

DRAWINGS

AsCas12a_SEQ2	--FDYQKKLDNREKERVAAQAVSVVGTIKDLKQGYLSQVIHEIVDLMIHQAVVLENL	995
CmaCas12a_SEQ7	--YDYRKALDVREYDNKEARRNWTKEGIRKMEGYLSLAVSKLADMIENNNAIIVMEDL	935
EbCas12a_SEQ6	--YDYQIKLKQOEGARQTARKWEKEIKKEIKEGYLSLVHIEISKMVIKYNNAIIAMEDL	964
TsCas12a_SEQ10	HVNDYQQKLDKKEQERDAARKSWTTVENIKELKEGYLSHVHKLALHIIKYNNAIVCLDEL	997
FnCas12a_SEQ3	MKTNYHDKLAAIEKDRDSARKDWKINNIIKEMKEGYLSQVVHETIAKLVIENNAIIVFDEL	1008
FtCas12a_SEQ4	MKTNYHDKLAAIEKDRDSARKDWKINNIIKEMKEGYLSQVVHETIAKLVIENNAIIVFDEL	1008
MbCas12a_SEQ8	MTTPYHKIILDKREIERLNARVWGGEIETIKELKSGYLSHVHQQISQMLKYNNAIIVLEDL	1082
Mb2Cas12a_SEQ11	VTTPYHKIILDKREIERLNARVWGGEIETIKELKSGYLSHVHQQINQMLKYNNAIIVLEDL	960
LbCas12a_SEQ1	IKTDYHSLLDKKEKERFEARQNWTSIENIKELKAGYSQVVHKKICELVKDYDAVIALEDL	927
PcCas12a_SEQ5	--IDYHDLLESRDKDRQERRNWQTTIEGIELKQGYLSQAVHRIAEIMVAYKAVVALEDL	967
BsCas12a_SEQ9	IETNYHALLDREDDRNKARKDWNTIENIKELKTGYLSQVVNVVAKLVLYKYNNAIICLEDL	908
Lb5Cas12a_SEQ12	IETDYHALLDREGEGRDKARKDWNTVENIRDLKAGYLSQVVNVVAKLVLYKYNNAIICLEDL	908
	*: * : . * * : * * : * * : * * : * * : * * : * * : *	
AsCas12a_SEQ2	NFGFKSKRTGTAEKAVYQOQFEKMLIDKLNCLVLKDY---- PAEKVGGVLPYQLTDQFTS	1051
CmaCas12a_SEQ7	NHGFKAGRSKI-EKQVYQKFESMLINKLYMVVKDK----- SIDQSGGALHGQOLANHVTT	990
EbCas12a_SEQ6	SYGFKKGRFKV-ERQVYQKFETMLINKLYLVFKDI---- SITENGGLLKGYQLTYIPEK	1019
TsCas12a_SEQ10	NFGFKRGRFKV-EKQVYQKFELIDKLNLYLVFKEK---- ELGEVGHYLTAYQLTAPFES	1052
FnCas12a_SEQ3	NFGFKRGRFKV-EKQVYQKLEKMLIEKLNLYLVFKDN---- EFDKTGGVLRAYQLTAPFET	1063
FtCas12a_SEQ4	NFGFKRGRFKV-EKQVYQKLEKMLIEKLNLYLVFKDN---- EFDKTGGVLRAYQLTAPFET	1063
MbCas12a_SEQ8	NFGFKRGRFKV-EKQIYQNFENALIKLNHLVLKDK---- ADDEIGSYKNALQLTNNFTD	1137
Mb2Cas12a_SEQ11	NFGFKRGRFKV-EKQIYQNFENALIKLNHLVLKDK---- ADDEIGSYKNALQLTNNFTD	1015
LbCas12a_SEQ1	NSGFKNRVRKV-EKQVYQKFELIDKLNLYLVFKDK---- ADDEIGSYKNALQLTNNFTD	982
PcCas12a_SEQ5	NMGFKRGRQKV-ESSVYQOQFEKMLIDKLNLYLVFKDK---- NPENATGGALKGYQITNKFES	1022
BsCas12a_SEQ9	NFGFKRGRQKV-EKQVYQKFELIDKLNLYLVFKDK---- RPEDIGGLRAYQFTAPFES	967
Lb5Cas12a_SEQ12	NFGFKRGRQKV-EKQVYQKFELIDKLNLYLVFKDK---- SPRELGGALNALQLTSKFES	967
	. *** * : * : * : * : * : * : * : * : * : *	

Fig. 1

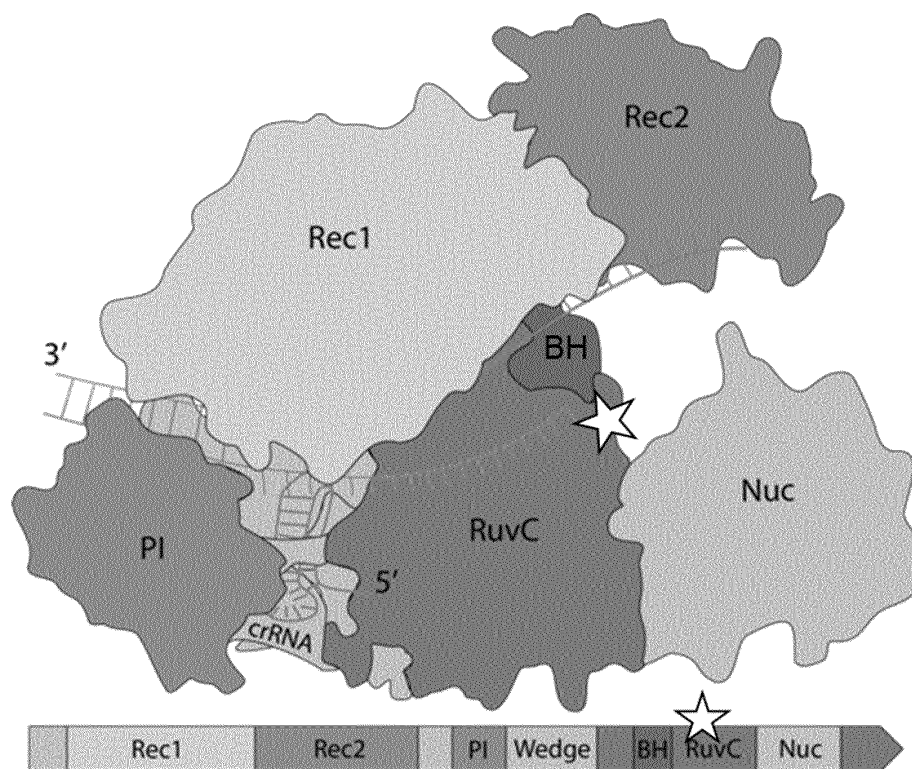


Fig. 2

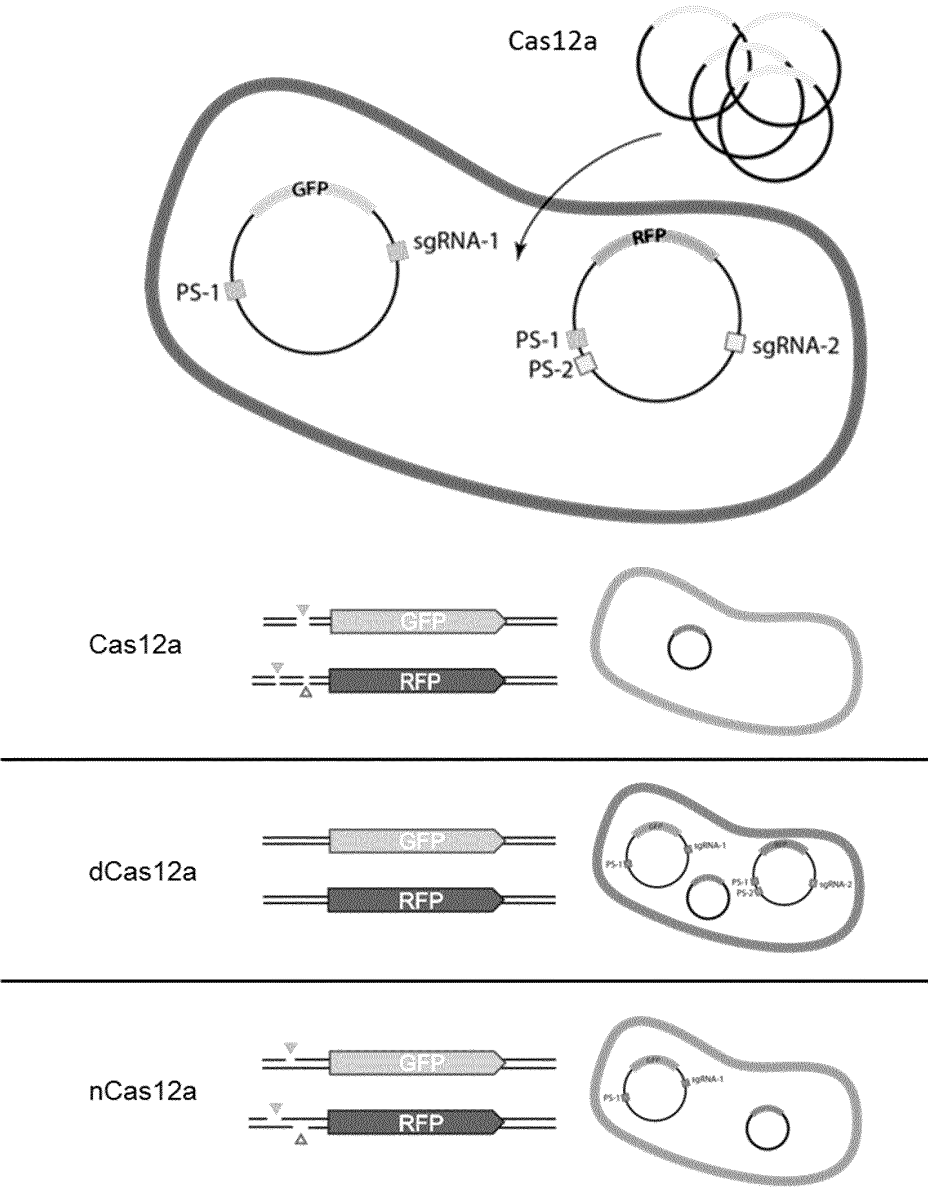


Fig. 3

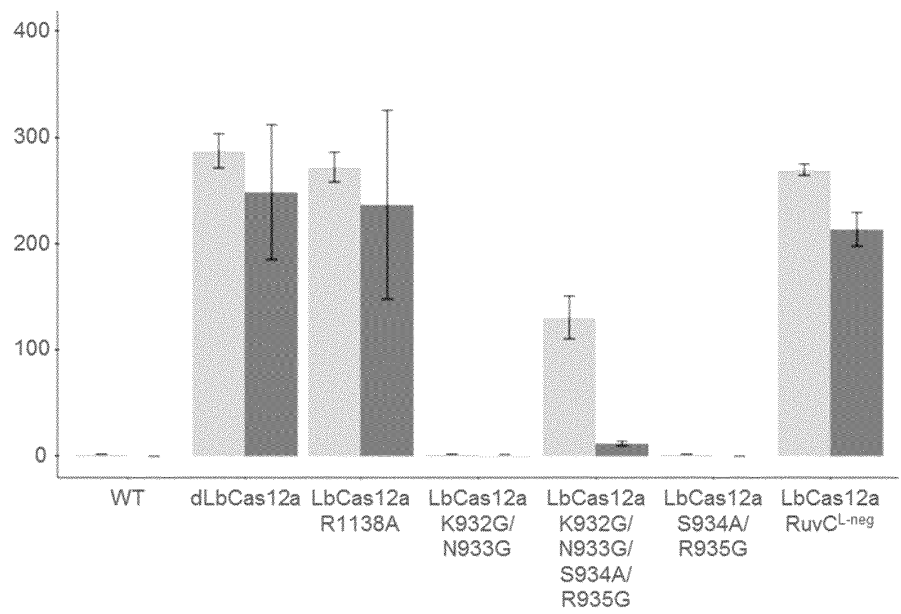


Fig. 4

928	930	932	934	936	938	940	942	944									
N	S	G	F	K	N	S	R	V	K	V	E	K	Q	V	Y	Q	LbCas12a WT
N	S	G	S	I	N	K	W	R	Y	P	E	A	Q	V	Y	Q	pRV26002
N	S	G	K	F	S	F	L	V	S	S	S	K	Q	V	Y	Q	pRV26004
N	S	G	I	I	M	L	K	H	A	S	G	C	Q	V	Y	Q	pRV26006
N	S	G	Y	W	L	H	R	Q	F	T	G	R	Q	V	Y	Q	pRV26008
N	S	G	F	K	N	K	W	R	Y	P	E	A	Q	V	Y	Q	pRV26010
N	S	G	F	K	N	F	L	V	S	S	S	K	Q	V	Y	Q	pRV26180
N	S	G	L	L	F	W	S	C	N	G	G	L	Q	V	Y	Q	pRV26182
N	S	G	F	K	N	W	S	C	N	G	G	L	Q	V	Y	Q	pRV26184

Fig. 5A

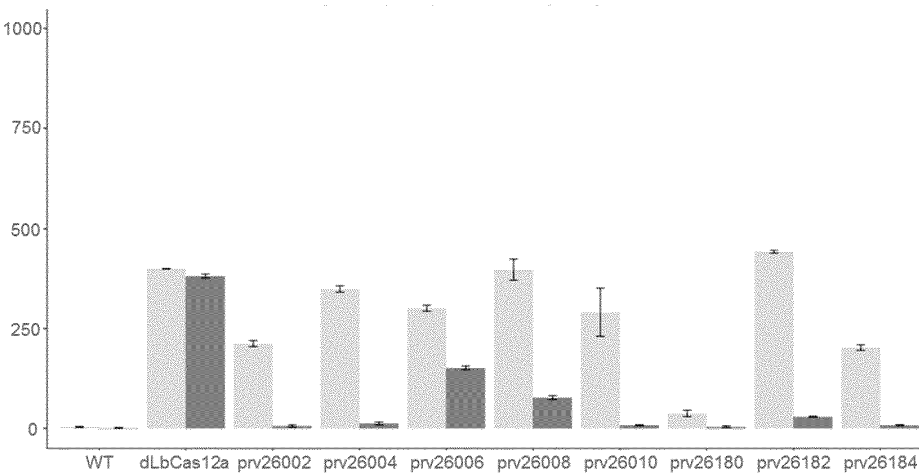


Fig. 5B

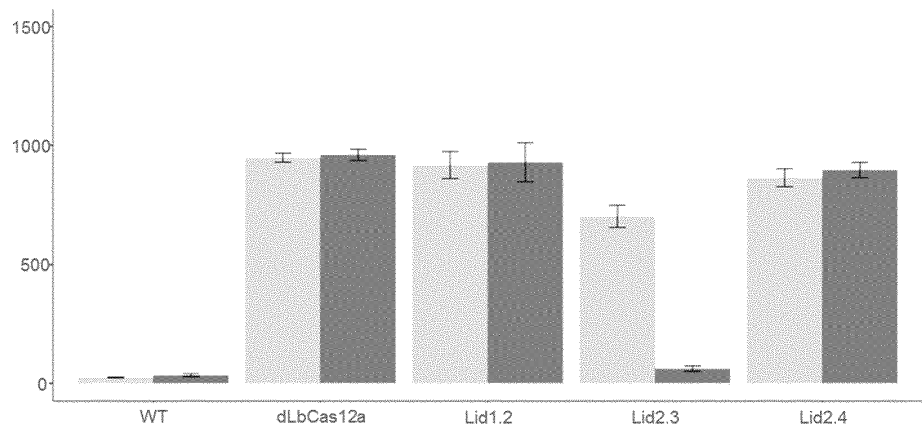


Fig. 5C

Variant	SEQ ID NO:	Sequence	Sequencing label	Abundance
Lid4.1	100	CLRR (SEQ ID NO: 107)	2213ZAA352	2
Lid4.2	101	YLGT SEQ ID NO: 108	2213ZAA353	2
Lid4.3	102	PITR SEQ ID NO: 109	2213ZAA354	3
Lid4.4	103	IFRR SEQ ID NO: 110	2213ZAA355	2
Lid4.5	104	PRSS SEQ ID NO: 111	2213ZAA356	4
Lid4.6	105	YIRR SEQ ID NO: 112	2213ZAA357	2
Lid4.7	106	TMFR SEQ ID NO: 113	2213ZAA358	2

Fig. 5D

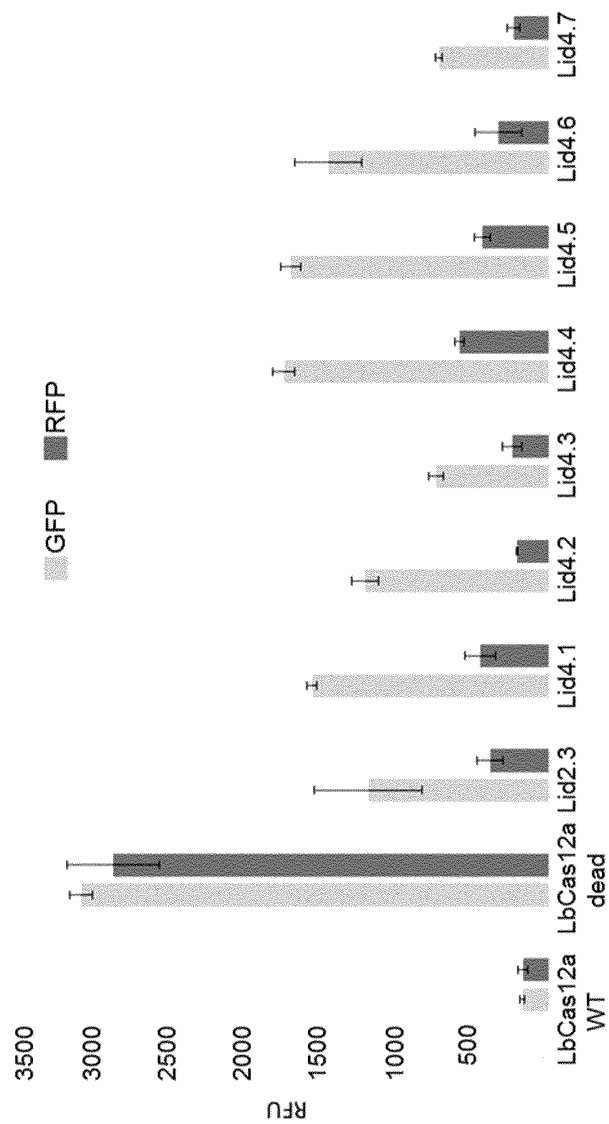


Fig. 5E

928930932934936938940942944

N S G F K N S R V K V E K Q V Y Q

N S G K F S F L V S S S K Q V Y Q

N S Y C S S V Y Q

LbCas12a WT

pRV26004

RuvC^{L-del1}

Fig. 6A

LbCas12a [100nM]	-	-	-	WT		dCas12a		pRV26004		RuvC ^{L-del1}	
crRNA [200nM]	-	-	-	+	+	+	+	+	+	+	+
pT [150fM]	+	+	+	-	+	-	+	-	+	-	+
pUC19 [150fM]	-	-	-	+	-	+	-	+	-	+	-
EcoRI	-	+	-	-	-	-	-	-	-	-	-
Nb.BbvCI	-	-	+	-	-	-	-	-	-	-	-

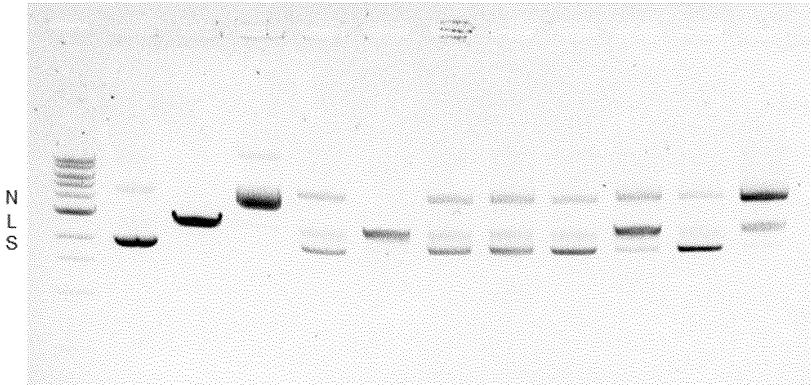


Fig. 6B

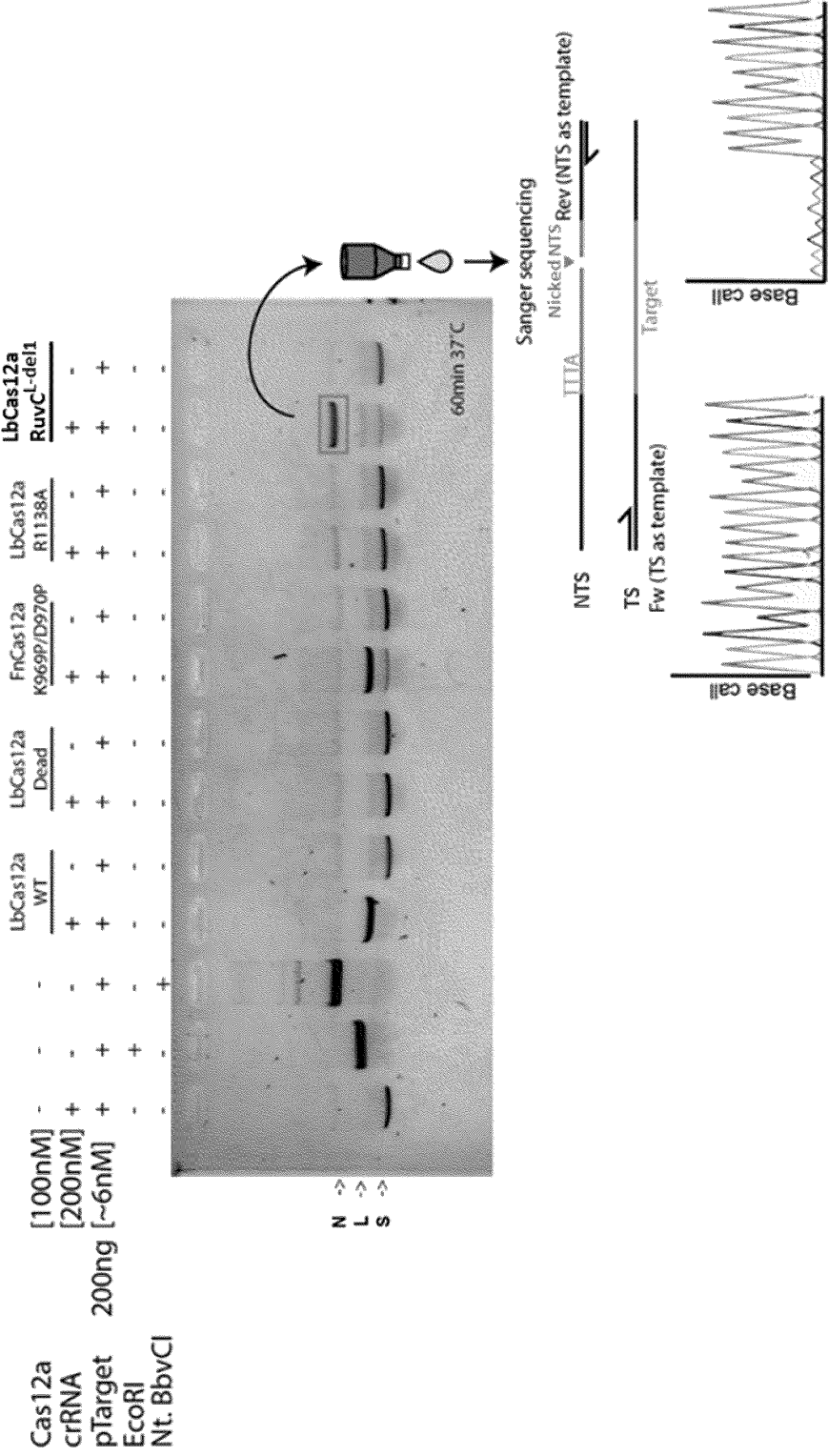


Fig. 6C

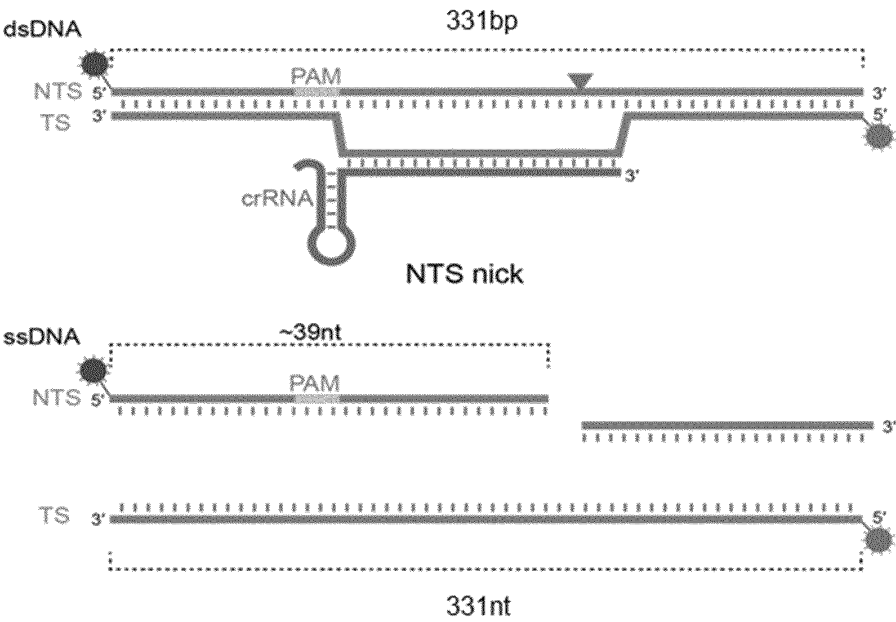


Fig. 6D

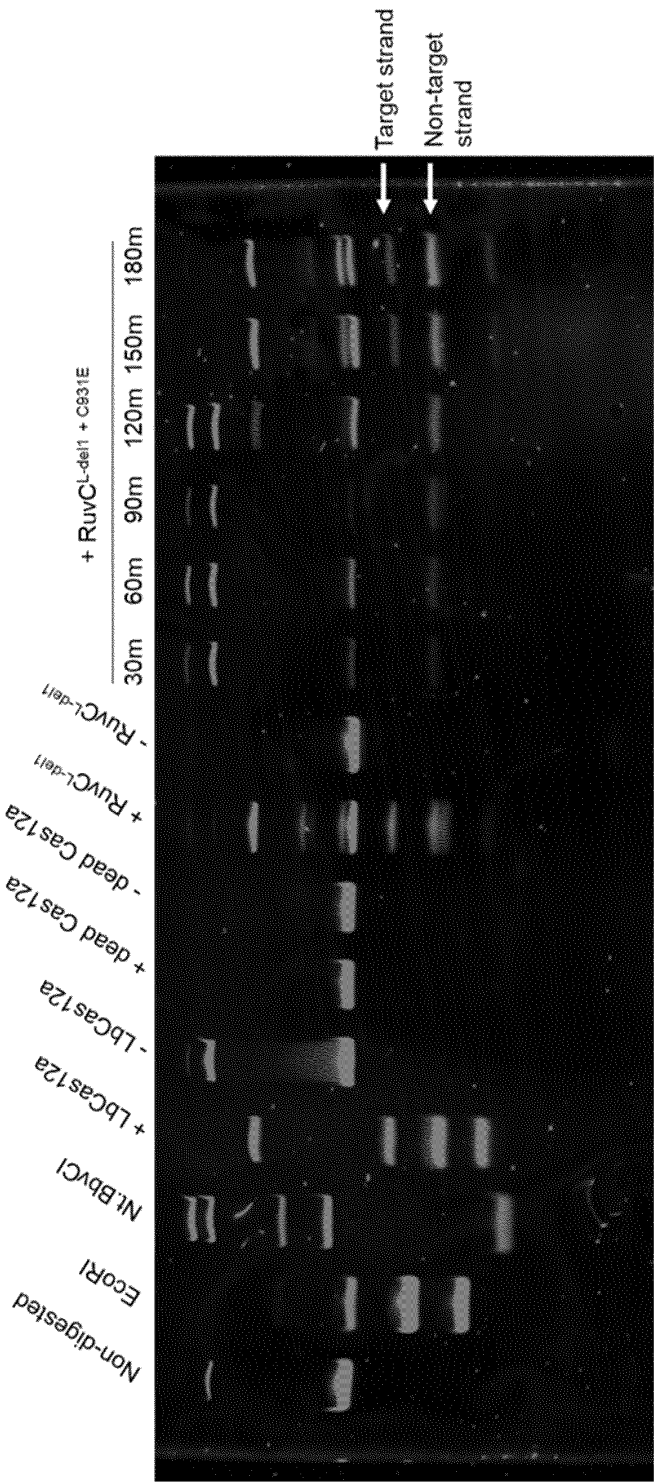


Fig. 6E

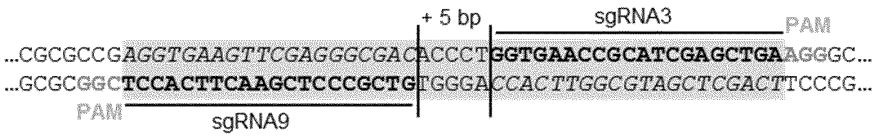


Fig. 7A

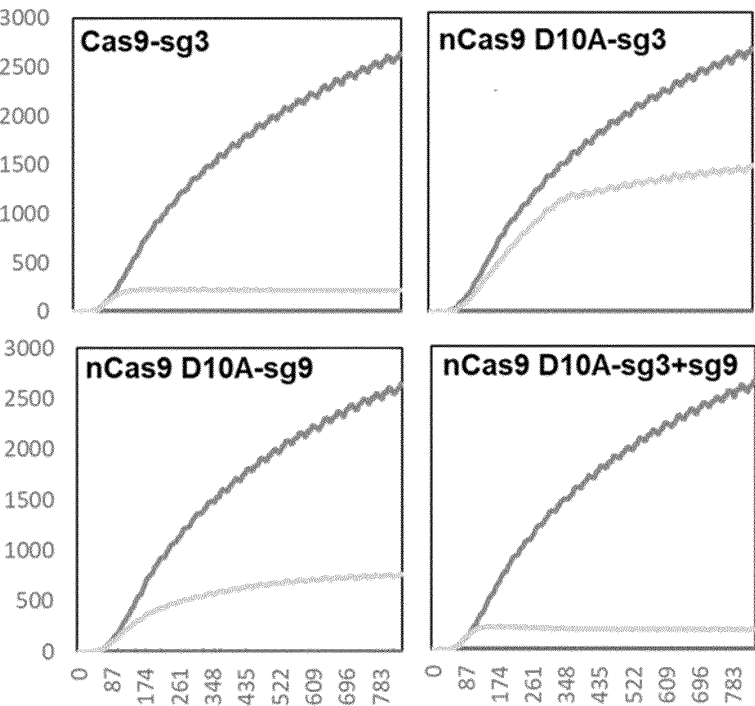


Fig. 7B

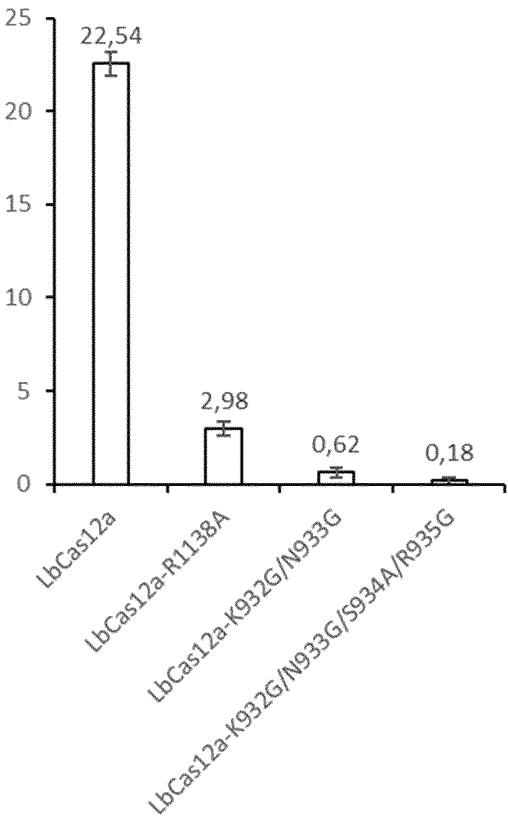


Fig. 8A

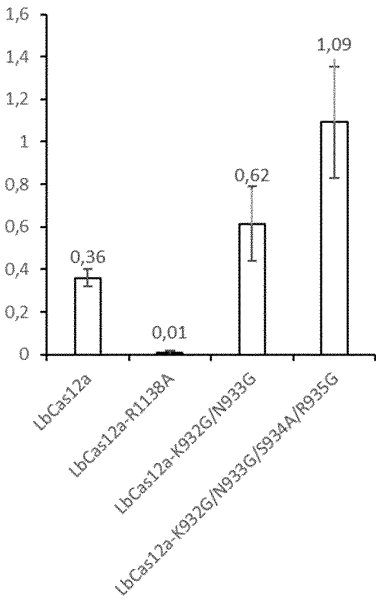


Fig. 8B

LbCas12a	I	II	III
WT	100.00	98.43	1.57
R1138A	13.21	99.44	0.56
K932G/N933G	2.73	49.09	50.91
K932G/N933G/S934A/R935G	0.78	13.81	86.19

Fig. 8C

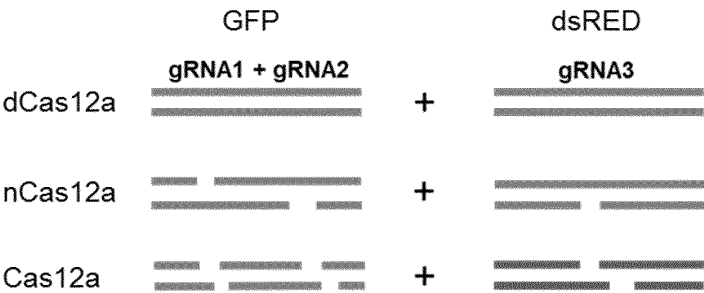


Fig. 9A

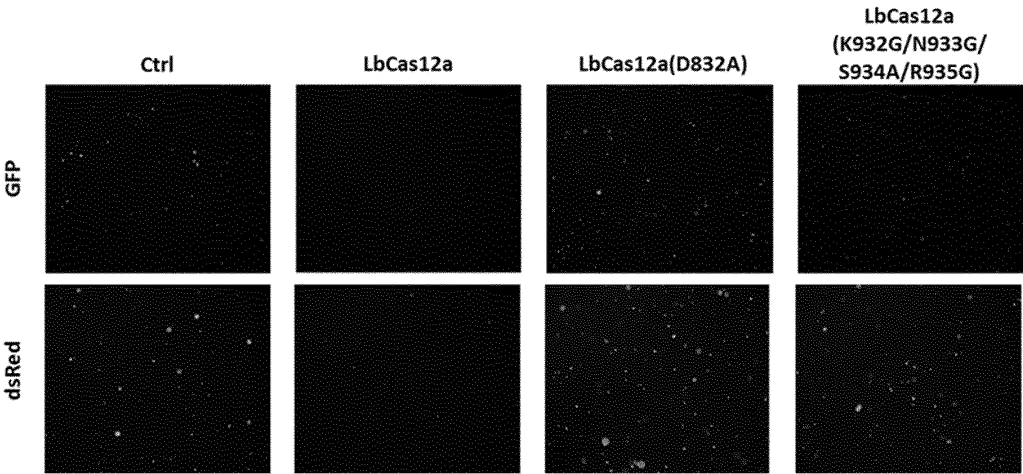


Fig. 9B

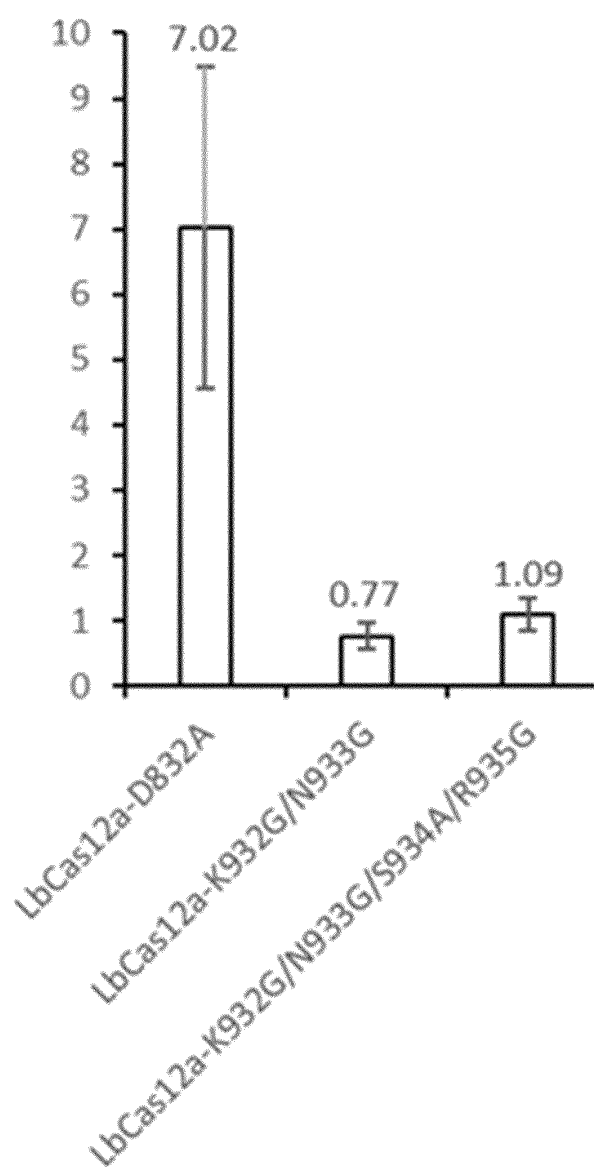


Fig. 10A

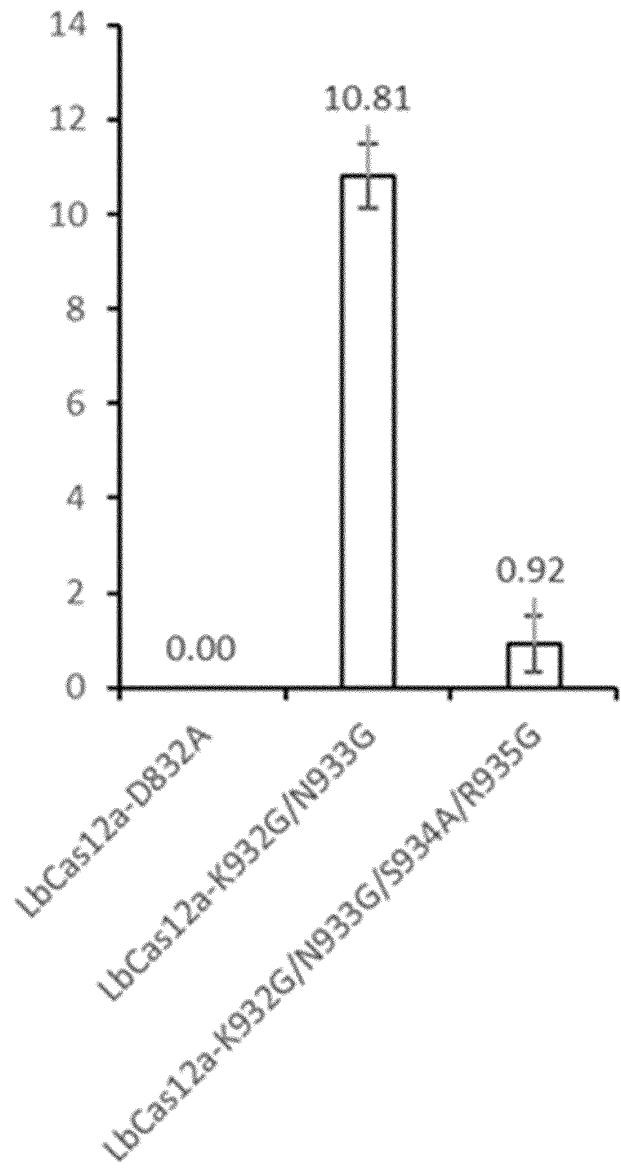


Fig. 10B

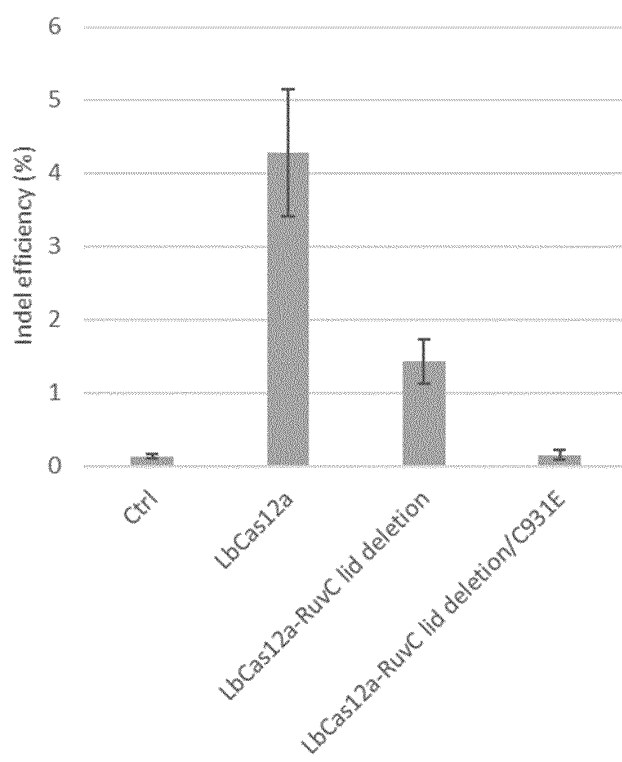


Fig. 11

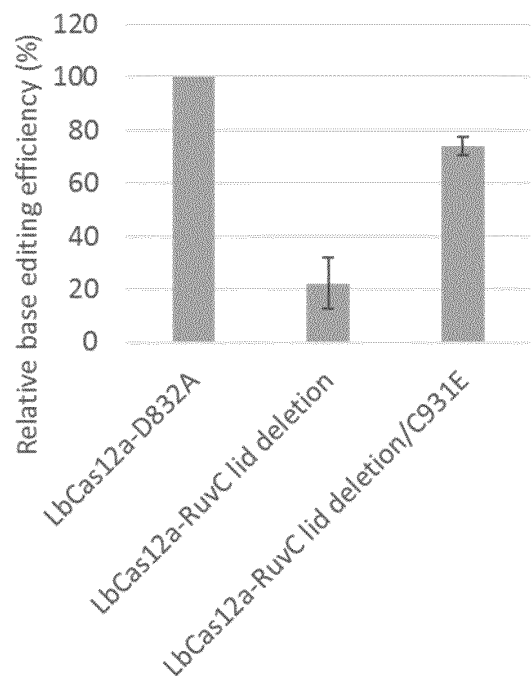


Fig. 12A

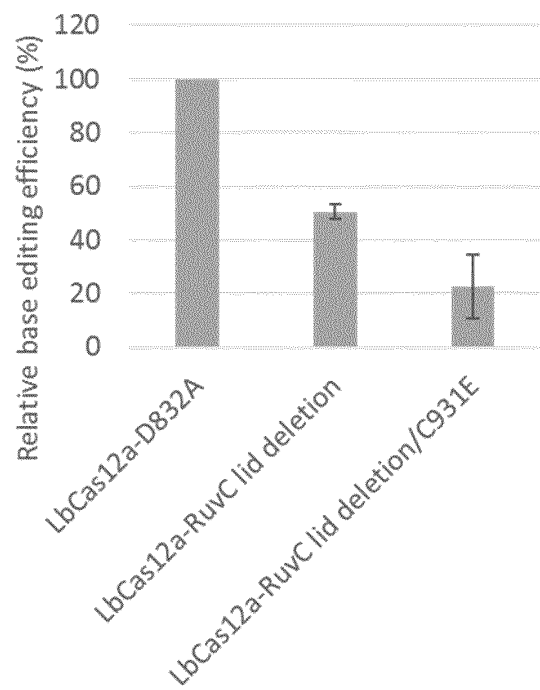


Fig. 12B

gRNA pair		Overhang type	Guide offset (bp)	Indel efficiency (% of sequencing reads)
cr-DEP1-G14	cr-DEP1-G3	5' overhang	-95	0
cr-DEP1-G17	cr-DEP1-G3	5' overhang	-54	1.49
cr-DEP1-G17	cr-DEP1-G16	5' overhang	-27	0.22
cr-DEP1-G6	cr-DEP1-G7	5' overhang	-6	0.18
cr-DEP1-G15	cr-DEP1-G7	5' overhang	6	0.17
cr-DEP1-G8	cr-DEP1-G7	3' overhang	62	0.02
cr-DEP1-G4	cr-DEP1-G16	3' overhang	38	0
cr-DEP1-G4	cr-DEP1-G3	3' overhang	11	0

Fig. 13

Guide	Indel efficiency (% of sequencing reads)			
	LbCas12a	LbCas12a-K932G/N933G/S934A/R935G	LbCas12a-RuvC ^{L-del1}	LbCas12a-RuvC ^{L-del1} /C931E
cr-DEP1-G17	3.840	0.037	0.860	0.107
cr-DEP1-G3	3.480	0.000	0.057	0.000
cr-DEP1-G17 + cr-DEP1-G3	3.609	0.645	1.278	0.915

Fig. 14

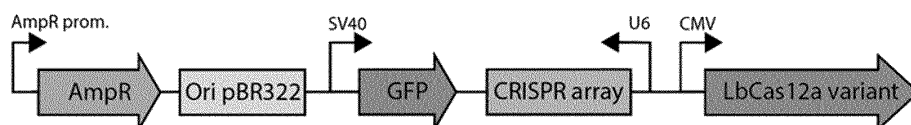


Fig. 15