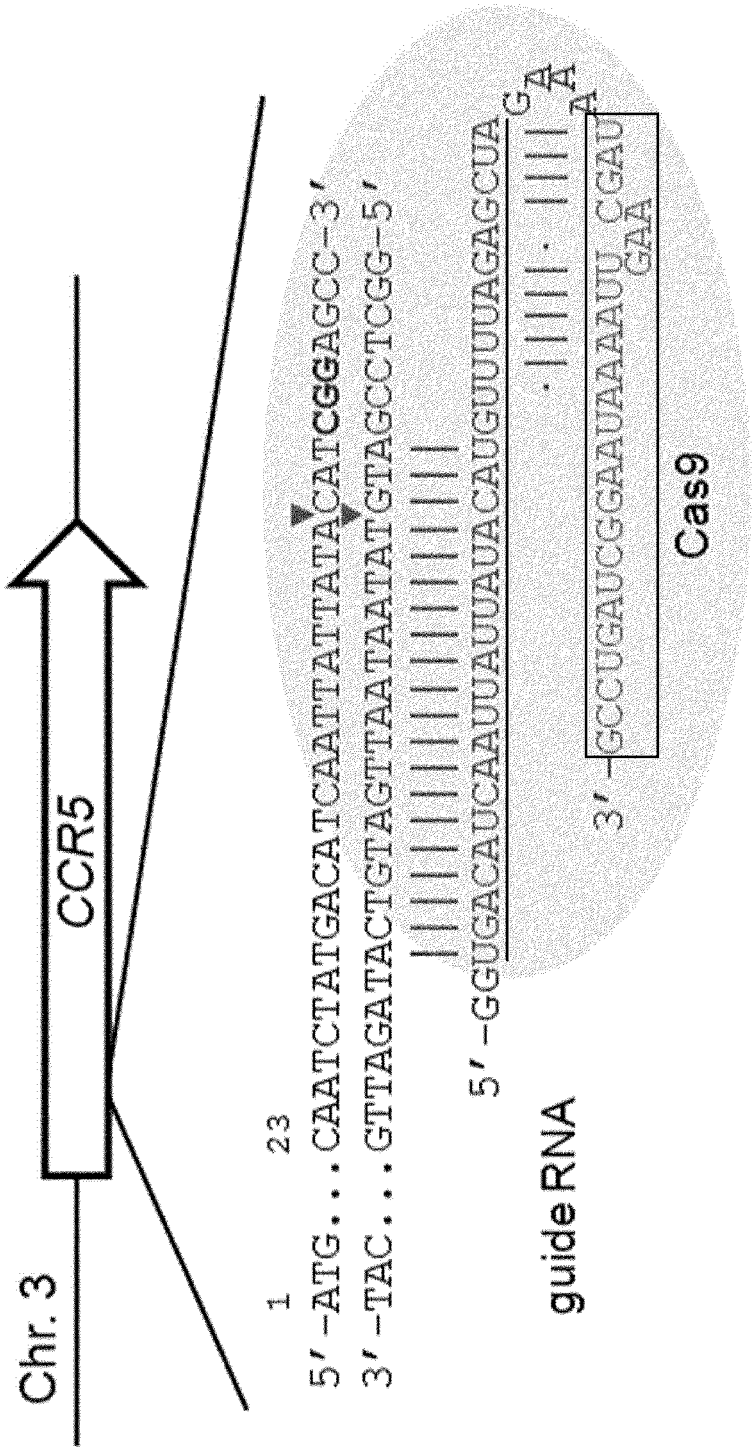
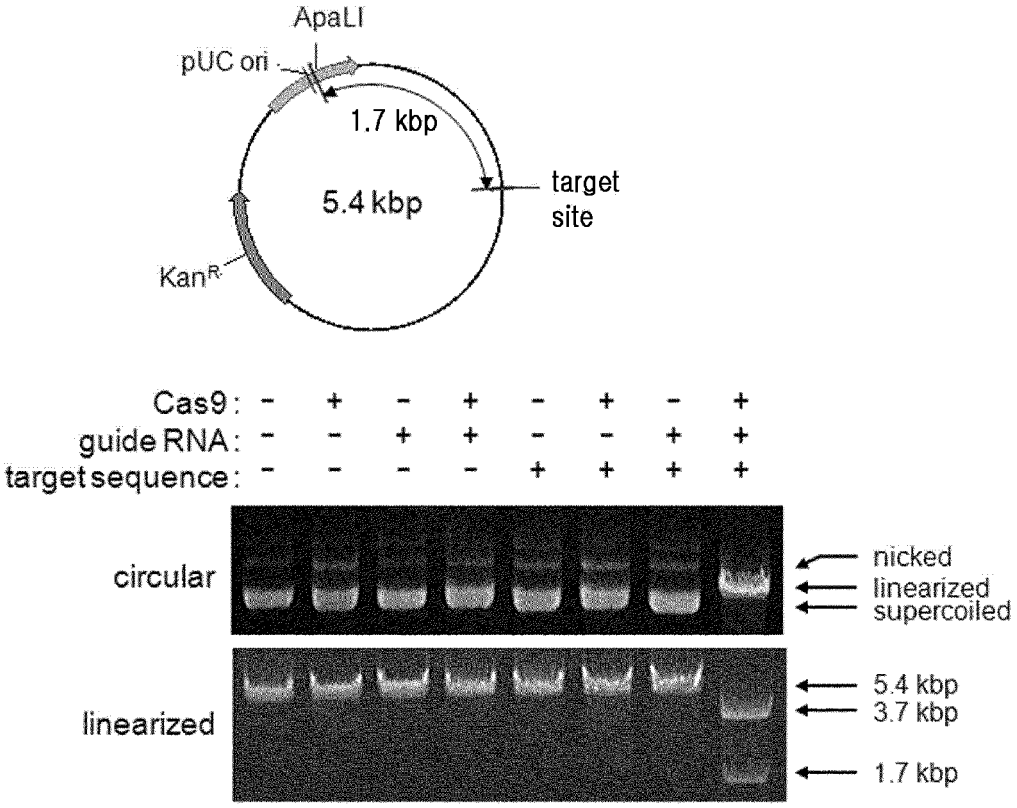
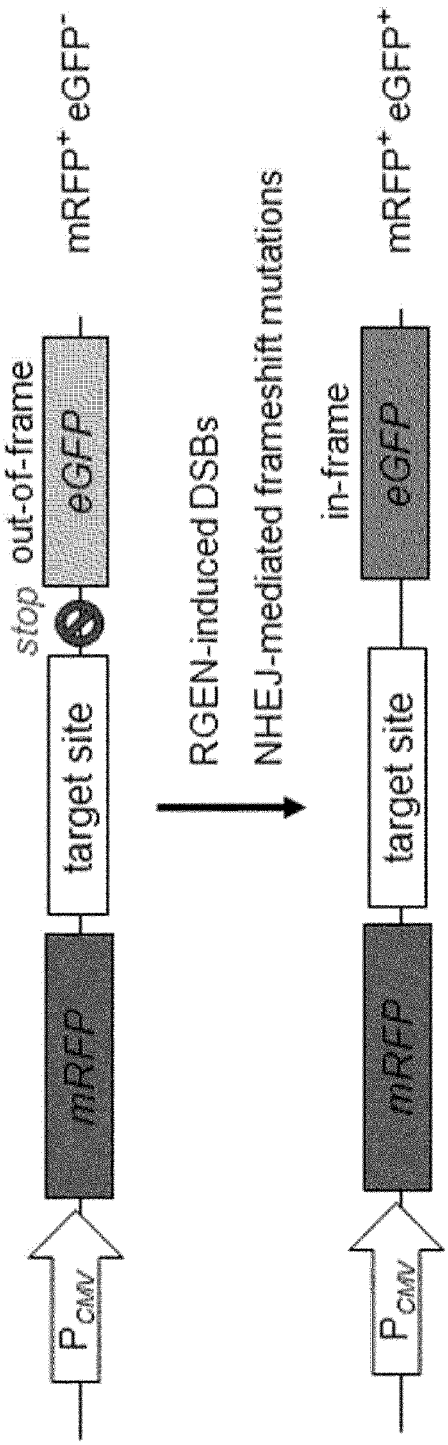
[Fig. 1a]


Chr. 3
CCR5
1
23
5′ –ATG. . . CAATCTATGACATCATTATTATACATCGGAGCC–3′
3′ –TAC. . . GTTAGATACTGTAGTTAATATATGTAGCCTCGG–5′
5′ –GGUGACAUCAAUUAUUAUACAUGUUUUAGAGCUA
guide RNA
3′ –GCCUGAUCGGAAUAAAAUU CGAU
GAA
Cas9

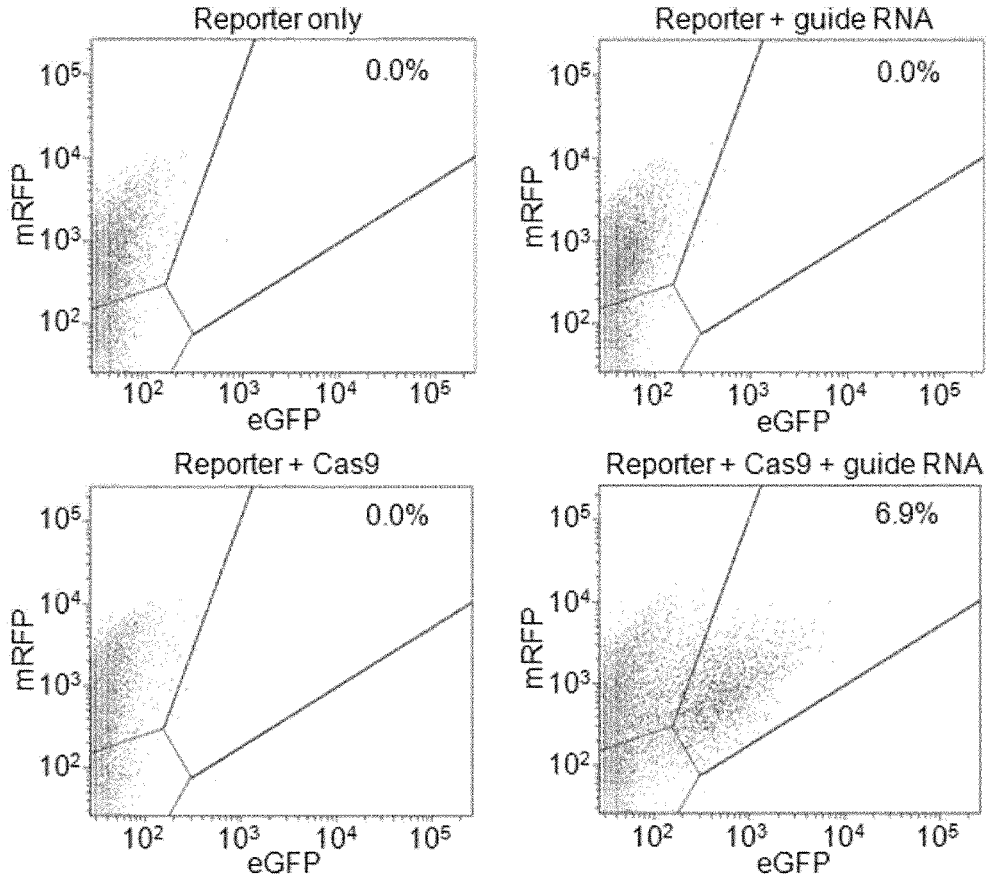
[Fig. 1b]


ApaLI
pUC ori
1.7 kbp
5.4 kbp
target site
Kan^R
Cas9 : - + - + - + - +
guide RNA : - - + + - - + +
target sequence : - - - - + + + +
circular
nicked
linearized
supercoiled
linearized
5.4 kbp
3.7 kbp
1.7 kbp

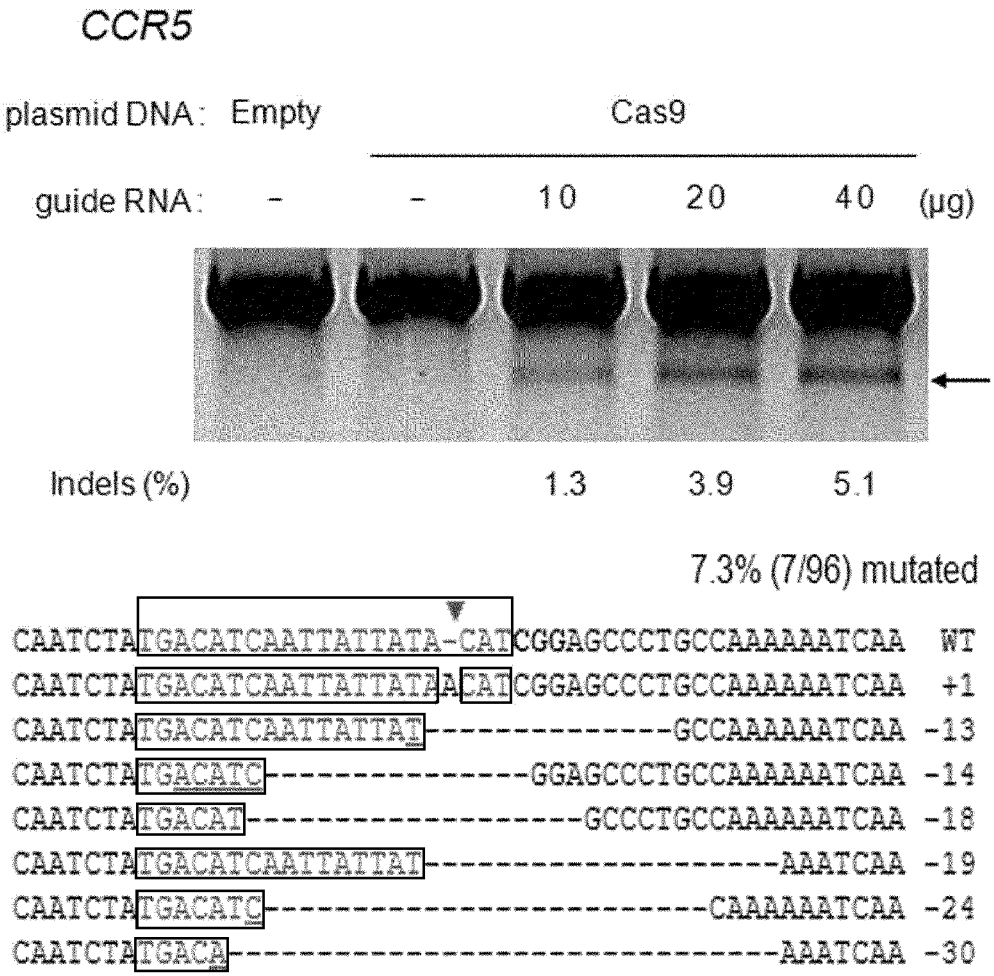
[Fig. 2a]


mRFP+ eGFP-
stop out-of-frame
eGFP
target site
mRFP
P CMV
RGEN-induced DSBs
NHEJ-mediated frameshift mutations
mRFP+ eGFP+
in-frame
eGFP
target site
mRFP
P CMV

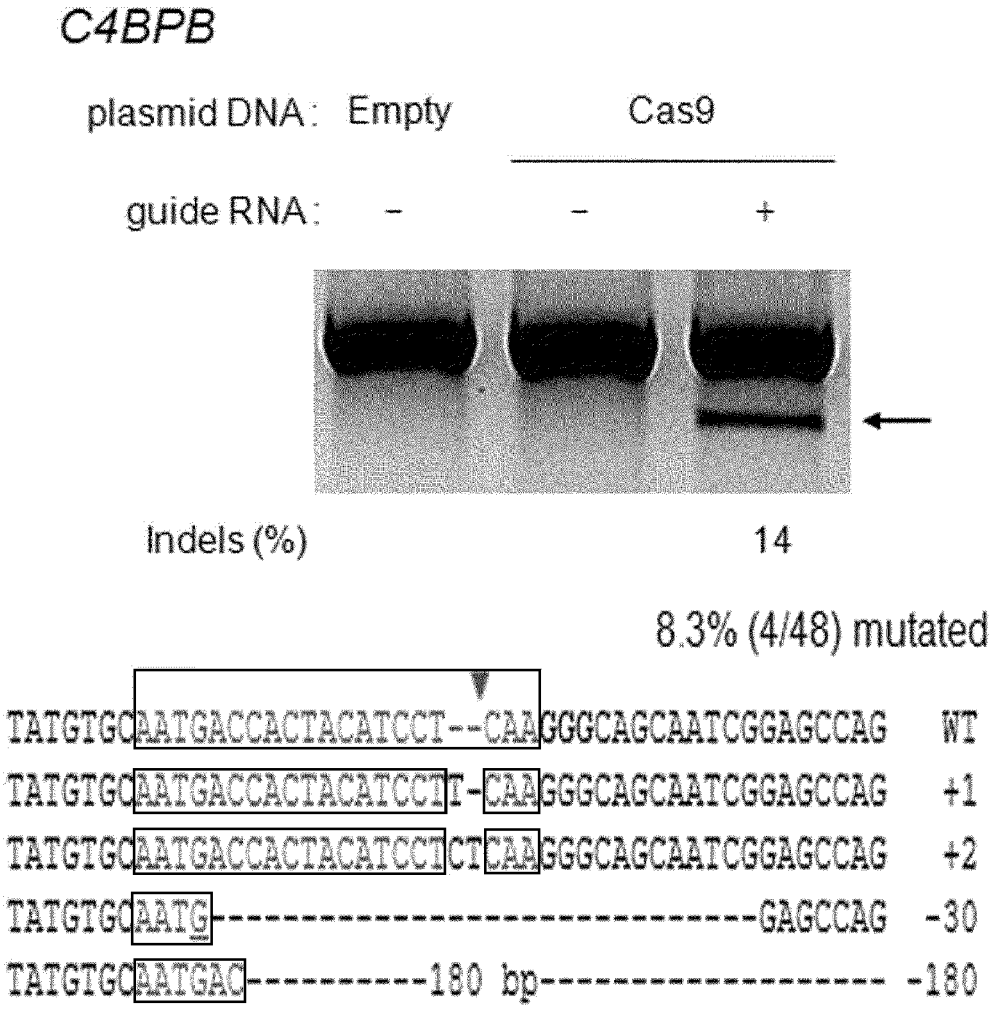
[Fig. 2b]


Reporter only
0.0%
Reporter + guide RNA
0.0%
Reporter + Cas9
0.0%
Reporter + Cas9 + guide RNA
6.9%
mRFP
eGFP
10^2
10^3
10^4
10^5

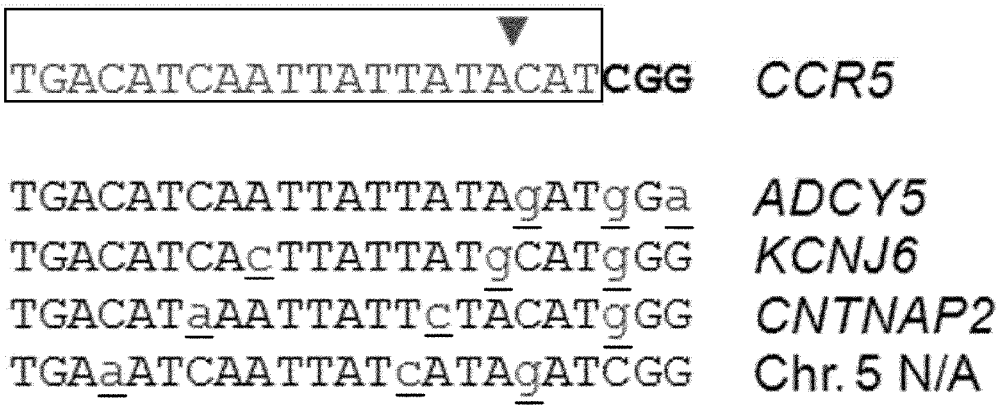
[Fig. 3a]


CCR5
plasmid DNA: Empty
Cas9
guide RNA: - - 10 20 40 (μg)
Indels (%) 1.3 3.9 5.1
7.3% (7/96) mutated
CAATCTATGACATCAATTATTATA-CATCGGAGCCCTGCCAAAAAATCAA WT
CAATCTATGACATCAATTATTATAACATCGGAGCCCTGCCAAAAAATCAA +1
CAATCTATGACATCAATTATTAT----------GCCAAAAAATCAA -13
CAATCTATGACATC----------GGAGCCCTGCCAAAAAATCAA -14
CAATCTATGACAT----------GCCCTGCCAAAAAATCAA -18
CAATCTATGACATCAATTATTAT----------AAATCAA -19
CAATCTATGACATC----------CAAAAAATCAA -24
CAATCTATGACA----------AAATCAA -30

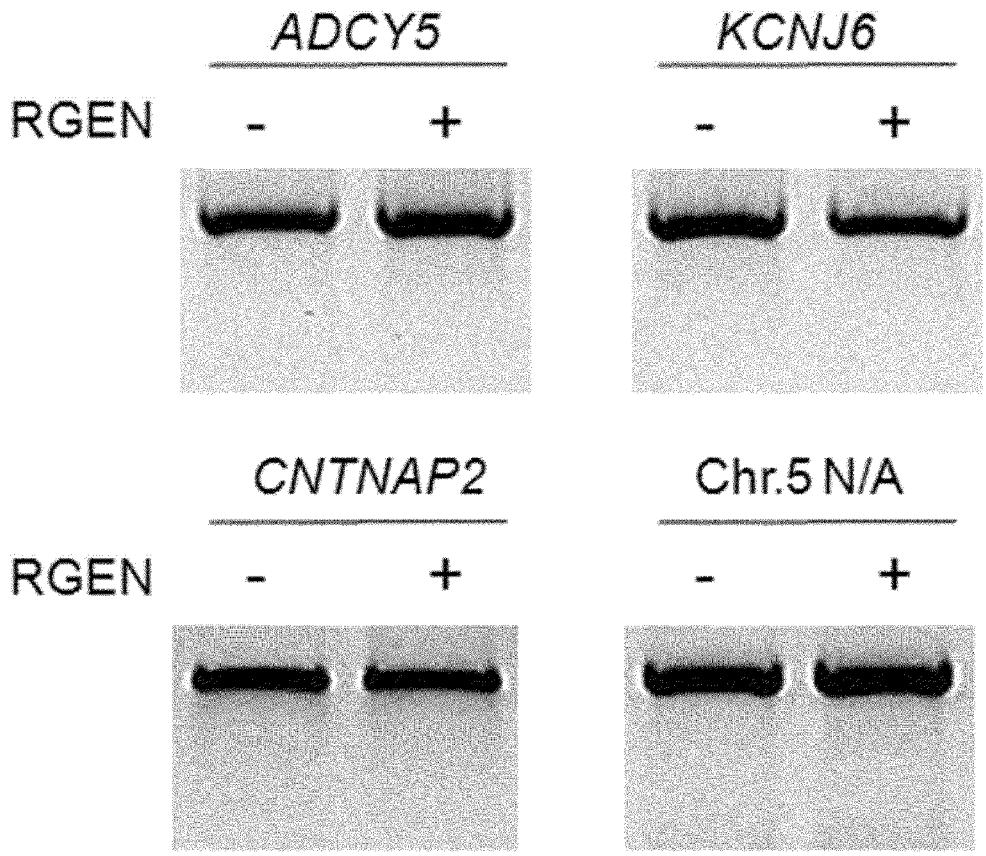
[Fig. 3b]


C4BPB
plasmid DNA: Empty Cas9
guide RNA: - - +
Indels (%) 14
8.3% (4/48) mutated
TATGTGCAATGACCACTACATCCT--CAAGGGCAGCAATCGGAGCCAG WT
TATGTGCAATGACCACTACATCCTT-CAAGGGCAGCAATCGGAGCCAG +1
TATGTGCAATGACCACTACATCCTCTCAAGGGCAGCAATCGGAGCCAG +2
TATGTGCAATG----------GAGCCAG -30
TATGTGCAATGAC----------180 bp---------- -180

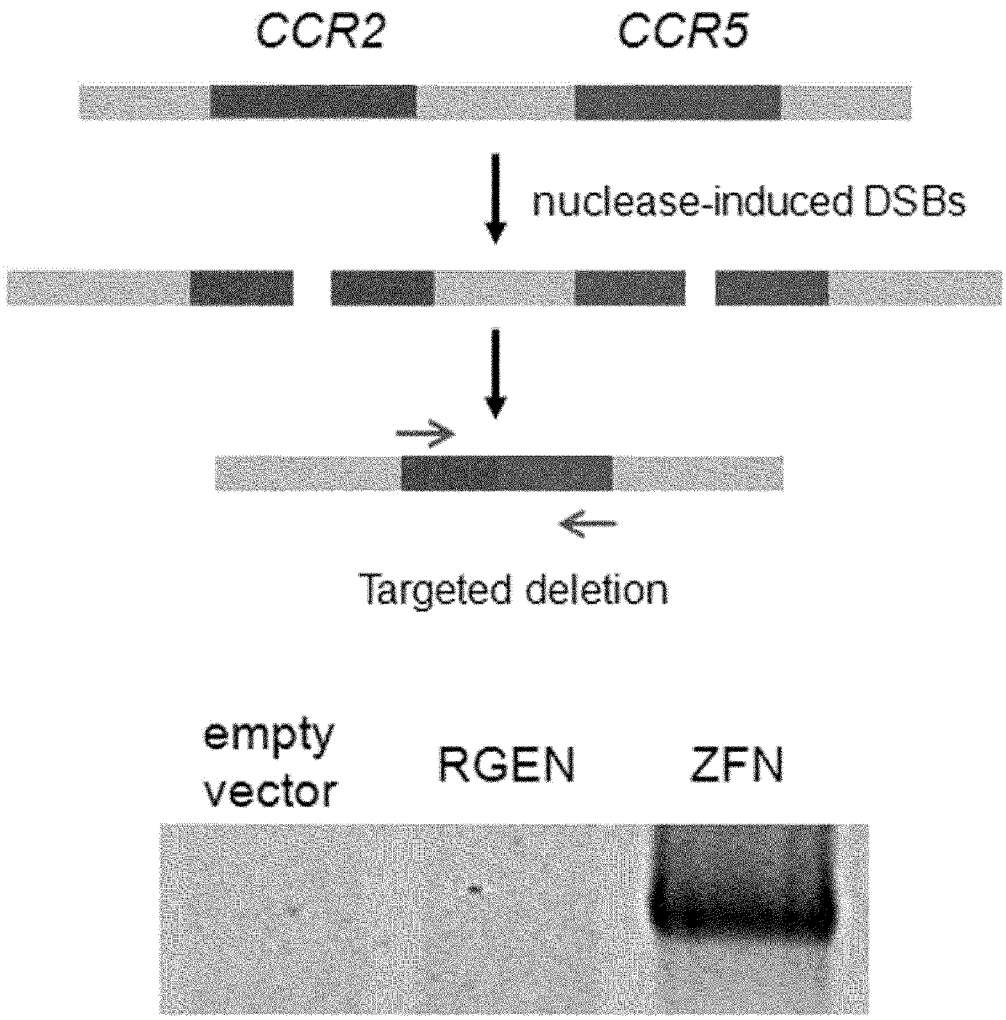
[Fig. 4a]


TGACATCAATTATTATACATCGG CCR5
TGACATCAATTATTATAgATgGa ADCY5
TGACATCAcTTATTATgCATgGG KCNJ6
TGACATaAATTATTcTACATgGG CNTNAP2
TGAaATCAATTATcATAgATCGG Chr. 5 N/A

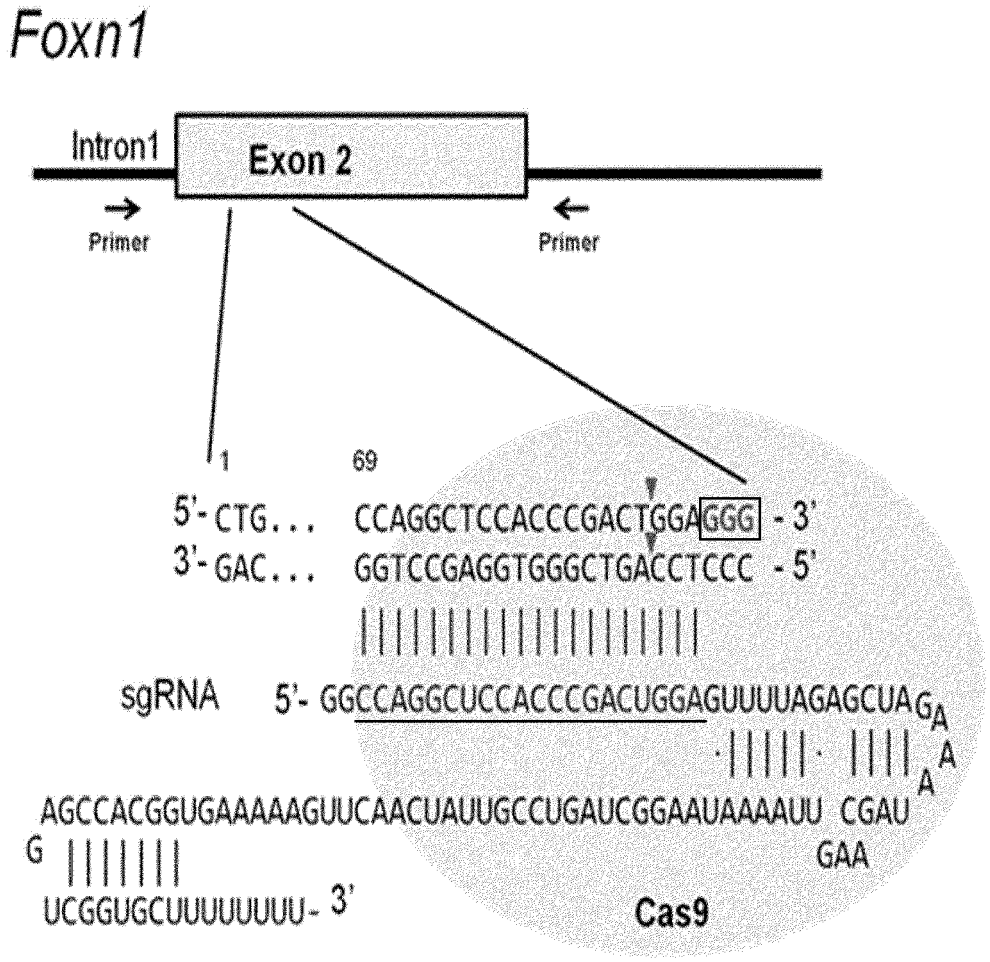
[Fig. 4b]



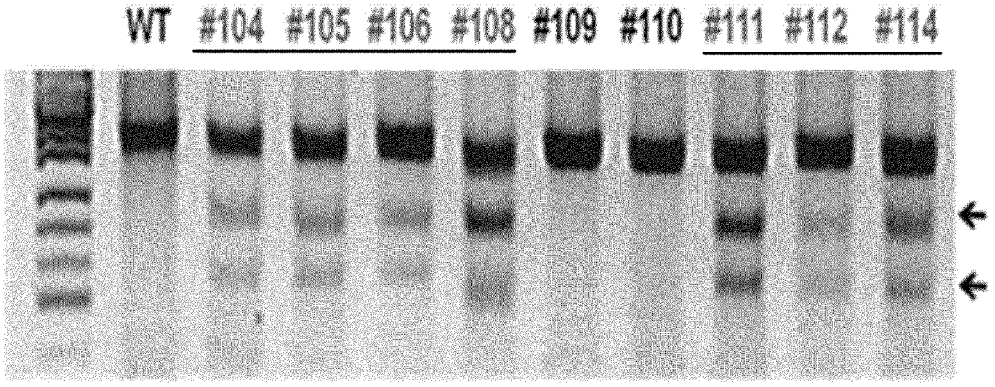
[Fig. 4c]


CCR2
CCR5
nuclease-induced DSBs
Targeted deletion
empty vector
RGEN
ZFN

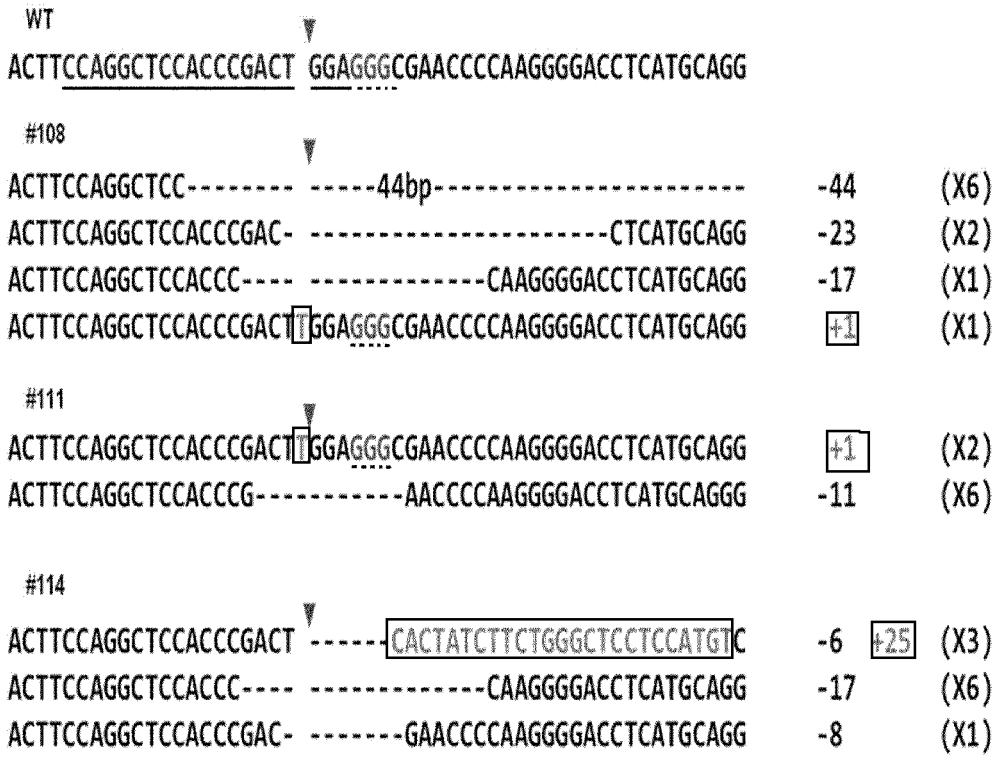
[Fig. 5a]


Foxn1
Intron1
Exon 2
Primer
Primer
1
69
5'- CTG... CCAGGCTCCACCCGACTGGAGGG - 3'
3'- GAC... GGTCCGAGGTGGGCTGACCTCCC - 5'
sgRNA 5'- GGCCAGGCUCCACCCGACUGGAGUUUUAGAGCUA
AGCCACGGUGAAAAAGUUCAACUAUUGCCUGAUCGGAAUAAAAUU CGAU
GAA
UCGGUGCUUUUUUUU- 3'
Cas9

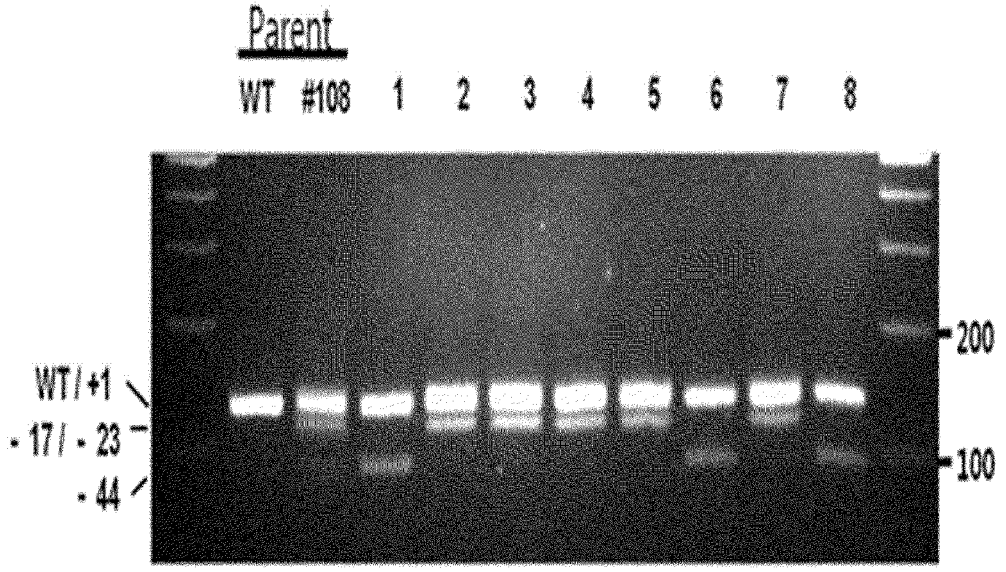
[Fig. 5b]


WT
#104
#105
#106
#108
#109
#110
#111
#112
#114

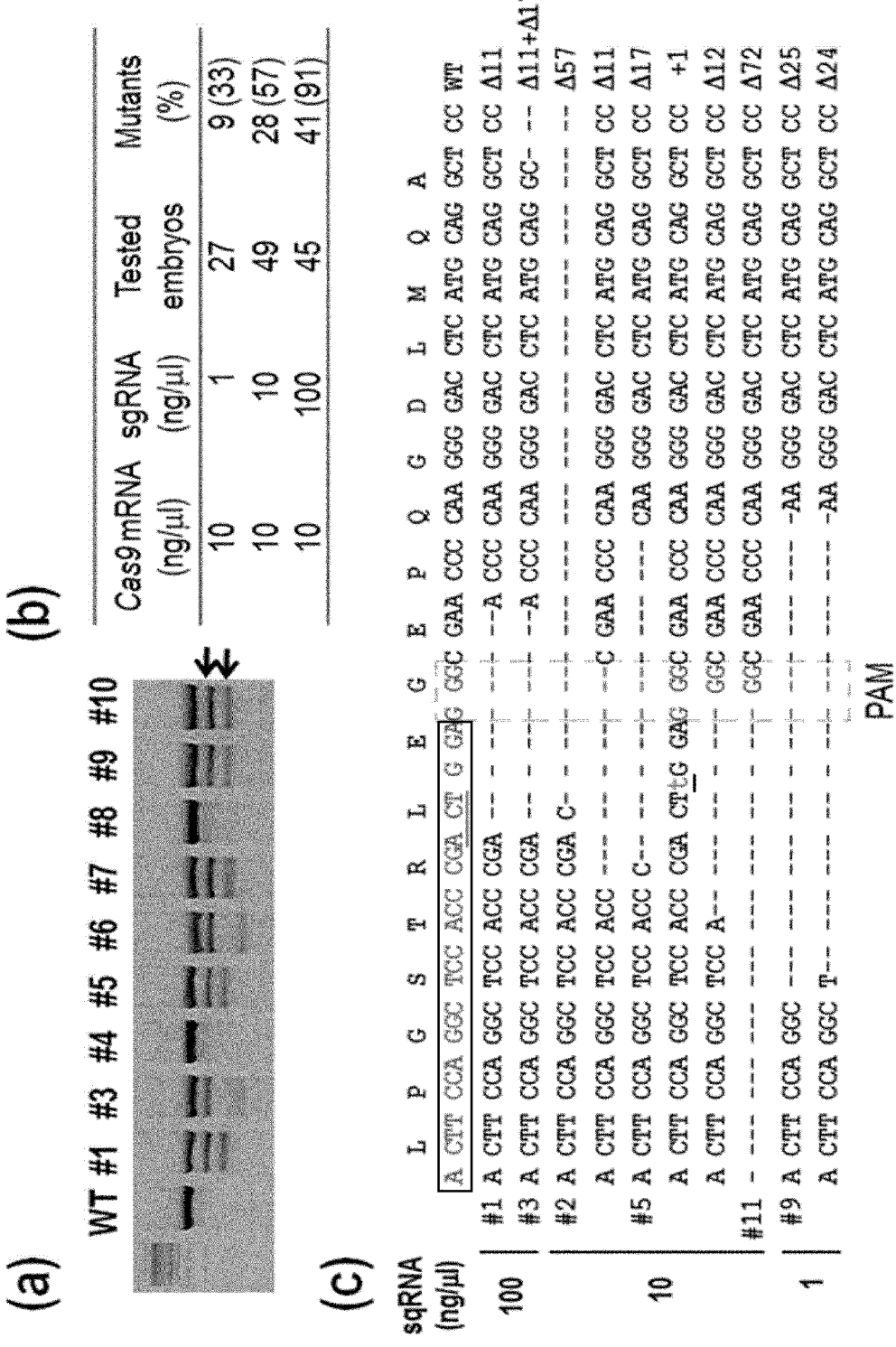
[Fig. 5c]


WT
ACTTCCAGGCTCCACCCGACT GGAGGGCGAACCCCAAGGGGACCTCATGCAGG
#108
ACTTCCAGGCTCC-------- -----44bp---------- -44 (X6)
ACTTCCAGGCTCCACCCGAC- ----------CTCATGCAGG -23 (X2)
ACTTCCAGGCTCCACCC---- ----------CAAGGGGACCTCATGCAGG -17 (X1)
ACTTCCAGGCTCCACCCGACTTGGAGGGCGAACCCCAAGGGGACCTCATGCAGG +1 (X1)
#111
ACTTCCAGGCTCCACCCGACTTGGAGGGCGAACCCCAAGGGGACCTCATGCAGG +1 (X2)
ACTTCCAGGCTCCACCCG----------AACCCCAAGGGGACCTCATGCAGGG -11 (X6)
#114
ACTTCCAGGCTCCACCCGACT ------CACTATCTTCTGGGCTCCTCCATGTC -6 +25 (X3)
ACTTCCAGGCTCCACCC---- ----------CAAGGGGACCTCATGCAGG -17 (X6)
ACTTCCAGGCTCCACCCGAC- -------GAACCCCAAGGGGACCTCATGCAGG -8 (X1)

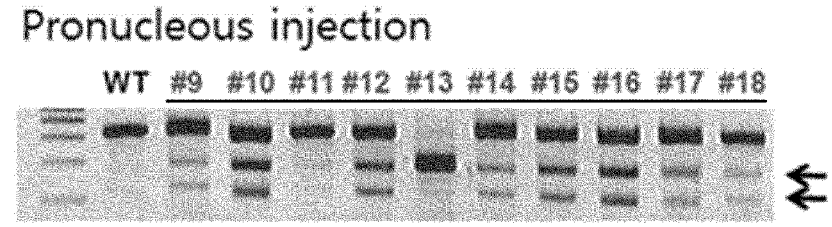
[Fig. 5d]


Parent
WT #108 1 2 3 4 5 6 7 8
WT / +1
- 17 / - 23
- 44
200
100

[Fig. 6]

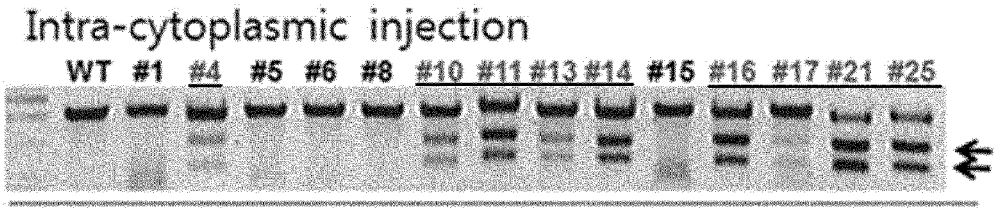

(a)
WT #1 #3 #4 #5 #6 #7 #8 #9 #10
(b)
Cas9 mRNA (ng/μl)
sgRNA (ng/μl)
Tested embryos
Mutants (%)
10 1 27 9 (33)
10 10 49 28 (57)
10 100 45 41 (91)
(c)
sqRNA (ng/μl)
L P G S T R L E G E P Q G D L M Q A
A CTT CCA GGC TCC ACC CGA CT G GAG GGC GAA CCC CAA GGG GAC CTC ATG CAG GCT CC WT
100
#1 A CTT CCA GGC TCC ACC CGA -- - --- --A CCC CAA GGG GAC CTC ATG CAG GCT CC Δ11
#3 A CTT CCA GGC TCC ACC CGA -- - --- --A CCC CAA GGG GAC CTC ATG CAG GC- -- Δ11+Δ17
10
#2 A CTT CCA GGC TCC ACC CGA C- - --- --- --- --- --- --- --- --- --- --- Δ57
A CTT CCA GGC TCC ACC --- -- - --- --C GAA CCC CAA GGG GAC CTC ATG CAG GCT CC Δ11
#5 A CTT CCA GGC TCC ACC C-- -- - --- --- --- --- CAA GGG GAC CTC ATG CAG GCT CC Δ17
A CTT CCA GGC TCC ACC CGA CTtG GAG GGC GAA CCC CAA GGG GAC CTC ATG CAG GCT CC +1
A CTT CCA GGC TCC A-- --- -- - --- GGC GAA CCC CAA GGG GAC CTC ATG CAG GCT CC Δ12
#11 - --- --- --- --- --- -- - --- GGC GAA CCC CAA GGG GAC CTC ATG CAG GCT CC Δ72
1
#9 A CTT CCA GGC --- --- --- -- - --- --- --- -AA GGG GAC CTC ATG CAG GCT CC Δ25
A CTT CCA GGC T-- --- --- -- - --- --- --- -AA GGG GAC CTC ATG CAG GCT CC Δ24
PAM

[Fig. 7a]



Cas9 protein (nM)	sgRNA (nM)	Tested embryos	Mutants (%)
2	4	20	3 (15%)
20	40	15	5 (33%)
200	400	17	15 (88%)

[Fig. 7b]

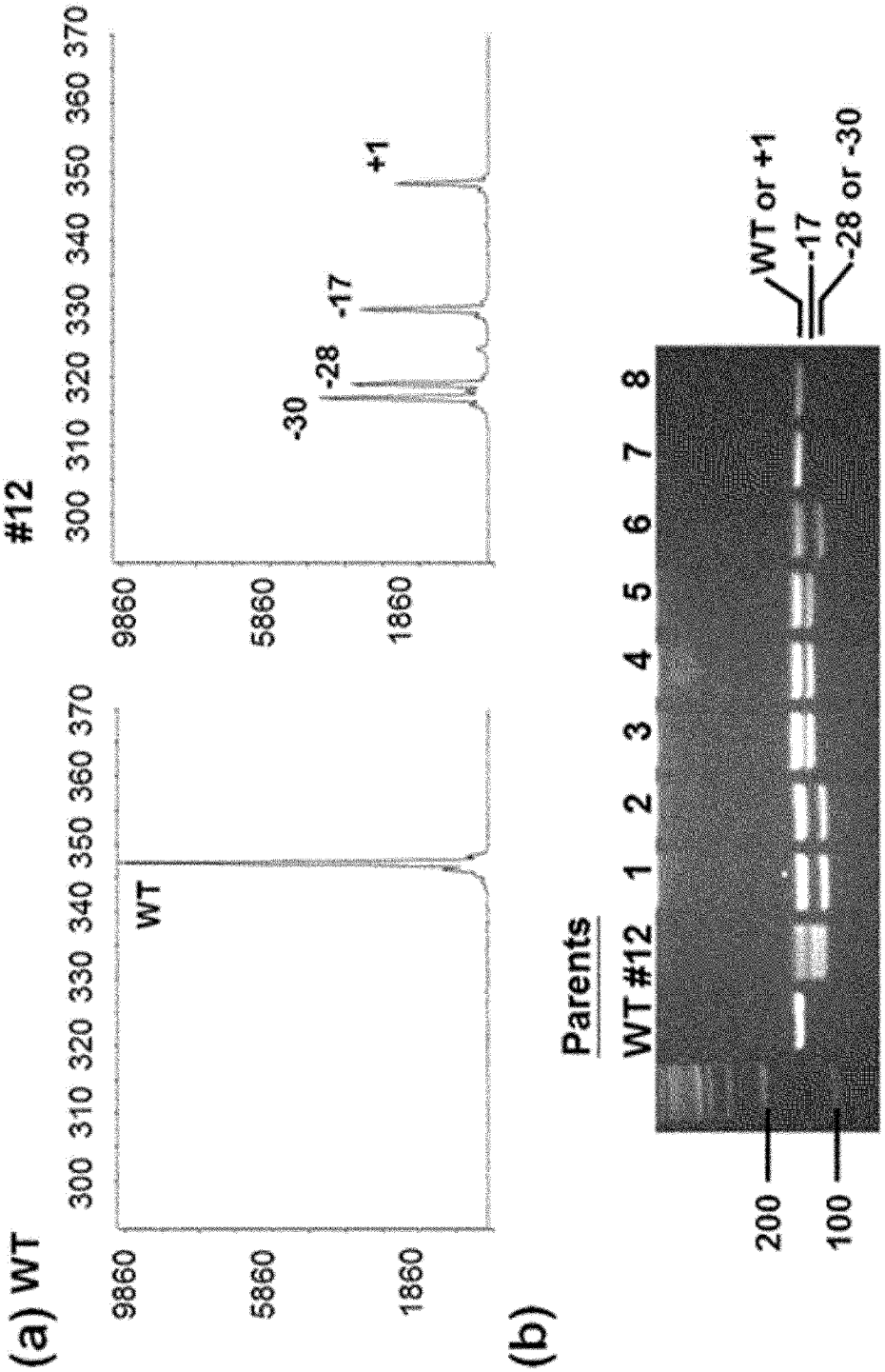


Cas9 protein (nM)	sgRNA (nM)	Tested embryos	Mutants (%)
2	4	18	1 (6%)
20	40	19	6 (32%)
200	400	14	10 (71%)

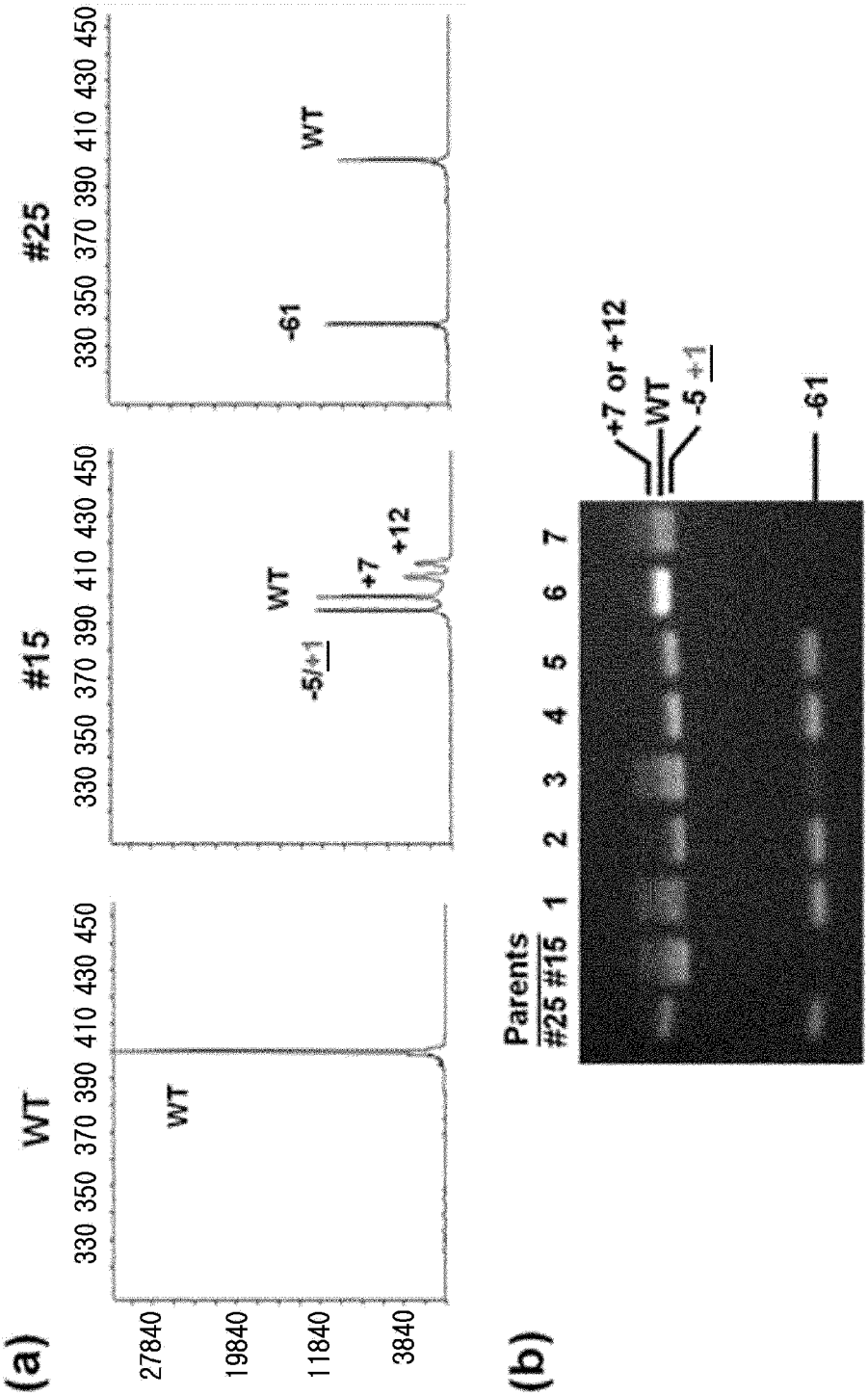
[Fig. 7c]

Sequence	Indels	Embryo no.
ACTTCCAGGCTCCACCCGACTGGAGGGCGAACCCCAAGGGGACCTCATGCAG	WT	
ACTTCCAGGCGAACCCC----------AAGGGGACCTCATGCAG	Δ18	2
ACTTCCAGGCTCCAC----------AAGGGGACCTCATGCAG	Δ20	1
ACTTCCAGGCTCCACCC----------AAGGGGACCTCATGCCC	Δ19	1
ACTTCCAGGCTCCACCC----------CAAGGGGACCTCATGCAG	Δ17	1
ACTTCCAGGCTCCACCCGA----------ACCCCAAGGGGACCTCATGCAG	Δ11	3
ACTTCCAGGCTCCACCCGAA--GGAGGGCGAACCCCAAGGGGACCTCATGCA	Δ3+1	1
ACTTCCAGGCTCCACCCGACT--AGGGCGAACCCCAAGGGGACCTCATGCAG	Δ2	1
ACTTCCAGGCTCCACCCGACTGGGAGGGCGAACCCCAAGGGGACCTCATGCA	+1	1
ACTTCCAGGCTCCACCCGACTTGGAGGGCGAACCCCAAGGGGACCTCATGCA	+1	10
ACTTCCAGGCTCCACCCGA------GGCGAACCCCAAGGGGACCTCATGCAG	Δ6	1
ACTTCCAGGCTCCACCCGA-----GGGCGAACCCCAAGGGGACCTCATGCAG	Δ5	2
ACTTCCAGGCTCCACC----------TCATGCAG	Δ28	1
----------AGGGCGAACCCCAAGGGGACCTCATGCAG	Δ126	1
Total		26

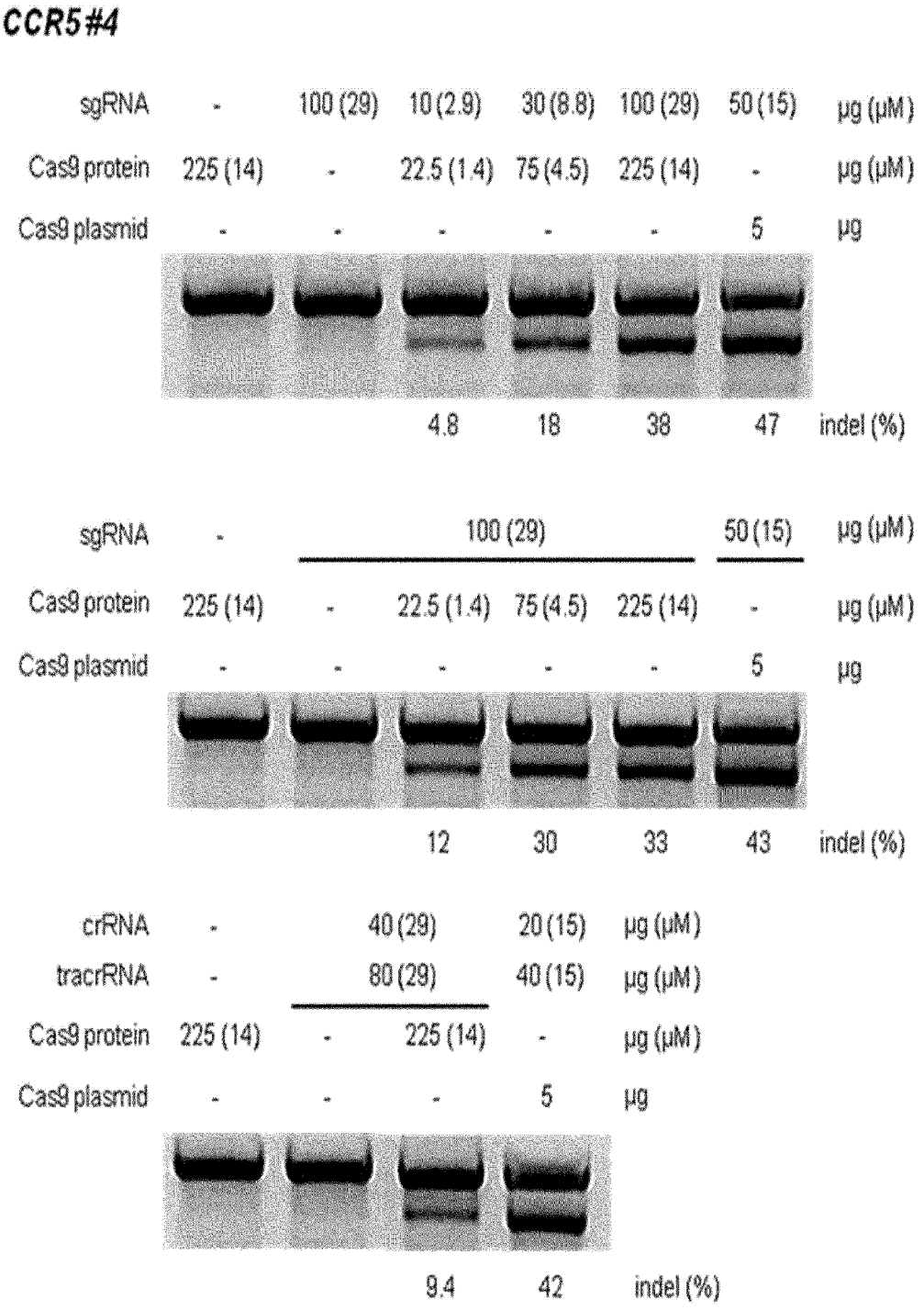
[Fig. 8]


(a) WT
#12
WT
-30
-28
-17
+1
9860
5860
1860
300 310 320 330 340 350 360 370
(b)
Parents
WT #12 1 2 3 4 5 6 7 8
WT or +1
-17
-28 or -30
200
100

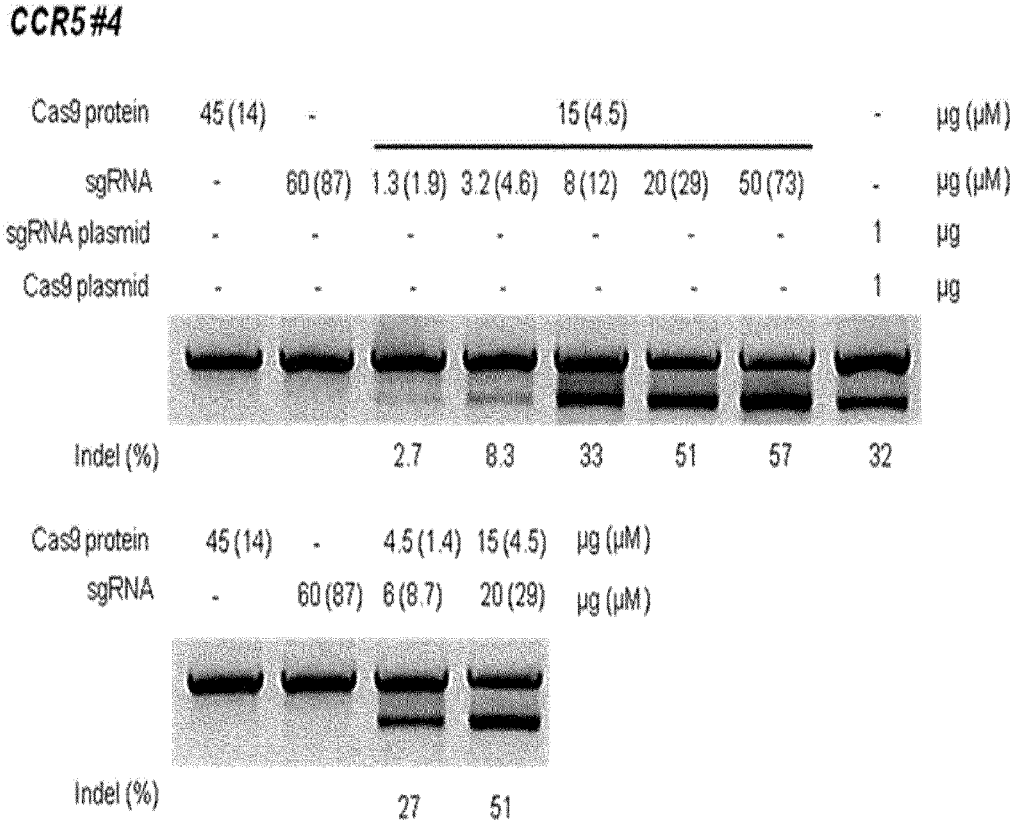
[Fig. 9]


(a)
WT
#15
#25
330 350 370 390 410 430 450
WT
-5/+1
+7
+12
-61
WT
27840
19840
11840
3840
(b)
Parents
#25 #15
1 2 3 4 5 6 7
+7 or +12
WT
-5 +1
-61

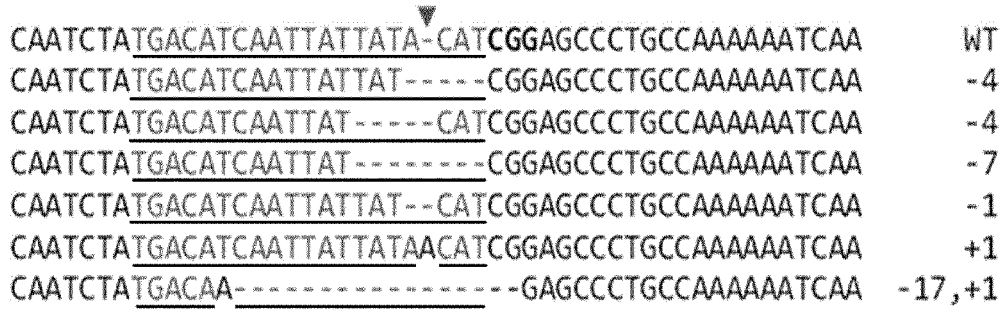
[Fig. 10a]


CCR5#4
sgRNA - 100 (29) 10 (2.9) 30 (8.8) 100 (29) 50 (15) μg (μM)
Cas9 protein 225 (14) - 22.5 (1.4) 75 (4.5) 225 (14) - μg (μM)
Cas9 plasmid - - - - - 5 μg
4.8 18 38 47 indel (%)
sgRNA - 100 (29) 50 (15) μg (μM)
Cas9 protein 225 (14) - 22.5 (1.4) 75 (4.5) 225 (14) - μg (μM)
Cas9 plasmid - - - - - 5 μg
12 30 33 43 indel (%)
crRNA - 40 (29) 20 (15) μg (μM)
tracrRNA - 80 (29) 40 (15) μg (μM)
Cas9 protein 225 (14) - 225 (14) - μg (μM)
Cas9 plasmid - - - 5 μg
9.4 42 indel (%)

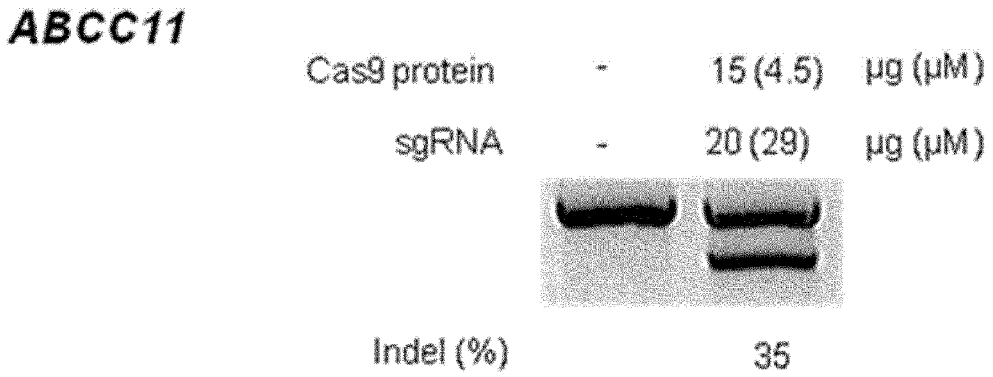
[Fig. 10b]



[Fig. 10c]

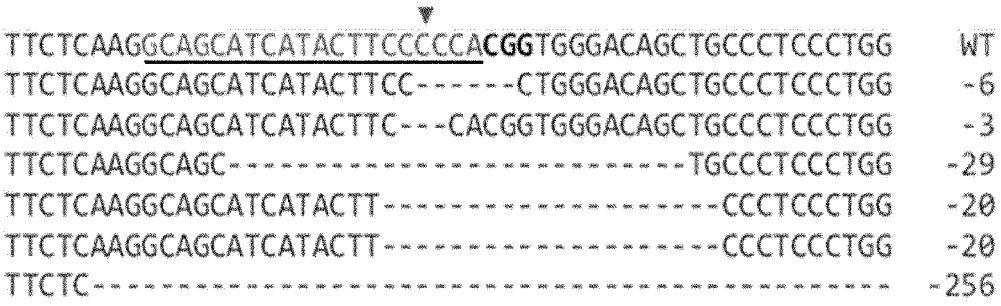


[Fig. 10d]

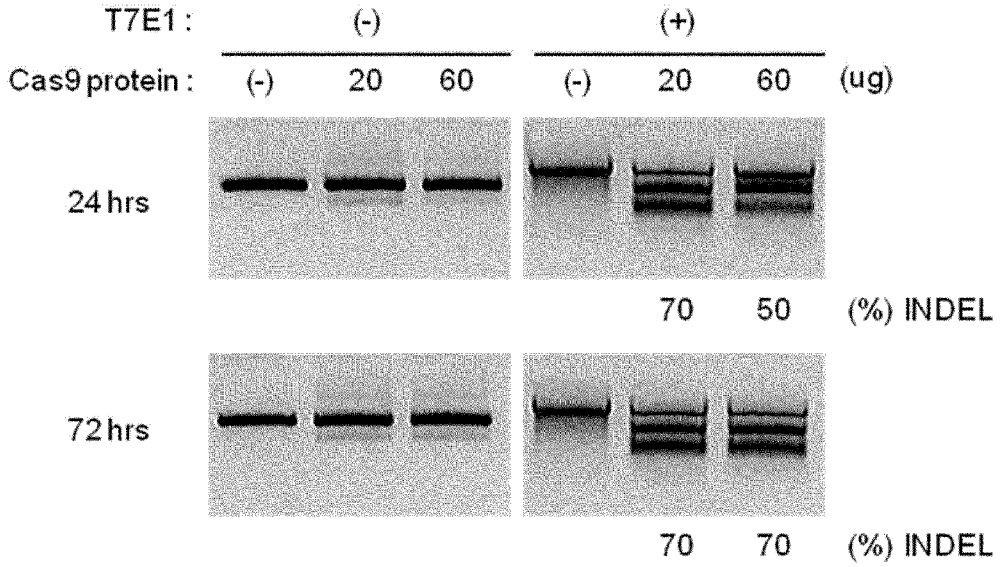


[Fig. 10e]

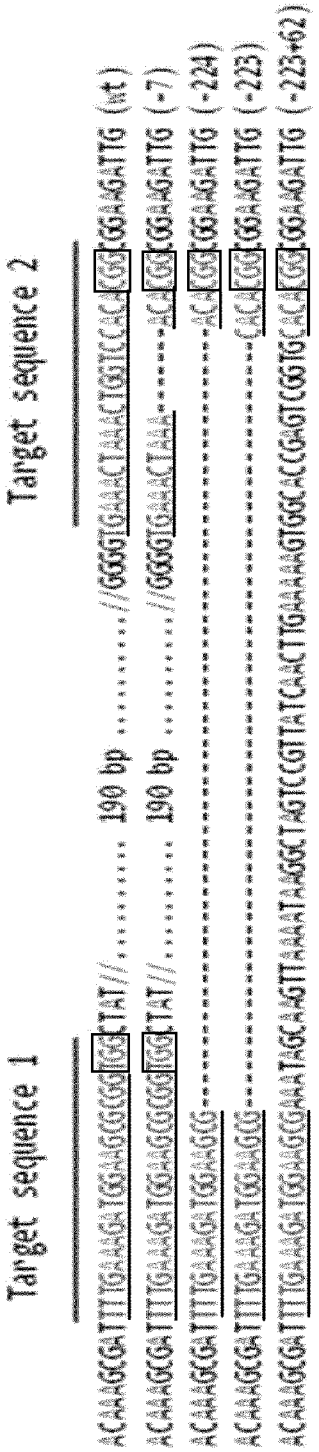
ABCC11



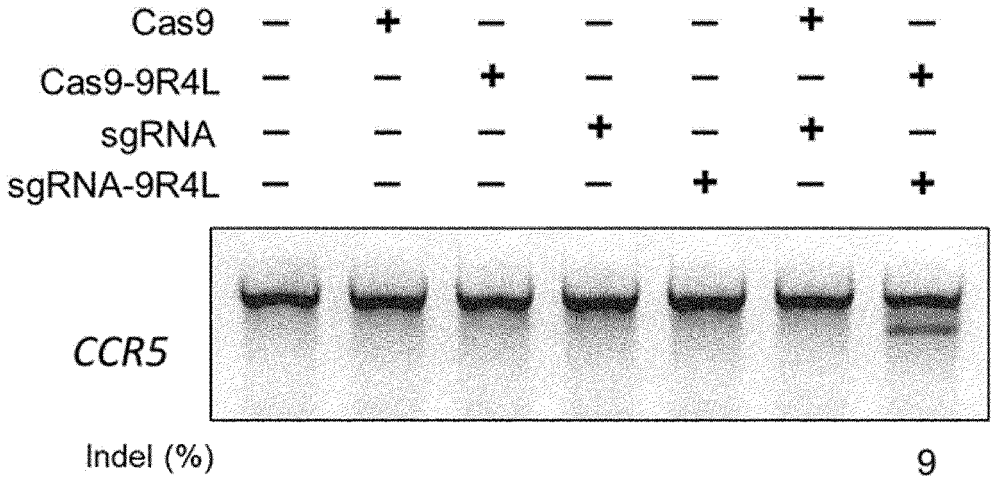
[Fig. 11]



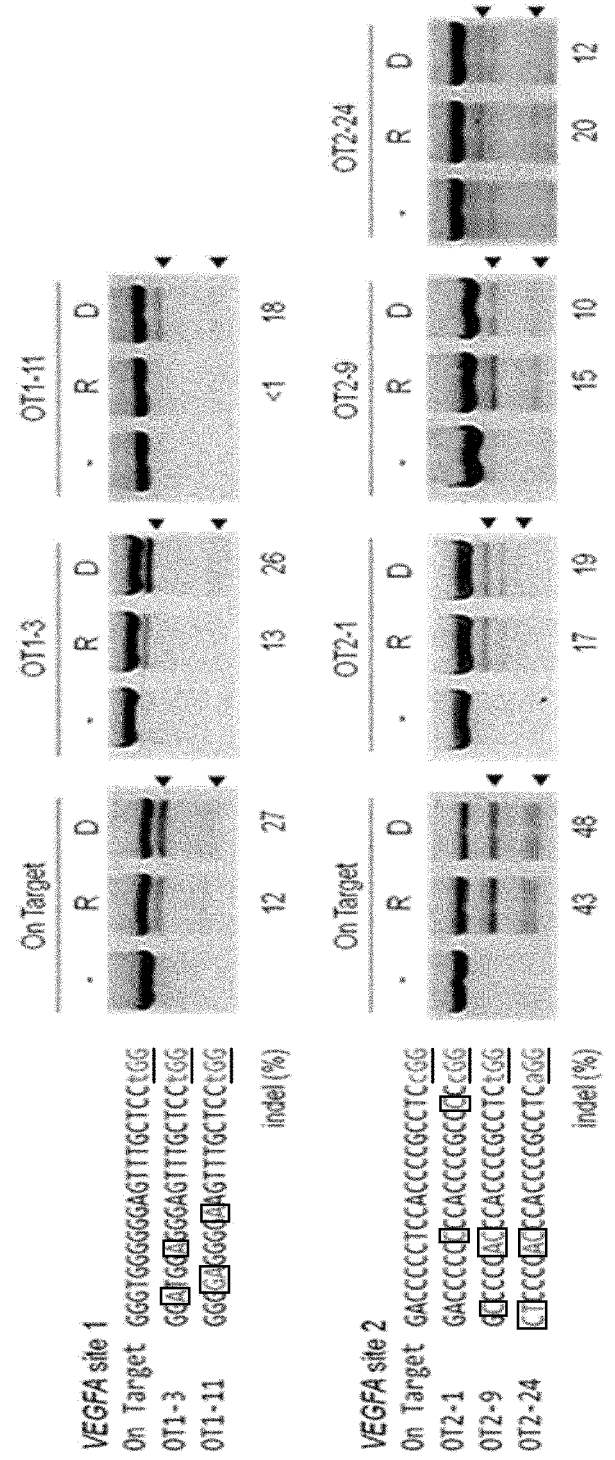
[Fig. 12]


Target sequence 1
Target sequence 2
ACAAAGCGATTTTGAAAGATGGAAGCGGCGGTCGTAT//..... 190 bp .....//GGGGTGAAACTAAACTGGTCCACACGGCGGAAGATTG (wt)
ACAAAGCGATTTTGAAAGATGGAAGCGGCGGTCGTAT//..... 190 bp .....//GGGGTGAAACTAAA----------ACACGGCGGAAGATTG (-7)
ACAAAGCGATTTTGAAAGATGGAAGCG--ACACGGCGGAAGATTG (-224)
ACAAAGCGATTTTGAAAGATGGAAGCG--CACACGGCGGAAGATTG (-223)
ACAAAGCGATTTTGAAAGATGGAAGCGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCACACGGCGGAAGATTG (-223+62)

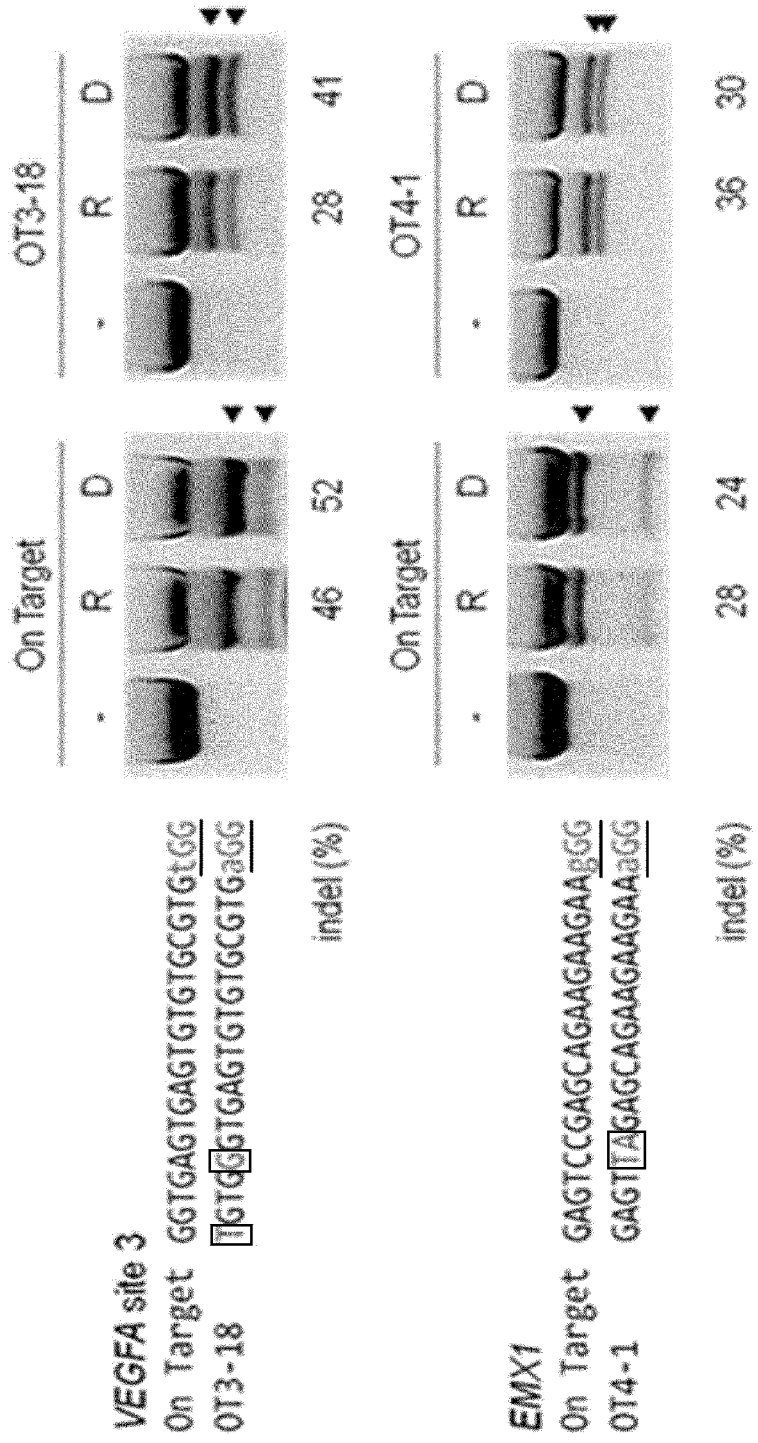
[Fig. 13]



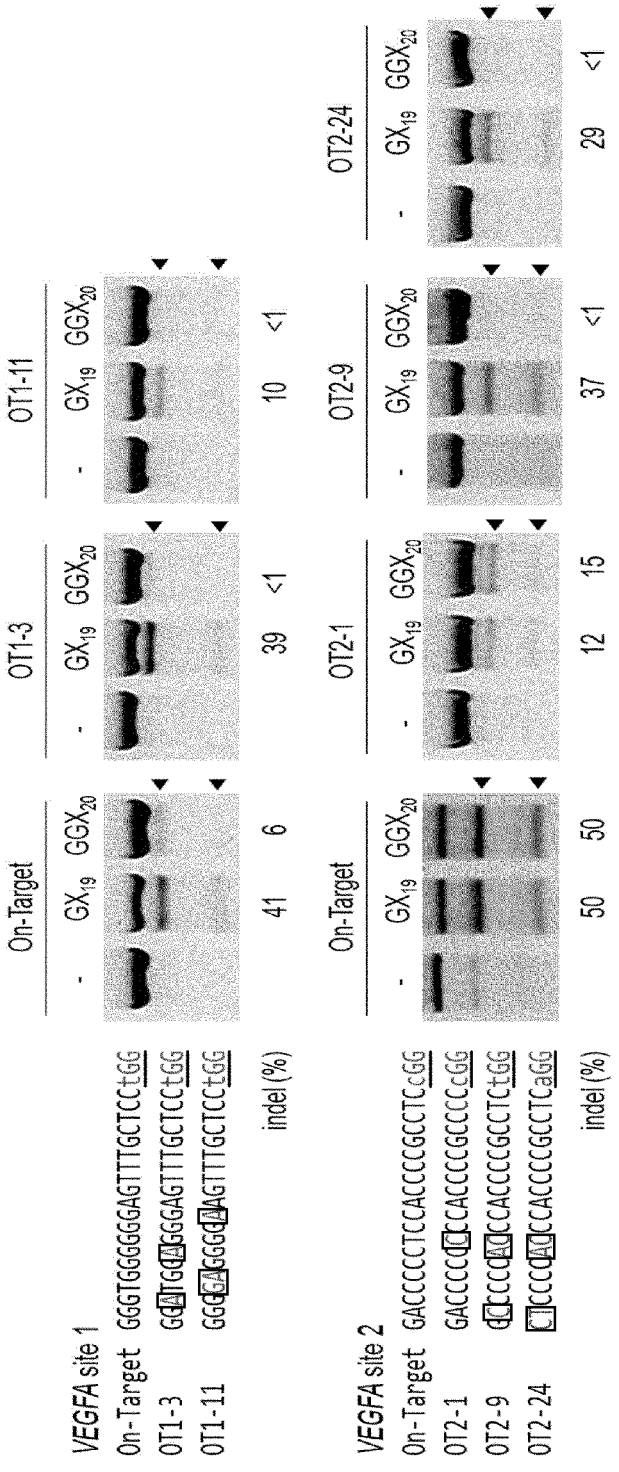
[Fig. 14a]


VEGFA site 1
On Target GGGTGGGGGGAGTTTGCTCCTGG
OT1-3 GGATGGGAGTTTGCTCCTGG
OT1-11 GGGAGGGATGTTTGCTCCTGG
indel (%)
On Target
OT1-3
OT1-11
- R D
12 27
13 26
<1 18
VEGFA site 2
On Target GACCCCCTCCACCCCGCCTCCGG
OT2-1 GACCCCCTCCACCCCGCCTCCGG
OT2-9 GTCCCCAACCACCCCGCCTCTGG
OT2-24 CTCCCCAACCACCCCGCCTCAGG
indel (%)
On Target
OT2-1
OT2-9
OT2-24
- R D
43 48
17 19
15 10
20 12

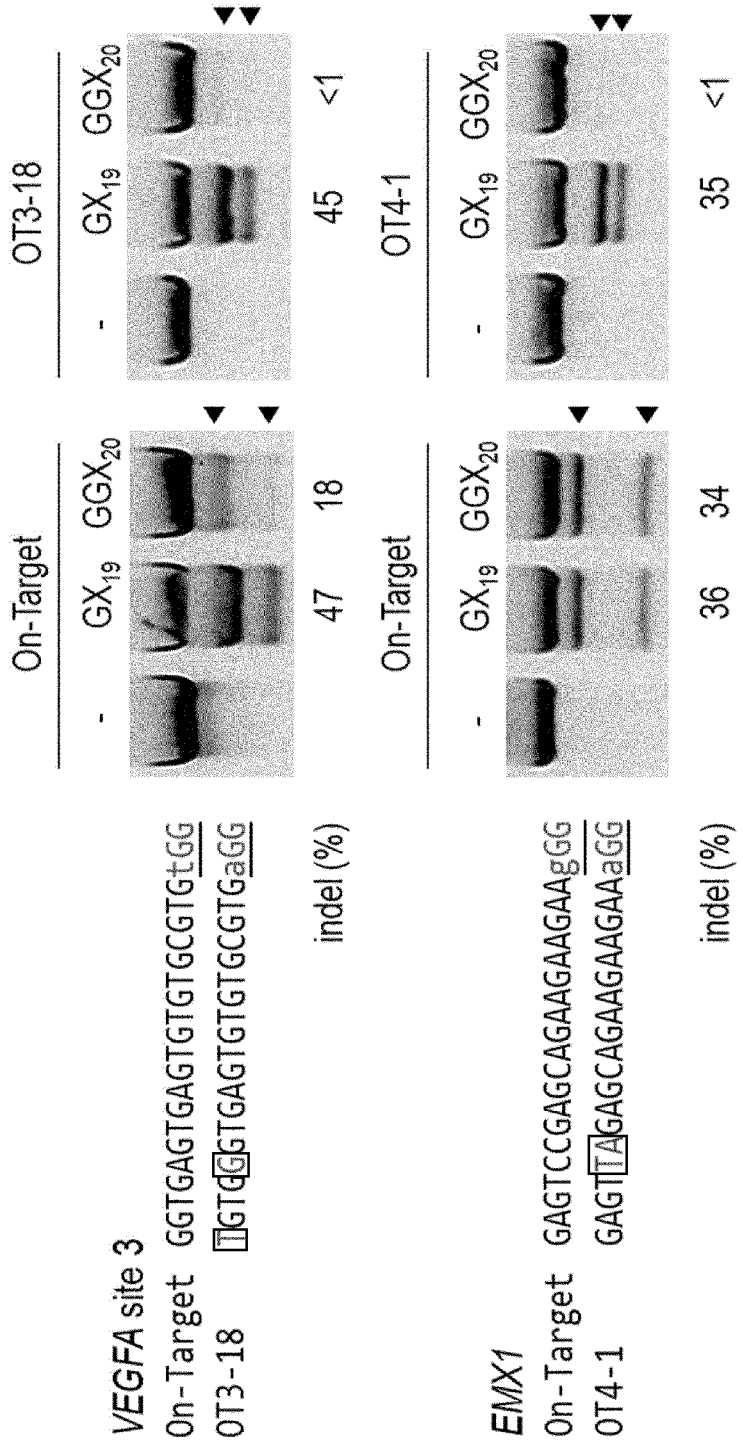
[Fig. 14b]


On Target
OT3-18
-
R
D
46
52
28
41
indel (%)
VEGFA site 3
On Target GGTGAGTGAGTGTGTGCGTGtGG
OT3-18 TGTGGGTGAGTGTGTGCGTGaGG
On Target
OT4-1
-
R
D
28
24
36
30
indel (%)
EMX1
On Target GAGTCCGAGCAGAAGAAGAAgGG
OT4-1 GAGTTAGAGCAGAAGAAGAAaGG

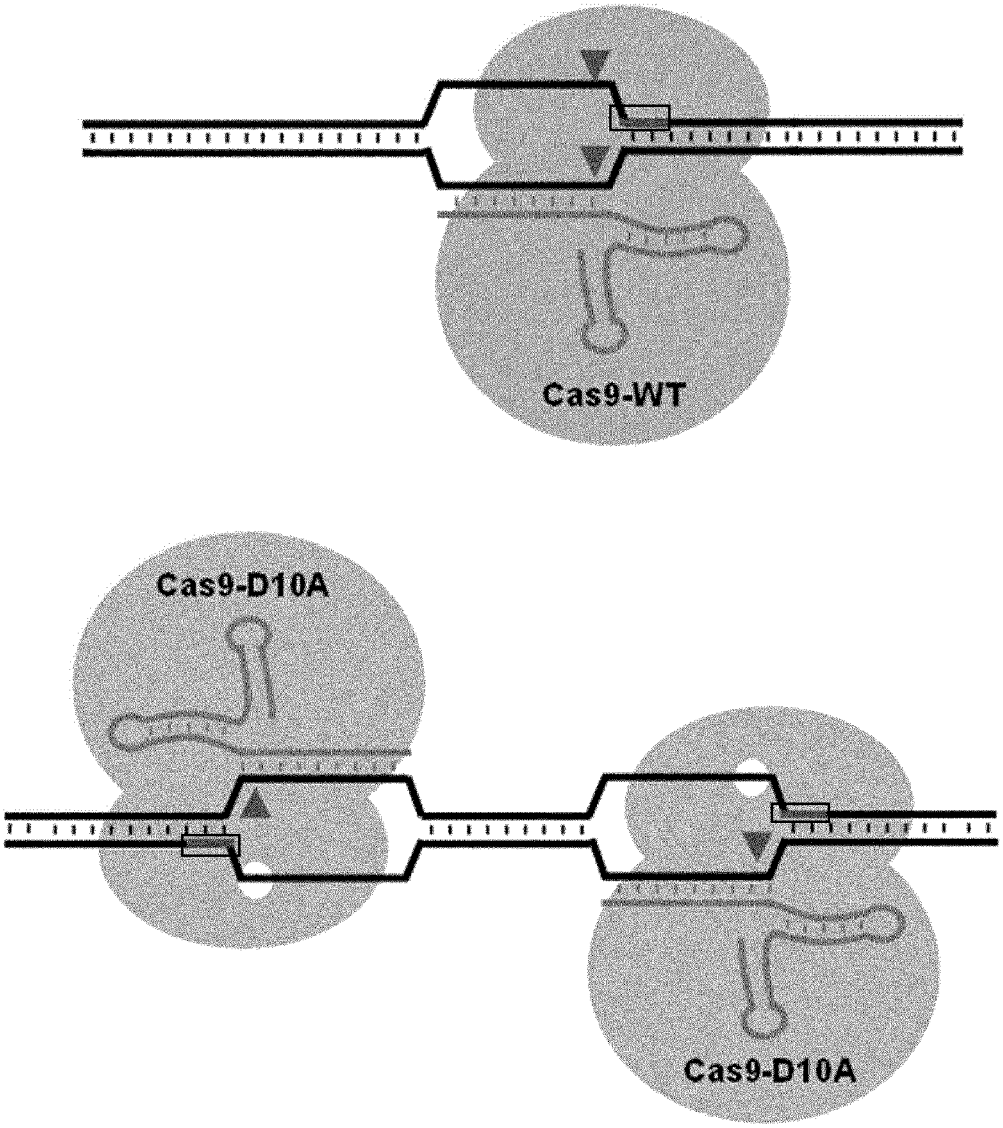
[Fig. 15a]


On-Target
OT1-3
OT1-11
- GX19 GGX20
VEGFA site 1
On-Target GGGTGGGGGGAGTTTGCTCCTGG
OT1-3 GGATGGAGGGAGTTTGCTCCTGG
OT1-11 GGGCAGGGGAAGTTTGCTCCTGG
indel (%) 41 6 39 <1 10 <1
On-Target
OT2-1
OT2-9
OT2-24
- GX19 GGX20
VEGFA site 2
On-Target GACCCCCTCCACCCCGCCTCCGG
OT2-1 GACCCCCTCCACCCCGCCCCCGG
OT2-9 CTCCCCAACCACCCCGCCTCTGG
OT2-24 CTCCCCAACCACCCCGCCTCAGG
indel (%) 50 50 12 15 37 <1 29 <1

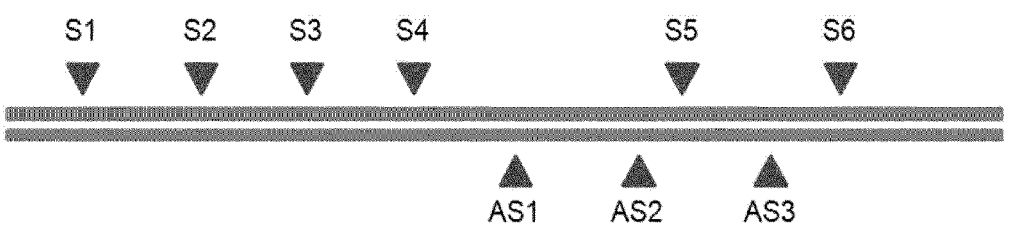
[Fig. 15b]


On-Target
OT3-18
-
GX19
GGX20
47
18
45
<1
On-Target
OT4-1
36
34
35
<1
indel (%)
VEGFA site 3
On-Target GGTGAGTGAGTGTGTGCGTGtGG
OT3-18 TGTGGTGAGTGTGTGCGTGaGG
EMX1
On-Target GAGTCCGAGCAGAAGAAGAAgGG
OT4-1 GAGTTAGAGCAGAAGAAGAAaGG

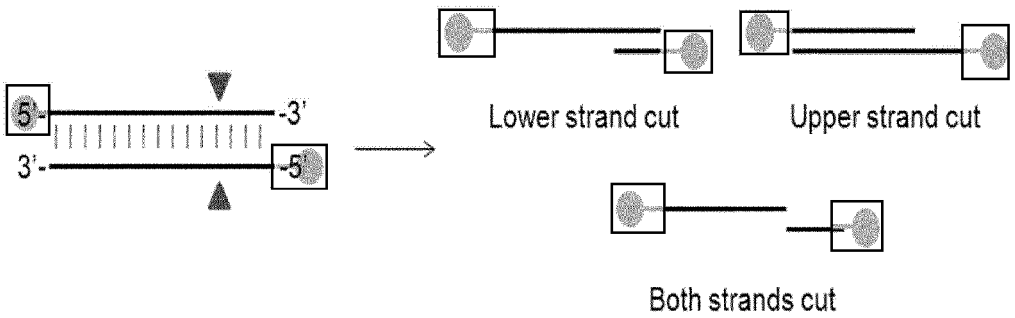
[Fig. 16a]



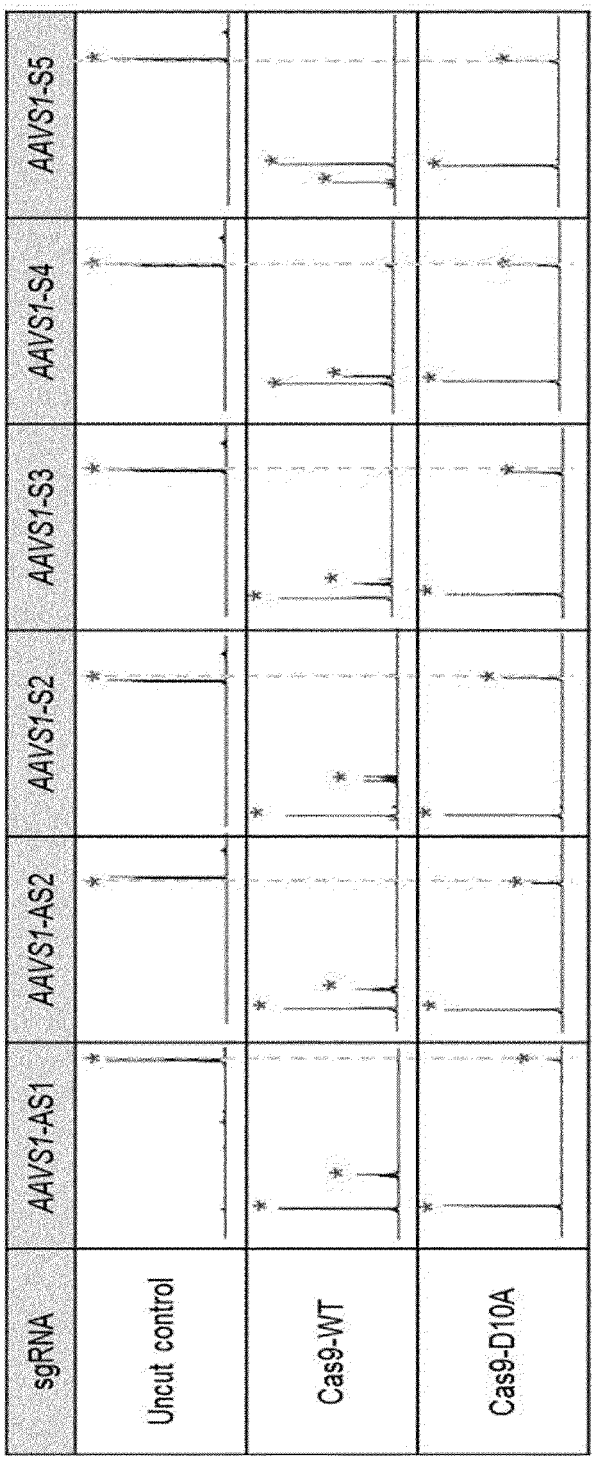
[Fig. 16b]



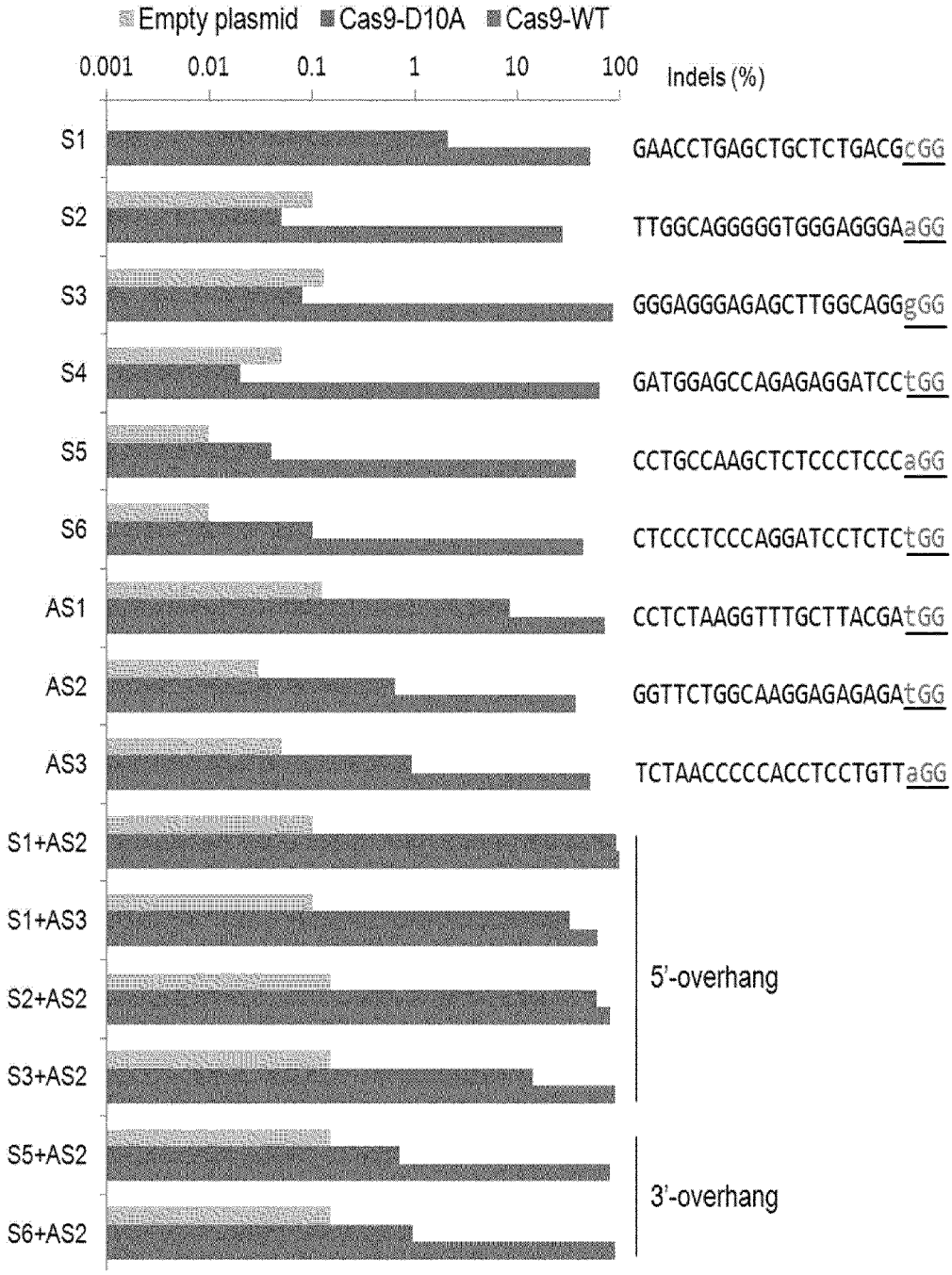
[Fig. 16c]



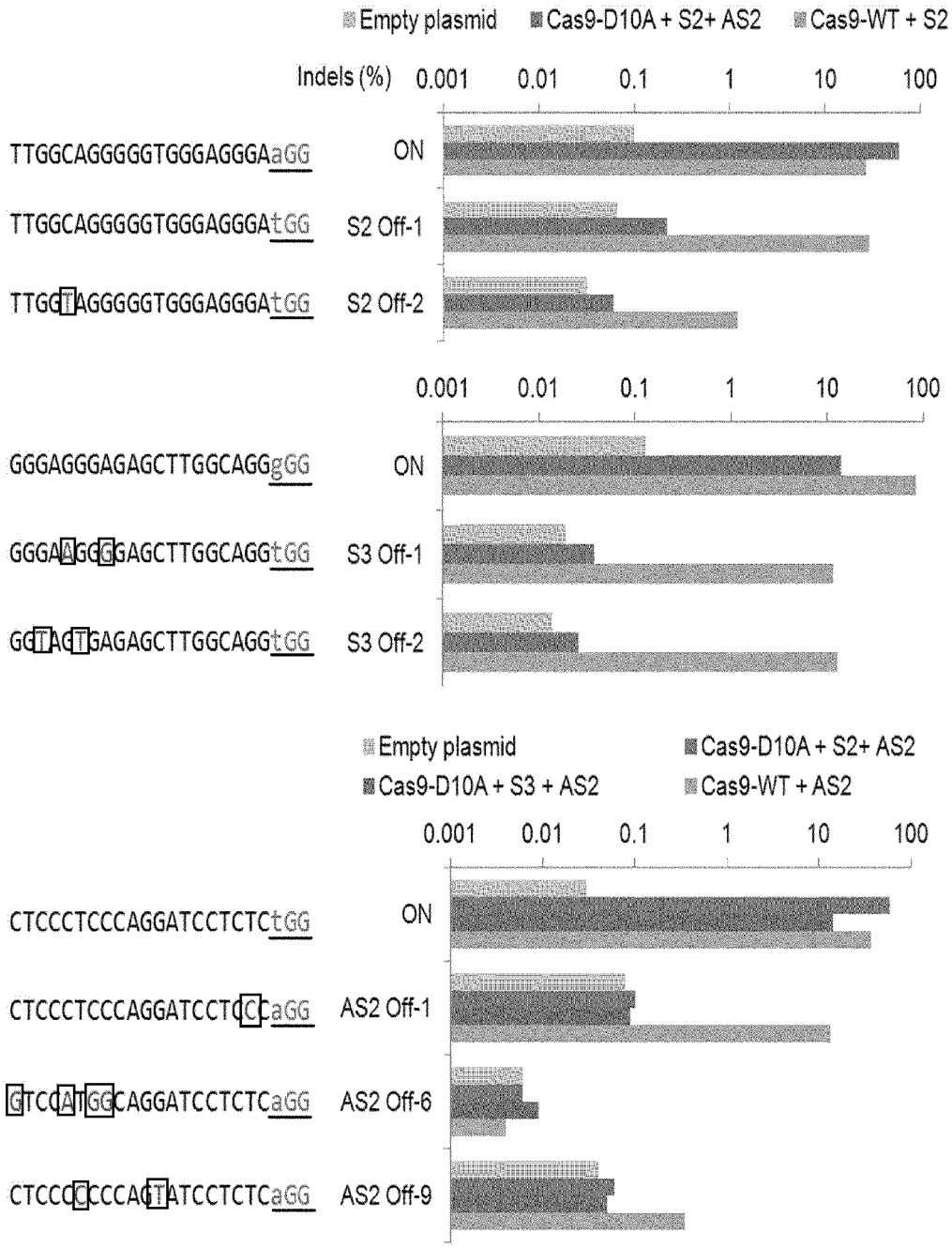
[Fig. 16d]


sgRNA
AAVS1-AS1
AAVS1-AS2
AAVS1-S2
AAVS1-S3
AAVS1-S4
AAVS1-S5
Uncut control
Cas9-WT
Cas9-D10A

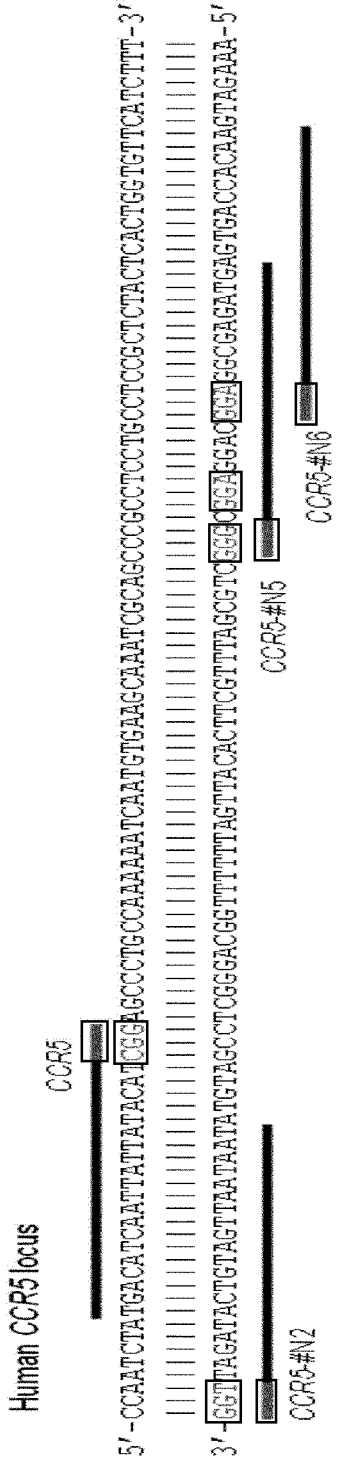
[Fig. 17a]


Empty plasmid
Cas9-D10A
Cas9-WT
0.001
0.01
0.1
1
10
100
Indels (%)
S1
GAACCTGAGCTGCTCTGACGcGG
S2
TTGGCAGGGGGTGGGAGGGAaGG
S3
GGGAGGGAGAGCTTGGCAGGgGG
S4
GATGGAGCCAGAGAGGATCCtGG
S5
CCTGCCAAGCTCTCCCTCCCaGG
S6
CTCCCTCCCAGGATCCTCTCtGG
AS1
CCTCTAAGGTTTGCTTACGAtGG
AS2
GGTTCTGGCAAGGAGAGAGAtGG
AS3
TCTAACCCCCACCTCCTGTTaGG
S1+AS2
S1+AS3
S2+AS2
S3+AS2
S5+AS2
S6+AS2
5'-overhang
3'-overhang

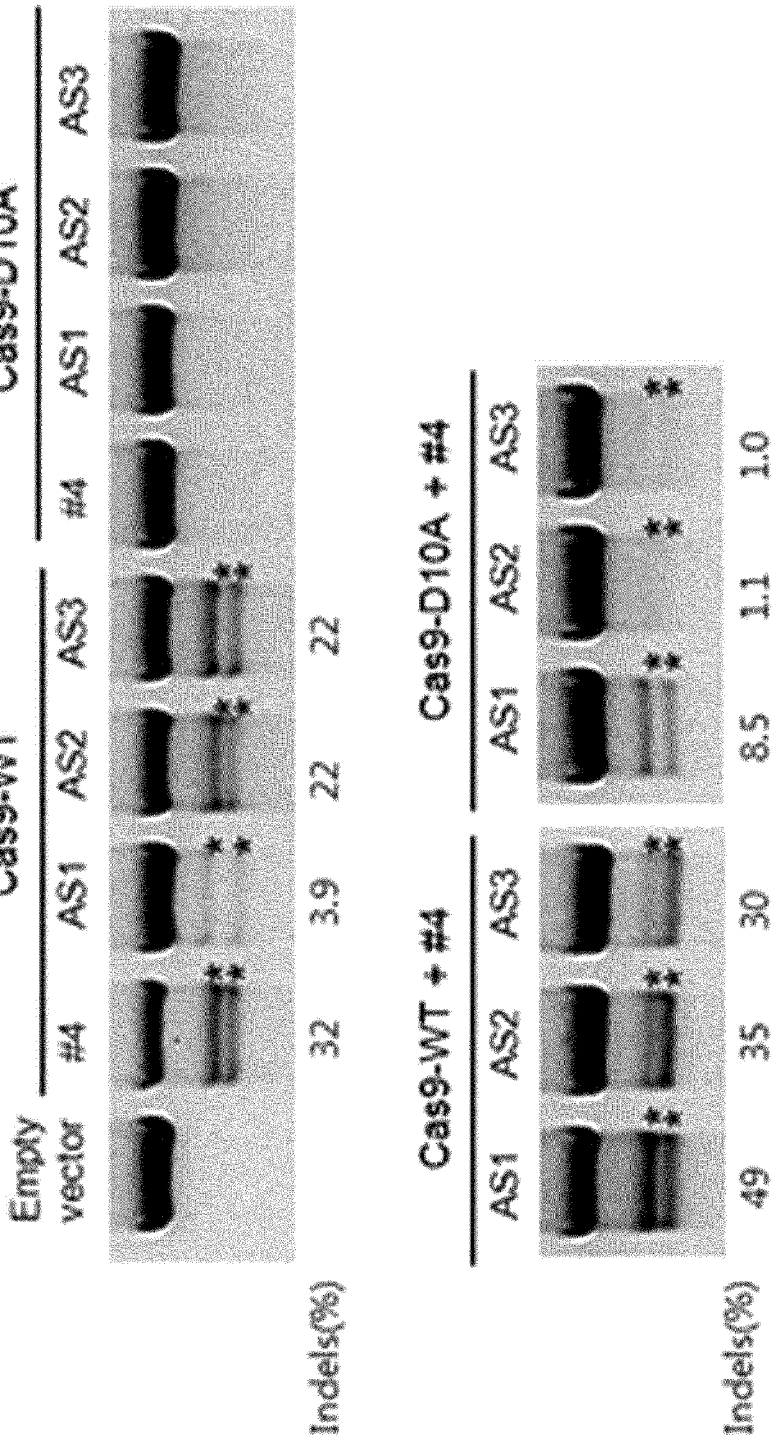
[Fig. 17b]


Empty plasmid
Cas9-D10A + S2+ AS2
Cas9-WT + S2
Indels (%)
0.001
0.01
0.1
1
10
100
TTGGCAGGGGGTGGGAGGGAaGG
ON
TTGGCAGGGGGTGGGAGGGAtGG
S2 Off-1
TTGGTAGGGGGTGGGAGGGAtGG
S2 Off-2
GGGAGGGAGAGCTTGGCAGGgGG
ON
GGGAAGGGGAGCTTGGCAGGtGG
S3 Off-1
GGTAGTGAGAGCTTGGCAGGtGG
S3 Off-2
Empty plasmid
Cas9-D10A + S2+ AS2
Cas9-D10A + S3 + AS2
Cas9-WT + AS2
CTCCCTCCCAGGATCCTCTCtGG
ON
CTCCCTCCCAGGATCCTCCCaGG
AS2 Off-1
GTCCATGGCAGGATCCTCTCaGG
AS2 Off-6
CTCCCCCCCAGTATCCTCTCaGG
AS2 Off-9

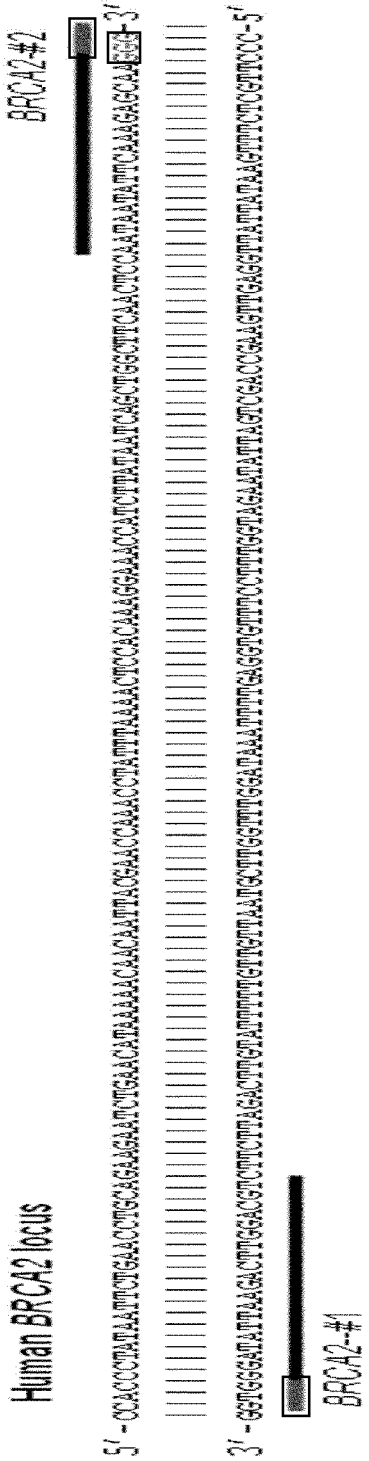
[Fig. 18a]


Human CCR5 locus
CCR5
5′-CCAATCTATGACATCAATTATTATACATCGGAGCCCTGCCAAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGTACCTCCTACTCACTGGTGTTCATCTTT-3′
3′-GGTTAGATACTGTAGTTAATAATATGTAGCCTCGGGACGGTTTTTTAGTTACACTTCGTTTAGCGTCGGGCCTGGAGGACGGAGGCGAGATGAGTGACCACACAGTAGAAA-5′
CCR5-#N2
CCR5-#N5
CCR5-#N6

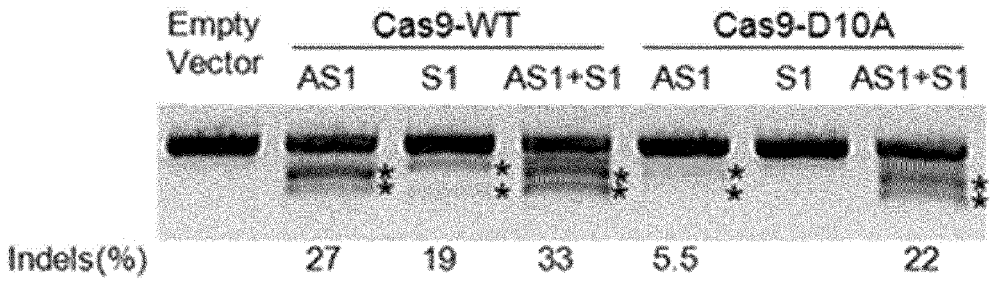
[Fig. 18b]


Empty vector
Cas9-WT
#4
AS1
AS2
AS3
Cas9-D10A
#4
AS1
AS2
AS3
Indels(%)
32
3.9
22
22
Cas9-WT + #4
AS1
AS2
AS3
Cas9-D10A + #4
AS1
AS2
AS3
Indels(%)
49
35
30
8.5
1.1
1.0

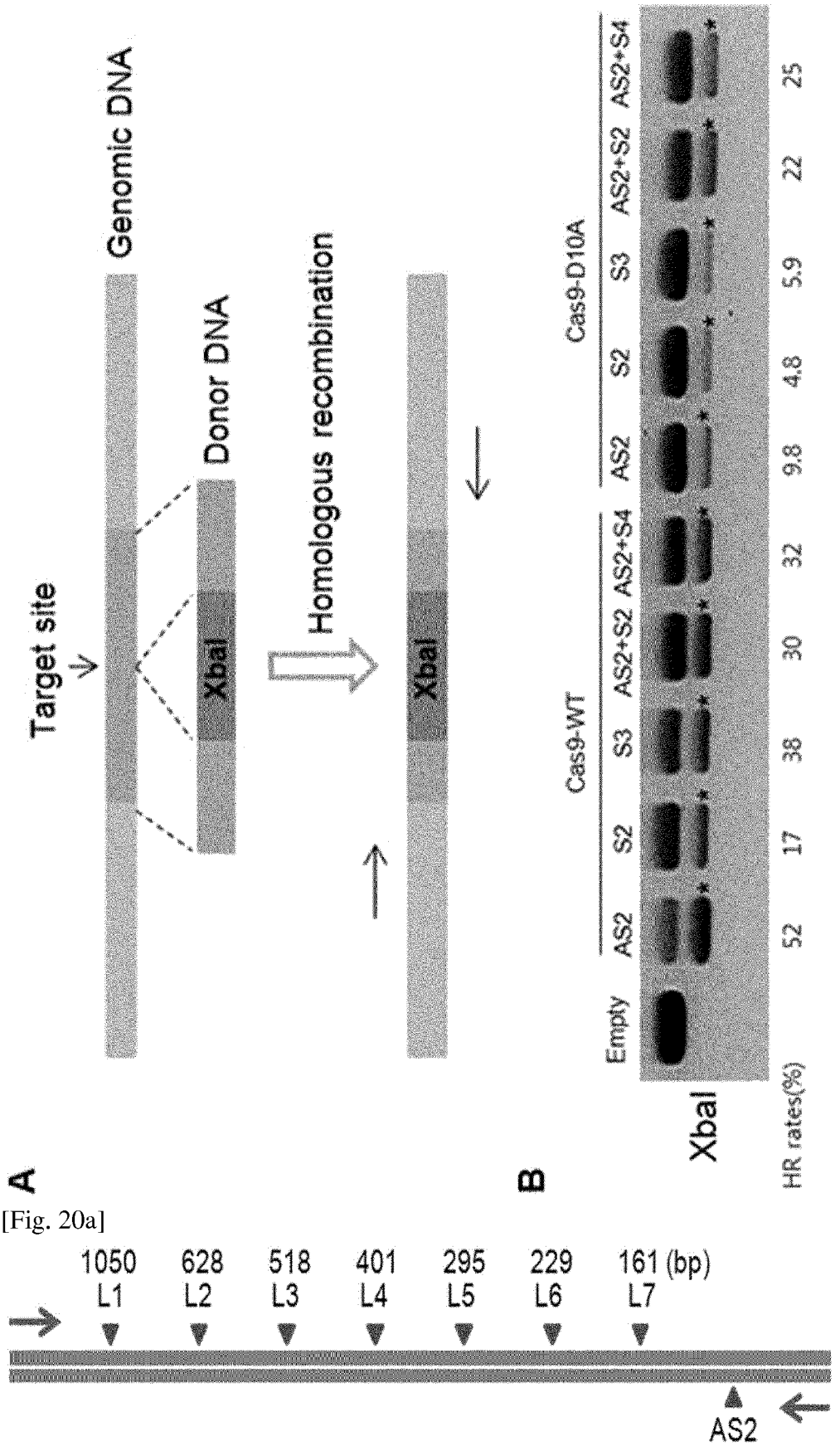
[Fig. 18c]



[Fig. 18d]

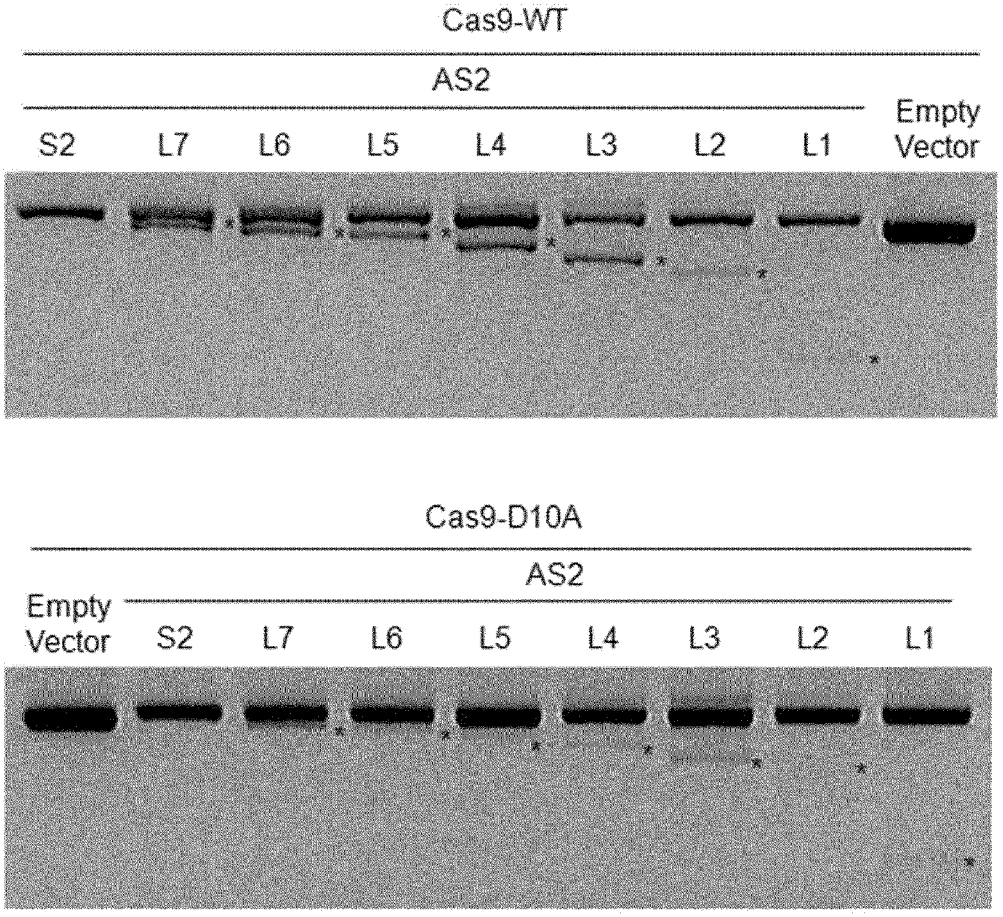


[Fig. 19]

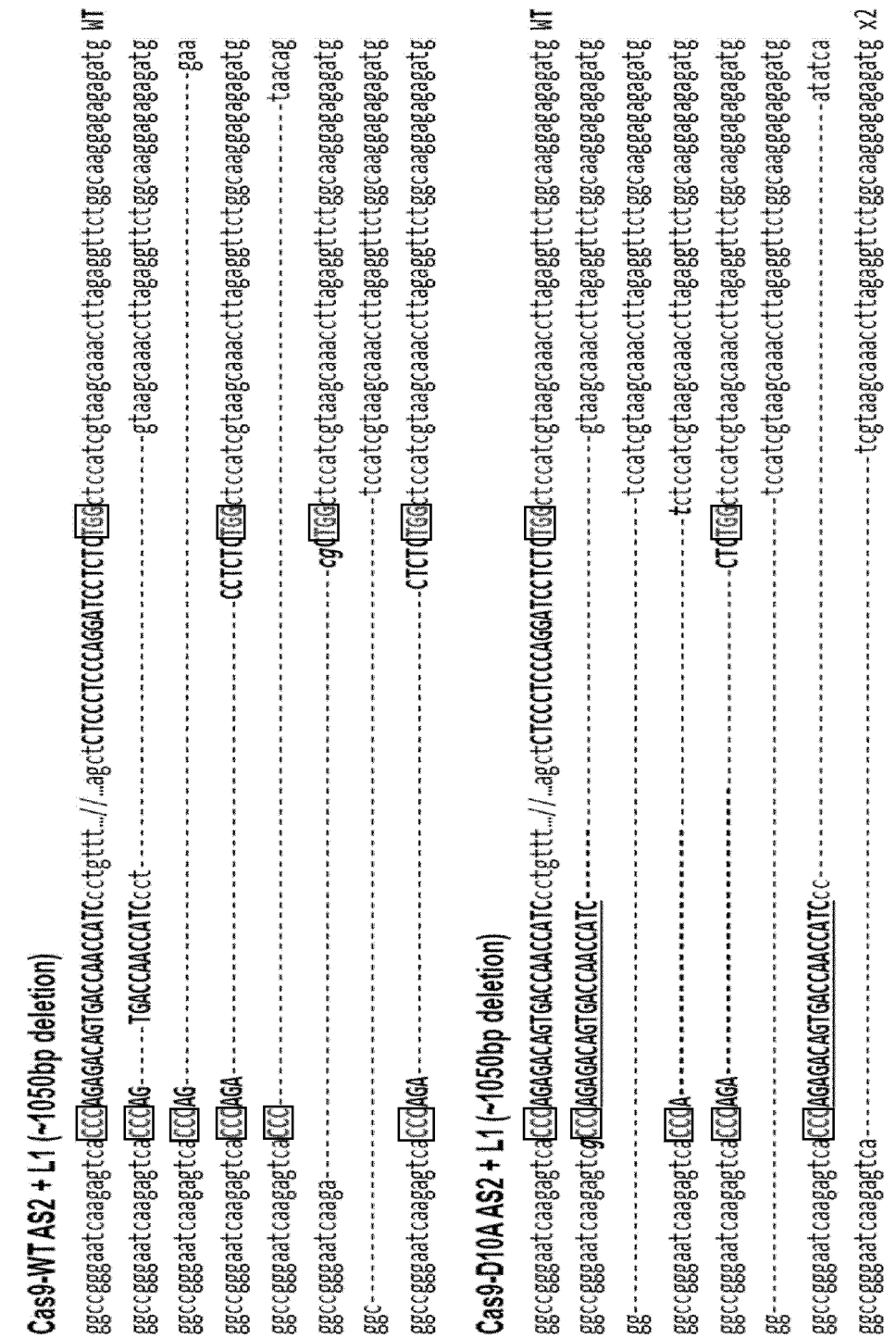


[Fig. 20a]

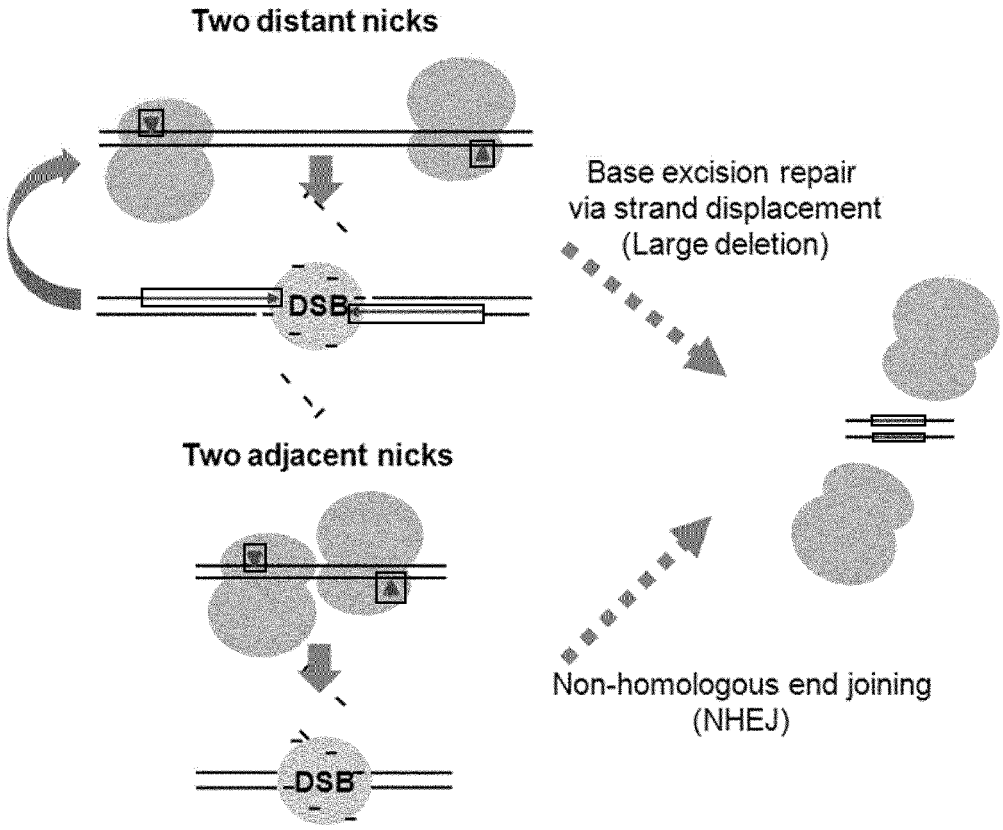
[Fig. 20b]


Cas9-WT
AS2
S2
L7
L6
L5
L4
L3
L2
L1
Empty Vector
Cas9-D10A
AS2
Empty Vector
S2
L7
L6
L5
L4
L3
L2
L1

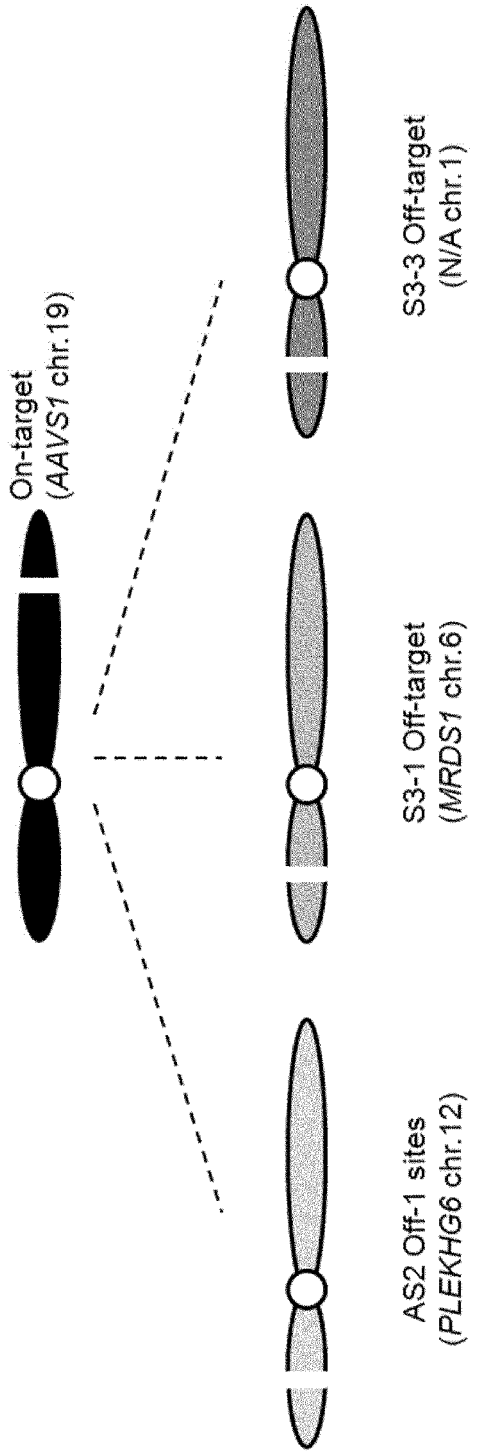
[Fig. 20c]


Cas9-WT AS2 + L1 (~1050bp deletion)
Cas9-D10A AS2 + L1 (~1050bp deletion)
WT
x2

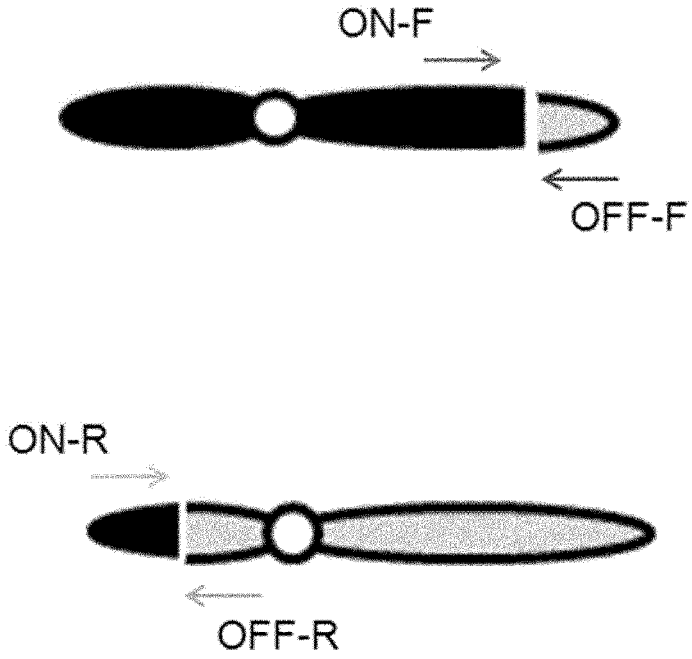
[Fig. 20d]


Two distant nicks
DSB
Base excision repair
via strand displacement
(Large deletion)
Two adjacent nicks
DSB
Non-homologous end joining
(NHEJ)

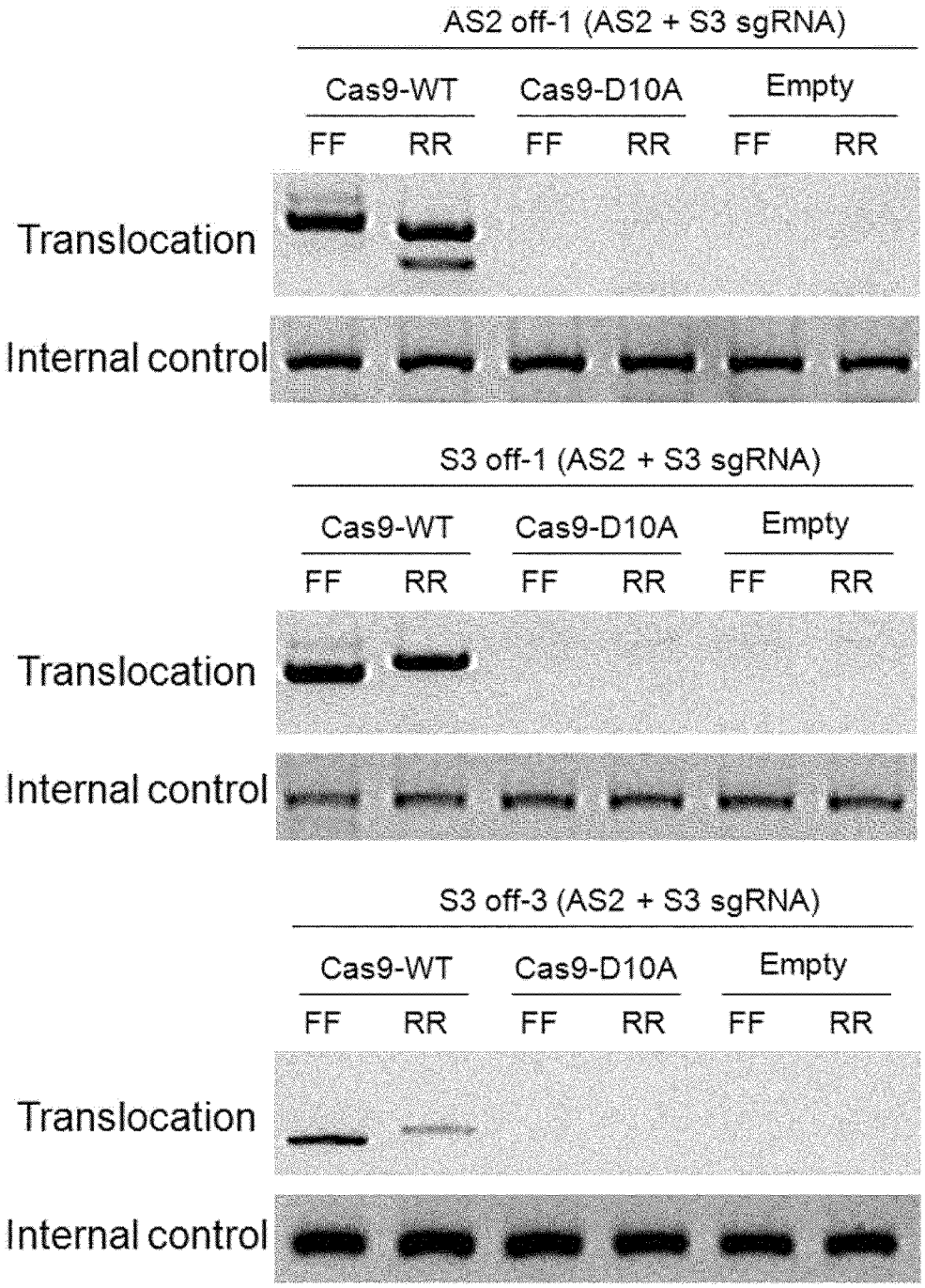
[Fig. 21a]


On-target
(AAVS1 chr.19)
S3-3 Off-target
(N/A chr.1)
S3-1 Off-target
(MRDS1 chr.6)
AS2 Off-1 sites
(PLEKHG6 chr.12)

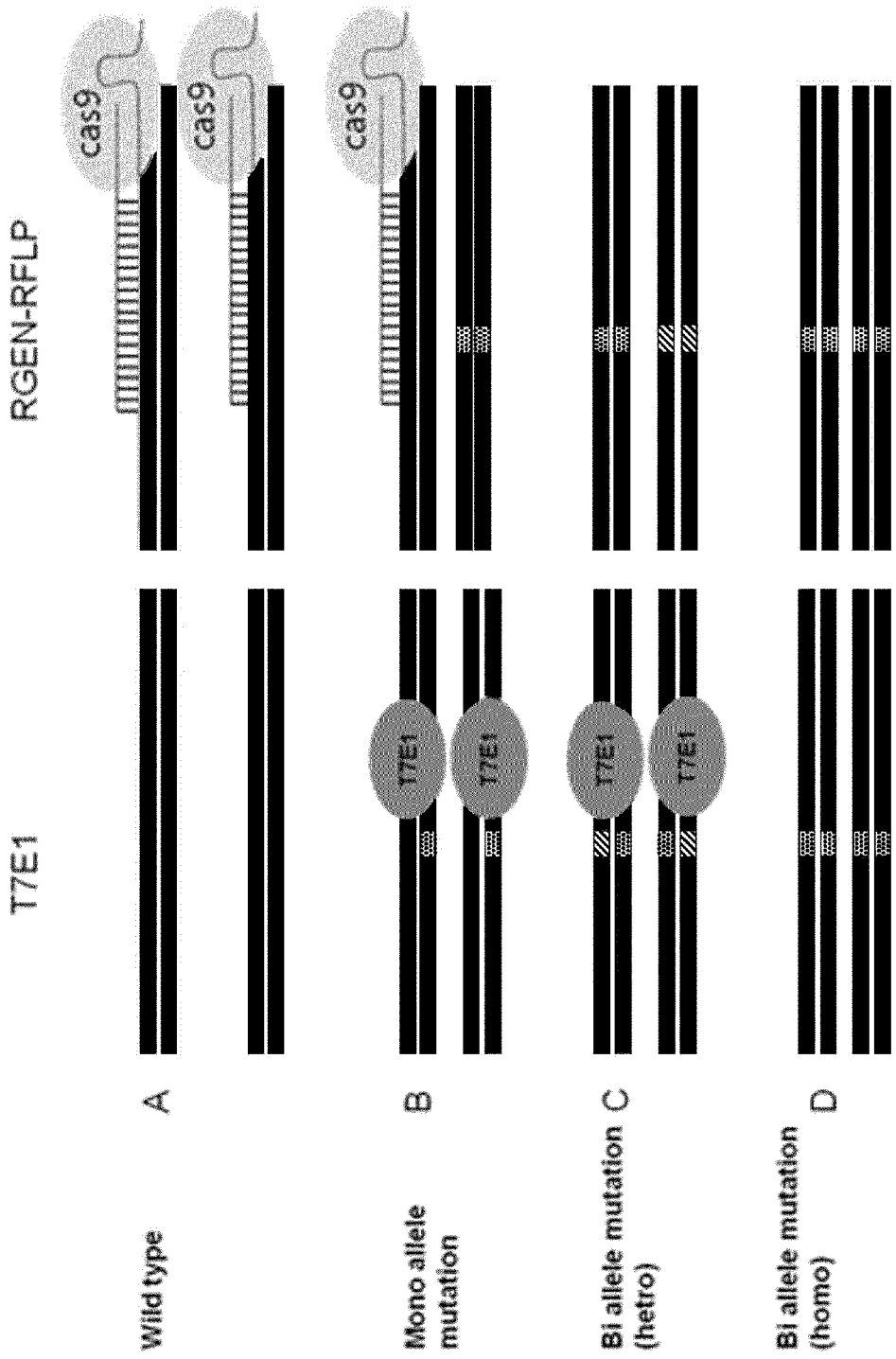
[Fig. 21b]



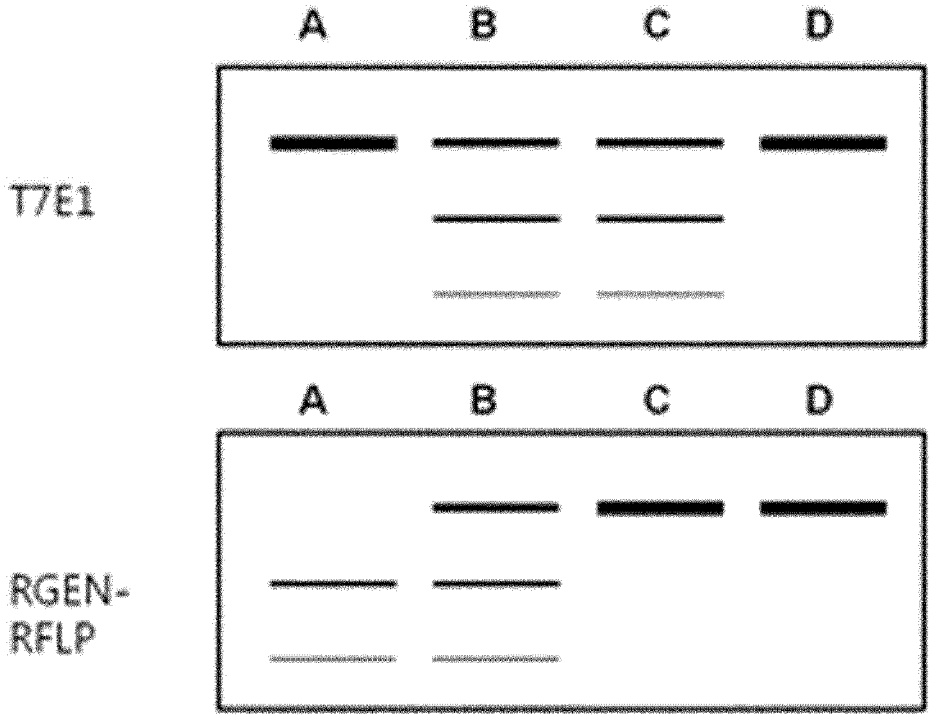
[Fig. 21c]


AS2 off-1 (AS2 + S3 sgRNA)
Cas9-WT
Cas9-D10A
Empty
FF
RR
FF
RR
FF
RR
Translocation
Internal control
S3 off-1 (AS2 + S3 sgRNA)
Cas9-WT
Cas9-D10A
Empty
FF
RR
FF
RR
FF
RR
Translocation
Internal control
S3 off-3 (AS2 + S3 sgRNA)
Cas9-WT
Cas9-D10A
Empty
FF
RR
FF
RR
FF
RR
Translocation
Internal control

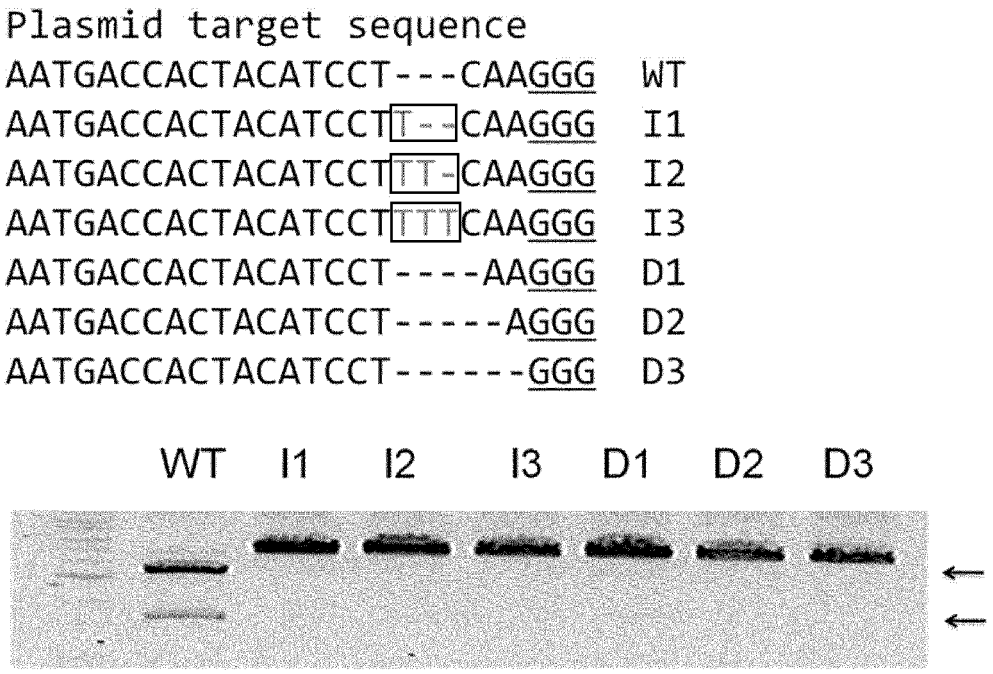
[Fig. 22a]


RGEN-RFLP
T7E1
cas9
cas9
cas9
T7E1
T7E1
T7E1
T7E1
A
B
C
D
Wild type
Mono allele mutation
Bi allele mutation (hetro)
Bi allele mutation (homo)

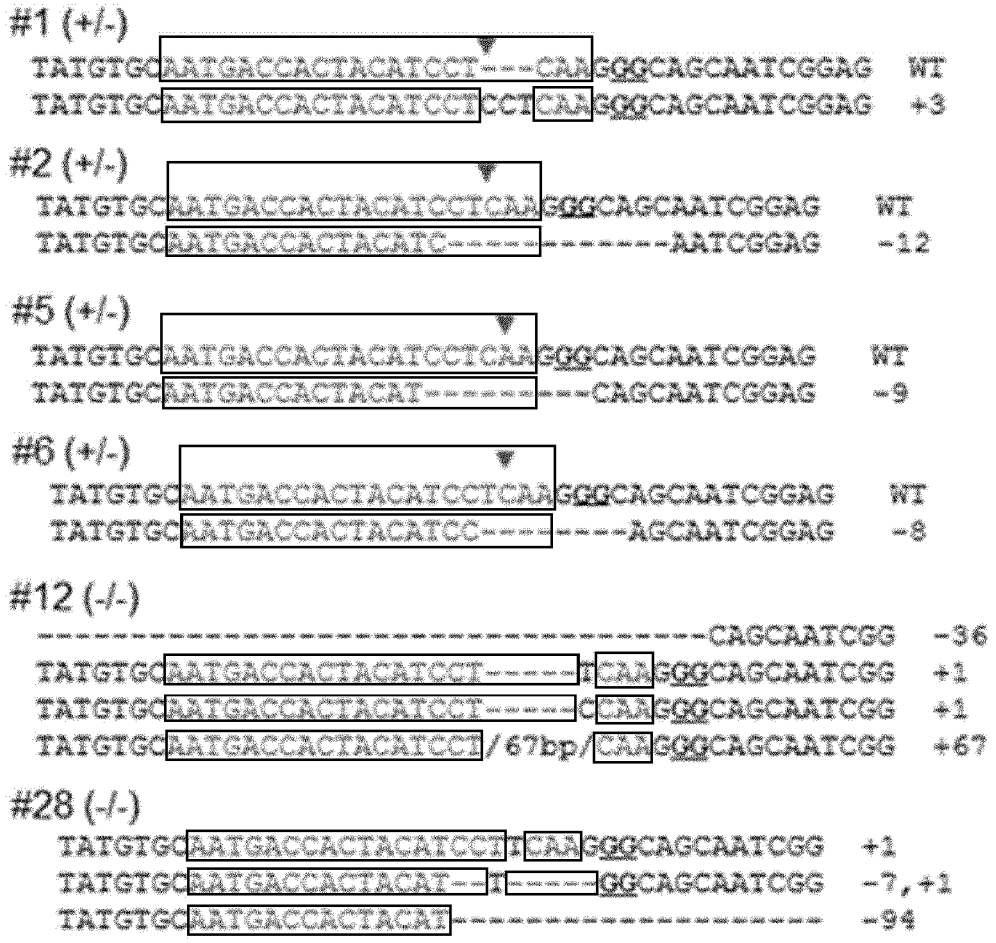
[Fig. 22b]



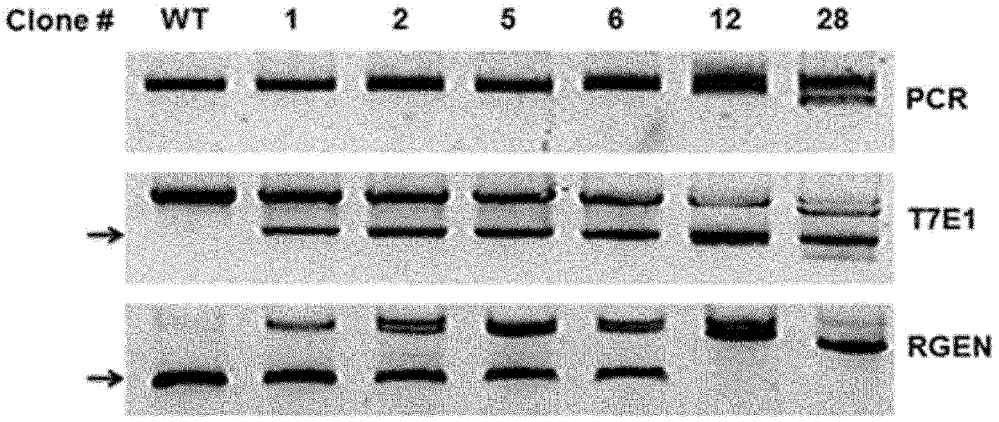
[Fig. 23]



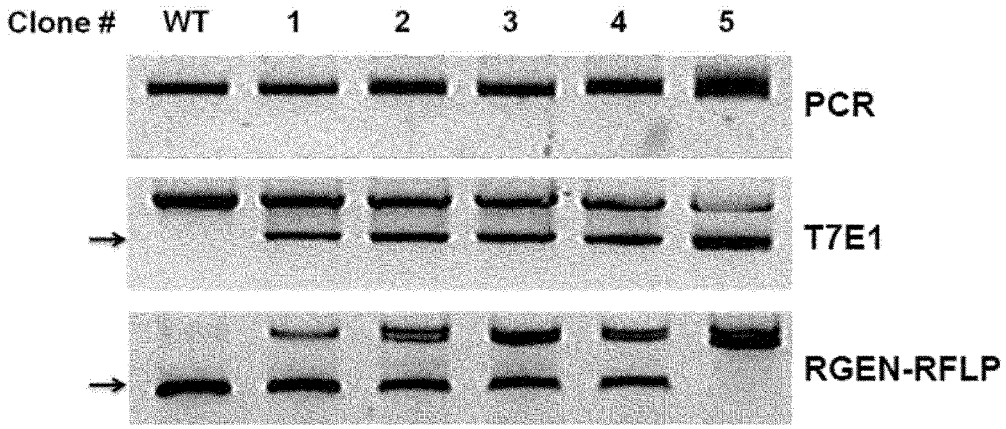
[Fig. 24a]



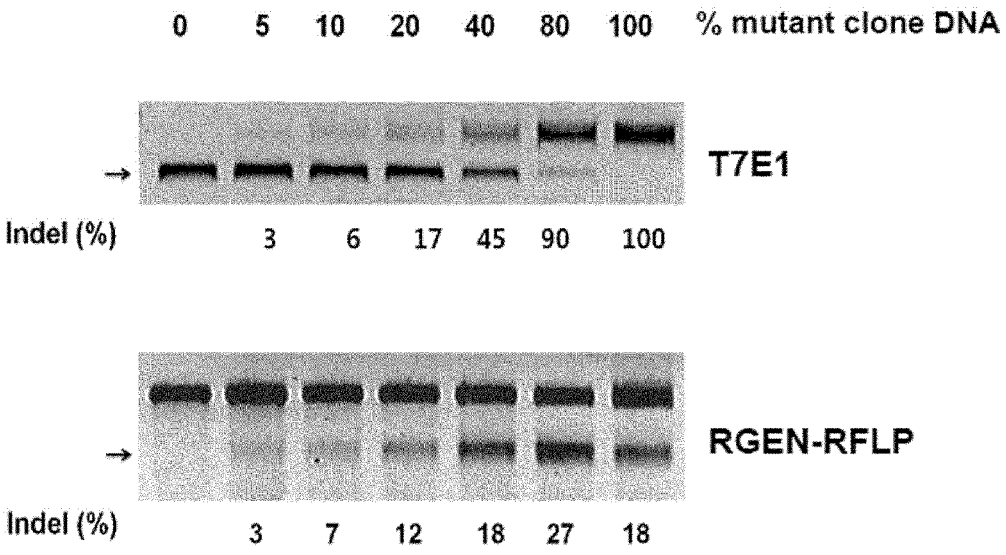
[Fig. 24b]



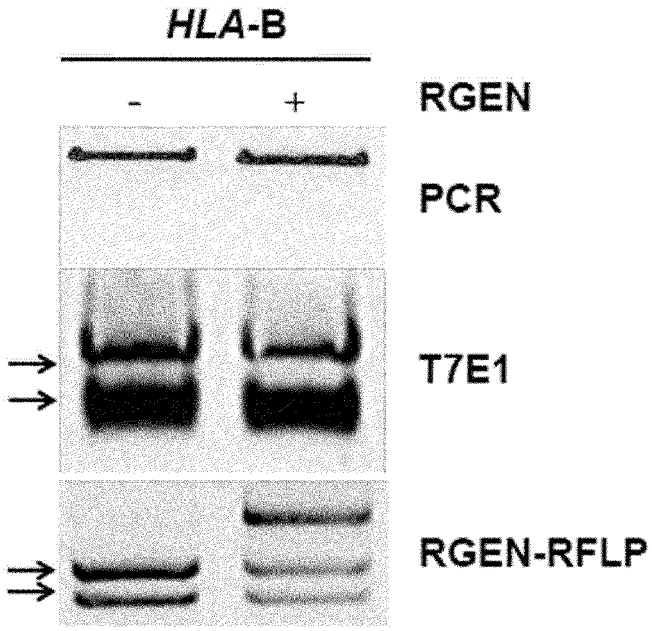
[Fig. 25a]

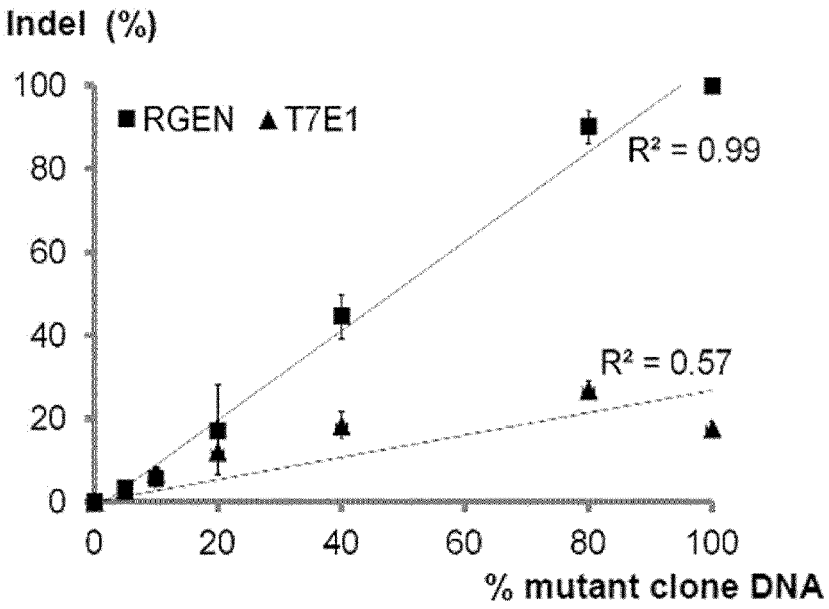


[Fig. 25b]

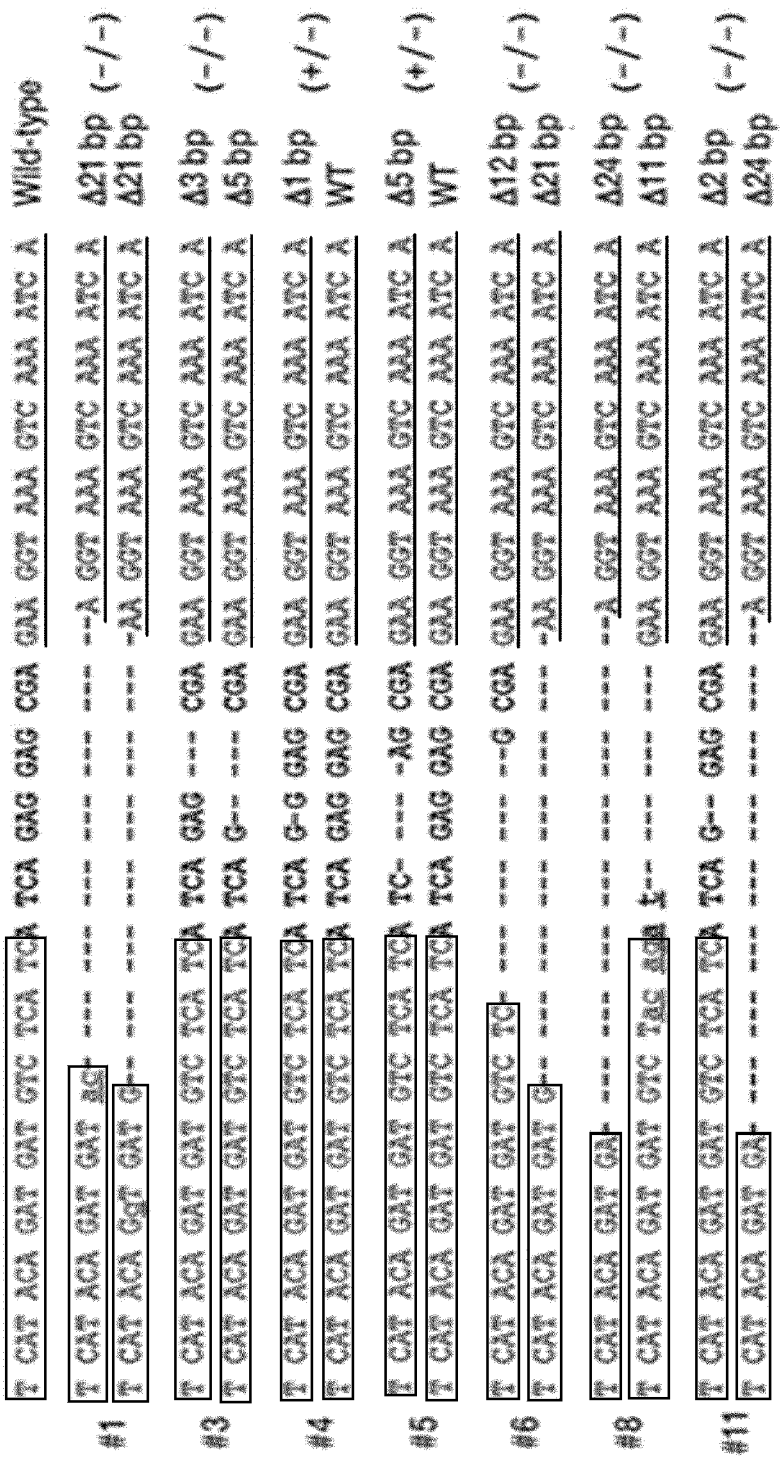


[Fig. 25c]

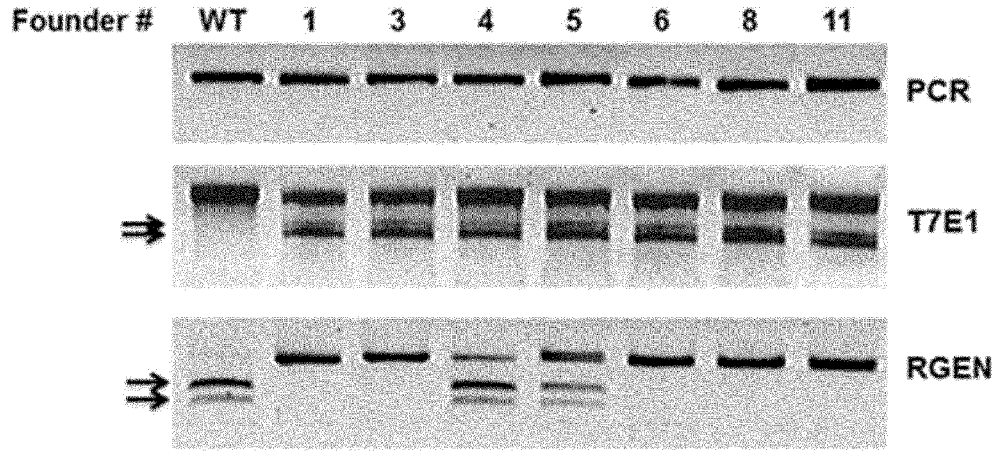




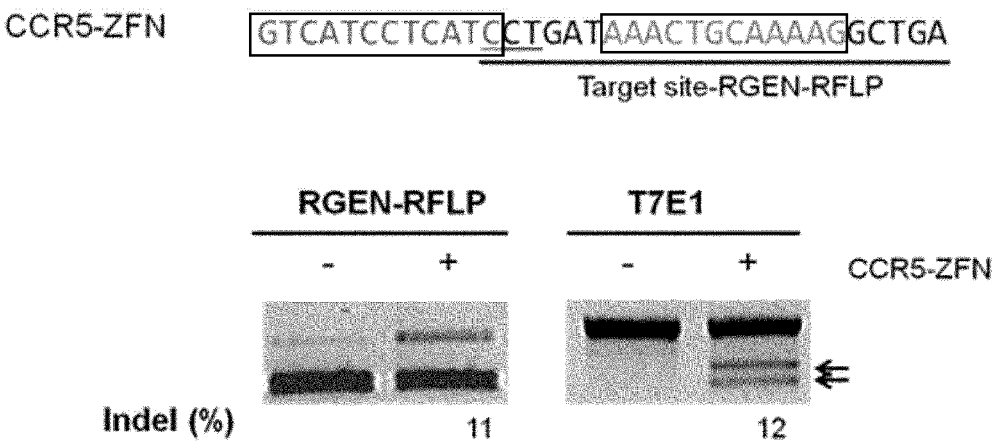
[Fig. 26a]


Wild-type
T CAT ACA GAT GAT GTC TCA TCA GAG GAG CGA GAA GGT AAA GTC AAA ATC A
#1
T CAT ACA GAT GAT GTC TCA TCA GAG GAG CGA --A GGT AAA GTC AAA ATC A
Δ21 bp (−/−)
T CAT ACA GAT GAT GTC TCA TCA GAG GAG CGA -AA GGT AAA GTC AAA ATC A
Δ21 bp
#3
T CAT ACA GAT GAT GTC TCA TCA GAG --- CGA GAA GGT AAA GTC AAA ATC A
Δ3 bp (−/−)
T CAT ACA GAT GAT GTC TCA TCA G-- --- CGA GAA GGT AAA GTC AAA ATC A
Δ5 bp
#4
T CAT ACA GAT GAT GTC TCA TCA G-G GAG CGA GAA GGT AAA GTC AAA ATC A
Δ1 bp (+/−)
T CAT ACA GAT GAT GTC TCA TCA GAG GAG CGA GAA GGT AAA GTC AAA ATC A
WT
#5
T CAT ACA GAT GAT GTC TCA TCA TC- --- -AG CGA GAA GGT AAA GTC AAA ATC A
Δ5 bp (+/−)
T CAT ACA GAT GAT GTC TCA TCA GAG GAG CGA GAA GGT AAA GTC AAA ATC A
WT
#6
T CAT ACA GAT GAT GTC TC- --- --- --G CGA GAA GGT AAA GTC AAA ATC A
Δ12 bp (−/−)
T CAT ACA GAT GAT G-- --- --- --- --- -AA GGT AAA GTC AAA ATC A
Δ21 bp
#8
T CAT ACA GAT GA- --- --- --- --- --- --- --A GGT AAA GTC AAA ATC A
Δ24 bp (−/−)
T CAT ACA GAT GAT GTC Tac aga t-- --- --- --- GAA GGT AAA GTC AAA ATC A
Δ11 bp
#11
T CAT ACA GAT GAT GTC TCA TCA G-- GAG CGA GAA GGT AAA GTC AAA ATC A
Δ2 bp (−/−)
T CAT ACA GAT GA- --- --- --- --- --- --- --A GGT AAA GTC AAA ATC A
Δ24 bp

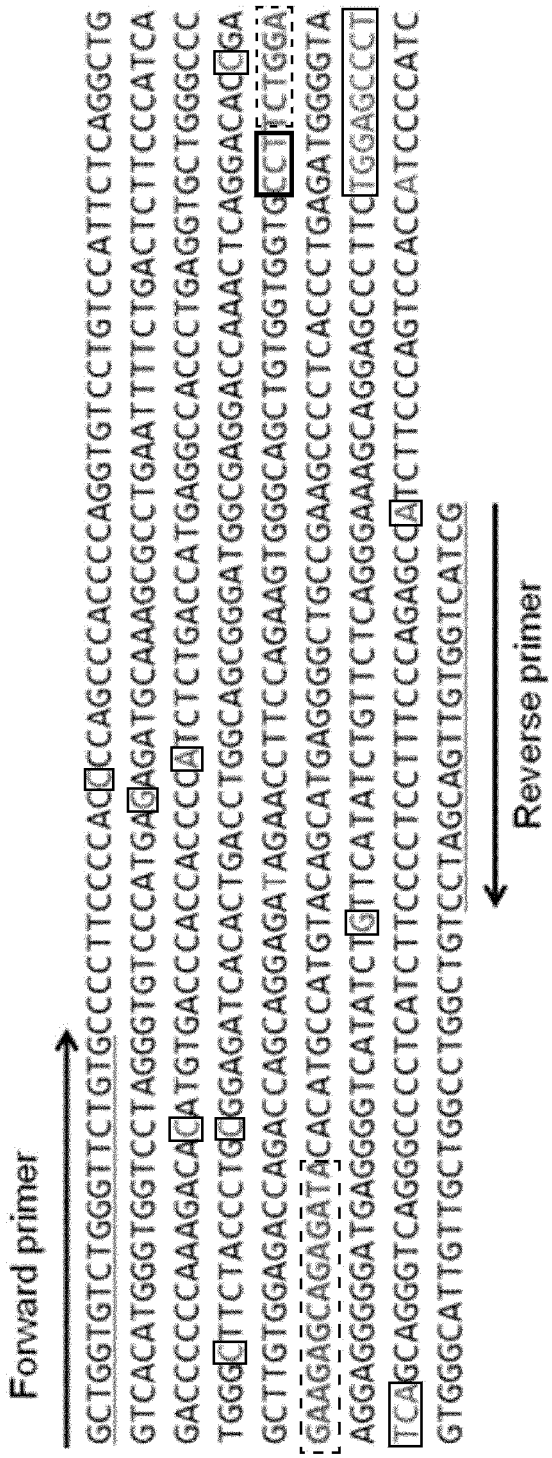
[Fig. 26b]



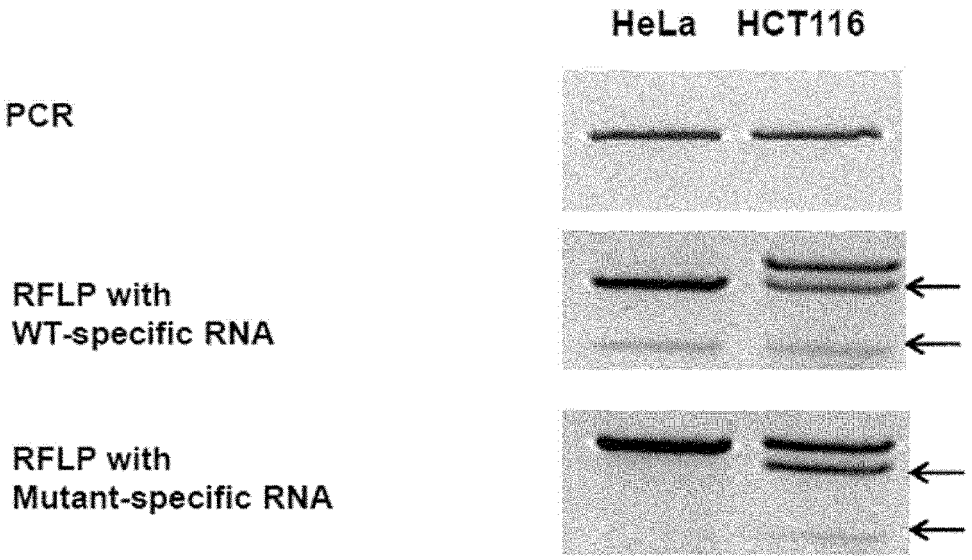
[Fig. 27]



[Fig. 28]



[Fig. 29a]



HeLa

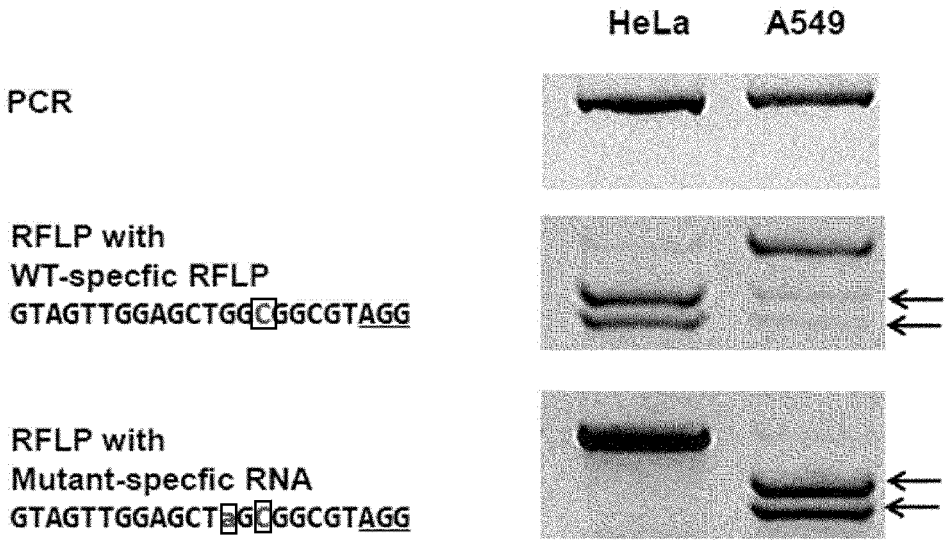
ACTACCACAGCTCCTTCTCTGAGTGG wild-type

HCT116

ACTACCACAGCTCCTTCTCTGAGTGG wild-type

ACTACCACAGCTCCT---CTGAGTGG c.133-135 del TCT

[Fig. 29b]



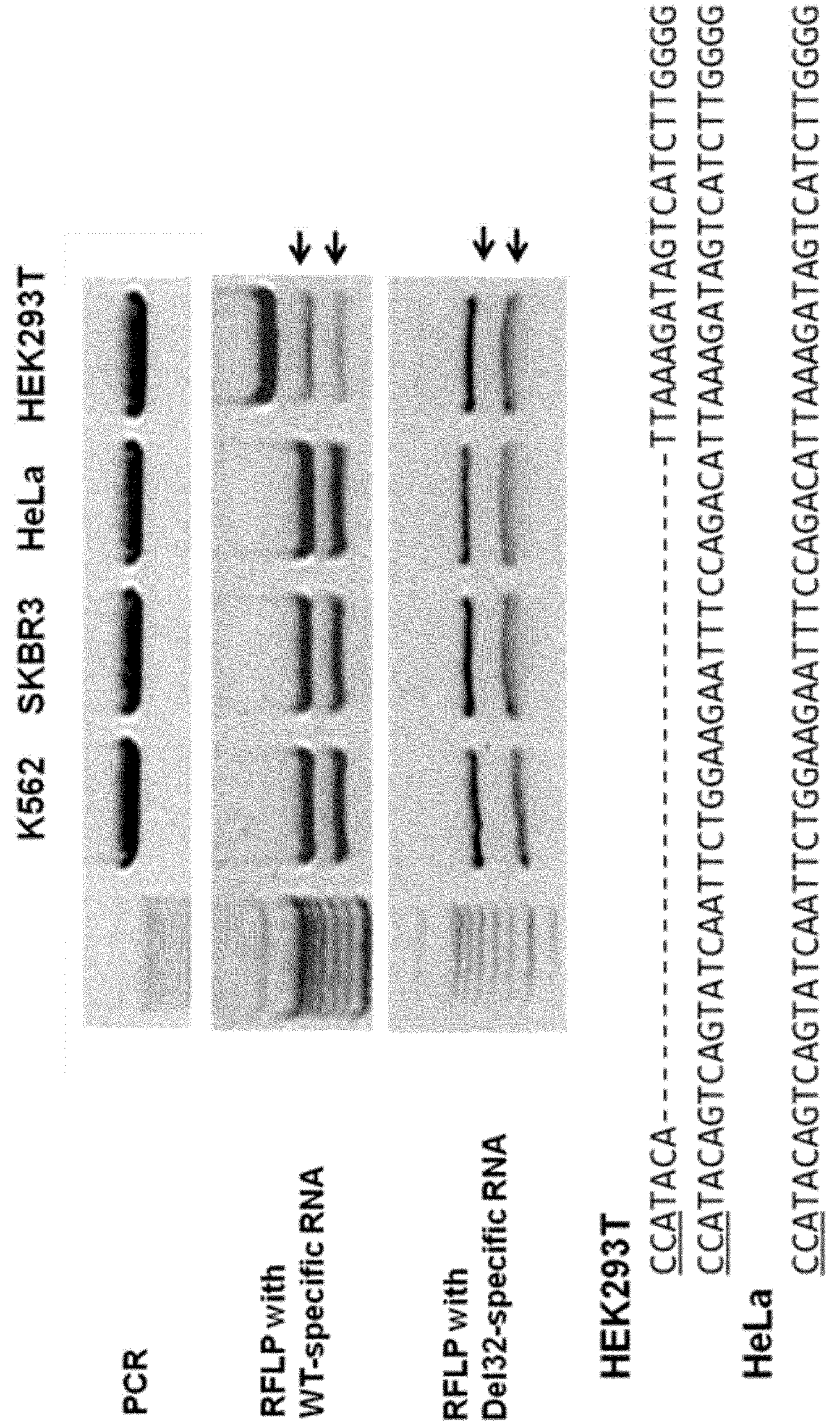
HeLa

GTAGTTGGAGCTGGTGGCGTAGG wild-type

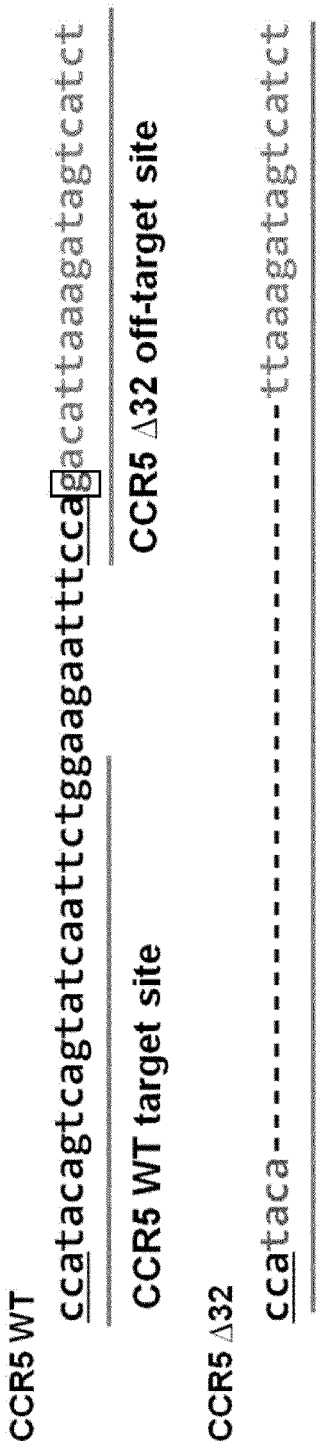
A549

GTAGTTGGAGCTAGTGGCGTAGG c.34G>A

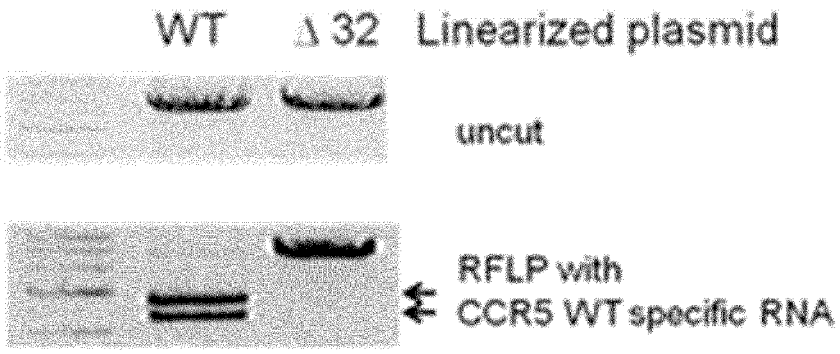
[Fig. 30a]


K562
SKBR3
HeLa
HEK293T
PCR
RFLP with
WT-specific RNA
RFLP with
Del32-specific RNA
HEK293T
CCATACA----------TTAAAGATAGTCATCTTGGGG
CCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCATCTTGGGG
HeLa
CCATACAGTCAGTATCAATTCTGGAAGAATTTCCAGACATTAAAGATAGTCATCTTGGGG

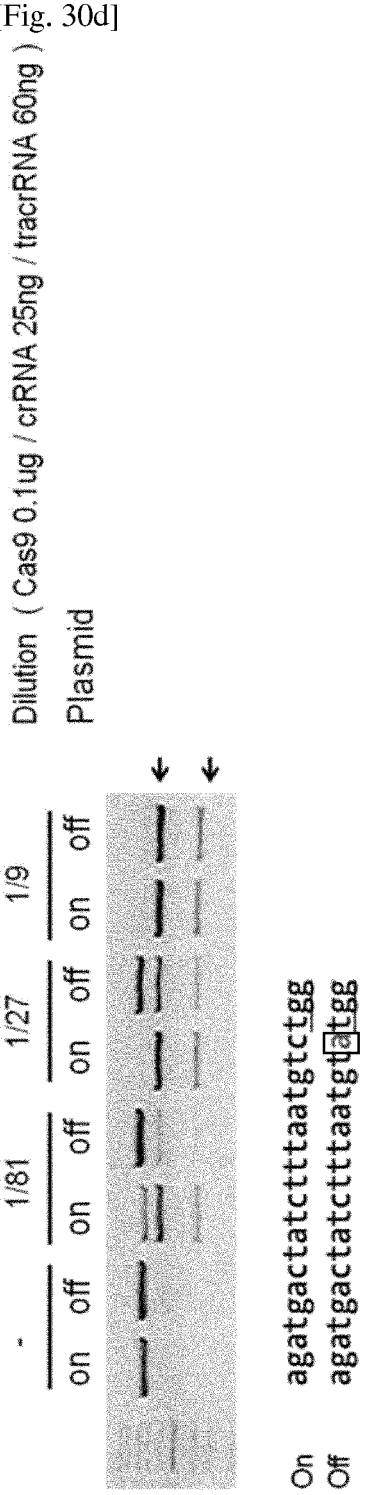
[Fig. 30b]



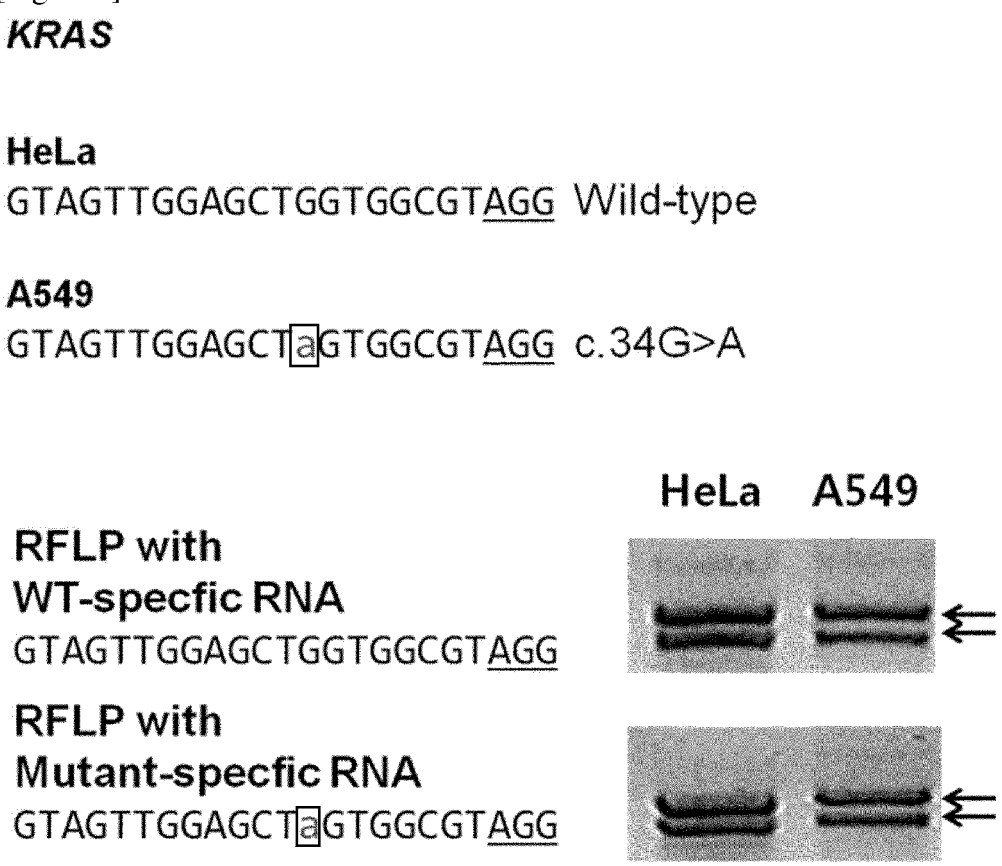
[Fig. 30c]



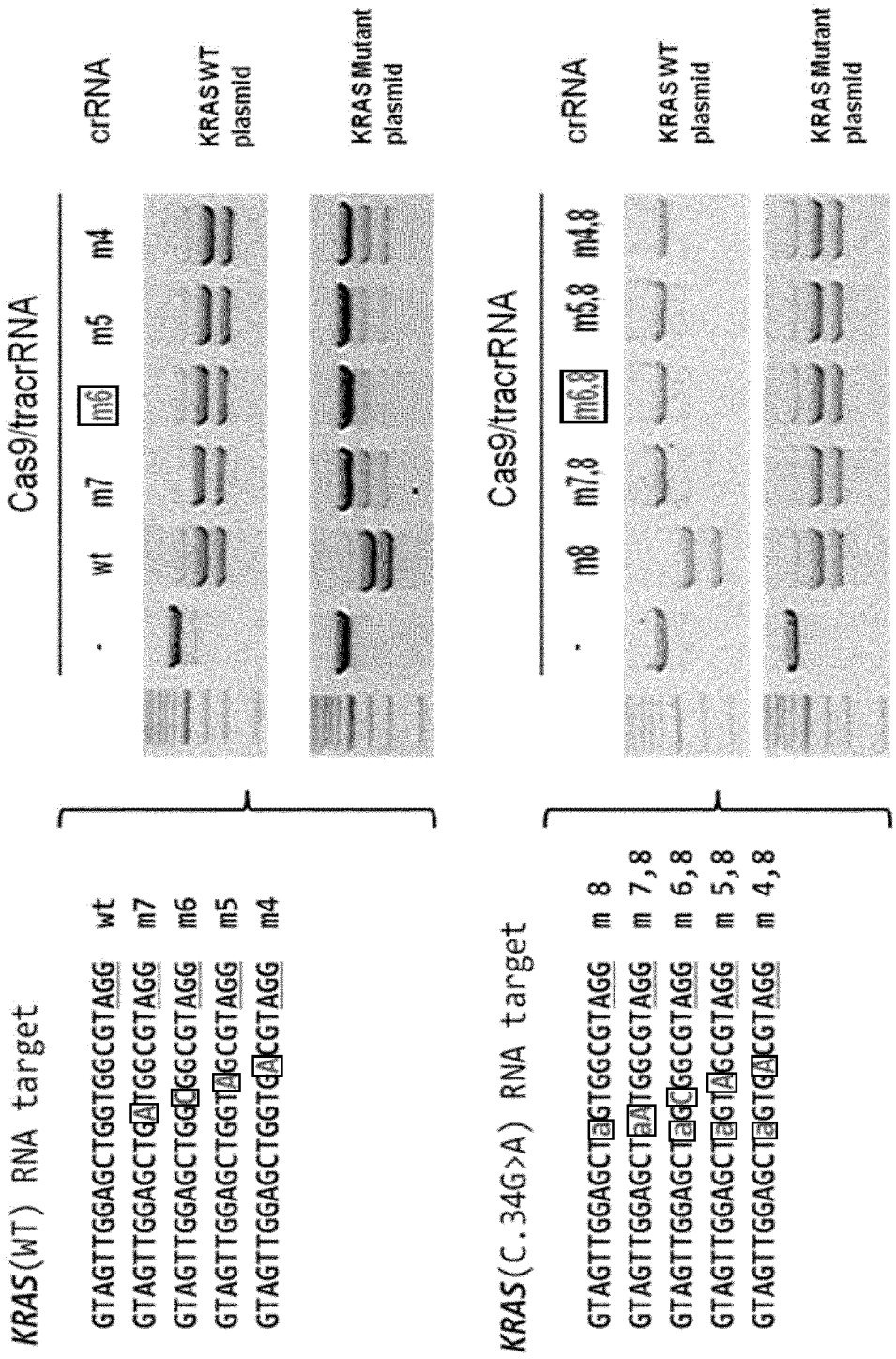
[Fig. 30d]


Dilution (Cas9 0.1ug / crRNA 25ng / tracrRNA 60ng)
Plasmid
-
1/81
1/27
1/9
on
off
on
off
on
off
on
off
On agatgactatctttaatgtctgg
Off agatgactatctttaatgtatgg

[Fig. 31a]


KRAS
HeLa
GTAGTTGGAGCTGGTGGCGTAGG Wild-type
A549
GTAGTTGGAGCTaGTGGCGTAGG c.34G>A
HeLa
A549
RFLP with
WT-specfic RNA
GTAGTTGGAGCTGGTGGCGTAGG
RFLP with
Mutant-specfic RNA
GTAGTTGGAGCTaGTGGCGTAGG

[Fig. 31b]


KRAS(WT) RNA target
GTAGTTGGAGCTGGTGGCGTAGG wt
GTAGTTGGAGCTGATGGCGTAGG m7
GTAGTTGGAGCTGGCGGCGTAGG m6
GTAGTTGGAGCTGGTAGCGTAGG m5
GTAGTTGGAGCTGGTGACGTAGG m4
Cas9/tracrRNA
- wt m7 m6 m5 m4
crRNA
KRAS WT plasmid
KRAS Mutant plasmid
KRAS(C.34G>A) RNA target
GTAGTTGGAGCTAGTGGCGTAGG m 8
GTAGTTGGAGCTAATGGCGTAGG m 7,8
GTAGTTGGAGCTAGCGGCGTAGG m 6,8
GTAGTTGGAGCTAGTAGCGTAGG m 5,8
GTAGTTGGAGCTAGTGACGTAGG m 4,8
Cas9/tracrRNA
- m8 m7,8 m6,8 m5,8 m4,8
crRNA
KRAS WT plasmid
KRAS Mutant plasmid

[Fig. 32a]

PIK3CA

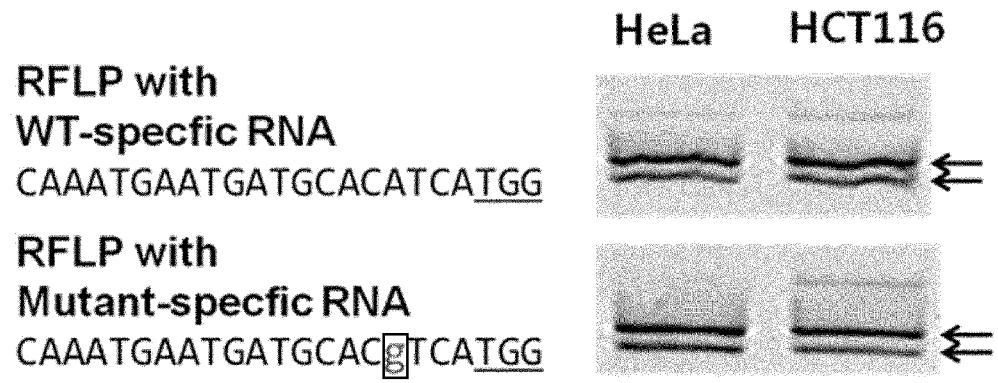
HeLa

CAAATGAATGATGCACATCATGG Wild-type

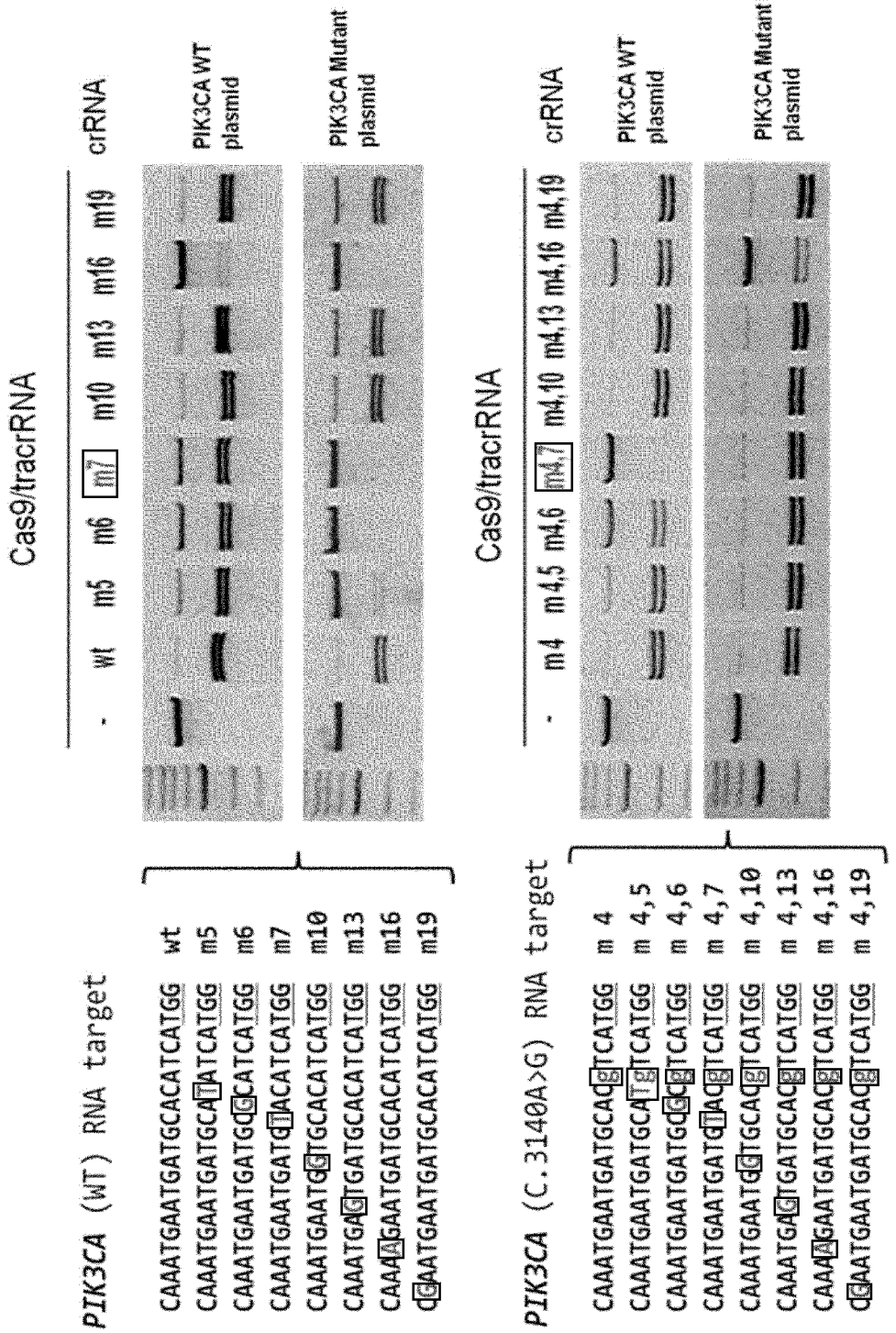
HCT116

CAAATGAATGATGCACATCATGG Wild-type

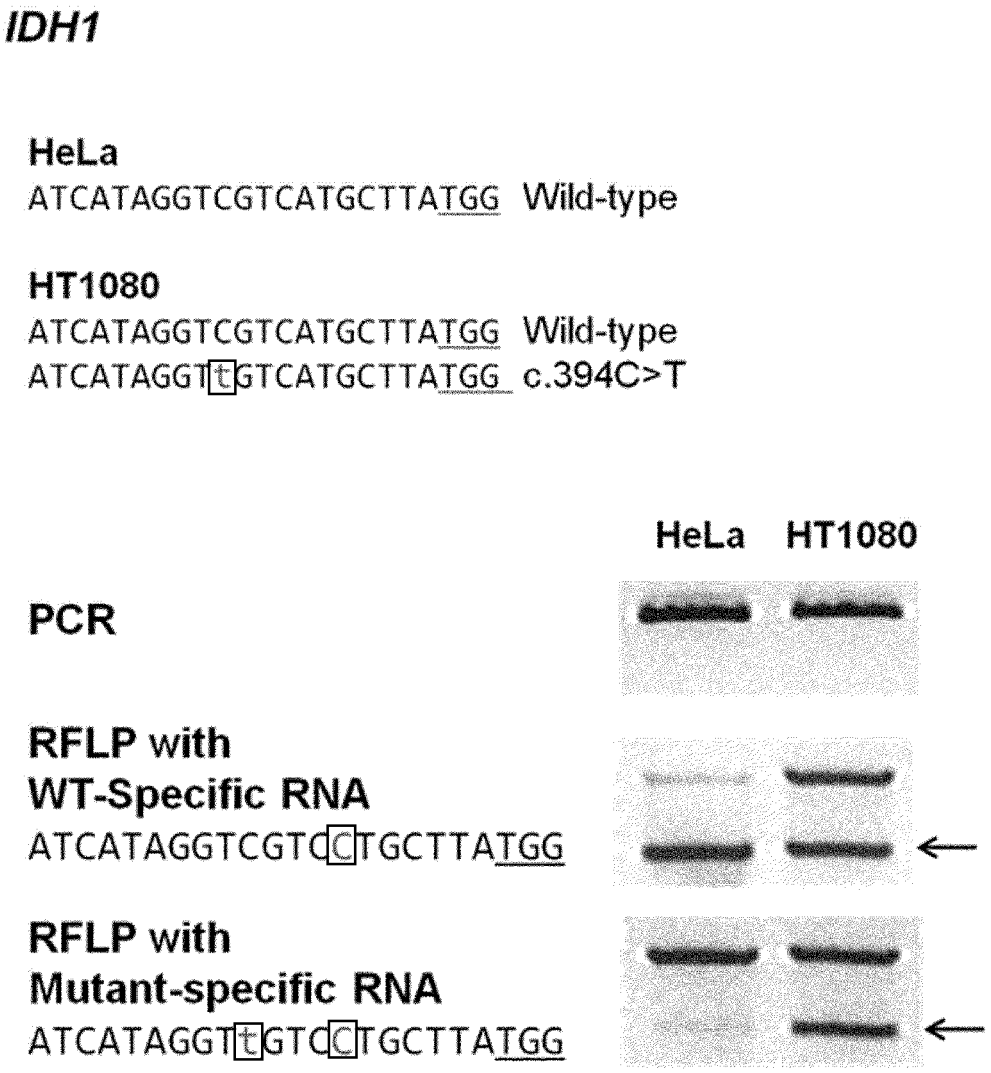
CAAATGAATGATGCACgTCATGG C.3140A>G



[Fig. 32b]


Cas9/tracrRNA
- wt m5 m6 m7 m10 m13 m16 m19 crRNA
PIK3CA WT plasmid
PIK3CA Mutant plasmid
PIK3CA (WT) RNA target
CAAATGAATGATGCACATCATGG wt
CAAATGAATGATGCATATCATGG m5
CAAATGAATGATGCGCATCATGG m6
CAAATGAATGATGTACATCATGG m7
CAAATGAATGGTGCACATCATGG m10
CAAATGAGTGATGCACATCATGG m13
CAAAAGAATGATGCACATCATGG m16
CGAATGAATGATGCACATCATGG m19
Cas9/tracrRNA
- m4 m4,5 m4,6 m4.7 m4,10 m4,13 m4,16 m4,19 crRNA
PIK3CA WT plasmid
PIK3CA Mutant plasmid
PIK3CA (C.3140A>G) RNA target
CAAATGAATGATGCACGTCATGG m 4
CAAATGAATGATGCATTTCATGG m 4,5
CAAATGAATGATGCGCTTCATGG m 4,6
CAAATGAATGATGTACGTCATGG m 4,7
CAAATGAATGGTGCACGTCATGG m 4,10
CAAATGAGTGATGCACGTCATGG m 4,13
CAAAAGAATGATGCACGTCATGG m 4,16
CGAATGAATGATGCACGTCATGG m 4,19

[Fig. 33a]


IDH1
HeLa
ATCATAGGTCGTCATGCTTATGG Wild-type
HT1080
ATCATAGGTCGTCATGCTTATGG Wild-type
ATCATAGGTtGTCATGCTTATGG c.394C>T
HeLa HT1080
PCR
RFLP with
WT-Specific RNA
ATCATAGGTCGTCCTGCTTATGG
RFLP with
Mutant-specific RNA
ATCATAGGTtGTCCTGCTTATGG

[Fig. 33b]

PIK3CA

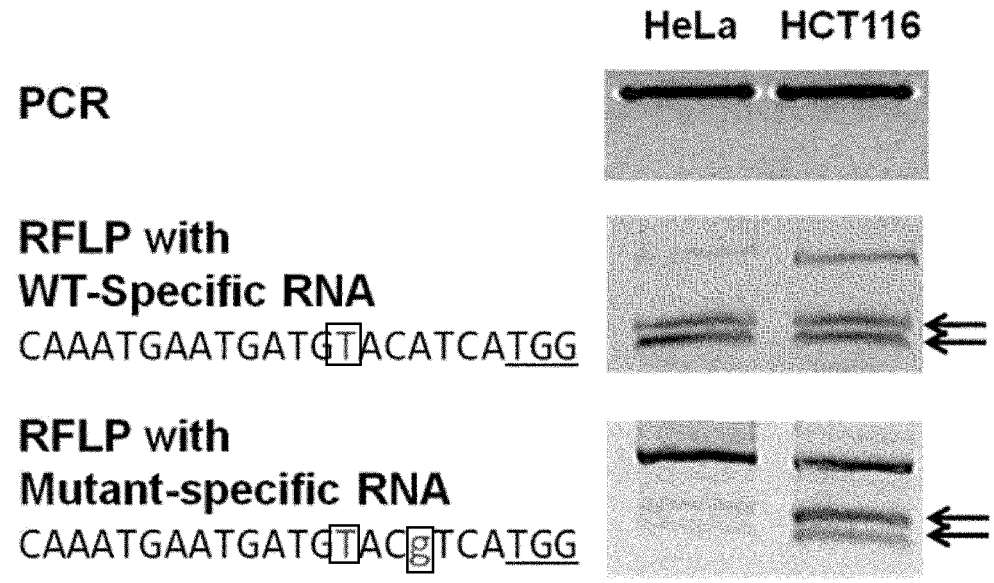
HeLa

CAAATGAATGATGCACATCATGG Wild-type

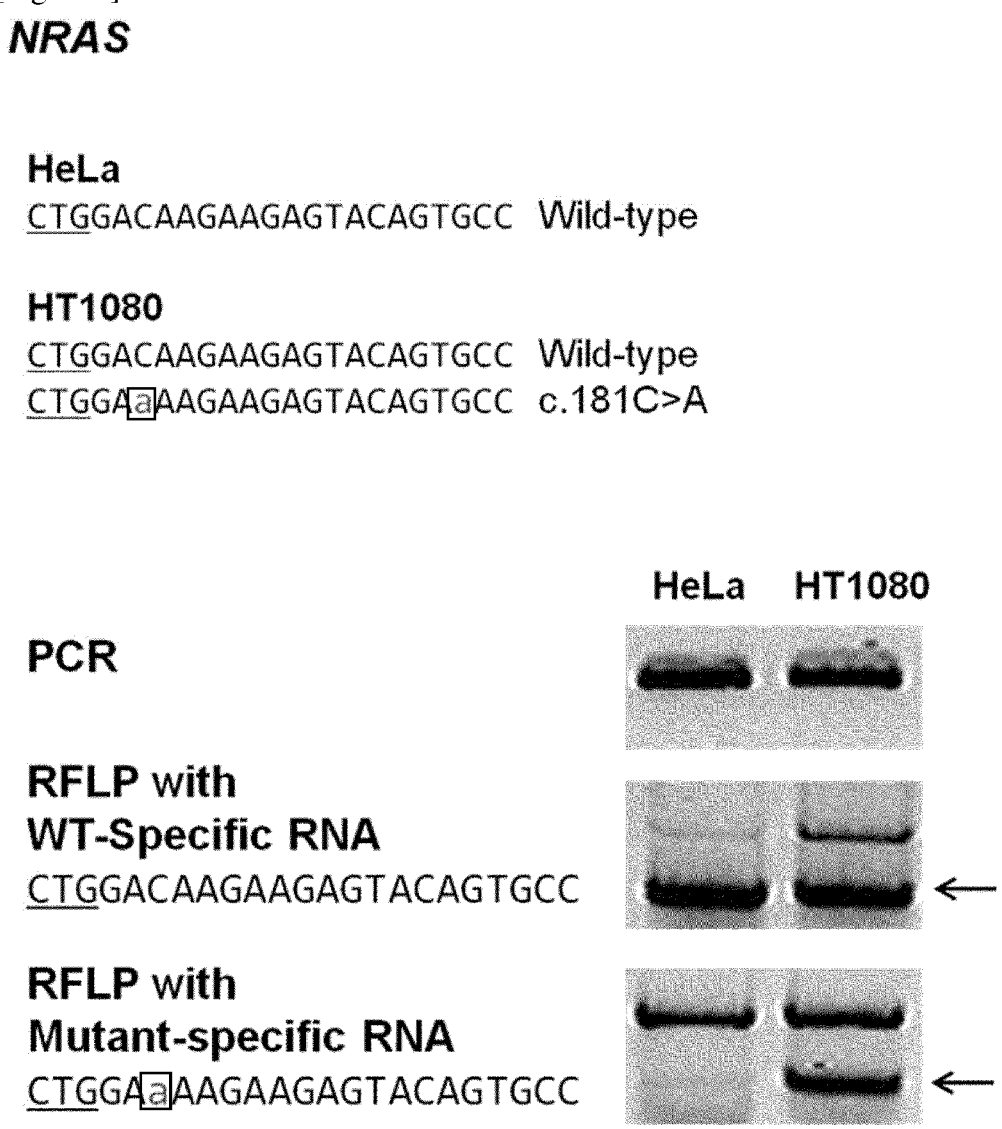
HCT116

CAAATGAATGATGCACATCATGG Wild-type

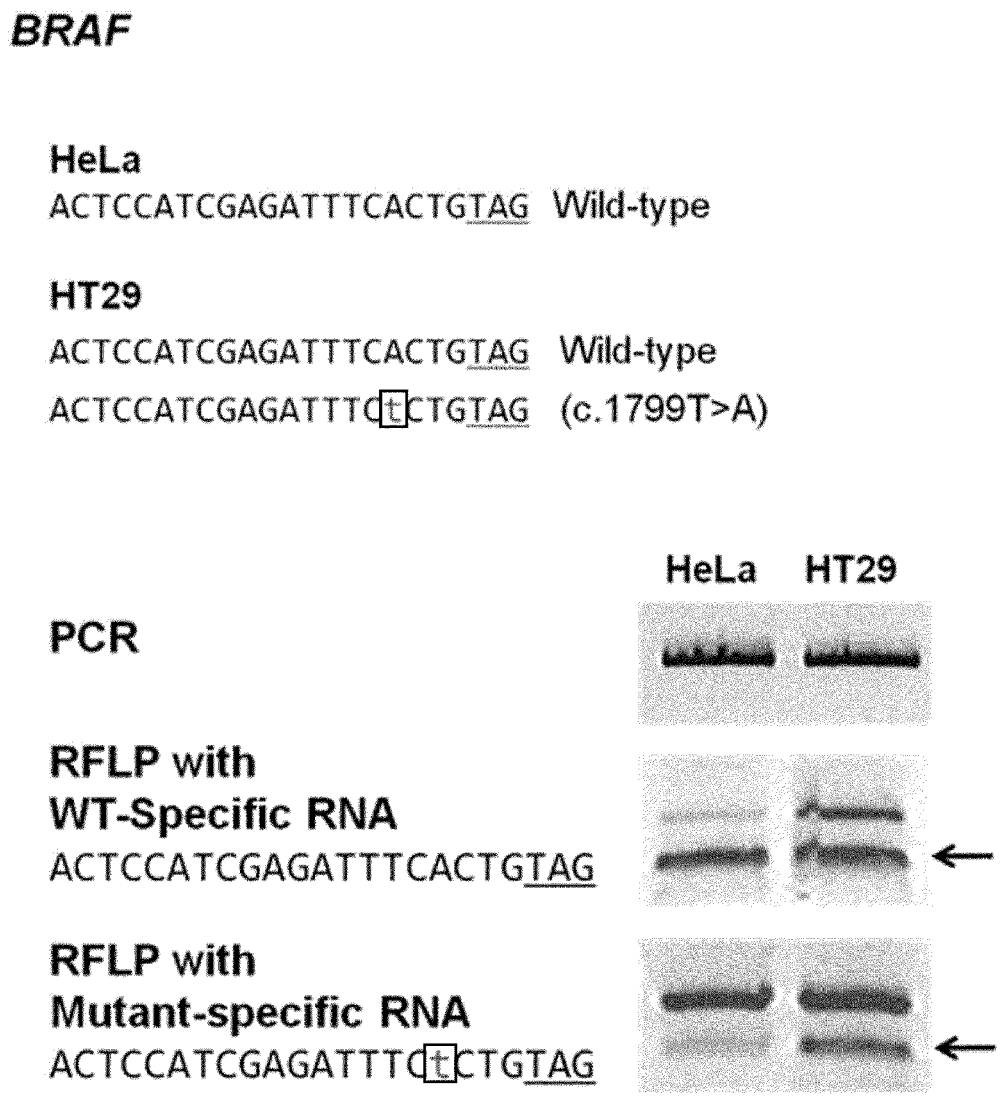
CAAATGAATGATGCACgTCATGG C.3140A>G



[Fig. 33c]


NRAS
HeLa
CTGGACAAGAAGAGTACAGTGCC Wild-type
HT1080
CTGGACAAGAAGAGTACAGTGCC Wild-type
CTGGAaAAGAAGAGTACAGTGCC c.181C>A
HeLa
HT1080
PCR
RFLP with
WT-Specific RNA
CTGGACAAGAAGAGTACAGTGCC
RFLP with
Mutant-specific RNA
CTGGAaAAGAAGAGTACAGTGCC

[Fig. 33d]


BRAF
HeLa
ACTCCATCGAGATTTCACTGTAG Wild-type
HT29
ACTCCATCGAGATTTCACTGTAG Wild-type
ACTCCATCGAGATTTCtCTGTAG (c.1799T>A)
HeLa
HT29
PCR
RFLP with
WT-Specific RNA
ACTCCATCGAGATTTCACTGTAG
RFLP with
Mutant-specific RNA
ACTCCATCGAGATTTCtCTGTAG