Fig. 1

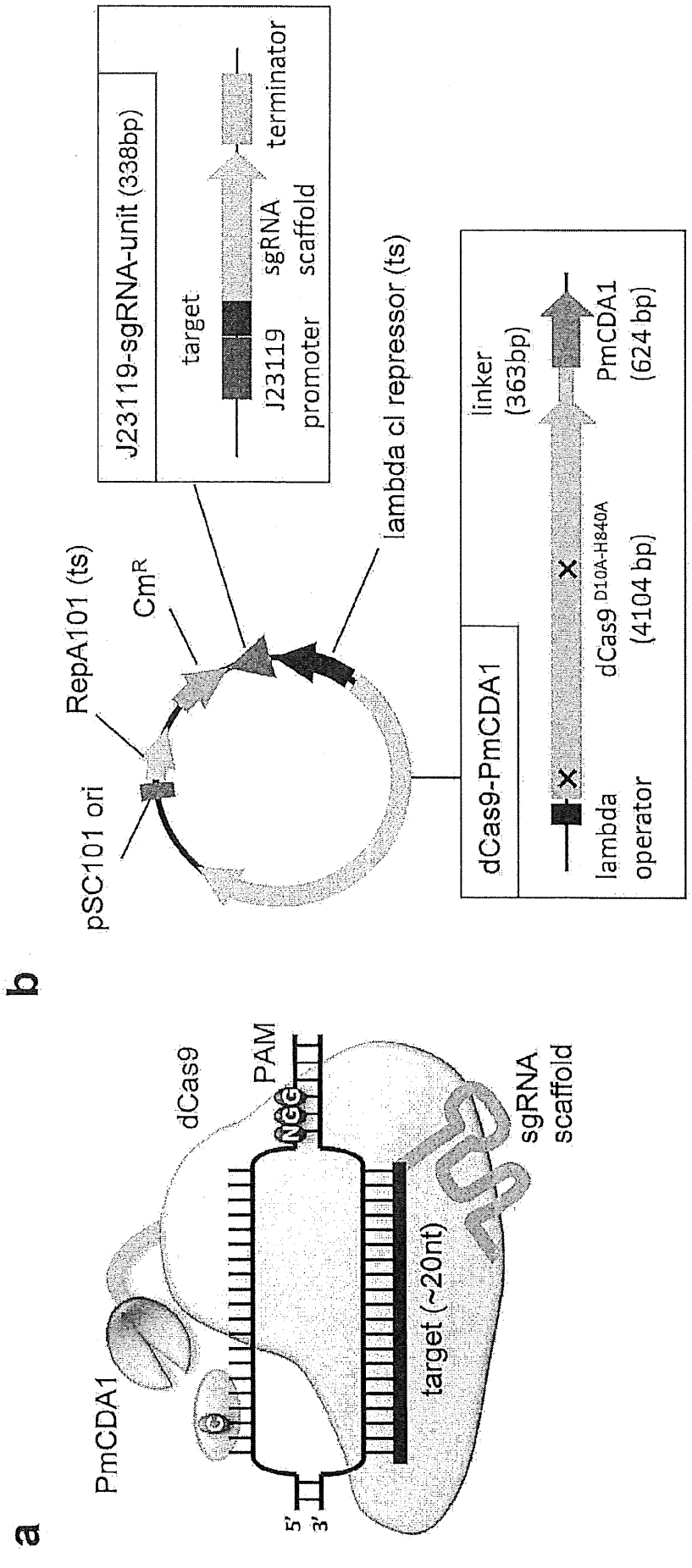

a
PmCDA1
dCas9
PAM
NGG
sgRNA
scaffold
target (~20nt)
5'
3'
b
pSC101 ori
RepA101 (ts)
Cm^R
lambda cI repressor (ts)
J23119-sgRNA-unit (338bp)
target
J23119
promoter
sgRNA
scaffold
terminator
dCas9-PmCDA1
linker
(363bp)
lambda
operator
dCas9 D10A-H840A
(4104 bp)
PmCDA1
(624 bp)

Fig. 2

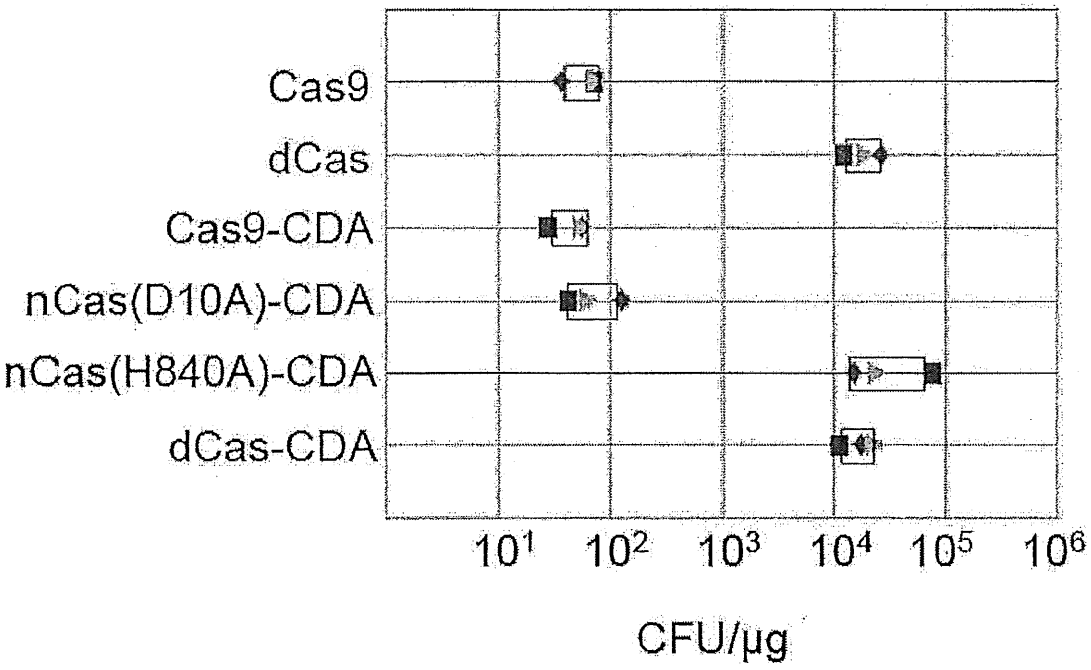

Cas9
dCas
Cas9-CDA
nCas(D10A)-CDA
nCas(H840A)-CDA
dCas-CDA
10^1
10^2
10^3
10^4
10^5
10^6
CFU/μg

Fig. 3

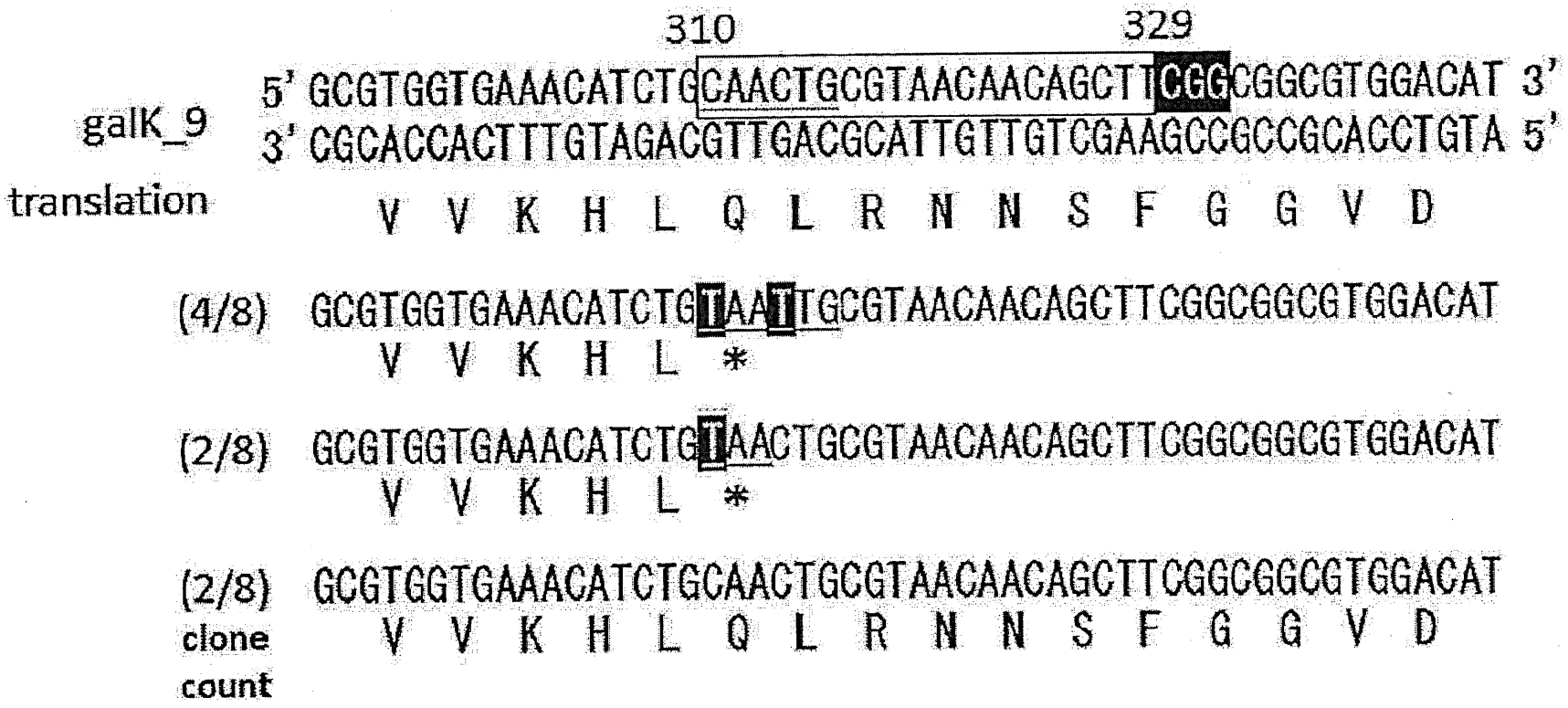

310
329
galK_9
5' GCGTGGTGAAACATCTGCAACTGCGTAACAACAGCTTCGGCGGCGTGGACAT 3'
3' CGCACCACTTTGTAGACGTTGACGCATTGTTGTCGAAGCCGCCGCACCTGTA 5'
translation
V V K H L Q L R N N S F G G V D
(4/8) GCGTGGTGAAACATCTGTAATTGCGTAACAACAGCTTCGGCGGCGTGGACAT
V V K H L *
(2/8) GCGTGGTGAAACATCTGTAACTGCGTAACAACAGCTTCGGCGGCGTGGACAT
V V K H L *
(2/8) GCGTGGTGAAACATCTGCAACTGCGTAACAACAGCTTCGGCGGCGTGGACAT
clone count
V V K H L Q L R N N S F G G V D

Fig. 4

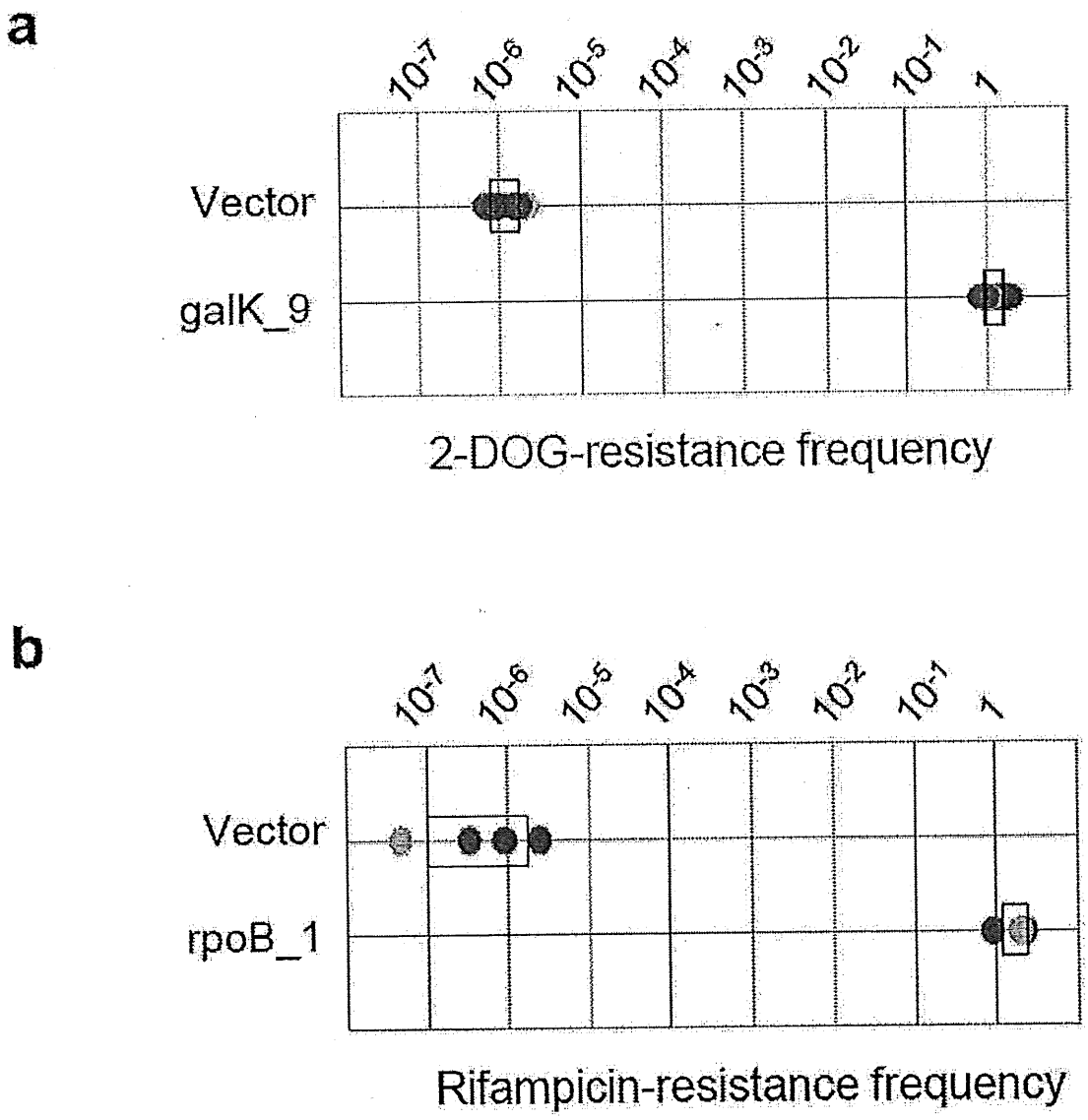
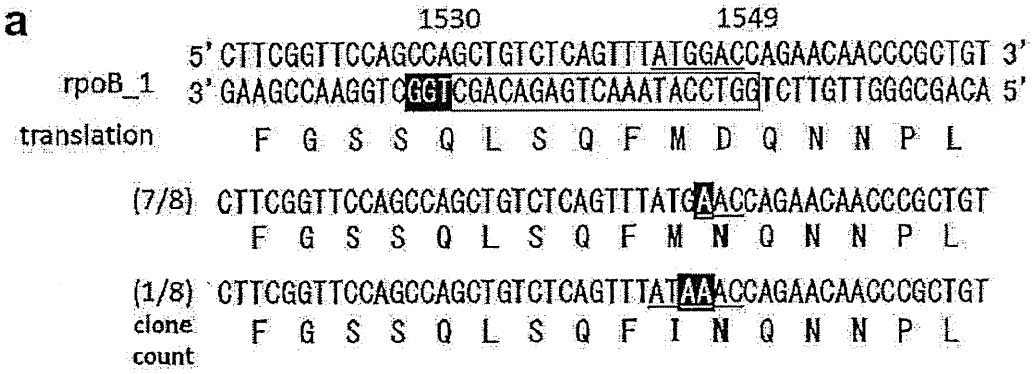

a
10-7
10-6
10-5
10-4
10-3
10-2
10-1
1
Vector
galK_9
2-DOG-resistance frequency
b
10-7
10-6
10-5
10-4
10-3
10-2
10-1
1
Vector
rpoB_1
Rifampicin-resistance frequency

Fig. 5

a



b

Strain	Clone	Sequence coverage	Parental/ variable mutation	Detected mutation				
				count	region	gene	reference	allele
dCas-CDA /rpoB_1	1	27.9	2	2	1591287 4172717	*yneO* *rpoB*	C GG	T AA
	2	22.1	3	3	2170816 4056442 4172717	*gatC* *yihN* *rpoB*	G C GG	A A AA
	3	51.1	1	1	4172717	*rpoB*	GG	AA

c

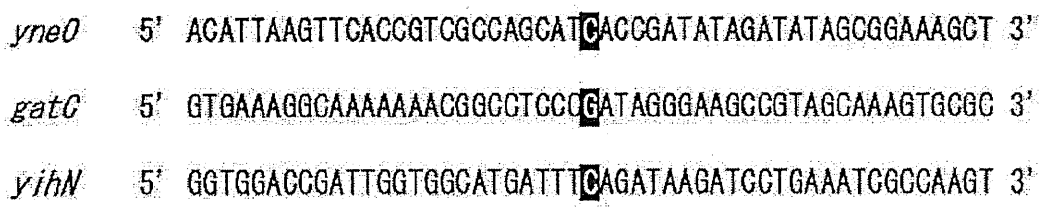


Fig. 6

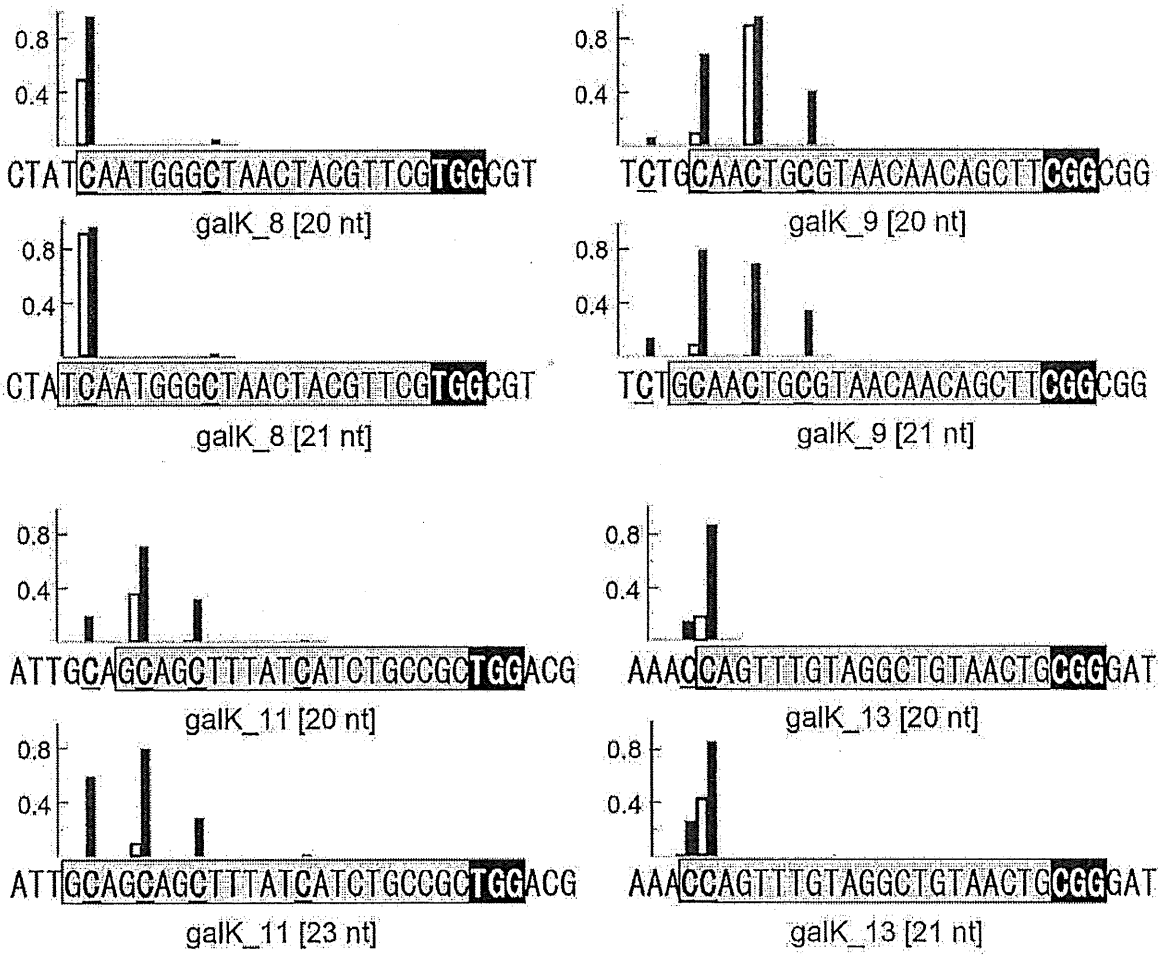

0.8
0.4
CTATCAATGGGCTAACTACGTTCGTGGCGT
galK_8 [20 nt]
0.8
0.4
CTATCAATGGGCTAACTACGTTCGTGGCGT
galK_8 [21 nt]
0.8
0.4
TCTGCAACTGCGTAACAACAGCTTCGGCGG
galK_9 [20 nt]
0.8
0.4
TCTGCAACTGCGTAACAACAGCTTCGGCGG
galK_9 [21 nt]
0.8
0.4
ATTGCAGCAGCTTTATCATCTGCCGCTGGACG
galK_11 [20 nt]
0.8
0.4
ATTGCAGCAGCTTTATCATCTGCCGCTGGACG
galK_11 [23 nt]
0.8
0.4
AAACCAGTTTGTAGGCTGTAACTGCGGGAT
galK_13 [20 nt]
0.8
0.4
AAACCAGTTTGTAGGCTGTAACTGCGGGAT
galK_13 [21 nt]

Fig. 7

Name	Position	Strand	GC contents	Missense or Nonsense	Mutation frequency (%)	Sequence (5'-> 3')	PAM
galK_1	25-44	+	9/20	M	71.8	TCTCTGTTTGCCAACGCATT	TGG
galK_2	51-70	+	12/20	M	90.4	CCCTGCCACTCACACCATTC	AGG
galK_3	58-77	+	12/20	M	93.9	ACTCACACCATTCAGGCGCC	TGG
galK_4	76-95	+	11/20	M	13.9	CCTGGCCGCGTGAATTTGAT	TGG
galK_5	186-205	-	10/20	M	1.4	CTGCCATCACGCGAACTTTA	CGG
galK_6	239-258	-	12/20	M	95.1	TGCGACAATGGGCGCATCGA	GGG
galK_7	254-273	+	7/20	M	22.0	TCGCACATGAAAACTATCAA	TGG
galK_8 [20 nt]	271-290	+	10/20	N	49.0	CAATGGGCTAACTACGTTCG	TGG
galK_8 [21 nt]	270-290	+	10/21	N	91.8	TCAATGGGCTAACTACGTTCG	TGG
galK_9 [20 nt]	310-329	+	9/20	N, M	90.4	CAACTGCGTAACAACAGCTT	CGG
galK_9 [21 nt]	309-329	+	10/21	N, M	1.1	GCAACTGCGTAACAACAGCTT	CGG
galK_10	392-411	-	12/20	M	2.8	GACCGCGACTTCCAGTGAAG	CGG
galK_11 [20 nt]	426-445	+	11/20	N, M	35.5	GCAGCTTTATCATCTGCCGC	TGG
galK_11 [23 nt]	423-445	+	13/23	N, M	9.2	GCAGCAGCTTTATCATCTGCCGC	TGG
galK_12	456-475	+	10/20	N	3.2	ACAAATCGCGCTTAACGGTC	AGG
galK_13 [20 nt]	490-509	+	9/20	N	17.2	CAGTTTGTAGGCTGTAACTG	CGG
galK_13 [21 nt]	489-509	+	10/21	N	42.9	CCAGTTTGTAGGCTGTAACTG	CGG
galK_14	520-539	+	10/20	N	61.7	GATCAGCTAATTTCCGCGCT	CGG

Fig. 8

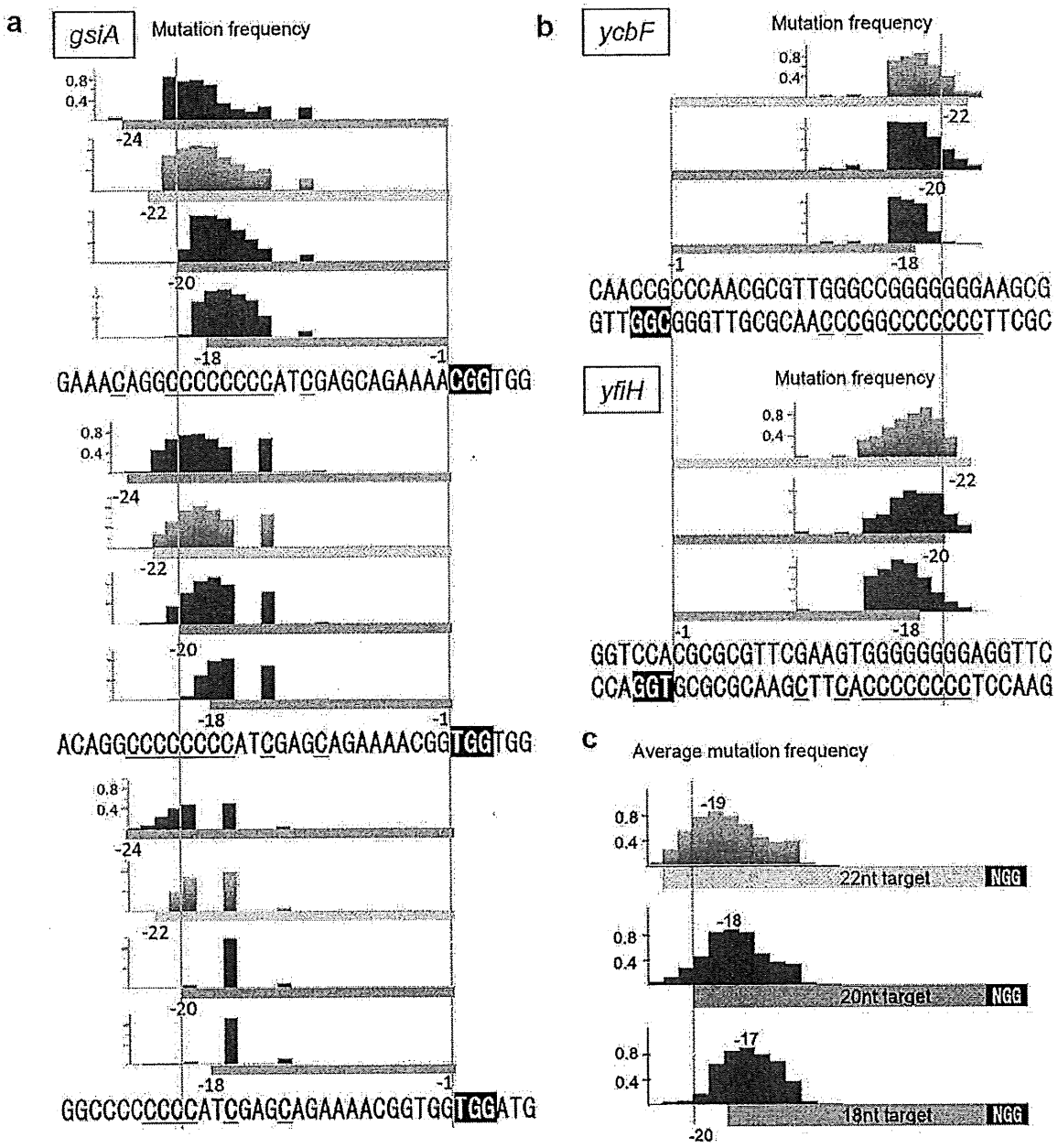
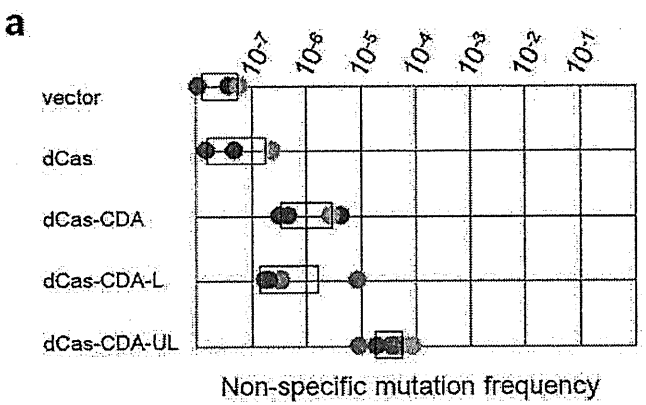

a
gsiA
Mutation frequency
0.8
0.4
-24
-22
-20
-18
-1
GAAACAGGCCCCCCCCATCGAGCAGAAAACGGTGG
ACAGGCCCCCCCCATCGAGCAGAAAACGGTGGTGG
GGCCCCCCCCATCGAGCAGAAAACGGTGGTGGATG
b
ycbF
Mutation frequency
CAACCGCCCAACGCGTTGGGCCGGGGGGGGAAGCG
GTTGGCGGGTTGCGCAACCCGGCCCCCCCTTCGC
yfiH
Mutation frequency
GGTCCACGCGCGTTCGAAGTGGGGGGGGAGGTTC
CCAGGTGCGCGCAAGCTTCACCCCCCCCTCCAAG
c
Average mutation frequency
-19
22nt target
NGG
-18
20nt target
NGG
-17
18nt target
NGG

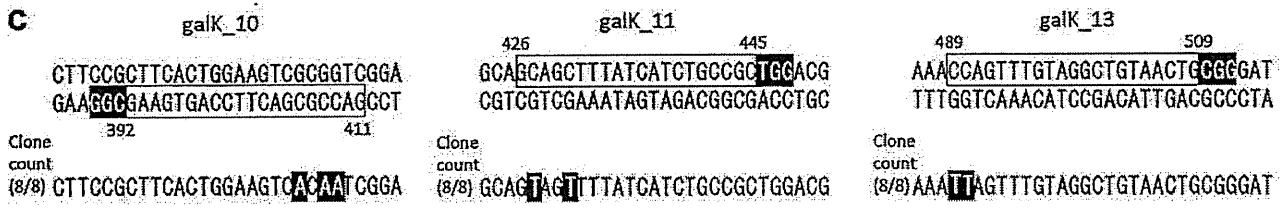
Fig. 9



b

Clone count of on-target mutation

	Single mutation	Double mutations	Triple mutations
dCas-CDA	1/8		
dCas-CDA-L	2/8		
dCas-CDA-UL		2/8	5/8



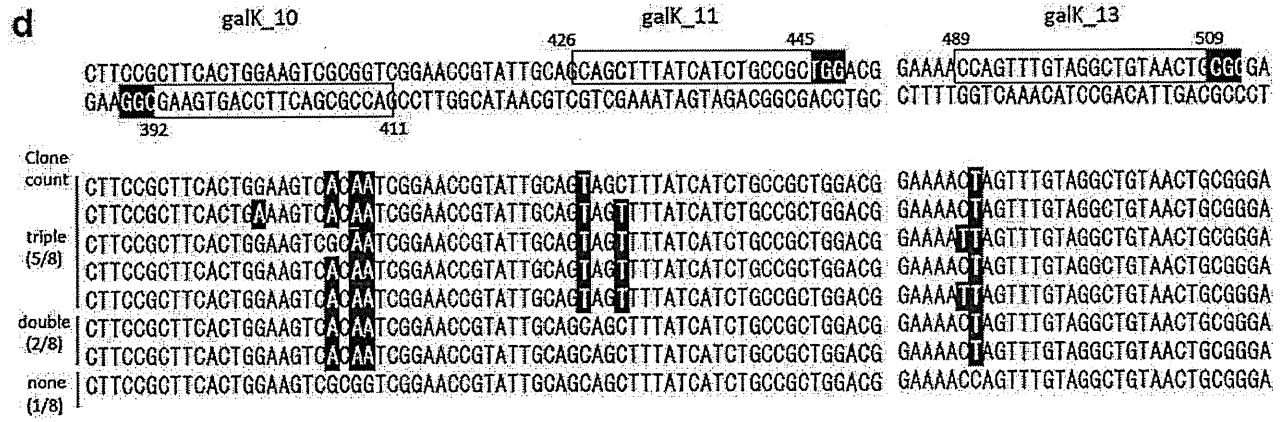


Fig. 10

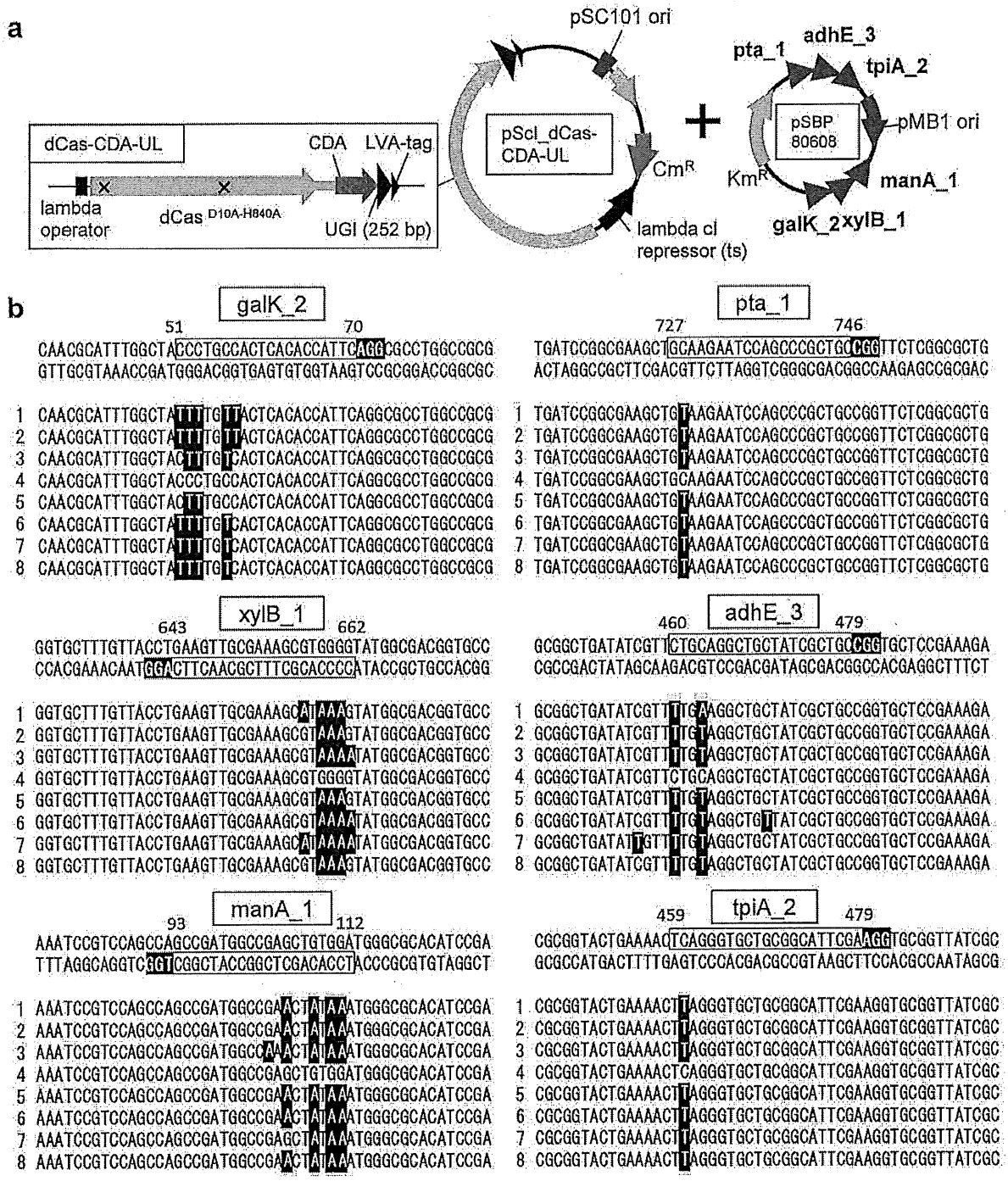

a
dCas-CDA-UL
CDA
LVA-tag
lambda operator
dCas D10A-H840A
UGI (252 bp)
pSC101 ori
pScI_dCas-CDA-UL
Cm^R
lambda cI repressor (ts)
pta_1
adhE_3
tpiA_2
pSBP 80608
pMB1 ori
Km^R
manA_1
galK_2
xylB_1
b
galK_2
51
70
pta_1
727
746
xylB_1
643
662
adhE_3
460
479
manA_1
93
112
tpiA_2
459
479

Fig. 11

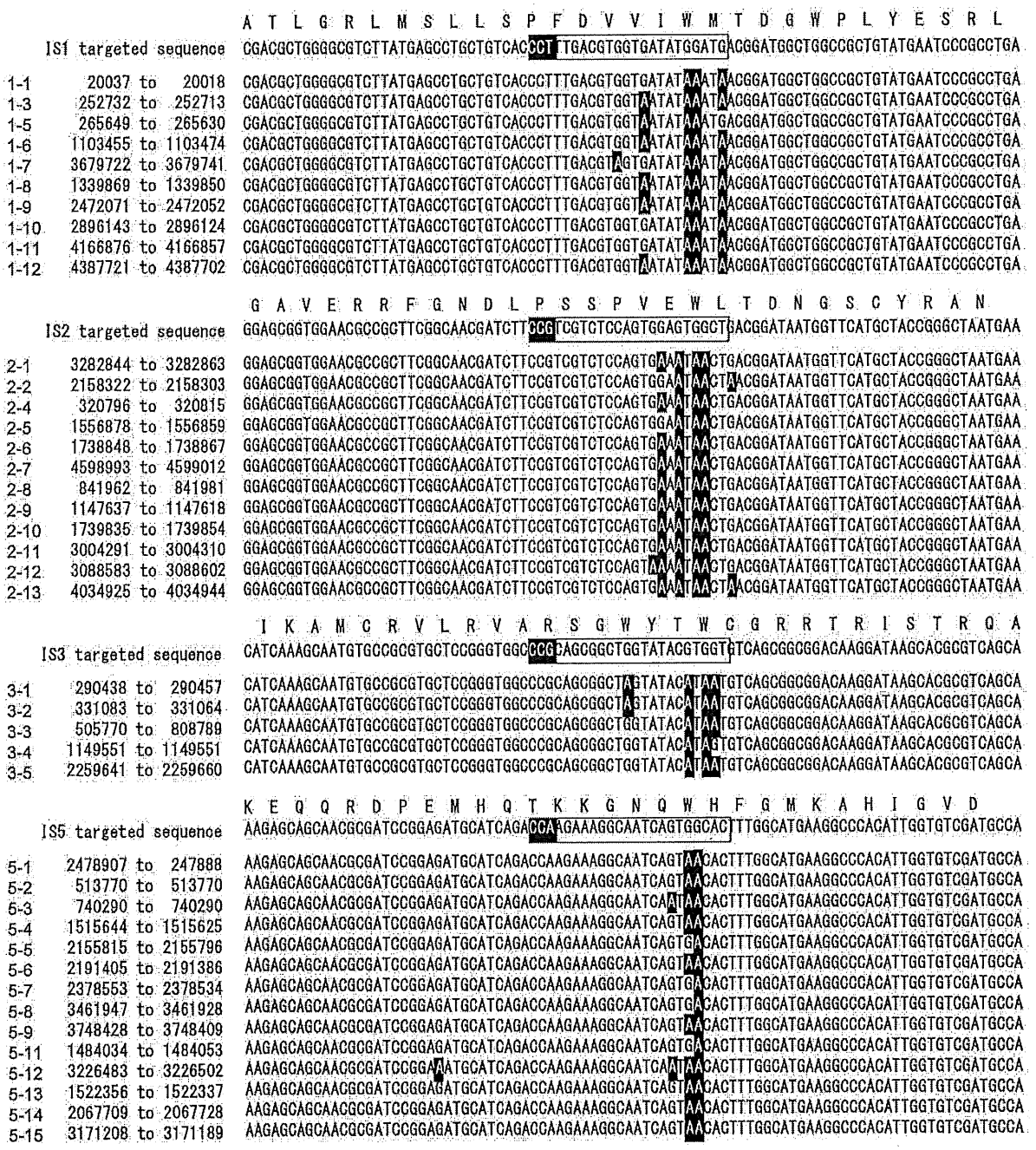

A T L G R L M S L L S P F D V V I W M T D G W P L Y E S R L
IS1 targeted sequence CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACGCTTGACGTGGTGATATGGATGACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-1 20037 to 20018 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTGATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-3 252732 to 252713 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTAATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-5 265649 to 265630 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTAATATAAATGACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-6 1103455 to 1103474 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTAATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-7 3679722 to 3679741 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTAGTGATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-8 1339869 to 1339850 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTAATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-9 2472071 to 2472052 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTAATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-10 2896143 to 2896124 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTGATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-11 4166876 to 4166857 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTGATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
1-12 4387721 to 4387702 CGACGCTGGGGCGTCTTATGAGCCTGCTGTCACCGTTTGACGTGGTAATATAAATAACGGATGGCTGGCCGCTGTATGAATCCGCCTGA
G A V E R R F G N D L P S S P V E W L T D N G S C Y R A N
IS2 targeted sequence GGAGCGGTGGAACGCCGGTTCGGCAACGATCTTCCGTCGTCTCCAGTGGAGTGGCTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-1 3282844 to 3282863 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-2 2158322 to 2158303 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGGAATAACTAACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-4 320796 to 320815 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-5 1556878 to 1556859 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGGAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-6 1738848 to 1738867 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-7 4598993 to 4599012 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-8 841962 to 841981 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-9 1147637 to 1147618 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-10 1739835 to 1739854 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-11 3004291 to 3004310 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-12 3088583 to 3088602 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTAAAATAACTGACGGATAATGGTTCATGCTACCGGGCTAATGAA
2-13 4034925 to 4034944 GGAGCGGTGGAACGCCGCTTCGGCAACGATCTTCCGTCGTCTCCAGTGAAATAACTAACGGATAATGGTTCATGCTACCGGGCTAATGAA
I K A M C R V L R V A R S G W Y T W C G R R T R I S T R Q A
IS3 targeted sequence CATCAAAGCAATGTGCCGCGTGCTCCGGGTGGCCCGCAGCGGCTGGTATACGTGGTGTCAGCGGCGGACAAGGATAAGCACGCGTCAGCA
3-1 290438 to 290457 CATCAAAGCAATGTGCCGCGTGCTCCGGGTGGCCCGCAGCGGCTAGTATACATAATGTCAGCGGCGGACAAGGATAAGCACGCGTCAGCA
3-2 331083 to 331064 CATCAAAGCAATGTGCCGCGTGCTCCGGGTGGCCCGCAGCGGCTAGTATACATAATGTCAGCGGCGGACAAGGATAAGCACGCGTCAGCA
3-3 505770 to 808789 CATCAAAGCAATGTGCCGCGTGCTCCGGGTGGCCCGCAGCGGCTGGTATACATAATGTCAGCGGCGGACAAGGATAAGCACGCGTCAGCA
3-4 1149551 to 1149551 CATCAAAGCAATGTGCCGCGTGCTCCGGGTGGCCCGCAGCGGGCTGGTATACATAGTGTCAGCGGCGGACAAGGATAAGCACGCGTCAGCA
3-5 2259641 to 2259660 CATCAAAGCAATGTGCCGCGTGCTCCGGGTGGCCCGCAGCGGCTGGTATACATAATGTCAGCGGCGGACAAGGATAAGCACGCGTCAGCA
K E Q Q R D P E M H Q T K K G N Q W H F G M K A H I G V D
IS5 targeted sequence AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTGGCACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-1 2478907 to 247888 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-2 513770 to 513770 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-3 740290 to 740290 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-4 1515644 to 1515625 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-5 2155815 to 2155796 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTGACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-6 2191405 to 2191386 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-7 2378553 to 2378534 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTGACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-8 3461947 to 3461928 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTGACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-9 3748428 to 3748409 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-11 1484034 to 1484053 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTGACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
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5-13 1522356 to 1522337 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-14 2067709 to 2067728 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA
5-15 3171208 to 3171189 AAGAGCAGCAACGCGATCCGGAGATGCATCAGACCAAGAAAGGCAATCAGTAACACTTTGGCATGAAGGCCCACATTGGTGTCGATGCCA

Fig. 12

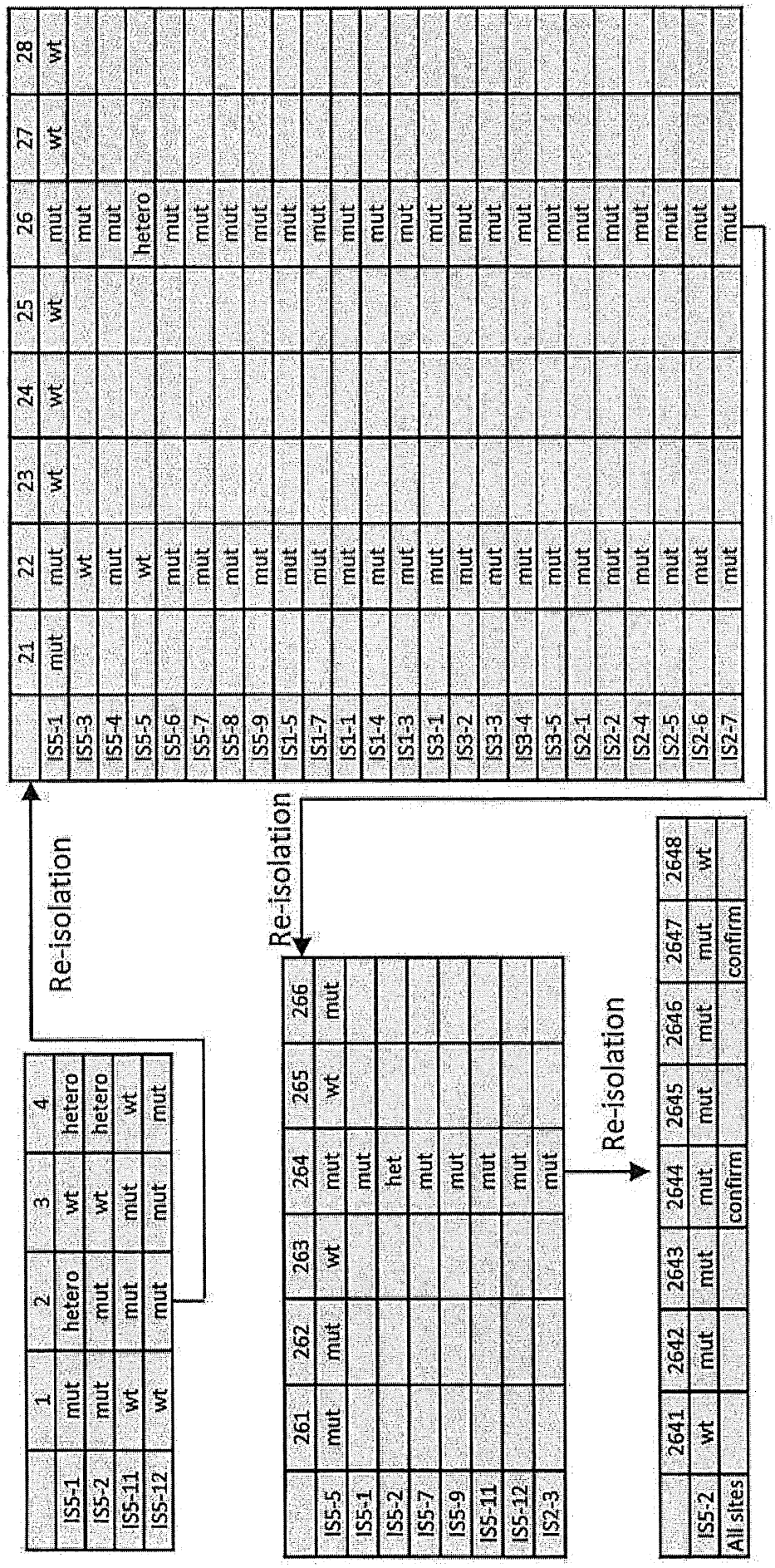
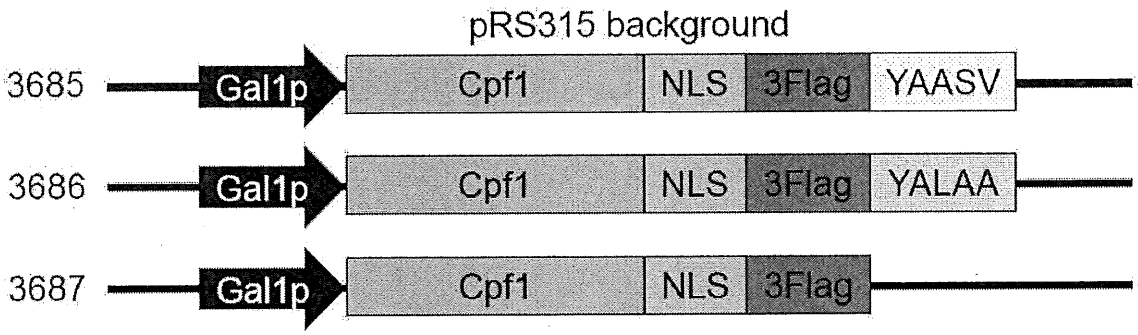

Re-isolation
Re-isolation
Re-isolation
IS5-1 mut hetero wt hetero
IS5-2 mut mut wt hetero
IS5-11 wt mut mut wt
IS5-12 wt mut mut mut
IS5-5 mut wt mut wt mut
IS5-1 mut mut
IS5-2 het mut
IS5-7 mut mut
IS5-9 mut mut
IS5-11 mut mut
IS5-12 mut mut
IS2-3 mut mut
IS5-2 wt mut mut confirm mut mut
All sites confirm
IS5-1 mut wt wt wt mut
IS5-3 mut mut
IS5-4 mut mut
IS5-5 wt hetero
IS5-6 mut mut
IS5-7 mut mut
IS5-8 mut mut
IS5-9 mut mut
IS1-5 mut mut
IS1-7 mut mut
IS1-1 mut mut
IS1-4 mut mut
IS1-3 mut mut
IS3-1 mut mut
IS3-2 mut mut
IS3-3 mut mut
IS3-4 mut mut
IS3-5 mut mut
IS2-1 mut mut
IS2-2 mut mut
IS2-4 mut mut
IS2-5 mut mut
IS2-6 mut mut
IS2-7 mut mut

Fig. 13


pRS315 background
3685
Gal1p
Cpf1
NLS
3Flag
YAASV
3686
Gal1p
Cpf1
NLS
3Flag
YALAA
3687
Gal1p
Cpf1
NLS
3Flag