Fig. 1-1

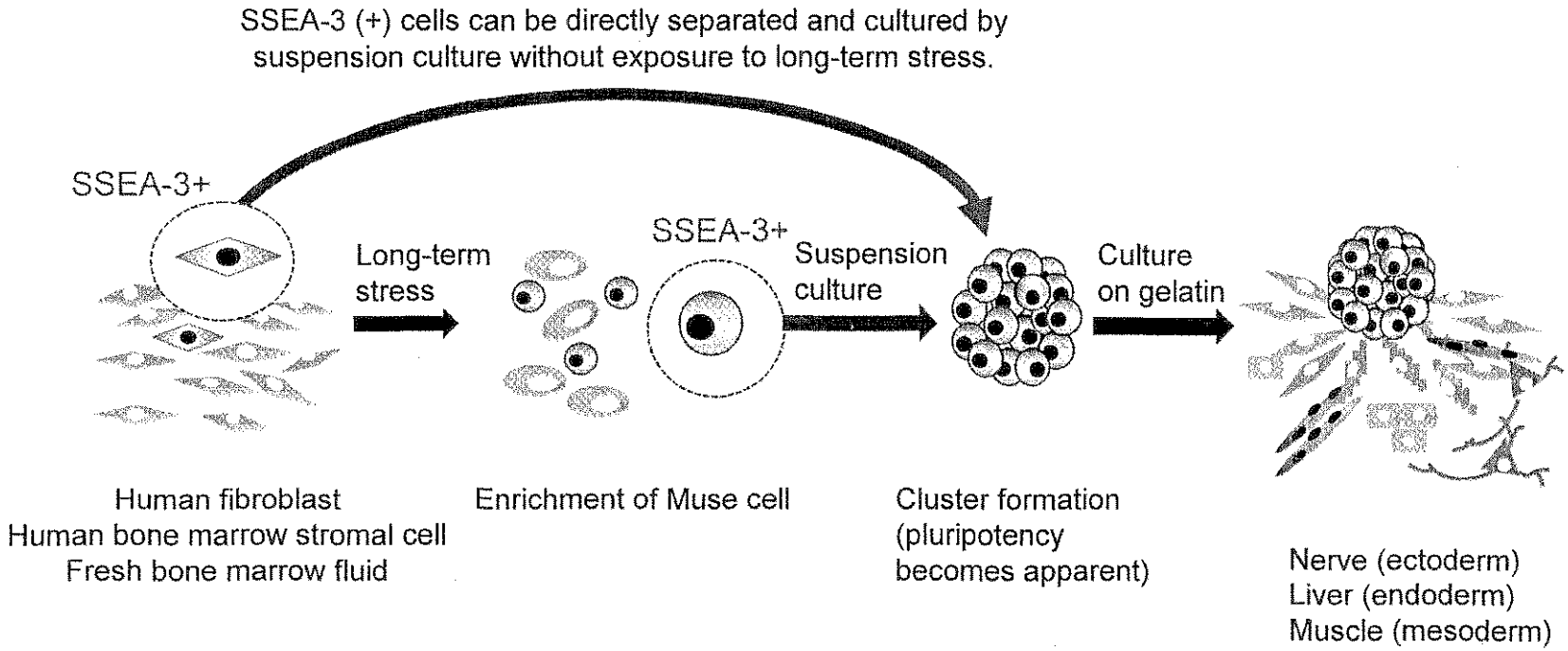

SSEA-3 (+) cells can be directly separated and cultured by suspension culture without exposure to long-term stress.
SSEA-3+
Long-term stress
SSEA-3+
Suspension culture
Culture on gelatin
Human fibroblast
Human bone marrow stromal cell
Fresh bone marrow fluid
Enrichment of Muse cell
Cluster formation (pluripotency becomes apparent)
Nerve (ectoderm)
Liver (endoderm)
Muscle (mesoderm)

Fig. 1-2

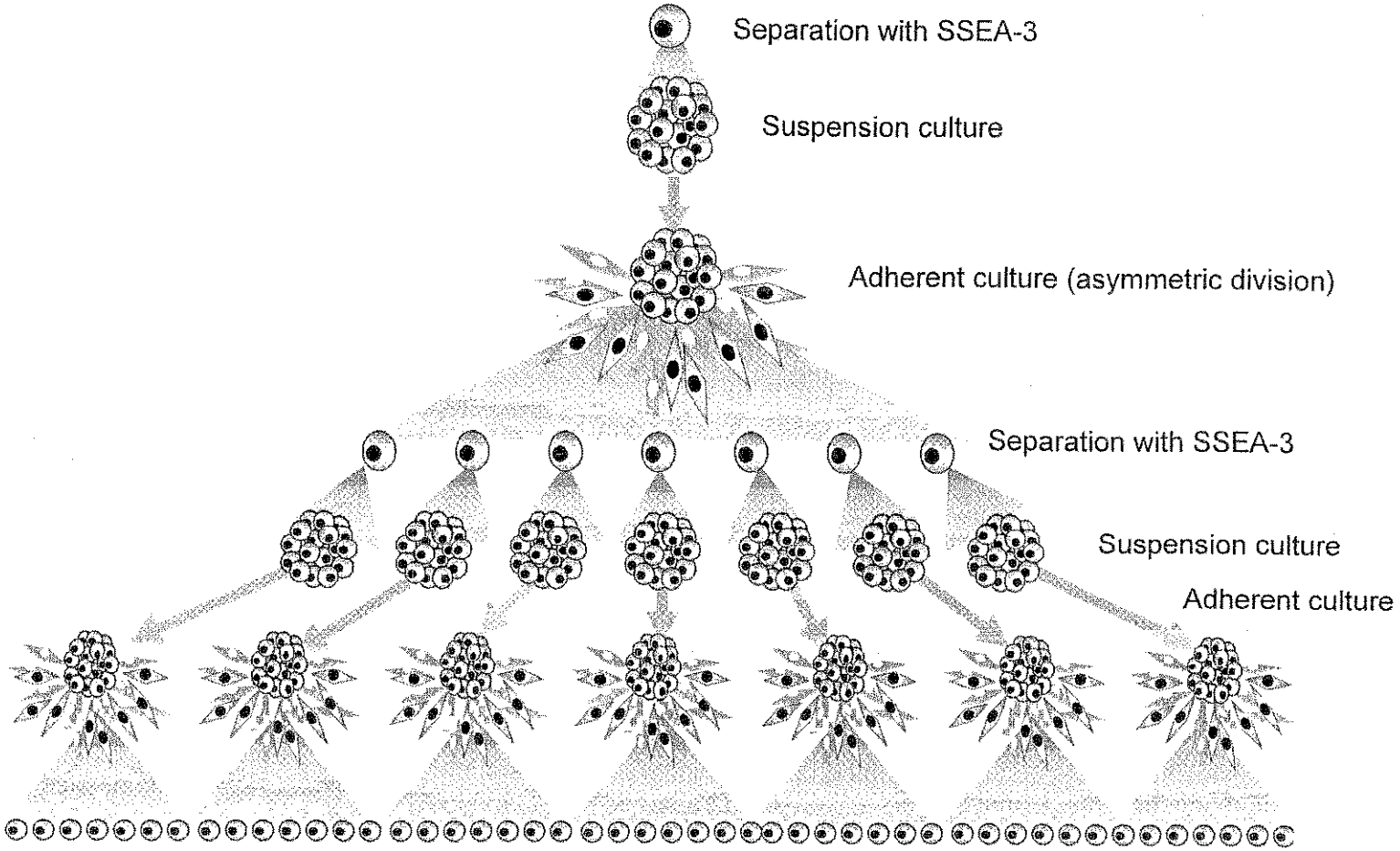

Separation with SSEA-3
Suspension culture
Adherent culture (asymmetric division)
Separation with SSEA-3
Suspension culture
Adherent culture

Fig. 2

Gene Symbol	Total	D24 EB body/D24 Native	NHDF EB body/NHDF Native	Gene Title
THBD	1267.42467	42.96487494	1224.459795	thrombomodulin
IL1RN	960.4457022	12.0038603	948.4418419	interleukin 1 receptor antagonist
FOS	954.4076469	88.7202031	865.6874438	v-fos FBJ murine osteosarcoma viral oncogene homolog
SLC16A6	849.437506	715.7799818	133.6575242	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)
TYRP1	768.3374259	11.67216252	756.6652634	tyrosinase-related protein 1
CACNA1A	685.5302732	3.084442121	682.445831	Calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
C16orf81	531.0858738	528.3969782	2.688895627	chromosome 16 open reading frame 81
CHI3L1	489.924397	373.8511267	116.0732704	chitinase 3-like 1 (cartilage glycoprotein-39)
PRSS35	441.7622409	371.6403738	70.12186703	protease, serine, 35
KYNU	420.8532931	182.1885786	238.6647145	kynureninase (L-kynurenine hydrolase)
SLC16A6	407.4159044	315.8163678	91.59953662	solute carrier family 16, member 6 (monocarboxylic acid transporter 7)
APOE	357.4991884	105.5807107	251.9184778	apolipoprotein E
THBD	347.7592226	34.12473279	313.6344898	thrombomodulin
SYTL5	320.0330513	15.80876986	304.2242814	synaptotagmin-like 5
CHI3L1	299.4142542	260.1989748	39.21527948	chitinase 3-like 1 (cartilage glycoprotein-39)
ABCA13	290.3969665	271.9782856	18.41868089	ATP-binding cassette, sub-family A (ABC1), member 13
ANGPTL4	284.3113469	11.53113972	272.7802072	angiopoietin-like 4
PTGS2	273.0706903	201.4865123	71.58417805	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
STC1	255.0878156	29.28287475	225.8049409	stanniocalcin 1
CCDC102B	253.1960755	37.22467468	215.9714008	coiled-coil domain containing 102B

Fig. 3

Gene Symbol	Total	D24 EB/hES	NHDF EB/hES	Gene Title
MMP1	36528.90821	6836.563387	29692.34482	matrix metallopeptidase 1 (interstitial collagenase)
EREG	19813.4402	18961.45781	851.9823837	epiregulin
CHI3L1	9641.34444	8842.305483	799.038957	chitinase 3-like 1 (cartilage glycoprotein-39)
---	7940.341316	7298.64809	641.6932268	Transcribed locus
CHI3L1	5714.359227	5385.877946	328.4812811	chitinase 3-like 1 (cartilage glycoprotein-39)
SRGN	4655.28423	4636.821662	18.46256778	serglycin
---	4397.855874	3959.205654	438.6502206	MRNA full length insert cDNA clone EUROIMAGE 1913076
RIN2	3611.379317	859.9132609	2751.466056	Ras and Rab interactor 2
LUM	3396.245648	1797.976375	1598.269272	lumican
CLCA2	3331.259662	747.5657674	2583.693895	CLCA family member 2, chloride channel regulator
IL8	3143.389365	2638.045747	505.3436182	interleukin 8
LOC401097	3012.485859	1860.366679	1152.119181	Similar to LOC166075
DPT	2807.405266	56.39203934	2751.013226	dermatopontin
ELTD1	2658.65006	1751.976082	906.6739784	EGF, latrophilin and seven transmembrane domain containing 1
IGFBP1	2423.035758	2405.299722	17.73603586	insulin-like growth factor binding protein 1
SLC16A4	2305.561138	1826.823338	478.7378001	solute carrier family 16, member 4 (monocarboxylic acid transporter 5)
SRGN	2293.730946	2279.537455	14.19349126	serglycin
GREM2	2053.928699	159.813291	1894.115408	gremlin 2, cysteine knot superfamily, homolog (Xenopus laevis)
IGFBP5	1960.755393	1915.425977	45.3294163	insulin-like growth factor binding protein 5
SQRDL	1918.477233	1217.387684	701.0895492	sulfide quinone reductase-like (yeast)

Fig. 4

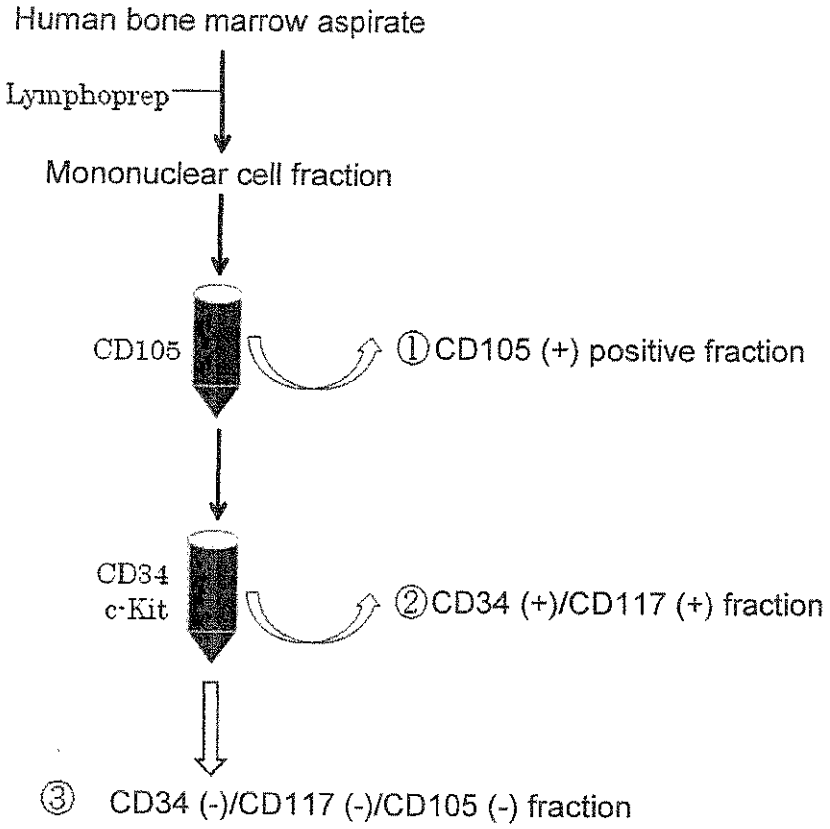

Human bone marrow aspirate
Lymphoprep
Mononuclear cell fraction
CD105
①CD105 (+) positive fraction
CD34
c-Kit
②CD34 (+)/CD117 (+) fraction
③ CD34 (-)/CD117 (-)/CD105 (-) fraction

Fig. 5

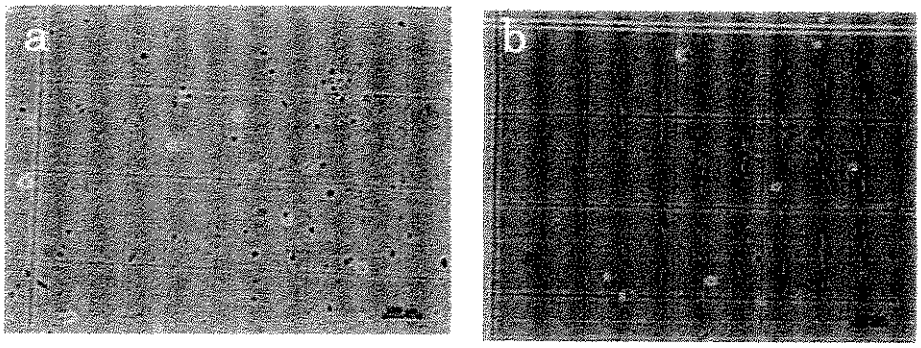


Fig. 6

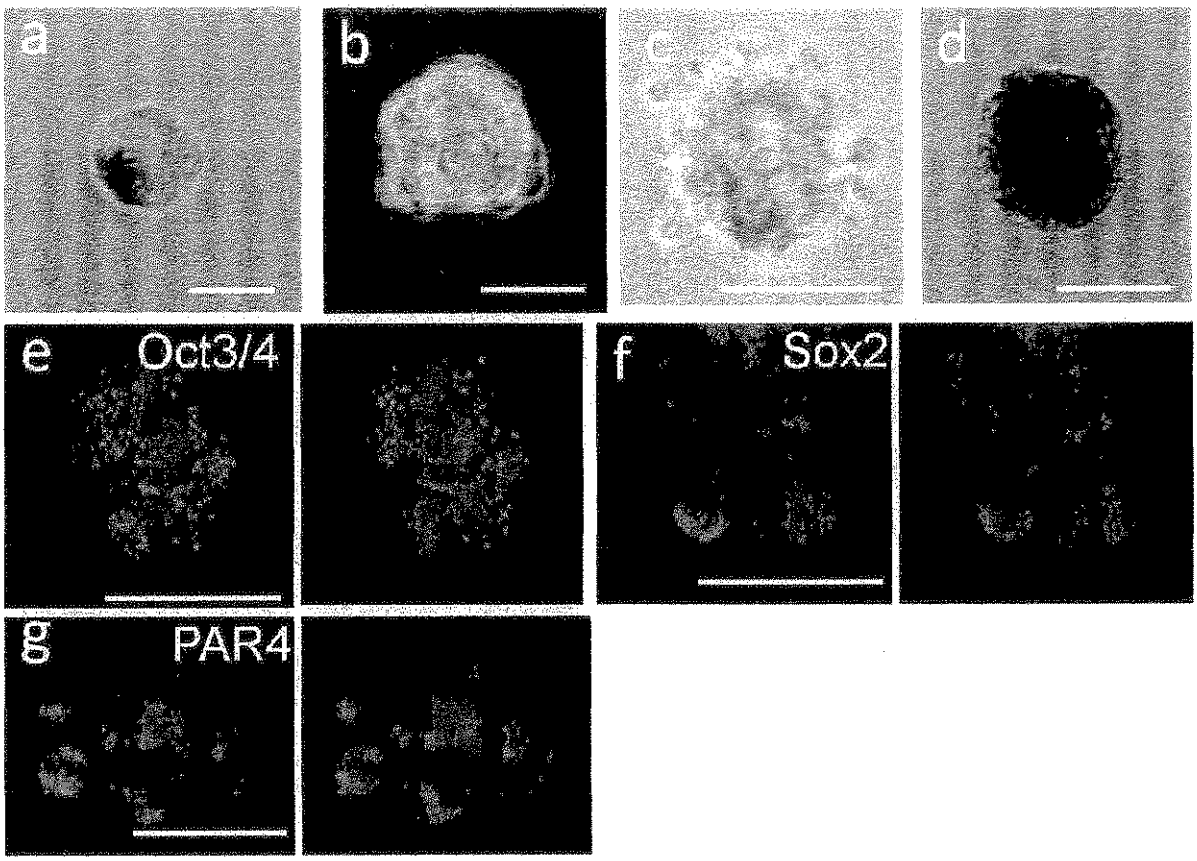


Fig. 7-1

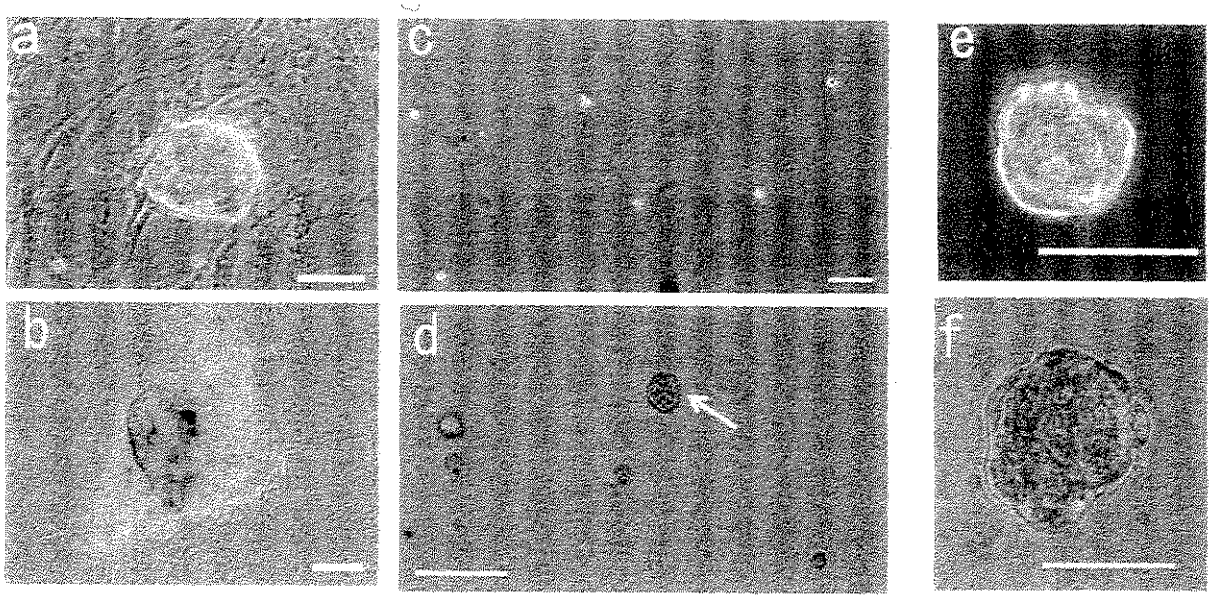


Fig. 7-2

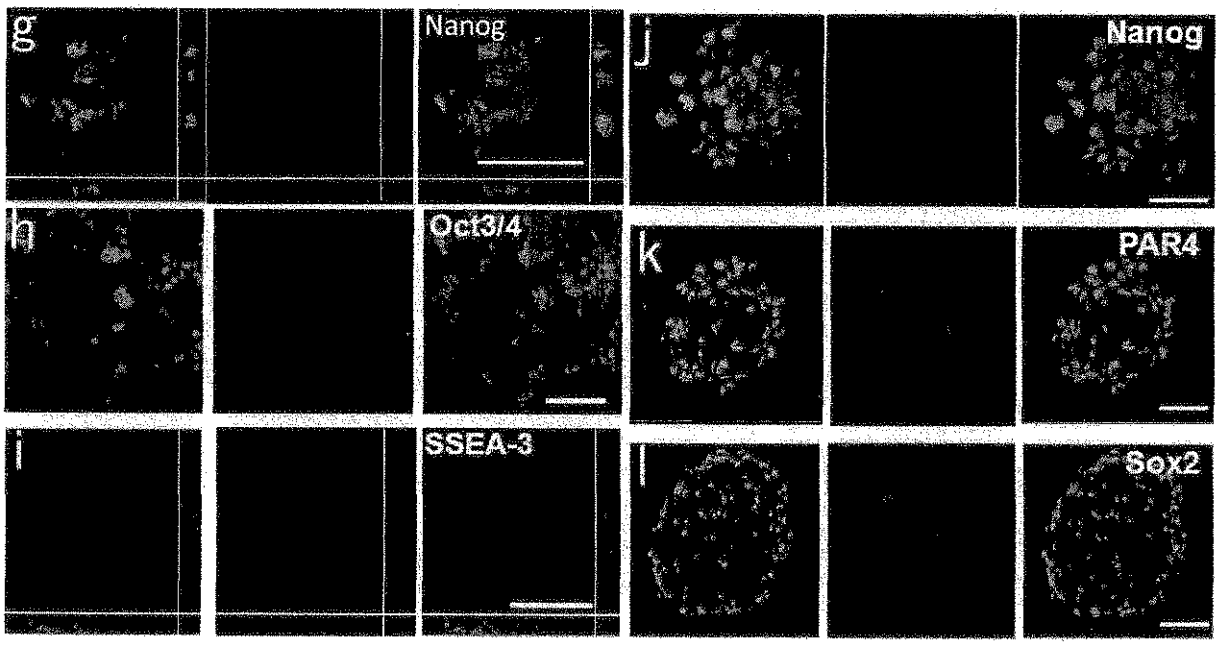


Fig. 7-3

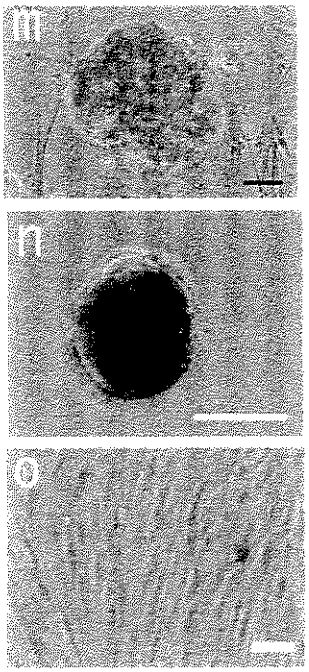


Fig. 7-4

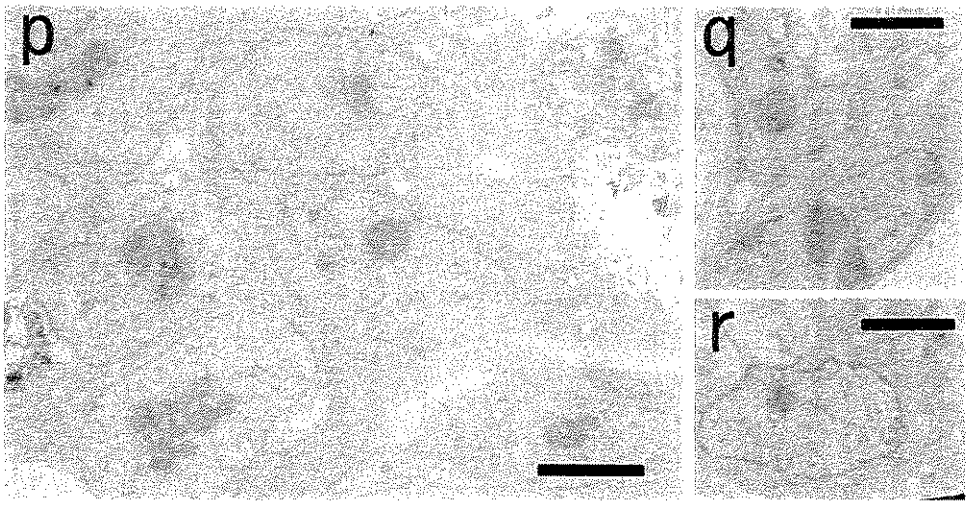


Fig. 8-1

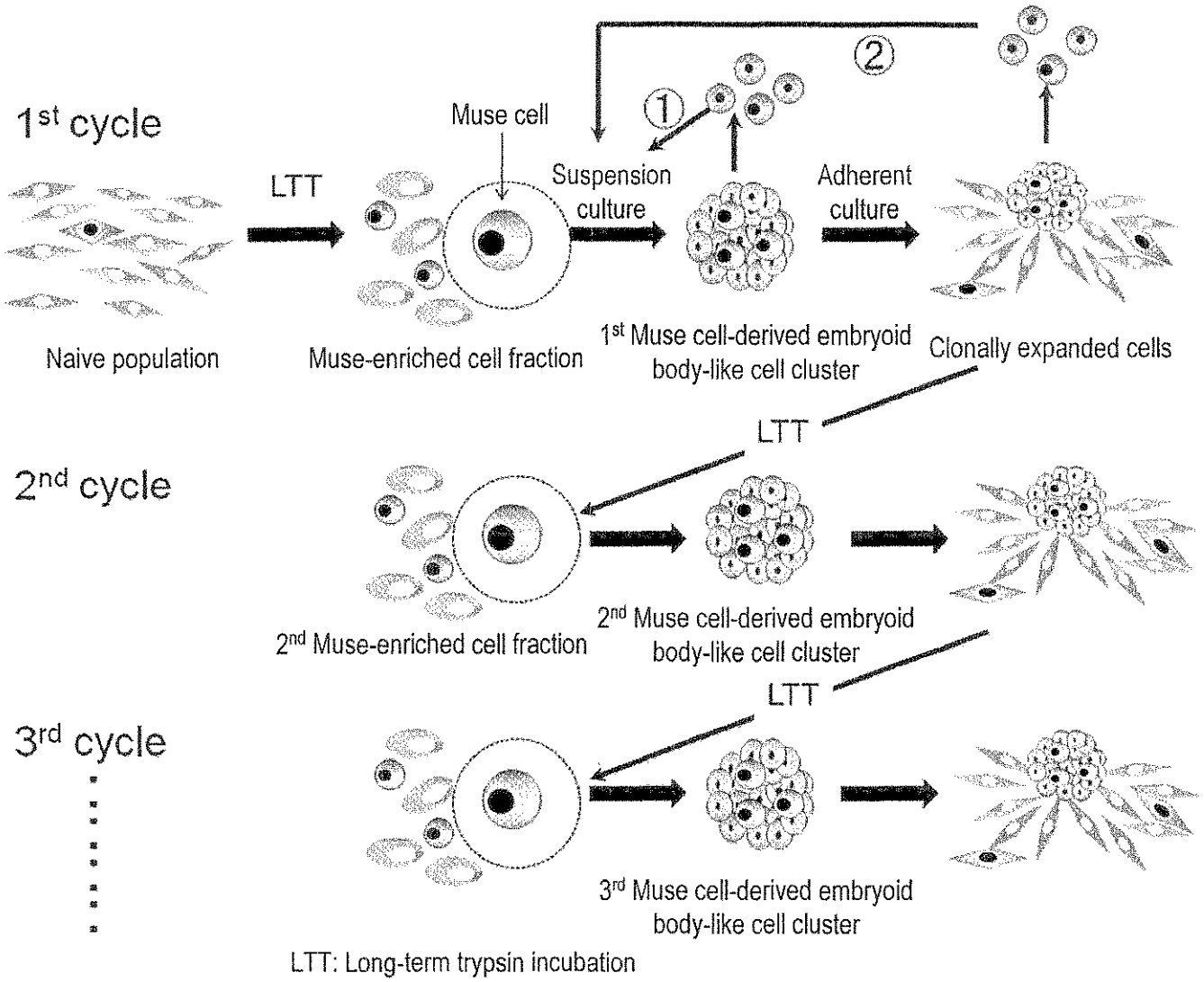

1st cycle
Muse cell
①
②
LTT
Suspension culture
Adherent culture
Naive population
Muse-enriched cell fraction
1st Muse cell-derived embryoid body-like cell cluster
Clonally expanded cells
LTT
2nd cycle
2nd Muse-enriched cell fraction
2nd Muse cell-derived embryoid body-like cell cluster
LTT
3rd cycle
3rd Muse cell-derived embryoid body-like cell cluster
LTT: Long-term trypsin incubation

Fig. 8-2

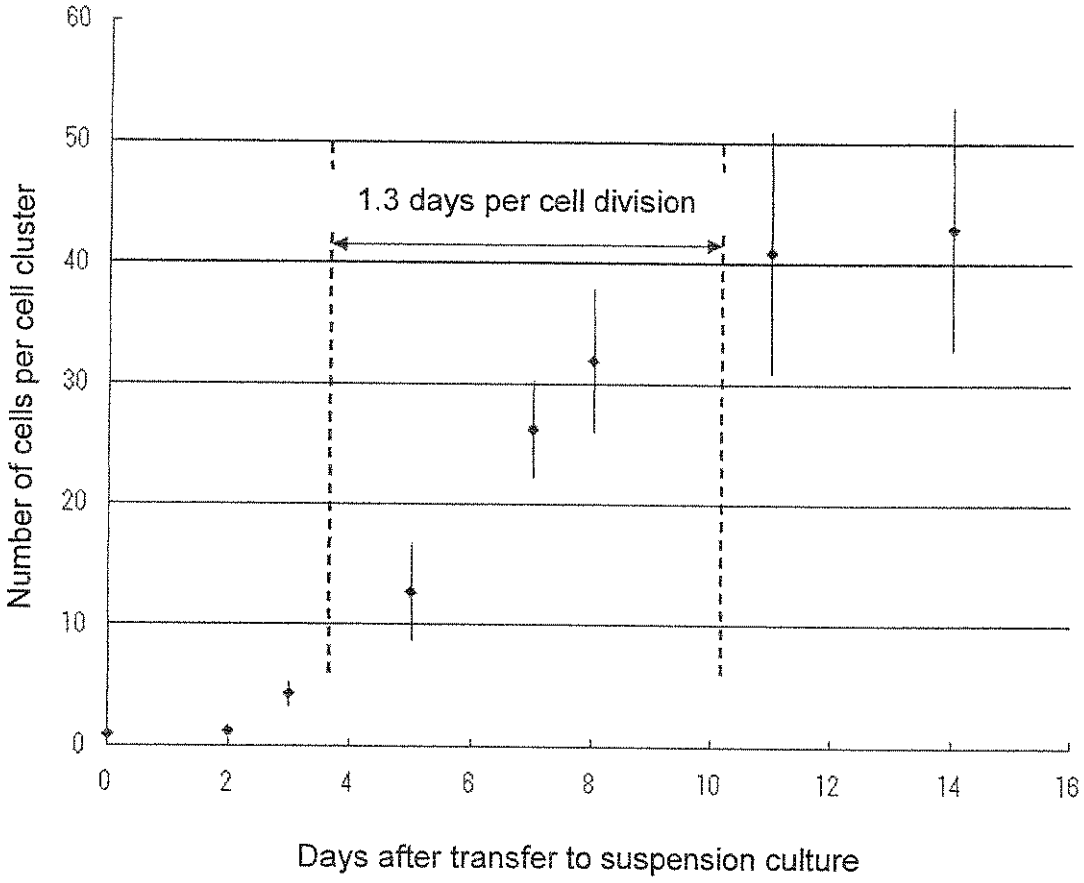

60
50
40
30
20
10
0
Number of cells per cell cluster
1.3 days per cell division
0
2
4
6
8
10
12
14
16
Days after transfer to suspension culture

Fig. 8-3

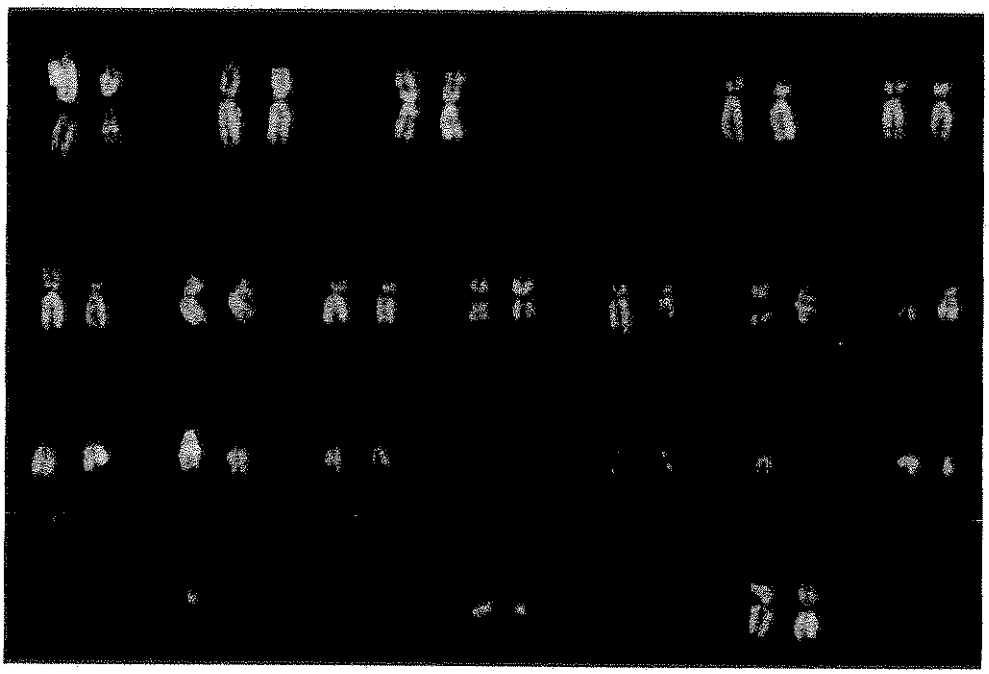


Fig. 9-1

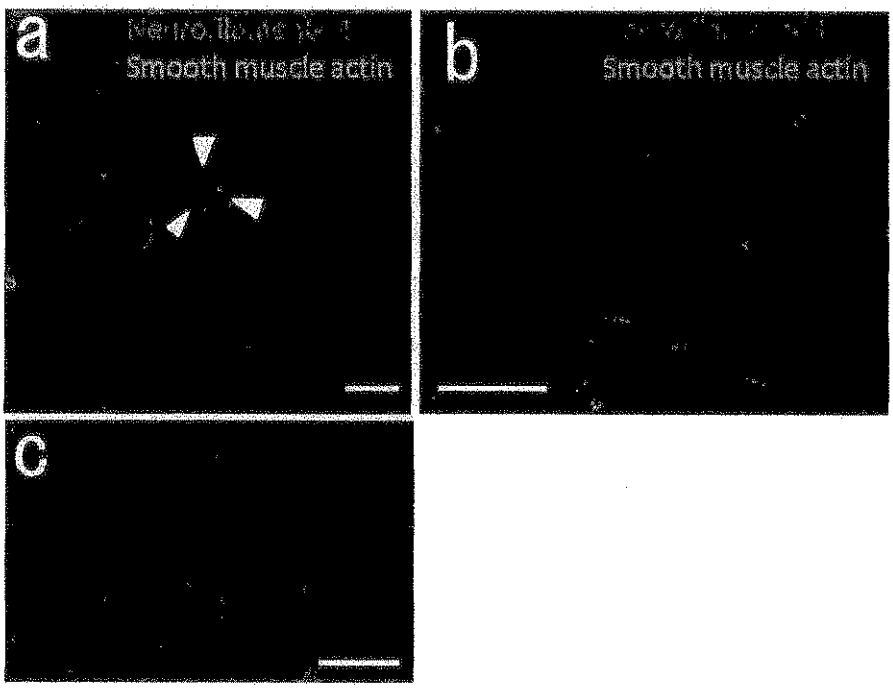


Fig. 9-2

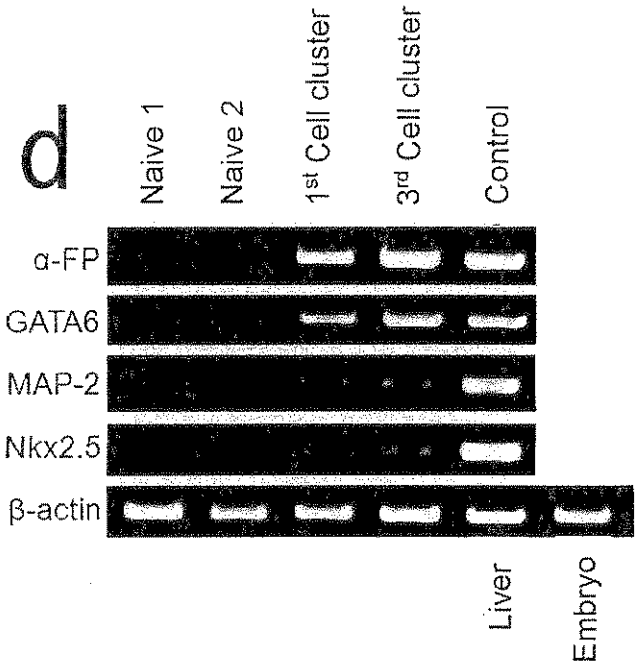

d
Naive 1
Naive 2
1st Cell cluster
3rd Cell cluster
Control
α-FP
GATA6
MAP-2
Nkx2.5
β-actin
Liver
Embryo

Fig. 9-3

M-Cluster : Muse cell-derived embryoid body-like cell cluster

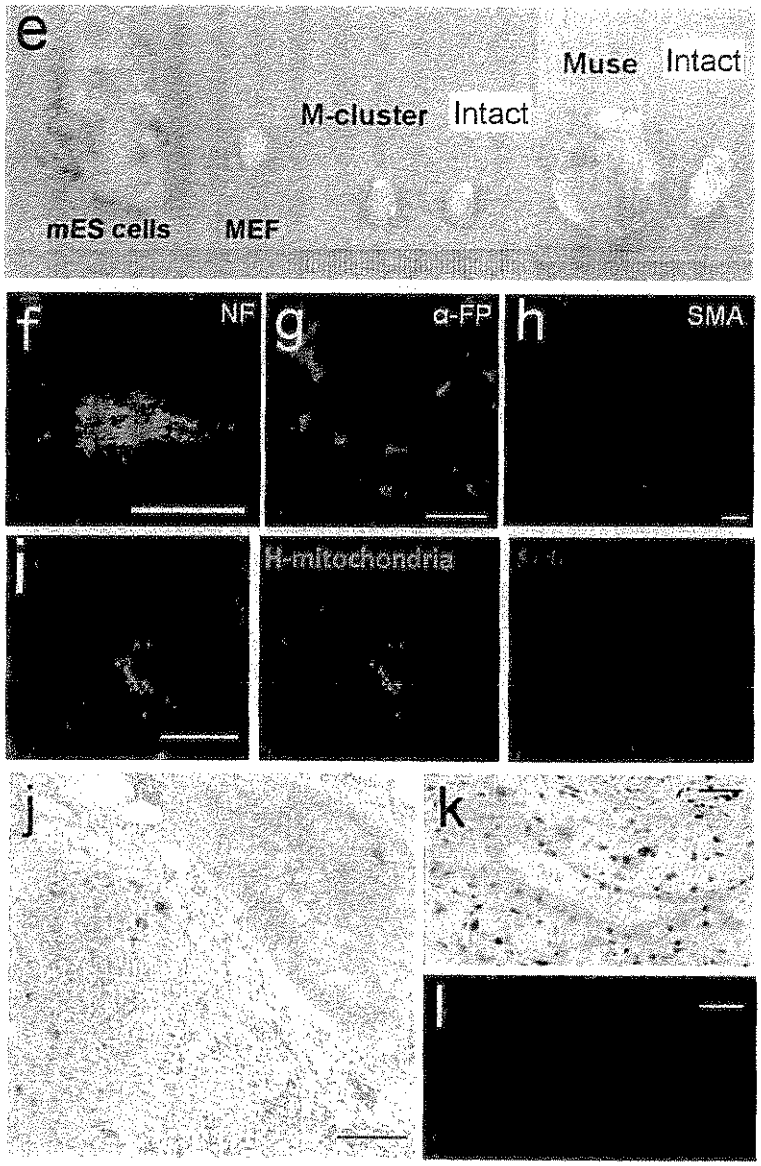


Fig. 10a

Symbol	Name	MUSE/Naive				Cell cluster/Naive			
		Fibro1	Fibro2	MSC-1	MSC-2	Fibro1	Fibro2	MSC-1	MSC-2
DAZL	Deleted in azoospermia-like			+		+	+		
DPPA2	Developmental pluripotency associated 2 (PESCRG1)	+	+	+	+	+			
DPPA4	Developmental pluripotency associated 4		−			+		+	
ERAS	ES cell expressed Ras	+	+	+	+	+	+	+	
GRB7	Growth factor receptor-bound protein 7	+	+	+		+			
HOXB1	Homeobox B1					+	+		
KLF4	Kruppel-like factor 4 (gut)	+	+	+					−
MYC	c-Myc	+	+	+	+				
PRDM1	PR domain containing 1, with ZNF domain					+	+	+	
SOX2	SRY (sex determining region Y)-box 2	+			+	+			+
SPAG9	Sperm associated antigen 9	+	+	+					
SPRY2	Sprouty homolog 2, antagonist of FGF signaling	+	+	+		+	+	+	+
TDGF1	Teratocarcinoma-derived growth factor 1	+	+	+	+	+	+		
ZFP42	Zinc finger protein 42 homolog (Rex1)		+	+	+				

\+ : − : No change

Fig. 10b

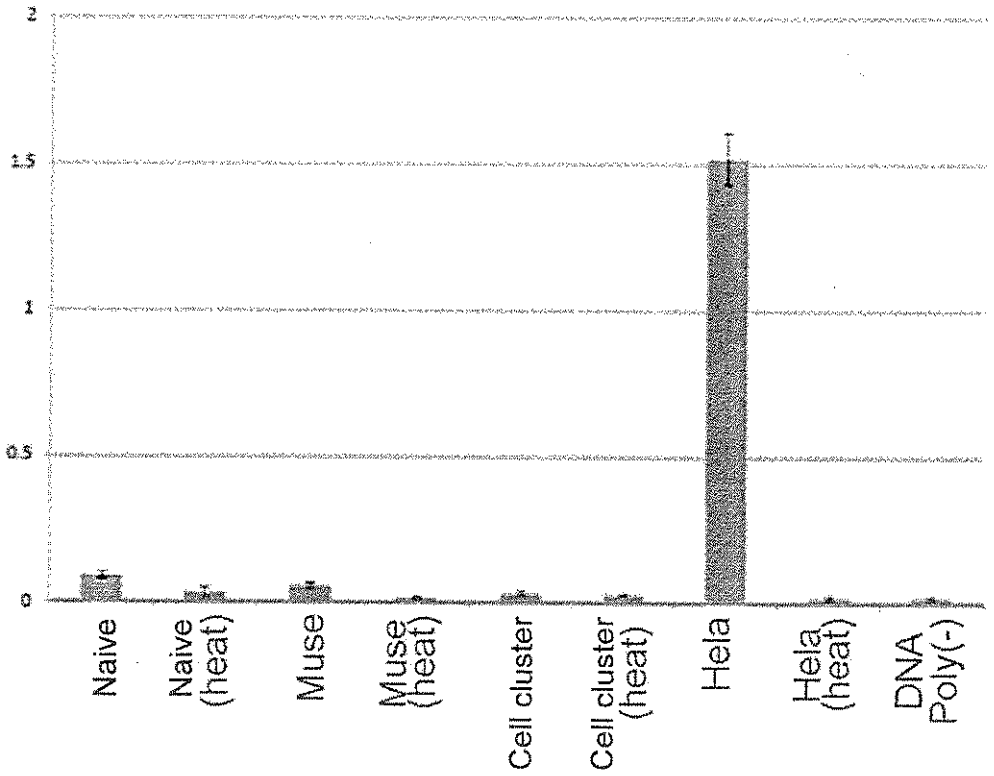

2
1.5
1
0.5
0
Naive
Naive (heat)
Muse
Muse (heat)
Cell cluster
Cell cluster (heat)
Hela
Hela (heat)
DNA Poly(-)

Fig. 11

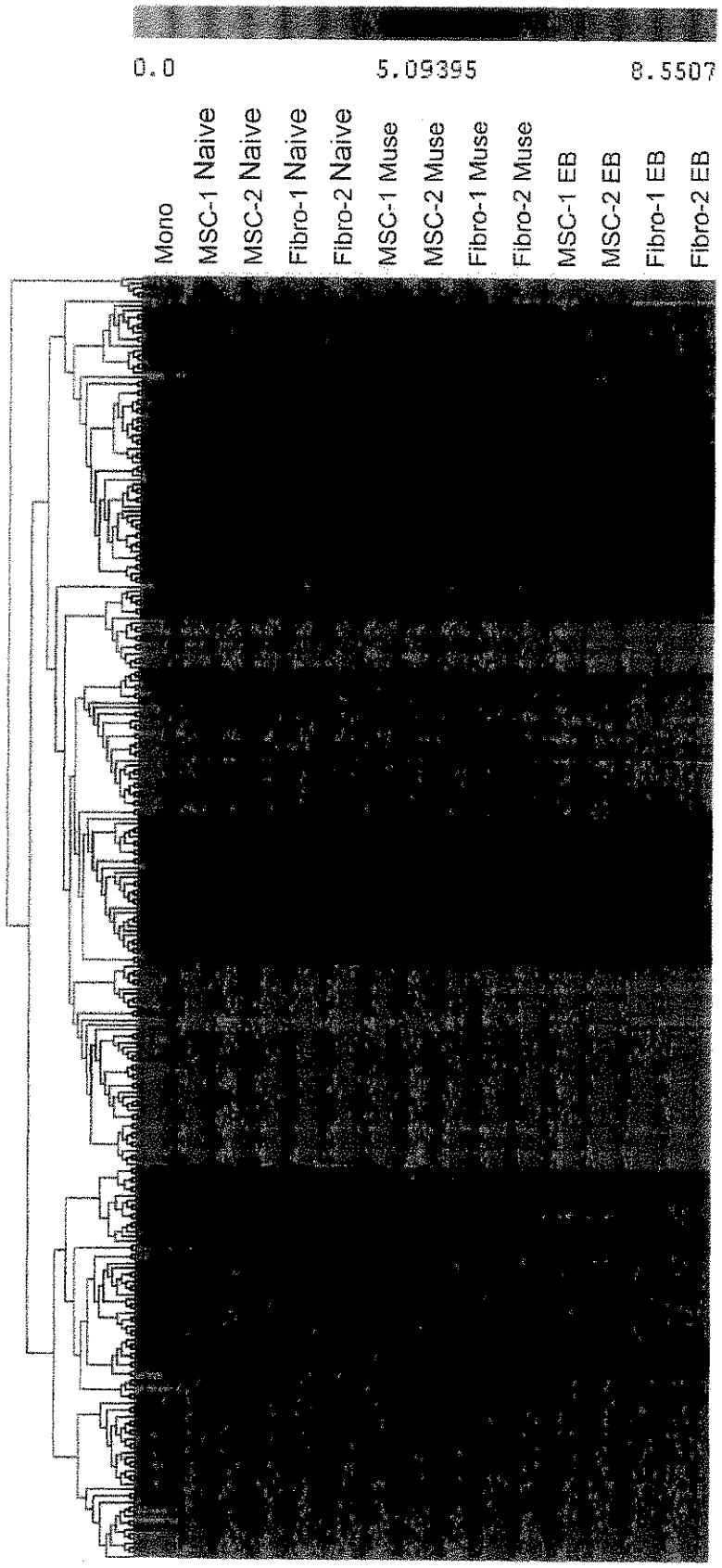

0.0
5.09395
8.5507
Mono
MSC-1 Naive
MSC-2 Naive
Fibro-1 Naive
Fibro-2 Naive
MSC-1 Muse
MSC-2 Muse
Fibro-1 Muse
Fibro-2 Muse
MSC-1 EB
MSC-2 EB
Fibro-1 EB
Fibro-2 EB

Fig. 12

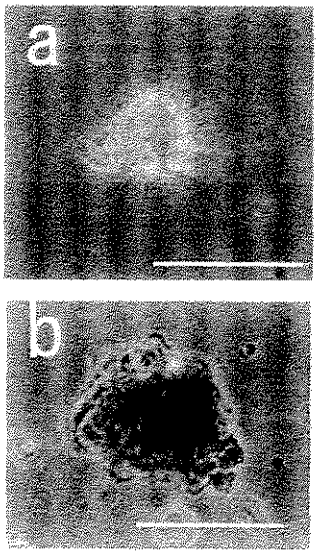


Fig. 13

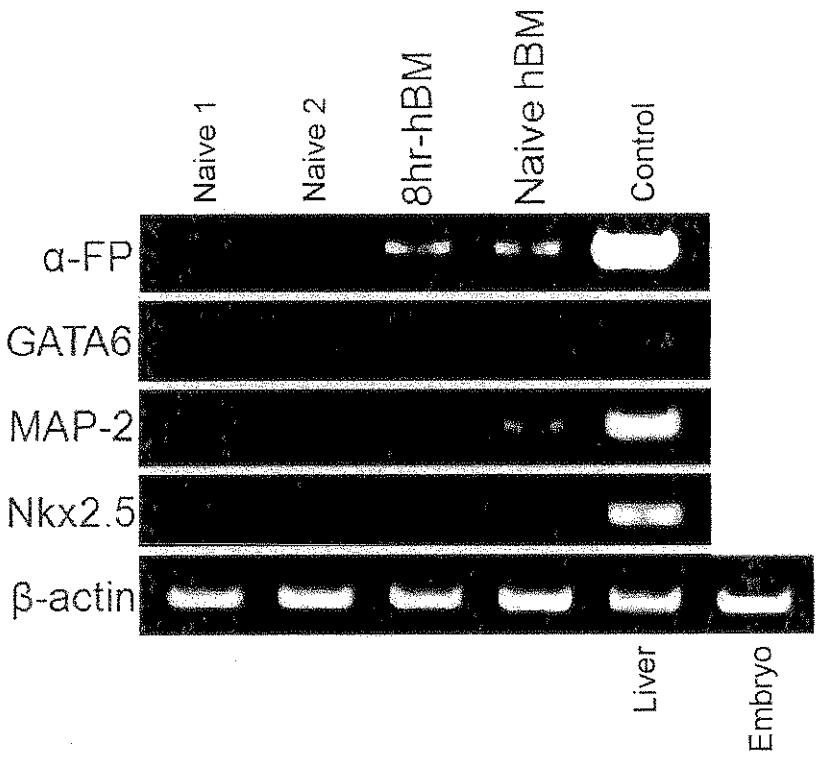

Naive 1
Naive 2
8hr-hBM
Naive hBM
Control
α-FP
GATA6
MAP-2
Nkx2.5
β-actin
Liver
Embryo

Fig. 14

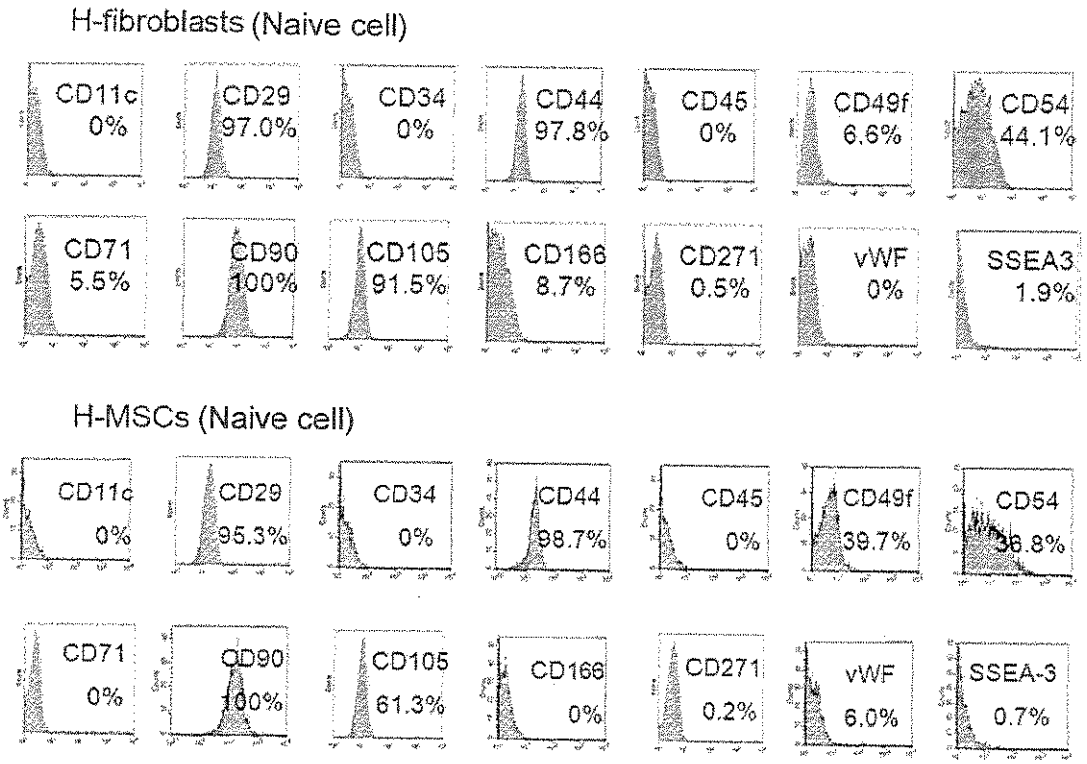

H-fibroblasts (Naive cell)
CD11c 0%
CD29 97.0%
CD34 0%
CD44 97.8%
CD45 0%
CD49f 6.6%
CD54 44.1%
CD71 5.5%
CD90 100%
CD105 91.5%
CD166 8.7%
CD271 0.5%
vWF 0%
SSEA3 1.9%
H-MSCs (Naive cell)
CD11c 0%
CD29 95.3%
CD34 0%
CD44 98.7%
CD45 0%
CD49f 39.7%
CD54 36.8%
CD71 0%
CD90 100%
CD105 61.3%
CD166 0%
CD271 0.2%
vWF 6.0%
SSEA-3 0.7%

Fig. 15-1

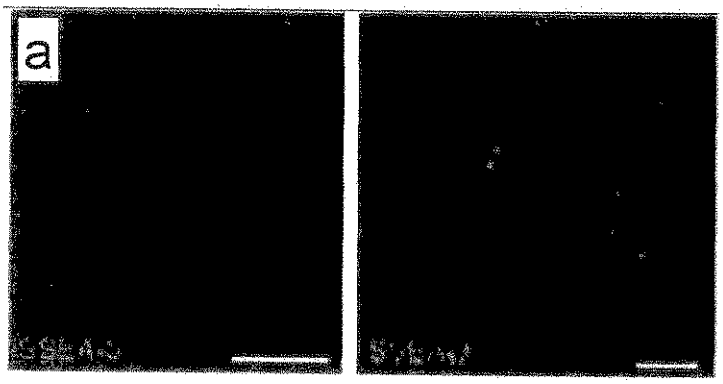


Fig. 15-2

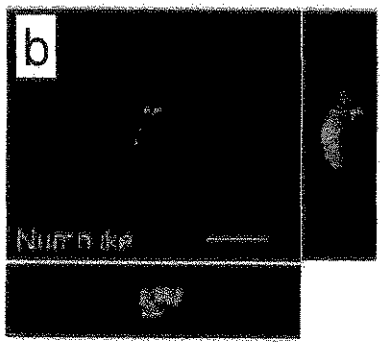


Fig. 15-3

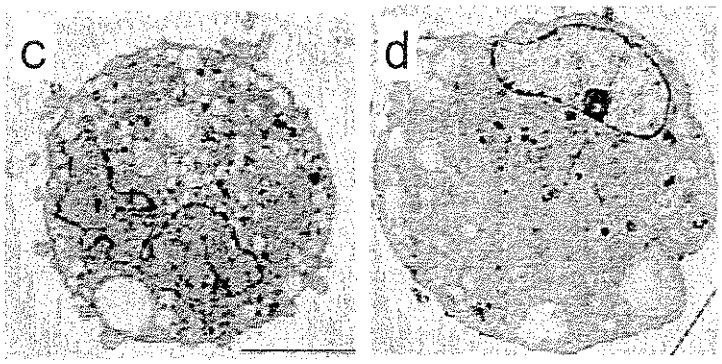


Fig. 15-4

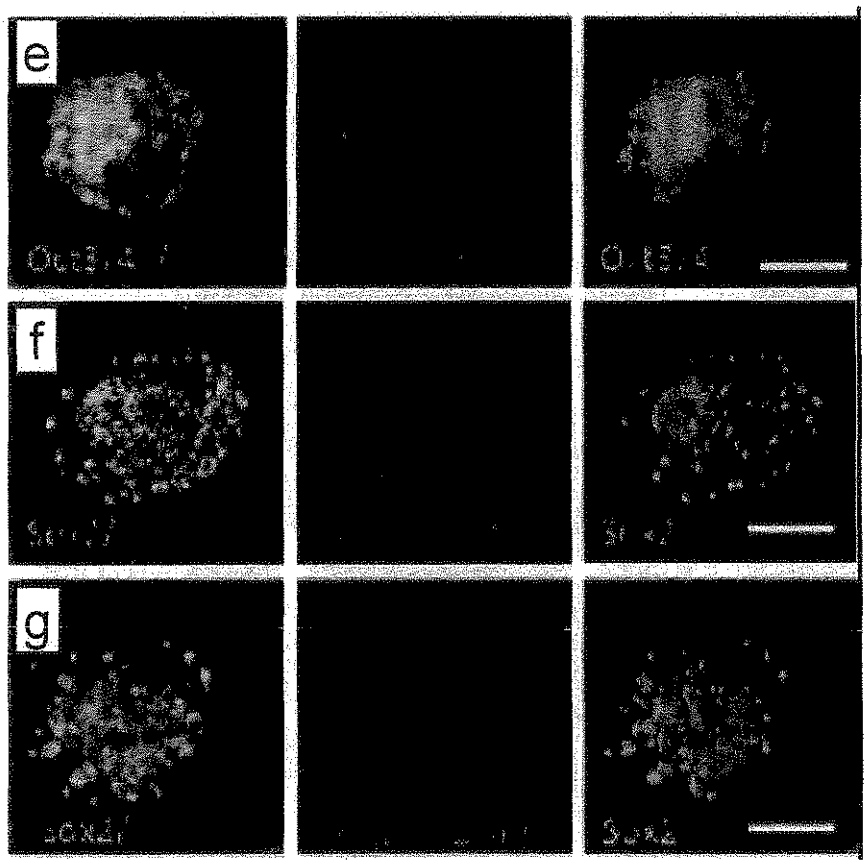


Fig. 16-1

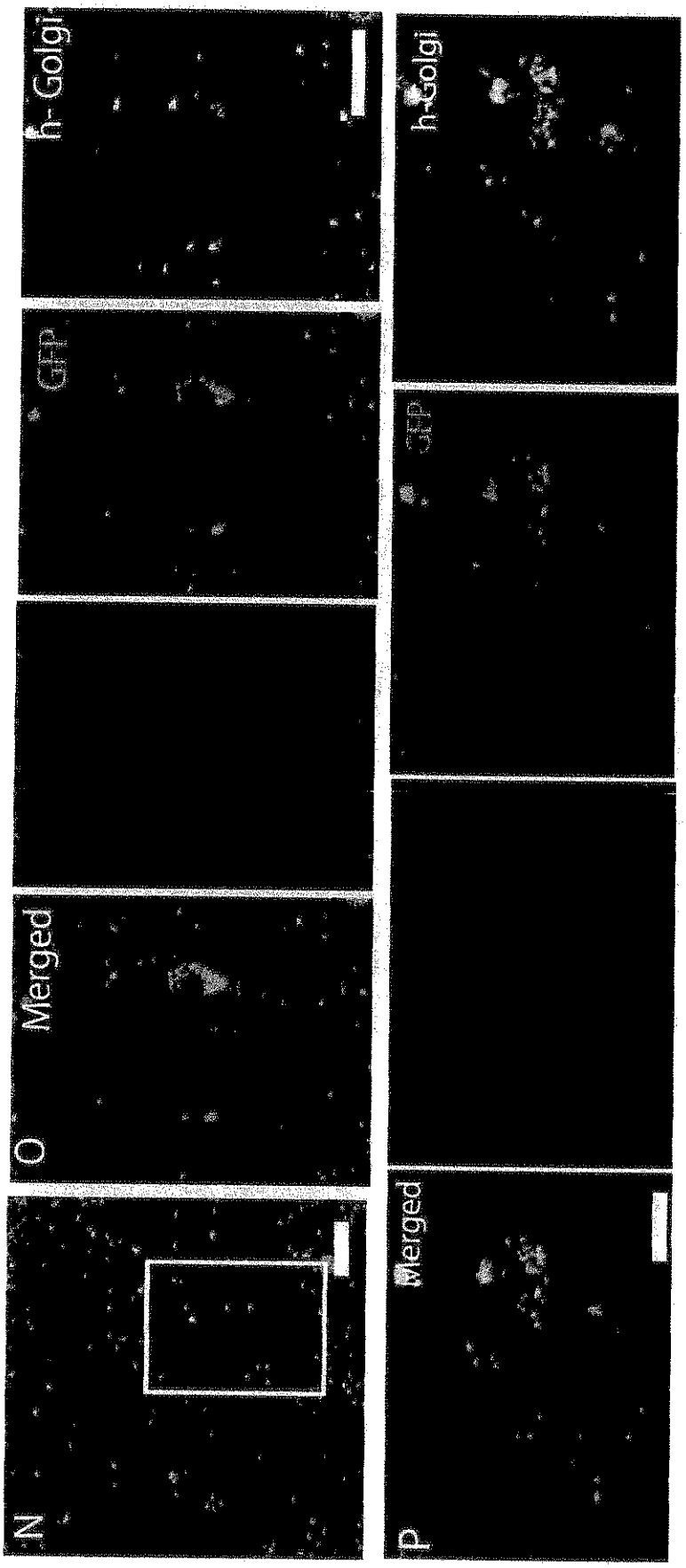


Fig. 16-2

Q

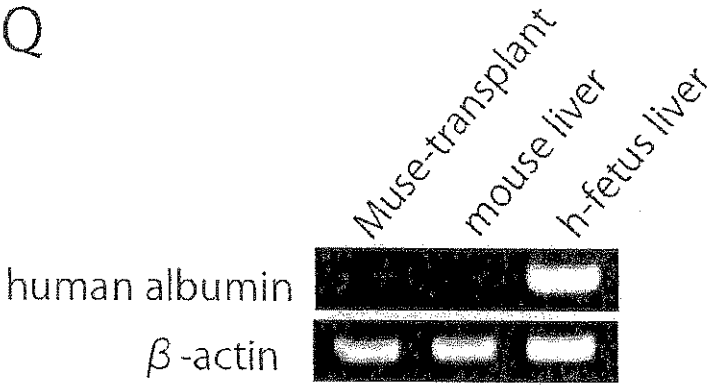

Muse-transplant
mouse liver
h-fetus liver
human albumin
β-actin

Fig. 16-3

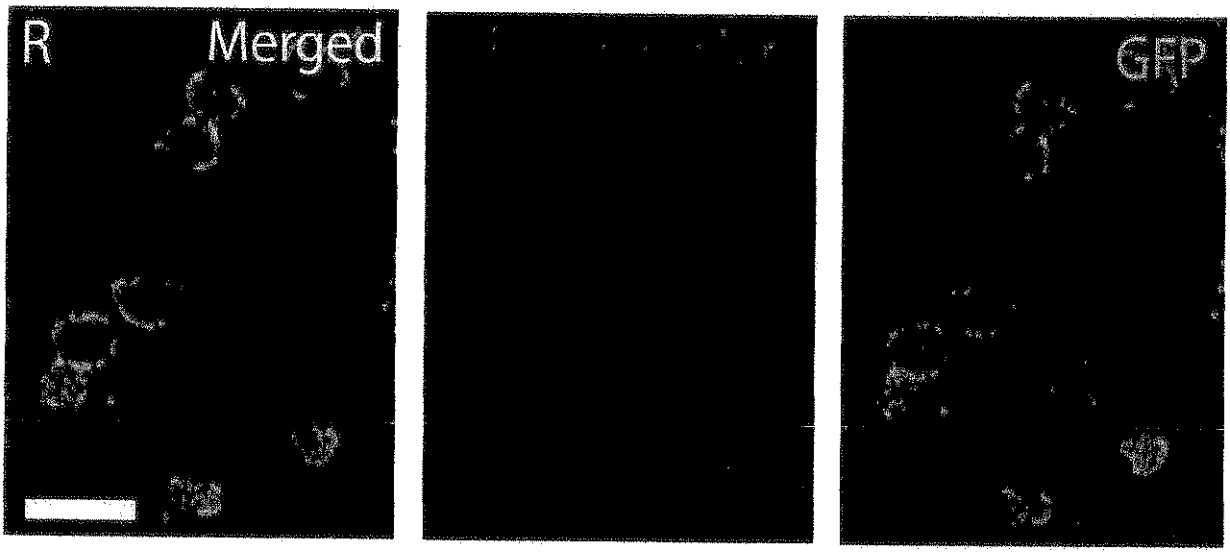


Fig. 17-1

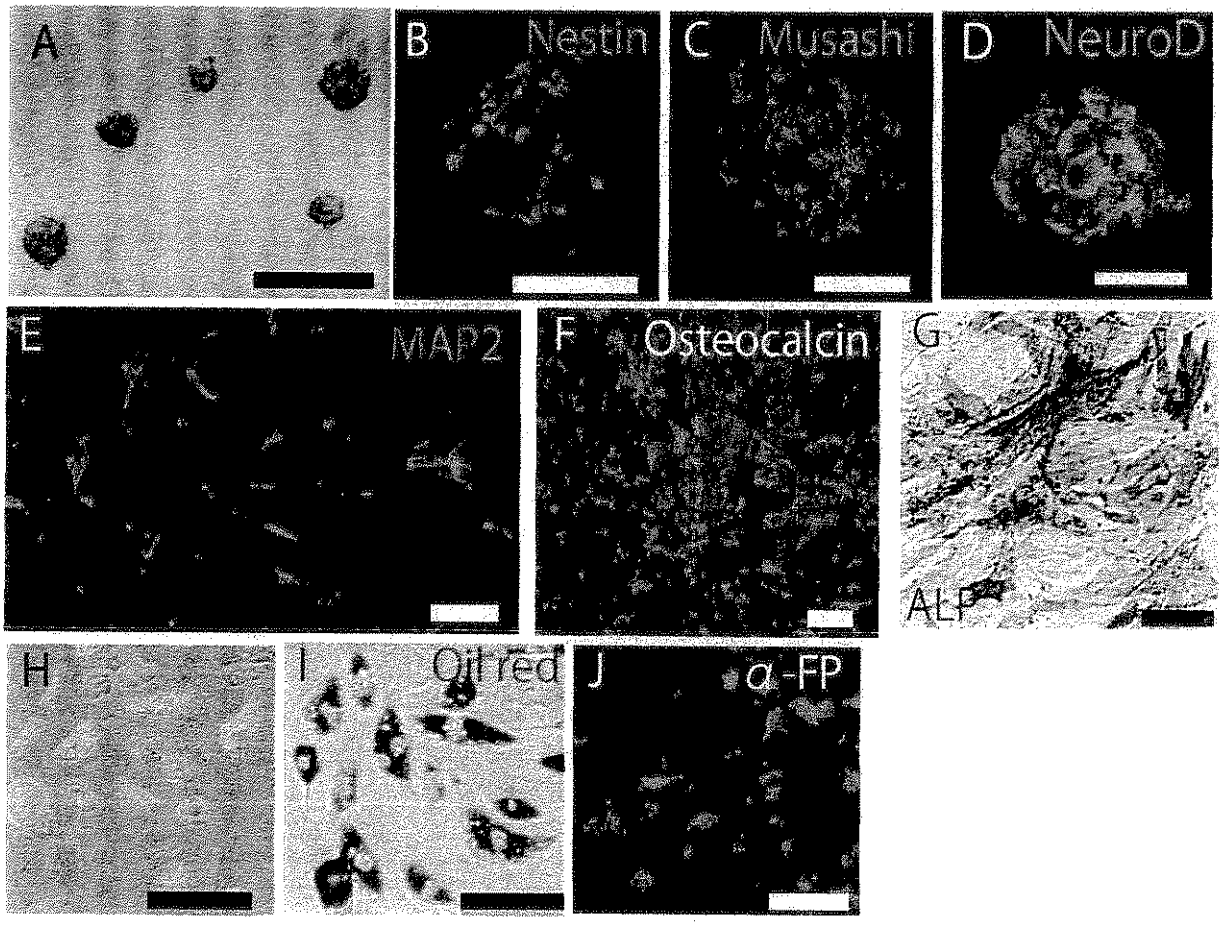

A
B Nestin
C Musashi
D NeuroD
E MAP2
F Osteocalcin
G ALP
H
I Oil red
J α-FP

Fig. 17-2

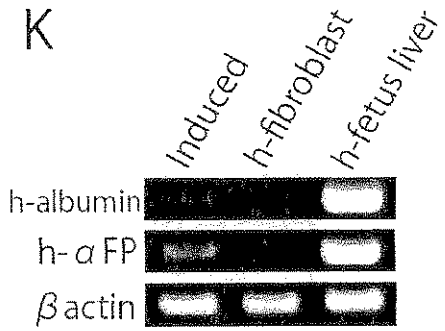


Fig. 18-1

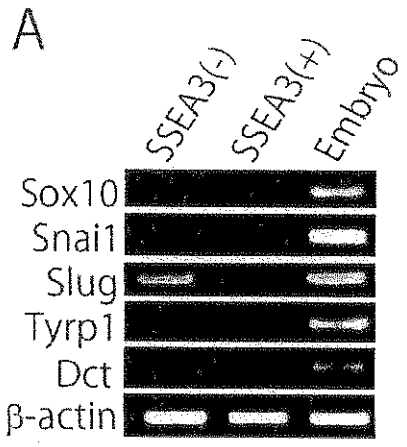


Fig. 18-2

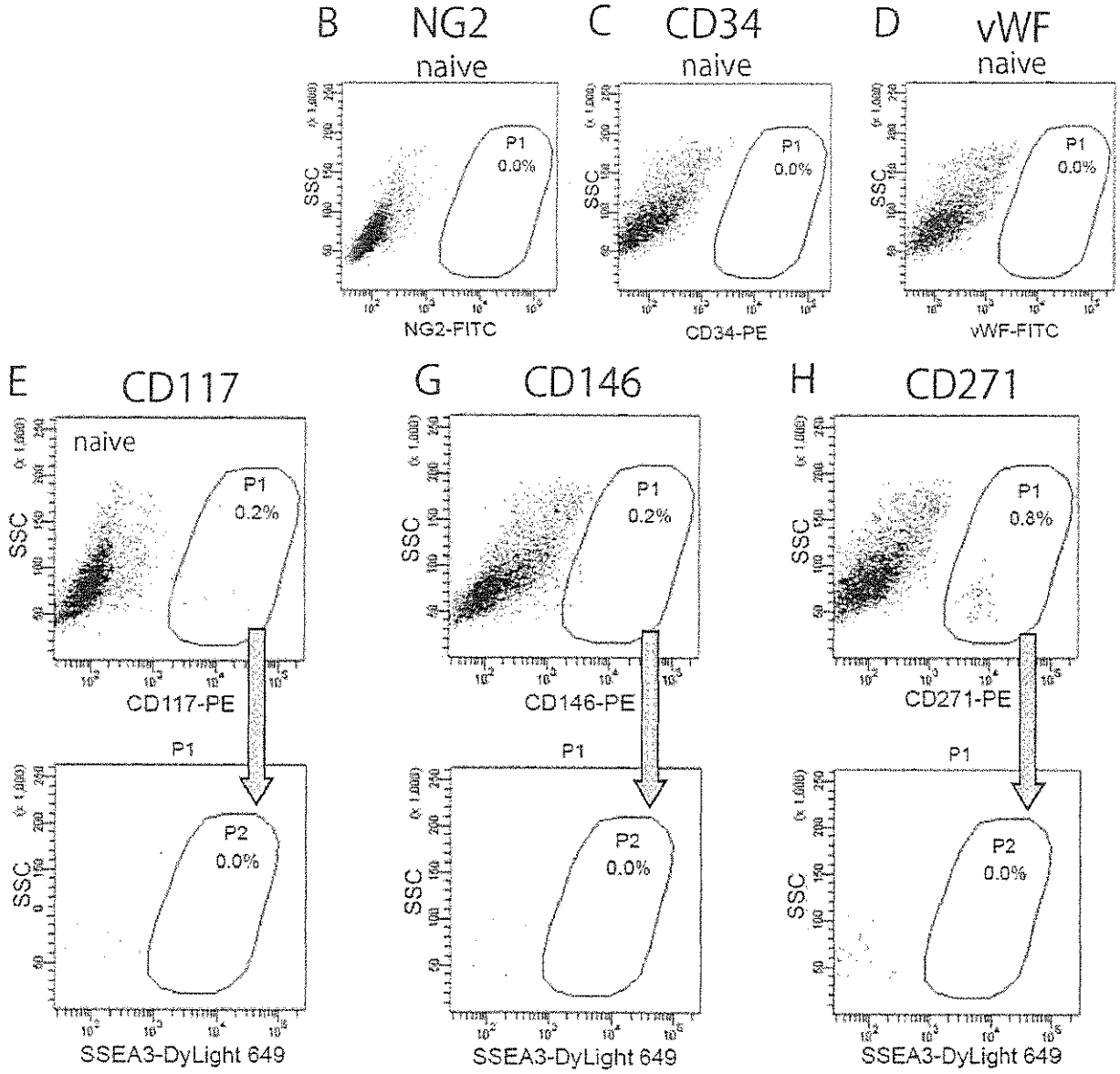

B
NG2
naive
P1
0.0%
SSC
NG2-FITC
C
CD34
naive
P1
0.0%
SSC
CD34-PE
D
vWF
naive
P1
0.0%
SSC
vWF-FITC
E
CD117
naive
P1
0.2%
SSC
CD117-PE
P1
P2
0.0%
SSC
SSEA3-DyLight 649
G
CD146
P1
0.2%
SSC
CD146-PE
P1
P2
0.0%
SSC
SSEA3-DyLight 649
H
CD271
P1
0.8%
SSC
CD271-PE
P1
P2
0.0%
SSC
SSEA3-DyLight 649

Fig. 18-3

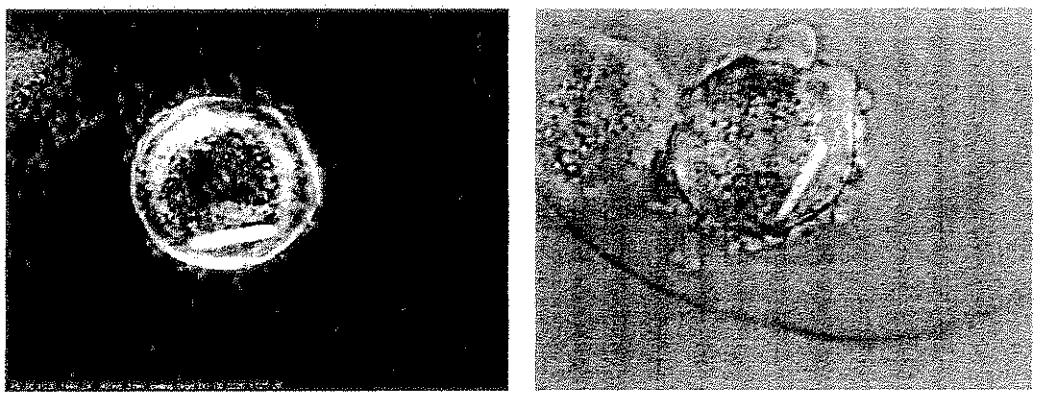


Fig. 19

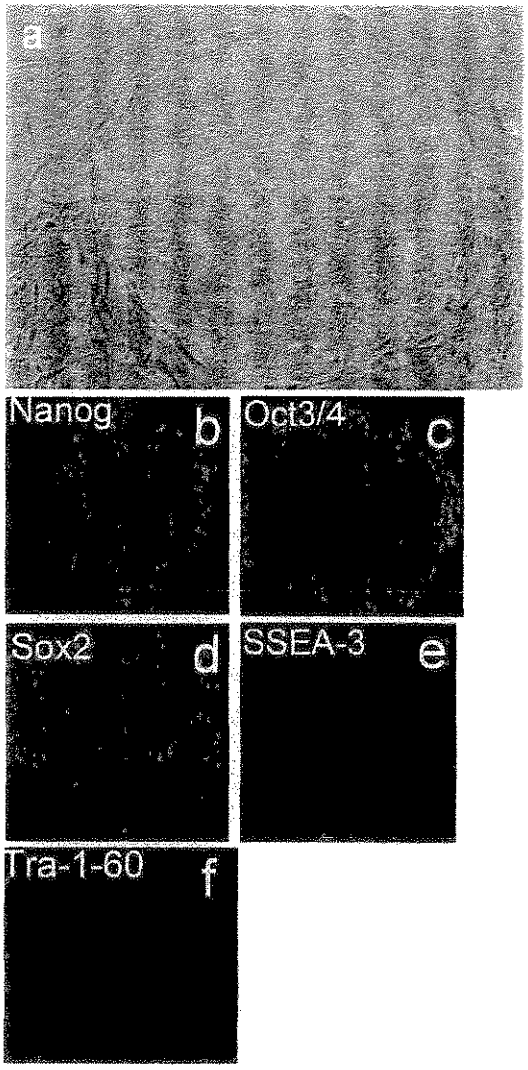


Fig. 20

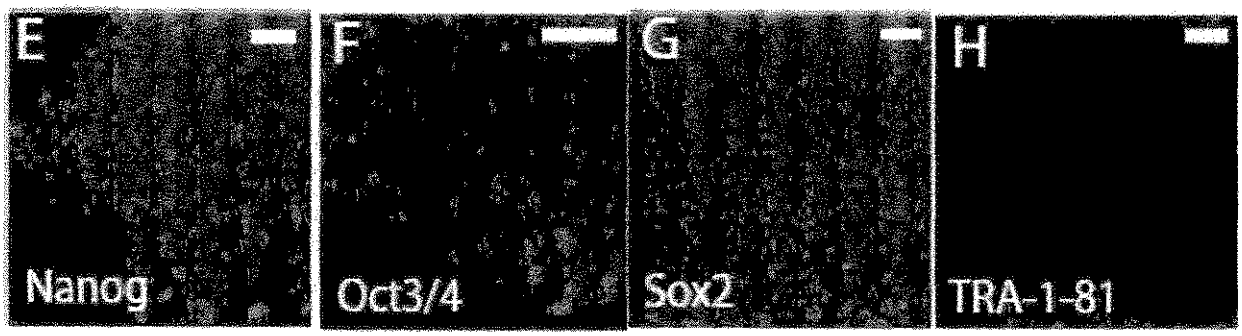


Fig. 21

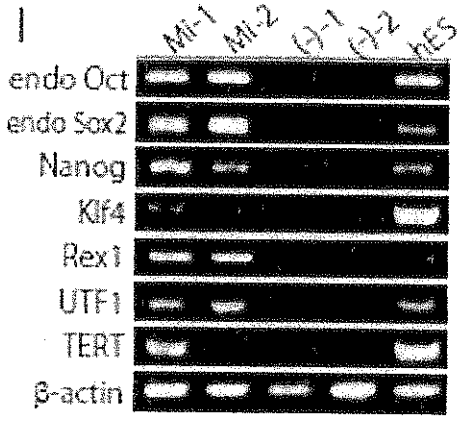

I
MI-1
MI-2
(-)-1
(-)-2
hES
endo Oct
endo Sox2
Nanog
Klf4
Rex1
UTF1
TERT
β-actin

Fig. 22-1

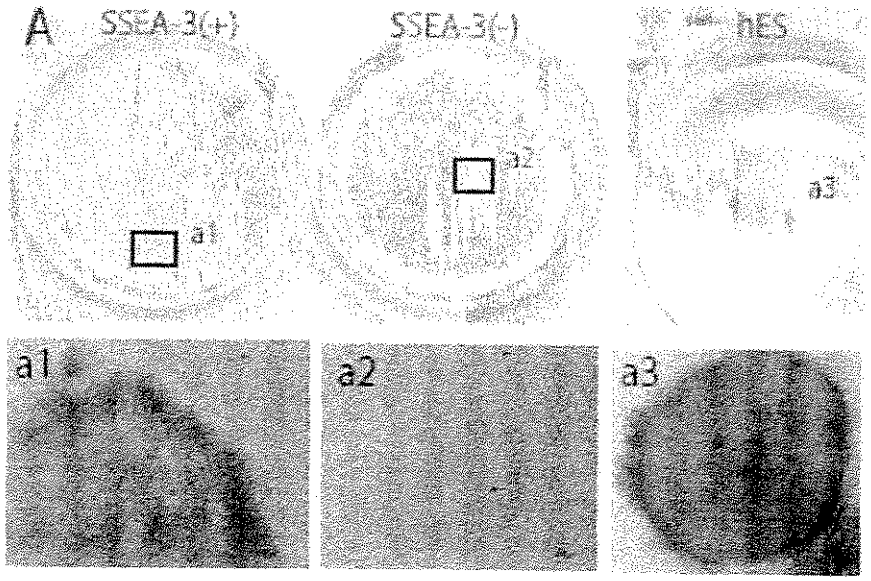


Fig. 22-2


B
SSEA3(+)
SSEA3(-)
hES
endo Oct
endo Sox2
Nanog
endo Klf4
Rex1
UTF1
β-actin

Fig. 22-3

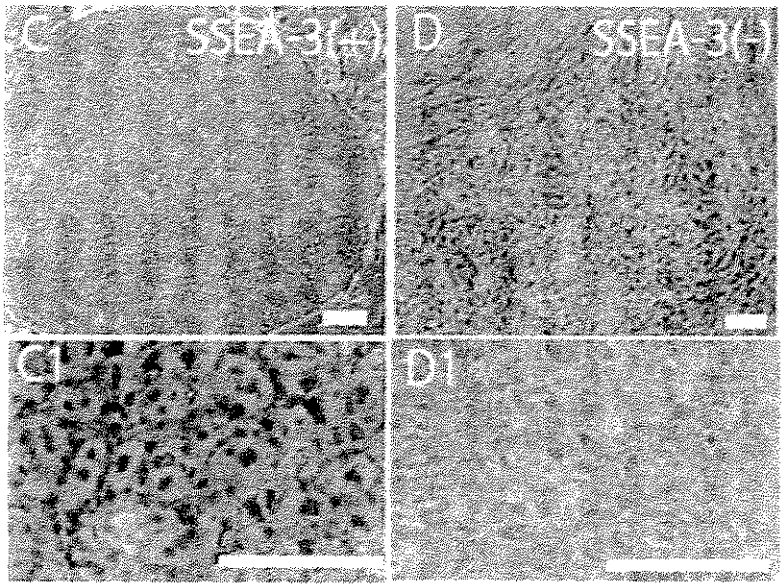


Fig. 23-1

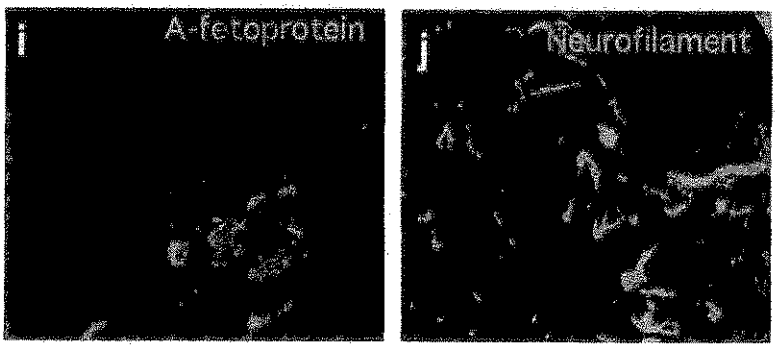


Fig. 23-2

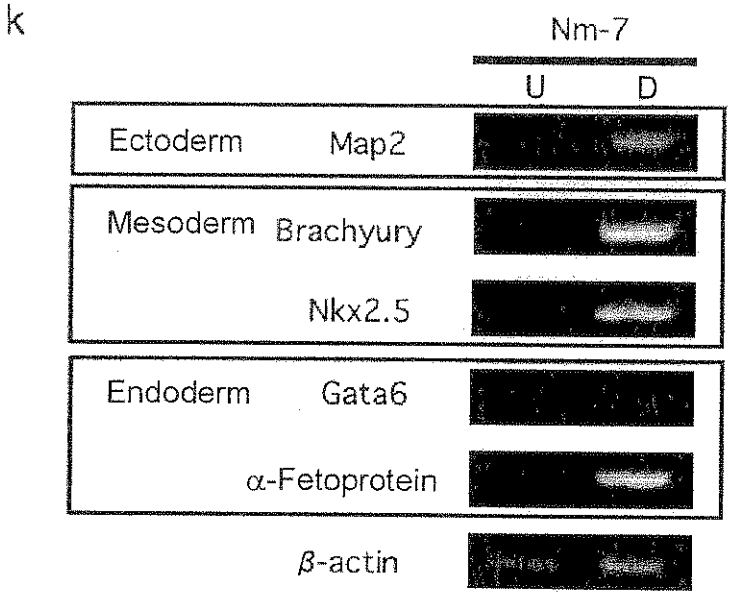

k
Nm-7
U
D
Ectoderm
Map2
Mesoderm
Brachyury
Nkx2.5
Endoderm
Gata6
α-Fetoprotein
β-actin

Fig. 23-3

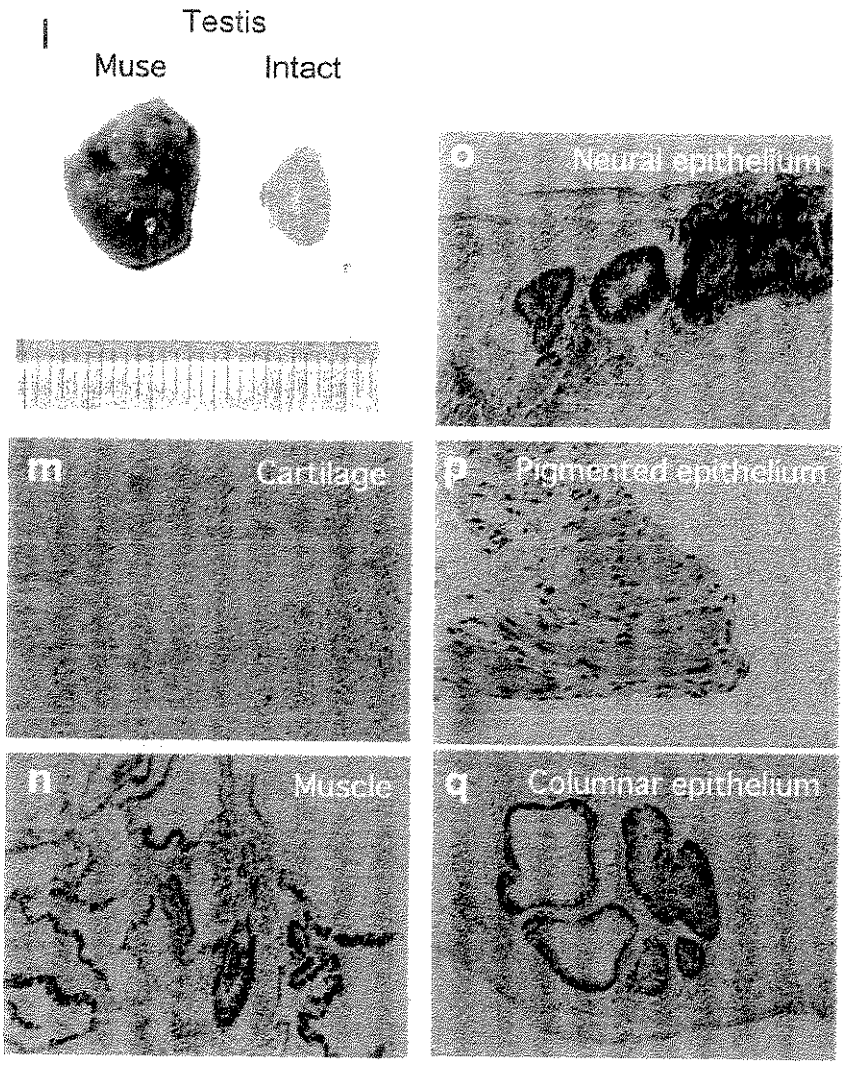

l
Testis
Muse
Intact
o
Neural epithelium
m
Cartilage
p
Pigmented epithelium
n
Muscle
q
Columnar epithelium

Fig. 24

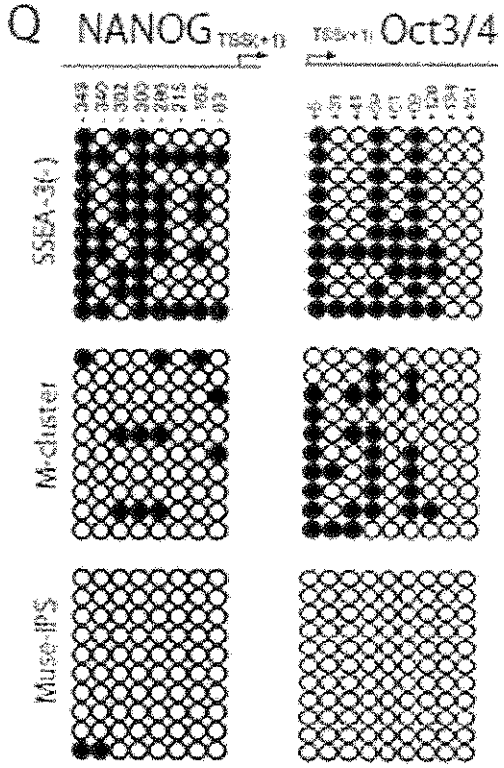


Fig. 25

		/Naive		/iPS
Symbol	Description	Cluster	iPS	Cluster
ABL1	C-abl oncogene 1, receptor tyrosine kinase			1.2E+00
ANAPC2	Anaphase promoting complex subunit 2			8.5E-01
ANAPC4	Anaphase promoting complex subunit 4			5.0E-01
ATM	Ataxia telangiectasia mutated			1.7E+00
ATR	Ataxia telangiectasia and Rad3 related			9.5E-01
BRCA1	Breast cancer 1, early onset			1.3E-01*
CCNB1	Cyclin B1			5.9E-02*
CCNB2	Cyclin B2			6.6E-02*
CCNC	Cyclin C			2.2E-01*
CCND1	Cyclin D1			3.7E+00**
CCND2	Cyclin D2			3.7E-02*
CCNE1	Cyclin E1			1.0E-01*
CCNF	Cyclin F			1.1E-01*
CCNG1	Cyclin G1			4.1E-01
CCNG2	Cyclin G2			1.1E+01**
CCNH	Cyclin H			6.6E-01
CCNT1	Cyclin T1			5.8E-01
CCNT2	Cyclin T2			4.0E-01
CDC16	Cell division cycle 16 homolog (S. cerevisiae)			3.3E-01*
CDC2	Cell division cycle 2, G1 to S and G2 to M			6.4E-02*
CDC20	Cell division cycle 20 homolog (S. cerevisiae)			3.3E-02*
CDC34	Cell division cycle 34 homolog (S. cerevisiae)			4.7E-01
CDK2	Cyclin-dependent kinase 2			3.0E-01*
CDK4	Cyclin-dependent kinase 4			6.4E-01
CDK5R1	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)			2.2E-01*
CDK5RAP1	CDK5 regulatory subunit associated protein 1			3.2E-01*
CDK6	Cyclin-dependent kinase 6			5.7E-01
CDK7	Cyclin-dependent kinase 7			3.5E-01
CDK8	Cyclin-dependent kinase 8			4.7E-01
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)			8.3E+01**
CDKN1B	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)			8.2E-01
CDKN2A	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)			3.7E+02**
CDKN2B	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)			7.8E+01**
CDKN3	Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase)			1.3E-01*
CHEK1	CHK1 checkpoint homolog (S. pombe)			1.7E-01*
CHEK2	CHK2 checkpoint homolog (S. pombe)			7.7E-02*
CUL1	Cullin 1			4.2E-01
E2F4	E2F transcription factor 4, p107/p130-binding			4.8E-01
GADD45A	Growth arrest and DNA-damage-inducible, alpha			5.1E+00**
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)			4.4E-02*
MAD2L2	MAD2 mitotic arrest deficient-like 2 (yeast)			8.2E-02*
MCM2	Minichromosome maintenance complex component 2			5.6E-02*
MCM3	Minichromosome maintenance complex component 3			5.7E-02*
MCM4	Minichromosome maintenance complex component 4			2.2E-01*
MCM5	Minichromosome maintenance complex component 5			3.3E-02*
MNAT1	Menage a trois homolog 1, cyclin H assembly factor (Xenopus laevis)			6.8E-01
MRE11A	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)			3.7E-01
PCNA	Proliferating cell nuclear antigen			2.0E-01*
RAD51	RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae)			7.0E-02*
RB1	Retinoblastoma 1 (including osteosarcoma)			4.0E+00**
RBBP8	Retinoblastoma binding protein 8			1.8E-01*
RBL1	Retinoblastoma-like 1 (p107)			2.3E-01*
RBL2	Retinoblastoma-like 2 (p130)			5.5E+00**
RPA3	Replication protein A3, 14kDa			1.3E-01*
SKP2	S-phase kinase-associated protein 2 (p45)			1.2E-01*
TERT	Telomerase reverse transcriptase			0.0E+00*
TFDP1	Transcription factor Dp-1			8.4E-01
TFDP2	Transcription factor Dp-2 (E2F dimerization partner 2)			6.6E-01
TP53	Tumor protein p53			3.3E-01*

Fig. 26

		/Naive		/iPS
Symbol	Description	Cluster	iPS	Cluster
ABCA13	ATP-binding cassette, sub-family A (ABC1), member 13			4.1E-01
ALPL	Alkaline phosphatase, liver/bone/kidney			2.9E-03*
ATRX	Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae)			9.9E-01
BMP4	Bone morphogenetic protein 4			1.4E-01*
BMPR1A	Bone morphogenetic protein receptor, type IA			6.6E-01
CBX7	Chromobox homolog 7			5.5E+00**
CTR9	Ctr9, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)			6.2E-01
DAZL	Deleted in azoospermia-like			5.8E-02*
DDX4	DEAD (Asp-Glu-Ala-Asp) box polypeptide 4			5.0E-01
DNMT1	DNA (cytosine-5-)-methyltransferase 1			2.1E-01*
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta			6.2E-04*
DPPA2	Developmental pluripotency associated 2			1.6E-03*
DPPA3	Developmental pluripotency associated 3			1.1E-02*
DPPA4	Developmental pluripotency associated 4			1.1E-04*
EPC1	Enhancer of polycomb homolog 1 (Drosophila)			8.2E-01
ERAS	ES cell expressed Ras			1.1E-01*
F11R	F11 receptor			2.0E-03*
FGFR1	Fibroblast growth factor receptor 1 (fms-related tyrosine kinase 2, Pfeiffer syndrome)			5.8E-01
FOXD3	Forkhead box D3			4.2E-04*
GDF3	Growth differentiation factor 3			0.0E+00*
GRB7	Growth factor receptor-bound protein 7			1.8E-03*
HAND1	Heart and neural crest derivatives expressed 1			1.9E-02*
HES1	Hairy and enhancer of split 1, (Drosophila)			1.4E+00
HEXIM1	Hexamethylene bis-acetamide inducible 1			2.8E+00
HOXB1	Homeobox B1			4.7E-02*
ID1	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein			3.3E+00**
ID3	Inhibitor of DNA binding 3, dominant negative helix-loop-helix protein			3.0E+00**
IFITM1	Interferon induced transmembrane protein 1 (9-27)			1.7E-01*
KCNK3	Potassium channel, subfamily K, member 3			3.4E+00**
KITLG	KIT ligand			1.2E+01**
KLF4	Kruppel-like factor 4 (gut)			1.0E+00
LIN28	Lin-28 homolog (C. elegans)			3.5E-04*
MSX2	Msh homeobox 2			3.8E-01
MYC	V-myc myelocytomatosis viral oncogene homolog (avian)			7.6E-01
NANOG	Nanog homeobox			4.0E-04*
NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)			9.4E-01
NKX1-2	NK1 homeobox 2			2.3E-03*
NR0B1	Nuclear receptor subfamily 0, group B, member 1			2.4E+01**
OCT3/4	POU class 5 homeobox 1			1.8E-02*
OTX2	Orthodenticle homeobox 2			0.0E+00*
PAN3	PAN3 polyA specific ribonuclease subunit homolog (S. cerevisiae)			4.5E-01
PODXL	Podocalyxin-like			3.8E-02*
PRDM1	PR domain containing 1, with ZNF domain			1.0E+01**
RAG1AP1	Recombination activating gene 1 activating protein 1			8.6E-01
SALL4	Sal-like 4 (Drosophila)			6.4E-04*
SIX4	SIX homeobox 4			9.9E-01
SOX2	SRY (sex determining region Y)-box 2			7.4E-04*
SPAG9	Sperm associated antigen 9			1.3E+00
SPRY1	Sprouty homolog 1, antagonist of FGF signaling (Drosophila)			5.1E-01
SPRY2	Sprouty homolog 2 (Drosophila)			1.9E+00
SSBP2	Single-stranded DNA binding protein 2			1.7E+00
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)			1.6E+00
TDGF1	Teratocarcinoma-derived growth factor 1			3.8E-05*
TFE3	Transcription factor binding to IGHM enhancer 3			1.1E+00
TRDMT1	TRNA aspartic acid methyltransferase 1			7.3E-01
UTF1	Undifferentiated embryonic cell transcription factor 1			0.0E+00*
VIM	Vimentin			1.4E+02**

Fig. 27

Animal	Reprogramming factor [b]	Cell type	Time (week)	Efficiency
Mouse	**Viral-integrating vectors**			
	O, K, S, M	MEFs	2-3	0.01-0.50%
	O, K, S, M	Hepatocyte	2-3	1-3%
	O, K, S, M	Gastric epithelial cell	2-3	1-3%
	O, K, S, M	B cell	2-3	0.01-0.10%
	O, K, S, M	β cell	3-4	0.1-0.2%
	O, K, S, M	Neural stem cell	1-2	3-5%
	O, K, S	MEFs	3-4	0.001-0.010%
	O, K, S	Hepatocyte	2-3	0.5-1.0%
	O, K, M	Neural stem cell	1-2	0.1-1.0%
	O, K,	Neural stem cell	2-3	0.1-1.0%
	O, M, S	Neural stem cell	3-4	0.1-1.0%
	O, K	Neural stem cell	2-3	0.1-0.2%
	O, M	Neural stem cell	3-4	0.1-0.2%
	Non-integrating viral vectors [c]			
	O, K, S, M	Hepatocyte	4-5	0.0001 to 0.0010%
	Plasmid-based vedtros [c]			
	O, K, S, M	MEFs	3-4	0.0001 to 0.0010%
Human	**Viral-integrating vectors**			
	O, K, S, M	Dermal fibroblast	4-5	0.001-0.002%
	O, K, L, N	Dermal fibroblast	2-3	N.D.
	O, K, S, M	Bone marrow stromal cell	2-3	N.D.
	O, K, N	Dermal fibroblast	2-3	N.D.
	O, K, L	Dermal fibroblast	2-3	N.D.
	O, S, K [d]	Dermal fibroblast	4	0.1-1.0%
	O, S [d]	Dermal fibroblast	4	0.001-0.010%
	O, S, L, N [e]	Dermal fibroblast		0.002%
	O, K, S, M, T,LT	Dermal fibroblast		0.0417%
	O, K, S, M, p, U	Dermal fibroblast		N.D.

MEFs, Mouse embryonic fibroblast ; N.D. , not determined.

[b] O, Oct4; S, Sox2; M, c-Myc; K, Klf4; N, Nanog; L, Lin28; T, hTERT; LT, Large T antigen; p, p53RNAi; U, Utf1
[c] Integration-free clones.
[d] Valpronic acid (VPA) used in association
[e] CHIR99021, PD0325901, A-83-01