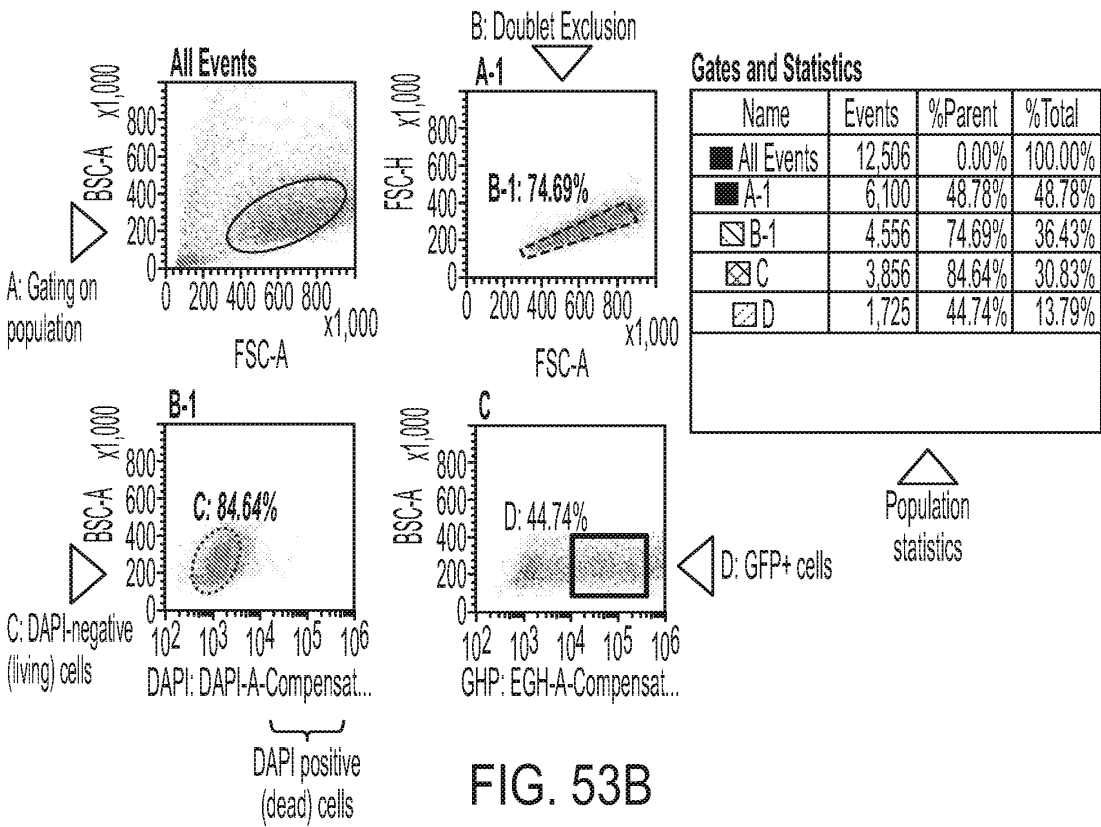


FIG. 52D



Line 1  
SEQ ID NO:

|     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |          |   |          |   |   |   |   |   |   |   |   |   |   |   |   |   |   |          |   |   |   |   |                       |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------|---|----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------|---|---|---|---|-----------------------|
| 868 | C | C | T | G | A | A | C | C | G | T | A | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -Reference            |
| 869 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -92.16% (28182 reads) |
| 870 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | <b>T</b> | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -1.54% (471 reads)    |
| 871 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | <b>T</b> | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -1.10% (335 reads)    |
| 872 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | <b>C</b> | T | T | T | C | -0.94% (288 reads)    |
| 869 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -0.61% (188 reads)    |
| 873 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | <b>C</b> | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -0.51% (156 reads)    |

Line 2

|     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |          |   |          |   |   |   |   |   |   |   |   |   |   |   |   |   |   |          |   |   |   |   |                       |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------|---|----------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----------|---|---|---|---|-----------------------|
| 868 | C | C | T | G | A | A | C | C | G | T | A | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -Reference            |
| 869 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -91.74% (18375 reads) |
| 870 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | <b>T</b> | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -1.34% (269 reads)    |
| 871 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | <b>T</b> | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -1.08% (217 reads)    |
| 872 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | <b>C</b> | T | T | T | C | -0.85% (171 reads)    |
| 869 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | <b>C</b> | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -0.59% (119 reads)    |
| 868 | C | C | T | G | A | A | C | C | G | T | A | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -0.57% (115 reads)    |
| 869 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | A        | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | A        | T | T | T | C | -0.53% (106 reads)    |
| 874 | A | A | C | C | G | T | A | T | A | T | C | T | A | T | C | C | T | <b>C</b> | T | G        | G | C | C | T | G | A | C | T | G | G | A | A | G | G | <b>C</b> | T | T | T | C | -0.23% (47 reads)     |

bold Substitutions

□ Insertions

- Deletions

---- Predicted cleavage position

FIG. 53C

SEQ ID NO: Line 1

|     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |                       |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----------------------|
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -Reference            |
| 876 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -97.45% (87424 reads) |
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.27% (240 reads)    |
| 877 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.24% (212 reads)    |

Line 2

|     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |                       |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----------------------|
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -Reference            |
| 876 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -97.16% (63793 reads) |
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.36% (239 reads)    |
| 877 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.22% (142 reads)    |

Line 3

|     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |                       |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----------------------|
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -Reference            |
| 876 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -97.32% (77514 reads) |
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.29% (228 reads)    |
| 877 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.22% (172 reads)    |
| 878 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | T | C | G | T | T | -0.21% (167 reads)    |

Line 4

|     |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |                       |
|-----|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----------------------|
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -Reference            |
| 876 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -96.94% (70061 reads) |
| 875 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | A | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.58% (419 reads)    |
| 877 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | C | C | G | T | T | -0.23% (165 reads)    |
| 878 | C | A | C | C | A | T | G | G | T | G | C | A | C | C | T | G | A | C | T | C | C | T | G | T | G | G | A | G | T | C | T | G | T | C | G | T | T | -0.21% (150 reads)    |

FIG. 53D

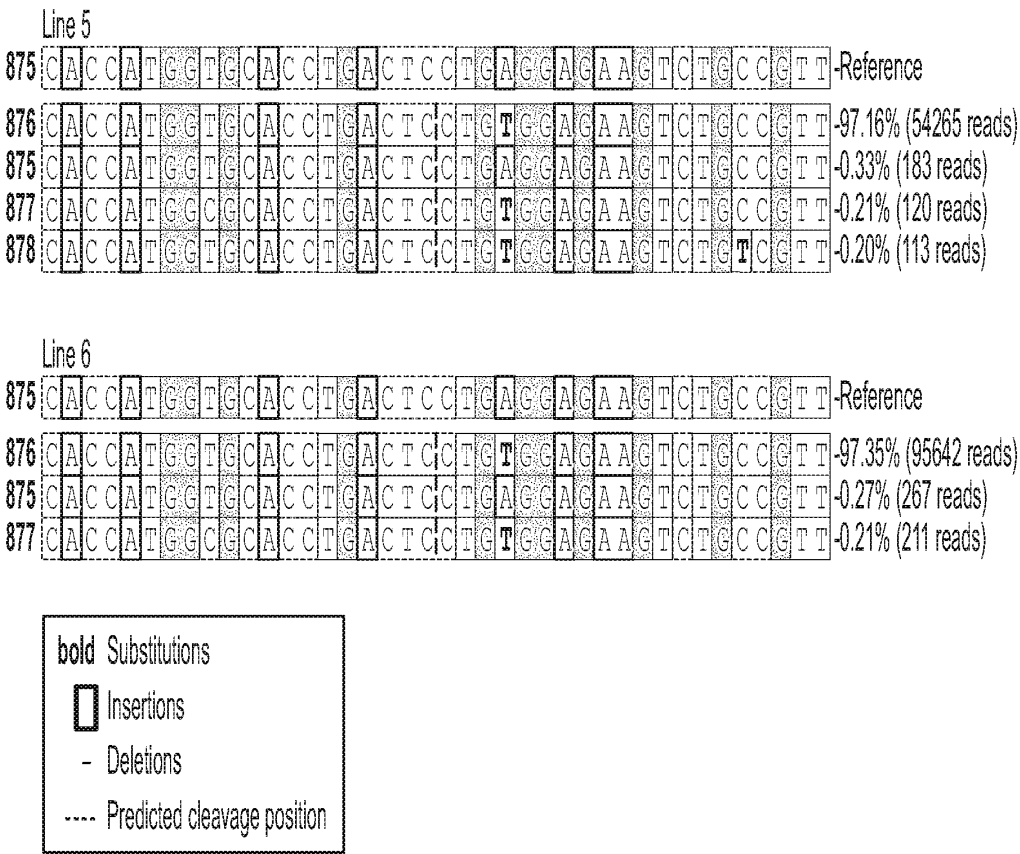
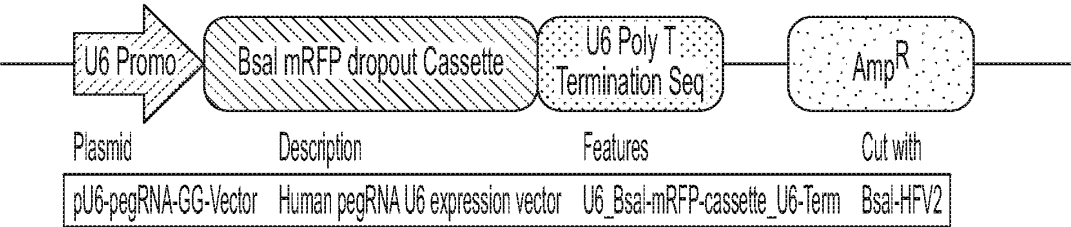


FIG. 53E

Protocol to simultaneously clone a spacer sequence (Target Site) and pegRNA 3' extension containing a PBS and RT template into a human U6 promotor pegRNA expression vector:

[Component 1] Undigested pU6-pegRNA-GG-Vector Schematic:



[Component 2] Ordering oligonucleotides to generate the spacer (target) sequence for human pegRNA vectors:

Top and bottom oligos to be annealed and then assembled into digested pU6-pegRNA-GG-Vector:

SEQ ID NO: 879 5' - CACCCNNNNNNNNNNNNNNNNNNNGTTTT - 3'

SEQ ID NO: 880 3' - NNNNNNNNNNNNNNNNNNNNCAAAATCTC - 5'

(The N20 mock protospacer sequence shown can be of variable length, as needed)

[Component 3] Ordering oligonucleotides to generate the SpCas9 sgRNA scaffold for human pegRNA vectors:

Top and bottom oligos to be annealed and then assembled into digested pU6-pegRNA-GG-Vector

{Phos} 5' - AGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGATCG - 3' SEQ ID NO: 881

SEQ ID NO: 882 3' - GATCTTTATCGTTCAATTTTATCCGATCAGCAATAGTTGAACCTTTTCACCGTGGCTCAGCCACG - 5' {Phos}

(Note that these oligos **must be 5' phosphorylated** to permit pegRNA vector assembly)

[Component 4] Ordering oligonucleotides to generate the pegRNA 3' extension containing a PBS and RT template sequence for human pegRNA vectors:

Top and bottom oligos to be annealed and then assembled into digested pU6-pegRNA-GG-Vector:

SEQ ID NO: 883 5' - GTGCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN - 3'

SEQ ID NO: 884 3' - NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN - 5'

(The N30 mock reverse transcriptase template sequence shown can be of variable length, as needed)

Schematic of final assembled U6 pegRNA expression vector from components 1, 2, 3, and 4:

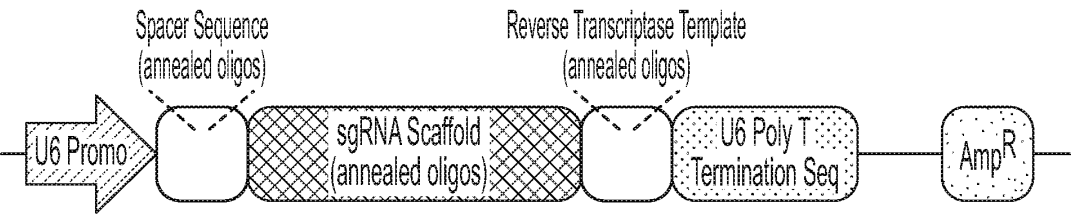


FIG. 54

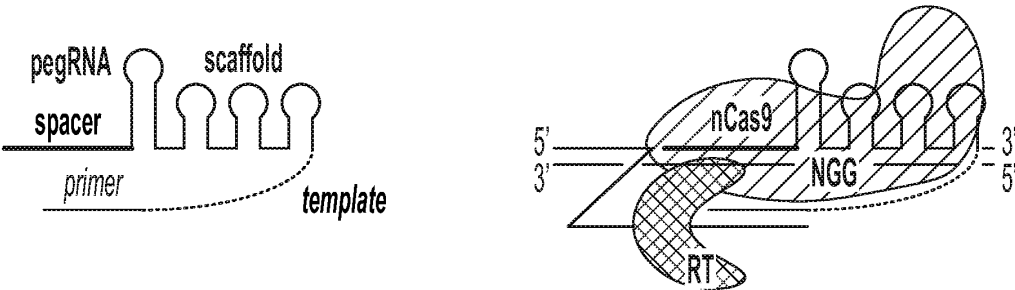


FIG. 55A

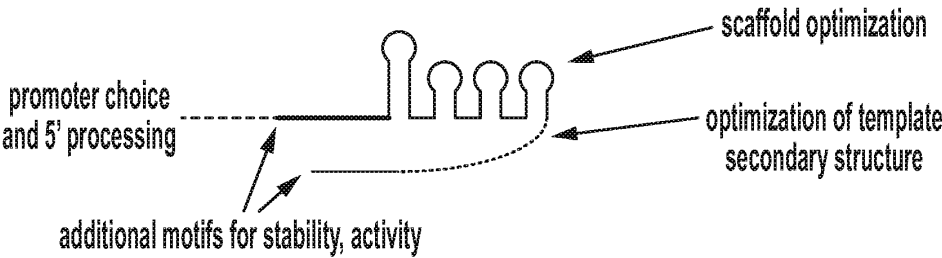


FIG. 55B

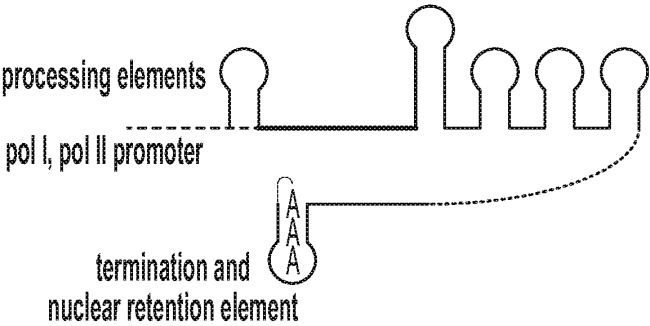


FIG. 55C

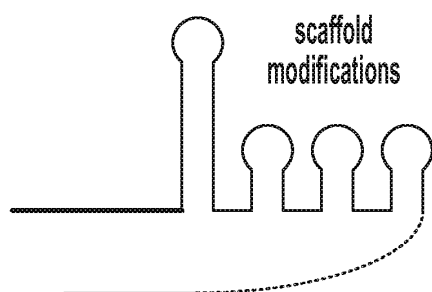


FIG. 55D

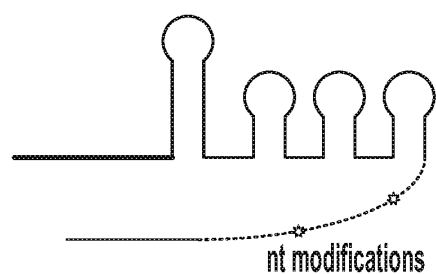


FIG. 55E

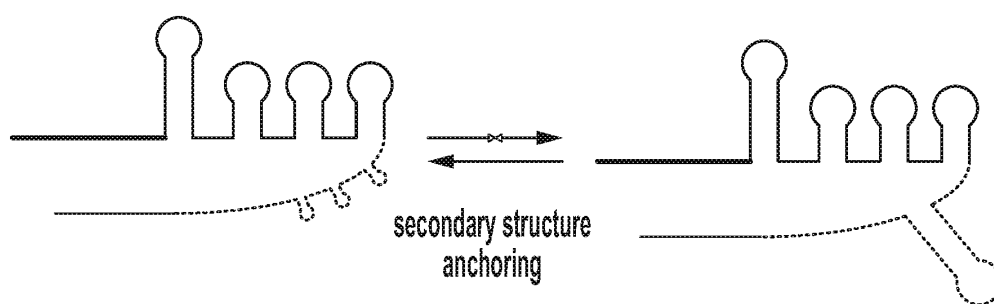


FIG. 55F

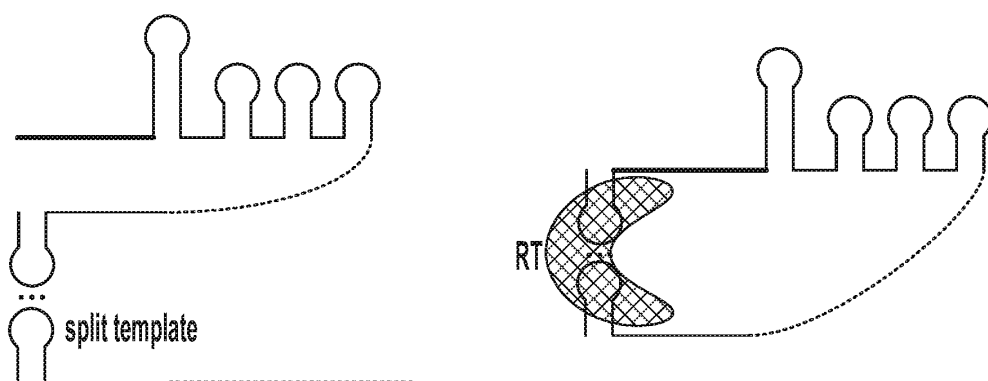
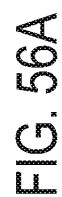


FIG. 55G



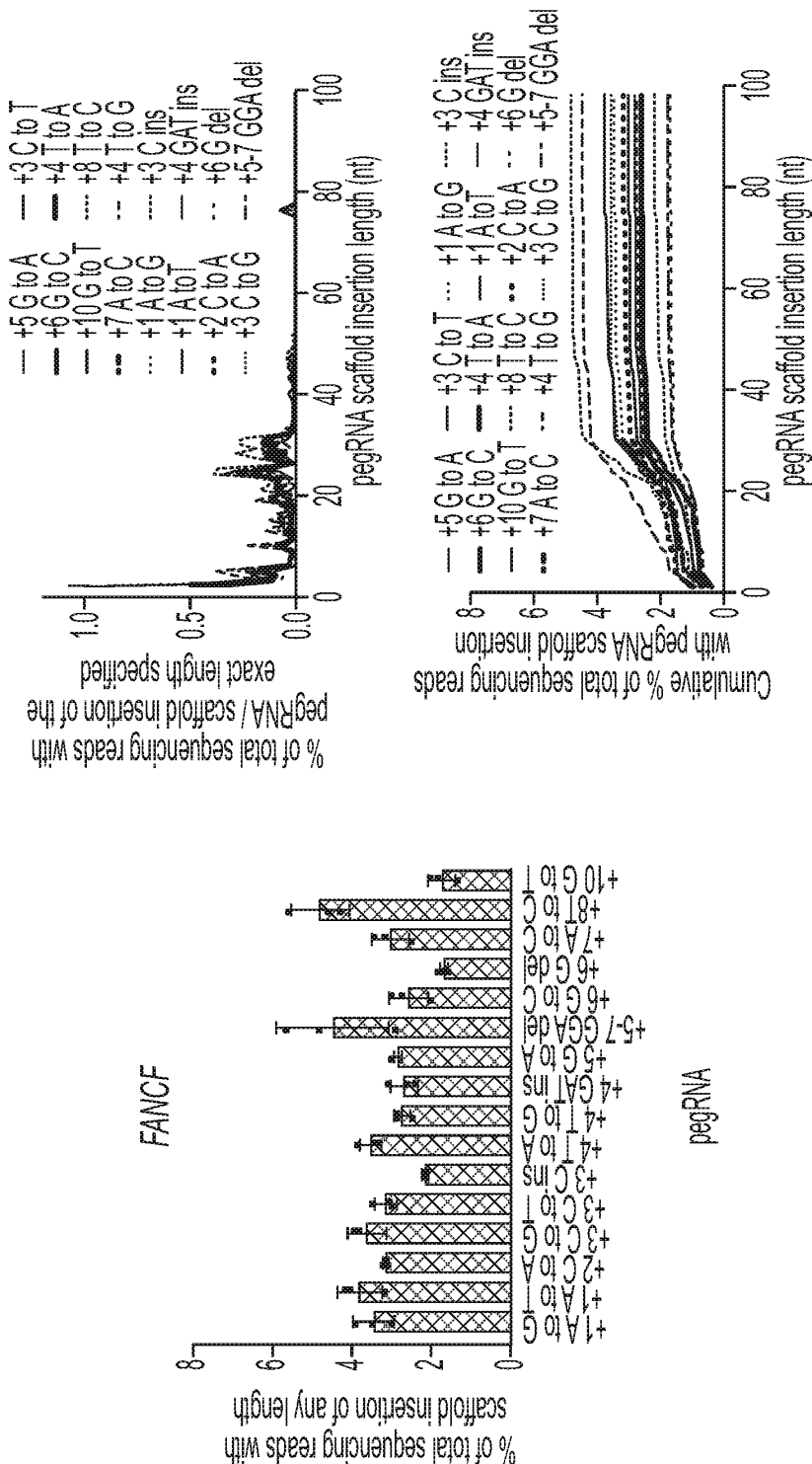


FIG. 56B

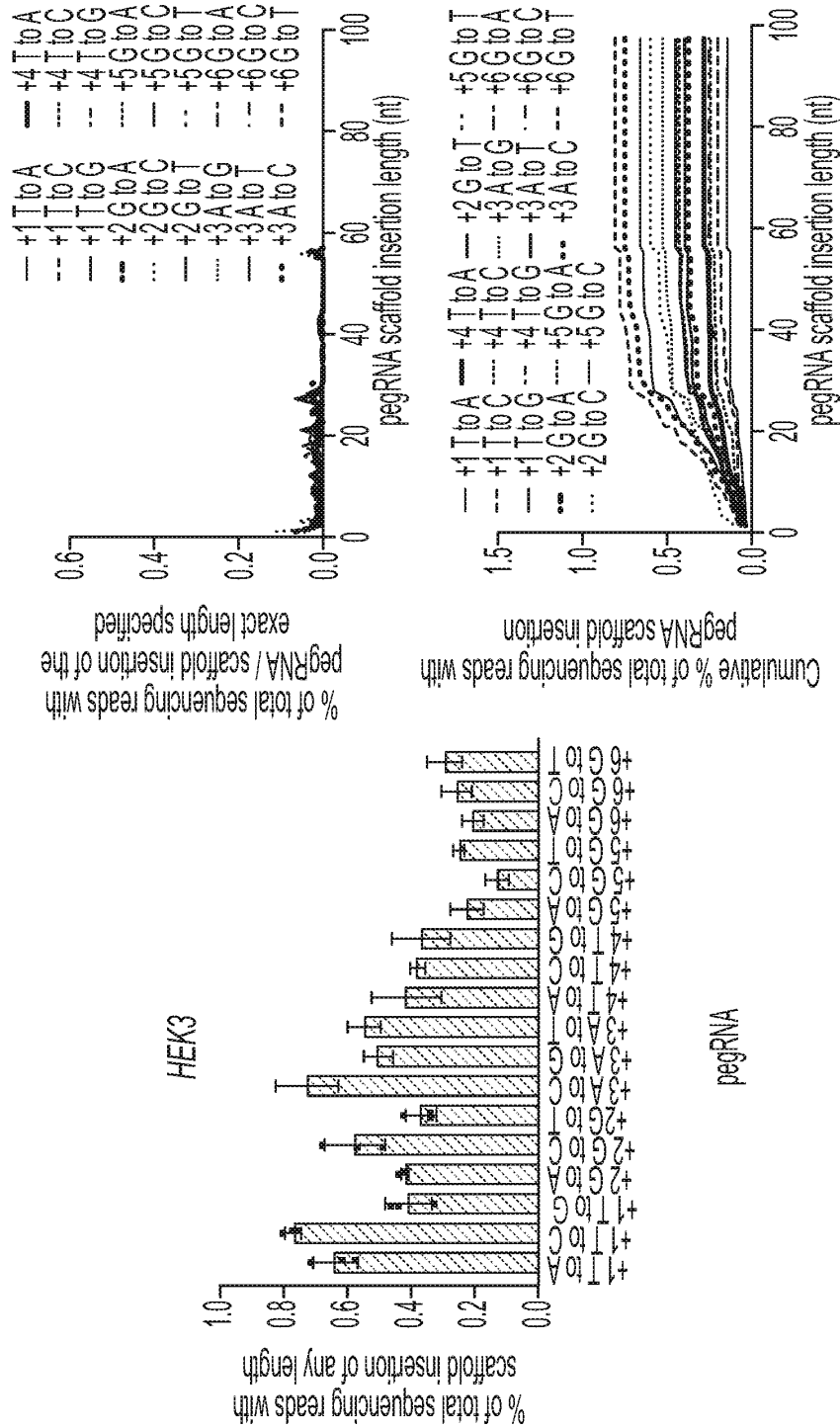


FIG. 56C

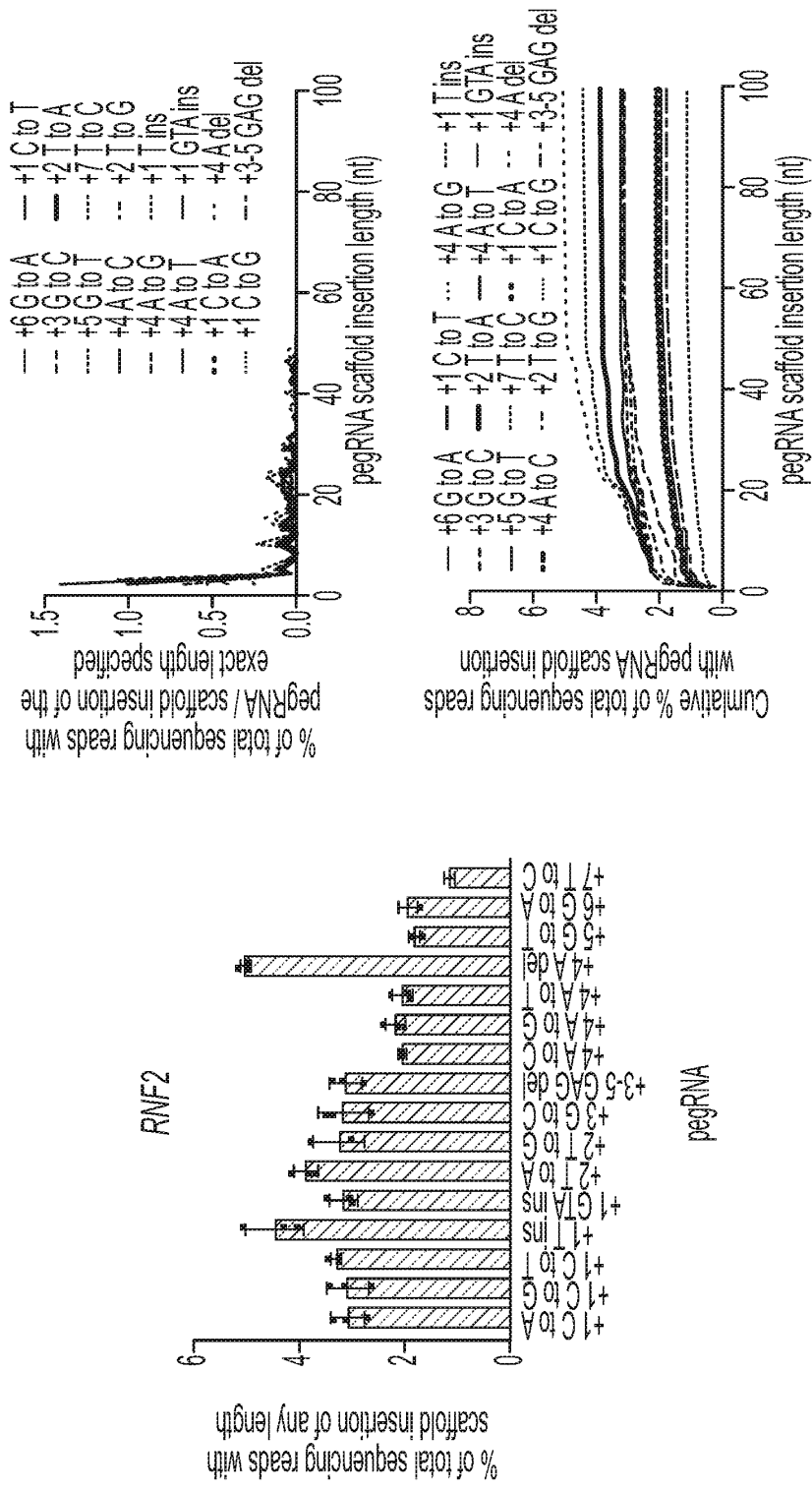


FIG. 56D

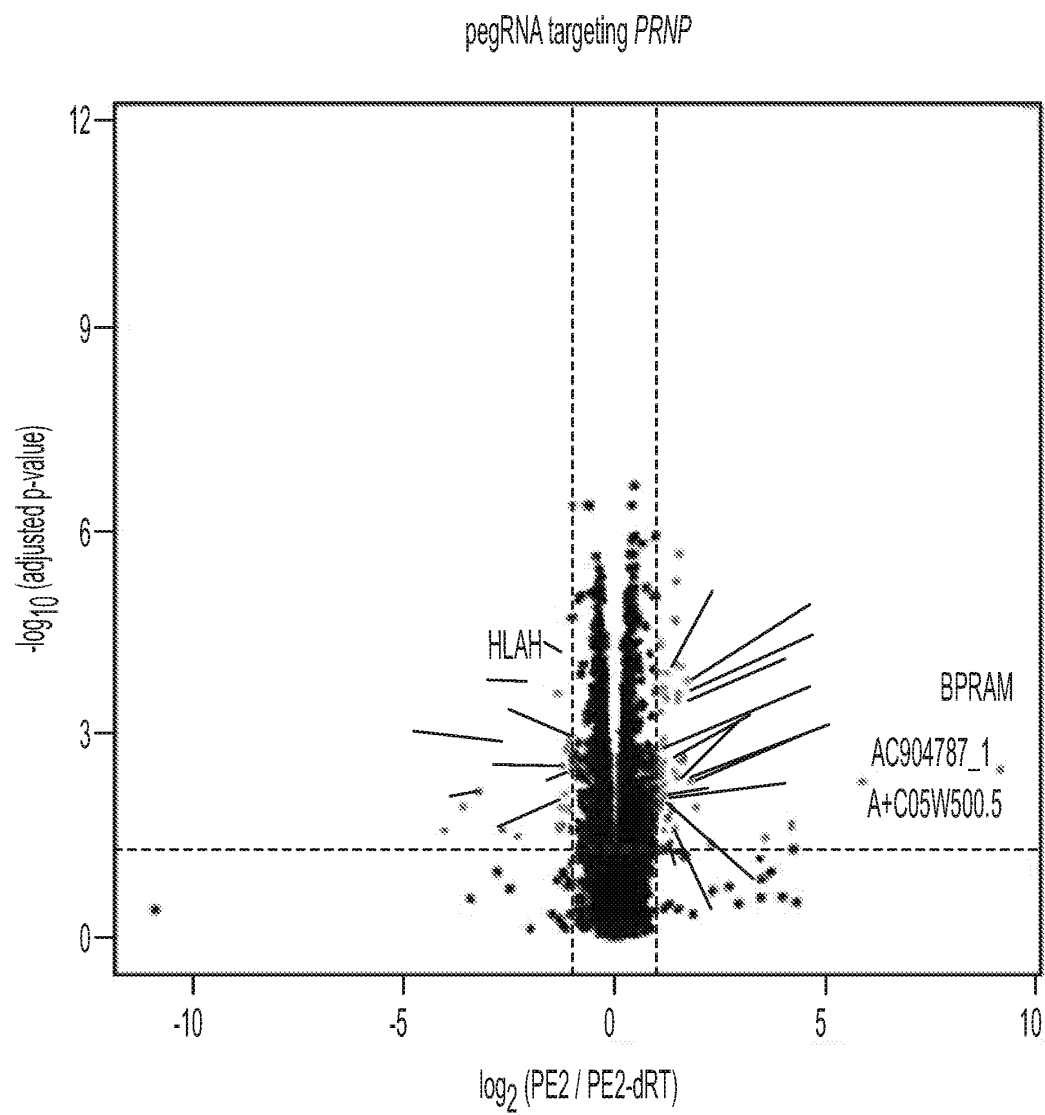


FIG. 57A

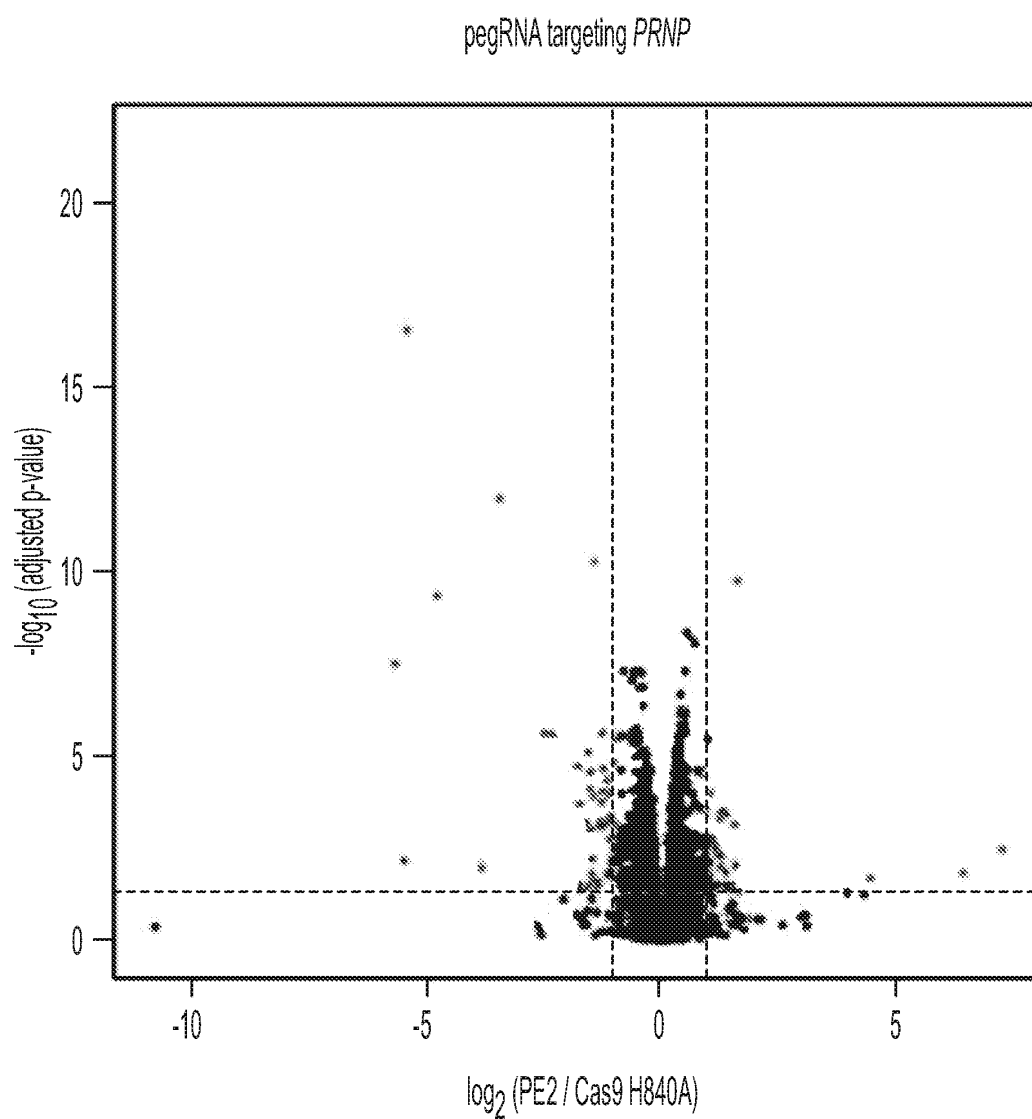
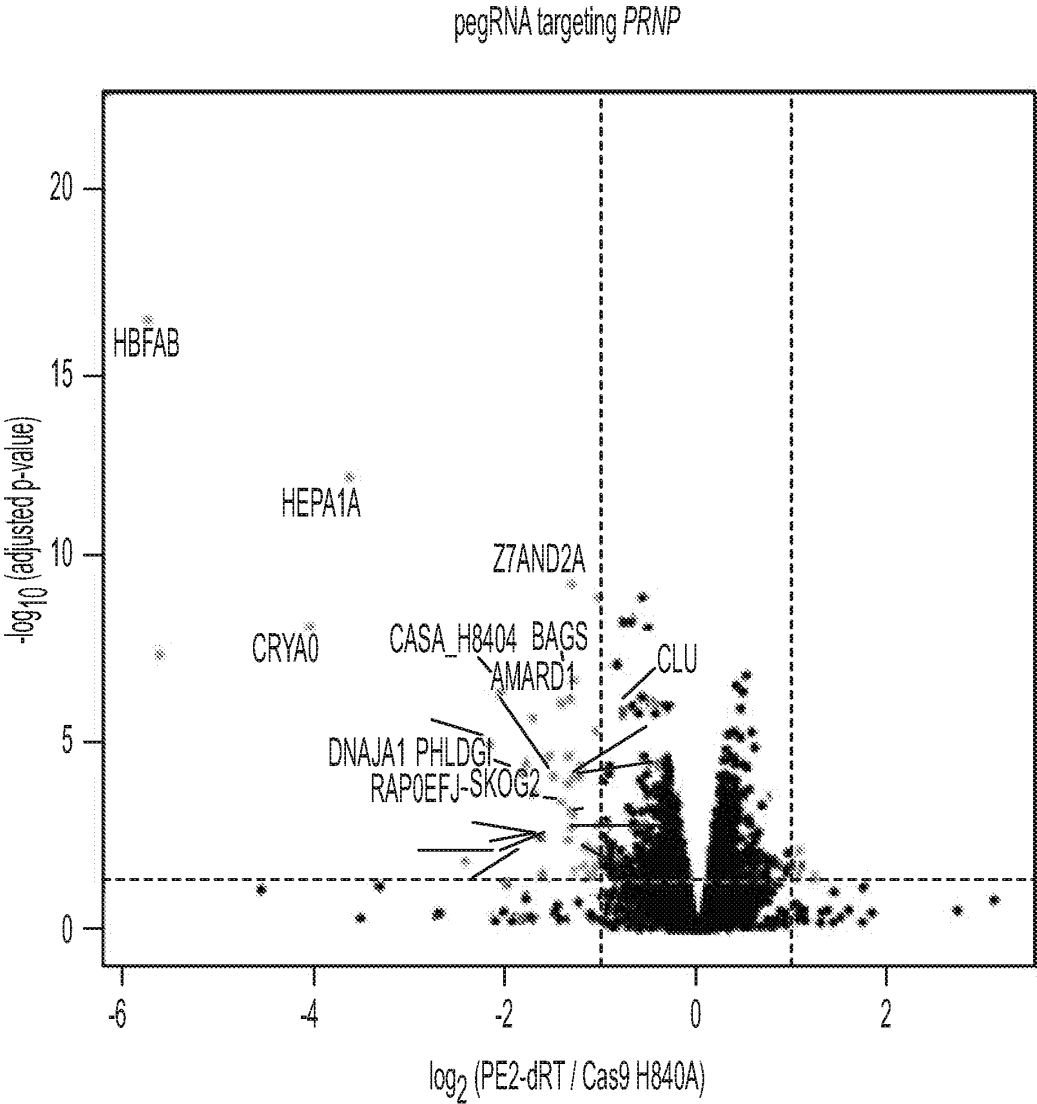


FIG. 57B



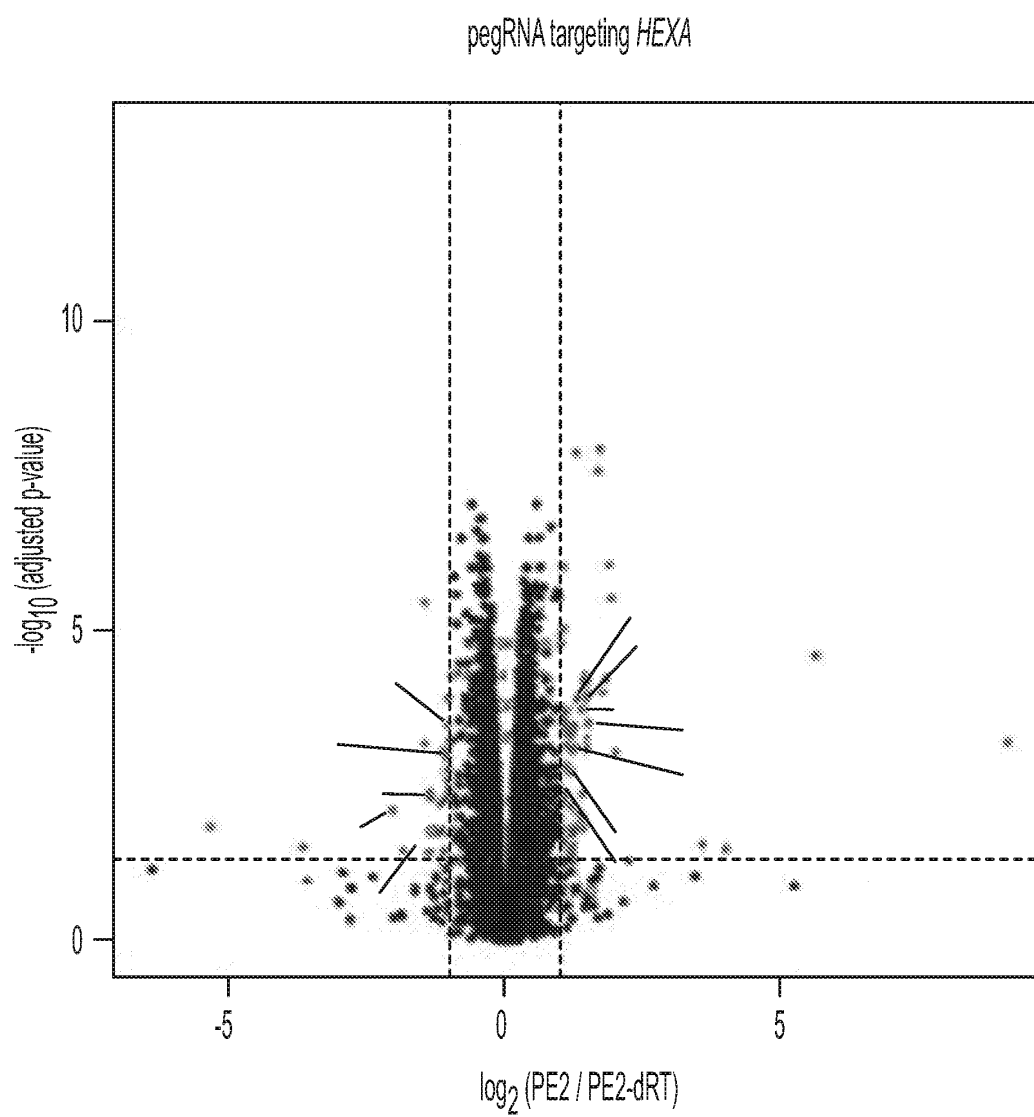


FIG. 57D

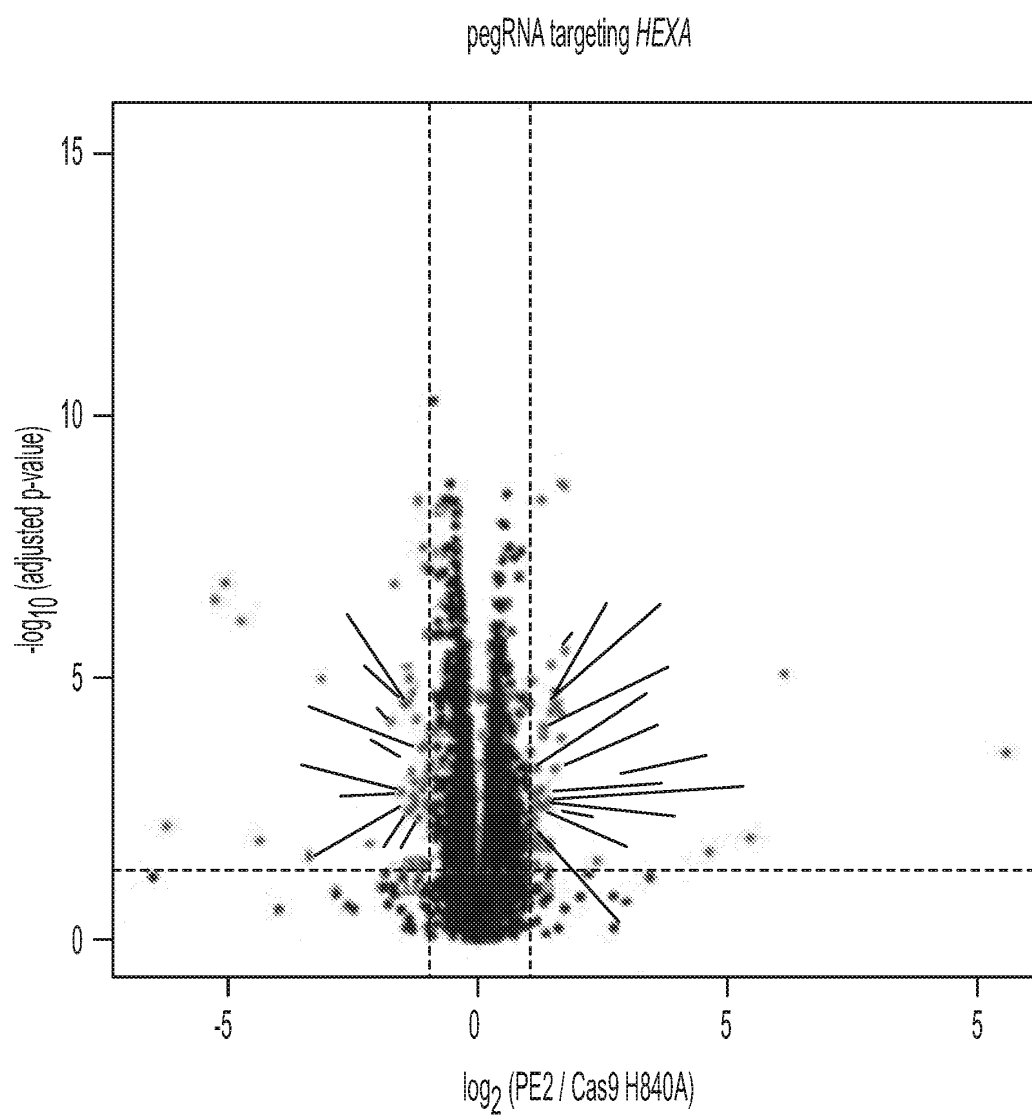


FIG. 57E

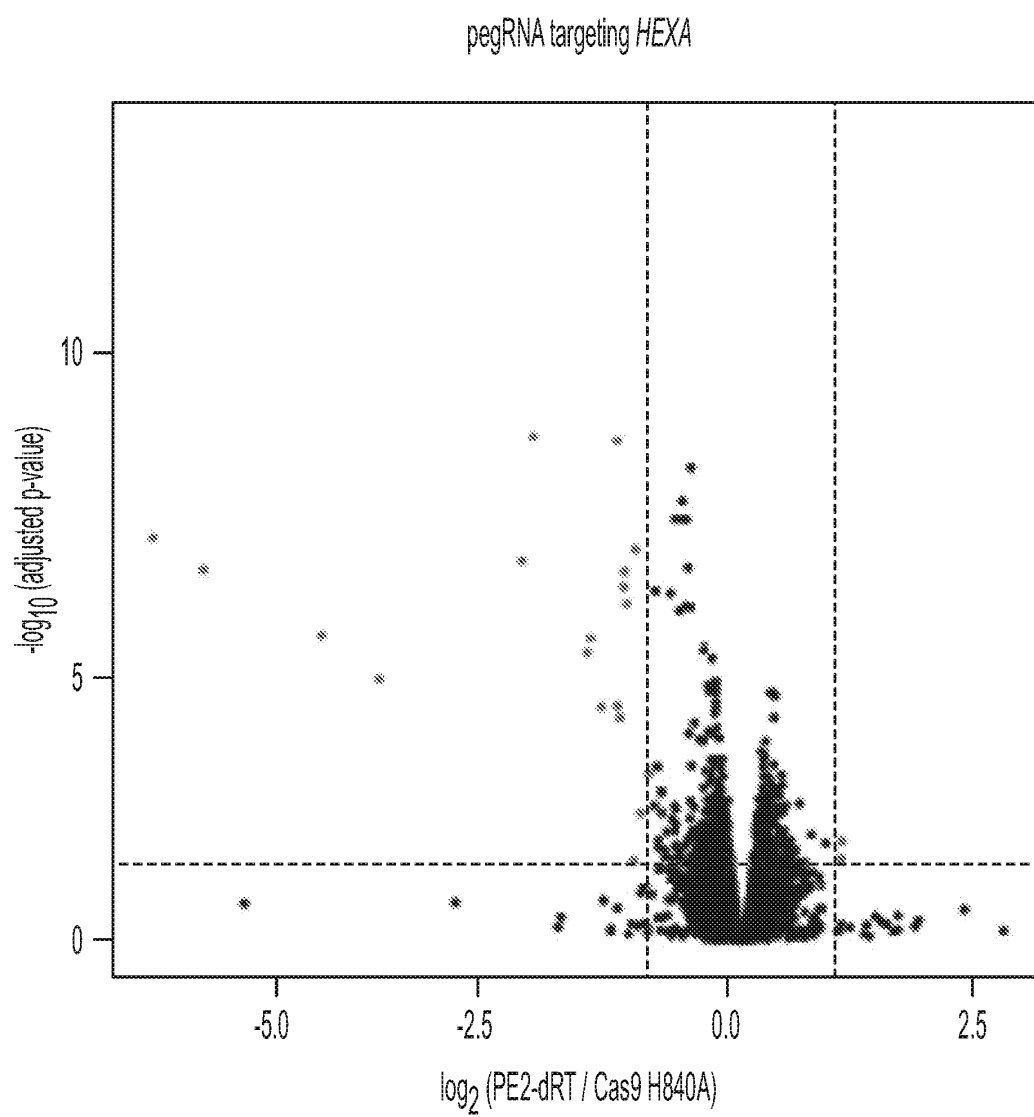


FIG. 57F

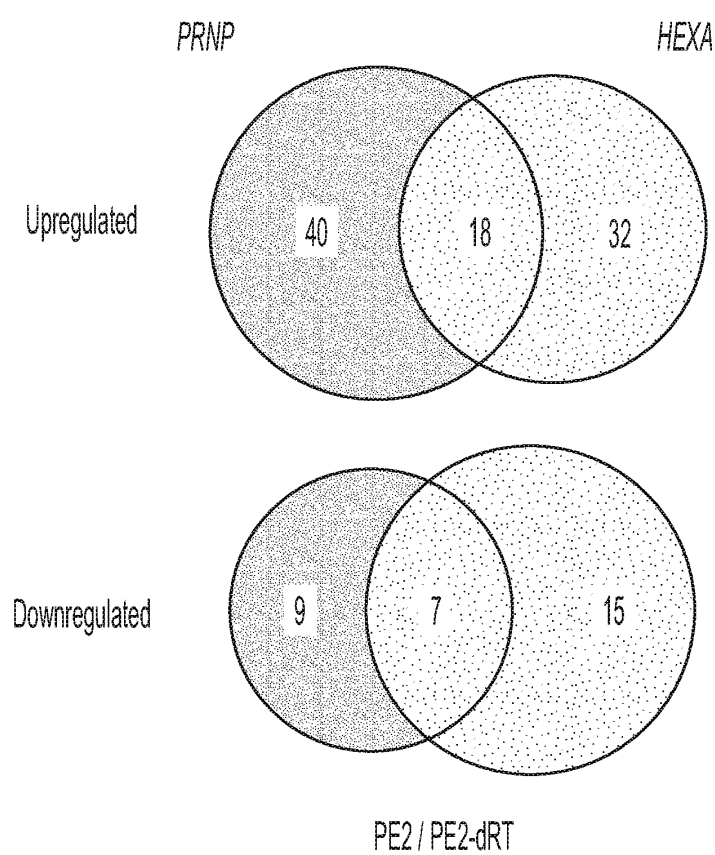


FIG. 57G

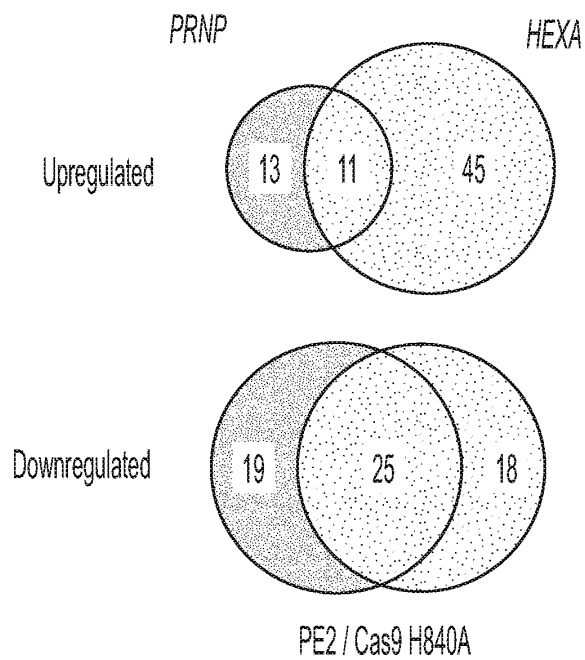


FIG. 57H

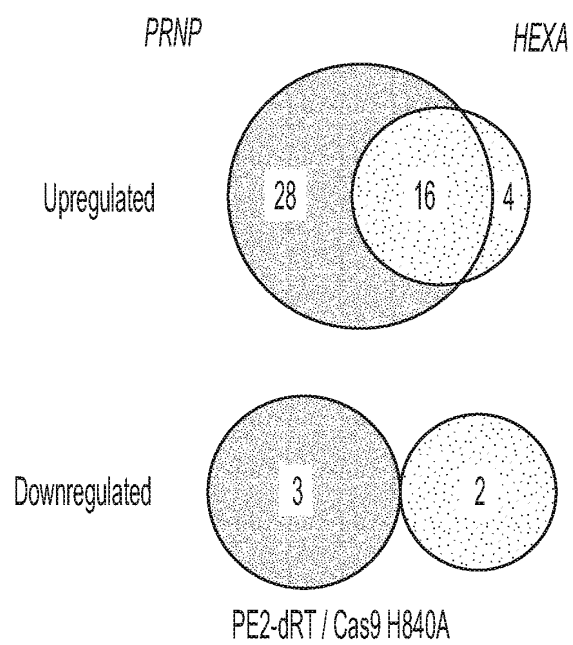


FIG. 57I

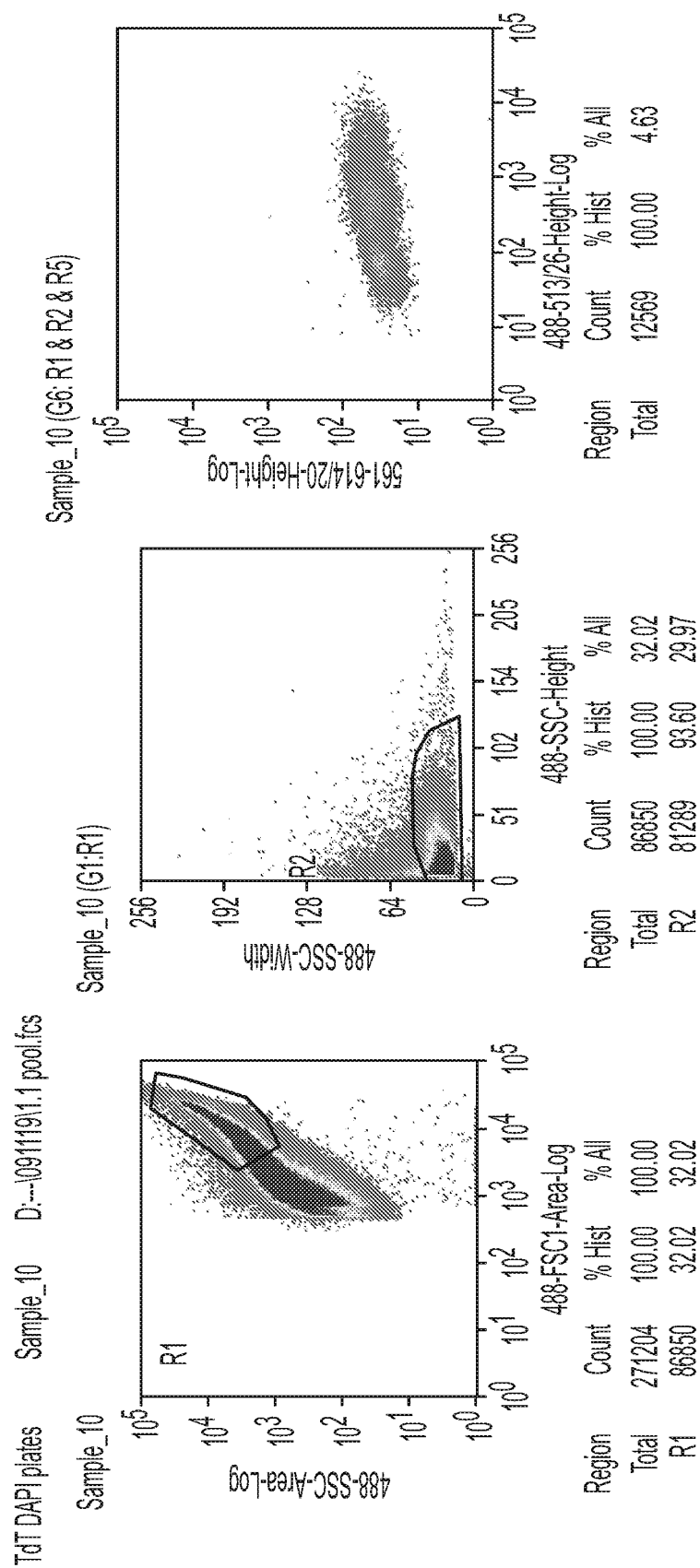


FIG. 58A

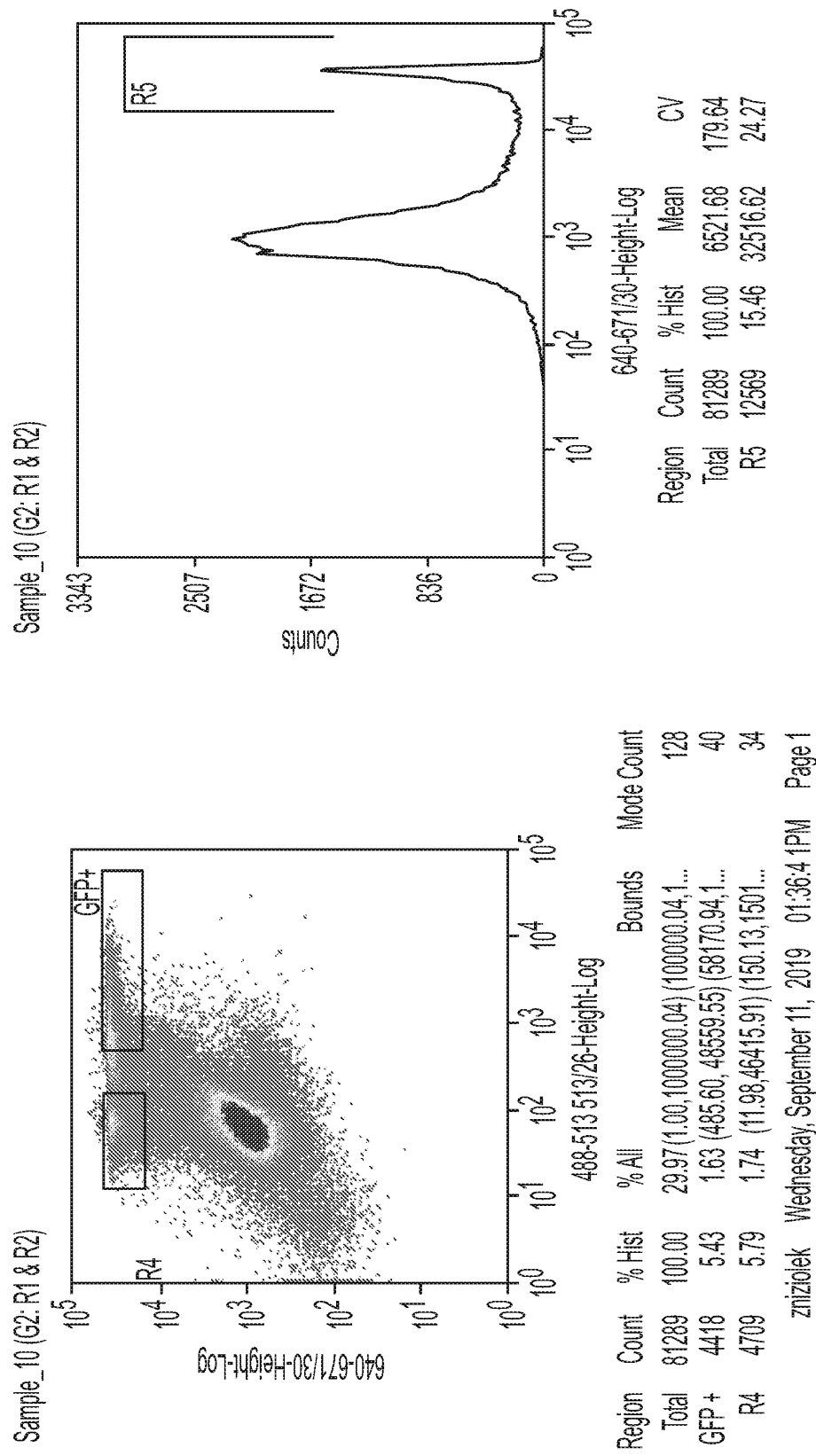


FIG. 58B

### Cloning overview

1. Digest pU6-pegRNA-GG-Vector plasmid (**component 1**) with BsaI and isolate the plasmid fragment (~2.2kb) containing the origin of replication, U6 promoter, U6 poly-T termination sequence, and Amp<sup>R</sup> gene
2. Order oligonucleotides for:
  - a. The desired spacer (target) sequence flanked by indicated overhangs (**component 2**)
    - i. Use the desired target's 5'-3' sequence for the top strand oligonucleotide (including the 5' CACC and 3' GTTTT overhangs) and use the reverse complement of the target sequence for the bottom strand oligonucleotide (including the 5' CTCTAAAC overhang). Spacer sequences must begin with a G nucleotide for efficient transcription initiation.
  - b. The desired pegRNA 3' extension template flanked by the indicated overhangs (**component 3**)
    - i. Use the RNA sense sequence as the top strand oligonucleotide (featuring the 5' GTGC overhang) and use the reverse complement of this sequence for the bottom strand oligonucleotide (featuring the 5' AAAA overhang).
  - c. SpCas9 sgRNA scaffold sequence featuring compatible golden gate overhangs (**component 4**)
    - i. These oligonucleotides are not the complete scaffold sequence, as overhangs from the remaining components contribute several missing nucleotides
    - ii. Note: these oligonucleotides must be 5' phosphorylated. Oligonucleotides can be 5' phosphorylated by the manufacturer or 5' phosphorylated enzymatically using T4 PNK (see protocol below)
3. Anneal top and bottom oligonucleotides for components 2, 3, and 4 in separate annealing reactions according to the protocol below. If the SpCas9 sgRNA scaffold sequence (component 4) was not phosphorylated, phosphorylate with T4 PNK.
4. Golden Gate assembly of isolated 2.2-kb fragment from component 1 with components 2, 3, and 4
5. Transform the ligation product into *E. coli*. The antibiotic resistance conferred by component 1 from the pU6-pegRNA-GG-vector plasmid is ampicillin and carbenicillin resistance.
6. Isolate and sequence plasmids from the resulting clonal transformants

## FIG. 59A

### pegRNA cloning protocol

#### Step 1: Digest pU6-pegRNA-GG-Vector plasmid (component 1)

Combine the following in a PCR tube:

|                                            |            |
|--------------------------------------------|------------|
| 2000 ng pU6-pegRNA-GG-Vector (component 1) | X µL       |
| Bsa1-HFv2 (NEB)                            | 1.0 µL     |
| 10x Cutsmart Buffer                        | 3.0 µL     |
| H <sub>2</sub> O                           | to 30.0 µL |
| Total reaction volume                      | 30.0 µL    |

Incubate at 37 °C for 4-16 hours

Isolate ~2.2-kb fragment from cut plasmid.

## FIG. 59B

**Steps 2 and 3: Order and anneal oligonucleotide parts (components 2, 3, and 4)**Materials

Annealing buffer: H<sub>2</sub>O supplemented with 10 mM Tris-Cl pH 8.5 and 50 mM NaCl

Complementary oligonucleotide pairs

Protocol

Combine the following in a PCR tube:

|                                           |              |
|-------------------------------------------|--------------|
| Top oligonucleotide, 100 $\mu$ M          | 1.0 $\mu$ L  |
| Bottom oligonucleotide, 100 $\mu$ M       | 1.0 $\mu$ L  |
| Annealing buffer (components 2, 3, and 4) | 23.0 $\mu$ L |
| <hr/>                                     |              |
| Total reaction volume                     | 25.0 $\mu$ L |

In thermocycler, heat at 95 °C for 3 minutes, then cool gradually (0.1 °C/s) to 22 °C

Dilute annealed oligonucleotides 1:4 by adding 75  $\mu$ L H<sub>2</sub>O. The final concentration of each oligonucleotide will be 1  $\mu$ M after this dilution. Do not dilute the sgRNA scaffold (component 4) if phosphorylating by PNK in step 2.5.

**FIG. 59C****Step 2.b.ii.: sgRNA scaffold phosphorylation (unnecessary if oligonucleotides were purchased phosphorylated)**Protocol

Combine the following in a PCR tube:

|                                              |               |
|----------------------------------------------|---------------|
| 4 $\mu$ M oligonucleotide duplex from step 1 | 6.25 $\mu$ L  |
| 10x T4 DNA ligase buffer (NEB)               | 2.50 $\mu$ L  |
| T4 PNK (NEB)                                 | 0.50 $\mu$ L  |
| H <sub>2</sub> O                             | 15.75 $\mu$ L |
| <hr/>                                        |               |
| Total reaction volume                        | 20.0 $\mu$ L  |

In thermocycler, incubate at 37 °C for 60 minutes

Following this phosphorylation, annealed scaffold oligonucleotides are now at a concentration of 1  $\mu$ M.

Proceed to step 3.

**FIG. 59D**

**Step 4: peg RNA assembly**pegRNA. Golden Gate assembly reaction

|                                                             |                               |
|-------------------------------------------------------------|-------------------------------|
| Digested pU6-pegRNA-GG plasmid-vector                       | 1.00 $\mu$ L @ 30 ng/ $\mu$ L |
| - Pre-cut, isolated 2.2-kb fragment                         |                               |
| Annealed protospacer oligonucleotides (component 2)         | 1.00 $\mu$ L @ 1 $\mu$ M      |
| Annealed pegRNA 3'-extension oligonucleotides (component 4) | 1.00 $\mu$ L @ 1 $\mu$ M      |
| sgRNA scaffold annealed oligonucleotides (component 3)      | 1.00 $\mu$ L @ 1 $\mu$ M      |
| - Oligonucleotides <i>must be phosphorylated</i>            |                               |
| <i>Bsa</i> I-HFv2 (NEB)                                     | 0.25 $\mu$ L                  |
| T4 DNA ligase (NEB)                                         | 0.50 $\mu$ L                  |
| 10x T4 DNA ligase buffer (NEB)                              | 1.00 $\mu$ L                  |
| H <sub>2</sub> O                                            | 4.25 $\mu$ L                  |
| <hr/>                                                       |                               |
| Total reaction volume:                                      | 10.0 $\mu$ L                  |

Incubate at room temperature for 10 min

Following incubation, perform the following program in a thermocycler:

5 min at 37 °C then 5 min at 80 °C then hold at 12 °C

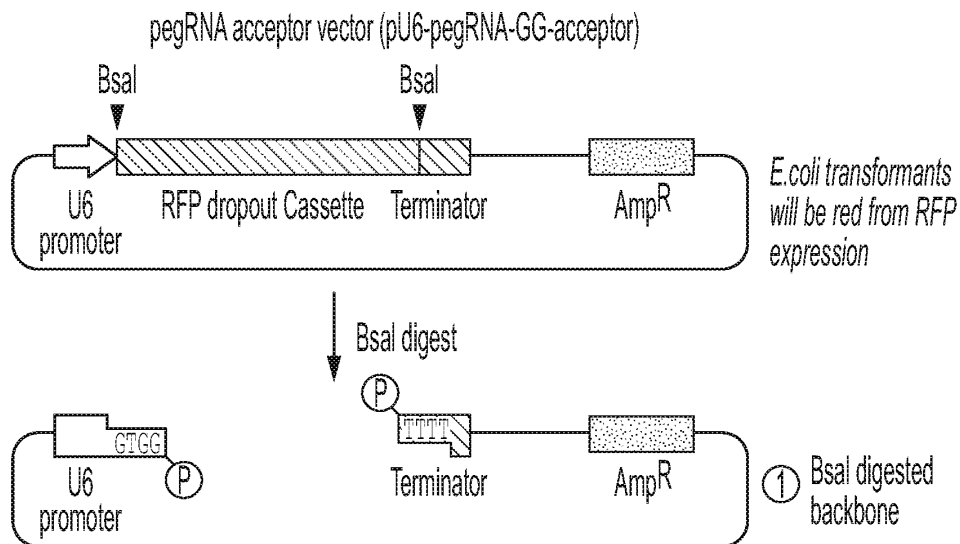
**FIG. 59E****Steps 5 and 6: Transformation of assembled plasmids**

Transform 1  $\mu$ L of the 10  $\mu$ L assembly reaction into 10  $\mu$ L of competent cells. The desired transformants will be resistant to ampicillin and carbenicillin.

The following diagram summarizes the pegRNA cloning protocol.

**FIG. 59F**

## pegRNA Cloning by Golden Gate Assembly



② pegRNA spacer annealed oligos

SEQ ID NO: 879

5' CACCNNNNNNNNNNNNNNNNNNNNGTTT 3'  
3' NNNNNNNNNNNNNNNNNNNNCAAAATCTC 5'

SEQ ID NO: 880

③ pegRNA 3' extension annealed oligos

SEQ ID NO: 883

[illegible]

SEQ ID NO: 884

④ pegRNA scaffold annealed oligos (5' phosphorylated)

SEQ ID NO: 881

5' [Phos]-AGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCGGTTATCAACTTGAAAAGTGGCACCGAGTCG 3'  
3' GATCTTTATCGTTCAAATTTTATCCGATCAGGCAATAGTTGAACTTTTTCACCGTGGCTCAGCCAG-[Phos] 5'

SEQ ID NO: 882

(Note that these oligos must be 5' phosphorylated to permit pegRNA vector assembly)

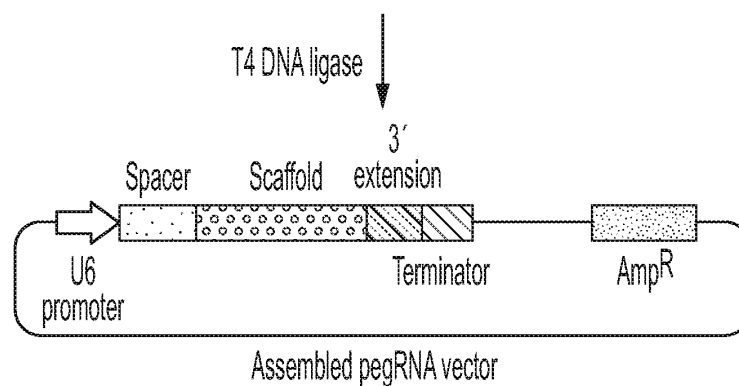


FIG. 59G

```

## sgRNA scaffold sequence search ##

import pandas as pd
import Bio as bio
from Bio import SeqIO
import glob

#generates list of fastq files to analyze
sources = glob.glob("*.fastq")

#reads the fastq files into a dictionary with the file names as keys
fastqdict = {}
for i in range(len(sources)):
    temp = list(SeqIO.parse(sources[i], "fastq"))
    fastqdict[sources[i]] = [str(temp[k].seq) for k in range(len(temp))]

#the referenced sequence to be searched for is entered into the following dictionary with
#an appropriate key
scaffdict =
{'HEK3': 'CAGAGGACCGACTCGGTCCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACCTCACGTGCTCAGTCTGGGCCGGTG', (SEQ ID NO: 885)}
{'EMX1': 'ATCACGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TGCTATTTCTAGCTCTAAAACCTTCTTCTGCTCGGACTCGGTG', (SEQ ID NO: 886)}
{'FANCF': 'TTTCCGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TTGCTATTTCTAGCTCTAAAACGGTGCTGCAGAAGGGATTCCGGTG', (SEQ ID NO: 887)}
{'RNF2': 'TCGTTGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACCGGTAATGACTAAGATGACGGTG'} (SEQ ID NO: 888)

#matches and counts iterative slices of the reference string to the appropriate fastq files
#reference key must be contained in the name of the fastq file
#generated values represent cumulative counts for a minimum degree of sgRNA integration
#i.e. a given value x means x reads contain y or more bases of the scaffold
resultdict = dict.fromkeys(sources)
for key in fastqdict:
    for scaffold in scaffdict:
        if scaffold in str(key):
            resultlist = []
            for j in range(len(scaffdict[scaffold])):

```

FIG. 60A

```
        extent = scaffold[scaffold][0:(j+1)]
        counter = 0
        for i in range(len(fastqdict[key])):
            if extent in fastqdict[key][i]:
                counter = counter + 1
        resultlist.append(counter)
        resultdict[key]=resultlist

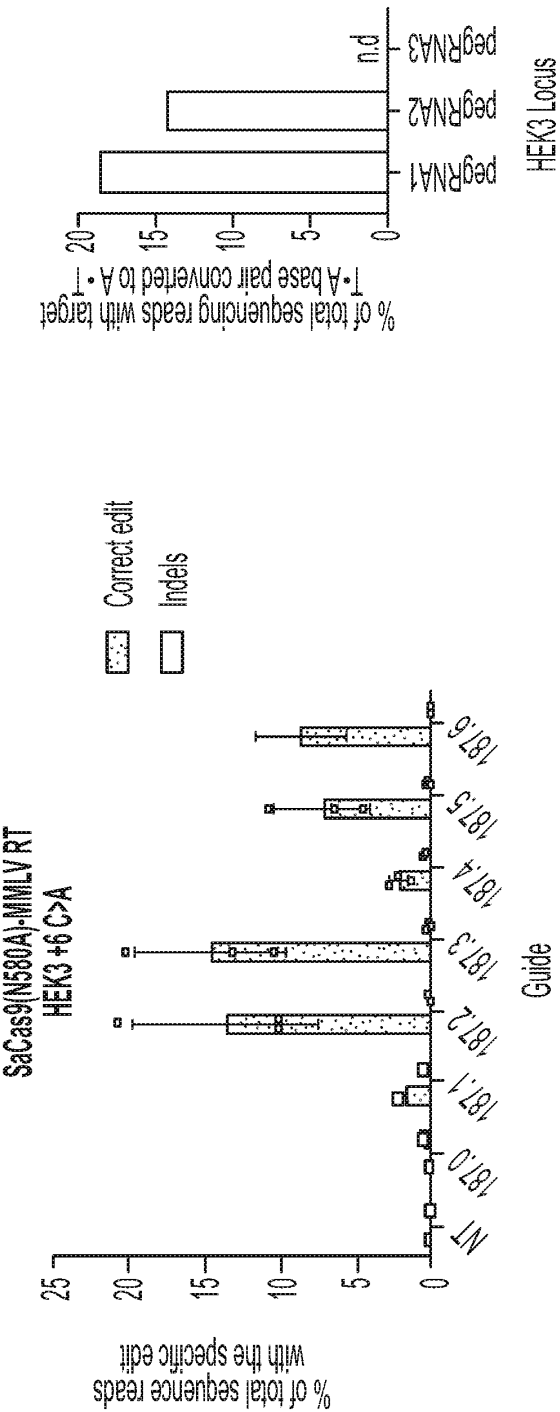
#writes the results into a dataframe indexed from 1
resultdf = pd.DataFrame.from_dict(resultdict)
resultdf = resultdf.reindex(sorted(resultdf.columns), axis=1)
resultdf.index = range(1,len(resultdf)+1)

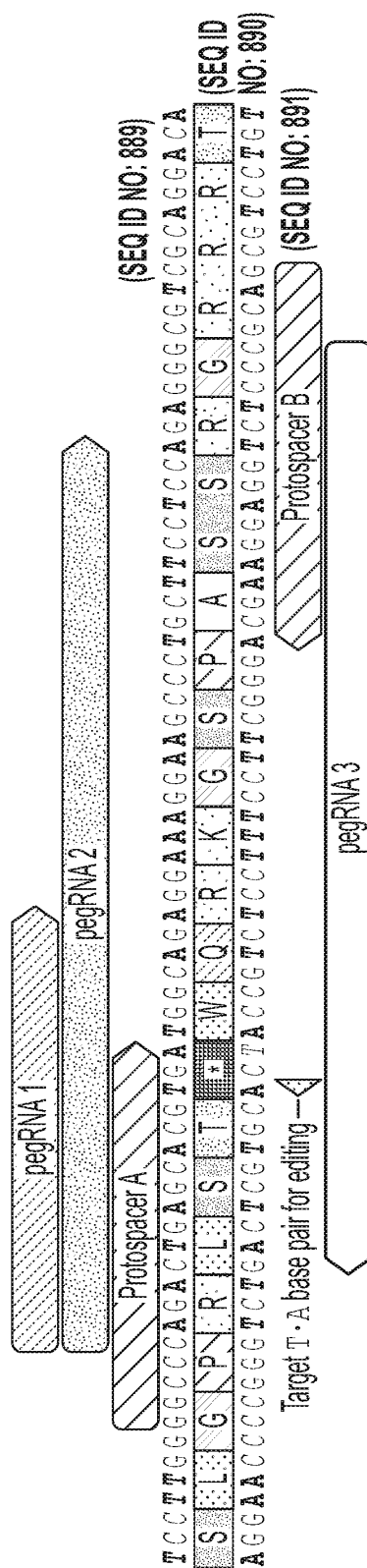
#converts the cumulative count values into specific counts
#i.e. a given value x means x reads contain exactly y bases of the scaffold
resultdf2=resultdf.copy()
for entry in resultdf:
    for i in range(1,len(resultdf[entry])+1):
        try:
            resultdf2[entry][i] = resultdf[entry][i]-resultdf[entry][i+1]
        except:
            resultdf2[entry][i] = resultdf[entry][i]

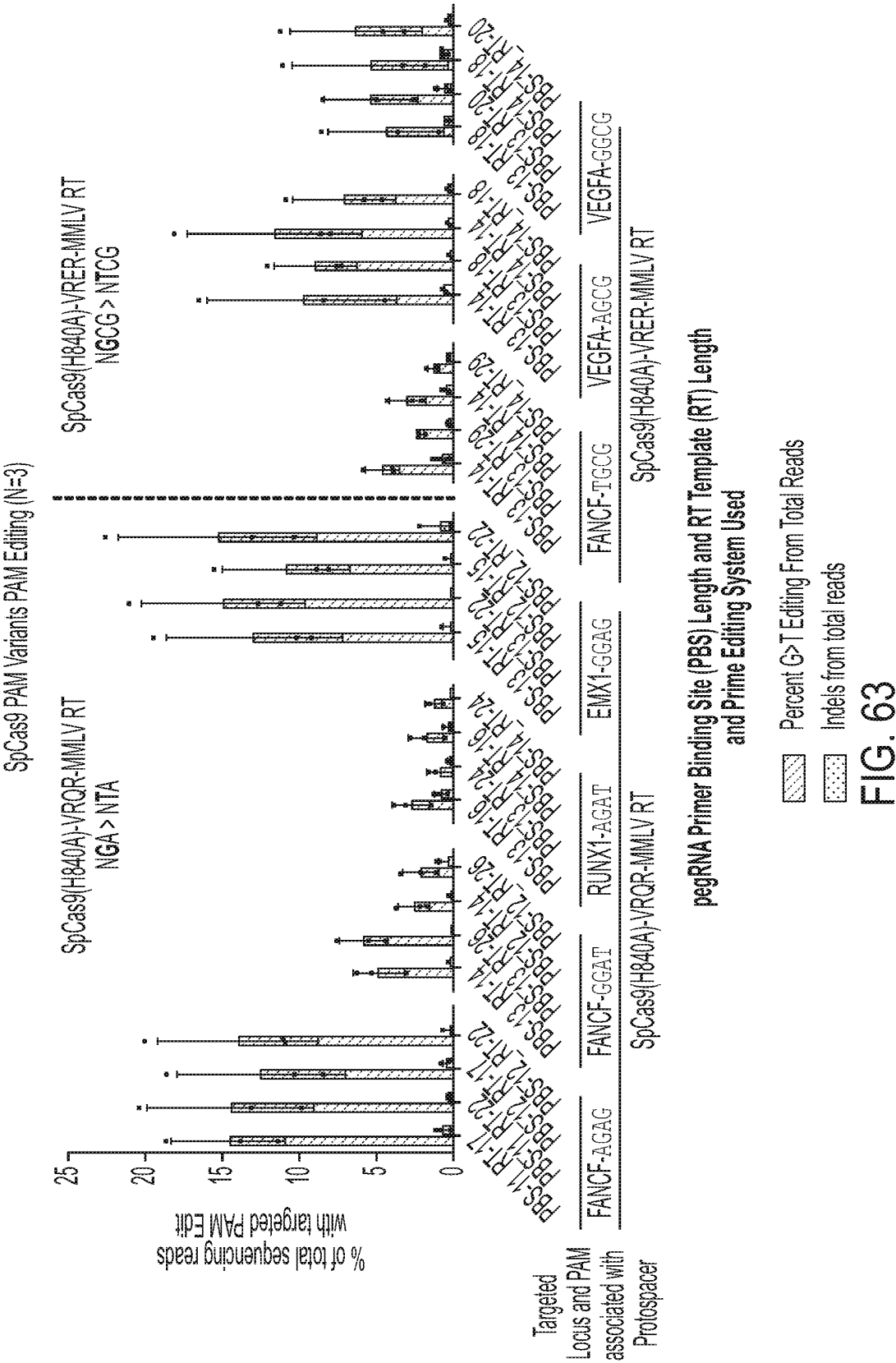
#converts the specific counts values into frequencies
resultdf3=resultdf2.copy()
for entry in resultdf3:
    resultdf3[entry]=resultdf2[entry].div(resultdf[entry][1])*100

#reads the results into excel files
resultdf.to_excel('cumulativecounts.xlsx')
resultdf2.to_excel('specificcounts.xlsx')
resultdf3.to_excel('specificfrequencies.xlsx')
```

FIG. 60B







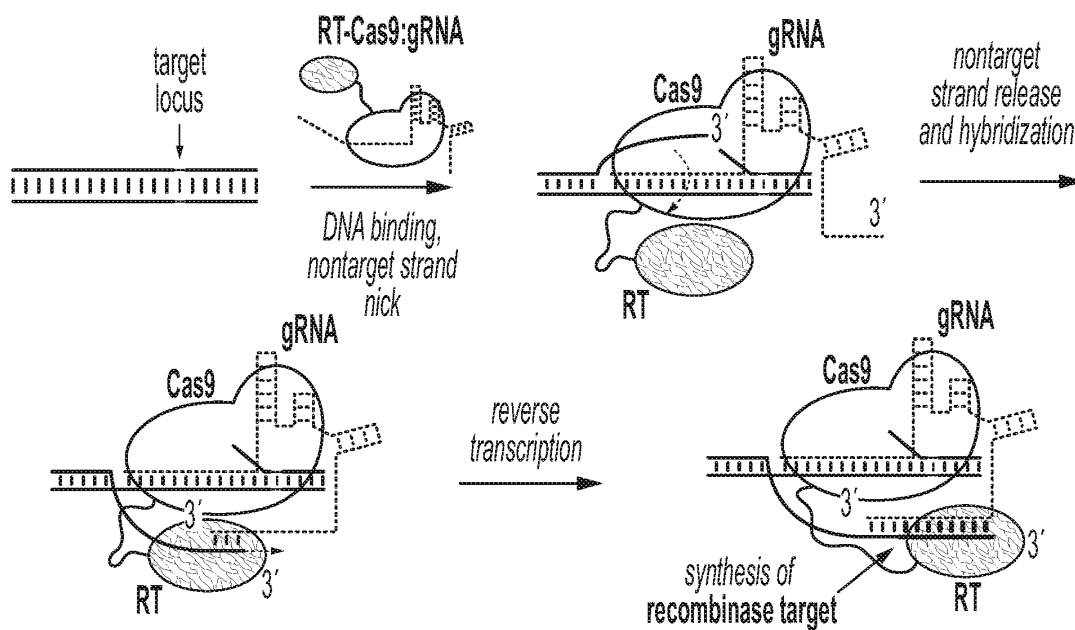


FIG. 64A

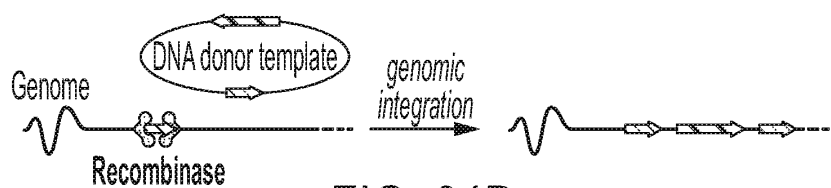


FIG. 64B



FIG. 64C



FIG. 64D



FIG. 64E

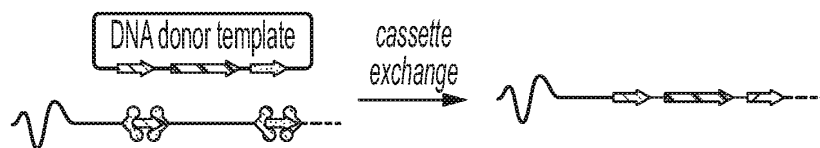


FIG. 64F

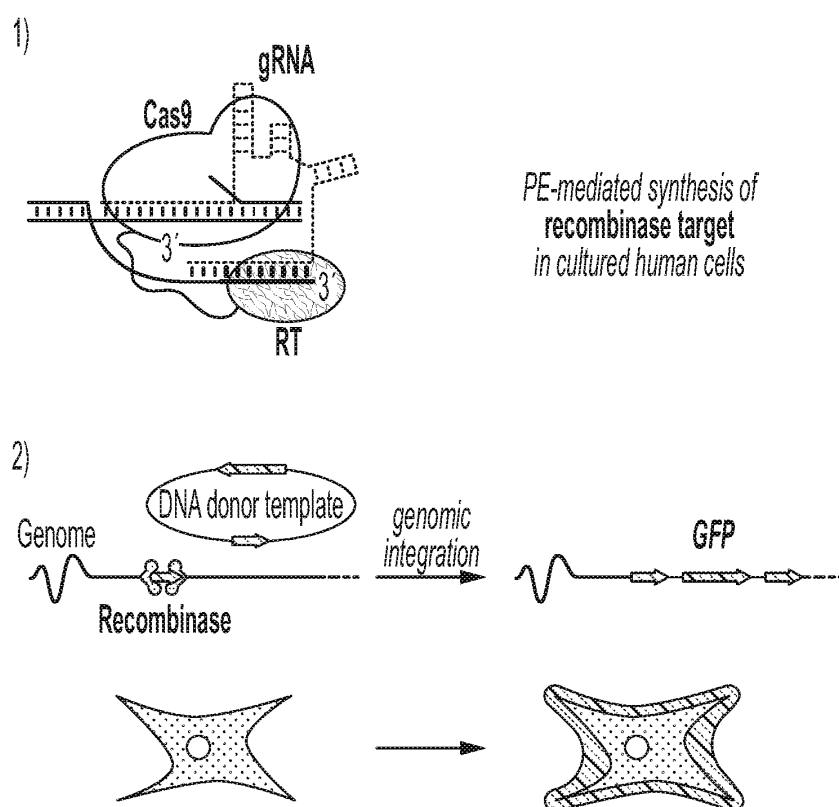
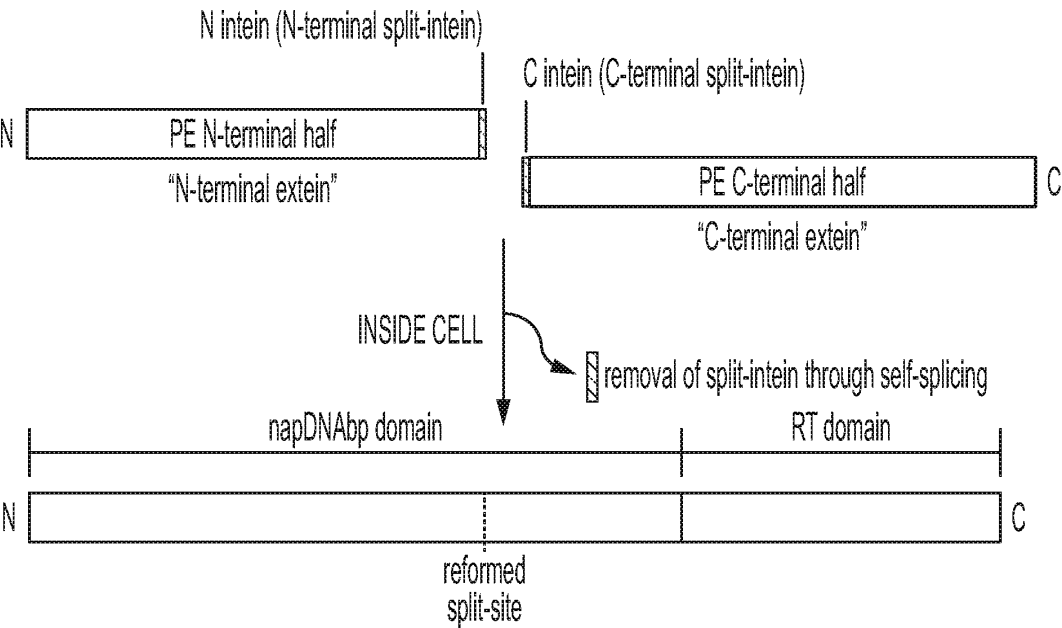


FIG. 65



Prime Editor

FIG. 66

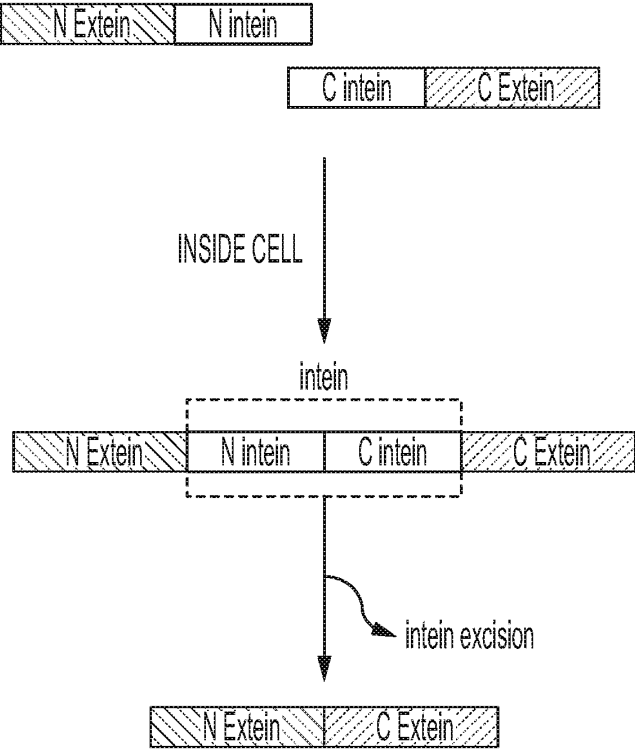
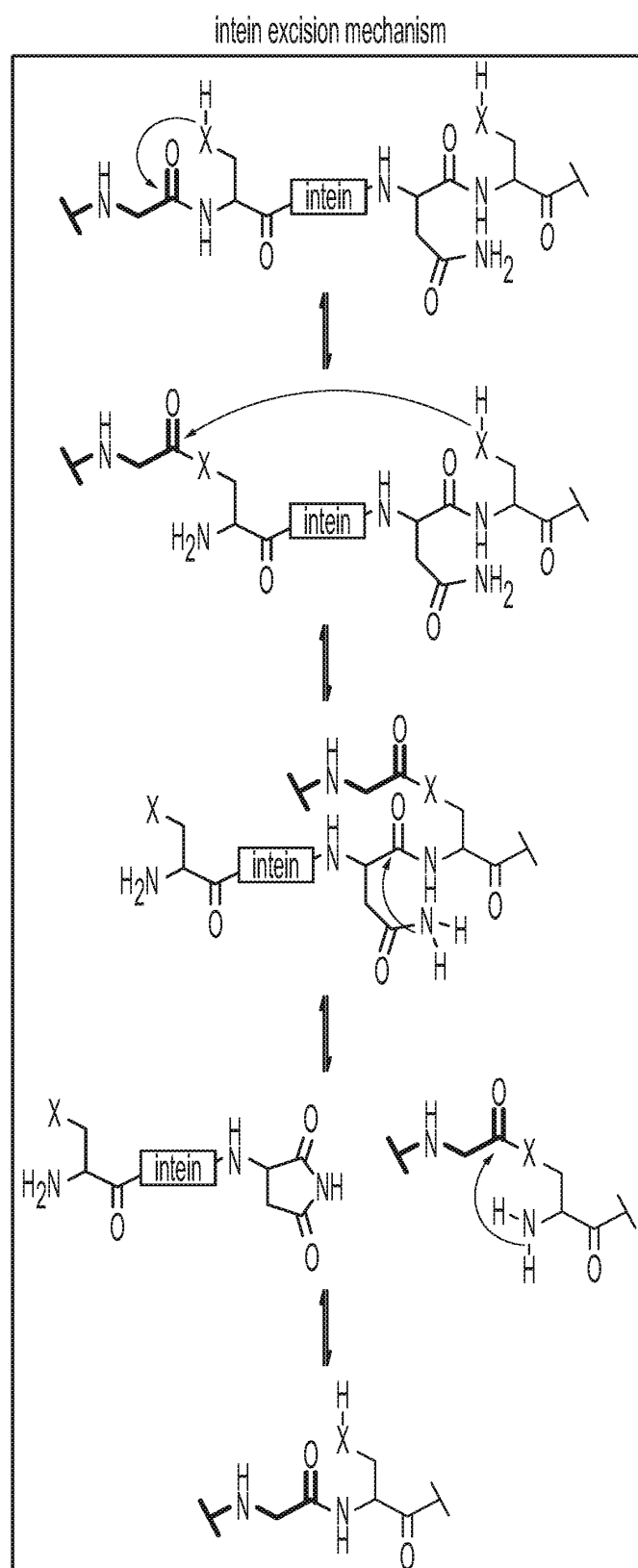


FIG. 67A



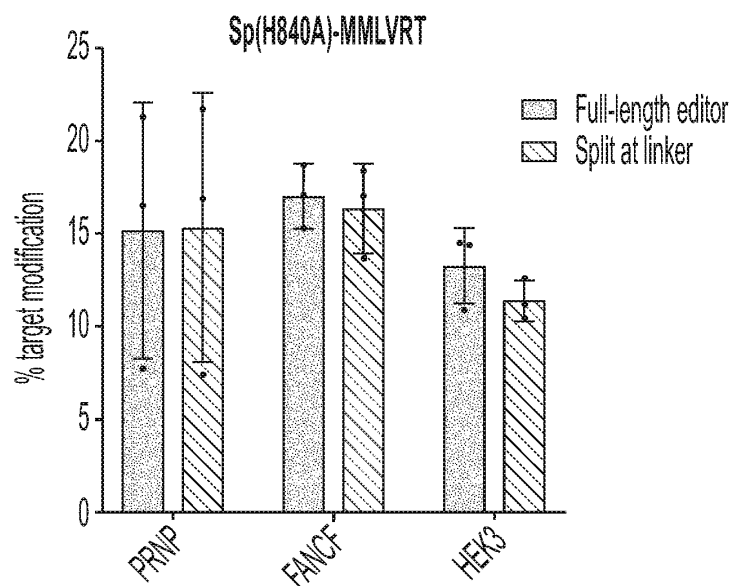


FIG. 68A

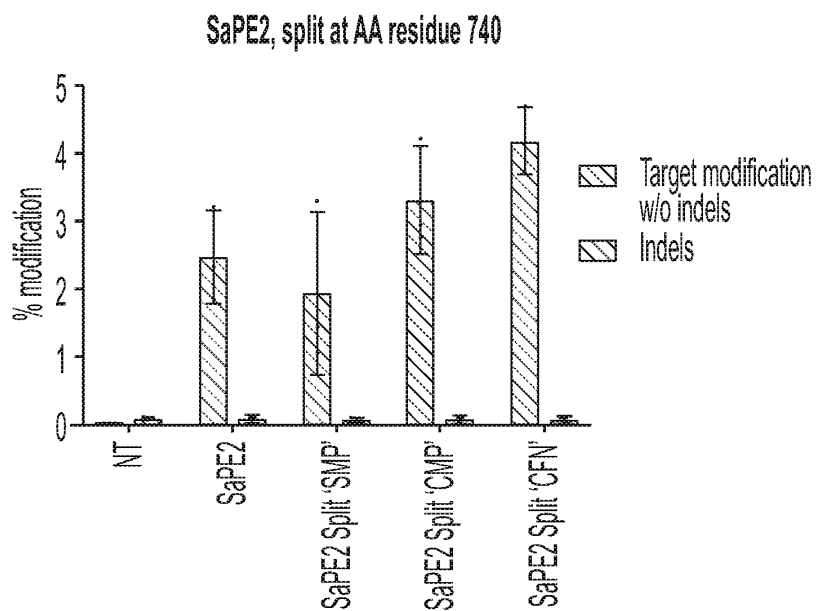


FIG. 68B

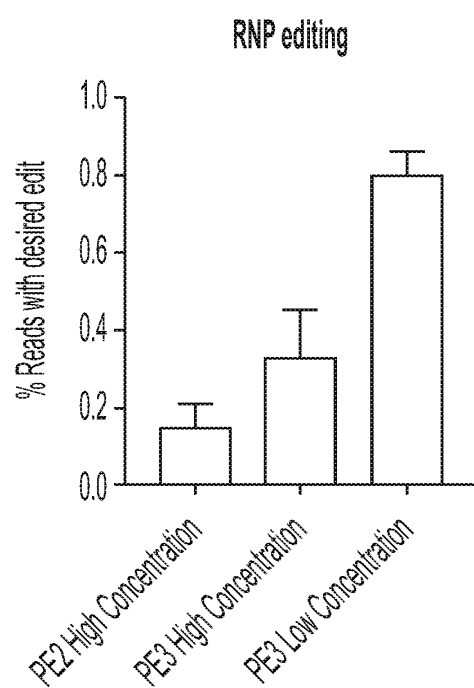


FIG. 68C

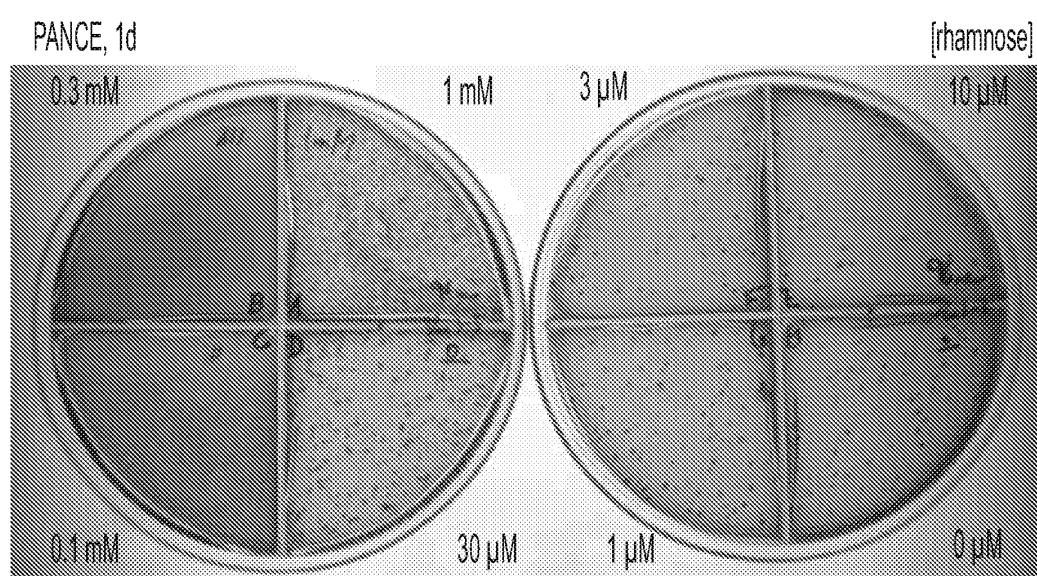


FIG. 69

Starting allele

(SEQ ID NO: 892)

5'- TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT  
ATAATAATGAGATACAAGATAAATTCAAAAGTACAAGATTTTACATAGGGTCAAATGTGCAGAGTA  
(SEQ ID NO: 893)

Edit Target (A•T to C•G)

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTCAGGTGTTTCGTTGTAACATATAAACTGAGTTCC  
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'  
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70A

Starting allele

(SEQ ID NO: 892)

5'- TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT  
ATAATAATGAGATACAAGATAAATTCAAAAGTACAAGATTTTACATAGGGTCAAATGTGCAGAGTA  
(SEQ ID NO: 893)

NGG PAM 1

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTCAGGTGTTTCGTTGTAACATATAAACTGAGTTCC  
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

NGG PAM 2

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'  
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70B

Starting allele

(SEQ ID NO: 892)

5'- TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT  
ATAATAATGAGATACAAGATAAATTCAAAAGTACAAGATTTTACATAGGGTCAAATGTGCAGAGTA  
(SEQ ID NO: 893)

nick for PAM 1

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTCAGGTGTTTCGTTGTAACATATAAACTGAGTTCC  
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

nick for PAM 2

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'  
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70C

Starting allele

(SEQ ID NO: 892)

5' - TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT  
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

Spacer

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATATAAACTGAGTTCC  
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'  
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGAATCTGTAACGACCTTTATTTCTTC

Spacer = GTCATCTTAGTCATTACCTG

FIG. 70D

Starting allele

(SEQ ID NO: 892)

5' - TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT  
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

PBS complement

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATATAAACTGAGTTCC  
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'  
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGAATCTGTAACGACCTTTATTTCTTC

PBS = GTAATGACTAAGATG

FIG. 70E

Desired allele

(SEQ ID NO: 892)

5' - TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT  
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

RT template complement

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTGCGGTGTTTCGTTGTAACATATATAAACTGAGTTCC  
TACGGGGAACCGTCAGTAGAATCAGTAATGGACGCCACAAGCAACATTGAGTATATTTGACTCAAGG

Edit

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'  
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGAATCTGTAACGACCTTTATTTCTTC

RT template = AACGAACACCGCAG

FIG. 70F



FIG. 70G

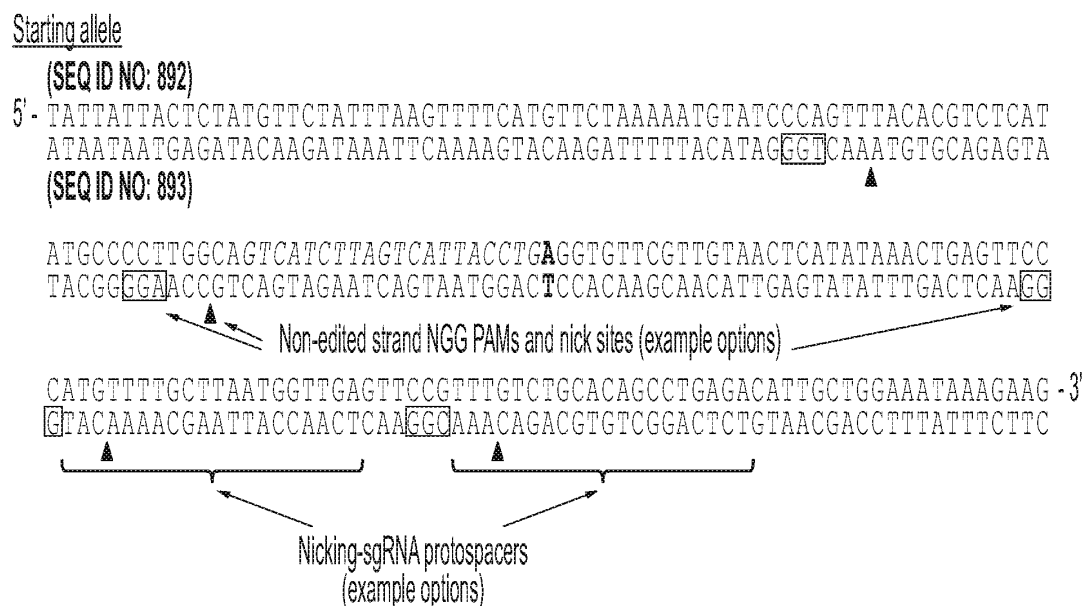


FIG. 70H

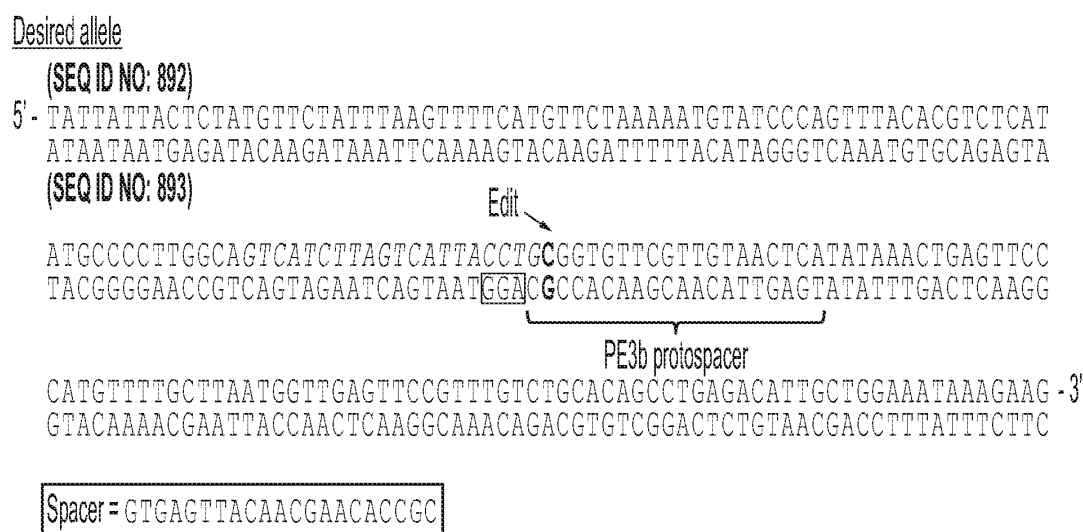


FIG. 70I



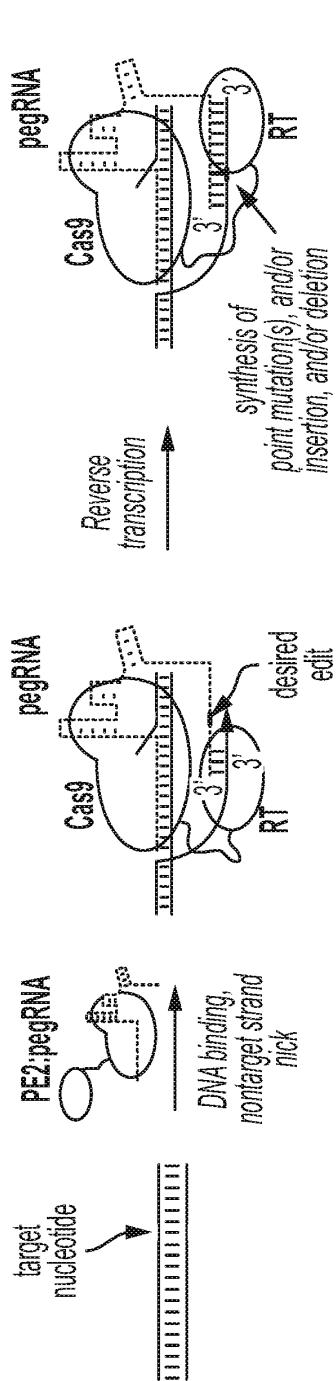


FIG. 72A

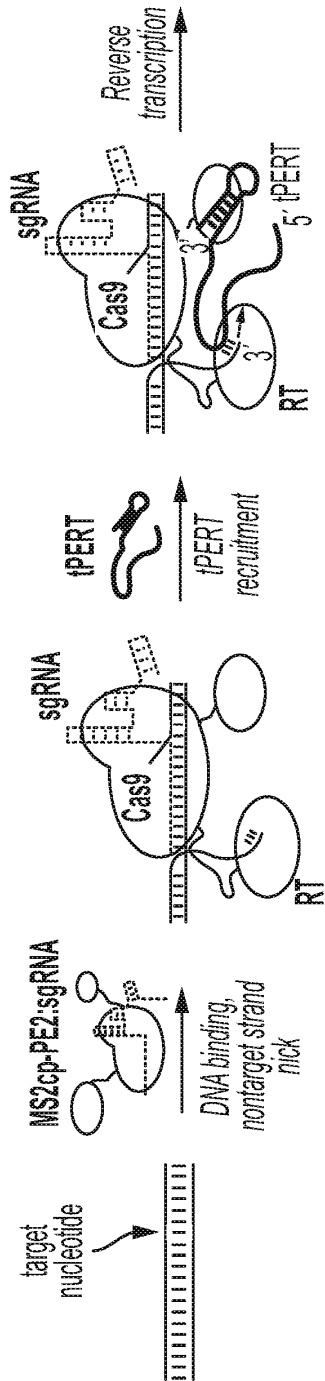
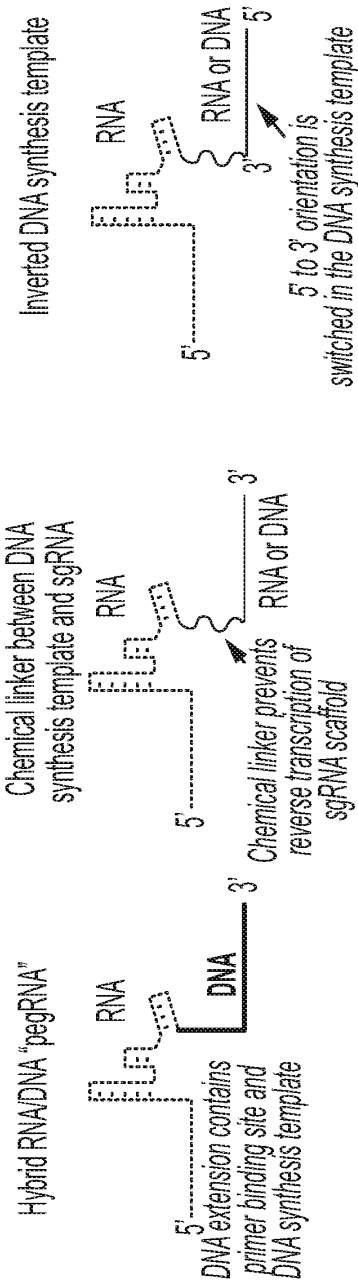


FIG. 72B



DNA-dependent DNA polymerase  
can be used in place of reverse transcriptase

FIG. 72C



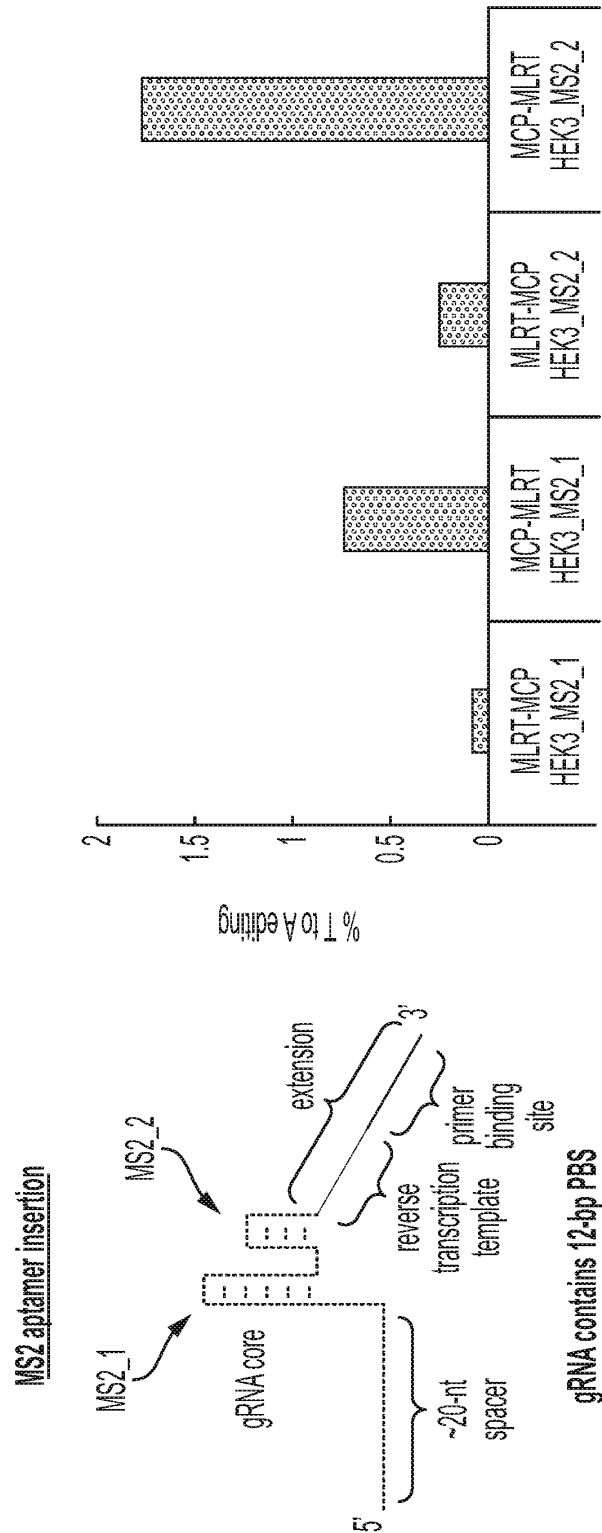


FIG. 74

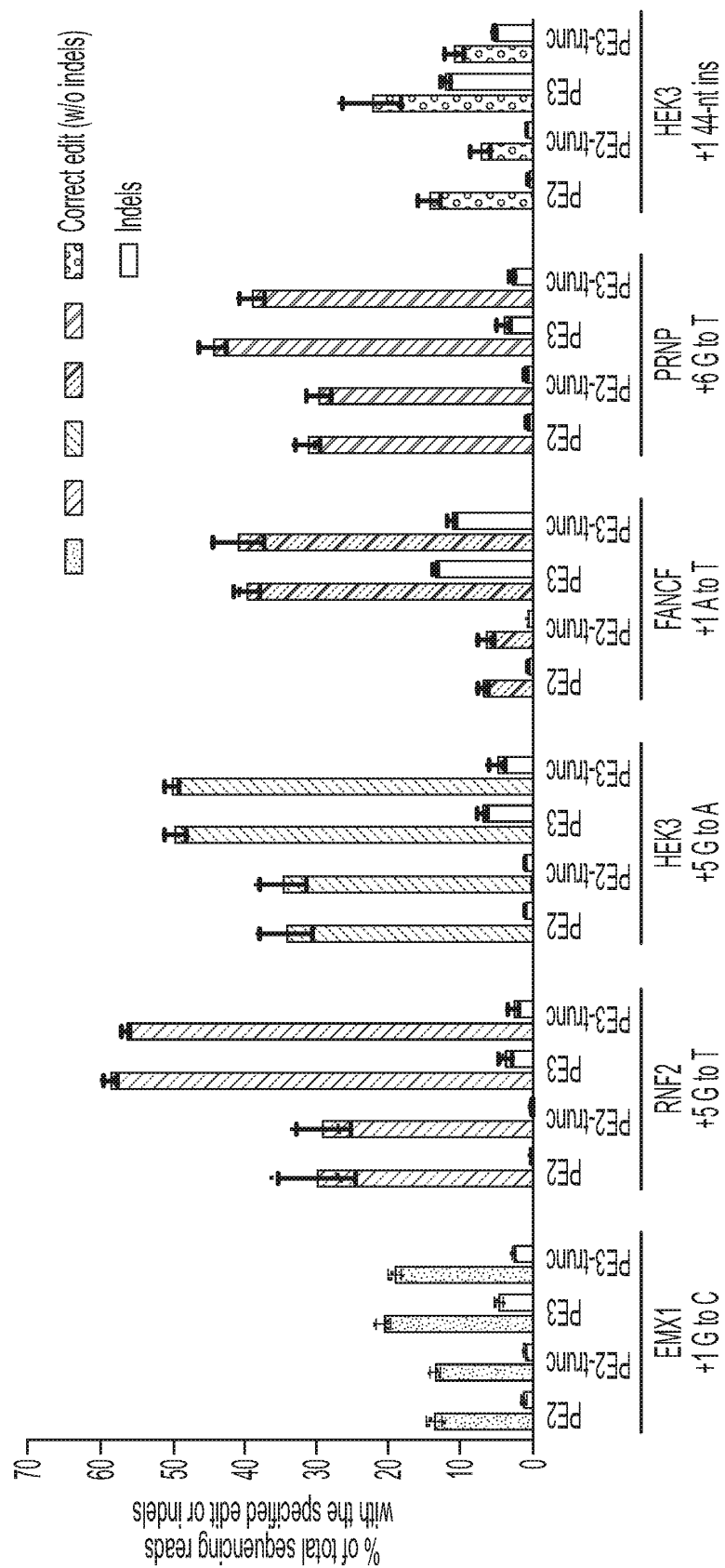


FIG. 75

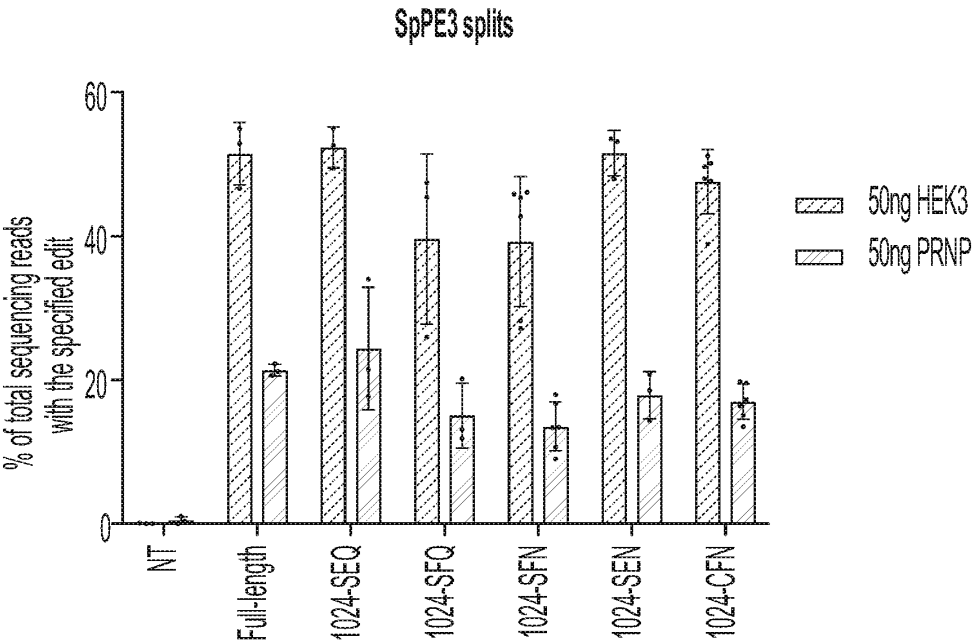


FIG. 76

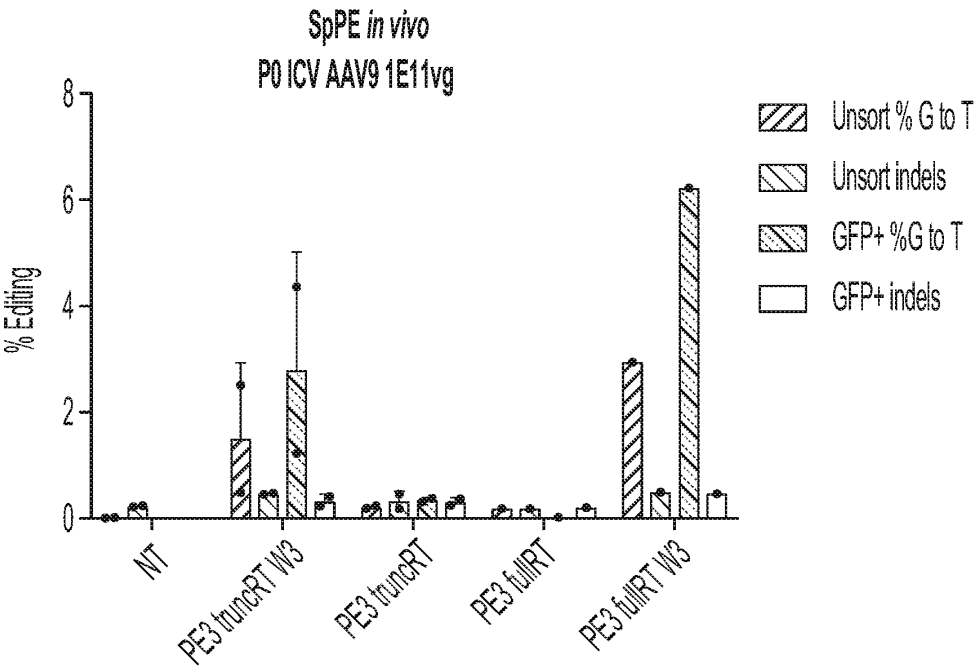


FIG. 77

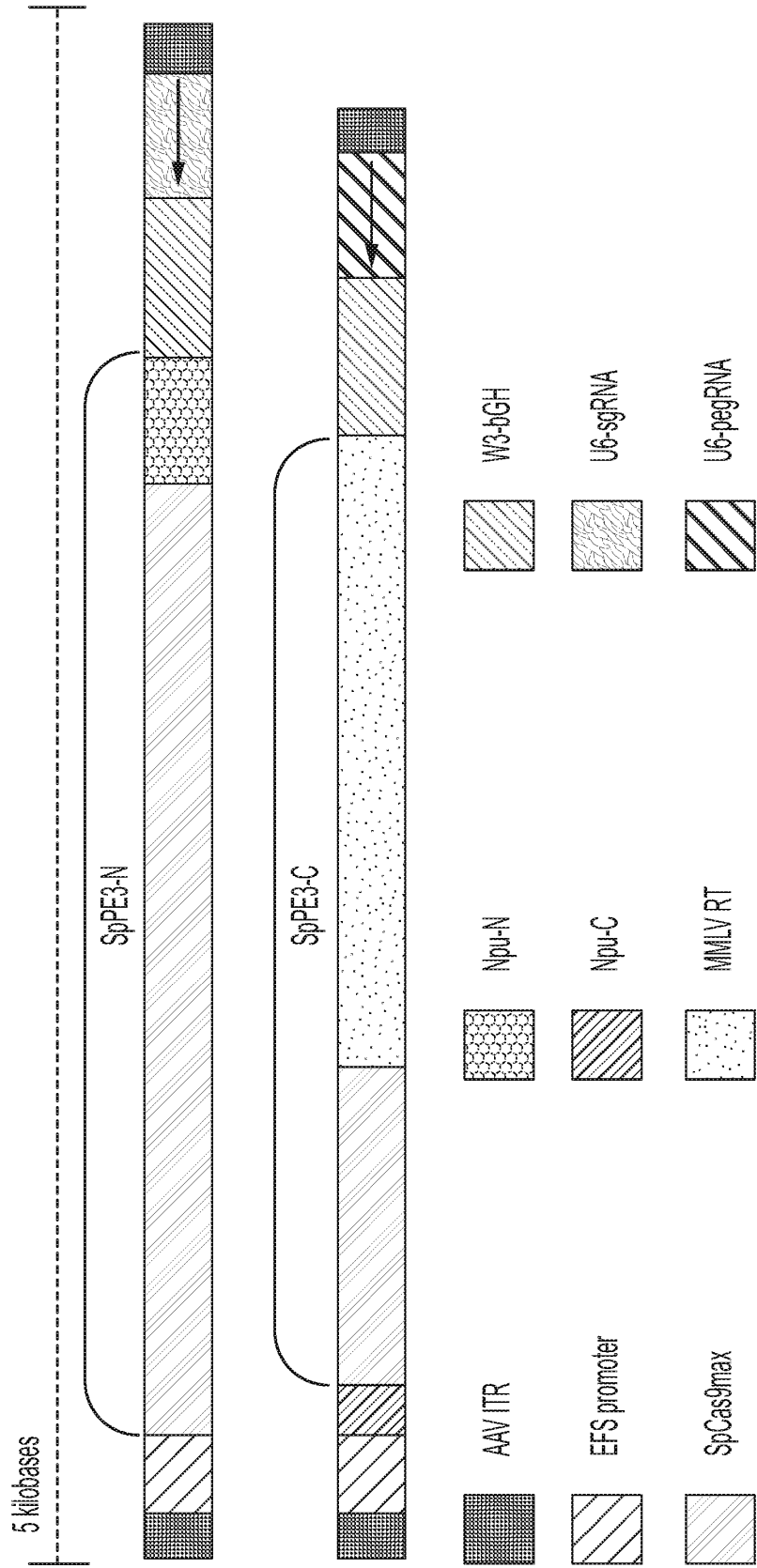


FIG. 78

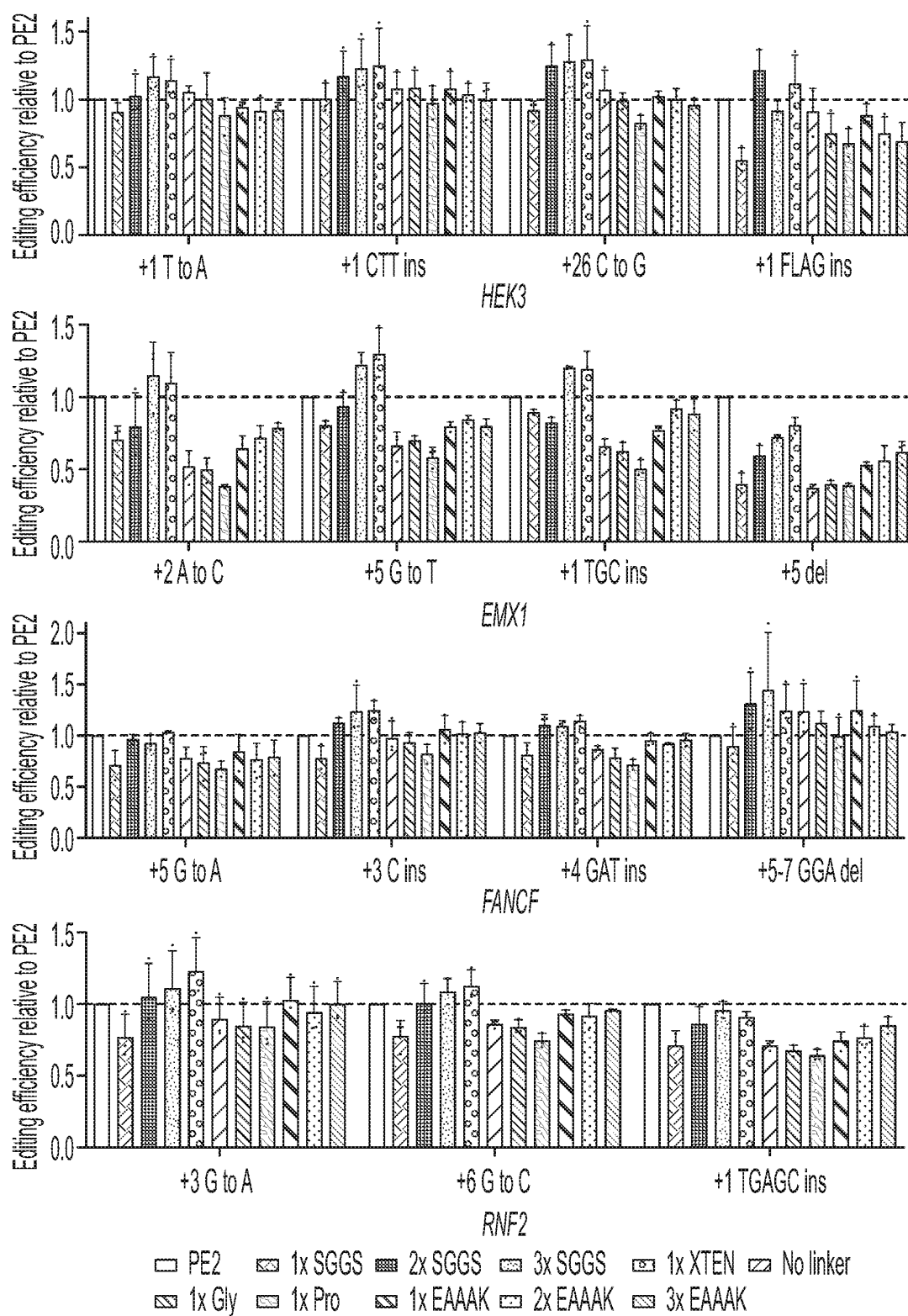


FIG. 79

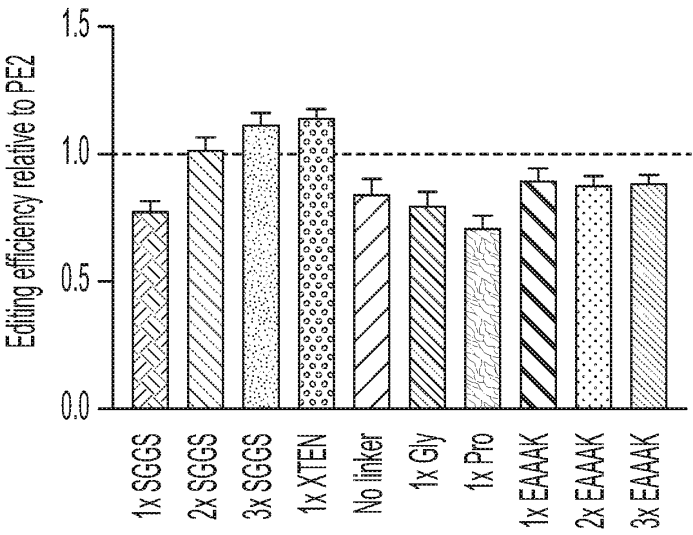


FIG. 80

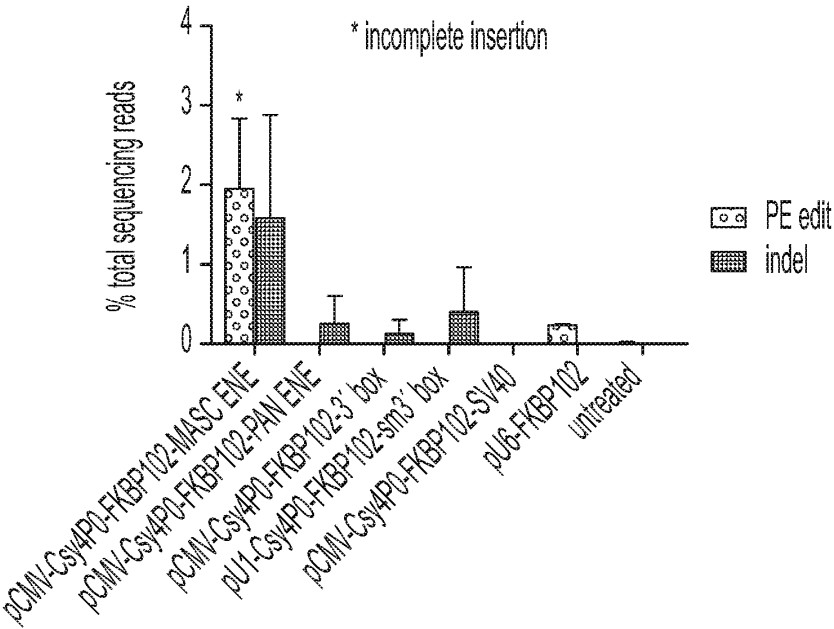


FIG. 81

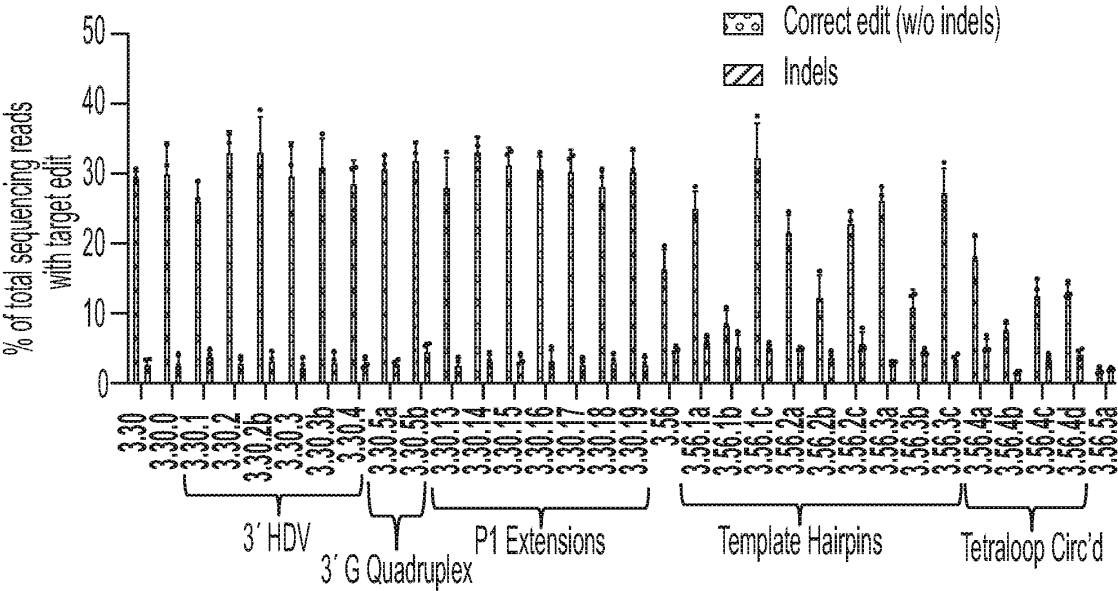


FIG. 82

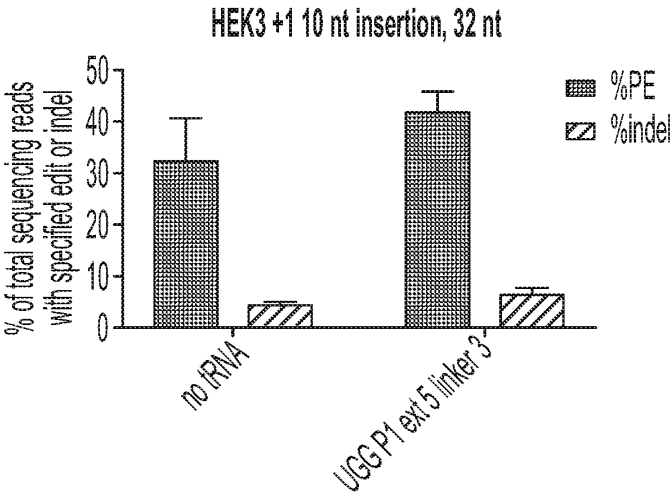


FIG. 83

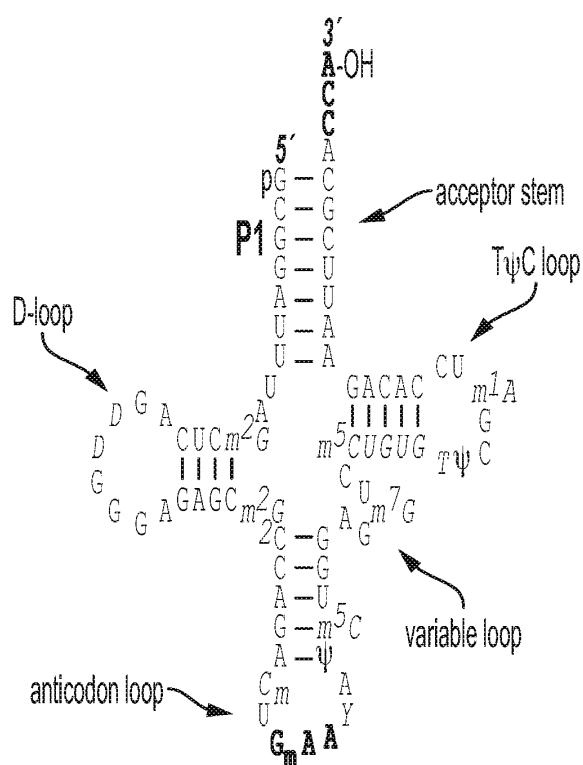


FIG. 84A

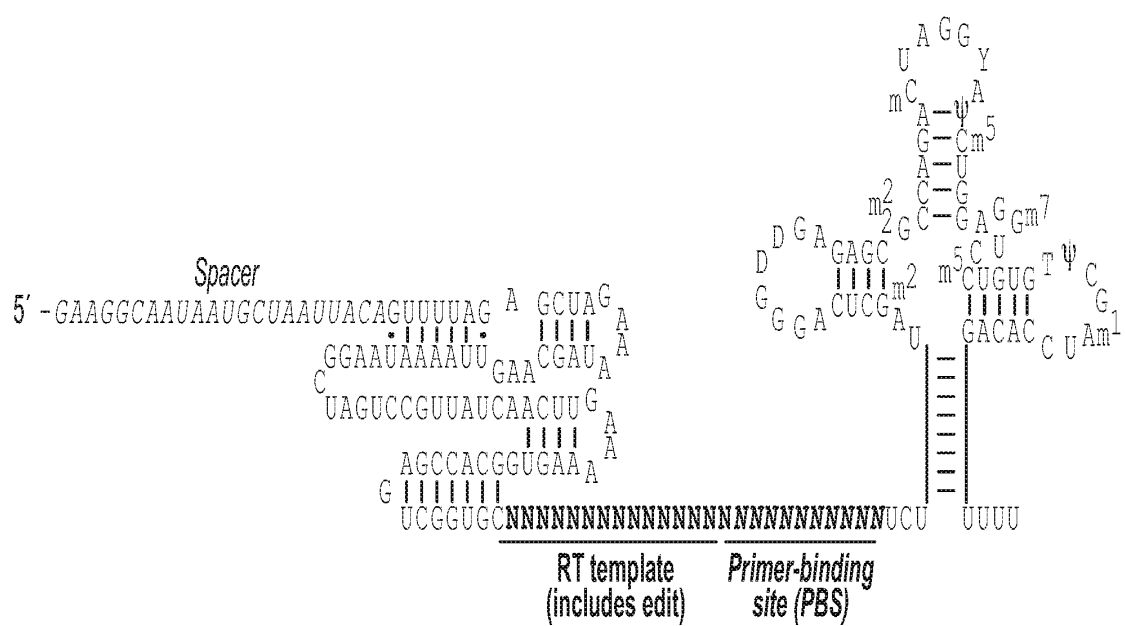


FIG. 84B

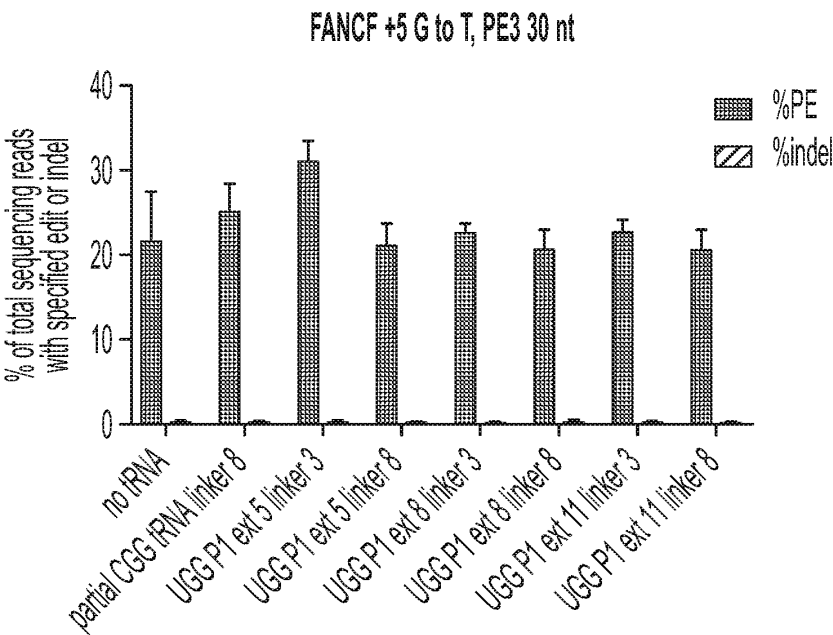


FIG. 85

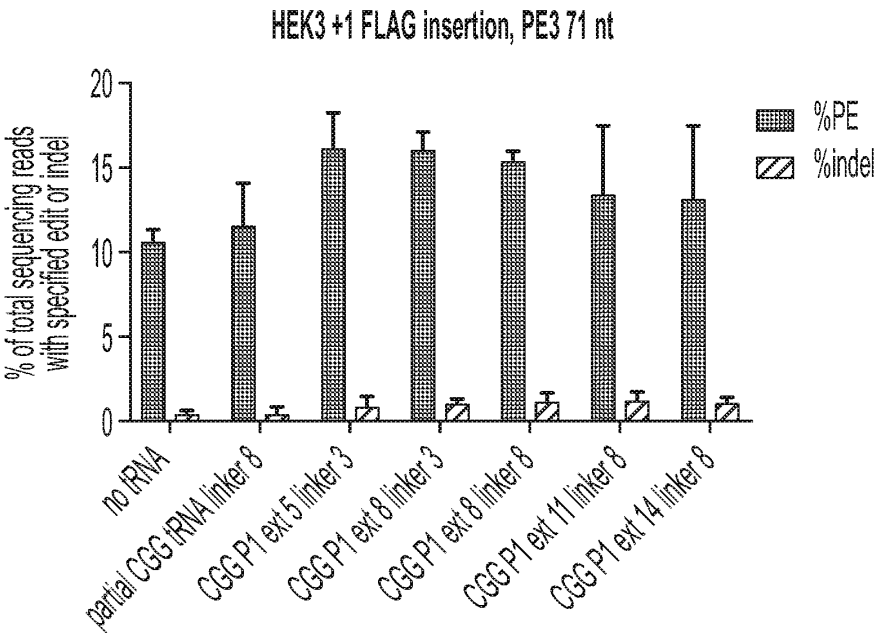


FIG. 86

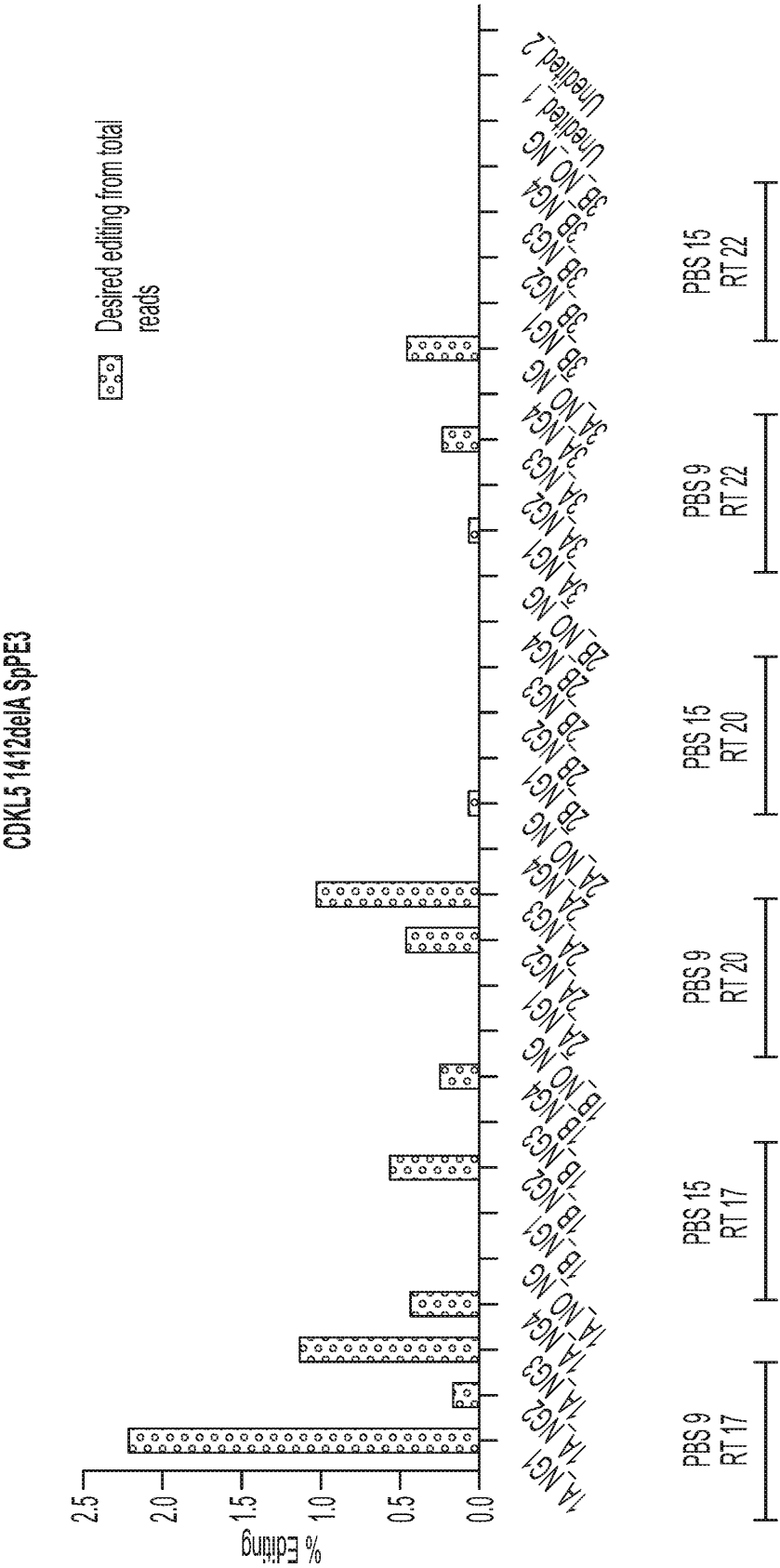
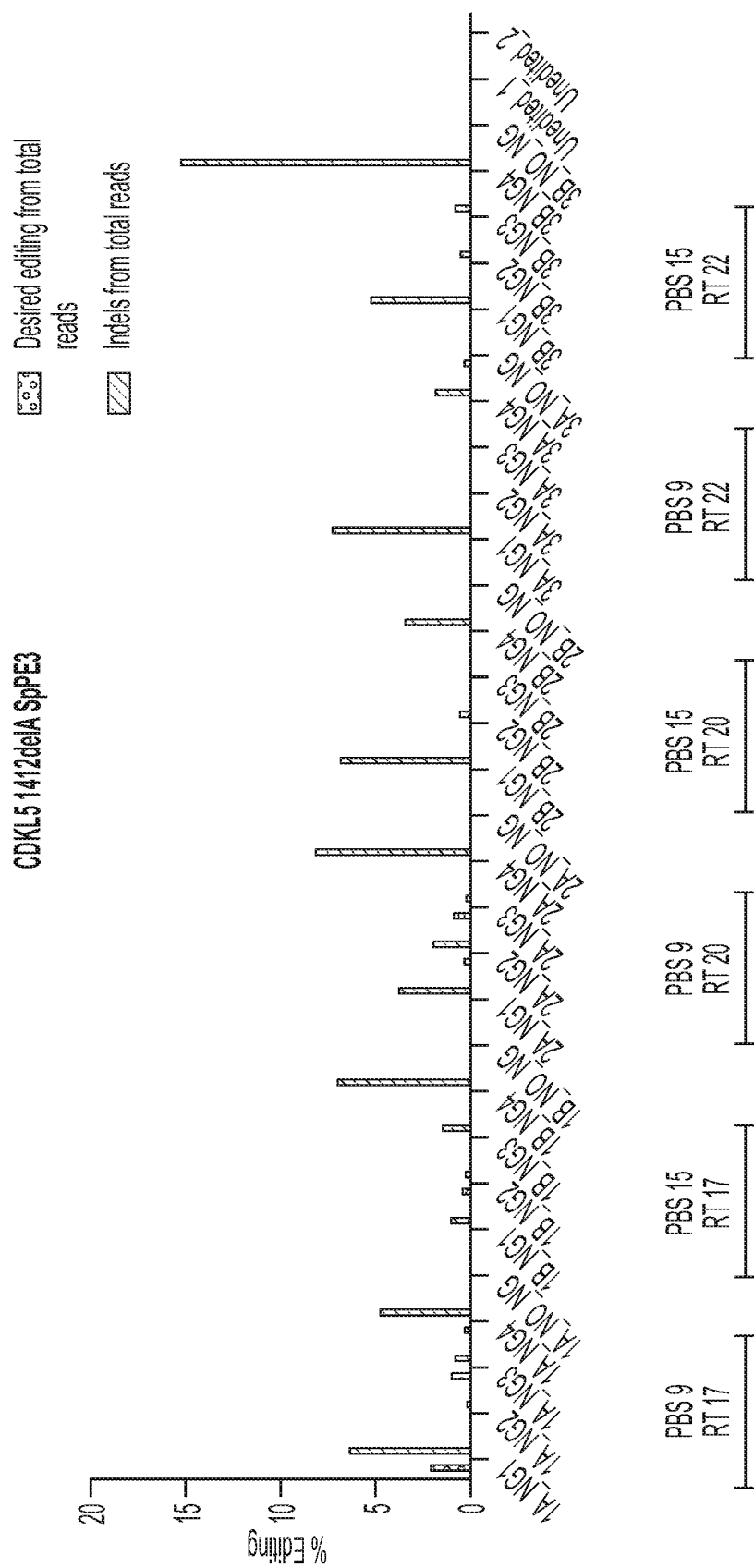


FIG. 87



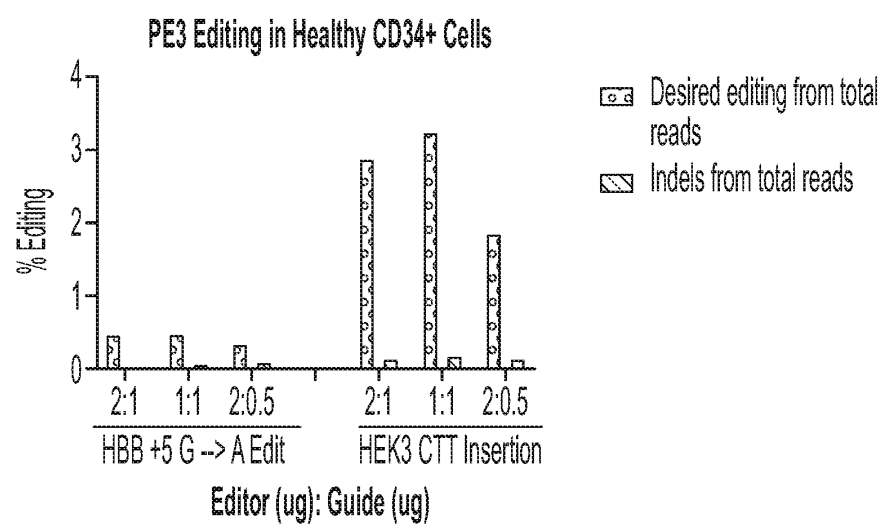


FIG. 89

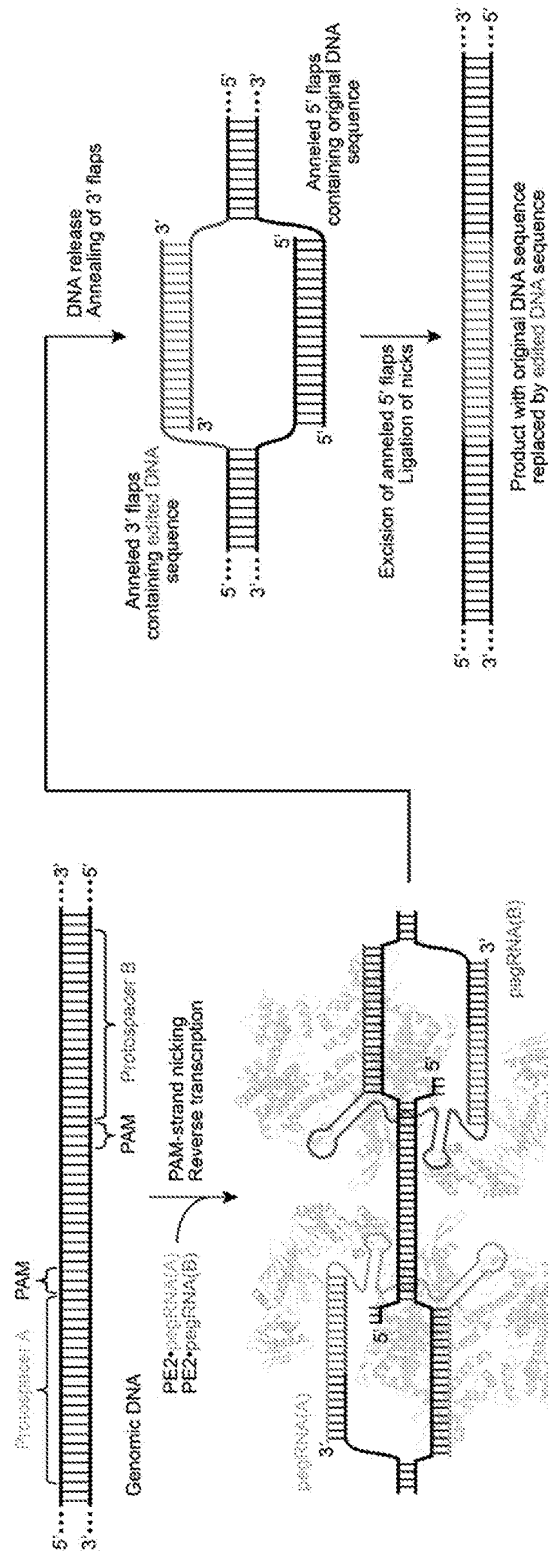


FIG. 90

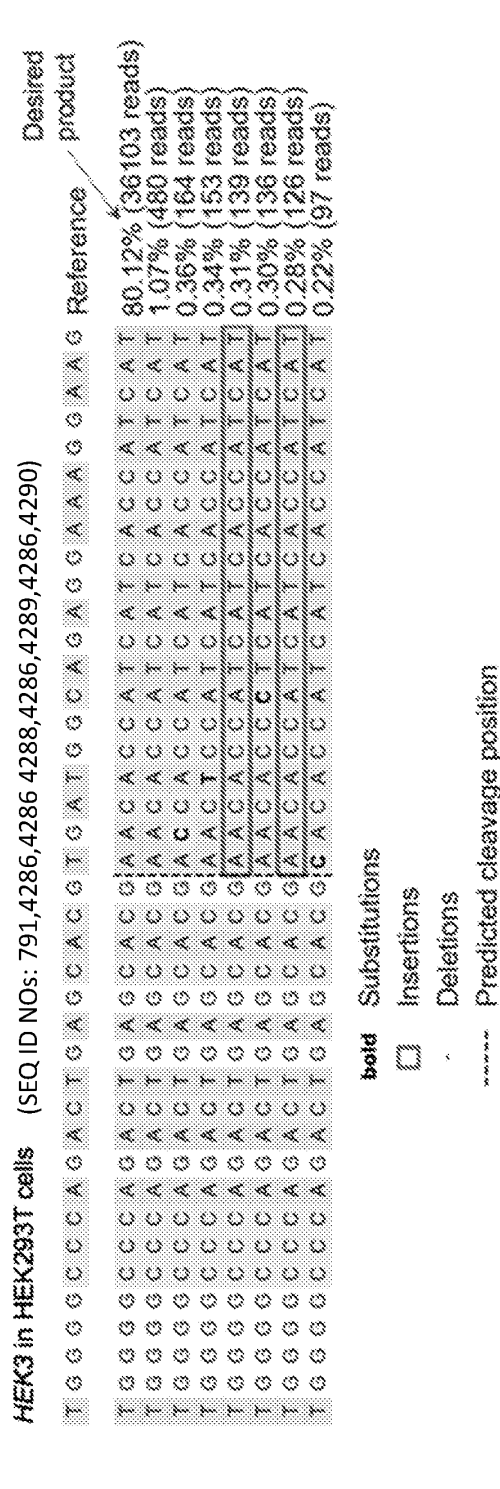


FIG. 91A

pegRNA1: (SEQ ID NO: 4291)  
5' – GGCCCAGACTGAGCAGCGTGAGTTTATAGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTC  
CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCAAATGATGGTGATGATGGTTCGTCAGTCTG – 3'

pegRNA2: (SEQ ID NO: 4292)  
5' – GTCACCCAGTATCCCGTGGTTTATAGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTC  
CGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCACACCATCATCACCATCAITTCGGGATACTGG – 3'

FIG. 91B

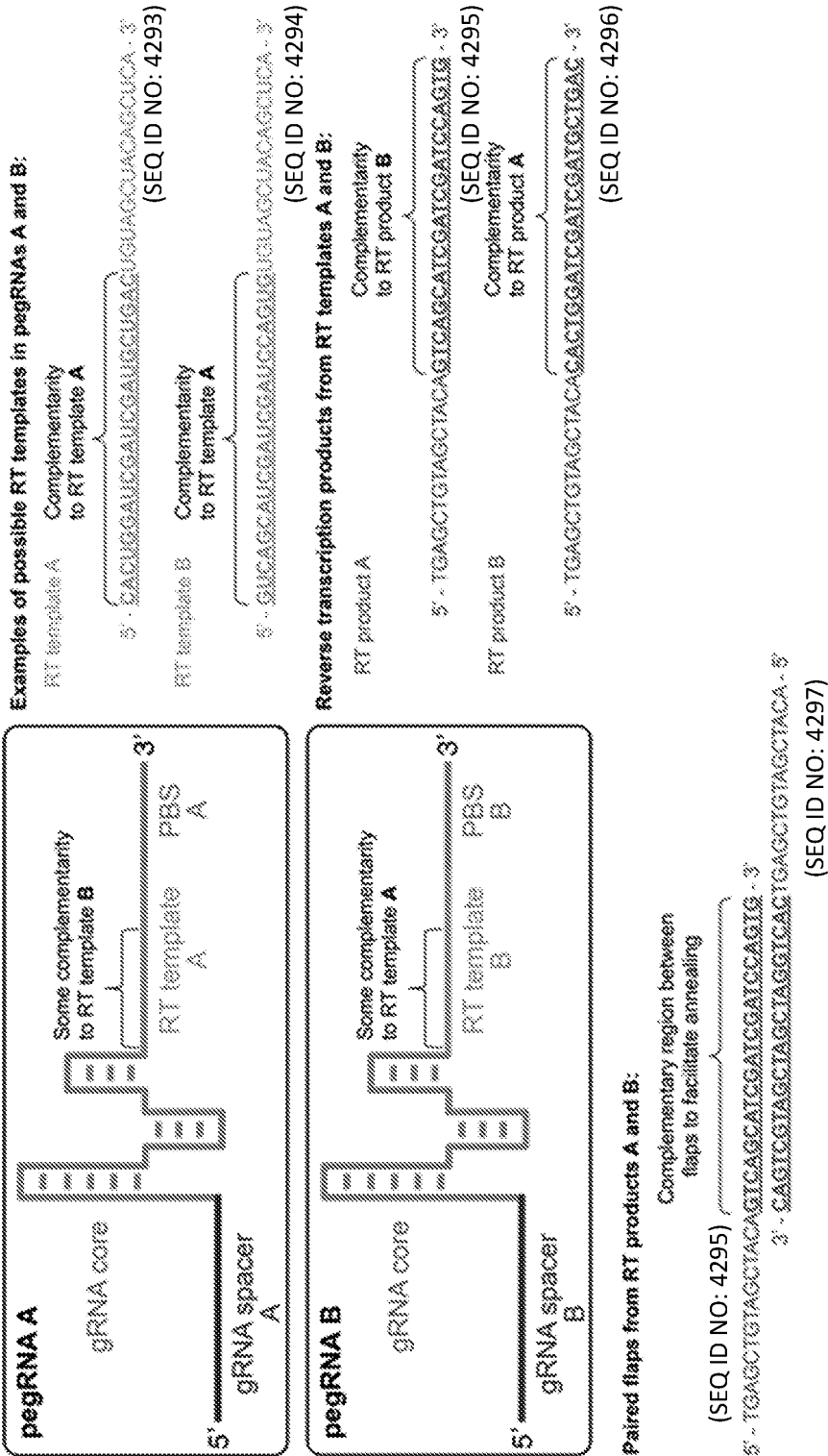


FIG. 92

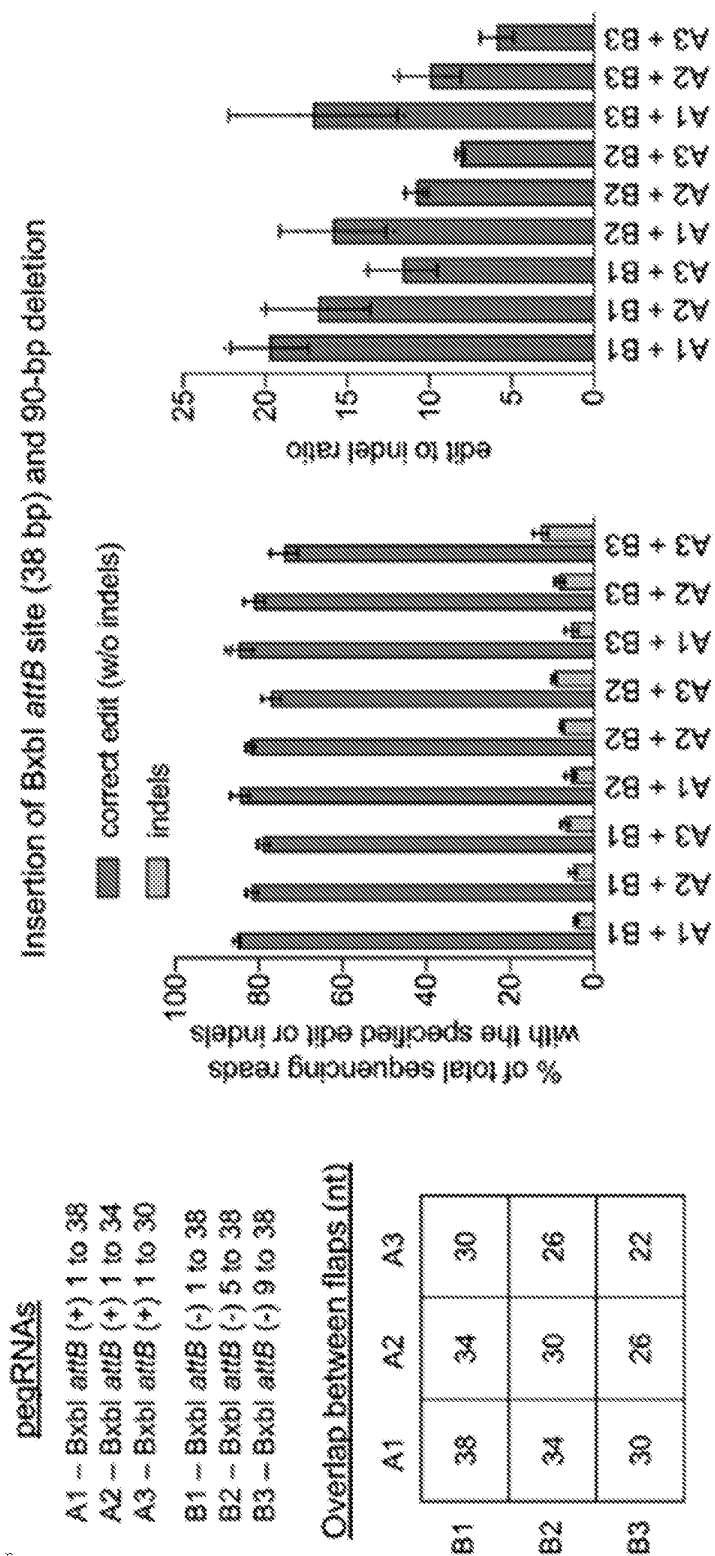


FIG. 93A

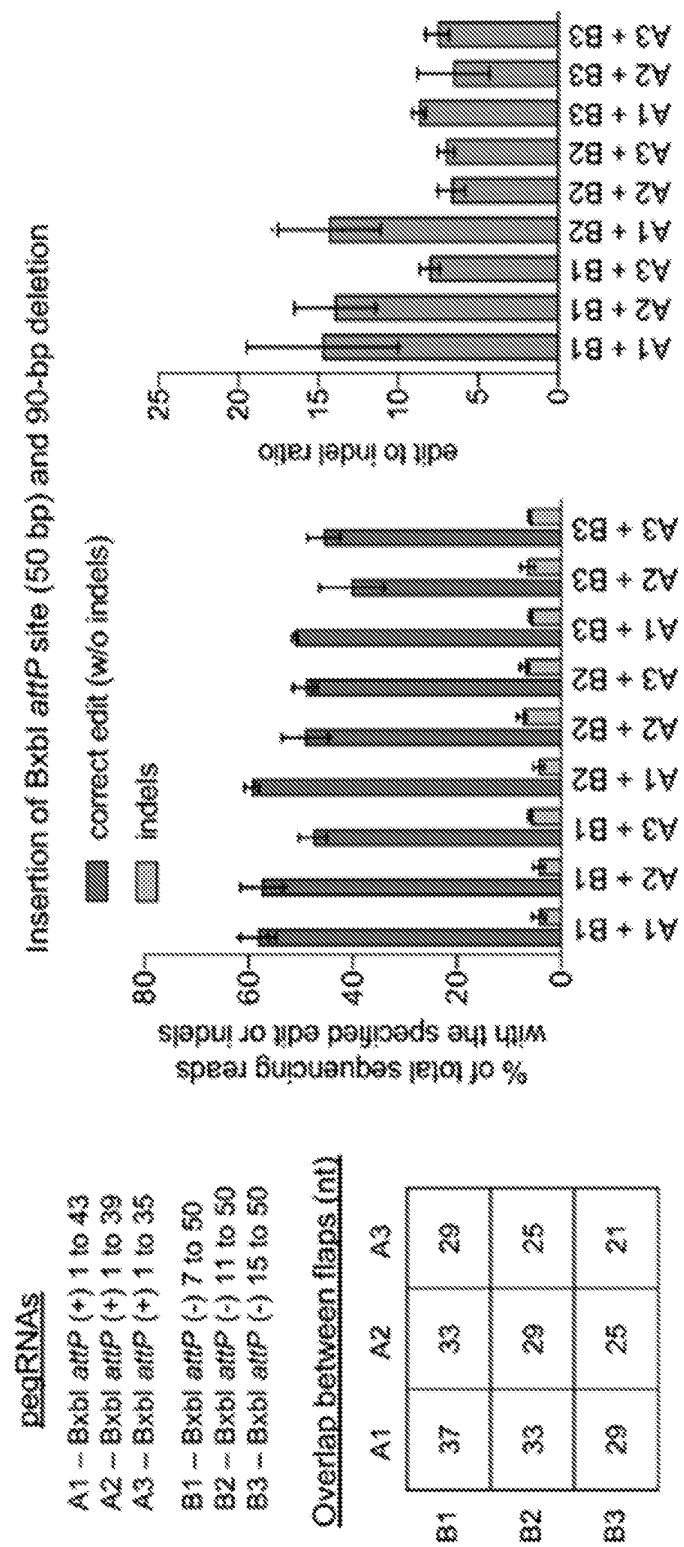


FIG. 93B

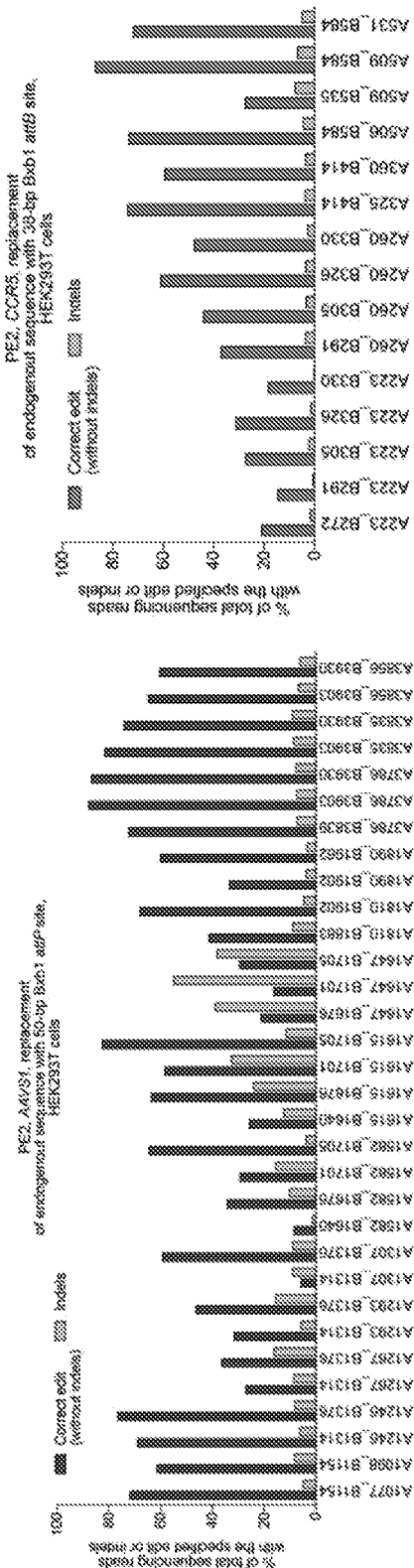


FIG. 94

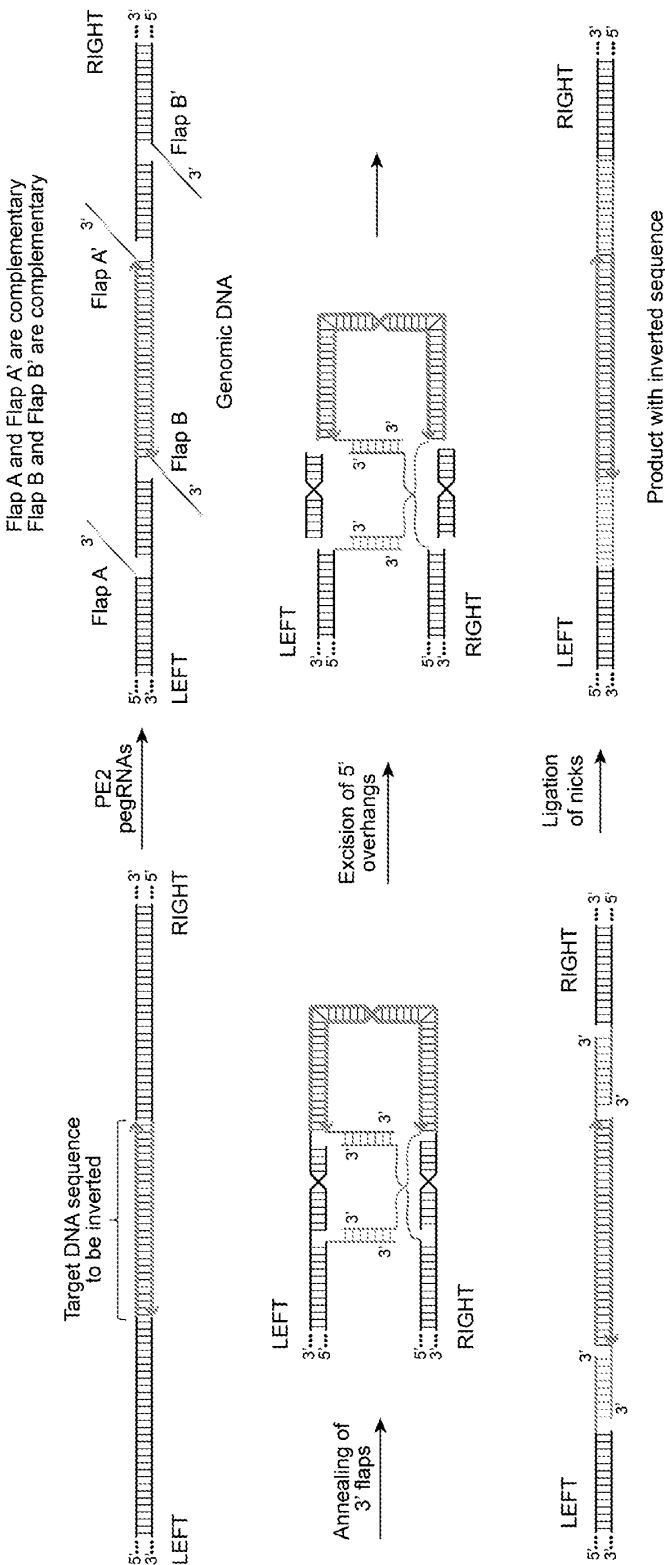
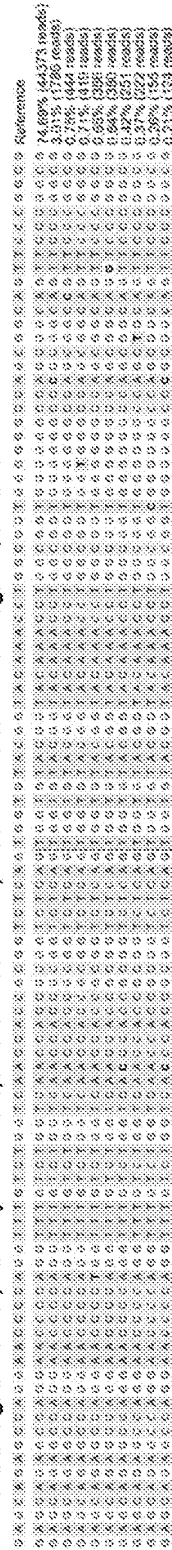


FIG. 95

HTS of PCR amplicons: (SEQ ID NOs: 4298,4298-4307)  
Reads aligned to expected junction A (contains attP site) ~ 90% of total reads aligned, 8-10% indels



(SEQ ID NOs: 4308,4308-4317)  
Reads aligned to expected junction B (contains attB site) ~ 93% of total reads aligned, 2.5% indels

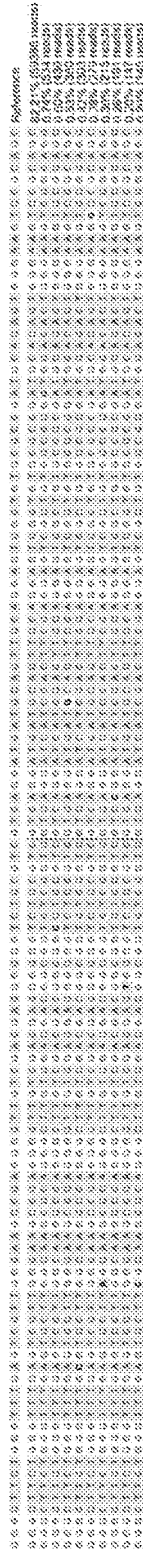


FIG. 96

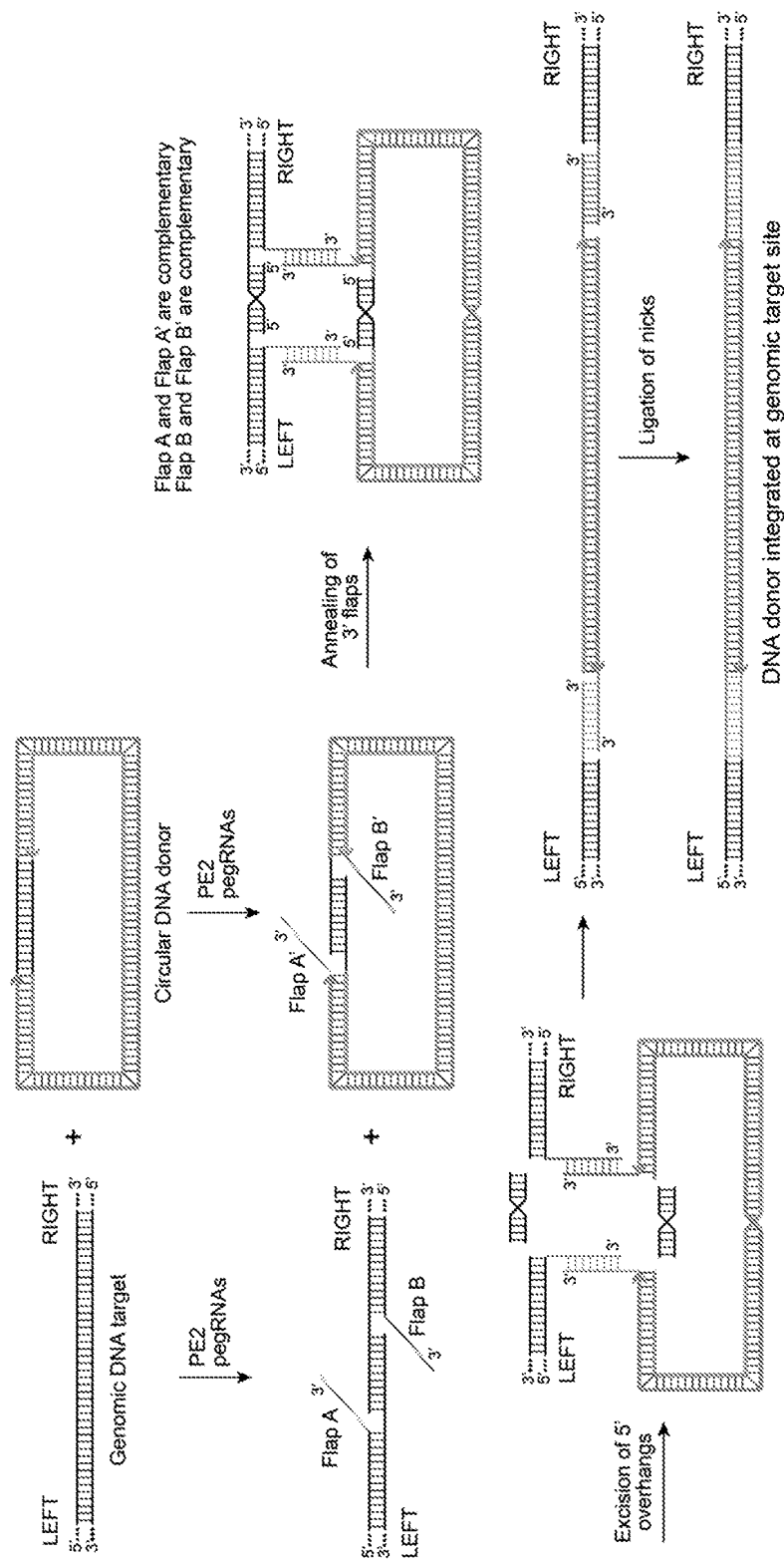


FIG. 97A

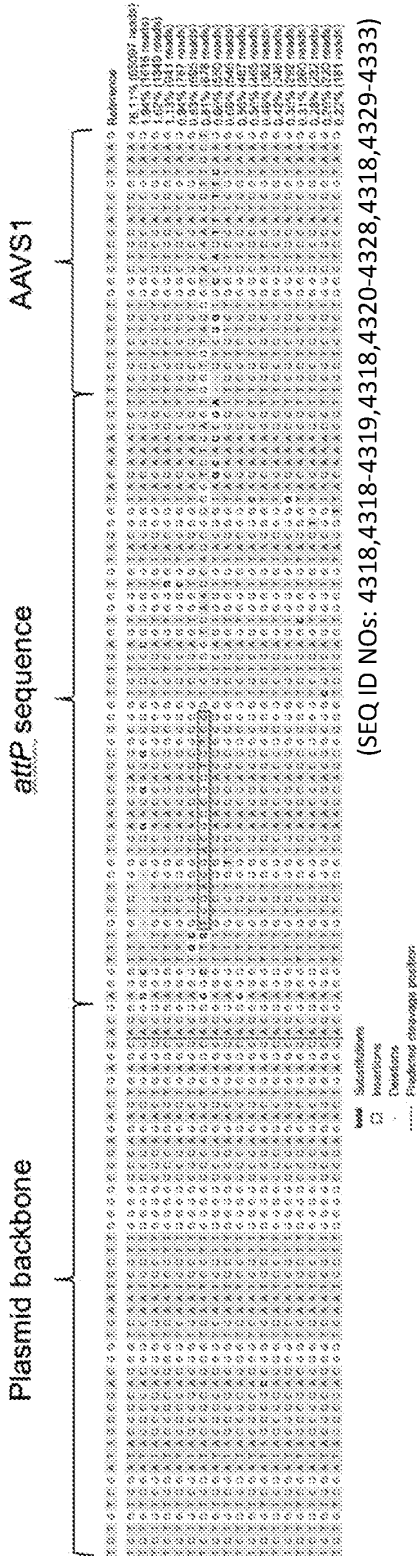


FIG. 97B

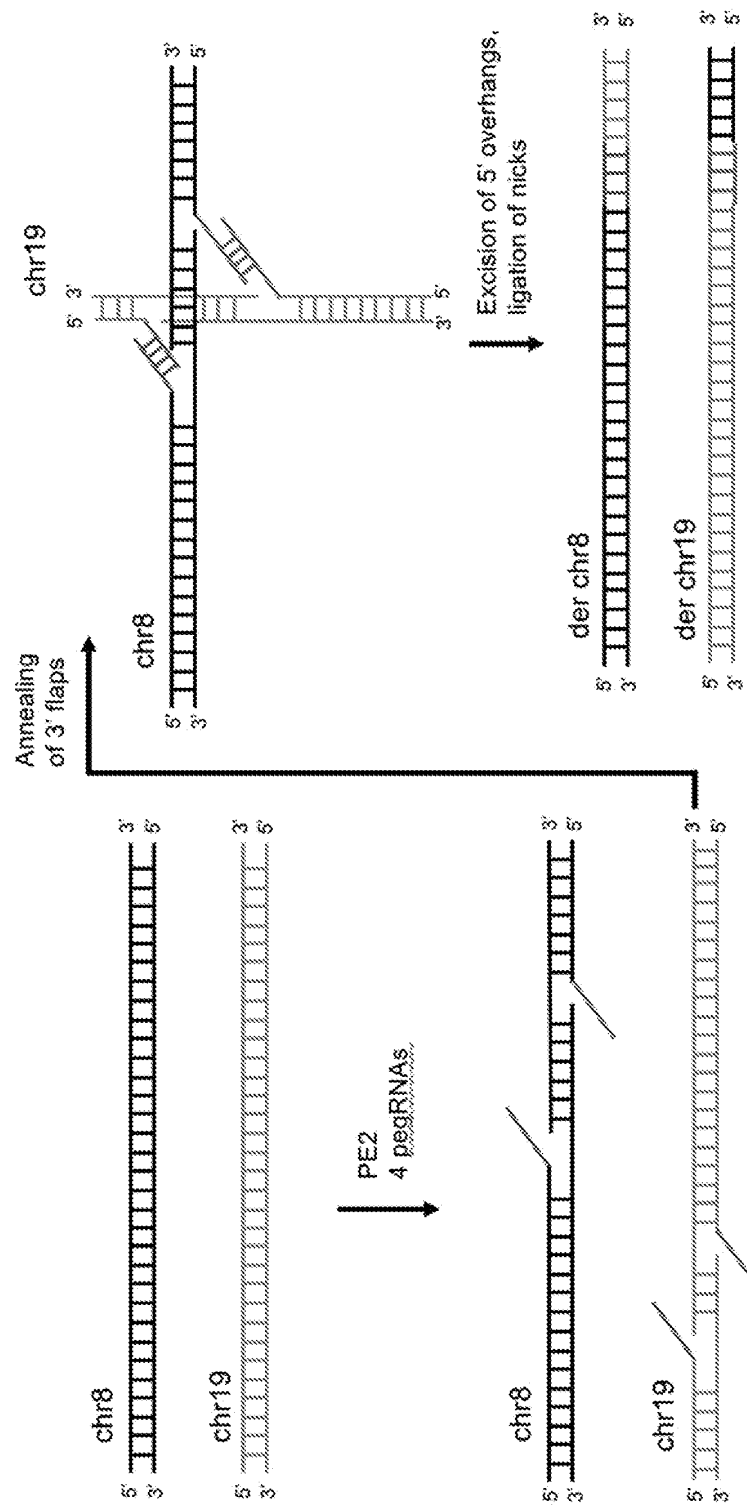


FIG. 98A

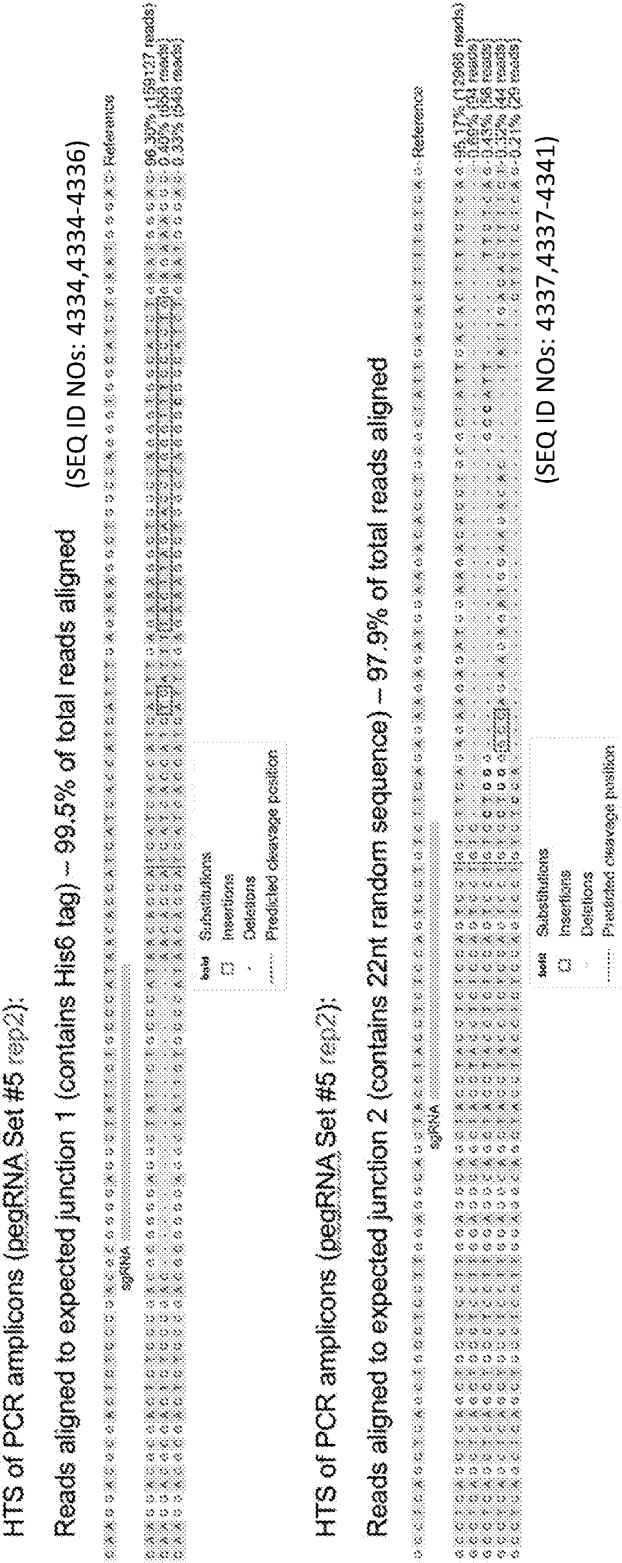


FIG. 98B

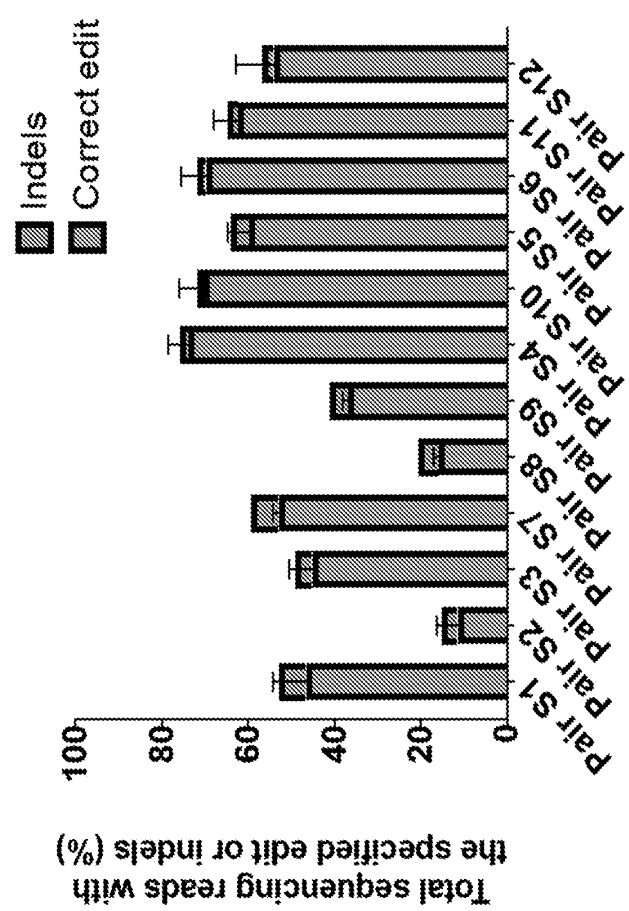


FIG. 99

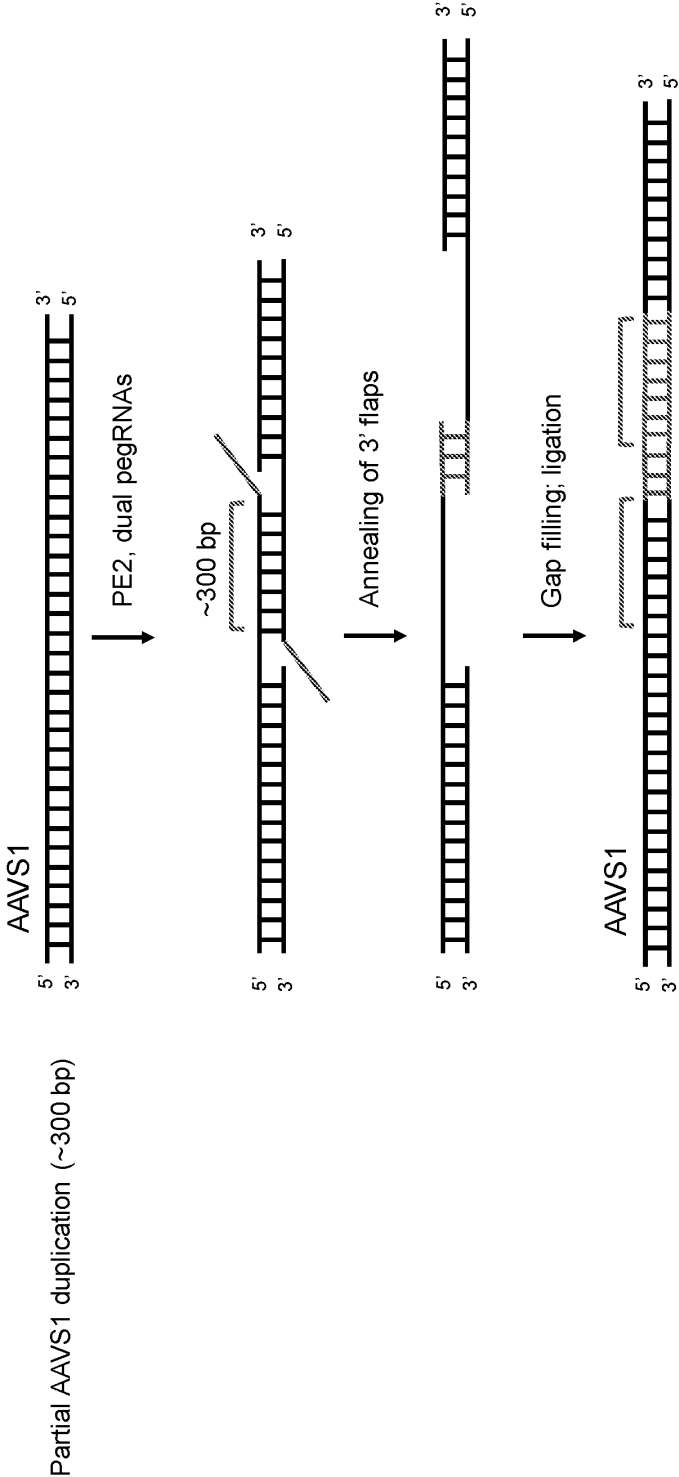
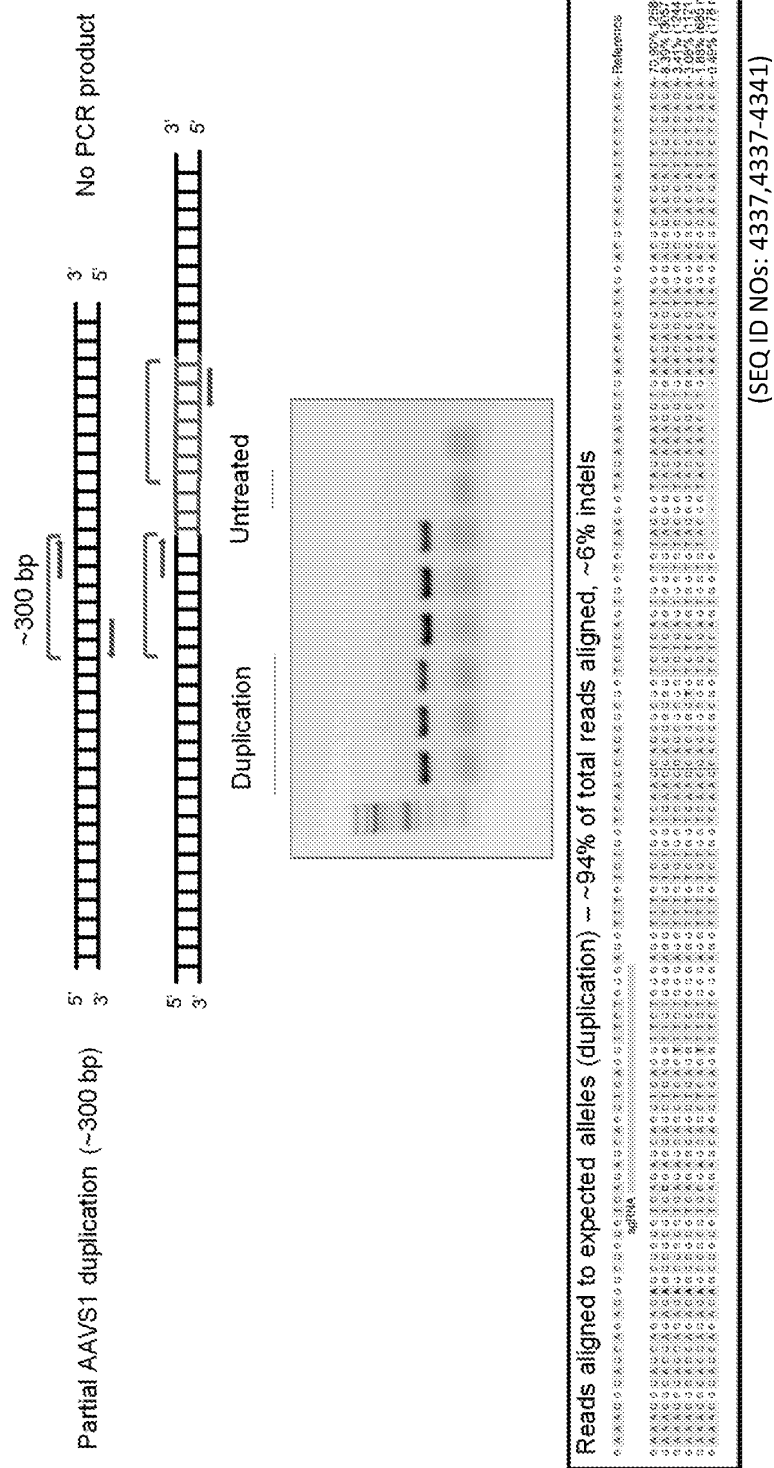


FIG. 100A



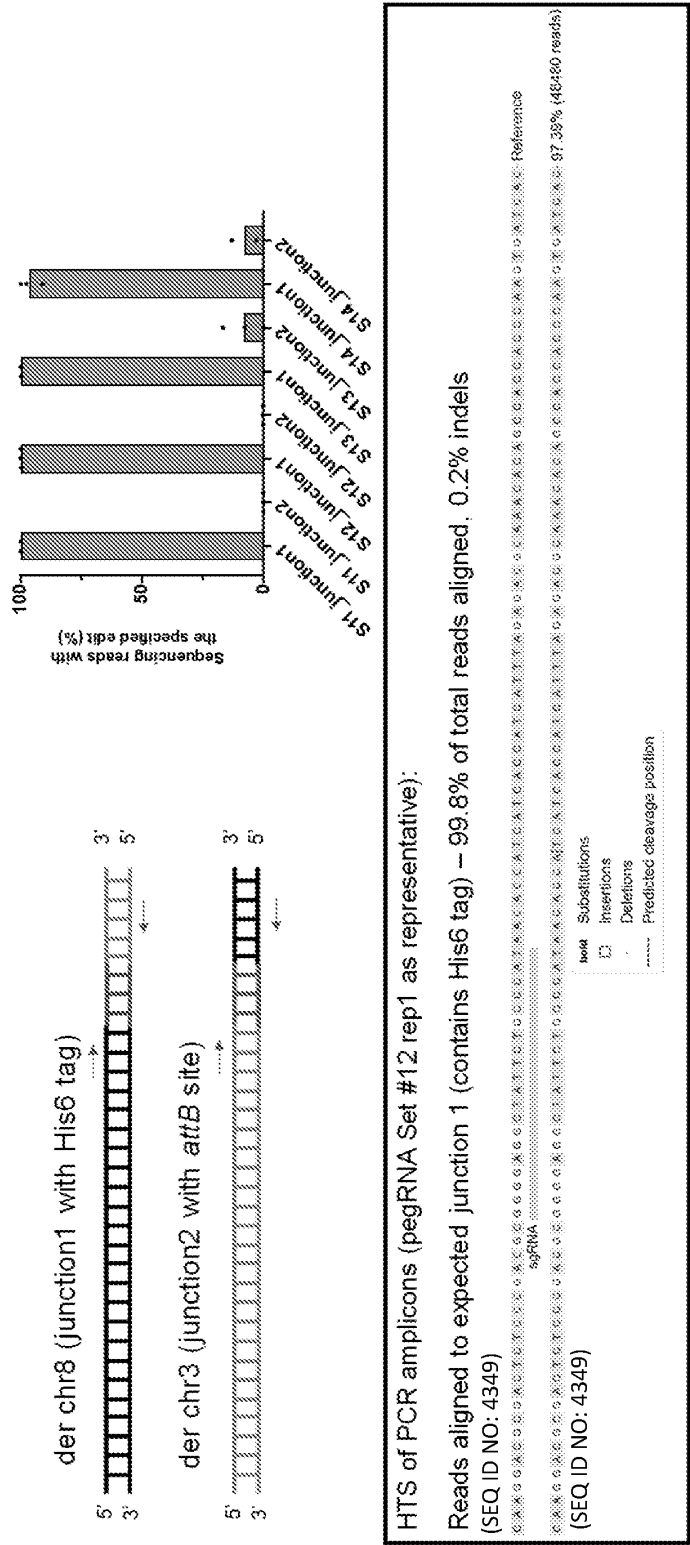


FIG. 101

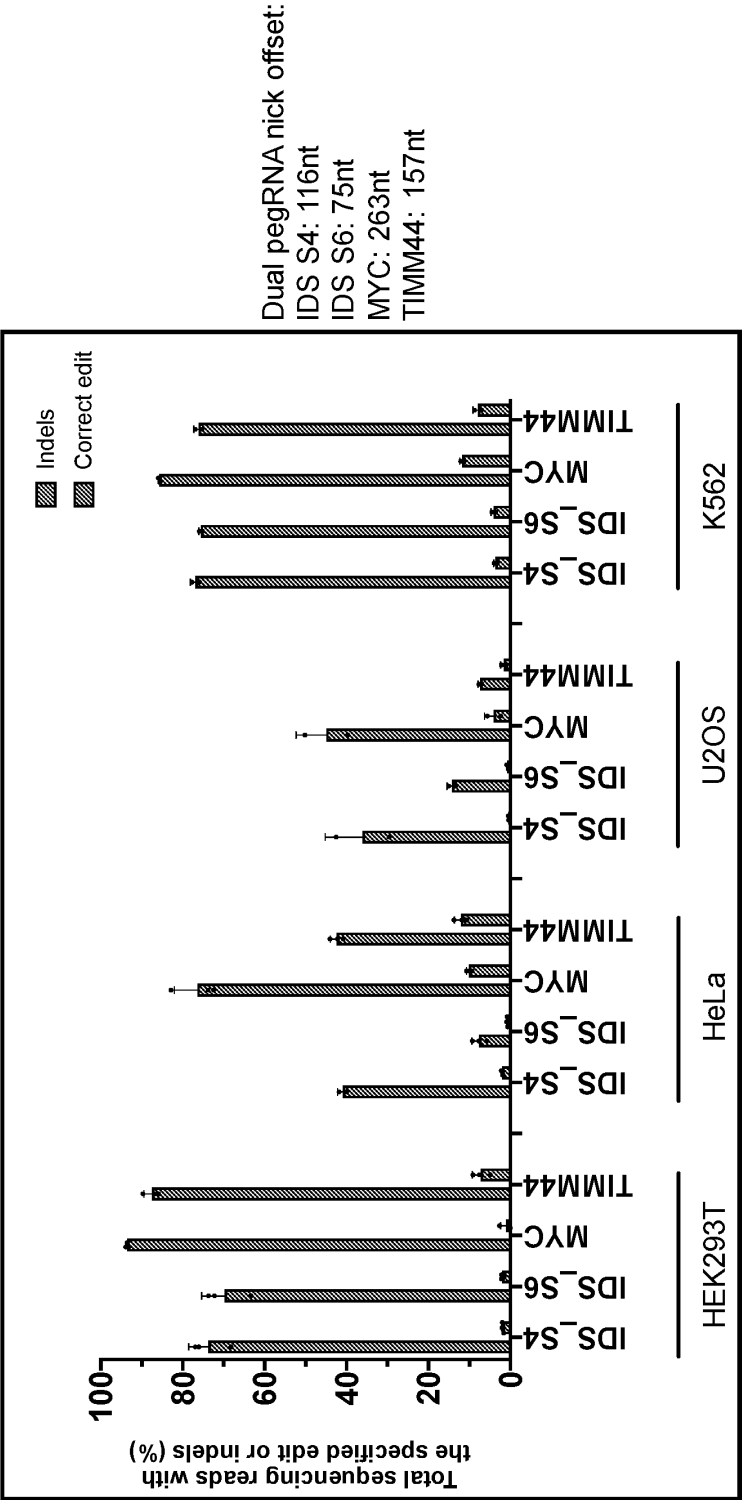


FIG. 102A

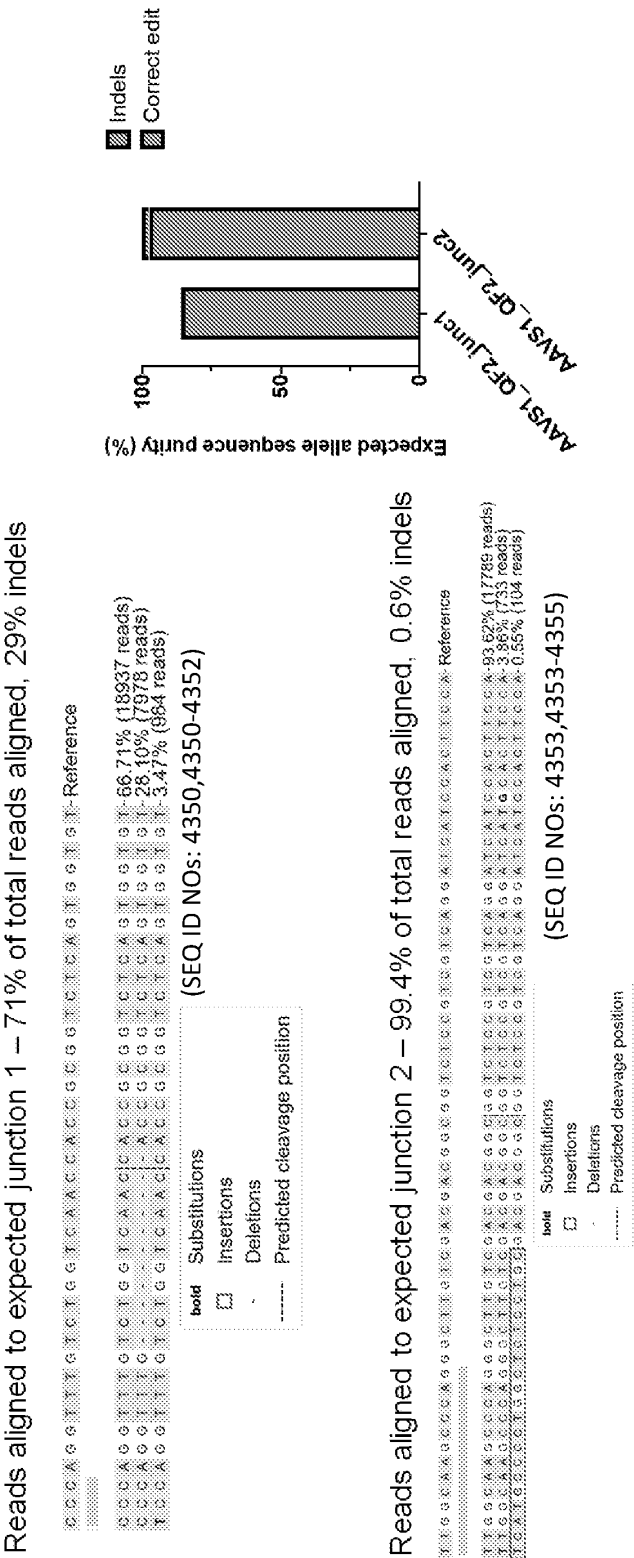


FIG. 102B

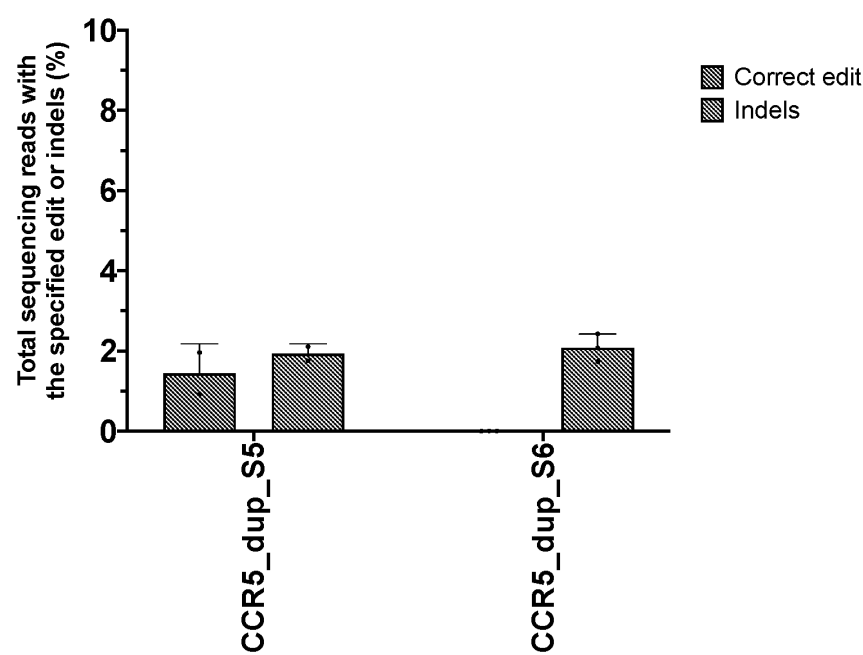


FIG. 103A

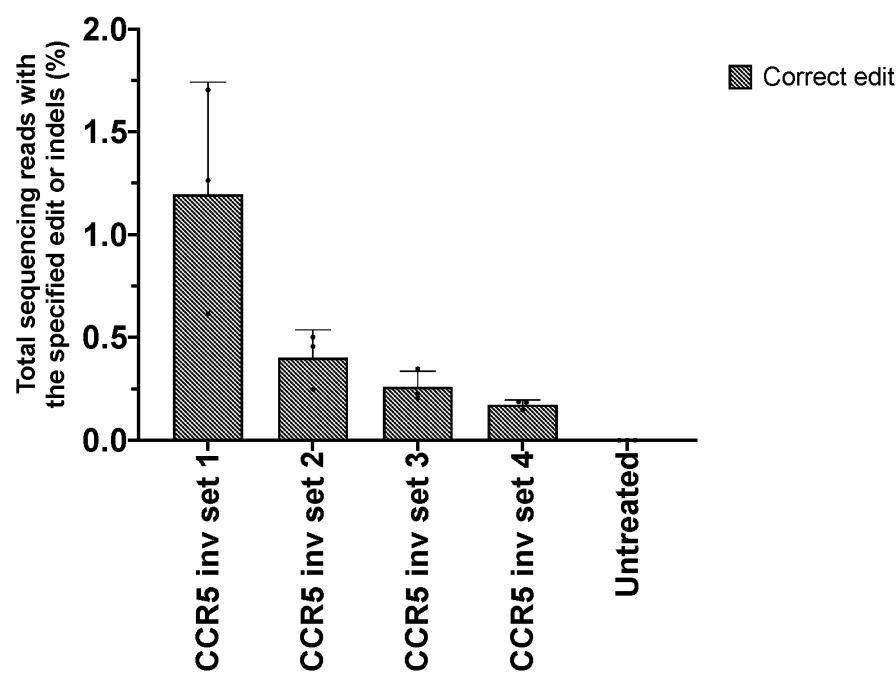


FIG. 103B

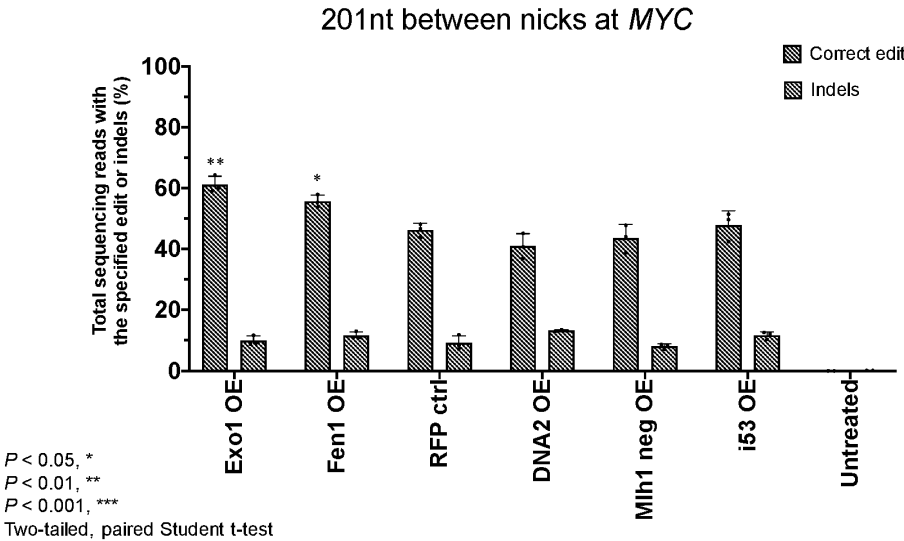


FIG. 104A

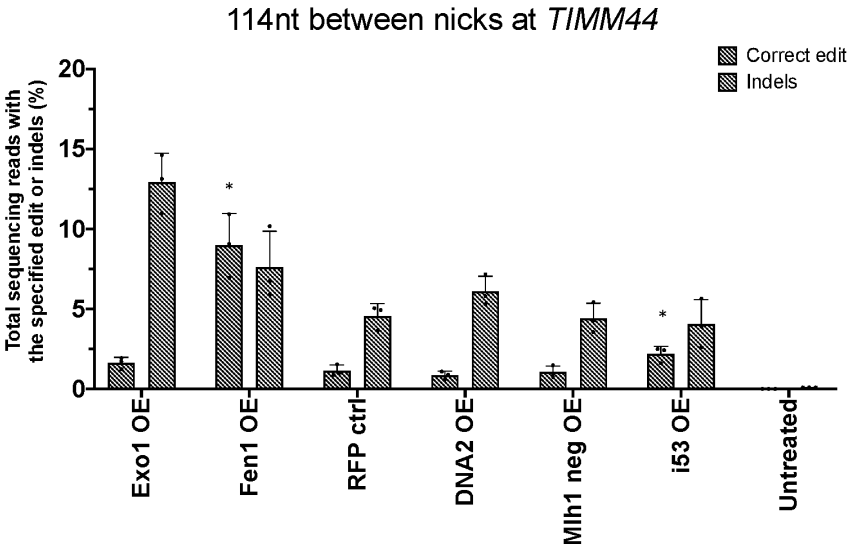


FIG. 104B

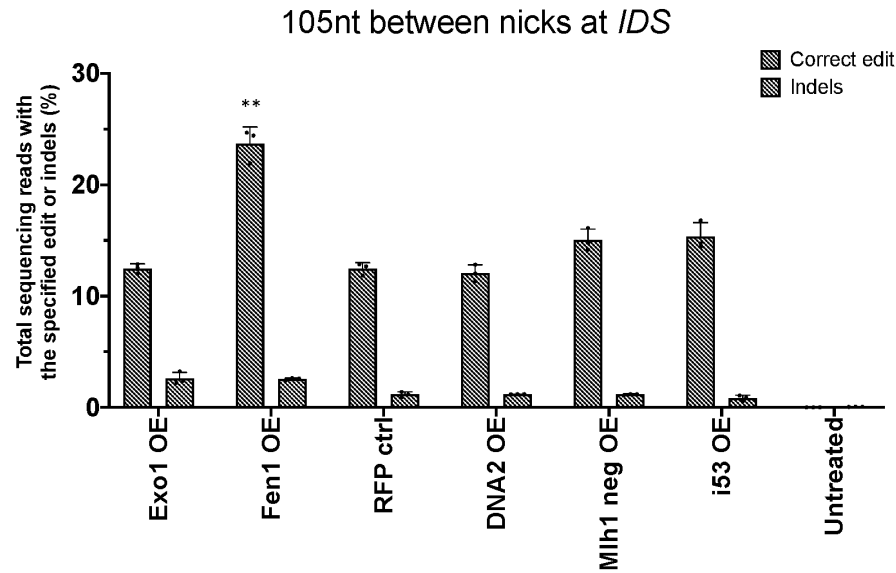


FIG. 104C

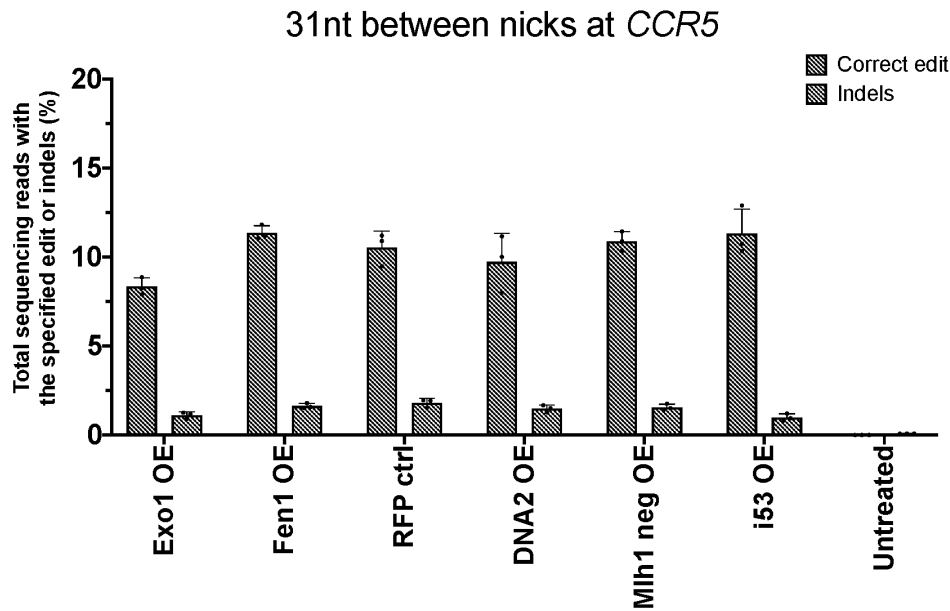


FIG. 104D

siExo1 knockdown experiment

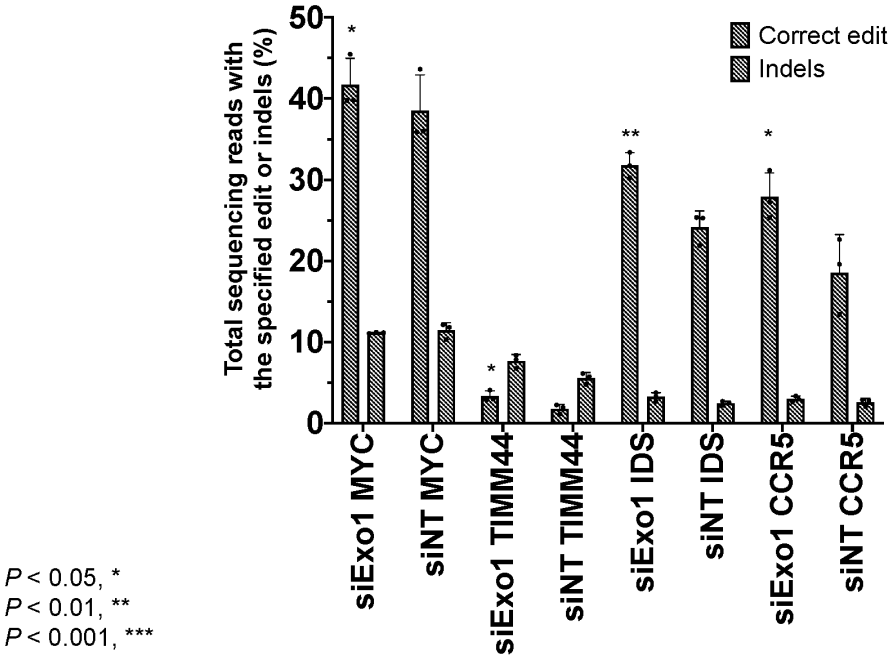


FIG. 104E

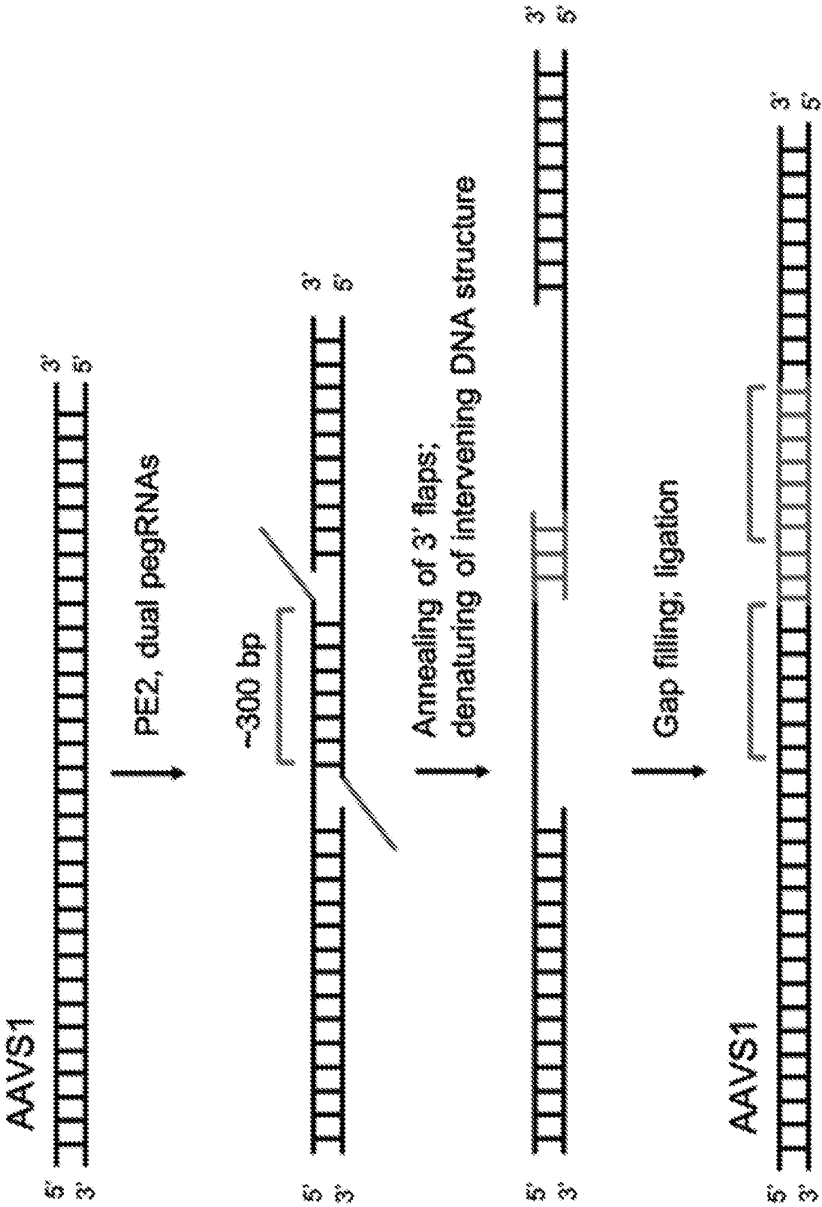


FIG. 105A

Reads aligned to expected alleles (duplication) -- ~94% of total reads aligned, ~6% indels



(SEQ ID NOS: 4342-4348)

FIG. 105B

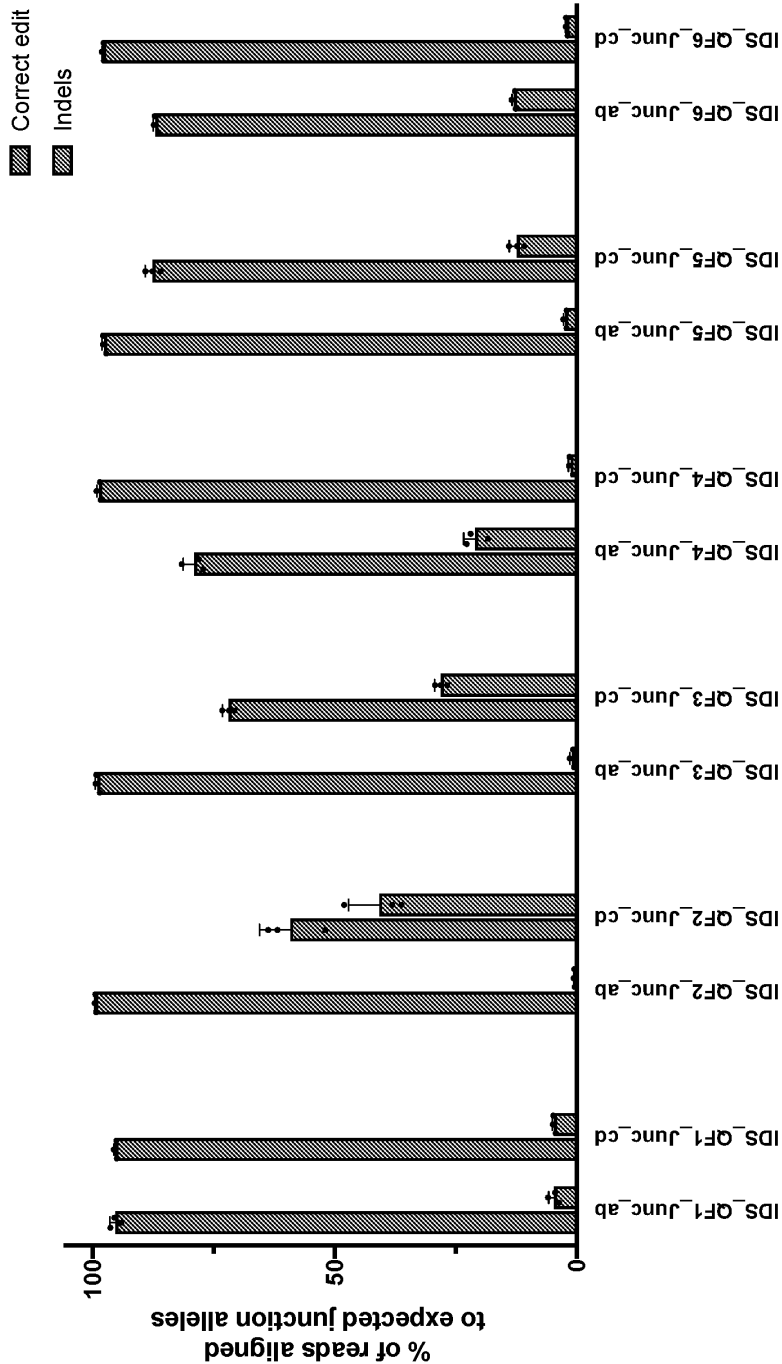


FIG. 106

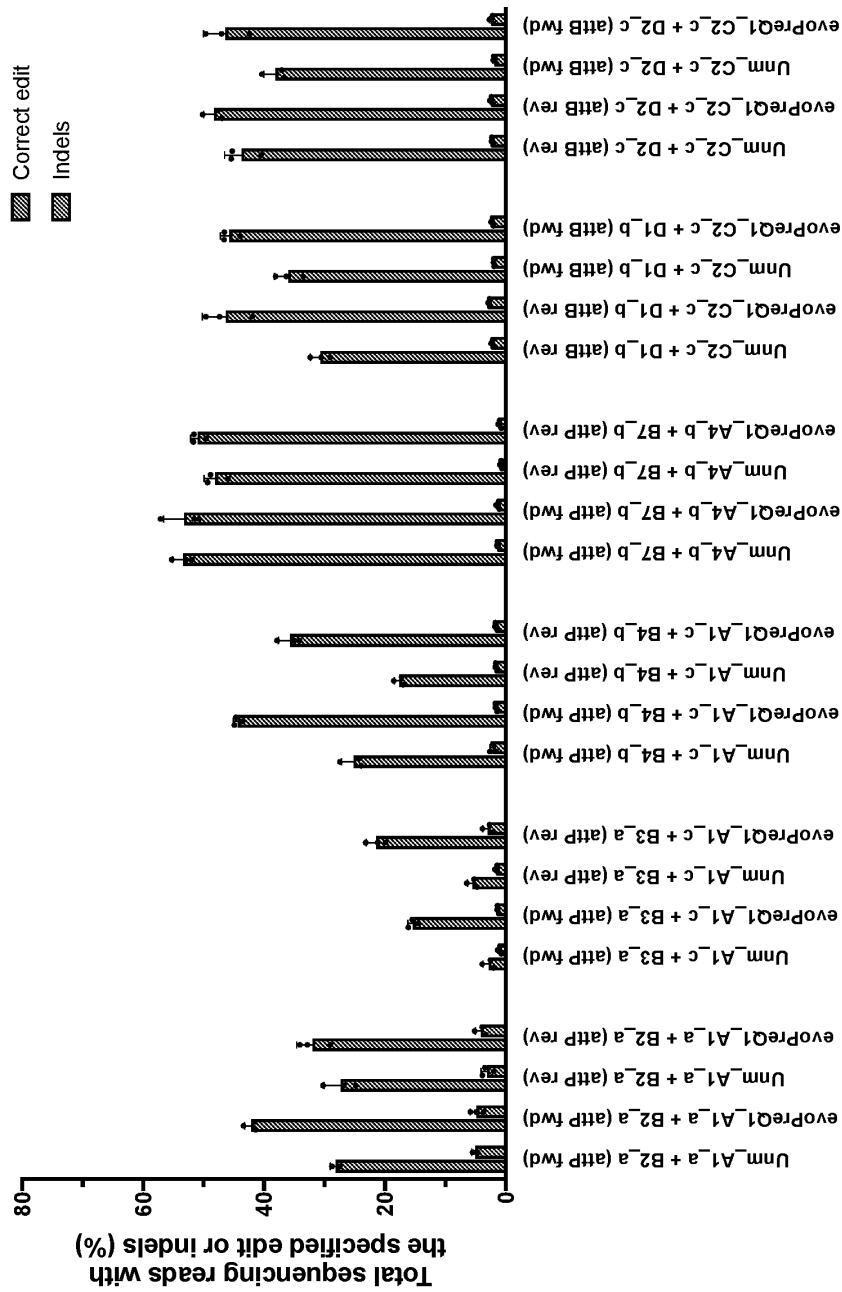


FIG. 107

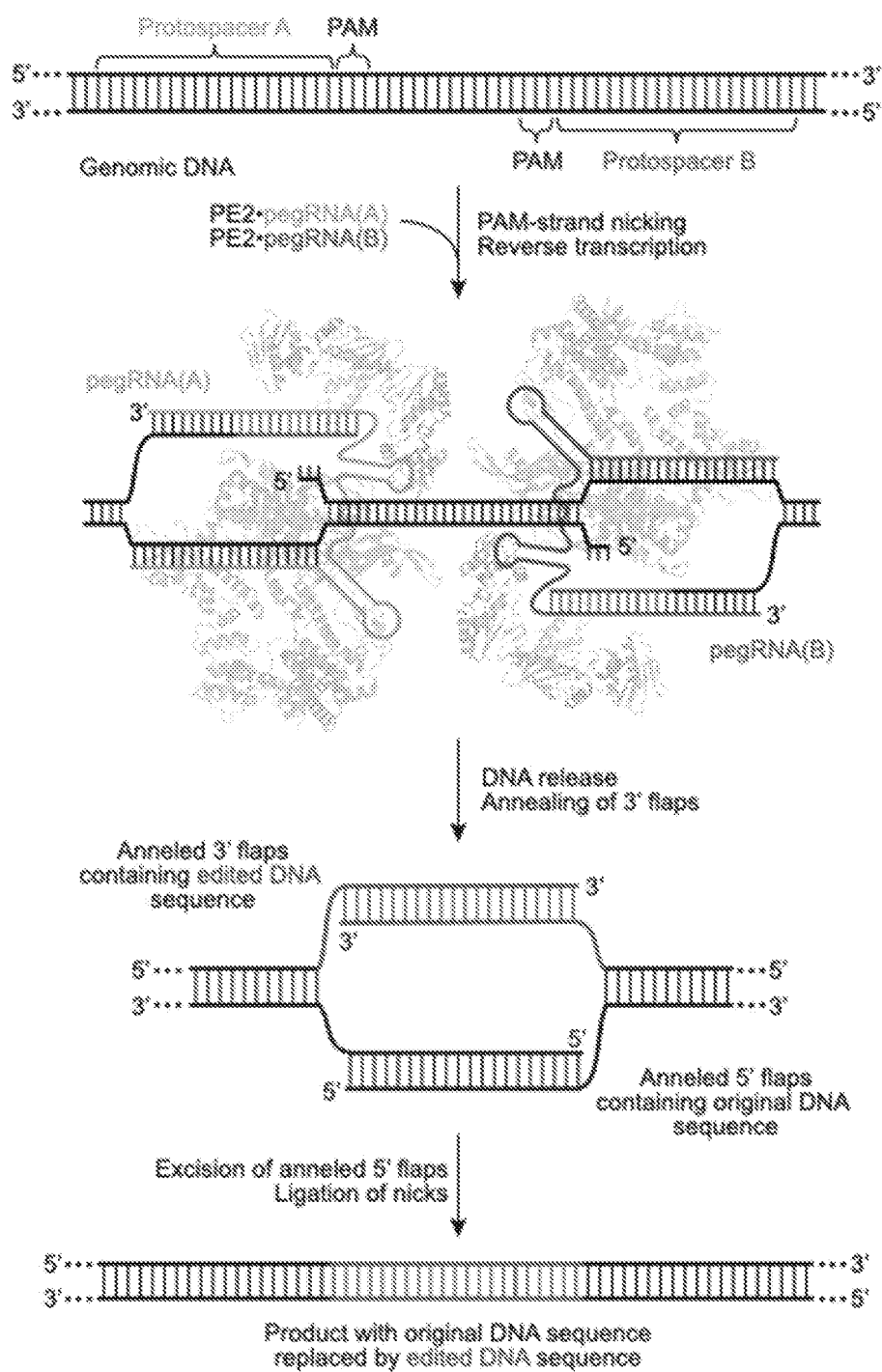


FIG. 108A

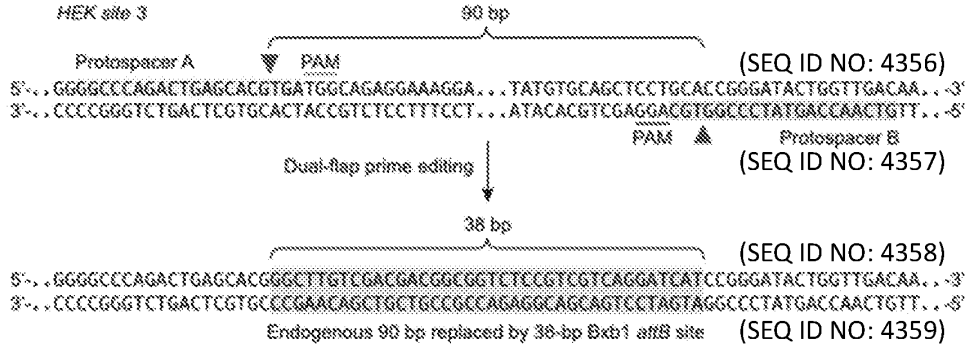


FIG. 108B

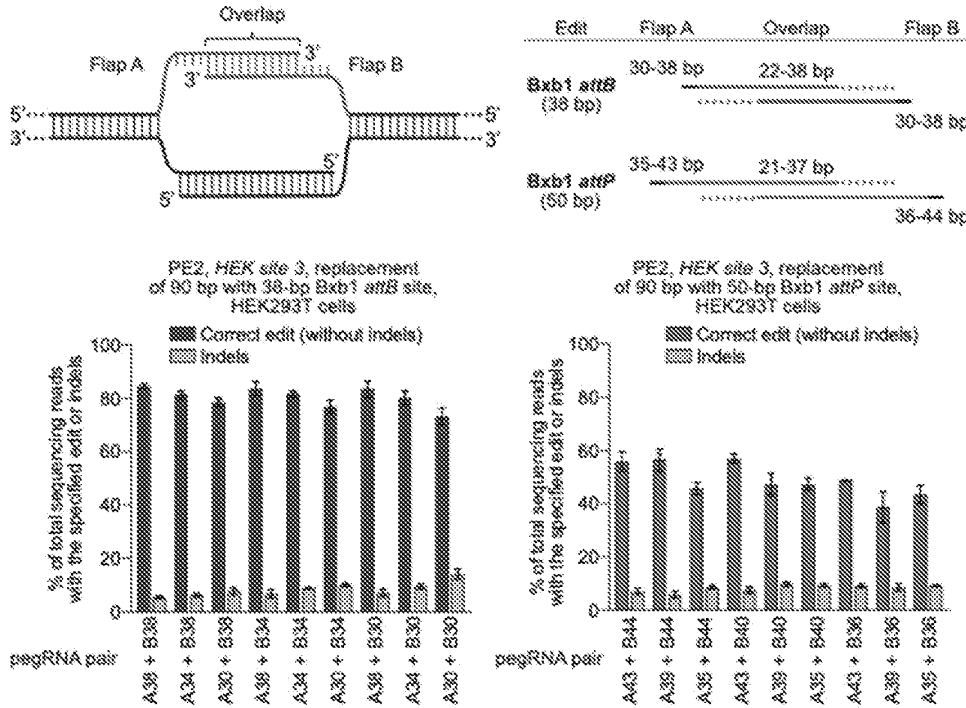


FIG. 108C

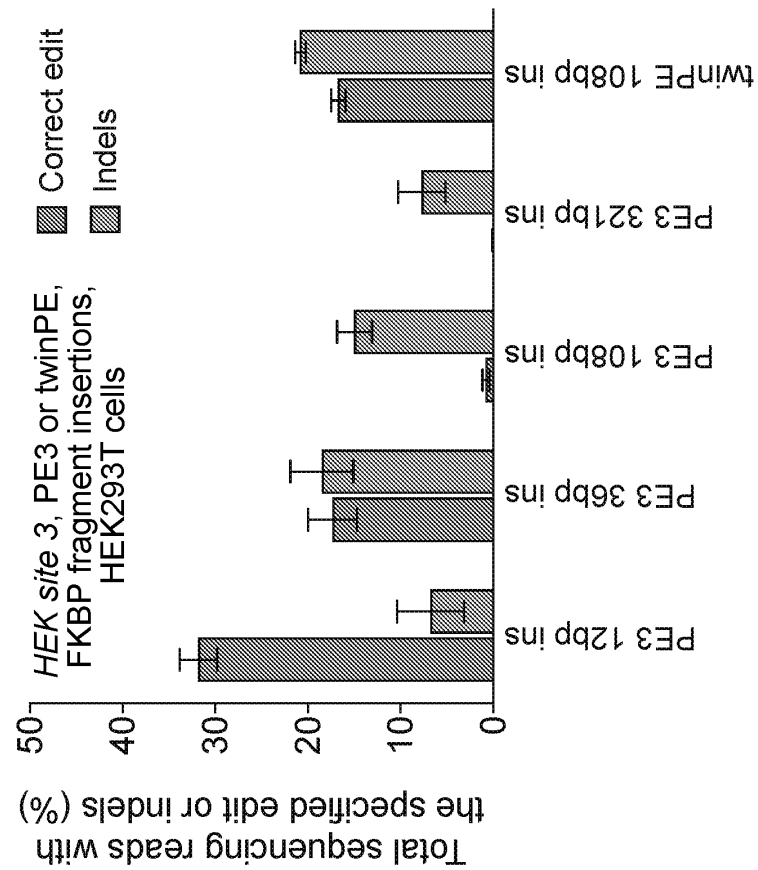


FIG. 109A

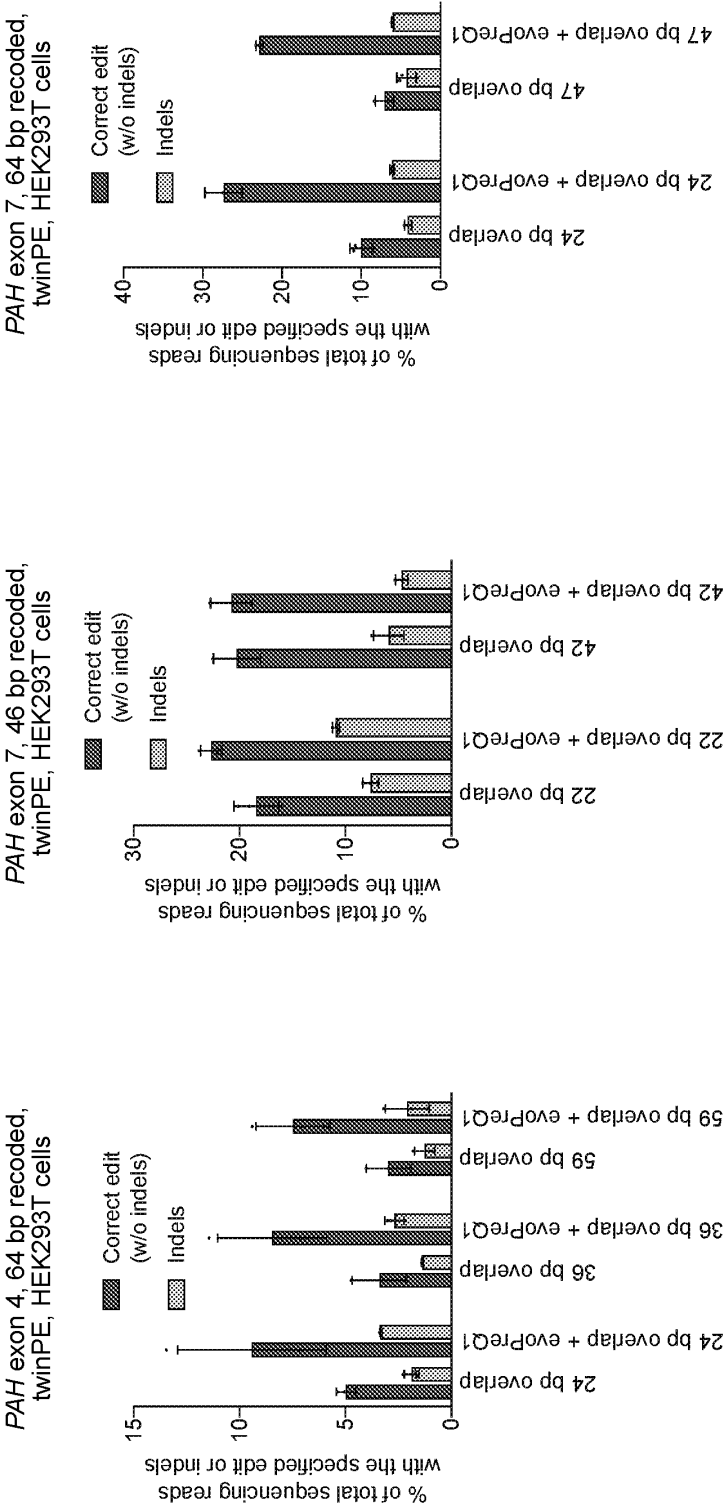


FIG. 109B

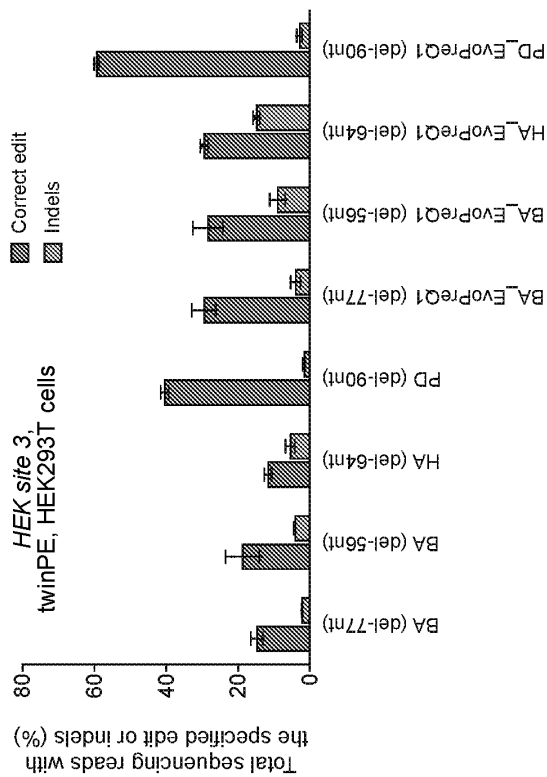


FIG. 109D

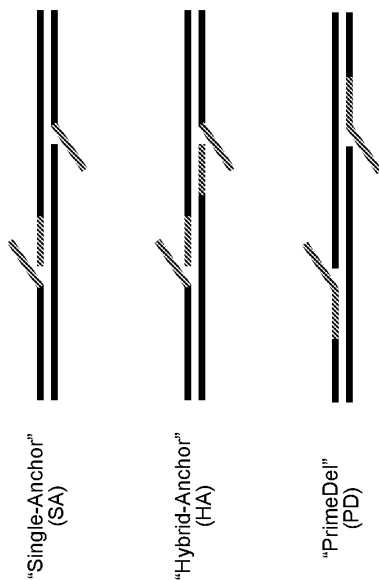
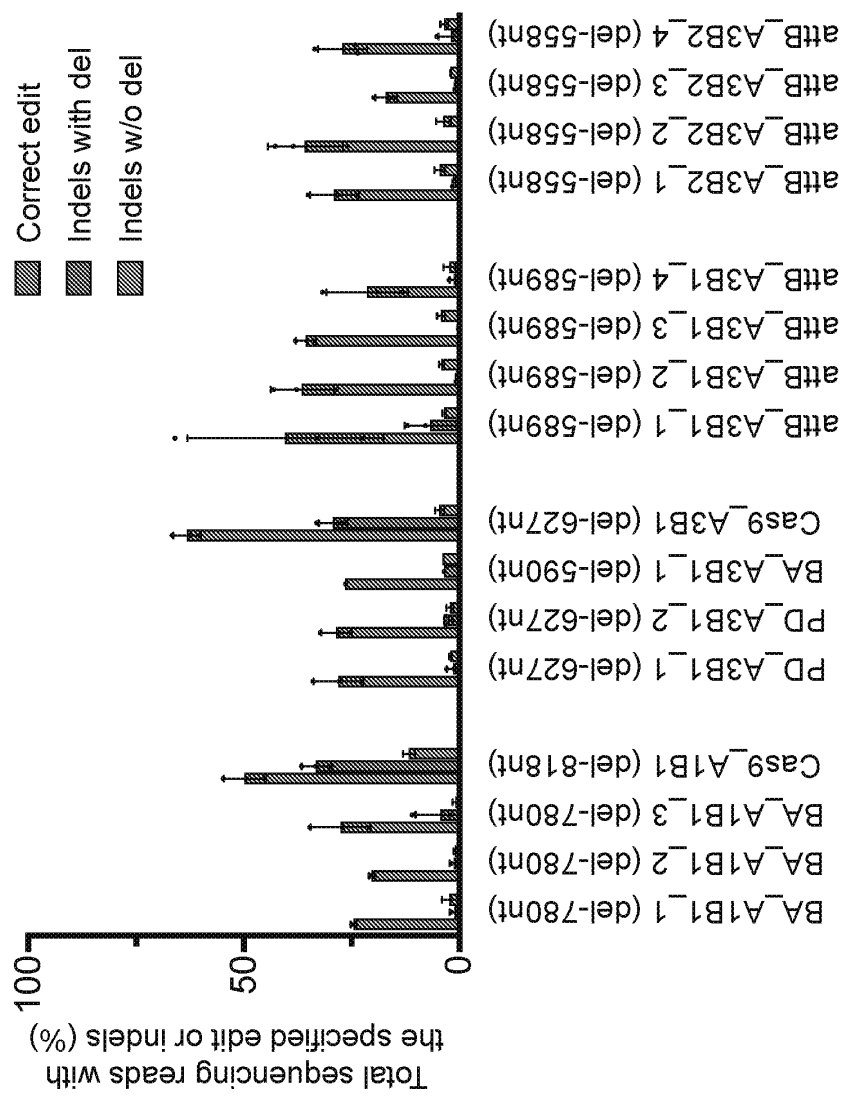


FIG. 109C



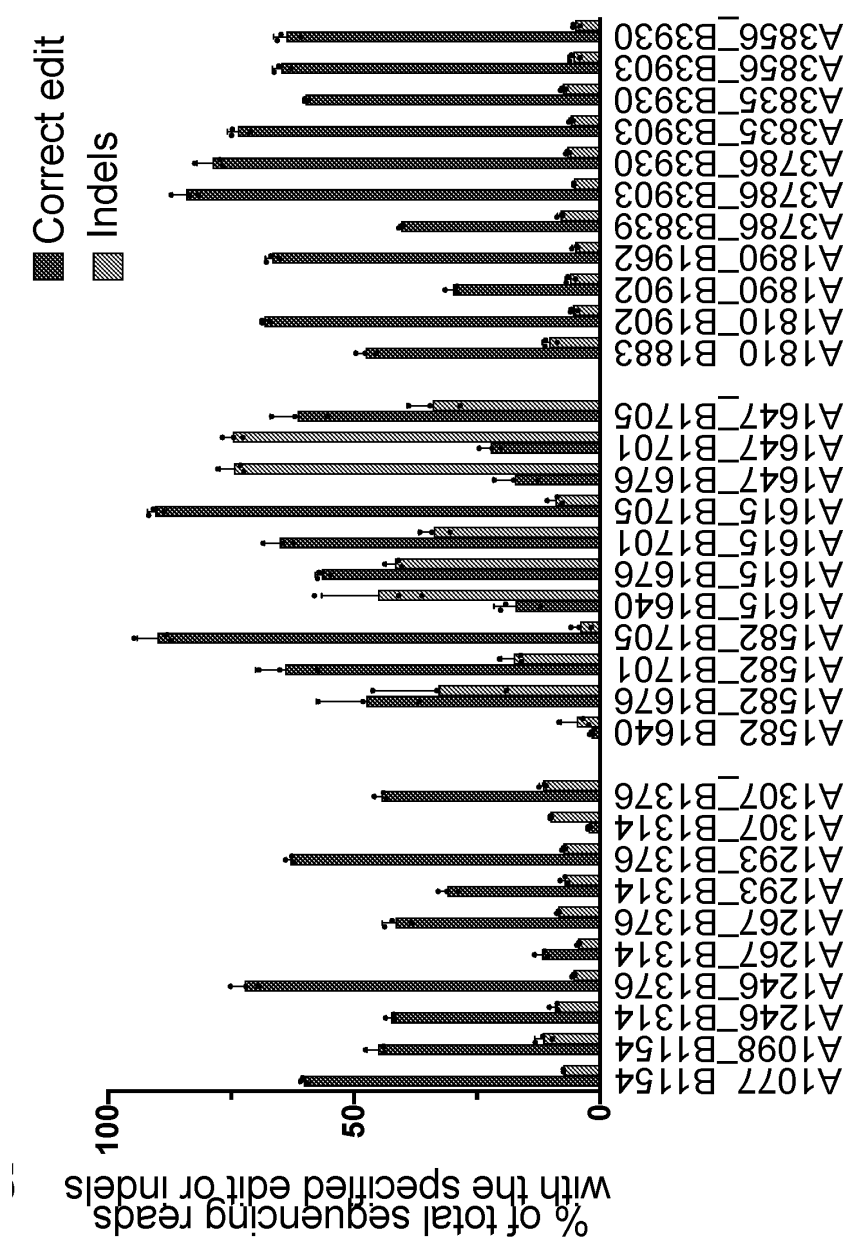


FIG. 110A

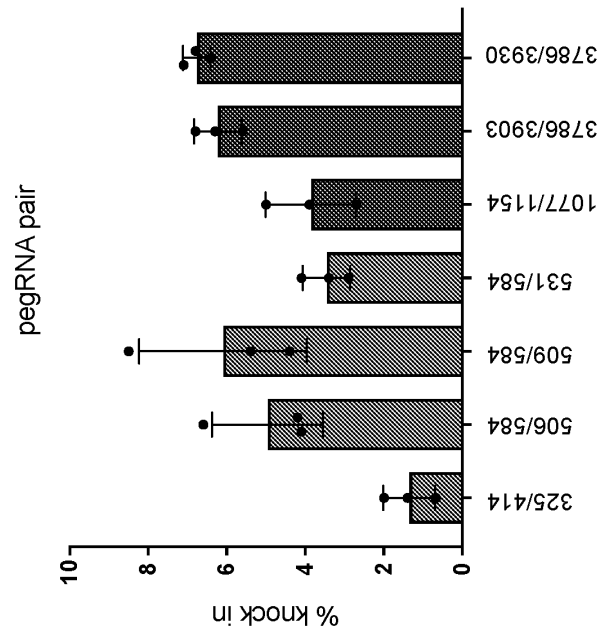


FIG. 110C

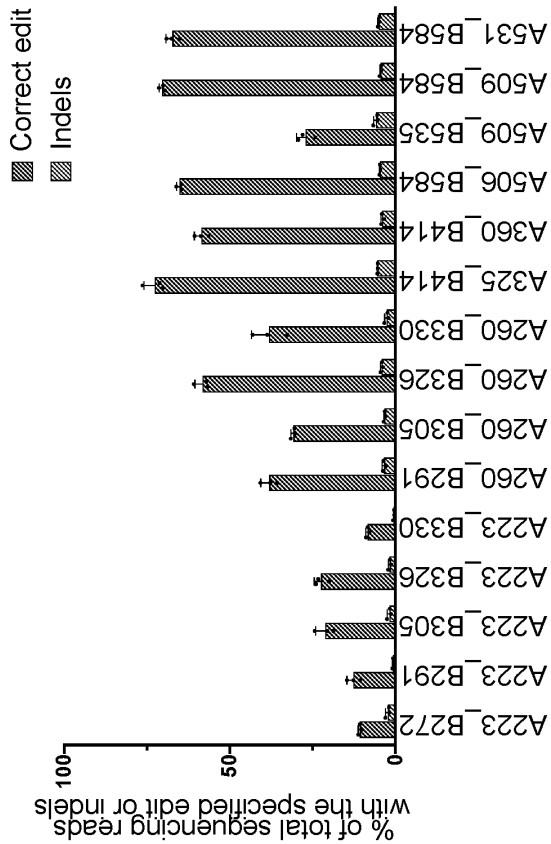
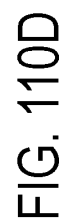


FIG. 110B



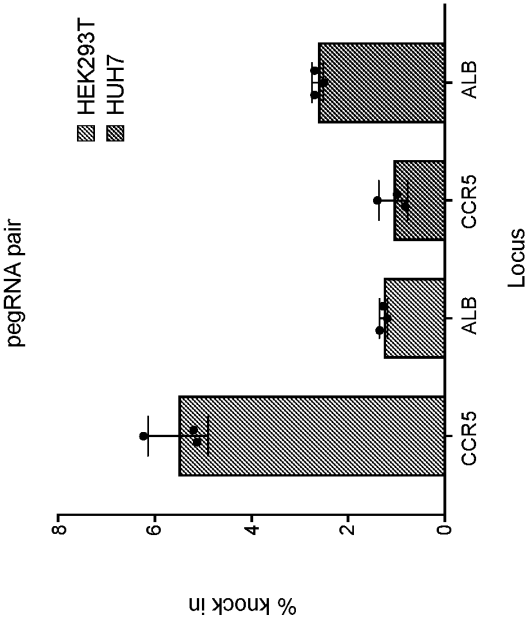


FIG. 110F

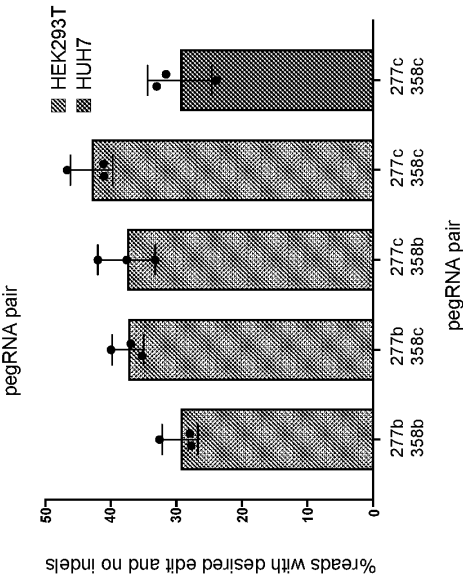


FIG. 110E

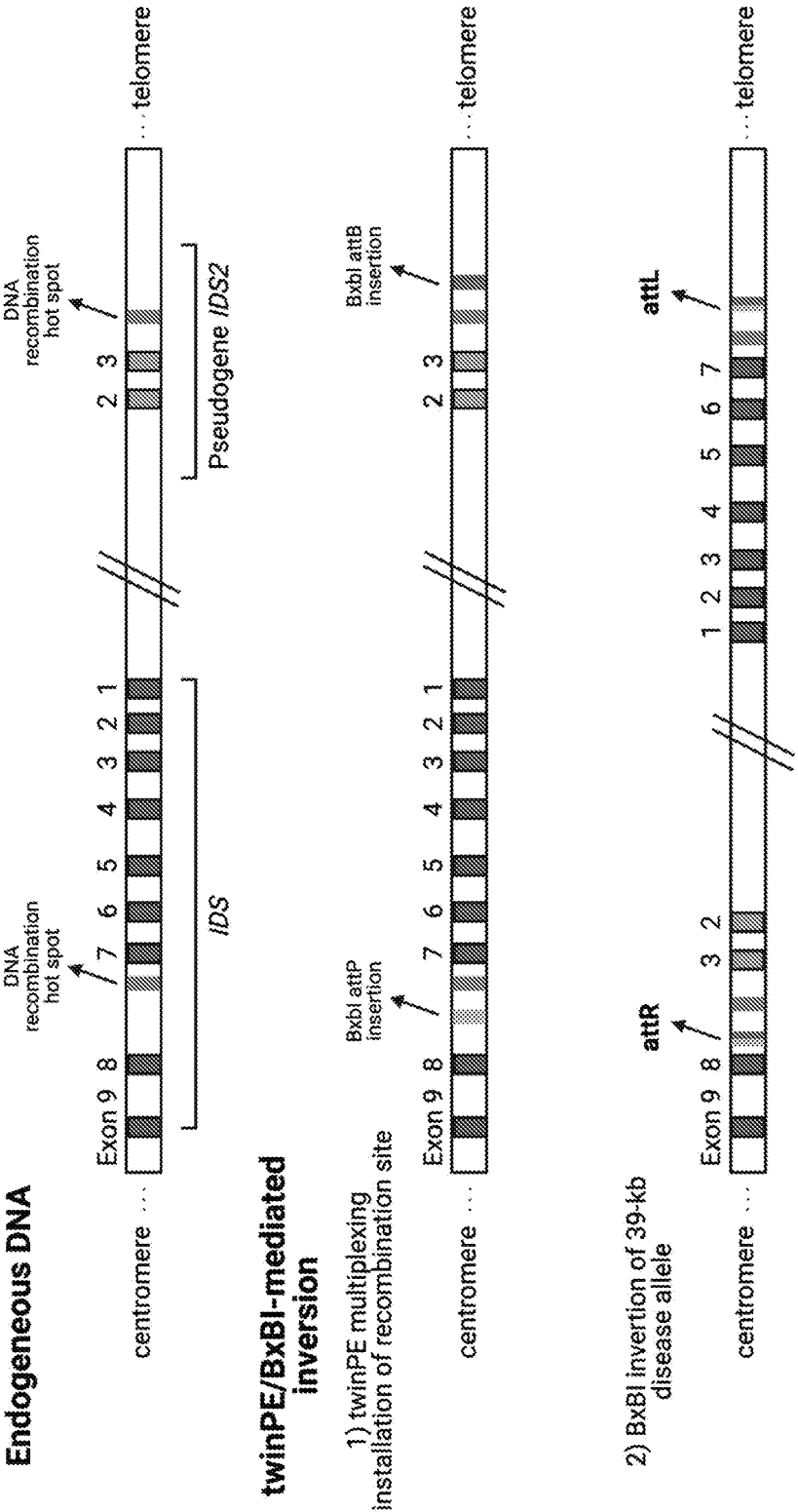


FIG. 111A

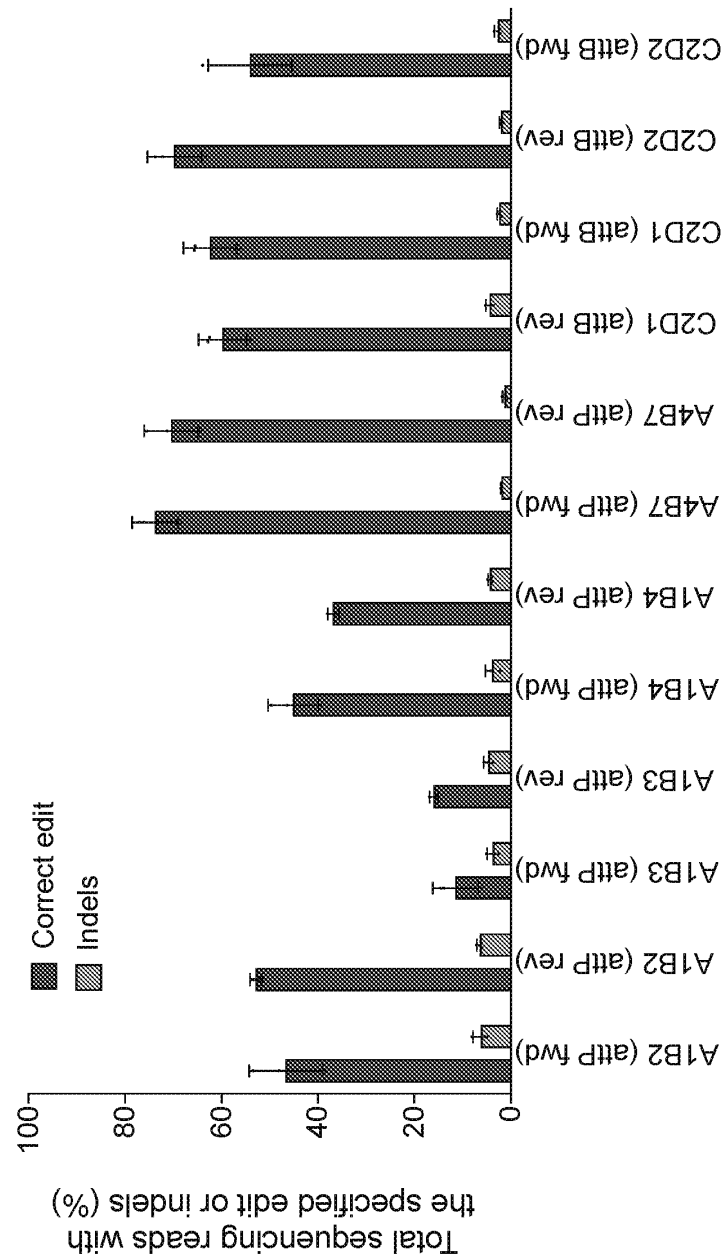
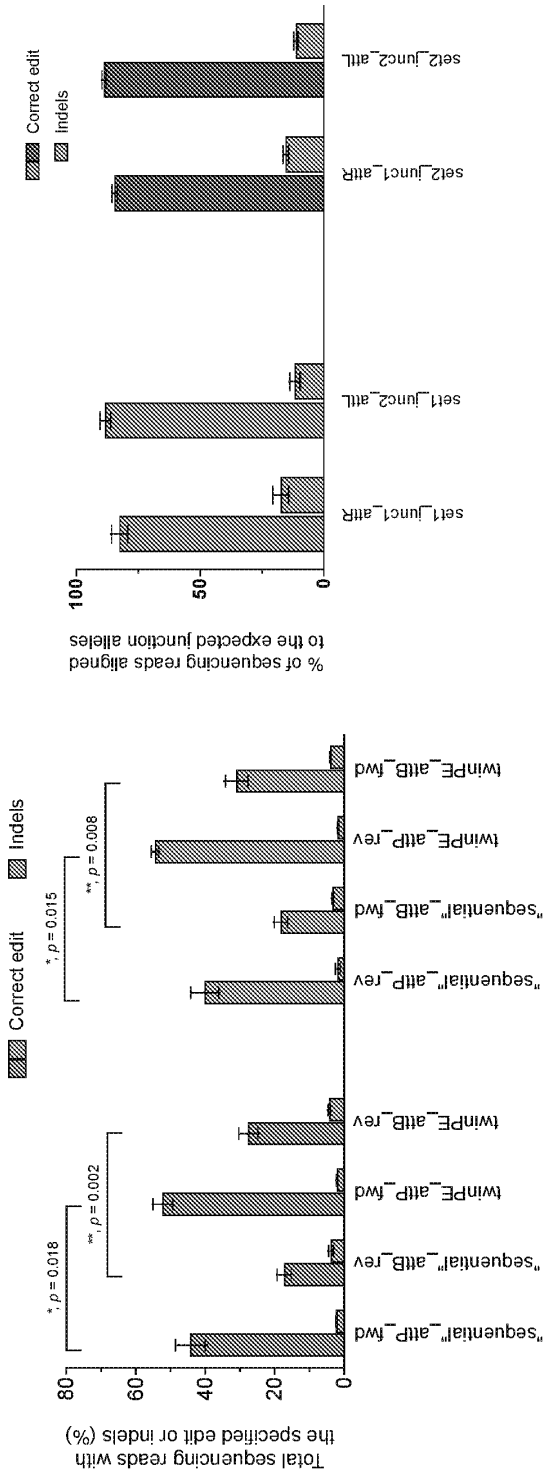


FIG. 111B



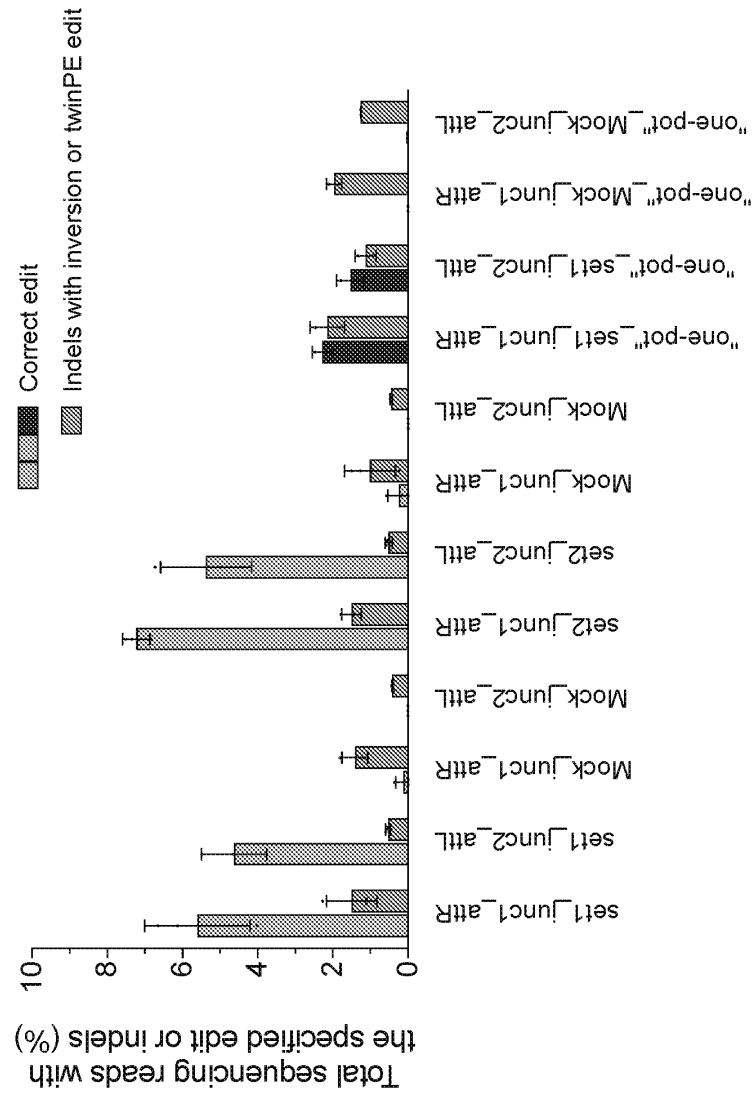


FIG. 111E

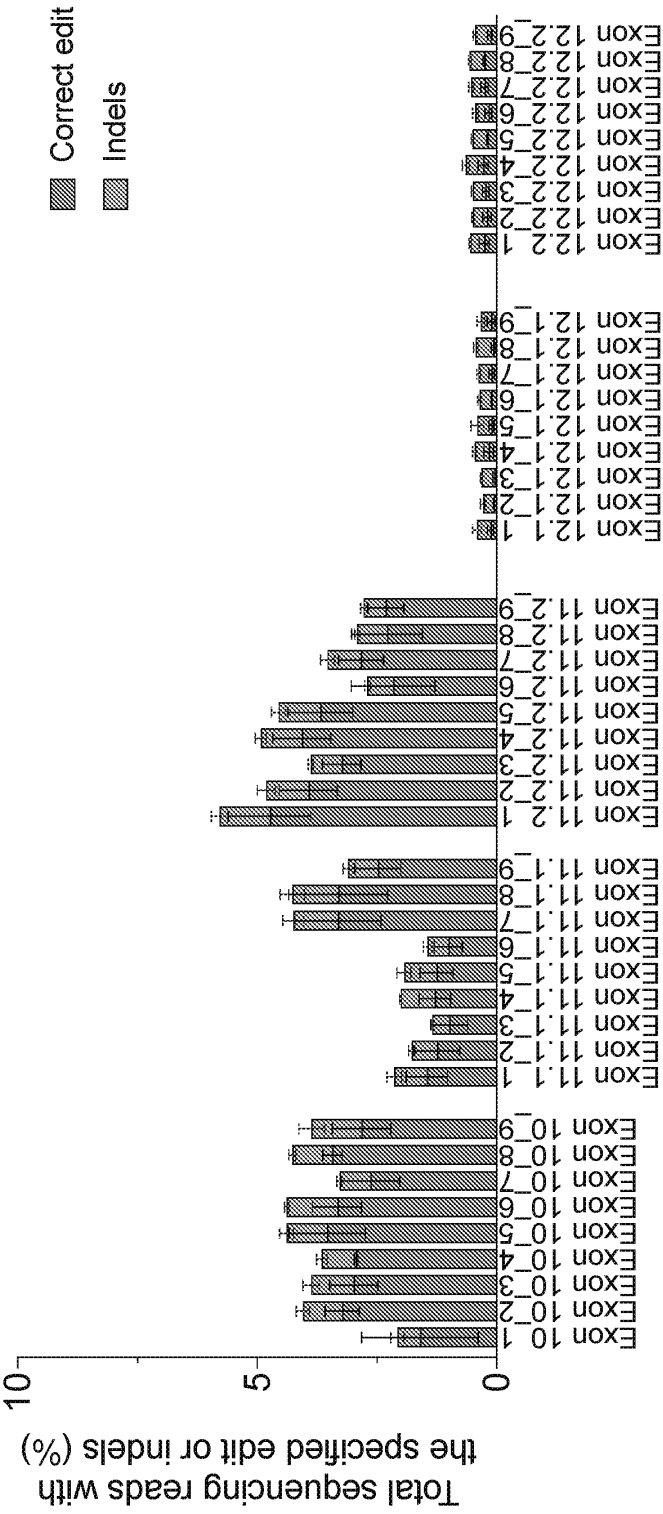


FIG. 112

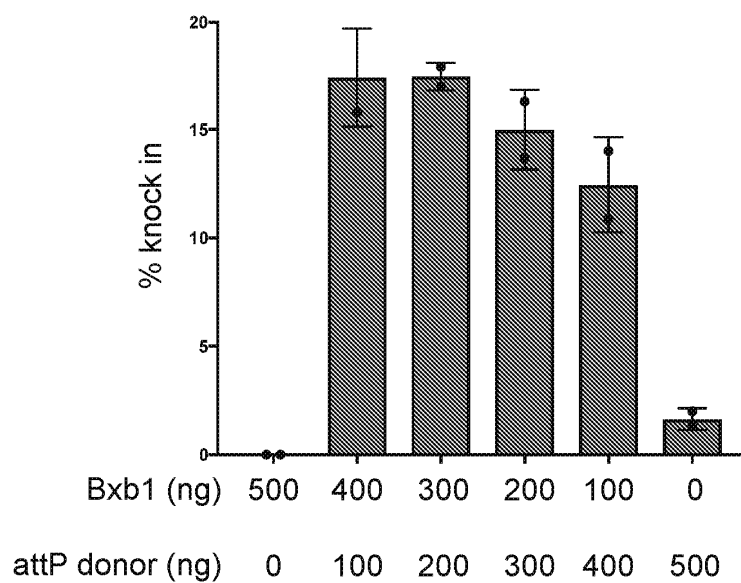


FIG. 113

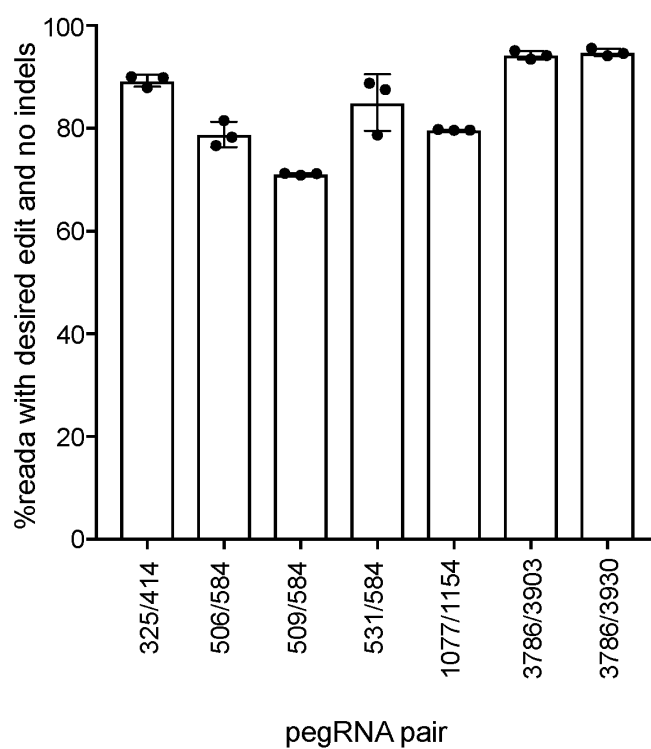


FIG. 114

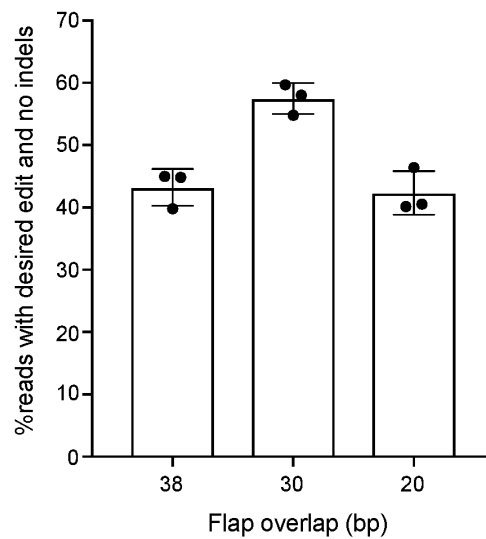


FIG. 115A

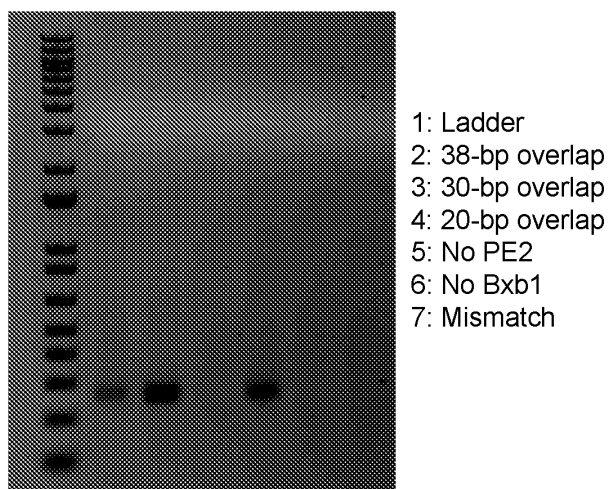
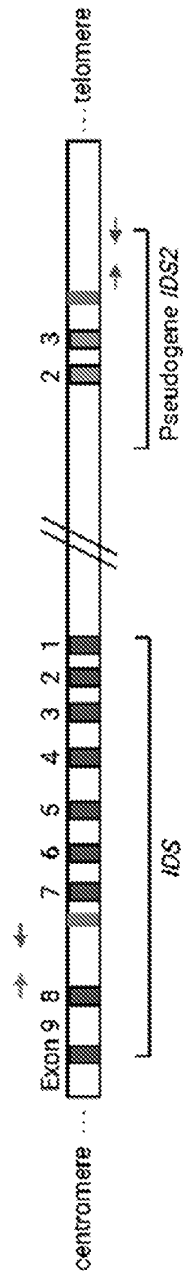


FIG. 115B



- ◆ The reverse primer (blue) that can bind to an identical sequence in both the non-inverted and inverted alleles and therefore amplify both edits with the same primer pair
- ◆ Primer pair 1 (green and blue) captures the unedited alleles (403 bp), twinPE edit (337 bp), and inverted alleles (326 bp) at junction 1
- ◆ Primer pair 2 (red and blue) captures the unedited alleles (346 bp), twinPE edit (326 bp), and inverted alleles (320 bp) at junction 2

FIG. 116