Fig. 1

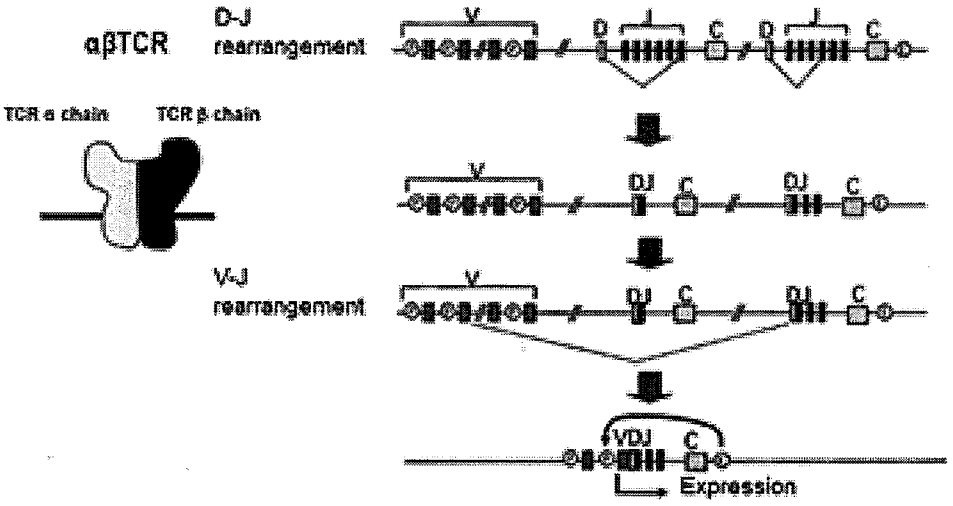

αβTCR
D-J
rearrangement
V
D
J
C
TCR α chain
TCR β chain
DJ
V-J
rearrangement
VDJ
Expression

Fig. 2

Fig. 2-1

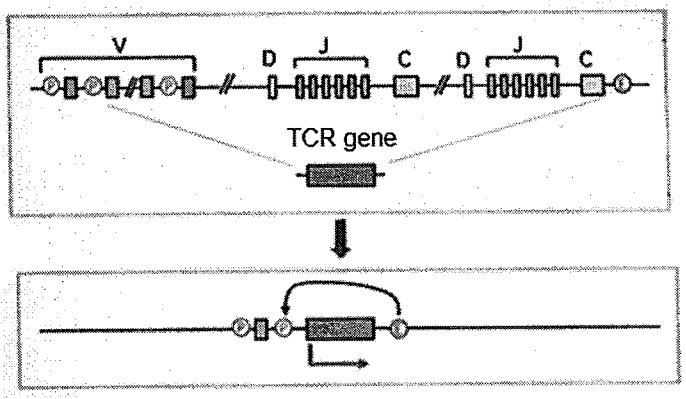


Fig. 2-2

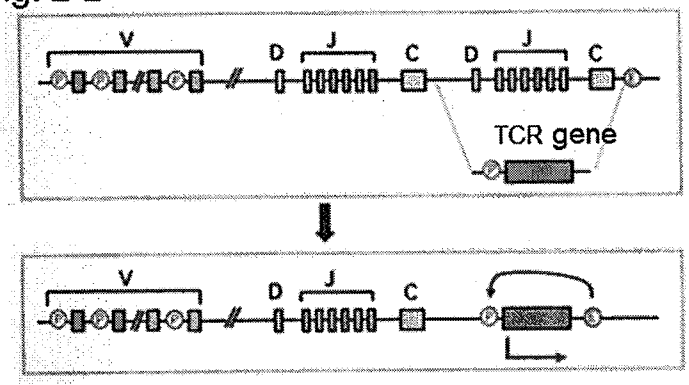


Fig. 2-3

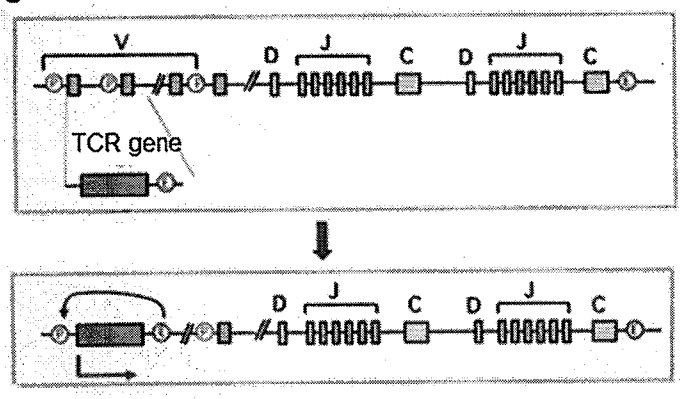


Fig. 3-1

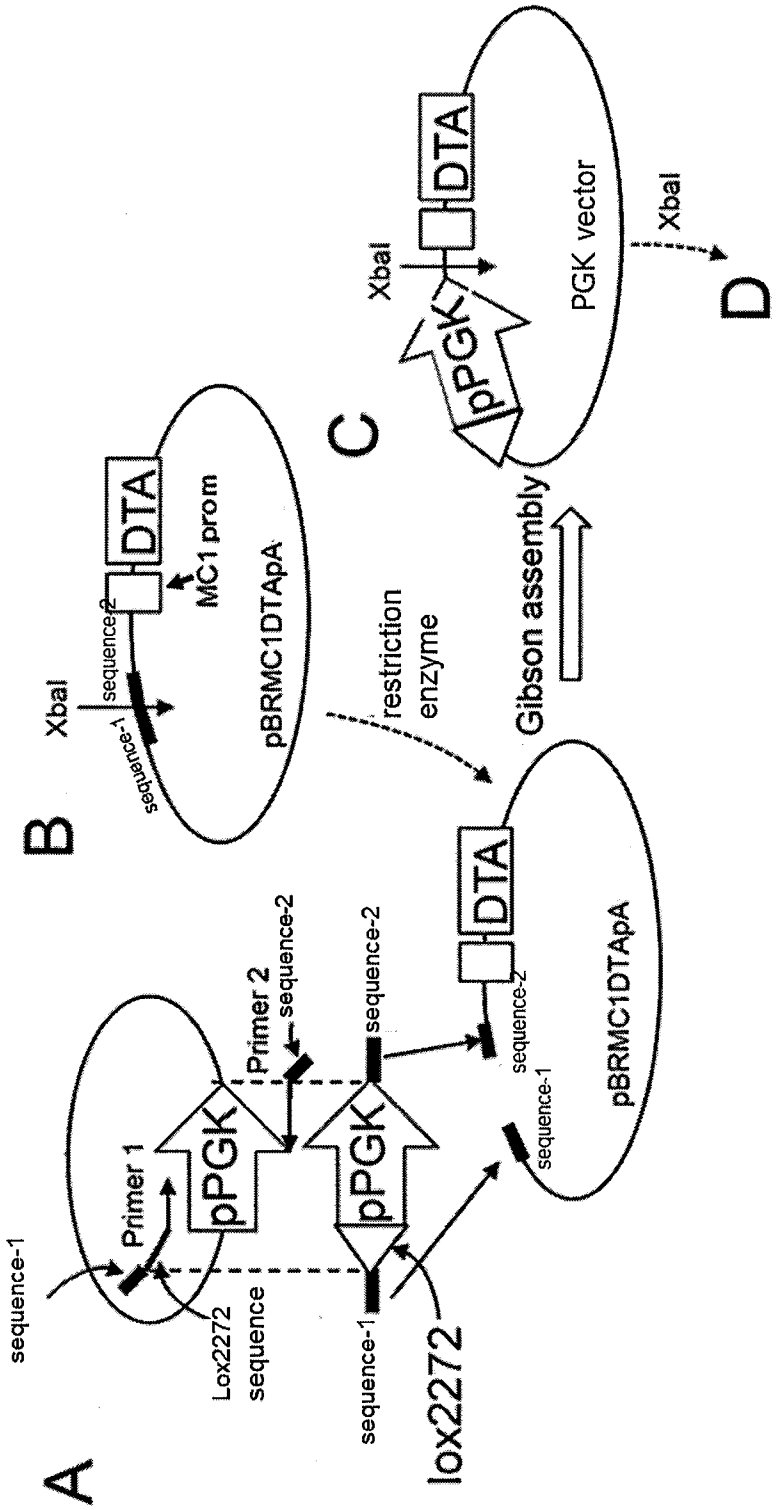

A
B
C
D
sequence-1
Primer 1
pPGK
Lox2272
sequence
Primer 2
sequence-2
sequence-2
sequence-1
lox2272
pPGK
DTA
sequence-2
sequence-1
pBRMC1DTApA
restriction
enzyme
Gibson assembly
XbaI
sequence-2
sequence-1
MC1 prom
DTA
pBRMC1DTApA
XbaI
pPGK
DTA
PGK vector
XbaI

Fig. 3-2

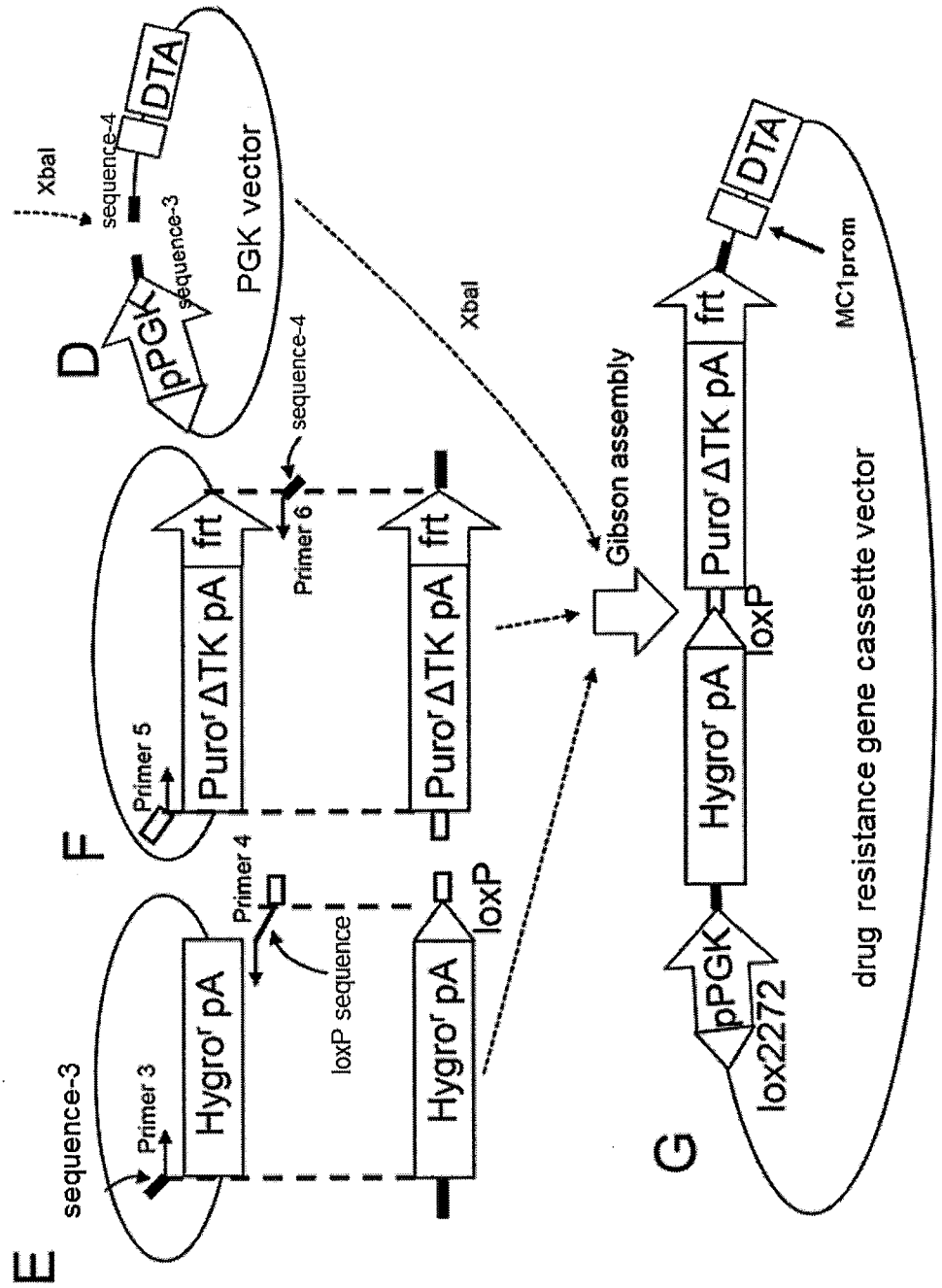

D
PGK vector
XbaI
sequence-4
DTA
sequence-3
pPGK
sequence-4
Primer 6
F
Primer 5
Puroʳ ΔTK pA
frt
Puroʳ ΔTK pA
frt
Primer 4
loxP sequence
loxP
E
sequence-3
Primer 3
Hygroʳ pA
Hygroʳ pA
XbaI
Gibson assembly
G
DTA
MC1prom
frt
Puroʳ ΔTK pA
loxP
Hygroʳ pA
pPGK
lox2272
drug resistance gene cassette vector

Fig. 4

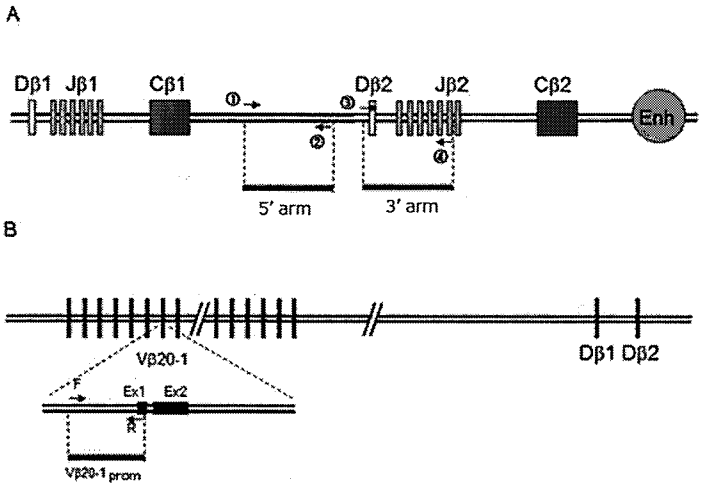

A
Dβ1 Jβ1
Cβ1
Dβ2
Jβ2
Cβ2
Enh
5' arm
3' arm
B
Vβ20-1
Dβ1 Dβ2
F
Ex1
Ex2
R
Vβ20-1 prom

Fig. 5

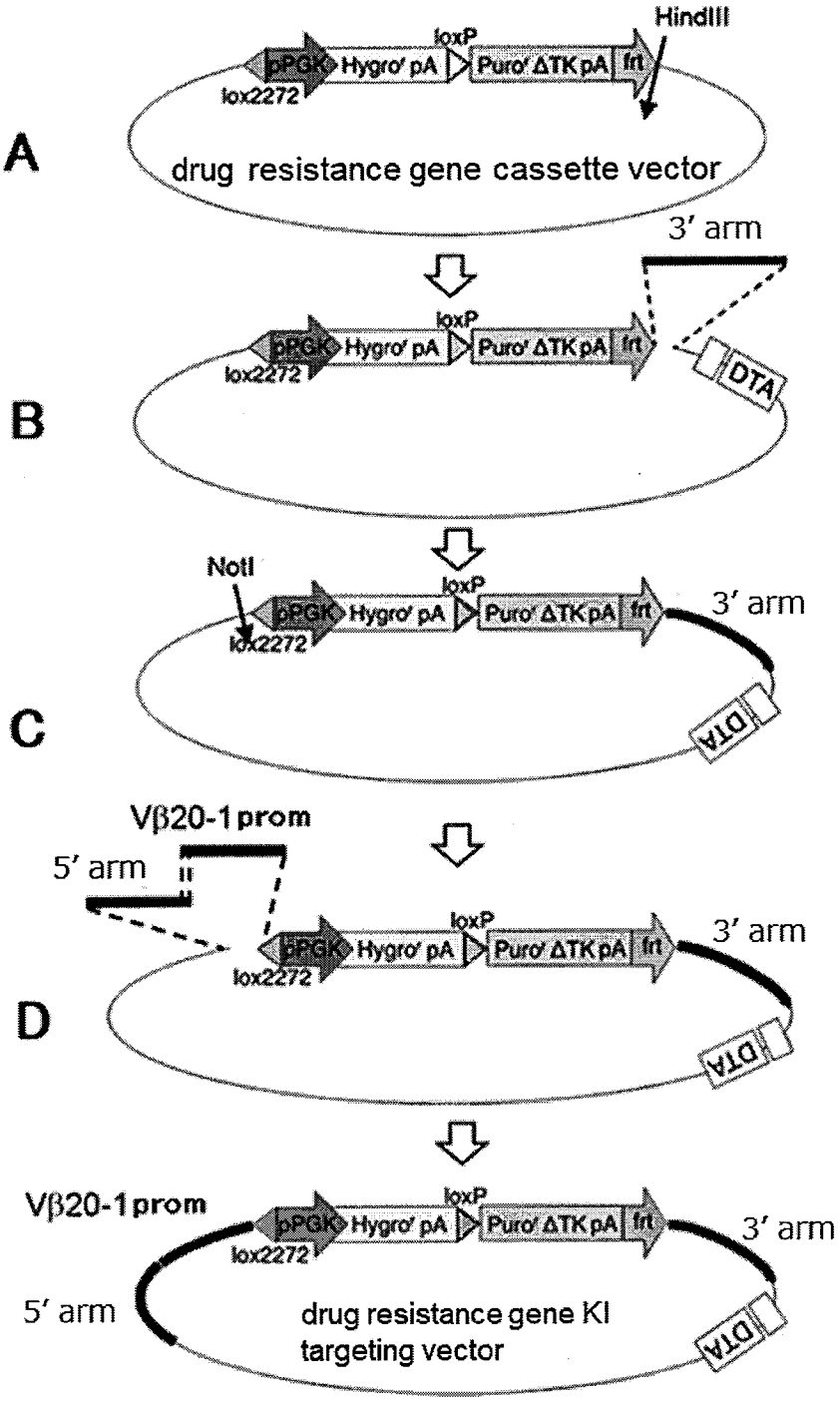

A
HindIII
loxP
pPGK Hygro' pA
Puro' ΔTK pA
frt
lox2272
drug resistance gene cassette vector
3' arm
B
DTA
NotI
C
Vβ20-1 prom
5' arm
D
drug resistance gene KI
targeting vector

Fig. 6

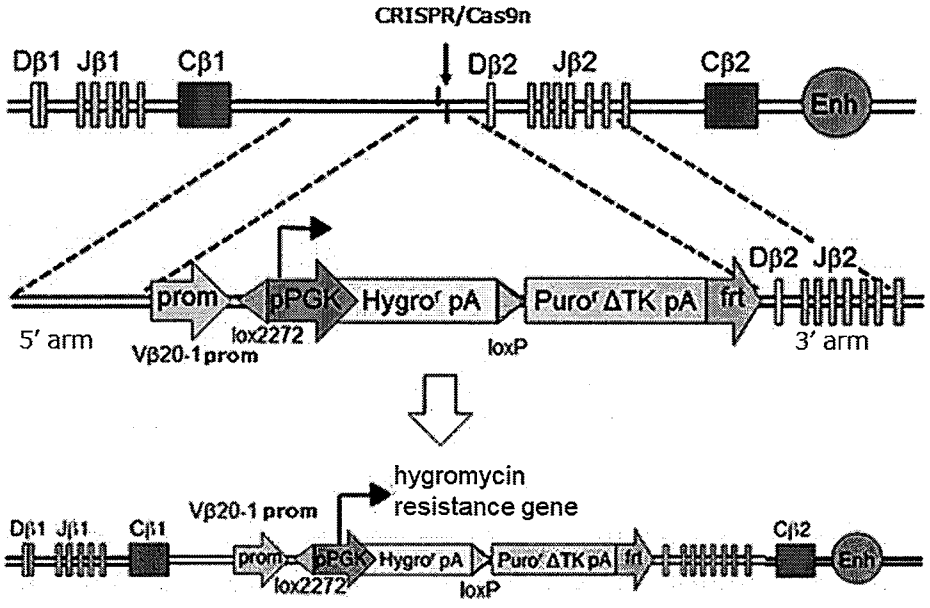

CRISPR/Cas9n
Dβ1 Jβ1 Cβ1
Dβ2 Jβ2 Cβ2
Enh
prom
pPGK
Hygroʳ pA
Puroʳ ΔTK pA
frt
Dβ2 Jβ2
5′ arm
Vβ20-1 prom
lox2272
loxP
3′ arm
hygromycin
resistance gene
Vβ20-1 prom
Dβ1 Jβ1 Cβ1
Cβ2
Enh
prom
pPGK
Hygroʳ pA
Puroʳ ΔTK pA
frt
lox2272
loxP

Fig. 7

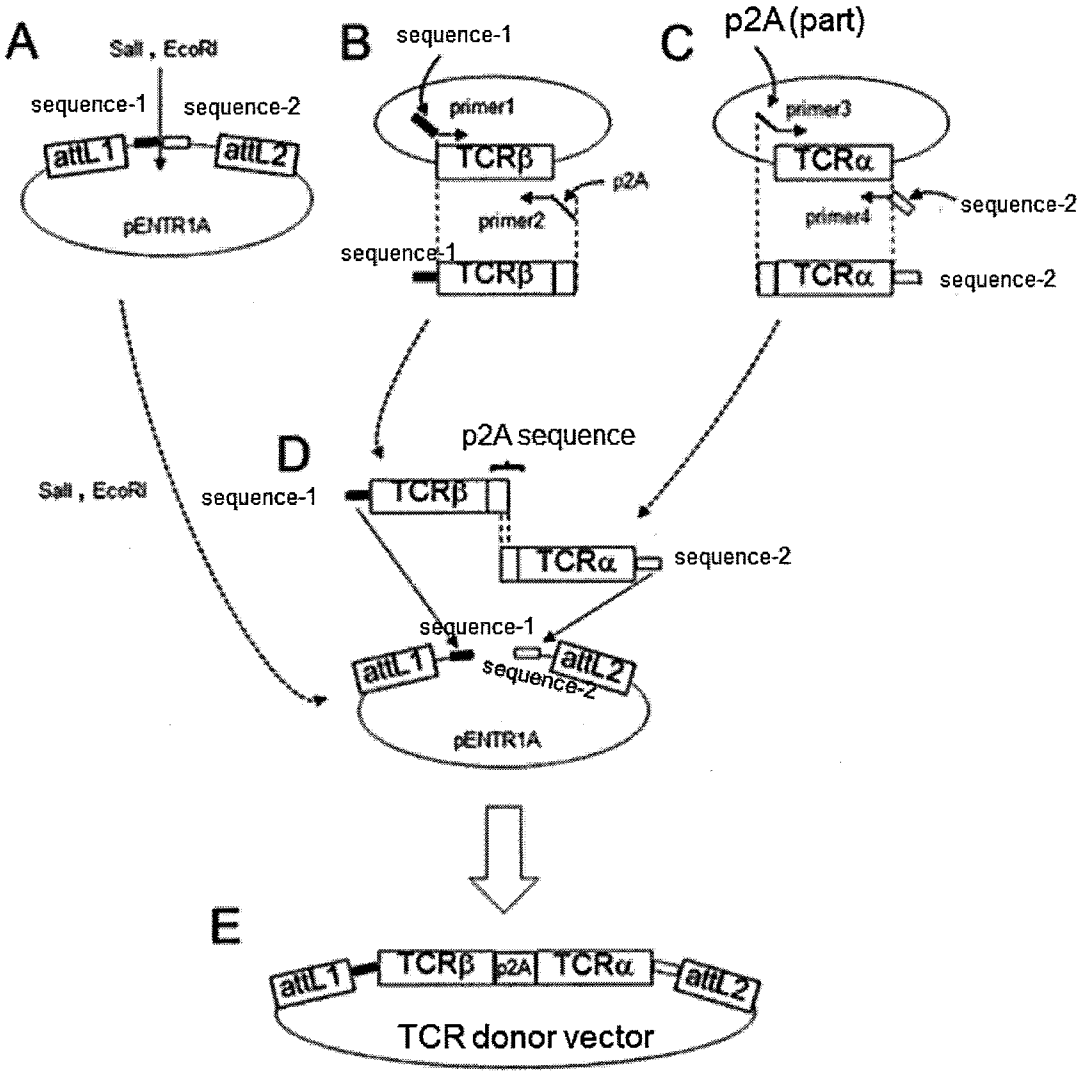

A
SalI , EcoRI
sequence-1
sequence-2
attL1
attL2
pENTR1A
B
sequence-1
primer1
TCRβ
p2A
primer2
sequence-1
TCRβ
C
p2A (part)
primer3
TCRα
primer4
sequence-2
TCRα
sequence-2
D
p2A sequence
SalI , EcoRI
sequence-1
TCRβ
TCRα
sequence-2
sequence-1
attL1
attL2
sequence-2
pENTR1A
E
attL1
TCRβ
p2A
TCRα
attL2
TCR donor vector

Fig. 8

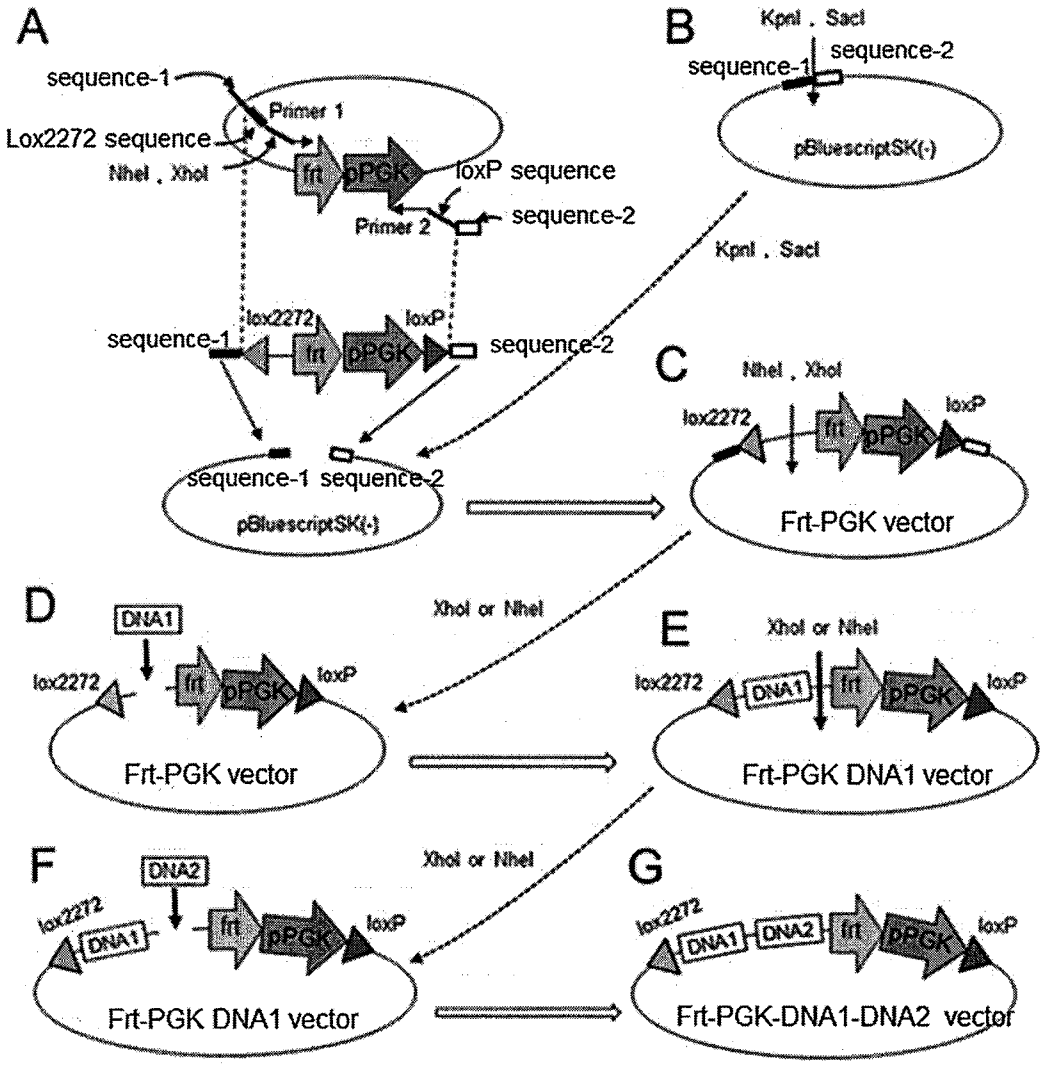

A
sequence-1
Lox2272 sequence
NheI , XhoI
Primer 1
frt
pPGK
loxP sequence
Primer 2
sequence-2
sequence-1
lox2272
loxP
sequence-2
sequence-1 sequence-2
pBluescriptSK(-)
B
KpnI , SacI
sequence-1
sequence-2
pBluescriptSK(-)
KpnI , SacI
C
NheI , XhoI
lox2272
loxP
Frt-PGK vector
D
DNA1
lox2272
loxP
Frt-PGK vector
XhoI or NheI
E
XhoI or NheI
lox2272
DNA1
loxP
Frt-PGK DNA1 vector
F
DNA2
lox2272
DNA1
loxP
Frt-PGK DNA1 vector
XhoI or NheI
G
lox2272
DNA1
DNA2
loxP
Frt-PGK-DNA1-DNA2 vector

Fig. 9

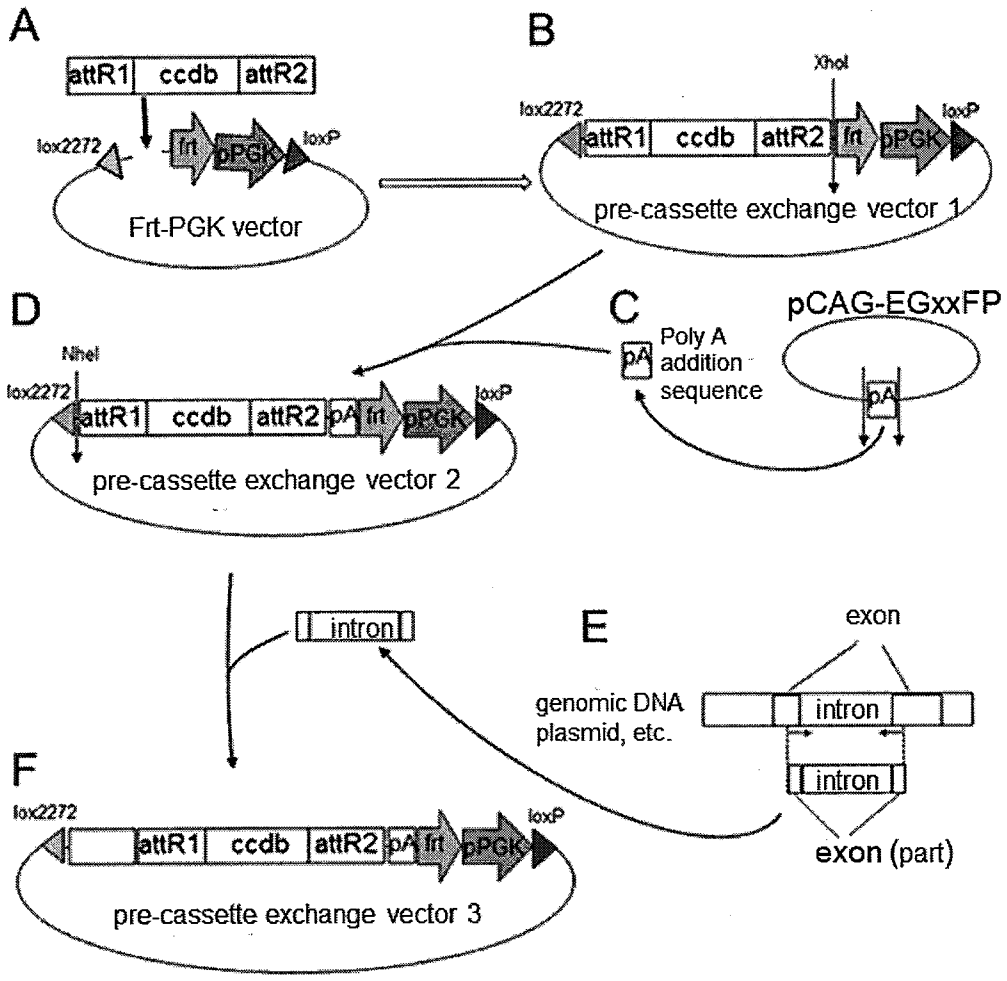

A
attR1
ccdb
attR2
lox2272
frt
pPGK
loxP
Frt-PGK vector
B
XhoI
lox2272
attR1
ccdb
attR2
frt
pPGK
loxP
pre-cassette exchange vector 1
C
pCAG-EGxxFP
pA
Poly A addition sequence
pA
D
NheI
lox2272
attR1
ccdb
attR2
pA
frt
pPGK
loxP
pre-cassette exchange vector 2
intron
E
exon
genomic DNA plasmid, etc.
intron
intron
exon (part)
F
lox2272
attR1
ccdb
attR2
pA
frt
pPGK
loxP
pre-cassette exchange vector 3

Fig. 10

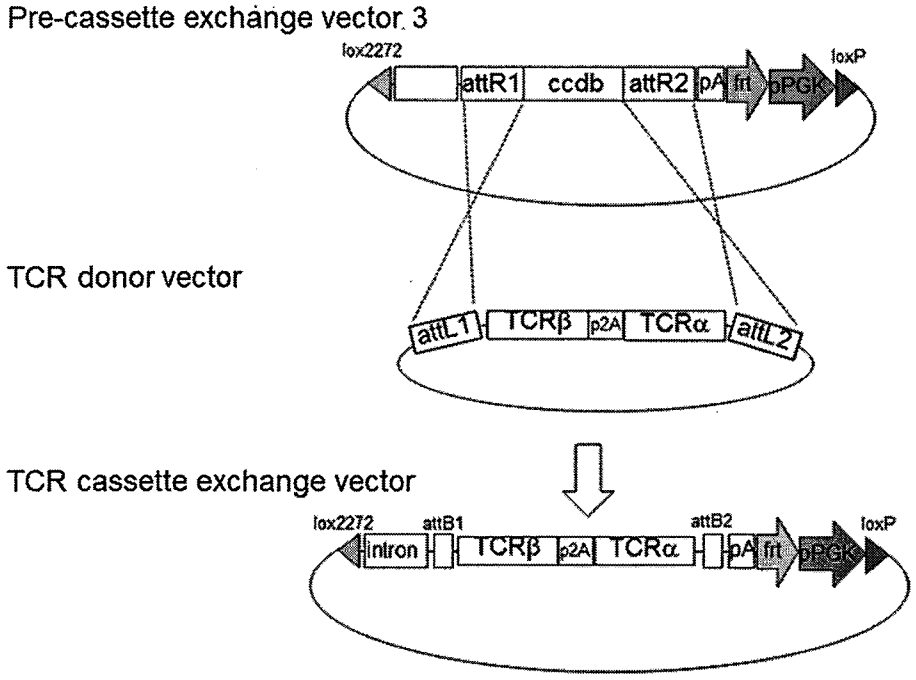

Pre-cassette exchange vector 3
lox2272
attR1
ccdb
attR2
pA
frt
pPGK
loxP
TCR donor vector
attL1
TCRβ
p2A
TCRα
attL2
TCR cassette exchange vector
lox2272
attB1
intron
TCRβ
p2A
TCRα
attB2
pA
frt
pPGK
loxP

Fig. 11

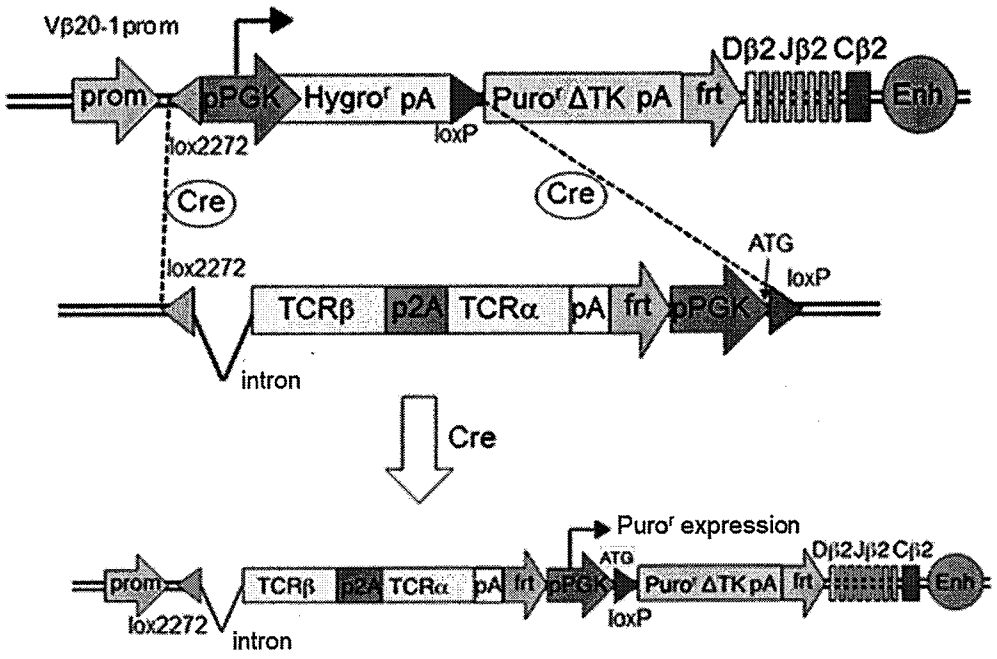

Vβ20-1prom
prom
pPGK
Hygro^r pA
Puro^r ΔTK pA
frt
Dβ2 Jβ2 Cβ2
Enh
lox2272
loxP
Cre
Cre
ATG
loxP
lox2272
TCRβ
p2A
TCRα
pA
frt
pPGK
intron
Cre
Puro^r expression
prom
TCRβ
p2A
TCRα
pA
frt
pPGK
ATG
Puro^r ΔTK pA
frt
Dβ2Jβ2 Cβ2
Enh
lox2272
intron
loxP

Fig. 12

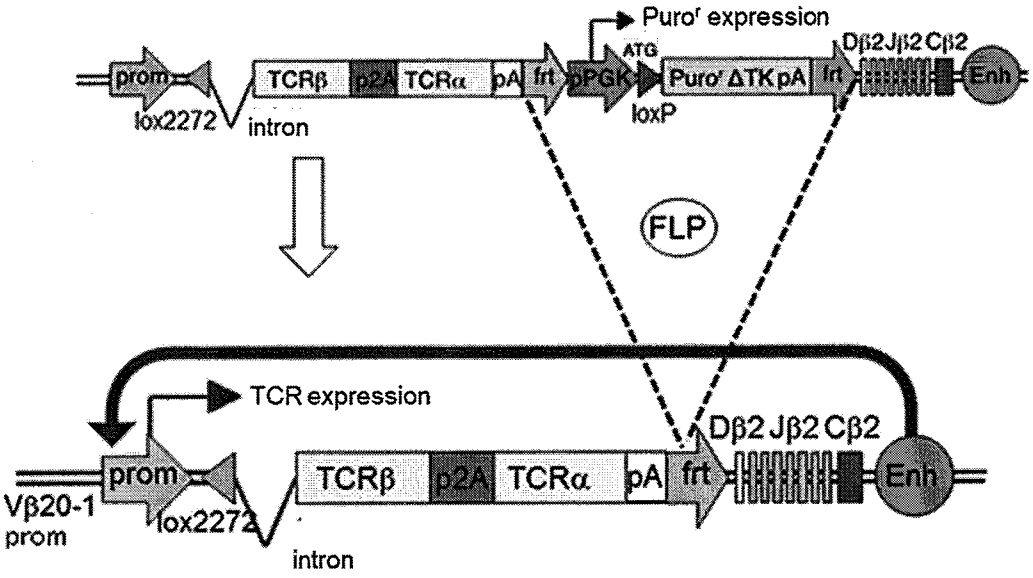

Puro^r expression
prom
TCRβ
p2A
TCRα
pA
frt
pPGK
ATG
Puro^r ΔTK pA
frt
Dβ2Jβ2 Cβ2
Enh
lox2272
intron
loxP
FLP
TCR expression
Dβ2 Jβ2 Cβ2
prom
TCRβ
p2A
TCRα
pA
frt
Enh
Vβ20-1
prom
lox2272
intron

Fig. 13

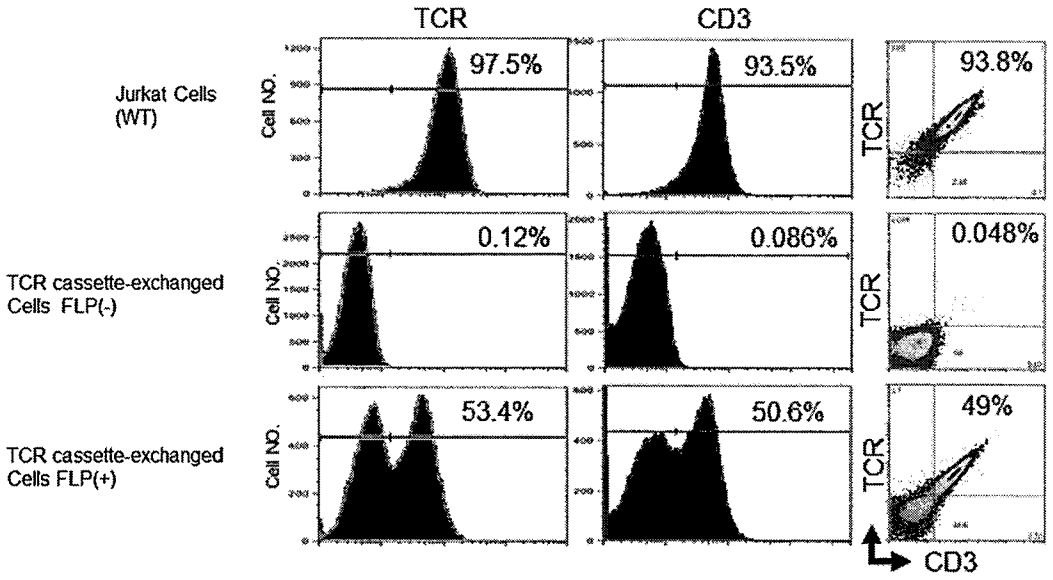

TCR
CD3
Jurkat Cells
(WT)
97.5%
93.5%
93.8%
TCR cassette-exchanged
Cells FLP(-)
0.12%
0.086%
0.048%
TCR cassette-exchanged
Cells FLP(+)
53.4%
50.6%
49%
Cell NO.
TCR
CD3

Fig. 14

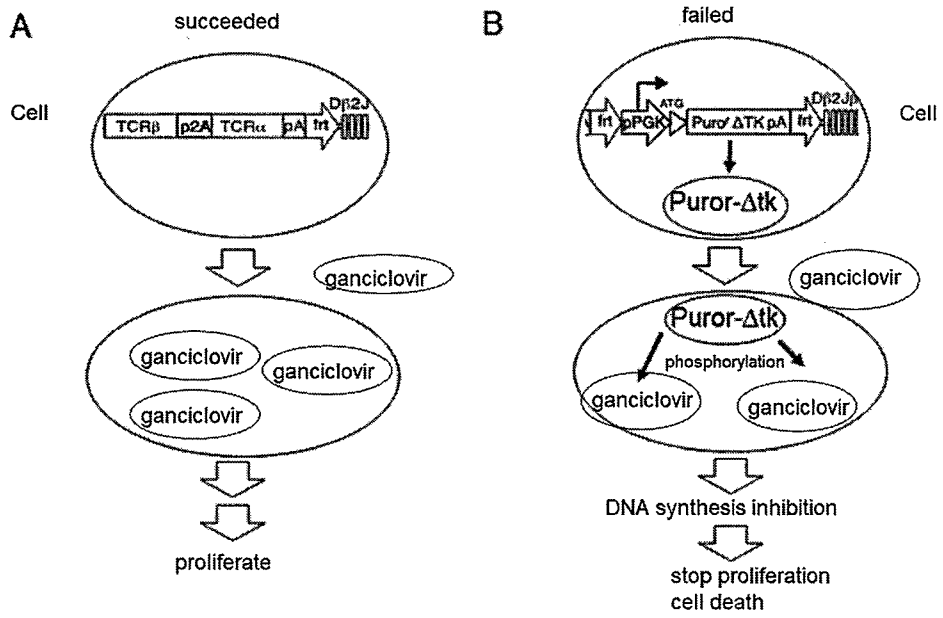

A
succeeded
Cell
TCRβ
p2A
TCRα
pA
frt
Dβ2J
ganciclovir
ganciclovir
ganciclovir
ganciclovir
proliferate
B
failed
Cell
frt
pPGK
ATG
Puror ΔTK pA
frt
Dβ2Jβ
Puror-Δtk
ganciclovir
Puror-Δtk
phosphorylation
ganciclovir
ganciclovir
DNA synthesis inhibition
stop proliferation
cell death

Fig. 15

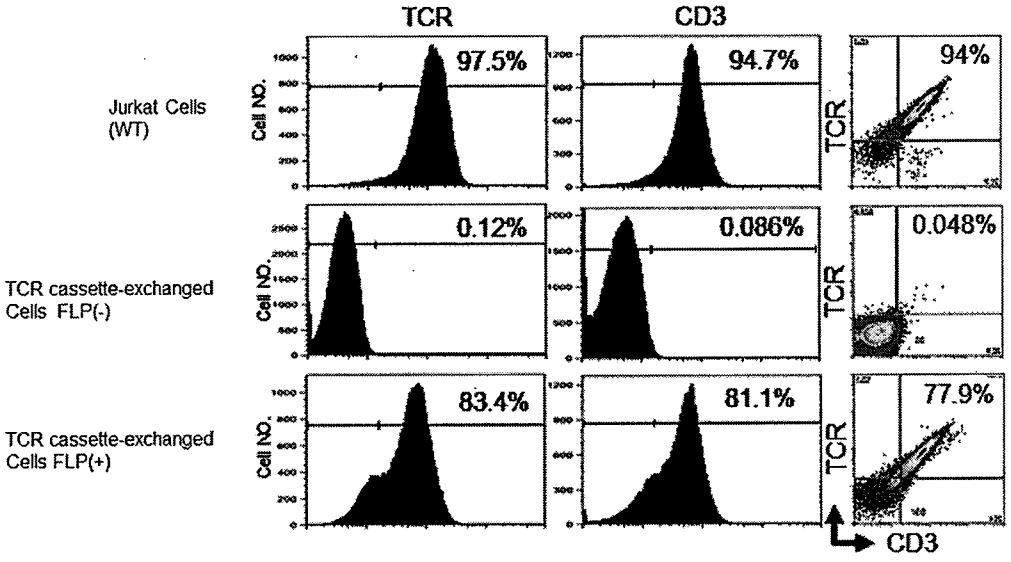

TCR
CD3
Jurkat Cells
(WT)
97.5%
94.7%
94%
TCR cassette-exchanged
Cells FLP(-)
0.12%
0.086%
0.048%
TCR cassette-exchanged
Cells FLP(+)
83.4%
81.1%
77.9%
Cell NO.
TCR
CD3

Fig. 16

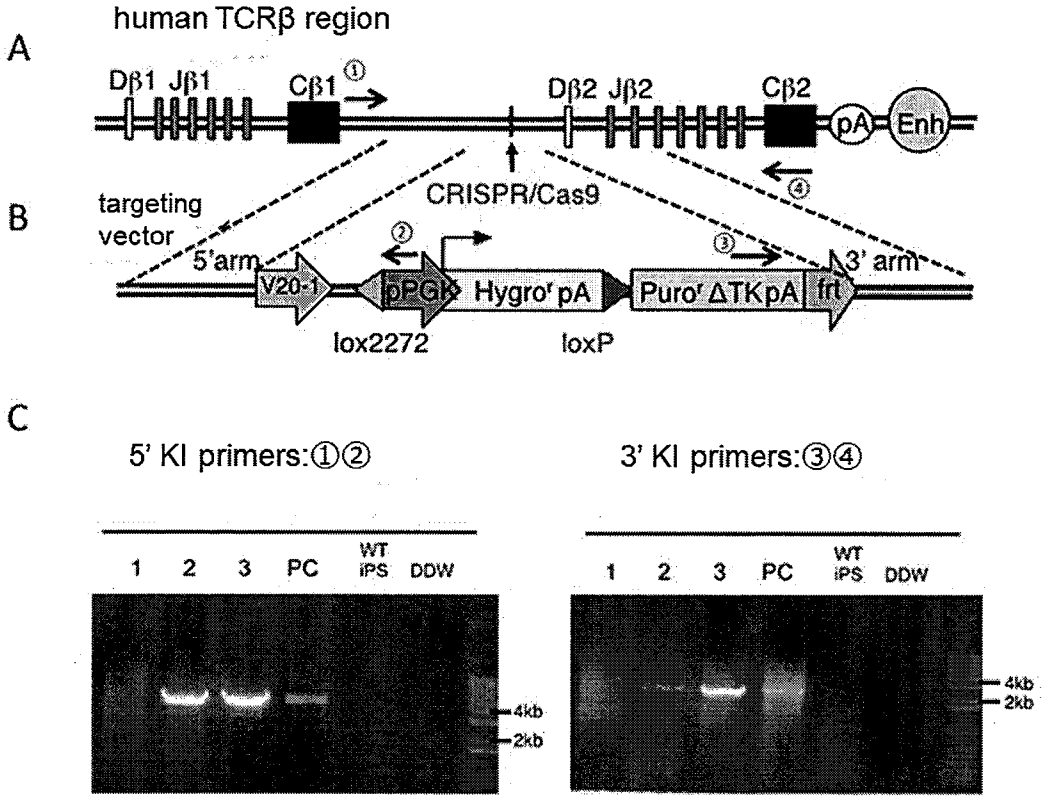

A
human TCRβ region
Dβ1 Jβ1
Cβ1
①
Dβ2 Jβ2
Cβ2
pA
Enh
B
targeting vector
5'arm
CRISPR/Cas9
②
③
④
3' arm
V20-1
pPGK
Hygro' pA
Puro' ΔTK pA
frt
lox2272
loxP
C
5' KI primers:①②
3' KI primers:③④
1
2
3
PC
WT iPS
DDW
4kb
2kb

Fig. 17

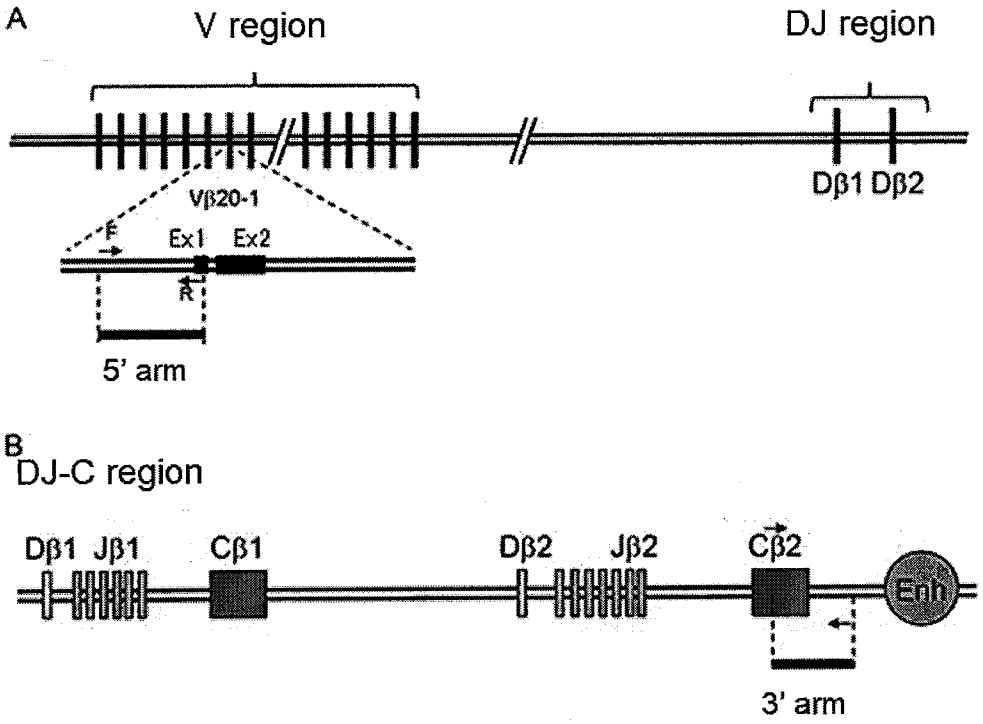

A
V region
DJ region
Vβ20-1
Dβ1 Dβ2
F
Ex1 Ex2
R
5' arm
B
DJ-C region
Dβ1 Jβ1 Cβ1 Dβ2 Jβ2 Cβ2 Enh
3' arm

Fig. 18

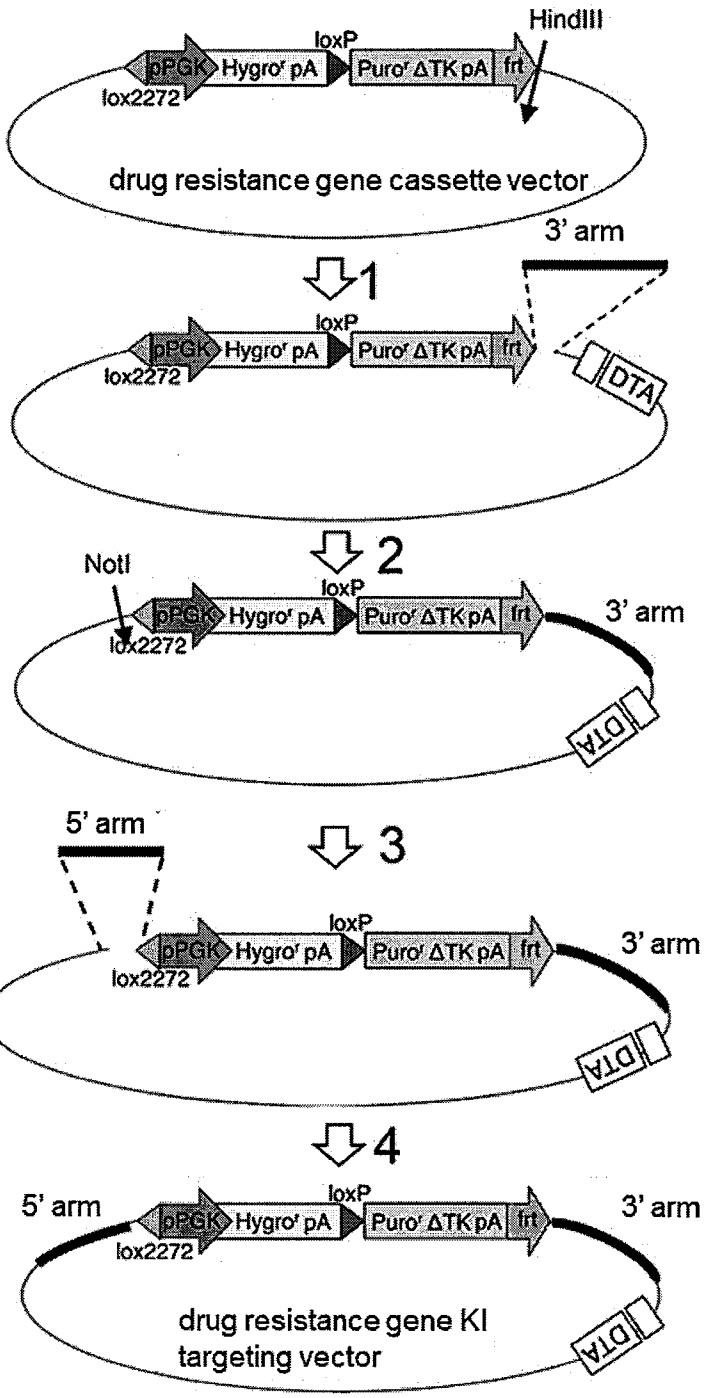

HindIII
loxP
pPGK
Hygror pA
Puror ΔTK pA
frt
lox2272
drug resistance gene cassette vector
3' arm
1
DTA
NotI
2
5' arm
3
4
drug resistance gene KI
targeting vector

Fig. 19

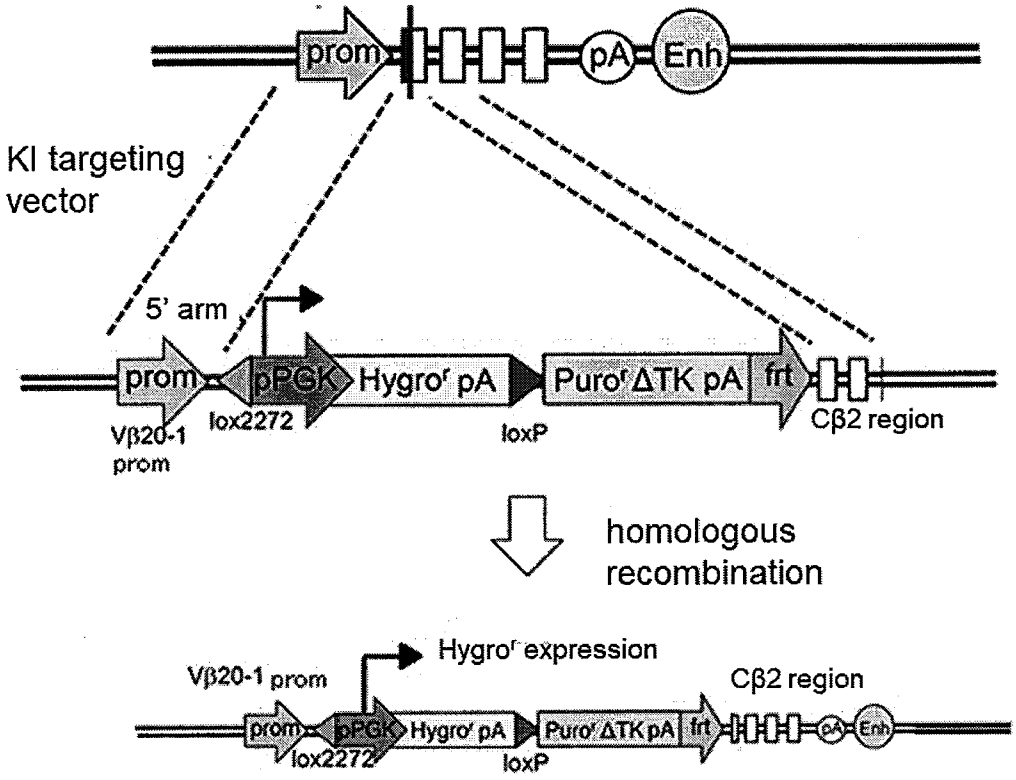

prom
pA
Enh
KI targeting vector
5' arm
prom
pPGK
Hygro^r pA
Puro^r ΔTK pA
frt
Vβ20-1 prom
lox2272
loxP
Cβ2 region
homologous recombination
Vβ20-1 prom
Hygro^r expression
Cβ2 region
prom
pPGK
Hygro^r pA
Puro^r ΔTK pA
frt
pA
Enh
lox2272
loxP

Fig. 20

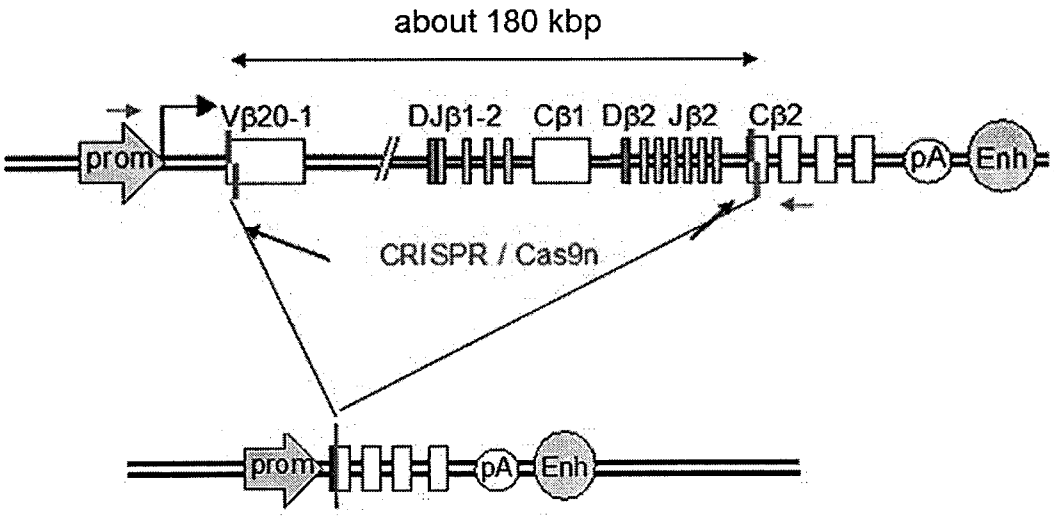

about 180 kbp
Vβ20-1
DJβ1-2
Cβ1
Dβ2
Jβ2
Cβ2
prom
pA
Enh
CRISPR / Cas9n
prom
pA
Enh

Fig. 21

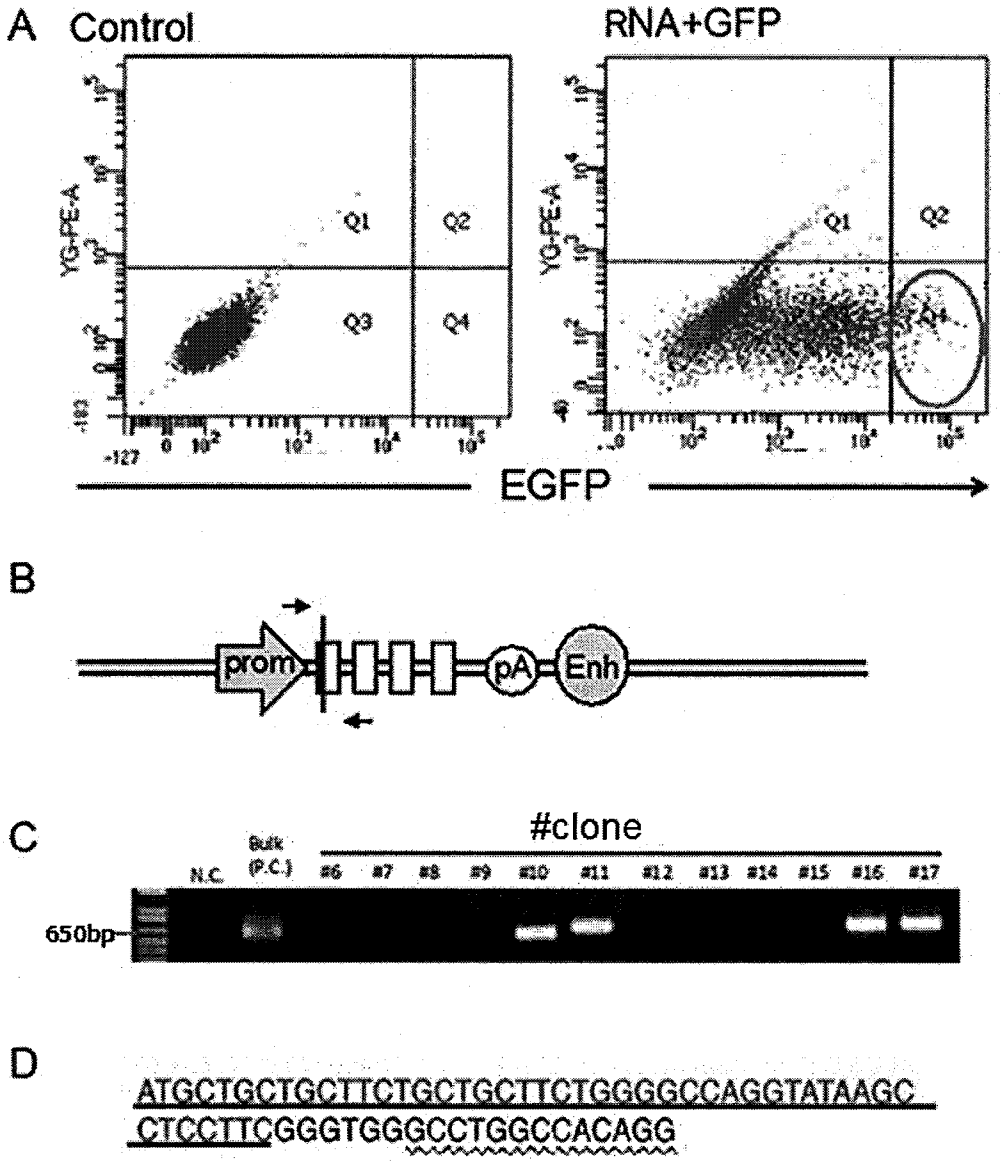

A
Control
RNA+GFP
YG-PE-A
Q1
Q2
Q3
Q4
EGFP
B
prom
pA
Enh
C
#clone
N.C.
Bulk (P.C.)
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
650bp
D
ATGCTGCTGCTTCTGCTGCTTCTGGGGCCAGGTATAAGC
CTCCTTCGGGTGGGCCTGGCCACAGG

Fig. 22

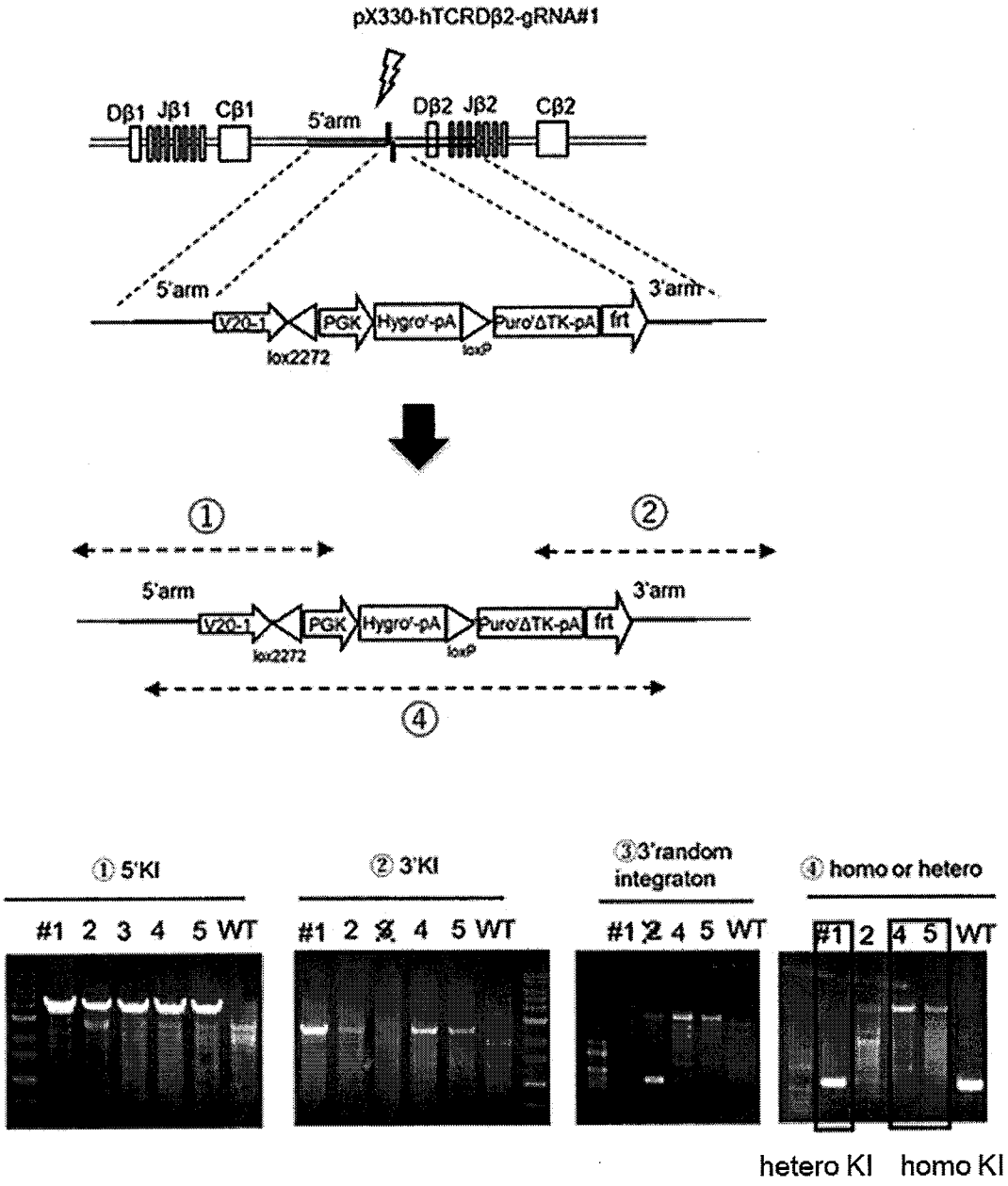

pX330-hTCRDβ2-gRNA#1
Dβ1 Jβ1 Cβ1
5'arm
Dβ2 Jβ2 Cβ2
5'arm
3'arm
V20-1
lox2272
PGK
Hygro'-pA
loxP
Puro'ΔTK-pA
frt
①
②
5'arm
3'arm
④
① 5'KI
② 3'KI
③3'random integraton
④ homo or hetero
#1 2 3 4 5 WT
#1 2 4 5 WT
#1 2 4 5 WT
#1 2 4 5 WT
hetero KI
homo KI

Fig. 23

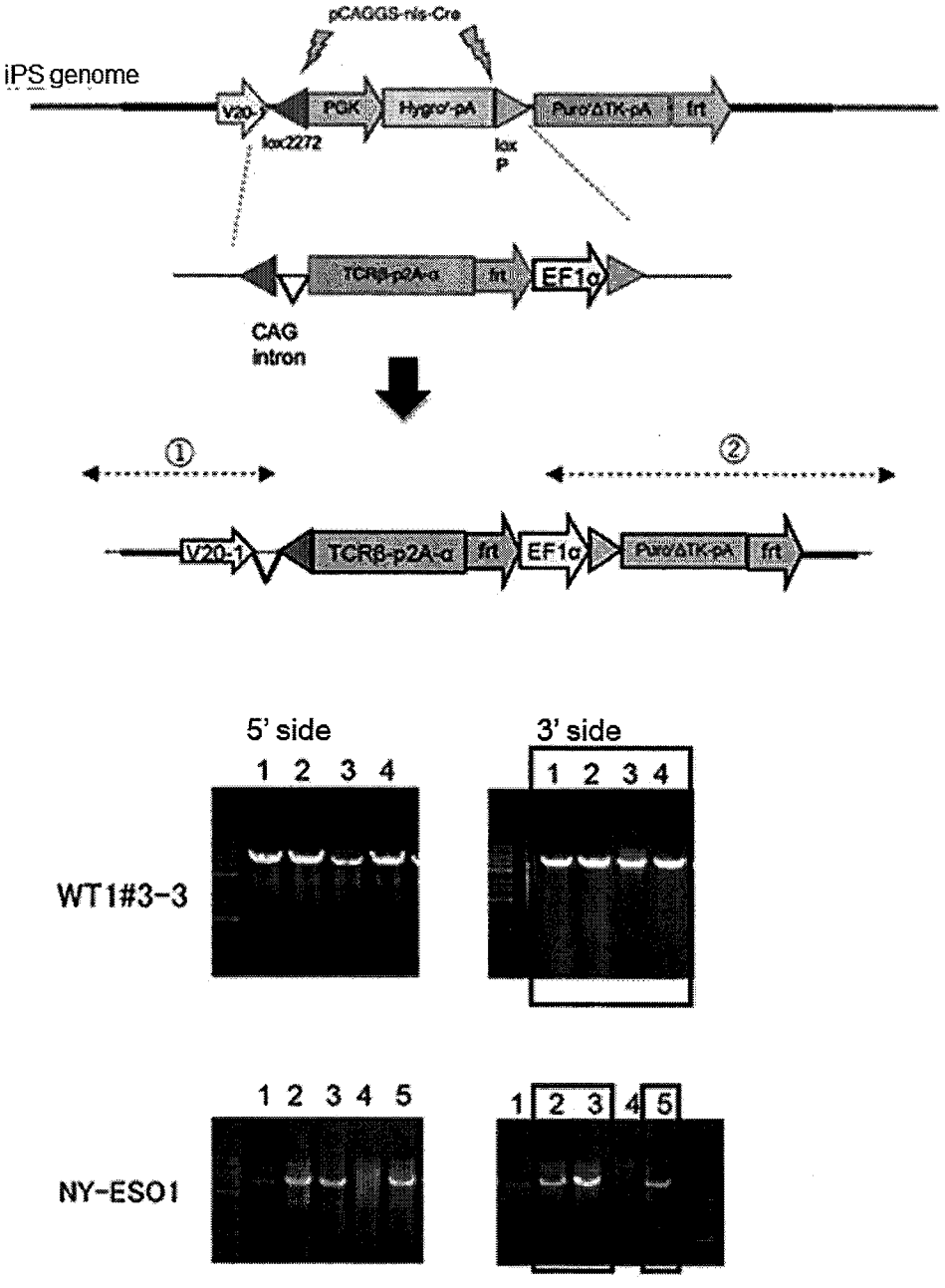

pCAGGS-nls-Cre
iPS genome
V20-1
PGK
Hygro'-pA
Puro'ΔTK-pA
frt
lox2272
lox P
TCRβ-p2A-α
frt
EF1α
CAG intron
①
②
V20-1
TCRβ-p2A-α
frt
EF1α
Puro'ΔTK-pA
frt
5' side
3' side
1 2 3 4
1 2 3 4
WT1#3-3
1 2 3 4 5
1 2 3 4 5
NY-ESO1

Fig. 24

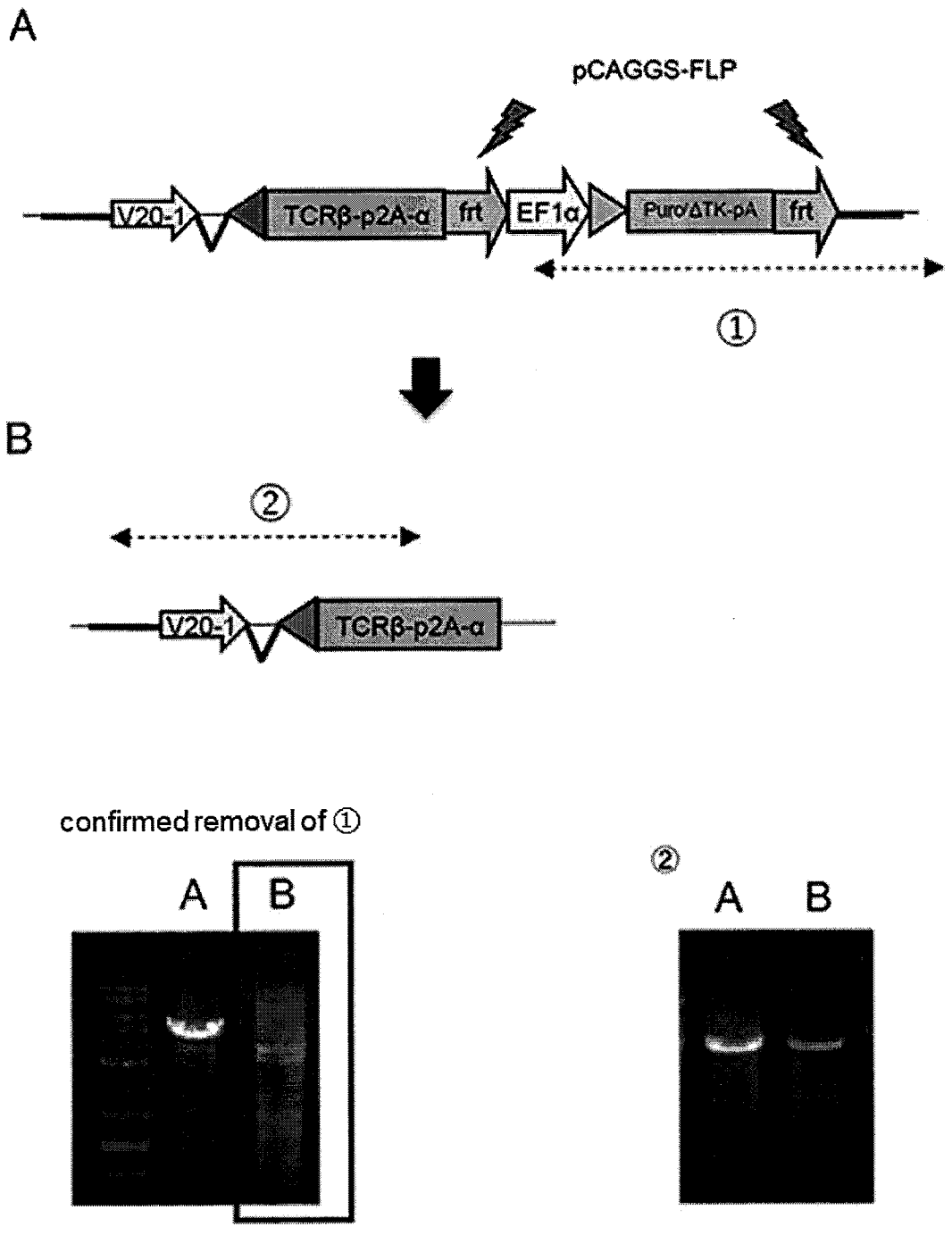

A
pCAGGS-FLP
V20-1
TCRβ-p2A-α
frt
EF1α
Puro/ΔTK-pA
frt
①
B
②
V20-1
TCRβ-p2A-α
confirmed removal of ①
A
B
②
A
B

Fig. 25

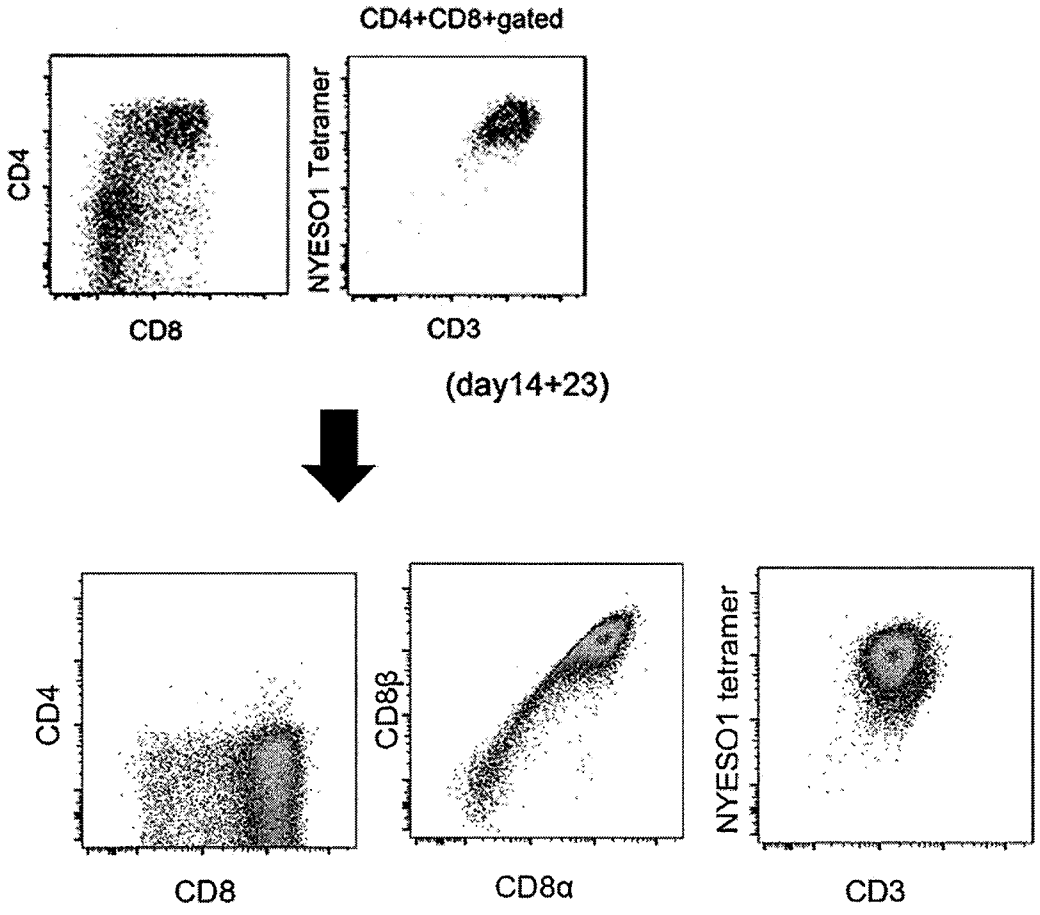

CD4+CD8+gated
CD4
CD8
NYESO1 Tetramer
CD3
(day14+23)
CD4
CD8
CD8β
CD8α
NYESO1 tetramer
CD3

Fig. 26

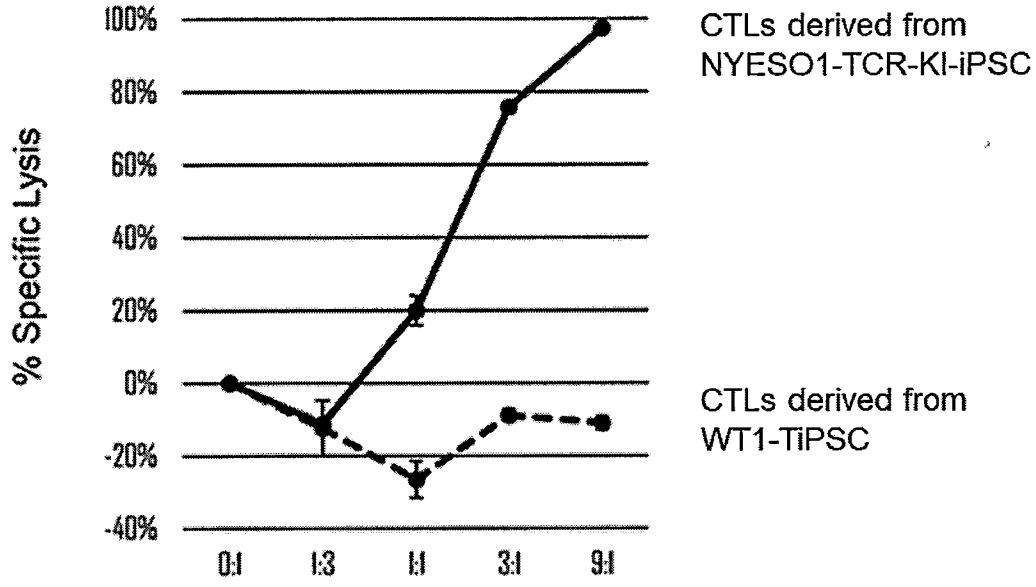

100%
80%
60%
40%
20%
0%
-20%
-40%
% Specific Lysis
0:1
1:3
1:1
3:1
9:1
CTLs derived from
NYESO1-TCR-KI-iPSC
CTLs derived from
WT1-TiPSC

Fig. 27

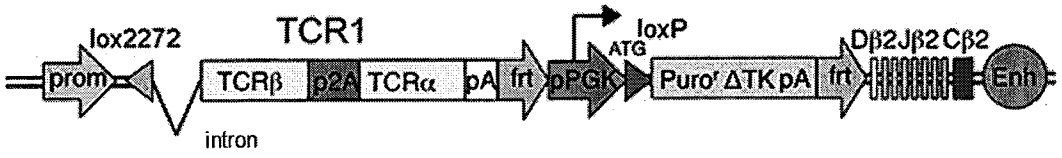

lox2272
TCR1
loxP
ATG
Dβ2Jβ2 Cβ2
prom
TCRβ
p2A
TCRα
pA
frt
pPGK
Puro' ΔTK pA
frt
Enh
intron

Fig. 28

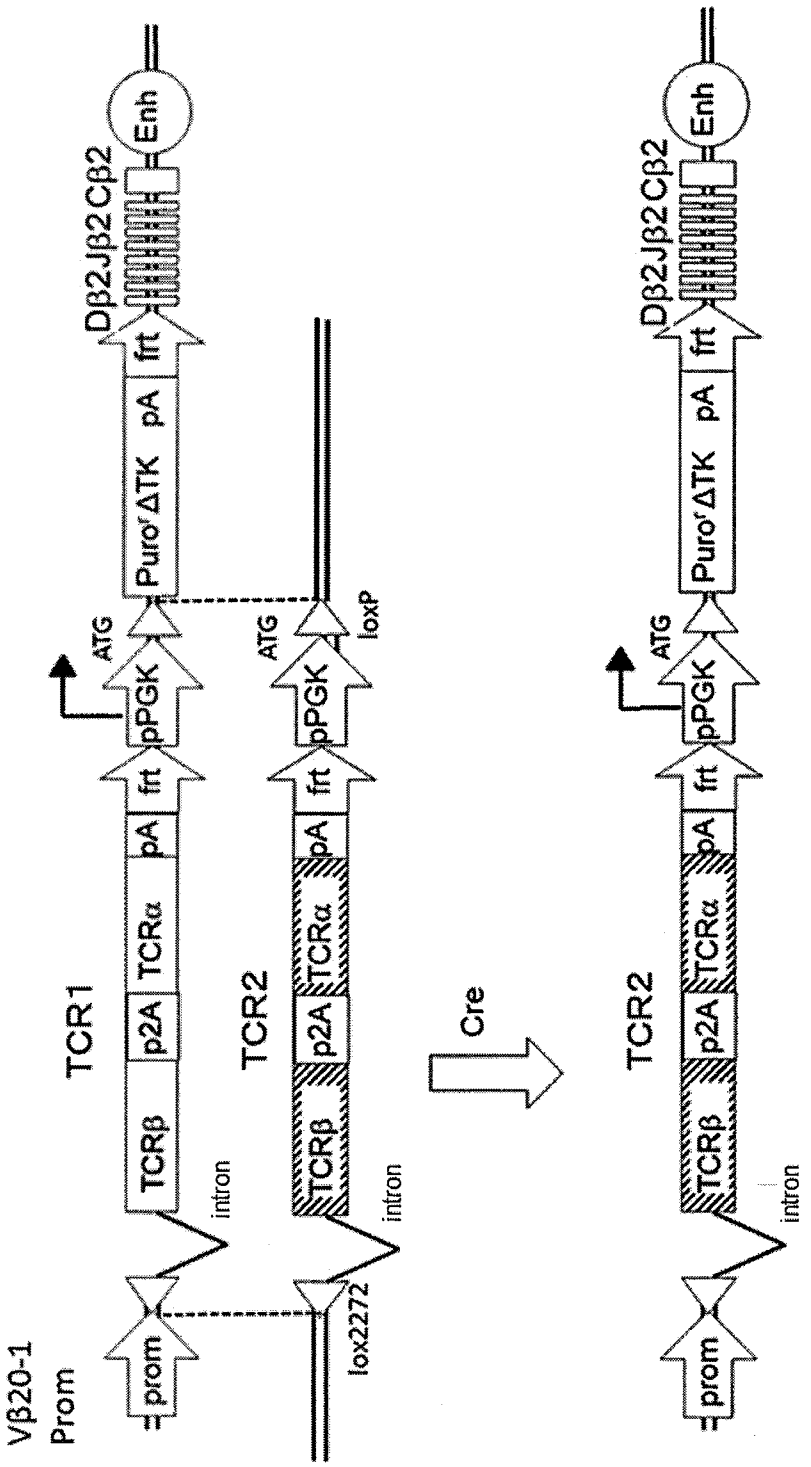
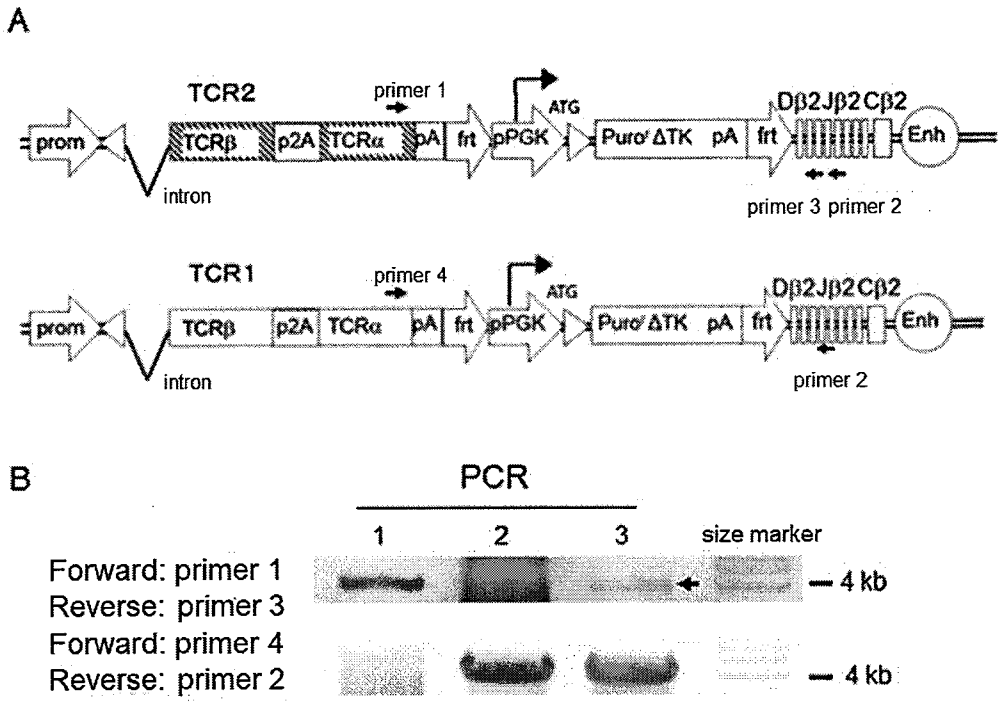

Vβ20-1
Prom
prom
intron
TCRβ
p2A
TCRα
pA
frt
pPGK
ATG
Puro^r ΔTK
pA
frt
Dβ2Jβ2 Cβ2
Enh
TCR1
lox2272
loxP
TCR2
Cre

Fig. 29


A
TCR2
primer 1
ATG
Dβ2Jβ2Cβ2
prom
TCRβ
p2A
TCRα
pA
frt
pPGK
Puro ΔTK
pA
frt
Enh
intron
primer 3 primer 2
TCR1
primer 4
ATG
Dβ2Jβ2Cβ2
prom
TCRβ
p2A
TCRα
pA
frt
pPGK
Puro ΔTK
pA
frt
Enh
intron
primer 2
B
PCR
1
2
3
size marker
Forward: primer 1
Reverse: primer 3
Forward: primer 4
Reverse: primer 2
4 kb
4 kb