Fig. 1

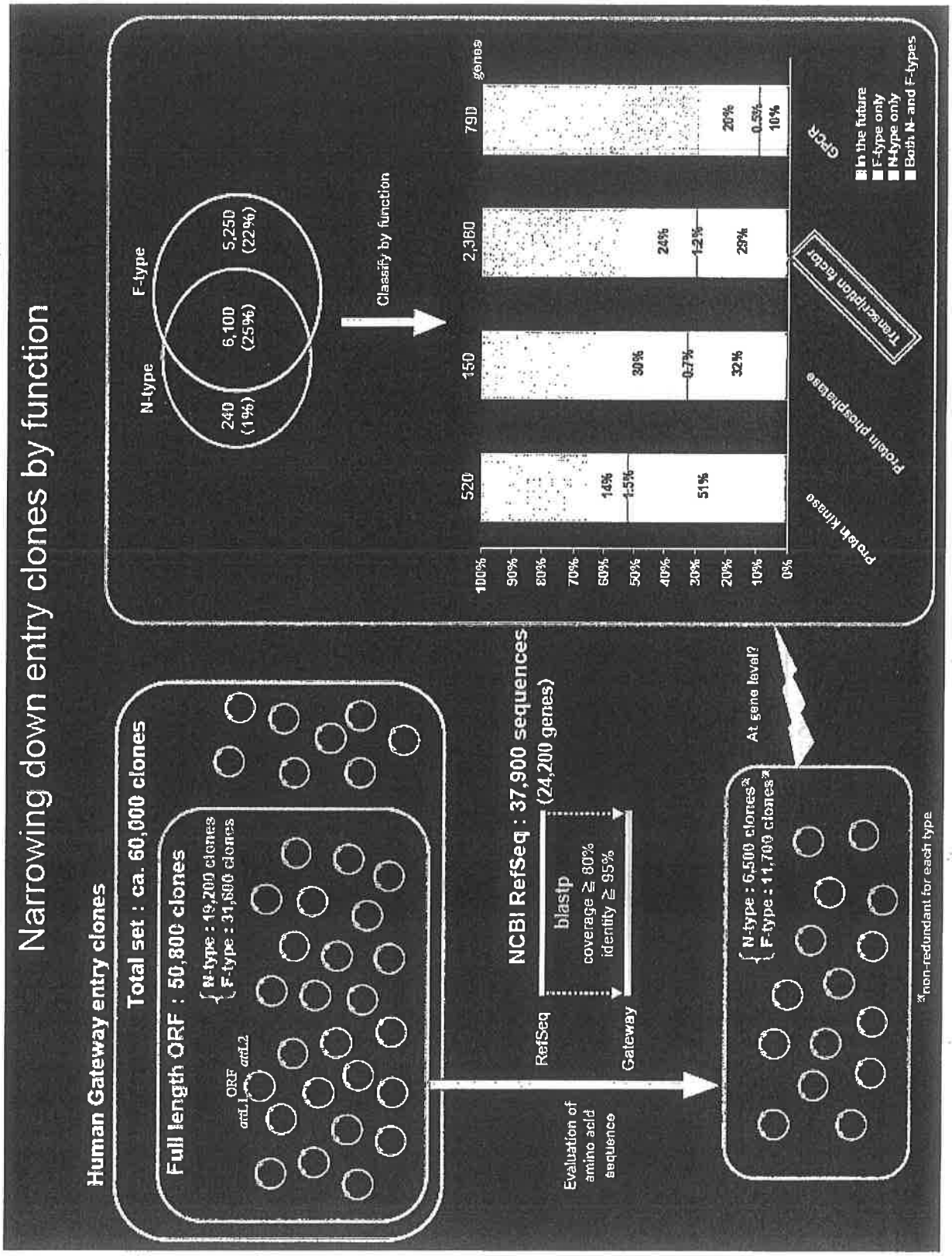

Narrowing down entry clones by function
Human Gateway entry clones
Total set : ca. 60,000 clones
Full length ORF : 50,800 clones
N-type : 19,200 clones
F-type : 31,600 clones
ORF
attL1
attL2
Evaluation of amino acid sequence
RefSeq
Gateway
NCBI RefSeq : 37,900 sequences
(24,200 genes)
blastp
coverage ≧ 80%
identity ≧ 95%
At gene level?
N-type : 6,500 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
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
14%
1.5%
30%
0.7%
24%
1.2%
20%
0.5%
10%
51%
32%
29%
Protein kinase
Protein phosphatase
Transcription factor
GPCR
In the future
F-type only
N-type only
Both N- and F-types

Fig. 2

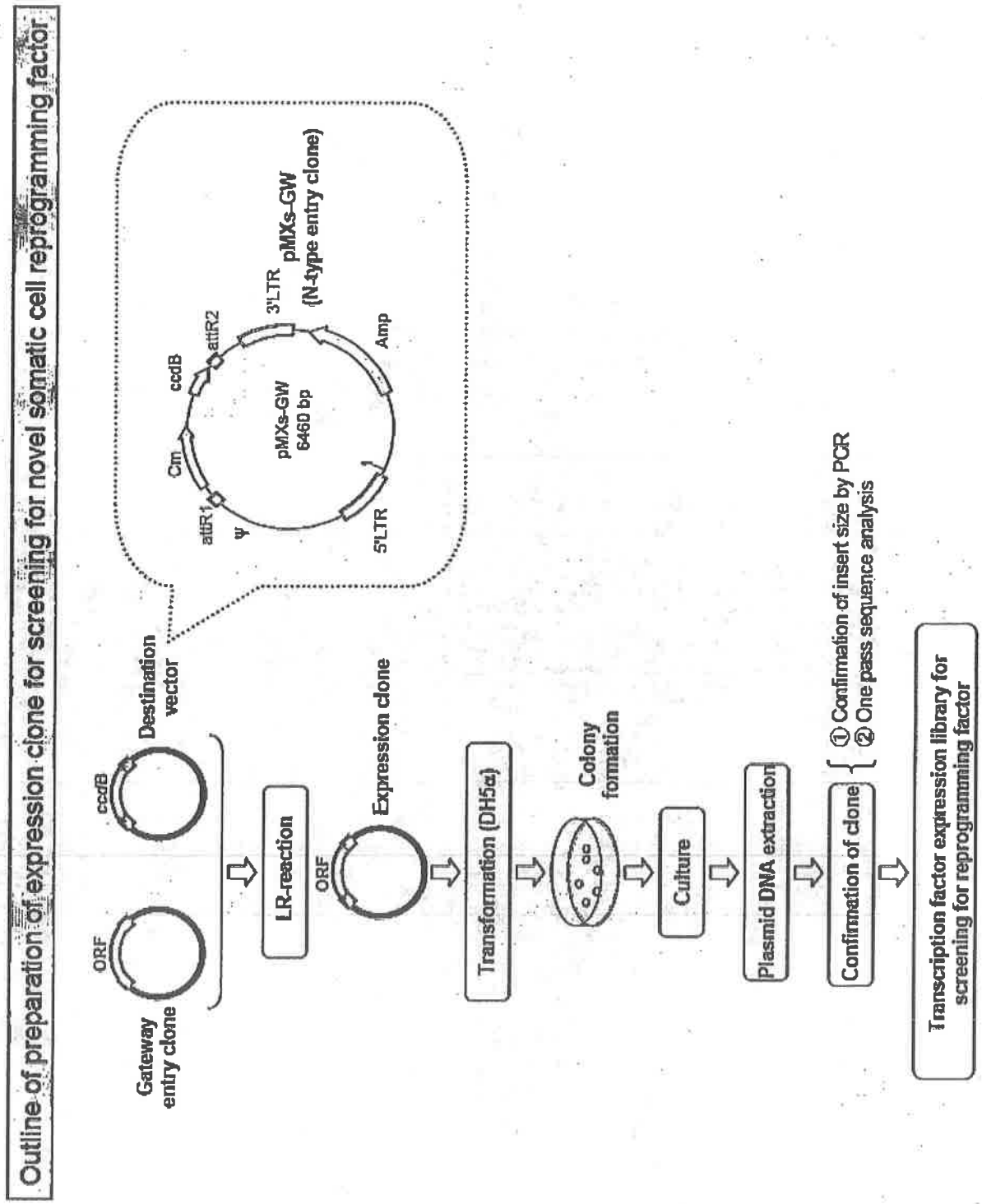

Outline of preparation of expression clone for screening for novel somatic cell reprogramming factor
Gateway entry clone
ORF
ccdB
Destination vector
LR-reaction
ORF
Expression clone
Transformation (DH5α)
Colony formation
Culture
Plasmid DNA extraction
Confirmation of clone
① Confirmation of insert size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

Fig. 3

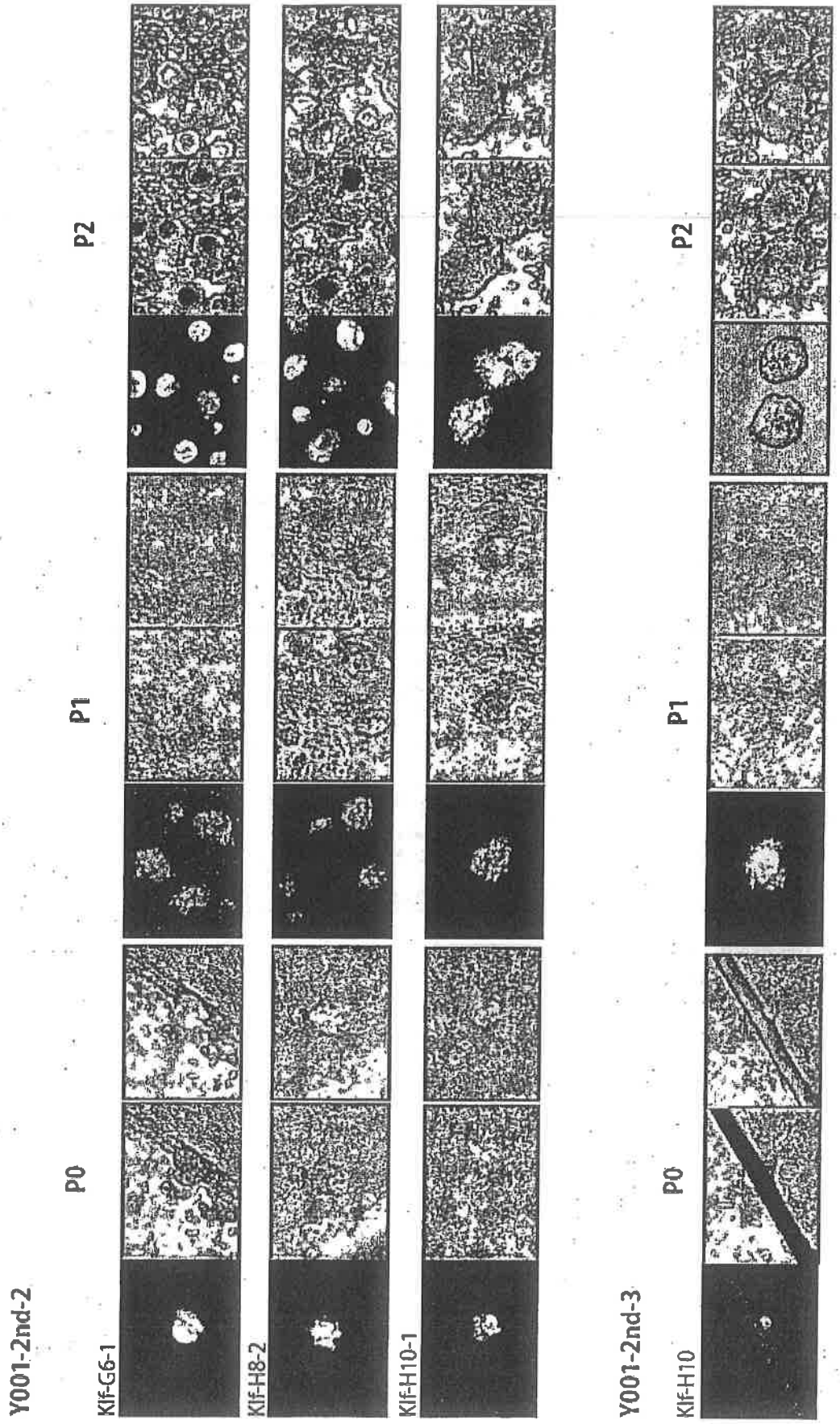

Y001-2nd-2
P0
P1
P2
Klf-G6-1
Klf-H8-2
Klf-H10-1
Y001-2nd-3
P0
P1
P2
Klf-H10

Fig. 4

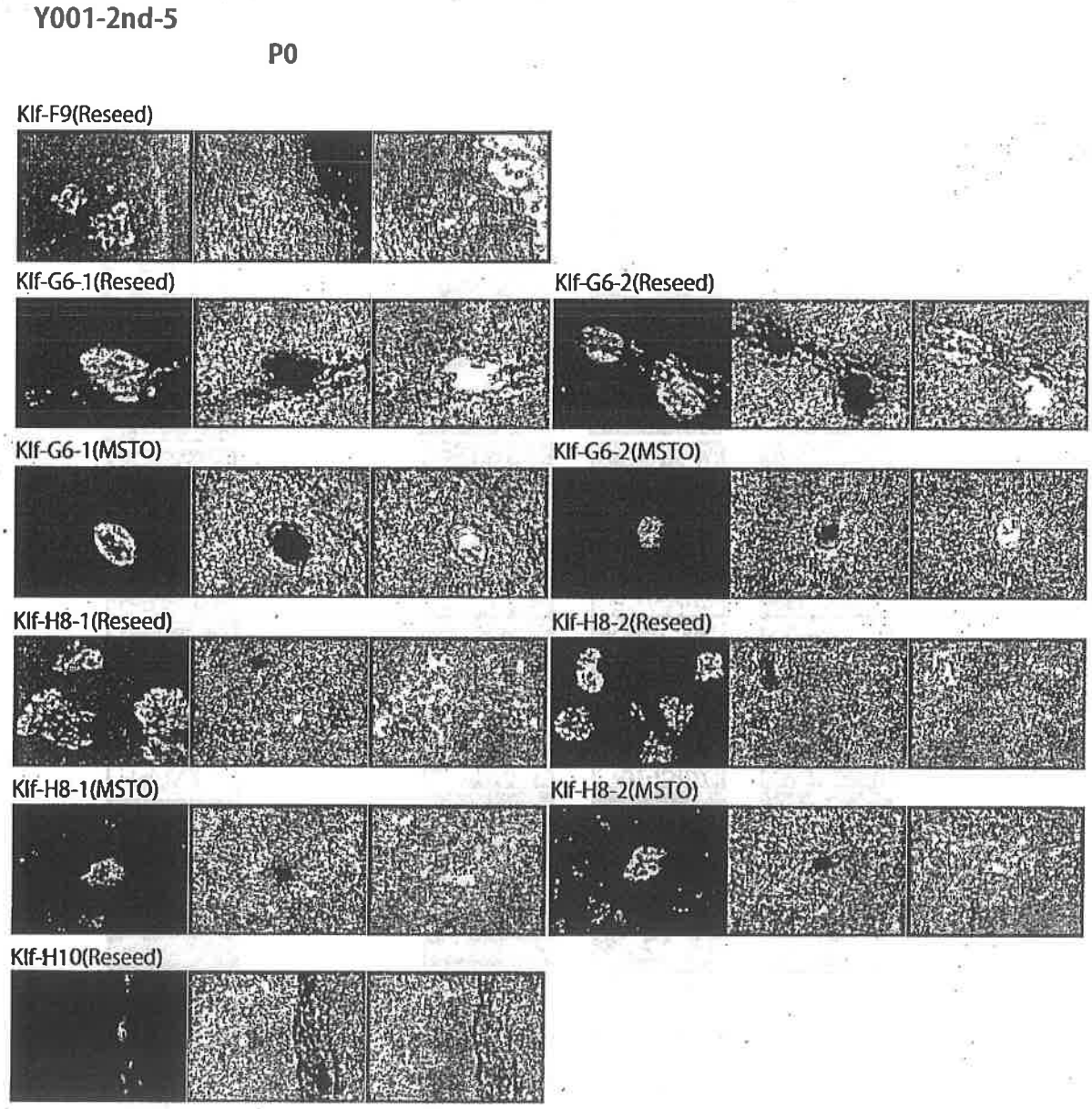

Y001-2nd-5
P0
Klf-F9(Reseed)
Klf-G6-1(Reseed)
Klf-G6-2(Reseed)
Klf-G6-1(MSTO)
Klf-G6-2(MSTO)
Klf-H8-1(Reseed)
Klf-H8-2(Reseed)
Klf-H8-1(MSTO)
Klf-H8-2(MSTO)
Klf-H10(Reseed)

Fig. 5

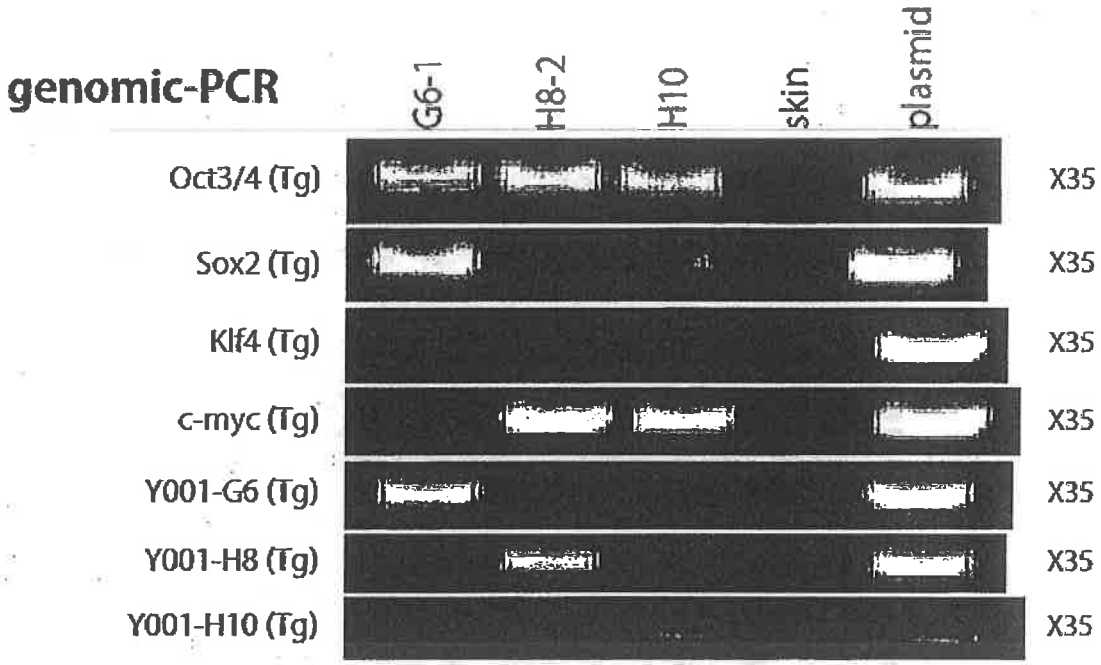

genomic-PCR
G6-1
H8-2
H10
skin
plasmid
Oct3/4 (Tg)
Sox2 (Tg)
Klf4 (Tg)
c-myc (Tg)
Y001-G6 (Tg)
Y001-H8 (Tg)
Y001-H10 (Tg)
X35
X35
X35
X35
X35
X35
X35

Fig. 6

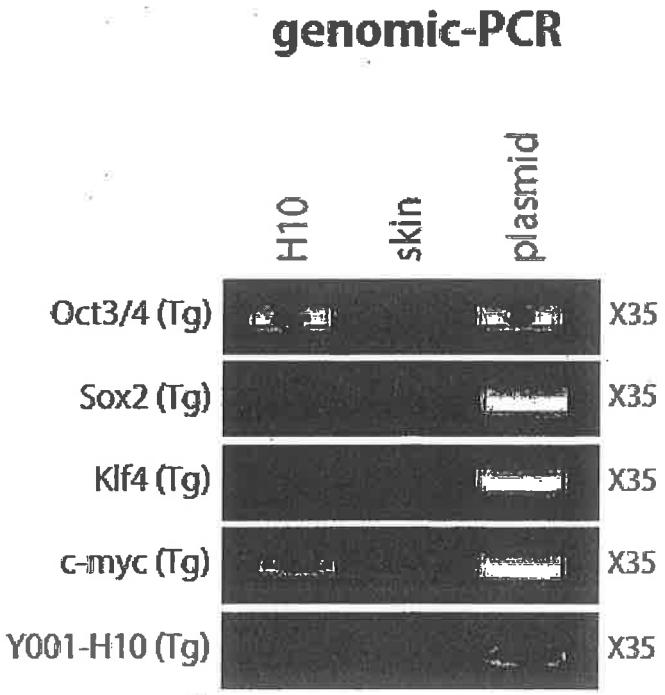

genomic-PCR
H10
skin
plasmid
Oct3/4 (Tg)
Sox2 (Tg)
Klf4 (Tg)
c-myc (Tg)
Y001-H10 (Tg)
X35
X35
X35
X35
X35

Fig. 7

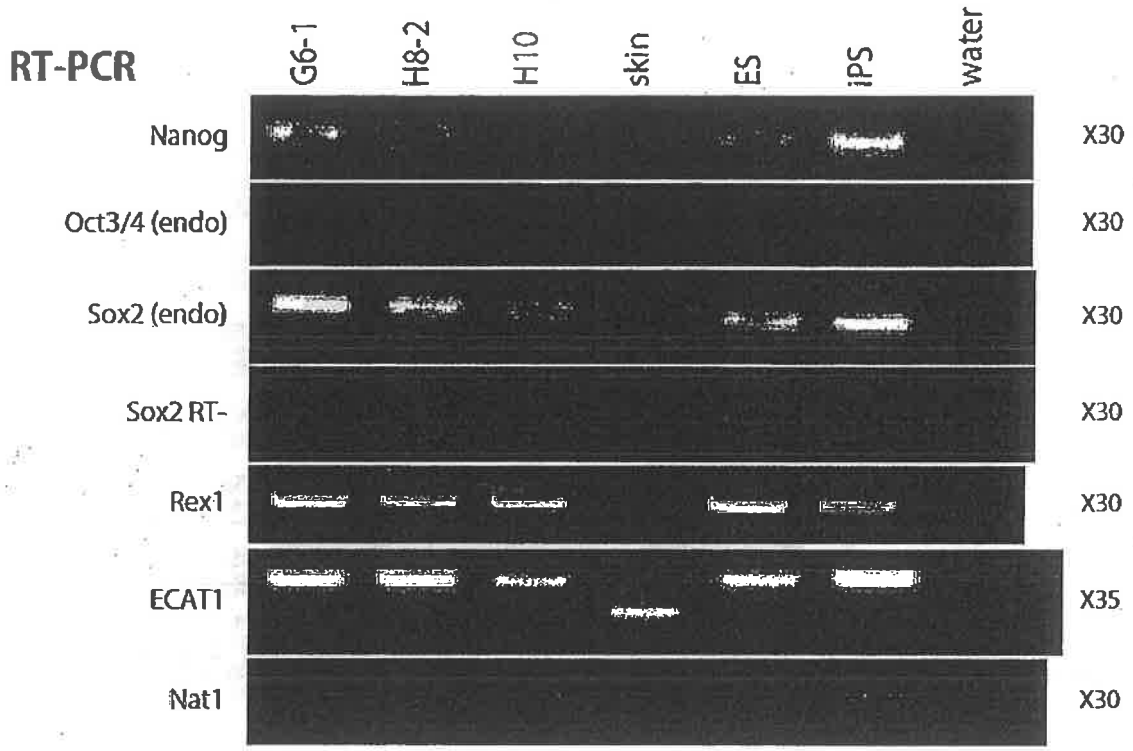

RT-PCR
G6-1
H8-2
H10
skin
ES
iPS
water
Nanog
Oct3/4 (endo)
Sox2 (endo)
Sox2 RT-
Rex1
ECAT1
Nat1
X30
X30
X30
X30
X30
X35
X30

Fig. 8

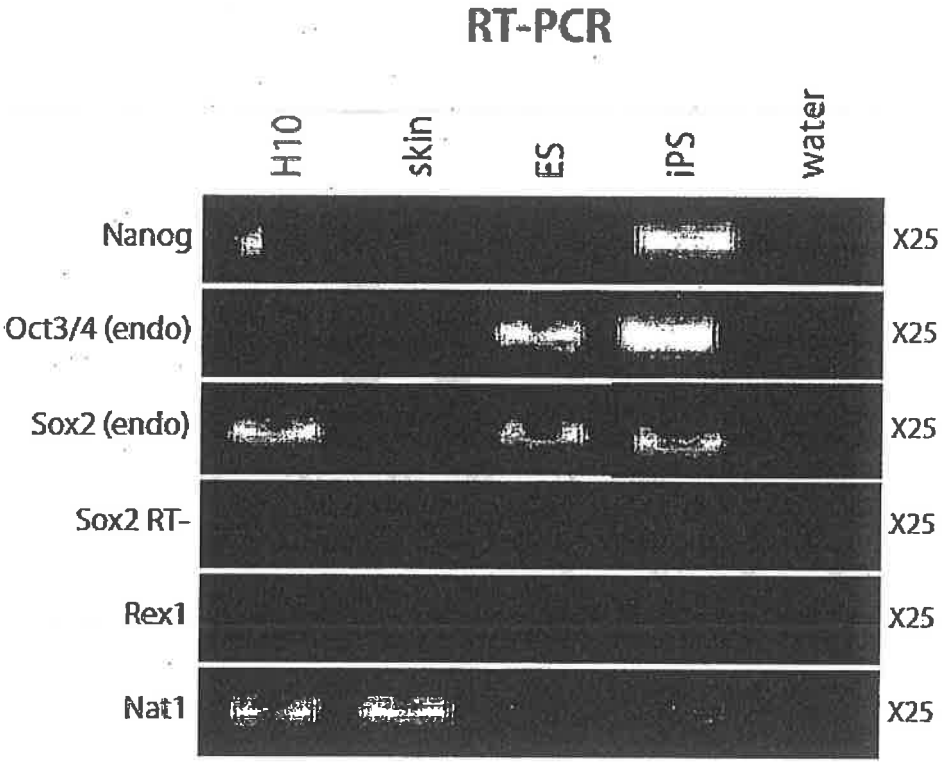

RT-PCR
H10
skin
ES
iPS
water
Nanog
Oct3/4 (endo)
Sox2 (endo)
Sox2 RT-
Rex1
Nat1
X25
X25
X25
X25
X25
X25

Fig. 9

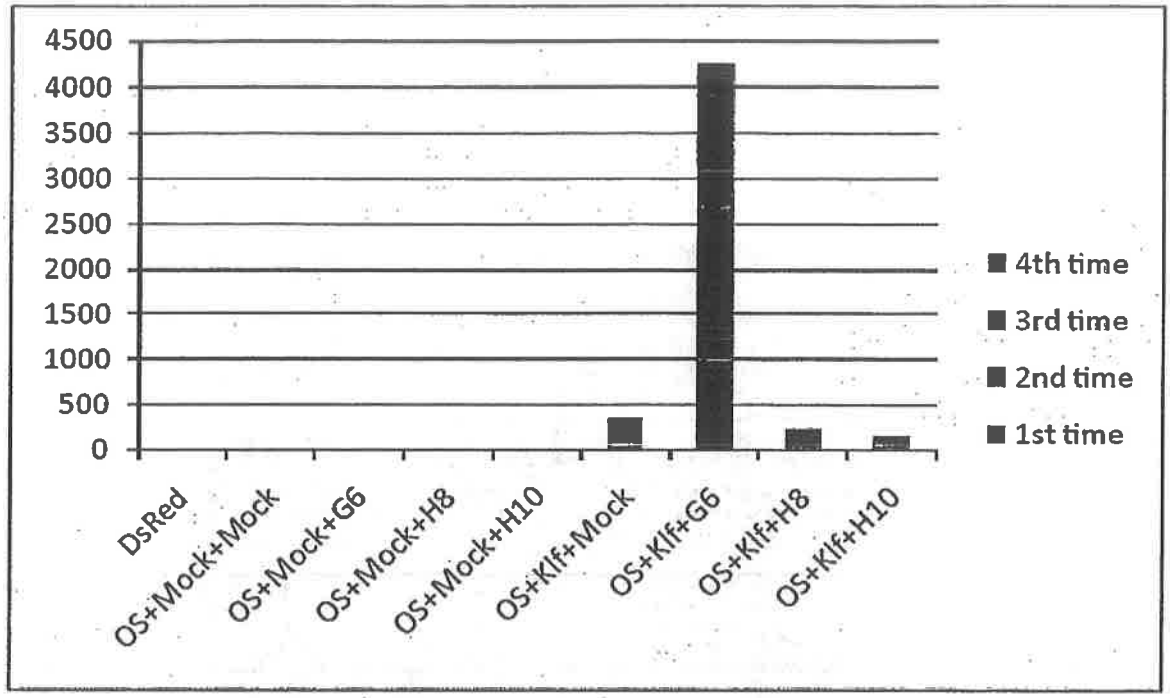

4500
4000
3500
3000
2500
2000
1500
1000
500
0
DsRed
OS+Mock+Mock
OS+Mock+G6
OS+Mock+H8
OS+Mock+H10
OS+Klf+Mock
OS+Klf+G6
OS+Klf+H8
OS+Klf+H10
4th time
3rd time
2nd time
1st time

Fig. 10

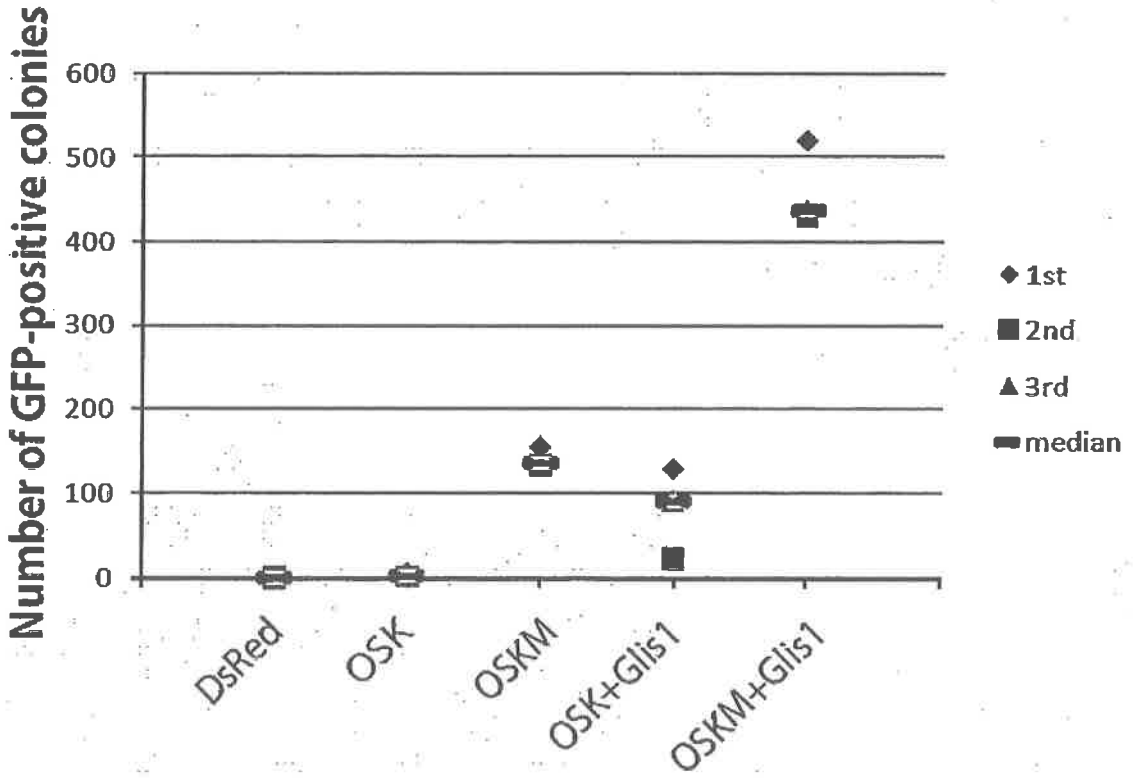

Number of GFP-positive colonies
600
500
400
300
200
100
0
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1
1st
2nd
3rd
median

Fig. 11

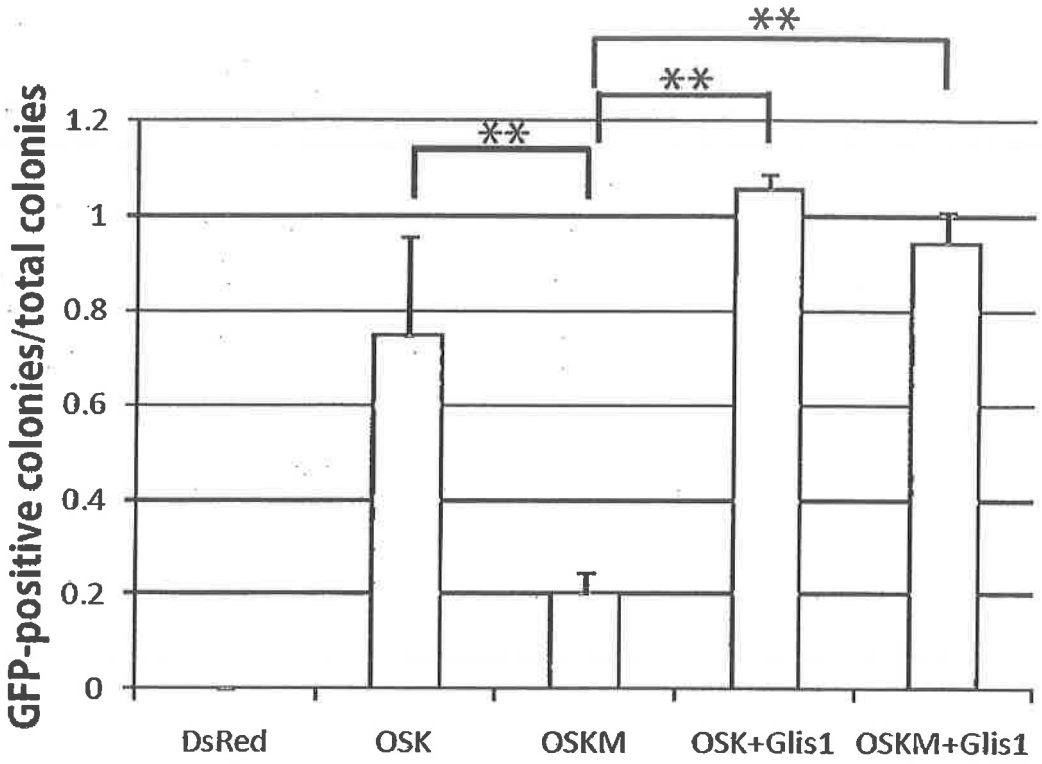

**
**
**
GFP-positive colonies/total colonies
1.2
1
0.8
0.6
0.4
0.2
0
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1

Fig. 12

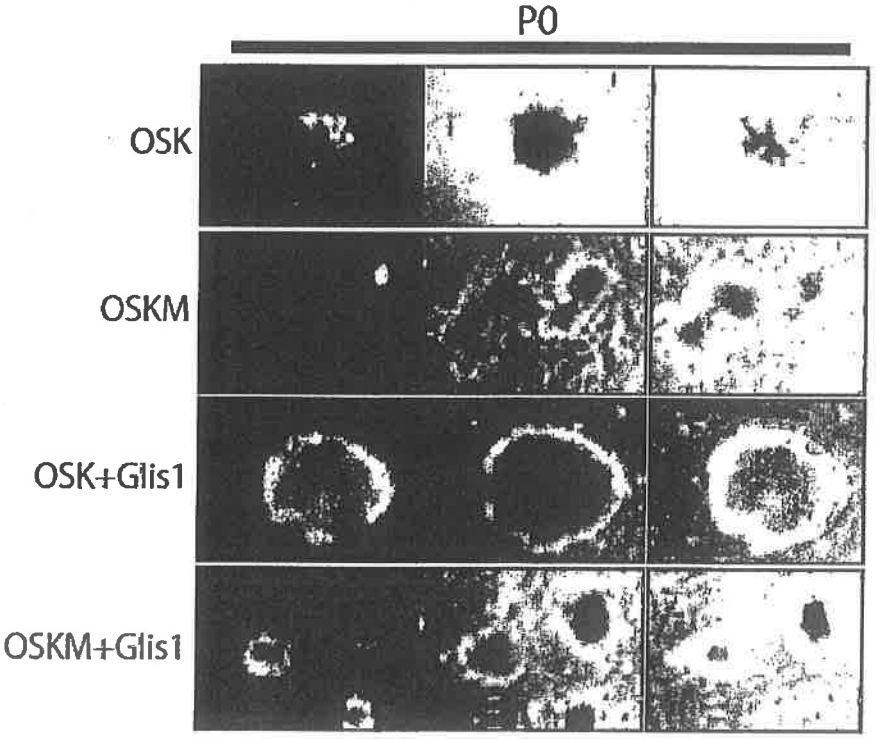

P0
OSK
OSKM
OSK+Glis1
OSKM+Glis1

Fig. 13

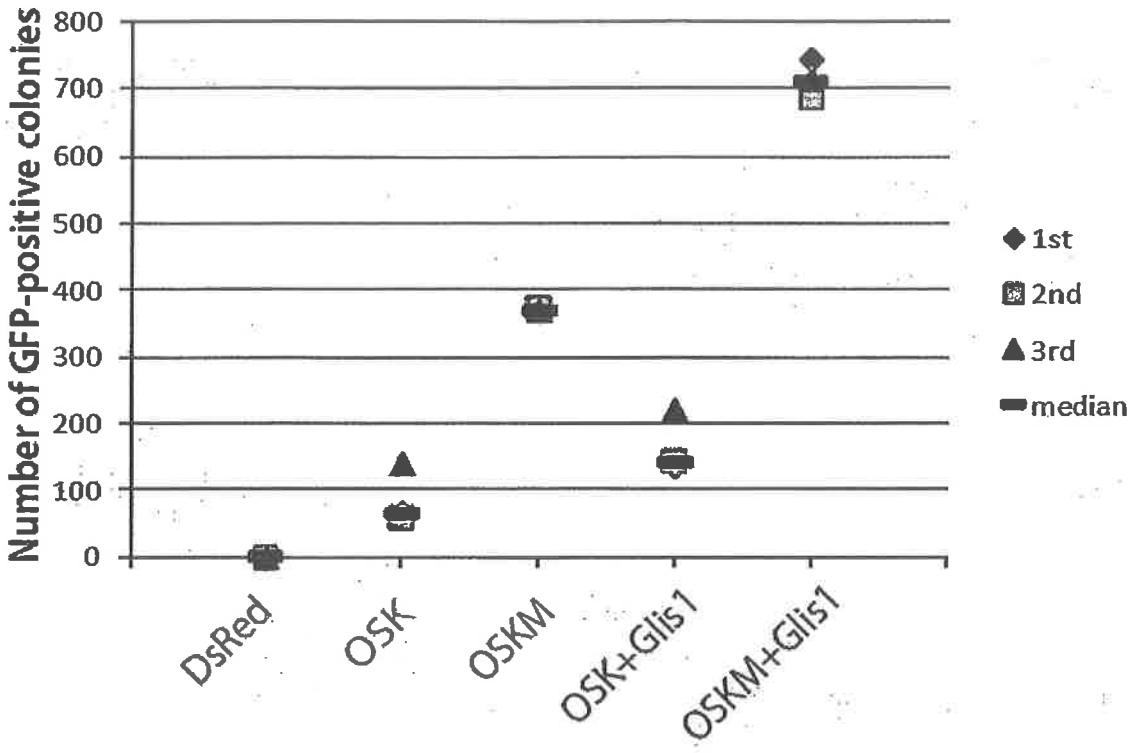

Number of GFP-positive colonies
800
700
600
500
400
300
200
100
0
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1
1st
2nd
3rd
median

Fig. 14

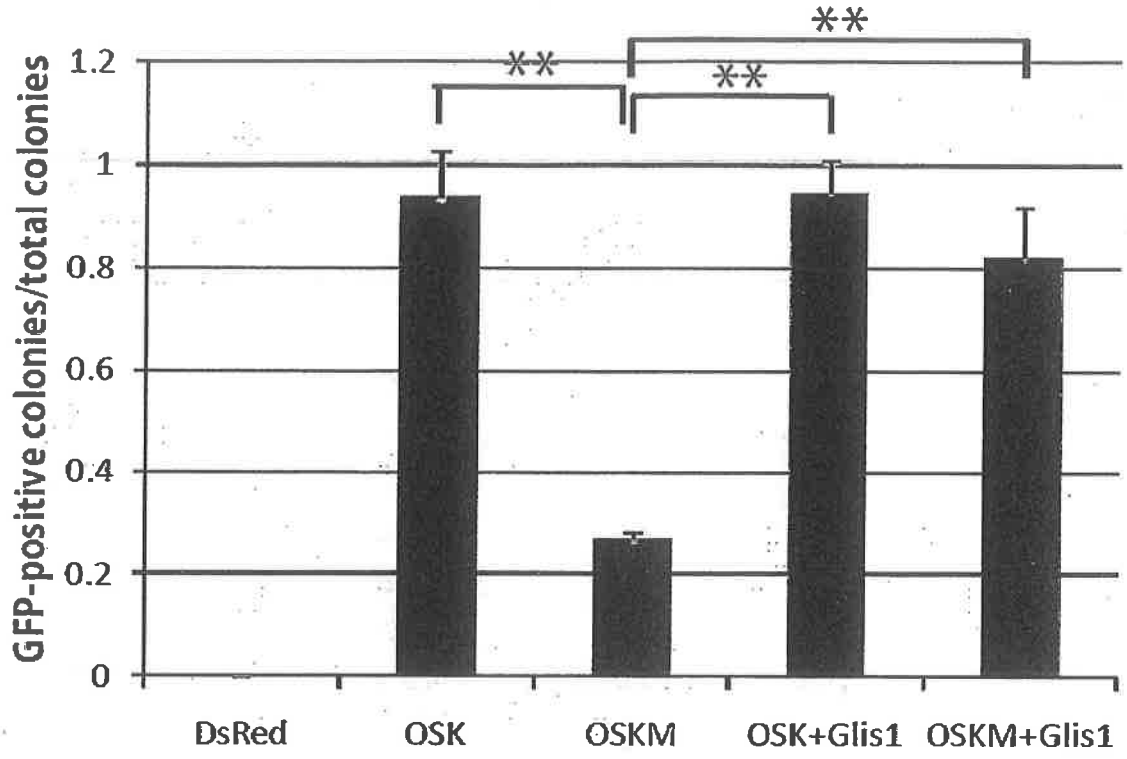

GFP-positive colonies/total colonies
1.2
1
0.8
0.6
0.4
0.2
0
**
**
**
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1

Fig. 15

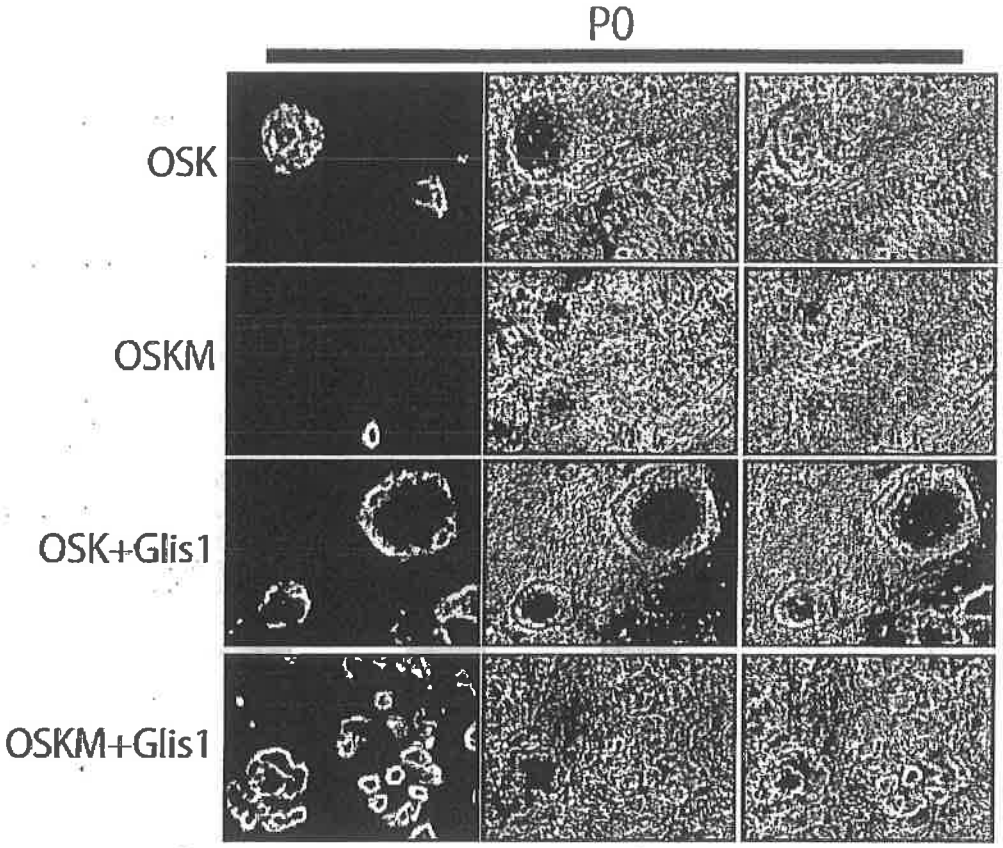

P0
OSK
OSKM
OSK+Glis1
OSKM+Glis1

Fig. 16

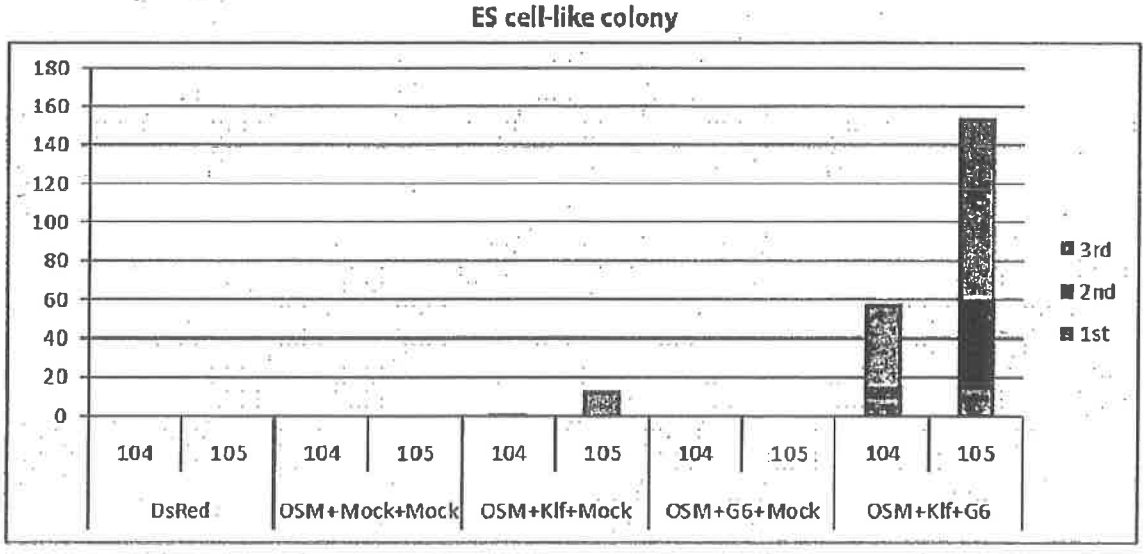

ES cell-like colony
180
160
140
120
100
80
60
40
20
0
104
105
DsRed
OSM+Mock+Mock
OSM+Klf+Mock
OSM+G6+Mock
OSM+Klf+G6
3rd
2nd
1st

Fig. 17

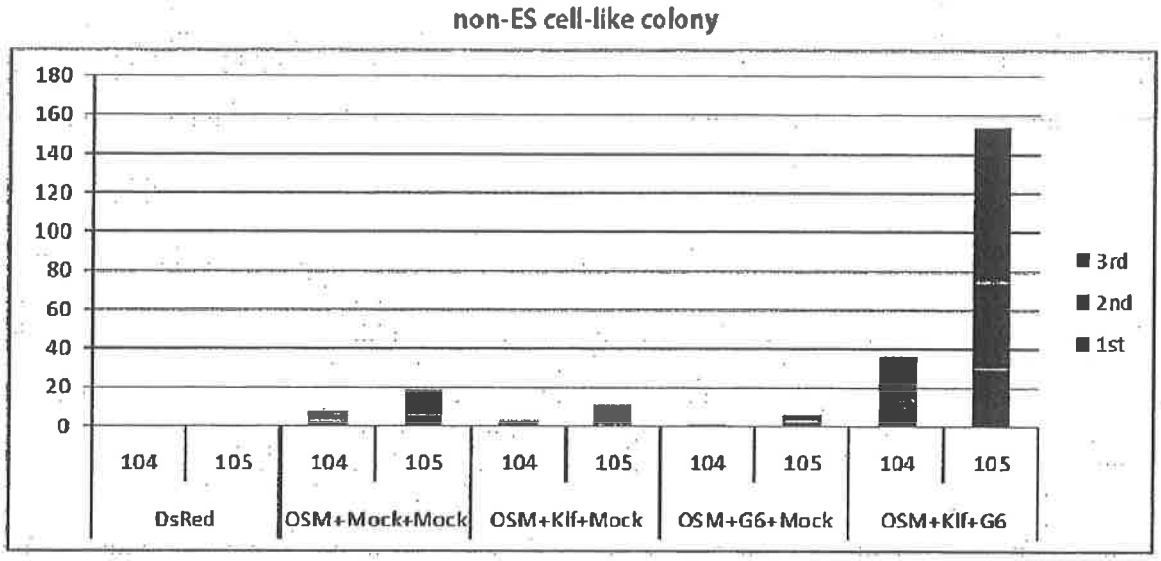

non-ES cell-like colony
180
160
140
120
100
80
60
40
20
0
104
105
104
105
104
105
104
105
104
105
DsRed
OSM+Mock+Mock
OSM+Klf+Mock
OSM+G6+Mock
OSM+Klf+G6
3rd
2nd
1st

Fig. 18

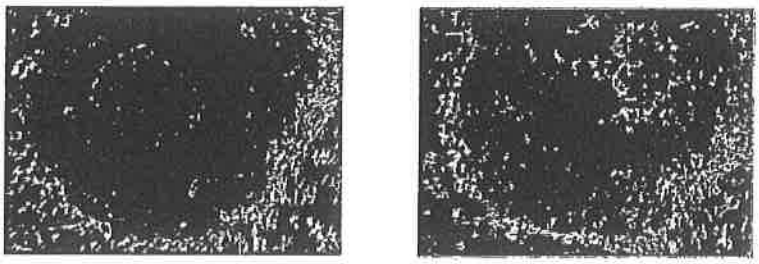


Fig. 19

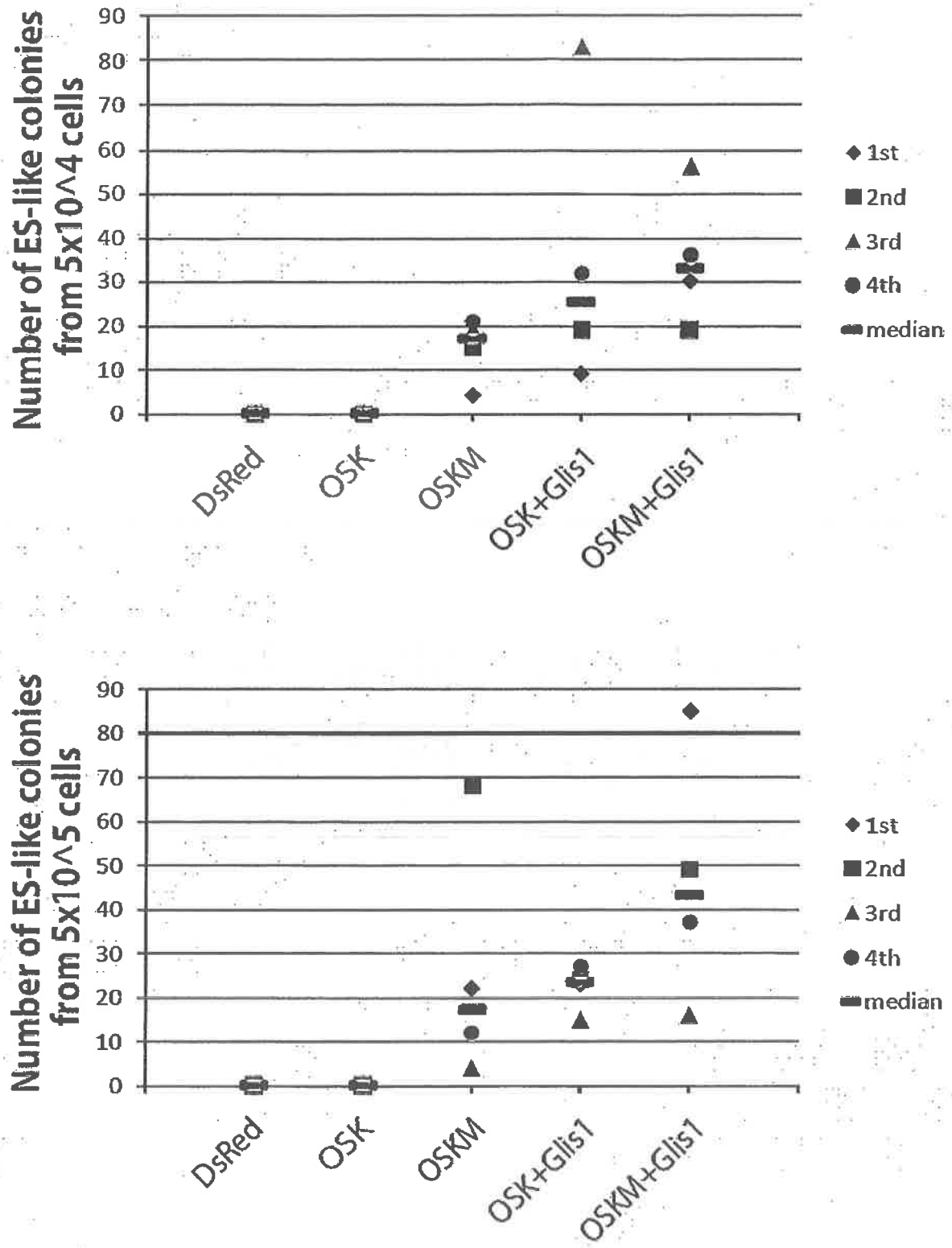
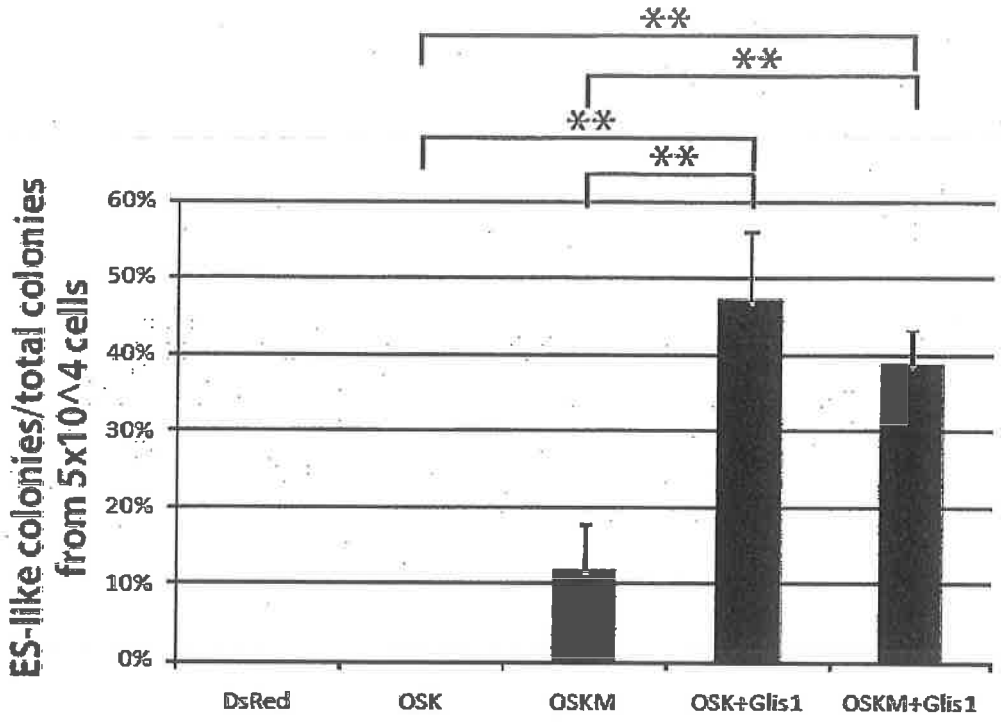

Number of ES-like colonies from 5x10^4 cells
90
80
70
60
50
40
30
20
10
0
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1
1st
2nd
3rd
4th
median
Number of ES-like colonies from 5x10^5 cells
90
80
70
60
50
40
30
20
10
0
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1
1st
2nd
3rd
4th
median

Fig. 20


**
**
**
**
ES-like colonies/total colonies from 5x10^4 cells
60%
50%
40%
30%
20%
10%
0%
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1

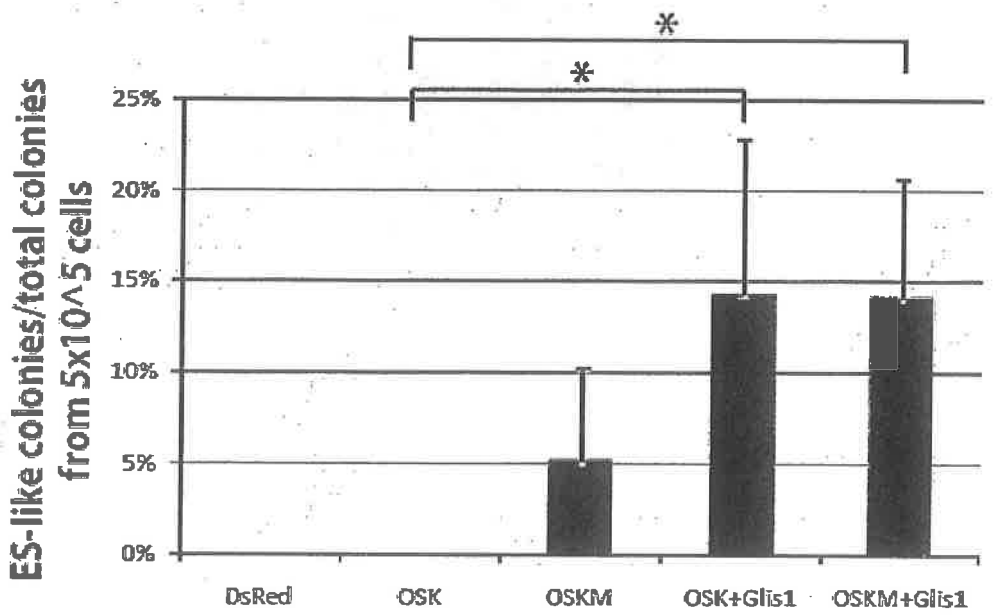

*
*
ES-like colonies/total colonies from 5x10^5 cells
25%
20%
15%
10%
5%
0%
DsRed
OSK
OSKM
OSK+Glis1
OSKM+Glis1

Fig. 21

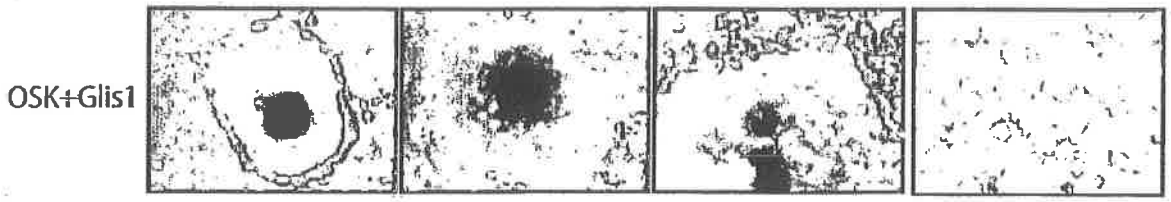

OSK+Glis1

Fig. 22

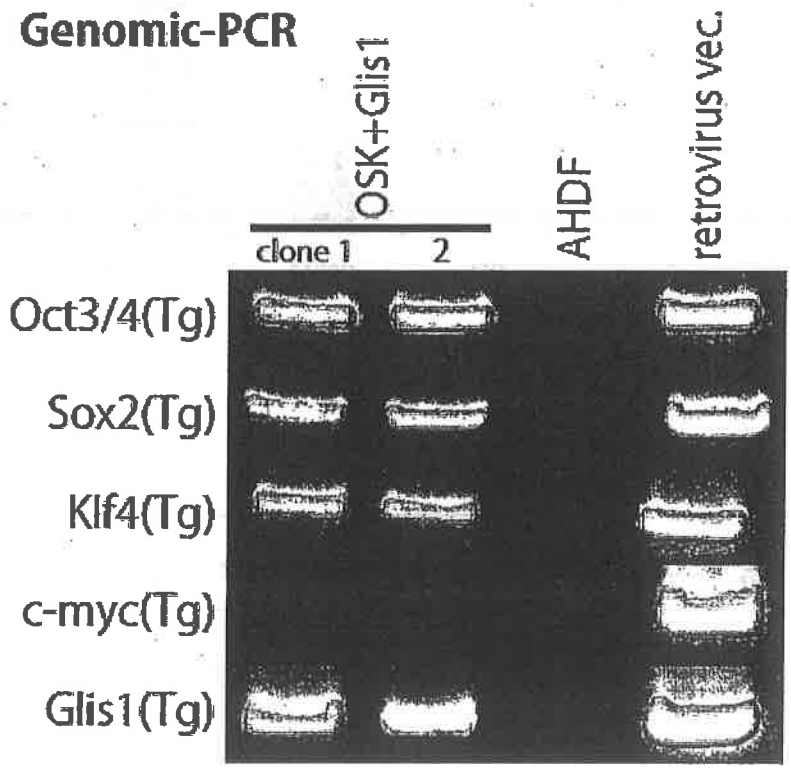

Genomic-PCR
OSK+Glis1
clone 1
2
AHDF
retrovirus vec.
Oct3/4(Tg)
Sox2(Tg)
Klf4(Tg)
c-myc(Tg)
Glis1(Tg)

Fig. 23

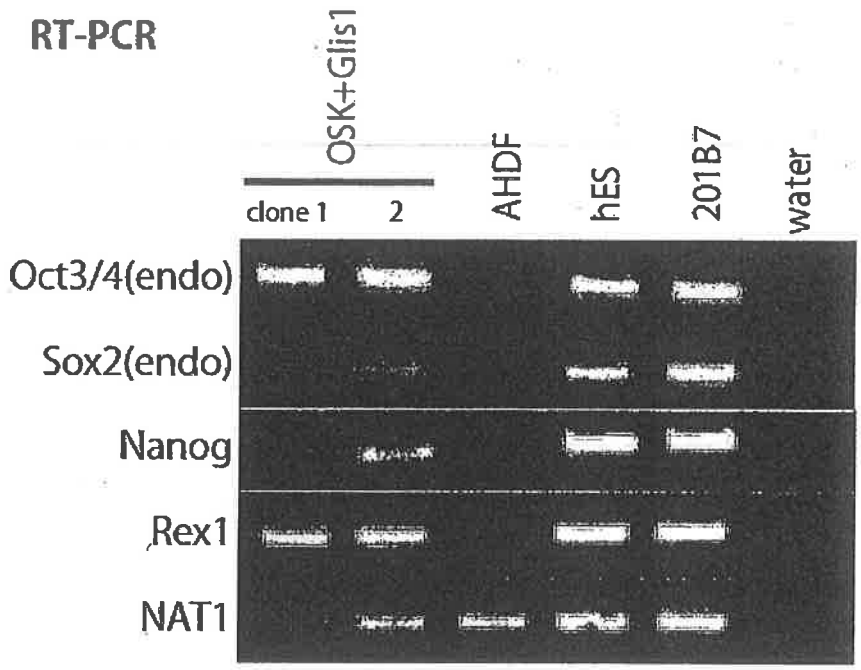

RT-PCR
OSK+Glis1
clone 1
2
AHDF
hES
201B7
water
Oct3/4(endo)
Sox2(endo)
Nanog
Rex1
NAT1

Fig. 24

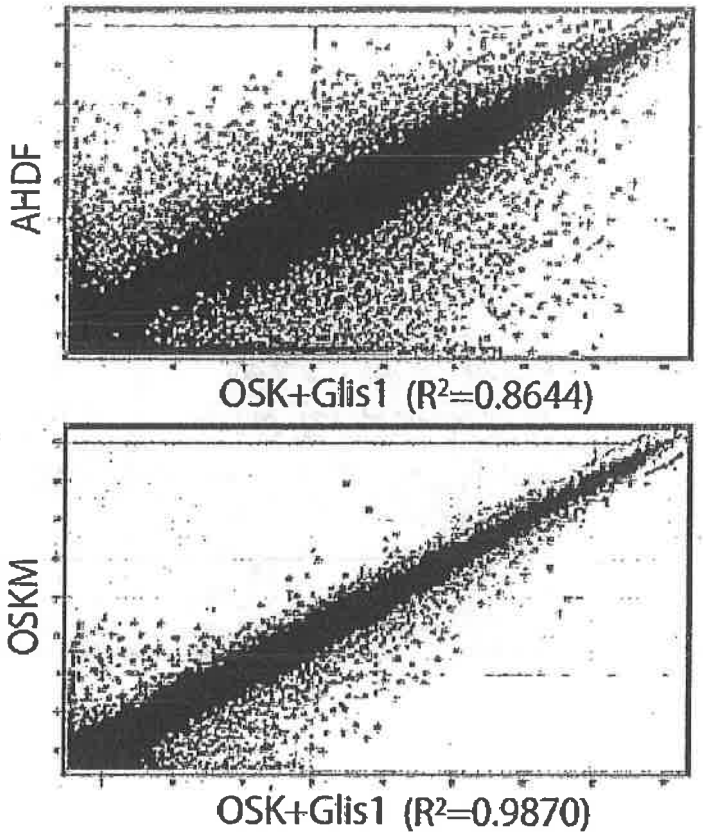


Fig. 25

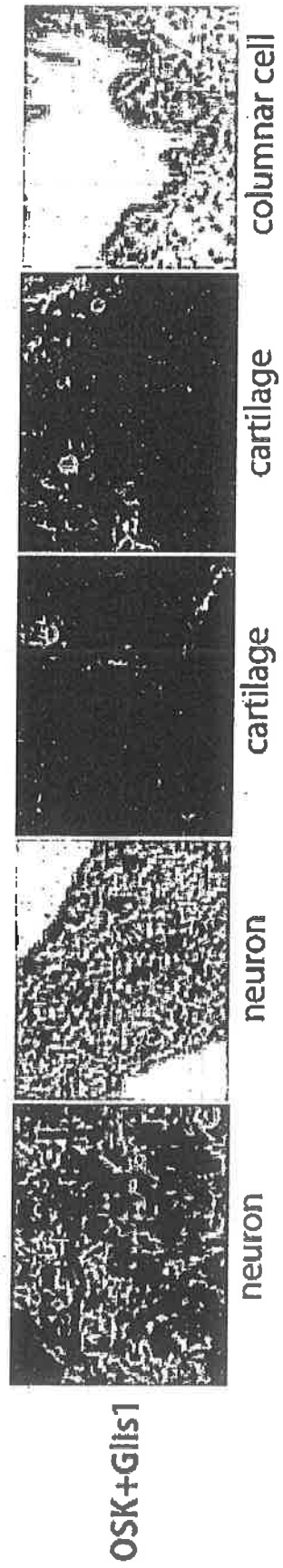

OSK+Glis1
neuron
neuron
cartilage
cartilage
columnar cell

Fig. 26


Glis1/NAT1
Relative expression level, /NAT1
Normalized Kidney =1.0
140
120
100
80
60
40
20
0
unfertilized egg
1cell
2cell
blast
kidney
placenta
brain
lung
liver
spleen
ovary
ES
MEF
skin

Fig. 27

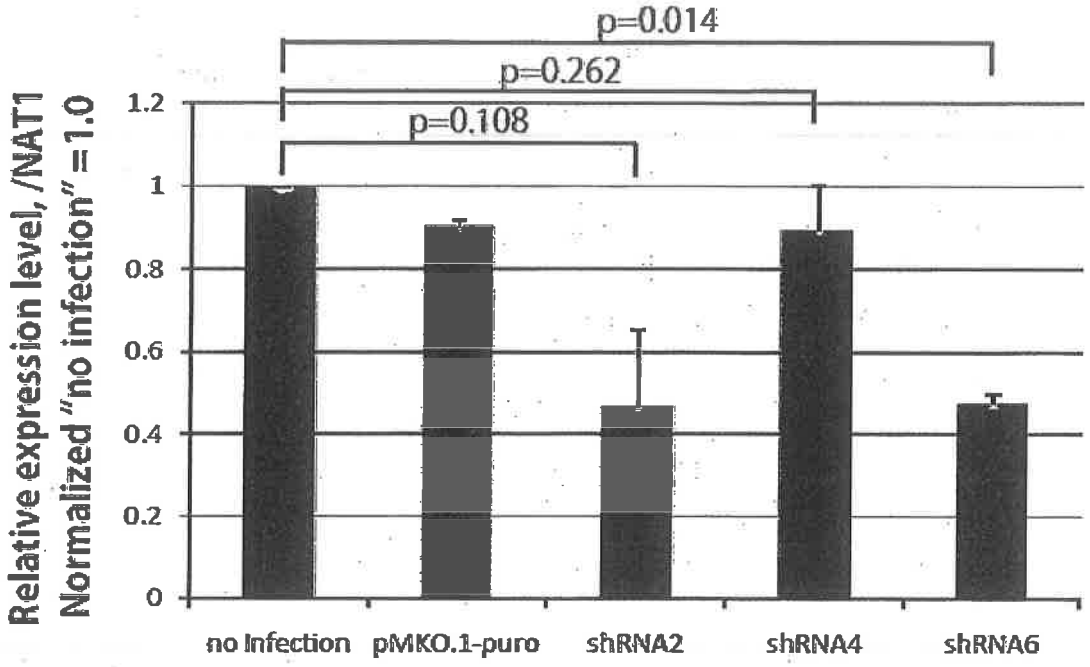
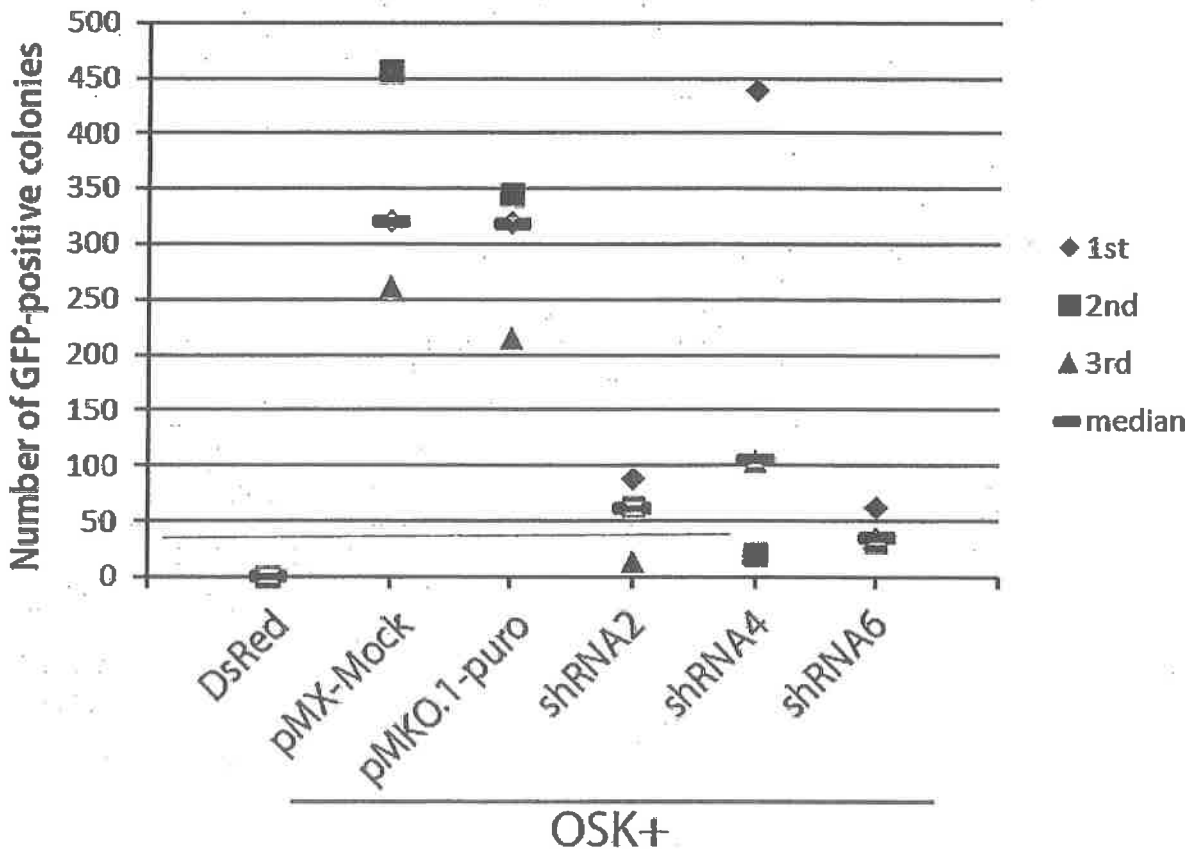

p=0.014
p=0.262
p=0.108
Relative expression level, /NAT1
Normalized "no infection" =1.0
1.2
1
0.8
0.6
0.4
0.2
0
no Infection
pMKO.1-puro
shRNA2
shRNA4
shRNA6

Fig. 28


Number of GFP-positive colonies
500
450
400
350
300
250
200
150
100
50
0
1st
2nd
3rd
median
DsRed
pMX-Mock
pMKO.1-puro
shRNA2
shRNA4
shRNA6
OSK+