Fig. 1

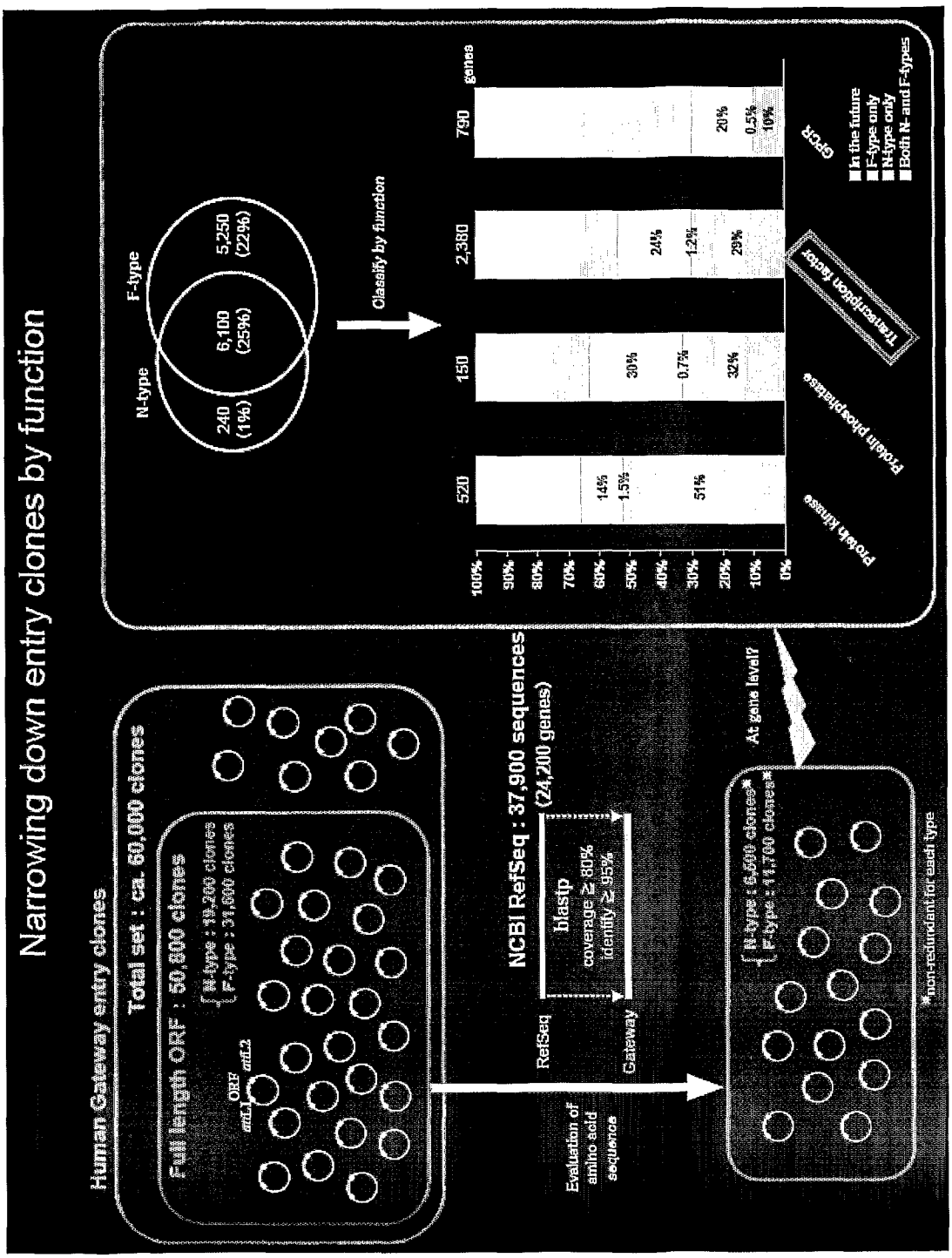

Narrowing down entry clones by function
Human Gateway entry clones
Total set : ca. 60,000 clones
Full length ORF : 50,800 clones
N-type : 19,200 clones
F-type : 31,600 clones
ORF
attL1
attL2
Evaluation of amino acid sequences
RefSeq
Gateway
NCBI RefSeq : 37,900 sequences
(24,200 genes)
blastp
coverage ≥ 80%
identity ≥ 95%
At gene level*
N-type : 6,600 clones*
F-type : 11,700 clones*
*non-redundant for each type
N-type
F-type
240 (1%)
6,100 (25%)
5,250 (22%)
Classify by function
520
150
2,380
790 genes
14%
1.5%
30%
51%
0.7%
24%
32%
1.2%
29%
20%
0.5%
16%
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Protein kinase
Protein phosphatase
Transcription factor
GPCR
In the future
F-type only
N-type only
Both N- and F-types

Fig. 2

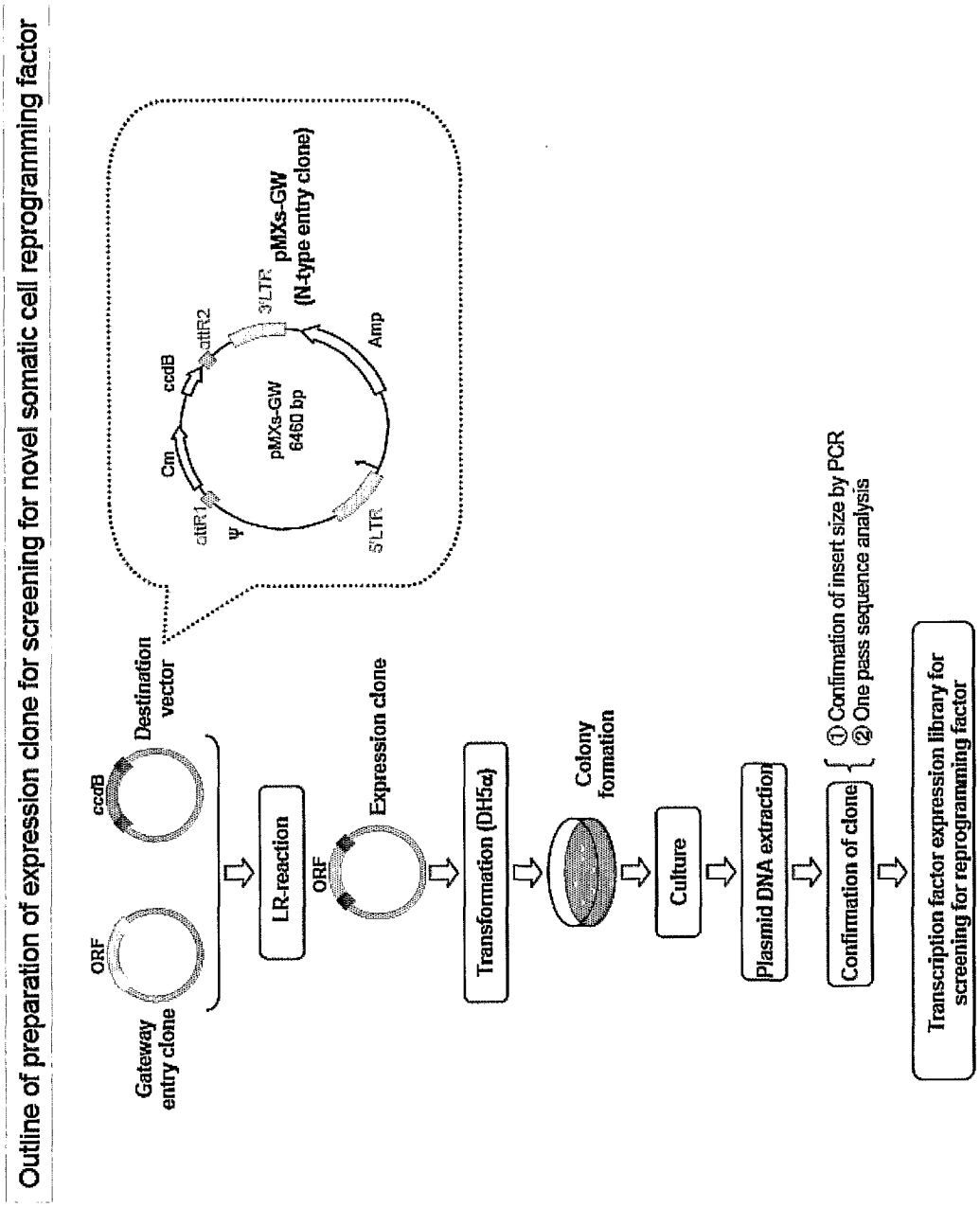

Outline of preparation of expression clone for screening for novel somatic cell reprogramming factor
Gateway entry clone
ORF
ccdB
Destination vector
LR-reaction
ORF
Expression clone
Transformation (DH5α)
Colony formation
Culture
Plasmid DNA extraction
Confirmation of clone
① Confirmation of insert size by PCR
② One pass sequence analysis
Transcription factor expression library for screening for reprogramming factor
Cm
ccdB
attR2
3'LTR
pMXs-GW (N-type entry clone)
attR1
Ψ
pMXs-GW 6460 bp
Amp
5'LTR

Fig. 3

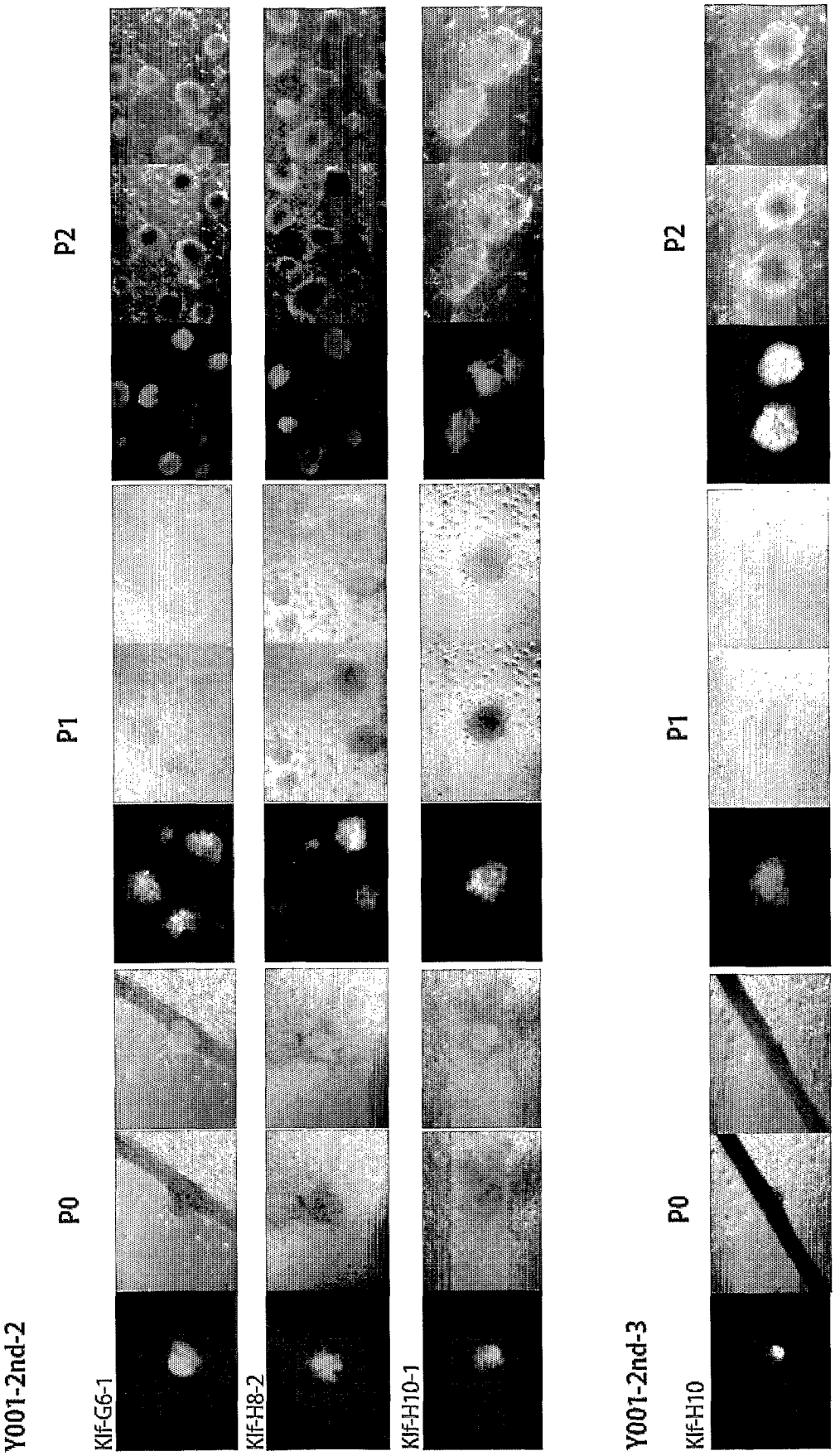

Y001-2nd-2
P0
P1
P2
Klf-G6-1
Klf-H8-2
Klf-H10-1
Y001-2nd-3
P0
P1
P2
Klf-H10

Fig. 4

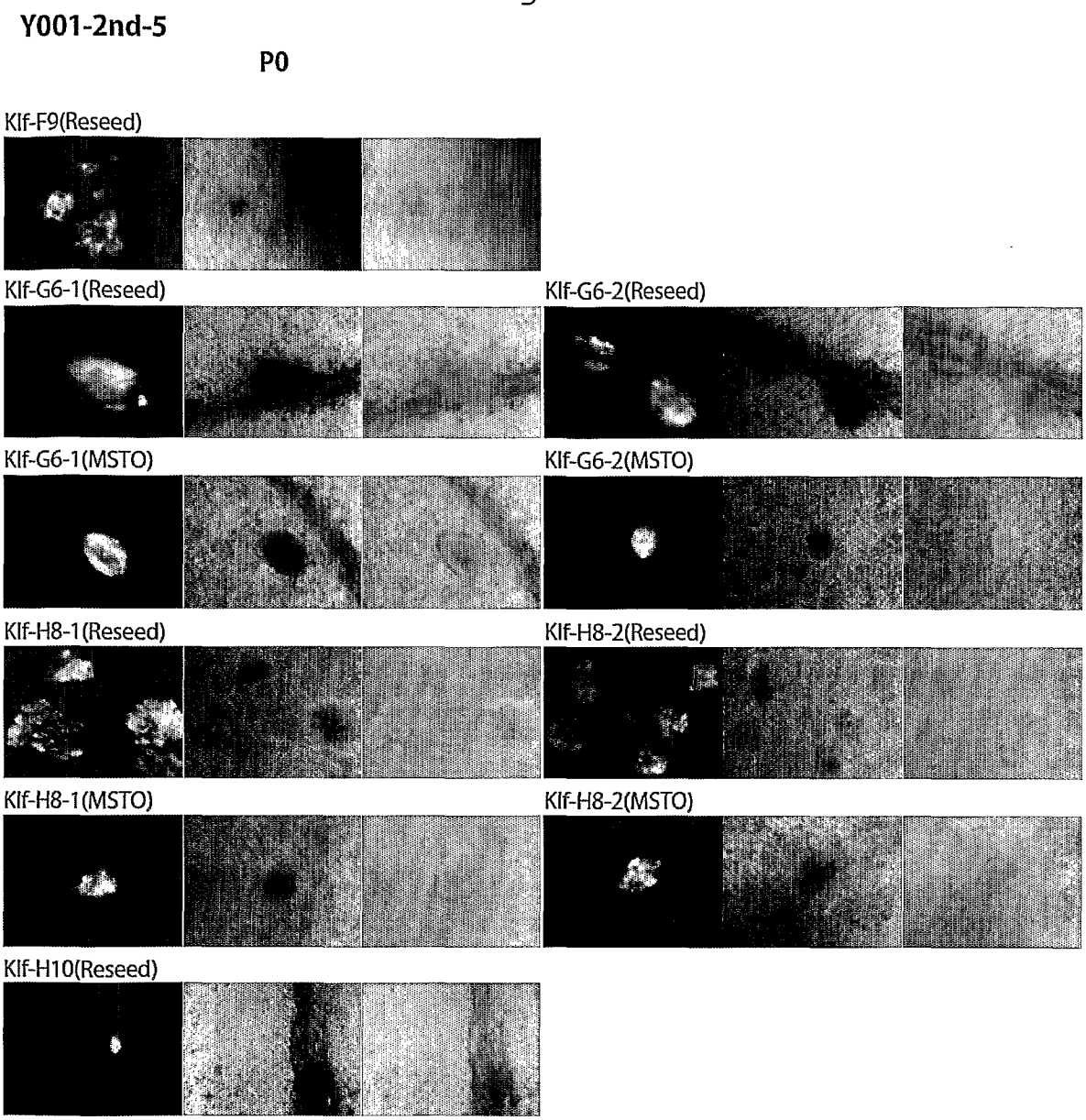

Y001-2nd-5
P0
Klf-F9(Reseed)
Klf-G6-1(Reseed)
Klf-G6-2(Reseed)
Klf-G6-1(MSTO)
Klf-G6-2(MSTO)
Klf-H8-1(Reseed)
Klf-H8-2(Reseed)
Klf-H8-1(MSTO)
Klf-H8-2(MSTO)
Klf-H10(Reseed)

Fig. 5

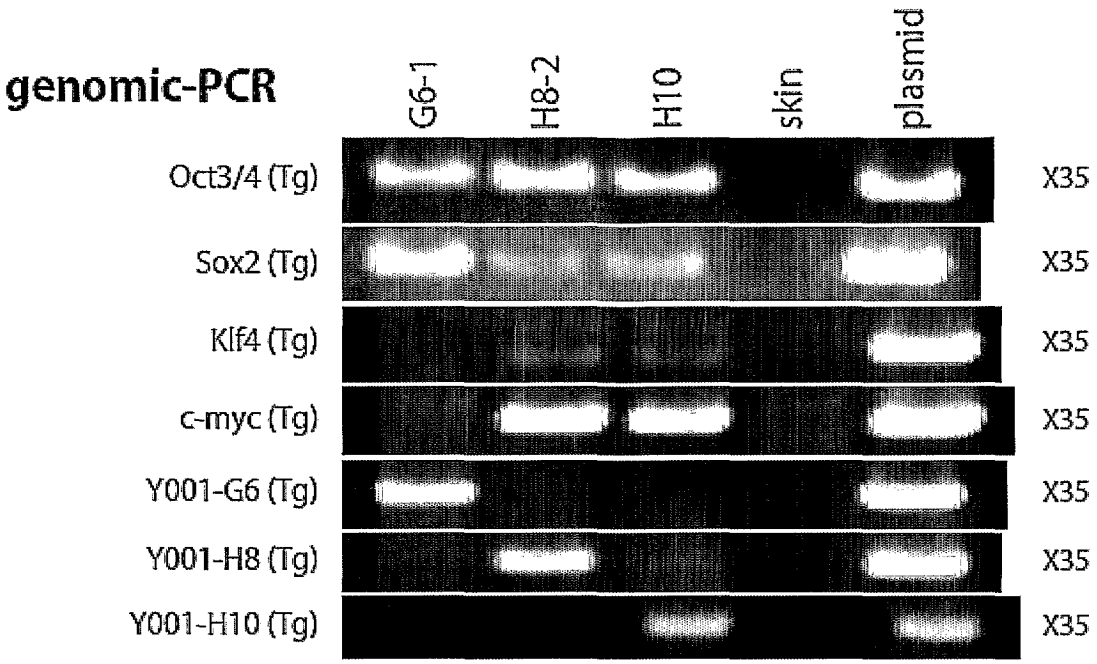

genomic-PCR
G6-1
H8-2
H10
skin
plasmid
Oct3/4 (Tg)
Sox2 (Tg)
Klf4 (Tg)
c-myc (Tg)
Y001-G6 (Tg)
Y001-H8 (Tg)
Y001-H10 (Tg)
X35
X35
X35
X35
X35
X35
X35

Fig. 6

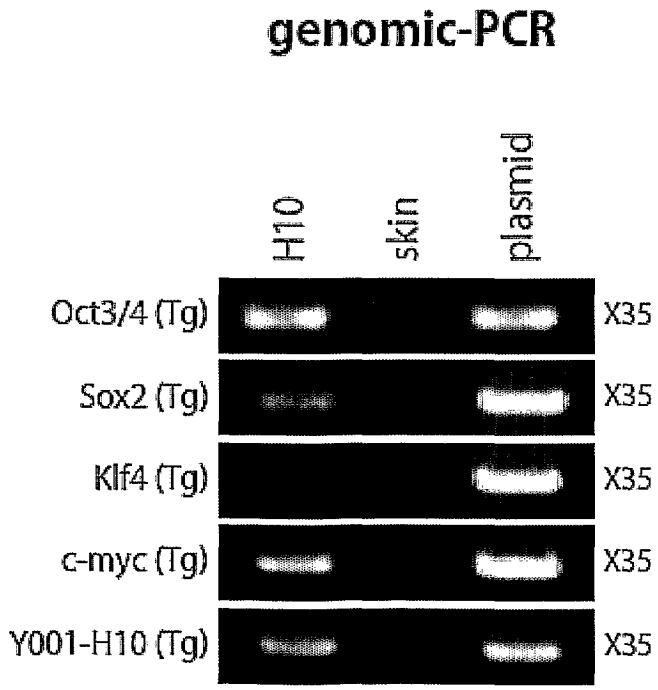

genomic-PCR
H10
skin
plasmid
Oct3/4 (Tg)
Sox2 (Tg)
Klf4 (Tg)
c-myc (Tg)
Y001-H10 (Tg)
X35
X35
X35
X35
X35

Fig. 7

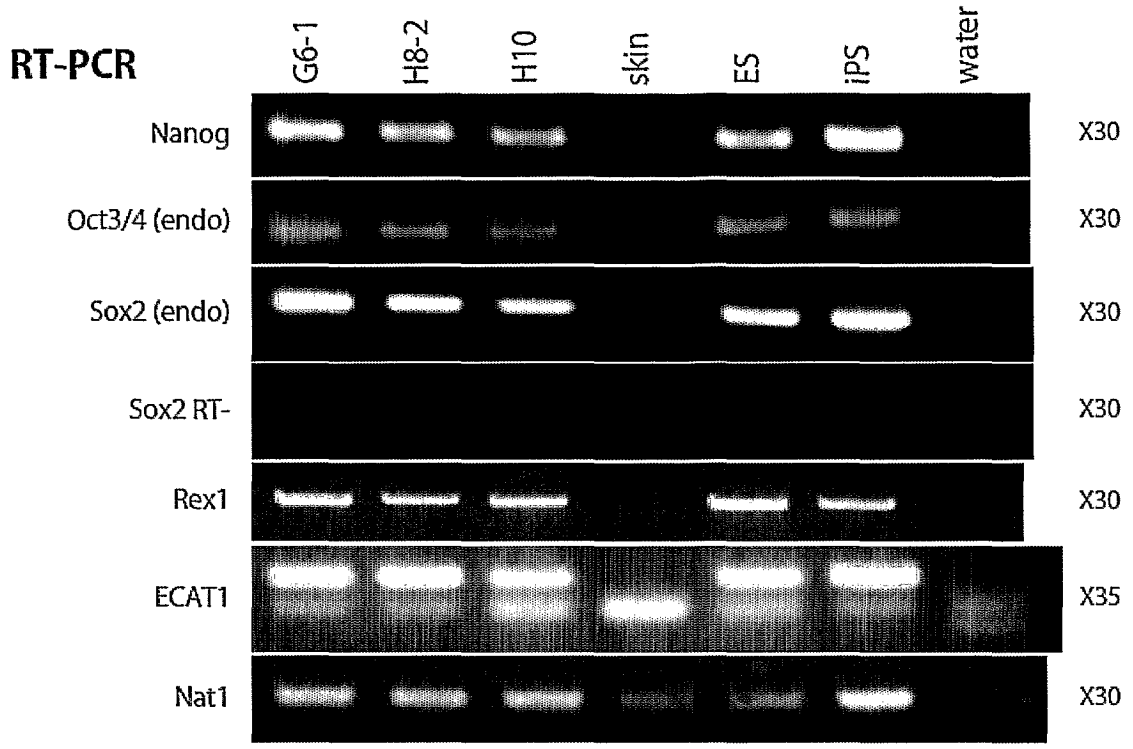

RT-PCR
G6-1
H8-2
H10
skin
ES
iPS
water
Nanog
Oct3/4 (endo)
Sox2 (endo)
Sox2 RT-
Rex1
ECAT1
Nat1
X30
X30
X30
X30
X30
X35
X30

Fig. 8

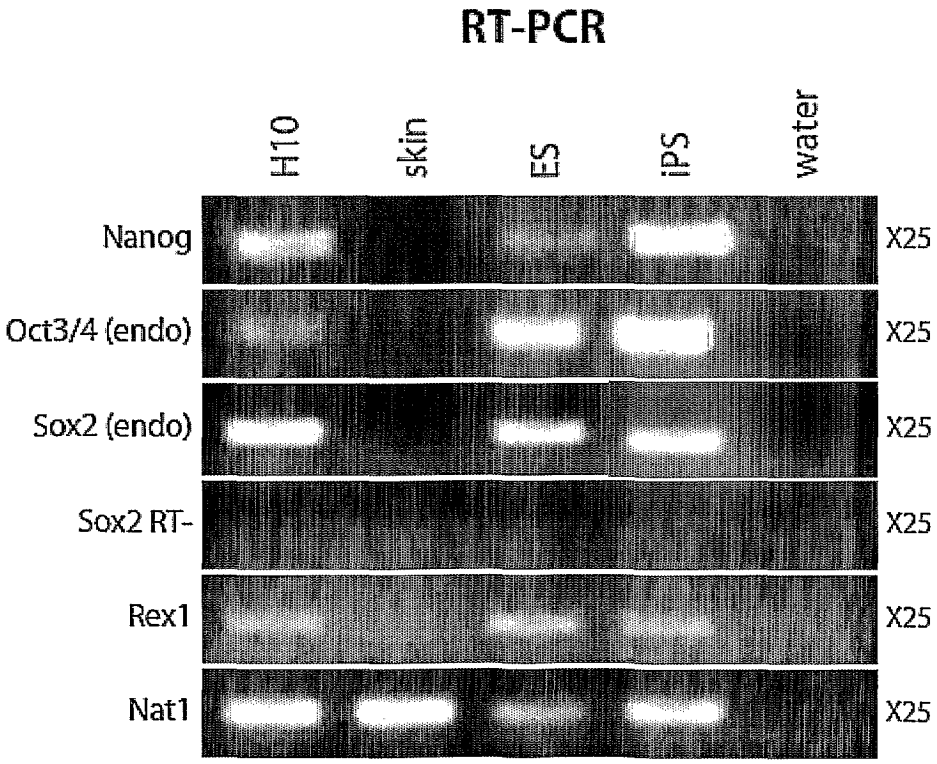

RT-PCR
H10
skin
ES
iPS
water
Nanog
Oct3/4 (endo)
Sox2 (endo)
Sox2 RT-
Rex1
Nat1
X25
X25
X25
X25
X25
X25

Fig. 9

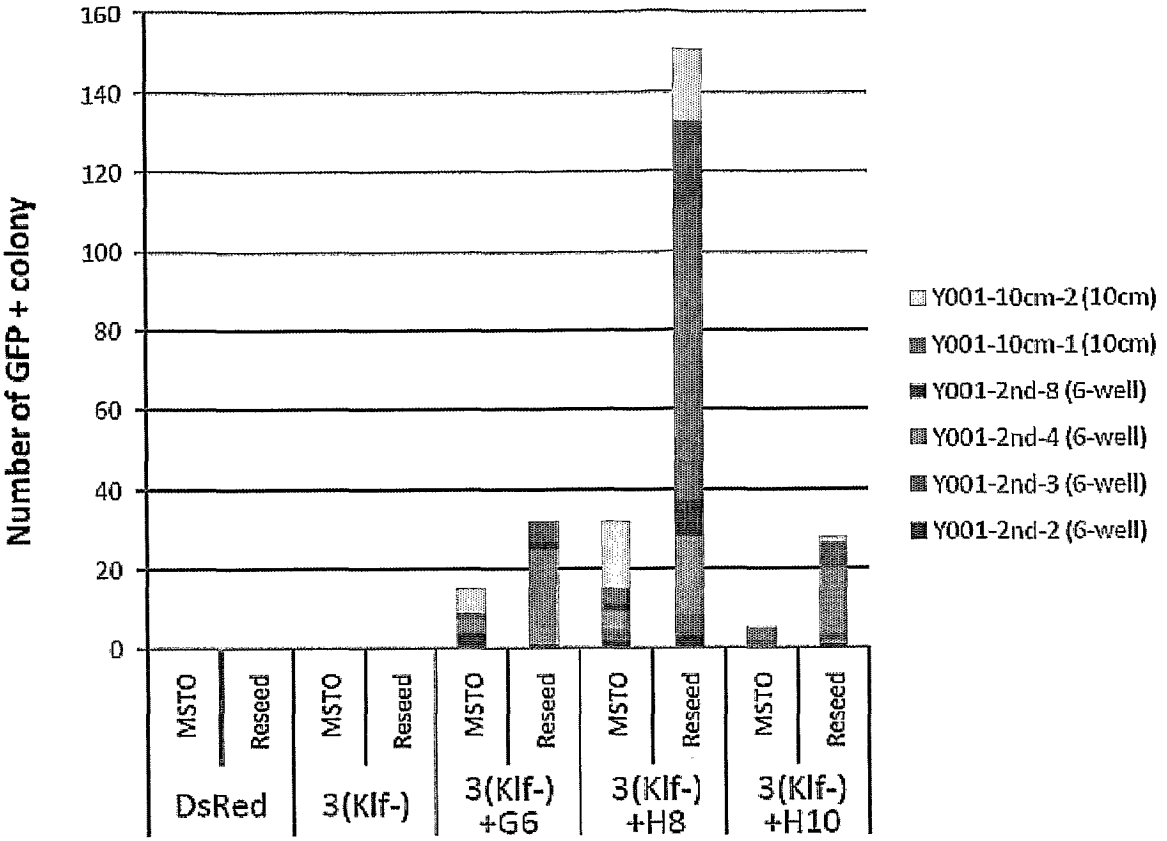

Number of GFP + colony
160
140
120
100
80
60
40
20
0
MSTO
Reseed
DsRed
3(Klf-)
3(Klf-) +G6
3(Klf-) +H8
3(Klf-) +H10
Y001-10cm-2 (10cm)
Y001-10cm-1 (10cm)
Y001-2nd-8 (6-well)
Y001-2nd-4 (6-well)
Y001-2nd-3 (6-well)
Y001-2nd-2 (6-well)

Fig. 10

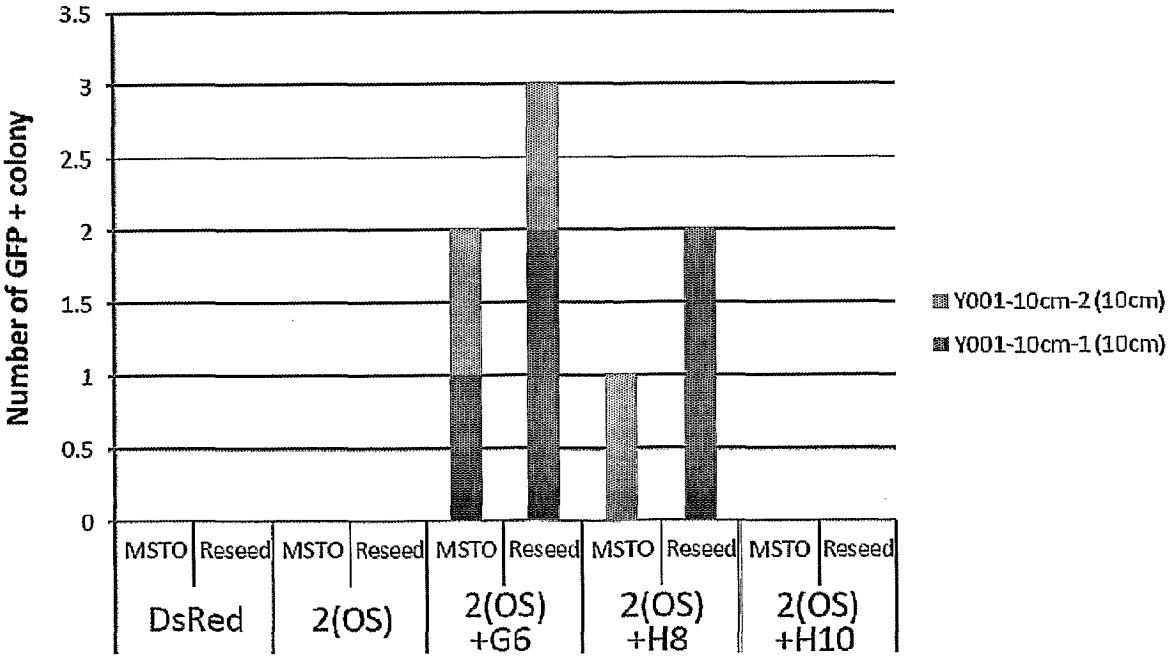

Number of GFP + colony
3.5
3
2.5
2
1.5
1
0.5
0
MSTO
Reseed
DsRed
2(OS)
2(OS) +G6
2(OS) +H8
2(OS) +H10
Y001-10cm-2 (10cm)
Y001-10cm-1 (10cm)

Fig. 11

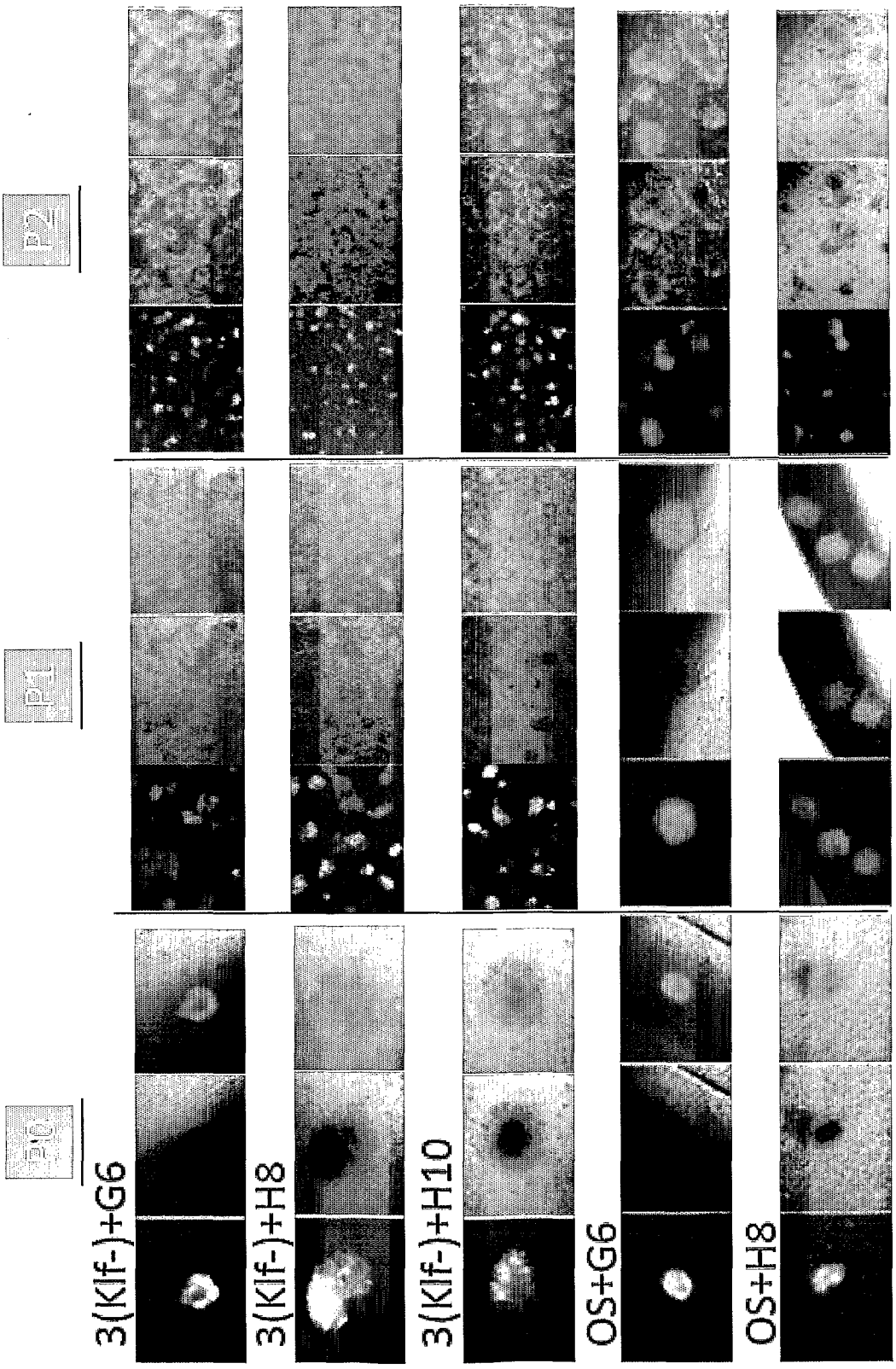


Fig. 12

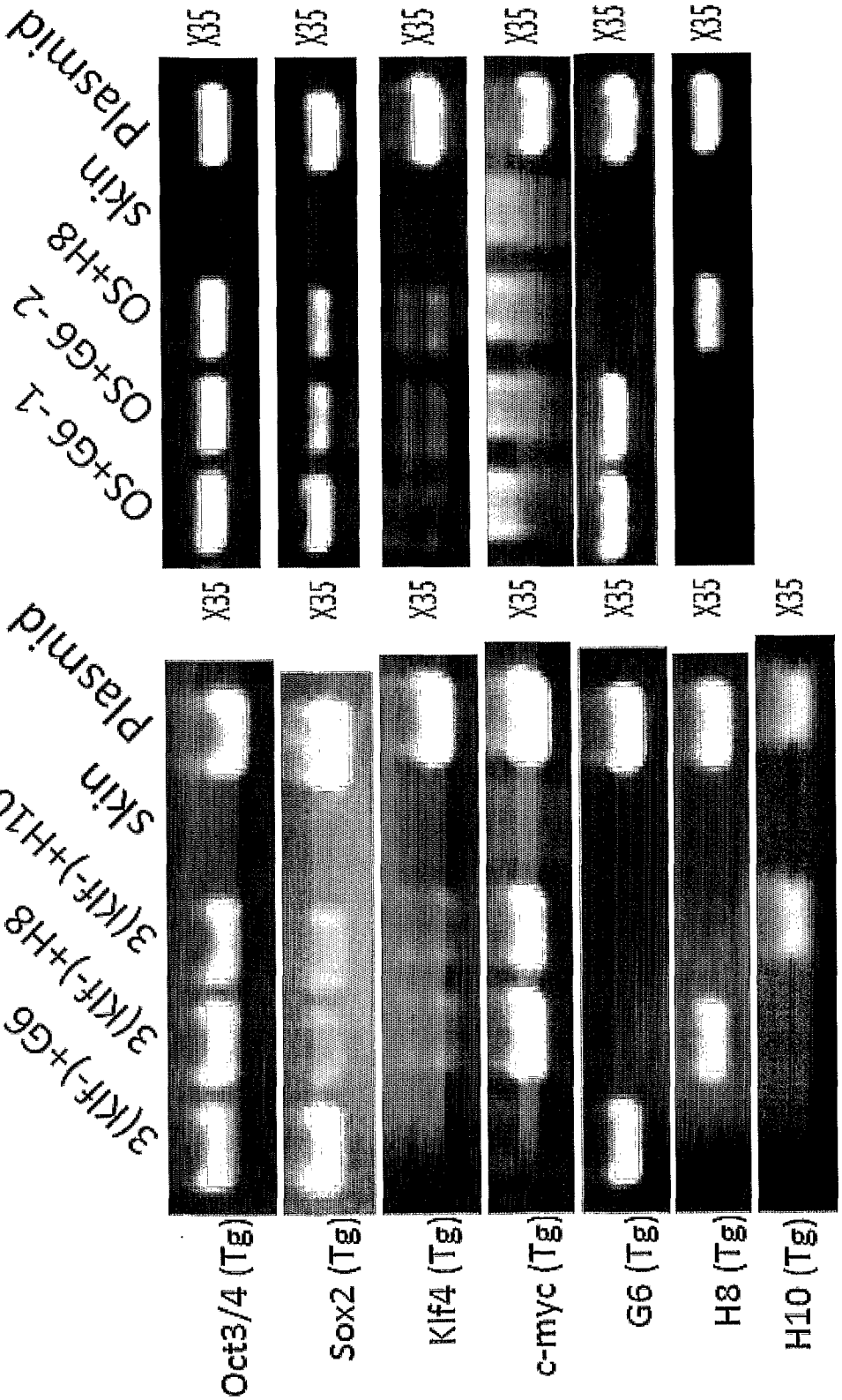

Oct3/4 (Tg)
Sox2 (Tg)
Klf4 (Tg)
c-myc (Tg)
G6 (Tg)
H8 (Tg)
H10 (Tg)
3(Klf-)+G6
3(Klf-)+H8
3(Klf-)+H10
skin
plasmid
X35
OS+G6-1
OS+G6-2
OS+H8
skin
plasmid
X35

Fig. 13

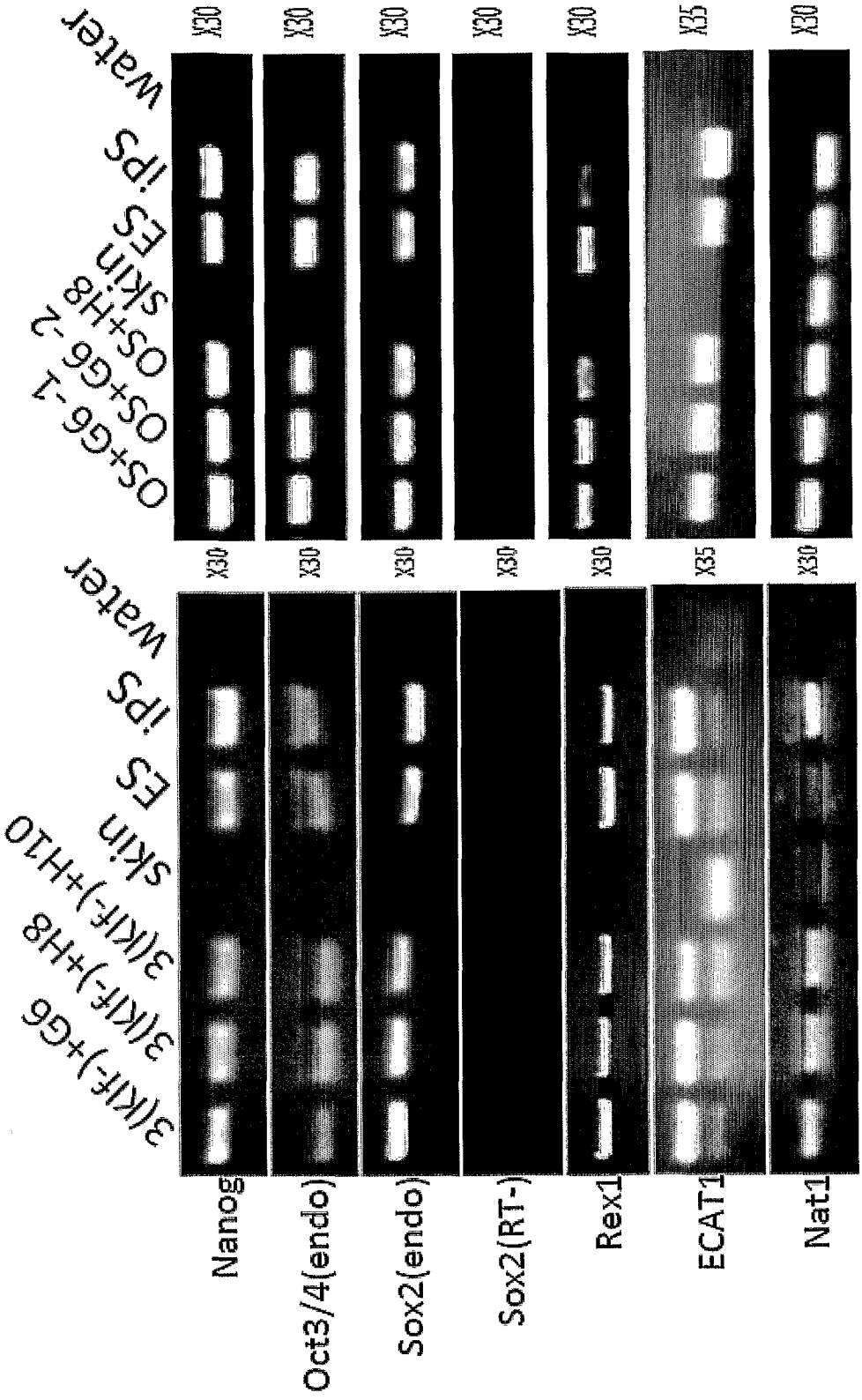

Nanog
Oct3/4(endo)
Sox2(endo)
Sox2(RT-)
Rex1
ECAT1
Nat1
3(KIf-)+G6
3(KIf-)+H8
3(KIf-)+H10
Skin
ES
iPS
Water
OS+G6-1
OS+G6-2
OS+H8
Skin
ES
iPS
Water
X30
X35

Fig. 14

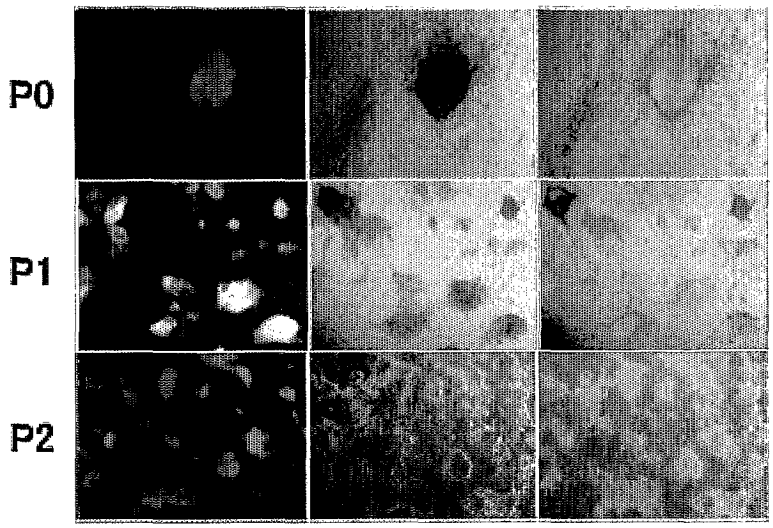


Fig. 15

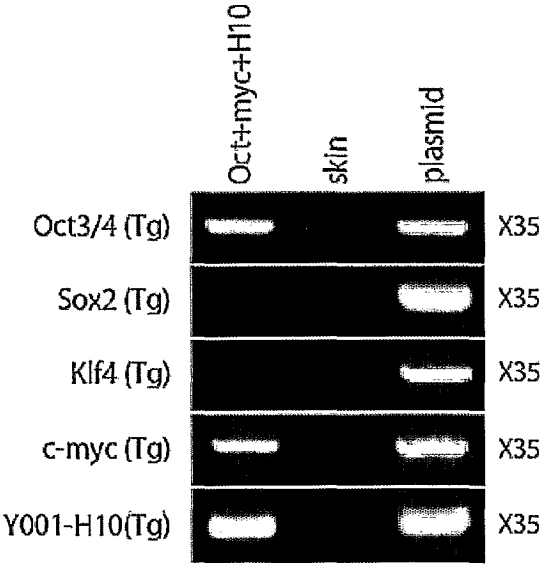


Fig. 16

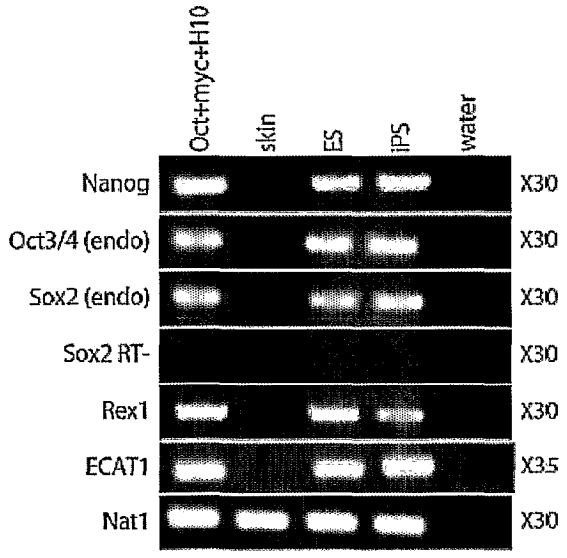


Fig. 17

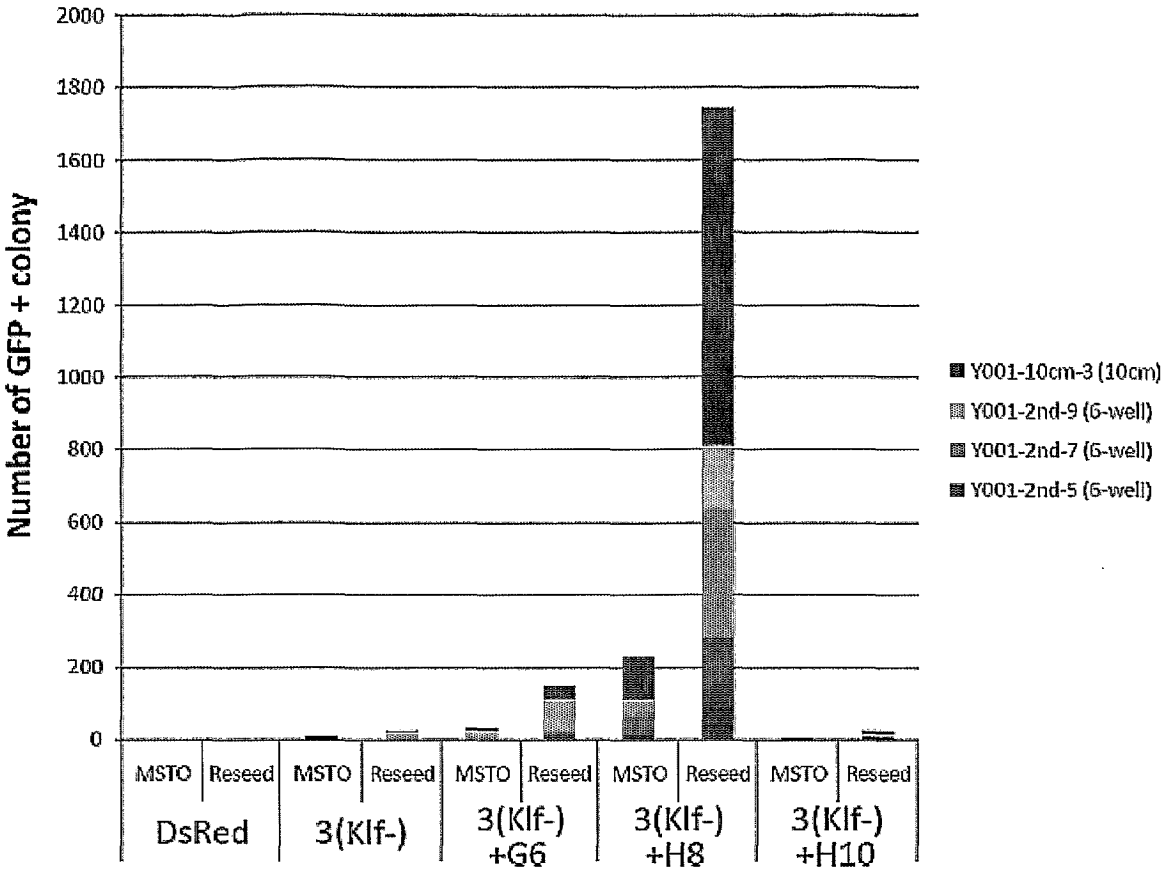

Number of GFP + colony
2000
1800
1600
1400
1200
1000
800
600
400
200
0
MSTO
Reseed
DsRed
3(Klf-)
3(Klf-) +G6
3(Klf-) +H8
3(Klf-) +H10
Y001-10cm-3 (10cm)
Y001-2nd-9 (6-well)
Y001-2nd-7 (6-well)
Y001-2nd-5 (6-well)

Fig. 18

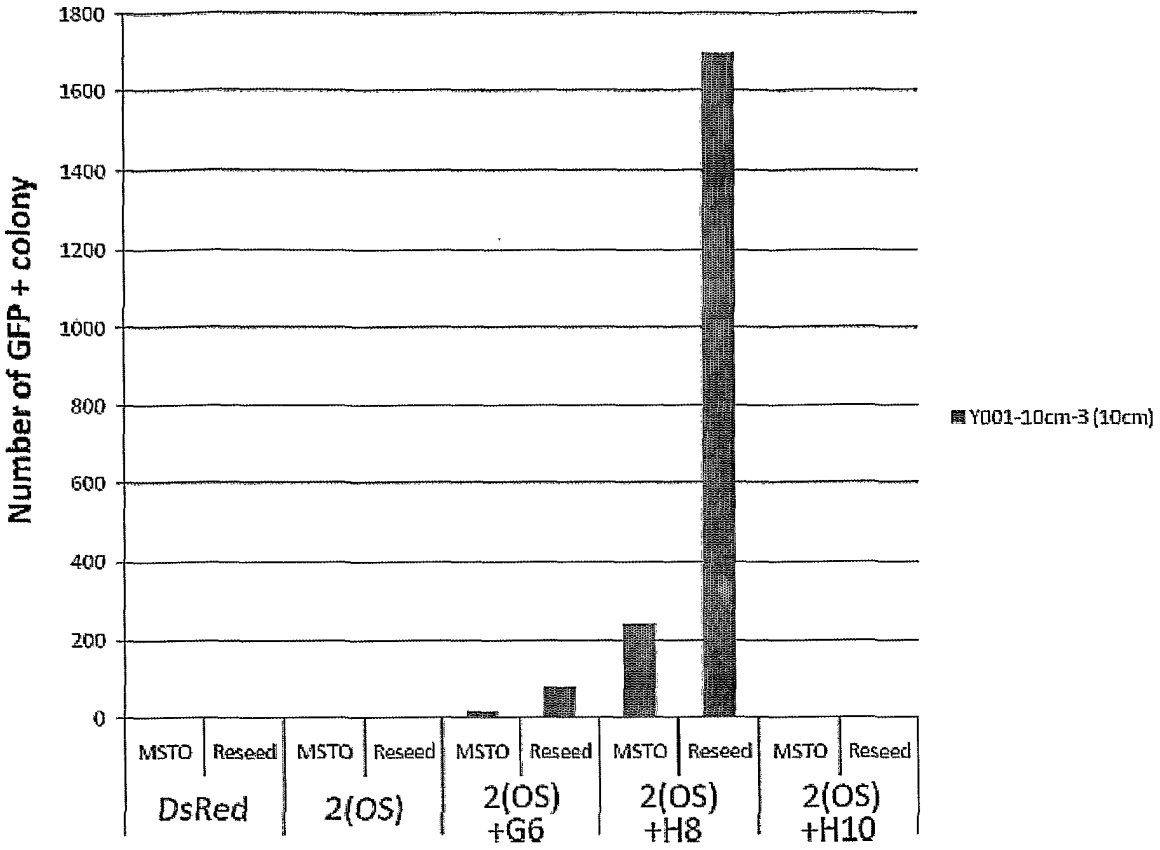

Number of GFP + colony
1800
1600
1400
1200
1000
800
600
400
200
0
MSTO
Reseed
DsRed
2(OS)
2(OS) +G6
2(OS) +H8
2(OS) +H10
Y001-10cm-3 (10cm)

Fig. 19

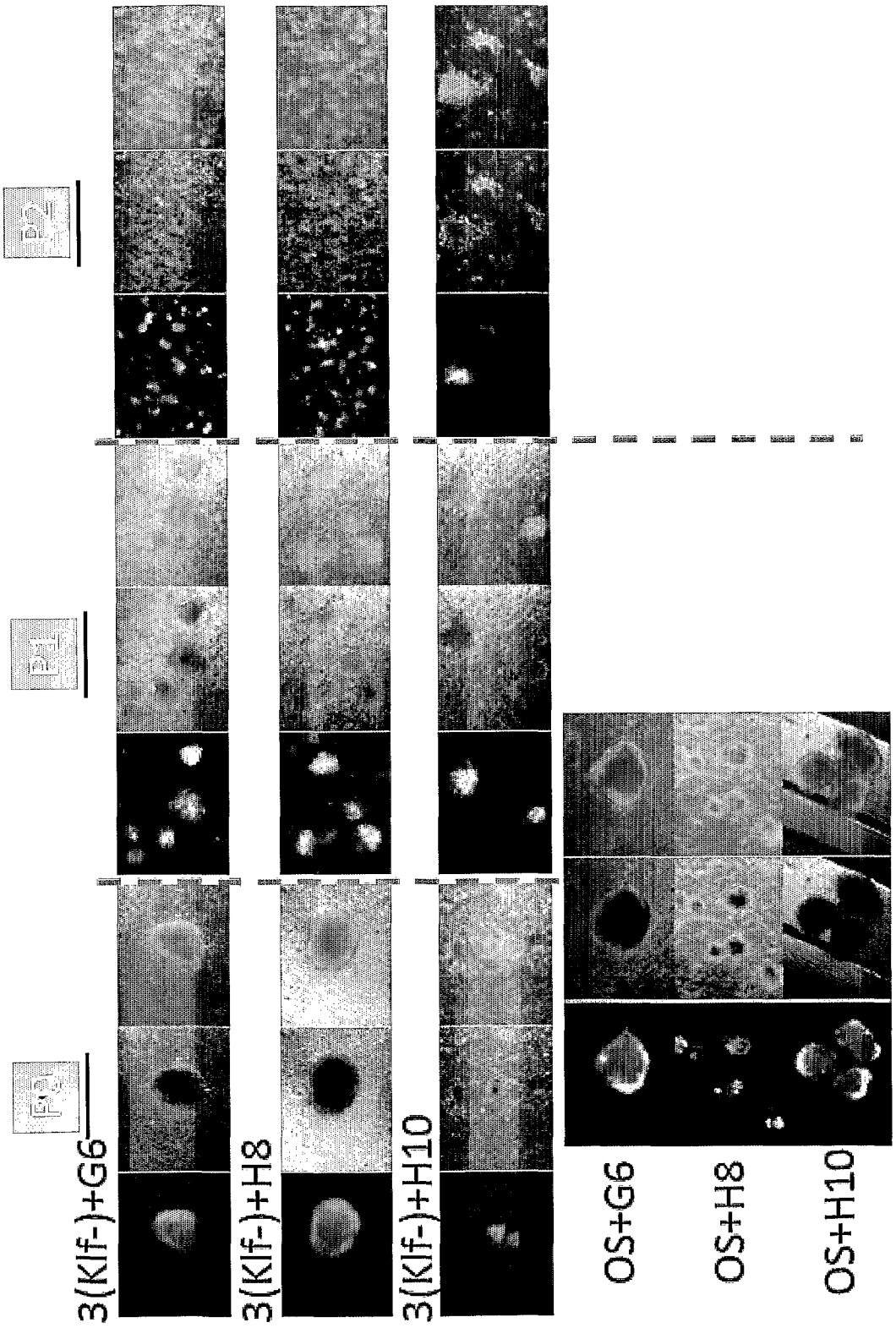


Fig. 20

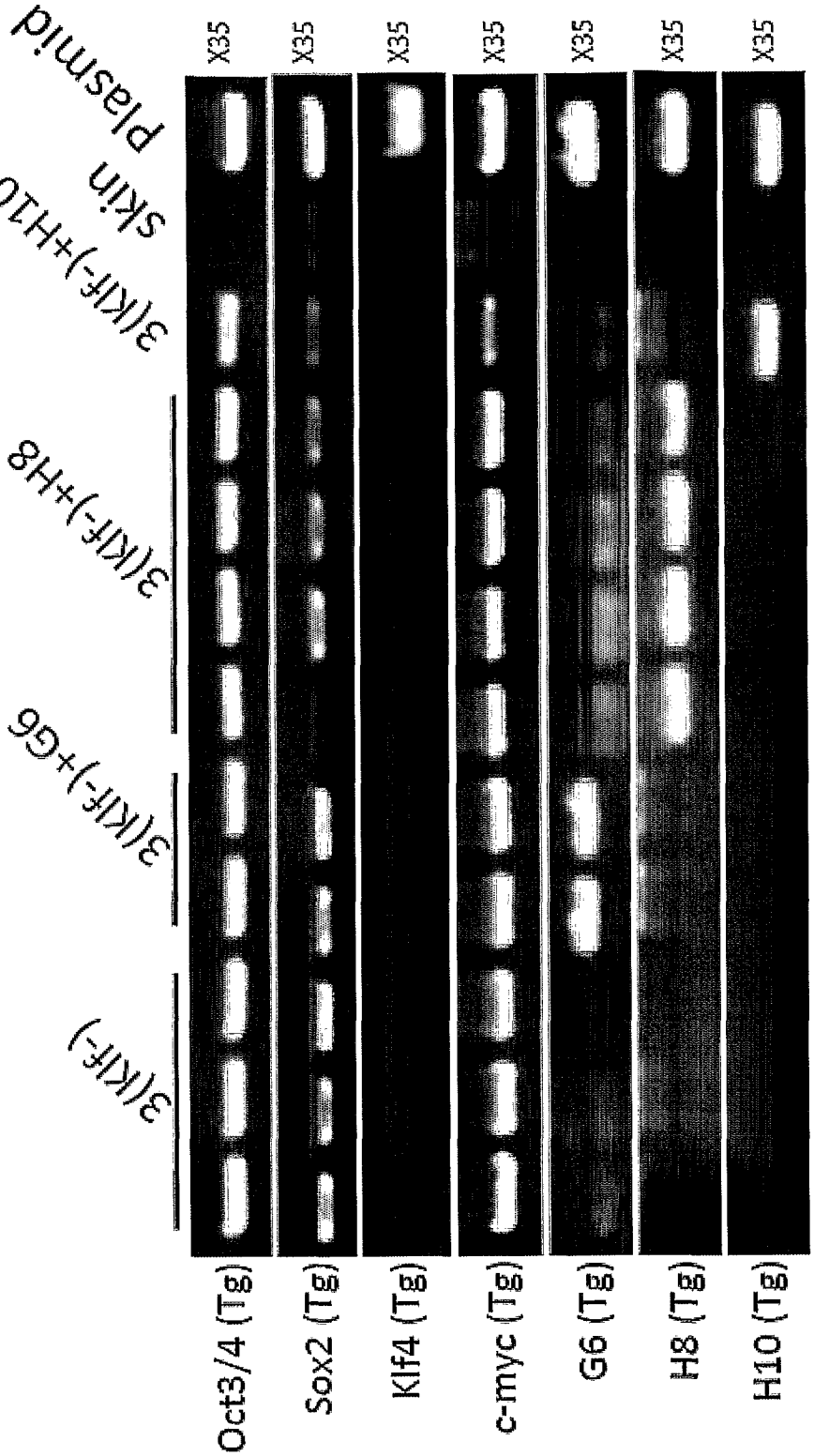

X35
Oct3/4 (Tg)
Sox2 (Tg)
Klf4 (Tg)
c-myc (Tg)
G6 (Tg)
H8 (Tg)
H10 (Tg)
3(Klf-)
3(Klf-)+G6
3(Klf-)+H8
3(Klf-)+H10
skin
plasmid

Fig. 21

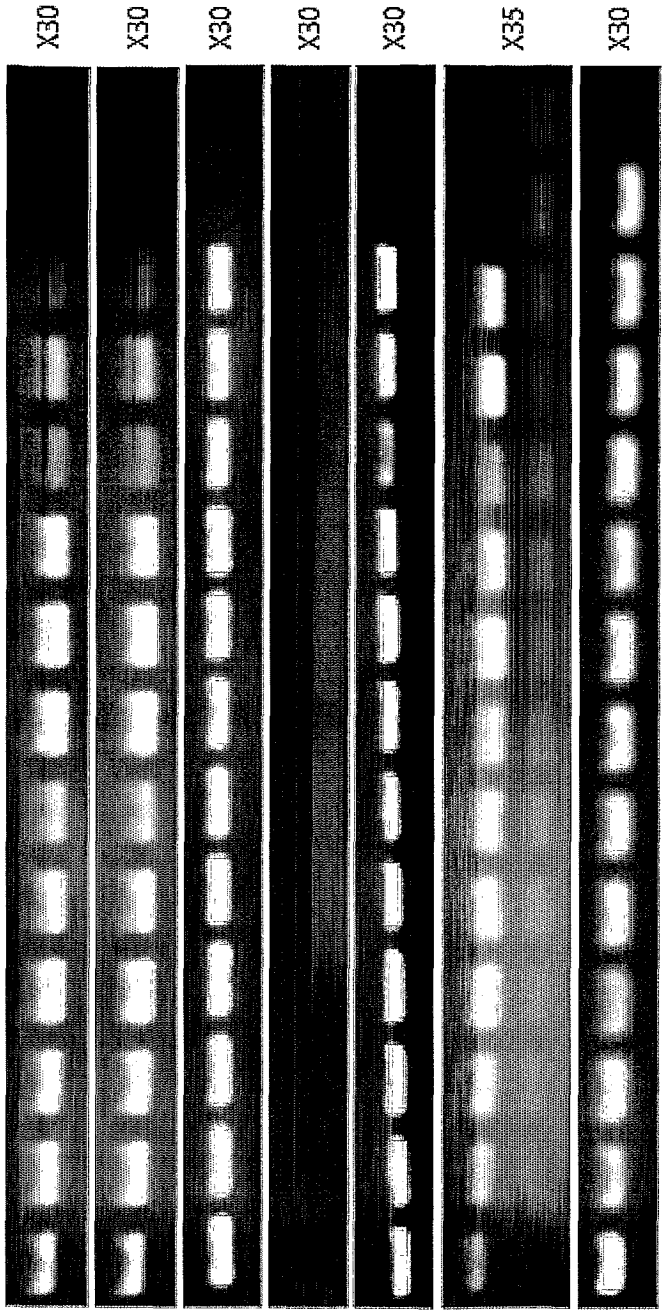

Nanog
Oct3/4(endo)
Sox2(endo)
Sox2(RT-)
Rex1
ECAT1
Nat1
X30
X30
X30
X30
X30
X35
X30
3(Klf-)+G6
3(Klf-)+H8
3(Klf-)+H10
3(Klf-)
iPS ES skin
water

Fig. 22

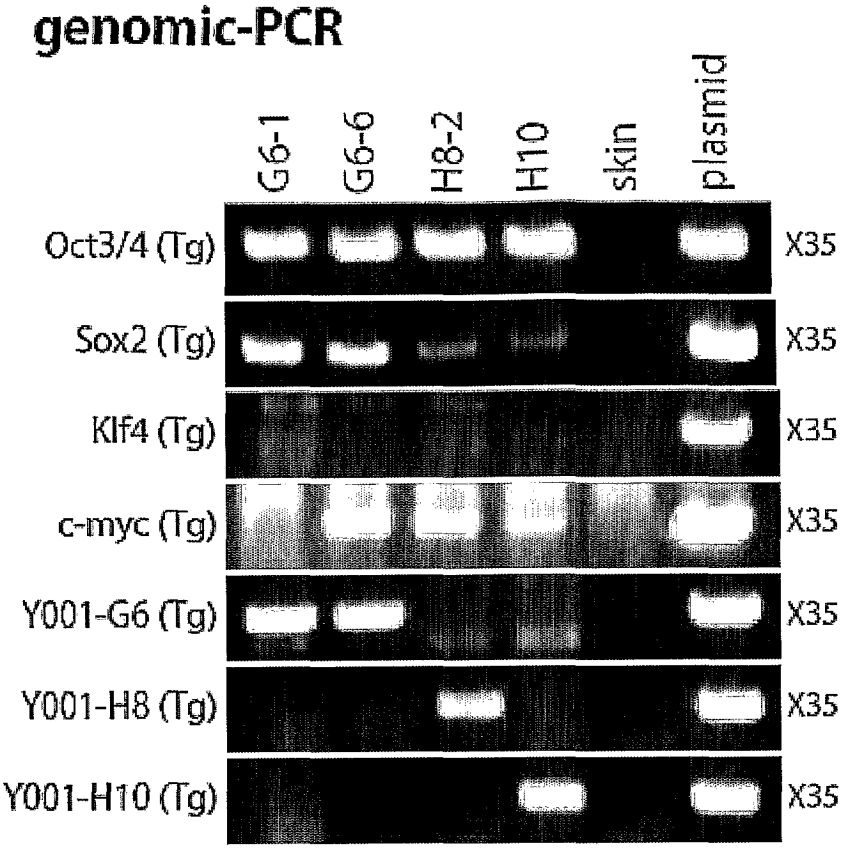

genomic-PCR
G6-1
G6-6
H8-2
H10
skin
plasmid
Oct3/4 (Tg)
Sox2 (Tg)
Klf4 (Tg)
c-myc (Tg)
Y001-G6 (Tg)
Y001-H8 (Tg)
Y001-H10 (Tg)
X35
X35
X35
X35
X35
X35
X35

Fig. 23

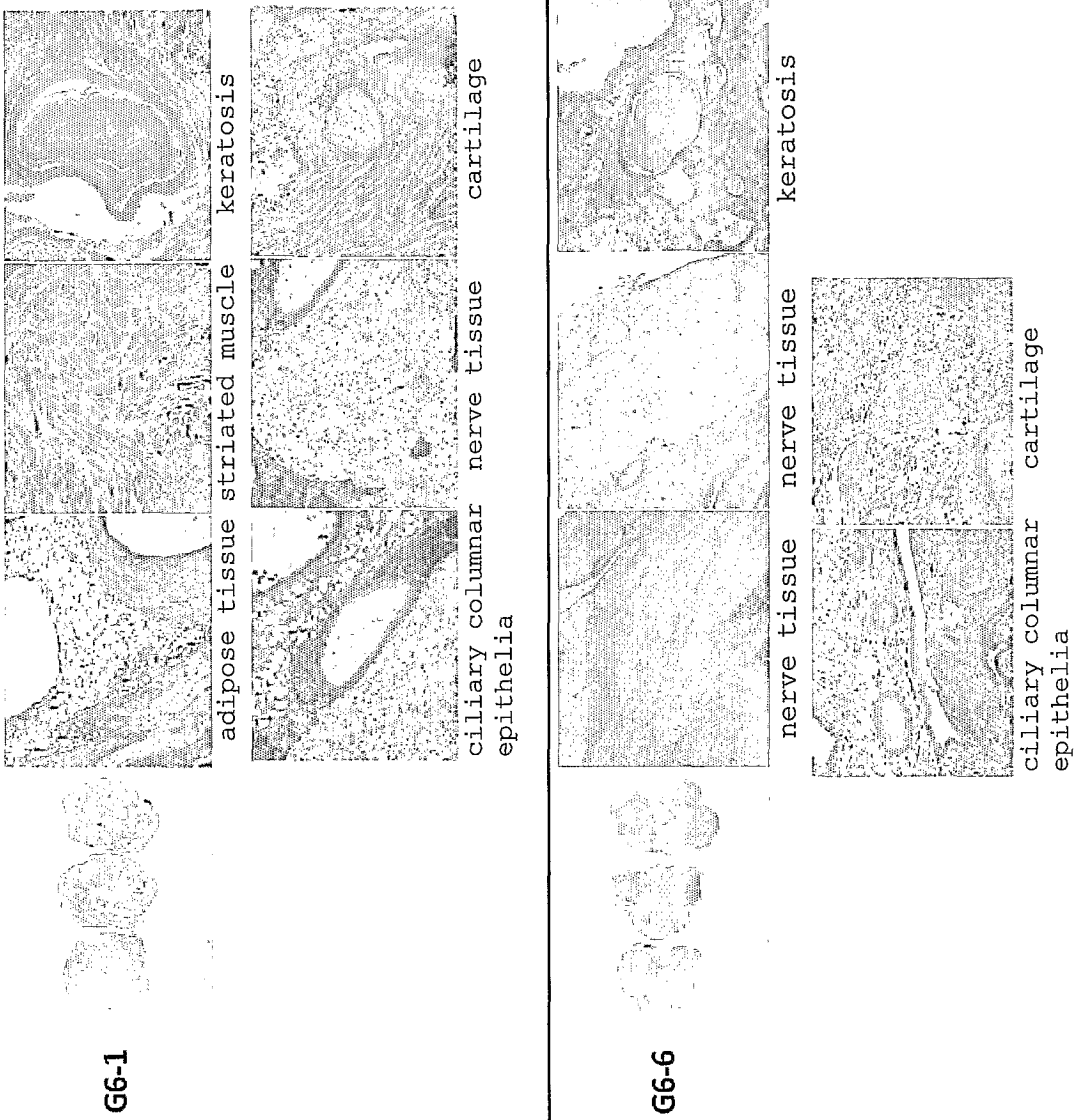

G6-1
adipose tissue
striated muscle
keratosis
ciliary columnar epithelia
nerve tissue
cartilage
G6-6
nerve tissue
nerve tissue
keratosis
ciliary columnar epithelia
cartilage

Fig. 24

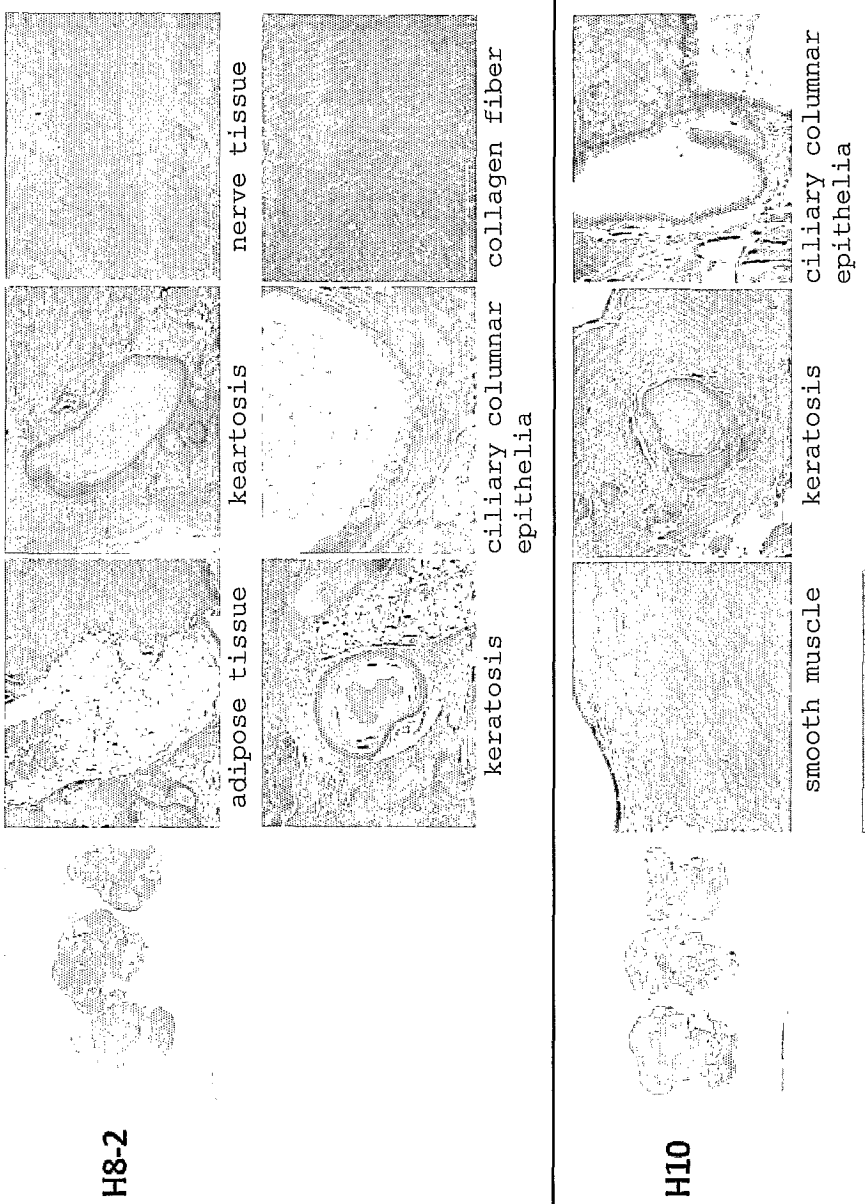

H8-2
adipose tissue
keratosis
keratosis
ciliary columnar epithelia
nerve tissue
collagen fiber
H10
smooth muscle
keratosis
ciliary columnar epithelia
nerve tissue

Fig. 25

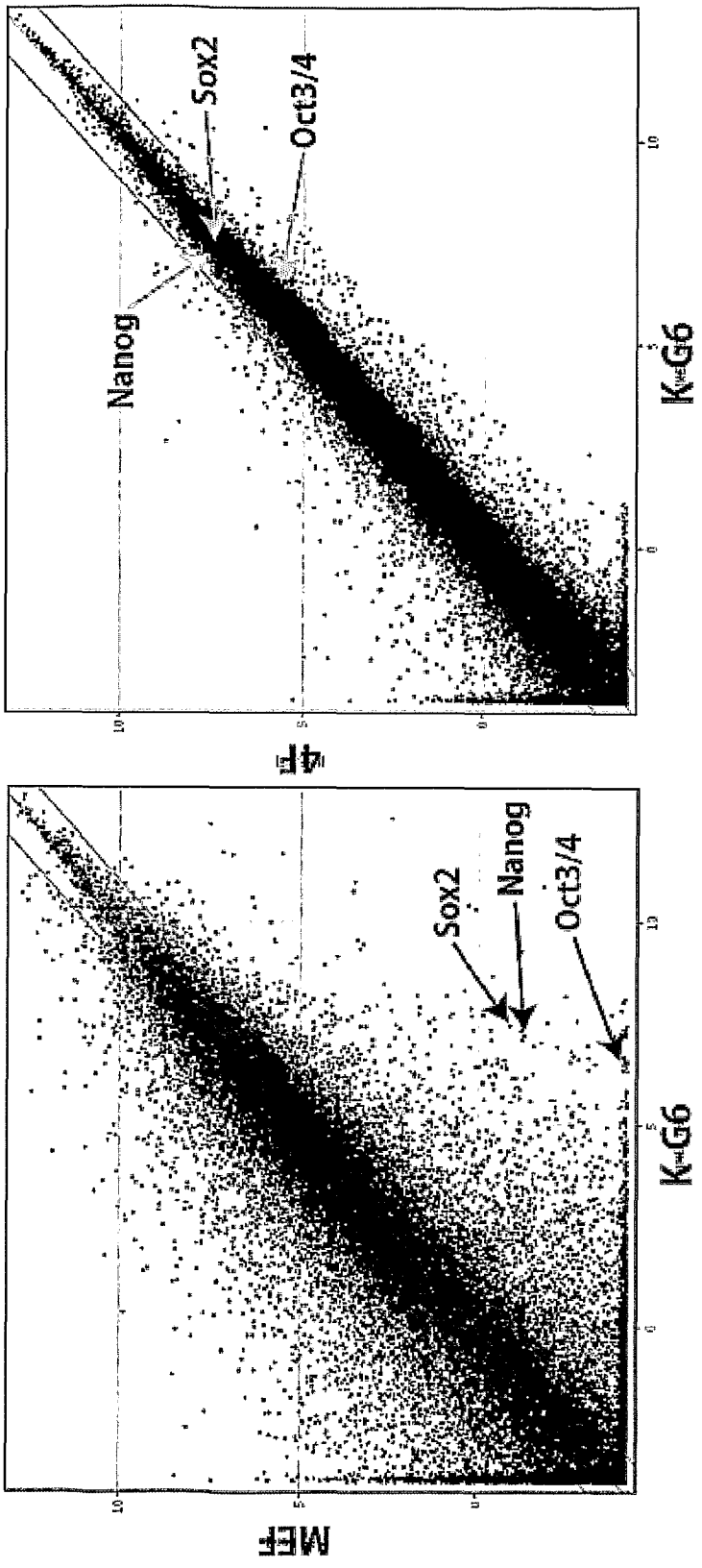


Fig. 26

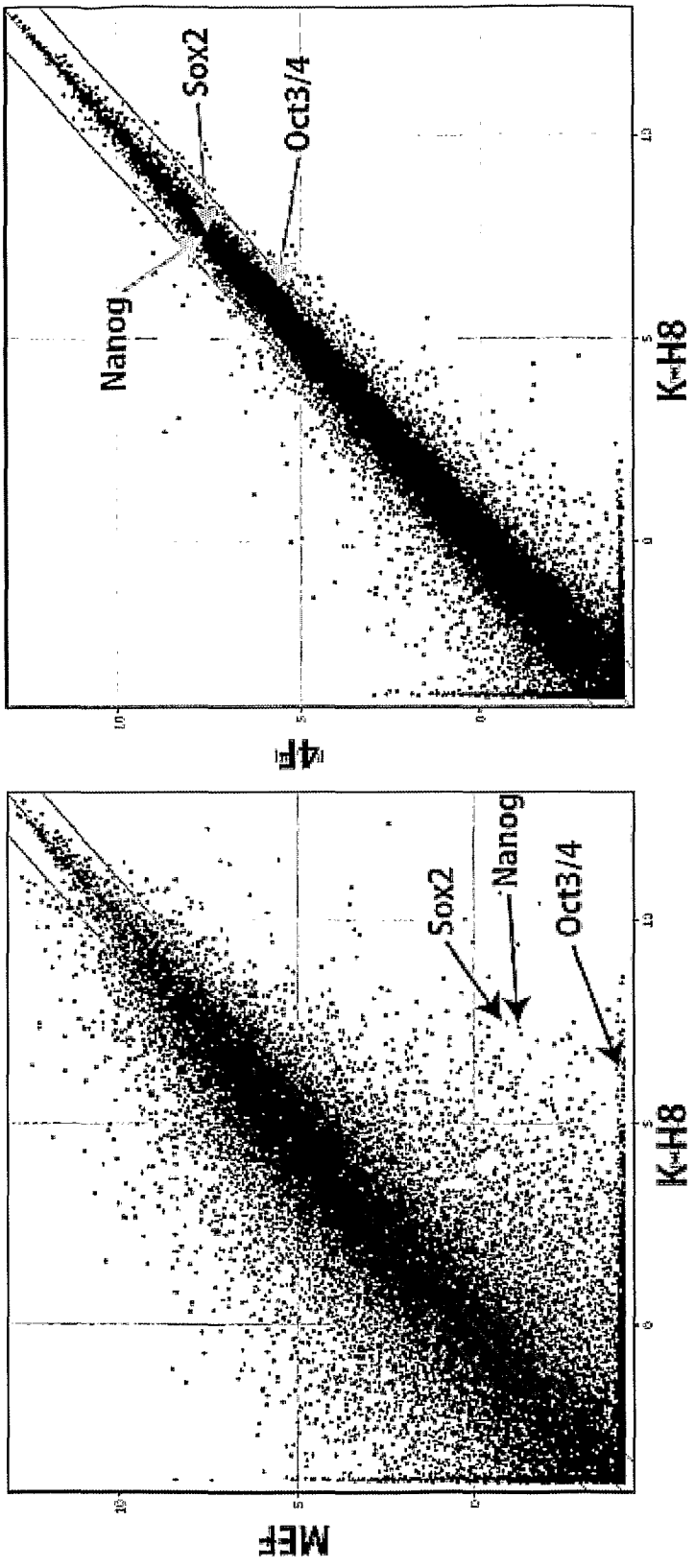


Fig. 27

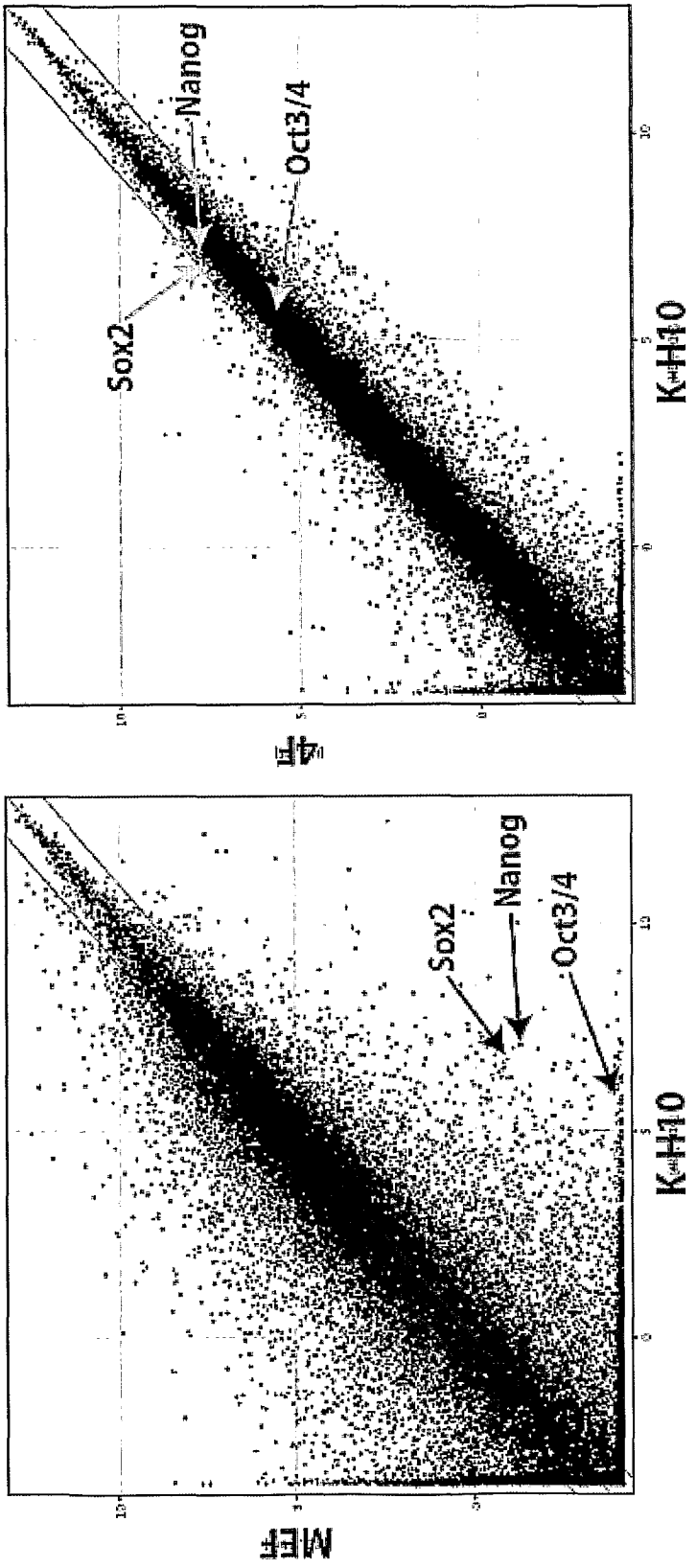


Fig. 28

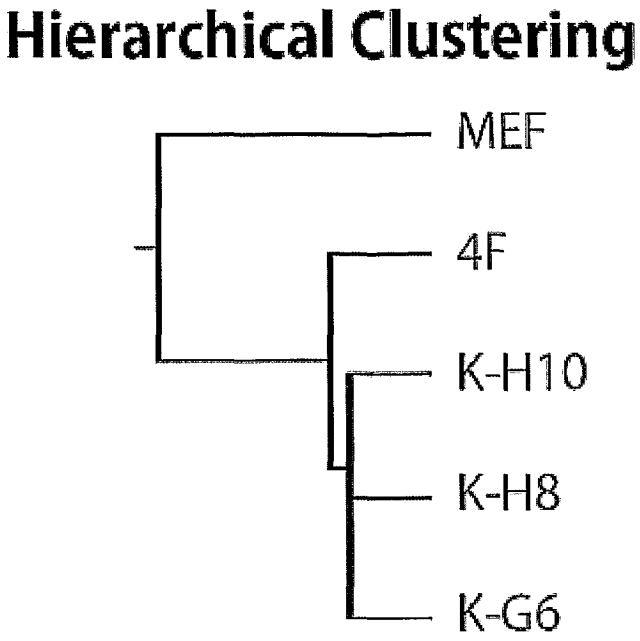

Hierarchical Clustering
MEF
4F
K-H10
K-H8
K-G6

Fig. 29

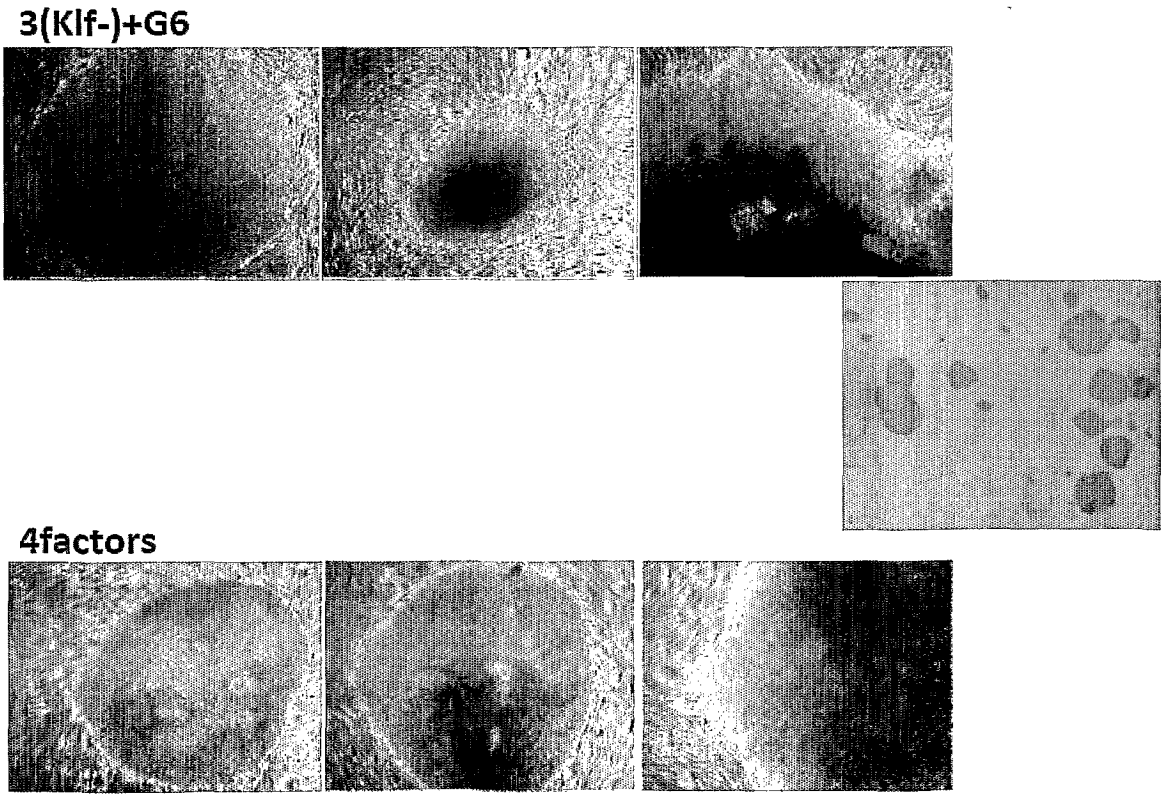

3(Klf-)+G6
4factors

Fig. 30

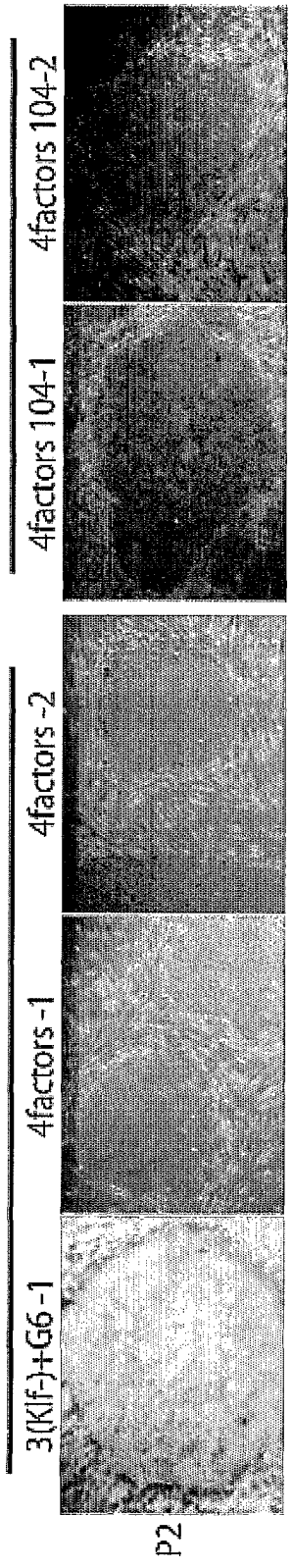

hiPS induction (2nd time)
3(Klf-)+G6-1
4factors-1
4factors-2
hiPS induction (3rd time)
4factors 104-1
4factors 104-2
P2

Fig. 31

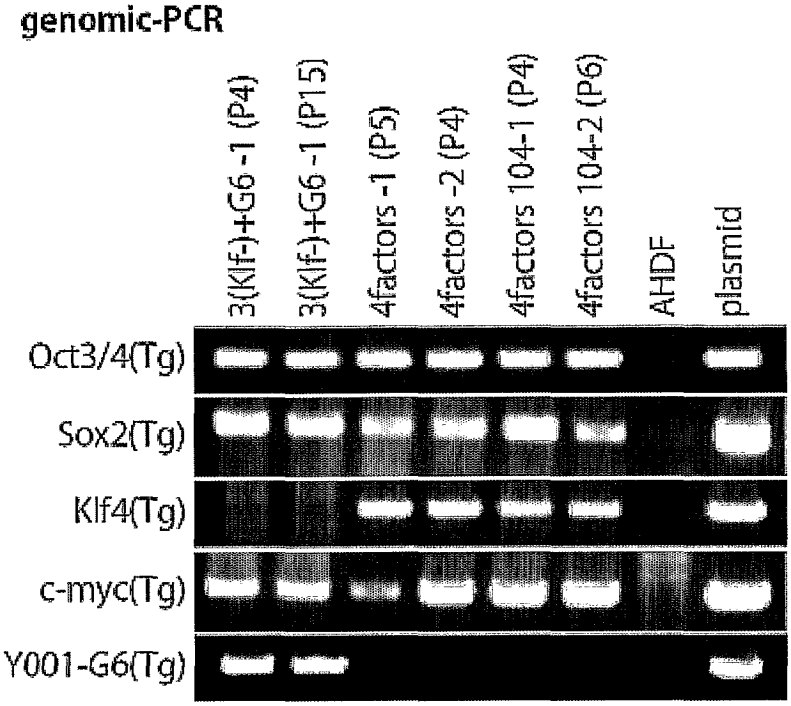

genomic-PCR
3(Klf-)+G6-1 (P4)
3(Klf-)+G6-1 (P15)
4factors-1 (P5)
4factors-2 (P4)
4factors 104-1 (P4)
4factors 104-2 (P6)
AHDF
plasmid
Oct3/4(Tg)
Sox2(Tg)
Klf4(Tg)
c-myc(Tg)
Y001-G6(Tg)

Fig. 32

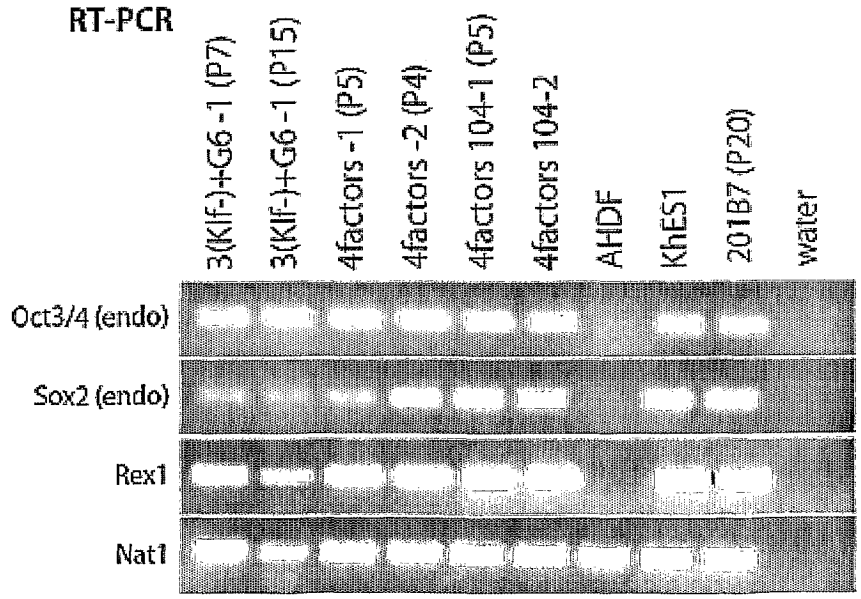

RT-PCR
3(Klf-)+G6-1 (P7)
3(Klf-)+G6-1 (P15)
4factors-1 (P5)
4factors-2 (P4)
4factors 104-1 (P5)
4factors 104-2
AHDF
KhES1
201B7 (P20)
water
Oct3/4 (endo)
Sox2 (endo)
Rex1
Nat1

Fig. 33

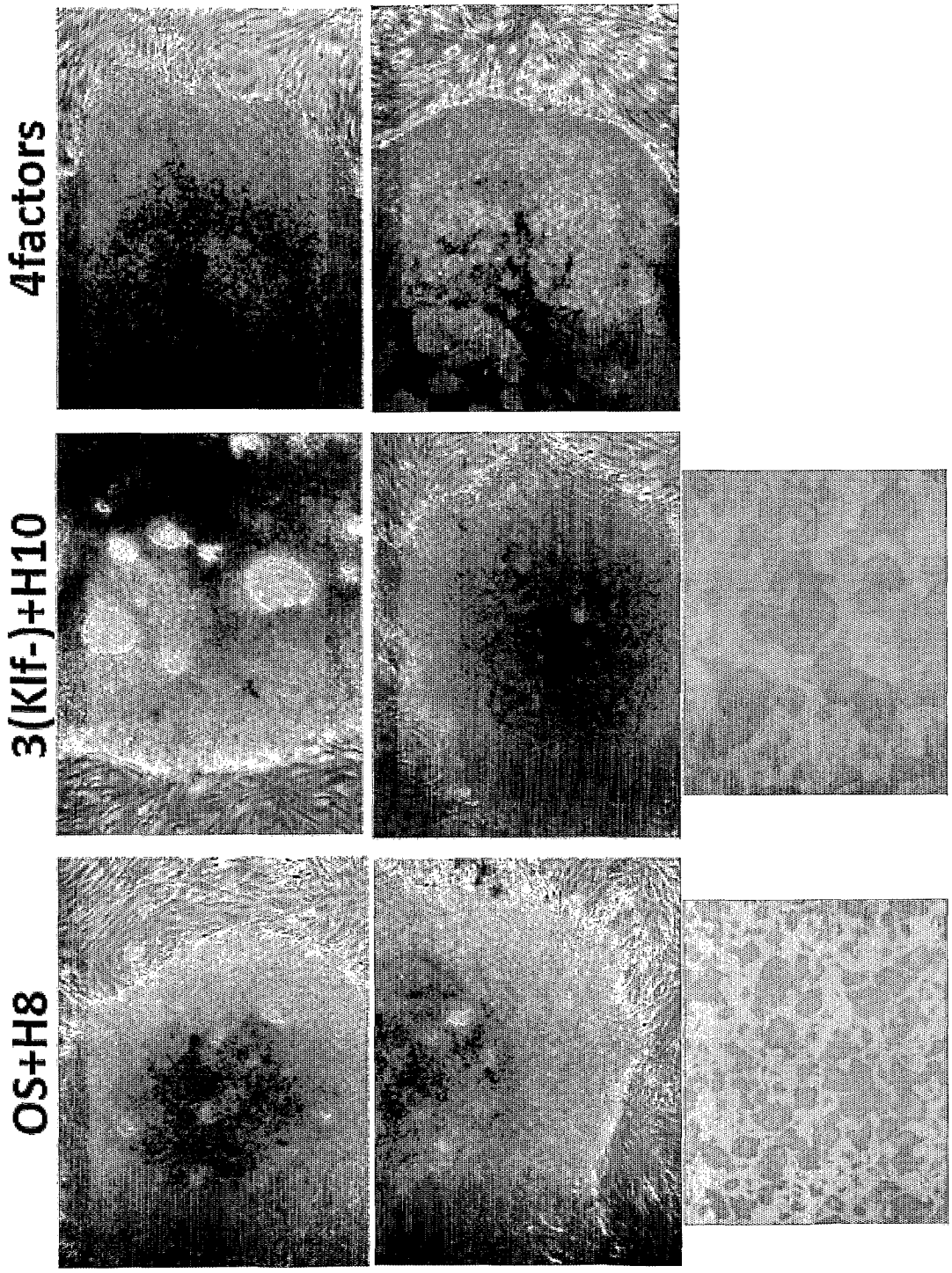


Fig. 34

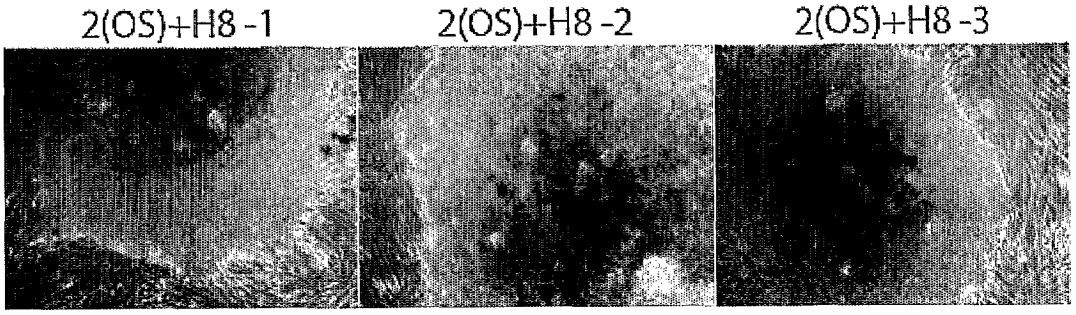


Fig. 35

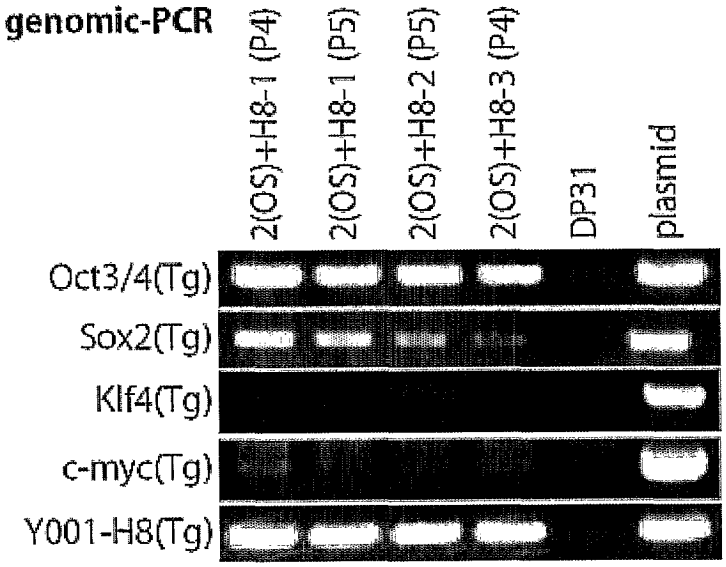


Fig. 36

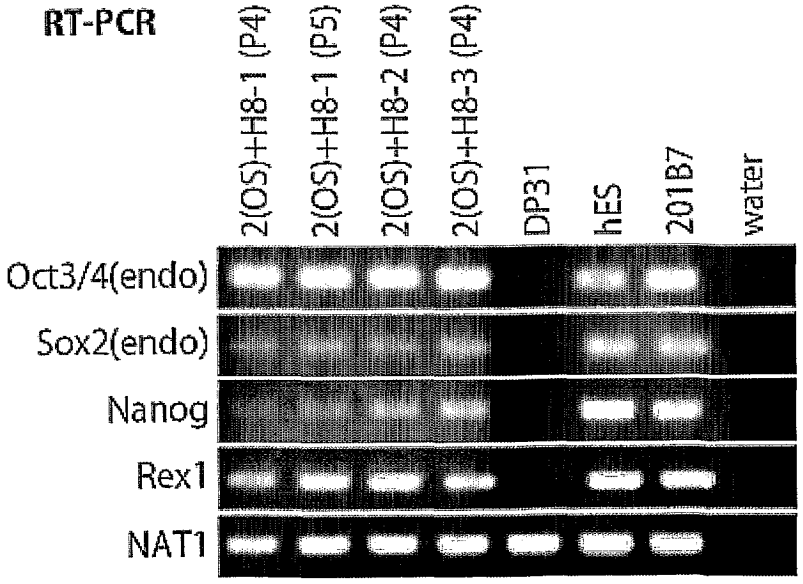


Fig. 37

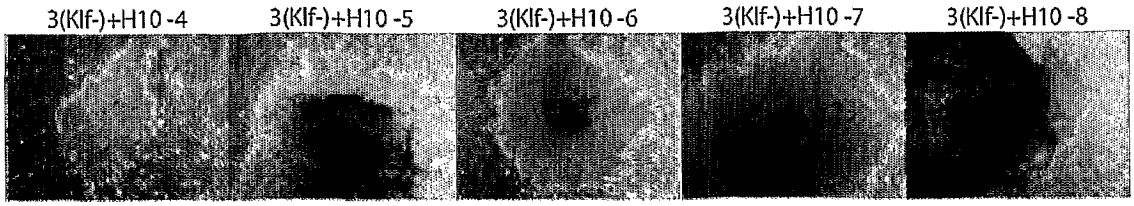


Fig. 38

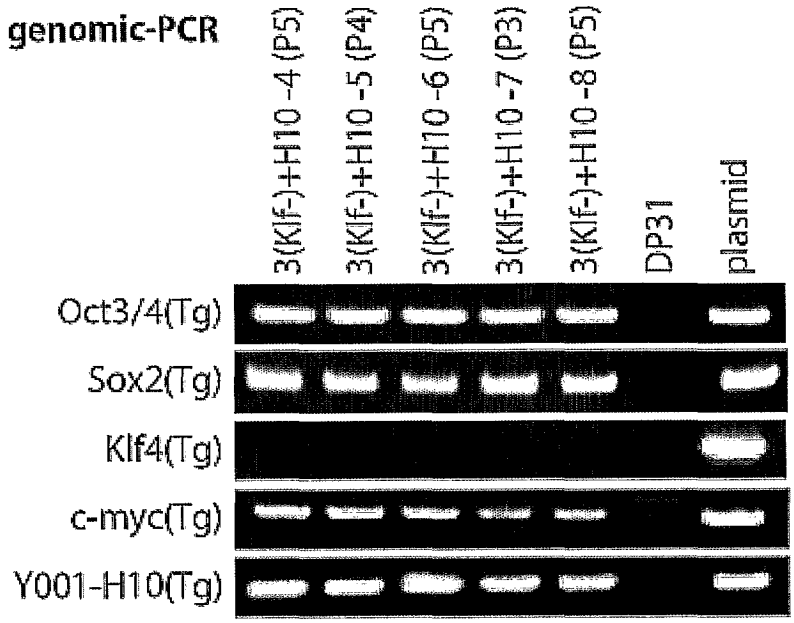


Fig. 39

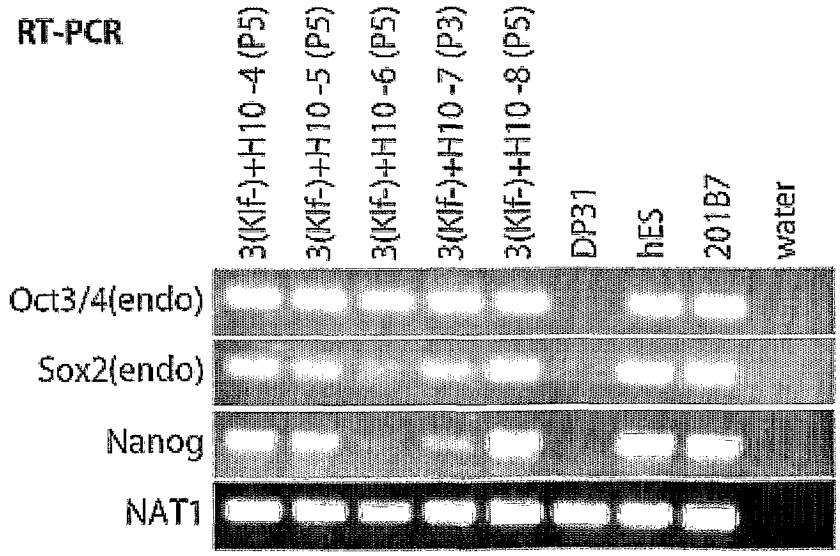
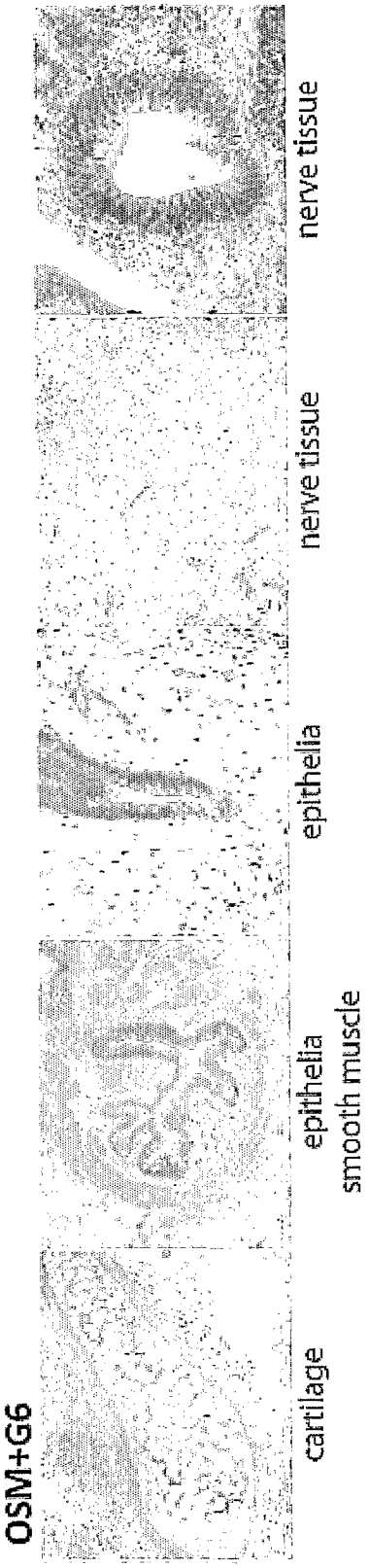


Fig. 40


OSM+G6
cartilage
epithelia
smooth muscle
epithelia
nerve tissue
nerve tissue