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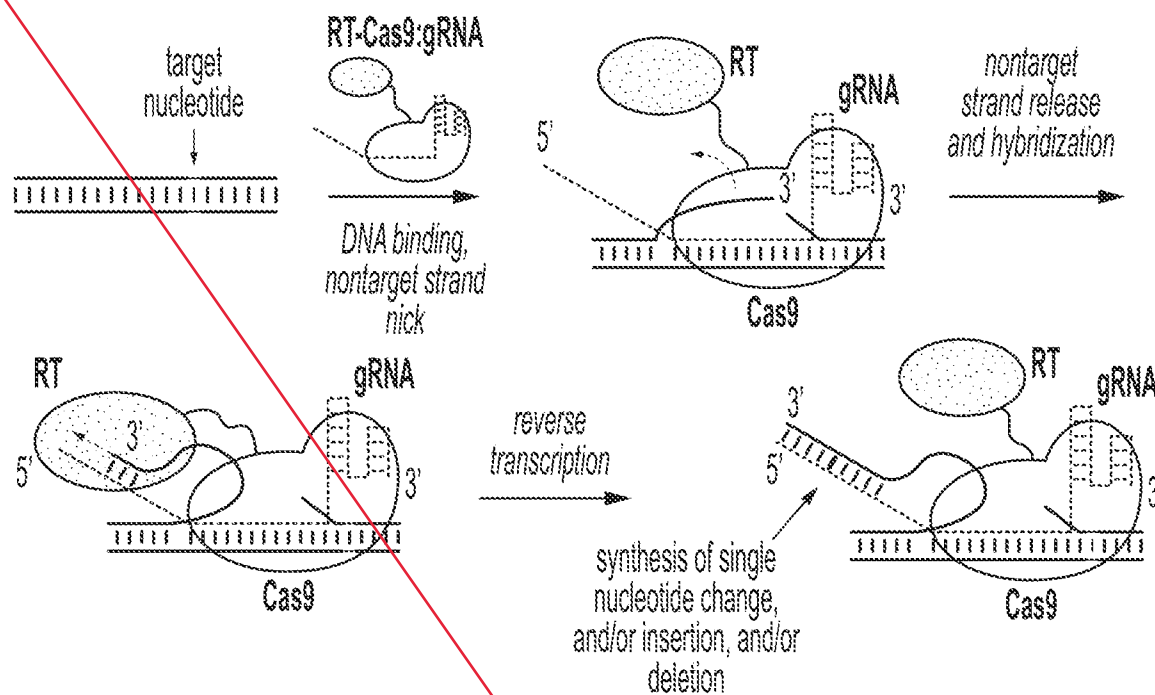


FIG. 1A

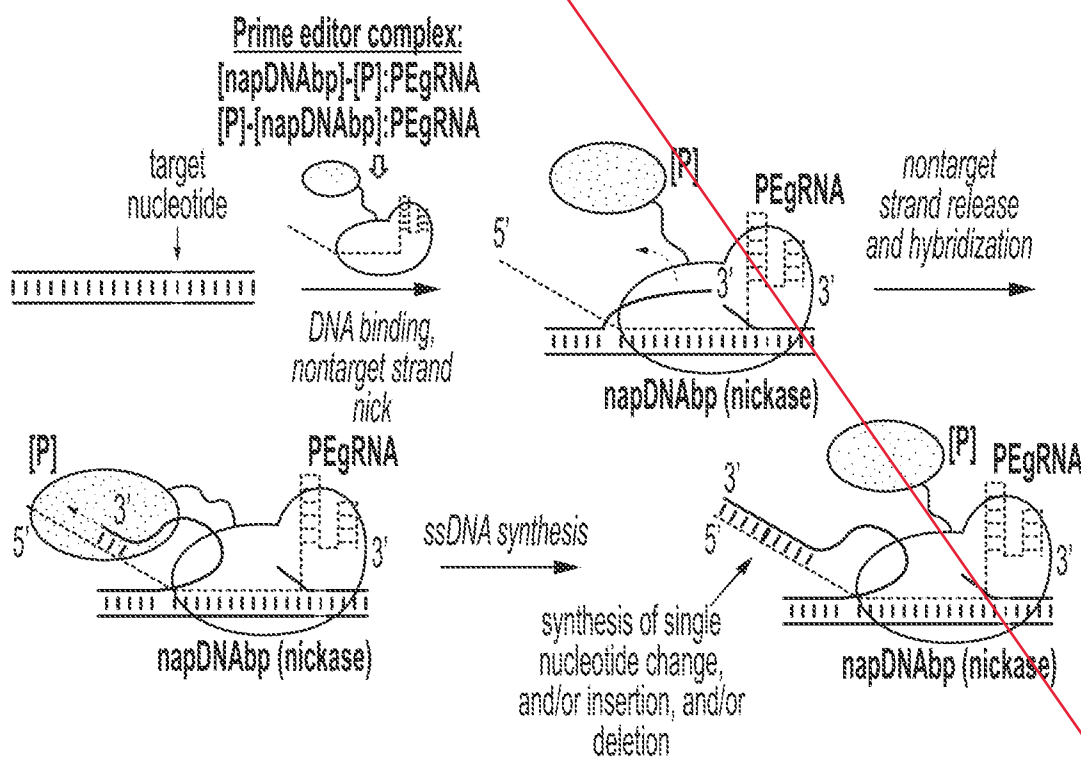


FIG. 1B

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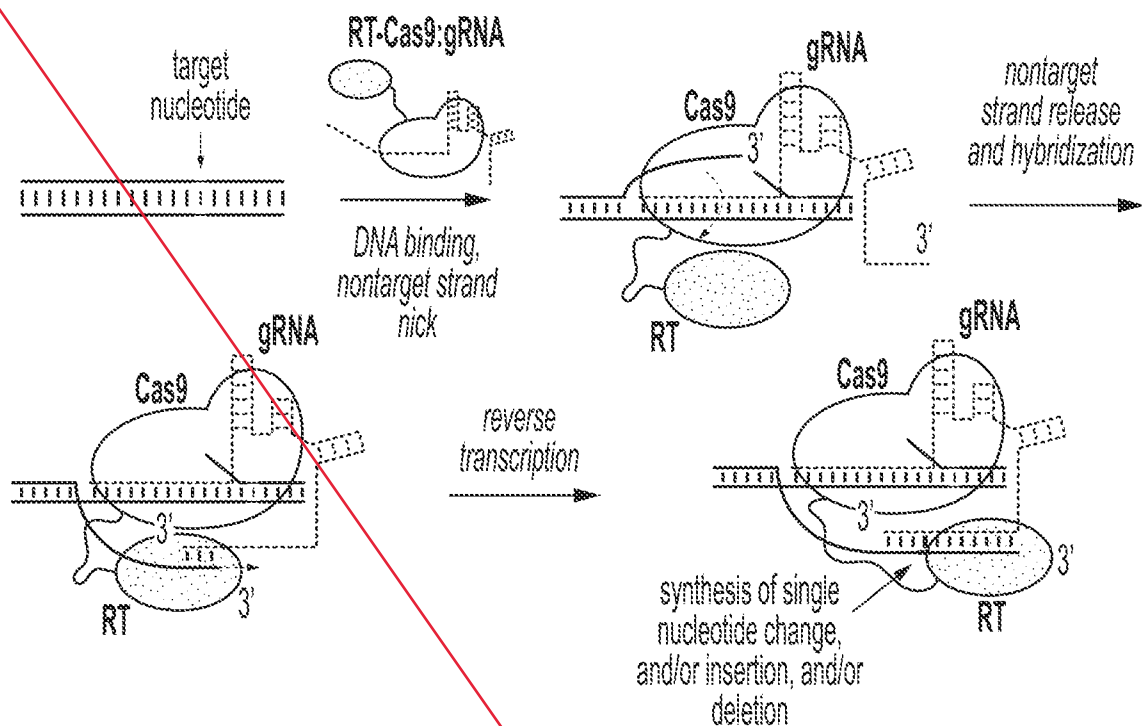


FIG. 1C

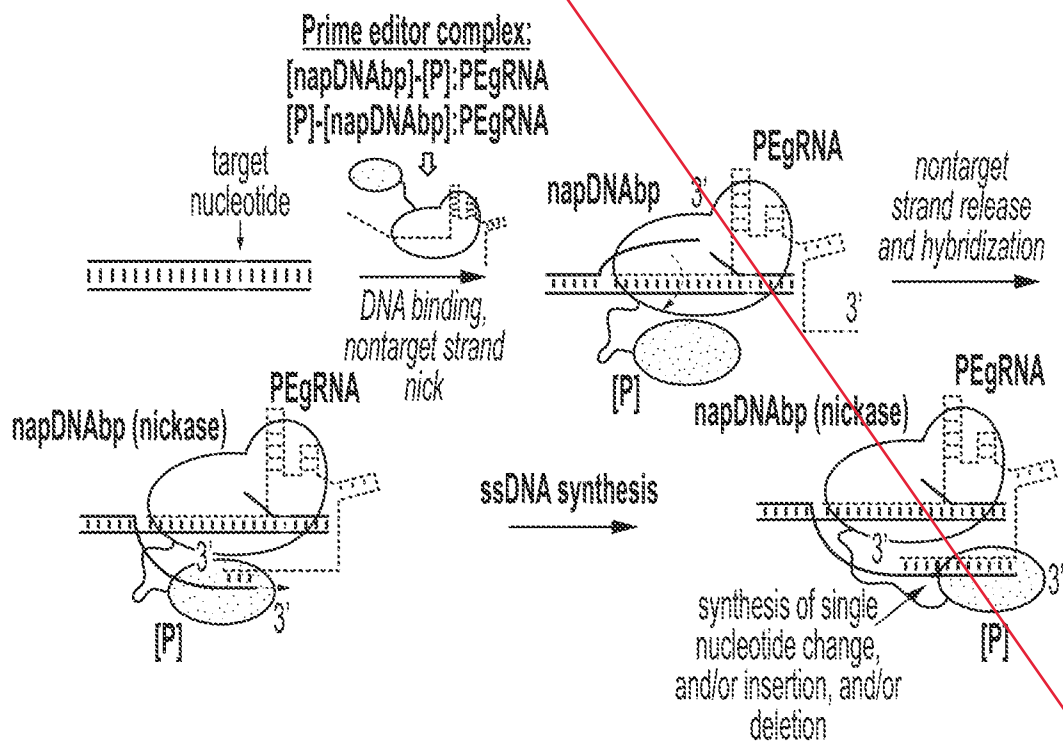


FIG. 1D

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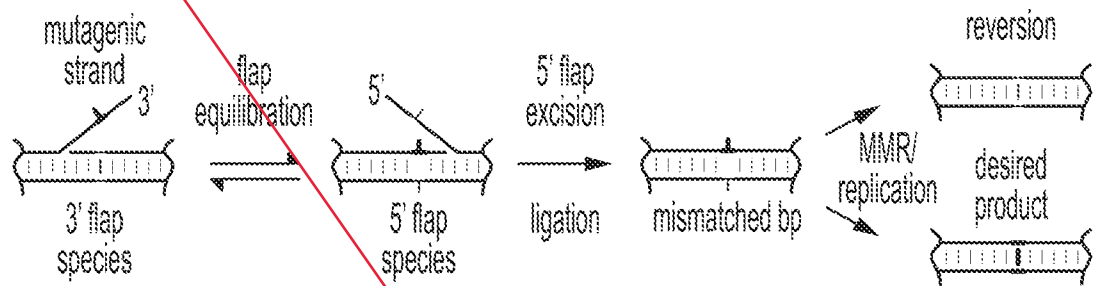


FIG. 1E

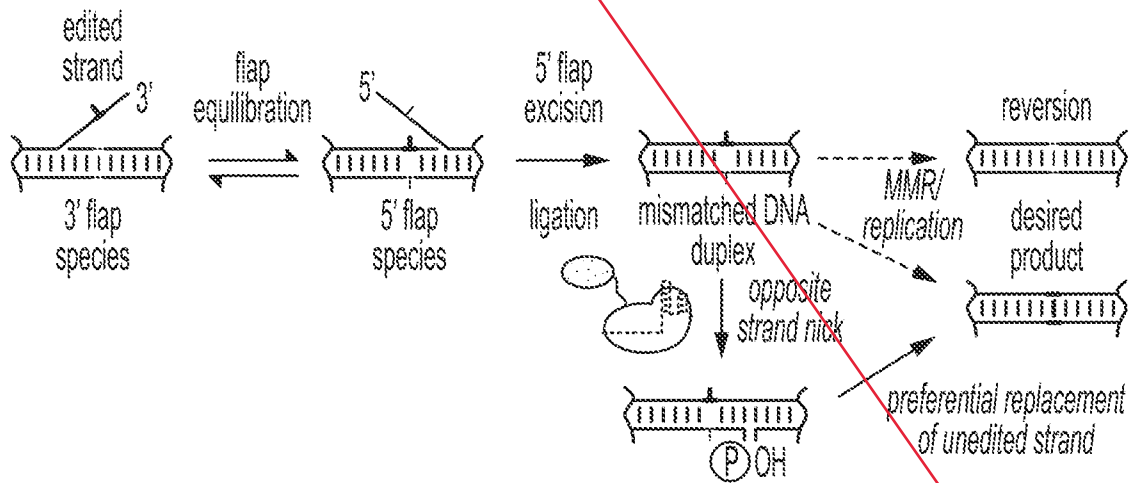


FIG. 1F

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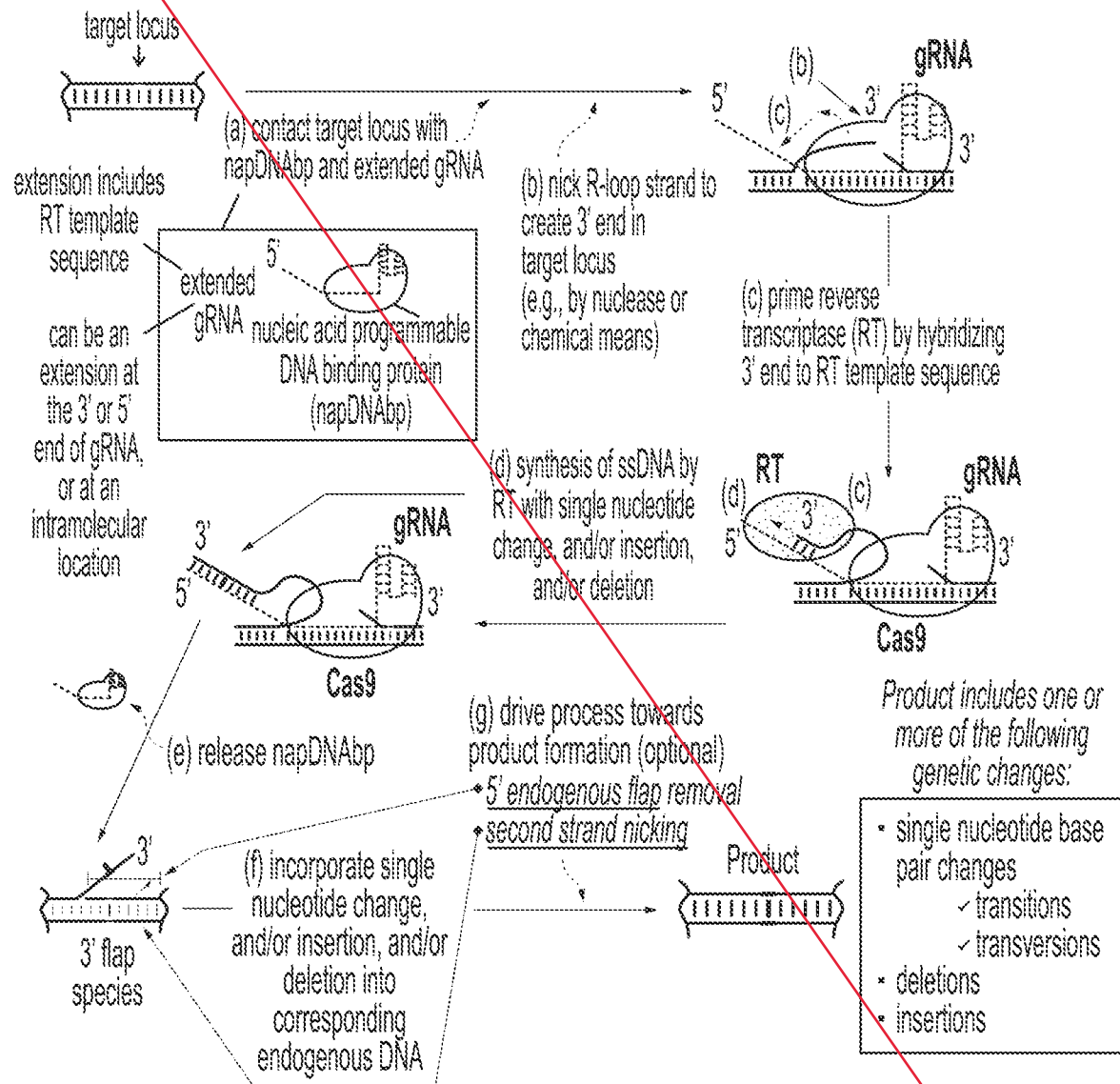


FIG. 1G

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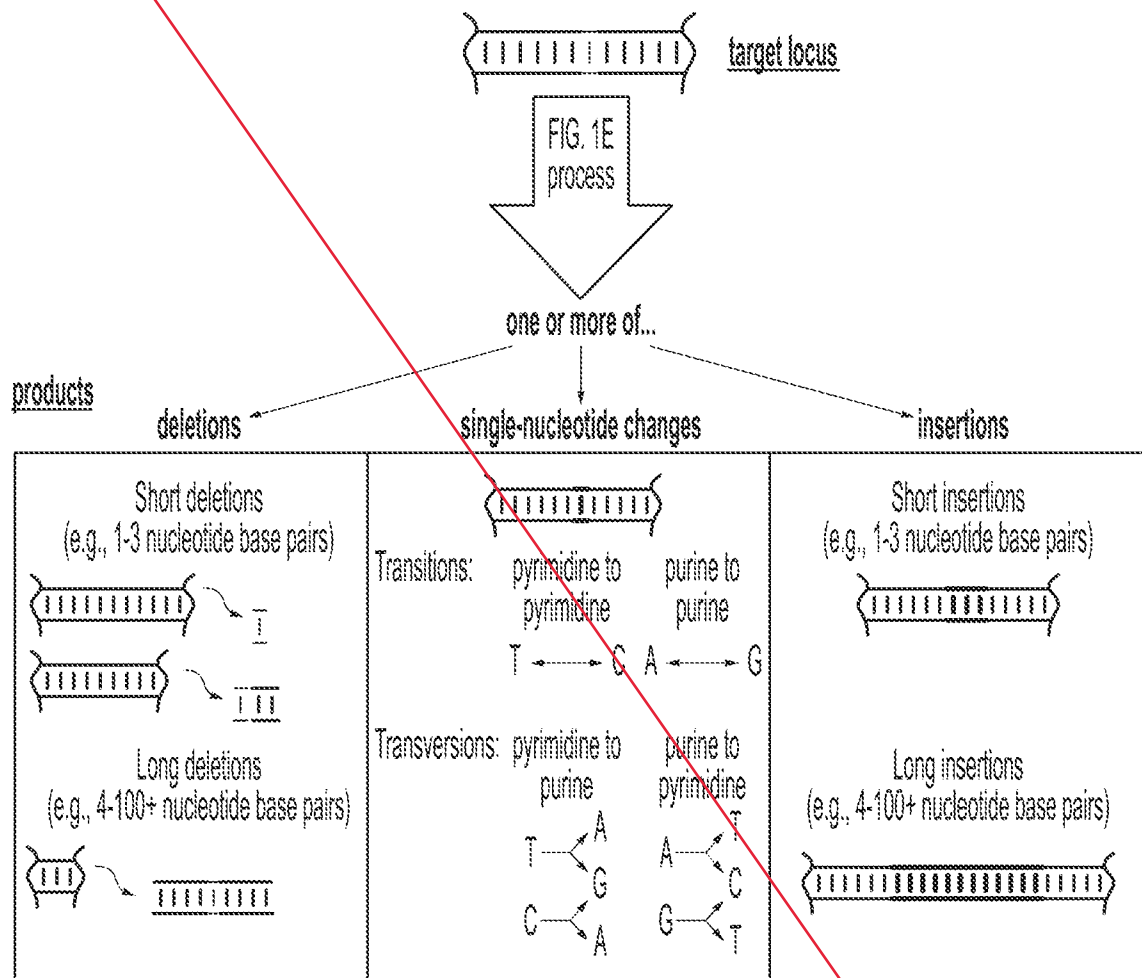


FIG. 1H

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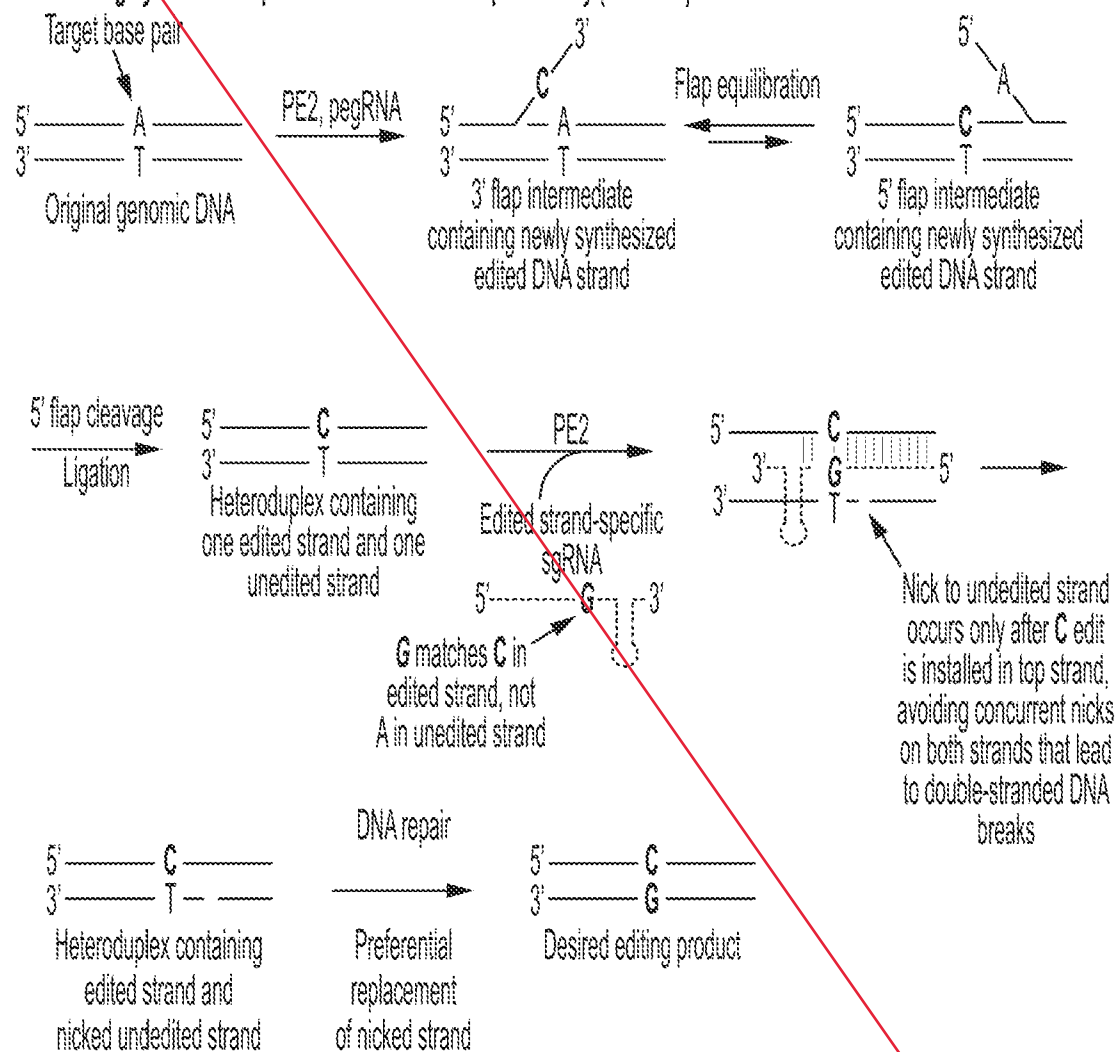
*PE3b temporal nicking.***PE3b editing system: edit-specific nicked of the complementary (unedited) DNA strand**

FIG. 11

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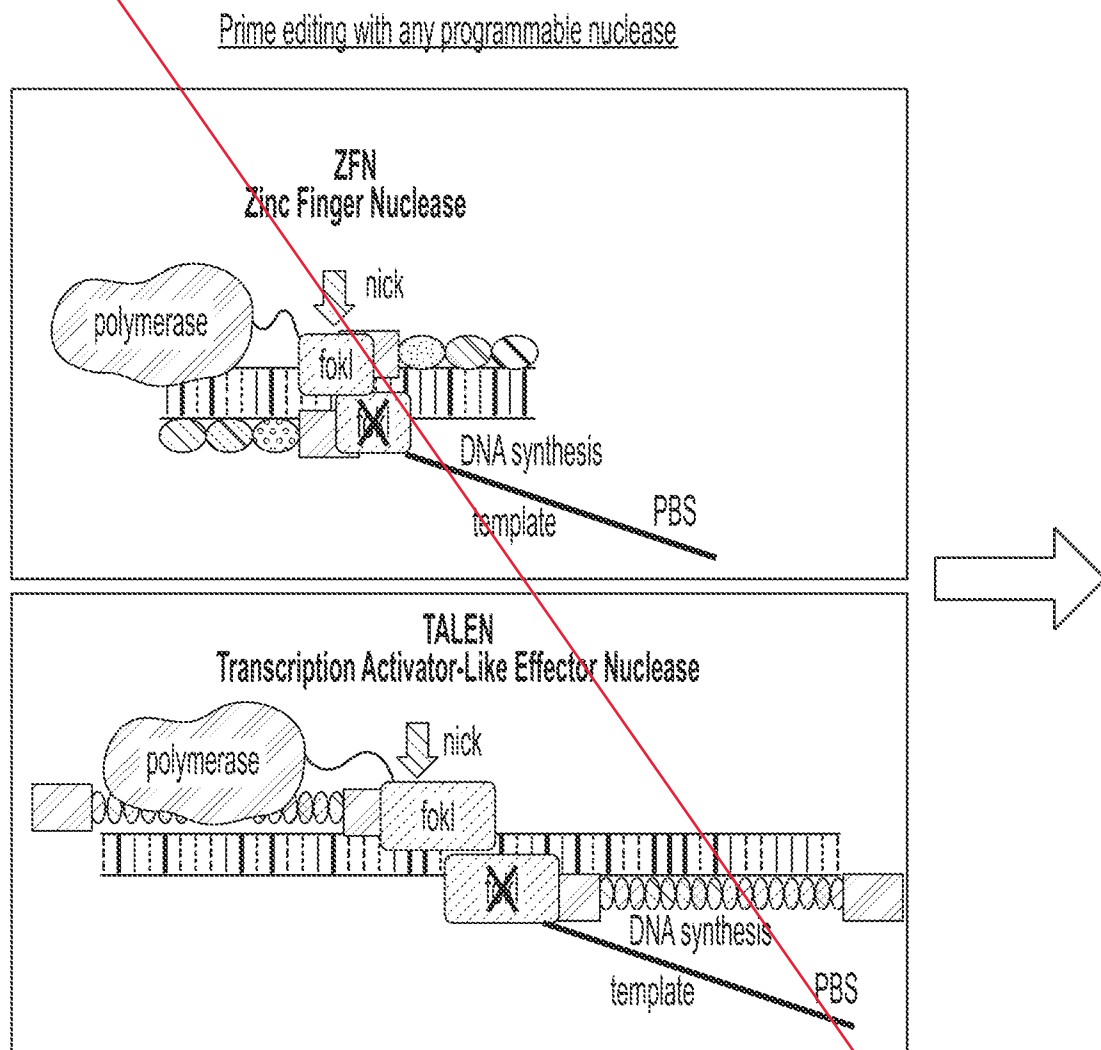


FIG. 1J

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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

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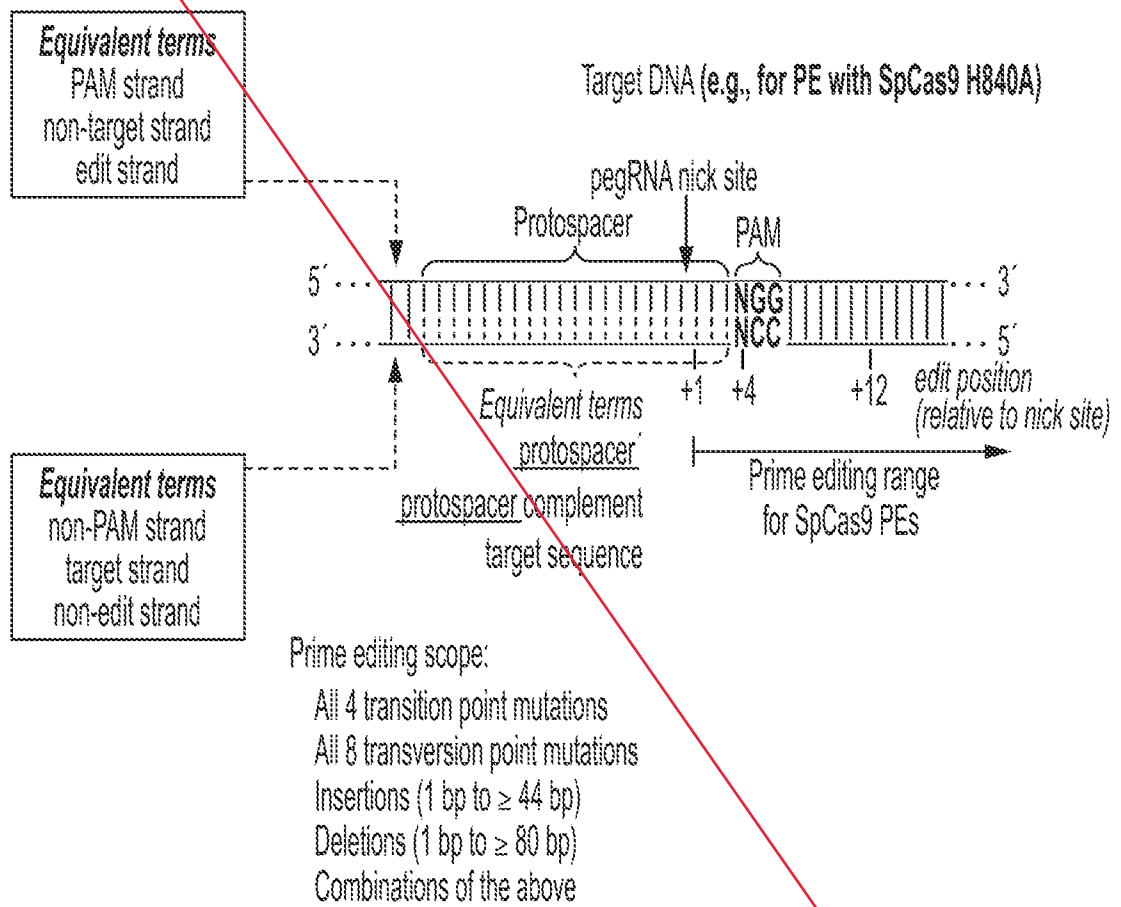


FIG. 1L

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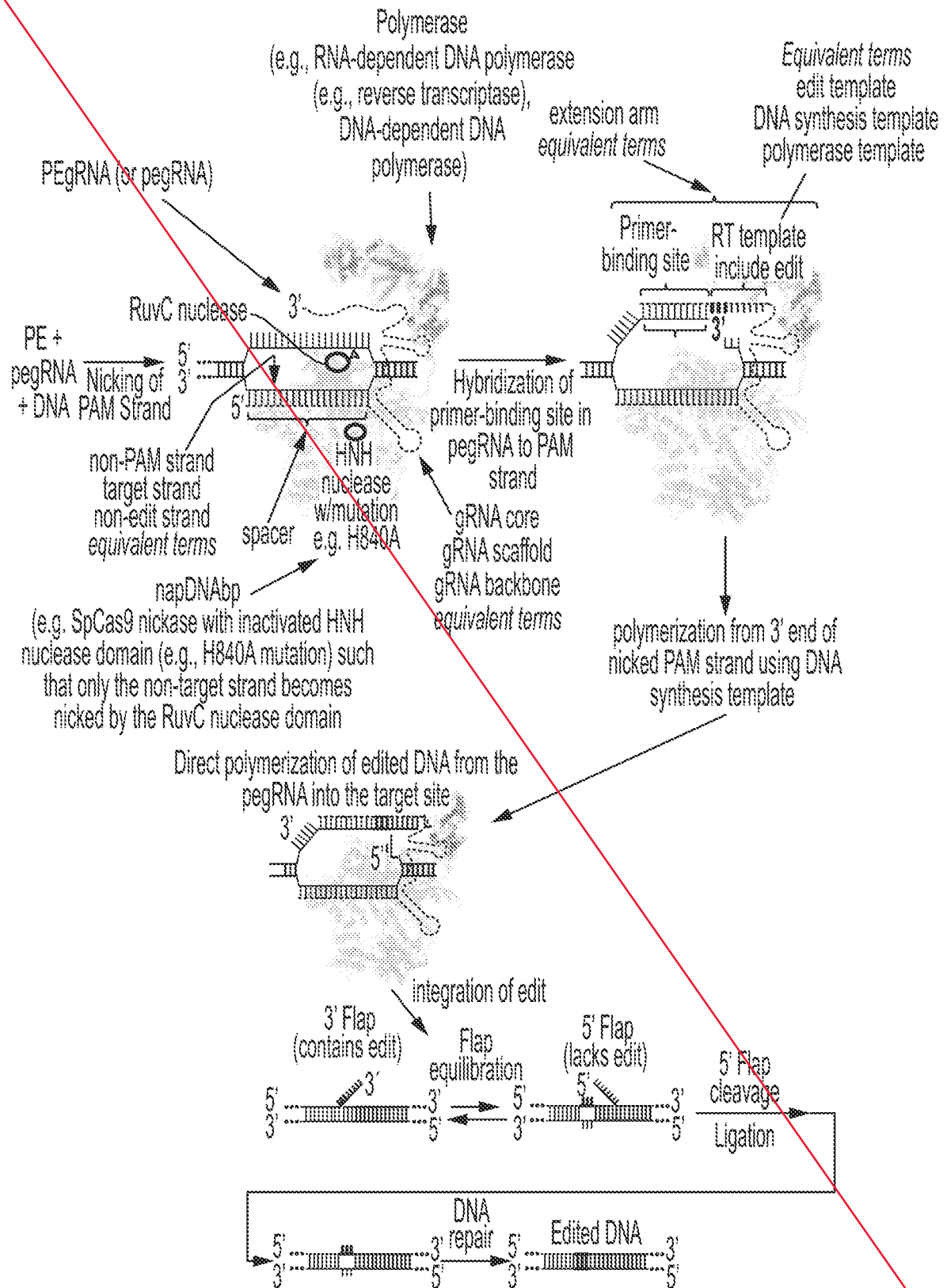
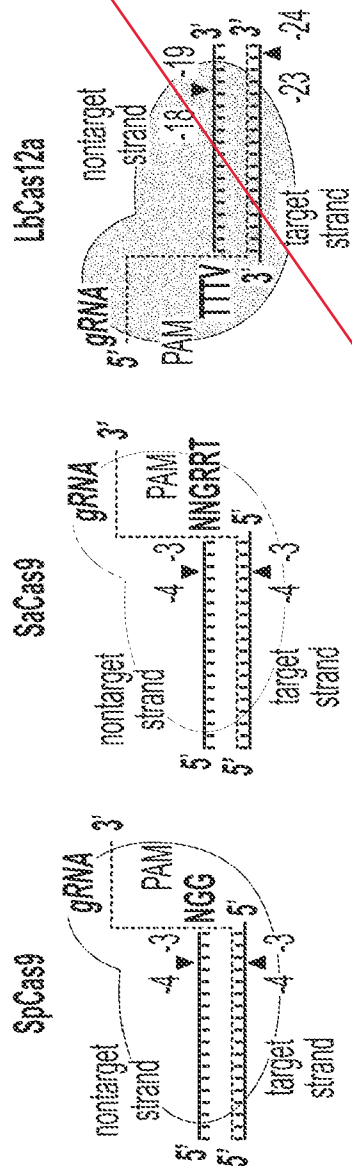
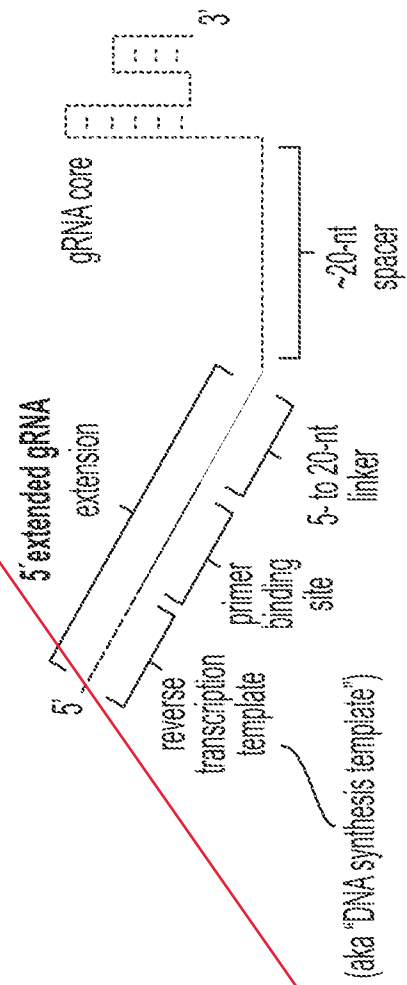


FIG. 1M



ALL

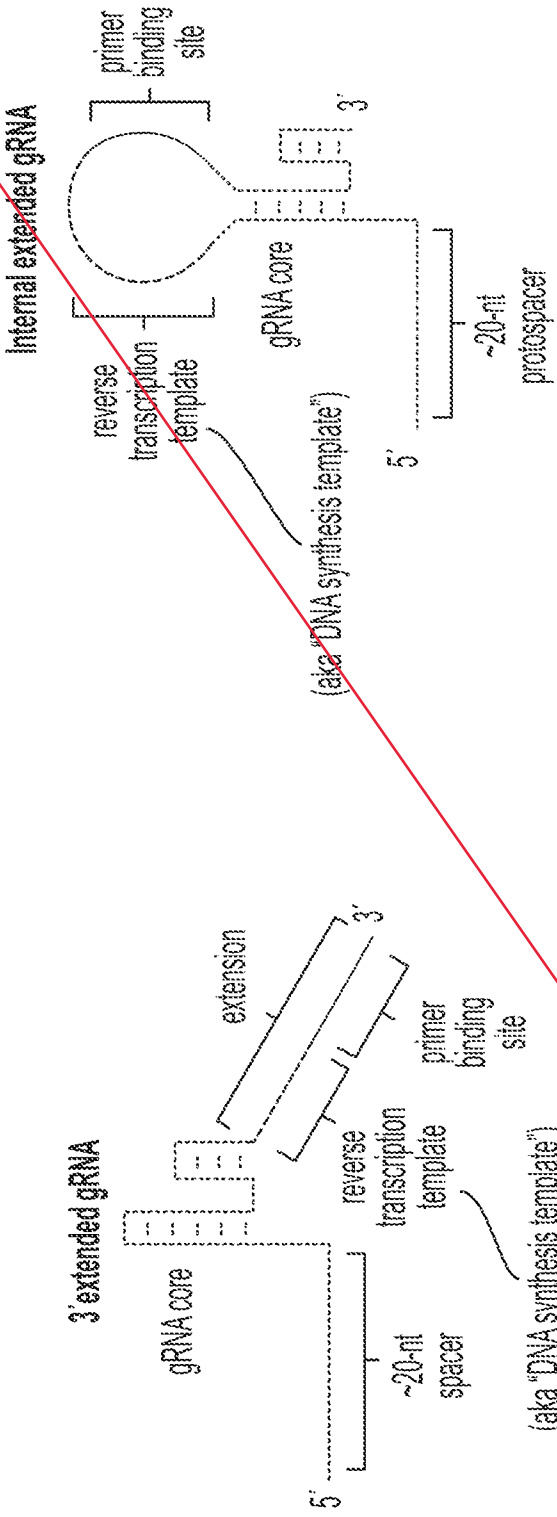


FIG. 3B

FIG. 3C

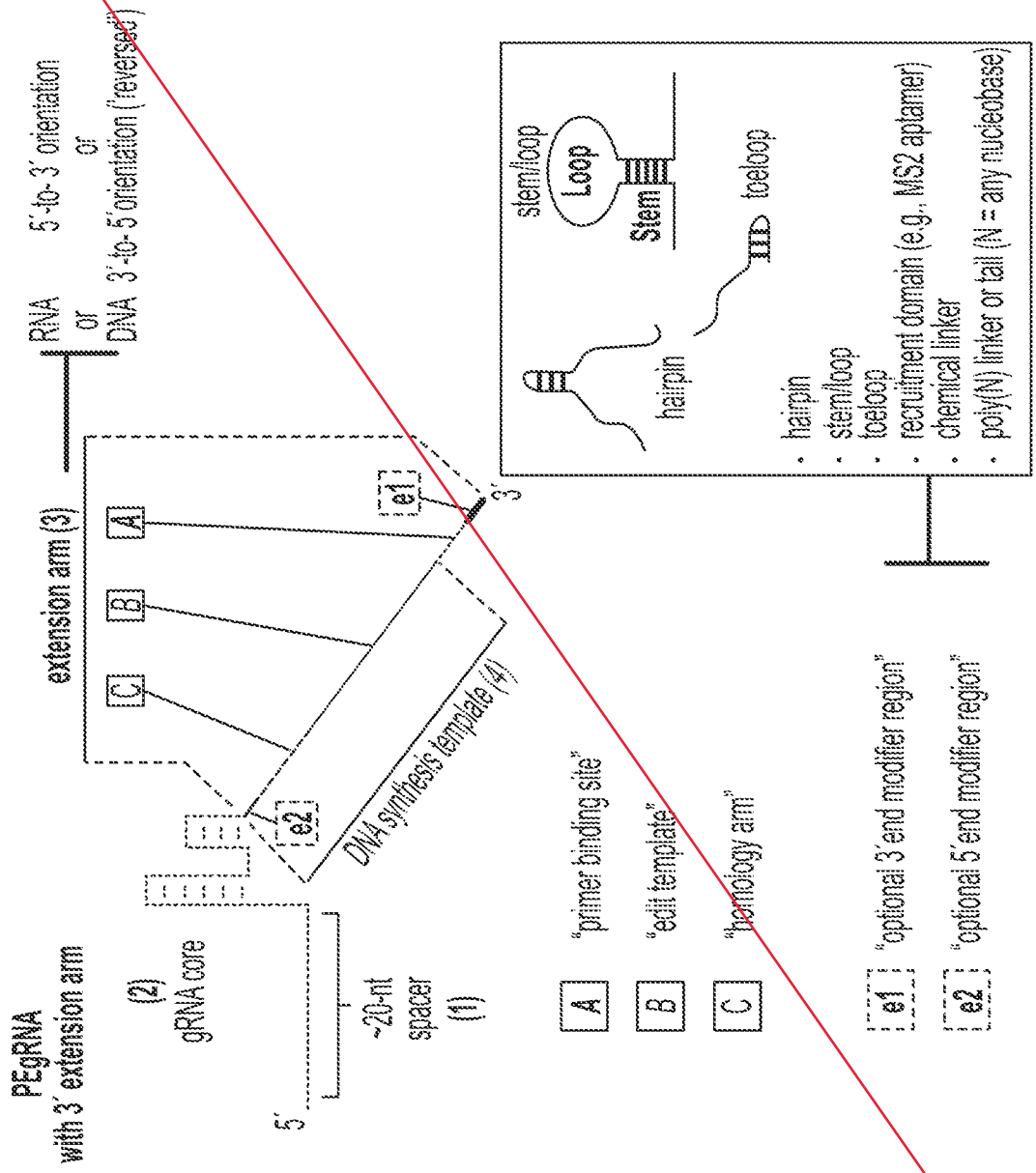


FIG. 3D

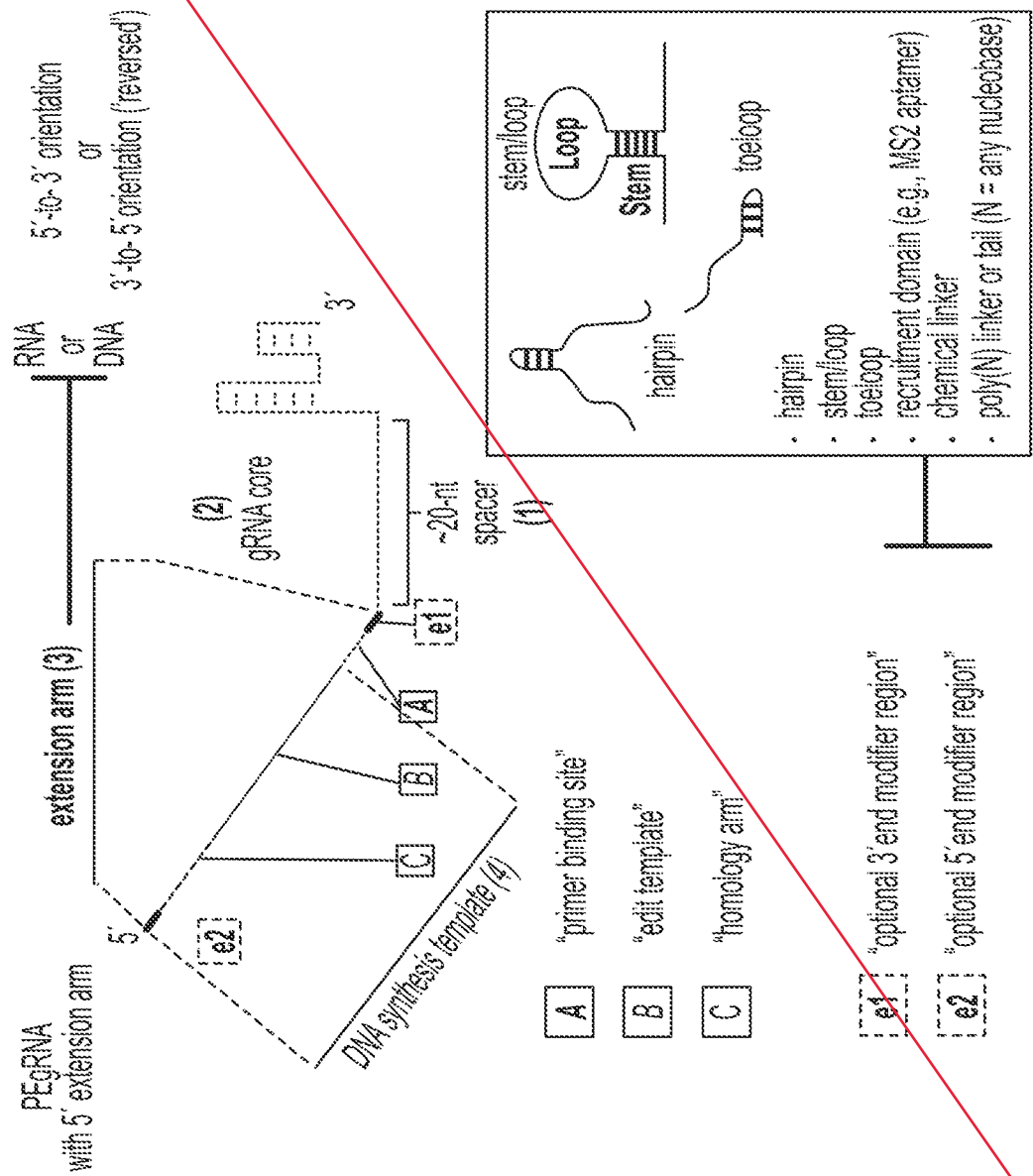
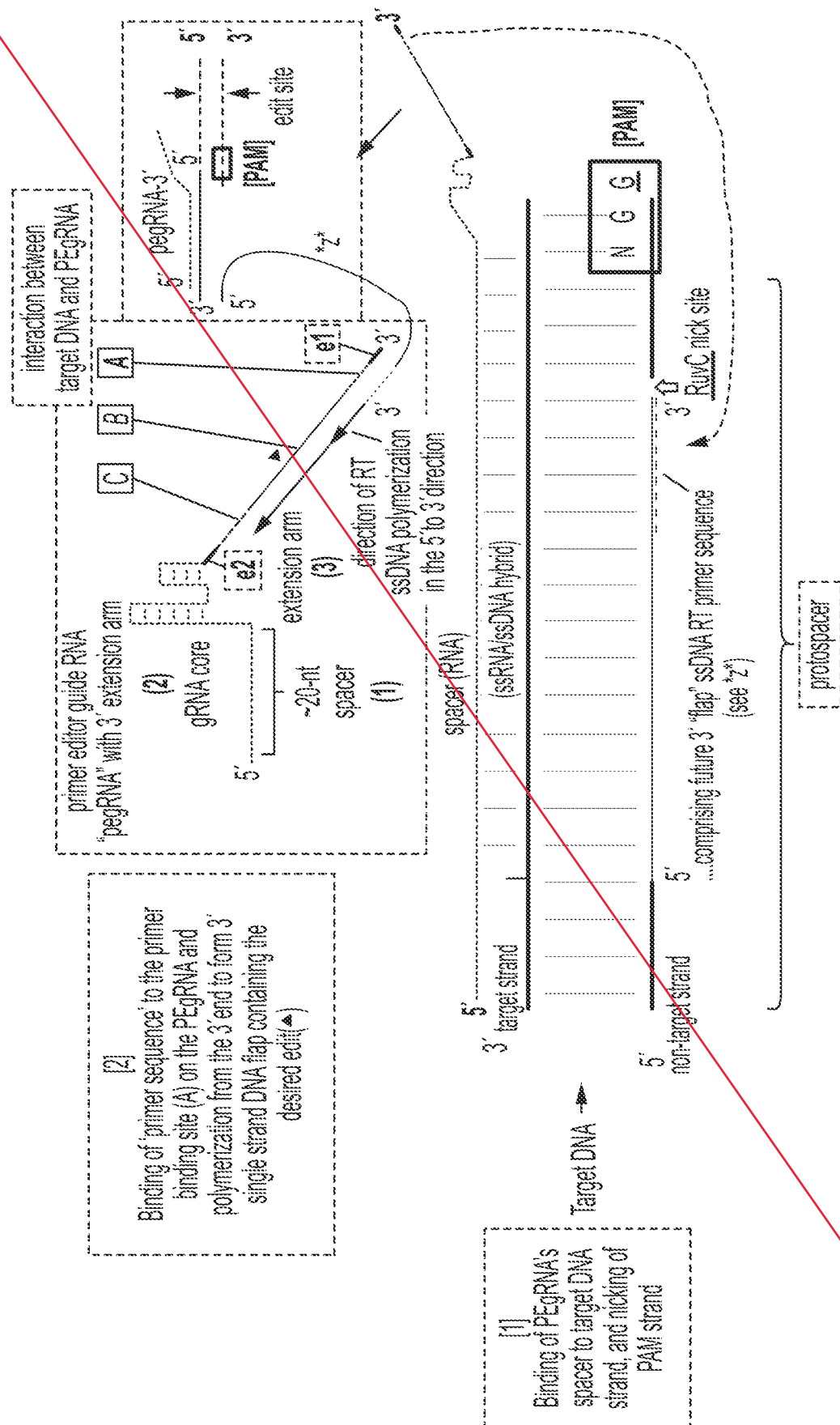
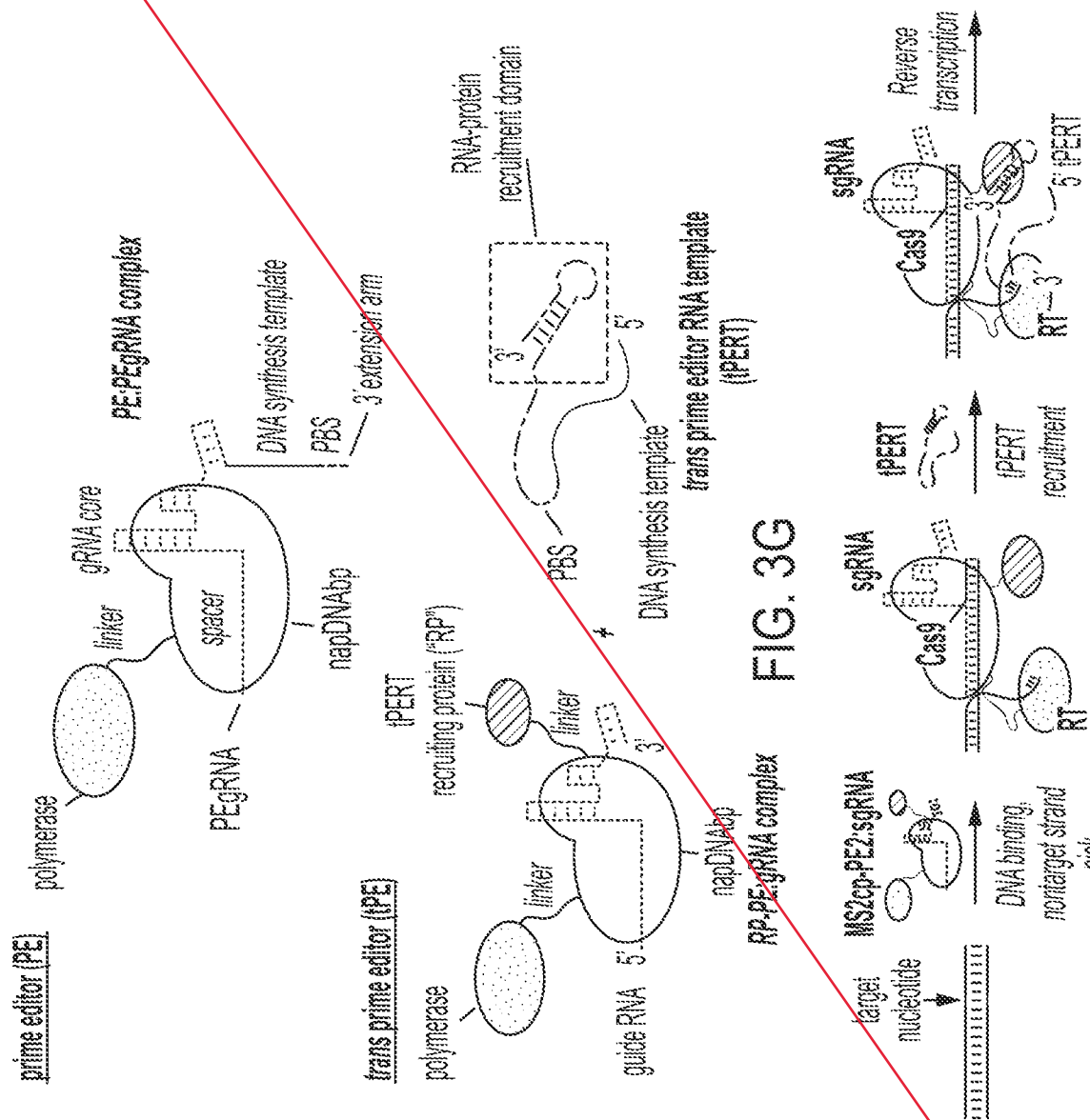


FIG. 3E



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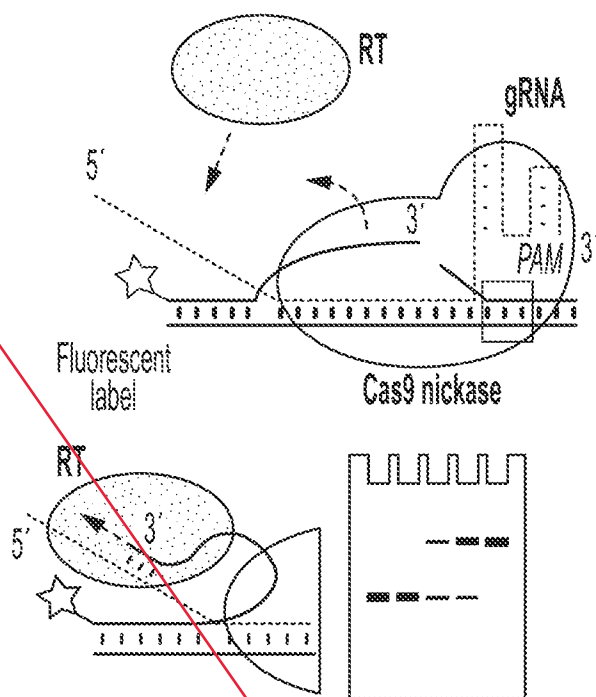


FIG. 4A

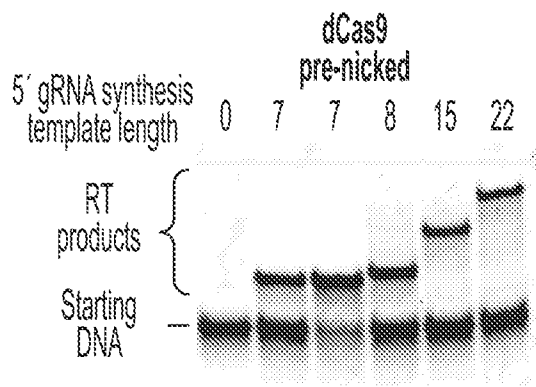


FIG. 4B

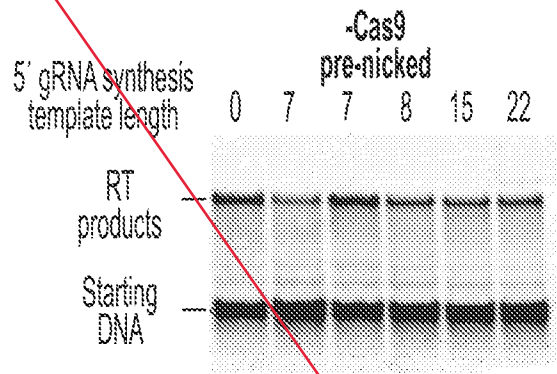


FIG. 4C

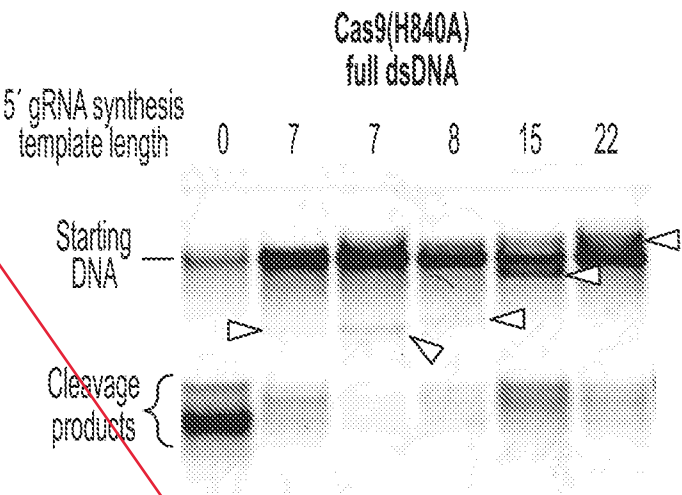


FIG. 4D

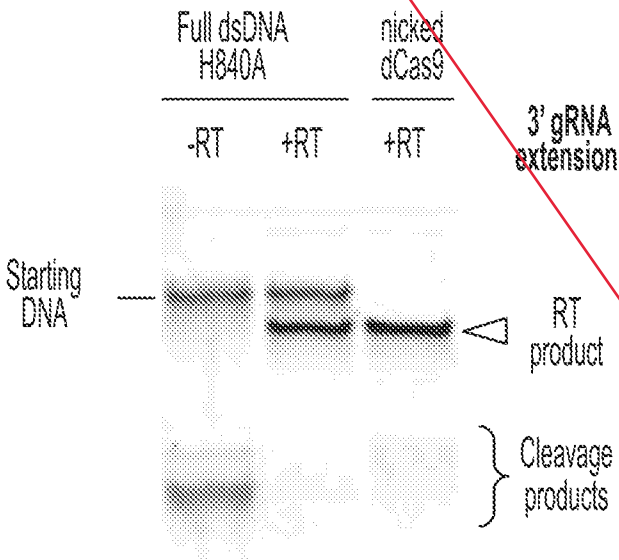
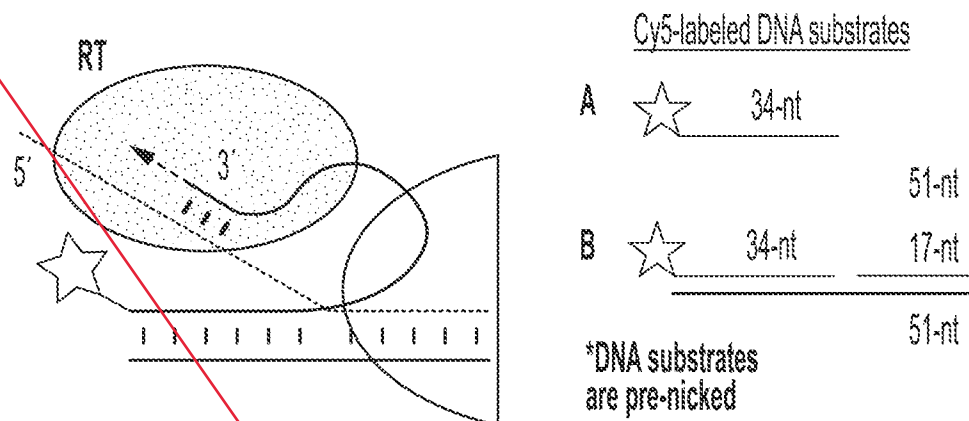


FIG. 4E

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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

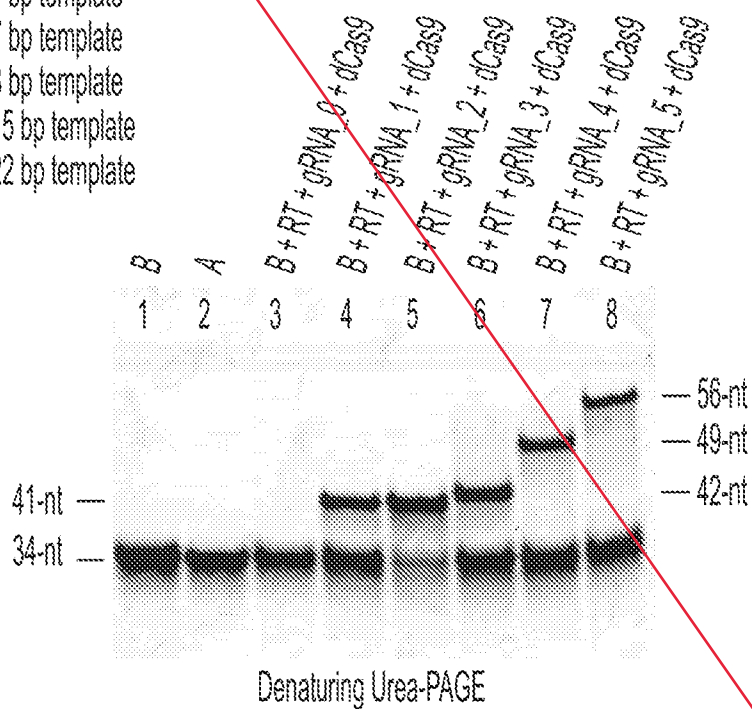


FIG. 5

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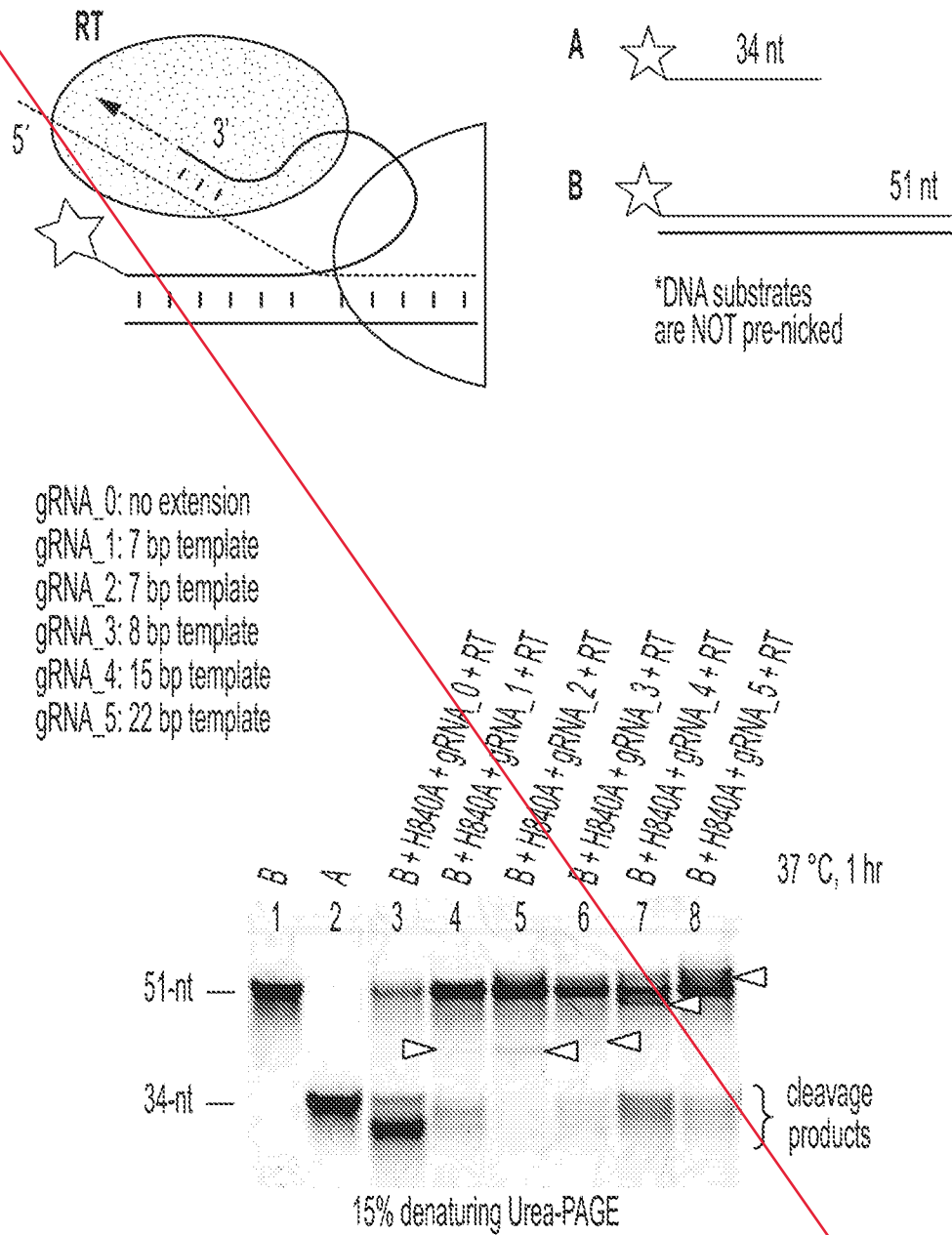
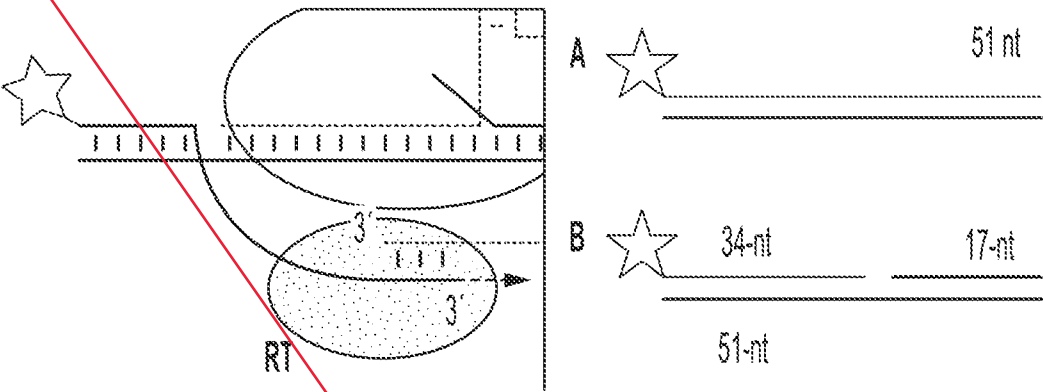


FIG. 6



gRNA_0: no extension
gRNA_1: 15 bp template

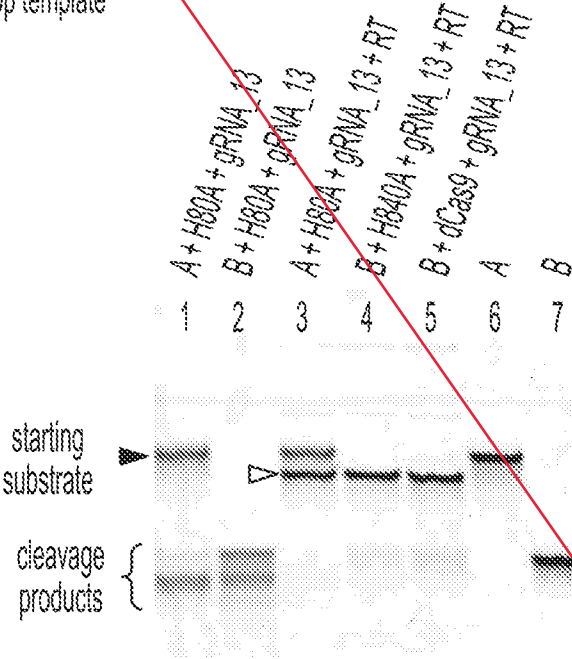


FIG. 7

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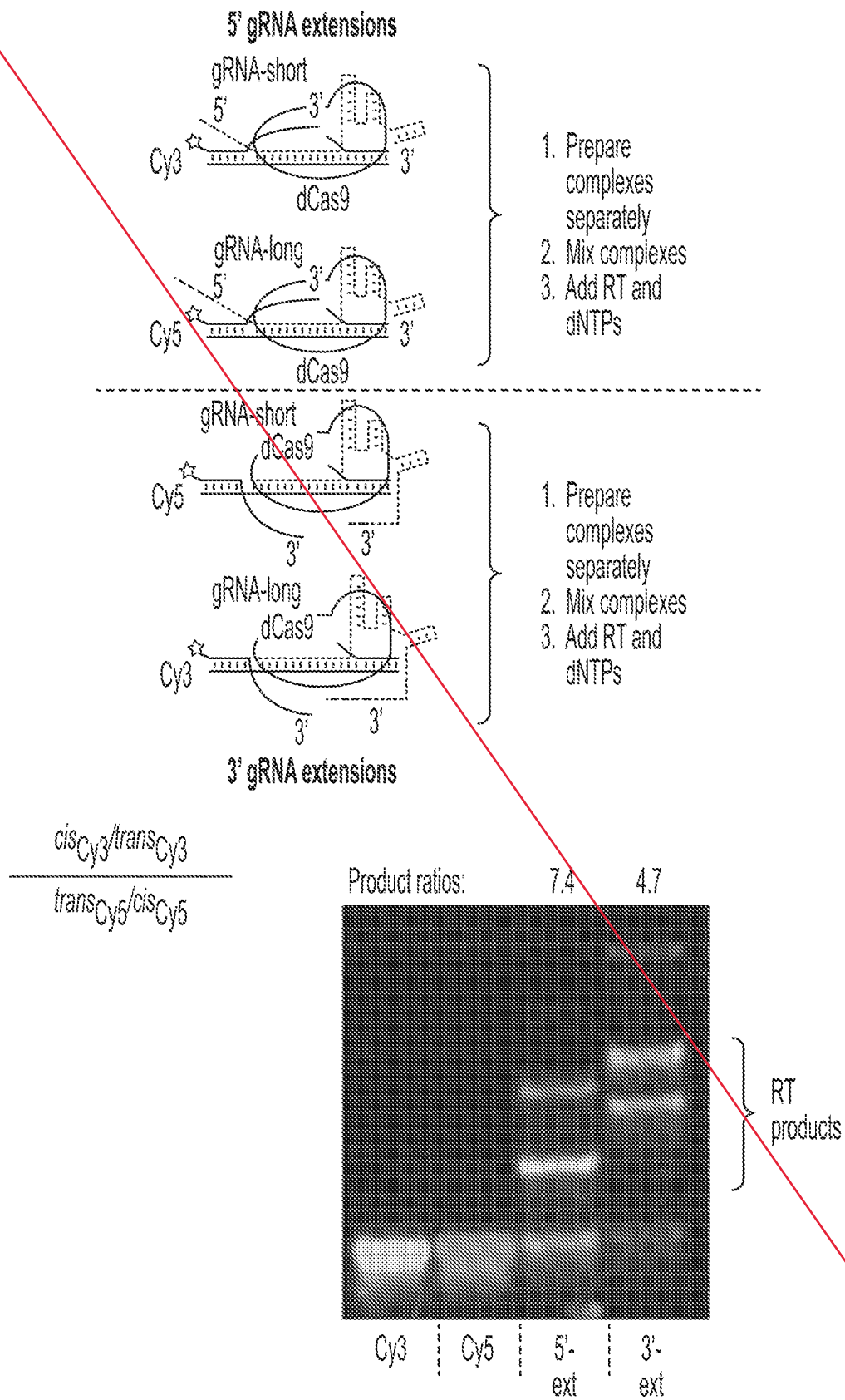


FIG. 8

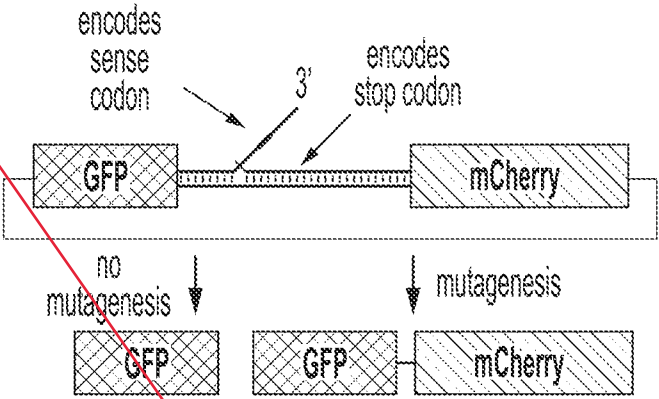


FIG. 9A

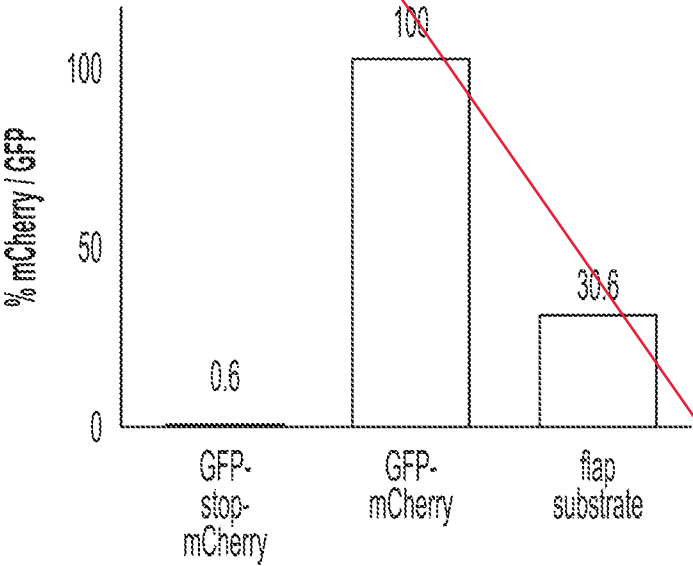


FIG. 9B

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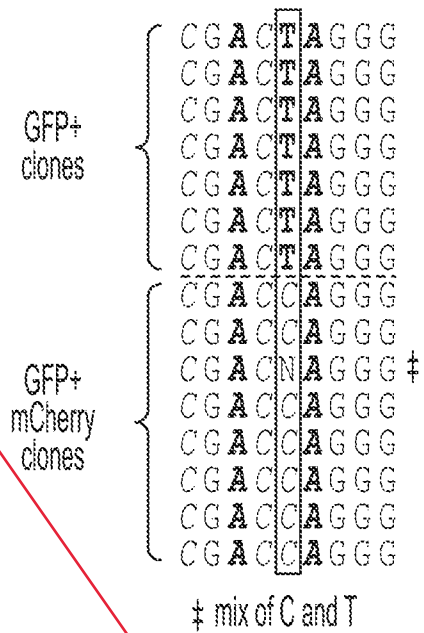


FIG. 9C

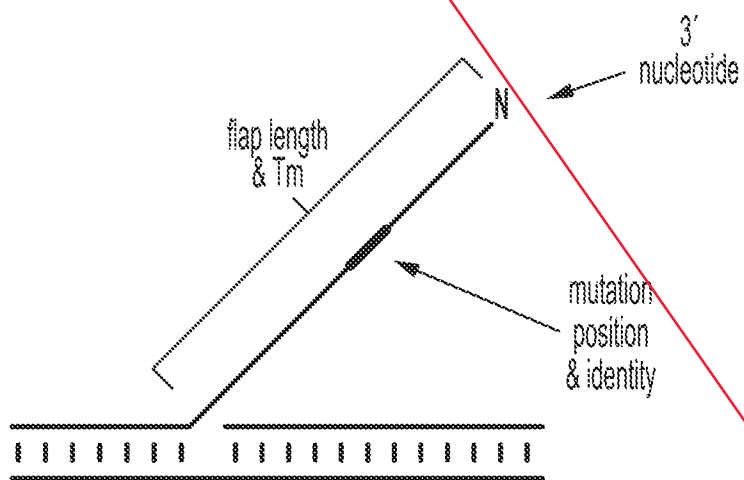


FIG. 9D

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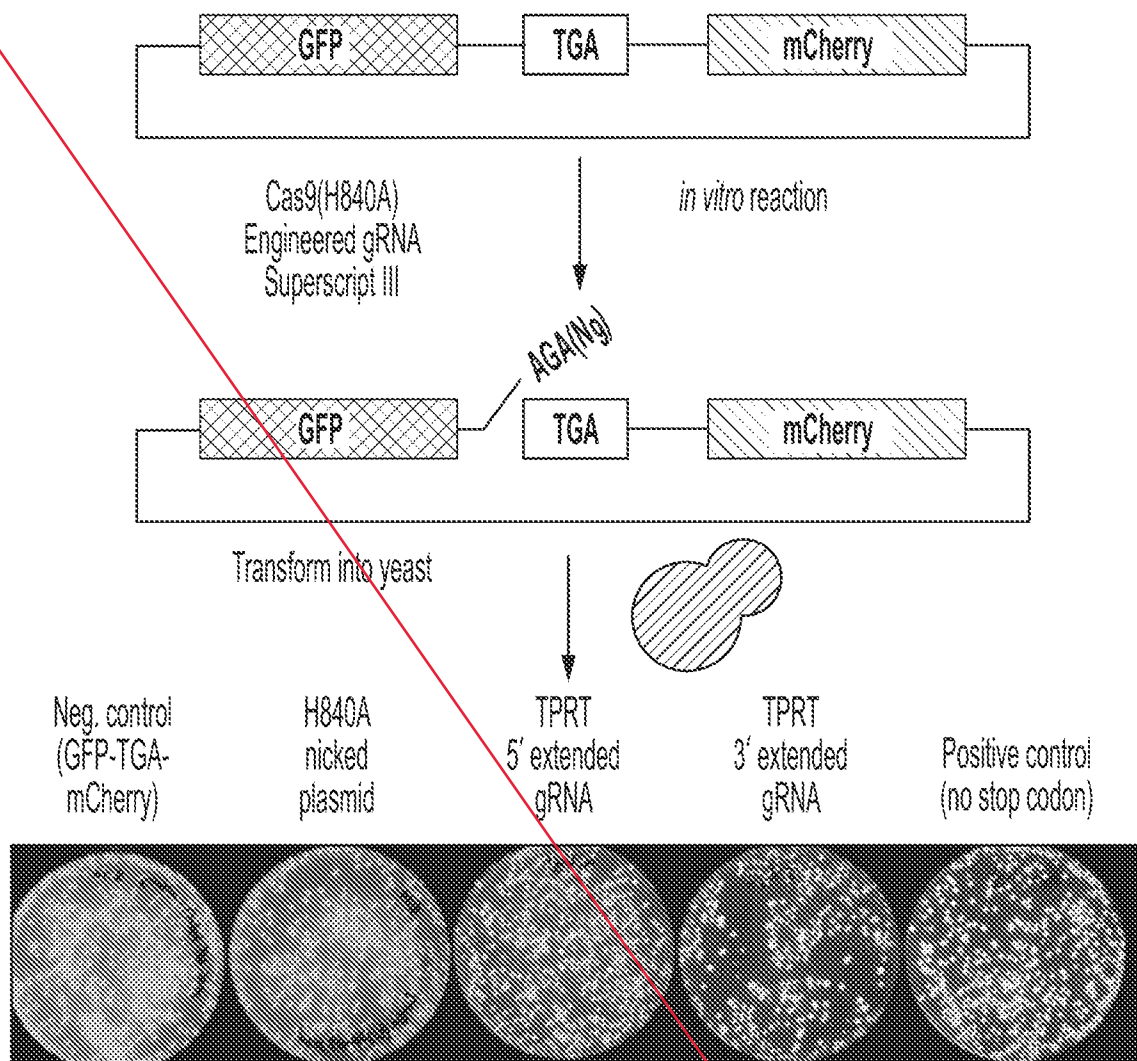


FIG. 10

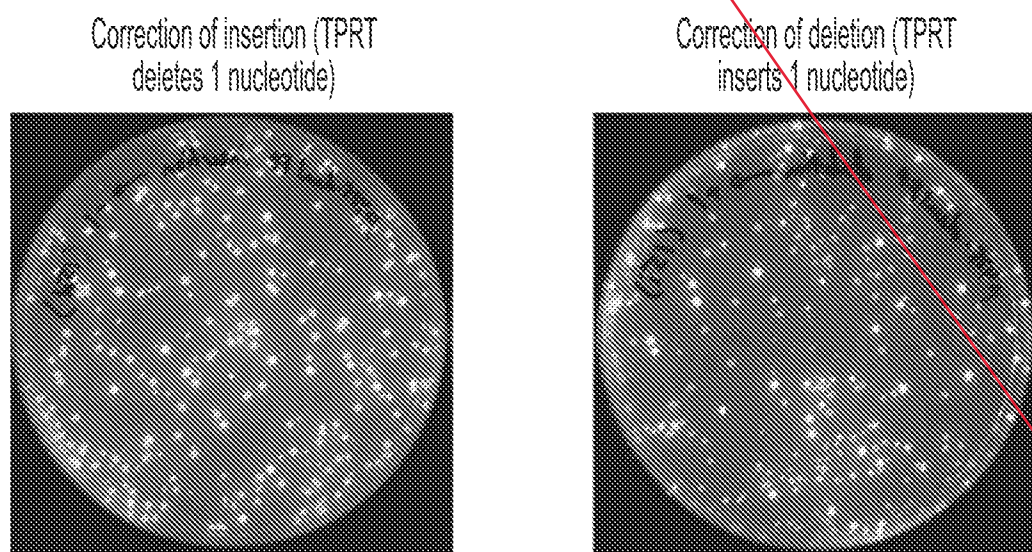


FIG. 11

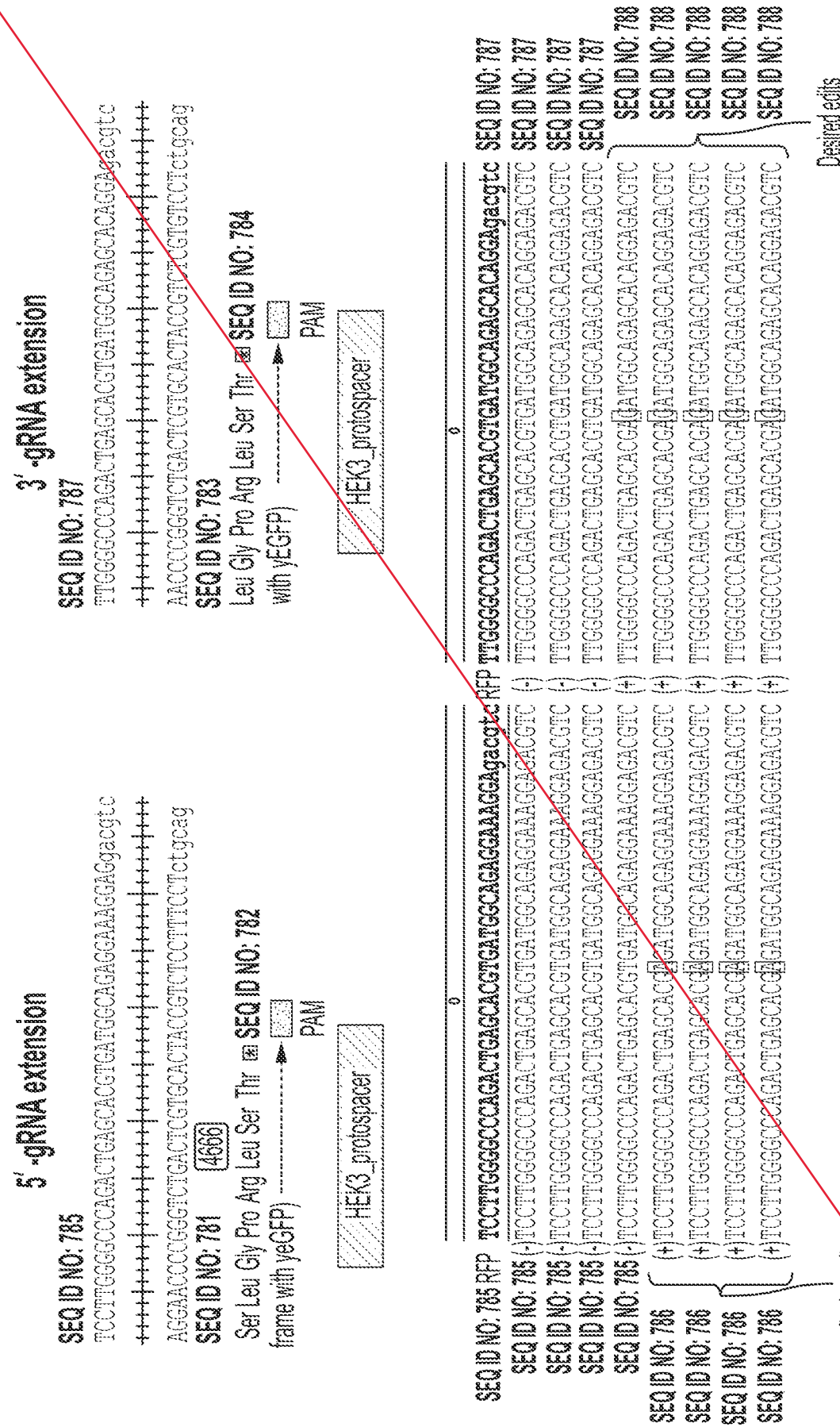


FIG. 12

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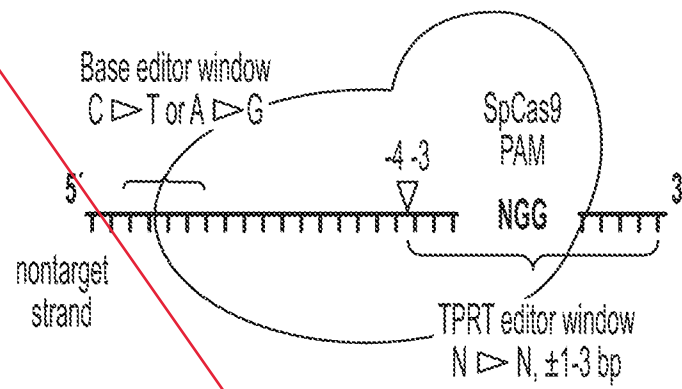


FIG. 13

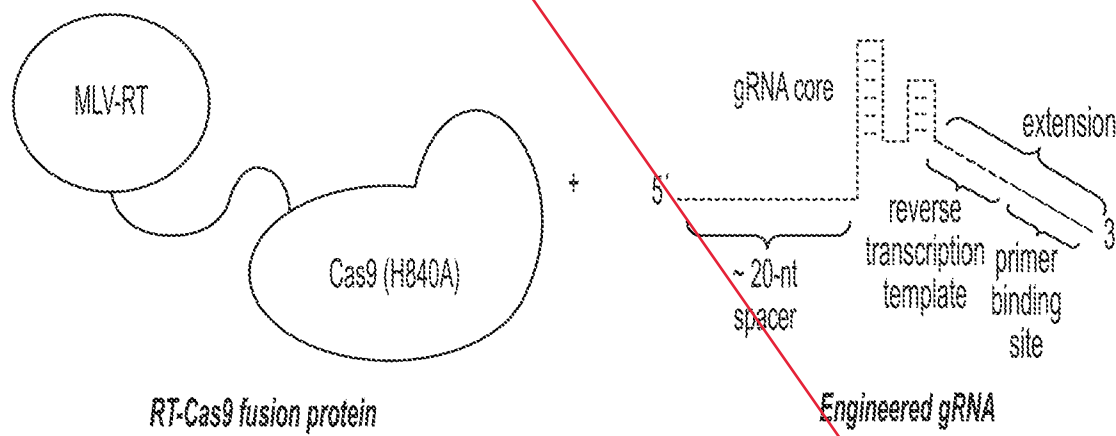
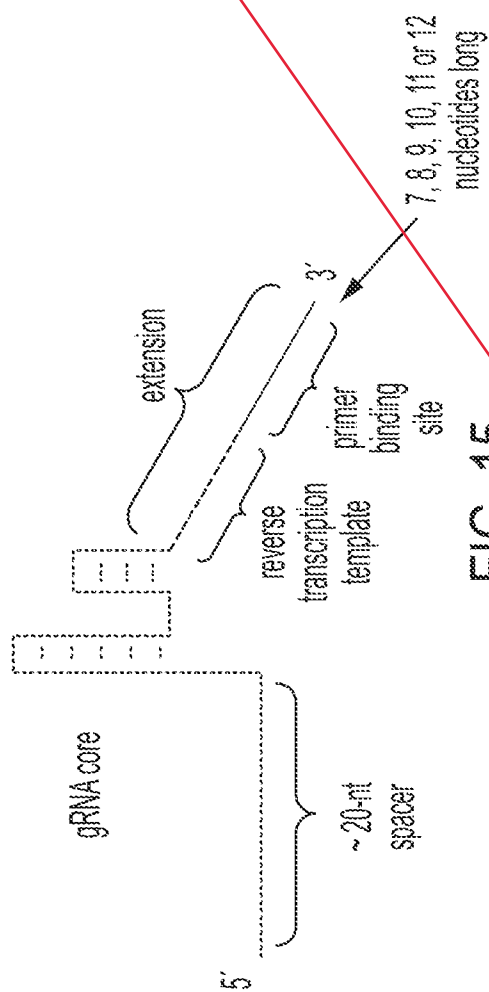


FIG. 14



SECRET

5' ATGTGGCTGGCTAGAAAGGCAATGCAATGAGAGAGAGCCCTGGAGACAGGGATCCACAGGGAACGCCCAATGCCAATAGTCTATTTCTGCTGGAAAGTAAGCAATG
3' TACACCGACGGAGACTTCCGTACTACTCTCTCTGGACCTCTGCTCCCTAGGGTCCCTTTGCGGGTACGTTAATCAGATAAGACGACGCTTCATTCGTAC

SEQ ID NO: 790

spacer 3

CAATTCAGGCTTGATGCTTTTTCGCTTCCAGCCCTGGGCTGGGTCAATCTTGGGGCCACACTGAGCACCGTGTATGGCAGAGGAAGGAGCCCG
GTAACATCCGAACTACGAAATAAACAGAGCTGGGACCGGACCCAGTATAGGAACCCGCGGTCTGACTGTGGCACACCGCTCTCTTTCTCTTGGG

C-18085

Spacer 2

12

TGCTTCTCCAGAGGGCGTGGGAGGACAGCGTTTCTCTAGACAGGGCGTAGTATGTGGAGCTCTCTGCACCGGATACTGGTTGACAGTTTGGCTGGG 3'
 ACGAAGAGGGTCTCCCGCAGCGTCTGTGCGAAGGATCTGTCCCGGATCATACACTGCAGGACCTGGCCCATGACCAATGTTCAACCGCAGCC 5'

~~spacer 4 rev~~

spacer_6

13

Speaker's Rev

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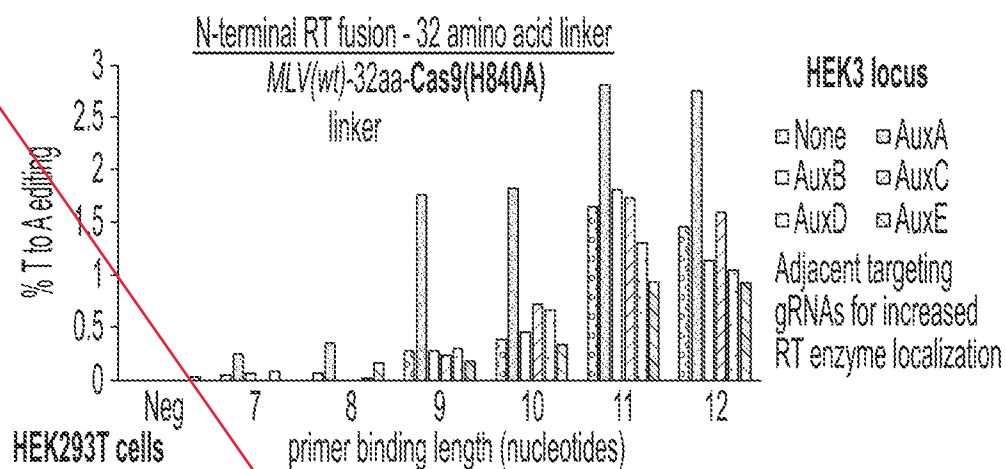


FIG. 17A

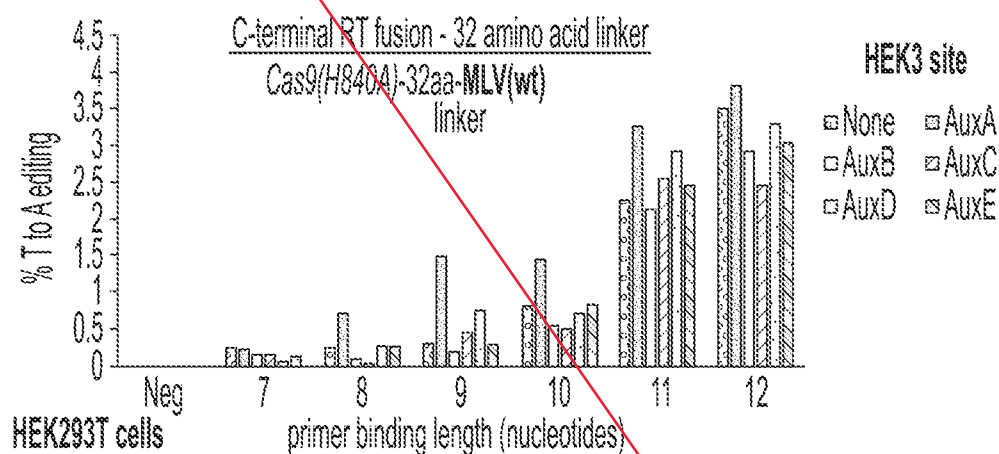


FIG. 17B

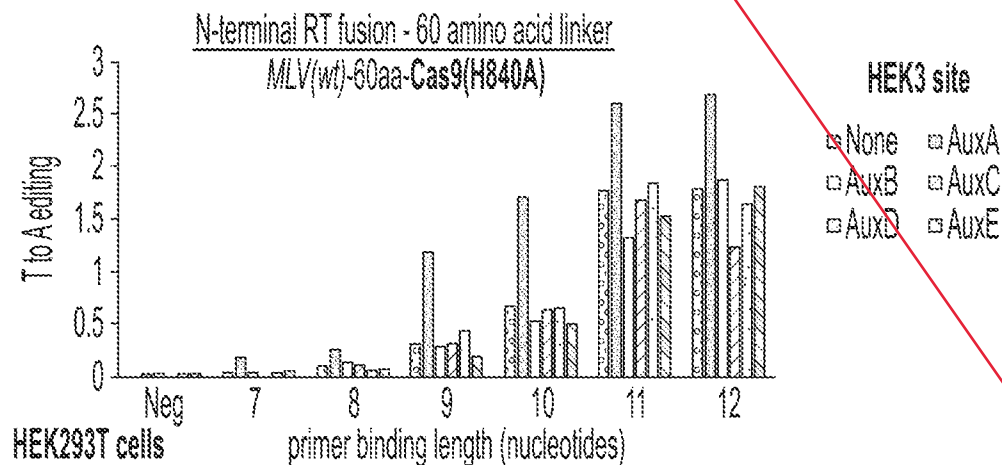


FIG. 17C

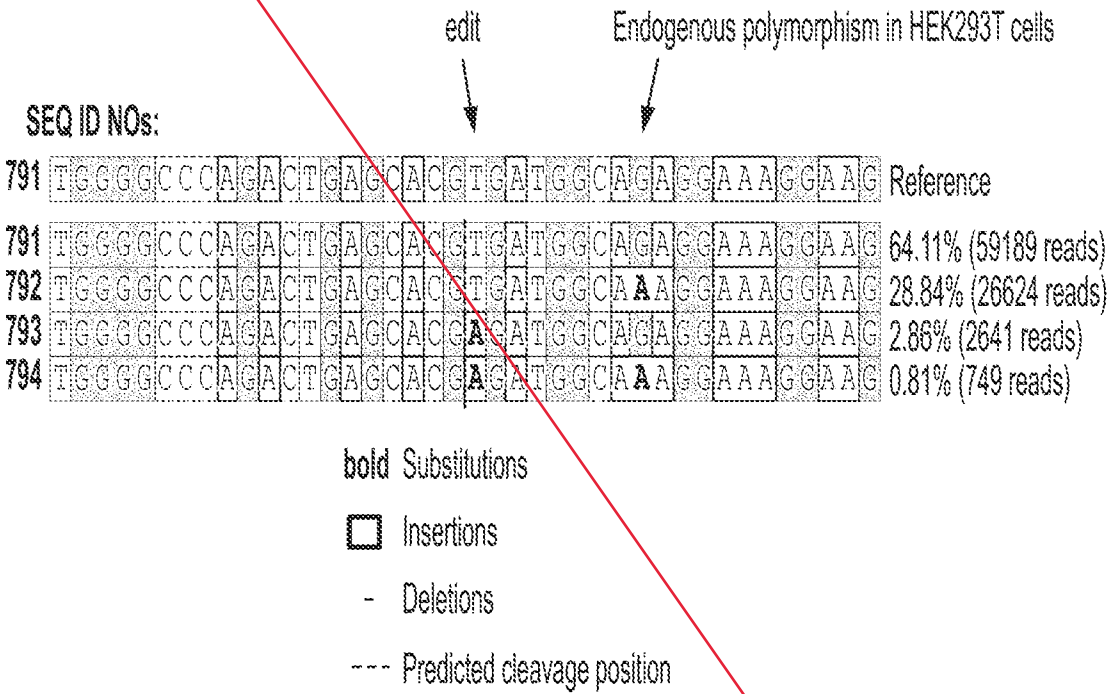


FIG. 18

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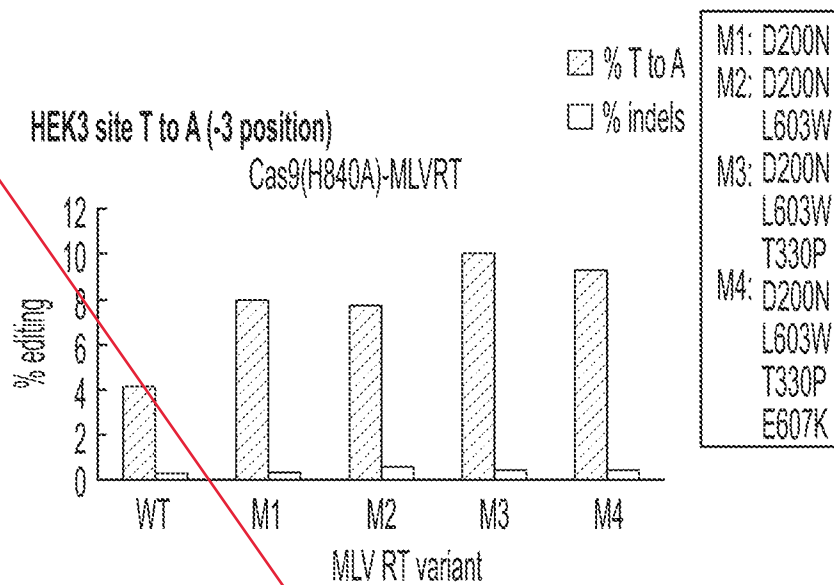


FIG. 19

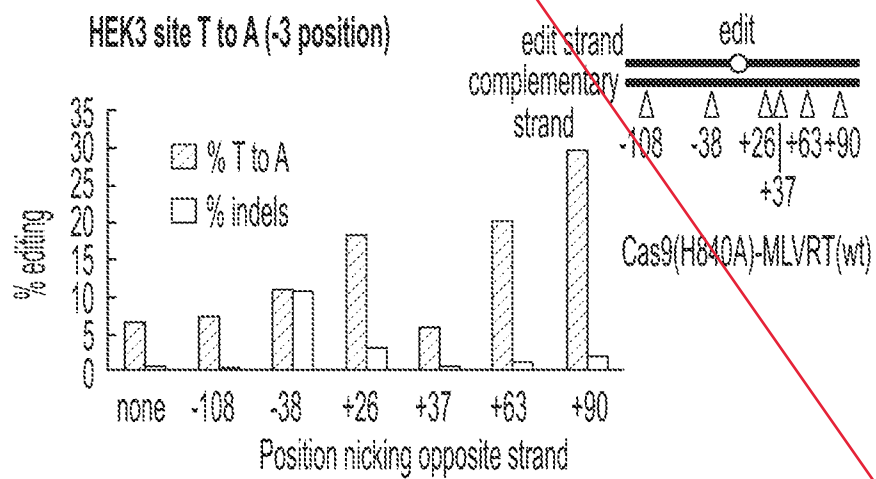


FIG. 20

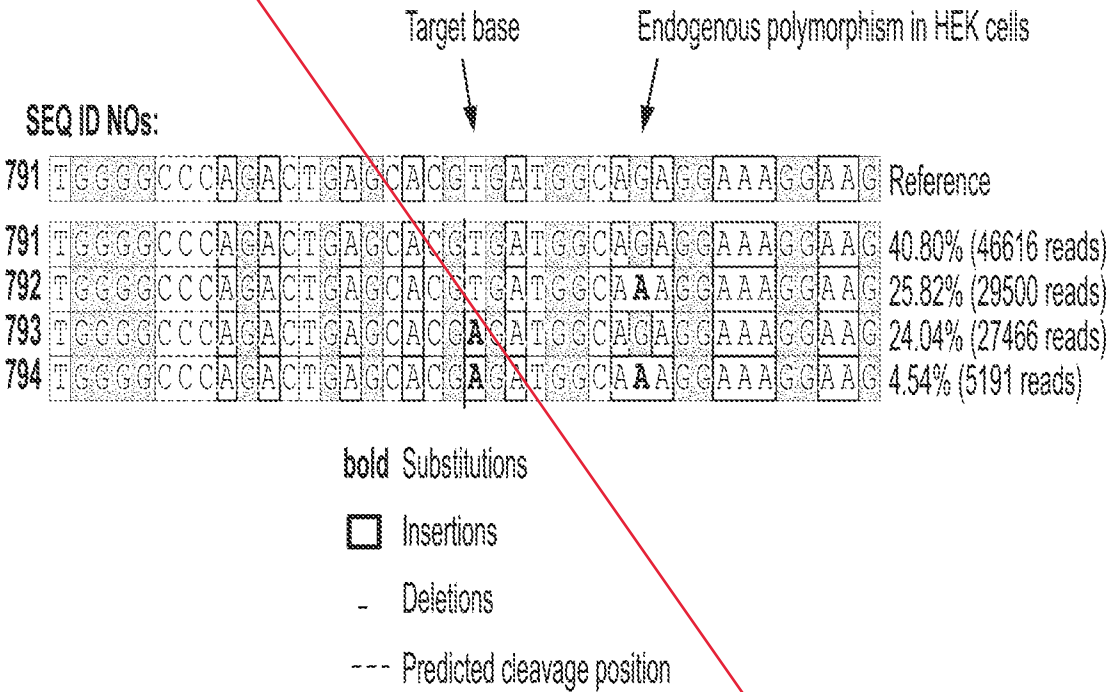


FIG. 21

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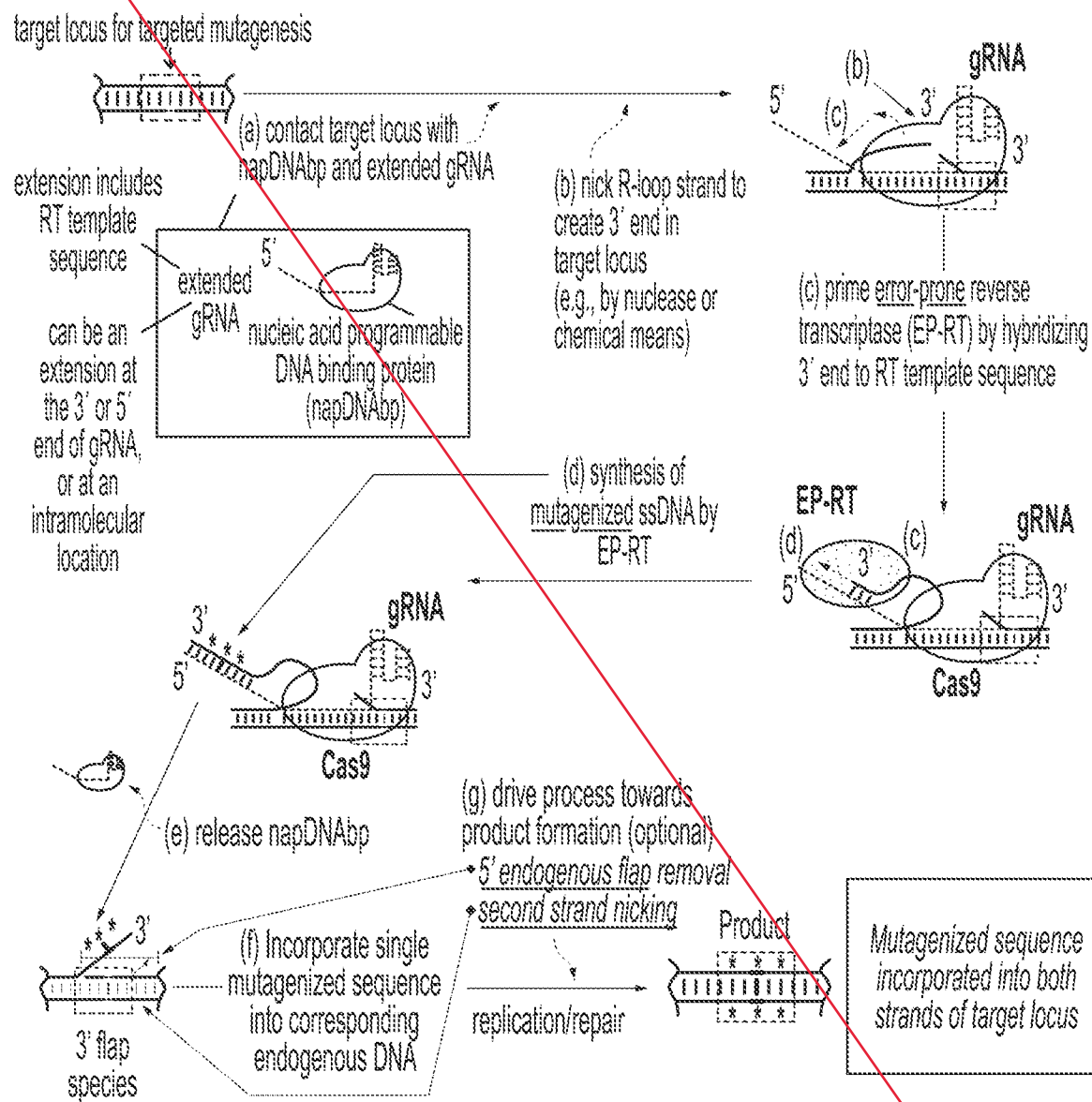
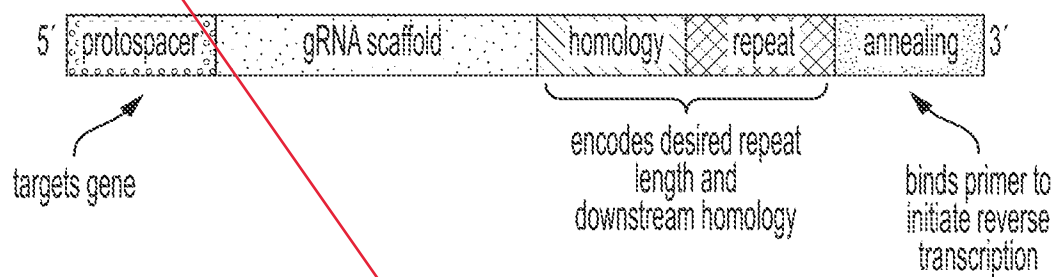


FIG. 22

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gRNA design for contracting trinucleotide repeat sequences



Trinucleotide repeat contraction with TPRT genome editing

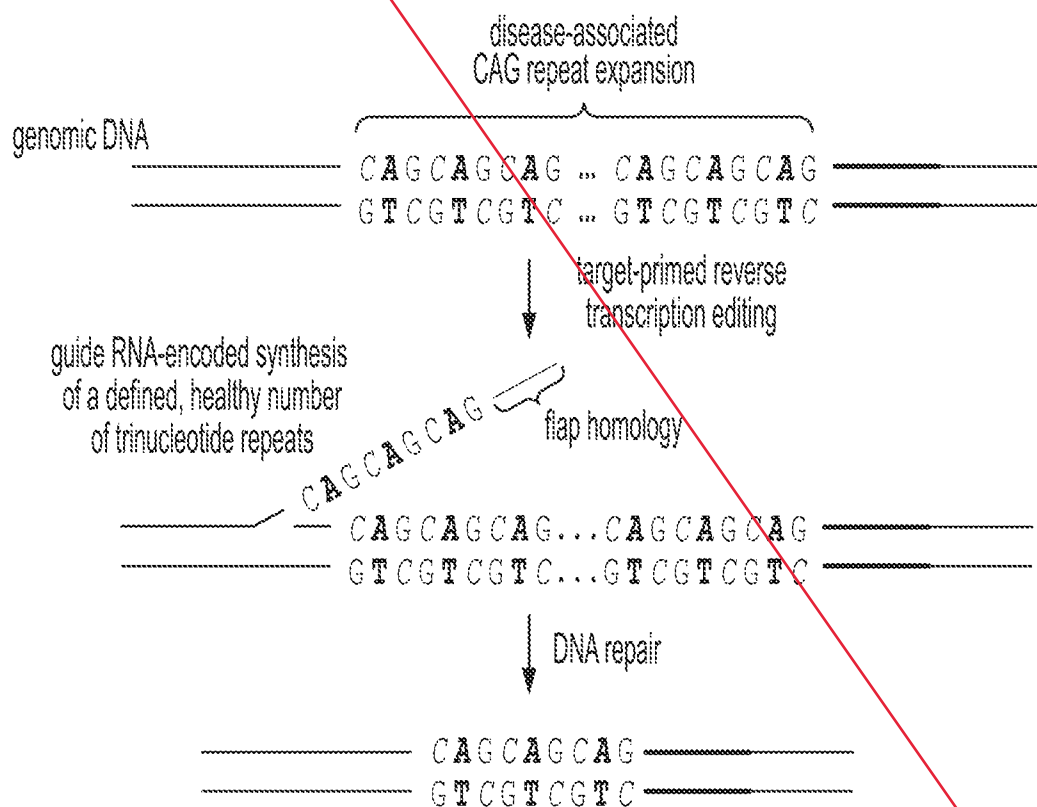


FIG. 23

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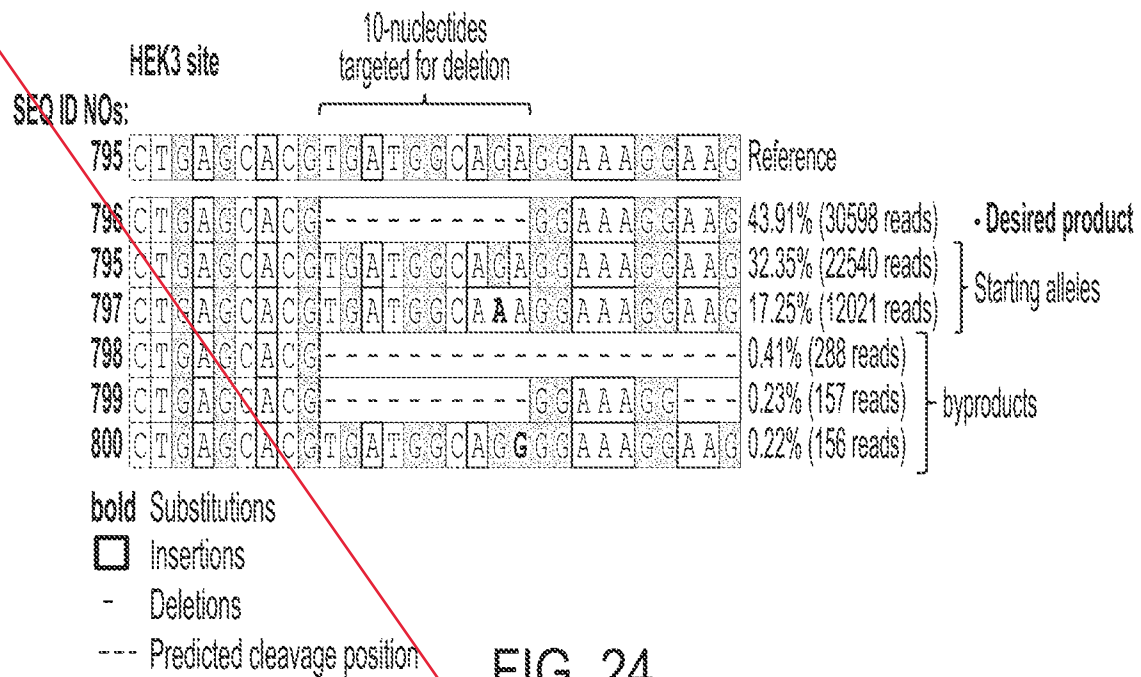


FIG. 24

gRNA design for peptide tagging genes at endogenous genomic loci

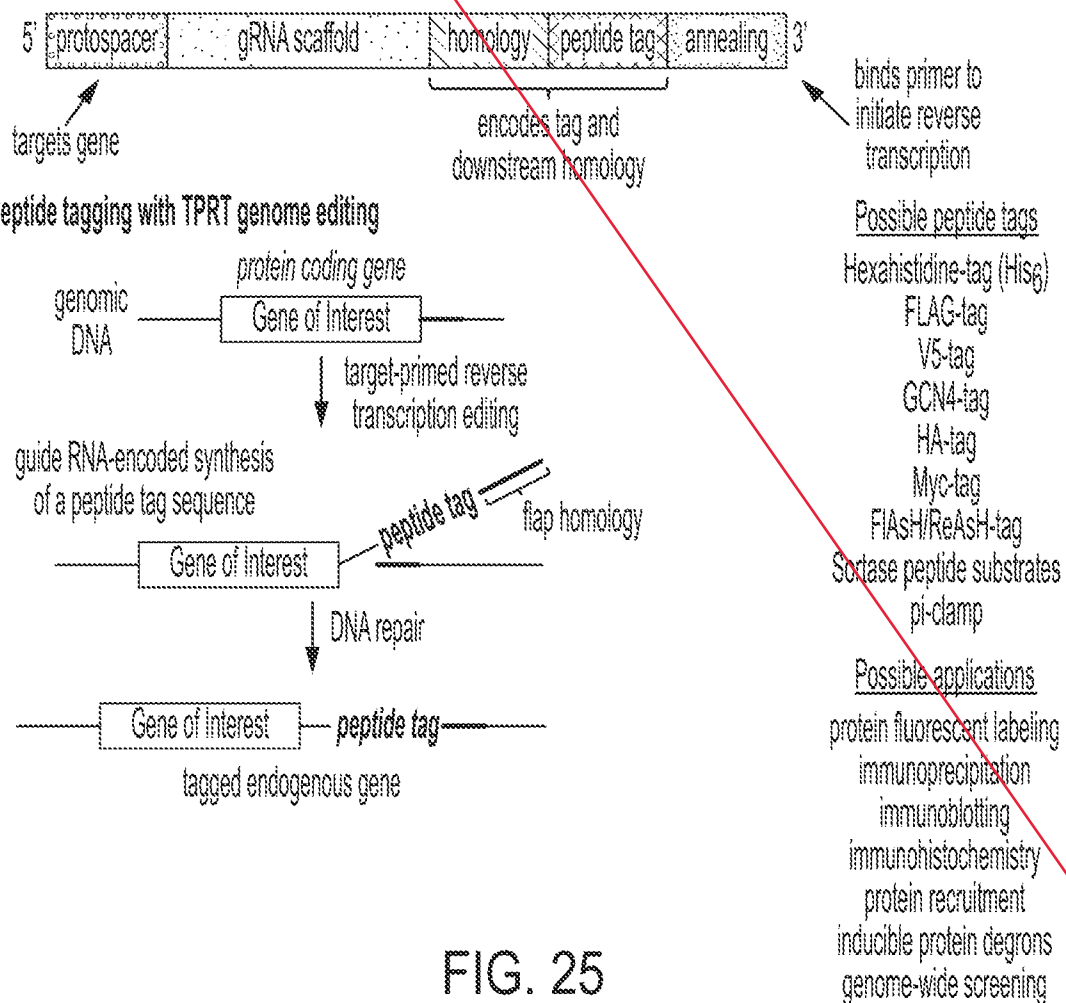


FIG. 25

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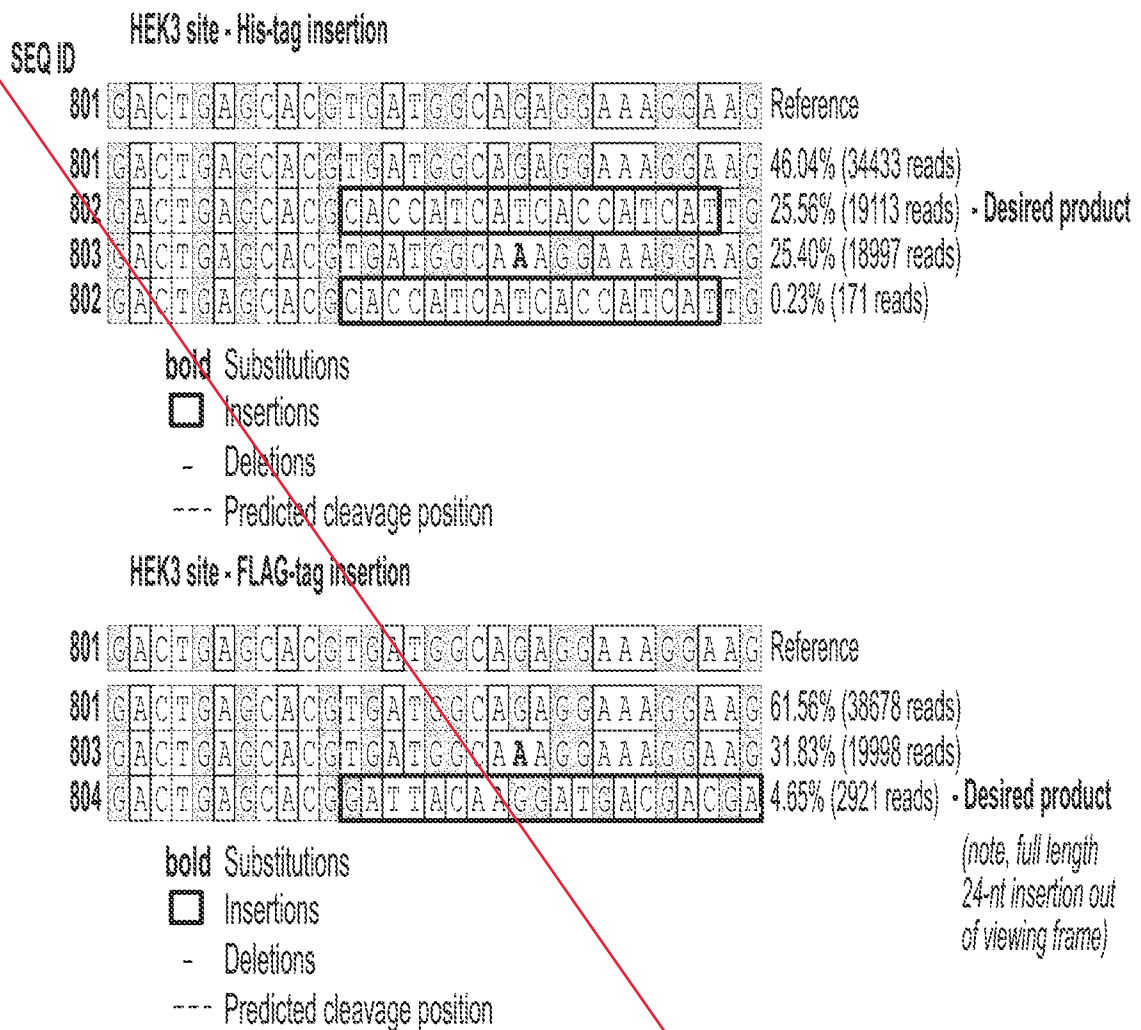


FIG. 26A

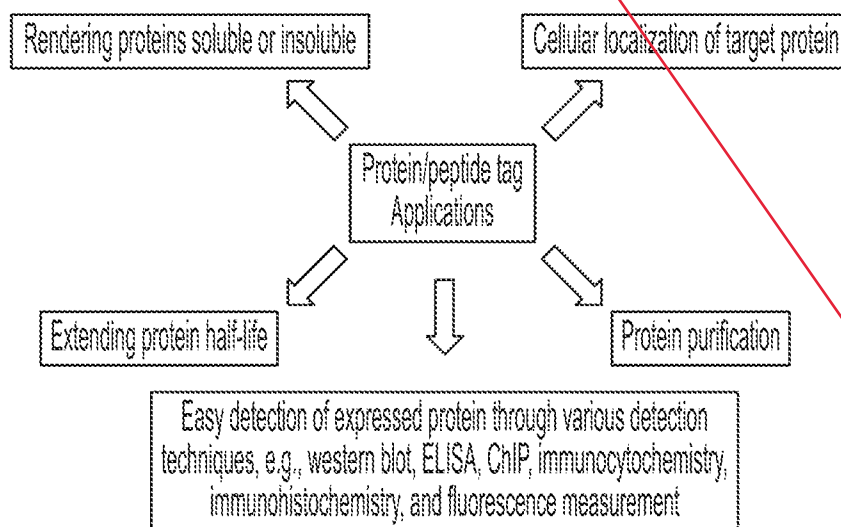


FIG. 26B

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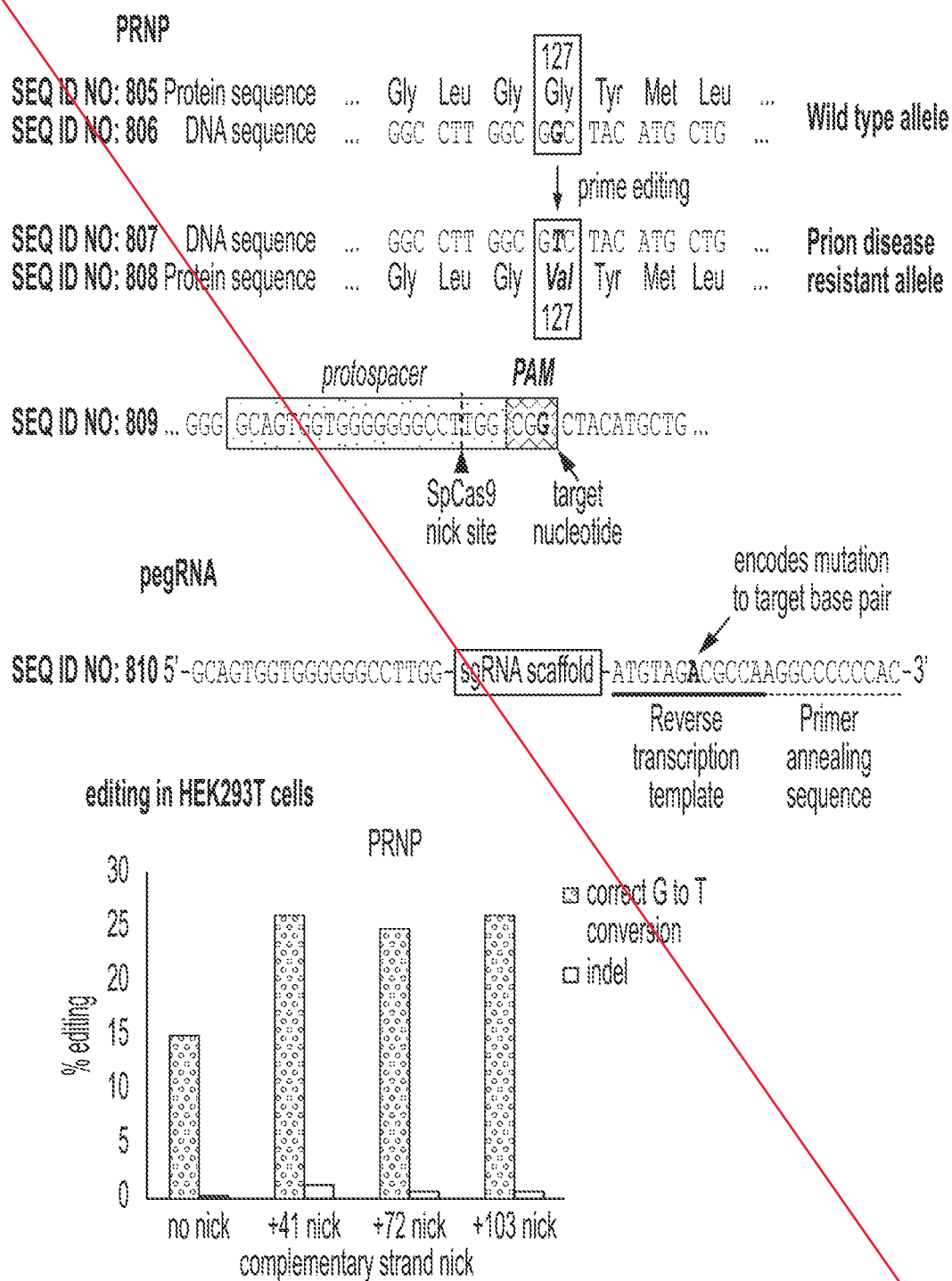


FIG. 27

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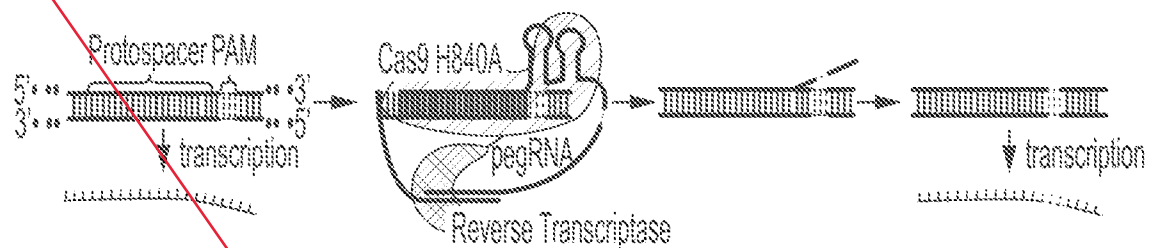


FIG. 28A

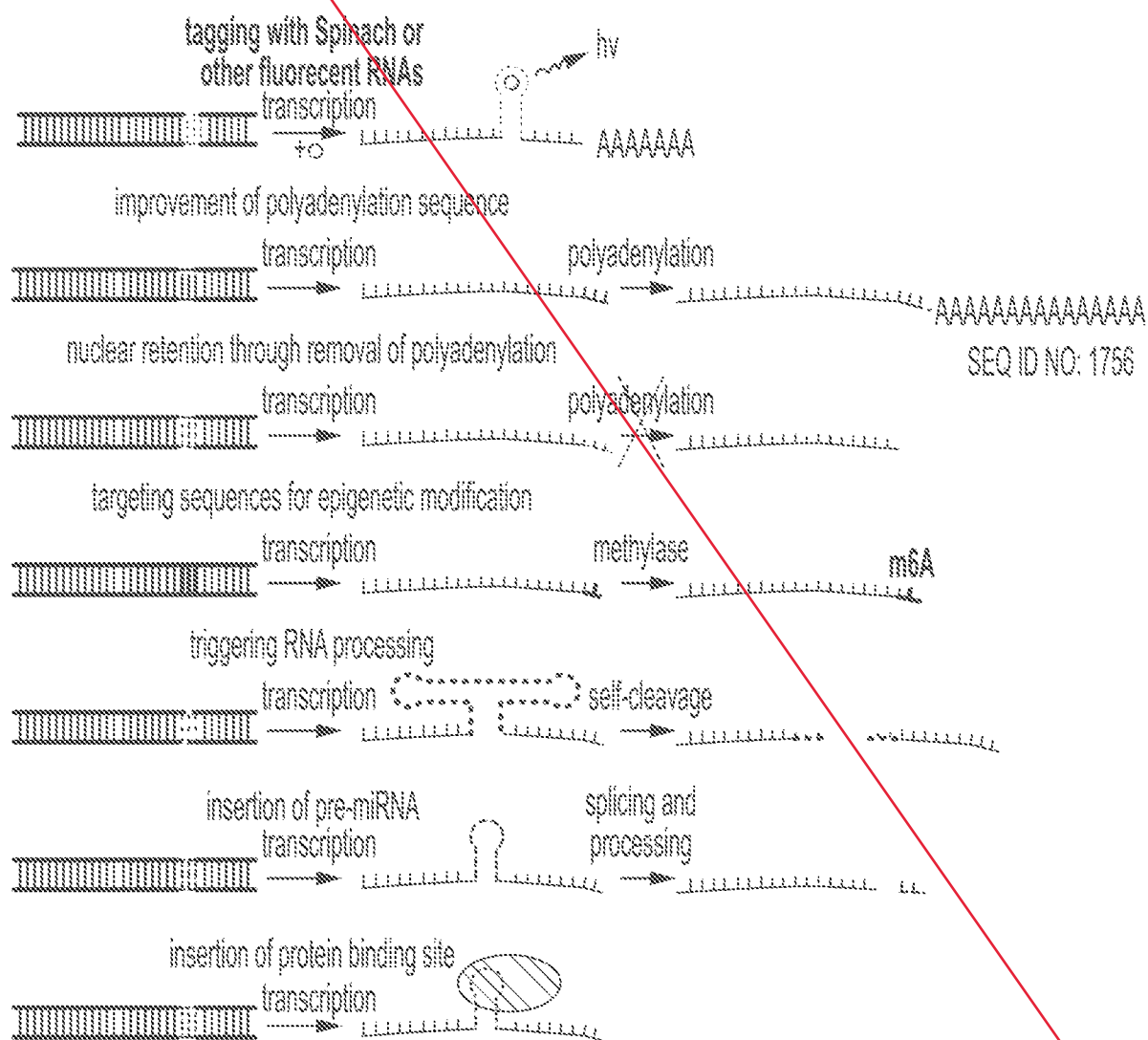


FIG. 28B

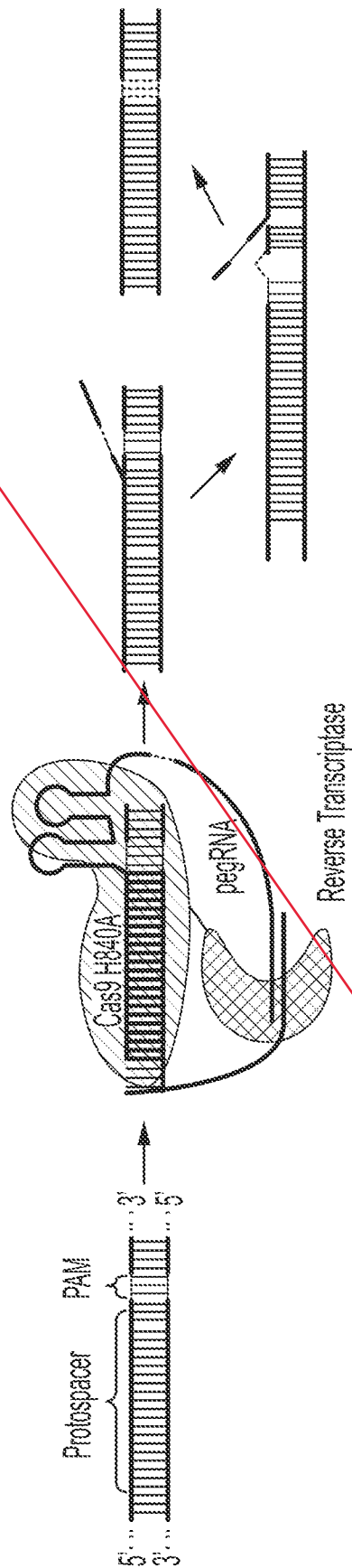


FIG. 29A

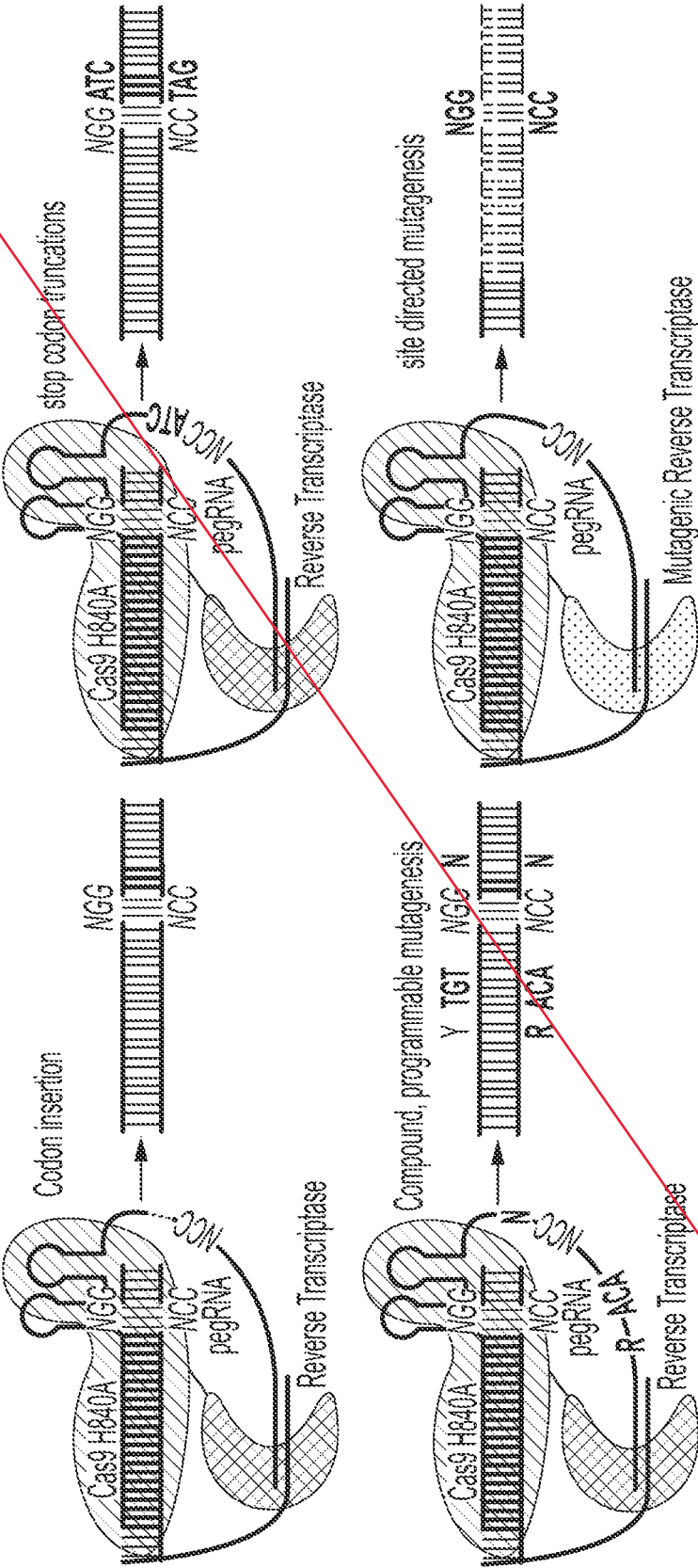
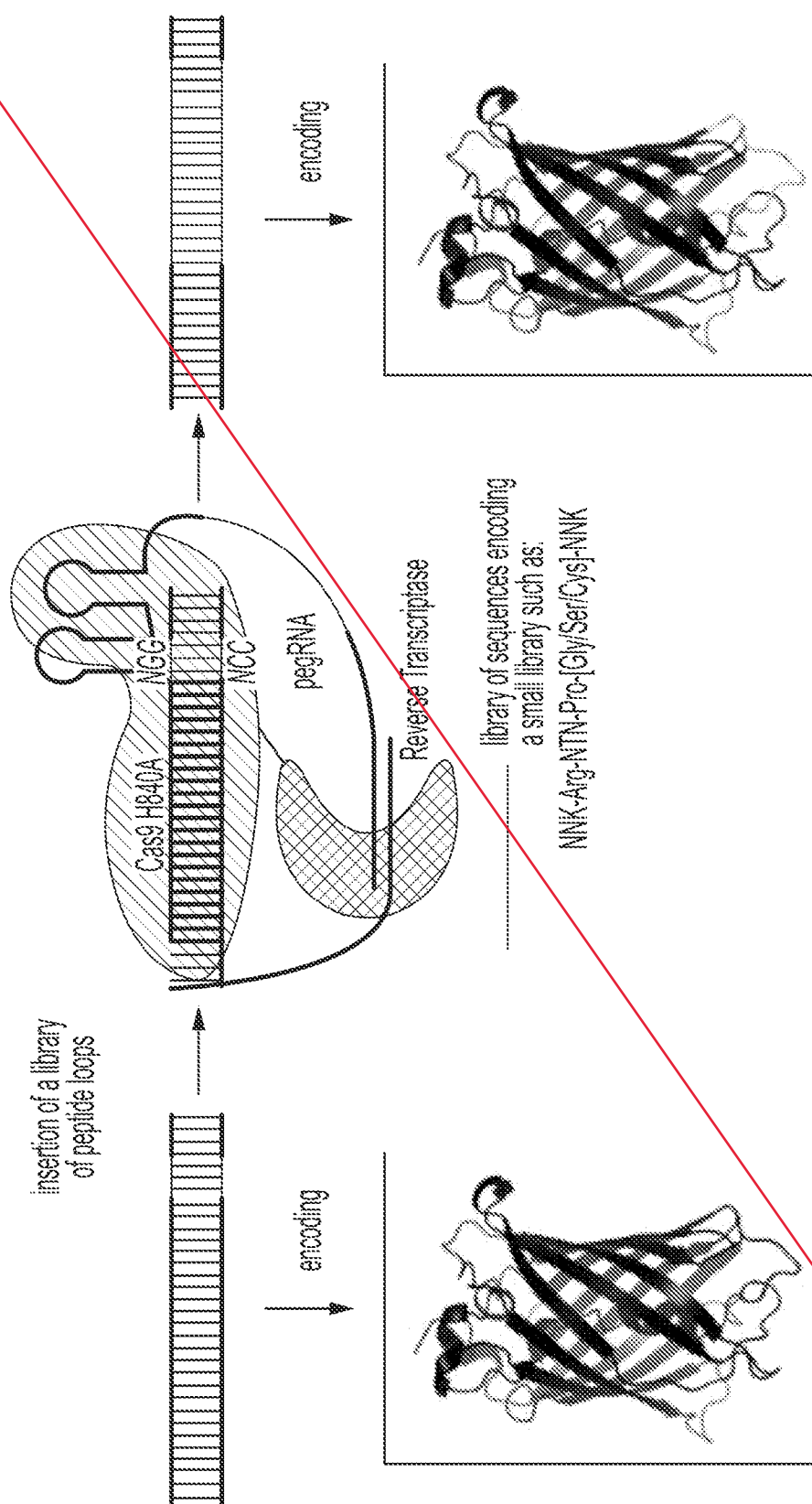


FIG. 29B

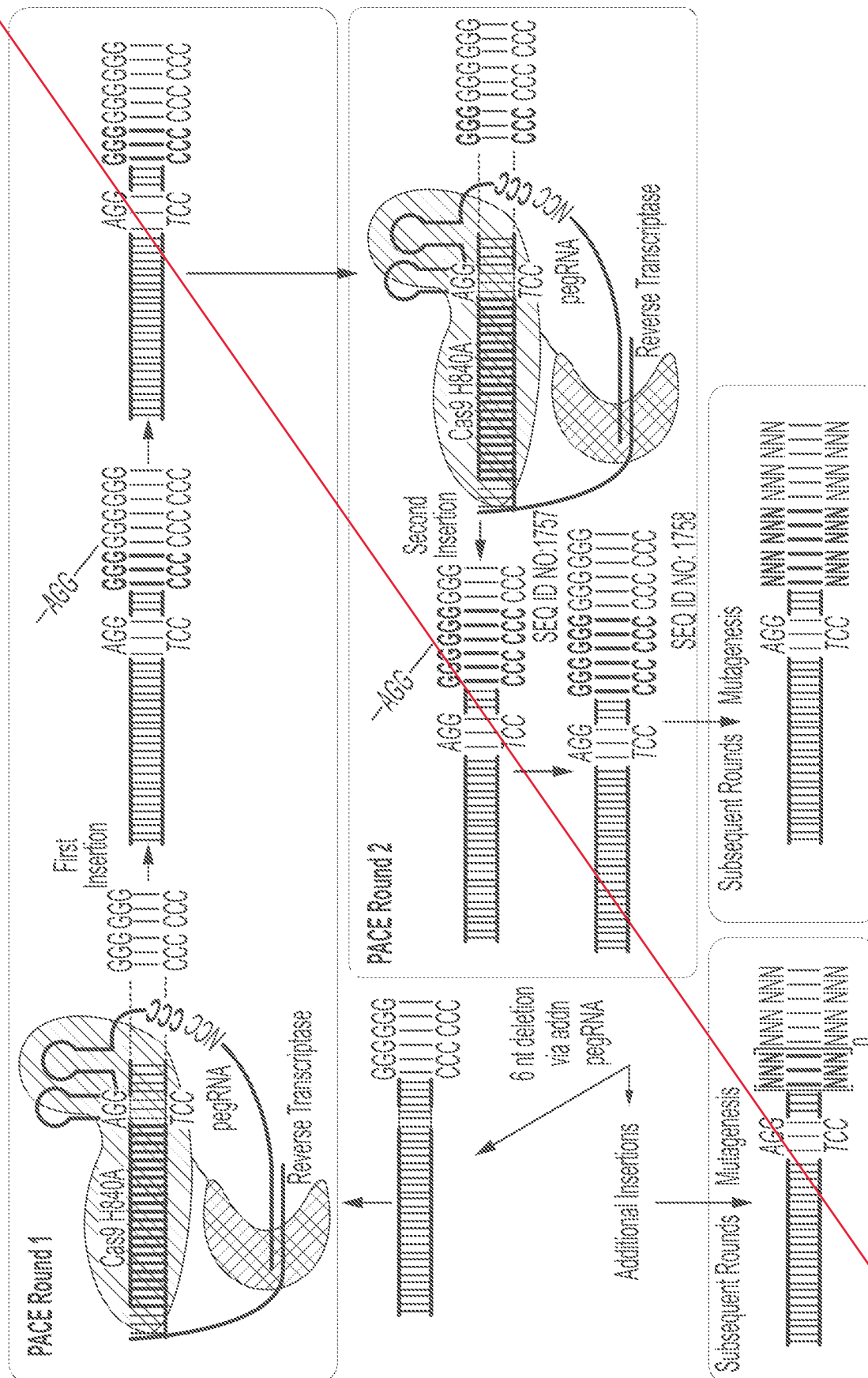
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F. G. 29C

programmable codon deletion and truncations		
SEQ ID NO: 811	GFP	NFKIRHNIEDGSVQLADHYQQNTPI
SEQ ID NO: 812	del15	NFKI-HNIEDGSVQLADHYQQNTPI
SEQ ID NO: 813	del16	NFKIR-NIEDGSVQLADHYQQNTPI
SEQ ID NO: 814	del18	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



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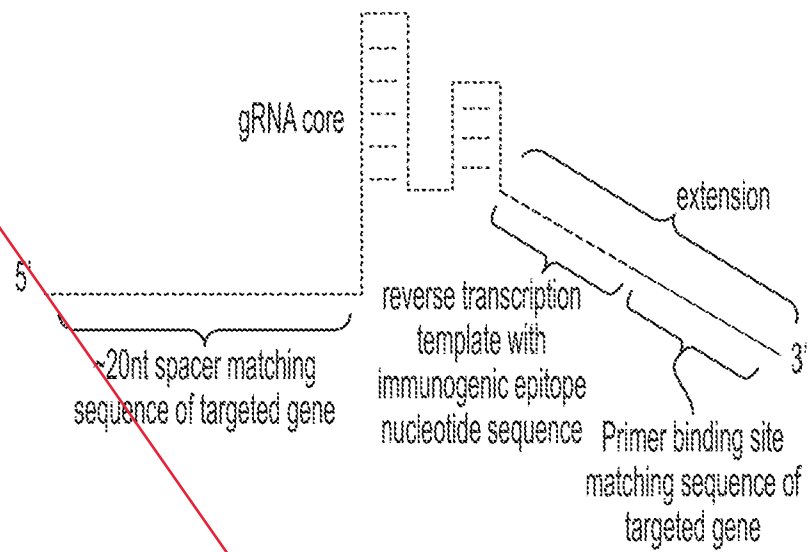


FIG. 31

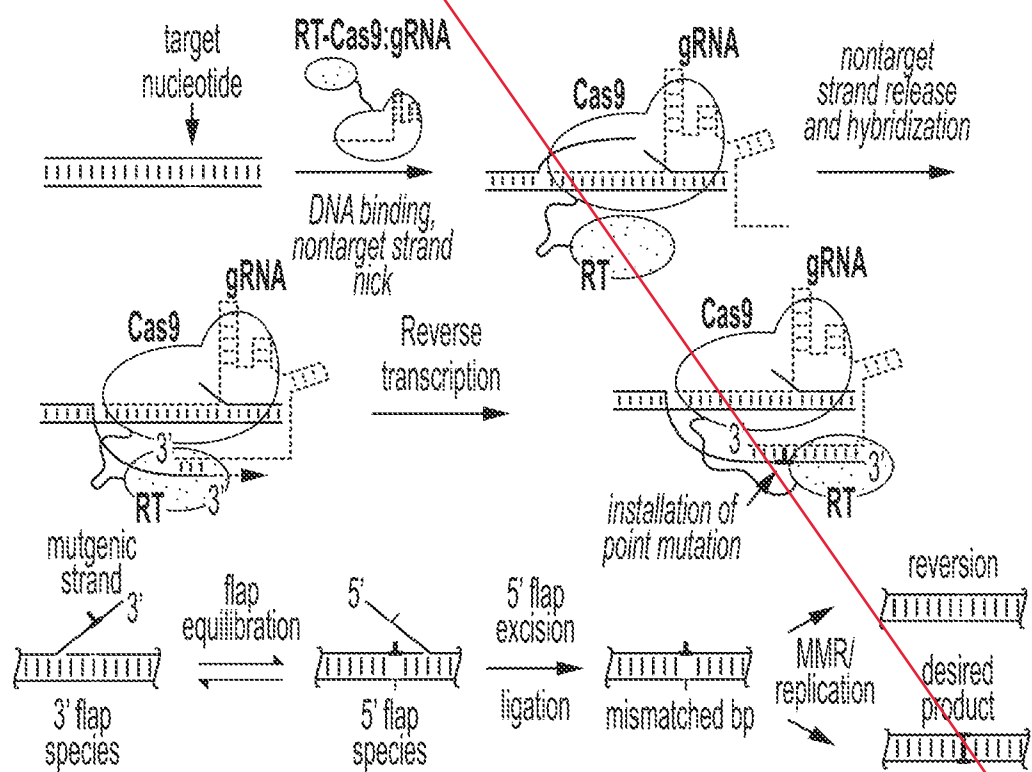


FIG. 32

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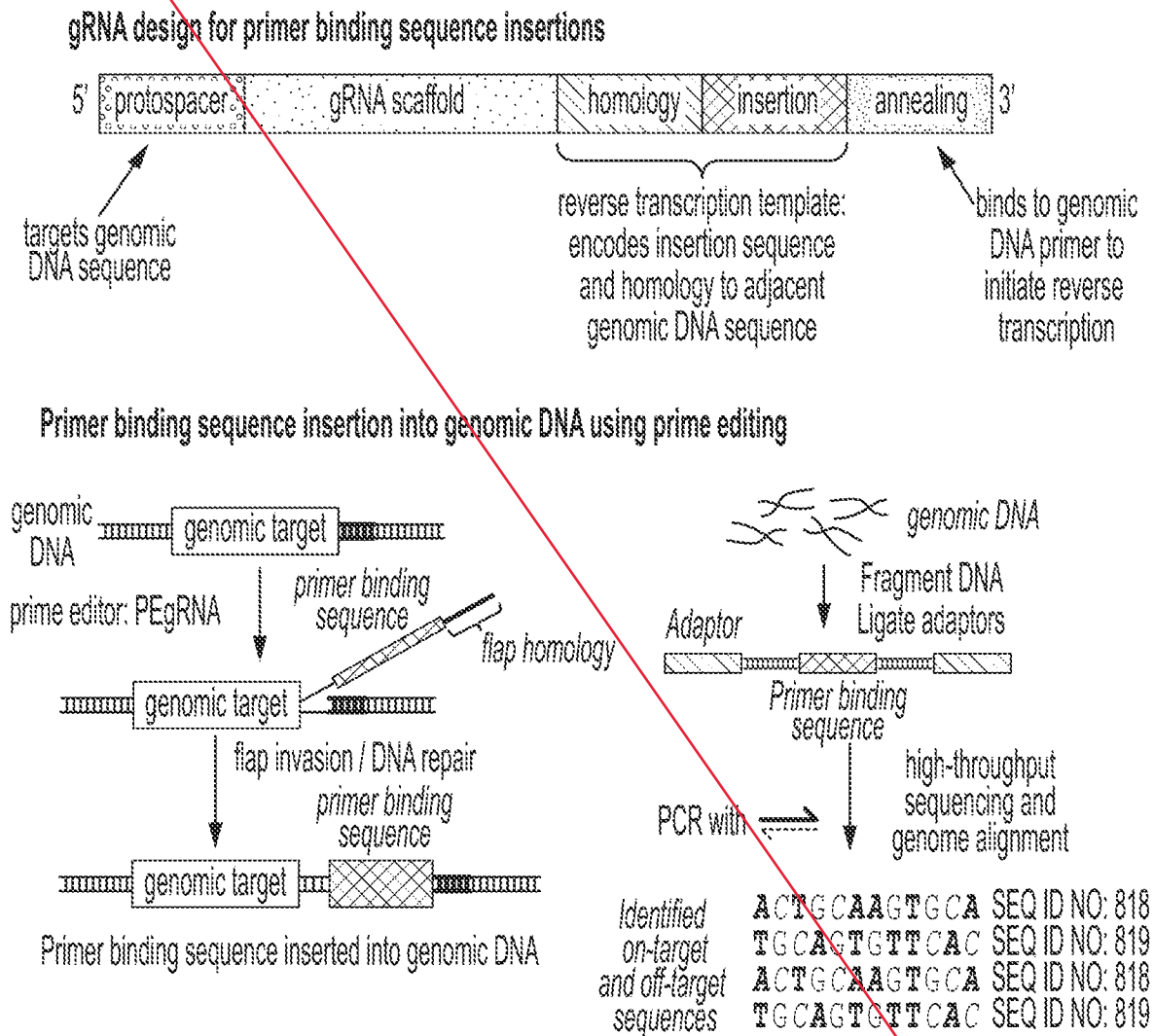


FIG. 33

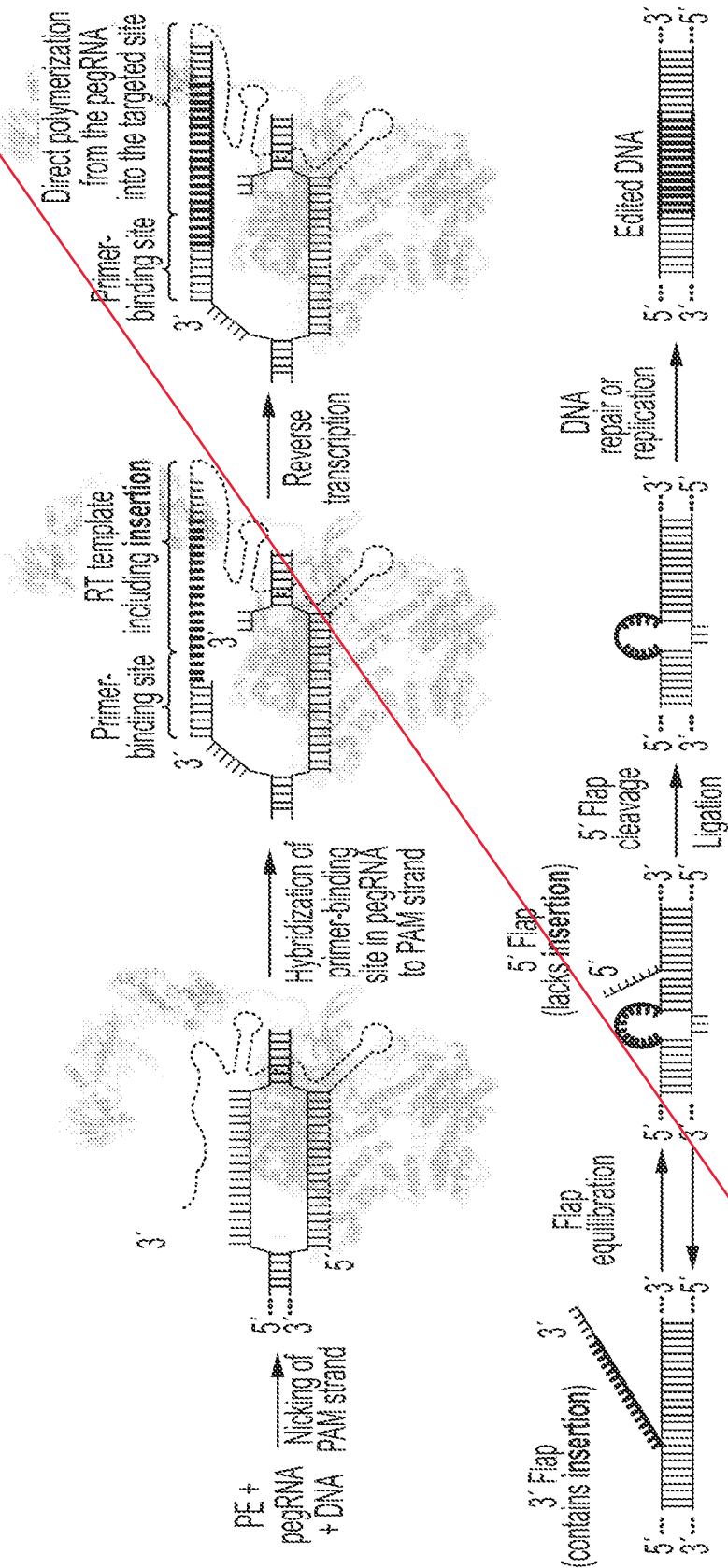


FIG. 34

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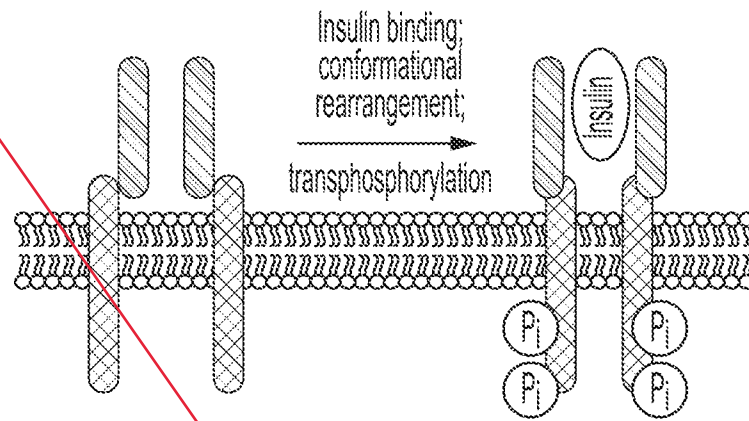


FIG. 35A

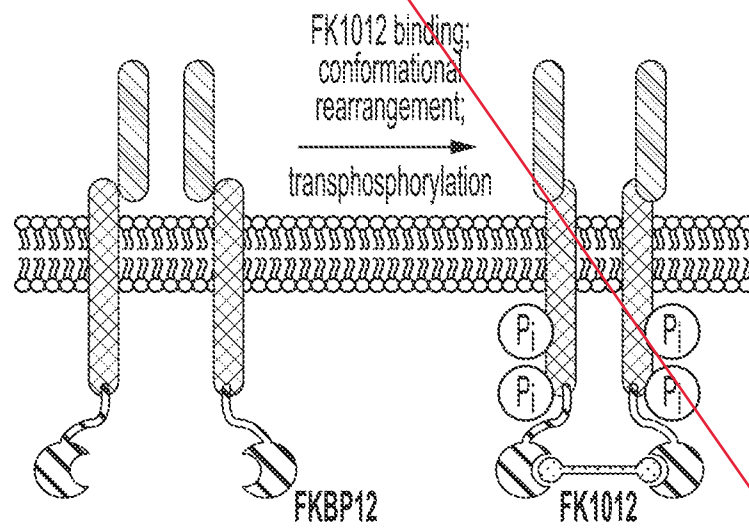


FIG. 35B

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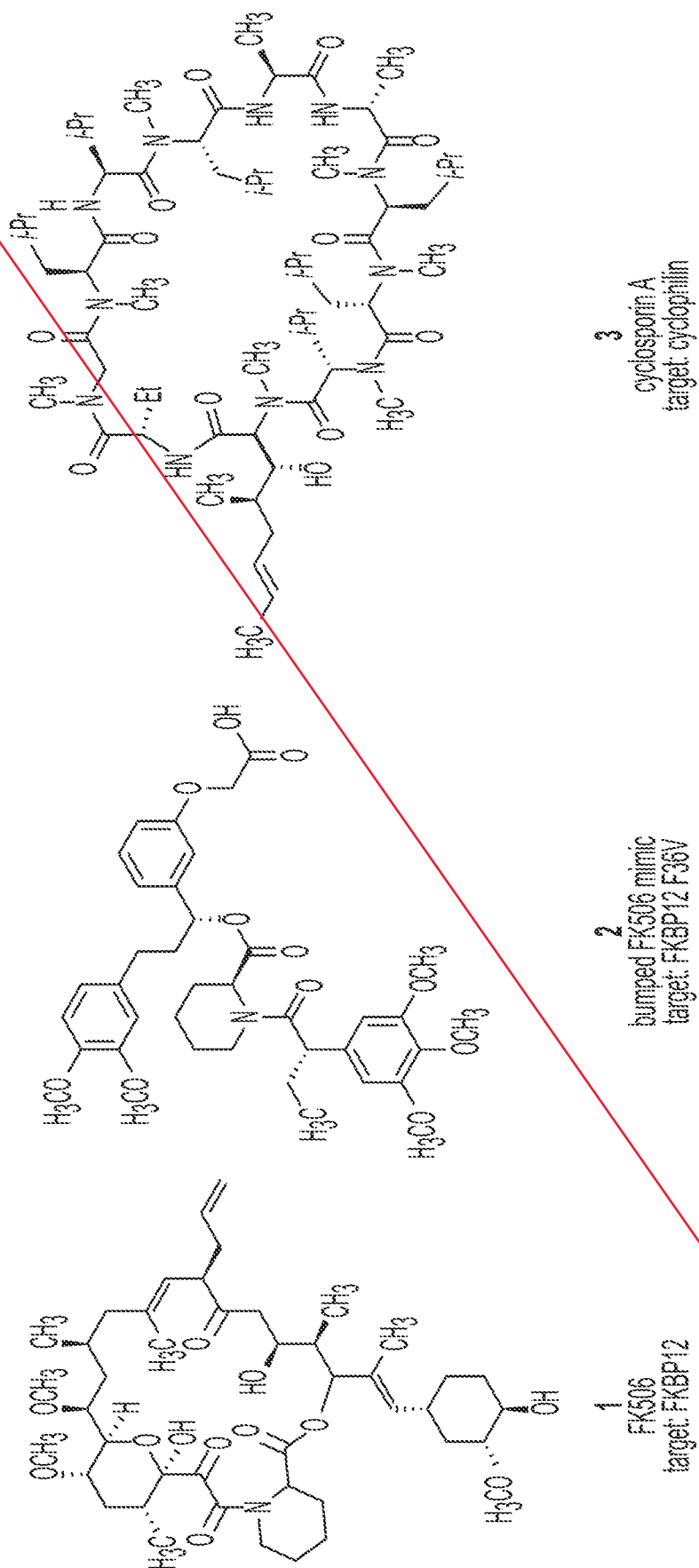


FIG. 36

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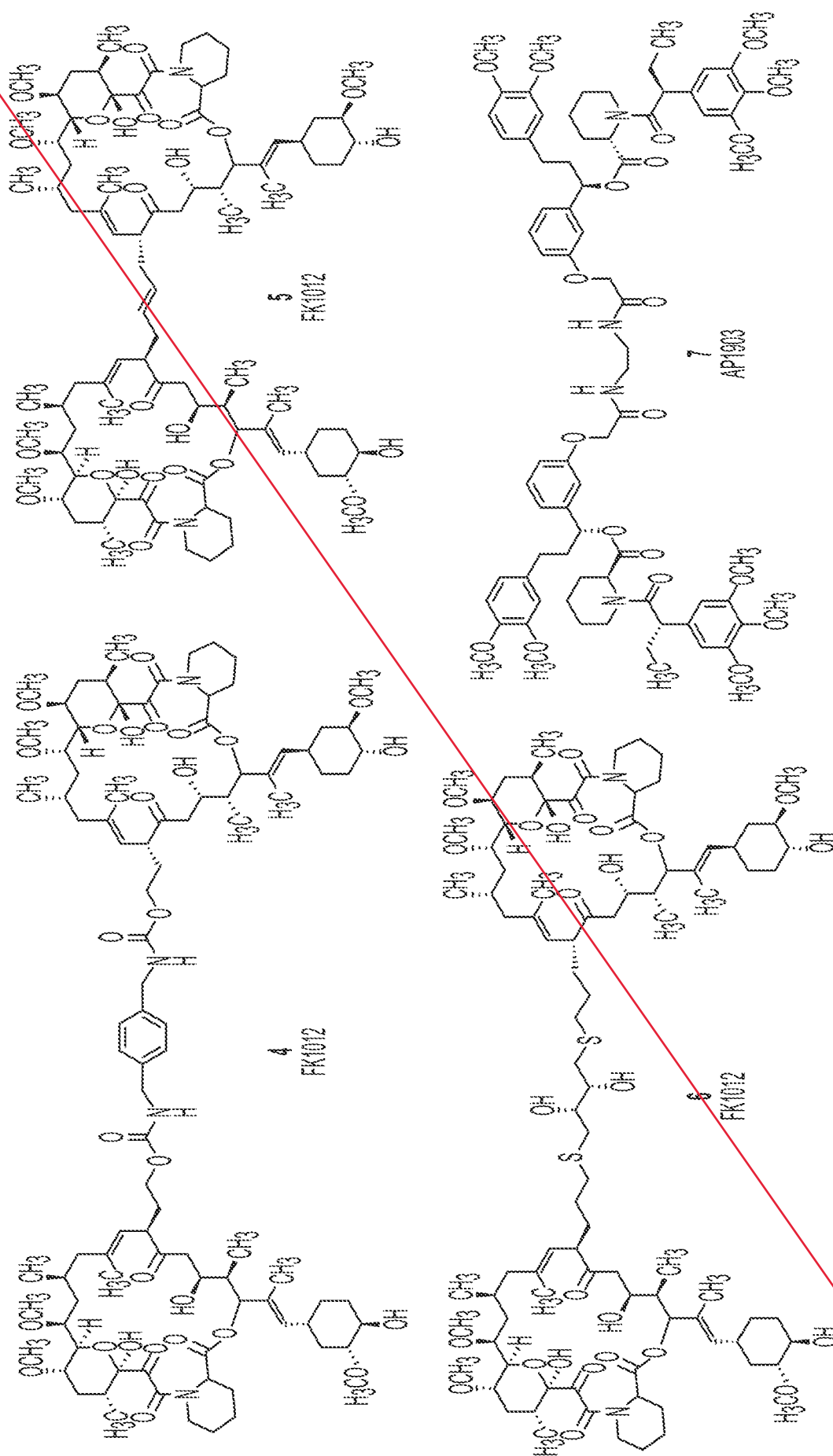


FIG. 37A

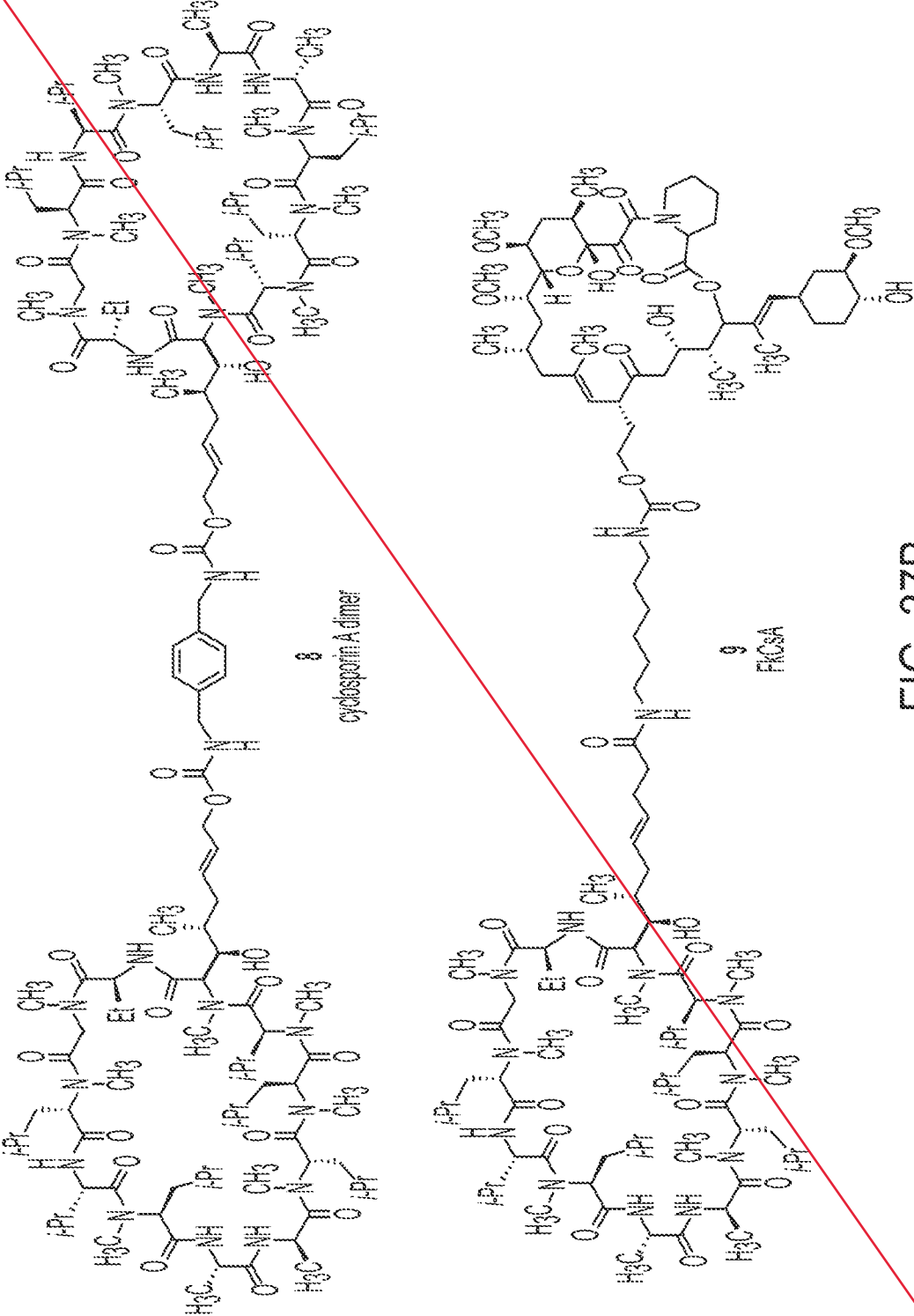


FIG. 37B

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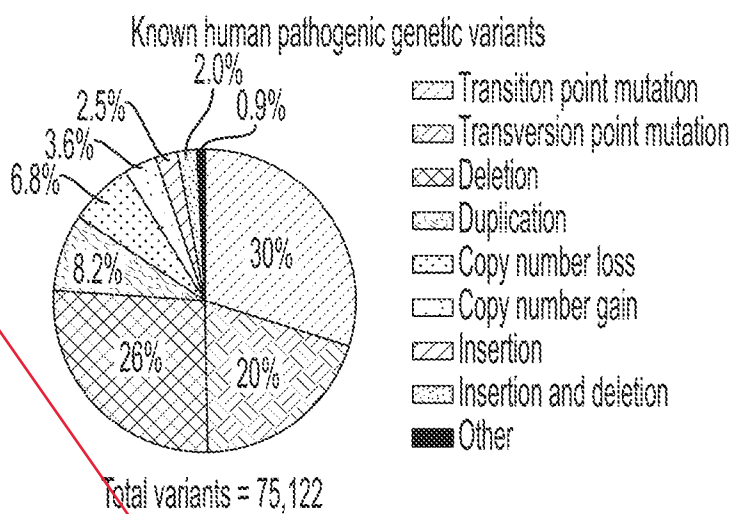


FIG. 38A

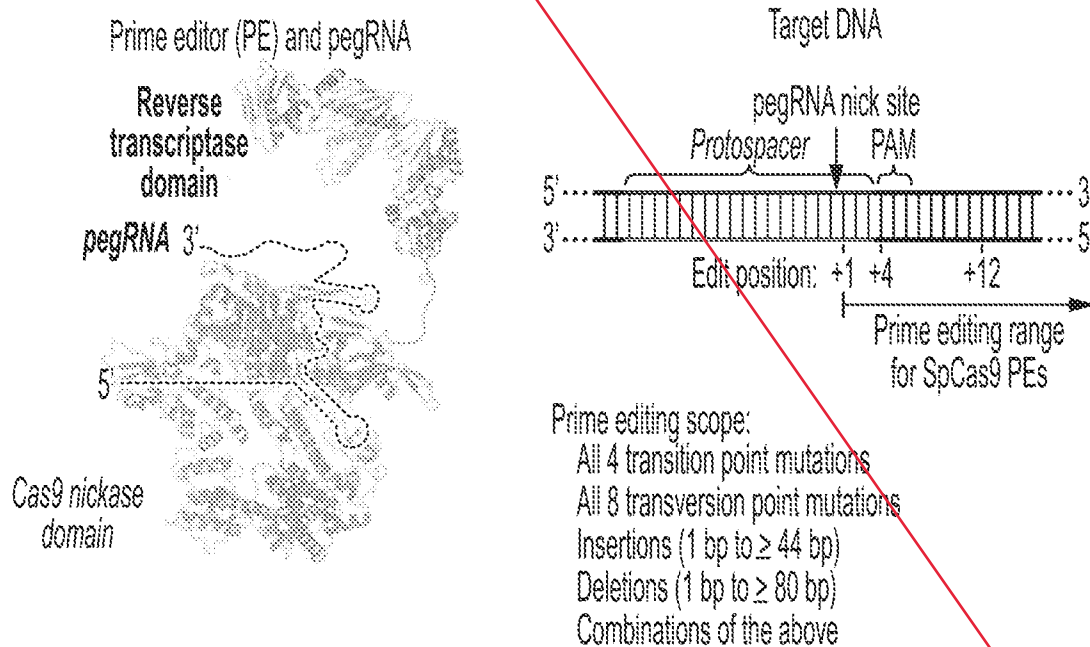


FIG. 38B

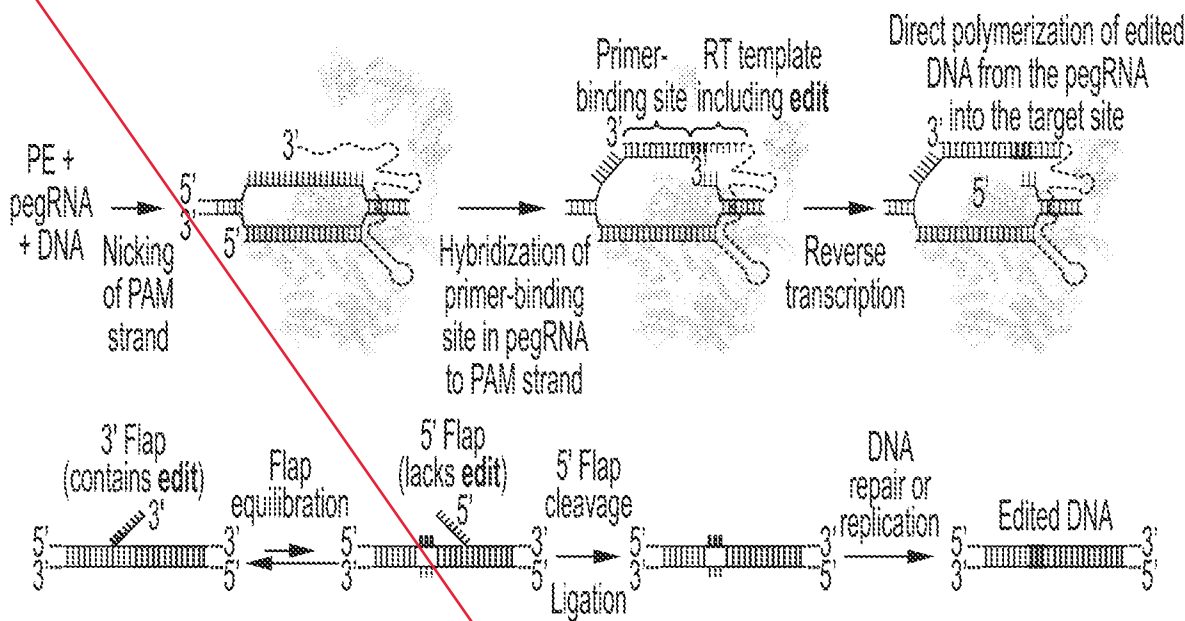


FIG. 38C

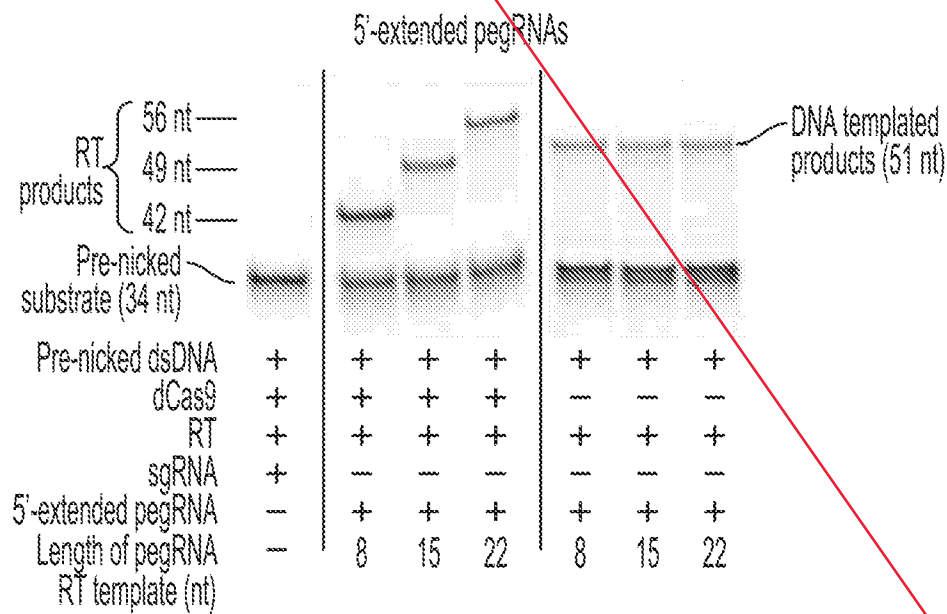


FIG. 38D

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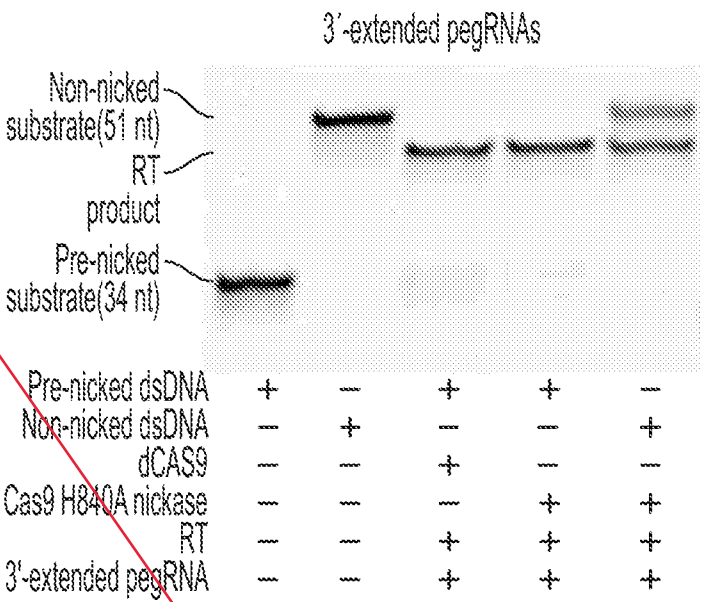


FIG. 38E

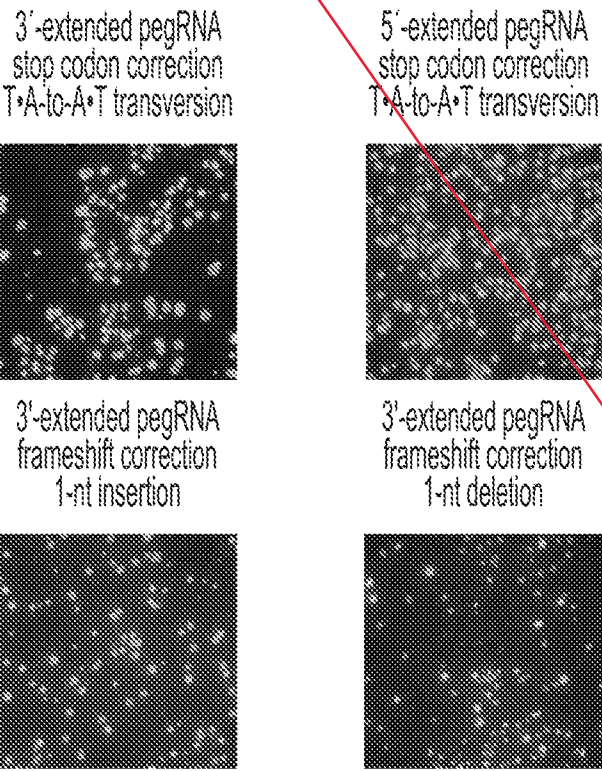
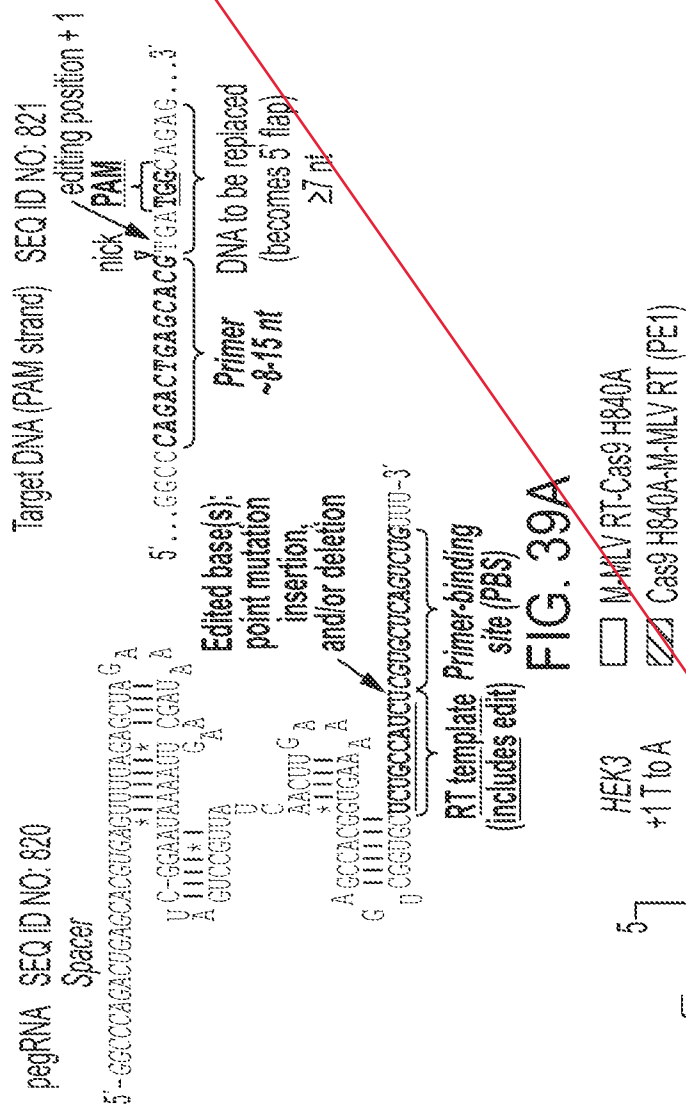
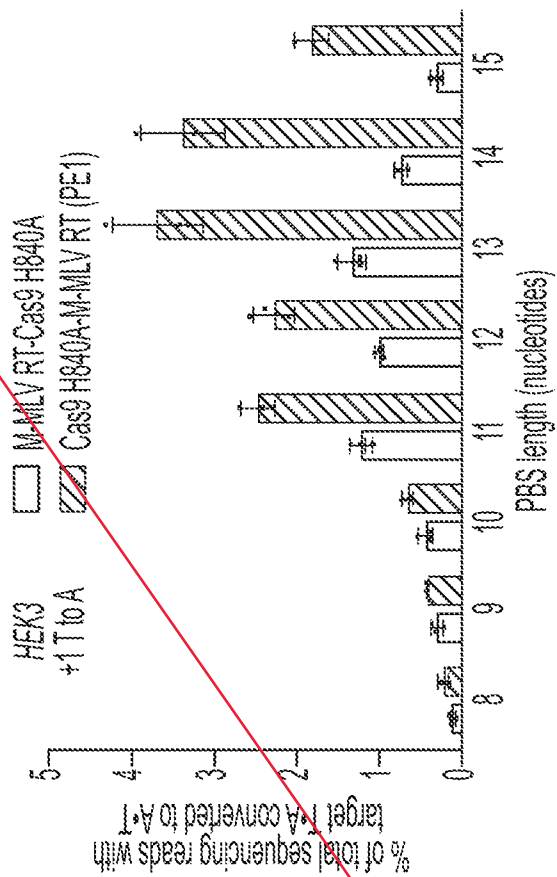
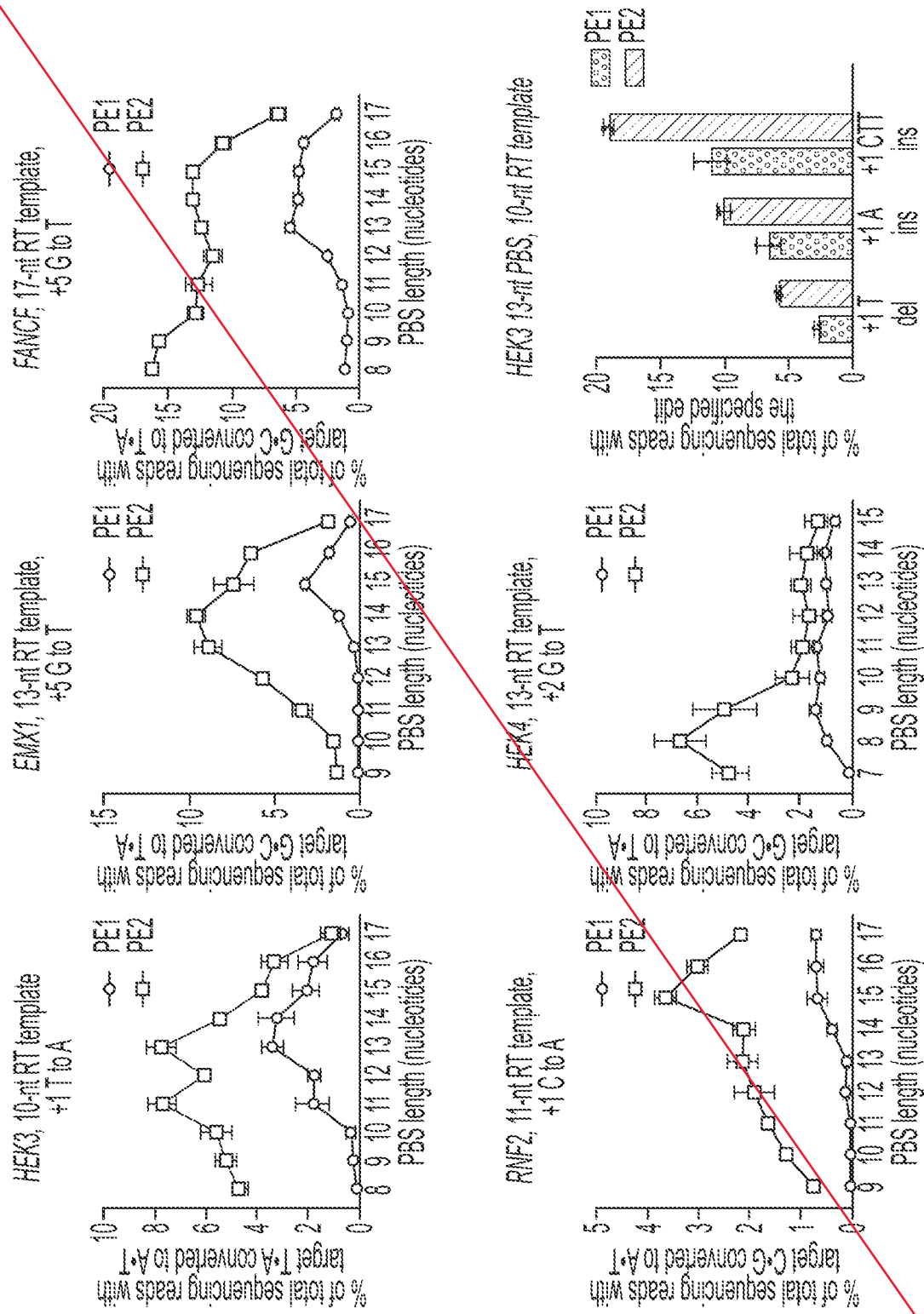


FIG. 38F



3



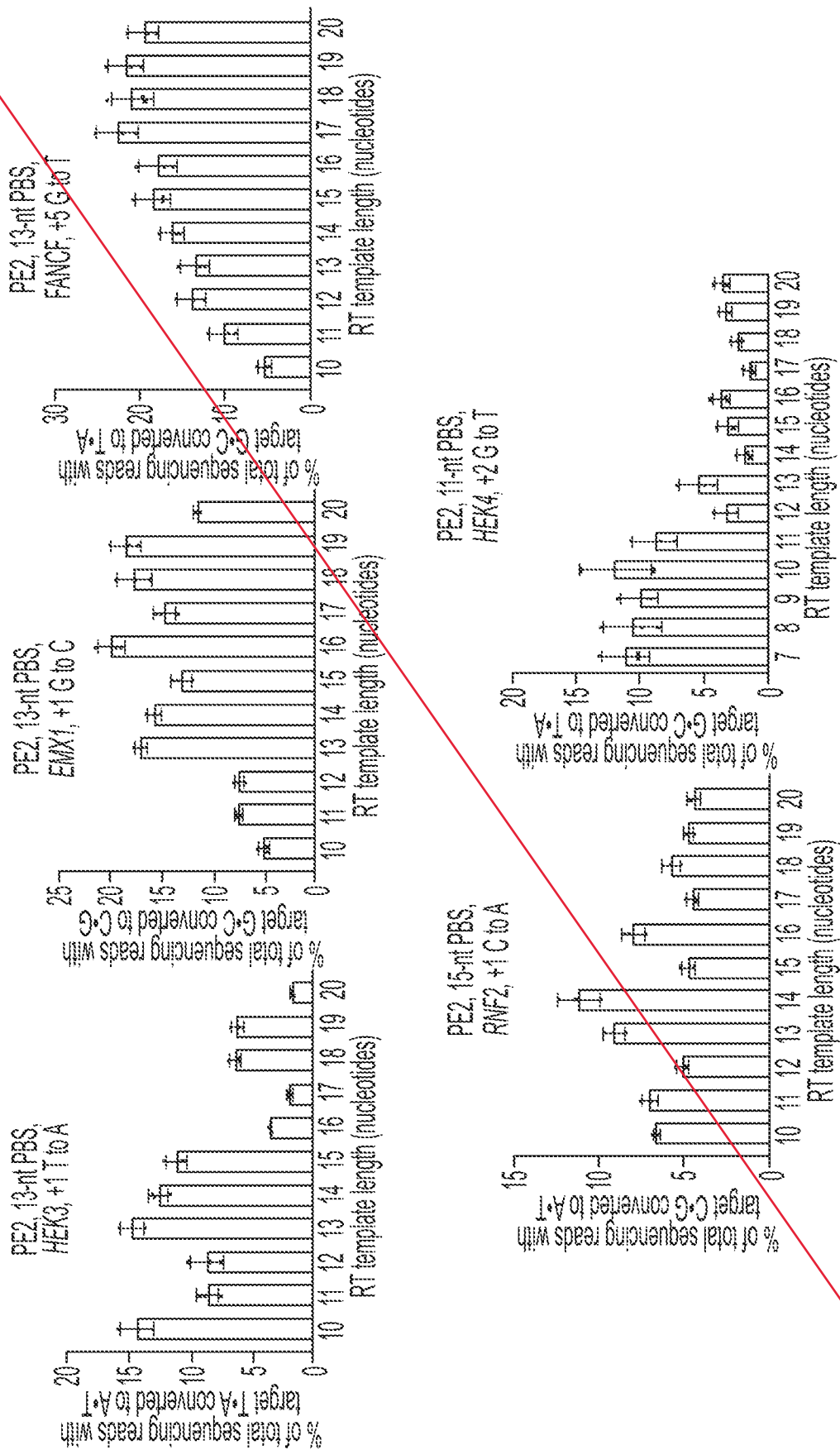


FIG. 39D

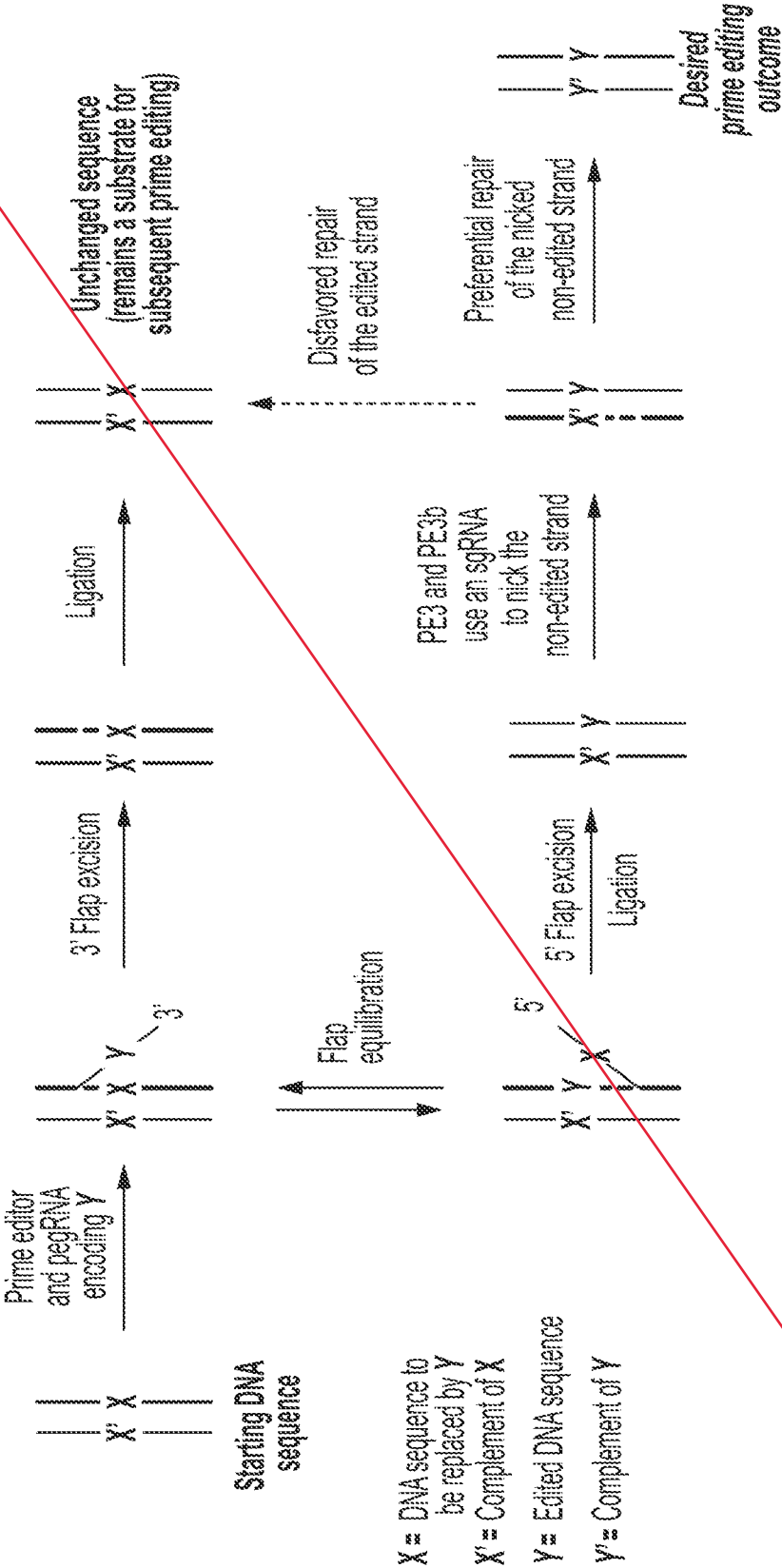
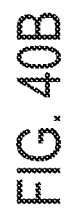


FIG. 40A



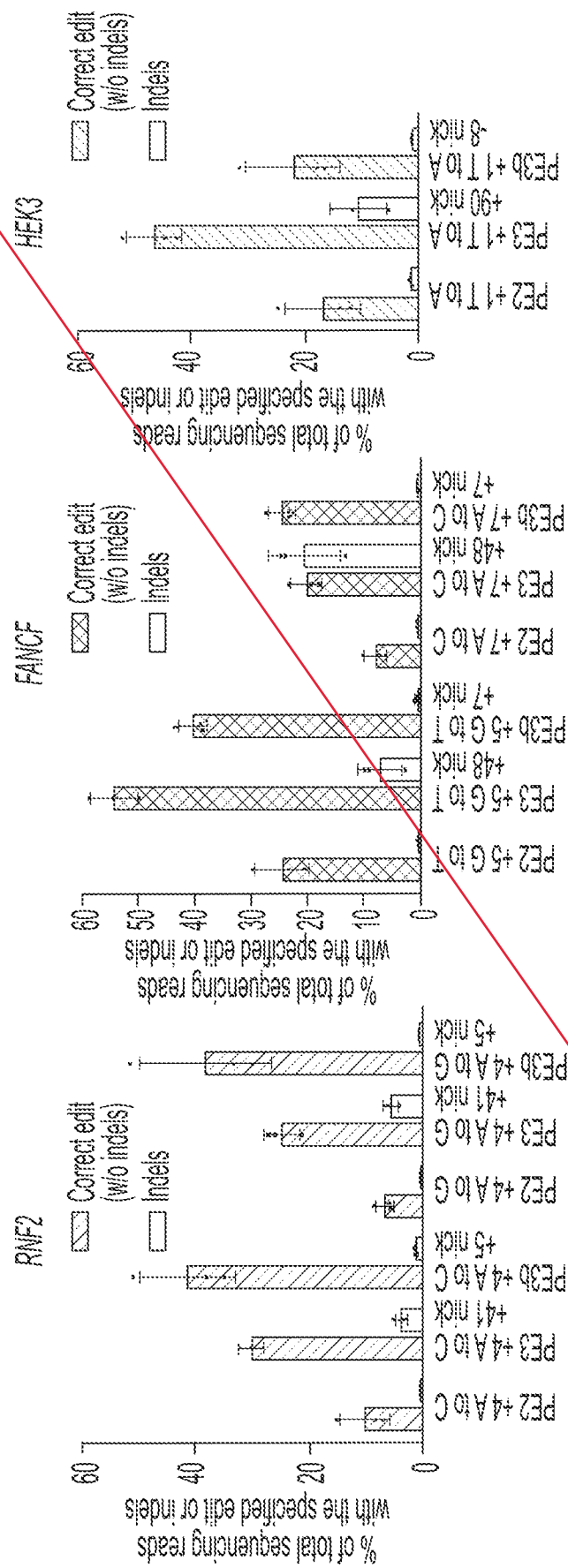


FIG. 40C

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PE3, 10-nt RT template,
nicking at +90, HEK3

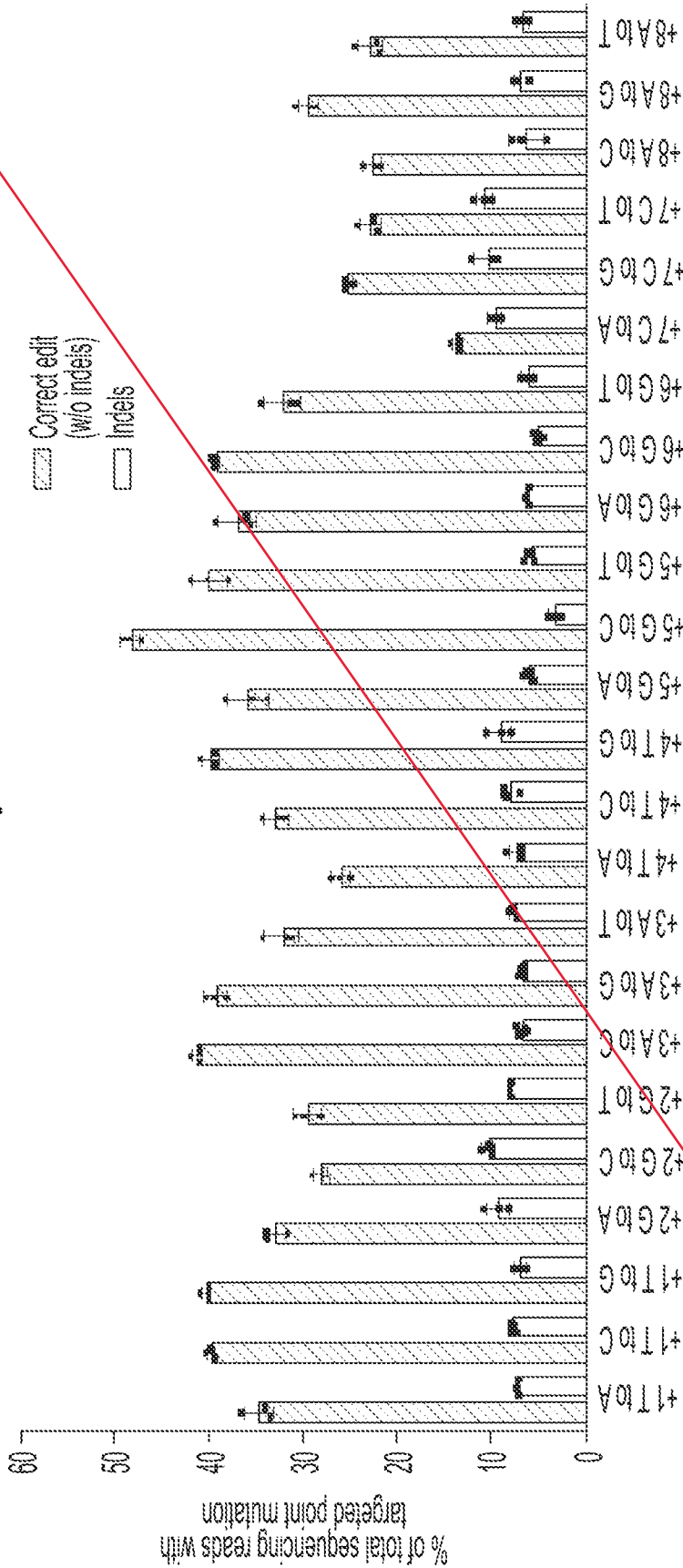


FIG. 41A

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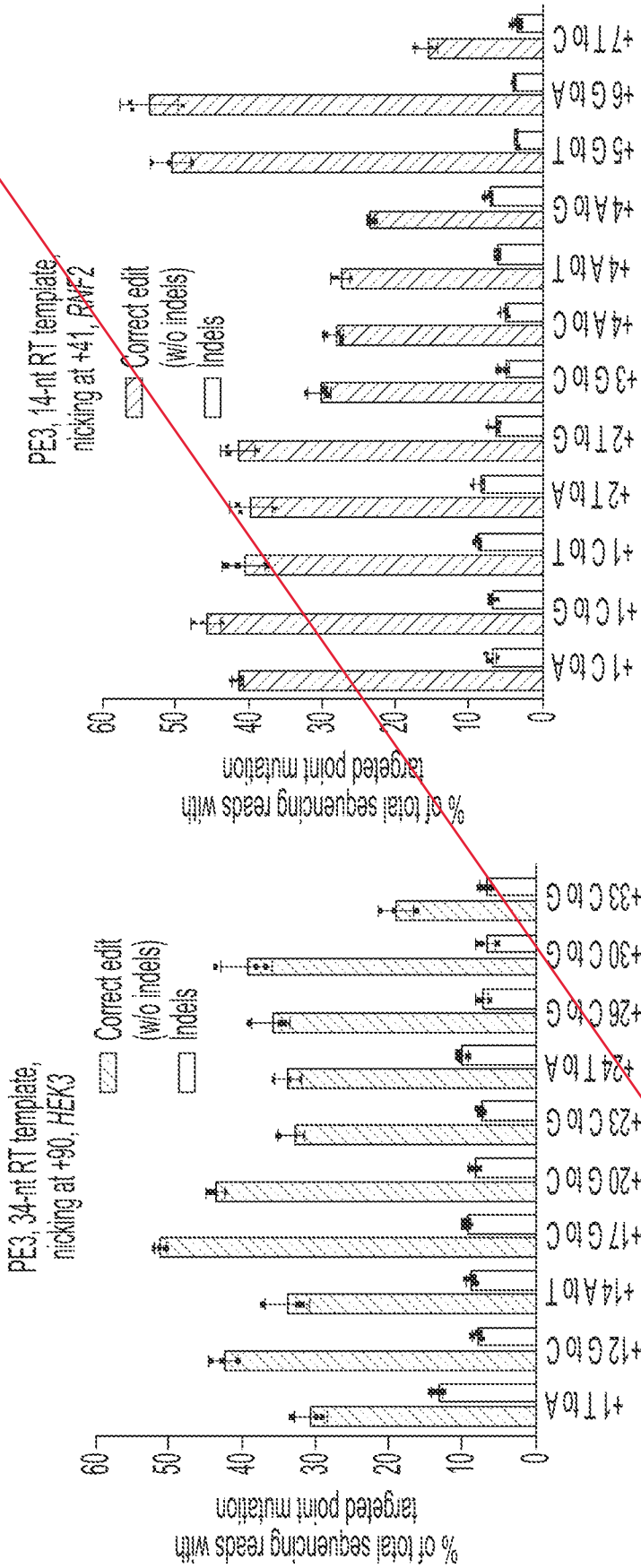


FIG. 41C

FIG. 41B

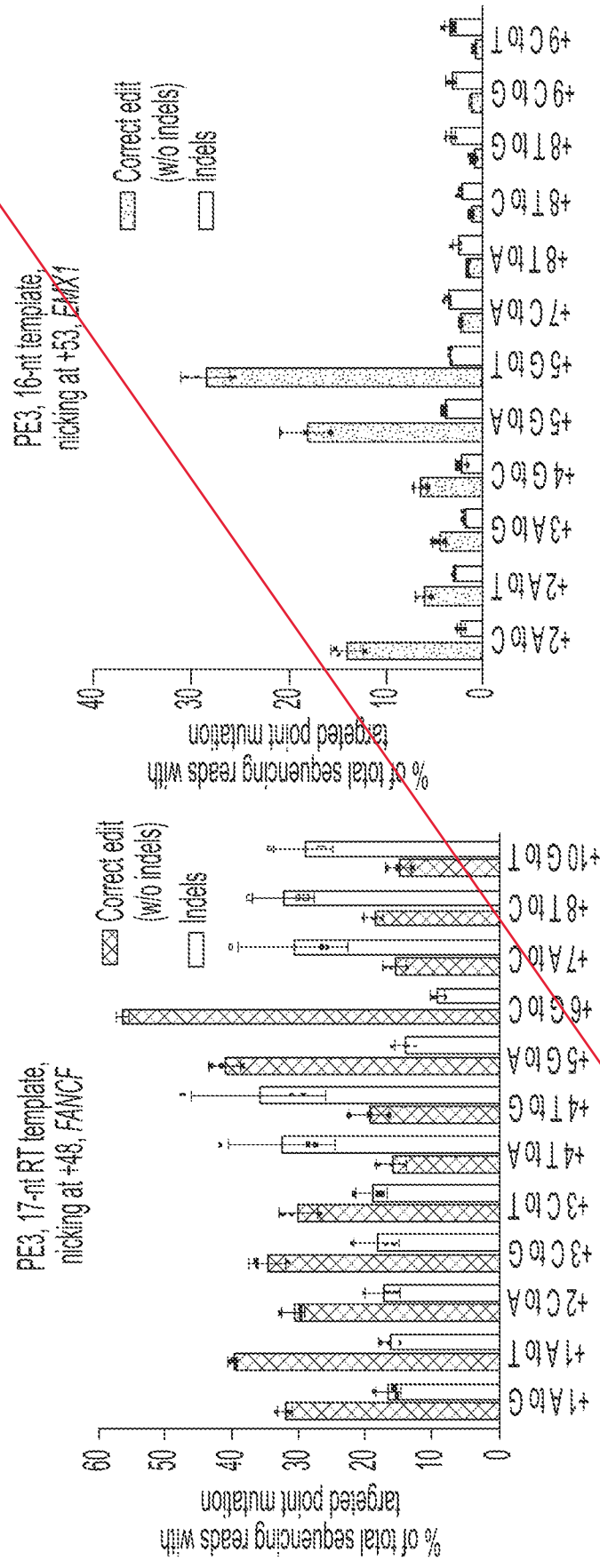


FIG. 41E

FIG. 41D

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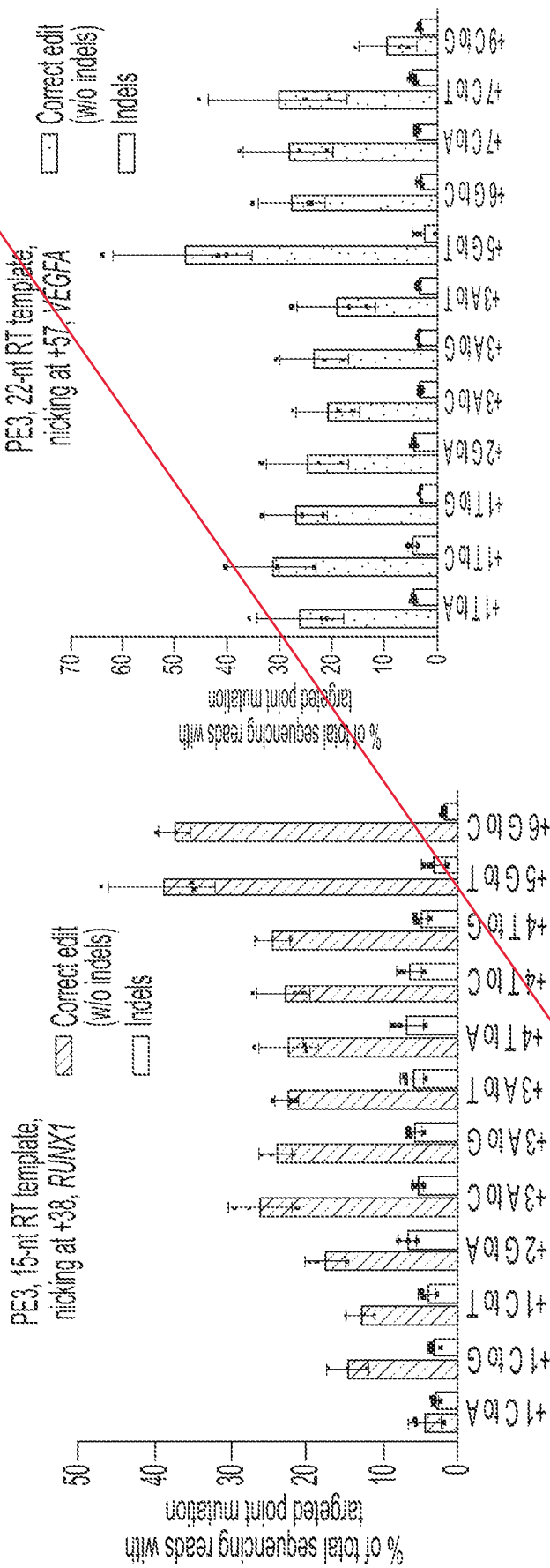


FIG. 41G

FIG. 41F

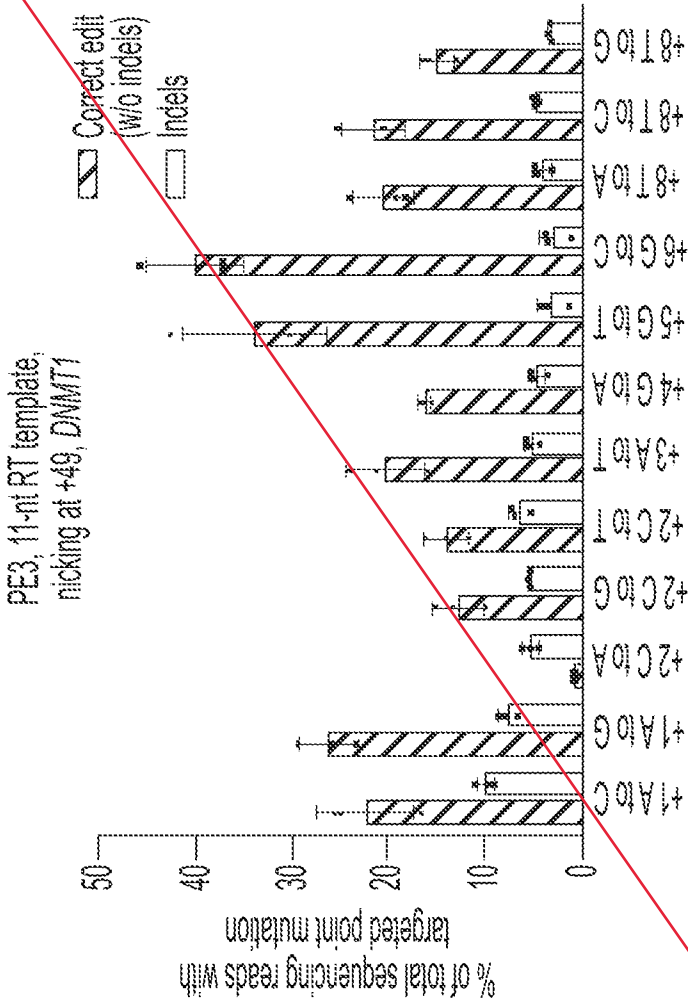


FIG. 41H

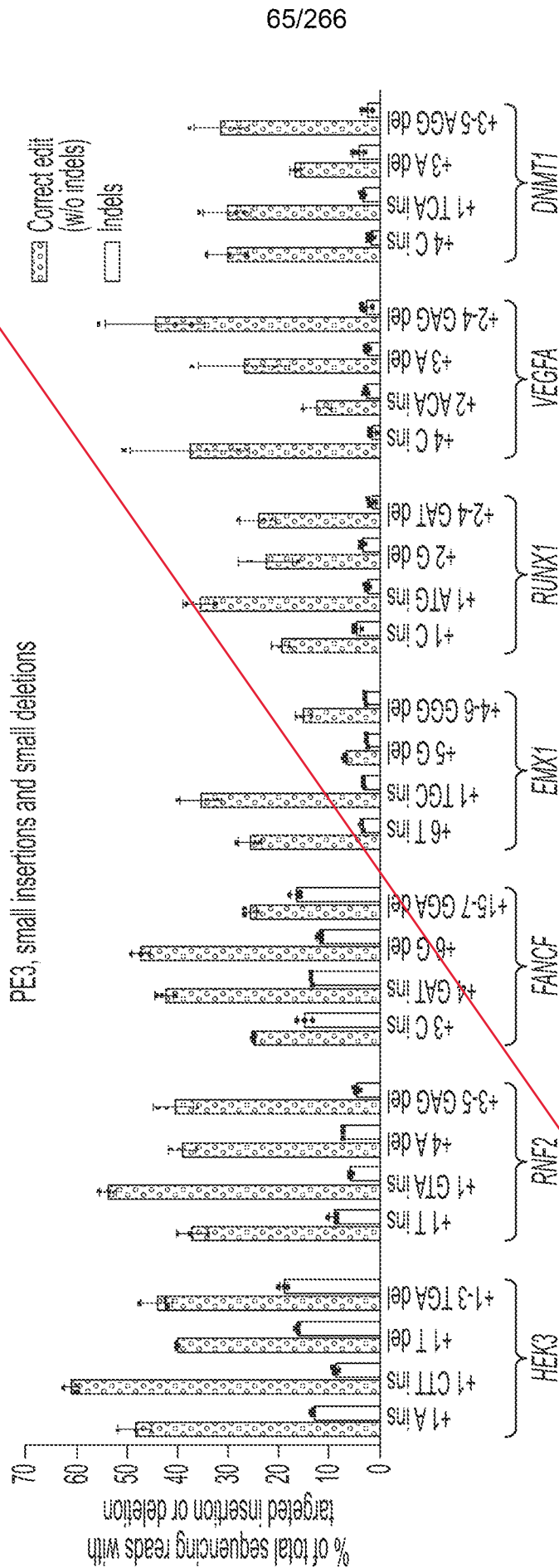


FIG. 41I

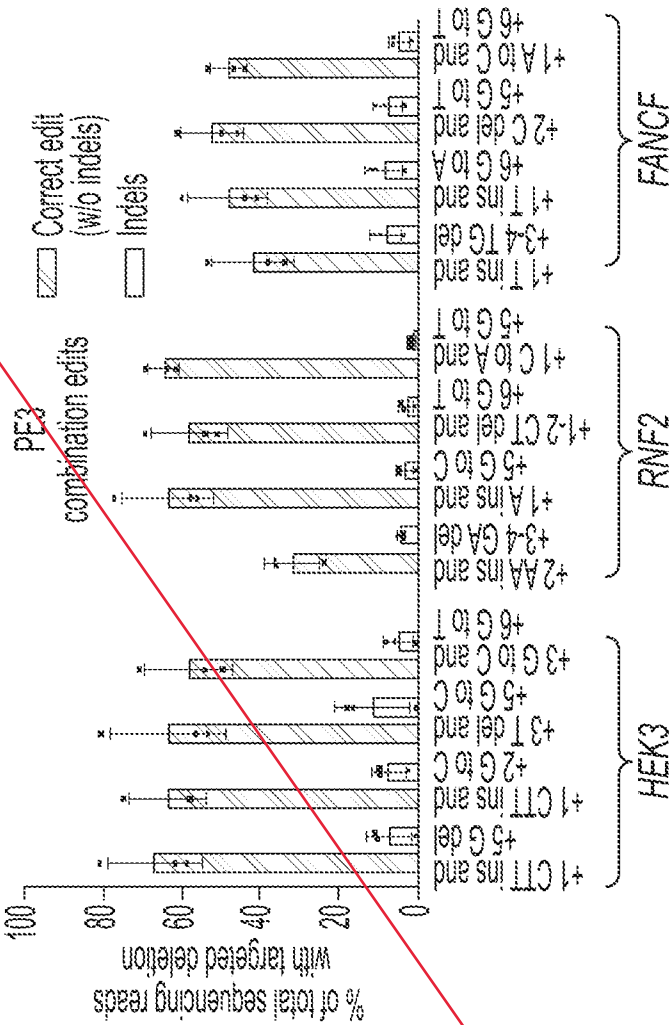


FIG. 41K

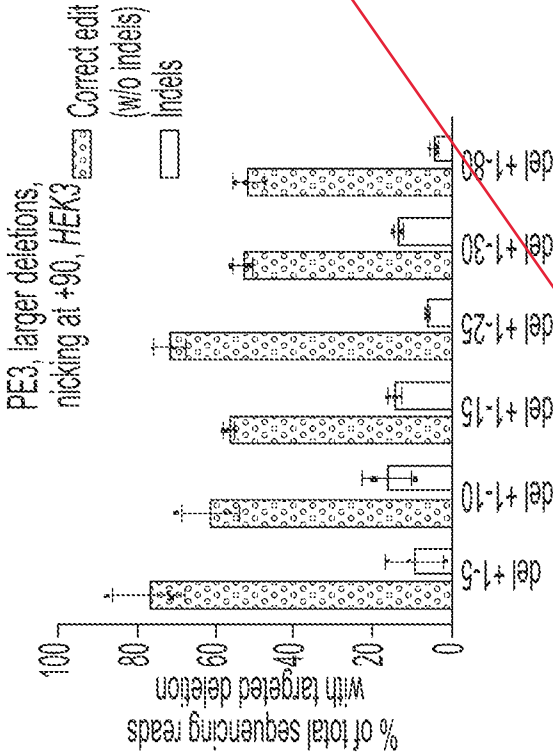


FIG. 41J

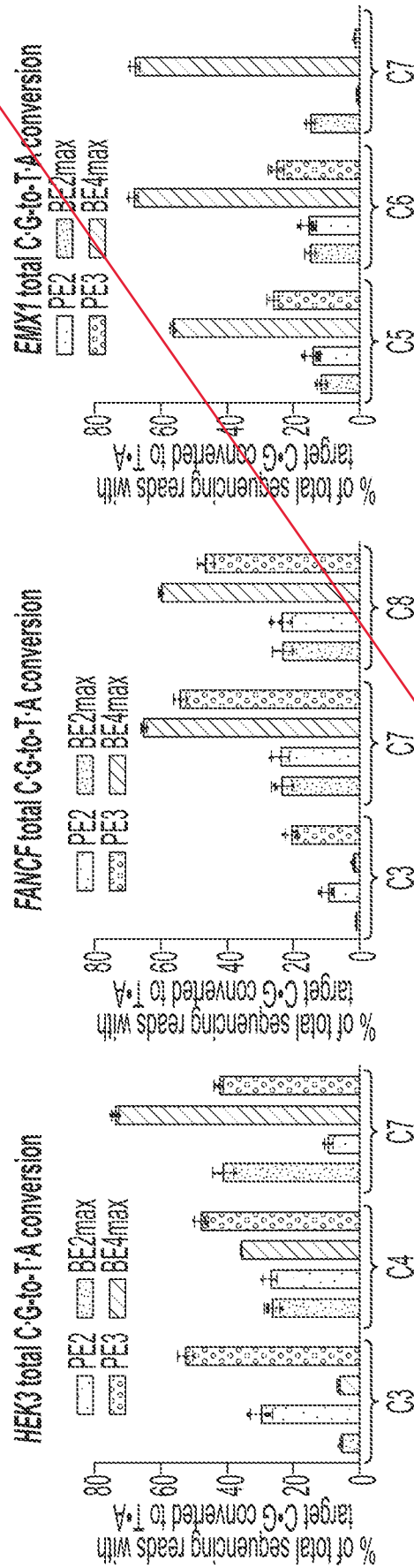


FIG. 42A

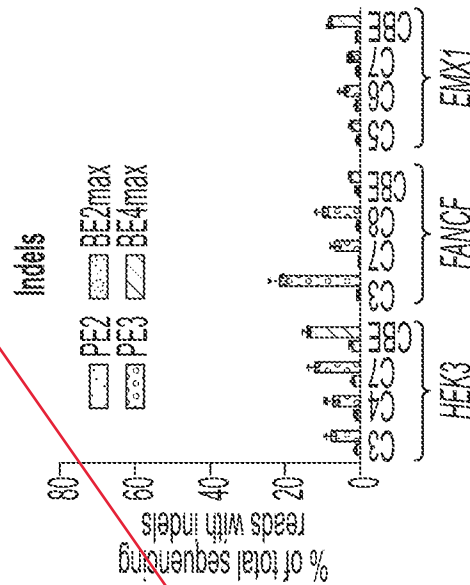


FIG. 42B

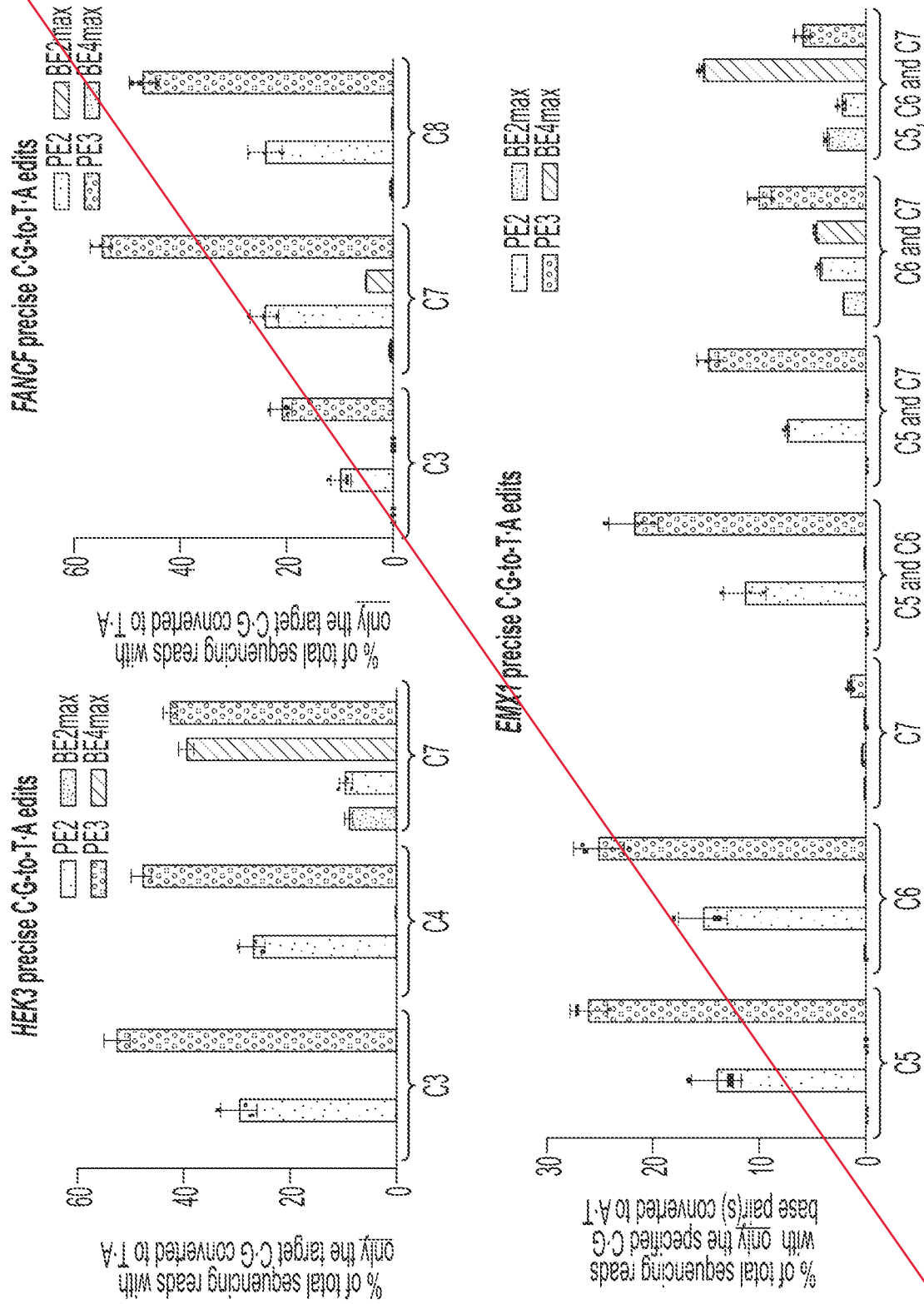


FIG. 42C

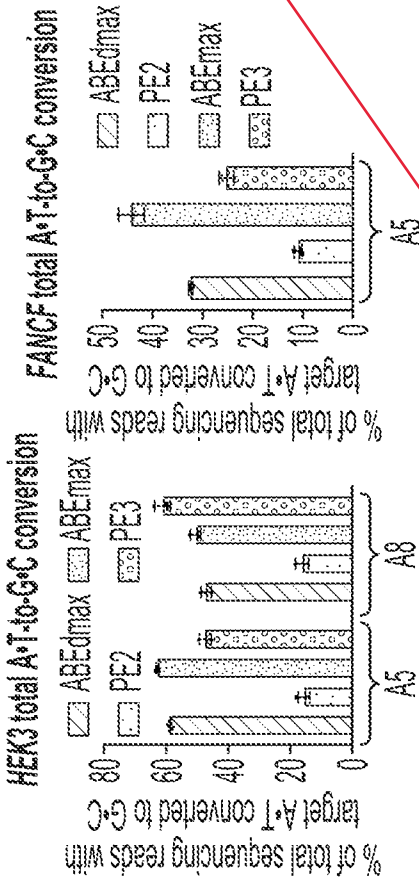


FIG. 42D

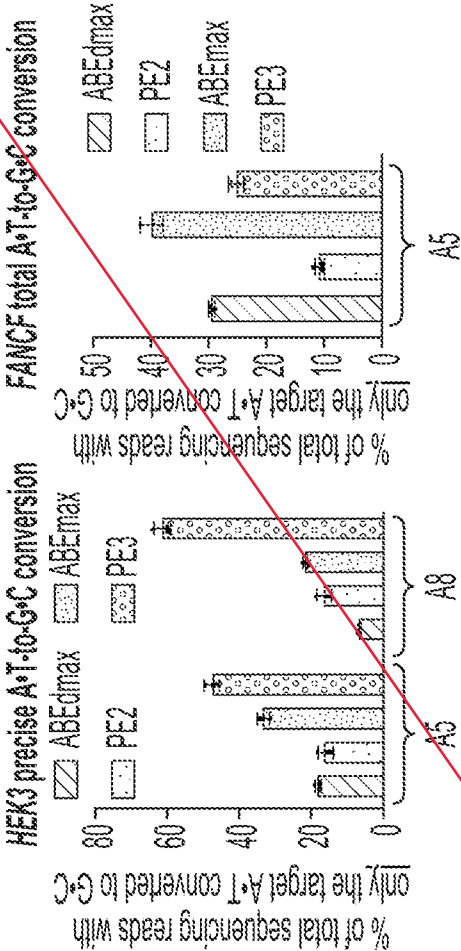


FIG. 42E

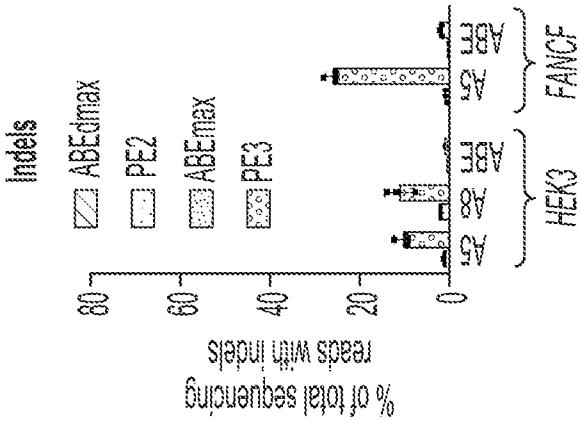


FIG. 42F

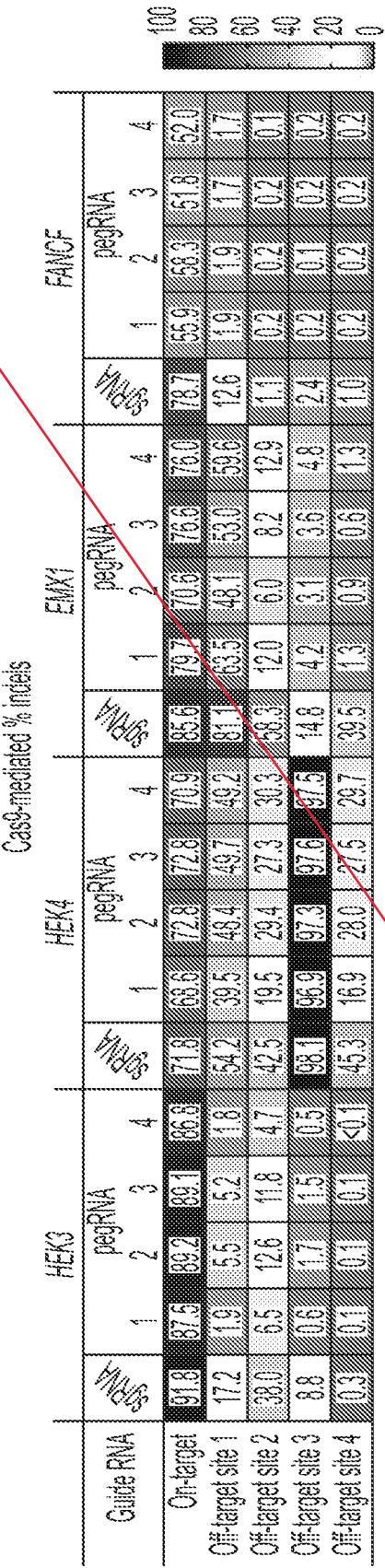


FIG. 42G

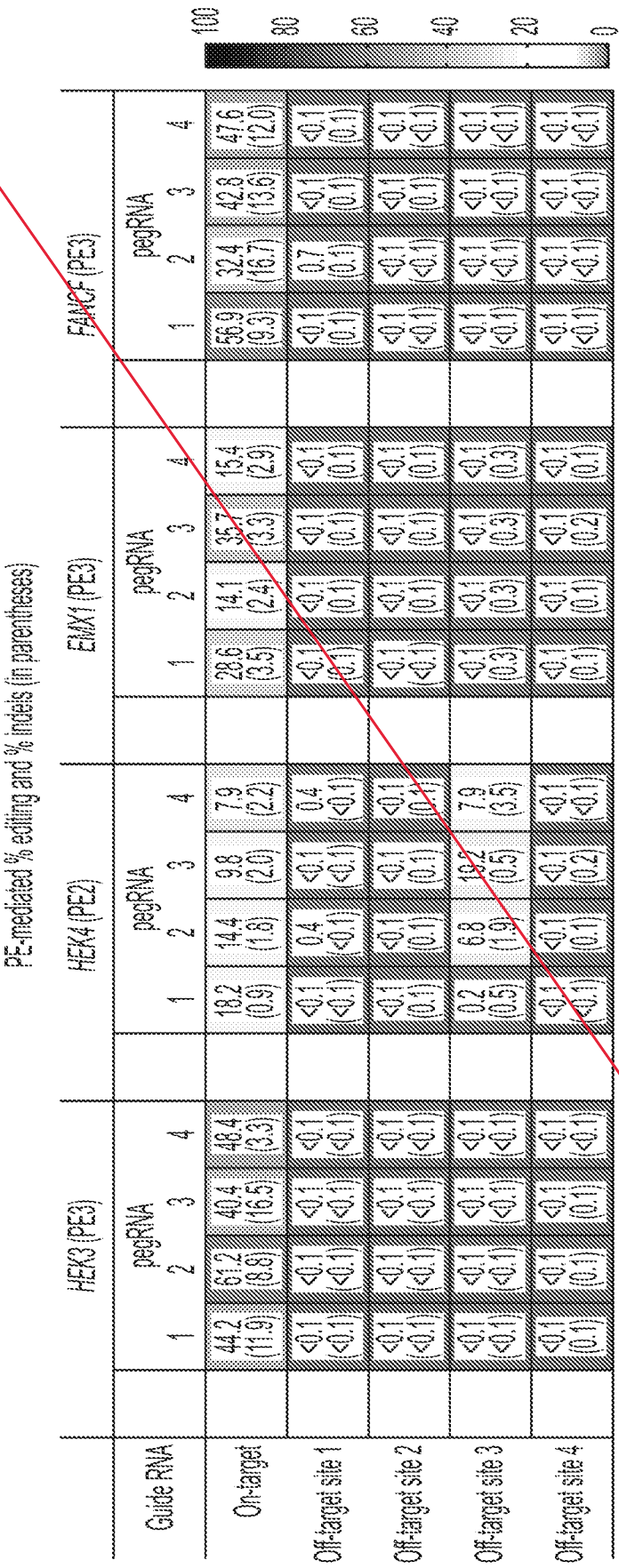


FIG. 42H

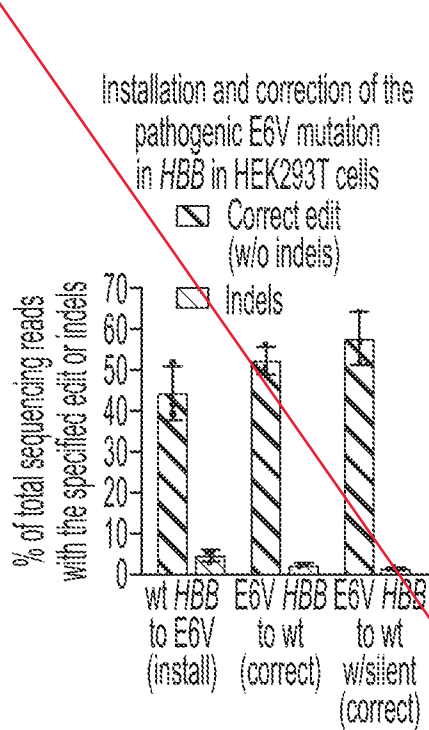


FIG. 43A

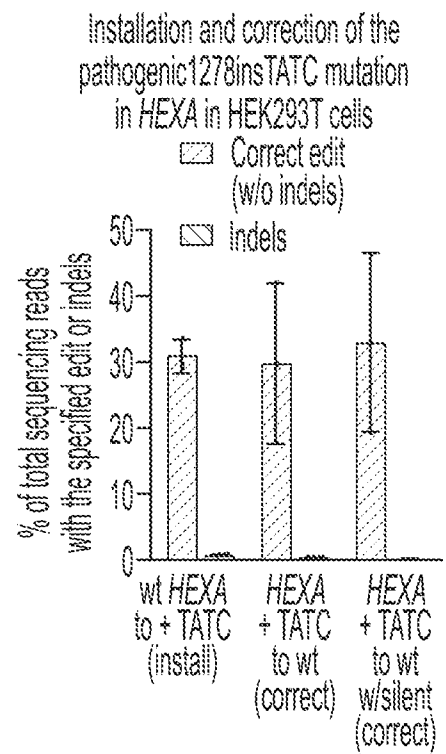


FIG. 43B

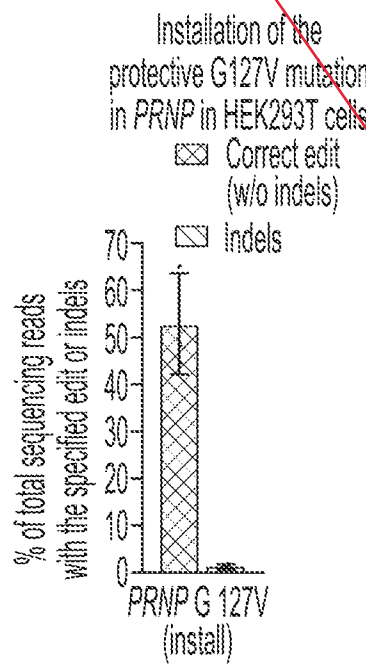


FIG. 43C

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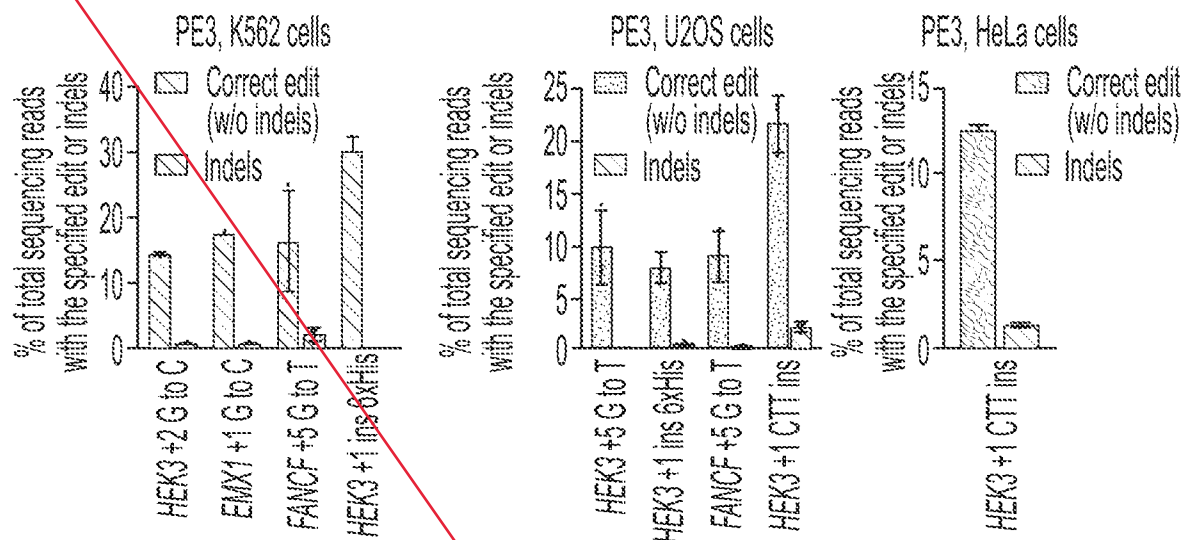


FIG. 43D

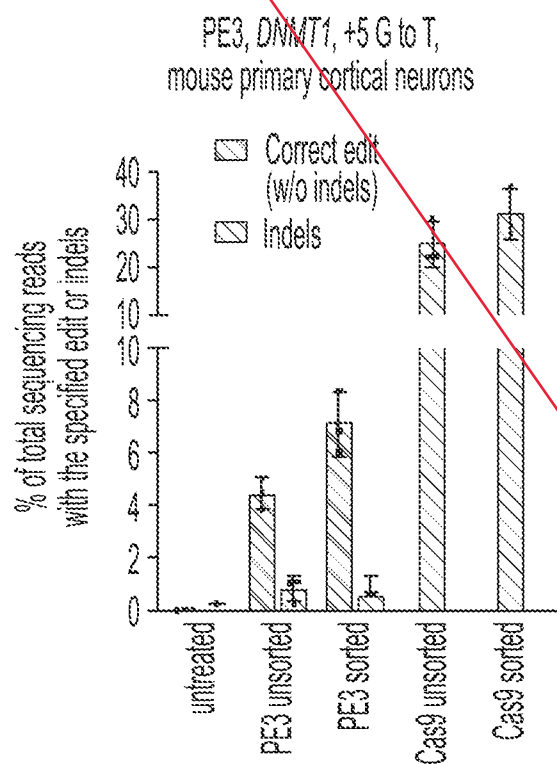


FIG. 43E

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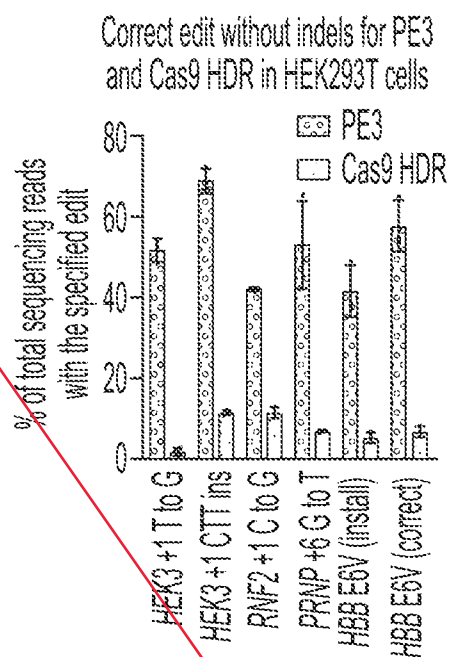


FIG. 43F

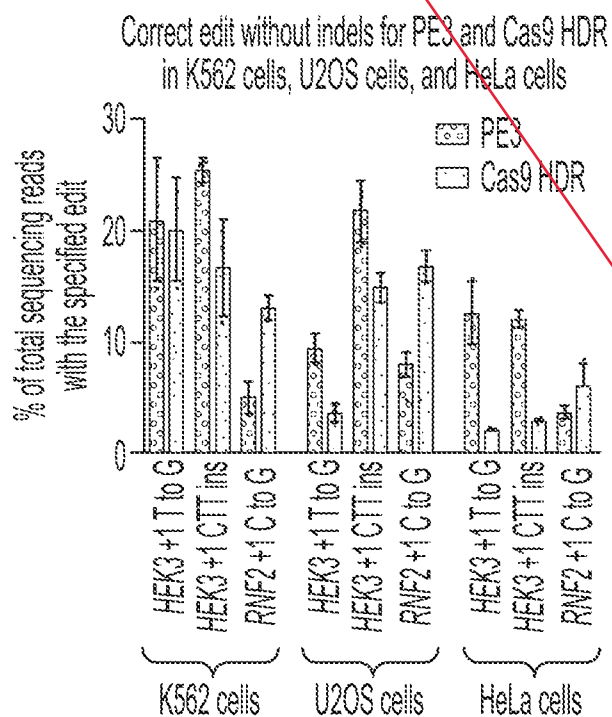


FIG. 43G

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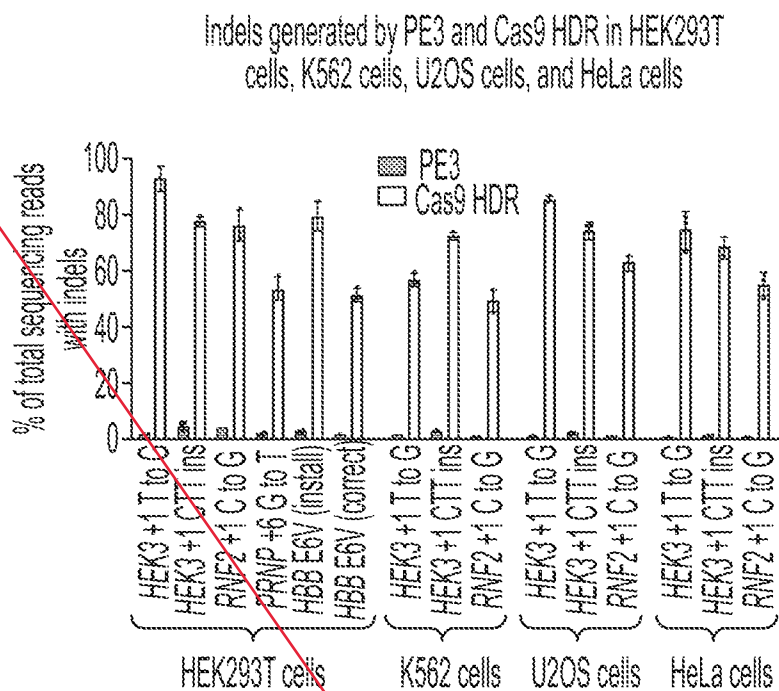


FIG. 43H



FIG. 43I

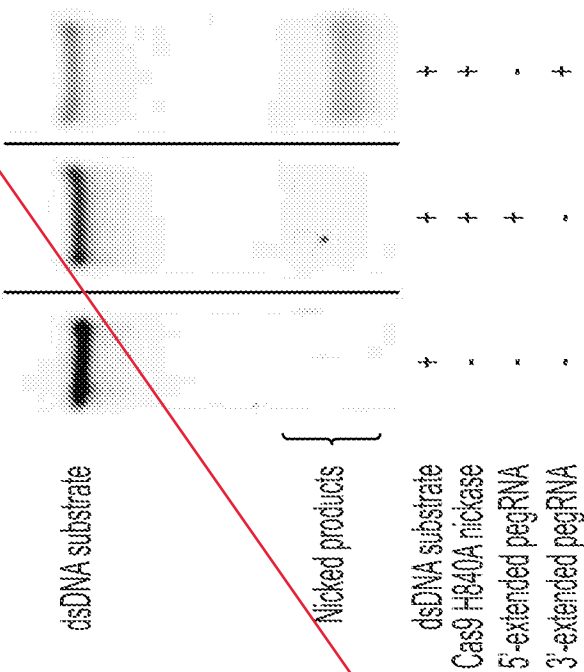


FIG. 44B

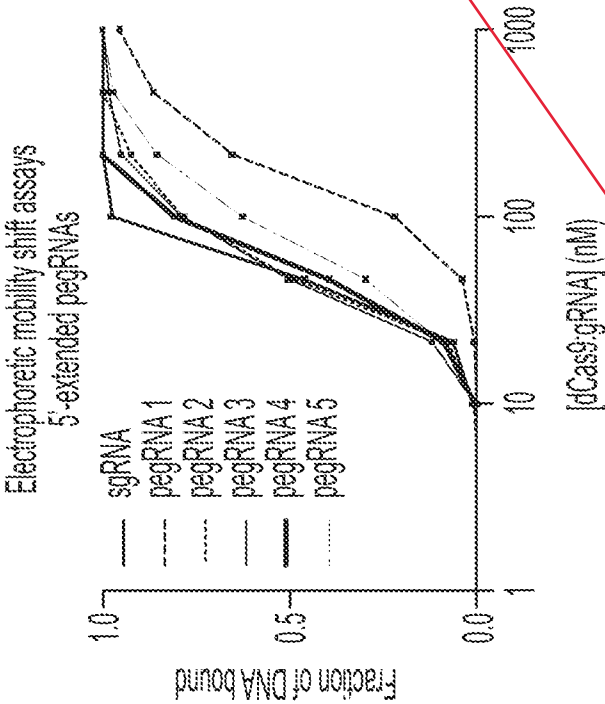


FIG. 44A

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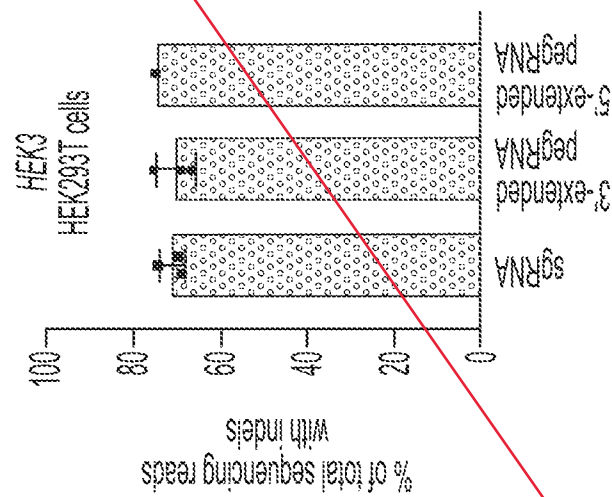


FIG. 44C

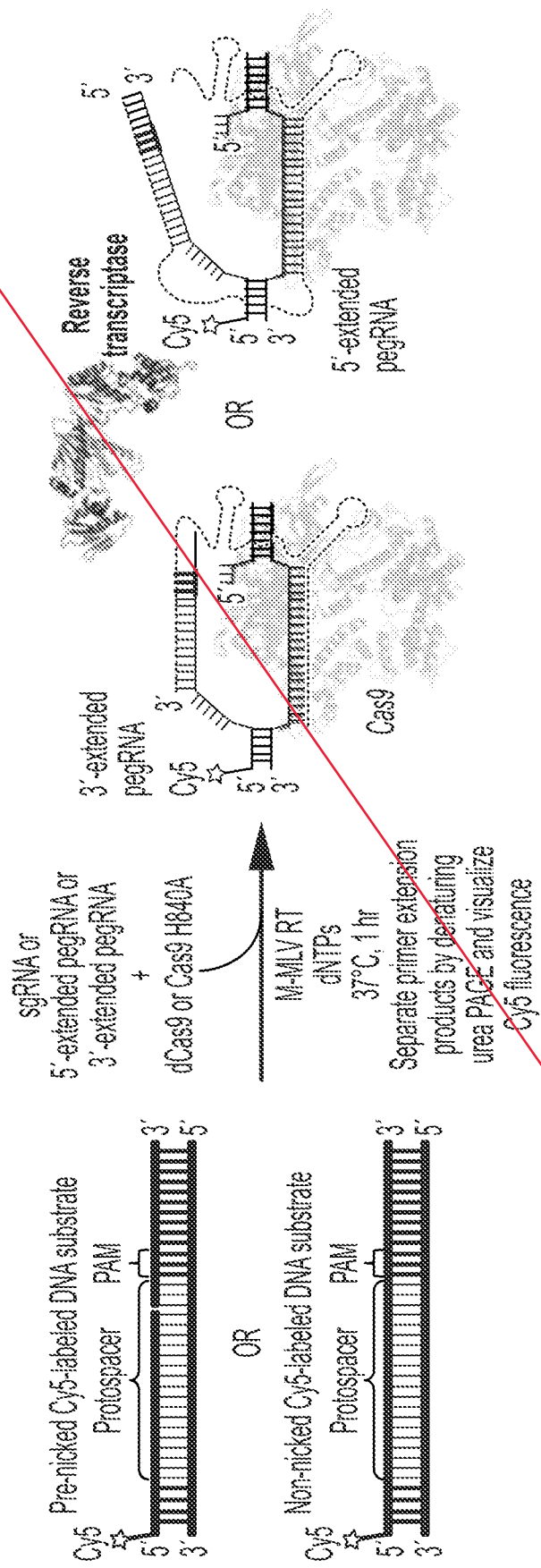


FIG. 44D

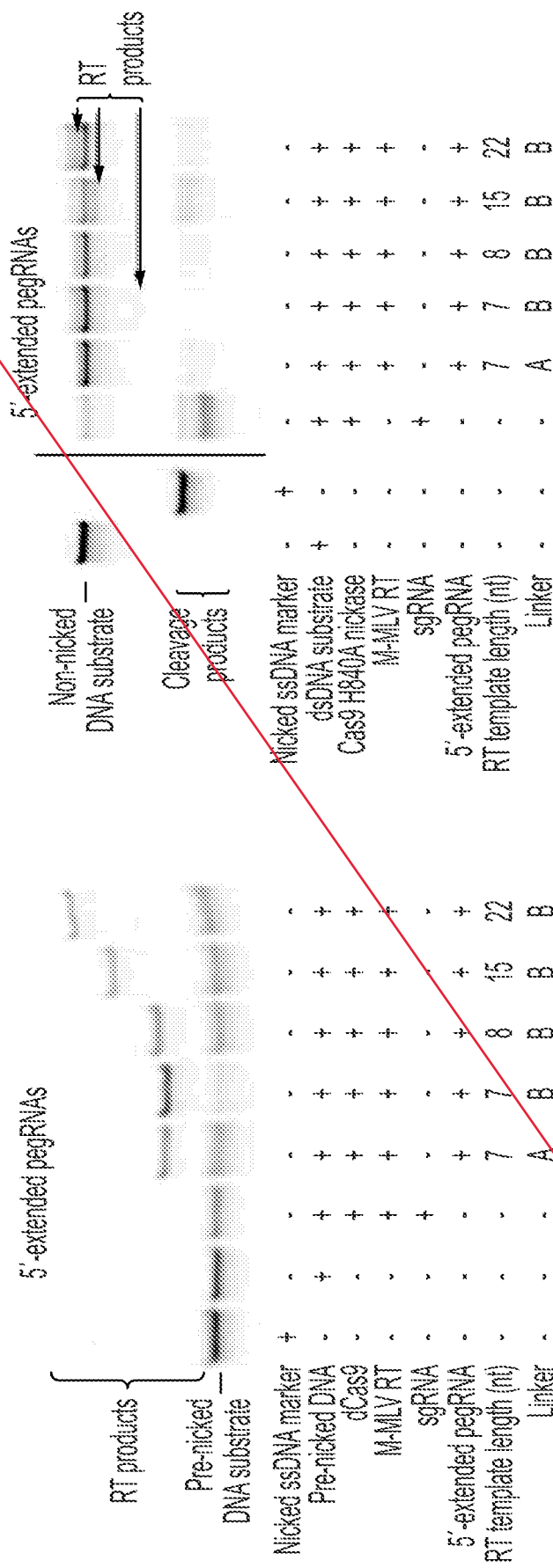
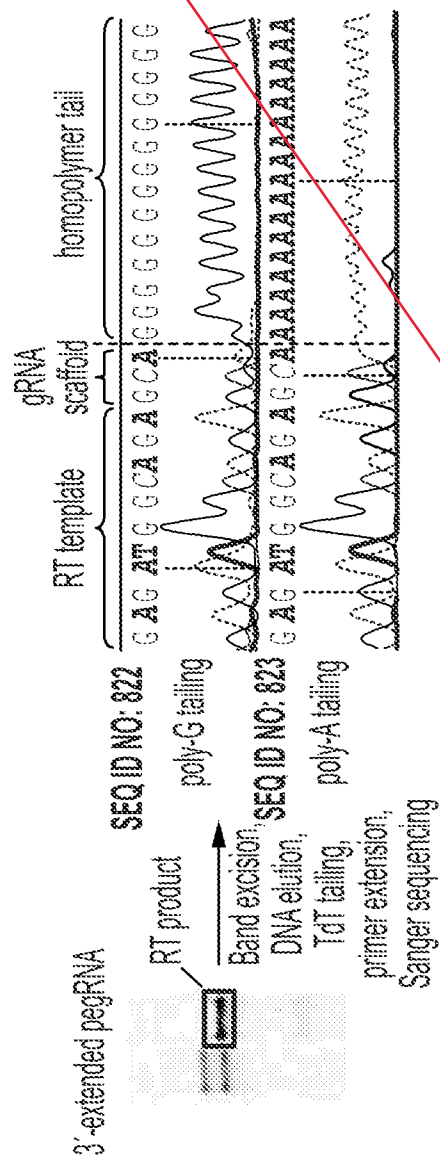
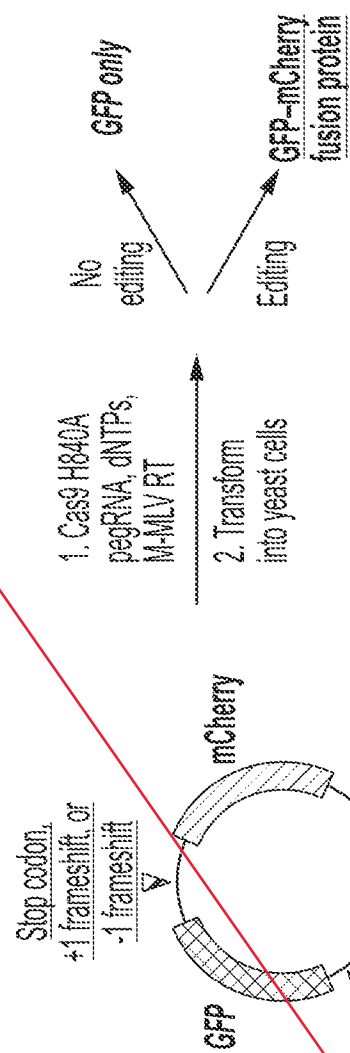


FIG. 44F

FIG. 44E



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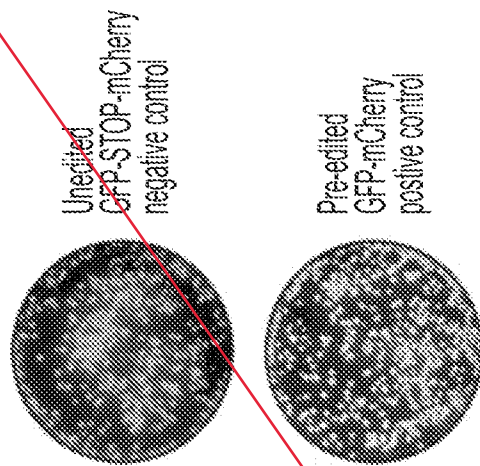


FIG. 45B

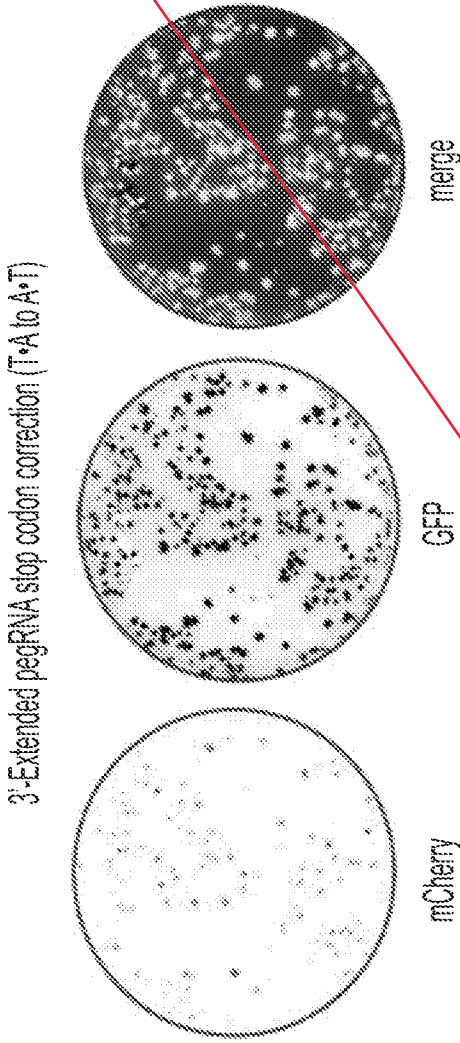


FIG. 45C

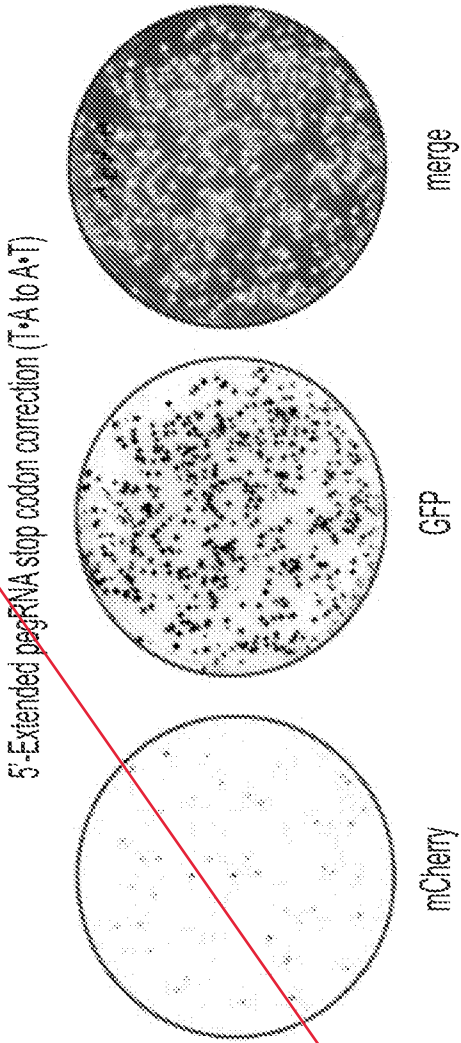


FIG. 45D

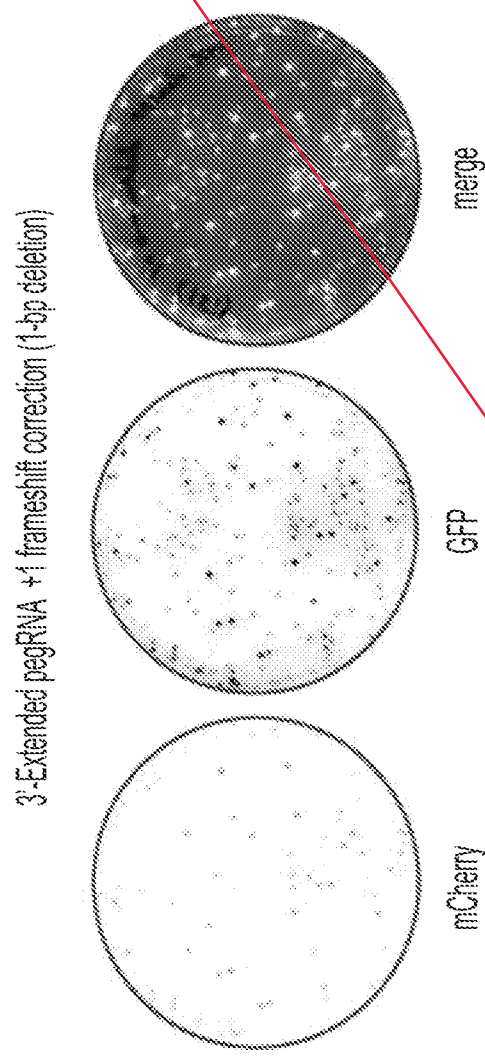


FIG. 45E

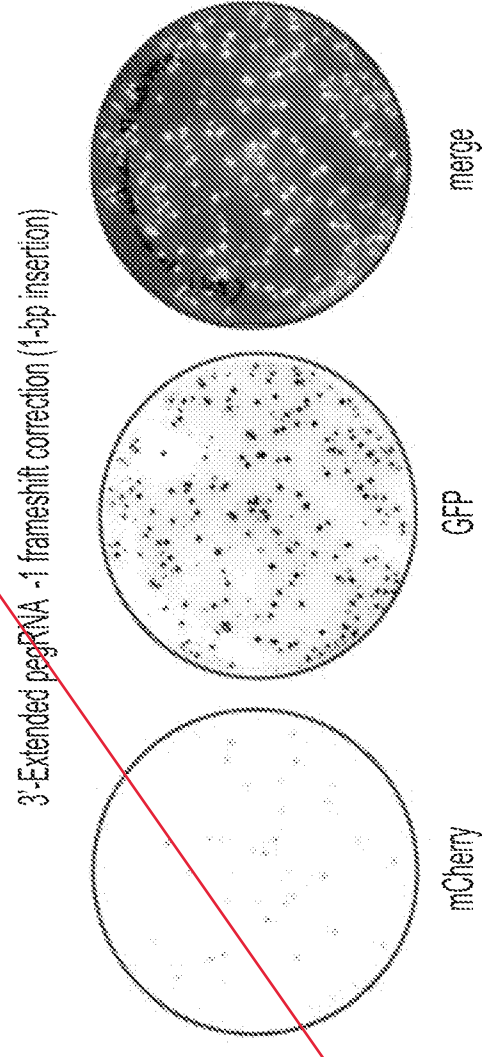


FIG. 45F

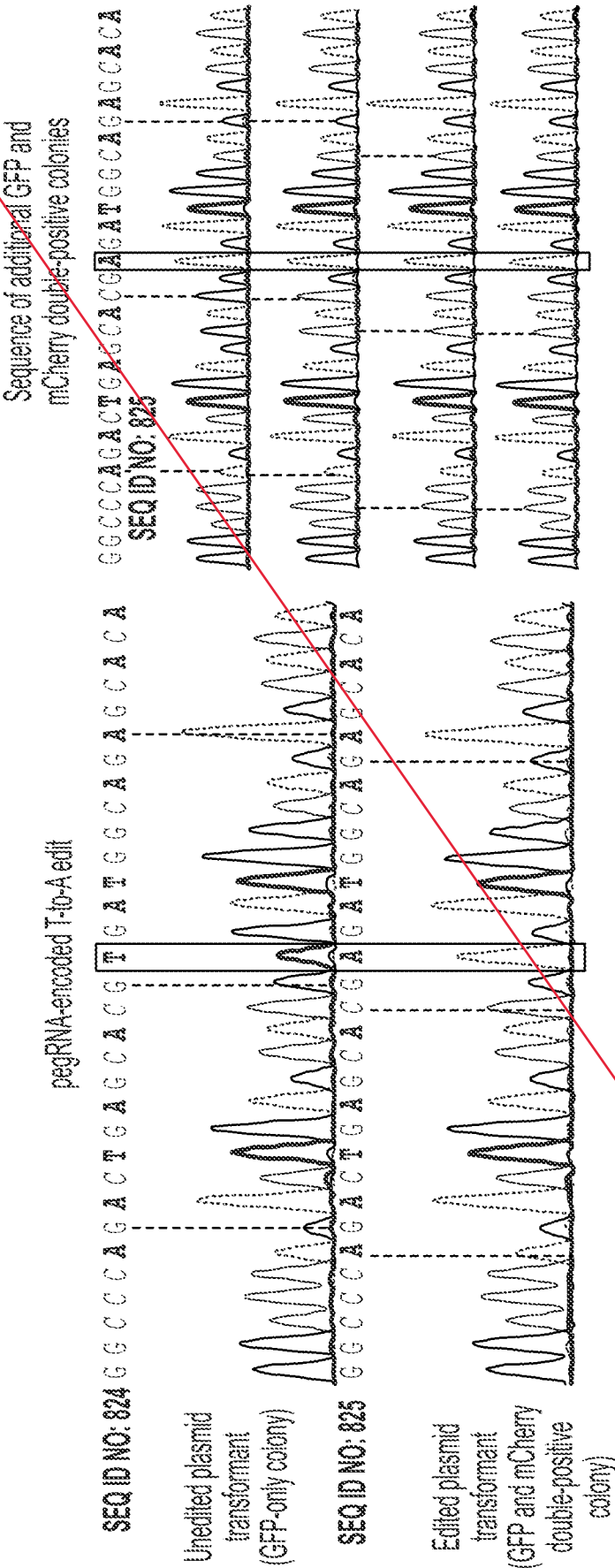


FIG. 45G

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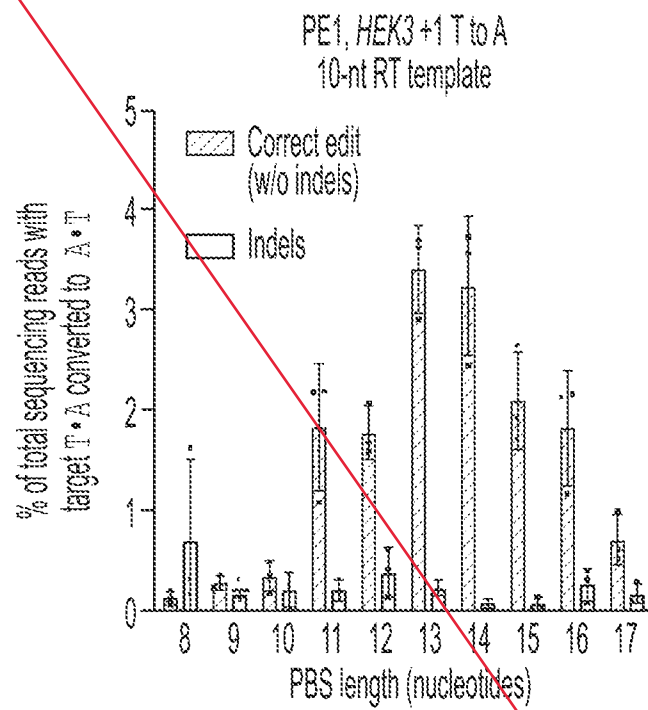


FIG. 46A

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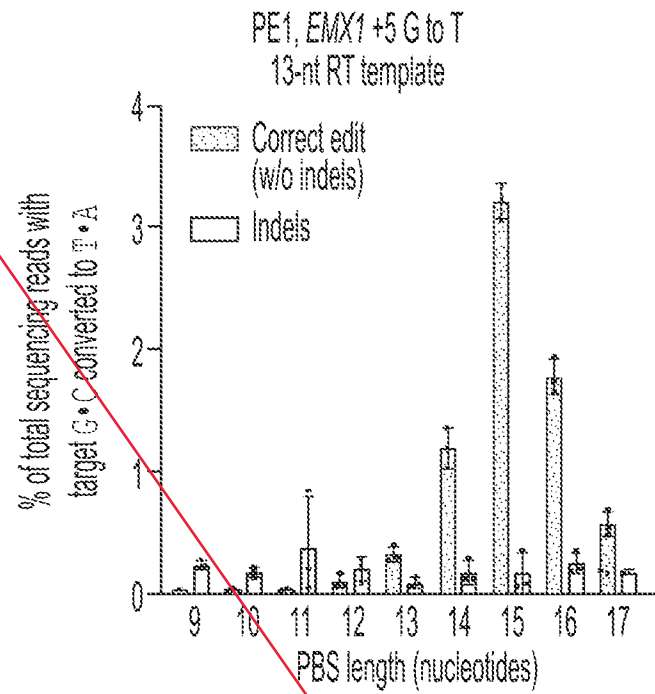


FIG. 46B

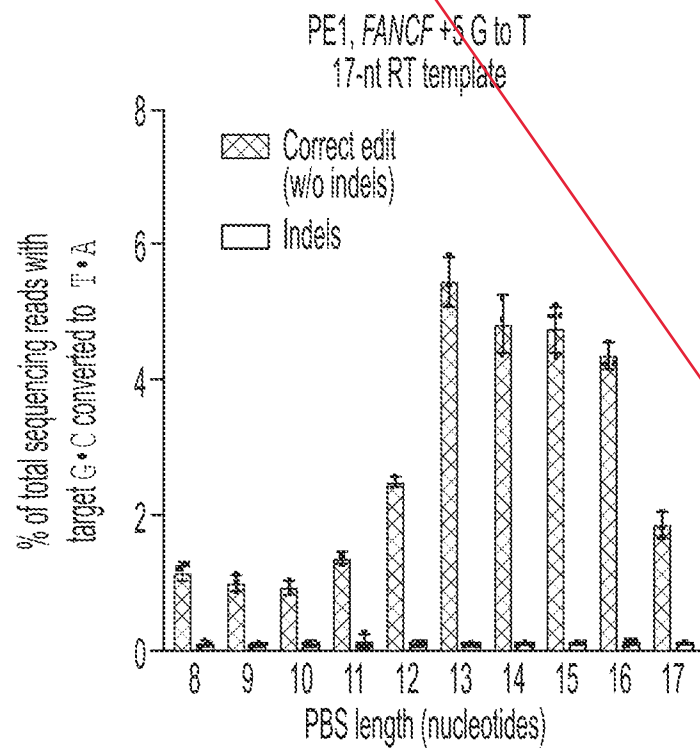


FIG. 46C

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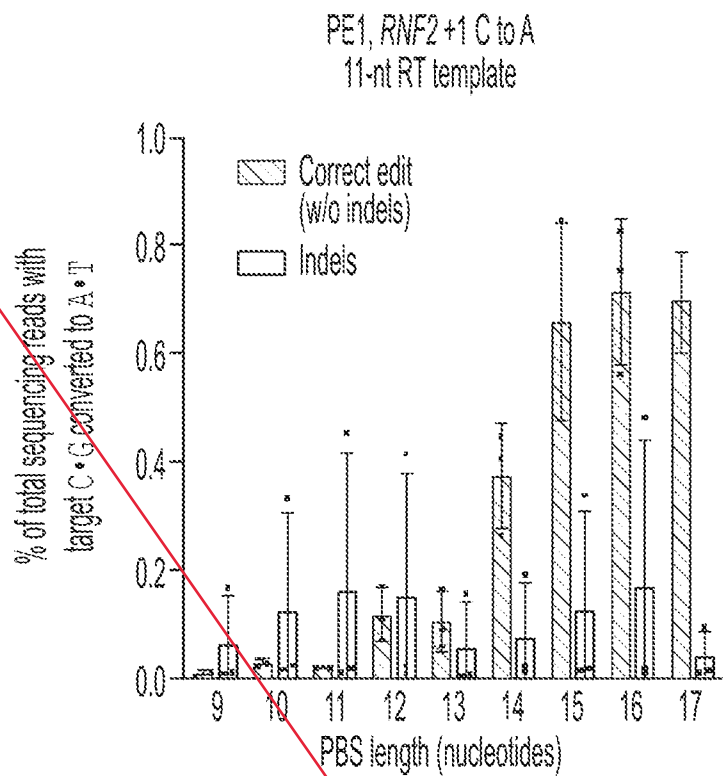


FIG. 46D

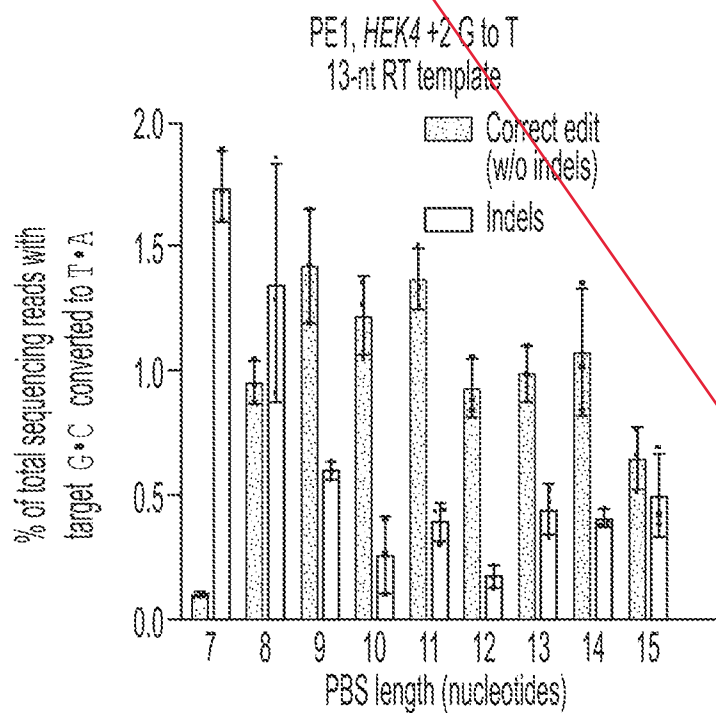


FIG. 46E

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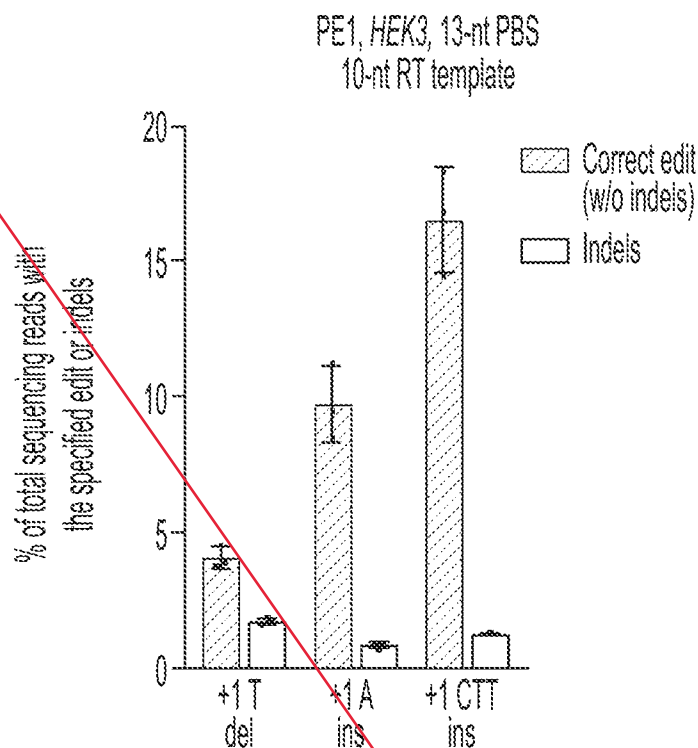


FIG. 46F

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

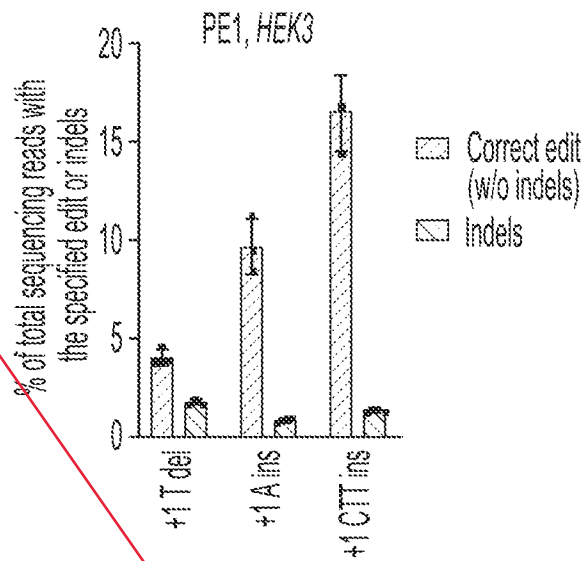


FIG. 47B

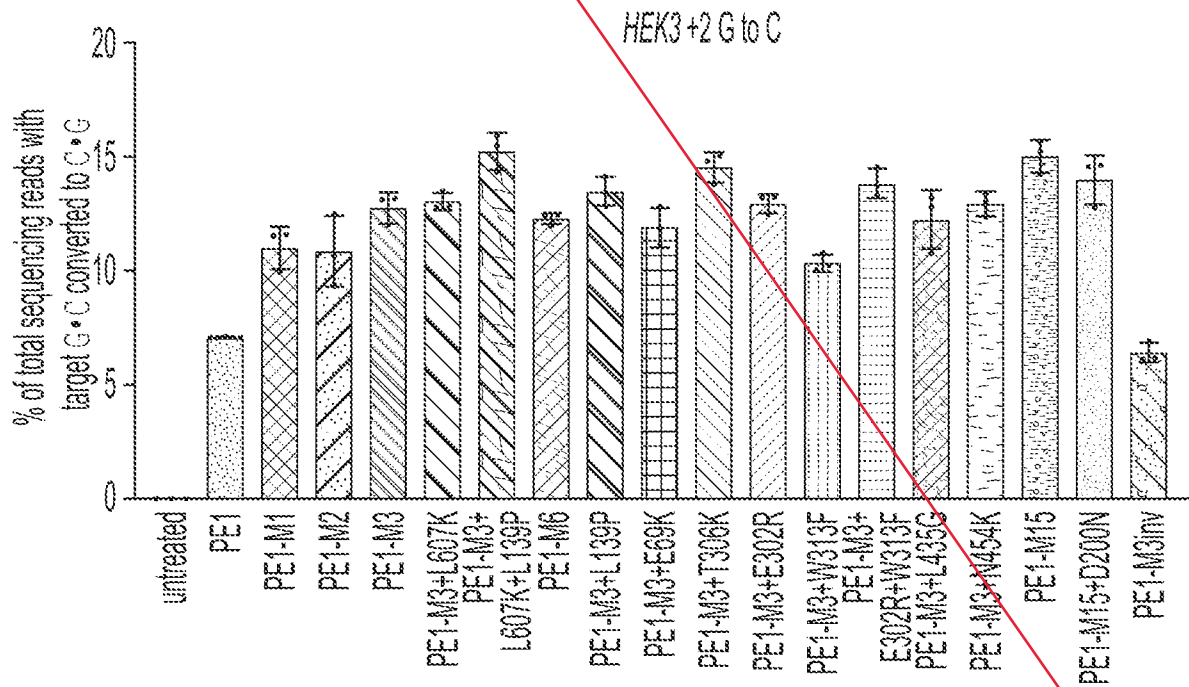


FIG. 47C

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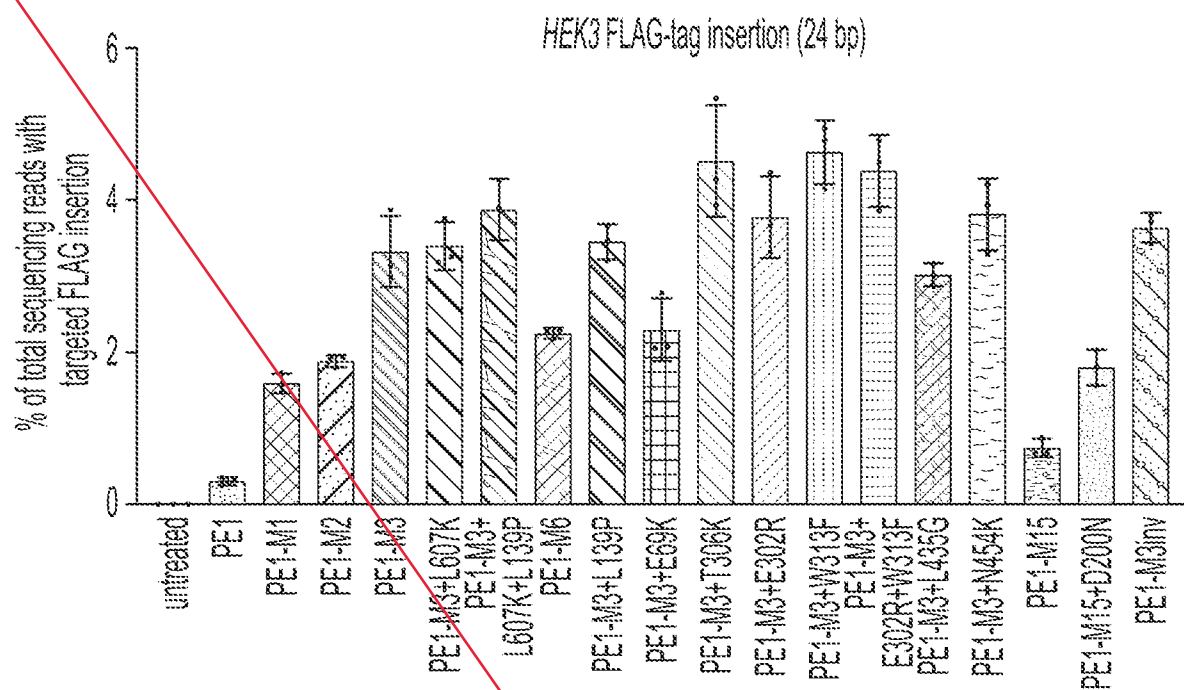


FIG. 47D

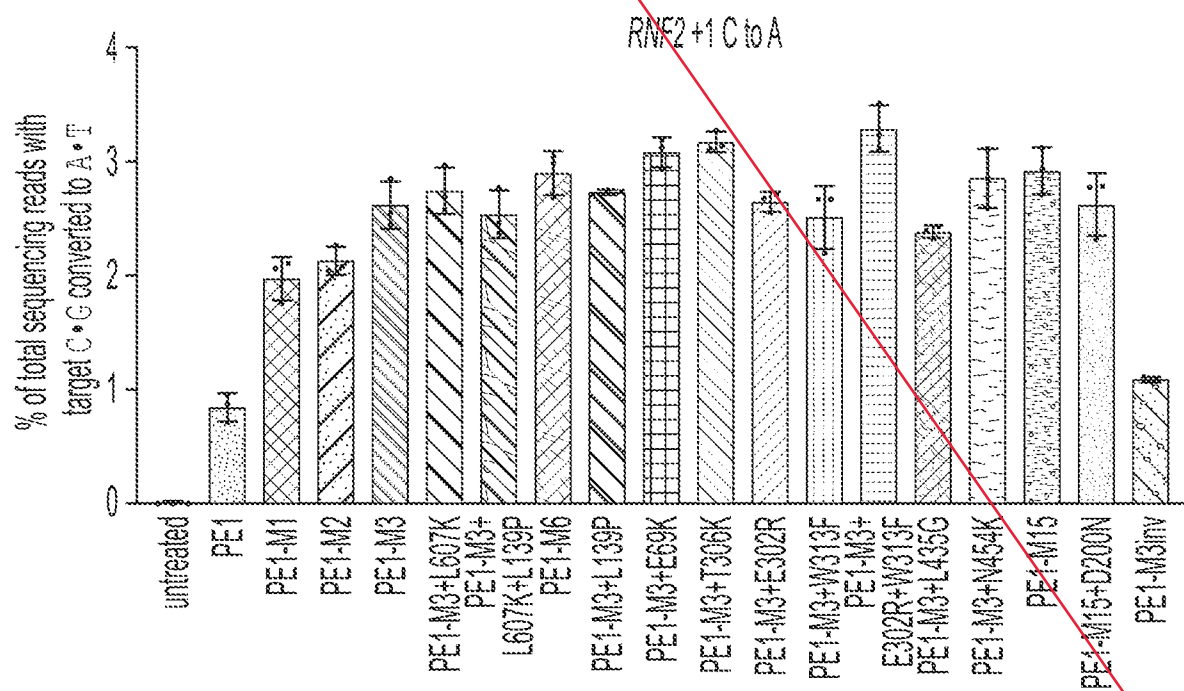


FIG. 47E

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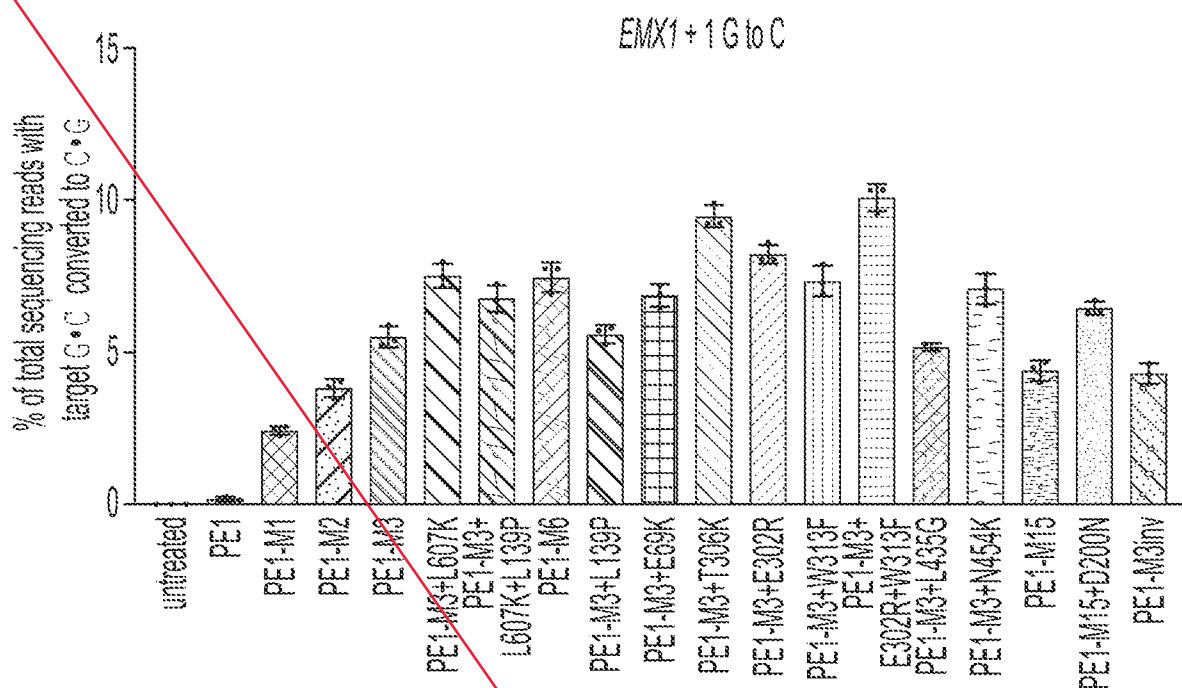


FIG. 47F

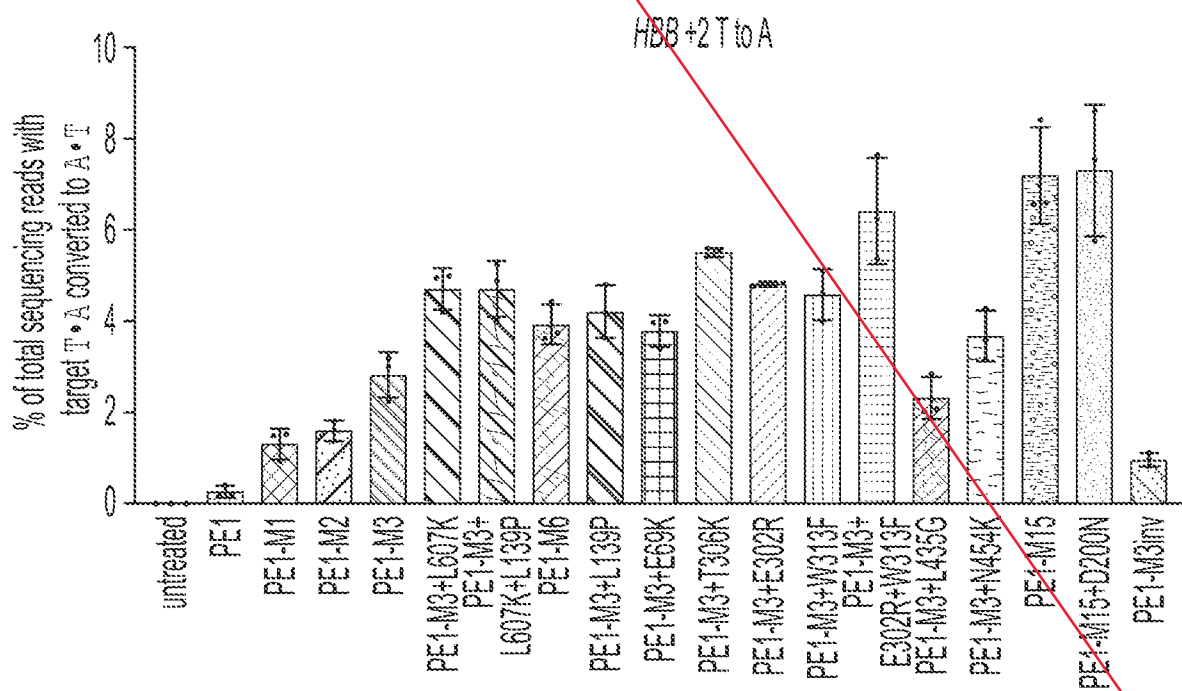


FIG. 47G

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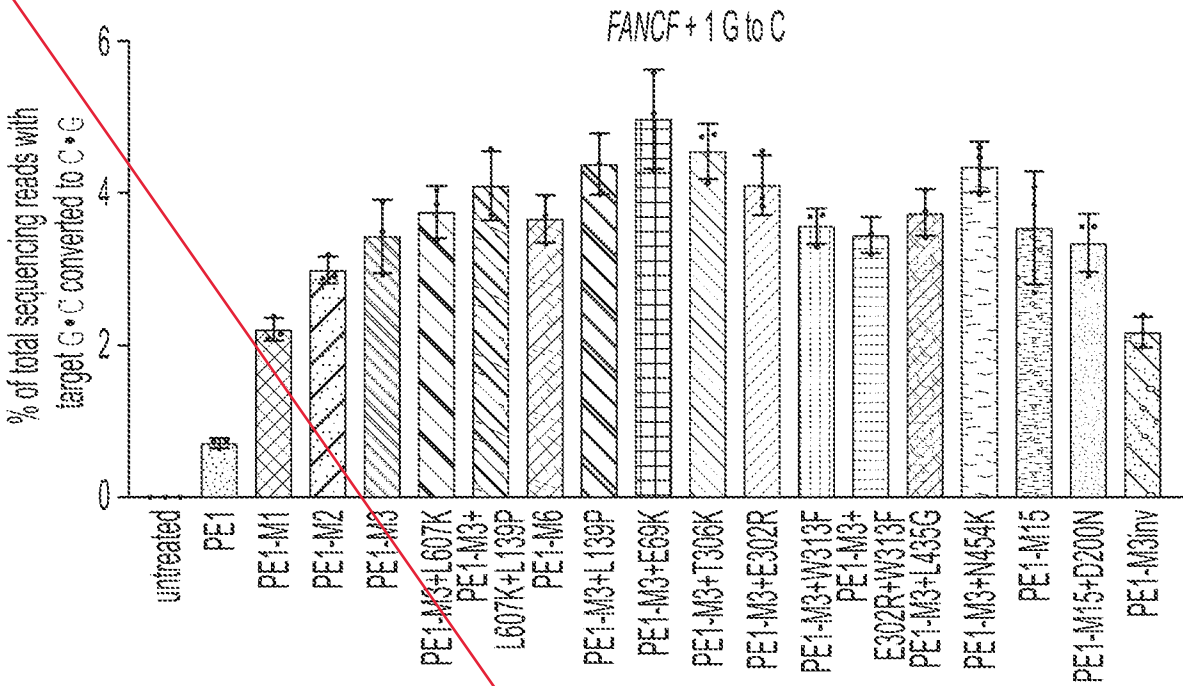


FIG. 47H

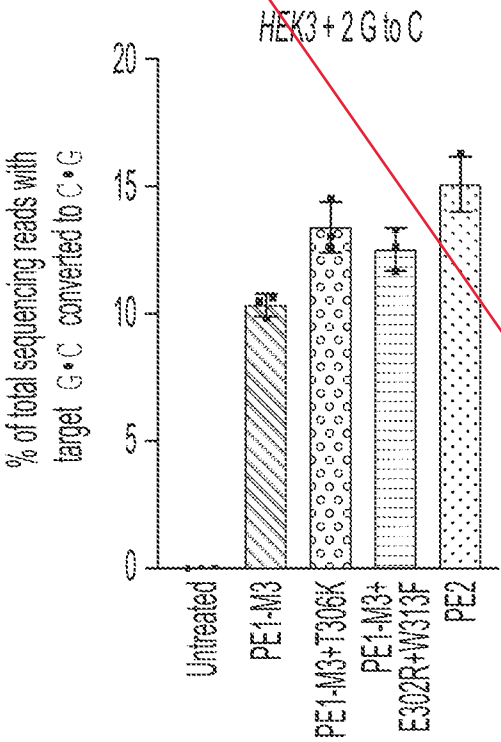


FIG. 47I

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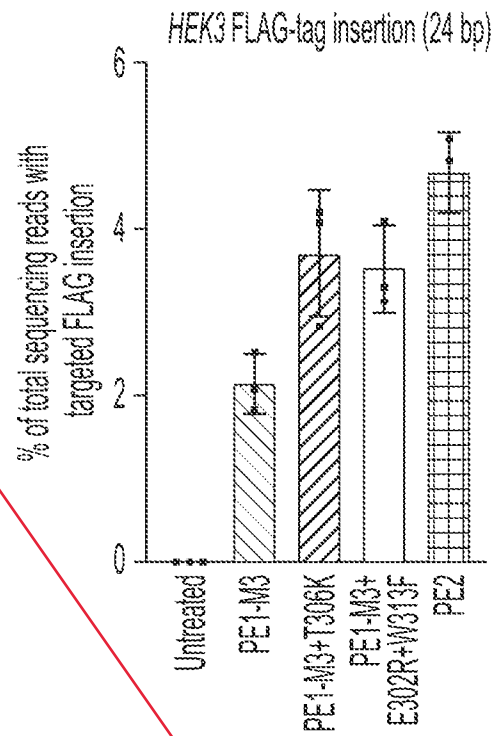


FIG. 47J

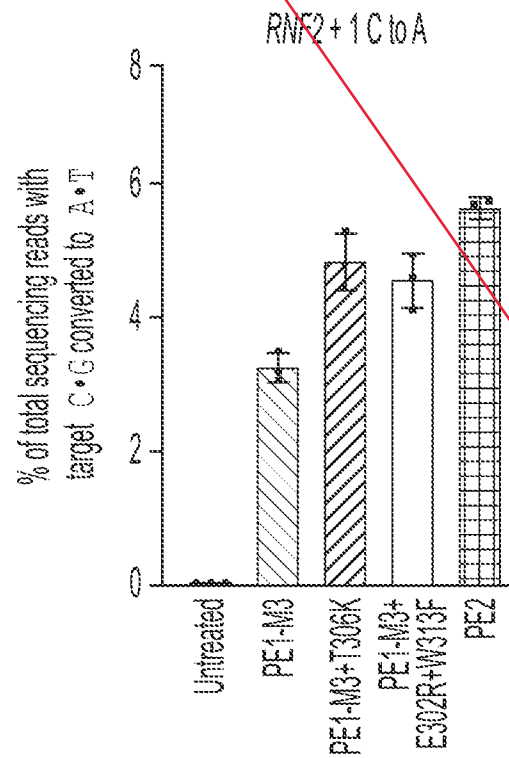


FIG. 47K

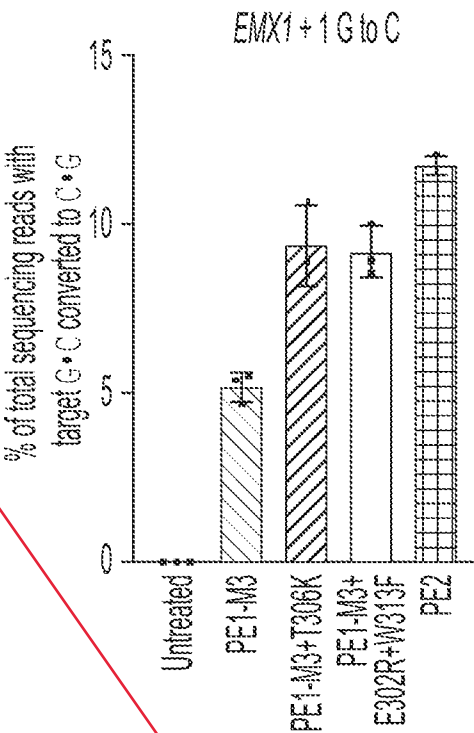


FIG. 47L

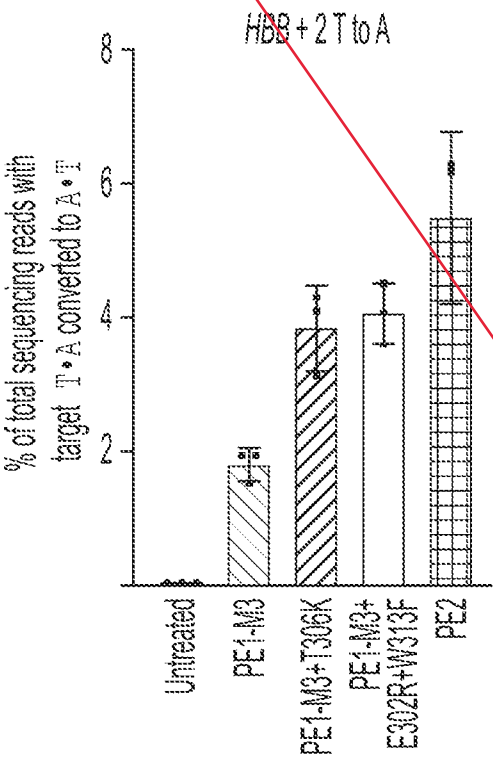


FIG. 47M

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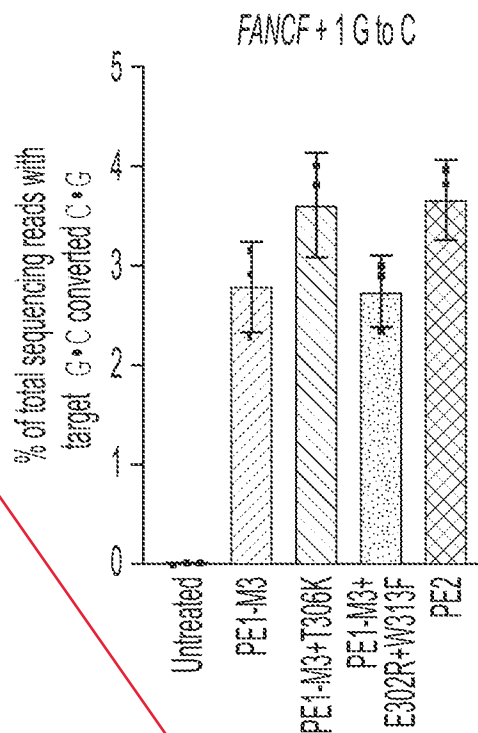


FIG. 47N

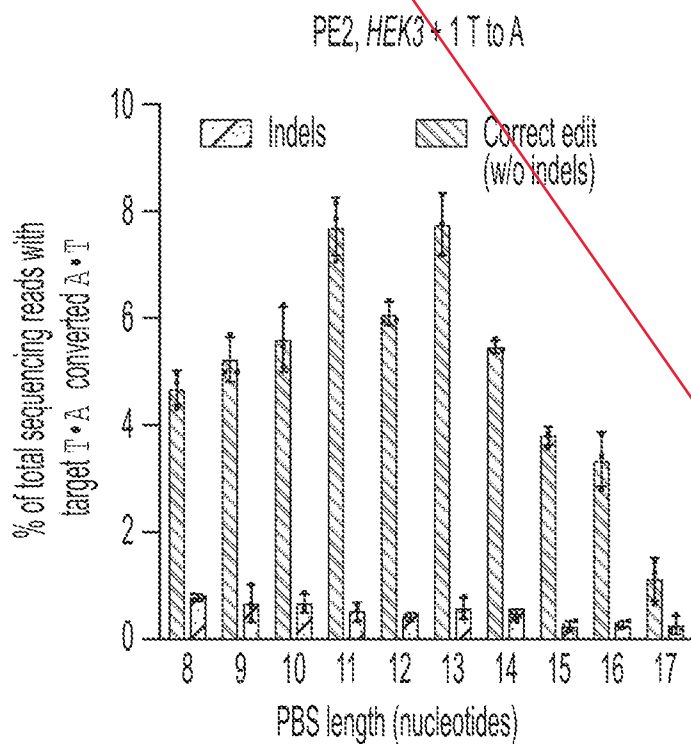


FIG. 47O

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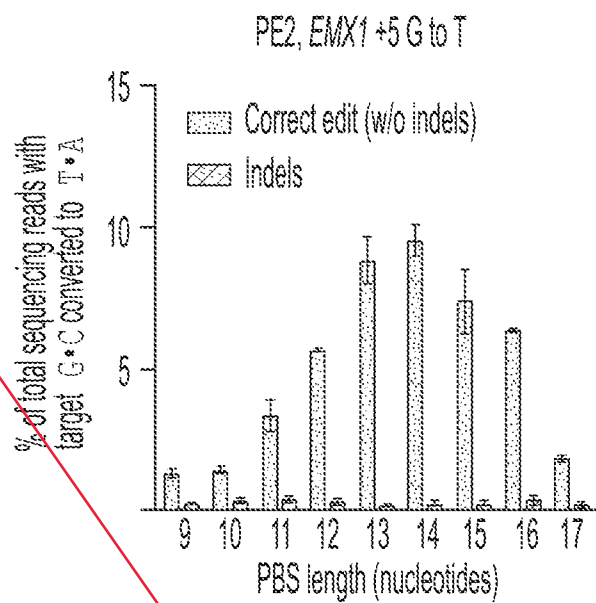


FIG. 47P

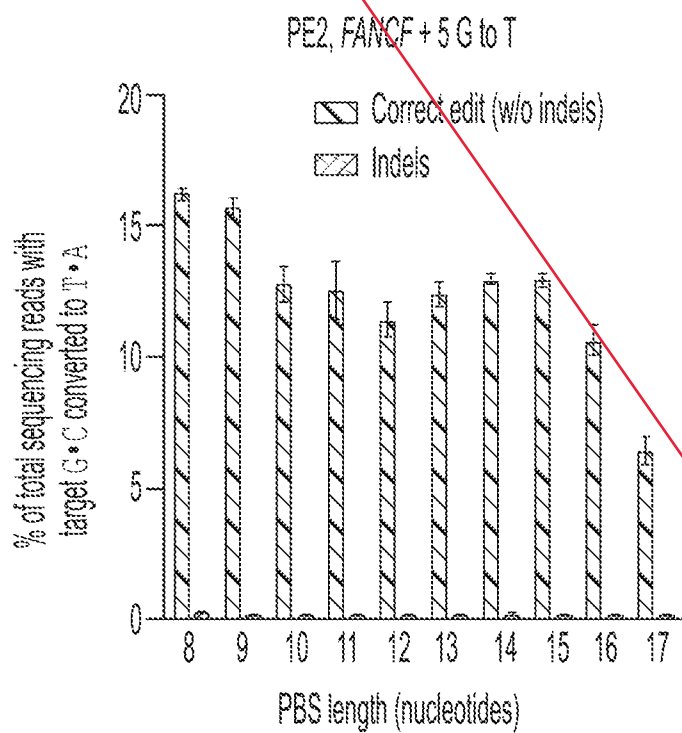


FIG. 47Q

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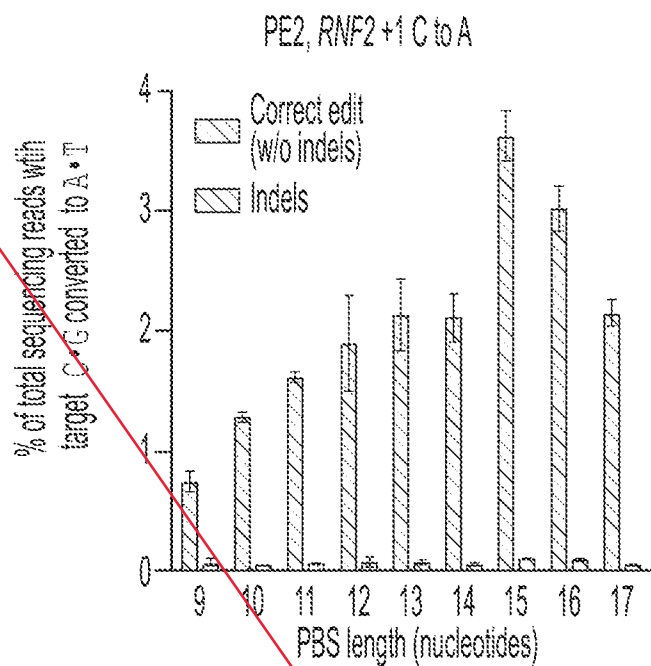


FIG. 47R

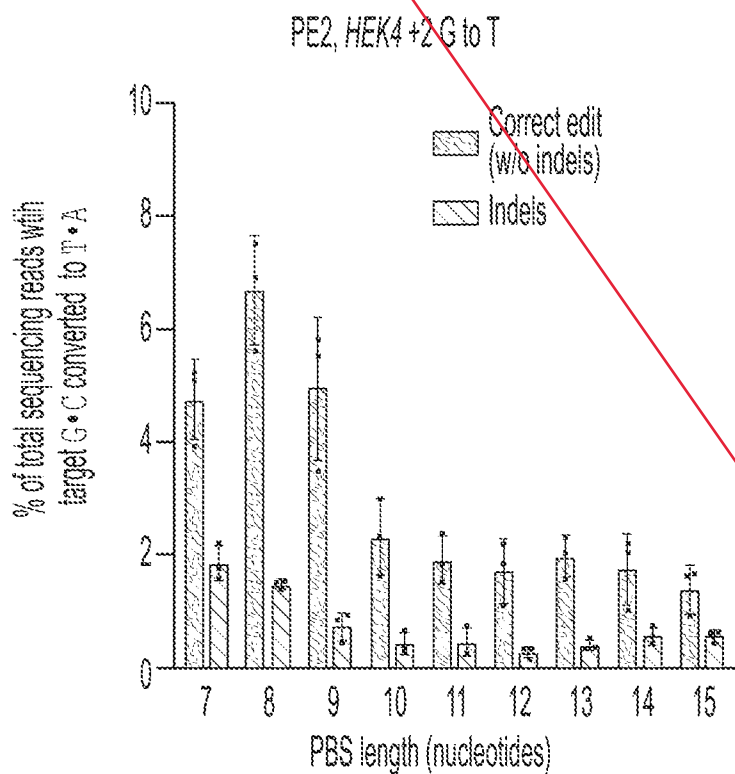


FIG. 47S

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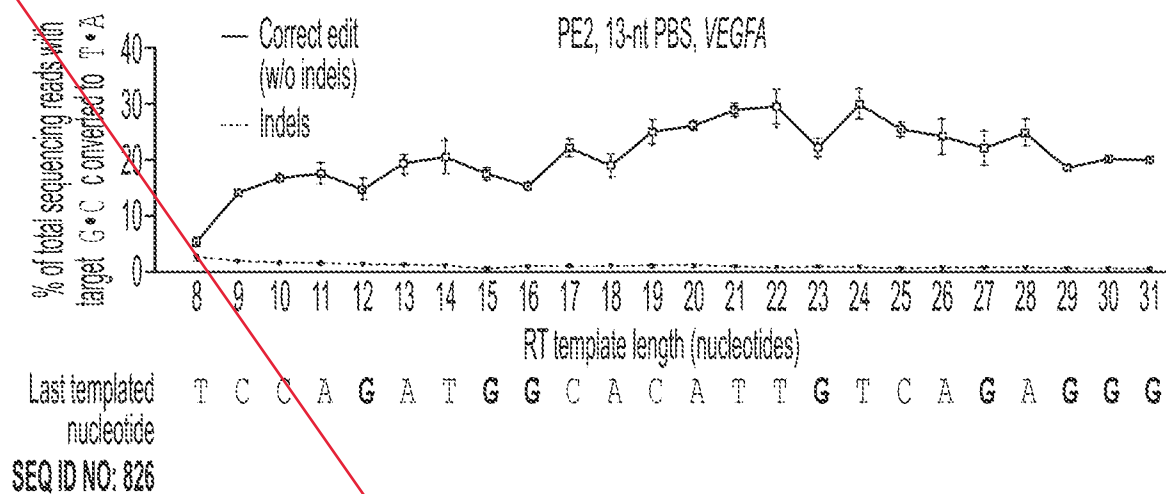


FIG. 48A

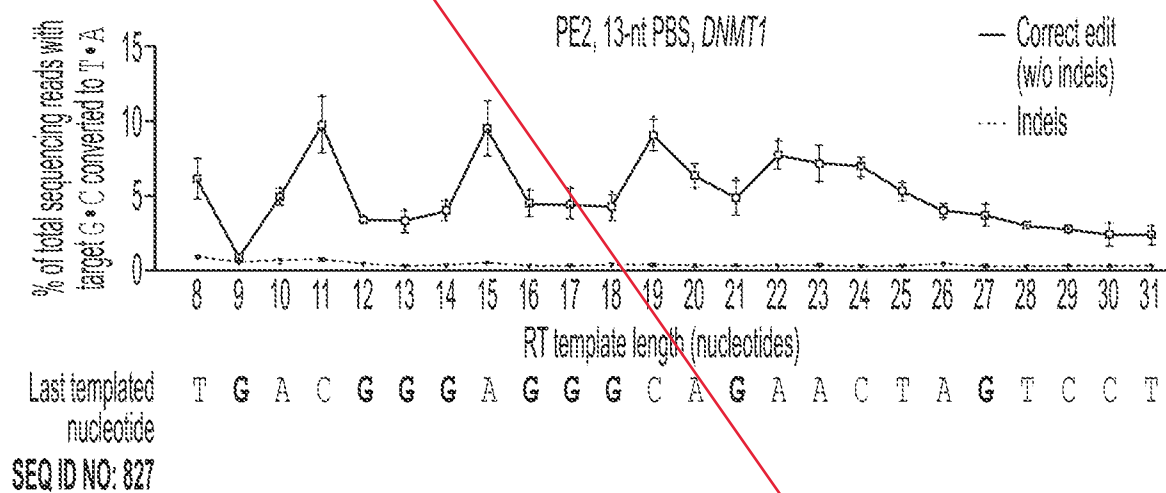


FIG. 48B

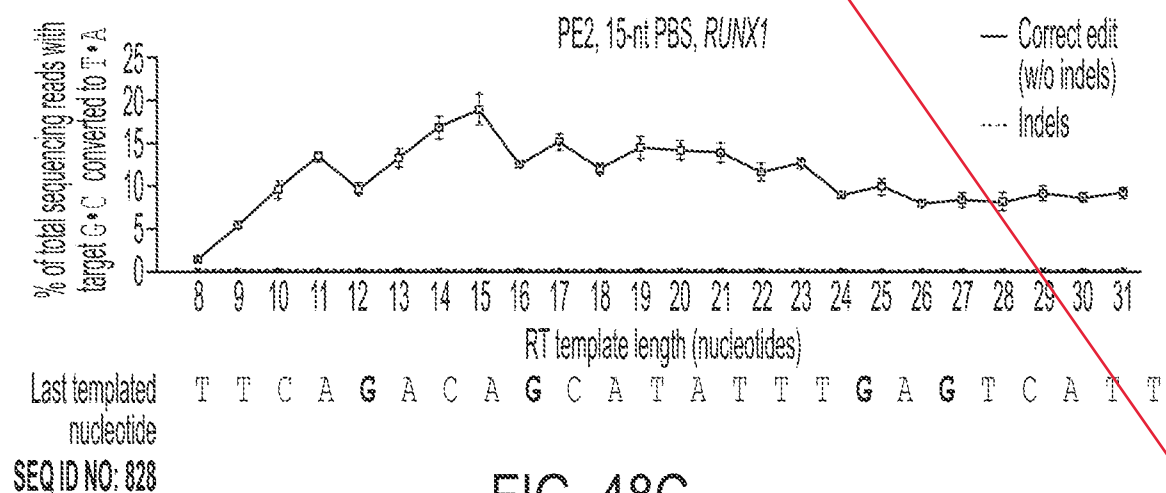


FIG. 48C

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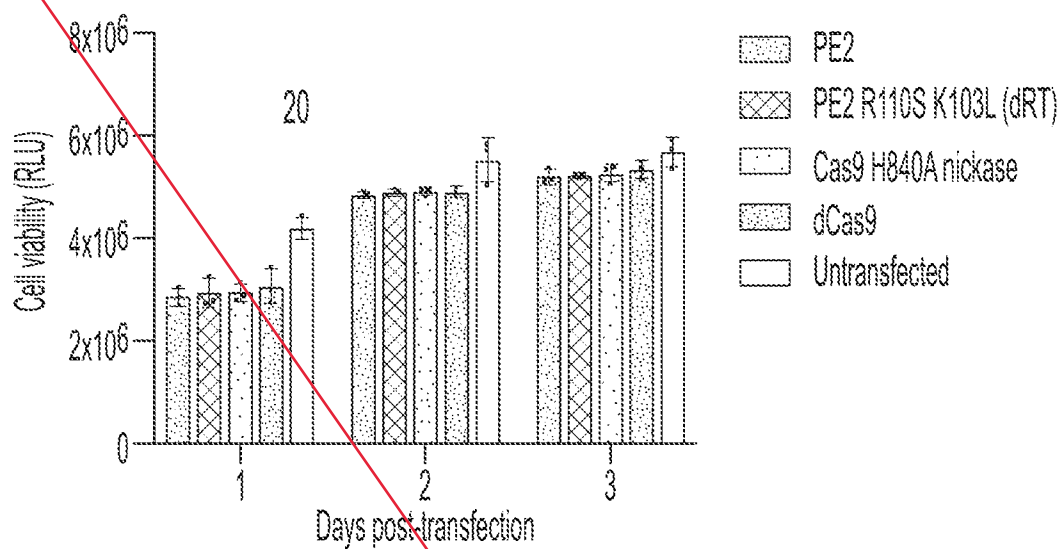


FIG. 49A

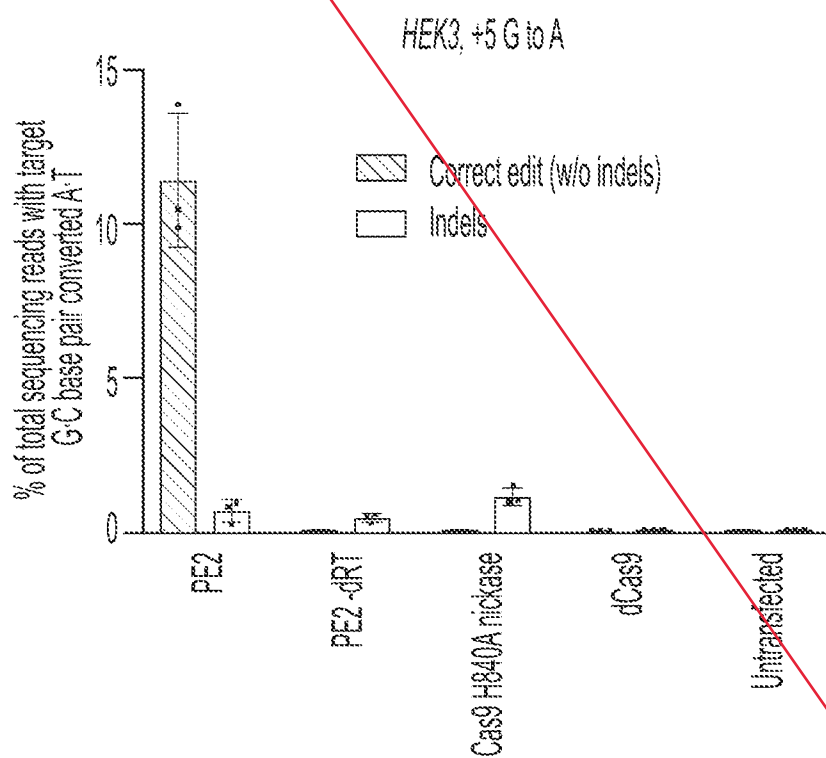


FIG. 49B

Correction of HBB EV in HEK293T cells with PE3

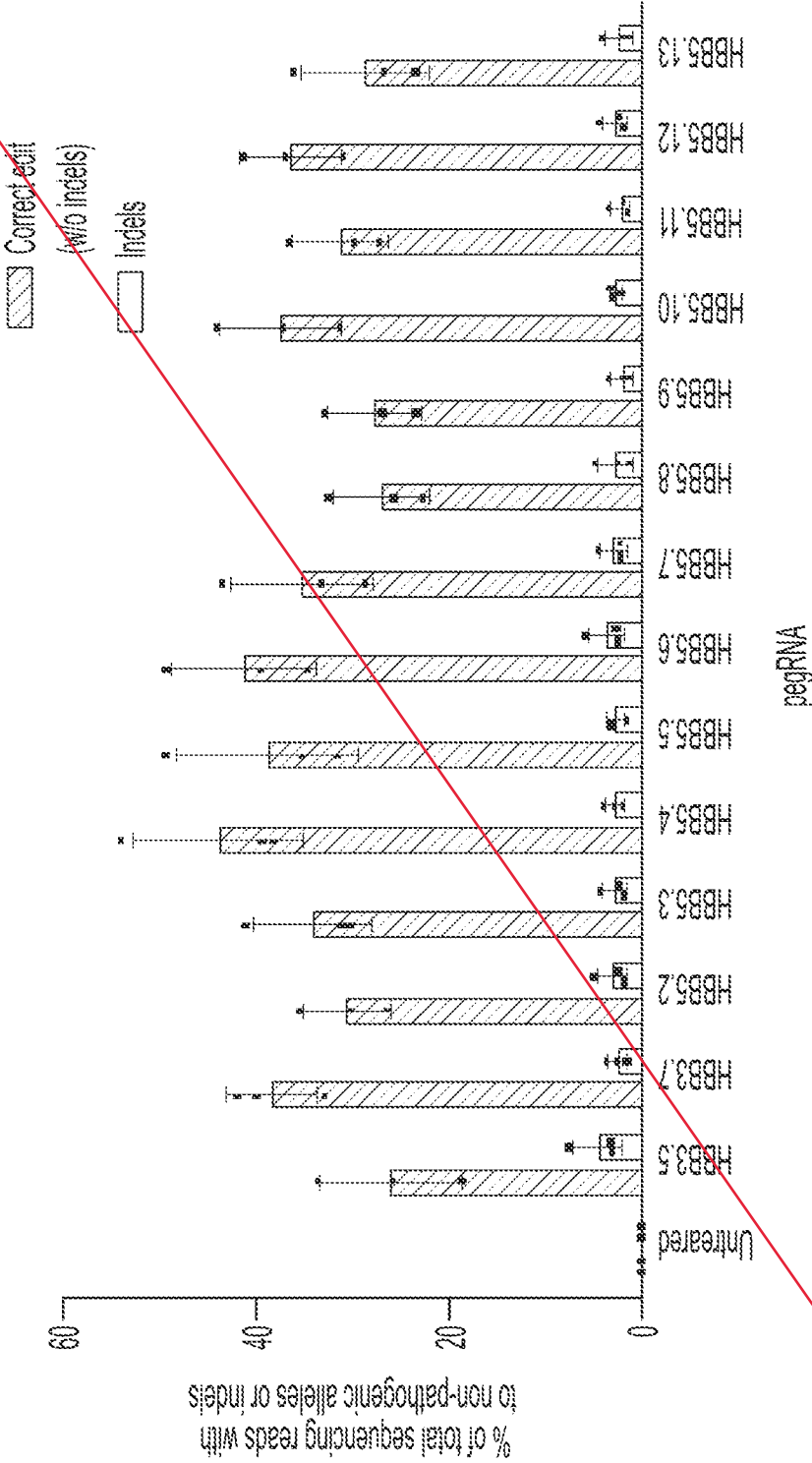


FIG. 50A

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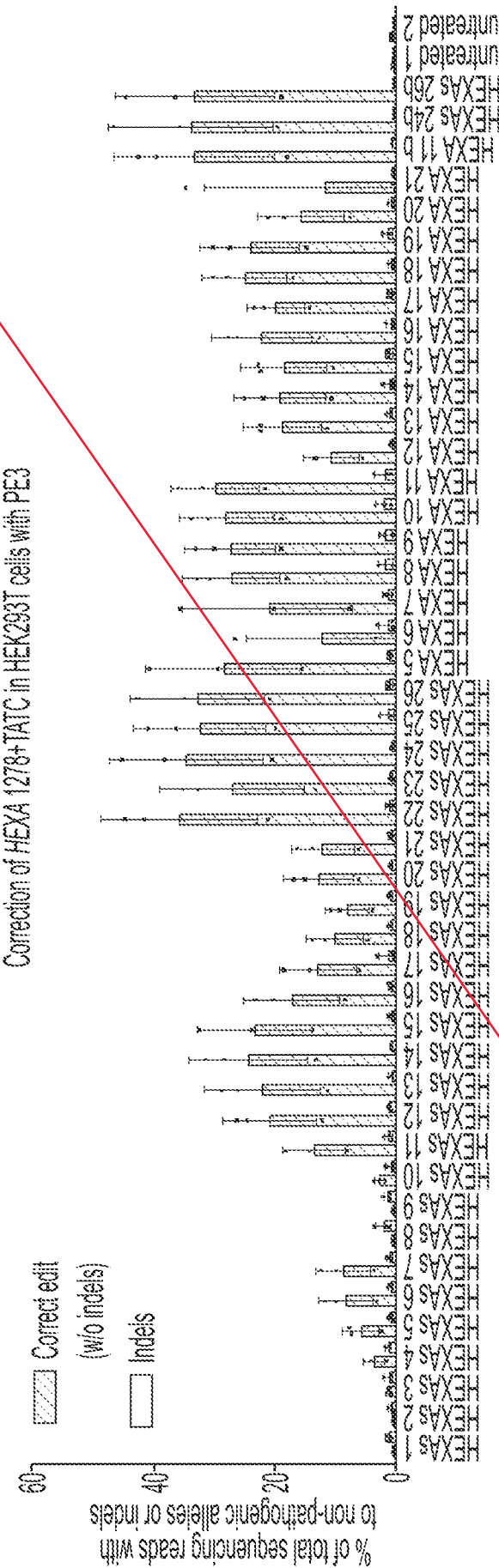


FIG. 50B

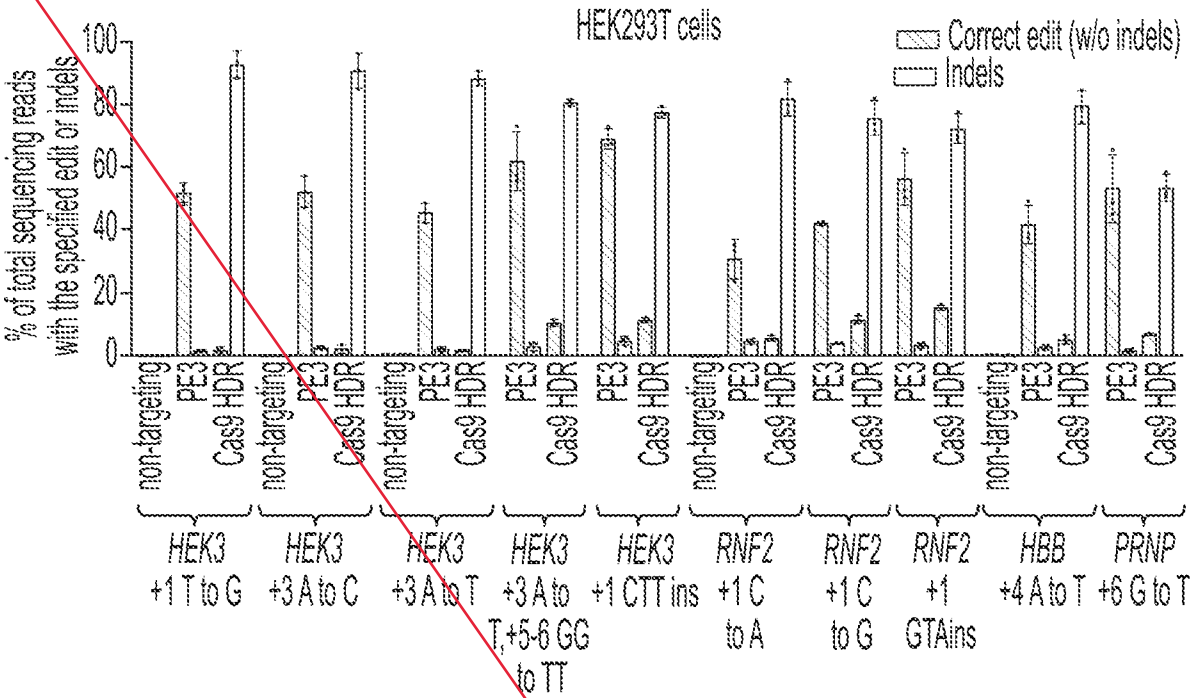


FIG. 51A

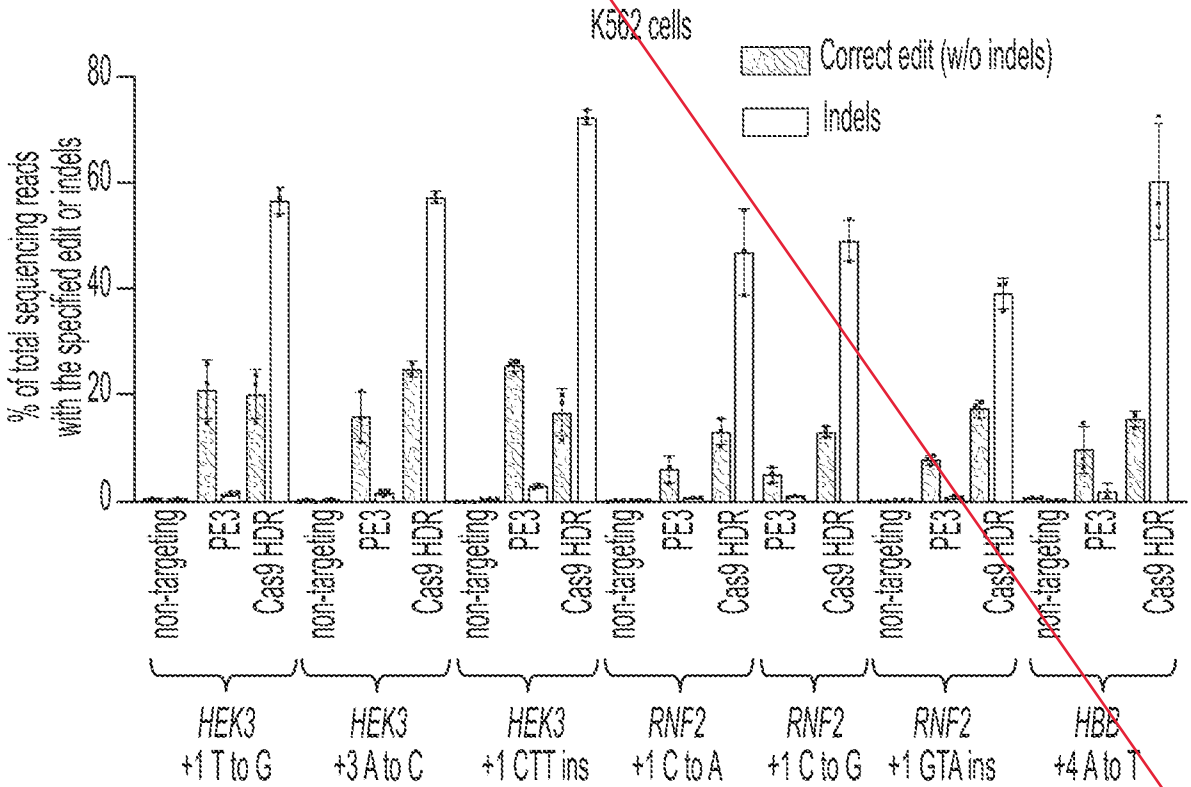


FIG. 51B

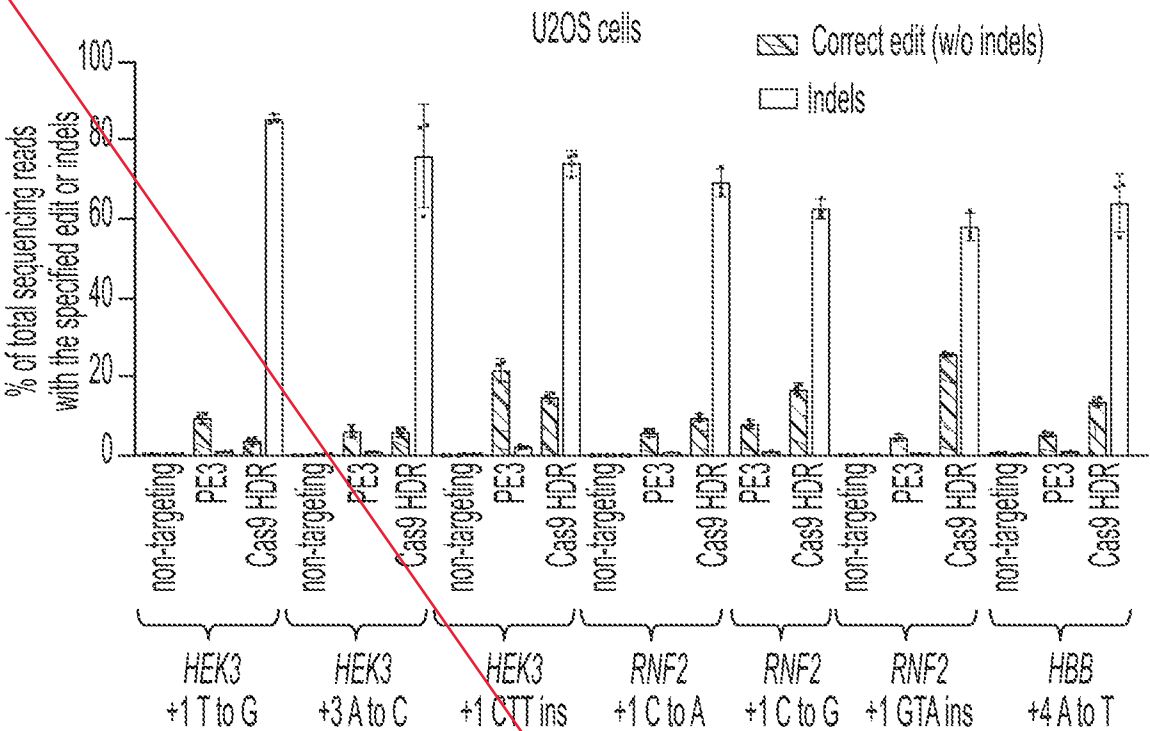


FIG. 51C

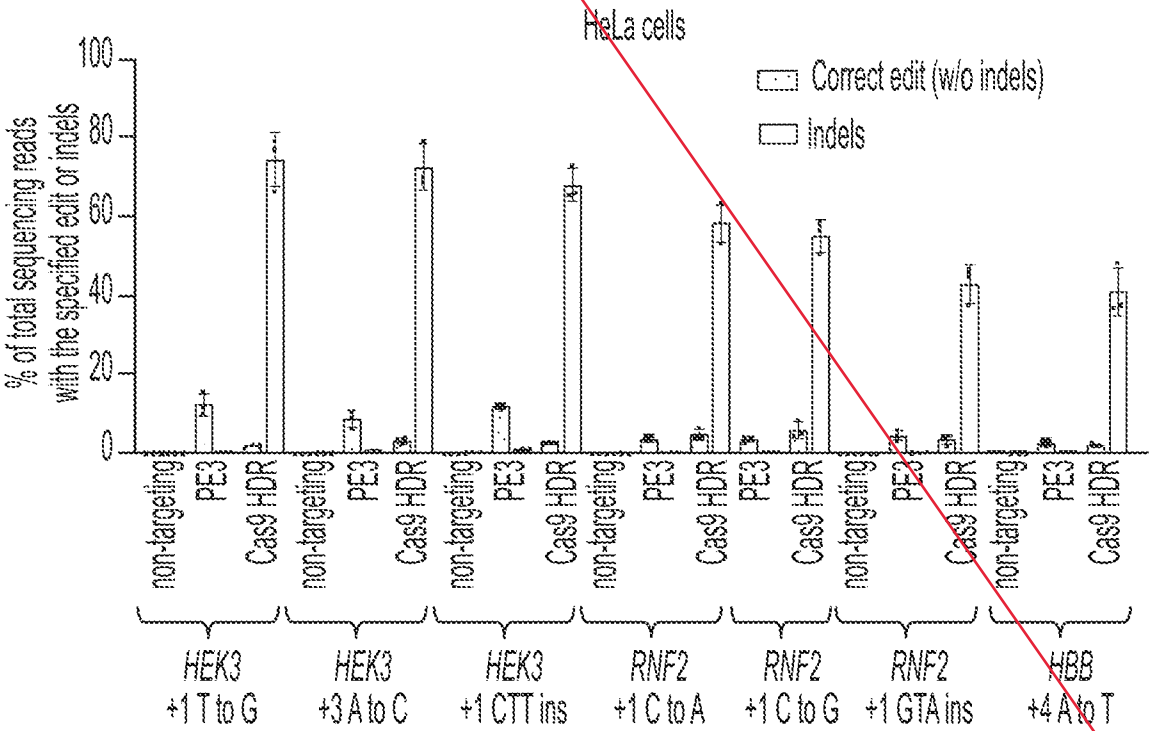


FIG. 51D

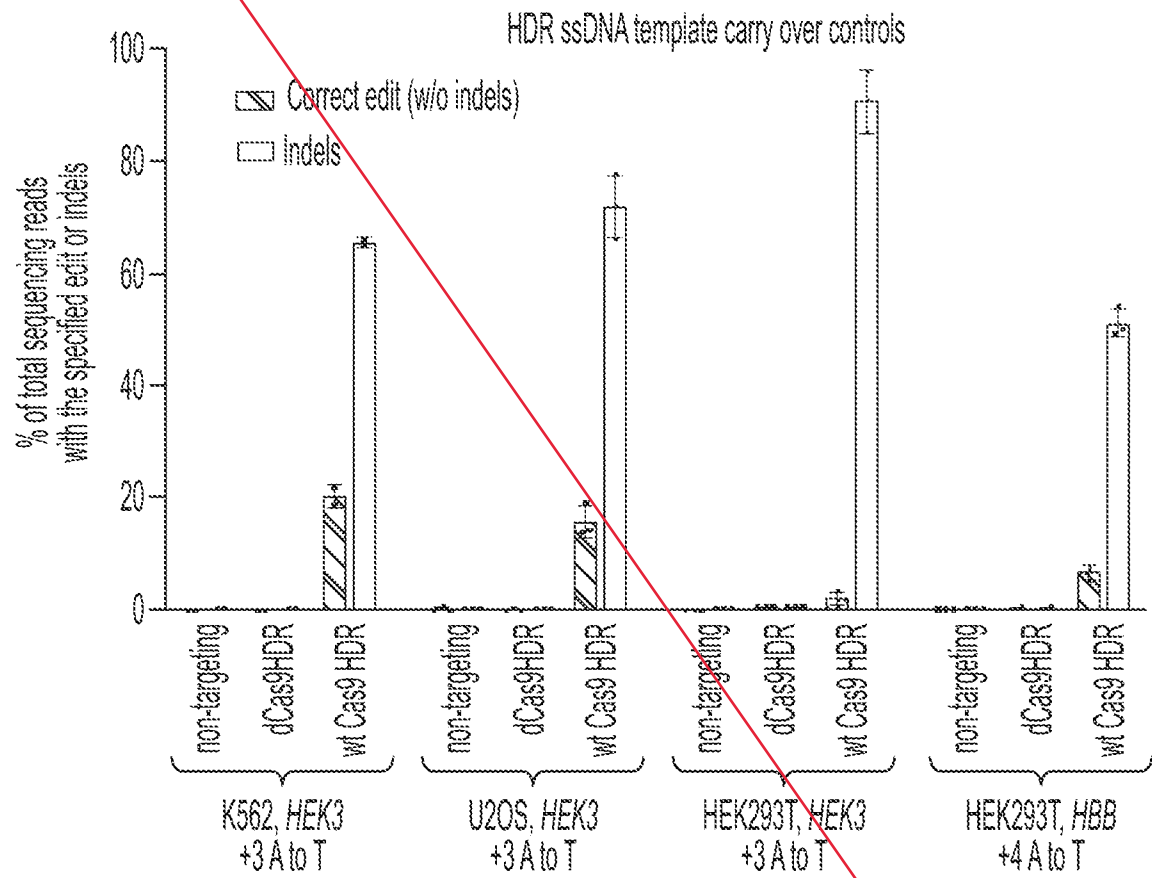


FIG. 51E

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SEQ ID NO:

791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -Reference

K562 HEK3 negative control (non-targeting pegRNA)

791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -93.54% (47190 reads)

791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -0.47% (238 reads)

829 TGGGGCCCAGACTGAGCACGTGATGGCAGCGGAAAGGAAG -0.21% (108 reads)

K562 HEK3 PE3+1 CTT insertion

791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -69.66% (39061 reads)

830 TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGGAAG -22.80% (12784 reads)

791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -0.32% (179 reads)

830 TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGGAAG -0.21% (120 reads)

831 TGGGGCCCAGACTGAGCACGCTTTTATGGCAGAGGAAAGGAAG -0.21% (116 reads)

K562 HEK3 Cas9-initiated HDR +1 CTT insertion

832 TGGGGCCCAGACTGAGCAC-TGATGGCAGAGGAAAGGAAG -17.15% (7256 reads)

791 TGGGGCCCAGACTGAGCACGTGATGGCAGAGGAAAGGAAG -13.86% (5864 reads)

830 TGGGGCCCAGACTGAGCACGCTTTGATGGCAGAGGAAAGGAAG -10.55% (4461 reads)

833 TGGGGCCCAGACTGAGCACA--TGATGGCAGAGGAAAGGAAG -9.72% (4111 reads)

834 TGGGGCCCAGACTGAGCACG--ATGGCAGAGGAAAGGAAG -5.14% (2174 reads)

835 TGGGGCCCAGACTGAGCACGCTGATGGCAGAGGAAAGGAAG -4.77% (2016 reads)

836 TGGGGCCCAGACTGAGCACG-GATGGCAGAGGAAAGGAAG -3.82% (1616 reads)

837 TGGGGCCCAGACTGAGCACGTTGATGGCAGAGGAAAGGAAG -3.17% (1342 reads)

838 TGGGGCCCAGACTGA-----TGATGGCAGAGGAAAGGAAG -1.92% (814 reads)

839 TGGGGCCCAGACTGAGCACGATGATGGCAGAGGAAAGGAAG -1.63% (689 reads)

840 TGGGGCCCAGACTGAGCACG-----CAGAGGAAAGGAAG -1.26% (535 reads)

841 TGGGGC-----TGATGGCAGAGGAAAGGAAG -0.78% (329 reads)

842 TGGGGCCCAGACTGAGCA-----GAGGAAAGGAAG -0.61% (257 reads)

843 TGGGGCCCAGACTGAGCACGCTGATGGCAGAGGAAAGGAAG -0.59% (250 reads)

844 TGGGGCC-----TGATGGCAGAGGAAAGGAAG -0.56% (237 reads)

FIG. 51F

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845	TGGGG	-----	TGATGGCAGAGGAAAGGAAG	-0.52% (219 reads)
846	TGGGGCCCA	GACTGAGCACG	-----AGGAAAGGAAG	-0.47% (197 reads)
847	TGGGGCCCA	GACTGAGCACG	-----AGAGGAAAGGAAG	-0.43 (181 reads)
848	TGGGGCCCA	GACTGAGCACG	----GGCAGAGGAAAGGAAG	-0.41 (173 reads)
849	TGGGGCCCA	GACTGAG	-----GAAAGGAAG	-0.40% (169 reads)
850	TGGGGC	-----	-----AGAGGAAAGGAAG	-0.38% (159 reads)
851	TGGGGCCCA	GACTGAGCACG	-----GAAAGGAAG	-0.37% (156 reads)
852	TGGGGCCCA	GA	---TGGCAGAGGAAAGGAAG	-0.35% (148 reads)
853	TGGGGCCCA	GACTGAGCACG	-----AAAGGAAG	-0.35% (146 reads)
854	TG	-----	---ATGGCAGAGGAAAGGAAG	-0.31% (132 reads)
855	TGGGGCCCA	GACTGAGC TT	TGATGGCAGAGGAAAGGAAG	-0.30% (125 reads)
856	TGGGGCCCA	GACTGAGCACG	-----AAG	-0.30% (125 reads)
857	TGGGGCCCA	GACTGAGCACG	-----GAGGAAAGGAAG	-0.29% (122 reads)
858	TGGGGCCCA	GACTGAGCACG	-----GAAG	-0.29% (121 reads)
859	TGGGGCCCA	GA	-----GGAAAGGAAG	-0.27% (114 reads)
860	TGGGGCCCA	G	TGATGGCAGAGGAAAGGAAG	-0.26% (112 reads)
861	TGG	-----	TGATGGCAGAGGAAAGGAAG	-0.26% (112 reads)
862	TGGGGCCCA	GACTGAGCACG	TGT ATGGCAGAGGAAAGGAAG	-0.26% (110 reads)
863	-----	-----	---ATGGCAGAGGAAAGGAAG	-0.24% (100 reads)
864	TGGGGCCCA	GACTG	-----TGATGGCAGAGGAAAGGAAG	-0.23% (96 reads)
865	TGGGGCCCA	GACTGAG	---TGATGGCAGAGGAAAGGAAG	-0.23% (96 reads)
866	TGGGGCCC	-----	TGATGGCAGAGGAAAGGAAG	-0.22% (93 reads)
867	TGGGGCCCA	GACTGAGCACG	----GCAGAGGAAAGGAAG	-0.21% (87 reads)

bold Substitutions

□ Insertions

- Deletions

---- Predicted

cleavage position

FIG. 51G

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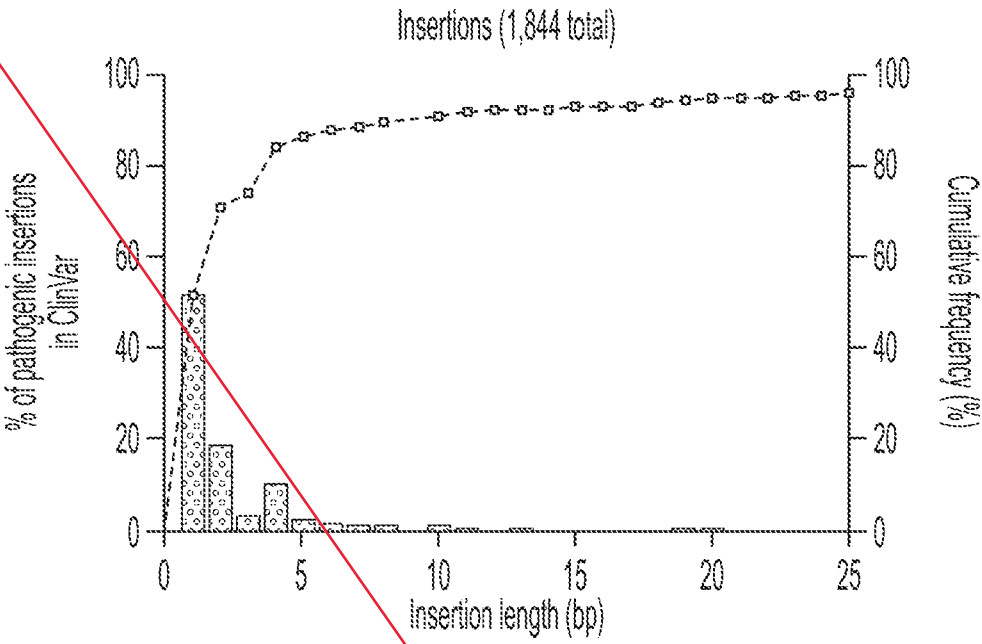


FIG. 52A

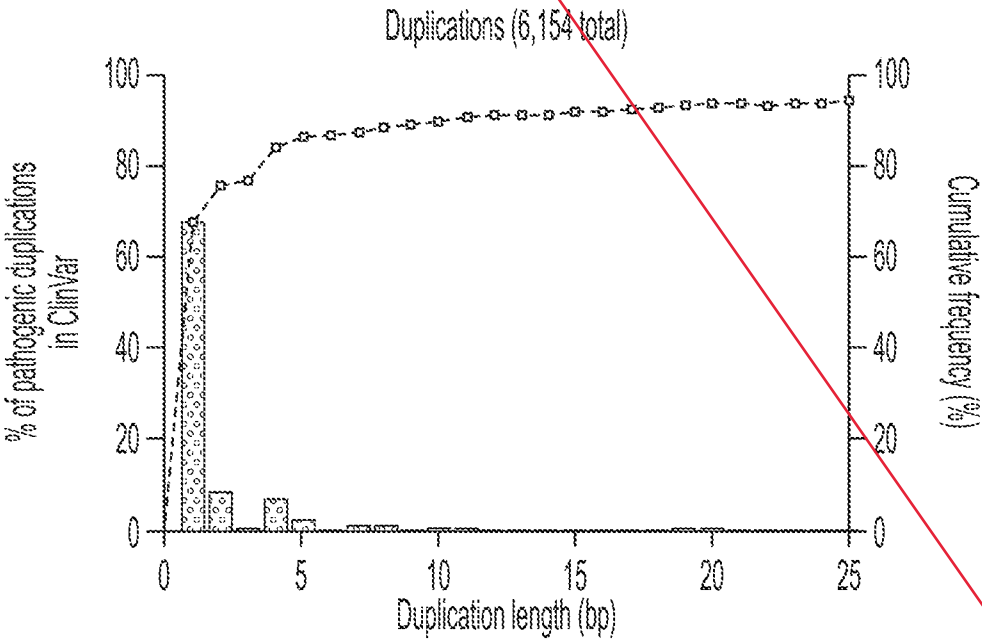


FIG. 52B

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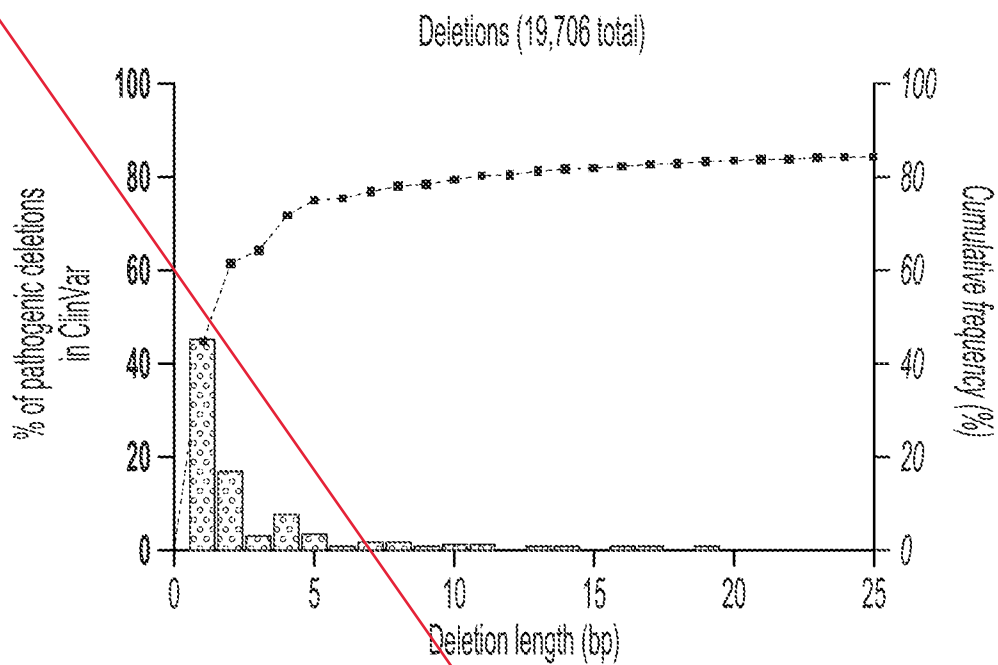


FIG. 52C

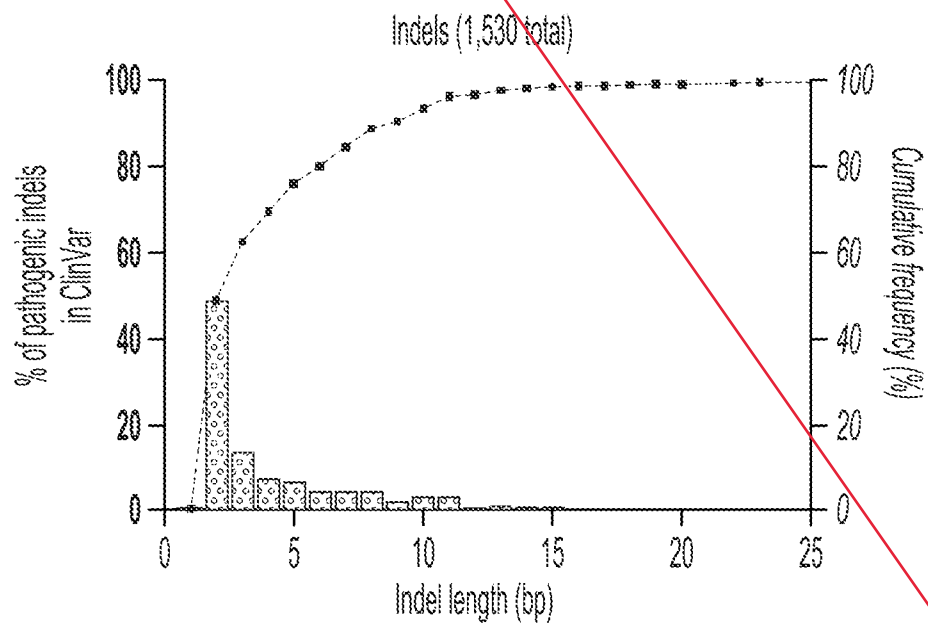
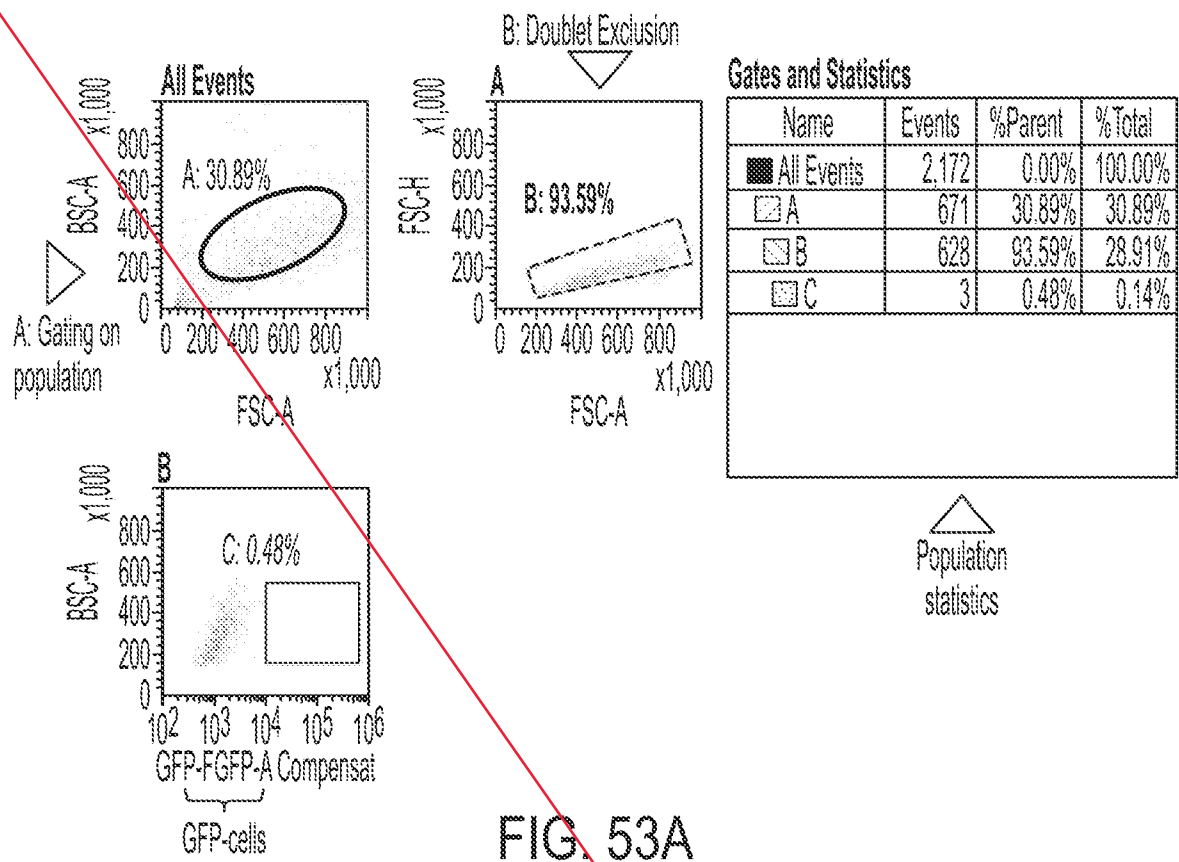
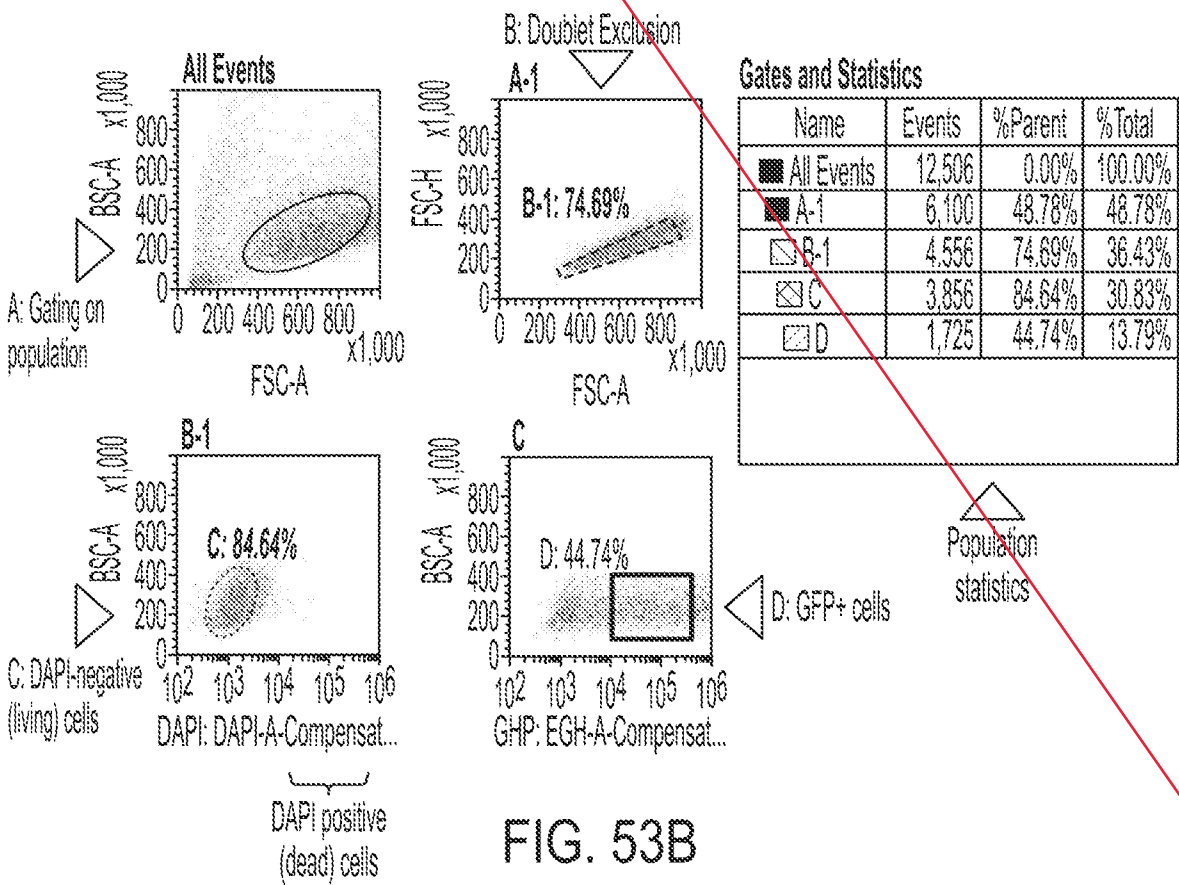


FIG. 52D

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SEQ ID NO: Line 1

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	T	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	T	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	C	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	A	T	C	T	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	T	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	T	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	C	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	A	T	C	T	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	A	T	C	T	G	C	C	T	G	A	C	T	G	G	A	A	G	G	C	T	T	T	C	-0.23% (47 reads)

bold Substitutions

 Insertions

- Deletions

---- Predicted cleavage position

FIG. 53C

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Line 1	
SEQ ID NO.	
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-Reference
876	CACCATGGGTGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-97.45% (87424 reads)
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-0.27% (240 reads)
877	CACCATGGCGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-0.24% (212 reads)
Line 2	
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-Reference
876	CACCATGGGTGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-97.16% (63793 reads)
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-0.36% (239 reads)
877	CACCATGGCGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-0.22% (142 reads)
Line 3	
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-Reference
876	CACCATGGGTGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-97.32% (77514 reads)
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-0.29% (228 reads)
877	CACCATGGCGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-0.22% (172 reads)
878	CACCATGGGTGCACCTGACTCCTGTGGAGAAAGTCTGTCTGTT-0.21% (167 reads)
Line 4	
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-Reference
876	CACCATGGGTGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-96.94% (70061 reads)
875	CACCATGGGTGCACCTGACTCCTGAGGAGAAAGTCTGCCGTT-0.58% (419 reads)
877	CACCATGGCGCACCTGACTCCTGTGGAGAAAGTCTGCCGTT-0.23% (165 reads)
878	CACCATGGGTGCACCTGACTCCTGTGGAGAAAGTCTGTCTGTT-0.21% (150 reads)

FIG. 53D

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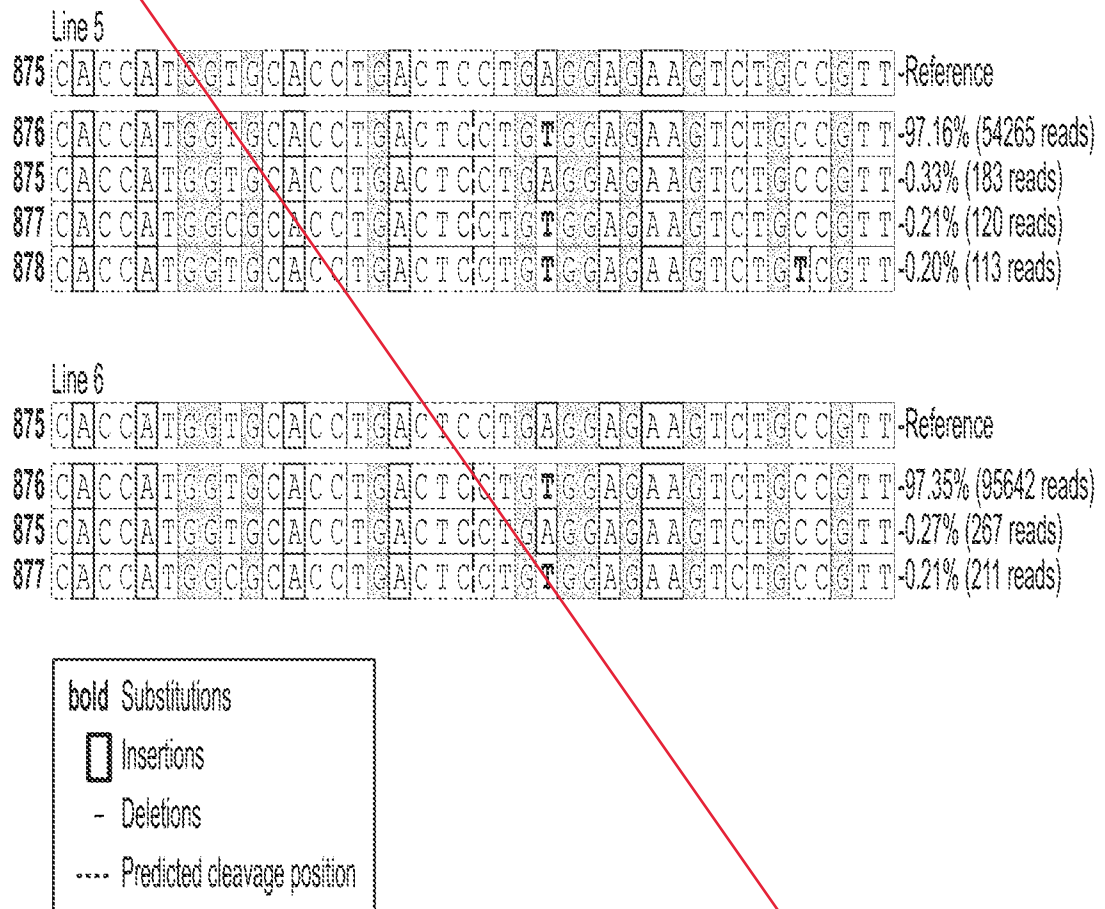
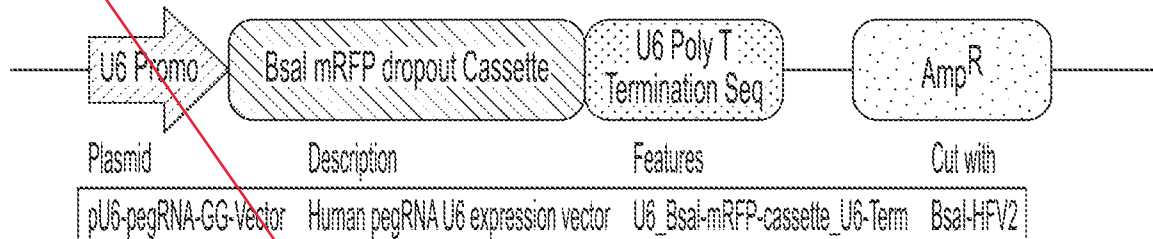


FIG. 53E

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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' - CACCNNNNNNNNNNNNNNNNNNCTTT - 3'

SEQ ID NO: 880 3' - NNNNNNNNNNNNNNNNNNNCAAAATCTC - 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' - AGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG - 3' SEQ ID NO: 881

SEQ ID NO: 882 3' - CATCTTTTATCGTTCAATTTTATTCGCATCAGGCAATAGTTGAACCTTTTCACCGTGGCTCAGCCACG - 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' - GTGCNNNNNNNNNNNNNNNNNNNNNNNN - 3'

SEQ ID NO: 884 3' - NNNNNNNNNNNNNNNNNNNNNNNNNNNNNAAAA - 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:

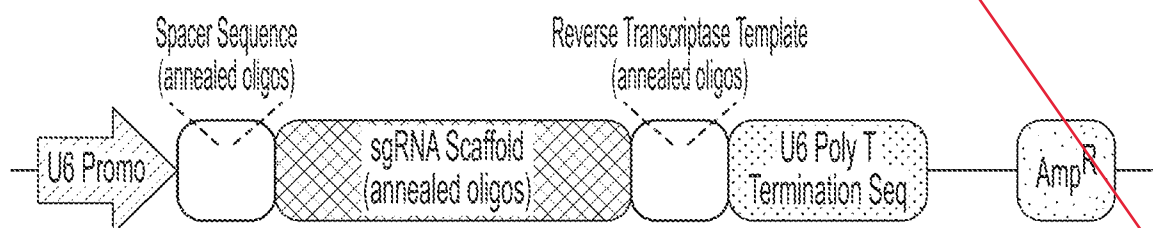


FIG. 54

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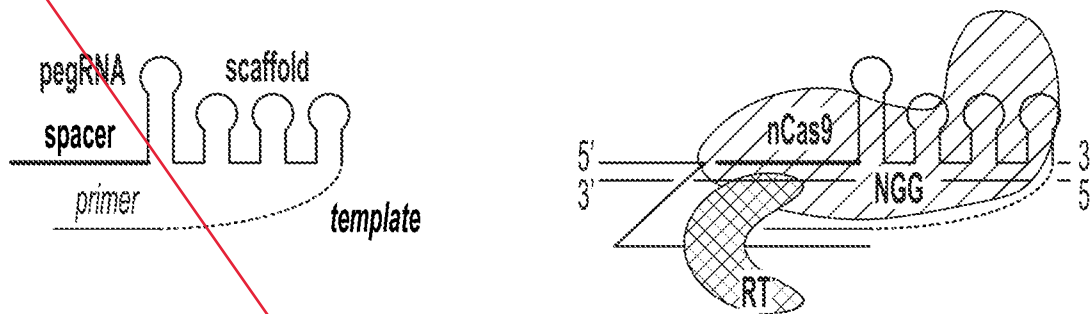


FIG. 55A

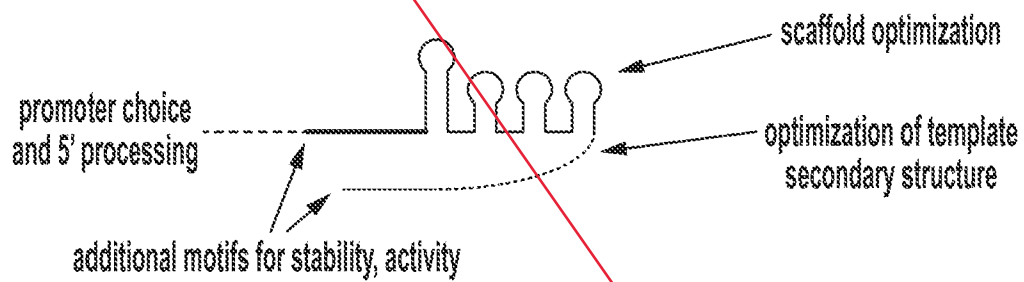


FIG. 55B

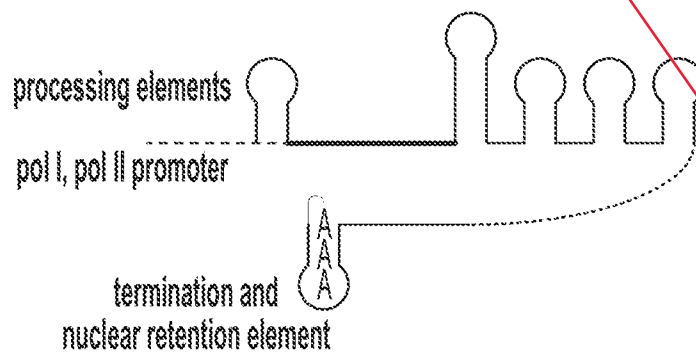


FIG. 55C

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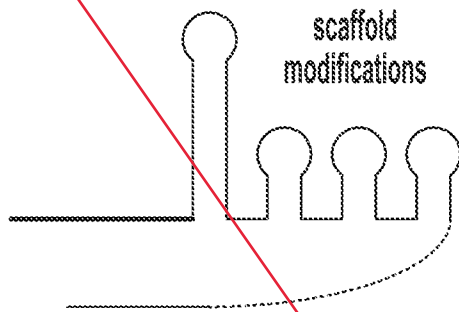


FIG. 55D

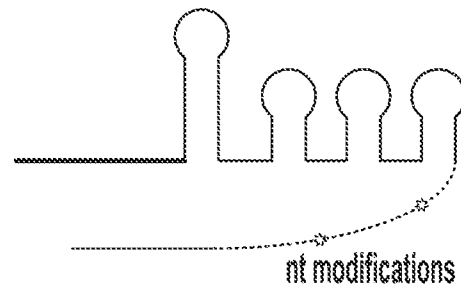


FIG. 55E

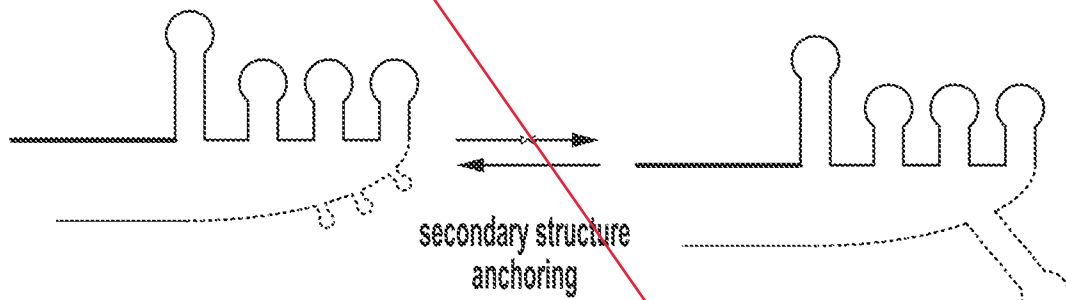


FIG. 55F

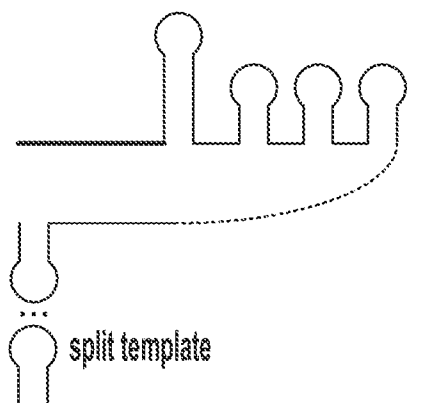
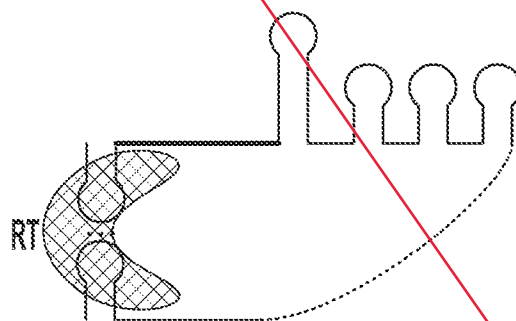
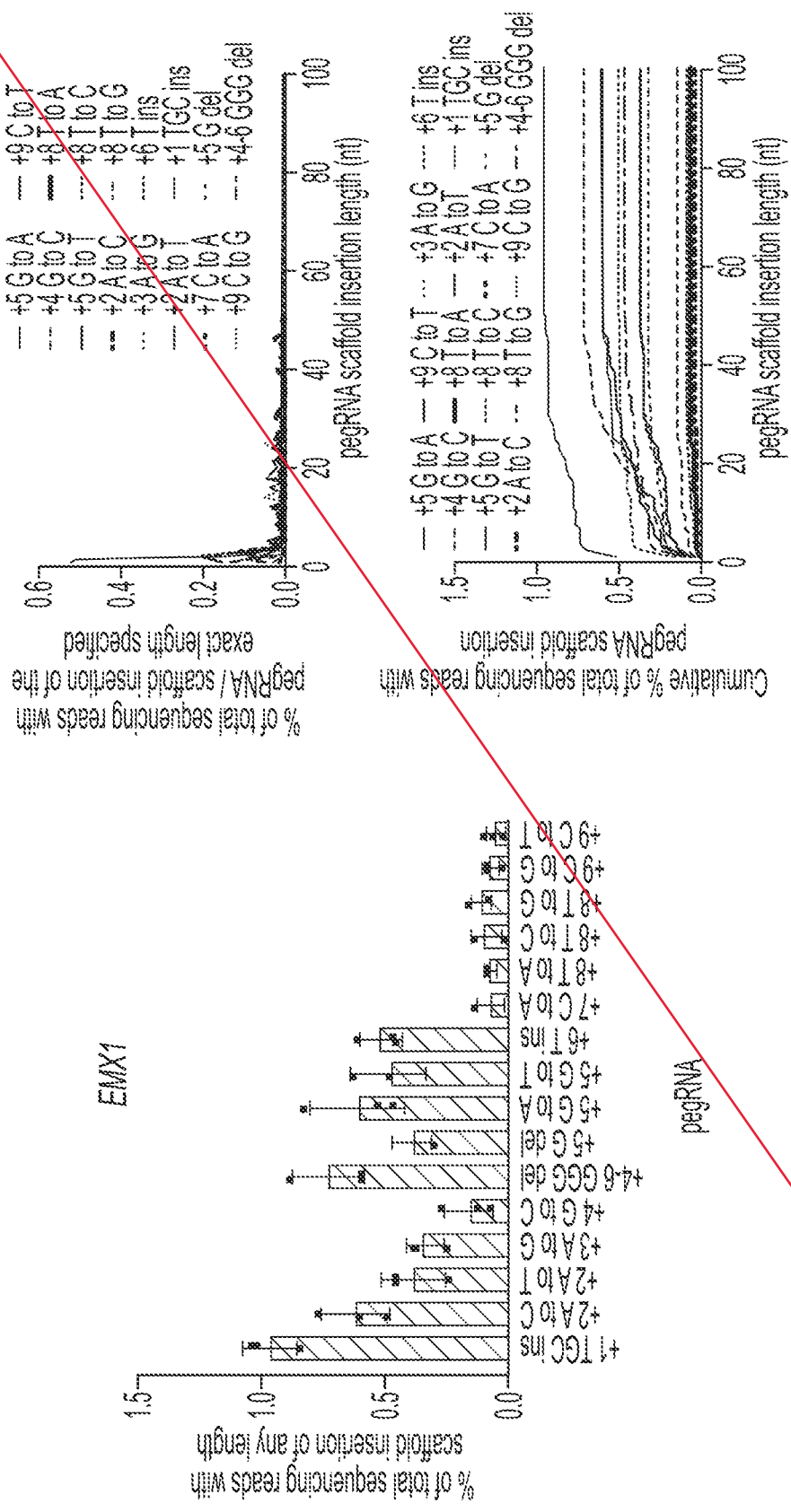
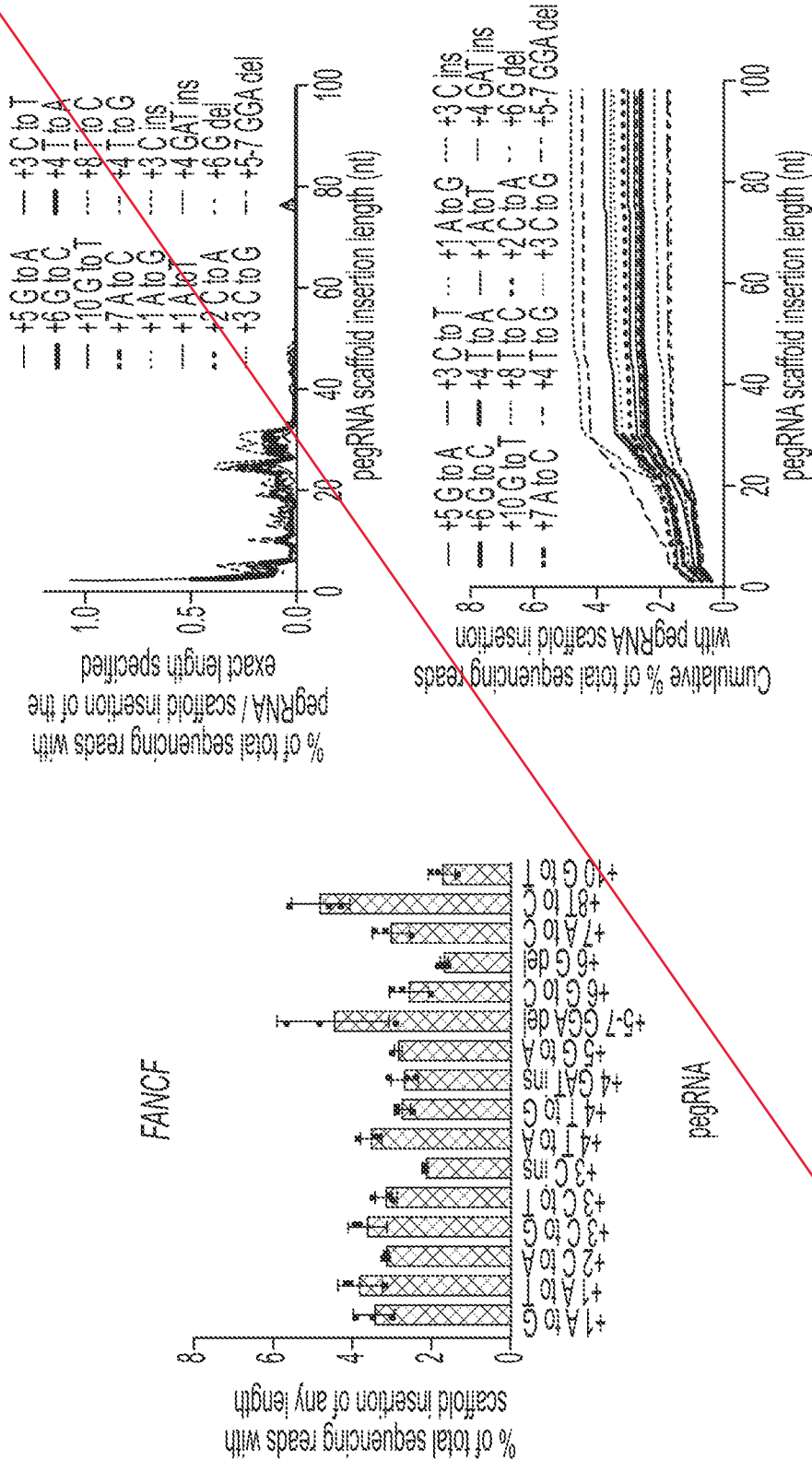


FIG. 55G





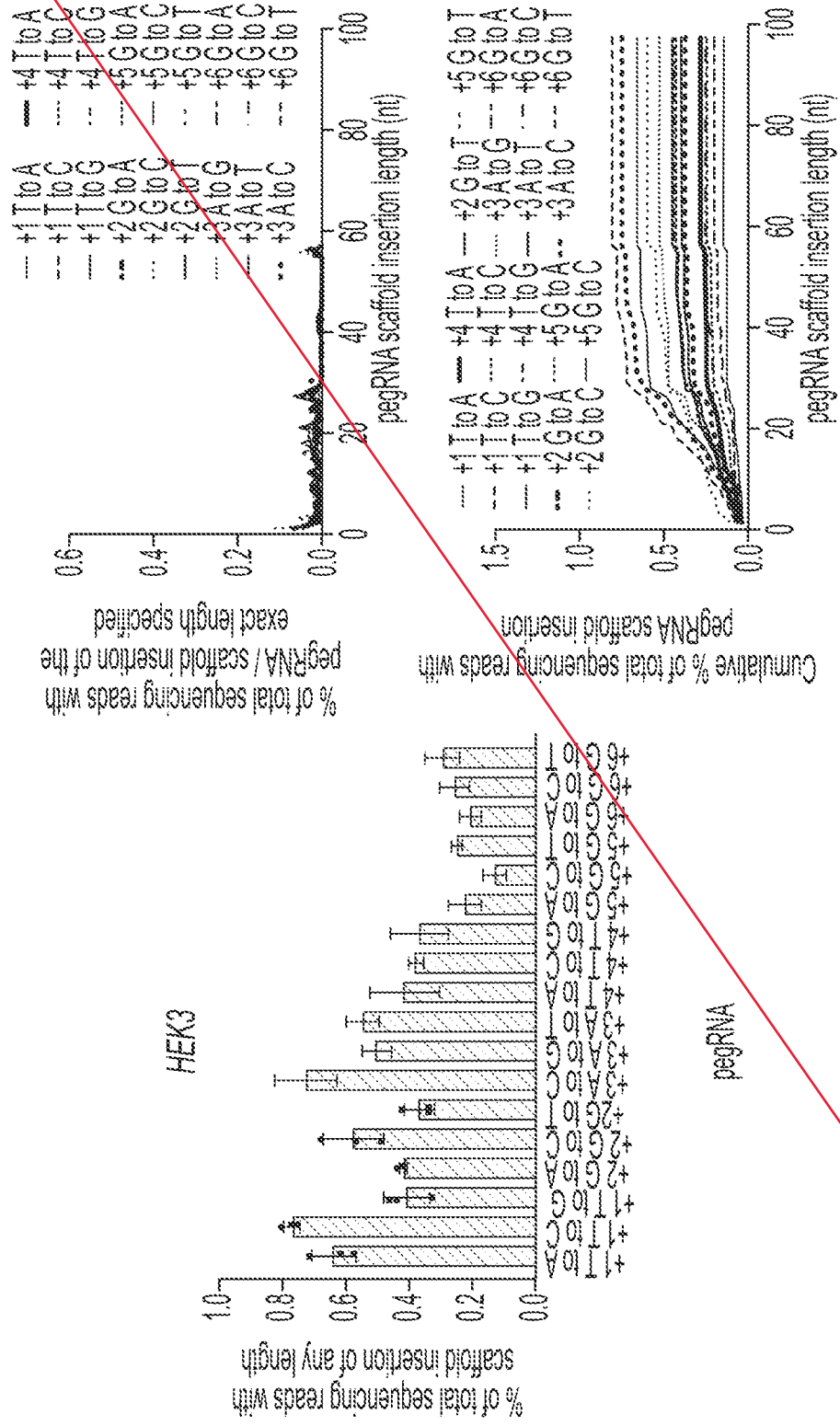


FIG. 56C

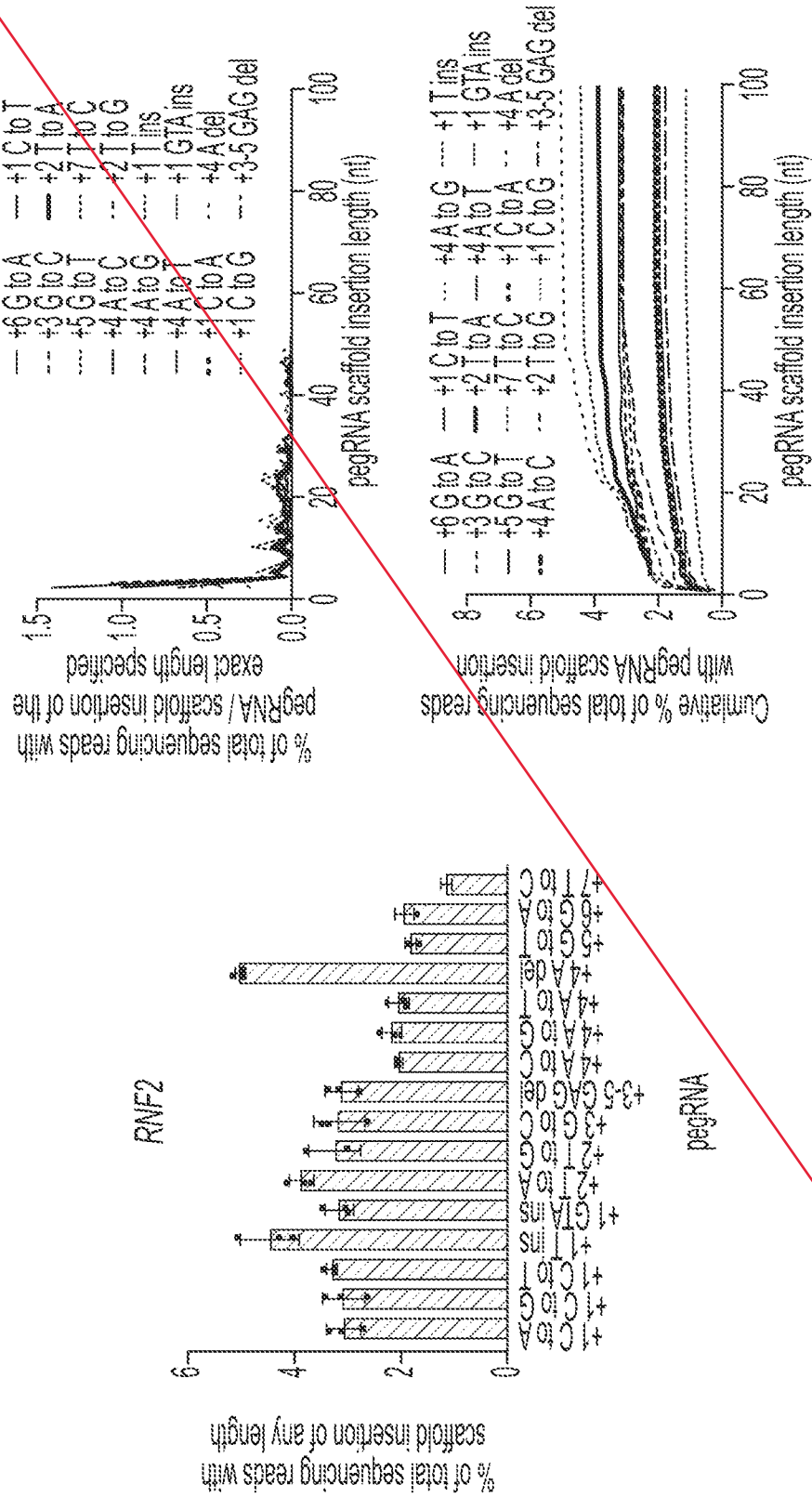


FIG. 56D

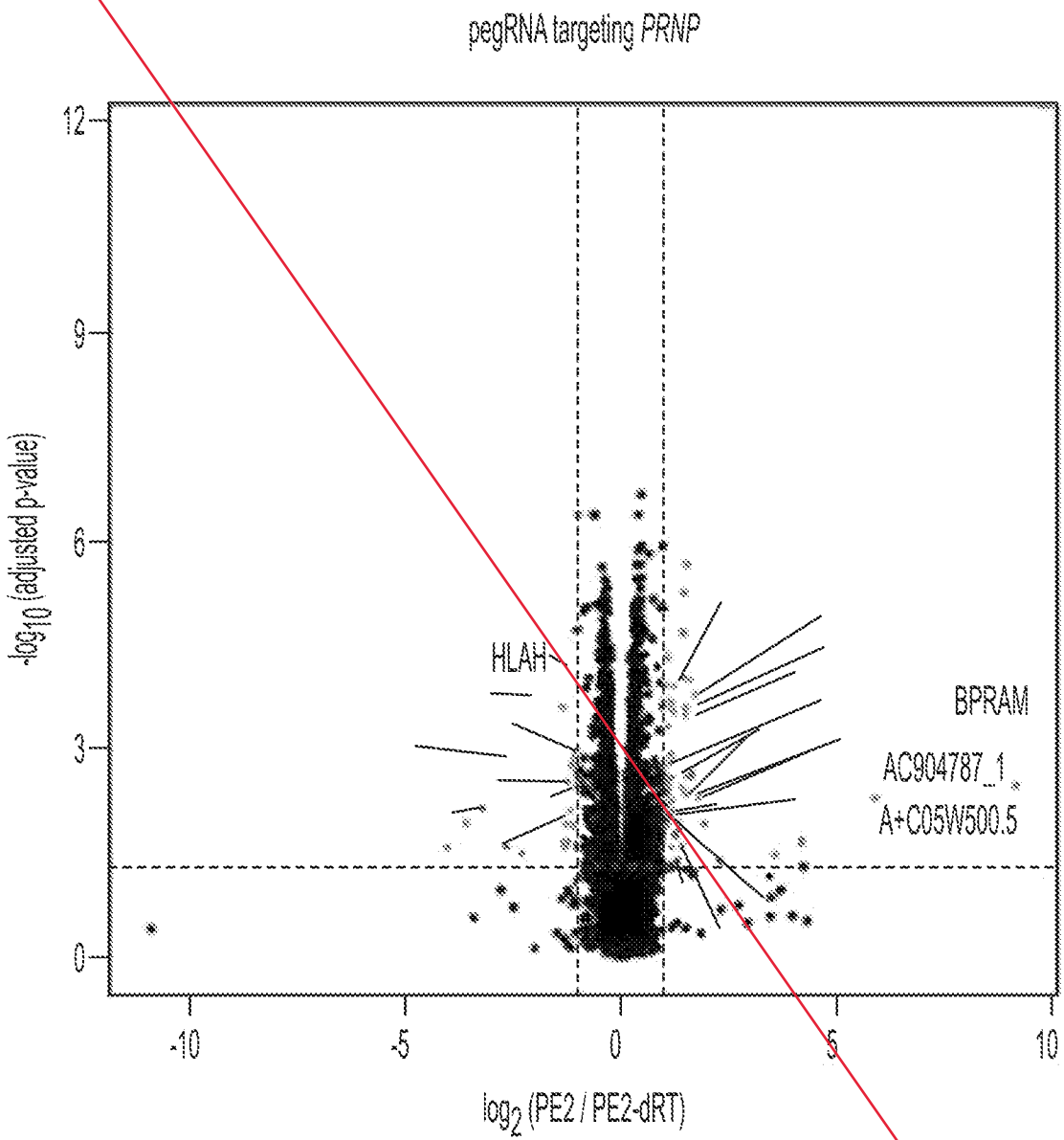


FIG. 57A

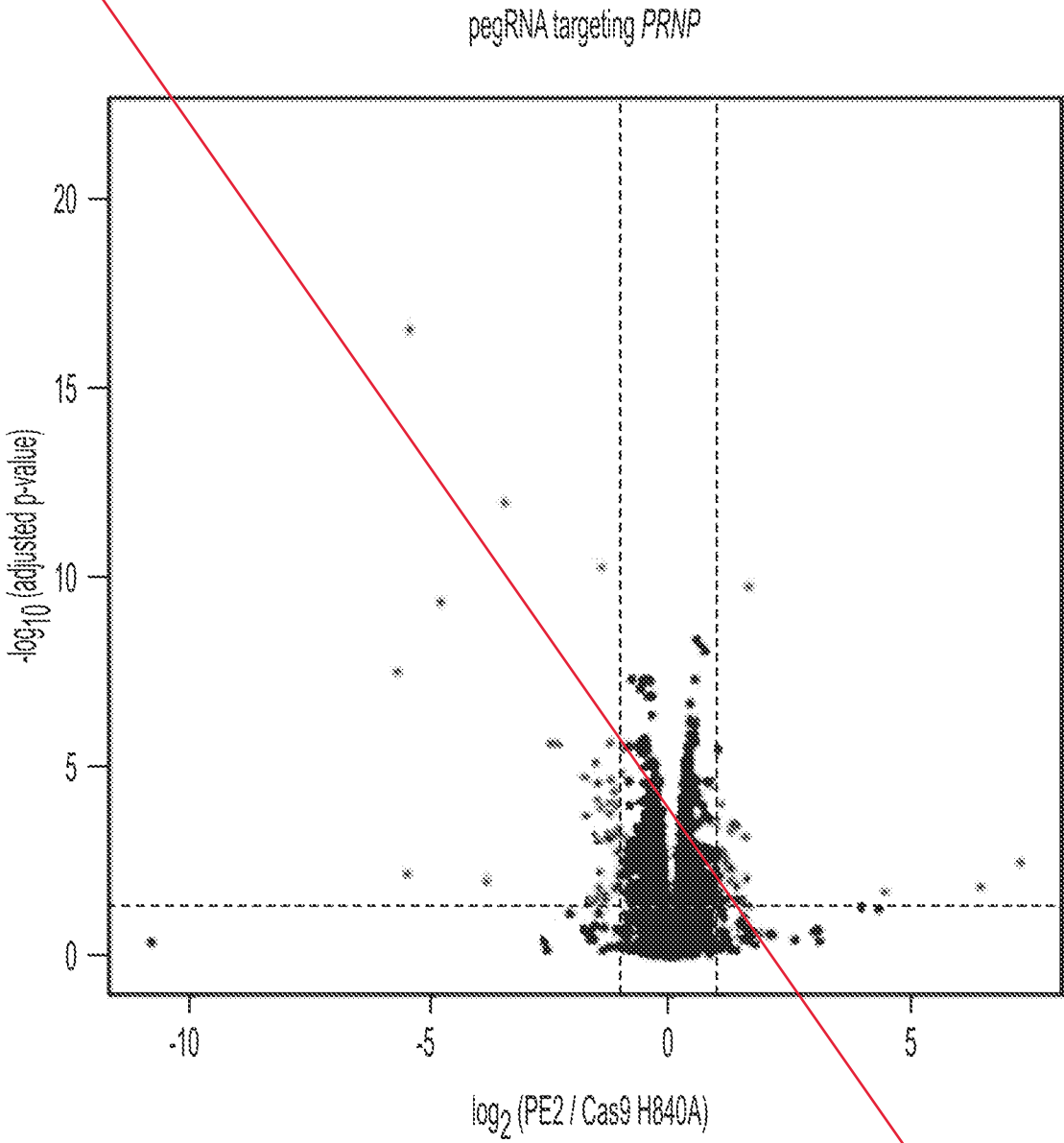
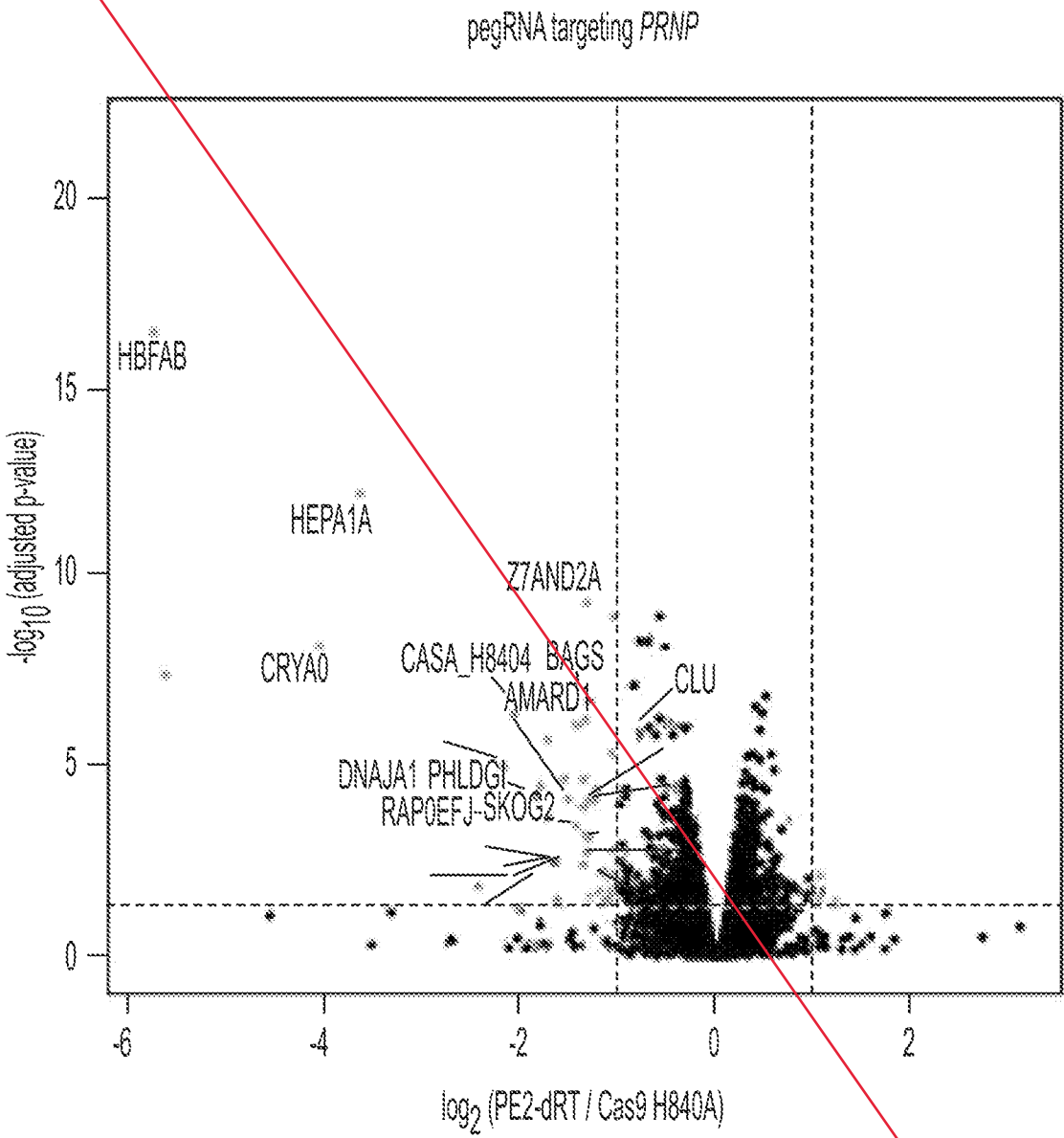


FIG. 57B



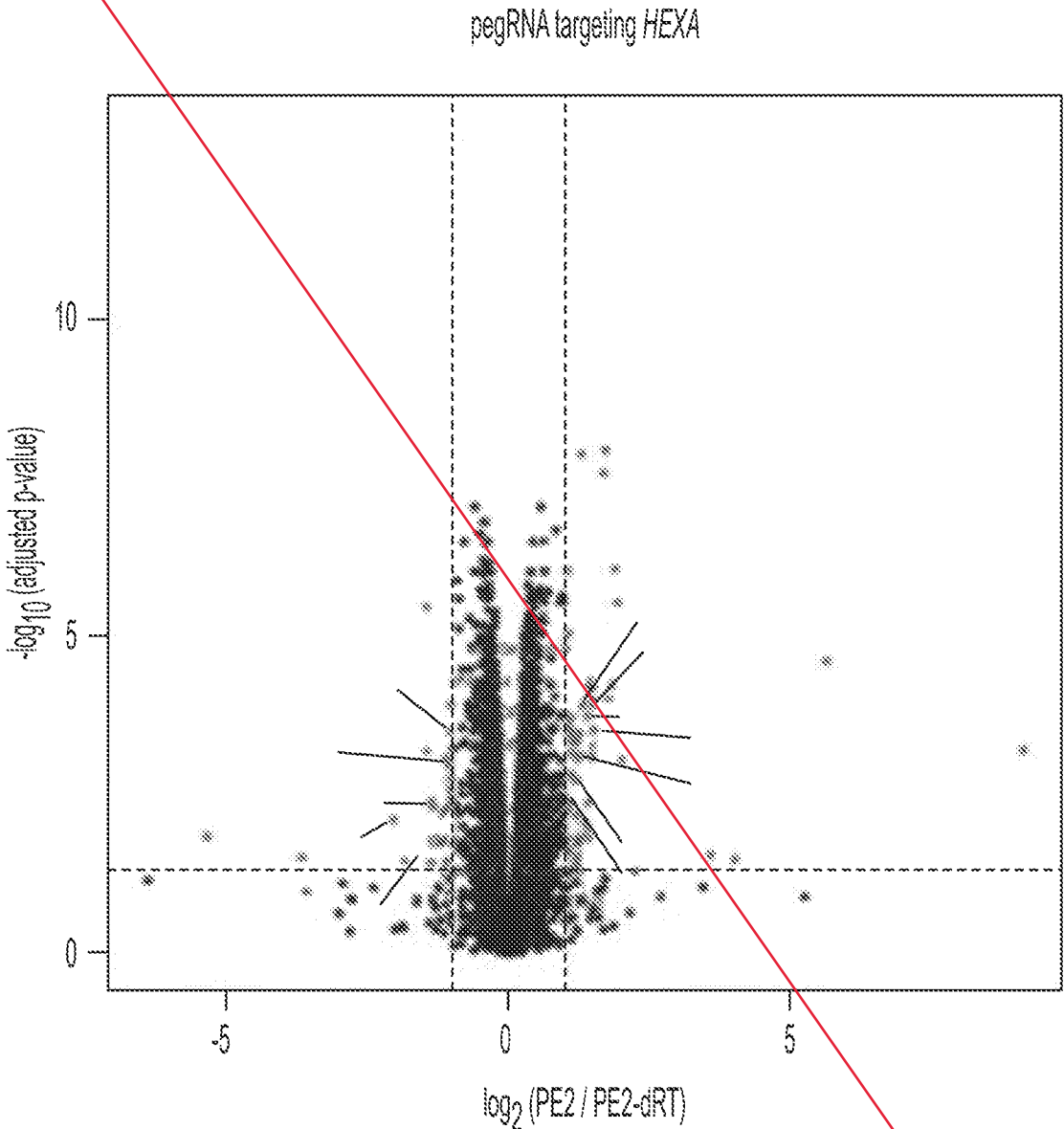


FIG. 57D

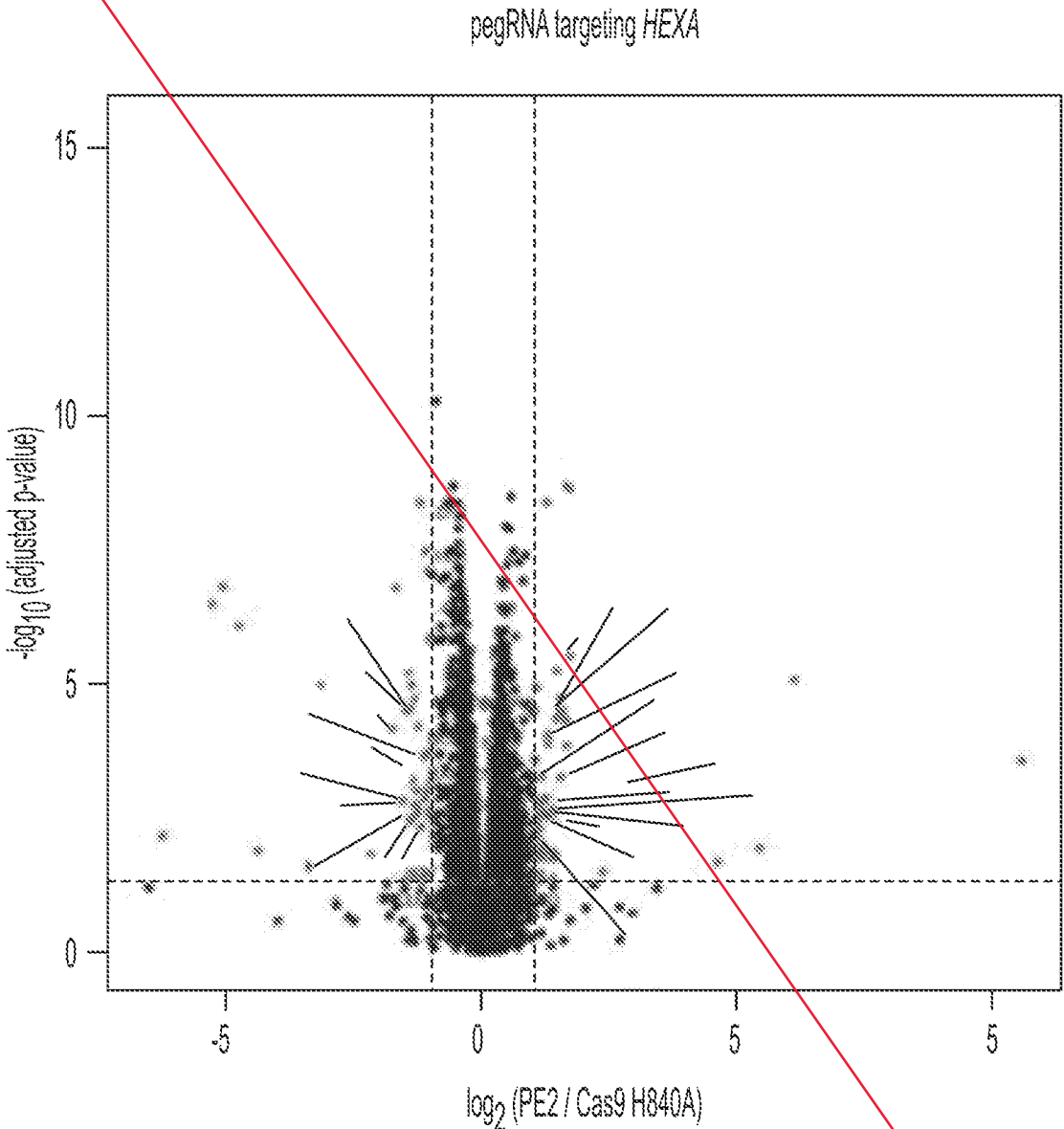


FIG. 57E

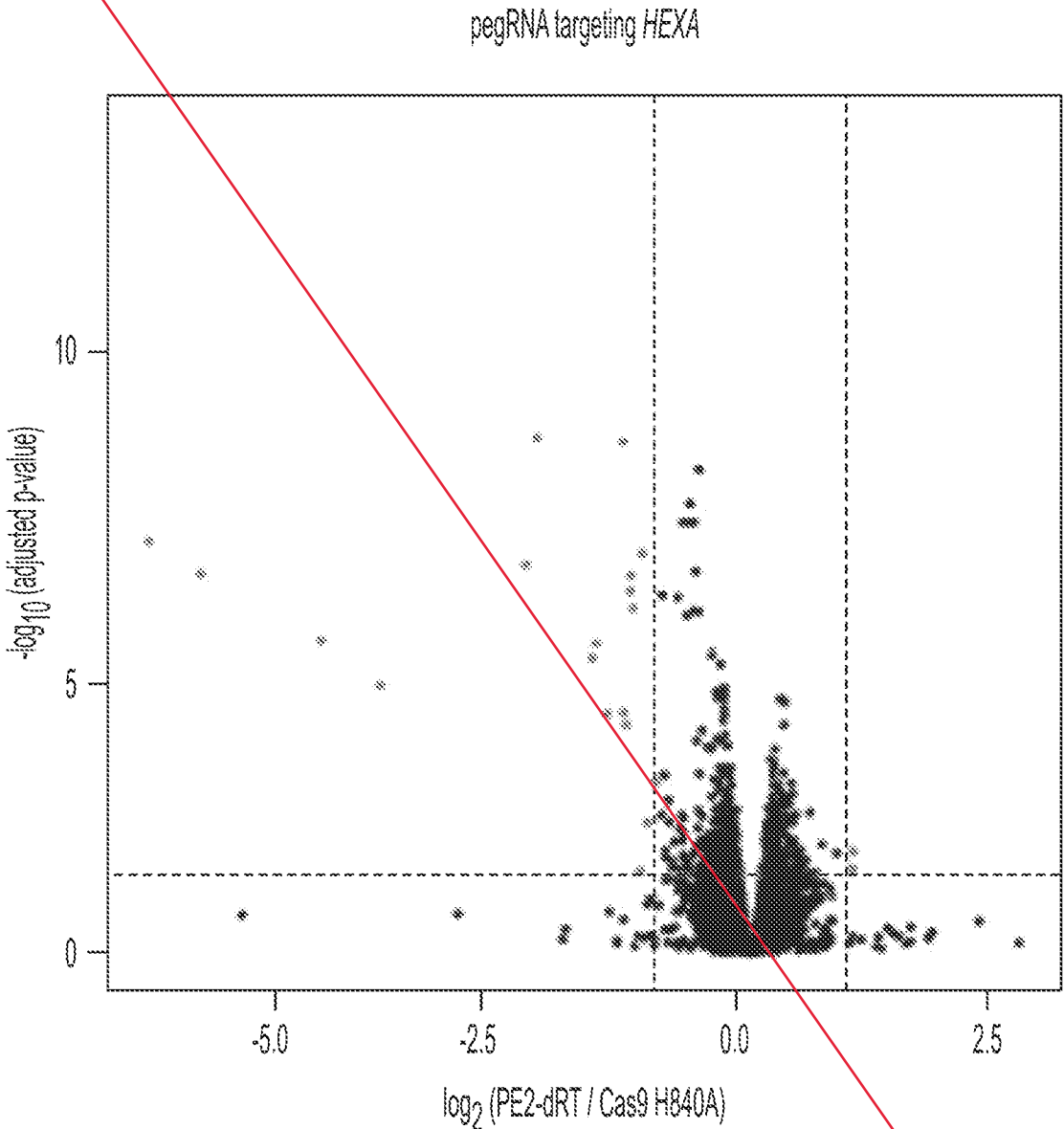


FIG. 57F

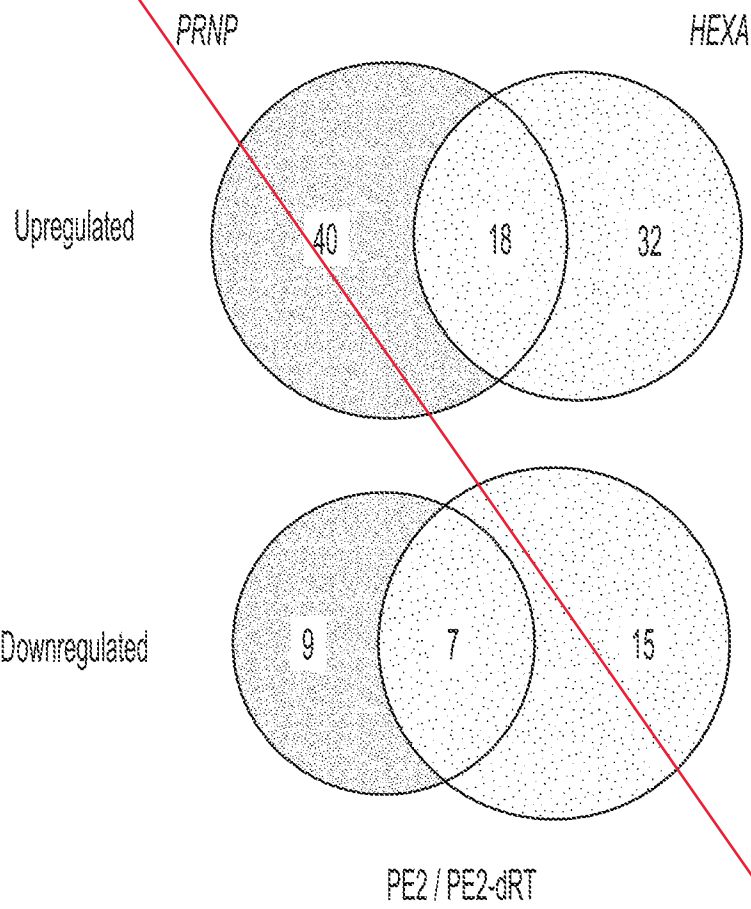


FIG. 57G

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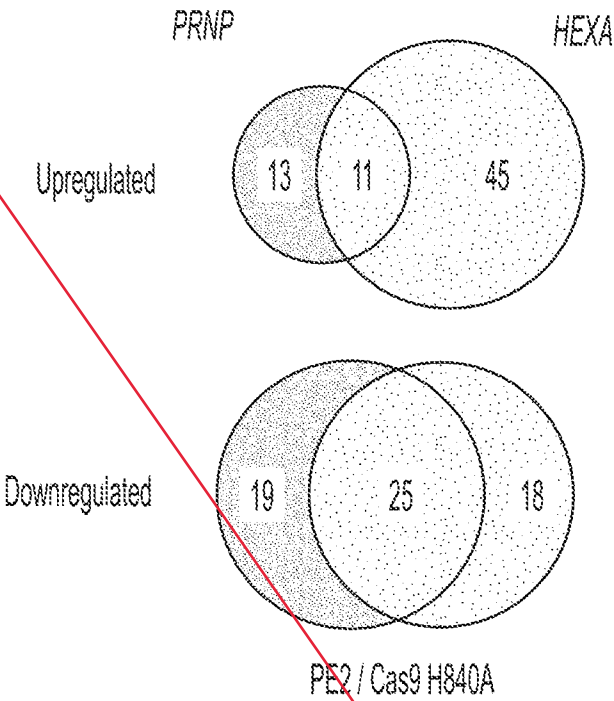


FIG. 57H



PE2-dRT / Cas9 H840A

FIG. 57I

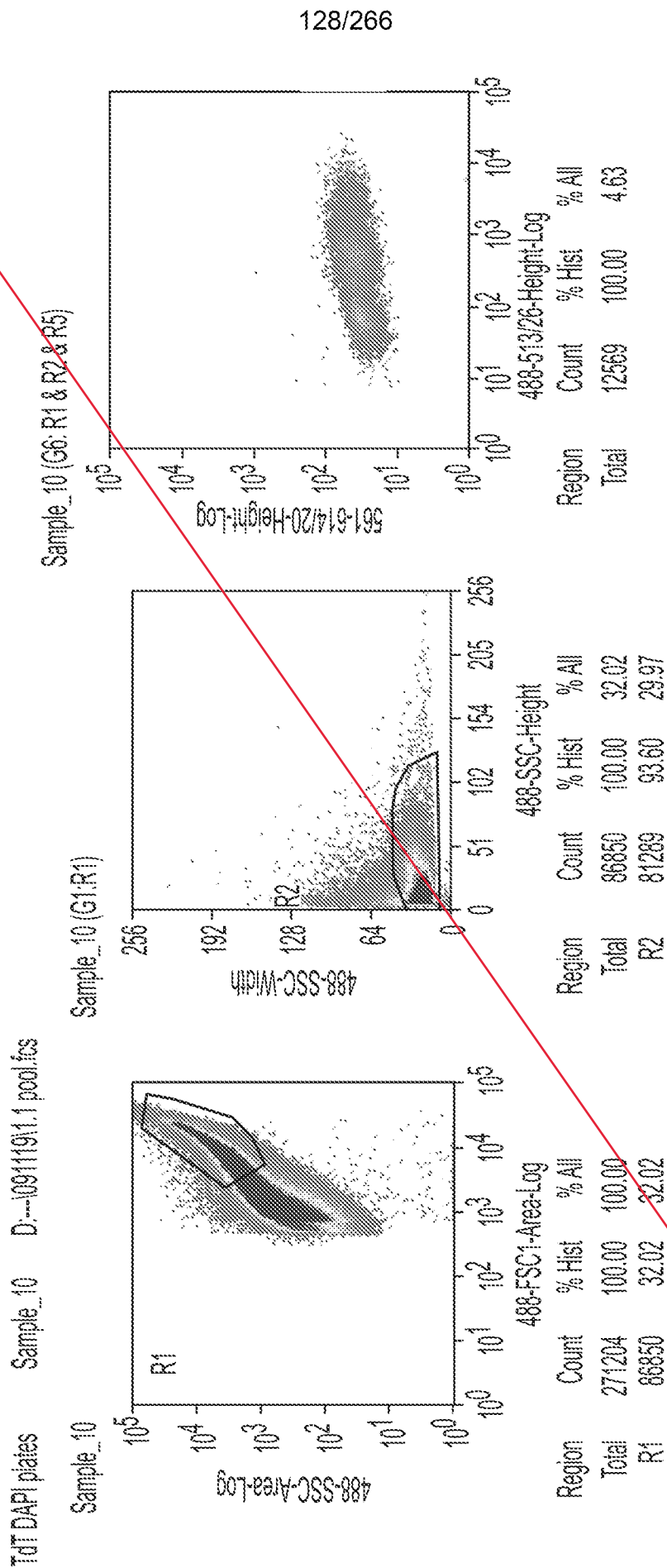


FIG. 58A

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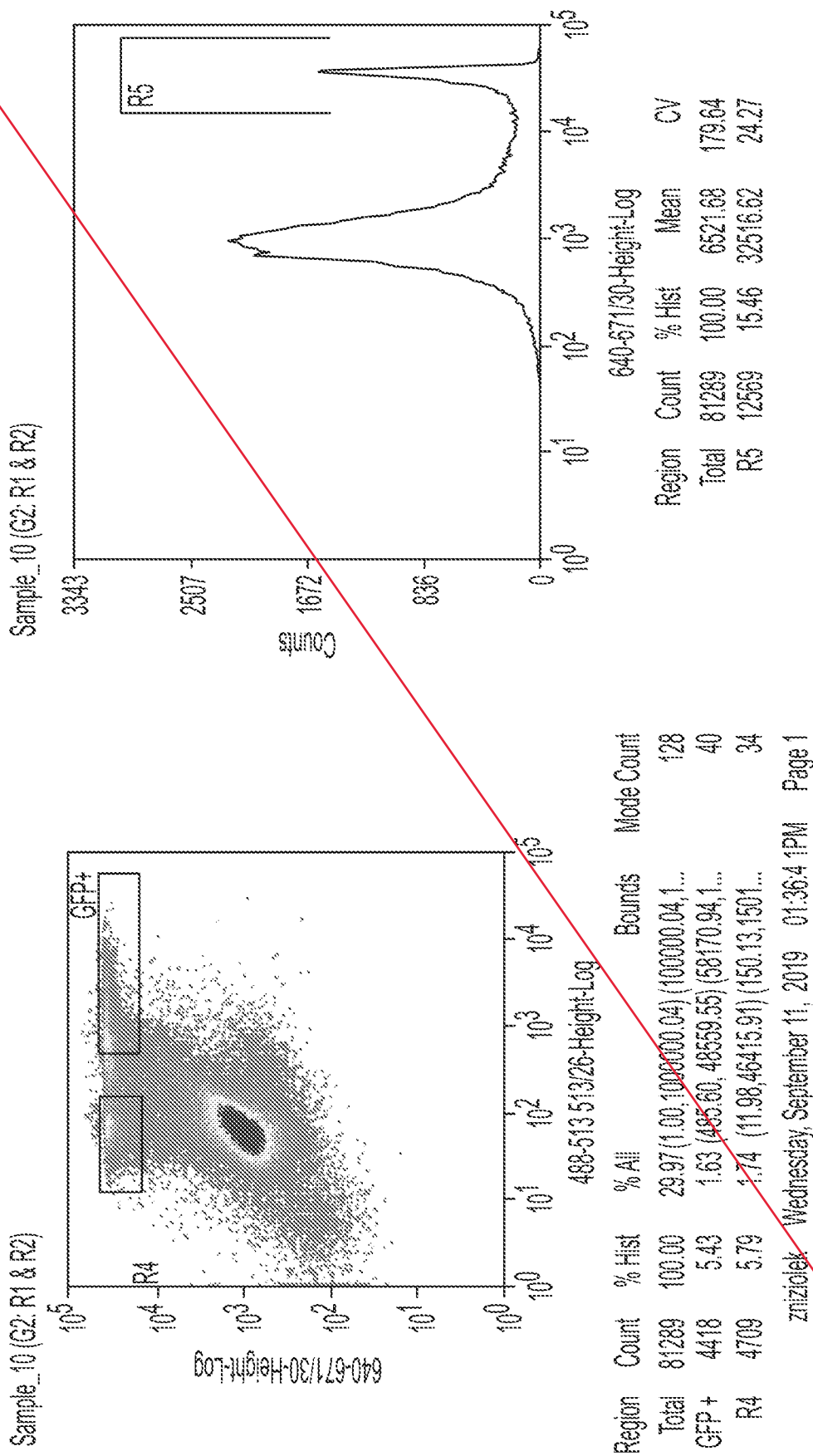


FIG. 58B

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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^R 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. 59ApegRNA 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
BsaI-HFv2 (NEB)	1.0 µL
10x Cutsmart Buffer	3.0 µL
H ₂ 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

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

Annealing buffer: H₂O 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 μ M	1.0 μ L
Bottom oligonucleotide, 100 μ M	1.0 μ L
Annealing buffer (components 2, 3, and 4)	23.0 μ L
<hr/>	
Total reaction volume	25.0 μ 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 μ L H₂O. The final concentration of each oligonucleotide will be 1 μ 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 μ M oligonucleotide duplex from step 1	6.25 μ L
10x T4 DNA ligase buffer (NEB)	2.50 μ L
T4 PNK (NEB)	0.50 μ L
H ₂ O	15.75 μ L
<hr/>	
Total reaction volume	20.0 μ L

In thermocycler, incubate at 37 °C for 60 minutes

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

Proceed to step 3.

FIG. 59D

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Step 4: peg RNA assemblypegRNA Golden Gate assembly reaction

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

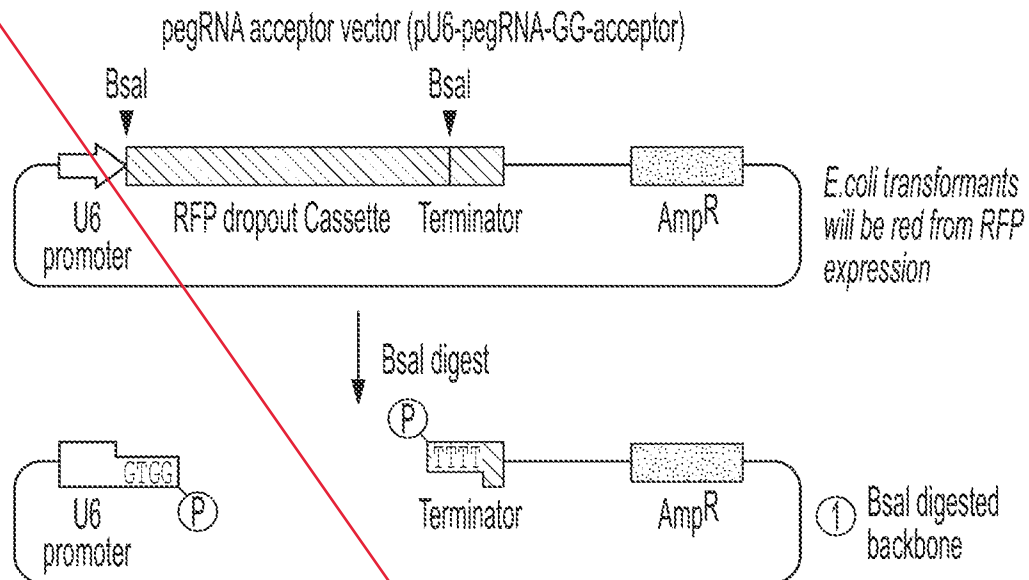
FIG. 59E**Steps 5 and 6: Transformation of assembled plasmids**

Transform 1 μ L of the 10 μ L assembly reaction into 10 μ 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

SEED NO. 879

[illegible]

SEQ ID NO: 880

③ pegRNA 3' extension annealed oligos

~~SECRET~~ NO. 883

~~5' GTGCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN~~ 3'

 NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNAAA 5'

SEQ ID NO: 884

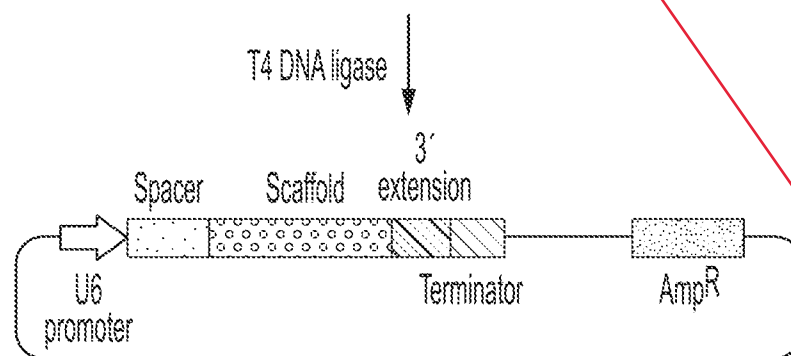
④ pegRNA scaffold annealed oligos (5' phosphorylated)

SEQ ID NO. 884

5' [Phos]-AGAGCTAGAAATAGCAAGTTAAAAATAAGGCTAGTCCGTTATCAACTTCAAAAAGTGGCACCCAGTCC 3'
3' GATCTTTATCCTTCAATTTTATTCGCATCAGCCAAATAGTTCGAACTTTTCACCGTGGCTCAGCCAGC-[Phos] 5'

SEQ ID NO: 882

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



Assembled pegRNA vector

FIG. 59G

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```

## 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':'CAGAGGACCGACTCGGTCCCACTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACCTCACGTGCTCAGTCTGGGCGCGTG', (SEQ ID NO: 885)
'EMX1':'ATCACGCACCGACTCGGTGCCACTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TGCTATTTCTAGCTCTAAAACCTTCTTCTTCTGCTCCGACTCGGTG', (SEQ ID NO: 886)
'FANCF':'TTTCCGCACCGACTCGGTGCCACTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TTGCTATTTCTAGCTCTAAAACGGTGCTGCAGAAGGGATTCCGGTG', (SEQ ID NO: 887)
'RNFB':'TCGTTGCACCGACTCGGTGCCACTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAAACCGTAATGACTAAGATGACGGTG', (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

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```

        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

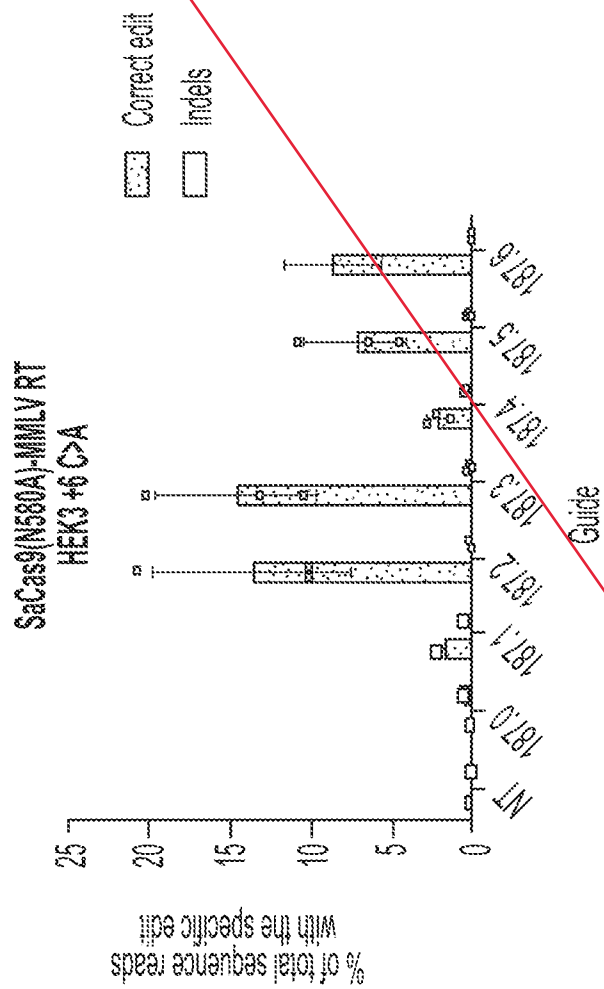


FIG. 61

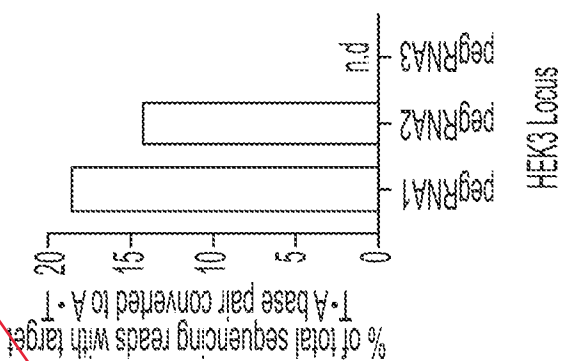


FIG. 62A

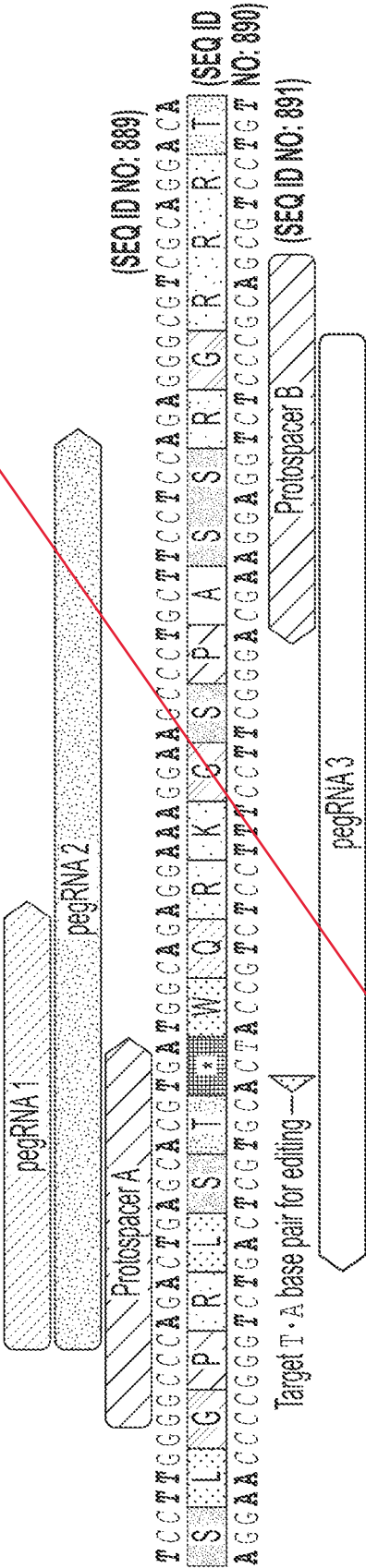
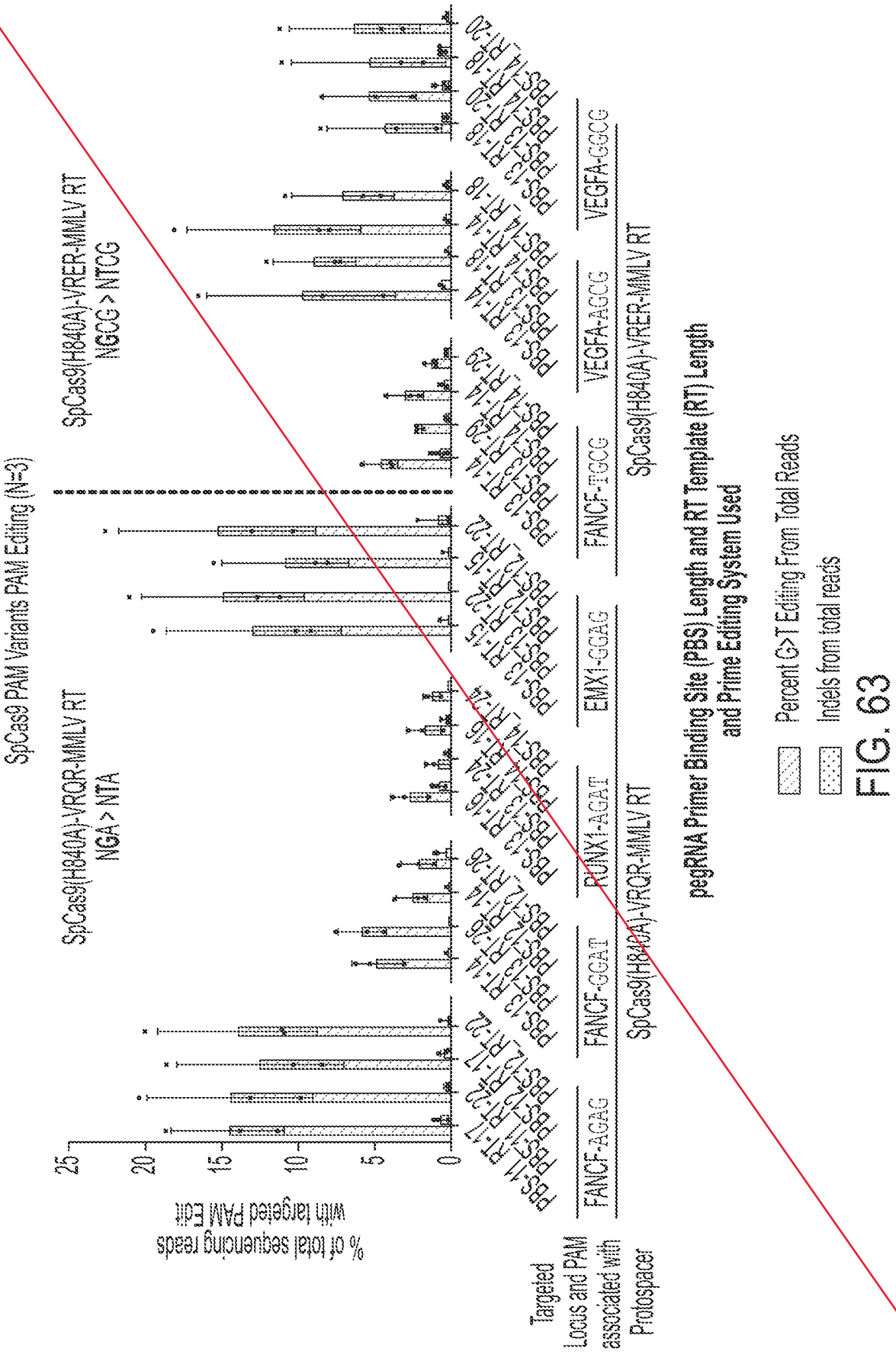


FIG. 62B



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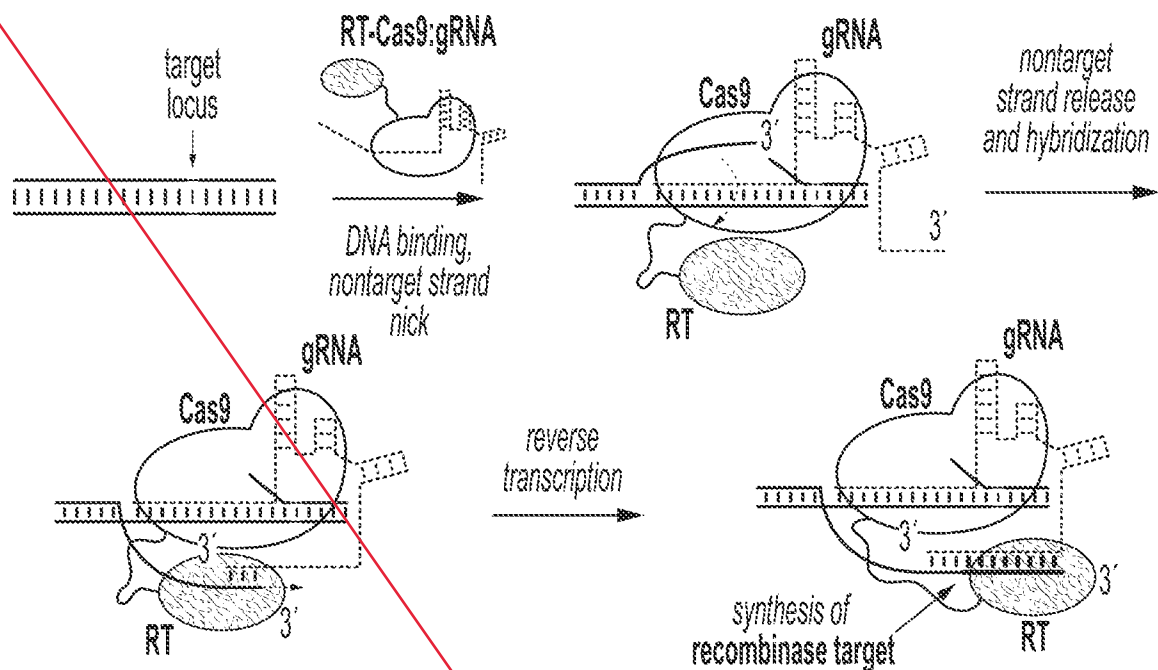


FIG. 64A

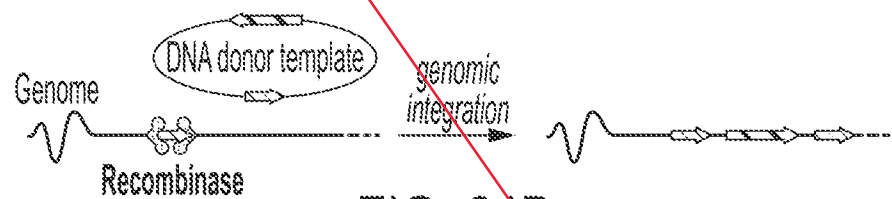


FIG. 64B

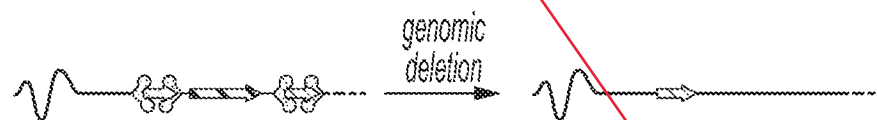


FIG. 64C



FIG. 64D

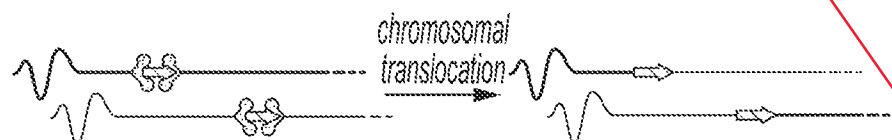


FIG. 64E

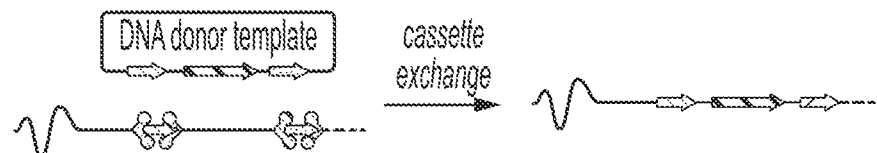
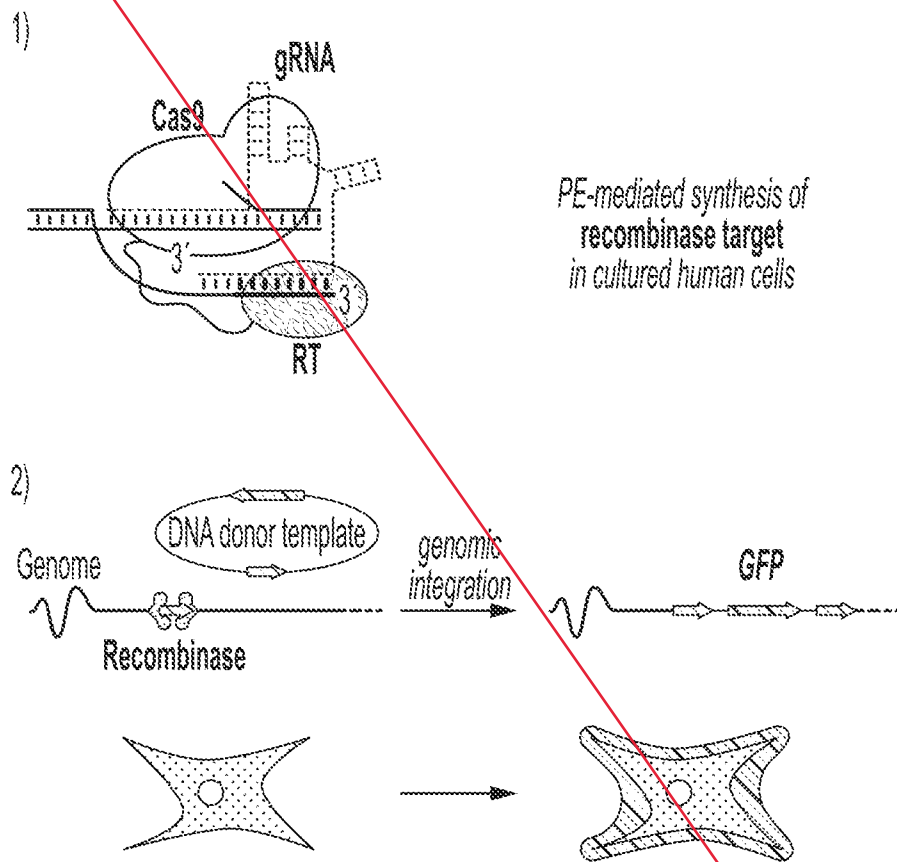


FIG. 64F

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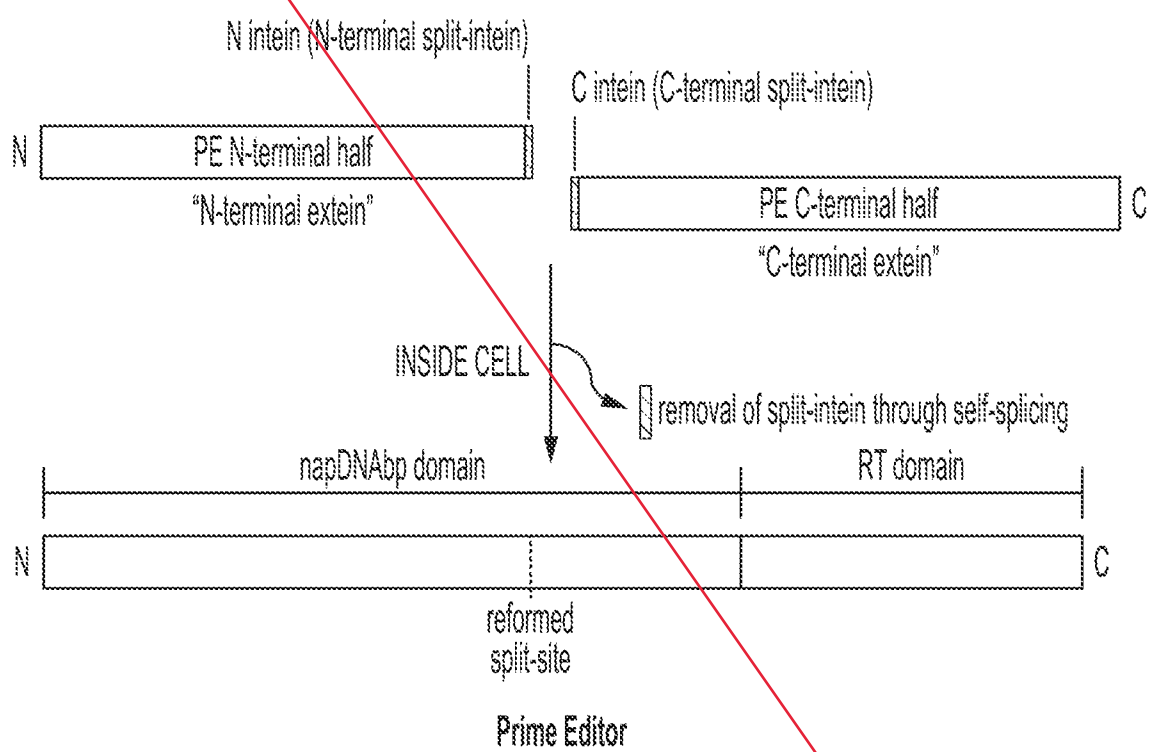


FIG. 66

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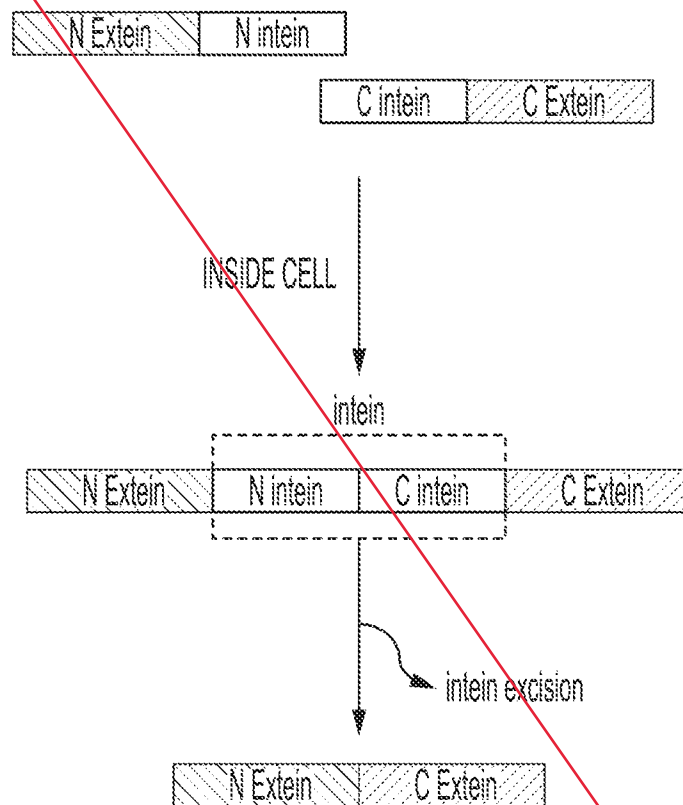


FIG. 67A

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intein excision mechanism

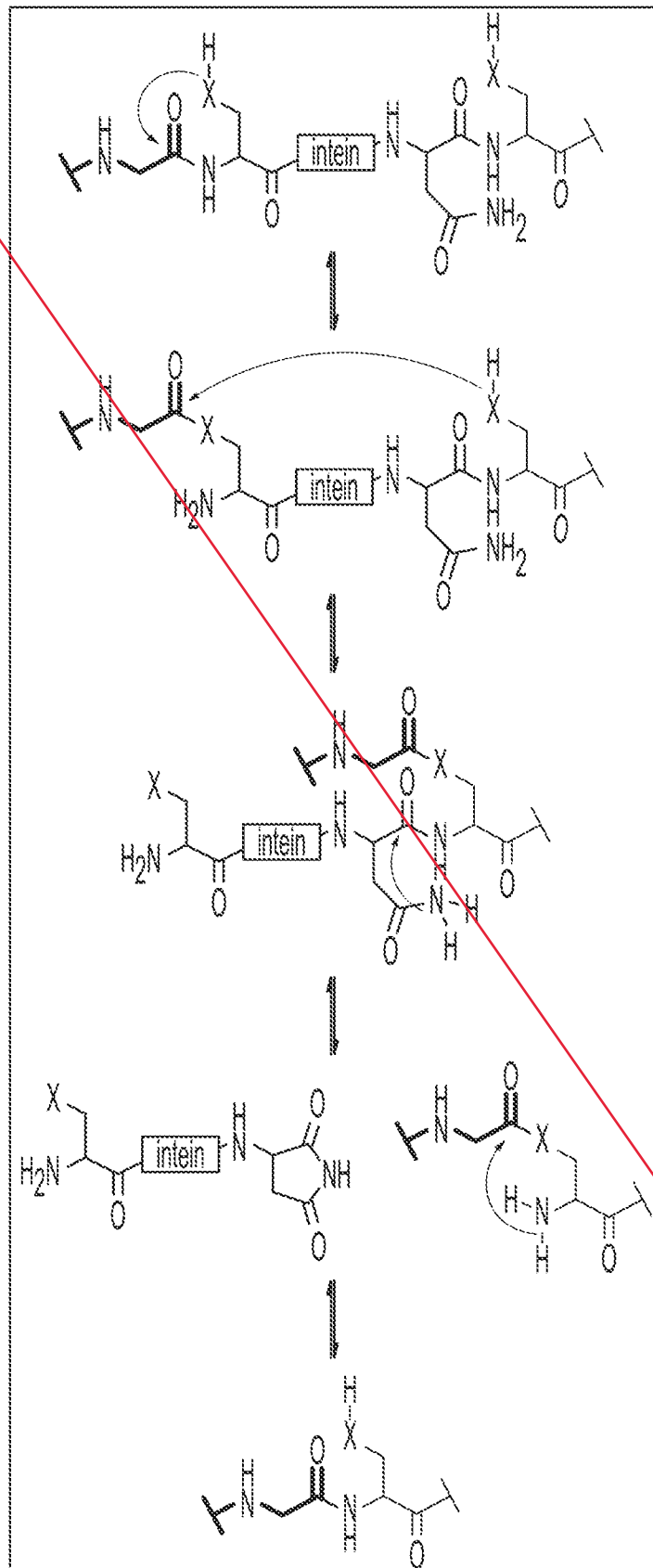


FIG. 67B

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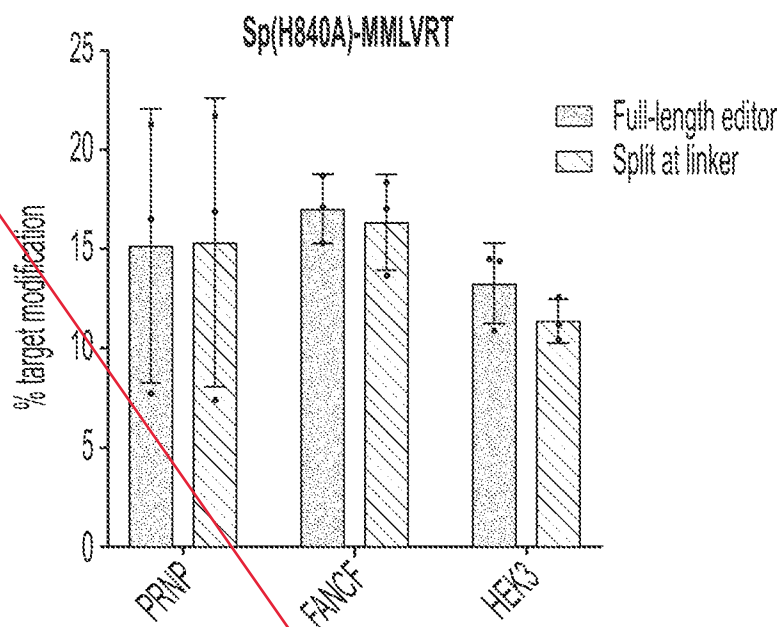


FIG. 68A

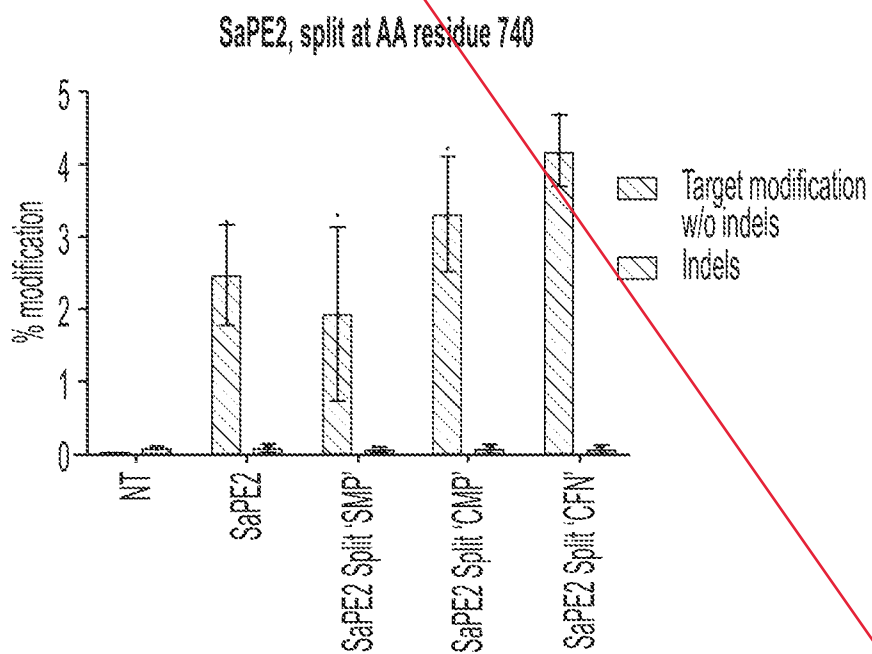


FIG. 68B

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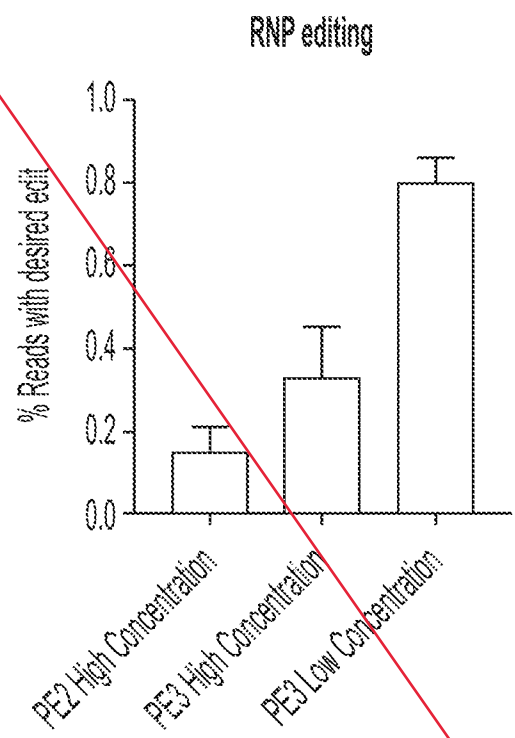


FIG. 68C

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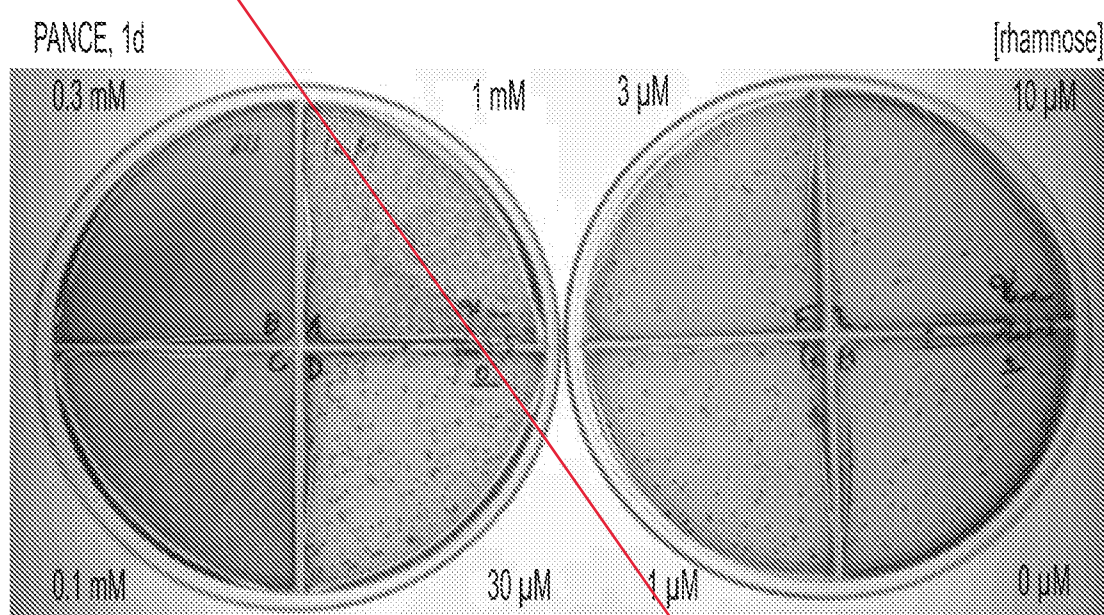


FIG. 69

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Starting allele

(SEQ ID NO: 892)

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

Edit Target (A•T to C•G)

ATGCCCCCTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATAAACTGAGTTCC
TACGGGGAAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70A

Starting allele

(SEQ ID NO: 892)

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

NGG PAM 1

ATGCCCCCTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATAAACTGAGTTCC
TACGGGGAAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

NGG PAM 2

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70B

Starting allele

(SEQ ID NO: 892)

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

nick for PAM 1

ATGCCCCCTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATAAACTGAGTTCC
TACGGGGAAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

nick for PAM 2

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70C

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Starting allele

(SEQ ID NO: 892)

5'-TATTATTACTCTATGTTCTATTTAAGTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAAGTACAAGATTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

Spacer

ATGCCCTTGGCAGTCATCTTAGTCATTACCTCGGGTGTTCGTTGTAACATATAAACTGAGTTCC
TACGGGGAAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCGGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCCAAACAGACGTGTGGACTCTGTAACGACCTTTATTTCTTC

(SEQ ID NO: 637)

Spacer = GTCATCTTAGTCATTACCTG

FIG. 70D

Starting allele

(SEQ ID NO: 892)

5'-TATTATTACTCTATGTTCTATTTAAGTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAAGTACAAGATTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

PBS complement

ATGCCCTTGGCAGTCATCTTAGTCATTACCTCGGGTGTTCGTTGTAACATATAAACTGAGTTCC
TACGGGGAAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCGGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCCAAACAGACGTGTGGACTCTGTAACGACCTTTATTTCTTC

PBS = GTAATGACTAAGATG

FIG. 70E

Desired allele

(SEQ ID NO: 892) 5'-

TATTATTACTCTATGTTCTATTTAAGTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAAGTACAAGATTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

RT template complement

ATGCCCTTGGCAGTCATCTTAGTCATTACCTCGGGTGTTCGTTGTAACATATAAACTGAGTTCC
TACGGGGAAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

Edit

CATGTTTTGCTTAATGGTTGAGTTCGGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCCAAACAGACGTGTGGACTCTGTAACGACCTTTATTTCTTC

SEQ ID NO: 1759

RT template = AACGAACACCGCAG

FIG. 70F

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FIG. 70G

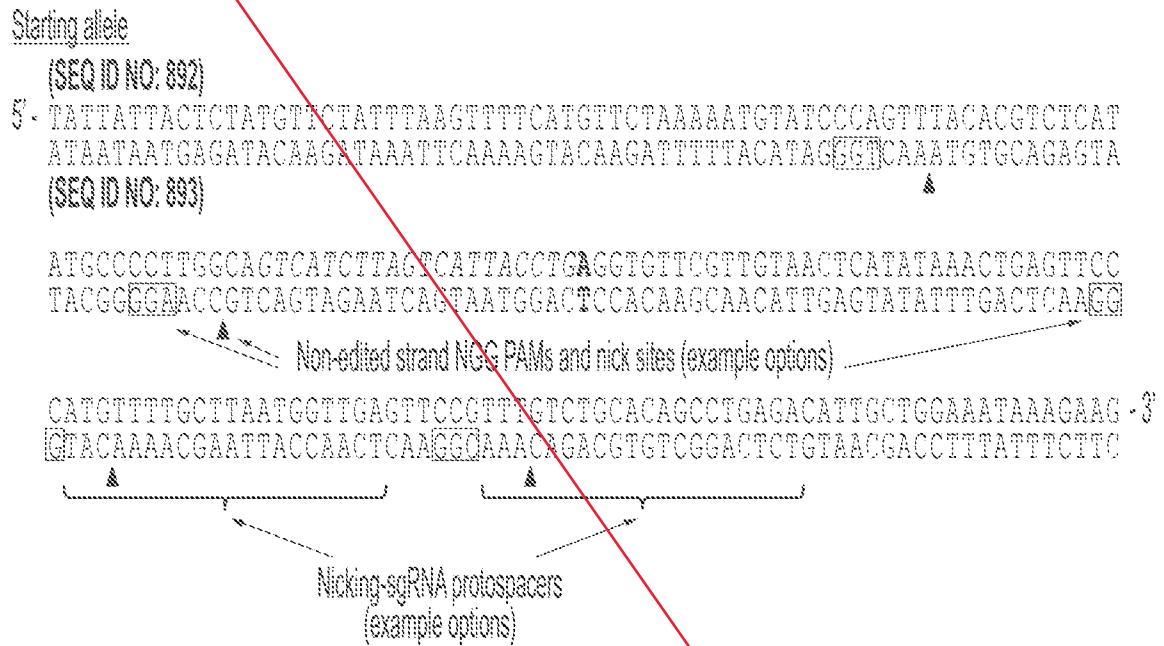
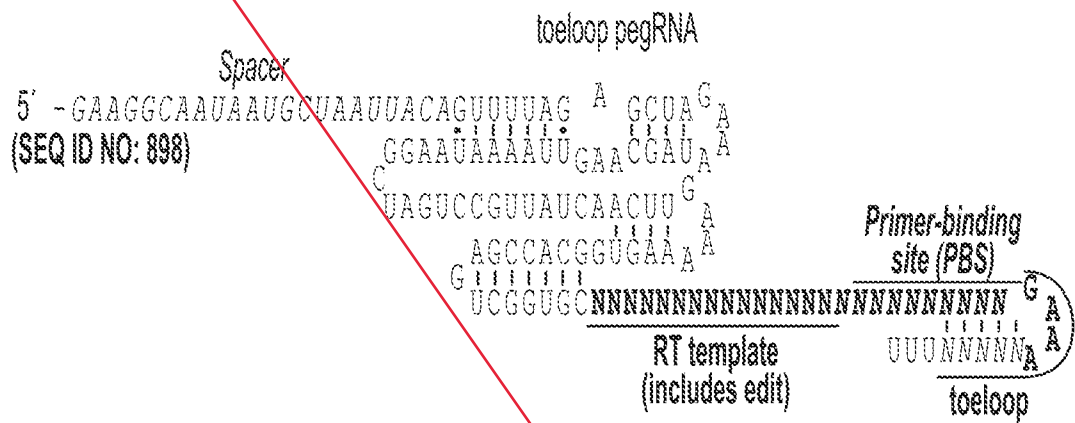


FIG. 70H



FIG. 70I

generic SpCas9 pegRNA



~~FIG. 71A~~

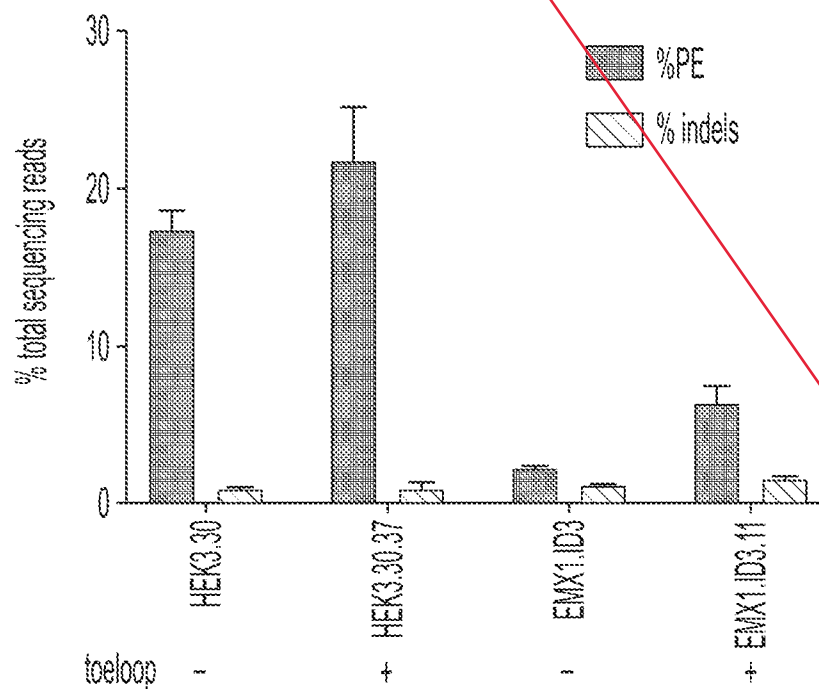


FIG. 71B

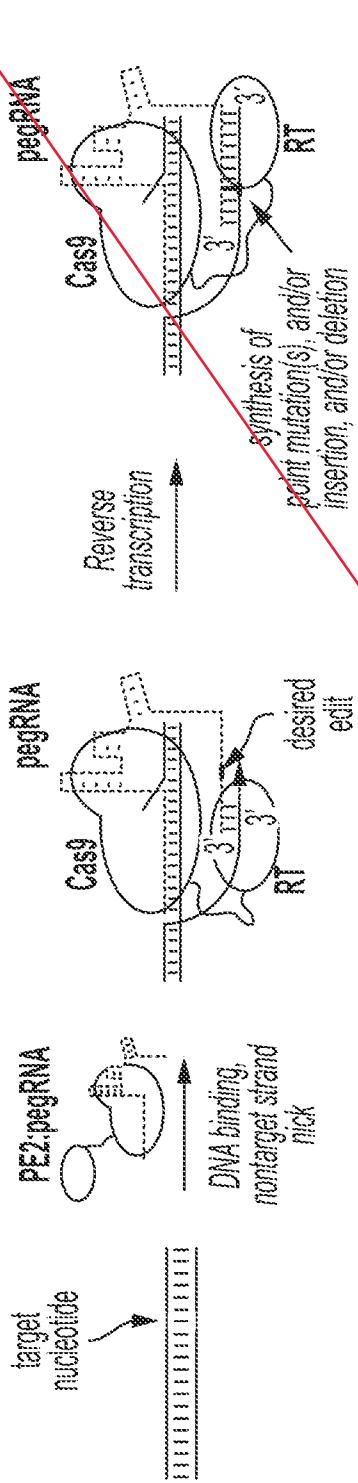


FIG. 72A

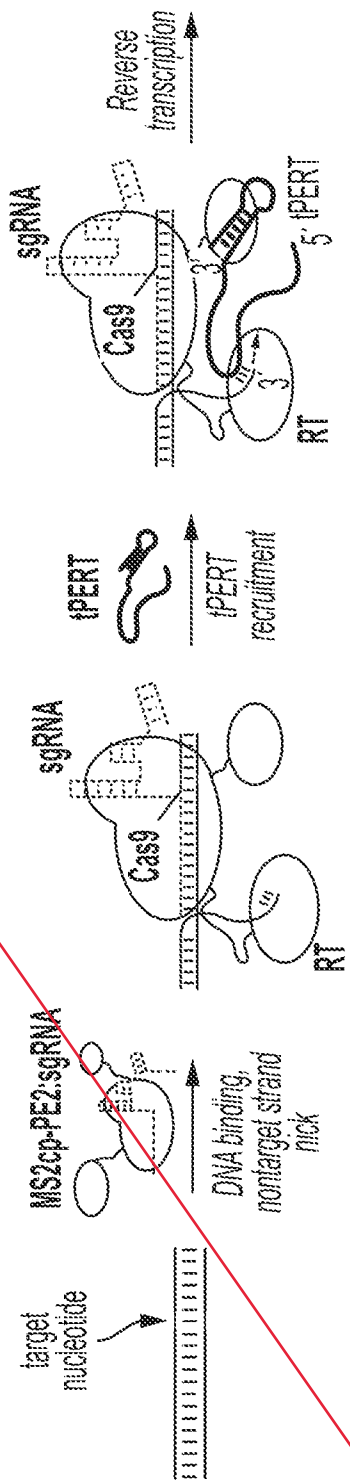


FIG. 72B

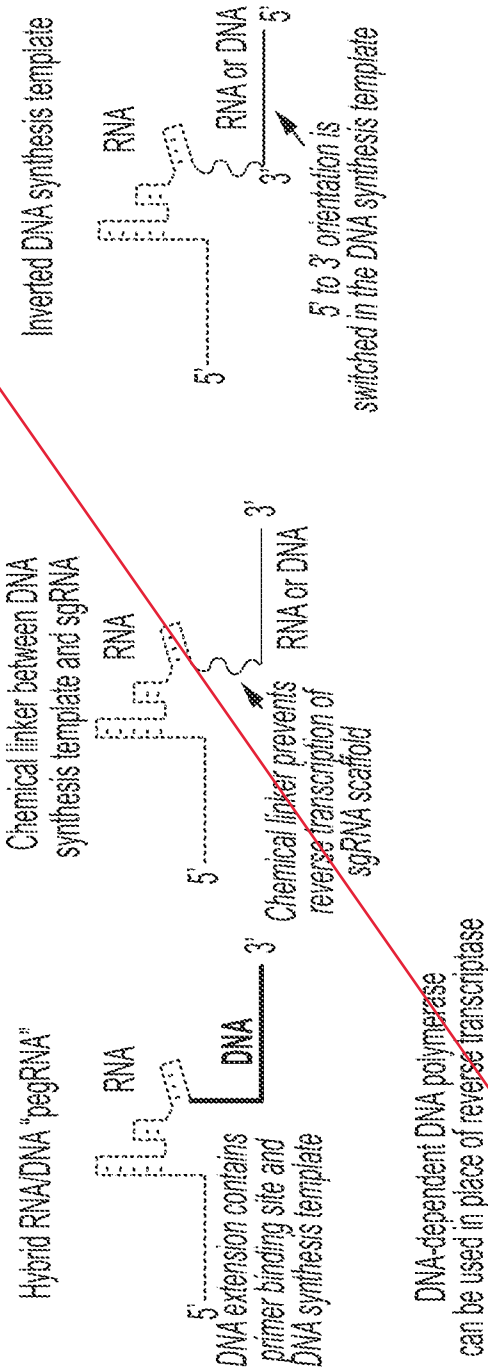
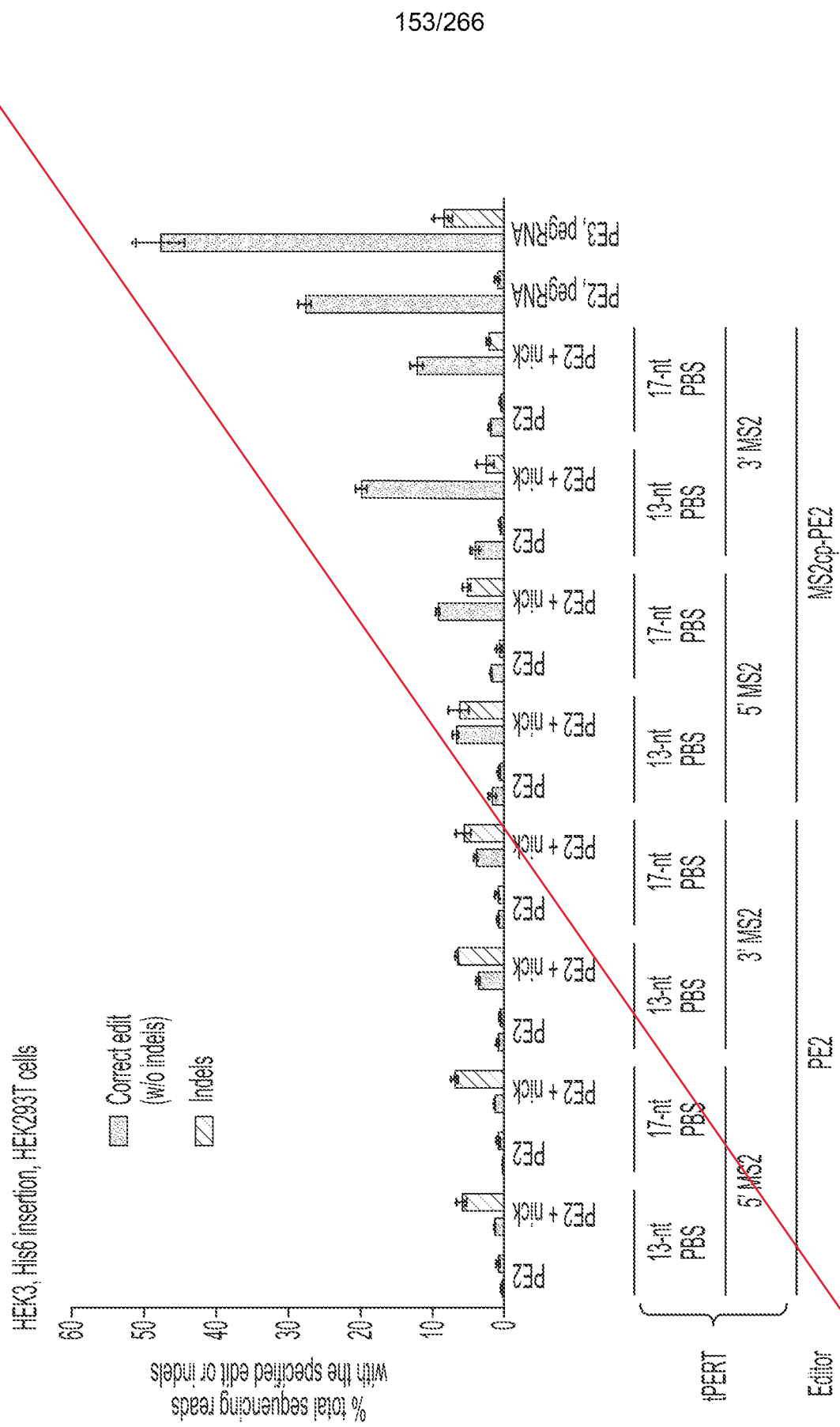


FIG. 72C



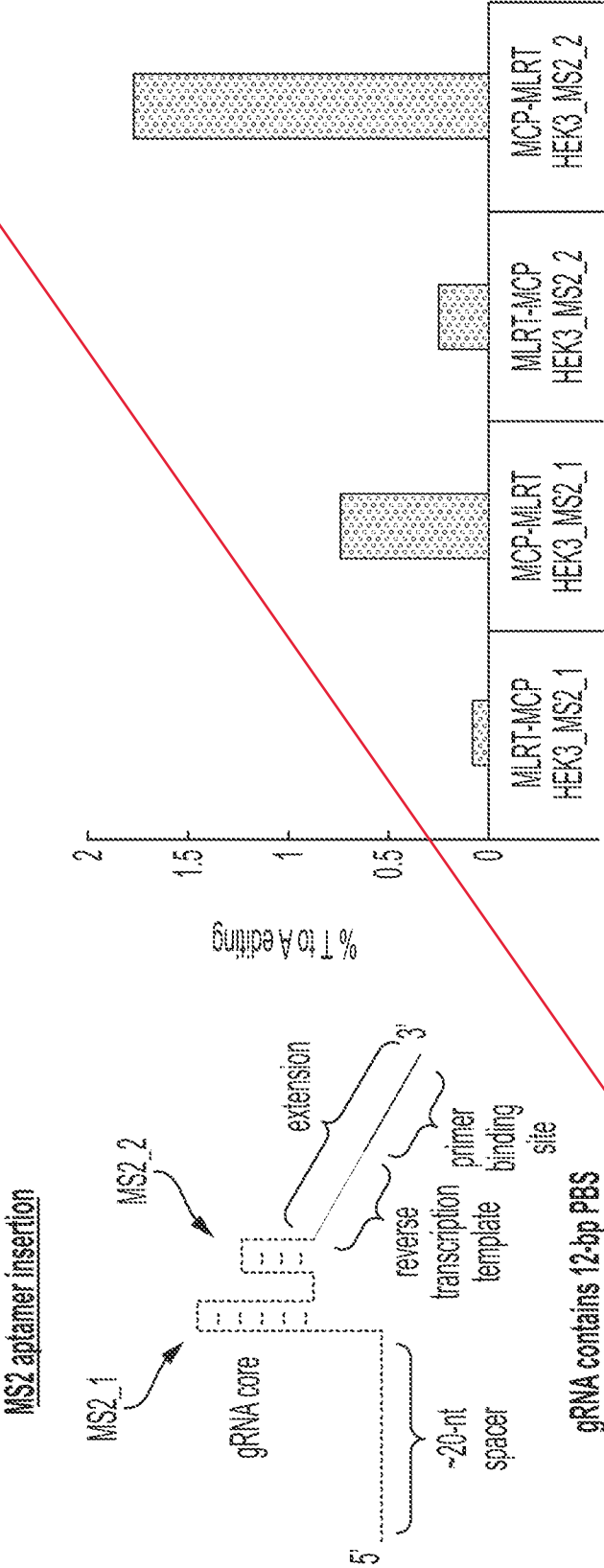


FIG. 74

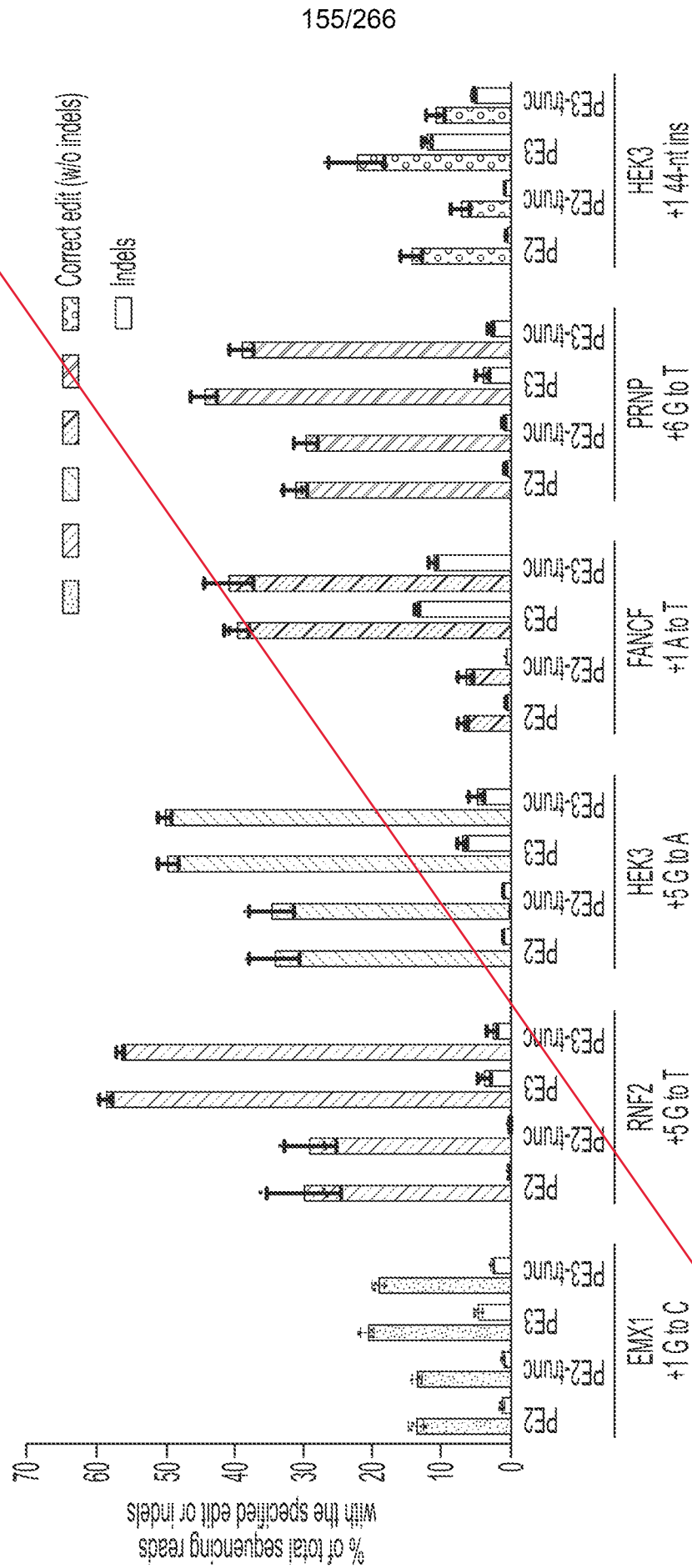


FIG. 75

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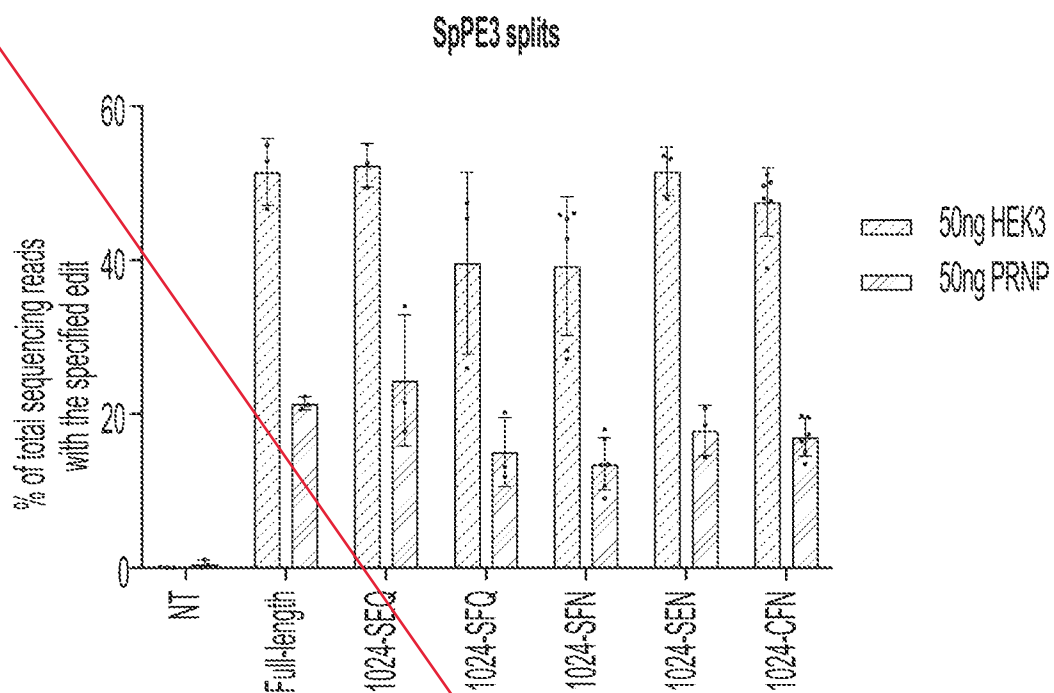


FIG. 76

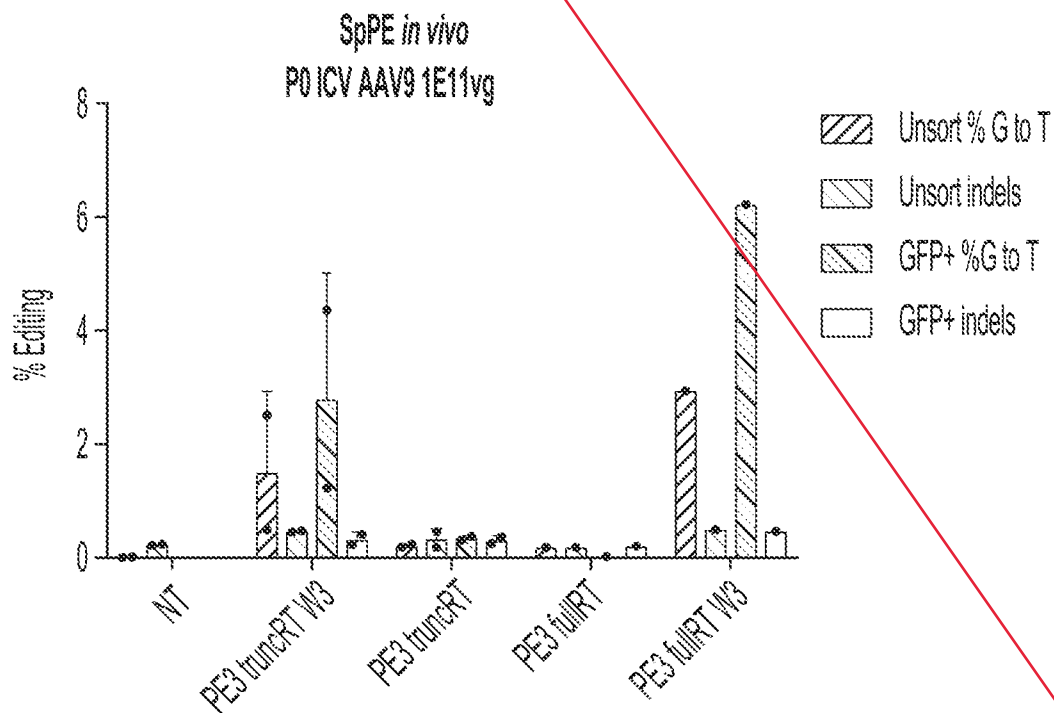


FIG. 77

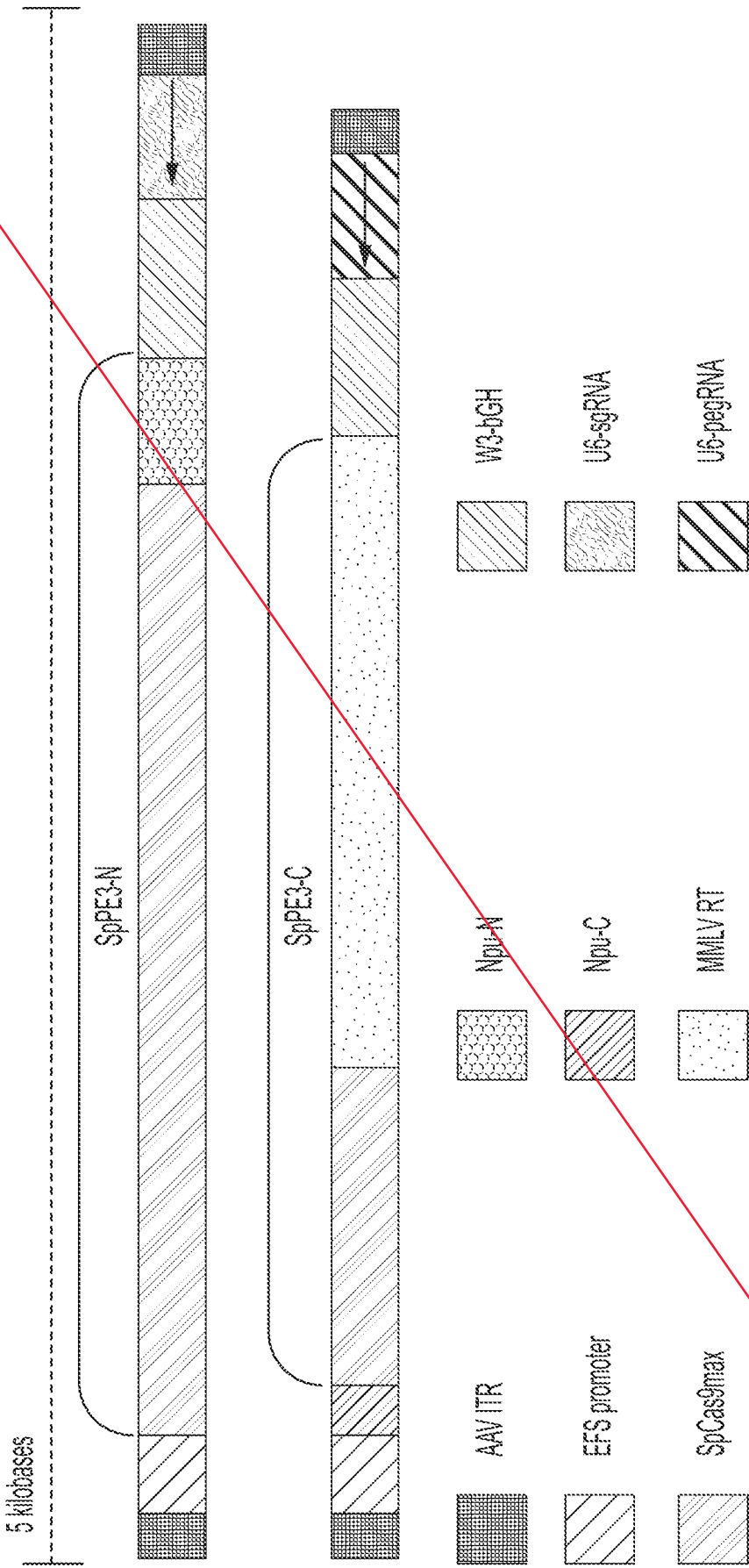


FIG. 78

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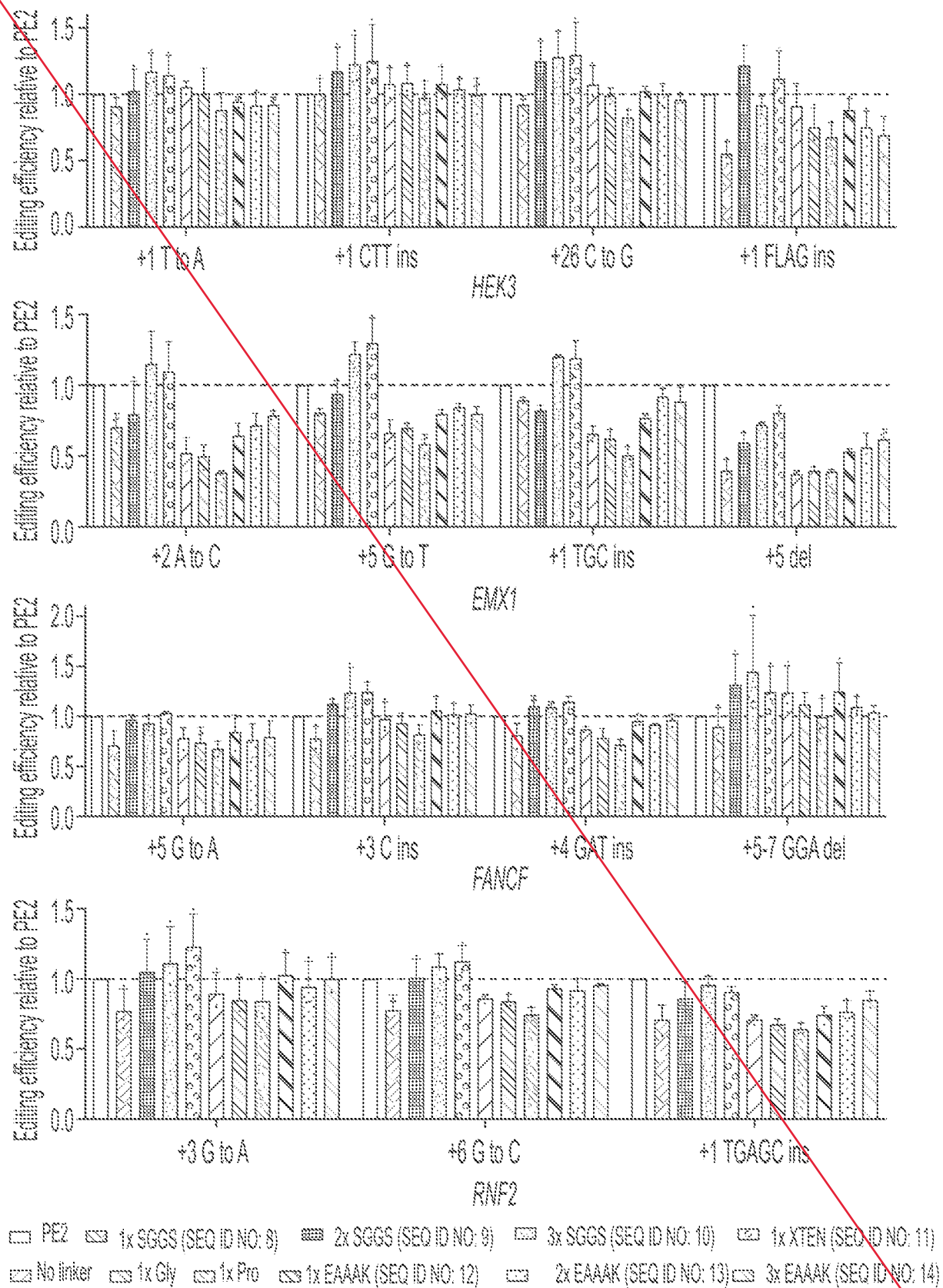


FIG. 79

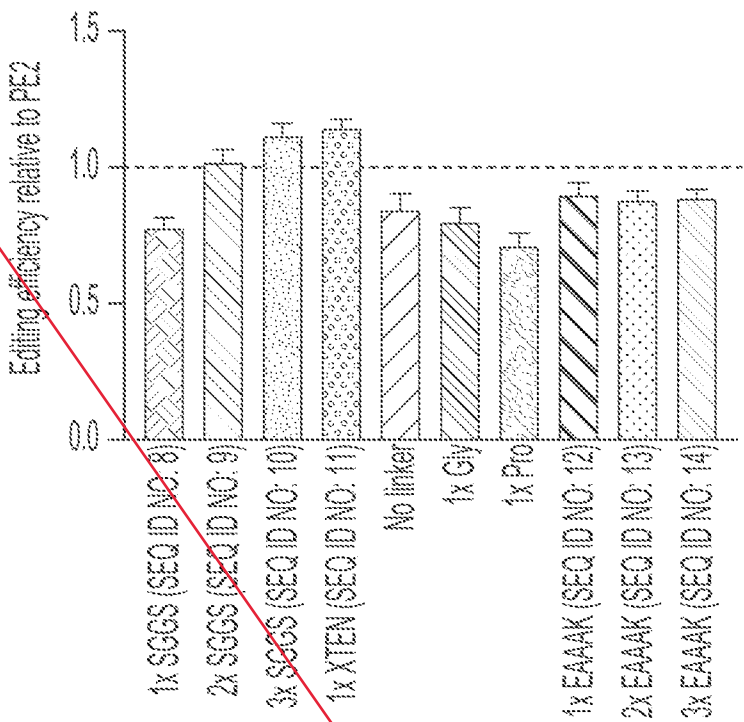


FIG. 80

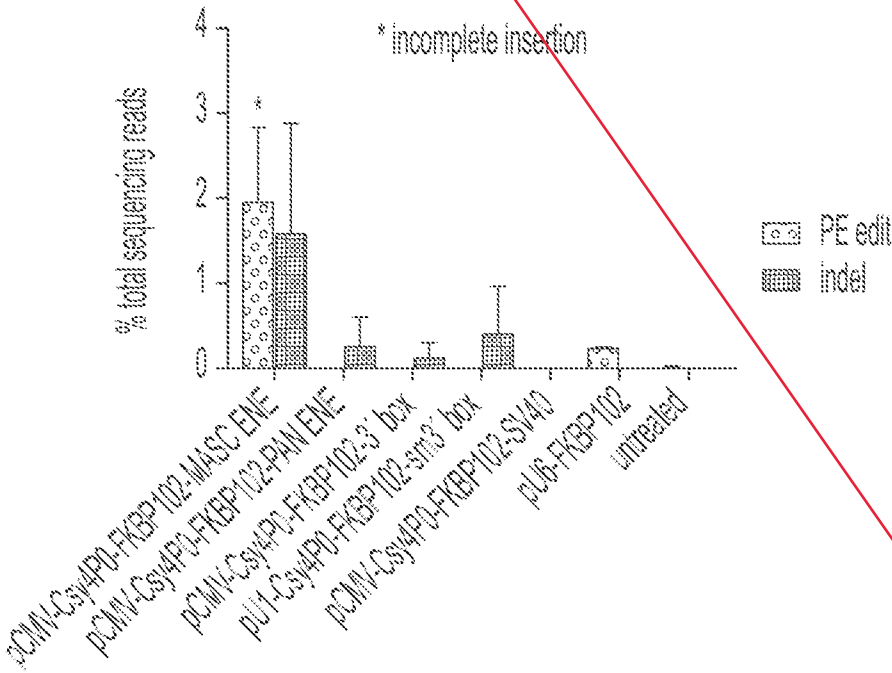


FIG. 81

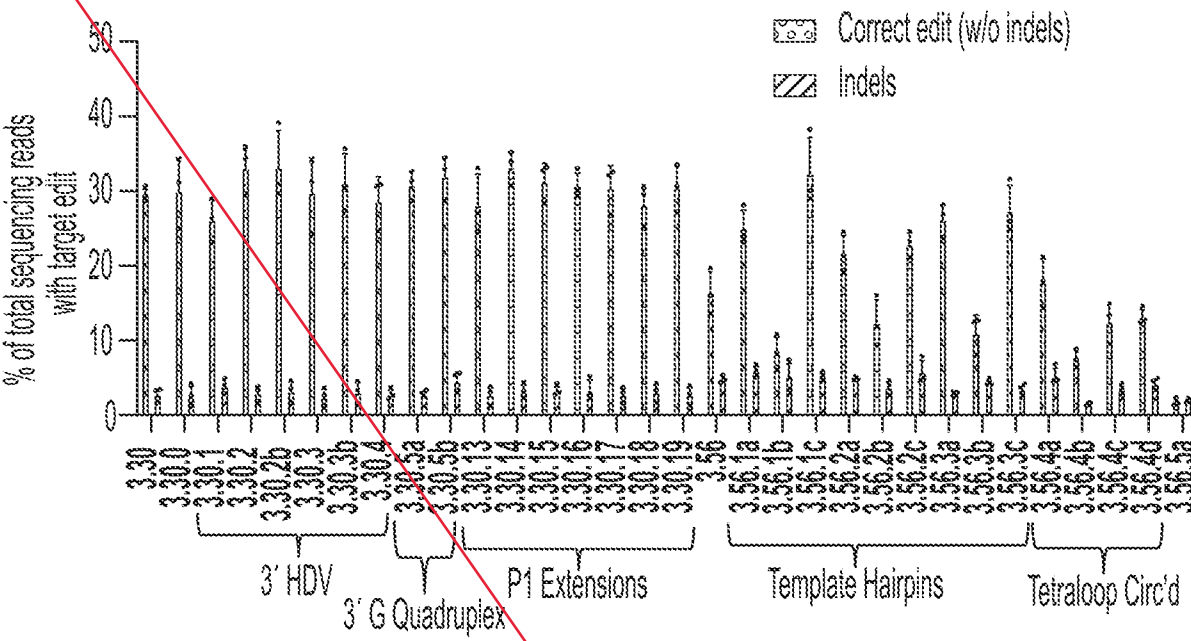


FIG. 82

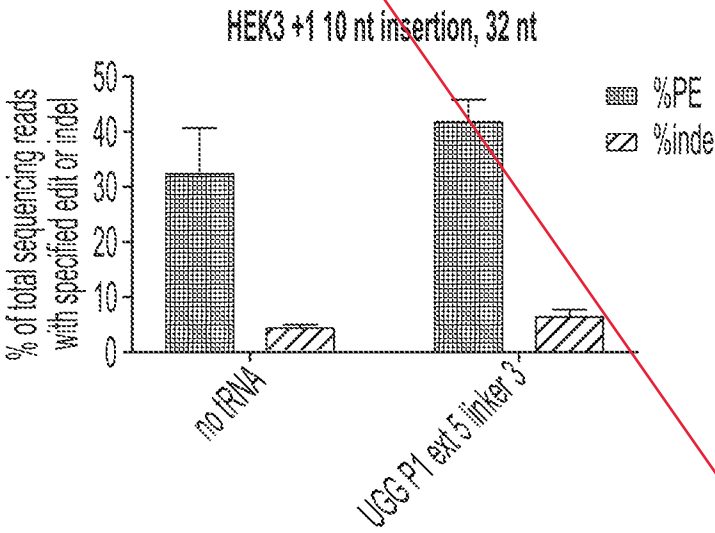


FIG. 83

Diagram illustrating the secondary structure of a tRNA molecule, labeled SEQ ID NO: 1760. The structure shows the characteristic L-shaped tertiary structure with various loops and stems. Key regions labeled include the acceptor stem, D-loop, anticodon loop, TψC loop, and variable loop. The sequence is written along the stems, with modifications like m¹A, m⁵C, m²G, and m⁷G indicated. The 3' end ends in a C-C-A-OH sequence, and the 5' end starts with a pG.

~~FIG. 84A~~

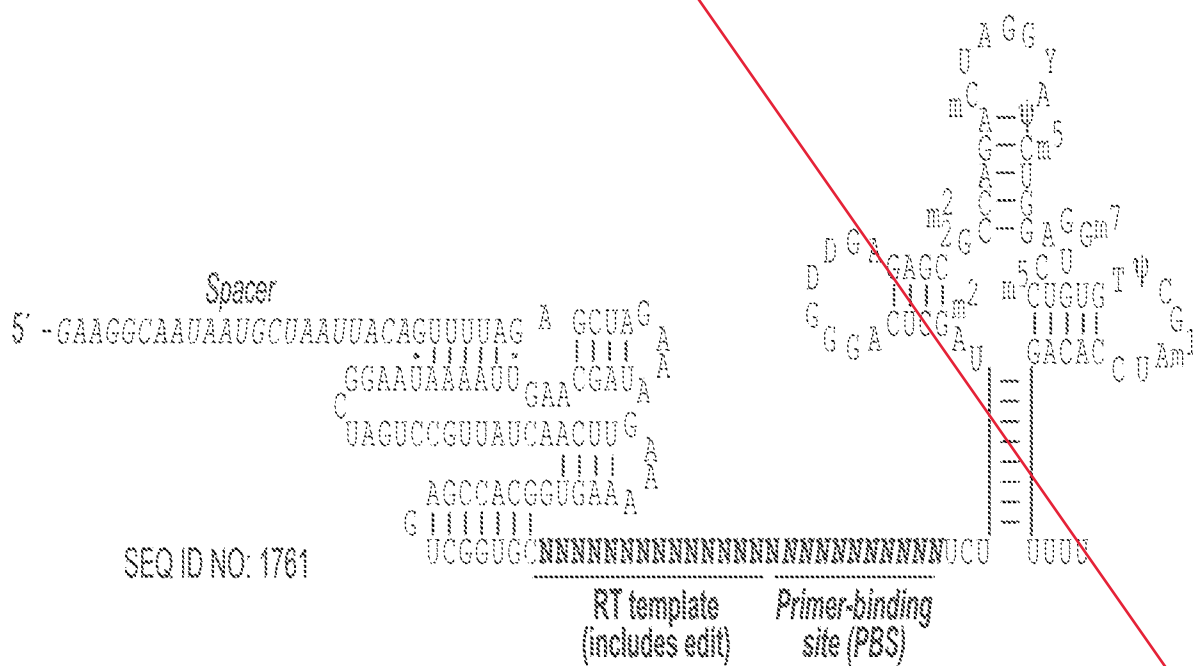


FIG. 84B

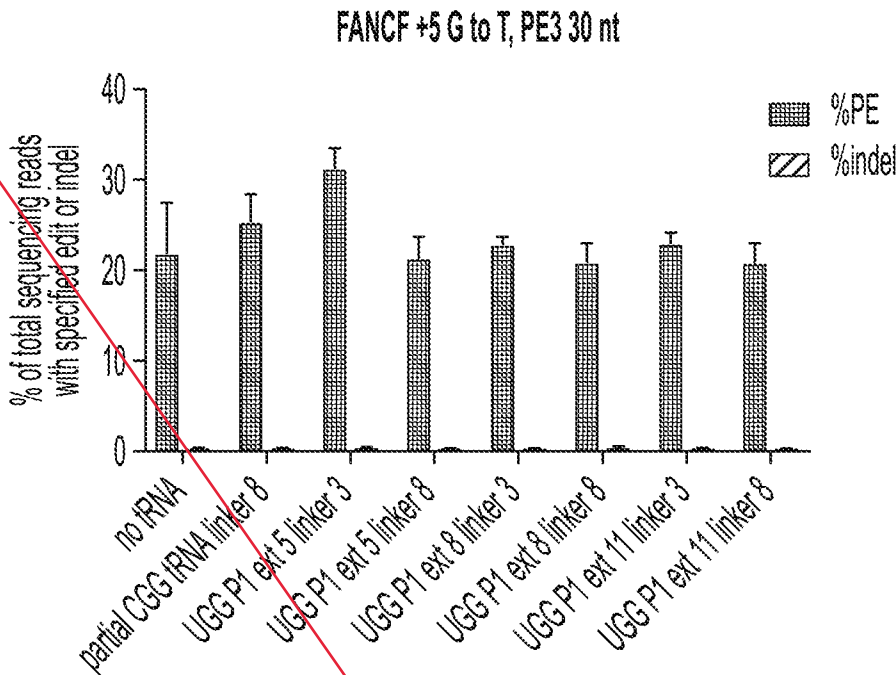


FIG. 85

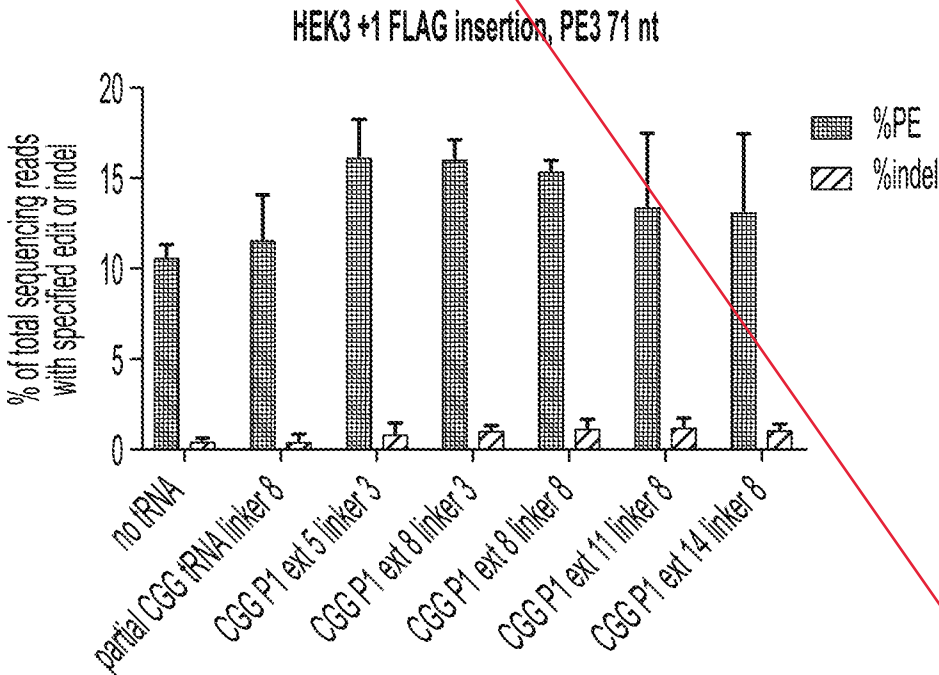


FIG. 86

CDKL5 1412delA SpPE3

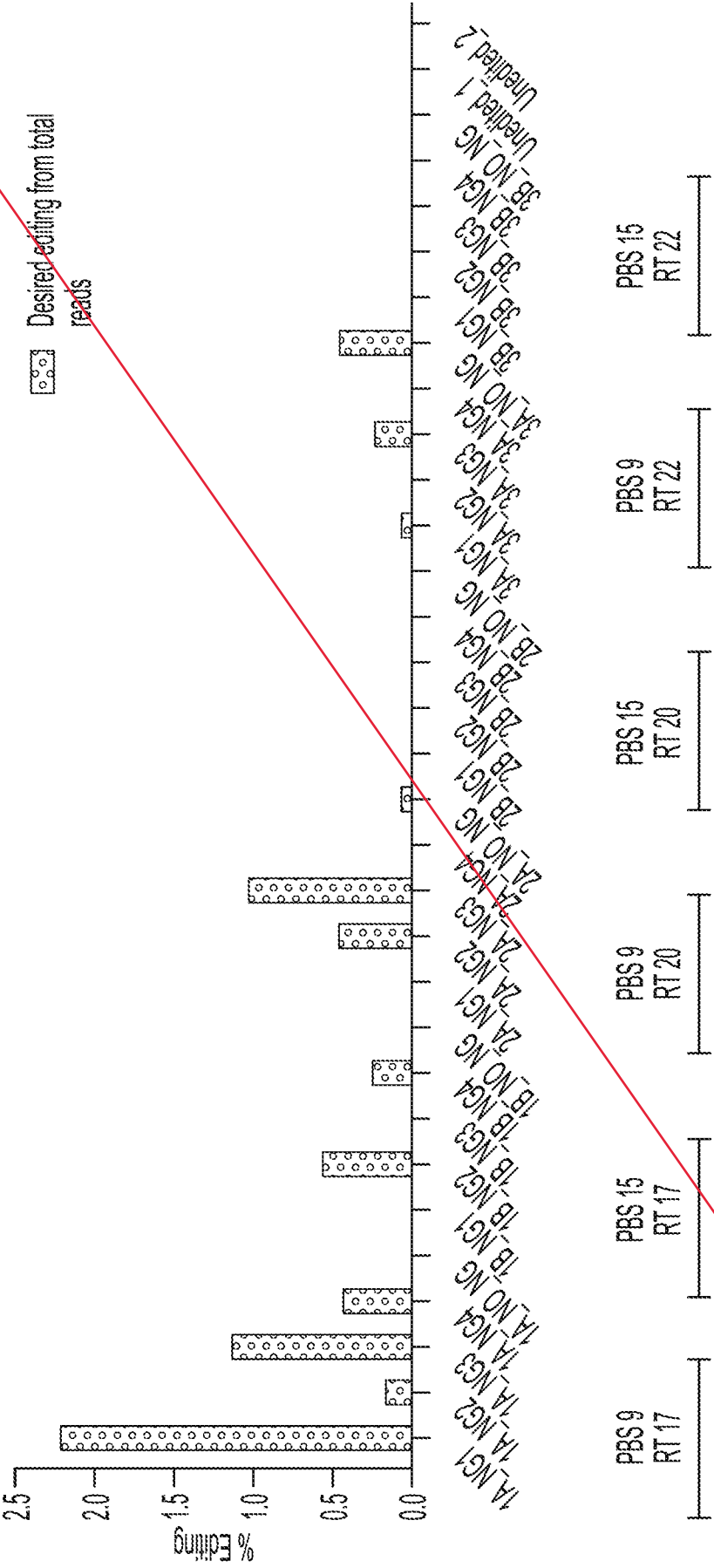
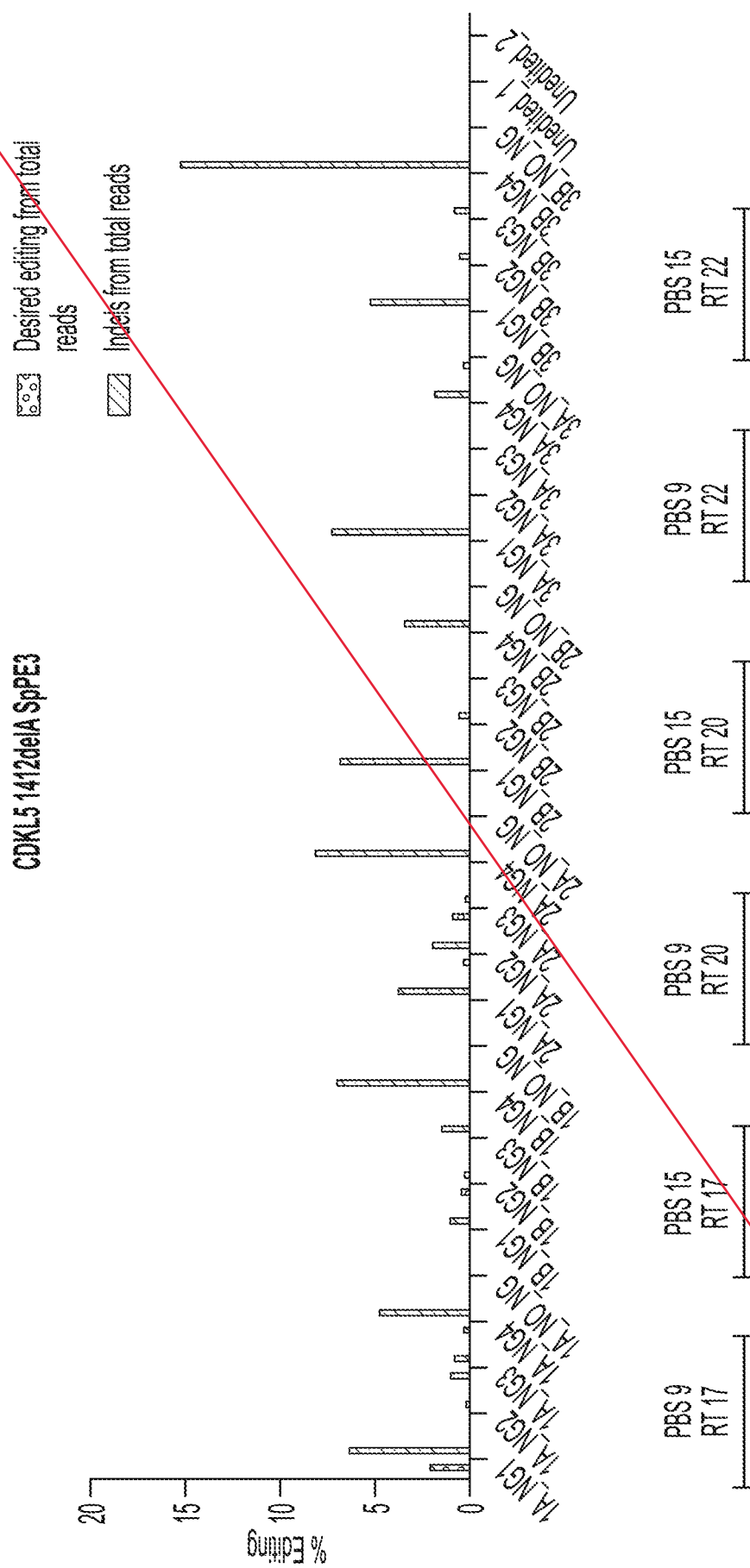


FIG. 87

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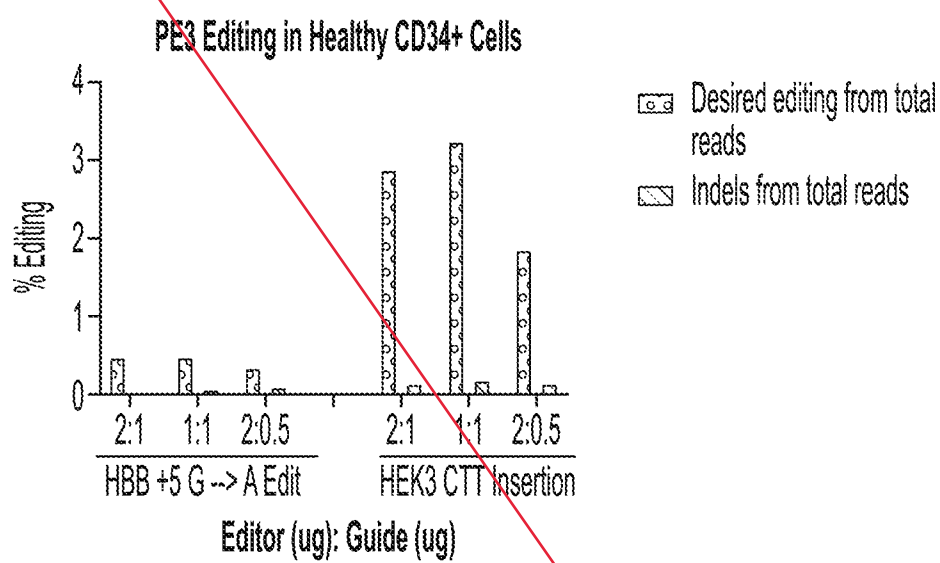


FIG. 89

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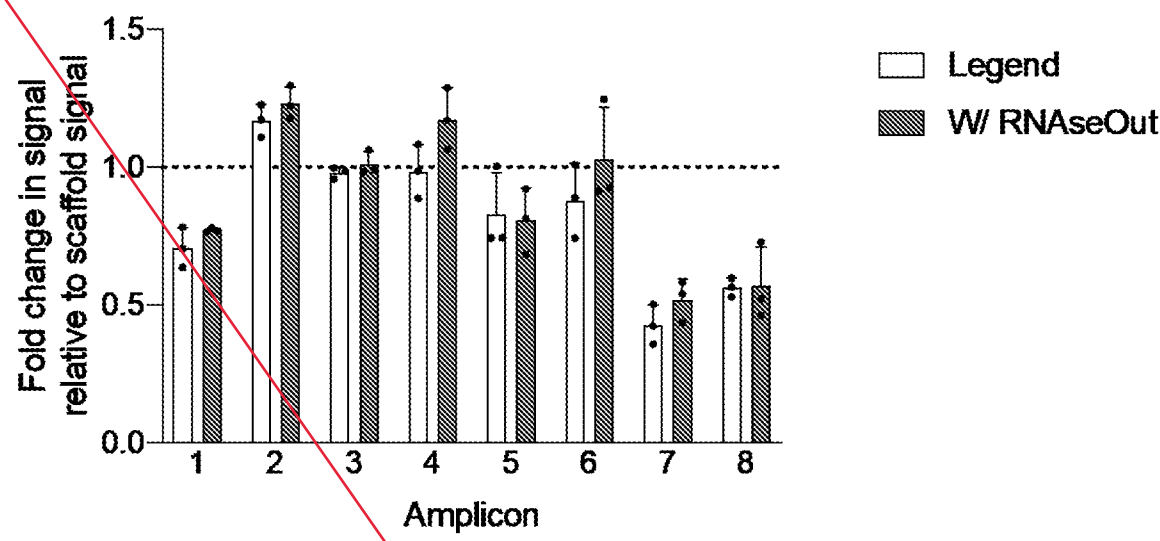


FIG. 90A

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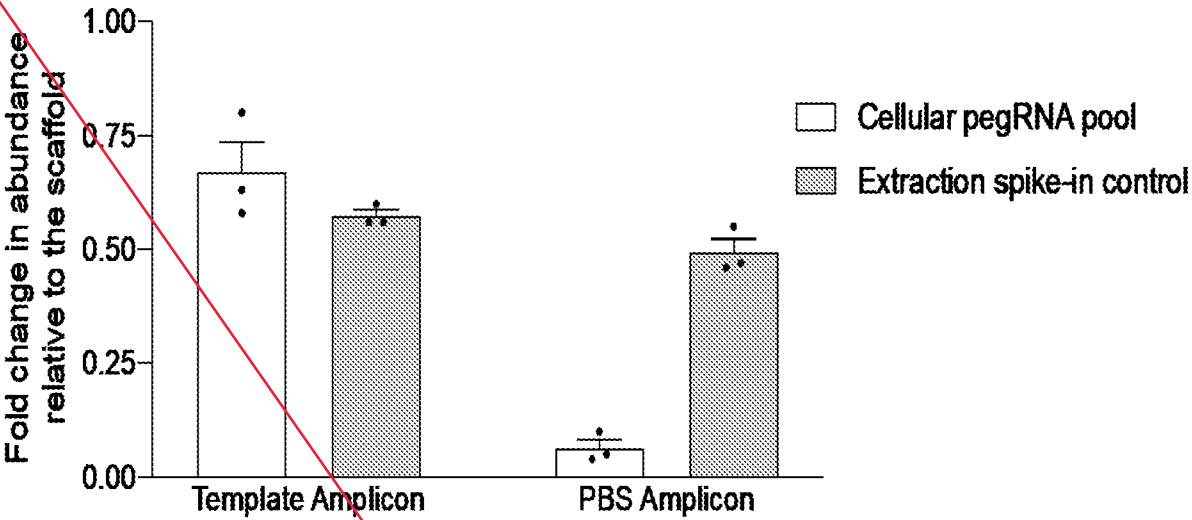


FIG. 90B

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pegRNA sequence: SEQ ID NO: 1762

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA
AAAGTGGCACCGAGTCGGTGCTGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGTCGTCATCCTTGT
ATCCGTGCTCAGTCTG

Template amplicon sequence: SEQ ID NO: 1763

AAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTGGAGGAAGCAGGGCTTCCTTTC
TCTGCCATCACITATCGTC

PBS amplicon sequence: SEQ ID NO: 1764

TGAAAAAGTGGCACCGAGTCGGTGCTGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGTCGTCATCC
TGTAATCCGTGCTCAGTCTGT

FIG. 90C

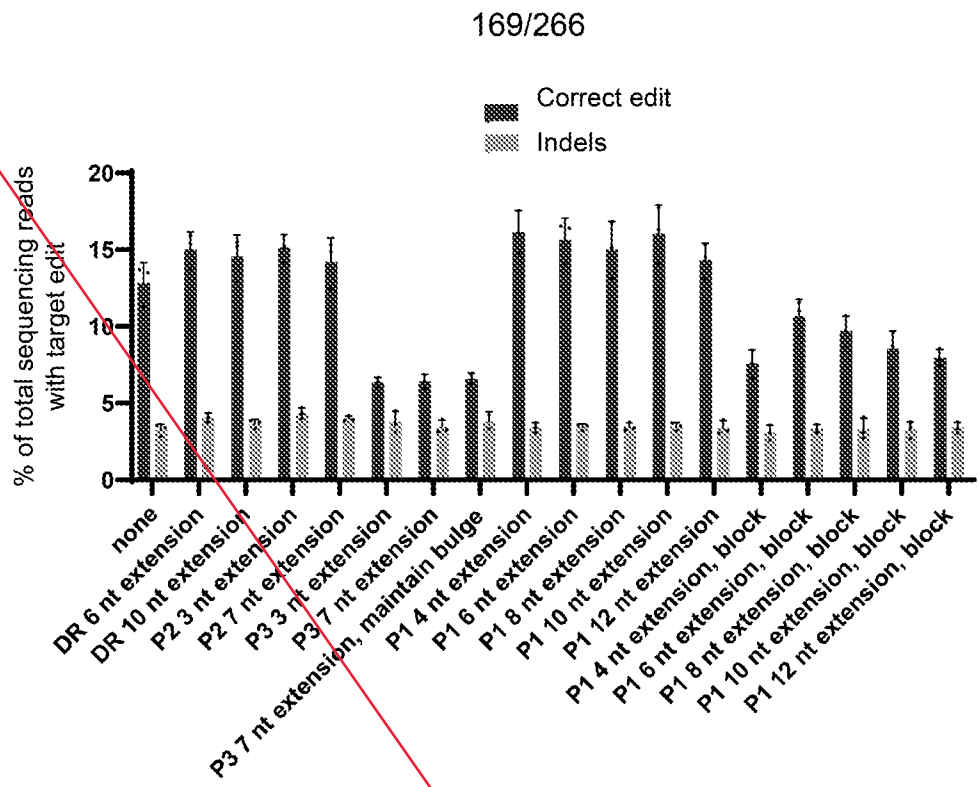


FIG. 91A

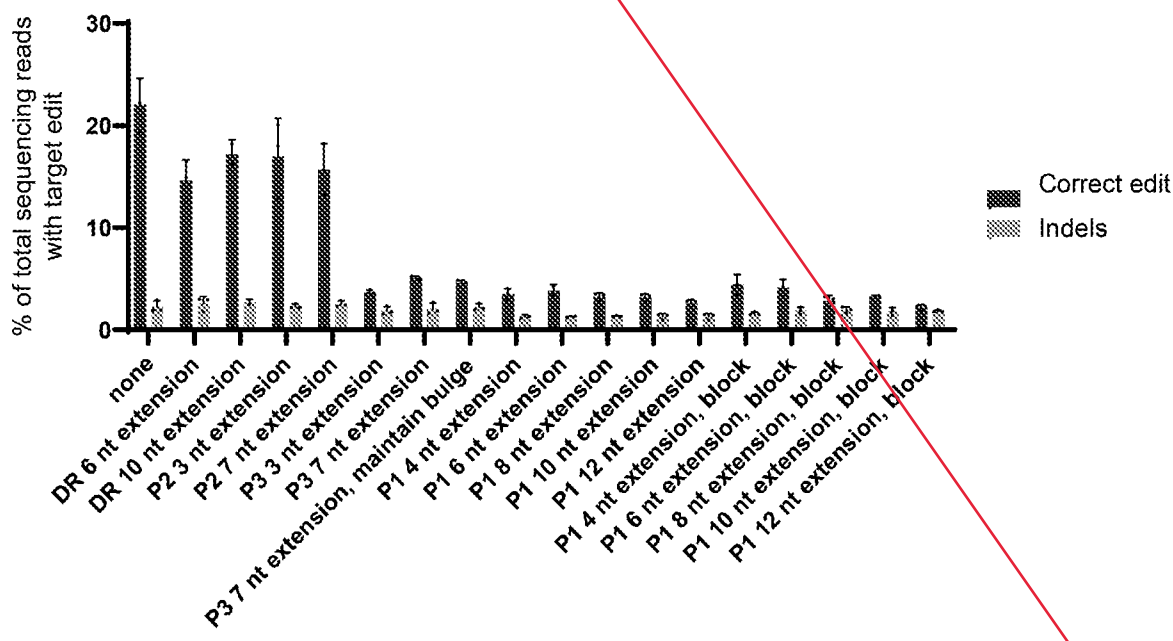


FIG. 91B

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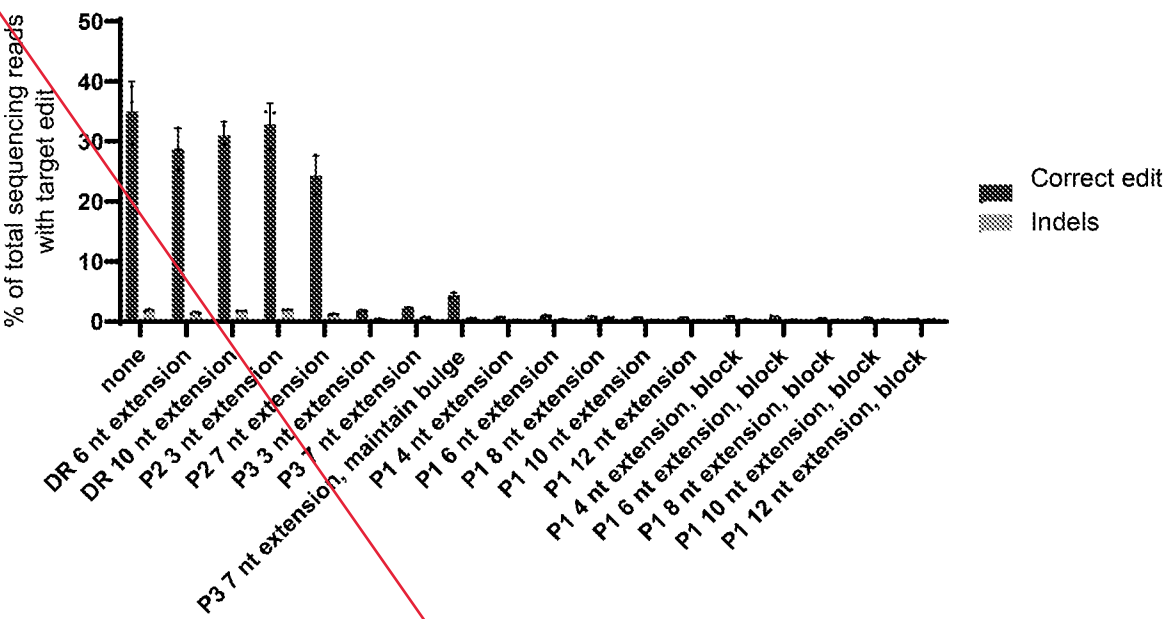


FIG. 91C

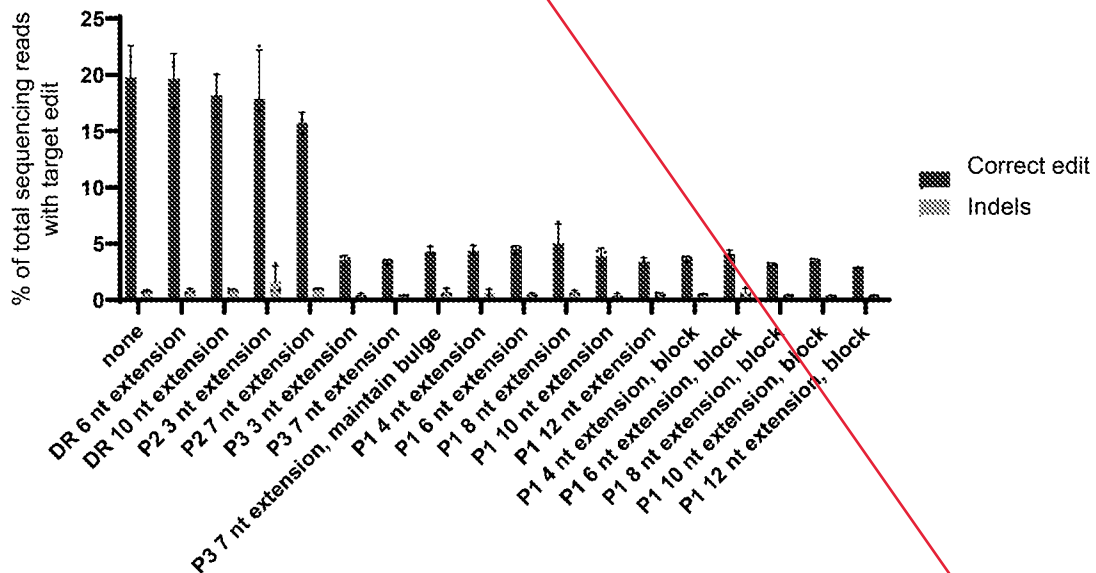


FIG. 91D

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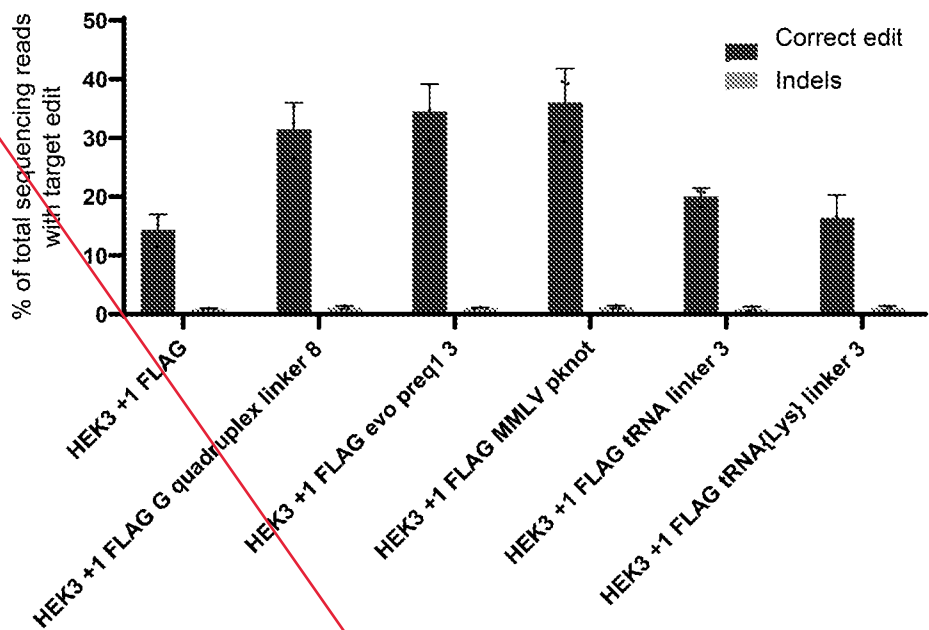


FIG. 92A

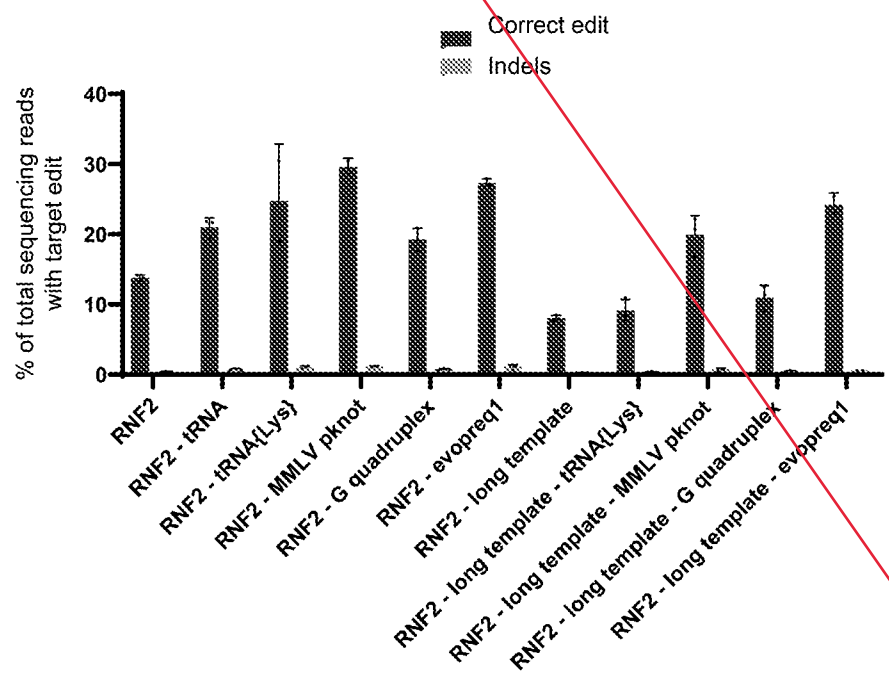


FIG. 92B

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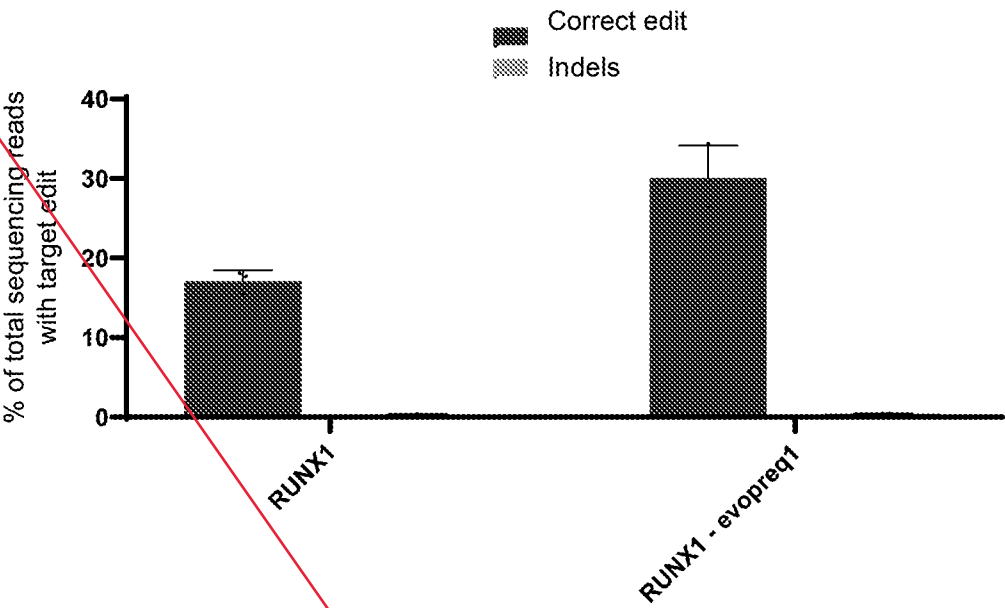


FIG. 92C

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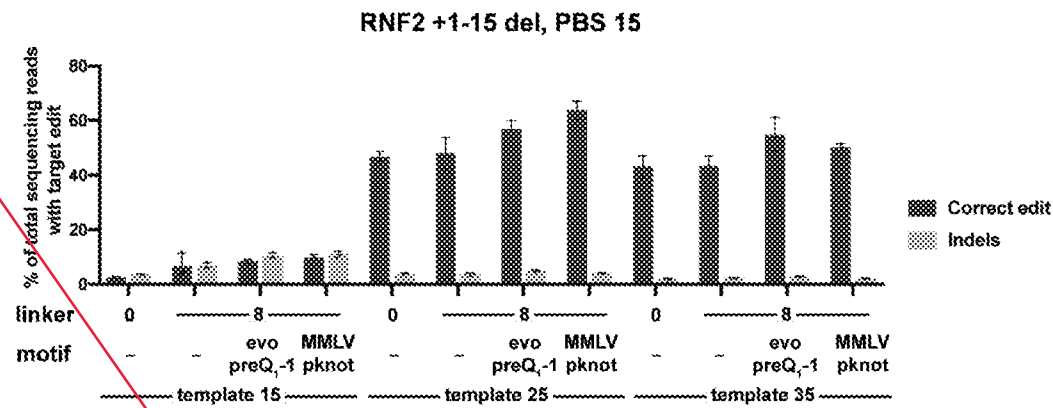


FIG. 93A

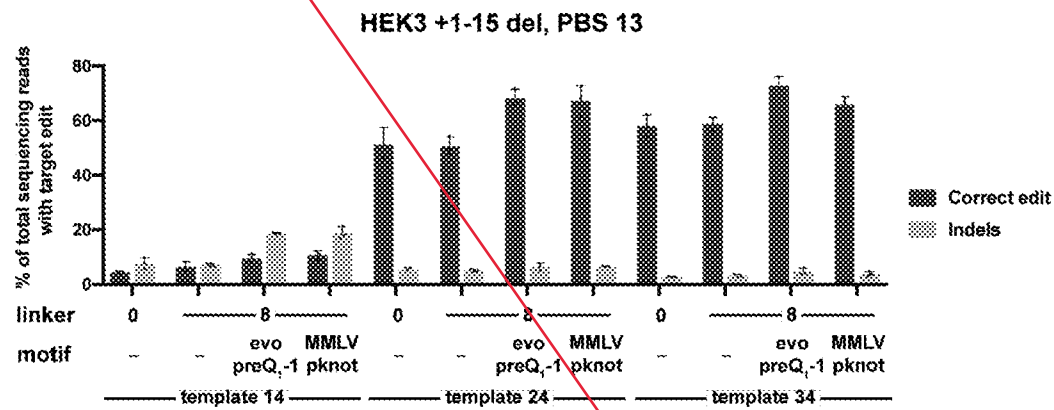


FIG. 93B

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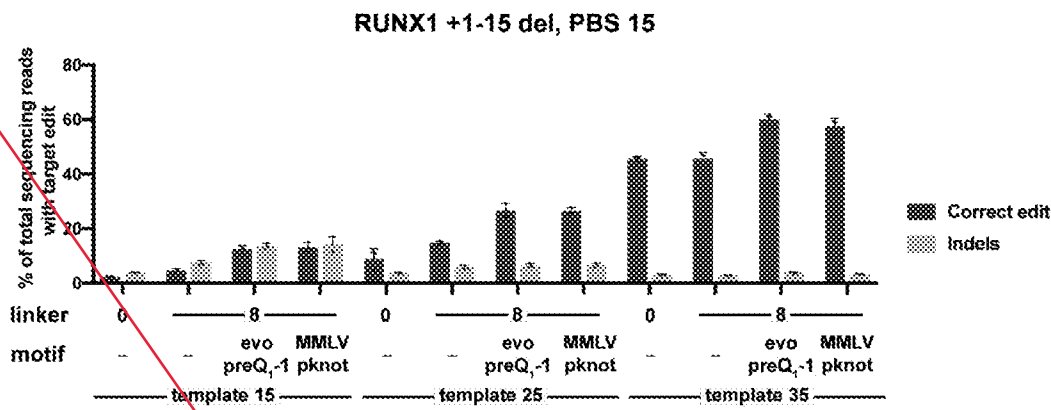


FIG. 93C

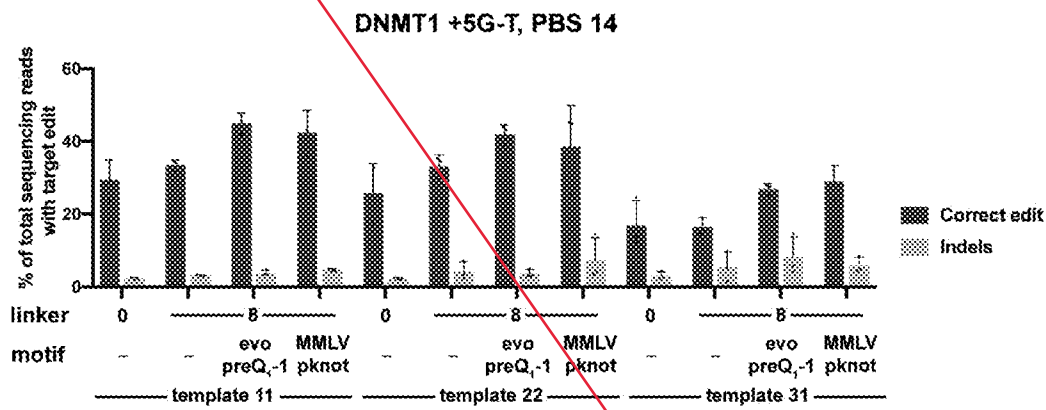


FIG. 93D

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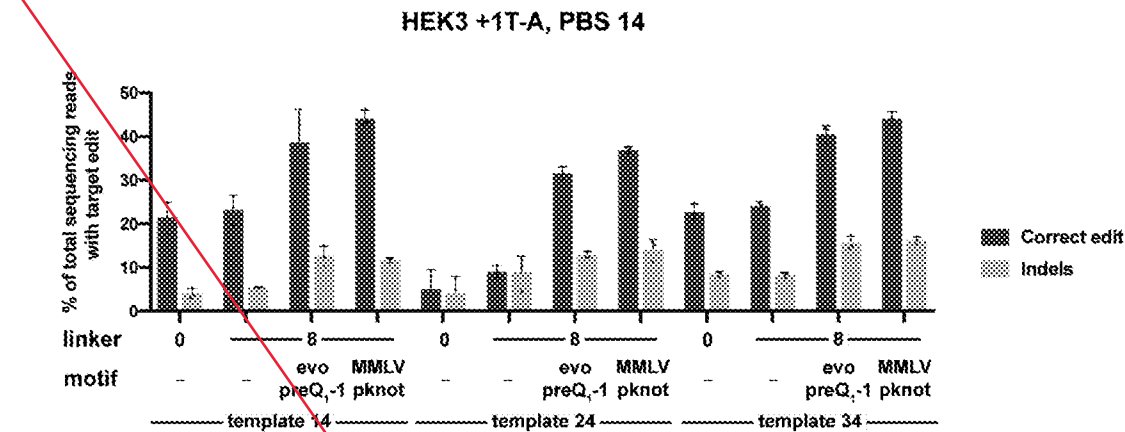


FIG. 93E

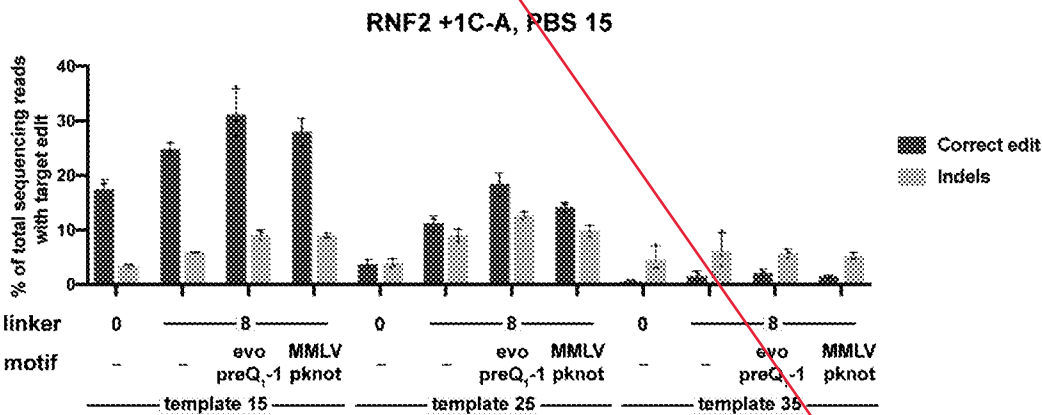


FIG. 93F

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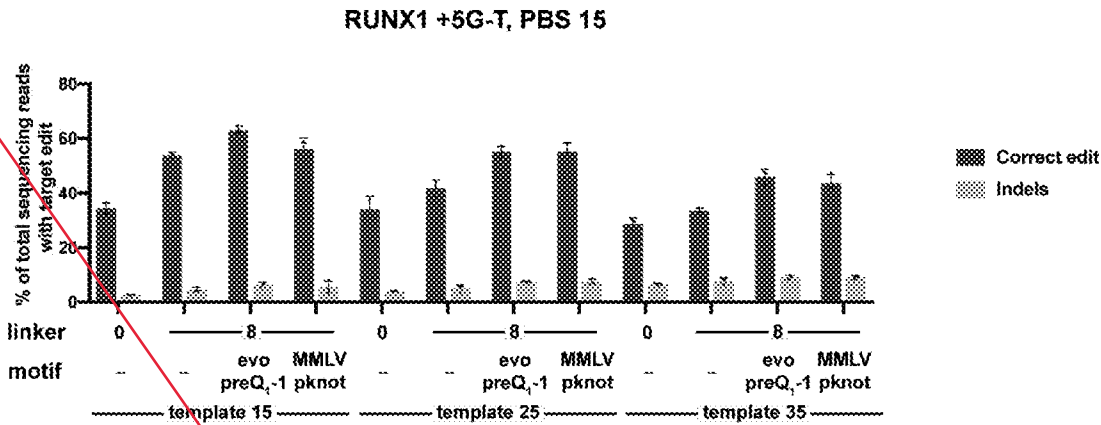


FIG. 93G

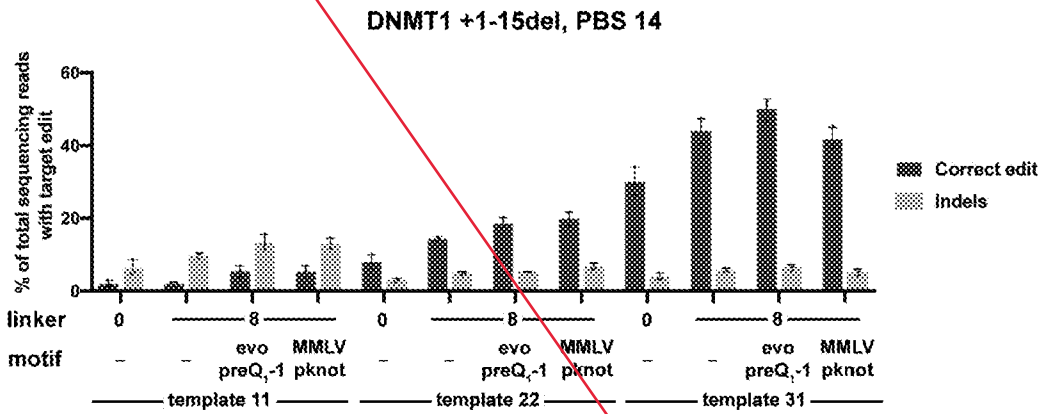


FIG. 93H

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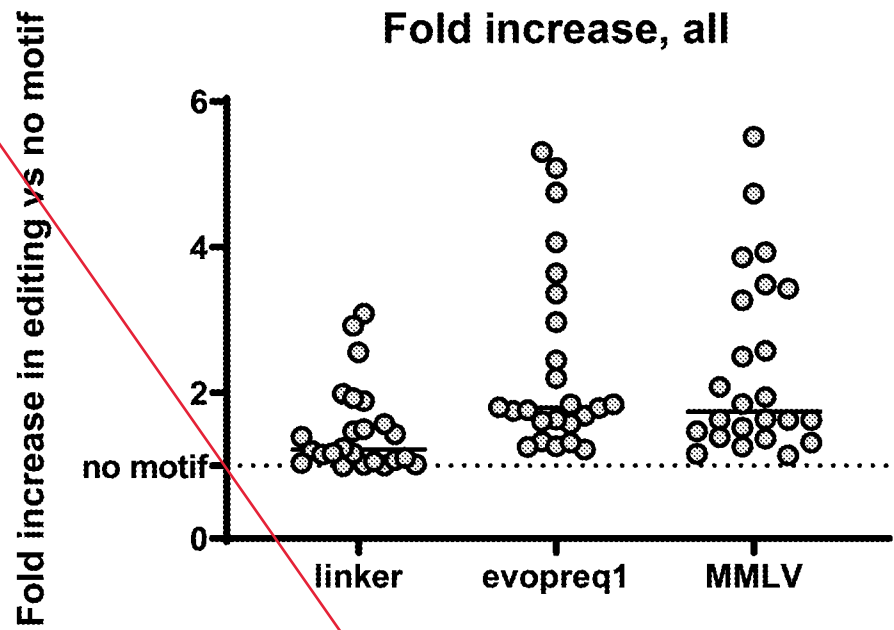


FIG. 94

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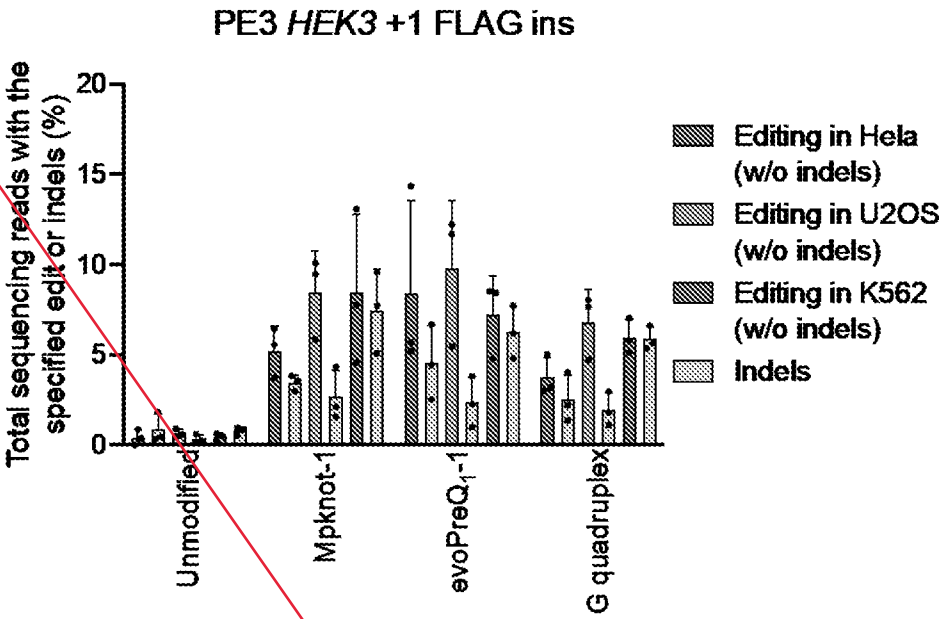


FIG. 95A

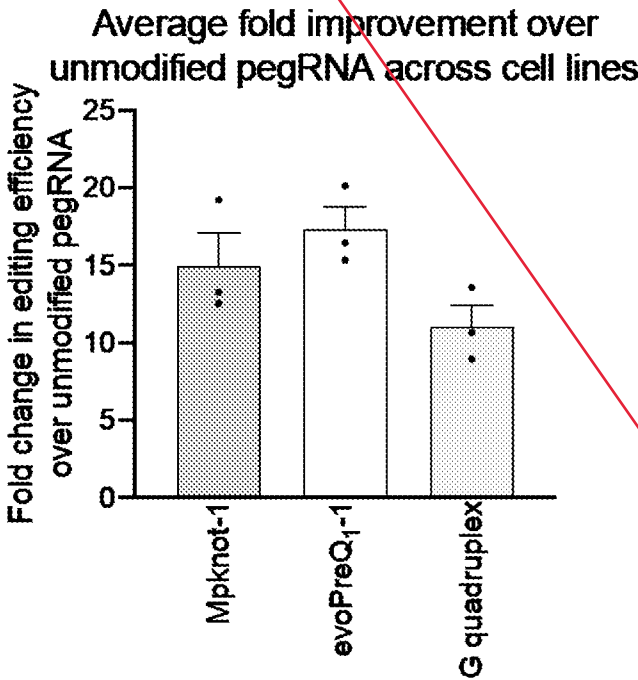


FIG. 95B

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PE2
HEK3 +1 FLAG

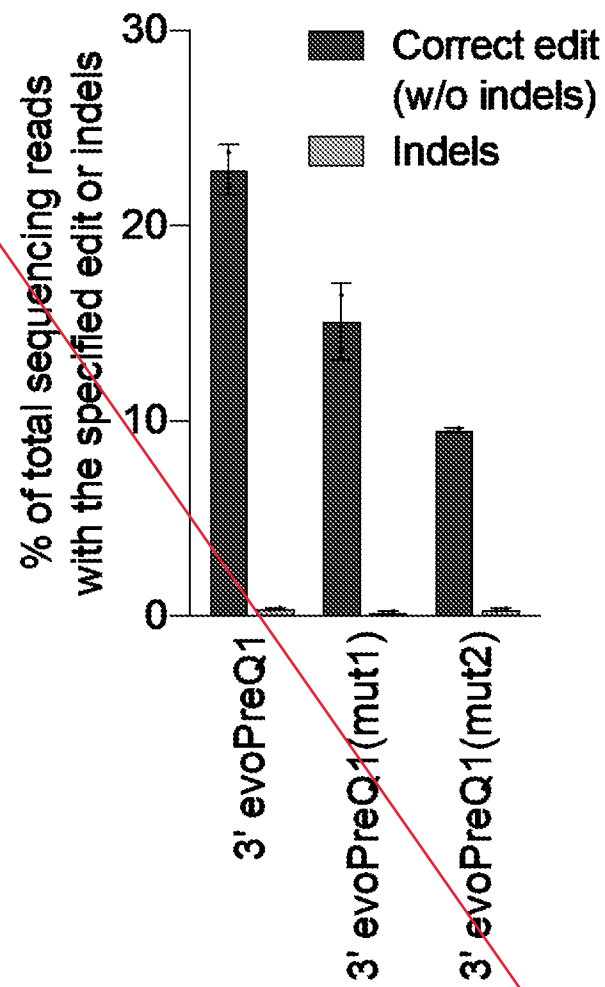


FIG. 96

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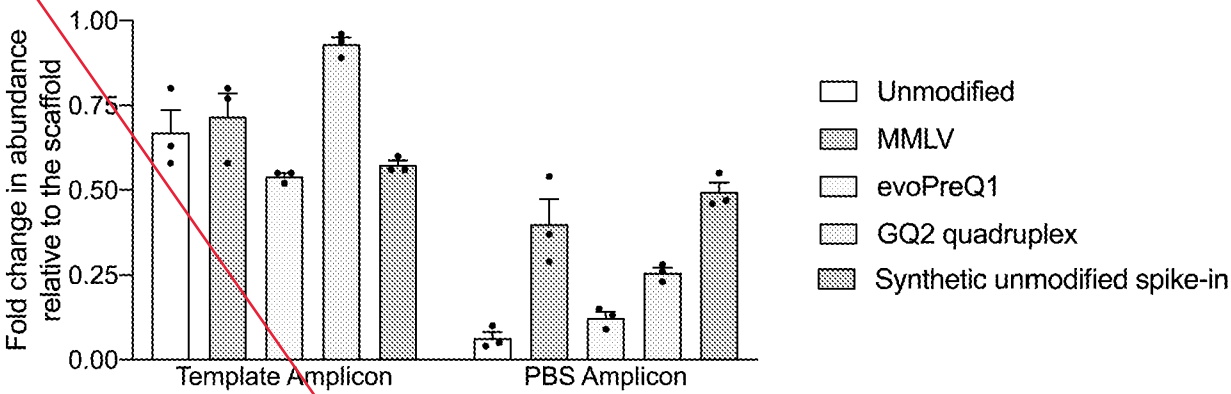
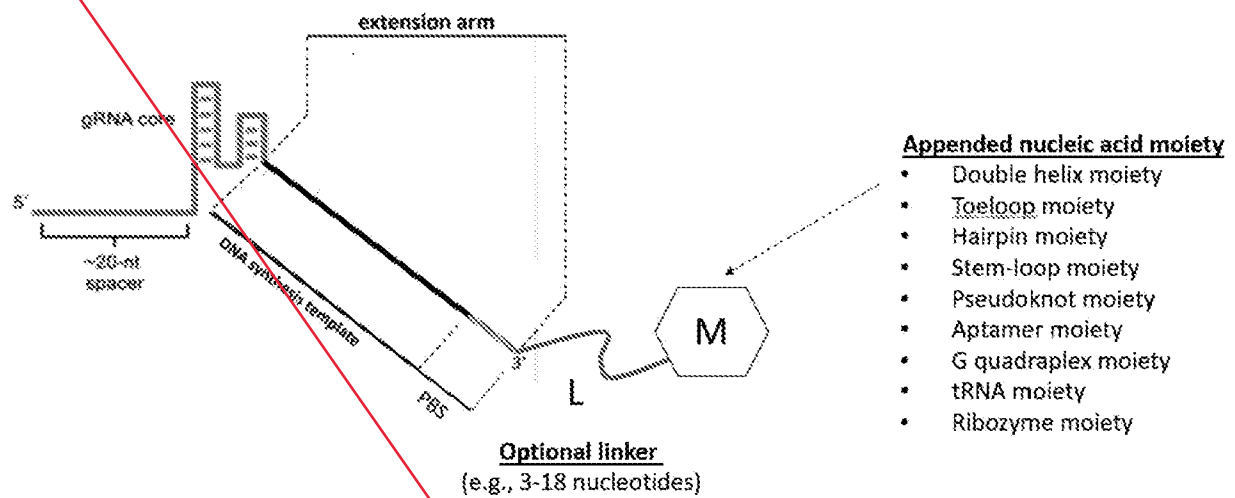


FIG. 97

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pegRNA appended at 3' end with nucleic acid moiety**FIG. 98**

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U6 promoter for enhanced transcription of pegRNAs

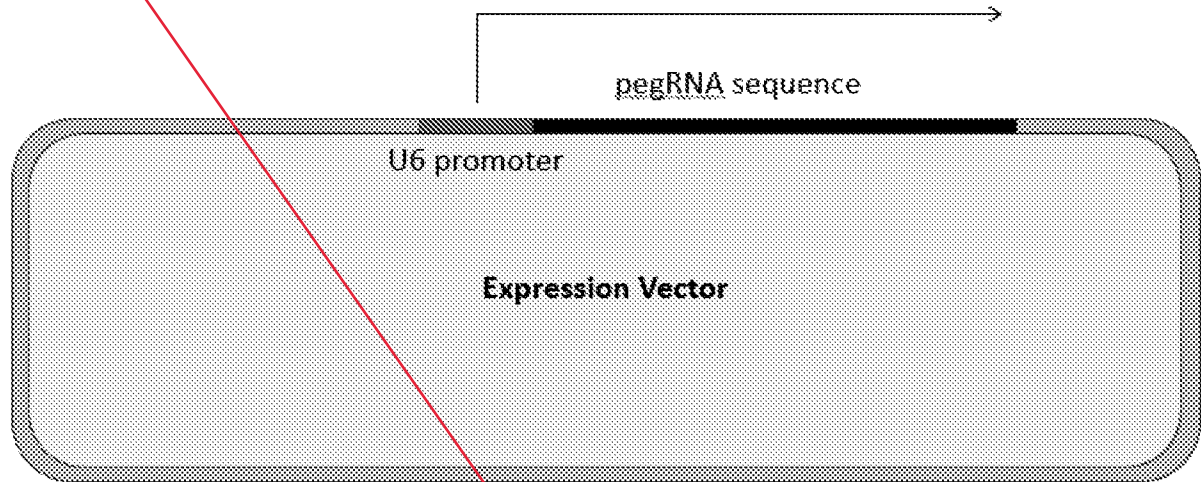


FIG. 99

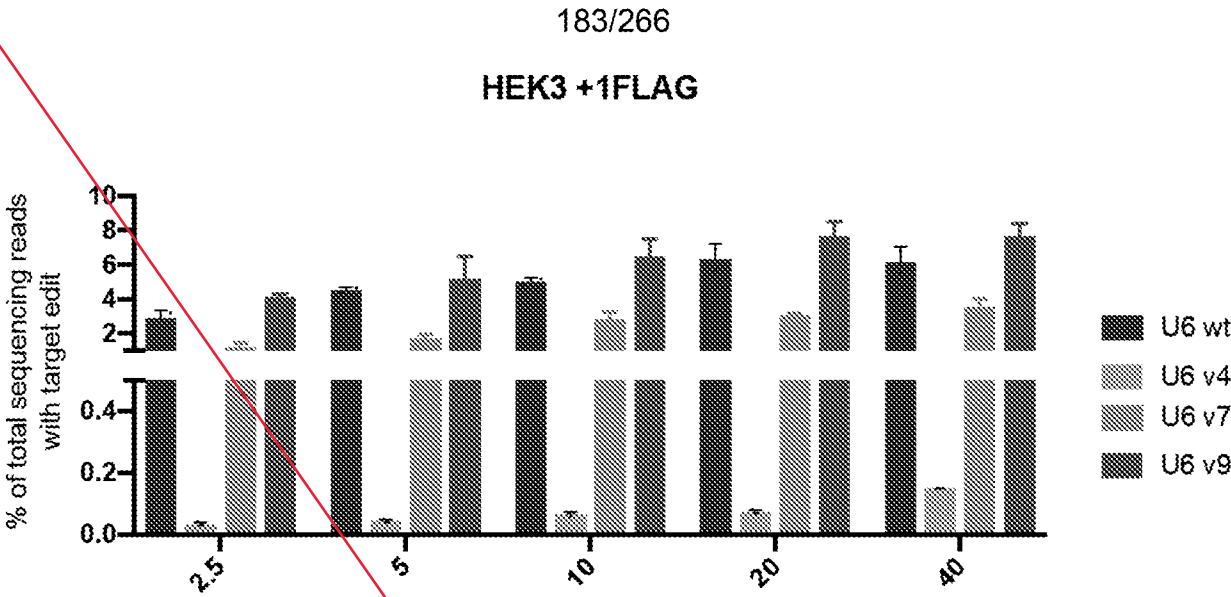


FIG. 100

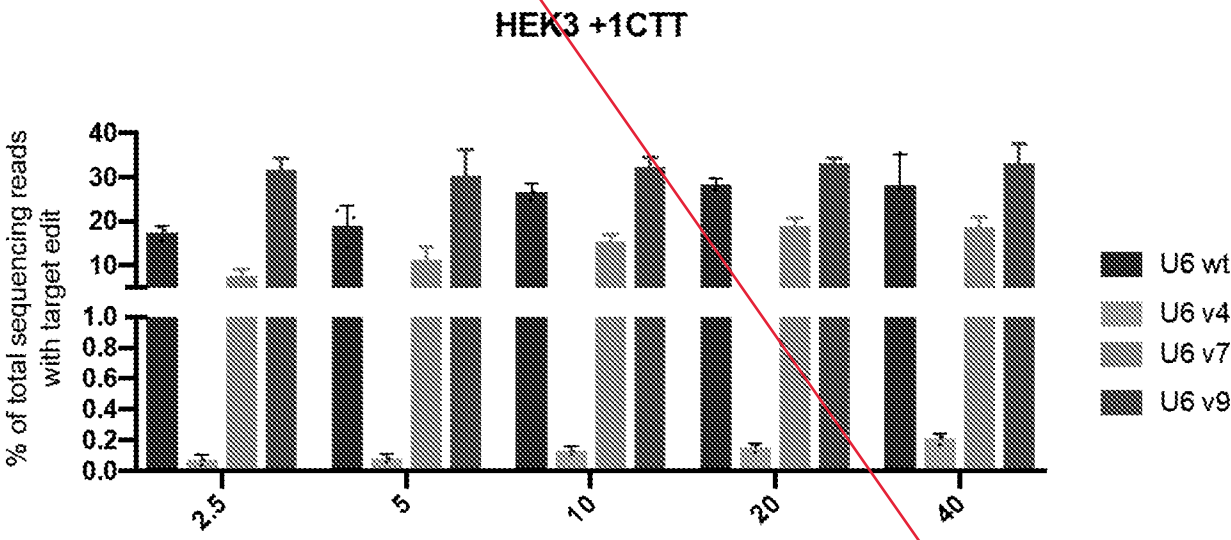


FIG. 100B

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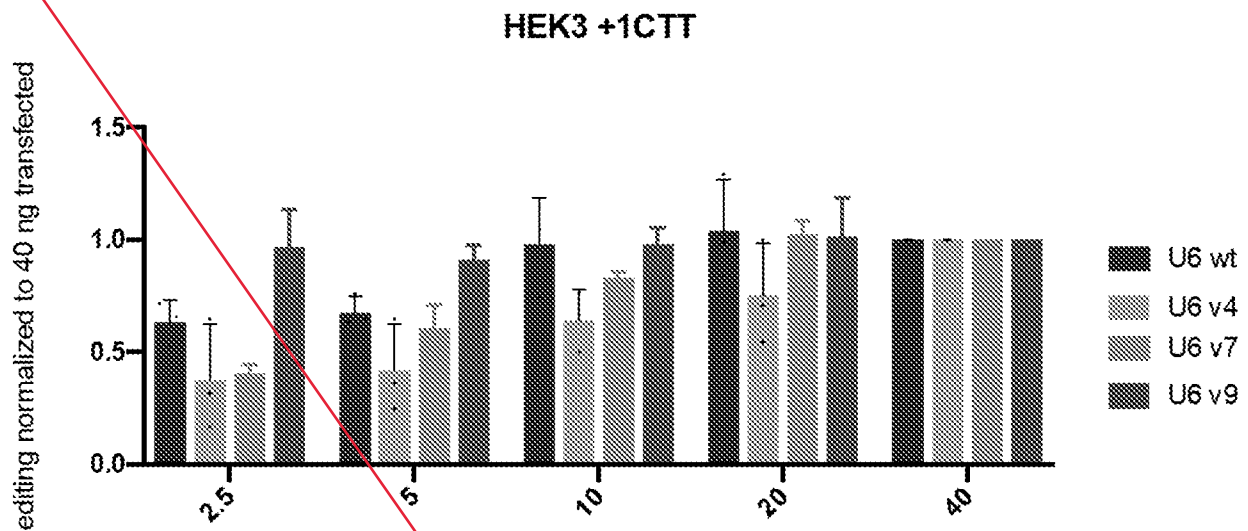


FIG. 100C

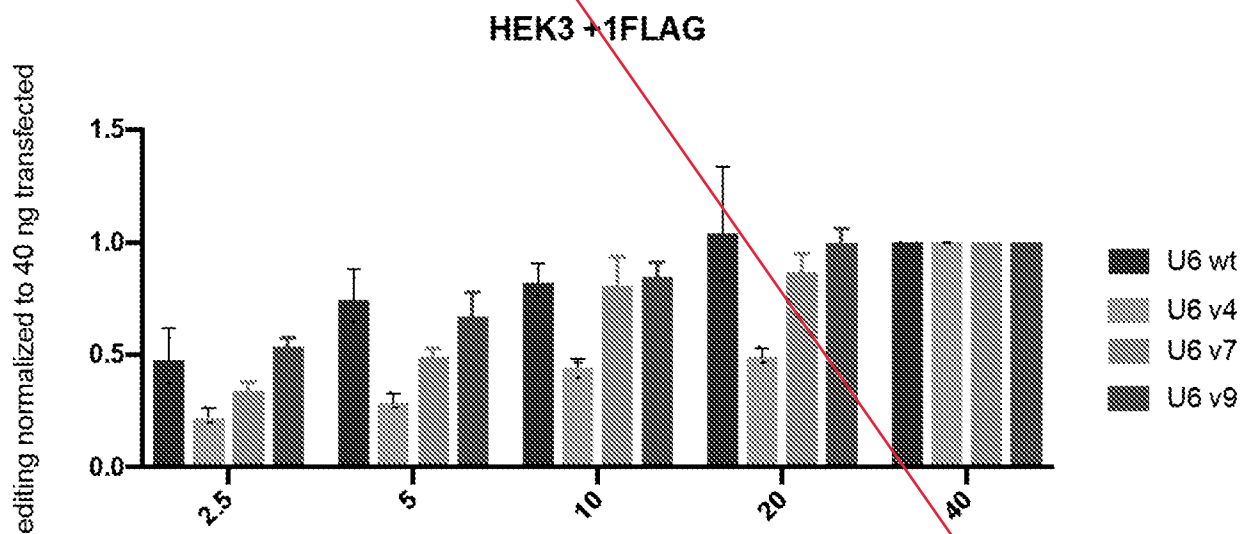


FIG. 100D

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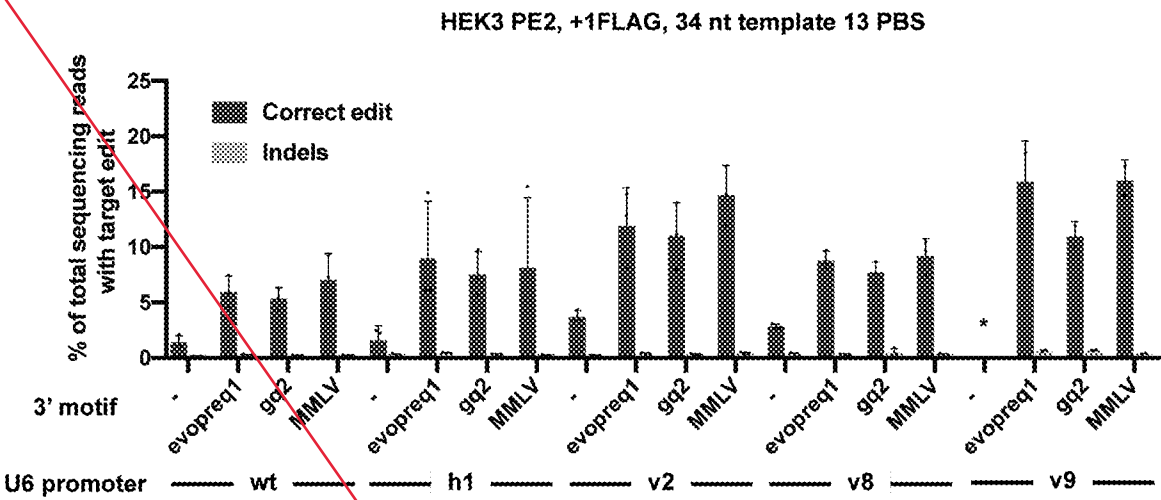


FIG. 100E

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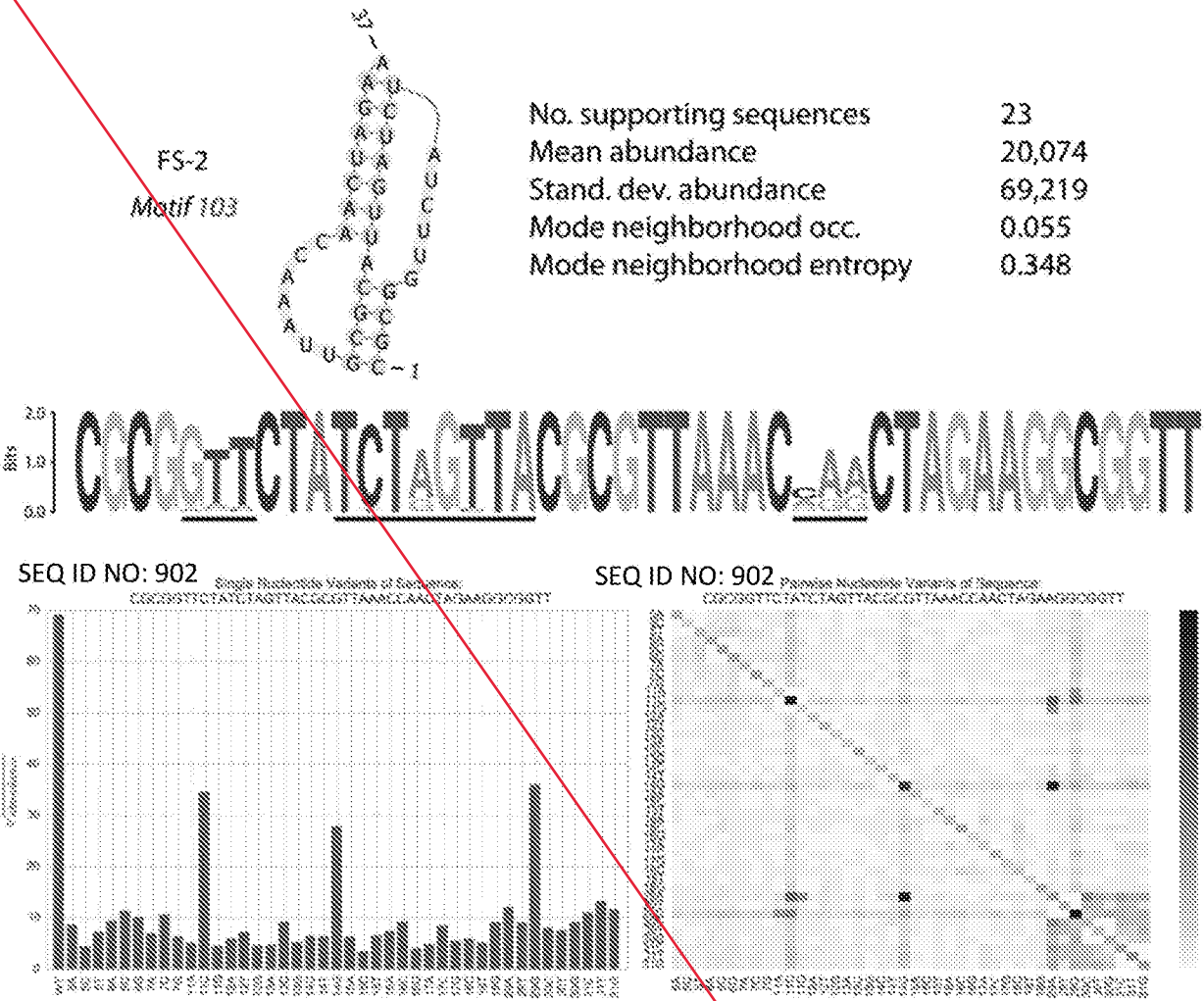


FIG. 101

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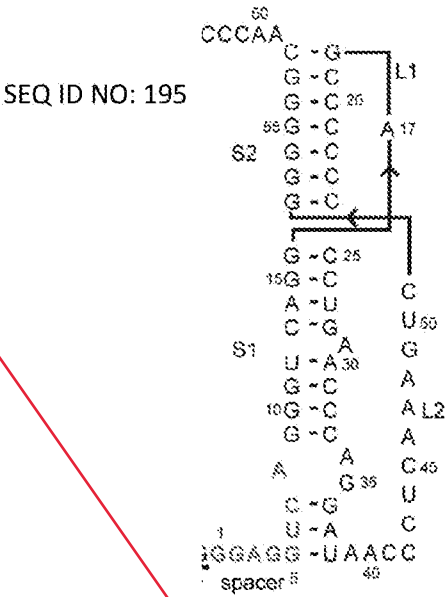
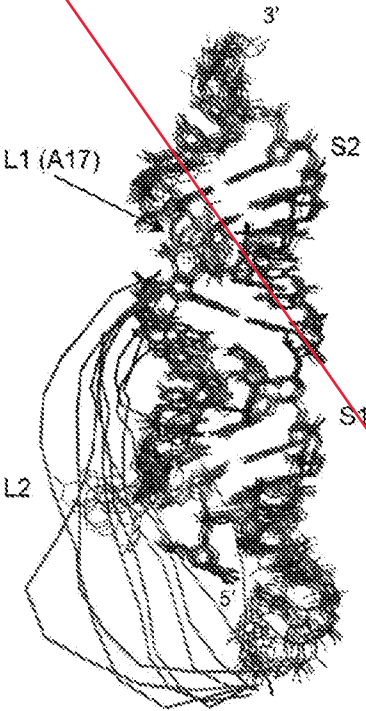


FIG. 102

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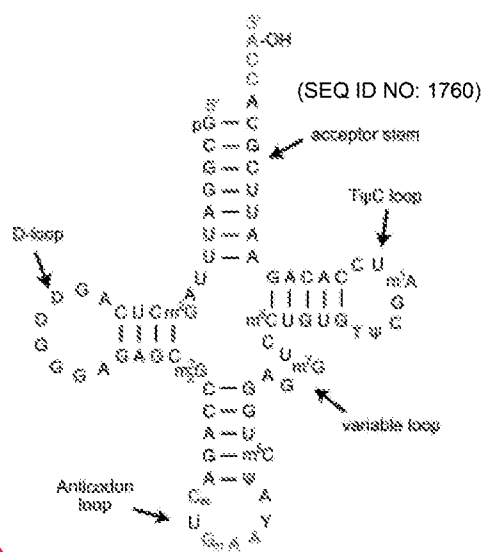


FIG. 103A

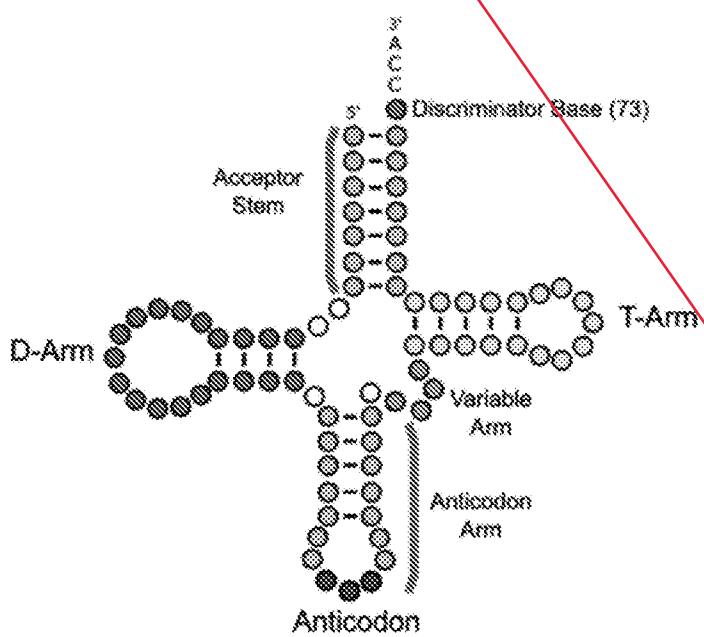


FIG. 103B

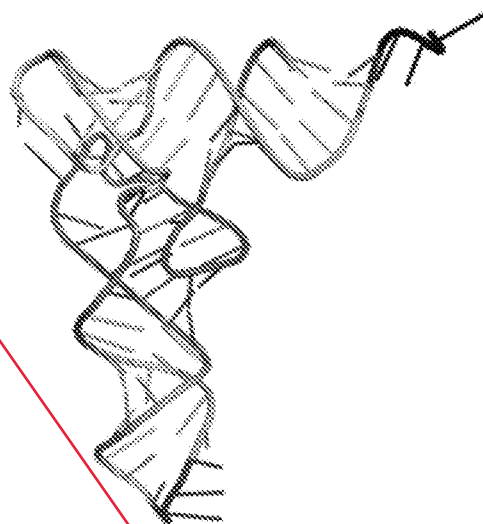


FIG. 103C

FIG. 104A

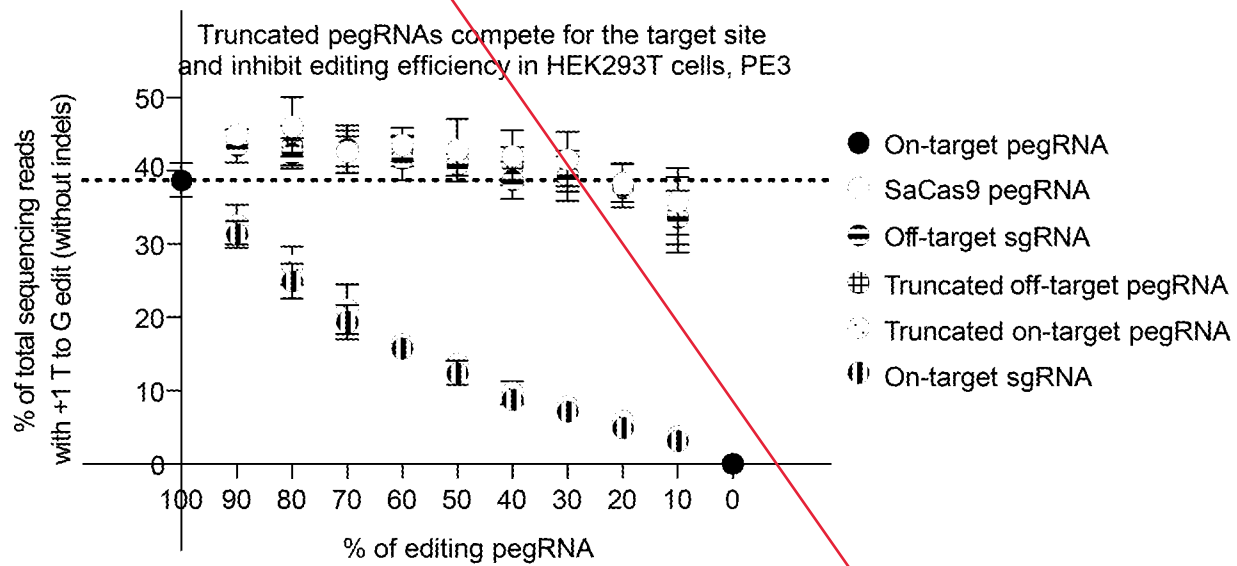


FIG. 104B

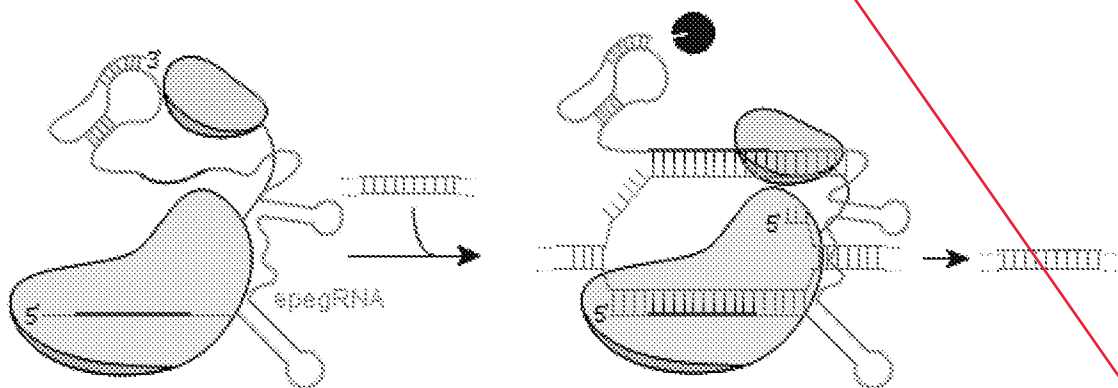


FIG. 104C

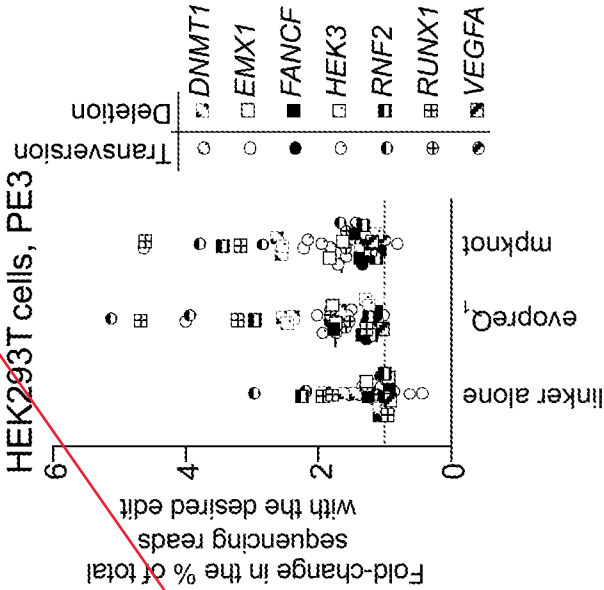


FIG. 105B

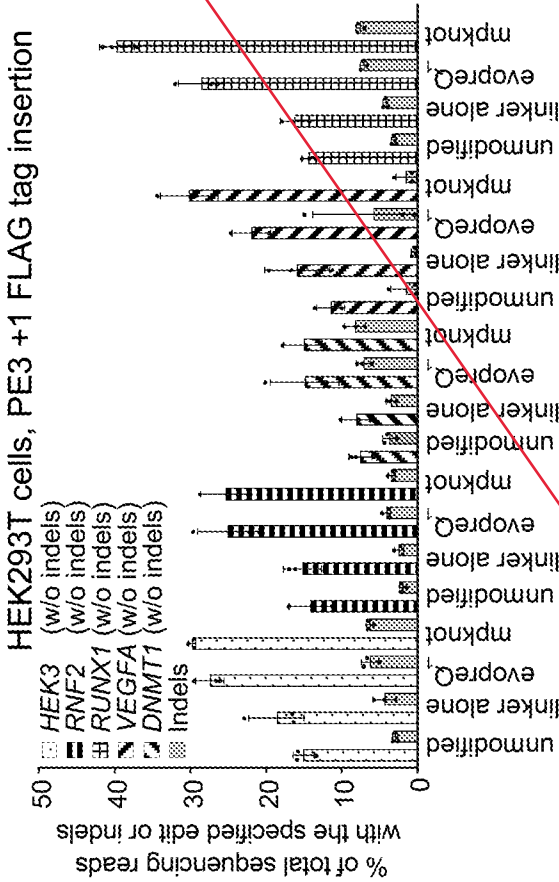


FIG. 105A

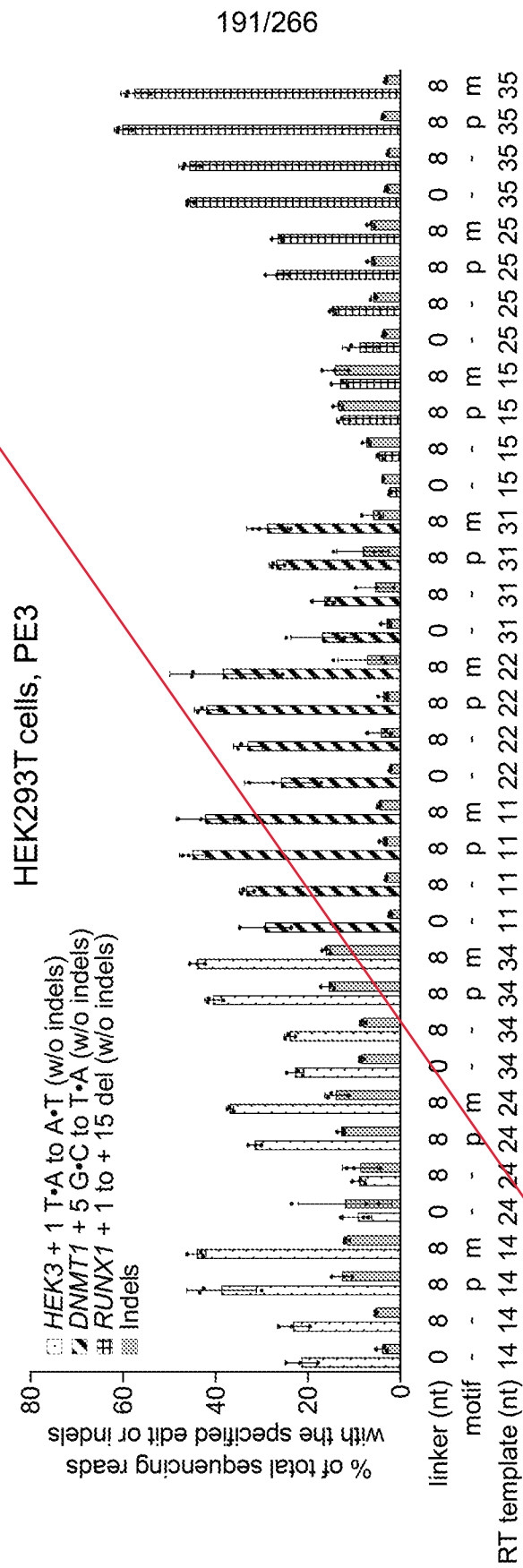


FIG. 105C

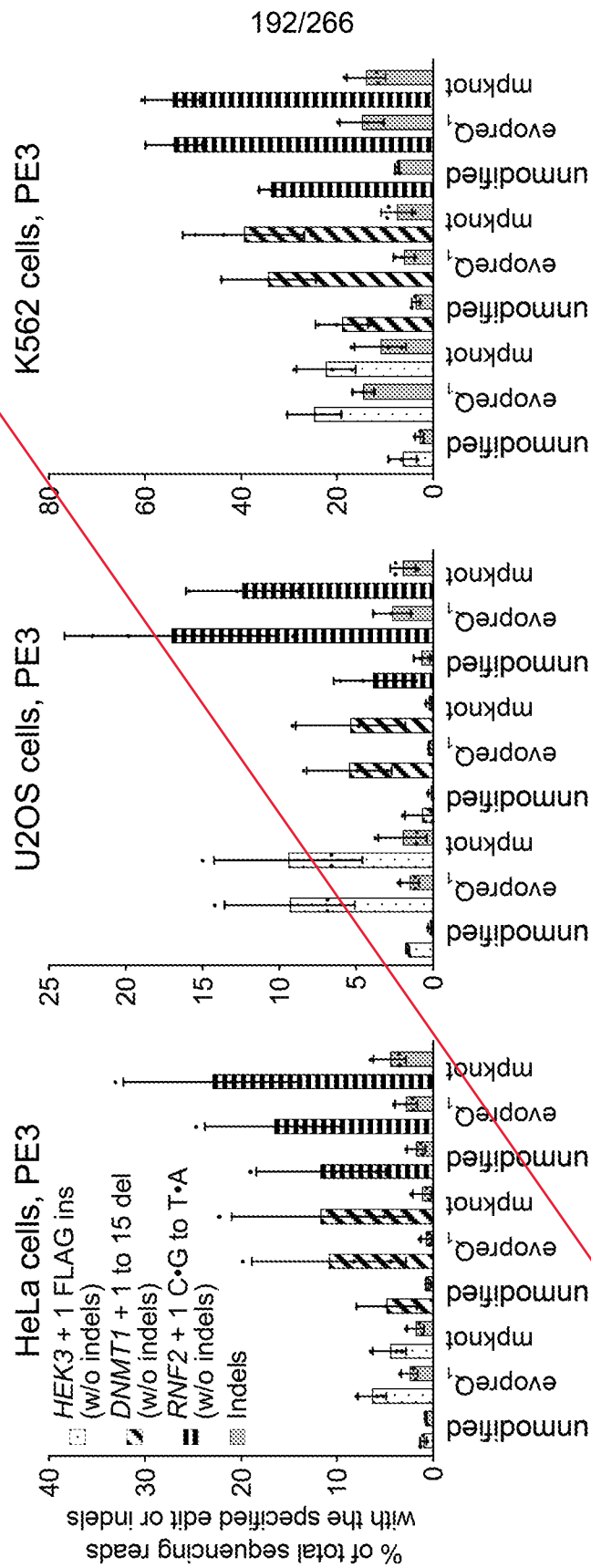


FIG. 105D

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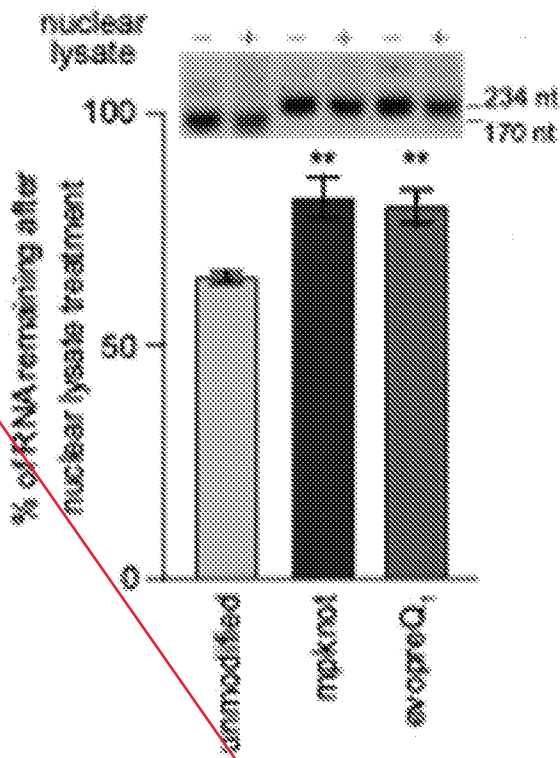


FIG. 106A

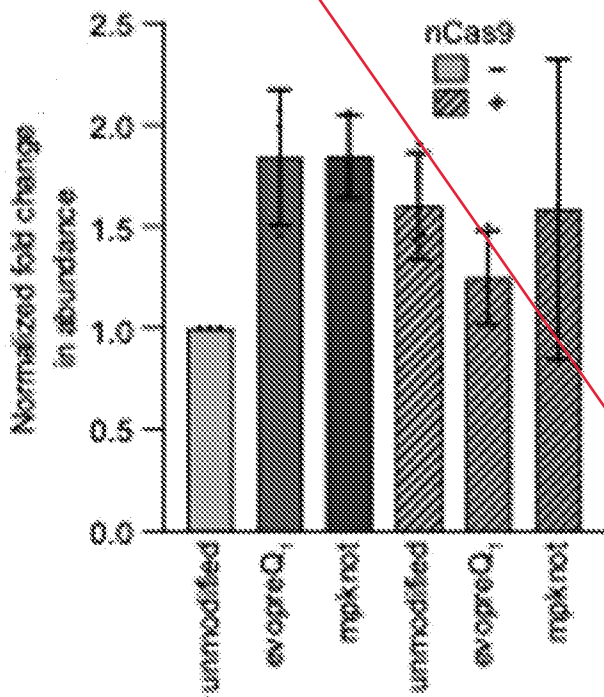


FIG. 106B

HEK293T, RNF2 + 1 C-G-to-A-T

Figure 1 consists of a histogram and two pie charts. The histogram shows the percentage of PCR intermediate-containing reads versus flap length (nt) for unmodified (white bars) and evopreQ₁ (black bars) DNA polymerases. The x-axis ranges from -10 to 60 nt, and the y-axis ranges from 0 to 100%. A red diagonal line is drawn across the histogram, indicating a threshold. The pie charts show the distribution of intermediates with and without the desired edit for each polymerase.

Flap length (nt)	unmodified (%)	evopreQ ₁ (%)
-10	0	0
-5	0	0
0	0	0
5	0	0
10	0	0
15	0	0
20	0	0
25	0	0
30	0	0
35	0	0
40	0	0
45	0	0
50	0	0
55	0	0
60	0	0

unmodified

- 76.7% Intermediates w/ desired edit
- 23.3% Intermediates w/o desired edit

evopreQ₁

- 90.2% Intermediates w/ desired edit
- 9.8% Intermediates w/o desired edit

HEK293T ~~cells~~, PE3

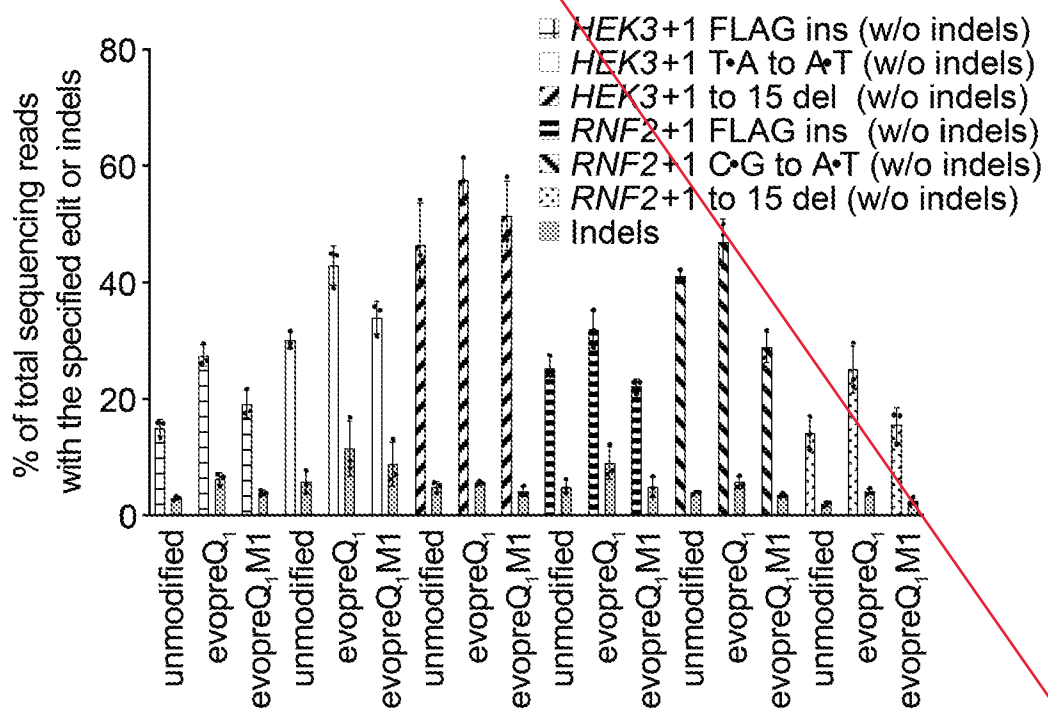


FIG. 106D

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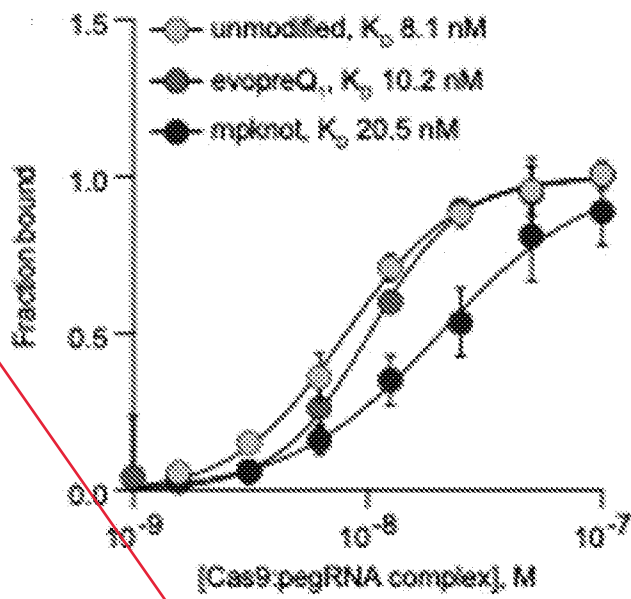


FIG. 106E

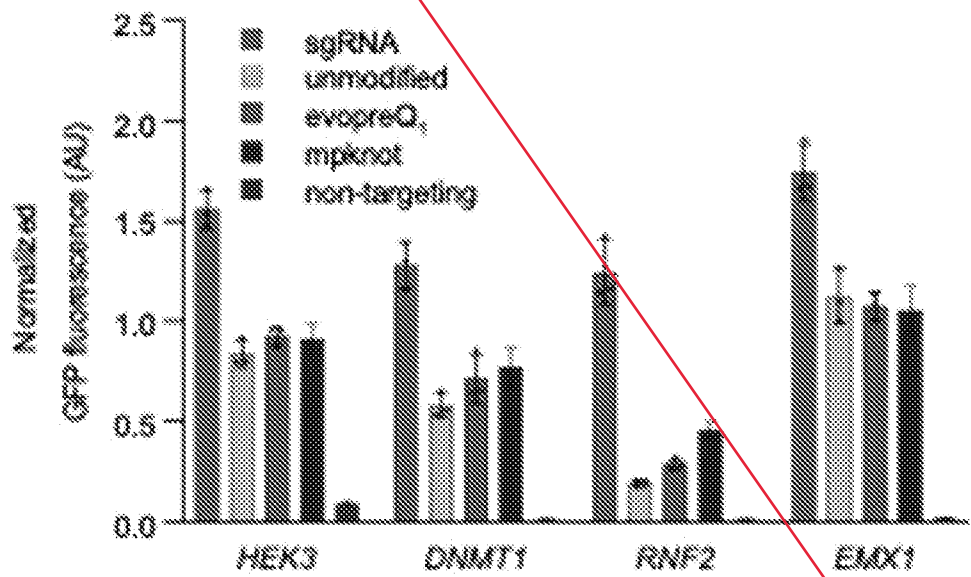


FIG. 106F

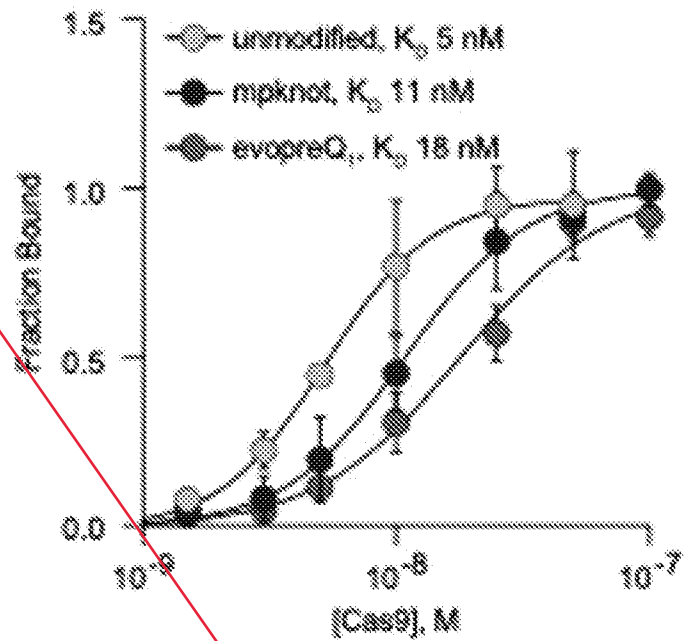


FIG. 106G

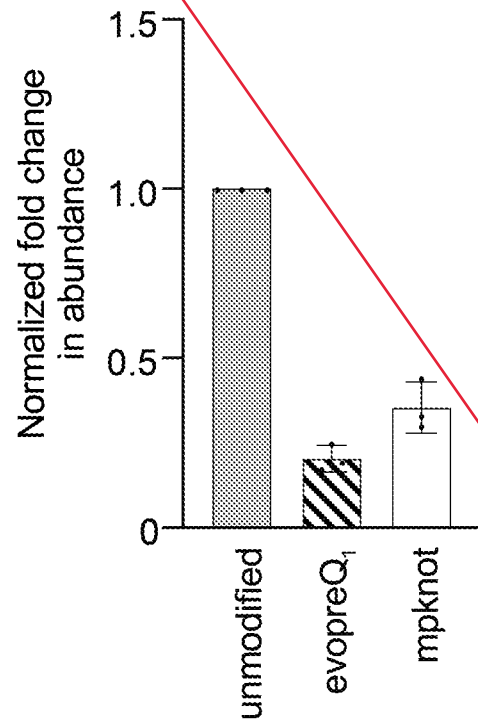


FIG. 106H

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Installation of the protective
G127V mutation in *PRNP*
in HEK293T cells

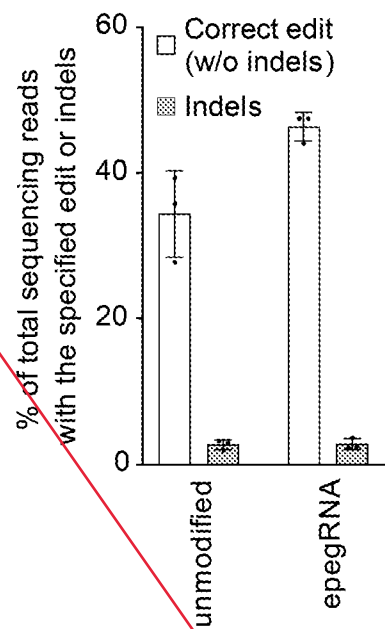


FIG. 107A

Correction of the pathogenic
1278insTATC mutation in
HEXA in HEK293T cells

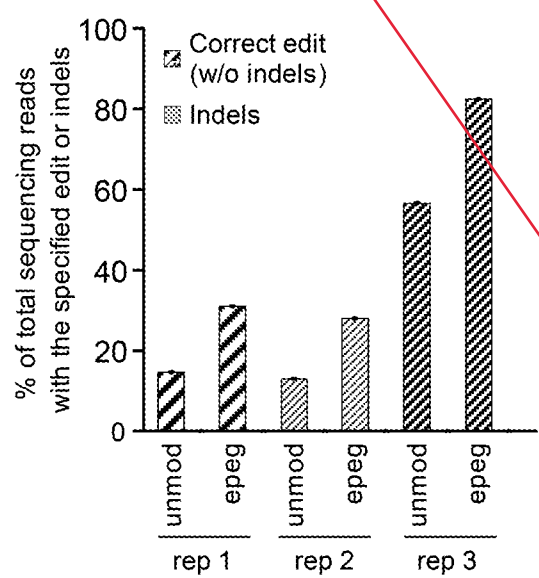


FIG. 107B

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Correction of the pathogenic
1278insTATC mutation in HEXA
in patient-derived fibroblasts

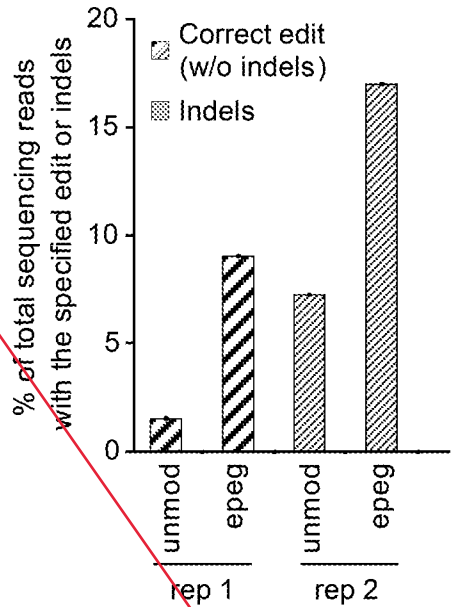


FIG. 107C

PE2 installation of nine pathogenic and protective alleles
with unoptimized pegRNAs and epegRNAs in HEK293T cells

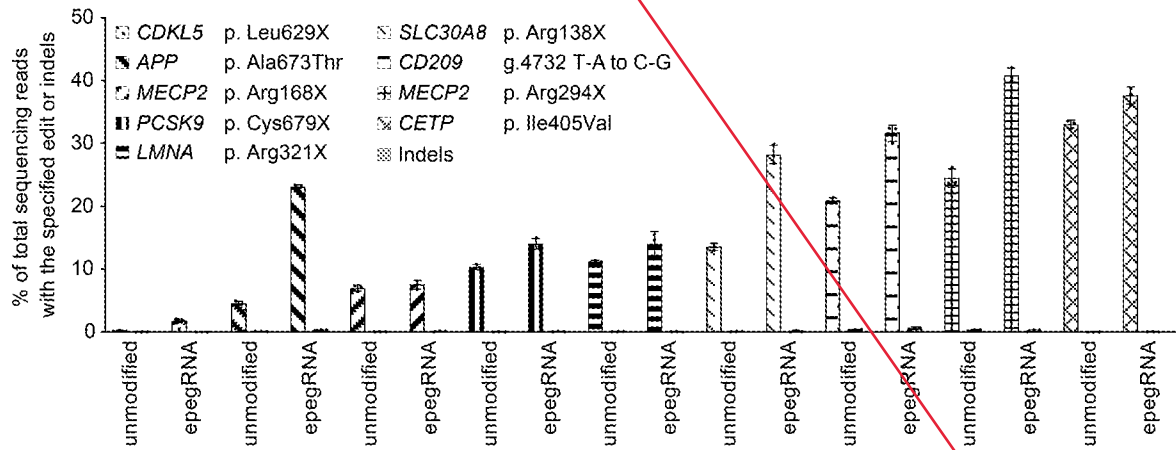


FIG. 107D

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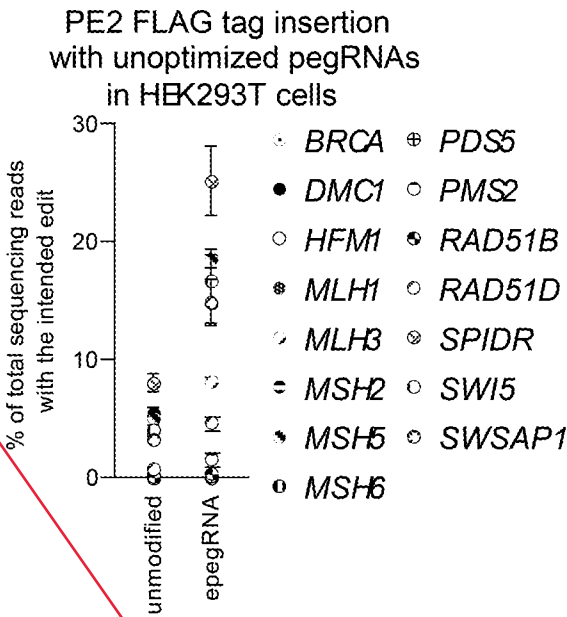
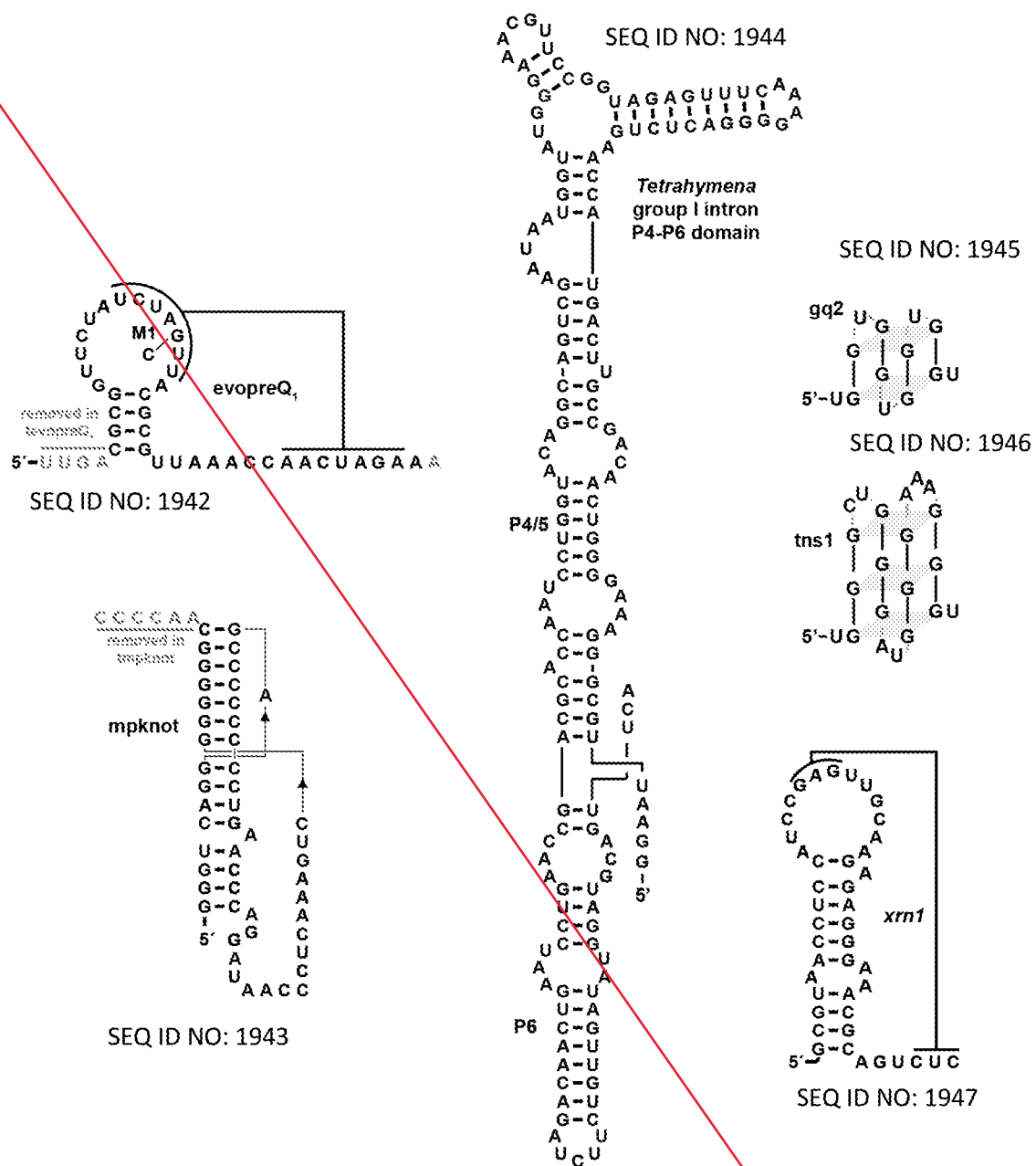


FIG. 107E

FIG. 108



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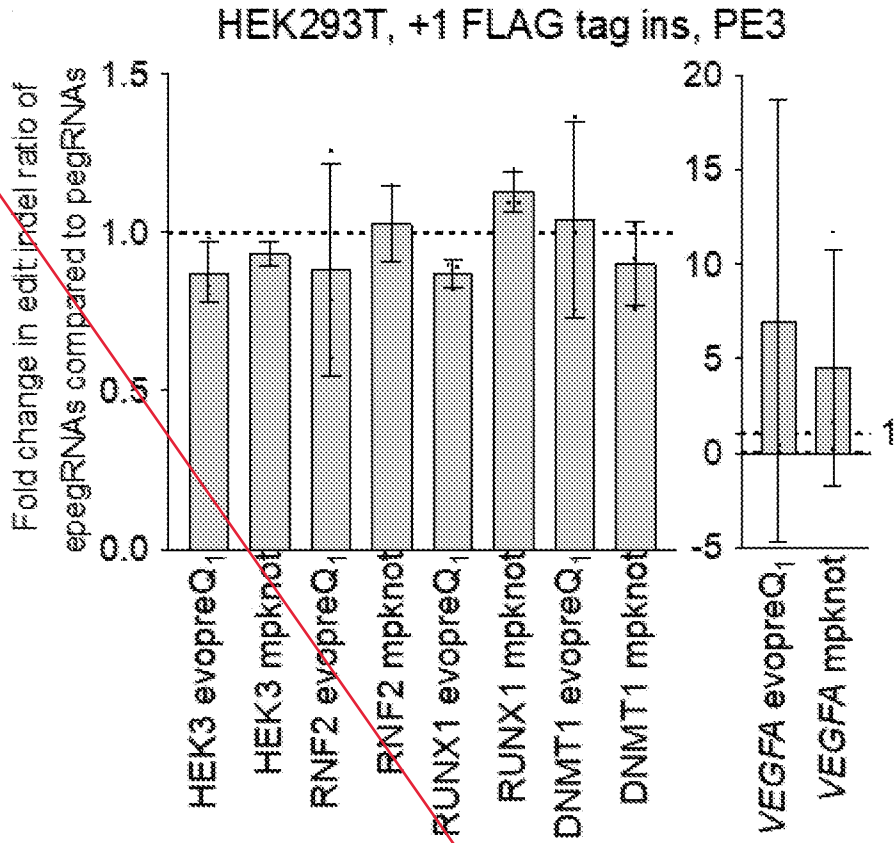


FIG. 109A

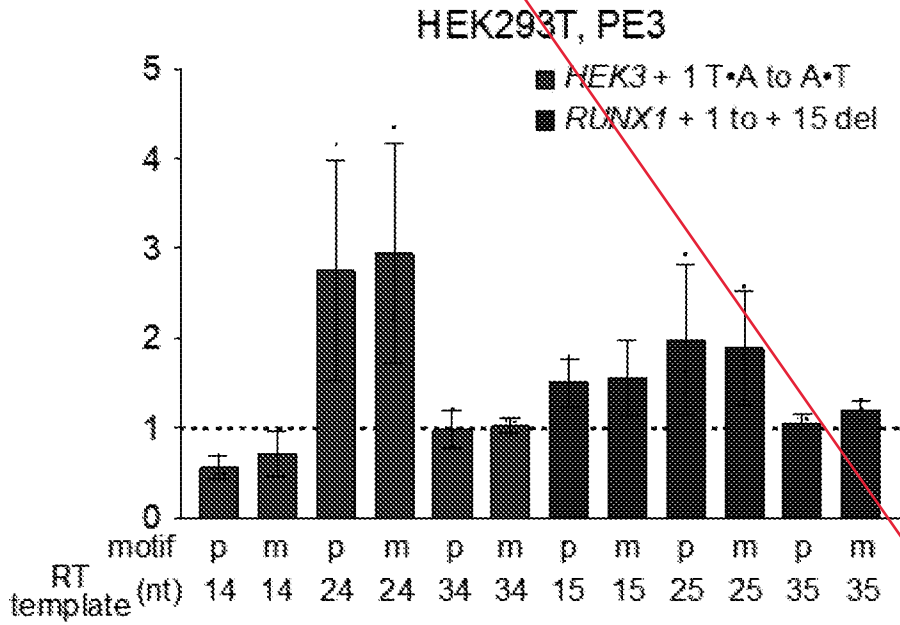


FIG. 109B

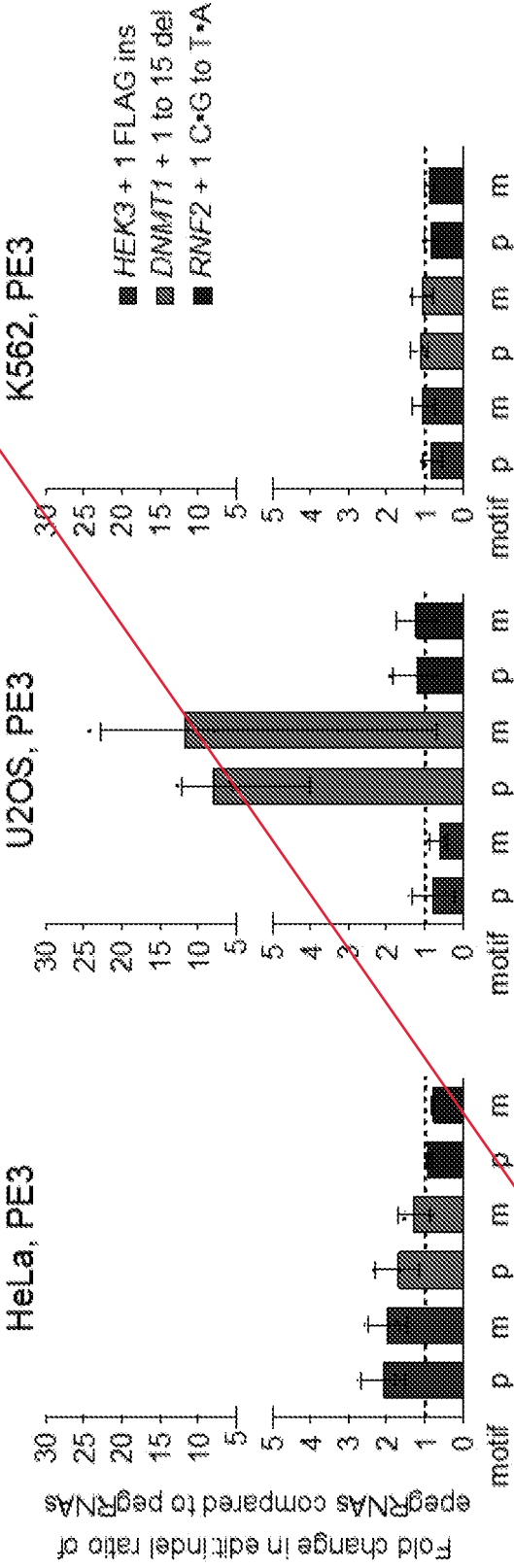


FIG. 109C

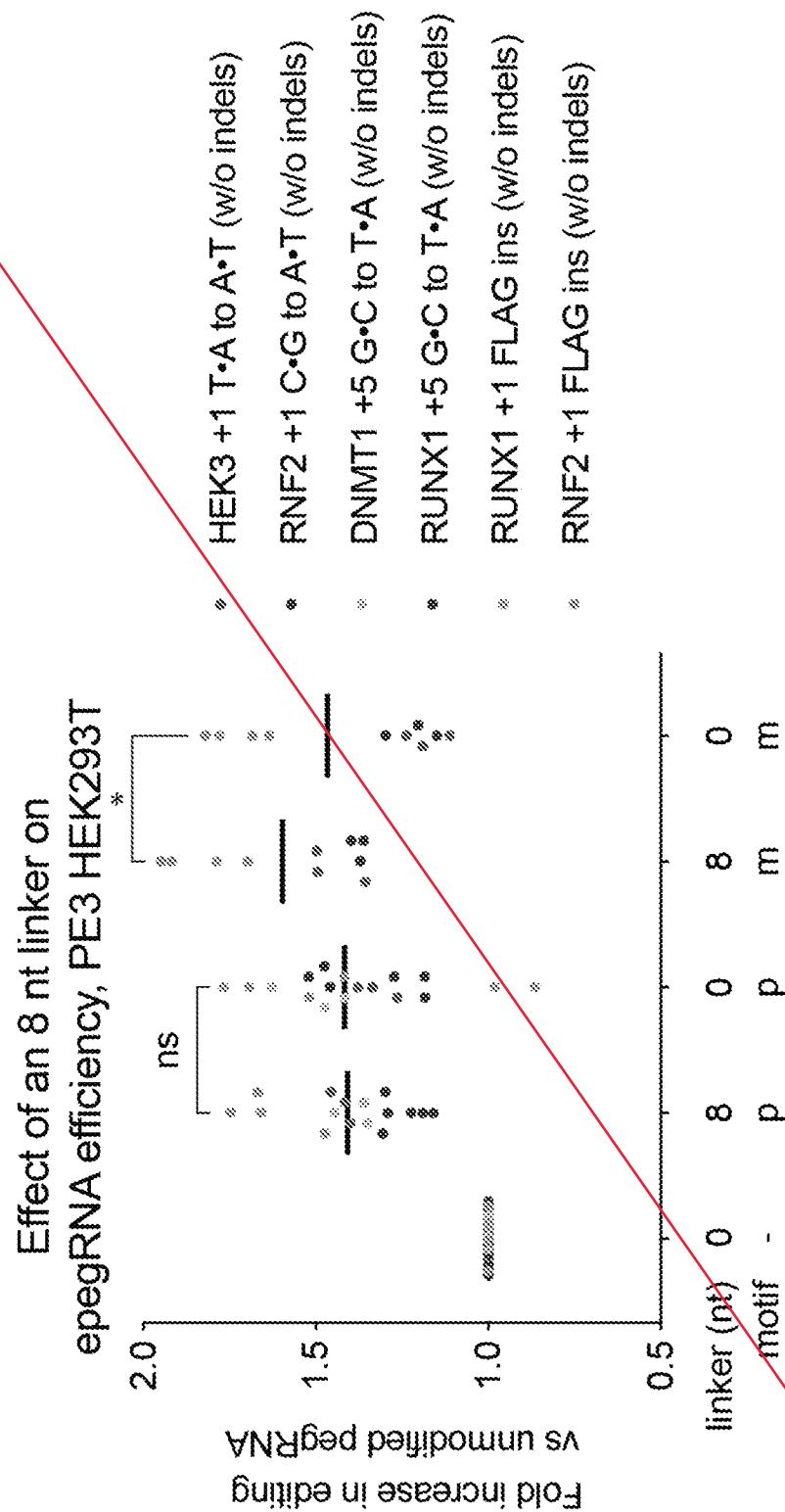


FIG. 110

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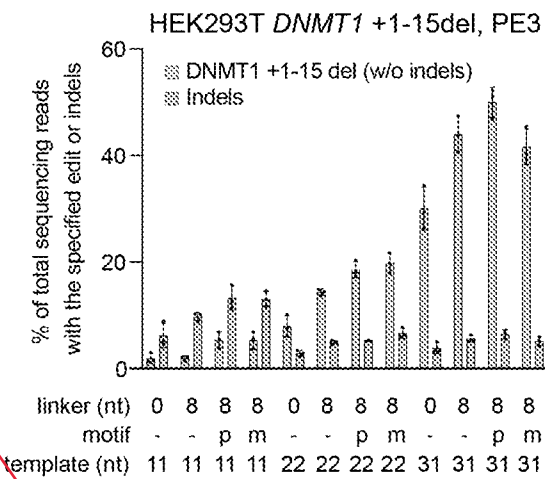


FIG. 111A

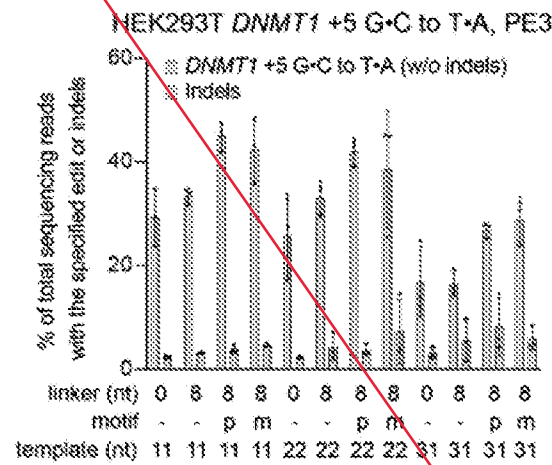


FIG. 111B

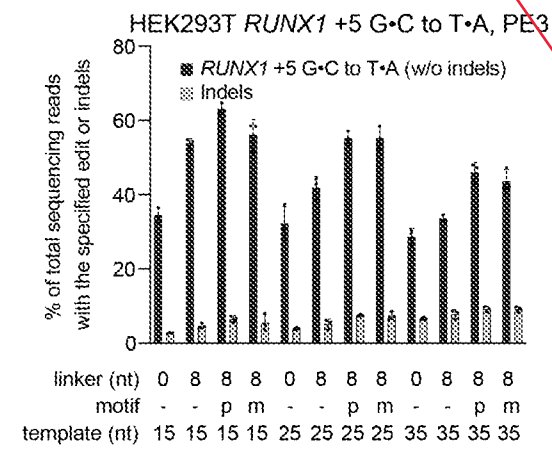


FIG. 111C

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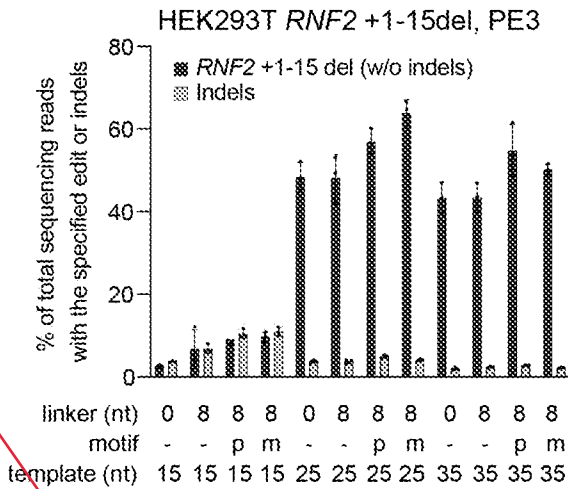


FIG. 111D

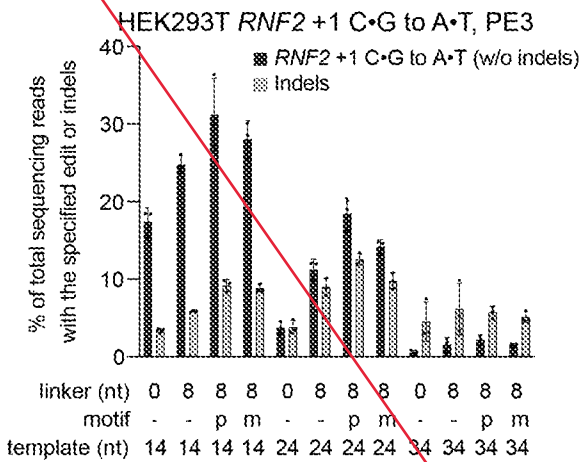


FIG. 111E

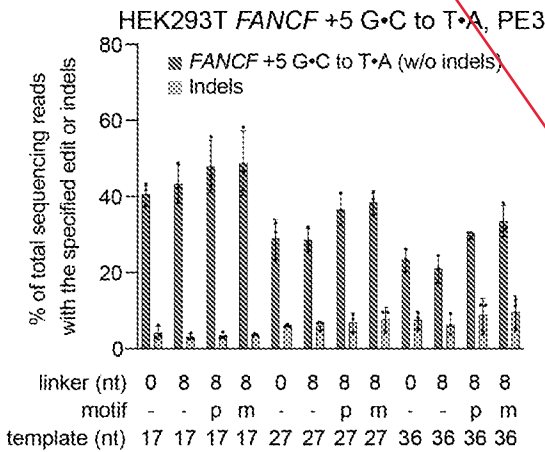


FIG. 111F

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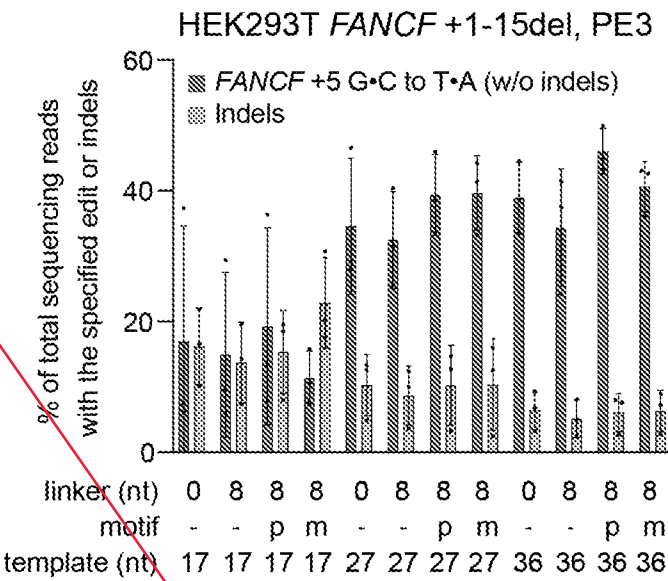


FIG. 111G

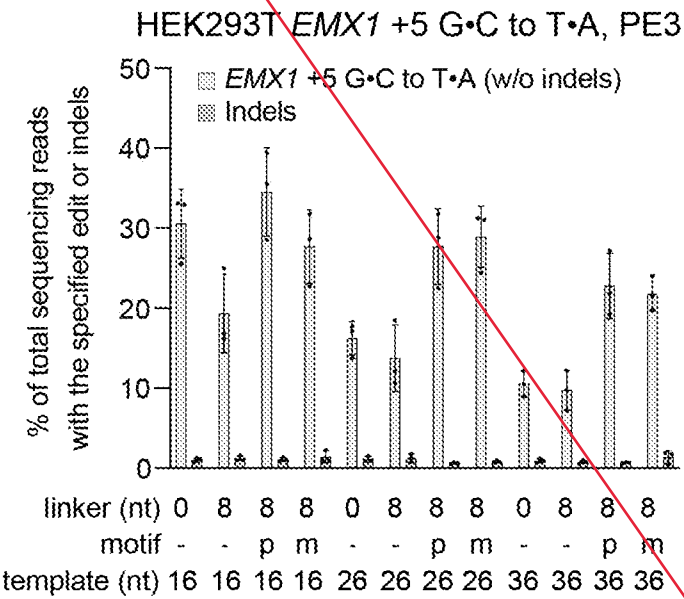


FIG. 111H

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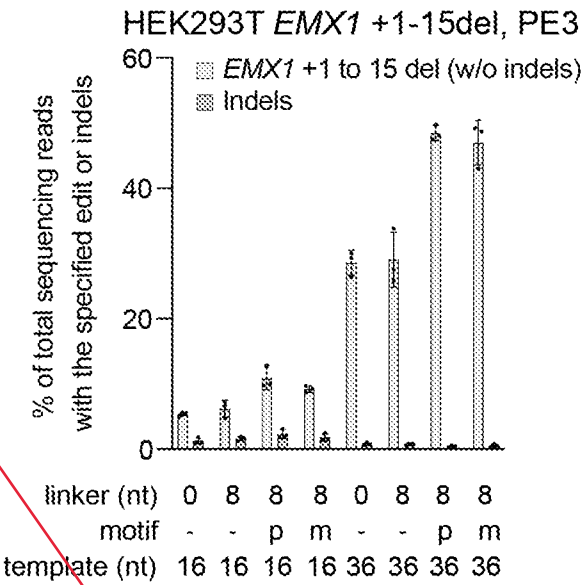


FIG. 111I

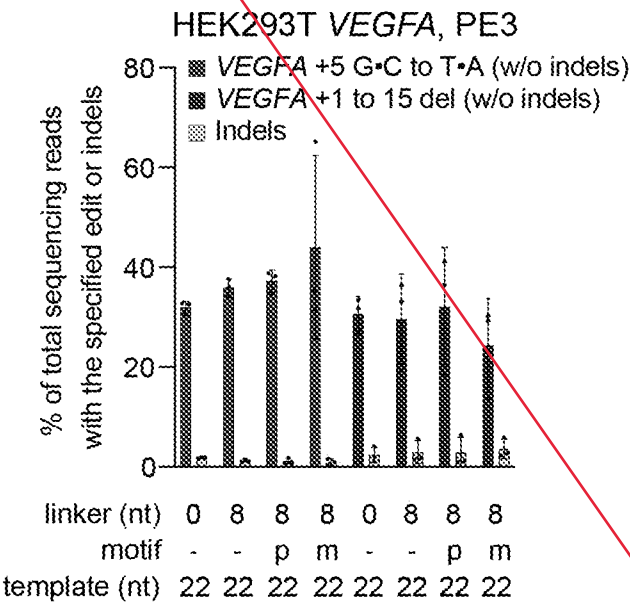


FIG. 111J

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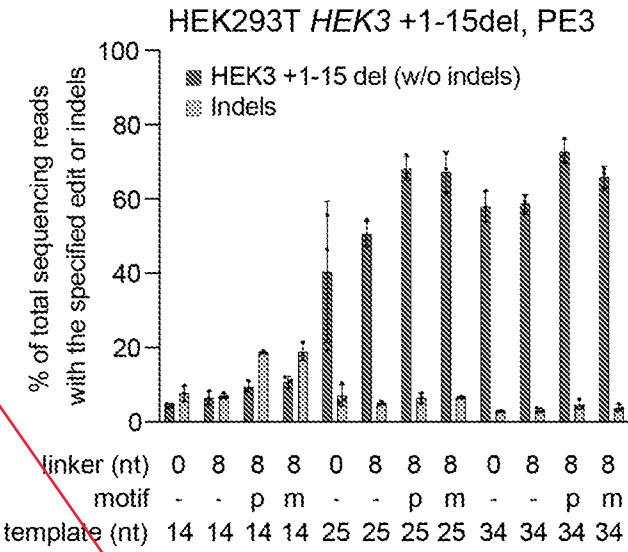


FIG. 111K

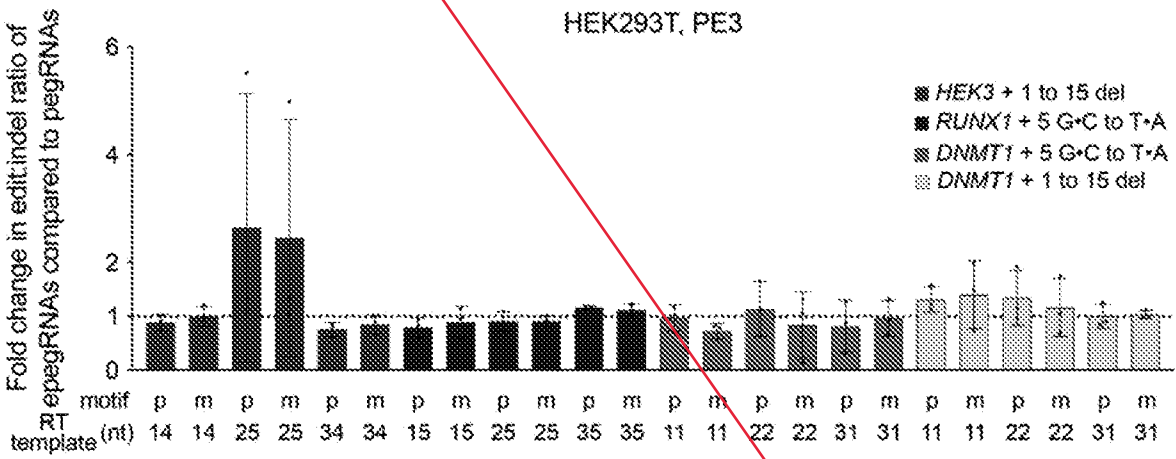


FIG. 112A

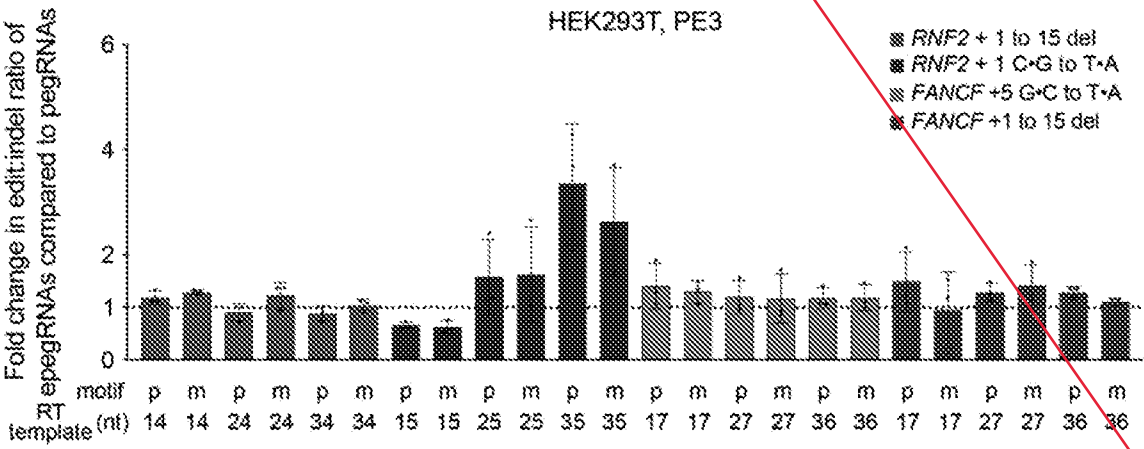


FIG. 112B

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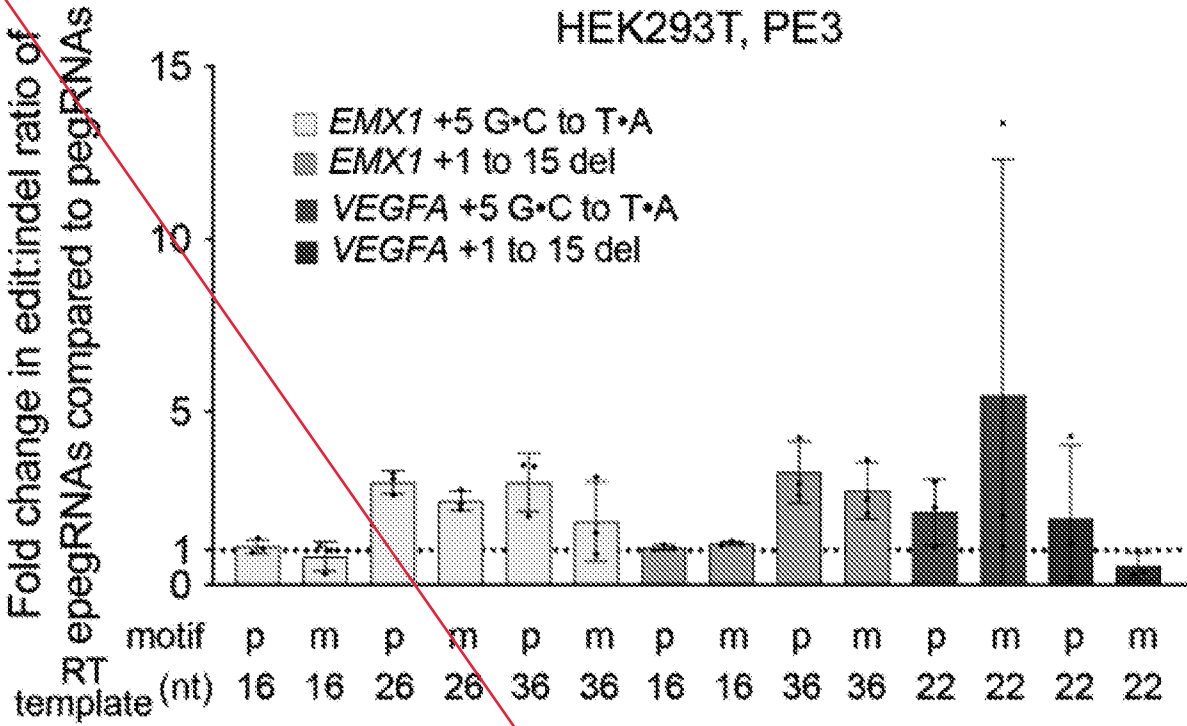


FIG. 112C

pegrNA site	HEK3(PE3)				EMX1(PE3)				FANCF(PE3)						
	...	pt min, -	pt min, m	del, -	del, p	...	pt min, -	pt min, p	del, -	del, m	...	pt min, -	pt min, m	del, -	del, m
On-target		21.5 (4.0)	44.1 (11.7)	57.9 (2.8)	72.7 (4.7)		30.5 (1.1)	24.5 (1.1)	28.6 (0.8)	47 (0.5)		40.7 (4.2)	48.8 (3.7)	38.9 (6.4)	40.6 (6.2)
Off-target 1		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 2		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 3		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 4		<0.1 (<0.1)	<0.1 (0.1)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (<0.1)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)

FIG. 113

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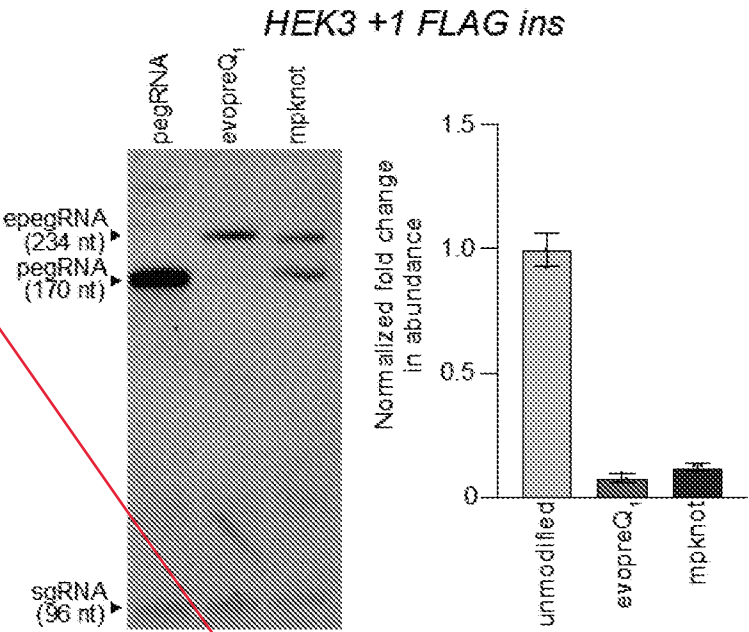


FIG. 114A

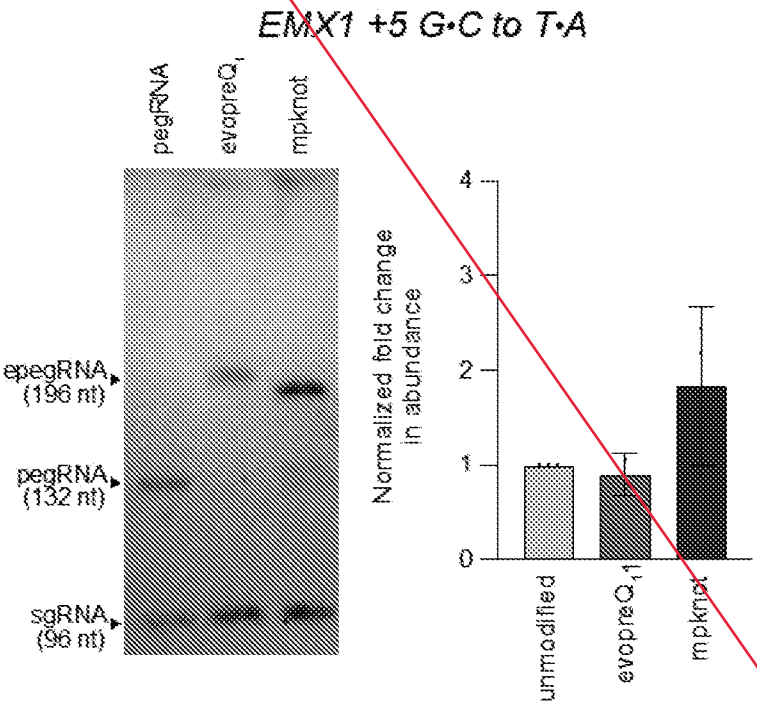


FIG. 114B

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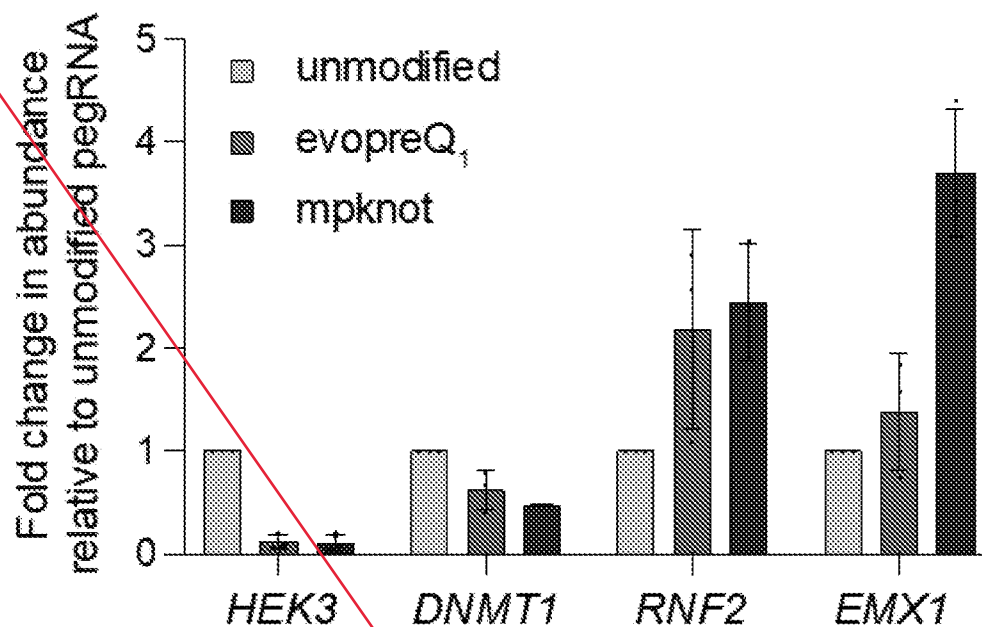


FIG. 114C

HEK3 +1 T•A-to-A•T
14-nt RT template

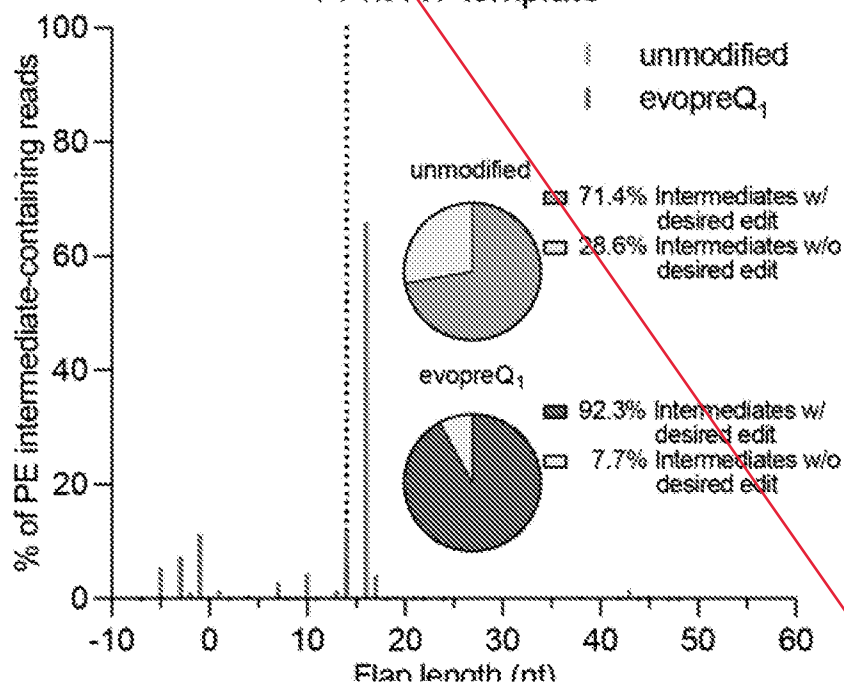


FIG. 115A

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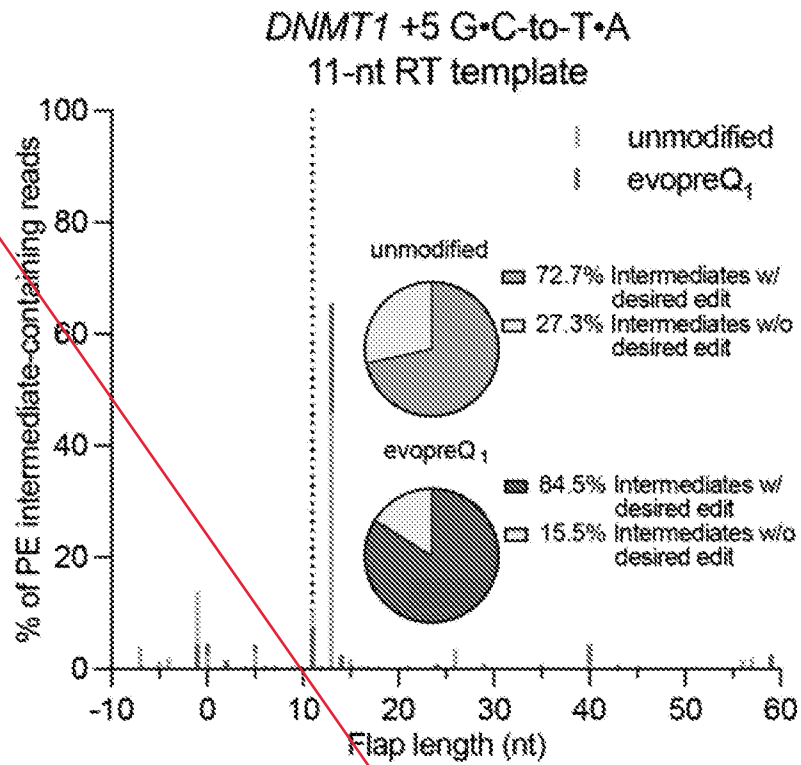


FIG. 115B

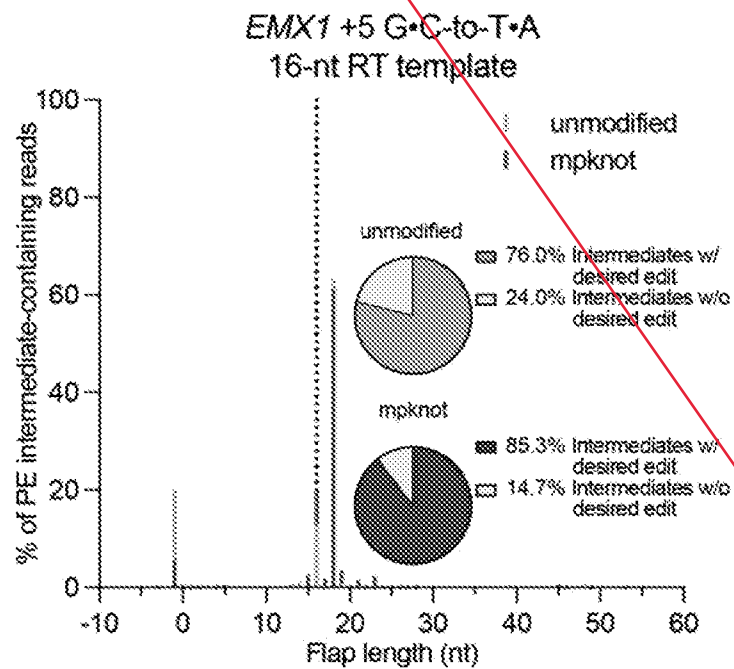


FIG. 115C

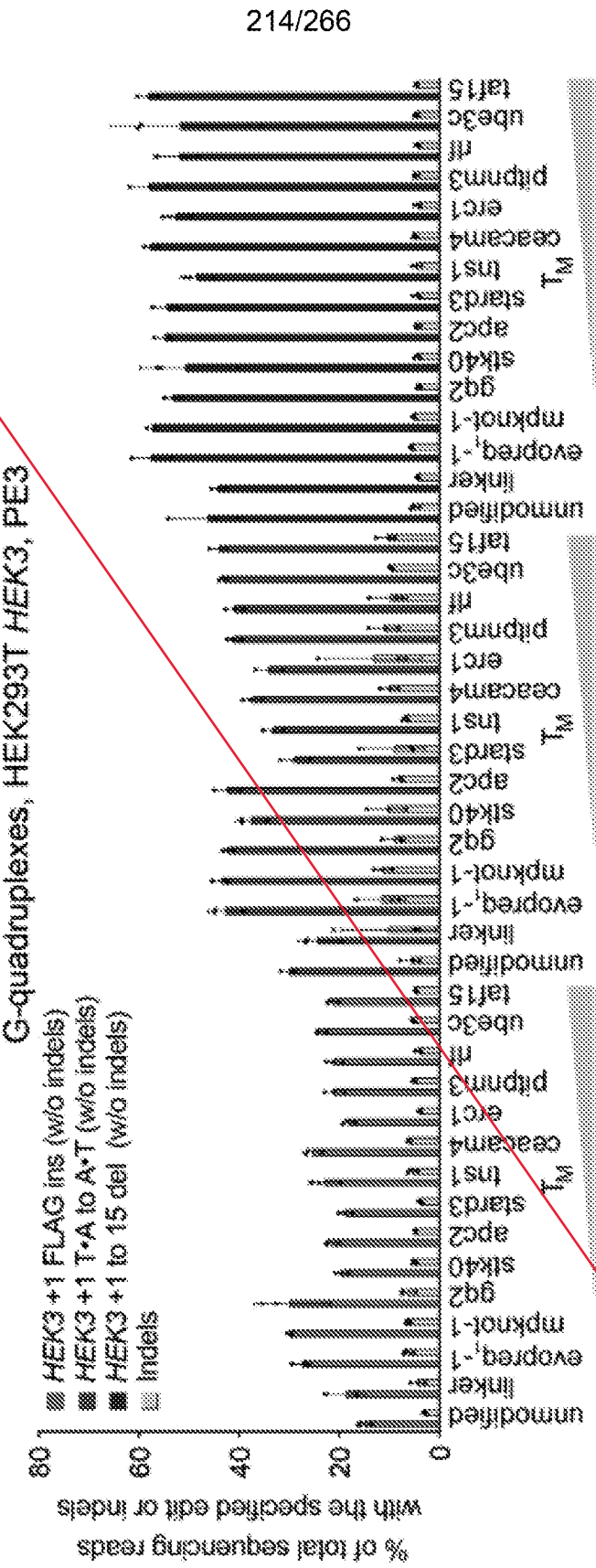


FIG. 116A

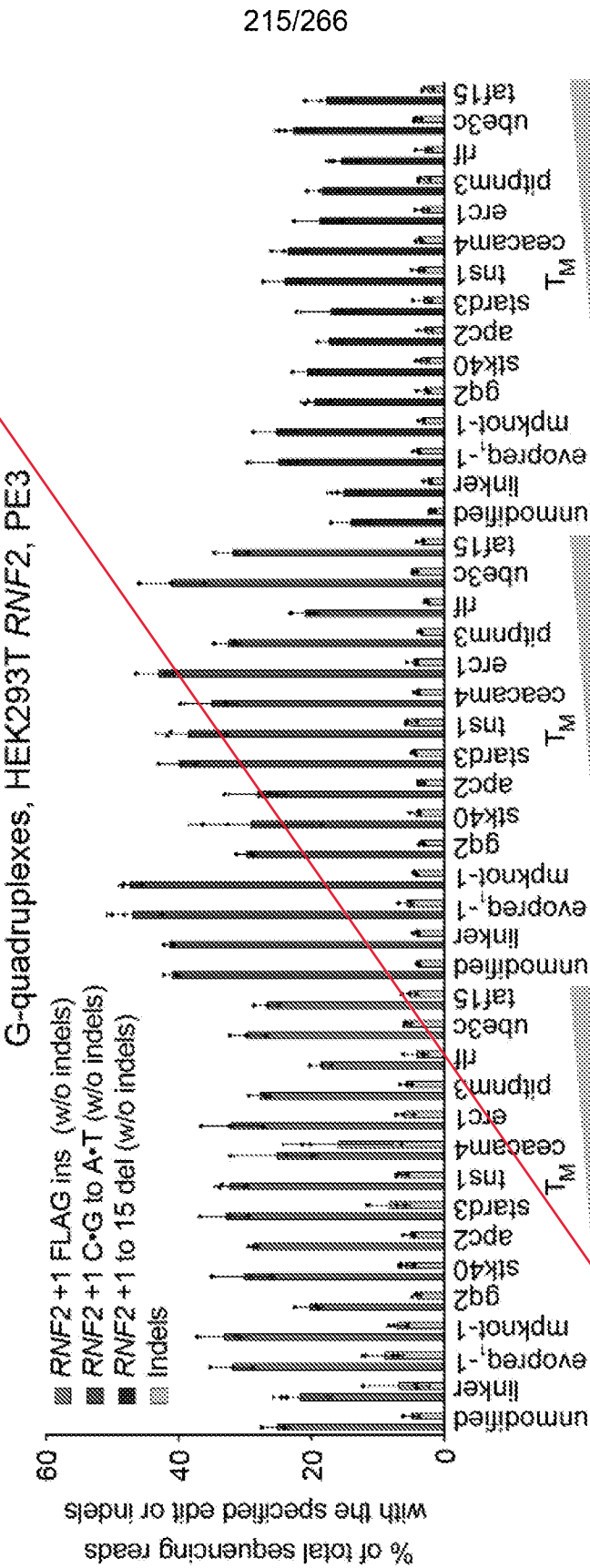


FIG. 116B

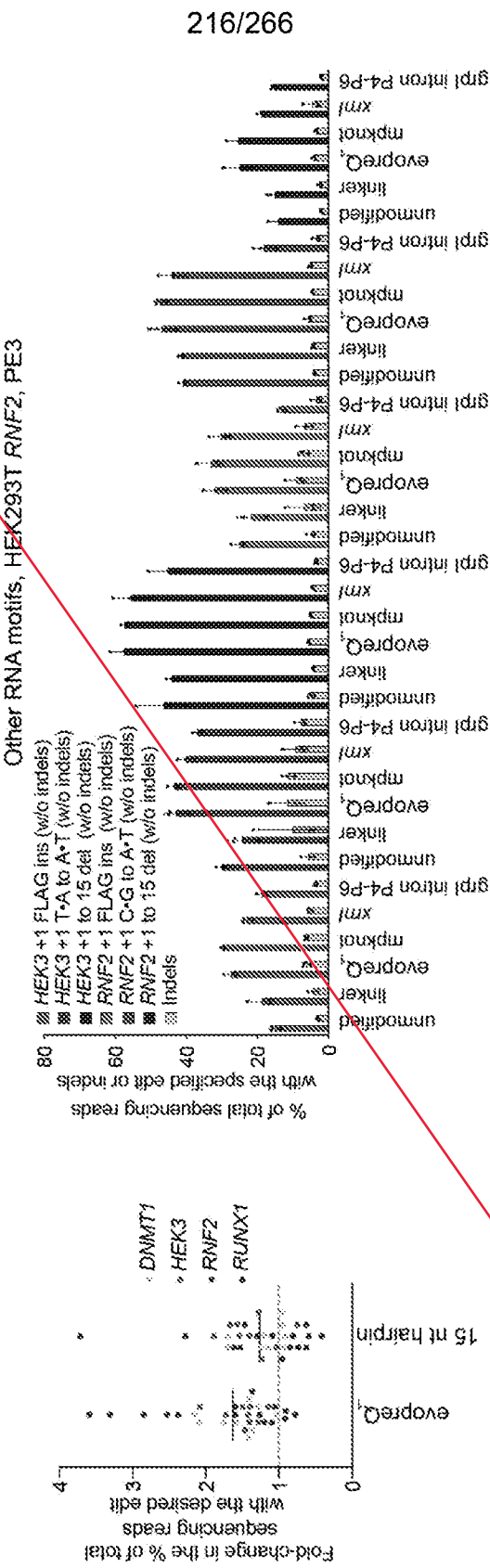


FIG. 116D

FIG. 116C

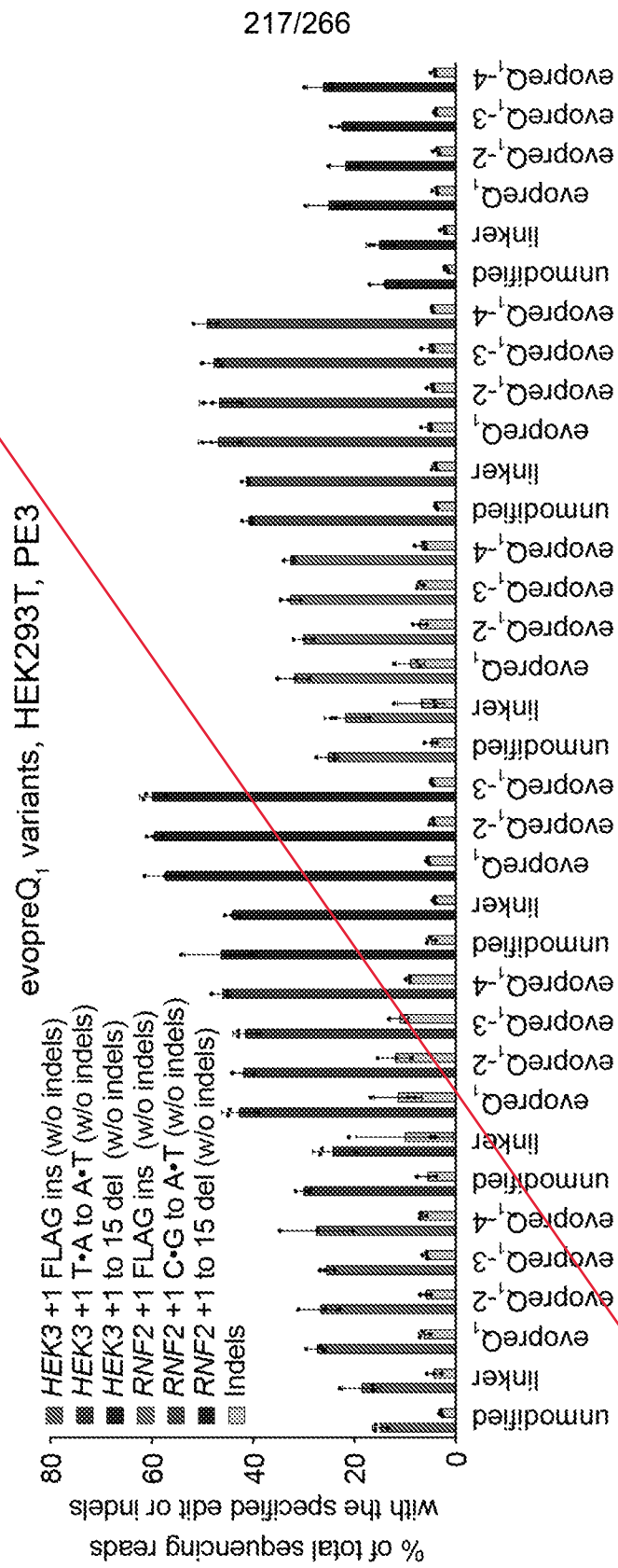


FIG. 117A

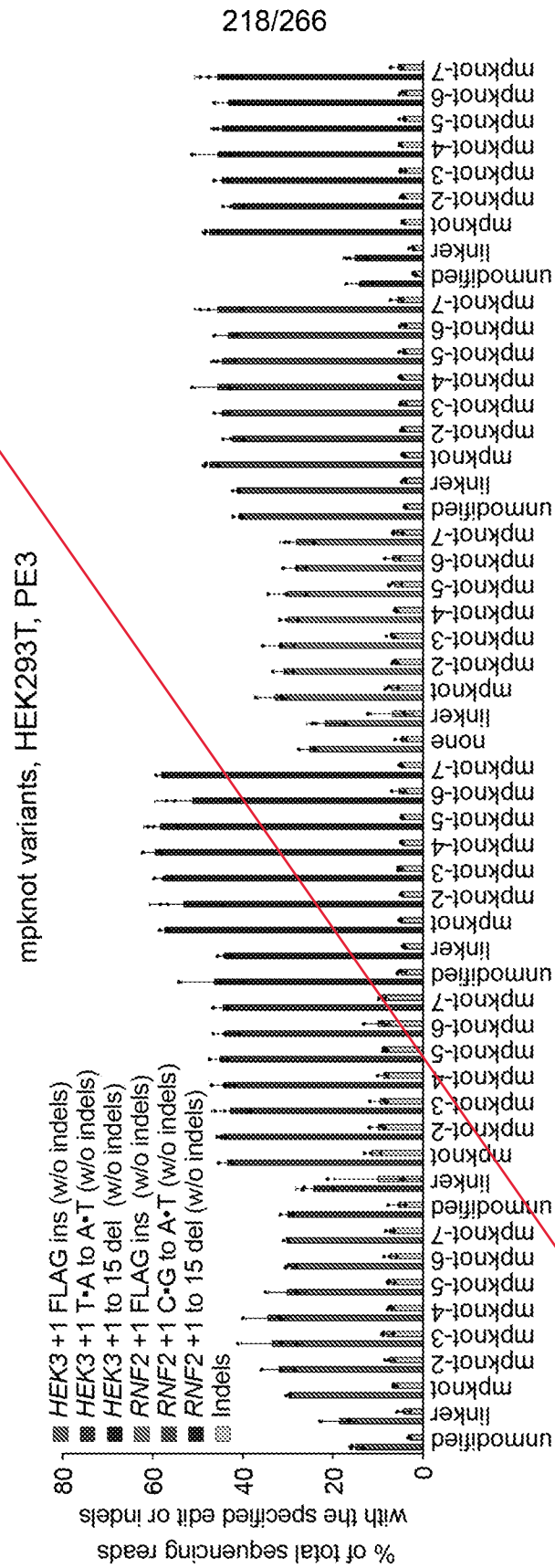


FIG. 117B

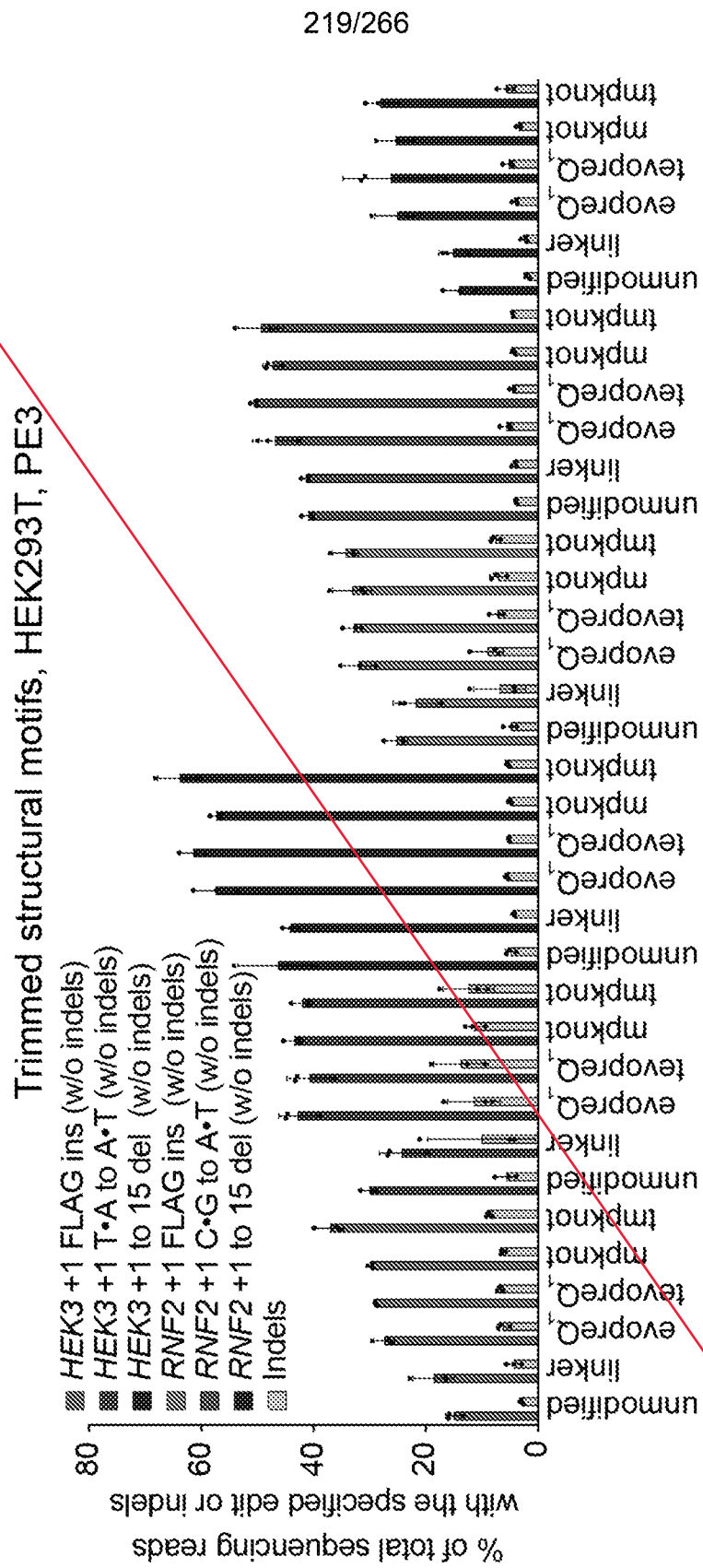


FIG. 117C

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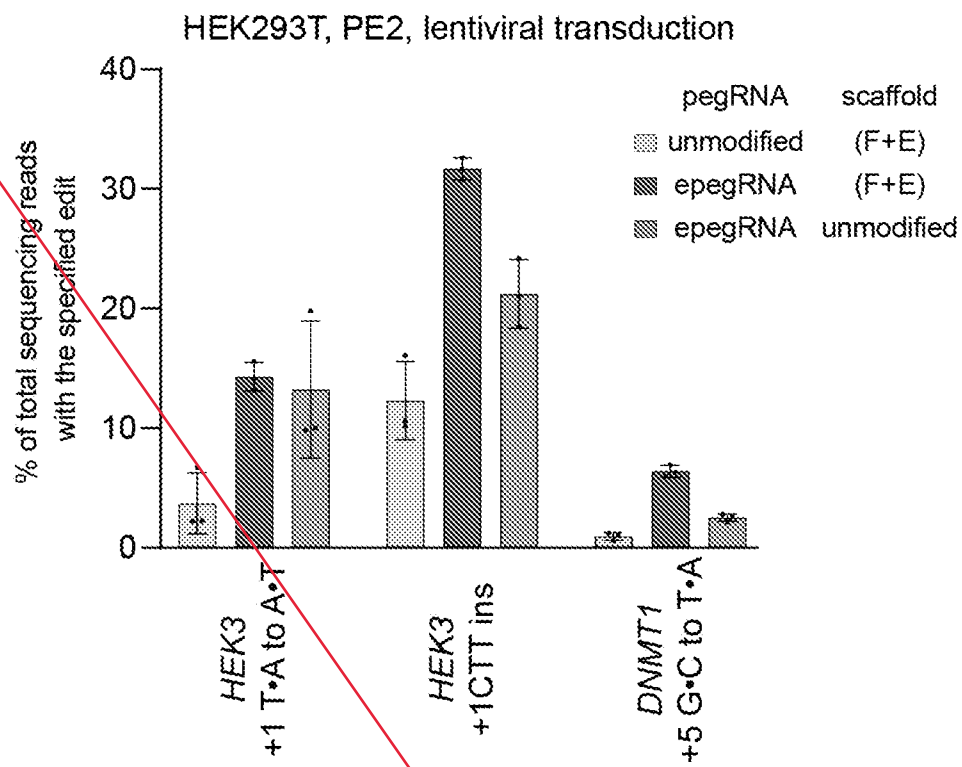


FIG. 118

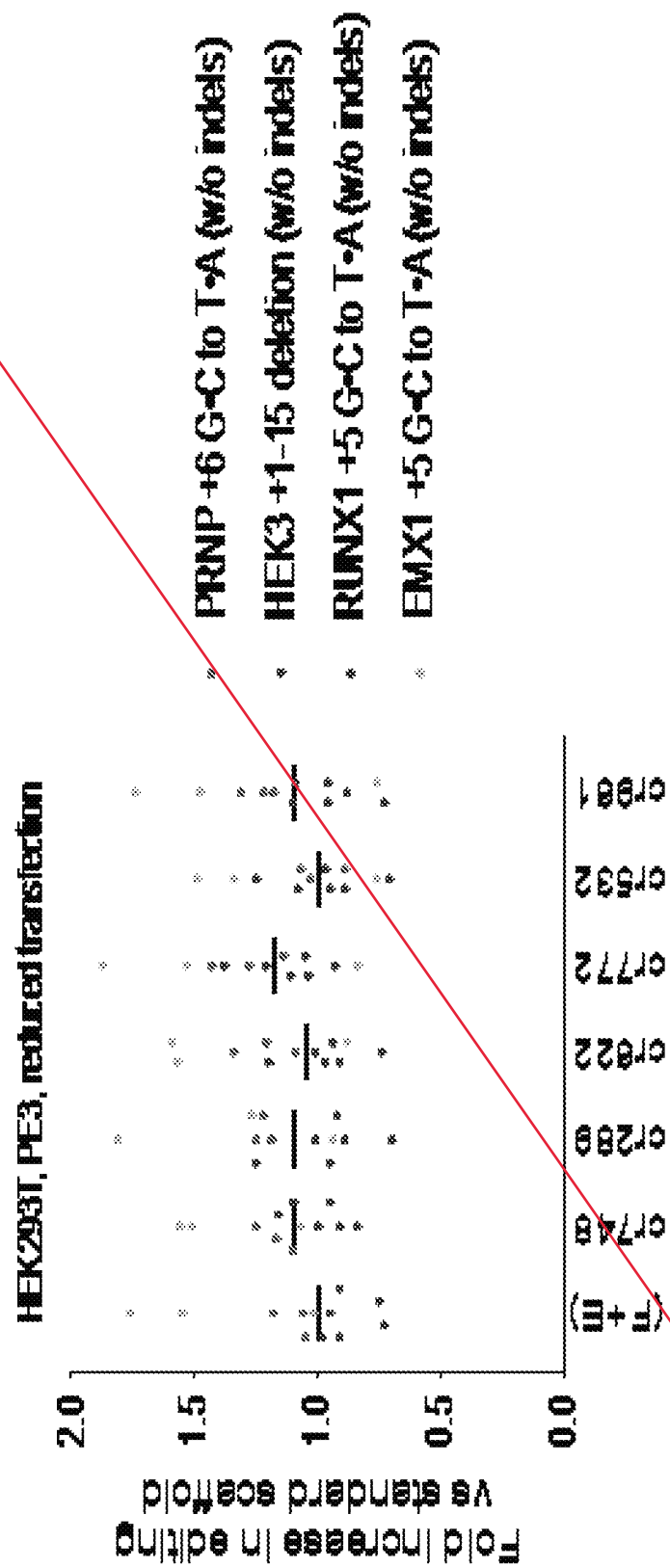


FIG. 119

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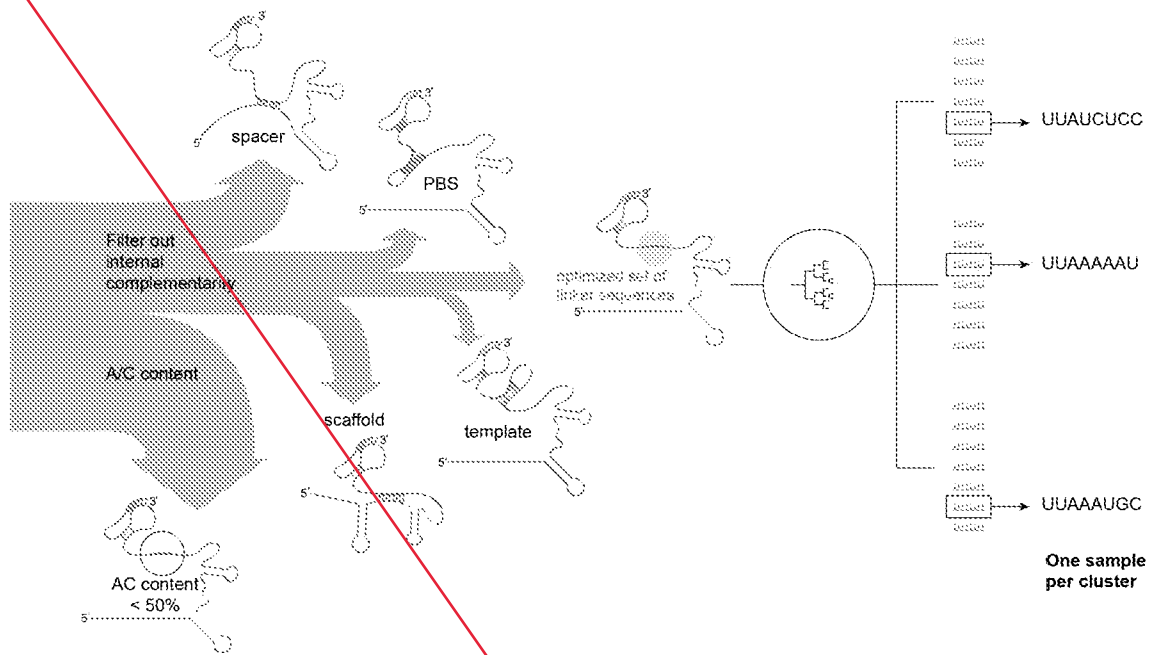


FIG. 120A

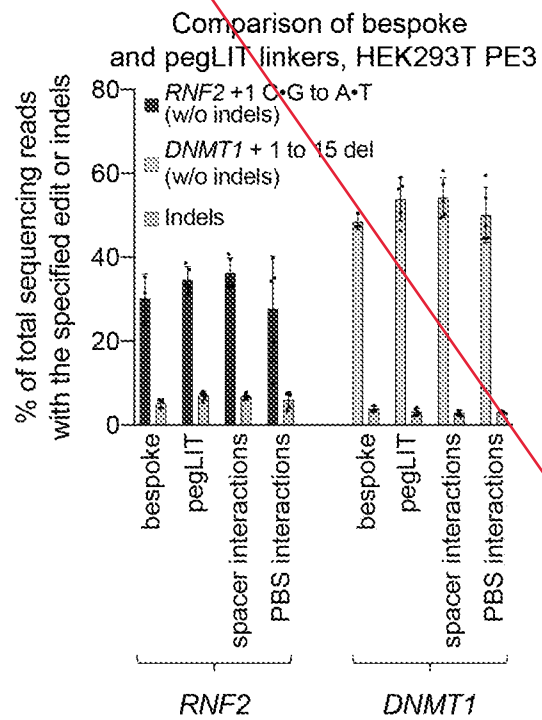


FIG. 120B

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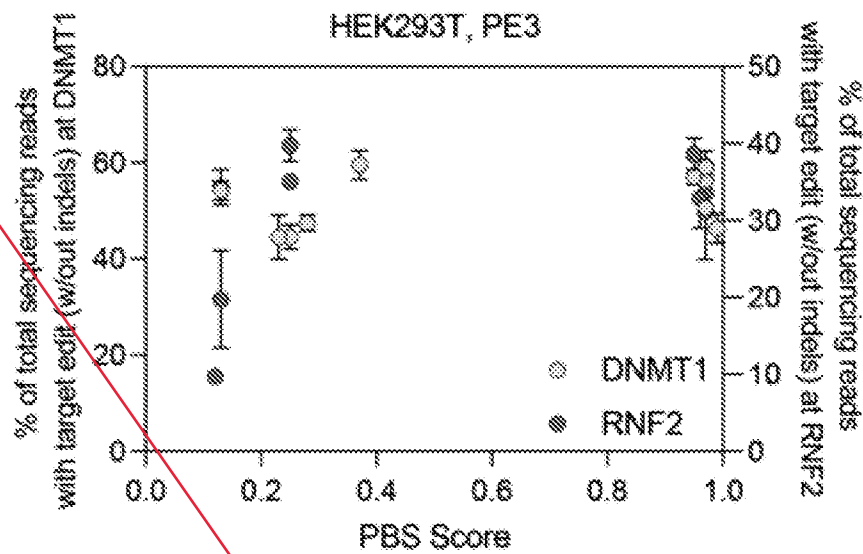


FIG. 120C

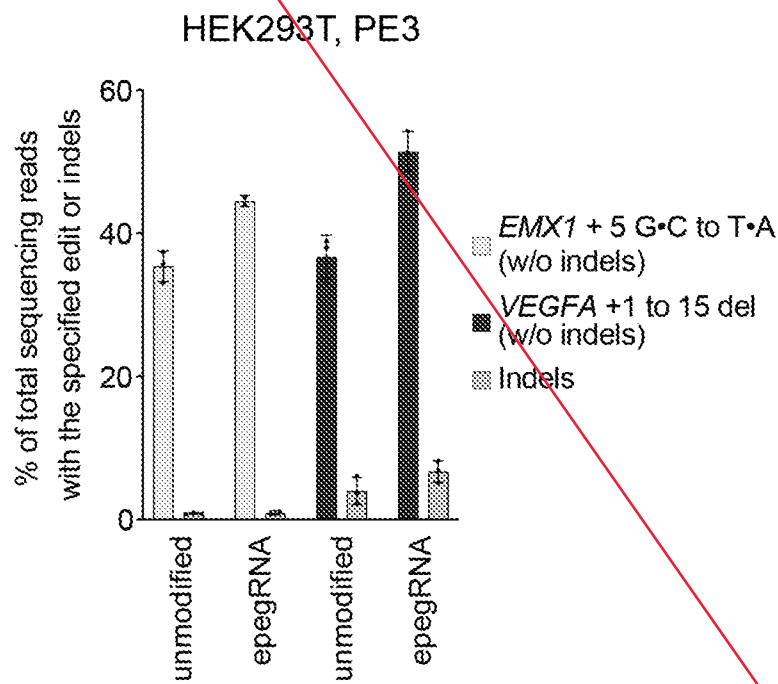


FIG. 120D

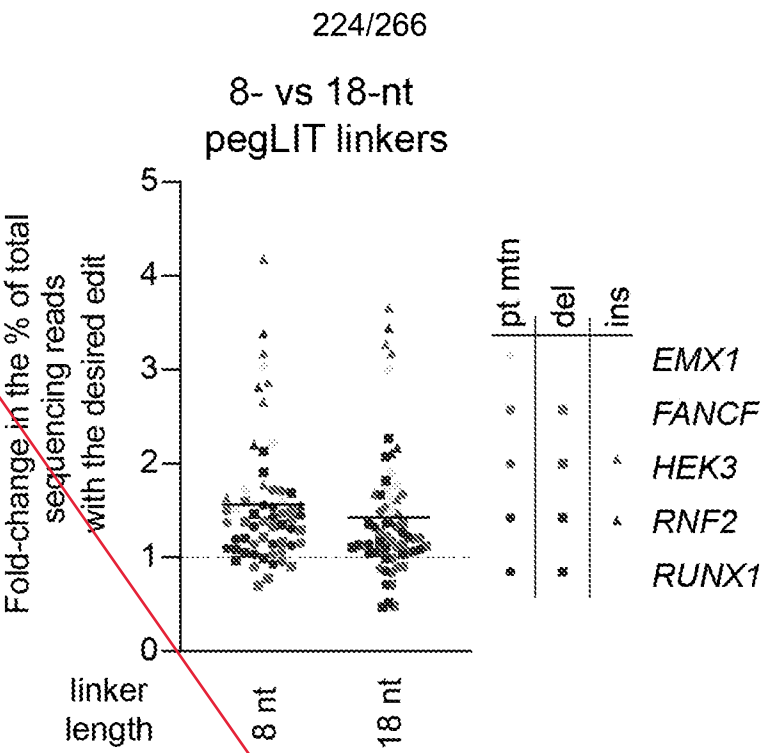


FIG. 120E

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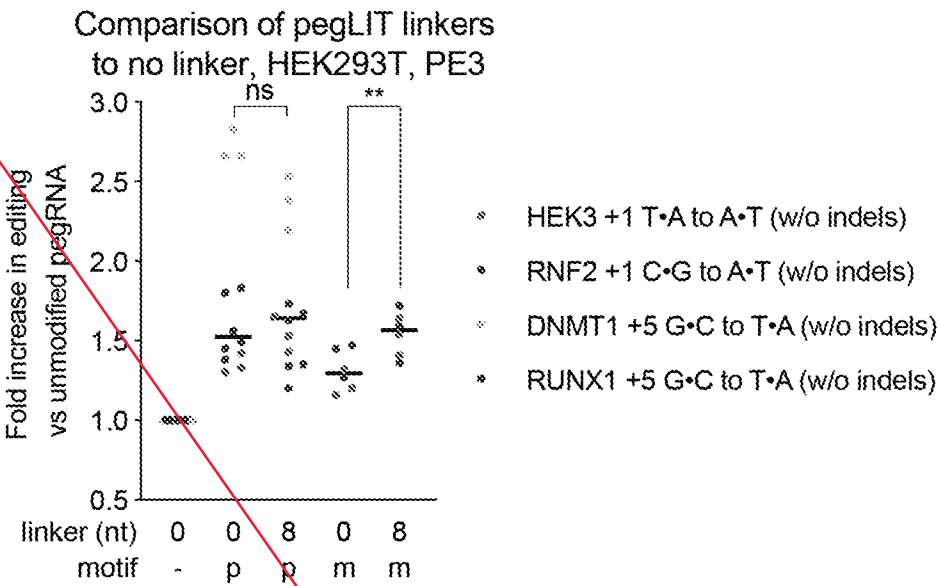


FIG. 120F

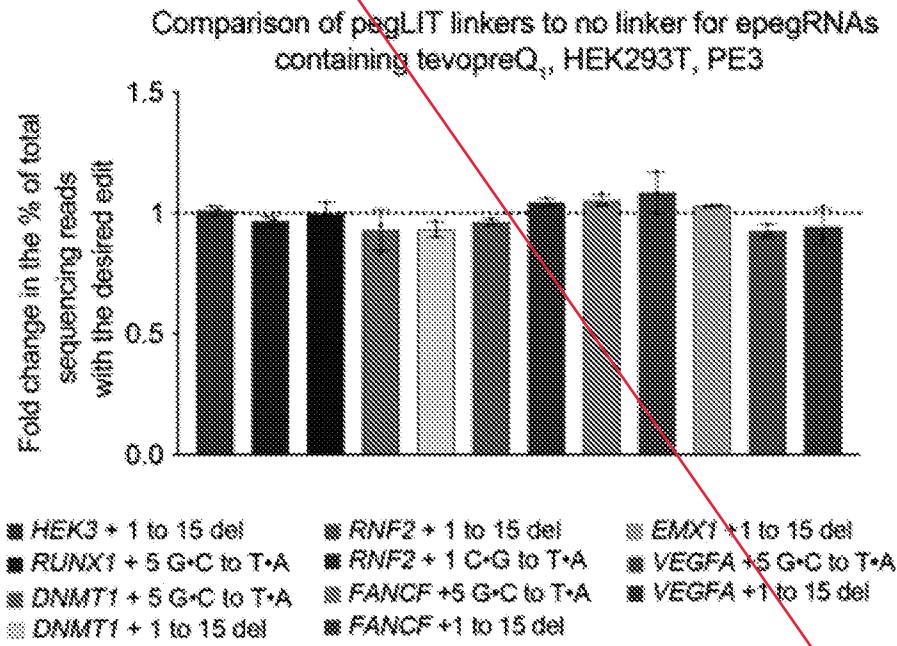


FIG. 120G

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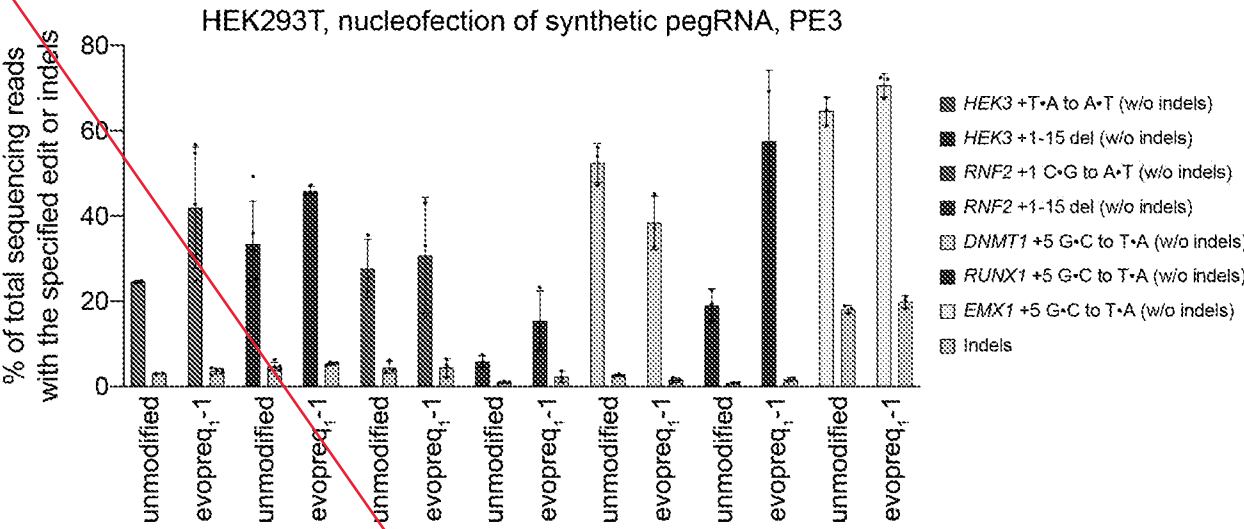


FIG. 121A

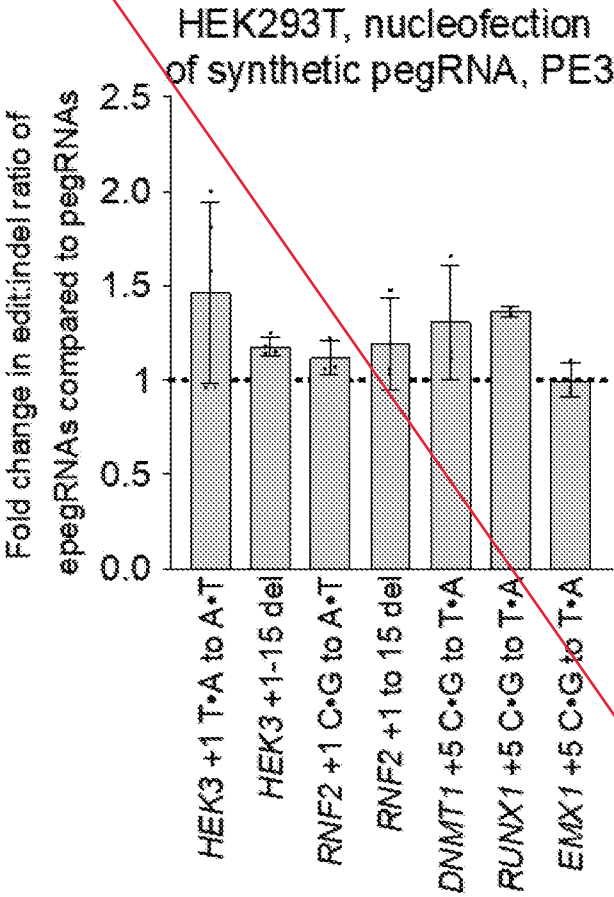


FIG. 121B

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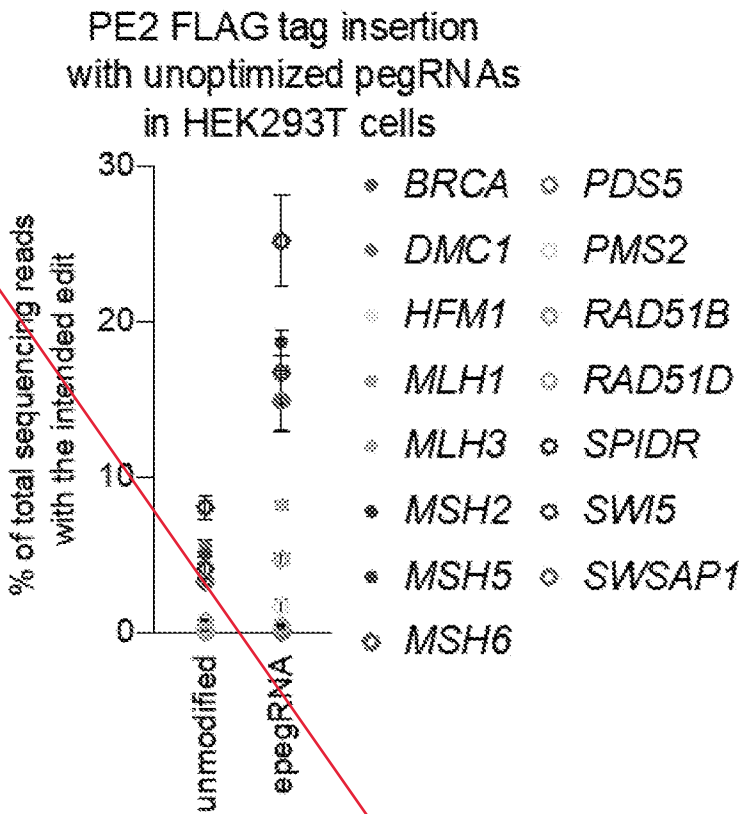


FIG. 122A

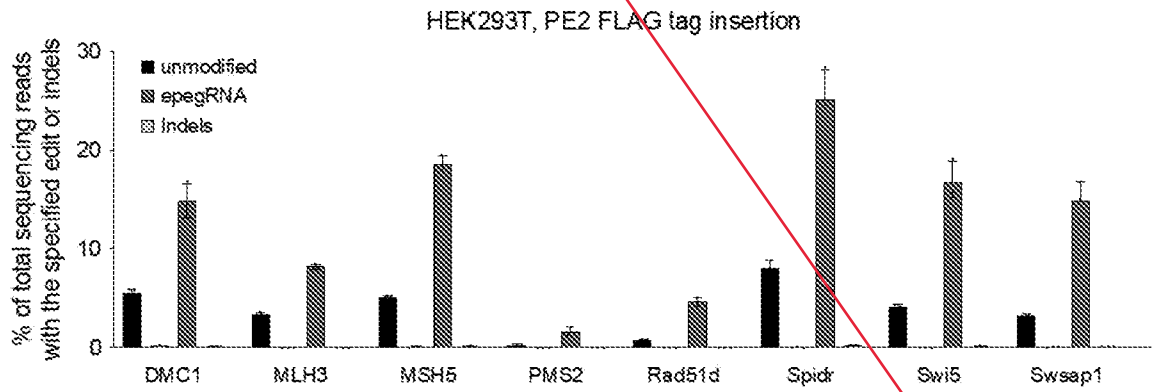


FIG. 122B

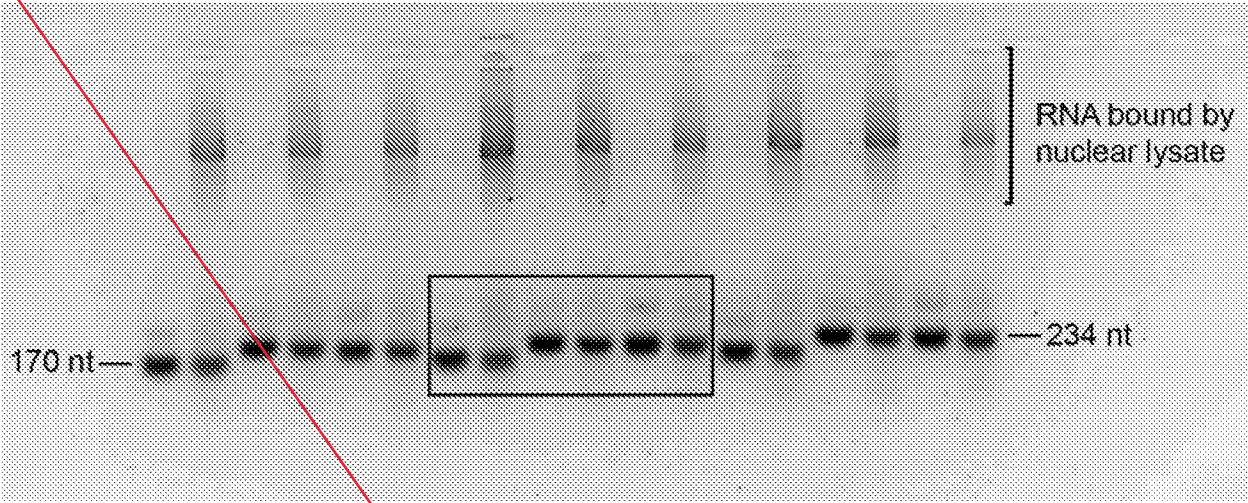


FIG. 123

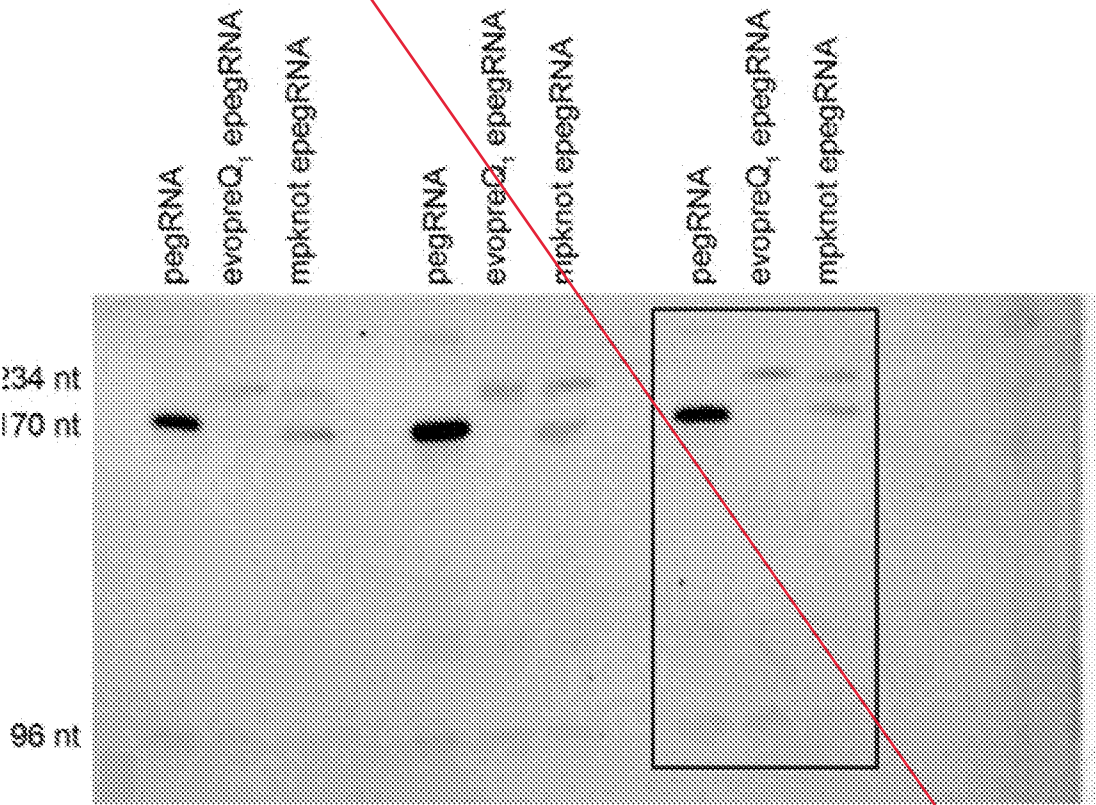


FIG. 124A

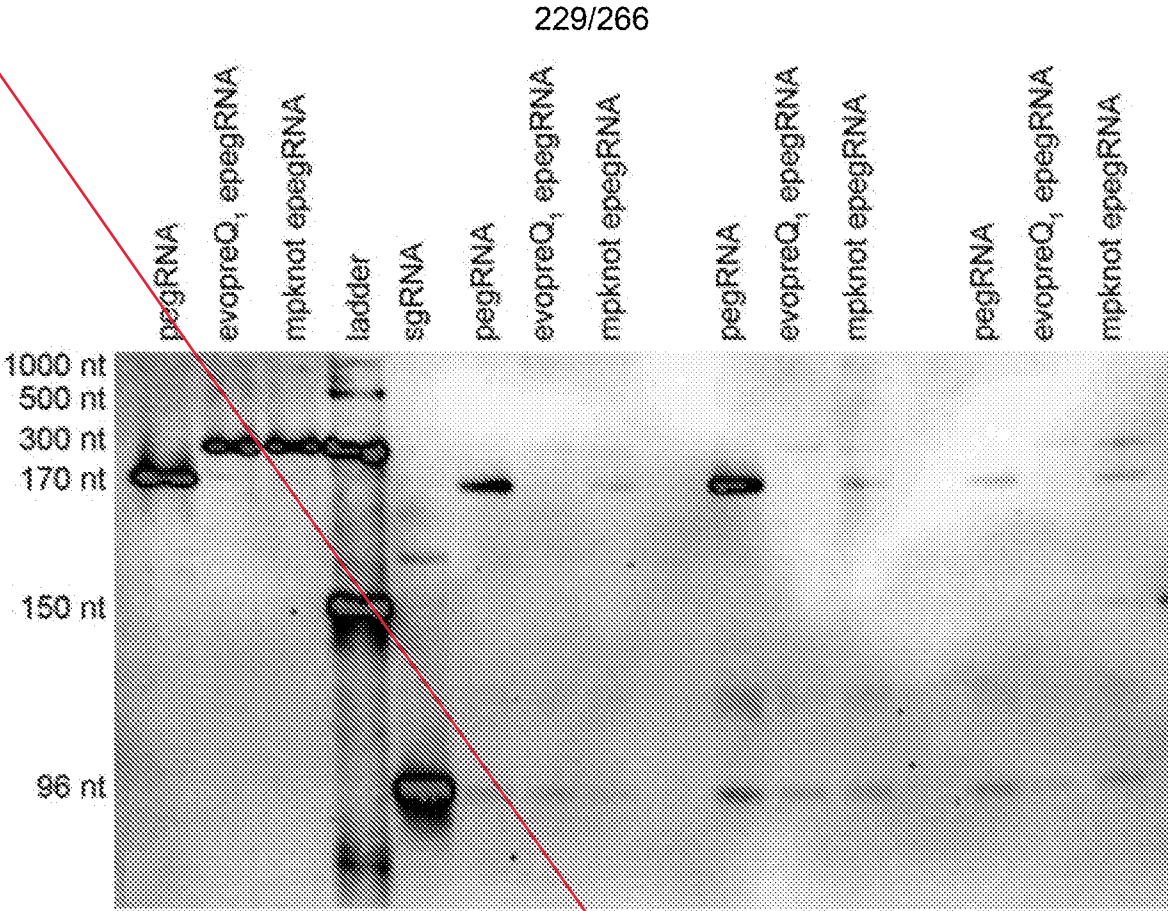


FIG. 124B

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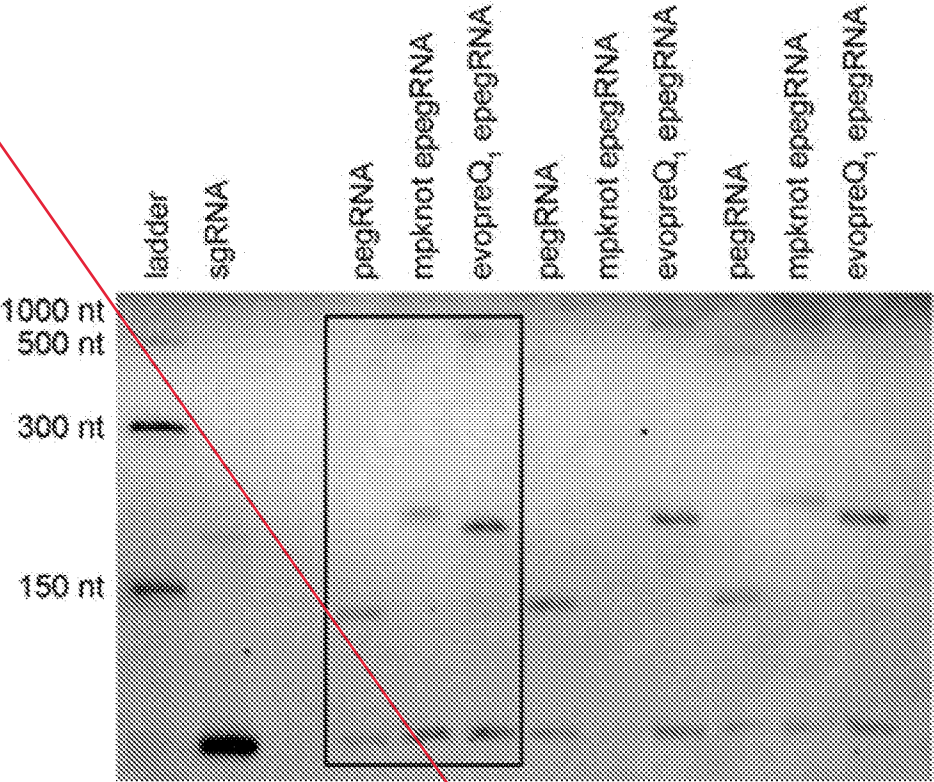


FIG. 124C

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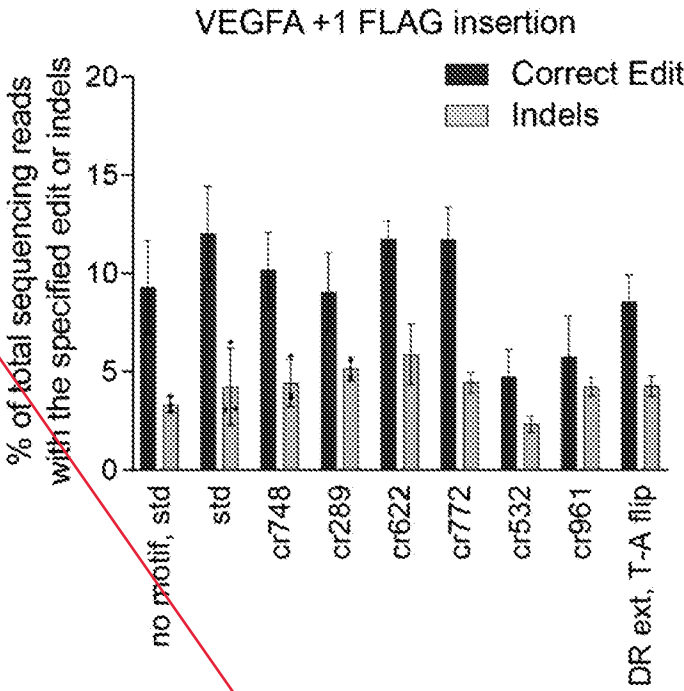


FIG. 125A

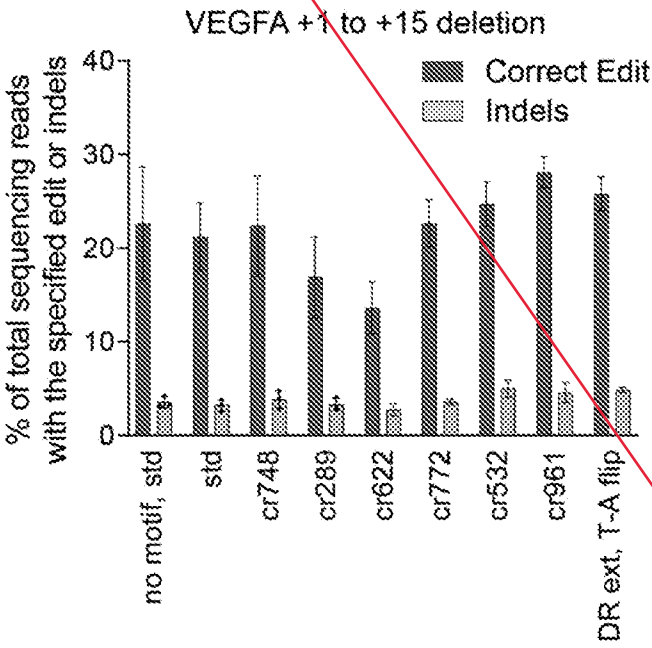


FIG. 125B

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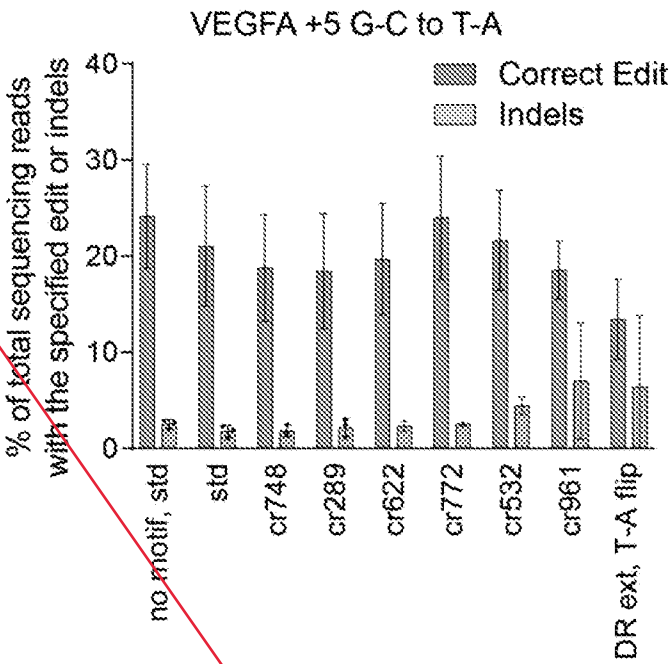


FIG. 125C

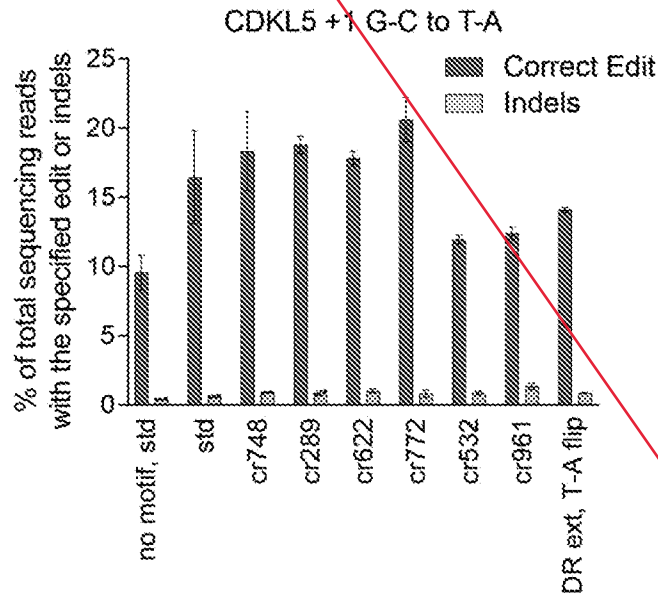


FIG. 125D

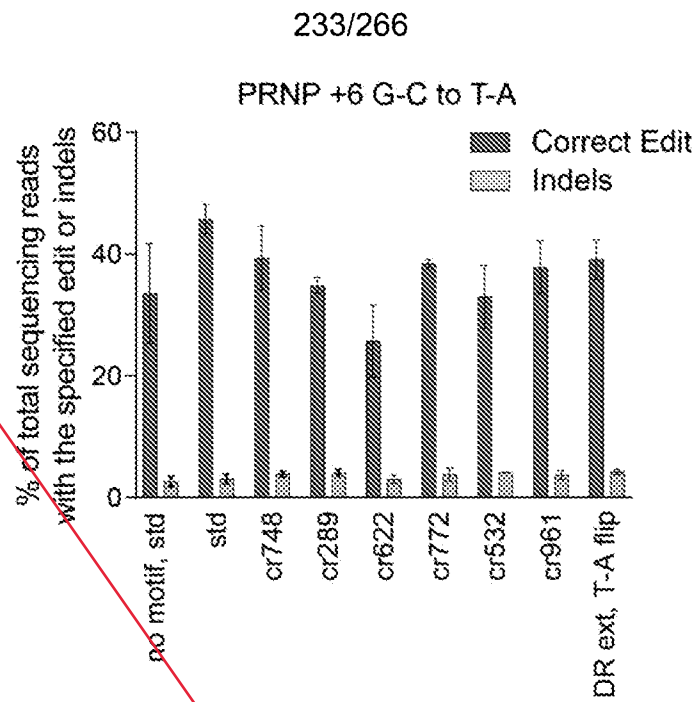
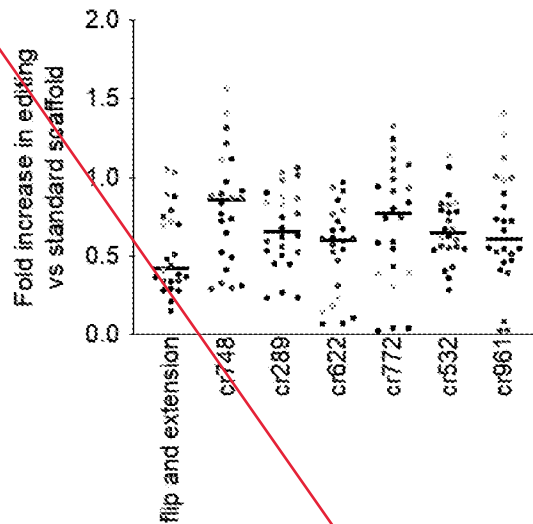


FIG. 125E

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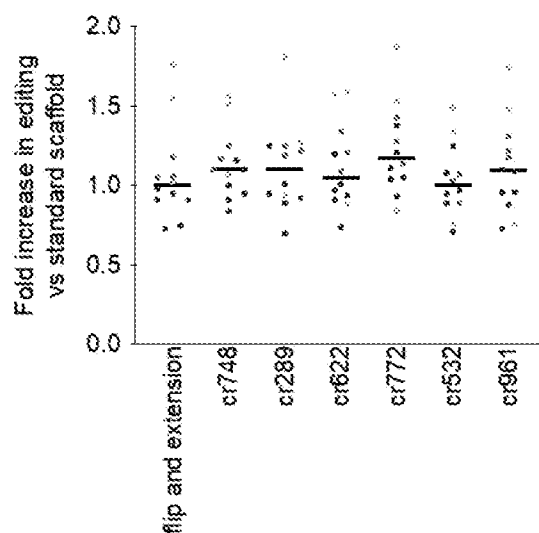
Comparison of scaffolds for epegRNAs
HeLa transfection, PE3



- PRNP +6 G•C to T•A (w/o indels)
- RUNX1 +5 G•C to T•A (w/o indels)
- EMX1 +5 G•C to T•A (w/o indels)
- DNMT1 +5 G•C to T•A (w/o indels)
- FANCF +5 G•C to T•A (w/o indels)
- VEGFA +5 G•C to T•A (w/o indels)
- HEK3 +1-15 deletion (w/o indels)
- RNF2 +1-15 deletion (w/o indels)

FIG. 126A

Comparison of scaffolds for epegRNAs
HEK293T [1/10 DNA transfected], PE3



- PRNP +6 G•C to T•A (w/o indels)
- RUNX1 +5 G•C to T•A (w/o indels)
- EMX1 +5 G•C to T•A (w/o indels)
- HEK3 +1-15 deletion (w/o indels)

FIG. 126B

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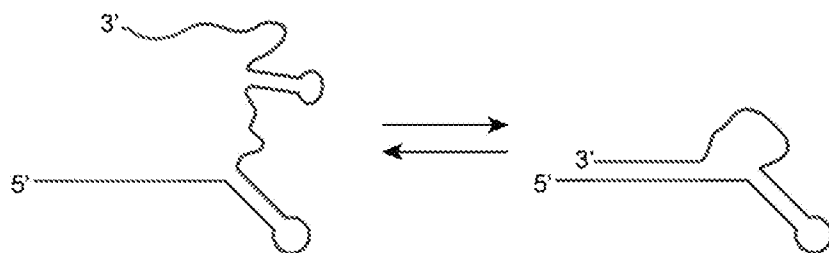


FIG. 127A

HEK293T [1/10 DNA transfected],
PE3, epegRNA,
scaffold comparison

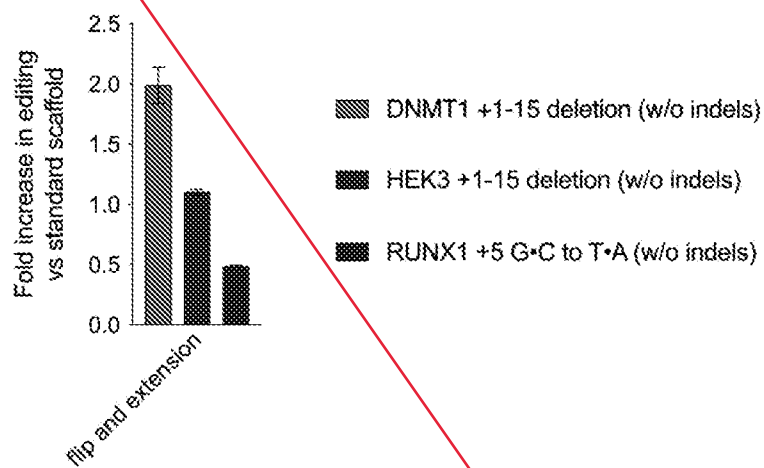
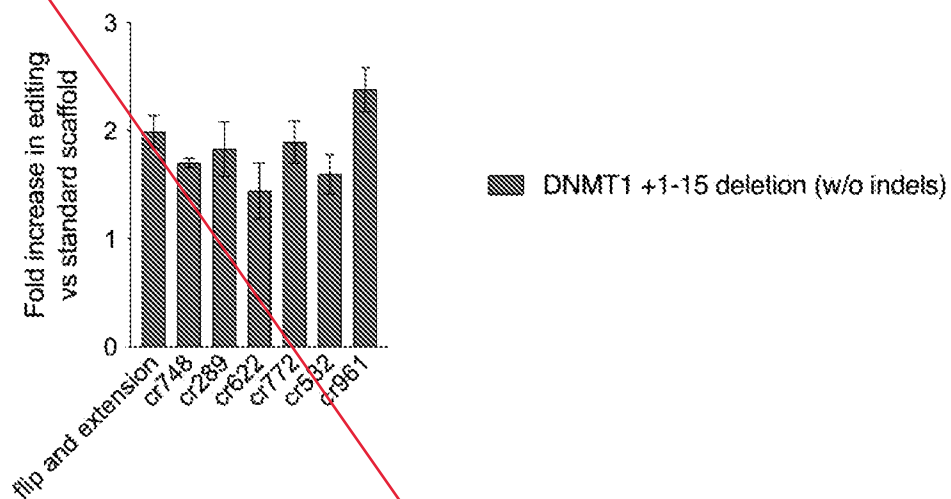


FIG. 127B

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HEK293T [1/10 DNA transfected], PE3,
epegRNA,
scaffold comparison

**FIG. 127C**

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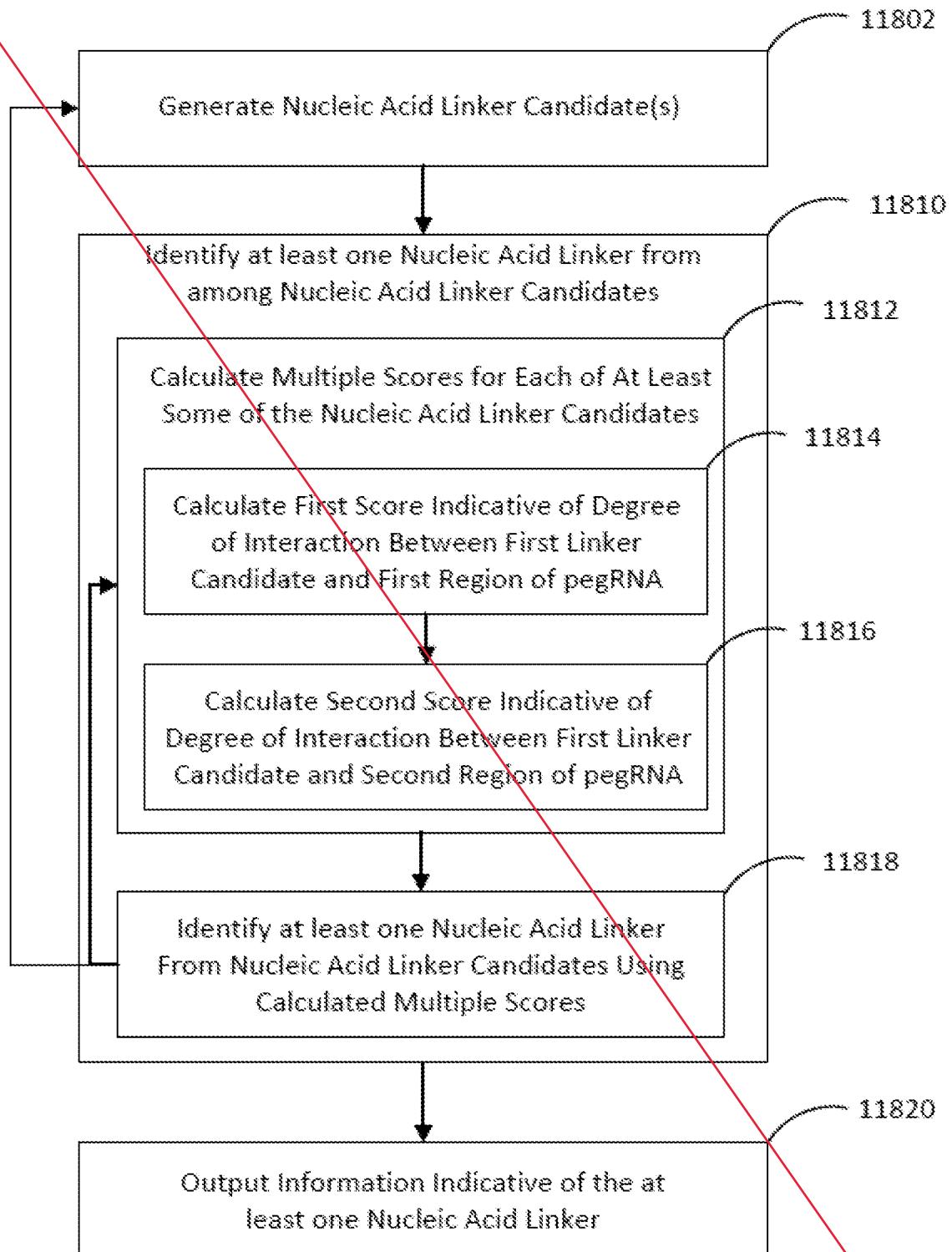


FIG. 128

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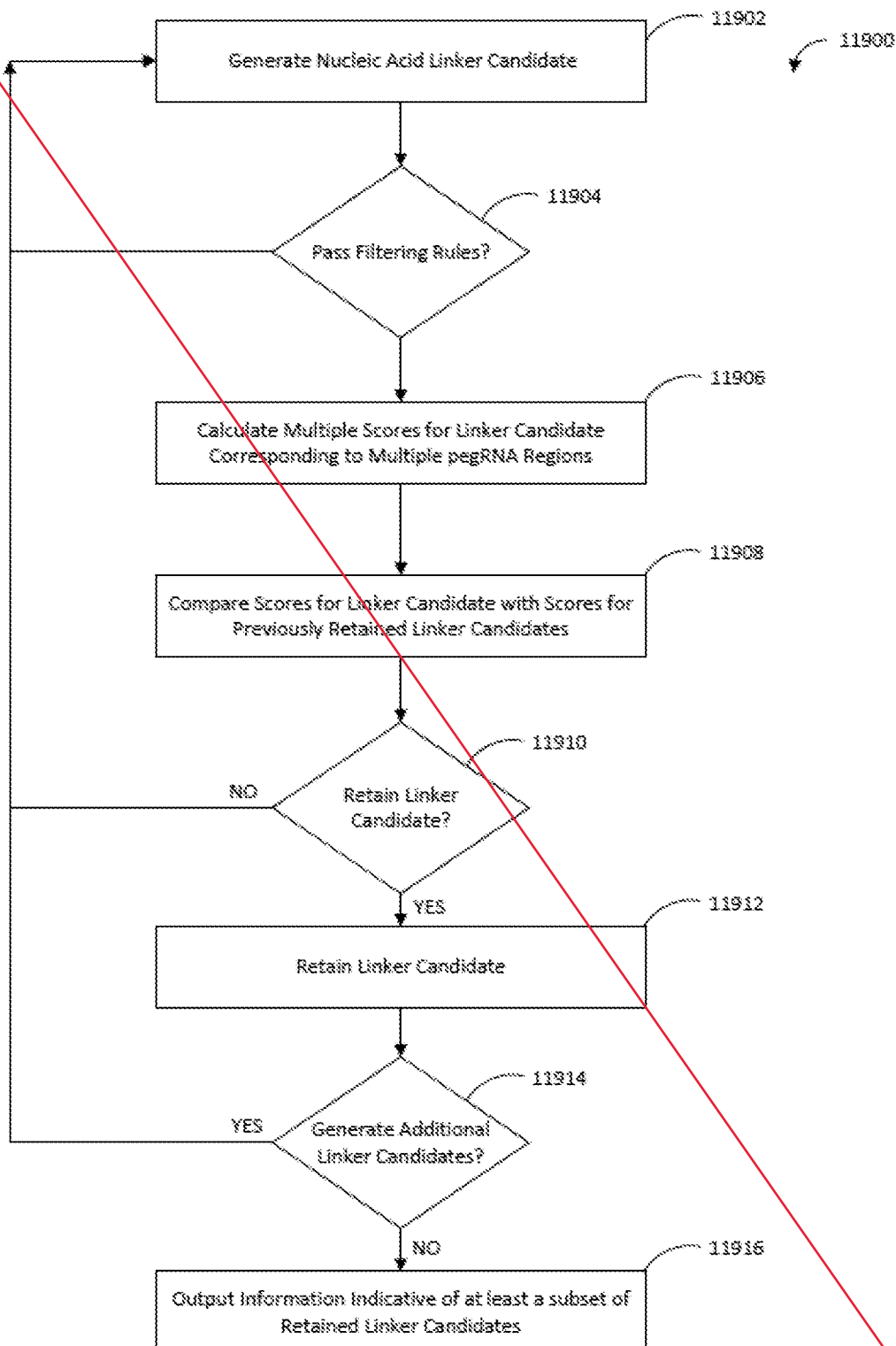


FIG. 129

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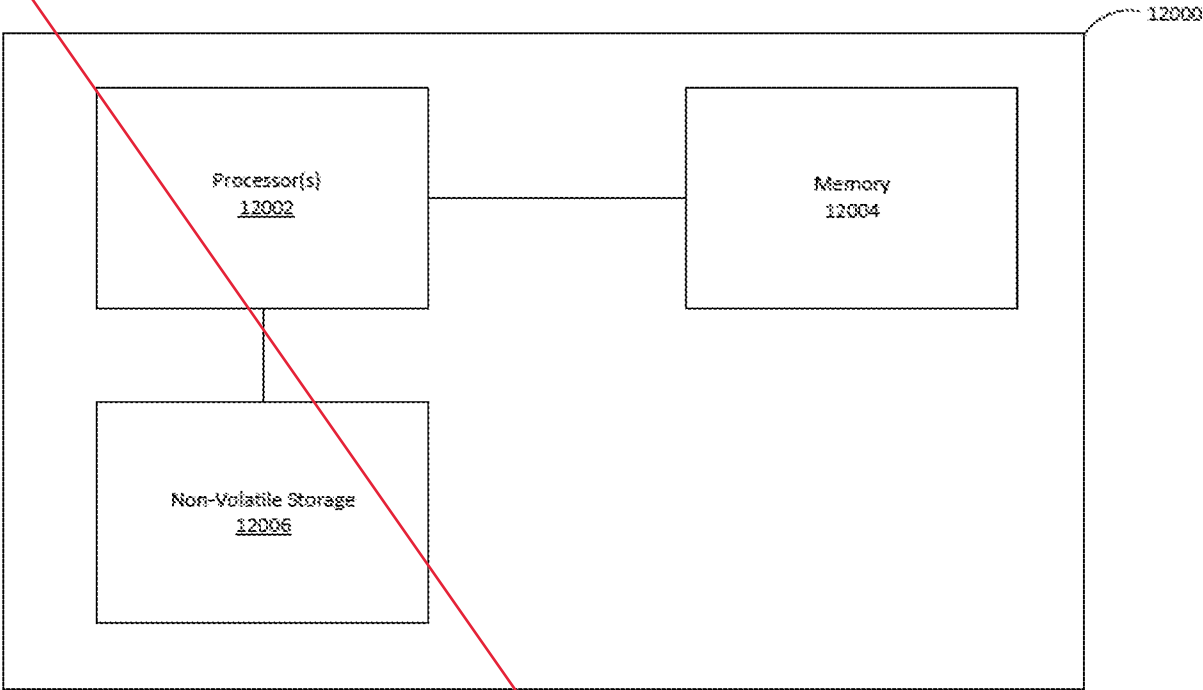
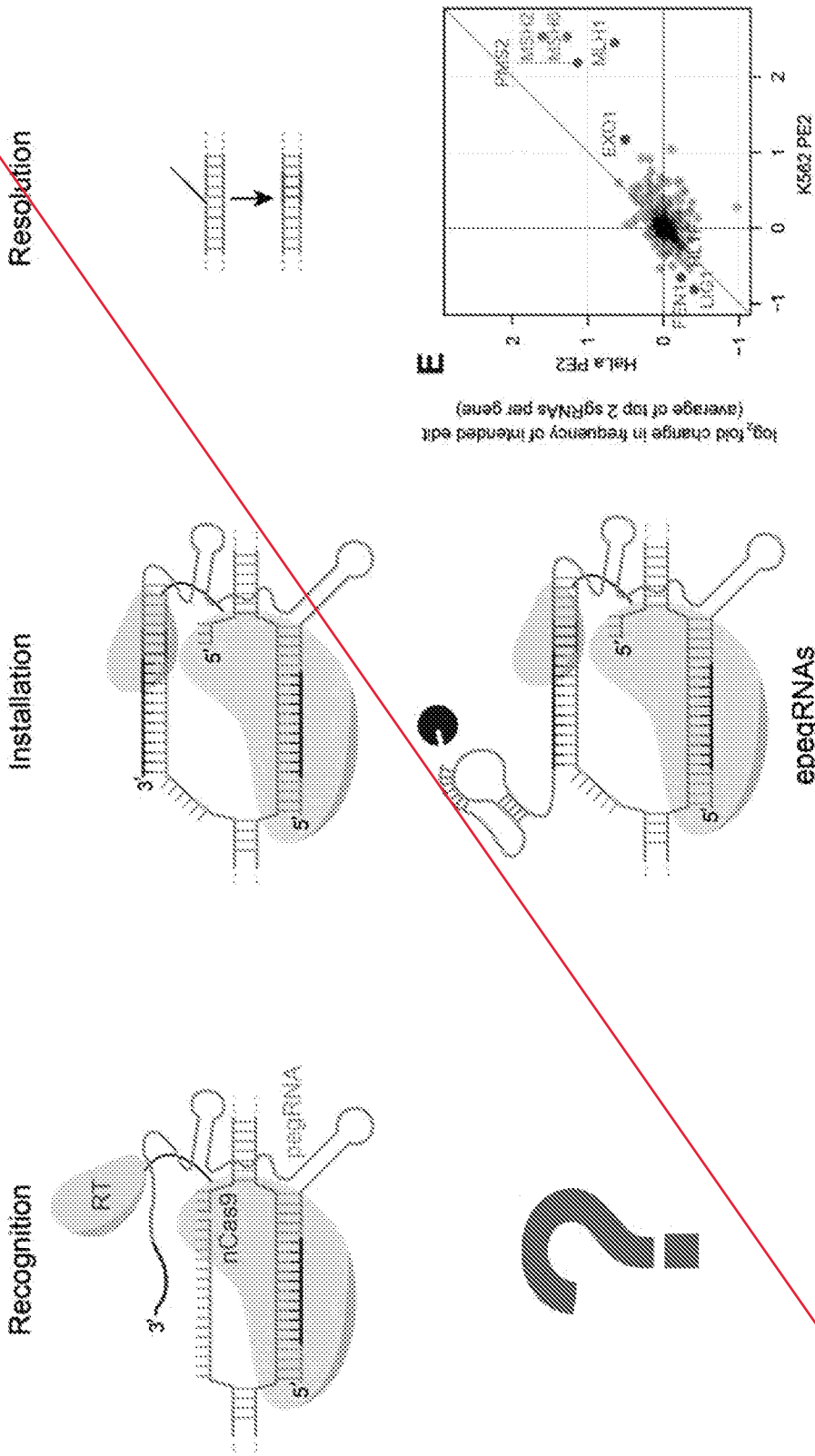


FIG. 130



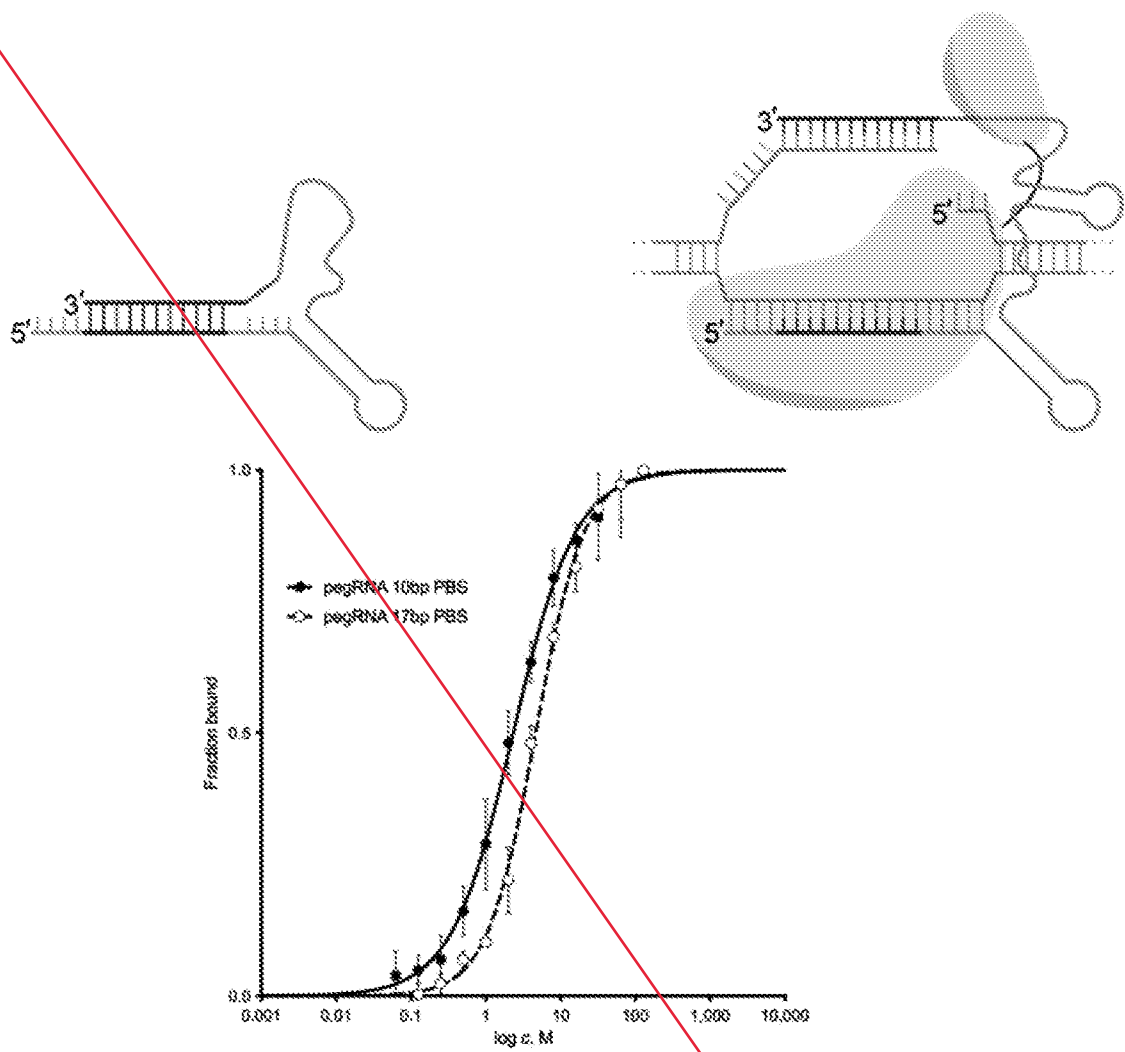


FIG. 132

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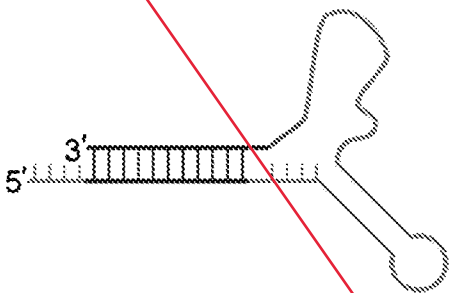
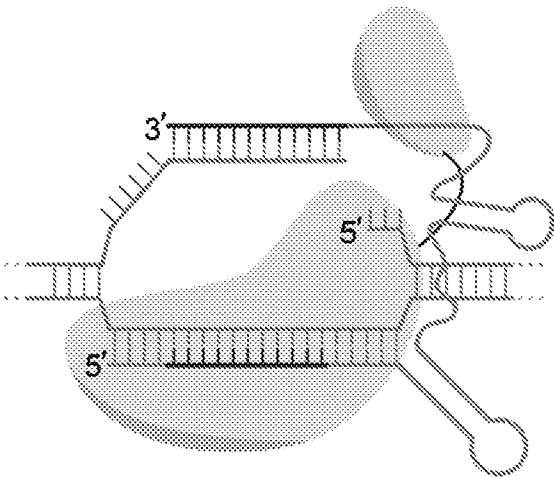


FIG. 133

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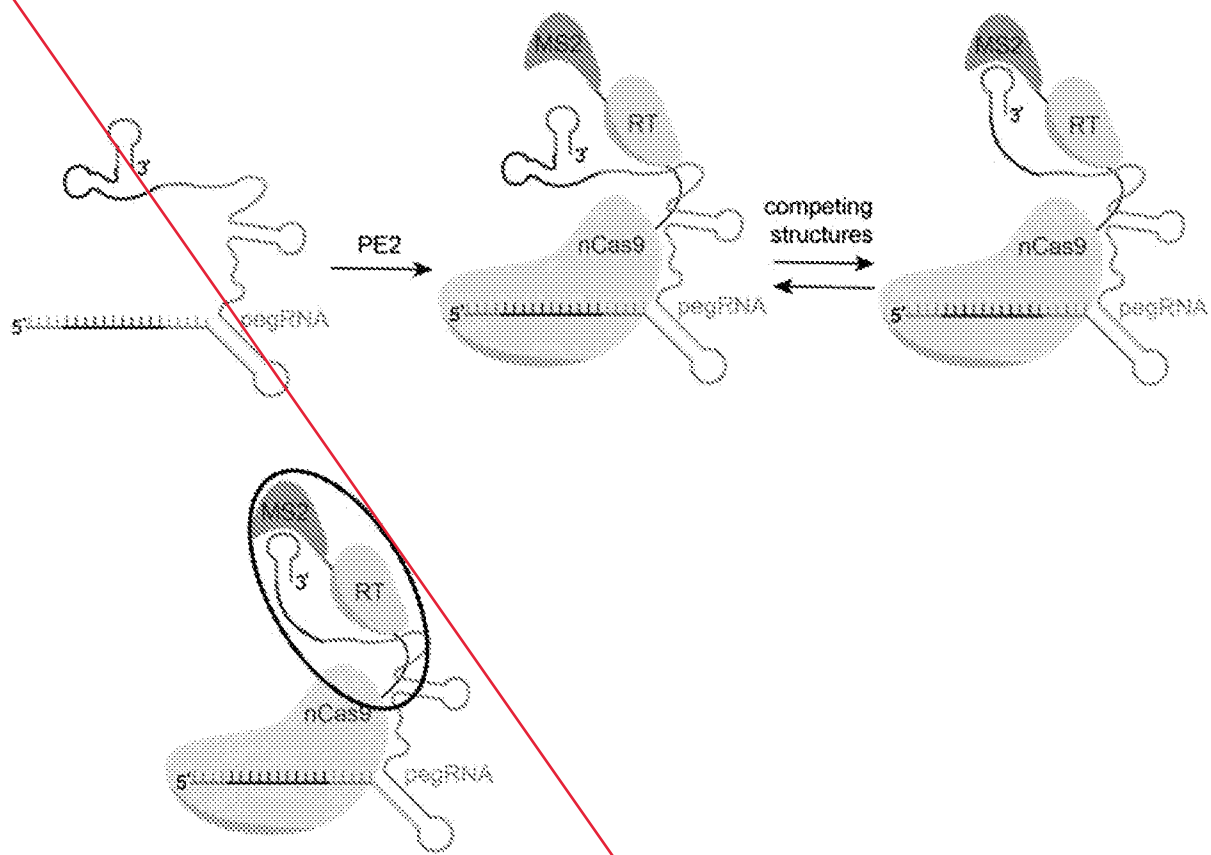
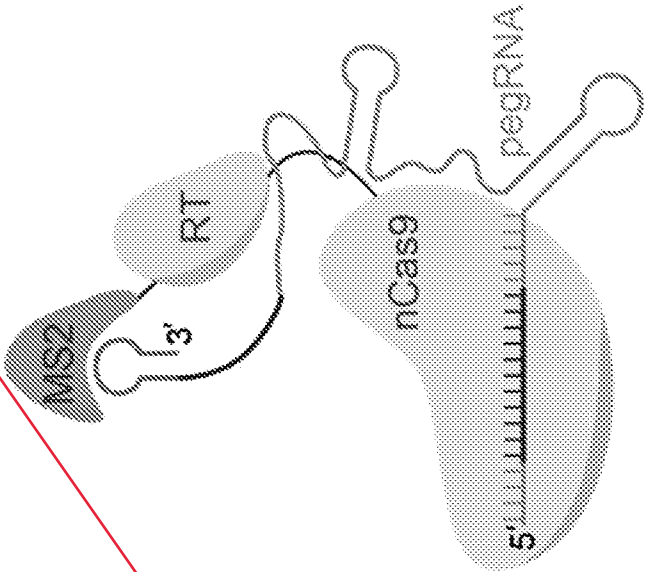
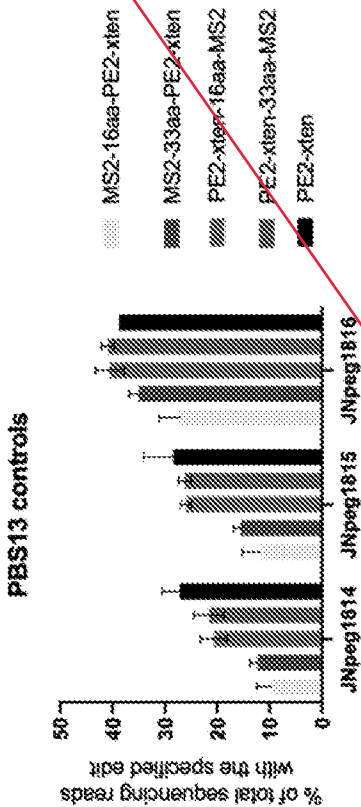


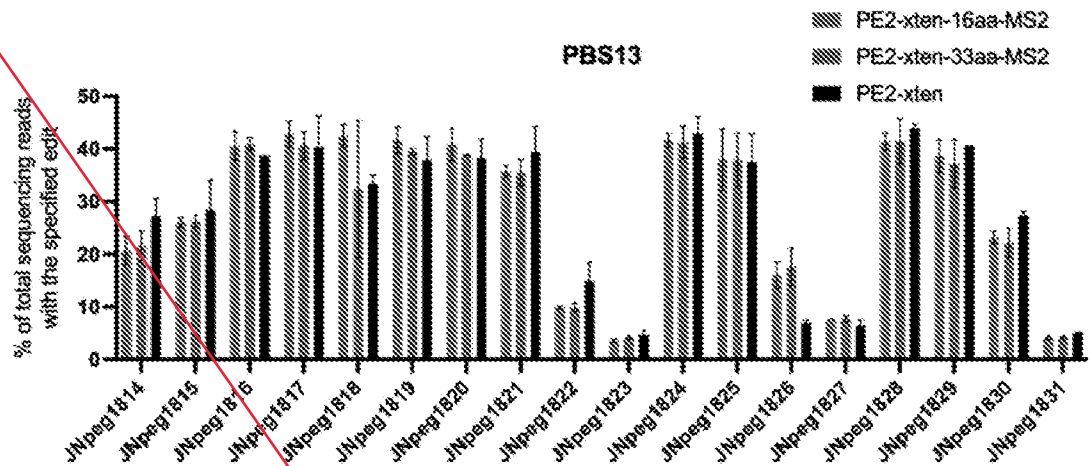
FIG. 134



1814: unmodified
1815: linker only
1816: epegRNA with MS2 aptamer

FIG. 135

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PBS = 13 all

1814: unmodified

1815: linker only

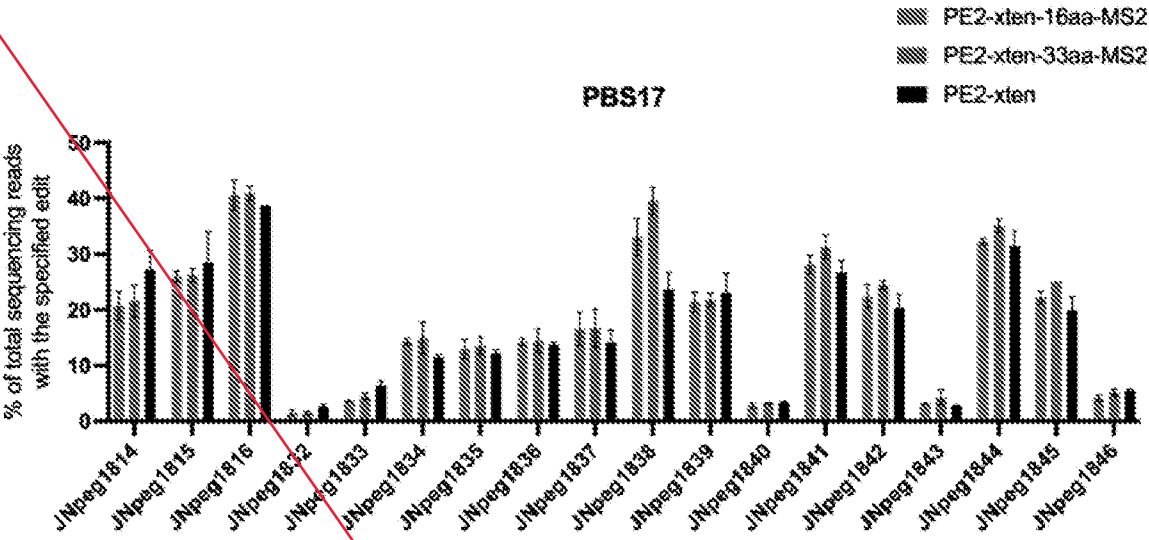
1816: epegRNA with MS2 aptamer

1817-1819: no toehold, MS2 aptamer

1820-1831: toeholds of various strengths, MS2 aptamer

FIG. 136

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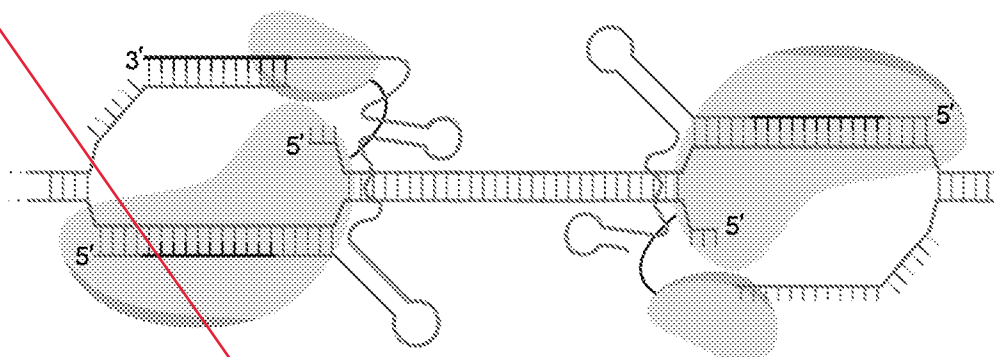


1814: unmodified PBS 13
1815: linker only PBS 13
1816: epegRNA with MS2 aptamer PBS 13
PBS 17 all
1832-1833: unmodified; linker alone
1834-1837: no toehold, MS2 aptamer
1838-1846: toeholds of various strengths, MS2 aptamer

FIG. 137

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PE3, pegRNA, ngRNA



PE3, sgRNA, pegRNA

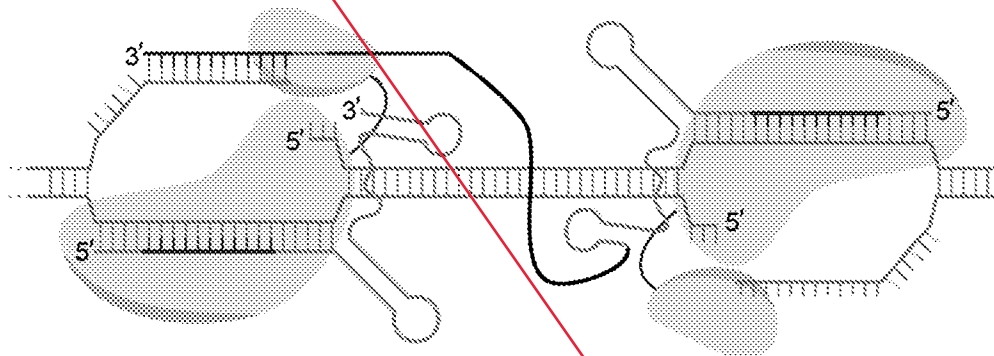


FIG. 138

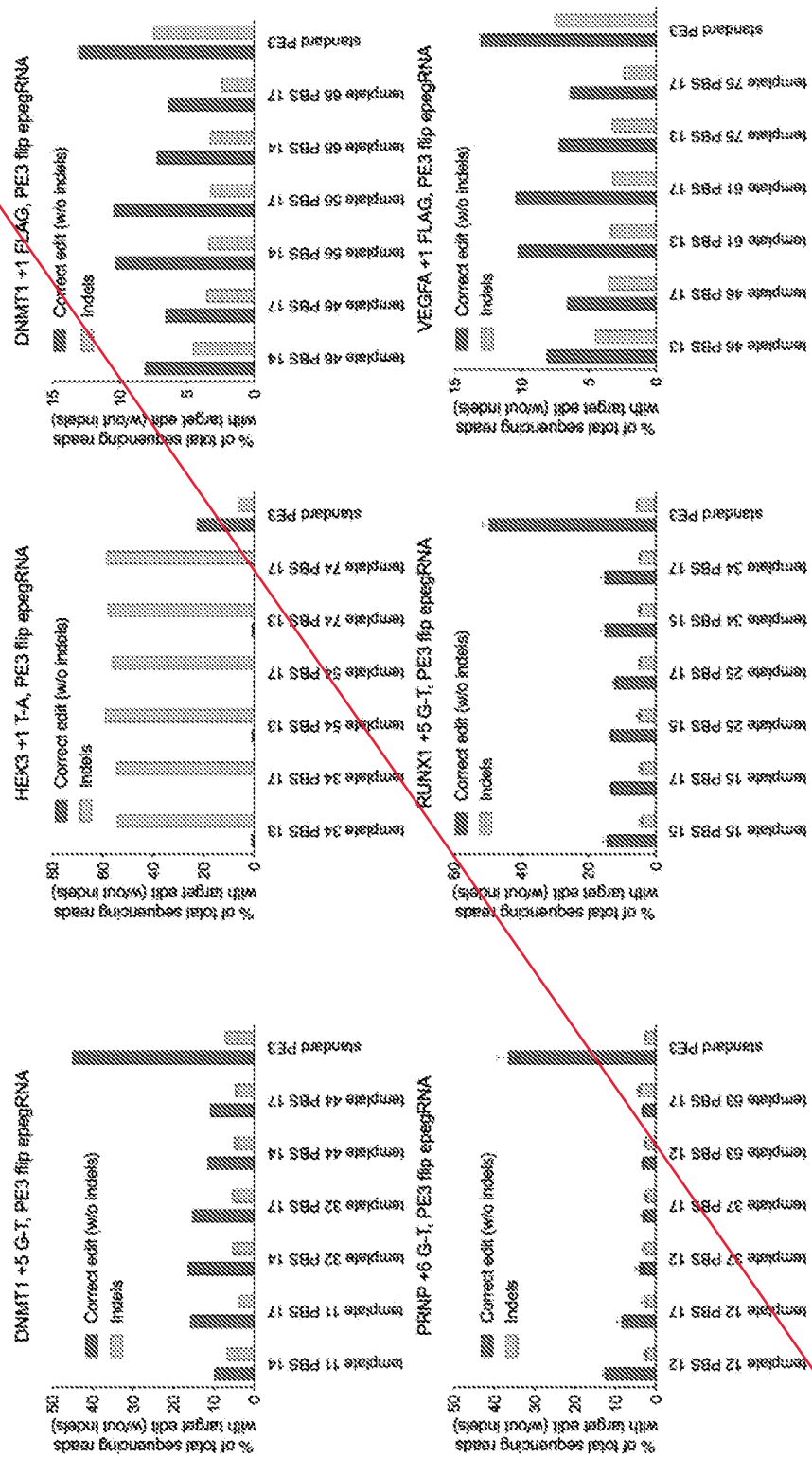


FIG. 139

Sequence score ordered by RNA stability, Set 10, 5 Data									
1.0	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
2	3	3	3	3	3	3	3	3	3
3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	9
10	10	10	10	10	10	10	10	10	10
11	11	11	11	11	11	11	11	11	11
12	12	12	12	12	12	12	12	12	12
13	13	13	13	13	13	13	13	13	13
14	14	14	14	14	14	14	14	14	14
15	15	15	15	15	15	15	15	15	15
16	16	16	16	16	16	16	16	16	16
17	17	17	17	17	17	17	17	17	17
18	18	18	18	18	18	18	18	18	18
19	19	19	19	19	19	19	19	19	19
20	20	20	20	20	20	20	20	20	20
21	21	21	21	21	21	21	21	21	21
22	22	22	22	22	22	22	22	22	22
23	23	23	23	23	23	23	23	23	23
24	24	24	24	24	24	24	24	24	24
25	25	25	25	25	25	25	25	25	25
26	26	26	26	26	26	26	26	26	26
27	27	27	27	27	27	27	27	27	27
28	28	28	28	28	28	28	28	28	28
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30	30	30	30	30	30	30	30	30	30
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36	36	36	36	36	36	36	36	36	36
37	37	37	37	37	37	37	37	37	37
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40	40	40	40	40	40	40	40	40	40
41	41	41	41	41	41	41	41	41	41
42	42	42	42	42	42	42	42	42	42
43	43	43	43	43	43	43	43	43	43
44	44	44	44	44	44	44	44	44	44
45	45	45	45	45	45	45	45	45	45
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47	47	47	47	47	47	47	47	47	47
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49	49	49	49	49	49	49	49	49	49
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51	51	51	51	51	51	51	51	51	51
52	52	52	52	52	52	52	52	52	52
53	53	53	53	53	53	53	53	53	53
54	54	54	54	54	54	54	54	54	54
55	55	55	55	55	55	55	55	55	55
56	56	56	56	56	56	56	56	56	56
57	57	57	57	57	57	57	57	57	57
58	58	58	58	58	58	58	58	58	58
59	59	59	59	59	59	59	59	59	59
60	60	60	60	60	60	60	60	60	60
61	61	61	61	61	61	61	61	61	61
62	62	62	62	62	62	62	62	62	62
63	63	63	63	63	63	63	63	63	63
64	64	64	64	64	64	64	64	64	64
65	65	65	65	65	65	65	65	65	65
66	66	66	66	66	66	66	66	66	66
67	67	67	67	67	67	67	67	67	67
68	68	68	68	68	68	68	68	68	68
69	69	69	69	69	69	69	69	69	69
70	70	70	70	70	70	70	70	70	70
71	71	71	71	71	71	71	71	71	71
72	72	72	72	72	72	72	72	72	72
73	73	73	73	73	73	73	73	73	73
74	74	74	74	74	74	74	74	74	74
75	75	75	75	75	75	75	75	75	75
76	76	76	76	76	76	76	76	76	76
77	77	77	77	77	77	77	77	77	77
78	78	78	78	78	78	78	78	78	78
79	79	79	79	79	79	79	79	79	79
80	80	80	80	80	80	80	80	80	80
81	81	81	81	81	81	81	81	81	81
82	82	82	82	82	82	82	82	82	82
83	83	83	83	83	83	83	83	83	83
84	84	84	84	84	84	84	84	84	84
85	85	85	85	85	85	85	85	85	85
86	86	86	86	86	86	86	86	86	86
87	87	87	87	87	87	87	87	87	87
88	88	88	88	88	88	88	88	88	88
89	89	89	89	89	89	89	89	89	89
90	90	90	90	90	90	90	90	90	90
91	91	91	91	91	91	91	91	91	91
92	92	92	92	92	92	92	92	92	92
93	93	93	93	93	93	93	93	93	93
94	94	94	94	94	94	94	94	94	94
95	95	95	95	95	95	95	95	95	95
96	96	96	96	96	96	96	96	96	96
97	97	97	97	97	97	97	97	97	97
98	98	98	98	98	98	98	98	98	98
99	99	99	99	99	99	99	99	99	99
100	100	100	100	100	100	100	100	100	100

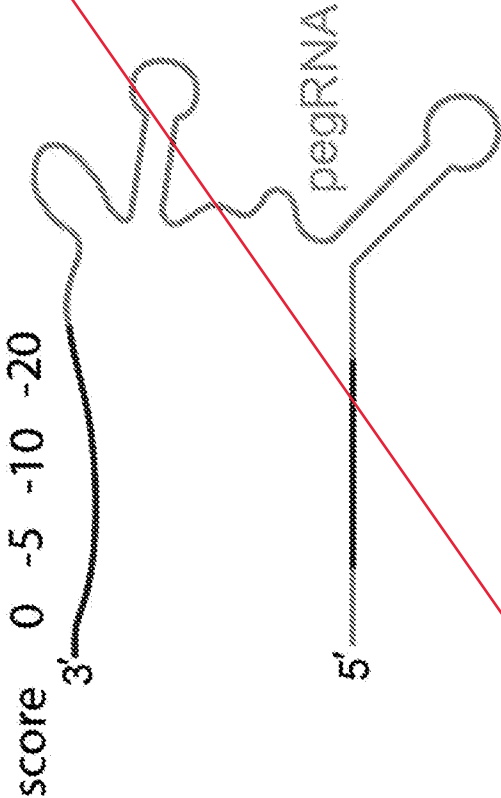


FIG. 140

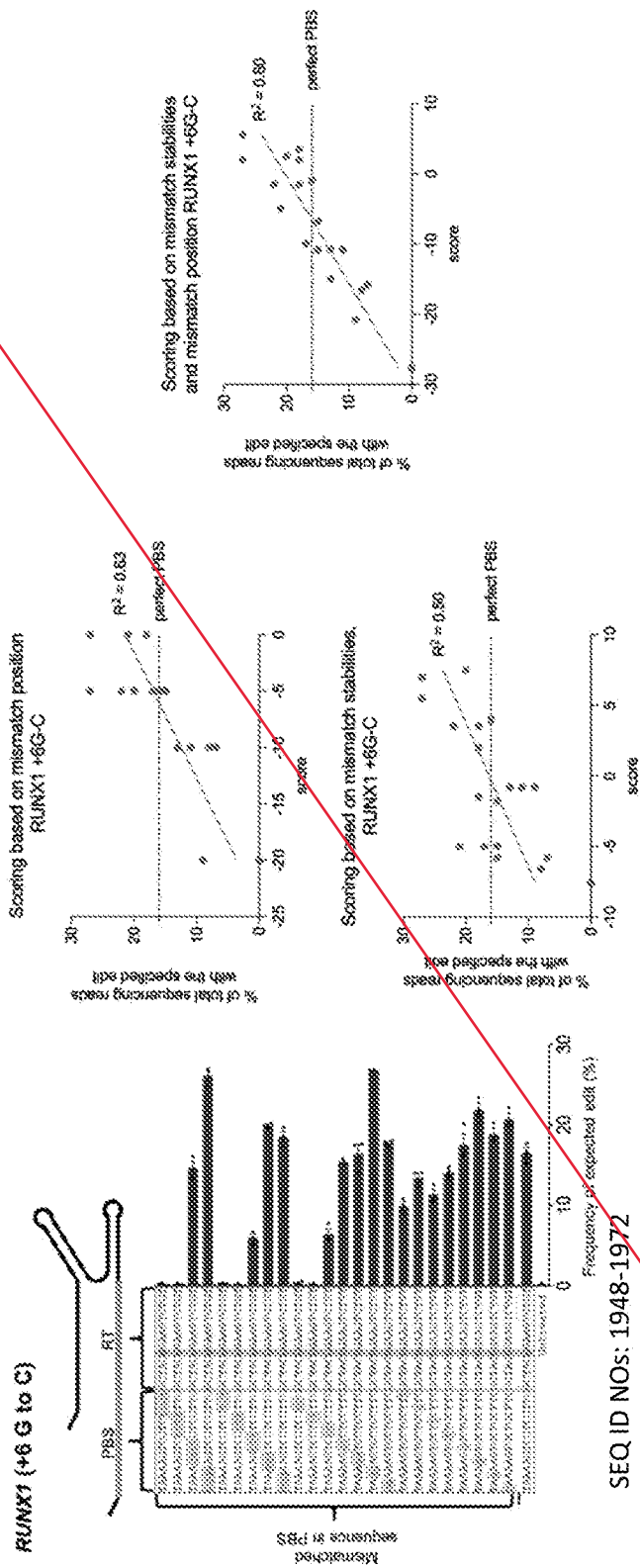


FIG. 141

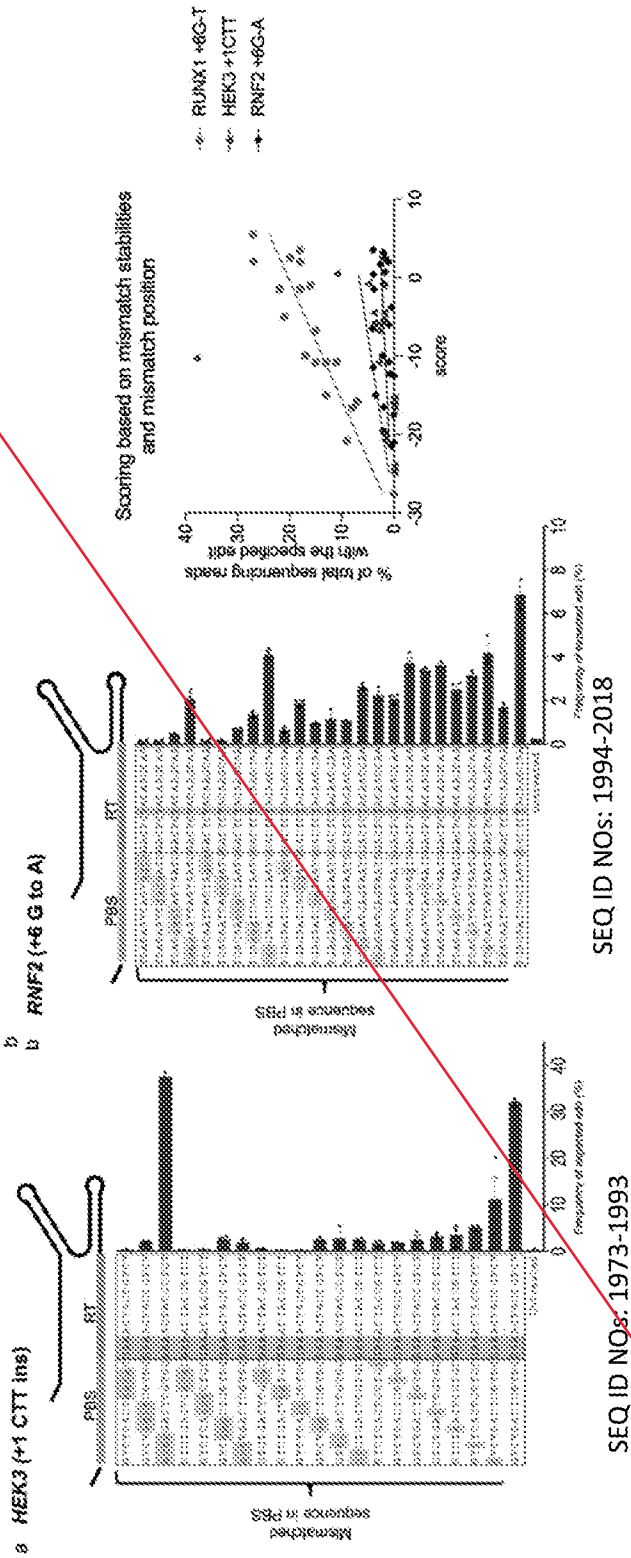


FIG. 142

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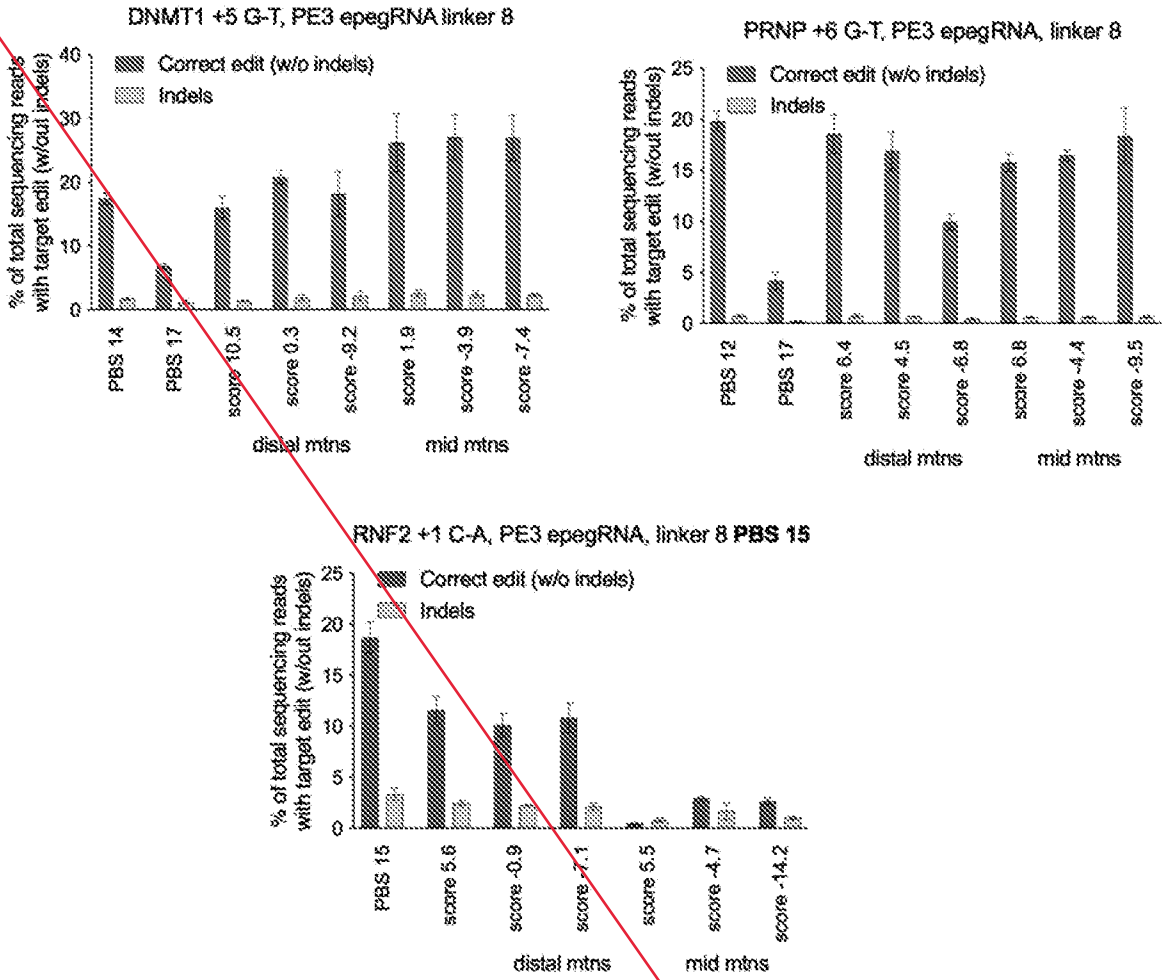


FIG. 143

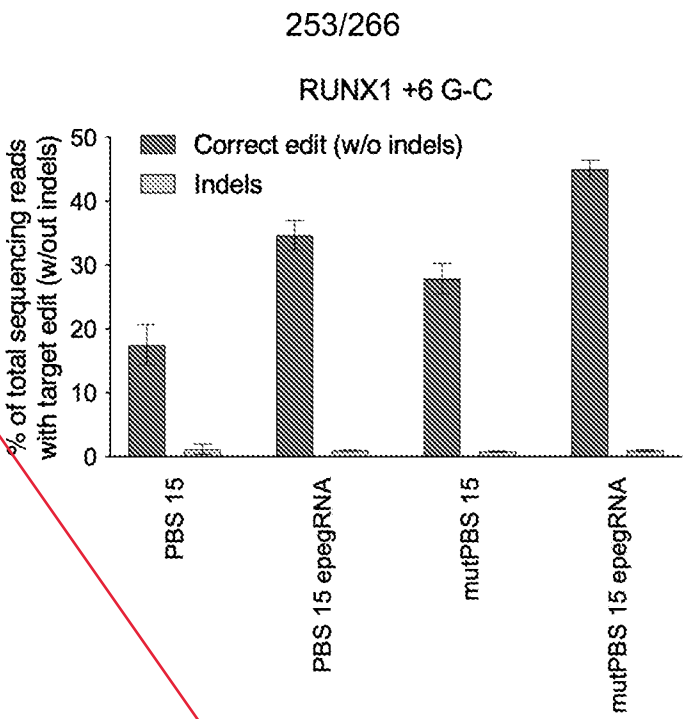


FIG. 144

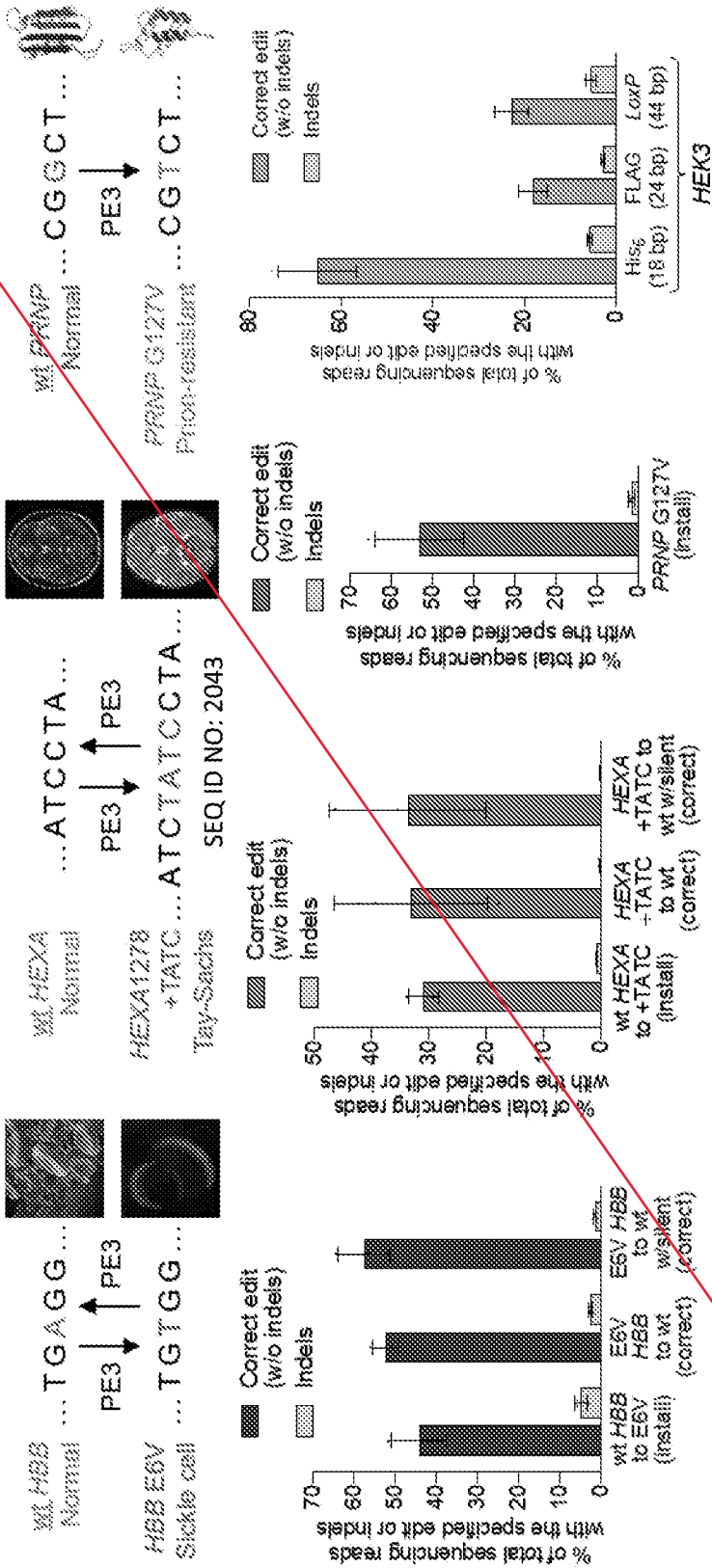


FIG. 145

SEQ ID NO: 2019

CDKL5 5'... TGGGGAATTGTGACAATATAGCTAT ... 3'

Prime editing in principle can directly install or correct mutations that cause CDD

CDKL5 1412delA 5'... TGGGGAATTGTG~CAATATAGCTAT ... 3'

SEQ ID NO: 2020

Spacer
5'-GAGGGACUCCUAGAGGACUGGUUUUAGAGCUA G A
U C-GGAUAAAUU CGAU A
A G U C C G U U A
1412delA
5'... GAGGGAATCTCTAGAGGACCTGGGGAATTGTGCAATATAGCTAT ... 3'
SEQ ID NO: 2022
Cas9-induced nick PAM
1412delA
Primer ~13 nt
DNA to be replaced by prime editing

One of many possible pegRNAs that programs a prime editor to insert the missing A at the target site

RT template that inserts the missing A in CDKL5

CDKL5 c.1412delA locus

SEQ ID NO: 2021

RT template (includes edit)
A G C C A C G G U C A A
U C C G U C A U A G C U A U A U U G U C A C A A U U C C C A G U C C U C U A G G A G U C U U U -3'

Primer-binding site (PBS)

FIG. 146

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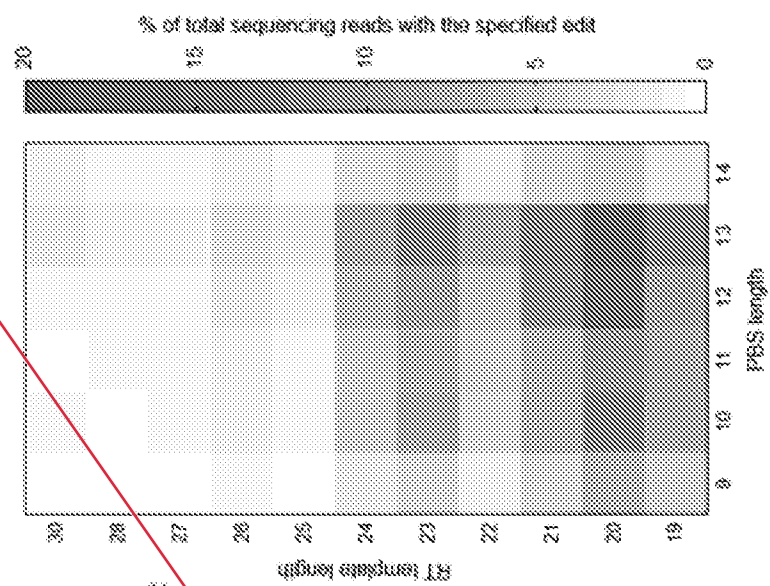
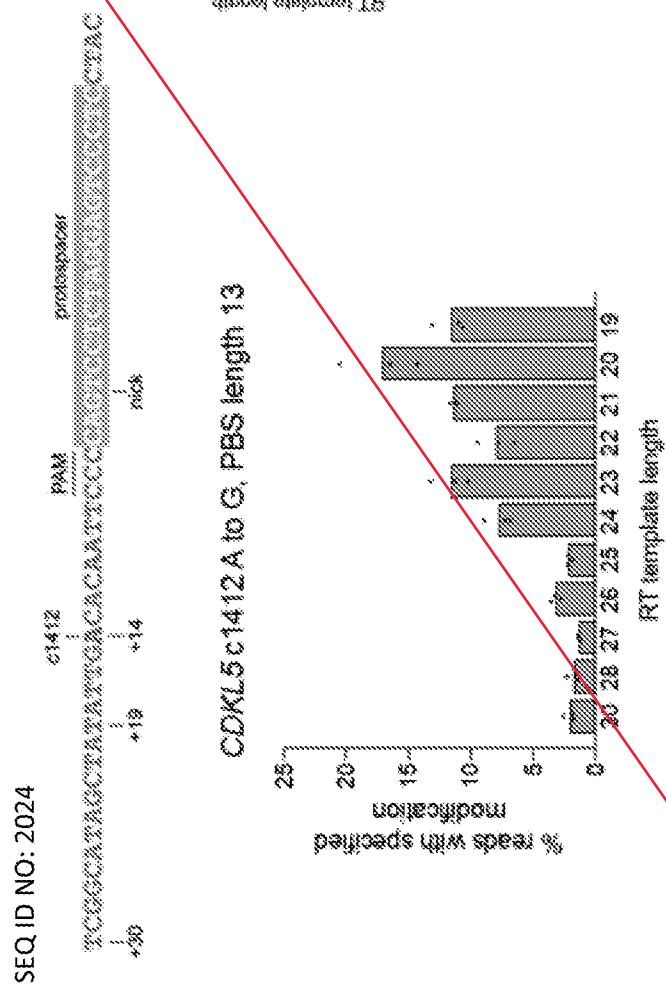


FIG. 147

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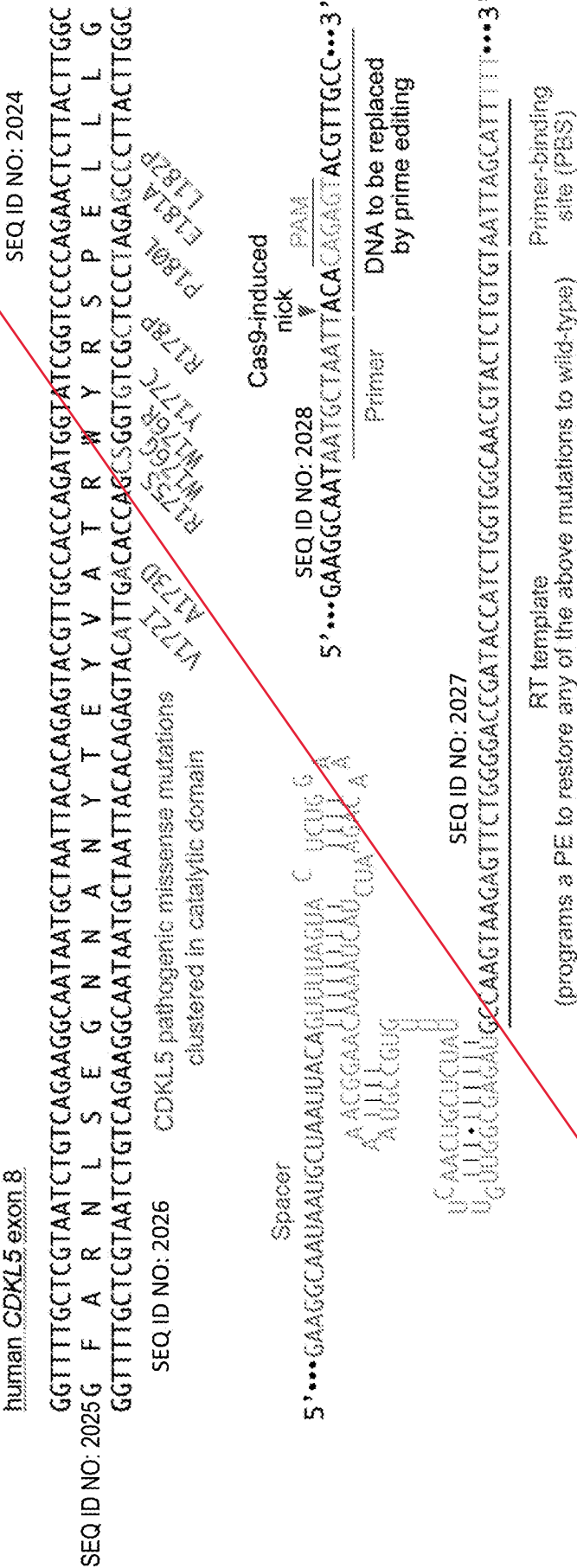


FIG. 148

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CDKL5 c1412delA prime editing
Transfection, N2A cells

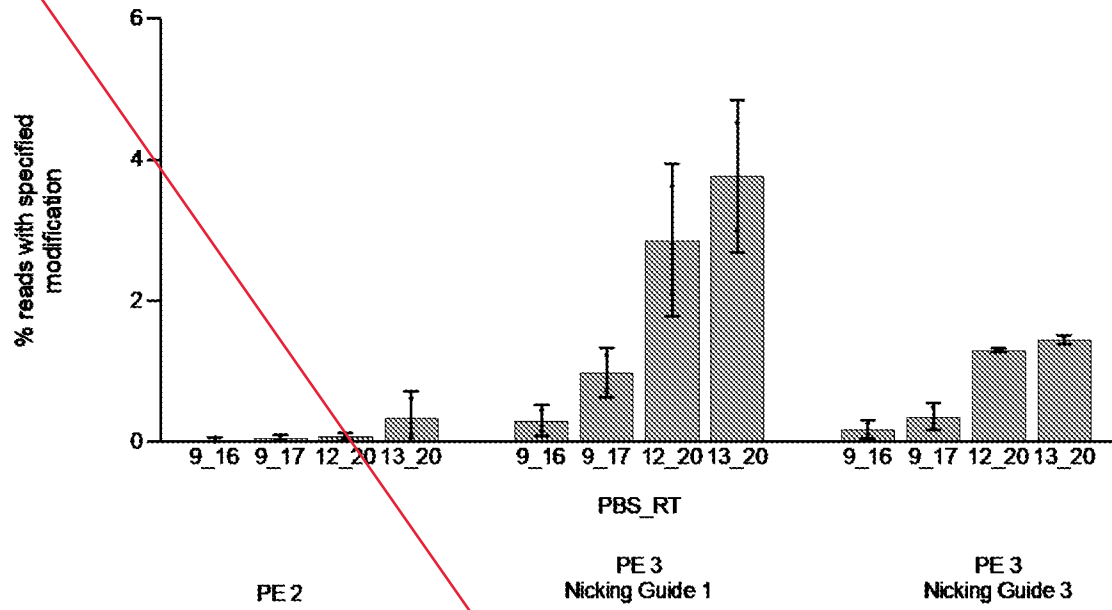


FIG. 150

1412delA insertion in N2A cells
epegRNA KAE072, no seed editing

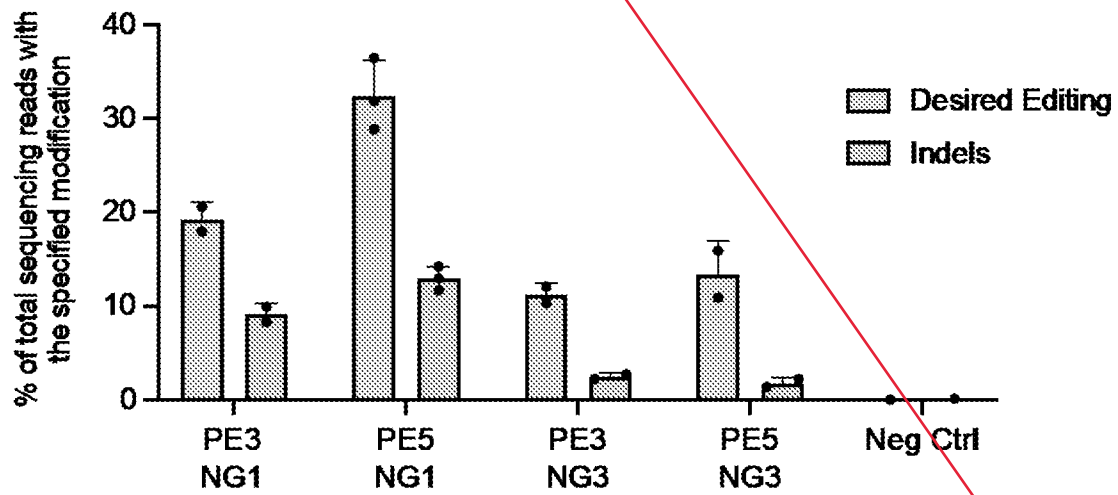


FIG. 151

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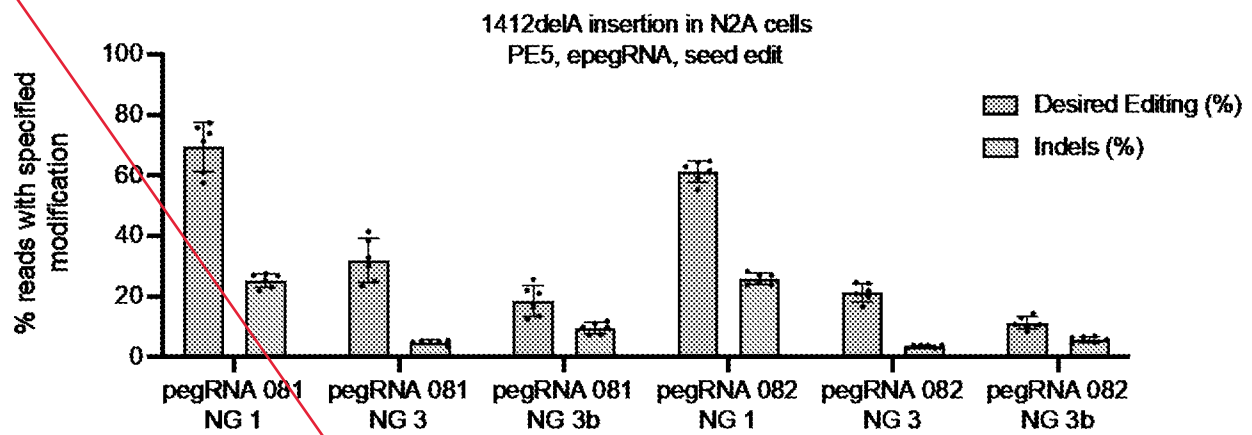


FIG. 152

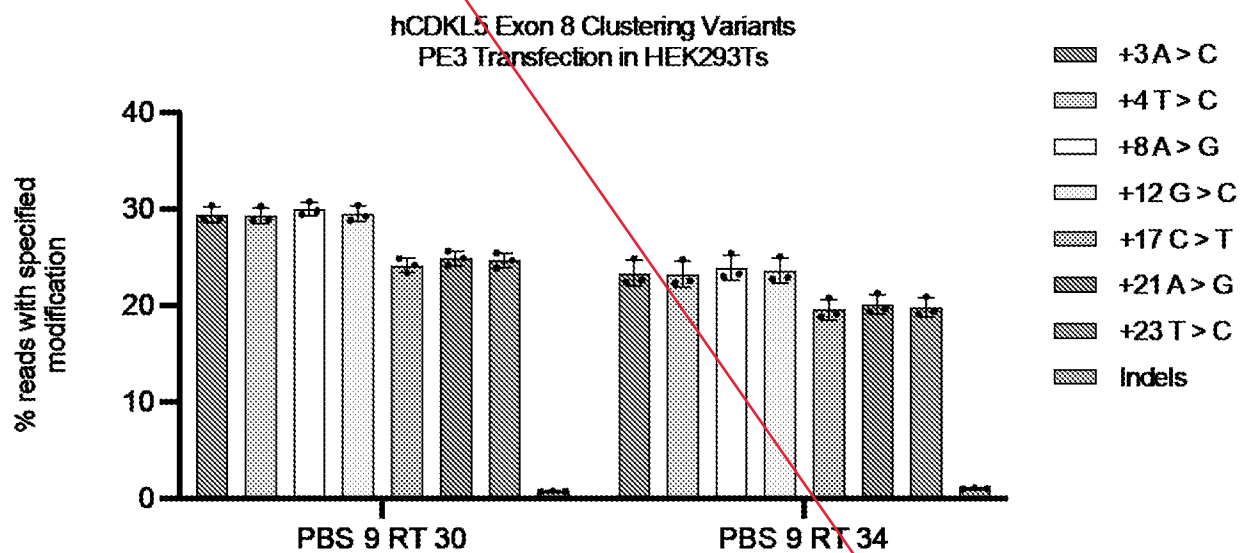


FIG. 153

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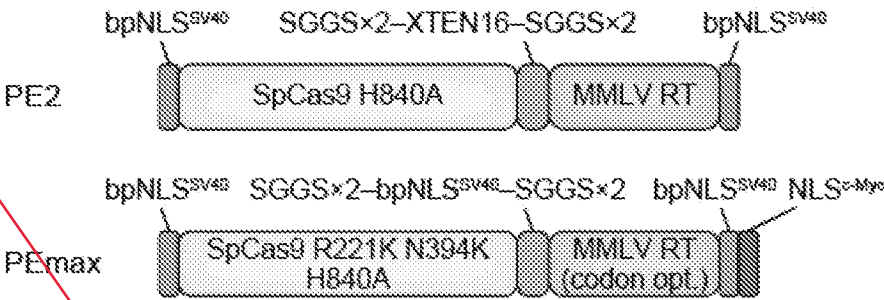


FIG. 154

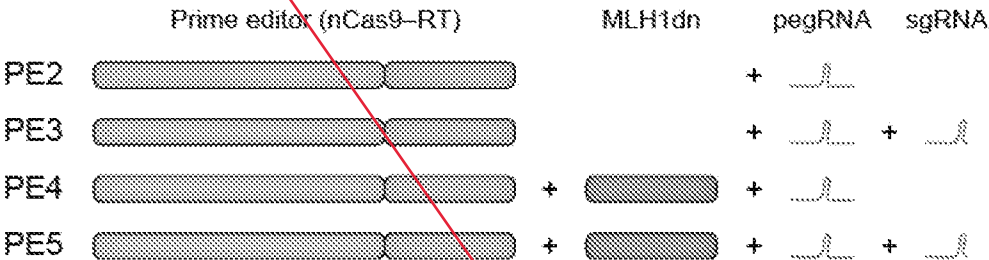


FIG. 155

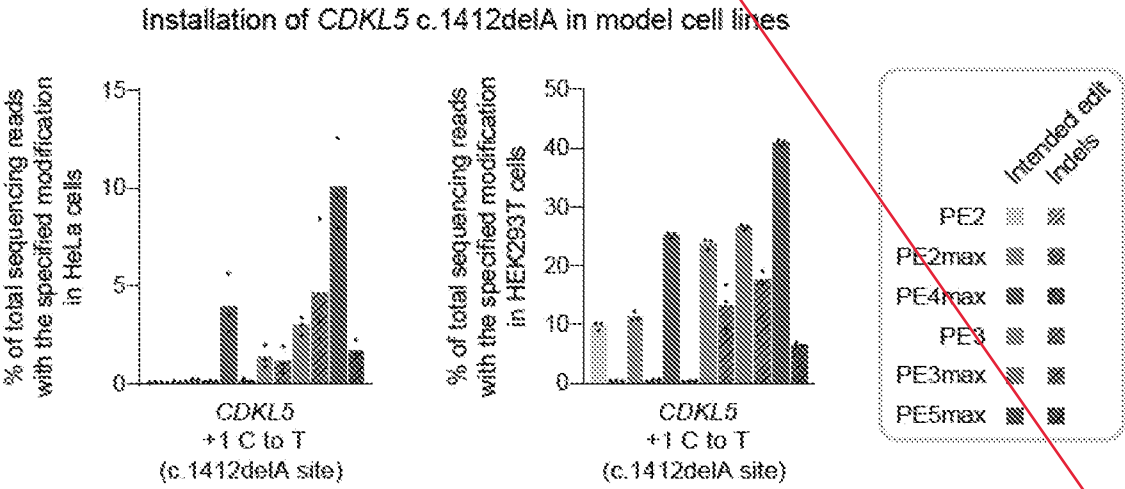


FIG. 156

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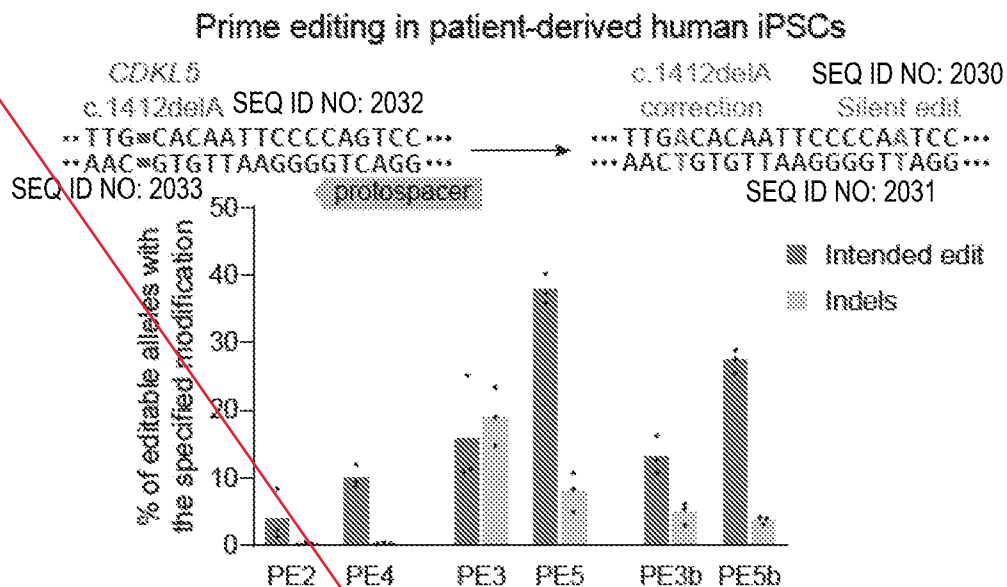


FIG. 157

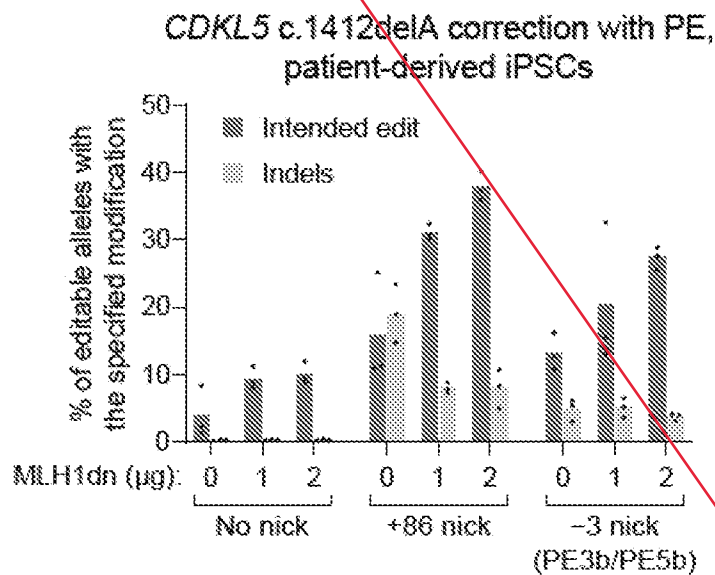


FIG. 158

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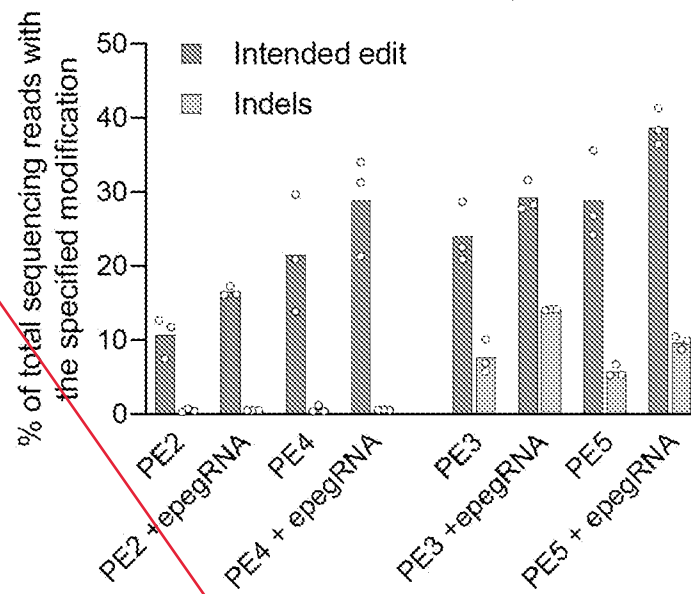
CDKL5 c.1412 A to G, HEK293T

FIG. 159

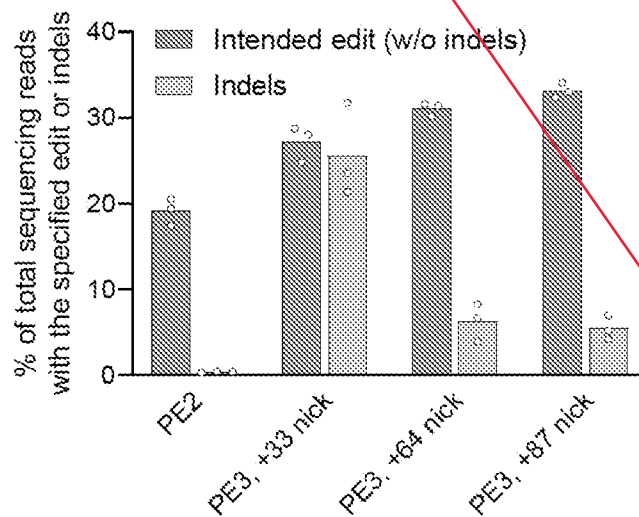
CDKL5 silent +1 C to T installation (c.1412delA site), HEK293T

FIG. 160

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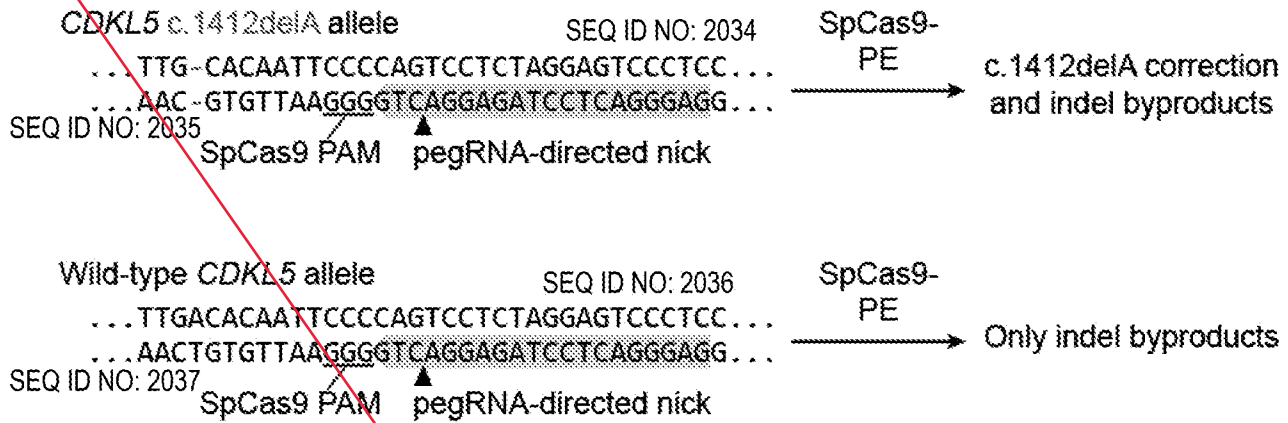


FIG. 161

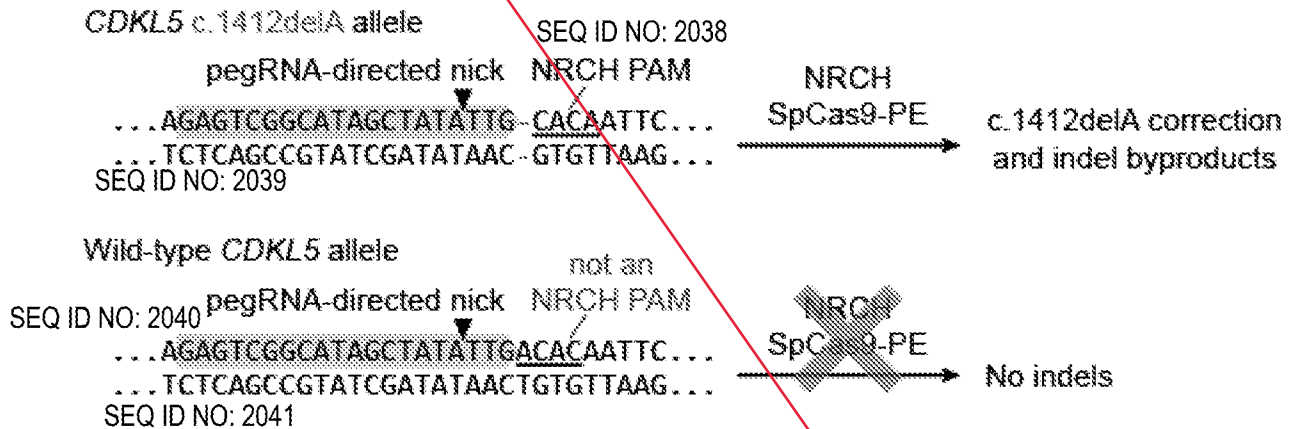


FIG. 162

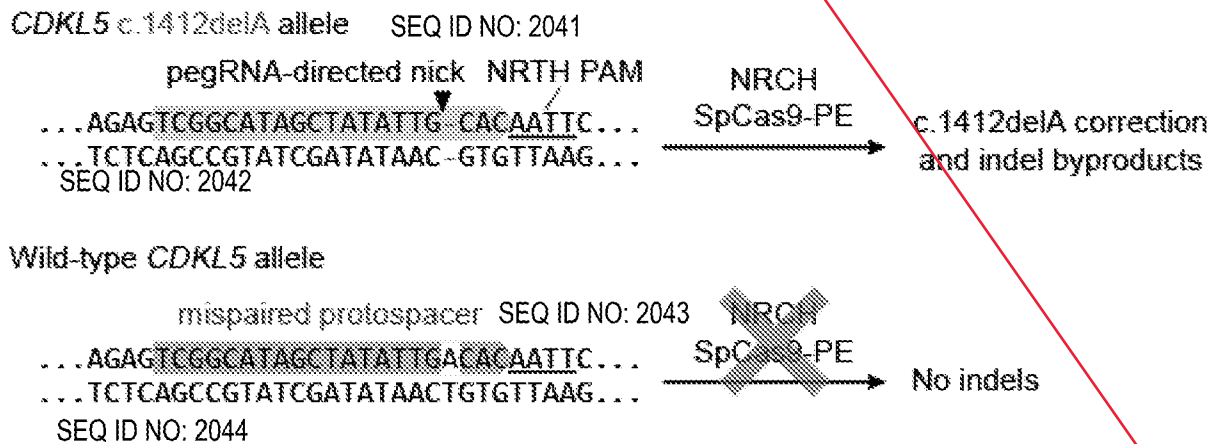


FIG. 163

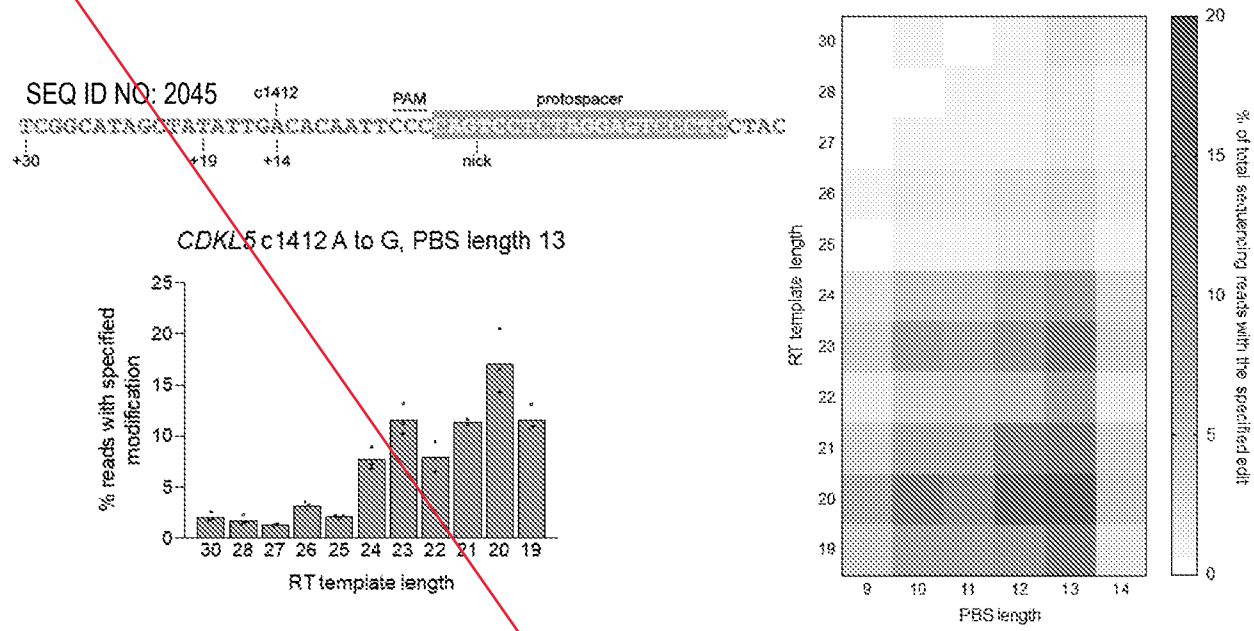


FIG. 164

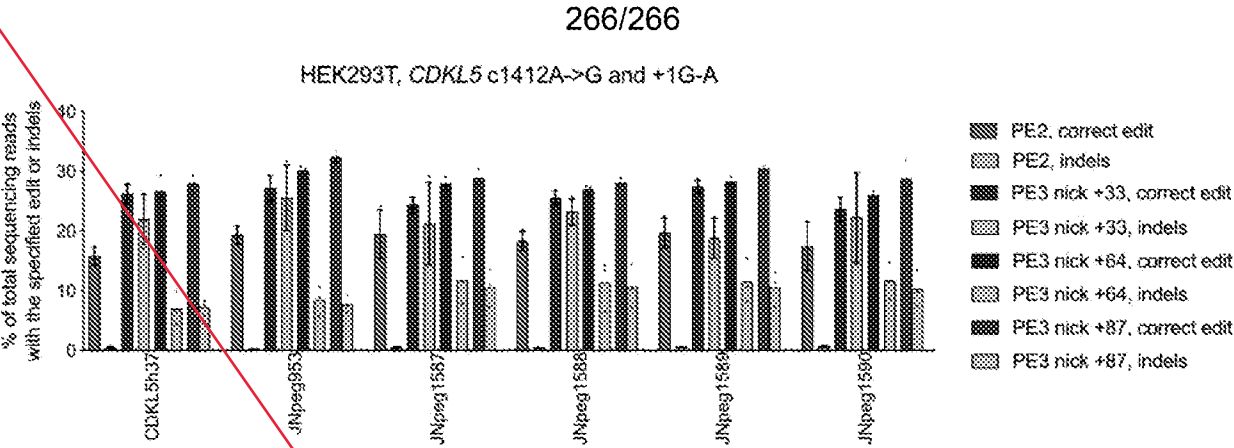


FIG. 165

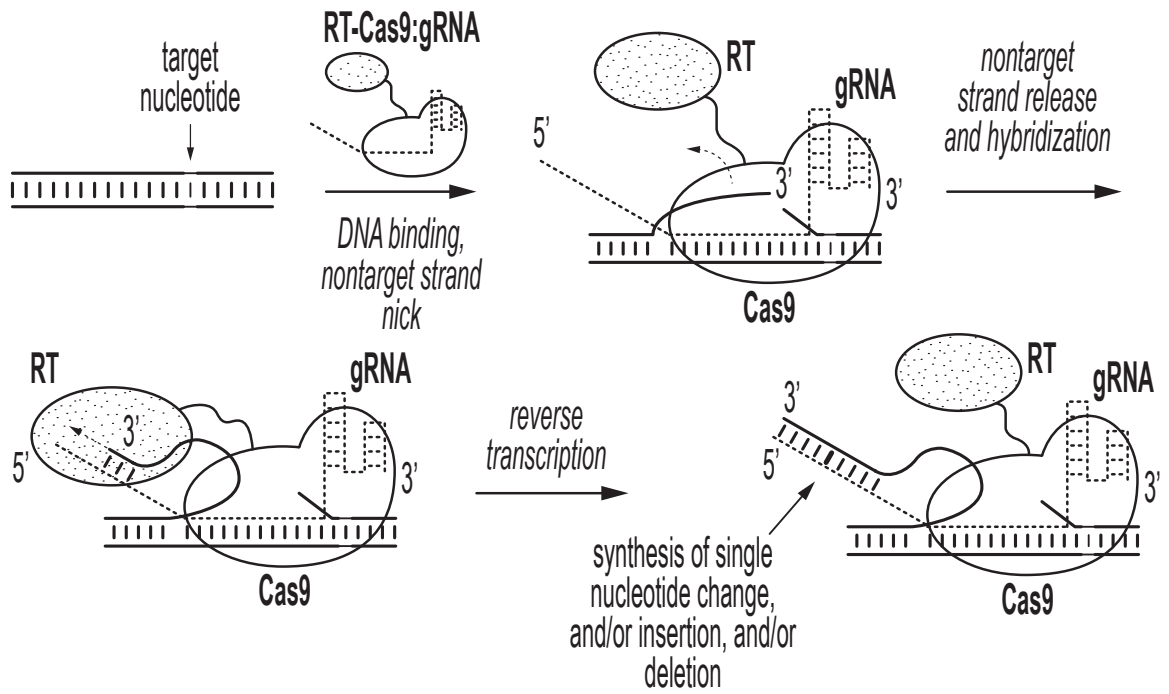


FIG. 1A

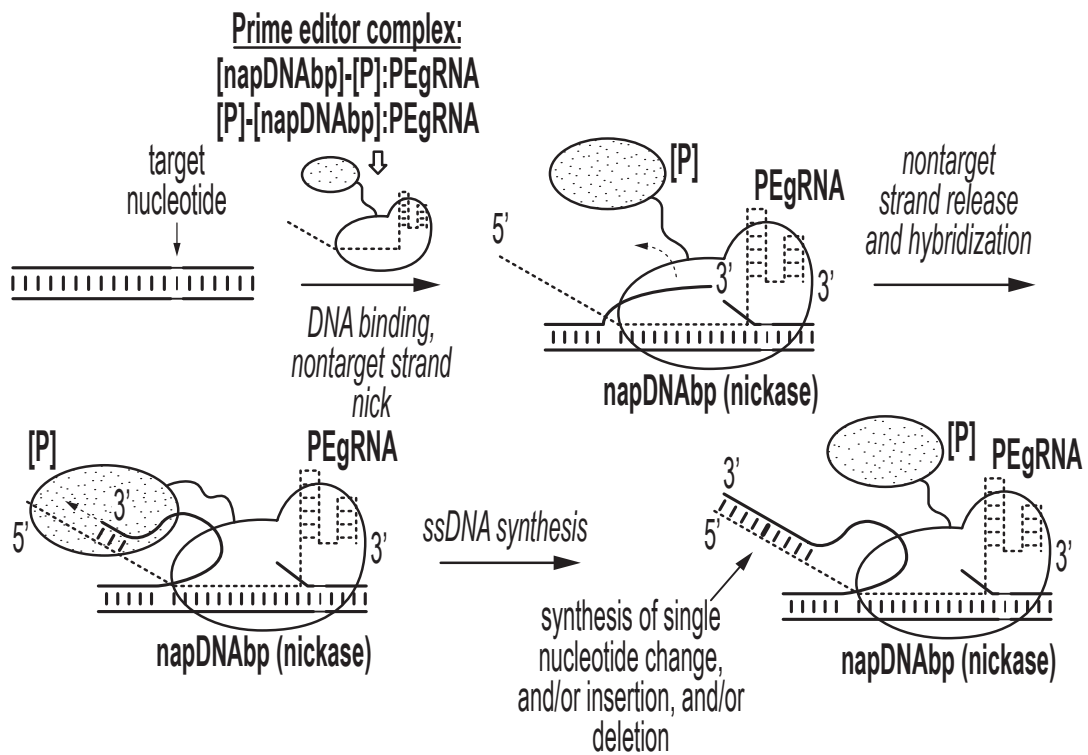


FIG. 1B

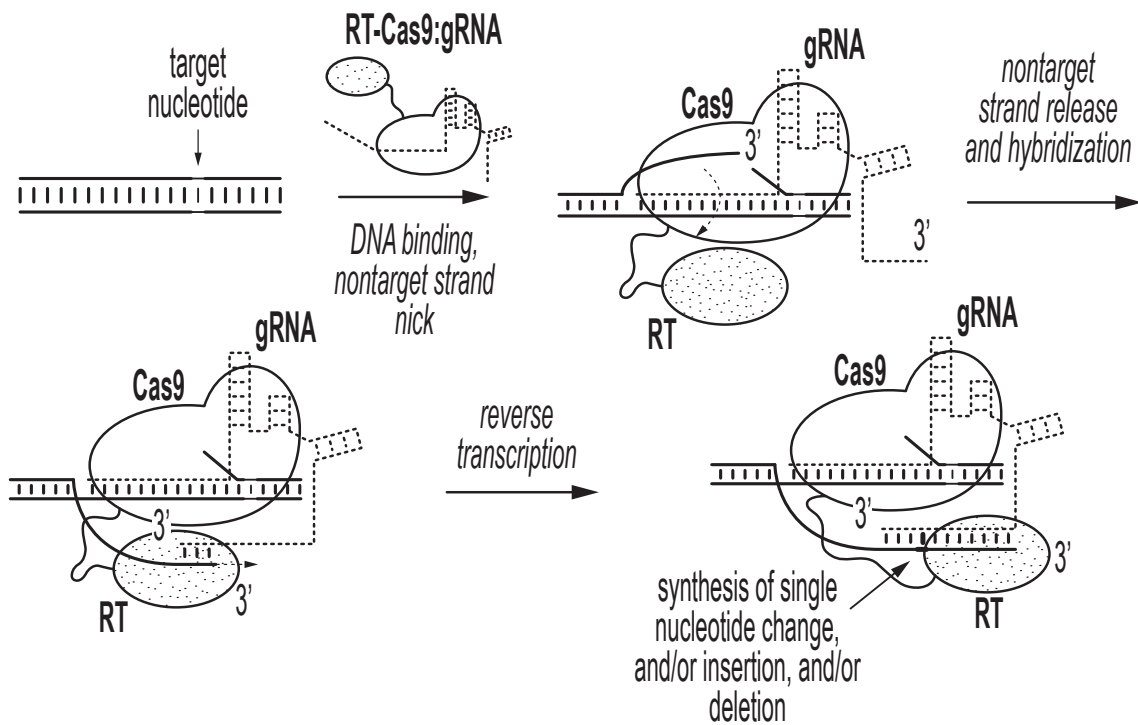


FIG. 1C

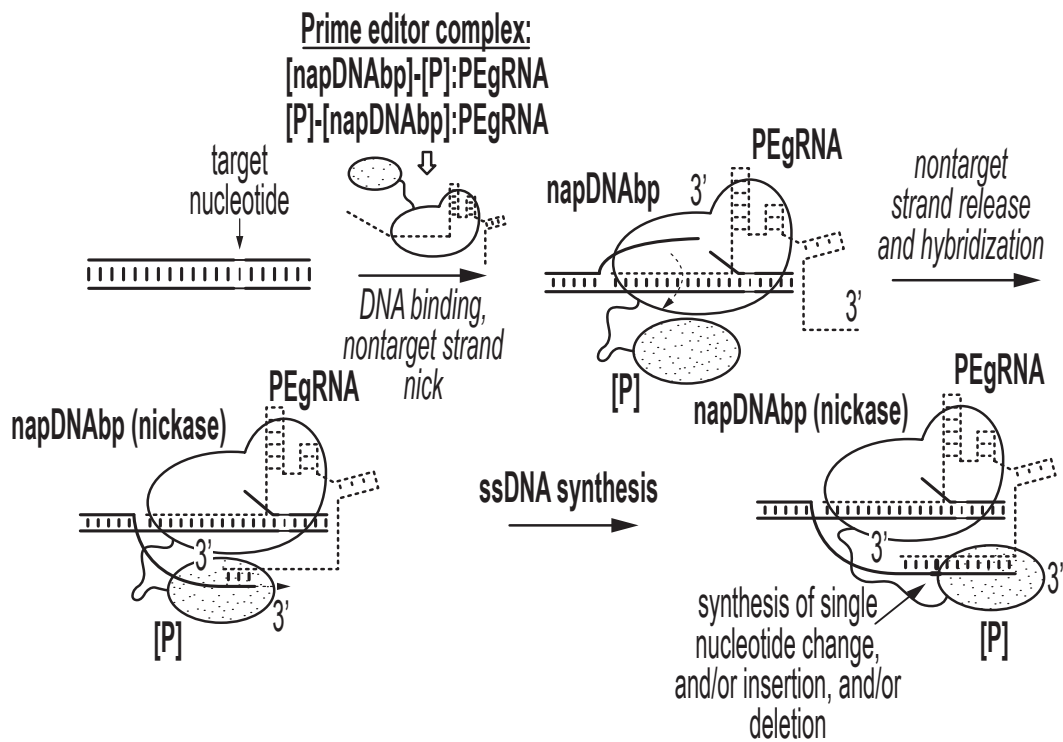


FIG. 1D

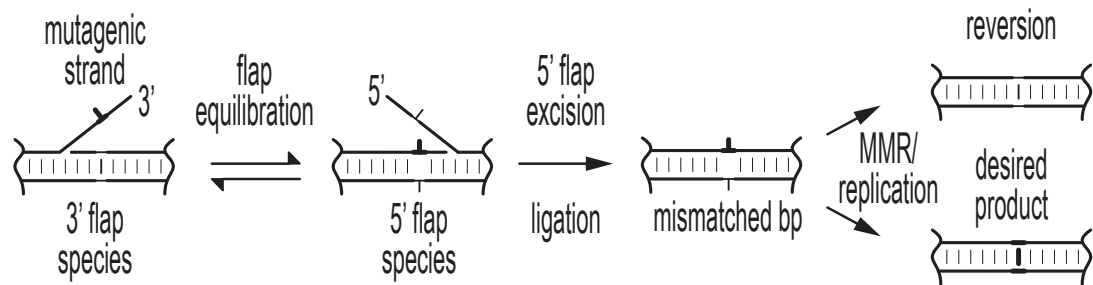


FIG. 1E

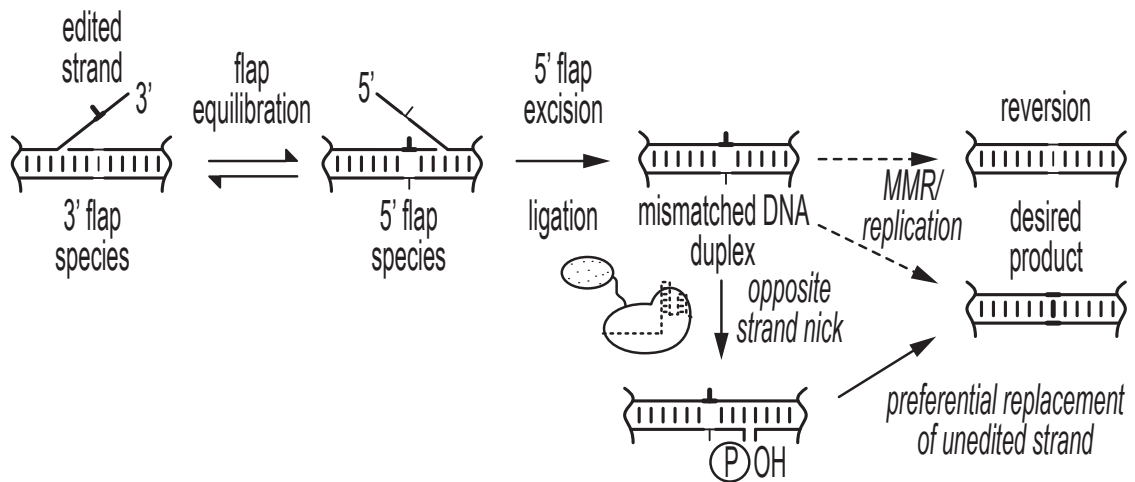


FIG. 1F

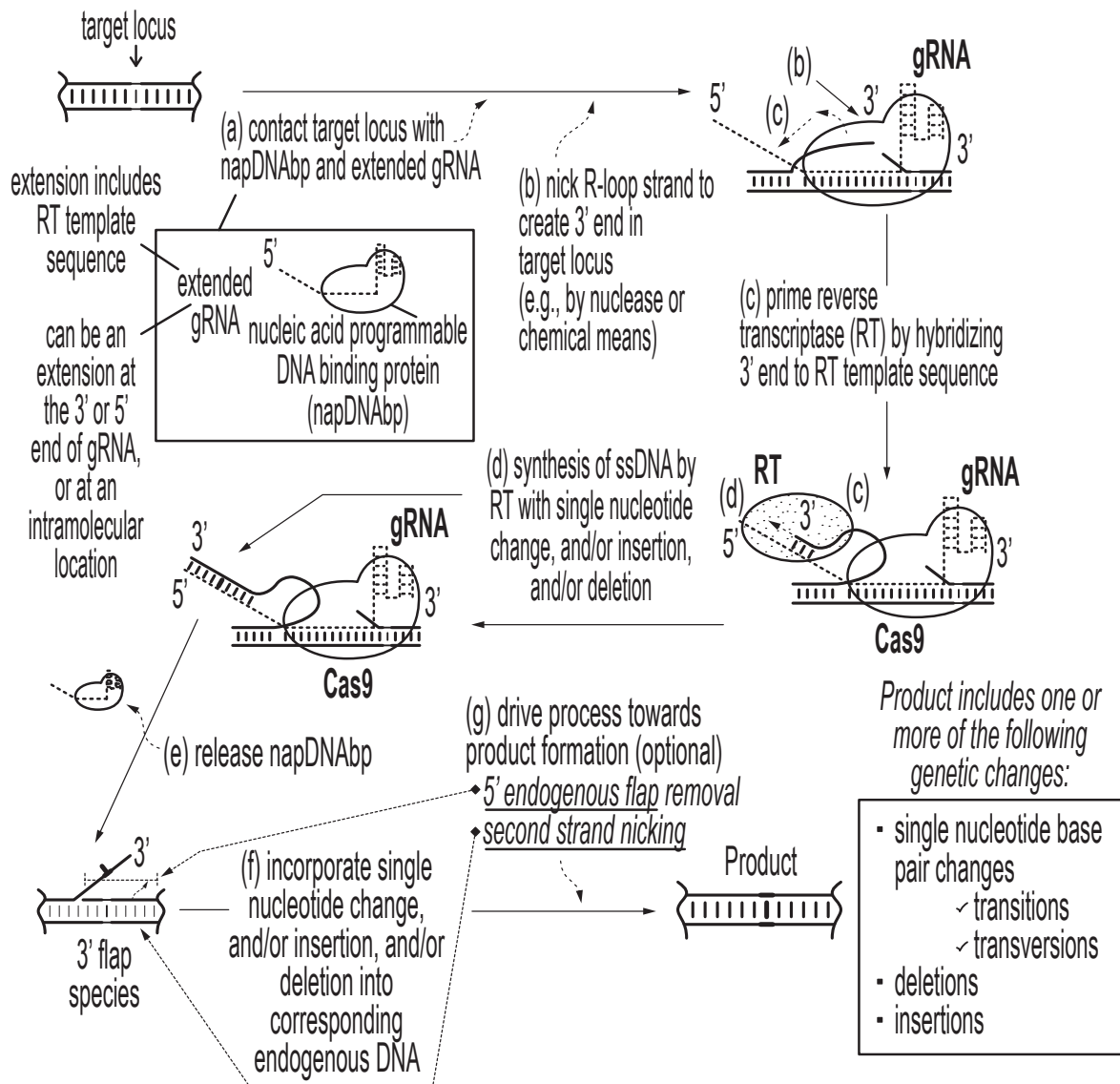


FIG. 1G

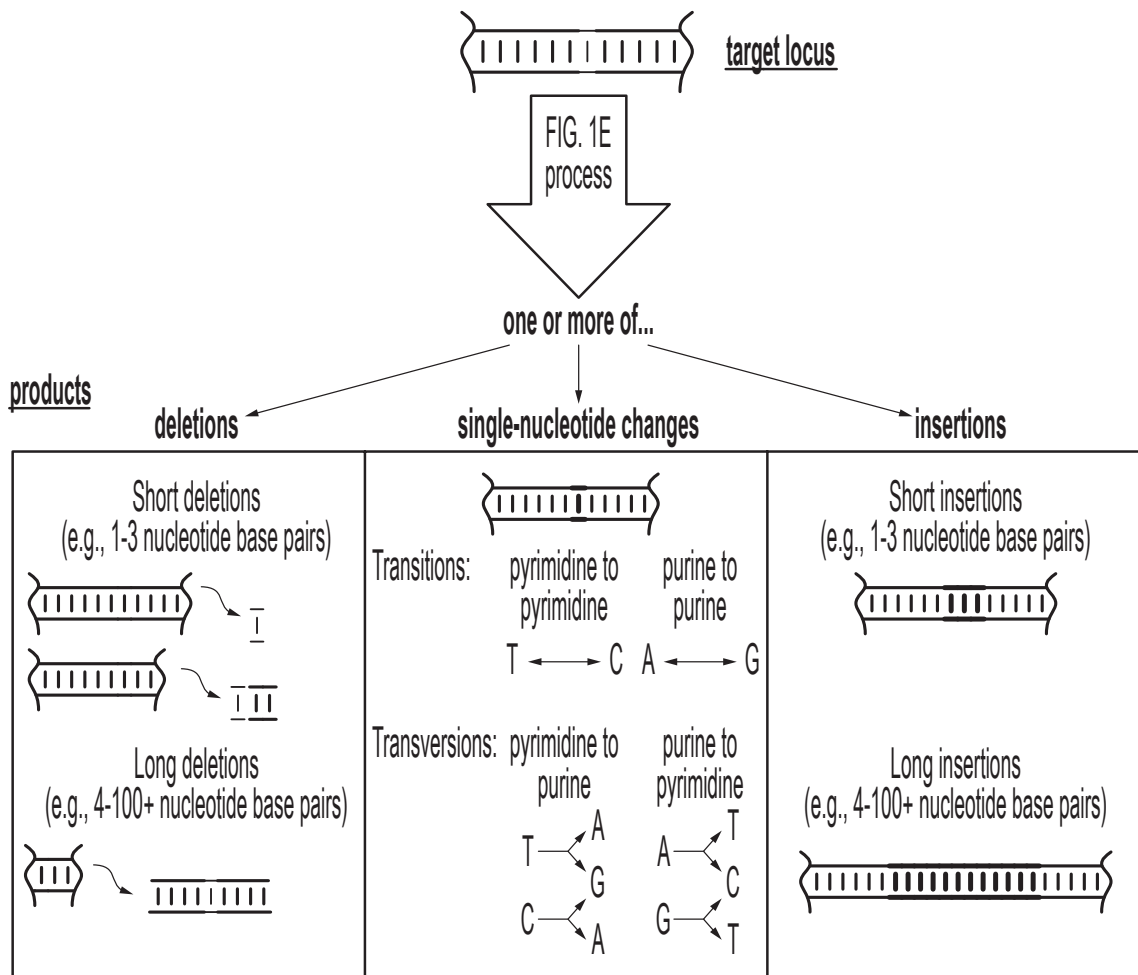


FIG. 1H

PE3b temporal nicking.

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

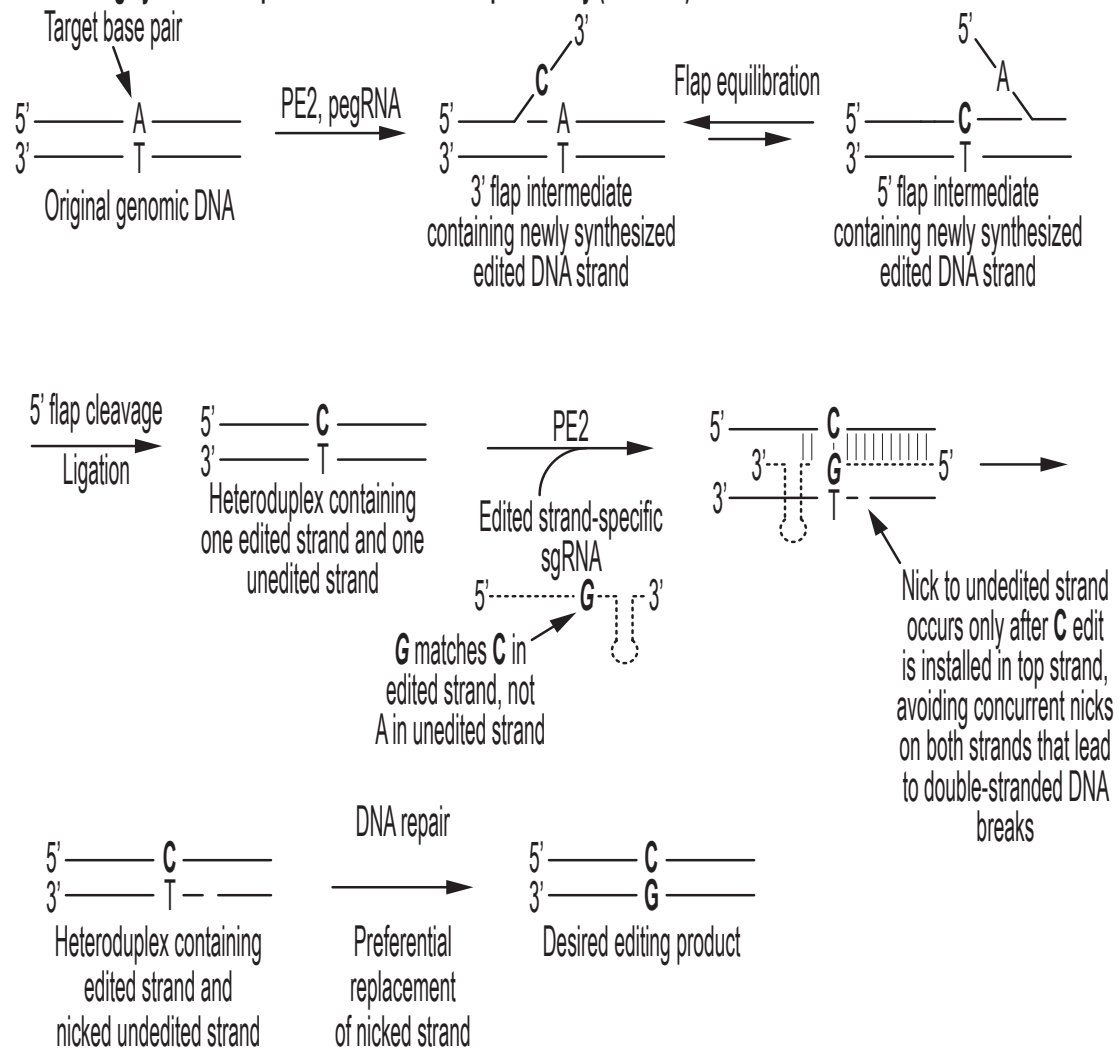


FIG. 11

Prime editing with any programmable nuclease

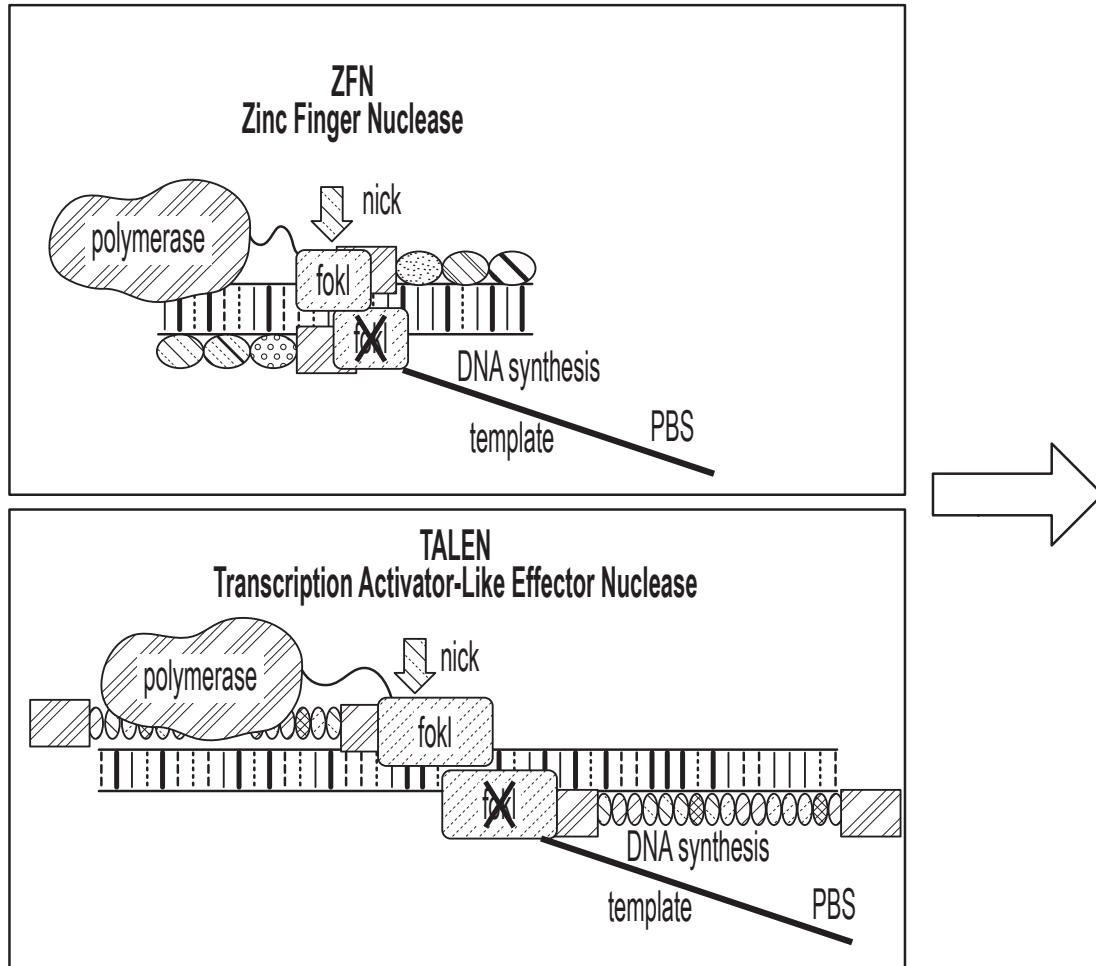
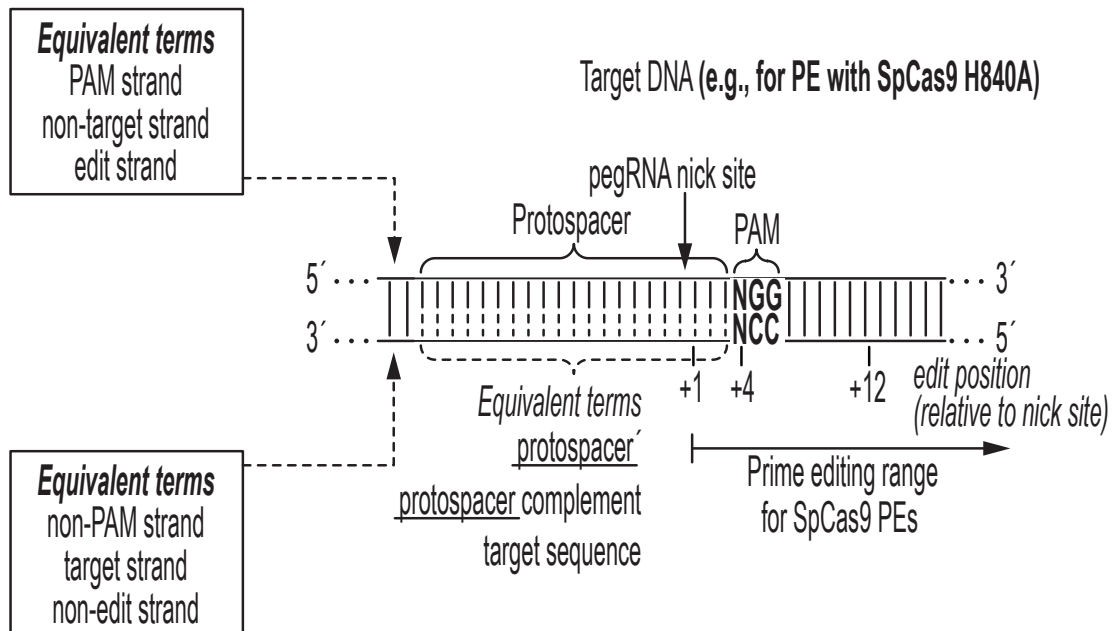


FIG. 1J

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



Prime editing scope:

- All 4 transition point mutations
- All 8 transversion point mutations
- Insertions (1 bp to ≥ 44 bp)
- Deletions (1 bp to ≥ 80 bp)
- Combinations of the above

FIG. 1L

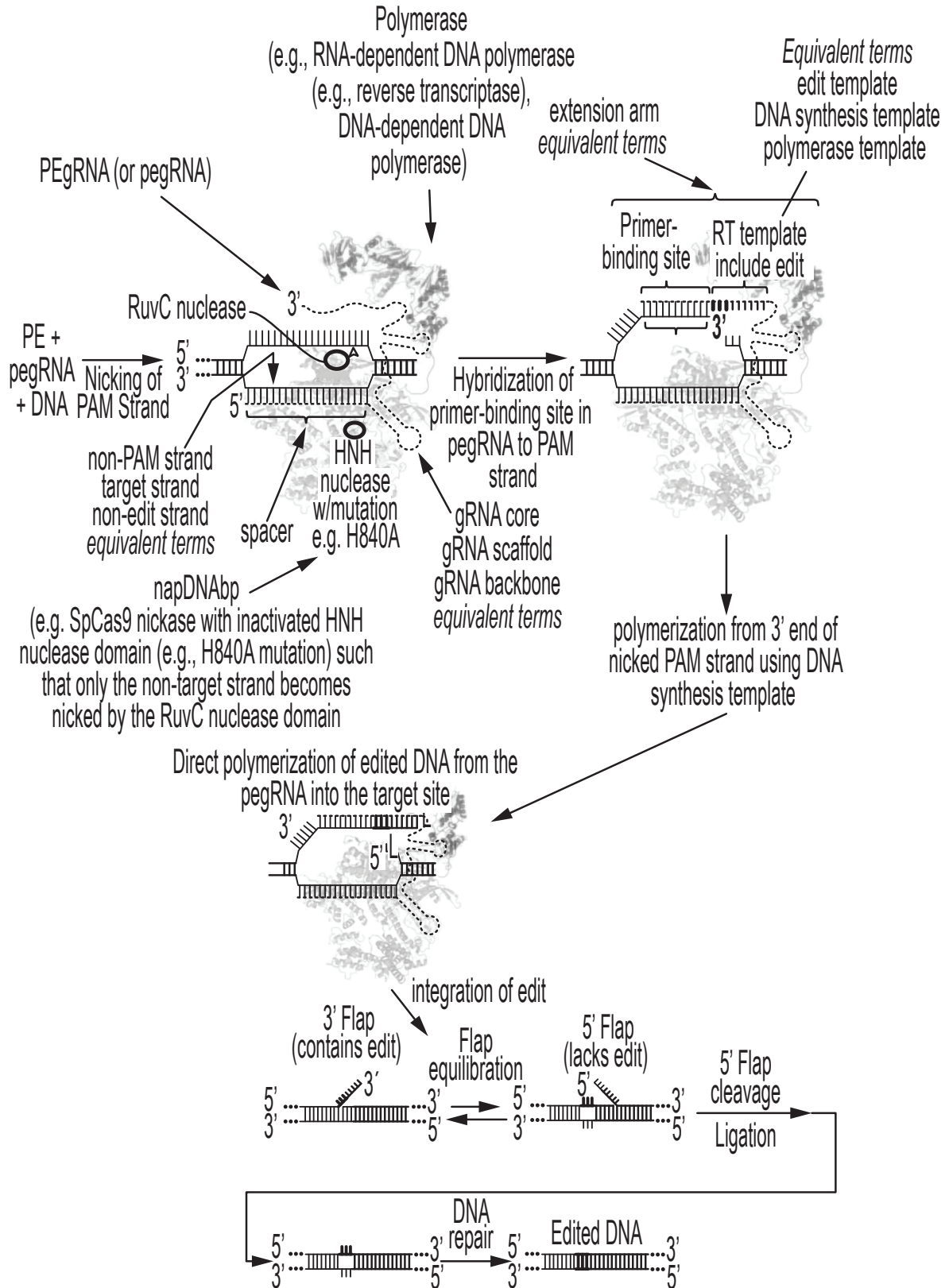


FIG. 1M

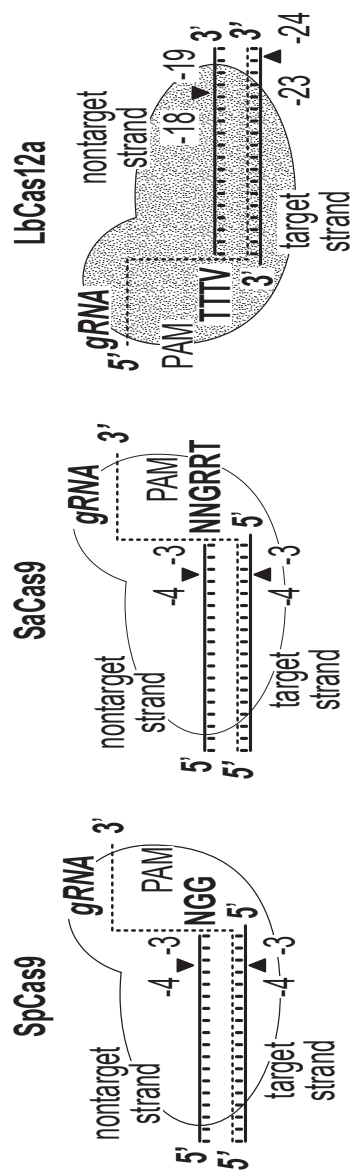


FIG. 2

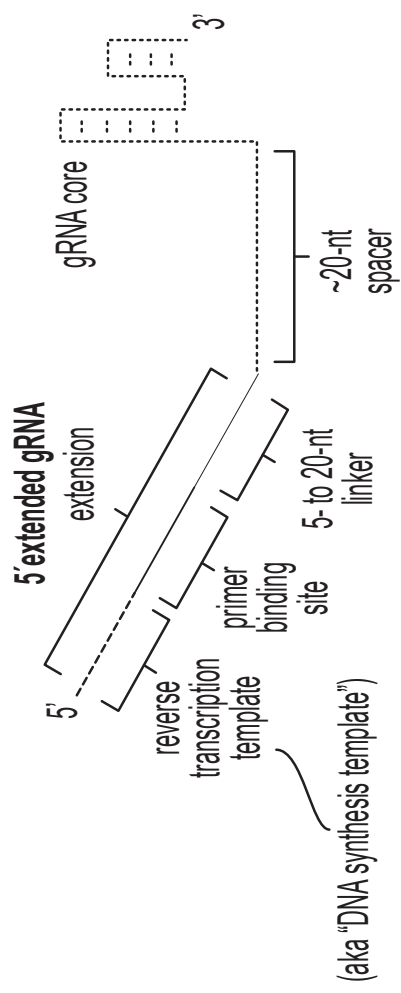


FIG. 3A

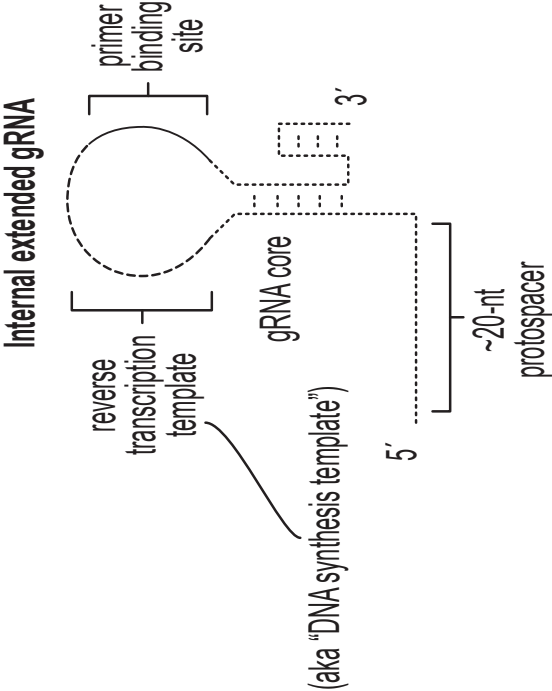


FIG. 3C

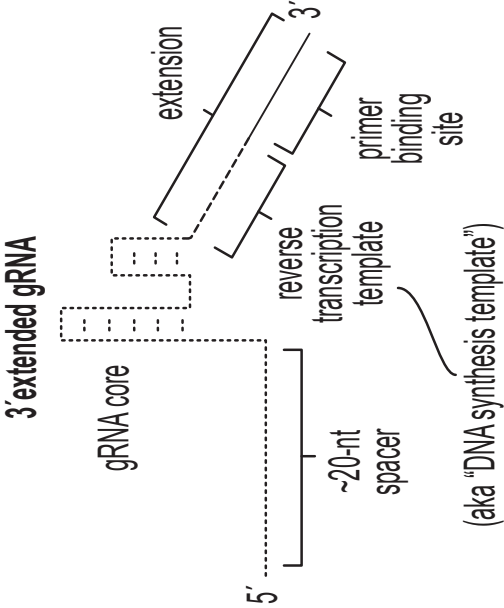


FIG. 3B

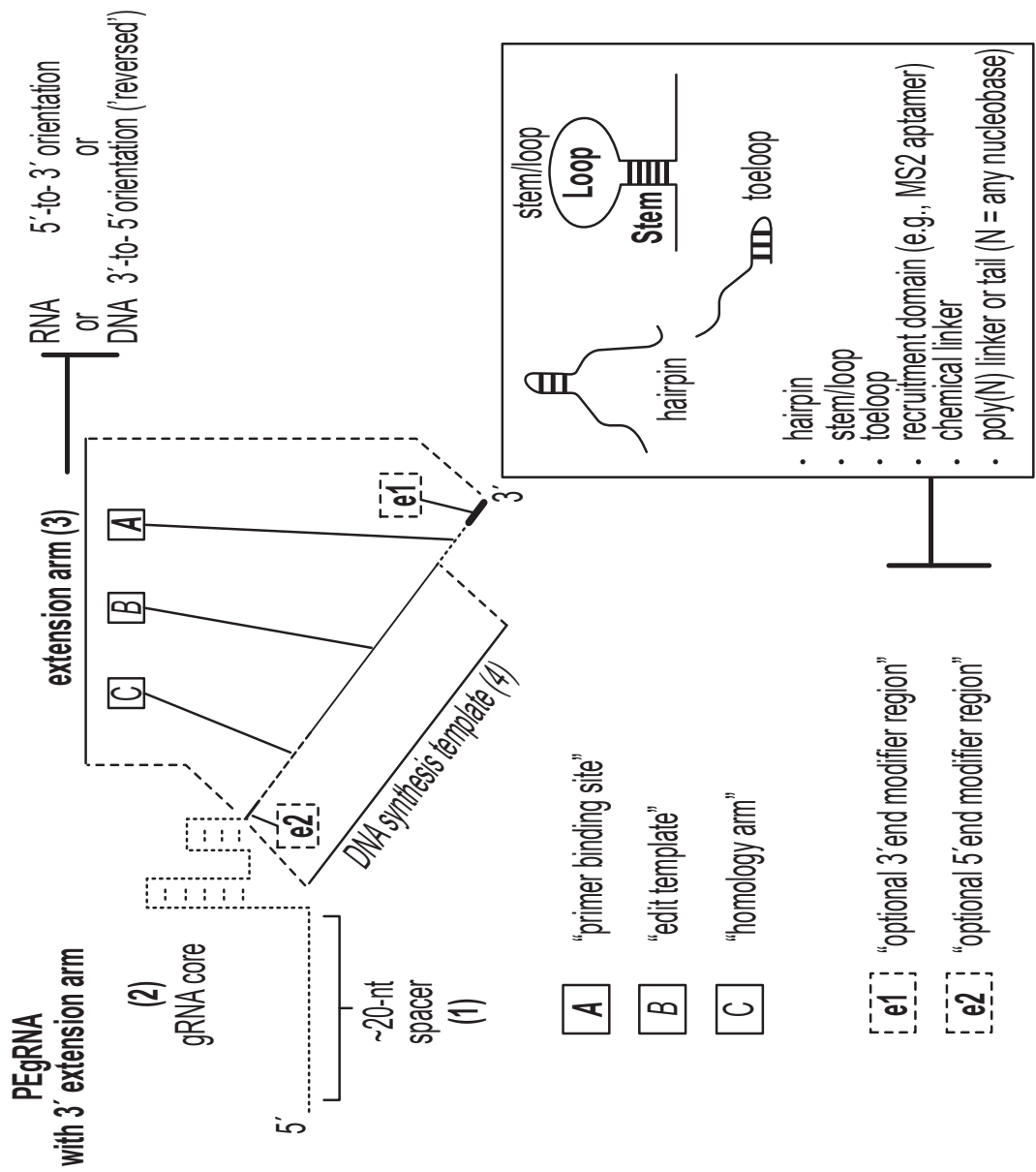


FIG. 3D

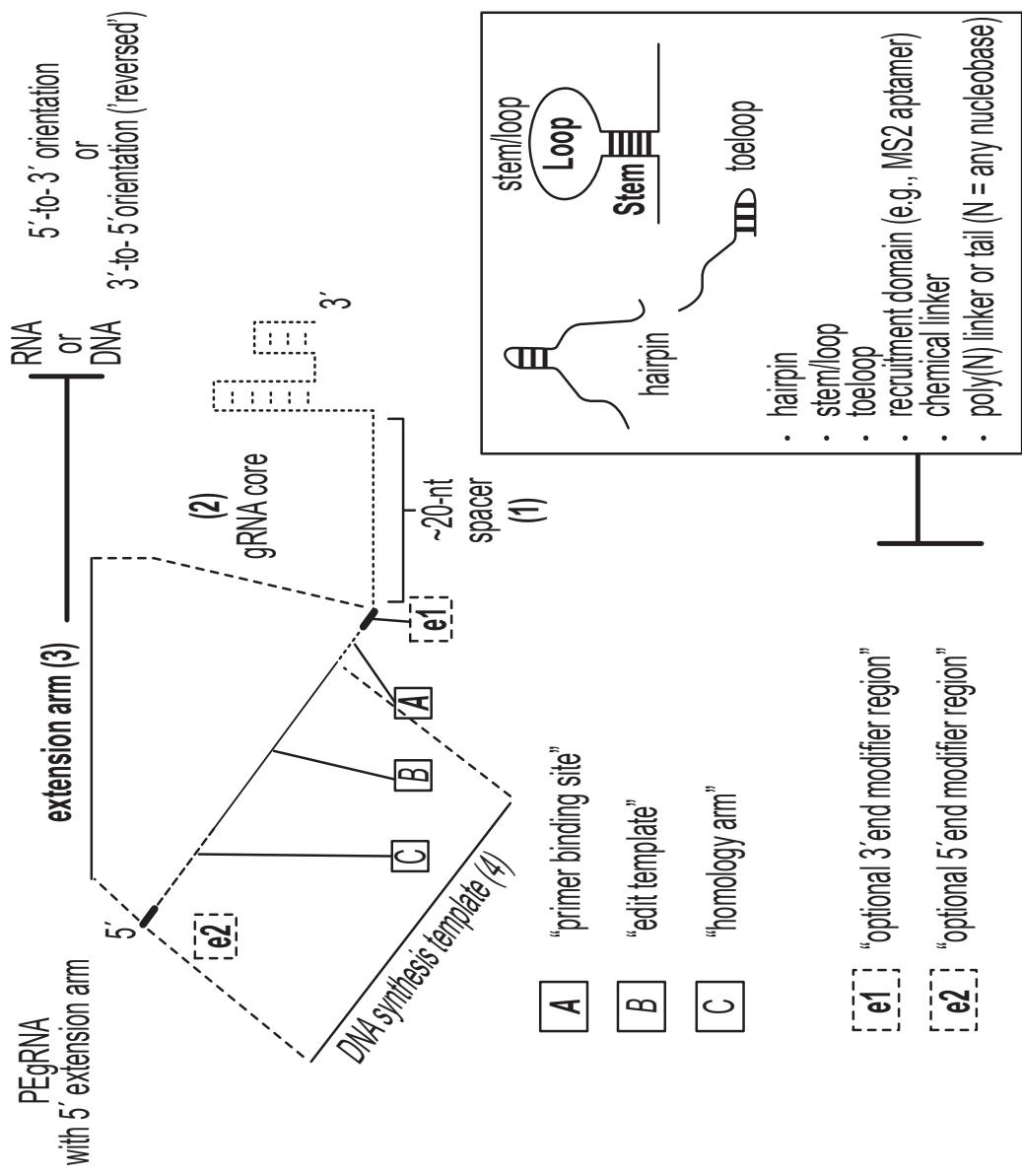


FIG. 3E



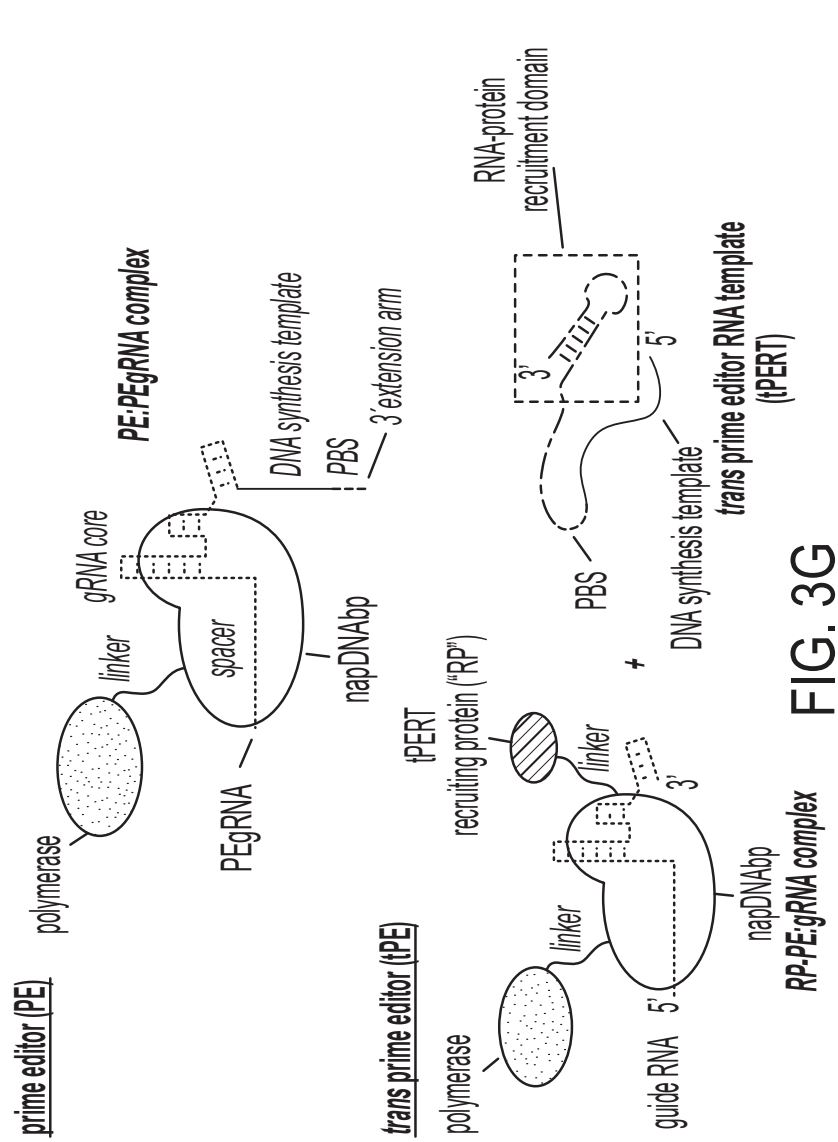


FIG. 3G

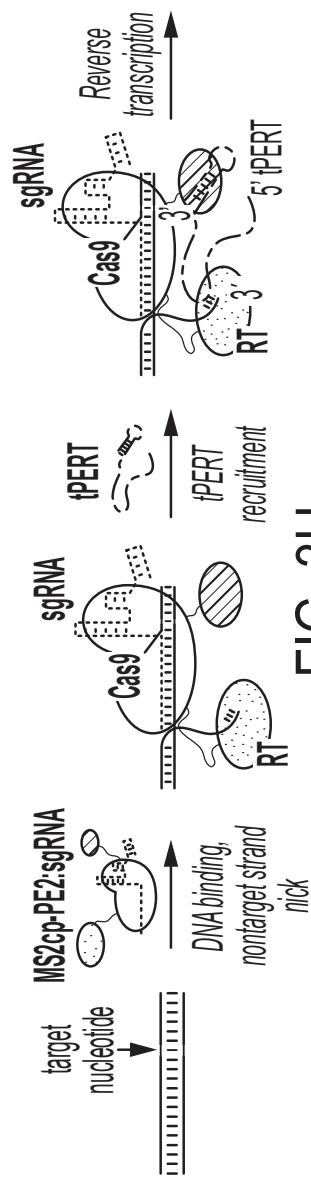


FIG. 3H

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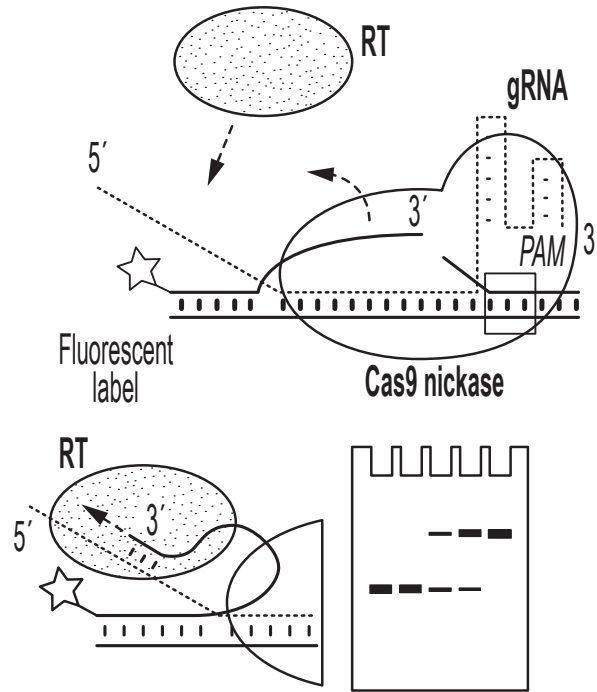


FIG. 4A

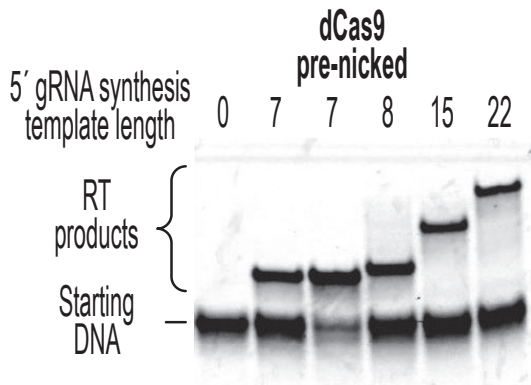


FIG. 4B

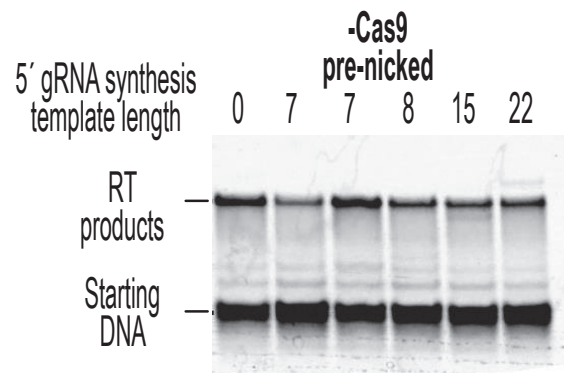


FIG. 4C

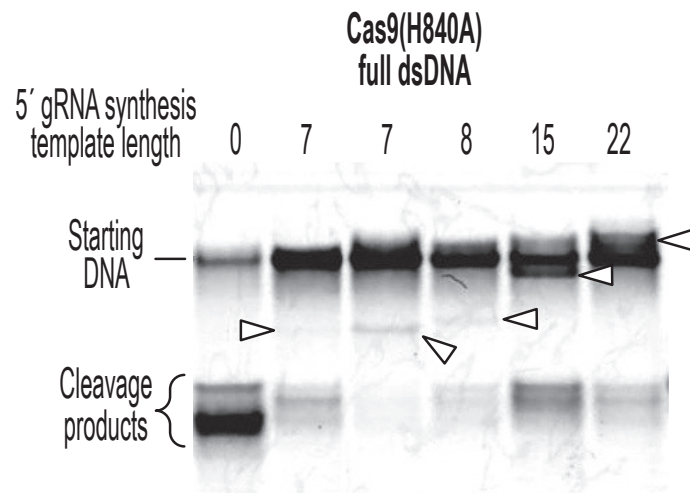


FIG. 4D

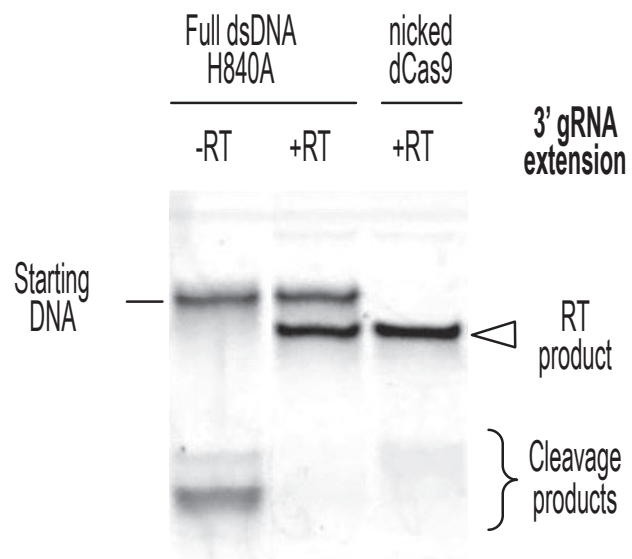


FIG. 4E

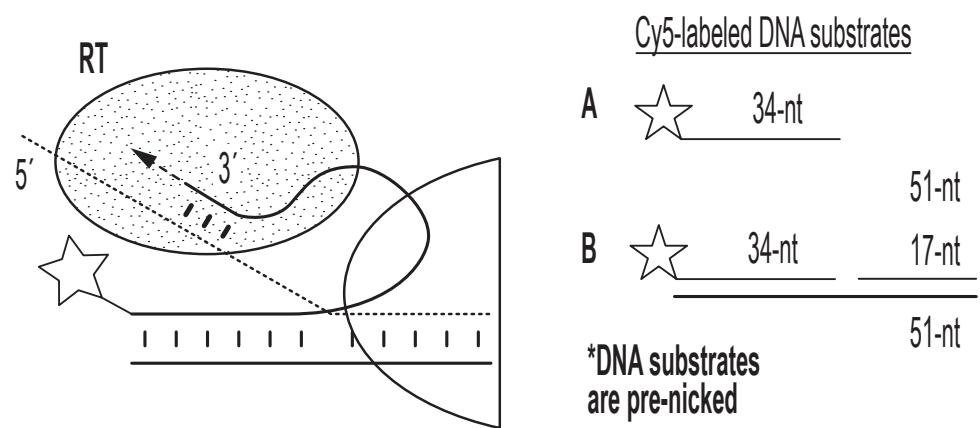
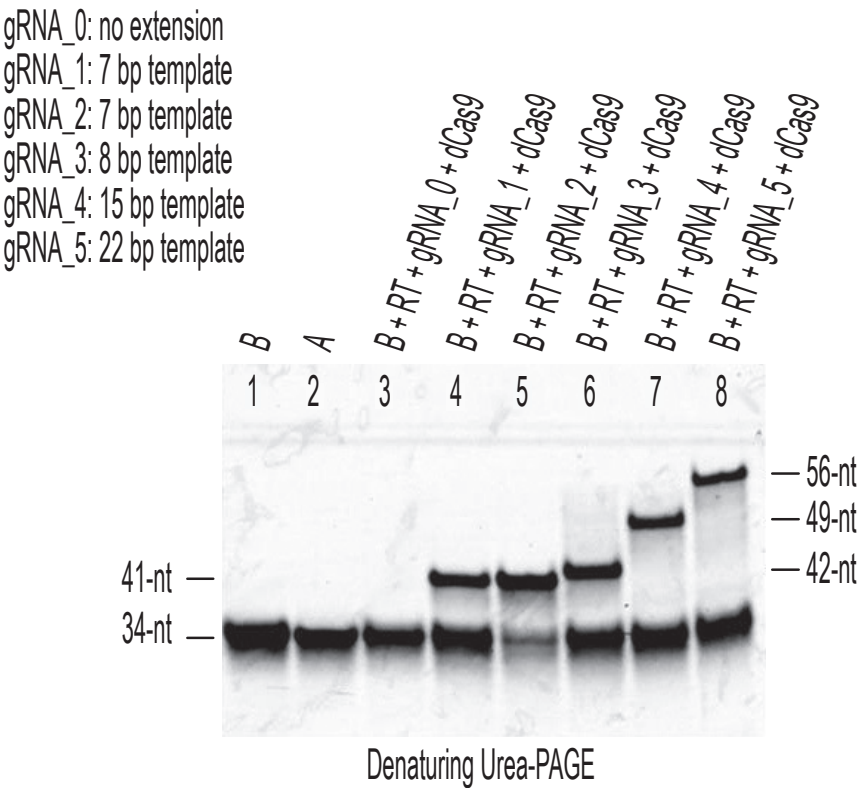
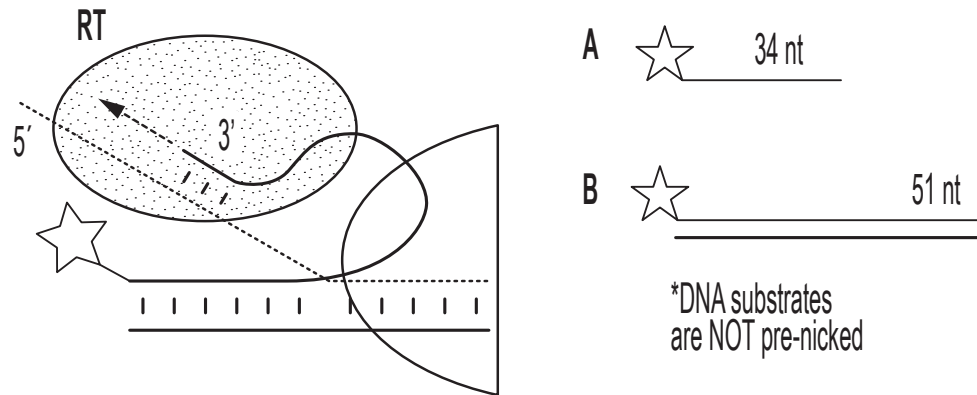


FIG. 5



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

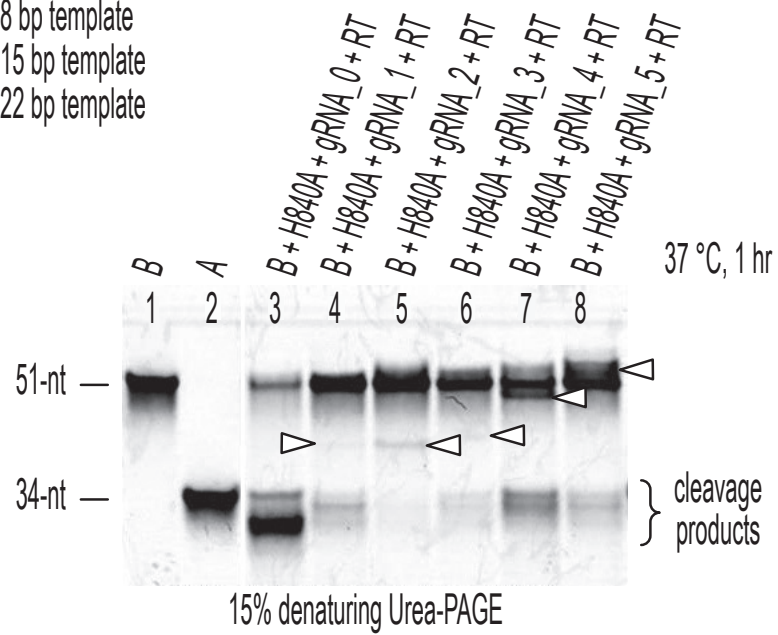
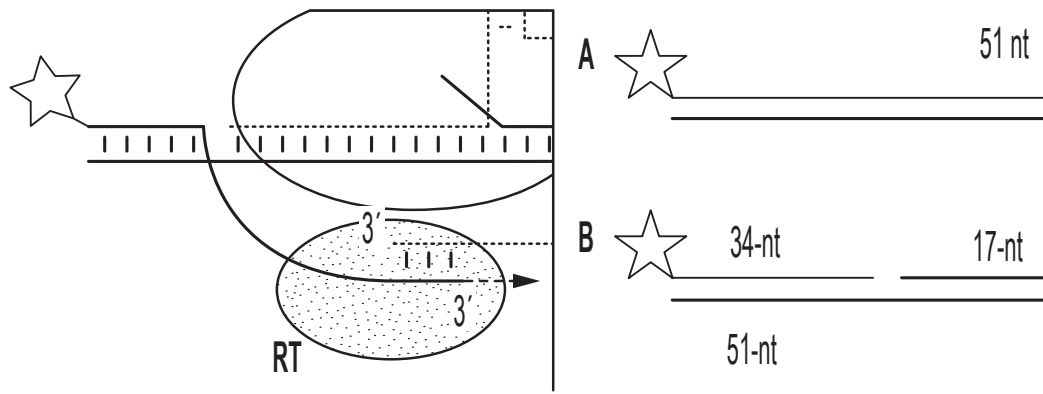


FIG. 6



gRNA_0: no extension
gRNA_1: 15 bp template

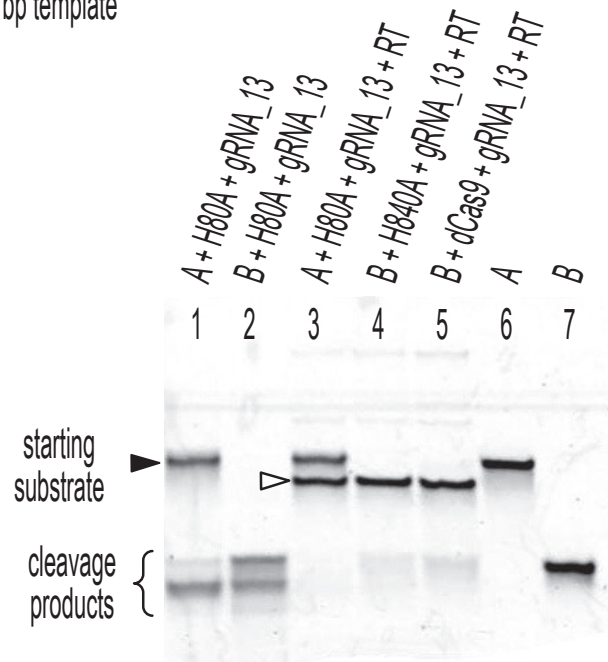


FIG. 7

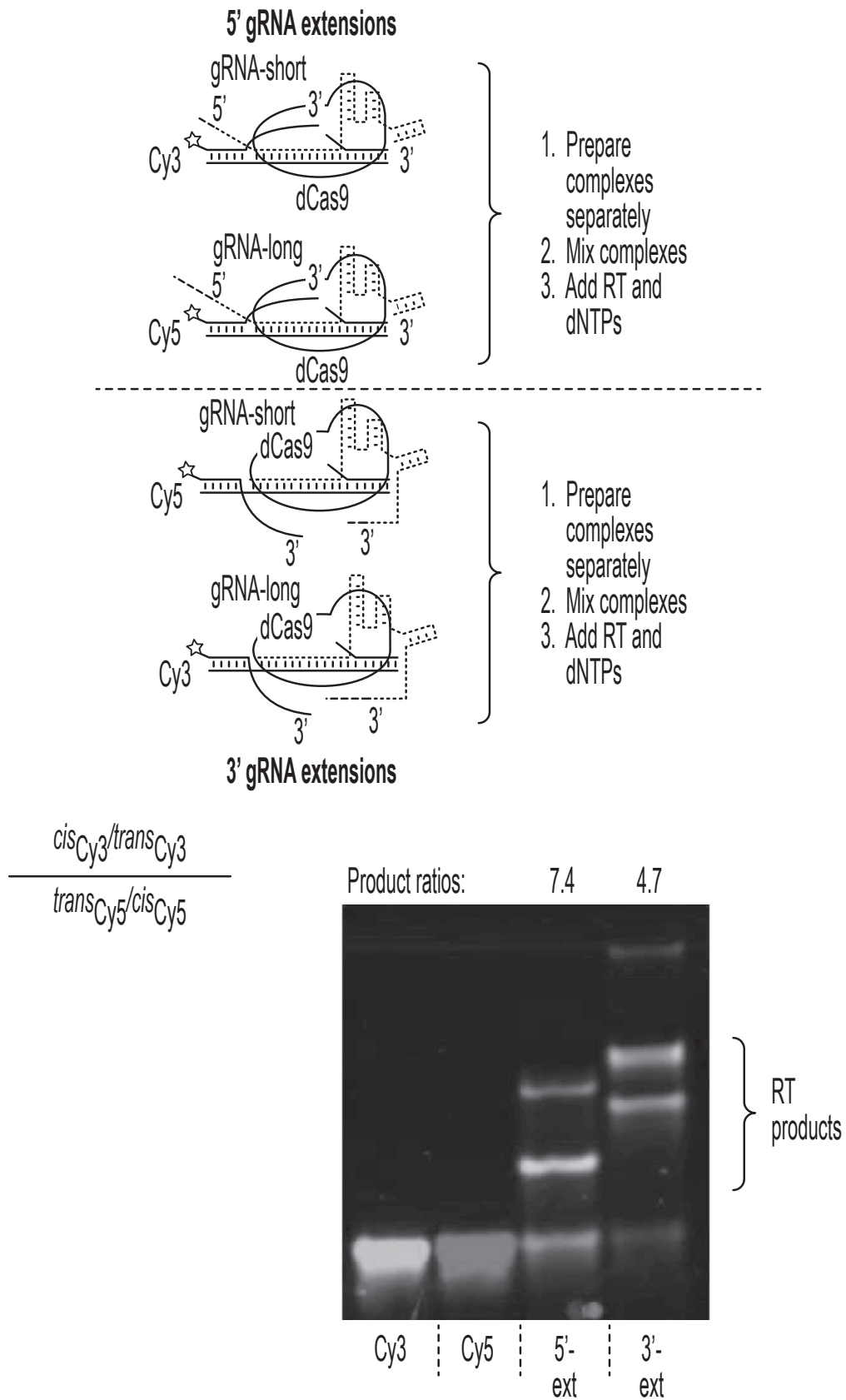


FIG. 8

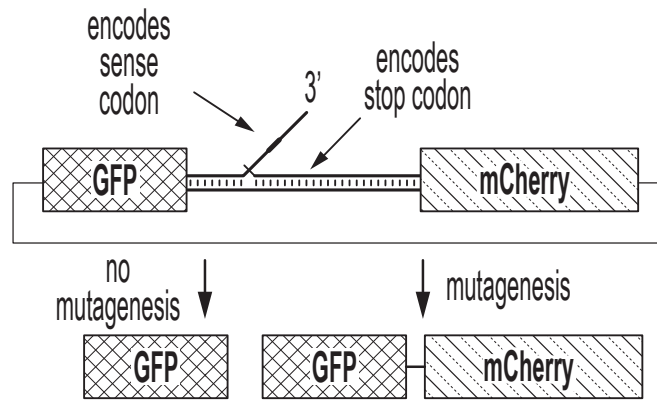


FIG. 9A

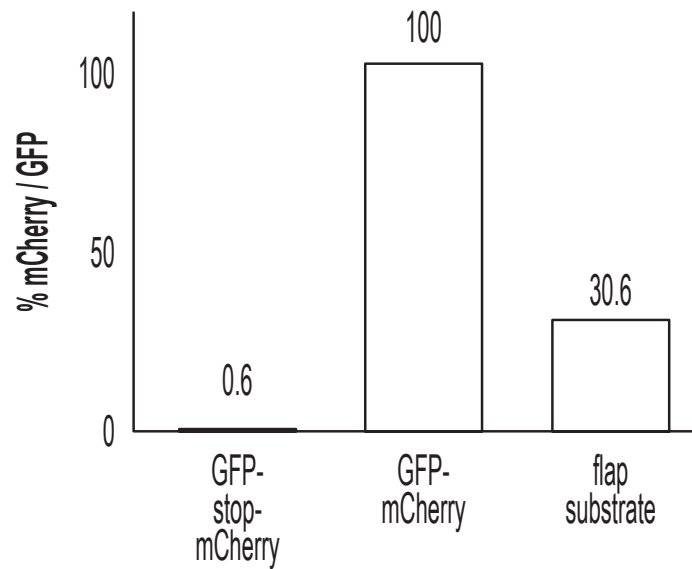


FIG. 9B

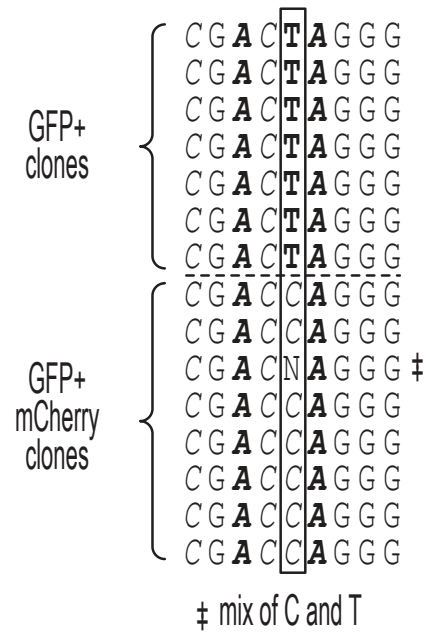


FIG. 9C

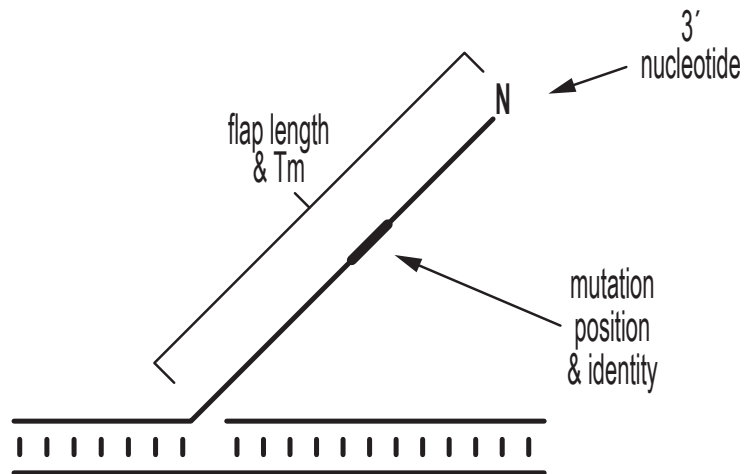


FIG. 9D

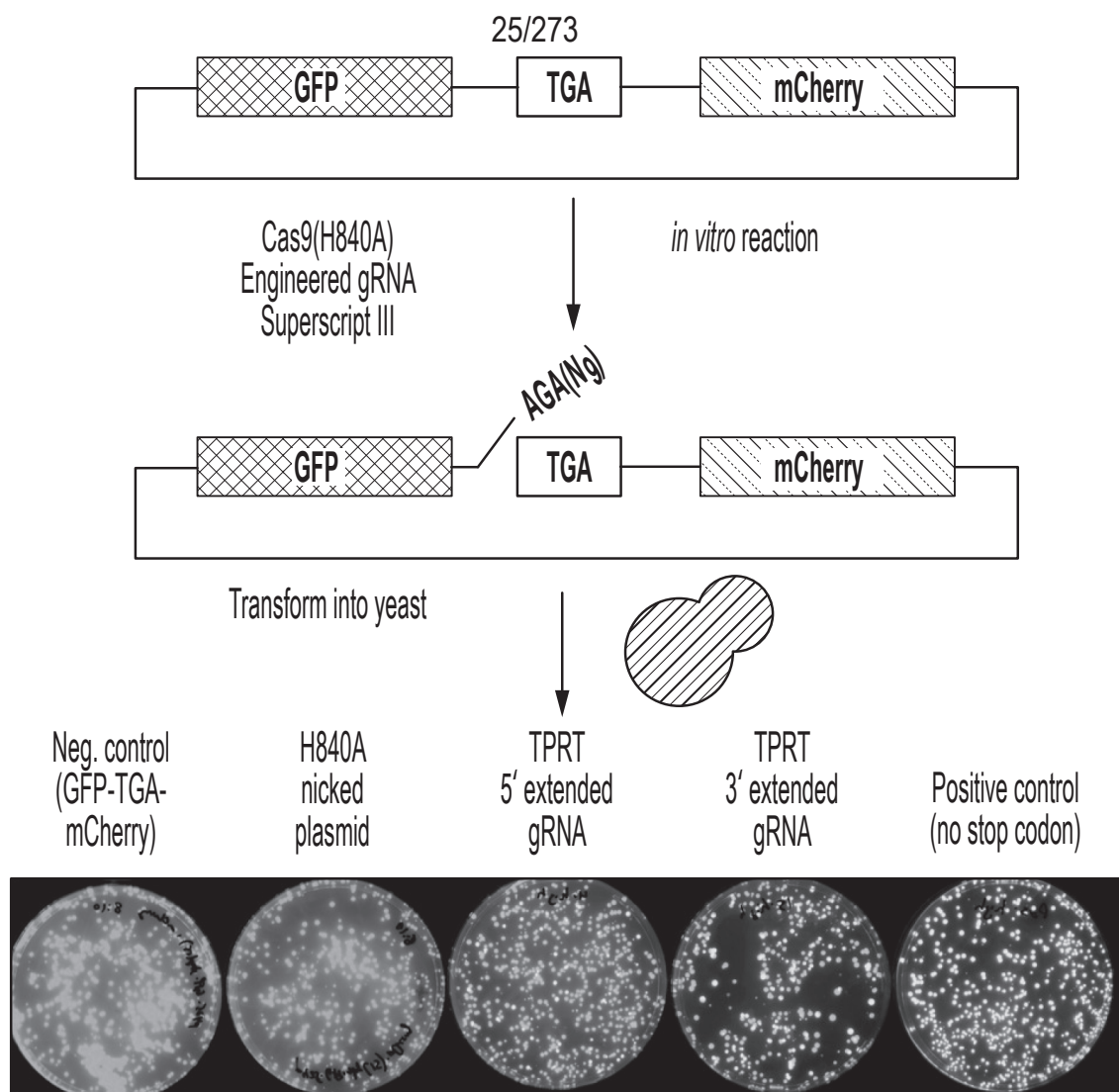


FIG. 10

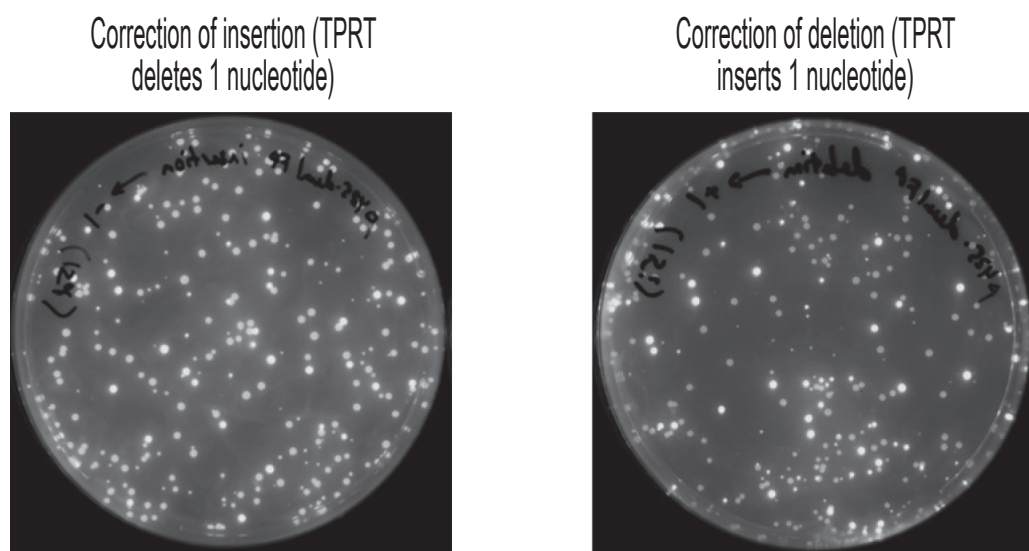


FIG. 11



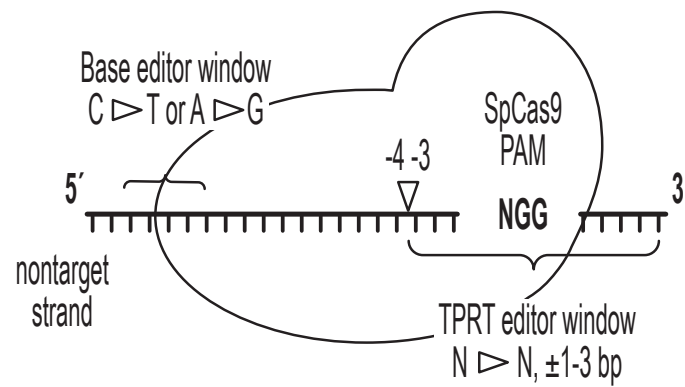


FIG. 13

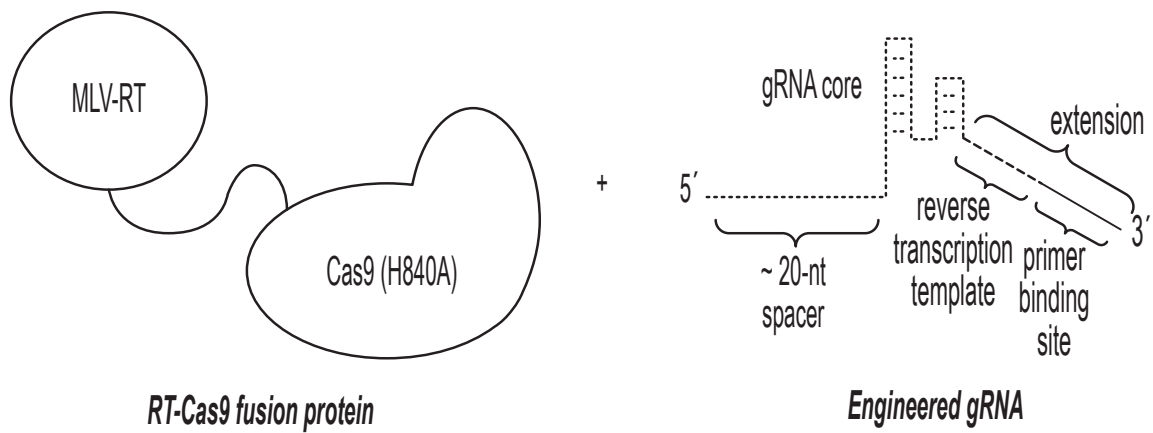


FIG. 14

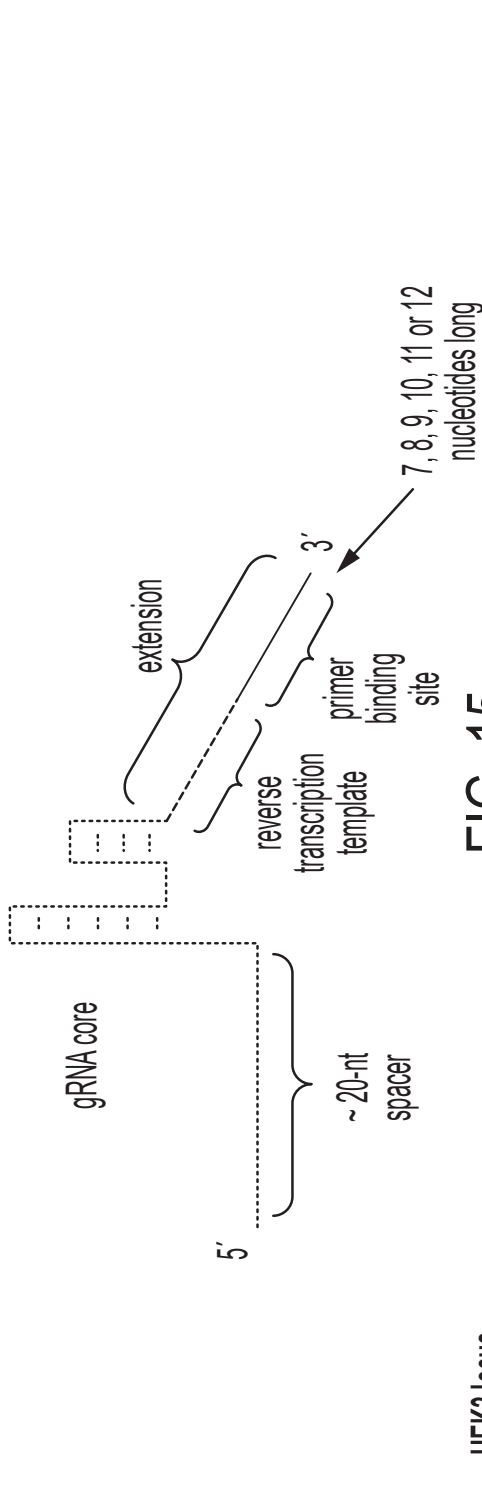


FIG. 15

HEK3 locus
SEQ ID NO: 789
SEQ ID NO: 790

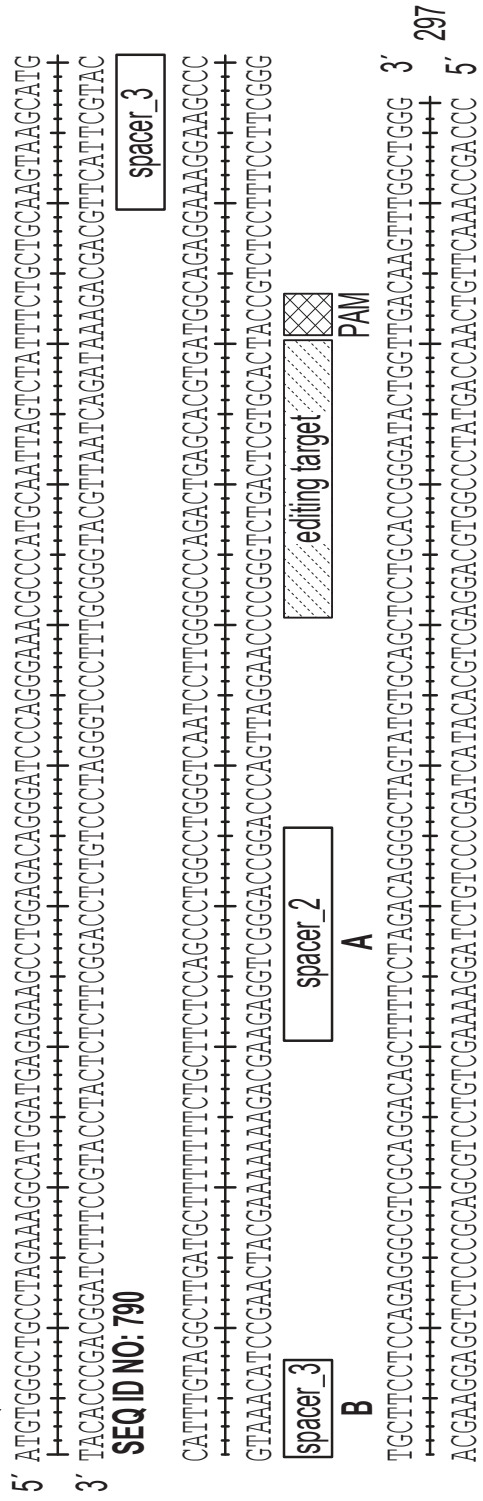


FIG. 16

A
B
C
D
E

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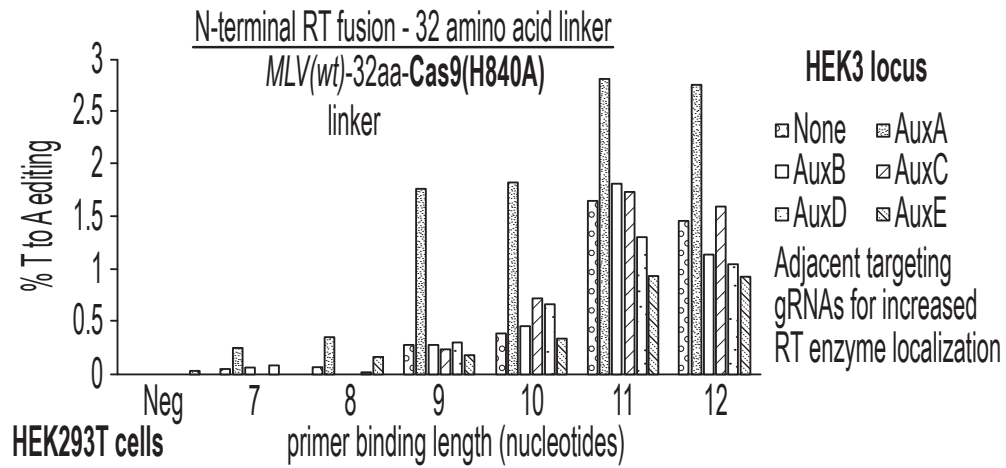


FIG. 17A

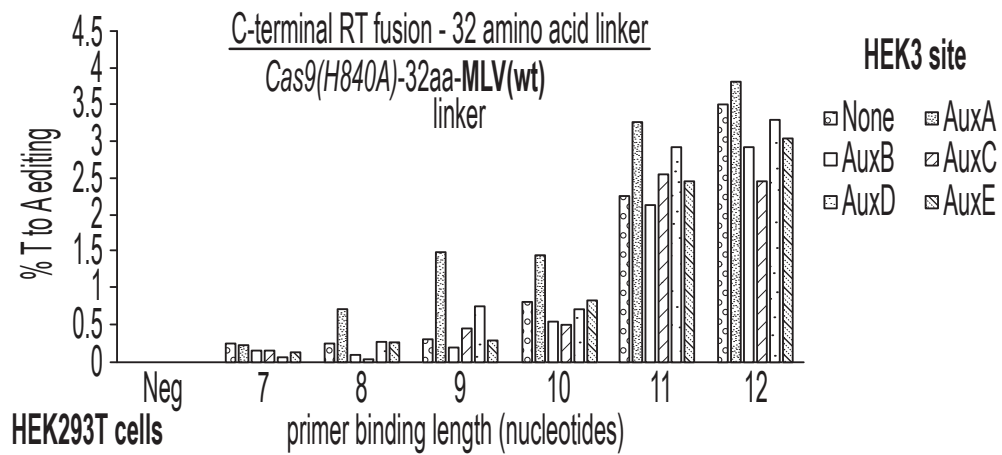


FIG. 17B

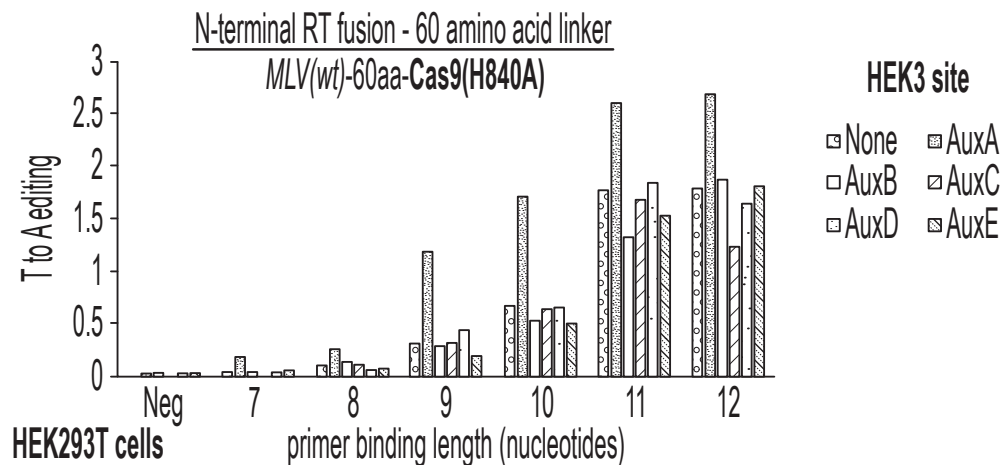


FIG. 17C

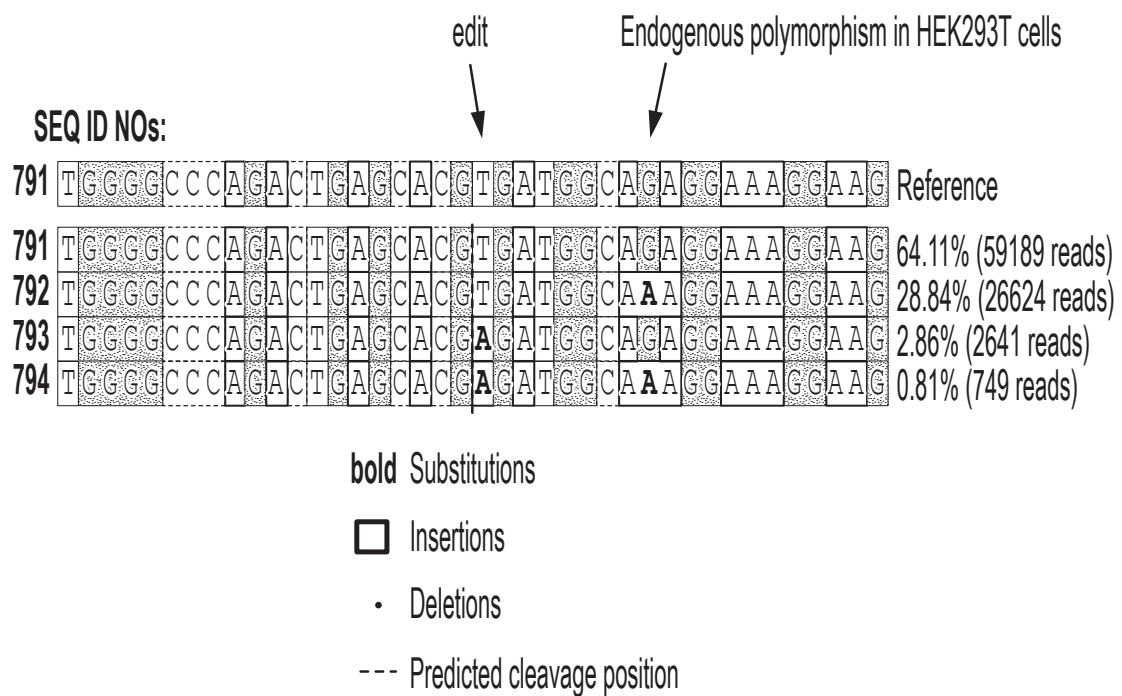


FIG. 18

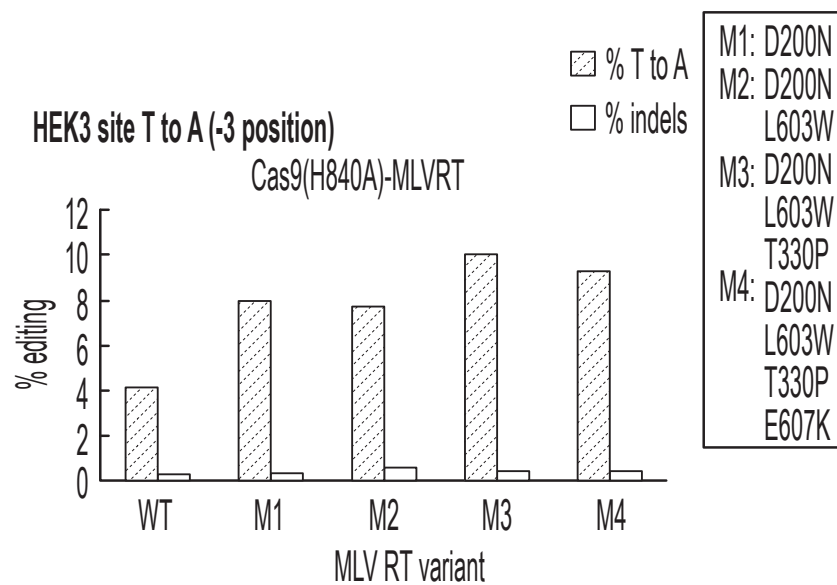


FIG. 19

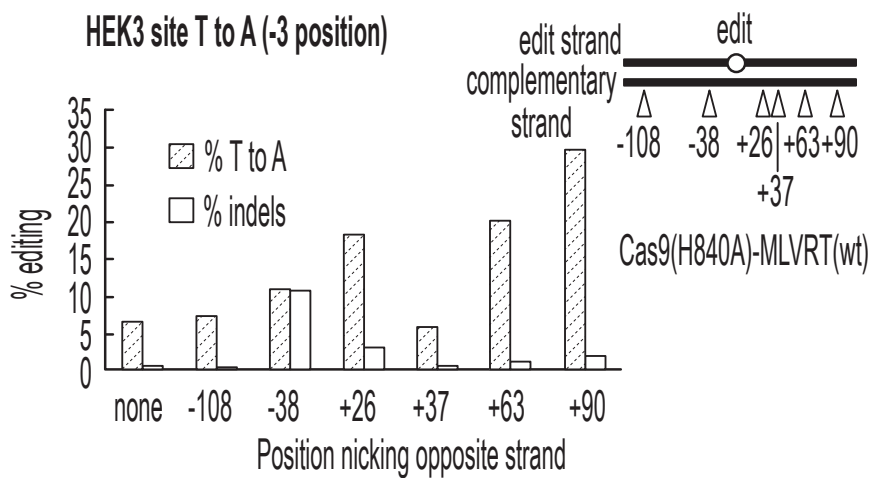


FIG. 20

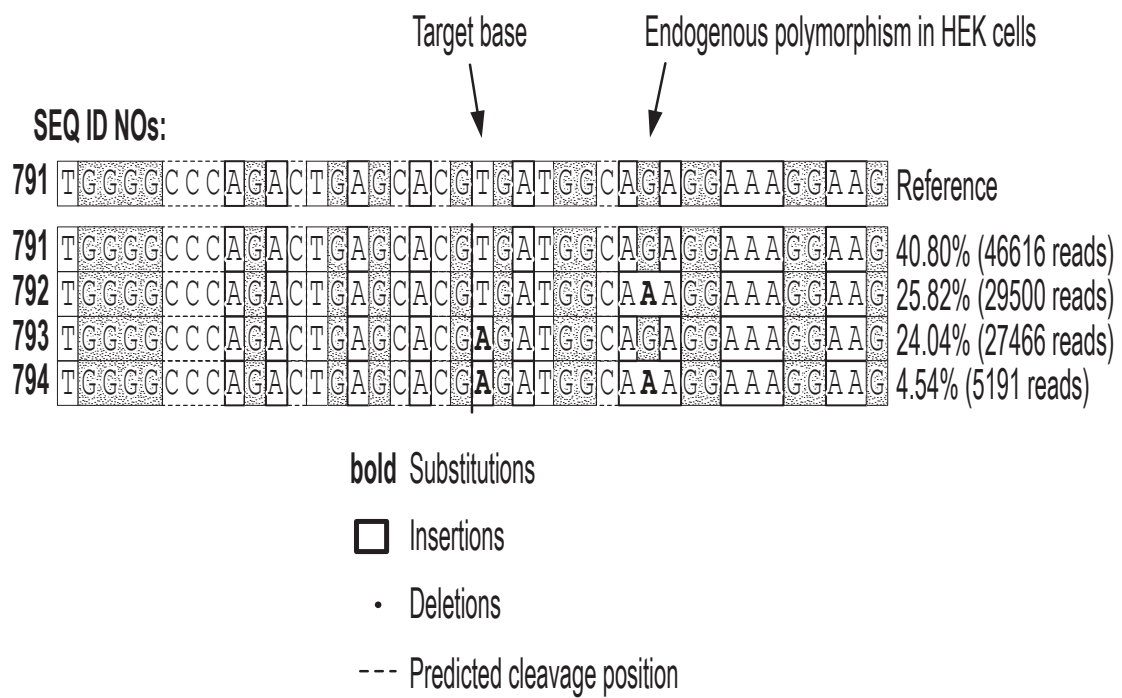


FIG. 21

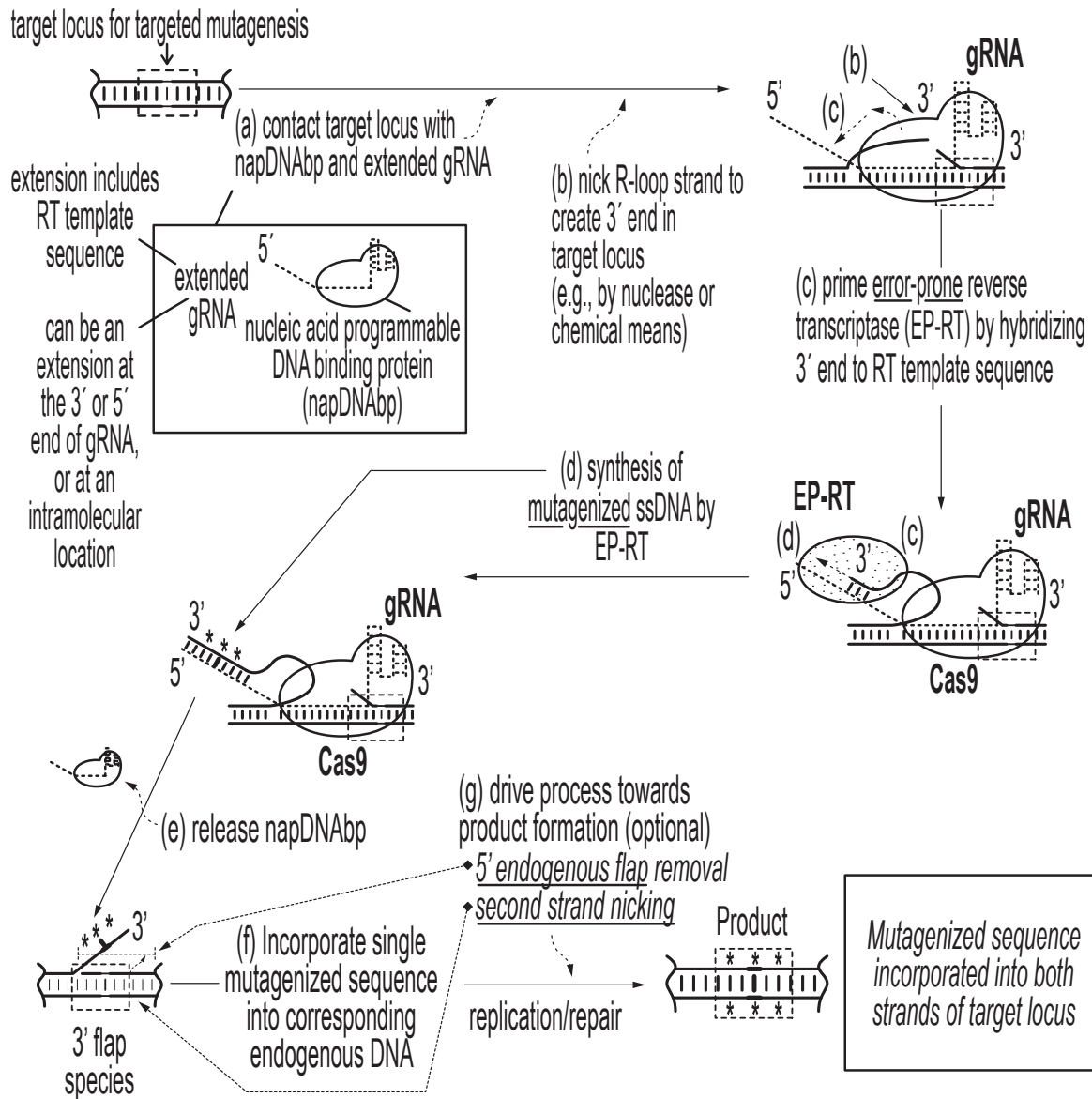
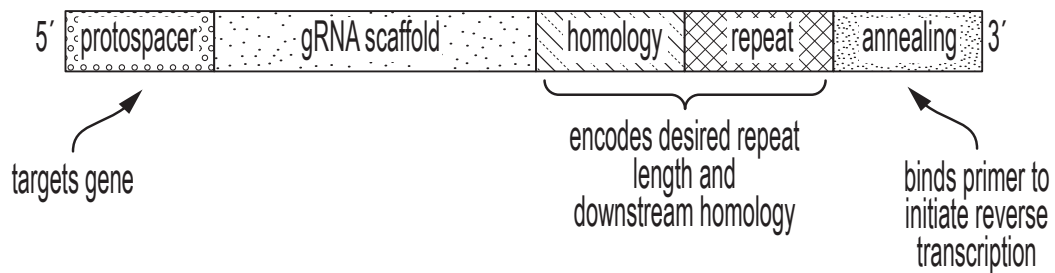


FIG. 22

gRNA design for contracting trinucleotide repeat sequences



Trinucleotide repeat contraction with TPRT genome editing

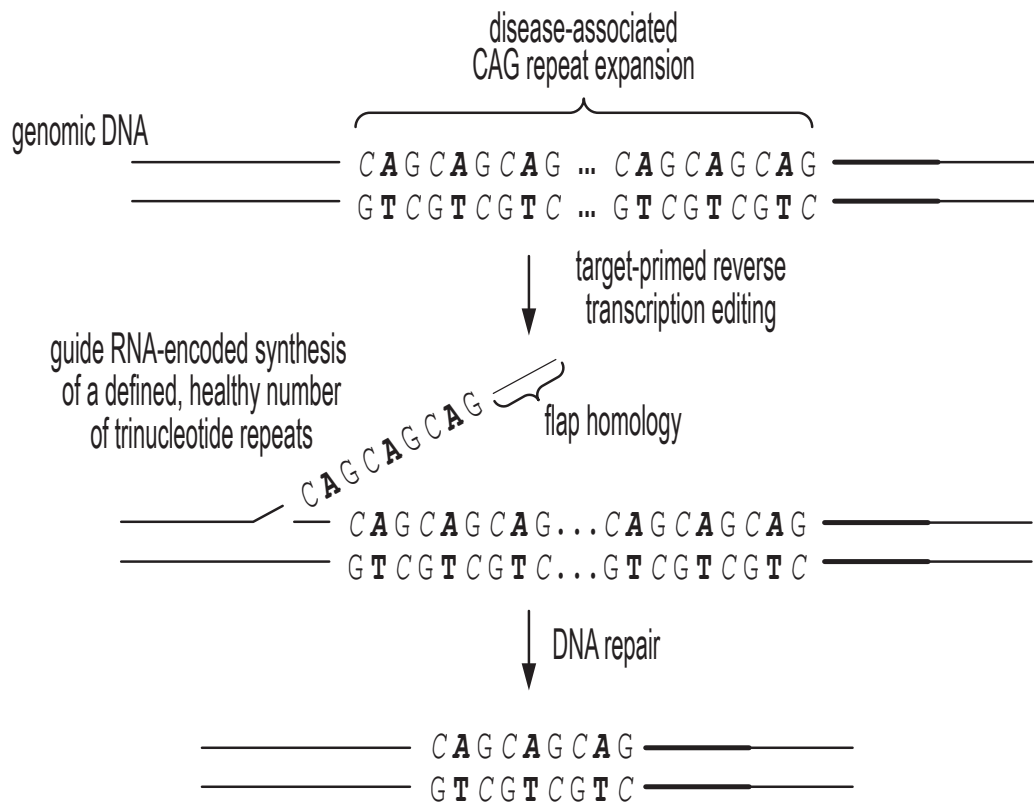


FIG. 23

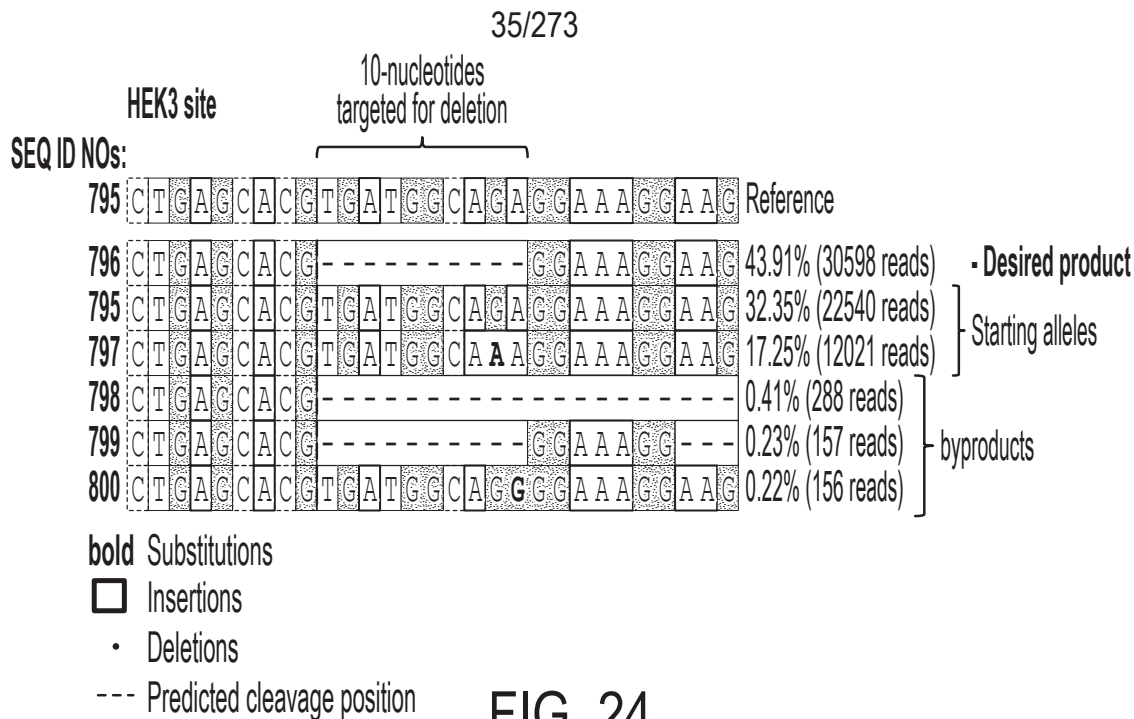


FIG. 24

gRNA design for peptide tagging genes at endogenous genomic loci

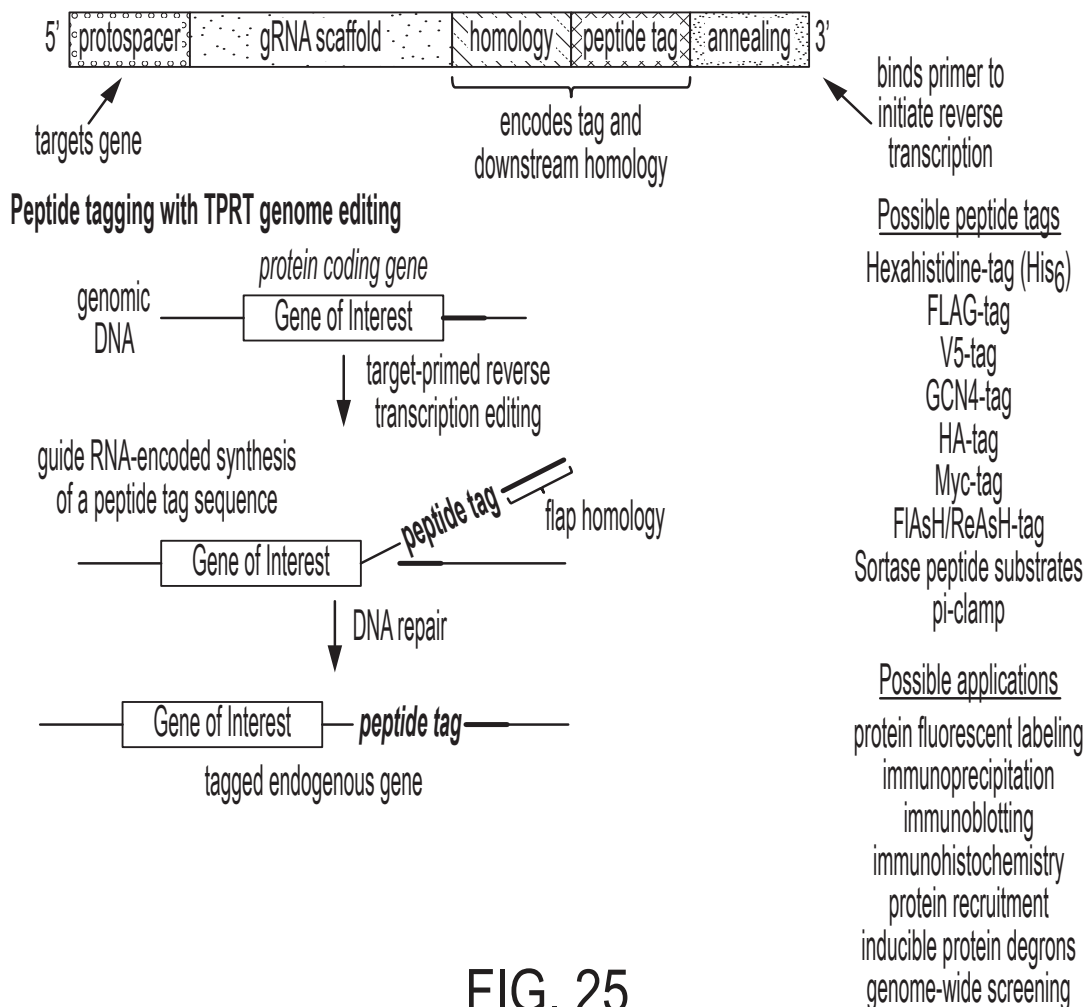


FIG. 25

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HEK3 site - His-tag insertion	
SEQ ID	
801	G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G Reference
801	G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G 46.04% (34433 reads)
802	G A C T G A G C A C G C A C C A T C A T C A C C A T C A T T G 25.56% (19113 reads) - Desired product
803	G A C T G A G C A C G T G A T G G C A A A G G A A A G G A A G 25.40% (18997 reads)
802	G A C T G A G C A C G C A C C A T C A T C A C C A T C A T T G 0.23% (171 reads)

bold Substitutions
□ Insertions
 • Deletions
 --- Predicted cleavage position

HEK3 site - FLAG-tag insertion

801	G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G Reference
801	G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G 61.56% (38678 reads)
803	G A C T G A G C A C G T G A T G G C A A A G G A A A G G A A G 31.83% (19998 reads)
804	G A C T G A G C A C G G A T T A C A A G G A T G A C G A C G A 4.65% (2921 reads) - Desired product

bold Substitutions
□ Insertions
 • Deletions
 --- Predicted cleavage position

(note, full length
 24-nt insertion out
 of viewing frame)

FIG. 26A

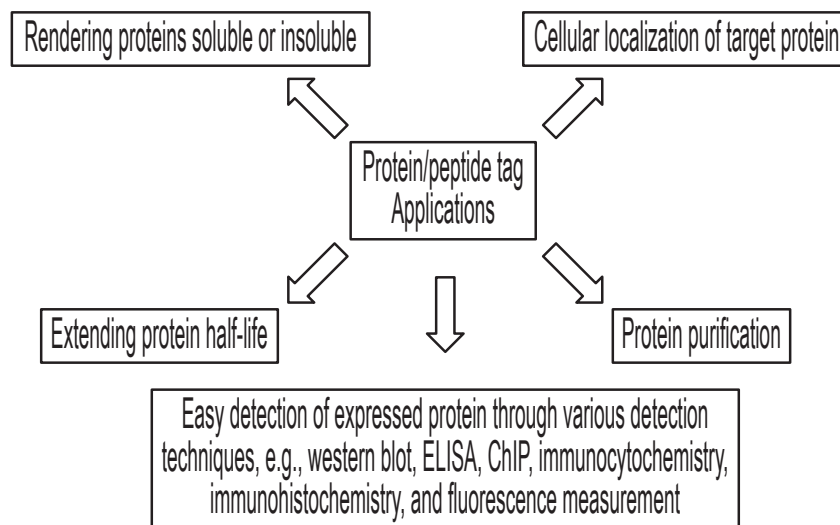


FIG. 26B

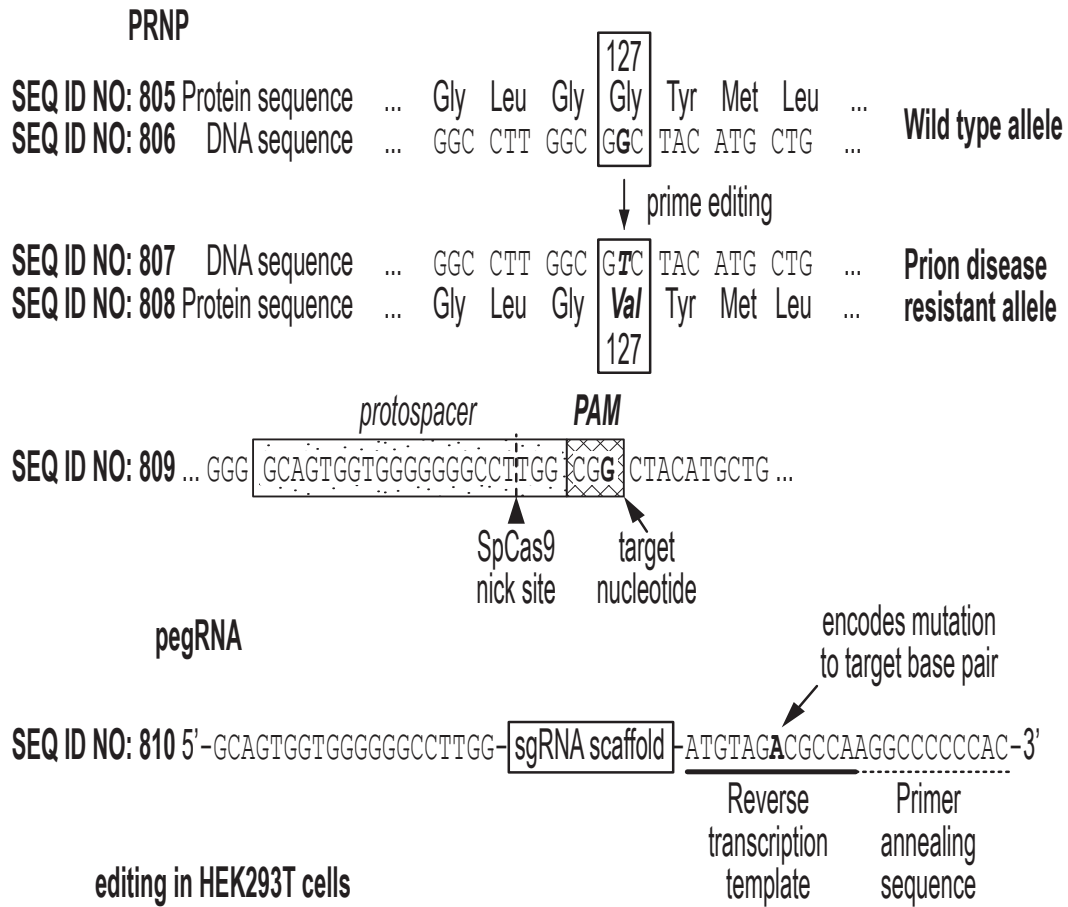
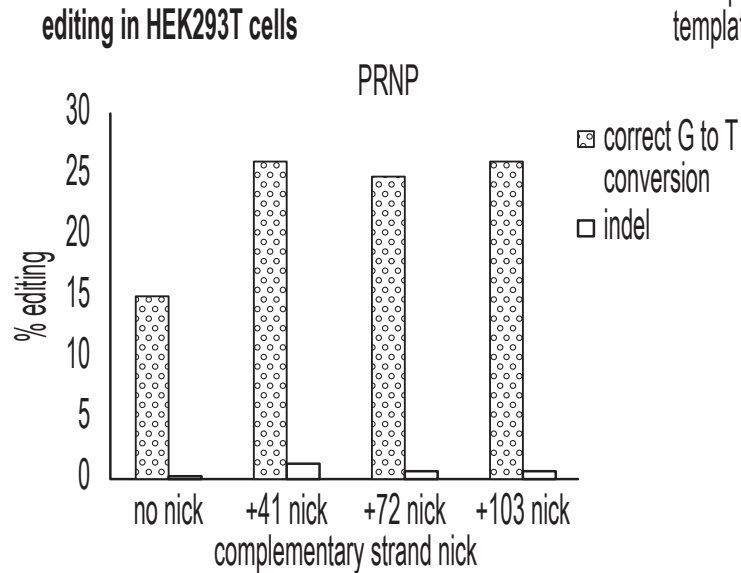


FIG. 27

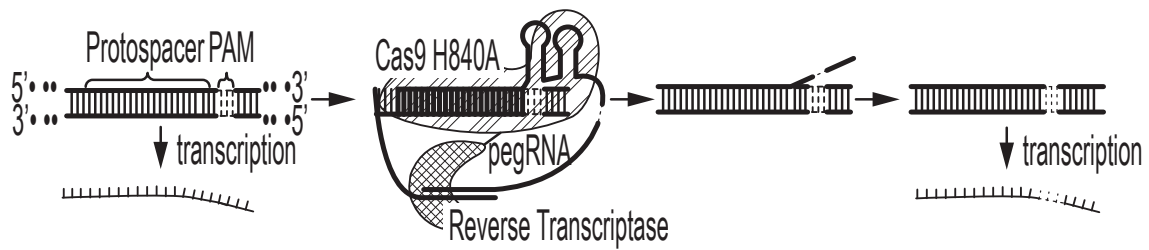


FIG. 28A

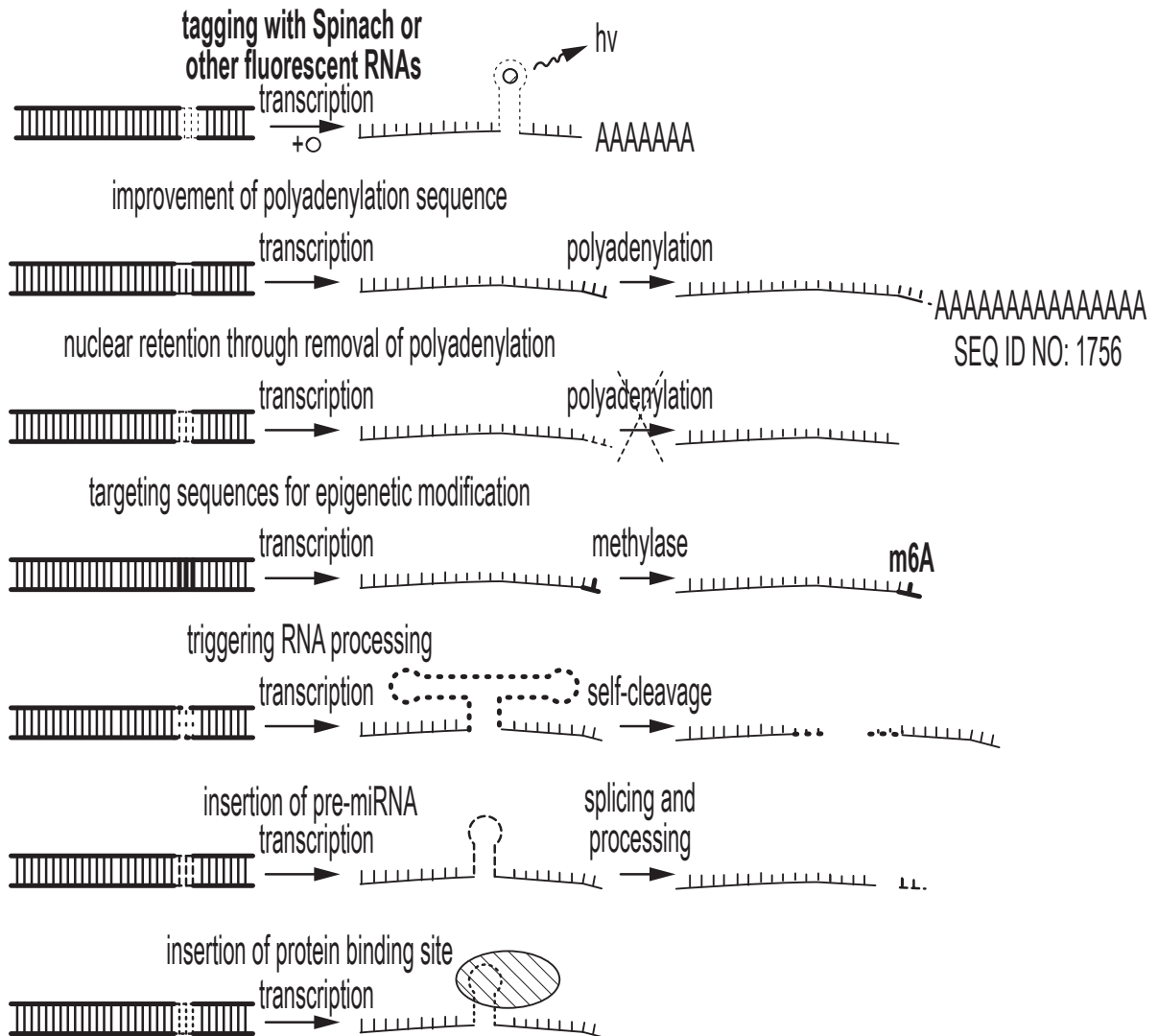


FIG. 28B

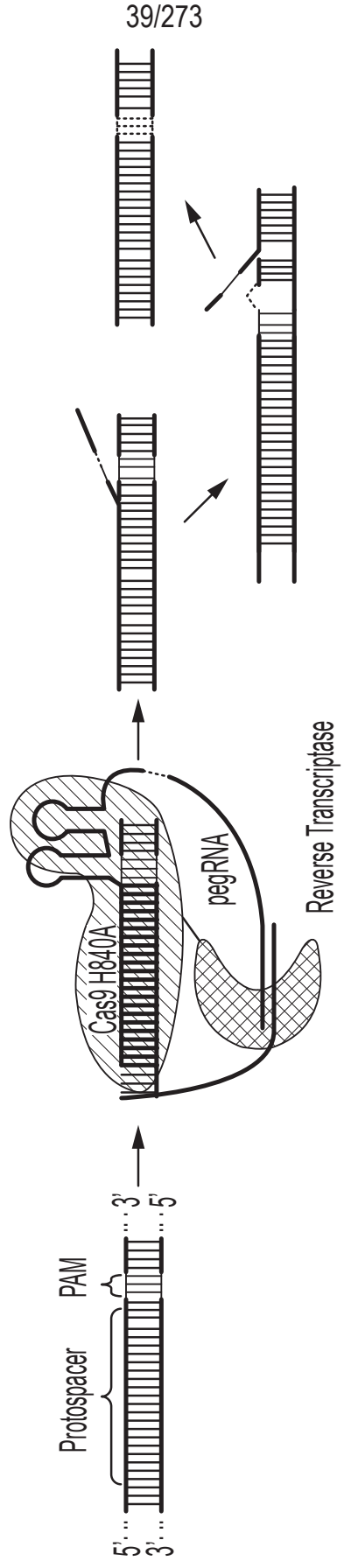


FIG. 29A

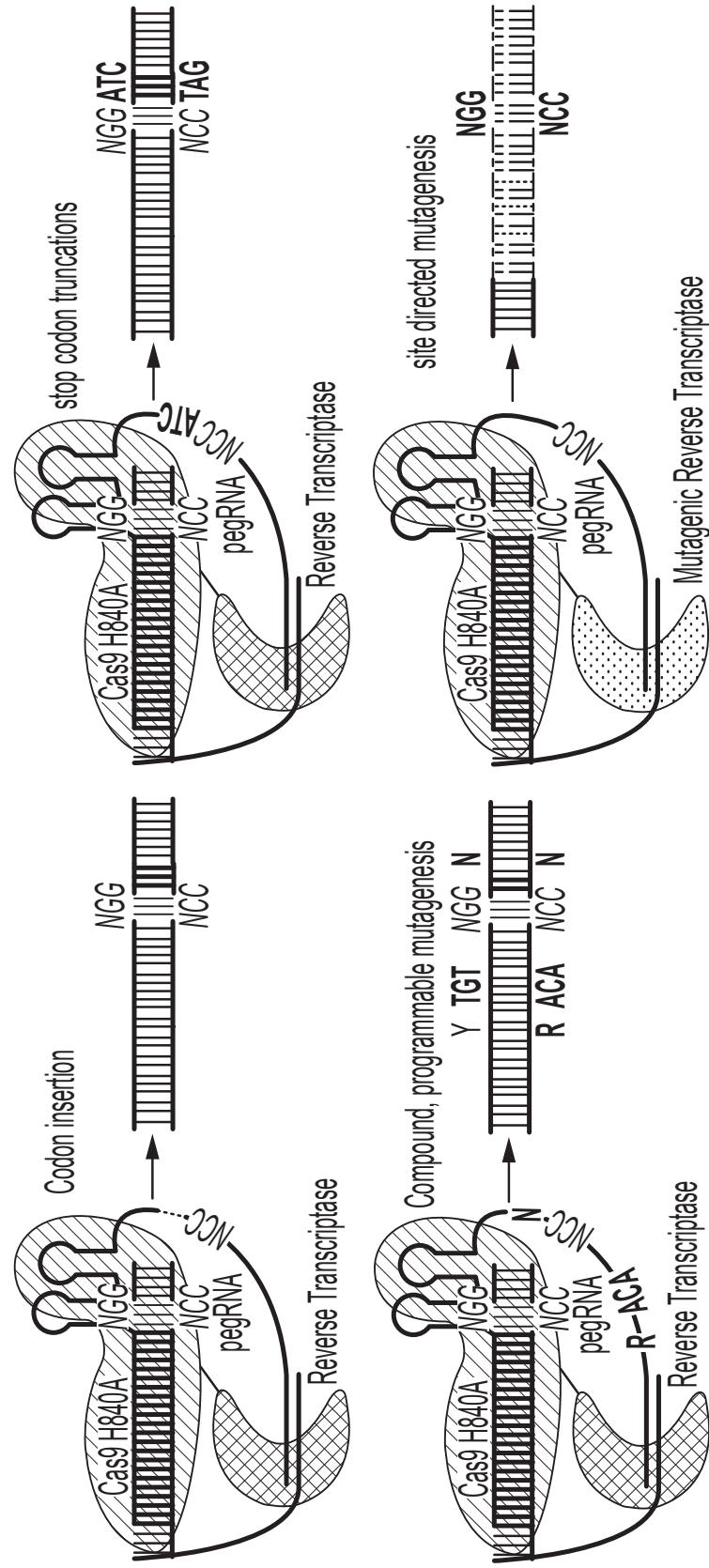


FIG. 29B

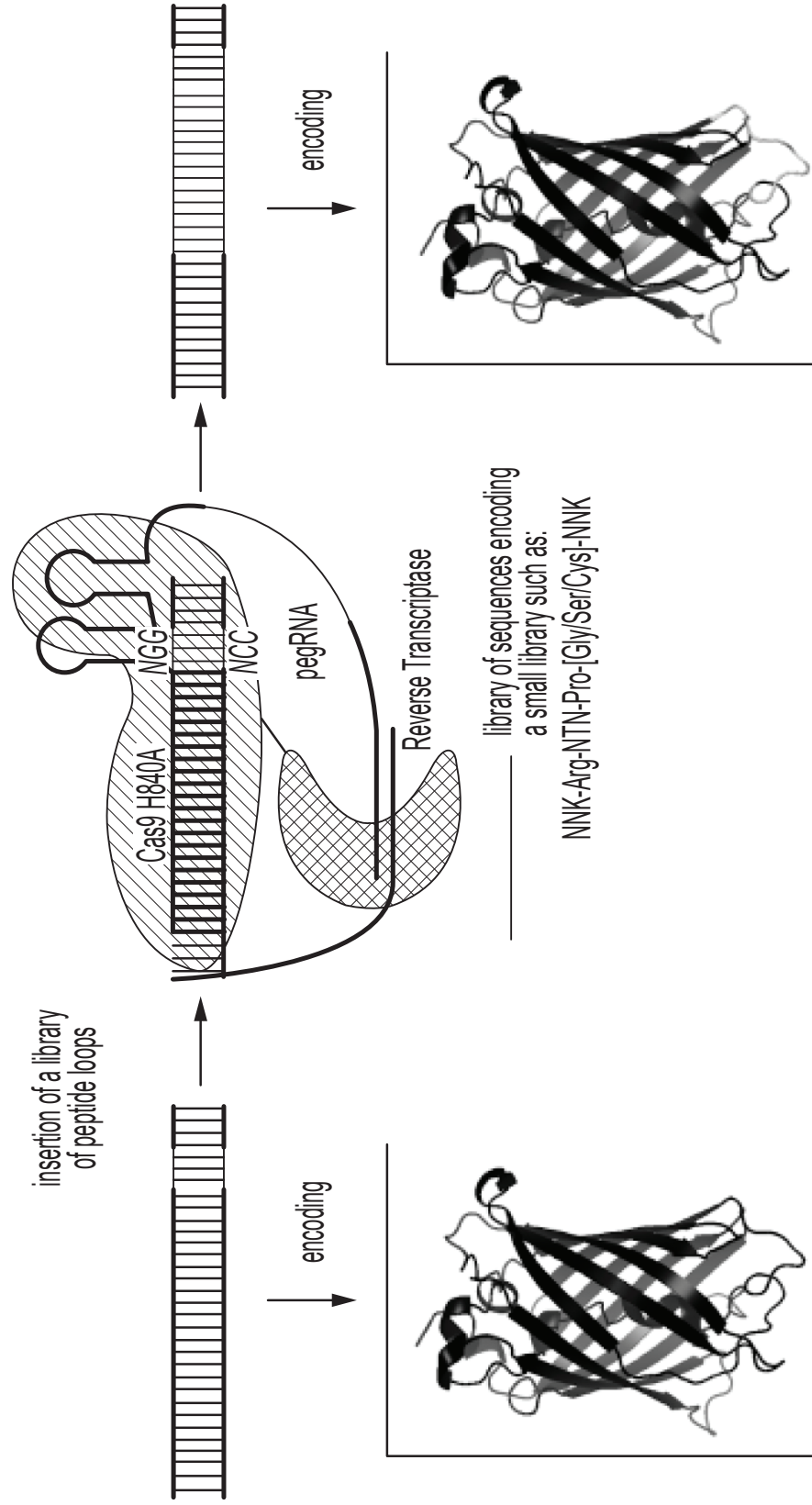


FIG. 29C

programmable codon deletion and truncations		
SEQ ID NO: 811	GFP	NFKIRHNIEDGSVQLADHYQQNTPI
SEQ ID NO: 812	del15	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

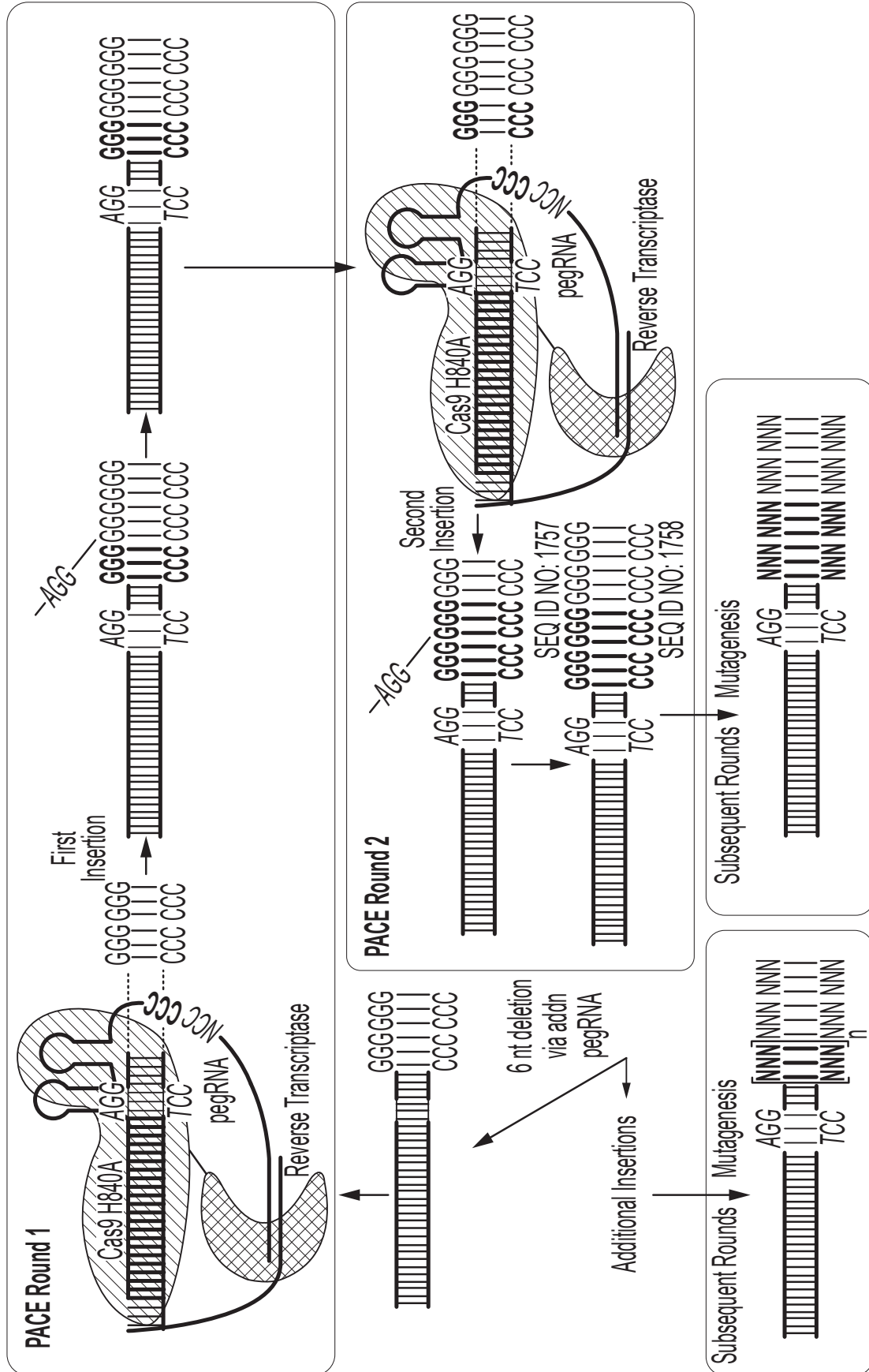


FIG. 30

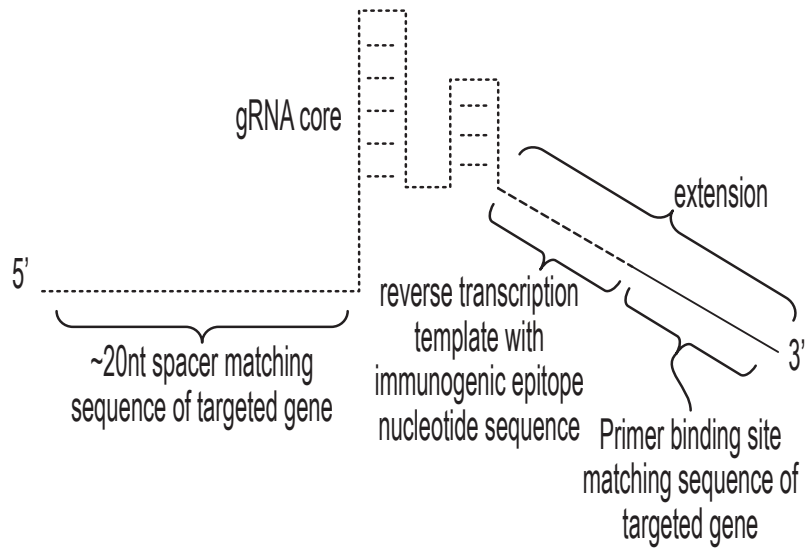


FIG. 31

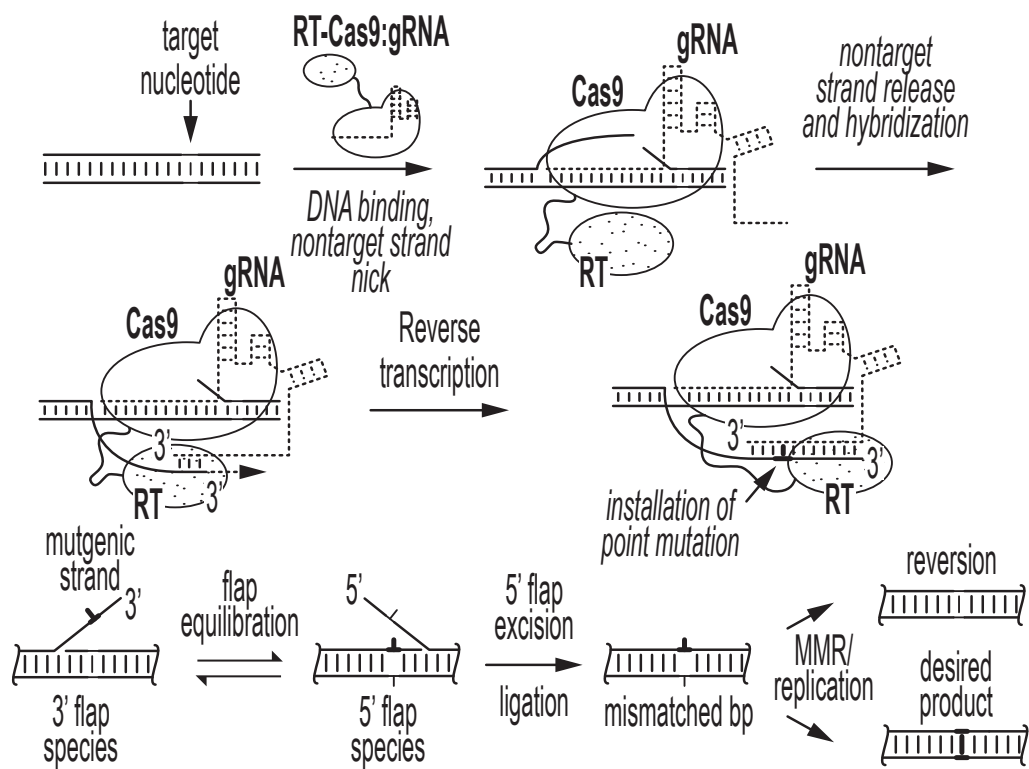


FIG. 32

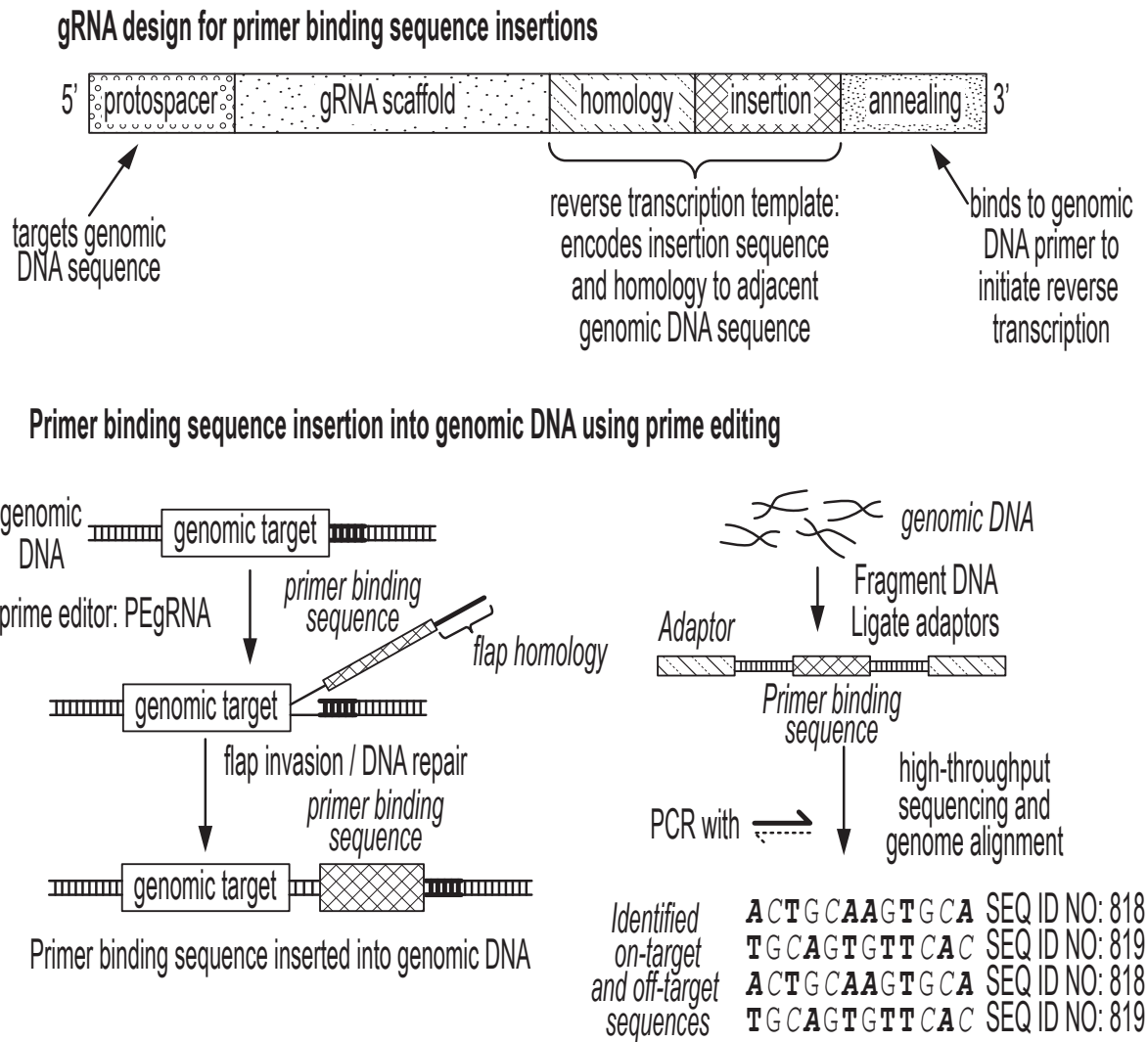


FIG. 33

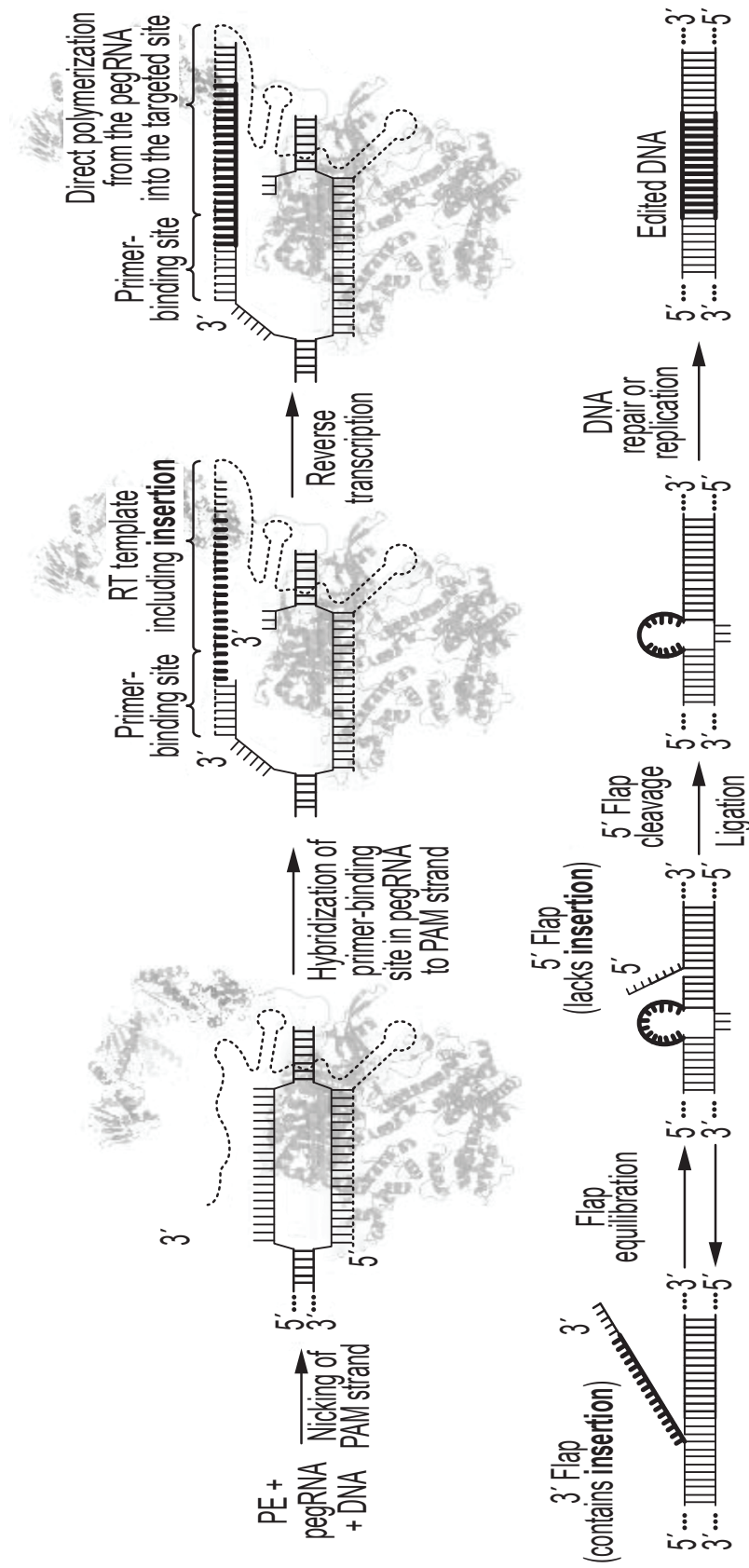


FIG. 34

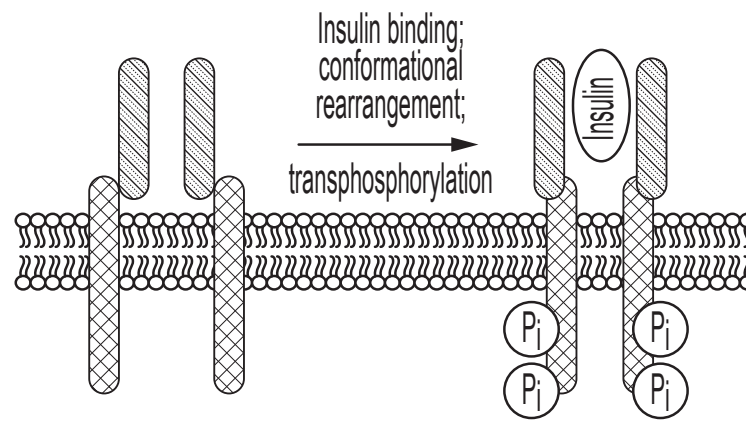


FIG. 35A

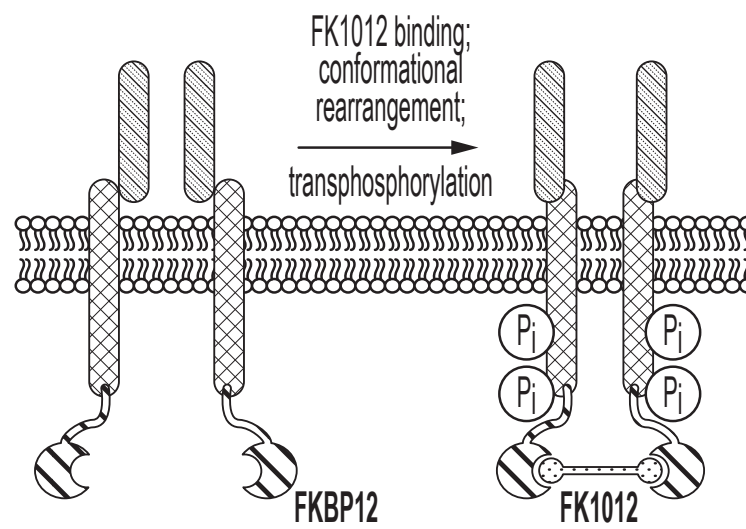


FIG. 35B

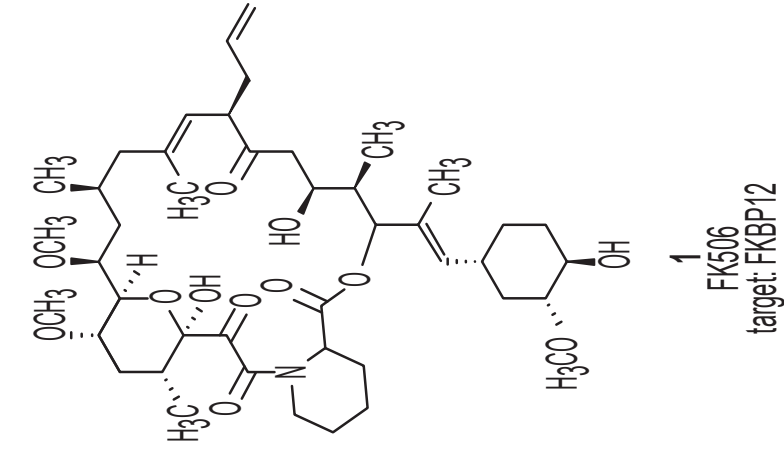
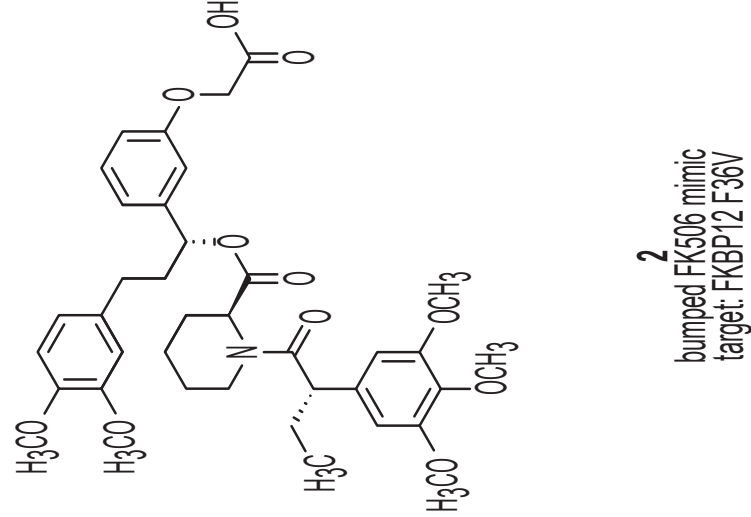
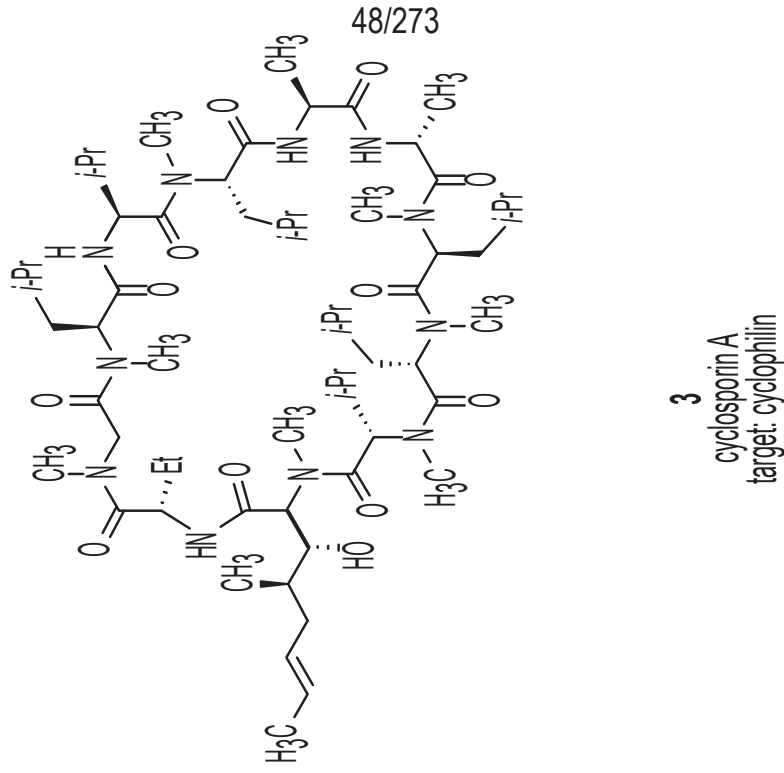


FIG. 36

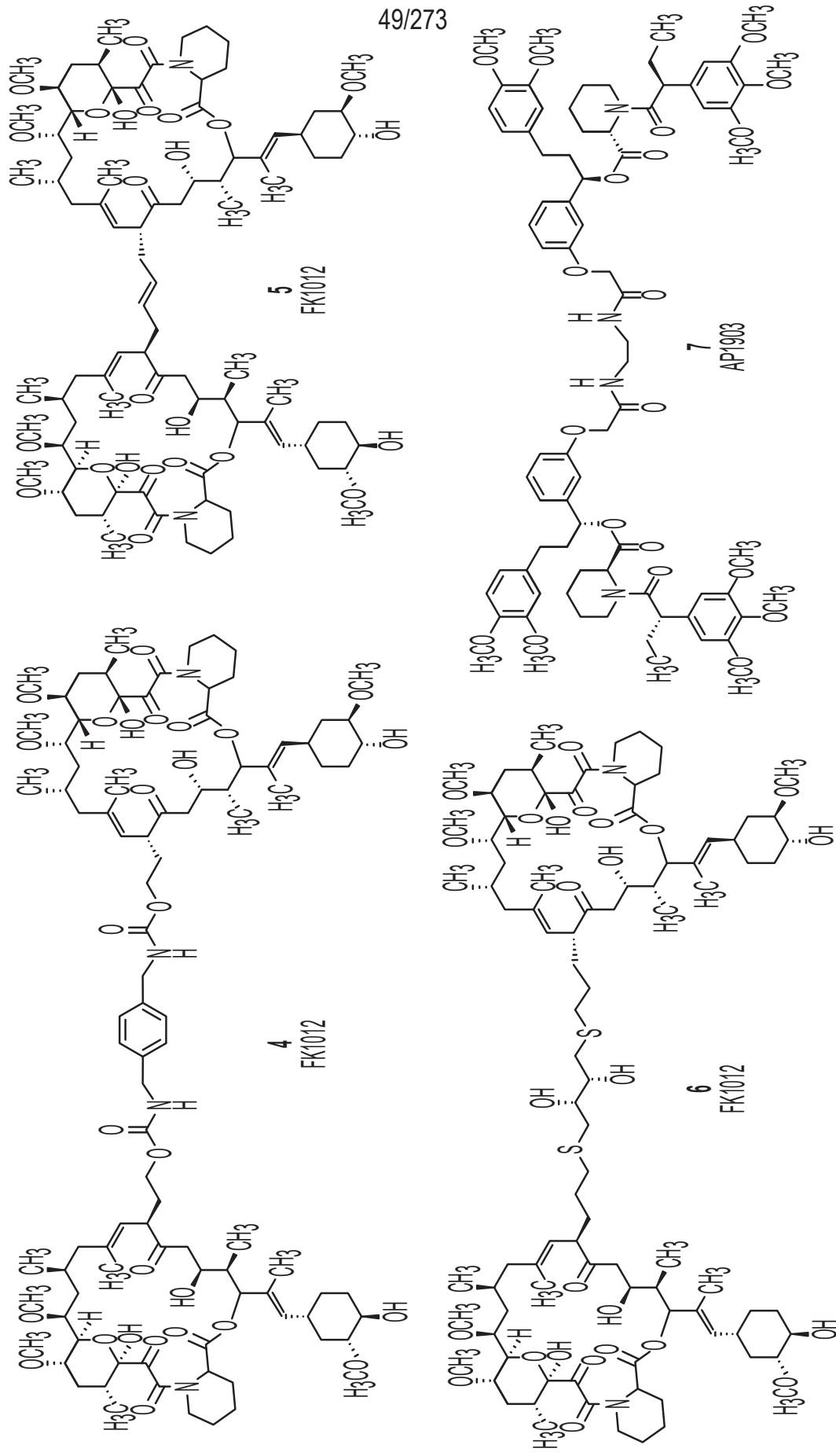


FIG. 37A

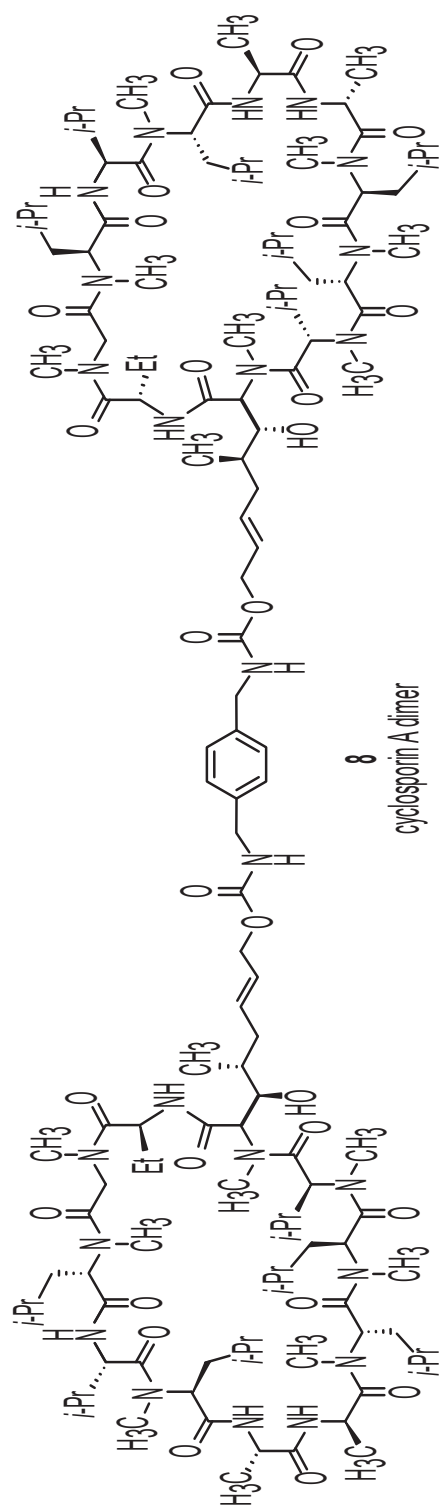
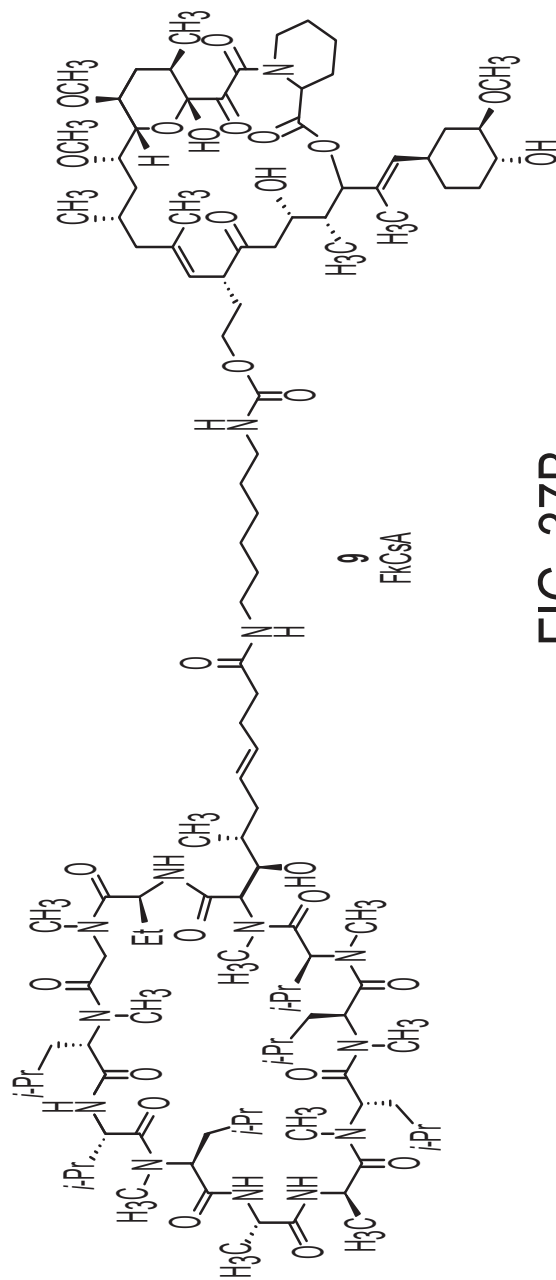


FIG. 37B

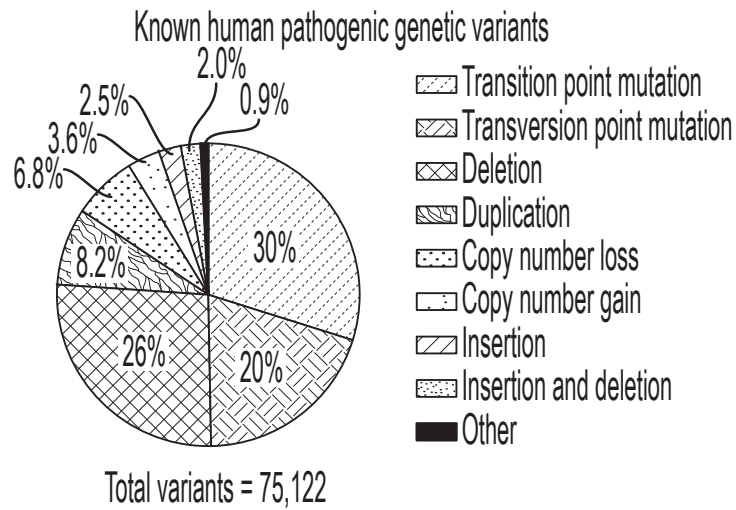


FIG. 38A

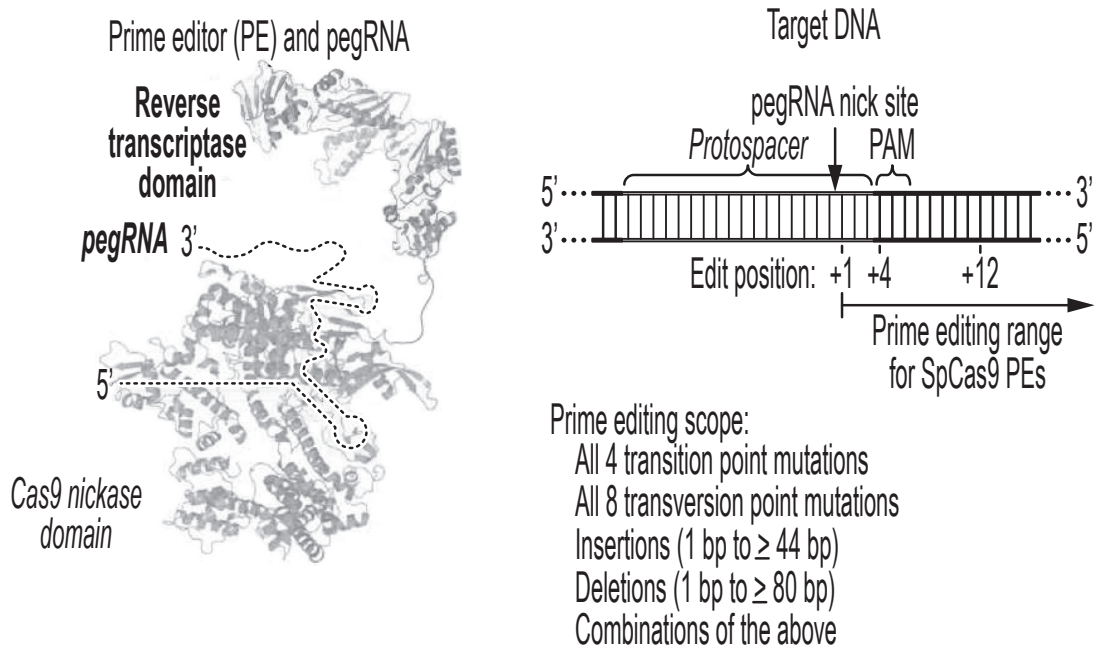


FIG. 38B

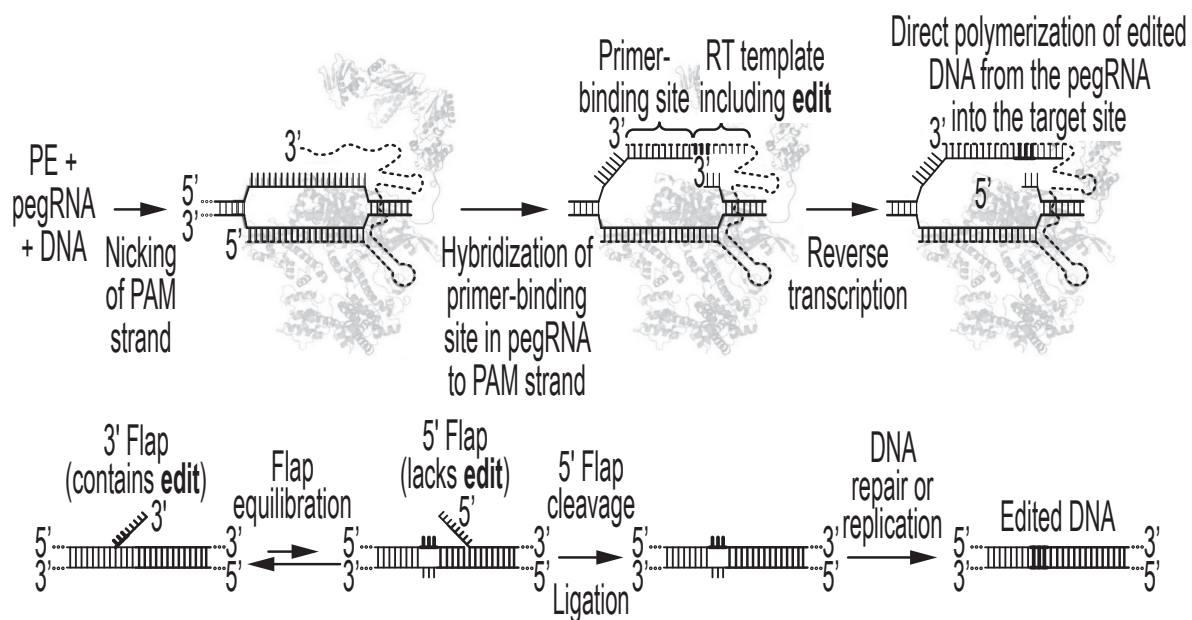


FIG. 38C

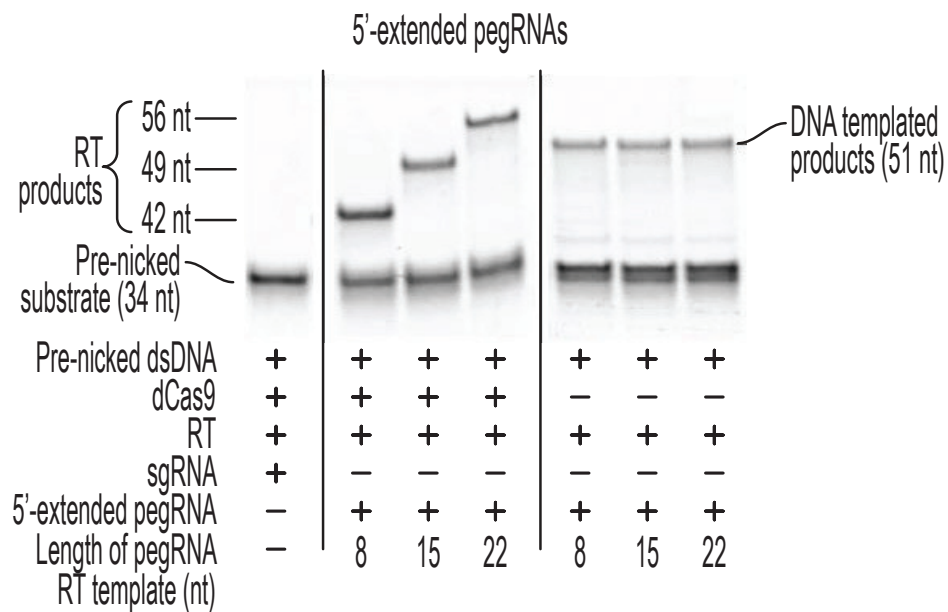


FIG. 38D

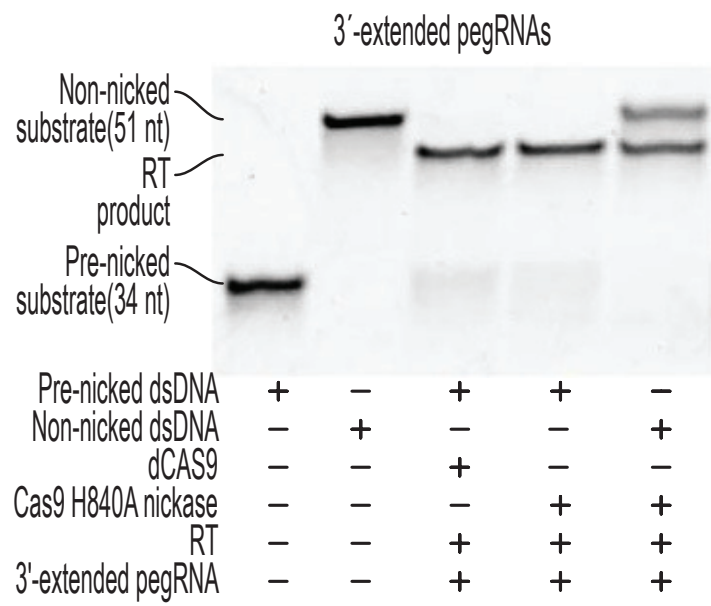


FIG. 38E

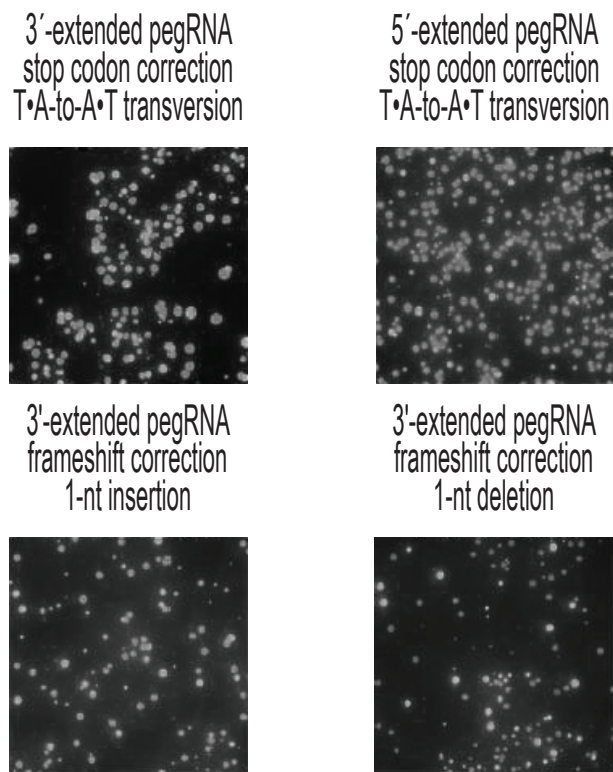


FIG. 38F

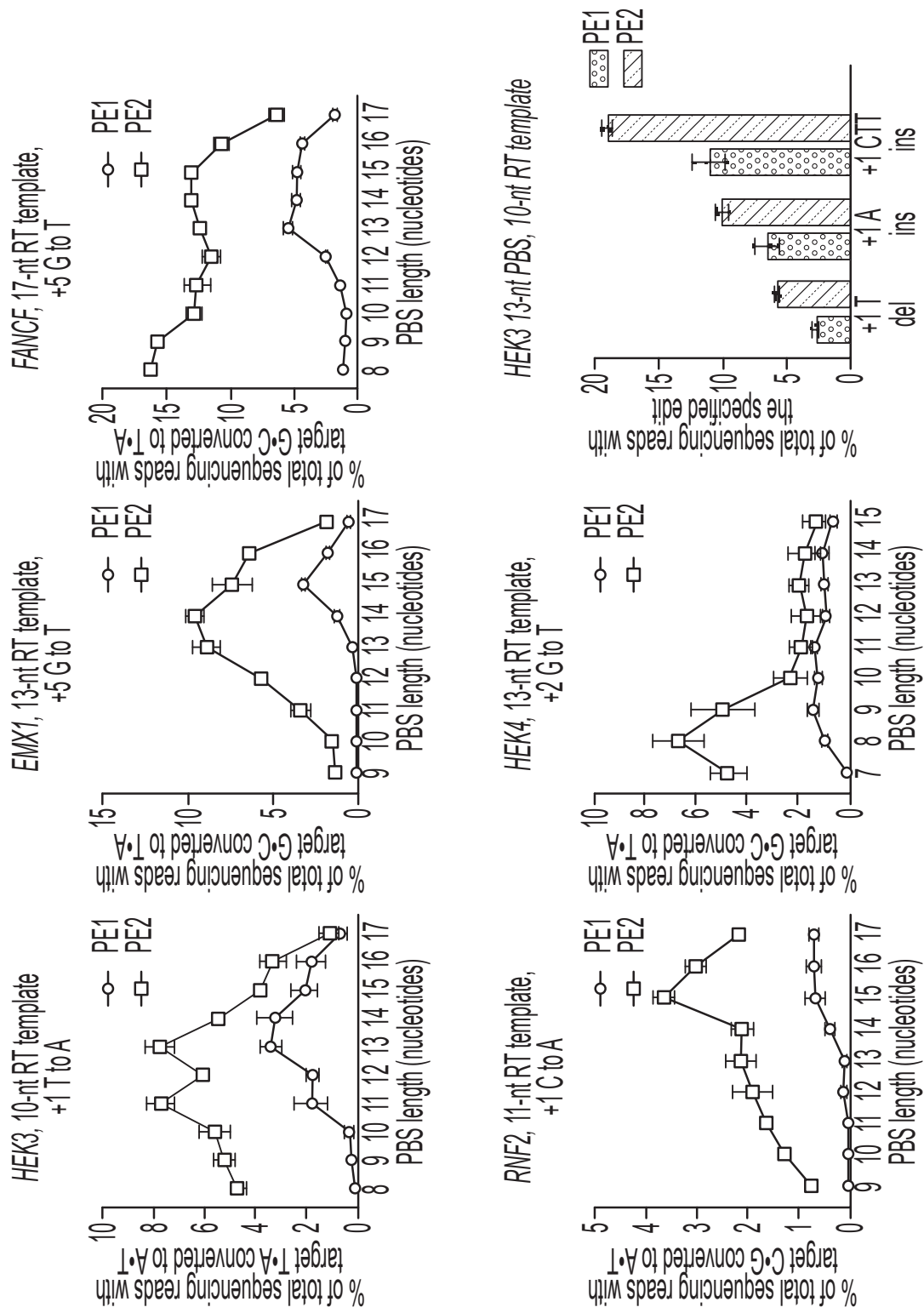


FIG. 39C

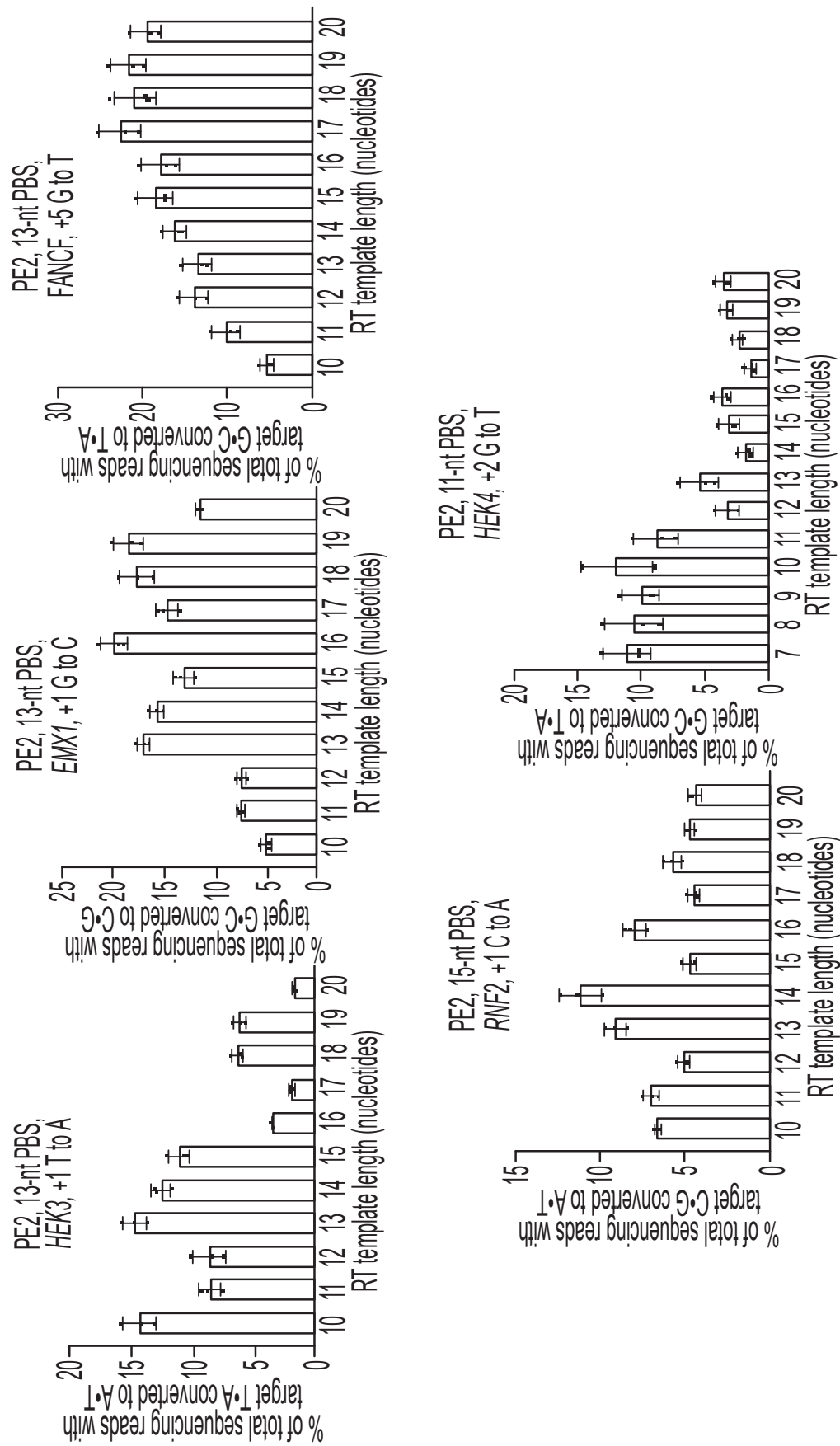


FIG. 39D

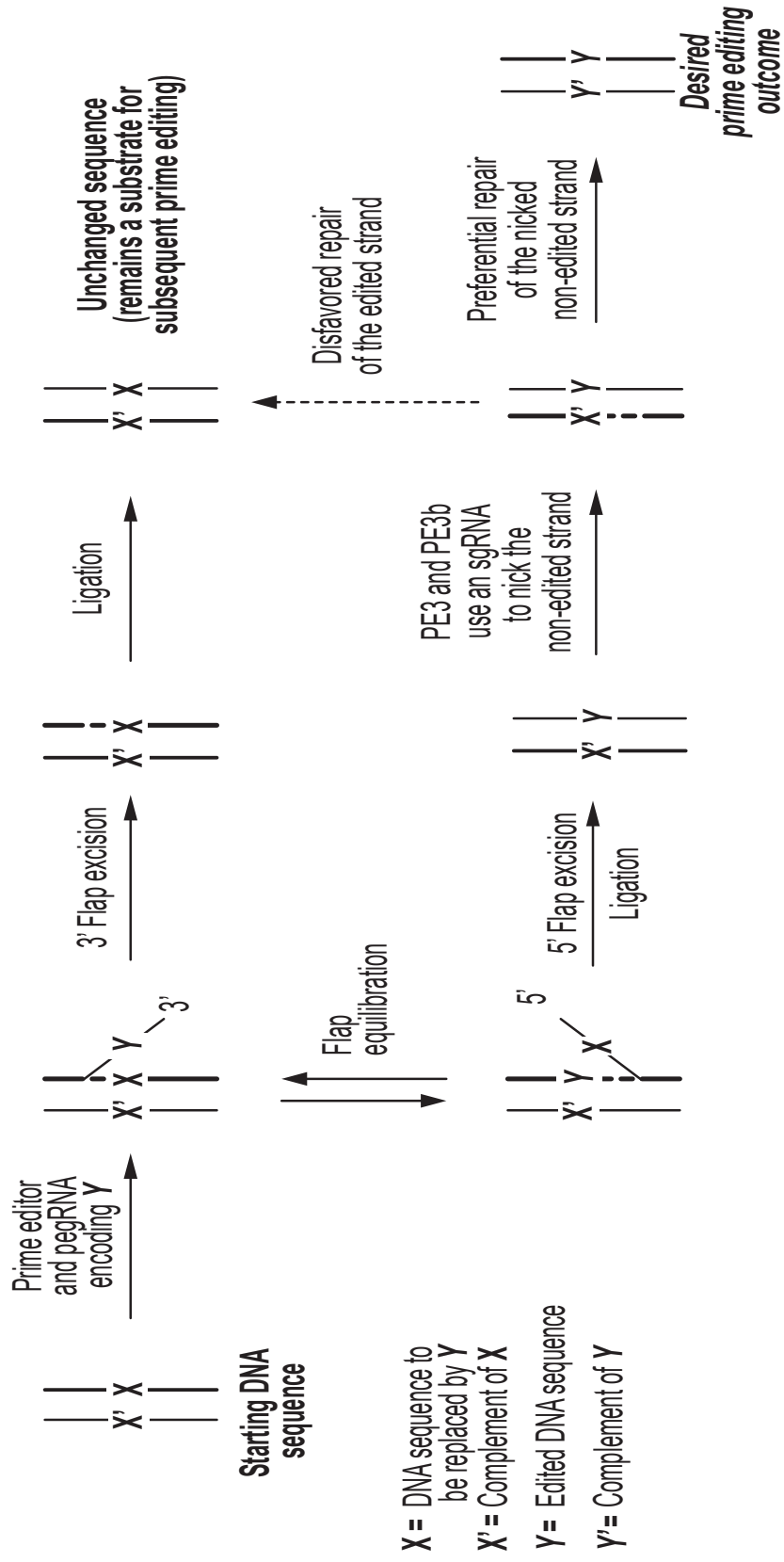


FIG. 40A

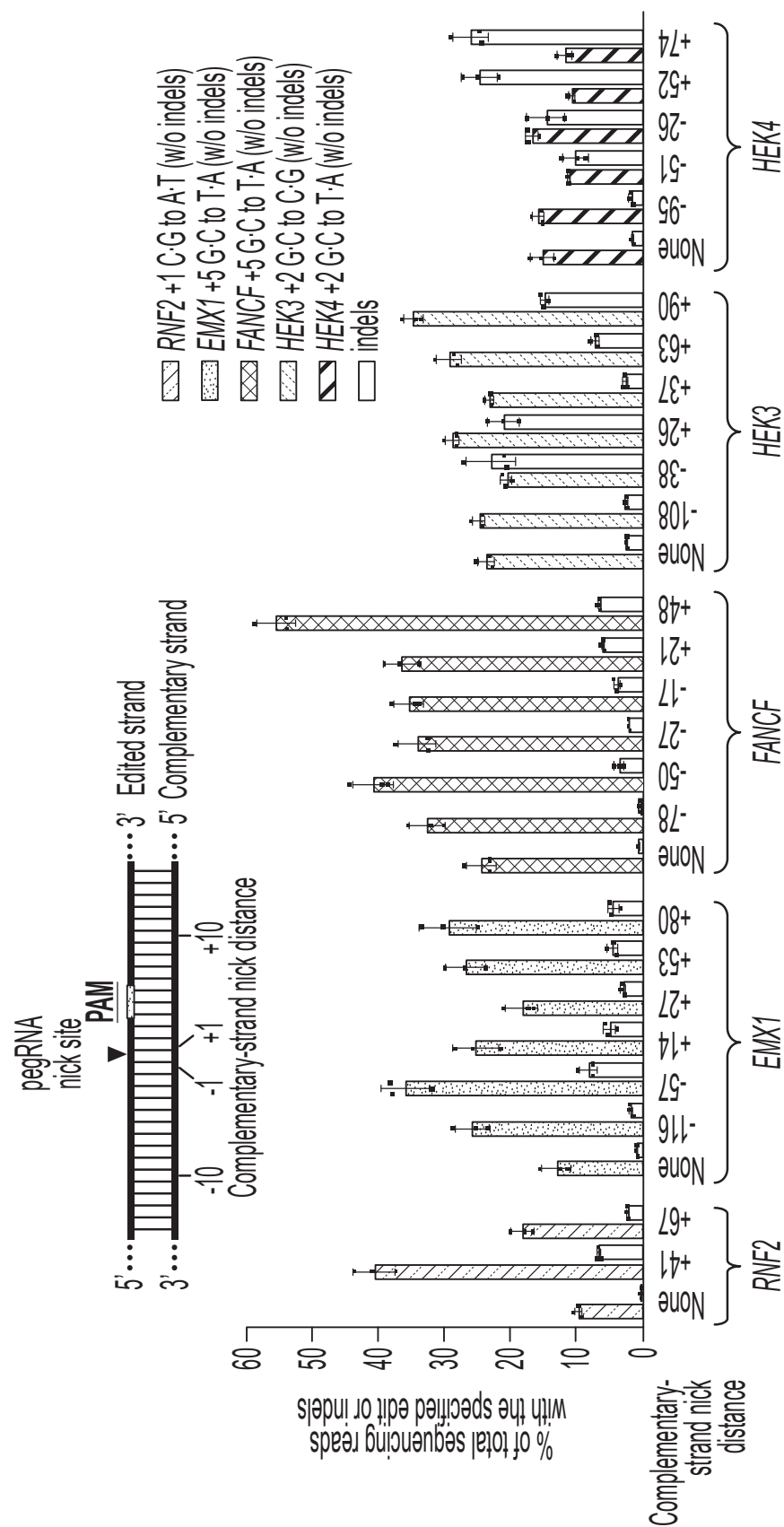


FIG. 40B

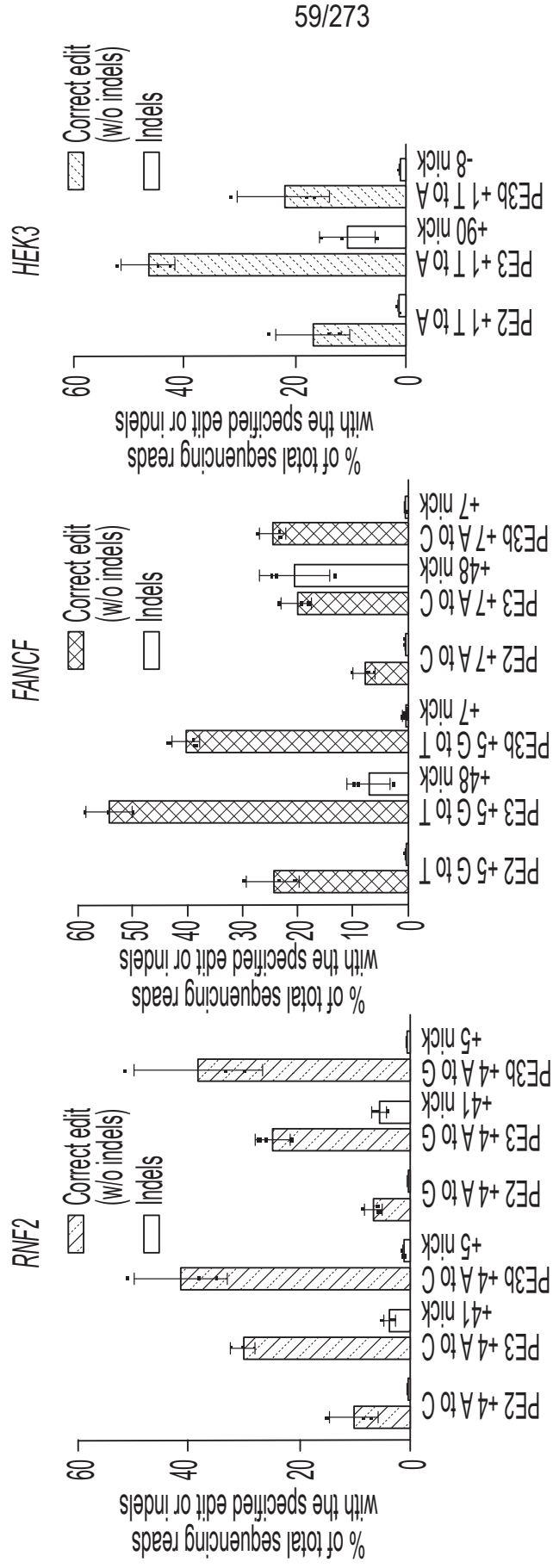


FIG. 40C

PE3, 10-nt RT template,
nicking at +90, HEK3

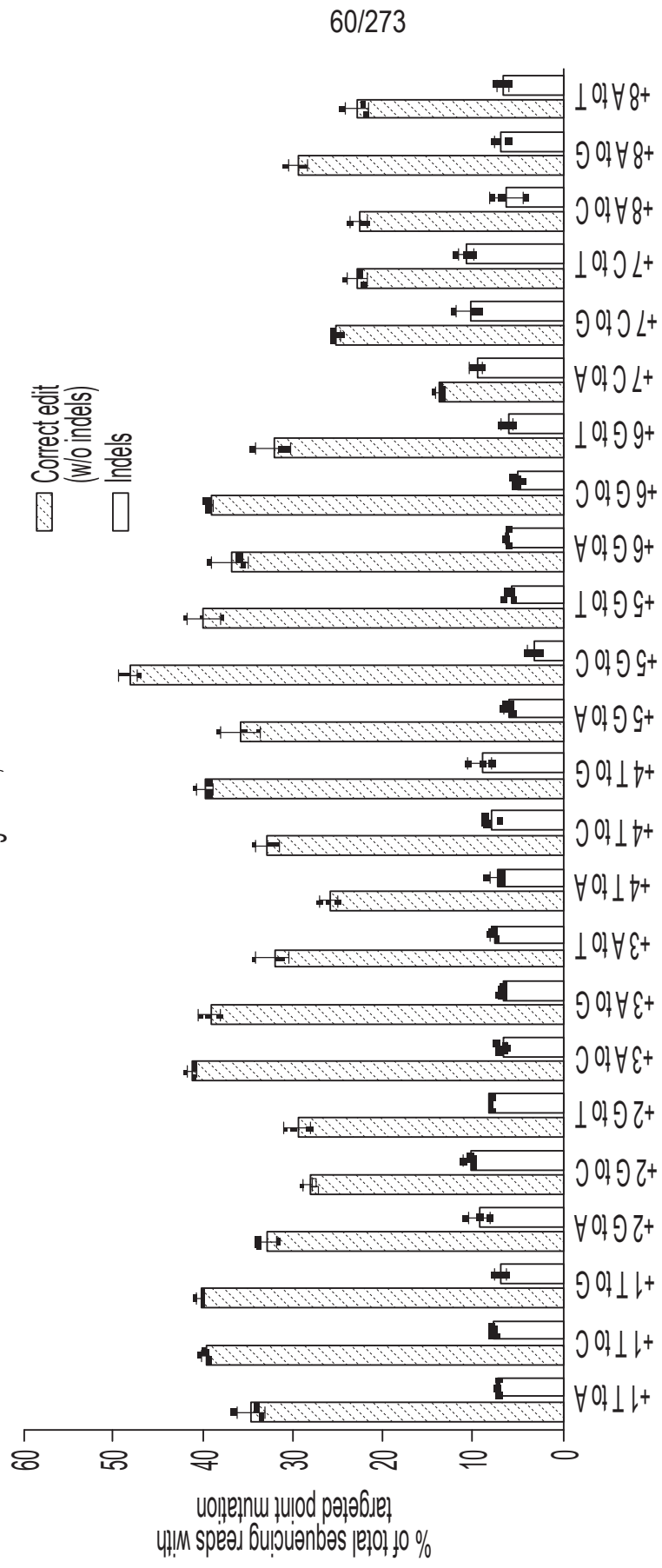


FIG. 41A

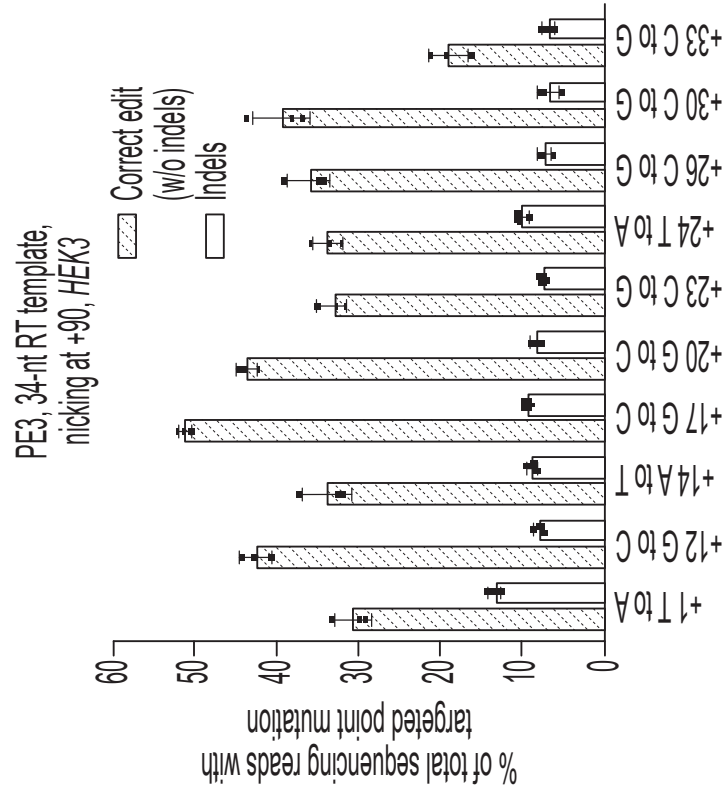


FIG. 41B

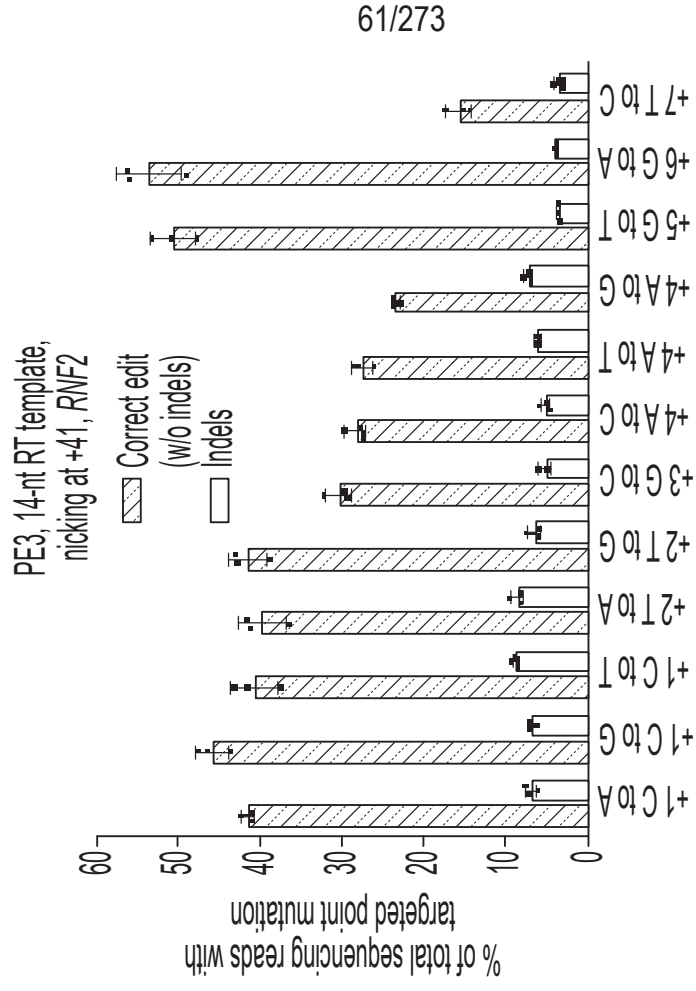


FIG. 41C

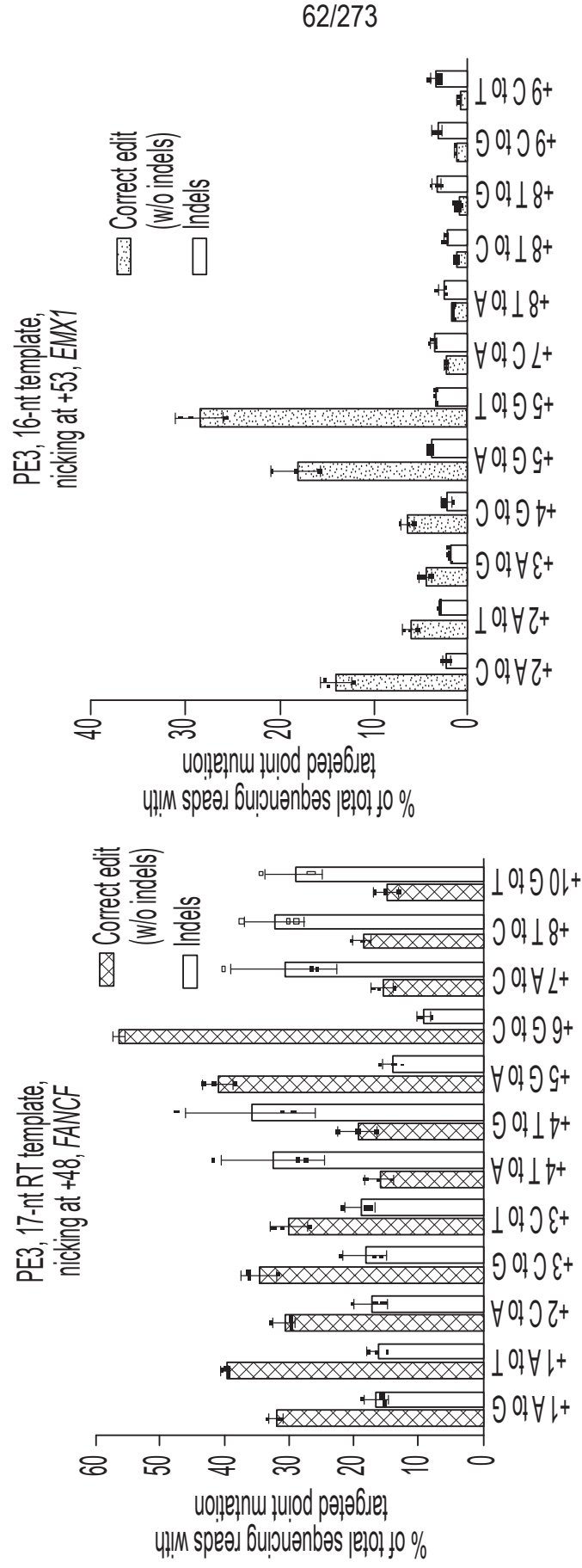


FIG. 41D

FIG. 41E

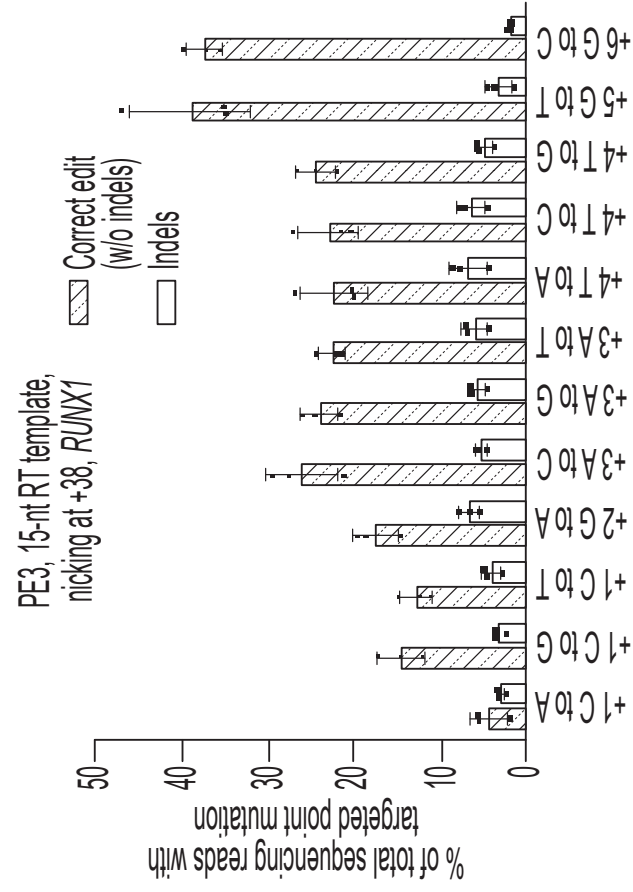


FIG. 41F

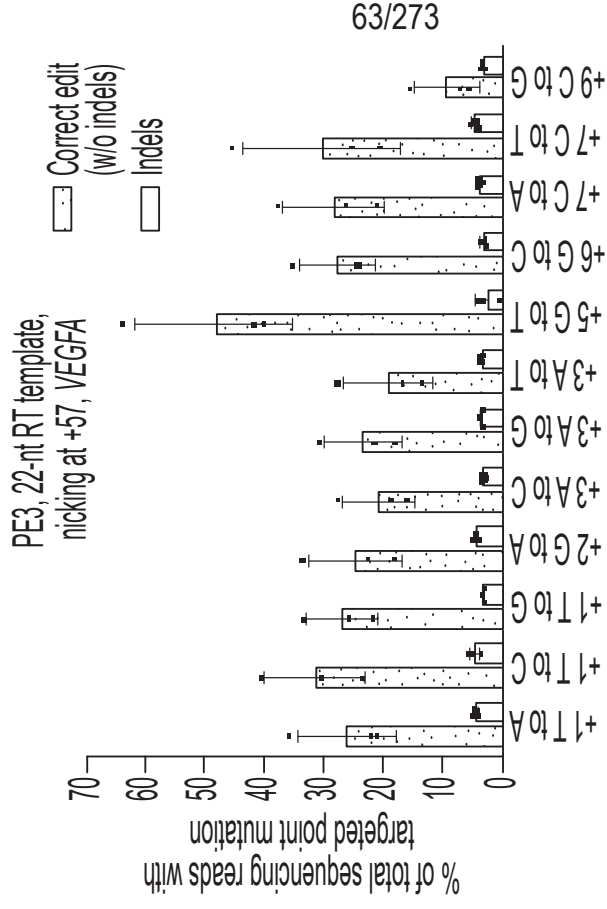


FIG. 41G

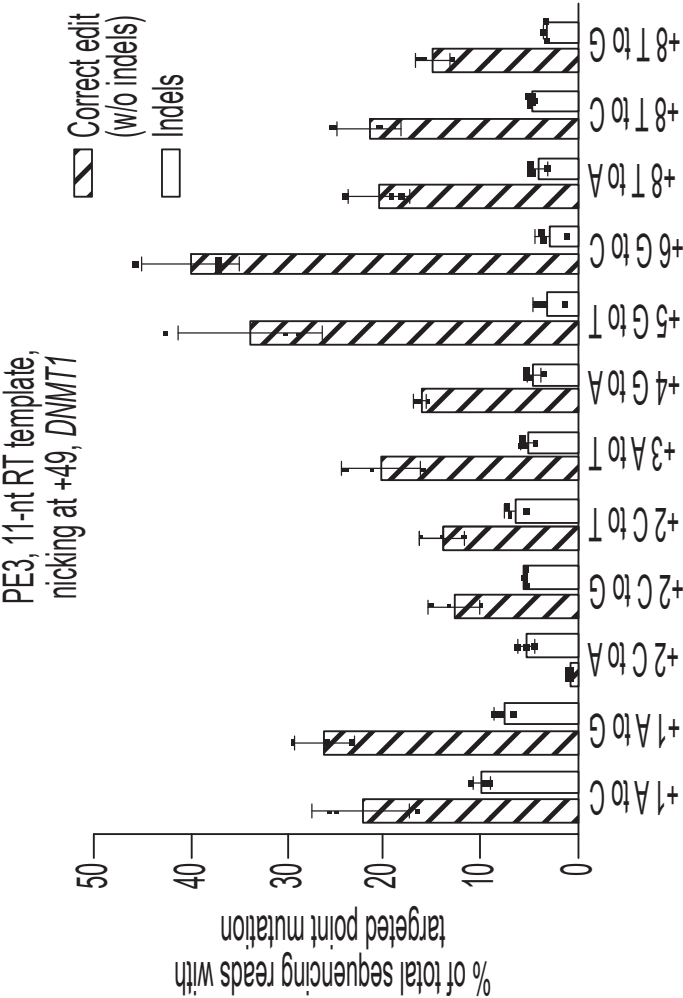


FIG. 41H

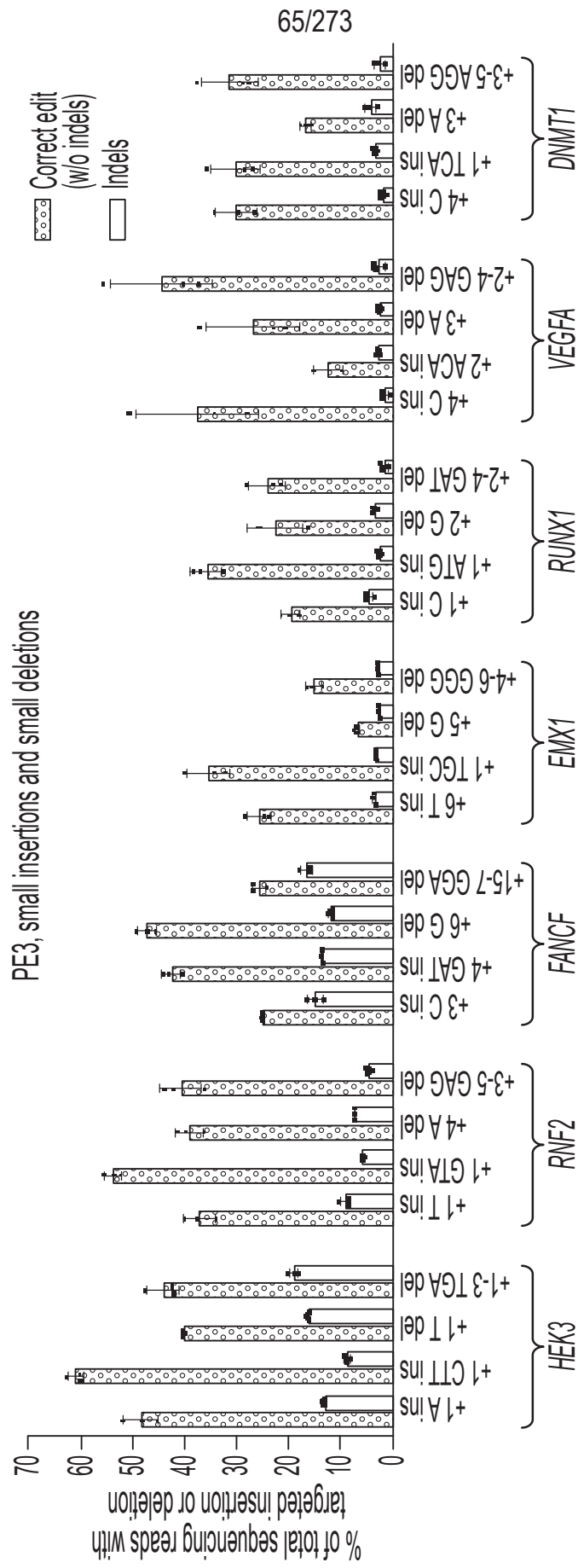


FIG. 411

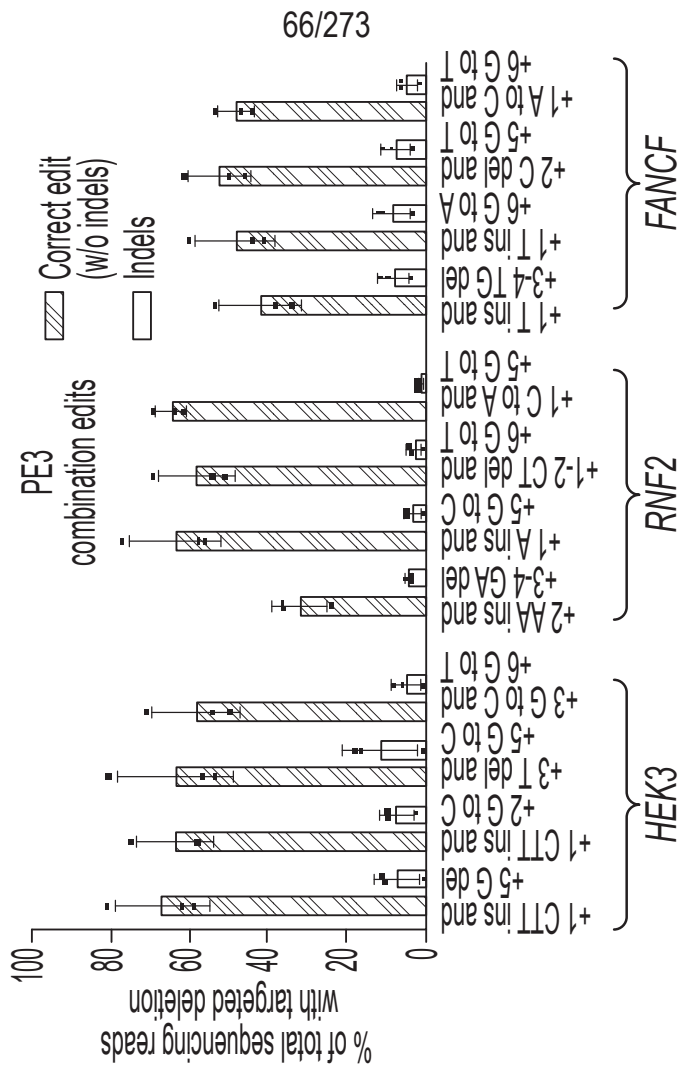


FIG. 41K

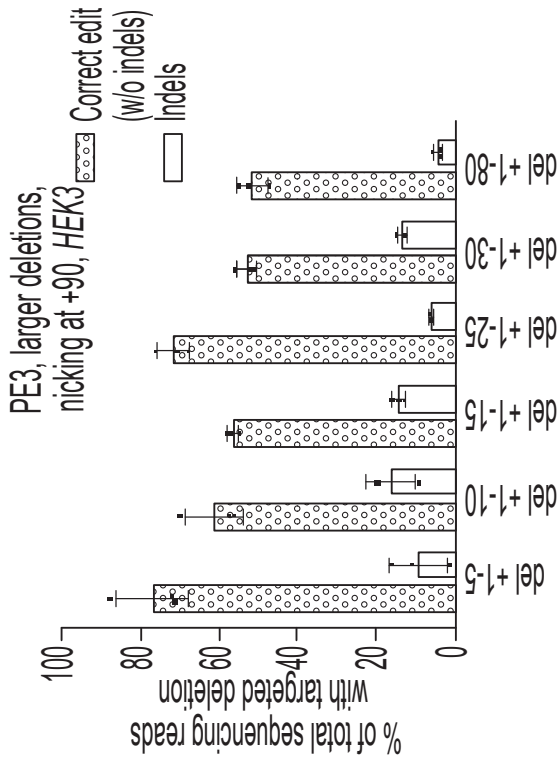


FIG. 41J

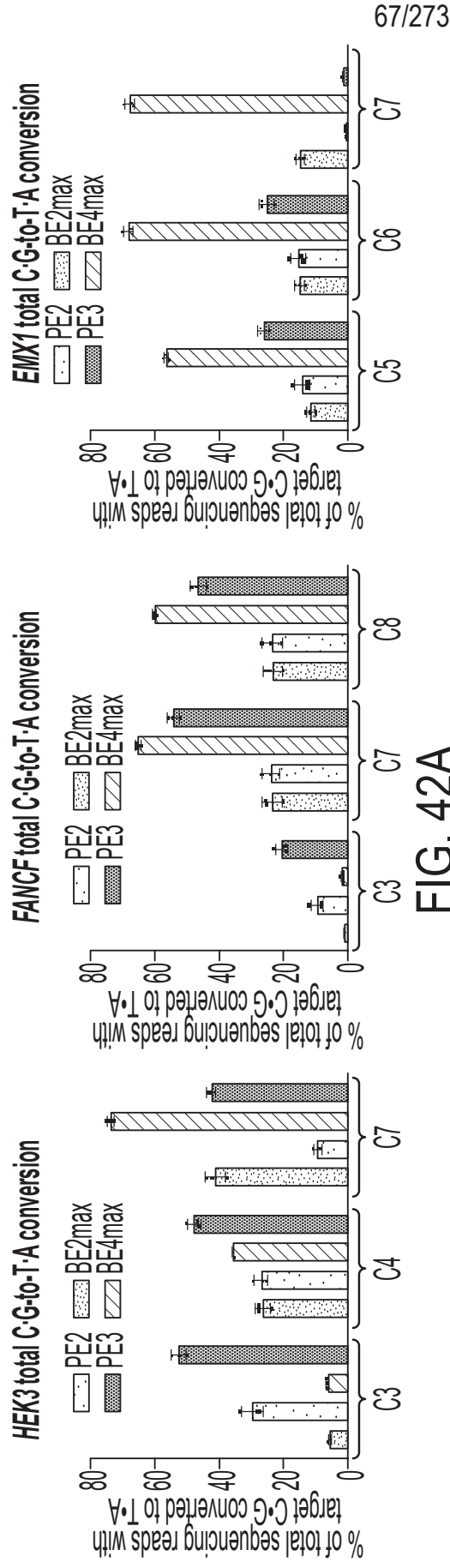


FIG. 42A

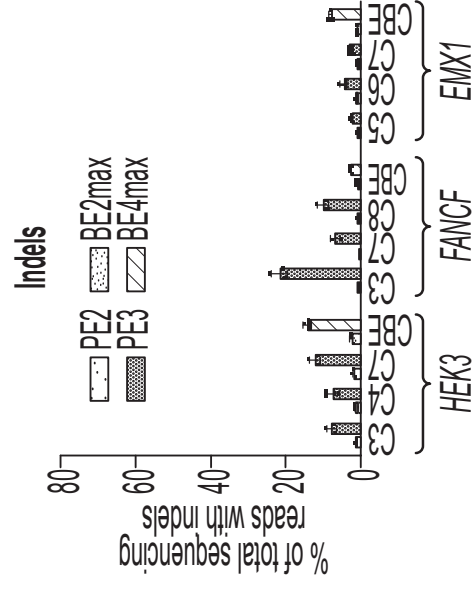


FIG. 42B

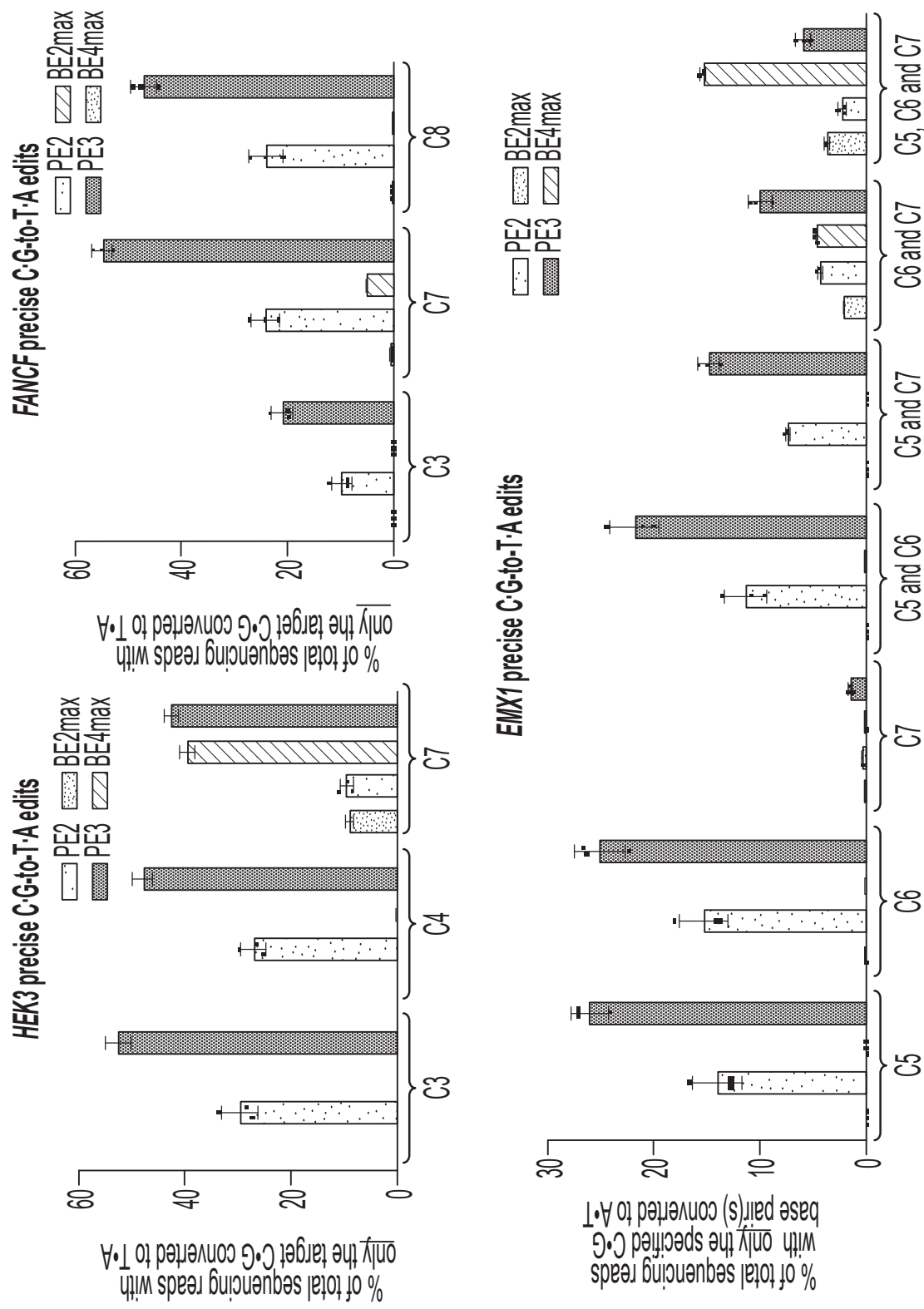


FIG. 42C

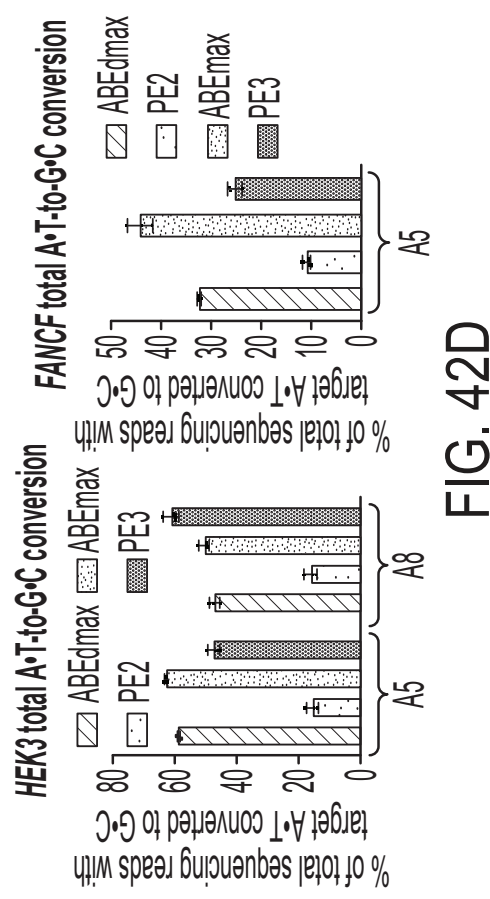


FIG. 42D

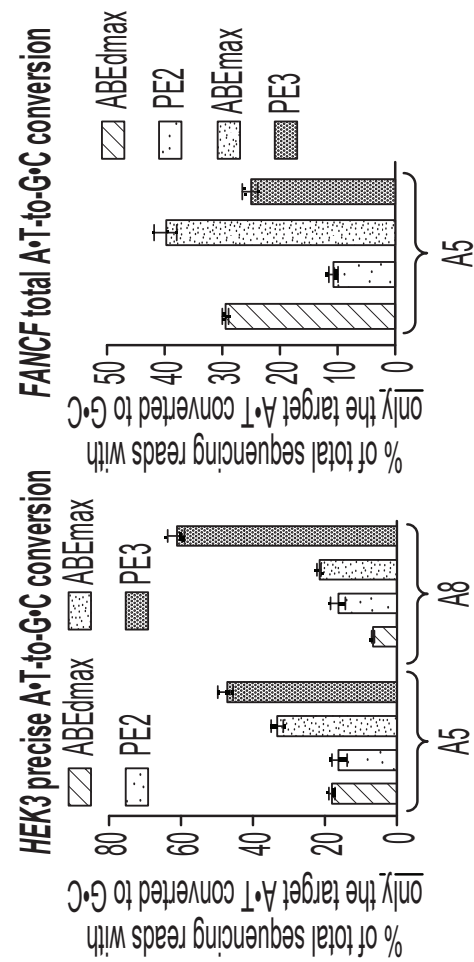


FIG. 42E

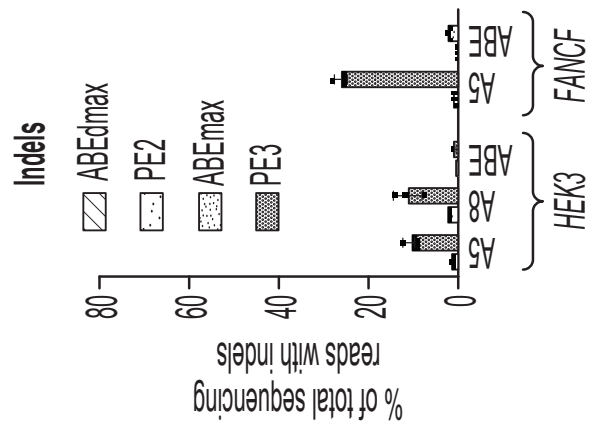


FIG. 42F

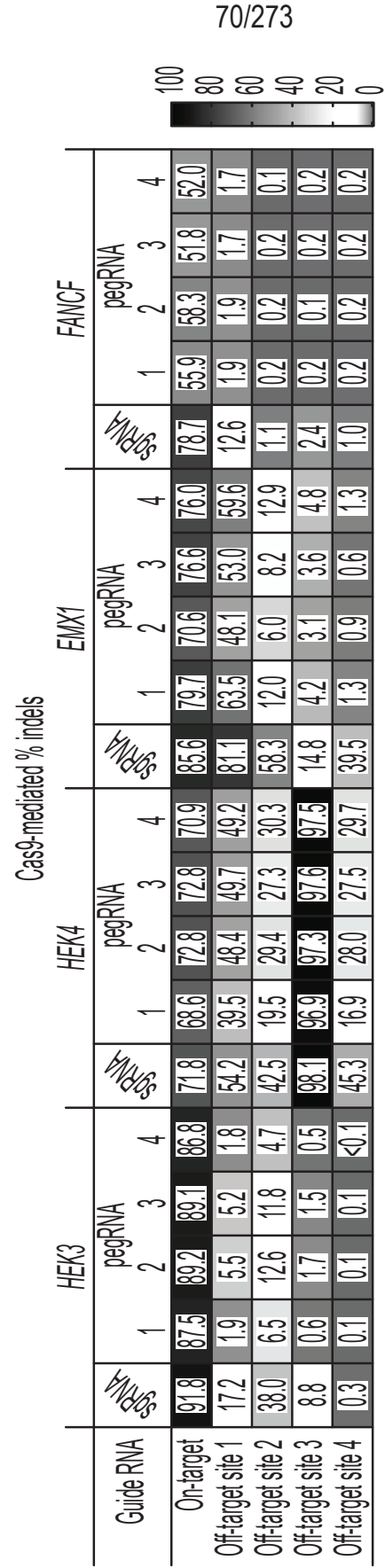


FIG. 42G

PE-mediated % editing and % indels (in parentheses)

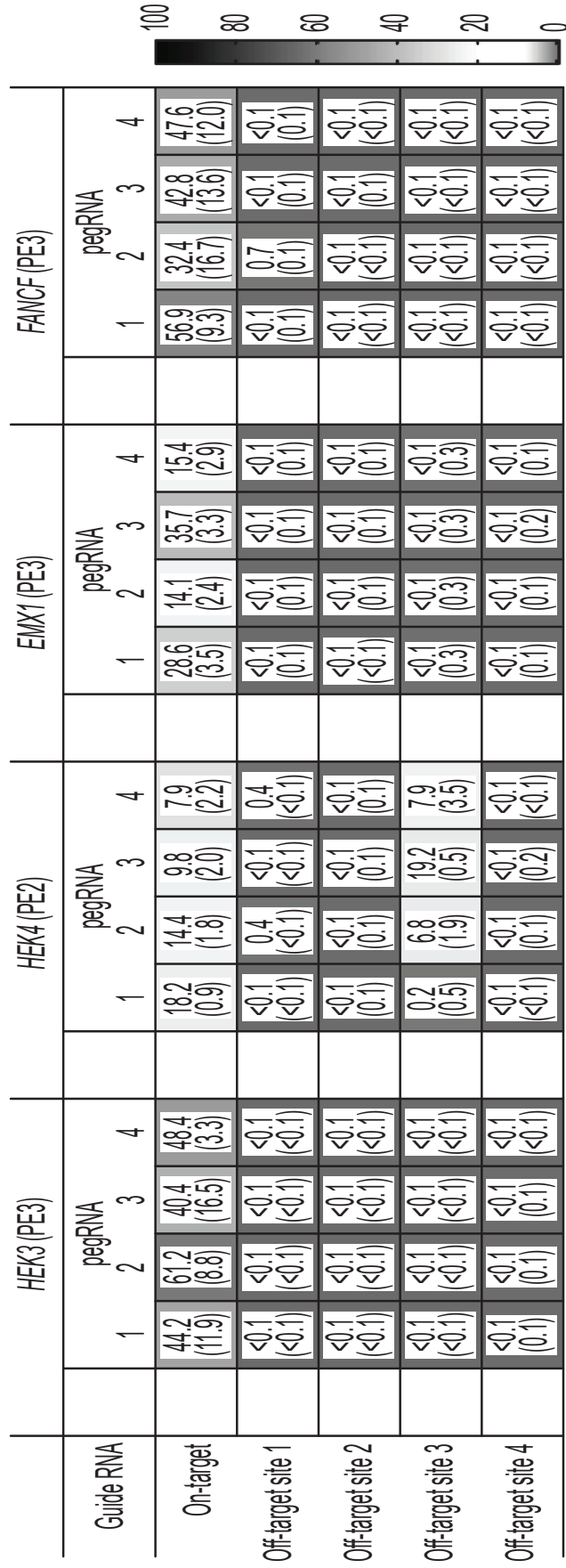


FIG. 42H

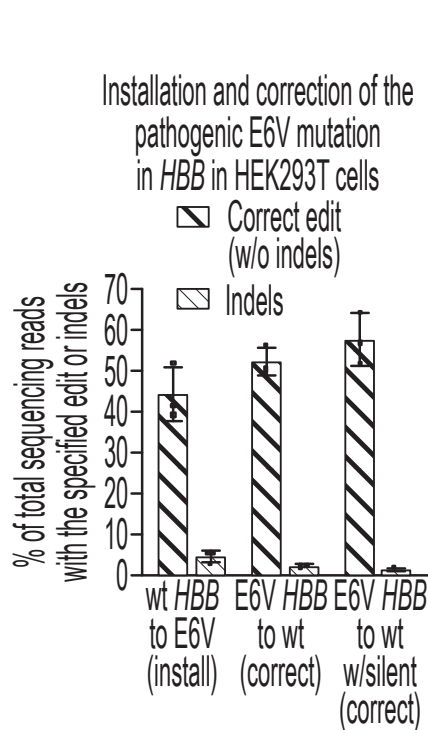


FIG. 43A

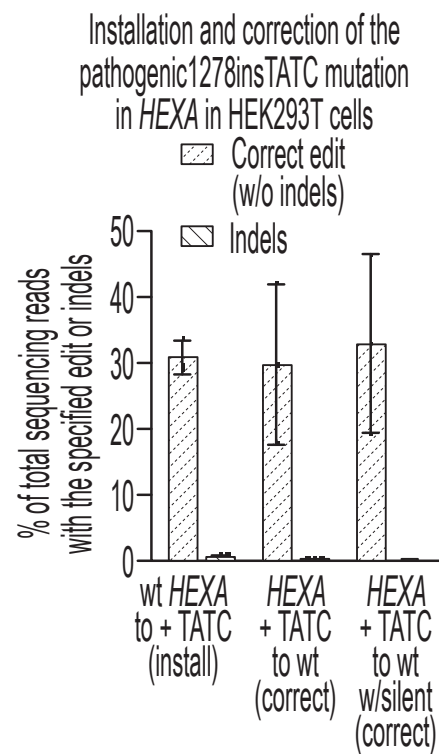


FIG. 43B

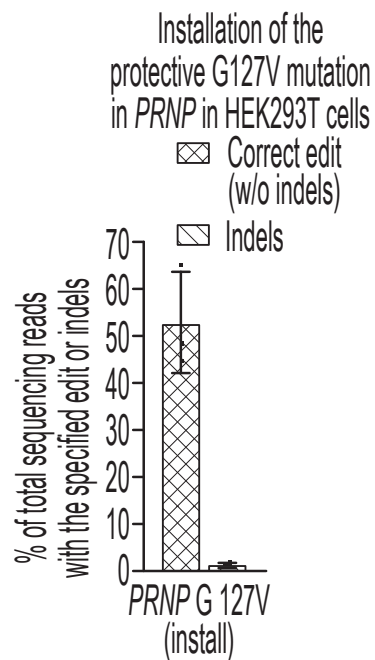


FIG. 43C

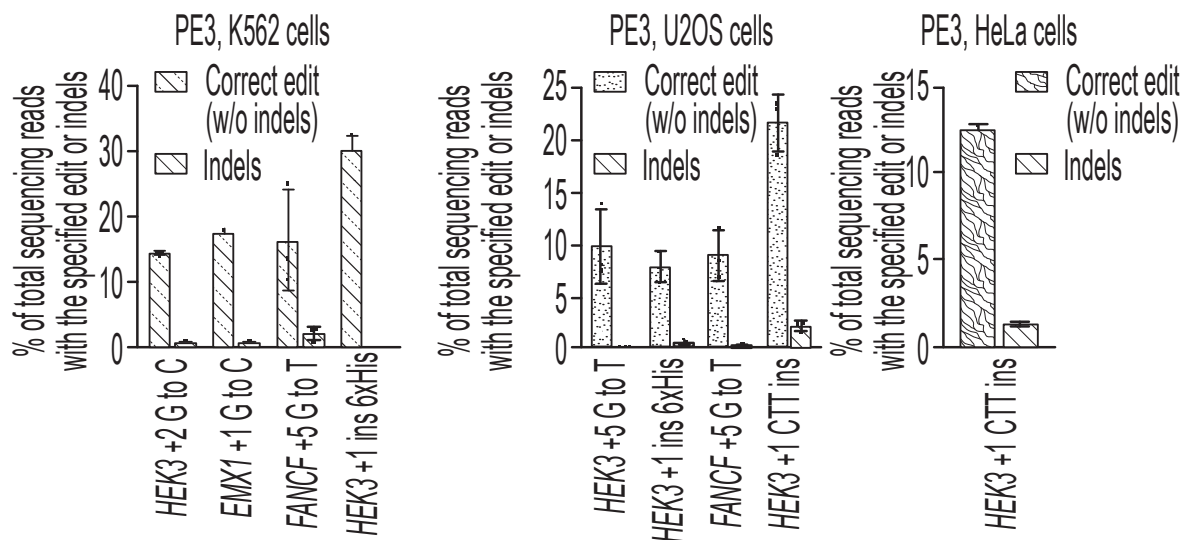


FIG. 43D

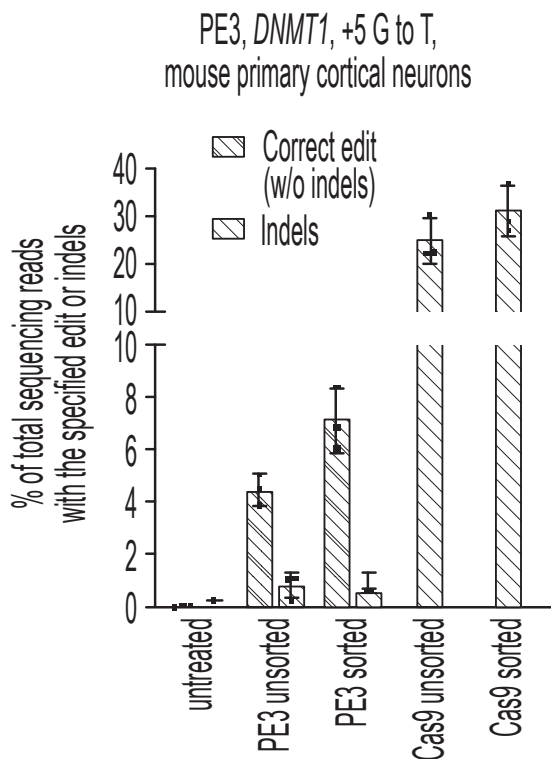


FIG. 43E

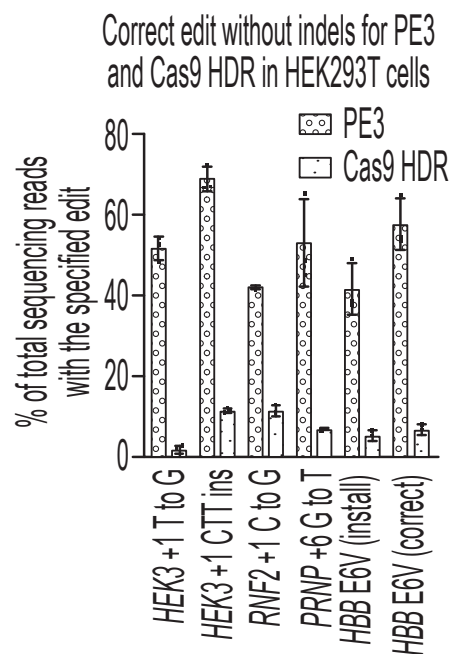


FIG. 43F

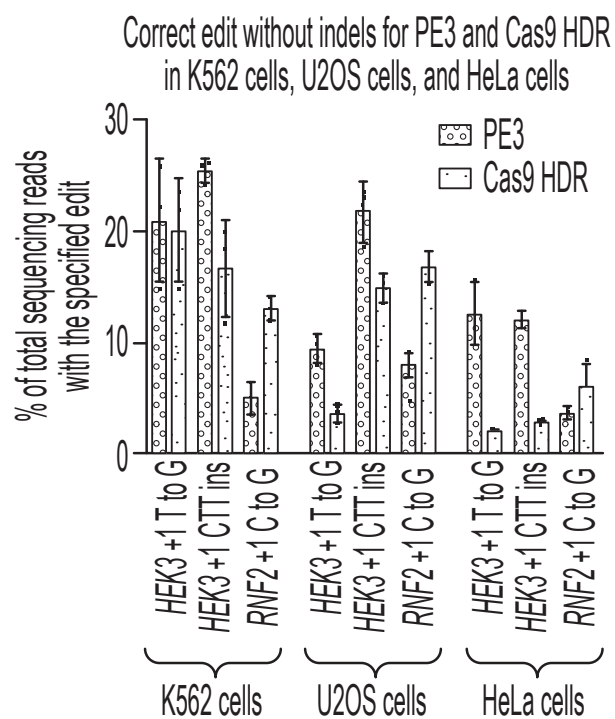


FIG. 43G

Indels generated by PE3 and Cas9 HDR in HEK293T cells, K562 cells, U2OS cells, and HeLa cells

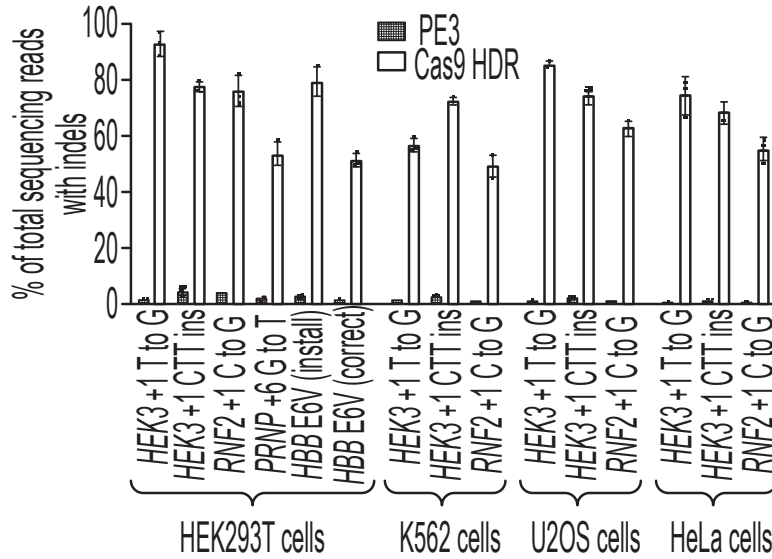


FIG. 43H

Insertion of a His₆ tag, FLAG tag, and extended *LoxP* sequence in *HEK3* in HEK293T cells

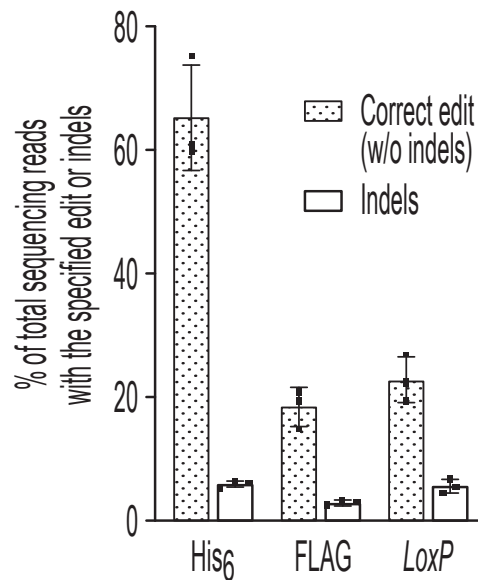


FIG. 43I

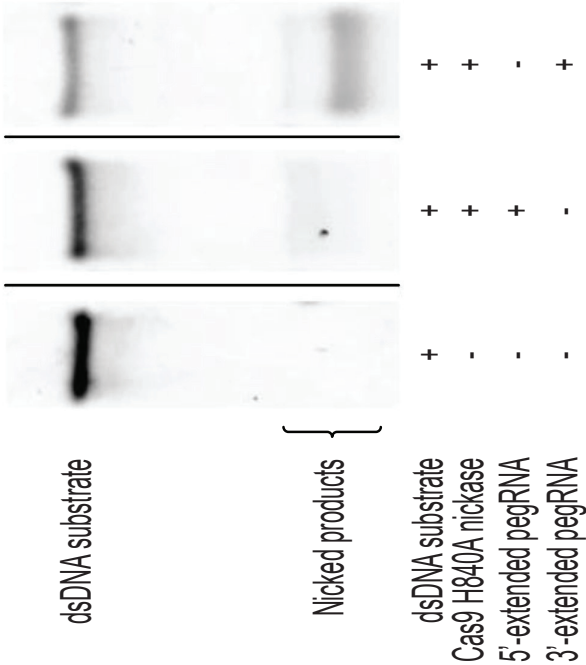


FIG. 44B

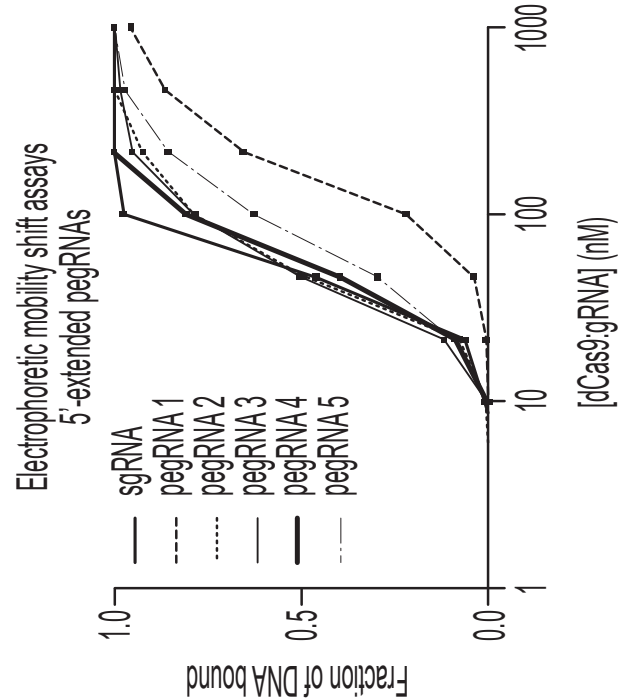


FIG. 44A

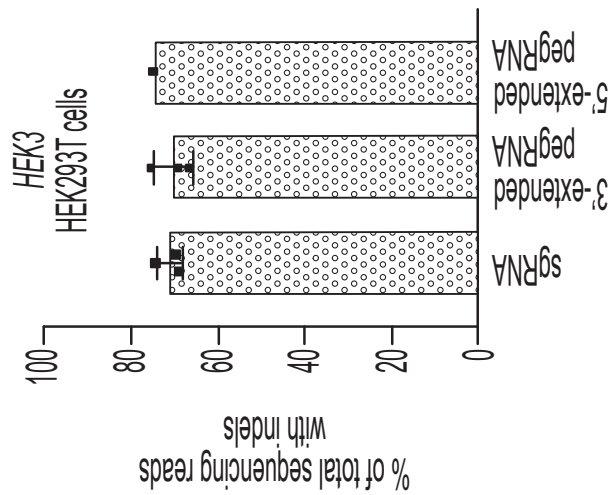


FIG. 44C

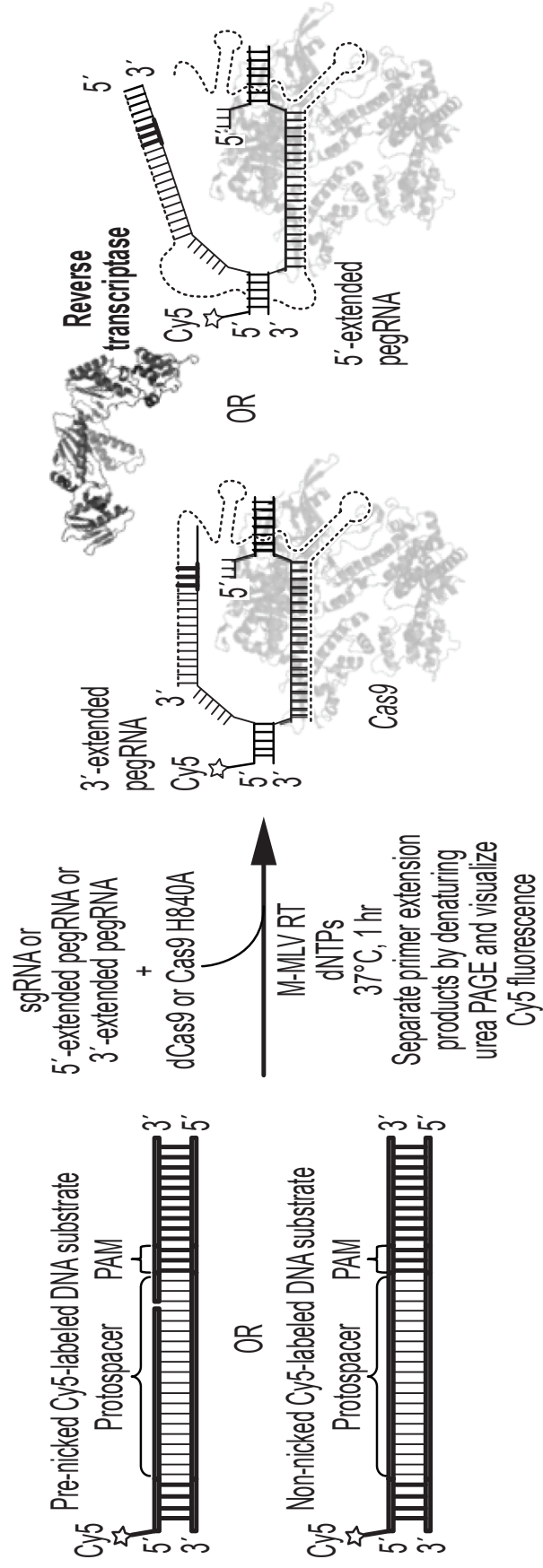


FIG. 44D



FIG. 44E

FIG. 44F

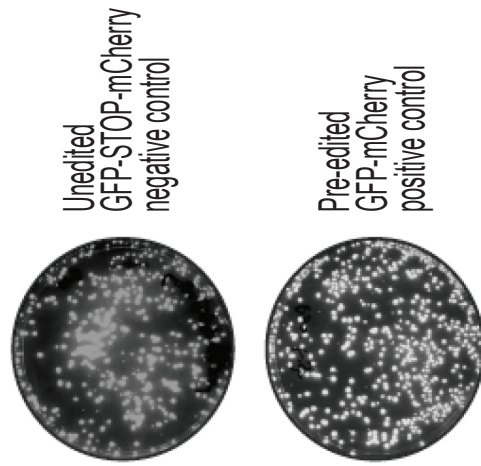


FIG. 45B

3'-Extended pegRNA stop codon correction (T•A to A•T)

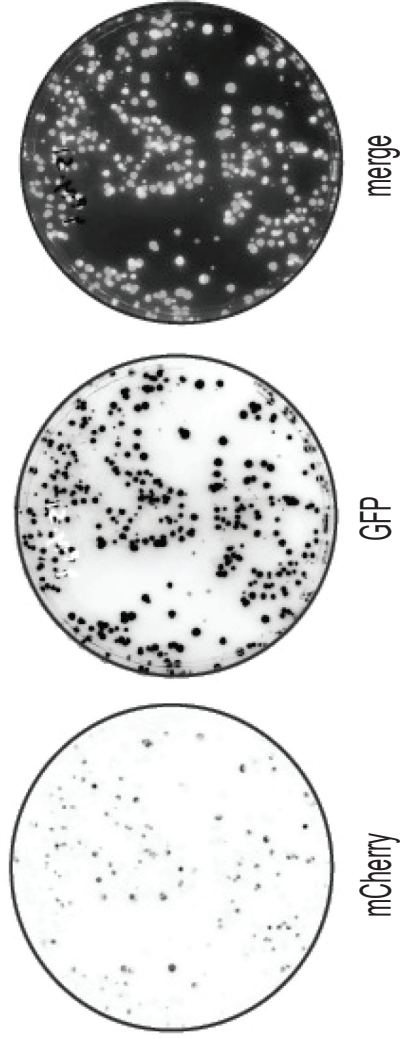


FIG. 45C

5'-Extended pegRNA stop codon correction (T•A to A•T)

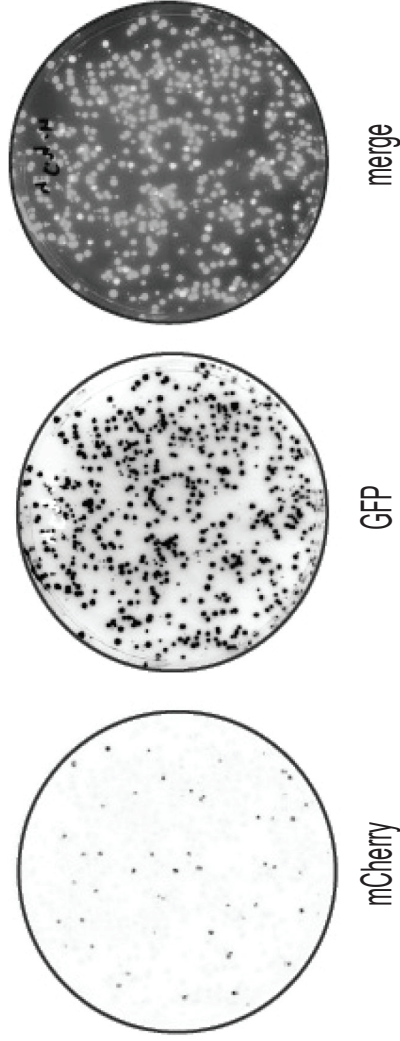


FIG. 45D

3'-Extended pegRNA +1 frameshift correction (1-bp deletion)

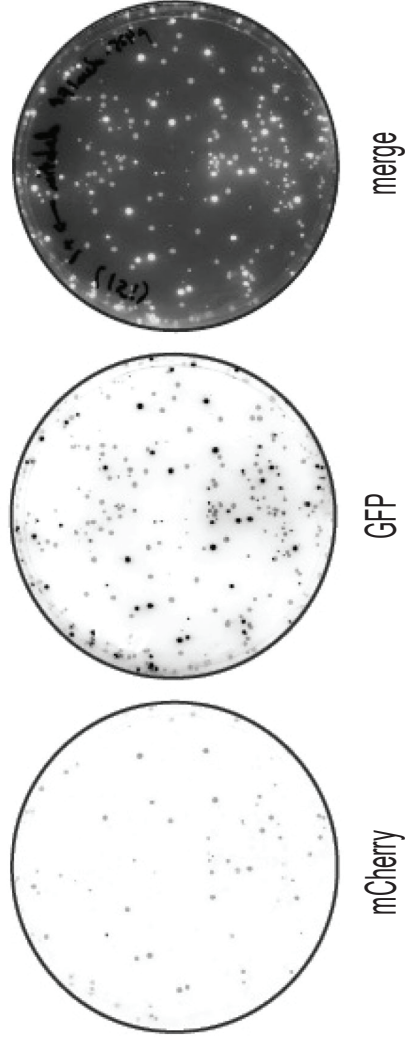


FIG. 45E

3'-Extended pegRNA -1 frameshift correction (1-bp insertion)

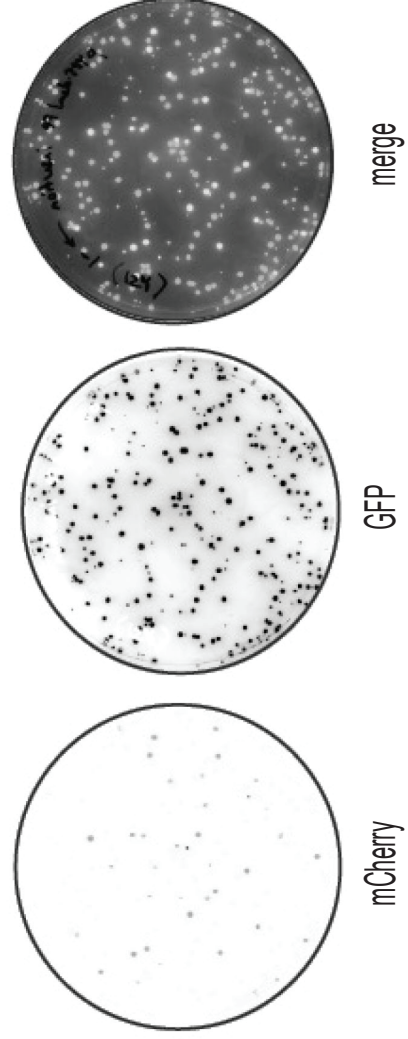


FIG. 45F

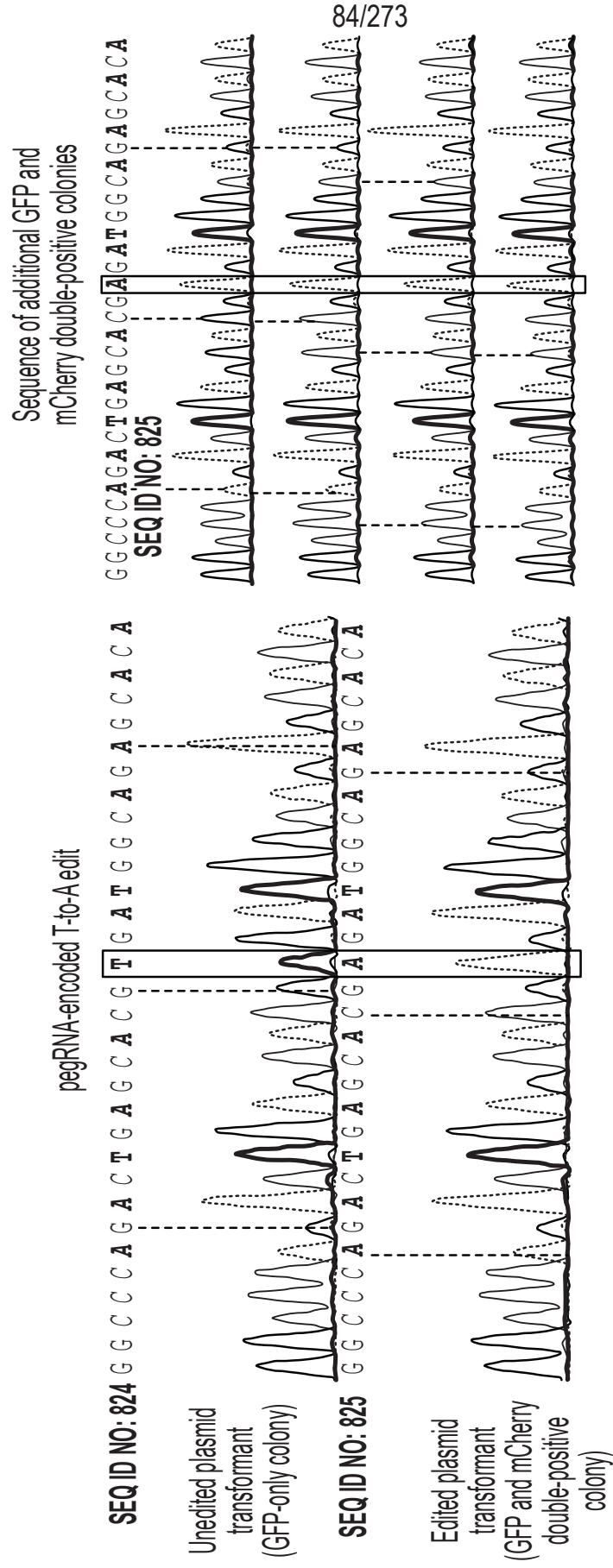


FIG. 45G

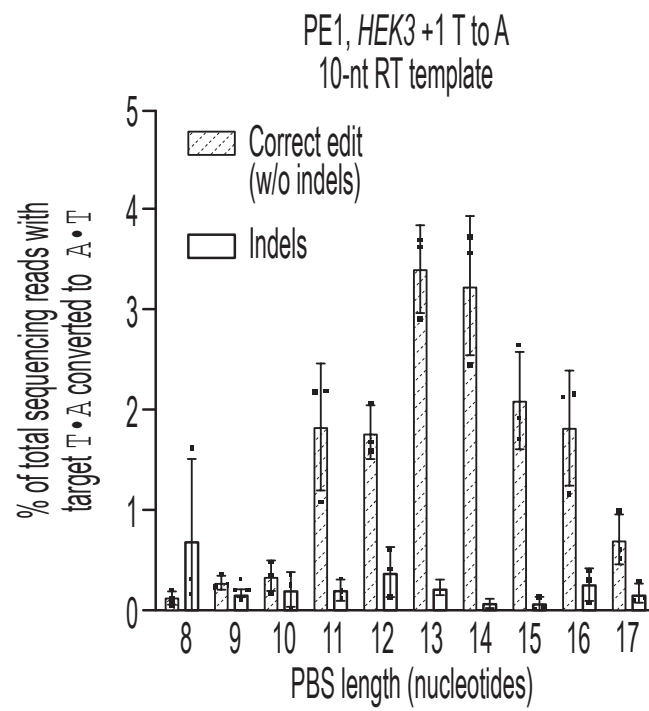


FIG. 46A

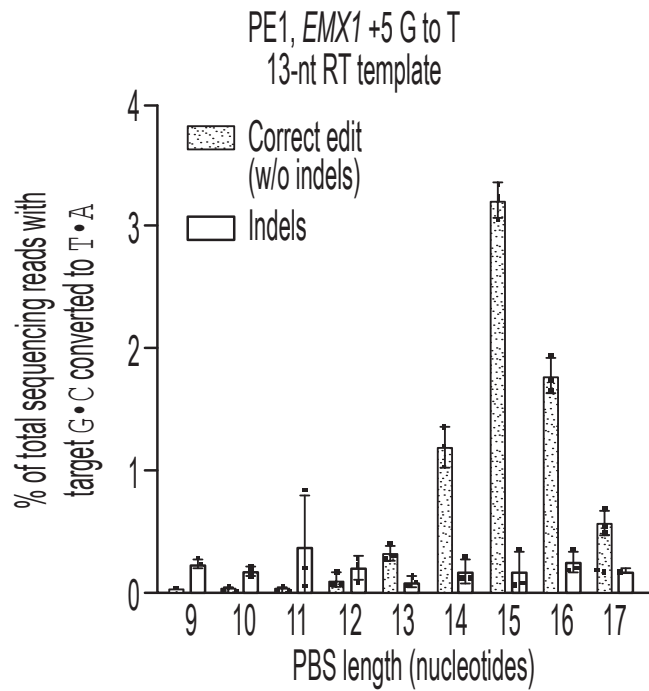


FIG. 46B

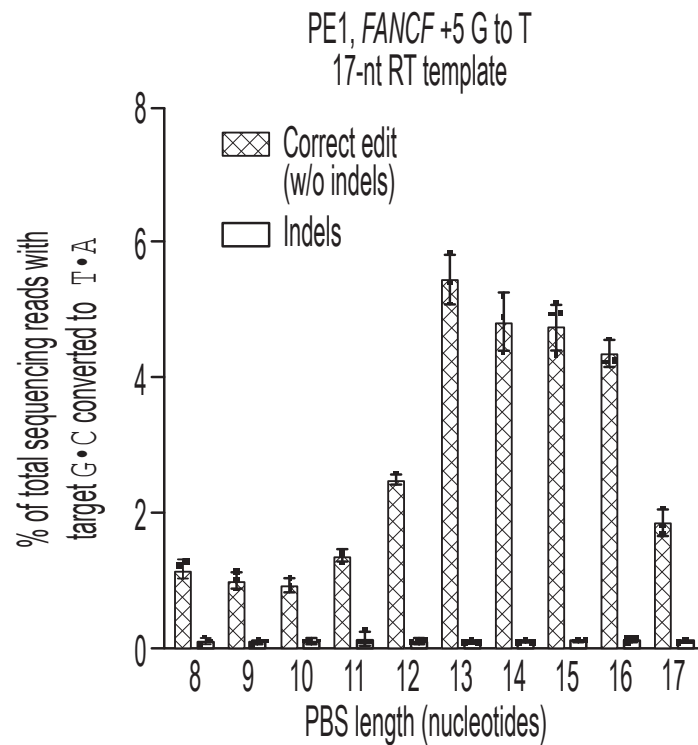


FIG. 46C

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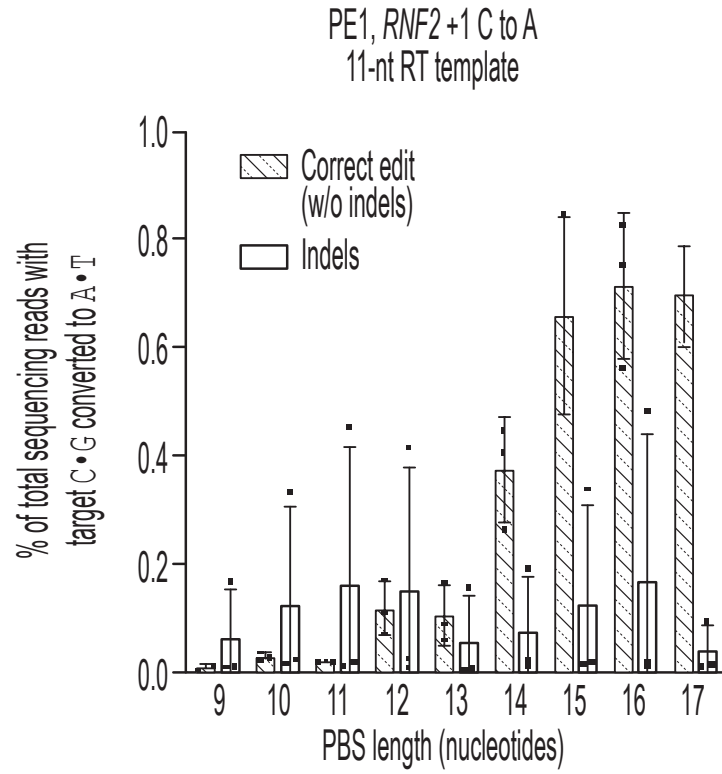


FIG. 46D

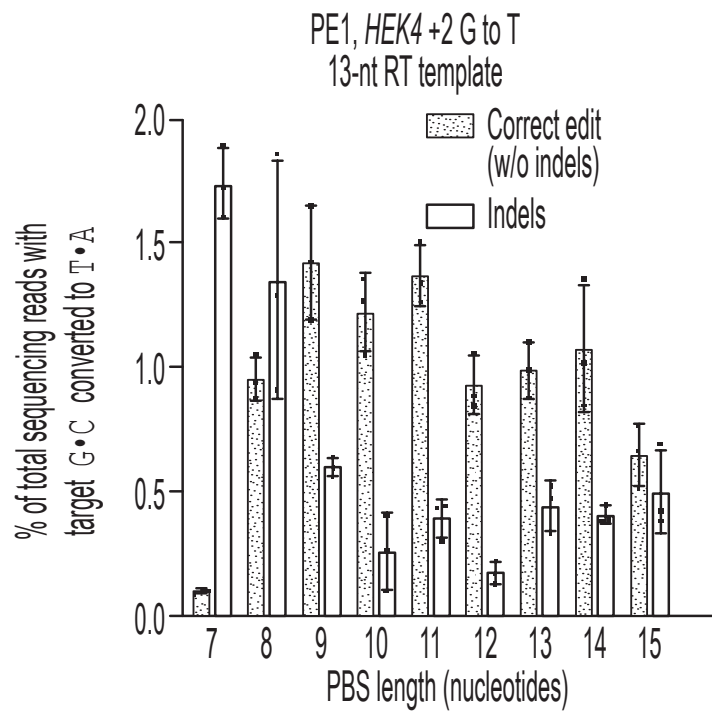


FIG. 46E

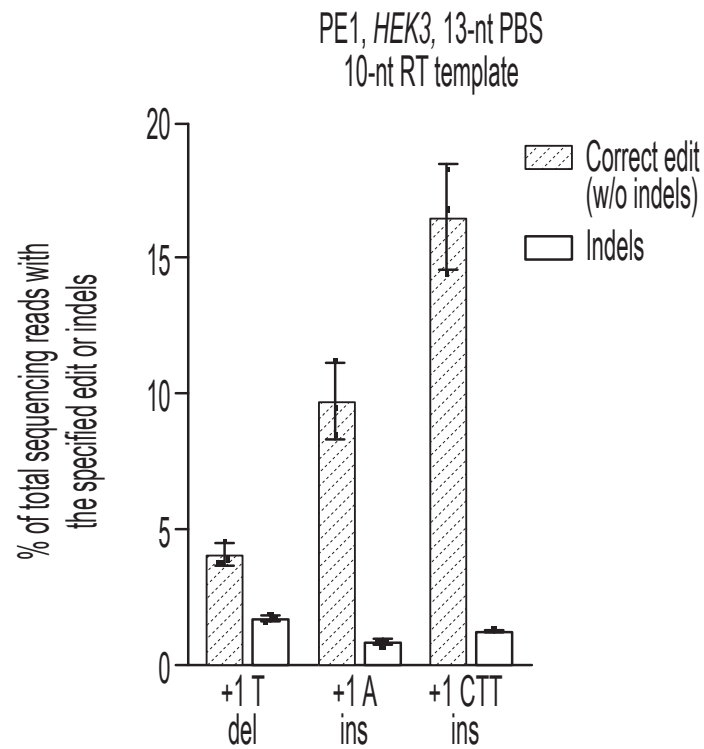


FIG. 46F

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

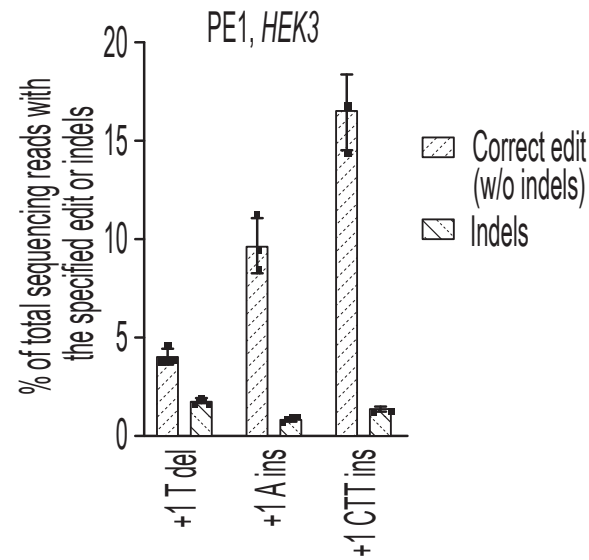


FIG. 47B

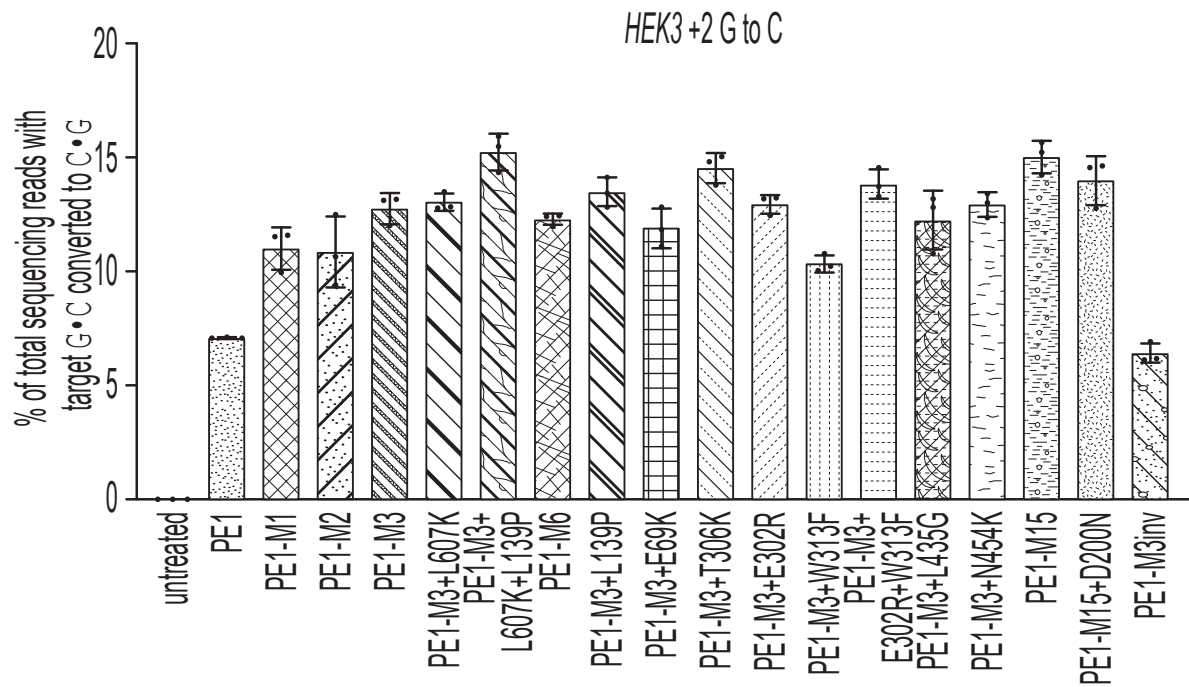


FIG. 47C

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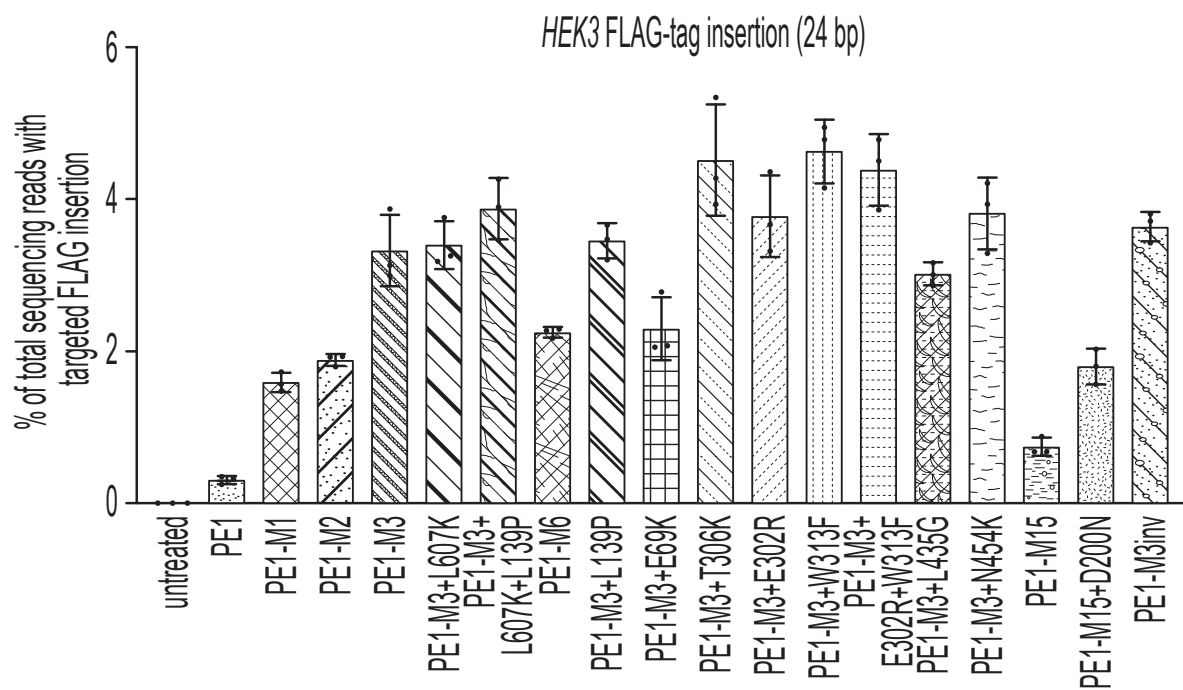


FIG. 47D

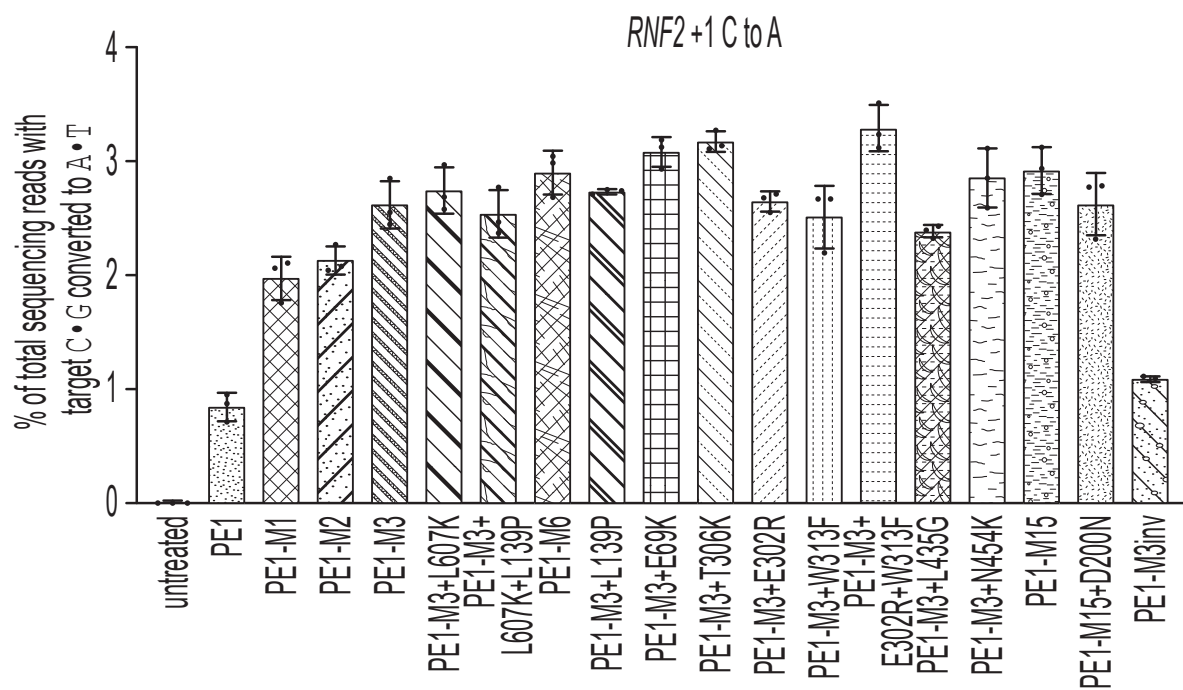


FIG. 47E

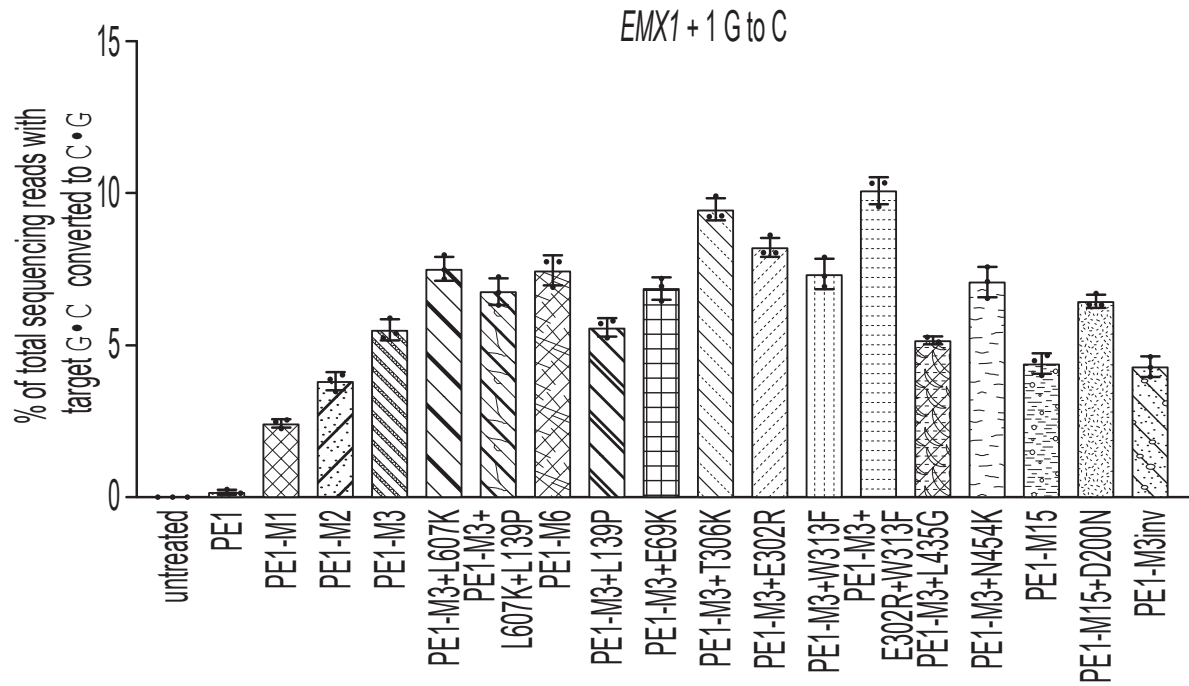


FIG. 47F

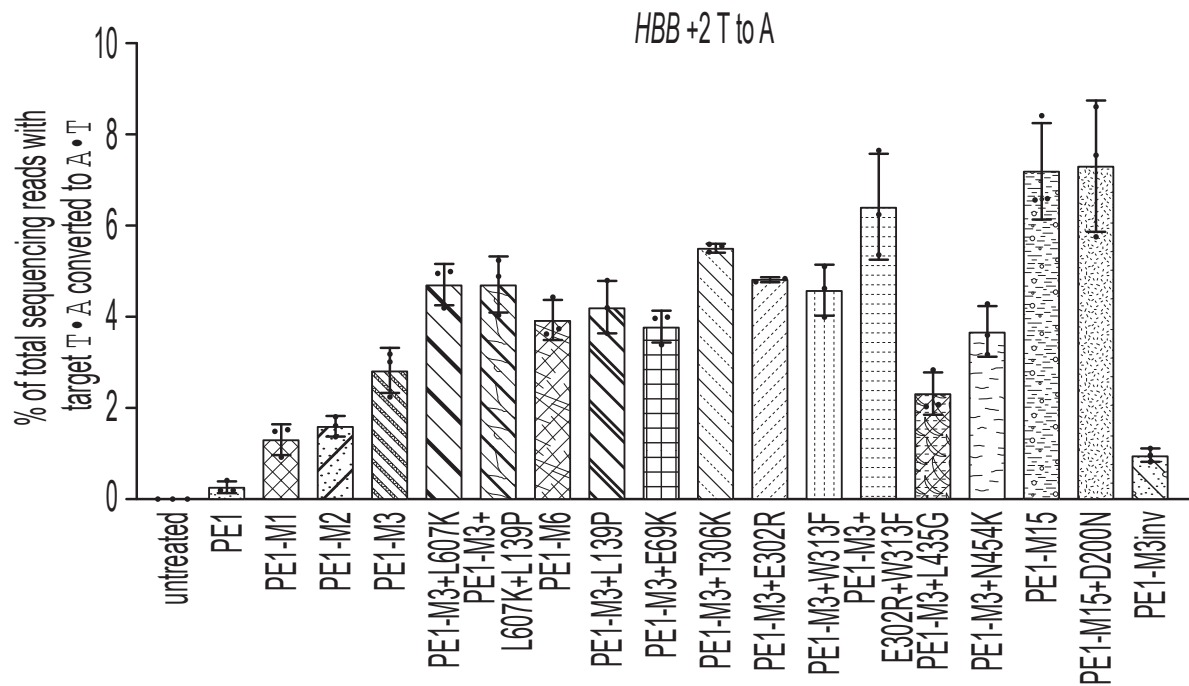


FIG. 47G

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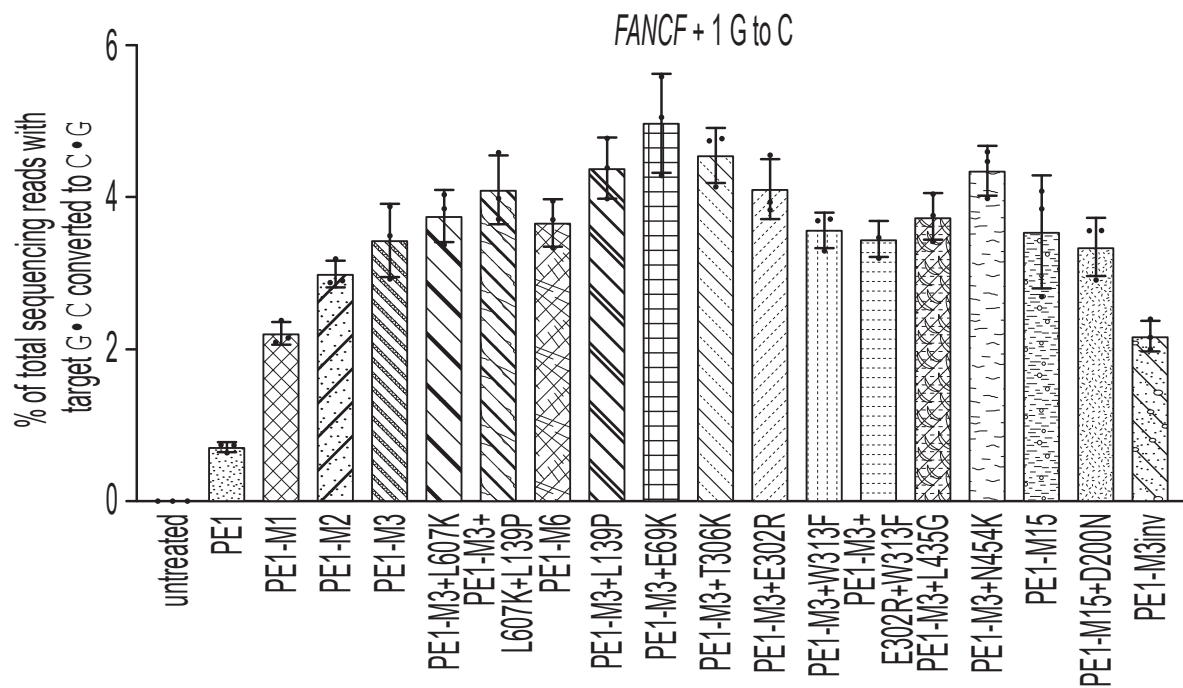


FIG. 47H

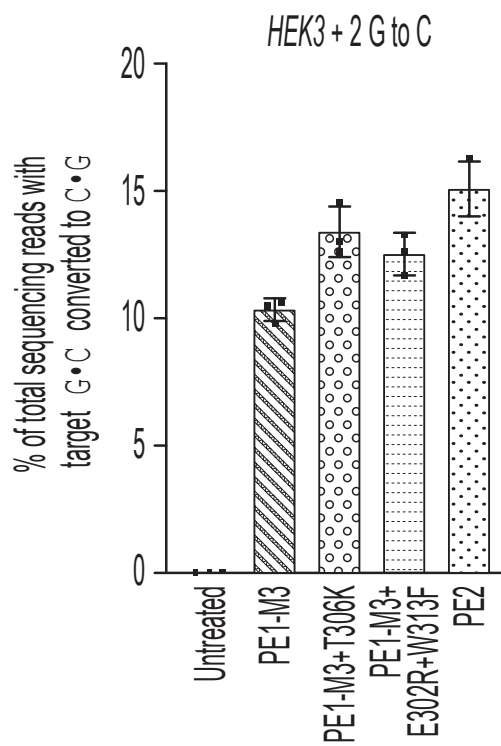


FIG. 47I

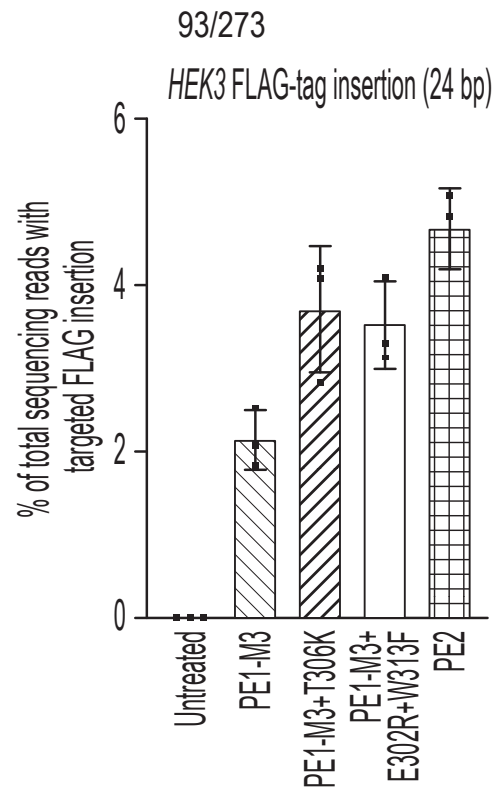


FIG. 47J

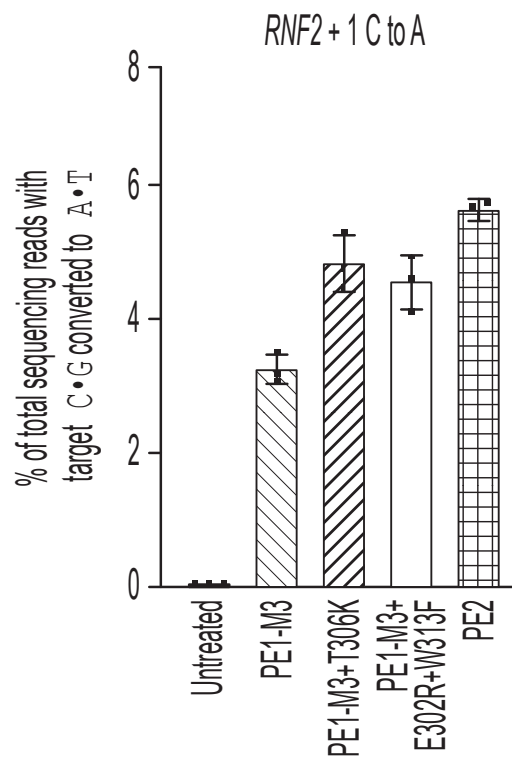


FIG. 47K

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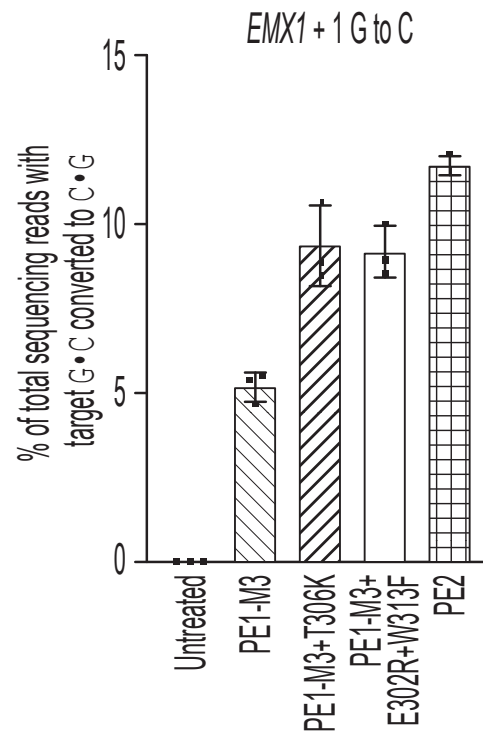


FIG. 47L

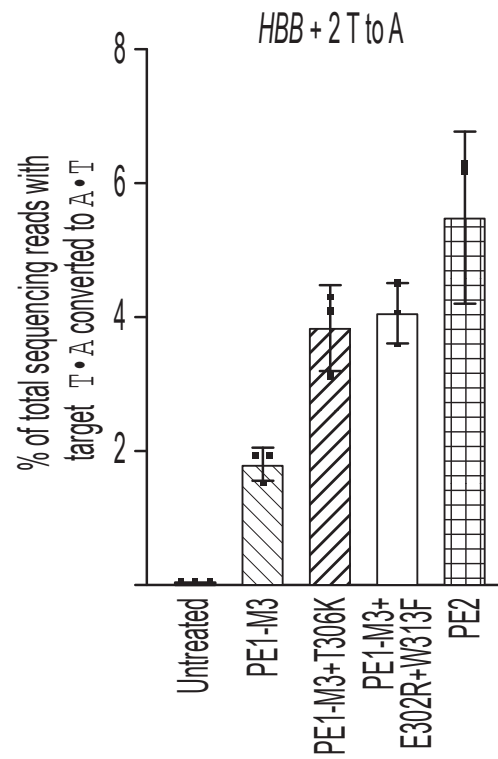


FIG. 47M

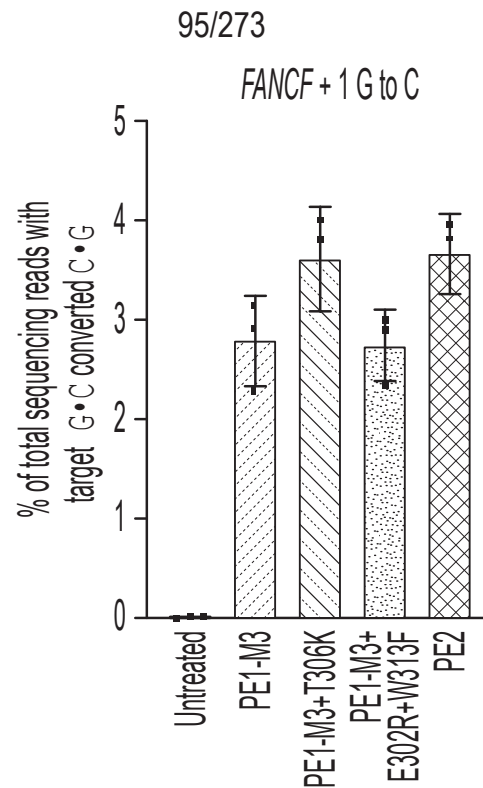


FIG. 47N

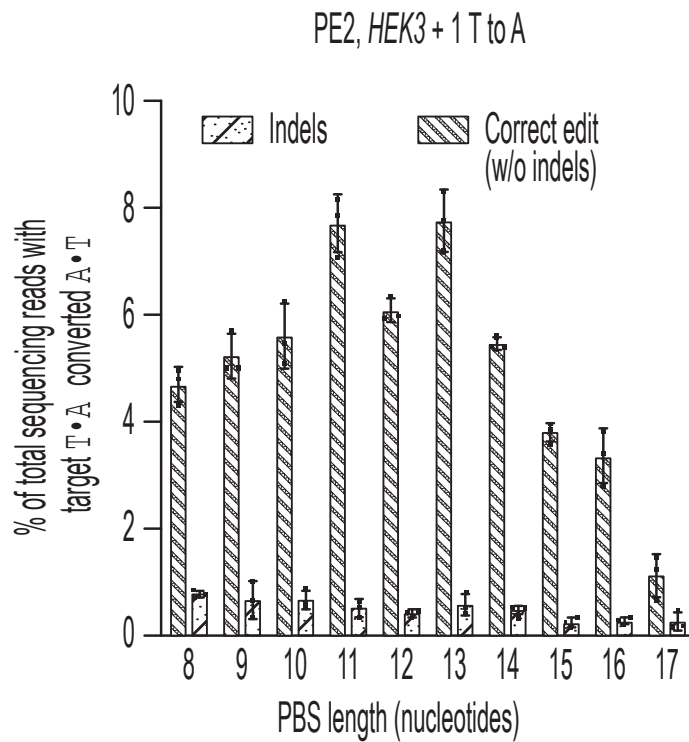


FIG. 47O

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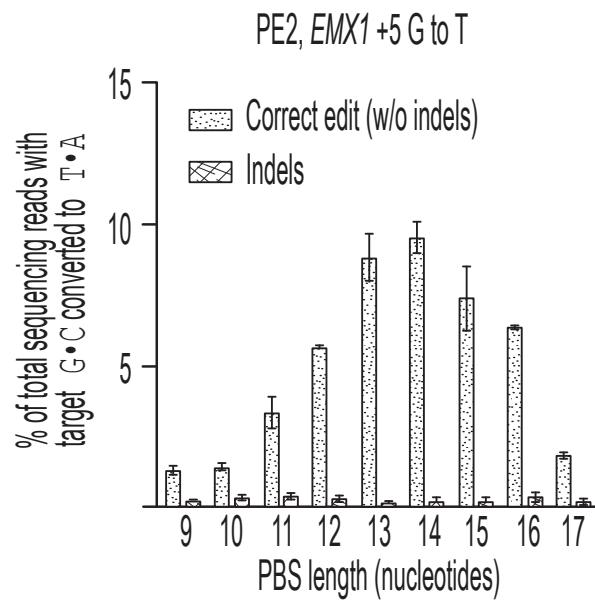


FIG. 47P

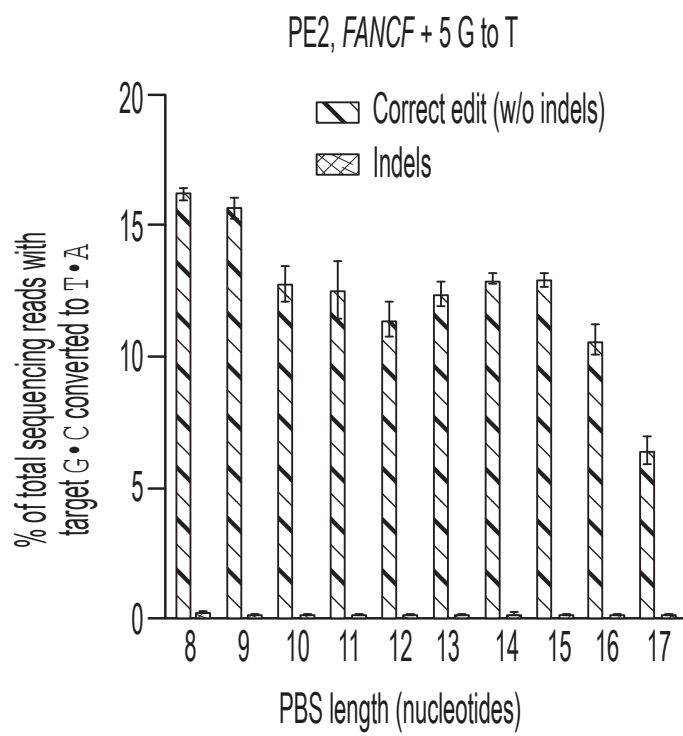


FIG. 47Q

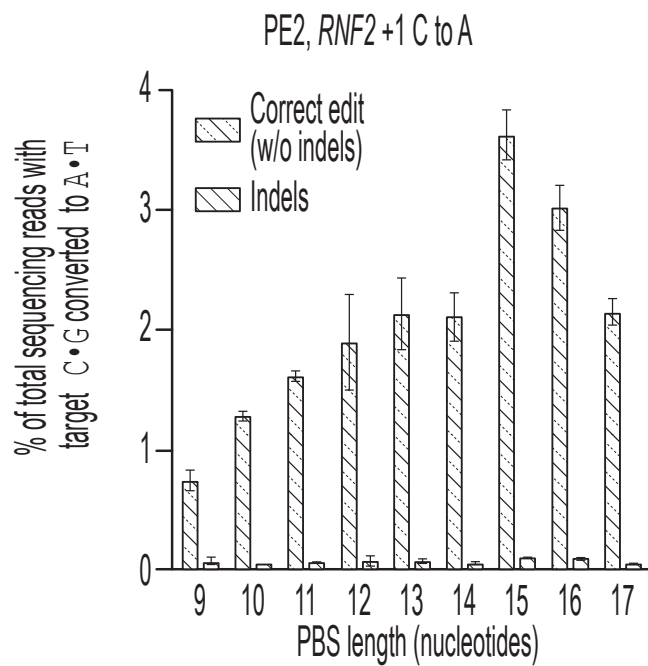


FIG. 47R

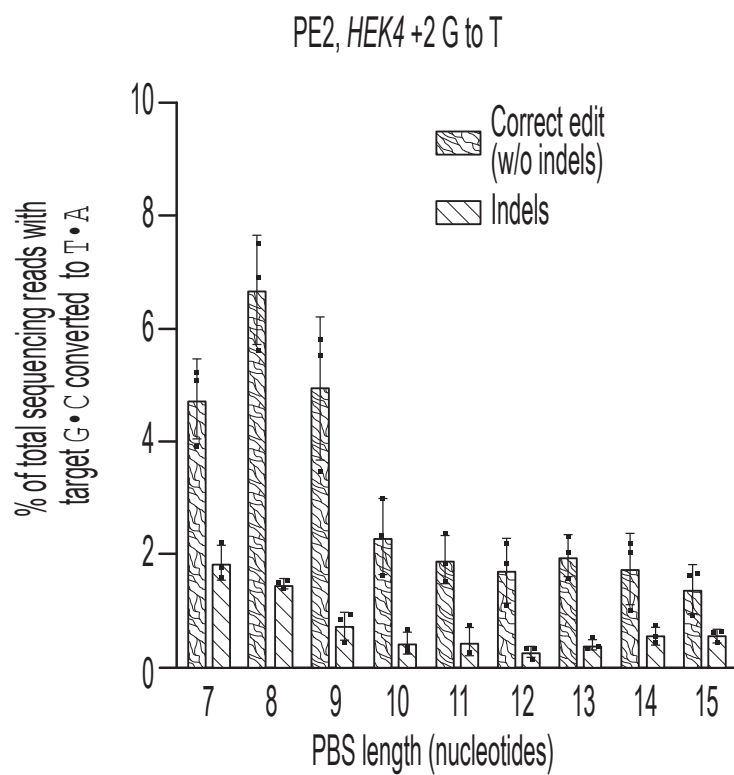


FIG. 47S

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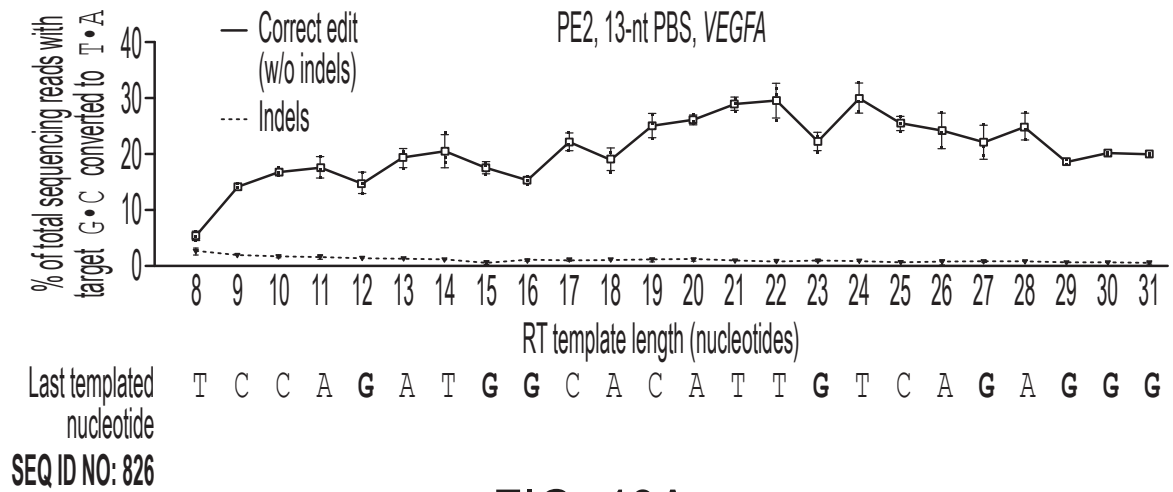


FIG. 48A

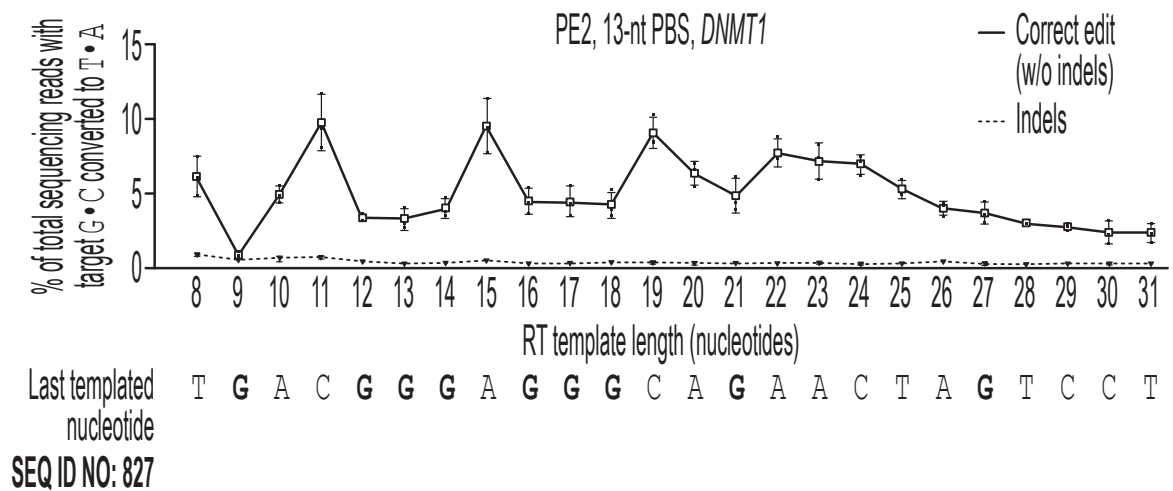


FIG. 48B

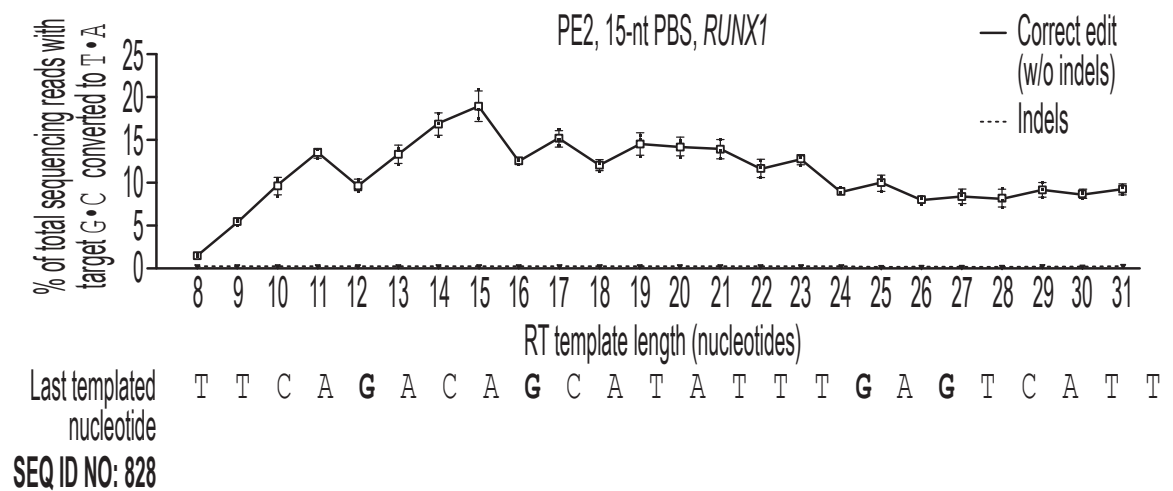


FIG. 48C

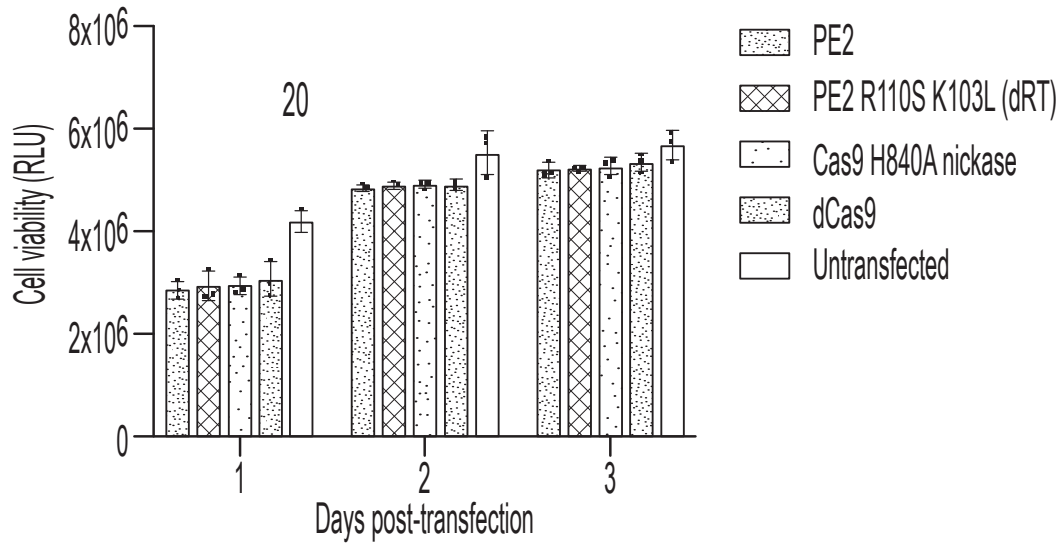


FIG. 49A

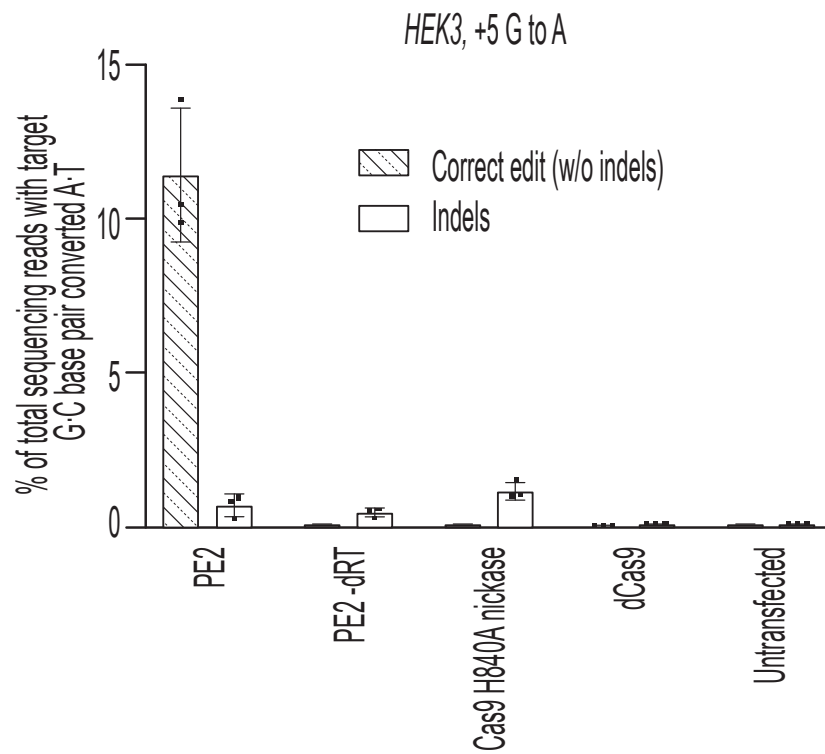


FIG. 49B

Correction of *HBB* E6V in HEK293T cells with PE3

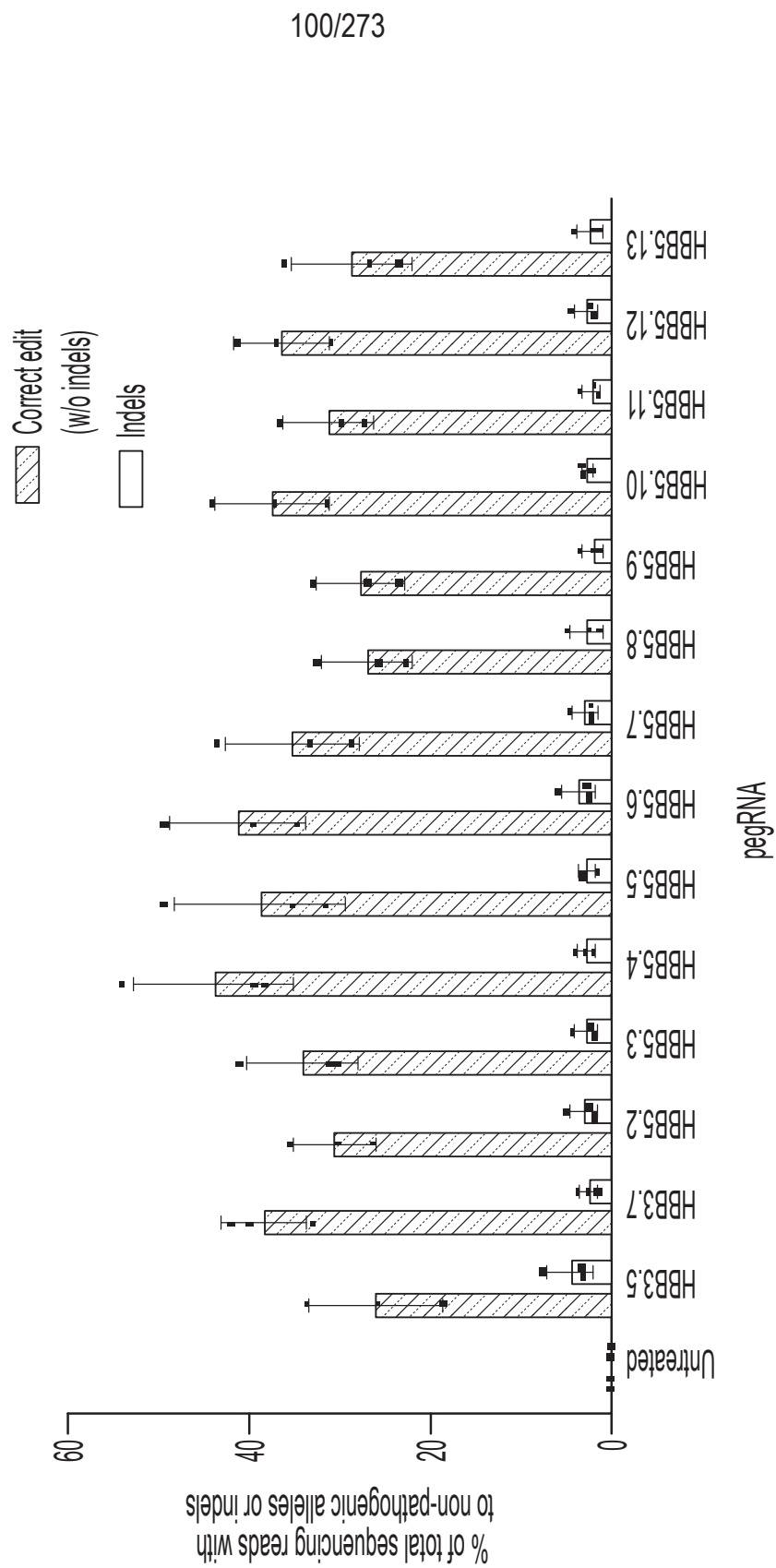


FIG. 50A

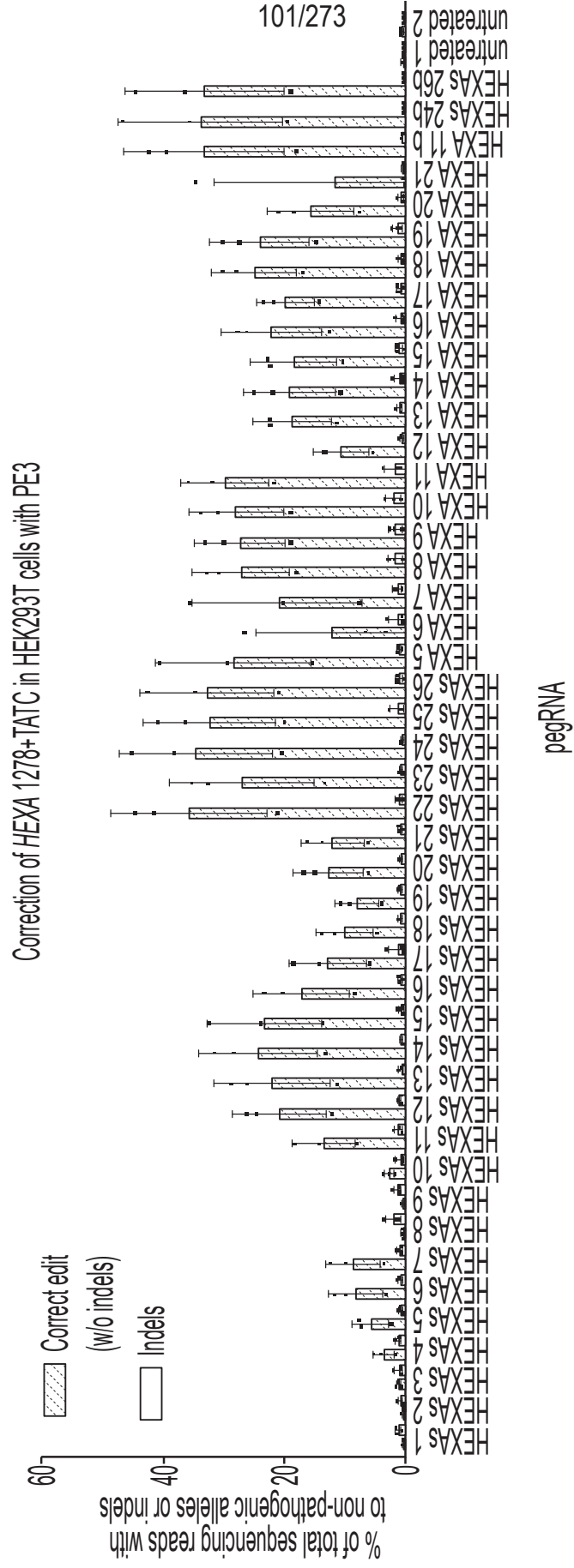


FIG. 50B

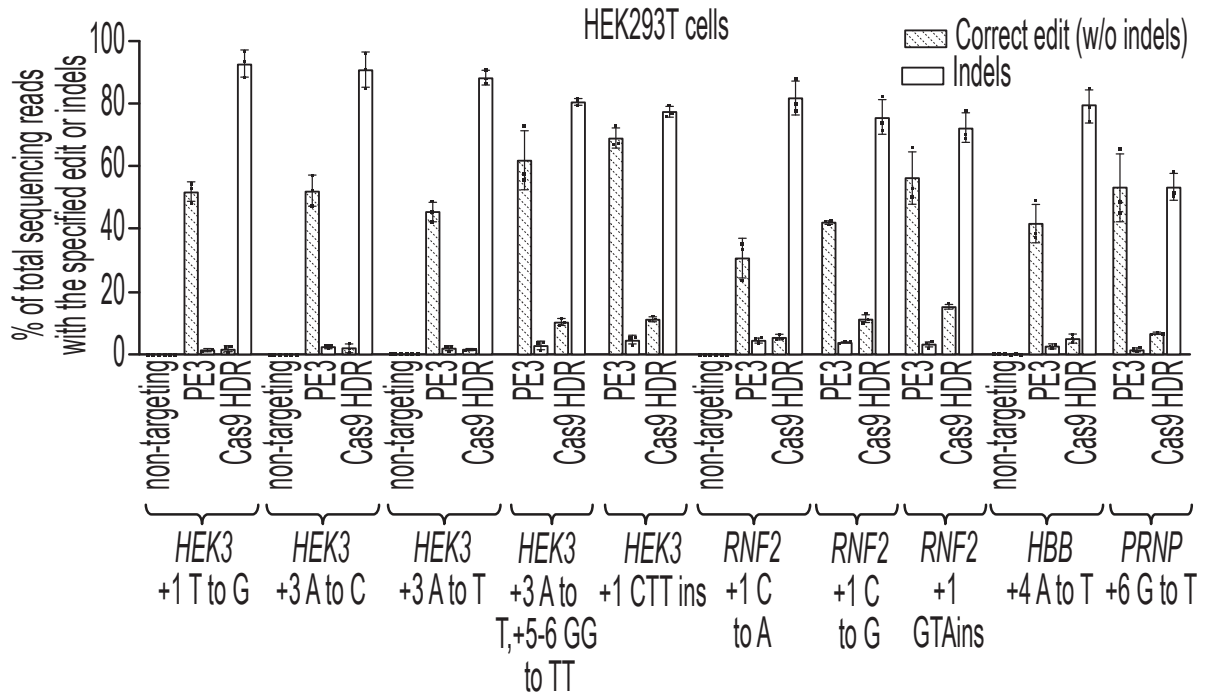


FIG. 51A

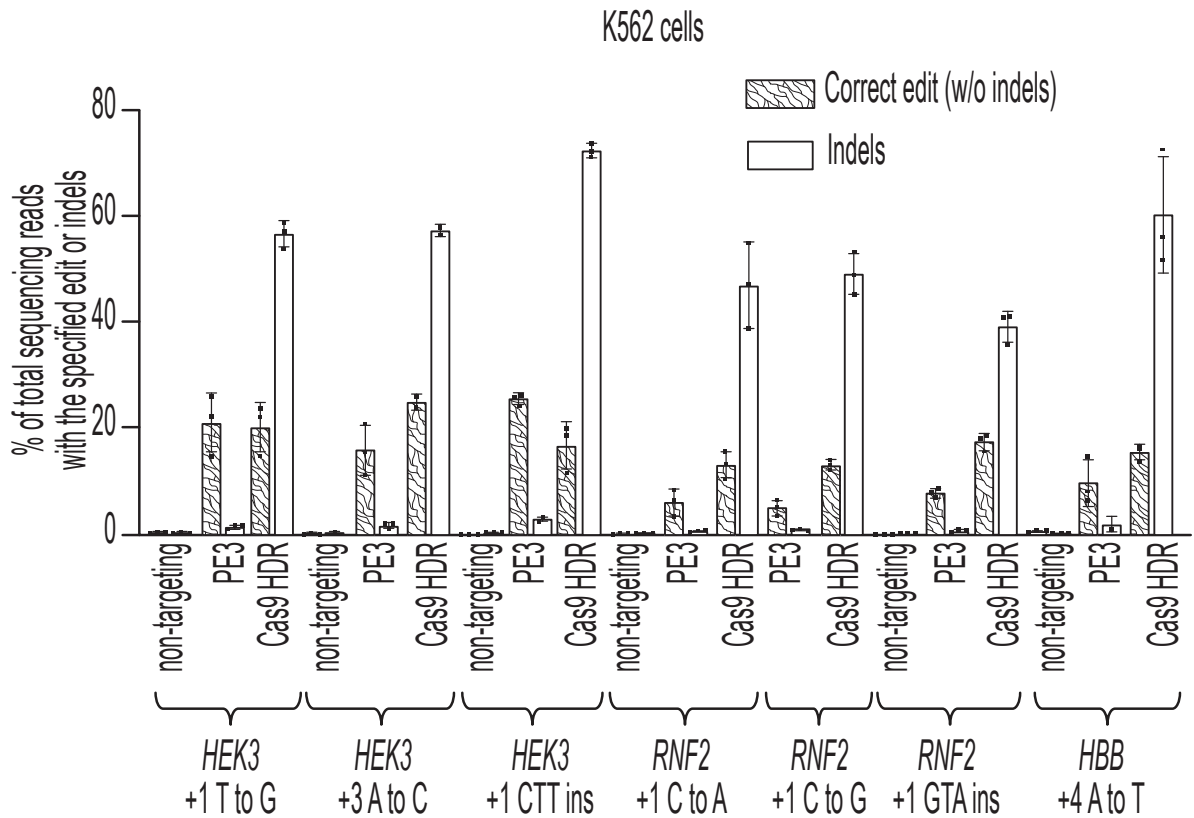


FIG. 51B

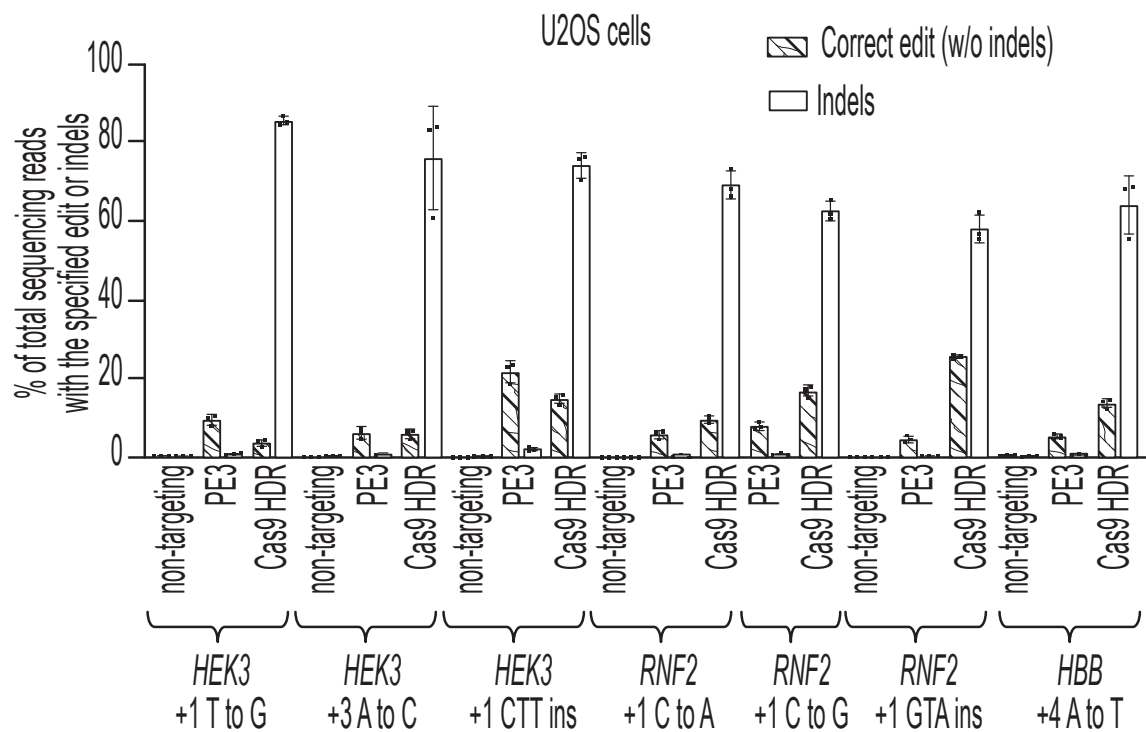


FIG. 51C

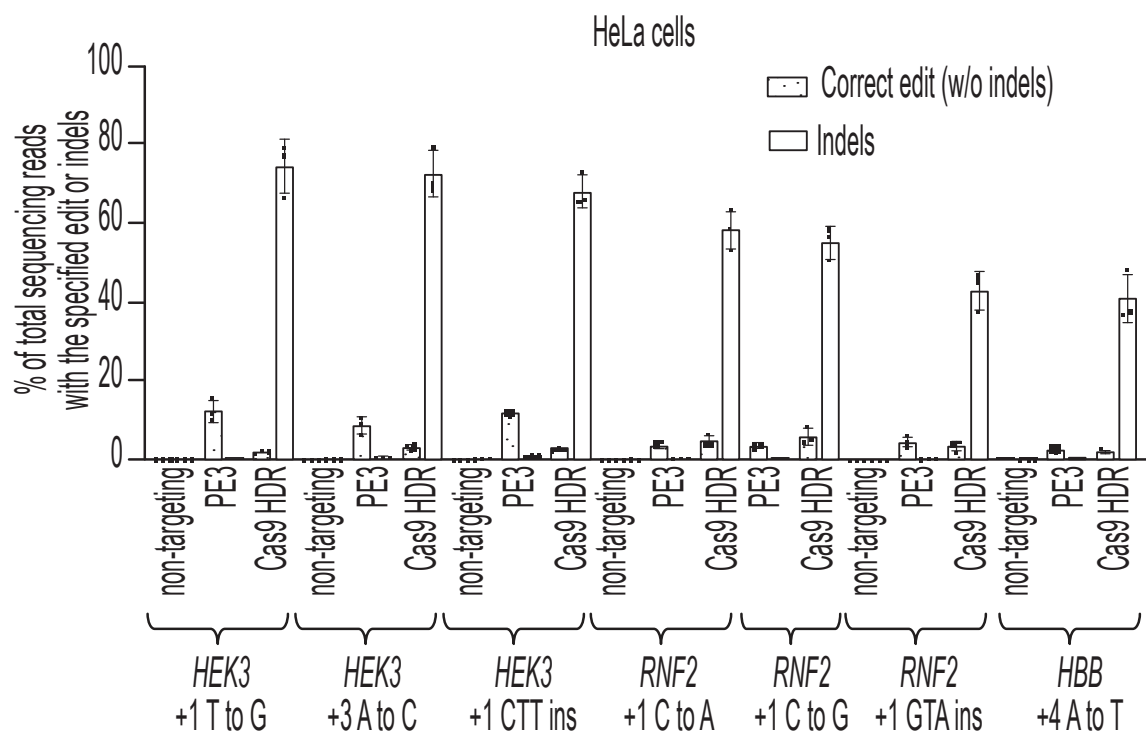


FIG. 51D

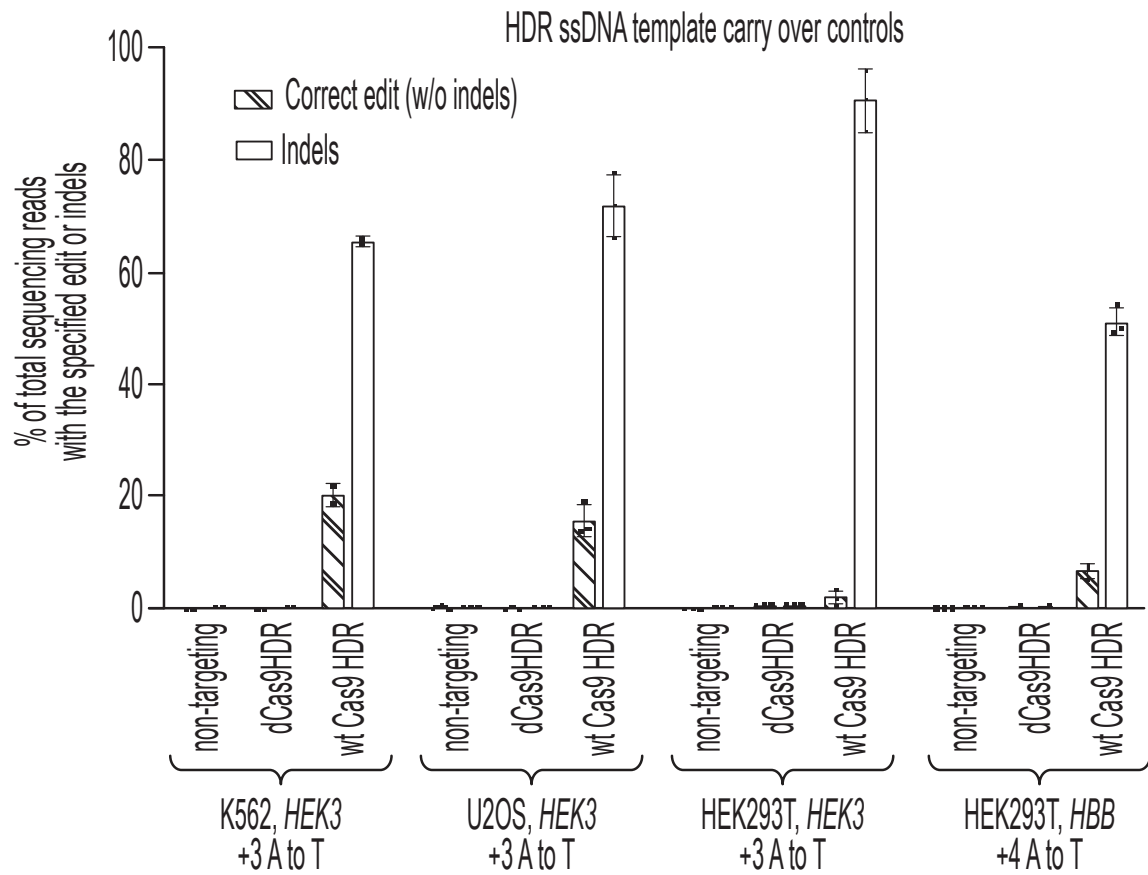


FIG. 51E

SEQ ID NO:

791 T G G G G C C C A G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G -Reference

K562 HEK3 negative control (non-targeting pegRNA)

791 T G G G G C C C A G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G -93.54% (47190 reads)

791 T G G G G C C C A G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G -0.47% (238 reads)

829 T G G G G C C C A G A C T G A G C A C G T G A T G G C A G C G G A A A G G A A G -0.21% (108 reads)

K562 HEK3 PE3+1 CTT insertion

791 T G G G G C C C A G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G -69.66% (39061 reads)

830 T G G G G C C C A G A C T G A G C A C G C T T T G A T G G C A G A G G A A A G G A A G -22.80% (12784 reads)

791 T G G G G C C C A G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G -0.32% (179 reads)

830 T G G G G C C C A G A C T G A G C A C G C T T T G A T G G C A G A G G A A A G G A A G -0.21% (120 reads)

831 T G G G G C C C A G A C T G A G C A C G C T T T T G A T G G C A G A G G A A A G G A A G -0.21% (116 reads)

K562 HEK3 Cas9-initiated HDR +1 CTT insertion

832 T G G G G C C C A G A C T G A G C A C - T G A T G G C A G A G G A A A G G A A G -17.15% (7256 reads)

791 T G G G G C C C A G A C T G A G C A C G T G A T G G C A G A G G A A A G G A A G -13.86% (5864 reads)

830 T G G G G C C C A G A C T G A G C A C G C T T T G A T G G C A G A G G A A A G G A A G -10.55% (4461 reads)

833 T G G G G C C C A G A C T G A G C A - - T G A T G G C A G A G G A A A G G A A G -9.72% (4111 reads)

834 T G G G G C C C A G A C T G A G C A C G - - A T G G C A G A G G A A A G G A A G -5.14% (2174 reads)

835 T G G G G C C C A G A C T G A G C A C G G T G A T G G C A G A G G A A A G G A A -4.77% (2016 reads)

836 T G G G G C C C A G A C T G A G C A C G - G A T G G C A G A G G A A A G G A A G -3.82% (1616 reads)

837 T G G G G C C C A G A C T G A G C A C G T T G A T G G C A G A G G A A A G G A A -3.17% (1342 reads)

838 T G G G G C C C A G A C T G A - - - - - T G G C A G A G G A A A G G A A G -1.92% (814 reads)

839 T G G G G C C C A G A C T G A G C A C G A T G A T G G C A G A G G A A A G G A A -1.63% (689 reads)

840 T G G G G C C C A G A C T G A G C A C G - - - - - C A G A G G A A A G G A A G -1.26% (535 reads)

841 T G G G G C - - - - - T G A T G G C A G A G G A A A G G A A G -0.78% (329 reads)

842 T G G G G C C C A G A C T G A G C A - - - - - G A G G A A A G G A A G -0.61% (257 reads)

843 T G G G G C C C A G A C T G A G C A C G C T G A T G G C A G A G G A A A G G A A -0.59% (250 reads)

844 T G G G G C - - - - - T G A T G G C A G A G G A A A G G A A G -0.56% (237 reads)

FIG. 51F

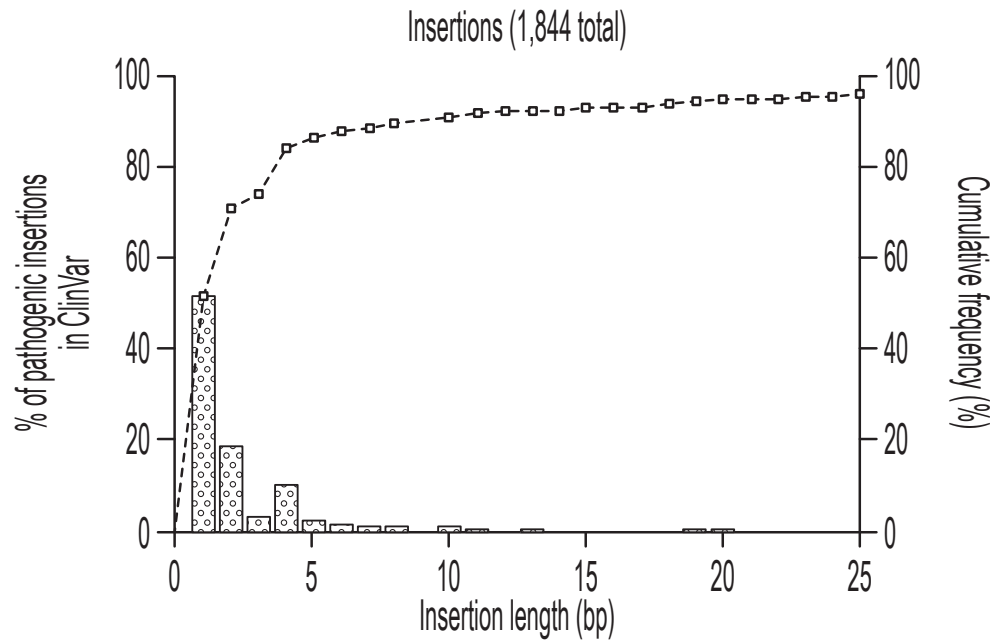


FIG. 52A

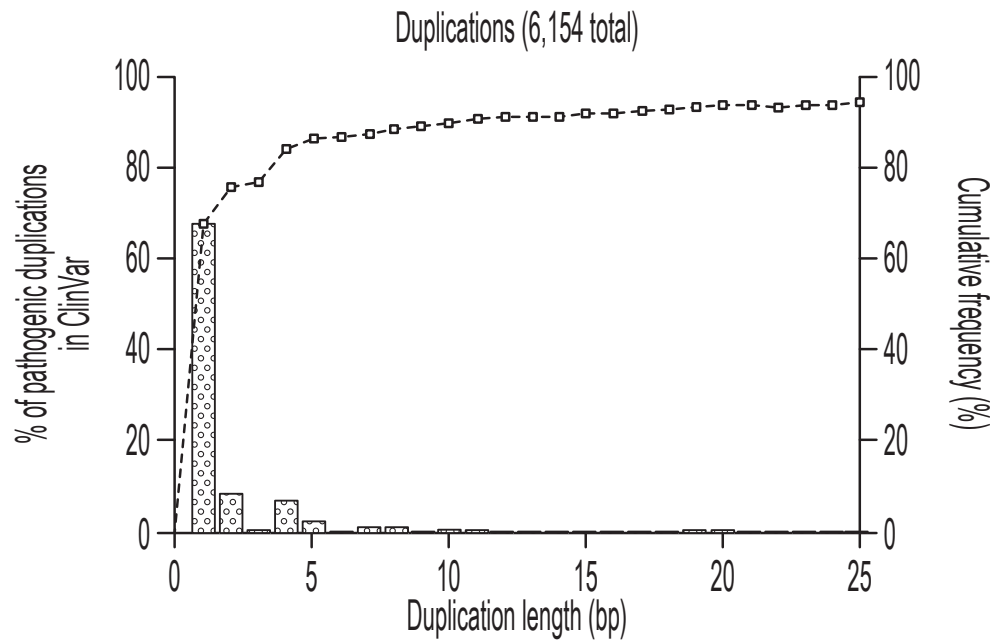


FIG. 52B

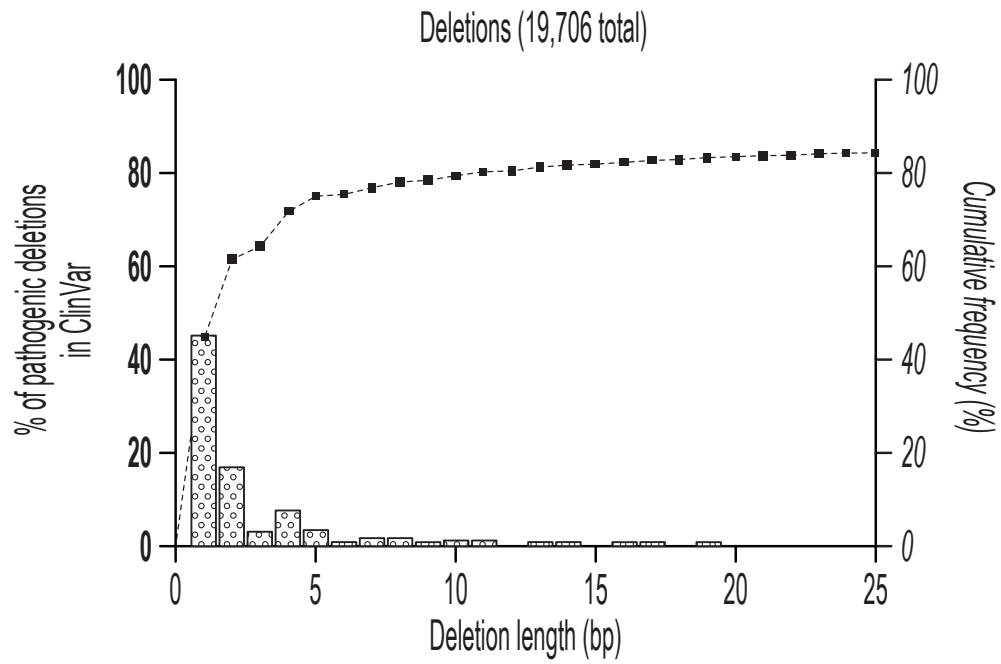


FIG. 52C

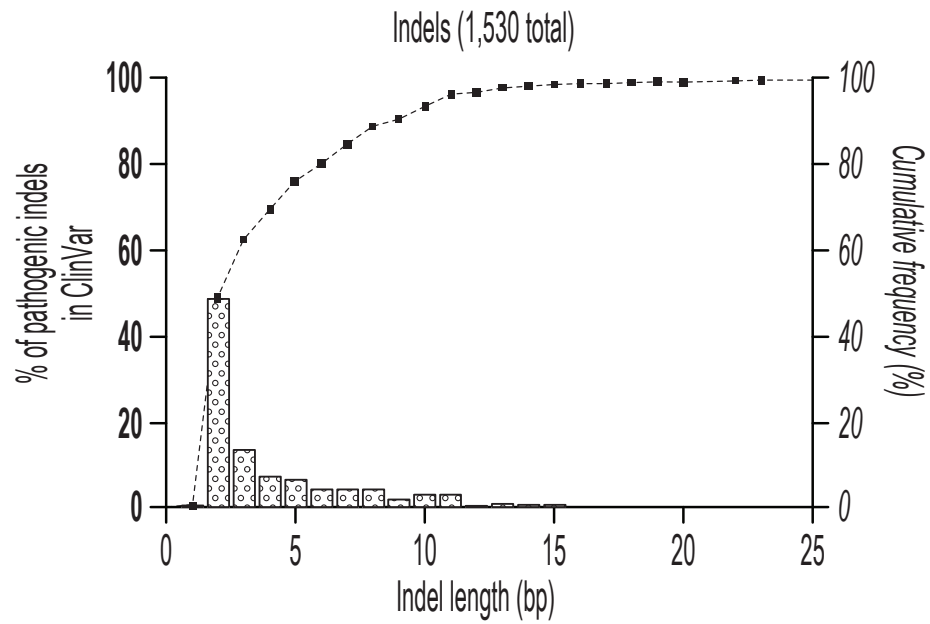


FIG. 52D

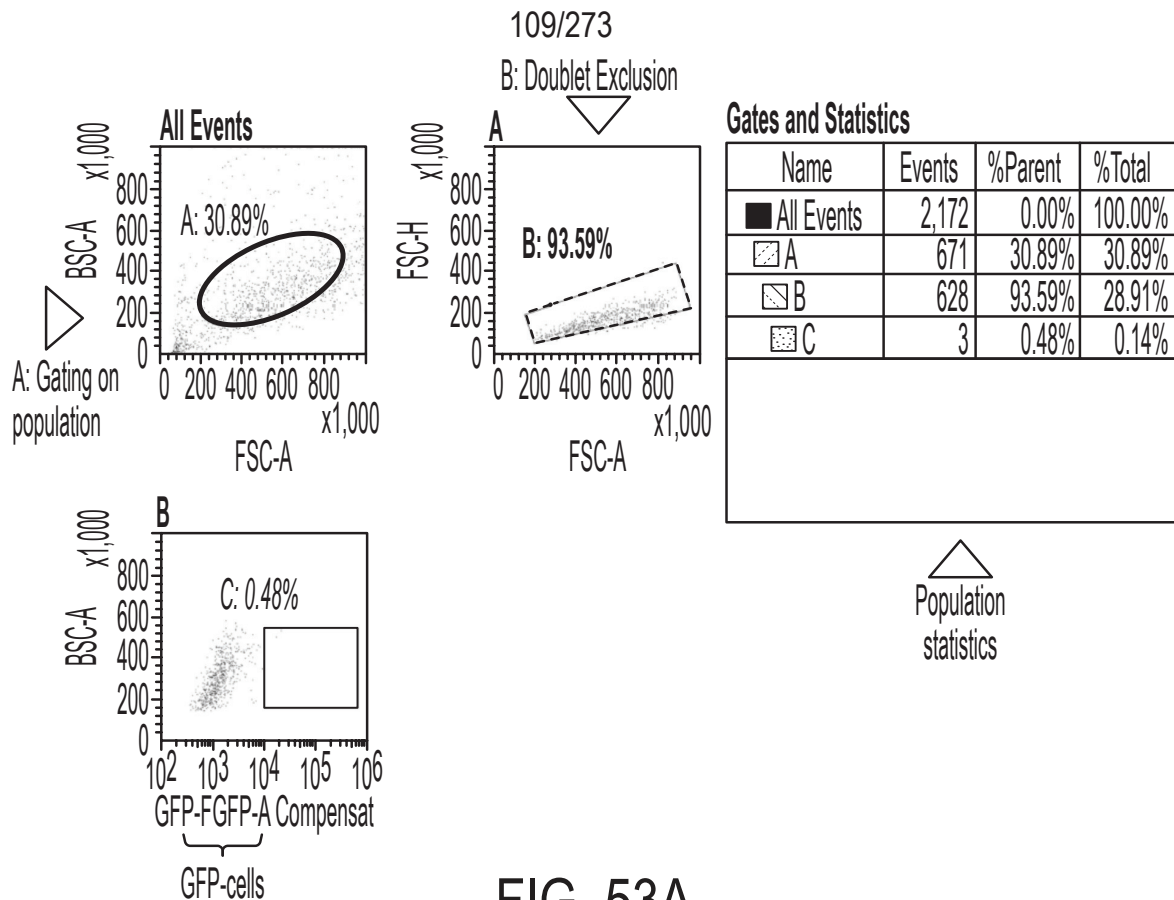


FIG. 53A

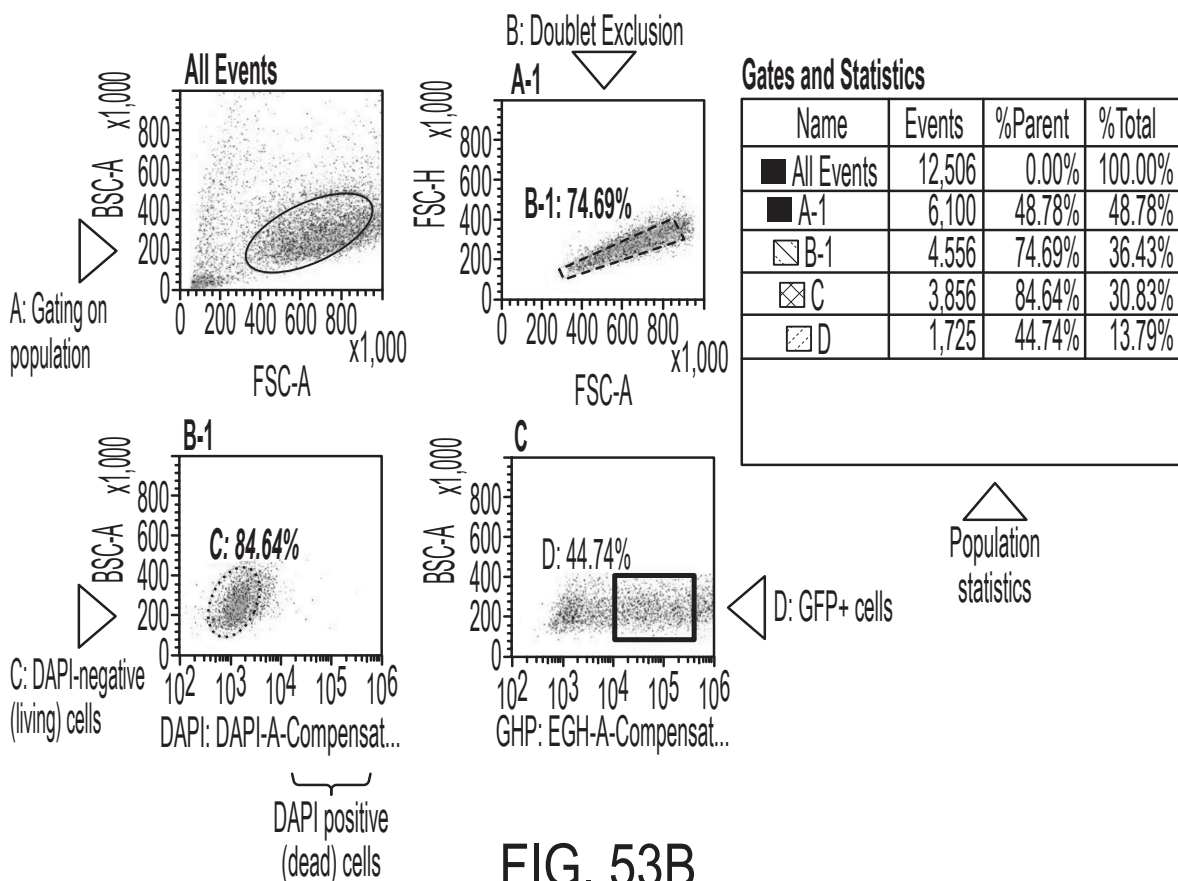


FIG. 53B

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	T	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	G	T	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	C	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	C	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	T	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	G	T	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	C	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	C	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	C	T	G	G	C	C	T	G	A	C	T	G	G	A	A	G	G	C	T	T	T	C	-0.23% (47 reads)

bold Substitutions

Insertions

- Deletions

---- Predicted cleavage position

FIG. 53C

Line 1
SEQ ID NO:

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	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	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	A	G	T	C	T	G	C	C	G	T	T	-0.27% (240 reads)
877	C	A	C	C	A	T	G	G	C	G	C	A	C	C	T	G	A	C	T	C	C	T	G	T	G	G	A	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	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	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	A	G	T	C	T	G	C	C	G	T	T	-0.36% (239 reads)
877	C	A	C	C	A	T	G	G	C	G	C	A	C	C	T	G	A	C	T	C	C	T	G	T	G	G	A	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	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	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	A	G	T	C	T	G	C	C	G	T	T	-0.29% (228 reads)
877	C	A	C	C	A	T	G	G	C	G	C	A	C	C	T	G	A	C	T	C	C	T	G	T	G	G	A	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	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	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	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	A	G	T	C	T	G	C	C	G	T	T	-0.58% (419 reads)
877	C	A	C	C	A	T	G	G	C	G	C	A	C	C	T	G	A	C	T	C	C	T	G	T	G	G	A	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	A	G	T	C	T	G	T	C	G	T	T	-0.21% (150 reads)

FIG. 53D

Line 5

875	C	A	C	C	A	T	G	G	T	G	C	A	C	C	T	G	A	C	T	C	T	G	A	G	G	A	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	T	G	T	G	G	A	G	A	G	T	C	T	G	C	C	G	T	T	-97.16% (54265 reads)
875	C	A	C	C	A	T	G	G	T	G	C	A	C	C	T	G	A	C	T	C	T	G	A	G	G	A	G	A	G	T	C	T	G	C	C	G	T	T	-0.33% (183 reads)
877	C	A	C	C	A	T	G	G	C	G	C	A	C	C	T	G	A	C	T	C	T	G	T	G	G	A	G	A	G	T	C	T	G	C	C	G	T	T	-0.21% (120 reads)
878	C	A	C	C	A	T	G	G	T	G	C	A	C	C	T	G	A	C	T	C	T	G	T	G	G	A	G	A	G	T	C	T	G	T	C	G	T	T	-0.20% (113 reads)

Line 6

875	C	A	C	C	A	T	G	G	T	G	C	A	C	C	T	G	A	C	T	C	T	G	A	G	G	A	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	T	G	T	G	G	A	G	A	G	T	C	T	G	C	C	G	T	T	-97.35% (95642 reads)
875	C	A	C	C	A	T	G	G	T	G	C	A	C	C	T	G	A	C	T	C	T	G	A	G	G	A	G	A	G	T	C	T	G	C	C	G	T	T	-0.27% (267 reads)
877	C	A	C	C	A	T	G	G	C	G	C	A	C	C	T	G	A	C	T	C	T	G	T	G	G	A	G	A	G	T	C	T	G	C	C	G	T	T	-0.21% (211 reads)

bold Substitutions

□ Insertions

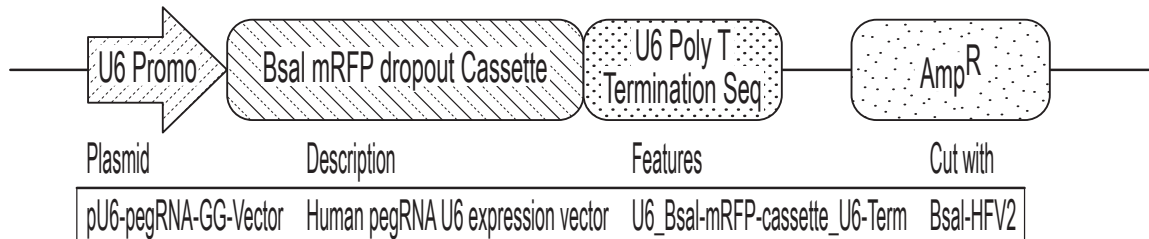
- Deletions

---- Predicted cleavage position

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' - CACCNNNNNNNNNNNNNNNNNGTTT - 3'

SEQ ID NO: 880 3' - NNNNNNNNNNNNNNNNNNCAAATCTC - 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' - AGAGCTAGAAATAGCAAGTTAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCG - 3' SEQ ID NO: 881
SEQ ID NO: 882 3' - GATCTTTATCGTTCAATTTTATTCGATCAGGCAATAGTTGAACCTTTTCACCGTGGCTCAGCCACG - 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:

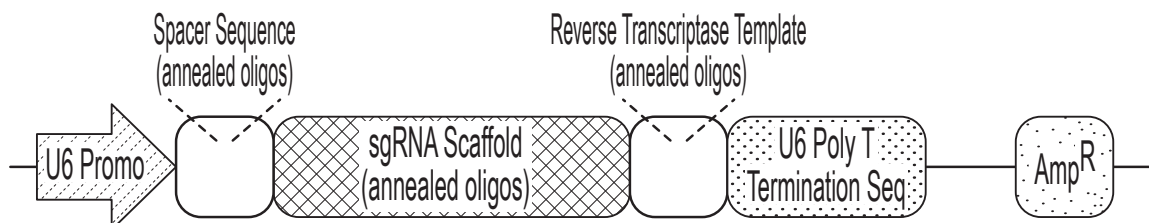


FIG. 54

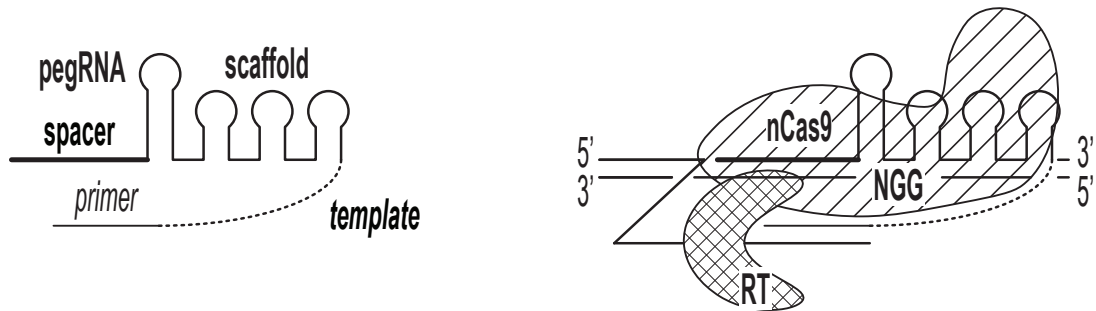


FIG. 55A

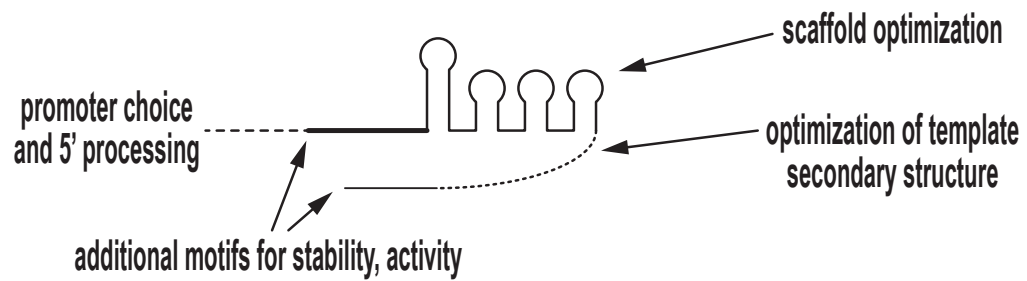


FIG. 55B

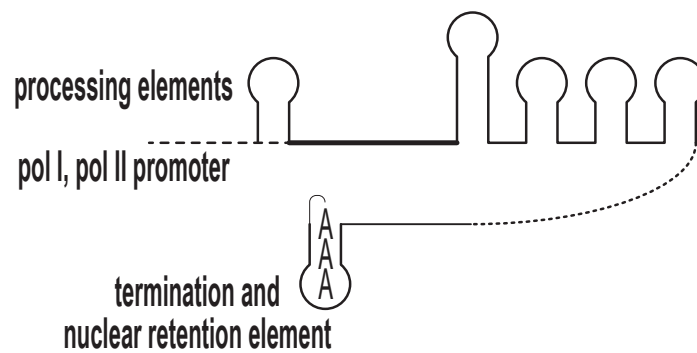


FIG. 55C

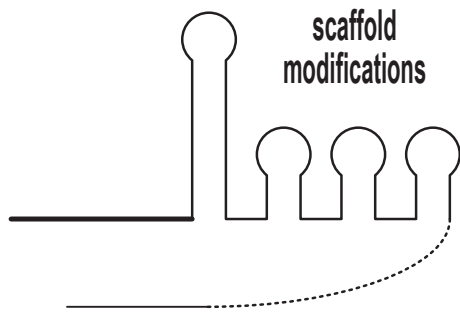


FIG. 55D

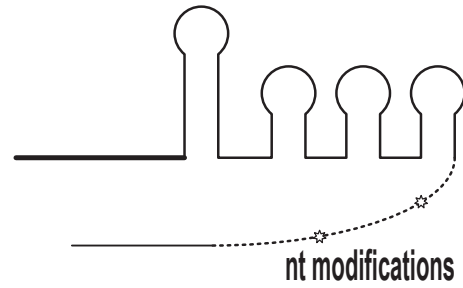


FIG. 55E

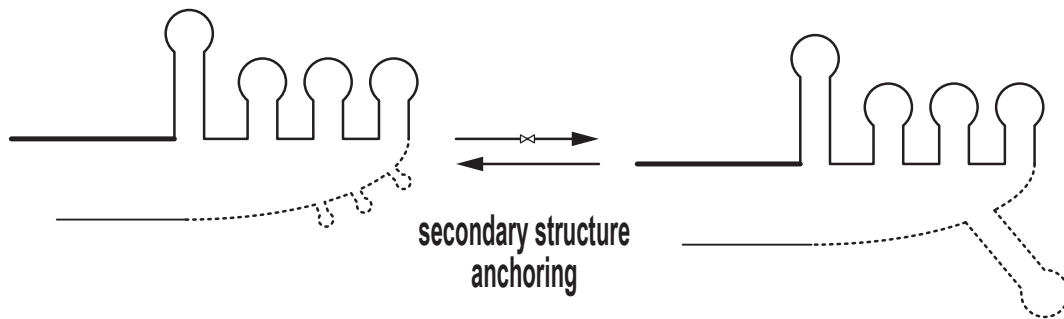


FIG. 55F

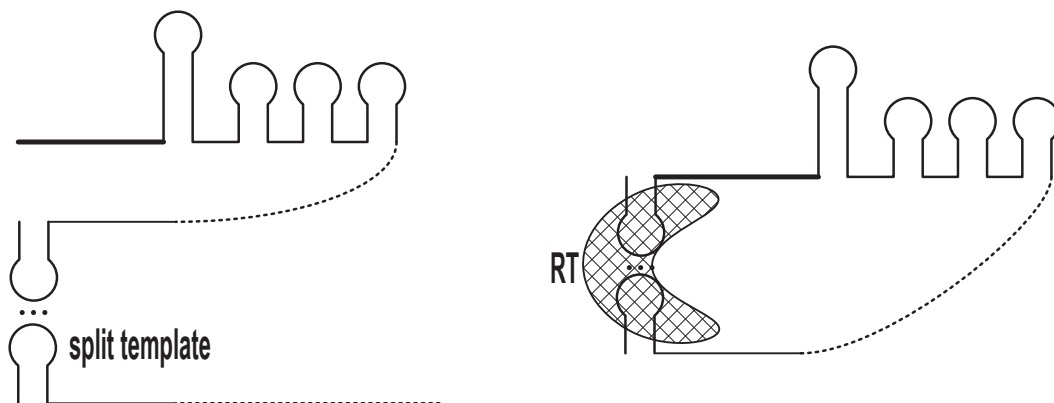


FIG. 55G

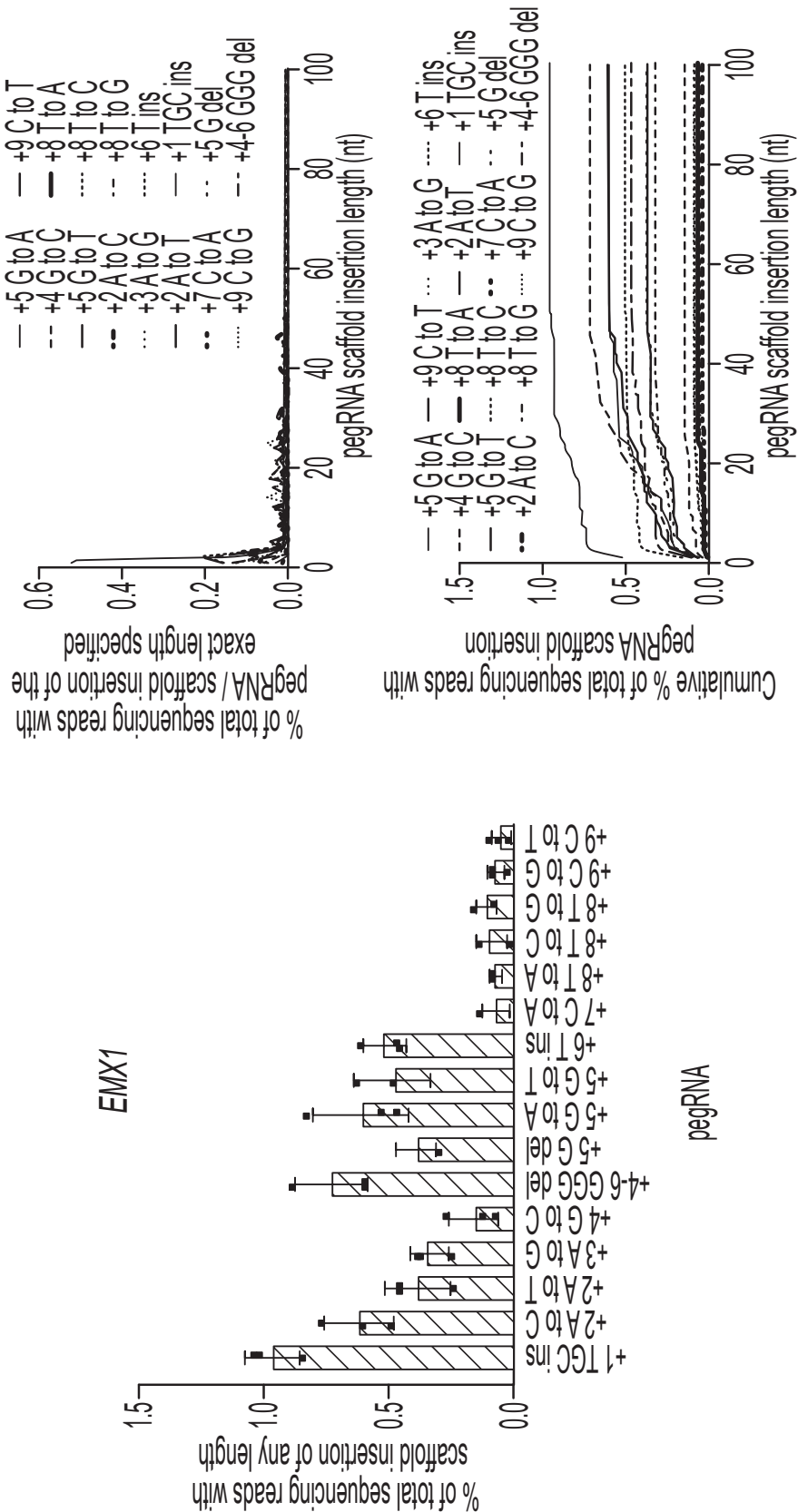


FIG. 56A



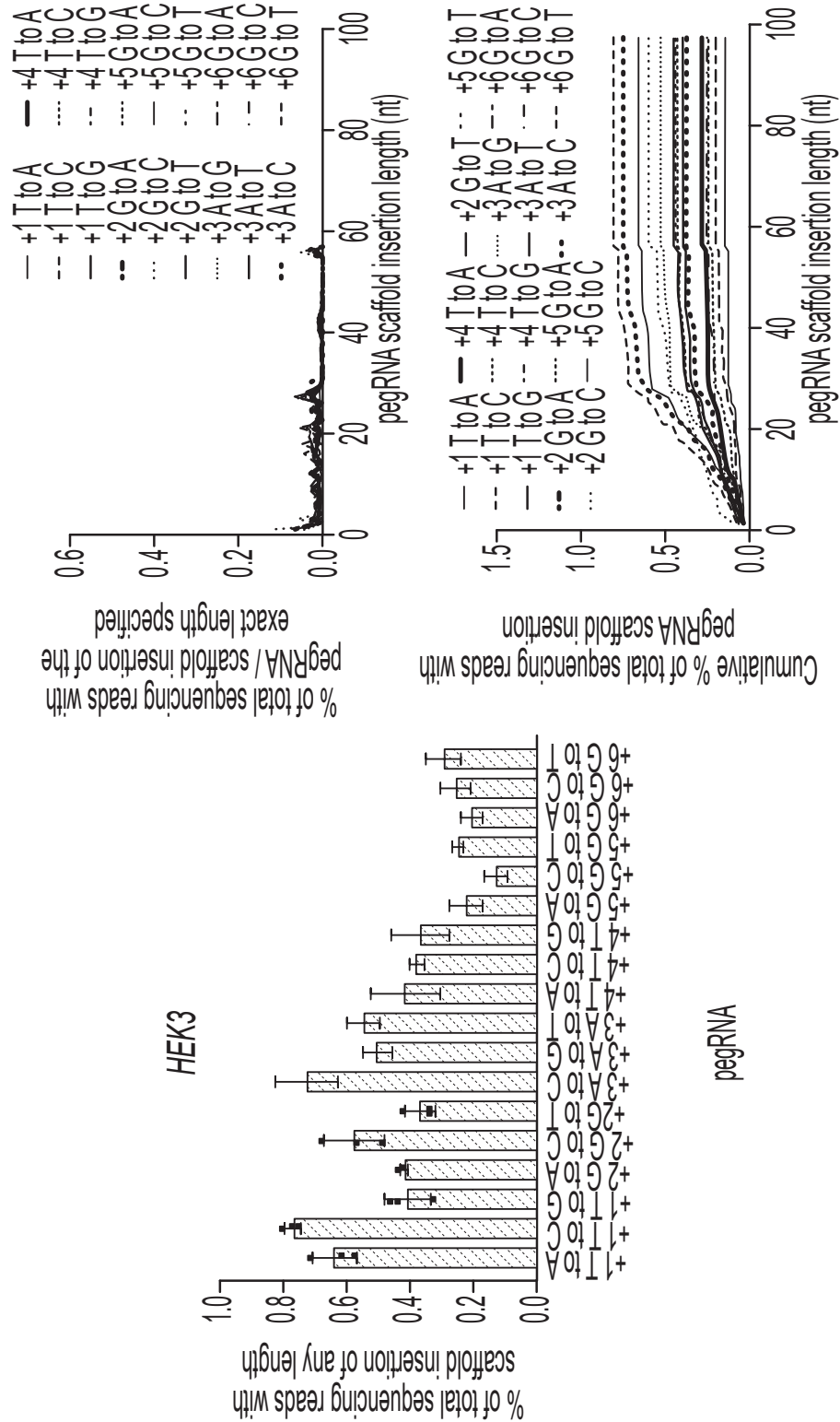


FIG. 56C

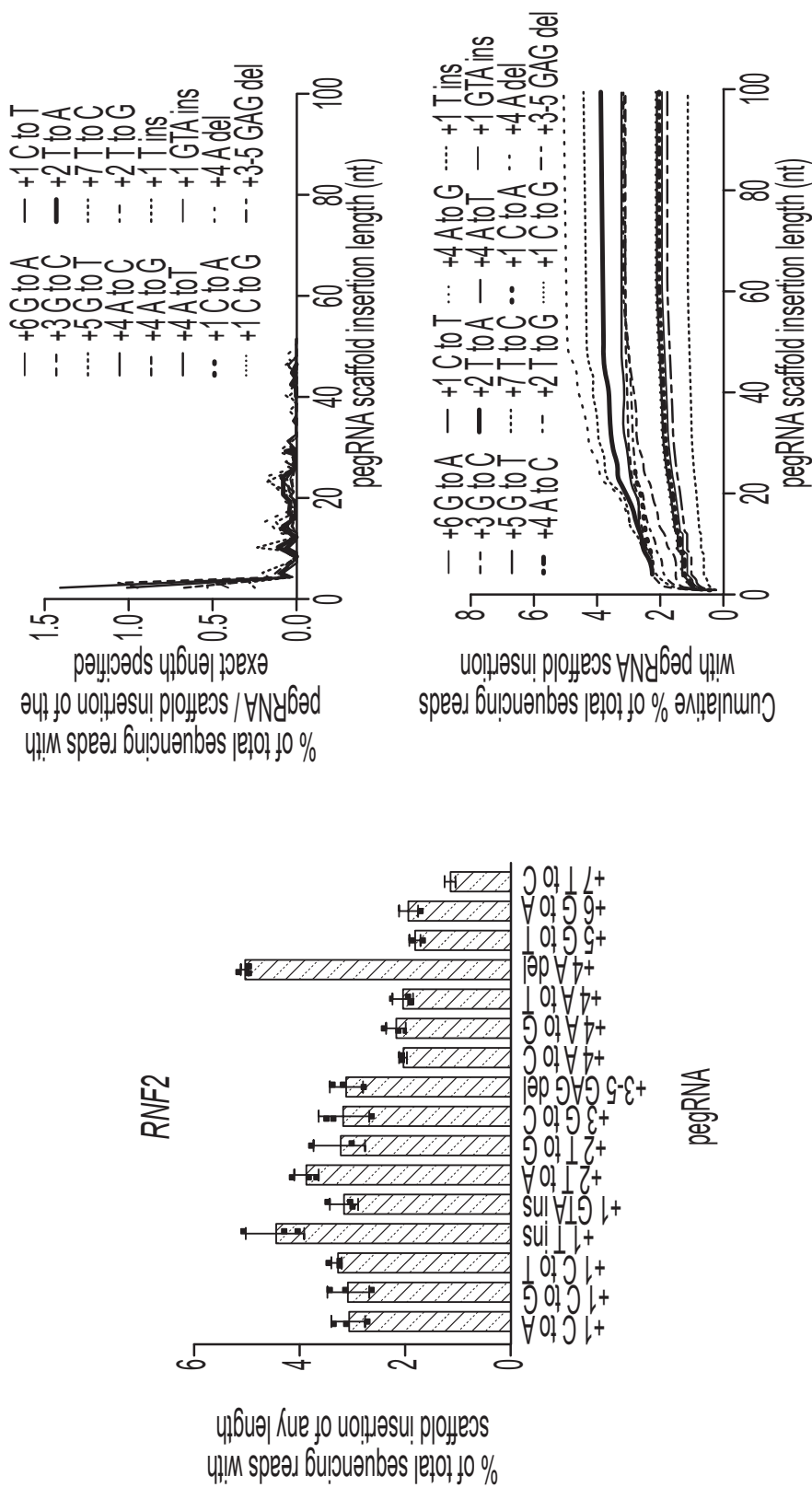


FIG. 56D

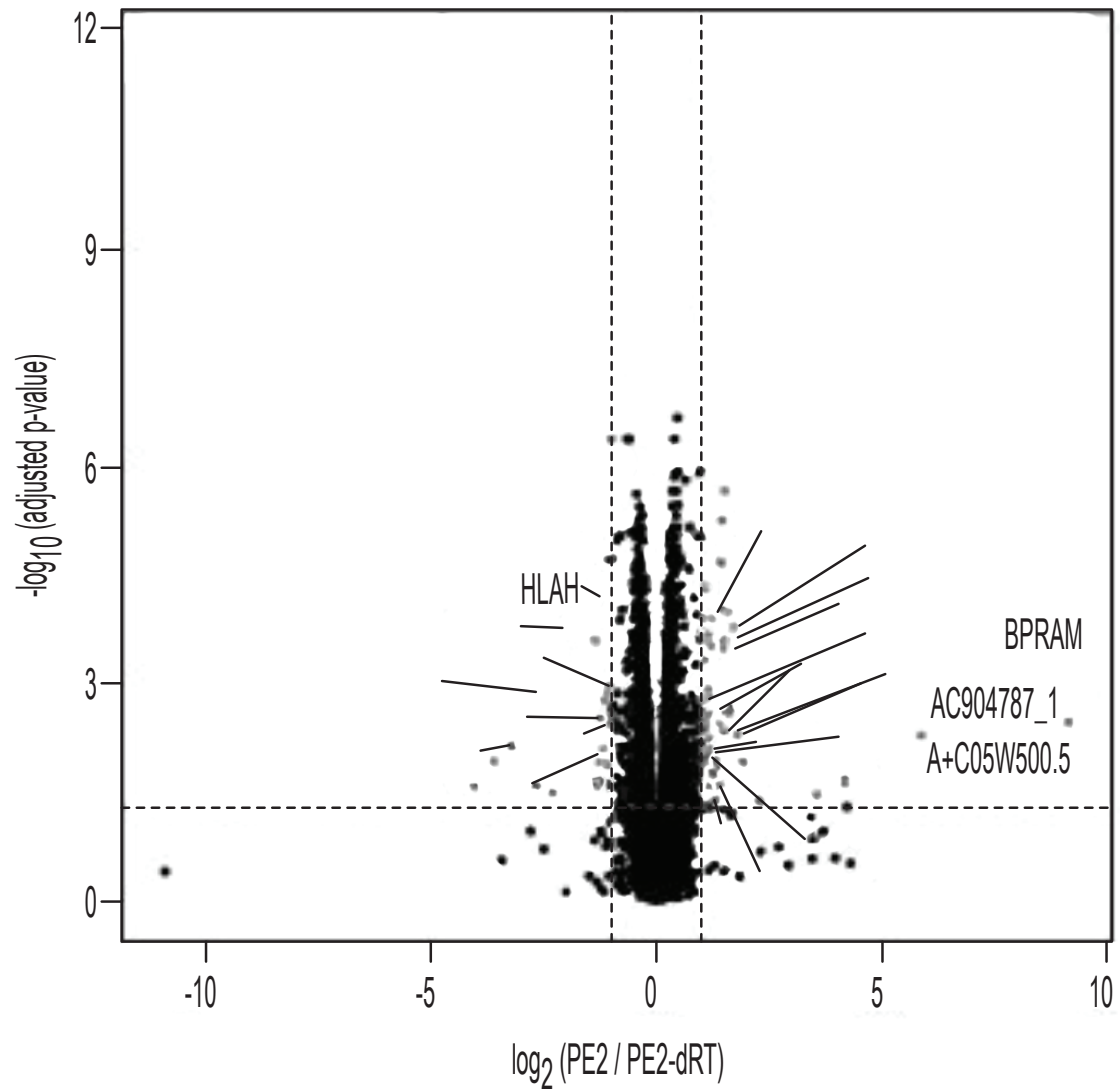
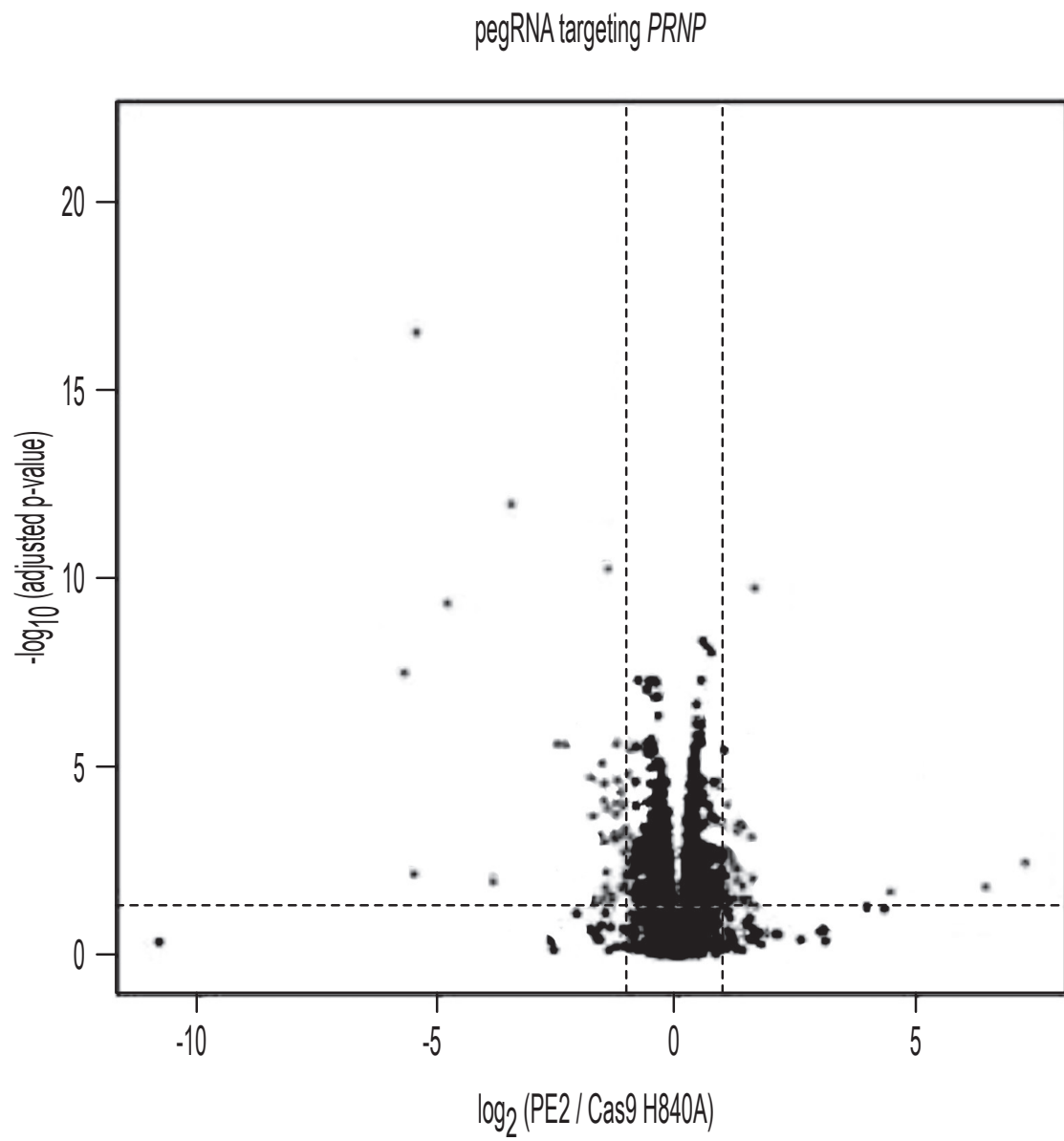
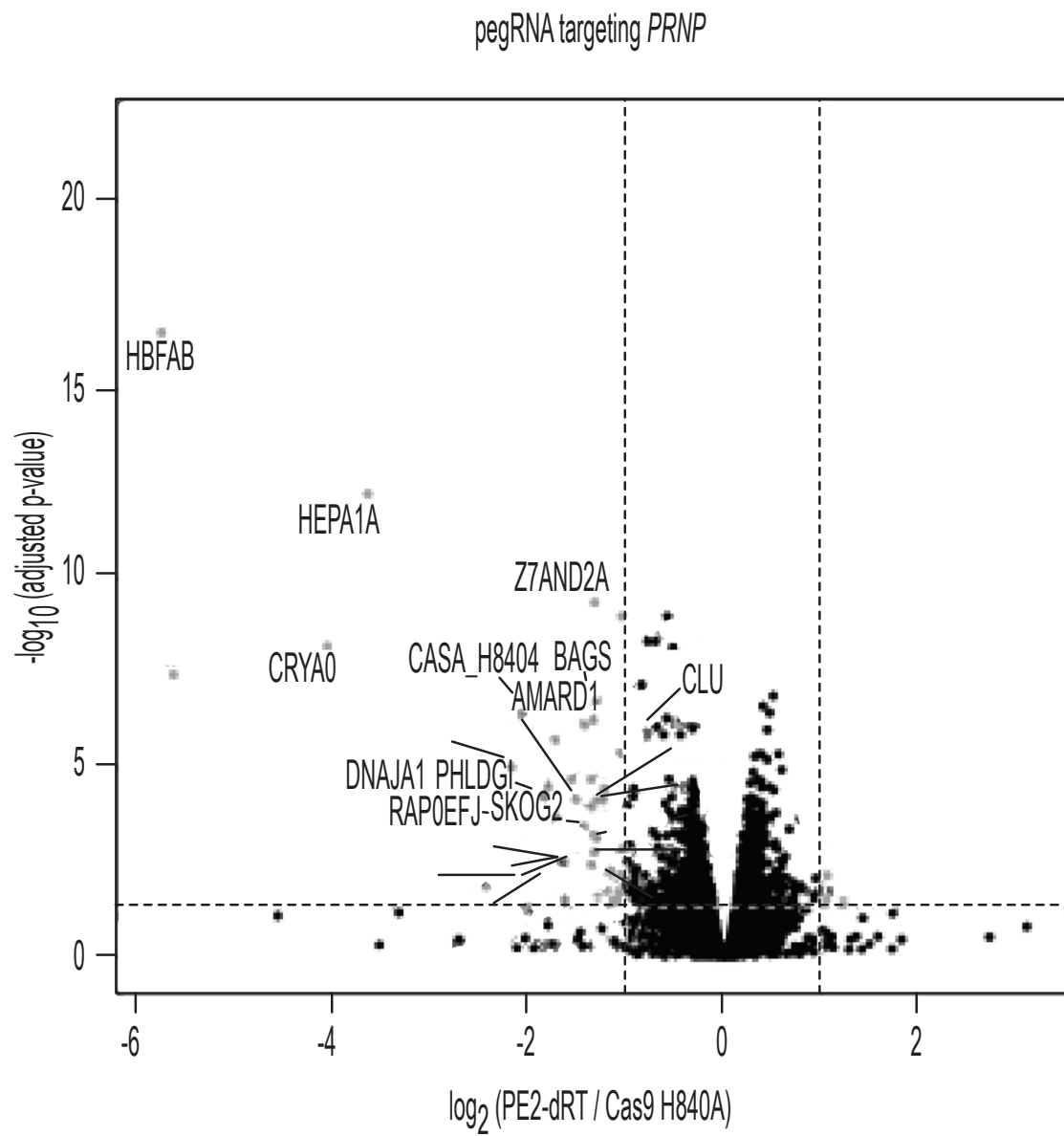
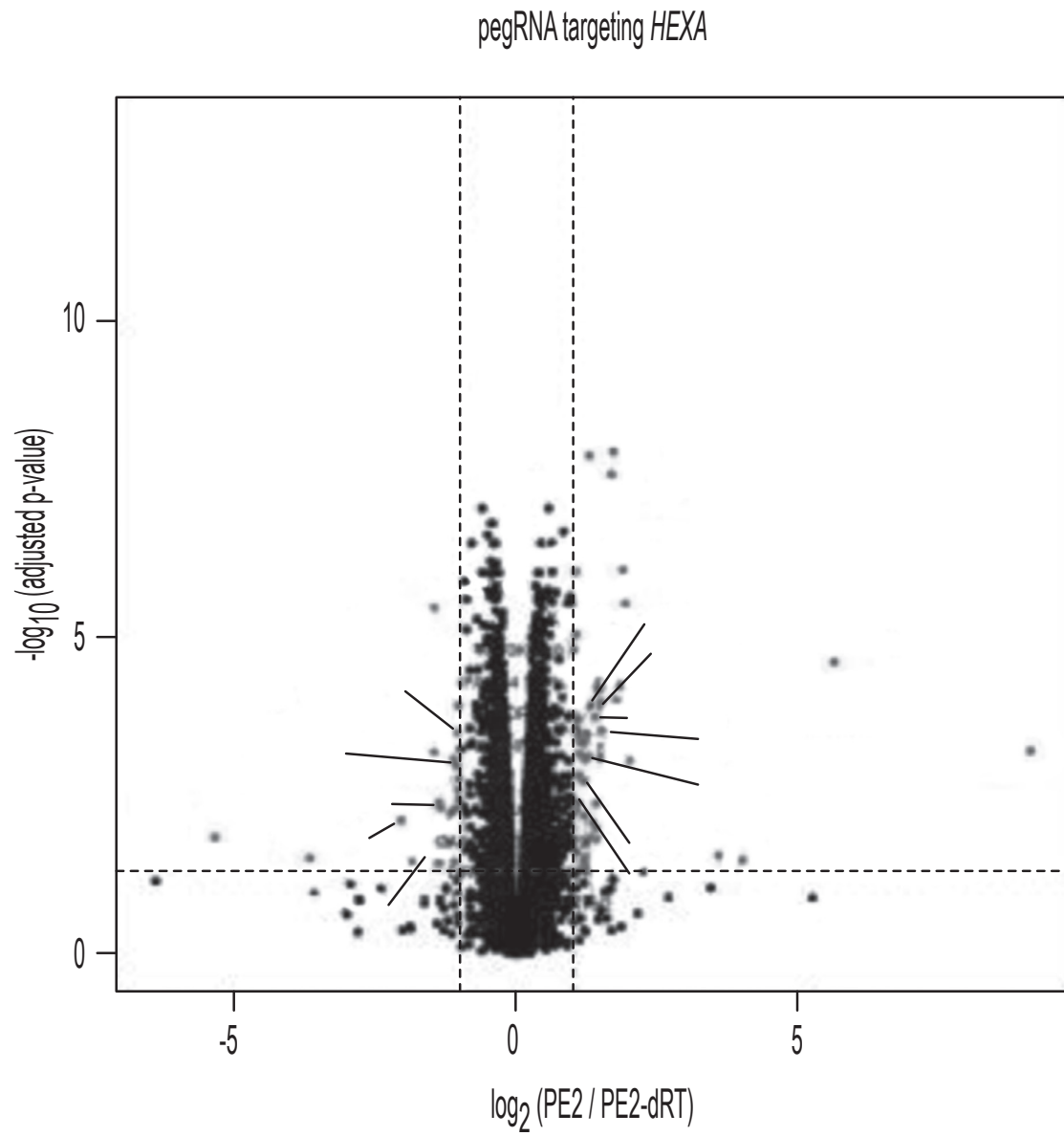
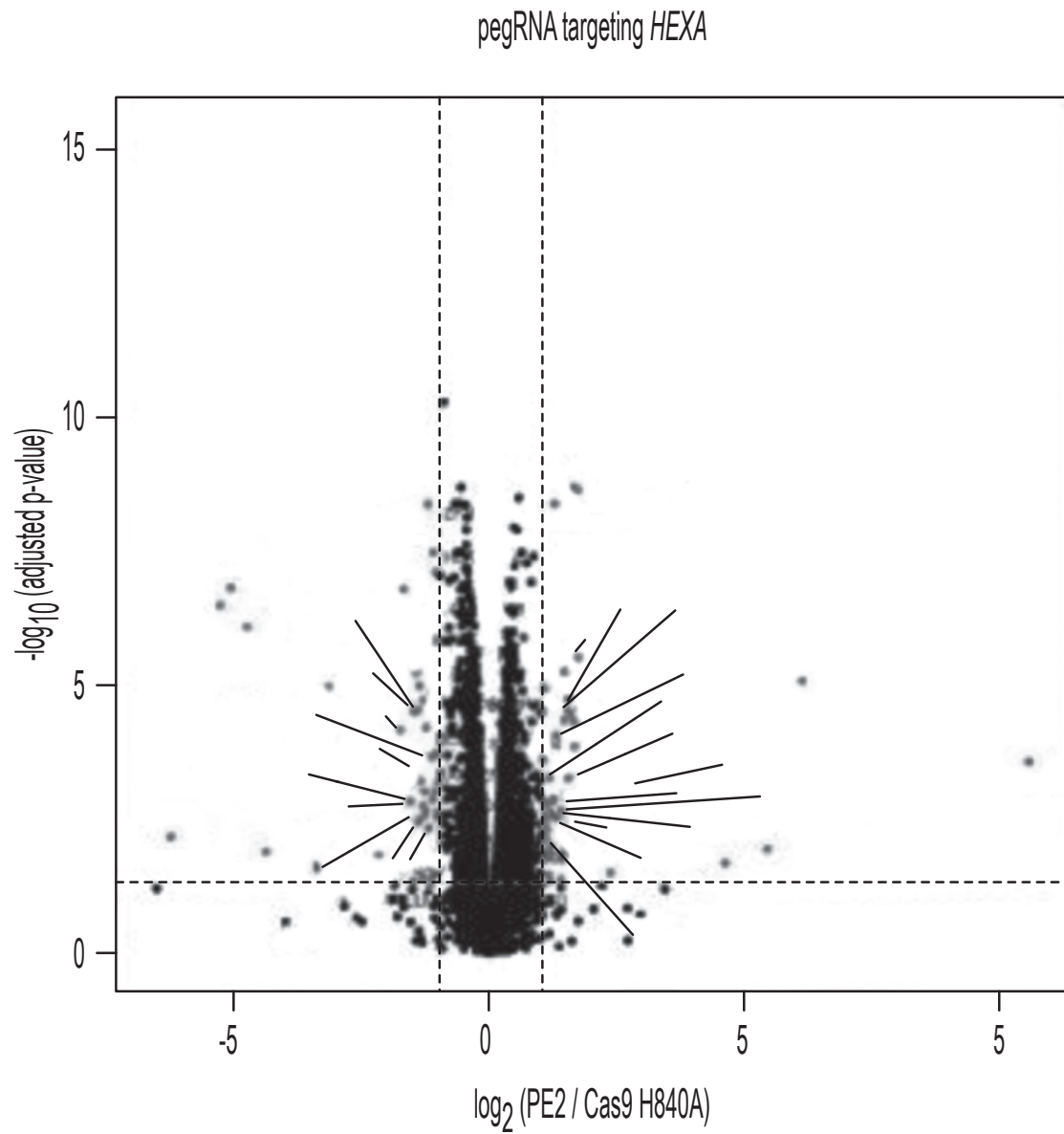
pegRNA targeting *PRNP*

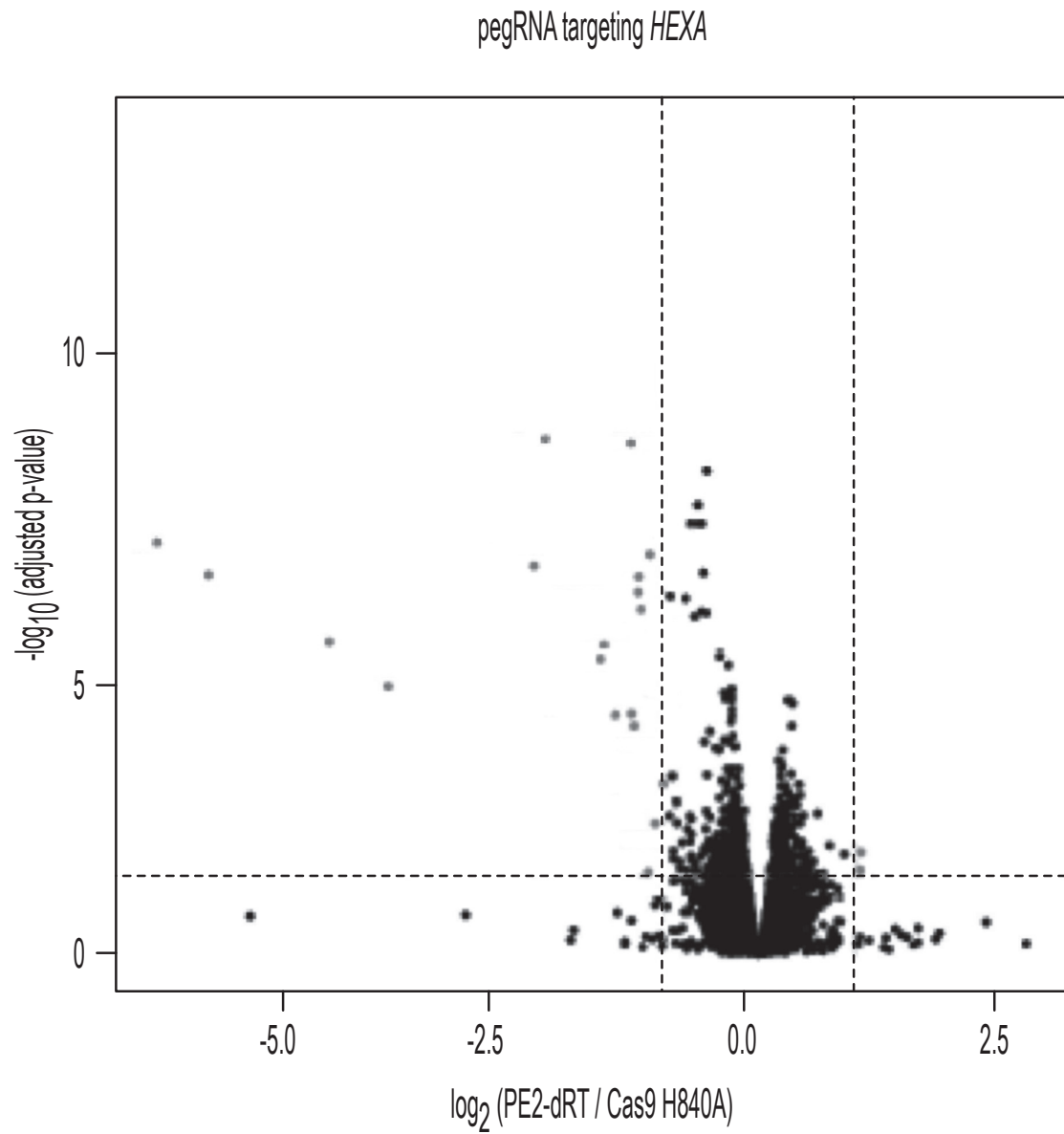
FIG. 57A

**FIG. 57B**



**FIG. 57D**

**FIG. 57E**

**FIG. 57F**

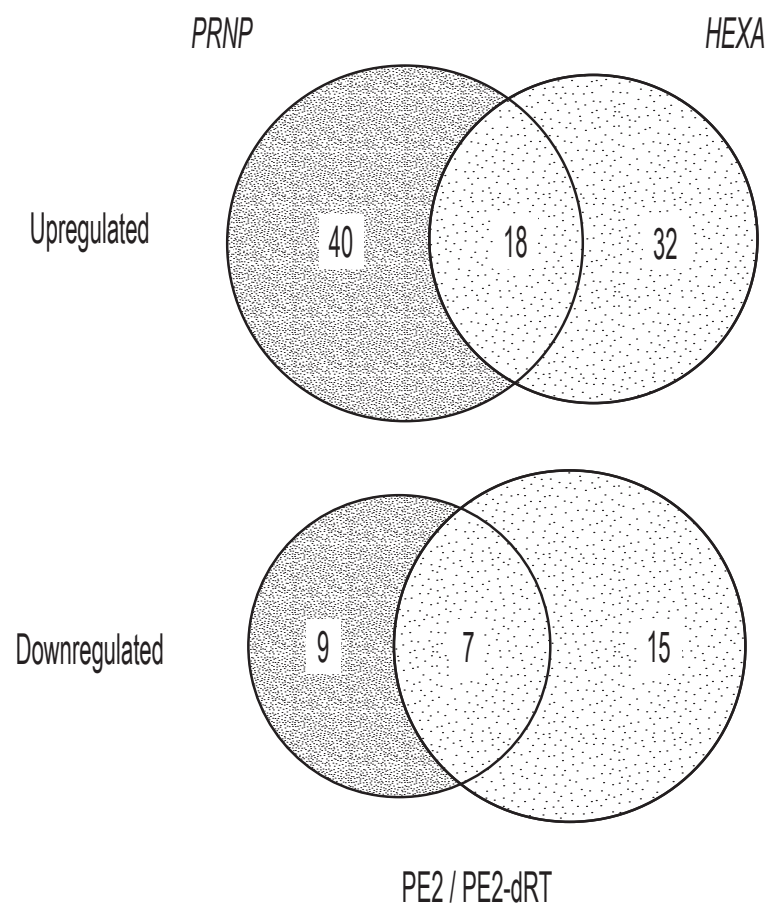


FIG. 57G

127/273

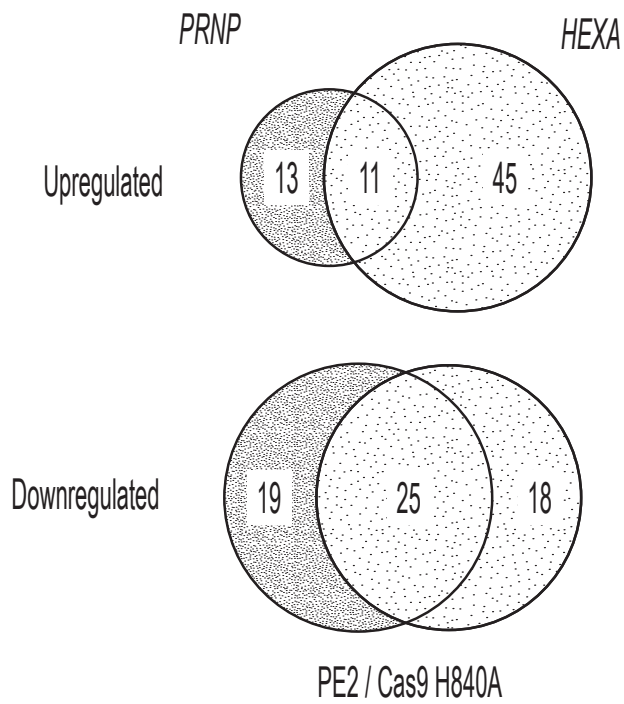


FIG. 57H

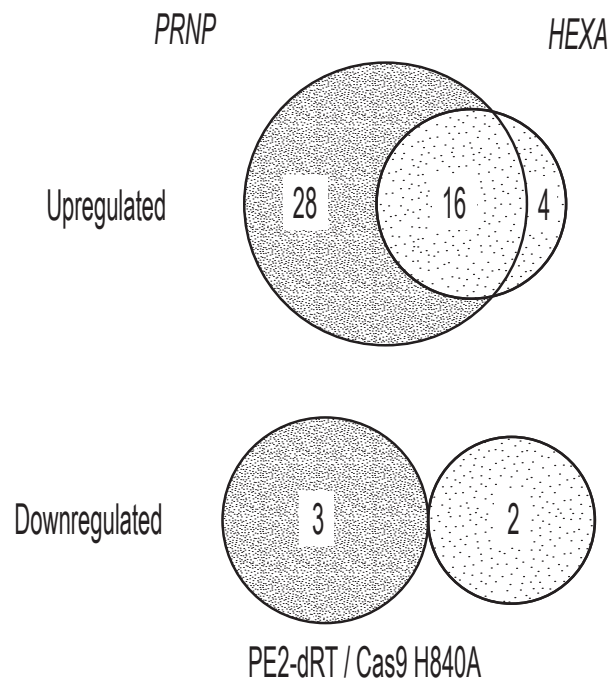


FIG. 57I

TdT DAPI plates Sample_10 D:---\091119\1.1 pool.fcs

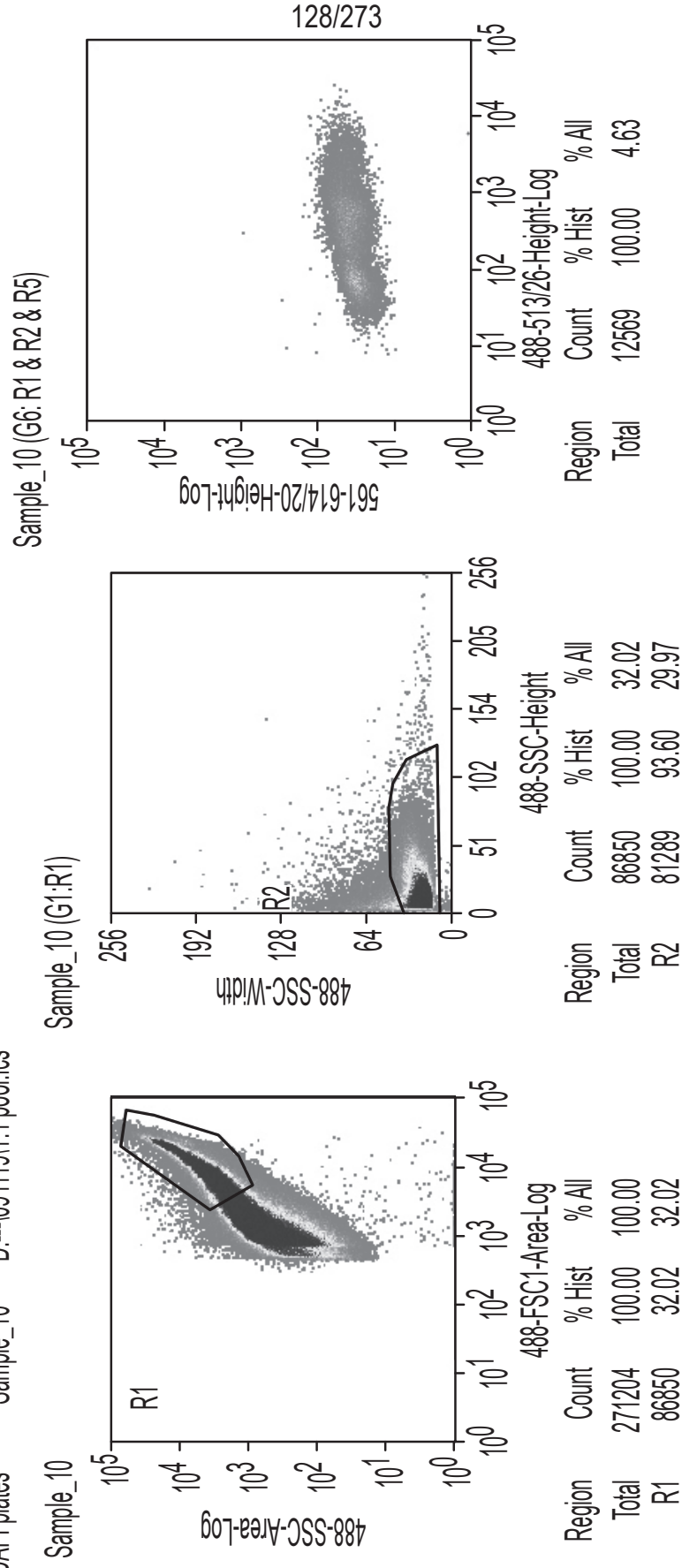
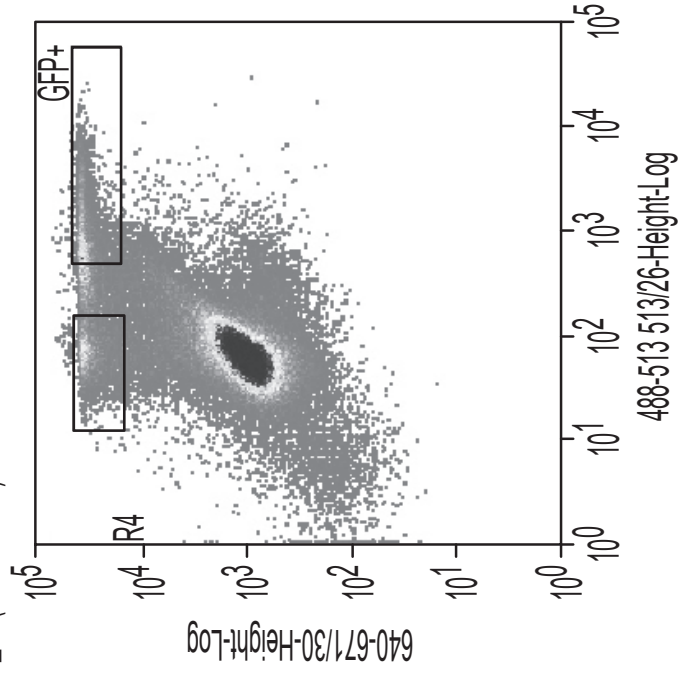


FIG. 58A

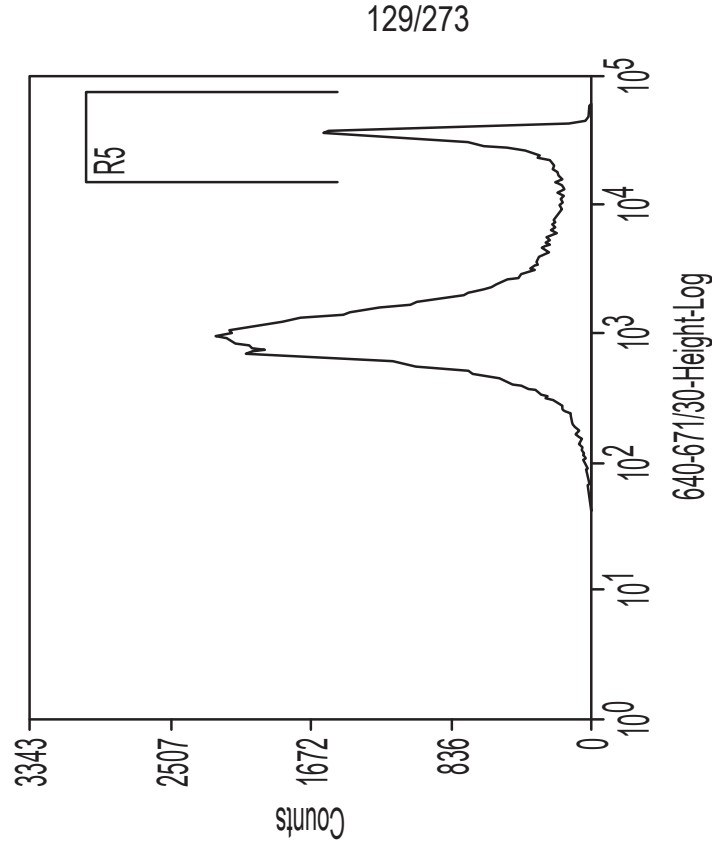
Sample_10 (G2: R1 & R2)



Region	Count	% Hist	% All	Bounds	Mode Count
Total	81289	100.00	29.97 (1.00, 1000000.04)	(100000.04, 1...	128
GFP +	4418	5.43	1.63 (485.60, 48559.55)	(58170.94, 1...	40
R4	4709	5.79	1.74 (11.98, 46415.91)	(150.13, 1501...	34

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Sample_10 (G2: R1 & R2)



Region	Count	% Hist	Mean	CV
Total	81289	100.00	6521.68	179.64
R5	12569	15.46	32516.62	24.27

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^R 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. 59ApegRNA 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 ₂ O	to 30.0 µL
<hr/>	
Total reaction volume	3.0 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: H₂O 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 μ M	1.0 μ L
Bottom oligonucleotide, 100 μ M	1.0 μ L
Annealing buffer (components 2, 3, and 4)	23.0 μ L
<hr/>	
Total reaction volume	25.0 μ 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 μ L H₂O. The final concentration of each oligonucleotide will be 1 μ 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 μ M oligonucleotide duplex from step 1	6.25 μ L
10x T4 DNA ligase buffer (NEB)	2.50 μ L
T4 PNK (NEB)	0.50 μ L
H ₂ O	15.75 μ L
<hr/>	
Total reaction volume	20.0 μ L

In thermocycler, incubate at 37 °C for 60 minutes

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

Proceed to step 3.

FIG. 59D

Step 4: peg RNA assemblypegRNA, Golden Gate assembly reaction

Digested pU6-pegRNA-GG plasmid-vector	1.00 μ L @ 30 ng/ μ L
- Pre-cut, isolated 2.2-kb fragment	
Annealed protospacer oligonucleotides (component 2)	1.00 μ L @ 1 μ M
Annealed pegRNA 3'-extension oligonucleotides (component 4)	1.00 μ L @ 1 μ M
sgRNA scaffold annealed oligonucleotides (component 3)	1.00 μ L @ 1 μ M
- Oligonucleotides <i>must be phosphorylated</i>	
<i>Bsa</i> I-HFv2 (NEB)	0.25 μ L
T4 DNA ligase (NEB)	0.50 μ L
10x T4 DNA ligase buffer (NEB)	1.00 μ L
H ₂ O	4.25 μ L
<hr/>	
Total reaction volume:	10.0 μ 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 μ L of the 10 μ L assembly reaction into 10 μ L of competent cells. The desired transformants will be resistant to ampicillin and carbenicillin.

The following diagram summarizes the pegRNA cloning protocol.

FIG. 59F


```

## 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
GCTATTTCTAGCTCTAAAACTCACGTGCTCAGTCTGGGCCGGTG', (SEQ ID NO: 885)
'EMX1': 'ATCACGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TGCTATTTCTAGCTCTAAAACTTCTTCTTCTGCTCGGACTCGGTG', (SEQ ID NO: 886)
'FANCF': 'TTTCCGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACT
TTGCTATTTCTAGCTCTAAACGGTGCTGCAGAAGGGATTCCGGTG', (SEQ ID NO: 887)
'RNFB2': 'TCGTTGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTT
GCTATTTCTAGCTCTAAACAGGTAATGACTAAGATGACGGTG'} (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

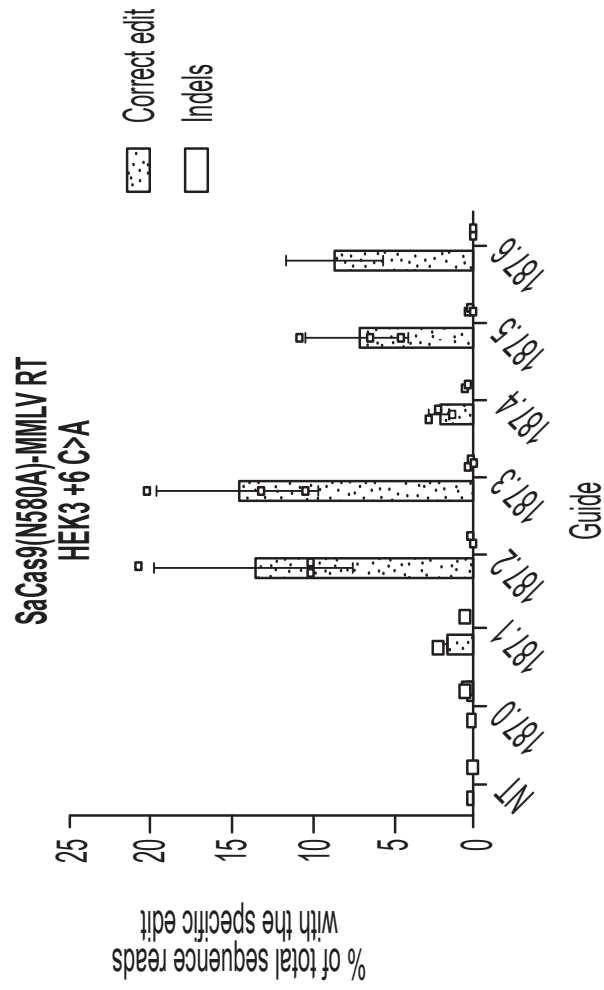


FIG. 61

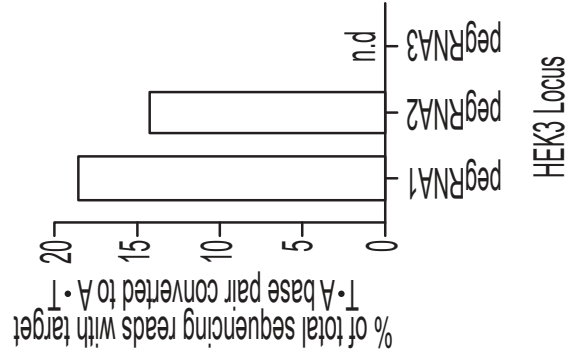


FIG. 62A

FIG. 62B

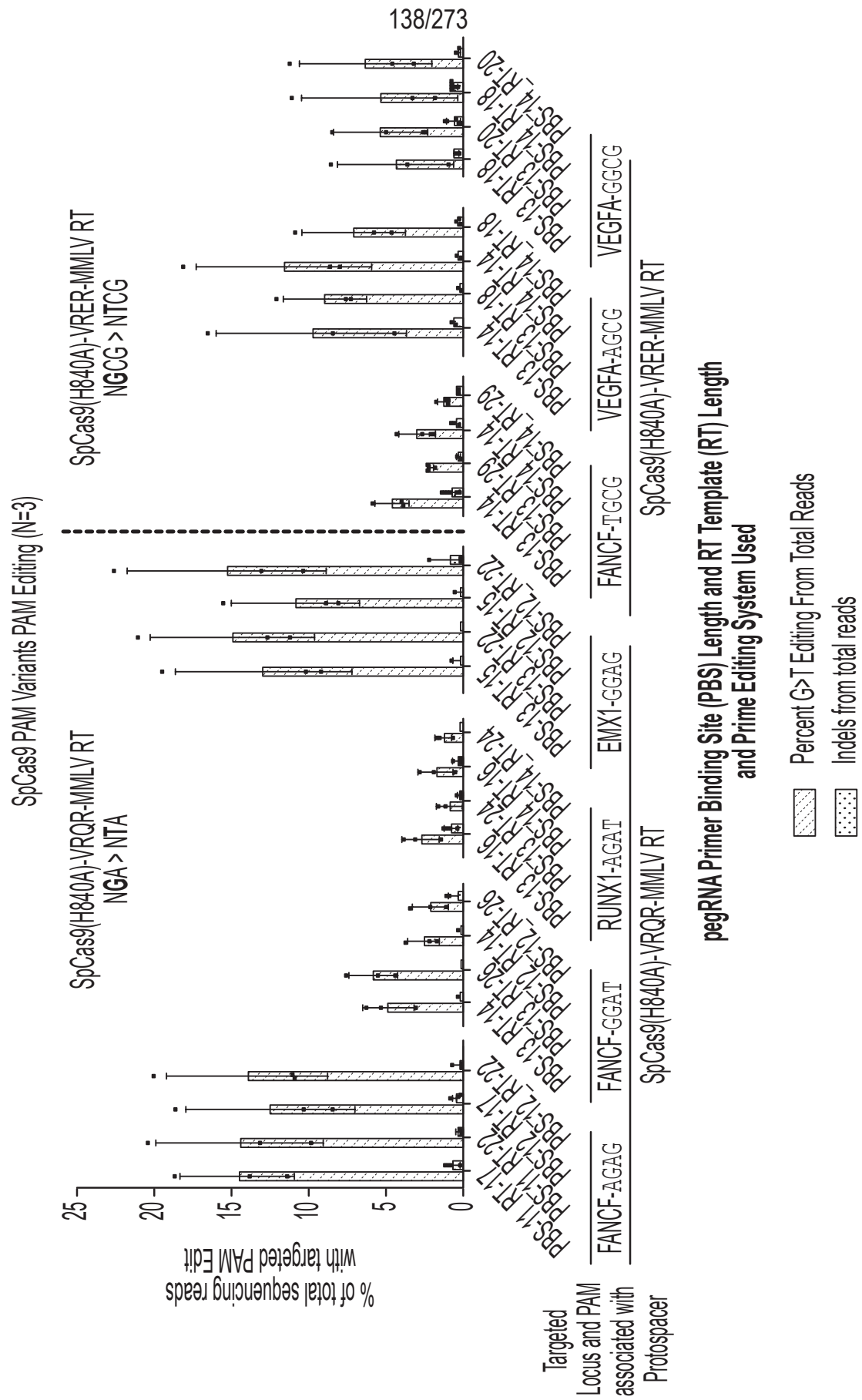


FIG. 63

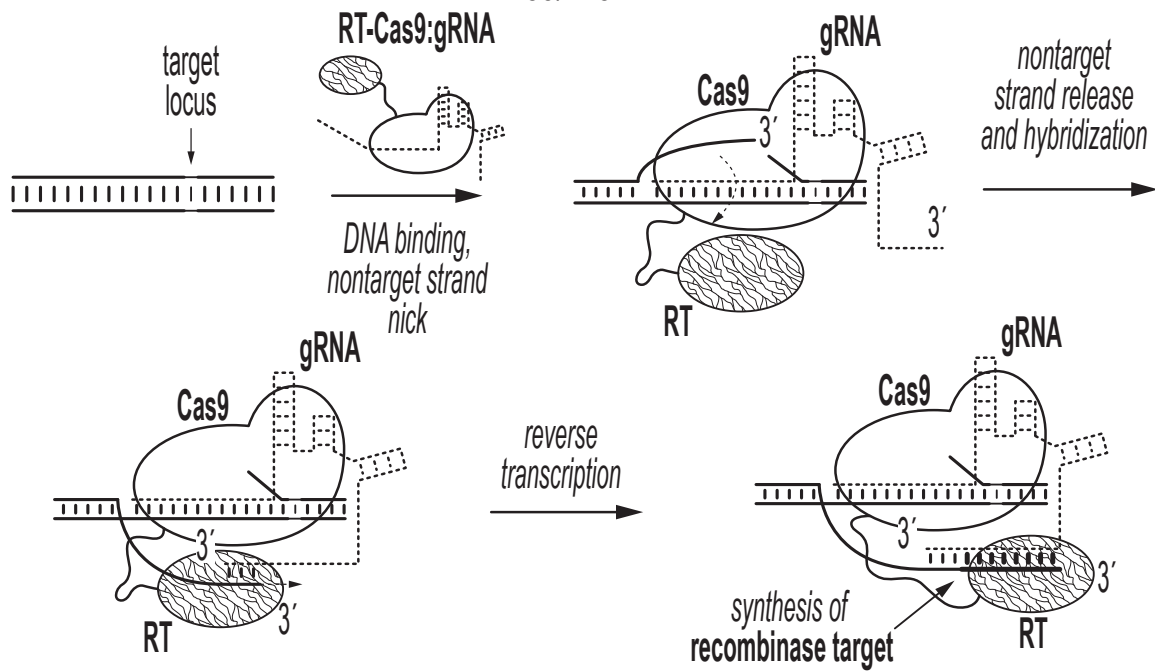


FIG. 64A



FIG. 64B



FIG. 64C



FIG. 64D



FIG. 64E

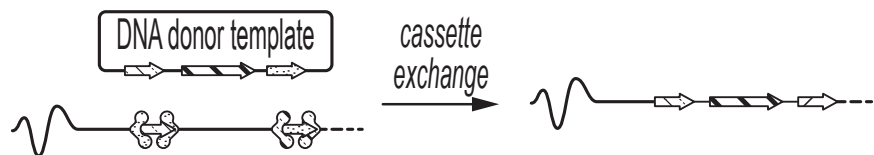


FIG. 64F

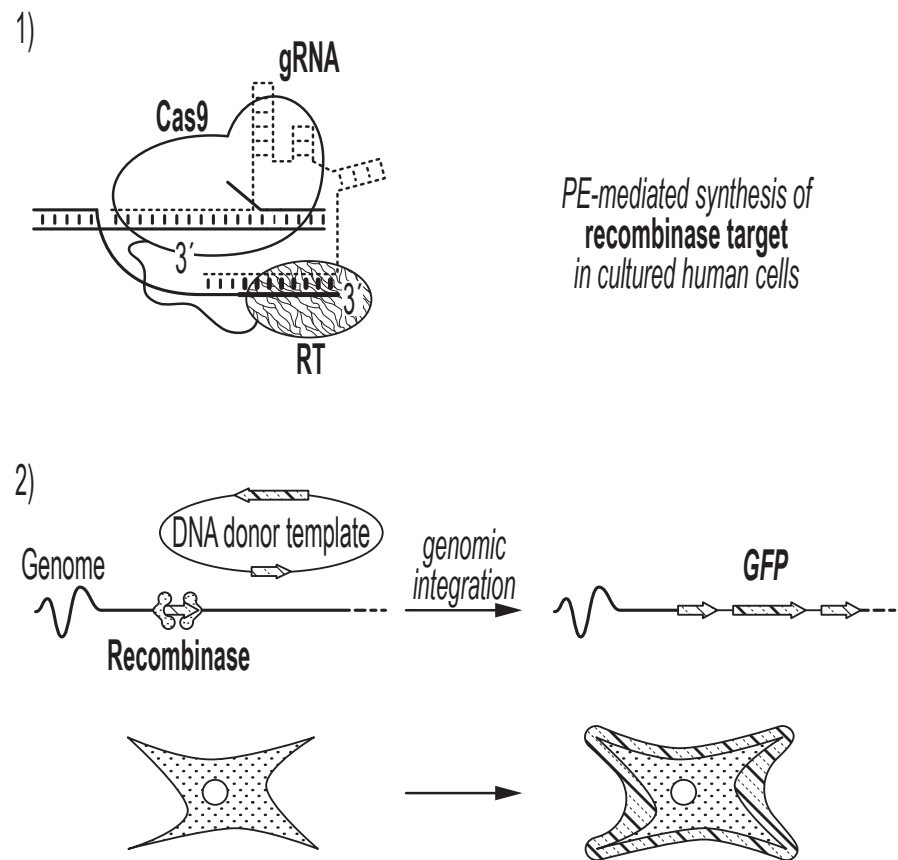
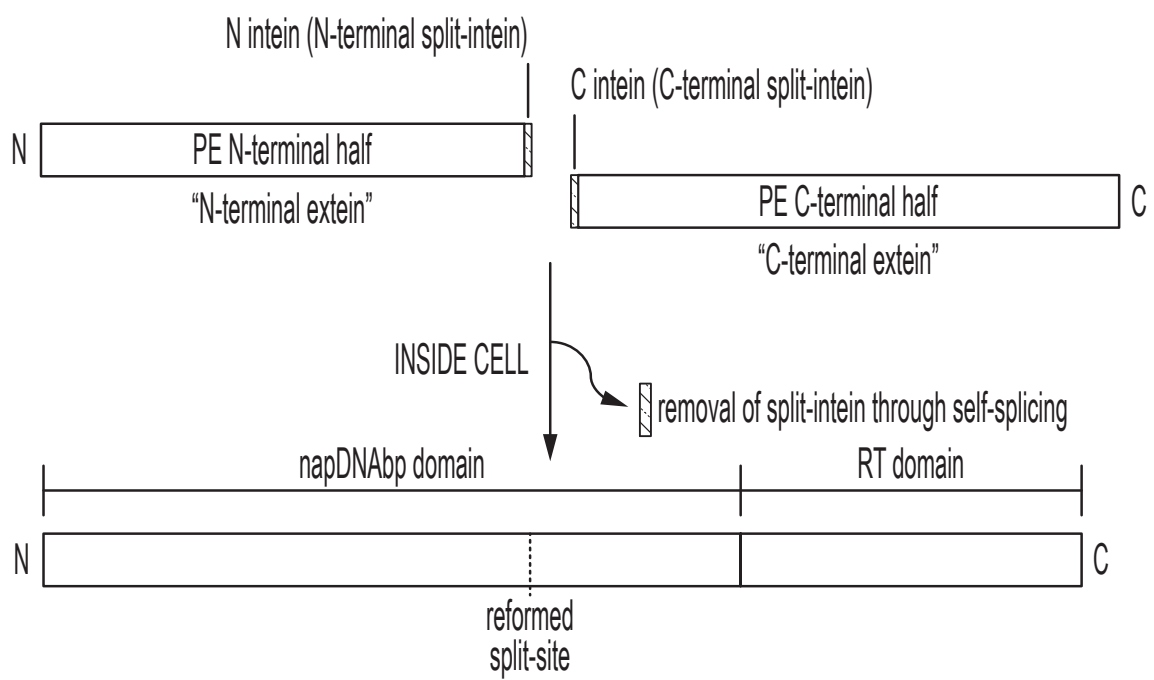


FIG. 65



Prime Editor

FIG. 66

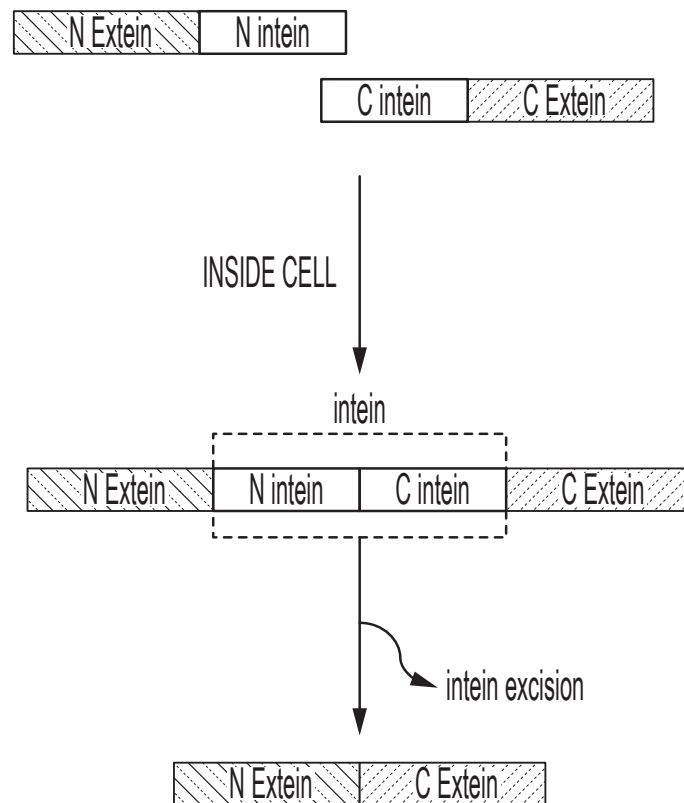


FIG. 67A

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intein excision mechanism

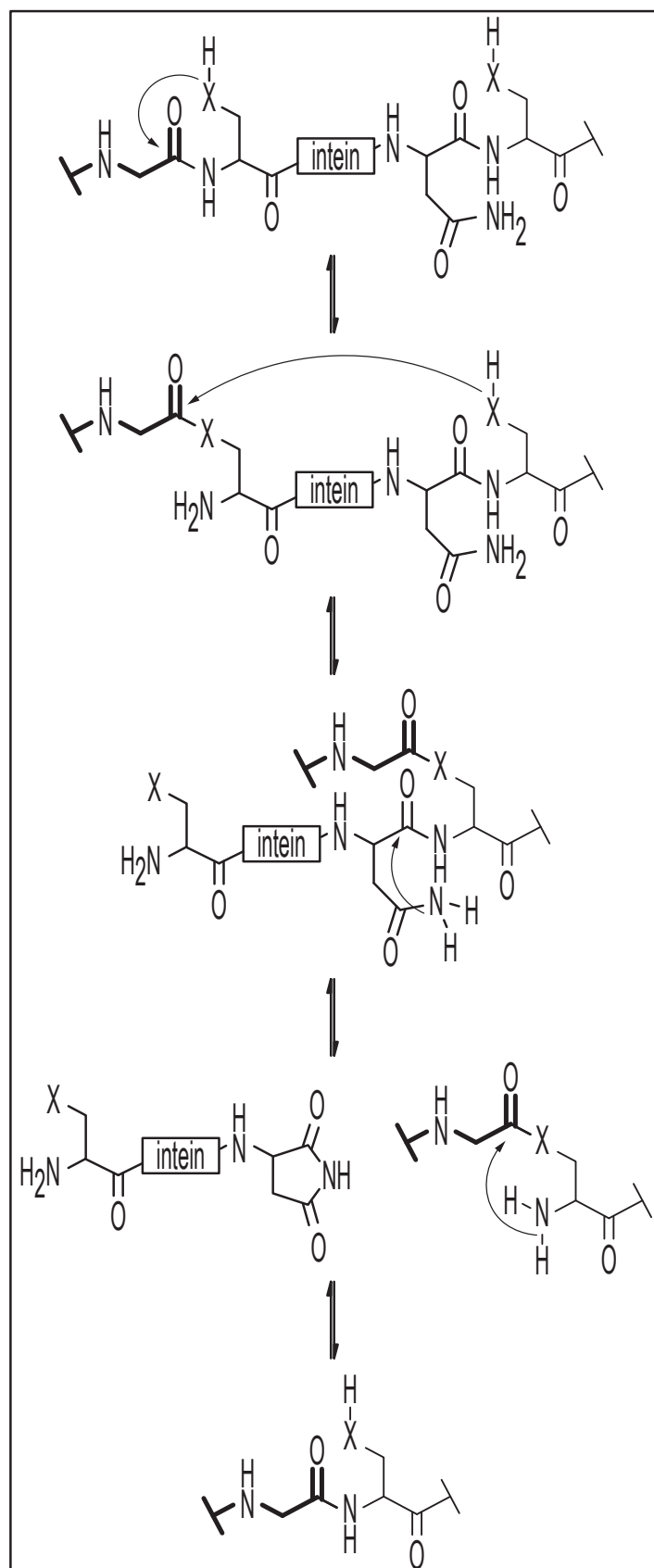
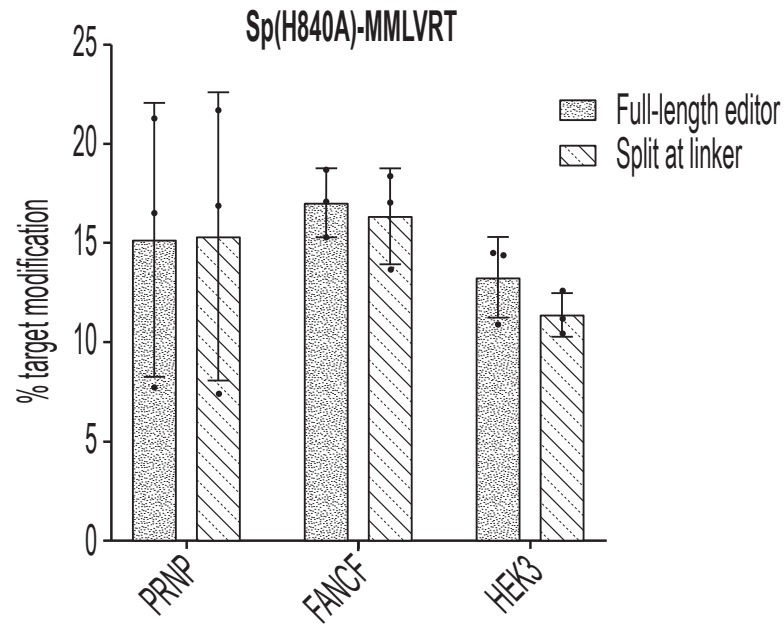
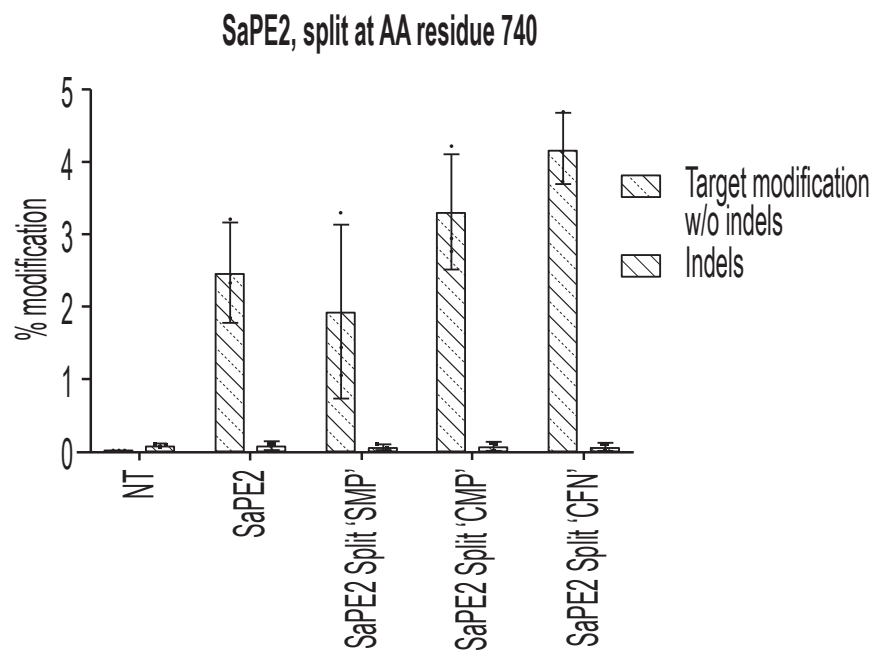
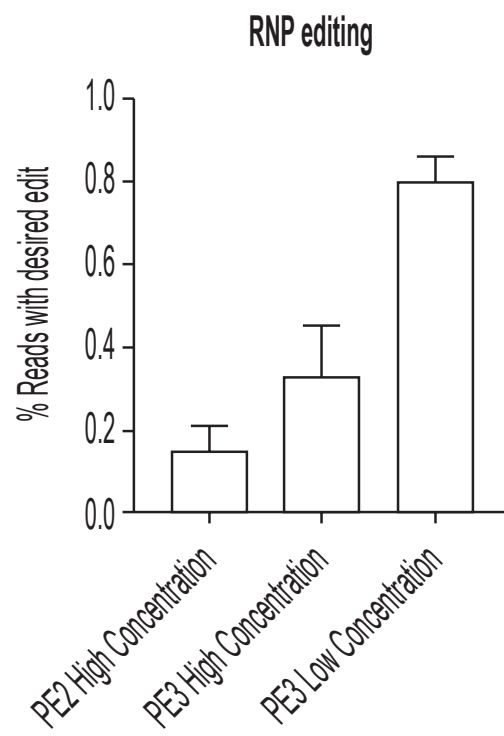


FIG. 67B

**FIG. 68A****FIG. 68B**

**FIG. 68C**

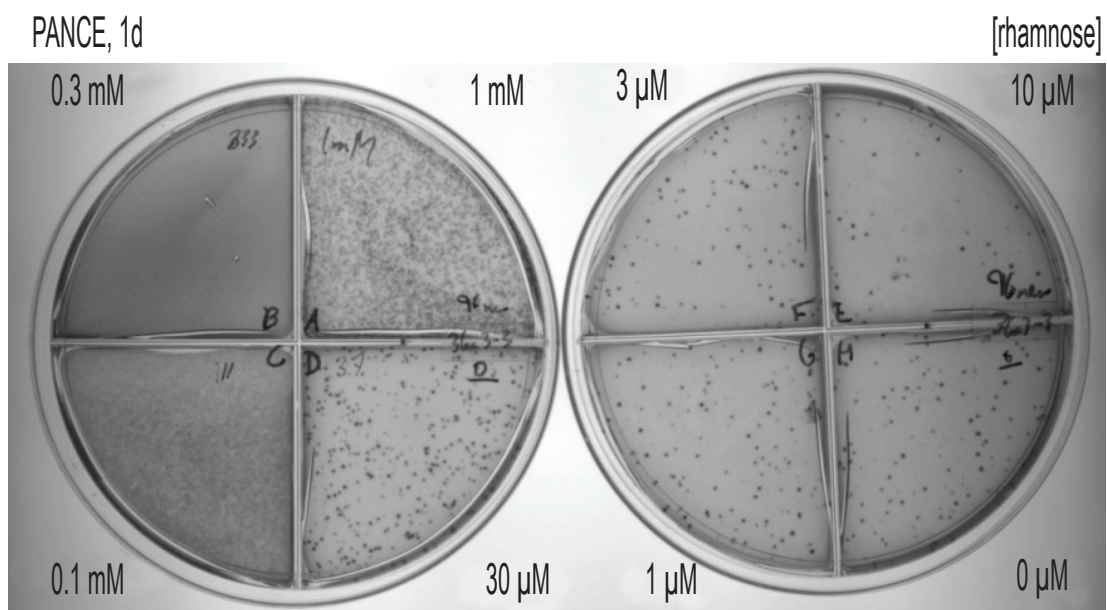


FIG. 69

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Starting allele

(SEQ ID NO: 892)

5' - TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

Edit Target (A•T to C•G)

ATGCCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATATAAACTGAGTTCC
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCGGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70A

Starting allele

(SEQ ID NO: 892)

5' - TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

NGG PAM 1

ATGCCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATATAAACTGAGTTCC
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

NGG PAM 2

CATGTTTTGCTTAATGGTTGAGTTCGGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70B

Starting allele

(SEQ ID NO: 892)

5' - TATTATTACTCTATGTTCTATTTAAGTTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

nick for PAM 1

ATGCCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATATAAACTGAGTTCC
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

nick for PAM 2

CATGTTTTGCTTAATGGTTGAGTTCGGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

FIG. 70C

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Starting allele

(SEQ ID NO: 892)

5'- TATTATTACTCTATGTTCTATTTAAGTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

Spacer

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATATAAACTGAGTTCC
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

(SEQ ID NO: 499)

Spacer = GTCATCTTAGTCATTACCTG

FIG. 70D

Starting allele

(SEQ ID NO: 892)

5'- TATTATTACTCTATGTTCTATTTAAGTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

PBS complement

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTGAGGTGTTTCGTTGTAACATATATAAACTGAGTTCC
TACGGGGAACCGTCAGTAGAATCAGTAATGGACTCCACAAGCAACATTGAGTATATTTGACTCAAGG

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

PBS = GTAATGACTAAGATG

FIG. 70E

(SEQ ID NO: 638)

Desired allele

(SEQ ID NO: 892)

5'- TATTATTACTCTATGTTCTATTTAAGTTTTCATGTTCTAAAAATGTATCCCAGTTTACACGTCTCAT
ATAATAATGAGATACAAGATAAATTCAAAGTACAAGATTTTTACATAGGGTCAAATGTGCAGAGTA

(SEQ ID NO: 893)

RT template complement

ATGCCCCTTGGCAGTCATCTTAGTCATTACCTGCGGTGTTTCGTTGTAACATATATAAACTGAGTTCC
TACGGGGAACCGTCAGTAGAATCAGTAATGGACGCCACAAGCAACATTGAGTATATTTGACTCAAGG

Edit

CATGTTTTGCTTAATGGTTGAGTTCCGTTTGTCTGCACAGCCTGAGACATTGCTGGAAATAAAGAAG -3'
GTACAAAACGAATTACCAACTCAAGGCAAACAGACGTGTCGGACTCTGTAACGACCTTTATTTCTTC

(SEQ ID NO: 1759)

RT template = AACGAACACCGCAG

FIG. 70F



FIG. 70G

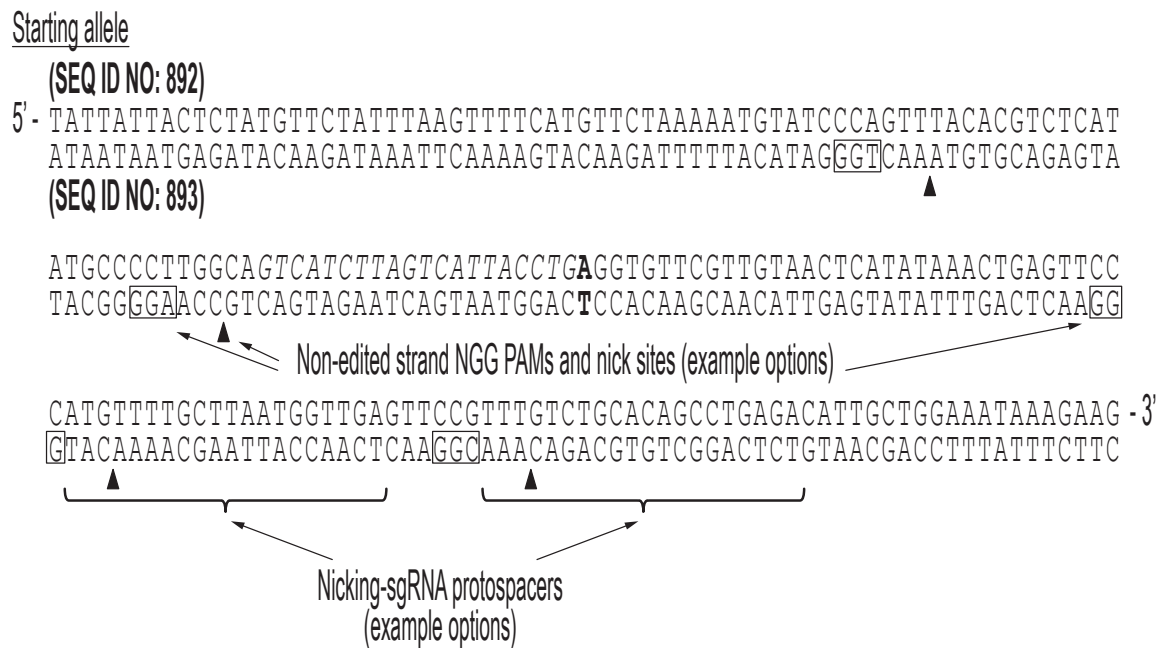


FIG. 70H

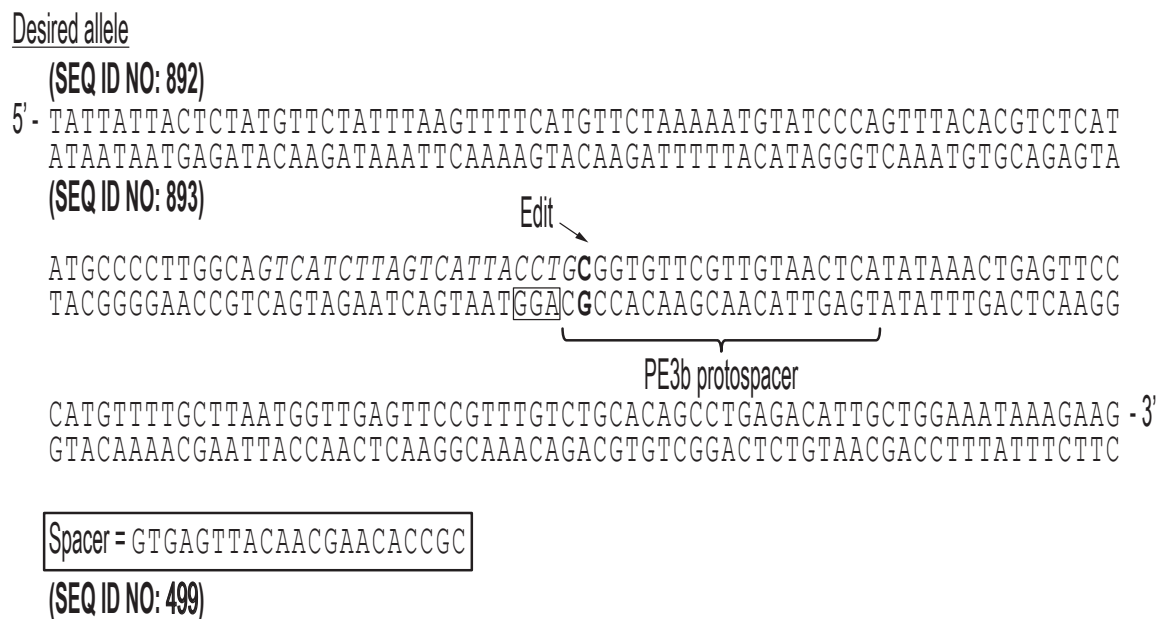


FIG. 70I

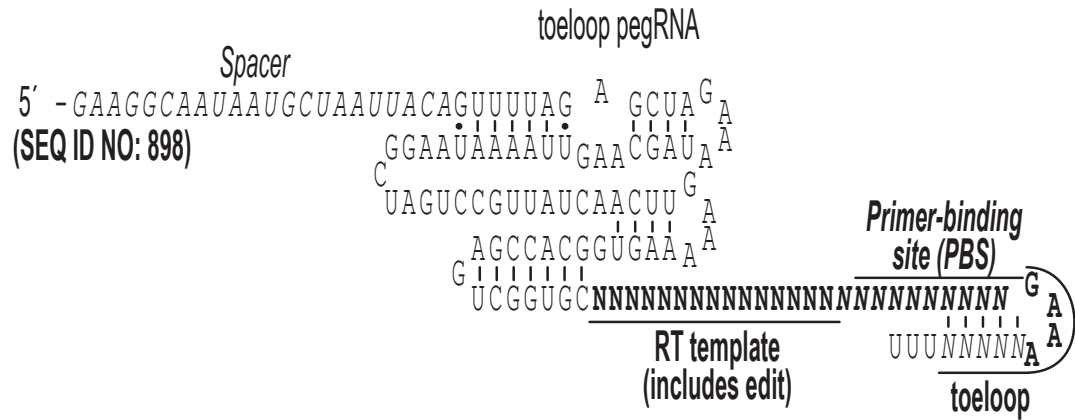


FIG. 71A

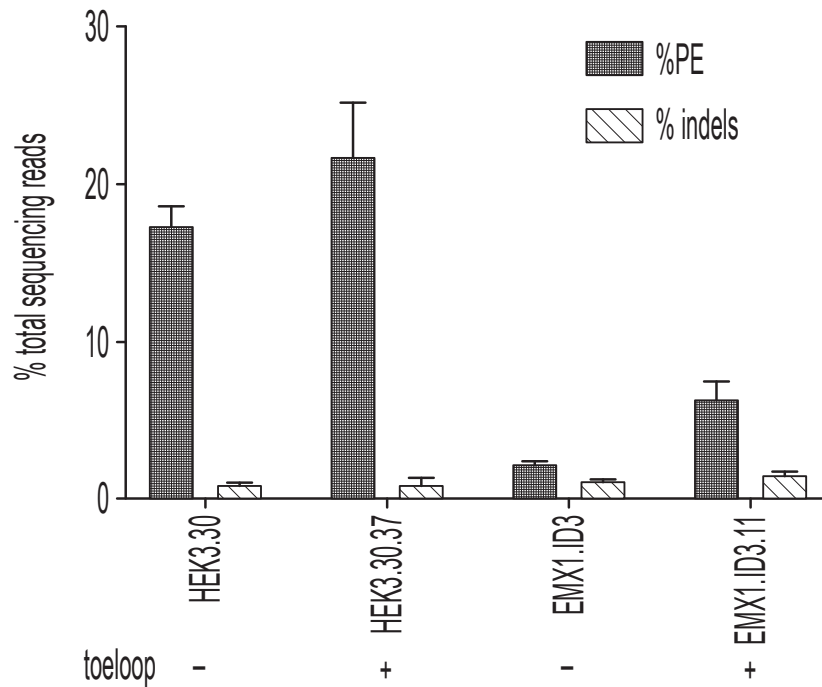


FIG. 71B

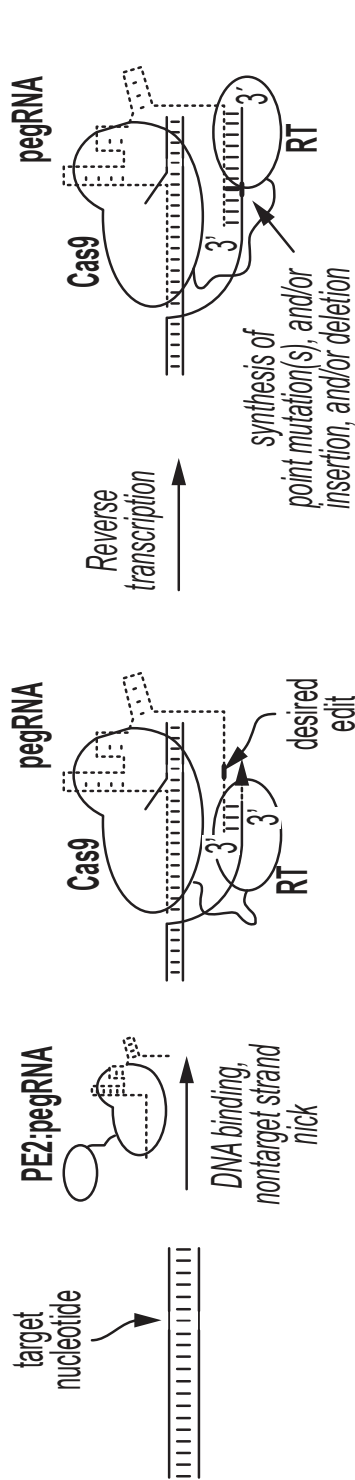


FIG. 72A

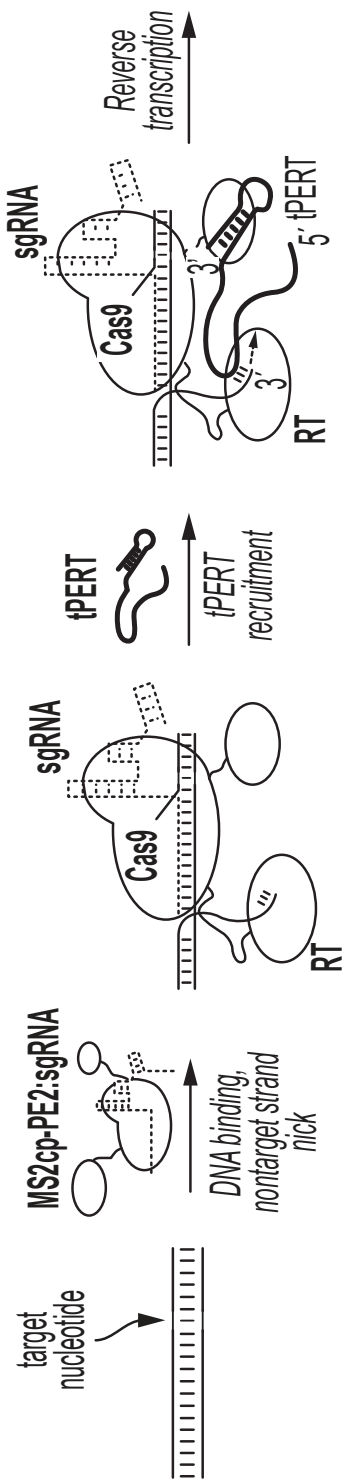
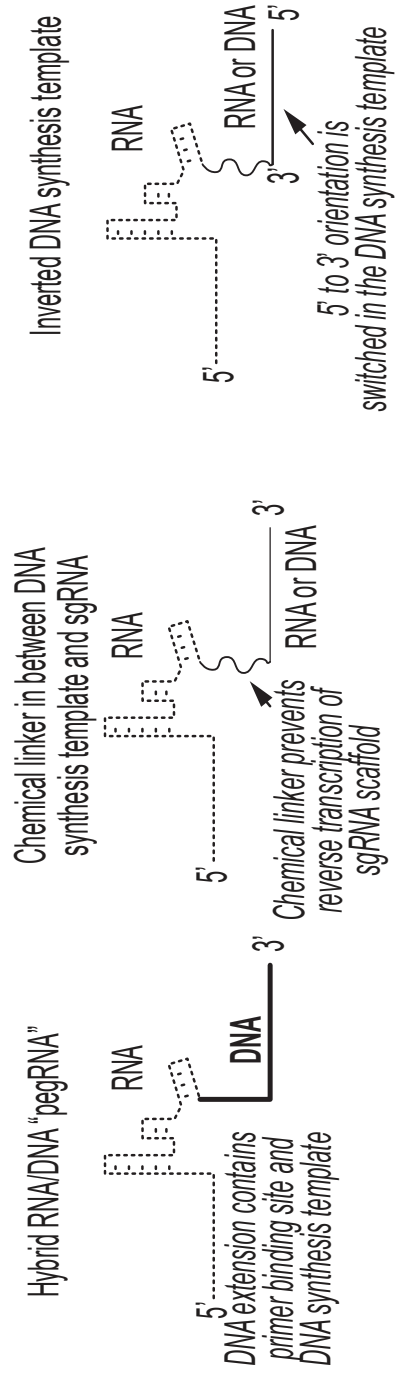
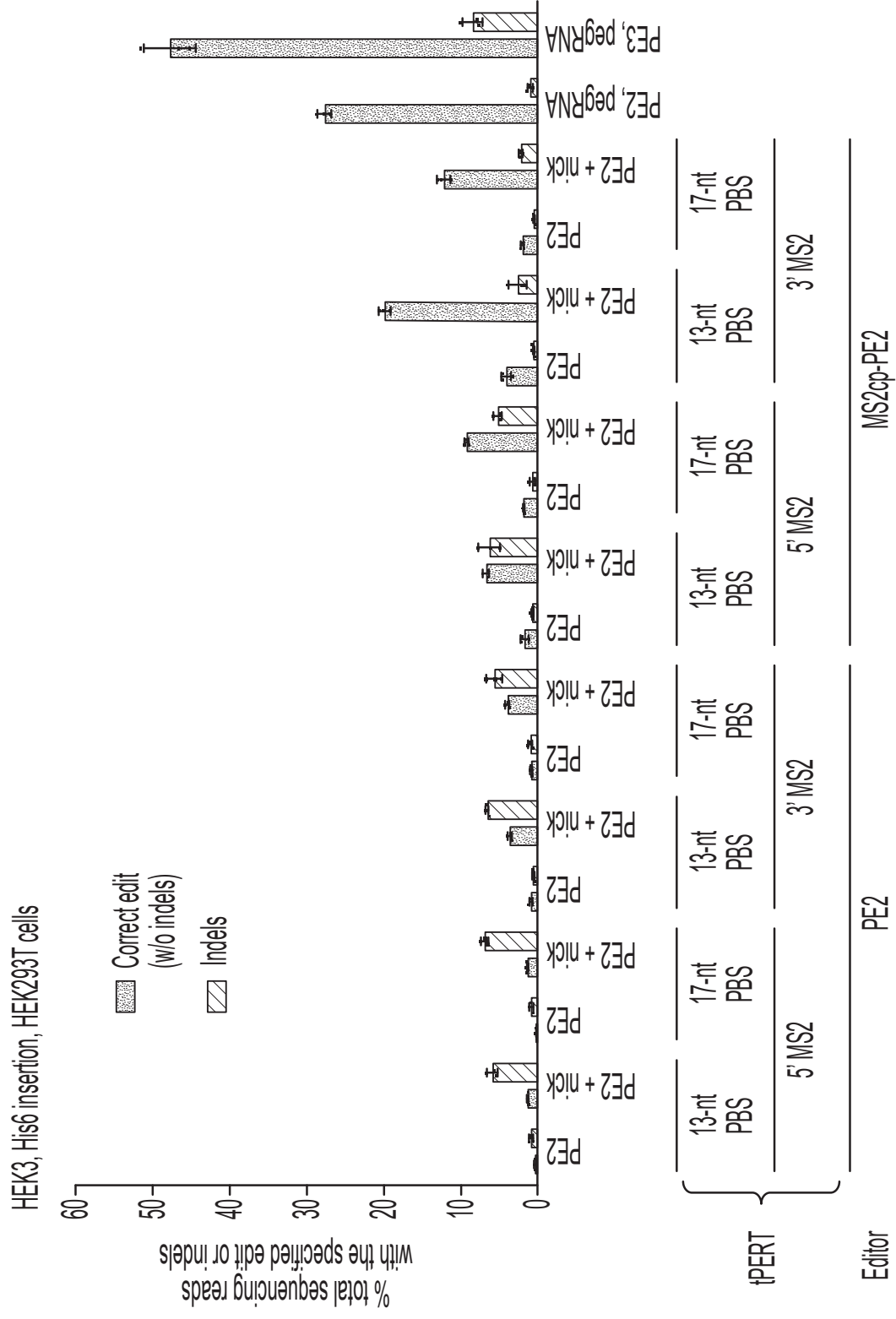


FIG. 72B



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

FIG. 72C



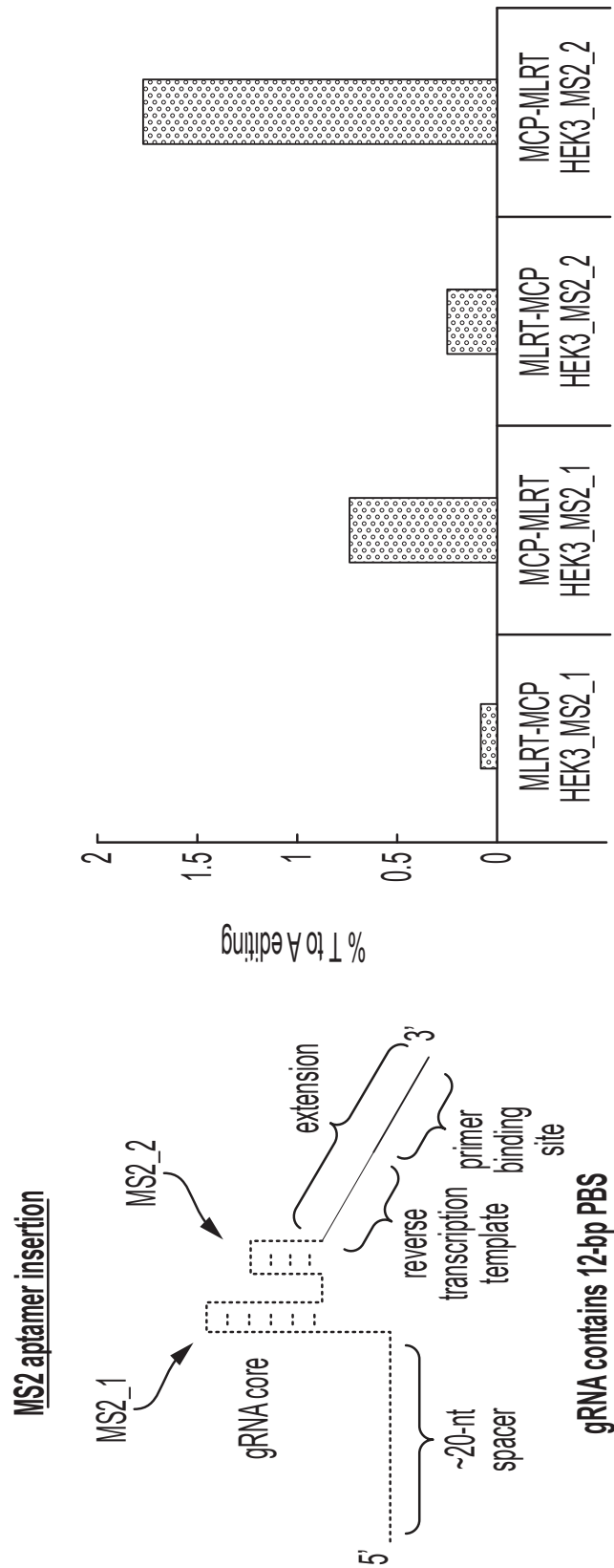


FIG. 74

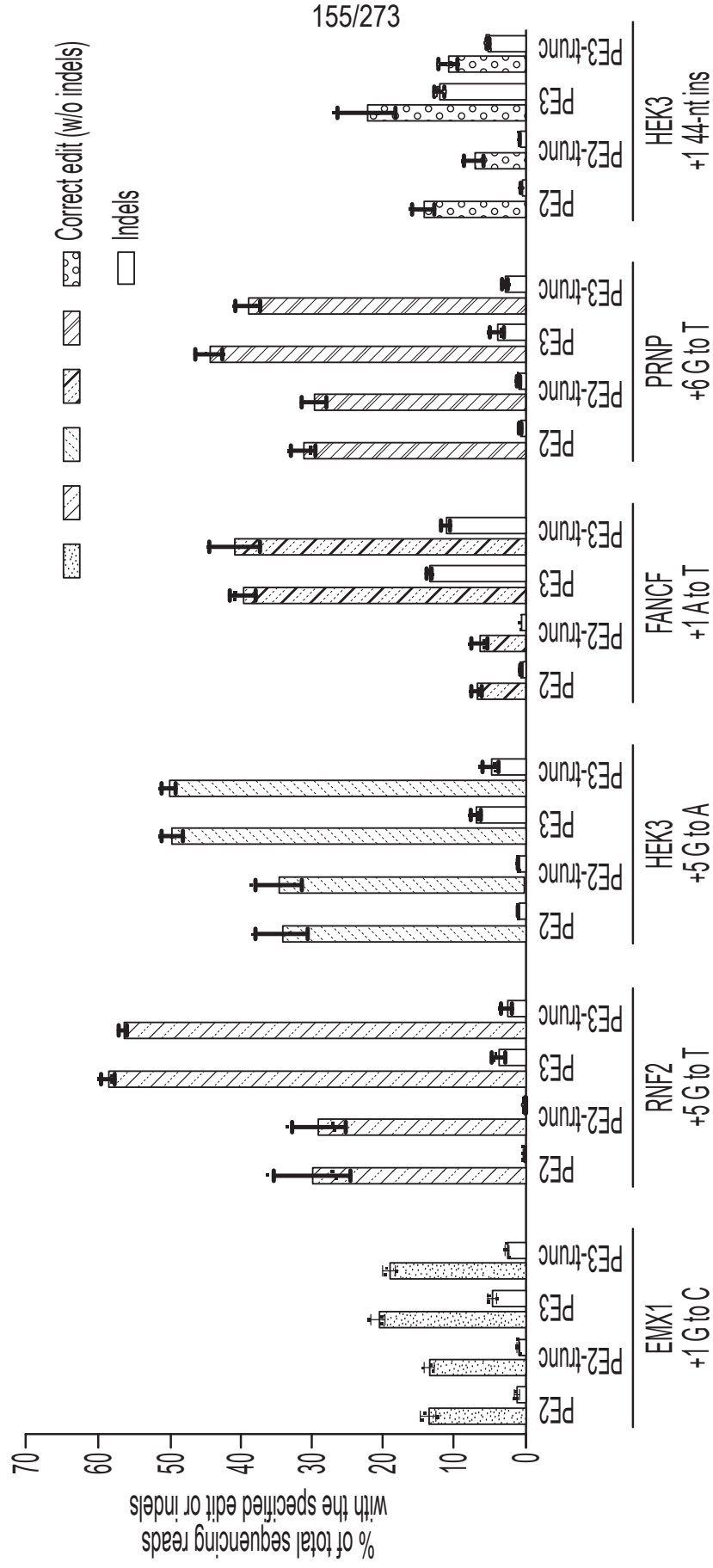


FIG. 75

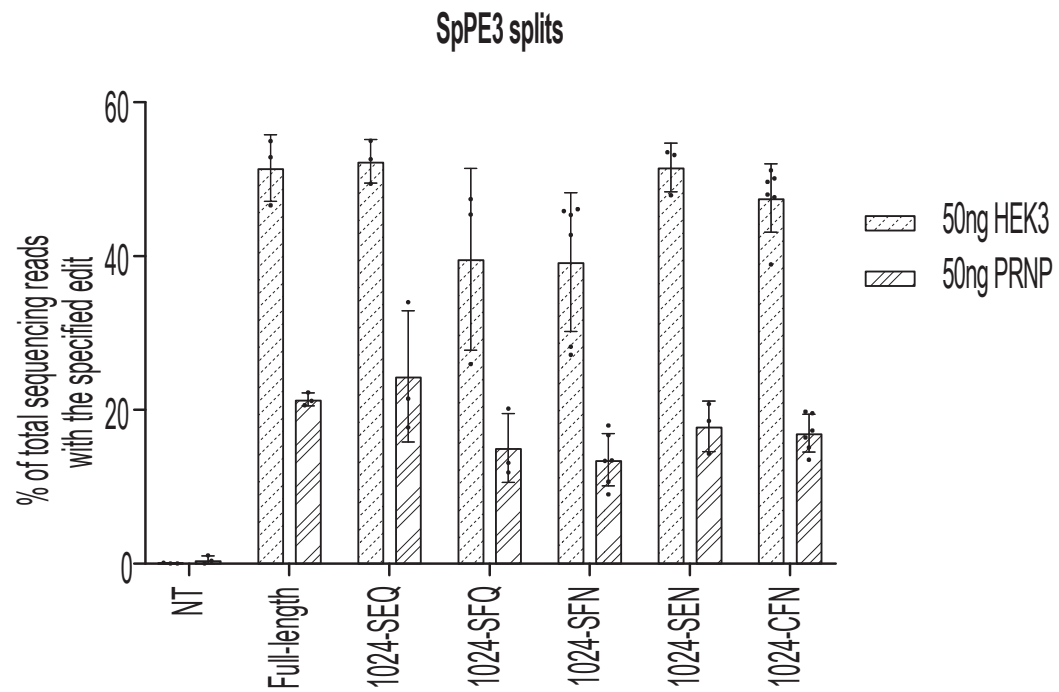


FIG. 76

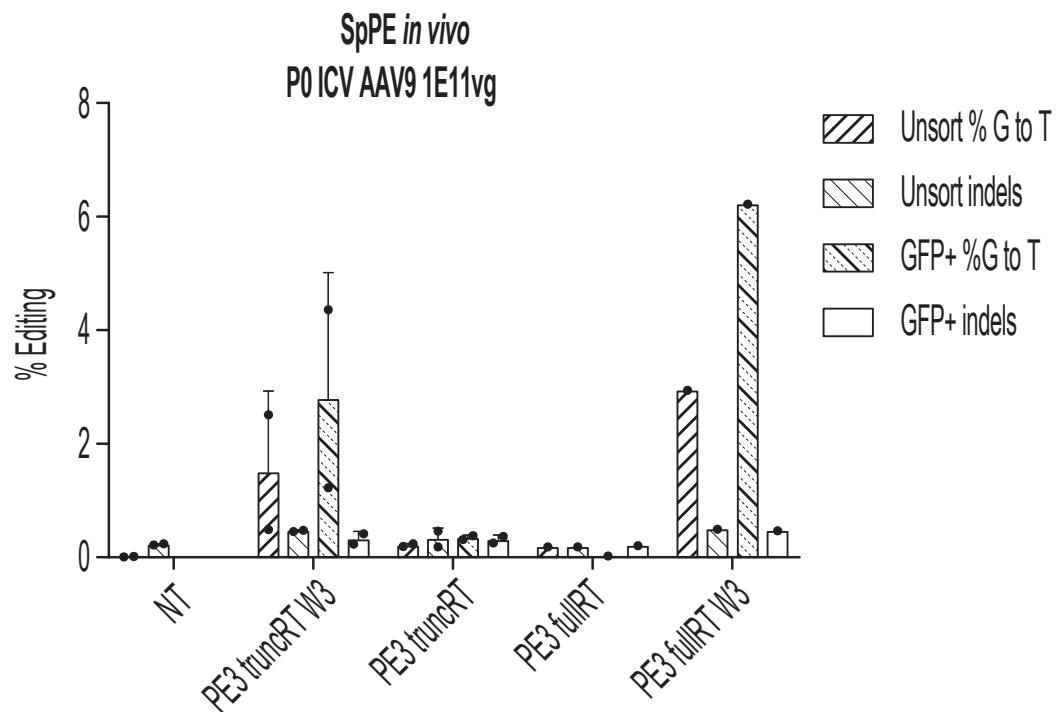


FIG. 77

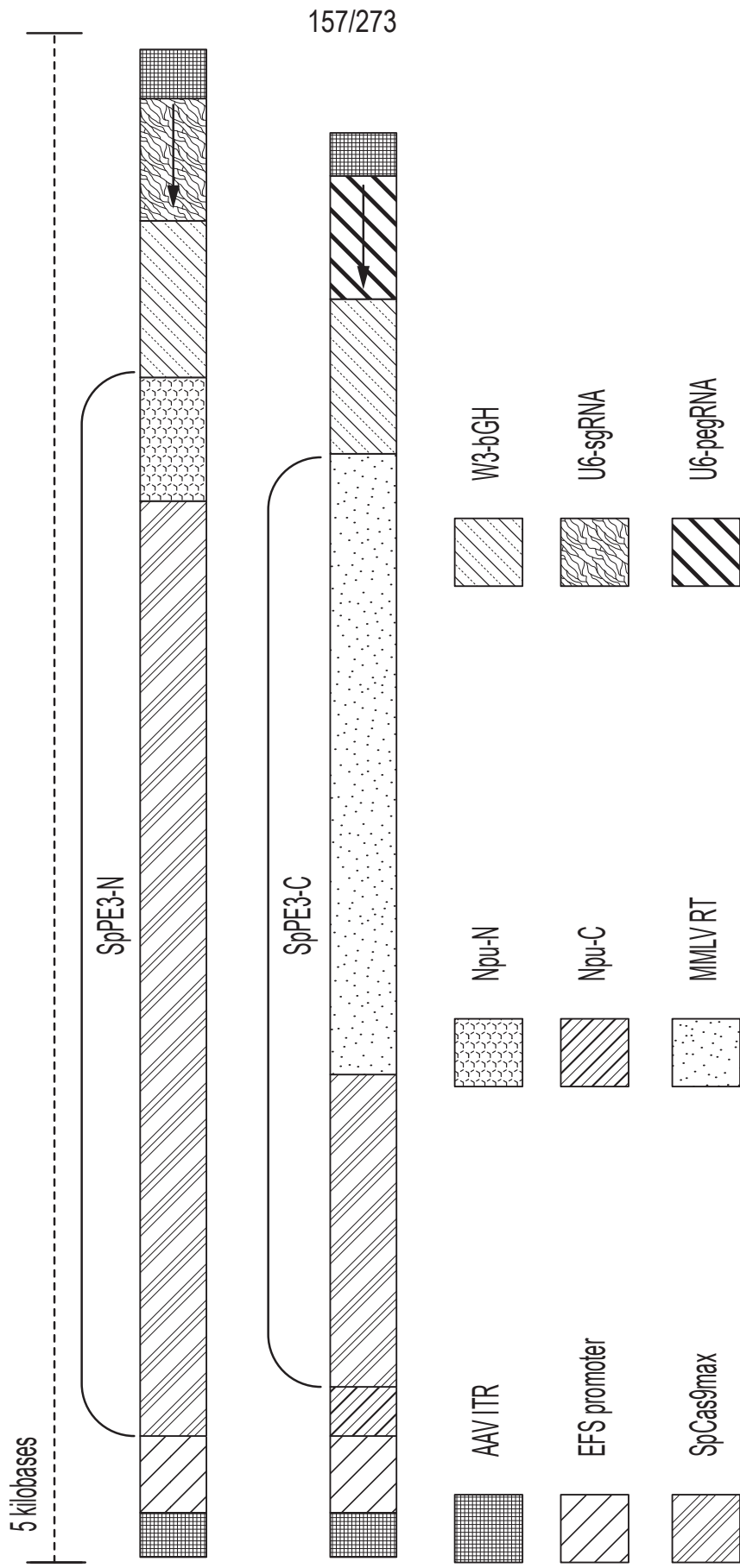


FIG. 78

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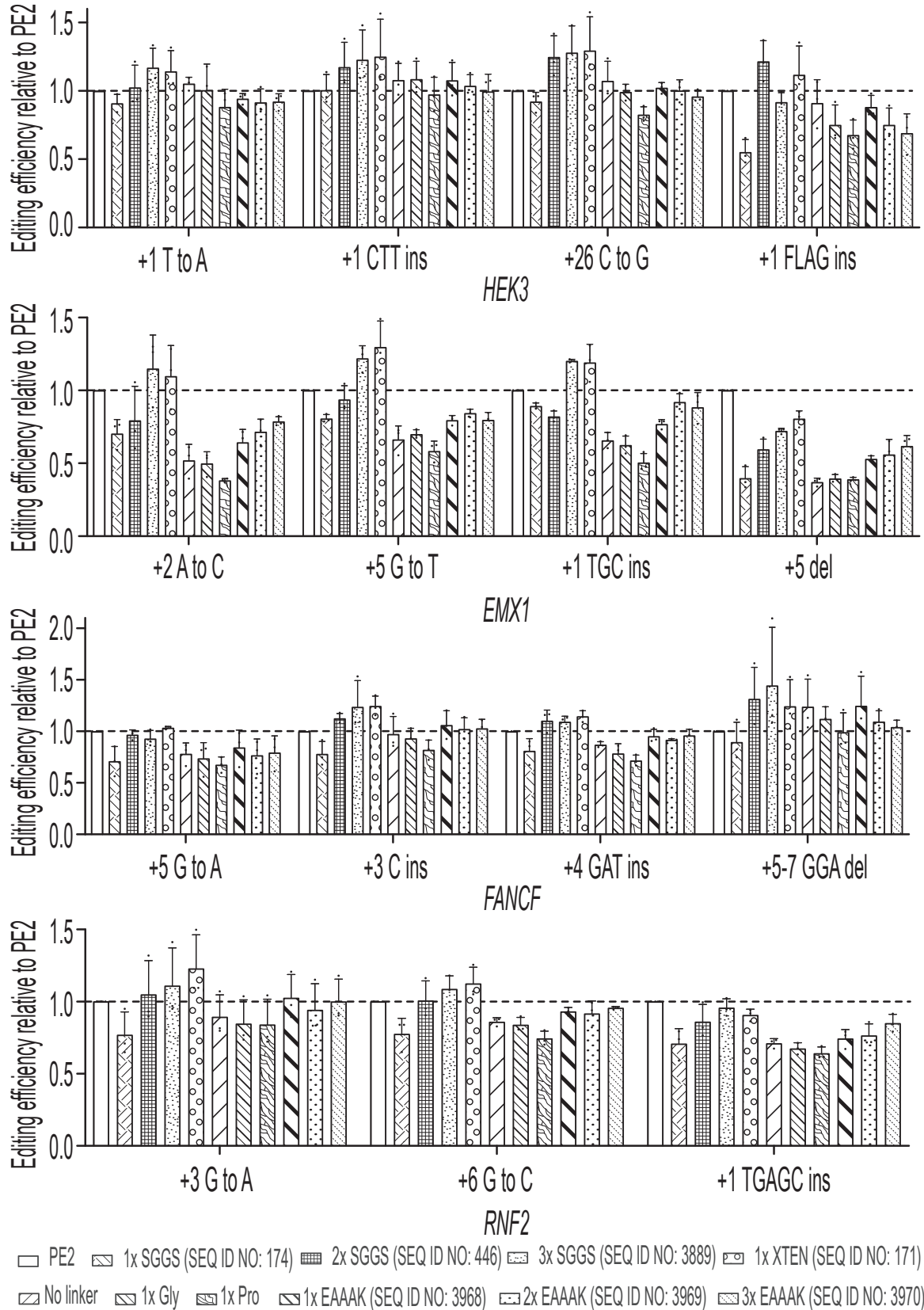


FIG. 79

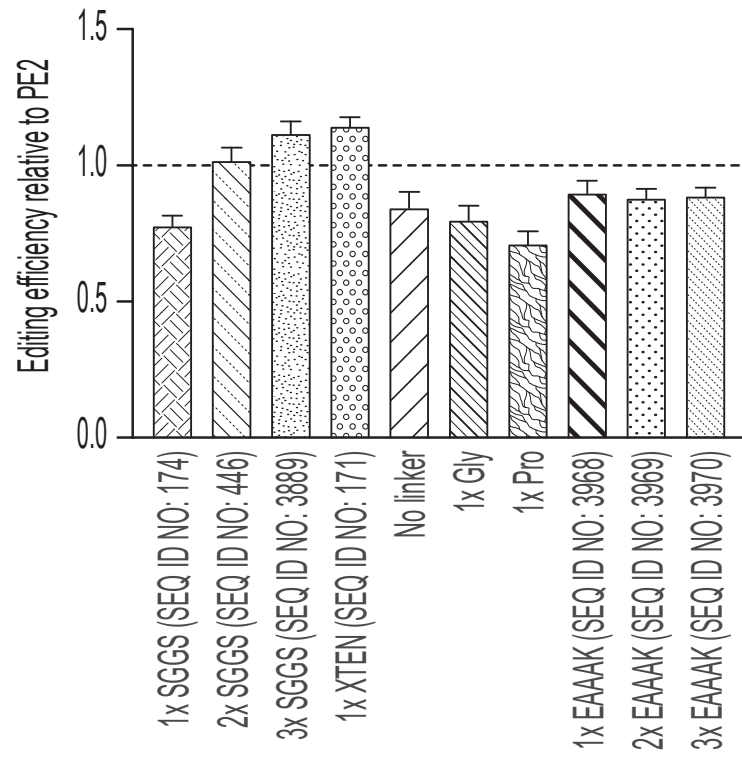


FIG. 80

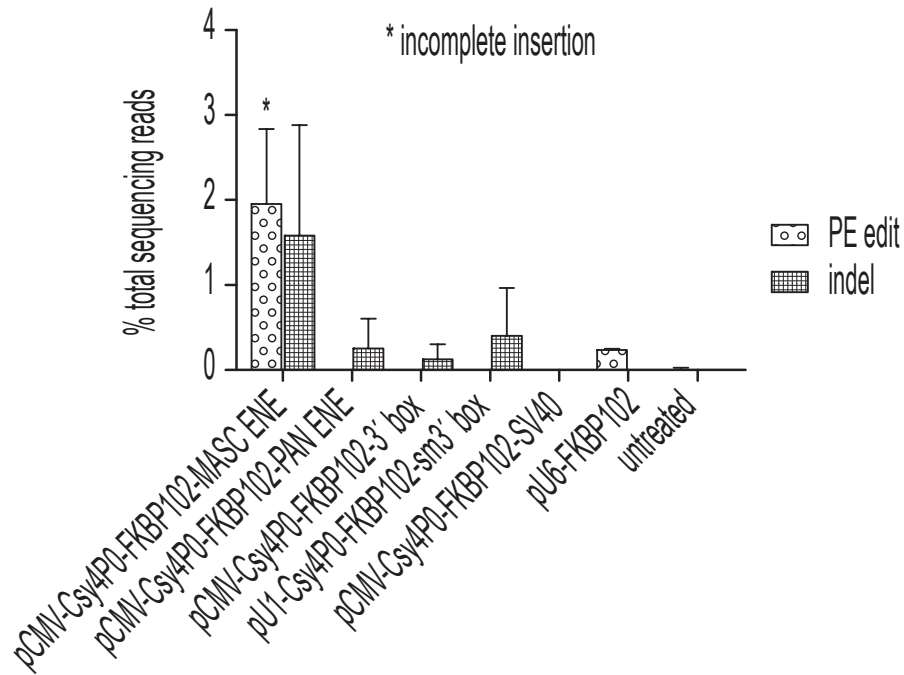


FIG. 81

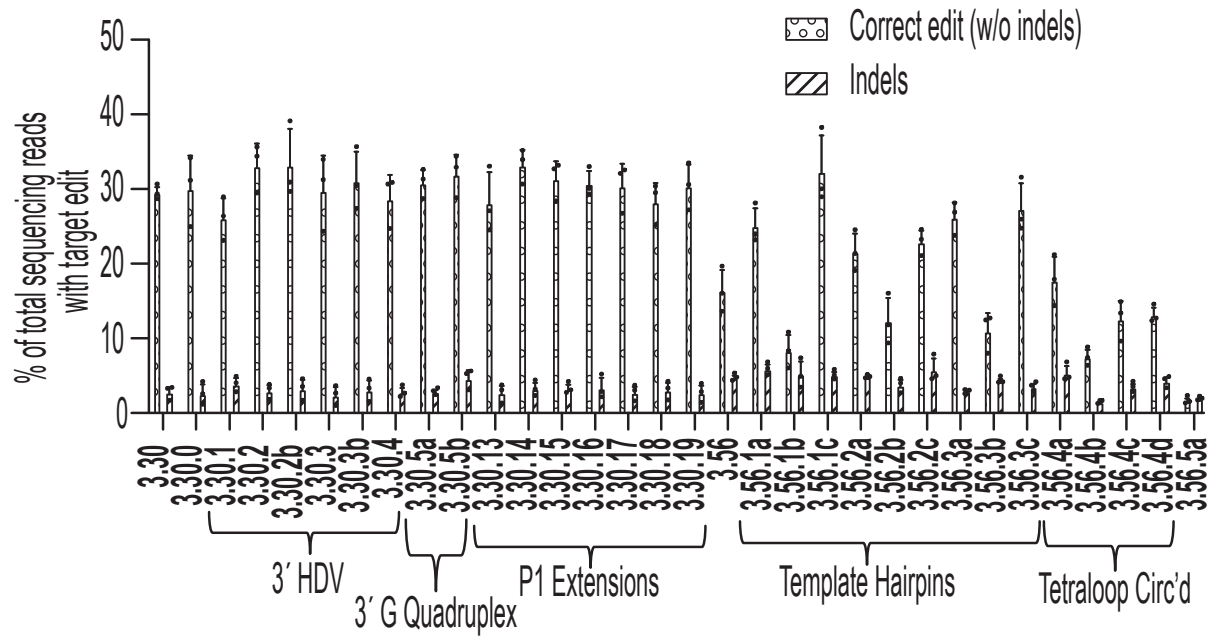


FIG. 82

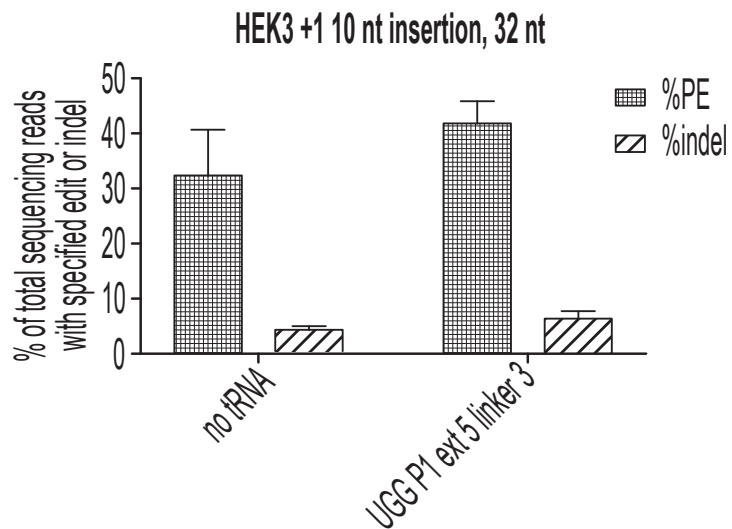


FIG. 83



FIG. 84B



FIG. 84B

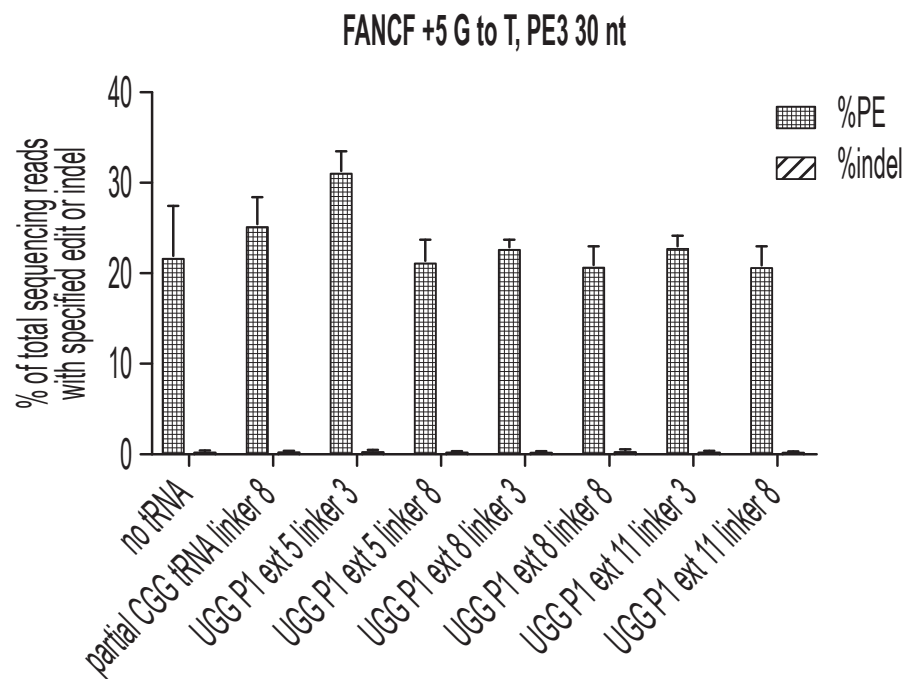


FIG. 85

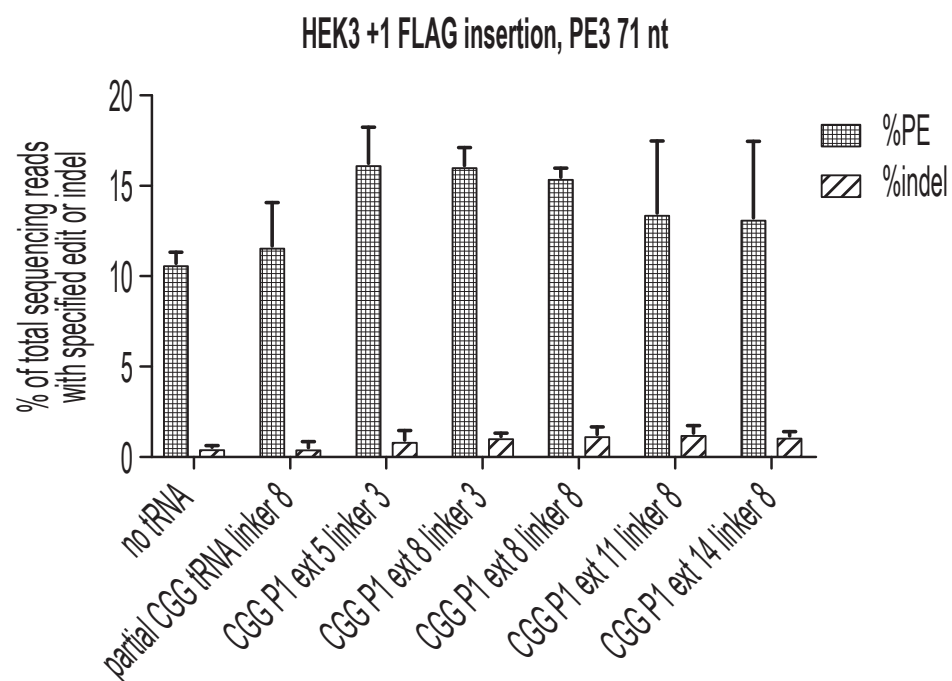


FIG. 86

CDKL5 1412delA SpPE3

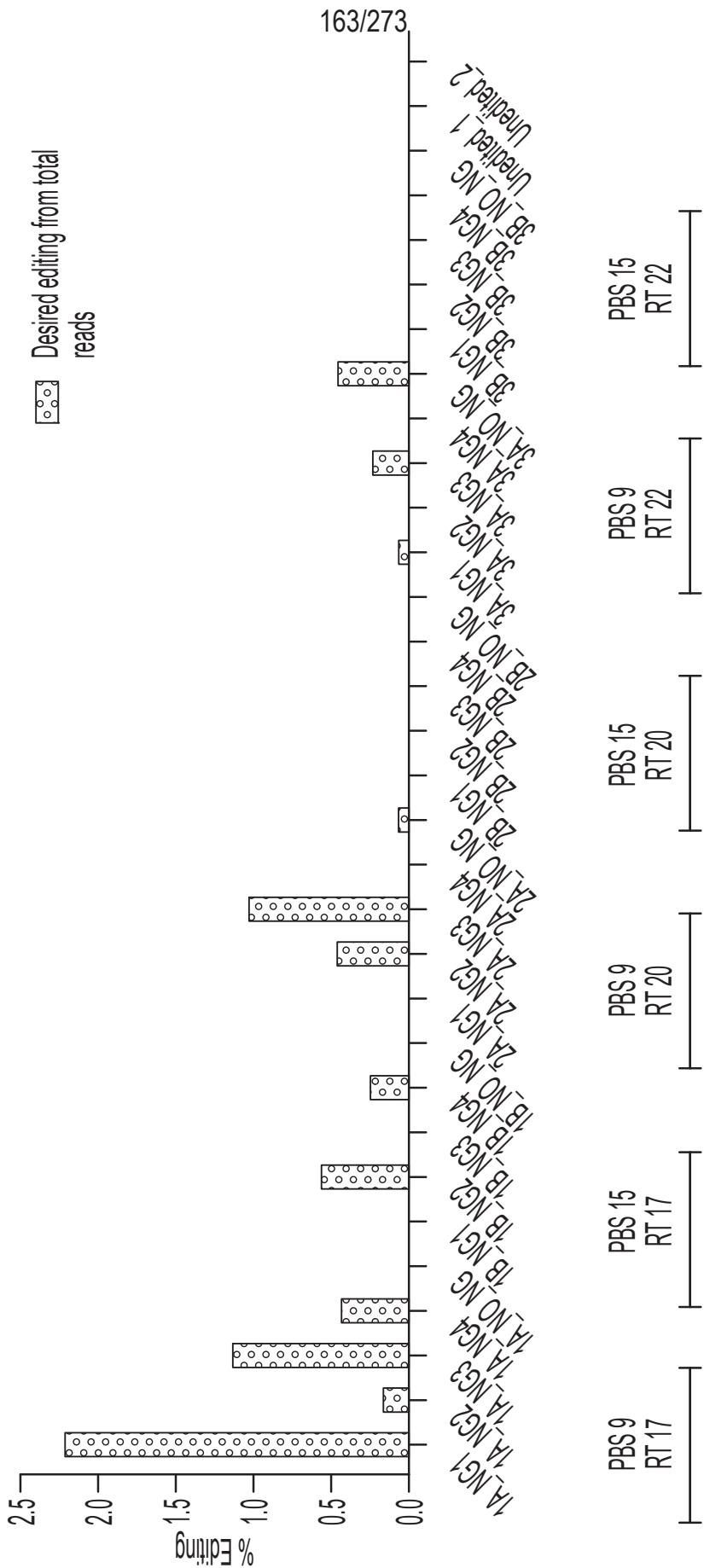
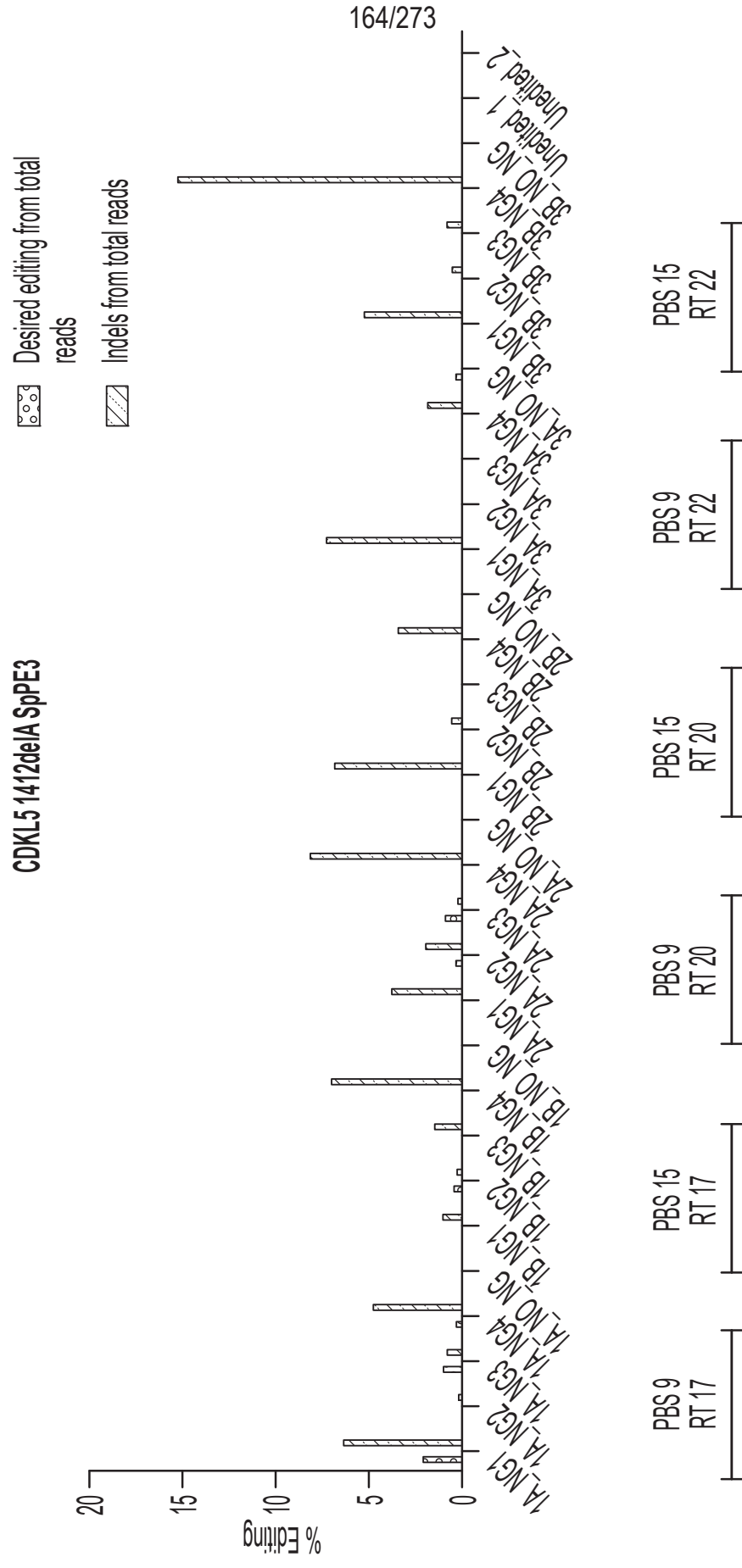


FIG. 87



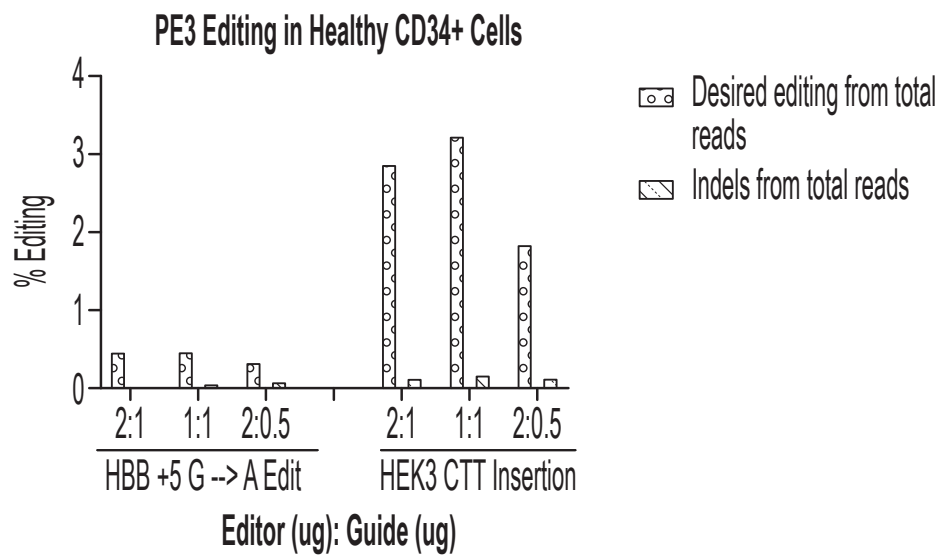


FIG. 89

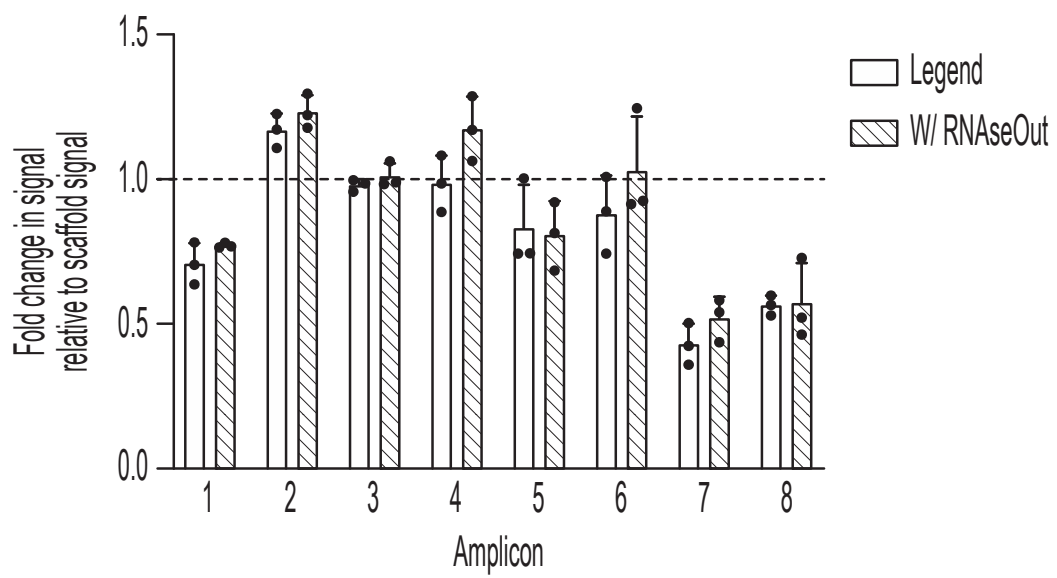


FIG. 90A

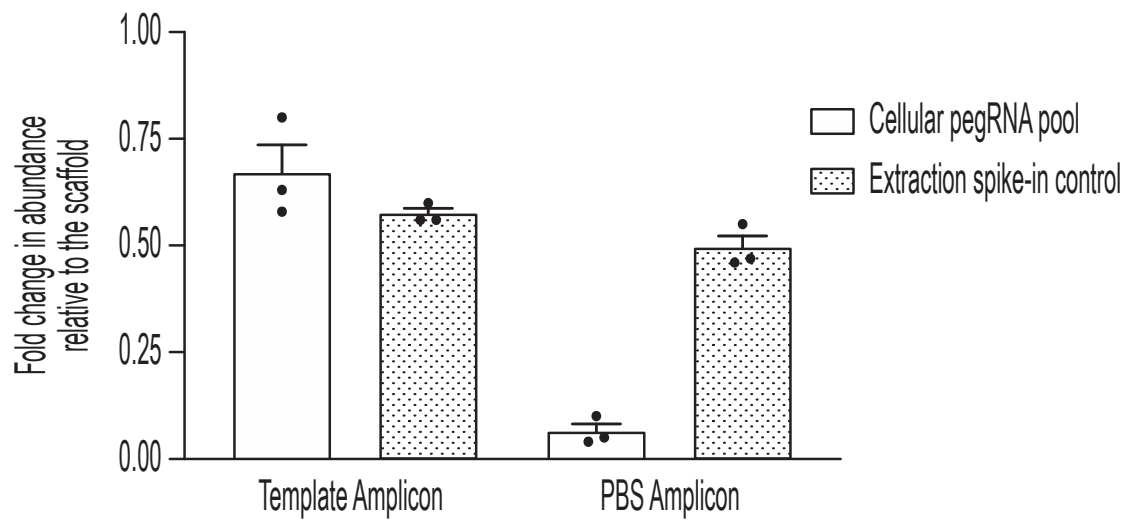


FIG. 90B

pegRNA sequence: SEQ ID NO: 1762

GGCCCAGACTGAGCACGTGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGA
AAAGTGGCACCAGATCGGTGCTGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGTCGTCATCCTTGT
ATCCGTGCTCAGTCTG

Template amplicon sequence: SEQ ID NO: 1763

AAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCAGATCGGTGCTGGAGGAAGCAGGGCTTCCTTTC
TCTGCCATCACTTATCGTC

PBS amplicon sequence: SEQ ID NO: 1764

TGAAAAAGTGGCACCAGATCGGTGCTGGAGGAAGCAGGGCTTCCTTTCCTCTGCCATCACTTATCGTCGTCATCC
TGTGAATCCGTGCTCAGTCTGT

FIG. 90C

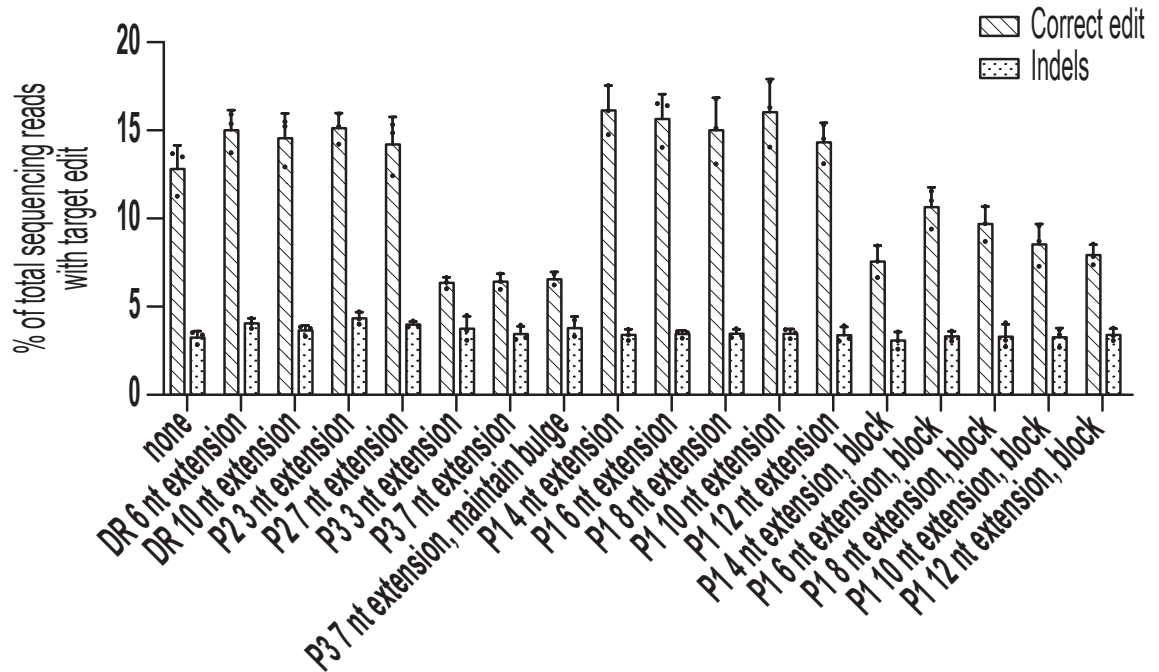


FIG. 91A

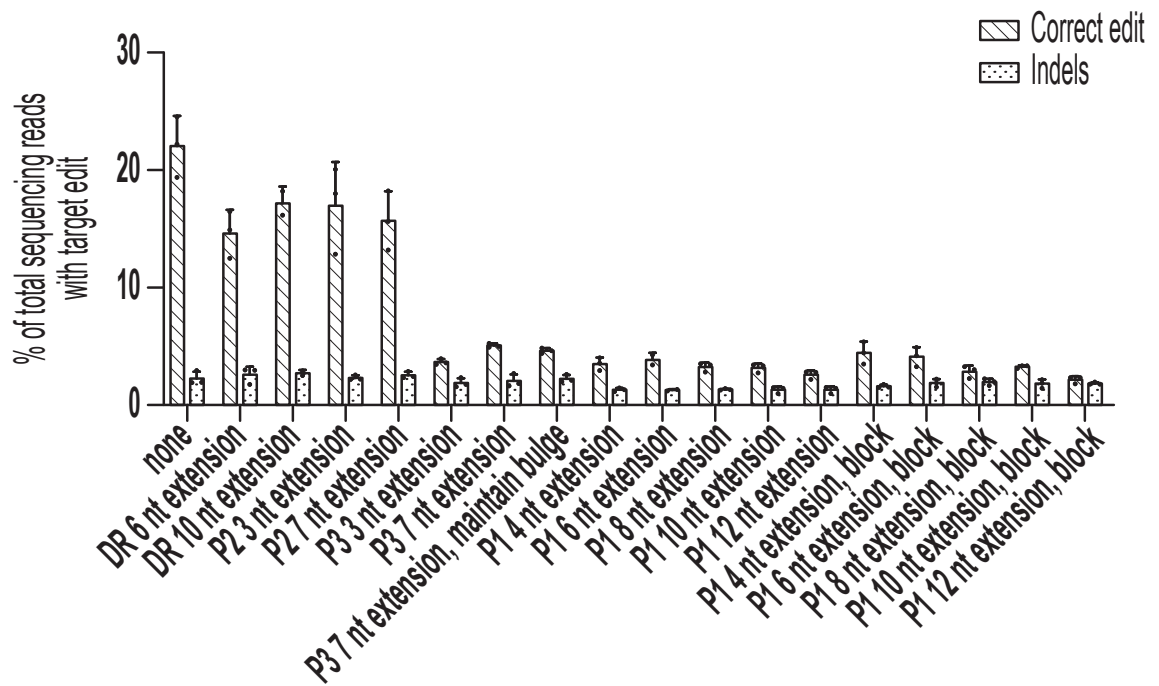


FIG. 91B

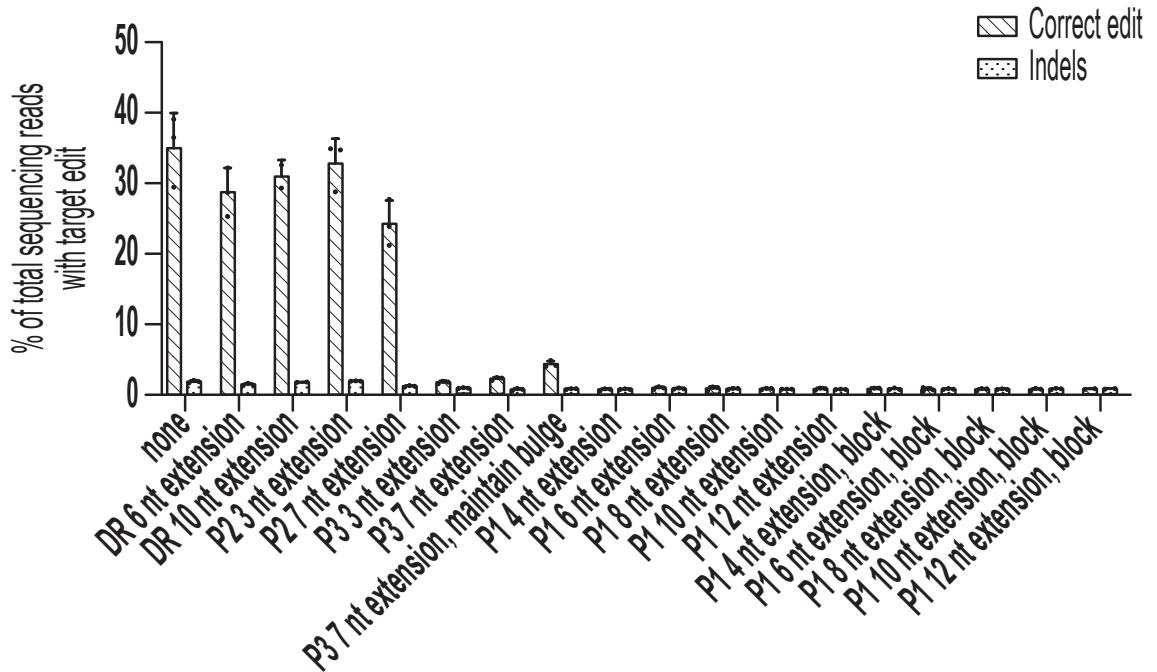


FIG. 91C

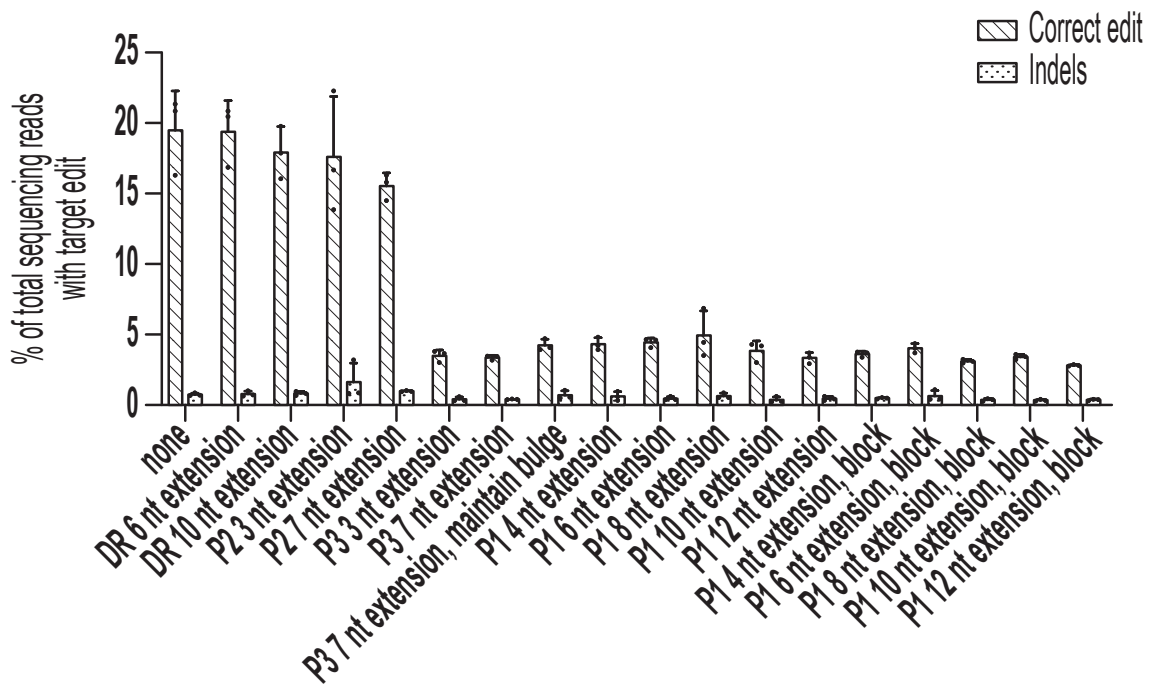


FIG. 91D

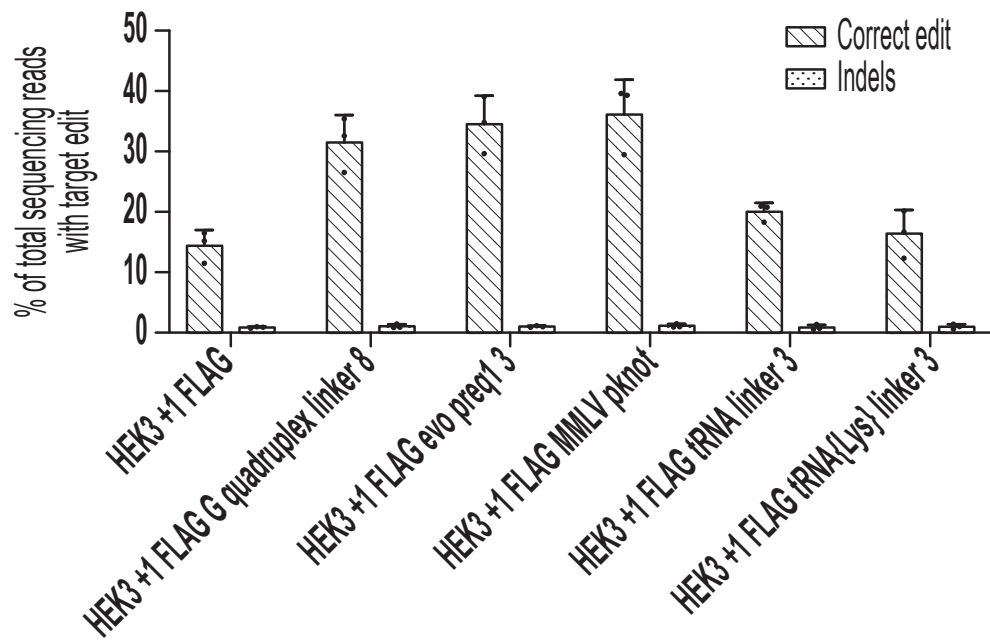


FIG. 92A

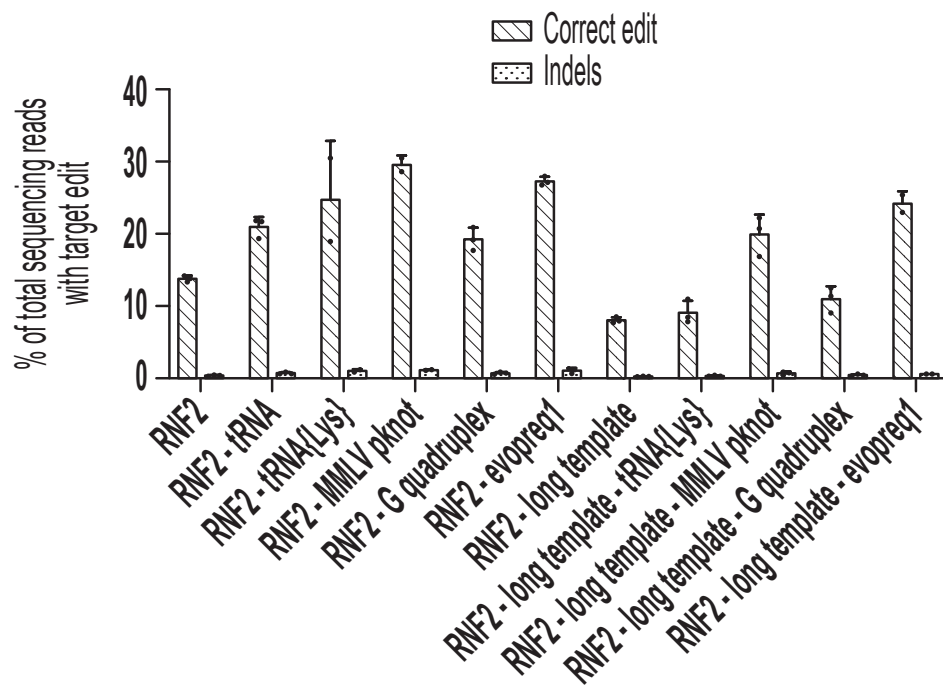


FIG. 92B

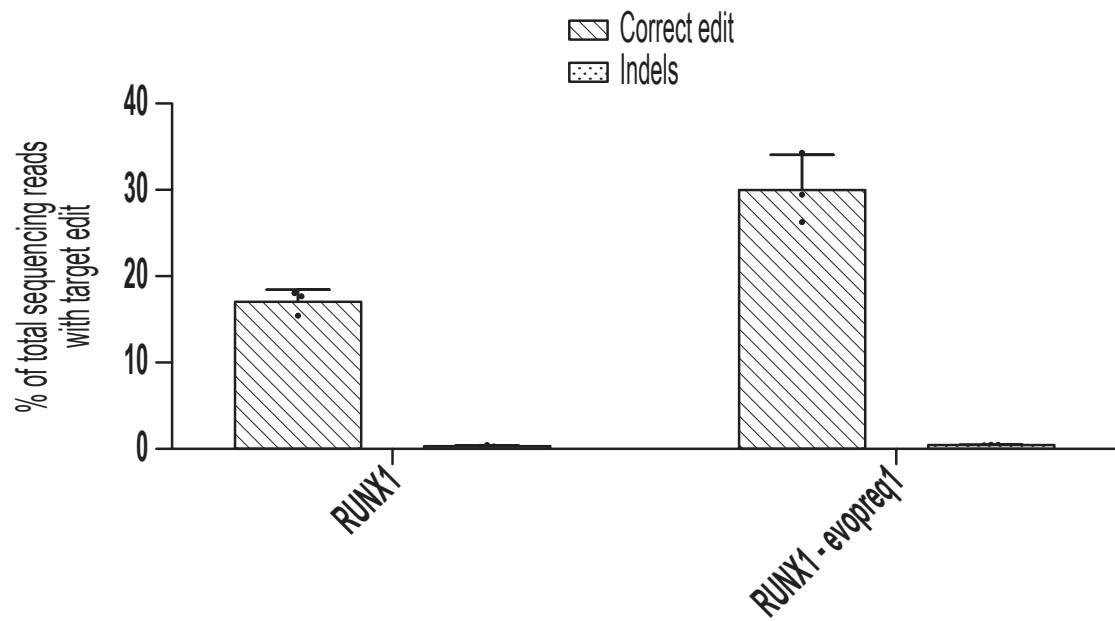


FIG. 92C

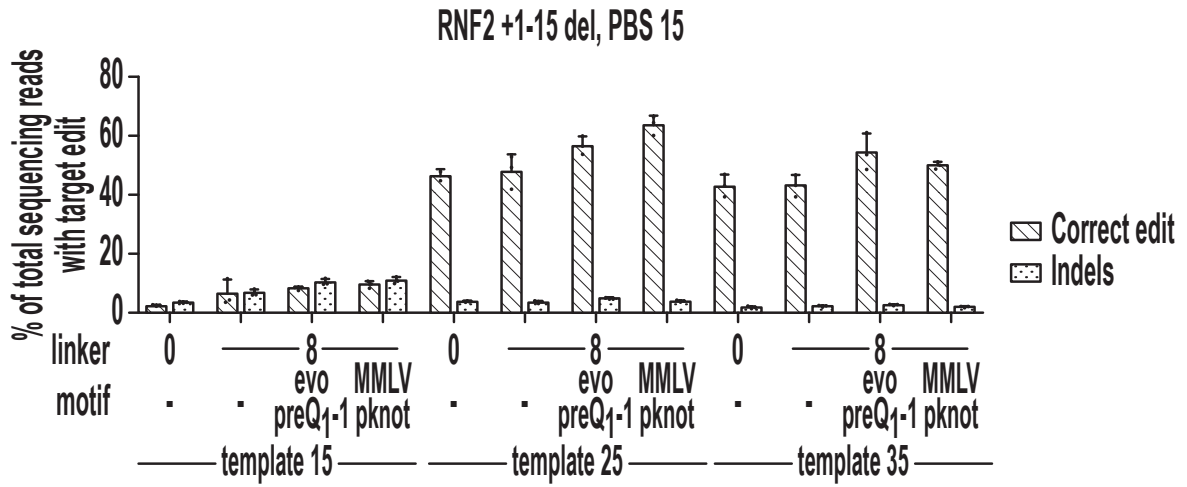


FIG. 93A

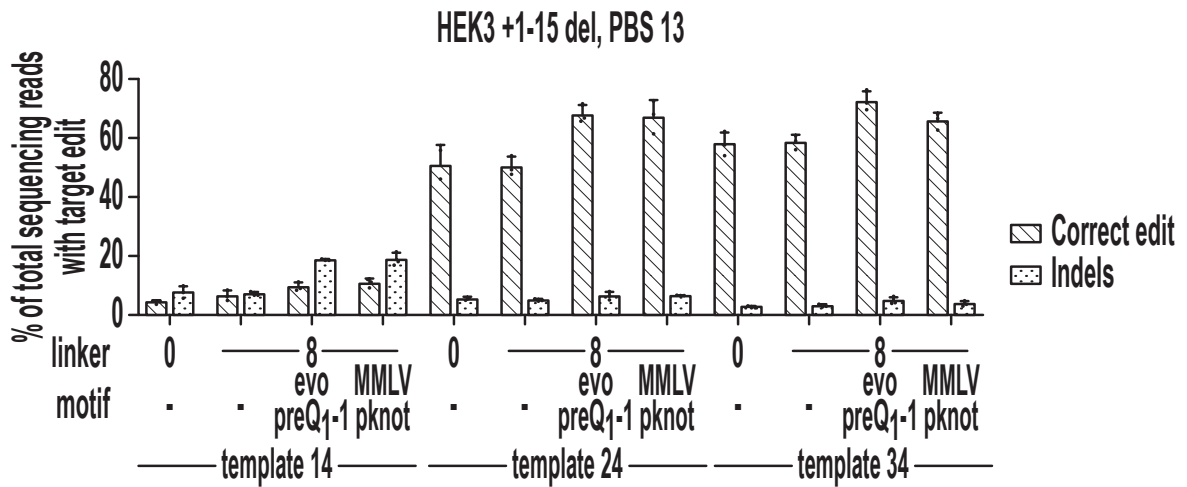


FIG. 93B

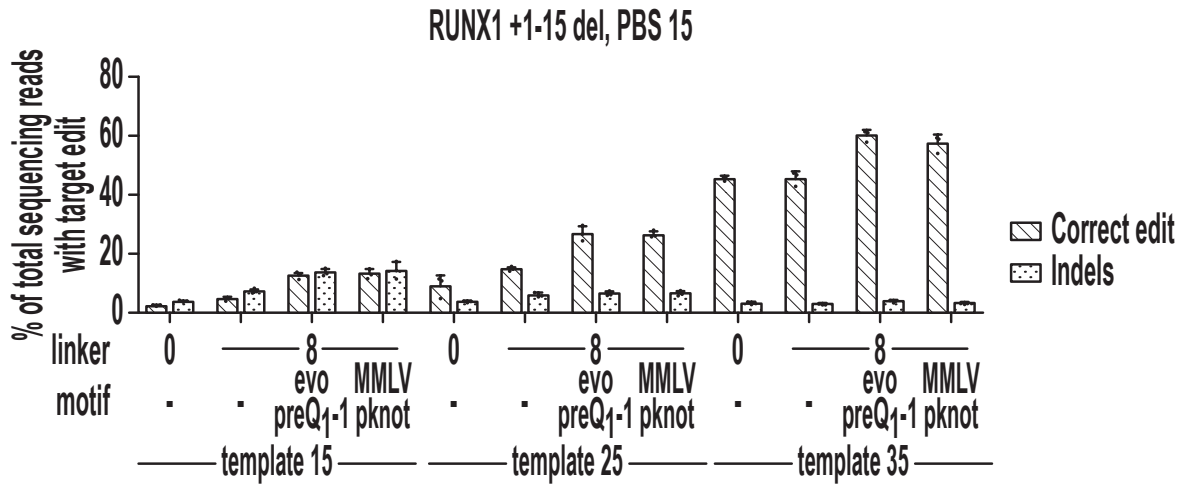


FIG. 93C

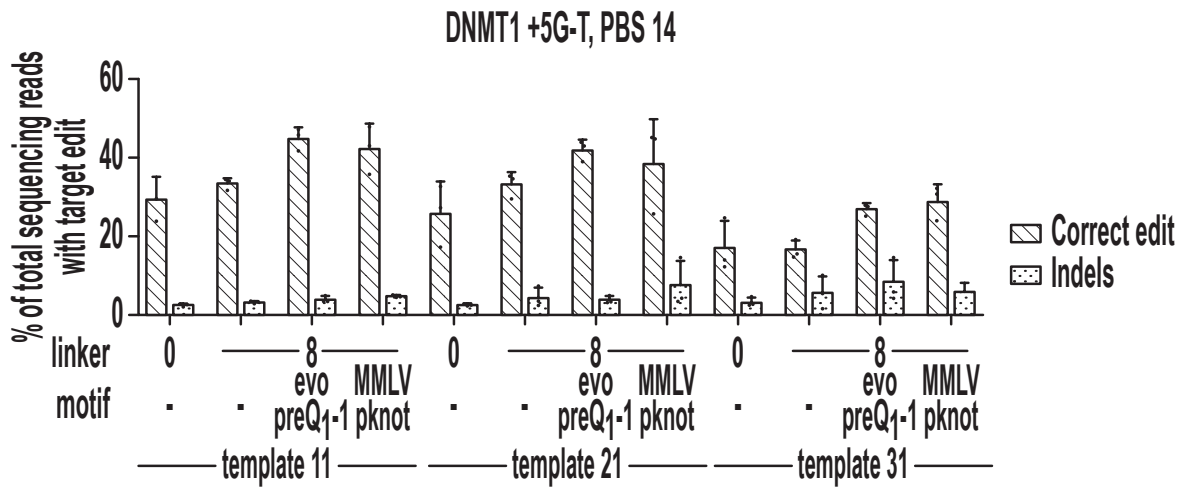


FIG. 93D

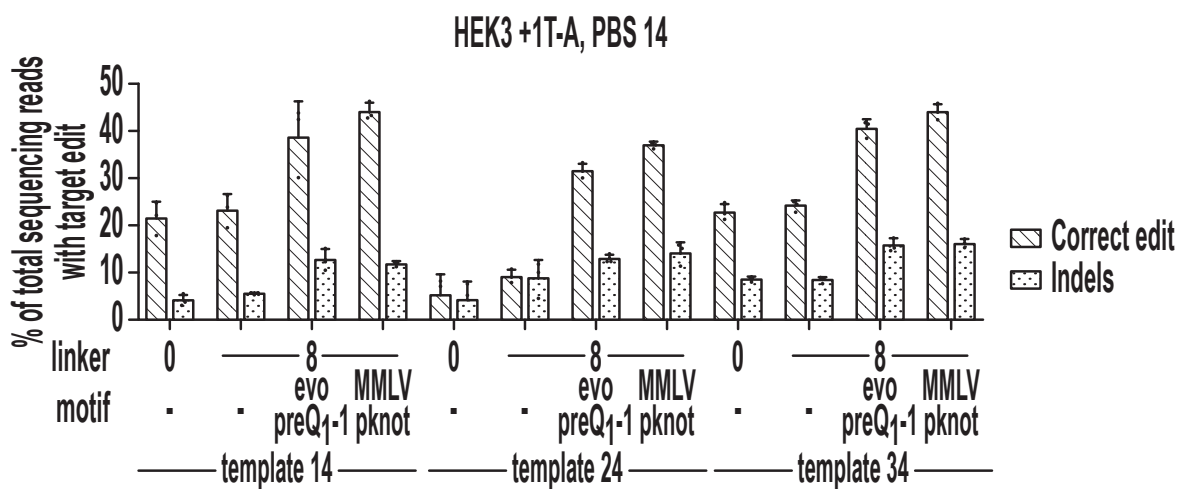


FIG. 93E

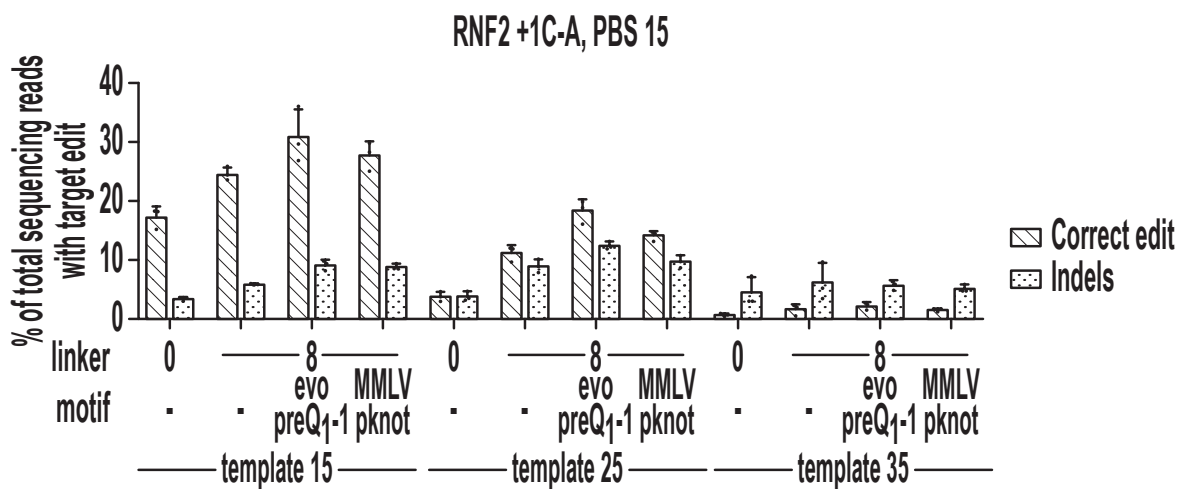


FIG. 93F

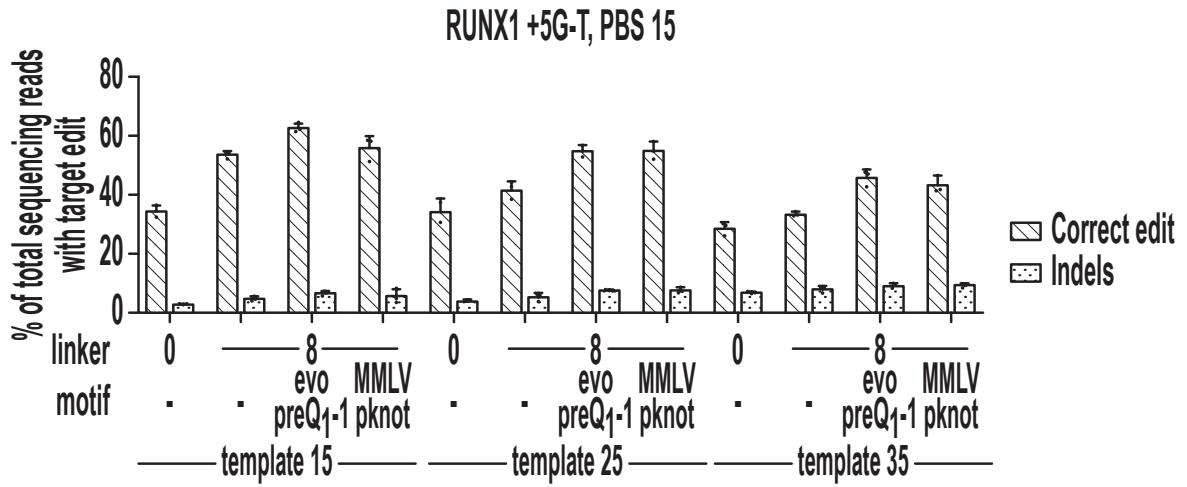


FIG. 93G

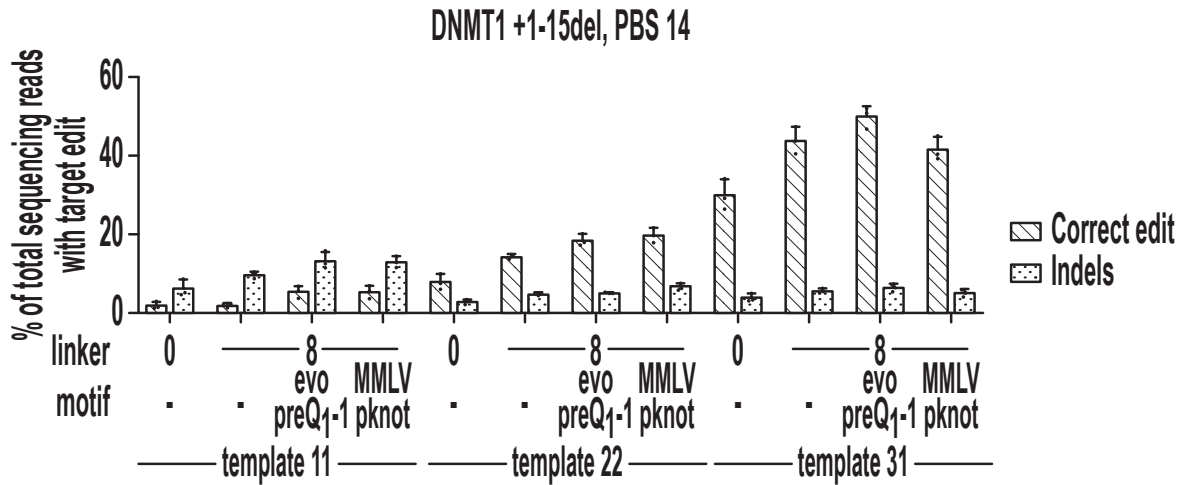


FIG. 93H

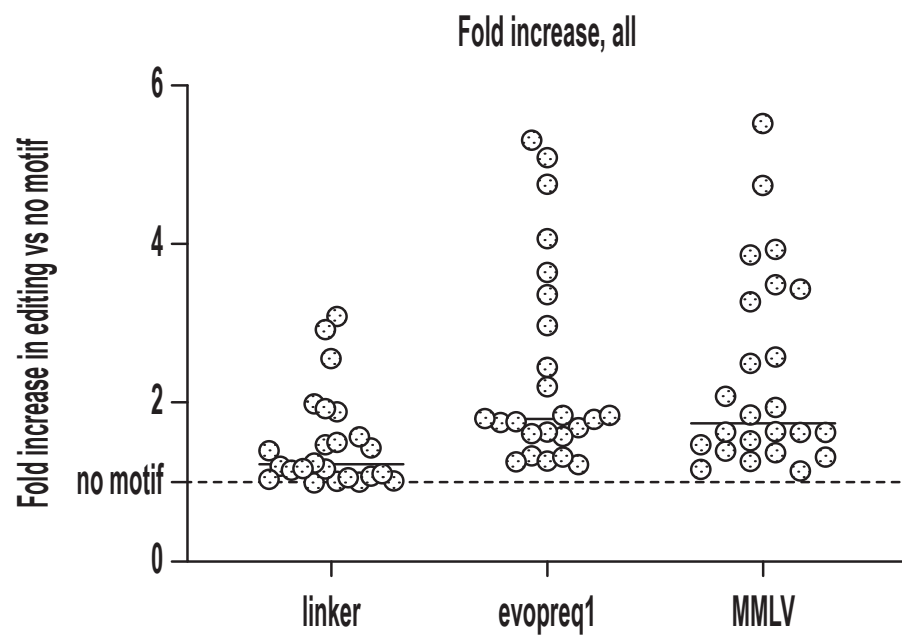


FIG. 94

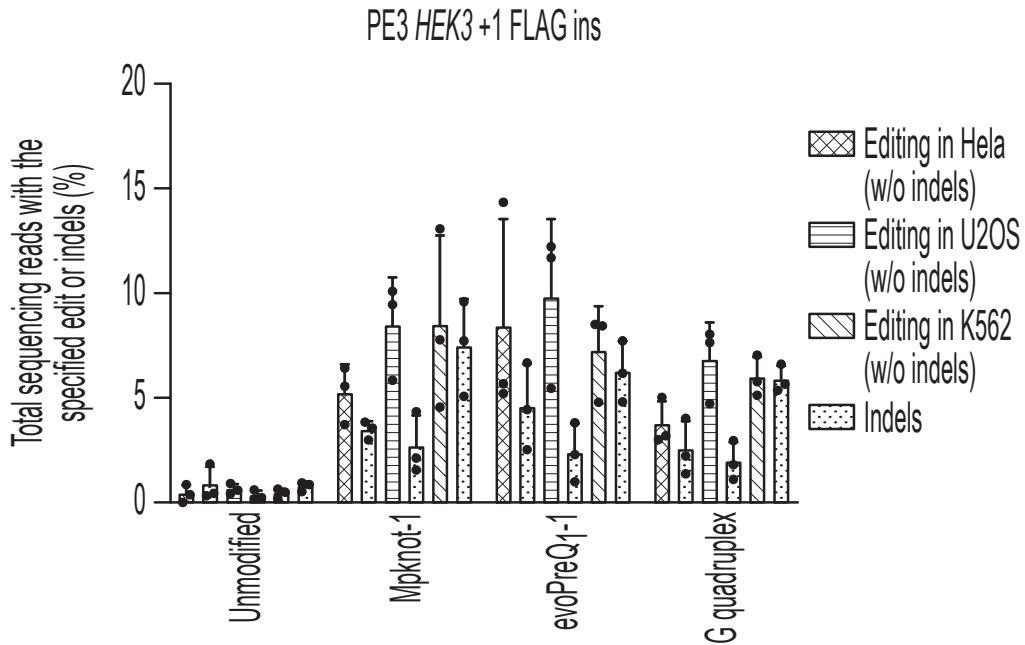


FIG. 95A

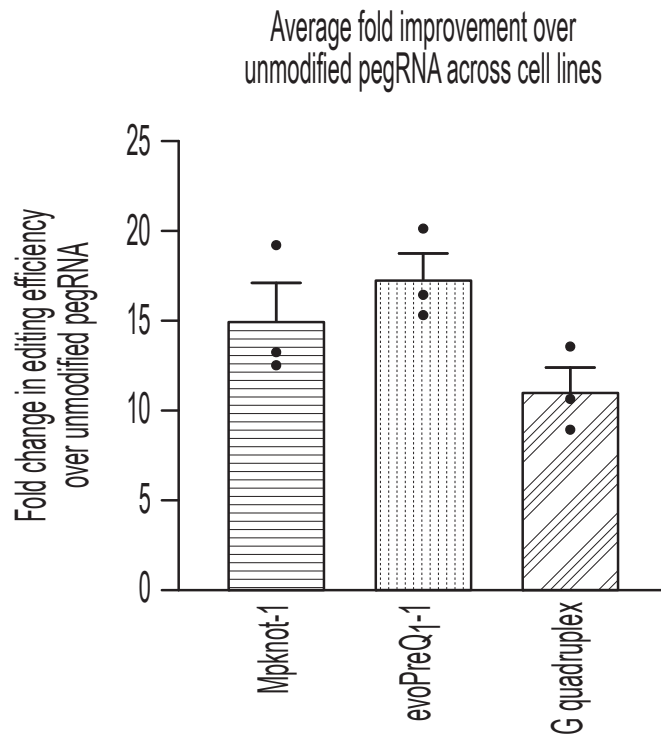


FIG. 95B

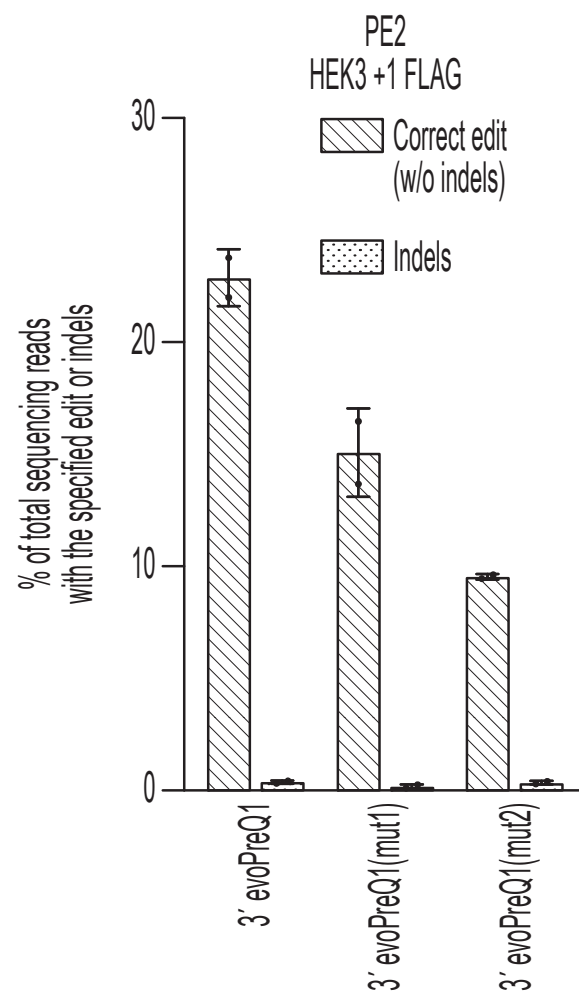


FIG. 96

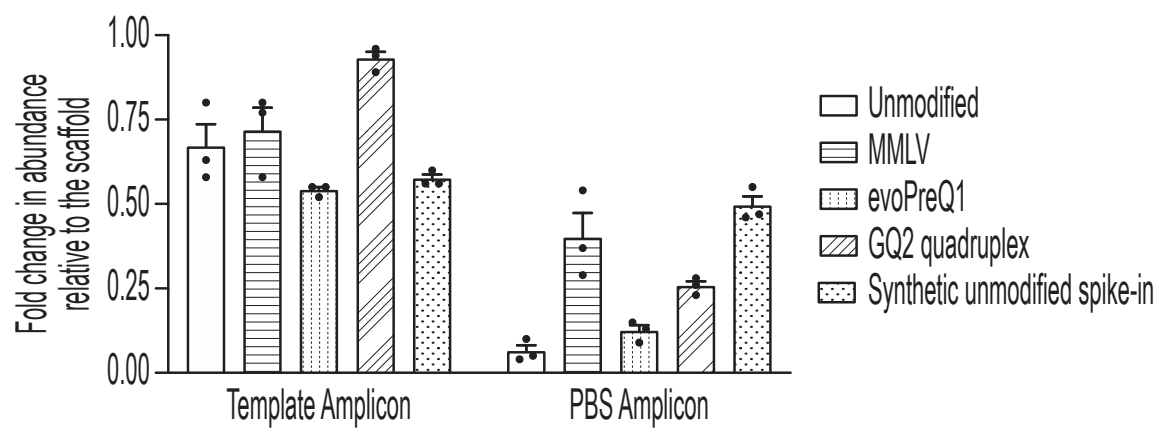


FIG. 97

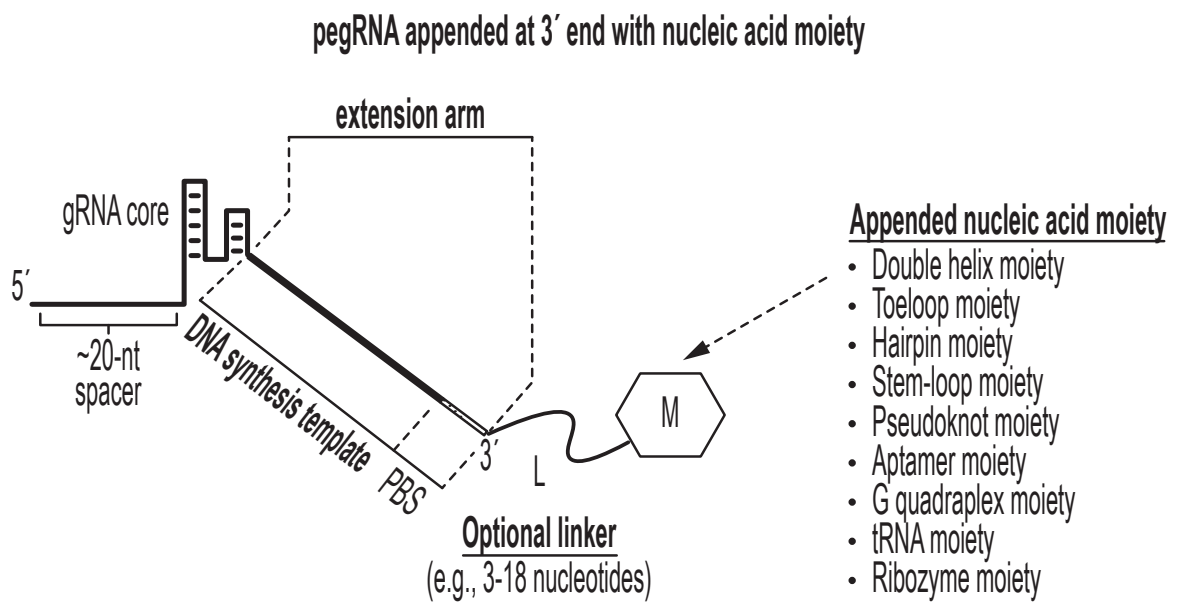


FIG. 98

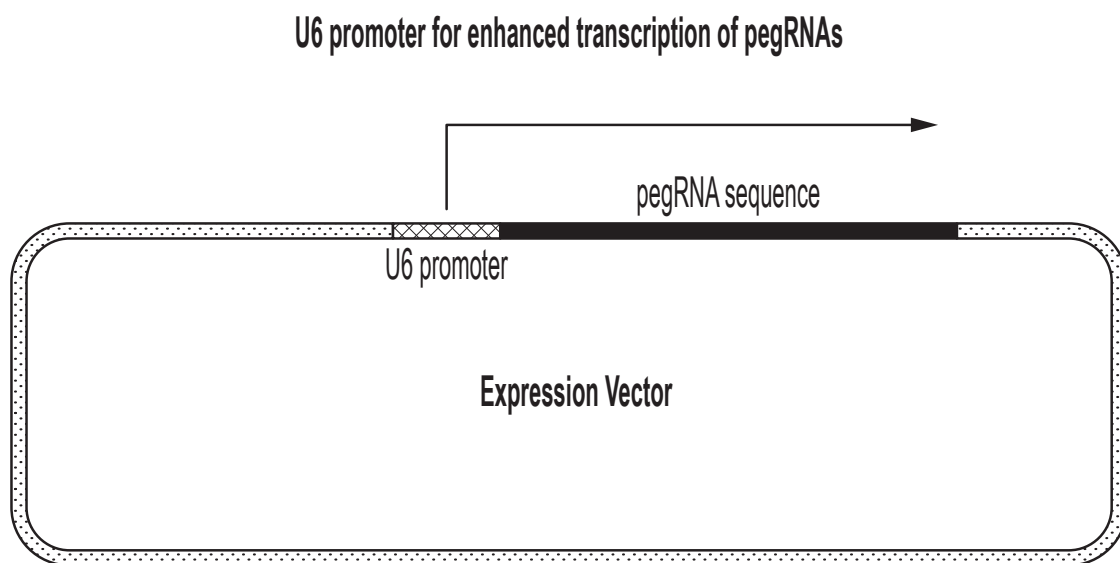


FIG. 99

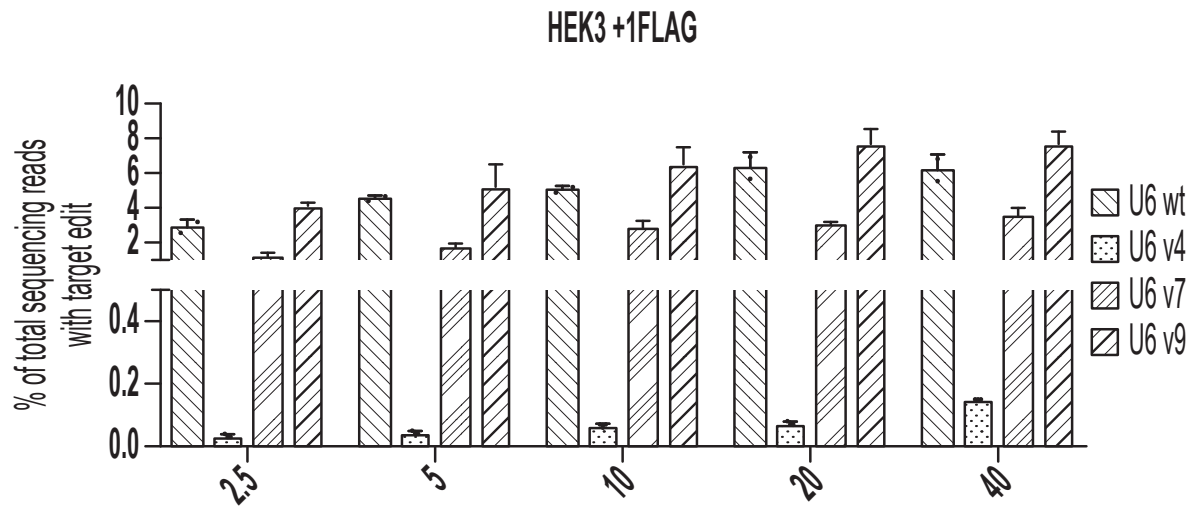


FIG. 100A

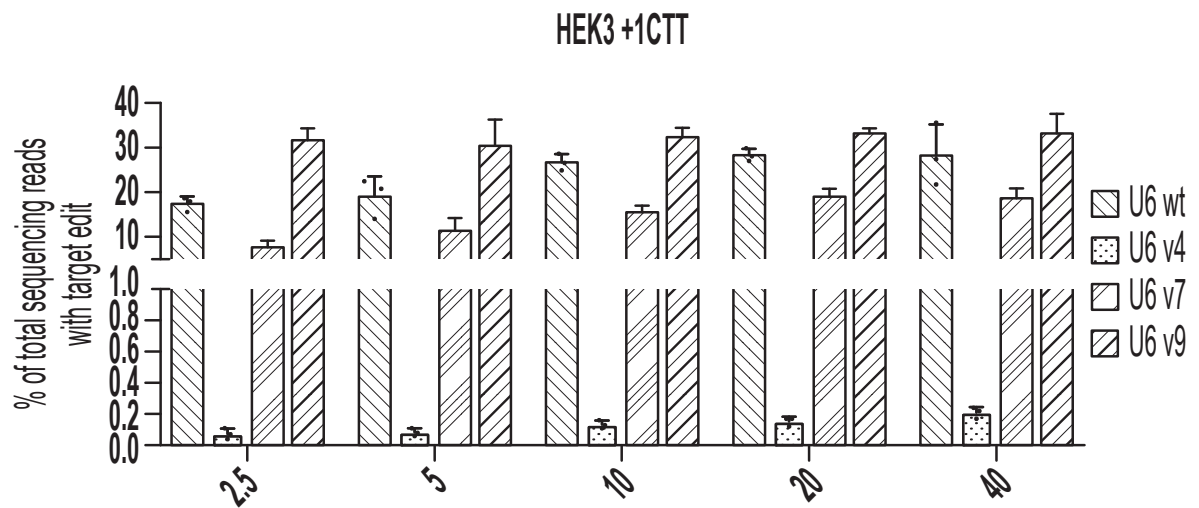


FIG. 100B

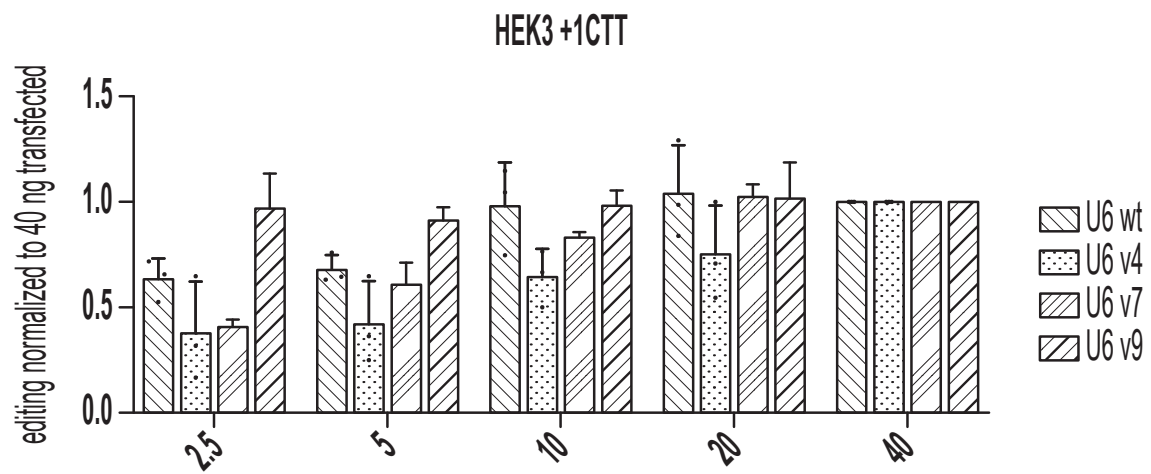


FIG. 100C

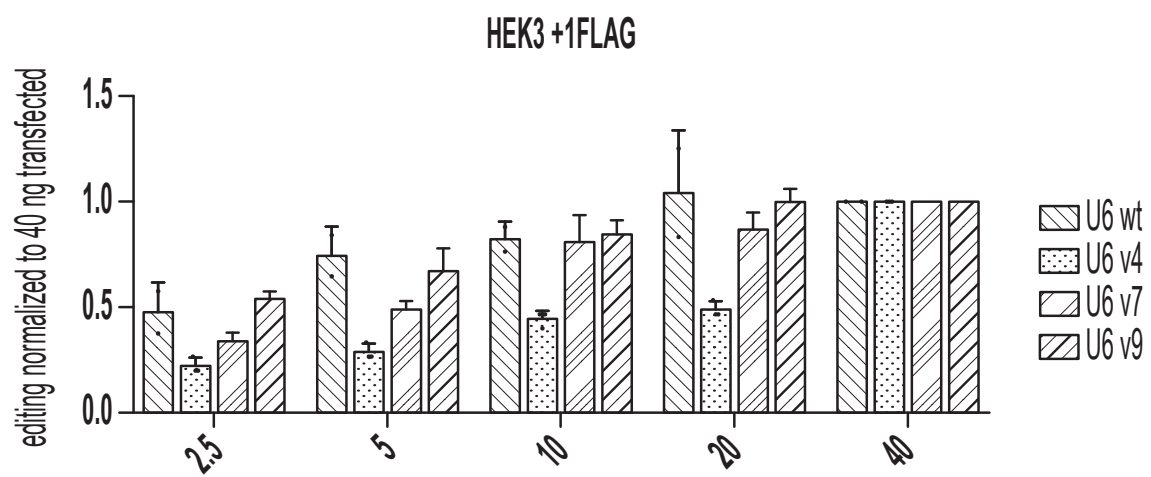


FIG. 100D

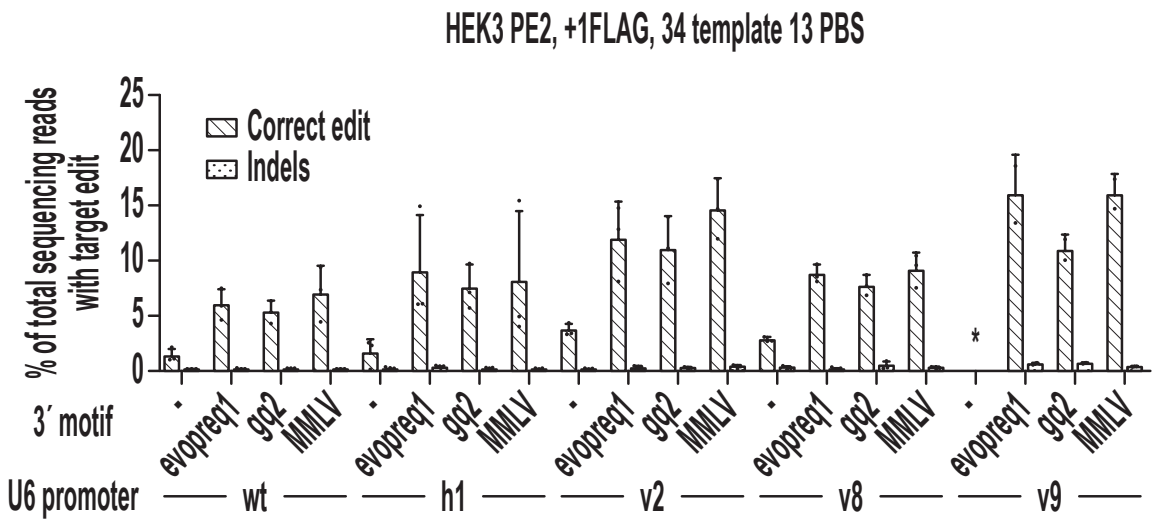


FIG. 100E

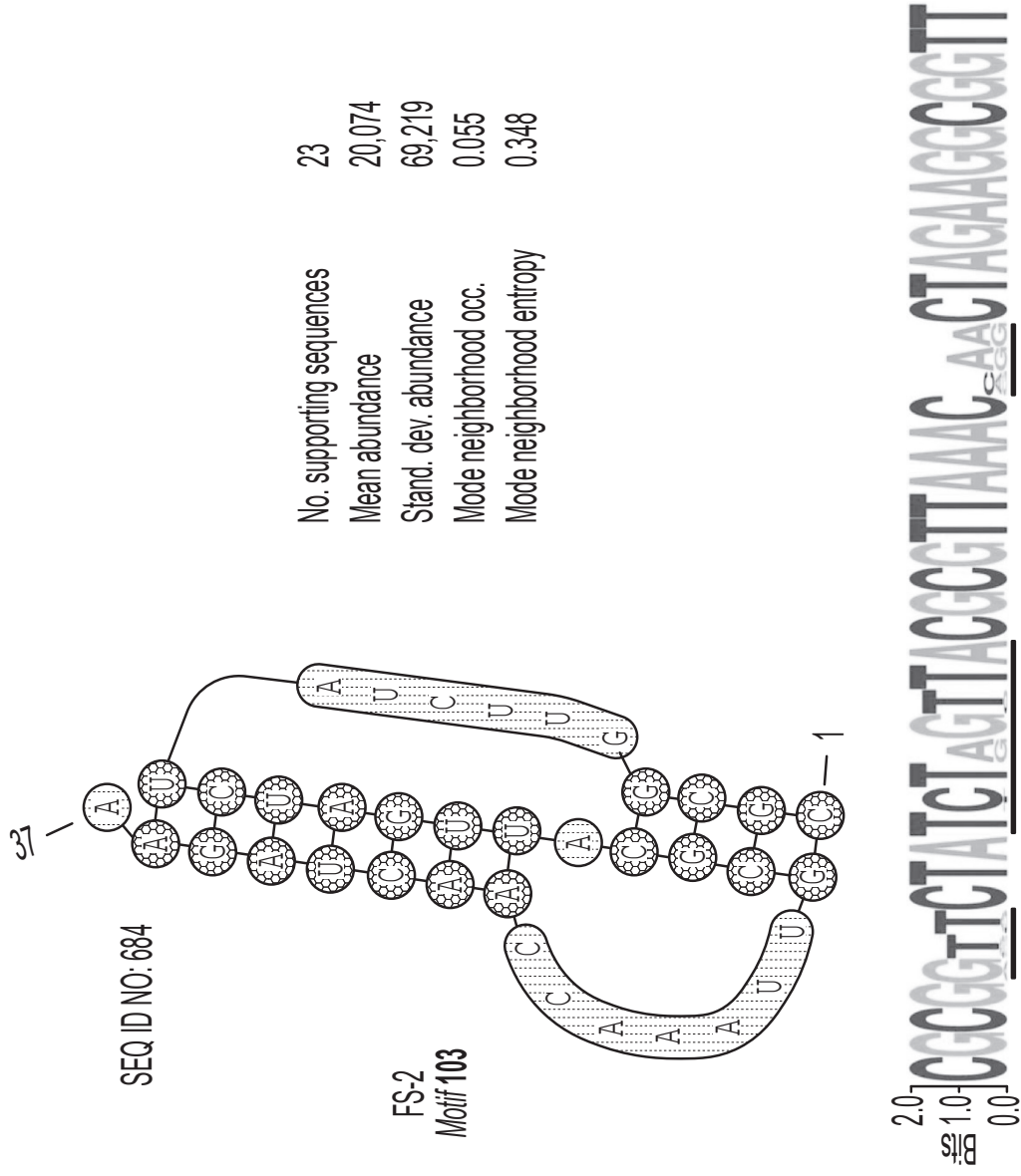


FIG. 101

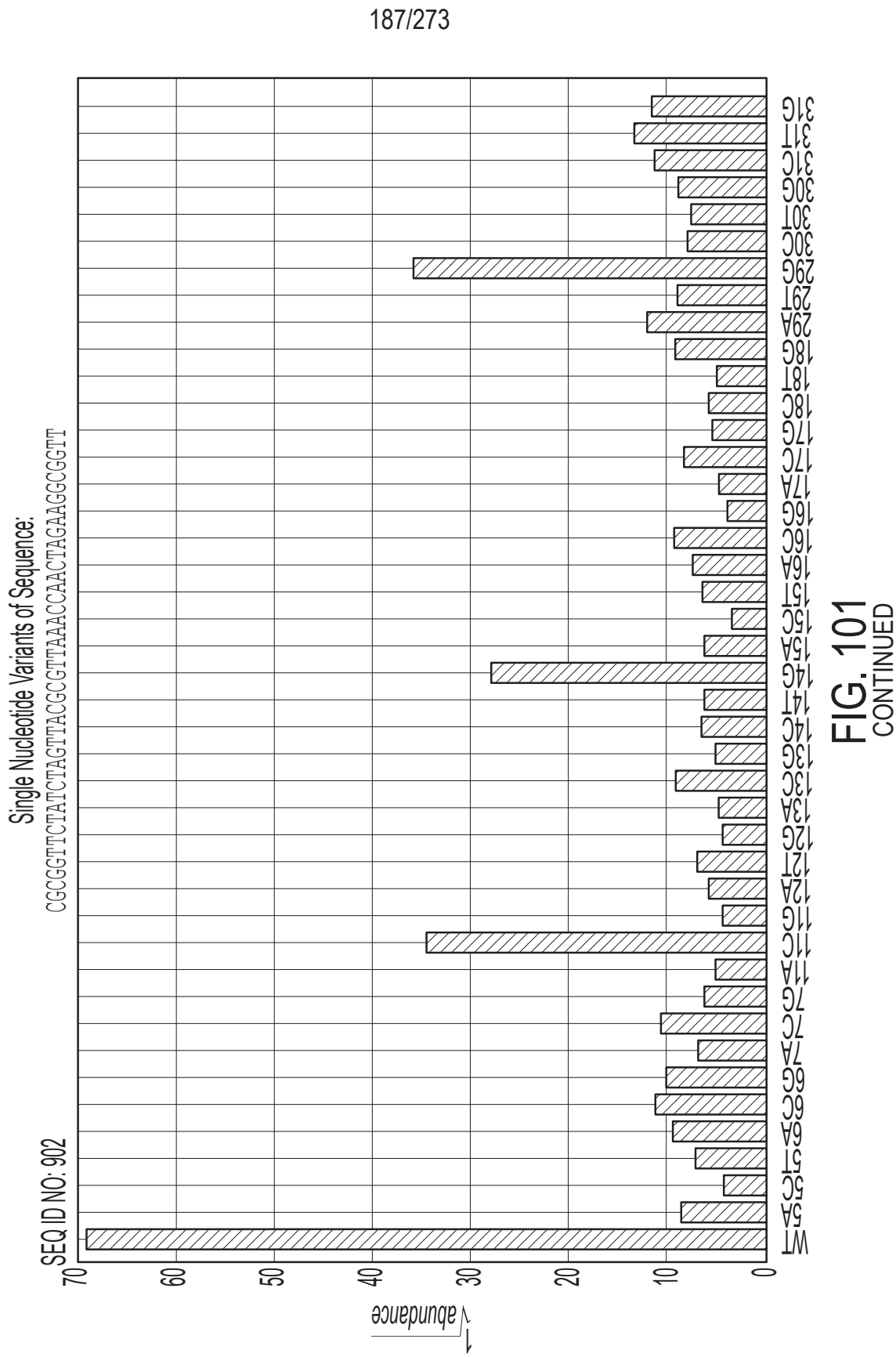


FIG. 101
CONTINUED

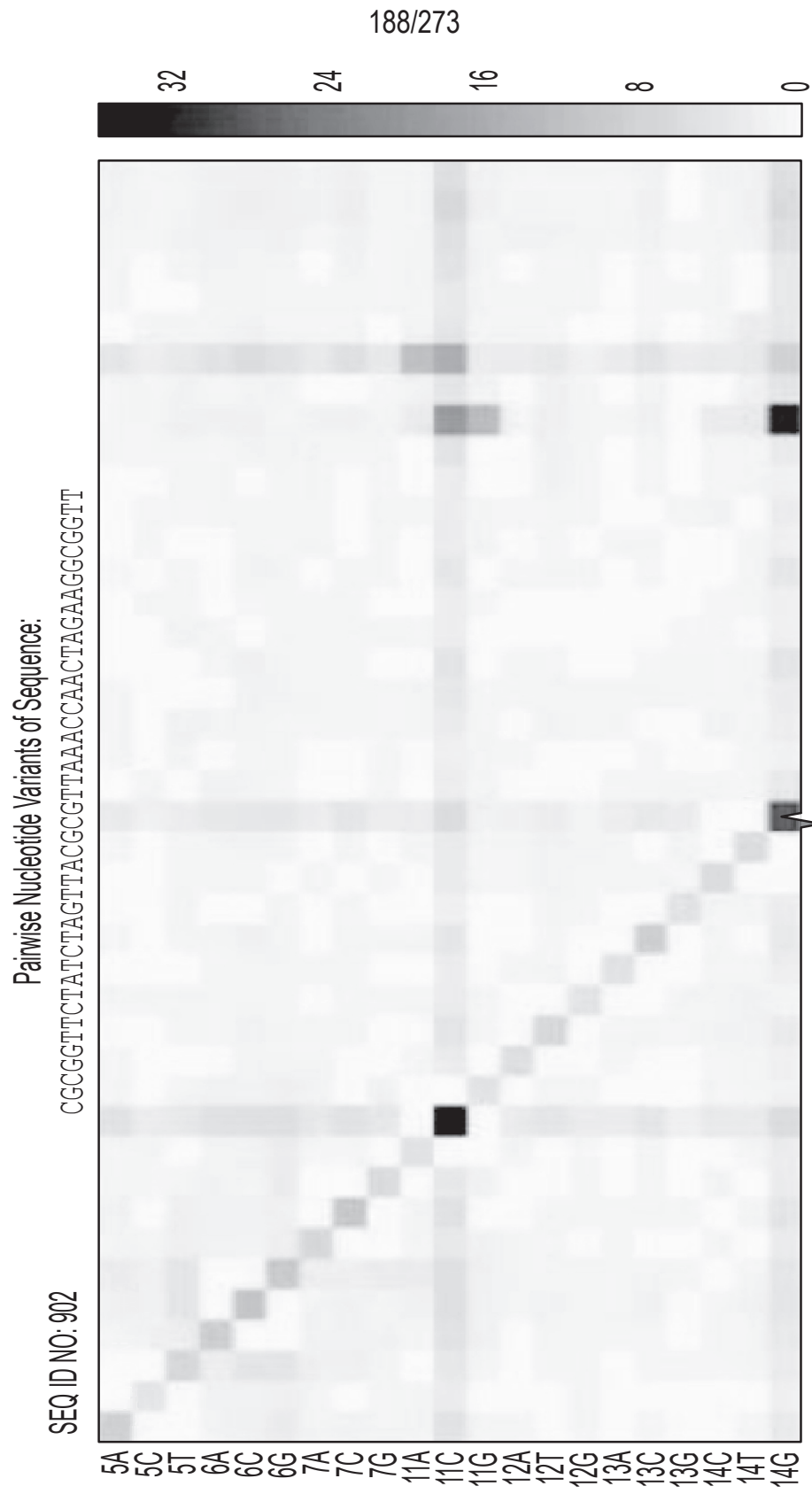


FIG. 101
CONTINUED

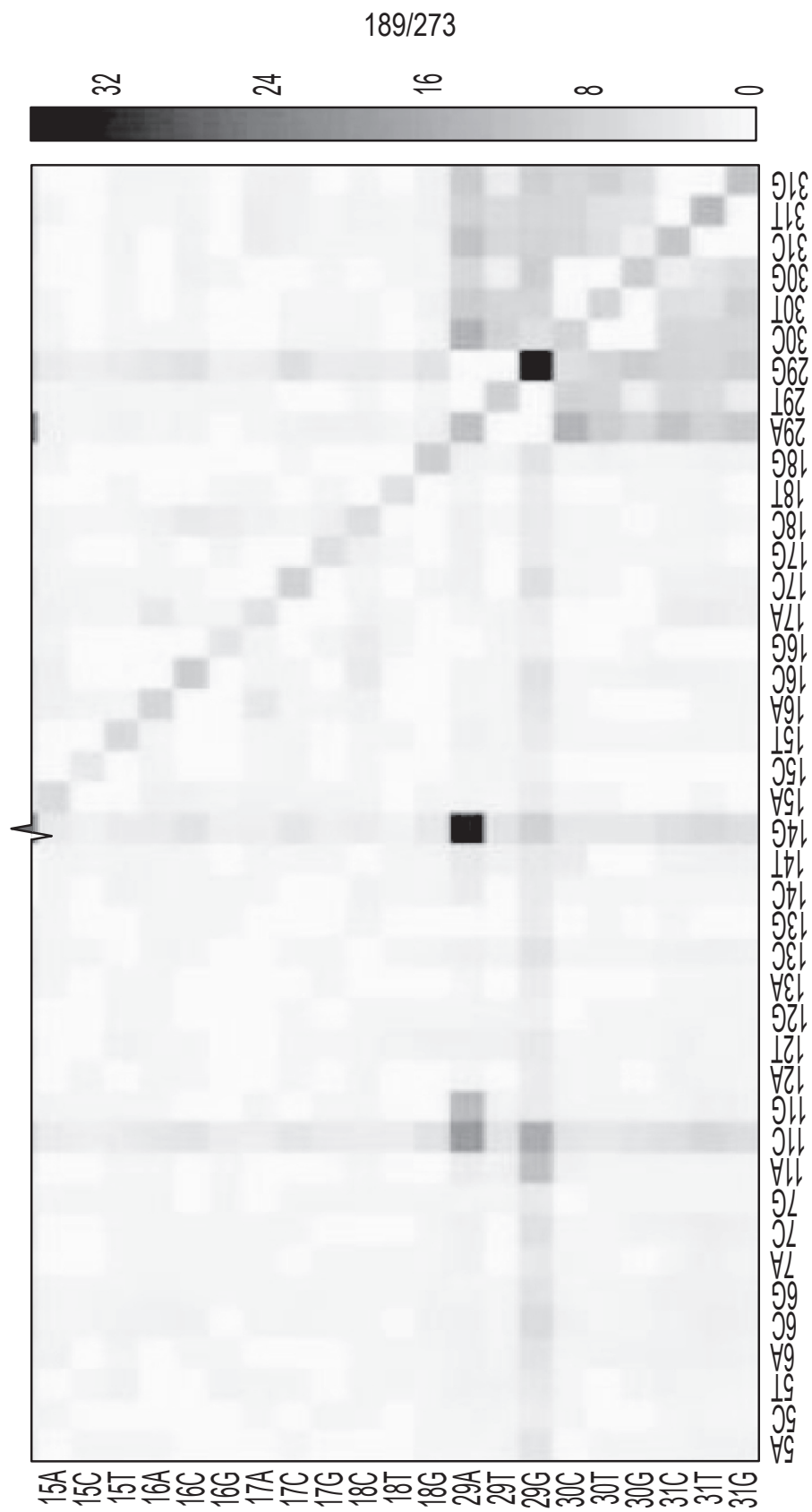


FIG. 101
CONTINUED

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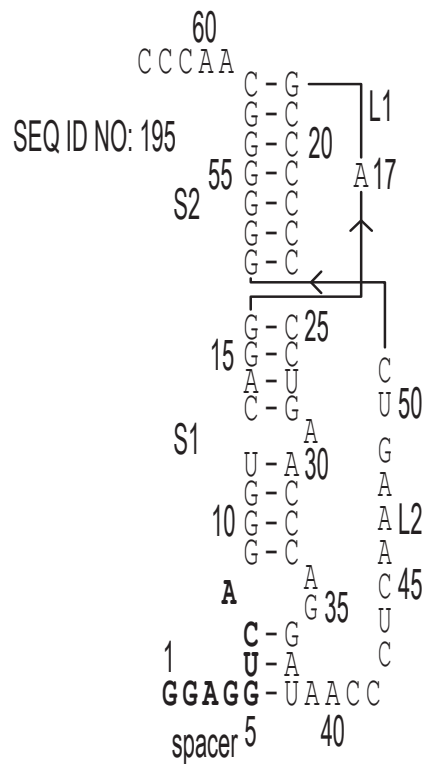
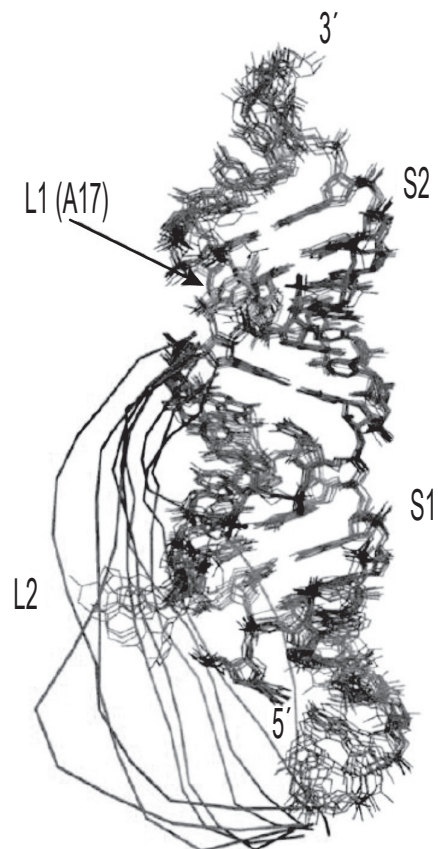


FIG. 102

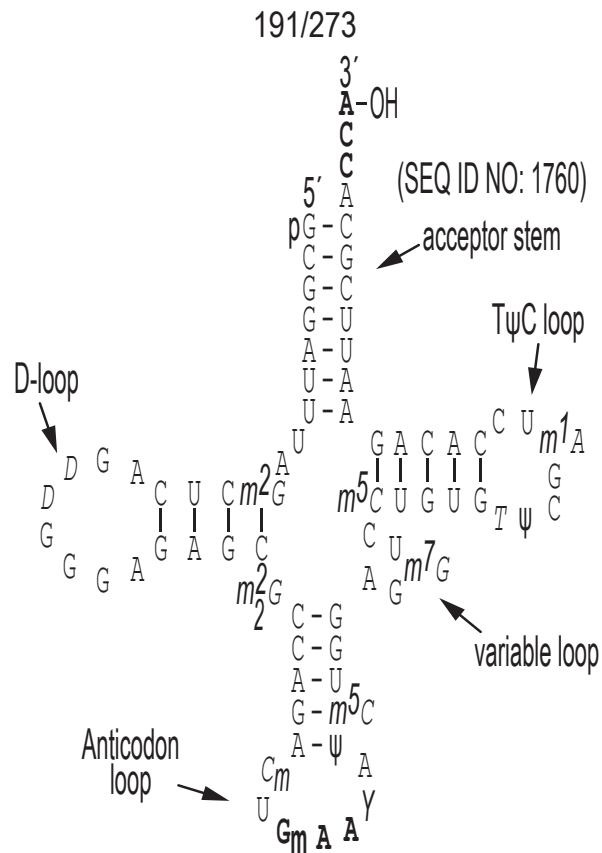


FIG. 103A

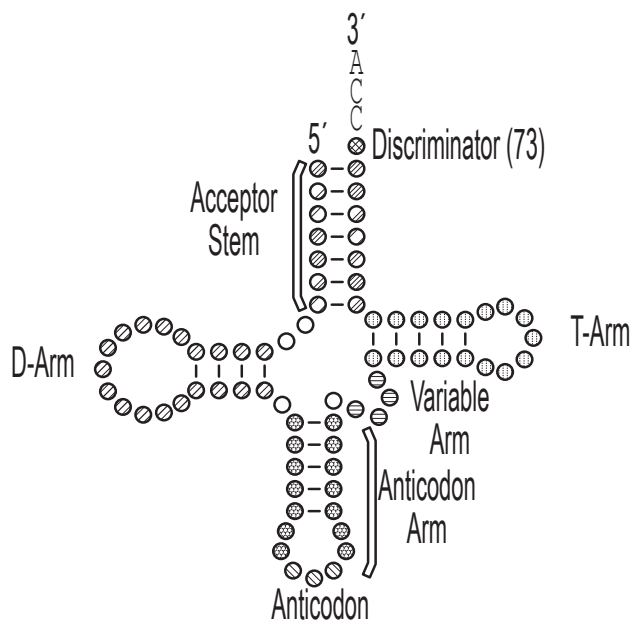


FIG. 103B



FIG. 103C

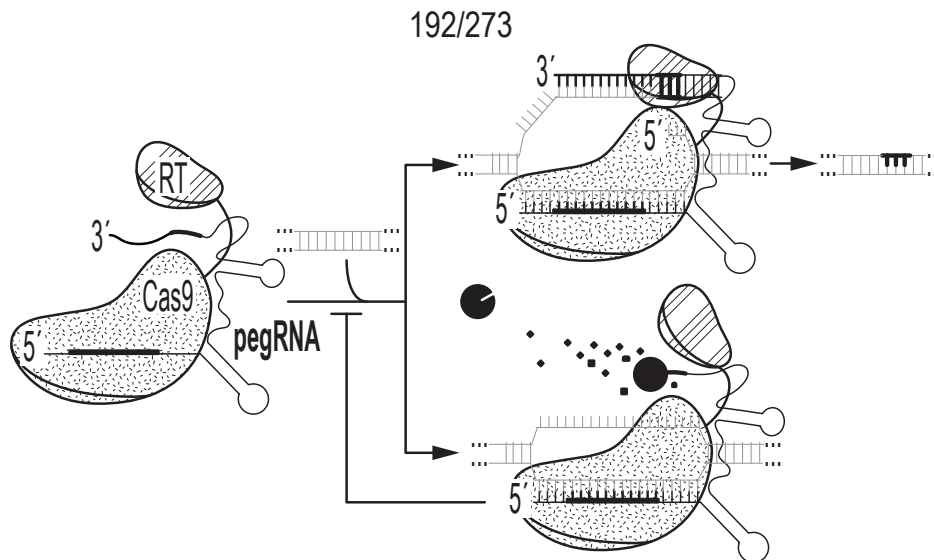


FIG. 104A

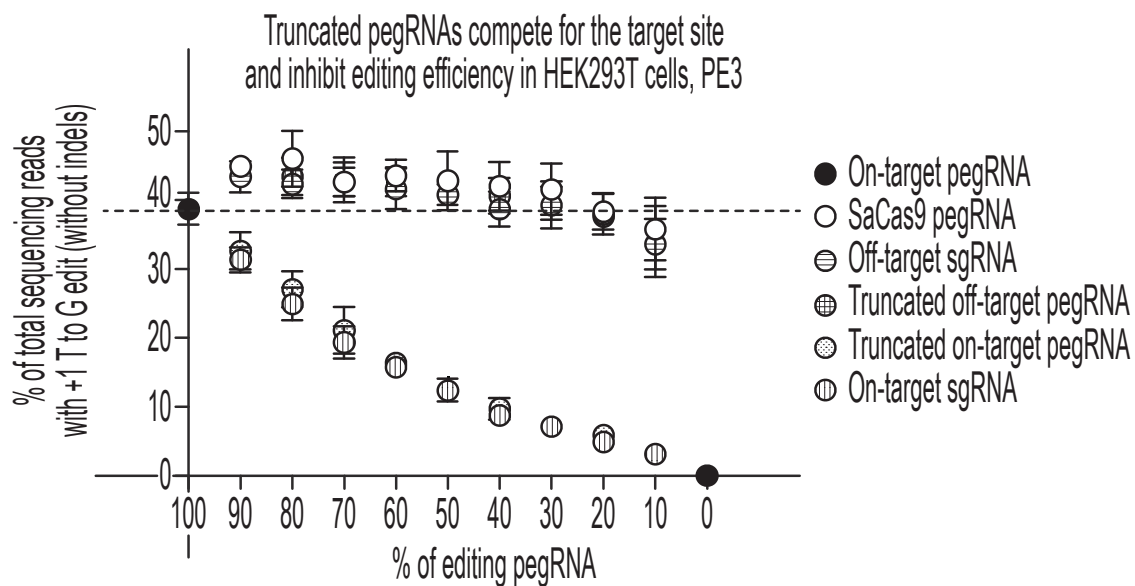


FIG. 104B

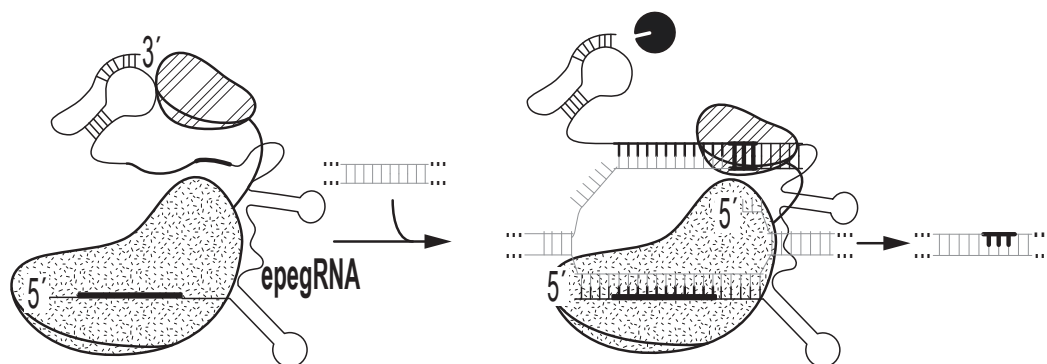


FIG. 104C

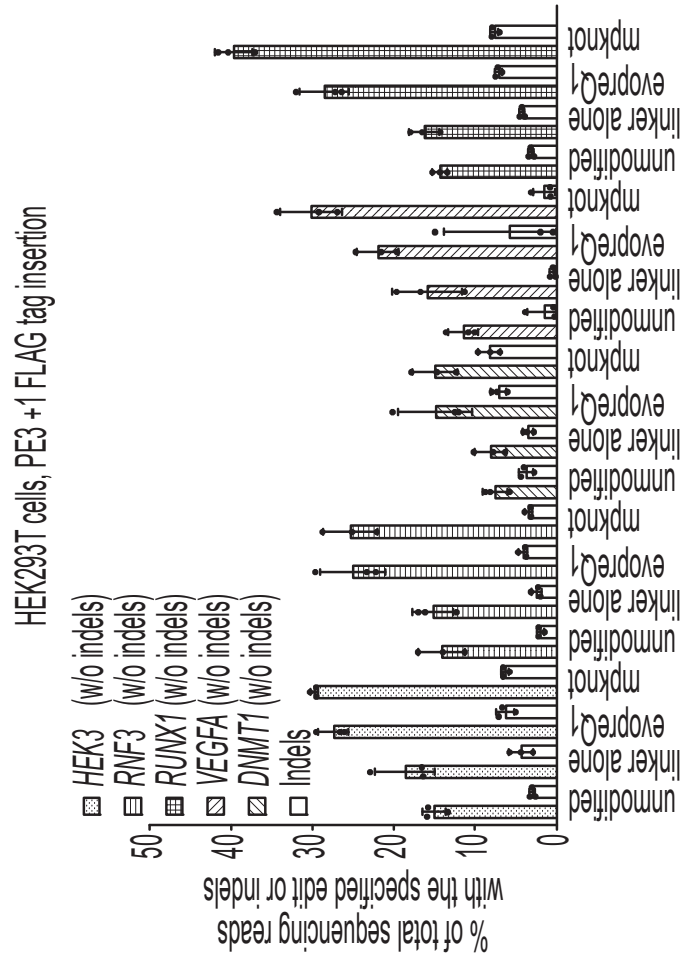
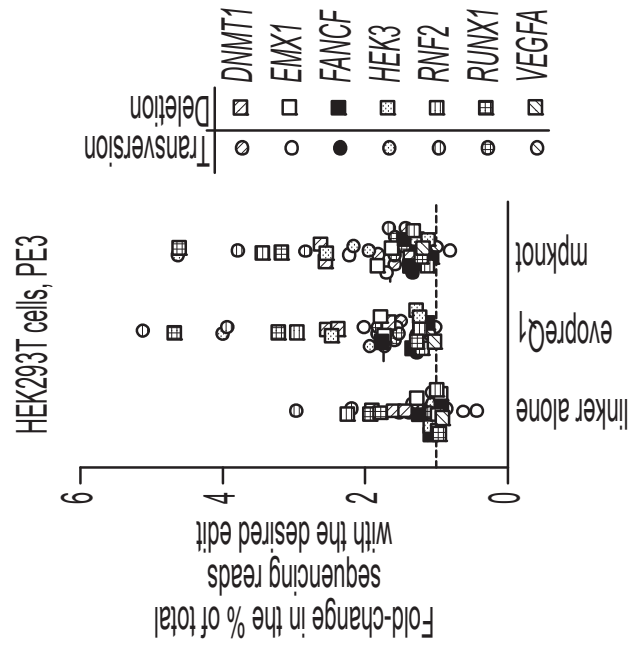


FIG. 105A

FIG. 105B

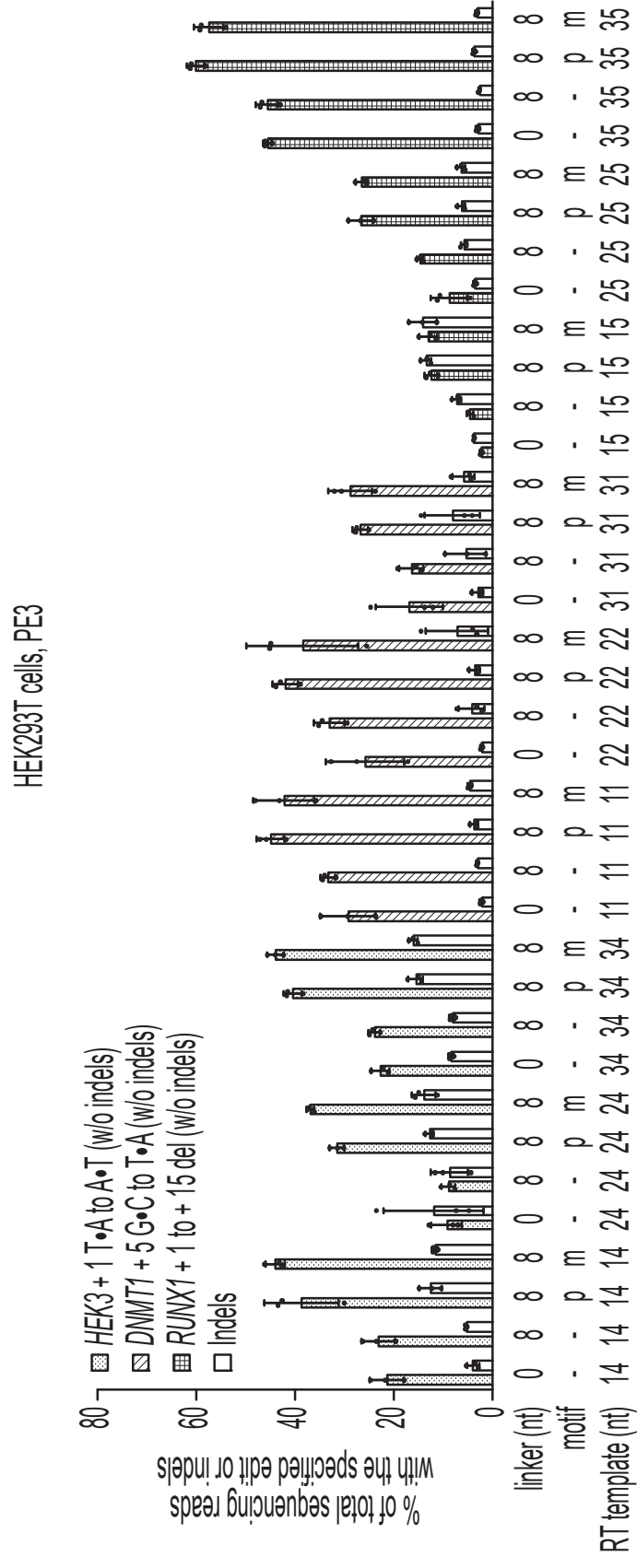


FIG. 105C

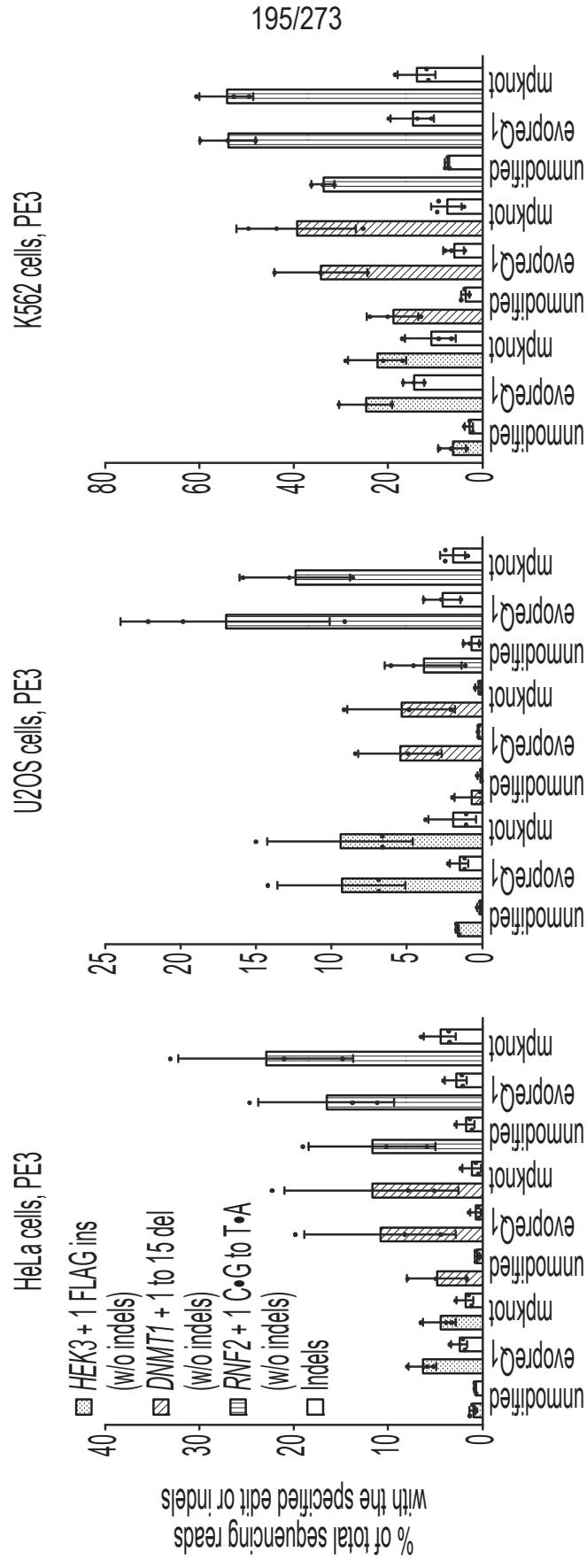


FIG. 105D

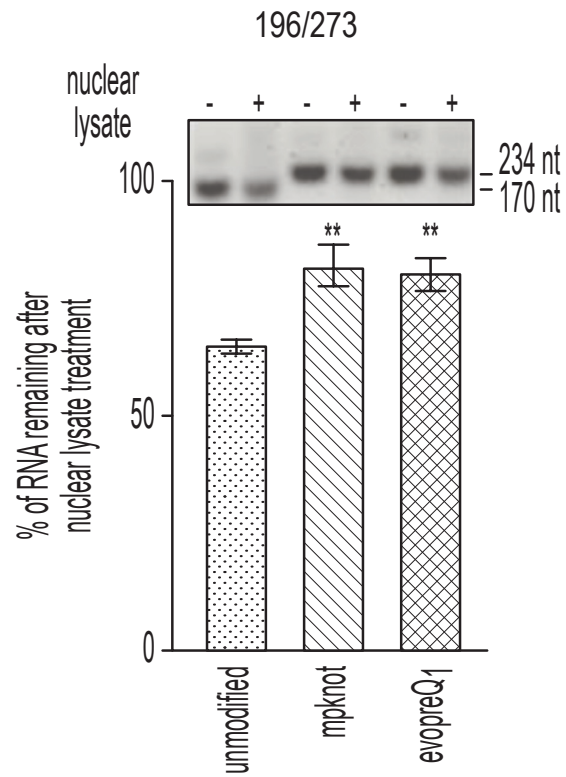


FIG. 106A

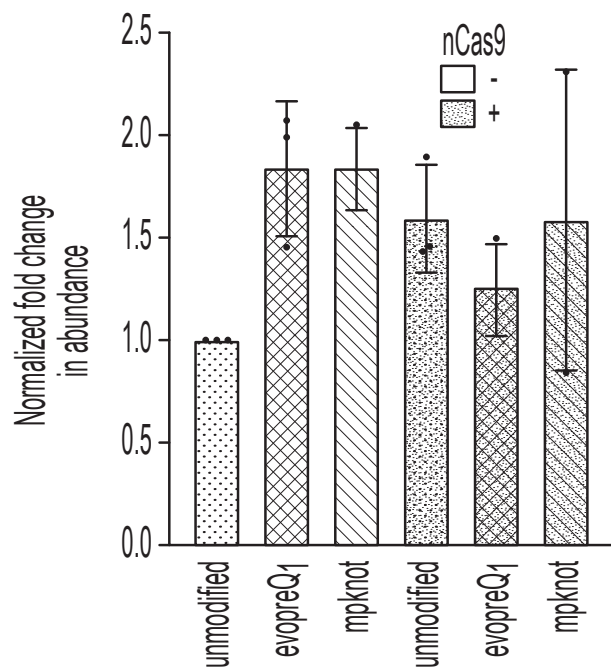


FIG. 106B

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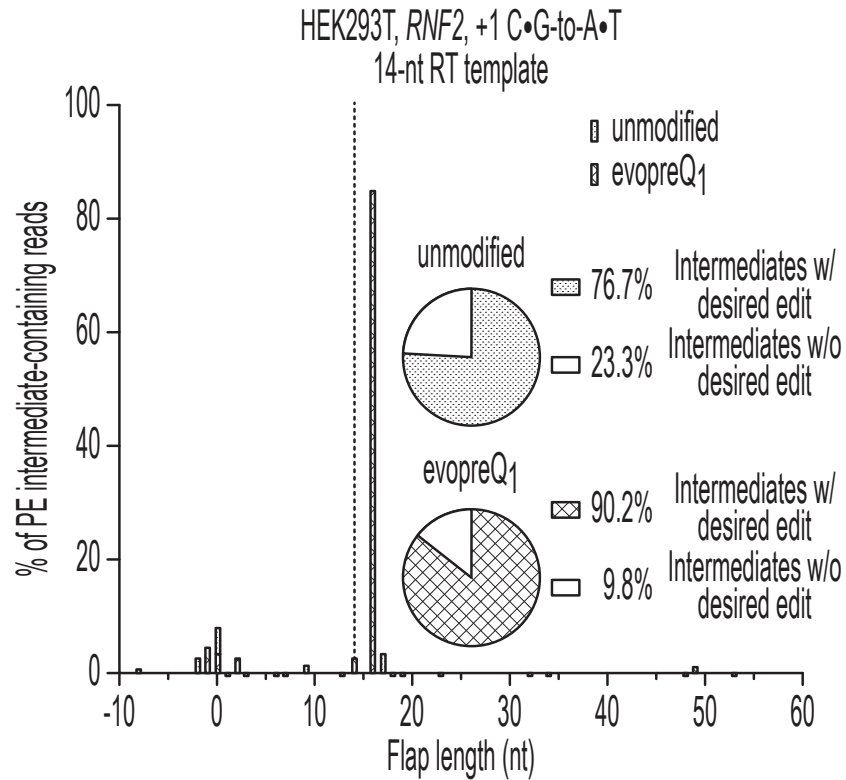


FIG. 106C

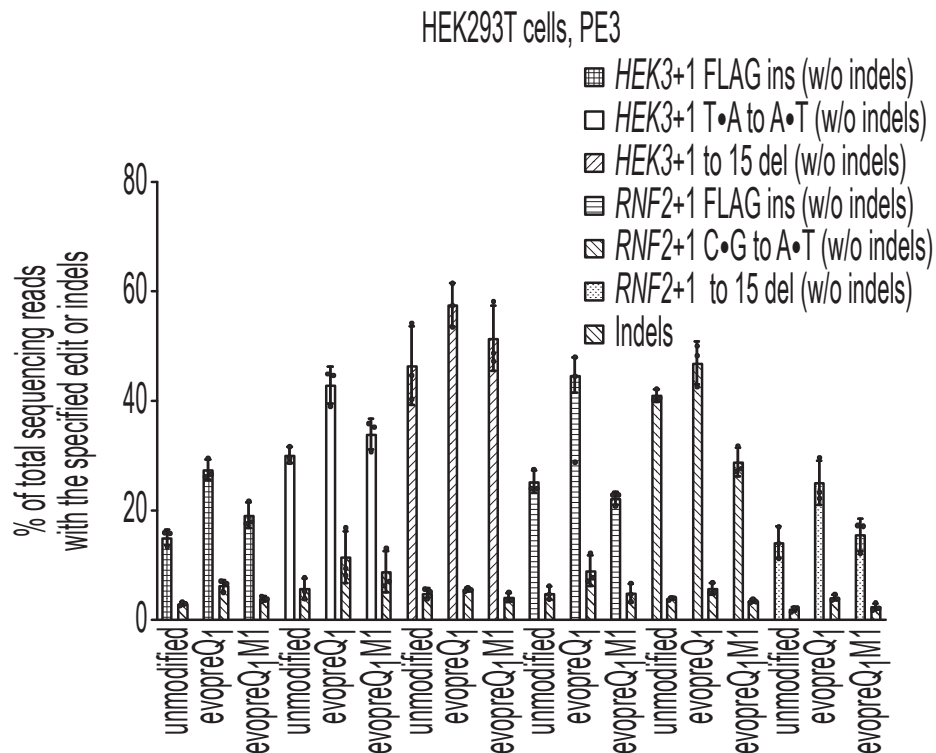


FIG. 106D

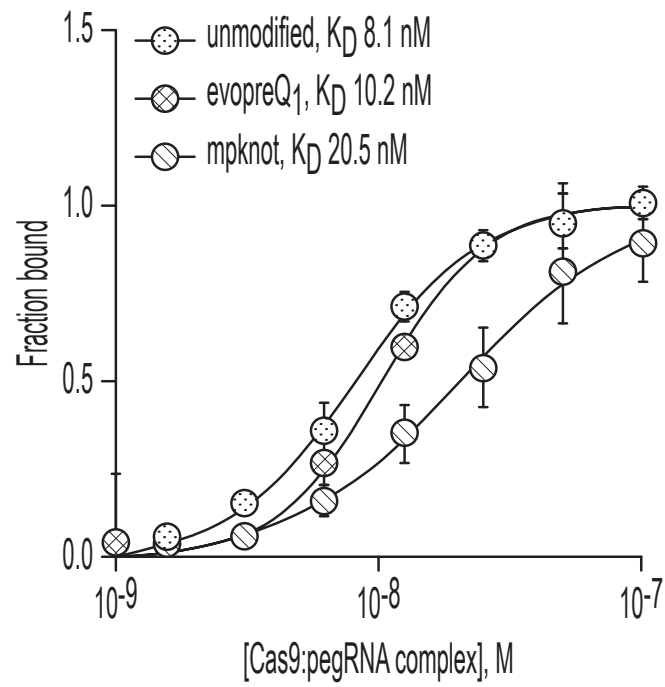


FIG. 106E

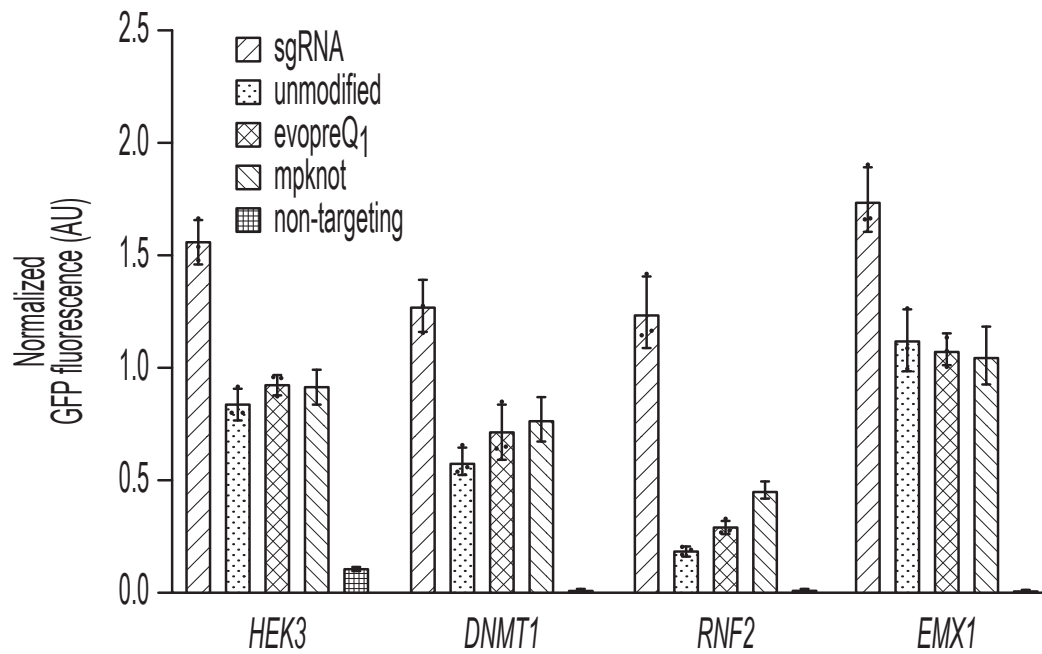


FIG. 106F

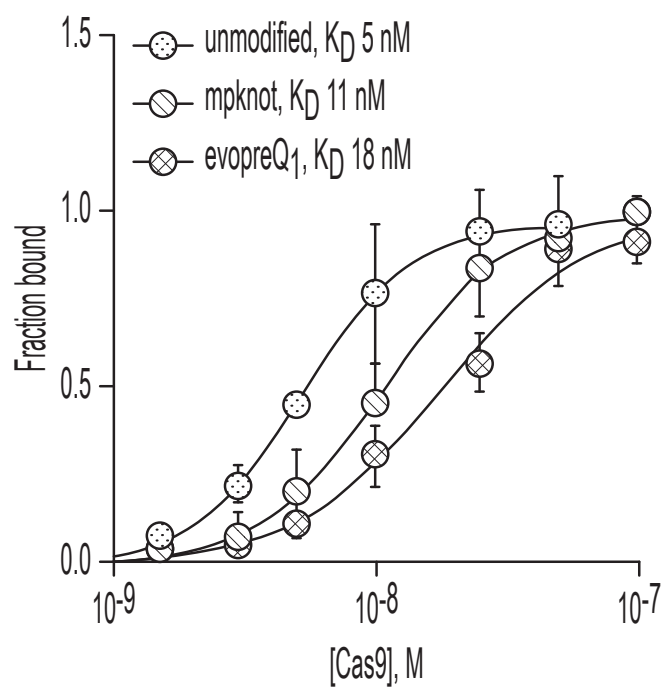


FIG. 106G

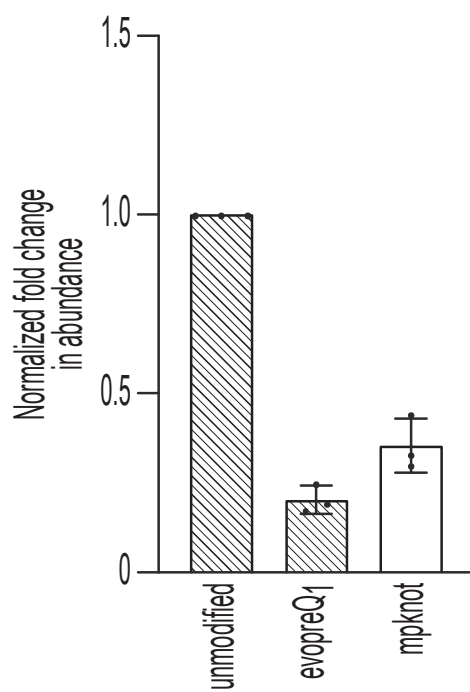


FIG. 106H

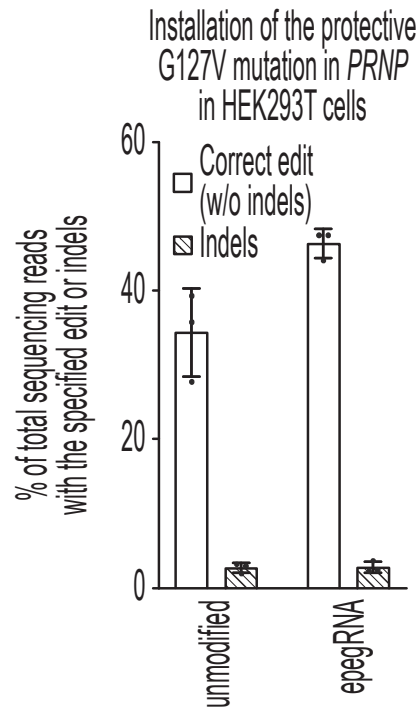


FIG. 107A

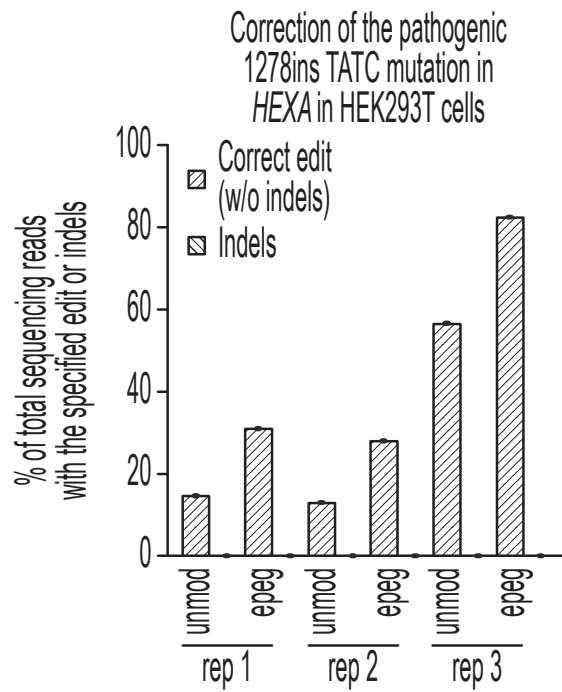


FIG. 107B

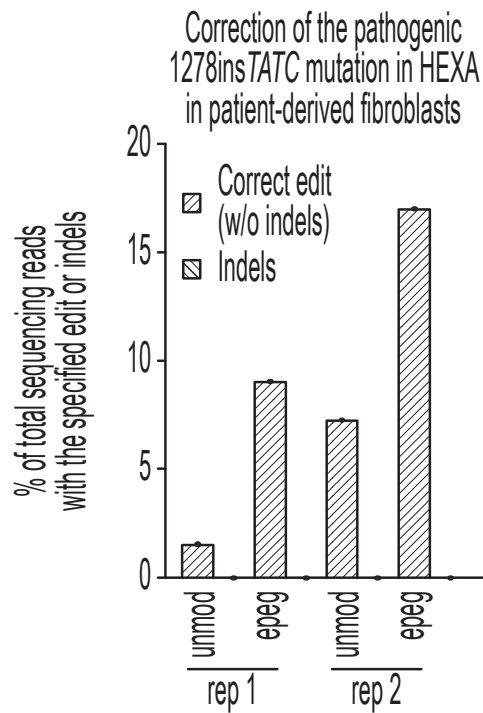


FIG. 107C

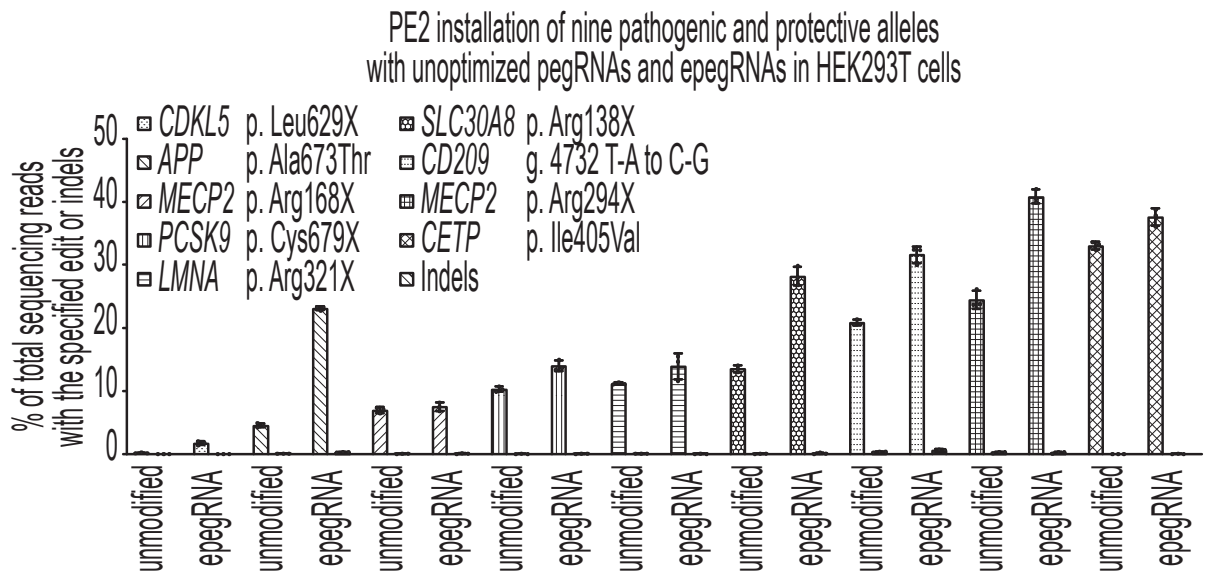


FIG. 107D

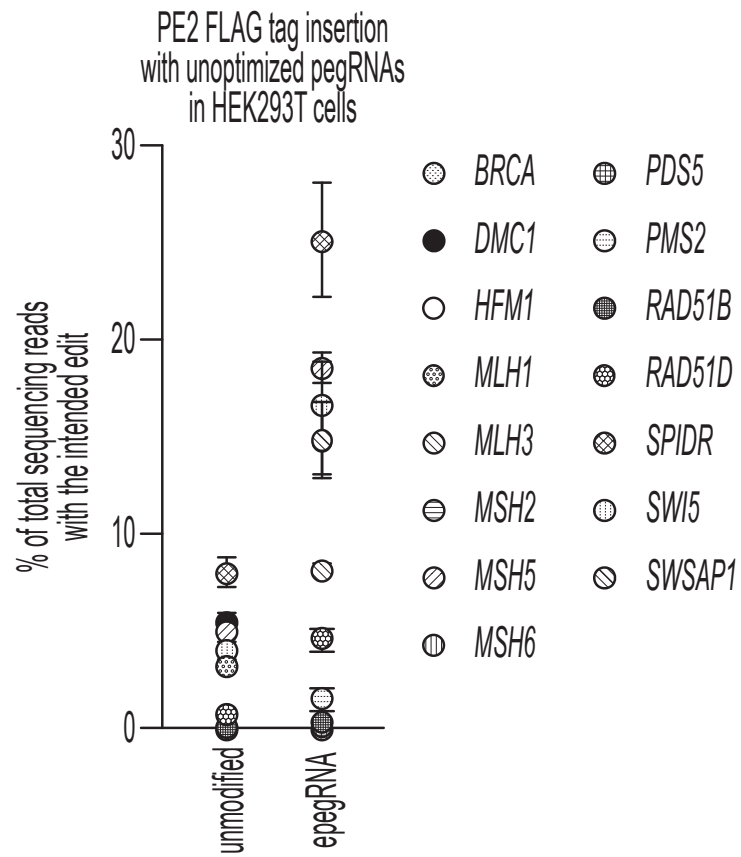
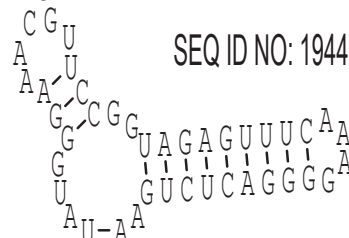


FIG. 107E

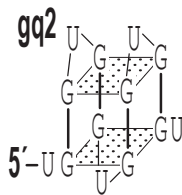
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SEQ ID NO: 1944

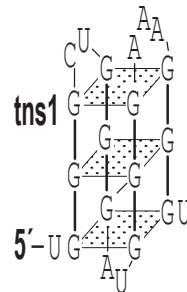
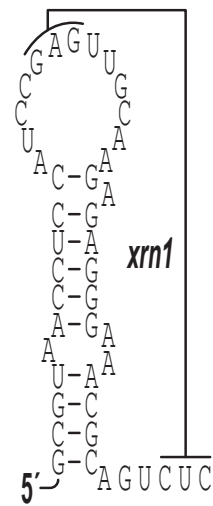


Tetrahymena
group I intron
P4-P6 domain

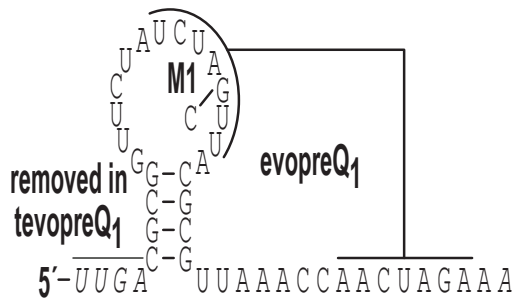
SEQ ID NO: 1945



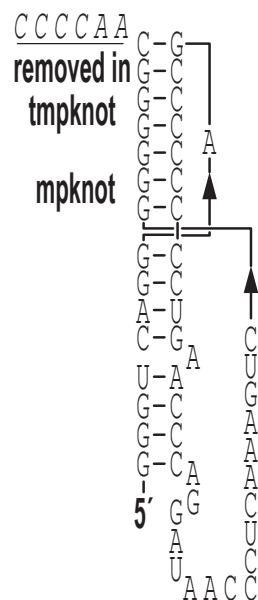
SEQ ID NO: 1946



SEQ ID NO: 1947



SEQ ID NO: 1942



SEQ ID NO: 1943



P6

FIG. 108

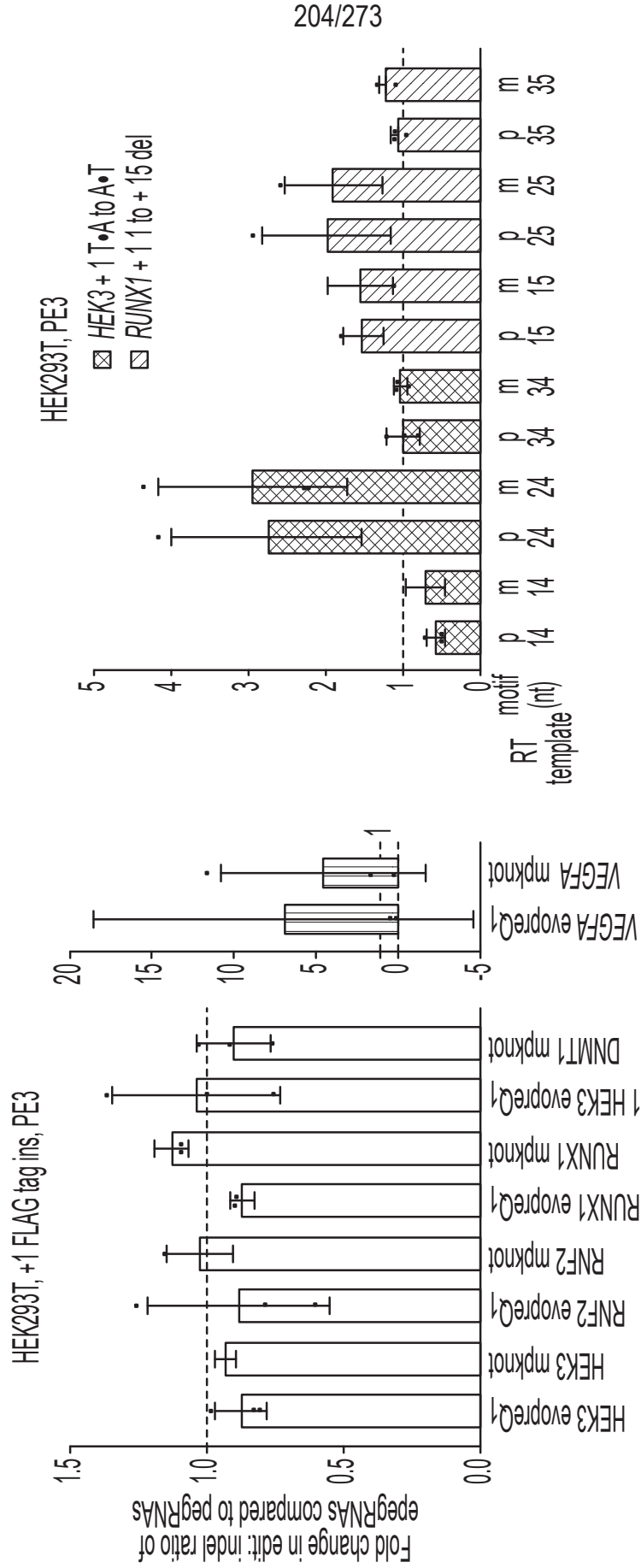


FIG. 109A

FIG. 109B

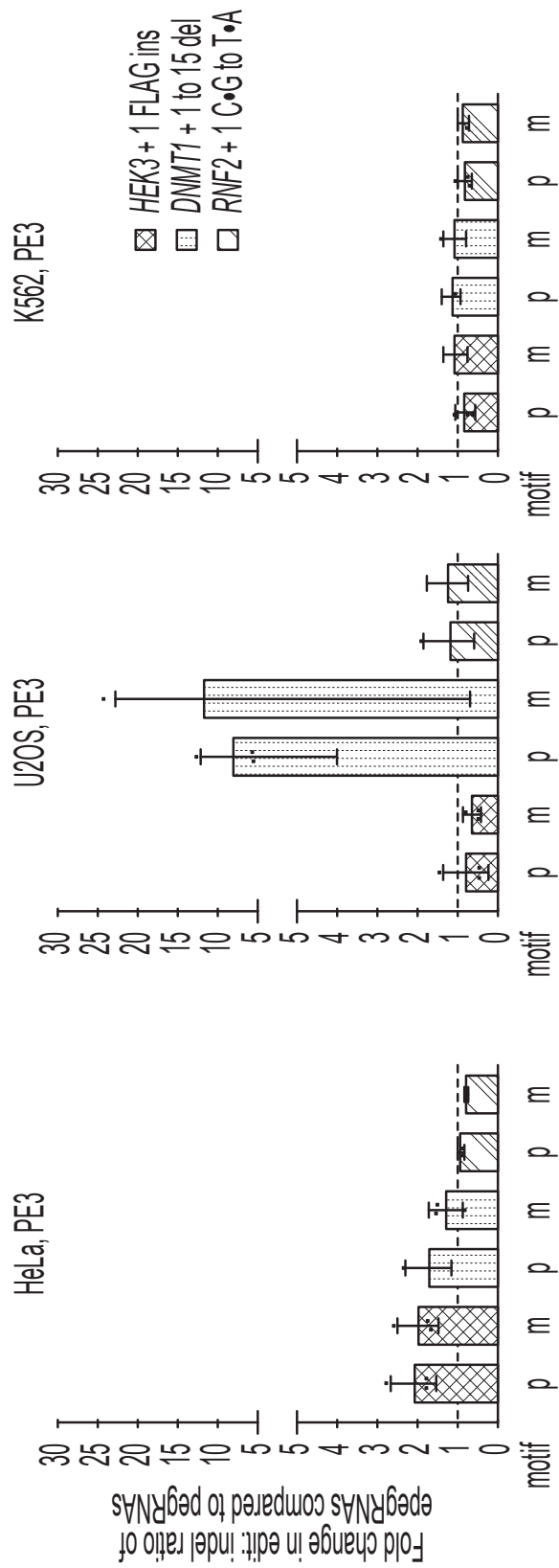


FIG. 109C

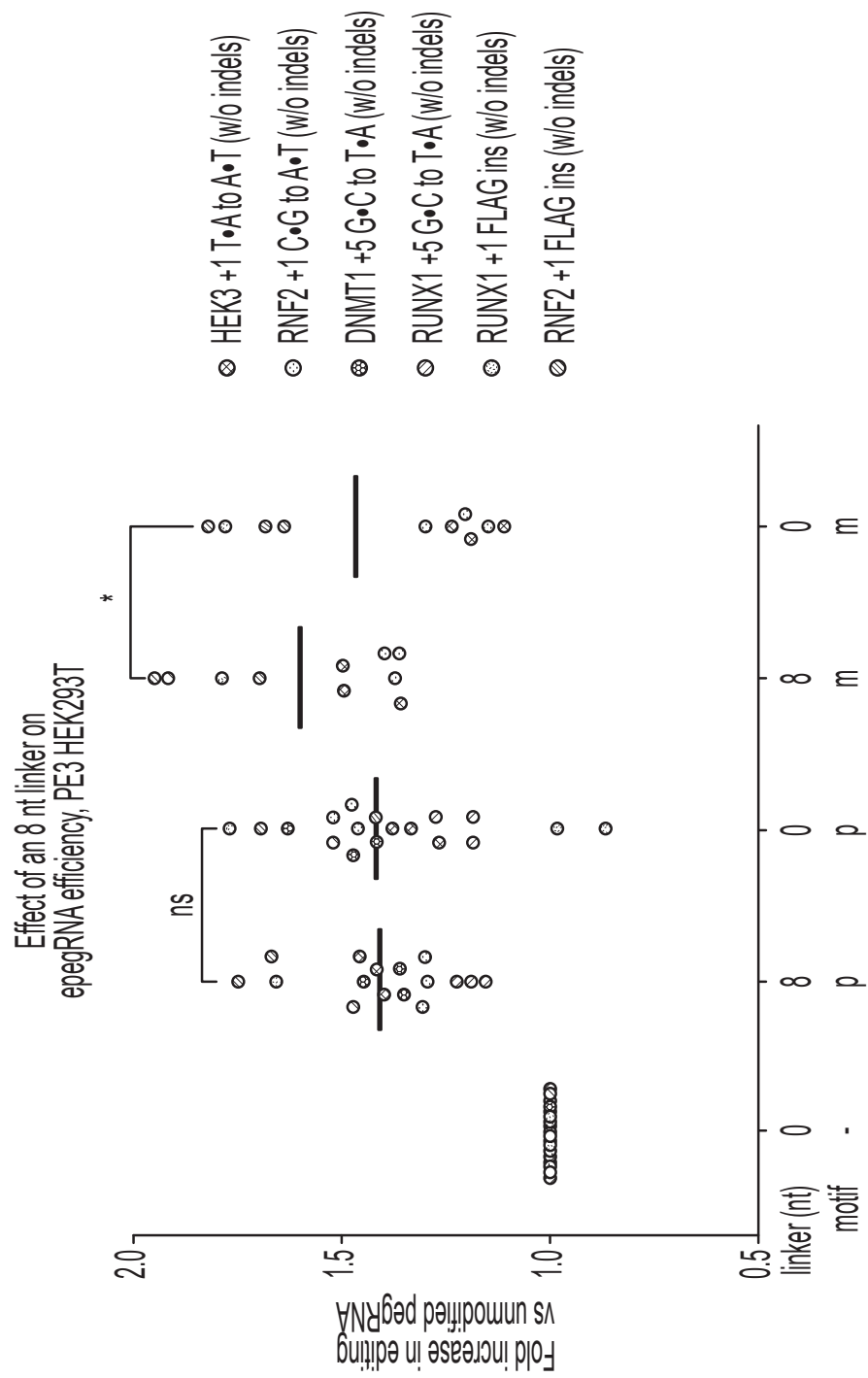


FIG. 110

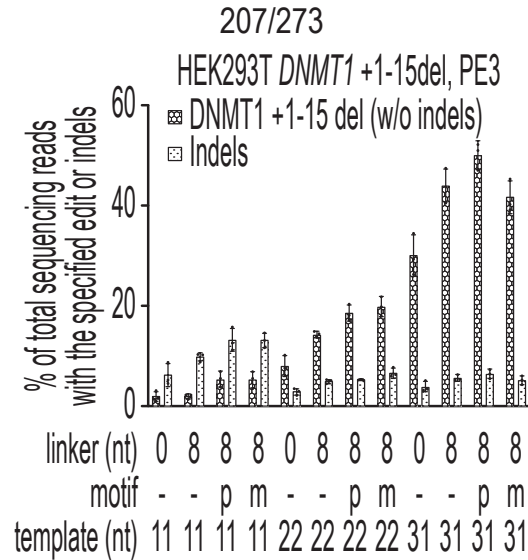


FIG. 111A

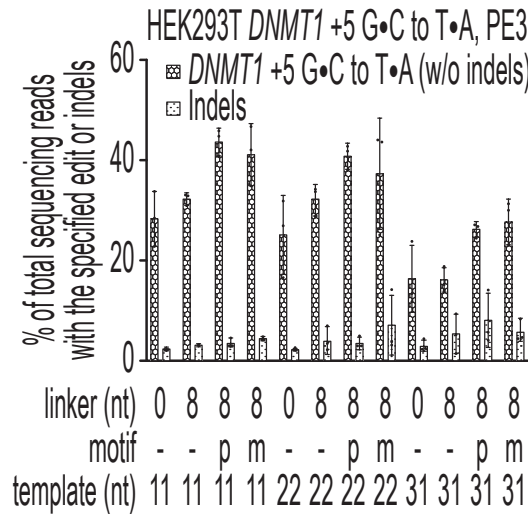


FIG. 111B

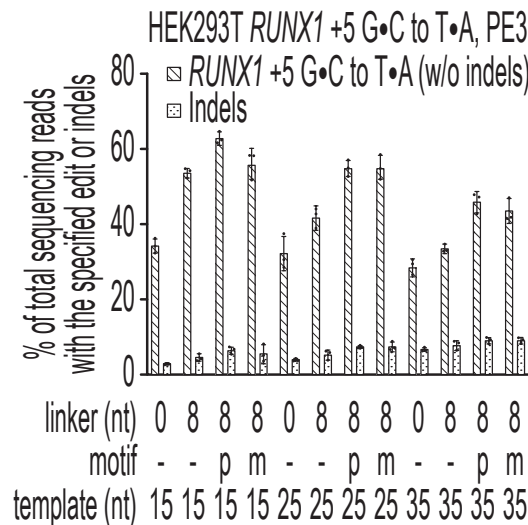


FIG. 111C

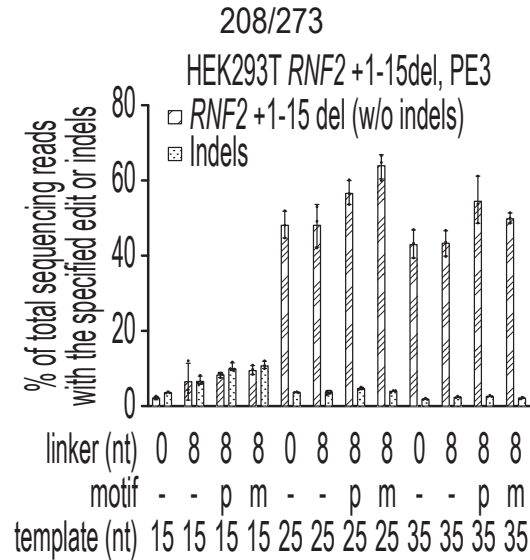


FIG. 111D

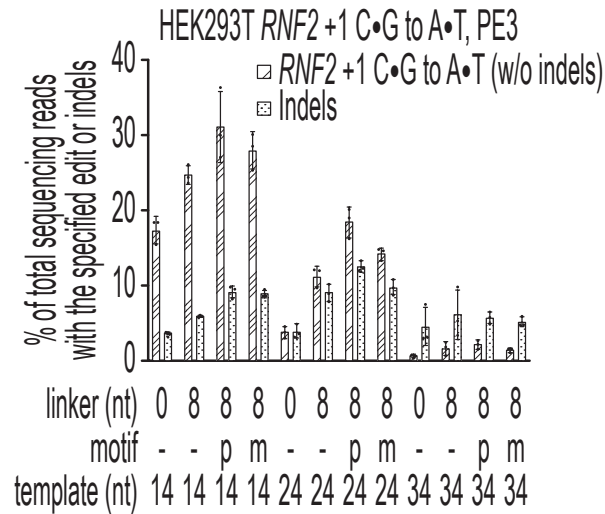


FIG. 111E

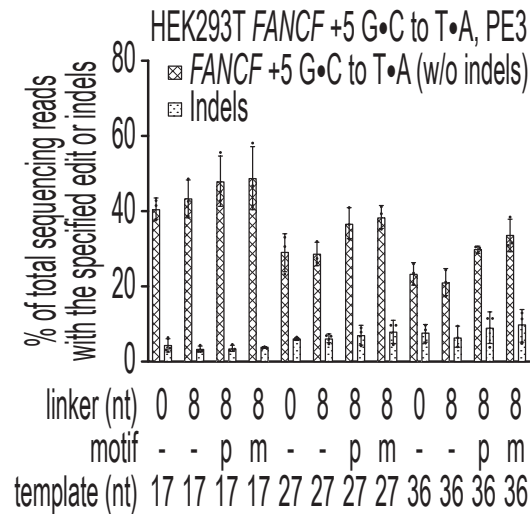


FIG. 111F

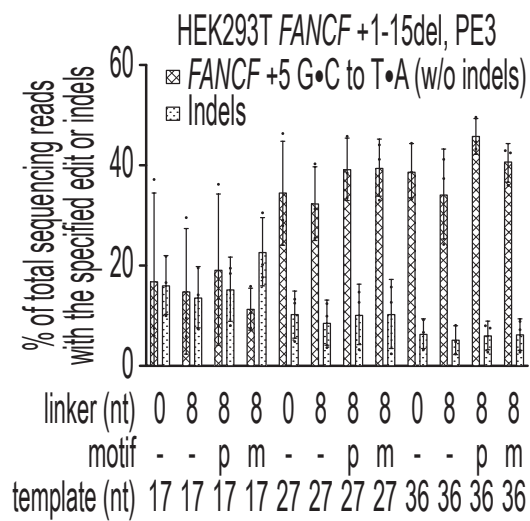


FIG. 111G

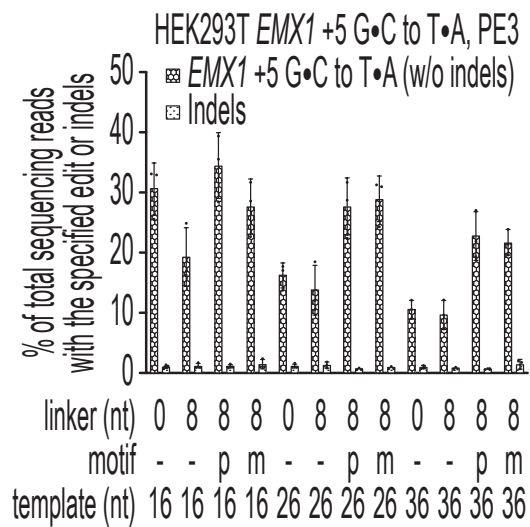


FIG. 111H

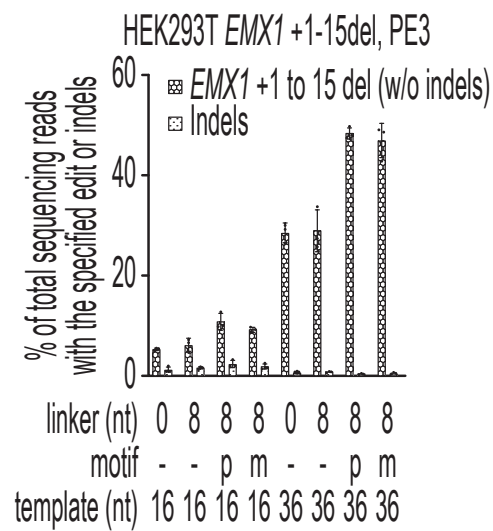


FIG. 111I

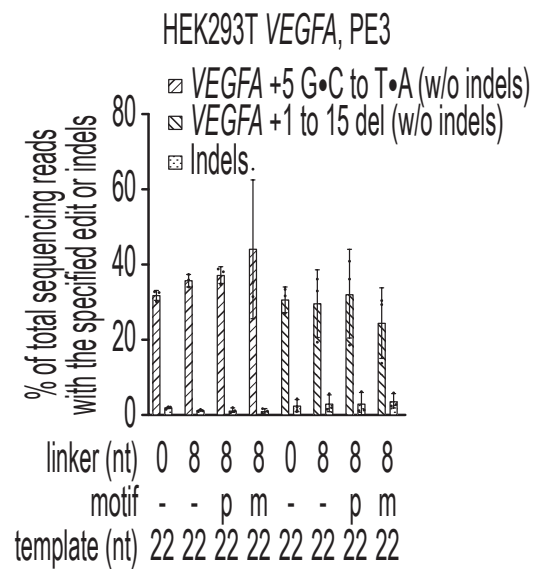


FIG. 111J

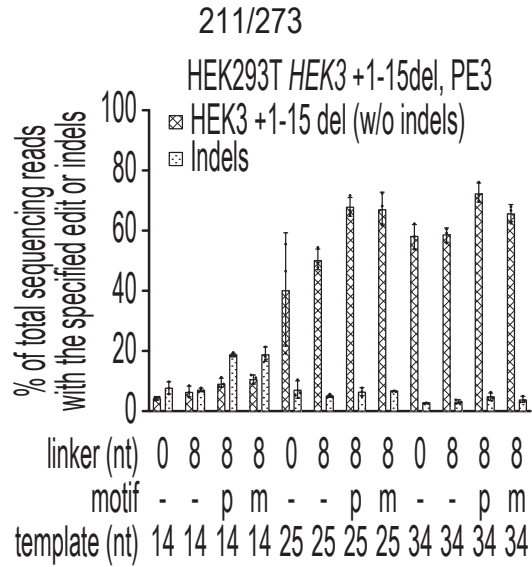


FIG. 111K

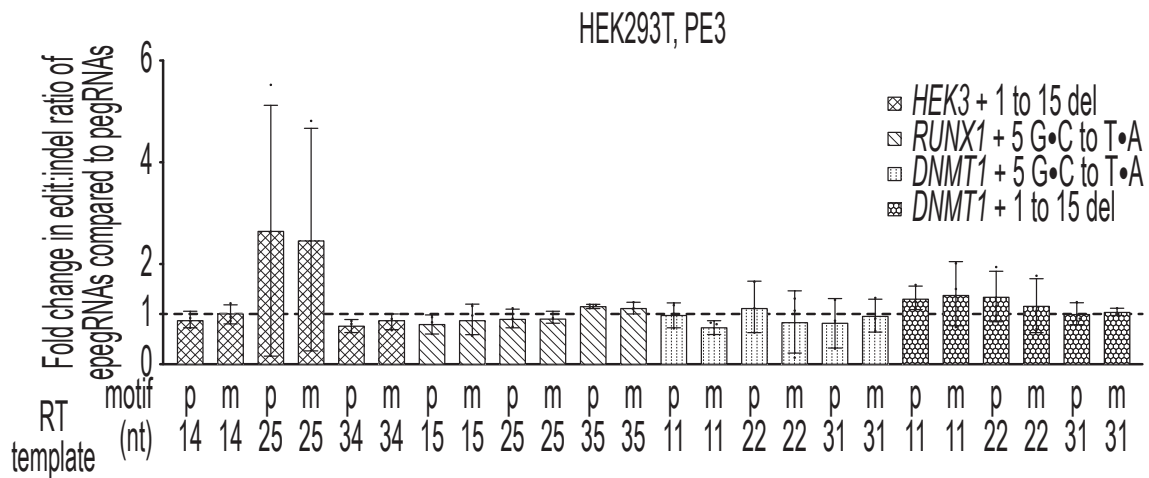


FIG. 112A

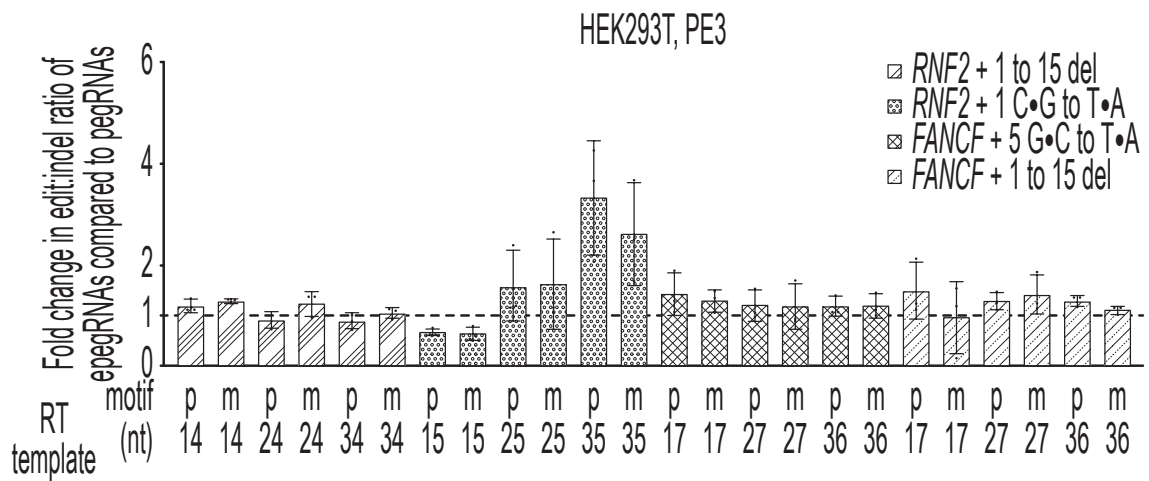


FIG. 112B

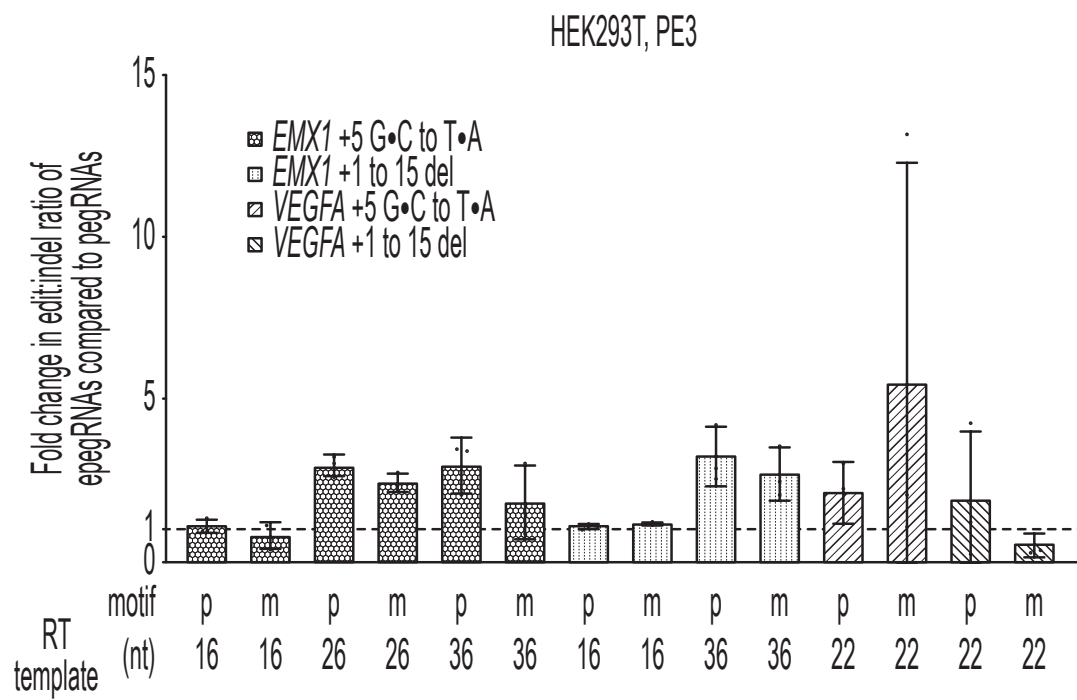


FIG. 112C

pegRNA site	HEK3 (PE3)					EMX1 (PE3)					FANCF (PE3)				
	...	pt mtn, -	pt mtn, m	del, -	del, p	...	pt mtn, -	pt mtn, p	del, -	del, m	...	pt mtn, -	pt mtn, m	del, -	del, m
On-target		21.5 (4.0)	44.1 (11.7)	57.9 (2.8)	72.7 (4.7)		30.5 (1.1)	34.5 (1.1)	28.6 (0.8)	47 (0.5)		40.7 (4.2)	48.8 (3.7)	38.9 (6.4)	40.6 (6.3)
Off-target 1		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 2		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 3		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)
Off-target 4		<0.1 (<0.1)	<0.1 (0.4)	<0.1 (0.1)	<0.1 (0.1)		<0.1 (0.1)	<0.1 (0.1)	<0.1 (<0.1)	<0.1 (0.1)		<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)	<0.1 (<0.1)

FIG. 113

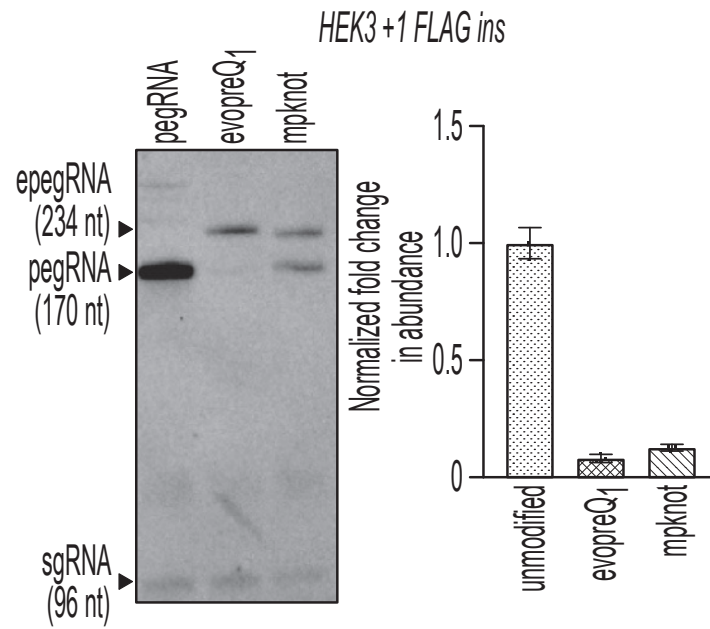


FIG. 114A

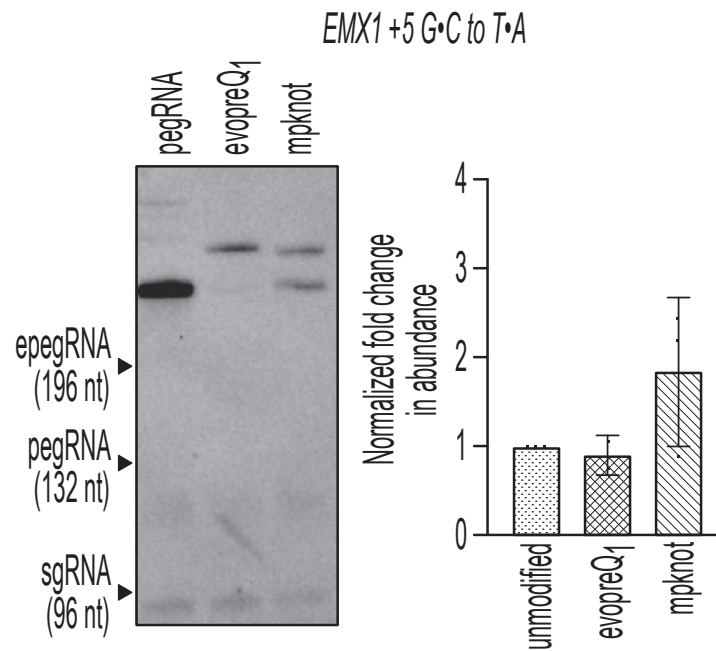


FIG. 114B

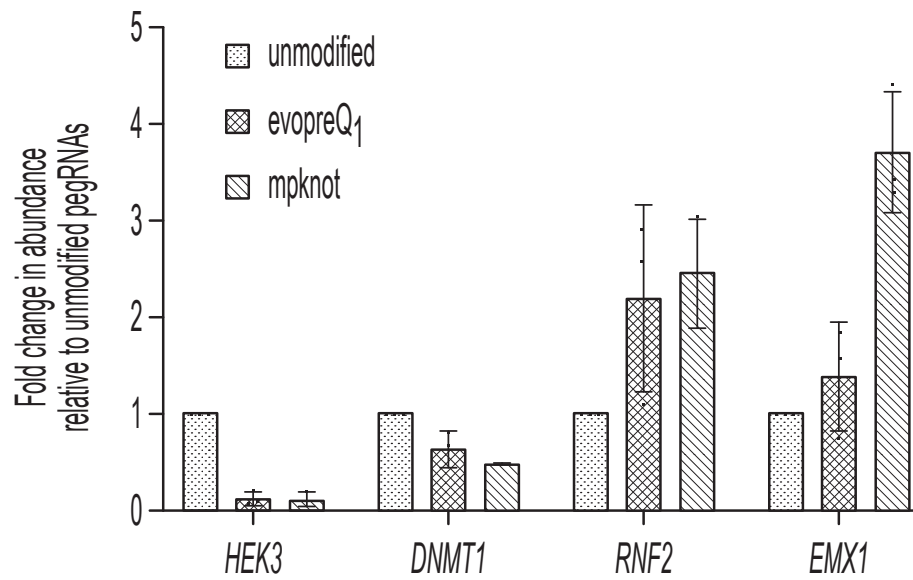


FIG. 114C

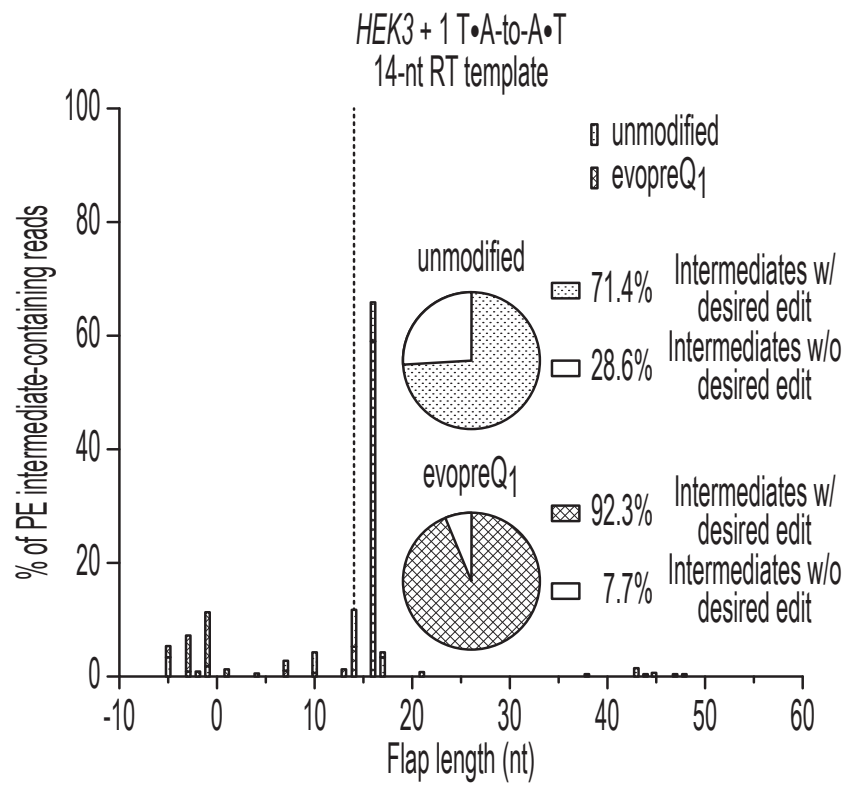


FIG. 115A

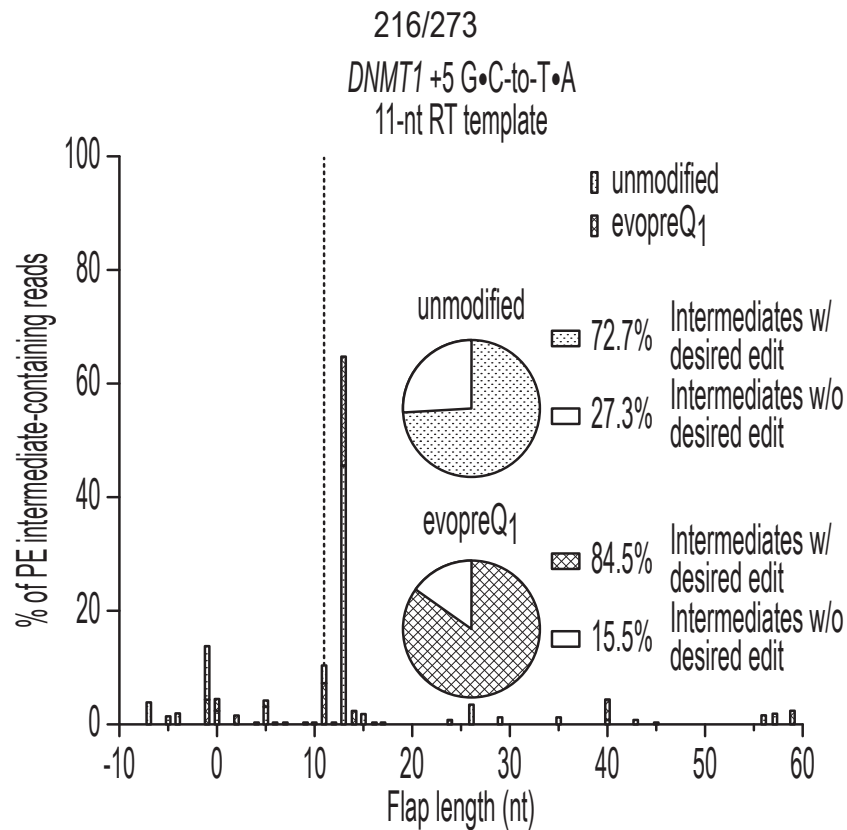


FIG. 115B

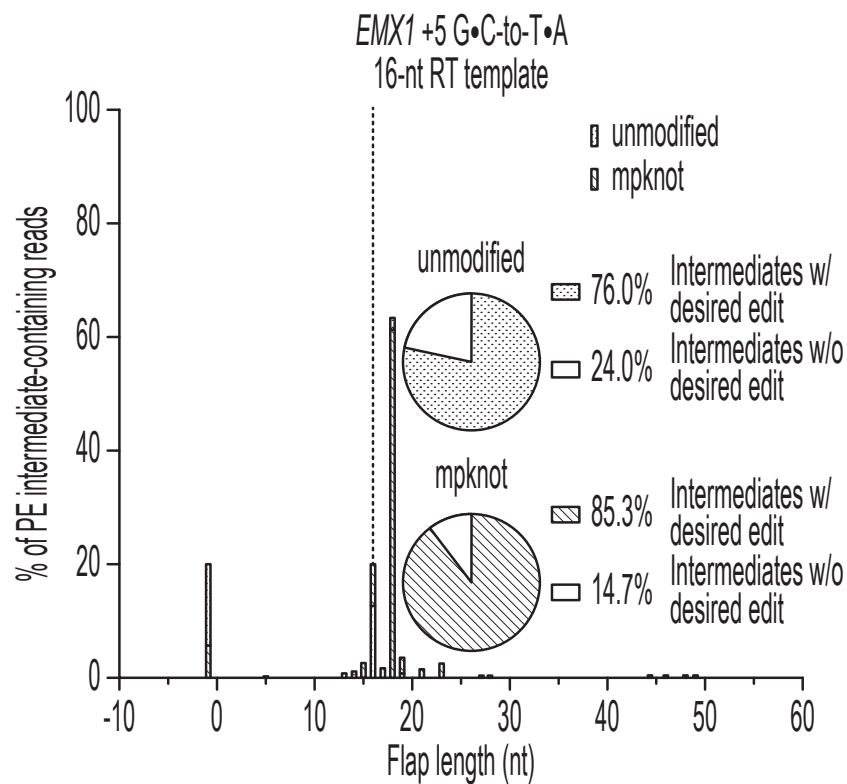


FIG. 115C

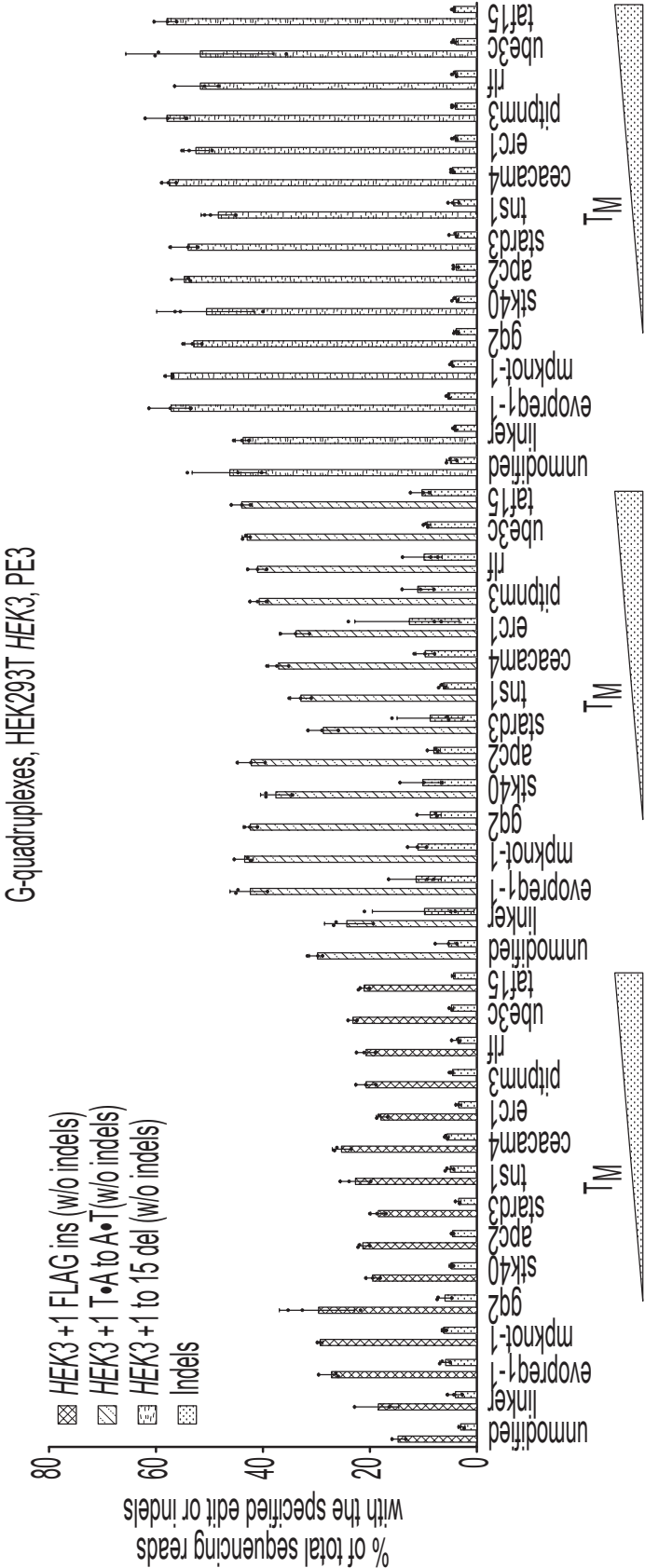


FIG. 116A

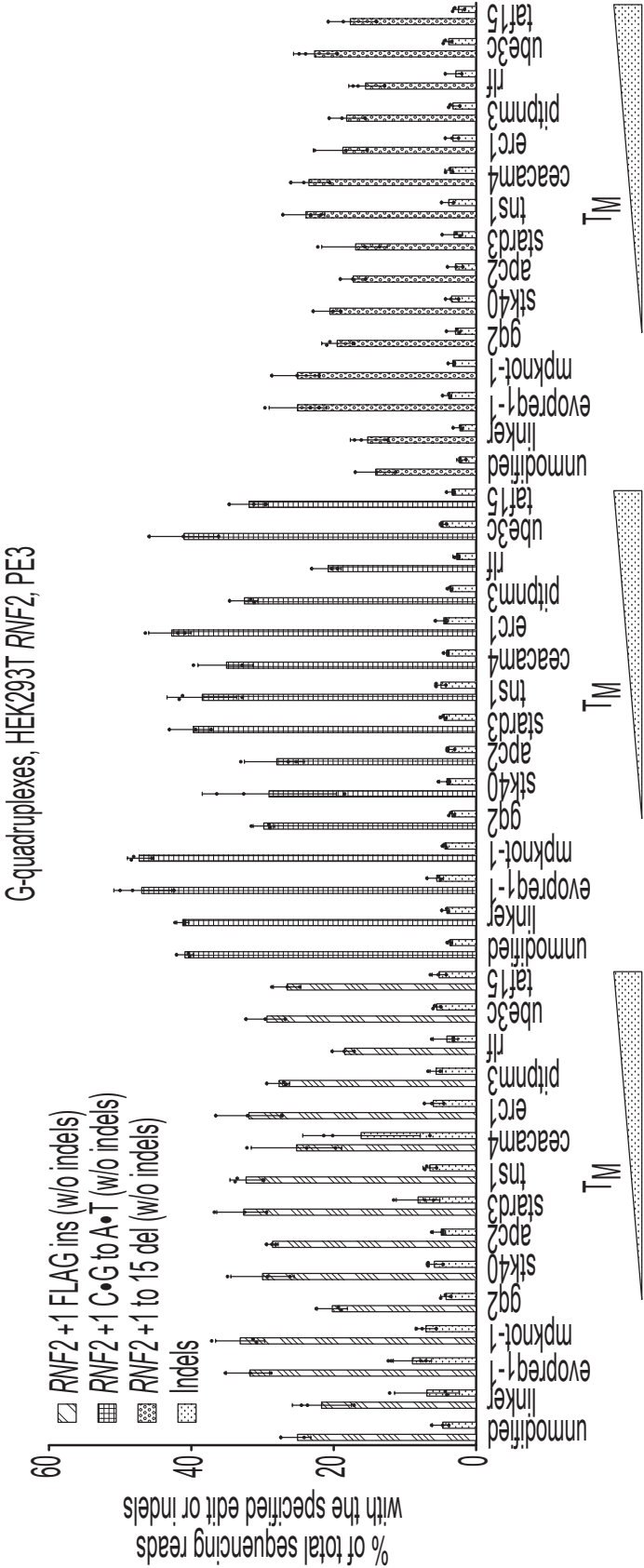


FIG. 116B

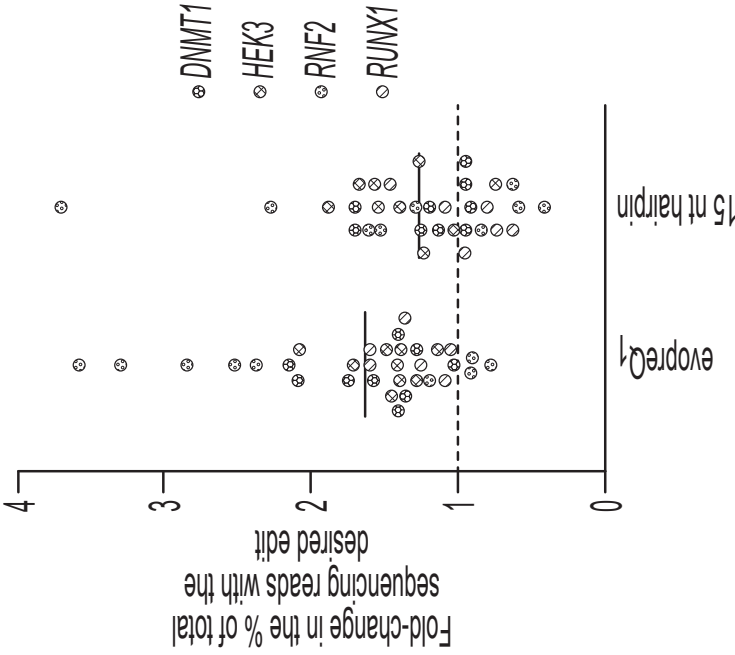


FIG. 116C

Other RNA motifs, HEK293T RNF2, PE3

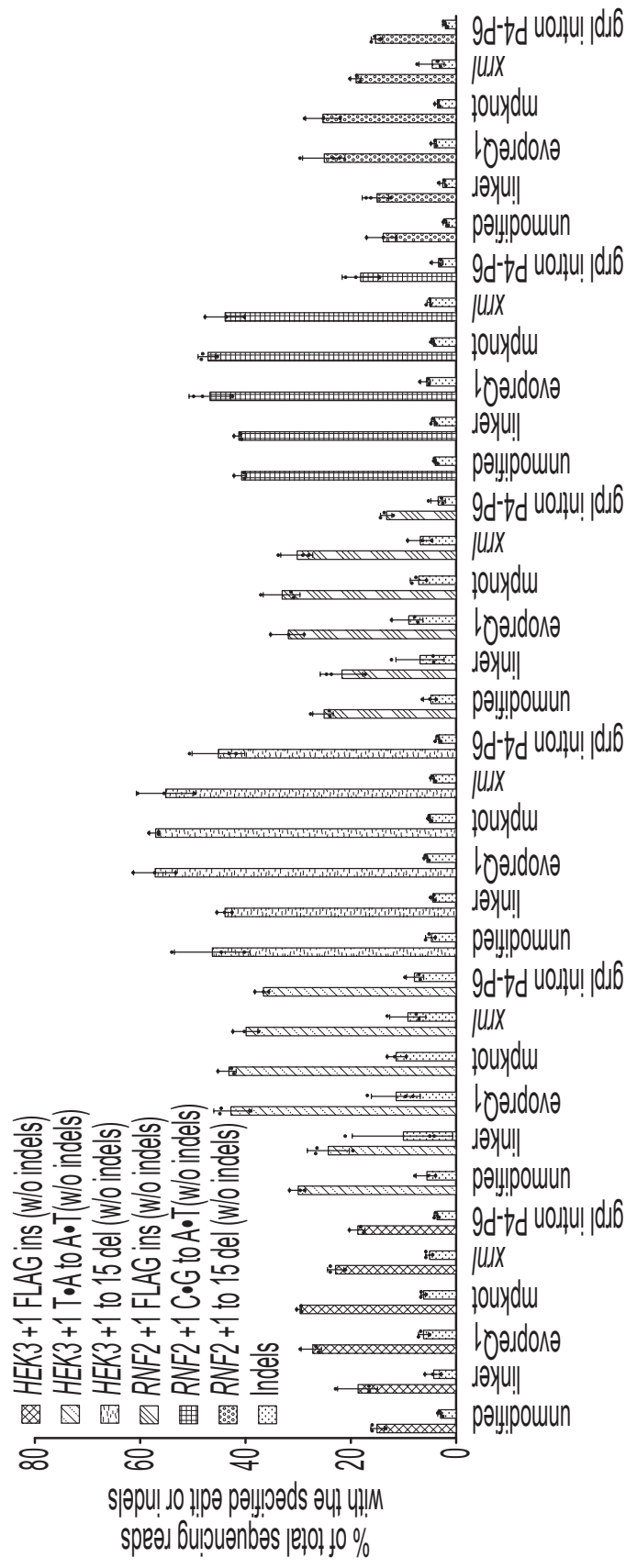


FIG. 116D



mpknot variants, HEK293T, PE3

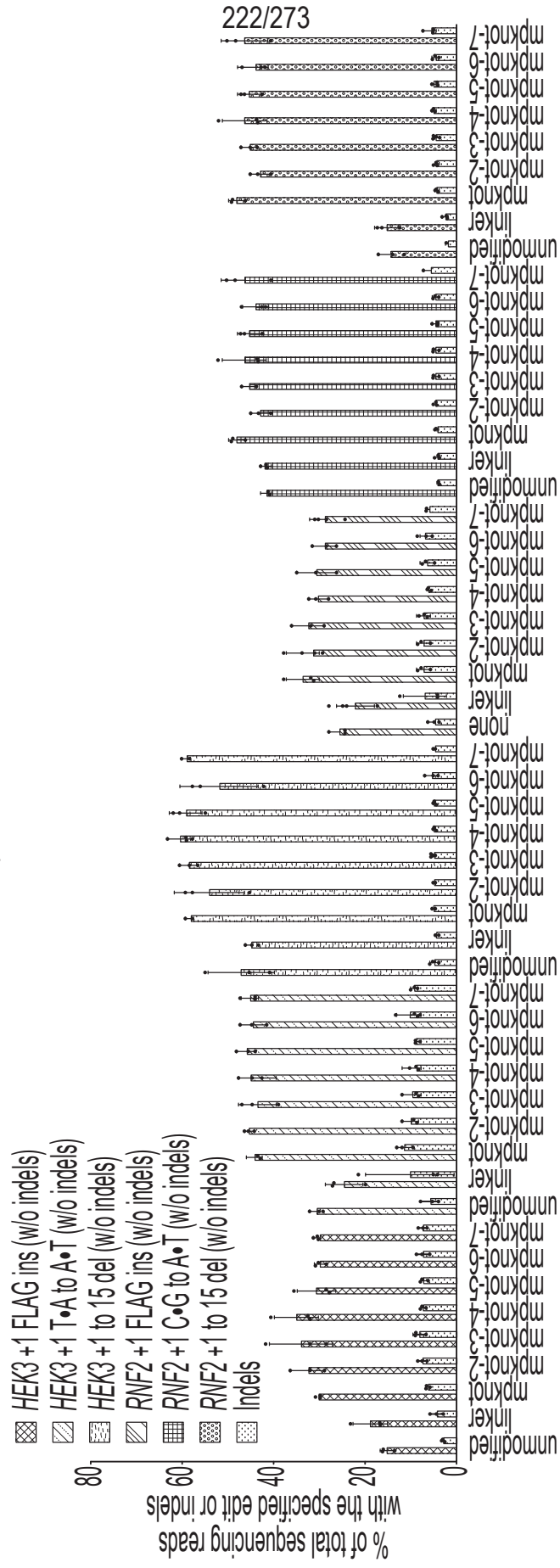


FIG. 117B

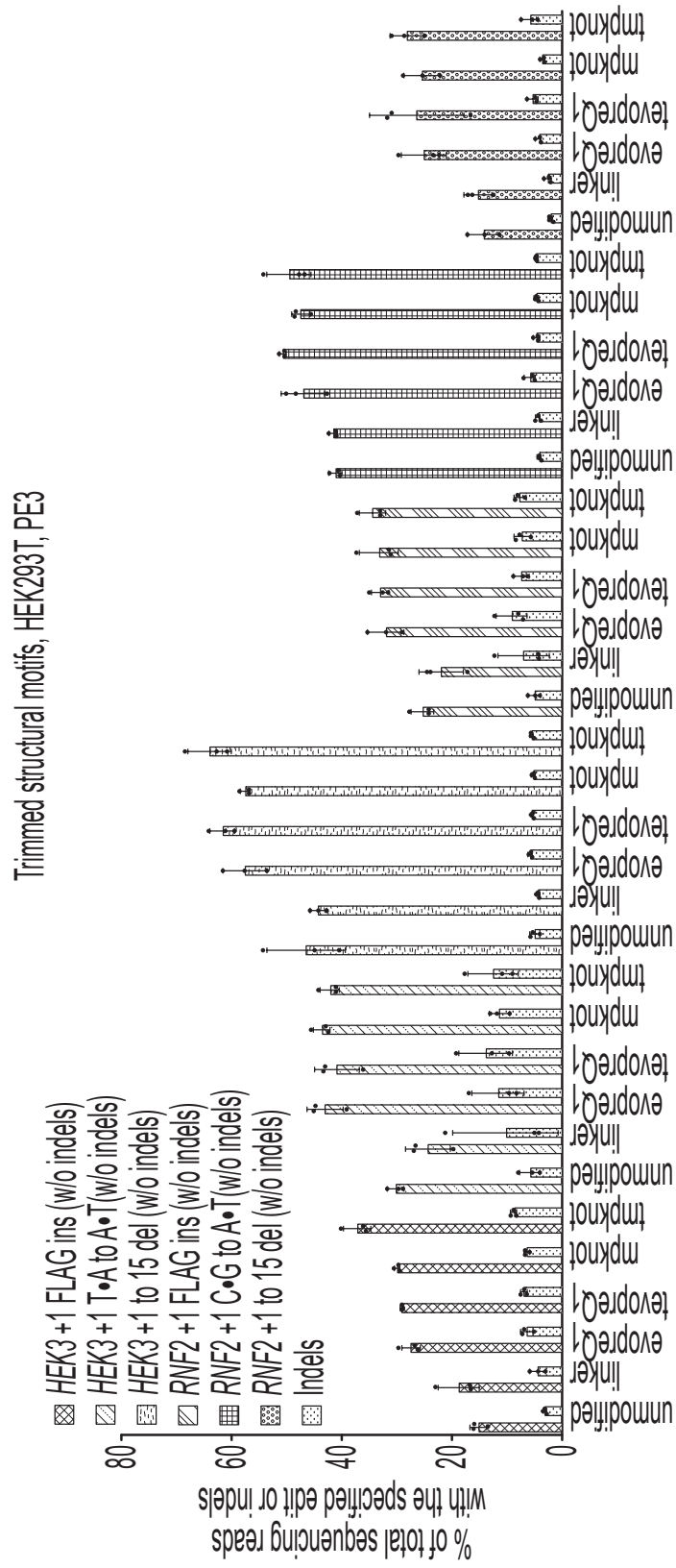


FIG. 117C

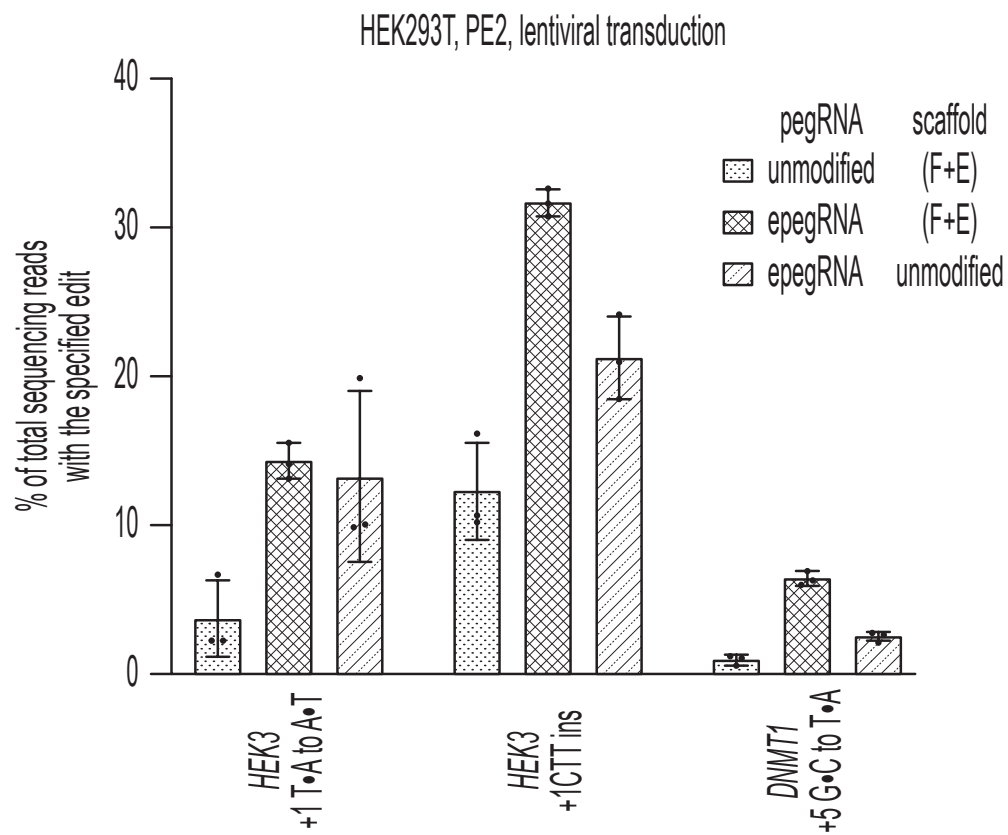


FIG. 118

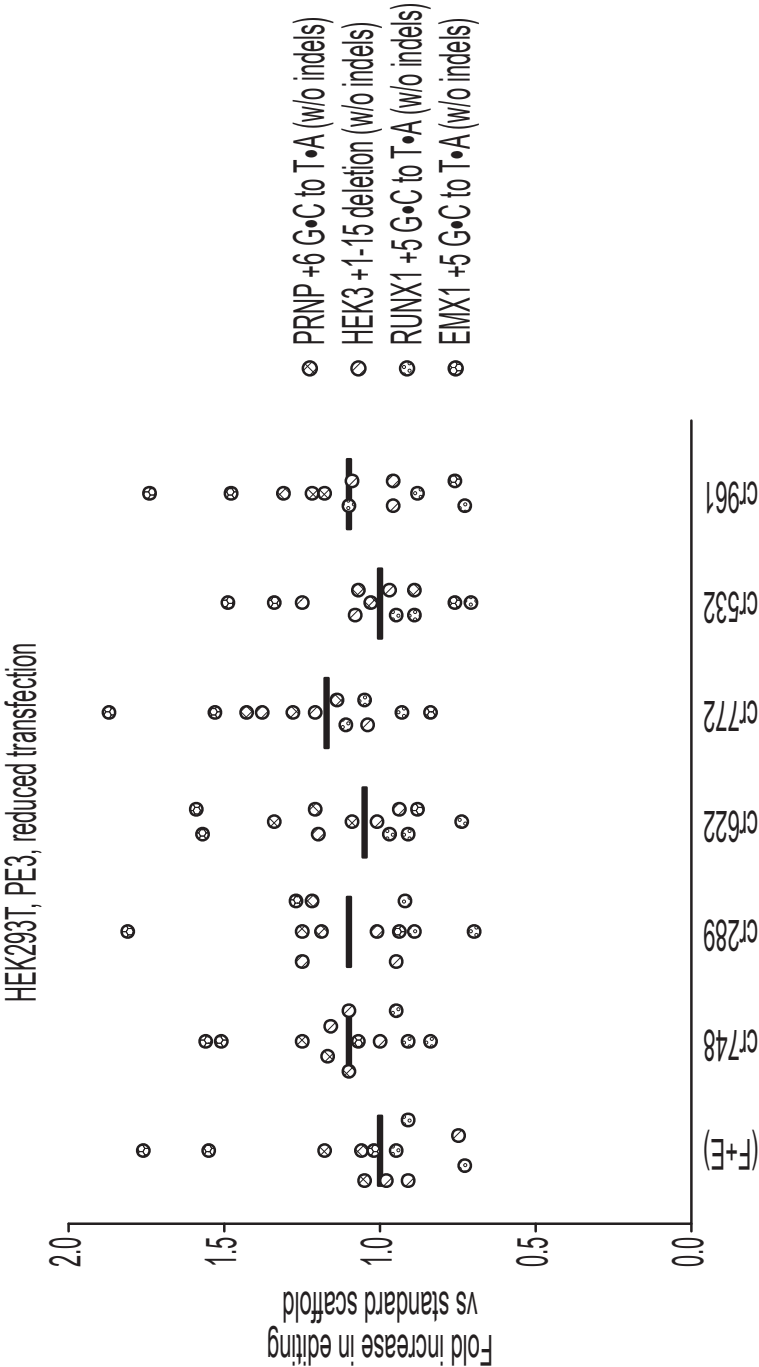


FIG. 119

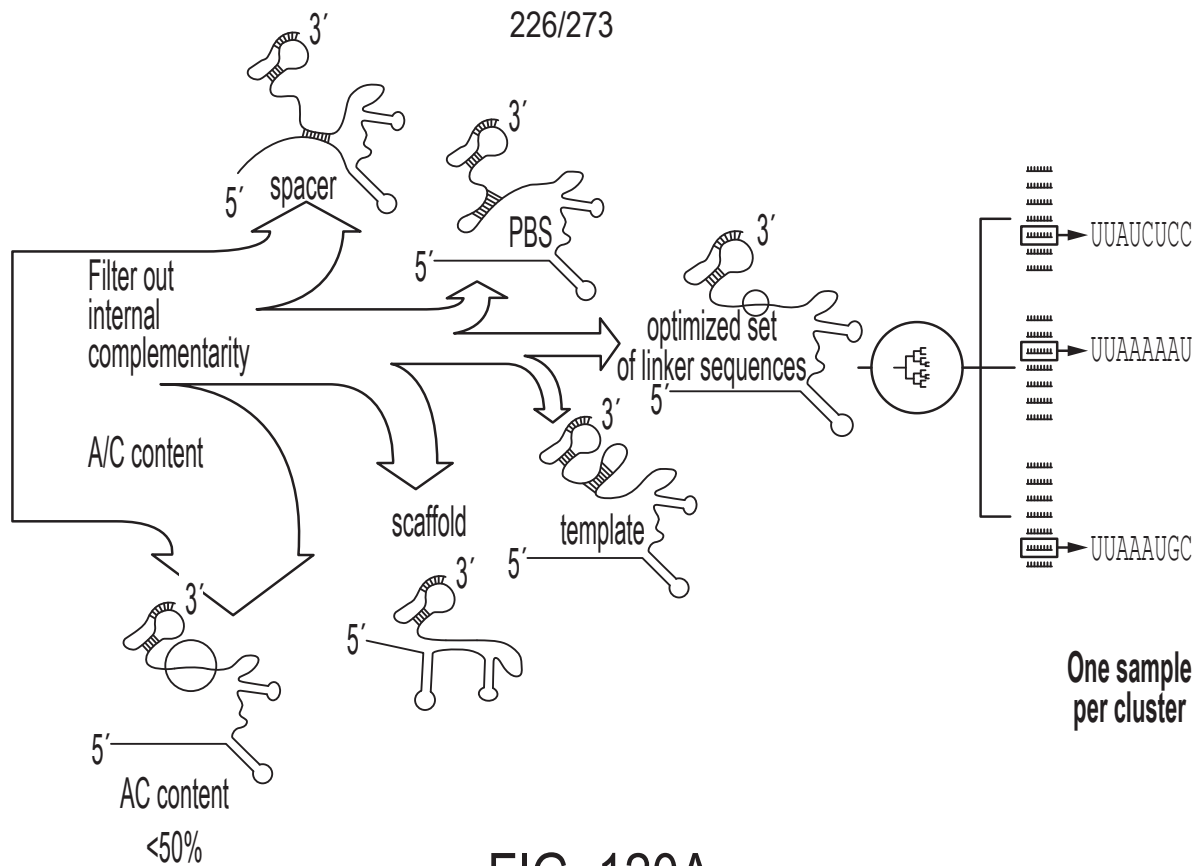


FIG. 120A

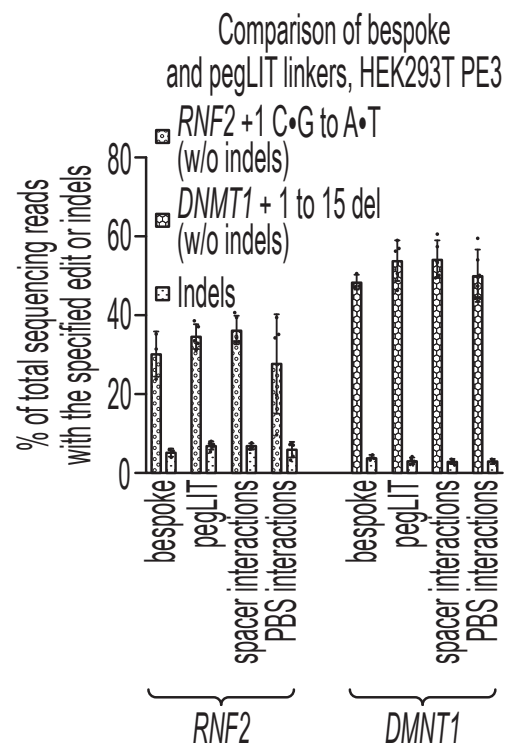


FIG. 120B

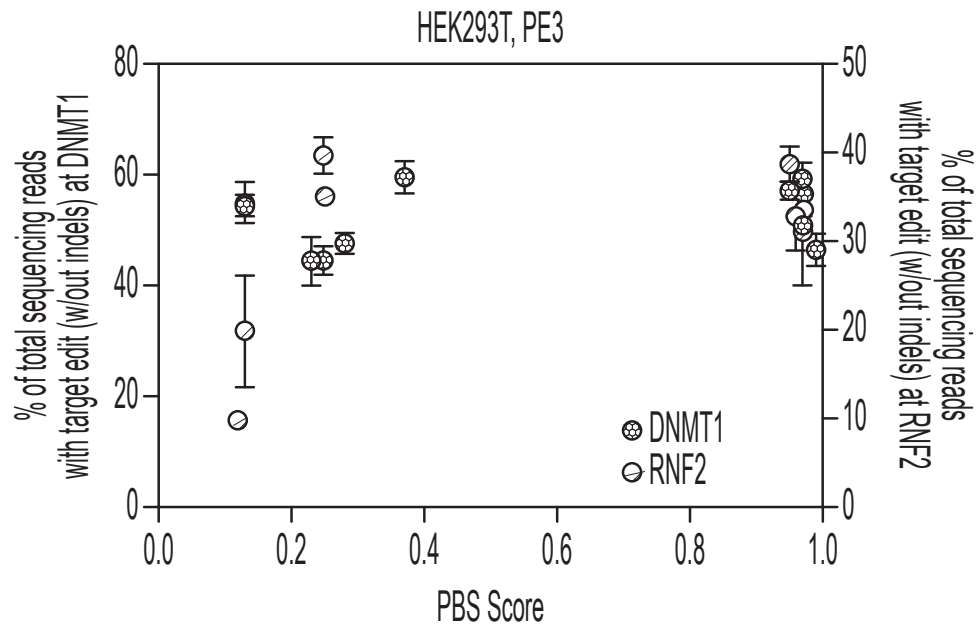


FIG. 120C

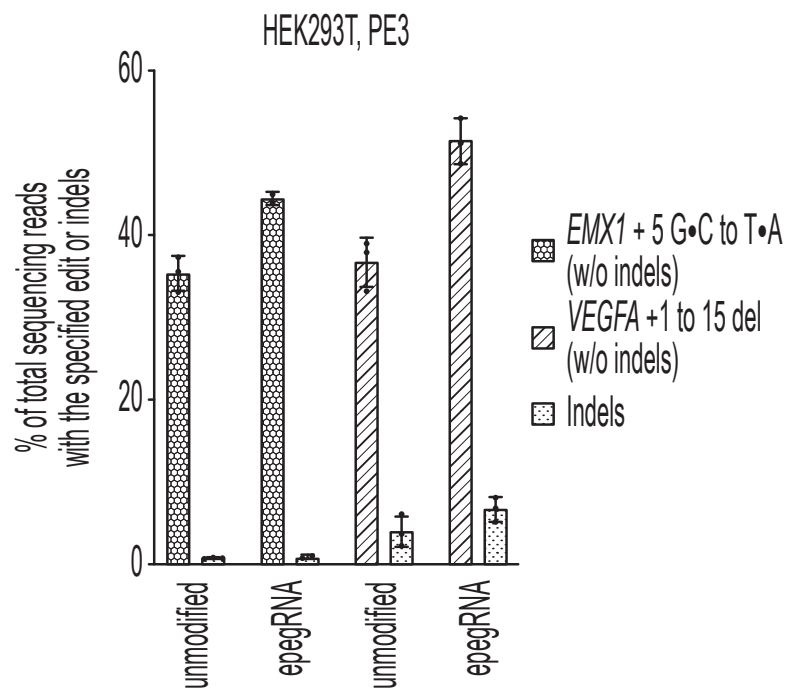


FIG. 120D

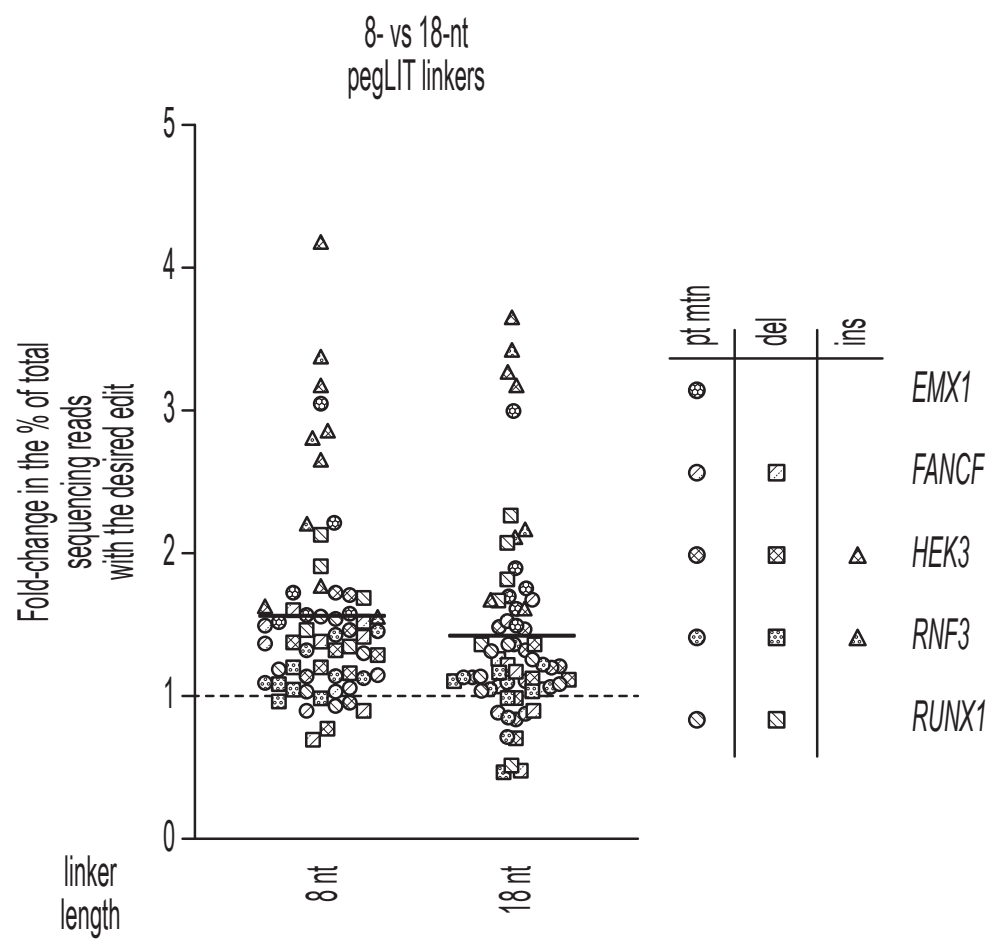


FIG. 120E

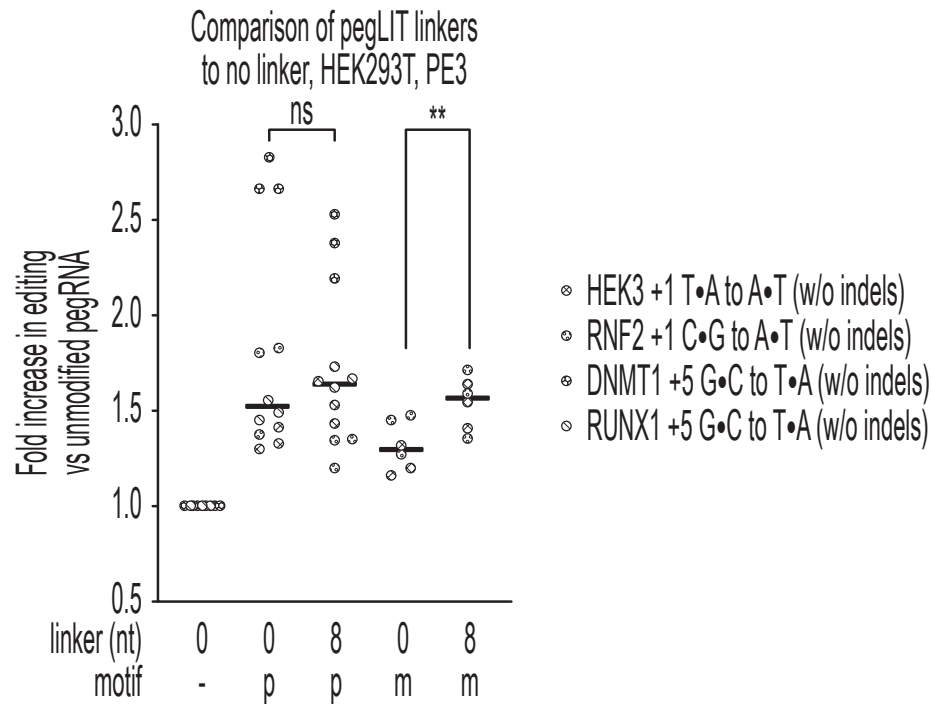


FIG. 120F

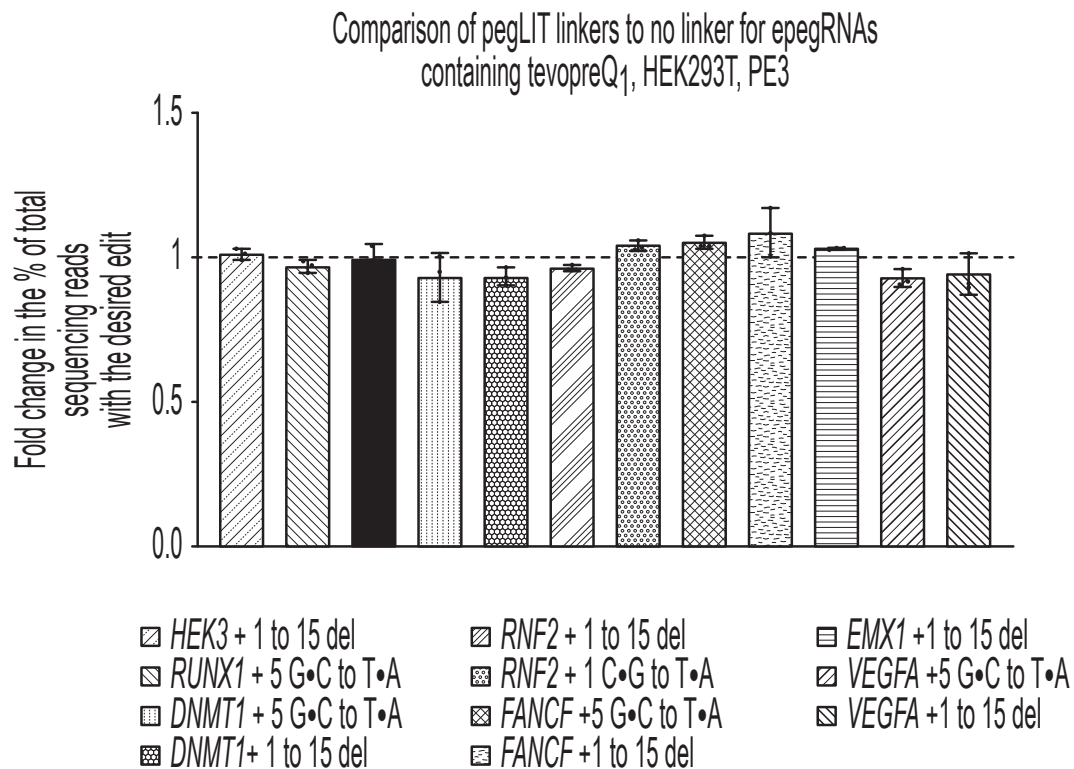


FIG. 120G

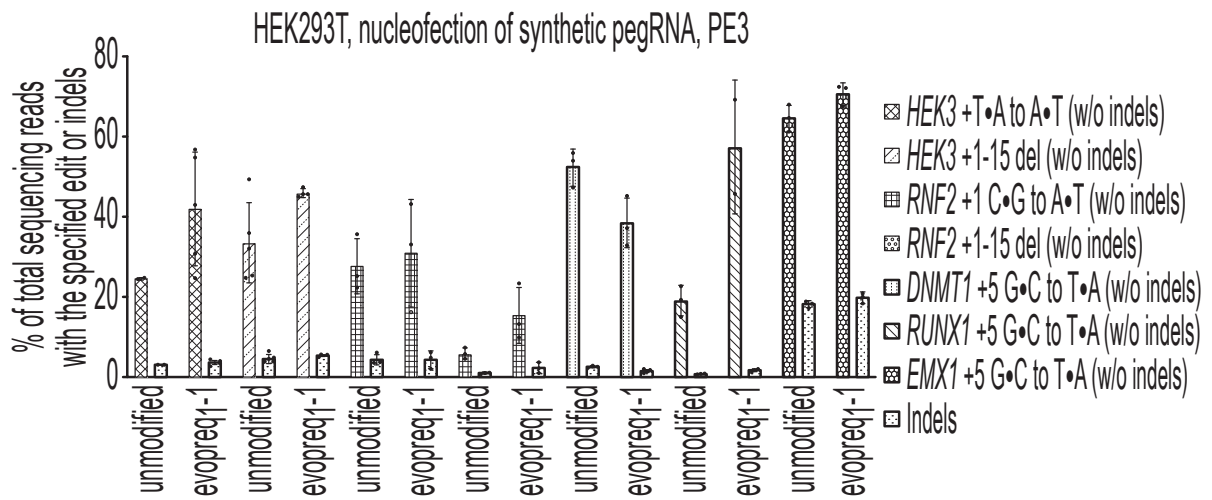


FIG. 121A

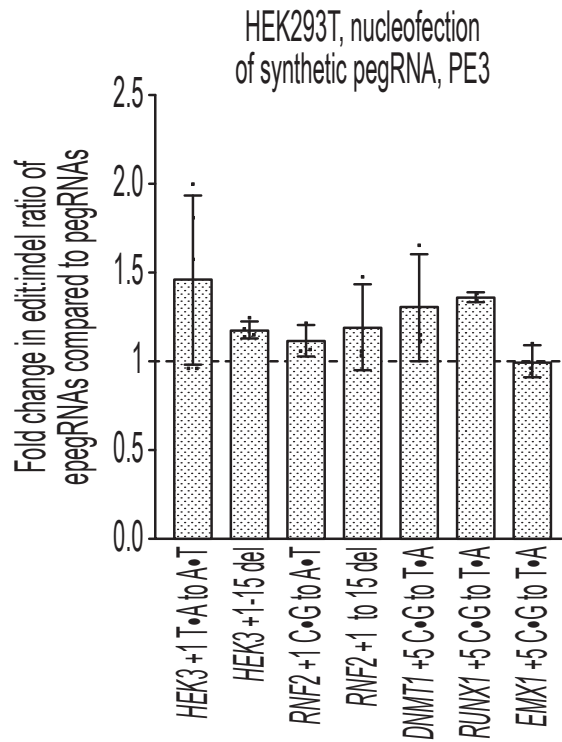


FIG. 121B

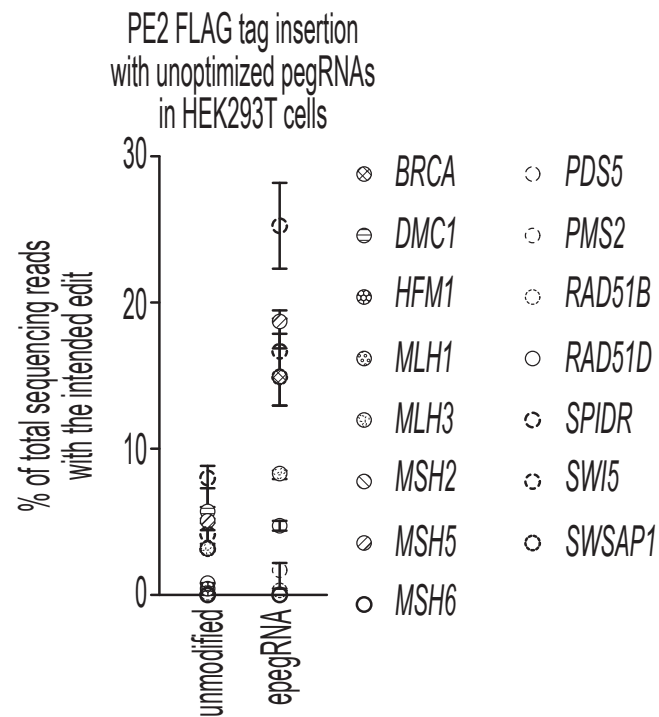


FIG. 122A

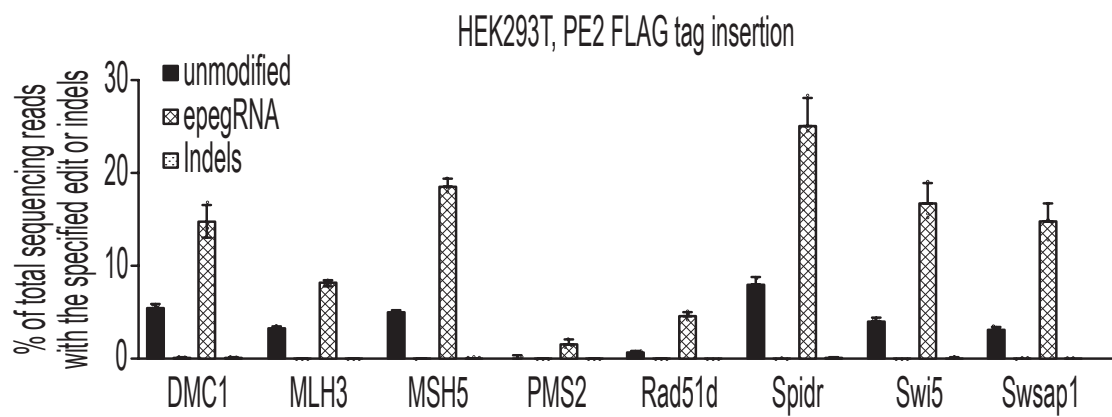


FIG. 122B

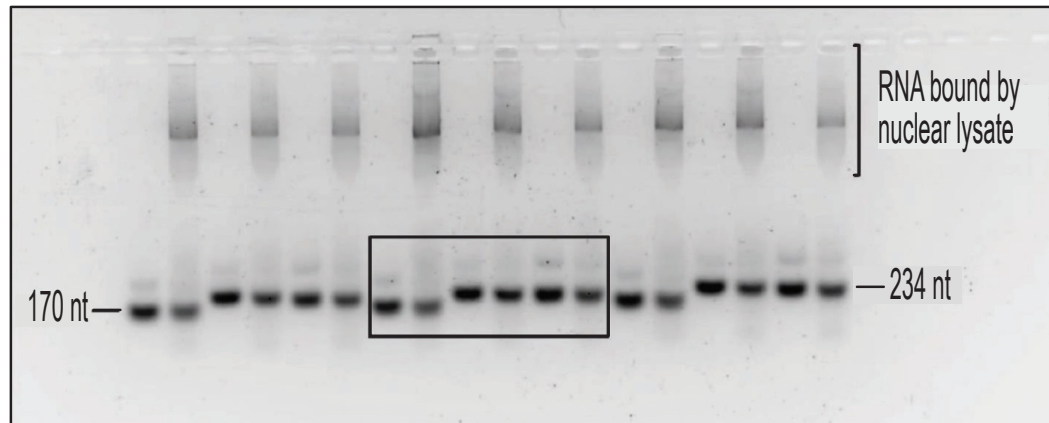


FIG. 123

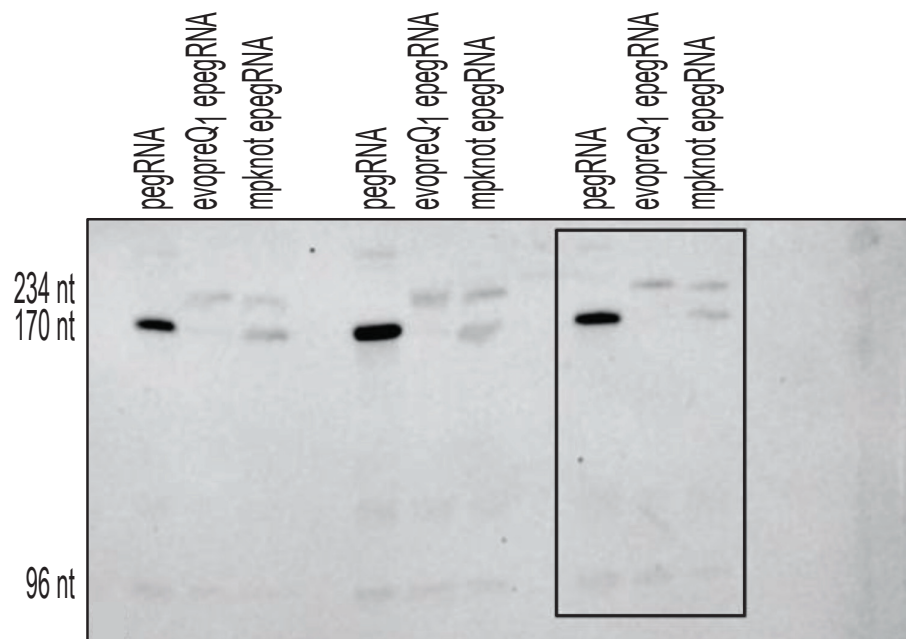


FIG. 124A

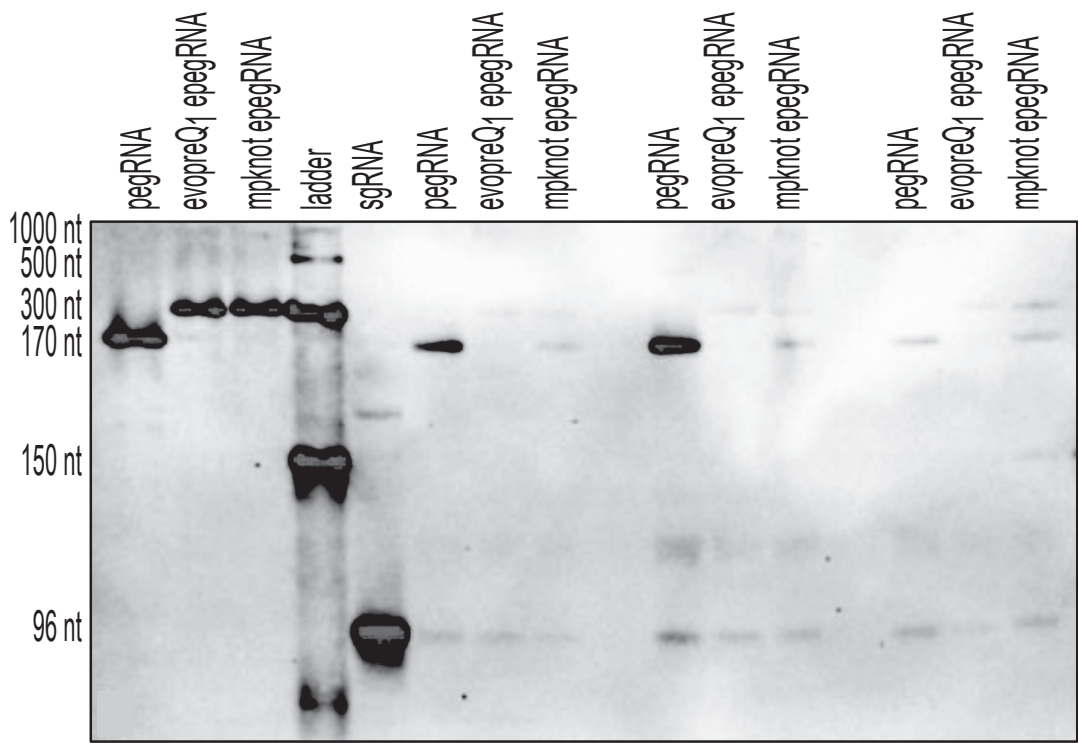


FIG. 124B

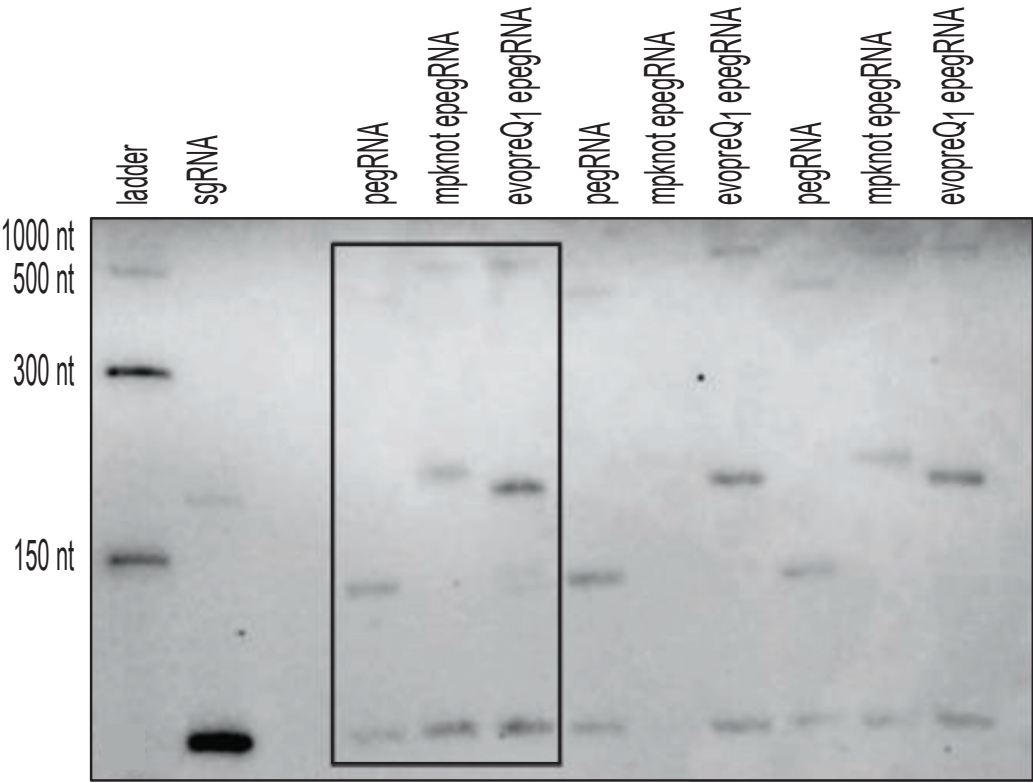


FIG. 124C

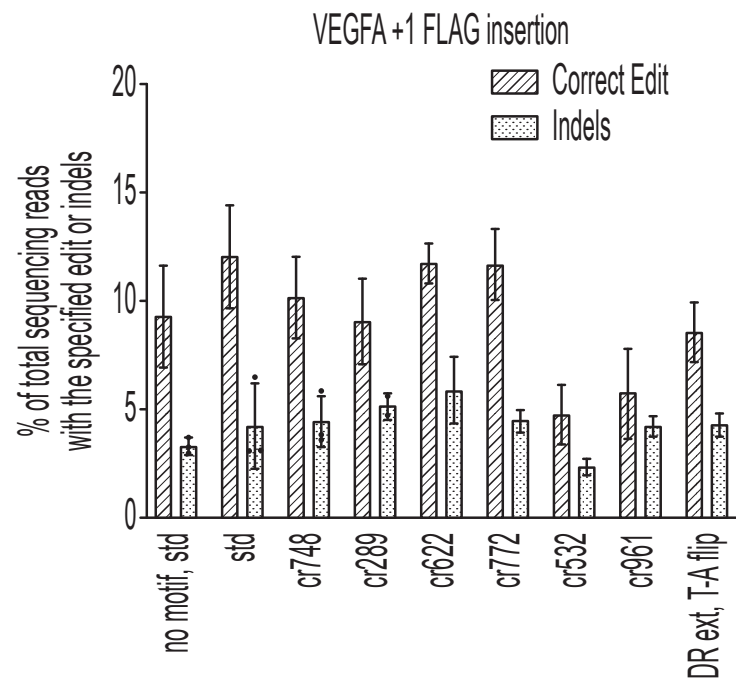


FIG. 125A

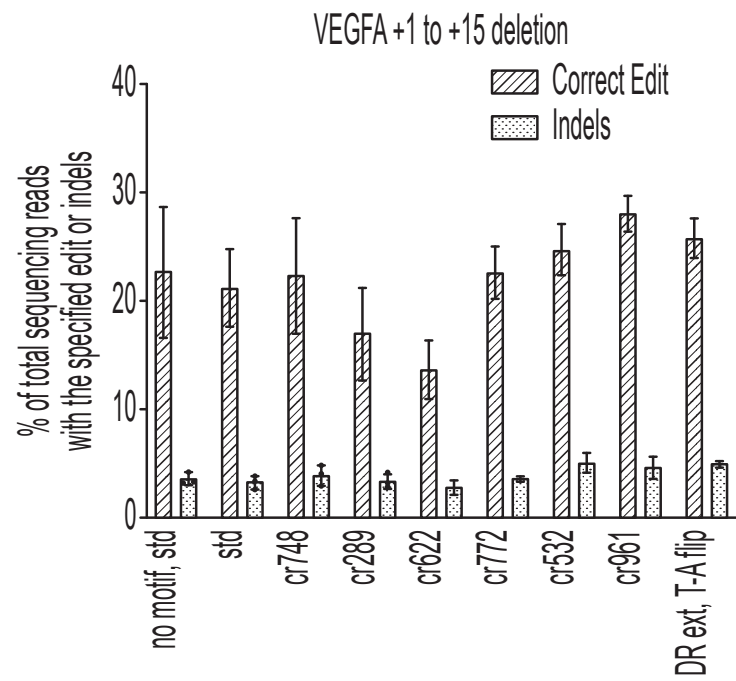


FIG. 125B

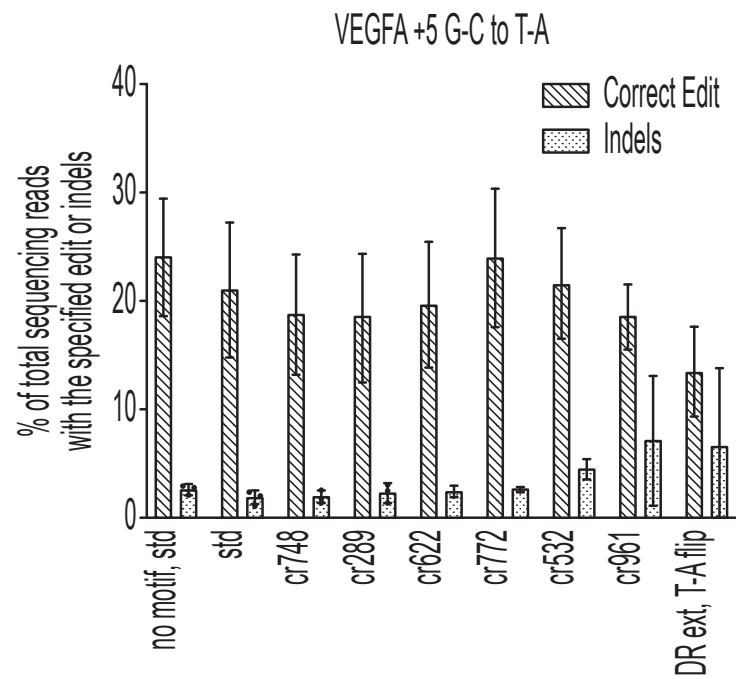


FIG. 125C

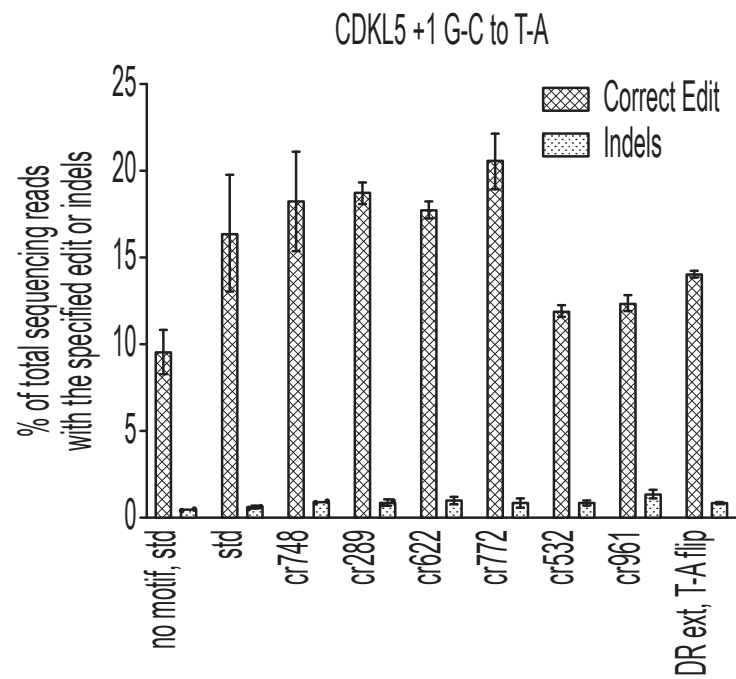


FIG. 125D

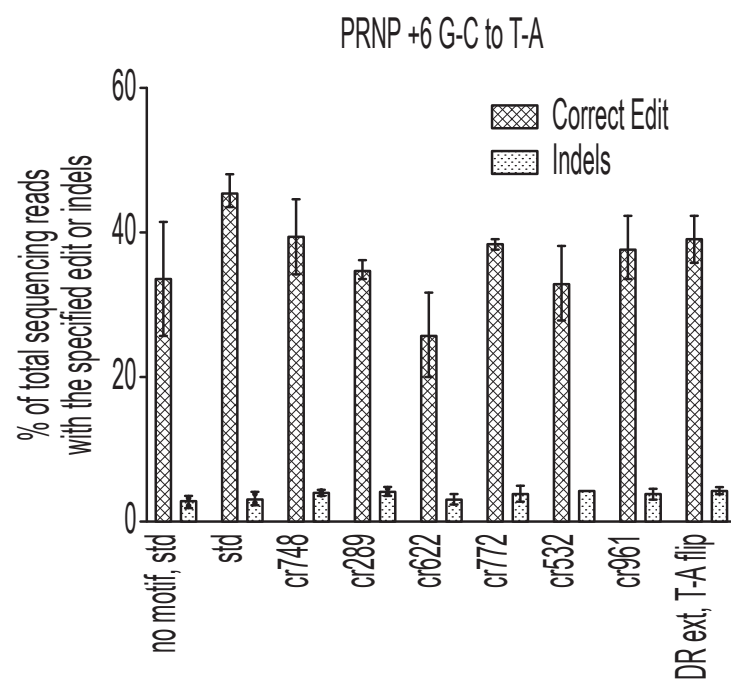


FIG. 125E

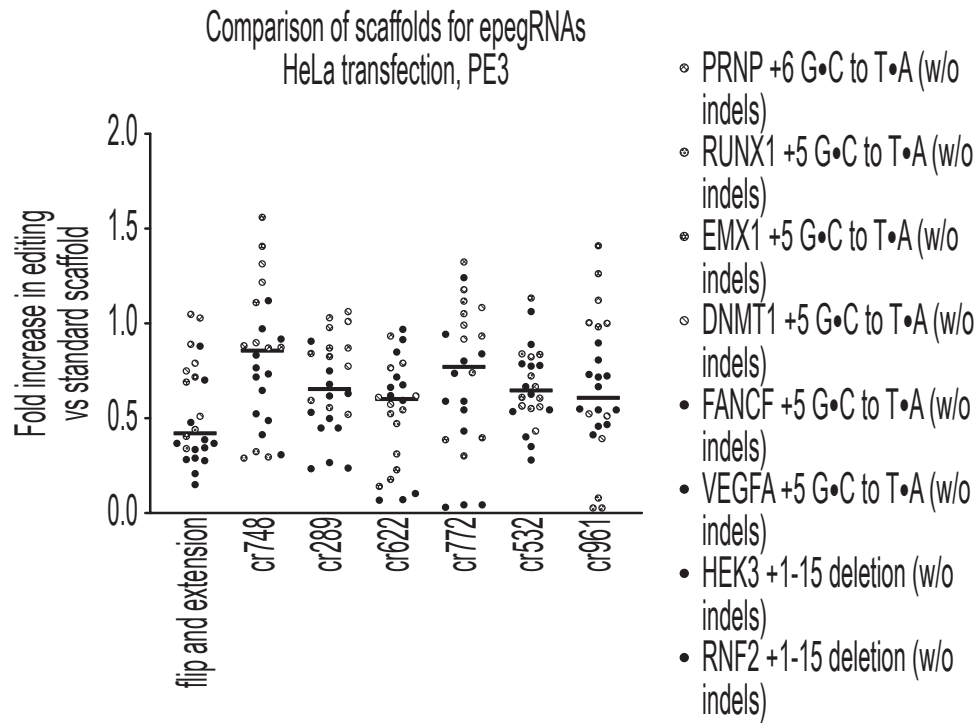


FIG. 126A

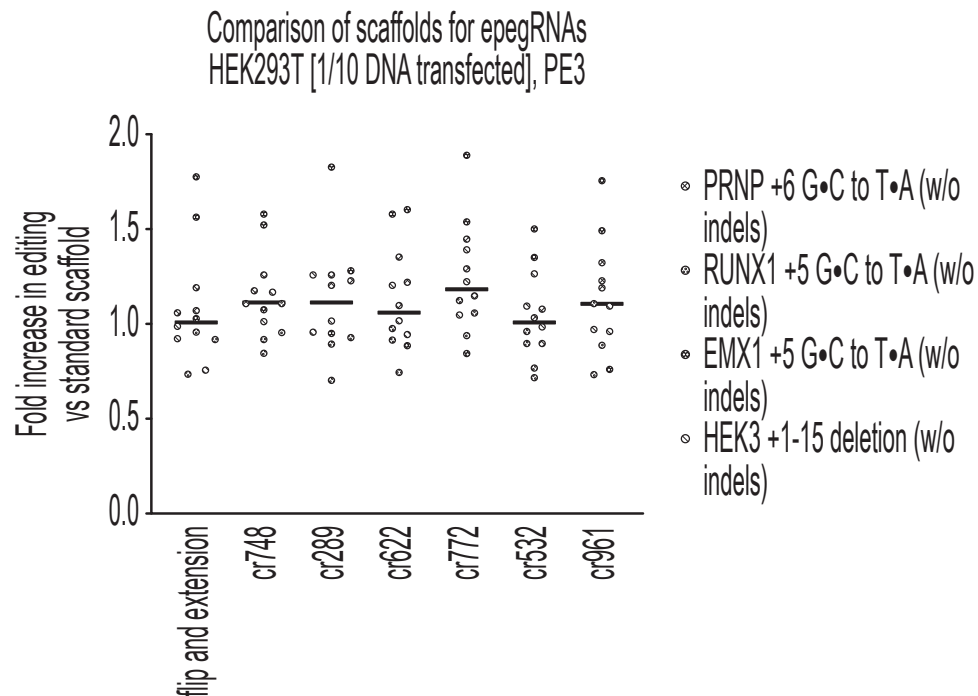


FIG. 126B

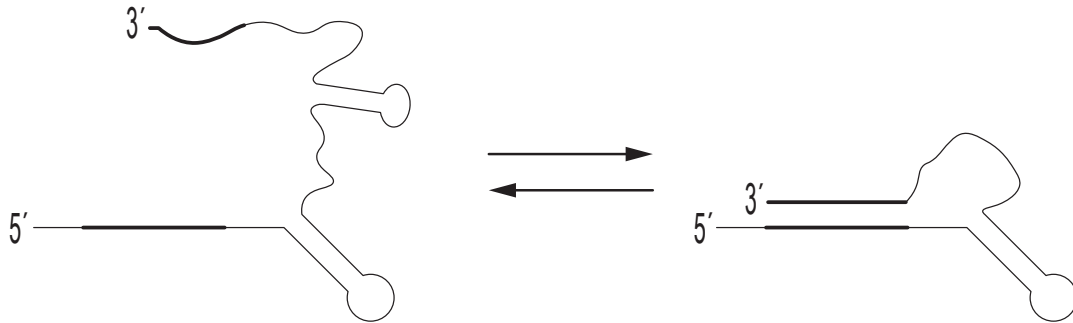


FIG. 127A

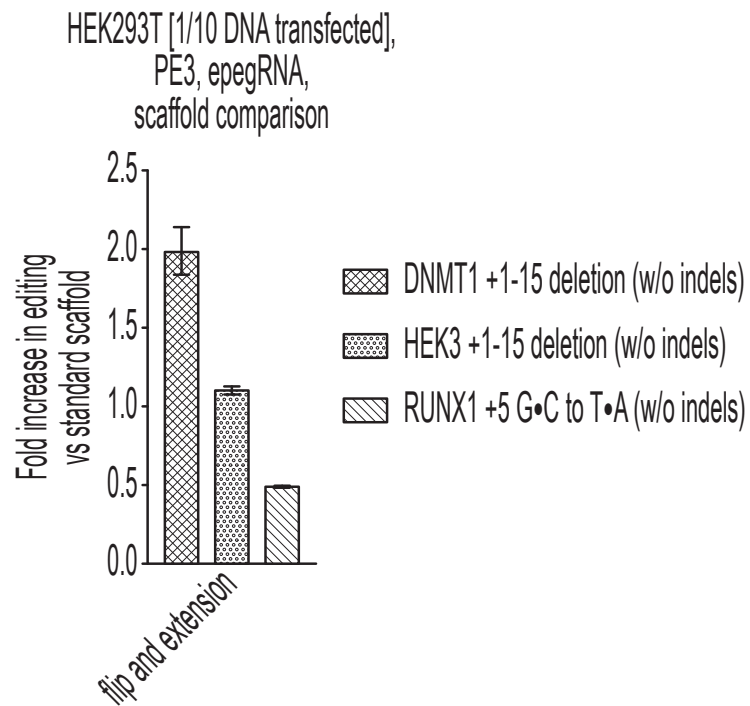


FIG. 127B

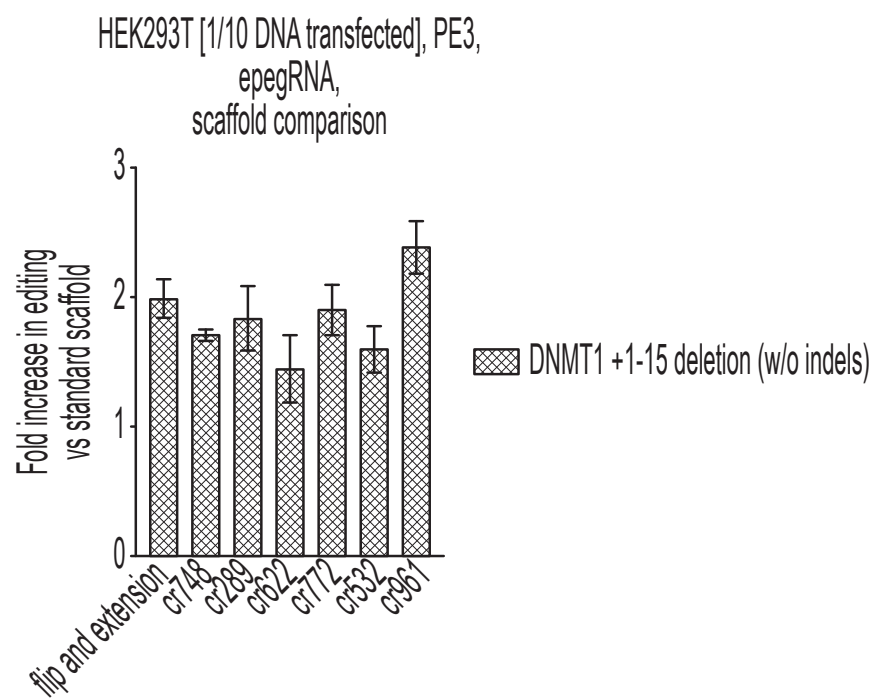


FIG. 127C

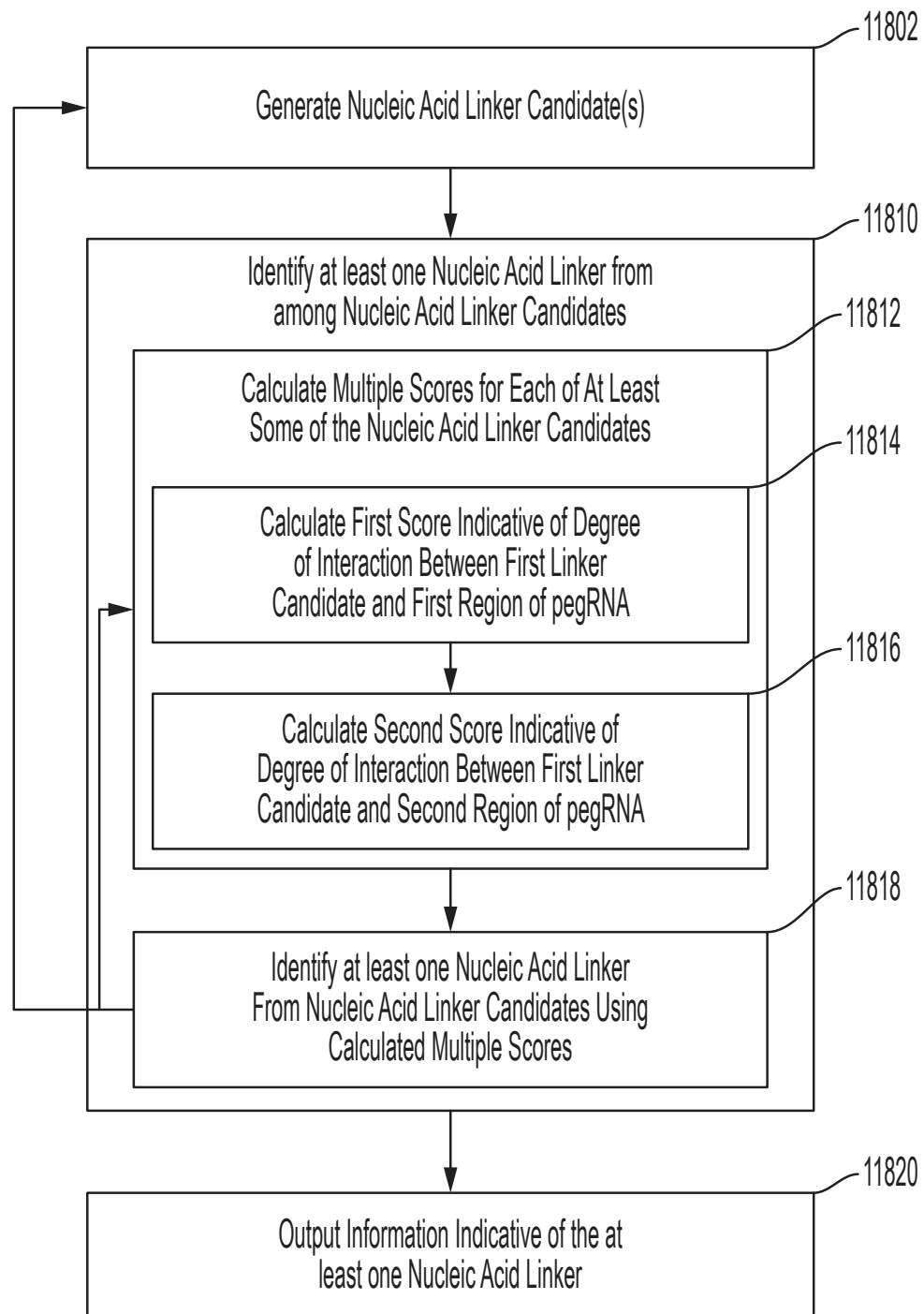


FIG. 128

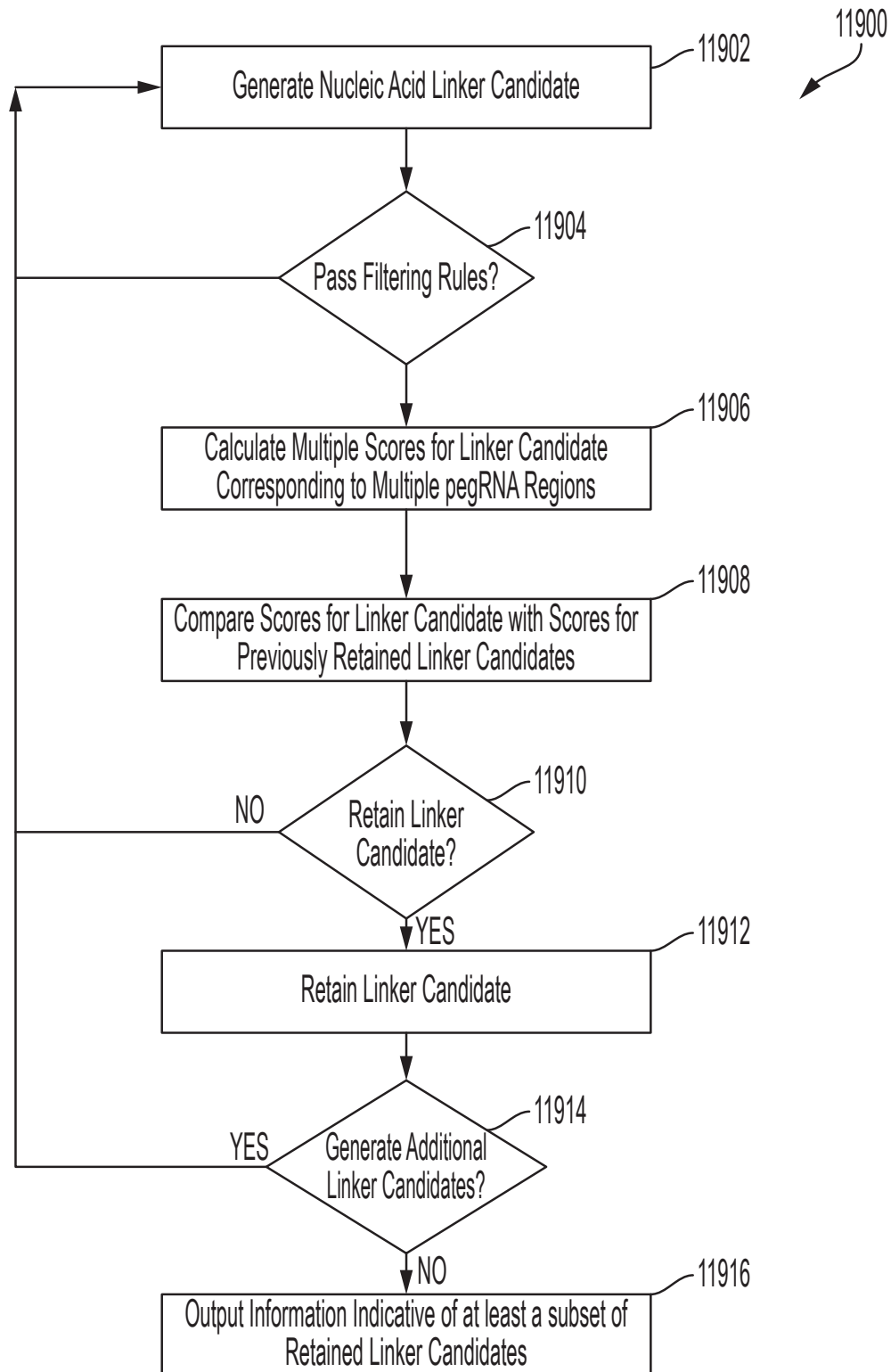


FIG. 129

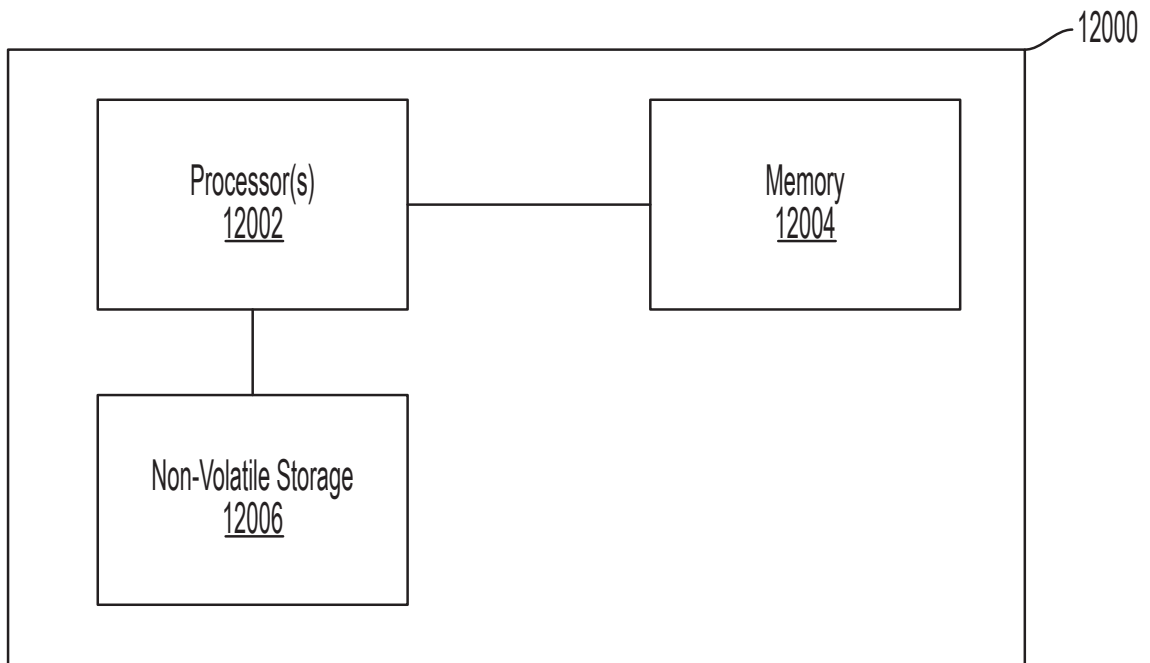
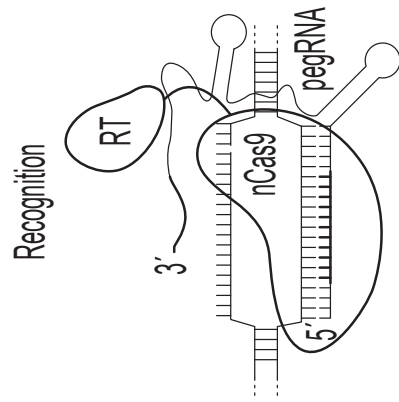
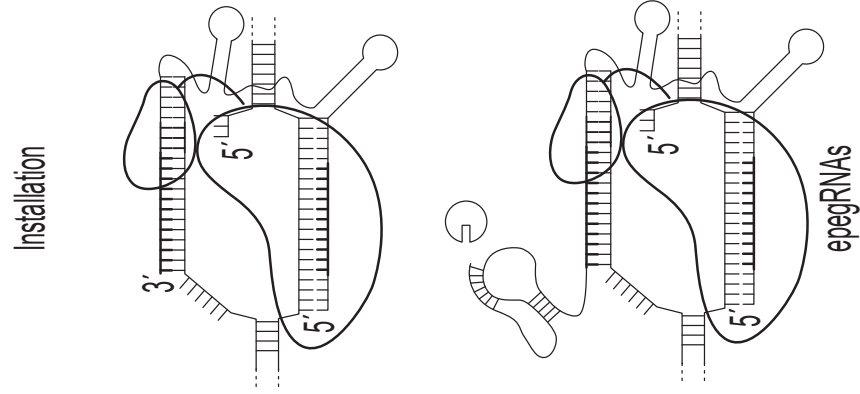
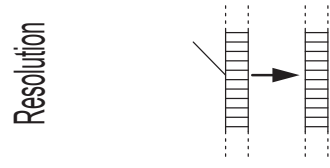


FIG. 130



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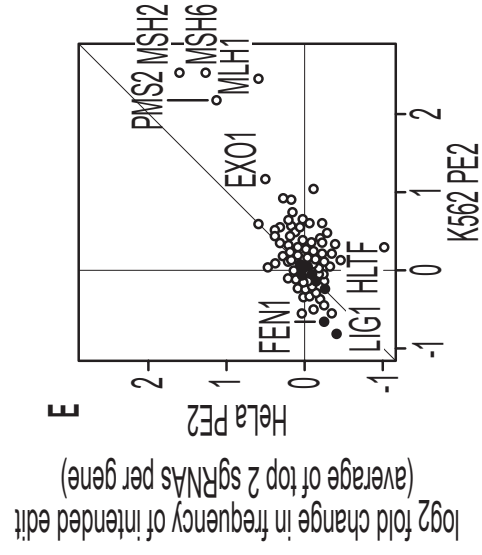


FIG. 131

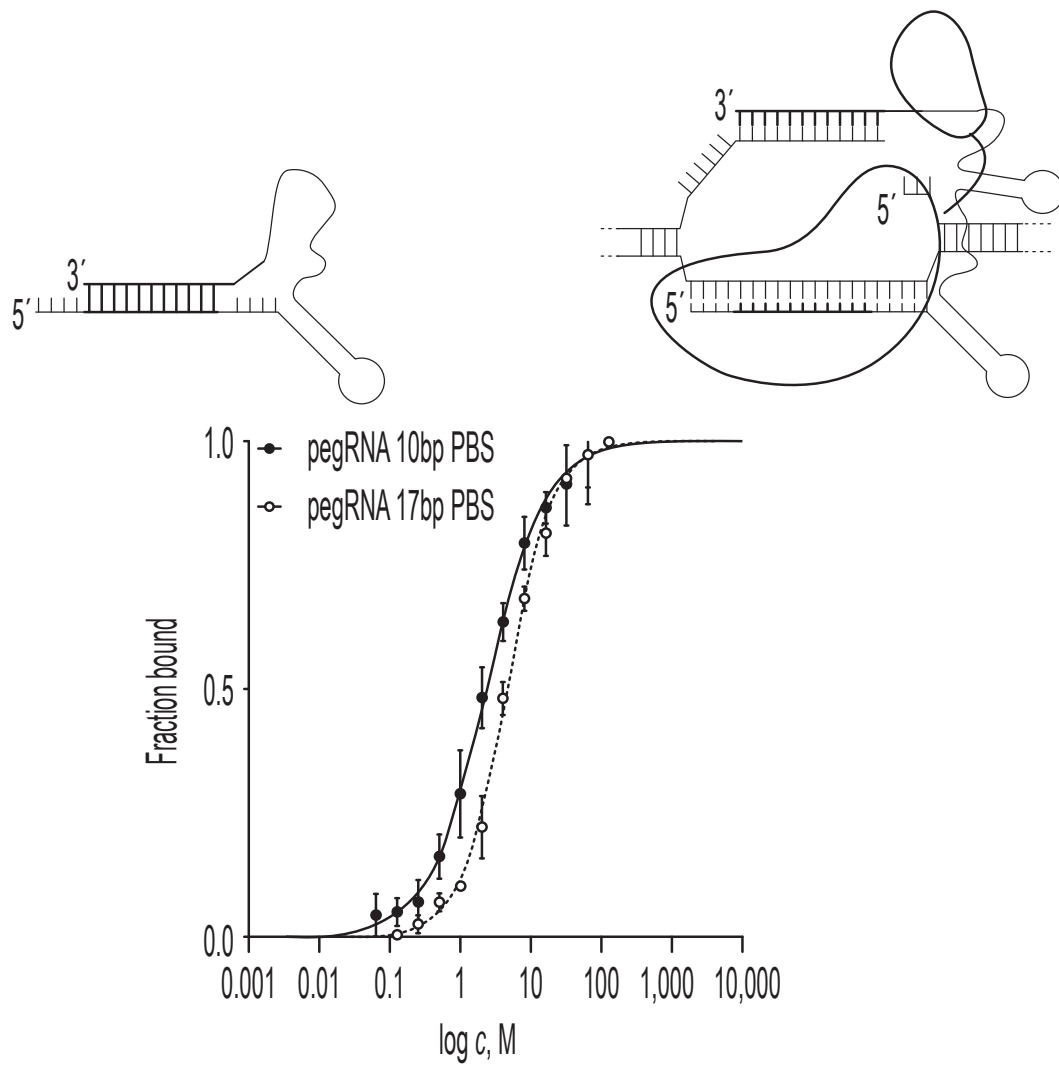


FIG. 132

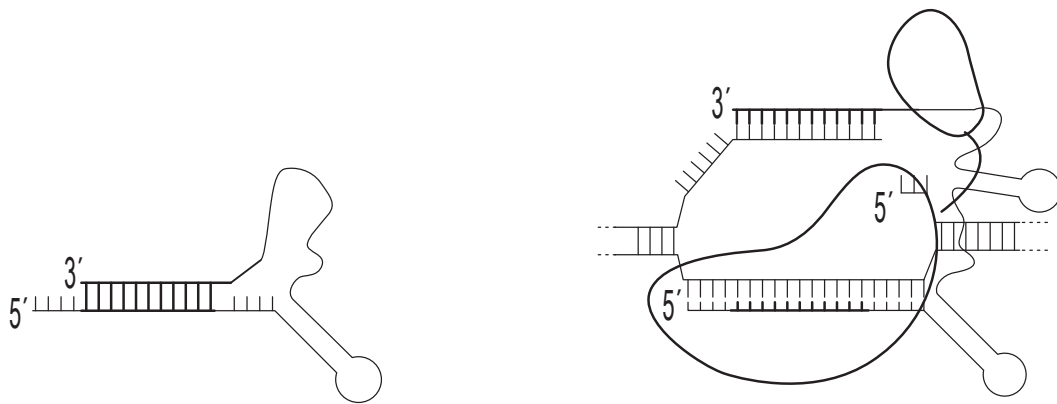


FIG. 133

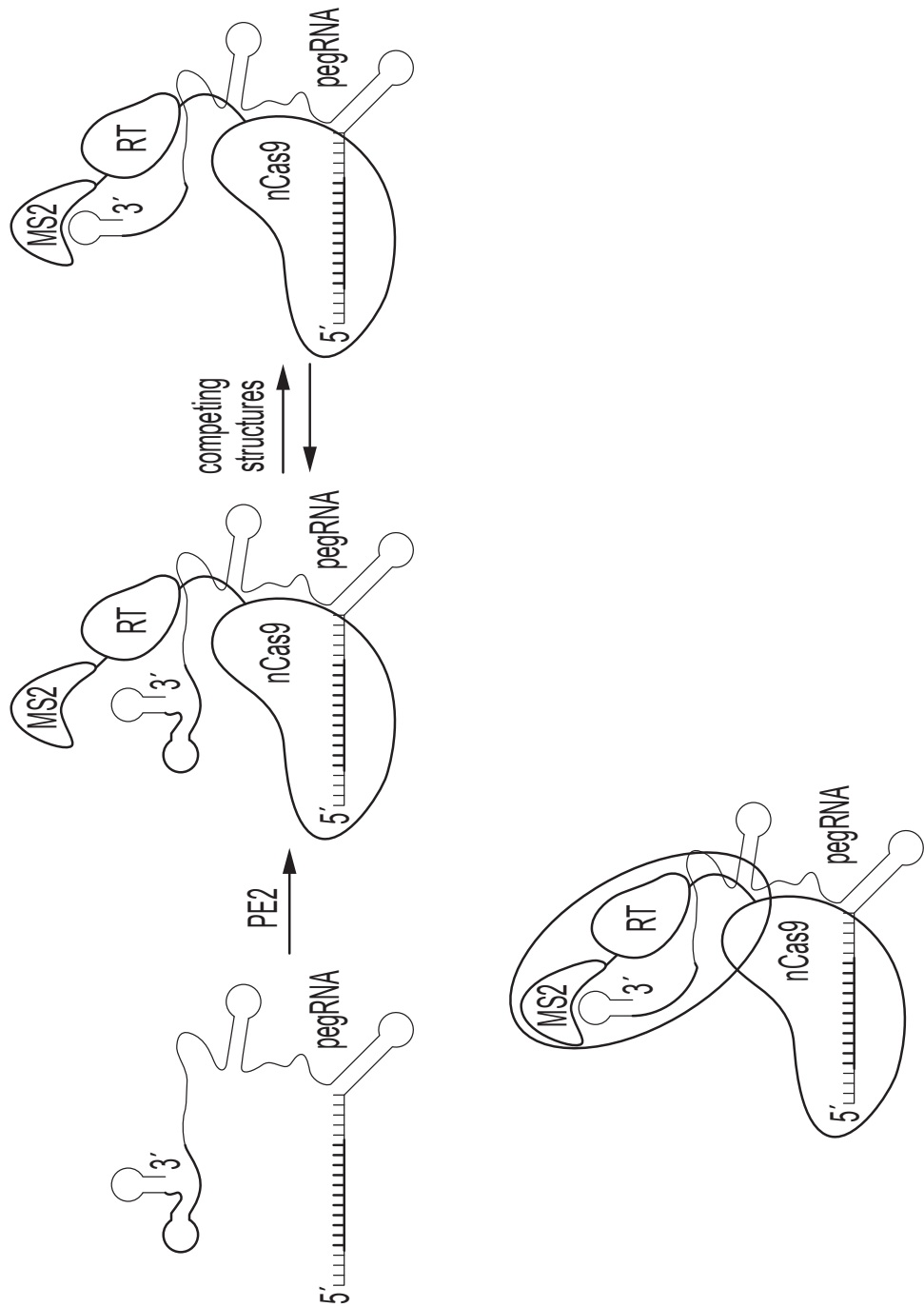


FIG. 134

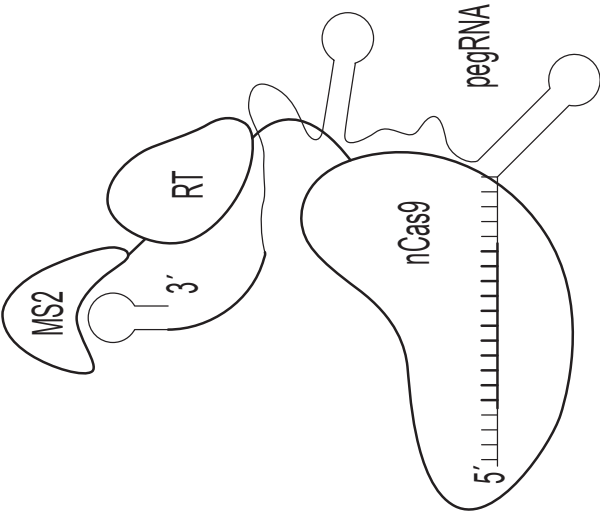
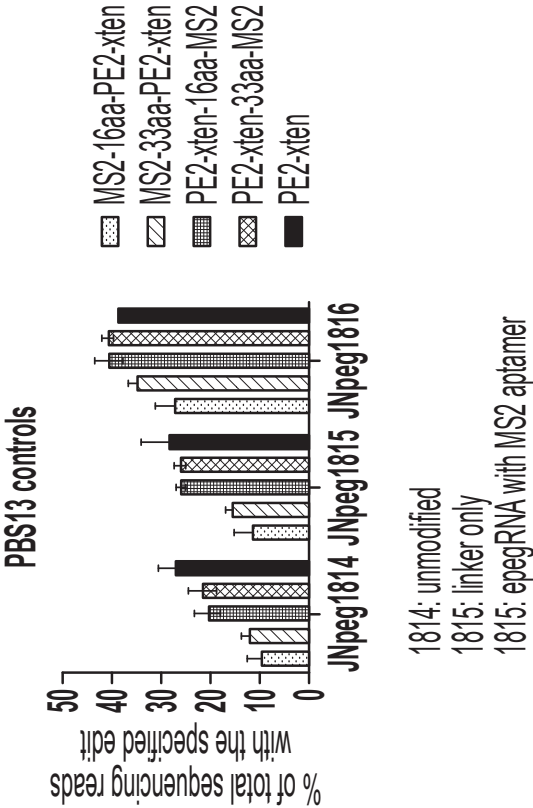
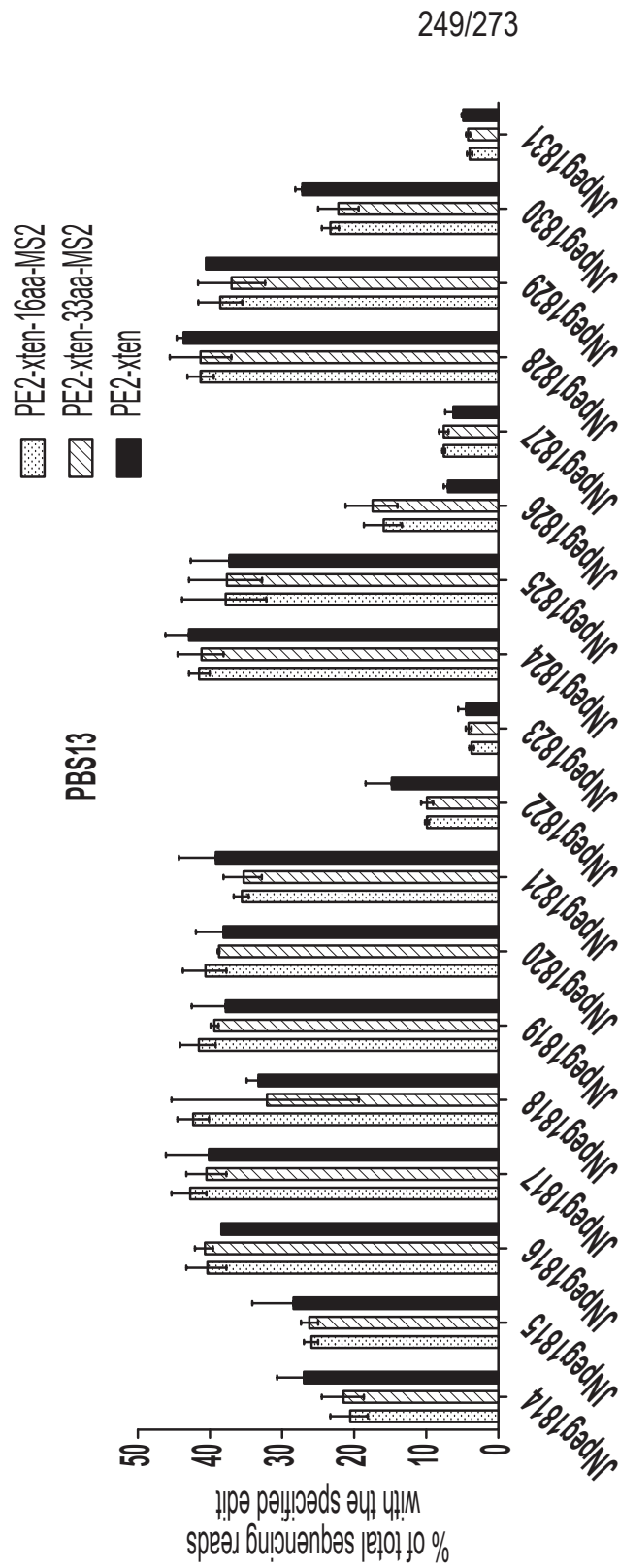
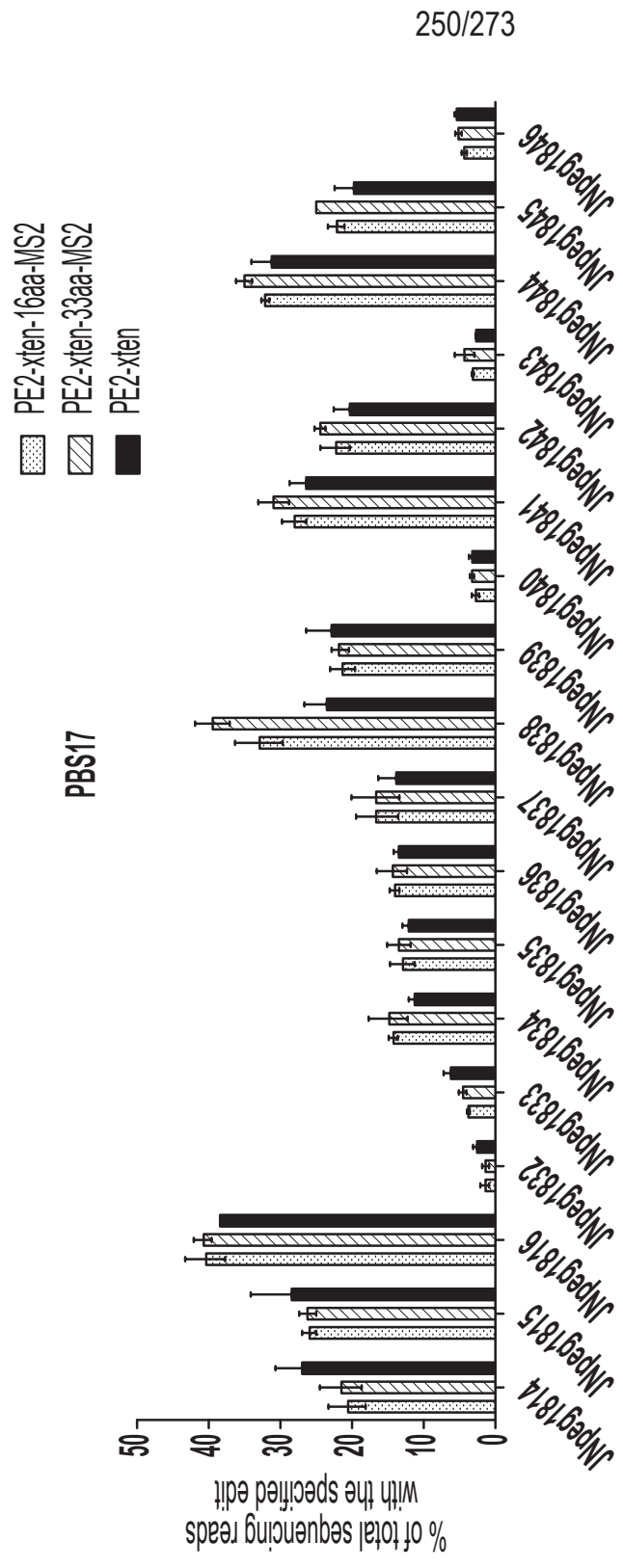


FIG. 135



PBS = 13 all
 1814: unmodified
 1815: linker only
 1816: epegRNA with MS2 aptamer
 1817-1819: no toehold, MS2 aptamer
 1820-1831: toeholds of various strengths, MS2 aptamer

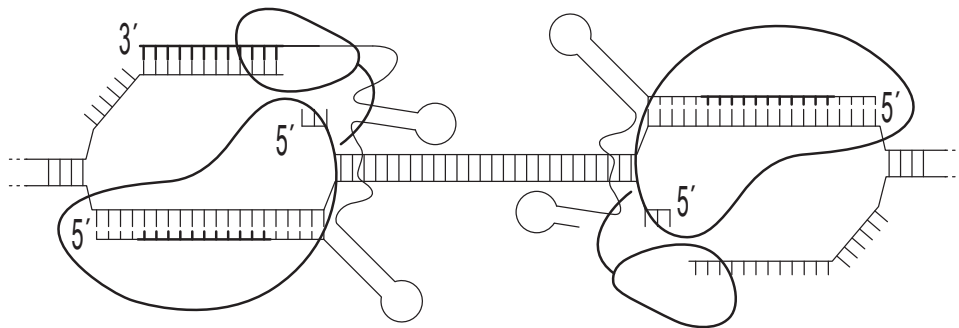
FIG. 136



1814: unmodified PBS 13
 1815: linker only PBS 13
 1816: epegRNA with MS2 aptamer PBS 13
 PBS 17 all
 1832-1833: unmodified; linker alone
 1834-1837: no toehold, MS2 aptamer
 1838-1846: toeholds of various strengths, MS2 aptamer

FIG. 137

PE3, pegRNA, ngRNA



PE3, sgRNA, pegRNA

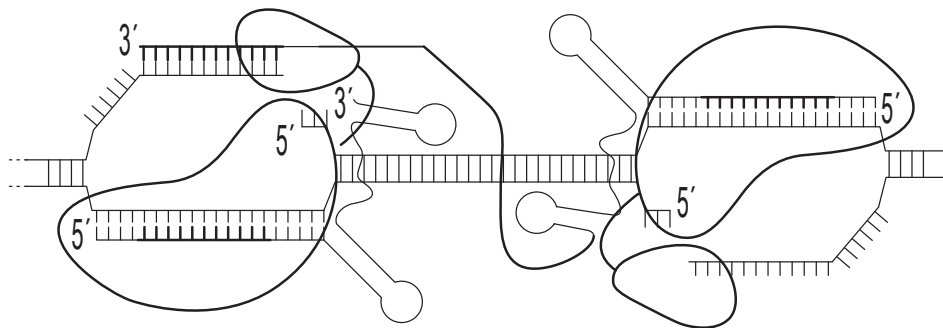


FIG. 138

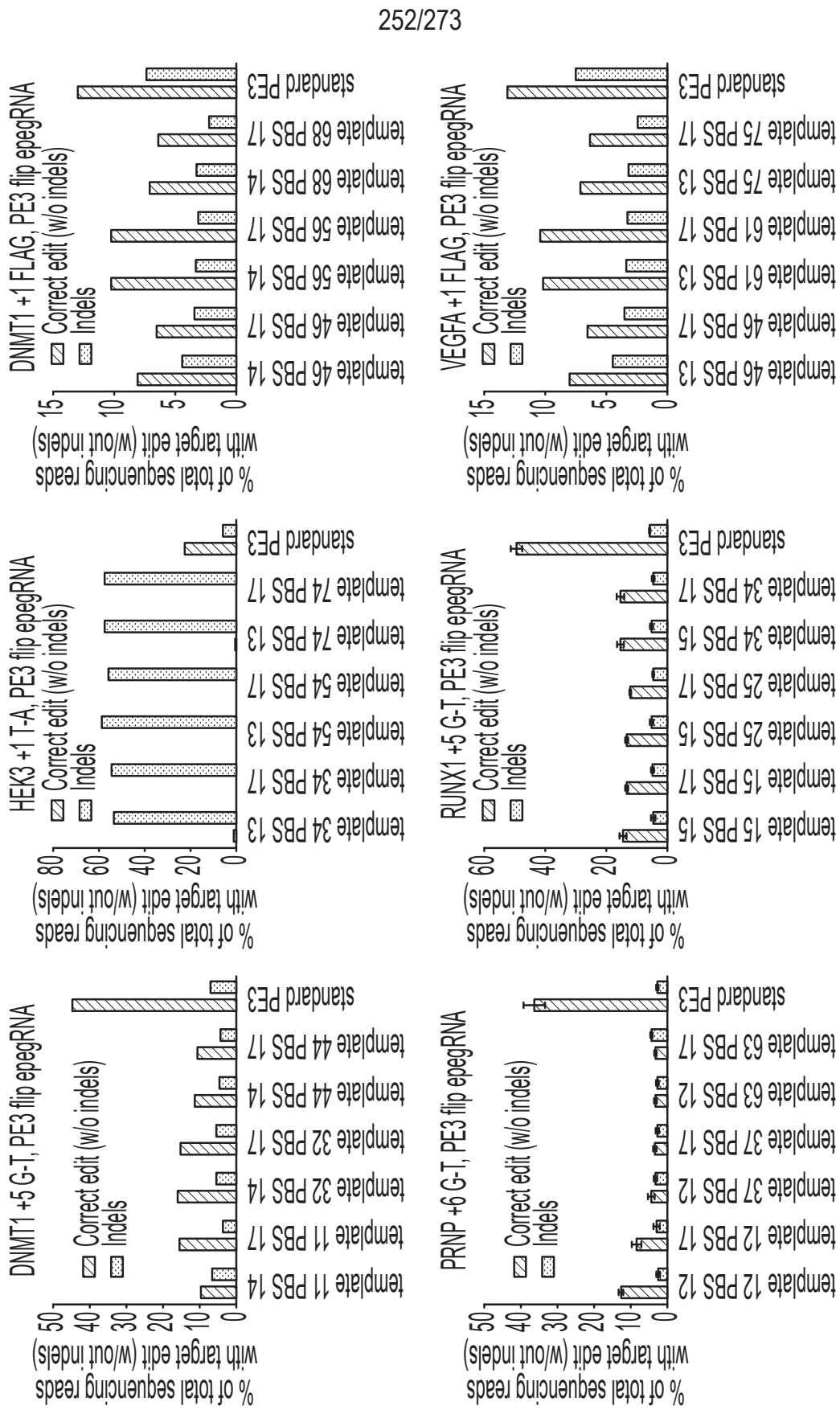


FIG. 139

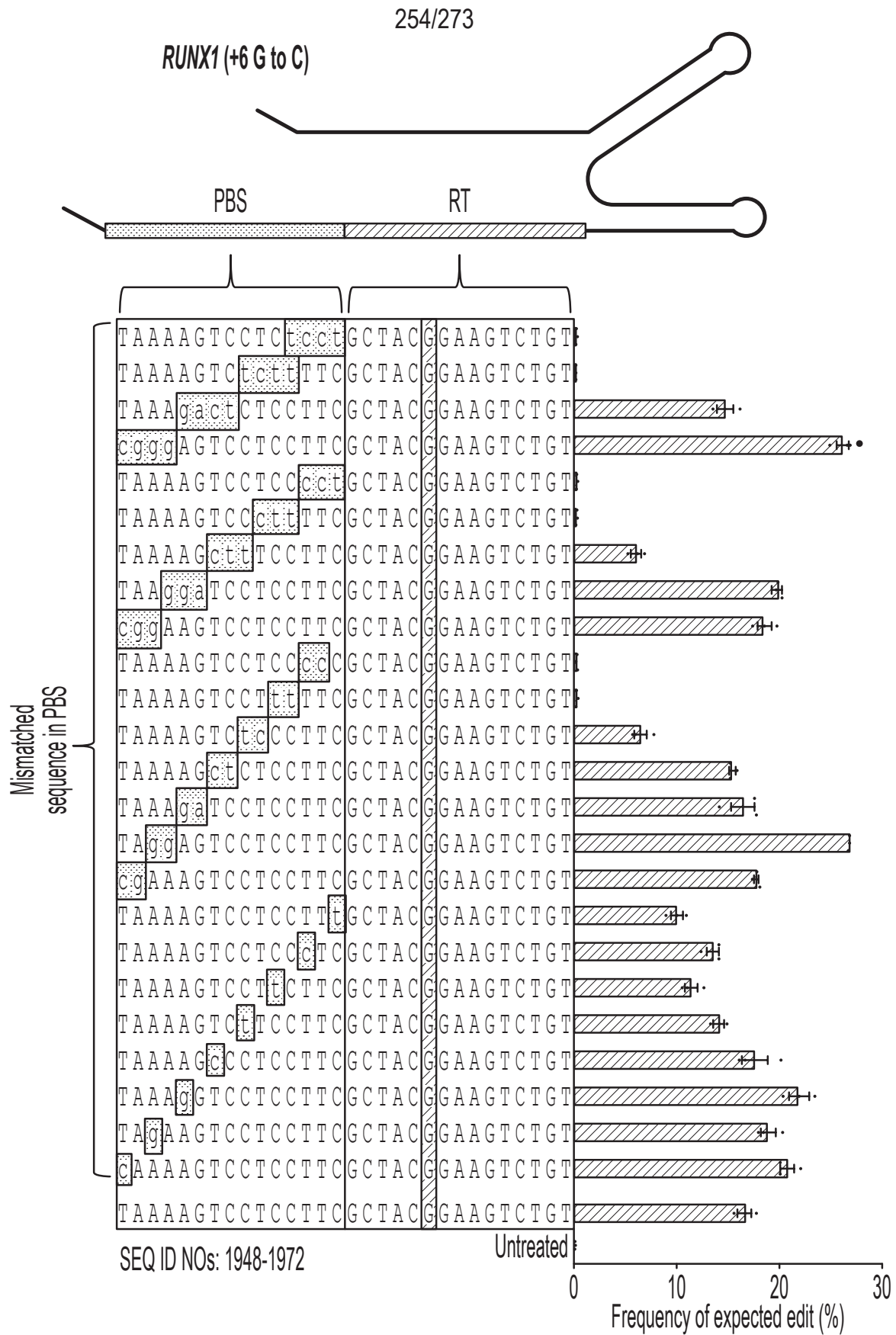


FIG. 141

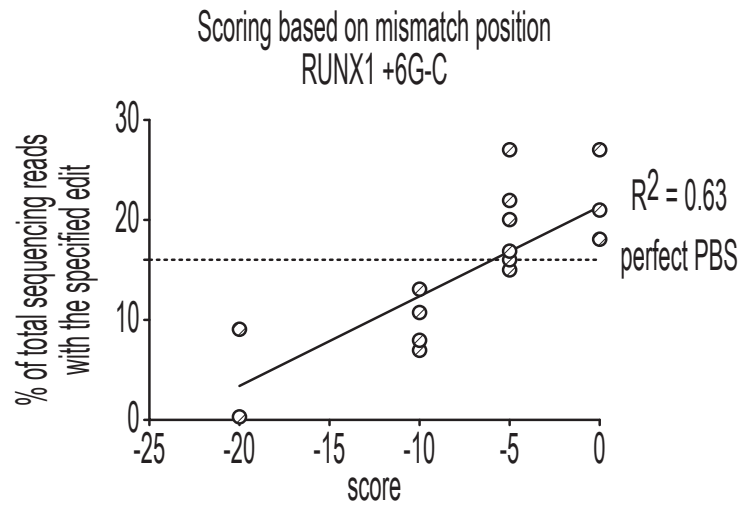
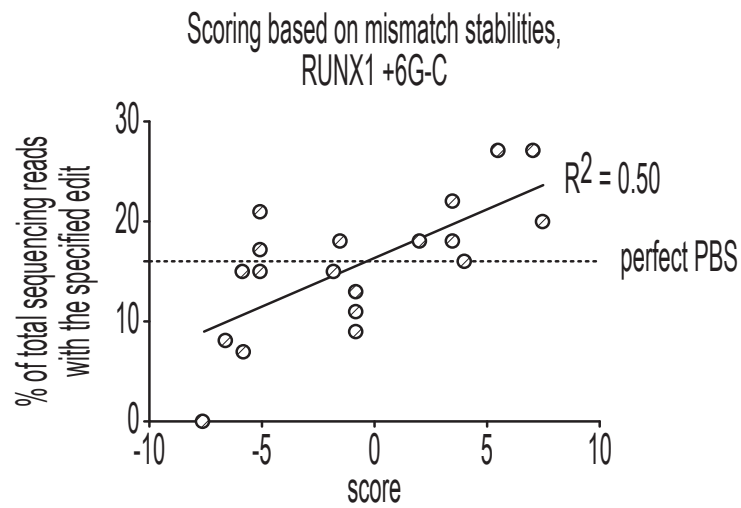
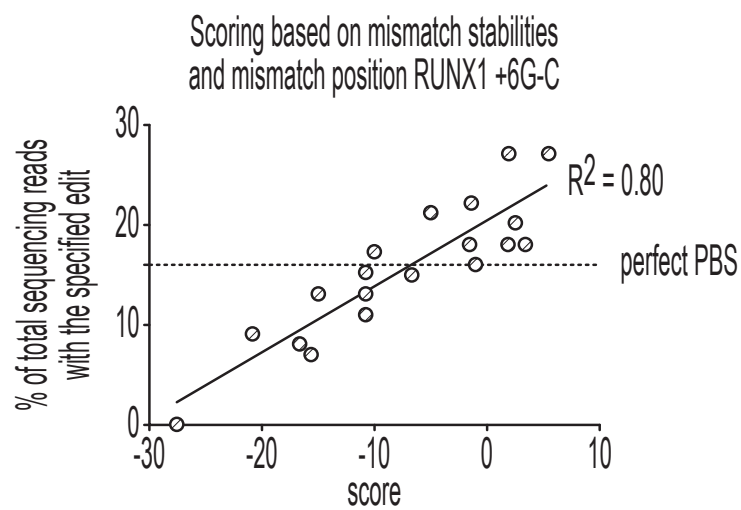


FIG. 141
CONTINUED

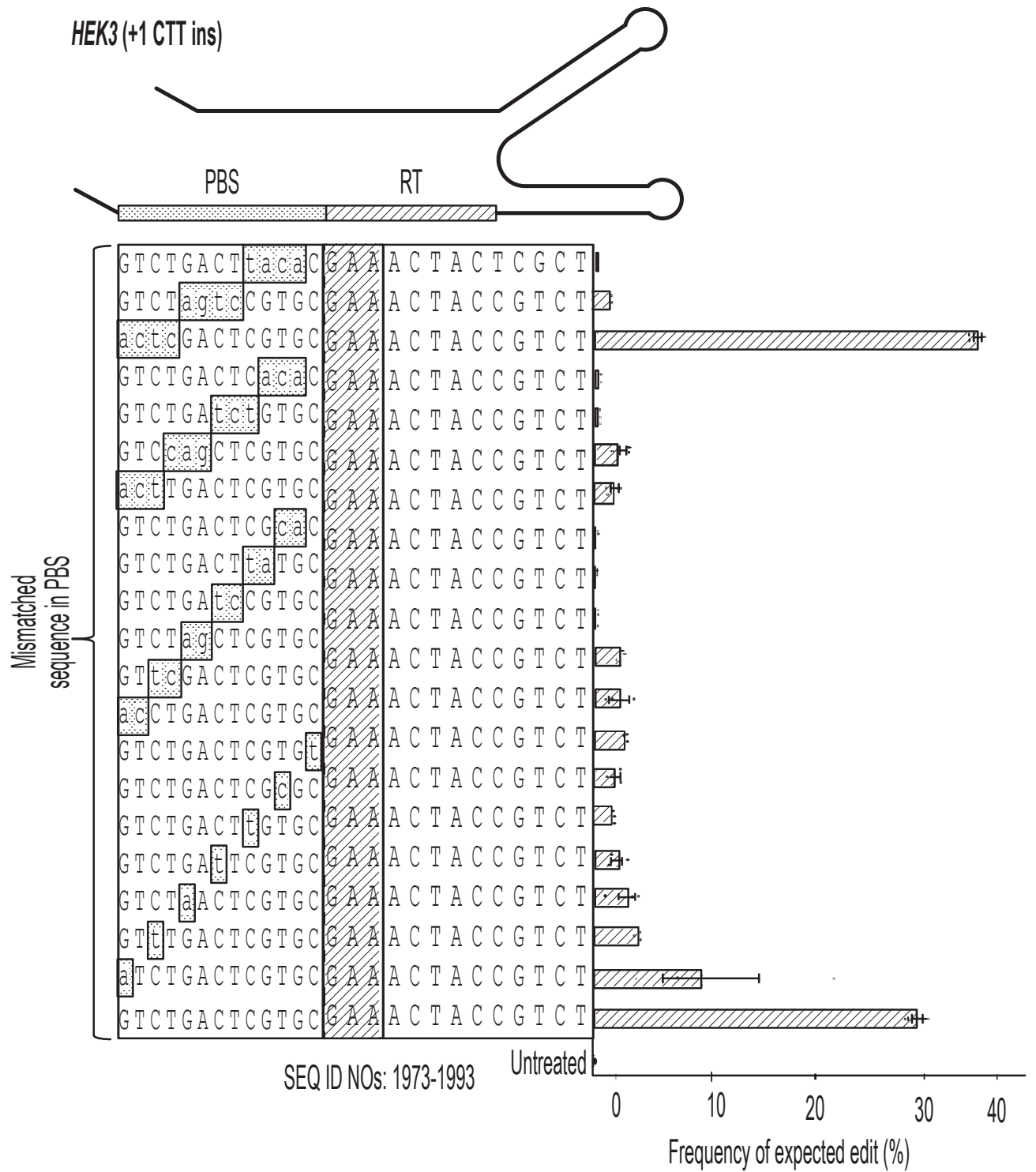


FIG. 142

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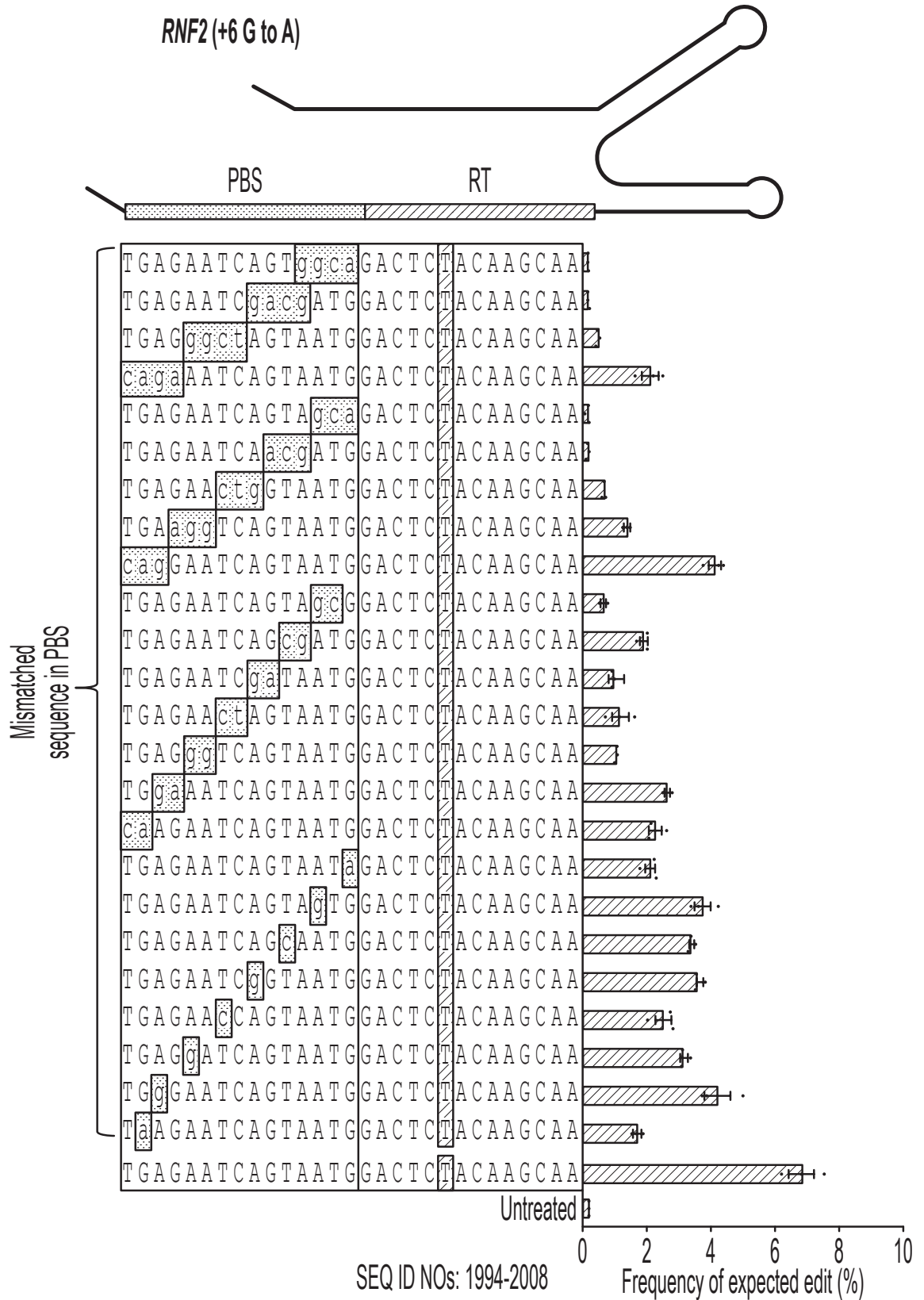


FIG. 142
CONTINUED

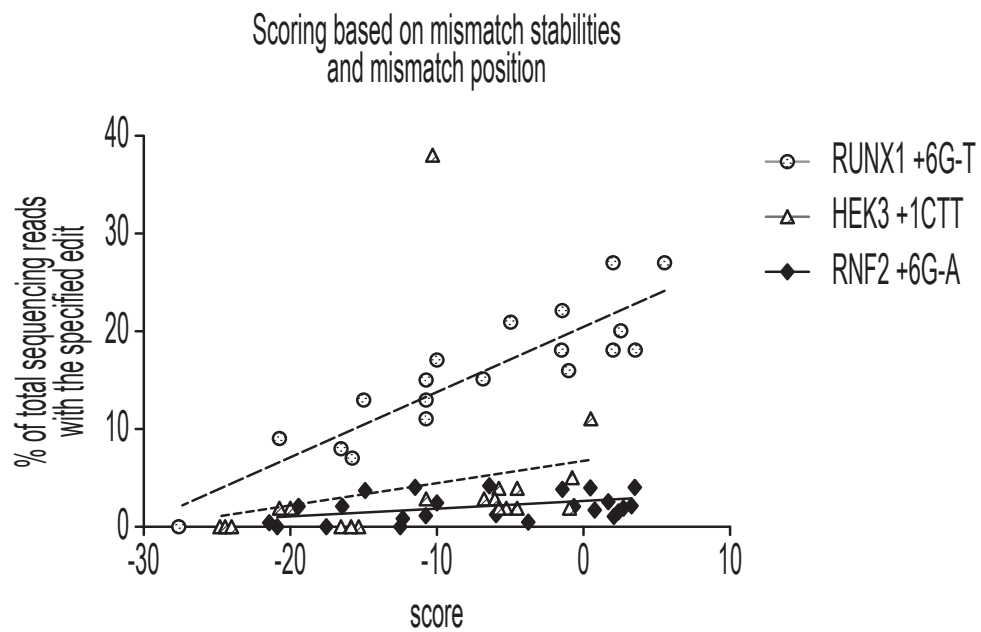


FIG. 142
CONTINUED

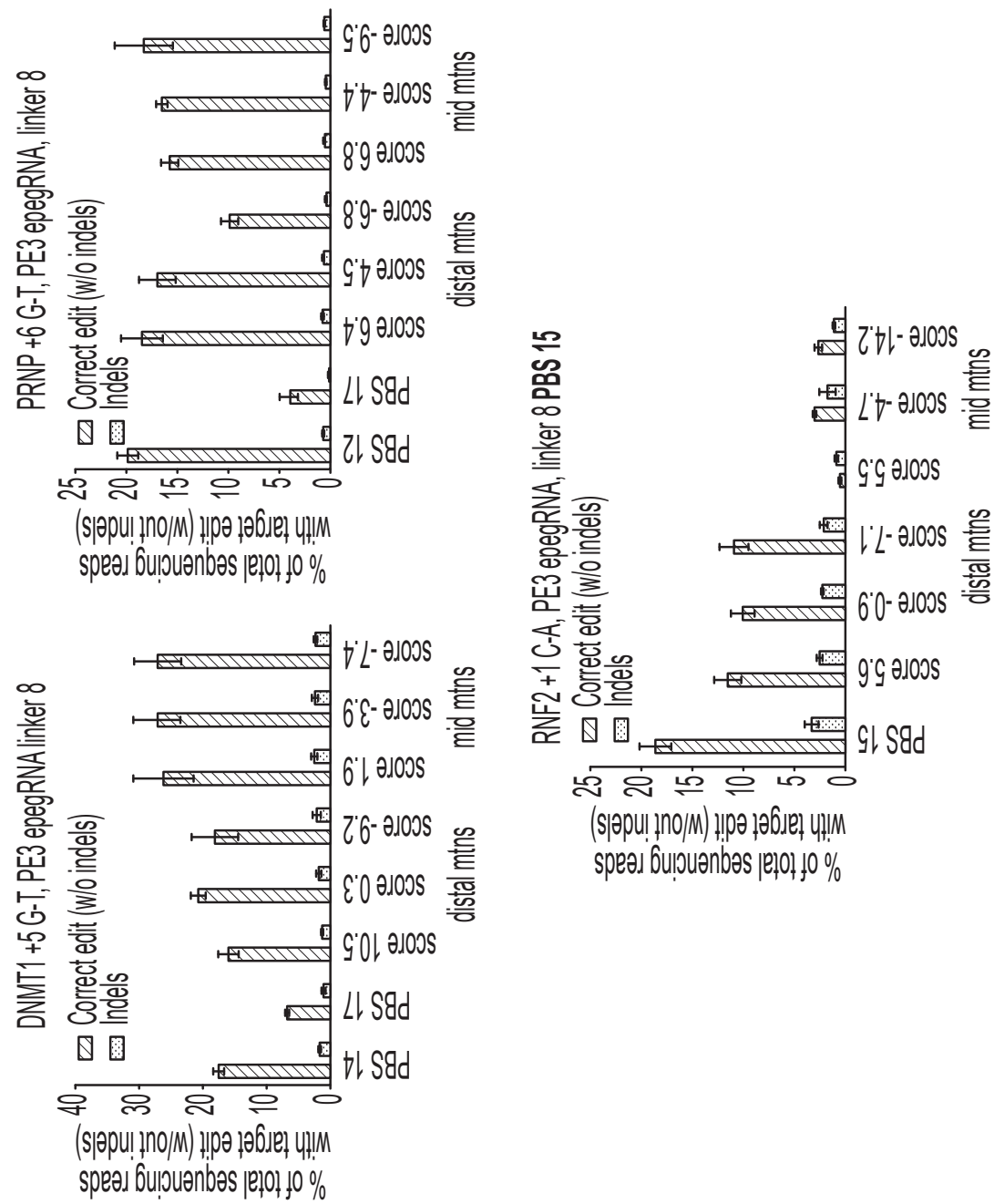


FIG. 143

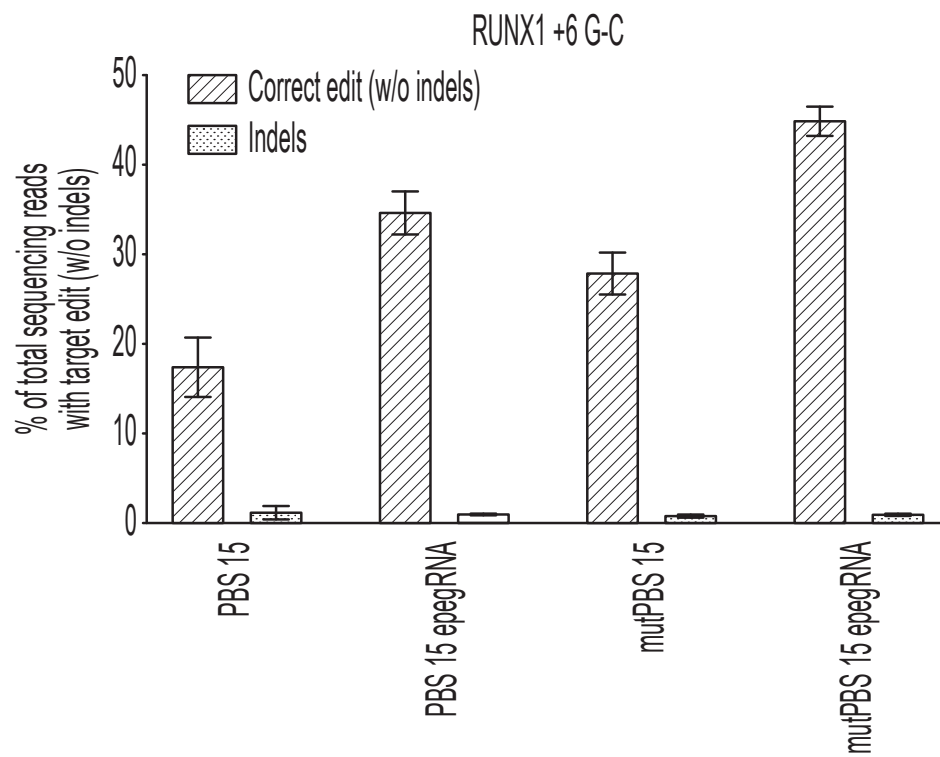


FIG. 144

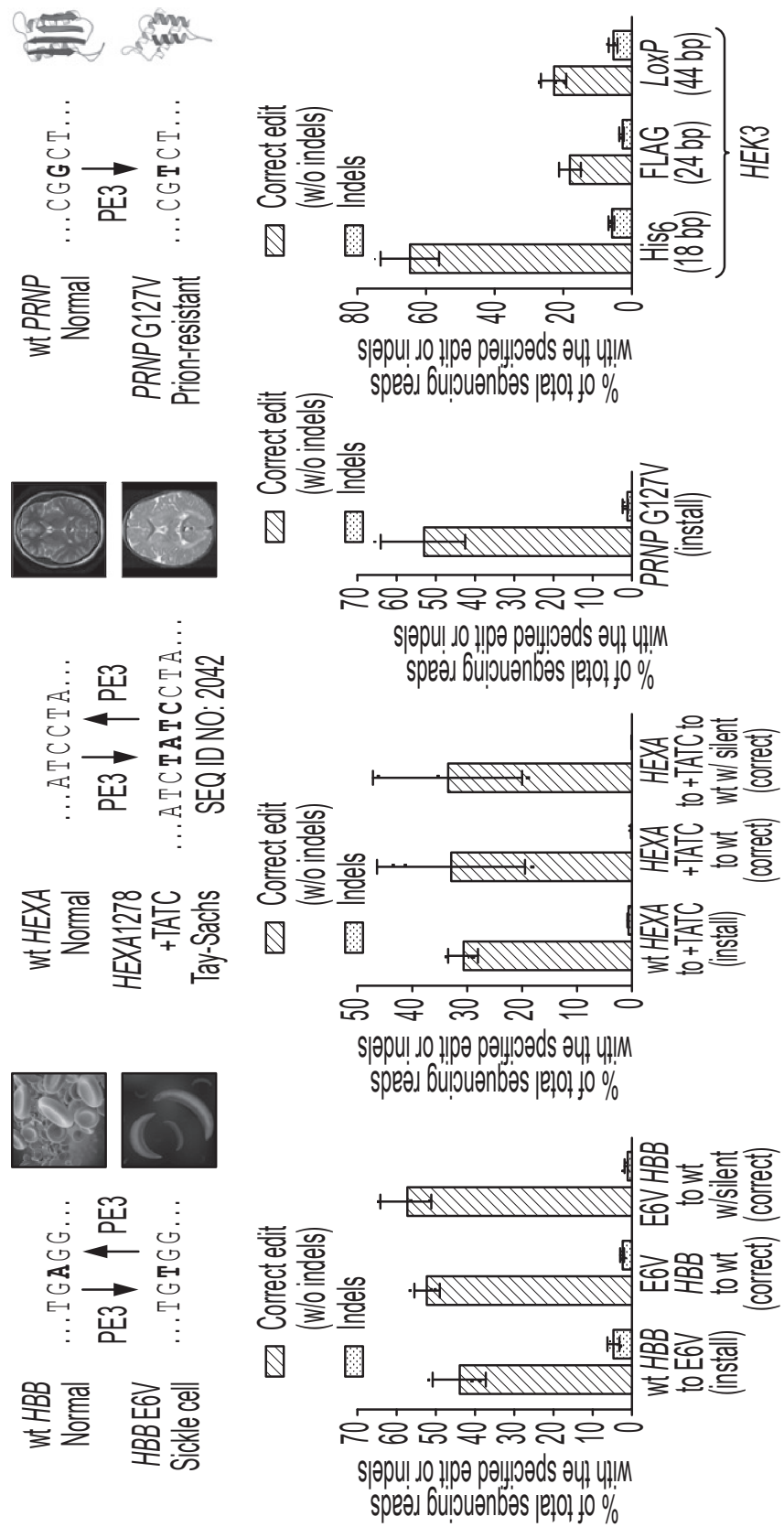


FIG. 145

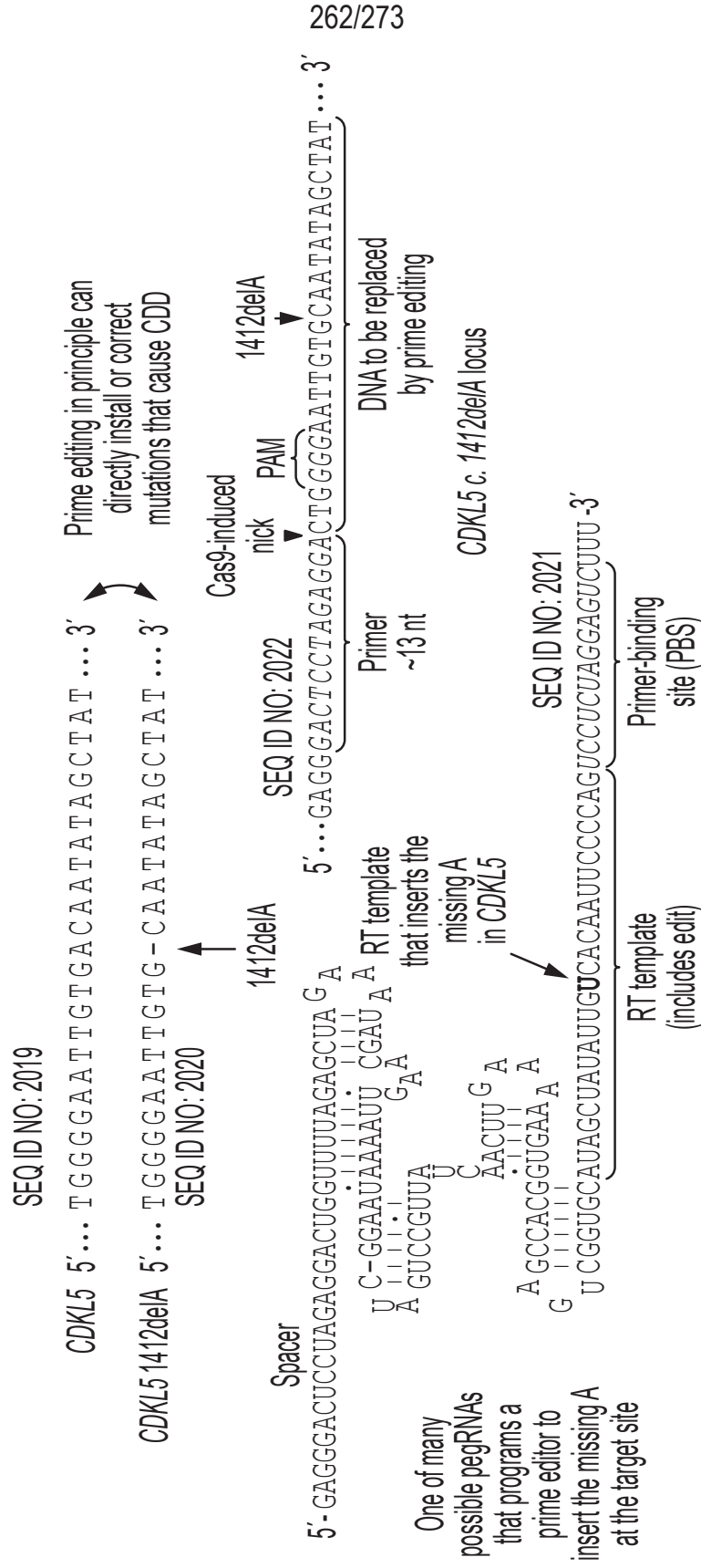


FIG. 146

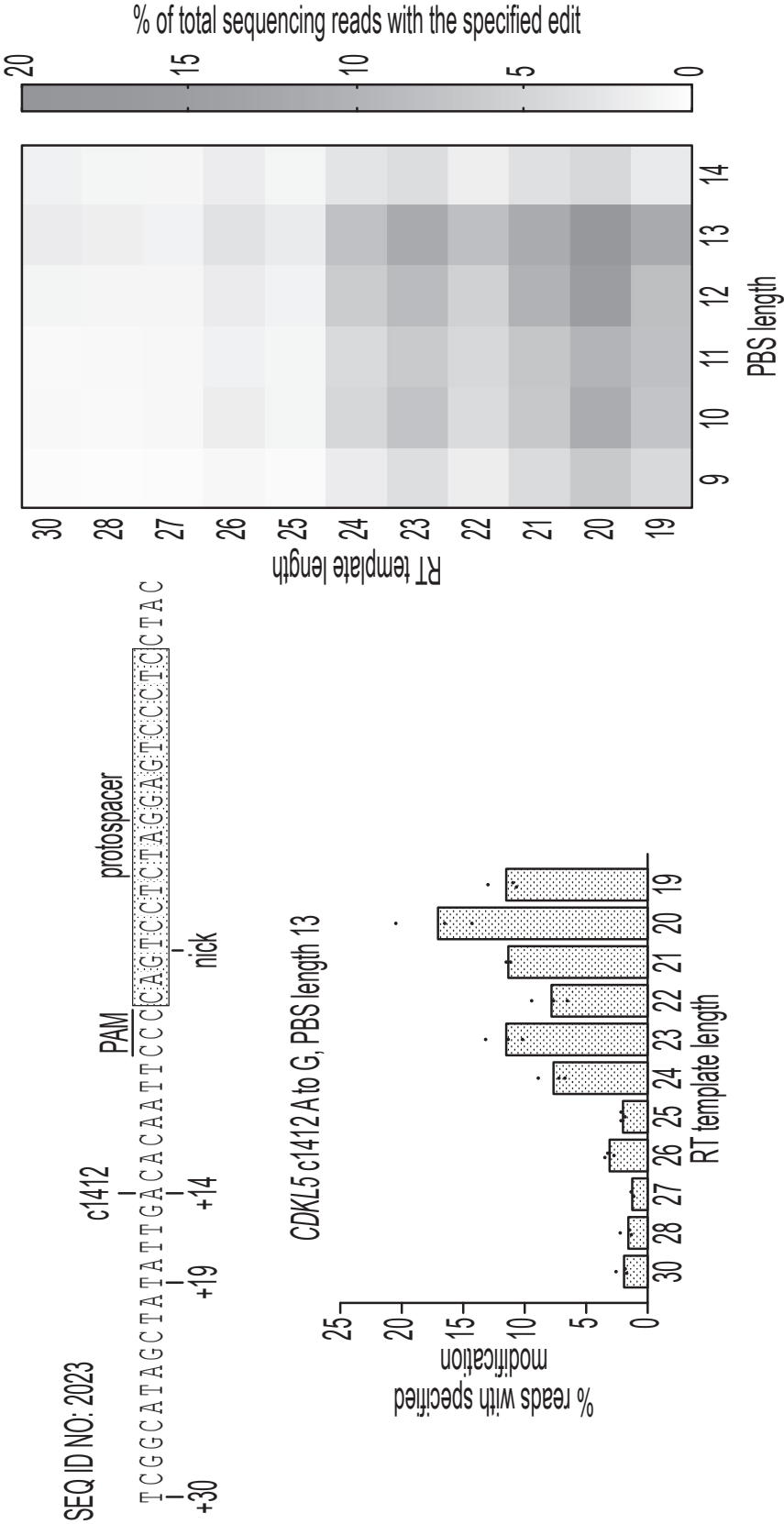


FIG. 147

FIG. 148

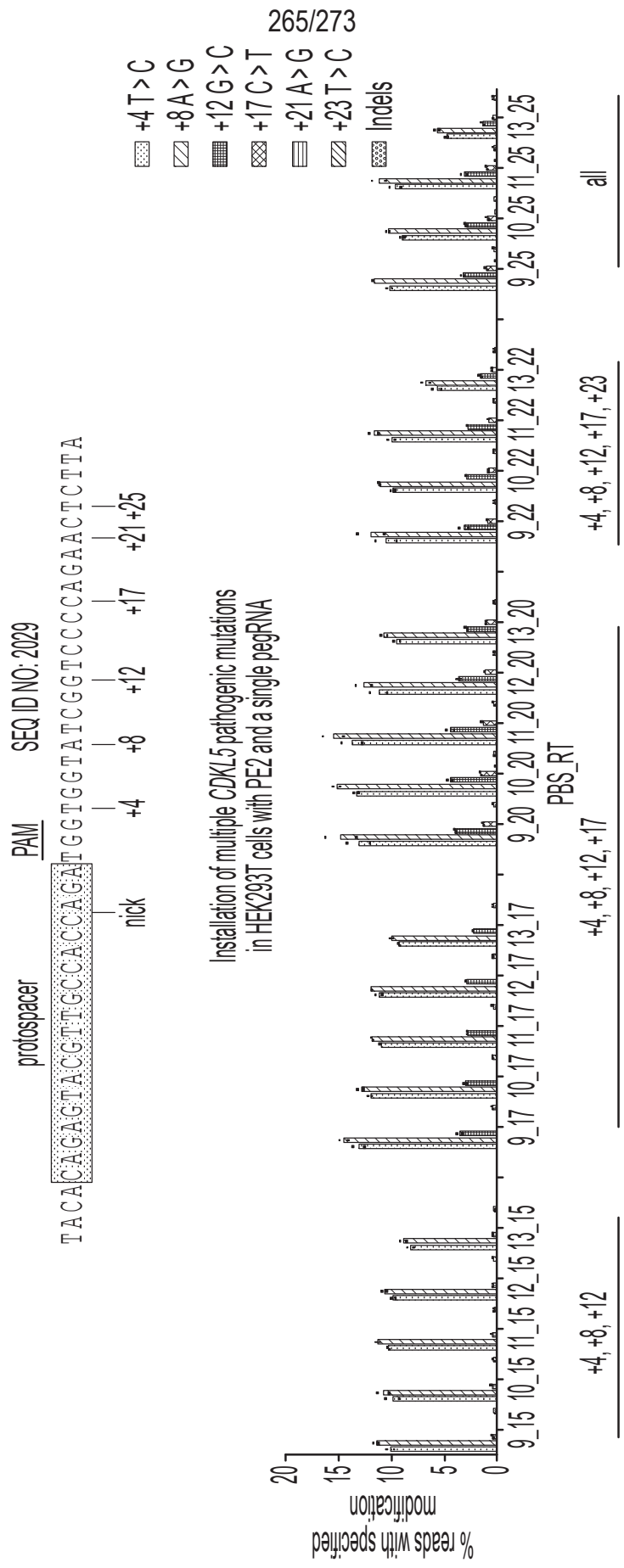


FIG. 149

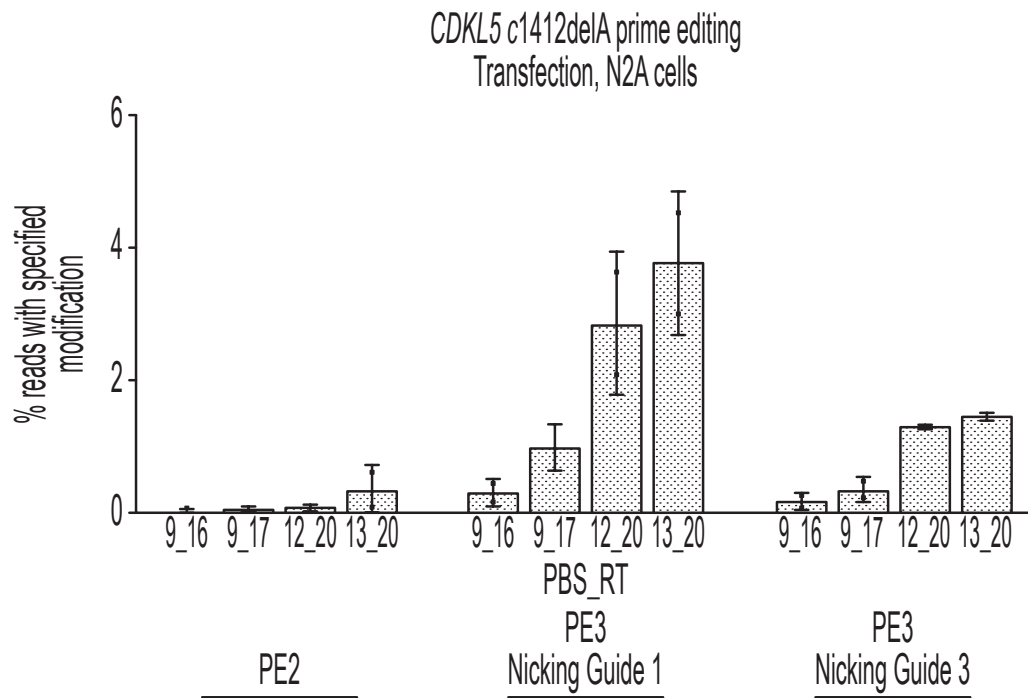


FIG. 150

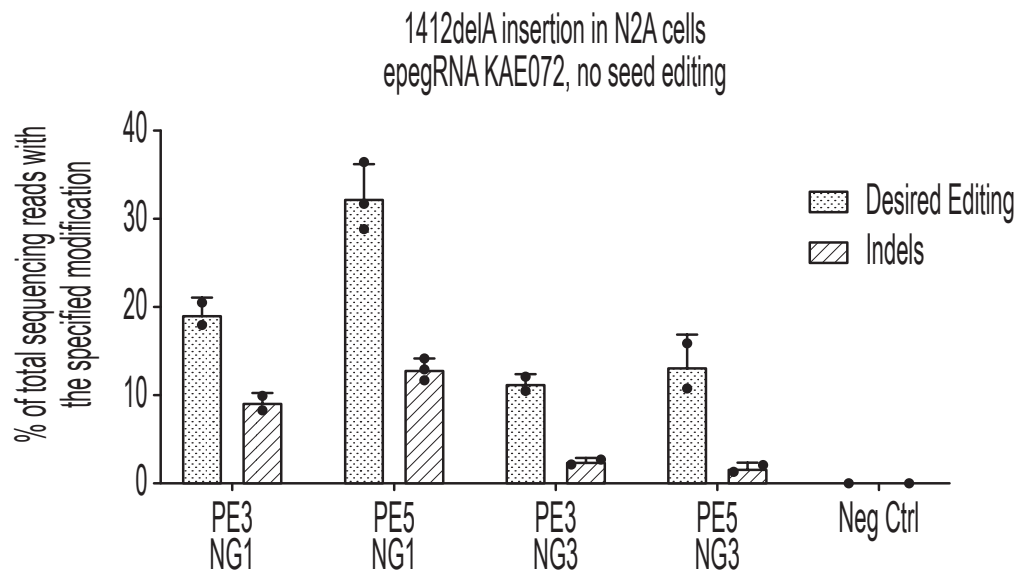


FIG. 151

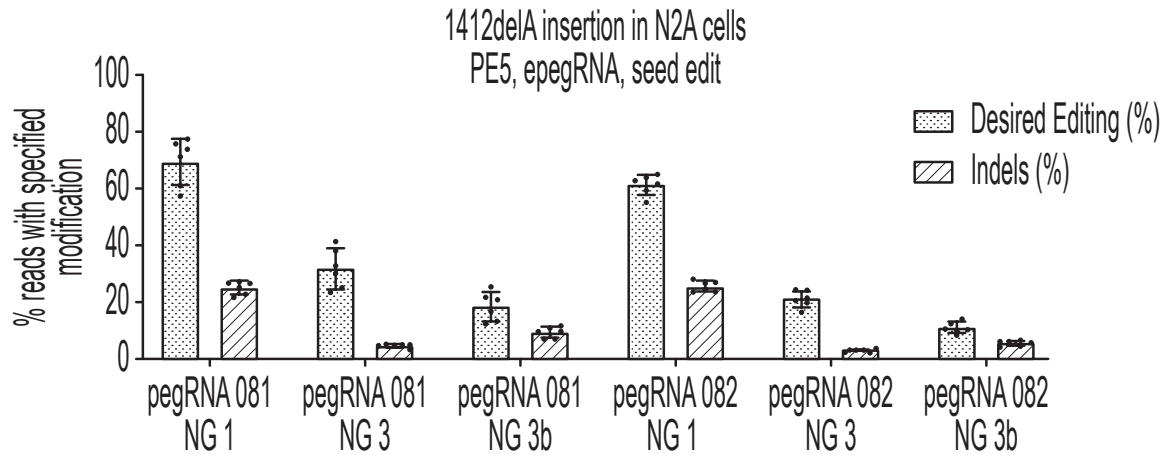


FIG. 152

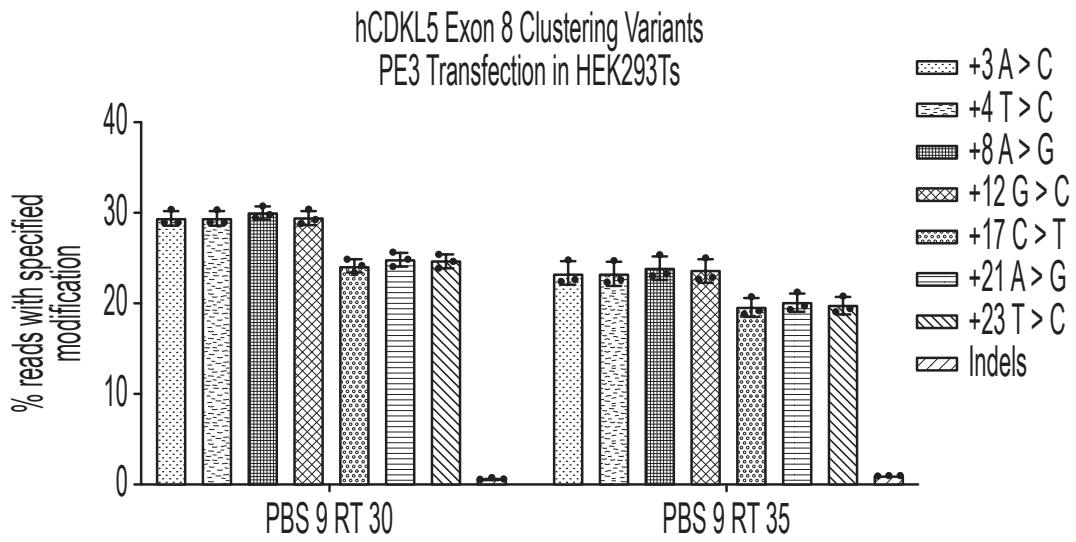


FIG. 153

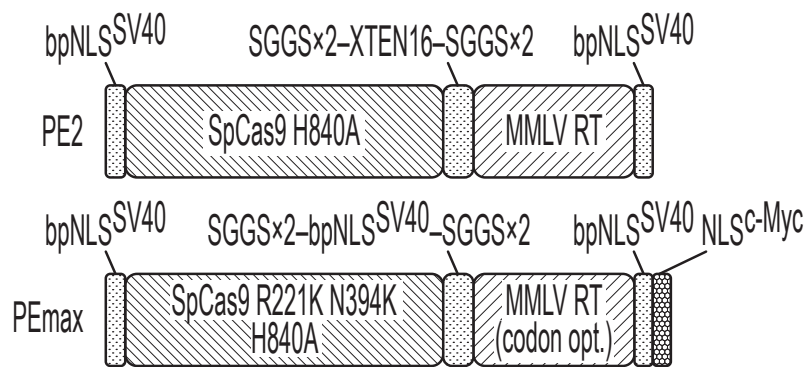


FIG. 154

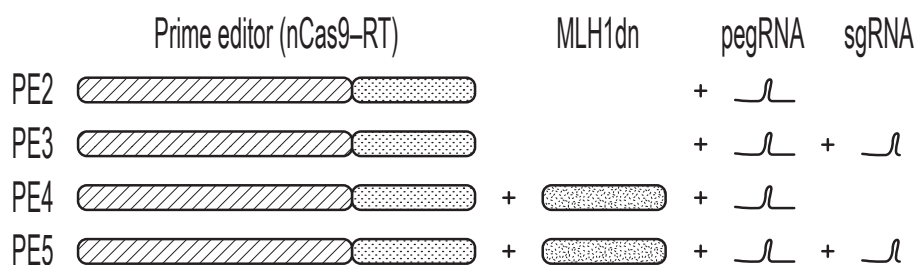


FIG. 155

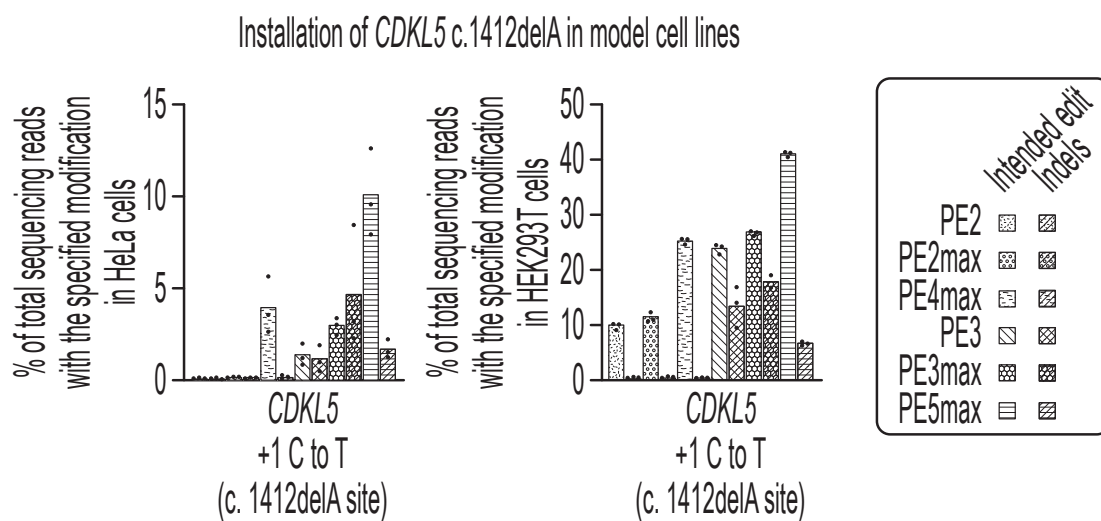


FIG. 156

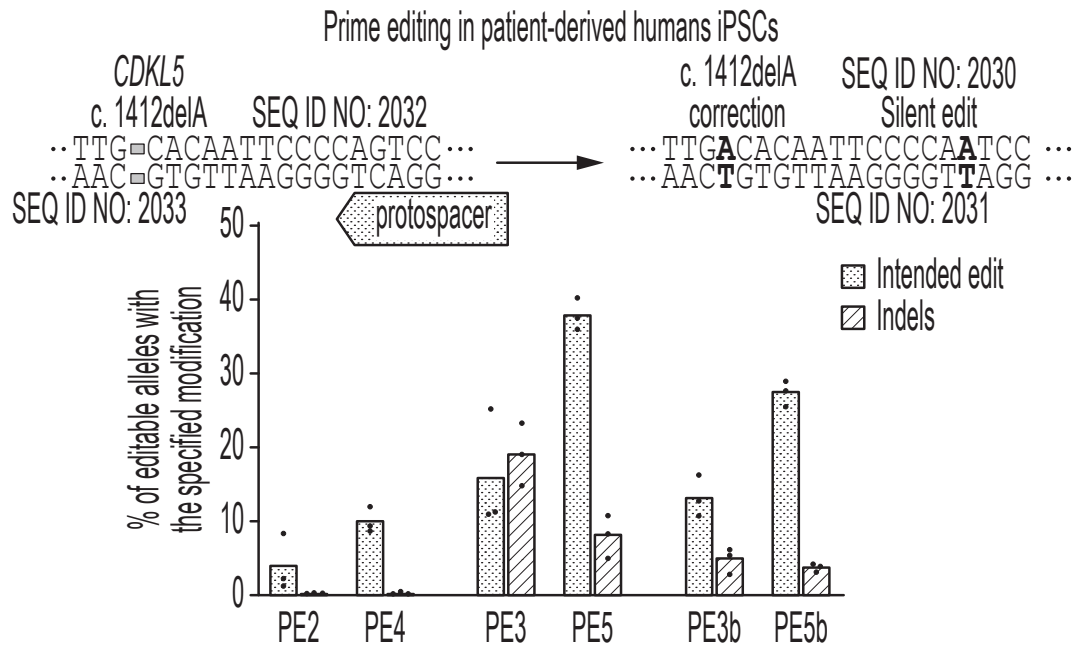


FIG. 157

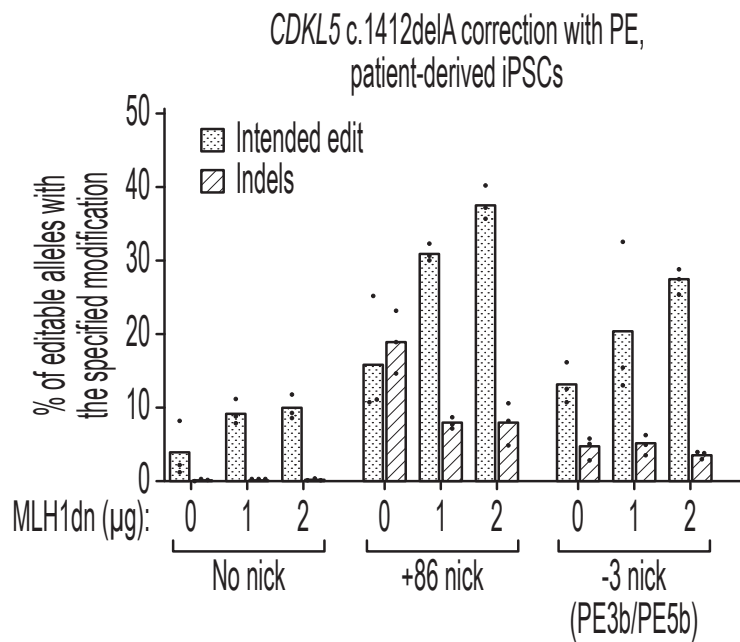


FIG. 158

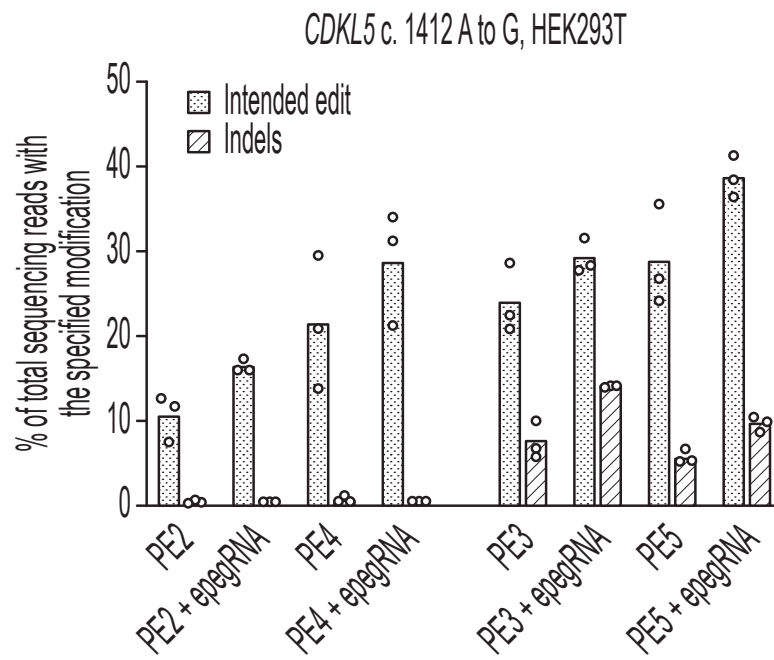


FIG. 159

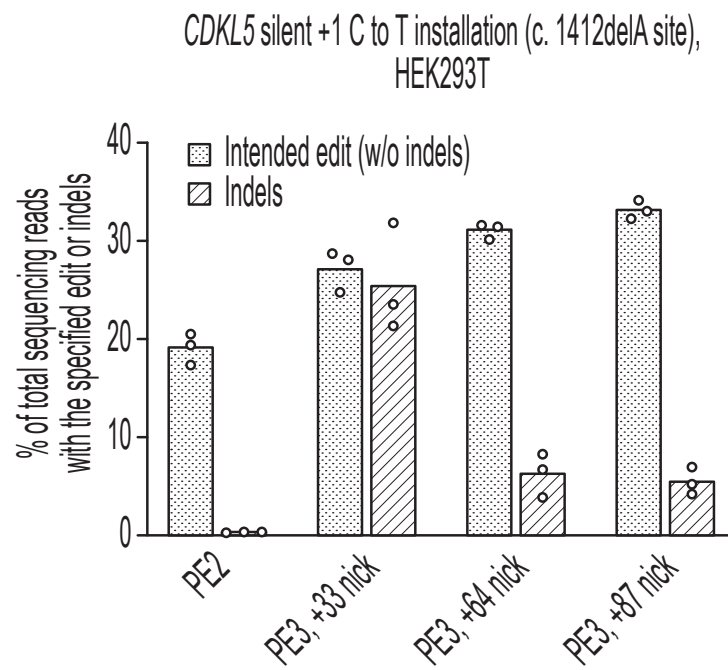


FIG. 160



FIG. 161

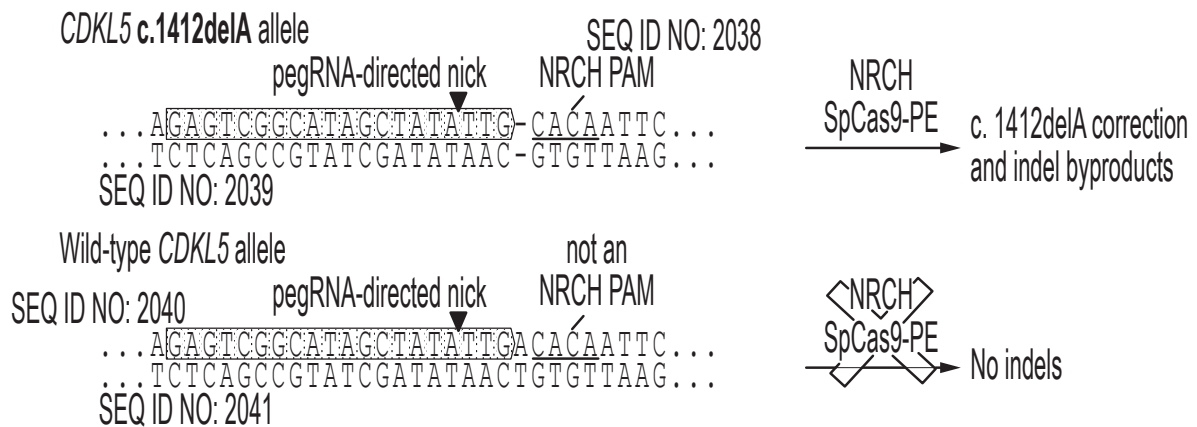


FIG. 162

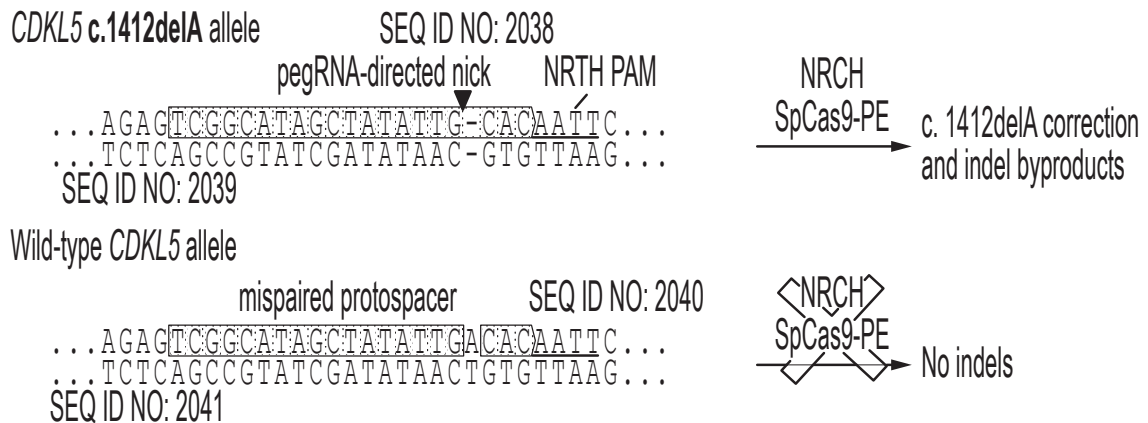


FIG. 163

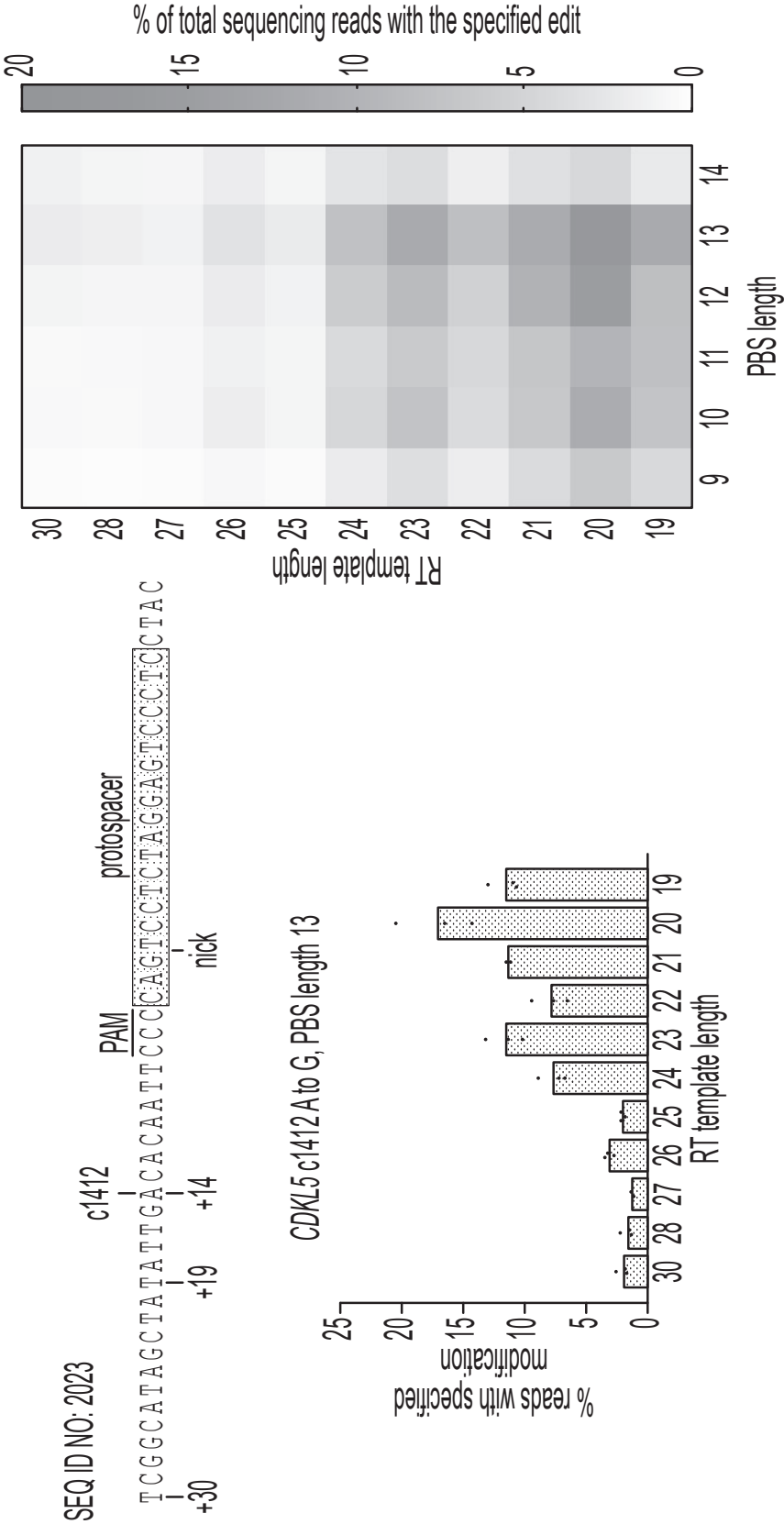


FIG. 164

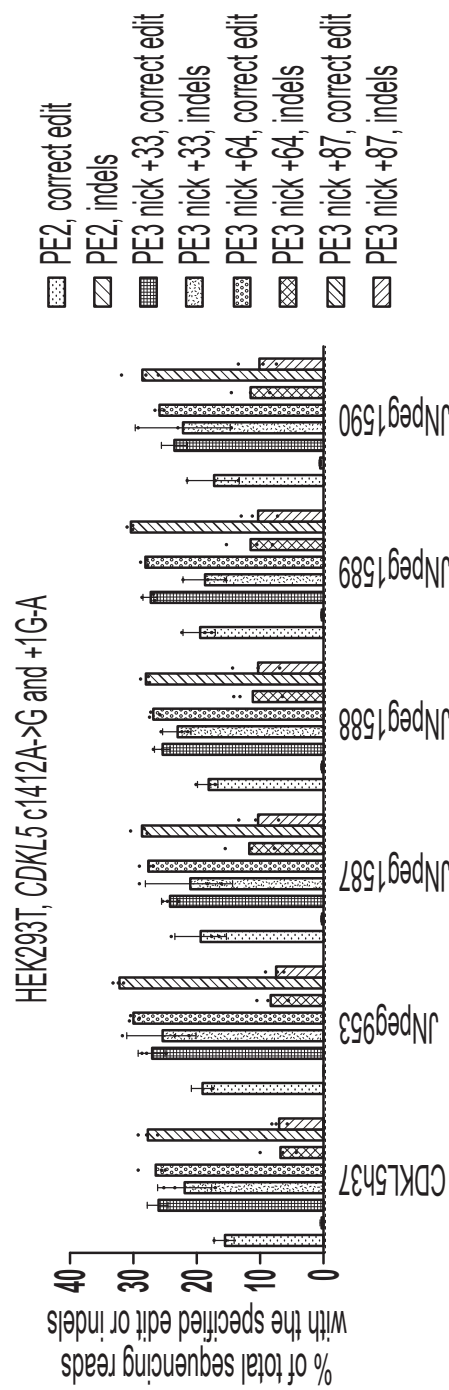


FIG. 165