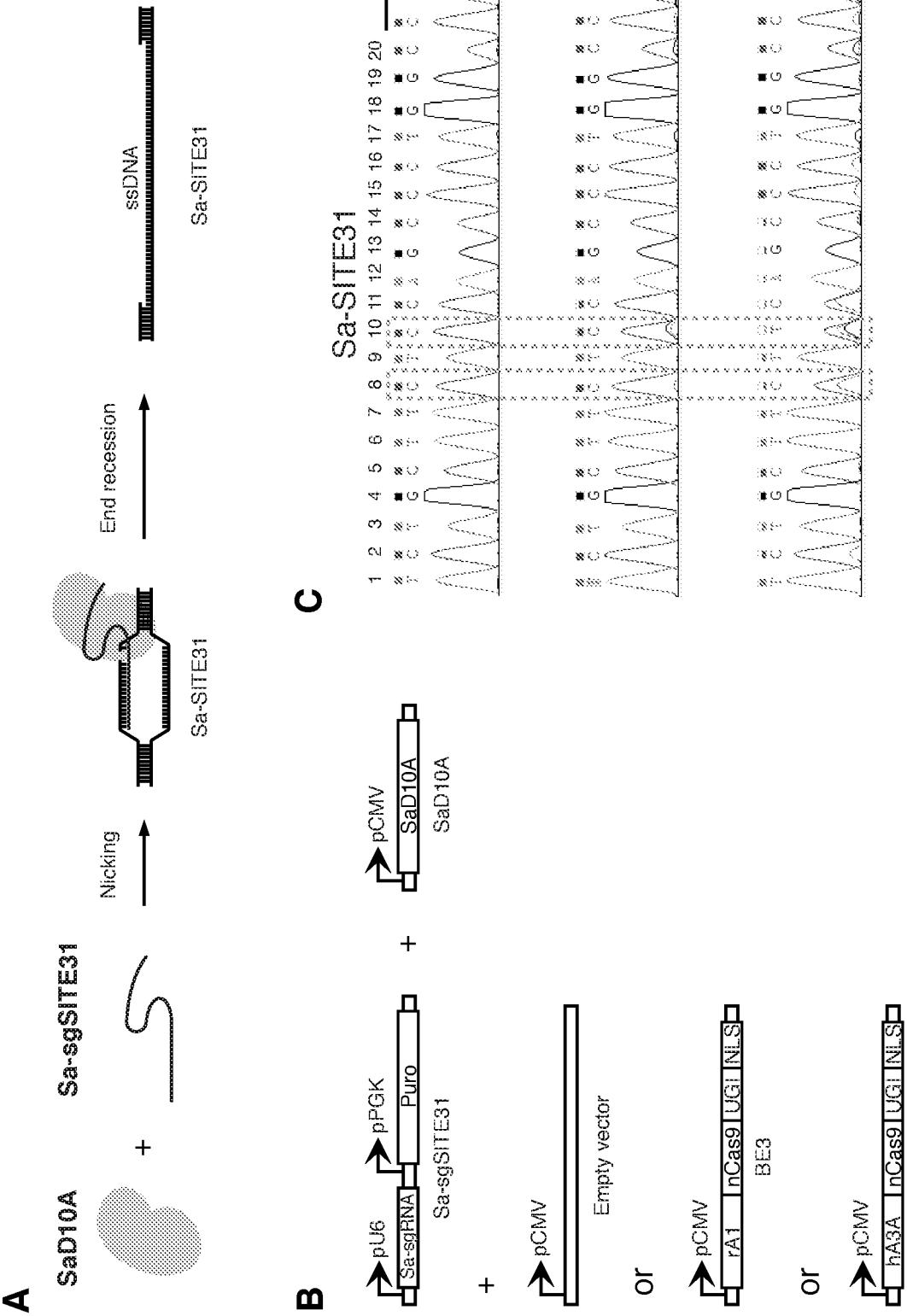

A
SaD10A
Sa-sgSITE31
Nicking
Sa-SITE31
End recession
ssDNA
Sa-SITE31
B
pU6
Sa-sgRNA
pPGK
Puro
Sa-sgSITE31
+
pCMV
SaD10A
SaD10A
+
pCMV
Empty vector
or
pCMV
rA1
nCas9
UGI
NLS
BE3
or
pCMV
hA3A
nCas9
UGI
NLS
hA3A-BE3
C
Sa-SITE31
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
Empty vector
BE3
hA3A-BE3

FIG. 1A-C

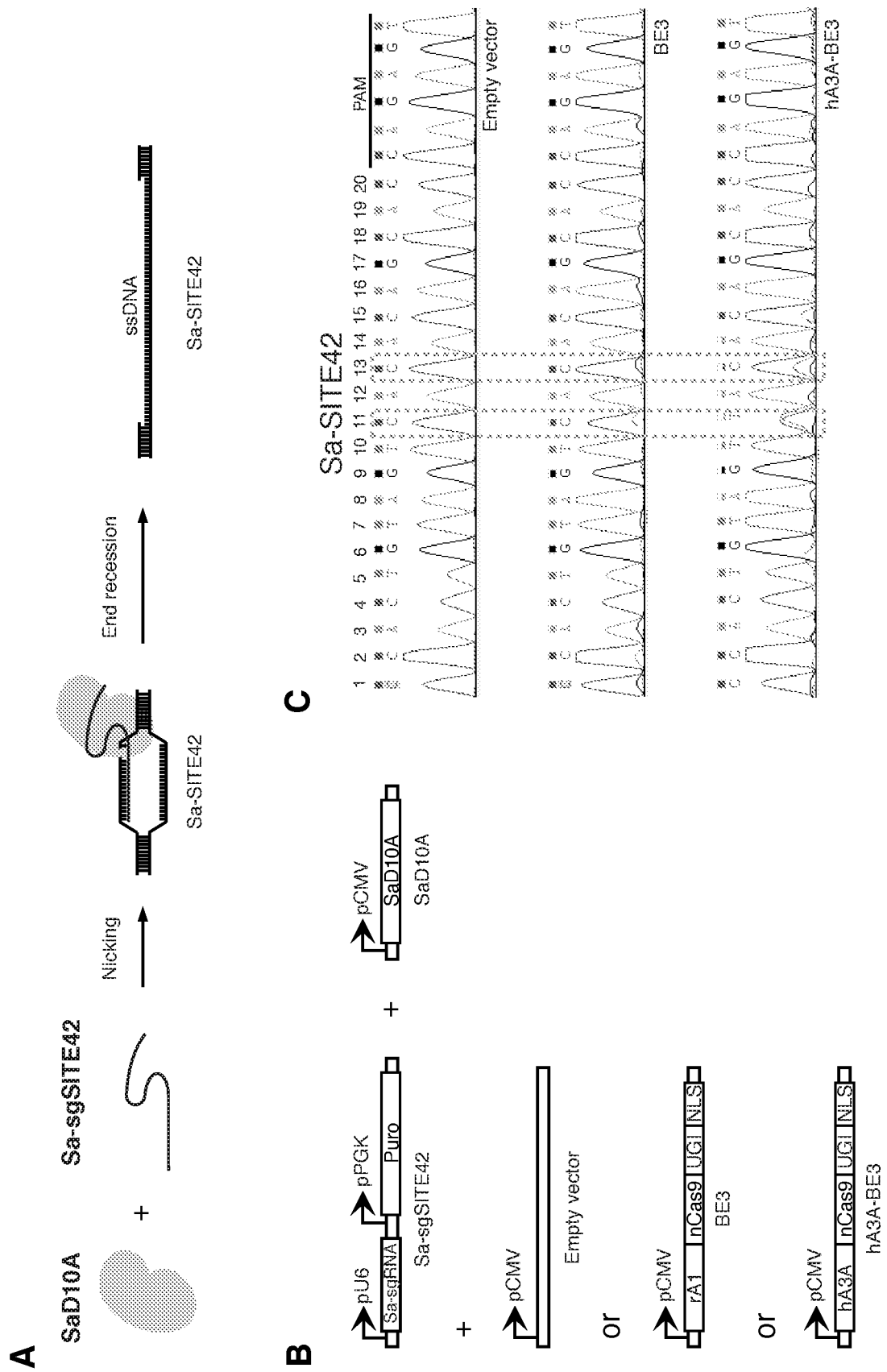

A
SaD10A
Sa-sgSITE42
Nicking
End recession
ssDNA
Sa-SITE42
Sa-SITE42
B
pU6
Sa-sgRNA
pPGK
Puro
Sa-sgSITE42
+
pCMV
SaD10A
SaD10A
+
pCMV
Empty vector
or
pCMV
rA1
nCas9
UGI
NLS
BE3
or
pCMV
hA3A
nCas9
UGI
NLS
hA3A-BE3
C
Sa-SITE42
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
Empty vector
BE3
hA3A-BE3

FIG. 2A-C

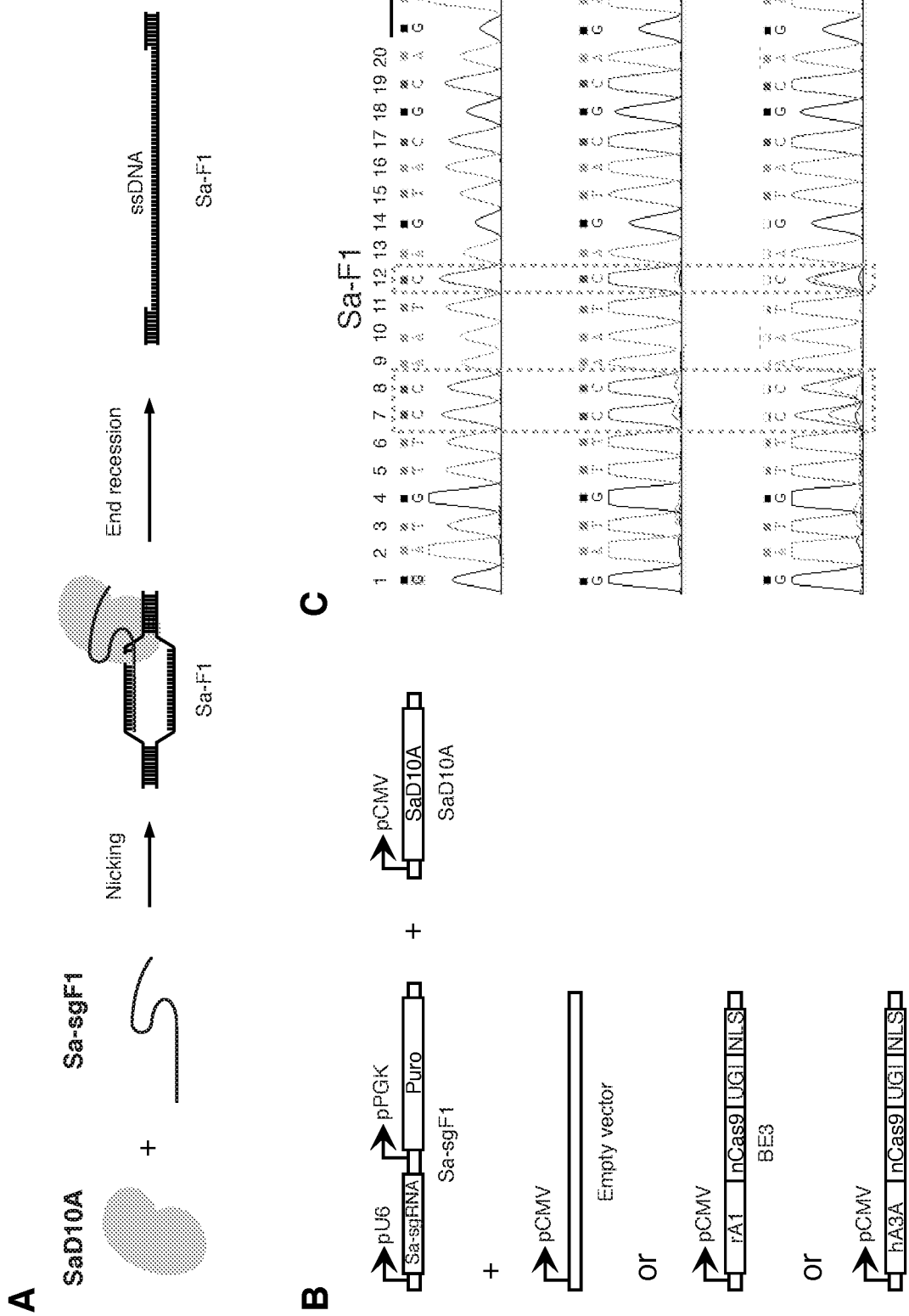

A
SaD10A
Sa-sgF1
+
Nicking
Sa-F1
End recession
ssDNA
Sa-F1
B
pU6
Sa-sgRNA
pPGK
Puro
Sa-sgF1
+
pCMV
SaD10A
SaD10A
+
pCMV
Empty vector
or
pCMV
rA1
nCas9
UGI
NLS
BE3
or
pCMV
hA3A
nCas9
UGI
NLS
hA3A-BE3
C
Sa-F1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
Empty vector
BE3
hA3A-BE3

FIG. 3A-C

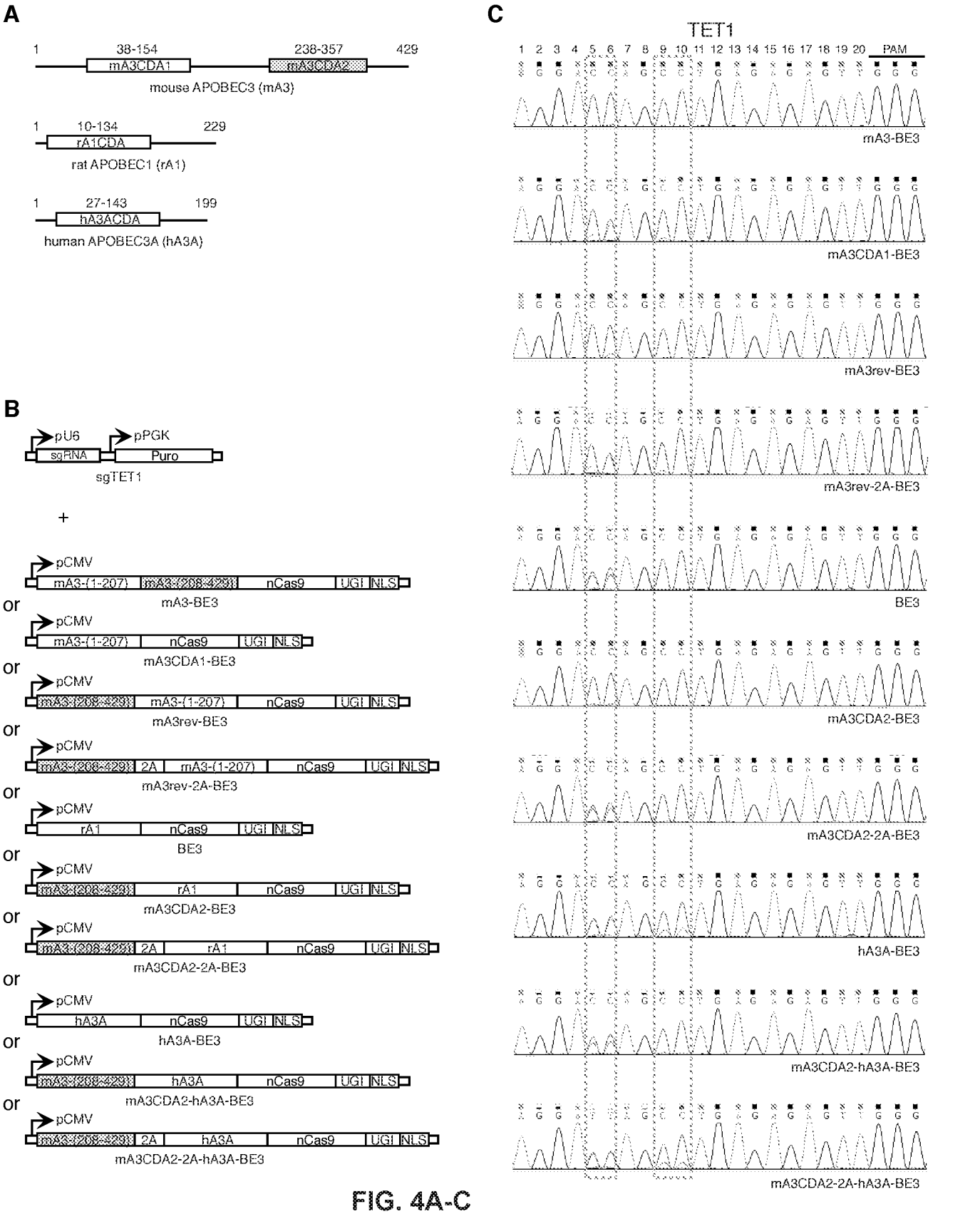

A
1
38-154
238-357
429
mA3CDA1
mA3CDA2
mouse APOBEC3 (mA3)
1
10-134
229
rA1CDA
rat APOBEC1 (rA1)
1
27-143
199
hA3ACDA
human APOBEC3A (hA3A)
B
pU6
pPGK
sgRNA
Puro
sgTET1
+
pCMV
mA3-(1-207)
mA3-(208-429)
nCas9
UGI
NLS
mA3-BE3
or
mA3CDA1-BE3
mA3rev-BE3
2A
mA3rev-2A-BE3
rA1
BE3
mA3CDA2-BE3
mA3CDA2-2A-BE3
hA3A
hA3A-BE3
mA3CDA2-hA3A-BE3
mA3CDA2-2A-hA3A-BE3
C
TET1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
mA3-BE3
mA3CDA1-BE3
mA3rev-BE3
mA3rev-2A-BE3
BE3
mA3CDA2-BE3
mA3CDA2-2A-BE3
hA3A-BE3
mA3CDA2-hA3A-BE3
mA3CDA2-2A-hA3A-BE3

FIG. 4A-C

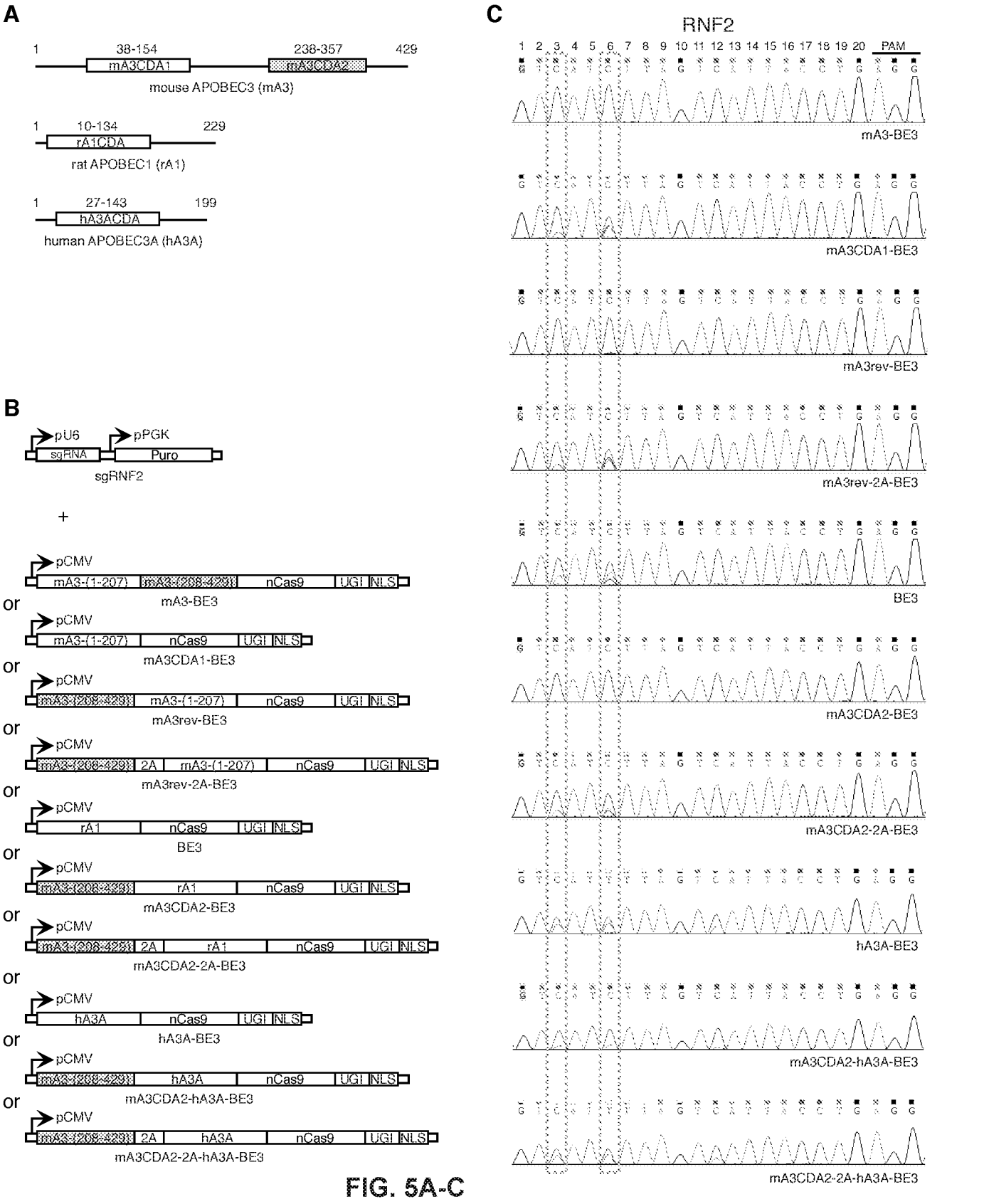

A
1
38-154
238-357
429
mA3CDA1
mA3CDA2
mouse APOBEC3 (mA3)
1
10-134
229
rA1CDA
rat APOBEC1 (rA1)
1
27-143
199
hA3ACDA
human APOBEC3A (hA3A)
B
pU6
pPGK
sgRNA
Puro
sgRNF2
+
pCMV
mA3-(1-207)
mA3-(208-429)
nCas9
UGI
NLS
mA3-BE3
or
mA3CDA1-BE3
mA3rev-BE3
2A
mA3rev-2A-BE3
rA1
BE3
mA3CDA2-BE3
mA3CDA2-2A-BE3
hA3A
hA3A-BE3
mA3CDA2-hA3A-BE3
mA3CDA2-2A-hA3A-BE3
C
RNF2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
mA3-BE3
mA3CDA1-BE3
mA3rev-BE3
mA3rev-2A-BE3
BE3
mA3CDA2-BE3
mA3CDA2-2A-BE3
hA3A-BE3
mA3CDA2-hA3A-BE3
mA3CDA2-2A-hA3A-BE3

FIG. 5A-C

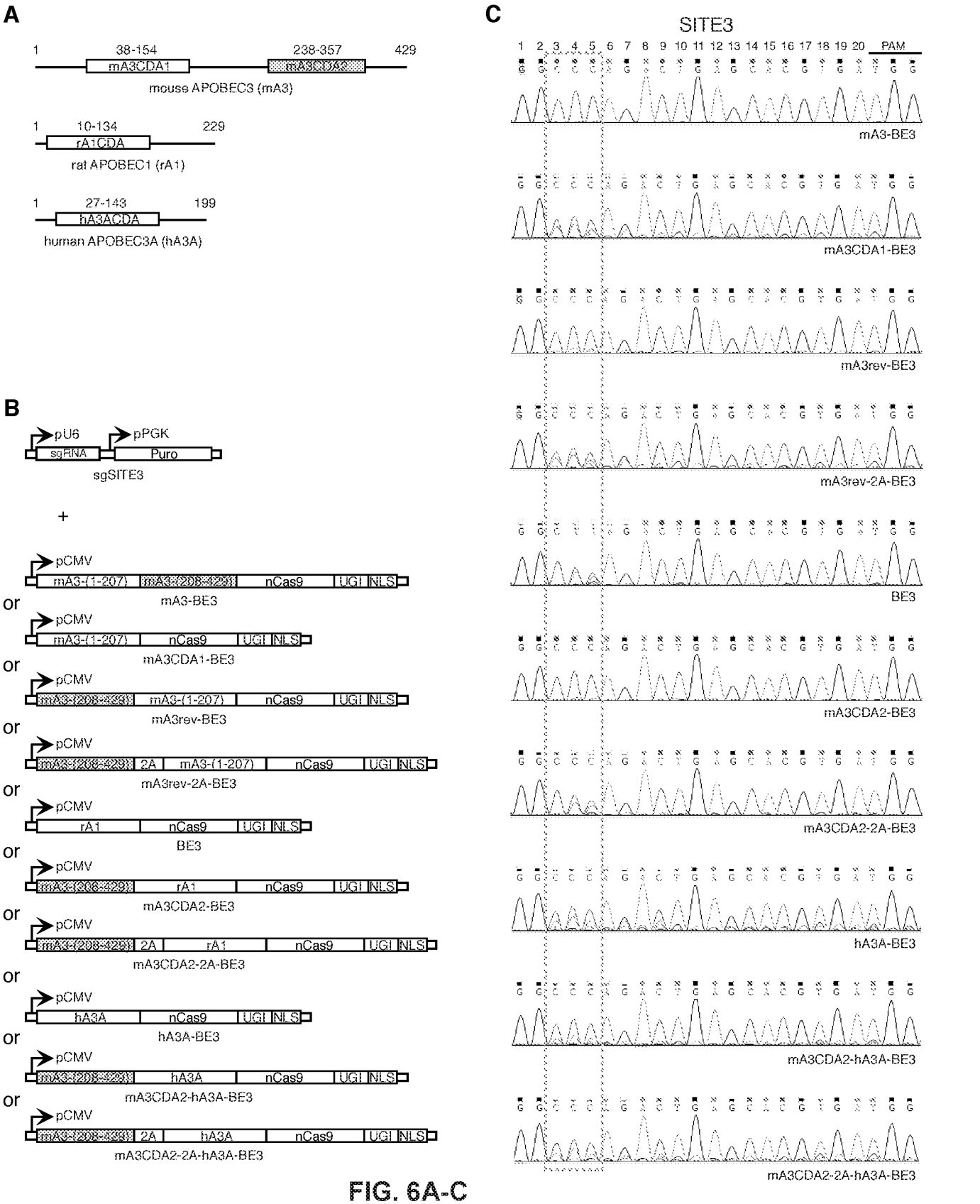

A
1
38-154
238-357
429
mA3CDA1
mA3CDA2
mouse APOBEC3 (mA3)
1
10-134
229
rA1CDA
rat APOBEC1 (rA1)
1
27-143
199
hA3ACDA
human APOBEC3A (hA3A)
B
pU6
pPGK
sgRNA
Puro
sgSITE3
+
pCMV
mA3-(1-207)
mA3-(208-429)
nCas9
UGI
NLS
mA3-BE3
or
mA3CDA1-BE3
mA3rev-BE3
2A
mA3rev-2A-BE3
rA1
BE3
mA3CDA2-BE3
mA3CDA2-2A-BE3
hA3A
hA3A-BE3
mA3CDA2-hA3A-BE3
mA3CDA2-2A-hA3A-BE3
C
SITE3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
mA3-BE3
mA3CDA1-BE3
mA3rev-BE3
mA3rev-2A-BE3
BE3
mA3CDA2-BE3
mA3CDA2-2A-BE3
hA3A-BE3
mA3CDA2-hA3A-BE3
mA3CDA2-2A-hA3A-BE3

FIG. 6A-C

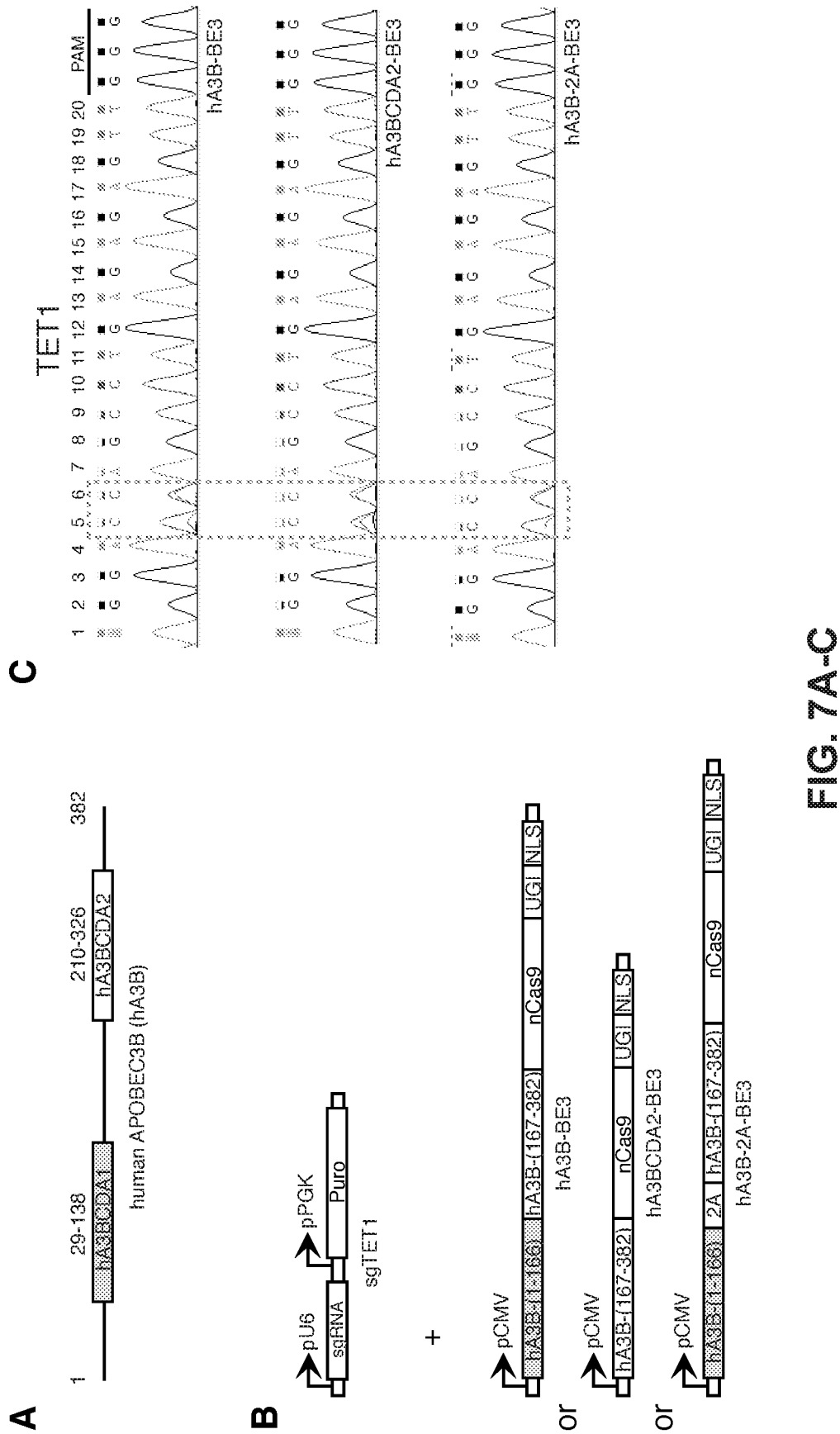

A
1
29-138
hA3BCDA1
210-326
hA3BCDA2
382
human APOBEC3B (hA3B)
B
pU6
sgRNA
pPGK
Puro
sgTET1
+
pCMV
hA3B-(1-166)
hA3B-(167-382)
nCas9
UGI
NLS
hA3B-BE3
or
pCMV
hA3B-(167-382)
nCas9
UGI
NLS
hA3BCDA2-BE3
or
pCMV
hA3B-(1-166)
2A
hA3B-(167-382)
nCas9
UGI
NLS
hA3B-2A-BE3
C
TET1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
hA3B-BE3
hA3BCDA2-BE3
hA3B-2A-BE3

FIG. 7A-C

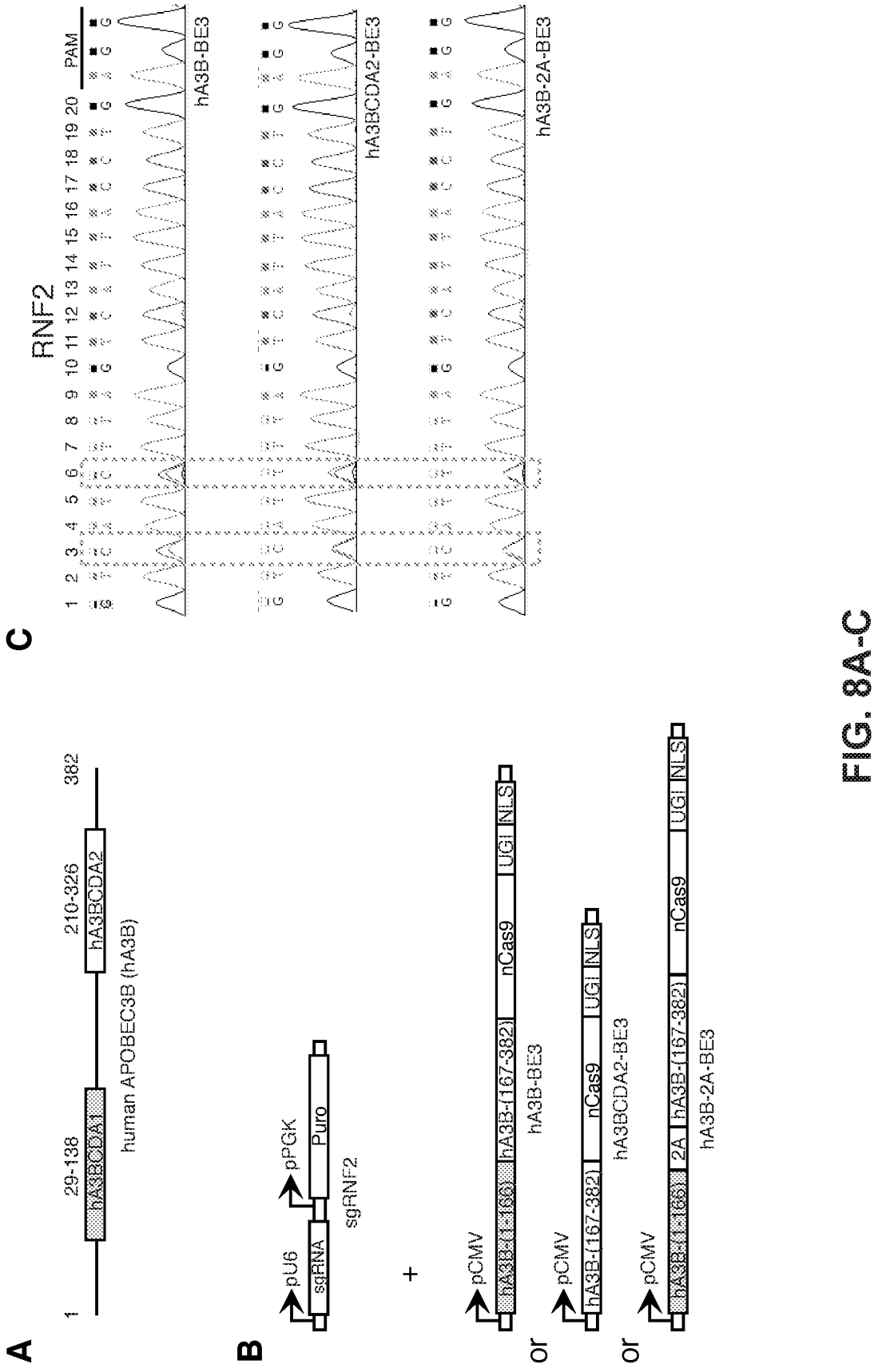

A
1
29-138
hA3BCDA1
210-326
hA3BCDA2
382
human APOBEC3B (hA3B)
B
pU6
sgRNA
pPGK
Puro
sgRNF2
+
pCMV
hA3B-(1-166)
hA3B-(167-382)
nCas9
UGI
NLS
hA3B-BE3
or
pCMV
hA3B-(167-382)
nCas9
UGI
NLS
hA3BCDA2-BE3
or
pCMV
hA3B-(1-166)
2A
hA3B-(167-382)
nCas9
UGI
NLS
hA3B-2A-BE3
C
RNF2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
hA3B-BE3
hA3BCDA2-BE3
hA3B-2A-BE3

FIG. 8A-C

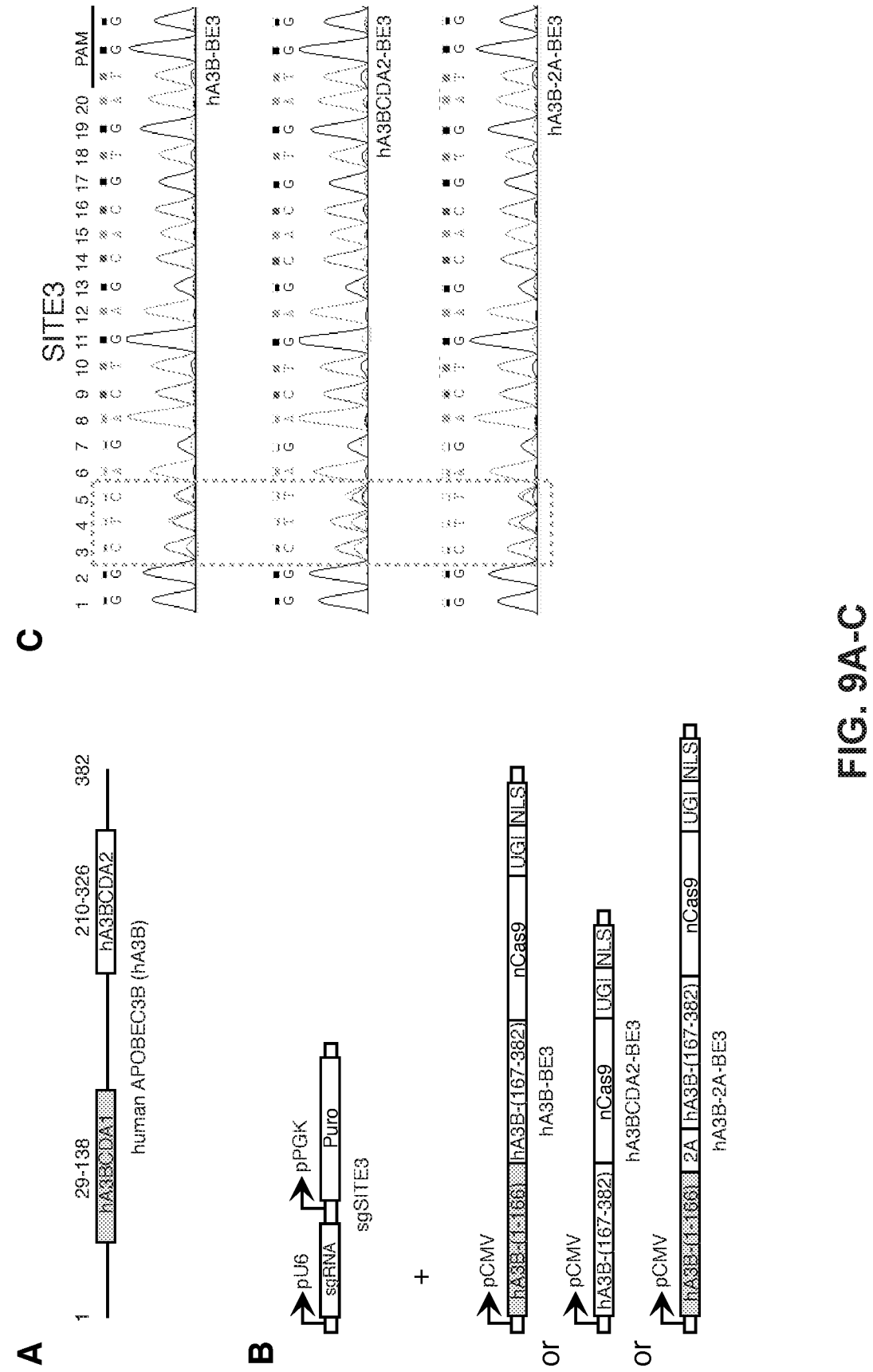

A
1
29-138
hA3BCDA1
210-326
hA3BCDA2
382
human APOBEC3B (hA3B)
B
pU6
sgRNA
pPGK
Puro
sgSITE3
+
pCMV
hA3B-(1-166)
hA3B-(167-382)
nCas9
UGI
NLS
hA3B-BE3
or
pCMV
hA3B-(167-382)
nCas9
UGI
NLS
hA3BCDA2-BE3
or
pCMV
hA3B-(1-166)
2A
hA3B-(167-382)
nCas9
UGI
NLS
hA3B-2A-BE3
C
SITE3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
hA3B-BE3
hA3BCDA2-BE3
hA3B-2A-BE3

FIG. 9A-C

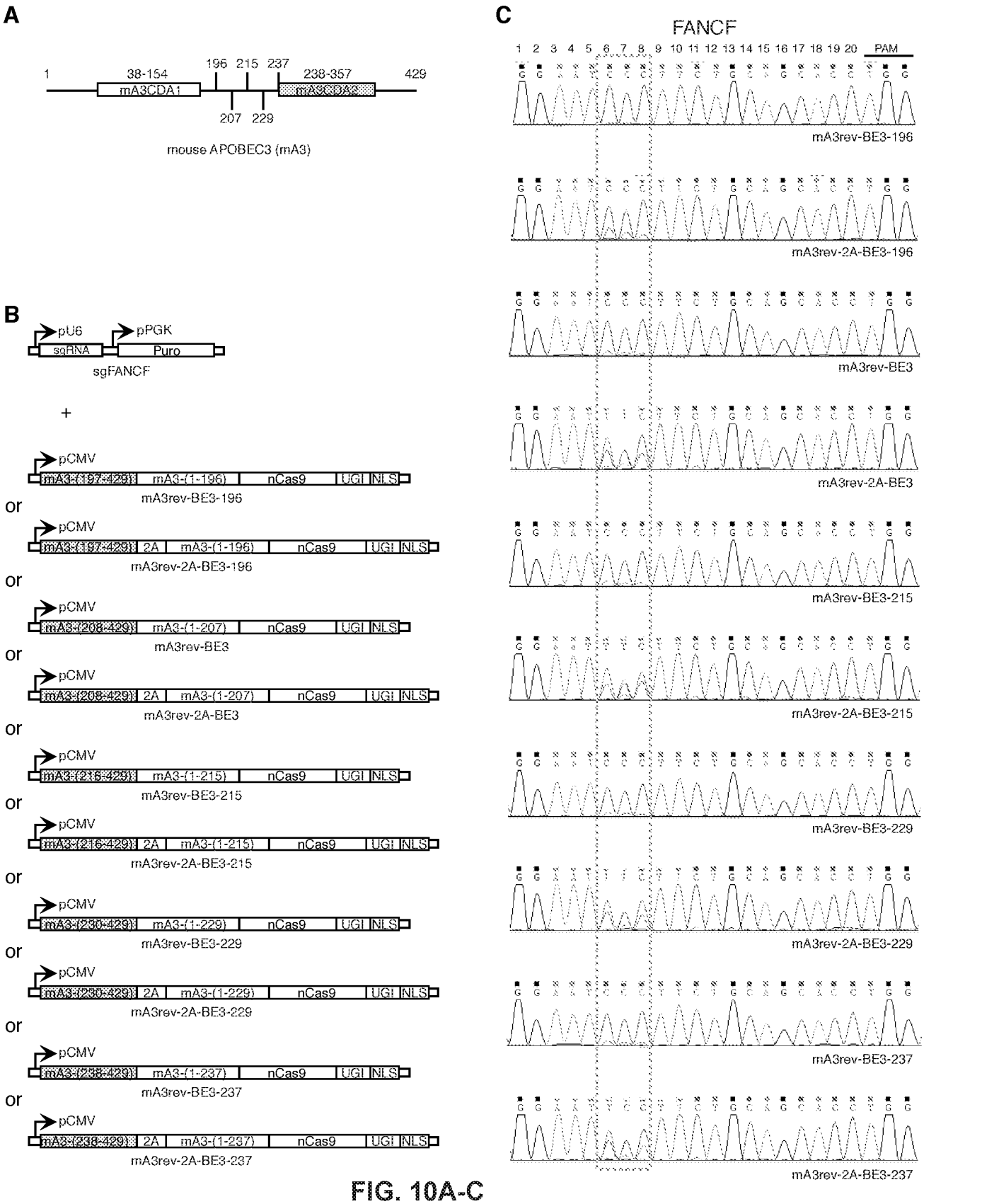

A
1
38-154
196 215 237
238-357
429
mA3CDA1
mA3CDA2
207 229
mouse APOBEC3 (mA3)
B
pU6
pPGK
sgRNA
Puro
sgFANCF
+
pCMV
mA3-(197-429)
mA3-(1-196)
nCas9
UGI
NLS
mA3rev-BE3-196
or
2A
mA3rev-2A-BE3-196
mA3-(208-429)
mA3-(1-207)
mA3rev-BE3
mA3rev-2A-BE3
mA3-(216-429)
mA3-(1-215)
mA3rev-BE3-215
mA3rev-2A-BE3-215
mA3-(230-429)
mA3-(1-229)
mA3rev-BE3-229
mA3rev-2A-BE3-229
mA3-(238-429)
mA3-(1-237)
mA3rev-BE3-237
mA3rev-2A-BE3-237
C
FANCF
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
mA3rev-BE3-196
mA3rev-2A-BE3-196
mA3rev-BE3
mA3rev-2A-BE3
mA3rev-BE3-215
mA3rev-2A-BE3-215
mA3rev-BE3-229
mA3rev-2A-BE3-229
mA3rev-BE3-237
mA3rev-2A-BE3-237

FIG. 10A-C

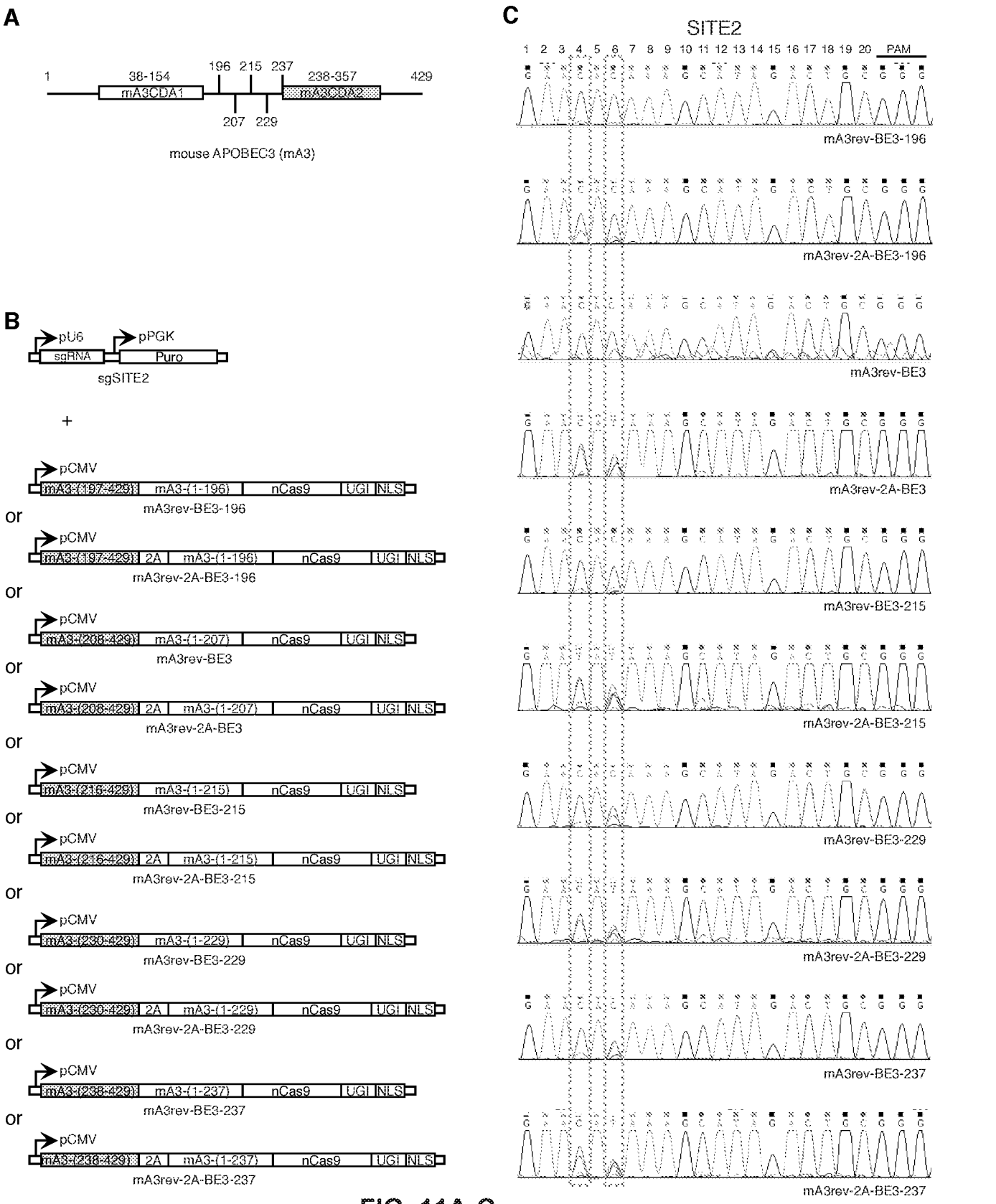

A
1
38-154
196 215 237
238-357
429
mA3CDA1
mA3CDA2
207 229
mouse APOBEC3 (mA3)
B
pU6
pPGK
sgRNA
Puro
sgSITE2
+
pCMV
mA3-(197-429)
mA3-(1-196)
nCas9
UGI
NLS
mA3rev-BE3-196
or
2A
mA3rev-2A-BE3-196
mA3-(208-429)
mA3-(1-207)
mA3rev-BE3
mA3rev-2A-BE3
mA3-(216-429)
mA3-(1-215)
mA3rev-BE3-215
mA3rev-2A-BE3-215
mA3-(230-429)
mA3-(1-229)
mA3rev-BE3-229
mA3rev-2A-BE3-229
mA3-(238-429)
mA3-(1-237)
mA3rev-BE3-237
mA3rev-2A-BE3-237
C
SITE2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
mA3rev-BE3-196
mA3rev-2A-BE3-196
mA3rev-BE3
mA3rev-2A-BE3
mA3rev-BE3-215
mA3rev-2A-BE3-215
mA3rev-BE3-229
mA3rev-2A-BE3-229
mA3rev-BE3-237
mA3rev-2A-BE3-237

FIG. 11A-C

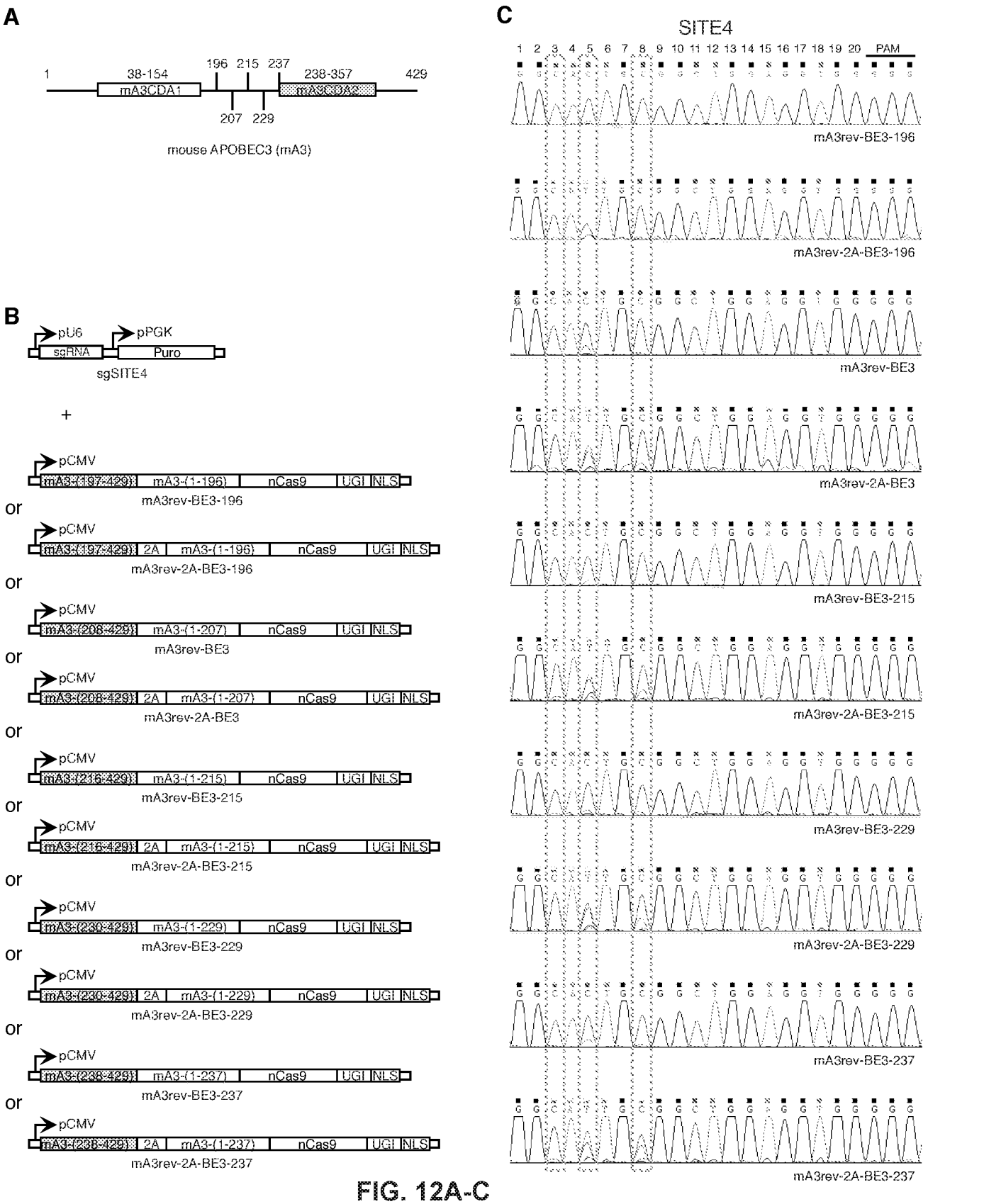

A
1
38-154
196 215 237
238-357
429
mA3CDA1
mA3CDA2
207 229
mouse APOBEC3 (mA3)
B
pU6
pPGK
sgRNA
Puro
sgSITE4
+
pCMV
mA3-(197-429)
mA3-(1-196)
nCas9
UGI
NLS
mA3rev-BE3-196
or
2A
mA3rev-2A-BE3-196
mA3-(208-429)
mA3-(1-207)
mA3rev-BE3
mA3rev-2A-BE3
mA3-(216-429)
mA3-(1-215)
mA3rev-BE3-215
mA3rev-2A-BE3-215
mA3-(230-429)
mA3-(1-229)
mA3rev-BE3-229
mA3rev-2A-BE3-229
mA3-(238-429)
mA3-(1-237)
mA3rev-BE3-237
mA3rev-2A-BE3-237
C
SITE4
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
mA3rev-BE3-196
mA3rev-2A-BE3-196
mA3rev-BE3
mA3rev-2A-BE3
mA3rev-BE3-215
mA3rev-2A-BE3-215
mA3rev-BE3-229
mA3rev-2A-BE3-229
mA3rev-BE3-237
mA3rev-2A-BE3-237

FIG. 12A-C

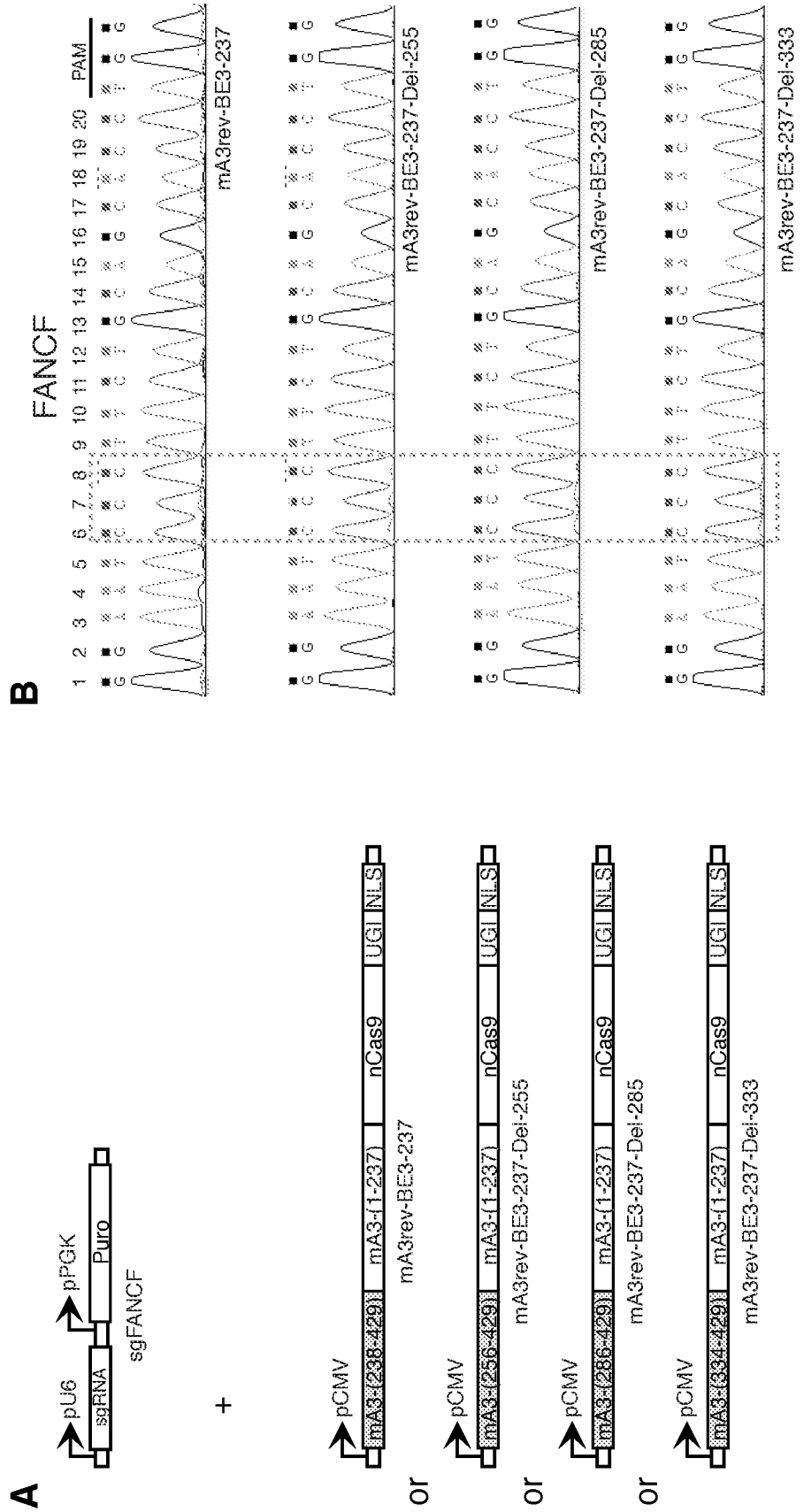

A
pU6
sgRNA
pPGK
Puro
sgFANCF
+
pCMV
mA3-(1-237)
nCas9
UGI
NLS
mA3rev-BE3-237
or
mA3-(238-429)
mA3rev-BE3-237-Del-255
mA3-(256-429)
mA3rev-BE3-237-Del-285
mA3-(286-429)
mA3rev-BE3-237-Del-333
mA3-(334-429)
B
FANCF
PAM
mA3rev-BE3-237
mA3rev-BE3-237-Del-255
mA3rev-BE3-237-Del-285
mA3rev-BE3-237-Del-333

FIG. 13A-B

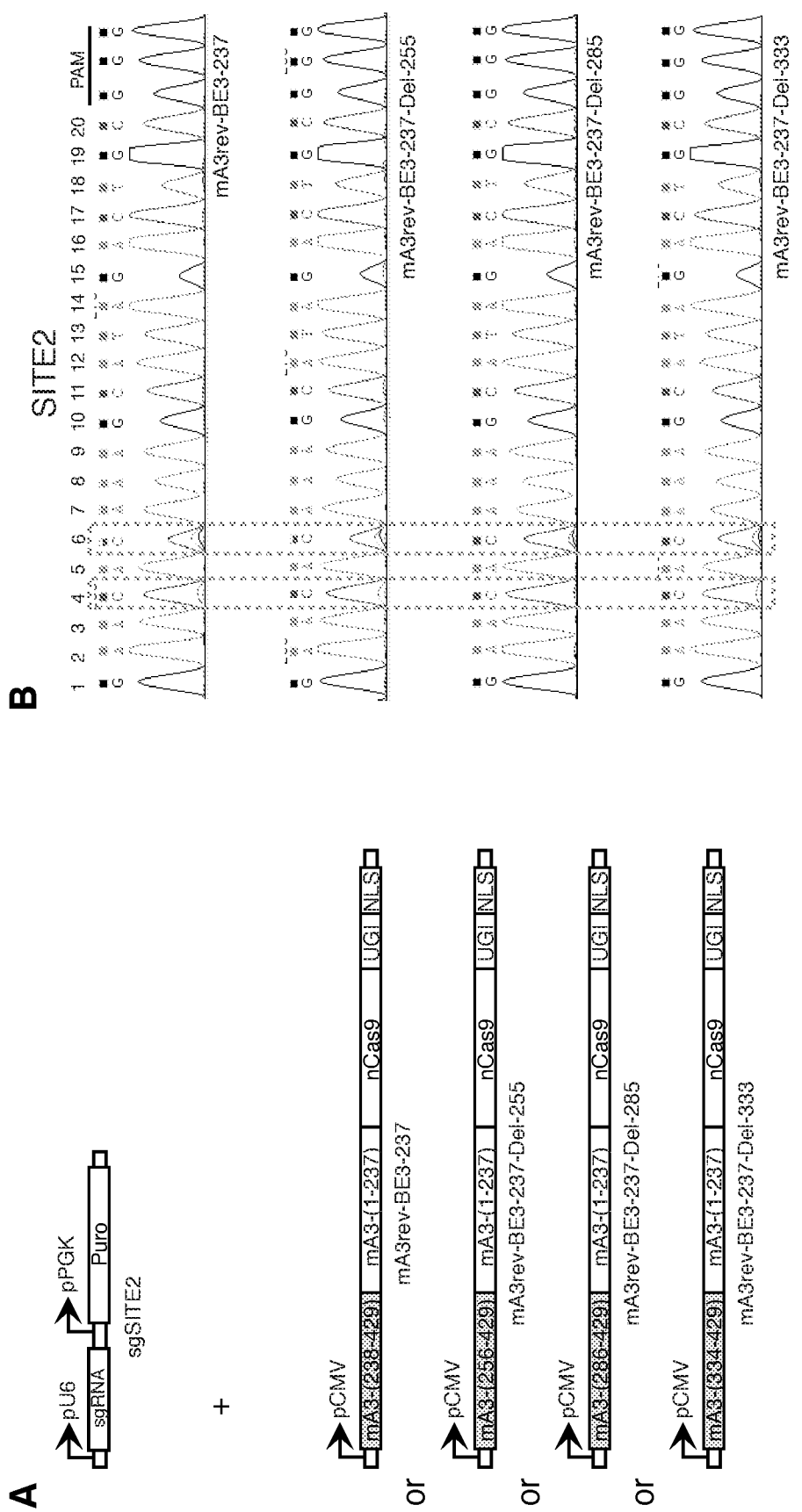

A
pU6
sgRNA
pPGK
Puro
sgSITE2
+
pCMV
mA3-(238-429)
mA3-(1-237)
nCas9
UGI
NLS
mA3rev-BE3-237
or
pCMV
mA3-(256-429)
mA3-(1-237)
nCas9
UGI
NLS
mA3rev-BE3-237-Del-255
or
pCMV
mA3-(286-429)
mA3-(1-237)
nCas9
UGI
NLS
mA3rev-BE3-237-Del-285
or
pCMV
mA3-(334-429)
mA3-(1-237)
nCas9
UGI
NLS
mA3rev-BE3-237-Del-333
B
SITE2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
mA3rev-BE3-237
mA3rev-BE3-237-Del-255
mA3rev-BE3-237-Del-285
mA3rev-BE3-237-Del-333

FIG. 14A-B

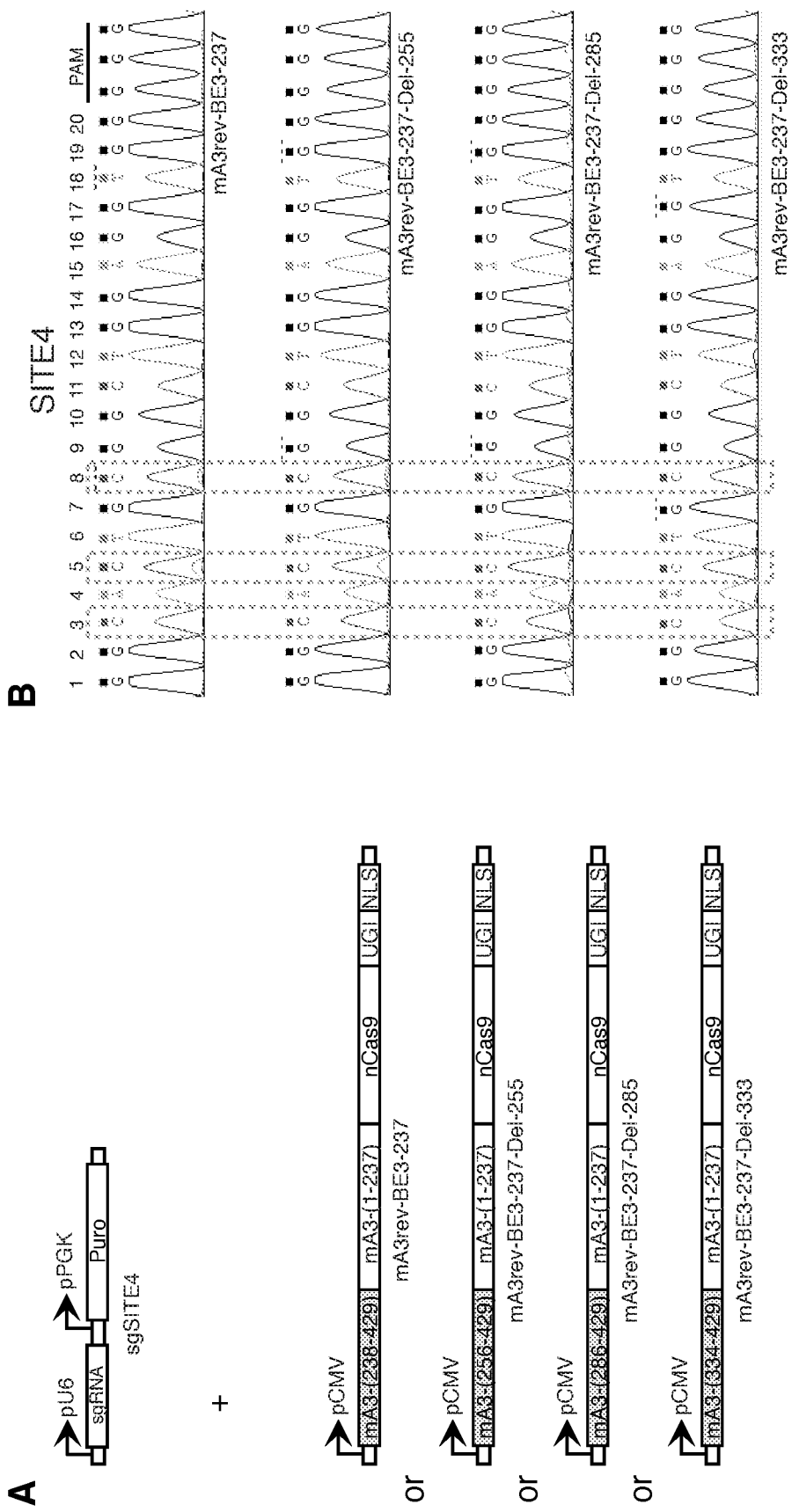

A
pU6
sgRNA
pPGK
Puro
sgSITE4
+
pCMV
mA3-(1-237)
nCas9
UGI
NLS
mA3rev-BE3-237
or
mA3-(238-429)
mA3rev-BE3-237-Del-255
mA3-(256-429)
mA3rev-BE3-237-Del-285
mA3-(286-429)
mA3rev-BE3-237-Del-333
mA3-(334-429)
B
SITE4
PAM
mA3rev-BE3-237
mA3rev-BE3-237-Del-255
mA3rev-BE3-237-Del-285
mA3rev-BE3-237-Del-333

FIG. 15A-B

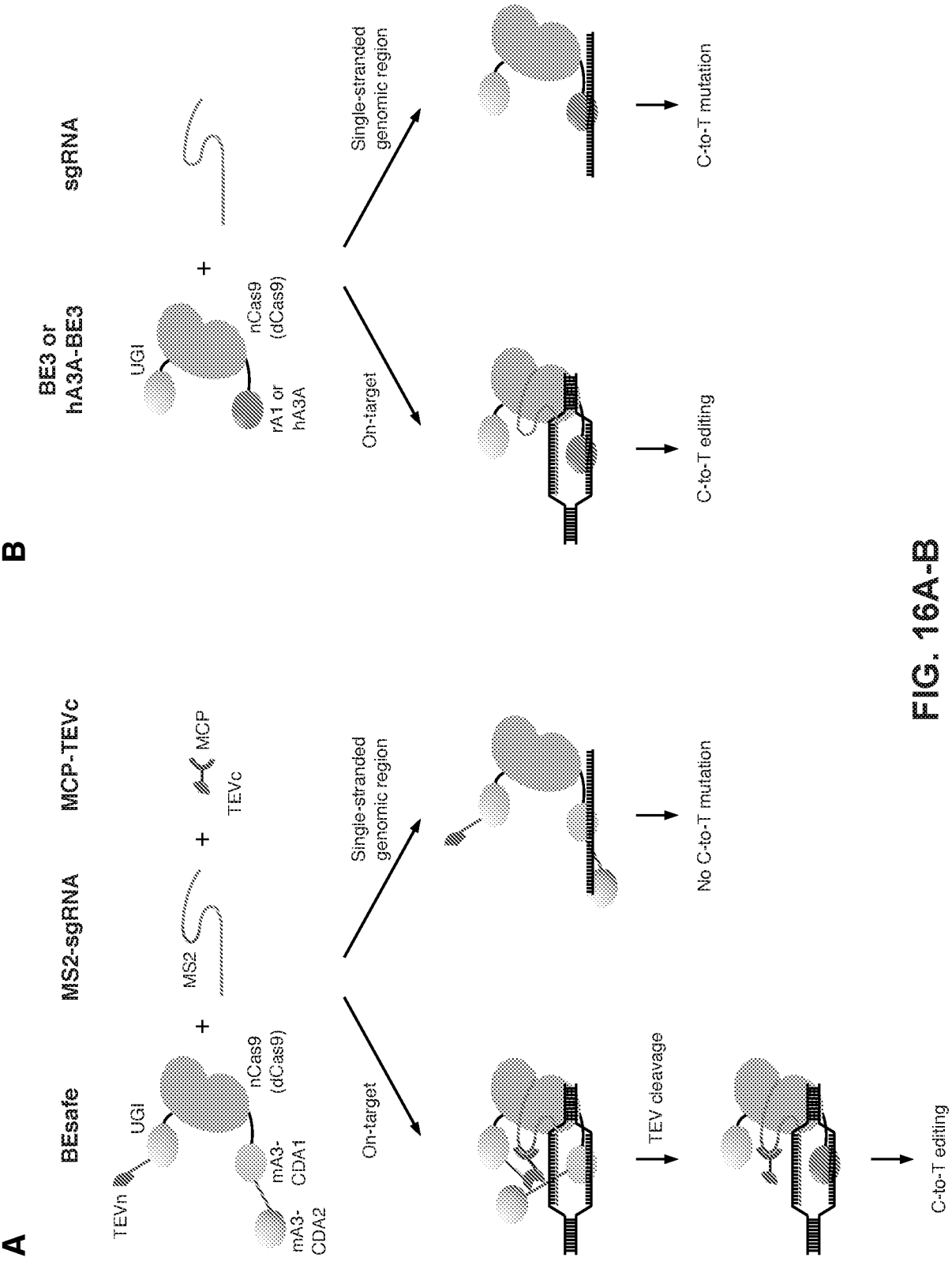

A
BEsafe
MS2-sgRNA
MCP-TEVc
TEVn
UGI
nCas9
(dCas9)
mA3-
CDA1
mA3-
CDA2
MS2
MCP
TEVc
On-target
Single-stranded
genomic region
No C-to-T mutation
TEV cleavage
C-to-T editing
B
BE3 or
hA3A-BE3
sgRNA
UGI
nCas9
(dCas9)
rA1 or
hA3A
On-target
Single-stranded
genomic region
C-to-T editing
C-to-T mutation

FIG. 16A-B

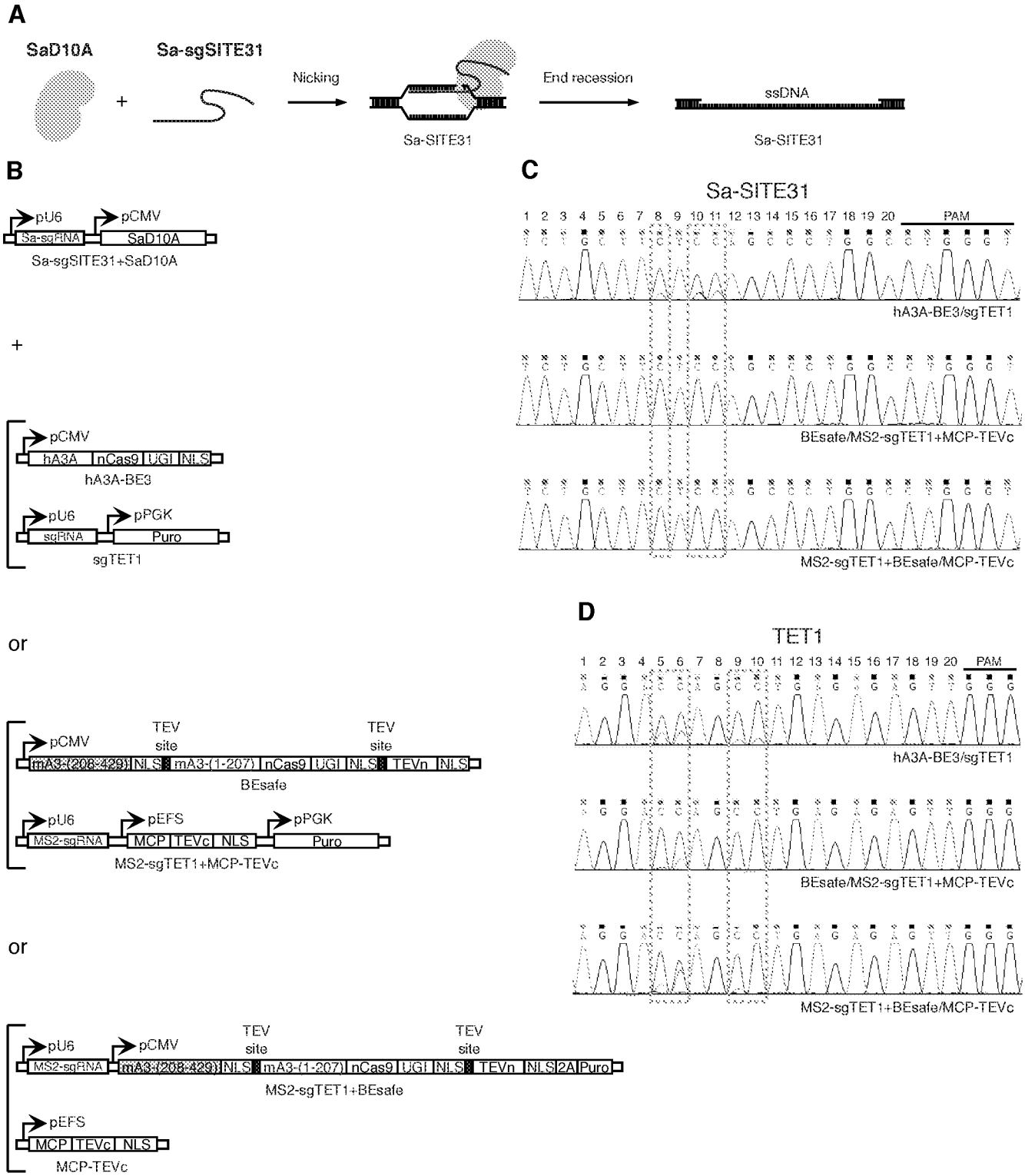

A
SaD10A
Sa-sgSITE31
Nicking
End recession
ssDNA
Sa-SITE31
Sa-SITE31
B
pU6
pCMV
Sa-sgRNA
SaD10A
Sa-sgSITE31+SaD10A
+
pCMV
hA3A
nCas9
UGI
NLS
hA3A-BE3
pU6
pPGK
sgRNA
Puro
sgTET1
or
TEV site
TEV site
pCMV
mA3-(208-429)
NLS
mA3-(1-207)
nCas9
UGI
NLS
TEVn
NLS
BEsafe
pU6
pEFS
pPGK
MS2-sgRNA
MCP
TEVc
NLS
Puro
MS2-sgTET1+MCP-TEVc
or
TEV site
TEV site
pU6
pCMV
MS2-sgRNA
mA3-(208-429)
NLS
mA3-(1-207)
nCas9
UGI
NLS
TEVn
NLS
2A
Puro
MS2-sgTET1+BEsafe
pEFS
MCP
TEVc
NLS
MCP-TEVc
C
Sa-SITE31
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
hA3A-BE3/sgTET1
BEsafe/MS2-sgTET1+MCP-TEVc
MS2-sgTET1+BEsafe/MCP-TEVc
D
TET1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
hA3A-BE3/sgTET1
BEsafe/MS2-sgTET1+MCP-TEVc
MS2-sgTET1+BEsafe/MCP-TEVc

FIG. 17A-D

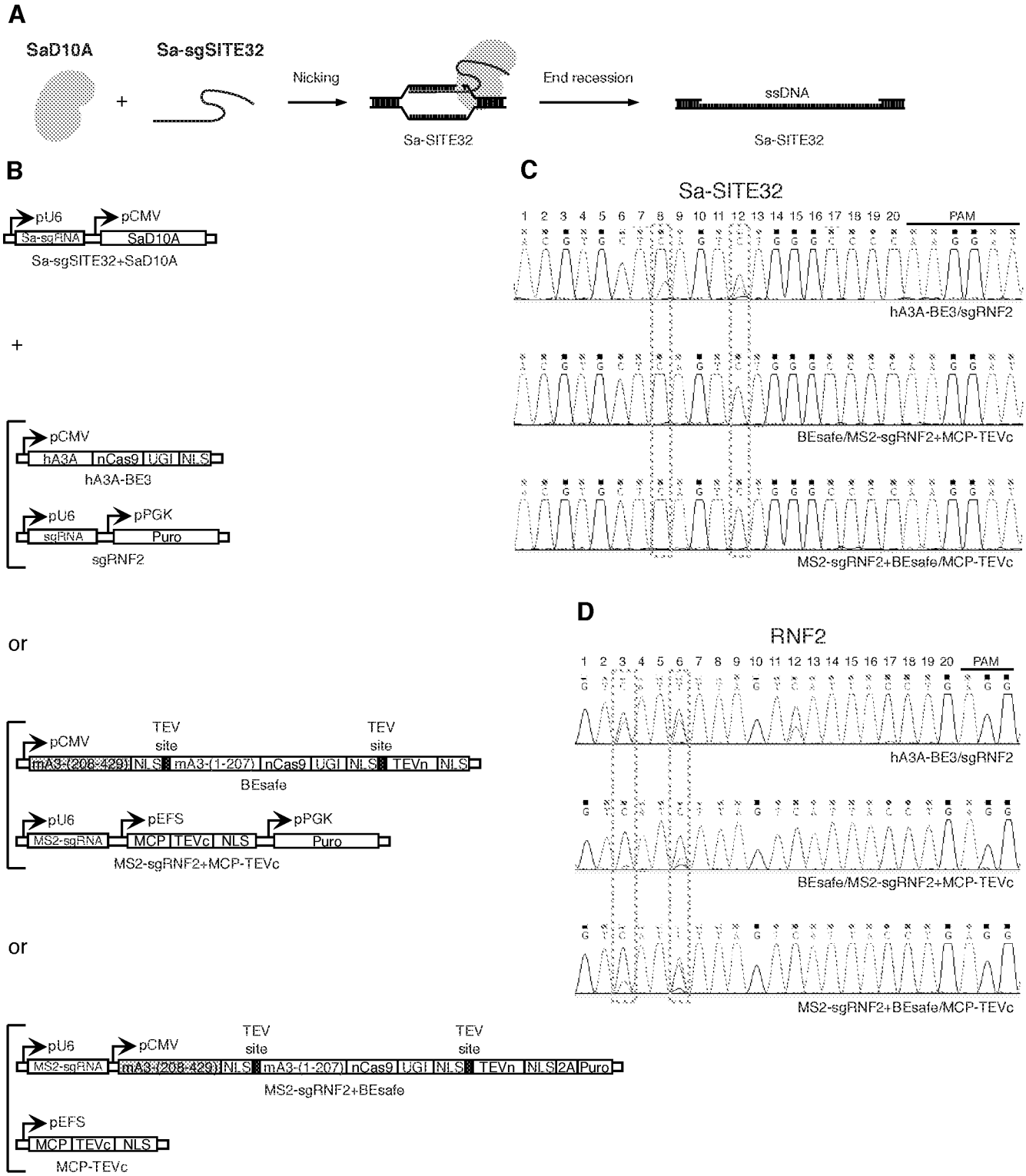

A
SaD10A
Sa-sgSITE32
Nicking
End recession
ssDNA
Sa-SITE32
Sa-SITE32
B
pU6
pCMV
Sa-sgRNA
SaD10A
Sa-sgSITE32+SaD10A
+
pCMV
hA3A
nCas9
UGI
NLS
hA3A-BE3
pU6
pPGK
sgRNA
Puro
sgRNF2
or
TEV site
TEV site
pCMV
mA3-(208-429)
NLS
mA3-(1-207)
nCas9
UGI
NLS
TEVn
NLS
BEsafe
pU6
pEFS
pPGK
MS2-sgRNA
MCP
TEVc
NLS
Puro
MS2-sgRNF2+MCP-TEVc
or
TEV site
TEV site
pU6
pCMV
MS2-sgRNA
mA3-(208-429)
NLS
mA3-(1-207)
nCas9
UGI
NLS
TEVn
NLS
2A
Puro
MS2-sgRNF2+BEsafe
pEFS
MCP
TEVc
NLS
MCP-TEVc
C
Sa-SITE32
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
hA3A-BE3/sgRNF2
BEsafe/MS2-sgRNF2+MCP-TEVc
MS2-sgRNF2+BEsafe/MCP-TEVc
D
RNF2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
hA3A-BE3/sgRNF2
BEsafe/MS2-sgRNF2+MCP-TEVc
MS2-sgRNF2+BEsafe/MCP-TEVc

FIG. 18A-D

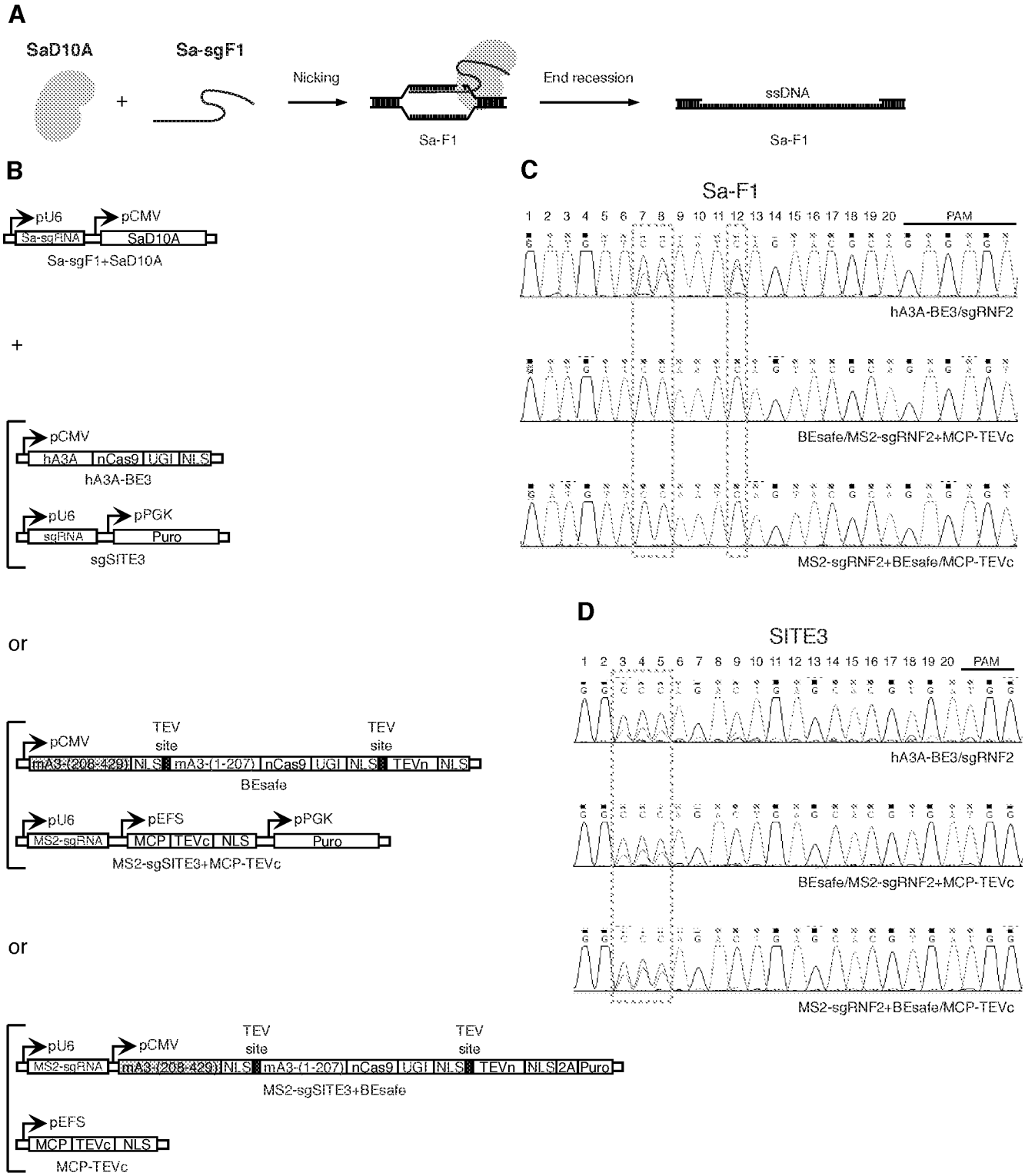

A
SaD10A
Sa-sgF1
Nicking
End recession
ssDNA
Sa-F1
Sa-F1
B
pU6
pCMV
Sa-sgRNA
SaD10A
Sa-sgF1+SaD10A
+
pCMV
hA3A
nCas9
UGI
NLS
hA3A-BE3
pU6
pPGK
sgRNA
Puro
sgSITE3
or
TEV site
TEV site
pCMV
mA3-(208-429)
NLS
mA3-(1-207)
nCas9
UGI
NLS
TEVn
NLS
BEsafe
pU6
pEFS
pPGK
MS2-sgRNA
MCP
TEVc
NLS
Puro
MS2-sgSITE3+MCP-TEVc
or
TEV site
TEV site
pU6
pCMV
MS2-sgRNA
mA3-(208-429)
NLS
mA3-(1-207)
nCas9
UGI
NLS
TEVn
NLS
2A
Puro
MS2-sgSITE3+BEsafe
pEFS
MCP
TEVc
NLS
MCP-TEVc
C
Sa-F1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
hA3A-BE3/sgRNF2
BEsafe/MS2-sgRNF2+MCP-TEVc
MS2-sgRNF2+BEsafe/MCP-TEVc
D
SITE3
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
PAM
hA3A-BE3/sgRNF2
BEsafe/MS2-sgRNF2+MCP-TEVc
MS2-sgRNF2+BEsafe/MCP-TEVc

FIG. 19A-D

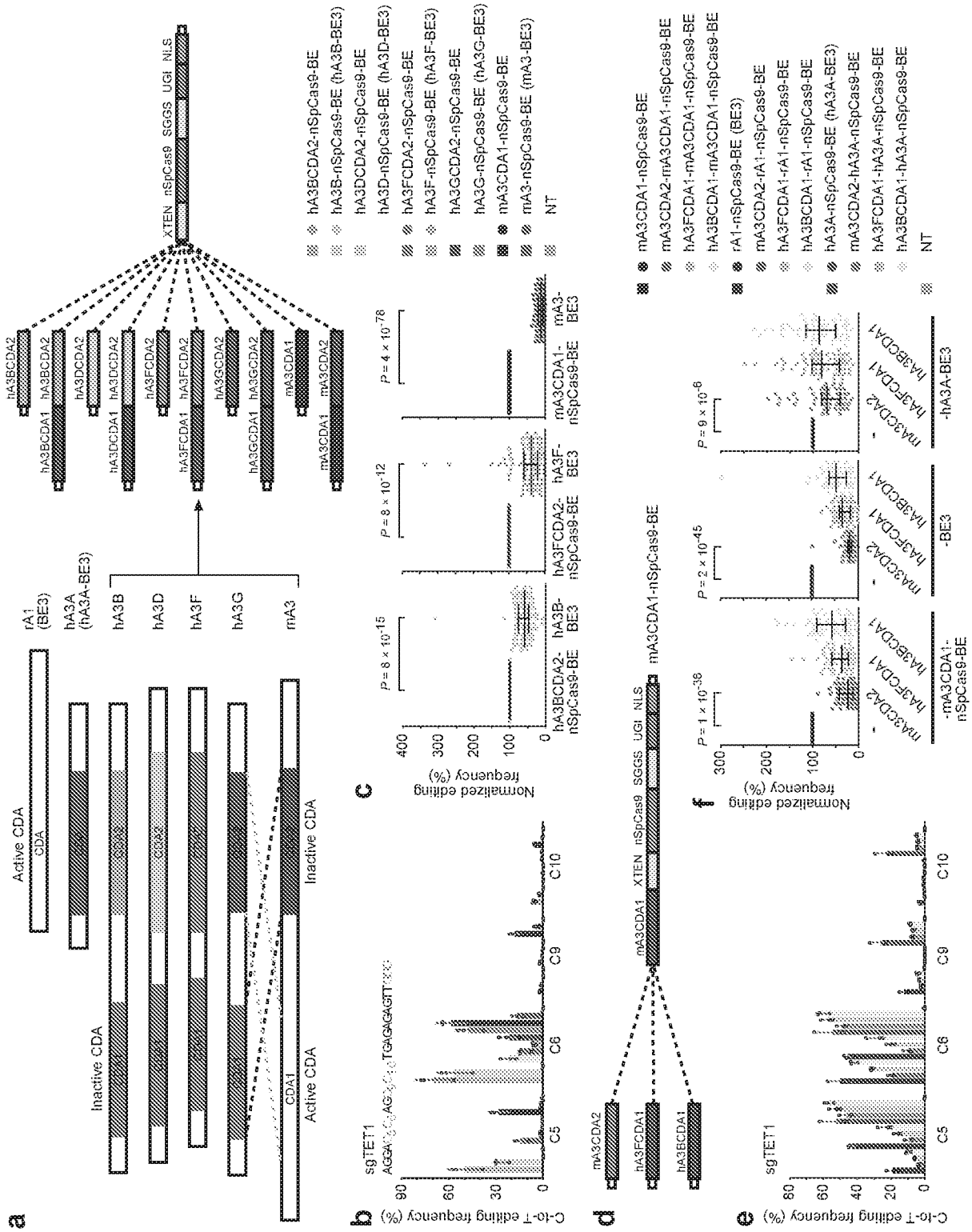

a
Active CDA
CDA
rA1 (BE3)
hA3A (hA3A-BE3)
hA3B
hA3D
hA3F
hA3G
mA3
Inactive CDA
Active CDA
Inactive CDA
CDA1
XTEN nSpCas9 SGGS UGI NLS
hA3BCDA2
hA3BCDA1
hA3DCDA2
hA3DCDA1
hA3FCDA2
hA3FCDA1
hA3GCDA2
hA3GCDA1
mA3CDA1
mA3CDA2
hA3BCDA2-nSpCas9-BE
hA3B-nSpCas9-BE (hA3B-BE3)
hA3DCDA2-nSpCas9-BE
hA3D-nSpCas9-BE (hA3D-BE3)
hA3FCDA2-nSpCas9-BE
hA3F-nSpCas9-BE (hA3F-BE3)
hA3GCDA2-nSpCas9-BE
hA3G-nSpCas9-BE (hA3G-BE3)
mA3CDA1-nSpCas9-BE
mA3-nSpCas9-BE (mA3-BE3)
NT
b
sgTET1
C-to-T editing frequency (%)
C5
C6
C9
C10
c
Normalized editing frequency (%)
P = 8 × 10-15
P = 8 × 10-12
P = 4 × 10-78
hA3BCDA2-nSpCas9-BE
hA3B-BE3
hA3FCDA2-nSpCas9-BE
hA3F-BE3
mA3CDA1-nSpCas9-BE
mA3-BE3
d
mA3CDA2
hA3FCDA1
hA3BCDA1
mA3CDA1
XTEN nSpCas9 SGGS UGI NLS
mA3CDA1-nSpCas9-BE
mA3CDA2-mA3CDA1-nSpCas9-BE
hA3FCDA1-mA3CDA1-nSpCas9-BE
hA3BCDA1-mA3CDA1-nSpCas9-BE
rA1-nSpCas9-BE (BE3)
mA3CDA2-rA1-nSpCas9-BE
hA3FCDA1-rA1-nSpCas9-BE
hA3BCDA1-rA1-nSpCas9-BE
hA3A-nSpCas9-BE (hA3A-BE3)
mA3CDA2-hA3A-nSpCas9-BE
hA3FCDA1-hA3A-nSpCas9-BE
hA3BCDA1-hA3A-nSpCas9-BE
NT
e
sgTET1
C-to-T editing frequency (%)
C5
C6
C9
C10
f
Normalized editing frequency (%)
P = 1 × 10-38
P = 2 × 10-45
P = 9 × 10-6
mA3CDA2
hA3FCDA1
hA3BCDA1
-mA3CDA1-nSpCas9-BE
-BE3
-hA3A-BE3

FIG. 20a-f

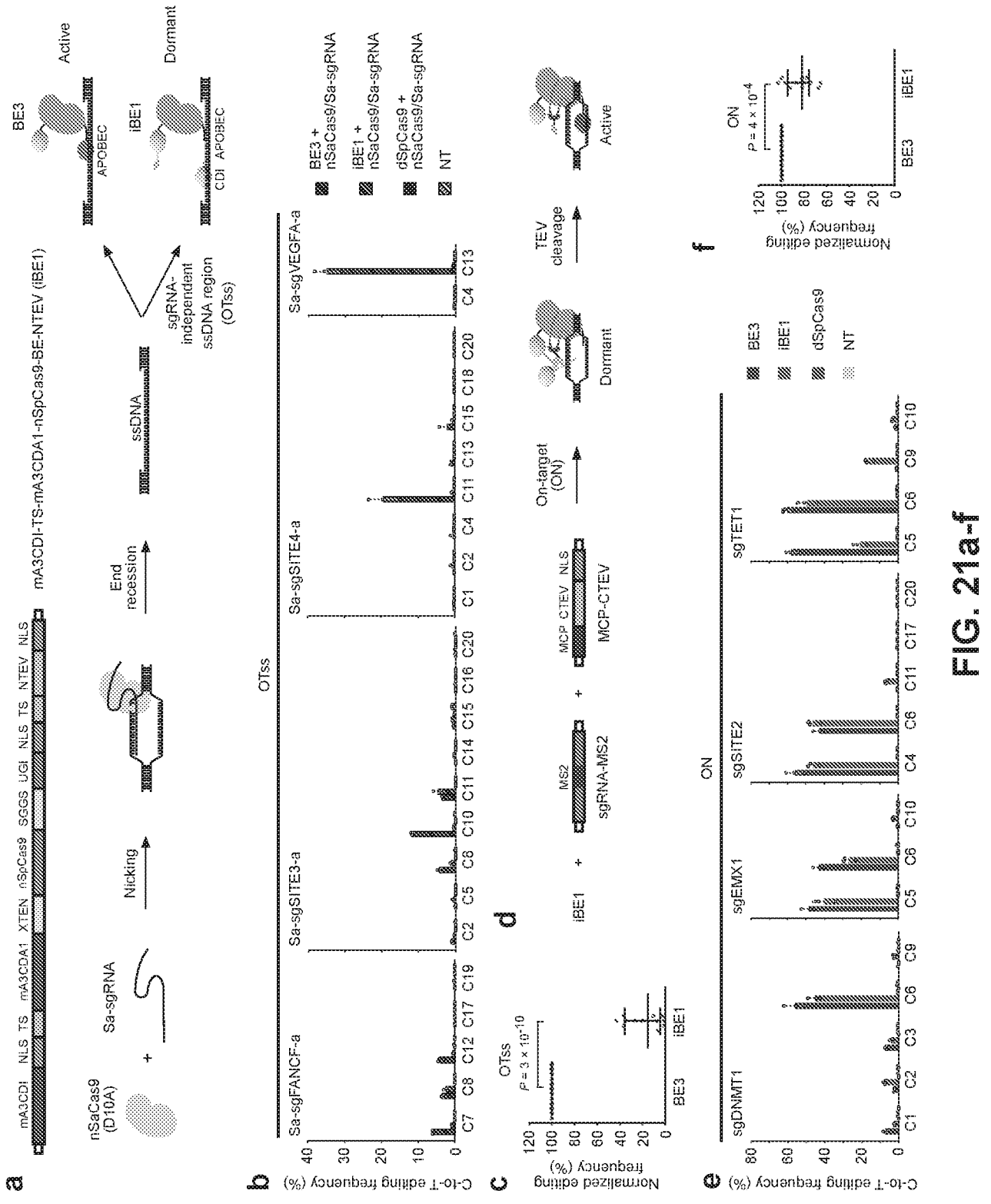

a
mA3CDI NLS TS mA3CDA1 XTEN nSpCas9 SGGS UGI NLS TS NTEV NLS
mA3CDI-TS-mA3CDA1-nSpCas9-BE-NTEV (iBE1)
nSaCas9 (D10A)
Sa-sgRNA
Nicking
End recession
ssDNA
sgRNA-independent ssDNA region (OTss)
BE3
APOBEC
Active
iBE1
CDI APOBEC
Dormant
b
Sa-sgFANCF-a
Sa-sgSITE3-a
Sa-sgSITE4-a
Sa-sgVEGFA-a
OTss
C-to-T editing frequency (%)
BE3 + nSaCas9/Sa-sgRNA
iBE1 + nSaCas9/Sa-sgRNA
dSpCas9 + nSaCas9/Sa-sgRNA
NT
c
OTss
P = 3 × 10−10
Normalized editing frequency (%)
BE3
iBE1
d
iBE1 + MS2
sgRNA-MS2
MCP CTEV NLS
MCP-CTEV
On-target (ON)
Dormant
TEV cleavage
Active
e
ON
sgDNMT1
sgEMX1
sgSITE2
sgTET1
C-to-T editing frequency (%)
BE3
iBE1
dSpCas9
NT
f
ON
P = 4 × 10−4
Normalized editing frequency (%)
BE3
iBE1

FIG. 21a-f

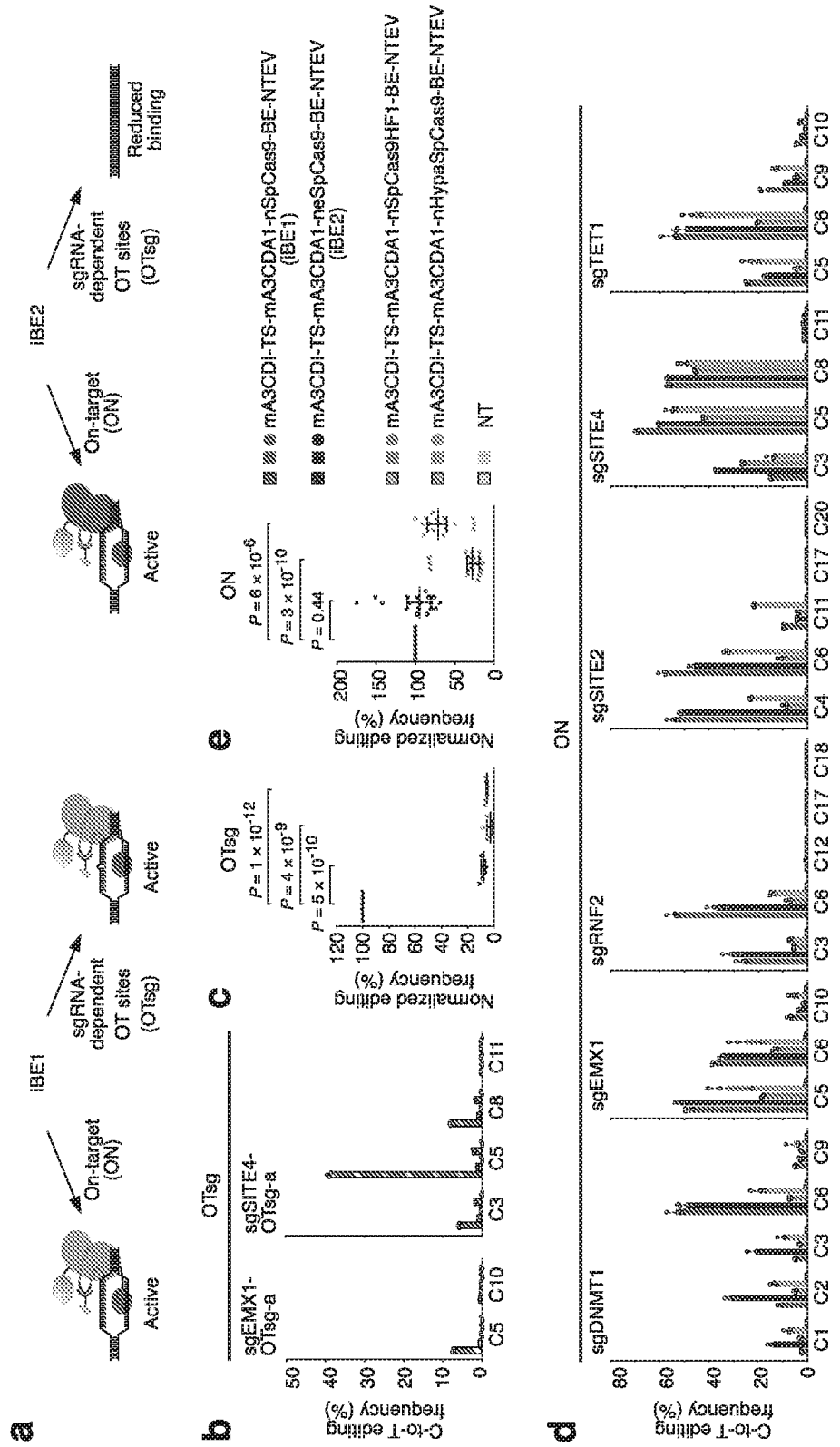

a
iBE1
On-target (ON)
Active
sgRNA-dependent OT sites (OTsg)
Active
iBE2
On-target (ON)
Active
sgRNA-dependent OT sites (OTsg)
Reduced binding
b
OTsg
sgEMX1-OTsg-a
sgSITE4-OTsg-a
C-to-T editing frequency (%)
C5 C10 C3 C6 C9 C5 C8 C11
c
OTsg
P = 1 × 10^-12
P = 4 × 10^-9
P = 5 × 10^-10
Normalized editing frequency (%)
e
ON
P = 6 × 10^-6
P = 3 × 10^-10
P = 0.44
Normalized editing frequency (%)
mA3CDI-TS-mA3CDA1-nSpCas9-BE-NTEV (iBE1)
mA3CDI-TS-mA3CDA1-neSpCas9-BE-NTEV (iBE2)
mA3CDI-TS-mA3CDA1-nSpCas9HF1-BE-NTEV
mA3CDI-TS-mA3CDA1-nHypaSpCas9-BE-NTEV
NT
d
ON
sgDNMT1
sgEMX1
sgRNF2
sgSITE2
sgSITE4
sgTET1
C-to-T editing frequency (%)
C1 C2 C3 C6 C9 C5 C6 C10 C3 C6 C12 C17 C18 C4 C6 C11 C17 C20 C3 C5 C8 C11 C5 C6 C9 C10

FIG. 22a-e

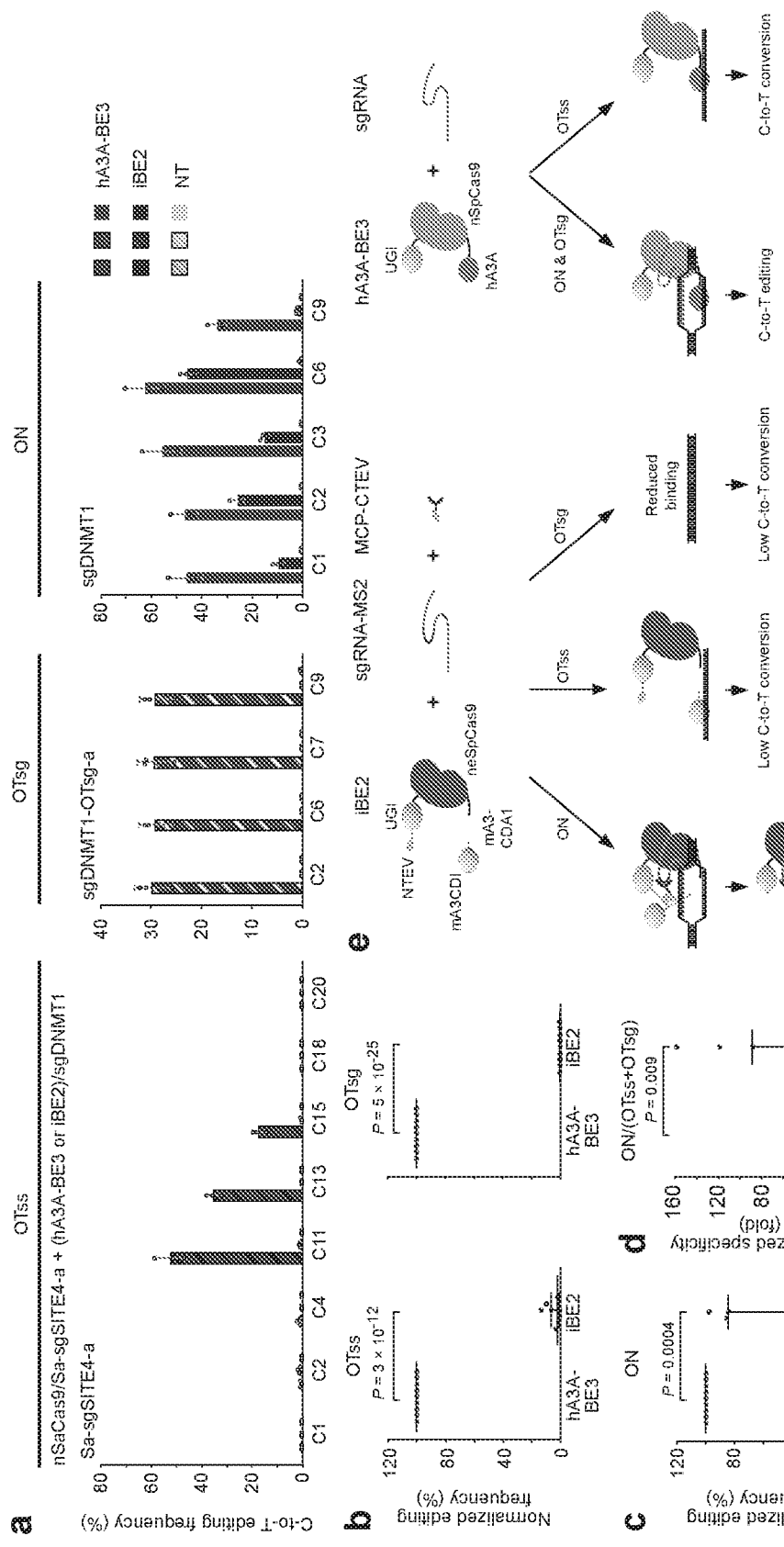

a
OTss
OTsg
ON
nSaCas9/Sa-sgSITE4-a + (hA3A-BE3 or iBE2)/sgDNMT1
Sa-sgSITE4-a
sgDNMT1-OTsg-a
sgDNMT1
C-to-T editing frequency (%)
hA3A-BE3
iBE2
NT
b
OTss
OTsg
P = 3 × 10-12
P = 5 × 10-25
Normalized editing frequency (%)
hA3A-BE3
iBE2
c
ON
P = 0.0004
Normalized editing frequency (%)
d
ON/(OTss+OTsg)
P = 0.009
Normalized specificity (fold)
e
iBE2
UGI
NTEV
mA3CDI
mA3-CDA1
neSpCas9
sgRNA-MS2
MCP-CTEV
ON
OTss
OTsg
Reduced binding
Low C-to-T conversion
C-to-T editing
hA3A-BE3
UGI
nSpCas9
hA3A
sgRNA
ON & OTsg
OTss
C-to-T editing
C-to-T conversion

FIG. 23a-e

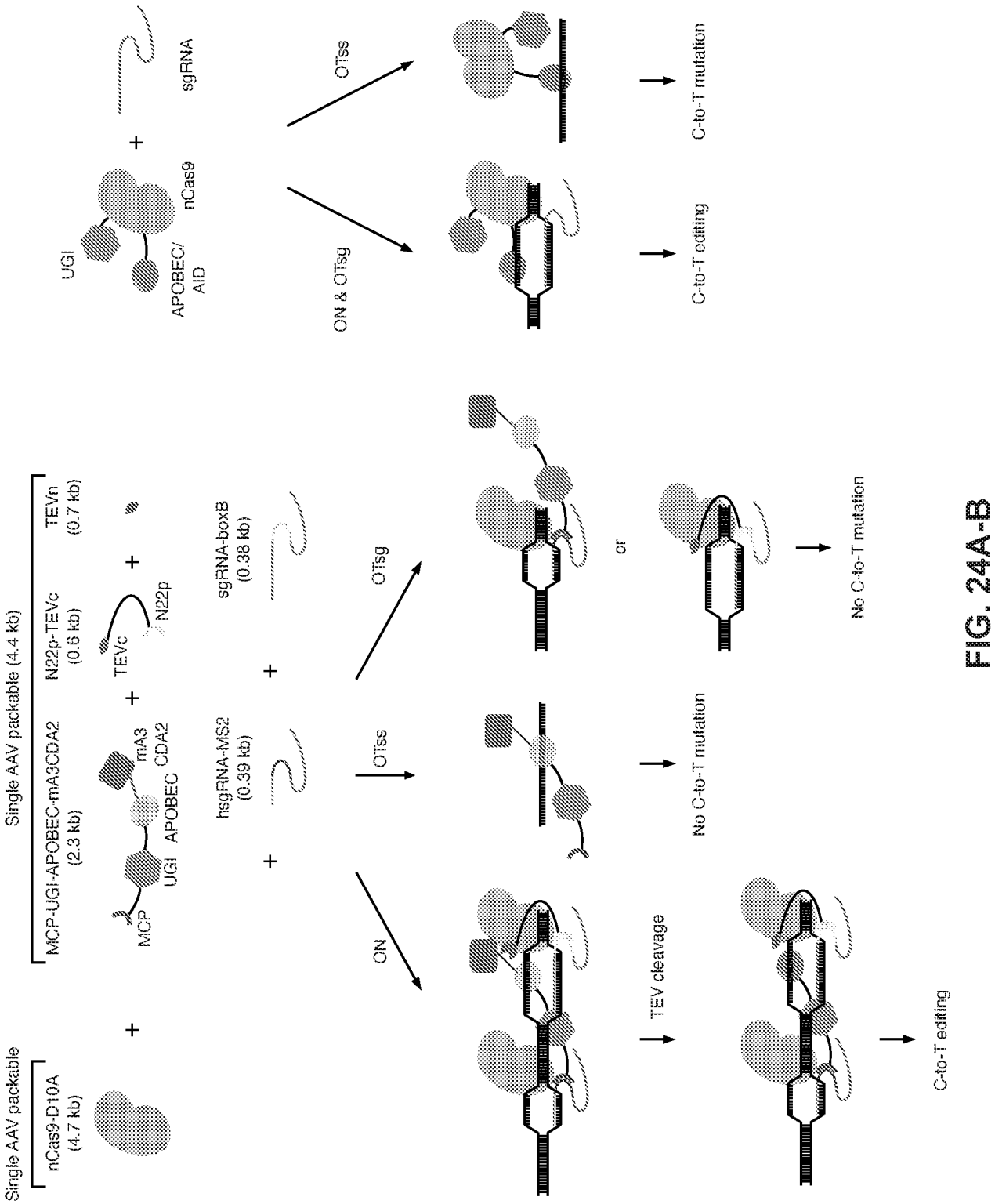

A
iBE system
Single AAV packable
nCas9-D10A
(4.7 kb)
Single AAV packable (4.4 kb)
MCP-UGI-APOBEC-mA3CDA2
(2.3 kb)
N22p-TEVc
(0.6 kb)
TEVn
(0.7 kb)
MCP
UGI
APOBEC
mA3
CDA2
TEVc
N22p
hsgRNA-MS2
(0.39 kb)
sgRNA-boxB
(0.38 kb)
ON
OTss
OTsg
TEV cleavage
No C-to-T mutation
C-to-T editing
or
No C-to-T mutation
B
Regular BEs
UGI
APOBEC/
AID
nCas9
sgRNA
ON & OTsg
OTss
C-to-T editing
C-to-T mutation

FIG. 24A-B

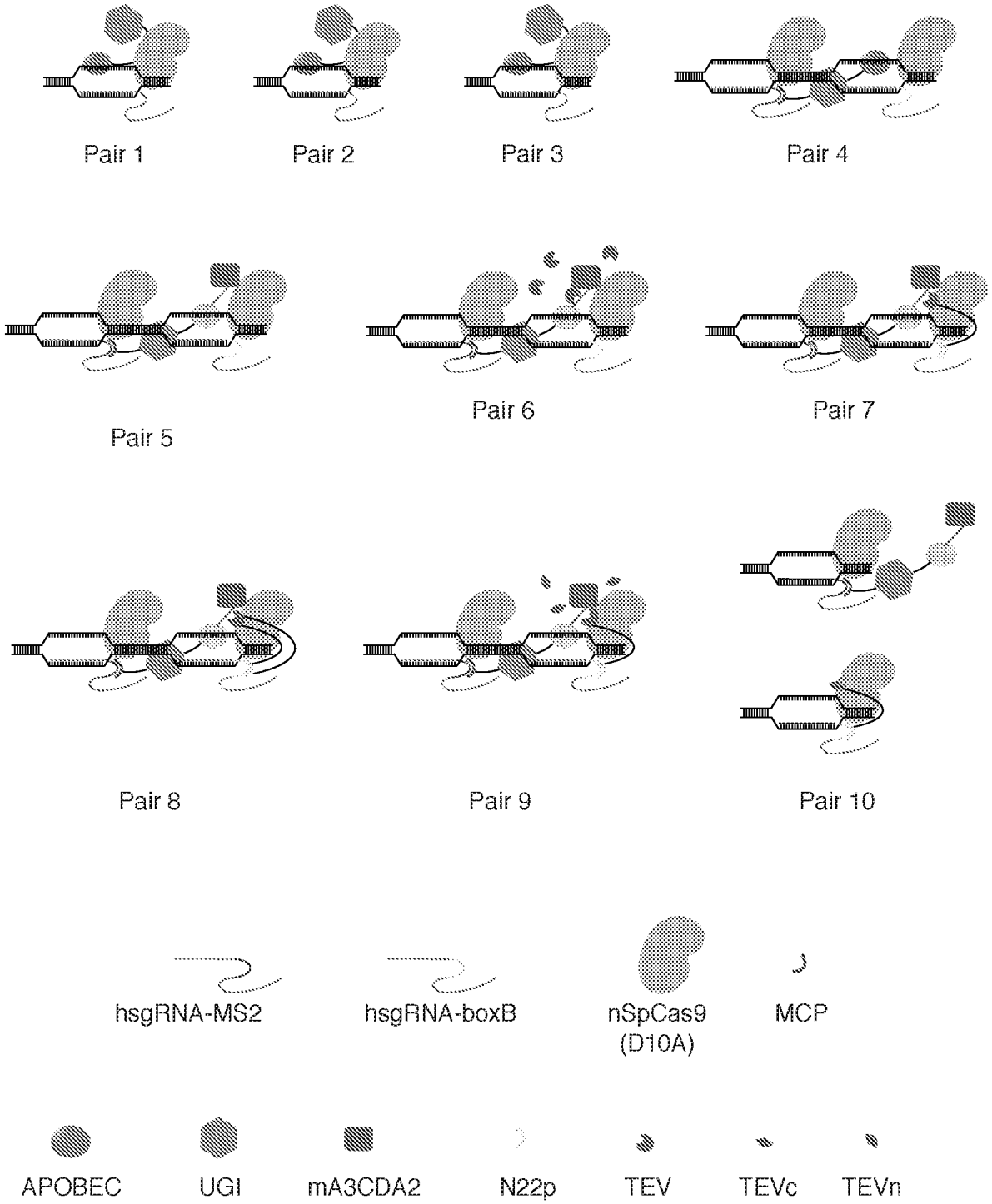

Pair 1
Pair 2
Pair 3
Pair 4
Pair 5
Pair 6
Pair 7
Pair 8
Pair 9
Pair 10
hsgRNA-MS2
hsgRNA-boxB
nSpCas9 (D10A)
MCP
APOBEC
UGI
mA3CDA2
N22p
TEV
TEVc
TEVn

FIG. 25


A
To generate OTss
pU6
pCMV
Sa-sgRNA
SaD10A
Sa-sgSITE31 + nSaCas9
+
nSaCas9
(D10A)
Sa-sgSITE31
Nicking
End recession
ssDNA
Sa-SITE31
Sa-SITE31
To induce on-target editing
Pair 1
pCMV
rA1
SpD10A
UGI
NLS
BE3
pU6
sgRNA
sgEMX1
or
Pair 2
pCMV
hA3A
SpD10A
UGI
NLS
hA3A-BE3
pU6
sgRNA
sgEMX1
or
Pair 3
pCMV
hA3A(Y130F)
SpD10A
UGI
NLS
hA3A-BE3-Y130F
pU6
sgRNA
sgEMX1
or
Pair 4
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgEMX1 + sgEMX1 + nSpCas9
pEFS
MCP
NLS
UGI
rA1
NLS
MCP-UGI-rA1
or
Pair 5
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgEMX1 + sgEMX1 + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
MCP-UGI-rA1-TS-mA3CDA2
or
Pair 6
TEV
site
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgEMX1 + sgEMX1 + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
2A
TEV
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-TEV
or
Pair 7
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgEMX1 + sgEMX1 + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
2A
N22p
TEV
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEV
or
Pair 8
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgEMX1 + sgEMX1 + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
N22p
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-N22p-TEVn
or
Pair 9
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgEMX1 + sgEMX1 + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-TEVn
or
Pair 10
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgGFP + sgRFP + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-TEVn

FIG. 26A

B

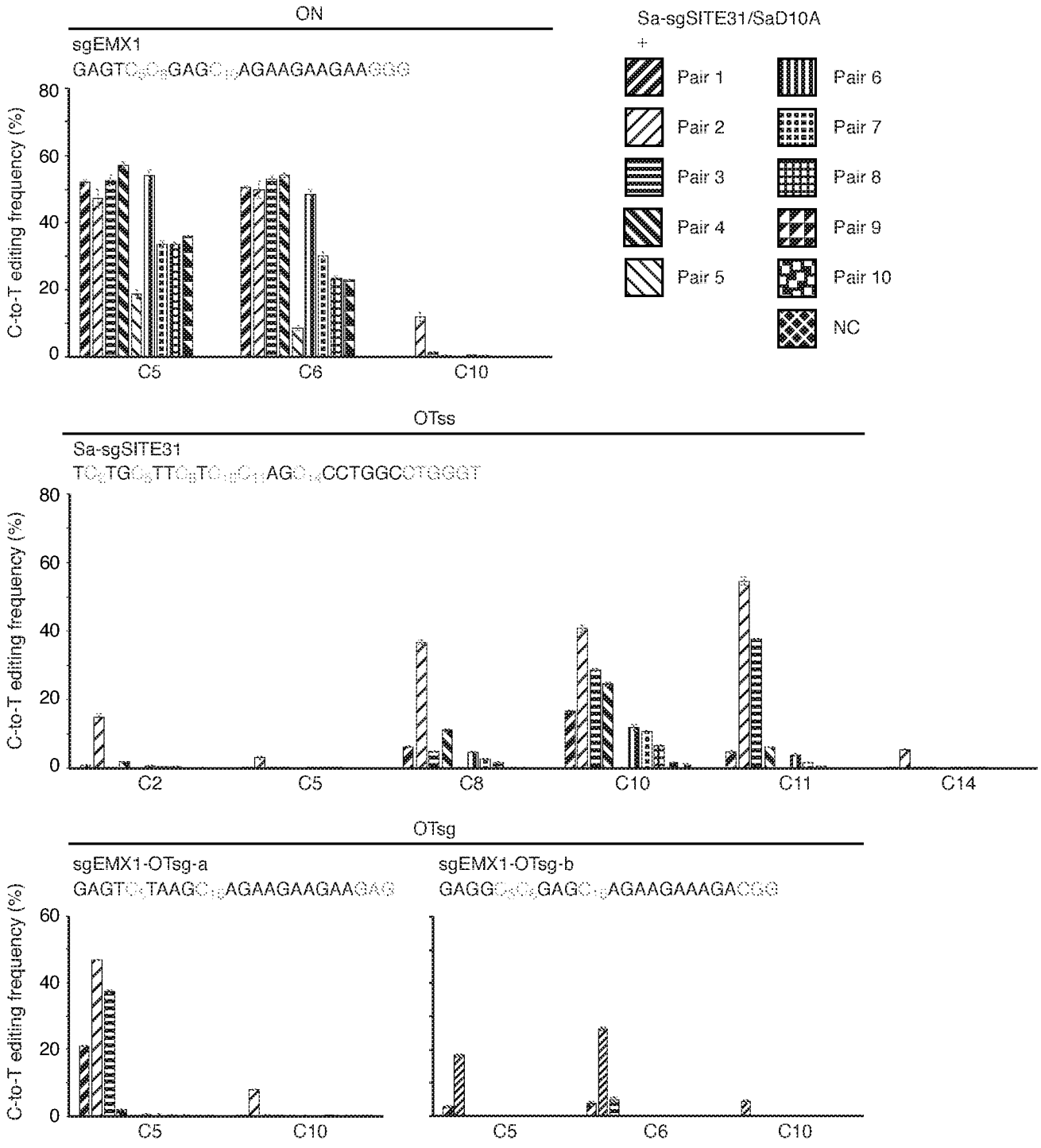

ON
sgEMX1
GAGTC5C6GAGC11AGAAGAAGAAGGG
C-to-T editing frequency (%)
80
60
40
20
0
C5
C6
C10
Sa-sgSITE31/SaD10A
+
Pair 1
Pair 2
Pair 3
Pair 4
Pair 5
Pair 6
Pair 7
Pair 8
Pair 9
Pair 10
NC
OTss
Sa-sgSITE31
TC2TGC5TTC8TC10C11AGC13CCTGGCCTGGGT
C-to-T editing frequency (%)
80
60
40
20
0
C2
C5
C8
C10
C11
C14
OTsg
sgEMX1-OTsg-a
GAGTC4TAAGC11AGAAGAAGAAGAG
C-to-T editing frequency (%)
60
40
20
0
C5
C10
sgEMX1-OTsg-b
GAGGC5C6GAGC11AGAAGAAAGACGG
C5
C6
C10

FIG. 26B


A
To generate OTss
pU6
pCMV
Sa-sgRNA
SaD10A
Sa-sgVEGFA-7 + nSaCas9
+
nSaCas9
(D10A)
Sa-sgVEGFA-7
Nicking
Sa-VEGFA-7
End recession
ssDNA
Sa-VEGFA-7
To induce on-target editing
Pair 1
pCMV
rA1
SpD10A
UGI
NLS
BE3
pU6
sgRNA
sgFANCF
or
Pair 2
pCMV
hA3A
SpD10A
UGI
NLS
hA3A-BE3
pU6
sgRNA
sgFANCF
or
Pair 3
pCMV
hA3A(Y130F)
SpD10A
UGI
NLS
hA3A-BE3-Y130F
pU6
sgRNA
sgFANCF
or
Pair 4
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgFANCF + sgFANCF + nSpCas9
pEFS
MCP
NLS
UGI
rA1
NLS
MCP-UGI-rA1
or
Pair 5
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgFANCF + sgFANCF + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
MCP-UGI-rA1-TS-mA3CDA2
or
Pair 6
TEV
site
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgFANCF + sgFANCF + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
2A
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-TEV
or
Pair 7
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgFANCF + sgFANCF + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
2A
N22p
TEV
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEV
or
Pair 8
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgFANCF + sgFANCF + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
N22p
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-N22p-TEVn
or
Pair 9
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgFANCF + sgFANCF + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-TEVn
or
Pair 10
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgGFP + sgRFP + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-TEVn

FIG. 27A

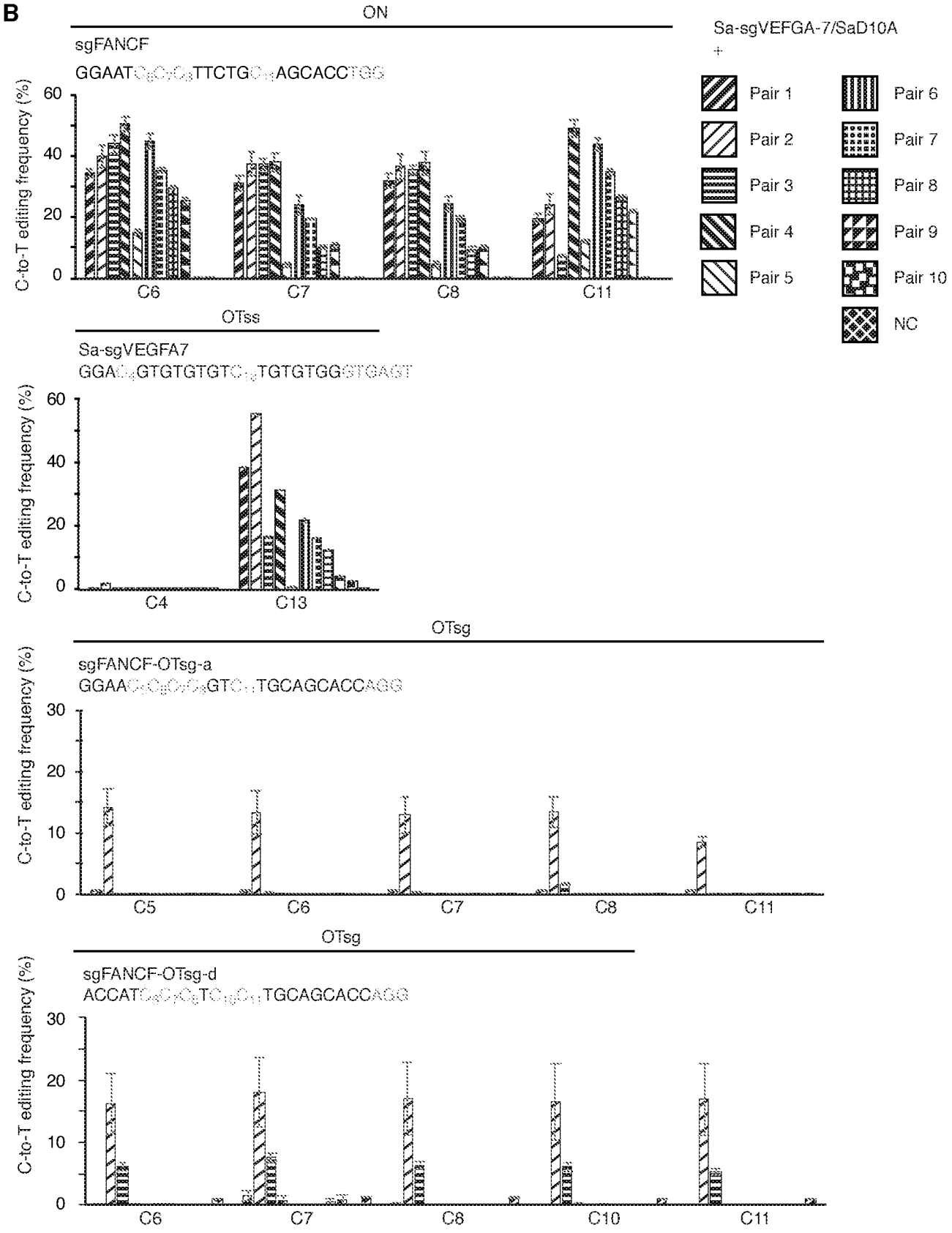

B
ON
sgFANCF
GGAATC6C7C8TTCTGC11AGCACCTGG
C-to-T editing frequency (%)
60
40
20
0
C6
C7
C8
C11
Sa-sgVEFGA-7/SaD10A
+
Pair 1
Pair 2
Pair 3
Pair 4
Pair 5
Pair 6
Pair 7
Pair 8
Pair 9
Pair 10
NC
OTss
Sa-sgVEGFA7
GGAC4GTGTGTGTC13TGTGTGGGTGAGT
C4
C13
OTsg
sgFANCF-OTsg-a
GGAAC4C5C7C8GTC11TGCAGCACCAGG
30
20
10
0
C5
C6
C7
C8
C11
OTsg
sgFANCF-OTsg-d
ACCATC6C7C8TC10C11TGCAGCACCAGG
C10

FIG. 27B

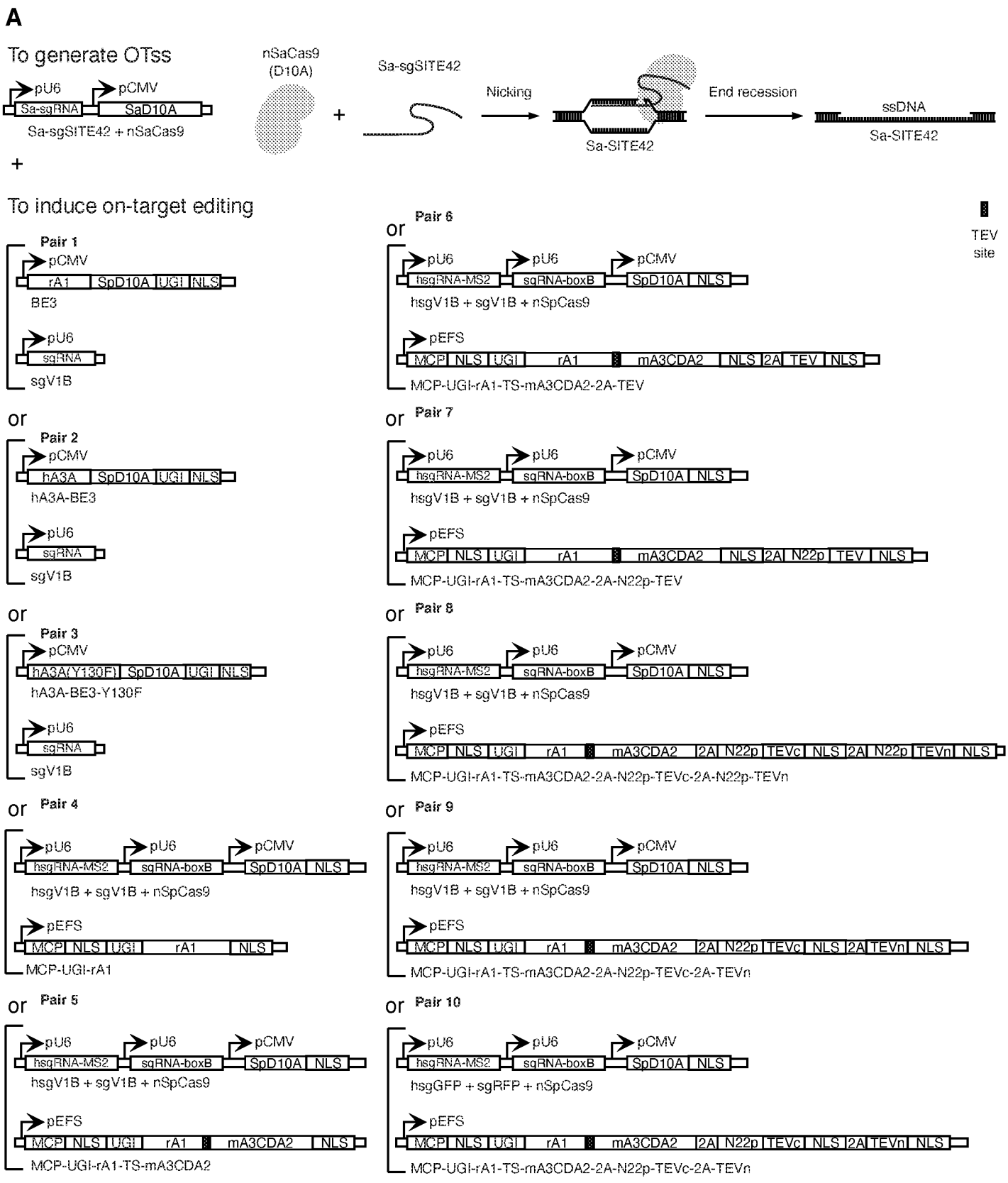

A
To generate OTss
pU6
pCMV
Sa-sgRNA
SaD10A
Sa-sgSITE42 + nSaCas9
+
nSaCas9
(D10A)
Sa-sgSITE42
Nicking
End recession
ssDNA
Sa-SITE42
Sa-SITE42
To induce on-target editing
TEV
site
Pair 1
pCMV
rA1
SpD10A
UGI
NLS
BE3
pU6
sgRNA
sgV1B
or
Pair 2
pCMV
hA3A
SpD10A
UGI
NLS
hA3A-BE3
pU6
sgRNA
sgV1B
or
Pair 3
pCMV
hA3A(Y130F)
SpD10A
UGI
NLS
hA3A-BE3-Y130F
pU6
sgRNA
sgV1B
or
Pair 4
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgV1B + sgV1B + nSpCas9
pEFS
MCP
NLS
UGI
rA1
NLS
MCP-UGI-rA1
or
Pair 5
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgV1B + sgV1B + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
MCP-UGI-rA1-TS-mA3CDA2
or
Pair 6
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgV1B + sgV1B + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
2A
TEV
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-TEV
or
Pair 7
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgV1B + sgV1B + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
NLS
2A
N22p
TEV
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEV
or
Pair 8
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgV1B + sgV1B + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
N22p
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-N22p-TEVn
or
Pair 9
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgV1B + sgV1B + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-TEVn
or
Pair 10
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
SpD10A
NLS
hsgGFP + sgRFP + nSpCas9
pEFS
MCP
NLS
UGI
rA1
mA3CDA2
2A
N22p
TEVc
NLS
2A
TEVn
NLS
MCP-UGI-rA1-TS-mA3CDA2-2A-N22p-TEVc-2A-TEVn

FIG. 28A

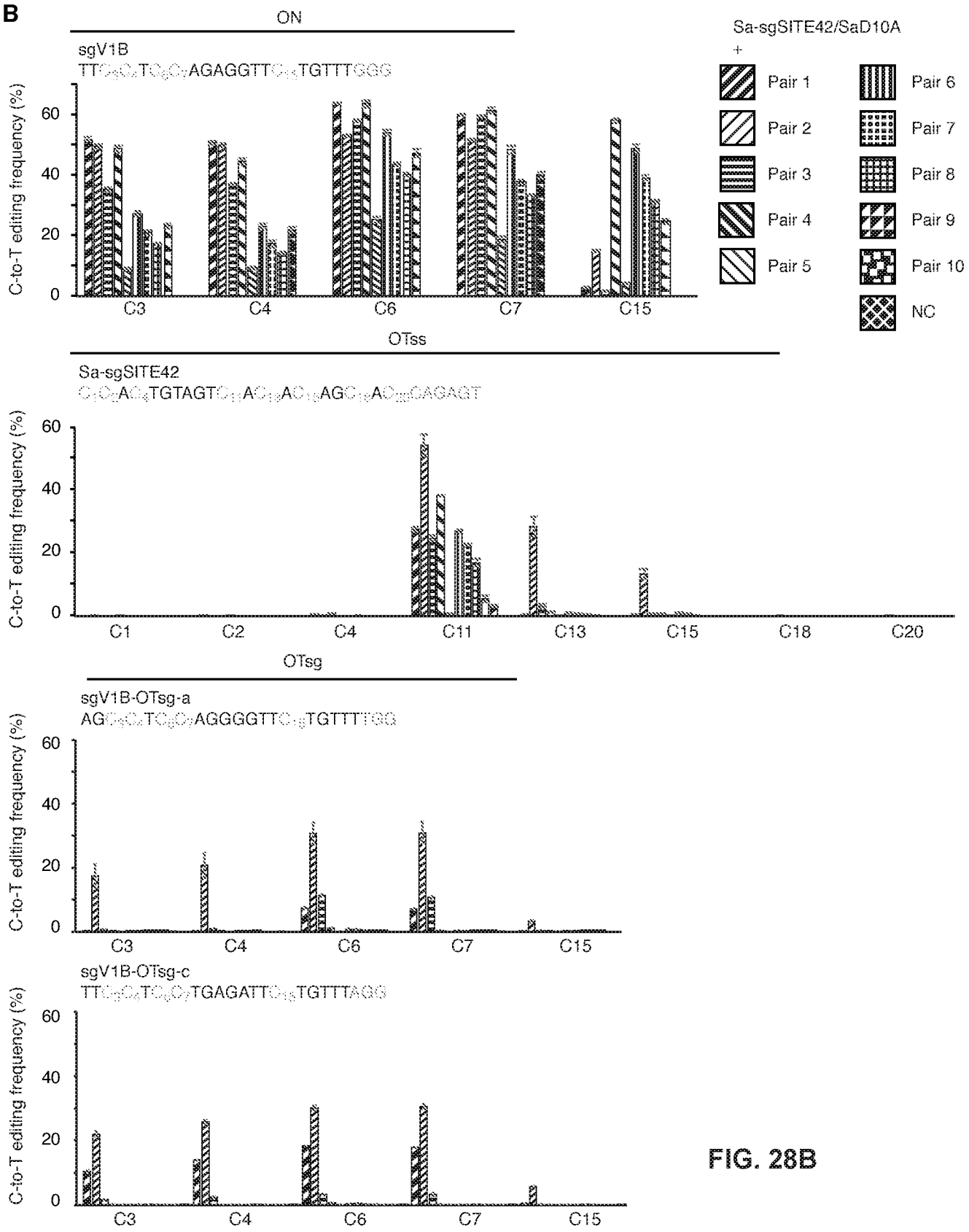

B
ON
sgV1B
Sa-sgSITE42/SaD10A
+
Pair 1
Pair 2
Pair 3
Pair 4
Pair 5
Pair 6
Pair 7
Pair 8
Pair 9
Pair 10
NC
C-to-T editing frequency (%)
0
20
40
60
C3
C4
C6
C7
C15
OTss
Sa-sgSITE42
C1
C2
C4
C11
C13
C15
C18
C20
OTsg
sgV1B-OTsg-a
sgV1B-OTsg-c

FIG. 28B

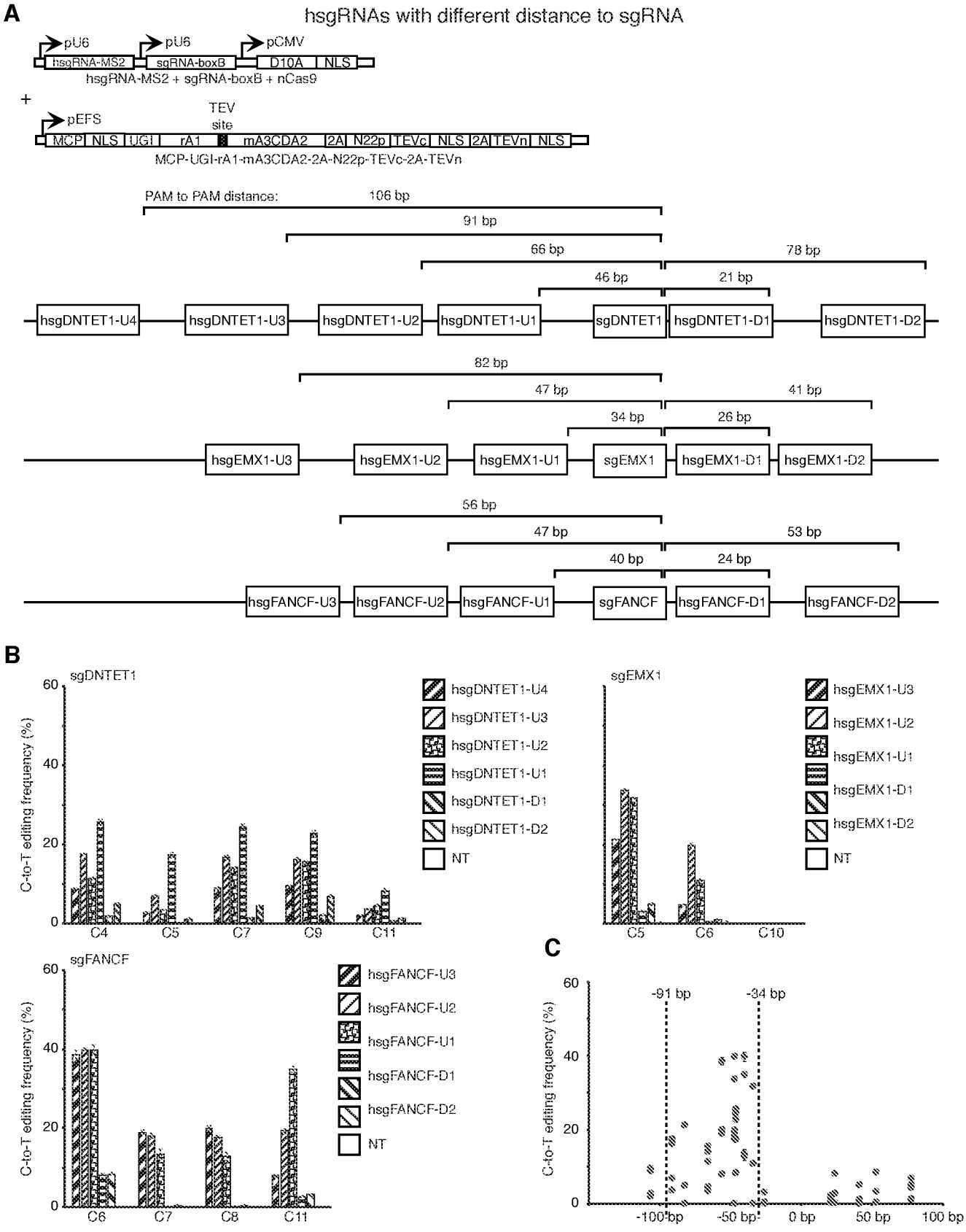

A
hsgRNAs with different distance to sgRNA
pU6
pU6
pCMV
hsgRNA-MS2
sgRNA-boxB
D10A
NLS
hsgRNA-MS2 + sgRNA-boxB + nCas9
+
pEFS
TEV site
MCP NLS UGI rA1 mA3CDA2 2A N22p TEVc NLS 2A TEVn NLS
MCP-UGI-rA1-mA3CDA2-2A-N22p-TEVc-2A-TEVn
PAM to PAM distance:
106 bp
91 bp
66 bp
78 bp
46 bp
21 bp
hsgDNTET1-U4
hsgDNTET1-U3
hsgDNTET1-U2
hsgDNTET1-U1
sgDNTET1
hsgDNTET1-D1
hsgDNTET1-D2
82 bp
47 bp
41 bp
34 bp
26 bp
hsgEMX1-U3
hsgEMX1-U2
hsgEMX1-U1
sgEMX1
hsgEMX1-D1
hsgEMX1-D2
56 bp
47 bp
53 bp
40 bp
24 bp
hsgFANCF-U3
hsgFANCF-U2
hsgFANCF-U1
sgFANCF
hsgFANCF-D1
hsgFANCF-D2
B
sgDNTET1
C-to-T editing frequency (%)
C4
C5
C7
C9
C11
hsgDNTET1-U4
hsgDNTET1-U3
hsgDNTET1-U2
hsgDNTET1-U1
hsgDNTET1-D1
hsgDNTET1-D2
NT
sgEMX1
C5
C6
C10
hsgEMX1-U3
hsgEMX1-U2
hsgEMX1-U1
hsgEMX1-D1
hsgEMX1-D2
NT
sgFANCF
C6
C7
C8
C11
hsgFANCF-U3
hsgFANCF-U2
hsgFANCF-U1
hsgFANCF-D1
hsgFANCF-D2
NT
C
-91 bp
-34 bp
-100 bp
-50 bp
0 bp
50 bp
100 bp

FIG. 29A-C

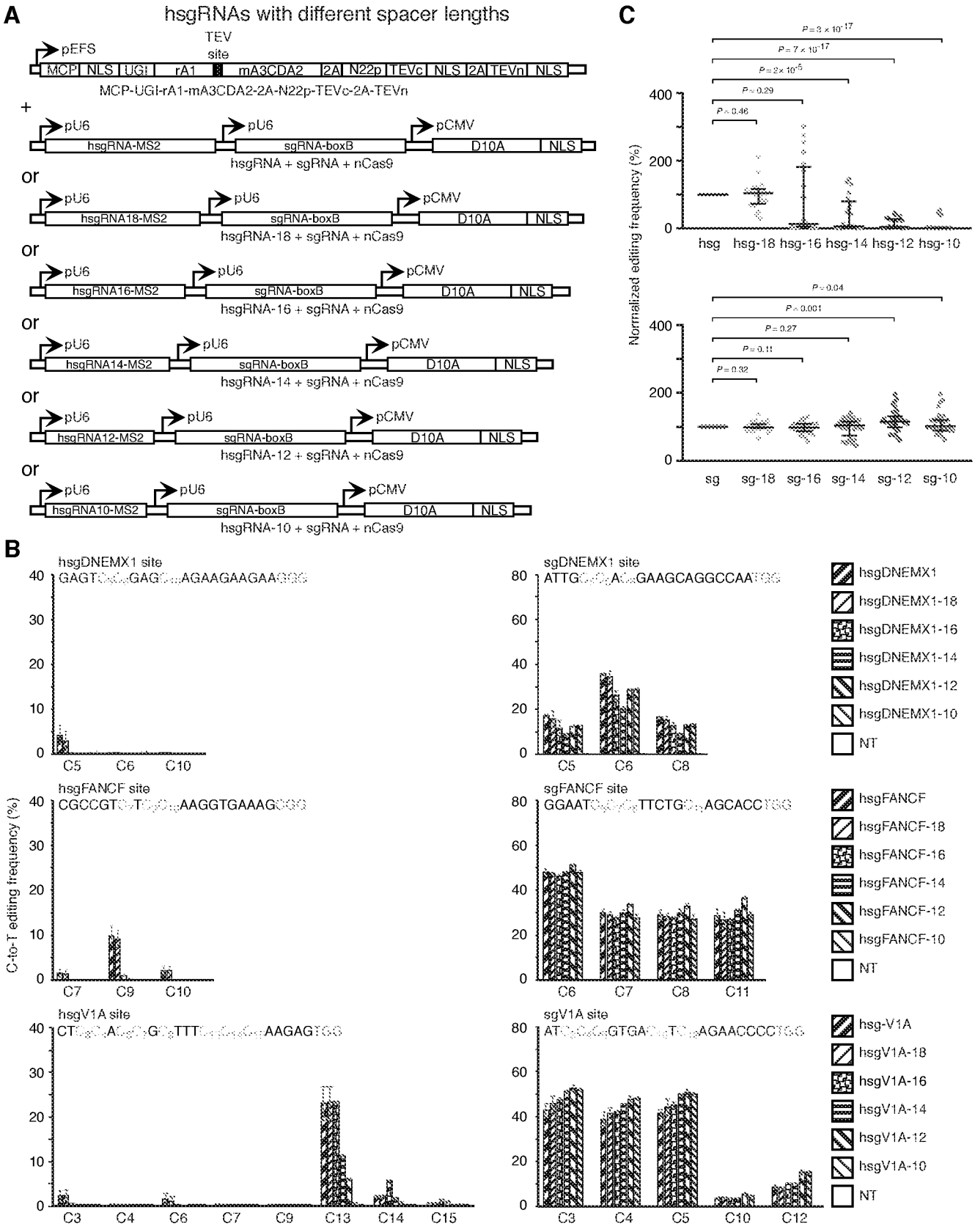

A
hsgRNAs with different spacer lengths
pEFS
TEV site
MCP NLS UGI rA1 mA3CDA2 2A N22p TEVc NLS 2A TEVn NLS
MCP-UGI-rA1-mA3CDA2-2A-N22p-TEVc-2A-TEVn
+
pU6 hsgRNA-MS2 pU6 sgRNA-boxB pCMV D10A NLS
hsgRNA + sgRNA + nCas9
or
pU6 hsgRNA18-MS2 pU6 sgRNA-boxB pCMV D10A NLS
hsgRNA-18 + sgRNA + nCas9
or
pU6 hsgRNA16-MS2 pU6 sgRNA-boxB pCMV D10A NLS
hsgRNA-16 + sgRNA + nCas9
or
pU6 hsgRNA14-MS2 pU6 sgRNA-boxB pCMV D10A NLS
hsgRNA-14 + sgRNA + nCas9
or
pU6 hsgRNA12-MS2 pU6 sgRNA-boxB pCMV D10A NLS
hsgRNA-12 + sgRNA + nCas9
or
pU6 hsgRNA10-MS2 pU6 sgRNA-boxB pCMV D10A NLS
hsgRNA-10 + sgRNA + nCas9
C
Normalized editing frequency (%)
hsg hsg-18 hsg-16 hsg-14 hsg-12 hsg-10
sg sg-18 sg-16 sg-14 sg-12 sg-10
B
hsgDNEMX1 site
sgDNEMX1 site
hsgFANCF site
sgFANCF site
hsgV1A site
sgV1A site
C-to-T editing frequency (%)
hsgDNEMX1
hsgDNEMX1-18
hsgDNEMX1-16
hsgDNEMX1-14
hsgDNEMX1-12
hsgDNEMX1-10
NT
hsgFANCF
hsgFANCF-18
hsgFANCF-16
hsgFANCF-14
hsgFANCF-12
hsgFANCF-10
NT
hsg-V1A
hsgV1A-18
hsgV1A-16
hsgV1A-14
hsgV1A-12
hsgV1A-10
NT

FIG. 30A-C

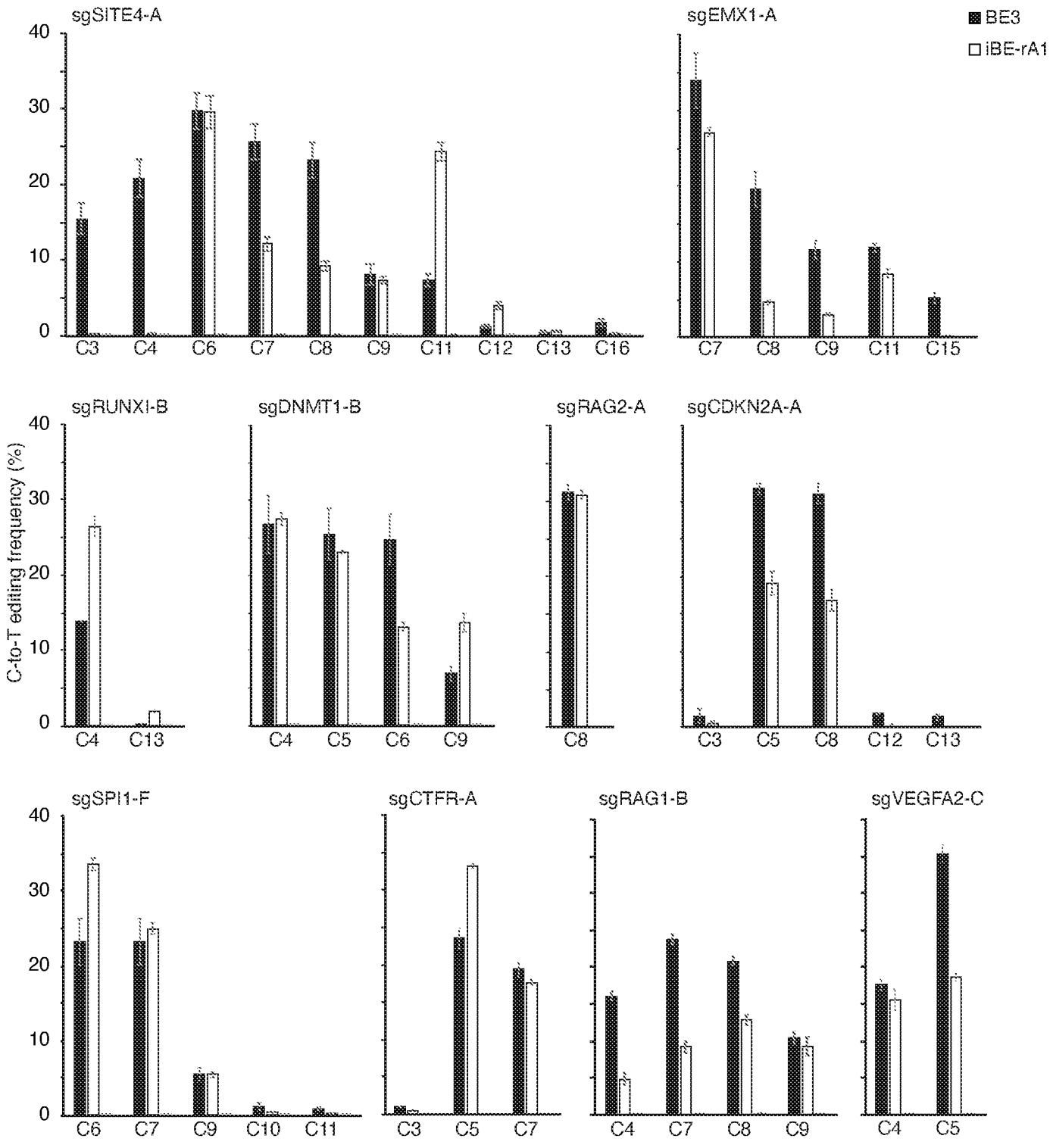

sgSITE4-A
sgEMX1-A
BE3
iBE-rA1
C-to-T editing frequency (%)
sgRUNX1-B
sgDNMT1-B
sgRAG2-A
sgCDKN2A-A
sgSPI1-F
sgCTFR-A
sgRAG1-B
sgVEGFA2-C
0
10
20
30
40
C3 C4 C6 C7 C8 C9 C11 C12 C13 C16
C7 C8 C9 C11 C15
C4 C13
C4 C5 C6 C9
C8
C3 C5 C8 C12 C13
C6 C7 C9 C10 C11
C3 C5 C7
C4 C7 C8 C9
C4 C5

FIG. 31

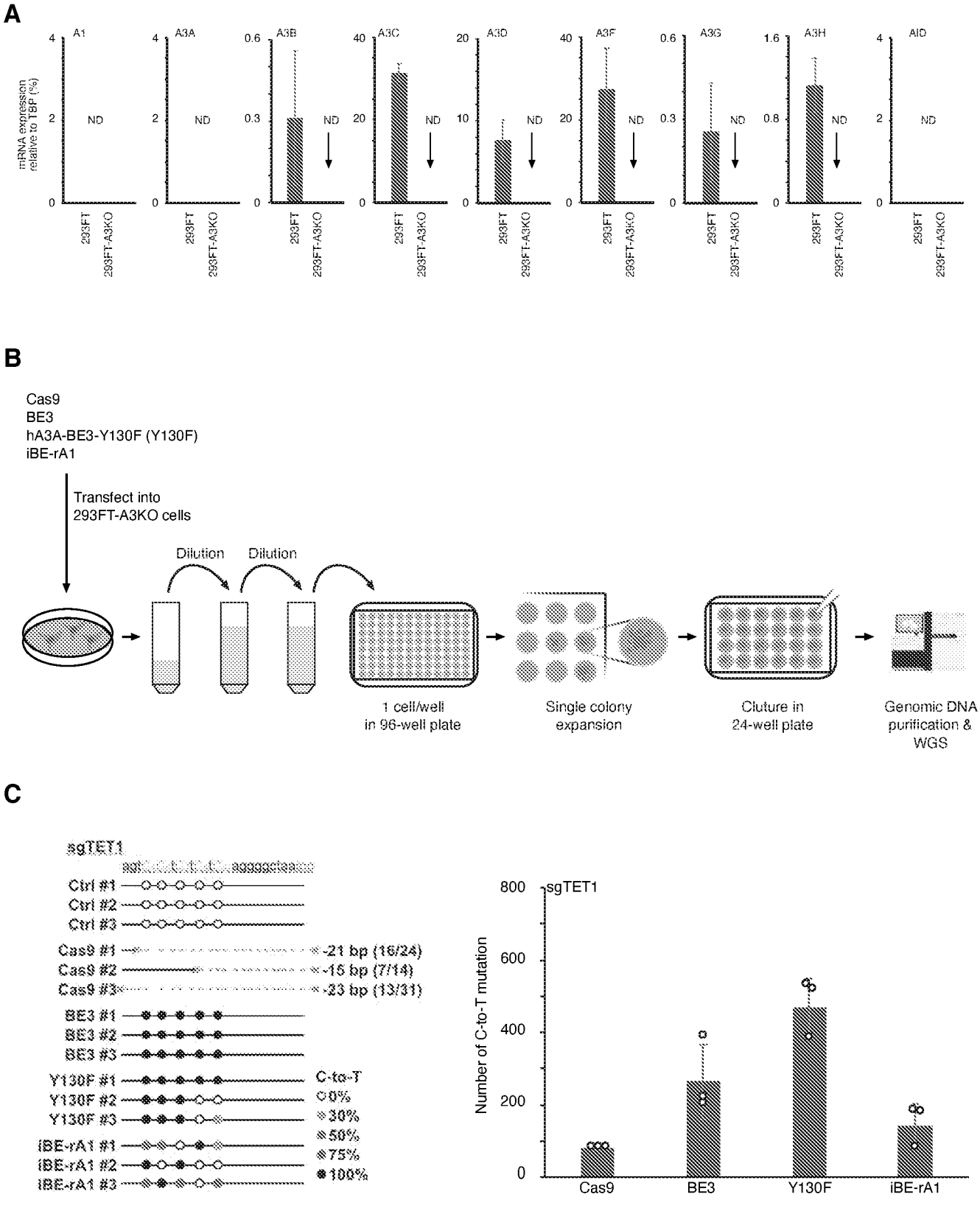

A
A1
A3A
A3B
A3C
A3D
A3F
A3G
A3H
AID
mRNA expression relative to TBP (%)
ND
293FT
293FT-A3KO
B
Cas9
BE3
hA3A-BE3-Y130F (Y130F)
iBE-rA1
Transfect into 293FT-A3KO cells
Dilution
Dilution
1 cell/well in 96-well plate
Single colony expansion
Cluture in 24-well plate
Genomic DNA purification & WGS
C
sgTET1
Ctrl #1
Ctrl #2
Ctrl #3
Cas9 #1
Cas9 #2
Cas9 #3
-21 bp (16/24)
-16 bp (7/14)
-23 bp (13/31)
BE3 #1
BE3 #2
BE3 #3
Y130F #1
Y130F #2
Y130F #3
iBE-rA1 #1
iBE-rA1 #2
iBE-rA1 #3
C-to-T
0%
30%
50%
75%
100%
sgTET1
Number of C-to-T mutation
Cas9
BE3
Y130F
iBE-rA1

FIG. 32A-C

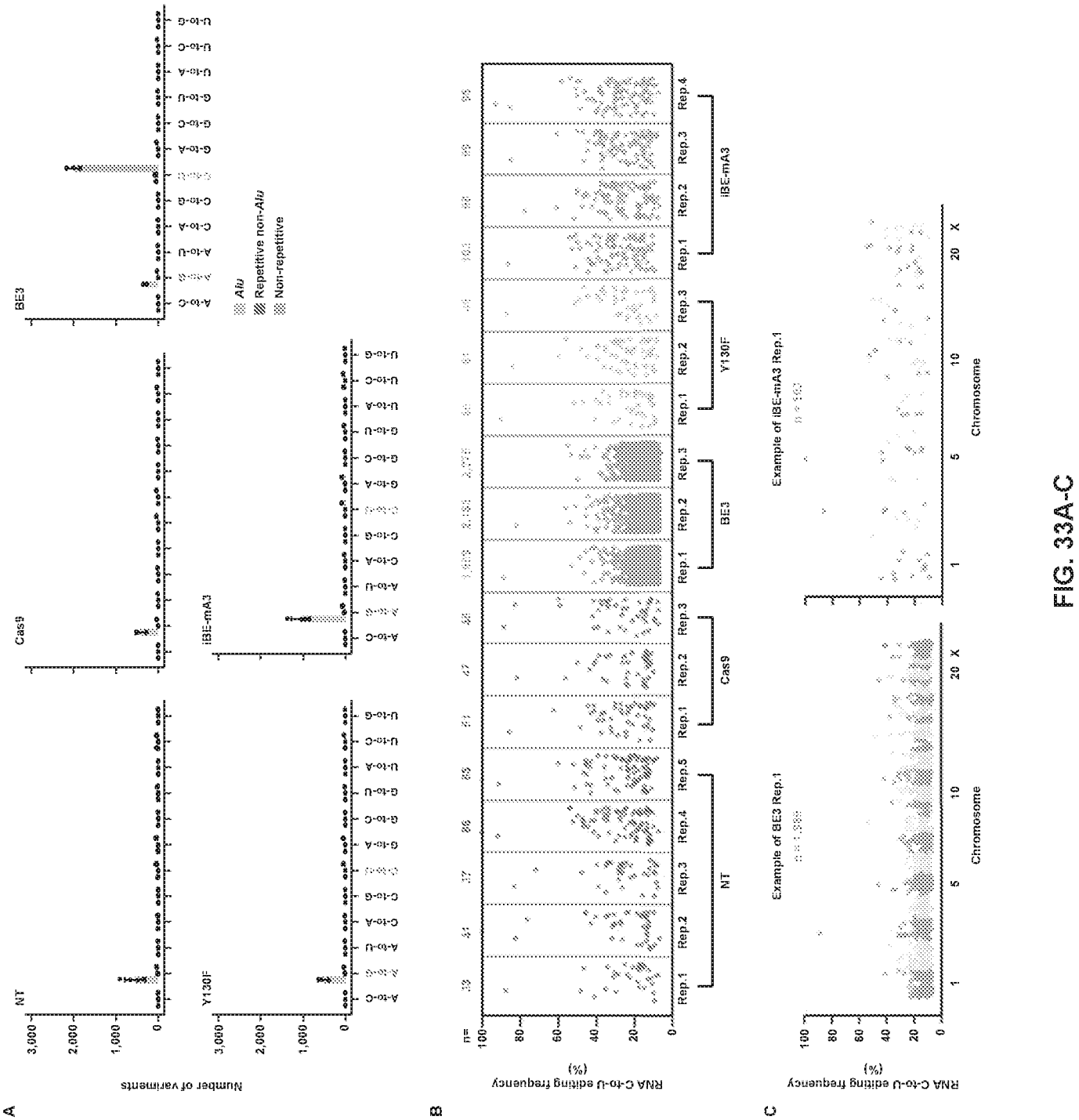

A
NT
Cas9
BE3
Y130F
iBE-mA3
Number of variments
Alu
Repetitive non-Alu
Non-repetitive
B
RNA C-to-U editing frequency (%)
Rep.1
Rep.2
Rep.3
Rep.4
Rep.5
NT
Cas9
BE3
Y130F
iBE-mA3
C
Example of BE3 Rep.1
Example of iBE-mA3 Rep.1
Chromosome

FIG. 33A-C

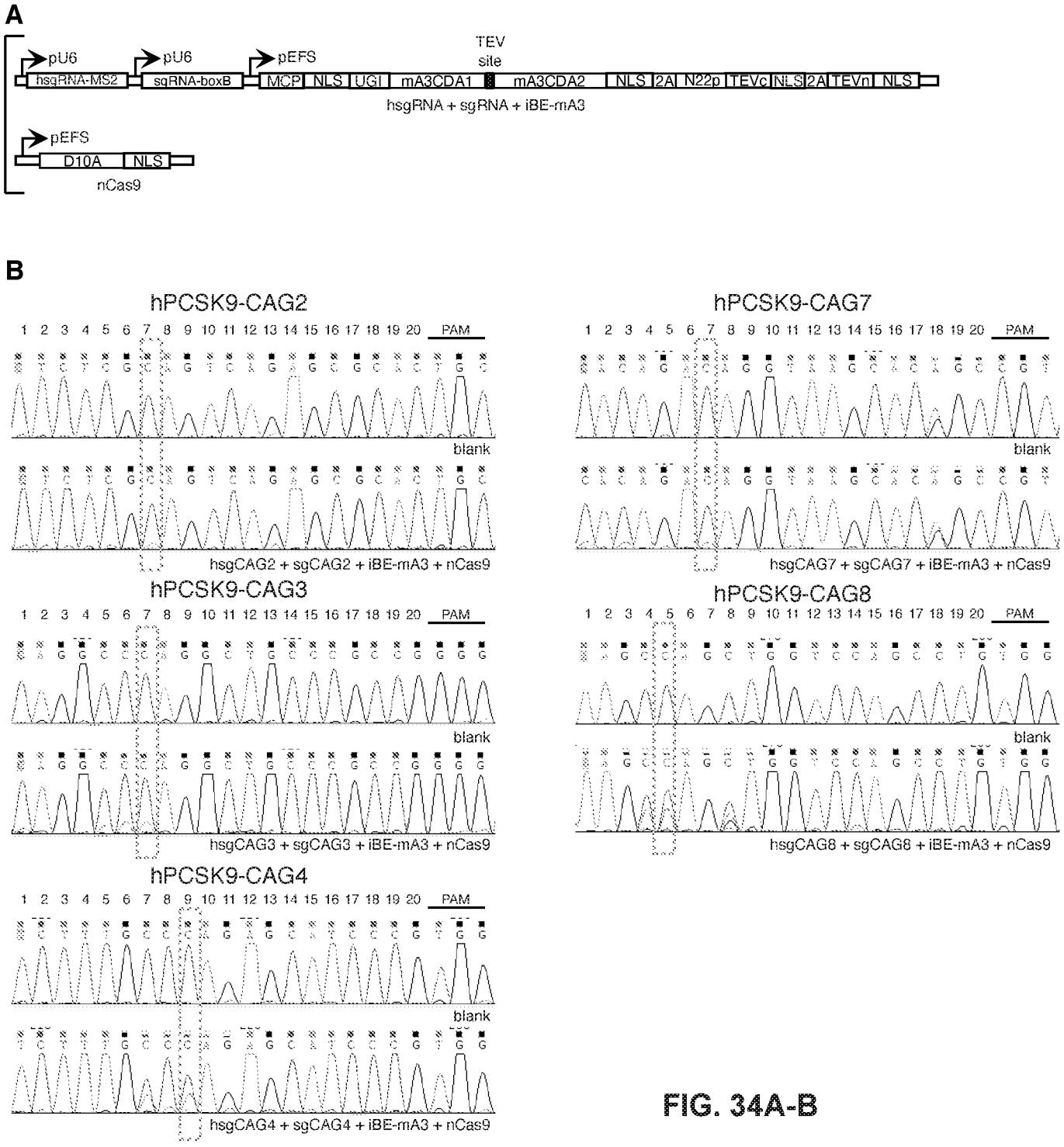

A
pU6
hsgRNA-MS2
pU6
sgRNA-boxB
pEFS
MCP
NLS
UGI
mA3CDA1
TEV site
mA3CDA2
NLS
2A
N22p
TEVc
NLS
2A
TEVn
NLS
hsgRNA + sgRNA + iBE-mA3
pEFS
D10A
NLS
nCas9
B
hPCSK9-CAG2
hPCSK9-CAG7
hPCSK9-CAG3
hPCSK9-CAG8
hPCSK9-CAG4
PAM
blank
hsgCAG2 + sgCAG2 + iBE-mA3 + nCas9
hsgCAG7 + sgCAG7 + iBE-mA3 + nCas9
hsgCAG3 + sgCAG3 + iBE-mA3 + nCas9
hsgCAG8 + sgCAG8 + iBE-mA3 + nCas9
hsgCAG4 + sgCAG4 + iBE-mA3 + nCas9

FIG. 34A-B

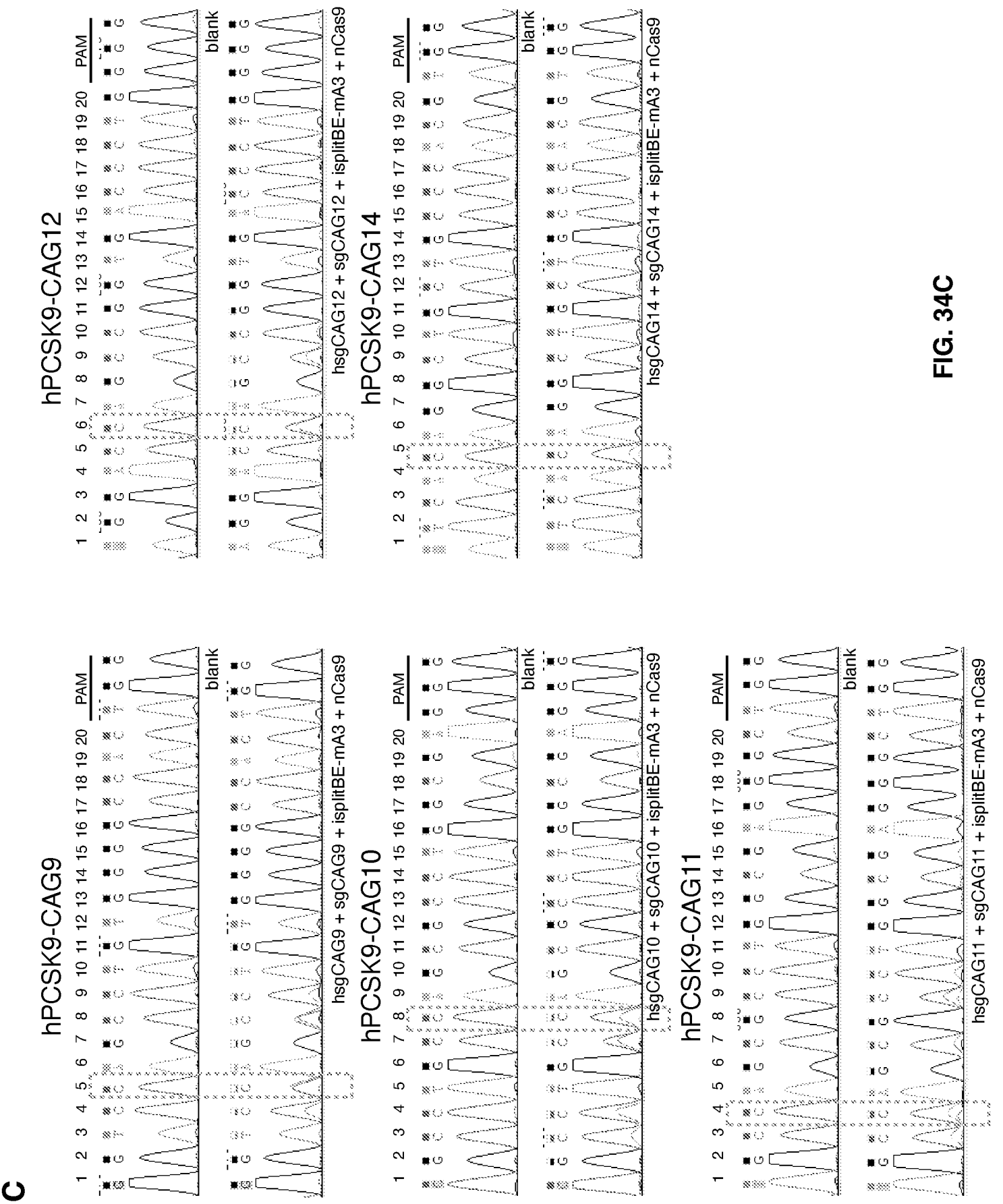

C
hPCSK9-CAG9
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
blank
hsgCAG9 + sgCAG9 + isplitBE-mA3 + nCas9
hPCSK9-CAG10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
blank
hsgCAG10 + sgCAG10 + isplitBE-mA3 + nCas9
hPCSK9-CAG11
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
blank
hsgCAG11 + sgCAG11 + isplitBE-mA3 + nCas9
hPCSK9-CAG12
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
blank
hsgCAG12 + sgCAG12 + isplitBE-mA3 + nCas9
hPCSK9-CAG14
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 PAM
blank
hsgCAG14 + sgCAG14 + isplitBE-mA3 + nCas9

FIG. 34C

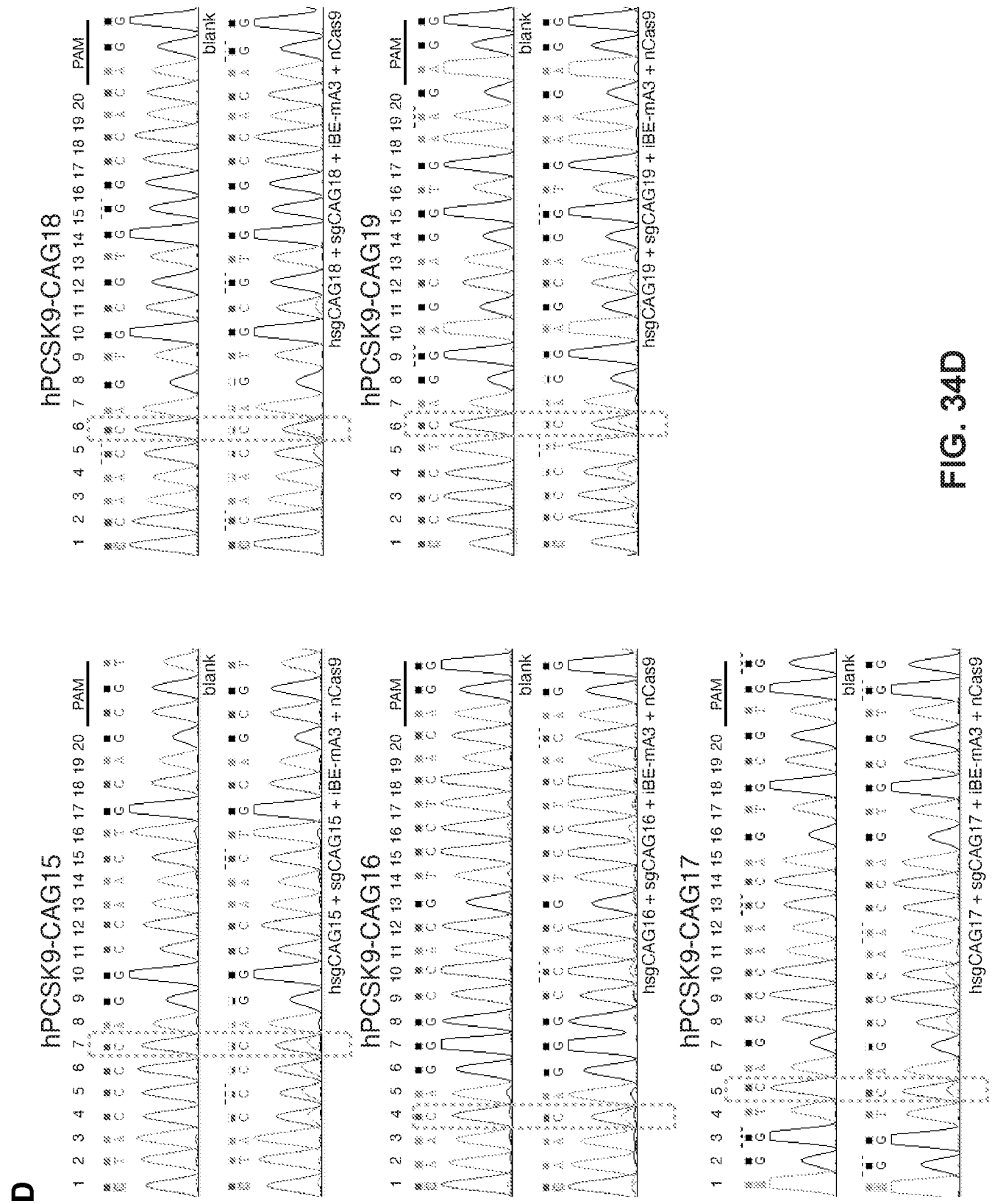
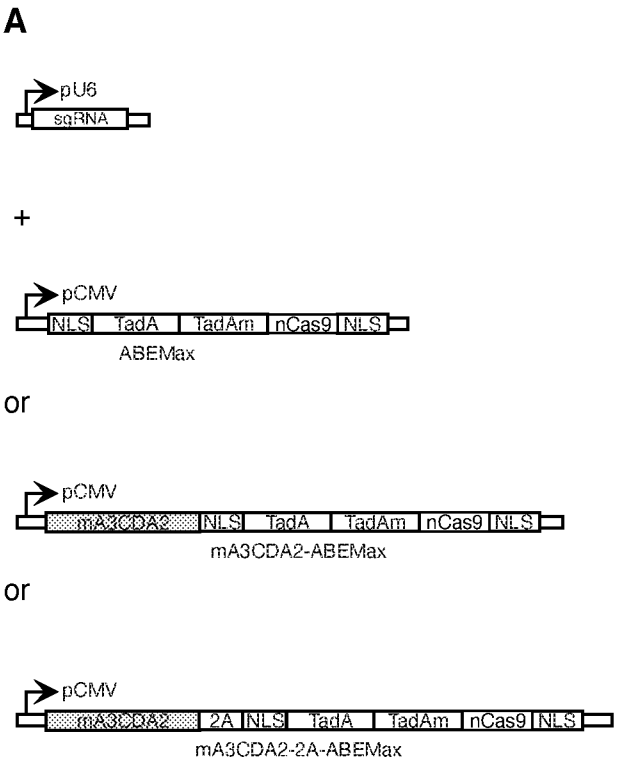

D
hPCSK9-CAG15
hPCSK9-CAG16
hPCSK9-CAG17
hPCSK9-CAG18
hPCSK9-CAG19
PAM
blank
hsgCAG15 + sgCAG15 + iBE-mA3 + nCas9
hsgCAG16 + sgCAG16 + iBE-mA3 + nCas9
hsgCAG17 + sgCAG17 + iBE-mA3 + nCas9
hsgCAG18 + sgCAG18 + iBE-mA3 + nCas9
hsgCAG19 + sgCAG19 + iBE-mA3 + nCas9

FIG. 34D


A
pU6
sgRNA
+
pCMV
NLS
TadA
TadAm
nCas9
NLS
ABEMax
or
pCMV
mA3CDA2
NLS
TadA
TadAm
nCas9
NLS
mA3CDA2-ABEMax
or
pCMV
mA3CDA2
2A
NLS
TadA
TadAm
nCas9
NLS
mA3CDA2-2A-ABEMax

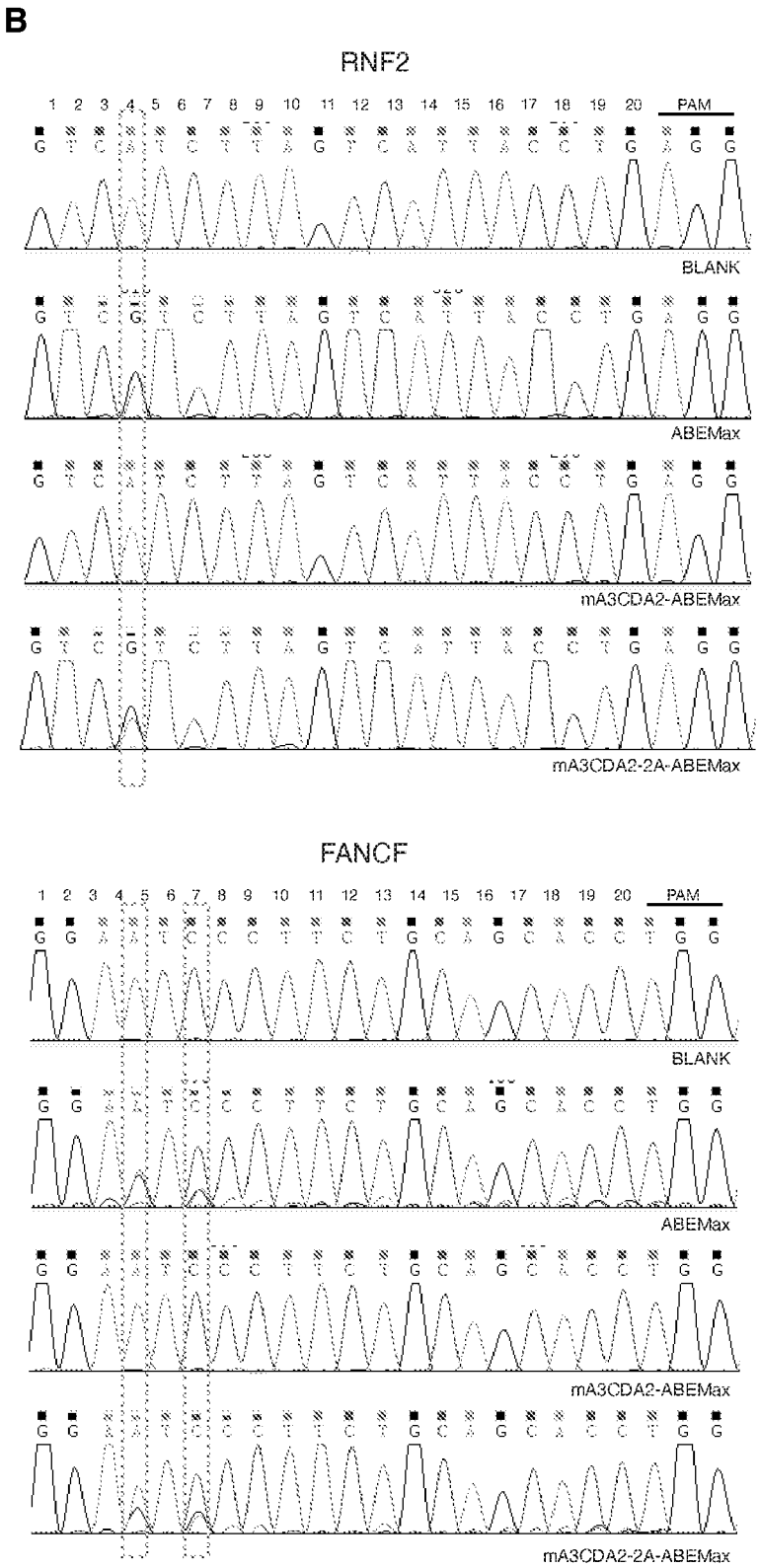
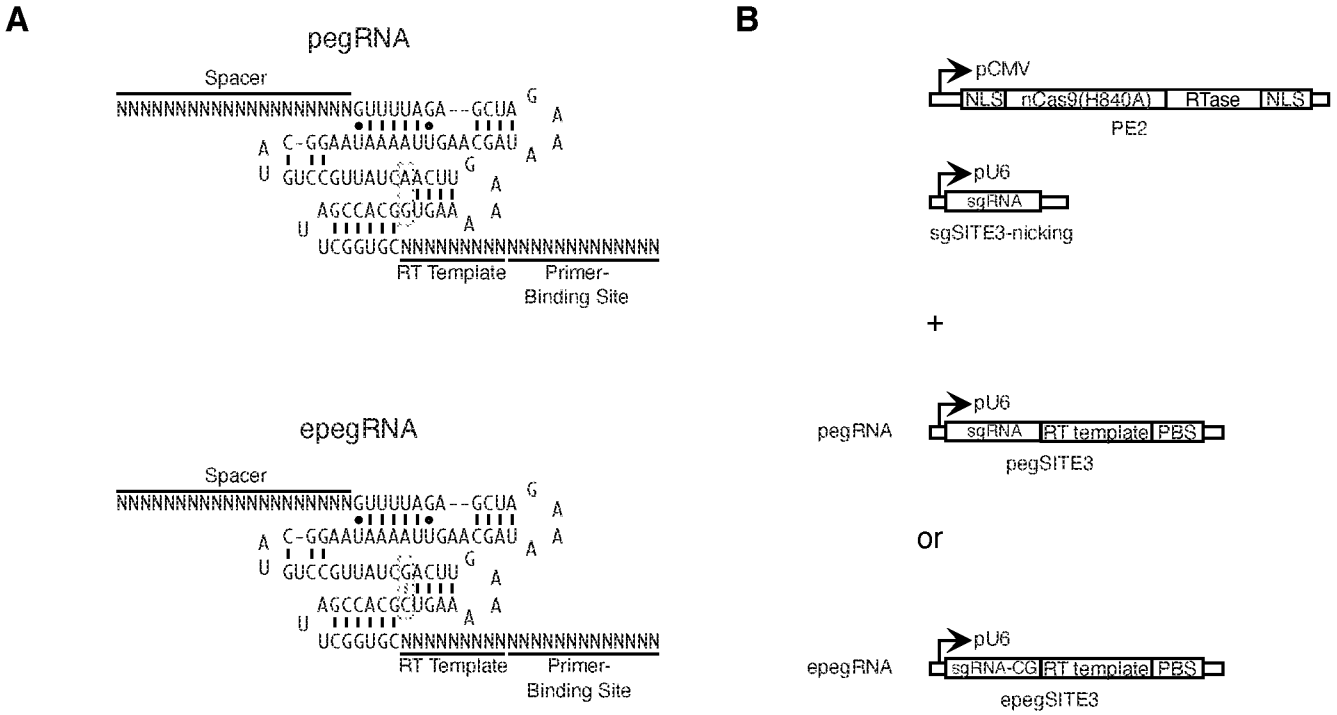

B
RNF2
PAM
BLANK
ABEMax
mA3CDA2-ABEMax
mA3CDA2-2A-ABEMax
FANCF
PAM
BLANK
ABEMax
mA3CDA2-ABEMax
mA3CDA2-2A-ABEMax

FIG. 35A-B


A
pegRNA
Spacer
RT Template
Primer-Binding Site
epegRNA
Spacer
RT Template
Primer-Binding Site
B
pCMV
NLS
nCas9(H840A)
RTase
NLS
PE2
pU6
sgRNA
sgSITE3-nicking
+
pegRNA
pU6
sgRNA
RT template
PBS
pegSITE3
or
epegRNA
pU6
sgRNA-CG
RT template
PBS
epegSITE3

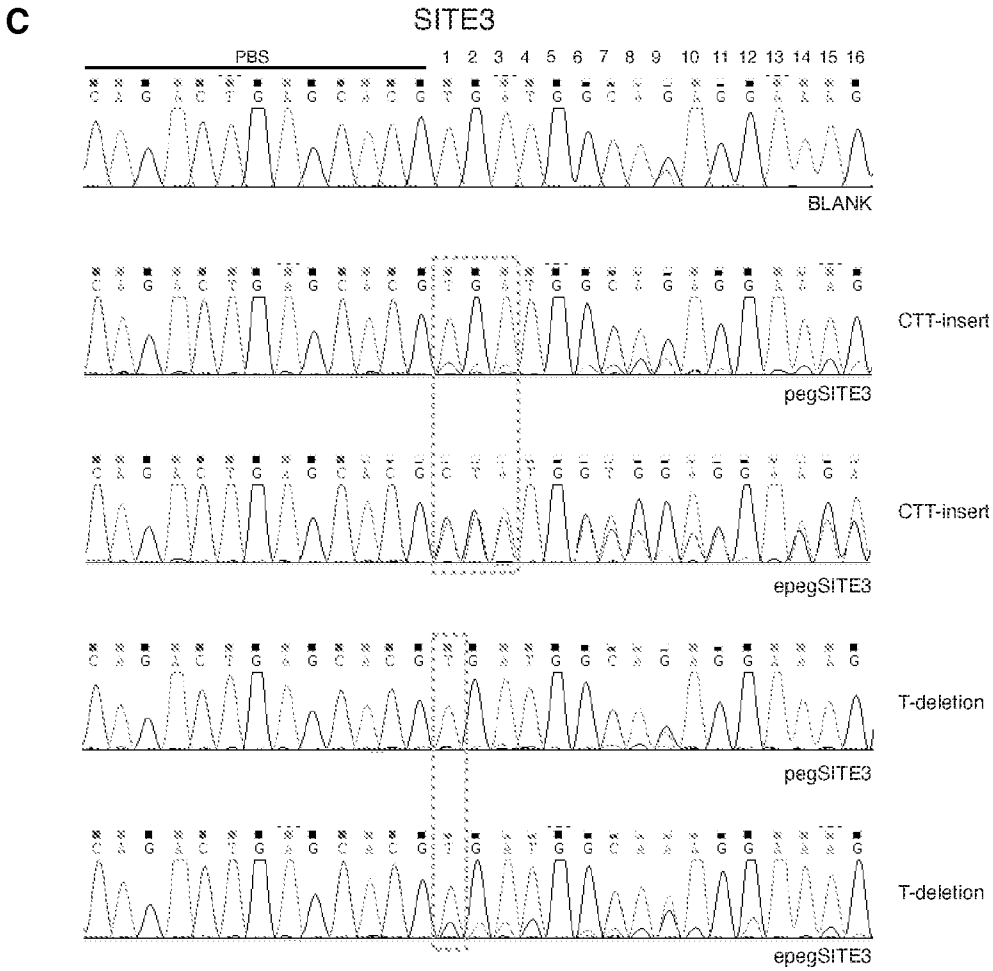

C
SITE3
PBS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
BLANK
CTT-insert
pegSITE3
CTT-insert
epegSITE3
T-deletion
pegSITE3
T-deletion
epegSITE3

FIG. 36A-C

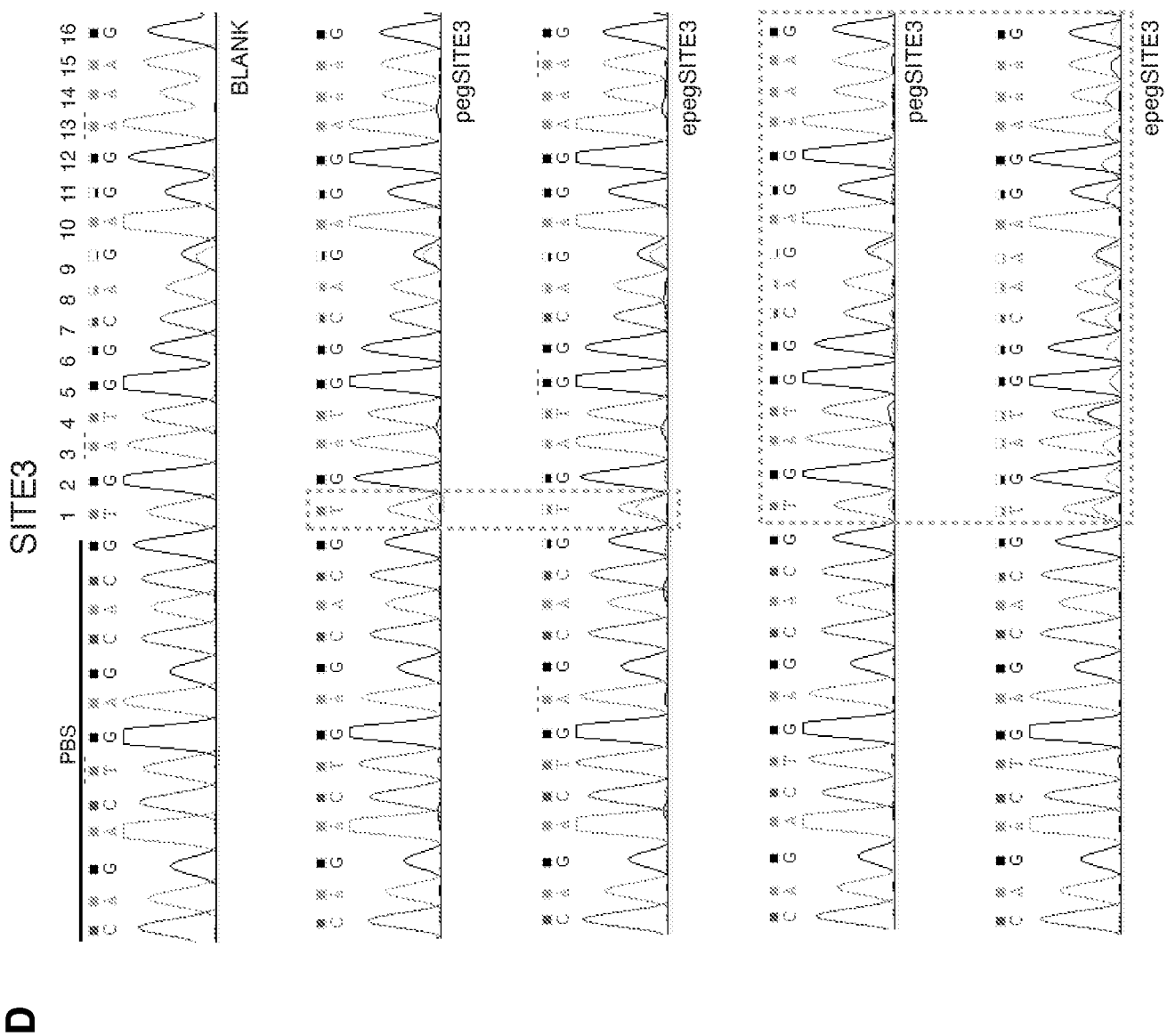
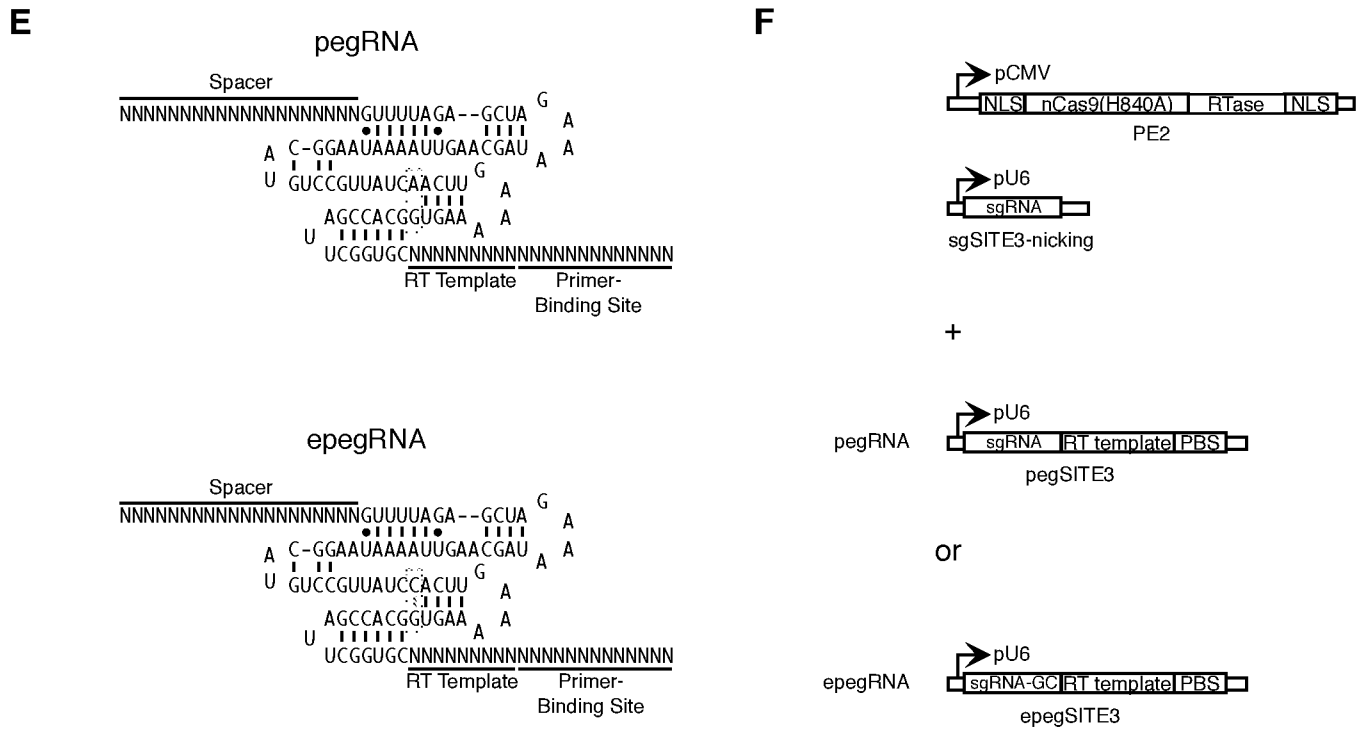

D
SITE3
PBS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
BLANK
T-A transversion
pegSITE3
T-A transversion
epegSITE3
44 bp insertion
pegSITE3
44 bp insertion
epegSITE3

FIG. 36D


E
pegRNA
Spacer
NNNNNNNNNNNNNNNNNNNNGUUUUAGA--GCUA
C-GGAAUAAAAUUGAACGAU
GUCCGUUAUCAACUU
AGCCACGGUGAA
UCGGUGCNNNNNNNNNNNNNNNNNNNN
RT Template
Primer-Binding Site
epegRNA
Spacer
NNNNNNNNNNNNNNNNNNNNGUUUUAGA--GCUA
C-GGAAUAAAAUUGAACGAU
GUCCGUUAUCCACUU
AGCCACGGUGAA
UCGGUGCNNNNNNNNNNNNNNNNNNNN
RT Template
Primer-Binding Site
F
pCMV
NLS
nCas9(H840A)
RTase
NLS
PE2
pU6
sgRNA
sgSITE3-nicking
+
pegRNA
pU6
sgRNA
RT template
PBS
pegSITE3
or
epegRNA
pU6
sgRNA-GC
RT template
PBS
epegSITE3

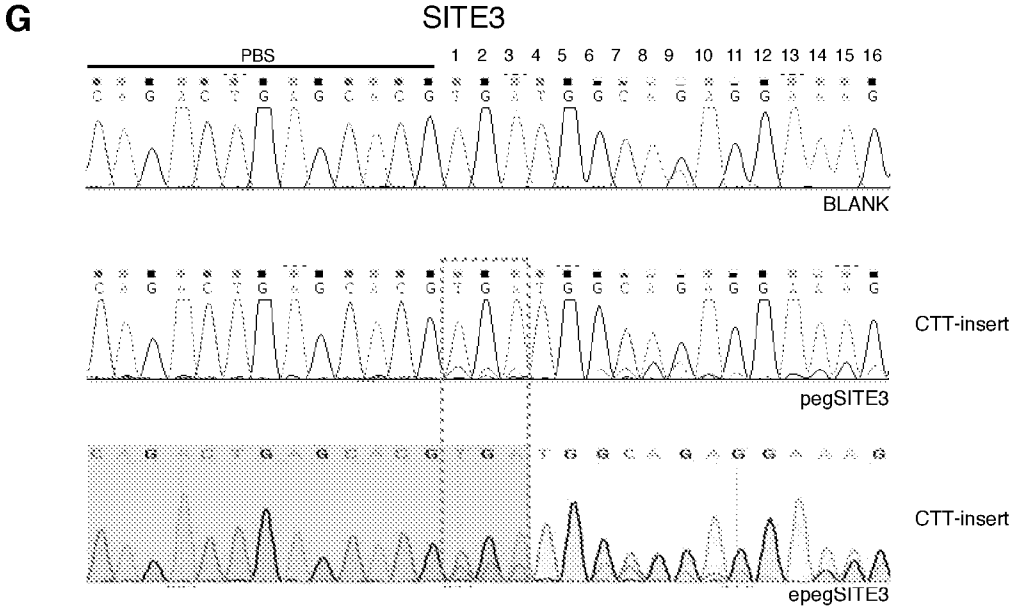
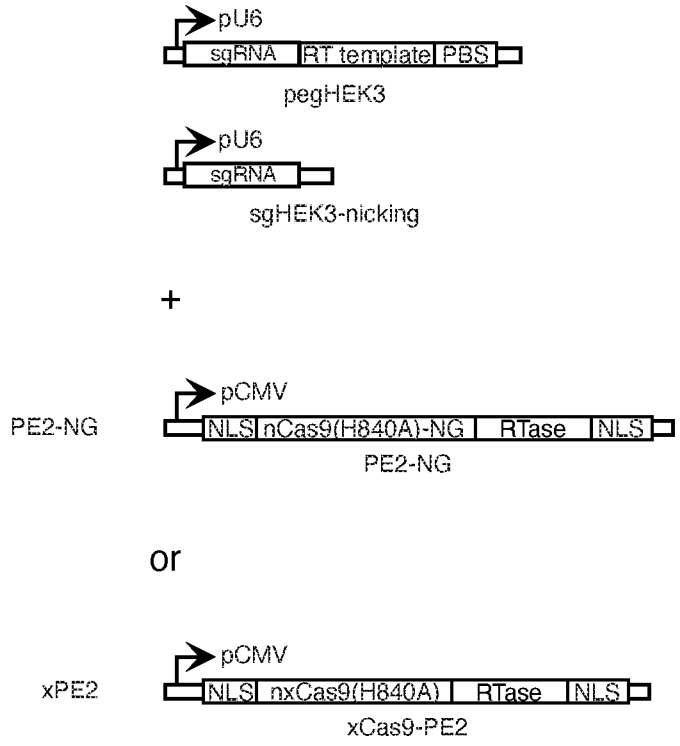

G
SITE3
PBS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
BLANK
CTT-insert
pegSITE3
CTT-insert
epegSITE3

FIG. 36E-G

**A**


pU6
sgRNA
RT template
PBS
pegHEK3
pU6
sgRNA
sgHEK3-nicking
+
PE2-NG
pCMV
NLS
nCas9(H840A)-NG
RTase
NLS
PE2-NG
or
xPE2
pCMV
NLS
nxCas9(H840A)
RTase
NLS
xCas9-PE2

**B**

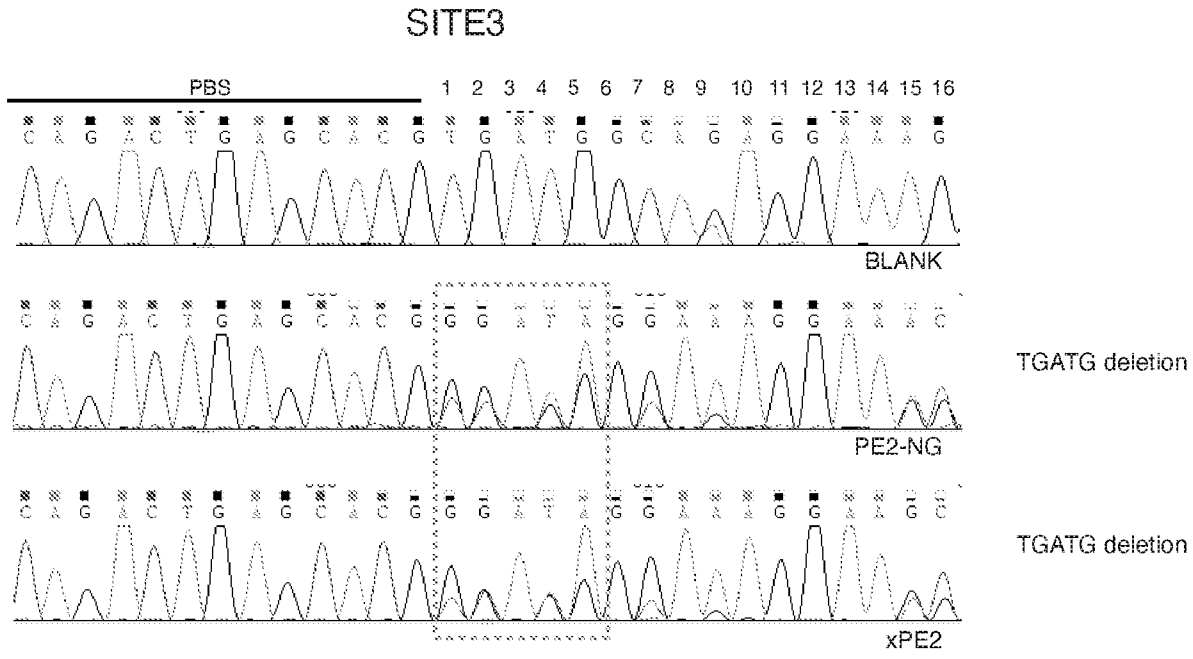

SITE3
PBS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
BLANK
TGATG deletion
PE2-NG
TGATG deletion
xPE2

FIG. 37A-B

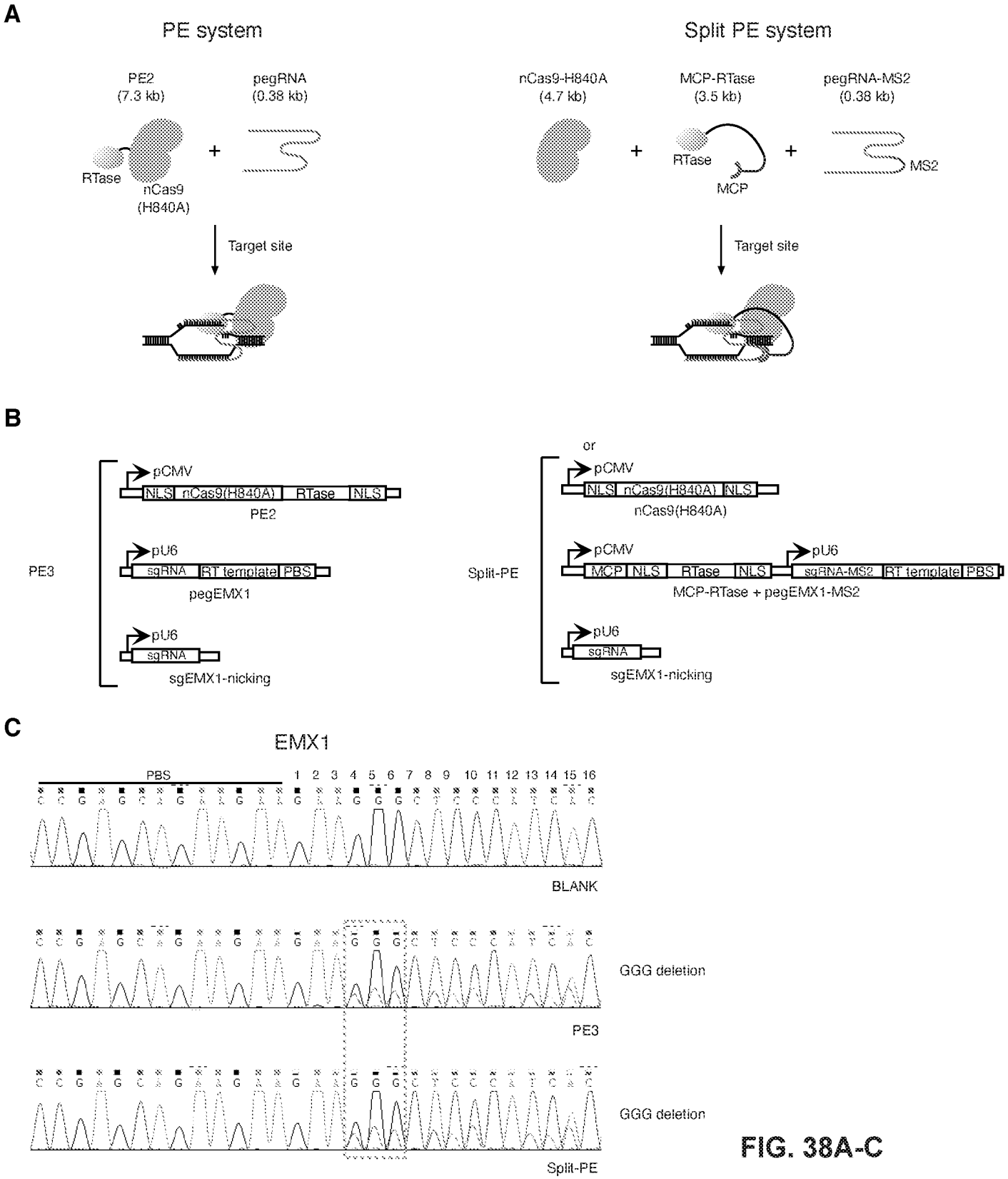

A
PE system
PE2
(7.3 kb)
pegRNA
(0.38 kb)
RTase
nCas9
(H840A)
Target site
Split PE system
nCas9-H840A
(4.7 kb)
MCP-RTase
(3.5 kb)
pegRNA-MS2
(0.38 kb)
RTase
MCP
MS2
Target site
B
PE3
pCMV
NLS nCas9(H840A) RTase NLS
PE2
pU6
sgRNA RT template PBS
pegEMX1
pU6
sgRNA
sgEMX1-nicking
or
Split-PE
pCMV
NLS nCas9(H840A) NLS
nCas9(H840A)
pCMV
MCP NLS RTase NLS
pU6
sgRNA-MS2 RT template PBS
MCP-RTase + pegEMX1-MS2
pU6
sgRNA
sgEMX1-nicking
C
EMX1
PBS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
BLANK
GGG deletion
PE3
GGG deletion
Split-PE

FIG. 38A-C

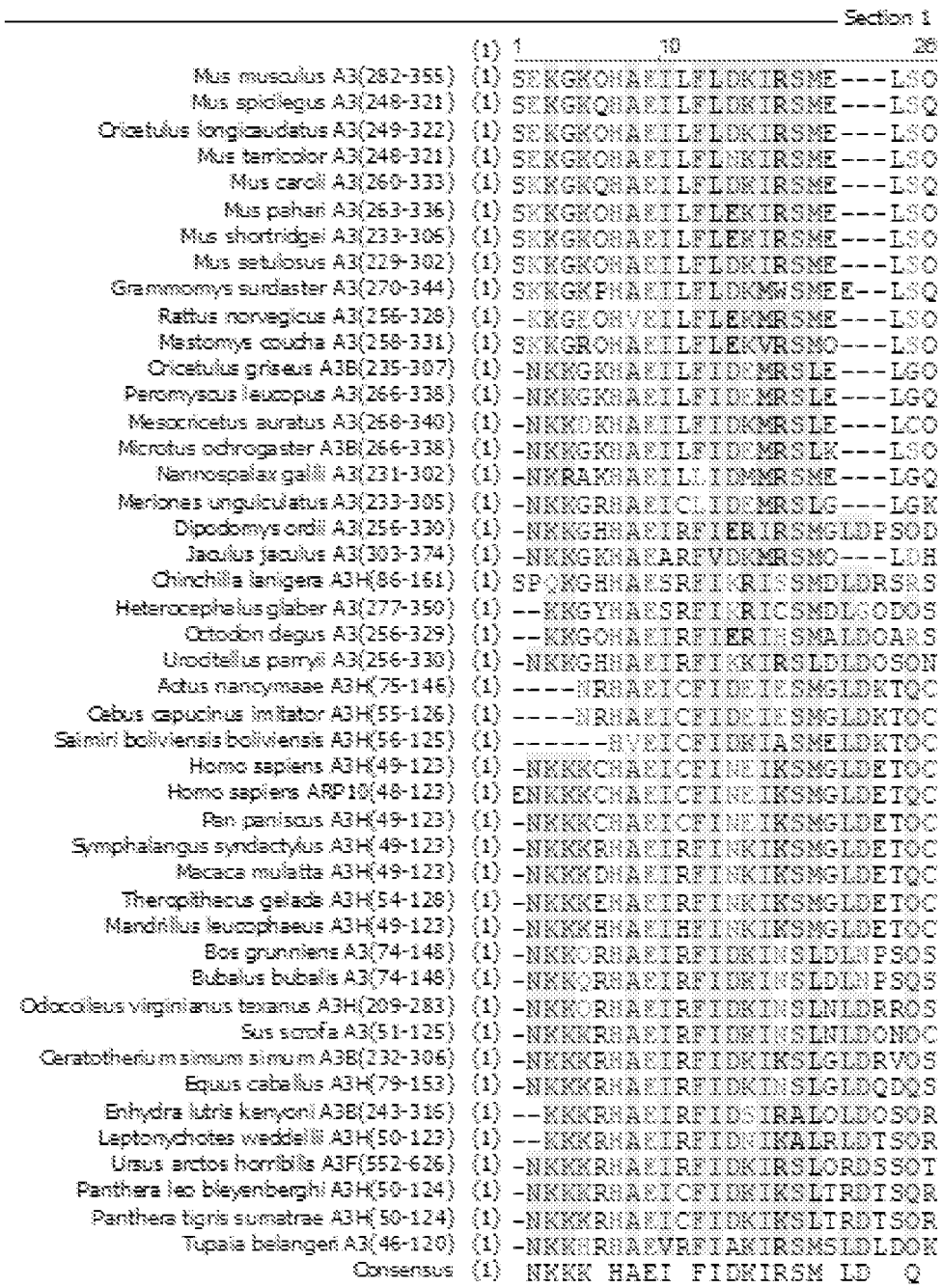

Section 1
(1) 1 10 20
Mus musculus A3(282-355) (1) SEKGKQNAEILFLDKIRSME---LSQ
Mus spicilegus A3(248-321) (1) SEKGKQNAEILFLDKIRSME---LSQ
Cricetulus longicaudatus A3(249-322) (1) SEKGKQNAEILFLDKIRSME---LSQ
Mus terricolor A3(248-321) (1) SEKGKQNAEILFLNKIRSME---LSQ
Mus caroli A3(260-333) (1) SEKGKQNAEILFLDKIRSME---LSQ
Mus pahari A3(263-336) (1) SEKGKQNAEILFLEKIRSME---LSQ
Mus shortridgei A3(233-306) (1) SEKGKQNAEILFLEKIRSME---LSQ
Mus setulosus A3(229-302) (1) SEKGKQNAEILFLDKIRSME---LSQ
Grammomys surdaster A3(270-344) (1) SEKGKPNAEILFLDKMSSMEE--LSQ
Rattus norvegicus A3(256-328) (1) -EKGEQNVEILFLEKMRSME---LSQ
Nastomys coucha A3(258-331) (1) SEKGRQNAEILFLEKVRSMQ---LSQ
Cricetulus griseus A3B(235-307) (1) -NKKGKNAEILFIDKMRSLE---LGQ
Peromyscus leucopus A3(266-338) (1) -NKKGKNAEILFIDKMRSLE---LGQ
Mesocricetus auratus A3(268-340) (1) -NKKQKNAEILFIDKMRSLE---LCQ
Microtus ochrogaster A3B(266-338) (1) -NKKGKNAEILFIDKMRSLK---LSQ
Nannospalax galili A3(231-302) (1) -NKRAKNAEILLIDKMRSME---LGQ
Meriones unguiculatus A3(233-305) (1) -NKKGRNAEICLIDKMRSLG---LGK
Dipodomys ordii A3(256-330) (1) -NKKGHNAEIRFIERIRSMGLDPSQD
Jaculus jaculus A3(303-374) (1) -NKKGKNAEARFVDKMRSMQ---LDH
Chinchilla lanigera A3H(86-161) (1) SPQKGHNAESRFIKRIGSMDLDRSRS
Heterocephalus glaber A3(277-350) (1) --KKGYNAESRFINRICSMDLGQDQS
Octodon degus A3(256-329) (1) --KKGQNAEIRFIERIKSMALDQARS
Urocitellus parryi A3(256-330) (1) -NKKGHNAEIRFIKKIRSLDLDQSQN
Actus nancymaae A3H(75-146) (1) ----NRNAEICFIDEIKSMGLDKTQC
Cebus capucinus imitator A3H(55-126) (1) ----NRNAEICFIDEIKSMGLDKTQC
Saimiri boliviensis boliviensis A3H(56-125) (1) ------RVEICFIDKIASMELDKTQC
Homo sapiens A3H(49-123) (1) -NKKKCNAEICFINEIKSMGLDETQC
Homo sapiens ARP10(48-123) (1) ENKKKCNAEICFINEIKSMGLDETQC
Pan paniscus A3H(49-123) (1) -NKKKCNAEICFINEIKSMGLDETQC
Symphalangus syndactylus A3H(49-123) (1) -NKKKRNAEIRFINKIKSMGLDETQC
Macaca mulatta A3H(49-123) (1) -NKKKDNAEIRFINKIKSMGLDETQC
Theropithecus gelada A3H(54-128) (1) -NKKKENAEIRFINKIKSMGLDETQC
Mandrillus leucophaeus A3H(49-123) (1) -NKKKHNAEIHFINKIKSMGLDETQC
Bos grunniens A3(74-148) (1) -NKKQRNAEIRFIDKIMSLDLNPSQS
Bubalus bubalis A3(74-148) (1) -NKKQRNAEIRFIDKIMSLDLNPSQS
Odocoileus virginianus texanus A3H(209-283) (1) -NKKQRNAEIRFIDKIMSLNLDRRQS
Sus scrofa A3(51-125) (1) -NKKKRNAEIRFIDKIMSLNLDQNQC
Ceratotherium simum simum A3B(232-306) (1) -NKKKRNAEIRFIDKIKSLGLDRVQS
Equus caballus A3H(79-153) (1) -NKKKRNAEIRFIDKIMSLGLDQDQS
Enhydra lutris kenyoni A3B(243-316) (1) --KKKRNAEIRFIDSIRALQLDQSQR
Leptonychotes weddellii A3H(50-123) (1) --KKKRNAEIRFIDKINALRLDTSQR
Ursus arctos horribilis A3F(552-626) (1) -NKKKRNAEIRFIDKIRSLQRDSSQT
Panthera leo bleyenberghi A3H(50-124) (1) -NKKKRNAEICFIDKIKSLTRDTSQR
Panthera tigris sumatrae A3H(50-124) (1) -NKKKRNAEICFIDKIKSLTRDTSQR
Tupaia belangeri A3(46-120) (1) -NKKNRNAEVRFIANIRSMSLDLDQK
Consensus (1) NKKK HAEI FIDKIRSM LD Q

FIG. 39A

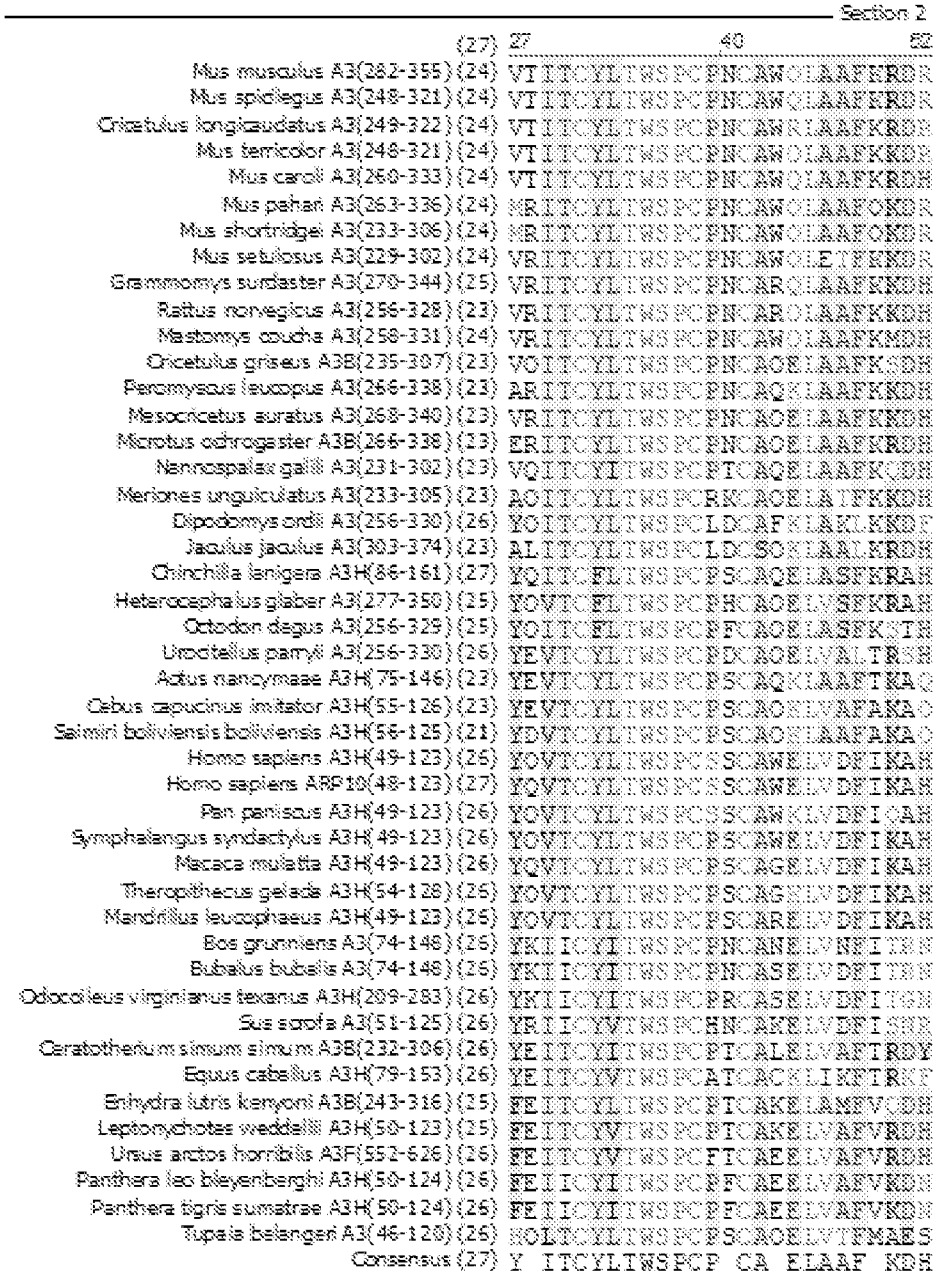

Section 2
(27) 27 40 52
Mus musculus A3(282-355) (24) VTITCYLTWSPCPNCAWQLAAFKRDR
Mus spicilegus A3(248-321) (24) VTITCYLTWSPCPNCAWQLAAFKRDR
Cricetulus longicaudatus A3(249-322) (24) VTITCYLTWSPCPNCAWRLAAFKRDR
Mus terricolor A3(248-321) (24) VTITCYLTWSPCPNCAWQLAAFKKDR
Mus caroli A3(260-333) (24) VTITCYLTWSPCPNCAWQLAAFKRDH
Mus pahari A3(263-336) (24) MRITCYLTWSPCPNCAWQLAAFQKDR
Mus shortridgei A3(233-306) (24) MRITCYLTWSPCPNCAWQLAAFQKDR
Mus setulosus A3(229-302) (24) VRITCYLTWSPCPNCAWQLETFKKDR
Grammomys surdaster A3(270-344) (25) VRITCYLTWSPCPNCARQLAAFKKDH
Rattus norvegicus A3(256-328) (23) VRITCYLTWSPCPNCARQLAAFKKDH
Mastomys coucha A3(258-331) (24) VRITCYLTWSPCPNCAWQLAAFKMDH
Cricetulus griseus A3B(235-307) (23) VQITCYLTWSPCPNCAQELAAFKSDH
Peromyscus leucopus A3(266-338) (23) ARITCYLTWSPCPNCAQKLAAFKKDH
Mesocricetus auratus A3(268-340) (23) VRITCYLTWSPCPNCAQELAAFKNDH
Microtus ochrogaster A3B(266-338) (23) ERITCYLTWSPCPNCAQELAAFKRDH
Nannospalax galili A3(231-302) (23) VQITCYITWSPCPTCAQELAAFKQDH
Meriones unguiculatus A3(233-305) (23) AQITCYLTWSPCRKCAQELATFKNDH
Dipodomys ordii A3(256-330) (26) YQITCYLTWSPCLDCAFRLAKLKNDT
Jaculus jaculus A3(303-374) (23) ALITCYLTWSPCLDCSQKLAALKRDH
Chinchilla lanigera A3H(86-161) (27) YQITCFLTWSPCPSCAQELASFKRAH
Heterocephalus glaber A3(277-350) (25) YQVTCFLTWSPCPHCAQELVSFKRAH
Octodon degus A3(256-329) (25) YQITCFLTWSPCPFCAQELASFKSIH
Urocitellus parryii A3(256-330) (26) YEVTCYLTWSPCPDCAQELVALIRSH
Actus nancymaae A3H(75-146) (23) YEVTCYLTWSPCPSCAQKLAAFTKAQ
Cebus capucinus imitator A3H(55-126) (23) YEVTCYLTWSPCPSCAQELVAFAKAQ
Saimiri boliviensis boliviensis A3H(56-125) (21) YDVTCYLTWSPCPSCAQELAAFAKAQ
Homo sapiens A3H(49-123) (26) YQVTCYLTWSPCPSCAWELVDFIKAH
Homo sapiens ARP10(48-123) (27) YQVTCYLTWSPCPSCAWELVDFIKAH
Pan paniscus A3H(49-123) (26) YQVTCYLTWSPCPSCAWKLVDFIQAH
Symphalangus syndactylus A3H(49-123) (26) YQVTCYLTWSPCPSCAWELVDFIKAH
Macaca mulatta A3H(49-123) (26) YQVTCYLTWSPCPSCAGELVDFIKAH
Theropithecus gelada A3H(54-128) (26) YQVTCYLTWSPCPSCAGELVDFIKAH
Mandrillus leucophaeus A3H(49-123) (26) YQVTCYLTWSPCPSCARELVDFIKAH
Bos grunniens A3(74-148) (26) YKIICYITWSPCPNCANELVNFITRN
Bubalus bubalis A3(74-148) (26) YKIICYITWSPCPNCASELVDFITRN
Odocoileus virginianus texanus A3H(209-283) (26) YKIICYITWSPCPRCASELVDFITGN
Sus scrofa A3(51-125) (26) YRIICYVTWSPCHNCAKELVDFISRR
Ceratotherium simum simum A3B(232-306) (26) YEITCYITWSPCPTCALELVAFTRDY
Equus caballus A3H(79-153) (26) YEITCYVTWSPCATCACKLIKFTRKF
Enhydra lutris kenyoni A3B(243-316) (25) FEITCYLTWSPCPTCAKELAMFVQDR
Leptonychotes weddellii A3H(50-123) (25) FEITCYVTWSPCPTCAKELVAFVRDR
Ursus arctos horribilis A3F(552-626) (26) FEITCYVTWSPCFTCAEELVAFVRDR
Panthera leo bleyenberghi A3H(50-124) (26) FEIICYITWSPCPFCAEELVAFVKDR
Panthera tigris sumatrae A3H(50-124) (26) FEIICYITWSPCPFCAEELVAFVKDR
Tupaia belangeri A3(46-120) (26) NQLTCYLTWSPCPSCAQELVTFMAES
Consensus (27) Y ITCYLTWSPCP CA ELAAF KDH

FIG. 39B

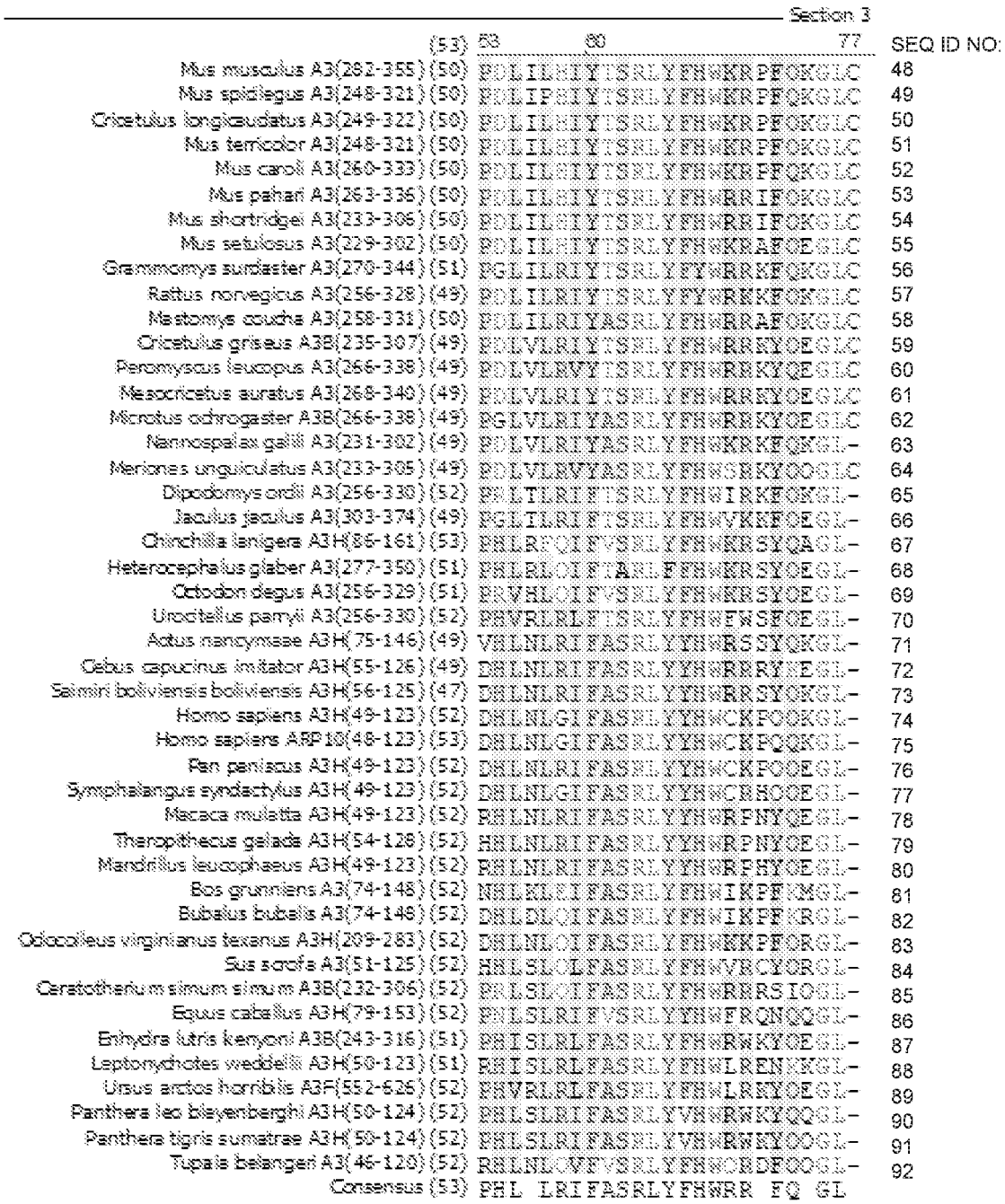

Section 3
(53) 58 60 77 SEQ ID NO:
Mus musculus A3(282-355) (50) PDLILRIYTSRLYFHWKRPFQKGLC 48
Mus spicilegus A3(248-321) (50) PDLIPRIYTSRLYFHWKRPFQKGLC 49
Cricetulus longicaudatus A3(249-322) (50) PDLILRIYTSRLYFHWKRPFQKGLC 50
Mus terricolor A3(248-321) (50) PDLILRIYTSRLYFHWKRPFQKGLC 51
Mus caroli A3(260-333) (50) PDLILRIYTSRLYFHWKRPFQKGLC 52
Mus pahari A3(263-336) (50) PDLILRIYTSRLYFHWRRIFQKGLC 53
Mus shortridgei A3(233-306) (50) PDLILRIYTSRLYFHWRRIFQKGLC 54
Mus setulosus A3(229-302) (50) PDLILRIYTSRLYFHWKRAFQEGLC 55
Grammomys surdaster A3(270-344) (51) PGLILRIYTSRLYFYWRRKFQKGLC 56
Rattus norvegicus A3(256-328) (49) PDLILRIYTSRLYFYWRRKFQKGLC 57
Mastomys coucha A3(258-331) (50) PDLILRIYASRLYFHWRRAFQKGLC 58
Cricetulus griseus A3B(235-307) (49) PDLVLRIYTSRLYFHWRRKYQEGLC 59
Peromyscus leucopus A3(266-338) (49) PDLVLRVYTSRLYFHWRRKYQEGLC 60
Mesocricetus auratus A3(268-340) (49) PDLVLRIYTSRLYFHWRRKYQEGLC 61
Microtus ochrogaster A3B(266-338) (49) PGLVLRIYASRLYFHWRRKYQEGLC 62
Nannospalax galili A3(231-302) (49) PDLVLRIYASRLYFHWKRKFQKGL- 63
Meriones unguiculatus A3(233-305) (49) PDLVLRVYASRLYFHWSRKYQQGLC 64
Dipodomys ordii A3(256-330) (52) PDLTLRIFTSRLYFHWIRKFQKGL- 65
Jaculus jaculus A3(303-374) (49) PGLILRIFTSRLYFHWVKKFQEGL- 66
Chinchilla lanigera A3H(86-161) (53) PHLRFQIFVSRLYFHWKRSYQAGL- 67
Heterocephalus glaber A3(277-350) (51) PHLRLQIFTARLFFHWKRSYQEGL- 68
Octodon degus A3(256-329) (51) PRVHLQIFVSRLYFHWKRSYQEGL- 69
Urocitellus parryii A3(256-330) (52) PHVRLRLFTSRLYFHWFWSFQEGL- 70
Actus nancymaae A3H(75-146) (49) VHLNLRIFASRLYYHWRSSYQKGL- 71
Cebus capucinus imitator A3H(55-126) (49) DHLNLRIFASRLYYHWRRRYQEGL- 72
Saimiri boliviensis boliviensis A3H(56-125) (47) DHLNLRIFASRLYYHWRRSYQKGL- 73
Homo sapiens A3H(49-123) (52) DHLNLGIFASRLYYHWCKPQQKGL- 74
Homo sapiens ARP10(48-123) (53) DHLNLGIFASRLYYHWCKPQQKGL- 75
Pan paniscus A3H(49-123) (52) DHLNLRIFASRLYYHWCKPQQEGL- 76
Symphalangus syndactylus A3H(49-123) (52) DHLNLGIFASRLYYHWCRHQQEGL- 77
Macaca mulatta A3H(49-123) (52) RHLNLRIFASRLYYHWRPNYQEGL- 78
Theropithecus gelada A3H(54-128) (52) HHLNLRIFASRLYYHWRPNYQEGL- 79
Mandrillus leucophaeus A3H(49-123) (52) RHLNLRIFASRLYYHWRPHYQEGL- 80
Bos grunniens A3(74-148) (52) NHLKLEIFASRLYFHWIKPFVMGL- 81
Bubalus bubalis A3(74-148) (52) DHLDLQIFASRLYFHWIKPFKRGL- 82
Odocoileus virginianus texanus A3H(209-283) (52) DHLNLQIFASRLYFHWKKPFQRGL- 83
Sus scrofa A3(51-125) (52) HHLSLQLFASRLYFHWVRCYQRGL- 84
Ceratotherium simum simum A3B(232-306) (52) PHLSLQIFASRLYFHWRRRSIQGL- 85
Equus caballus A3H(79-153) (52) PHLSLRIFVSRLYYHWFRQNQQGL- 86
Enhydra lutris kenyoni A3B(243-316) (51) PHISLRLFASRLYFHWRWKYQEGL- 87
Leptonychotes weddellii A3H(50-123) (51) RHISLRLFASRLYFHWLRENKKGL- 88
Ursus arctos horribilis A3F(552-626) (52) PHVRLRLFASRLYFHWLRNYQEGL- 89
Panthera leo blayenberghi A3H(50-124) (52) PHLSLRIFASRLYVHWRWKYQQGL- 90
Panthera tigris sumatrae A3H(50-124) (52) PHLSLRIFASRLYVHWRWNYQQGL- 91
Tupaia belangeri A3(46-120) (52) RHLNLQVFVSRLYFHWQRDFQQGL- 92
Consensus (53) PHL LRIFASRLYFHWRR FQ GL

FIG. 39C

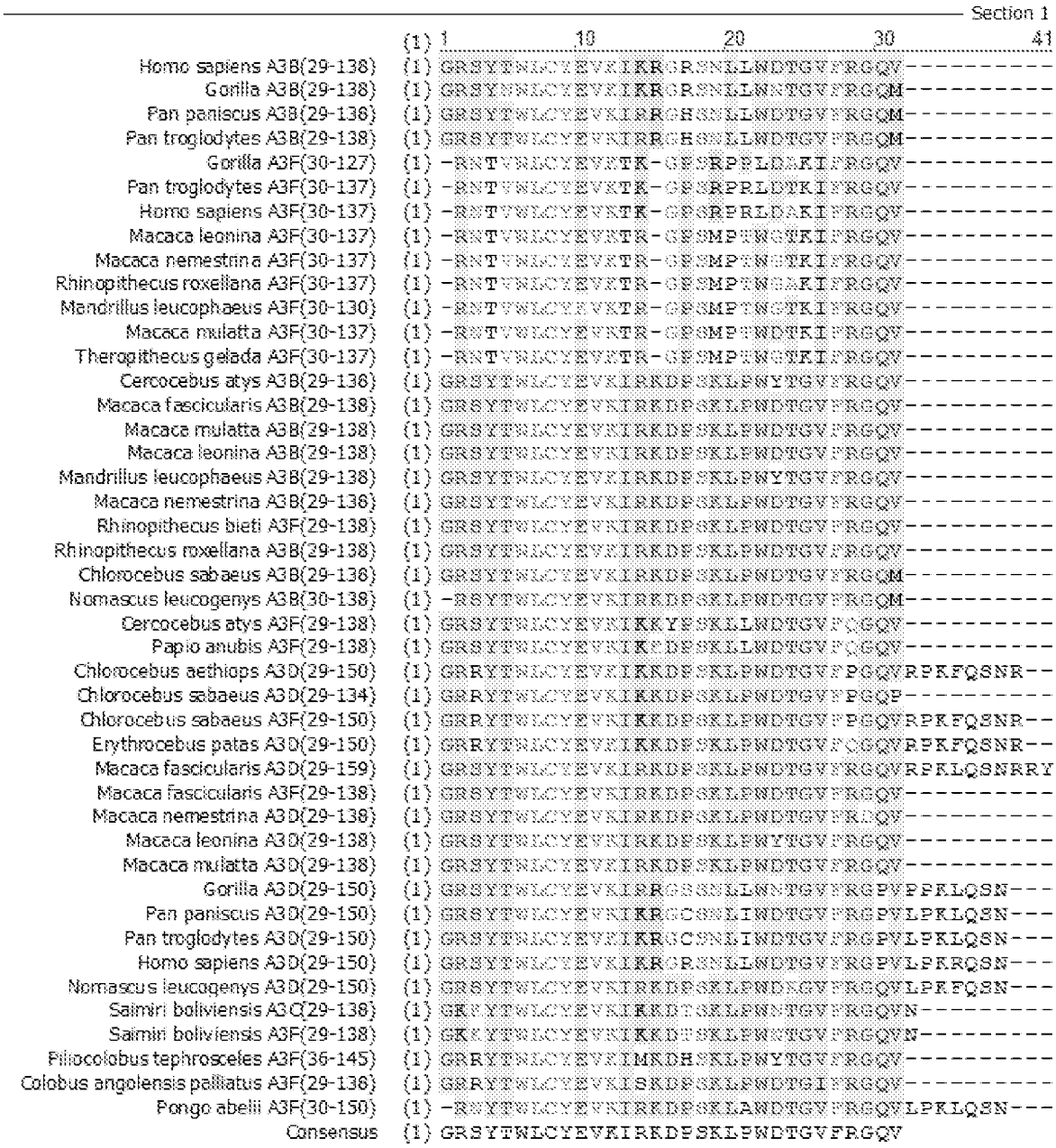

Section 1
(1) 1 10 20 30 41
Homo sapiens A3B(29-138) (1) GRSYTWLCYEVKIKRGRSNLLWDTGVFRGQV----------
Gorilla A3B(29-138) (1) GRSYTWLCYEVKIKRGRSNLLWNTGVFRGQM----------
Pan paniscus A3B(29-136) (1) GRSYTWLCYEVKIRRGHSNLLWDTGVFRGQM----------
Pan troglodytes A3B(29-138) (1) GRSYTWLCYEVKIRRGHSNLLWDTGVFRGQM----------
Gorilla A3F(30-127) (1) -RNTVWLCYEVNTK-GPSRPPLDAKIFRGQV----------
Pan troglodytes A3F(30-137) (1) -RNTVWLCYEVKTK-GPSRPRLDTKIFRGQV----------
Homo sapiens A3F(30-137) (1) -RNTVWLCYEVNTK-GPGRPRLDAKIFRGQV----------
Macaca leonina A3F(30-137) (1) -RNTVWLCYEVNTR-GPSMPTWGTKIFRGQV----------
Macaca nemestrina A3F(30-137) (1) -RNTVWLCYEVKTR-GPSMPTWGTKIFRGQV----------
Rhinopithecus roxellana A3F(30-137) (1) -RNTVWLCYEVKTR-GPSMPTWGAKIFRGQV----------
Mandrillus leucophaeus A3F(30-130) (1) -RNTVWLCYEVKTR-GPGMPTWGTKIFRGQV----------
Macaca mulatta A3F(30-137) (1) -RNTVWLCYEVNTR-GPGMPTWDTKIFRGQV----------
Theropithecus gelada A3F(30-137) (1) -RNTVWLCYEVNTR-GPSMPTWGTKIFRGQV----------
Cercocebus atys A3B(29-136) (1) GRSYTWLCYEVKIRKDPSKLPWYTGVFRGQV----------
Macaca fascicularis A3B(29-138) (1) GRSYTWLCYEVNIRKDPSKLPWDTGVFRGQV----------
Macaca mulatta A3B(29-138) (1) GRSYTWLCYEVNIRKDPSKLPWDTGVFRGQV----------
Macaca leonina A3B(29-138) (1) GRSYTWLCYEVNIRKDPSKLPWDTGVFRGQV----------
Mandrillus leucophaeus A3B(29-138) (1) GRSYTWLCYEVKIRKDPSKLPWYTGVFRGQV----------
Macaca nemestrina A3B(29-136) (1) GRSYTWLCYEVKIRKDPSKLPWDTGVFRGQV----------
Rhinopithecus bieti A3F(29-138) (1) GRSYTWLCYEVNIRKDPSKLPWDTGVFRGQV----------
Rhinopithecus roxellana A3B(29-138) (1) GRSYTWLCYEVNIRKDPSKLPWDTGVFRGQV----------
Chlorocebus sabaeus A3B(29-136) (1) GRSYTWLCYEVKIRKDPSKLPWDTGVFRGQM----------
Nomascus leucogenys A3B(30-138) (1) -RSYTWLCYEVNIRKDPSKLPWDTGVFRGQM----------
Cercocebus atys A3F(29-138) (1) GRSYTWLCYEVNIKKYPSKLLWDTGVFQGQV----------
Papio anubis A3F(29-138) (1) GRSYTWLCYEVNIKKDPSKLLWDTGVFQGQV----------
Chlorocebus aethiops A3D(29-150) (1) GRRYTWLCYEVKIKKDPSKLPWDTGVFPGQVRPKFQSNR--
Chlorocebus sabaeus A3D(29-134) (1) GRRYTWLCYEVKIKKDPSKLPWDTGVFPGQP----------
Chlorocebus sabaeus A3F(29-150) (1) GRRYTWLCYEVNIKKDPSKLPWDTGVFPGQVRPKFQSNR--
Erythrocebus patas A3D(29-150) (1) GRRYTWLCYEVKIKKDPSKLPWDTGVFQGQVRPKFQSNR--
Macaca fascicularis A3D(29-159) (1) GRSYTWLCYEVKIRKDPSKLPWDTGVFRGQVRPKLQSNRRY
Macaca fascicularis A3F(29-138) (1) GRSYTWLCYEVNIRKDPSKLPWDTGVFRGQV----------
Macaca nemestrina A3D(29-138) (1) GRSYTWLCYEVKIRKDPSKLPWDTGVFRDQV----------
Macaca leonina A3D(29-138) (1) GRSYTWLCYEVNIRKDPSKLPWYTGVFRGQV----------
Macaca mulatta A3D(29-138) (1) GRSYTWLCYEVKIRKDPSKLPWDTGVFRGQV----------
Gorilla A3D(29-150) (1) GRSYTWLCYEVKIRRGRSNLLWNTGVFRGPVPPKLQSN---
Pan paniscus A3D(29-150) (1) GRSYTWLCYEVNIKRGCSNLIWDTGVFRGPVLPKLQSN---
Pan troglodytes A3D(29-150) (1) GRSYTWLCYEVKIKRGCSNLIWDTGVFRGPVLPKLQSN---
Homo sapiens A3D(29-150) (1) GRSYTWLCYEVKIKRGRSNLLWDTGVFRGPVLPKRQSN---
Nomascus leucogenys A3D(29-150) (1) GRSYTWLCYEVNIRKDPSKLPWDTGVFRGQVLPKFQSN---
Saimiri boliviensis A3C(29-138) (1) GRSYTWLCYEVKIKKDTSKLPWNTGVFRGQVN----------
Saimiri boliviensis A3F(29-138) (1) GKSYTWLCYEVNIKKDTSKLPWNTGVFRGQVN----------
Piliocolobus tephrosceles A3F(36-145) (1) GRRYTWLCYEVKIMKDHSKLPWYTGVFRGQV----------
Colobus angolensis palliatus A3F(29-136) (1) GRRYTWLCYEVKISKDPSKLPWDTGIFRGQV----------
Pongo abelii A3F(30-150) (1) -RSYTWLCYEVNIRKDPSKLAWDTGVFRGQVLPKLQSN---
Consensus (1) GRSYTWLCYEVKIRKDPSKLPWDTGVFRGQV

FIG. 40A

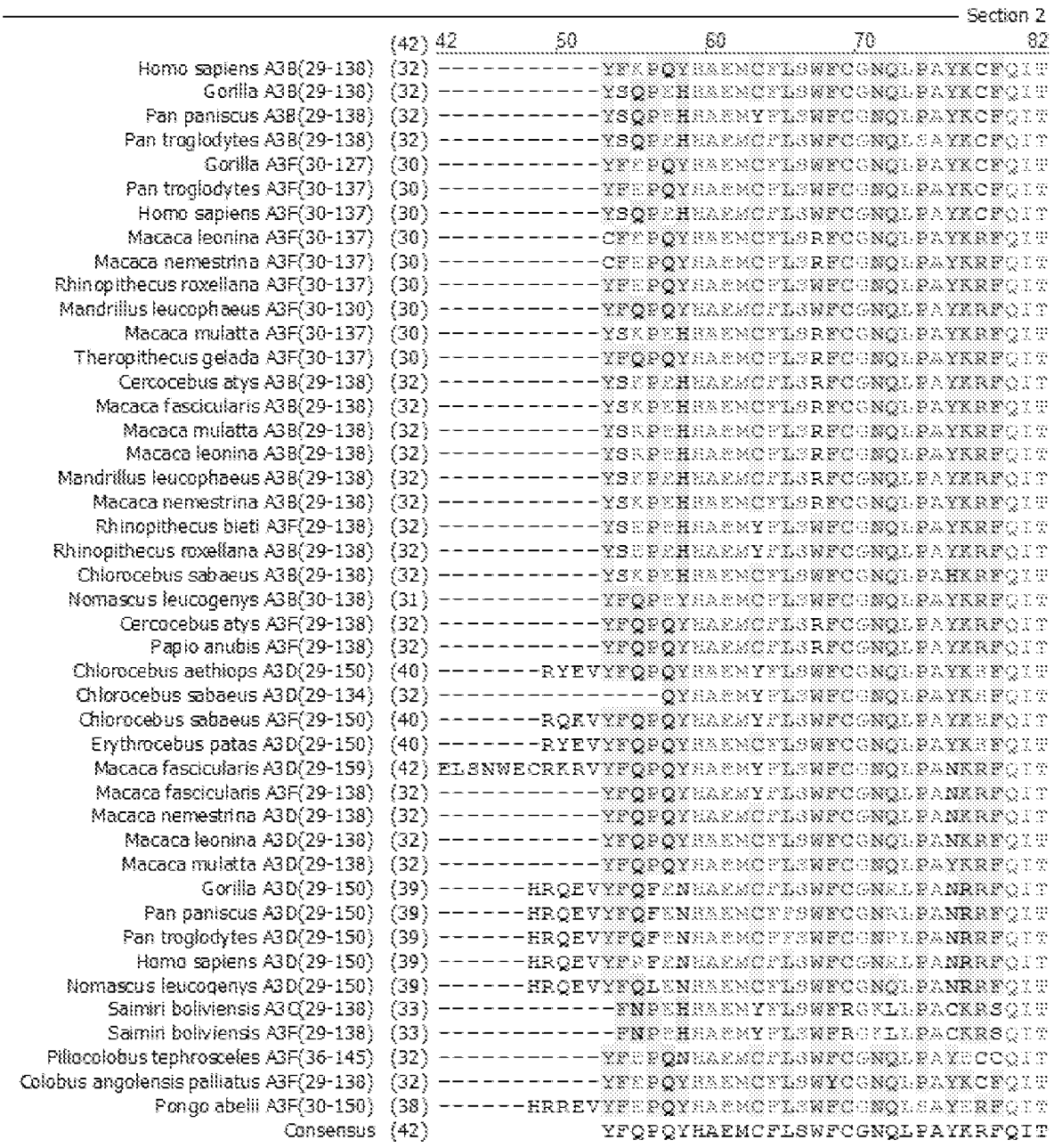

Section 2
(42) 42 50 60 70 82
Homo sapiens A3B(29-138) (32)
Gorilla A3B(29-138) (32)
Pan paniscus A3B(29-138) (32)
Pan troglodytes A3B(29-138) (32)
Gorilla A3F(30-127) (30)
Pan troglodytes A3F(30-137) (30)
Homo sapiens A3F(30-137) (30)
Macaca leonina A3F(30-137) (30)
Macaca nemestrina A3F(30-137) (30)
Rhinopithecus roxellana A3F(30-137) (30)
Mandrillus leucophaeus A3F(30-130) (30)
Macaca mulatta A3F(30-137) (30)
Theropithecus gelada A3F(30-137) (30)
Cercocebus atys A3B(29-138) (32)
Macaca fascicularis A3B(29-138) (32)
Macaca mulatta A3B(29-138) (32)
Macaca leonina A3B(29-138) (32)
Mandrillus leucophaeus A3B(29-138) (32)
Macaca nemestrina A3B(29-138) (32)
Rhinopithecus bieti A3F(29-138) (32)
Rhinopithecus roxellana A3B(29-138) (32)
Chlorocebus sabaeus A3B(29-138) (32)
Nomascus leucogenys A3B(30-138) (31)
Cercocebus atys A3F(29-138) (32)
Papio anubis A3F(29-138) (32)
Chlorocebus aethiops A3D(29-150) (40)
Chlorocebus sabaeus A3D(29-134) (32)
Chlorocebus sabaeus A3F(29-150) (40)
Erythrocebus patas A3D(29-150) (40)
Macaca fascicularis A3D(29-159) (42)
Macaca fascicularis A3F(29-138) (32)
Macaca nemestrina A3D(29-138) (32)
Macaca leonina A3D(29-138) (32)
Macaca mulatta A3D(29-138) (32)
Gorilla A3D(29-150) (39)
Pan paniscus A3D(29-150) (39)
Pan troglodytes A3D(29-150) (39)
Homo sapiens A3D(29-150) (39)
Nomascus leucogenys A3D(29-150) (39)
Saimiri boliviensis A3C(29-138) (33)
Saimiri boliviensis A3F(29-138) (33)
Piliocolobus tephrosceles A3F(36-145) (32)
Colobus angolensis palliatus A3F(29-138) (32)
Pongo abelii A3F(30-150) (38)
Consensus (42)

FIG. 40B

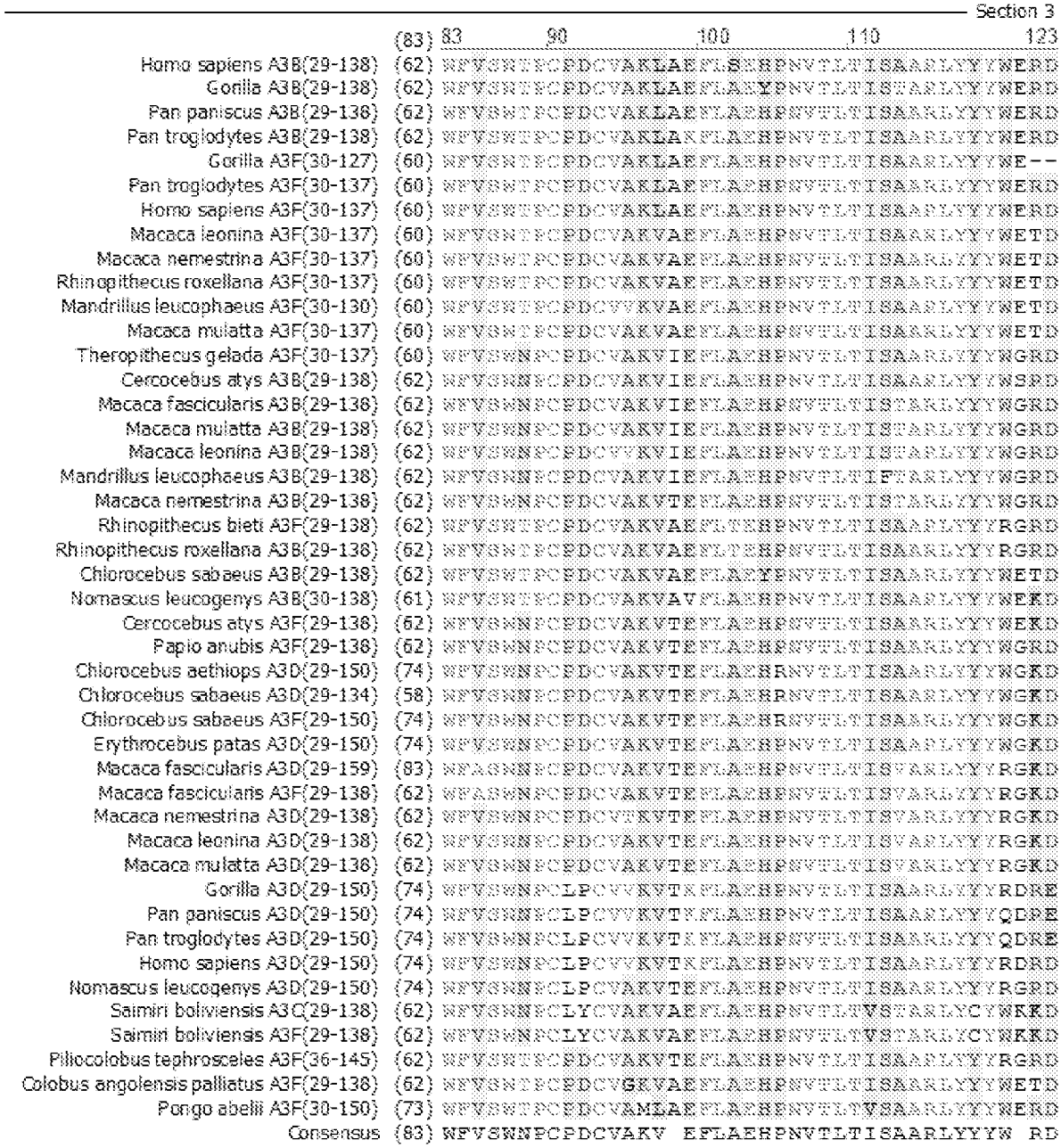

Section 3
(83) 83 90 100 110 123
Homo sapiens A3B(29-138) (62) WFVSWNPCPDCVAKLAEFLSEHPNVTLTISAARLYYYWERD
Gorilla A3B(29-138) (62) WFVSWNPCPDCVAKLAEFLAEYPNVTLTISVARLYYYWERD
Pan paniscus A3B(29-138) (62) WFVSWNPCPDCVAKLAEFLAEHPNVTLTISAARLYYYWERD
Pan troglodytes A3B(29-138) (62) WFVSWNPCPDCVAKLAEFLAEHPNVTLTISAARLYYYWERD
Gorilla A3F(30-127) (60) WFVSWNPCPDCVAKLAEFLAEHPNVTLTISAARLYYYWE--
Pan troglodytes A3F(30-137) (60) WFVSWNPCPDCVAKLAEFLAEHPNVTLTISAARLYYYWERD
Homo sapiens A3F(30-137) (60) WFVSWNPCPDCVAKLAEFLAEHPNVTLTISAARLYYYWERD
Macaca leonina A3F(30-137) (60) WFVSWNPCPDCVAKVAEFLAEHPNVTLTISAARLYYYWETD
Macaca nemestrina A3F(30-137) (60) WFVSWNPCPDCVAKVAEFLAEHPNVTLTISAARLYYYWETD
Rhinopithecus roxellana A3F(30-137) (60) WFVSWNPCPDCVAKVAEFLAEHPNVTLTISAARLYYYWETD
Mandrillus leucophaeus A3F(30-130) (60) WFVSWNPCPDCVVKVAEFLAEHPNVTLTISAARLYYYWETD
Macaca mulatta A3F(30-137) (60) WFVSWNPCPDCVAKVAEFLAEHPNVTLTISAARLYYYWETD
Theropithecus gelada A3F(30-137) (60) WFVSWNPCPDCVAKVIEFLAEHPNVTLTISAARLYYYWGRD
Cercocebus atys A3B(29-138) (62) WFVSWNPCPDCVAKVIEFLAEHPNVTLTISAARLYYYWSPD
Macaca fascicularis A3B(29-138) (62) WFVSWNPCPDCVAKVIEFLAEHPNVTLTISTARLYYYWGRD
Macaca mulatta A3B(29-138) (62) WFVSWNPCPDCVAKVIEFLAEHPNVTLTISTARLYYYWGRD
Macaca leonina A3B(29-138) (62) WFVSWNPCPDCVVKVIEFLAEHPNVTLTISTARLYYYWGRD
Mandrillus leucophaeus A3B(29-138) (62) WFVSWNPCPDCVAKVIEFLAEHPNVTLTIFTARLYYYWGRD
Macaca nemestrina A3B(29-138) (62) WFVSWNPCPDCVAKVTEFLAEHPNVTLTISTARLYYYWGRD
Rhinopithecus bieti A3F(29-138) (62) WFVSWNPCPDCVAKVAEFLTEHPNVTLTISAARLYYYRGRD
Rhinopithecus roxellana A3B(29-138) (62) WFVSWNPCPDCVAKVAEFLTEHPNVTLTISAARLYYYRGRD
Chlorocebus sabaeus A3B(29-138) (62) WFVSWNPCPDCVAKVAEFLAEYPNVTLTISAARLYYYWETD
Nomascus leucogenys A3B(30-138) (61) WFVSWNPCPDCVAKVAVFLAEHPNVTLTISAARLYYYWEKD
Cercocebus atys A3F(29-138) (62) WFVSWNPCPDCVAKVTEFLAEHPNVTLTISAARLYYYWEKD
Papio anubis A3F(29-138) (62) WFVSWNPCPDCVAKVTEFLAEHPNVTLTISAARLYYYWGRD
Chlorocebus aethiops A3D(29-150) (74) WFVSWNPCPDCVAKVTEFLAEHRNVTLTISAARLYYYWGKD
Chlorocebus sabaeus A3D(29-134) (58) WFVSWNPCPDCVAKVTEFLAEHRNVTLTISAARLYYYWGKD
Chlorocebus sabaeus A3F(29-150) (74) WFVSWNPCPDCVAKVTEFLAEHRNVTLTISAARLYYYWGKD
Erythrocebus patas A3D(29-150) (74) WFVSWNPCPDCVAKVTEFLAEHPNVTLTISAARLYYYWGKD
Macaca fascicularis A3D(29-159) (83) WFASWNPCPDCVAKVTEFLAEHPNVTLTISVARLYYYRGKD
Macaca fascicularis A3F(29-138) (62) WFASWNPCPDCVAKVTEFLAEHPNVTLTISVARLYYYRGKD
Macaca nemestrina A3D(29-138) (62) WFVSWNPCPDCVTKVTEFLAEHPNVTLTISVARLYYYRGKD
Macaca leonina A3D(29-138) (62) WFVSWNPCPDCVAKVTEFLAEHPNVTLTISVARLYYYRGKD
Macaca mulatta A3D(29-138) (62) WFVSWNPCPDCVAKVTEFLAEHPNVTLTISVARLYYYRGKD
Gorilla A3D(29-150) (74) WFVSWNPCLPCVVKVTKFLAEHPNVTLTISAARLYYYRDRE
Pan paniscus A3D(29-150) (74) WFVSWNPCLPCVVKVTYFLAEHPNVTLTISAARLYYYQDPE
Pan troglodytes A3D(29-150) (74) WFVSWNPCLPCVVKVTKFLAEHPNVTLTISAARLYYYQDRE
Homo sapiens A3D(29-150) (74) WFVSWNPCLPCVVKVTKFLAEHPNVTLTISAARLYYYRDRD
Nomascus leucogenys A3D(29-150) (74) WFVSWNPCLPCVAKVTEFLAEHPNVTLTISAARLYYYRGRD
Saimiri boliviensis A3C(29-138) (62) WFVSWNPCLYCVAKVAEFLAEHPNVTLTVSTARLYCYWKKD
Saimiri boliviensis A3F(29-138) (62) WFVSWNPCLYCVAKVAEFLAEHPNVTLTVSTARLYCYWKKD
Piliocolobus tephrosceles A3F(36-145) (62) WFVSWNPCPDCVAKVTEFLAEHPNVTLTISAARLYYYRGRD
Colobus angolensis palliatus A3F(29-138) (62) WFVSWNPCPDCVGKVAEFLAEHPNVTLTISAARLYYYWETD
Pongo abelii A3F(30-150) (73) WFVSWNPCPDCVAMLAEFLAEHPNVTLTVSAARLYYYWERD
Consensus (83) WFVSWNPCPDCVAKV EFLAEHPNVTLTISAARLYYYW RD

FIG. 40C

Section 4

| | | 124 131 | SEQ ID NO: |
|---|---|---|---|
| Homo sapiens A3B(29-138) | (103) | YRRALCRL | 93 |
| Gorilla A3B(29-138) | (103) | YRRALCRL | 94 |
| Pan paniscus A3B(29-138) | (103) | YRRALCRL | 95 |
| Pan troglodytes A3B(29-138) | (103) | YRRALCRL | 96 |
| Gorilla A3F(30-127) | (99) | -------- | 97 |
| Pan troglodytes A3F(30-137) | (101) | YRRALCRL | 98 |
| Homo sapiens A3F(30-137) | (101) | YRRALCRL | 99 |
| Macaca leonina A3F(30-137) | (101) | YRRALCRL | 100 |
| Macaca nemestrina A3F(30-137) | (101) | YRRALCRL | 101 |
| Rhinopithecus roxellana A3F(30-137) | (101) | YRRALCRL | 102 |
| Mandrillus leucophaeus A3F(30-130) | (101) | Y------- | 103 |
| Macaca mulatta A3F(30-137) | (101) | YRRALCRL | 104 |
| Theropithecus gelada A3F(30-137) | (101) | WRRALRRL | 105 |
| Cercocebus atys A3B(29-138) | (103) | WQRALCRL | 106 |
| Macaca fascicularis A3B(29-138) | (103) | WQRALCRL | 107 |
| Macaca mulatta A3B(29-138) | (103) | WQRALCRL | 108 |
| Macaca leonina A3B(29-138) | (103) | WQRALCRL | 109 |
| Mandrillus leucophaeus A3B(29-138) | (103) | WQRALCRL | 110 |
| Macaca nemestrina A3B(29-138) | (103) | WQRALCRL | 111 |
| Rhinopithecus bieti A3F(29-136) | (103) | WRRALCRL | 112 |
| Rhinopithecus roxellana A3B(29-138) | (103) | WRRALCRL | 113 |
| Chlorocebus sabaeus A3B(29-138) | (103) | YRRALCRL | 114 |
| Nomascus leucogenys A3B(30-136) | (102) | WQRALCRL | 115 |
| Cercocebus atys A3F(29-138) | (103) | XRRALRRL | 116 |
| Papio anubis A3F(29-138) | (103) | WRRALRRL | 117 |
| Chlorocebus aethiops A3D(29-150) | (115) | WRRALCRL | 118 |
| Chlorocebus sabaeus A3D(29-134) | (99) | WRRALCRL | 119 |
| Chlorocebus sabaeus A3F(29-150) | (115) | WRRALCRL | 120 |
| Erythrocebus patas A3D(29-150) | (115) | WRRALCRL | 121 |
| Macaca fascicularis A3D(29-159) | (124) | WRRALRRL | 122 |
| Macaca fascicularis A3F(29-136) | (103) | WRRALRRL | 123 |
| Macaca nemestrina A3D(29-136) | (103) | WRRALRRL | 124 |
| Macaca leonina A3D(29-138) | (103) | WRRALRRL | 125 |
| Macaca mulatta A3D(29-138) | (103) | WRRALCRL | 126 |
| Gorilla A3D(29-150) | (115) | WRRYLRRL | 127 |
| Pan paniscus A3D(29-150) | (115) | WRRYLRRL | 128 |
| Pan troglodytes A3D(29-150) | (115) | WRRYLRRL | 129 |
| Homo sapiens A3D(29-150) | (115) | WRWYLLRL | 130 |
| Nomascus leucogenys A3D(29-150) | (115) | WRRALRRL | 131 |
| Saimiri boliviensis A3C(29-138) | (103) | WRRALRKL | 132 |
| Saimiri boliviensis A3F(29-138) | (103) | WRRALRKL | 133 |
| Piliocolobus tephrosceles A3F(36-145) | (103) | WRRALRRL | 134 |
| Colobus angolensis palliatus A3F(29-136) | (103) | YRRALCRL | 135 |
| Consensus | (124) | WRRALCRL | |

FIG. 40D