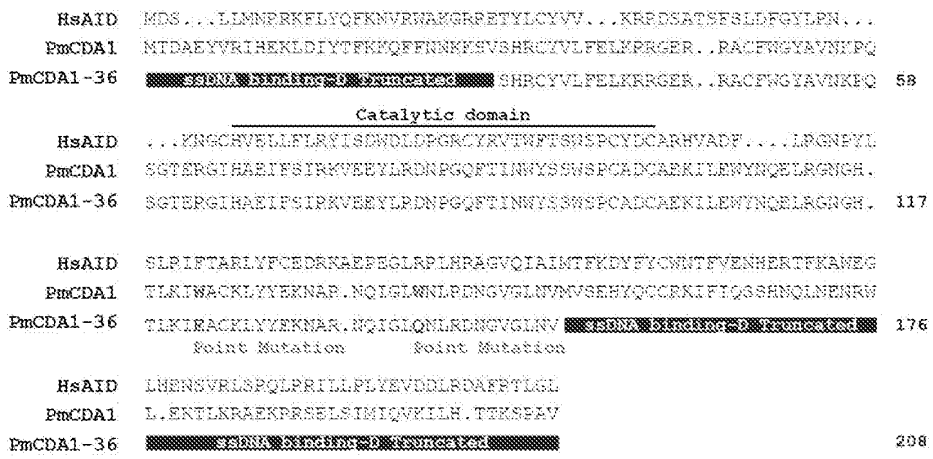
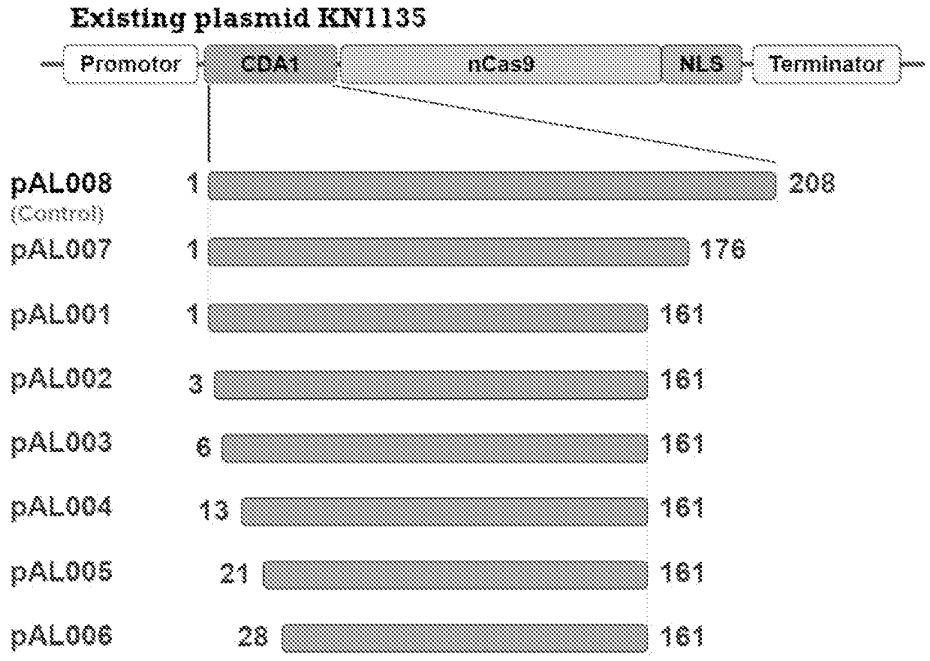
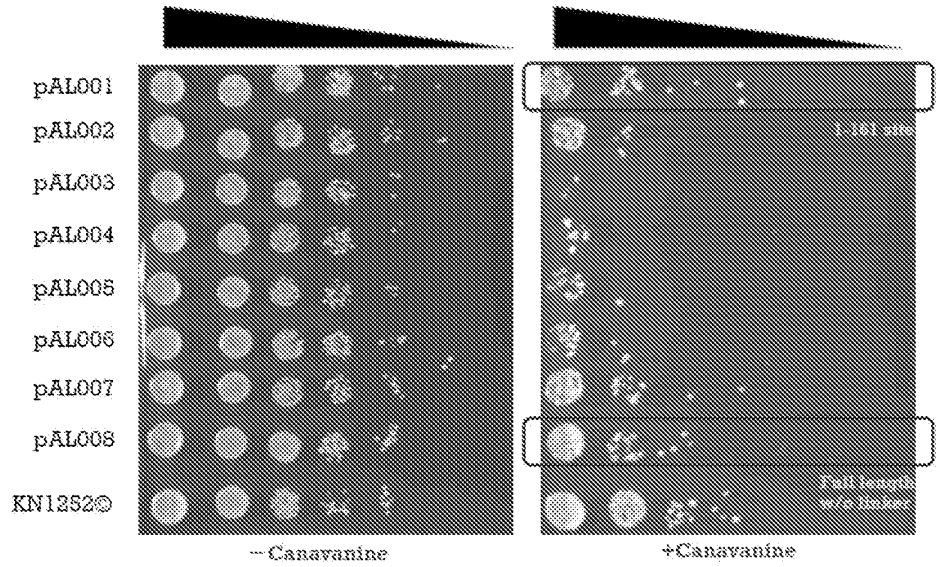
[図1]


HsAID
PmCDA1
PmCDA1-36
Catalytic domain
Point Mutation
Point Mutation

[図2]

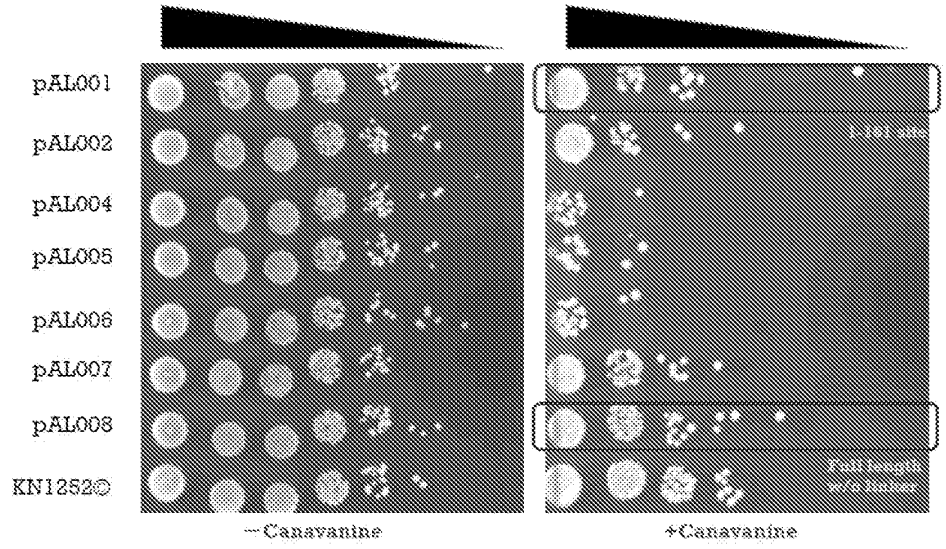

Existing plasmid KN1135
Promotor
CDA1
nCas9
NLS
Terminator
pAL008
(Control)
pAL007
pAL001
pAL002
pAL003
pAL004
pAL005
pAL006

[図3]



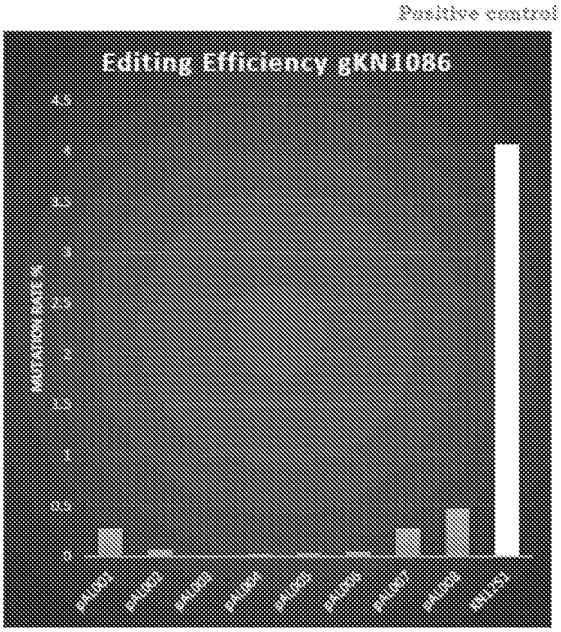
Galactoses Induction(0.02%)
gRNA: KN1086

[図4]

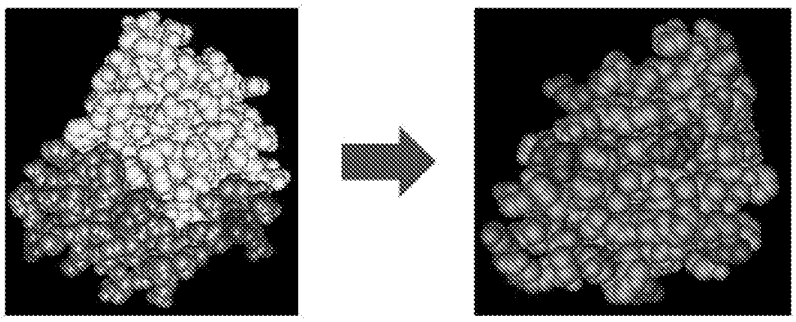


Galactoses Induction(0.02%)
gRNA: KN1086

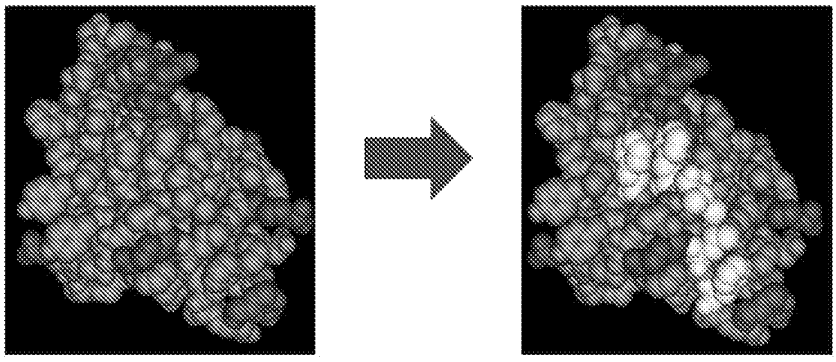
[図5]



[図6]

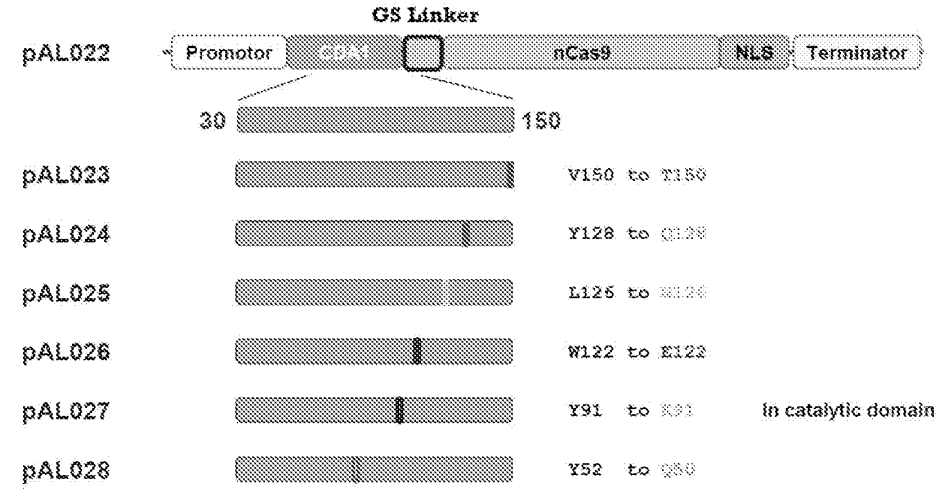


[図7]

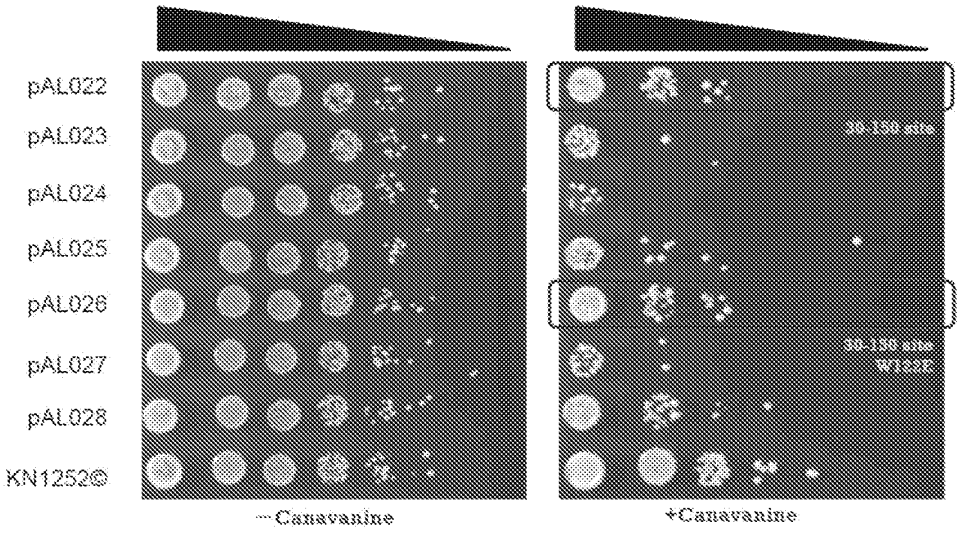


V150 to T150 Y128 to Q128
L126 to N126 W122 to E122
Y91 to K91

[図8]

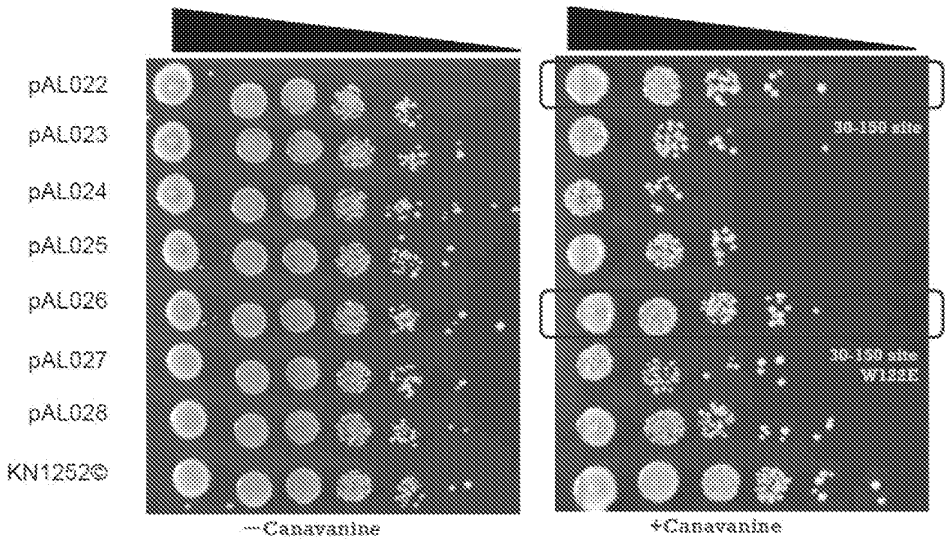


[図9]



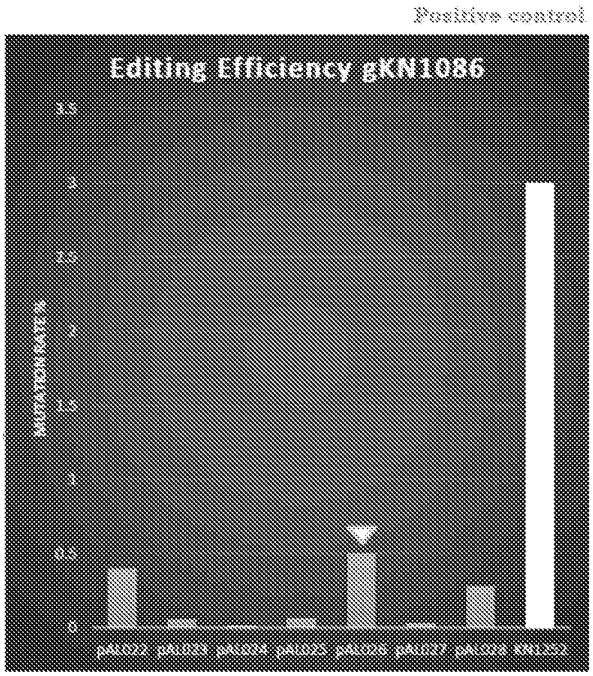
Galactoses Induction(0.02%)
gRNA: KN1086

[図10]

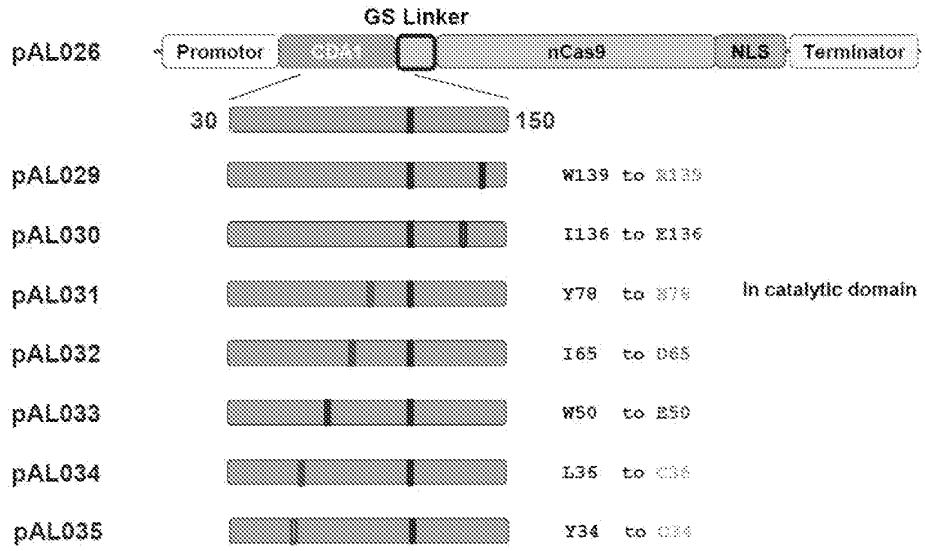


Galactoses Induction(0.02%)
gRNA: KN1150

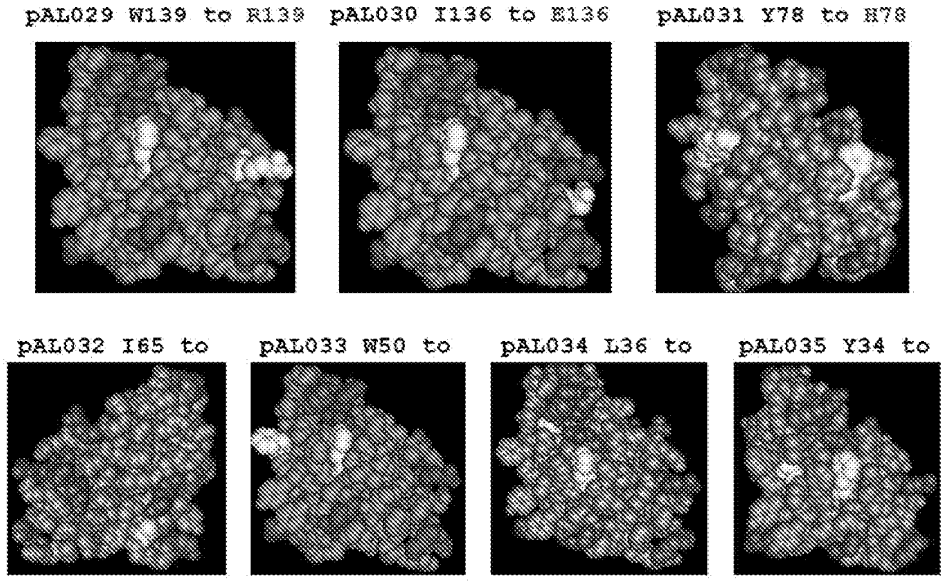
[図11]



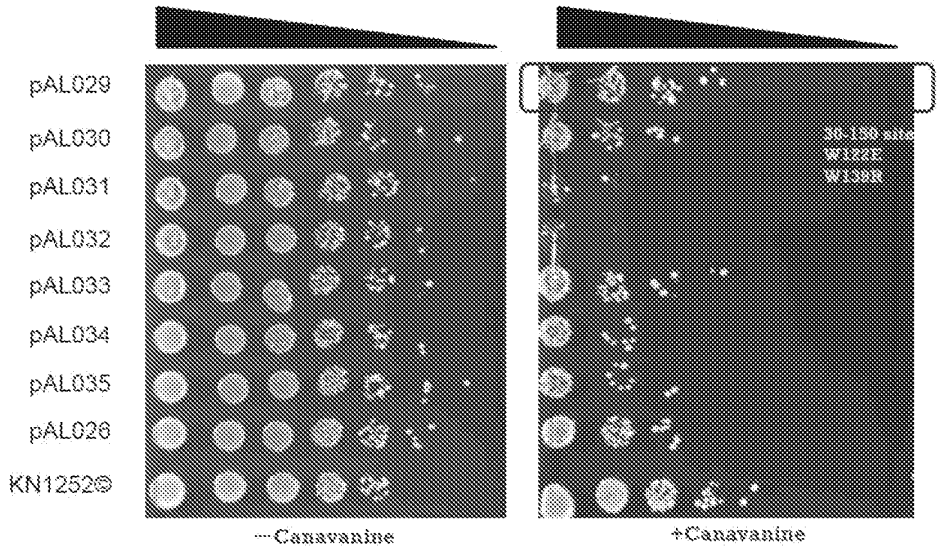
[図12]


GS Linker
pAL026
Promotor
CDA1
nCas9
NLS
Terminator
30
150
pAL029
W139 to R139
pAL030
I136 to E136
pAL031
Y78 to H78
In catalytic domain
pAL032
I65 to D65
pAL033
W50 to R50
pAL034
L36 to C36
pAL035
Y34 to C34

[図13]

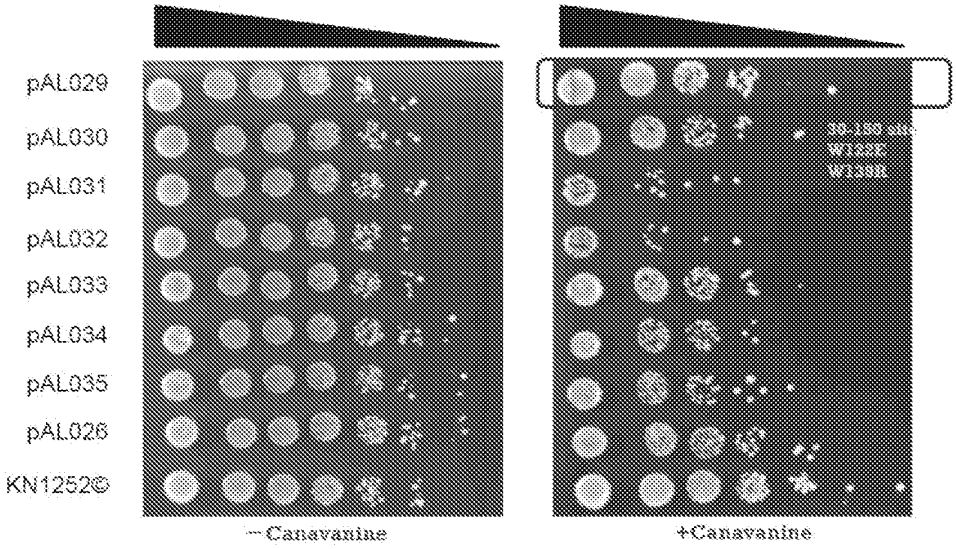

pAL029 W139 to R139
pAL030 I136 to E136
pAL031 Y78 to H78
pAL032 I65 to
pAL033 W50 to
pAL034 L36 to
pAL035 Y34 to

[図14]



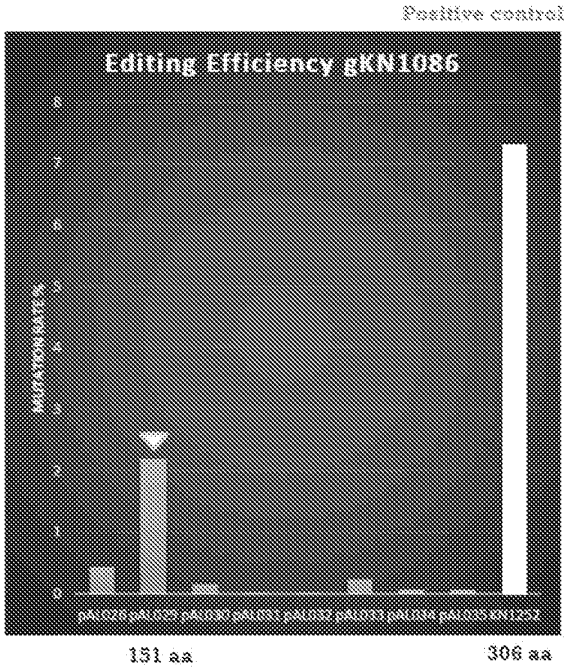
Galactoses Induction(0.02%)
gRNA: KN1086

[図15]

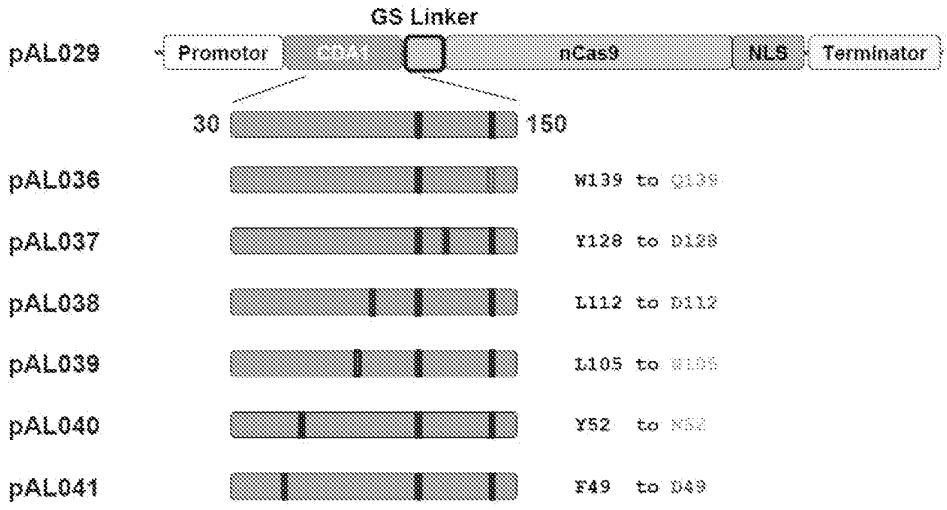


Galactoses Induction(0.02%)
gRNA: KN1150

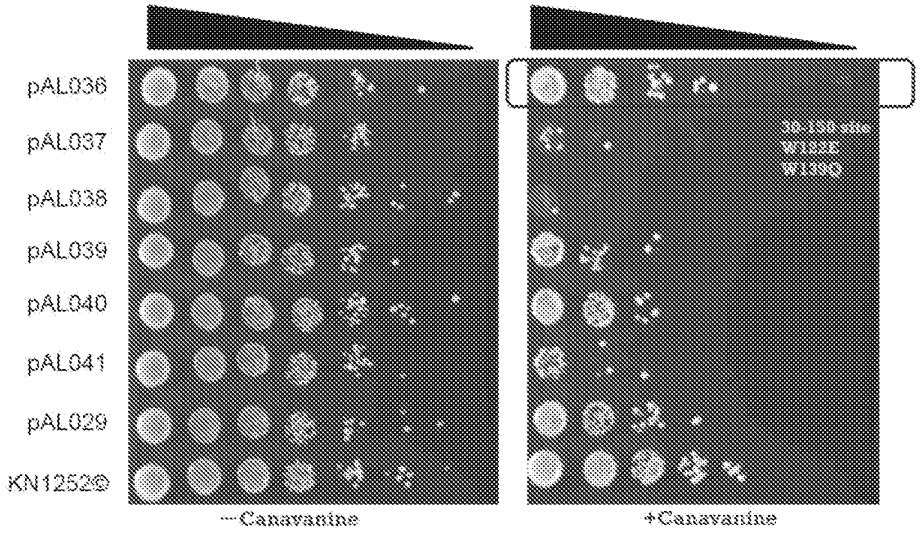
[図16]


Positive control
Editing Efficiency gKN1086
151 aa
306 aa

[図17]

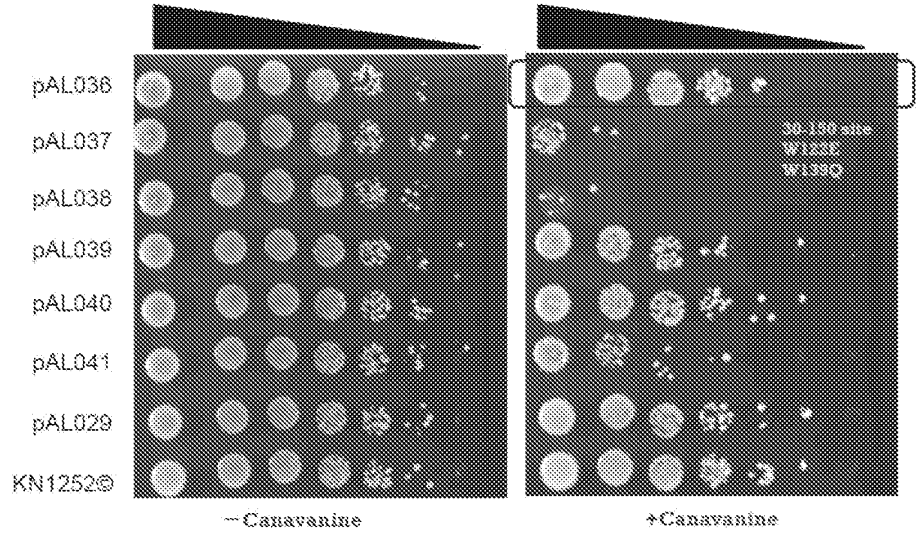

GS Linker
pAL029
Promotor
nCas9
NLS
Terminator
30
150
pAL036
W139 to Q139
pAL037
Y128 to D128
pAL038
L112 to D112
pAL039
L105 to
pAL040
Y52 to
pAL041
F49 to D49

[図18]



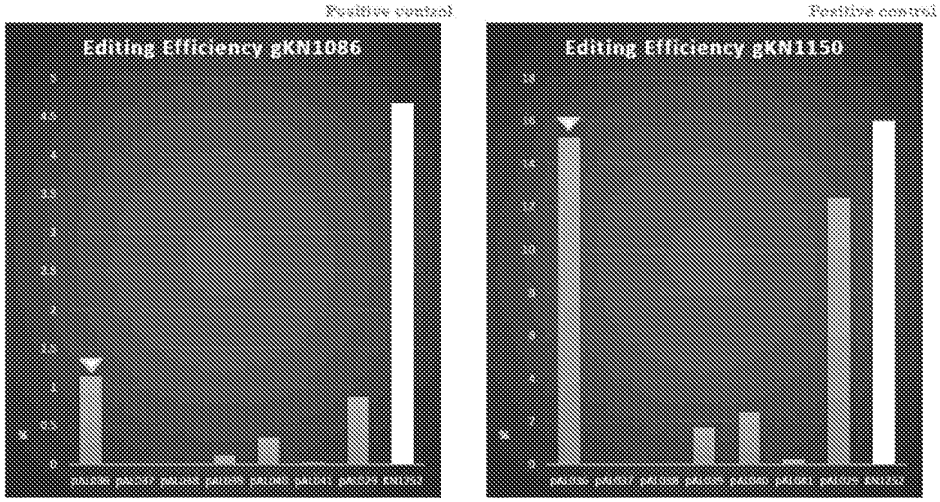
Galactoses Induction(0.02%)
gRNA: KN1086

[図19]

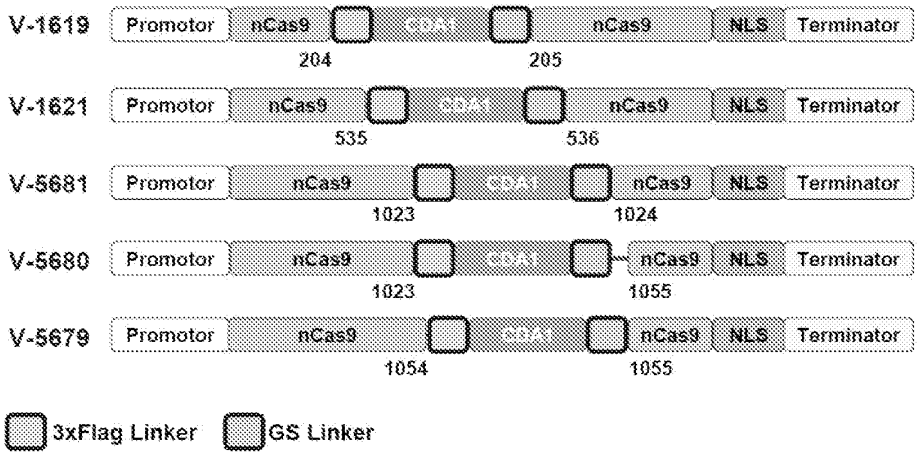


Galactoses Induction(0.02%)
gRNA: KN1150

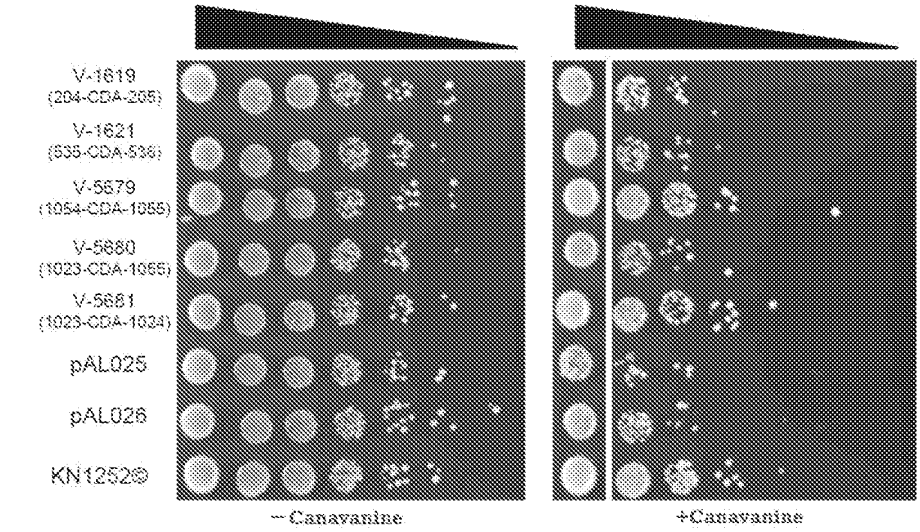
[図20]



[図21]

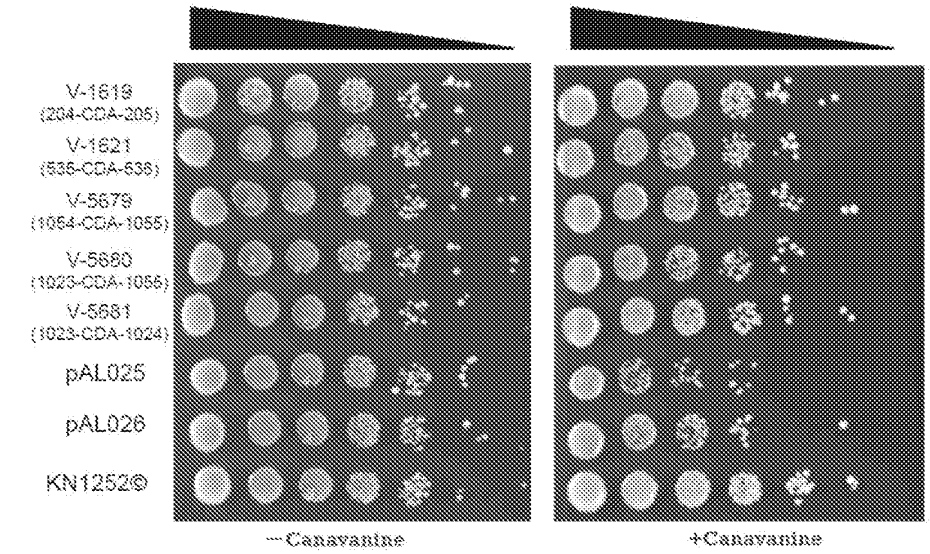


[図22]



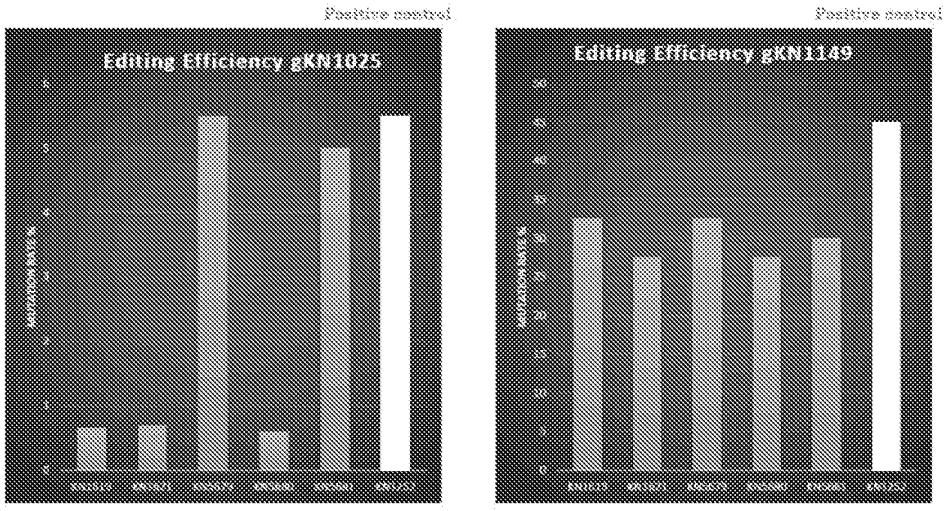
Galactoses Induction(0.02%)
gRNA: KN1025

[図23]

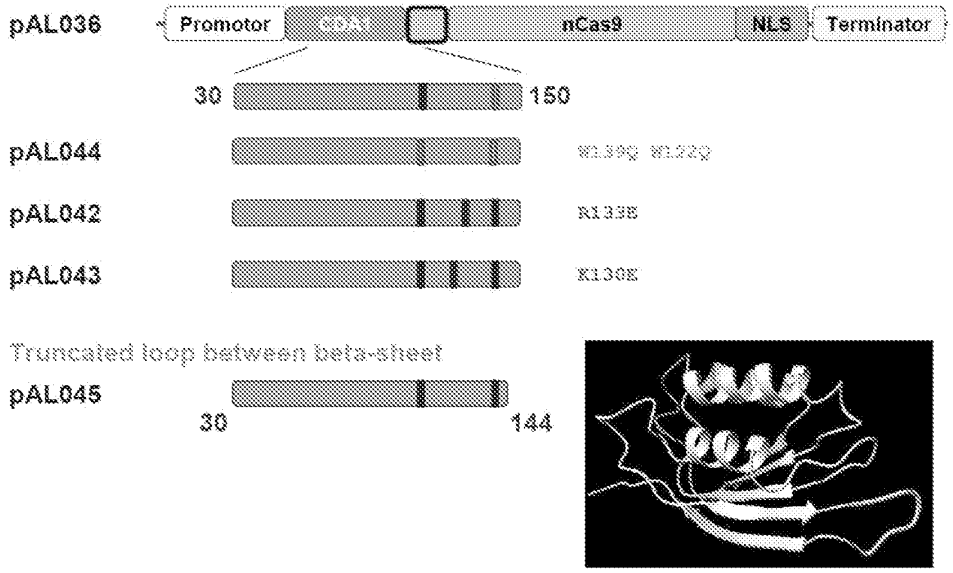


Galactoses Induction(0.02%)
gRNA: KN1149

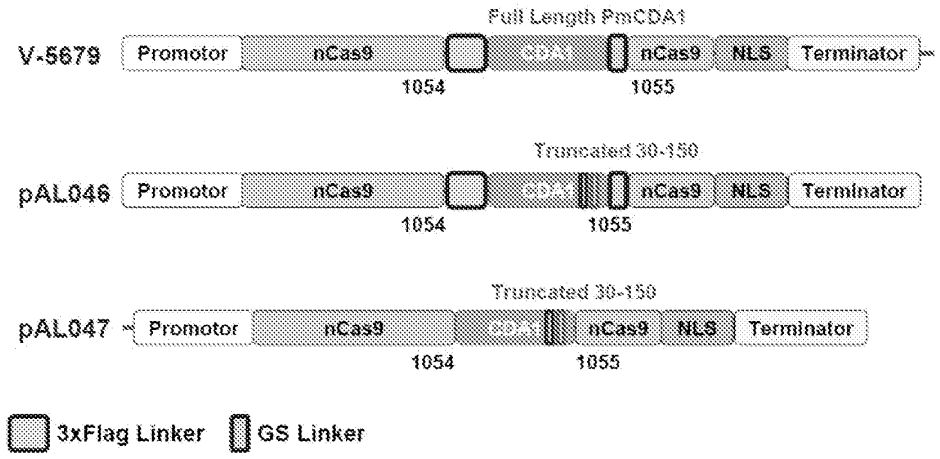
[図24]



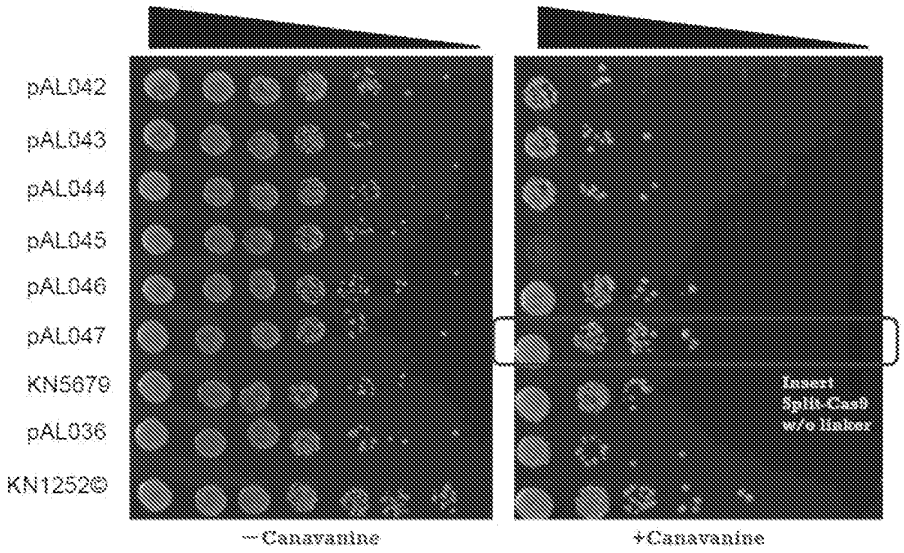
[図25]


pAL036
Promotor
CDA1
nCas9
NLS
Terminator
30
150
pAL044
pAL042
pAL043
Truncated loop between beta-sheet
pAL045
30
144

[図26]

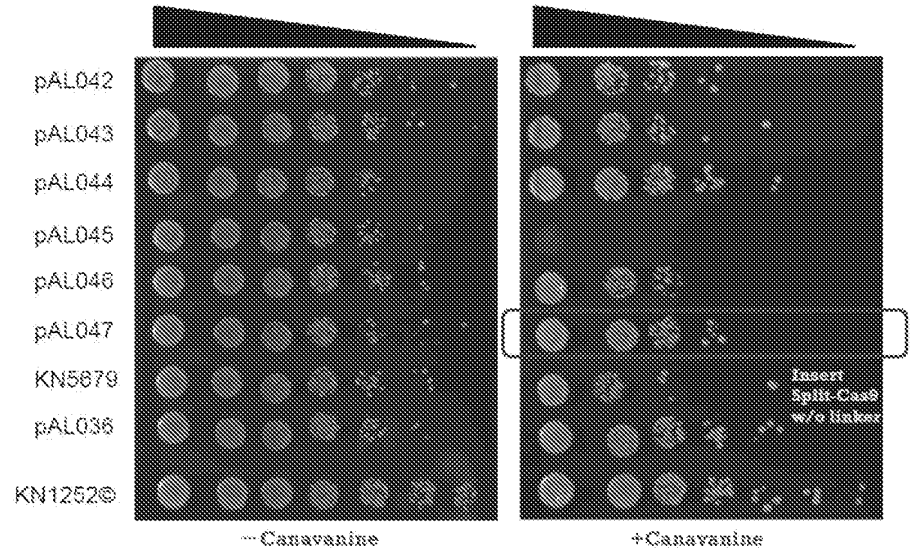

Full Length PmCDA1
V-5679
Promotor
nCas9
CDA1
nCas9
NLS
Terminator
1054
1055
Truncated 30-150
pAL046
Promotor
nCas9
CDA1
nCas9
NLS
Terminator
1054
1055
Truncated 30-150
pAL047
Promotor
nCas9
CDA1
nCas9
NLS
Terminator
1054
1055
3xFlag Linker
GS Linker

[図27]



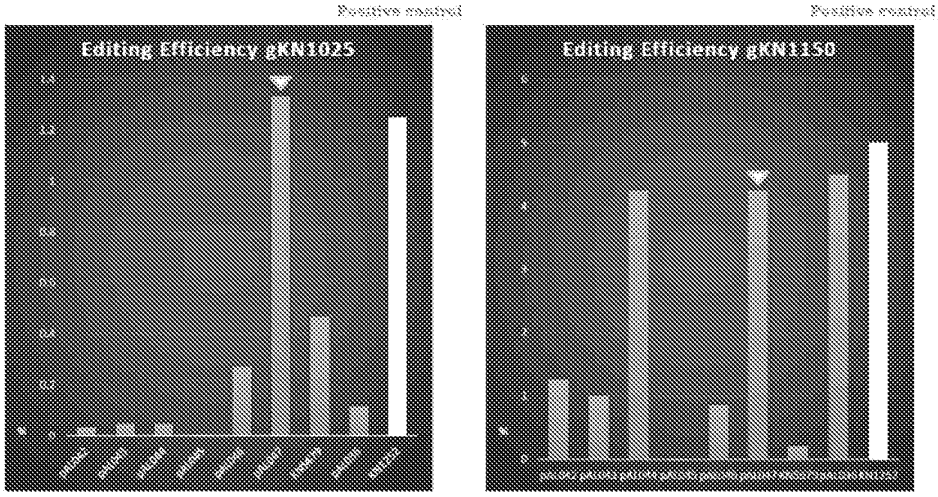
Galactoses Induction(0.02%)
gRNA: KN1025

[図28]

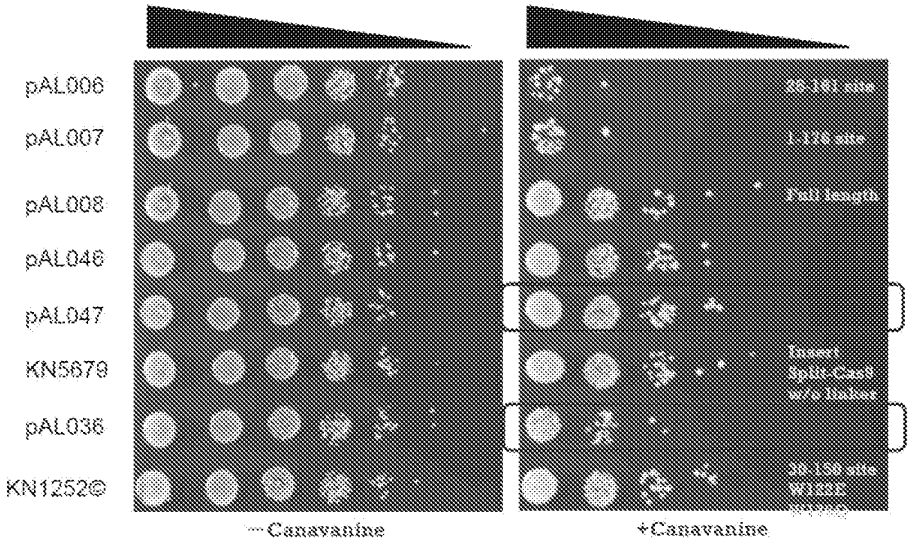


Galactoses Induction(0.02%)
gRNA: KN1130

[図29]

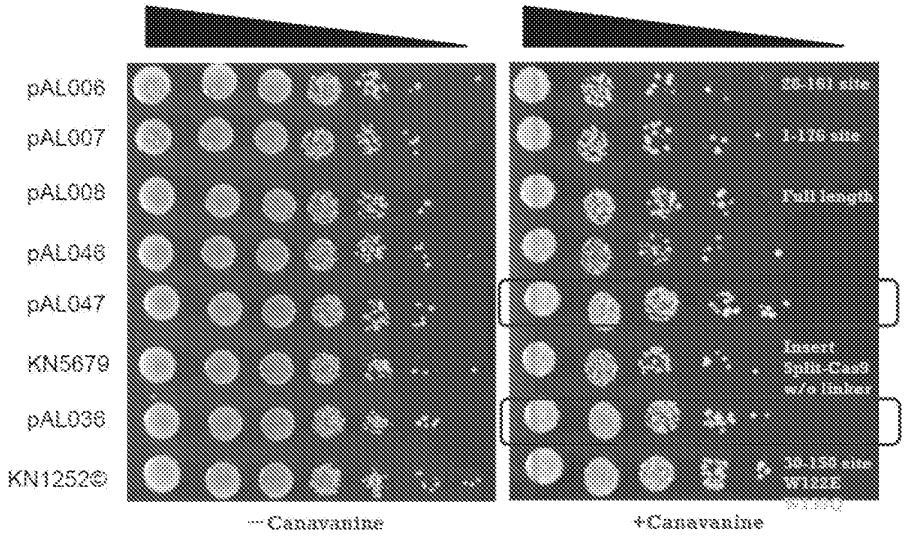


[図30]



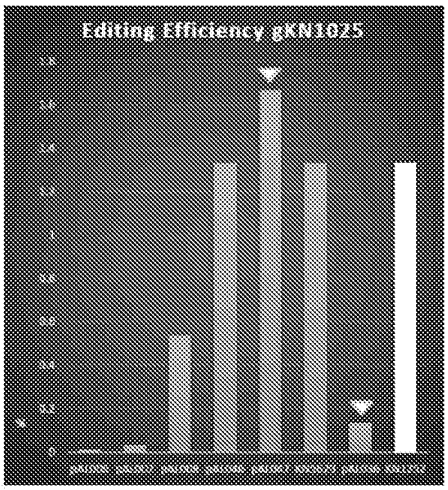
Galactoses Induction(0.02%)
gRNA: KN1025

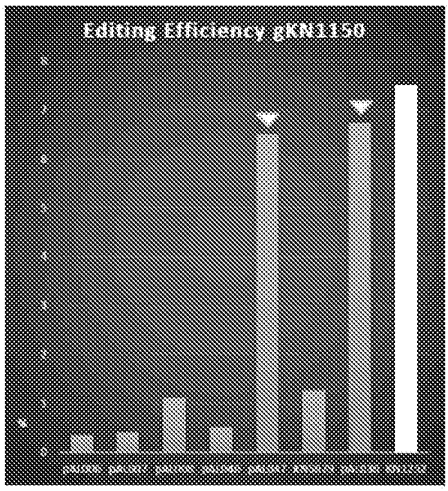
[図31]



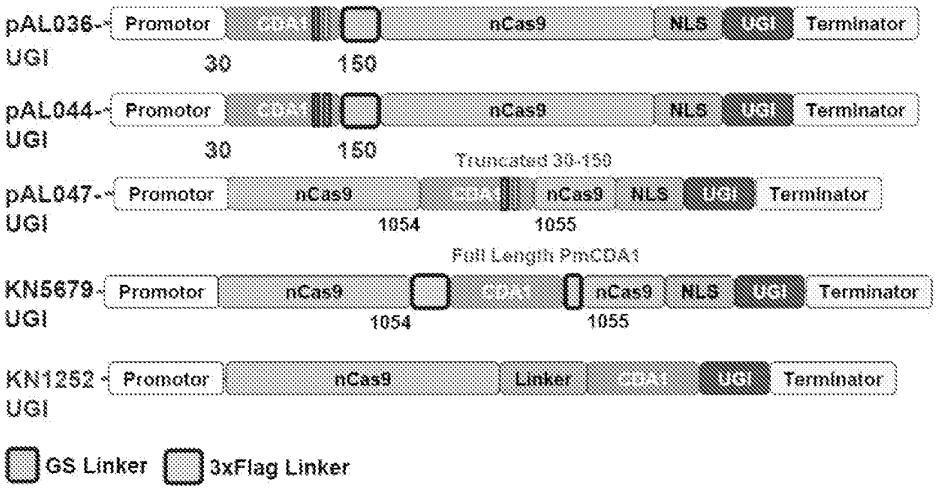
Galactoses Induction(0.02%)
gRNA: KN1150

[図32]

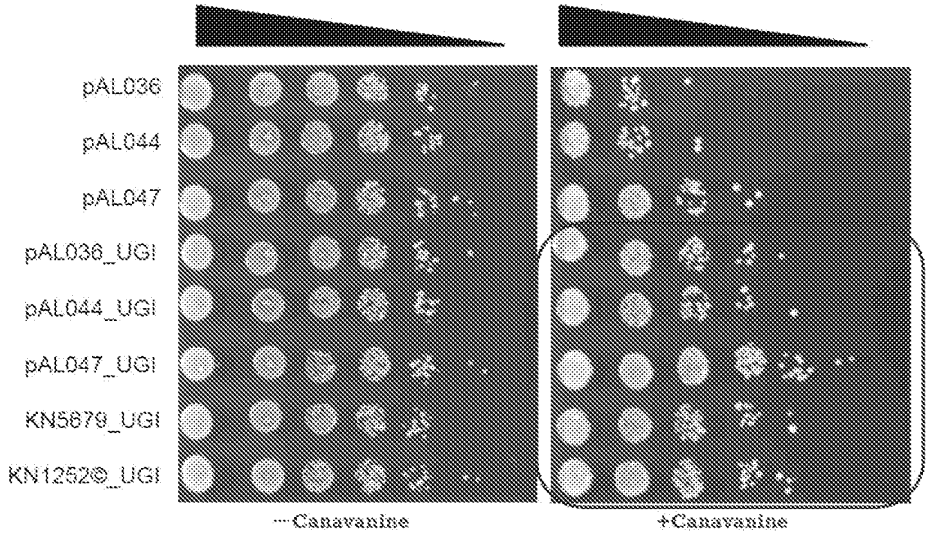




[図33]

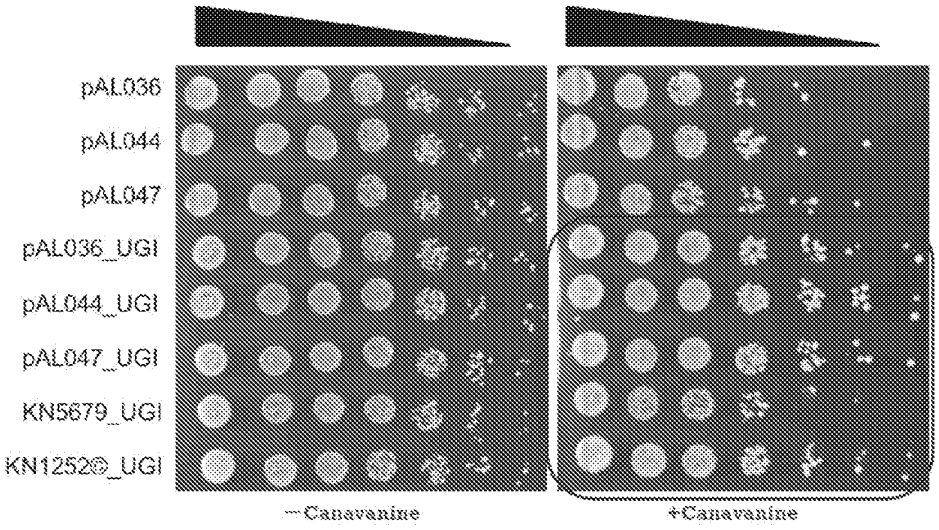


[図34]



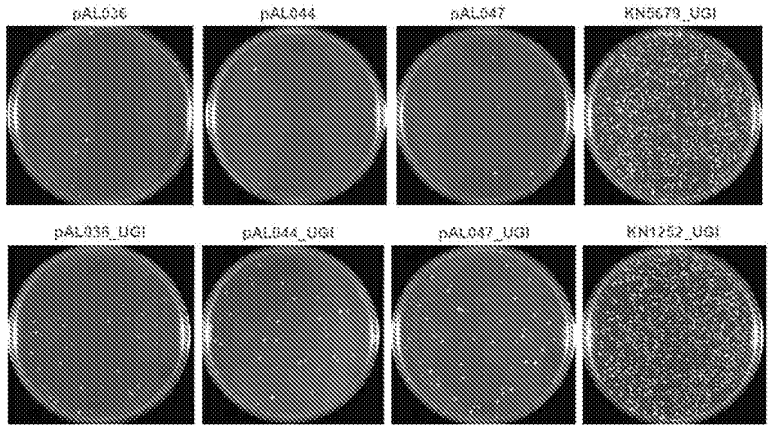
Galactoses Induction(0.02%)
gRNA: KN1025

[図35]



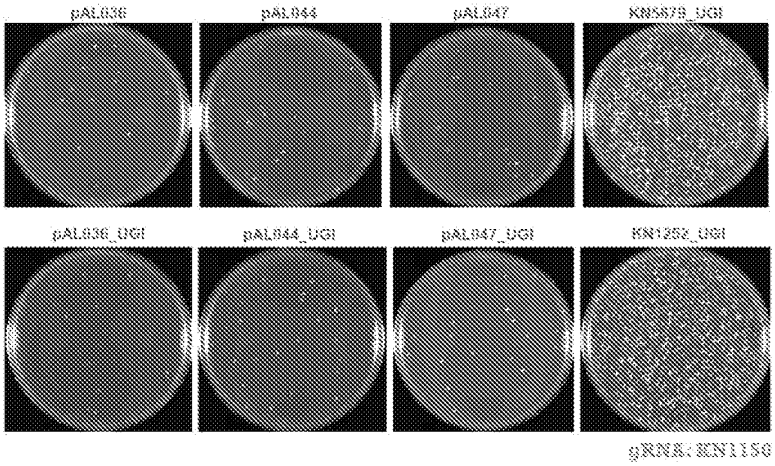
Galactoses Induction(0.02%)
gRNA: KN1150

[図36]



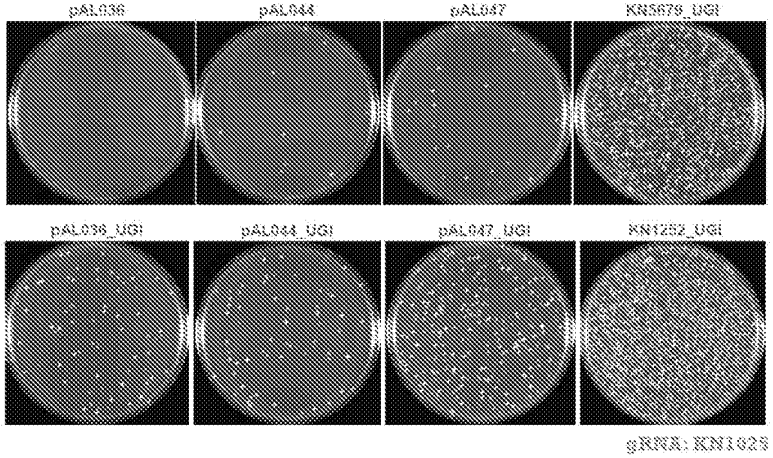
gRNA: KN1025

[図37]

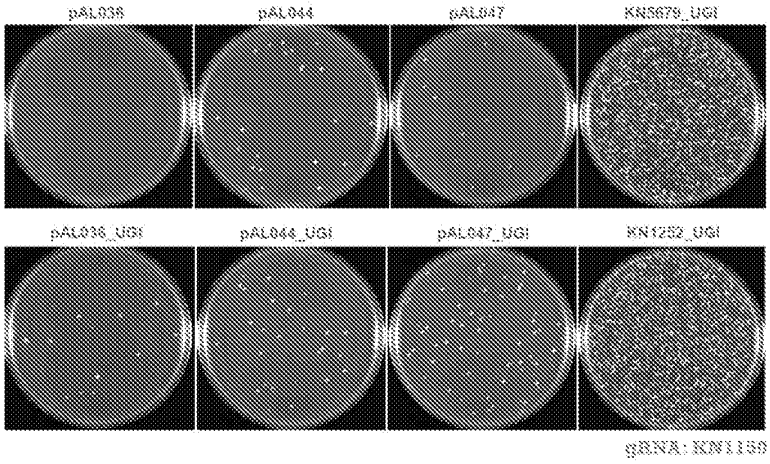


gRNA: KN1150

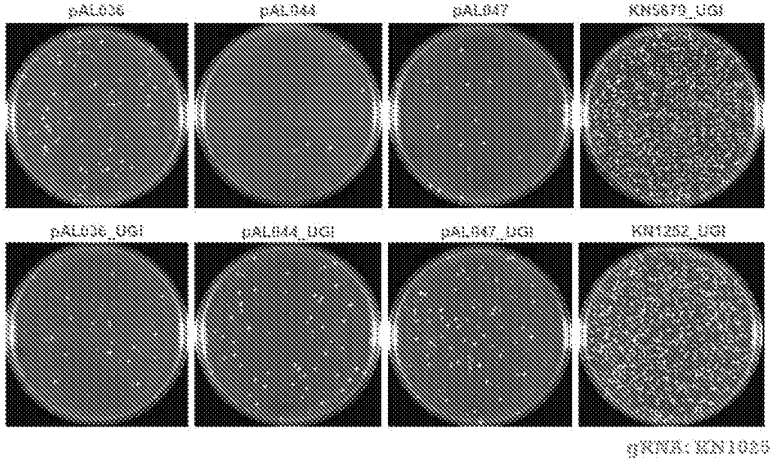
[図38]


pAL036
pAL044
pAL047
KN5679_UGI
pAL036_UGI
pAL044_UGI
pAL047_UGI
KN1252_UGI
gRNA: KN1028

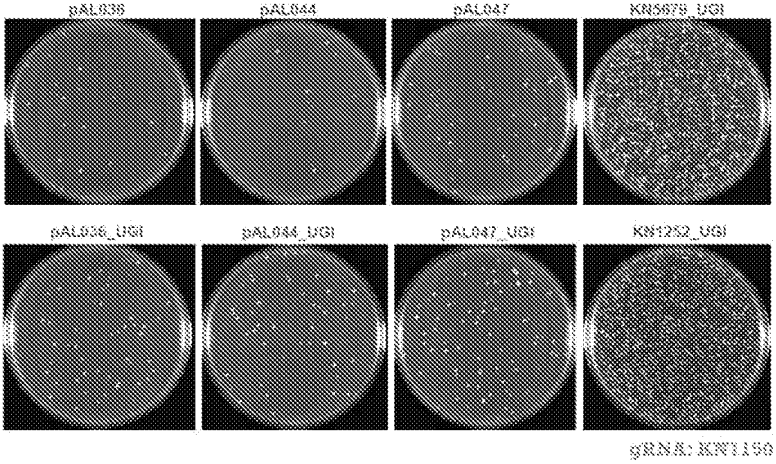
[図39]


pAL036
pAL044
pAL047
KN5679_UGI
pAL036_UGI
pAL044_UGI
pAL047_UGI
KN1252_UGI
gRNA: KN1130

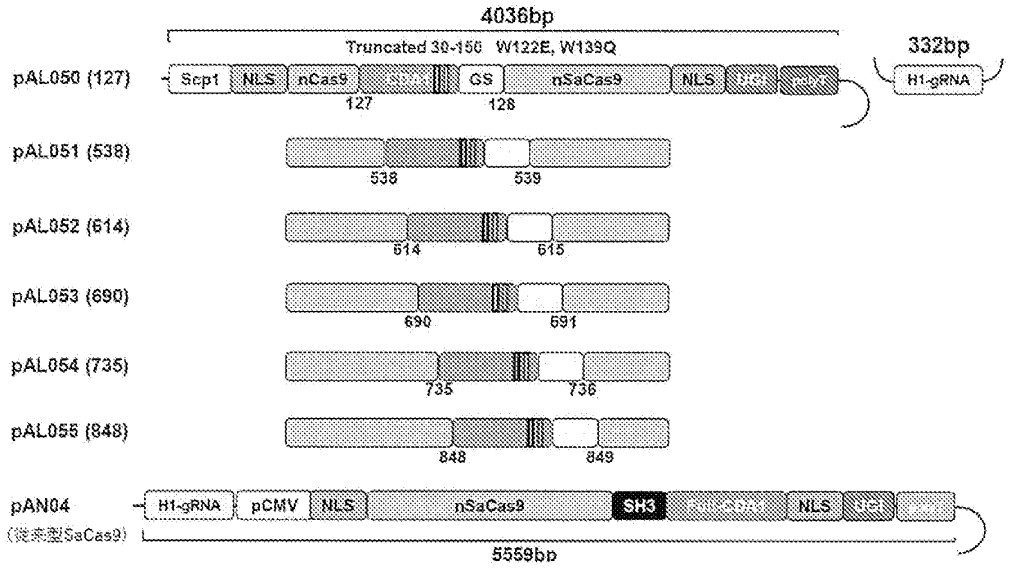
[図40]


pAL036
pAL044
pAL047
KN5679_UGI
pAL036_UGI
pAL044_UGI
pAL047_UGI
KN1252_UGI
gRNA: KN1085

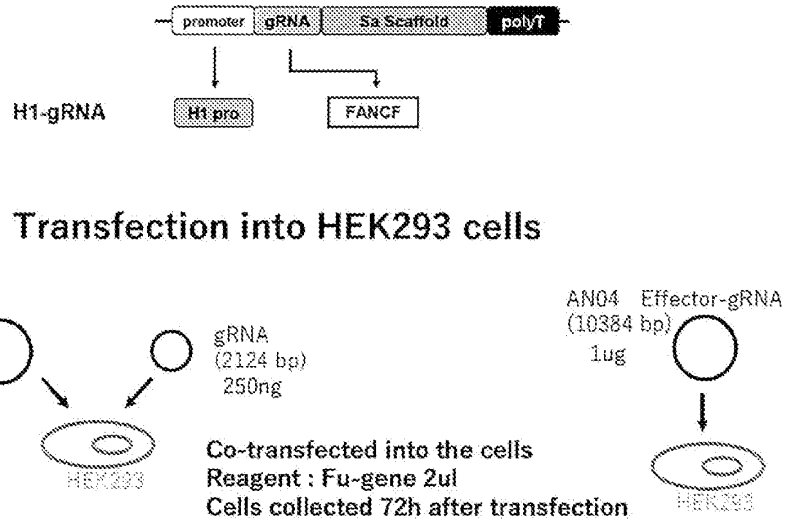
[図41]


pAL036
pAL044
pAL047
KR5679_UGI
pAL036_UGI
pAL044_UGI
pAL047_UGI
KN1252_UGI
gRNA: KN150

[図42]


4036bp
Truncated 30-150 W122E, W139Q
332bp
pAL050 (127)
Scp1
NLS
nCas9
GS
nSaCas9
NLS
H1-gRNA
127
128
pAL051 (538)
538
539
pAL052 (614)
614
615
pAL053 (690)
690
691
pAL054 (735)
735
736
pAL055 (848)
848
849
pAN04
(従来型SaCas9)
H1-gRNA
pCMV
NLS
nSaCas9
SH3
NLS
5559bp

[図43]



[図44]

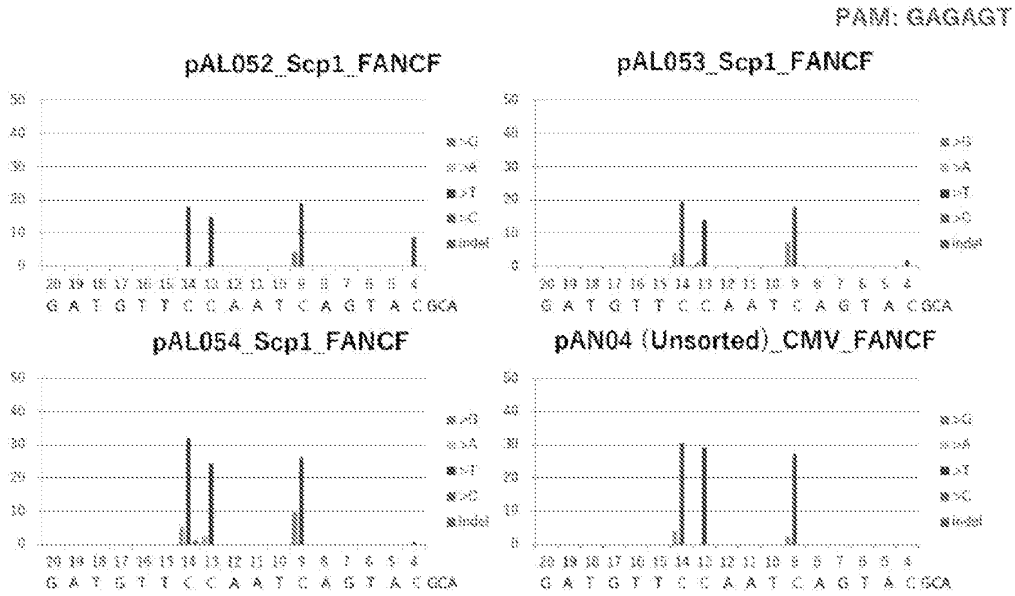
PAM: GAGAGT

GATGTTCCAATCAGTAOGCA

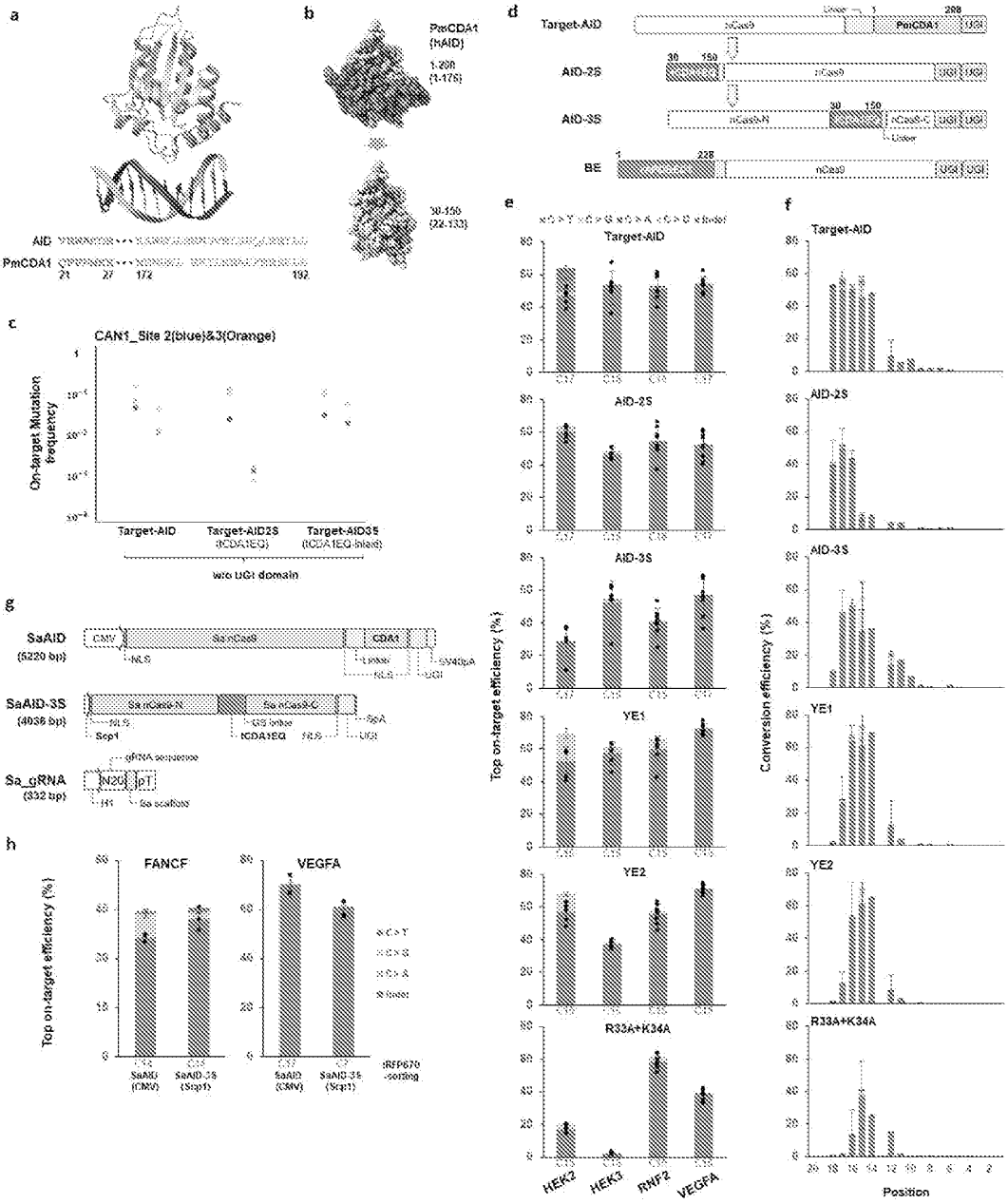
	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117
24 well	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4
Reference	G	A	T	G	T	T	C	C	A	A	T	C	A	G	T	A	C
S0_H1								>T 0.57				>T 0.5 >G 0.91					
S1_H1							>T 1.67	>T 2.82				>T 3.28					>T 1.29
S2_H1							>T 18.23	>T 14.84 >A 1.49				>T 19.00 >G 4.27 >A 1.27					>T 0.80
S3_H1							>T 15.42 >G 4.27 >A 0.67 >Del 0.65	> T 13.97 >G 1.28 >A 1.93				>T 17.86 >G 7.41 >A 1.92 >Del 0.52					>T 1.71
S4_H1							>T 31.96 >G 5.3 >A 2.57 >Del 1.12	>T 24.40 >G 2.31 >A 2.70				>T 26.10 >G 6.51 >A 2.44					>T 0.49
S5_H1																	
AN04_Unsorting							>T 30.66 >G 4.02 >A 0.52	>T 29.15				>T 27.37 >G 2.43 >A 0.53					
NC																	

>5% >10% >20%

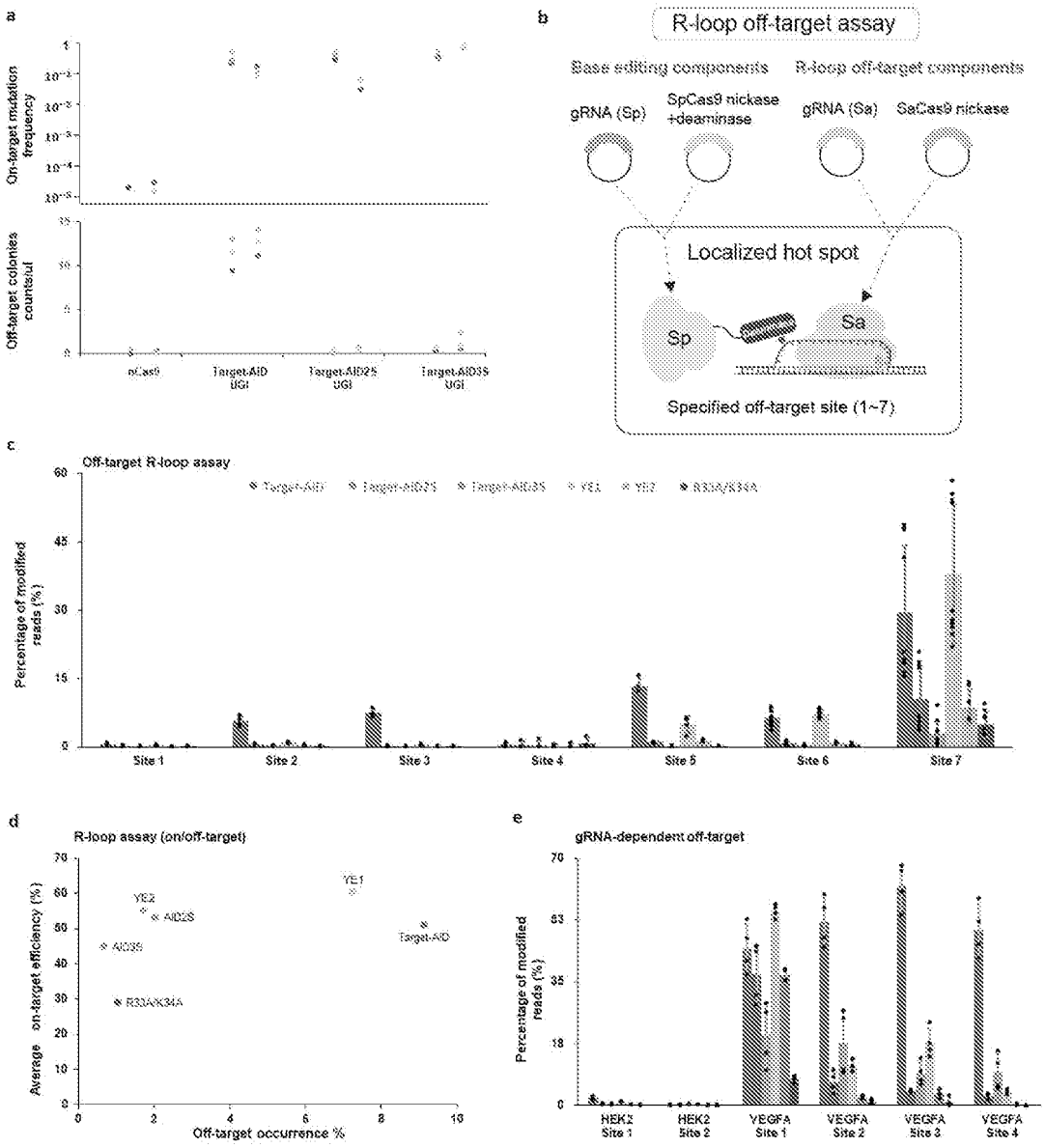
[図45]


PAM: GAGAGT
pAL052_Scp1_FANCF
pAL053_Scp1_FANCF
pAL054_Scp1_FANCF
pAN04 (Unsorted)_CMV_FANCF

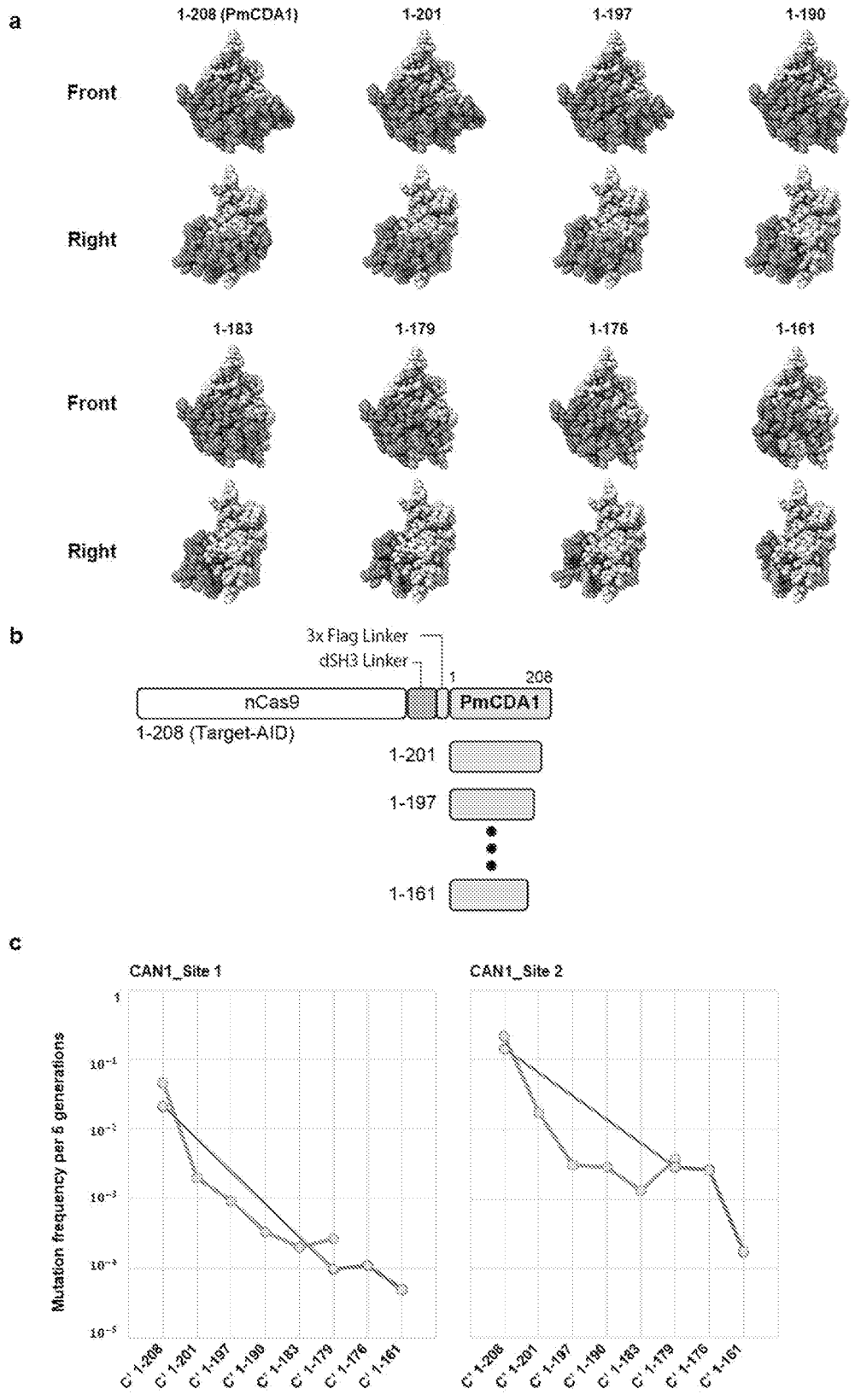
[図46]


a
b
PmCDA1
(hAID)
1-208
(1-176)
50-155
(22-133)
AID
PmCDA1
21
27
172
192
c
CAN1_Site 2(blue)&3(Orange)
On-target Mutation frequency
Target-AID
Target-AID2S
(hCDA1EQ)
Target-AID3S
(hCDA1EQ-linker)
w/o UGI domain
d
Target-AID
nCas9
PmCDA1
UGI
AID-2S
AID-3S
nCas9-N
nCas9-C
Linker
BE
hCDA1
e
Target-AID
AID-2S
AID-3S
YE1
YE2
R33A+K34A
Top on-target efficiency (%)
HEK2
HEK3
RNF2
VEGFA
f
Conversion efficiency (%)
Position
g
SaAID
(5220 bp)
CMV
Sa nCas9
CDA1
NLS
UGI
SV40pA
SaAID-3S
(4036 bp)
Sa nCas9-N
Sa nCas9-C
GS linker
hCDA1EQ
Scp1
SpA
gRNA sequence
Sa_gRNA
(332 bp)
N20
pT
Sa scaffold
h
FANCF
VEGFA
Top on-target efficiency (%)
SaAID (CMV)
SaAID-3S (Scp1)
tRFP670 -sorting

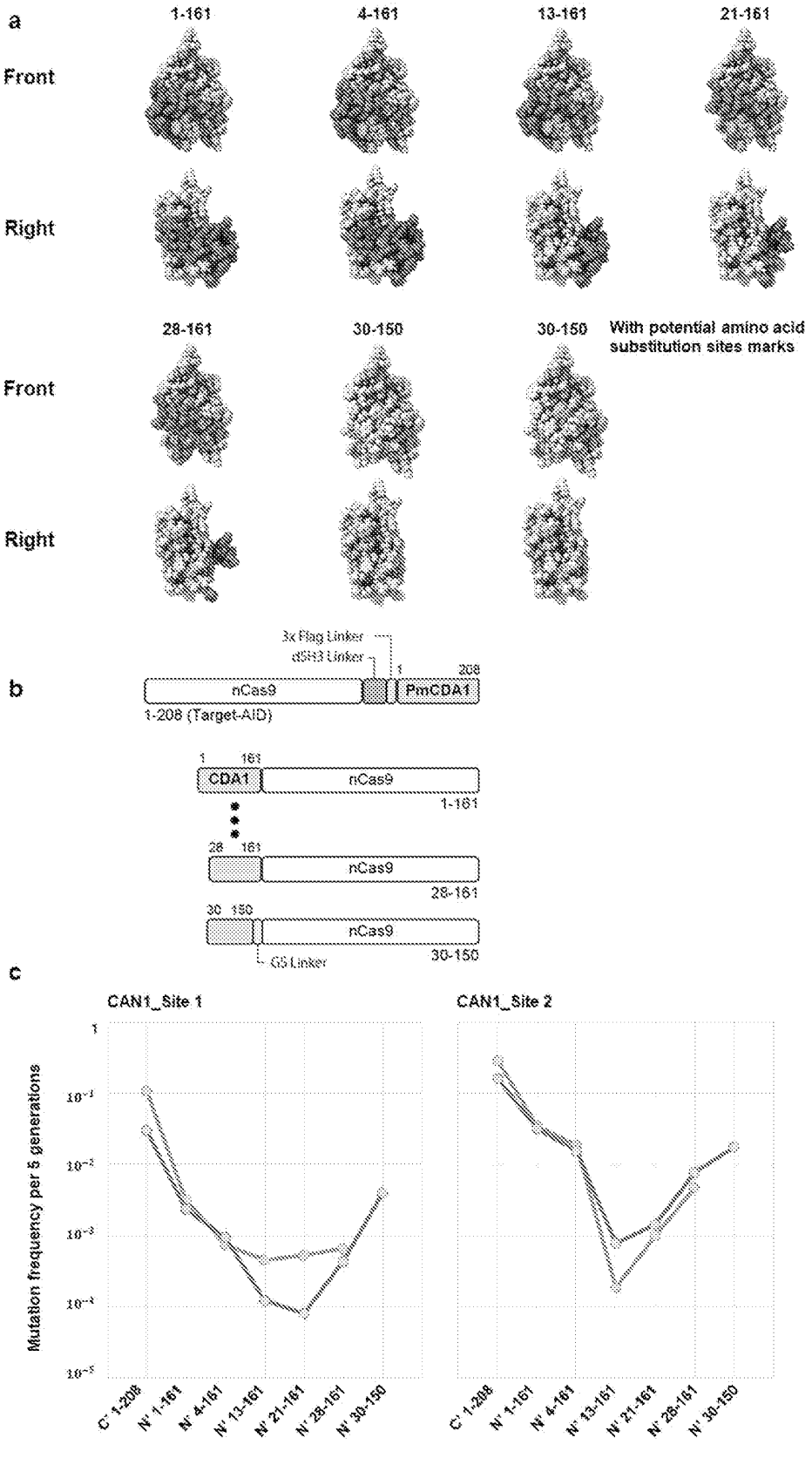
[図47]


a
On-target mutation frequency
Off-target colonies count/ul
nCas9
Target-AID uGI
Target-AID2S uGI
Target-AID3S uGI
b
R-loop off-target assay
Base editing components
R-loop off-target components
gRNA (Sp)
SpCas9 nickase +deaminase
gRNA (Sa)
SaCas9 nickase
Localized hot spot
Sp
Sa
Specified off-target site (1~7)
c
Off-target R-loop assay
Target-AID
Target-AID2S
Target-AID3S
YE1
YE2
R33A/K34A
Percentage of modified reads (%)
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site 7
d
R-loop assay (on/off-target)
Average on-target efficiency (%)
Off-target occurrence %
YE1
YE2
AID2S
AID3S
R33A/K34A
Target-AID
e
gRNA-dependent off-target
Percentage of modified reads (%)
HEK2 Site 1
HEK2 Site 2
VEGFA Site 1
VEGFA Site 2
VEGFA Site 3
VEGFA Site 4

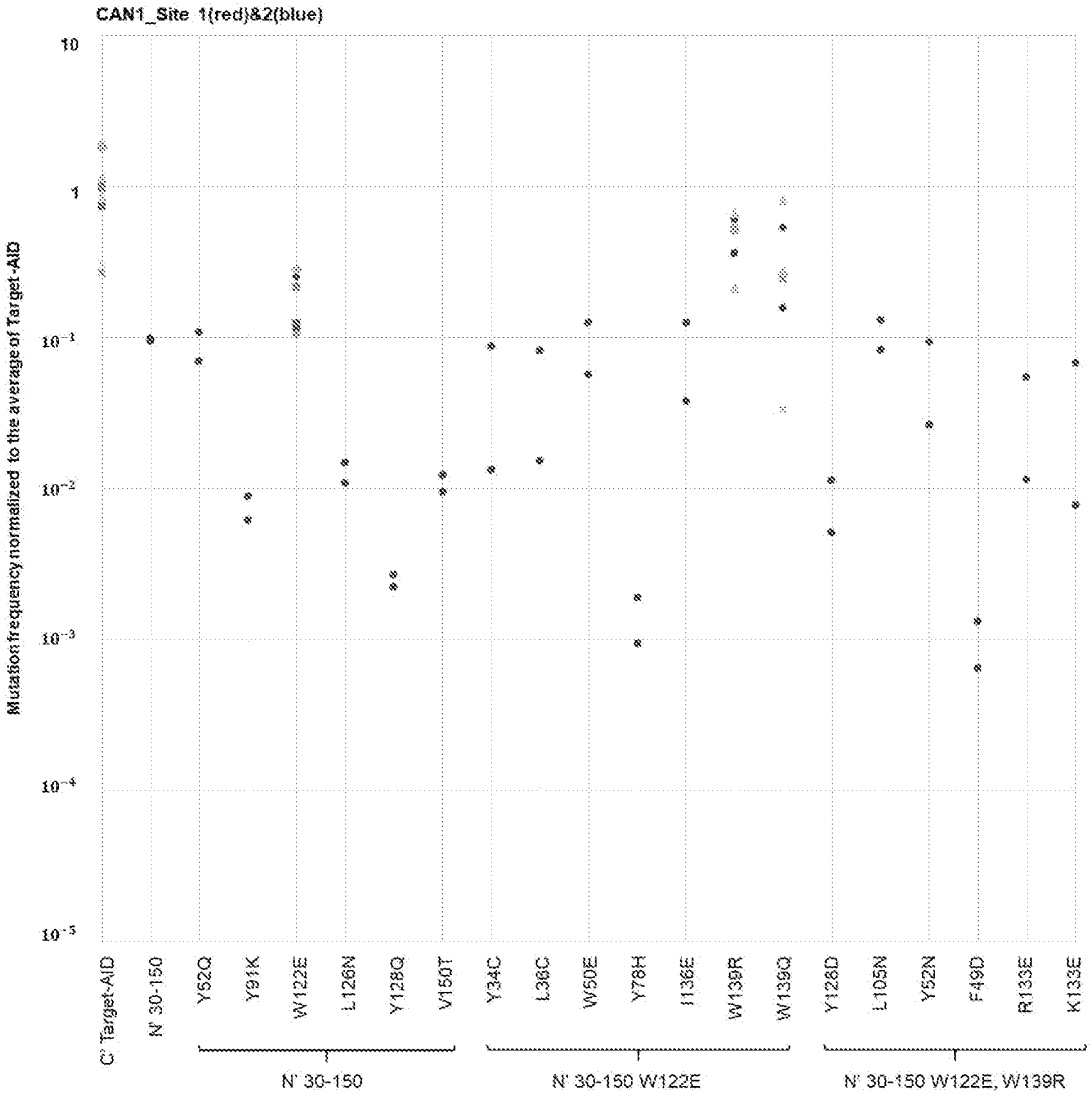
[図48]


a
1-208 (PmCDA1)
1-201
1-197
1-190
Front
Right
1-183
1-179
1-176
1-161
Front
Right
b
3x Flag Linker
dSH3 Linker
1
208
nCas9
PmCDA1
1-208 (Target-AID)
1-201
1-197
1-161
c
CAN1_Site 1
CAN1_Site 2
Mutation frequency per 6 generations
1
10⁻⁴
10⁻³
10⁻³
10⁻⁴
10⁻⁵
C' 1-208
C' 1-201
C' 1-197
C' 1-190
C' 1-183
C' 1-179
C' 1-176
C' 1-161

[図49]

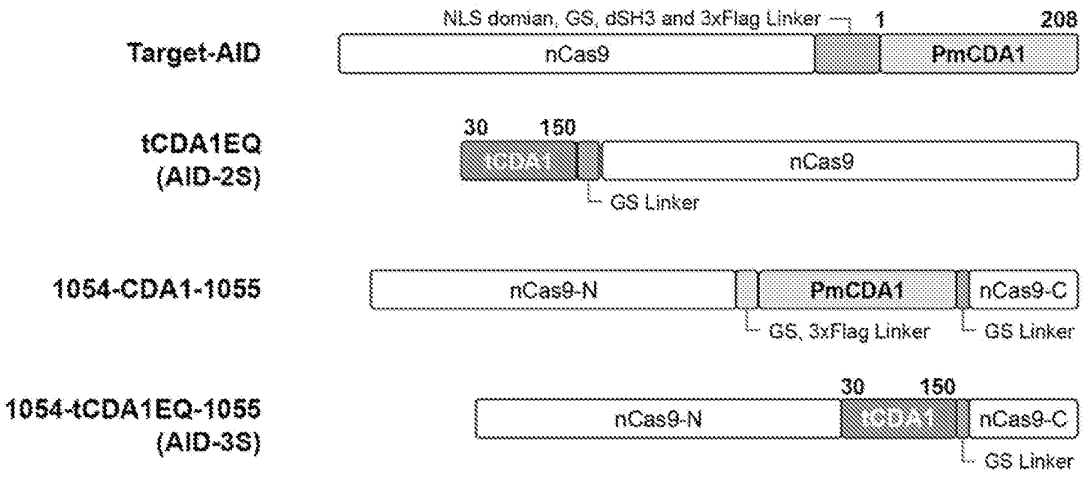

a
1-161
4-161
13-161
21-161
Front
Right
28-161
30-150
30-150
With potential amino acid substitution sites marks
Front
Right
b
3x Flag Linker
dSH3 Linker
1
208
nCas9
PmCDA1
1-208 (Target-AID)
1
161
CDA1
nCas9
1-161
28
161
nCas9
28-161
30
150
nCas9
30-150
G5 Linker
c
CAN1_Site 1
CAN1_Site 2
Mutation frequency per 5 generations
C' 1-208
N' 1-161
N' 4-161
N' 13-161
N' 21-161
N' 28-161
N' 30-150

[図50]

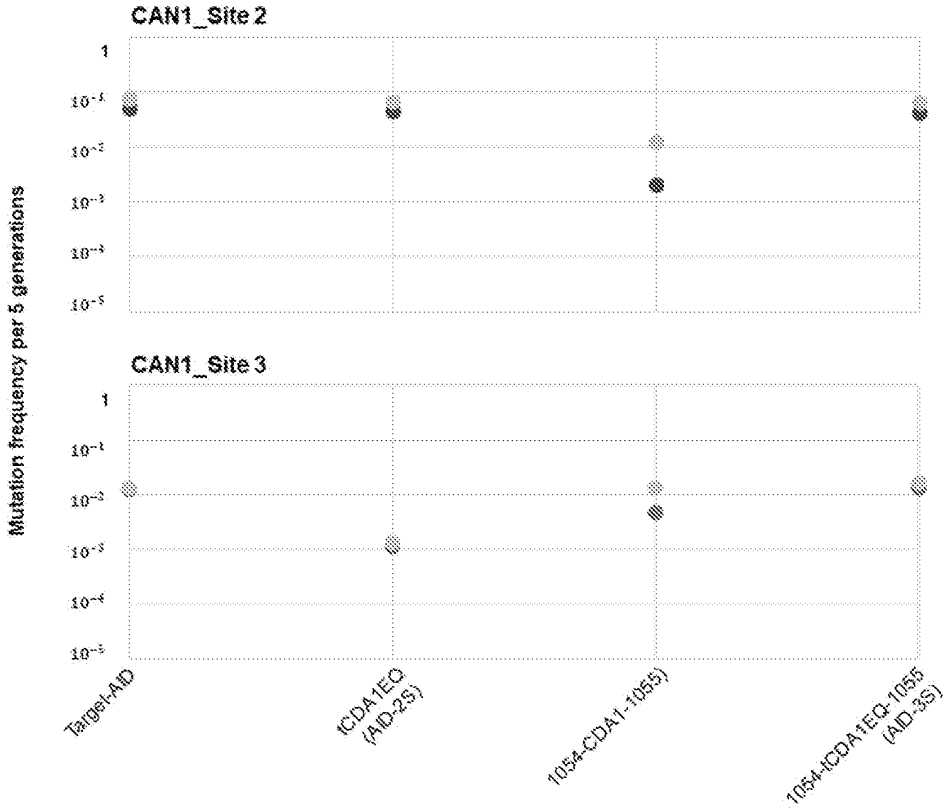

CAN1_Site 1(red)&2(blue)
Mutation frequency normalized to the average of Target-AID
10
1
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
C' Target-AID
N' 30-150
Y52Q
Y91K
W122E
L126N
Y128Q
V150T
Y34C
L36C
W50E
Y78H
I136E
W139R
W139Q
Y128D
L105N
Y52N
F49D
R133E
K133E
N' 30-150
N' 30-150 W122E
N' 30-150 W122E, W139R

[図51]

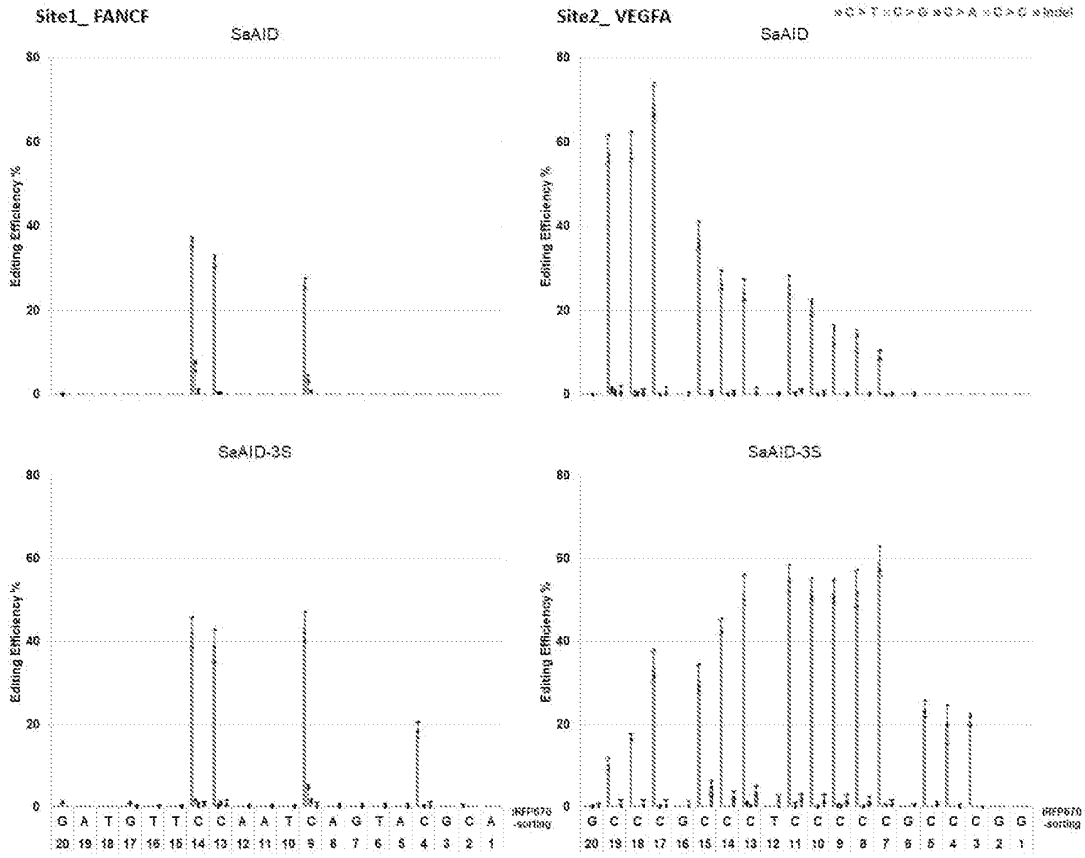
a


NLS domian, GS, dSH3 and 3xFlag Linker
1
208
Target-AID
nCas9
PmCDA1
30
150
tCDA1EQ
(AID-2S)
tCDA1
nCas9
GS Linker
1054-CDA1-1055
nCas9-N
PmCDA1
nCas9-C
GS, 3xFlag Linker
GS Linker
1054-tCDA1EQ-1055
(AID-3S)
30
150
nCas9-N
tCDA1
nCas9-C
GS Linker

b


CAN1_Site 2
CAN1_Site 3
Mutation frequency per 5 generations
1
10⁻¹
10⁻²
10⁻³
10⁻⁴
10⁻⁵
Target-AID
tCDA1EQ (AID-2S)
1054-CDA1-1055)
1054-tCDA1EQ-1055 (AID-3S)

[図52]


Site1_ FANCF
SaAID
Site2_ VEGFA
SaAID
SaAID-3S
SaAID-3S
Editing Efficiency %
0
20
40
60
80
G A T G T T C C A A T C A G T A C G C A
20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1
G C C C G C C C T C C C C C G C C C G G
20 19 18 17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1