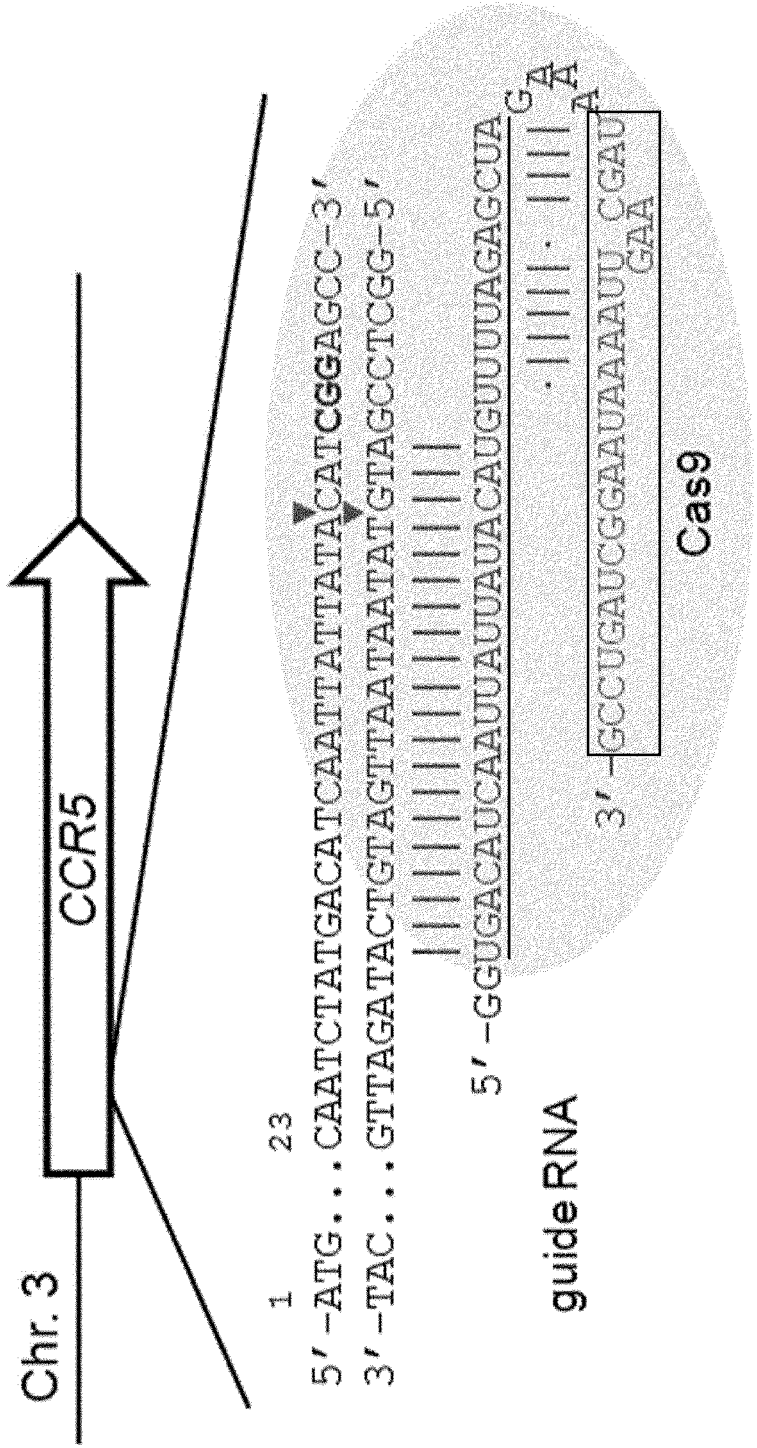
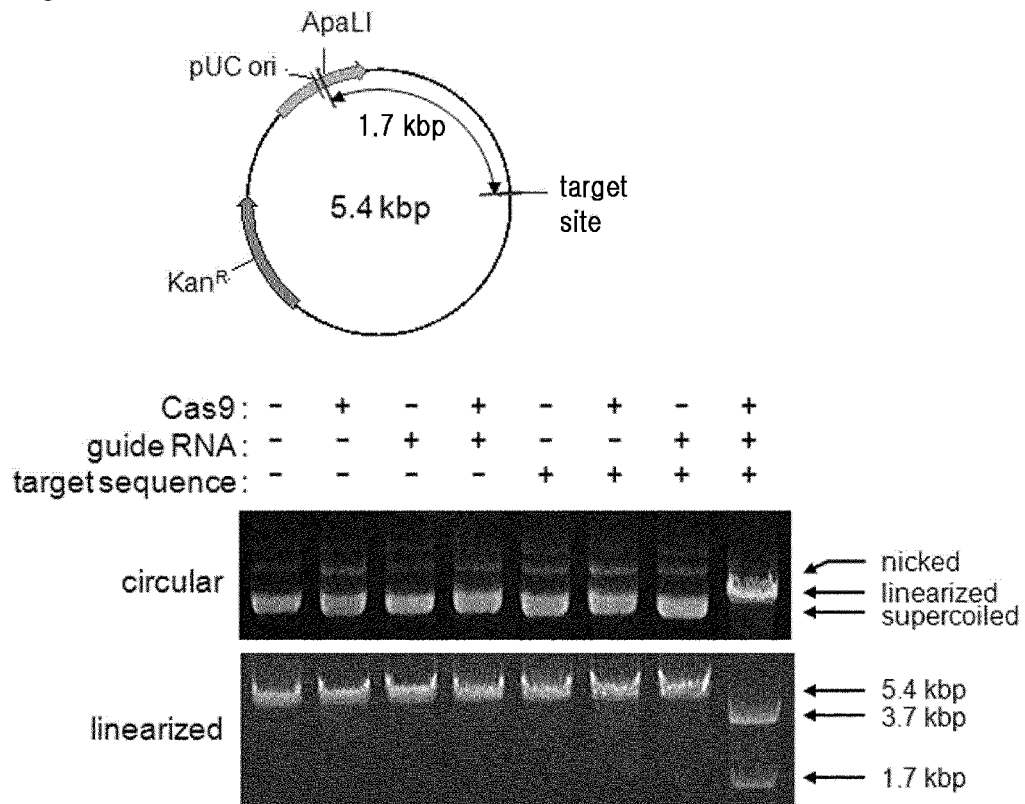


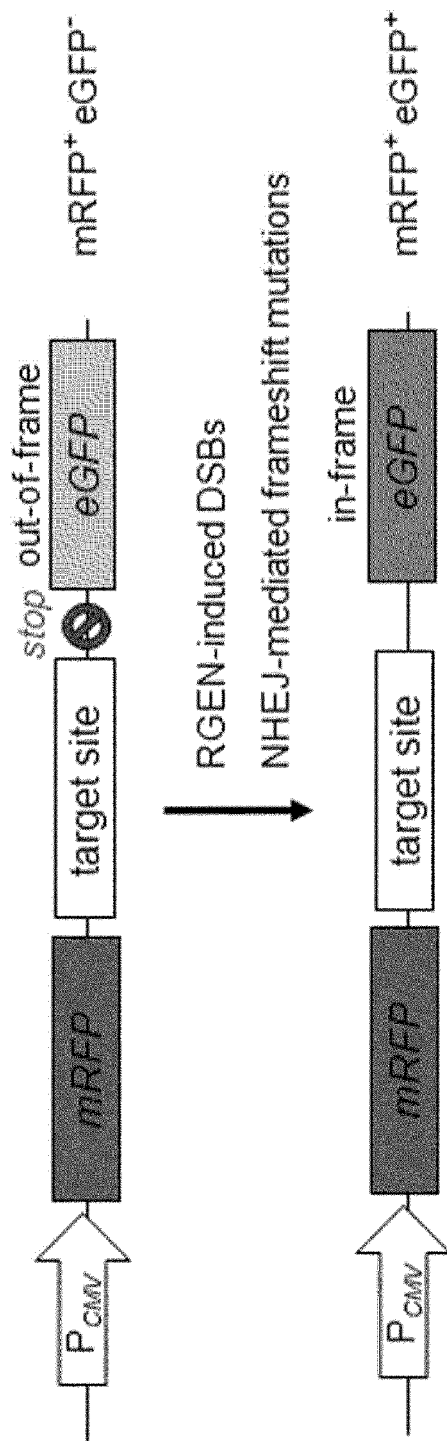
[Fig. 1a]



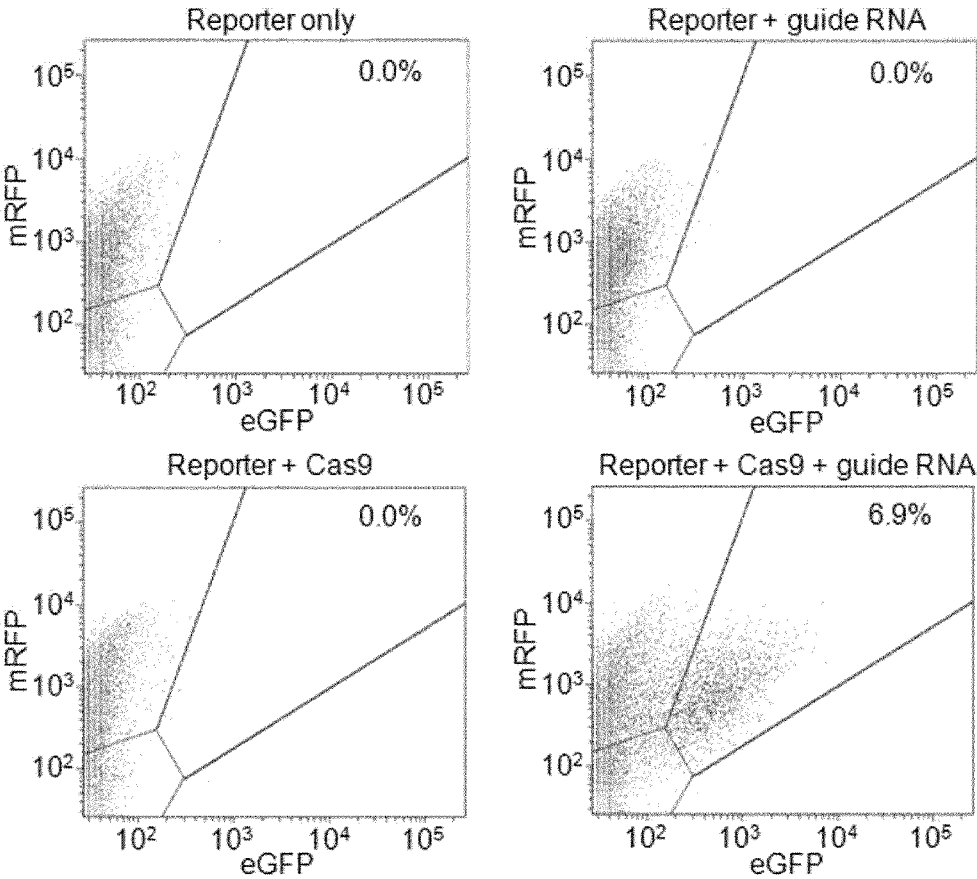
[Fig. 1b]



[Fig. 2a]

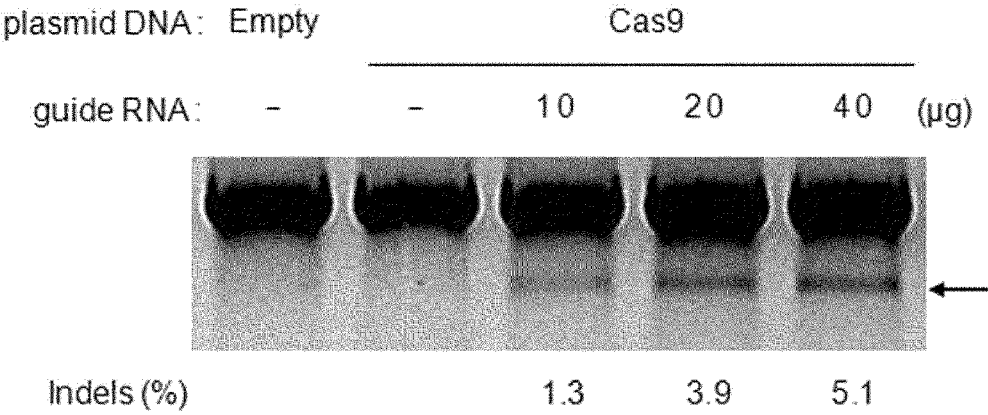


[Fig. 2b]



[Fig. 3a]

CCR5



7.3% (7/96) mutated

```
CAATCTATGACATCAATTATTATA-CATCGGAGCCCTGCCAAAAAATCAA WT
CAATCTATGACATCAATTATTATAA-CATCGGAGCCCTGCCAAAAAATCAA +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

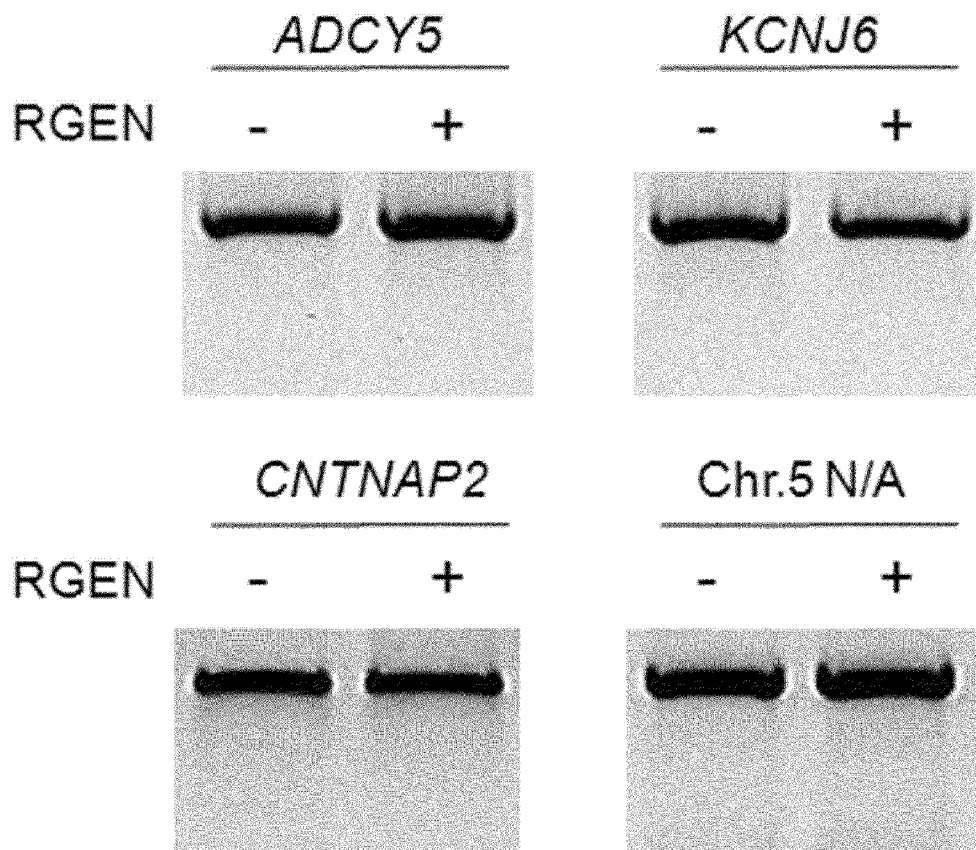
8.3% (4/48) mutated

TATGTGCAATGACCACTACATCCT--CAAGGGGCAGCAATCGGAGCCAG WT
 TATGTGCAATGACCACTACATCCTT-CAAGGGGCAGCAATCGGAGCCAG +1
 TATGTGCAATGACCACTACATCCTCTCAAGGGGCAGCAATCGGAGCCAG +2
 TATGTGCAATG-----GAGCCAG -30
 TATGTGCAATGAC-----180 bp----- -180

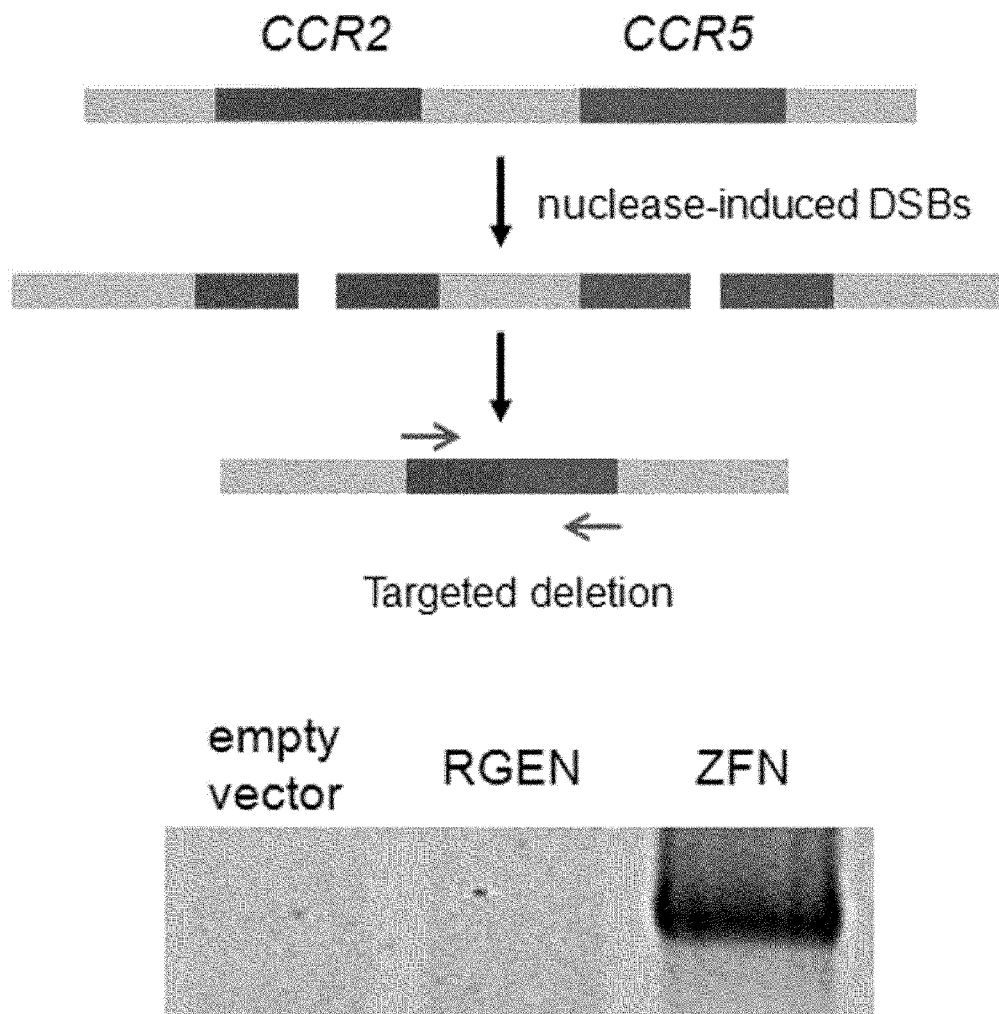
[Fig. 4a]

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

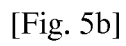
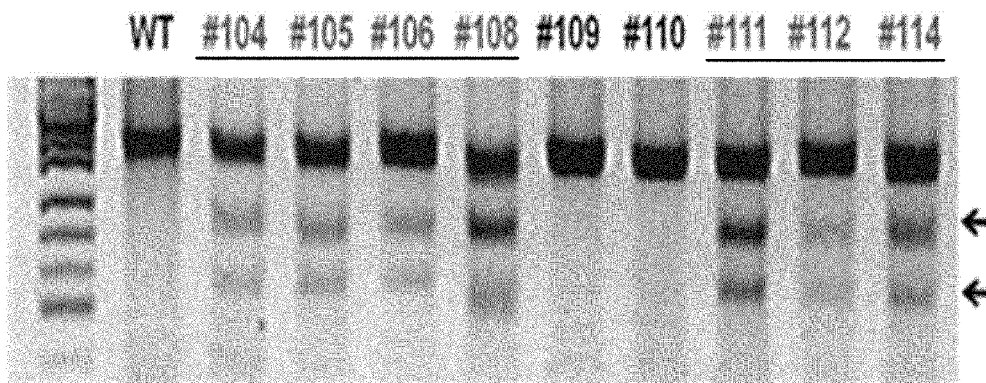
[Fig. 4b]



[Fig. 4c]



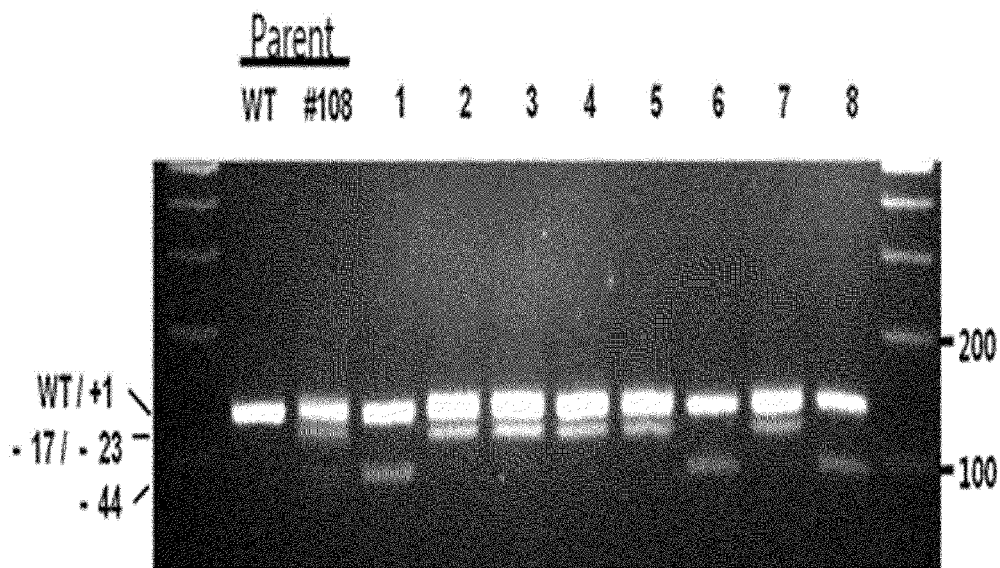
Foxn1



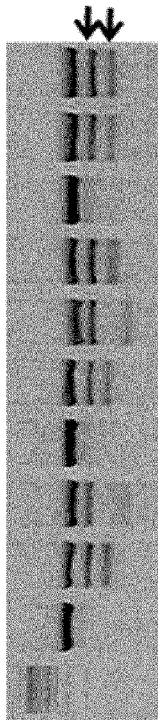
[Fig. 5c]

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

[Fig. 5d]



(b)



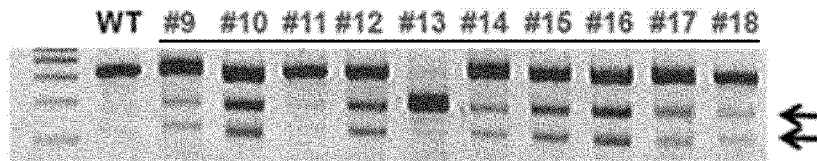
Cas9 mRNA (ng/μl)	sgRNA (ng/μl)	Tested embryos	Mutants (%)
10	1	27	9 (33)
10	10	49	28 (57)
10	100	45	41 (91)



sqRNA (ng/ μ l)	L	P	G	G	S	T	R	L	E	G	E	P	Q	G	D	L	M	Q	A				
100	<div style="border: 1px solid black; padding: 2px;">A CTT CCA GGC TCC ACC CCA CT G GGC GGC GAA CCC CAA GGG GAC CTC ATG CAG GCT CC WT</div>																						
	#1	A	CTT	CCA	GGC	TCC	ACC	CGA	--	--	--	--	A	CCC	CAA	GGG	GAC	CTC	ATG	CAG	GCT	CC Δ 11	
	#3	A	CTT	CCA	GGC	TCC	ACC	CGA	--	--	--	--	A	CCC	CAA	GGG	GAC	CTC	ATG	CAG	GC--	-- Δ 11+ Δ 17	
	#2	A	CTT	CCA	GGC	TCC	ACC	CGA	C-	--	--	--	--	--	--	--	--	--	--	--	--	-- Δ 57	
10		A	CTT	CCA	GGC	TCC	ACC	--	--	--	--	C	GAA	CCC	CAA	GGG	GAC	CTC	ATG	CAG	GCT	CC Δ 11	
	#5	A	CTT	CCA	GGC	TCC	ACC	C--	--	--	--	--	--	--	CAA	GGG	GAC	CTC	ATG	CAG	GCT	CC Δ 17	
		A	CTT	CCA	GGC	TCC	ACC	CGA	CTGG	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 Δ 12	
1	#11	-	--	--	--	--	--	--	--	--	--	GGC	GAA	CCC	CAA	GGG	GAC	CTC	ATG	CAG	GCT	CC Δ 72	
	#9	A	CTT	CCA	GGC	--	--	--	--	--	--	--	--	--	--	AA	GGG	GAC	CTC	ATG	CAG	GCT	CC Δ 25
		A	CTT	CCA	GGC	T--	--	--	--	--	--	--	--	--	--	AA	GGG	GAC	CTC	ATG	CAG	GCT	CC Δ 24
		PAM																					

[Fig. 7a]

Pronucleous injection



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]

Intra-cytoplasmic injection

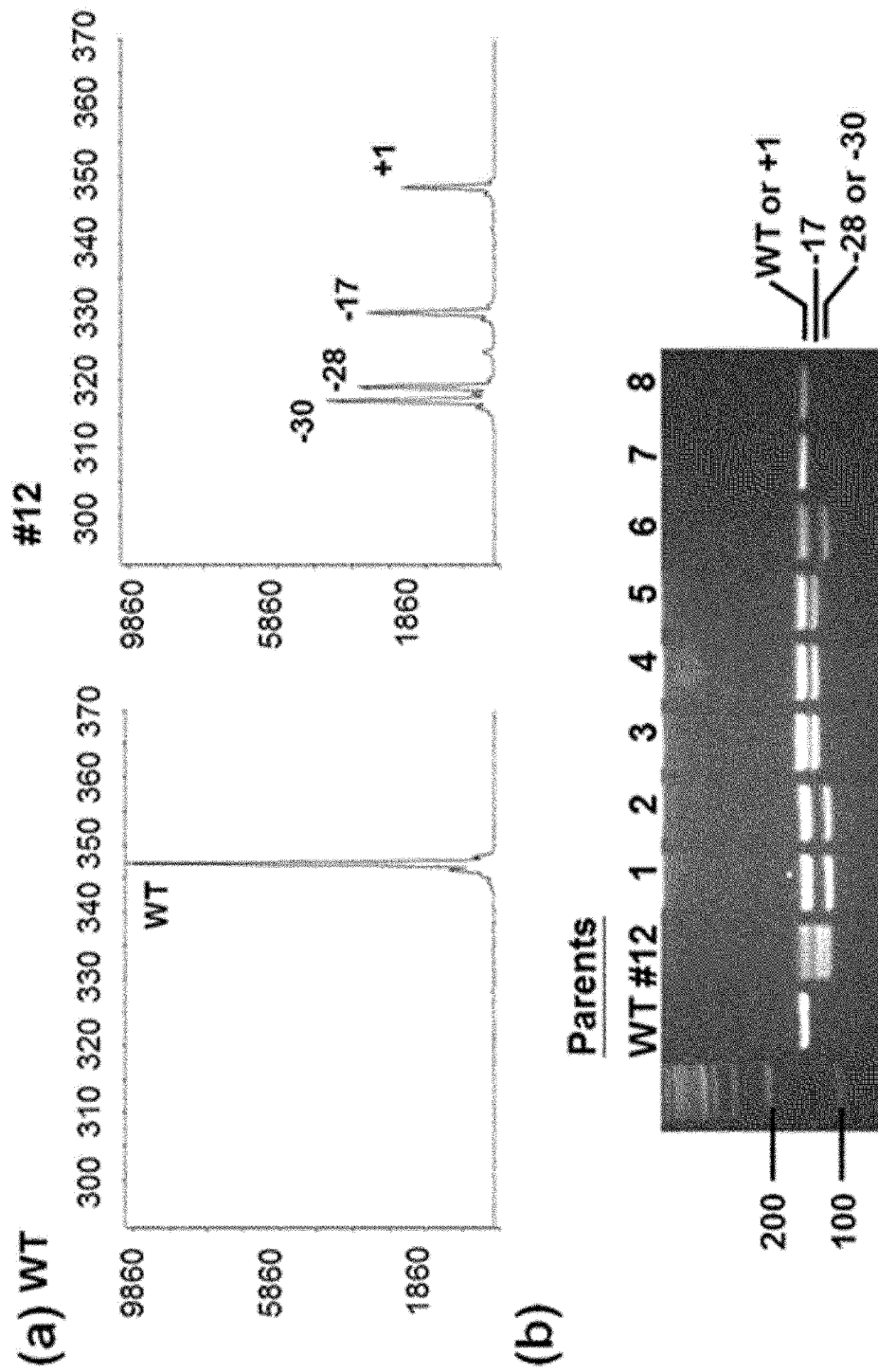


Cas9 protein (nM)	sgRNA (nM)	Tested embryos	Mutants (%)
2	4	18	1 (6%)
20	40	19	6 (32%)
200	400	14	10 (71%)

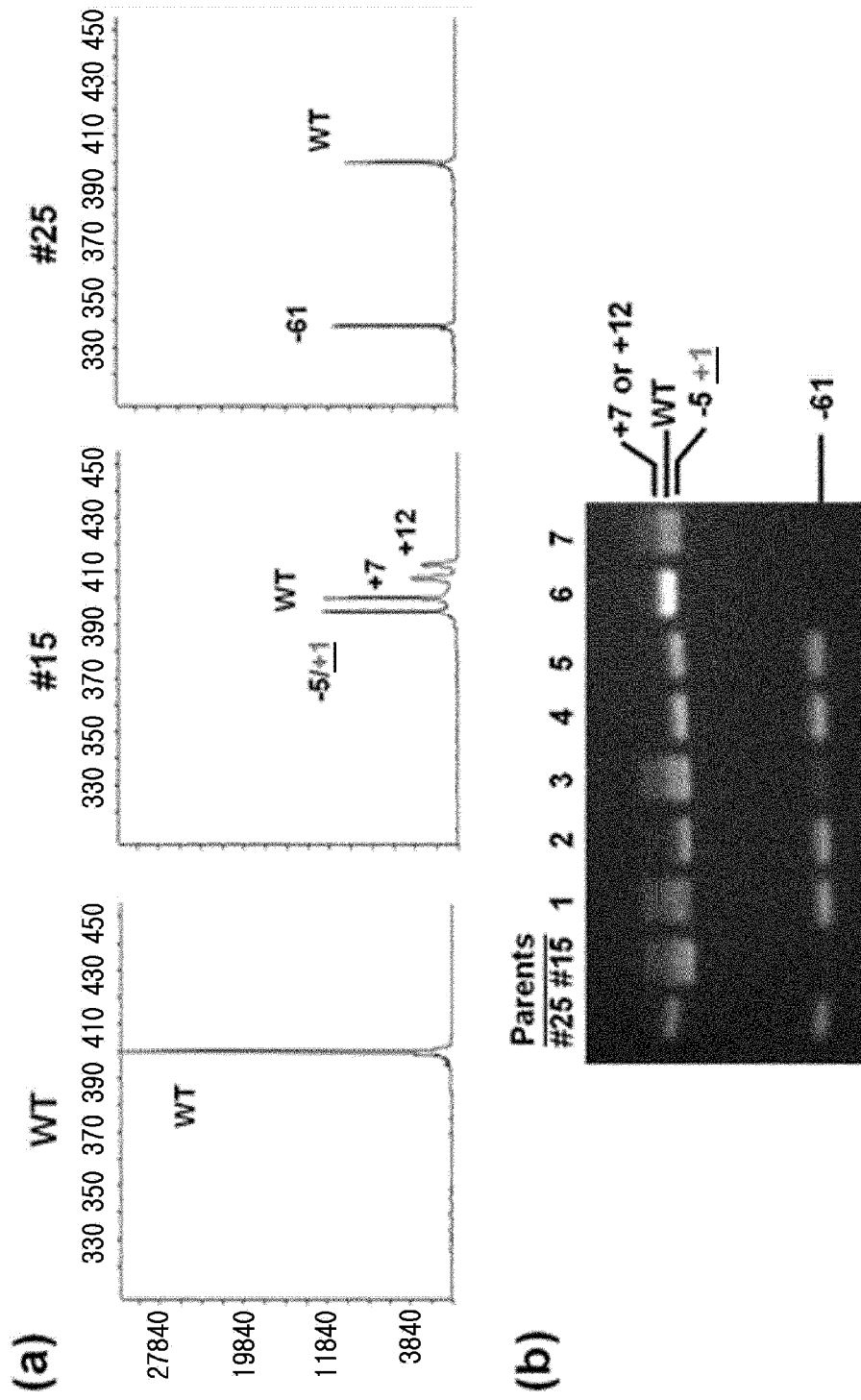
[Fig. 7c]

Sequence	Indels	Embryo no.
<u>ACTTCCAGGCTCCACCCGACTGGA</u> GGGCGAACCCCAAGGGACCTCATGCCAG	WT	
ACTTCCAGGCGAACCC--AAGGGACCTCATGCCAG	Δ18	2
ACTTCCAGGCTCCAC--AAGGGACCTCATGCCAG	Δ20	1
ACTTCCAGGCTCCACCC--AAGGGACCTCATGCC	Δ19	1
ACTTCCAGGCTCCACCC--CAAGGGACCTCATGCCAG	Δ17	1
ACTTCCAGGCTCCACCCGA--ACCCCAAGGGACCTCATGCCAG	Δ11	3
ACTTCCAGGCTCCACCCGAA--GGAGGGCGAACCCCAAGGGACCTCATGCCA	Δ3+1	1
ACTTCCAGGCTCCACCCGACT--AGGGCGAACCCCAAGGGACCTCATGCCAG	Δ2	1
ACTTCCAGGCTCCACCCGACTGGGAGGGCGAACCCCAAGGGACCTCATGCCA	+1	1
ACTTCCAGGCTCCACCCGACTTGGAGGGCGAACCCCAAGGGACCTCATGCCA	+1	10
ACTTCCAGGCTCCACCCGA--GGCGAACCCCAAGGGACCTCATGCCAG	Δ6	1
ACTTCCAGGCTCCACCCGA--GGCGAACCCCAAGGGACCTCATGCCAG	Δ5	2
ACTTCCAGGCTCCACCC--TCATGCCAG	Δ28	1
-----AGGGCGAACCCCAAGGGACCTCATGCCAG	Δ126	1
Total		26

[Fig. 8]



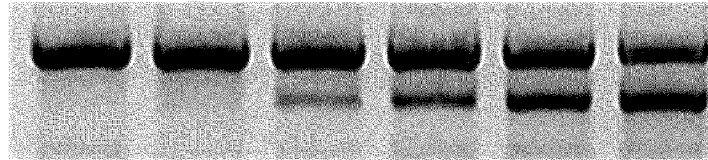
[Fig. 9]



[Fig. 10a]

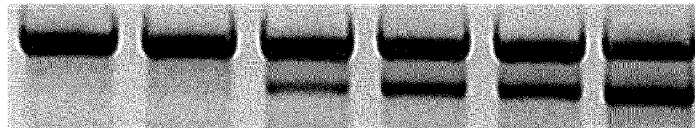
CCR5#4

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 (%)

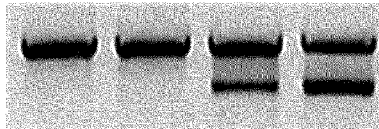
[Fig. 10b]

CCR5#4

[illegible]

Indel (%)	2.7	8.3	33	51	57	32
-----------	-----	-----	----	----	----	----

Cas9 protein	45 (14)	-	4.5 (1.4)	15 (4.5)	μg (μM)
sgRNA	-	60 (87)	6 (8.7)	20 (29)	μg (μM)



Indel (%)	27	51
-----------	----	----

[Fig. 10c]

CCR5

CAATCTATGACATCAATTATTATA-CATCGGAGCCCTGCCAAAAAATCAA WT
 CAATCTATGACATCAATTATTAT----CGGAGCCCTGCCAAAAAATCAA -4
 CAATCTATGACATCAATTAT----CATCGGAGCCCTGCCAAAAAATCAA -4
 CAATCTATGACATCAATTAT-----CGGAGCCCTGCCAAAAAATCAA -7
 CAATCTATGACATCAATTATTAT--CATCGGAGCCCTGCCAAAAAATCAA -1
 CAATCTATGACATCAATTATTATAACATCGGAGCCCTGCCAAAAAATCAA +1
 CAATCTATGACAA-----GAGCCCTGCCAAAAAATCAA -17,+1

[Fig. 10d]

ABCC11

Cas9 protein	-	15 (4.5)	µg (µM)
sgRNA	-	20 (29)	µg (µM)



Indel (%)	35
-----------	----

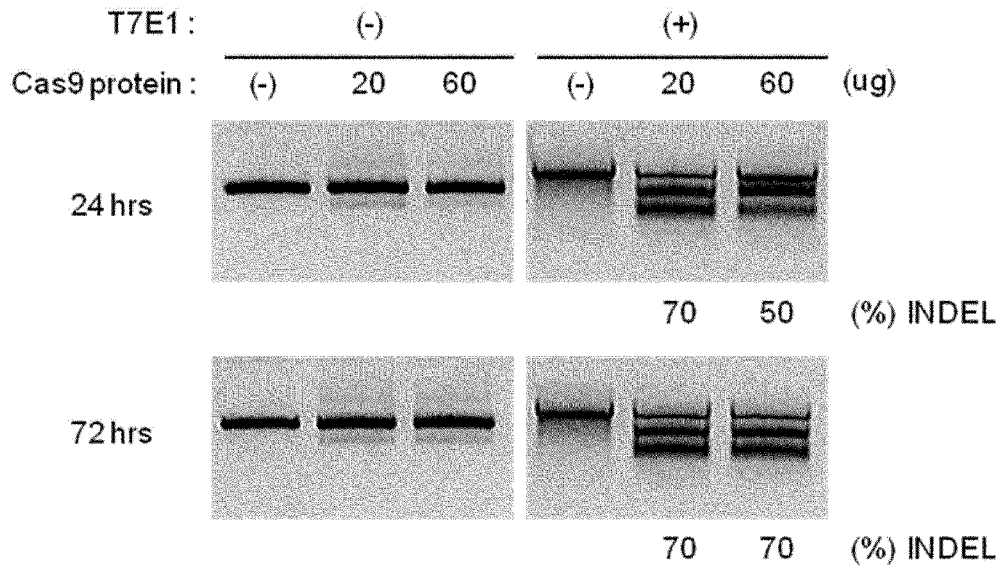
[Fig. 10e]

ABCC11

▼

TTCTCAAGGCAGCATCATACTTCCCCA	CGGT GGGACAGCTGCCCTCCCTGG	WT
TTCTCAAGGCAGCATCATACTTCC-----	CTGGGACAGCTGCCCTCCCTGG	-6
TTCTCAAGGCAGCATCATACTTC--	CACGGTGGGACAGCTGCCCTCCCTGG	-3
TTCTCAAGGCAGC-----	TGCCCTCCCTGG	-29
TTCTCAAGGCAGCATCATACTT-----	CCCTCCCTGG	-20
TTCTCAAGGCAGCATCATACTT-----	CCCTCCCTGG	-20
TTCTC-----		-256

[Fig. 11]



[Fig. 12]

Target sequence 1		Target sequence 2
ACAAAGCGATTITGAAGAIGGAAGCGCGTGGTTAT//.....	190 bp	//GGGTGAACATAAAGTGGTCCACACGGTGGAGATTG (nt)
ACAAAGCGATTITGAAGAIGGAAGCGCGTGGTTAT//.....	190 bp	ACCGGTGAACATAAAGTGGTGGAGATTG (-7)
ACAAAGCGATTITGAAGAIGGAAGCGCGTGGTTAT//.....	190 bp	ACCGGTGAACATAAAGTGGTGGAGATTG (-224)
ACAAAGCGATTITGAAGAIGGAAGCGCGTGGTTAT//.....	190 bp	ACCGGTGAACATAAAGTGGTGGAGATTG (-223)
ACAAAGCGATTITGAAGAIGGAAGCGCGTGGTTAT//.....	190 bp	ACCGGTGAACATAAAGTGGTGGAGATTG (-223+62)

[Fig. 13]

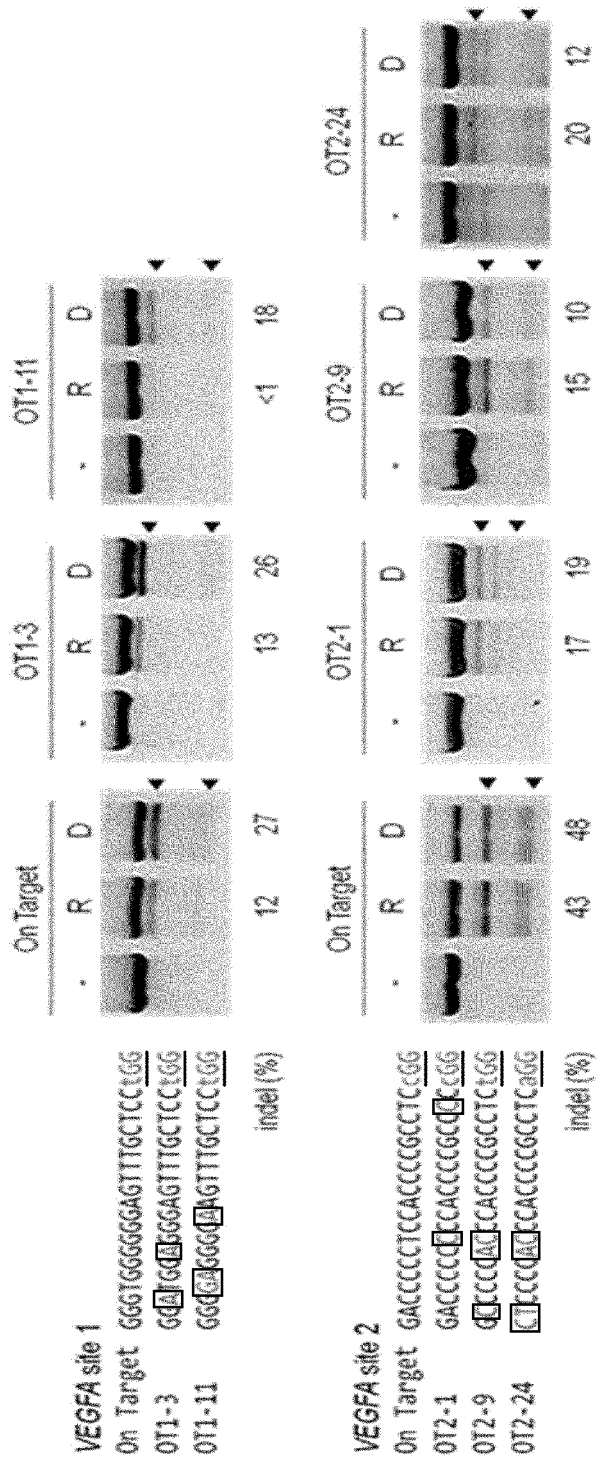
Cas9	-	+	-	-	-	+	-
Cas9-9R4L	-	-	+	-	-	-	+
sgRNA	-	-	-	+	-	+	-
sgRNA-9R4L	-	-	-	-	+	-	+

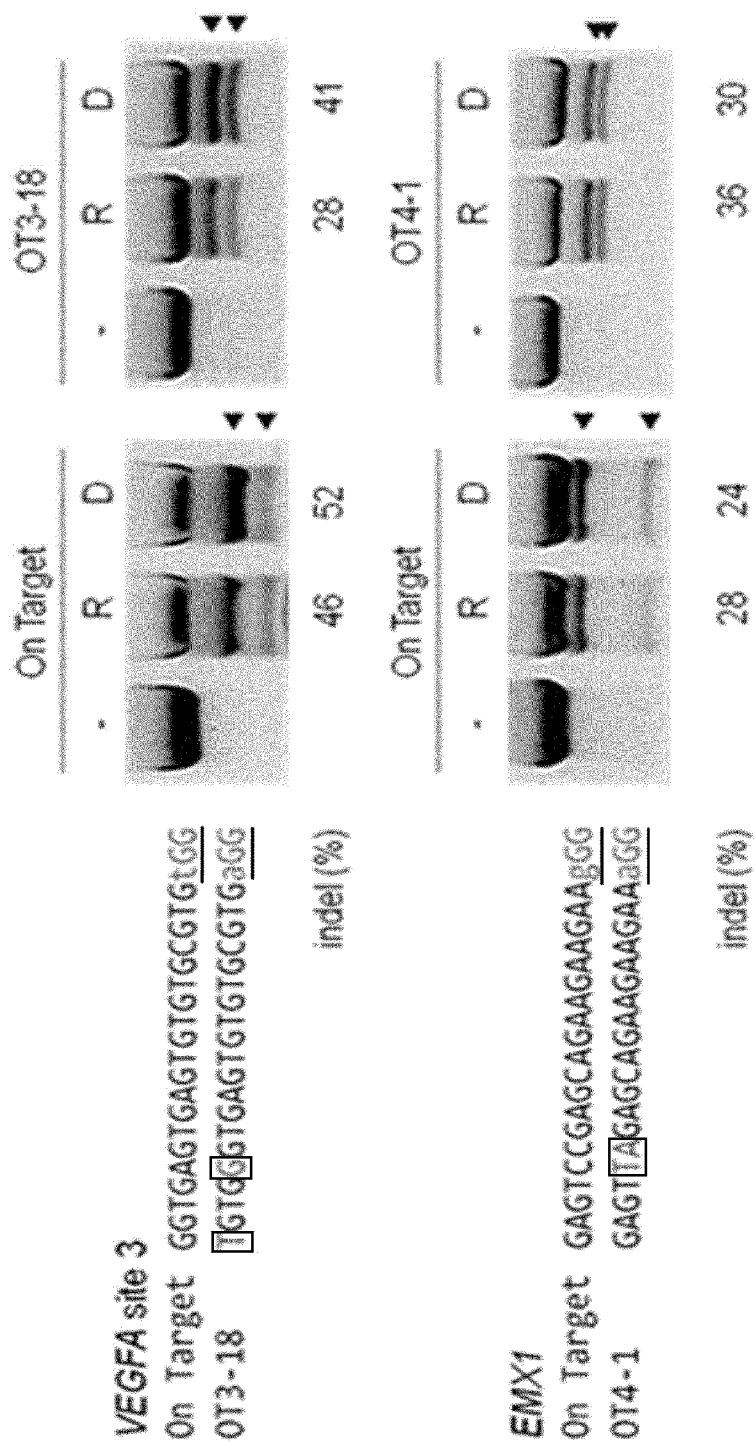


Indel (%)

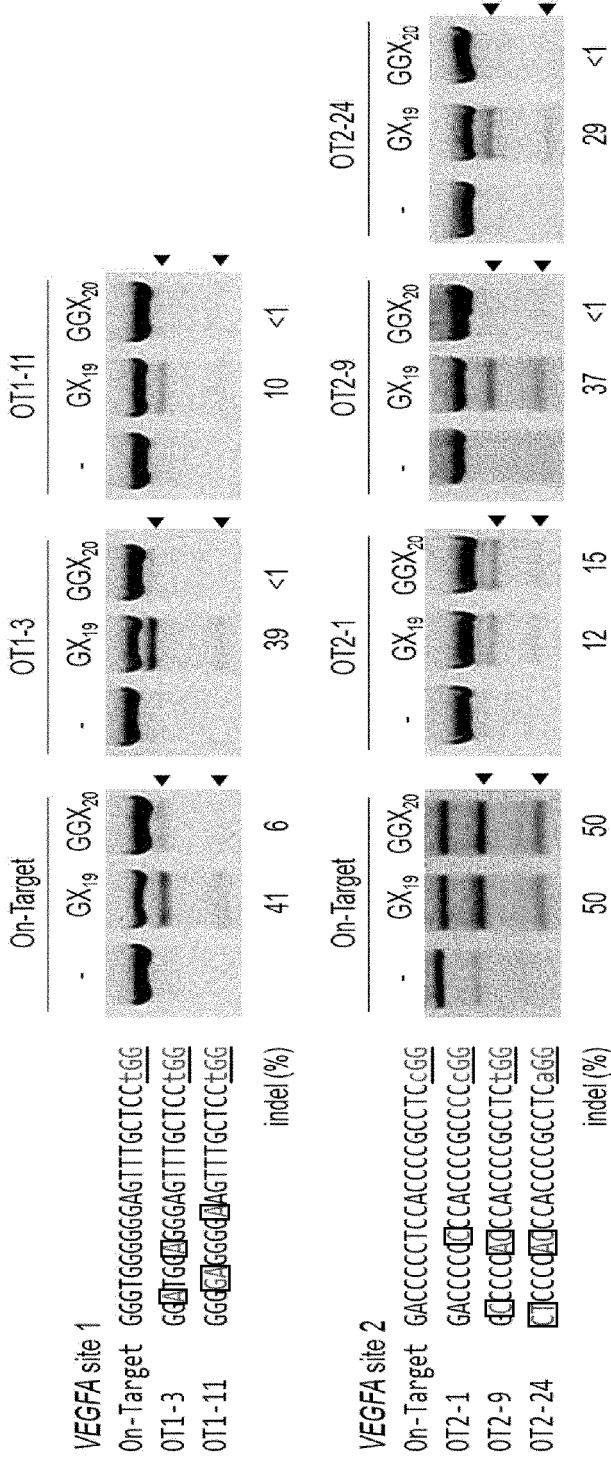
9

[Fig. 14a]

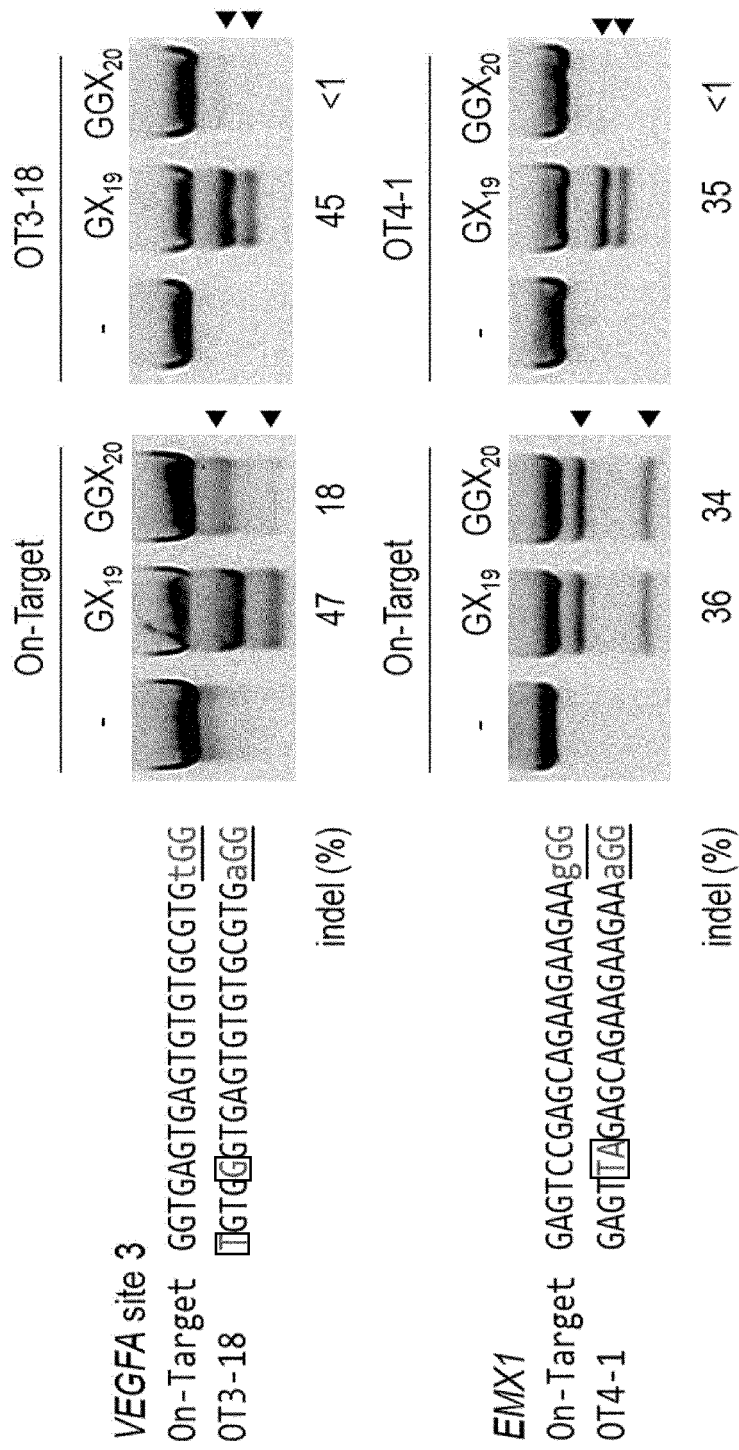




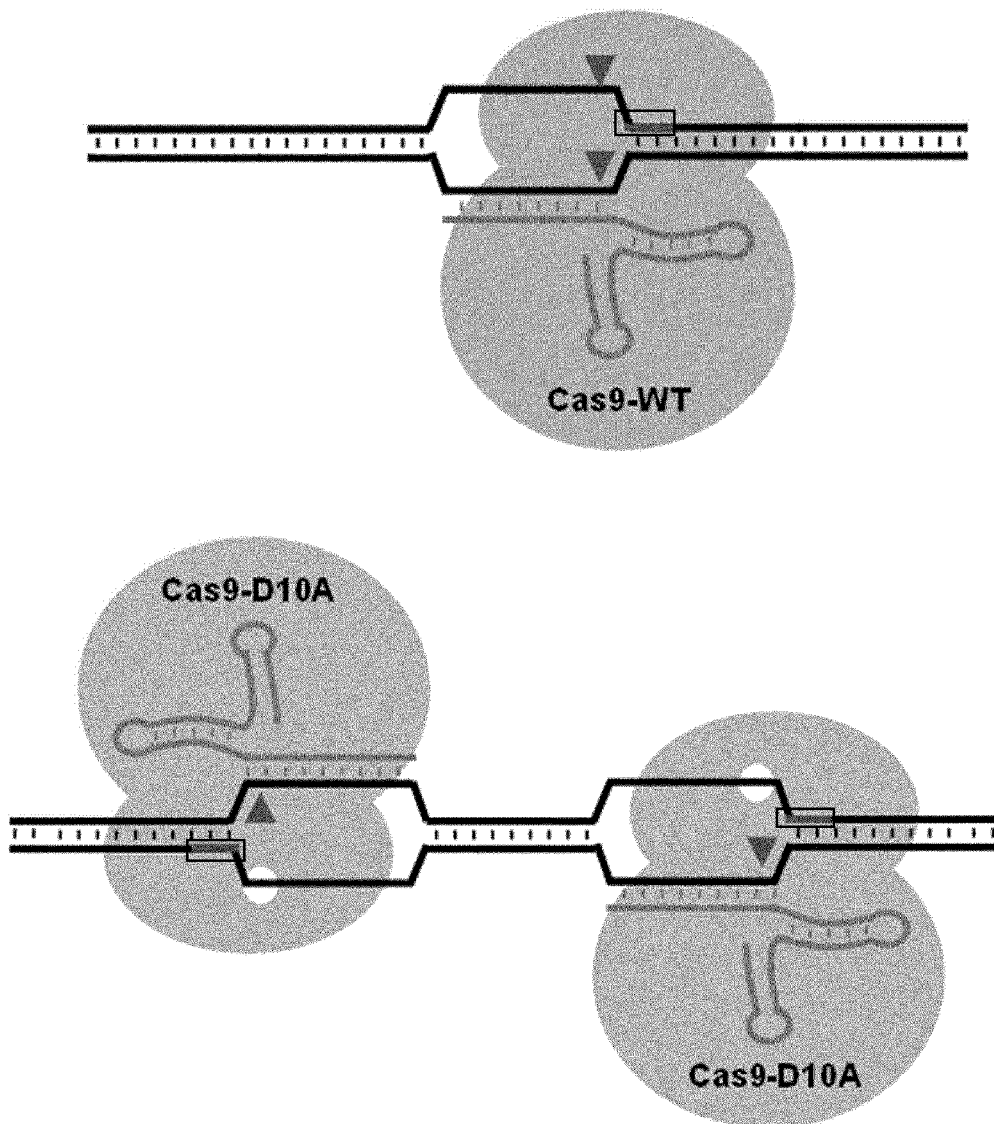
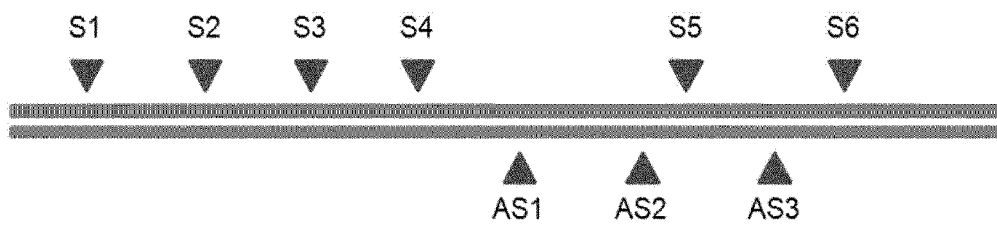
[Fig. 15a]



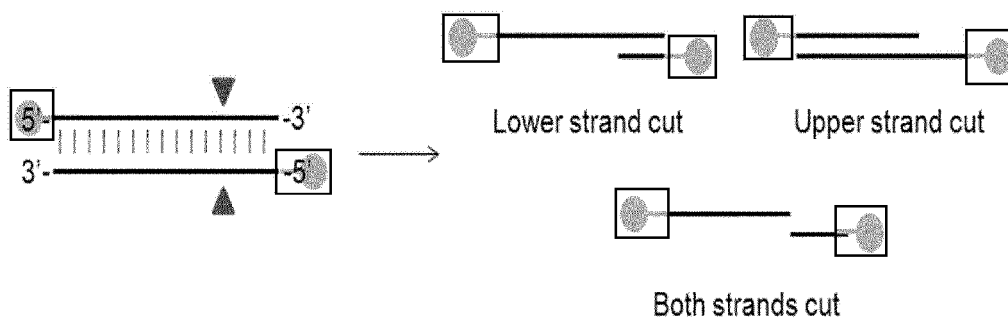
[Fig. 15b]



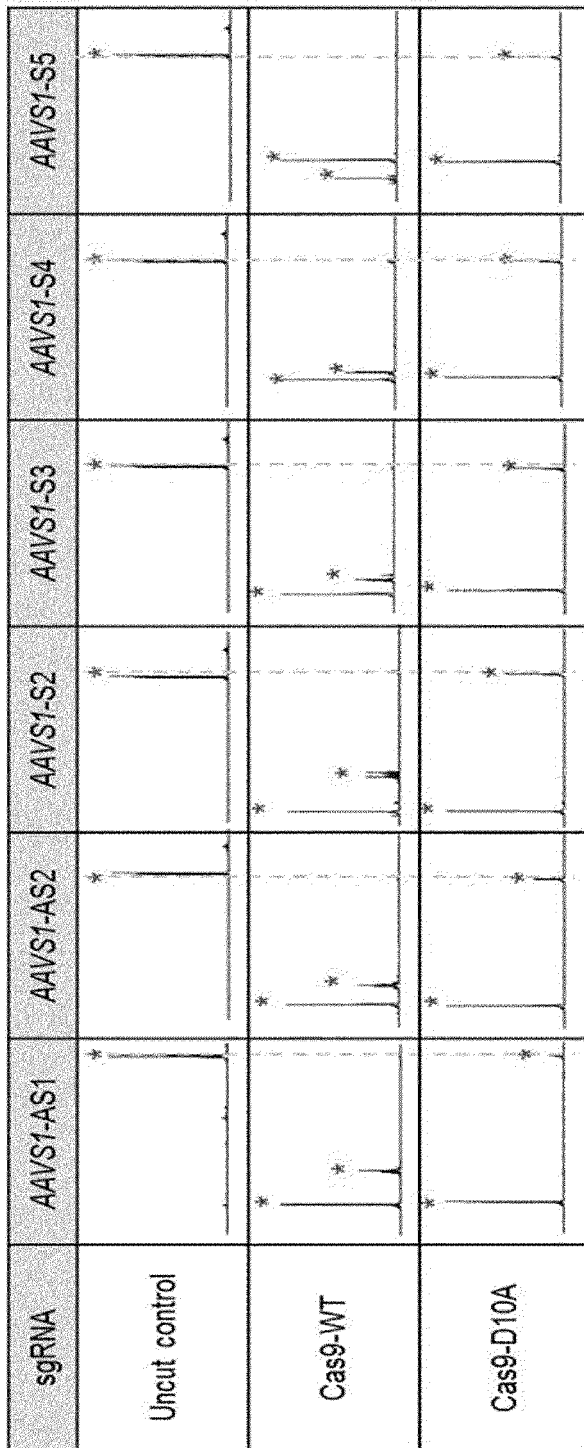
[Fig. 16a]

[Fig. 16b]
AAVS1 locus

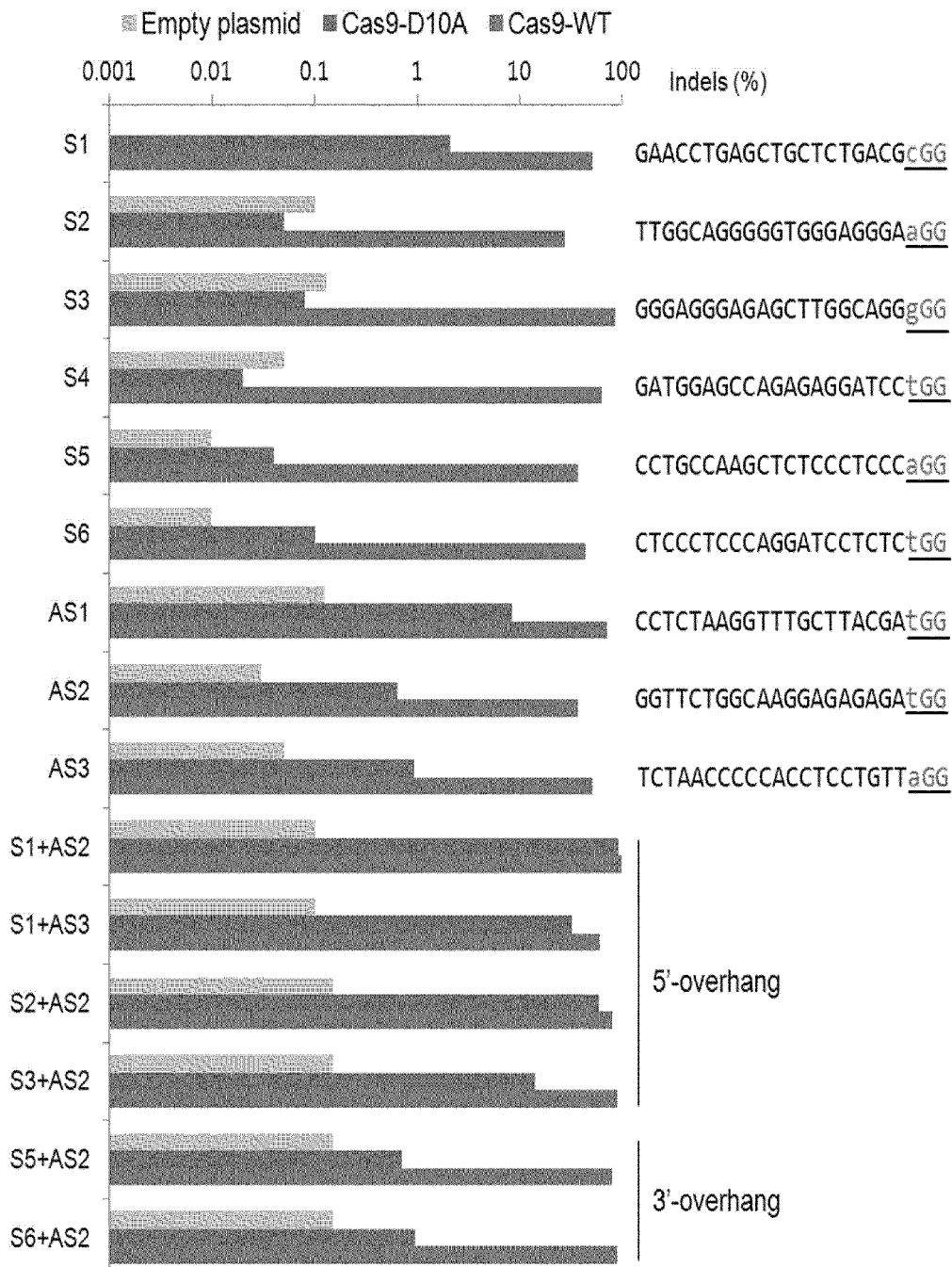
[Fig. 16c]



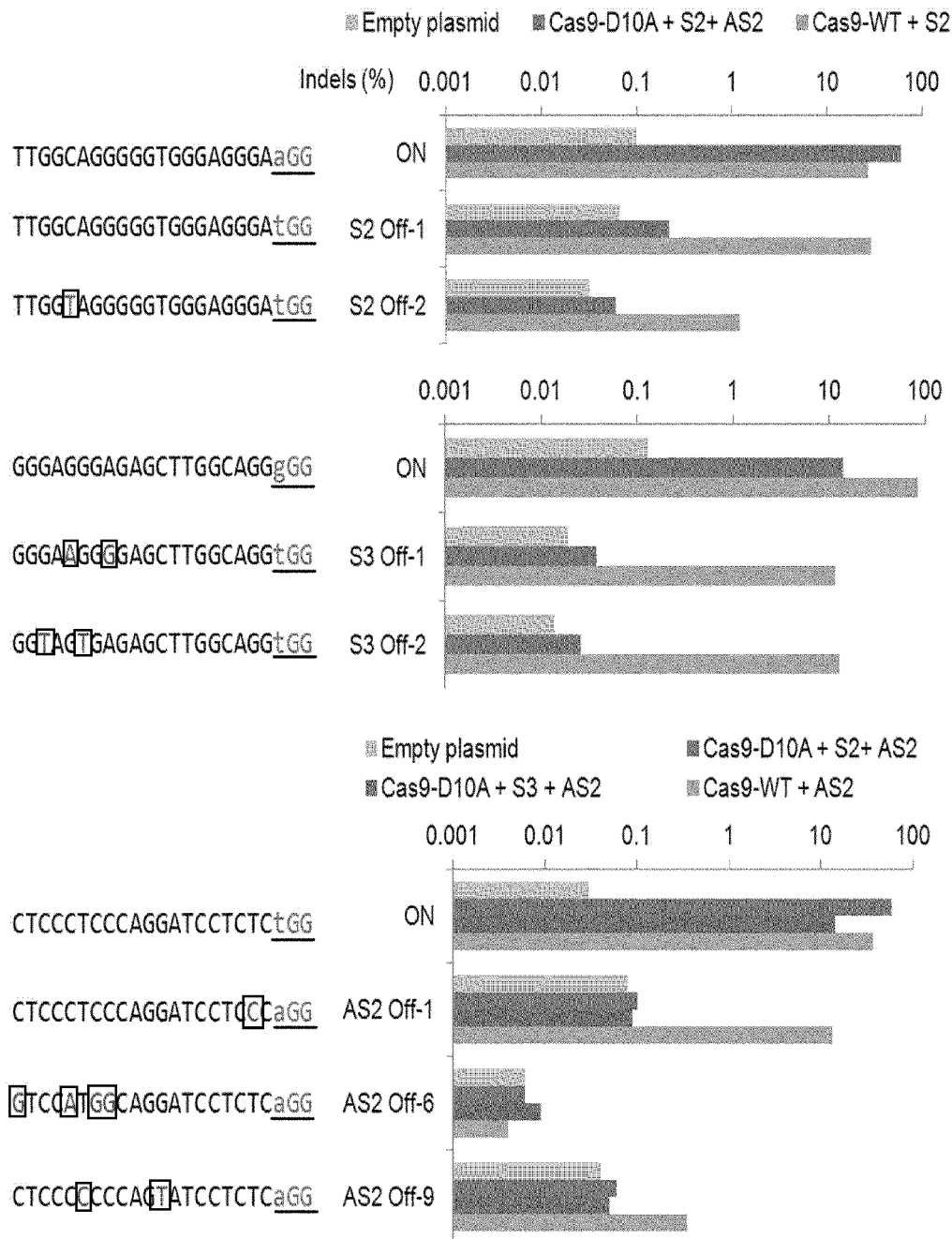
[Fig. 16d]



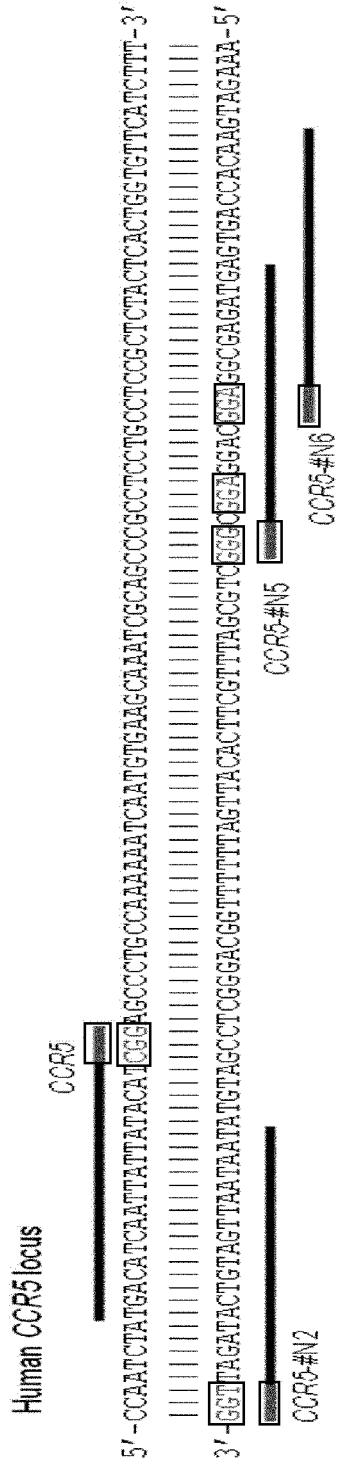
[Fig. 17a]



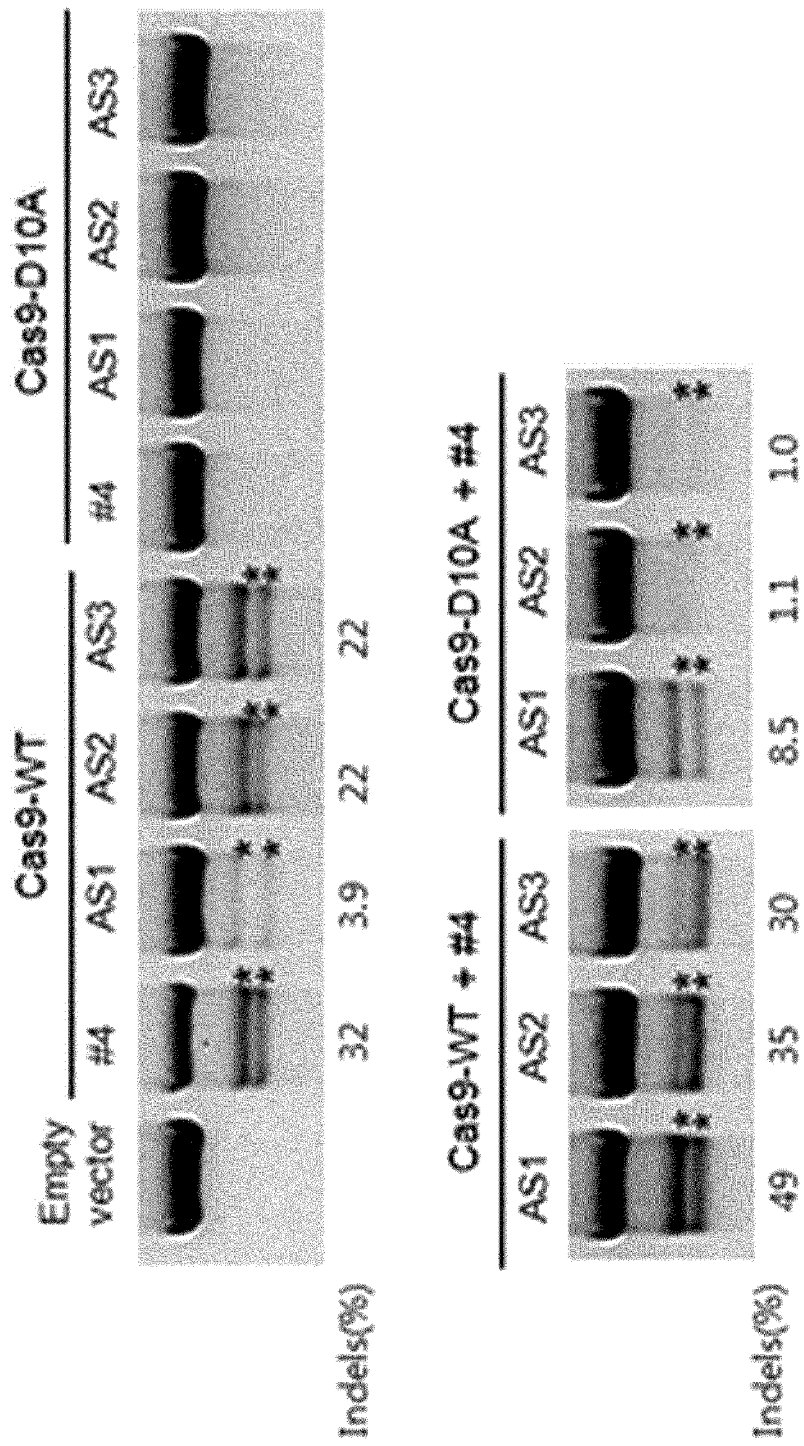
[Fig. 17b]



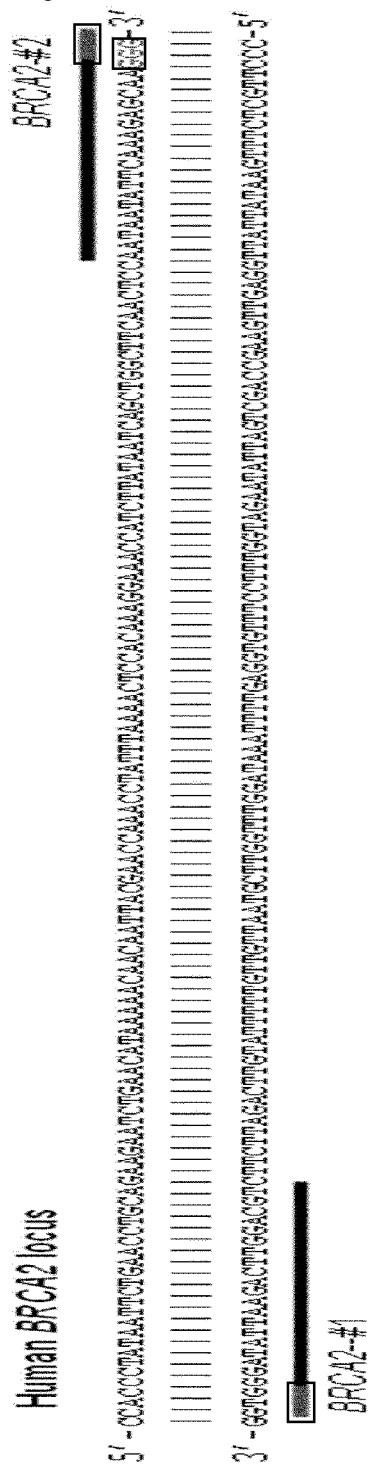
[Fig. 18a]



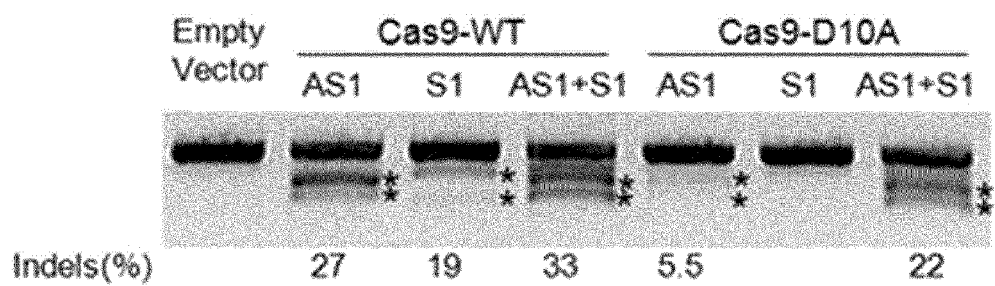
[Fig. 18b]



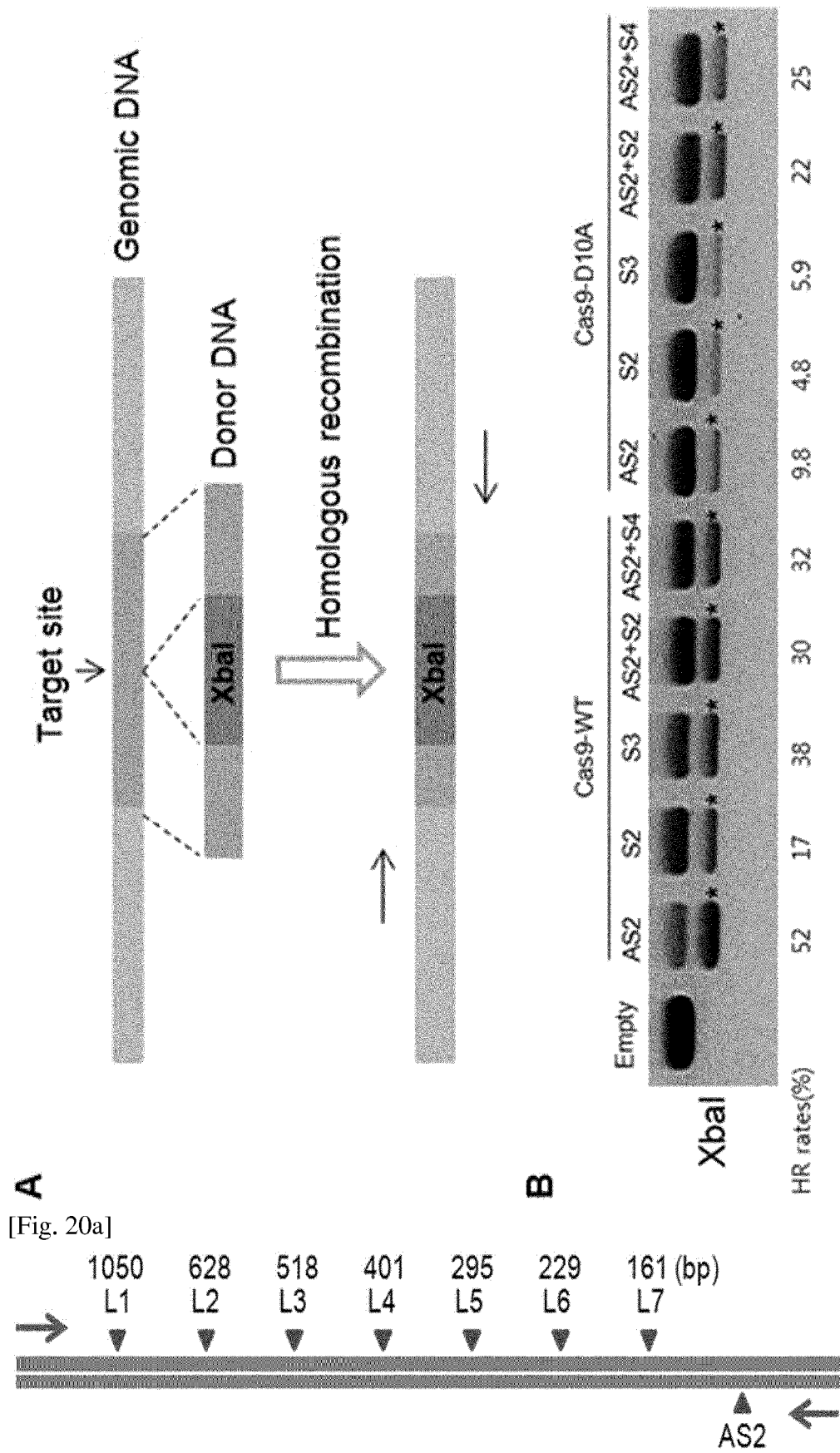
[Fig. 18c]



[Fig. 18d]

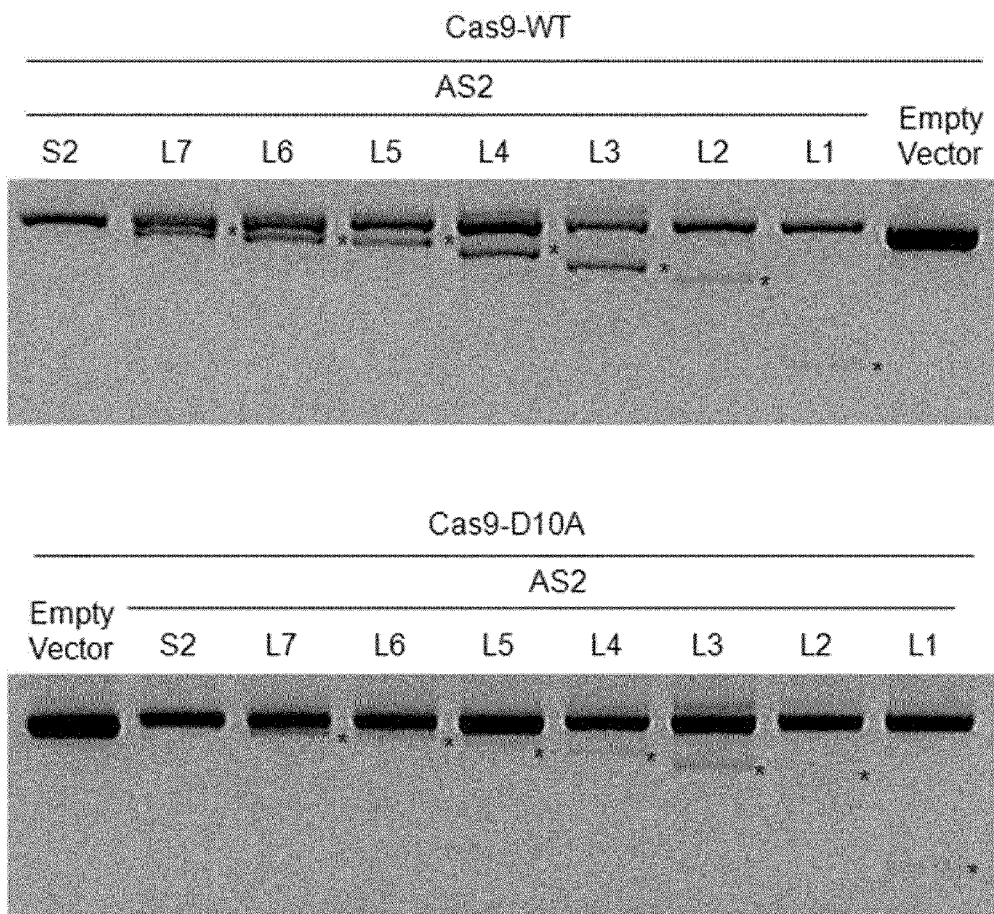


[Fig. 19]



[Fig. 20a]

[Fig. 20b]



[Fig. 20c]

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

```

ggccggggaatcaagagtgca[CCC]AGAGACAGTGACCAACCATCctgttt...//...agctCTCCCTCCCAAGGATCCCTCT[CGG]ctccatcgtaagcaaaccttagagggttctggccaaggagagagatg WT
ggccggggaatcaagagtgca[CCC]AG-----TGACCAACCATCct-----gtaagcaaaccttagagggttctggccaaggagagagatg
ggccggggaatcaagagtgca[CCC]AG-----gaa
ggccggggaatcaagagtgca[CCC]AGA-----CCTCT[CGG]ctccatcgtaagcaaaccttagagggttctggccaaggagagagatg
ggccggggaatcaagagtgca[CCC]-----taacag
ggccggggaatcaaga-----cg[CGG]ctccatcgtaagcaaaccttagagggttctggccaaggagagagatg
ggc-----tccatcgtaagcaaaccttagagggttctggccaaggagagagatg
ggccggggaatcaagagtgca[CCC]AGA-----CTCT[CGG]ctccatcgtaagcaaaccttagagggttctggccaaggagagagatg

```

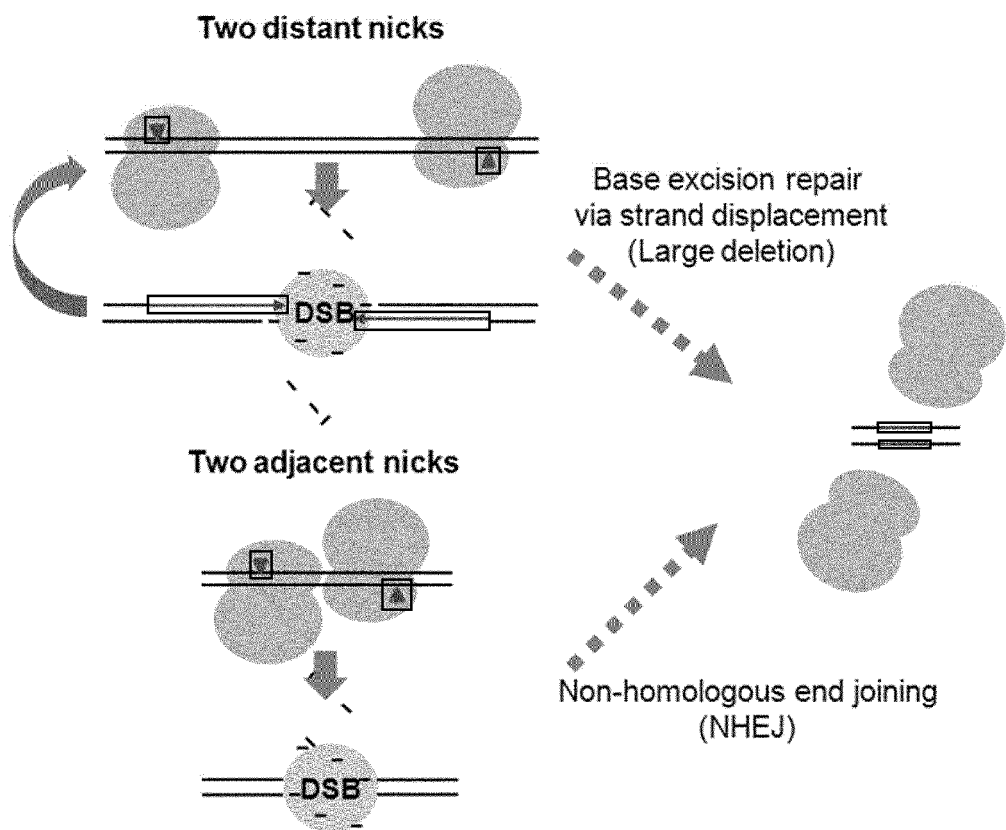
Cas9-D10A AS2 + L1 (~1050bp deletion)

```

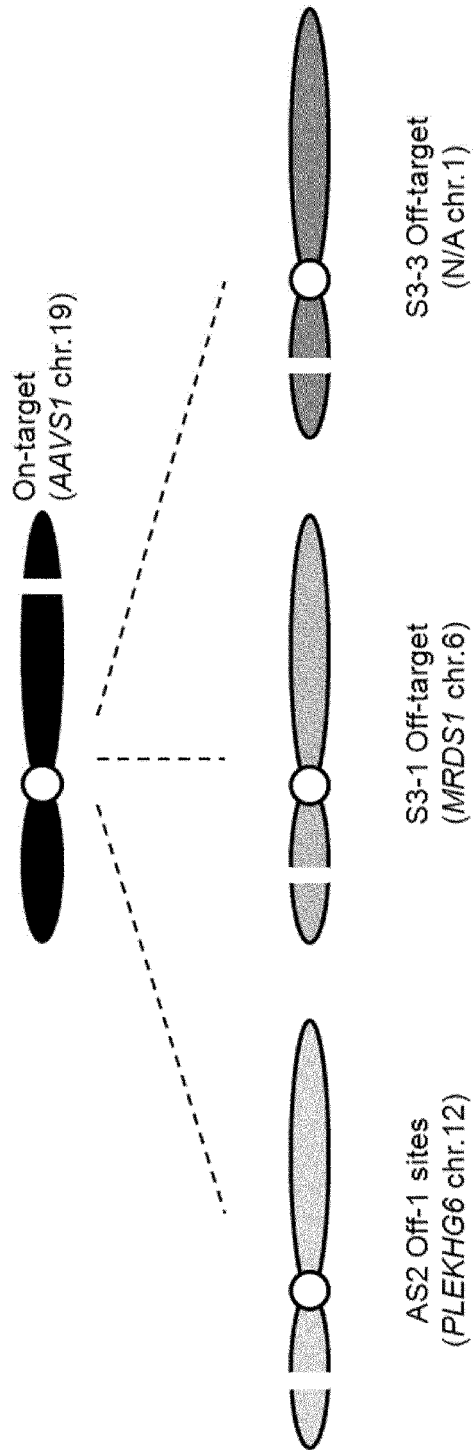
ggccggggaatcaagagtgca[CCC]AGAGACAGTGACCAACCATCctgttt...//...agctCTCCCTCCCAAGGATCCCTCT[CGG]ctccatcgtaagcaaaccttagagggttctggccaaggagagagatg WT
ggccggggaatcaagagtgca[CCC]AGAGACAGTGACCAACCATC-----gtaagcaaaccttagagggttctggccaaggagagagatg
gg-----tccatcgtaagcaaaccttagagggttctggccaaggagagagatg
ggccggggaatcaagagtgca[CCC]A-----tctccatcgtaagcaaaccttagagggttctggccaaggagagagatg
ggccggggaatcaagagtgca[CCC]AGA-----CT[CGG]ctccatcgtaagcaaaccttagagggttctggccaaggagagagatg
gg-----tccatcgtaagcaaaccttagagggttctggccaaggagagagatg
ggccggggaatcaagagtgca[CCC]AGAGACAGTGACCAACCATCcc-----atata
ggccggggaatcaagagtgca-----tcgtaagcaaaccttagagggttctggccaaggagagagatg x2

```

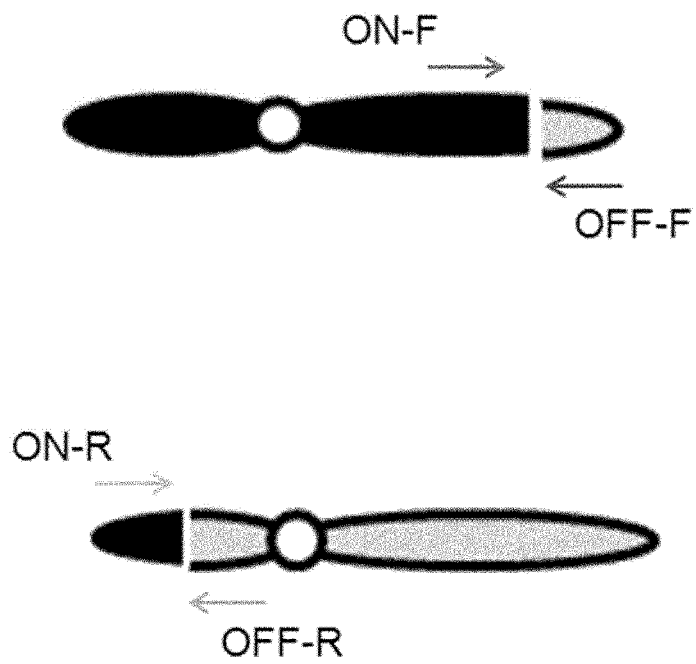
[Fig. 20d]



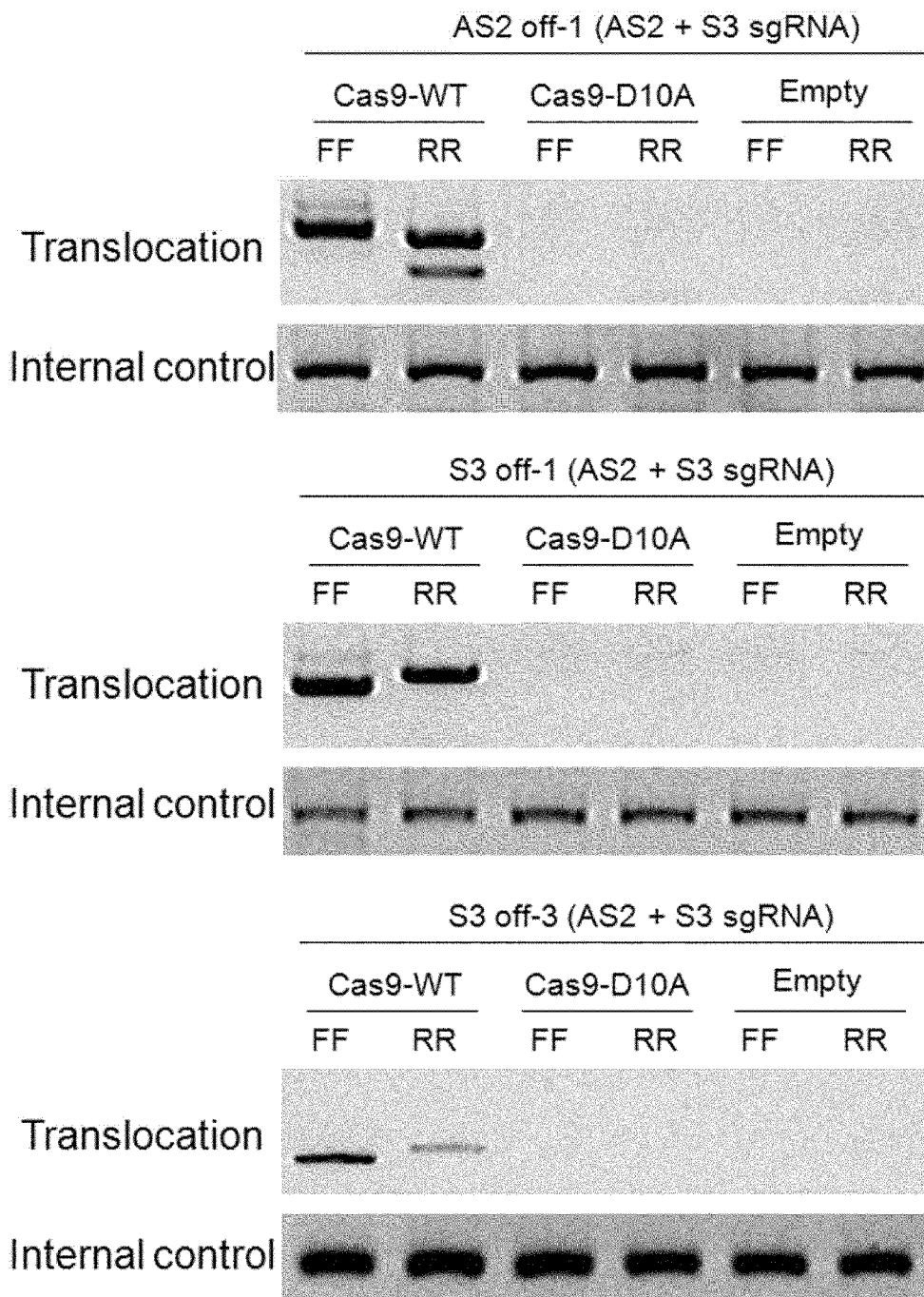
[Fig. 21a]



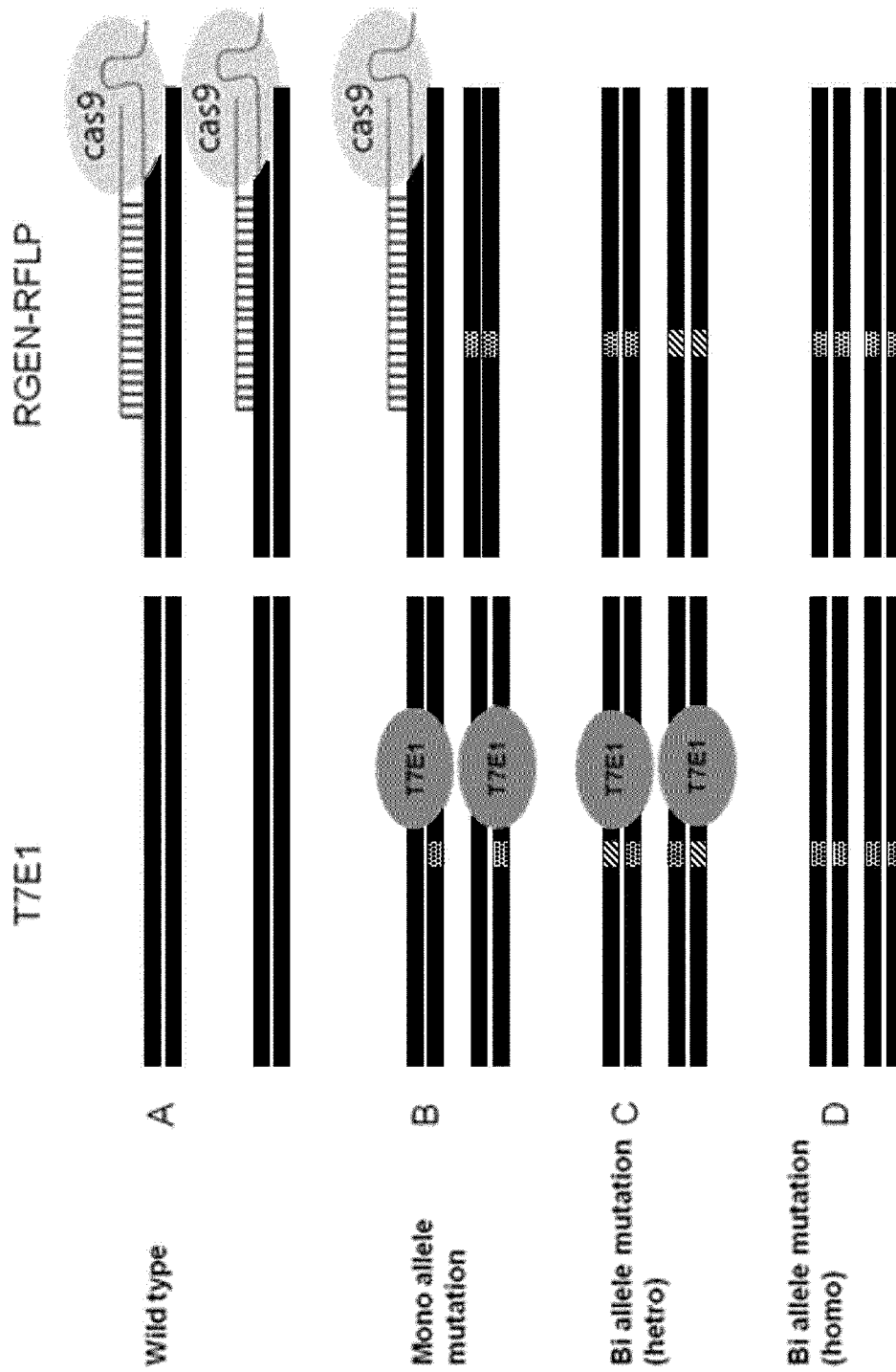
[Fig. 21b]



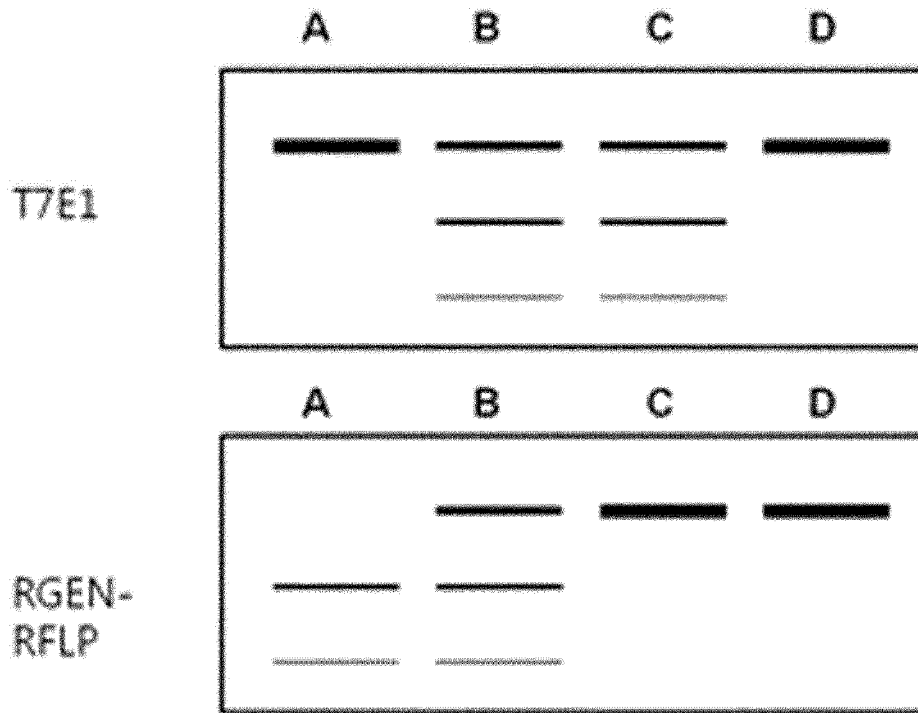
[Fig. 21c]



[Fig. 22a]



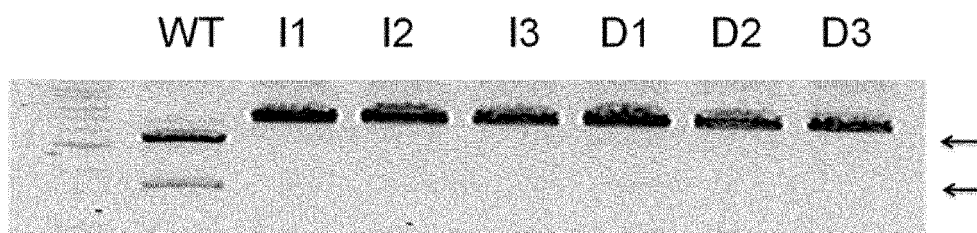
[Fig. 22b]



[Fig. 23]

Plasmid target sequence

AATGACCACTACATCCT---CAAGGG	WT
AATGACCACTACATCCTT---CAAGGG	I1
AATGACCACTACATCCTTT---CAAGGG	I2
AATGACCACTACATCCTTTTCAAGGG	I3
AATGACCACTACATCCT----AAGGG	D1
AATGACCACTACATCCT-----AGGG	D2
AATGACCACTACATCCT-----GGG	D3



[Fig. 24a]

#1 (+/-)

```

TATGTGCAATGACCACTACATCCT---CAAGGGCAGCAATCGGAG WT
TATGTGCAATGACCACTACATCCTCCTCAAGGGCAGCAATCGGAG +3

```

#2 (+/-)

```

TATGTGCAATGACCACTACATCCTCAAGGGCAGCAATCGGAG WT
TATGTGCAATGACCACTACATC-----AATCGGAG -12

```

#5 (+/-)

```

TATGTGCAATGACCACTACATCCTCAAGGGCAGCAATCGGAG WT
TATGTGCAATGACCACTACAT-----CAGCAATCGGAG -9

```

#6 (+/-)

```

TATGTGCAATGACCACTACATCCTCAAGGGCAGCAATCGGAG WT
TATGTGCAATGACCACTACATCC-----AGCAATCGGAG -8

```

#12 (-/-)

```

-----CAGCAATCGG -36
TATGTGCAATGACCACTACATCCT---TCAAGGGCAGCAATCGG +1
TATGTGCAATGACCACTACATCCT---TCAAGGGCAGCAATCGG +1
TATGTGCAATGACCACTACATCCT/ 67bp/CAAGGGCAGCAATCGG +67

```

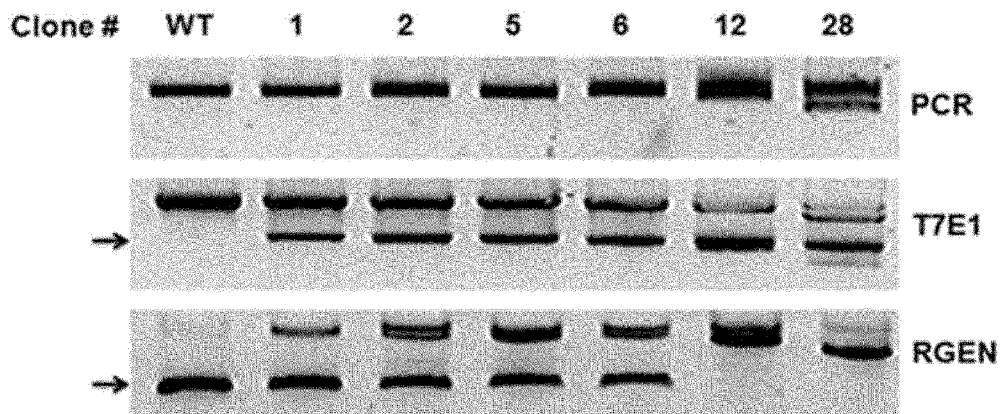
#28 (-/-)

```

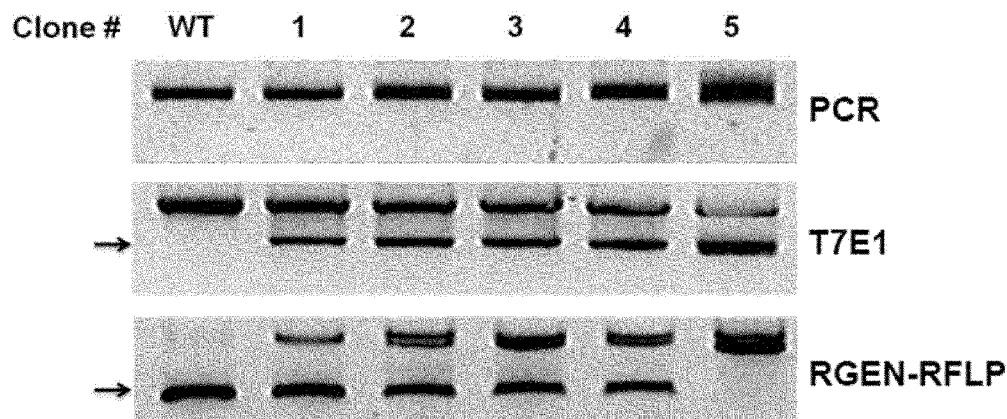
TATGTGCAATGACCACTACATCCTTCAAGGGCAGCAATCGG +1
TATGTGCAATGACCACTACAT---T---GGCAGCAATCGG -7, +1
TATGTGCAATGACCACTACAT----- -94

```

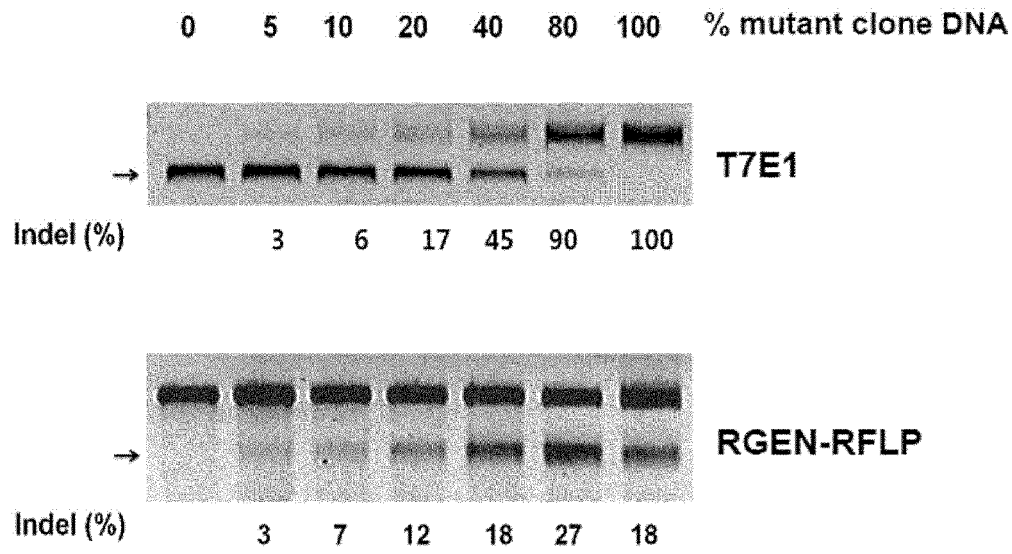
[Fig. 24b]



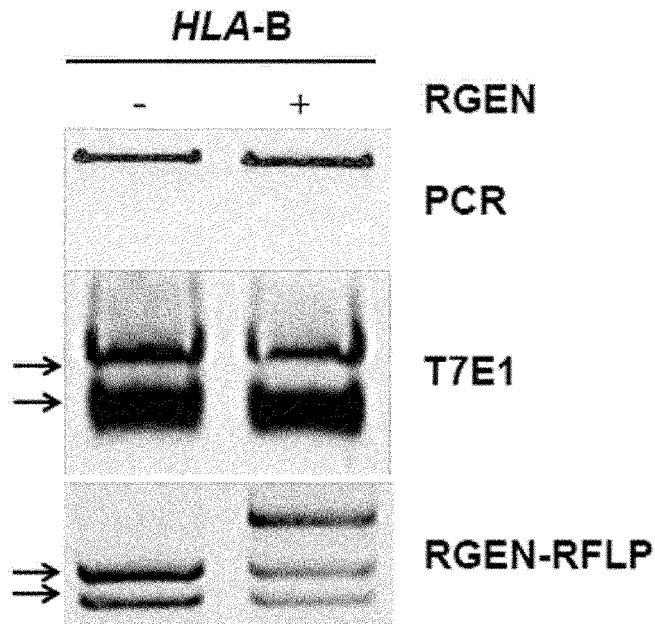
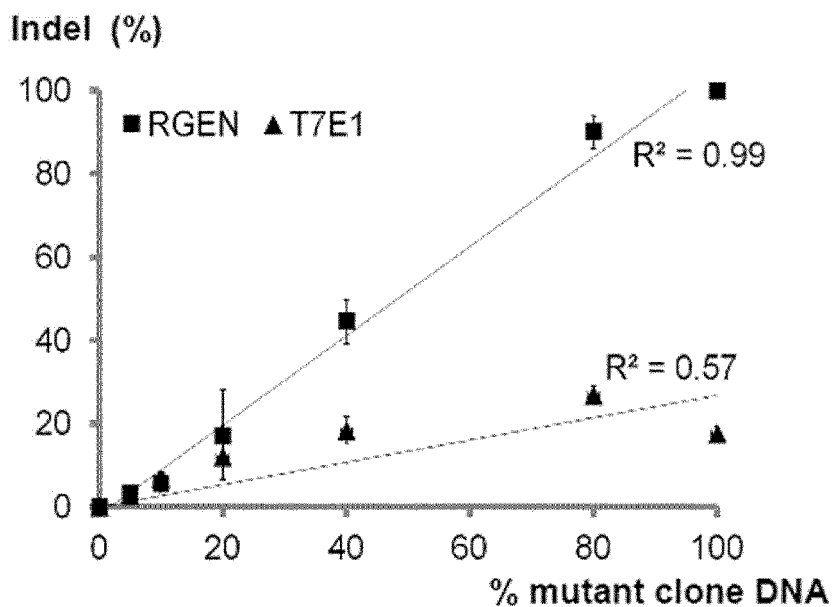
[Fig. 25a]



[Fig. 25b]



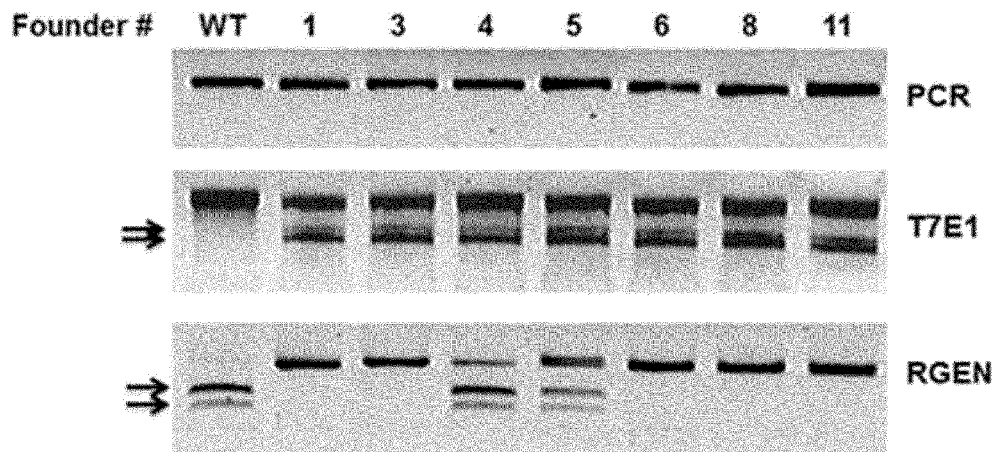
[Fig. 25c]



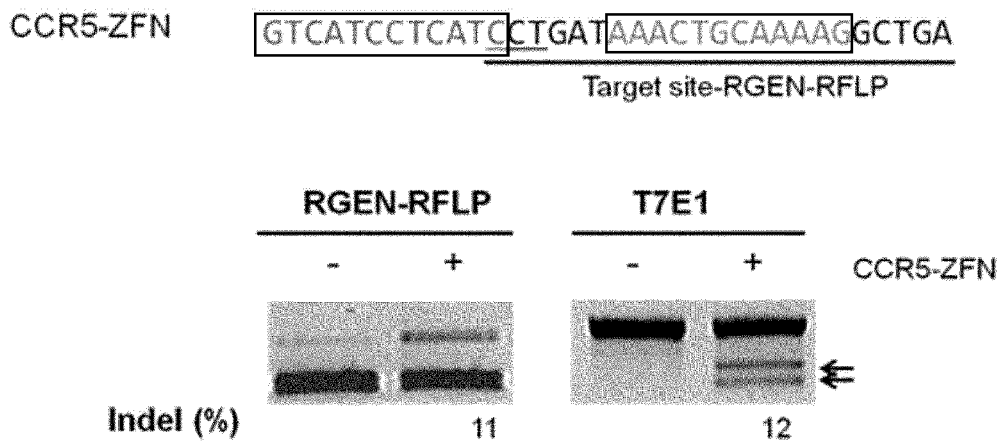
[Fig. 26a]

	T	CAT	ACA	GAT	GAT	GTC	TCA	TCA	TCA	GAG	GAG	CGA	GAA	GGT	AAA	GTC	AAA	ATC	A	Wild-type
#1	T	CAT	ACA	GAT	GAT	AC-	---	---	---	---	---	---	--A	GGT	AAA	GTC	AAA	ATC	A	Δ21 bp (-/-)
	T	CAT	ACA	GGT	GAT	G--	---	---	---	---	---	---	-AA	GGT	AAA	GTC	AAA	ATC	A	Δ21 bp
#3	T	CAT	ACA	GAT	GAT	GTC	TCA	TCA	TCA	GAG	---	CGA	GAA	GGT	AAA	GTC	AAA	ATC	A	Δ3 bp (-/-)
	T	CAT	ACA	GAT	GAT	GTC	TCA	TCA	TCA	G--	---	CGA	GAA	GGT	AAA	GTC	AAA	ATC	A	Δ5 bp
#4	T	CAT	ACA	GAT	GAT	GTC	TCA	TCA	TCA	G-G	GAG	CGA	GAA	GGT	AAA	GTC	AAA	ATC	A	Δ1 bp (+/-)
	T	CAT	ACA	GAT	GAT	GTC	TCA	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	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	GAT	GA-	---	---	---	---	---	---	--A	GGT	AAA	GTC	AAA	ATC	A	Δ24 bp (-/-)
	T	CAT	ACA	GAT	GAT	GTC	TAG	AGA	T--	---	---	---	GAA	GGT	AAA	GTC	AAA	ATC	A	Δ11 bp
#11	T	CAT	ACA	GAT	GAT	GTC	TCA	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

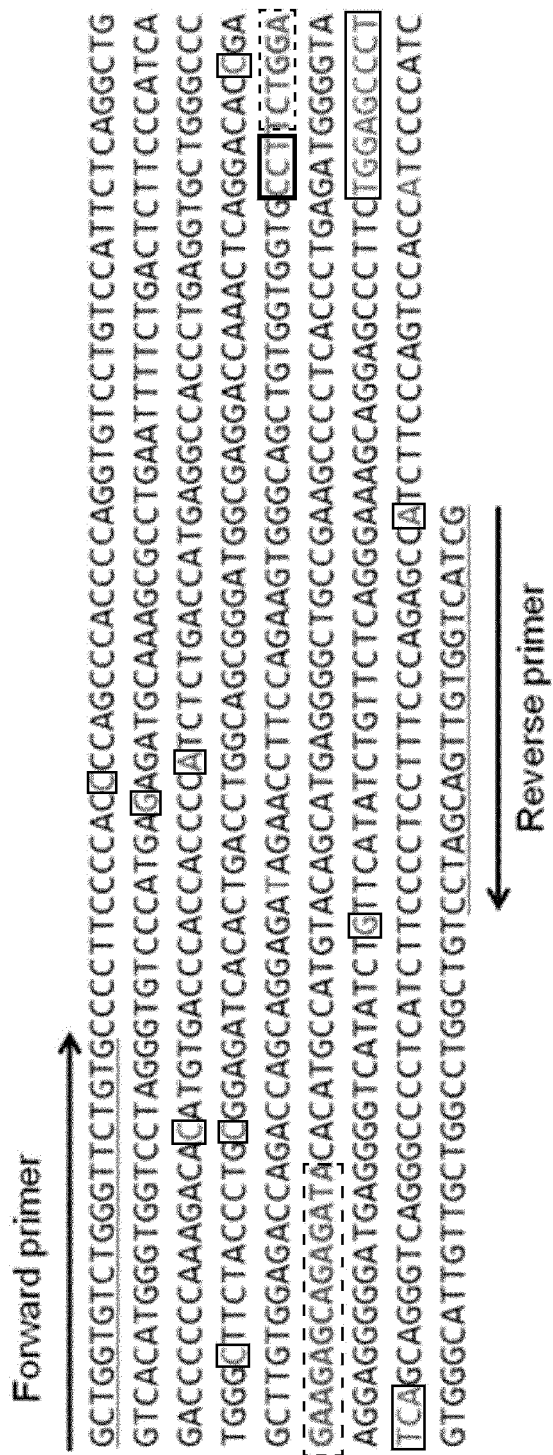
[Fig. 26b]



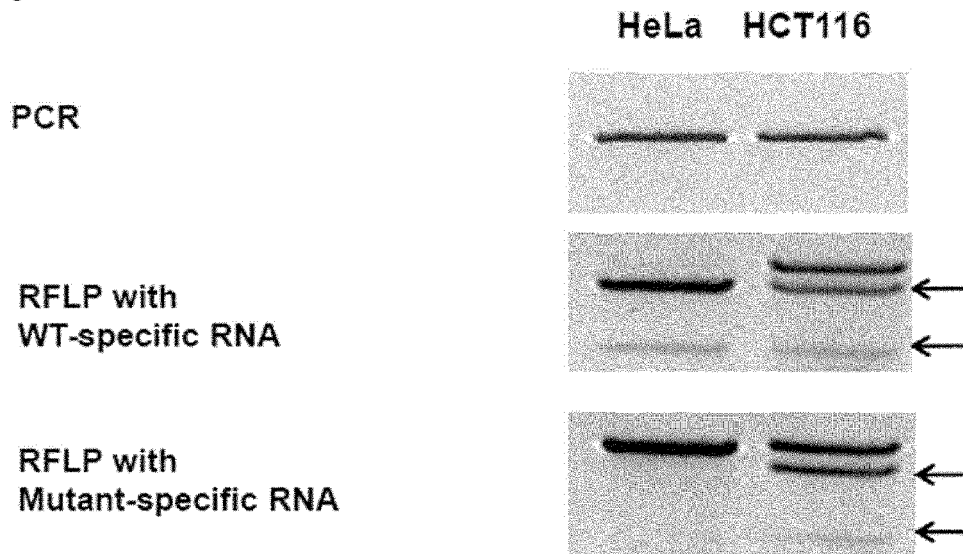
[Fig. 27]



[Fig. 28]



[Fig. 29a]

**HeLa**

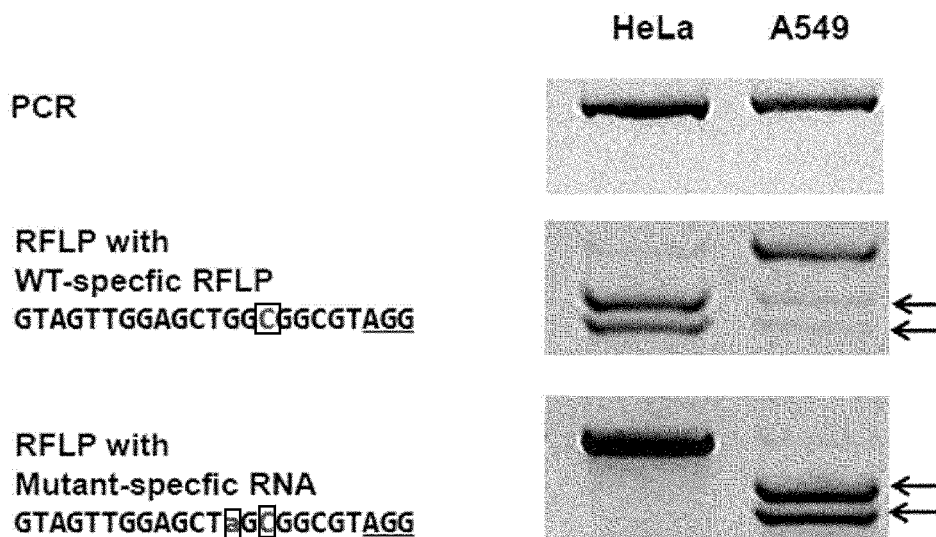
ACTACCACAGCTCCTTCTCTGAGTGG wild-type

HCT116

ACTACCACAGCTCCTTCTCTGAGTGG wild-type

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

[Fig. 29b]

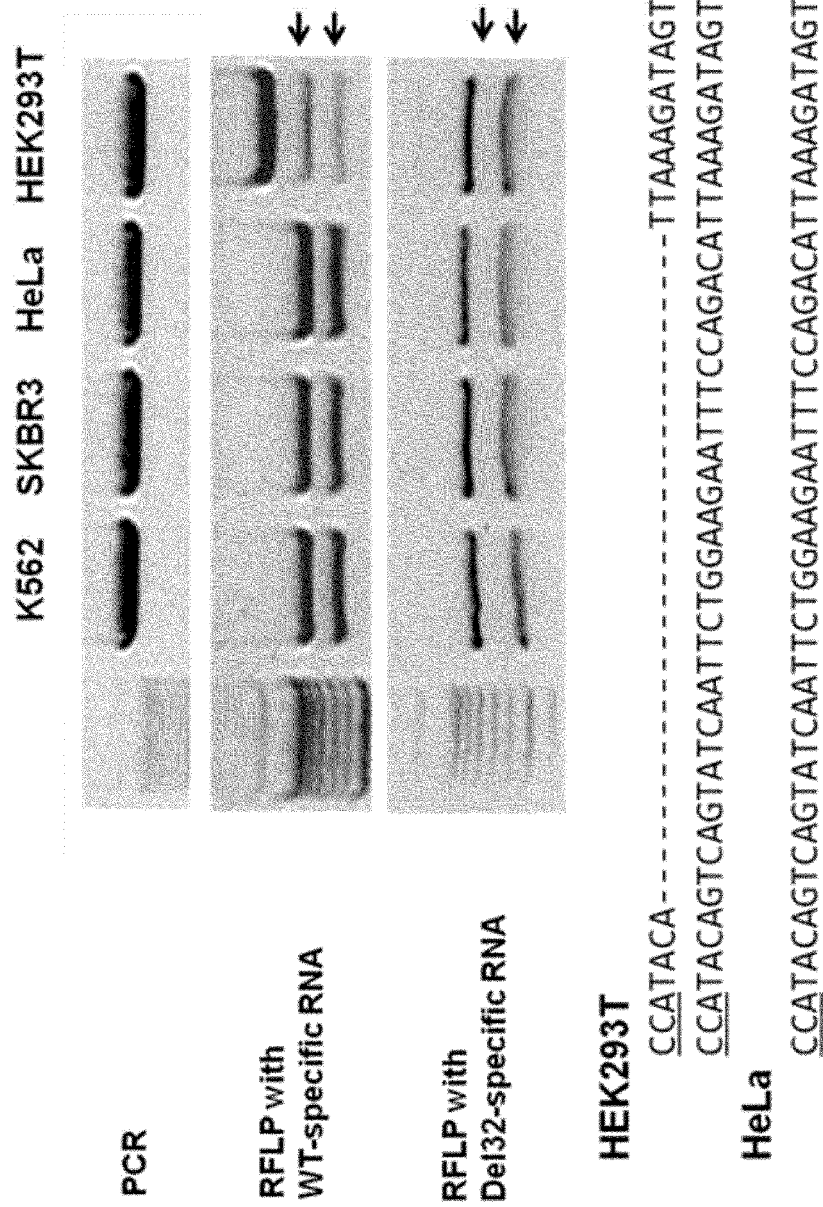
**HeLa**

GTAGTTGGAGCTGGTGGCGTAGG wild-type

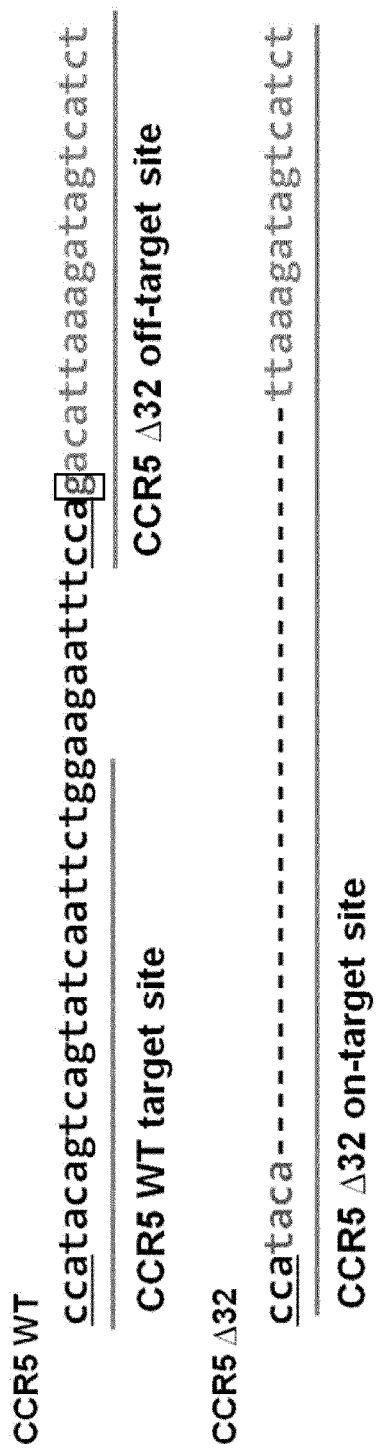
A549

GTAGTTGGAGCTGGTGGCGTAGG c.34G>A

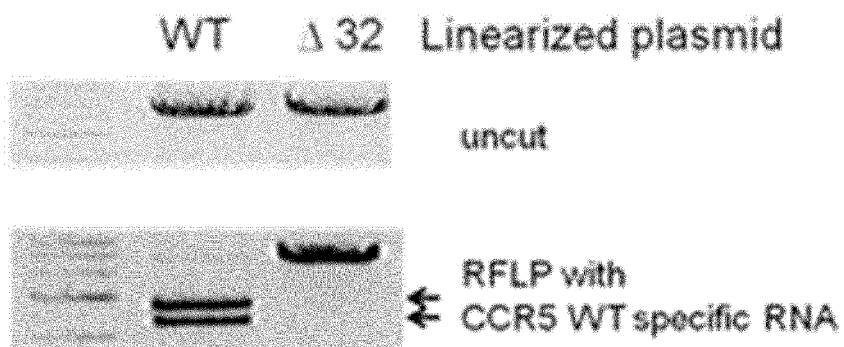
[Fig. 30a]



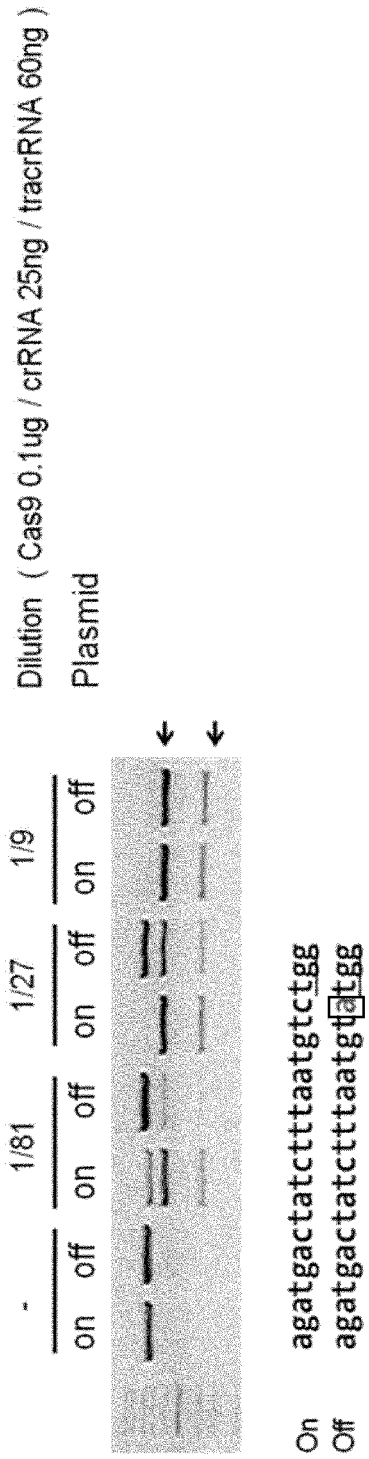
[Fig. 30b]



[Fig. 30c]



[Fig. 30d]



[Fig. 31a]

KRAS**HeLa**

GTAGTTGGAGCTGGTGGCGTAGG Wild-type

A549

GTAGTTGGAGCTaGTGGCGTAGG c.34G>A

RFLP with
WT-specific RNA
GTAGTTGGAGCTGGTGGCGTAGG

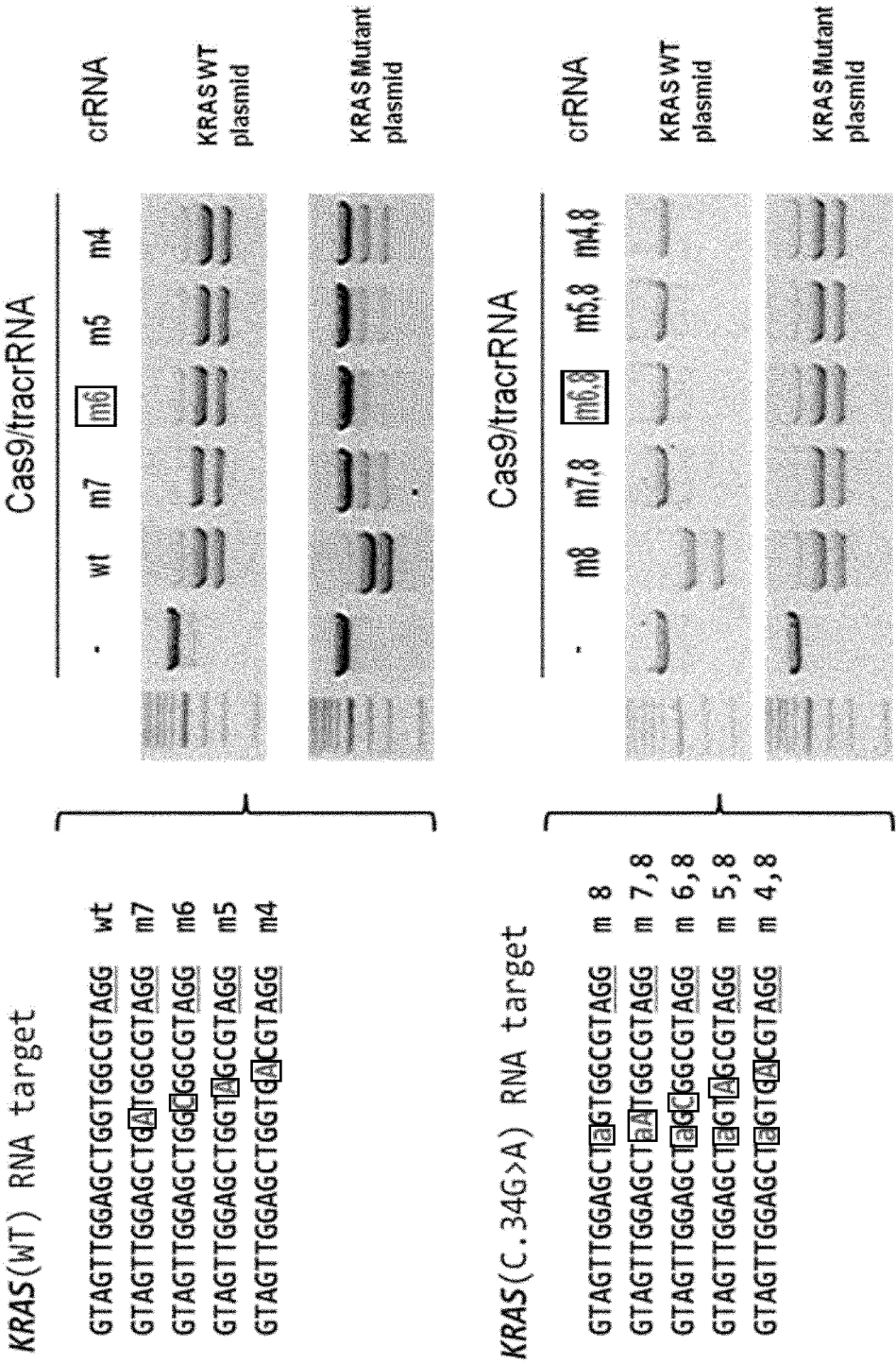
HeLa A549



RFLP with
Mutant-specific RNA
GTAGTTGGAGCTaGTGGCGTAGG



[Fig. 31b]



[Fig. 32a]

PIK3CA**HeLa**CAAATGAATGATGCACATCATGG Wild-type**HCT116**CAAATGAATGATGCACATCATGG Wild-typeCAAATGAATGATGCACGTCATGG C.3140A>G

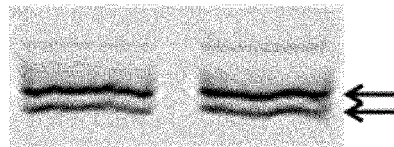
RFLP with

WT-specific RNA

CAAATGAATGATGCACATCATGG

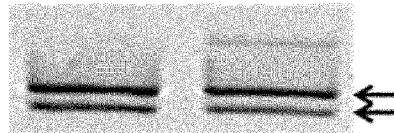
HeLa

HCT116

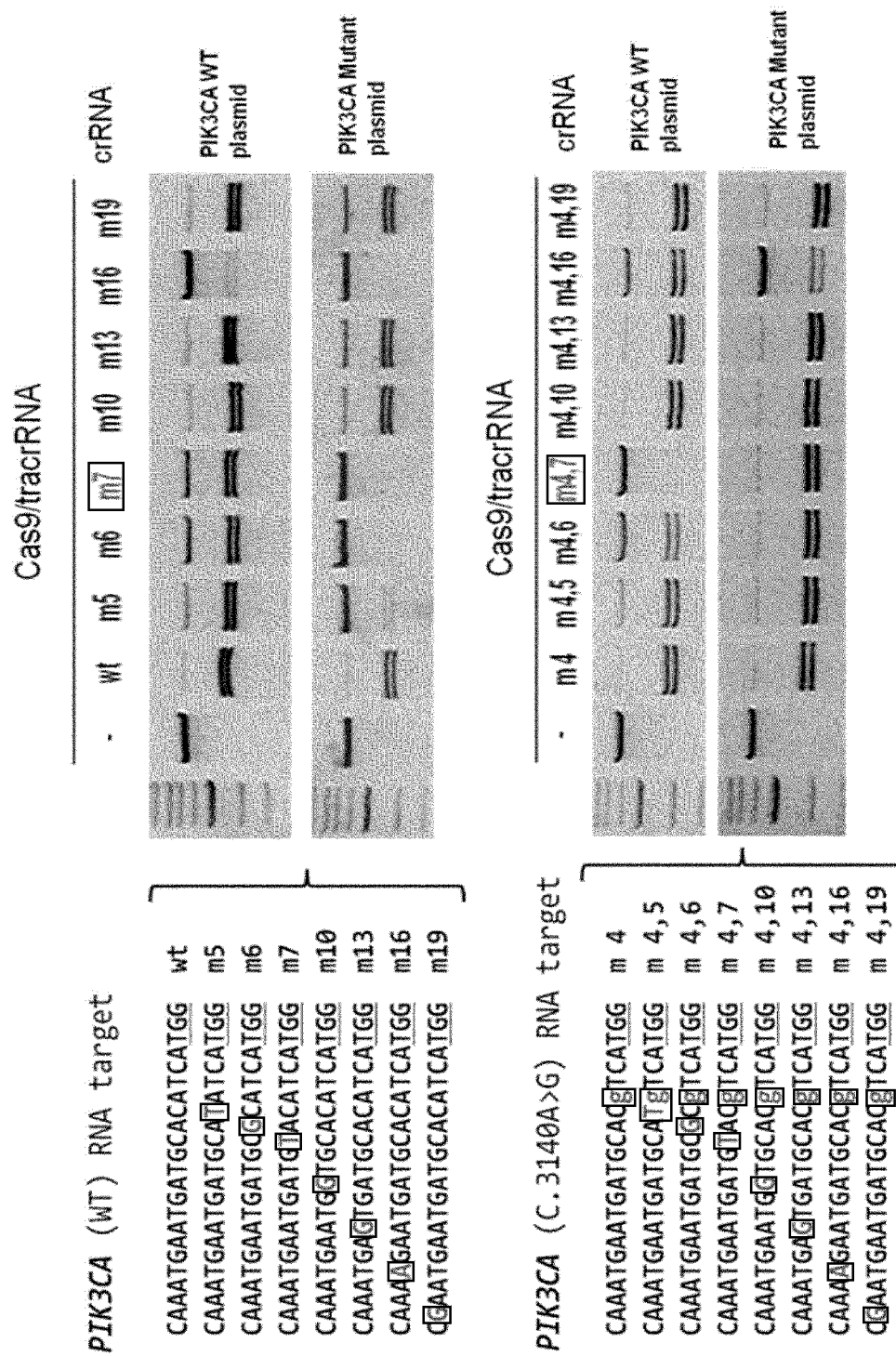


RFLP with

Mutant-specific RNA

CAAATGAATGATGCACGTCATGG

[Fig. 32b]



[Fig. 33a]

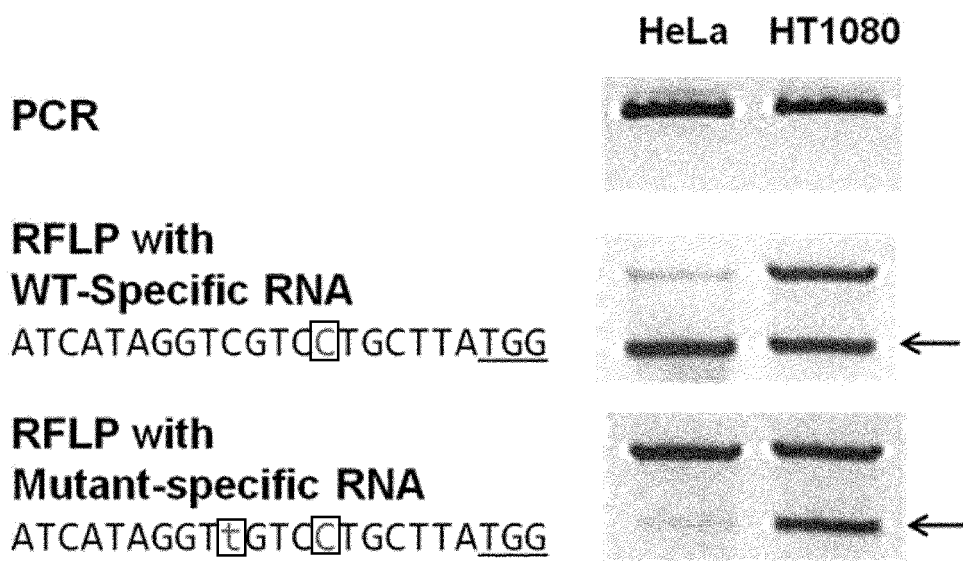
IDH1**HeLa**

ATCATAGGTCGTCATGCTTATGG Wild-type

HT1080

ATCATAGGTCGTCATGCTTATGG Wild-type

ATCATAGGTtGTCATGCTTATGG c.394C>T



[Fig. 33b]

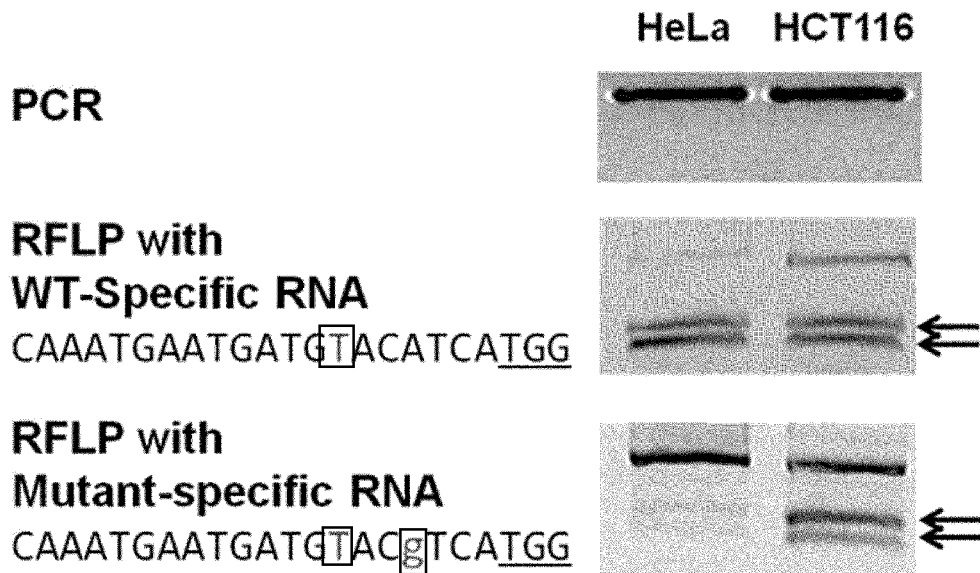
PIK3CA**HeLa**

CAAATGAATGATGCACATCATGG Wild-type

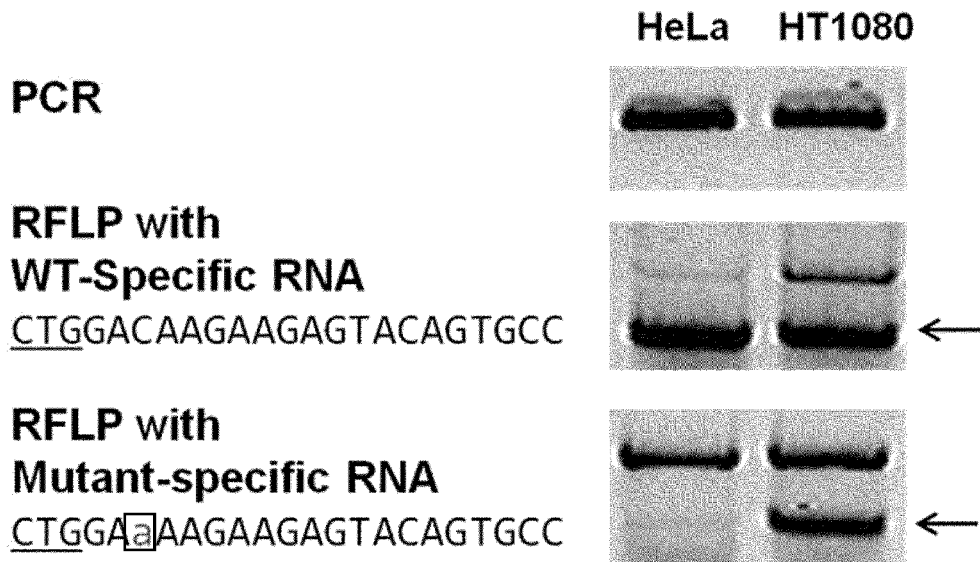
HCT116

CAAATGAATGATGCACATCATGG Wild-type

CAAATGAATGATGCACgTCATGG C.3140A>G



[Fig. 33c]

NRAS**HeLa**CTGGACAAGAAGAGTACAGTGCC Wild-type**HT1080**CTGGACAAGAAGAGTACAGTGCC Wild-typeCTGGGAaAAGAAGAGTACAGTGCC c.181C>A

[Fig. 33d]

BRAF**HeLa**ACTCCATCGAGATTTCACTGTAG Wild-type**HT29**ACTCCATCGAGATTTCACTGTAG Wild-typeACTCCATCGAGATTTCTTCTGTAG (c.1799T>A)