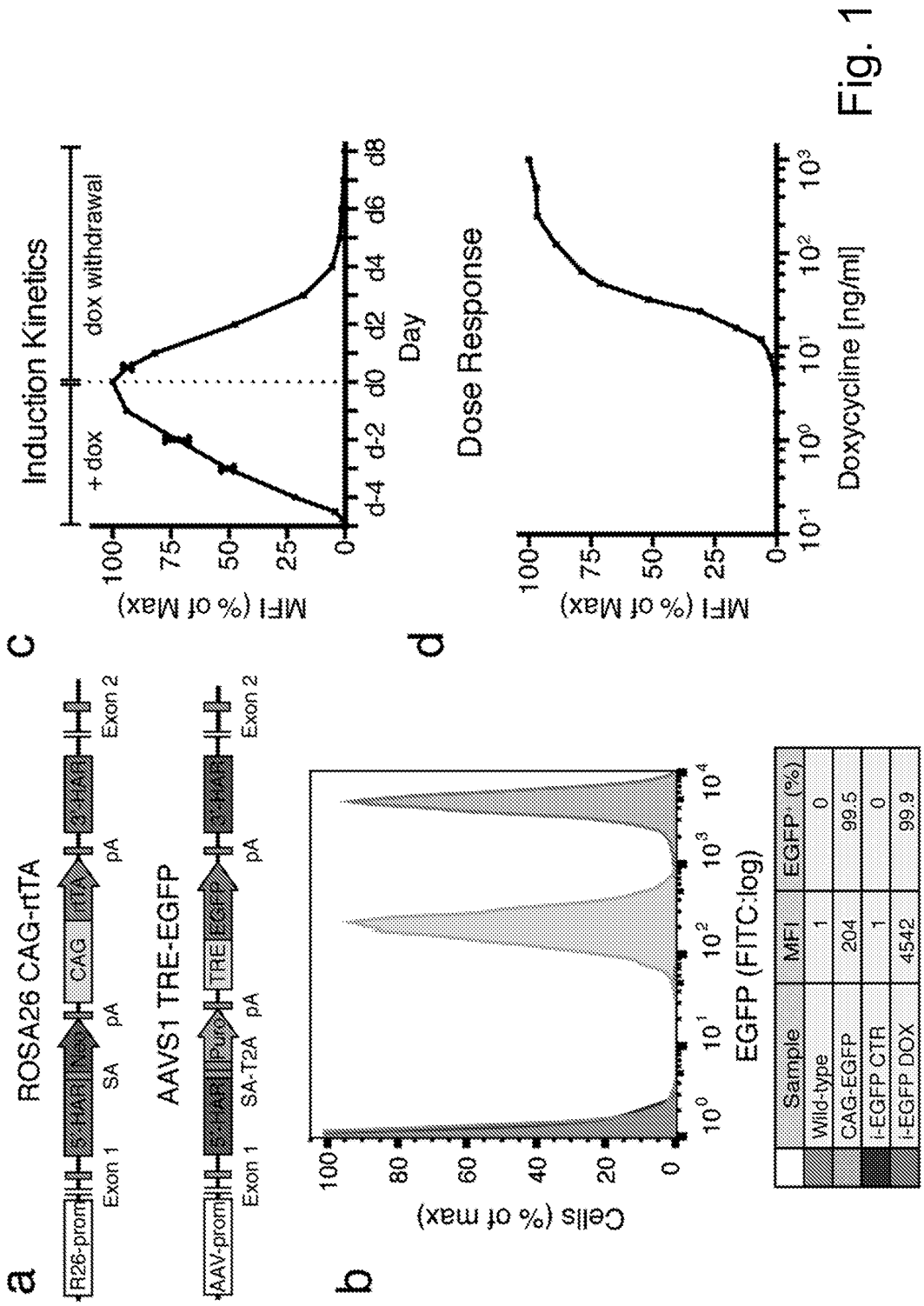

a
ROSA26 CAG-rtTA
R26-prom
5'-HA
rtTA
CAG
2A-HA
Exon 1
SA
pA
Exon 2
AAVS1 TRE-EGFP
AAV-prom
5'-HA
Puro
TRE
EGFP
3'-HA
Exon 1
SA-T2A
pA
Exon 2
b
Cells (% of max)
EGFP (FITC-log)
Sample
MFI
EGFP+ (%)
Wild-type
1
0
CAG-EGFP
204
99.5
i-EGFP CTR
1
0
i-EGFP DOX
4542
99.9
c
Induction Kinetics
+ dox
dox withdrawal
MFI (% of Max)
Day
d-4
d-2
d0
d2
d4
d6
d8
d
Dose Response
MFI (% of Max)
Doxycycline [ng/ml]

Fig. 1

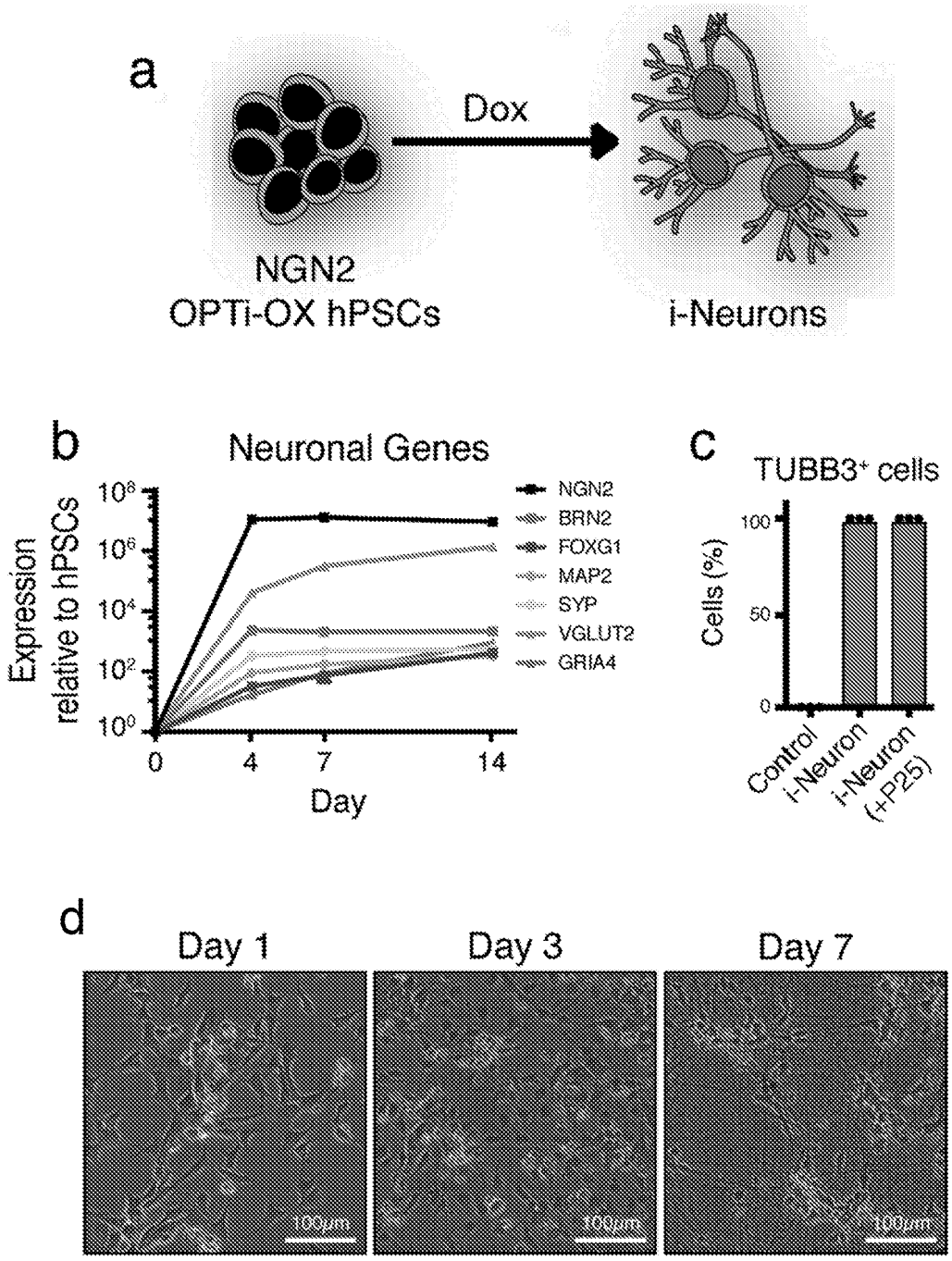

a
Dox
NGN2
OPTi-OX hPSCs
i-Neurons
b
Neuronal Genes
Expression relative to hPSCs
10^8
10^6
10^4
10^2
10^0
0
4
7
14
Day
NGN2
BRN2
FOXG1
MAP2
SYP
VGLUT2
GRIA4
c
TUBB3+ cells
Cells (%)
100
50
0
Control
i-Neuron
i-Neuron (+P25)
d
Day 1
Day 3
Day 7
100μm
100μm
100μm

Fig. 2

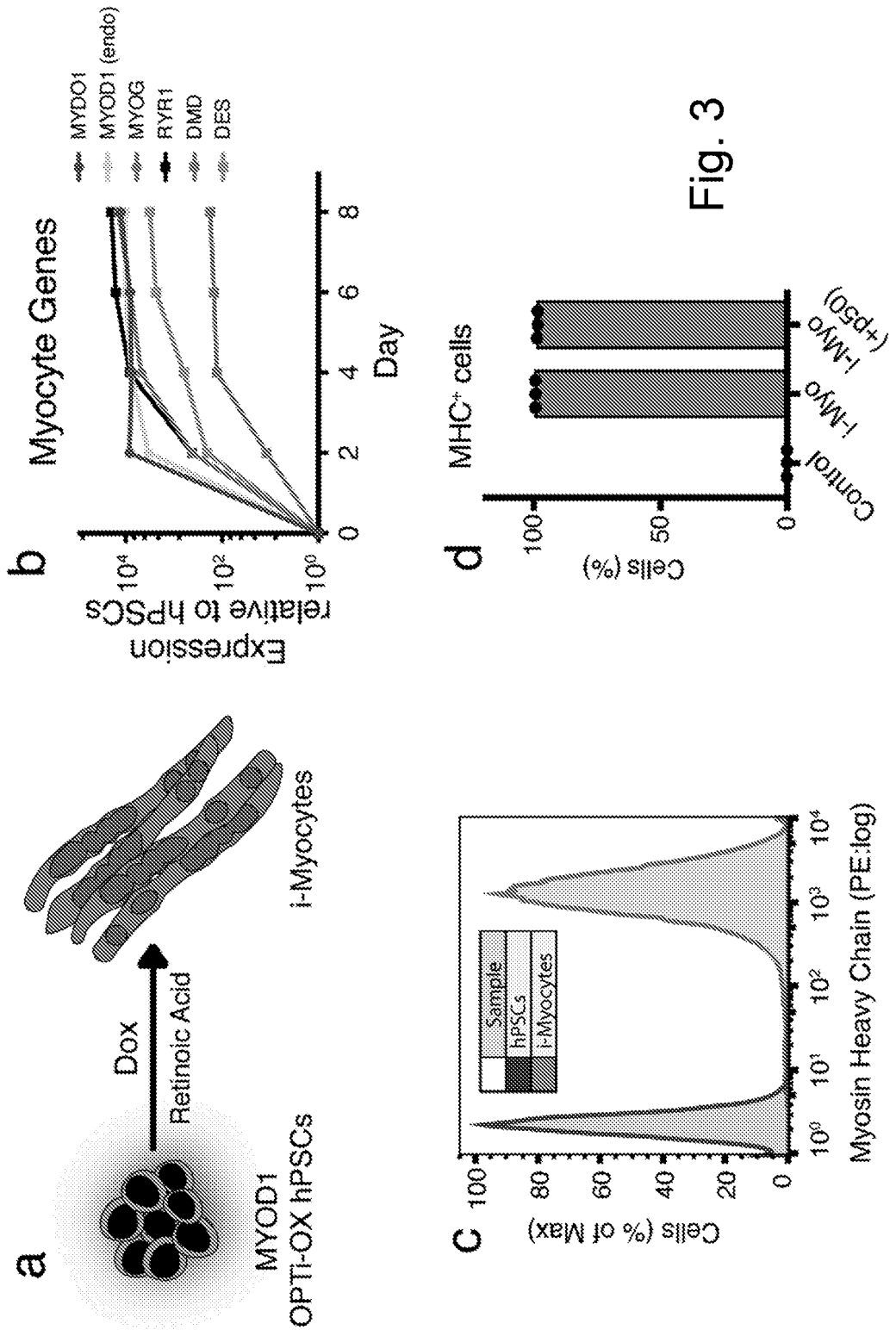

a
MYOD1
OPTI-OX hPSCs
Dox
Retinoic Acid
i-Myocytes
b
Myocyte Genes
MYOD1
MYOD1 (endo)
MYOG
RYR1
DMD
DES
Expression relative to hPSCs
Day
c
Cells (% of Max)
Sample
hPSCs
i-Myocytes
Myosin Heavy Chain (PE:log)
d
MHC+ cells
Cells (%)
Control
i-Myo
i-Myo (+p53i)

Fig. 3

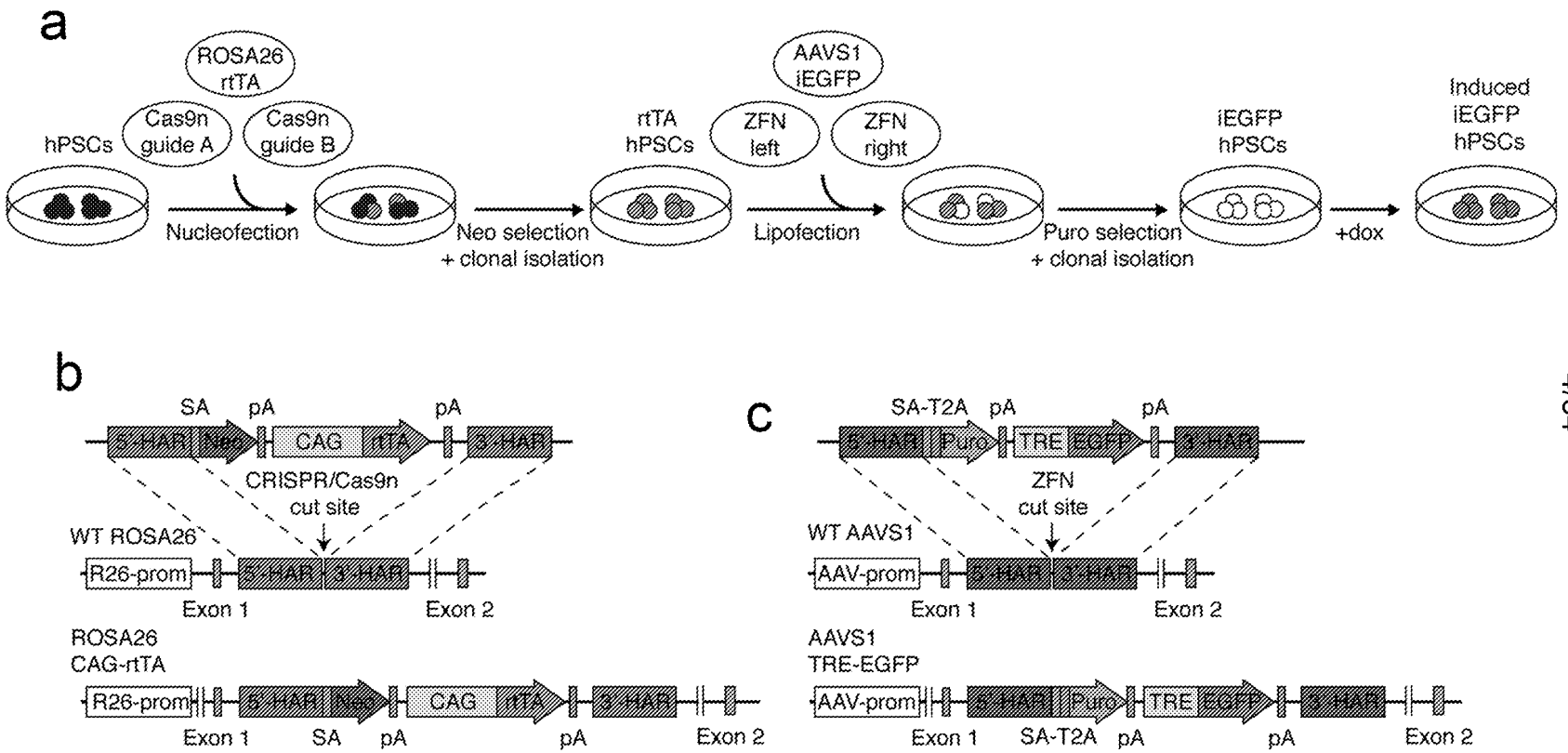

a
ROSA26
rtTA
Cas9n
guide A
Cas9n
guide B
hPSCs
Nucleofection
Neo selection
+ clonal isolation
rtTA
hPSCs
AAVS1
iEGFP
ZFN
left
ZFN
right
Lipofection
Puro selection
+ clonal isolation
iEGFP
hPSCs
+dox
Induced
iEGFP
hPSCs
b
SA
pA
pA
5' HAR
Neo
CAG
rtTA
3' HAR
CRISPR/Cas9n
cut site
WT ROSA26
R26-prom
5' HAR
3' HAR
Exon 1
Exon 2
ROSA26
CAG-rtTA
R26-prom
5' HAR
Neo
CAG
rtTA
3' HAR
Exon 1
SA
pA
pA
Exon 2
c
SA-T2A
pA
pA
5' HAR
Puro
TRE
EGFP
3' HAR
ZFN
cut site
WT AAVS1
AAV-prom
5' HAR
3' HAR
Exon 1
Exon 2
AAVS1
TRE-EGFP
AAV-prom
5' HAR
Puro
TRE
EGFP
3' HAR
Exon 1
SA-T2A
pA
pA
Exon 2

Fig. 4

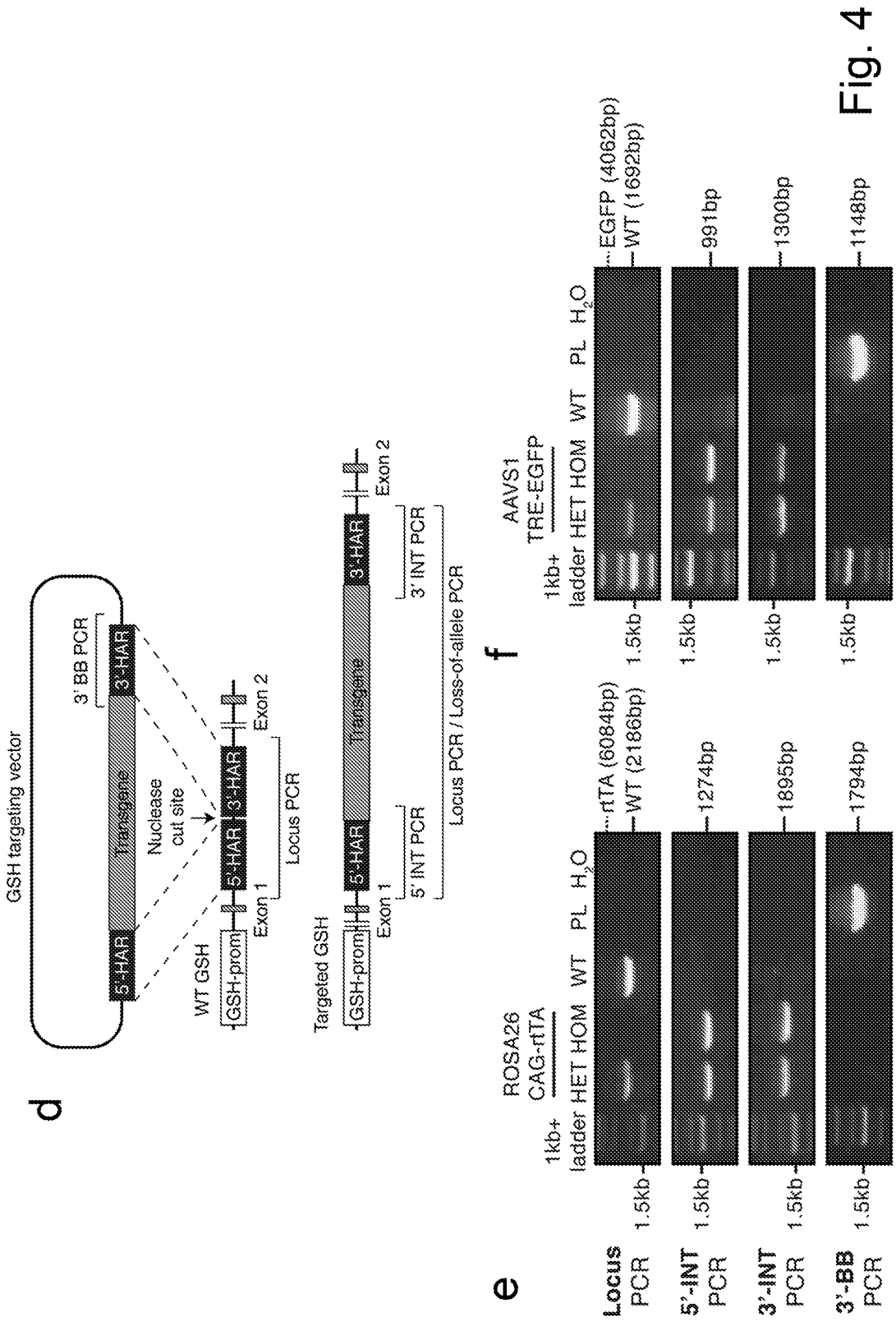

d
GSH targeting vector
3' BB PCR
5'-HAR
Transgene
3'-HAR
Nuclease cut site
WT GSH
GSH-prom
5'-HAR
3'-HAR
Exon 1
Exon 2
Locus PCR
Targeted GSH
GSH-prom
5'-HAR
Transgene
3'-HAR
Exon 1
Exon 2
5' INT PCR
3' INT PCR
Locus PCR / Loss-of-allele PCR
e
ROSA26
CAG-rtTA
1kb+ ladder
HET
HOM
WT
PL
H2O
rtTA (6084bp)
WT (2186bp)
Locus PCR
5'-INT PCR
3'-INT PCR
3'-BB PCR
1.5kb
1274bp
1895bp
1794bp
f
AAVS1
TRE-EGFP
EGFP (4062bp)
WT (1692bp)
991bp
1300bp
1148bp

Fig. 4

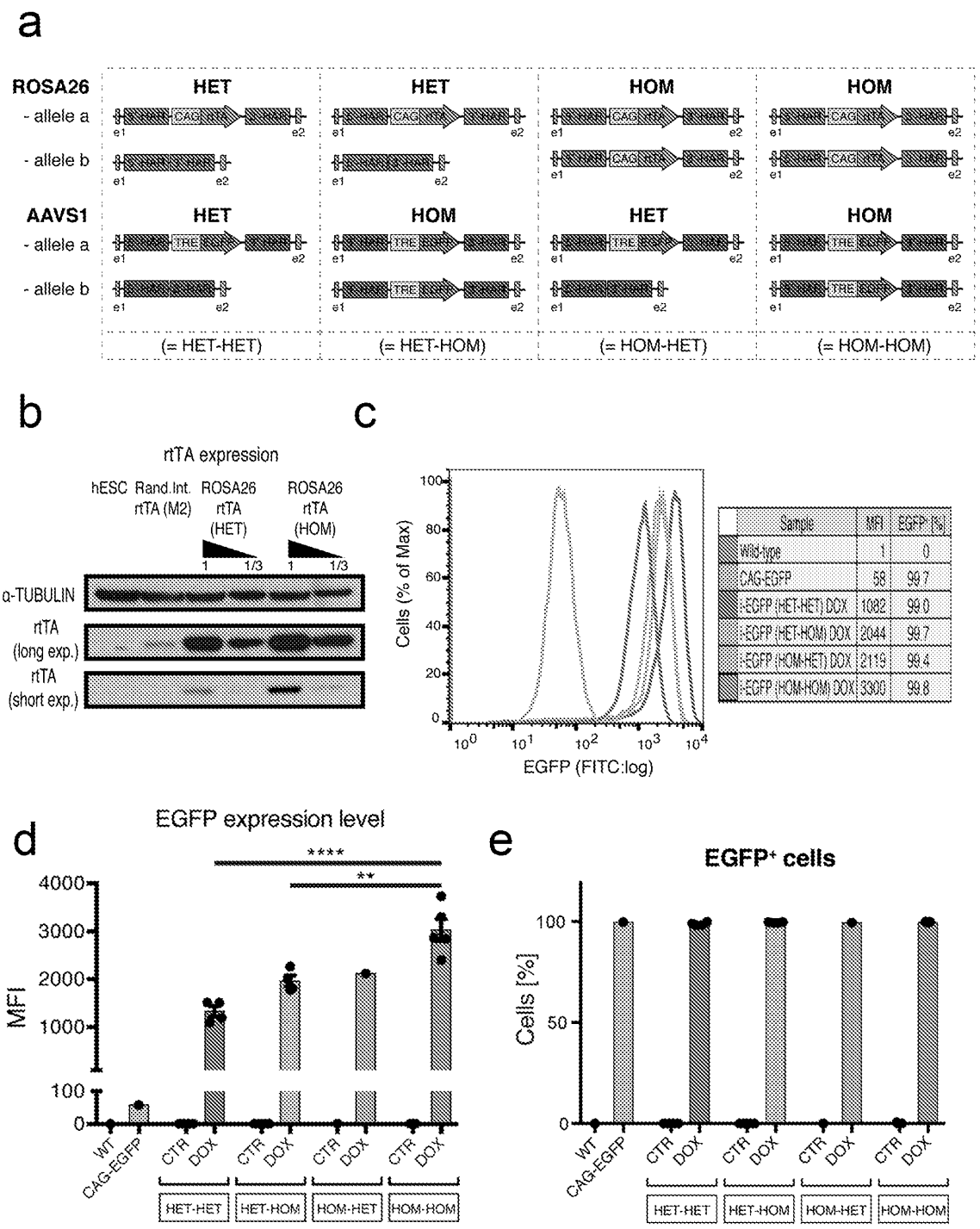

a
ROSA26
- allele a
- allele b
AAVS1
- allele a
- allele b
HET
HOM
(= HET-HET)
(= HET-HOM)
(= HOM-HET)
(= HOM-HOM)
b
rtTA expression
hESC
Rand.Int. rtTA (M2)
ROSA26 rtTA (HET)
ROSA26 rtTA (HOM)
α-TUBULIN
rtTA (long exp.)
rtTA (short exp.)
c
Cells (% of Max)
EGFP (FITC:log)
Sample | MFI | EGFP (%)
Wild-type | 1 | 0
CAG-EGFP | 58 | 99.7
-EGFP (HET-HET) DOX | 1082 | 99.0
-EGFP (HET-HOM) DOX | 2044 | 99.7
-EGFP (HOM-HET) DOX | 2119 | 99.4
-EGFP (HOM-HOM) DOX | 3300 | 99.8
d
EGFP expression level
MFI
****
**
WT
CAG-EGFP
CTR
DOX
HET-HET
HET-HOM
HOM-HET
HOM-HOM
e
EGFP+ cells
Cells [%]

Fig. 5

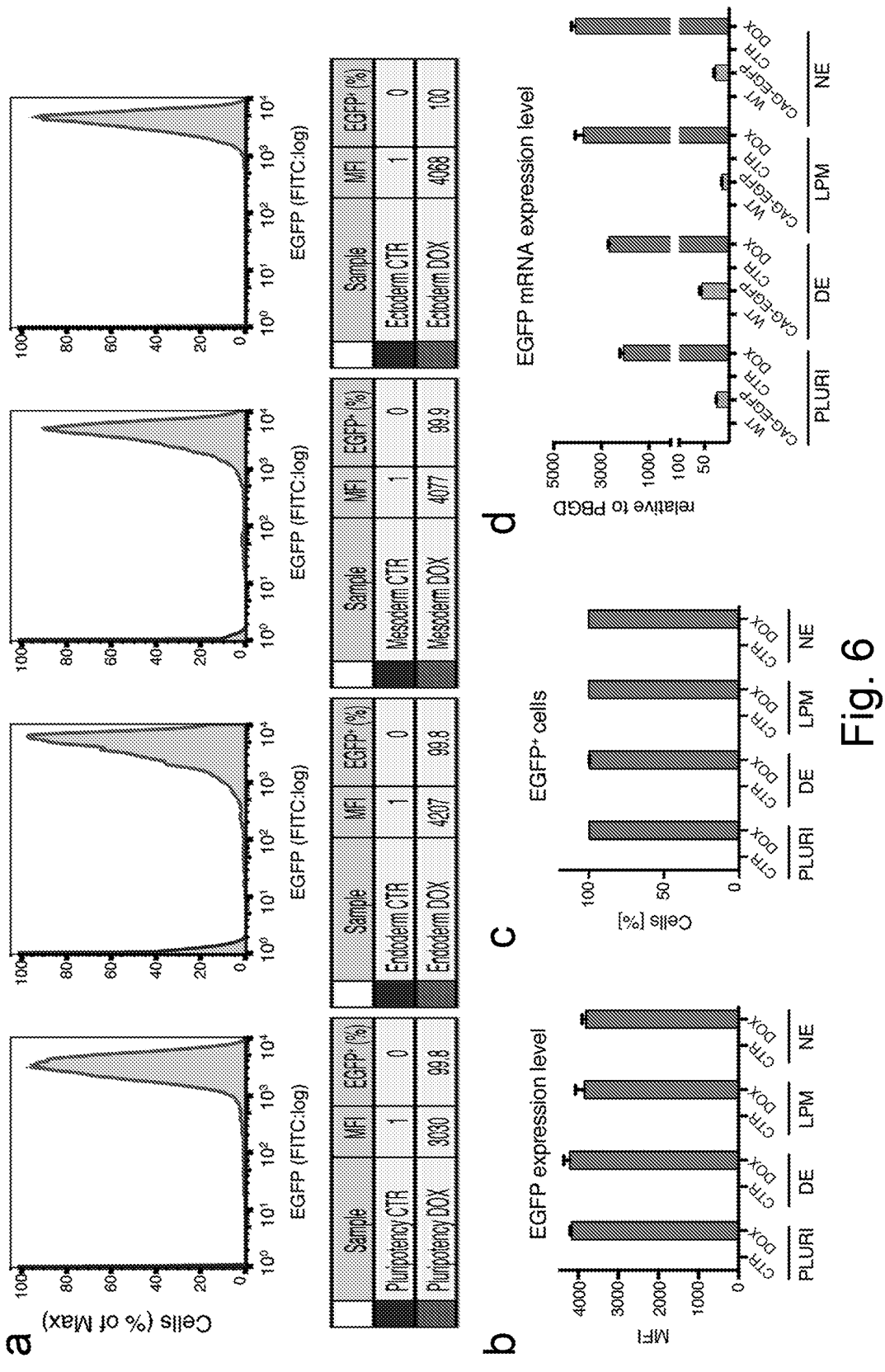

a
Cells (% of Max)
EGFP (FITC:log)
Sample | MFI | EGFP+ (%)
Pluripotency CTR | 1 | 0
Pluripotency DOX | 3030 | 99.8
Endoderm CTR | 1 | 0
Endoderm DOX | 4237 | 99.8
Mesoderm CTR | 1 | 0
Mesoderm DOX | 4077 | 99.9
Ectoderm CTR | 1 | 0
Ectoderm DOX | 4068 | 100
b
EGFP expression level
MFI
PLURI DE LPM NE
CTR DOX
c
EGFP+ cells
Cells [%]
d
EGFP mRNA expression level
relative to PBGD
WT CAG-EGFP CTR DOX
PLURI DE LPM NE

Fig. 6

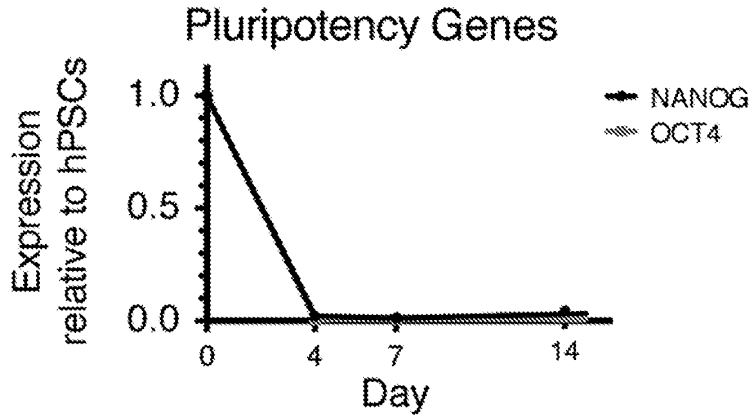

Pluripotency Genes
Expression relative to hPSCs
1.0
0.5
0.0
0
4
7
14
Day
NANOG
OCT4

Fig. 7

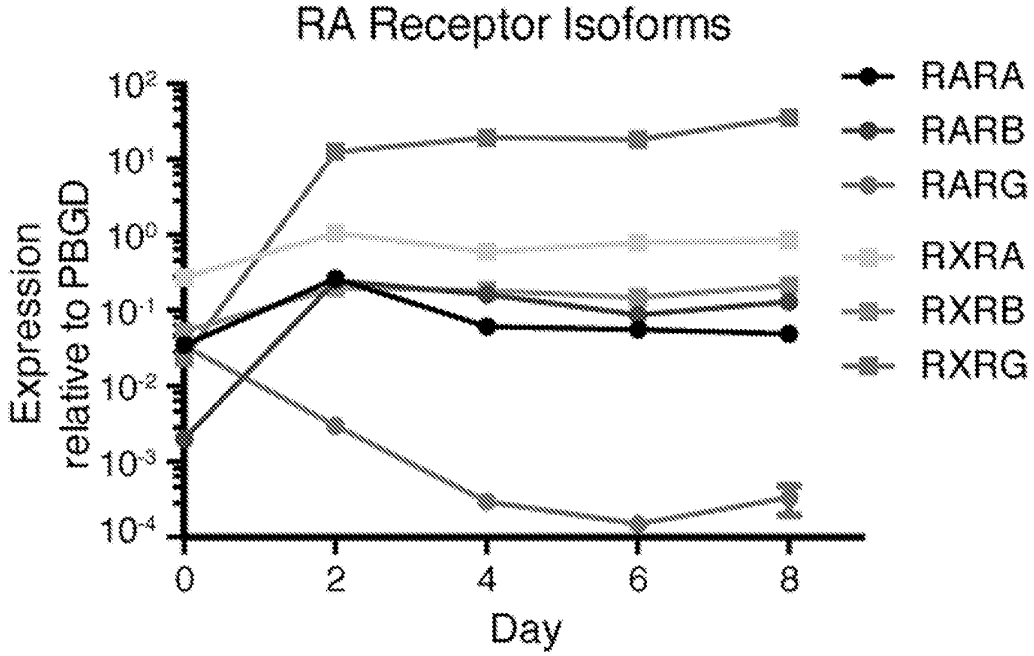

RA Receptor Isoforms
Expression relative to PBGD
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
2
4
6
8
Day
RARA
RARB
RARG
RXRA
RXRB
RXRG

Fig. 8

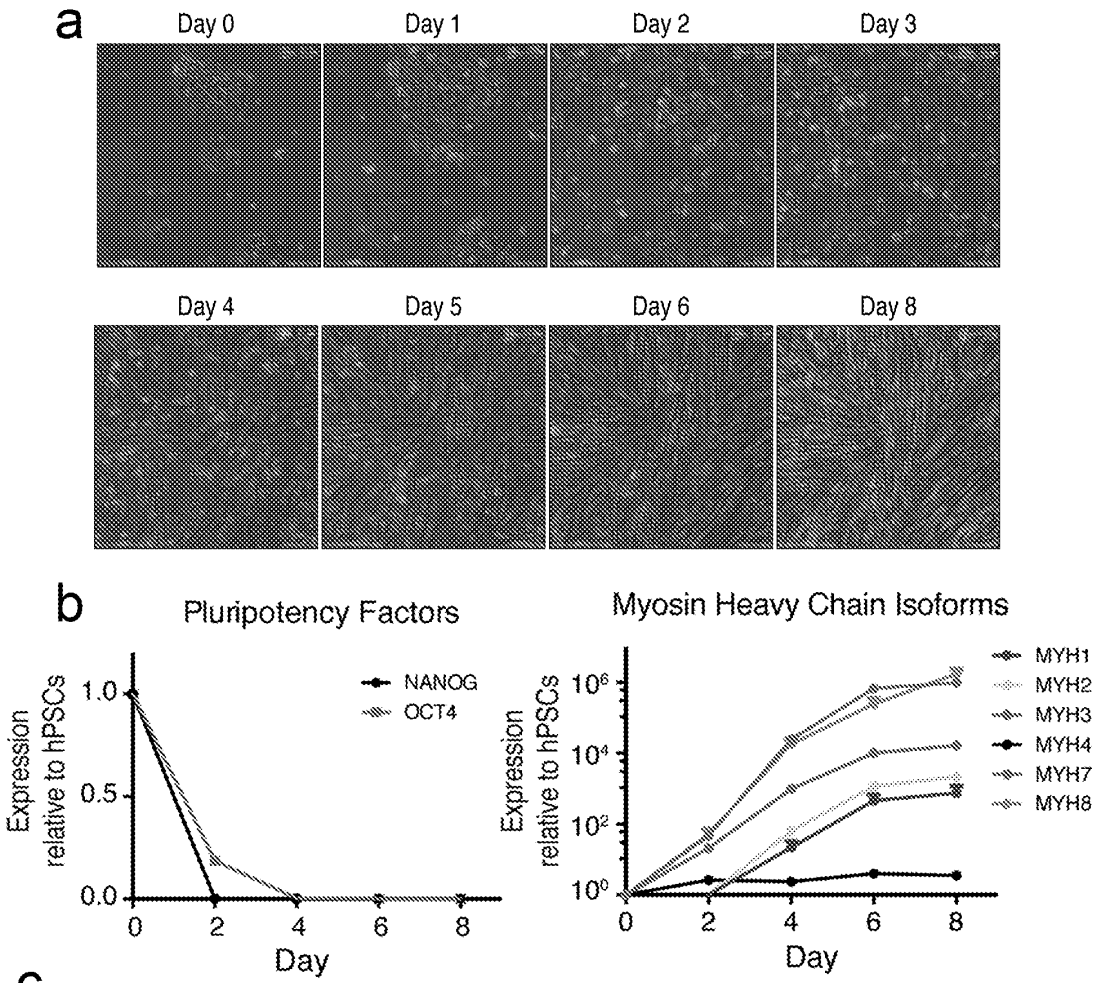

a
Day 0
Day 1
Day 2
Day 3
Day 4
Day 5
Day 6
Day 8
b
Pluripotency Factors
Expression relative to hPSCs
1.0
0.5
0.0
NANOG
OCT4
0
2
4
6
8
Day
Myosin Heavy Chain Isoforms
Expression relative to hPSCs
10^6
10^4
10^2
10^0
MYH1
MYH2
MYH3
MYH4
MYH7
MYH8
0
2
4
6
8
Day
c
Phalloidin-488
NCAM
DES
MYH
TTN
ACTN2
TNNT
PAX3/7
DAPI
MYOG

Fig. 9

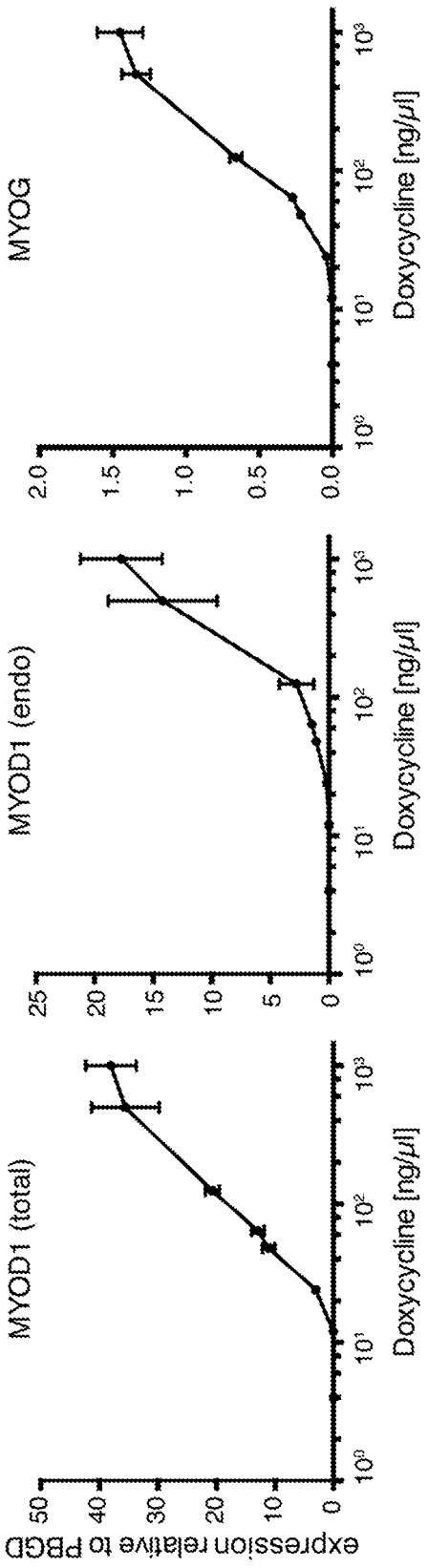

MYOD1 (total)
MYOD1 (endo)
MYOG
expression relative to PBGD
Doxycycline [ng/µl]
0
10
20
30
40
50
5
15
25
0.0
0.5
1.0
1.5
2.0
10⁰
10¹
10²
10³

Fig. 10

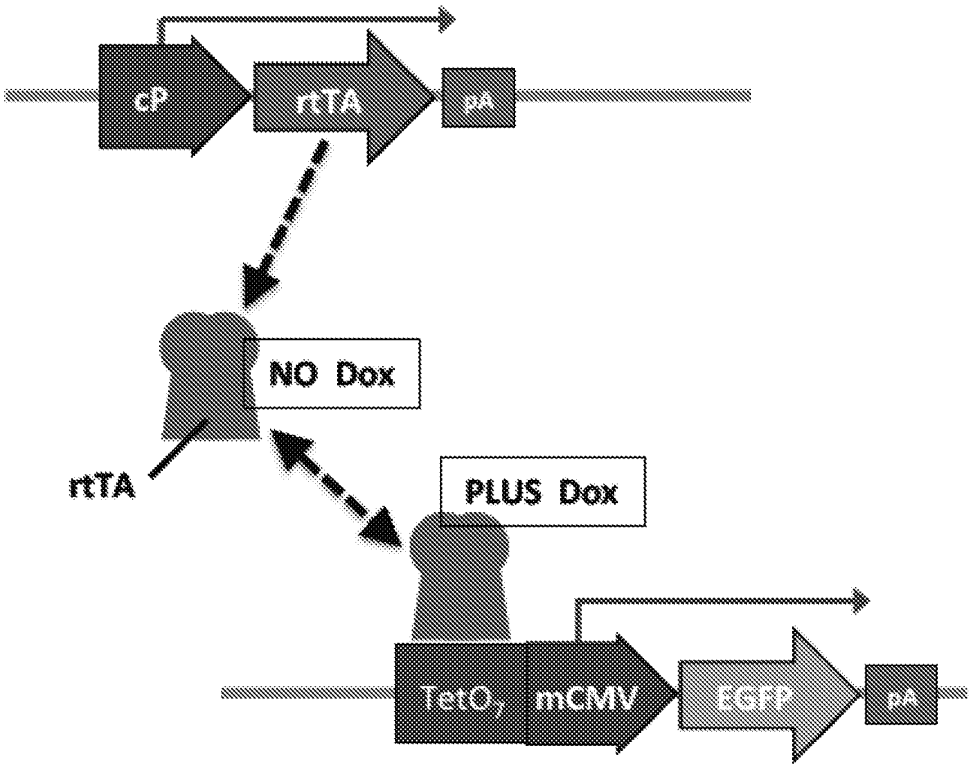


Fig. 11

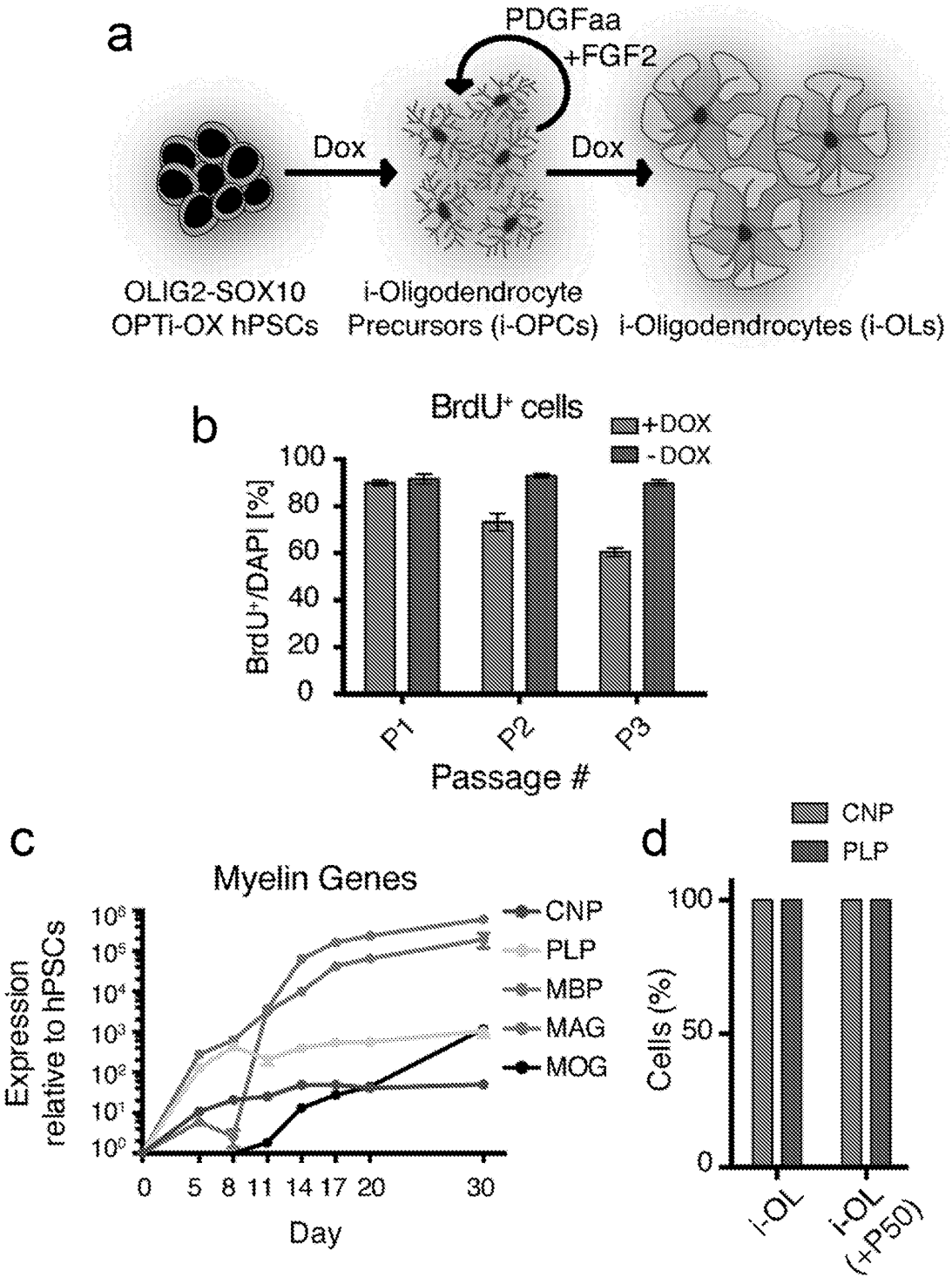
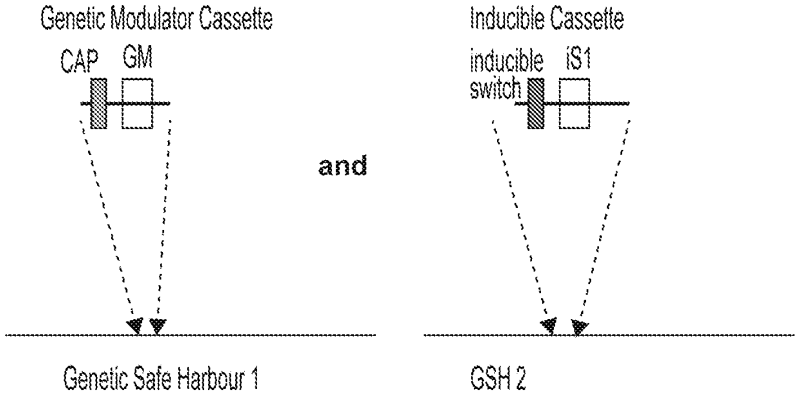

a
PDGFaa
+FGF2
Dox
Dox
OLIG2-SOX10
OPTi-OX hPSCs
i-Oligodendrocyte
Precursors (i-OPCs)
i-Oligodendrocytes (i-OLs)
b
BrdU+ cells
+DOX
-DOX
BrdU+/DAPI [%]
100
80
60
40
20
0
P1
P2
P3
Passage #
c
Myelin Genes
Expression relative to hPSCs
CNP
PLP
MBP
MAG
MOG
0 5 8 11 14 17 20 30
Day
d
CNP
PLP
Cells (%)
100
50
0
i-OL
i-OL (+P50)

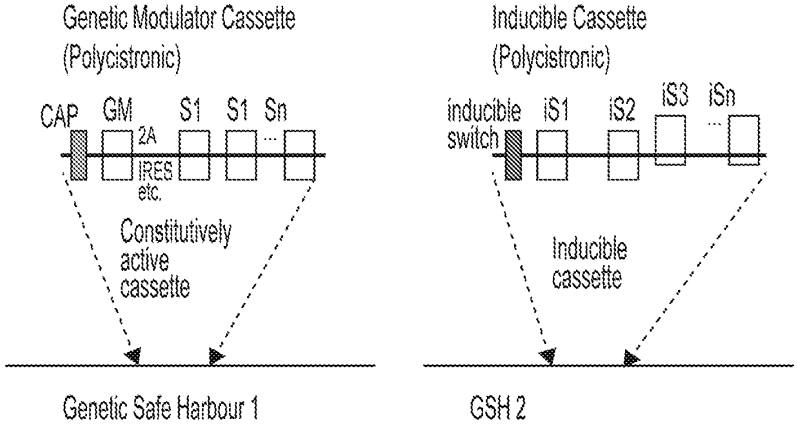
Fig. 12

CAP = Constitutively active promoter
GM = Genetic modulator (e.g. rtTA)
S1-n = Constitutively expressed genetic sequence 1-n
iS1-n = inducibly expressed genetic sequence

Principle Invention



Extension to polycistronic vector contructs



Extension to multiple GSH

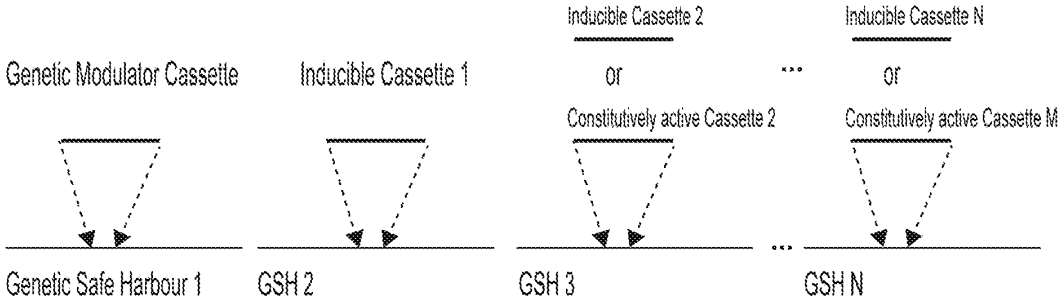


Fig. 13

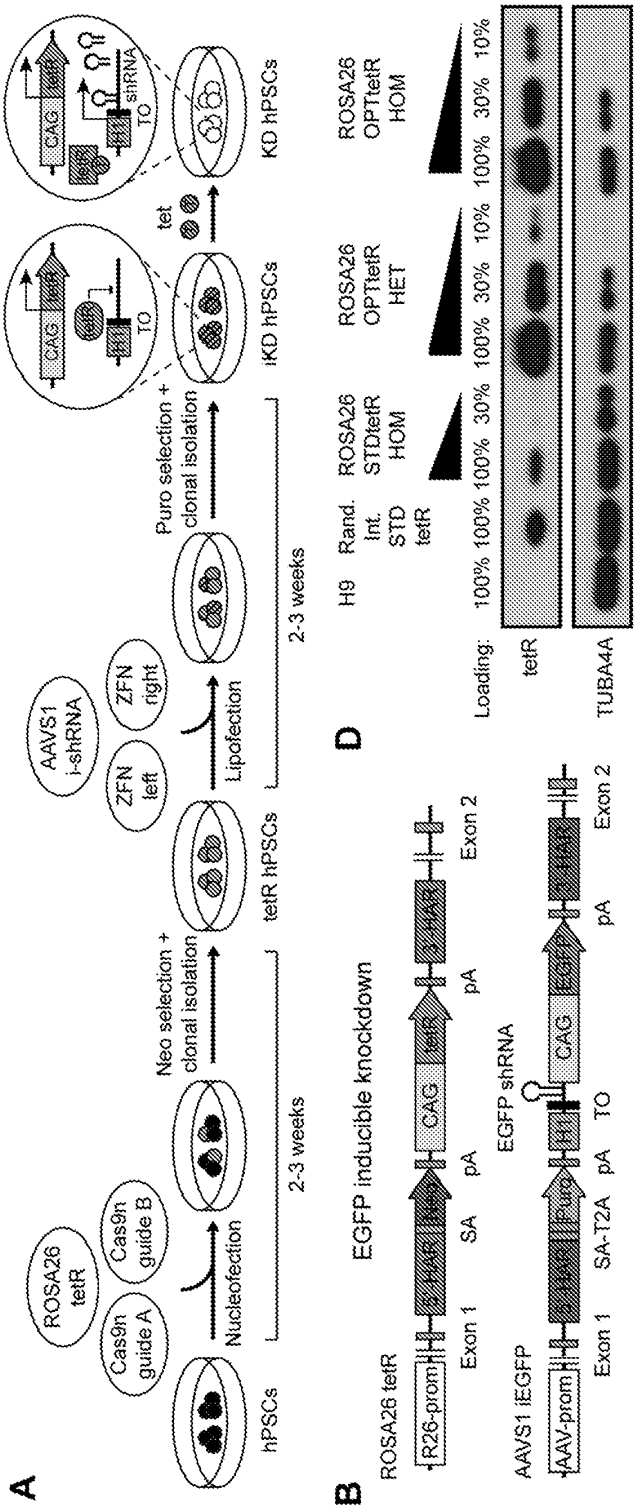

A
hPSCs
ROSA26 tetR
Cas9n guide A
Cas9n guide B
Nucleofection
2-3 weeks
tetR hPSCs
Neo selection + clonal isolation
AAVS1 i-shRNA
ZFN left
ZFN right
Lipofection
2-3 weeks
iKD hPSCs
Puro selection + clonal isolation
tet
KD hPSCs
CAG
rtTA
TO
shRNA
B
EGFP inducible knockdown
ROSA26 tetR
R26-prom
Exon 1
SA
pA
CAG
tetR
pA
Exon 2
AAVS1 iEGFP
AAV-prom
Exon 1
SA-T2A
pA
TO
EGFP shRNA
CAG
EGFP
pA
Exon 2
D
H9
Rand. Int. STD tetR
ROSA26 STDtetR HOM
ROSA26 OPTetR HET
ROSA26 OPTetR HOM
Loading:
100% 100% 100% 30% 100% 30% 10% 100% 30% 10%
tetR
TUBA4A

Fig. 14

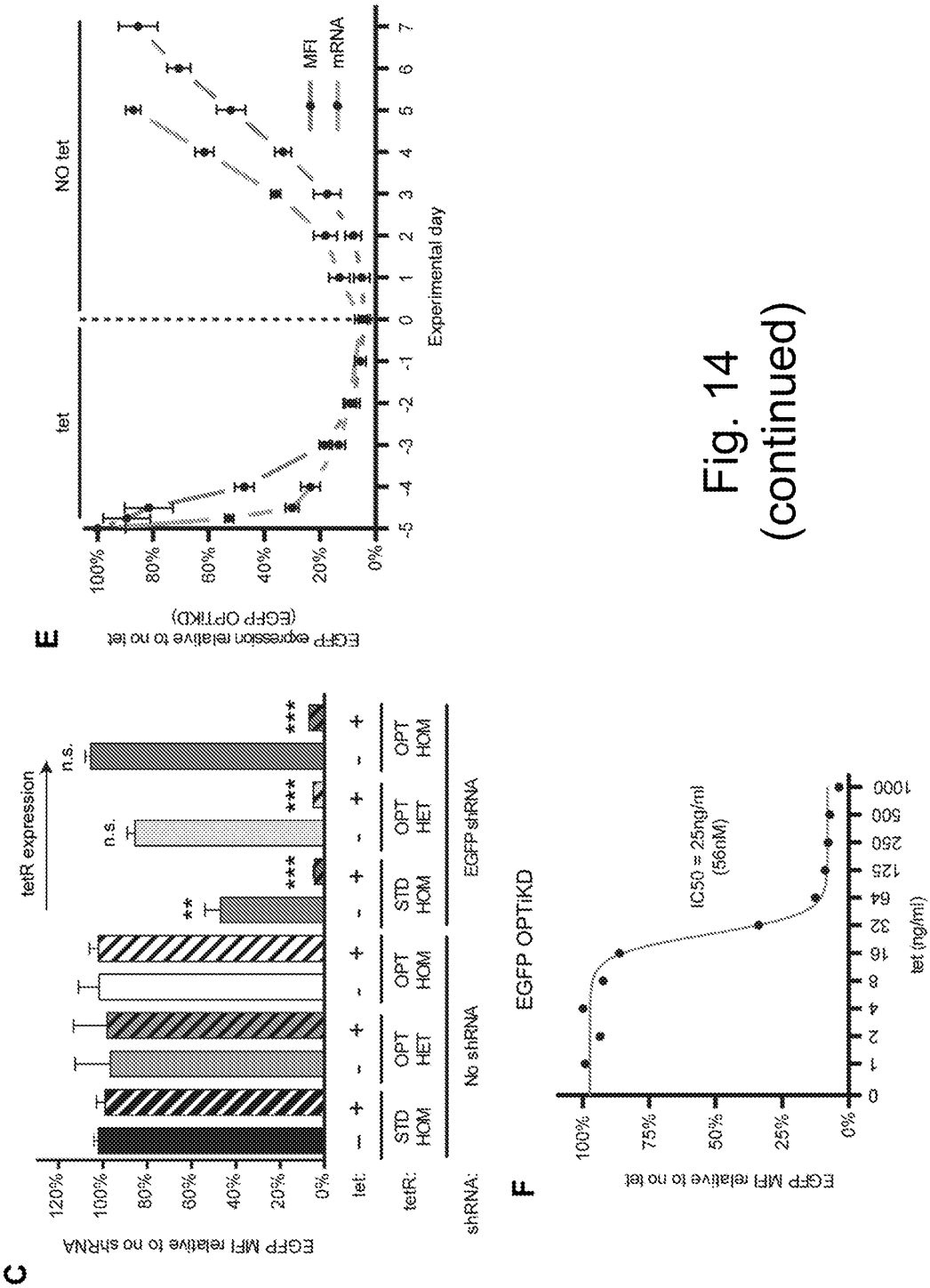


Fig. 14
(continued)

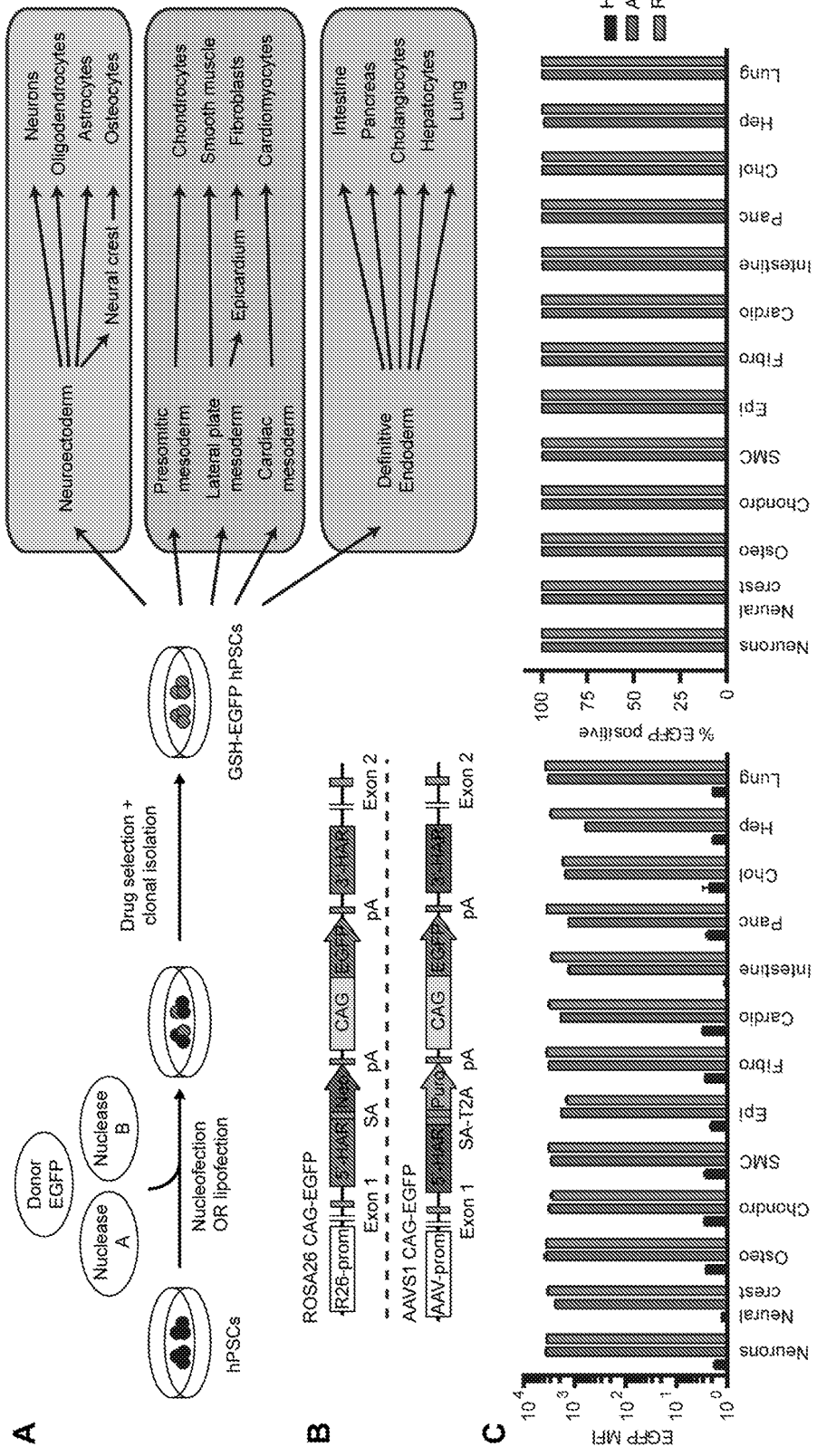

A
hPSCs
Donor EGFP
Nuclease A
Nuclease B
Nucleofection OR lipofection
Drug selection + clonal isolation
GSH-EGFP hPSCs
Neuroectoderm
Neurons
Oligodendrocytes
Astrocytes
Osteocytes
Neural crest
Presomitic mesoderm
Lateral plate mesoderm
Cardiac mesoderm
Chondrocytes
Smooth muscle
Fibroblasts
Cardiomyocytes
Epicardium
Definitive Endoderm
Intestine
Pancreas
Cholangiocytes
Hepatocytes
Lung
B
ROSA26 CAG-EGFP
R26-prom
CAG
EGFP
Exon 1
SA
pA
Exon 2
AAVS1 CAG-EGFP
AAV-prom
Puro
SA-T2A
C
EGFP MFI
% EGFP positive
H9
AAV
ROSA
Neurons
Neural crest
Osteo
Chondro
SMC
Epi
Fibro
Cardio
Intestine
Panc
Chol
Hep
Lung

Fig. 15

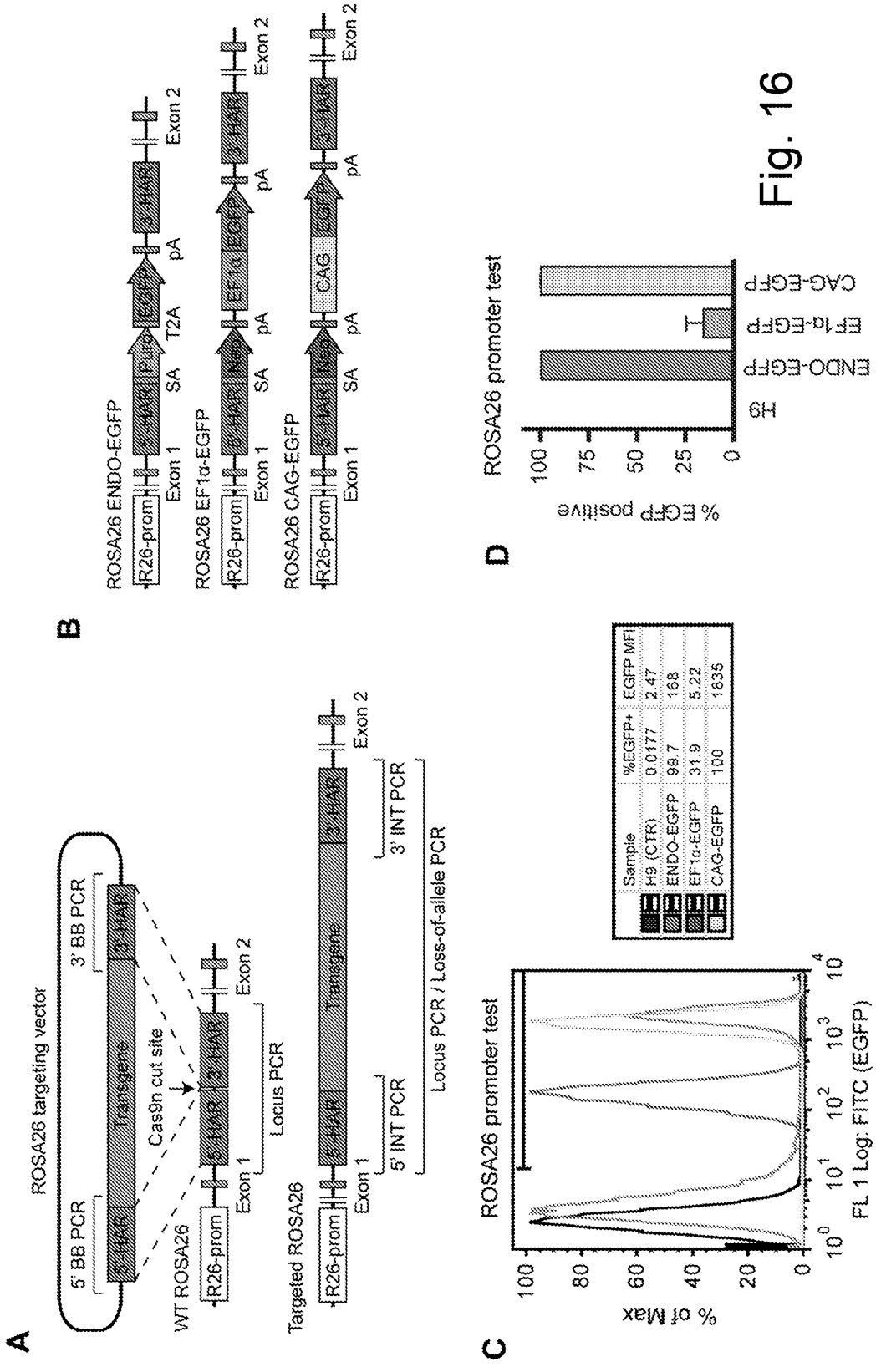

A
ROSA26 targeting vector
5' BB PCR
3' BB PCR
5' HAR
Transgene
3' HAR
Cas9n cut site
WT ROSA26
R26-prom
Exon 1
Exon 2
Locus PCR
Targeted ROSA26
5' INT PCR
3' INT PCR
Locus PCR / Loss-of-allele PCR
B
ROSA26 ENDO-EGFP
ROSA26 EF1α-EGFP
ROSA26 CAG-EGFP
Puro
EGFP
SA
T2A
pA
EF1α
CAG
C
ROSA26 promoter test
% of Max
FL 1 Log: FITC (EGFP)
Sample
%EGFP+
EGFP MFI
H9 (CTR)
0.0177
2.47
ENDO-EGFP
99.7
168
EF1α-EGFP
31.9
5.22
CAG-EGFP
100
1835
D
ROSA26 promoter test
% EGFP positive
H9
ENDO-EGFP
EF1α-EGFP
CAG-EGFP

Fig. 16

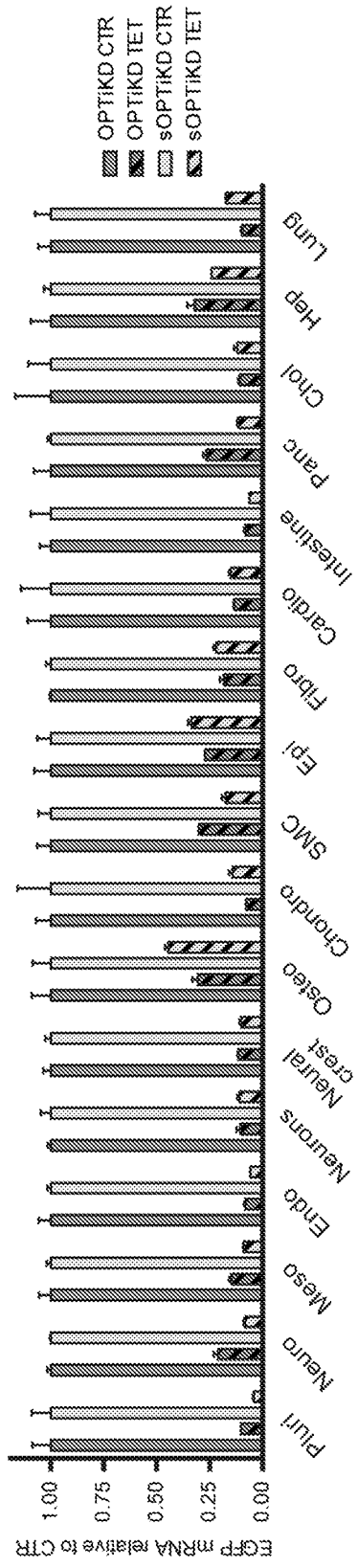

OPTiKD CTR
OPTiKD TET
sOPTiKD CTR
sOPTiKD TET
EGFP mRNA relative to CTR
0.00
0.25
0.50
0.75
1.00
Pluri
Neuro
Meso
Endo
Neurons
Neural crest
Osteo
Chondro
SMC
Epi
Fibro
Cardio
Intestine
Panc
Chol
Hep
Lung

Fig. 17

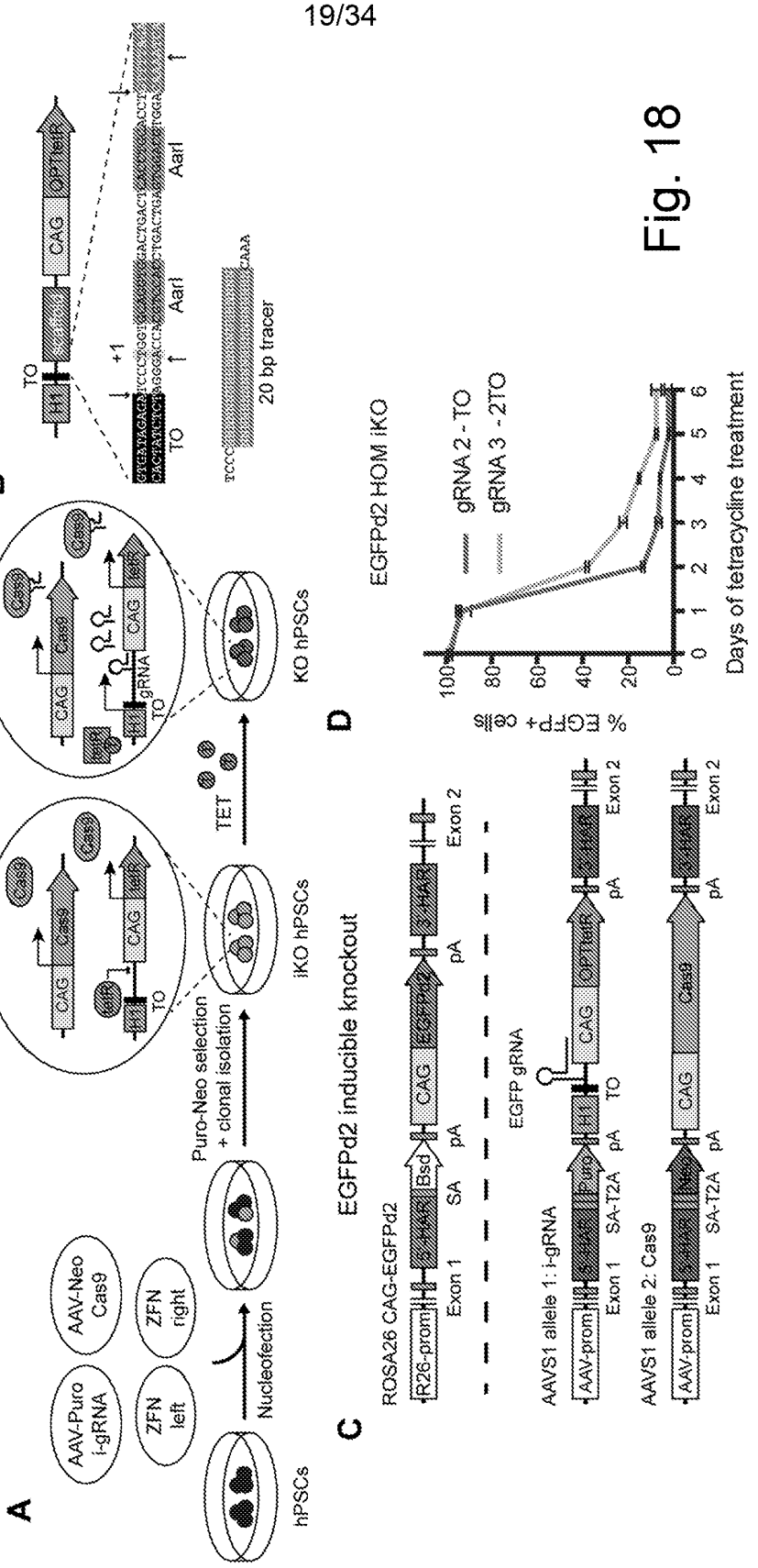

A
AAV-Puro i-gRNA
AAV-Neo Cas9
ZFN left
ZFN right
Nucleofection
hPSCs
Puro-Neo selection + clonal isolation
iKO hPSCs
TET
KO hPSCs
B
TO
+1
AarI
20 bp tracer
C
EGFPd2 inducible knockout
ROSA26 CAG-EGFPd2
R26-prom
Bsd
CAG
EGFPd2
Exon 1
SA
pA
Exon 2
EGFP gRNA
AAVS1 allele 1: i-gRNA
AAV-prom
Puro
SA-T2A
rtTA
DPTetR
TO
AAVS1 allele 2: Cas9
Cas9
D
EGFPd2 HOM iKO
gRNA 2 - TO
gRNA 3 - 2TO
% EGFP+ cells
Days of tetracycline treatment

Fig. 18

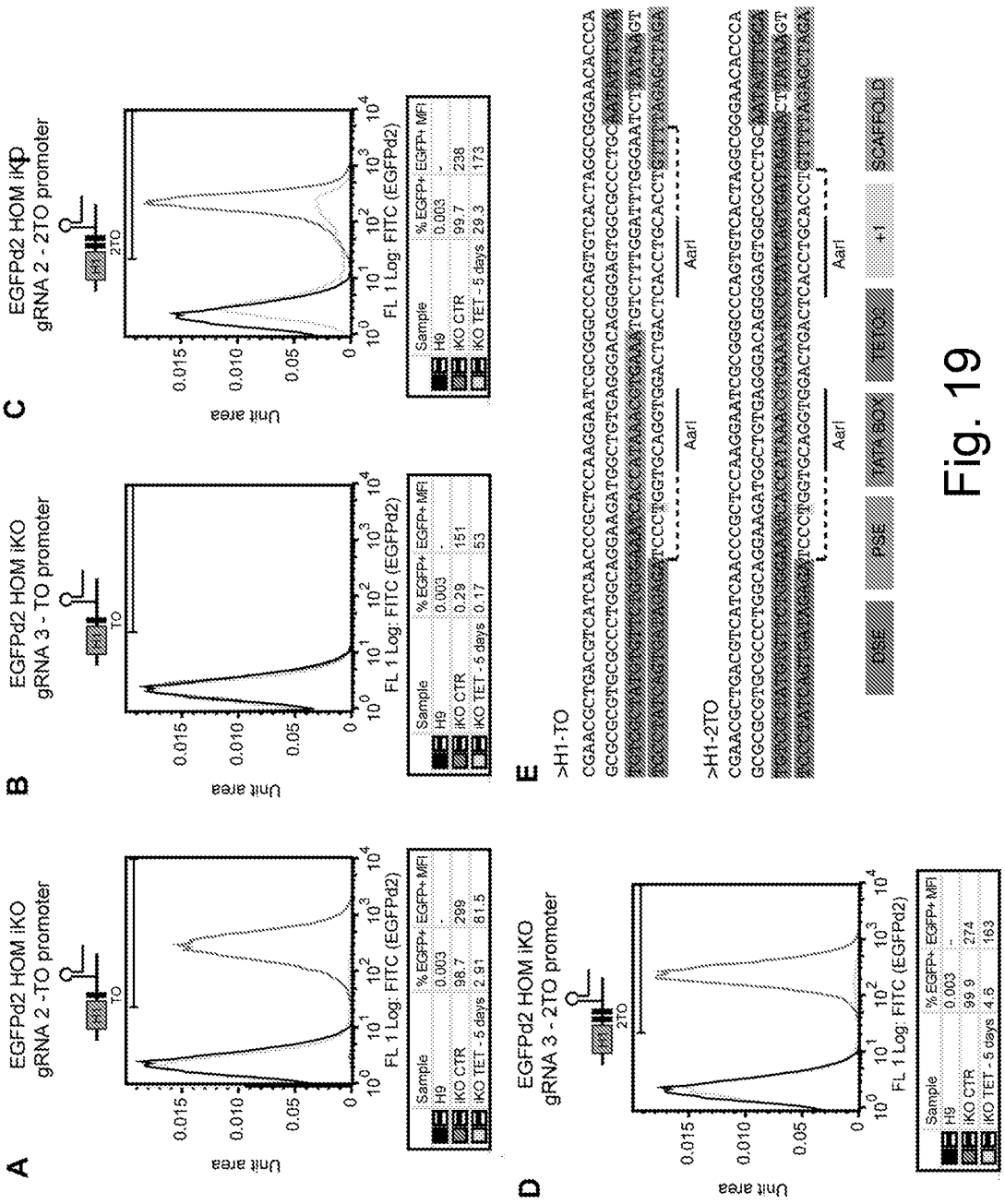

A
EGFPd2 HOM iKO
gRNA 2 -TO promoter
TO
Unit area
FL 1 Log: FITC (EGFPd2)
Sample | % EGFP+ | EGFP+ MFI
H9 | 0.003 | -
iKO CTR | 98.7 | 289
iKO TET - 5 days | 2.91 | 81.5
B
EGFPd2 HOM iKO
gRNA 3 - TO promoter
TO
Unit area
FL 1 Log: FITC (EGFPd2)
Sample | % EGFP+ | EGFP+ MFI
H9 | 0.003 | -
iKO CTR | 0.29 | 151
iKO TET - 5 days | 0.17 | 53
C
EGFPd2 HOM iKO
gRNA 2 - 2TO promoter
2TO
Unit area
FL 1 Log: FITC (EGFPd2)
Sample | % EGFP+ | EGFP+ MFI
H9 | 0.003 | -
iKO CTR | 99.7 | 238
iKO TET - 5 days | 29.3 | 173
D
EGFPd2 HOM iKO
gRNA 3 - 2TO promoter
2TO
Unit area
FL 1 Log: FITC (EGFPd2)
Sample | % EGFP+ | EGFP+ MFI
H9 | 0.003 | -
iKO CTR | 99.9 | 274
iKO TET - 5 days | 4.6 | 183
E
>H1-TO
AarI
>H1-2TO
AarI
AarI
PSE
TRE-BDX
TETON
+1
SCAFFOLD

Fig. 19

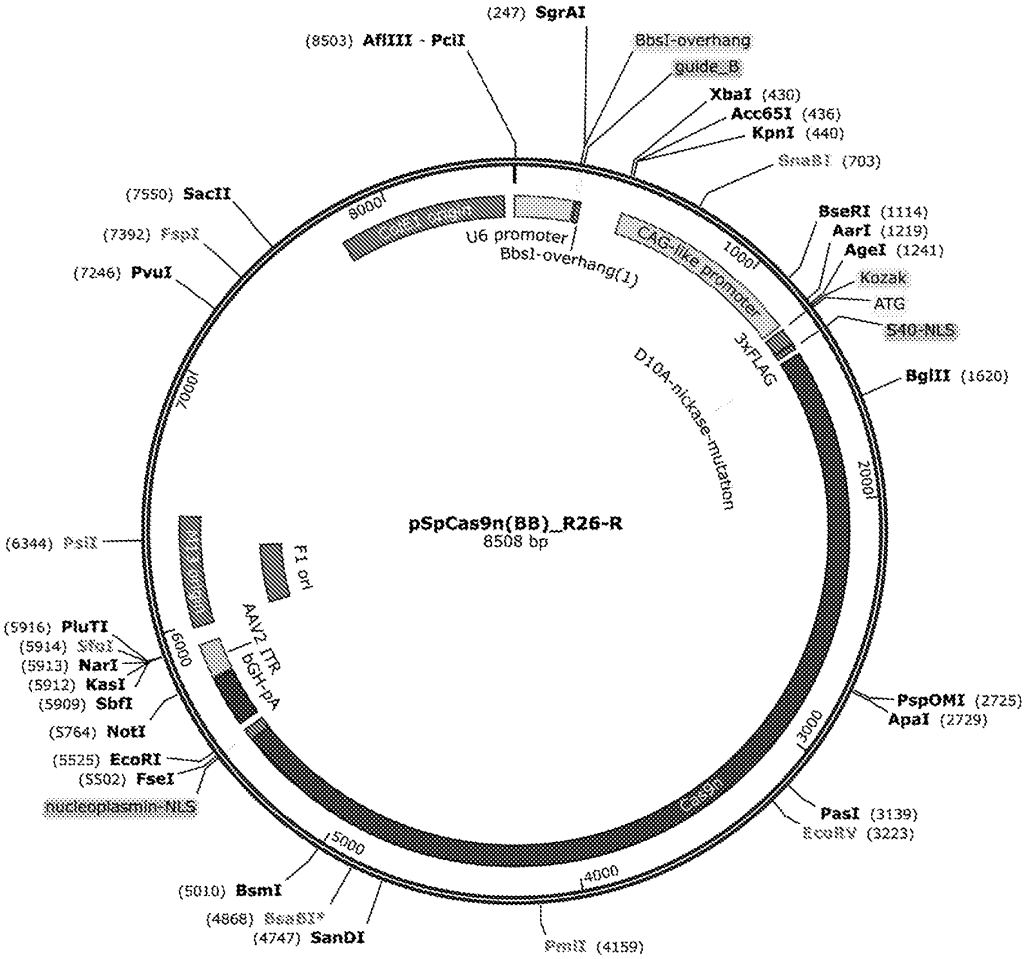

(247) SgrAI
(8503) AflIII - PciI
BbsI-overhang
guide_B
XbaI (430)
Acc65I (436)
KpnI (440)
SnaBI (703)
BseRI (1114)
AarI (1219)
AgeI (1241)
Kozak
ATG
S40-NLS
BglII (1620)
(7550) SacII
(7392) FspI
(7246) PvuI
(6344) PsiI
(5916) PluTI
(5914) SfoI
(5913) NarI
(5912) KasI
(5909) SbfI
(5764) NotI
(5525) EcoRI
(5502) FseI
nucleoplasmin-NLS
(5010) BsmI
(4868) BsaBI*
(4747) SanDI
PmlI (4159)
PspOMI (2725)
ApaI (2729)
PasI (3139)
EcoRV (3223)
U6 promoter
Bbsl-overhang(1)
CAG-like promoter
3xFLAG
D10A-nickase-mutation
pSpCas9n(BB)_R26-R
8508 bp
F1 ori
AAV2 ITR
bGH-pA
1000
2000
3000
4000
5000
6000
7000
8000

Fig. 20

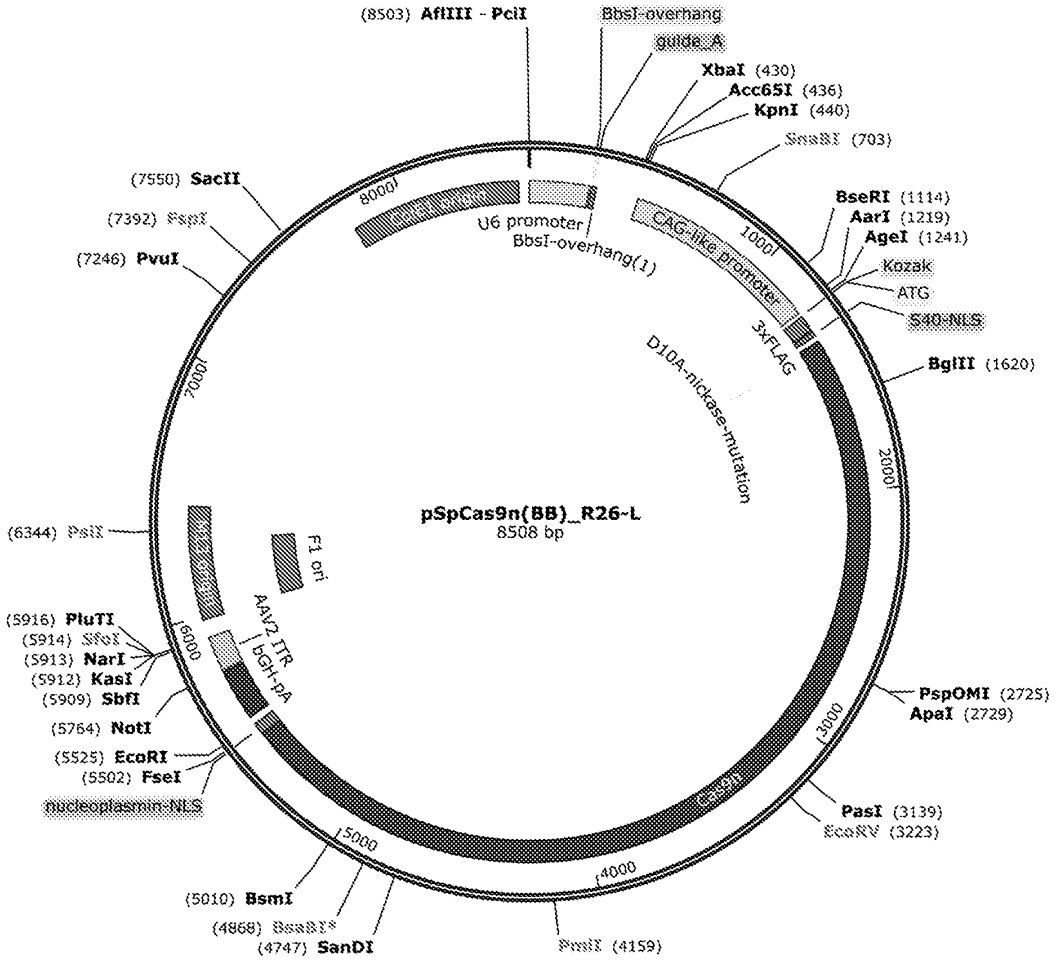

(8503) AflIII - PciI
BbsI-overhang
guide_A
XbaI (430)
Acc65I (436)
KpnI (440)
SnaBI (703)
BseRI (1114)
AarI (1219)
AgeI (1241)
Kozak
ATG
S40-NLS
BglII (1620)
PspOMI (2725)
ApaI (2729)
PasI (3139)
EcoRV (3223)
PmlI (4159)
(4747) SanDI
(4868) BsaBI*
(5010) BsmI
(5502) FseI
(5525) EcoRI
(5764) NotI
(5909) SbfI
(5912) KasI
(5913) NarI
(5914) SfoI
(5916) PluTI
(6344) PsiI
(7246) PvuI
(7392) PspI
(7550) SacII
U6 promoter
BbsI-overhang(1)
CAG-like promoter
3xFLAG
D10A-nickase-mutation
pSpCas9n(BB)_R26-L
8508 bp
F1 ori
AAV2 ITR
bGH-pA
nucleoplasmin-NLS
SV40 NLS
1000
2000
3000
4000
5000
6000
7000
8000

Fig. 21

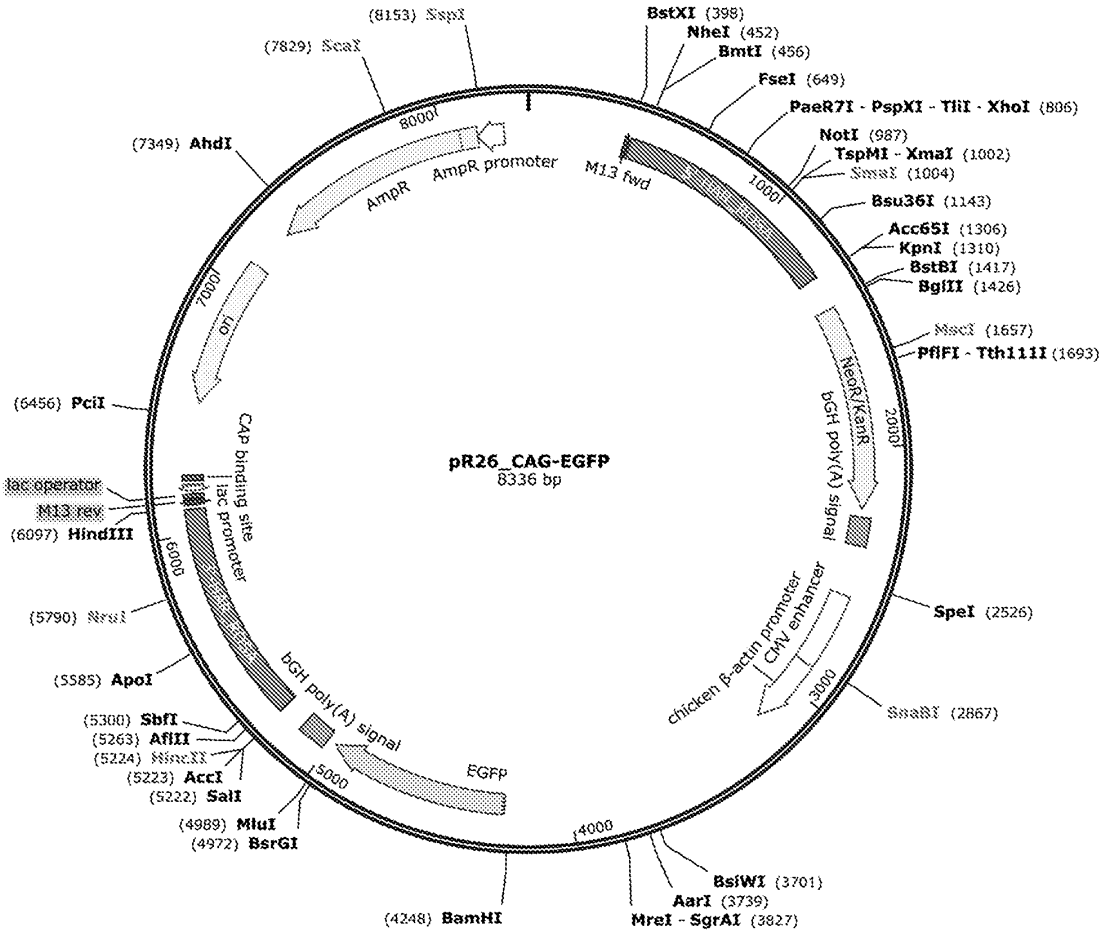

pR26_CAG-EGFP
8336 bp
(8153) SspI
(7829) ScaI
(7349) AhdI
(6456) PciI
(6097) HindIII
(5790) NruI
(5585) ApoI
(5300) SbfI
(5263) AflII
(5224) MfeI
(5223) AccI
(5222) SalI
(4989) MluI
(4972) BsrGI
(4248) BamHI
(3827) MreI - SgrAI
(3739) AarI
(3701) BsiWI
(2867) SnaBI
(2526) SpeI
(1693) PflFI - Tth111I
(1657) MscI
(1426) BglII
(1417) BstBI
(1310) KpnI
(1306) Acc65I
(1143) Bsu36I
(1004) SmaI
(1002) TspMI - XmaI
(987) NotI
(806) PaeR7I - PspXI - TliI - XhoI
(649) FseI
(456) BmtI
(452) NheI
(398) BstXI
8000
AmpR
AmpR promoter
M13 fwd
1000
Neo(r)/KanR
bGH poly(A) signal
2000
ori
7000
CAP binding site
lac operator
M13 rev
lac promoter
6000
bGH poly(A) signal
EGFP
5000
chicken β-actin promoter
CMV enhancer
3000
4000

Fig. 22

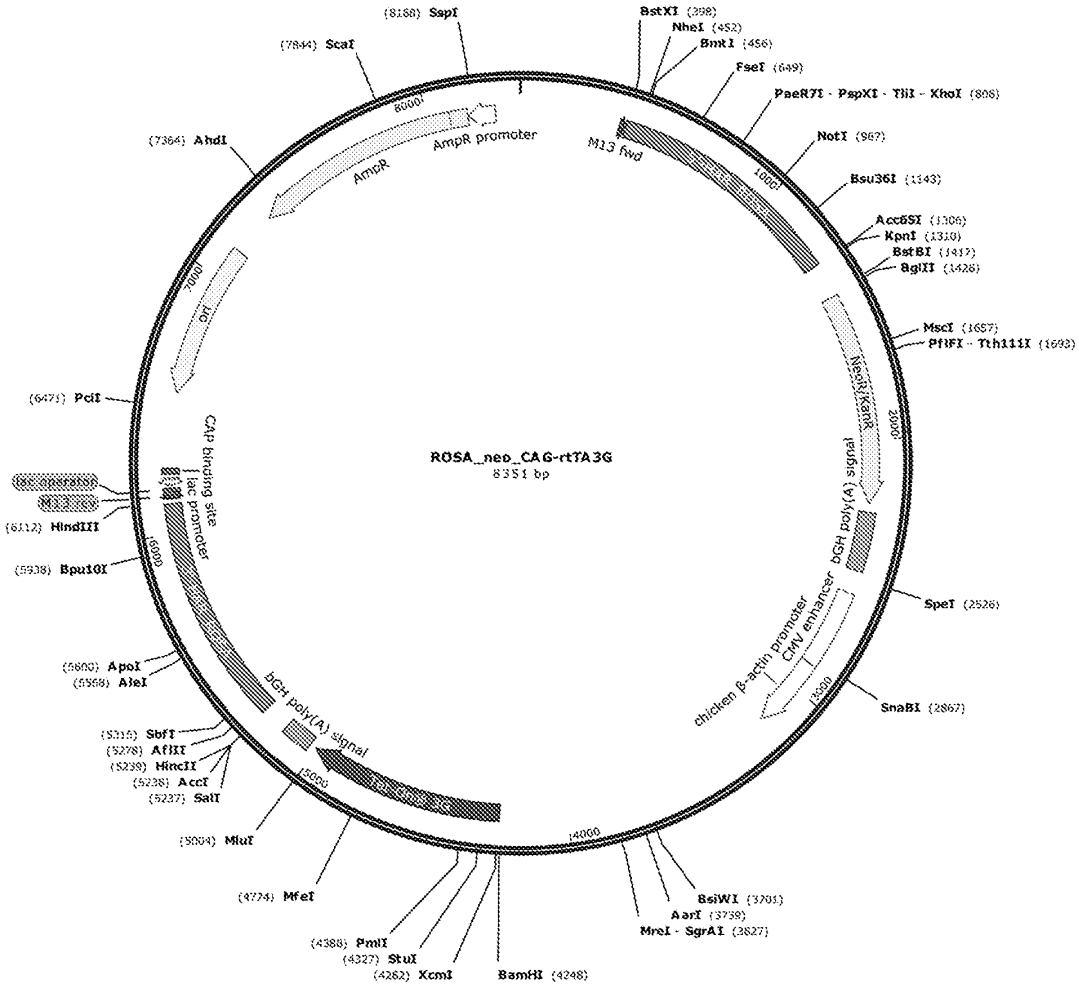

ROSA_neo_CAG-rtTA3G
8351 bp
(8188) SapI
(7844) ScaI
(7384) AhdI
(6471) PciI
(6112) HindIII
(5938) Bpu10I
(5600) ApoI
(5568) AleI
(5315) SbfI
(5278) AflII
(5238) HincII
(5236) AccI
(5237) SalI
(5004) MluI
(4724) MfeI
(4388) PmlI
(4327) StuI
(4262) XcmI
BamHI (4248)
(3738) AarI
MreI - SgrAI (3827)
SnaBI (2867)
SpeI (2526)
NotI (987)
Bsu36I (1143)
Acc65I (1306)
KpnI (1310)
BstBI (1417)
BglII (1428)
MscI (1657)
PflFI - Tth111I (1698)
BstXI (398)
NheI (452)
BmtI (456)
FseI (649)
PaeR7I - PspXI - XhoI (808)
AmpR promoter
AmpR
M13 fwd
Ori
CAP binding site
lac promoter
bGH poly(A) signal
chicken β-actin promoter
CMV enhancer
bGH poly(A) signal
Neo/KanR

Fig. 23

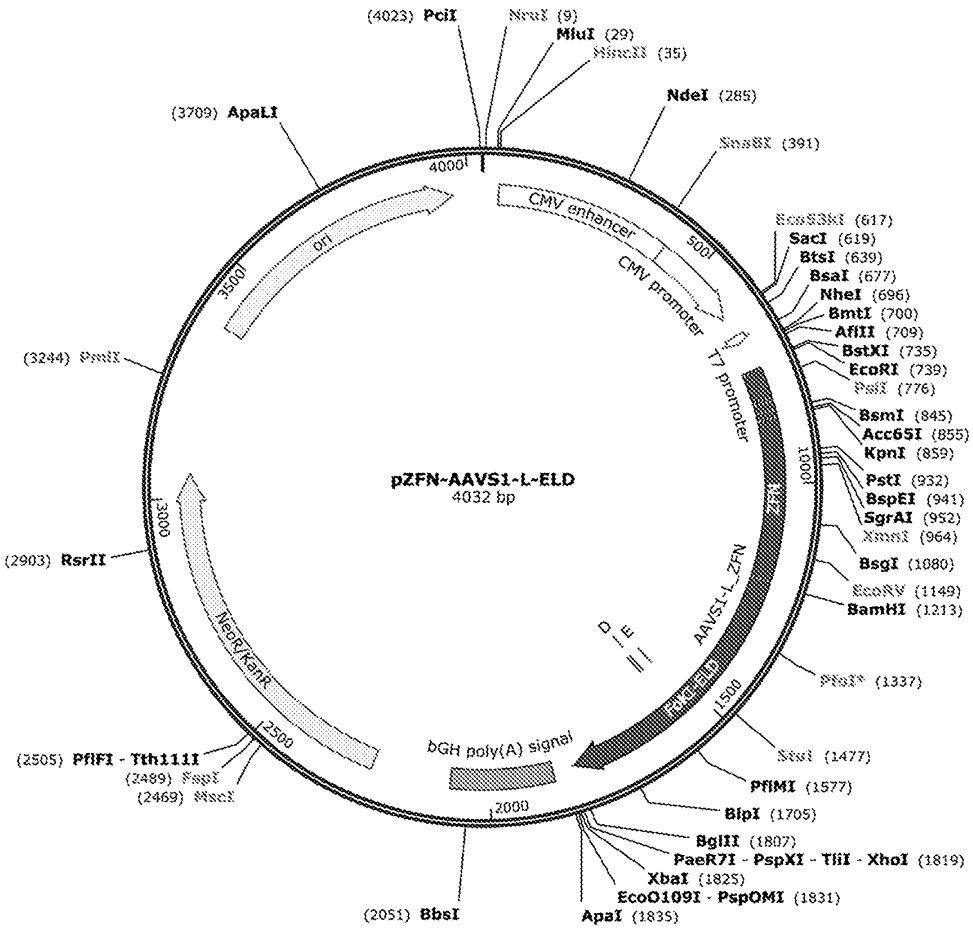

pZFN-AAVS1-L-ELD
4032 bp
(4023) PciI
NruI (9)
MluI (29)
NsiI (35)
NdeI (285)
SmaI (391)
BamHI (617)
SacI (619)
BtsI (639)
BsaI (677)
NheI (696)
BmtI (700)
AflII (709)
BstXI (735)
EcoRI (739)
PstI (776)
BsmI (845)
Acc65I (855)
KpnI (859)
PstI (932)
BspEI (941)
SgrAI (952)
XmaI (964)
BsgI (1080)
EcoRV (1149)
BamHI (1213)
PleI* (1337)
SbfI (1477)
PflMI (1577)
BlpI (1705)
BglII (1807)
PaeR7I - PspXI - TliI - XhoI (1819)
XbaI (1825)
EcoO109I - PspOMI (1831)
ApaI (1835)
(2051) BbsI
bGH poly(A) signal
AAVS1-L_ZFN
T7 promoter
CMV promoter
CMV enhancer
ori
NeoR/KanR
(2505) PflFI - Tth111I
(2489) PspI
(2469) MscI
(2903) RsrII
(3244) PmlI
(3709) ApaLI
4000
500
1000
1500
2000
2500
3000
3500

Fig. 24

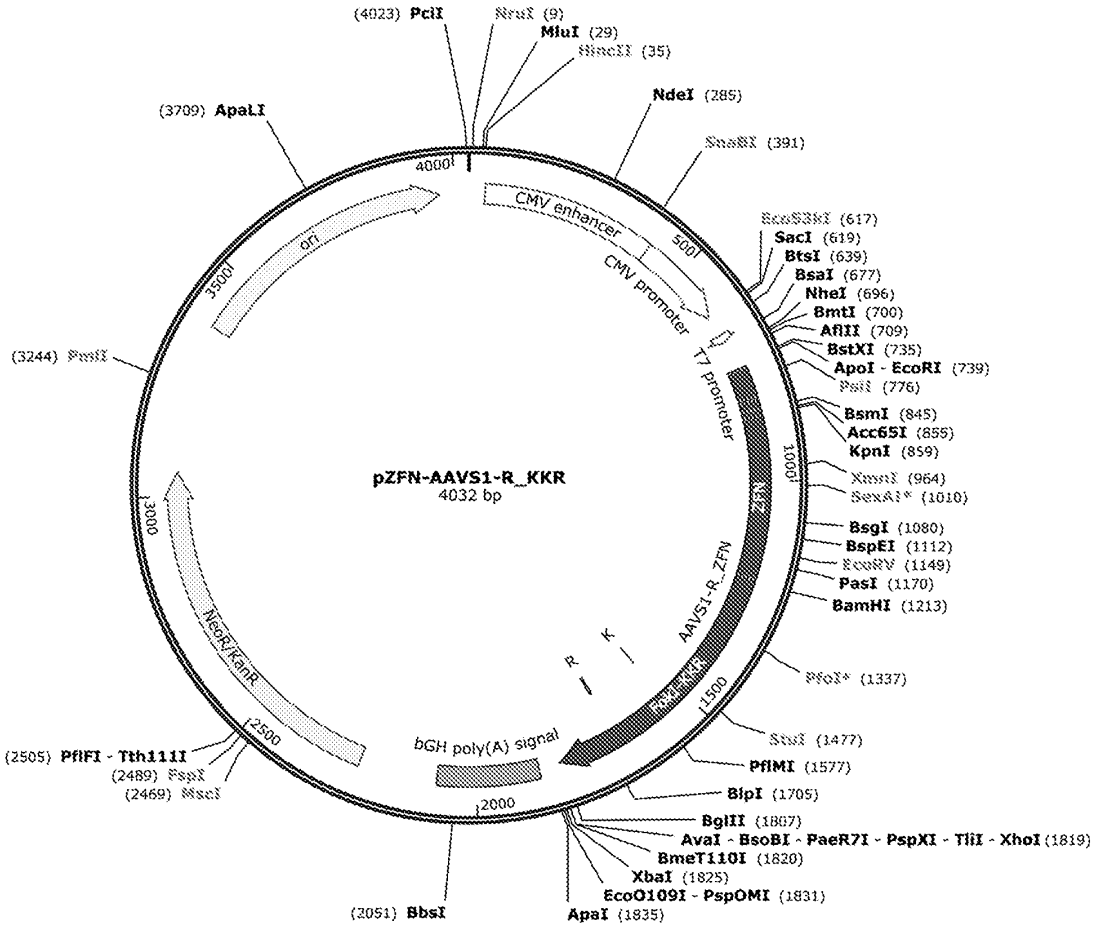

pZFN-AAVS1-R_KKR
4032 bp
CMV enhancer
CMV promoter
T7 promoter
AAVS1-R_ZFN
R
K
bGH poly(A) signal
NeoR/KanR
ori
(4023) PciI
NruI (9)
MluI (29)
NheI (35)
NdeI (285)
SnaBI (391)
(3709) ApaLI
EcoRI (617)
SacI (619)
BtsI (639)
BsaI (677)
NheI (696)
BmtI (700)
AflII (709)
BstXI (735)
ApoI - EcoRI (739)
PsiI (776)
BsmI (845)
Acc65I (855)
KpnI (859)
XmnI (964)
SexAI* (1010)
BsgI (1080)
BspEI (1112)
EcoRV (1149)
PasI (1170)
BamHI (1213)
PfoI* (1337)
StuI (1477)
PflMI (1577)
BipI (1705)
BglII (1807)
AvaI - BsoBI - PaeR7I - PspXI - TliI - XhoI (1819)
BmeT110I (1820)
XbaI (1825)
EcoO109I - PspOMI (1831)
ApaI (1835)
(2051) BbsI
(2505) PflFI - Tth111I
(2489) PspXI
(2469) MscI
(3244) PmlI
4000
500
1000
1500
2000
2500
3000
3500

Fig. 25

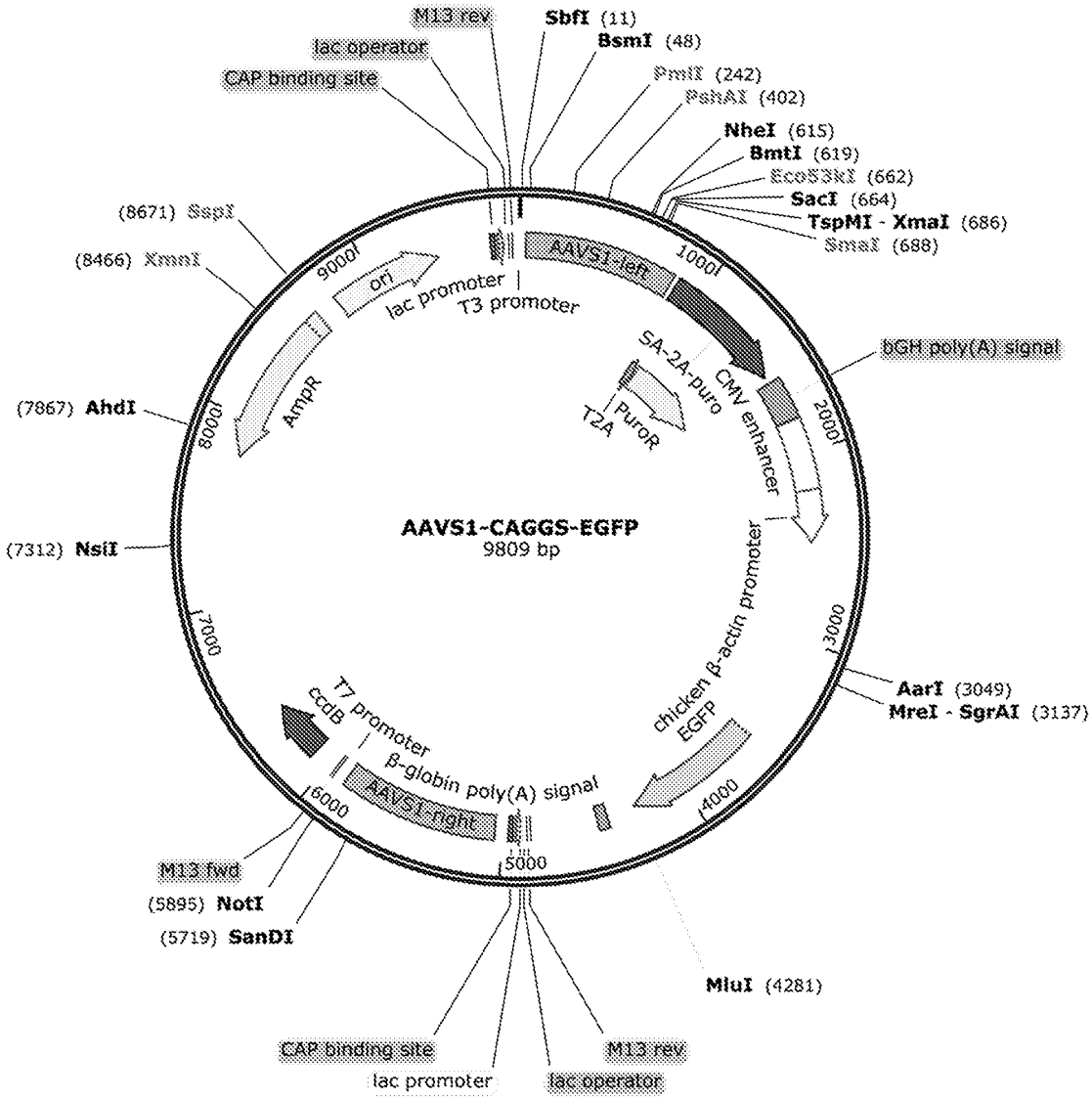

M13 rev
SbfI (11)
BsmI (48)
lac operator
CAP binding site
PmlI (242)
PshAI (402)
NheI (615)
BmtI (619)
EcoS3NI (662)
SacI (664)
TspMI - XmaI (686)
SmaI (688)
(8671) SspI
(8466) XmnI
9000
ori
lac promoter
T3 promoter
AAVS1 left
10000
bGH poly(A) signal
SA-2A-puro
T2A
PuroR
CMV enhancer
(7867) AhdI
8000
AmpR
AAVS1~CAGGS-EGFP
9809 bp
(7312) NsiI
2000
7000
3000
AarI (3049)
MreI - SgrAI (3137)
T7 promoter
ccdB
chicken β-actin promoter
EGFP
β-globin poly(A) signal
AAVS1 right
6000
4000
5000
M13 fwd
(5895) NotI
(5719) SanDI
MluI (4281)
CAP binding site
lac promoter
lac operator
M13 rev

Fig. 26

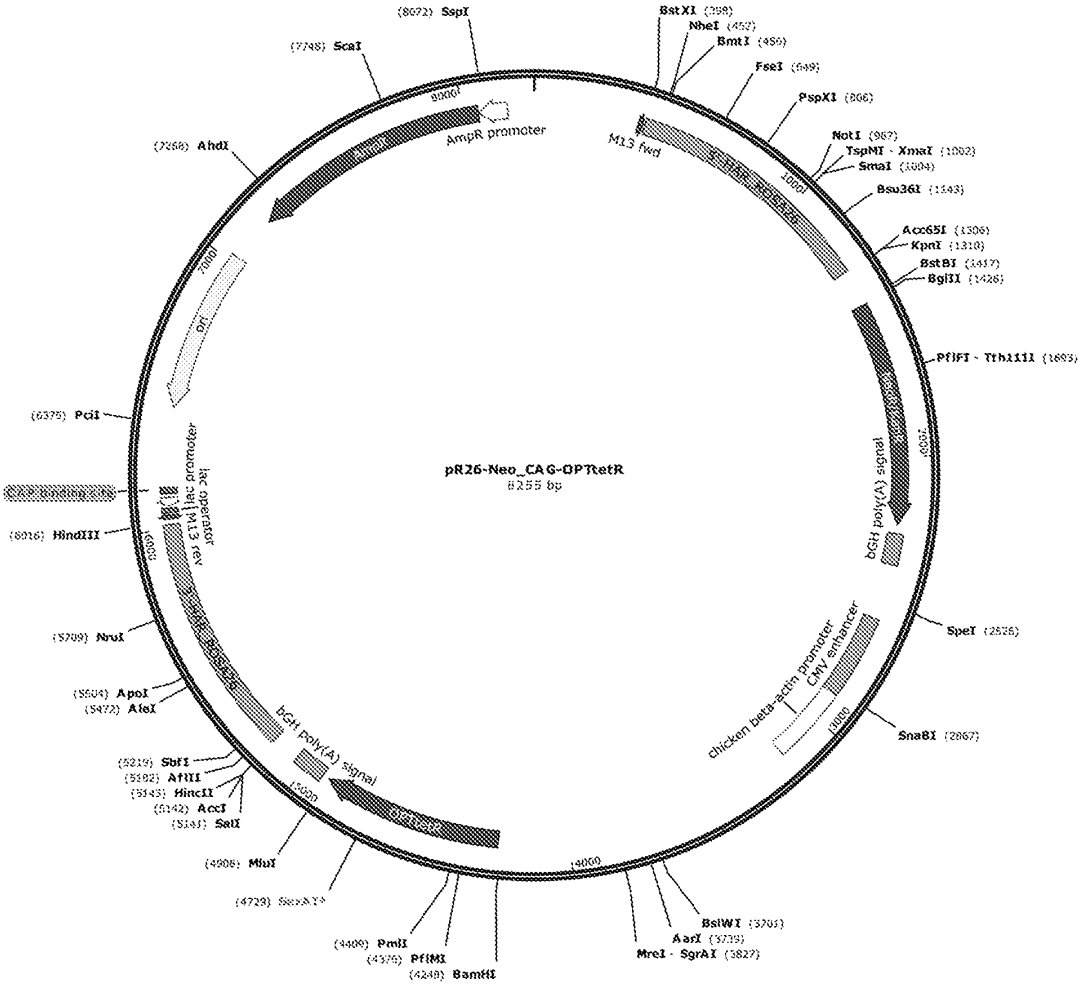

pR26-Neo_CAG-OPTetR
8255 bp
(8072) SspI
(7748) ScaI
(7268) AhdI
(6375) PciI
(6016) HindIII
(5709) NruI
(5504) ApoI
(5472) AleI
(5219) SbfI
(5182) AflII
(5143) HincII
(5142) AccI
(5141) SalI
(4908) MluI
(4729) SexAI*
(4409) PmlI
(4376) PflMI
(4248) BamHI
(3827) MreI - SgrAI
(3739) AarI
(3701) BsiWI
(2867) SnaBI
(2626) SpeI
(1693) PflFI - Tth111I
(1448) BglII
(1437) BstBI
(1318) KpnI
(1306) Acc65I
(1143) Bsu36I
(1004) SmaI
(1002) TspMI - XmaI
(967) NotI
(806) PspXI
(649) FseI
(490) BmtI
(452) NheI
(398) BstXI
AmpR promoter
M13 fwd
bGH poly(A) signal
chicken beta-actin promoter
CMV enhancer
bGH poly(A) signal
lac operator
lac promoter
M13 rev
ori

Fig. 27

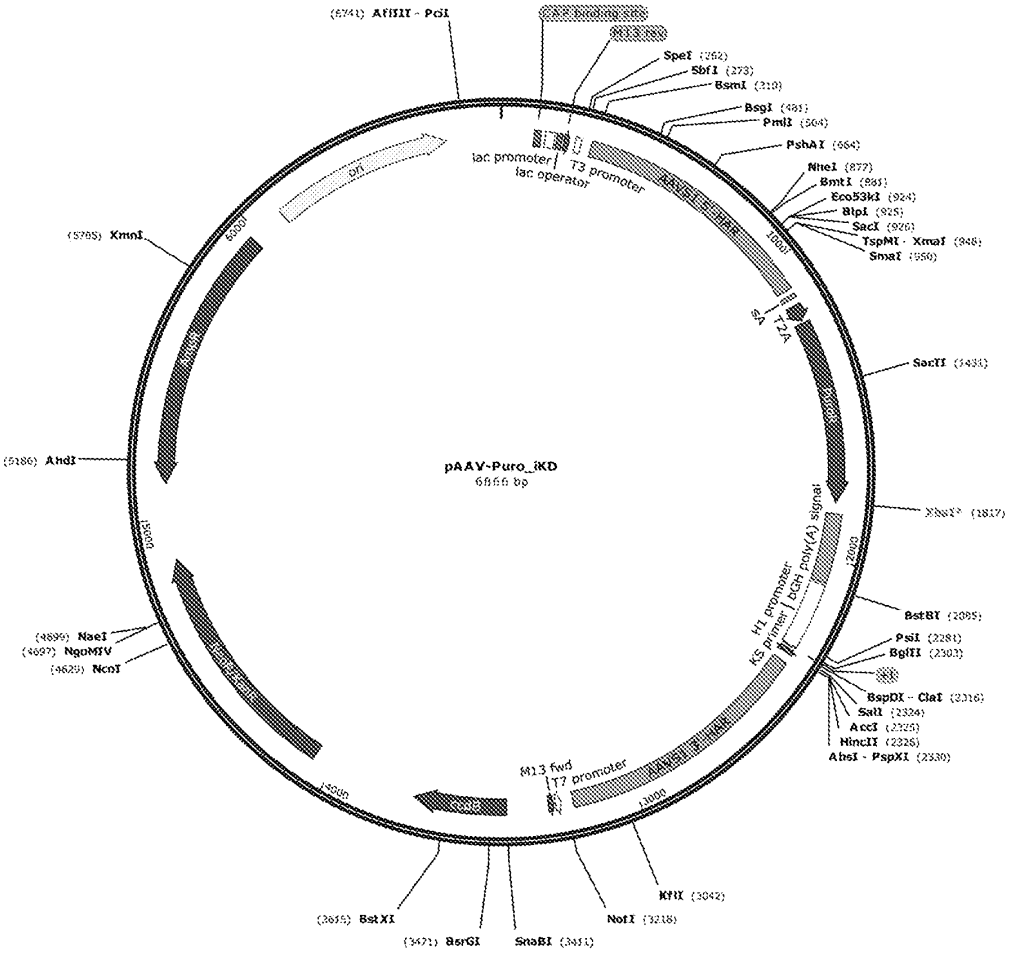

pAAV-Puro_iKD
6666 bp
(6741) AflIII - PciI
SpeI (262)
SbfI (273)
BsmI (310)
BsgI (481)
PmlI (504)
PshAI (664)
NheI (877)
BmtI (885)
Eco53kI (924)
BlpI (925)
SacI (926)
TspMI - XmaI (948)
SmaI (950)
SexAI (1434)
XbaI (1817)
BstBI (2085)
PsiI (2281)
BglII (2303)
BspDI - ClaI (2316)
SalI (2324)
AccI (2325)
HincII (2326)
AhdI - PspXI (2330)
KflI (3042)
NotI (3238)
SnaBI (3451)
BsrGI (3471)
BstXI (3655)
(5186) AhdI
(4896) NaeI
(4897) NgoMIV
(4629) NcoI
(5785) XmnI
ori
lac promoter
lac operator
T3 promoter
SA
T2A
H1 promoter
KS primer
bGH poly(A) signal
M13 fwd
T7 promoter
M13 rev
AmpR promoter
AmpR
f1 ori
1000
2000
3000
4000
5000
6000

Fig. 28

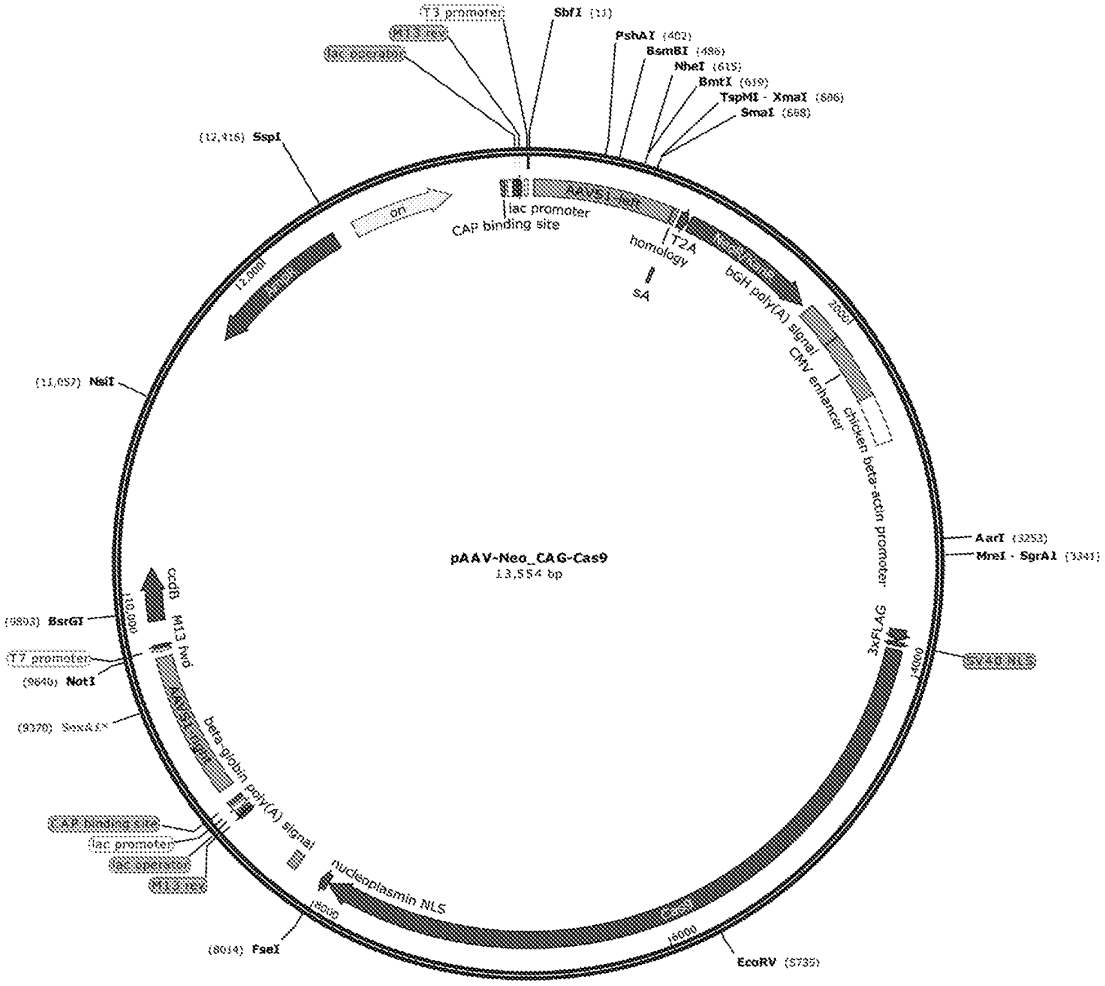

pAAV-Neo_CAG-Cas9
13,554 bp
T3 promoter
SbfI (1)
PshAI (402)
BsmBI (486)
NheI (615)
BmtI (619)
TspMI - XmaI (806)
SmaI (668)
(12,416) SspI
ori
lac promoter
CAP binding site
T2A
homology
sA
bGH poly(A) signal
CMV enhancer
chicken beta-actin promoter
3xFLAG
(11,057) NsiI
AarI (3253)
MreI - SgrAI (3341)
ccdB
(9803) BsrGI
T7 promoter
(9646) NotI
(9370) SmaAI
beta-globin poly(A) signal
nucleoplasmin NLS
(8014) FseI
EcoRV (5735)

Fig. 29


pAAV-Puro_siKD
9585 bp
(9460) PciI
SbfI (273)
BsmI (330)
PshAI (664)
NheI (877)
BmtI (885)
Eco53kI (924)
SacI (926)
TspMI - XmaI (946)
SmaI (950)
lac promoter
T3 promoter
T2A homology
SA
H1 promoter
gRNA scaffold
BstBI (2085)
PsiI (2281)
SalI (2422)
AccI (2423)
HincII (2424)
NdeI (2667)
chicken beta-actin promoter
CMV enhancer
SV40 NLS
bGH poly(A) signal
MluI (4814)
NruI - SgrAI (3733)
NotI (5937)
KflI (5761)
BstXI (6334)
BsrGI (6198)
T7 promoter
(7905) AhdI

Fig. 30


pAAV-Puro_sKO-2TO
9585 bp
(9860) PciI
SbfI (273)
BsmI (310)
PshAI (664)
NheI (677)
BmtI (681)
Eco53kI (924)
SacI (926)
TspMI - XmaI (948)
SmaI (950)
ori
lac promoter
T3 promoter
M13 rev
AAS1 left
homology
T2A
SA
SA-2A-puro
SA-2A-puro
AAS1 right
bGH poly(A) signal
H1 promoter
gRNA scaffold
BstBI (2085)
PsiI (2281)
SalI (2422)
AccI (2423)
NruI (2424)
NdeI (2667)
chicken beta-actin promoter
CMV enhancer
MreI - SgrAI (3733)
SV40 NLS
bGH poly(A) signal
T7 promoter
(5781) KfiI
(5937) NotI
(6190) BsrGI
(6334) BstXI
(7908) AhdI
(4814) MluI

Fig. 31

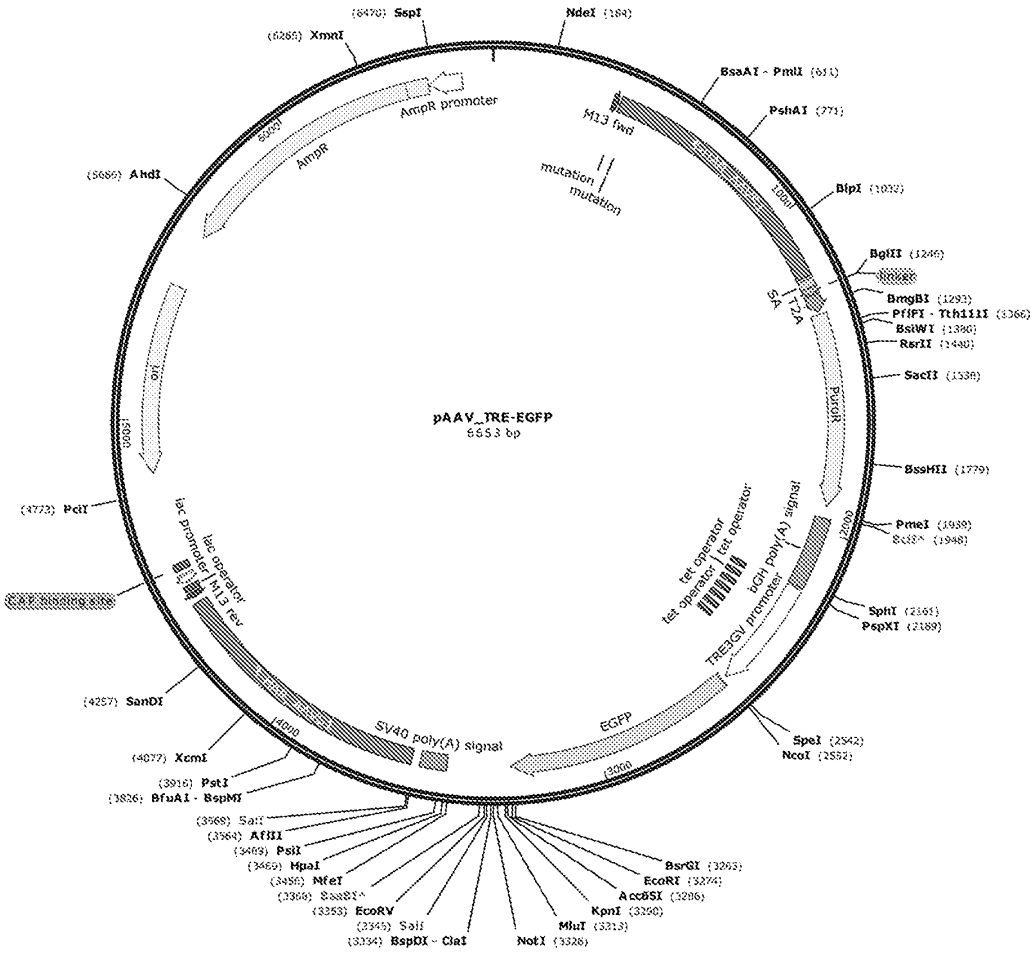

pAAV_TRE-EGFP
6653 bp
(6470) SspI
NdeI (184)
(6265) XmnI
BsaAI - PmlI (611)
PshAI (771)
BlpI (1032)
BglII (1246)
BmgBI (1293)
PflFI - Tth111I (1366)
BstWI (1380)
RsrII (1440)
SacII (1538)
BssHII (1779)
PmeI (1938)
SphI (2161)
PspXI (2189)
SpeI (2542)
NcoI (2552)
BsrGI (3263)
EcoRI (3274)
Acc65I (3286)
KpnI (3290)
MluI (3313)
NotI (3328)
(3334) BspDI - ClaI
(3345) SalI
(3353) EcoRV
(3366) SmaI^
(3369) NheI
(3456) HpaI
(3469) PsiI
(3564) AflII
(3568) SalI
(3826) BfuAI - BspMI
(3916) PstI
(4077) XcmI
(4257) SanDI
(4773) PciI
(5686) AhdI
AmpR promoter
AmpR
M13 fwd
mutation
mutation
IRES
T2A
SA
WPRE
bGH poly(A) signal
TRE3GV promoter
tet operator
tet operator
tet operator
EGFP
SV40 poly(A) signal
M13 rev
lac operator
lac promoter
ori

Fig. 32

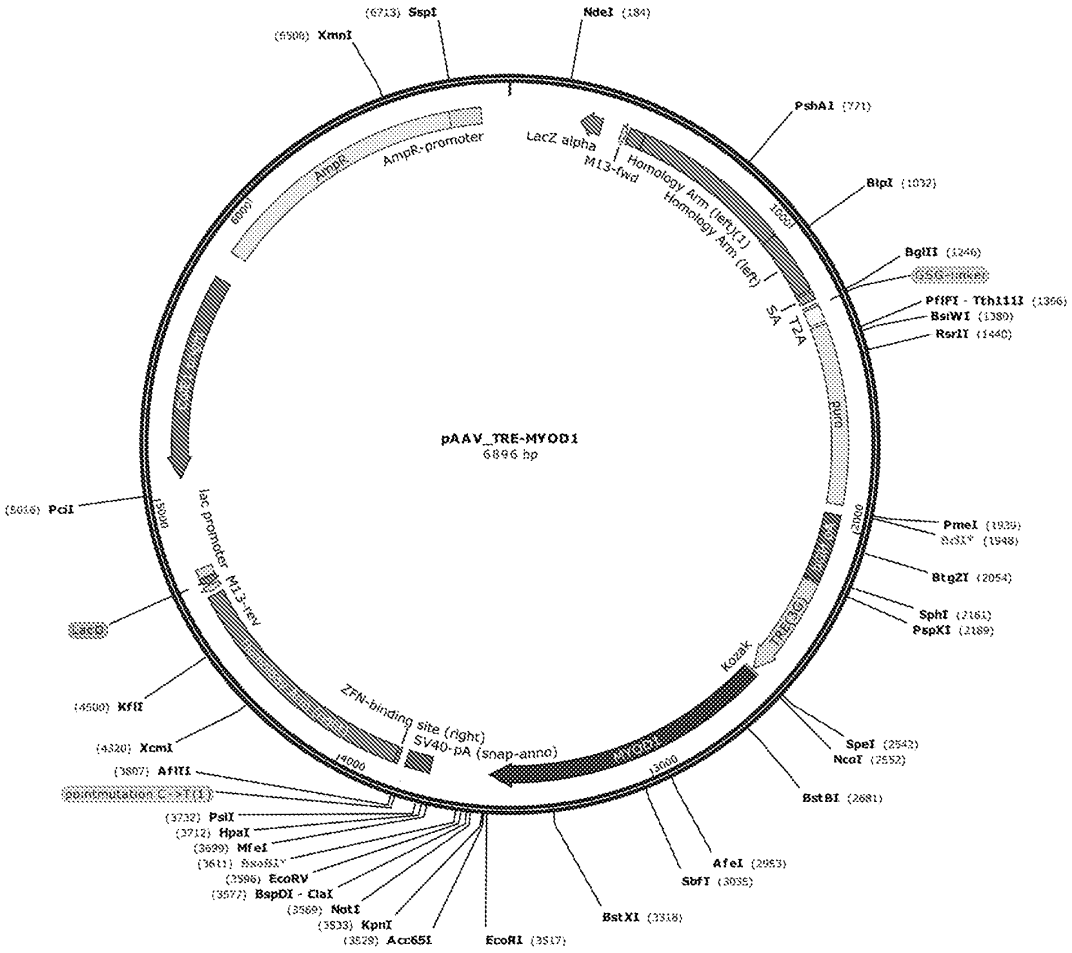

pAAV_TRE-MYOD1
6896 bp
(6713) SspI
NdeI (184)
(6508) XmnI
PshAI (971)
BlpI (1032)
BglII (1246)
PflFI - Tth111I (1366)
BsiWI (1380)
RsrII (1440)
PmeI (1939)
Bst1107I (1948)
Btg2I (2054)
SphI (2161)
PspXI (2189)
SpeI (2542)
NcoI (2552)
BstBI (2681)
AfeI (2953)
SbfI (3035)
BstXI (3318)
EcoRI (3517)
(3528) Acc65I
(3533) KpnI
(3569) NotI
(3577) BspDI - ClaI
(3586) EcoRV
(3611) SnaBI
(3699) MfeI
(3712) HpaI
(3732) PsiI
(3807) AflII
(4320) XcmI
(4500) KflI
(5016) PciI
LacZ alpha
M13-fwd
Homology Arm (left)(1)
Homology Arm (left)
SA
T2A
puro
TRE3GS
Kozak
ZFN-binding site (right)
SV40-pA (snap-anno)
lac promoter M13-rev
AmpR
AmpR-promoter
ori
1000
2000
3000
4000
5000
6000

Fig. 33