Fig. 1

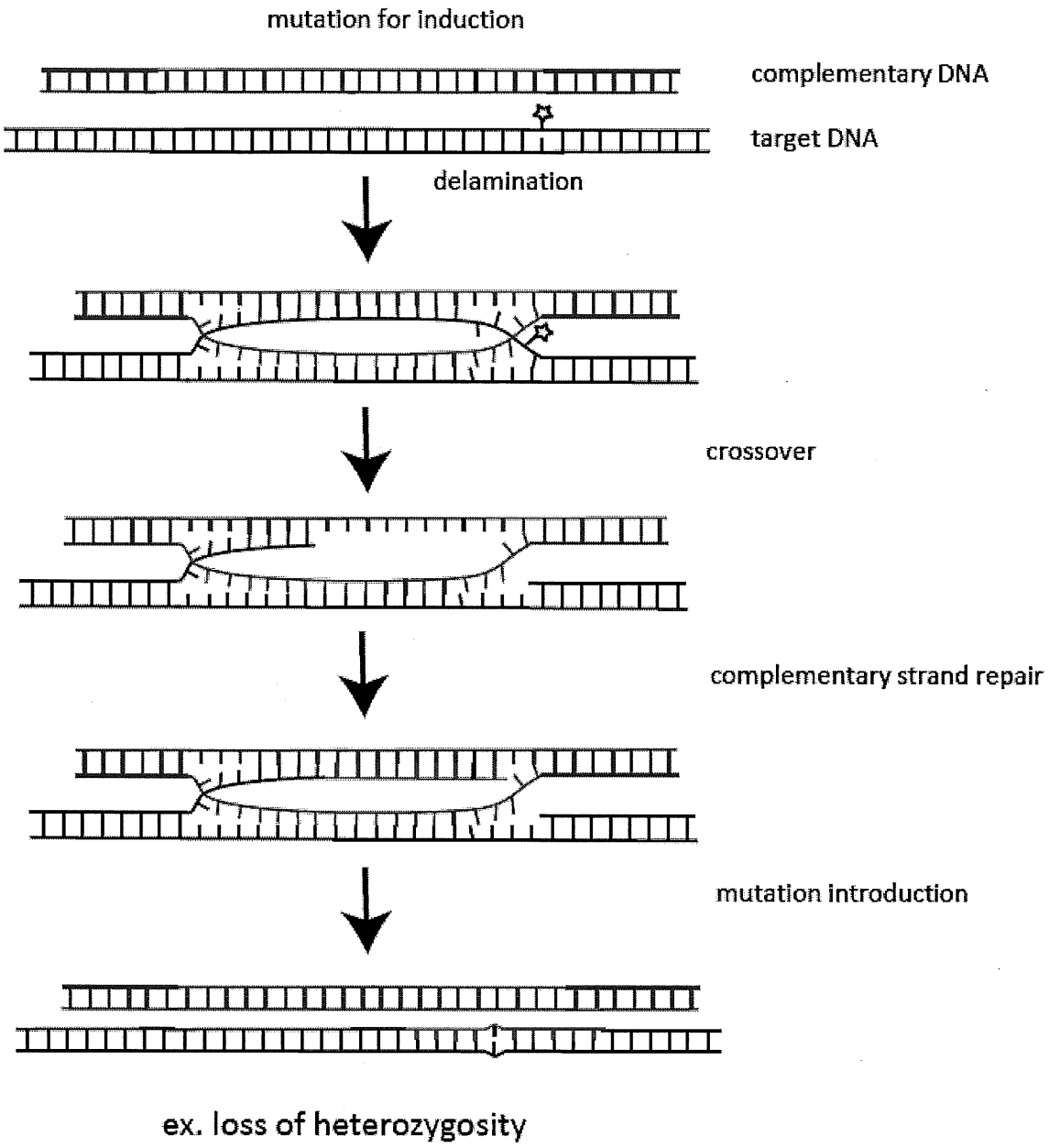

mutation for induction
complementary DNA
target DNA
delamination
crossover
complementary strand repair
mutation introduction
ex. loss of heterozygosity

Fig. 2

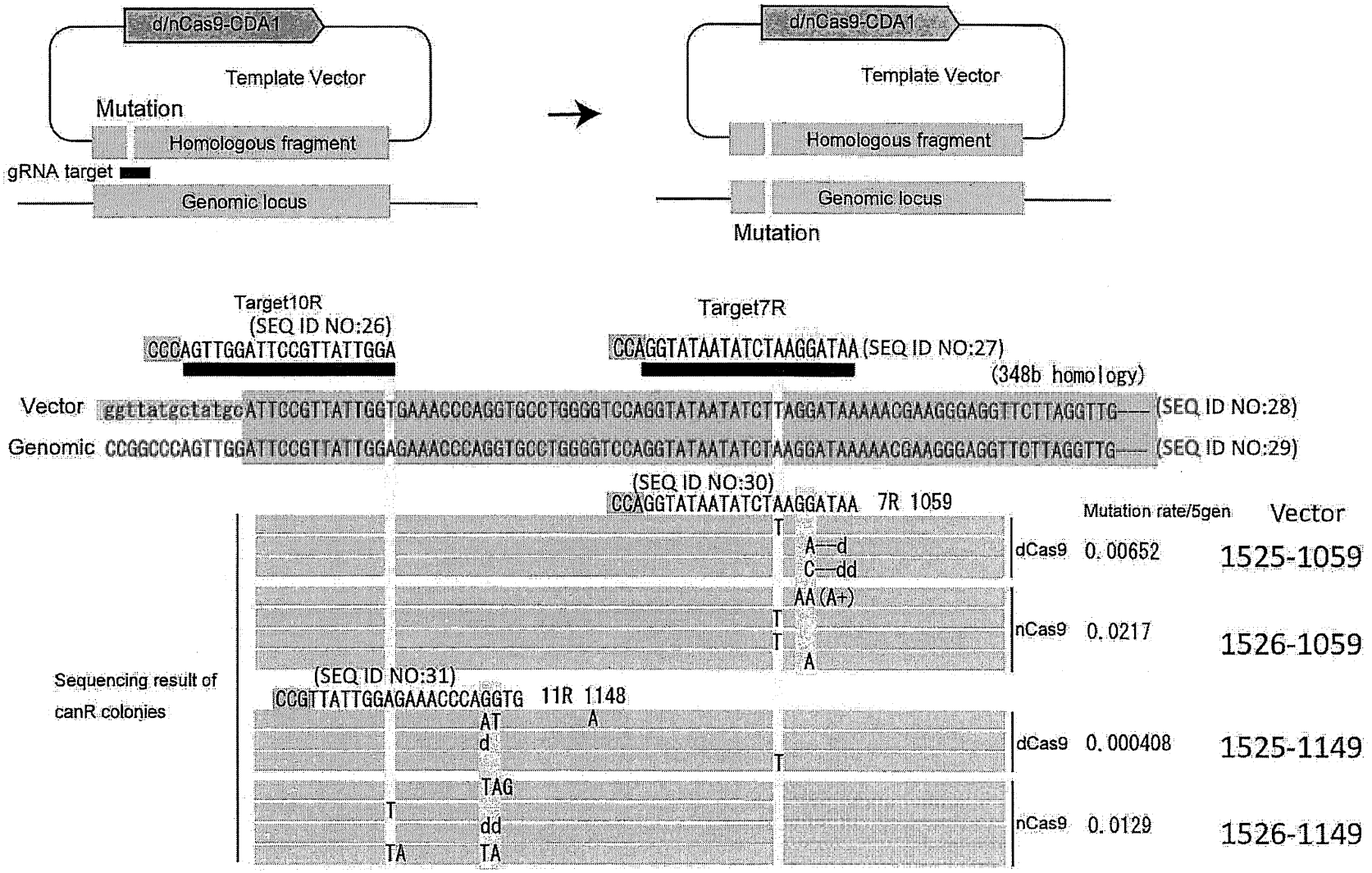

d/nCas9-CDA1
Template Vector
Mutation
Homologous fragment
gRNA target
Genomic locus
d/nCas9-CDA1
Template Vector
Homologous fragment
Genomic locus
Mutation
Target10R
(SEQ ID NO:26)
CCCAGTTGGATTCCGTTATTGGA
Target7R
CCAGGTATAATATCTAAGGATAA (SEQ ID NO:27)
(348b homology)
Vector ggttatgctatgcATTCCGTTATTGGTGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTTAGGATAAAAACGAAGGGAGGTTCTTAGGTTG—— (SEQ ID NO:28)
Genomic CCGGCCCAGTTGGATTCCGTTATTGGAGAAACCCAGGTGCCTGGGGTCCAGGTATAATATCTAAGGATAAAAACGAAGGGAGGTTCTTAGGTTG—— (SEQ ID NO:29)
(SEQ ID NO:30)
CCAGGTATAATATCTAAGGATAA 7R 1059
Mutation rate/5gen
Vector
dCas9 0.00652 1525-1059
nCas9 0.0217 1526-1059
Sequencing result of canR colonies
(SEQ ID NO:31)
CCGTTATTGGAGAAACCCAGGTG 11R 1148
dCas9 0.000408 1525-1149
nCas9 0.0129 1526-1149

Fig. 3

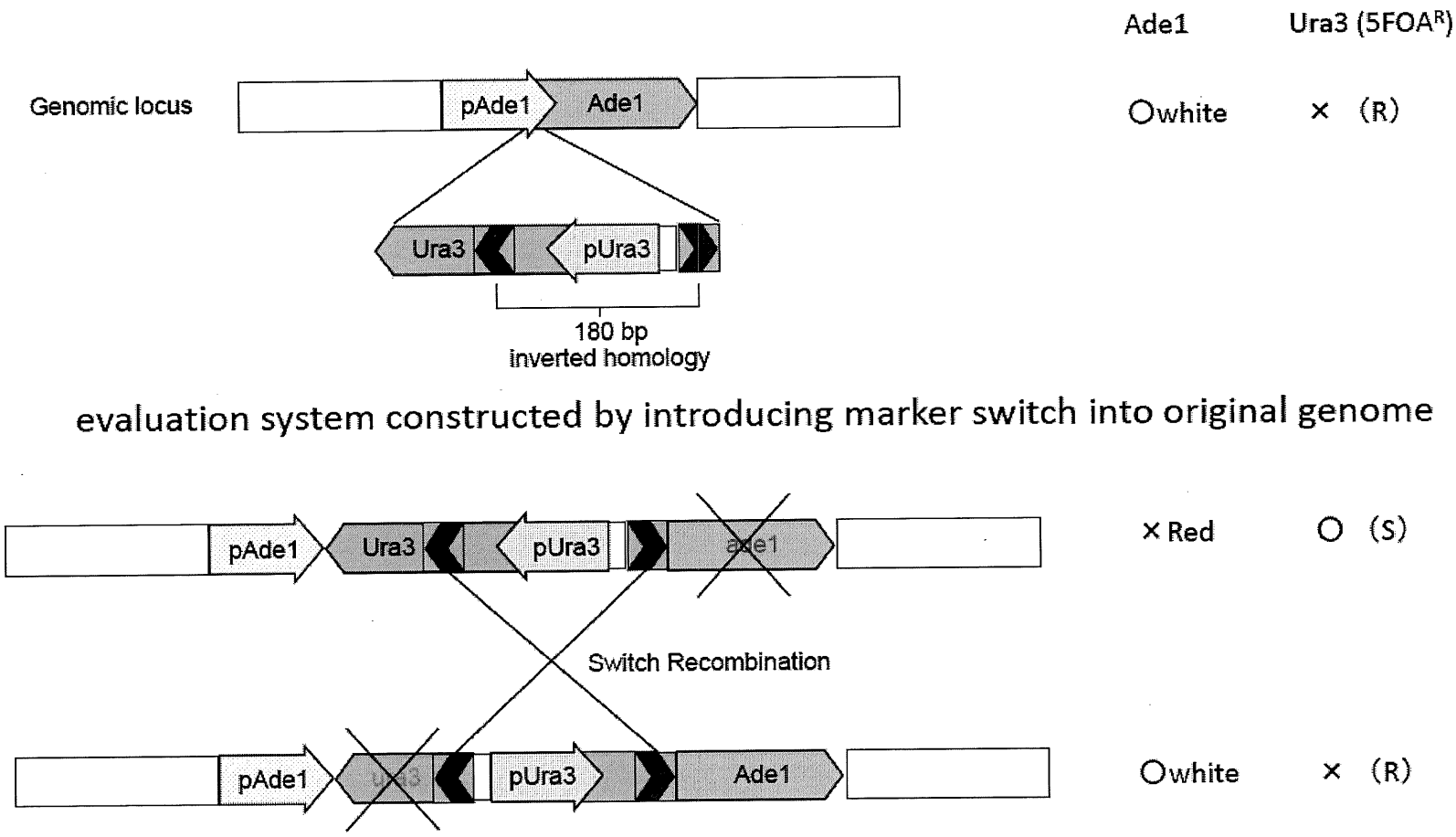

Ade1
Ura3 (5FOA^R)
Genomic locus
pAde1
Ade1
○white
× (R)
Ura3
pUra3
180 bp
inverted homology
evaluation system constructed by introducing marker switch into original genome
pAde1
Ura3
pUra3
ade1
× Red
○ (S)
Switch Recombination
pAde1
pUra3
Ade1
○white
× (R)

Fig. 4

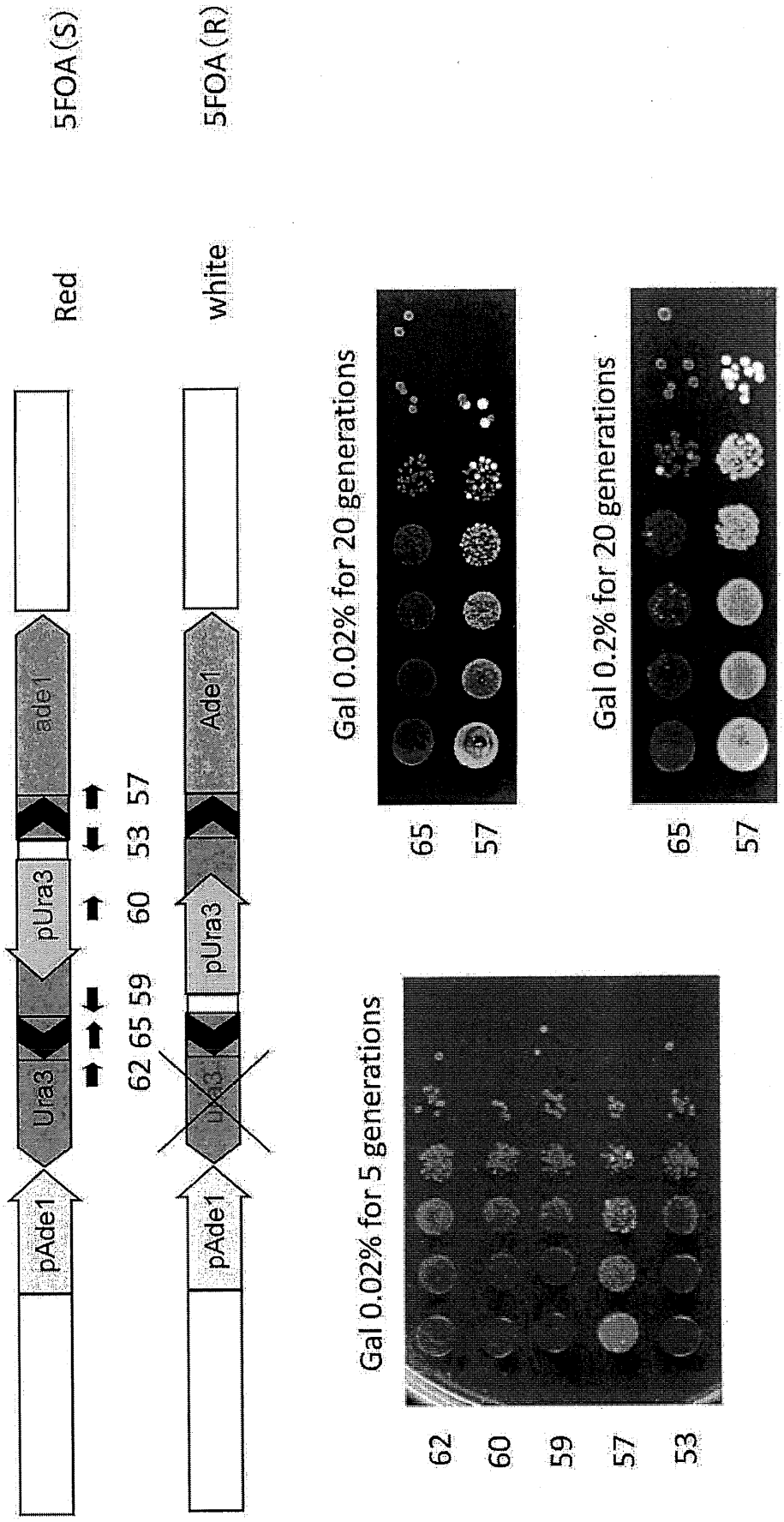

5FOA(S)
Red
5FOA(R)
white
pAde1
Ura3
pUra3
ade1
Ade1
62 65 59 60 53 57
Gal 0.02% for 20 generations
65
57
Gal 0.2% for 20 generations
65
57
Gal 0.02% for 5 generations
62
60
59
57
53

Fig. 5

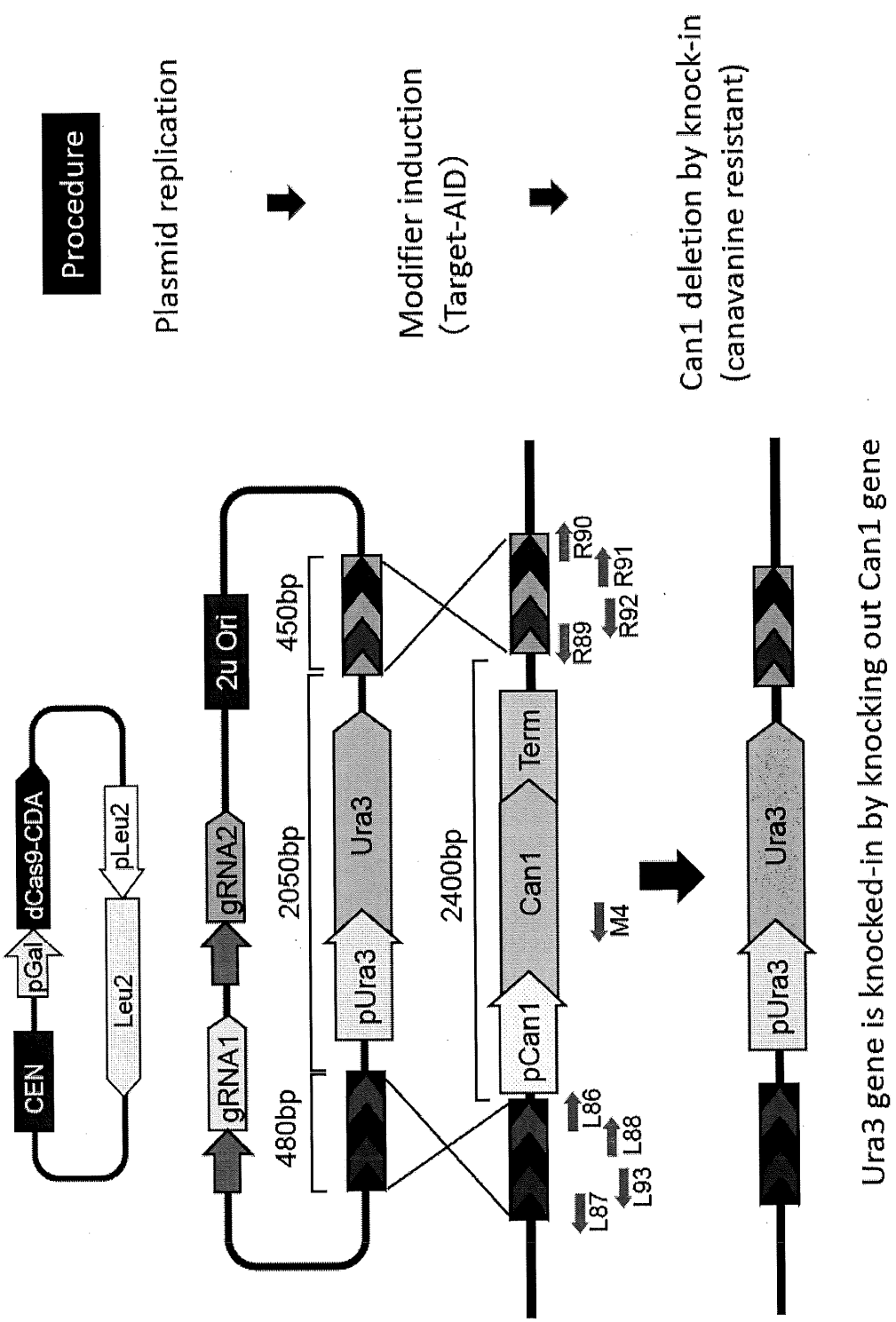

Procedure
Plasmid replication
Modifier induction
(Target-AID)
Can1 deletion by knock-in
(canavanine resistant)
CEN
pGal
dCas9-CDA
Leu2
pLeu2
gRNA1
gRNA2
2u Ori
480bp
2050bp
450bp
pUra3
Ura3
2400bp
pCan1
Can1
Term
L86
L88
L87
L93
M4
R90
R91
R89
R92
pUra3
Ura3
Ura3 gene is knocked-in by knocking out Can1 gene

Fig. 6

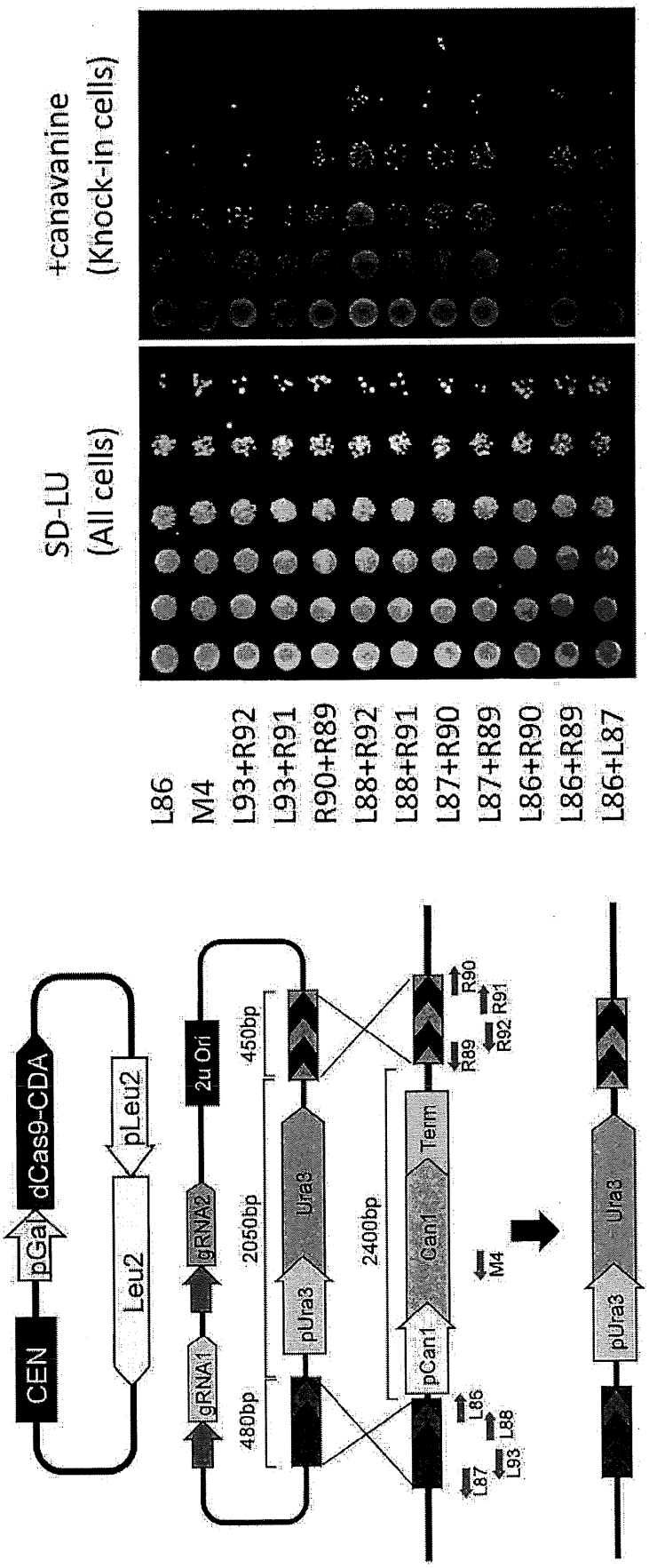
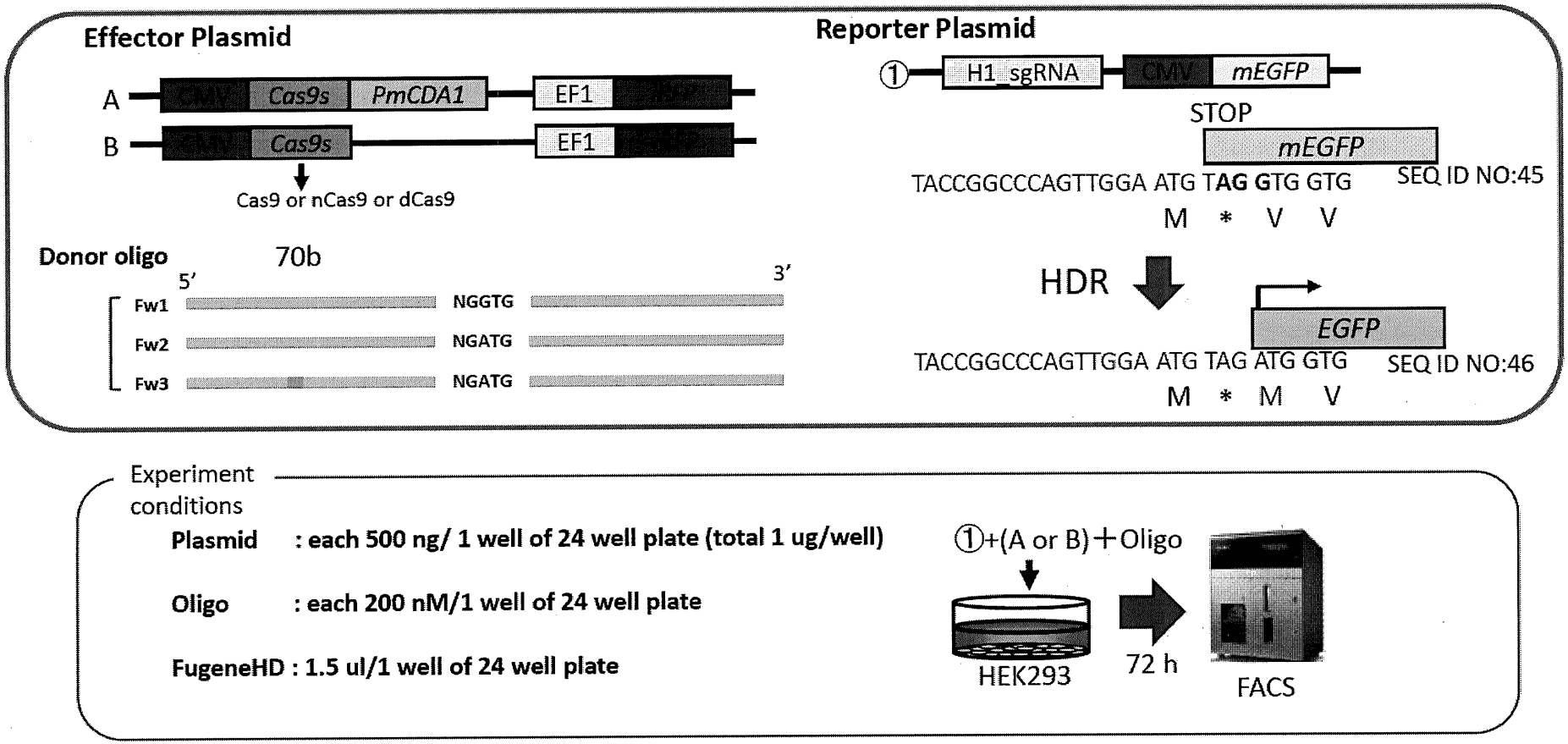

SD-LU
(All cells)
+canavanine
(Knock-in cells)
L86
M4
L93+R92
L93+R91
R90+R89
L88+R92
L88+R91
L87+R90
L87+R89
L86+R90
L86+R89
L86+L87
CEN
pGal
dCas9-CDA
Leu2
pLeu2
gRNA1
gRNA2
2u Ori
480bp
2050bp
450bp
pUra3
Ura3
pCan1
Can1
Term
2400bp
L86
L88
L93
L87
M4
R90
R91
R92
R89
pUra3
Ura3

Fig. 7


Effector Plasmid
A
CMV
Cas9s
PmCDA1
EF1
EGFP
B
CMV
Cas9s
EF1
EGFP
Cas9 or nCas9 or dCas9
Donor oligo
70b
5'
3'
Fw1
NGGTG
Fw2
NGATG
Fw3
NGATG
Reporter Plasmid
①
H1 sgRNA
CMV
mEGFP
STOP
mEGFP
TACCGGCCCAGTTGGA ATG TAG GTG GTG
SEQ ID NO:45
M * V V
HDR
EGFP
TACCGGCCCAGTTGGA ATG TAG ATG GTG
SEQ ID NO:46
M * M V

Experiment conditions
Plasmid : each 500 ng/ 1 well of 24 well plate (total 1 ug/well)
Oligo : each 200 nM/1 well of 24 well plate
FugeneHD : 1.5 ul/1 well of 24 well plate
①+(A or B)+Oligo
HEK293
72 h
FACS

Fig. 8

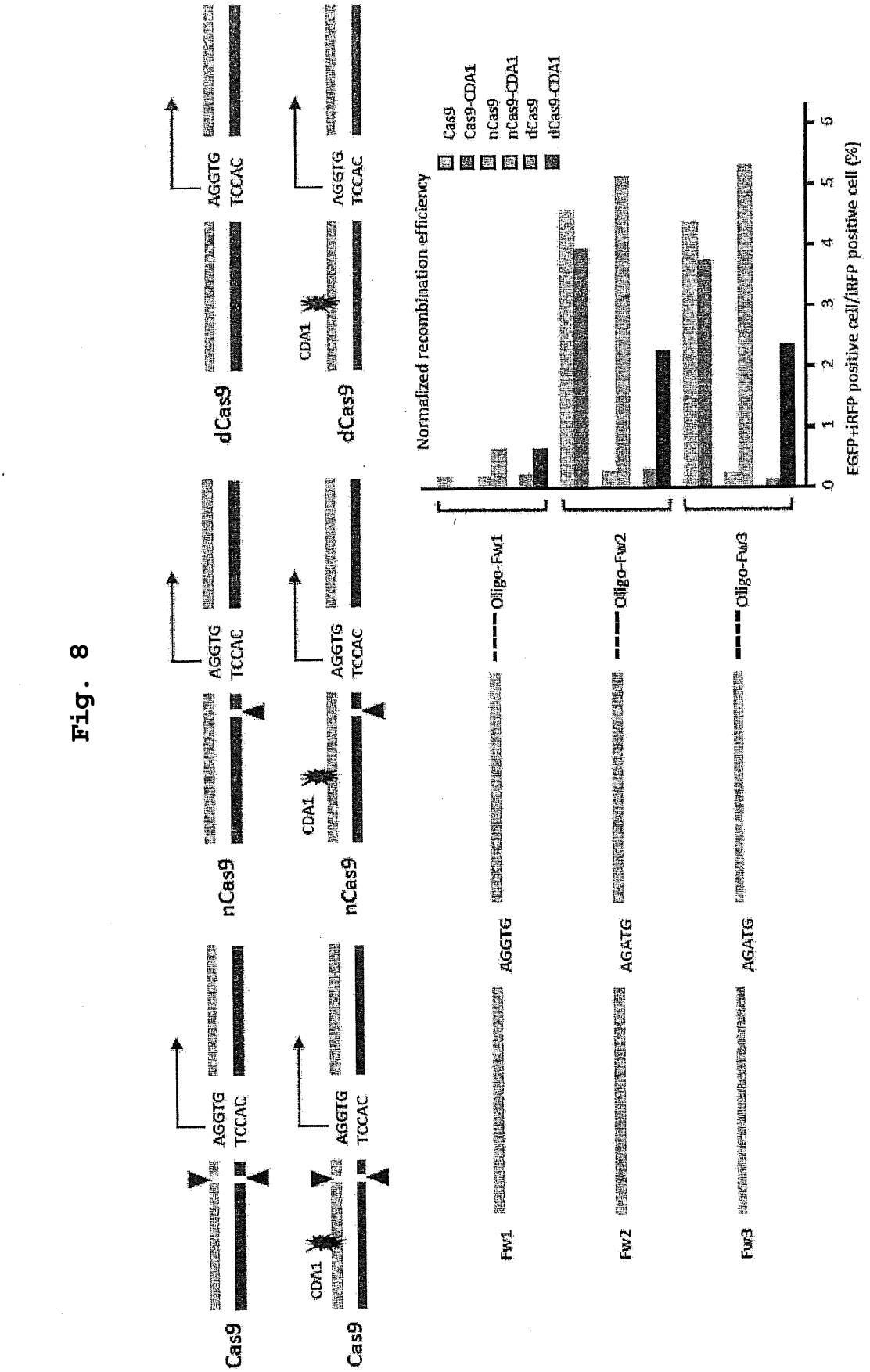
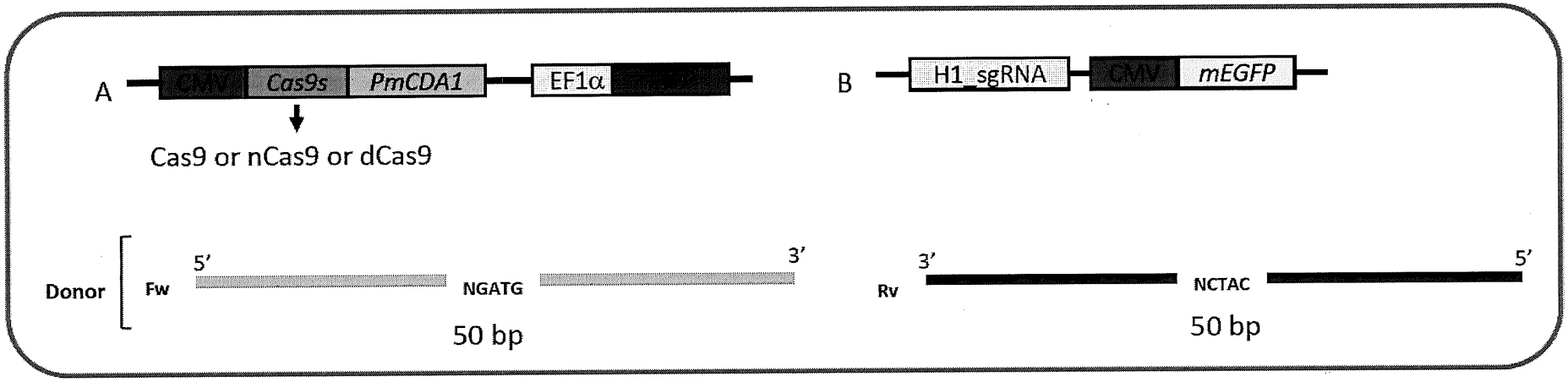

Cas9
nCas9
dCas9
CDA1
AGGTG
TCCAC
Fw1
Fw2
Fw3
AGGTG
AGATG
AGATG
Oligo-Fw1
Oligo-Fw2
Oligo-Fw3
Normalized recombination efficiency
Cas9
Cas9-CDA1
nCas9
nCas9-CDA1
dCas9
dCas9-CDA1
0
1
2
3
4
5
6
EGFP+iRFP positive cell/iRFP positive cell (%)

Fig. 9



Experiment conditions

- **Plasmid : each 500 ng/ 1 well of 24 well plate (total 1 ug/well)**
- **Oligo : each 200 nM/1 well of 24 well plate**
- **FugeneHD : 1.5 ul/1 well of 24 well plate**

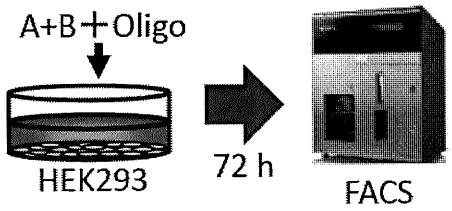


Fig. 10

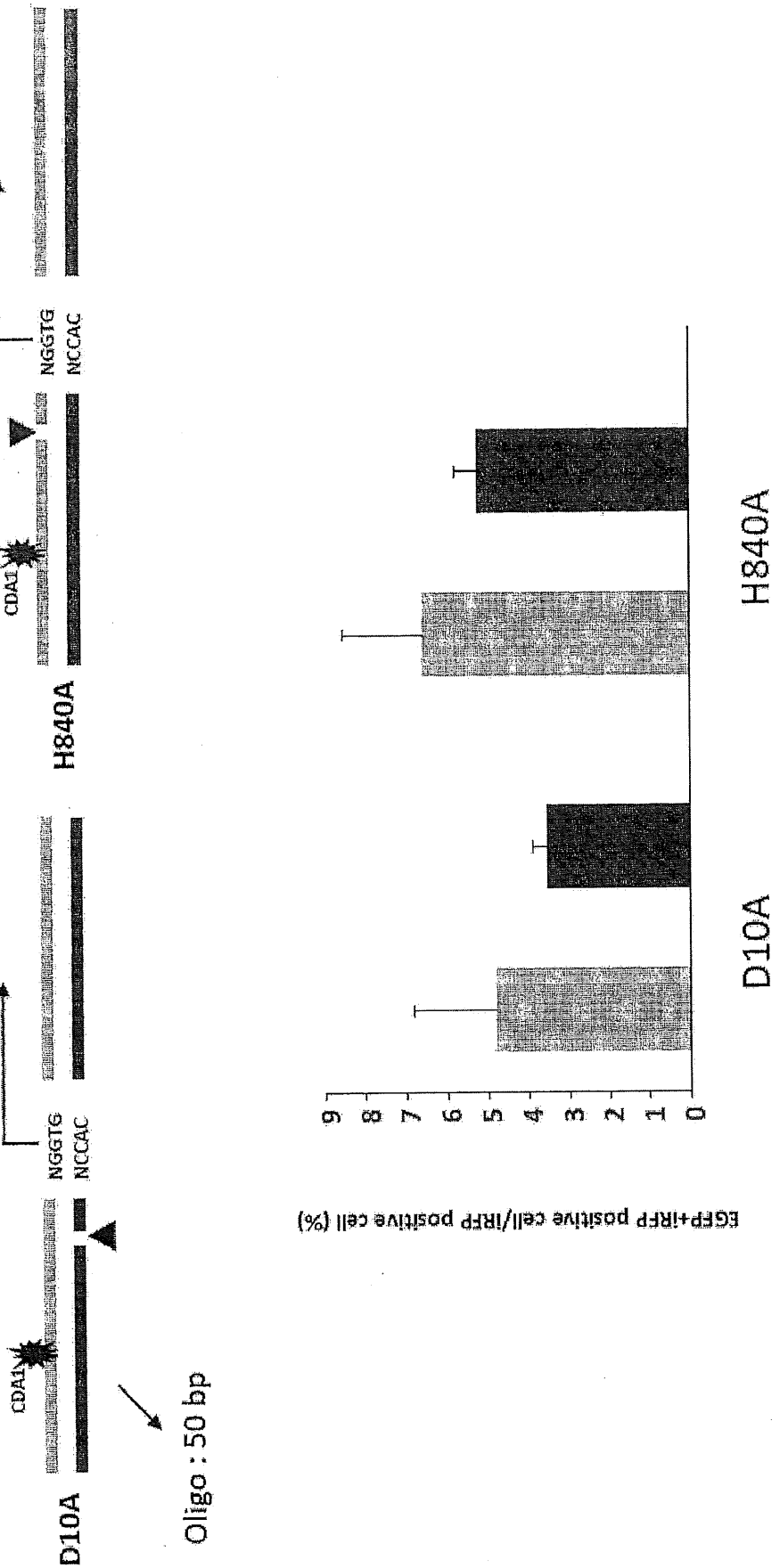

D10A
CDA1
NGGTG
NCCAC
Oligo : 50 bp
H840A
CDA1
NGGTG
NCCAC
EGFP+iRFP positive cell/iRFP positive cell (%)
0
1
2
3
4
5
6
7
8
9
D10A
H840A

Fig. 11

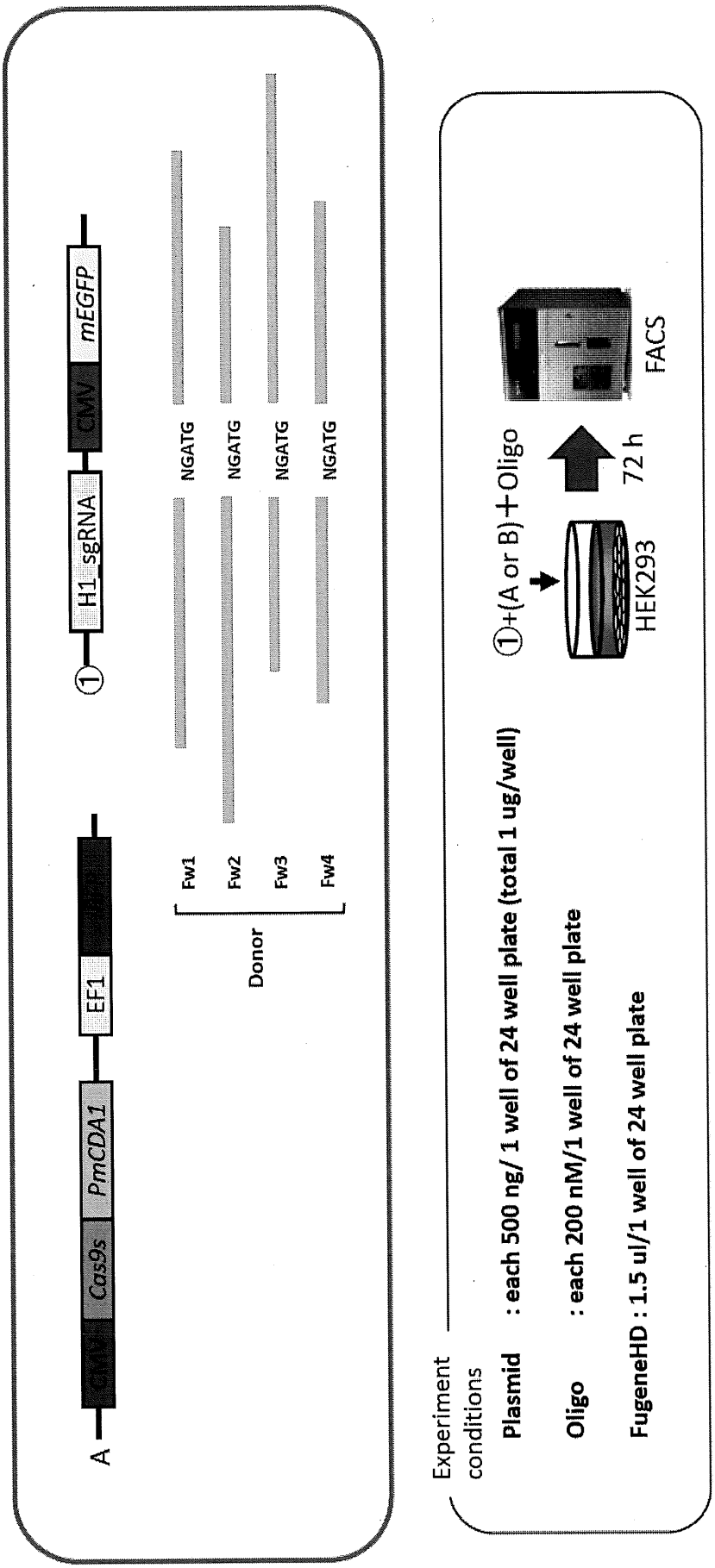
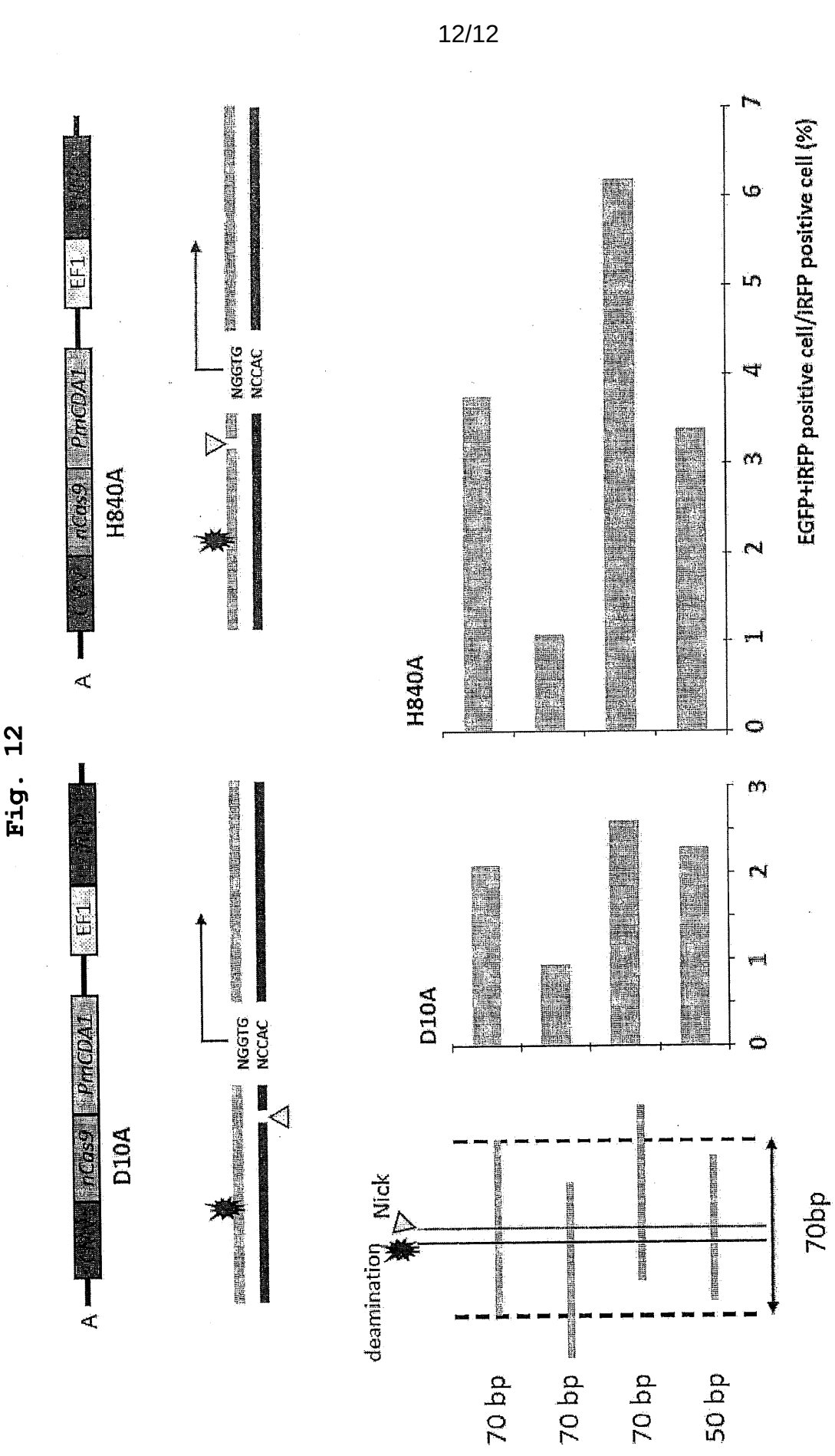

A
CMV
Cas9s
PmCDA1
EF1
①
H1_sgRNA
CMV
mEGFP
Donor
Fw1
Fw2
Fw3
Fw4
NGATG
NGATG
NGATG
NGATG
Experiment conditions
Plasmid : each 500 ng/ 1 well of 24 well plate (total 1 ug/well)
Oligo : each 200 nM/1 well of 24 well plate
FugeneHD : 1.5 ul/1 well of 24 well plate
①+(A or B)+Oligo
HEK293
72 h
FACS

Fig. 12


A
nCas9
PmCDA1
EF1
D10A
H840A
NGGTG
NCCAC
deamination
Nick
70 bp
70 bp
70 bp
50 bp
70bp
0
1
2
3
4
5
6
7
EGFP+iRFP positive cell/iRFP positive cell (%)