

Fig. 1

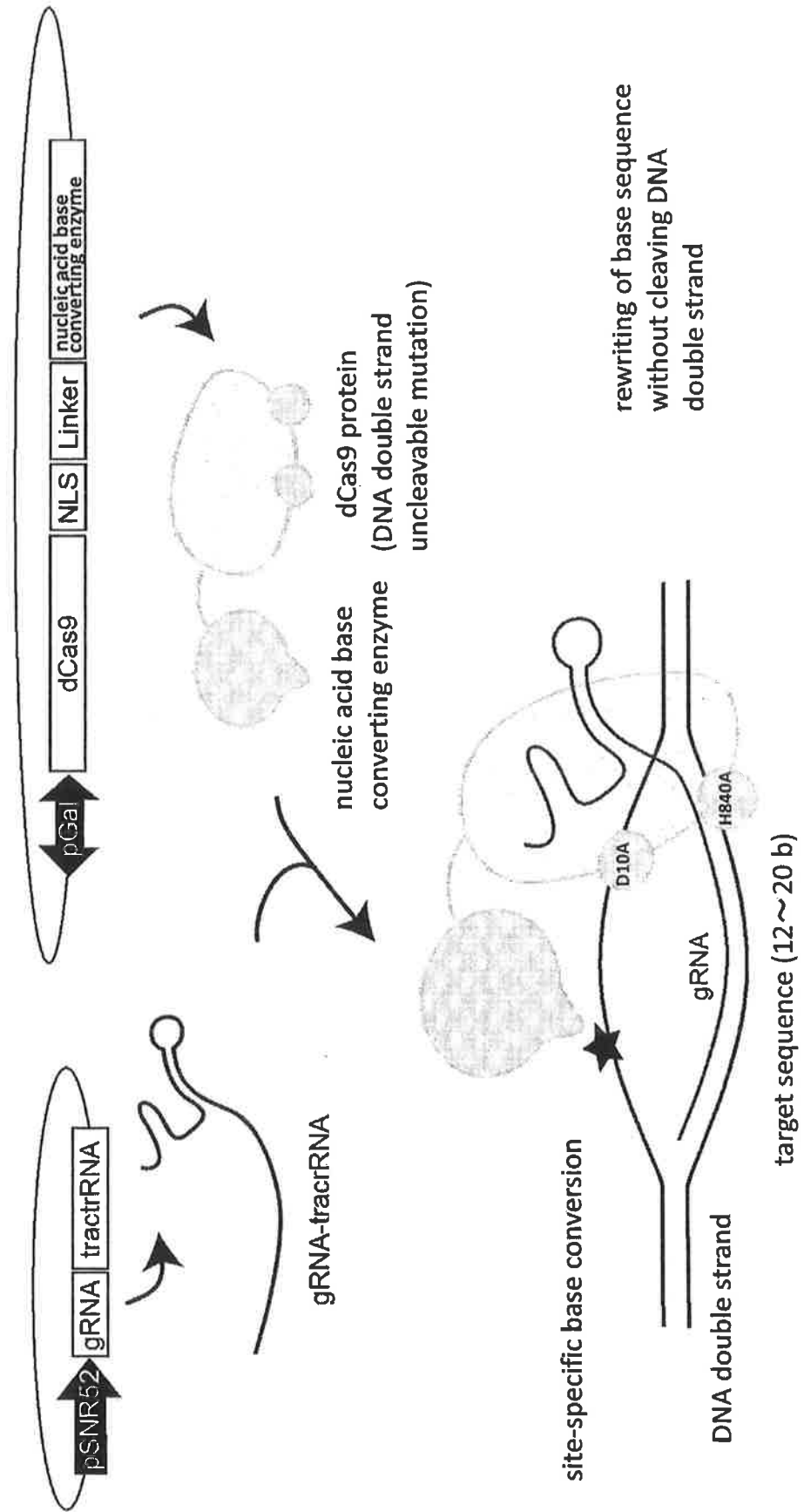


Fig. 2

gRNA target=187th – 206th base sequence in CAN1 gene ORF verification of Can1 mutation rate on Canavanine plate

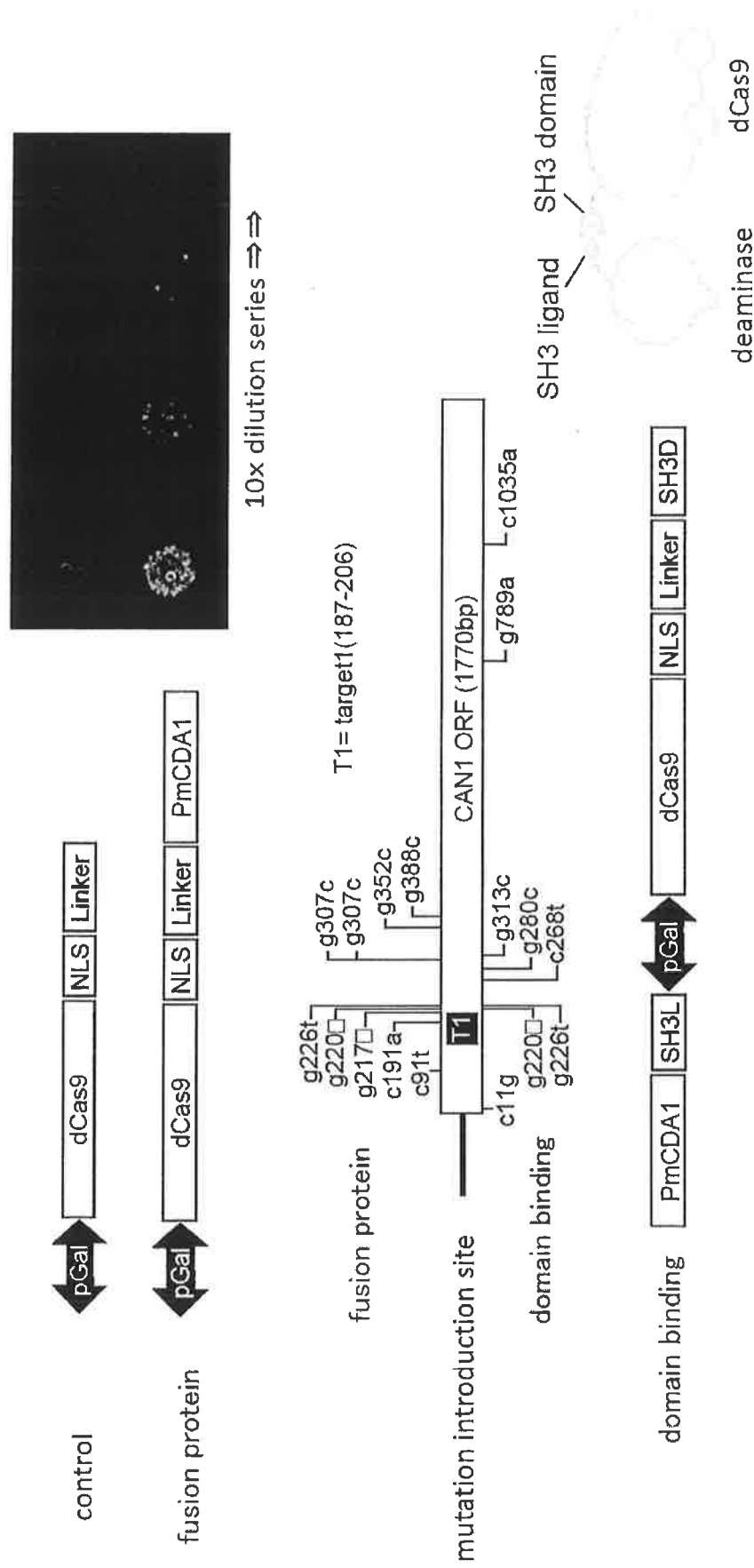


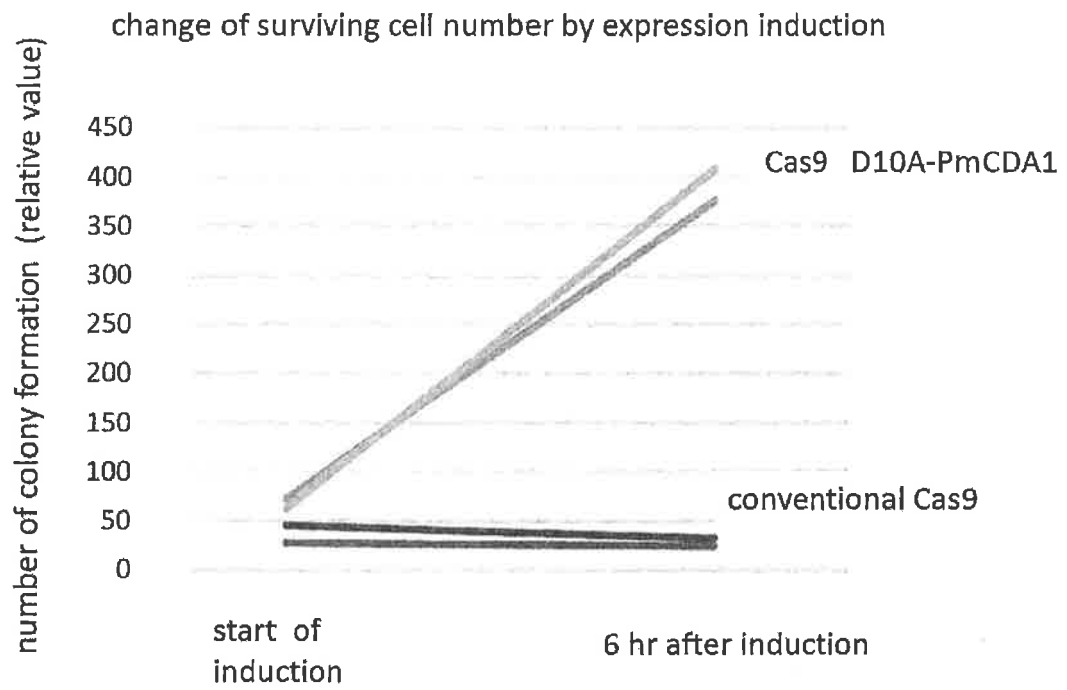
Fig. 3

Fig. 5

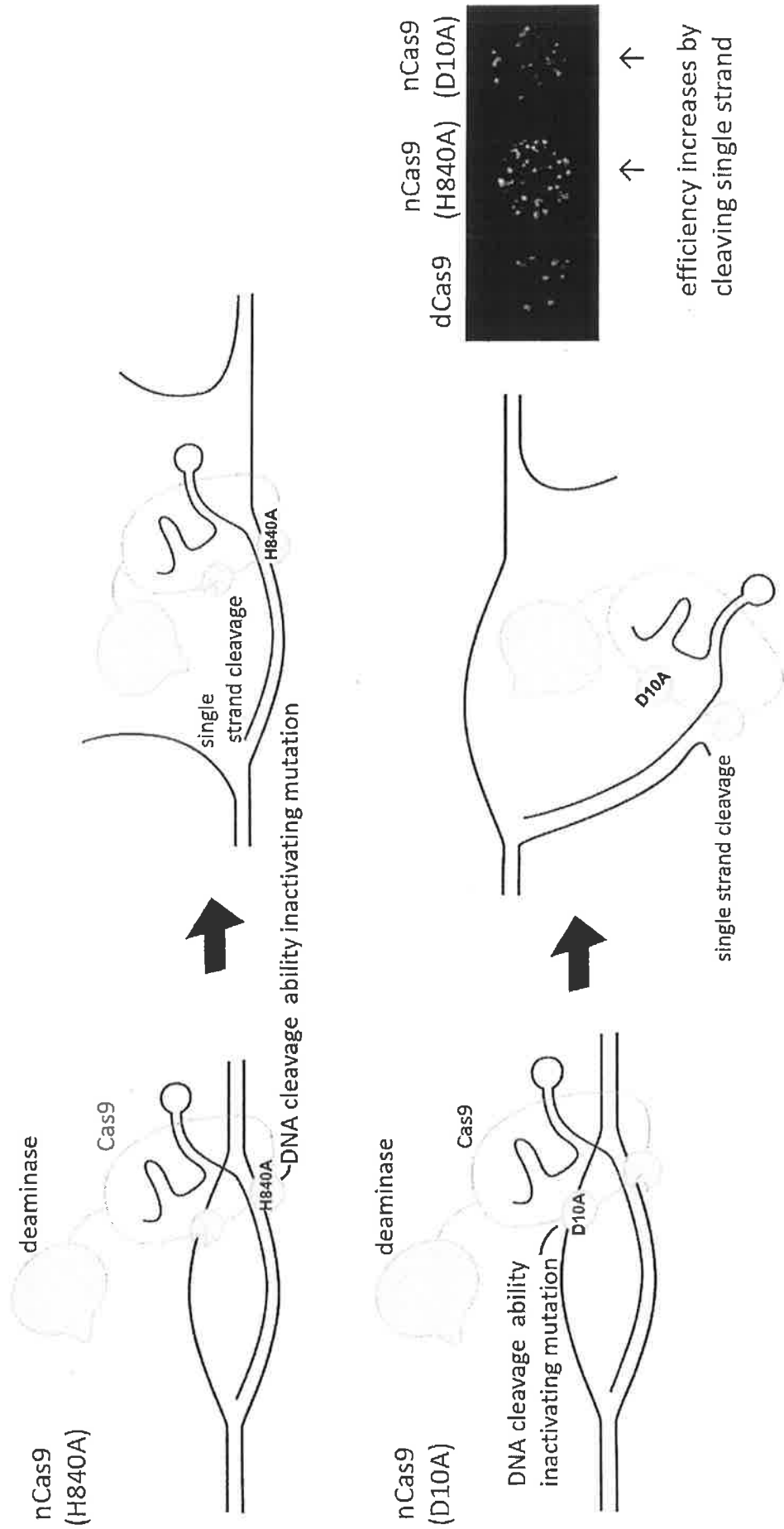
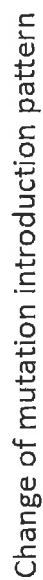
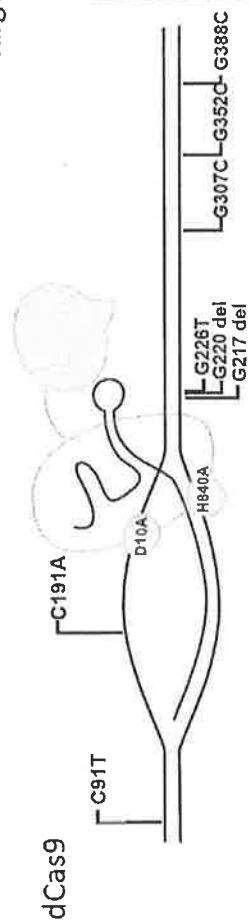
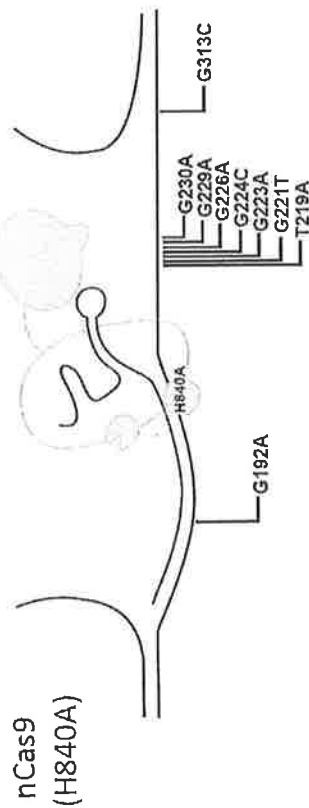
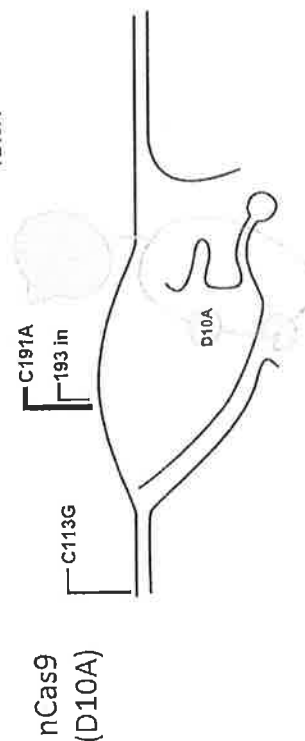


Fig. 6



target sequence

187 AAATGGCGAGGATACGTTCTCTATGGAGGATGGCA TAGGT 206
TTTACCGGTCTATGCAAGAGATACGTCCTACCGGATCGA

1024-T1	
1118-9	C191A
1118-10	G226T
1118-11	C091T
1130-9	G226T
1130-10	G307C
1130-11	G352C
1130-13	G217del
1130-14	G307C
1130-15	G388C
1130-16	G220del

1043-T1	
0110-1	G313C
0110-2	G223A
0110-3	T219A
0110-4	G223A
0111-1	G313C
0111-2	C191T
0111-5	G215C
0111-6	G257C

1042-T1	
0110-6	C191A
0110-7	in 194
0110-8	C113G
0110-10	C191A
0110-11	C191A
0110-12	C191A
0110-13	in 193
0111-7	del 188-209
0111-8	in 192
0111-9	del 188-209
0111-10	C191T
0111-11	C191A

Fig. 7

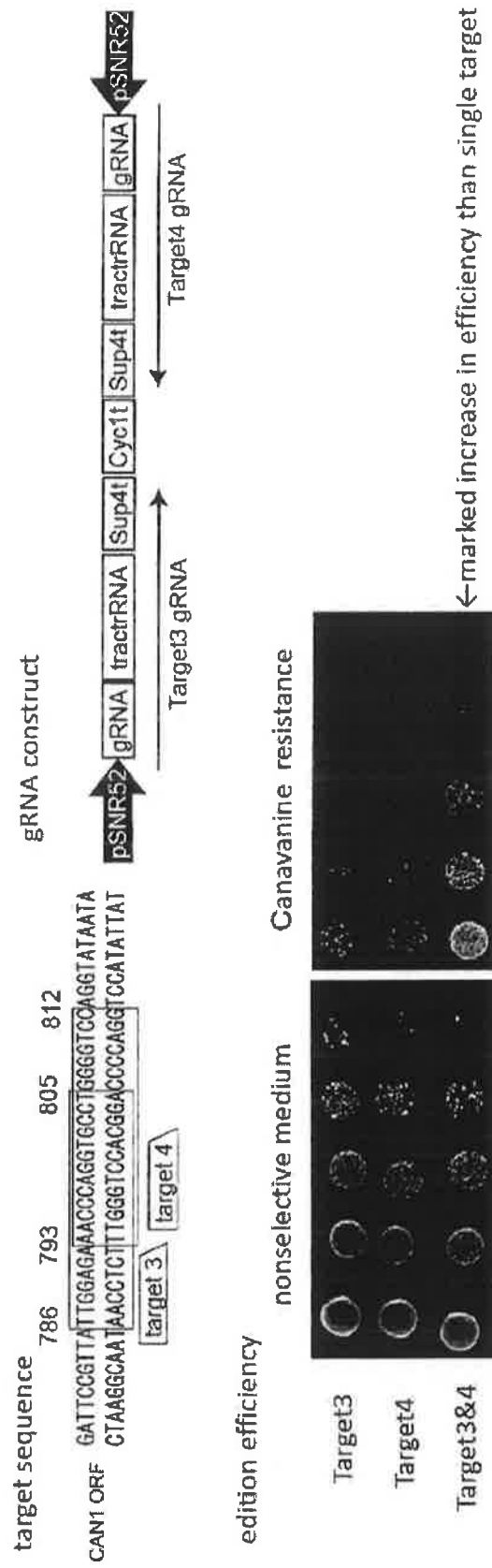


Fig. 8

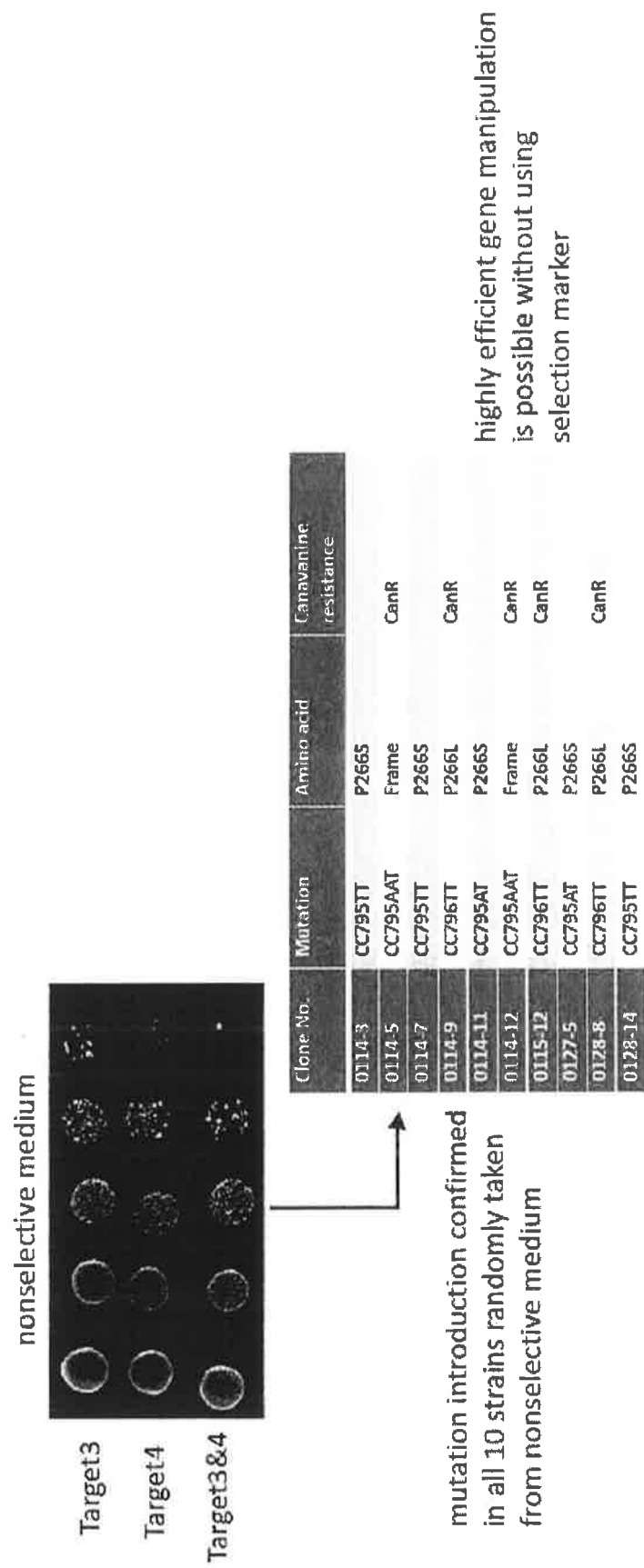


Fig. 9

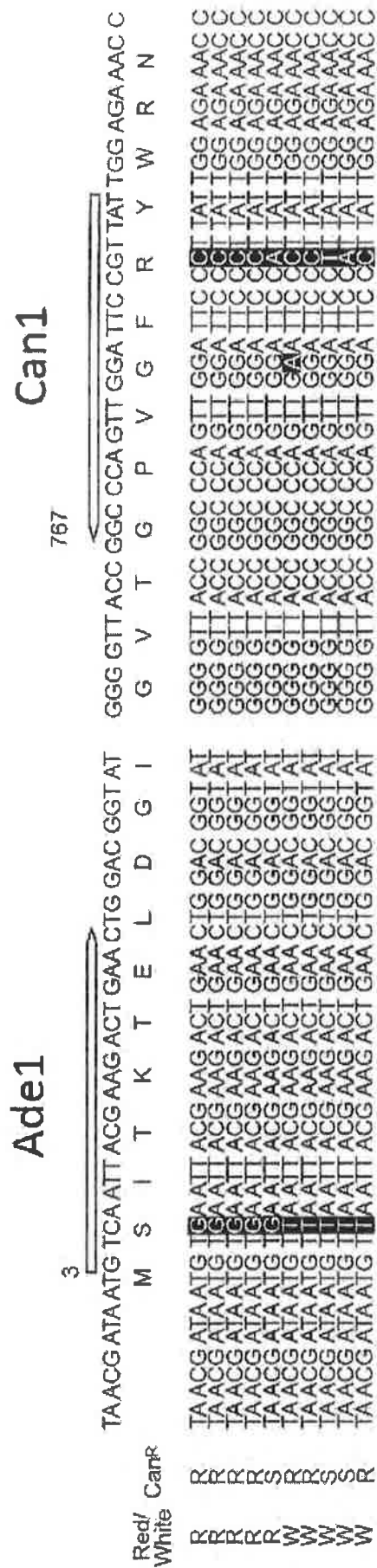
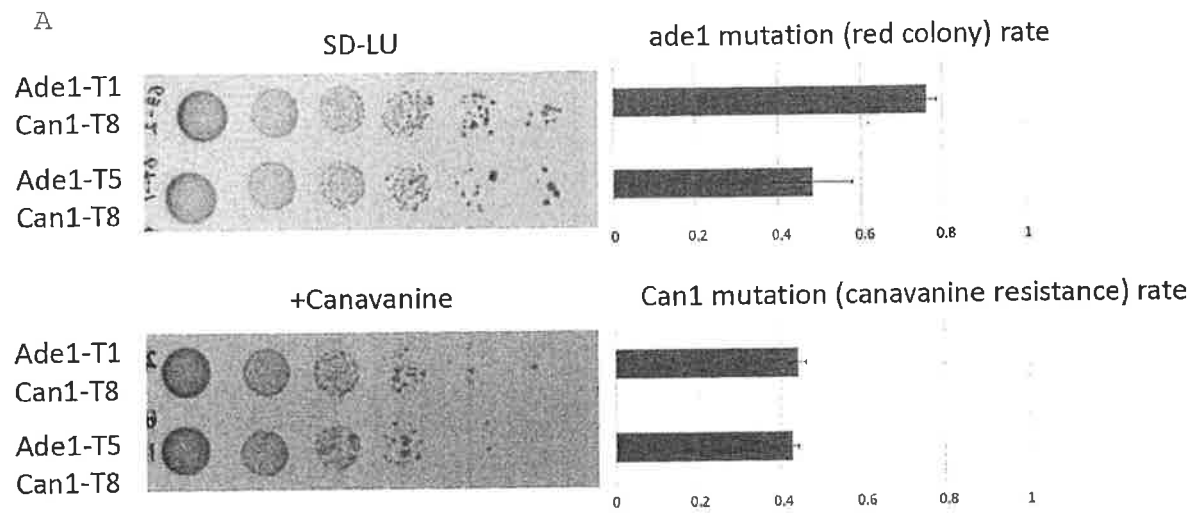


Fig. 10**B**

WT-Ade1

ACGATAATGTCAATTACGAAG

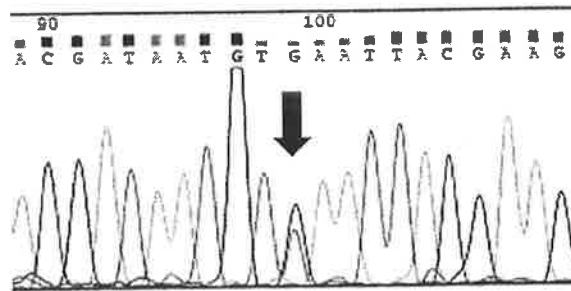
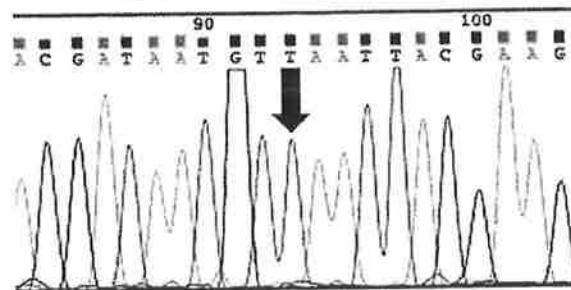
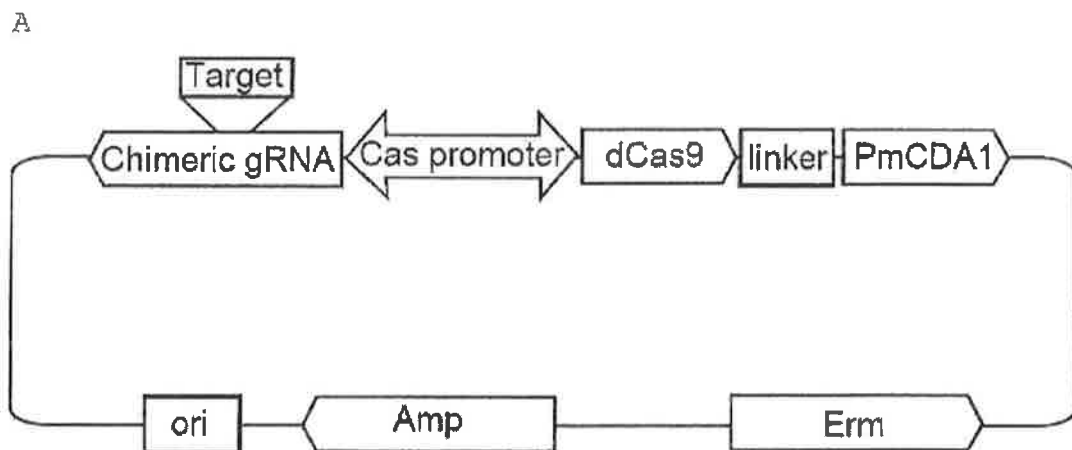
heteromutation
(white colony)homomutation
(red colony)

Fig. 11



B

galK TCAATGGGCTAACTACGTTC-3'

1 TCAATGGGCTAACTACGTTC

2 TTAATGGGCTAACTACGTTC

3 TTAATGGGCTAACTACGTTC

C

rpoB GCTGTCTCAGTTTATGGACC-3'

[CGACAGAGTCAAATACCTGG-5']

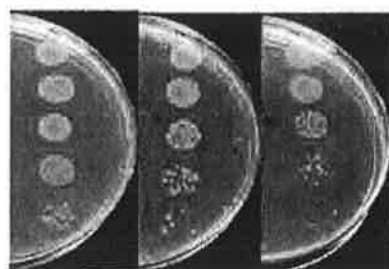
none [1 GCTGTCTCAGTTTATGGACC
2 GCTGTCTCAGTTTATGGACC

Rif25 [1 GCTGTCTCAGTTTATGAACC
2 GCTGTCTCAGTTTATGAACC

Rif50 [1 GCTGTCTCAGTTTATAAACC
2 GCTGTCTCAGTTTATGAACC

[CGACAGAGTCAAATACCTGG-5']

DH5a



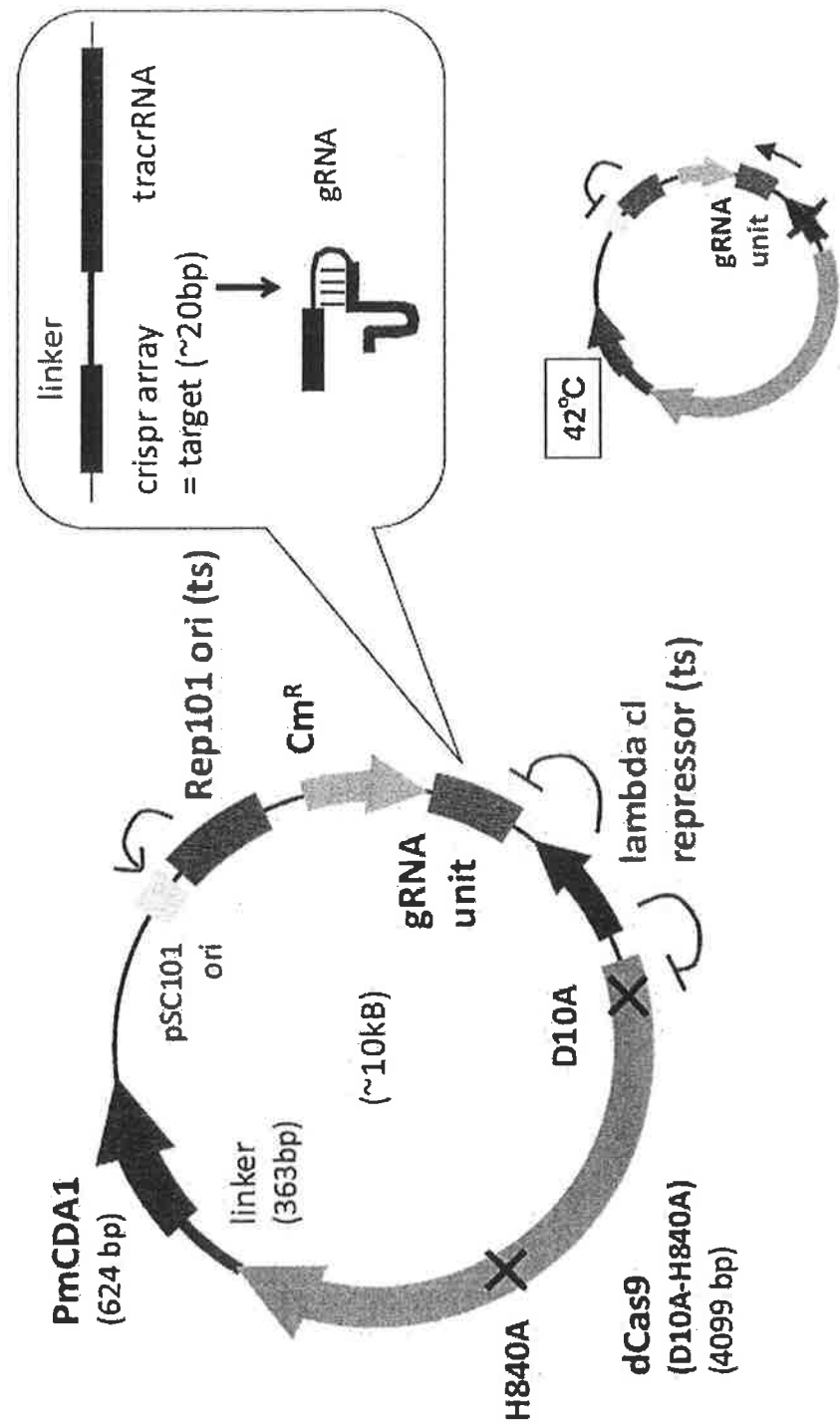
none

Rif 25
(ug/ml)

Rif 50
(ug/ml)

Fig. 13

mutation-introduced plasmid produced
(temperature sensitive)



at high temperature (42°C):

- dCas9-PmCDA1 expression • gRNA transcription are induced
- replication of plasmid ceases

Fig. 14

mutation introduction method (temperature sensitive plasmid)

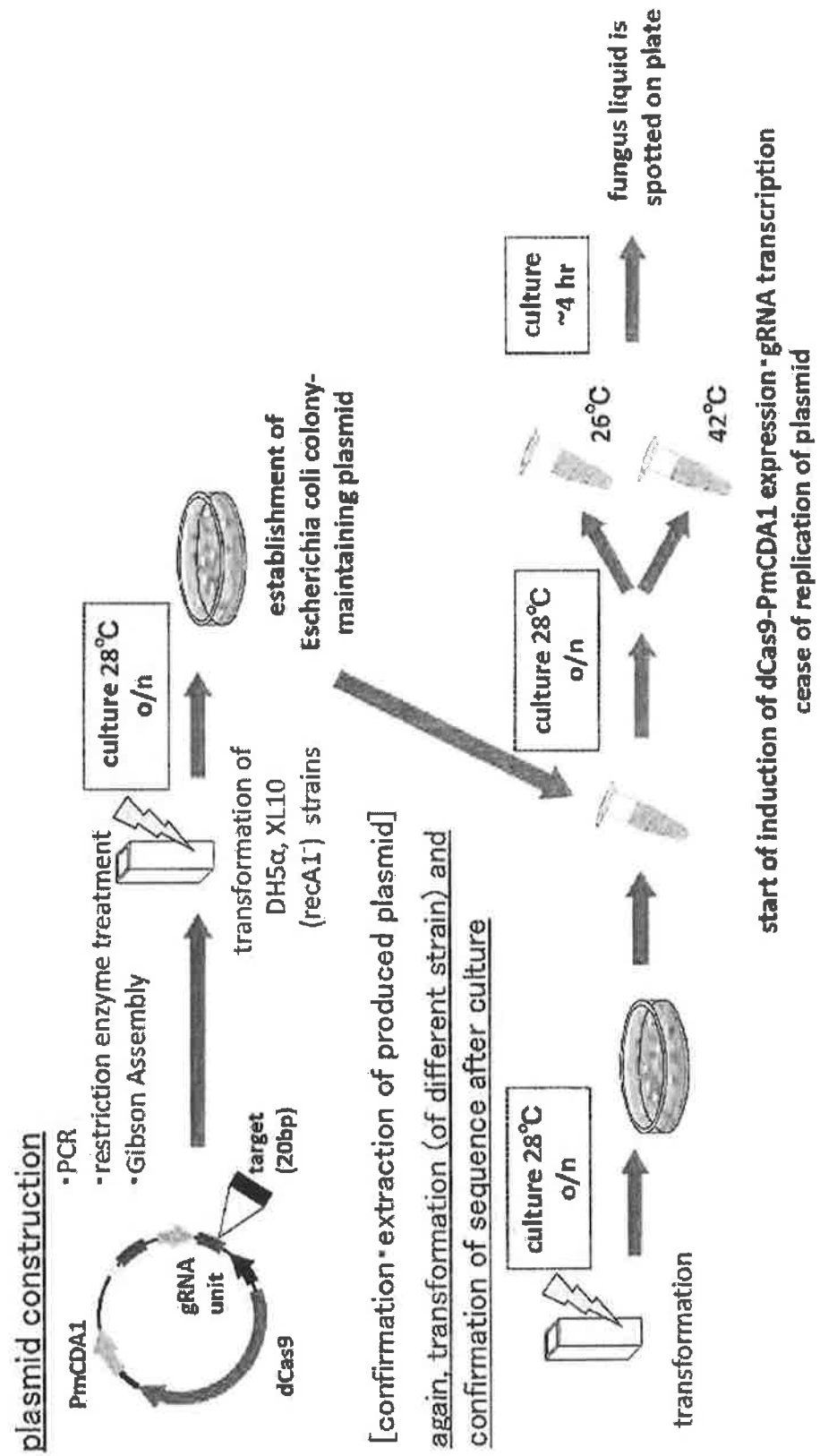


Fig. 15

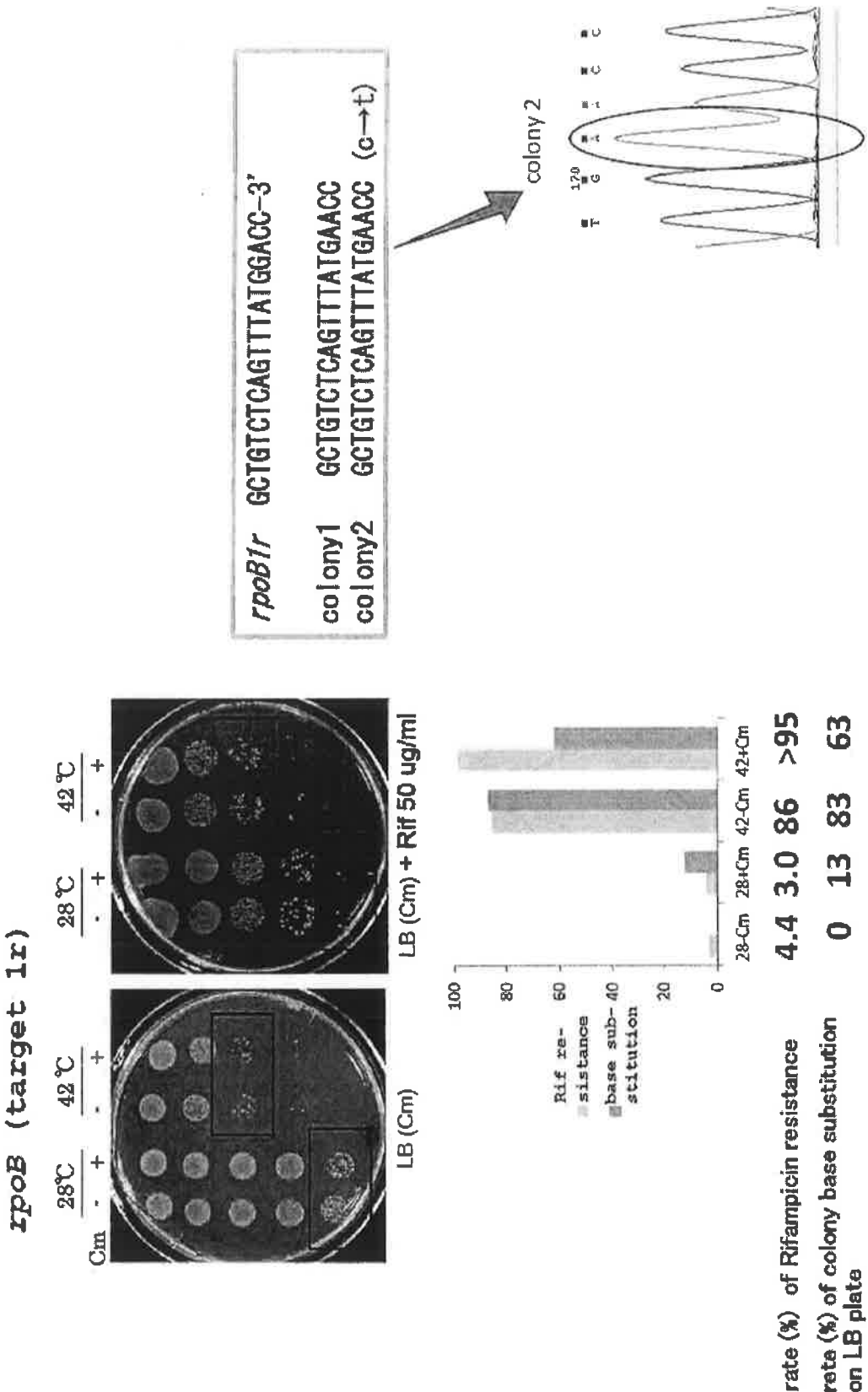


Fig. 16

galK (target 8, target 12)

