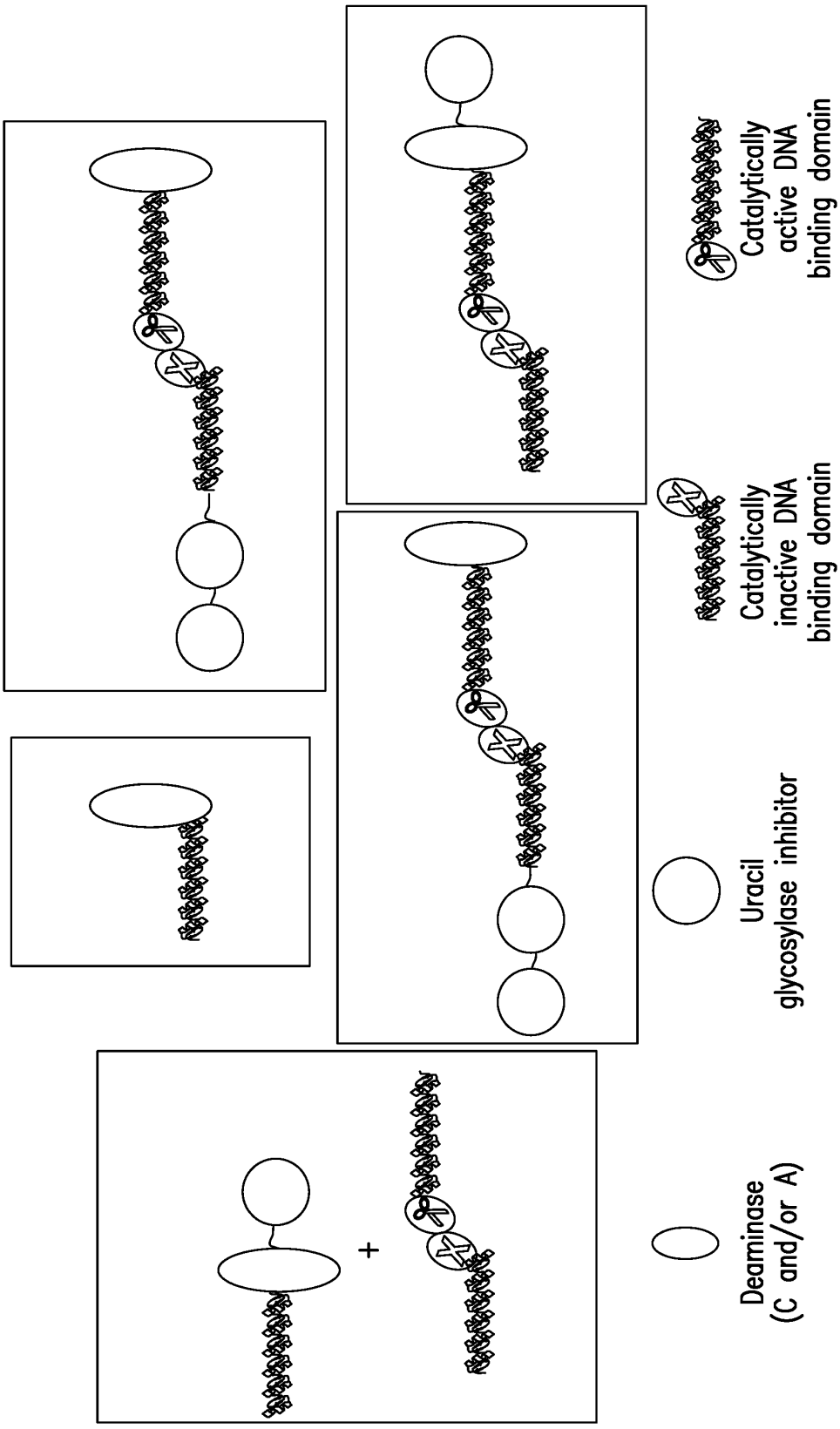

Catalytically active DNA binding domain
Catalytically inactive DNA binding domain
Uracil glycosylase inhibitor
Deaminase (C and/or A)

FIG. 1A

Cont. On FIG.1B-2

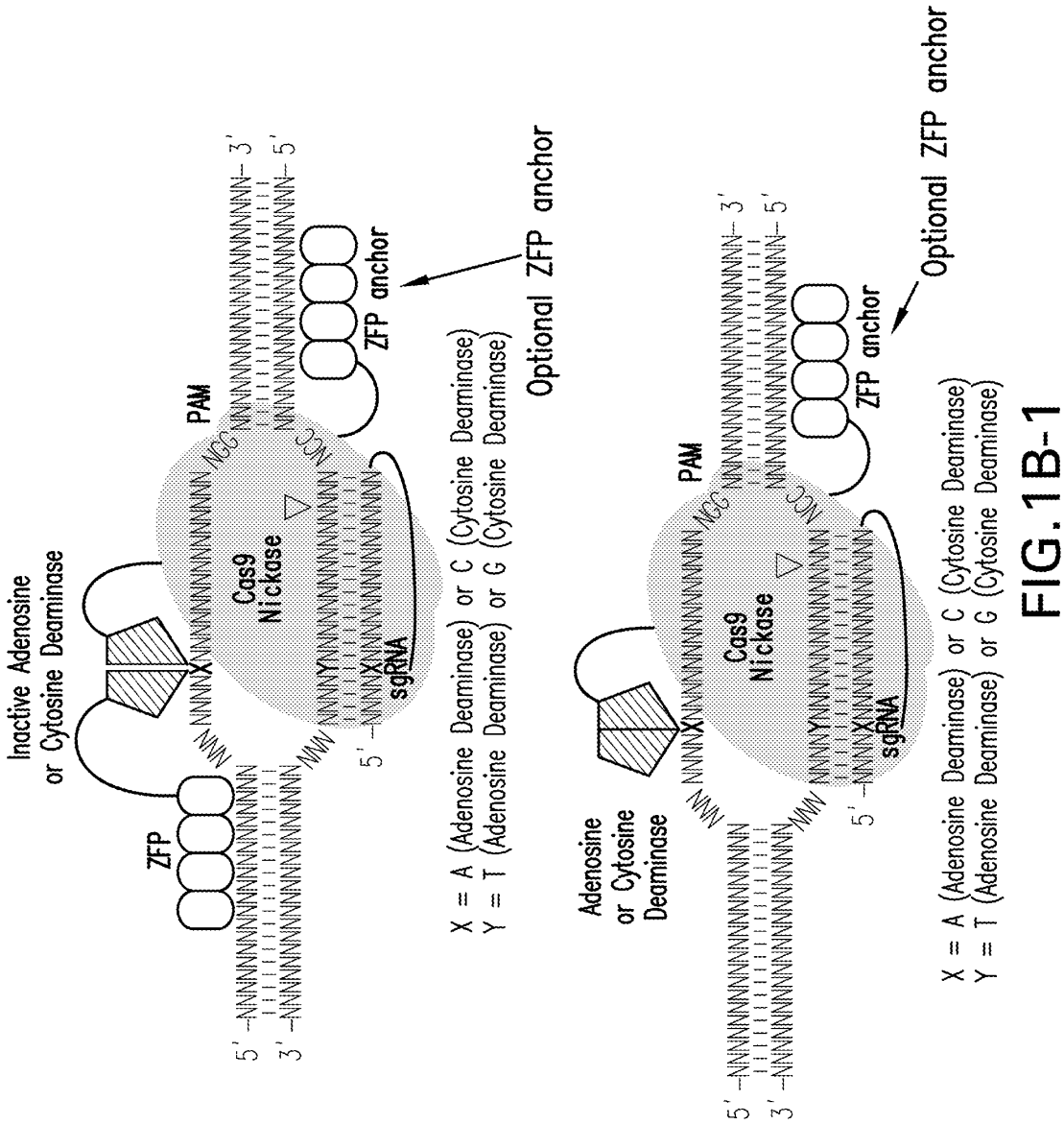


FIG.1B-1

Cont. On FIG.1B-3

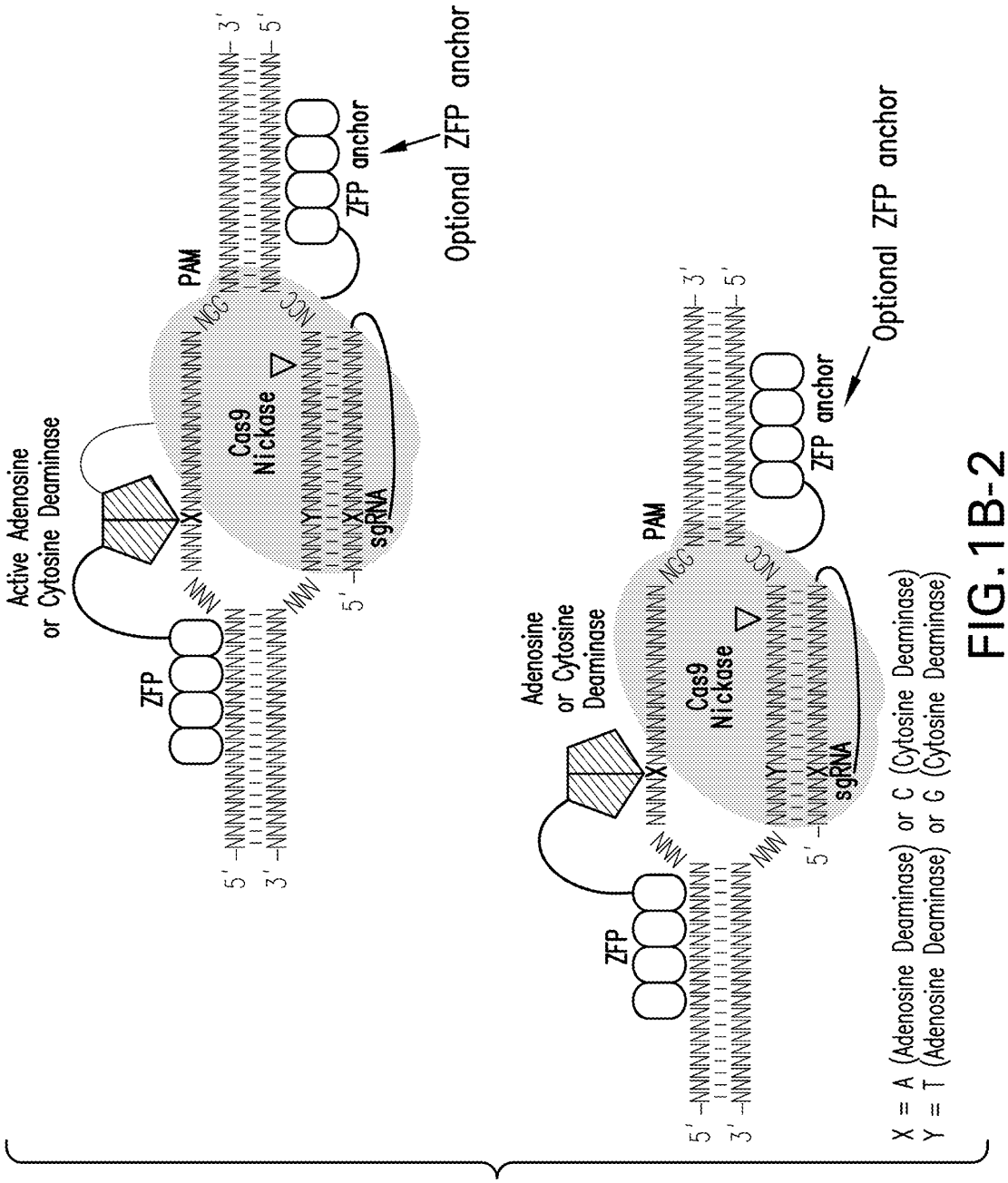


FIG.1B-2

Cont. From FIG.1B-1

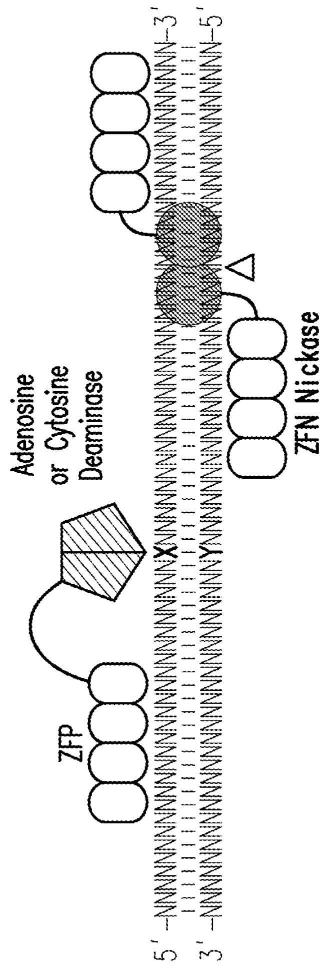


FIG.1B-3

Cont. From FIG.1B-2

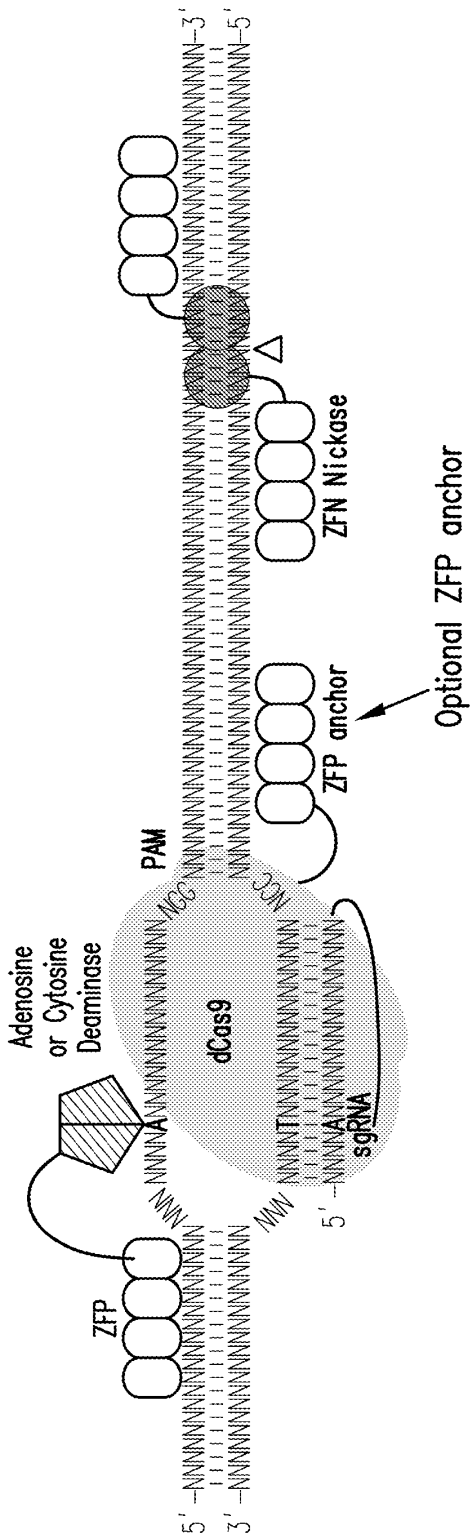
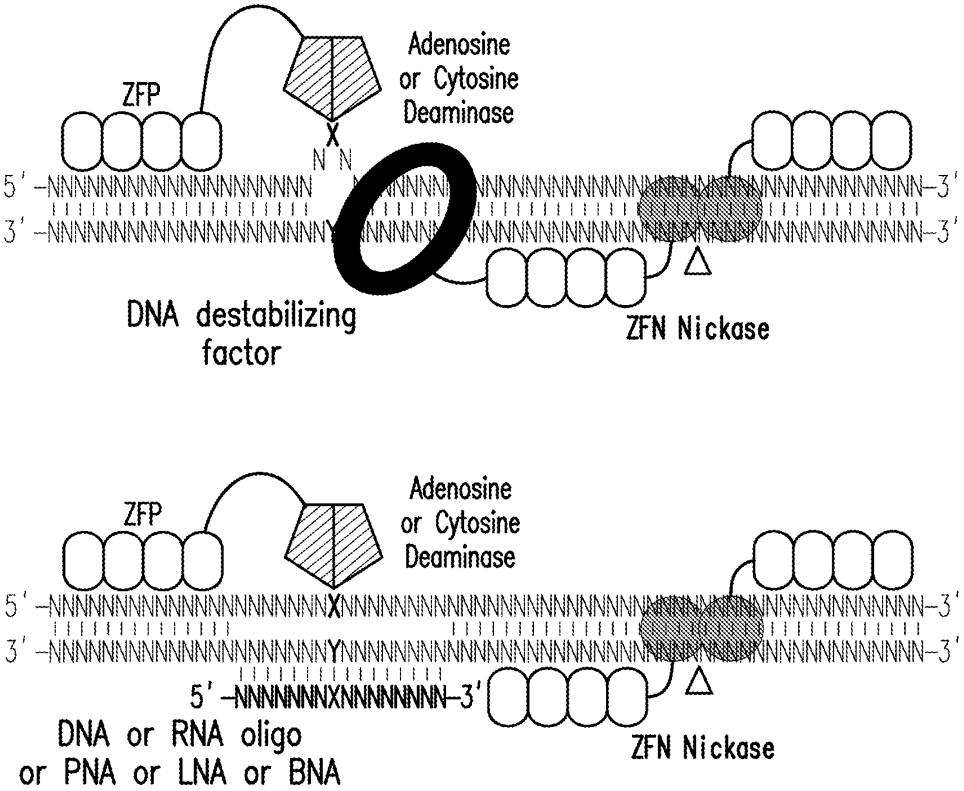

ZFN Nickase
Optional ZFP anchor
ZFP anchor
PAM
Adenosine or Cytosine Deaminase
dCas9
sgRNA
ZFP

FIG. 1C



X = A (Adenosine Deaminase) or C (Cytosine Deaminase)
Y = T (Adenosine Deaminase) or G (Cytosine Deaminase)

FIG.1D

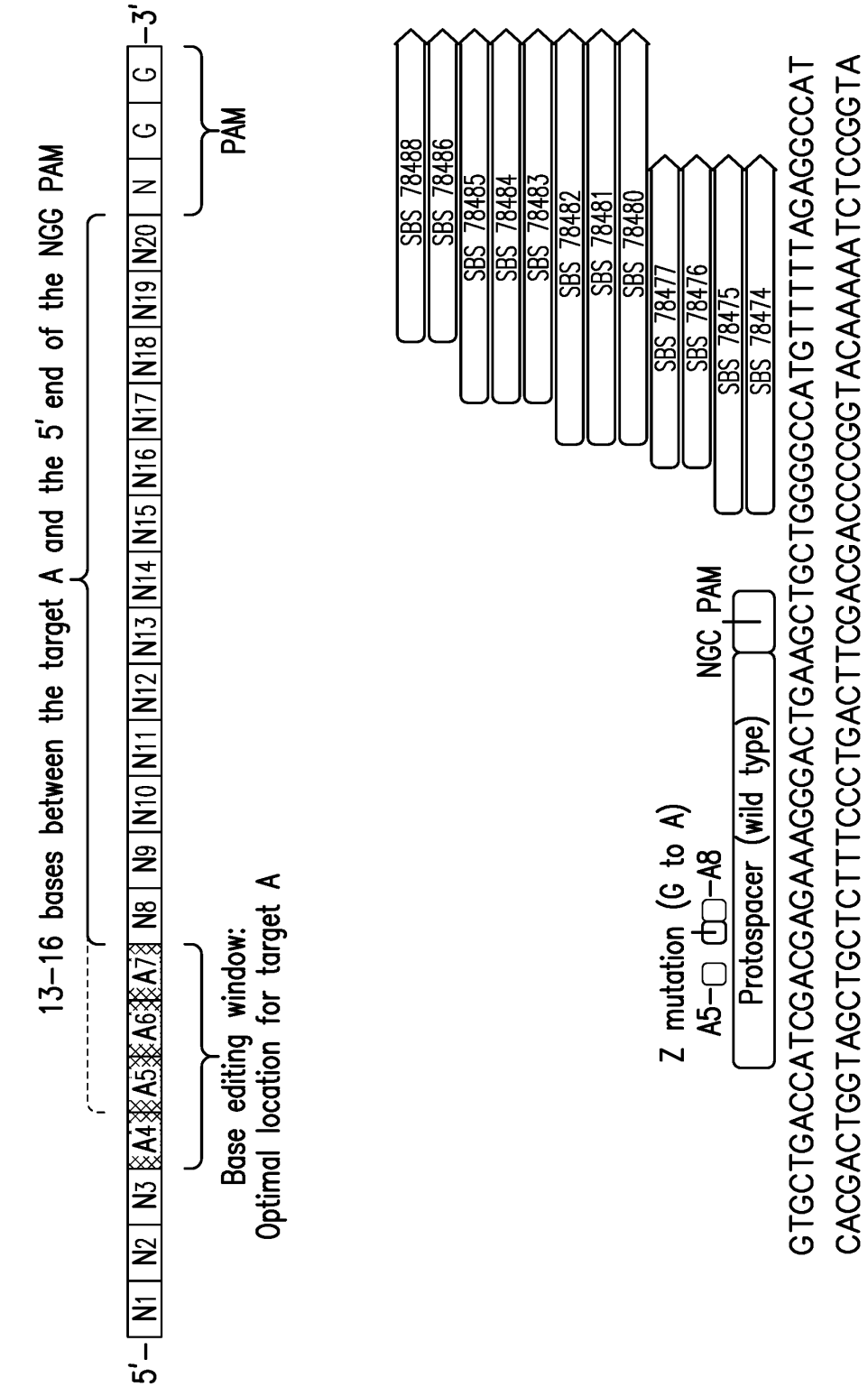

13–16 bases between the target A and the 5' end of the NGG PAM
5'–
N1 N2 N3 A4 A5 A6 A7 N8 N9 N10 N11 N12 N13 N14 N15 N16 N17 N18 N19 N20 N G G
–3'
PAM
Base editing window:
Optimal location for target A
SBS 78488
SBS 78486
SBS 78485
SBS 78484
SBS 78483
SBS 78482
SBS 78481
SBS 78480
SBS 78477
SBS 78476
SBS 78475
SBS 78474
Z mutation (G to A)
A5–□ □–A8
NGC PAM
Protospacer (wild type)
GTGCTGACCATCGACGAGAAAGGGACTGAAGCTGCTGGGGCCATGTTTTTAGAGGCCAT
CACGACTGGTAGCTGCTCTTTCCCTGACTTCGACGACCCCGGTACAAAAATCTCCGGTA

FIG. 2

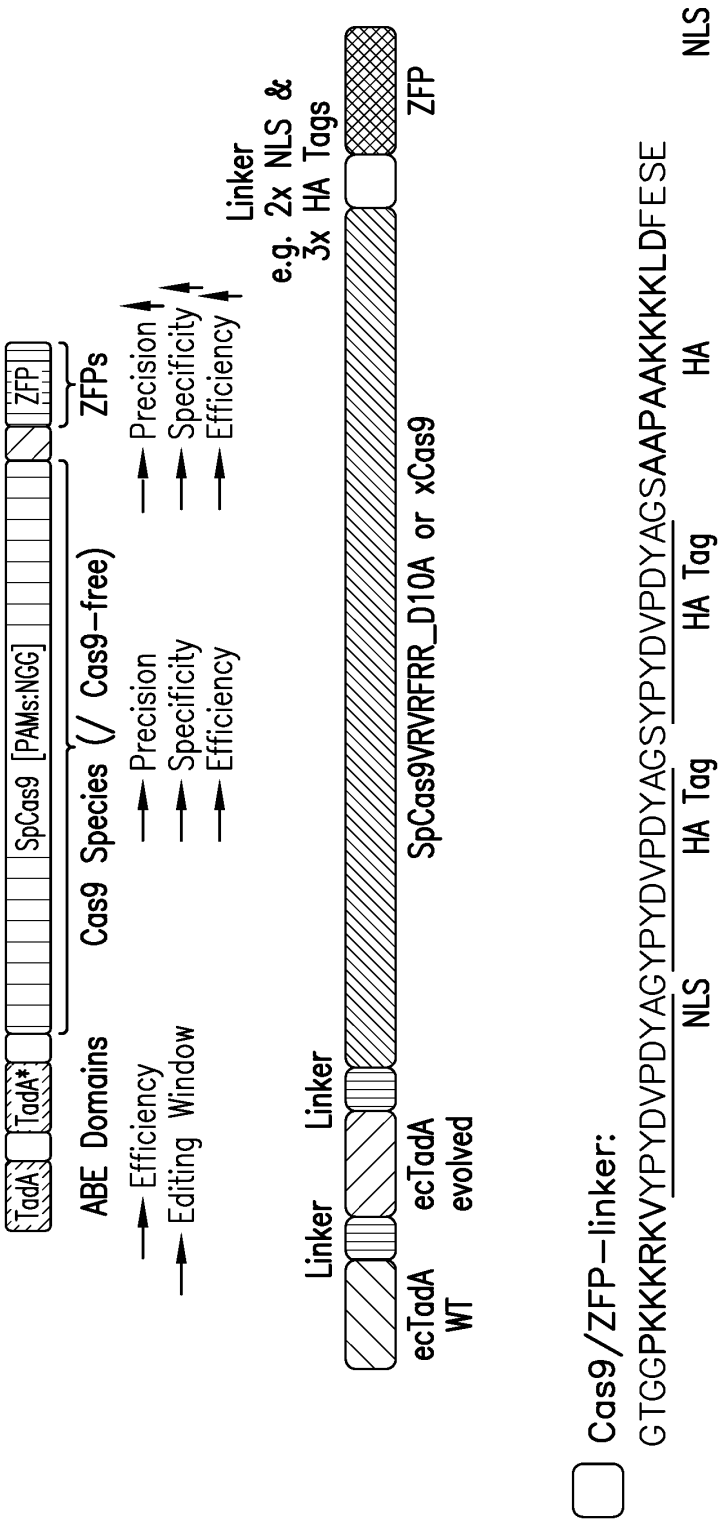

TadA
TadA*
SpCas9 [PAMs:NGG]
ZFP
ABE Domains
Cas9 Species (/ Cas9-free)
ZFPs
Efficiency
Editing Window
Precision
Specificity
Efficiency
Precision
Specificity
Efficiency
Linker
e.g. 2x NLS &
3x HA Tags
Linker
Linker
ecTadA
WT
ecTadA
evolved
SpCas9VRVRFRR_D10A or xCas9
ZFP
Cas9/ZFP-linker:
GTGGPKKKRKVYPYDVPDYAGYPYDVPDYAGSYPYDVPDYAGSAAPAAKKKKLDFESE
NLS
HA Tag
HA Tag
HA
NLS
TadA linker: SGGSSGGSSGSETPGTSESATPESSGGSSGGS

FIG.3A

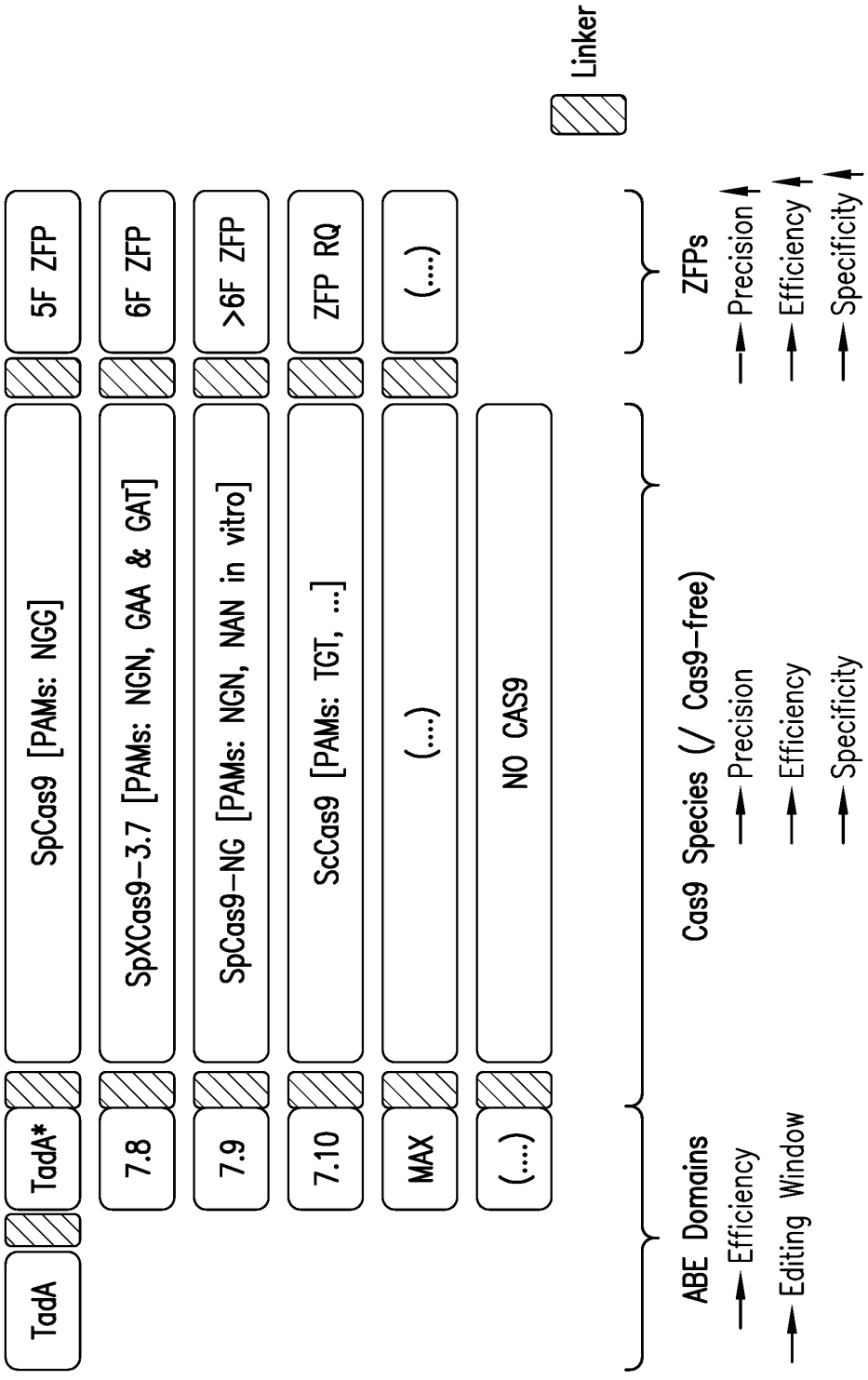

TadA
TadA*
SpCas9 [PAMs: NGG]
5F ZFP
7.8
SpXCas9-3.7 [PAMs: NGN, GAA & GAT]
6F ZFP
7.9
SpCas9-NG [PAMs: NGN, NAN in vitro]
>6F ZFP
7.10
ScCas9 [PAMs: TGT, ...]
ZFP RQ
MAX
(....)
(....)
NO CAS9
(....)
Linker
ABE Domains
Efficiency
Editing Window
Cas9 Species (/ Cas9-free)
Precision
Efficiency
Specificity
ZFPs
Precision
Efficiency
Specificity

FIG.3B

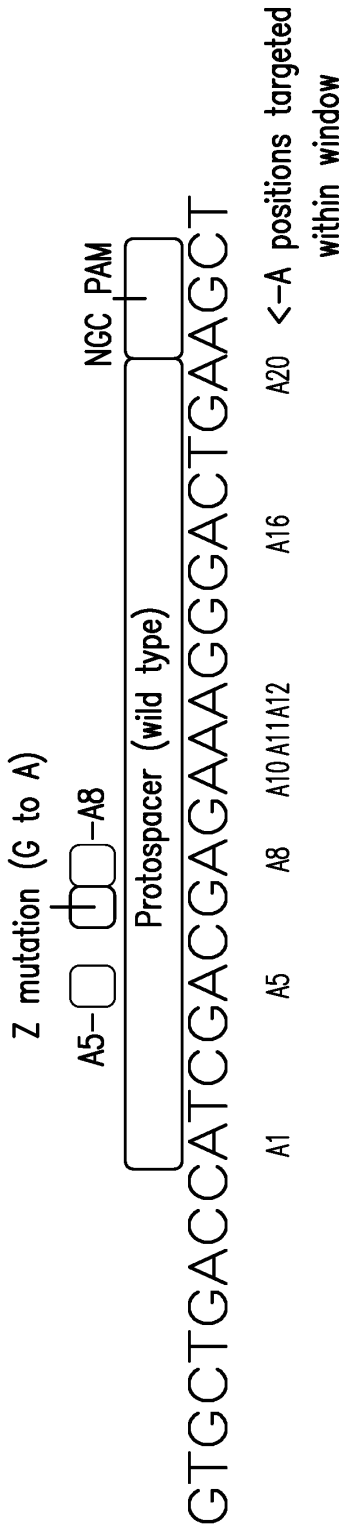

Z mutation (G to A)
A5-
-A8
Protospacer (wild type)
NGC PAM
GTGCTGACCATCGACGAGAAAGGGACTGAAGCT
A1
A5
A8
A10 A11 A12
A16
A20
<-A positions targeted within window

FIG.4

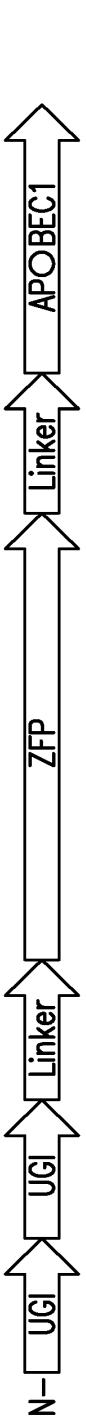


FIG.5A

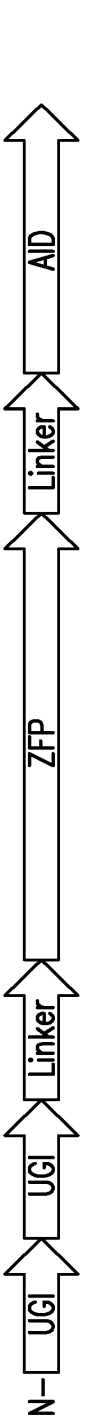


FIG.5B

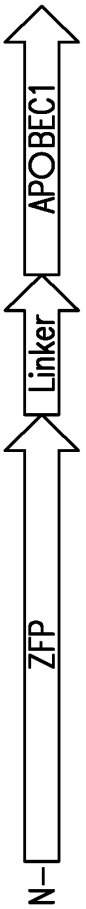


FIG.5C

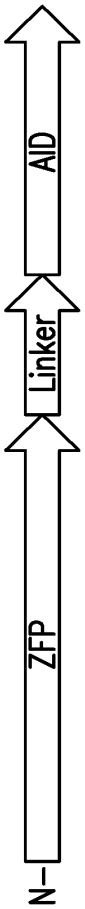


FIG.5D

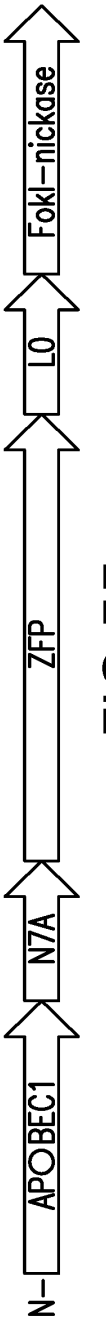


FIG.5E

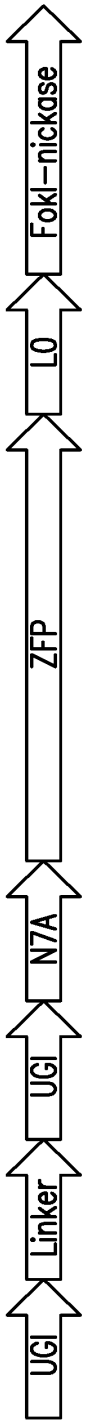


FIG.5F


N–
TadA
Linker
TadA*
Linker
ZFP

FIG.6A


N–
TadA
Linker
TadA*
Linker
ZFP
Linker
FokI–nickase

FIG.6B

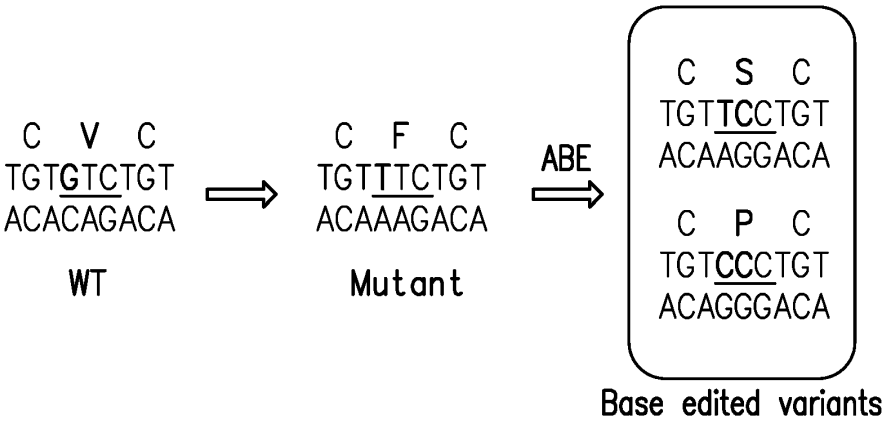

C V C
TGTGTCTGT
ACACAGACA
WT
C F C
TGTTTCTGT
ACAAAGACA
Mutant
ABE
C S C
TGTTCCTGT
ACAAGGACA
C P C
TGTCCCTGT
ACAGGGACA
Base edited variants

FIG.7A

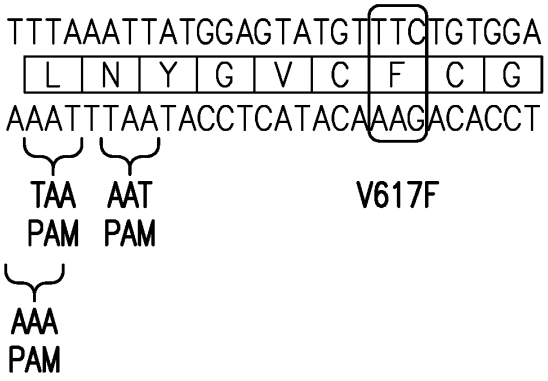

TTTAAATTATGGAGTATGTTTCTGTGGA
L N Y G V C F C G
AAATTTAATACCTCATACAAAGACACCT
TAA PAM
AAT PAM
AAA PAM
V617F

FIG.7B

600 ng	T	V617 (V617F) G (>T)	T	C	T
ABEmax-Cas9NG_AAT_PAM	0.64	NA	0.59	NA	6.78
ABEmax-Cas9NG_ZFP_2_AAT_PAM	0.27	NA	0.50	NA	6.94
ABEmax-Cas9NG_ZFP_4_AAT_PAM	0.26	NA	0.71	NA	8.65
ABEmax-Cas9NG_ZFP_6_AAT_PAM	0.67	NA	1.29	NA	13.11
ABEmax-Cas9NG_ZFP_7_AAT_PAM	0.52	NA	1.14	NA	10.94
ABEmax-Cas9NG_TAA_PAM	0.43	NA	0.25	NA	0.06
ABEmax-Cas9NG_ZFP_2_TAA_PAM	5.48	NA	1.30	NA	0.24
ABEmax-Cas9NG_ZFP_4_TAA_PAM	4.18	NA	1.19	NA	0.16
ABEmax-Cas9NG_ZFP_6_TAA_PAM	3.06	NA	0.93	NA	0.18
ABEmax-Cas9NG_ZFP_7_TAA_PAM	2.11	NA	0.69	NA	0.09
ABEmax-Cas9NG_AAA_PAM	2.76	NA	0.17	NA	0.10
ABEmax-Cas9NG_ZFP_2_AAA_PAM	4.65	NA	0.17	NA	0.15
ABEmax-Cas9NG_ZFP_4_AAA_PAM	6.90	NA	0.17	NA	0.09
ABEmax-Cas9NG_ZFP_6_AAA_PAM	4.76	NA	0.21	NA	0.15
ABEmax-Cas9NG_ZFP_7_AAA_PAM	4.58	NA	0.16	NA	0.17

800 ng	T	V617 (V617F) G (>T)	T	C	T
ABEmax-Cas9NG_AAT_PAM	0.38	NA	0.78	NA	7.35
ABEmax-Cas9NG_ZFP_2_AAT_PAM	0.76	NA	1.37	NA	15.24
ABEmax-Cas9NG_ZFP_4_AAT_PAM	0.58	NA	1.16	NA	15.16
ABEmax-Cas9NG_ZFP_6_AAT_PAM	0.59	NA	1.57	NA	19.77
ABEmax-Cas9NG_ZFP_7_AAT_PAM	0.64	NA	1.25	NA	17.68
ABEmax-Cas9NG_TAA_PAM	0.45	NA	0.17	NA	0.13
ABEmax-Cas9NG_ZFP_2_TAA_PAM	7.85	NA	1.69	NA	0.25
ABEmax-Cas9NG_ZFP_4_TAA_PAM	4.92	NA	1.31	NA	0.40
ABEmax-Cas9NG_ZFP_6_TAA_PAM	4.84	NA	1.13	NA	0.12
ABEmax-Cas9NG_ZFP_7_TAA_PAM	3.45	NA	0.97	NA	0.19

FIG.7C

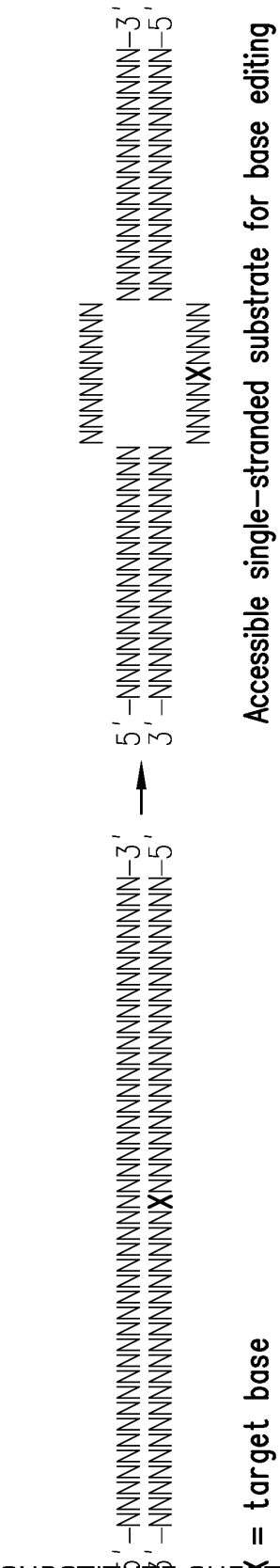
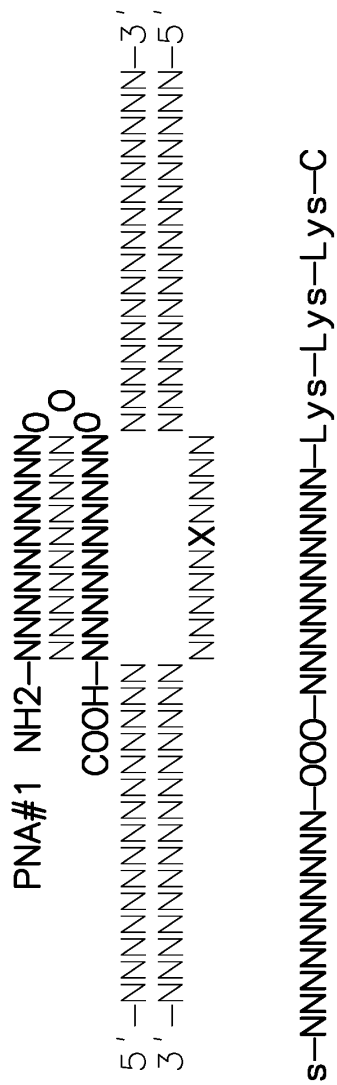
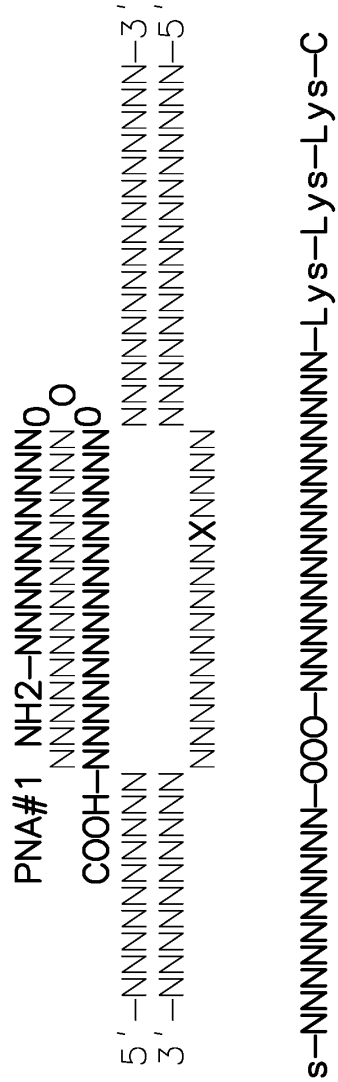

5′–NNNNNNNNNNNNNNNNNNNN–3′
3′–NNNNNNNNNNNNNNNNNNNN–5′
NNNNNNNN
NNNNXNNNN
Accessible single–stranded substrate for base editing
5′–NNNNNNNNNNNNNNNNNNNN–3′
3′–NNNNNNNNNNNNNNNNXNNN–5′
X = target base

FIG.8A



PNA#1: N–Lys–Lys–Lys–NNNNNNNNNN–OOO–NNNNNNNNNN–Lys–Lys–Lys–C

FIG.8B



PNA#2: N–Lys–Lys–Lys–NNNNNNNNNN–OOO–NNNNNNNNNN–Lys–Lys–Lys–C

FIG.8C

FIG.8D

PNA#3: N–Lys–Lys–Lys–NNNNNNNNNN–OOO–NNNNNNNNNN–Lys–Lys–Lys–C
PNA#4: N–Lys–Lys–Lys–NNNNNNNNNN–OOO–NNNNNNNNNN–Lys–Lys–Lys–C

FIG.8E

PNA#5: N–Lys–Lys–Lys–NNNNNNNNNNNNNNN–Lys–Lys–Lys–C

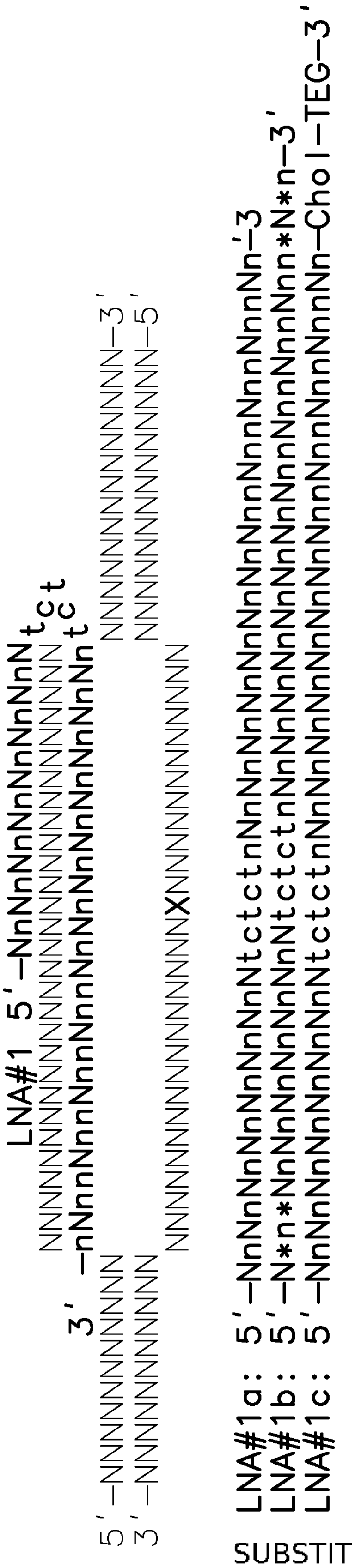


FIG.8F

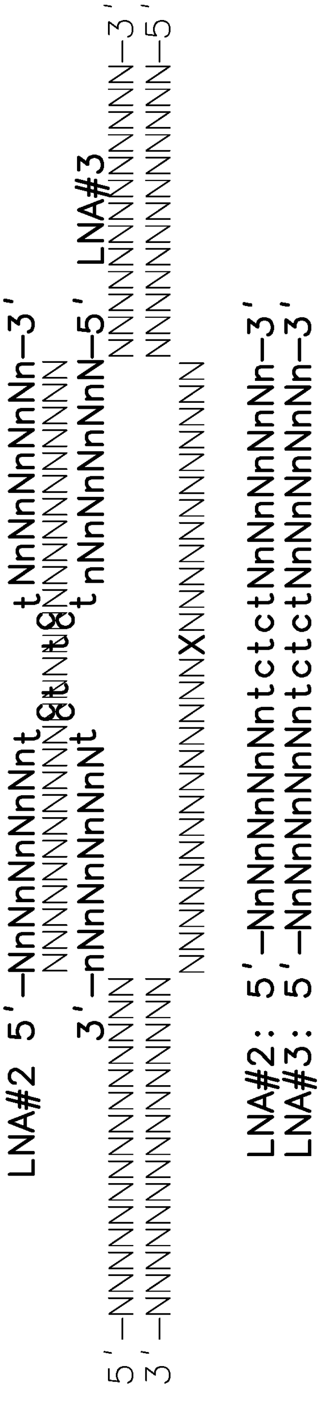


FIG.8G