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 synthesize repair through favored cellular repair of the non-edited strand)

FIG. 1K

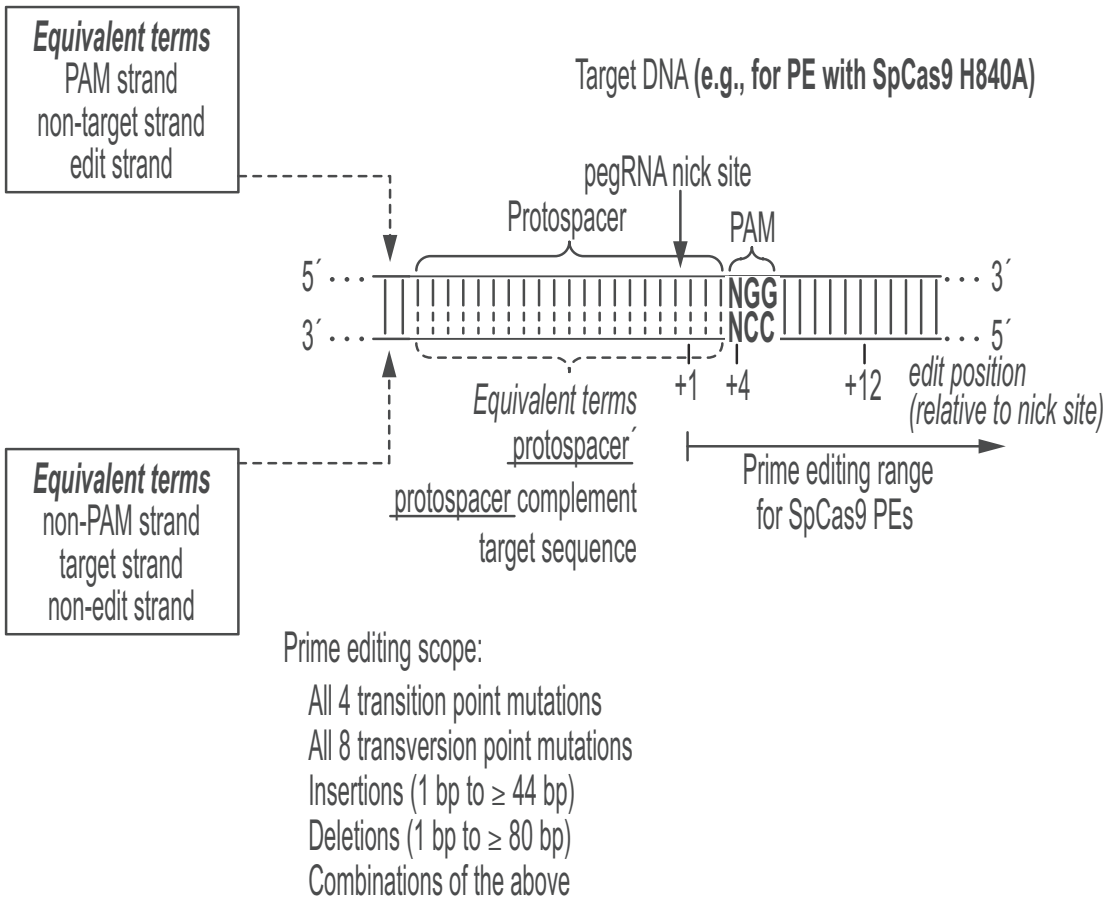

Equivalent terms
PAM strand
non-target strand
edit strand
Target DNA (e.g., for PE with SpCas9 H840A)
pegRNA nick site
Protospacer
PAM
5´
3´
NGG
NCC
3´
5´
+1
+4
+12
edit position
(relative to nick site)
Equivalent terms
protospacer´
protospacer complement
target sequence
Prime editing range
for SpCas9 PEs
Equivalent terms
non-PAM strand
target strand
non-edit strand
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

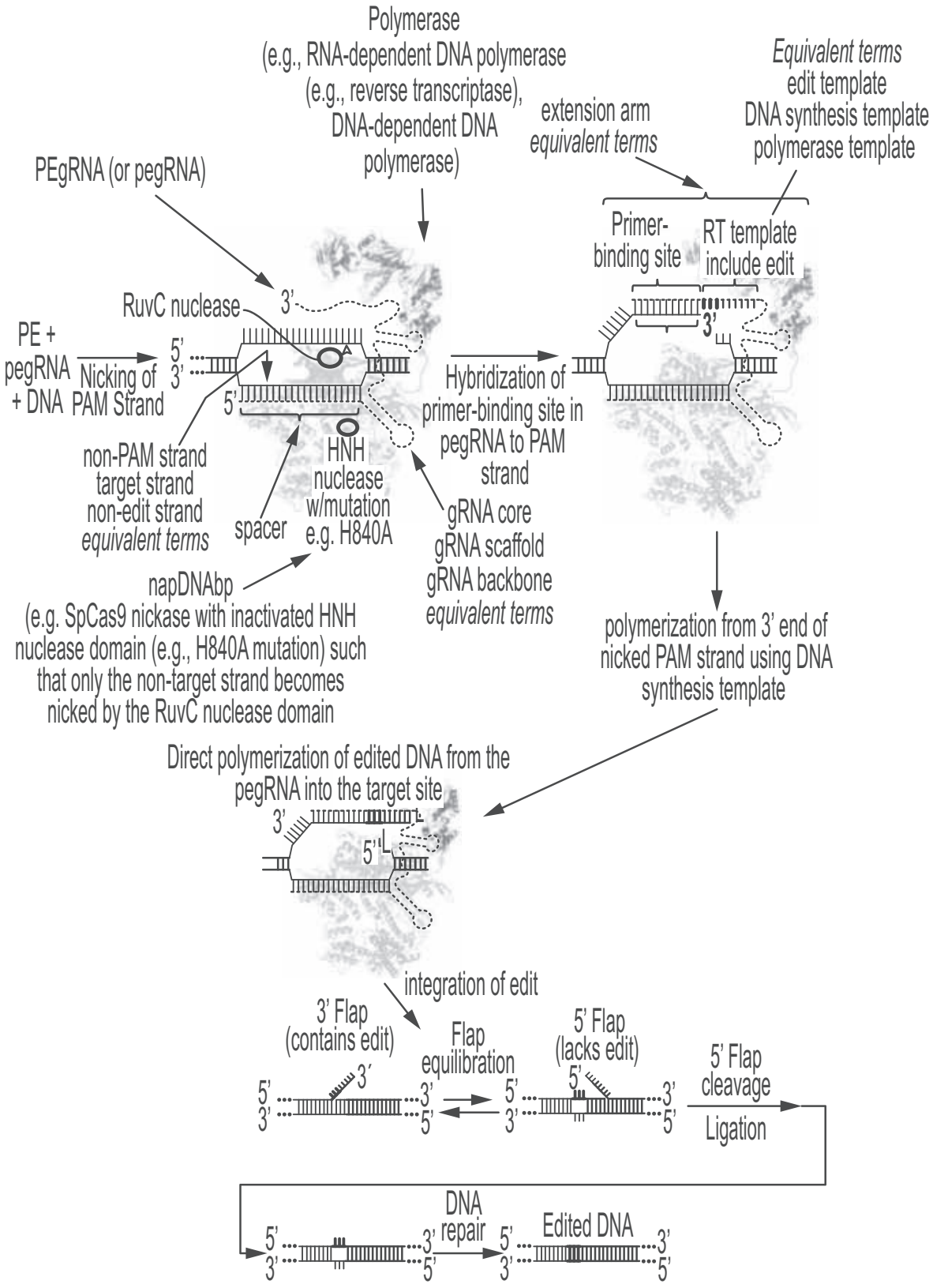

Polymerase
(e.g., RNA-dependent DNA polymerase
(e.g., reverse transcriptase),
DNA-dependent DNA
polymerase)
PEgRNA (or pegRNA)
extension arm
equivalent terms
Equivalent terms
edit template
DNA synthesis template
polymerase template
Primer-
binding site
RT template
include edit
RuvC nuclease
PE +
pegRNA
+ DNA
Nicking of
PAM Strand
non-PAM strand
target strand
non-edit strand
equivalent terms
spacer
HNH
nuclease
w/mutation
e.g. H840A
Hybridization of
primer-binding site in
pegRNA to PAM
strand
gRNA core
gRNA scaffold
gRNA backbone
equivalent terms
napDNAbp
(e.g. SpCas9 nickase with inactivated HNH
nuclease domain (e.g., H840A mutation) such
that only the non-target strand becomes
nicked by the RuvC nuclease domain
polymerization from 3' end of
nicked PAM strand using DNA
synthesis template
Direct polymerization of edited DNA from the
pegRNA into the target site
integration of edit
3' Flap
(contains edit)
Flap
equilibration
5' Flap
(lacks edit)
5' Flap
cleavage
Ligation
DNA
repair
Edited DNA

FIG. 1M

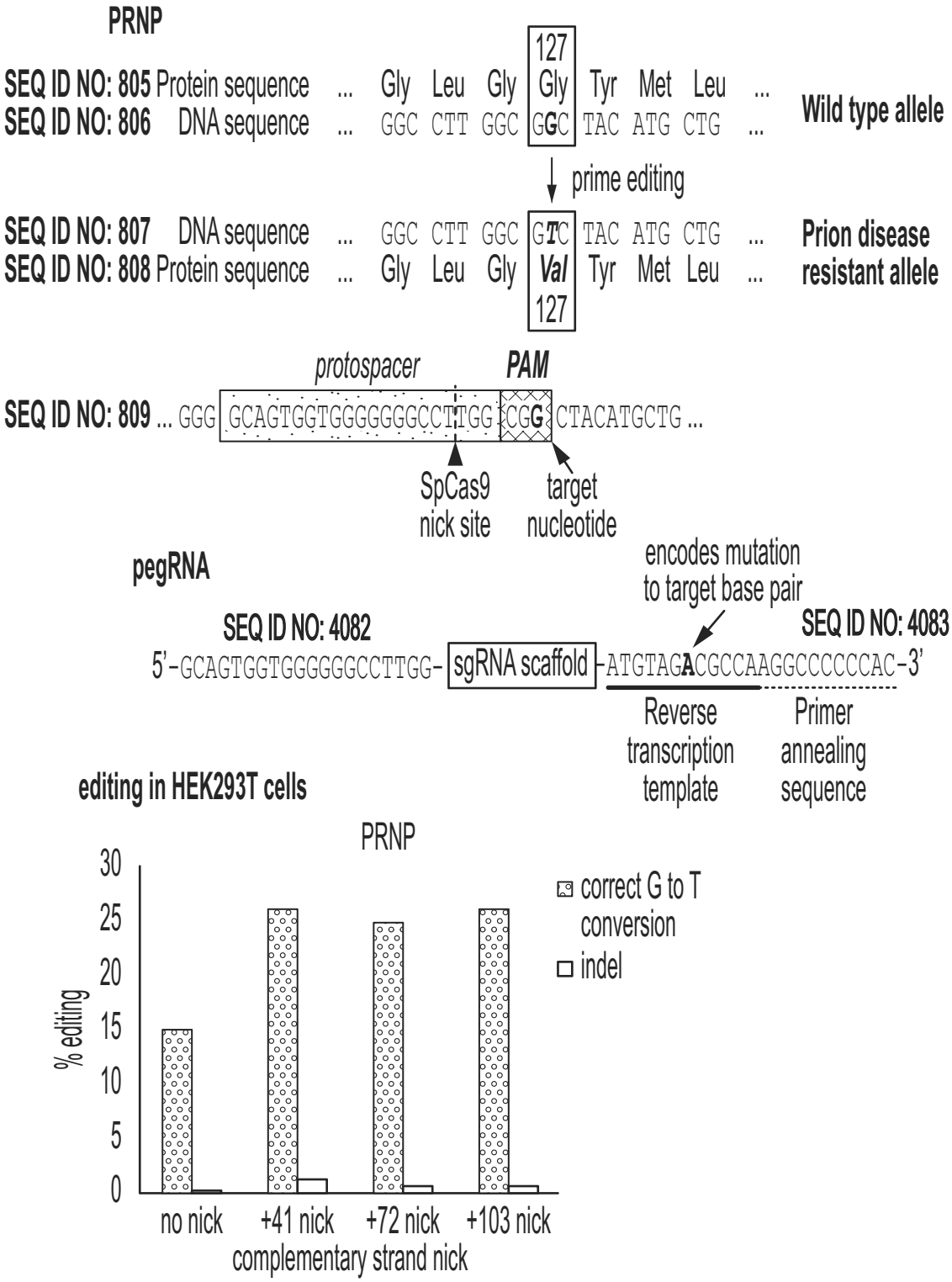

PRNP
SEQ ID NO: 805 Protein sequence ... Gly Leu Gly Gly Tyr Met Leu ...
127
SEQ ID NO: 806 DNA sequence ... GGC CTT GGC GGC TAC ATG CTG ...
Wild type allele
prime editing
SEQ ID NO: 807 DNA sequence ... GGC CTT GGC GTC TAC ATG CTG ...
SEQ ID NO: 808 Protein sequence ... Gly Leu Gly Val Tyr Met Leu ...
127
Prion disease resistant allele
protospacer
PAM
SEQ ID NO: 809 ... GGG GCAGTGGTGGGGGGGCCTTGG CGG CTACATGCTG ...
SpCas9 nick site
target nucleotide
pegRNA
encodes mutation to target base pair
SEQ ID NO: 4082
SEQ ID NO: 4083
5'-GCAGTGGTGGGGGGCCTTGG- sgRNA scaffold -ATGTAGACGCCAAGGCCCCCAC-3'
Reverse transcription template
Primer annealing sequence
editing in HEK293T cells
PRNP
% editing
30
25
20
15
10
5
0
correct G to T conversion
indel
no nick
+41 nick
+72 nick
+103 nick
complementary strand nick

FIG. 27

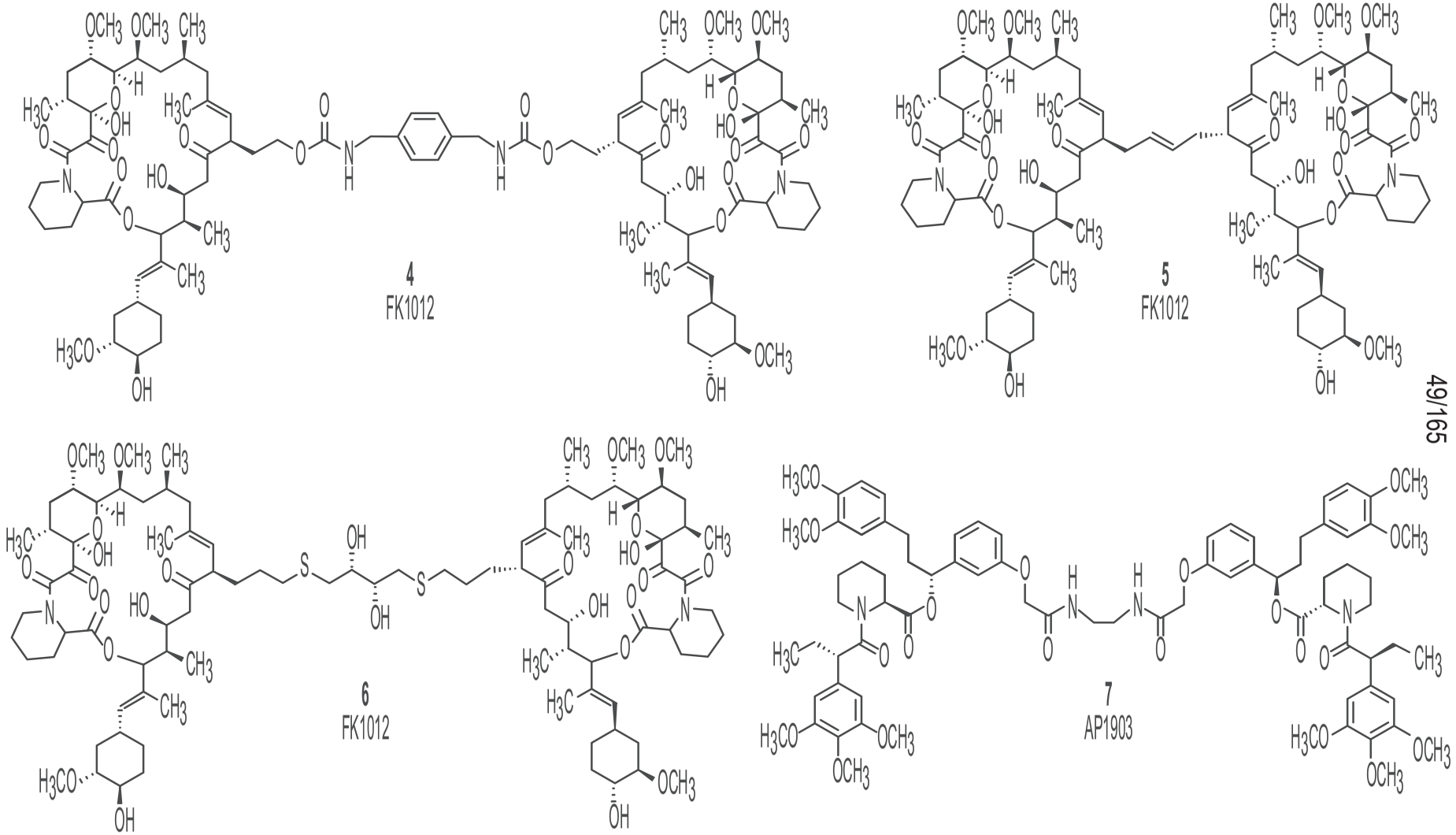
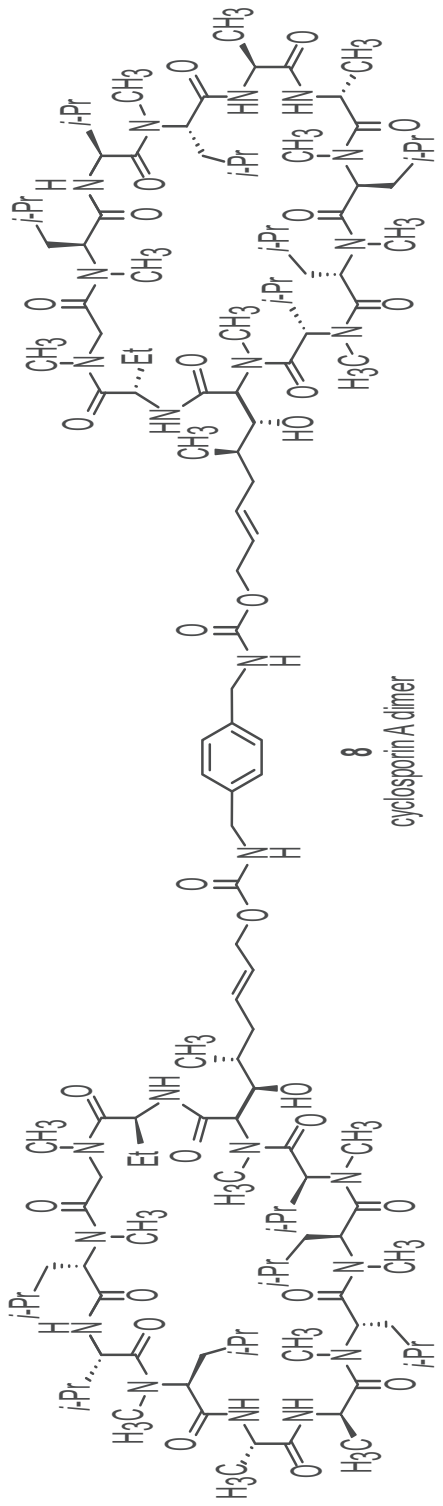
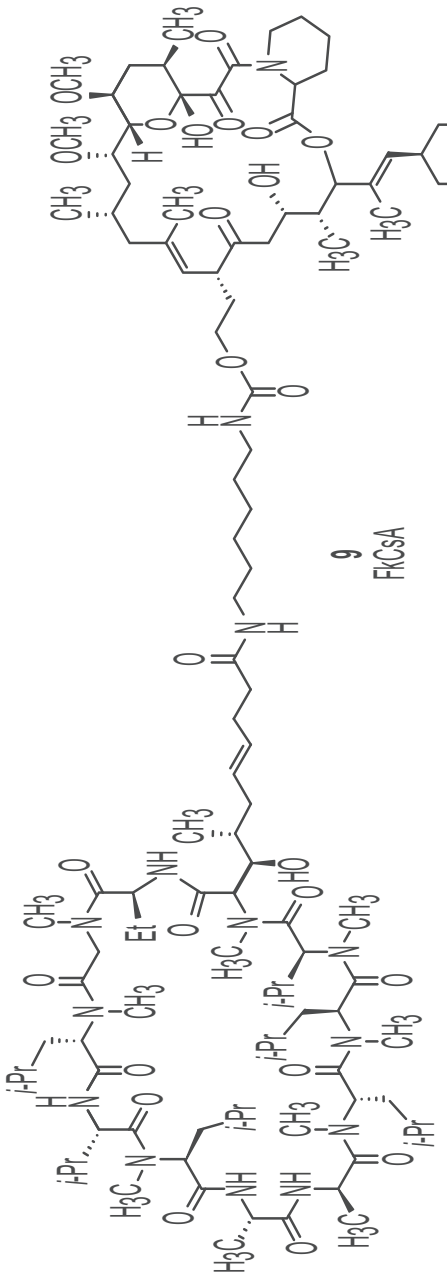


FIG. 37A



8
cyclosporin A dimer



9
FkCsA

FIG. 37B

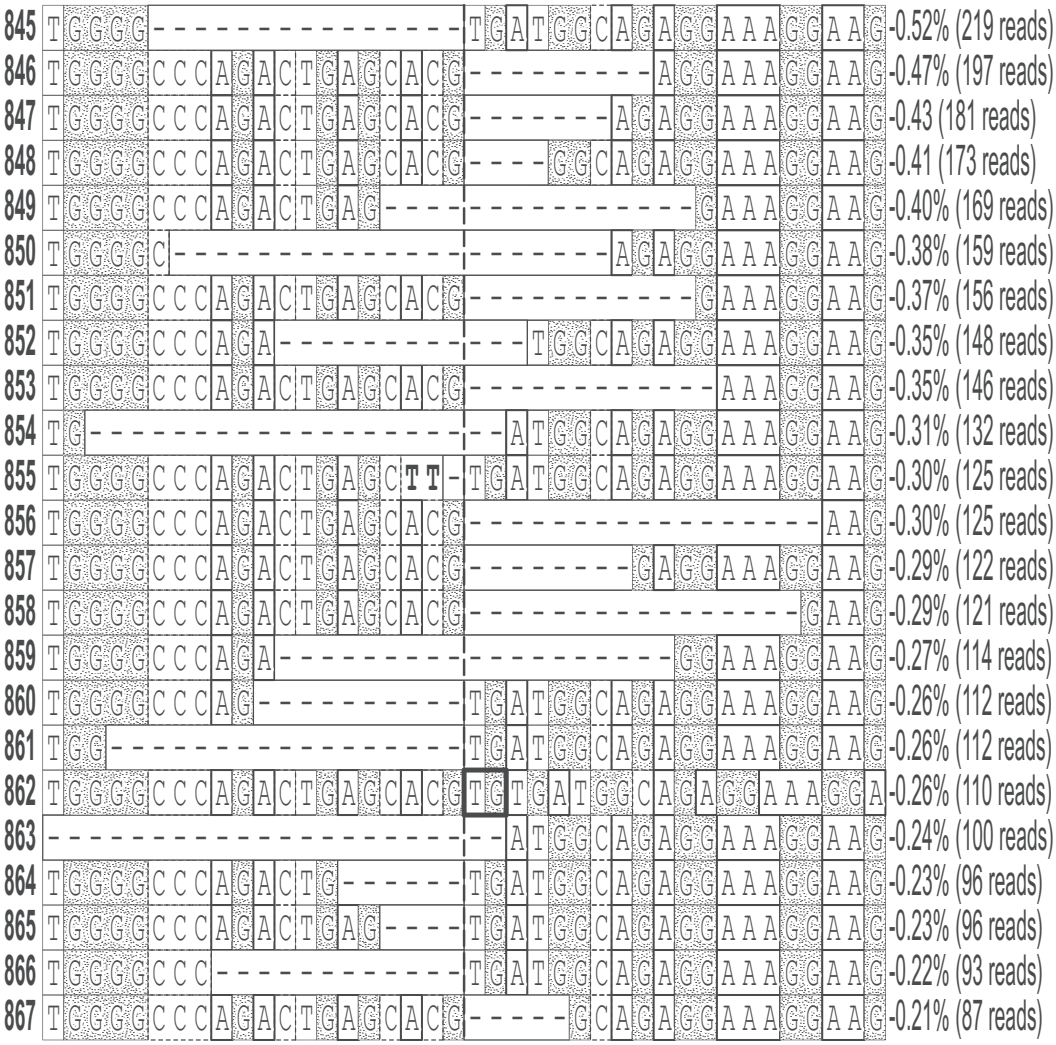

845 -0.52% (219 reads)
846 -0.47% (197 reads)
847 -0.43 (181 reads)
848 -0.41 (173 reads)
849 -0.40% (169 reads)
850 -0.38% (159 reads)
851 -0.37% (156 reads)
852 -0.35% (148 reads)
853 -0.35% (146 reads)
854 -0.31% (132 reads)
855 -0.30% (125 reads)
856 -0.30% (125 reads)
857 -0.29% (122 reads)
858 -0.29% (121 reads)
859 -0.27% (114 reads)
860 -0.26% (112 reads)
861 -0.26% (112 reads)
862 -0.26% (110 reads)
863 -0.24% (100 reads)
864 -0.23% (96 reads)
865 -0.23% (96 reads)
866 -0.22% (93 reads)
867 -0.21% (87 reads)
bold Substitutions
Insertions
- Deletions
---- Predicted
cleavage position

FIG. 51G

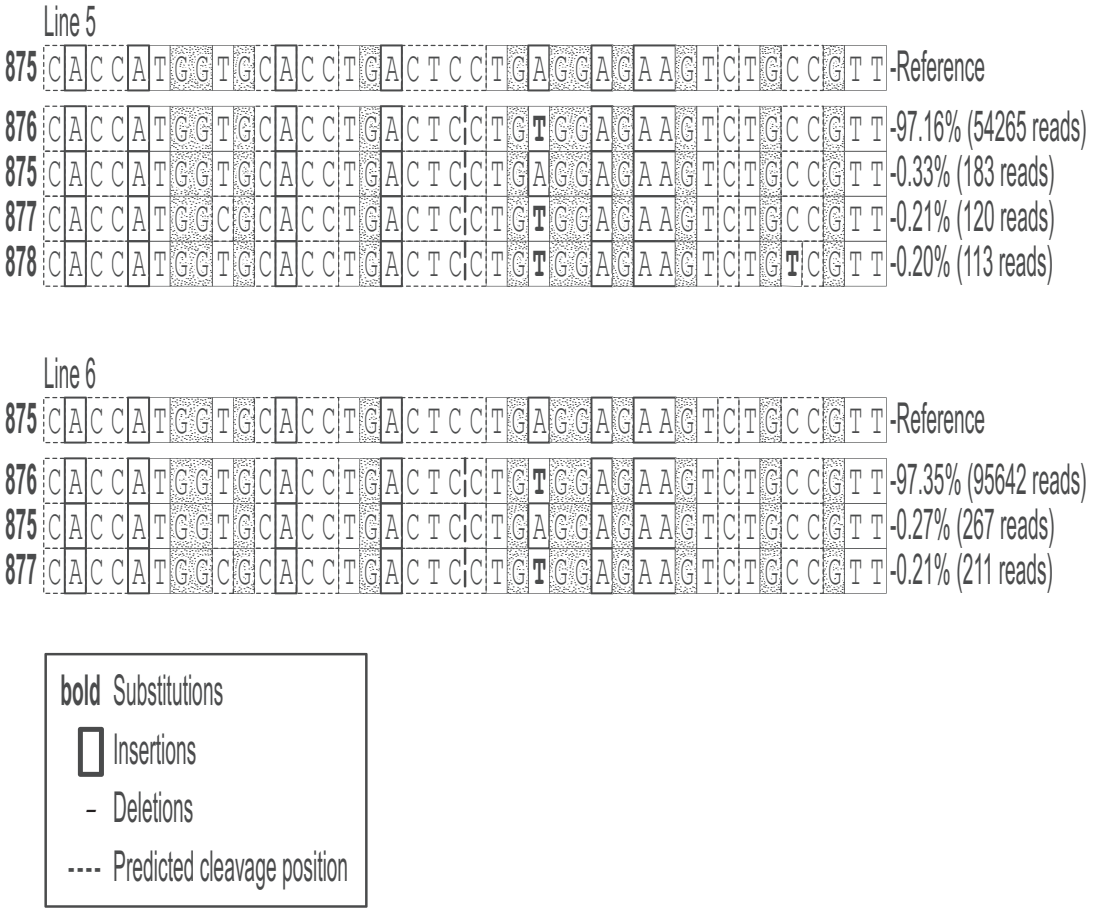
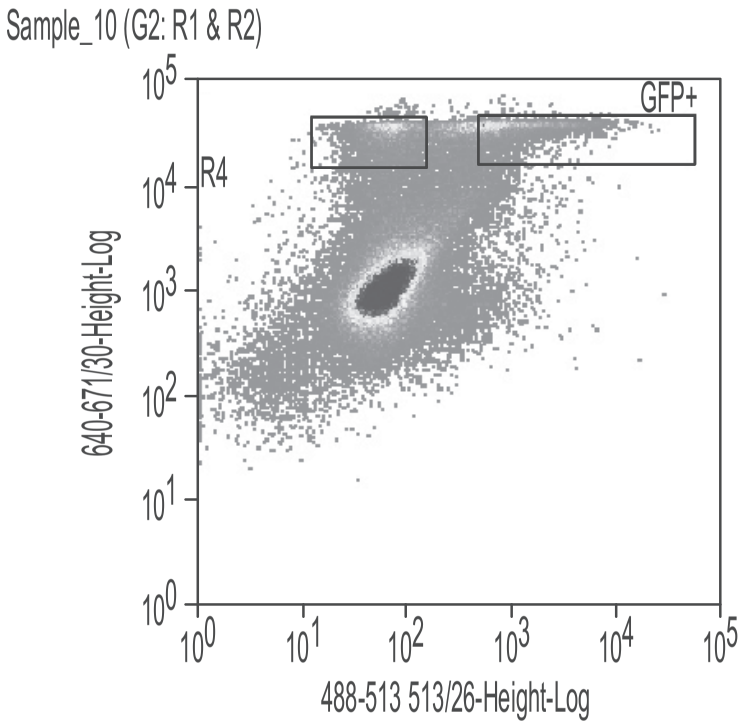
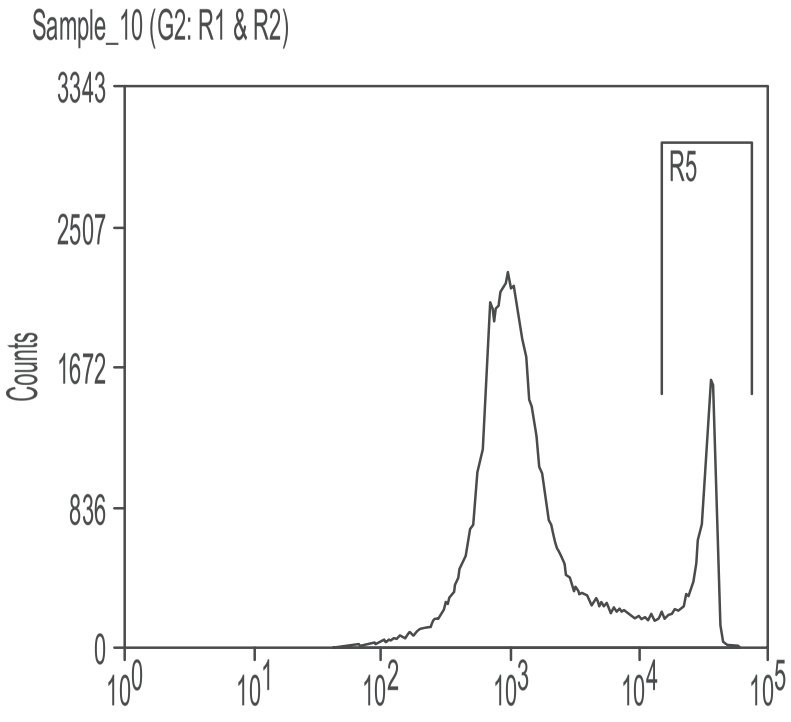

Line 5
875 CACCATGGTGCACCTGACTCCCTGAGGAGAAGTCTGCCGTT -Reference
876 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -97.16% (54265 reads)
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -0.33% (183 reads)
877 CACCATGGCGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -0.21% (120 reads)
878 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGTCGTT -0.20% (113 reads)
Line 6
875 CACCATGGTGCACCTGACTCCCTGAGGAGAAGTCTGCCGTT -Reference
876 CACCATGGTGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -97.35% (95642 reads)
875 CACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCCGTT -0.27% (267 reads)
877 CACCATGGCGCACCTGACTCCTGTGGAGAAGTCTGCCGTT -0.21% (211 reads)
bold Substitutions
Insertions
- Deletions
---- Predicted cleavage position

FIG. 53E



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

FIG. 58B