Fig. 1

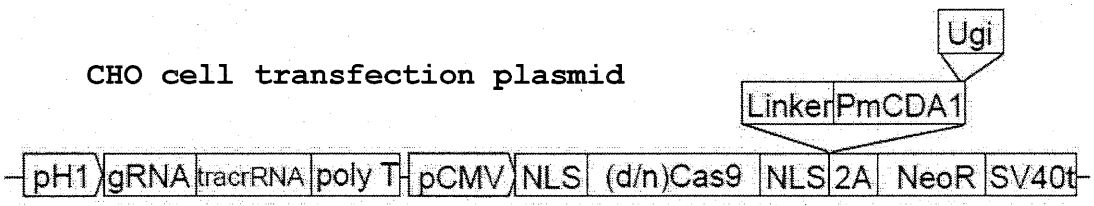

CHO cell transfection plasmid
Ugi
Linker
PmCDA1
pH1
gRNA
tracrRNA
poly T
pCMV
NLS
(d/n)Cas9
NLS
2A
NeoR
SV40t

Fig. 2

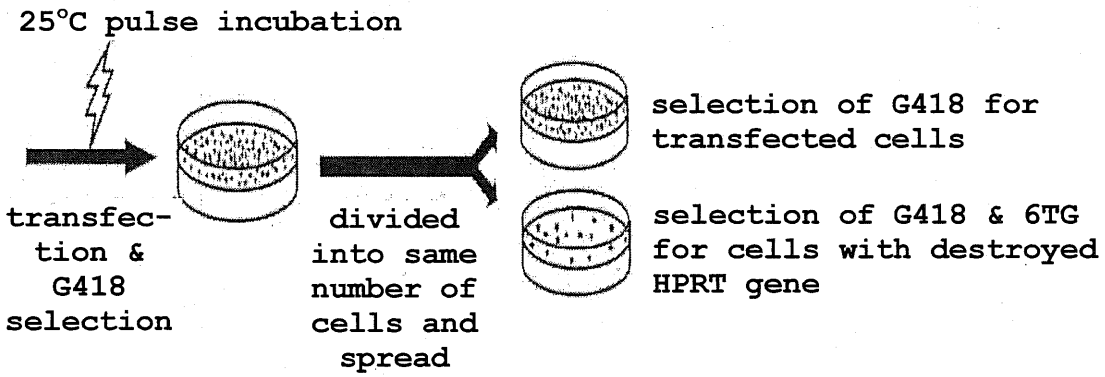
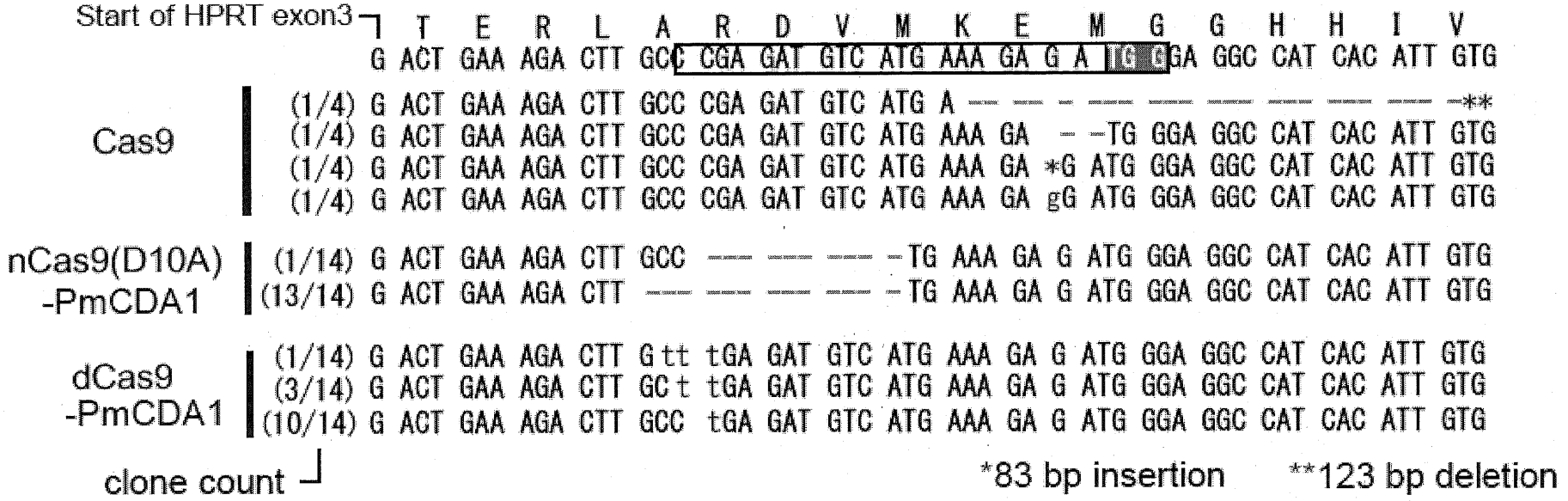

25°C pulse incubation
transfec-
tion &
G418
selection
divided
into same
number of
cells and
spread
selection of G418 for
transfected cells
selection of G418 & 6TG
for cells with destroyed
HPRT gene

Fig. 3


Start of HPRT exon3
T E R L A R D V M K E M G G H H I V
G ACT GAA AGA CTT GCC CGA GAT GTC ATG AAA GA G A TG GGA GGC CAT CAC ATT GTG
Cas9
(1/4) G ACT GAA AGA CTT GCC CGA GAT GTC ATG A — — — — — — — — — — —**
(1/4) G ACT GAA AGA CTT GCC CGA GAT GTC ATG AAA GA — —TG GGA GGC CAT CAC ATT GTG
(1/4) G ACT GAA AGA CTT GCC CGA GAT GTC ATG AAA GA *G ATG GGA GGC CAT CAC ATT GTG
(1/4) G ACT GAA AGA CTT GCC CGA GAT GTC ATG AAA GA gG ATG GGA GGC CAT CAC ATT GTG
nCas9(D10A)
-PmCDA1
(1/14) G ACT GAA AGA CTT GCC — — — — —TG AAA GA G ATG GGA GGC CAT CAC ATT GTG
(13/14) G ACT GAA AGA CTT — — — — — —TG AAA GA G ATG GGA GGC CAT CAC ATT GTG
dCas9
-PmCDA1
(1/14) G ACT GAA AGA CTT G tt tGA GAT GTC ATG AAA GA G ATG GGA GGC CAT CAC ATT GTG
(3/14) G ACT GAA AGA CTT GC t tGA GAT GTC ATG AAA GA G ATG GGA GGC CAT CAC ATT GTG
(10/14) G ACT GAA AGA CTT GCC tGA GAT GTC ATG AAA GA G ATG GGA GGC CAT CAC ATT GTG
clone count
*83 bp insertion
**123 bp deletion