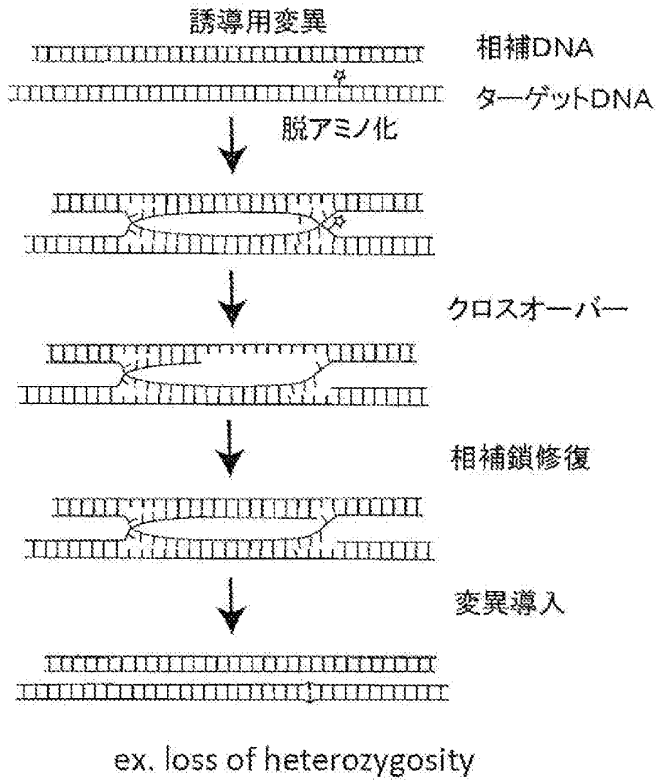
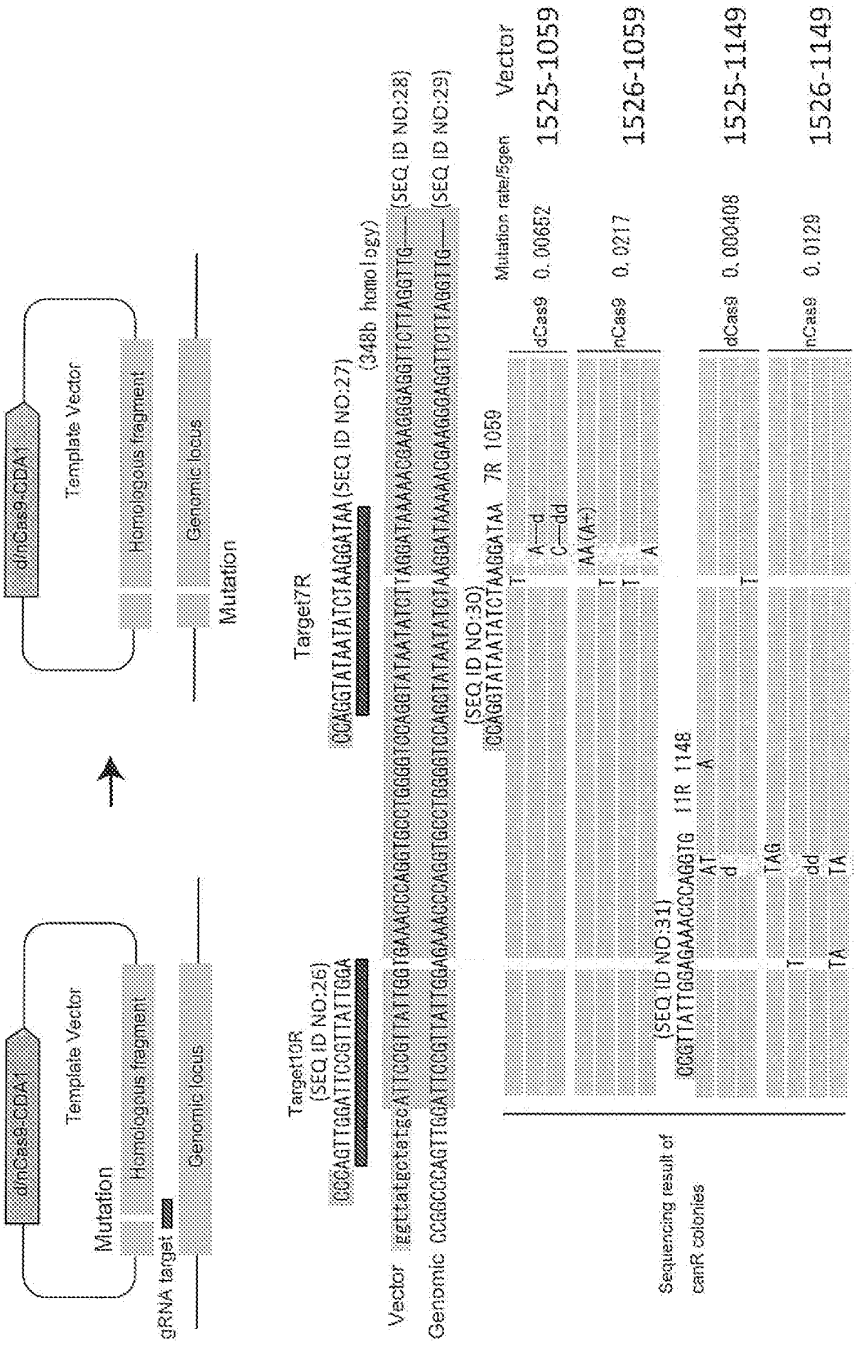
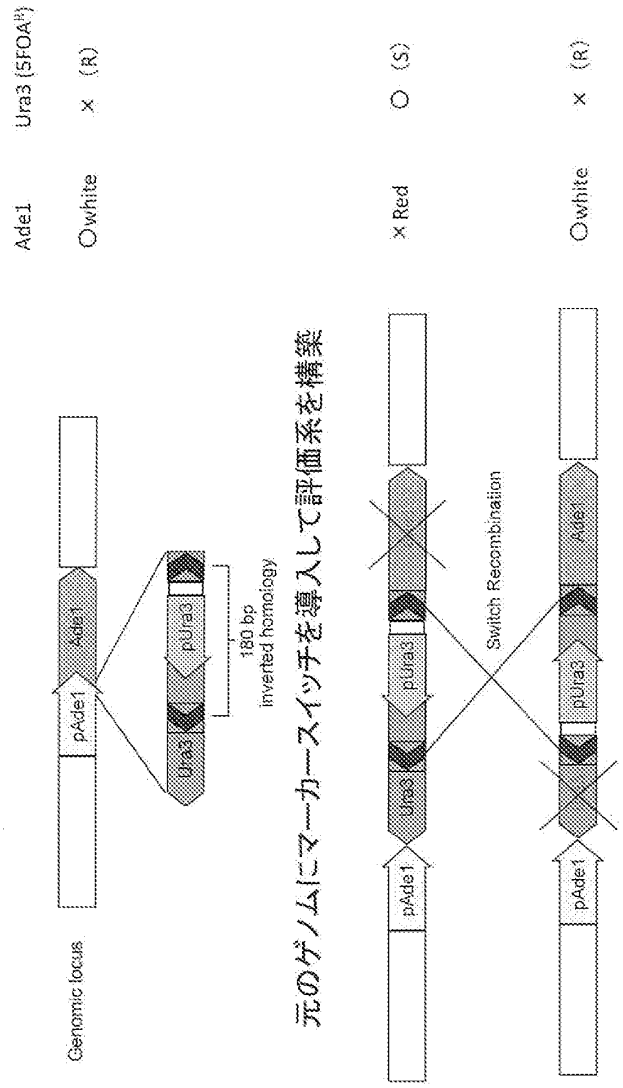
[図1]


誘導用変異
相補DNA
ターゲットDNA
脱アミノ化
クロスオーバー
相補鎖修復
変異導入
ex. loss of heterozygosity

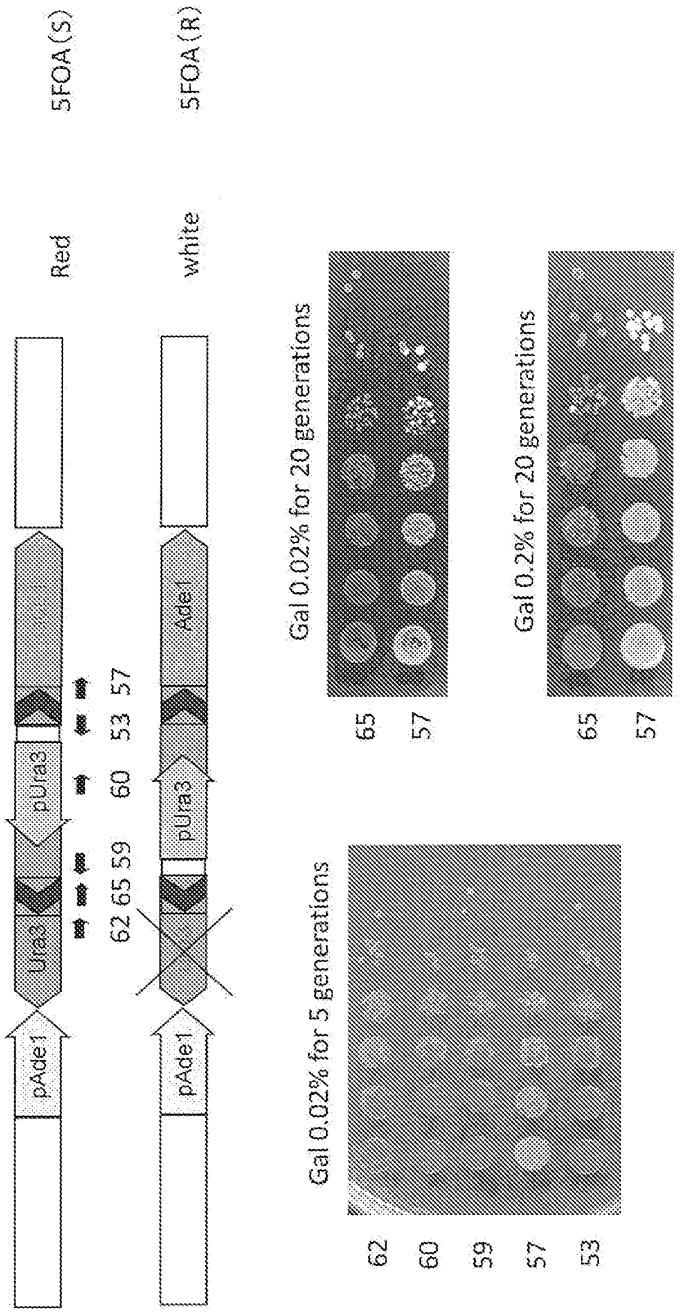
[図2]


dnCas9-CDA1
Template Vector
Mutation
Homologous fragment
Genomic locus
gRNA target
Mutation
Target13R
(SEQ ID NO:26)
Target7R
(SEQ ID NO:27)
(348b homology)
(SEQ ID NO:28)
(SEQ ID NO:29)
(SEQ ID NO:30)
Vector
Genomic
(SEQ ID NO:31)
Sequencing result of
canR colonies
Mutation rate/Sgen
Vector
dCas9 0.00652 1525-1059
nCas9 0.0217 1526-1059
dCas9 0.000408 1525-1149
nCas9 0.0129 1526-1149

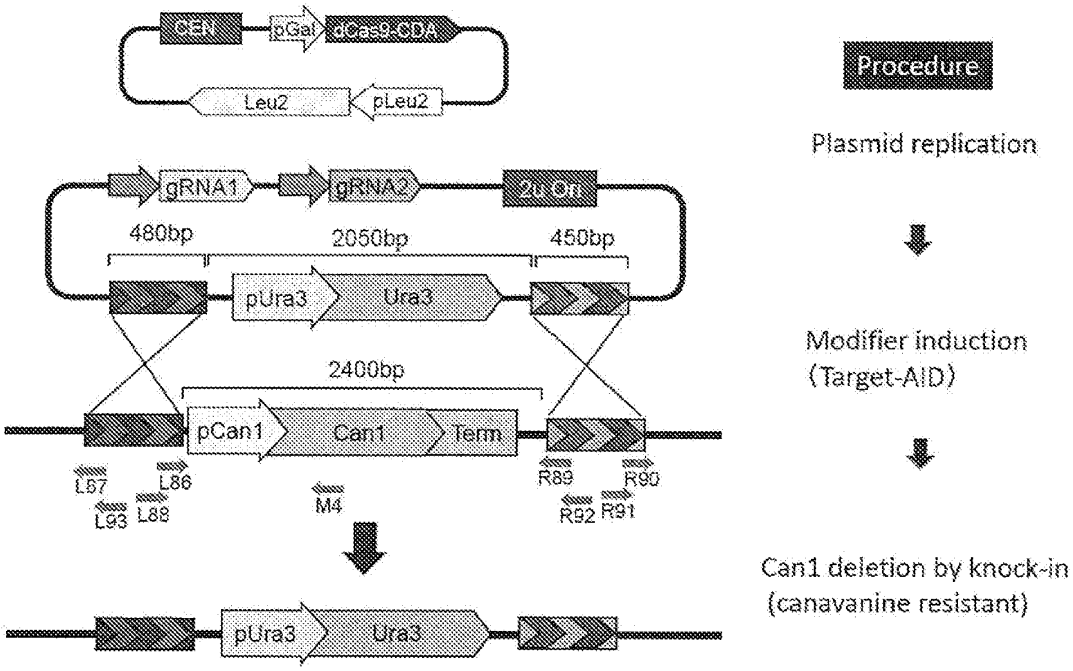
[図3]


Ade1
Ura3 (5FOA^R)
○white
× (R)
元のゲノムにマーカースイッチを導入して評価系を構築
Genomic locus
pAde1
Ade1
pUra3
Ura3
180 bp
inverted homology
× Red
○ (S)
Switch Recombination
○white
× (R)

[図4]

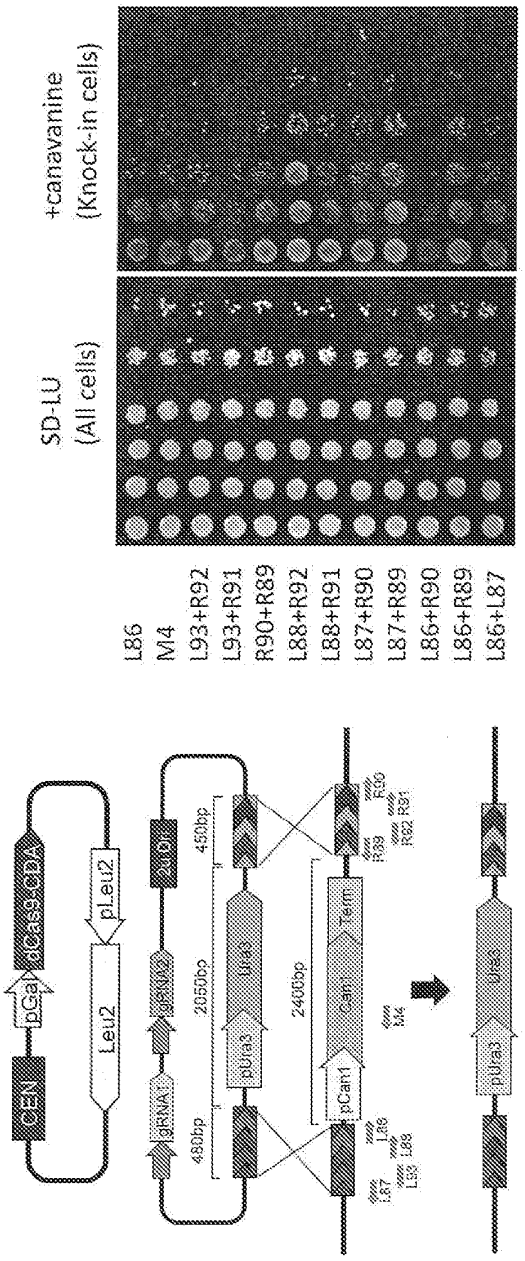

5FOA(S)
5FOA(R)
Red
white
pAde1
Ura3
pUra3
62 65 59 60 53 57
pAde1
Ade1
pUra3
Gal 0.02% for 20 generations
65
57
Gal 0.2% for 20 generations
65
57
Gal 0.02% for 5 generations
62
60
59
57
53

[図5]

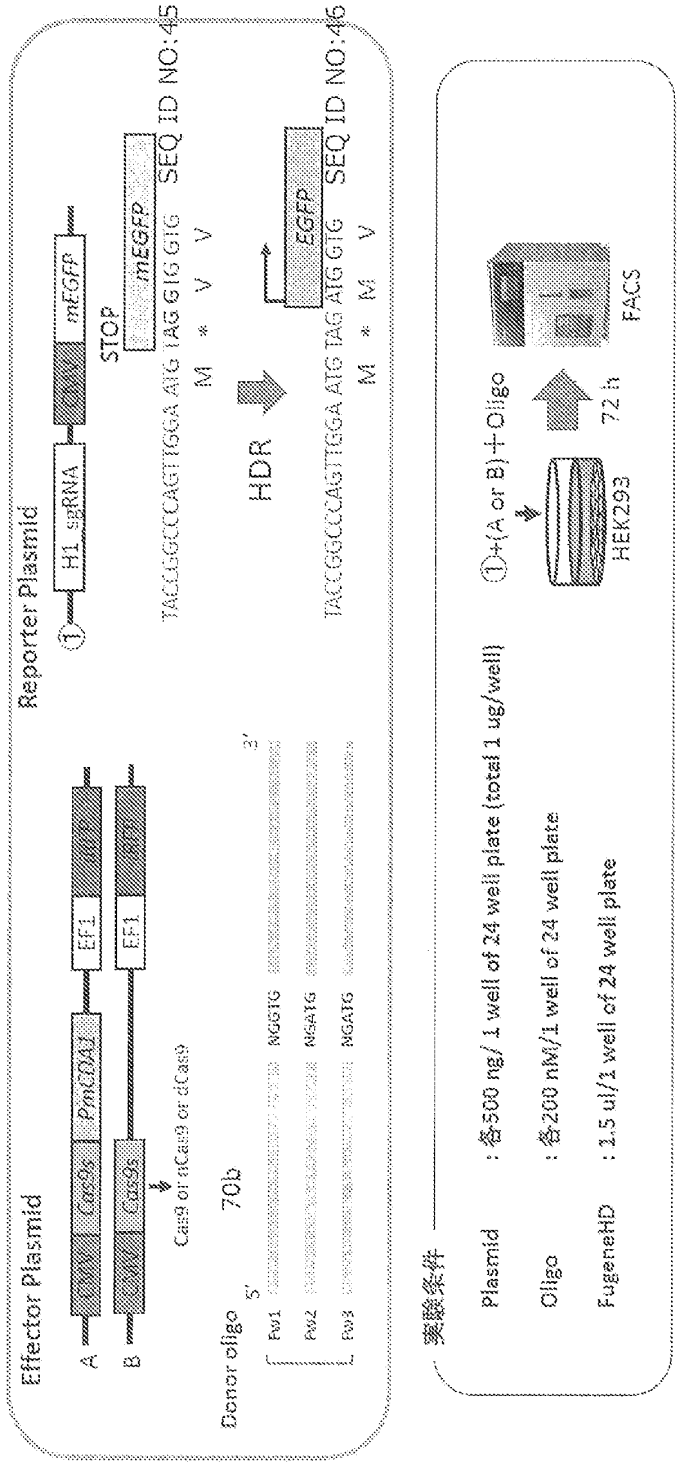


Can1遺伝子をノックアウトしてUra3遺伝子をノックイン

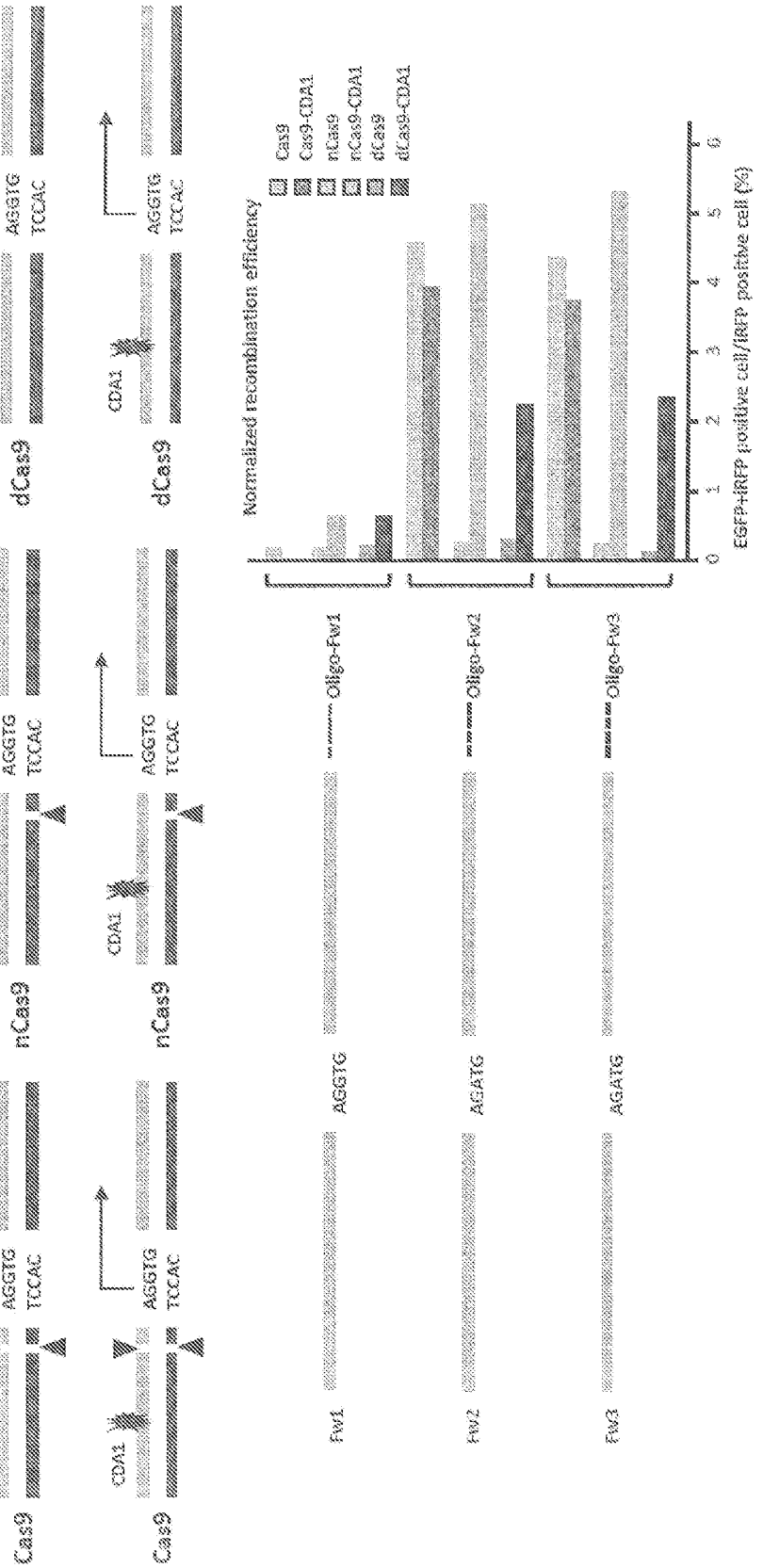
[図6]


+canavanine
(Knock-in cells)
SD-LU
(All 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
pLeu2
Leu2
gRNA2
gRNA1
2μori
480bp
2050bp
450bp
pUra3
Ura3
2400bp
Cen1
Term
pCan1
R90
R89
R88
R92
R91
L86
L88
L87
L93
M4
Ura3
pUra3

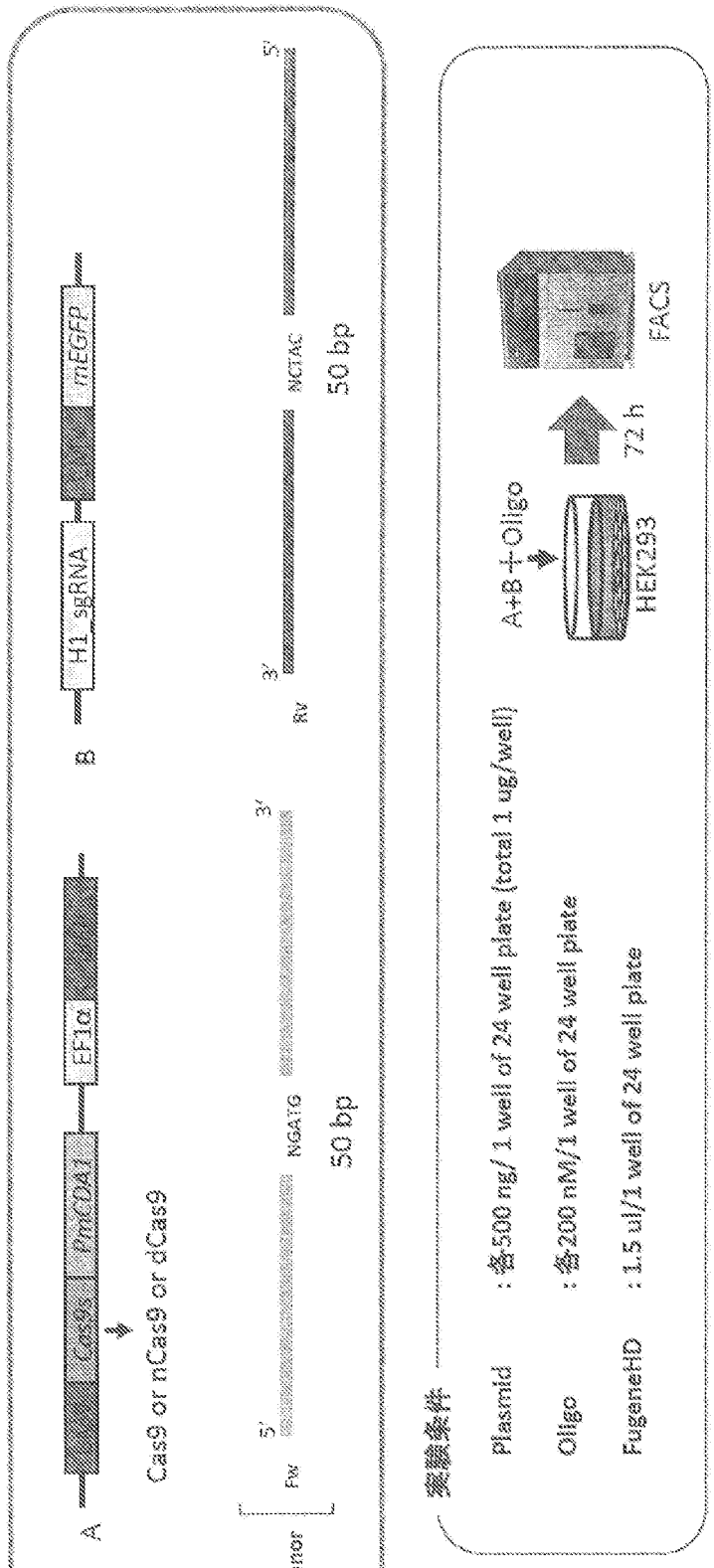
[図7]


Effector Plasmid
Reporter Plasmid
A
B
EF1
PmCDA1
Cas9n
Cas9n
EF1
Cas9
Cas9 or nCas9 or dCas9
Donor oligo
70b
5'
3'
Fw1
Fw2
Fw3
NGGTG
NGATG
NGATG
H1
sgRNA
mEGFP
STOP
mEGFP
SEQ ID NO:45
TACGGCCCAGTTGGA ATG TAG GTG GTG
M * V V
HDR
EGFP
SEQ ID NO:46
TACGGCCCAGTTGGA ATG TAG GTG GTG
M * M V
実験条件
Plasmid : 各500 ng/ 1 well of 24 well plate (total 1 ug/well)
Oligo : 各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

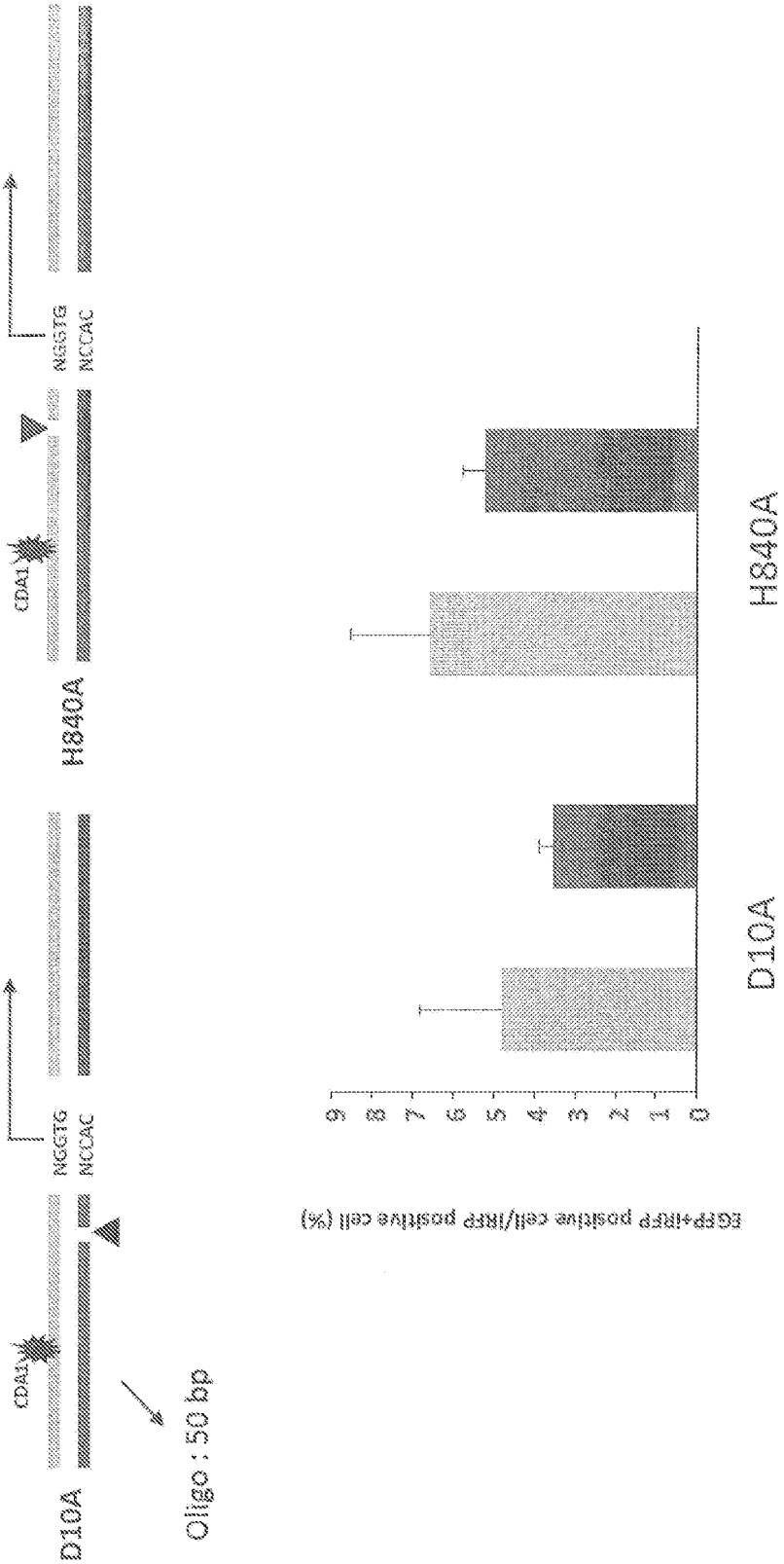
[図8]


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 (%)

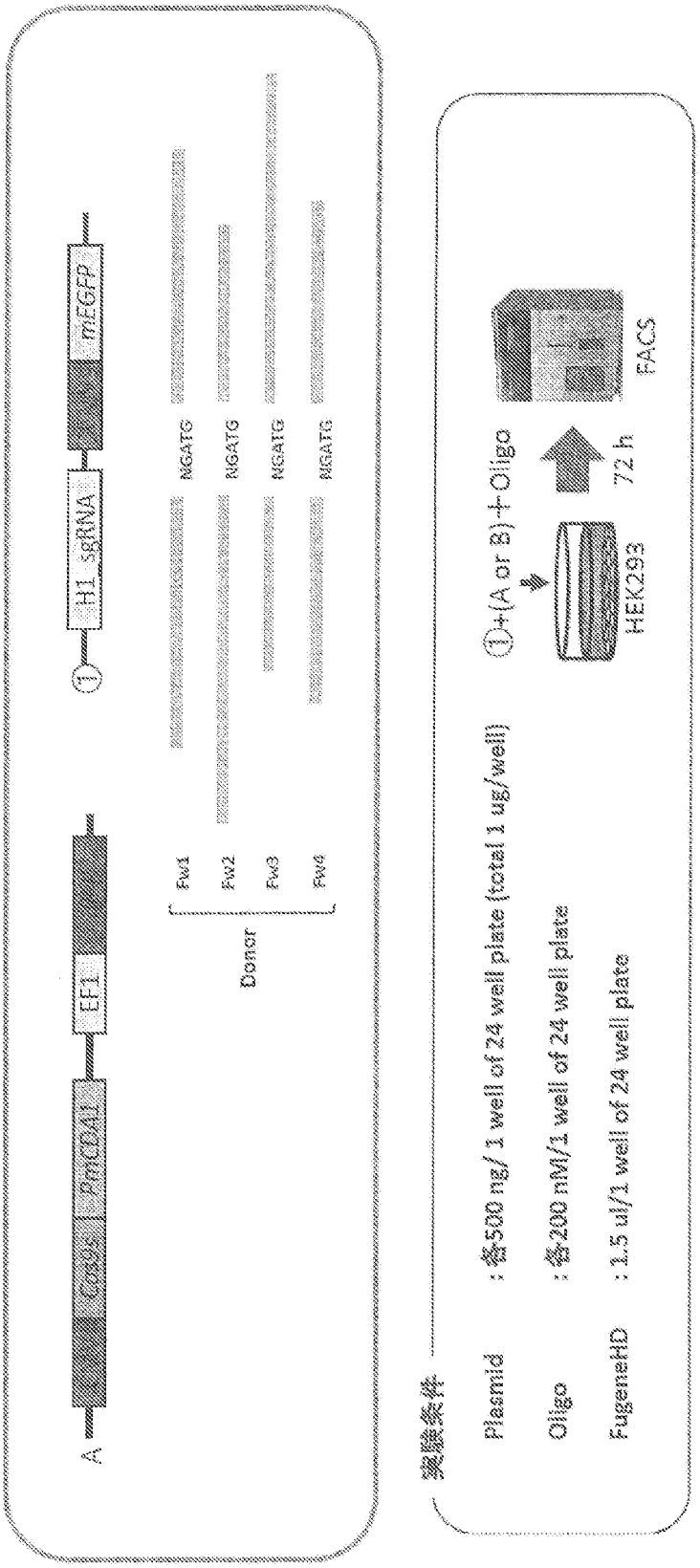
[図9]


A
Cas9
PmCDA1
EF1α
Cas9 or nCas9 or dCas9
B
H1
sgRNA
mEGFP
Donor
Fw
5'
3'
NGATG
50 bp
Rv
3'
5'
NCTAC
50 bp
実験条件
Plasmid : 各500 ng/ 1 well of 24 well plate (total 1 ug/well)
Oligo : 各200 nM/1 well of 24 well plate
FugeneHD : 1.5 ul/1 well of 24 well plate
A+B+Oligo
HEK293
72 h
FACS

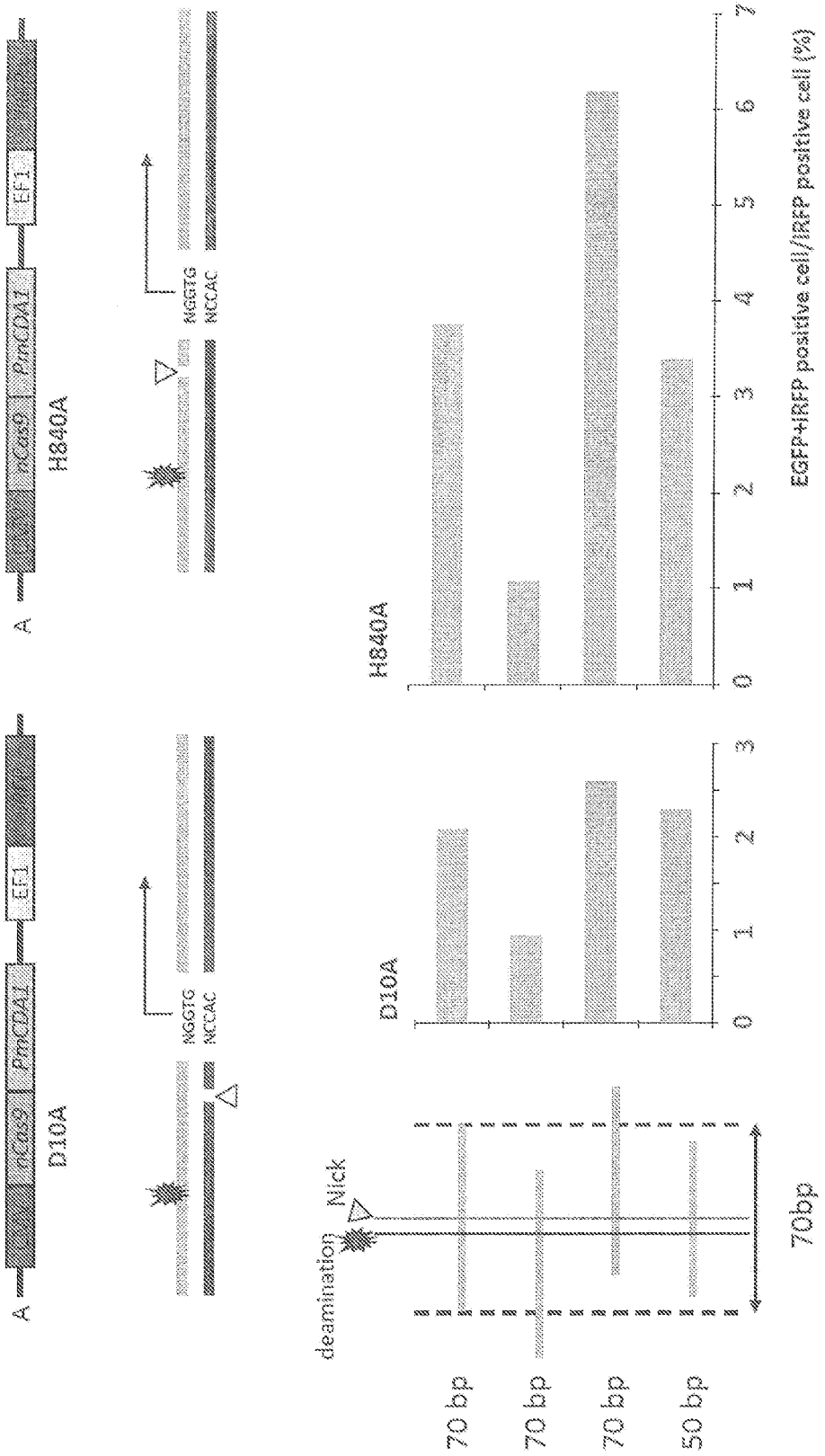
[図10]


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

[図11]


A
Cas9
PmCDA1
EF1
①
H1 sgRNA
mEGFP
Donor
Fw1
Fw2
Fw3
Fw4
MGATG
MGATG
MGATG
MGATG
実験条件
Plasmid
：各500 ng/ 1 well of 24 well plate (total 1 ug/well)
Oligo
：各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

[図12]


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