

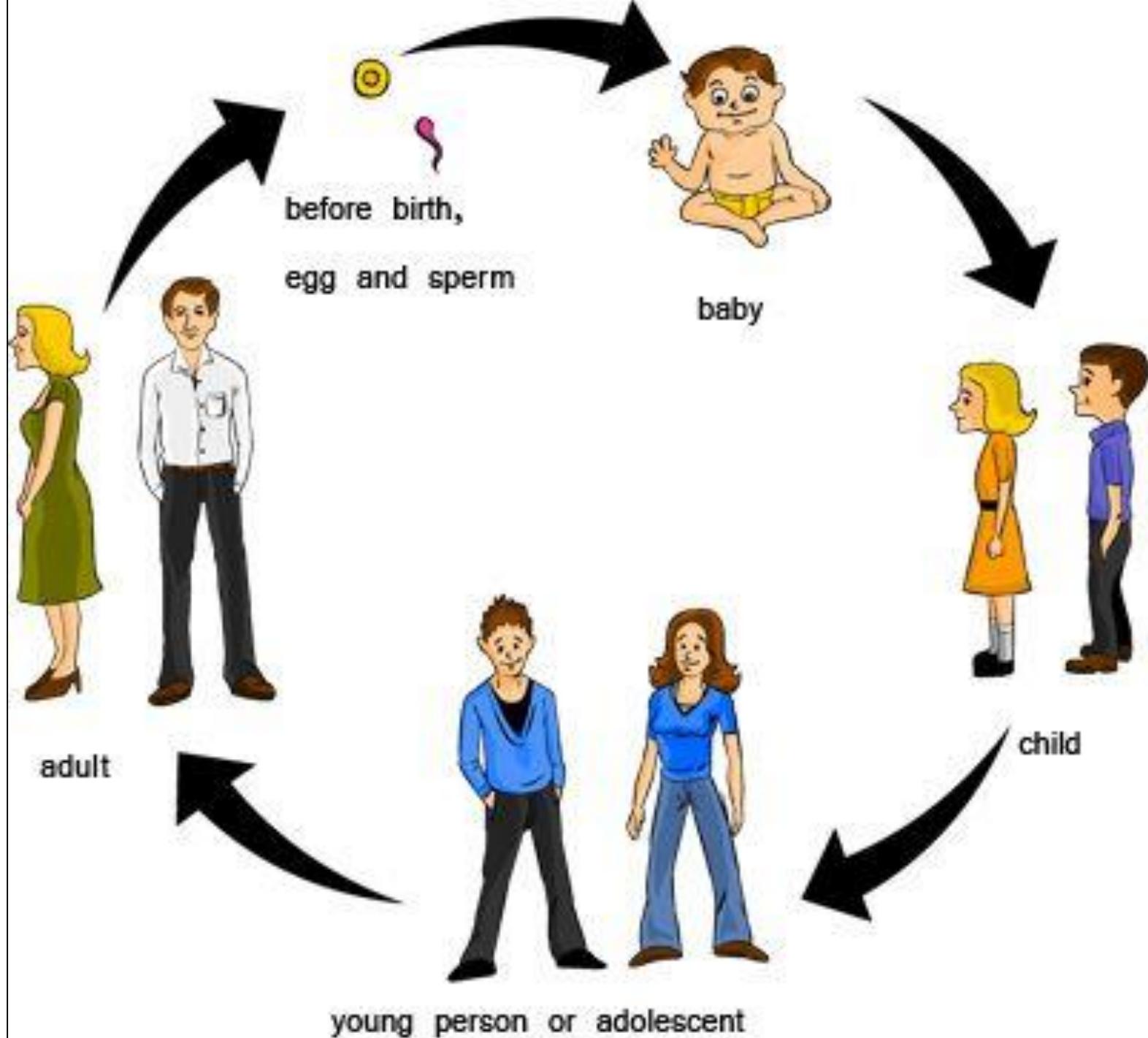
Lecture 5-11

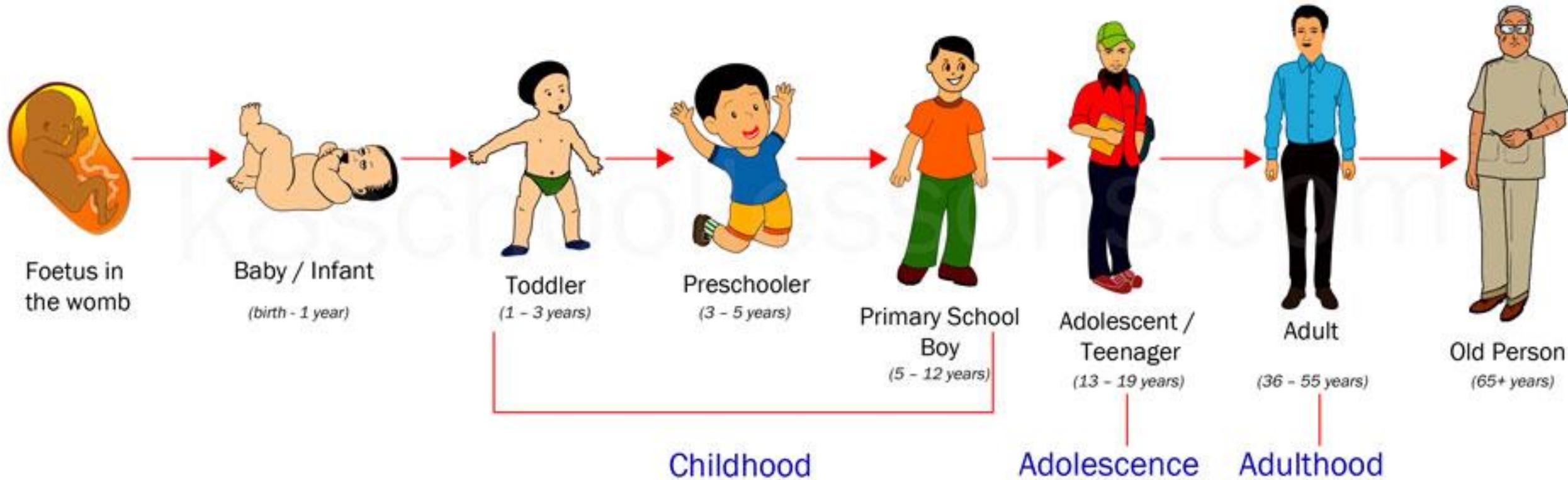
BT 632

Stem Cells, Cancer and Therapy (3-0-0-6)

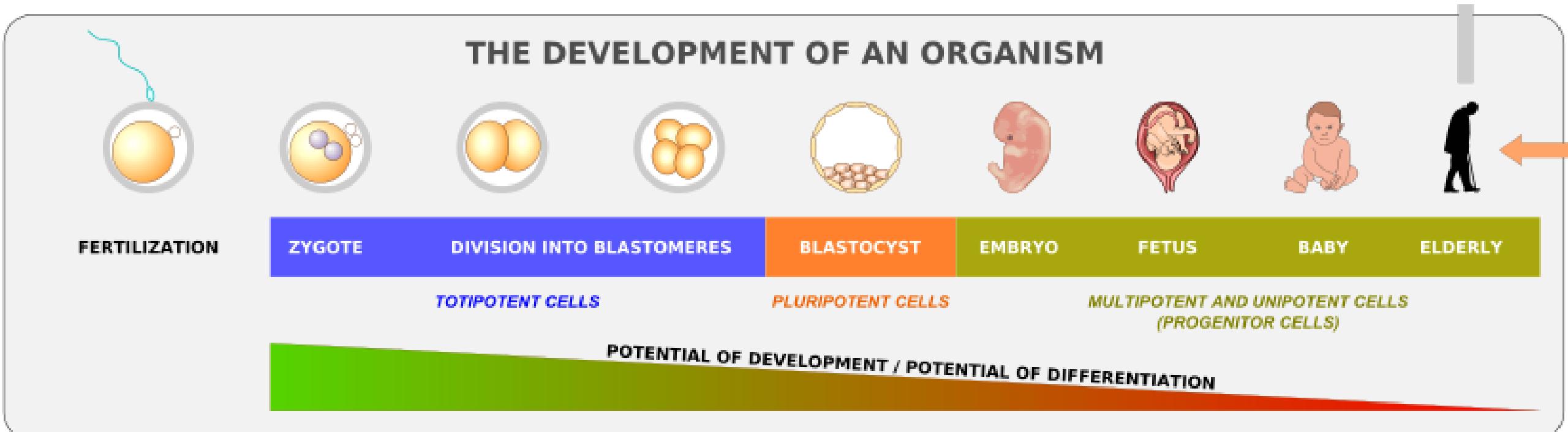
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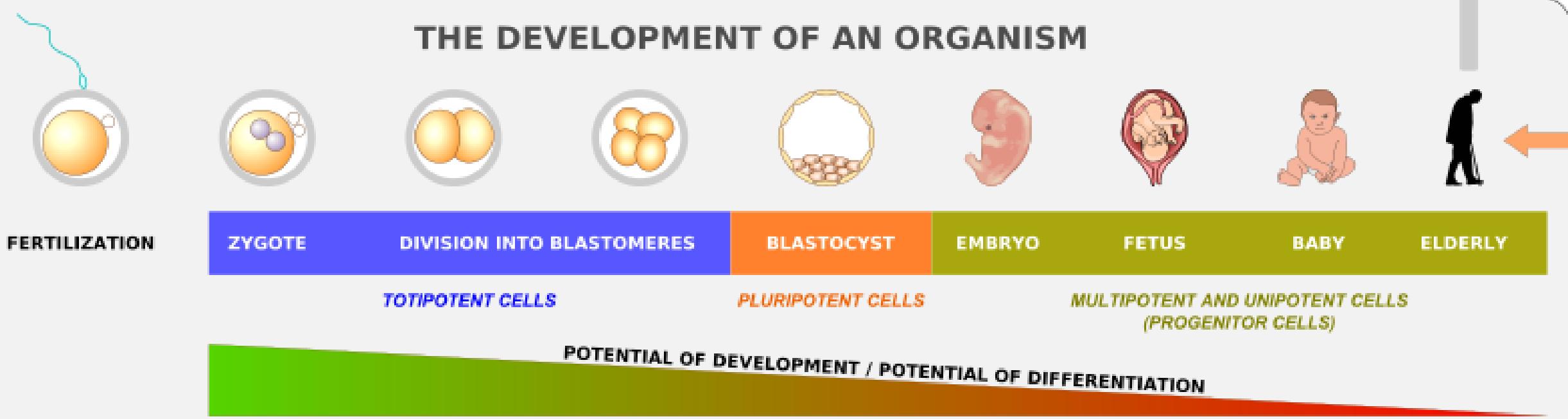




Human development



THE DEVELOPMENT OF AN ORGANISM



DIFFERENTIATION



PLASTICITY

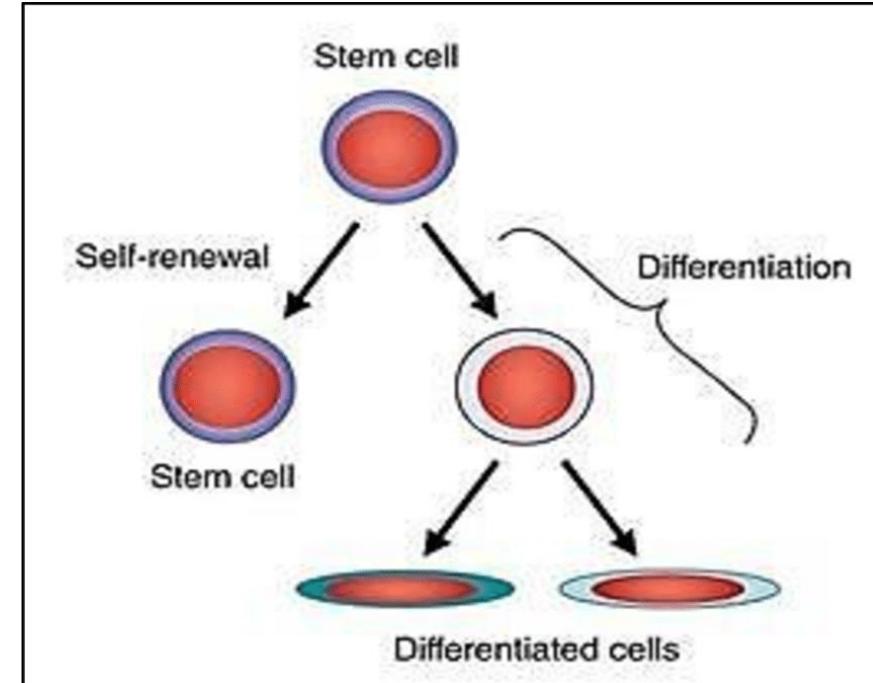


Stem cells

An undifferentiated / non-specialized cell found in multicellular organisms that has the ability to self-renew and when coaxed has the capability to differentiate into more than one specialized cell types.

Stem cells and its special characteristics

- Undifferentiated cells
- Ability to **self-renew** *in vitro*
- Ability to **differentiate** into multiple **OR** all cell types of the three germ layers
- Source for **autologous** (self) and **allogenic** (non-self) transplantation

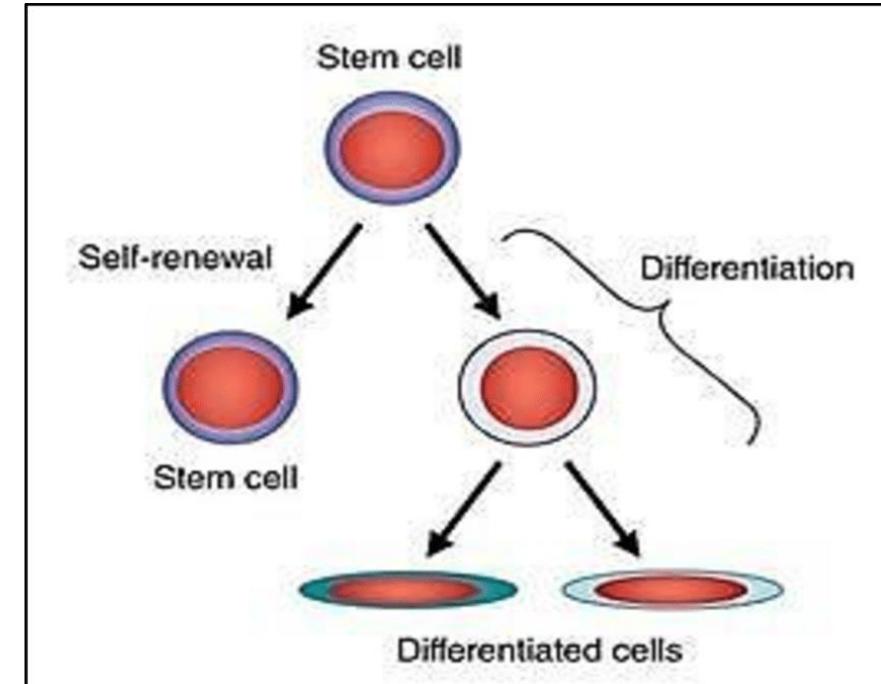


Self-renewal: the ability to go through numerous cycles of cell growth and cell division, known as cell proliferation, while maintaining the undifferentiated state.

Potency: the capacity to differentiate into specialized cell types.

Stem cells and its special characteristics

- Undifferentiated cells
- Ability to **self-renew** *in vitro*
- Ability to **differentiate** into multiple **OR** all cell types of the three germ layers
- Source for **autologous** (self) and **allogenic** (non-self) transplantation



Symmetric division gives rise to two identical daughter cells both endowed with stem cell properties.

Asymmetric division, on the other hand, produces only one stem cell and a progenitor cell with limited self-renewal potential. Progenitors can go through several rounds of cell division before terminally differentiating into a mature cell.

Types of Stem Cells

based on source

Embryo-derived stem cells

derived from an embryo
(produced by IVF)

E.g.

Embryonic stem cells
Epiblast stem cells
Embryonic germ cells

Fetal stem cells

derived from fetus
(aborted fetuses)

E.g.

Fetal blood and
fetal tissues

Adult stem cells

derived from adult
(tissues of adults)

E.g.

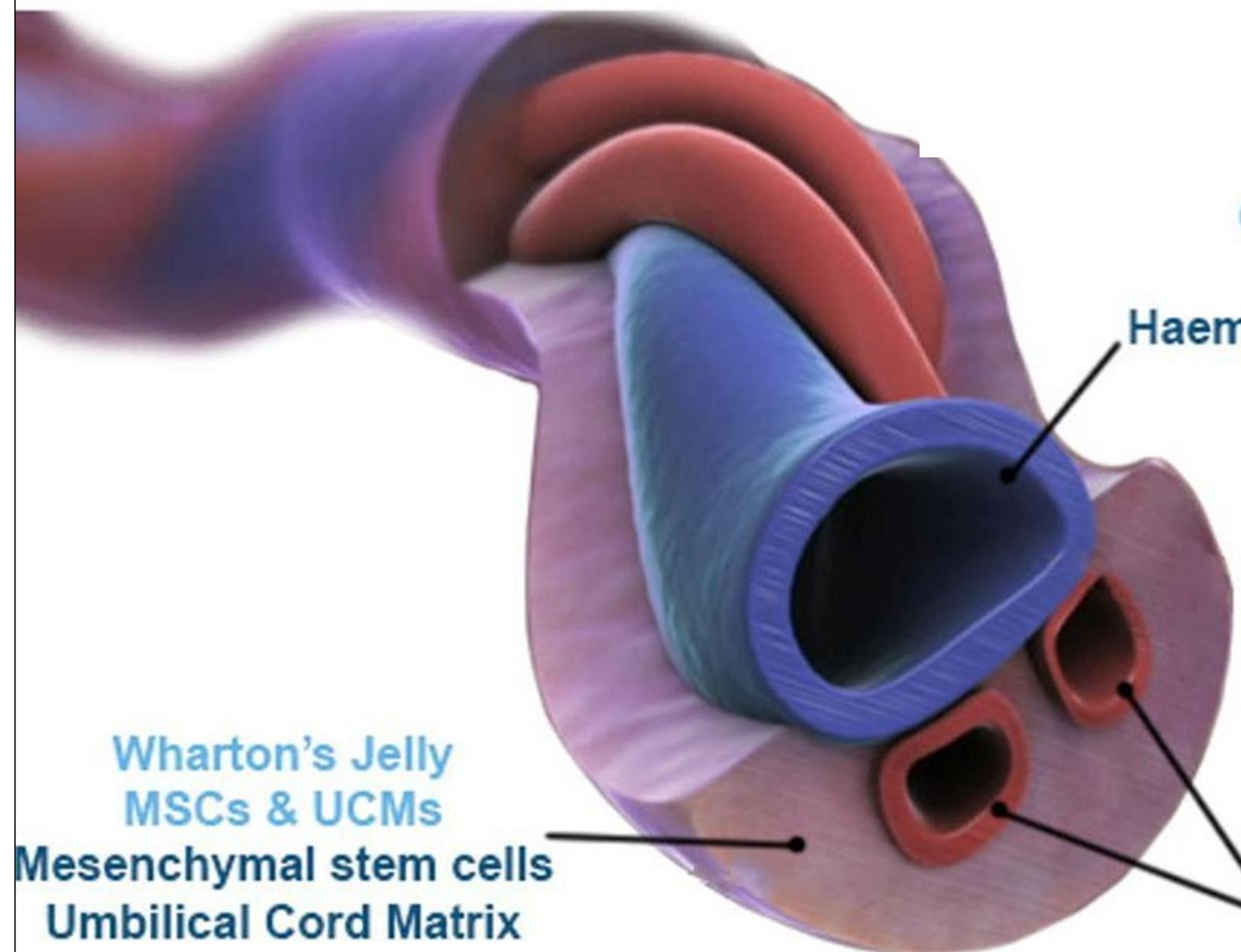
**HSCs, MSCs,
NSCs, ADSCs,
etc.**

Induced pluripotent stem cells

Generated artificially
in a lab

E.g.
iPSCs

Umbilical cord blood stem cells → derived from umbilical cord
HSCs (cord blood), MSCs (Wharton's jelly)

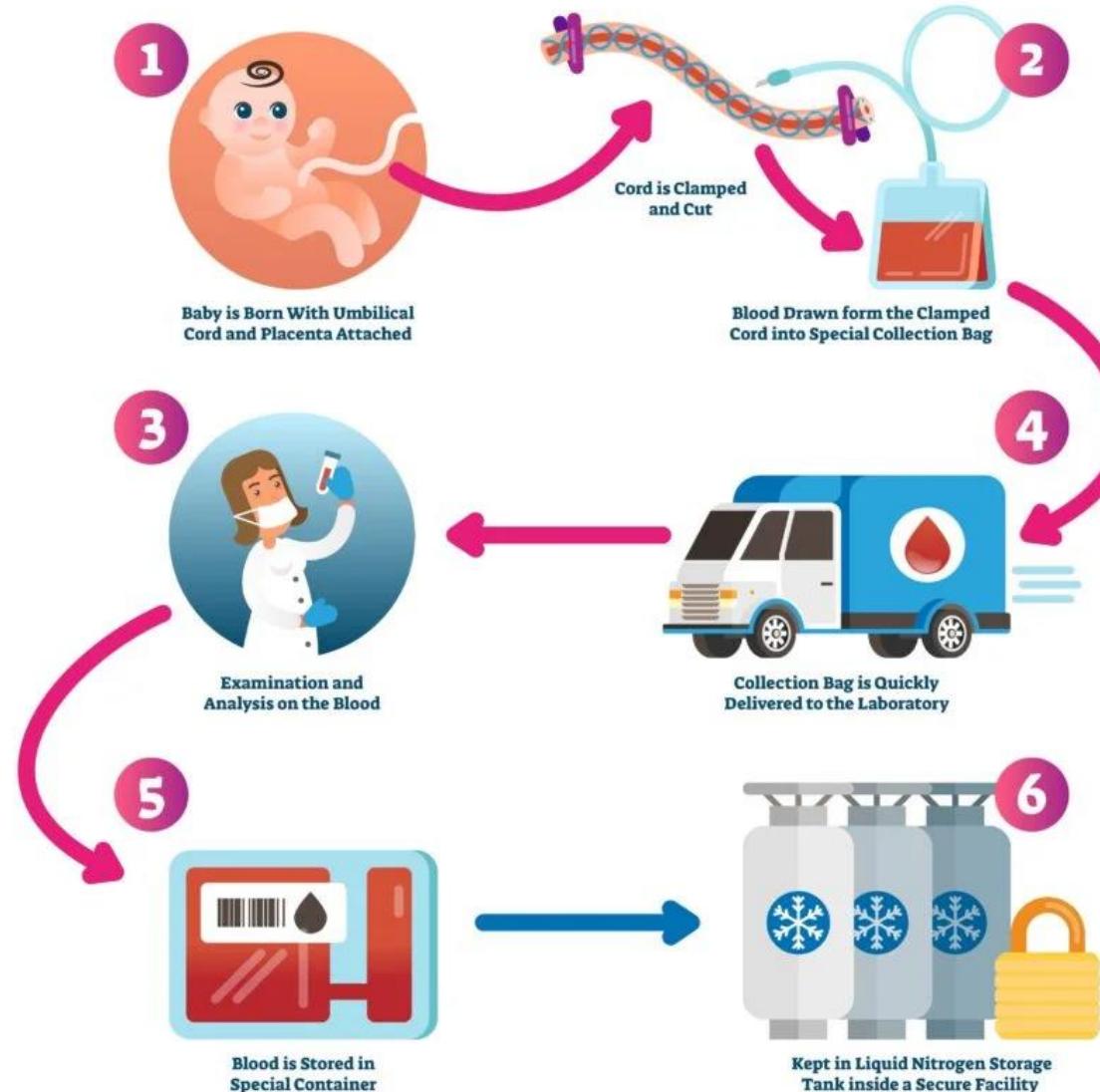


Wharton's Jelly
MSCs & UCMs
Mesenchymal stem cells
Umbilical Cord Matrix

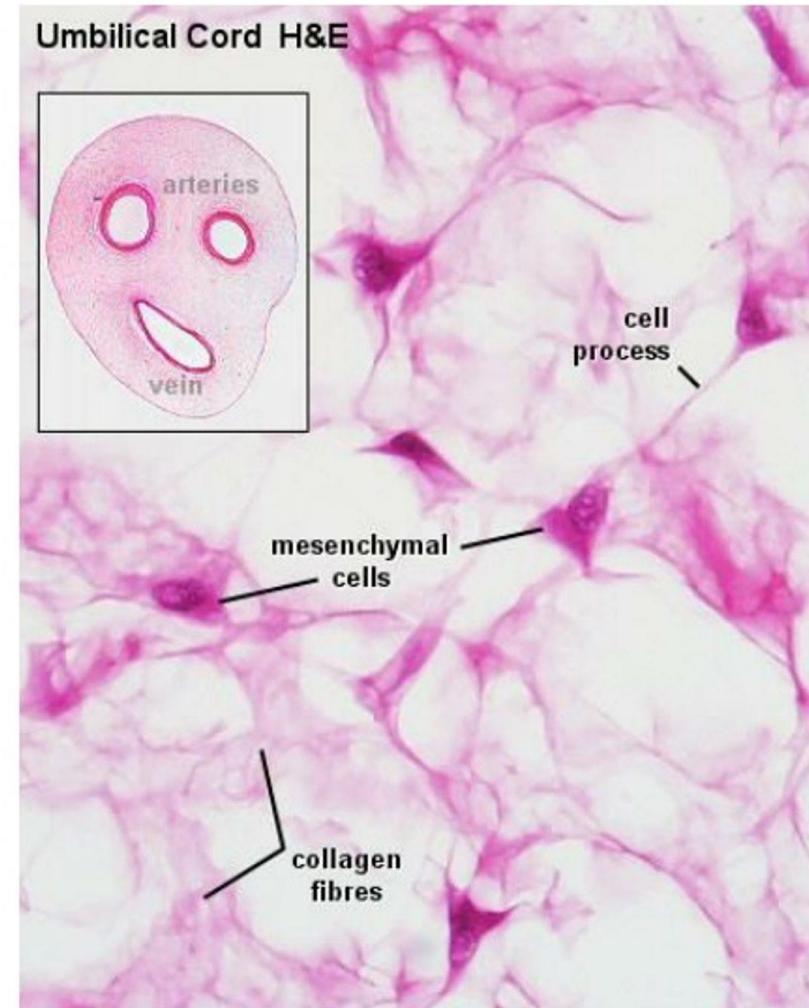
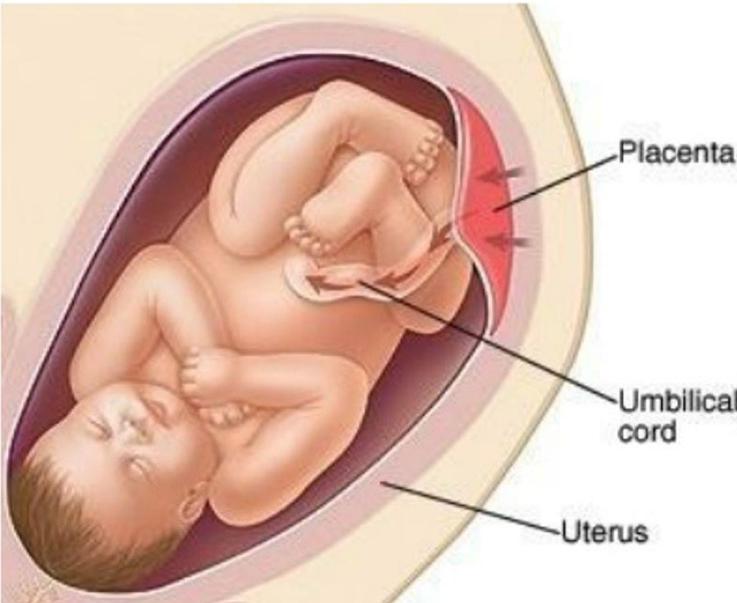
Cord Blood Vein
HSCs
Haematopoietic stem cells

Cord Blood Arterie

CORD BLOOD BANKING



Wharton's jelly in the umbilical cord

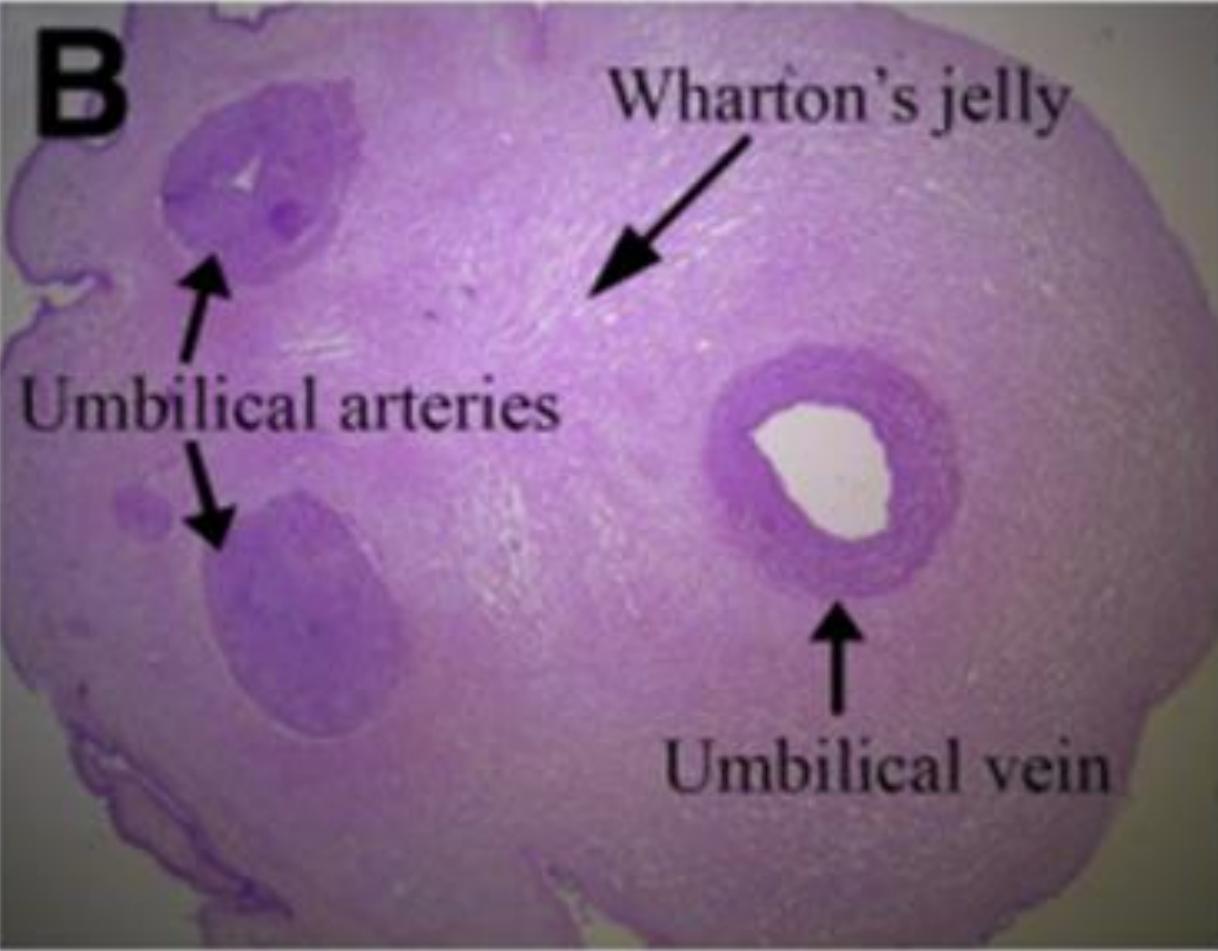


Wharton's jelly is a mucous connective tissue surrounding and protecting the umbilical cord vessels (2 arteries and one vein) against compression, bending twisting etc. It originates from the extraembryonic mesoderm and composed of mesenchymal cells and ECM (collagen, hyaluronan and proteoglycans). Some fibroblasts and macrophages also appear. Hyaluronan makes this tissue highly hydrated, collagen makes it resistant. It is a postnatal source of fetal stem cells.

A



Umbilical cord



Umbilical arteries

Wharton's jelly

Umbilical vein

Mesenchymal stem cells derived from Wharton's jelly: Comparative phenotype analysis between tissue and in vitro expansion

July 2012 . [Bio-medical Materials and Engineering](#)... 22(4):243-54

DOI: [10.3233/BME-2012-0714](https://doi.org/10.3233/BME-2012-0714)

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Talar Margossian · Loic Reppel · Nehman Makdissi · [Show all 6 authors](#) ·
Céline Huselstein

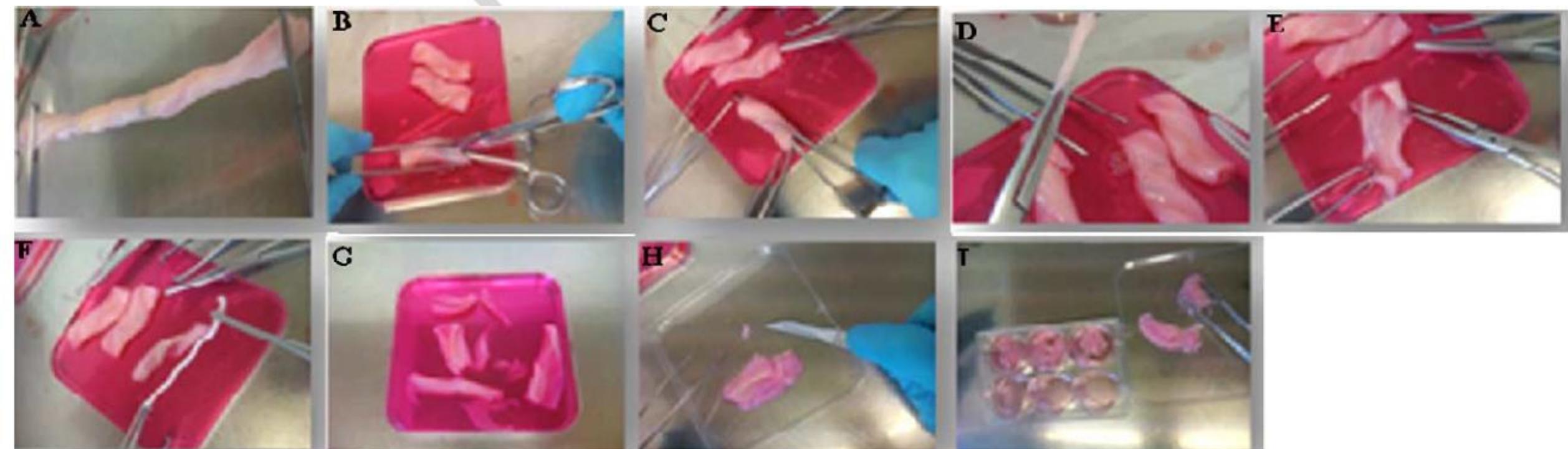
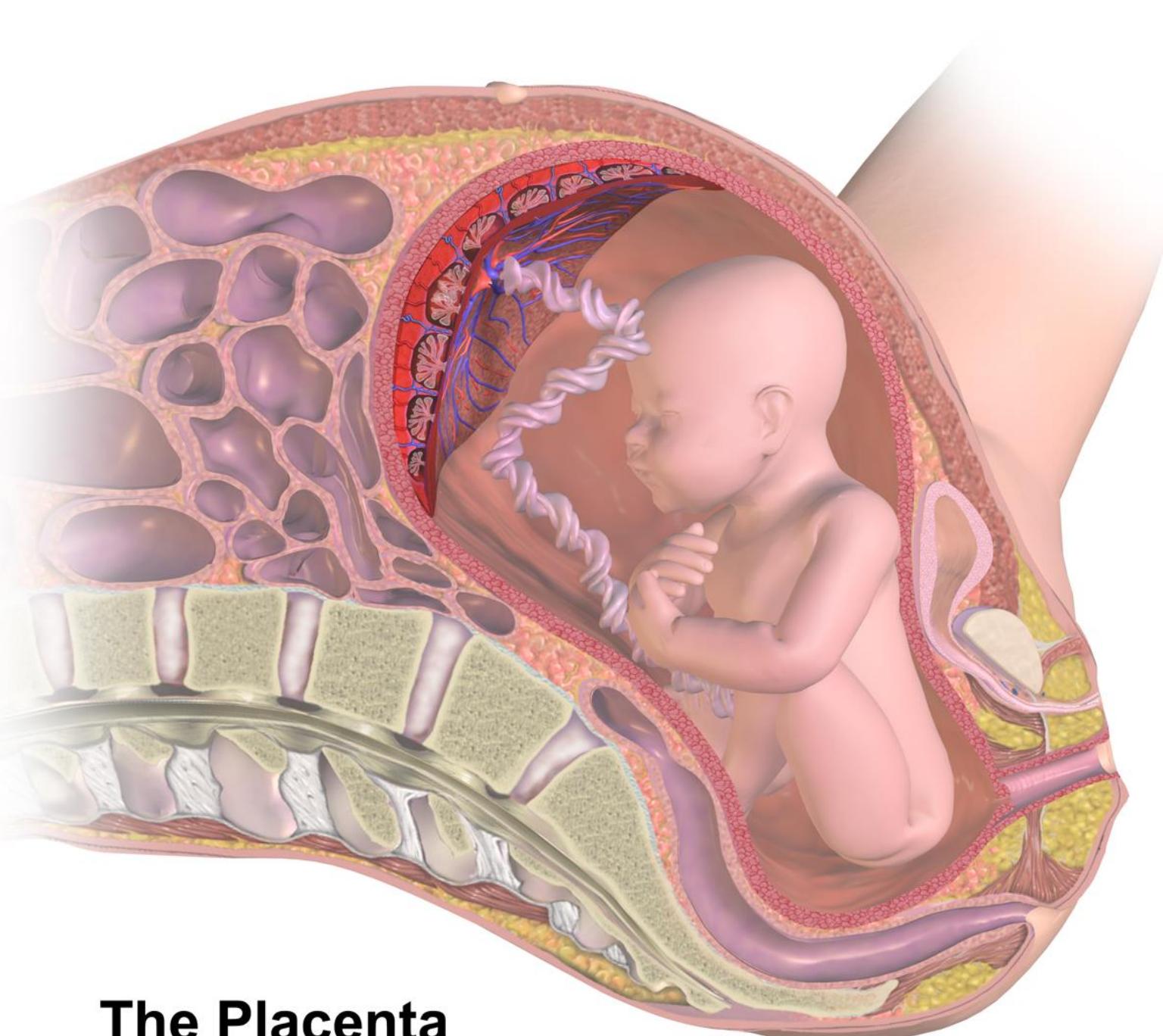


Fig. 1. Isolation of Wharton's jelly from human umbilical cord. (A) Cord removed from T75 flask and washed with alcohol. (B) Insertion of the clamp into the umbilical vein. (C) Longitudinal incision in the wall of the cord. (D and E) Detachment of the umbilical vein. (F) Withdraw of umbilical arteries. (G) Pieces of Wharton's jelly after removal of arteries and vein. (H) Cut into small pieces of 2–3 mm. (I) Distribution of pieces in six well plates. (Colors are visible in the online version of the article; <http://dx.doi.org/10.3233/BME-2012-0714>.)

Fetal Growth From 8 to 40 Weeks



- ❑ A fetus is defined as a later stage of development of an unborn child that takes place after the ninth week of conception



The Placenta



The **placenta** is a temporary organ that connects the developing fetus via the **umbilical cord** to the uterine wall to:

- Allow nutrient uptake
- Thermo-regulation
- Waste elimination
- Gas exchange via the mother's blood supply
- To fight against internal infection
- Produce hormones which support pregnancy.

Types of Stem Cells

based on potency or differentiation ability

Totipotent cells

present in embryo

E.g.

Fertilized zygote
Morula

Pluripotent stem cells

derived from an embryo
(produced by IVF)

E.g.

Embryonic stem cells
Epiblast stem cells
Embryonic germ cells
iPSCs

Multipotent stem cells

derived from fetal, UC
and adult tissues

E.g.

HSCs, MSCs,
NSCs, ADSCs,
UCBSCs,
etc.

Unipotent stem cells

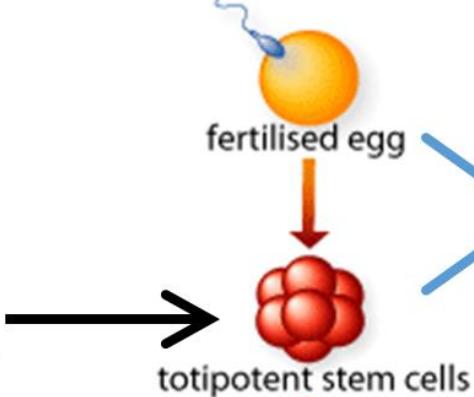
present in adult tissues

E.g.

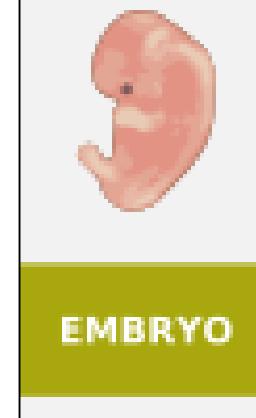
Skin stem cells,
muscle stem cells,
etc.

Plasticity

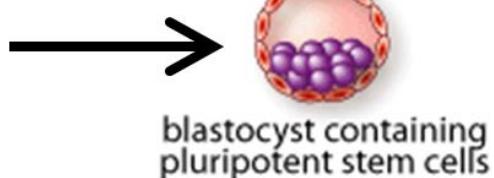
These cells can form the three germ layers and placenta



Totipotent
(*toti* – entirely)

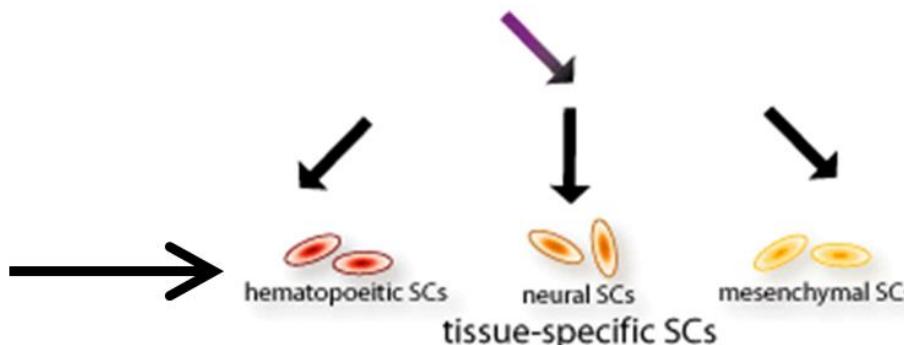


These cells can form only the three germ layers



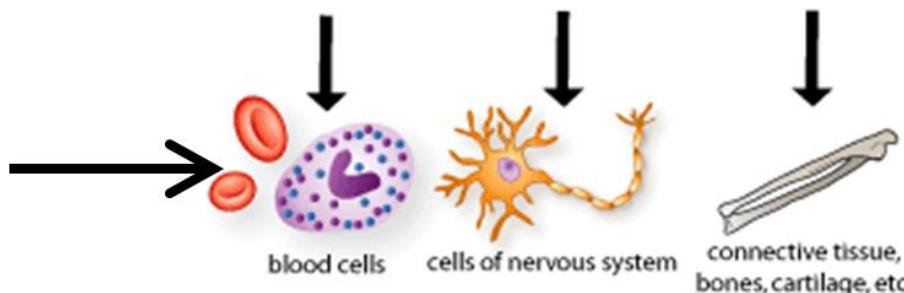
Pluripotent
(*plurimus* – very many)

These cells can form limited cell types

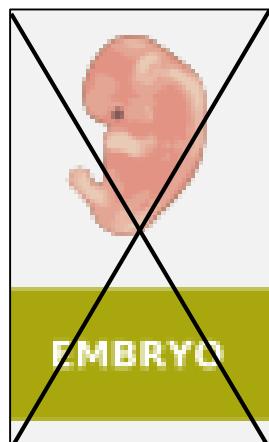


Multipotent

These cells can form itself



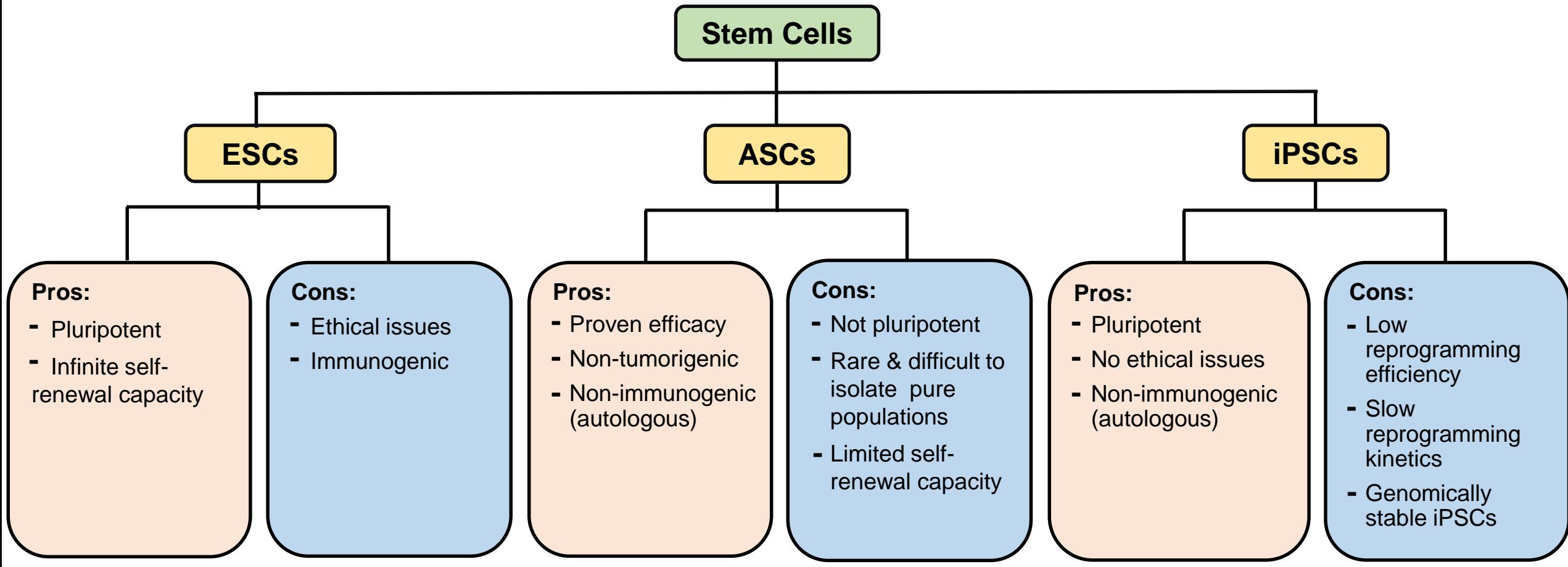
Unipotent



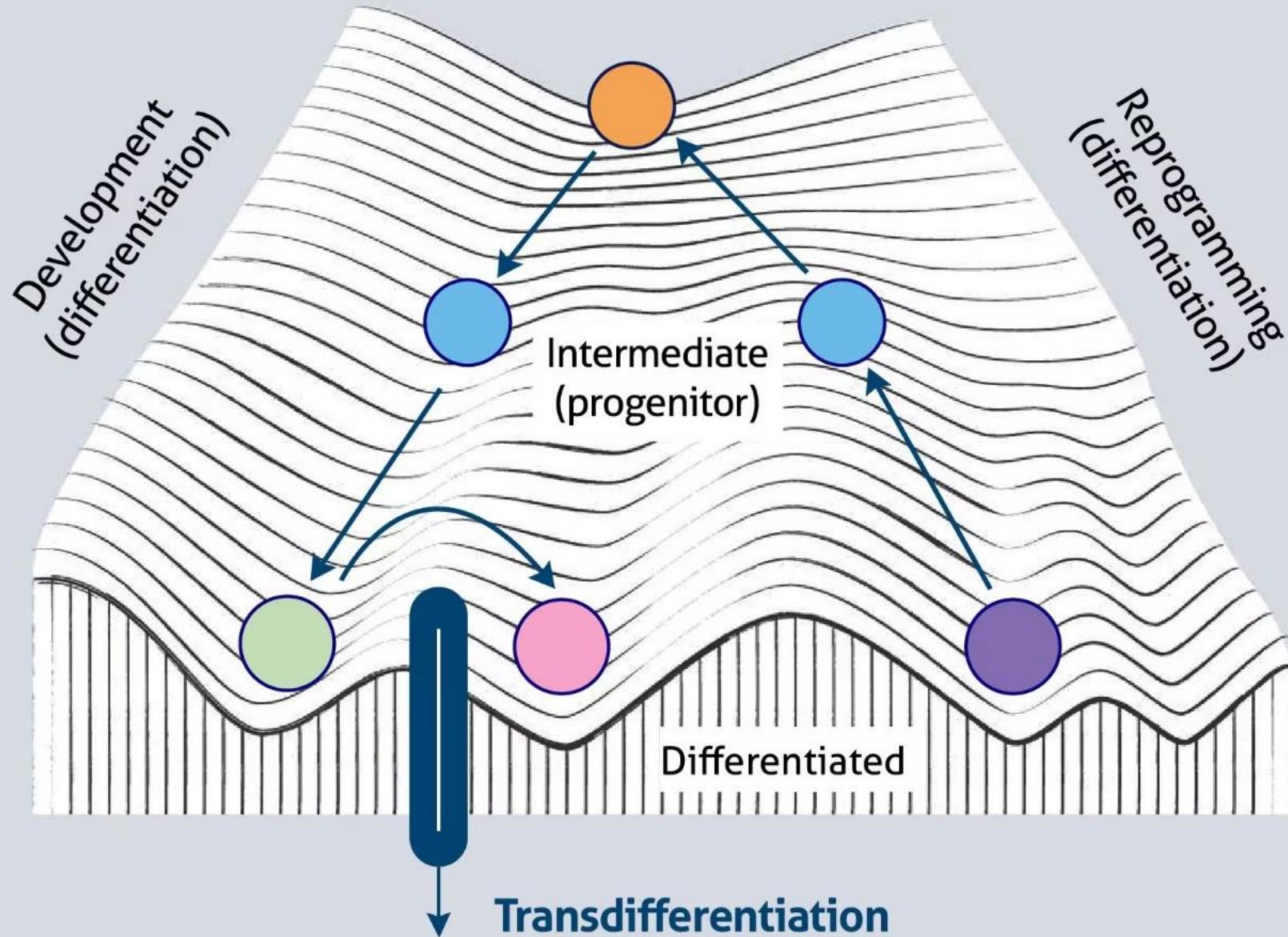
Three germ layers

Ectoderm	Mesoderm	Endoderm	Germ cells
			
Keratinocyte	Cardiac muscle cell	Smooth muscle cell (in gut)	Egg
			
Neuronal cell	Skeletal muscle cell	Pancreatic cell	Spem
			
Pigment cell	Kidney tubule cell	Lung cell (alveolar cell)	

Types of stem cells

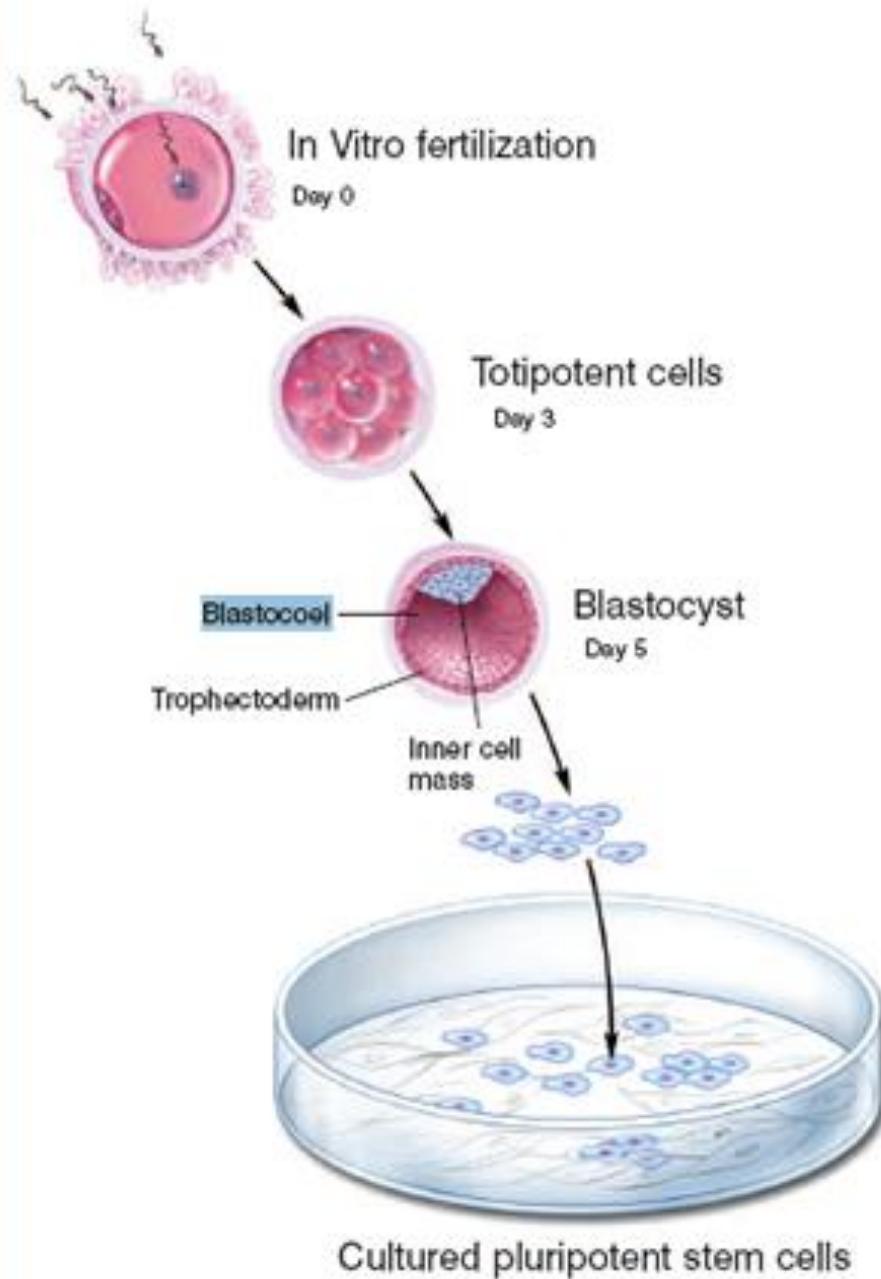


Pluripotent



**Who isolated ES cells
and
from where and how?**

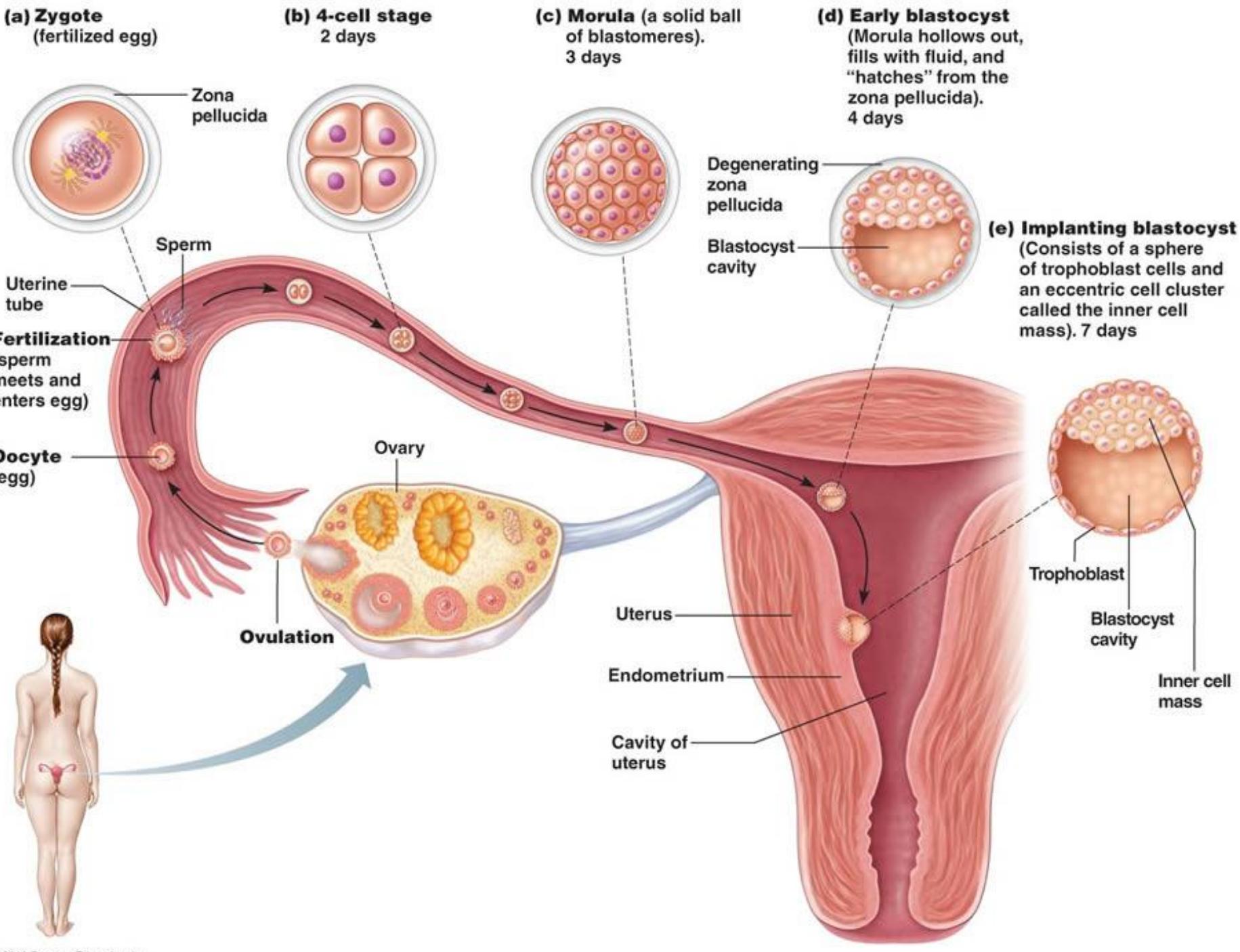
How Human Embryonic Stem Cells Are Derived



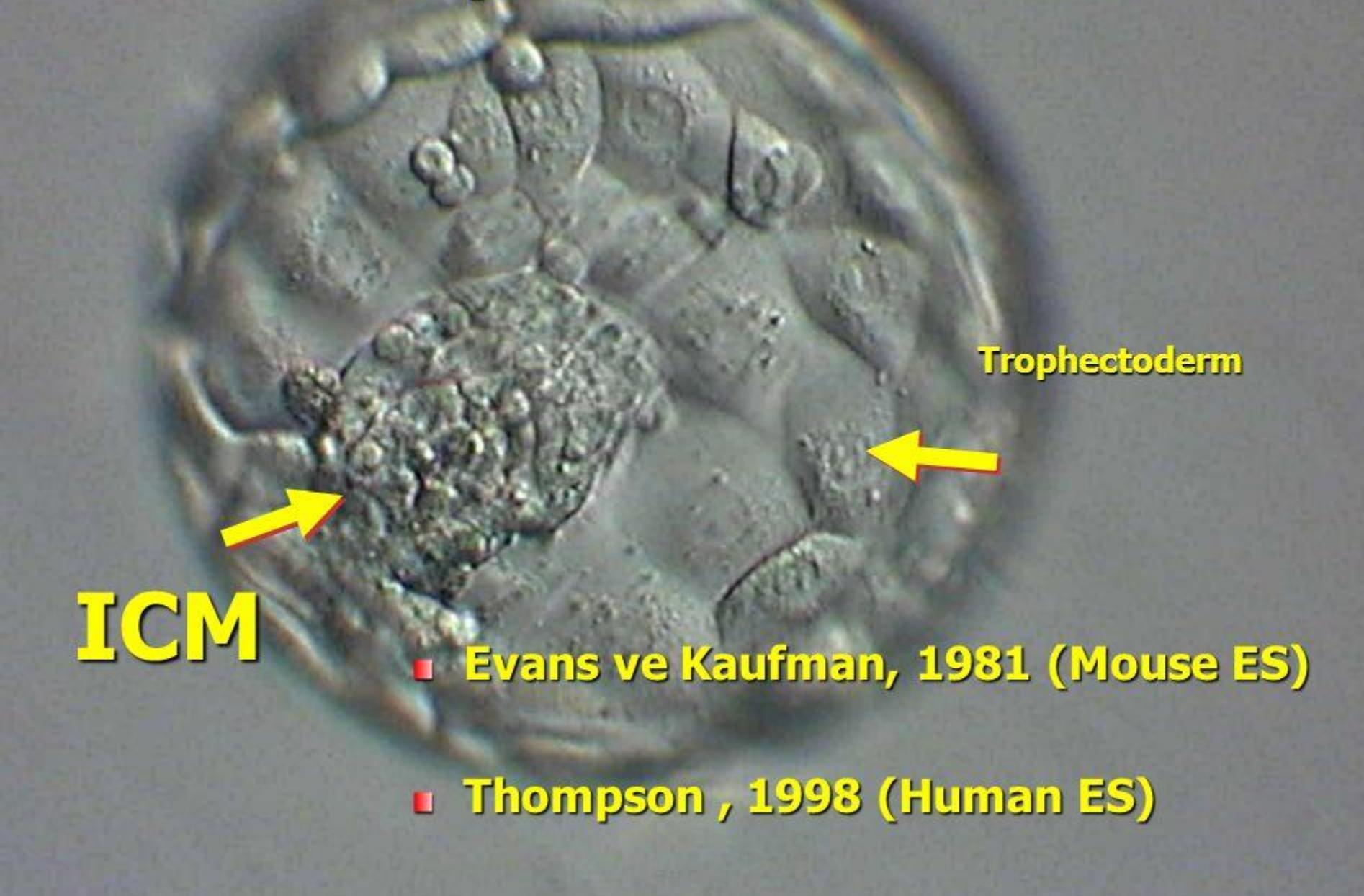
Zona pellucida

(also egg coat or pellucid zone)

- is a **glycoprotein layer** surrounding the plasma membrane of mammalian oocytes.
- This structure **binds spermatozoa**.
- In humans, **five days** after the fertilization, the blastocyst performs zona hatching; the zona pellucida degenerates and decomposes, to be replaced by the underlying layer of trophoblastic cells.



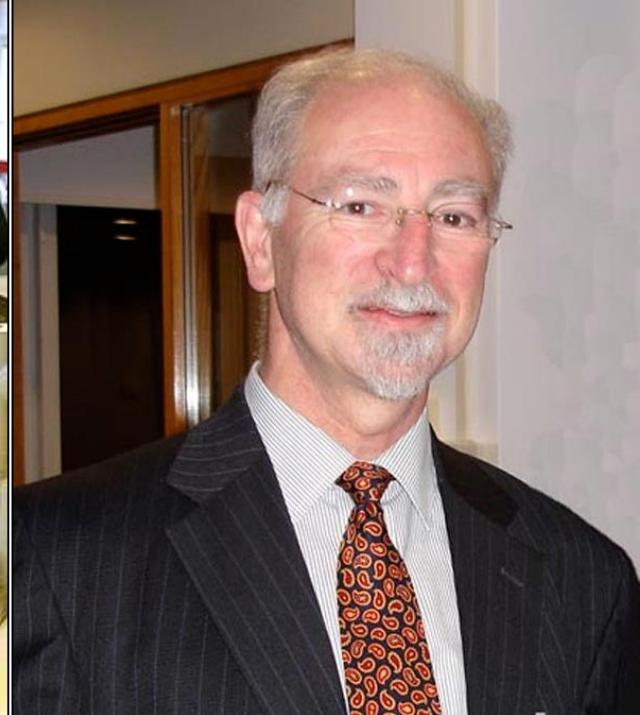
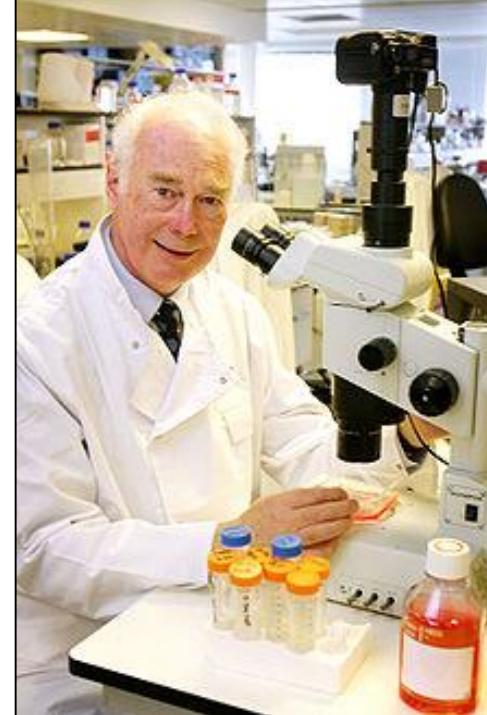
Embryonic Stem Cells



Establishment in culture of pluripotential cells from mouse embryos

M. J. Evans* & M. H. Kaufman†

Departments of Genetics* and Anatomy†, University of Cambridge,
Downing Street, Cambridge CB2 3EH, UK



Nature Vol. 292 9 July 1981

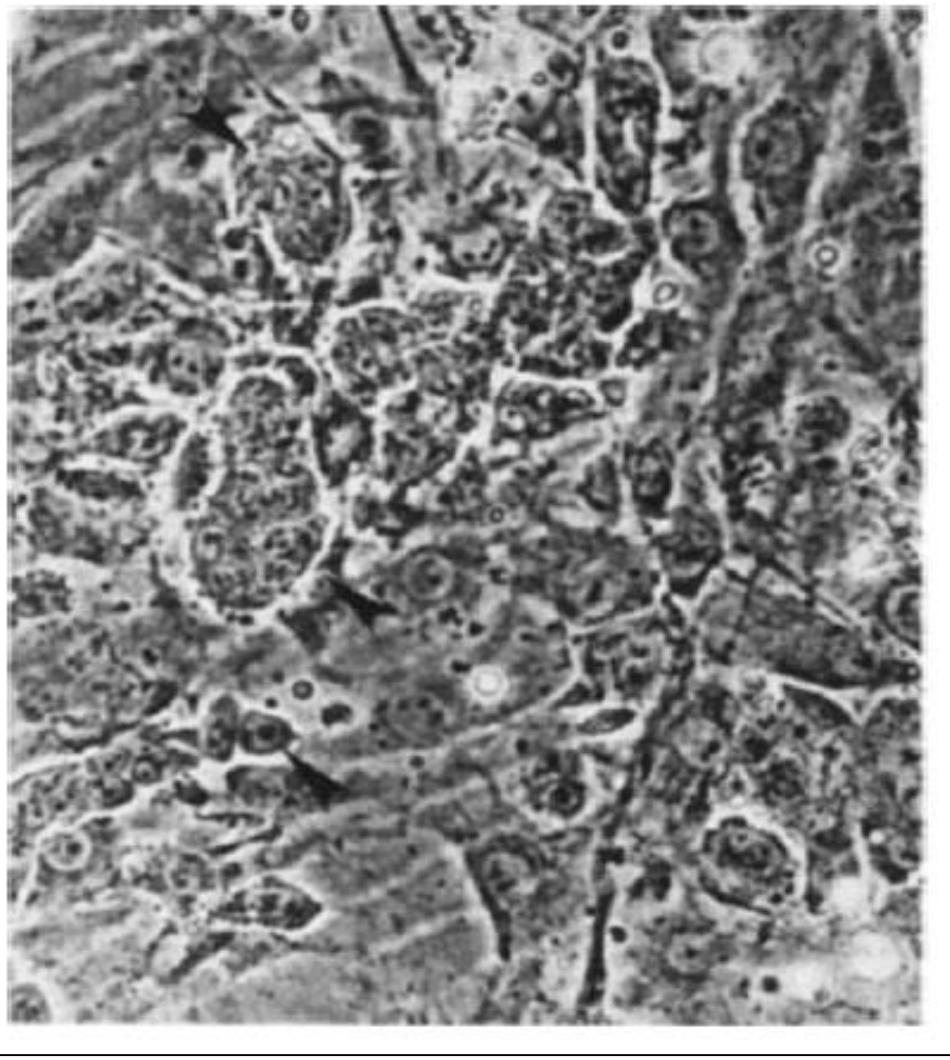
Martin J. Evans

Mathew H. Kaufman

Sir Martin John Evans, an English biologist, who with Matthew Kaufman, was the first to culture mice embryonic stem cells and cultivate them in a laboratory in 1981.

He is also known, along with Mario Capecchi and Oliver Smithies, for his work in the development of the knockout mouse and the related technology of gene targeting, a method of using embryonic stem cells to create specific gene modifications in mice.

In 2007, the three shared the **Nobel Prize in Physiology or Medicine** in recognition of their discovery and contribution to the efforts to develop new treatments for illnesses in humans.



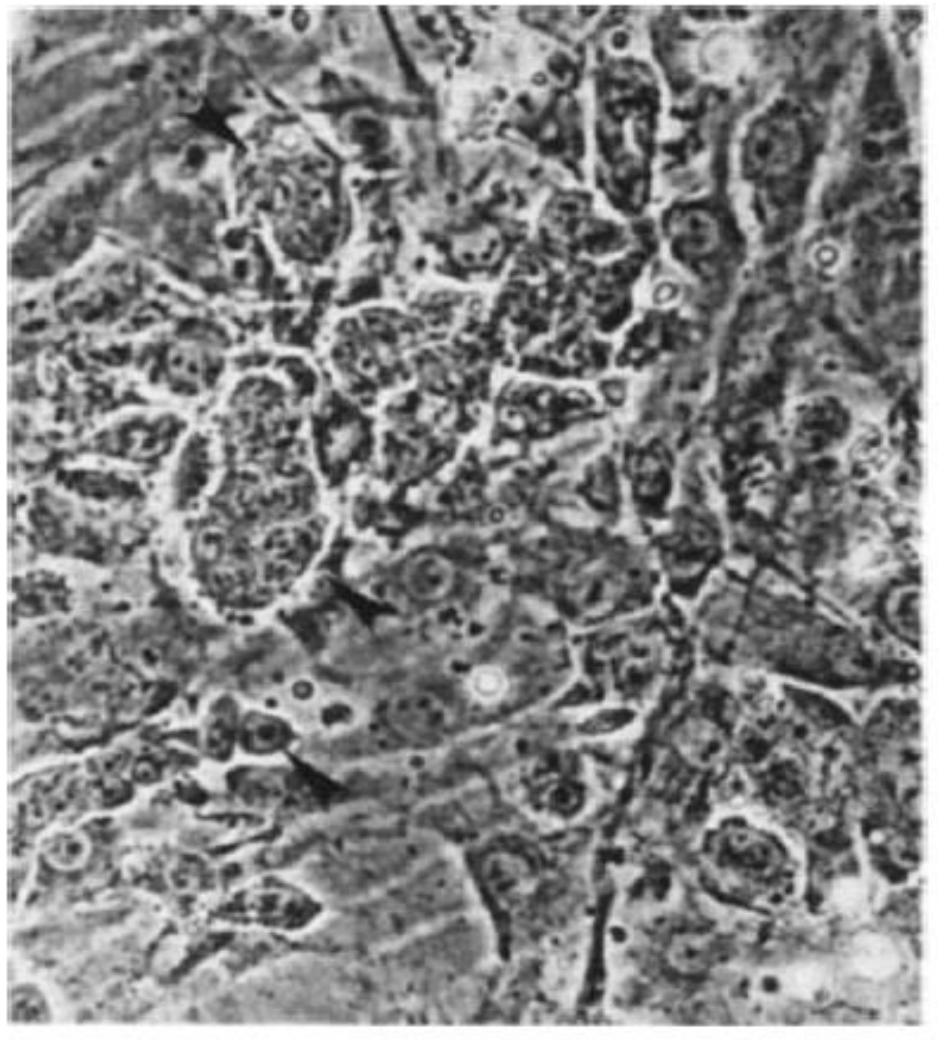
Embryo derived EK cells from 129 SvE strain mice

Fig. 1 Groups of pluripotential embryo cells (arrowed) growing in monolayer culture on a background of mitomycin C-inhibited STO cells. The isolation of a definite cell line from a blastocyst takes only ~ 3 weeks and the pluripotential cell colonies are visible within 5 days of passage. We have had 30% yield of lines from blastocysts in one experiment. Two of the lines have been rigorously cloned by single-cell isolation but most were only colony-picked—this makes no difference.

Evan and Kaufmann, 1981; Nature

3 important points for successful isolation of ES cells

- 1. The exact stage at which pluripotential cells capable of growth in tissue culture exist in the embryo.**
- 2. Explantation of a sufficiently large number of these precursor cells from each embryo.**
- 3. Tissue culture in conditions most conducive to multiplication rather than differentiation of these embryonic cells.**



Embryo derived EK cells from 129 SvE strain mice

Fig. 1 Groups of pluripotential embryo cells (arrowed) growing in monolayer culture on a background of mitomycin C-inhibited STO cells. The isolation of a definite cell line from a blastocyst takes only ~ 3 weeks and the pluripotential cell colonies are visible within 5 days of passage. We have had 30% yield of lines from blastocysts in one experiment. Two of the lines have been rigorously cloned by single-cell isolation but most were only colony-picked—this makes no difference.



Fig. 2 Karyotype of an embryo-derived pluripotential cell line, 40XY. Over 80% of the spreads of this clonal line possessed 40 chromosomes and had a clearly identifiable Y chromosome.

**Normal karyotype;
30 passages**

Evan and Kaufmann, 1981; Nature

Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells

(embryonic stem cells/inner cell masses/differentiation *in vitro*/embryonal carcinoma cells/growth factors)

GAIL R. MARTIN

Department of Anatomy, University of California, San Francisco, California 94143

Communicated by J. Michael Bishop, September 14, 1981

Proc. Natl. Acad. Sci. USA
Vol. 78, No. 12, pp. 7634–7638, December 1981
Developmental Biology

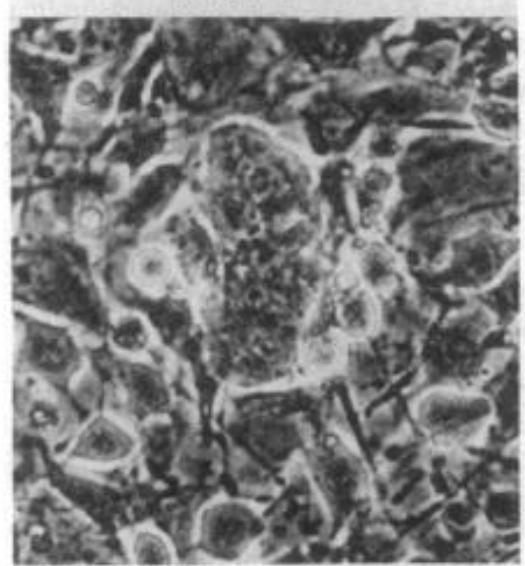
Coined the term “Embryonic Stem” Cells



Martin GR, 1981; PNAS

Gail Roberta Martin

PSA-1



ESC-ICR

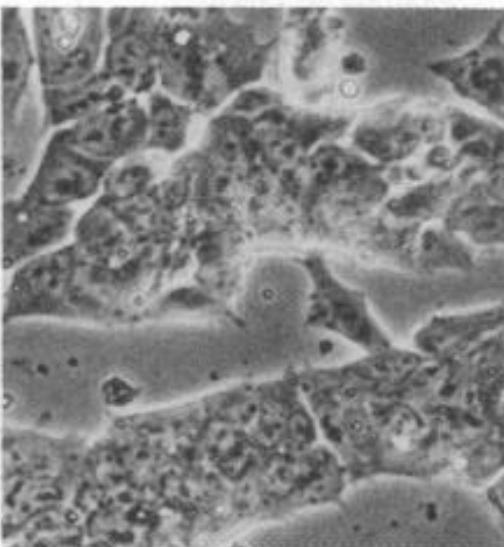
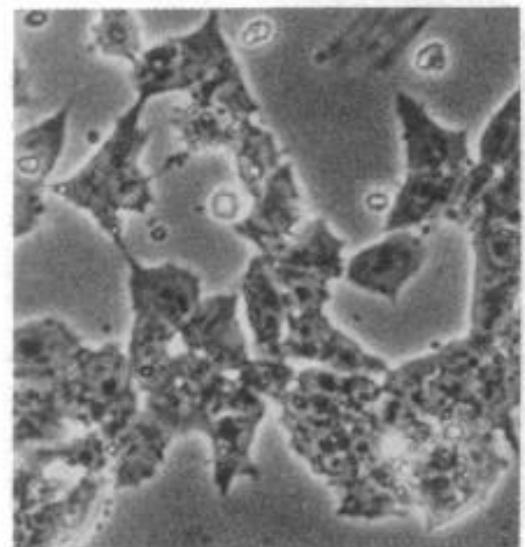
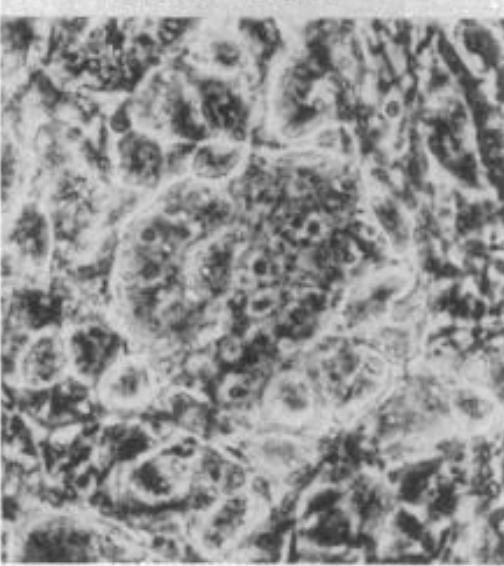


FIG. 2. Morphological similarity of embryo-derived ESC-ICR cells to PSA-1 embryonal carcinoma cells. (*Upper*) Cells growing on a fibroblastic feeder layer. (*Lower*) Mass cultures of the cells seeded in the absence of feeder cells. (Phase-contrast microscopy; approximately $\times 250$.)

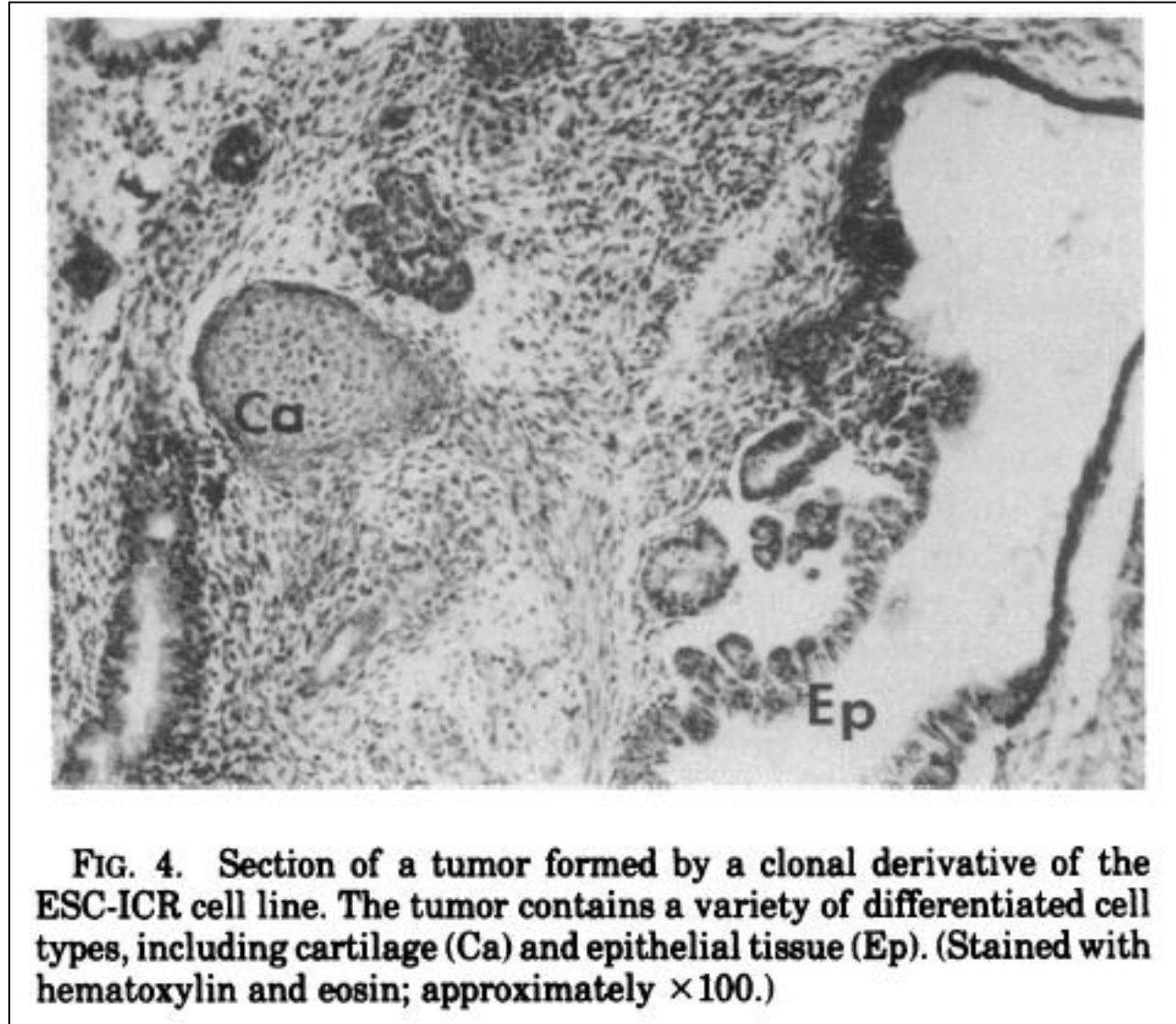


FIG. 4. Section of a tumor formed by a clonal derivative of the ESC-ICR cell line. The tumor contains a variety of differentiated cell types, including cartilage (Ca) and epithelial tissue (Ep). (Stained with hematoxylin and eosin; approximately $\times 100$.)

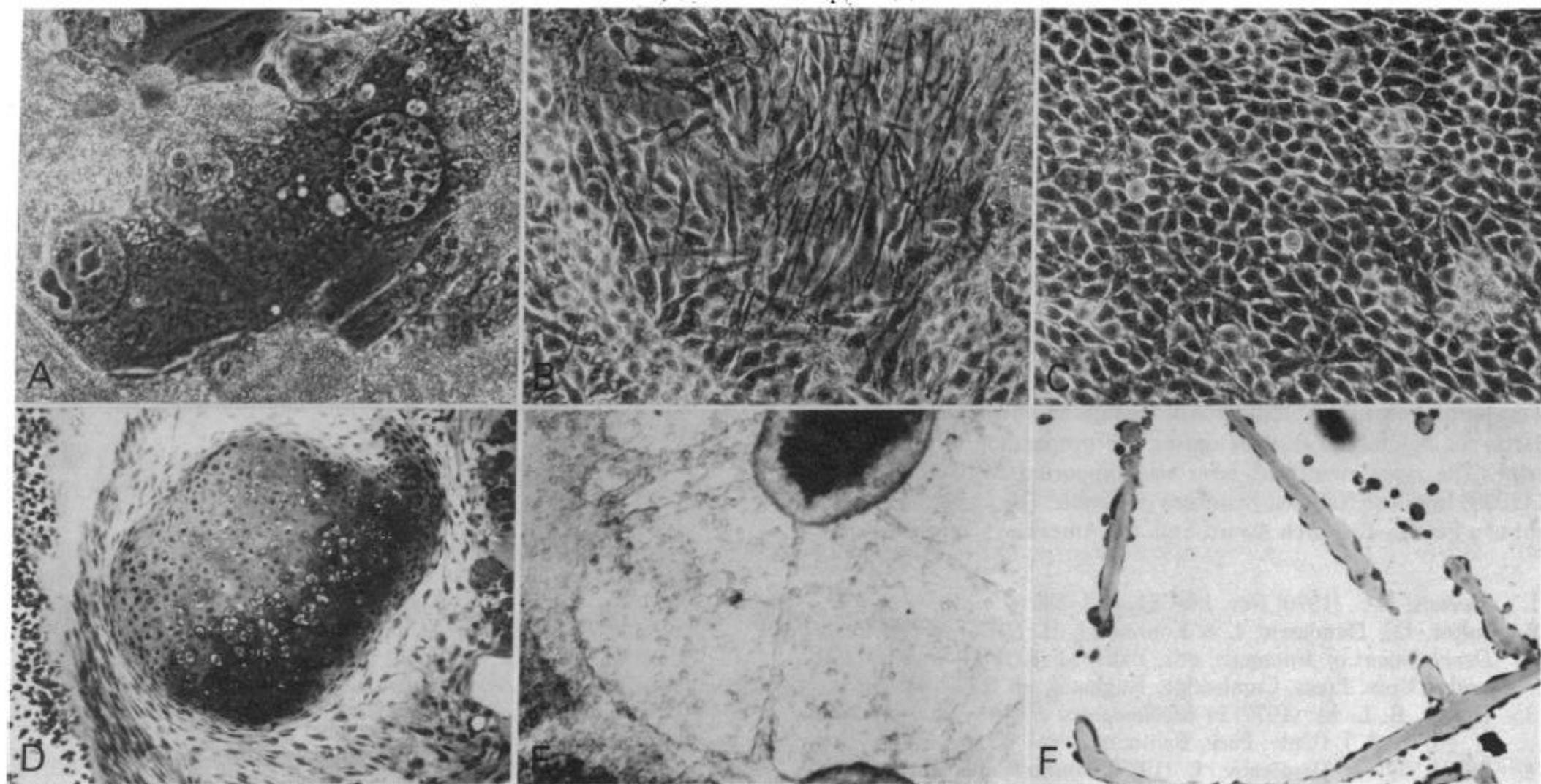


FIG. 5. Differentiation of ESC-ICR cells *in vitro*. A variety of cell types are apparent during the 6 weeks after the reattachment to tissue culture dishes of embryoid bodies formed by ESC-ICR cells. (*Upper*) Phase-contrast microscopy of live cells. (Approximately $\times 160$). (A) Giant cells, (B) neuron-like cells, (C) endodermal cells. (*Lower*) (D) Section of plastic-embedded culture showing cartilage. (Approximately $\times 100$.) (E) Live cells forming tubules. (Approximately $\times 35$.) (F) Section of area shown in E after embedding in plastic. Tubules are filled with a granular, acellular deposit. (Approximately $\times 100$.)

LIF

(Leukemia Inhibitory Factor)

Williams et al., 1988

Isolation and culture of inner cell mass cells from human blastocysts

Ariff Bongso¹, Chui-Yee Fong, Soon-Chye Ng and Shan Ratnam

Department of Obstetrics and Gynaecology, National University Hospital, Kent Ridge, Singapore 0511

¹To whom correspondence should be addressed



Totipotent non-committed inner cell mass (ICM) cells from human blastocysts, if demonstrated to be capable of proliferating *in vitro* without differentiation, will have several beneficial uses, not only in the treatment of neurodegenerative and genetic disorders, but also as a model in studying the events involved in embryogenesis and genomic manipulation. Nine patients admitted to an in-vitro fertilization programme donated 21 spare embryos for this study. All 21 embryos were grown from the 2-pronuclear until blastocyst stages on a human tubal epithelial monolayer in commercial Earle's medium (Medicult, Denmark) supplemented with 10% human serum. The medium was changed after blastocyst formation to Chang's medium supplemented with 1000 units/ml of human leukaemia inhibitory factor (HLIF) and the embryos left undisturbed for 72 h to allow the hatched ICM and trophoblast to attach to the feeder monolayer. Nineteen of the 21 embryos from nine patients produced healthy ICM lumps which could be separated and grown *in vitro*. Two of the lumps differentiated into fibroblasts while the remaining 17 (eight patients) produced cells with typical stem cell-like morphology, were alkaline phosphatase positive and could be maintained for two passages. It was possible to retain the stem cell-like morphology, alkaline phosphatase positiveness and normal karyotype through the two passages in all of them using repeated doses of HLIF every 48 to 72 h. This is the first report on the successful isolation of human ICM cells and their continued culture for at least two passages *in vitro*.

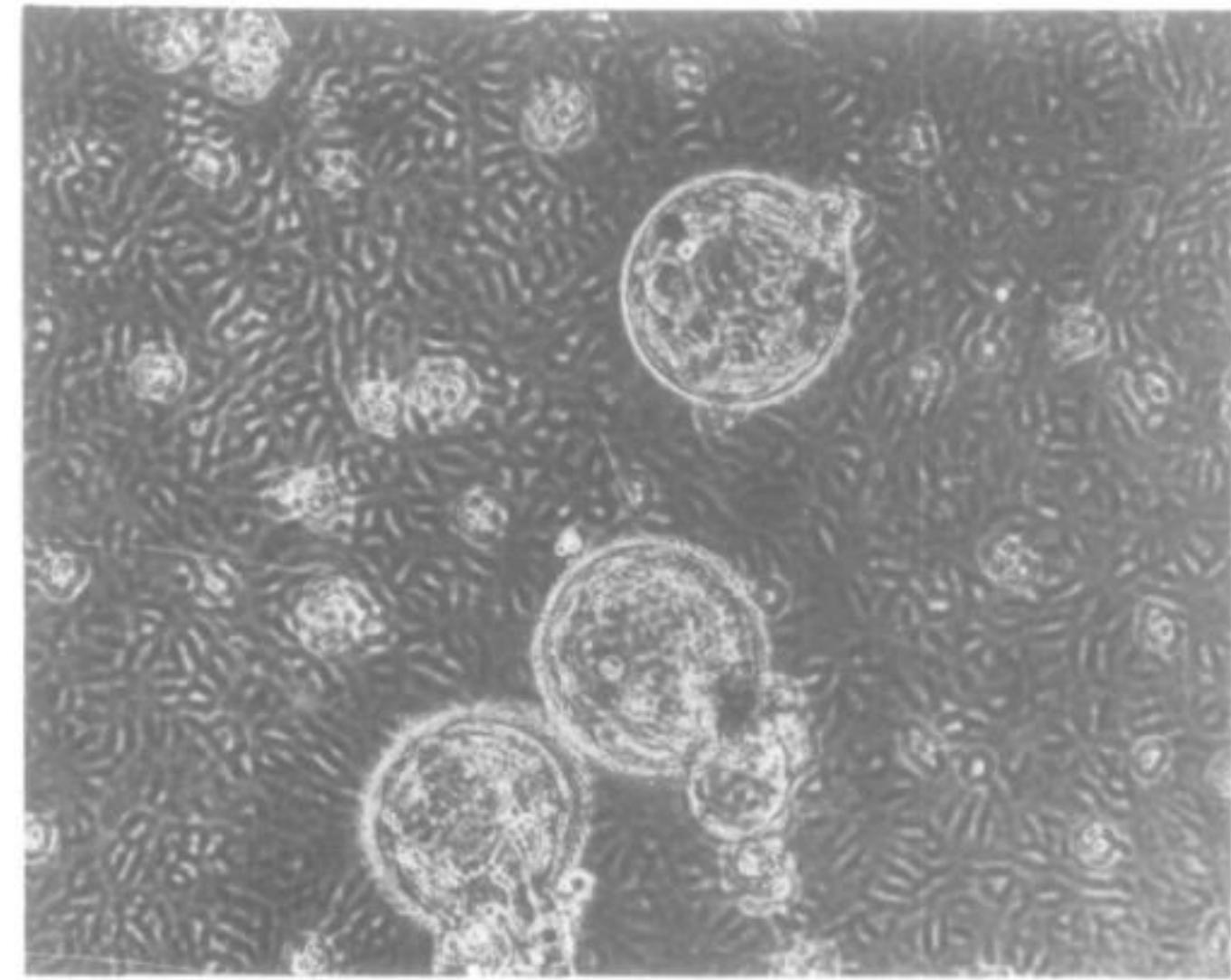


Fig. 1. Human blastocysts (144 h post-insemination) hatching on human tubal ampullary epithelial monolayer ($\times 100$).

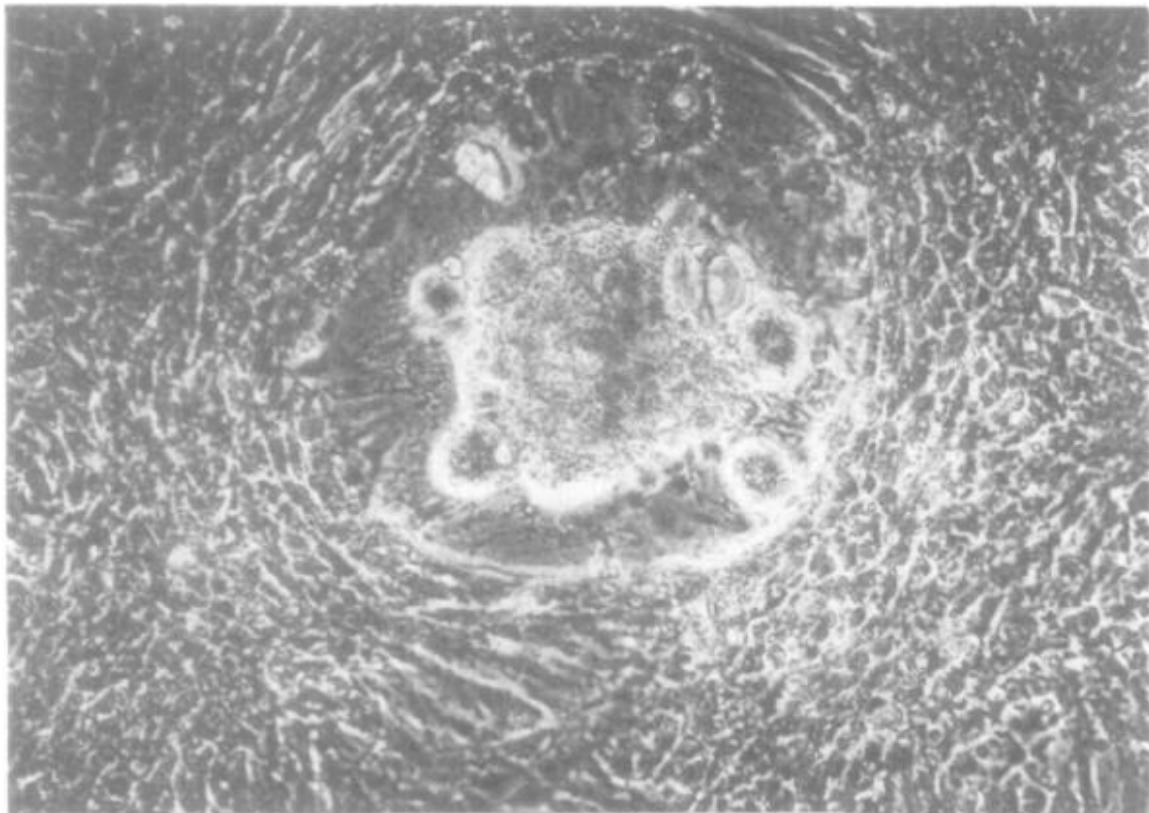


Fig. 2. Inner cell mass (ICM) lump with peripheral trophoblast-like cell outgrowths 2 days after hatching.

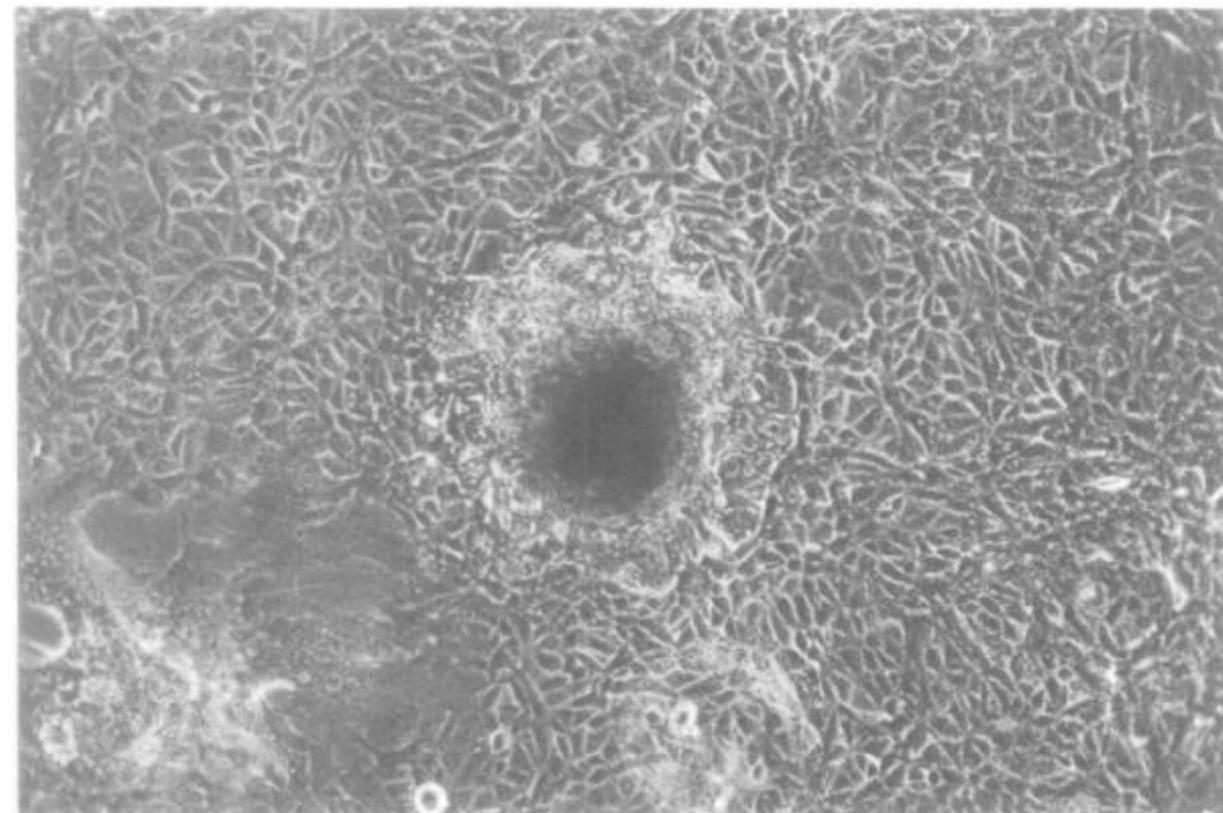


Fig. 3. Inner cell mass lump with peripheral trophoblast-like cells attached to ampullary epithelial monolayer 5 days after hatching.

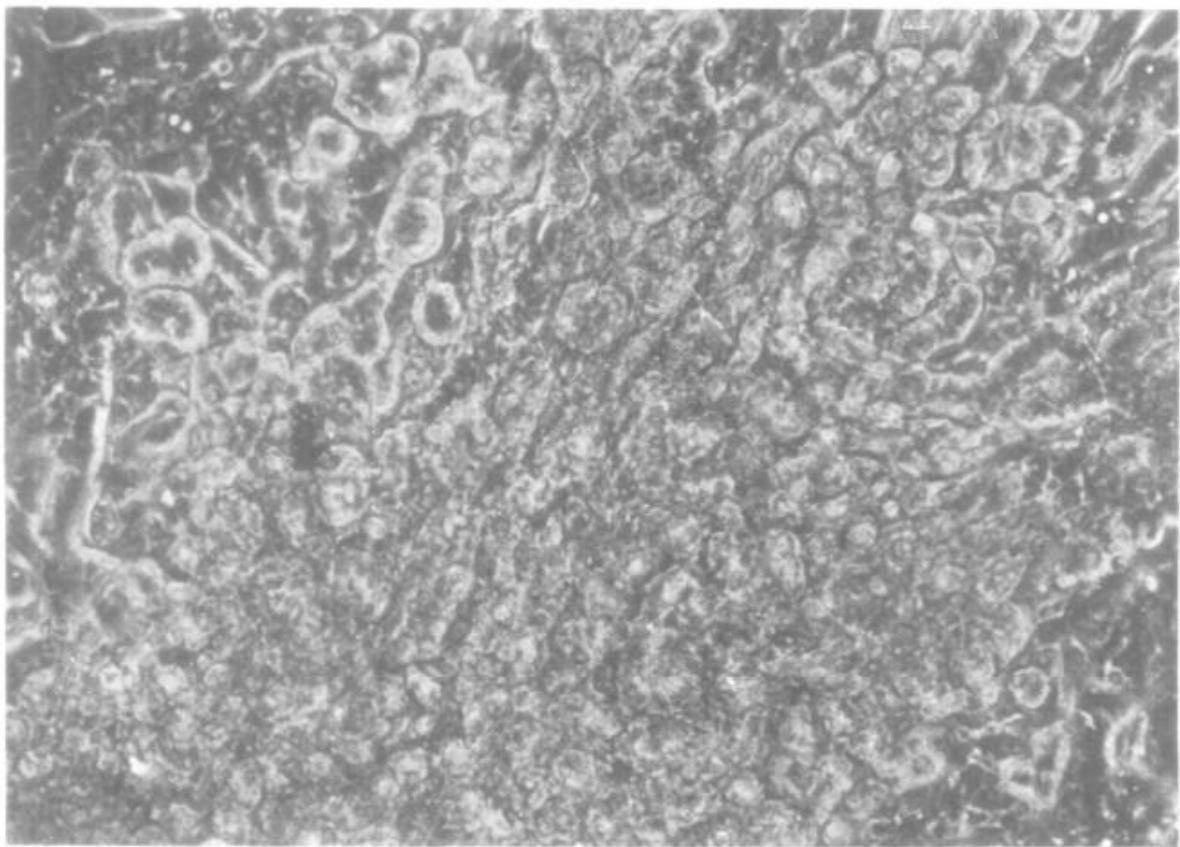


Fig. 6. High magnification of inner cell mass cells. Note epithelioid monolayer with circular small and large cell nests typical of ES-like cells ($\times 600$).

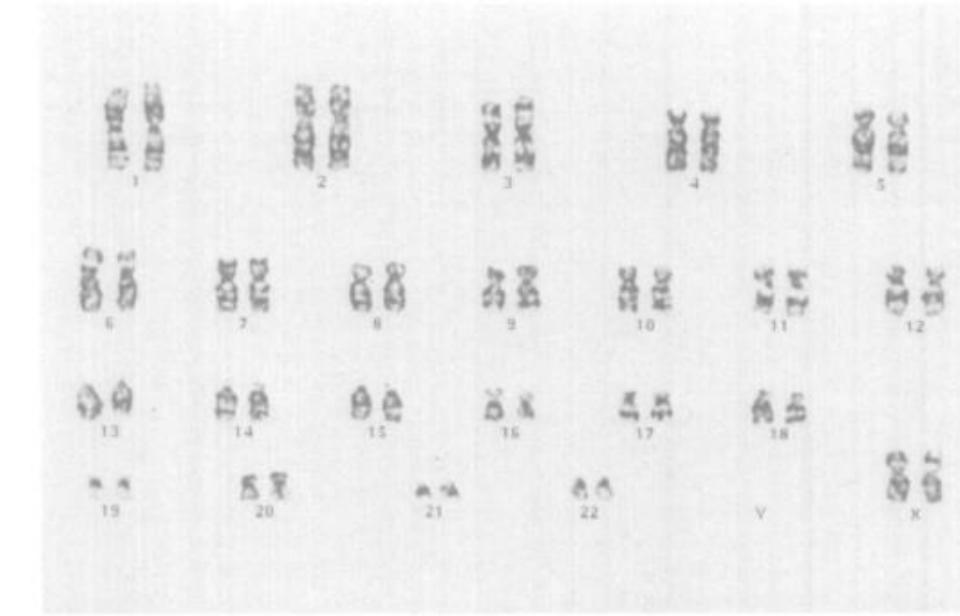


Fig. 7. Giemsa-banded karyotype of an ES-like cell from primary culture showing a normal 46XX karyotype.

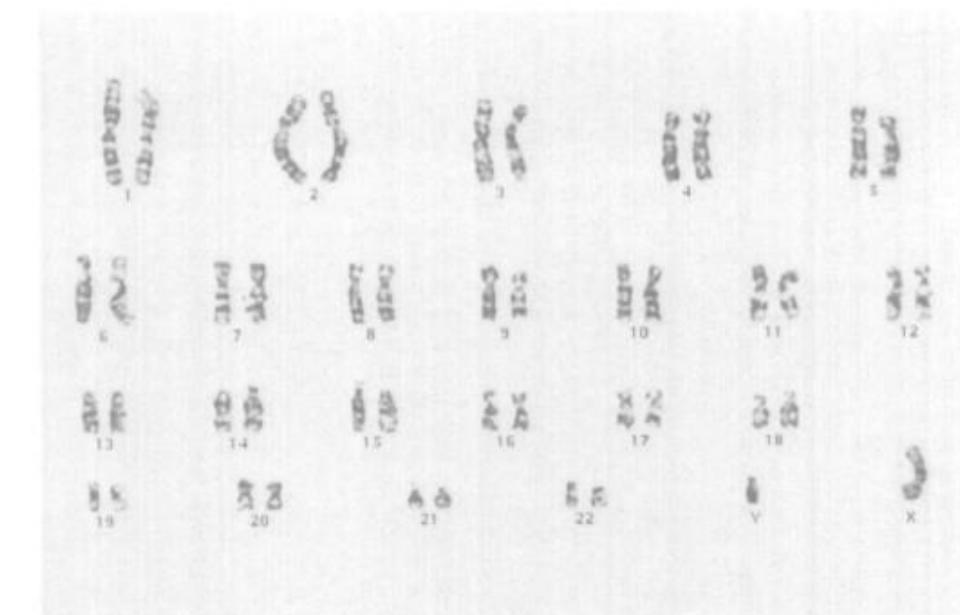


Fig. 8. Giemsa-banded karyotype of an ES-like cell from the first passage showing a normal 46XY karyotype.

Embryonic Stem Cell Lines Derived from Human Blastocysts

James A. Thomson,* Joseph Itskovitz-Eldor, Sander S. Shapiro,
Michelle A. Waknitz, Jennifer J. Swiergiel, Vivienne S. Marshall,
Jeffrey M. Jones

SCIENCE VOL 282 6 NOVEMBER 1998

Fresh and frozen cleavage stage embryos
produced by IVF for clinical purposes

Embryos were cultured to the blastocyst stage

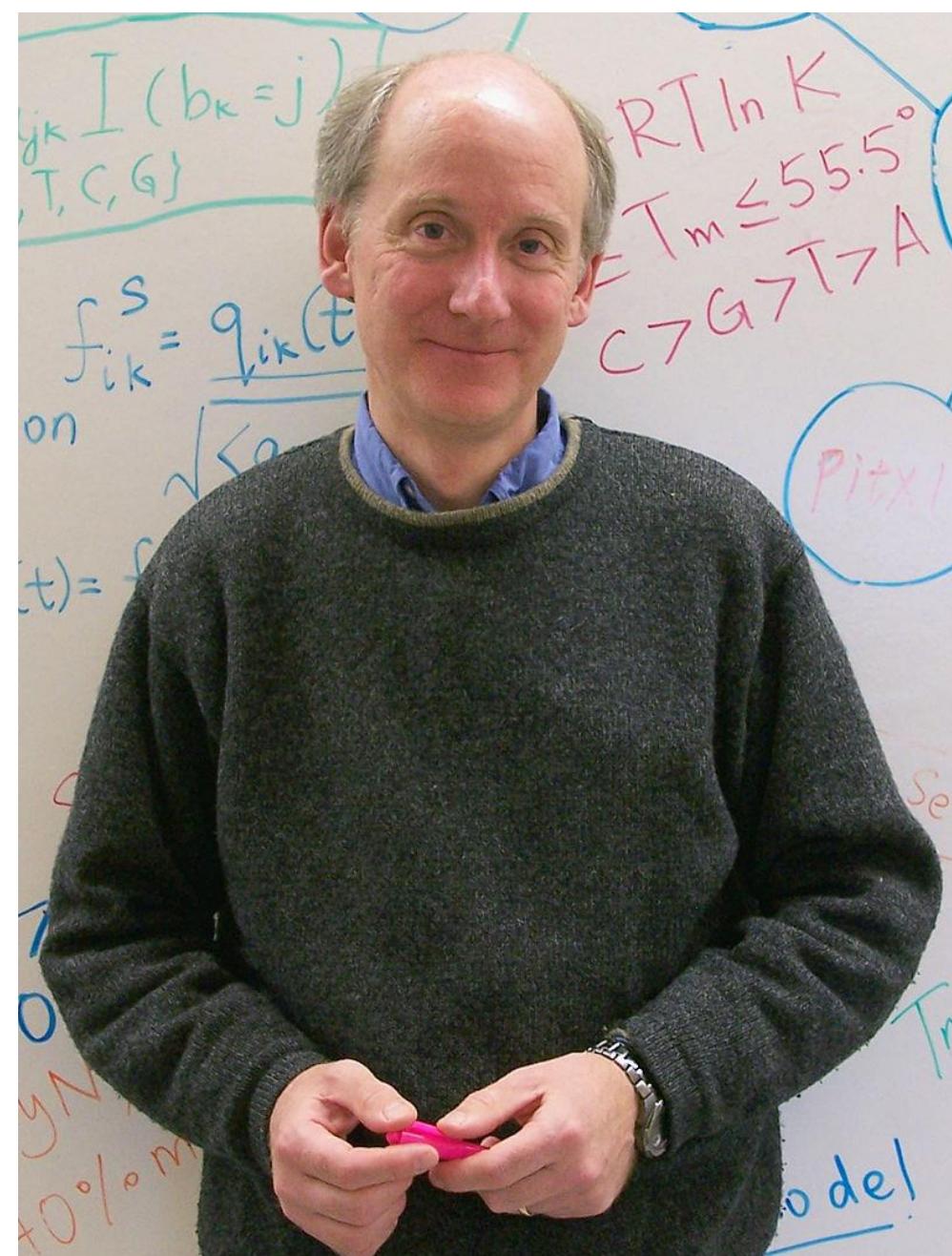
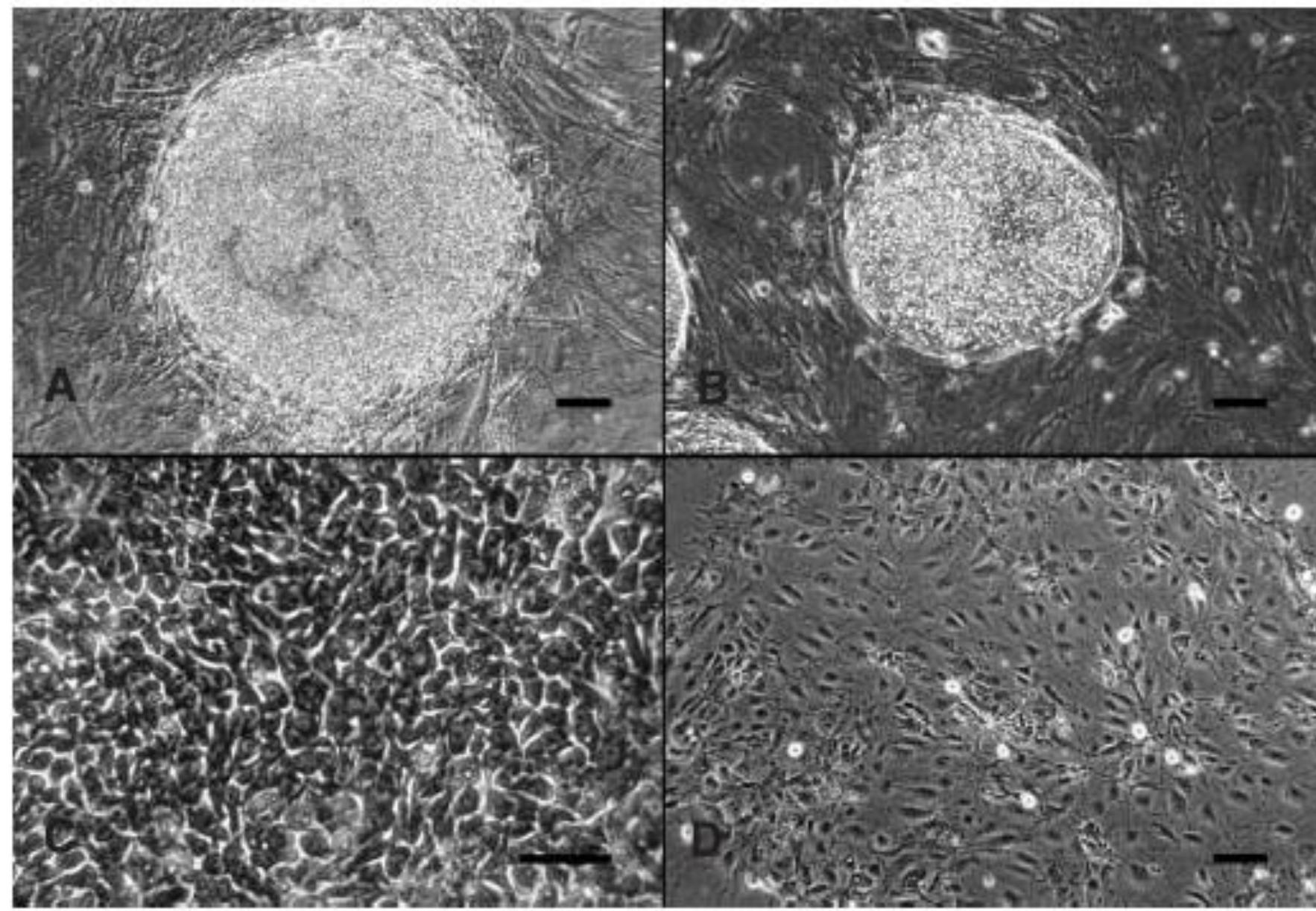


Fig. 1. Derivation of the H9 cell line. (A) Inner cell mass-derived cells attached to mouse embryonic fibroblast feeder layer after 8 days of culture, 24 hours before first dissociation. Scale bar, 100 μ m. (B) H9 colony. Scale bar, 100 μ m. (C) H9 cells. Scale bar, 50 μ m. (D) Differentiated H9 cells, cultured for 5 days in the absence of mouse embryonic fibroblasts, but in the presence of human LIF (20 ng/ml; Sigma). Scale bar, 100 μ m.



cultured for more than 30 passages

Thomson et al. 1998; Science

Fig. 3. Expression of cell surface markers by H9 cells. Scale bar, 100 μ m. (A) Alkaline phosphatase. (B) SSEA-1. Undifferentiated cells failed to stain for SSEA-1 (large colony, left). Occasional colonies consisted of non-stained, central, undifferentiated cells surrounded by a margin of stained, differentiated, epithelial cells (small colony, right). (C) SSEA-3. Some small colonies stained uniformly for SSEA-3 (colony left of center), but most colonies contained a mixture of weakly stained cells and a majority of non-stained cells (colony right of center). (D) SSEA-4. (E) TRA-1-60. (F) TRA-1-81. Similar results were obtained for cell lines H1, H7, H13, and H14.

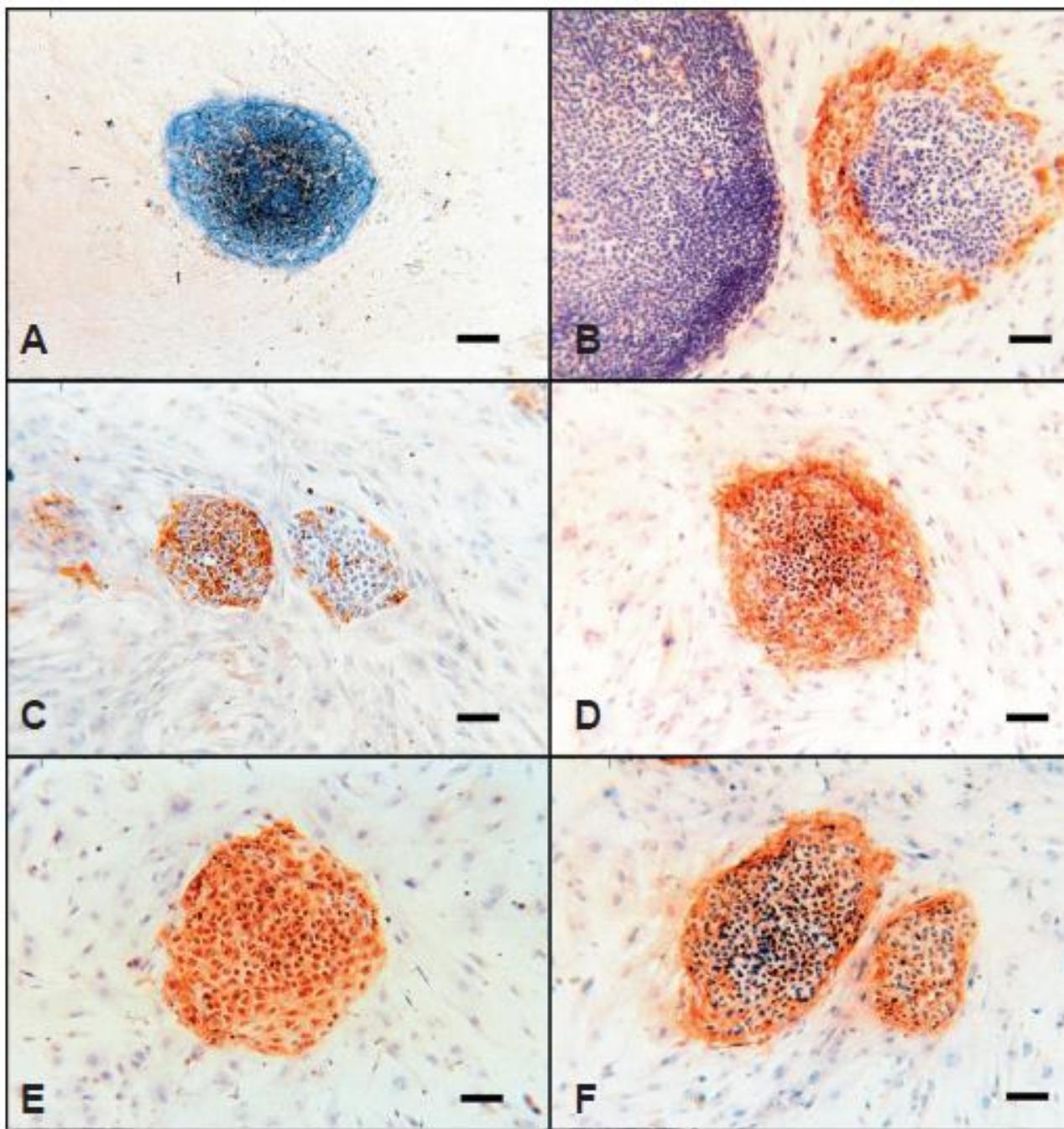
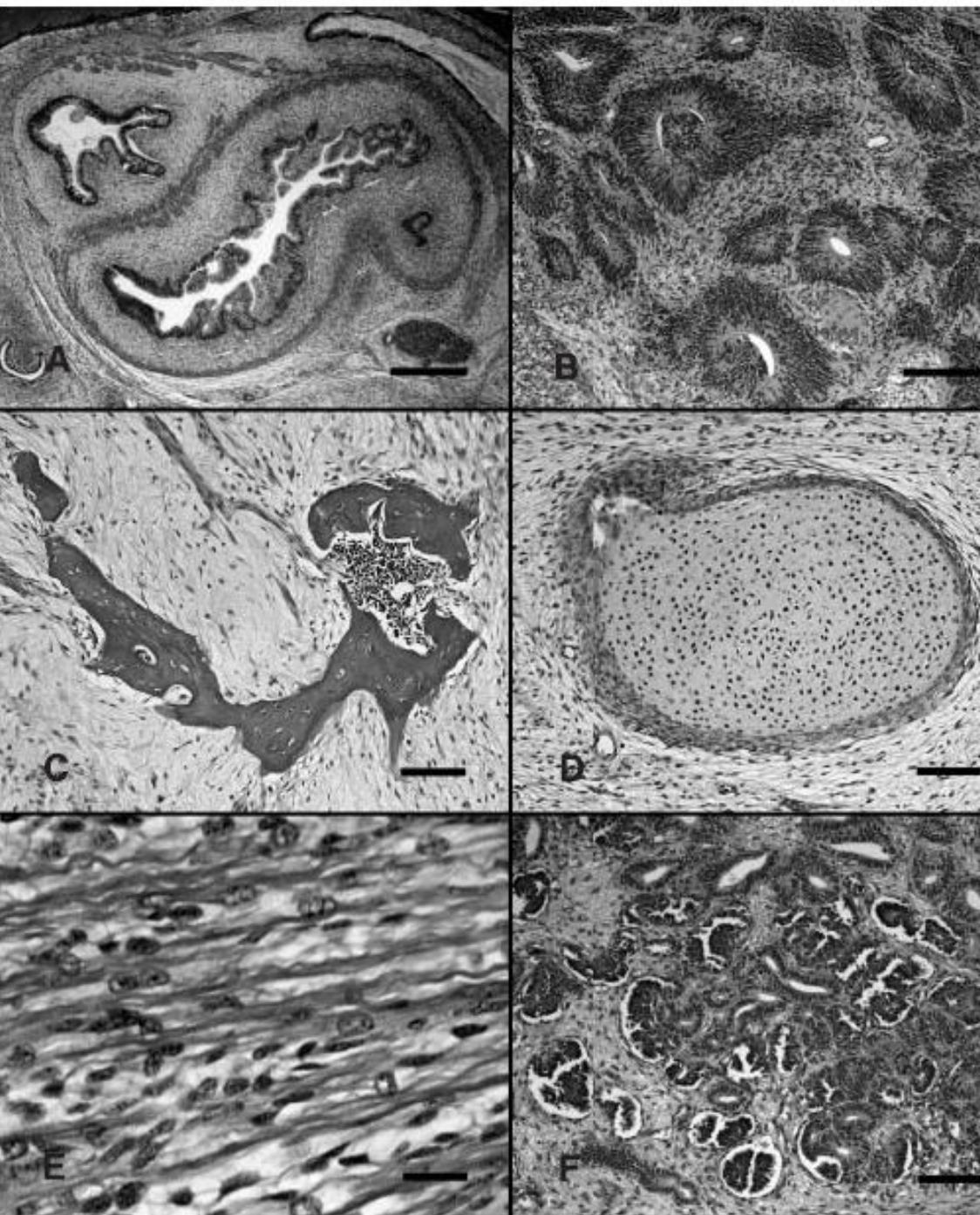


Fig. 4. Teratomas formed by the human ES cell lines in SCID-beige mice. Human ES cells after 4 to 5 months of culture (passages 14 to 16) from about 50% confluent six-well plates were injected into the rear leg muscles of 4-week-old male SCID-beige mice (two or more mice per cell line). Seven to eight weeks after injection, the resulting teratomas were examined histologically. (A) Gutlike structures. Cell line H9. Scale bar, 400 μ m. (B) Rosettes of neural epithelium. Cell line H14. Scale bar, 200 μ m. (C) Bone. Cell line H14. Scale bar, 100 μ m. (D) Cartilage. Cell line H9. Scale bar, 100 μ m. (E) Striated muscle. Cell line H13. Scale bar, 25 μ m. (F) Tubules interspersed with structures resembling fetal glomeruli. Cell line H9. Scale bar, 100 μ m.

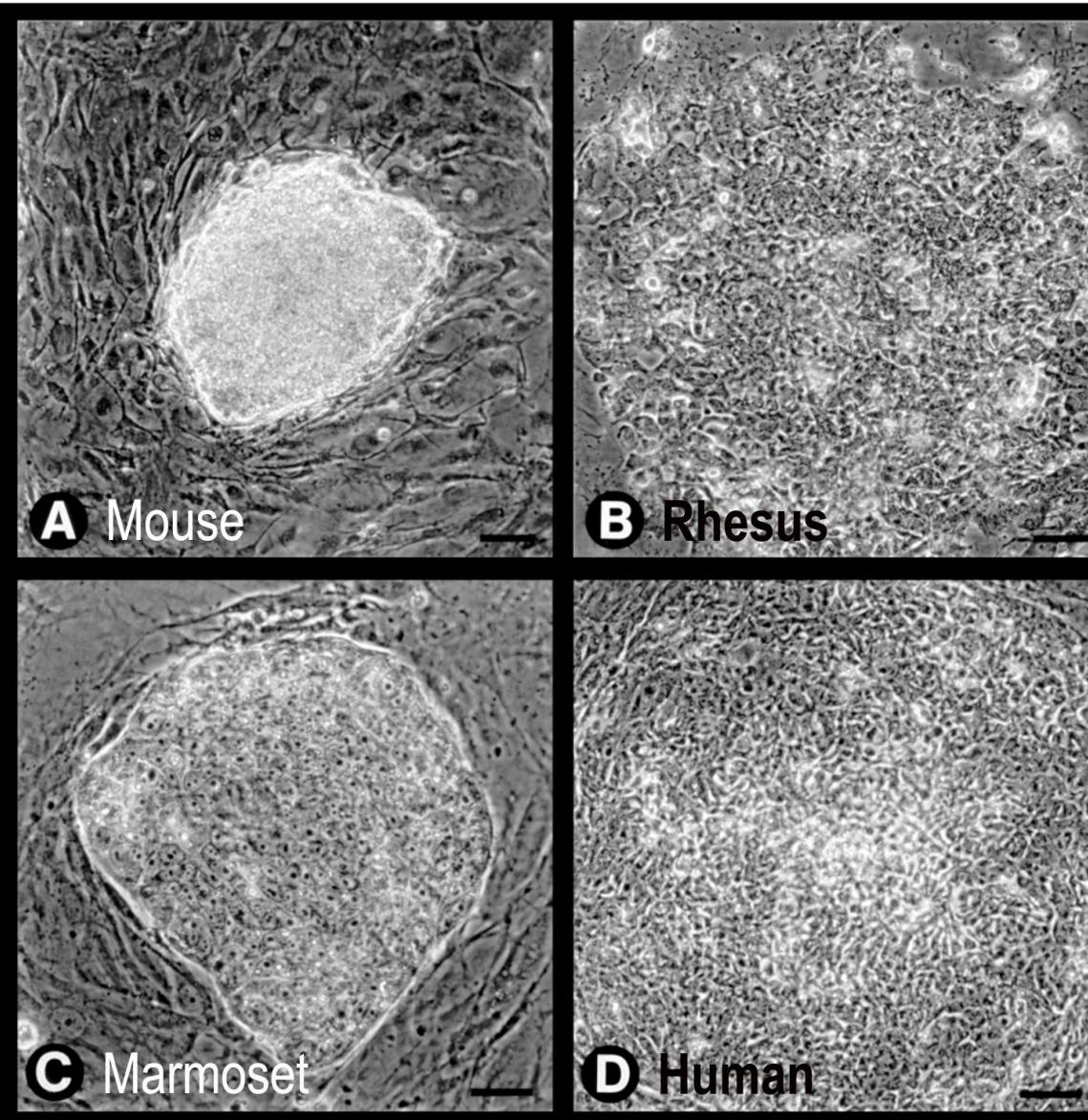


Thomson et al. 1998; Science

The first significant studies that explored the role of **Fibroblast Growth Factor (FGF)** in the growth and maintenance of **human embryonic stem cells (hESCs)** were conducted in the early 2000s. These studies helped establish the crucial role of FGF in regulating the self-renewal and pluripotency of hESCs.

One of the most influential early studies was published in **2001** by **James A. Thomson** and colleagues, who were the first to isolate and culture human embryonic stem cells. This landmark work established the foundational techniques for the culturing of human embryonic stem cells and provided insights into the factors necessary for their maintenance in an undifferentiated state.

ES cells isolated from other species



Embryonic stem cell colonies cultured by Dr. James Thomson.

Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer

Masahito Tachibana,¹ Paula Amato,² Michelle Sparman,¹ Nuria Marti Gutierrez,¹ Rebecca Tippner-Hedges,¹ Hong Ma,¹ Eunju Kang,¹ Alimujiang Fulati,¹ Hyo-Sang Lee,^{1,6} Hathaitip Sritanaudomchai,³ Keith Masterson,² Janine Larson,² Deborah Eaton,² Karen Sadler-Fredd,² David Battaglia,² David Lee,² Diana Wu,² Jeffrey Jensen,^{1,4} Phillip Patton,² Sumita Gokhale,⁵ Richard L. Stouffer,^{1,2} Don Wolf,¹ and Shoukhrat Mitalipov^{1,2,*}

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⁵Boston University School of Medicine, 72 East Concord Street, Boston, MA 02118, USA

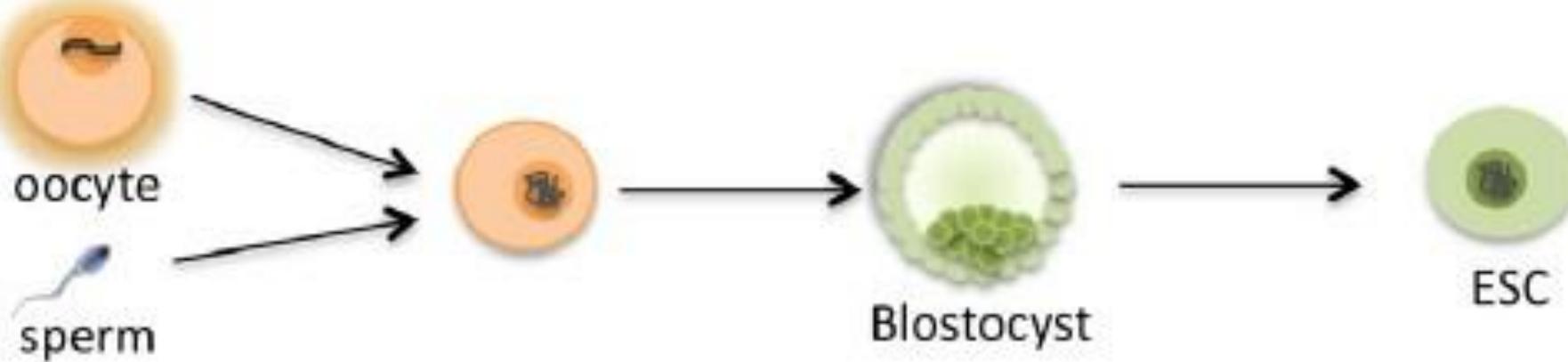
⁶Present address: Laboratory Animal Center, Osong Medical Innovation Foundation, Chungbuk 363-951, Republic of Korea

*Correspondence: mitalipo@ohsu.edu

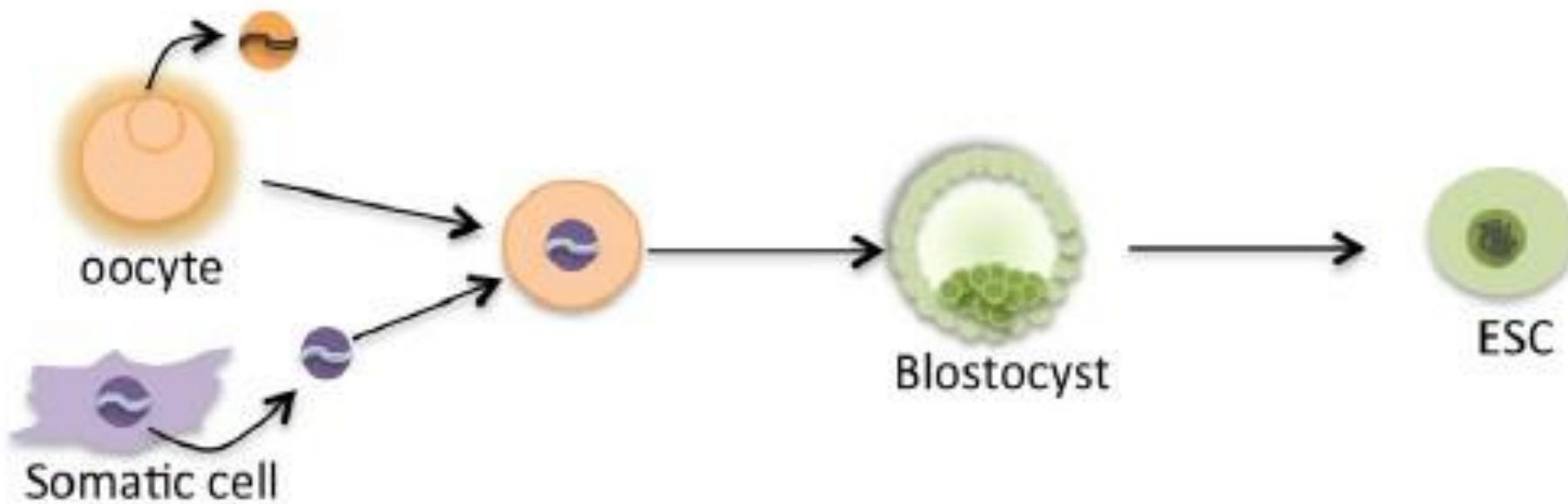
<http://dx.doi.org/10.1016/j.cell.2013.05.006>

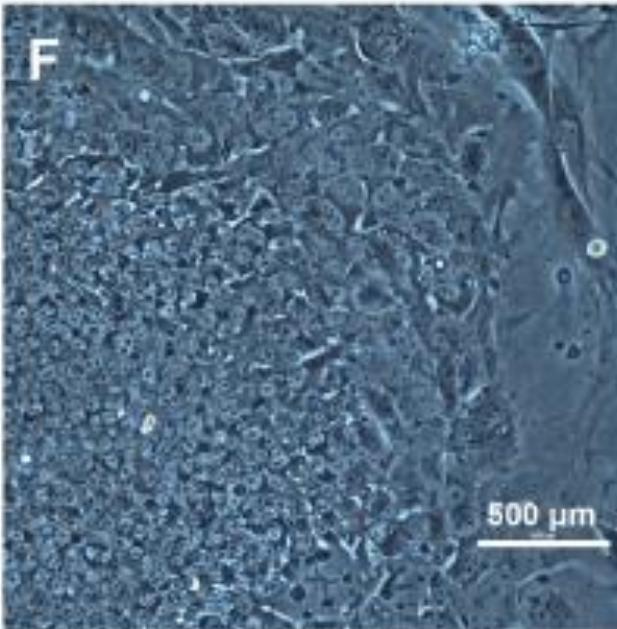
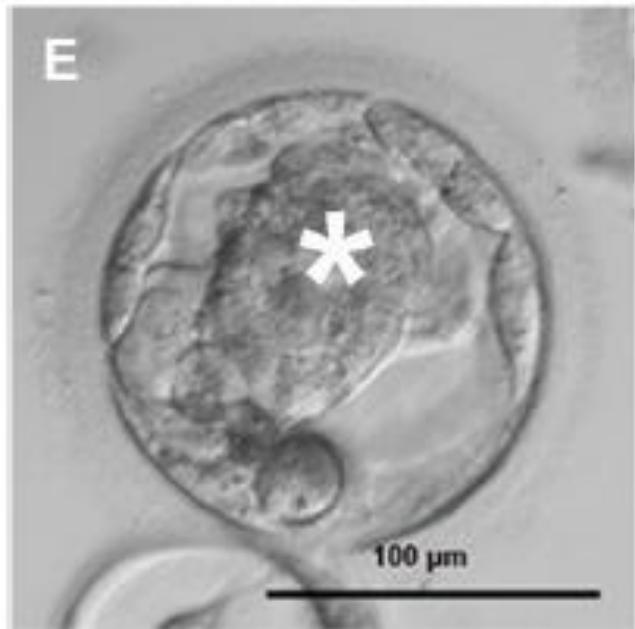
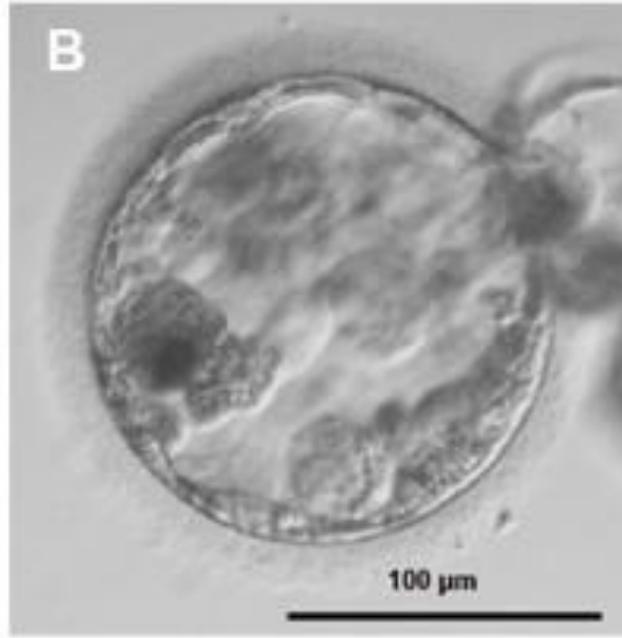
Cell 153, 1228–1238, June 6, 2013

a. Fertilization



b. Nuclear transfer





**used fetal and infantile somatic
cells to produce their ESC**

Human Somatic Cell Nuclear Transfer Using Adult Cells

Young Gie Chung,^{1,2,9,*} Jin Hee Eum,^{2,9} Jeoung Eun Lee,² Sung Han Shim,³ Vicken Sepilian,⁴ Seung Wook Hong,⁴ Yumie Lee,¹ Nathan R. Treff,⁵ Young Ho Choi,⁶ Erin A. Kimbrel,⁷ Ralph E. Dittman,⁸ Robert Lanza,⁷ and Dong Ryul Lee^{1,2,3,*}

¹Research Institute for Stem Cell Research, CHA Health Systems, Los Angeles, CA 90036, USA

²CHA Stem Cell Institute, CHA University, Seoul 135-081, Korea

³Department of Biomedical Science, CHA University 135-081, Seoul 135-081, Korea

⁴CHA Fertility Center, Los Angeles, CA 90036, USA

⁵Reproductive Medicine Associates of New Jersey, Basking Ridge, NJ 07920, USA

⁶Department of Veterinary Physiology & Pharmacology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA

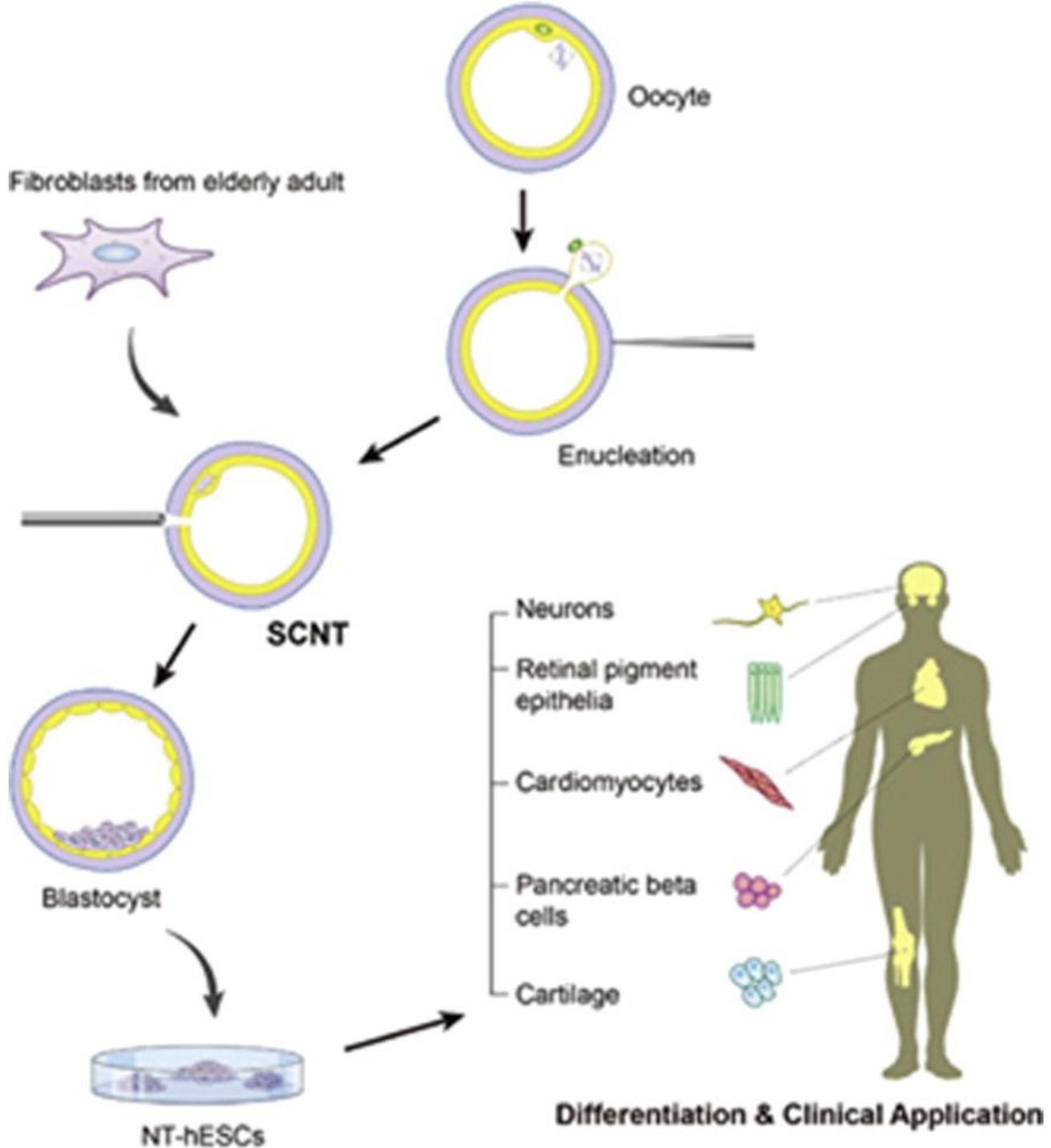
⁷Advanced Cell Technology, Marlborough, MA 01752, USA

⁸Stem Cell Source LLC, Houston, TX 77056, USA

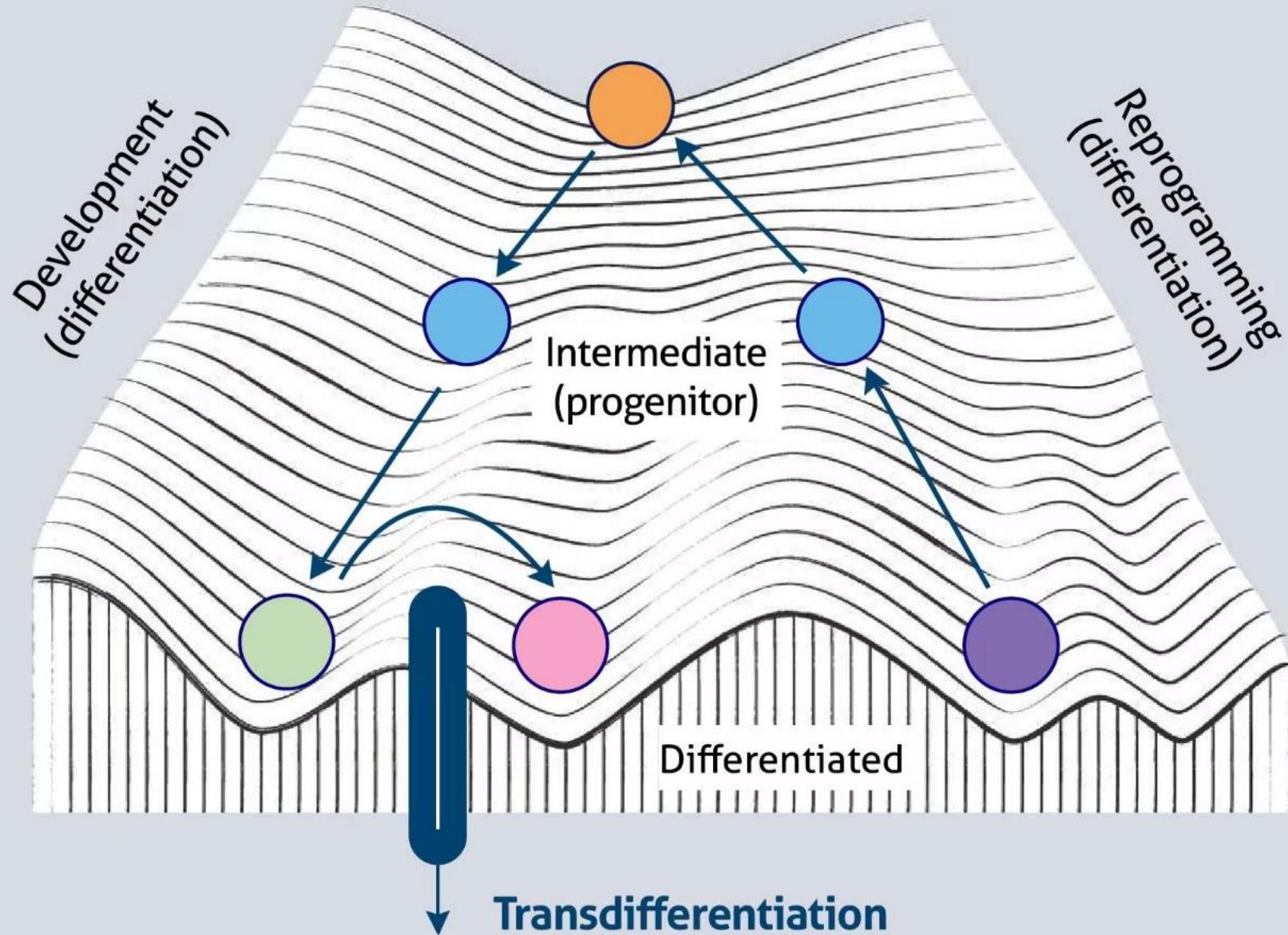
Cell Stem Cell 14, 777–780, June 5, 2014

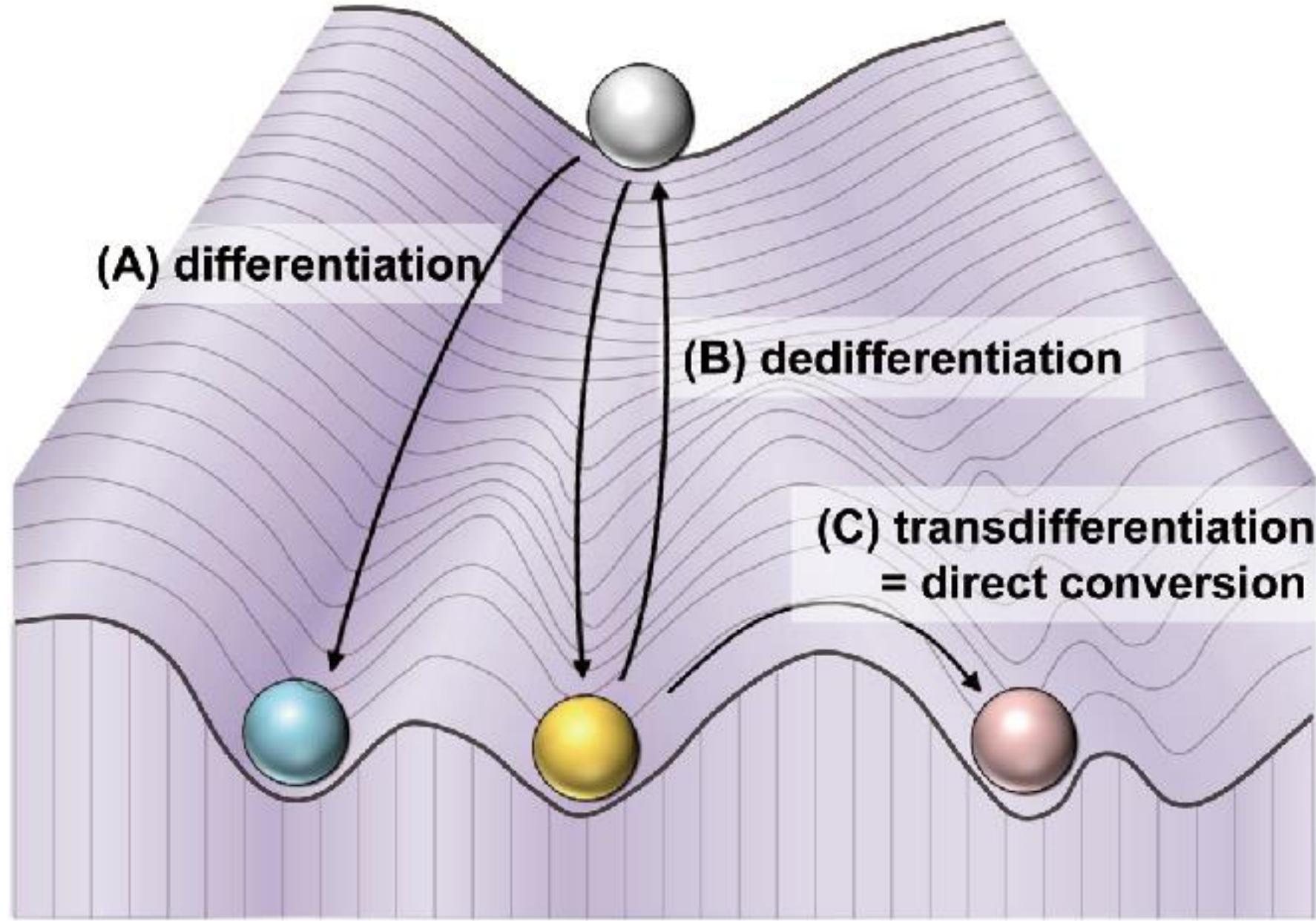
35 year old male

75 year old male



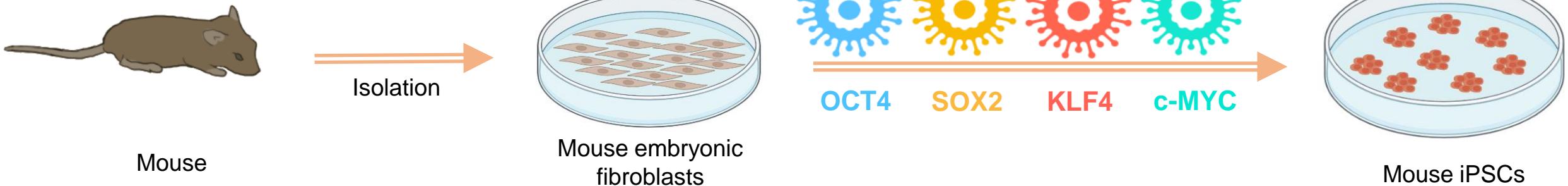
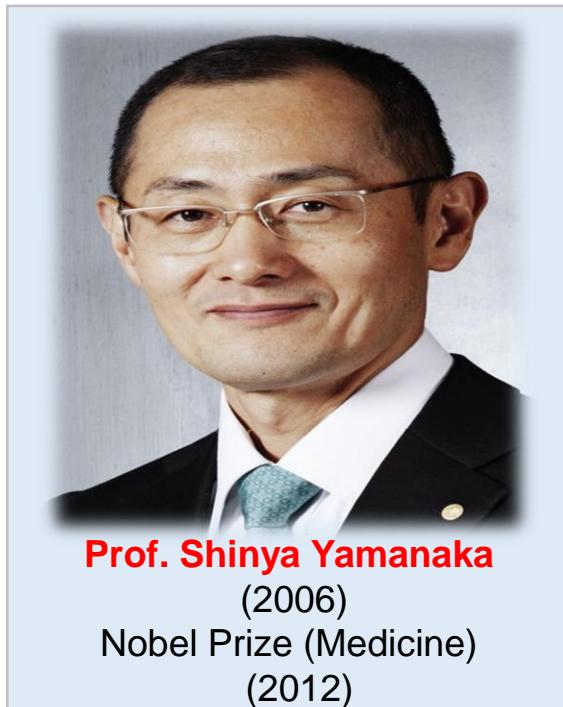
Pluripotent





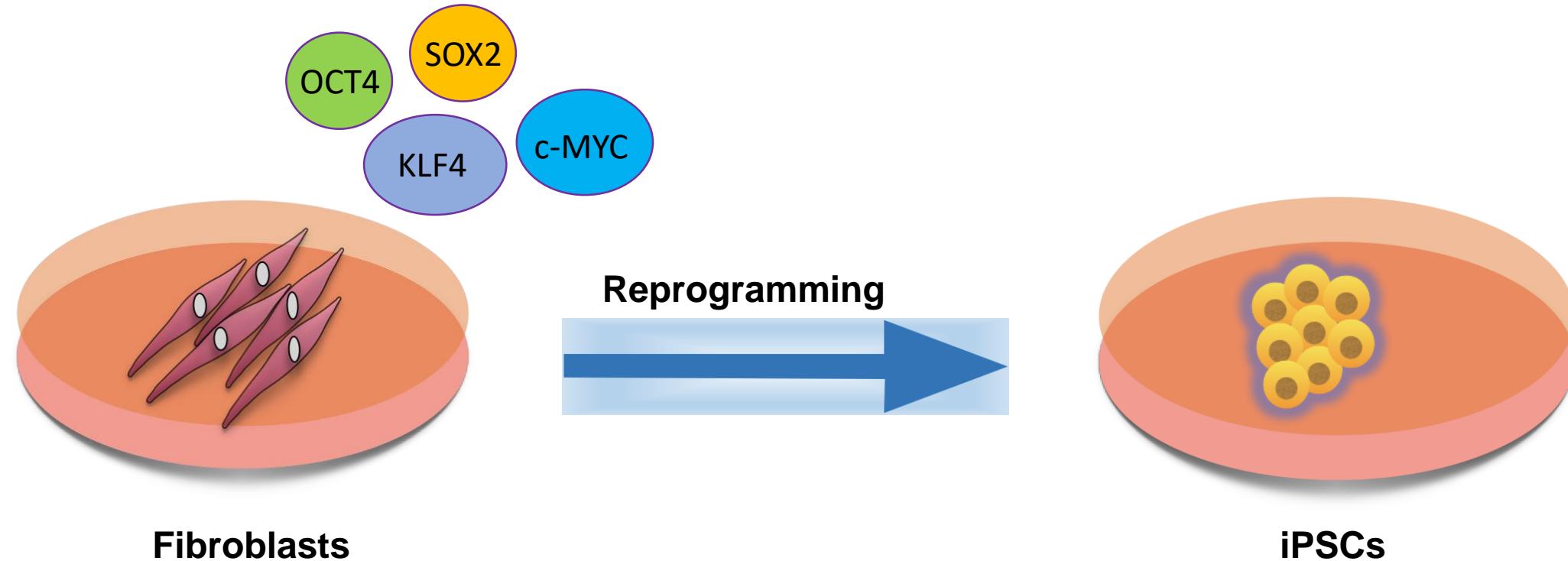
Induced Pluripotent Stem Cells (iPSCs)

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors



- Derived by reprogramming somatic cells to pluripotent state

Induced Pluripotent Stem Cells



24 genes

Induced Pluripotent Stem Cells (iPSCs)

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors



Kazutoshi Takahashi,¹ Koji Tanabe,¹ Mari Ohnuki,¹ Megumi Narita,^{1,2} Tomoko Ichisaka,^{1,2} Kiichiro Tomoda,³ and Shinya Yamanaka^{1,2,3,4,*}

¹Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

²CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

³Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA

⁴Institute for Integrated Cell-Material Sciences, Kyoto University, Kyoto 606-8507, Japan

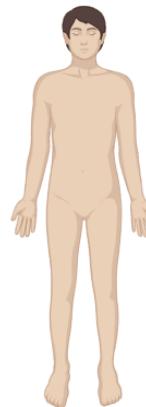
*Correspondence: yamanaka@frontier.kyoto-u.ac.jp

DOI 10.1016/j.cell.2007.11.019

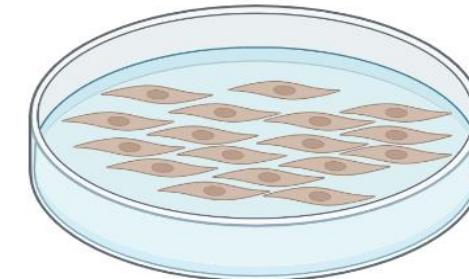
Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells

Junying Yu,^{1,2*} Maxim A. Vodyanik,² Kim Smuga-Otto,^{1,2} Jessica Antosiewicz-Bourget,^{1,2} Jennifer L. Frane, Shulan Tian, Jeff Nie, Gudrun A. Jonsdottir, Victor Ruotti, Ron Stewart,³ Igor I. Slukvin,^{2,4} James A. Thomson^{1,2,5*}

Yamanaka factors
OCT4, SOX2, KLF4, c-MYC

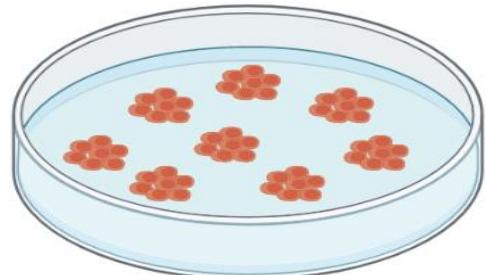
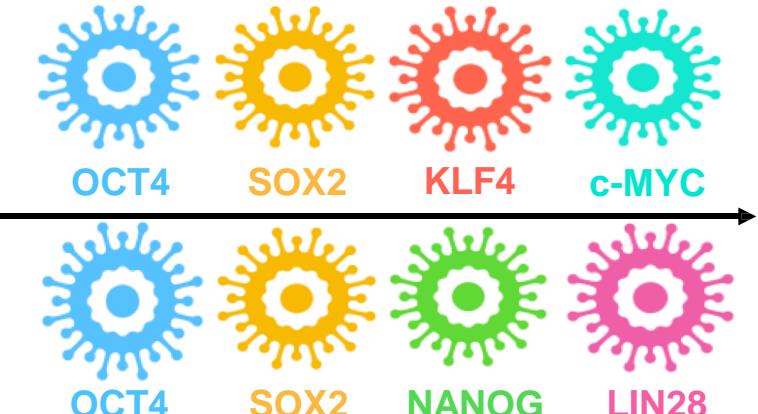


Isolation



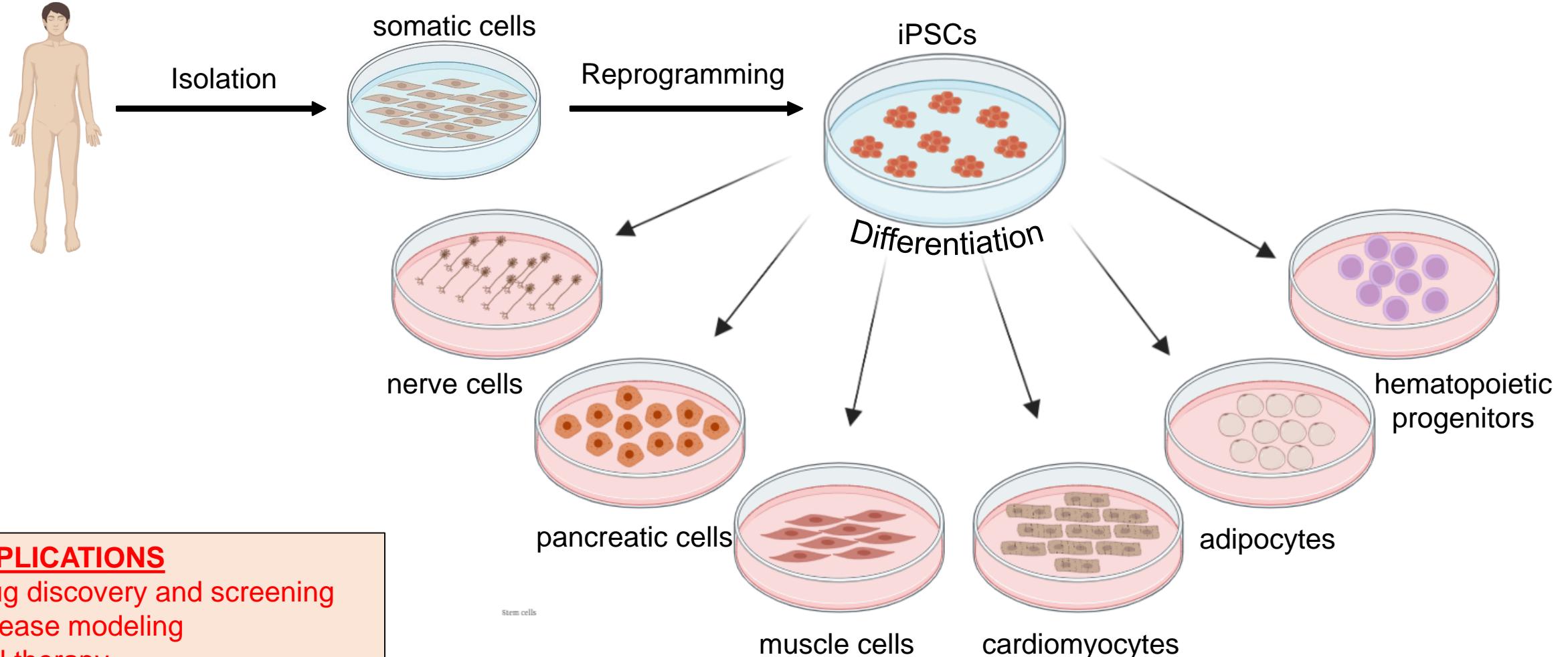
Human

Thomson factors
OCT4, SOX2, NANOG, LIN28



Human iPSCs

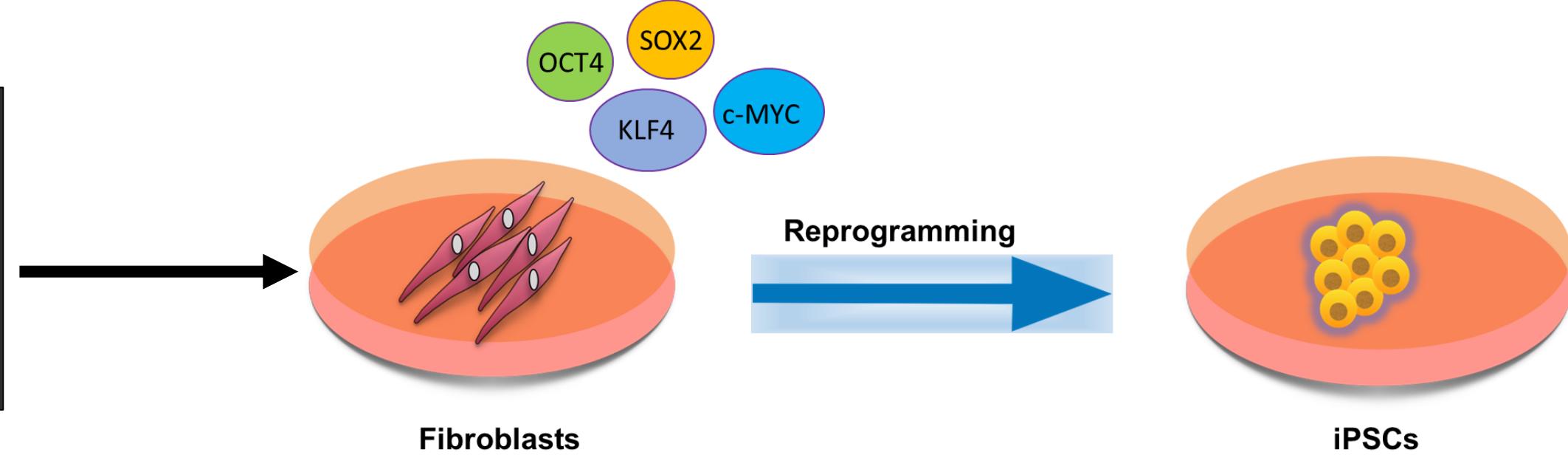
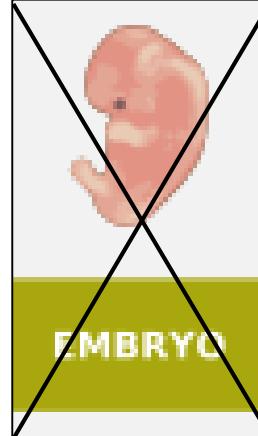
Induced Pluripotent Stem Cells (iPSCs)



Can differentiate into all 3 germ layers: Ectoderm, Endoderm and Mesoderm

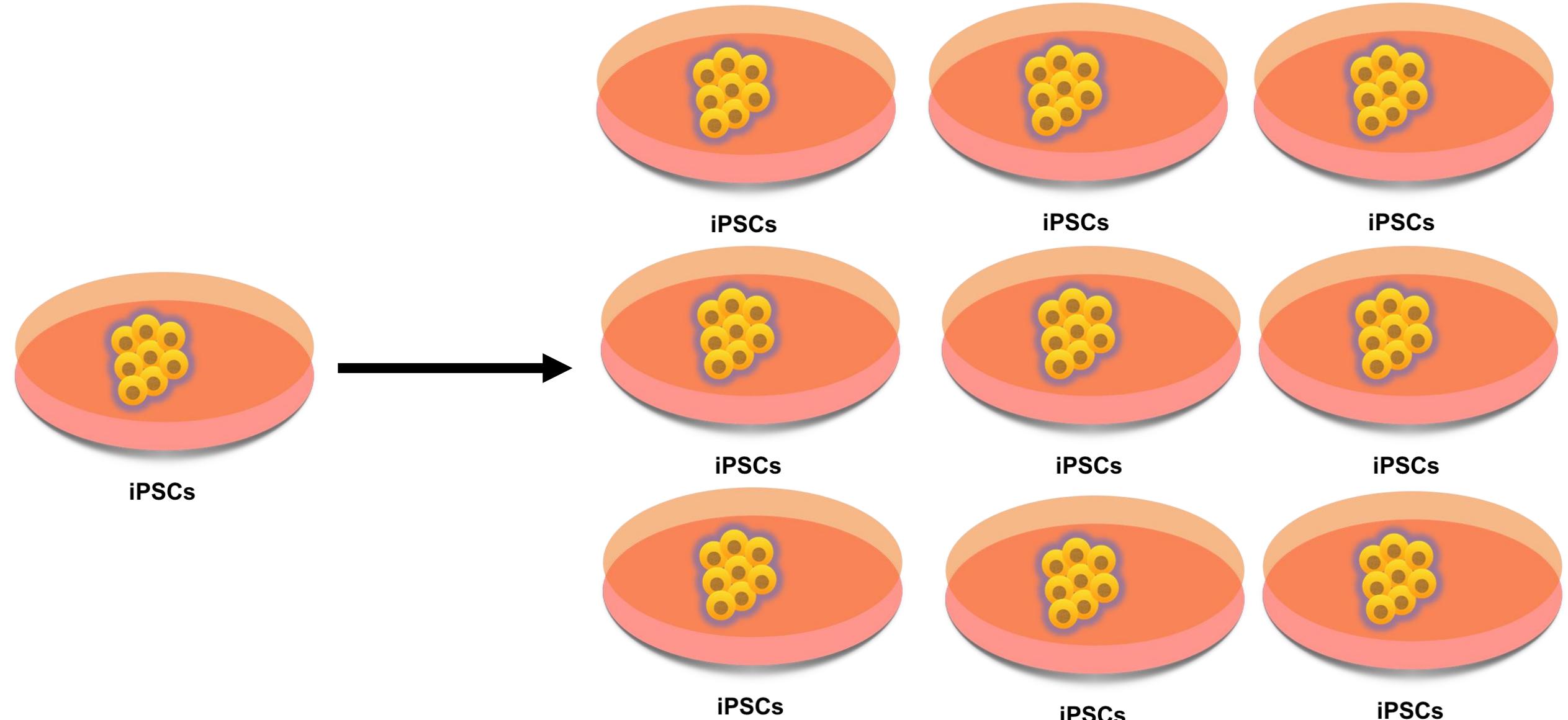
Advantages of iPSCs

- No destruction of embryo (non-invasive source)



Advantages of iPSCs

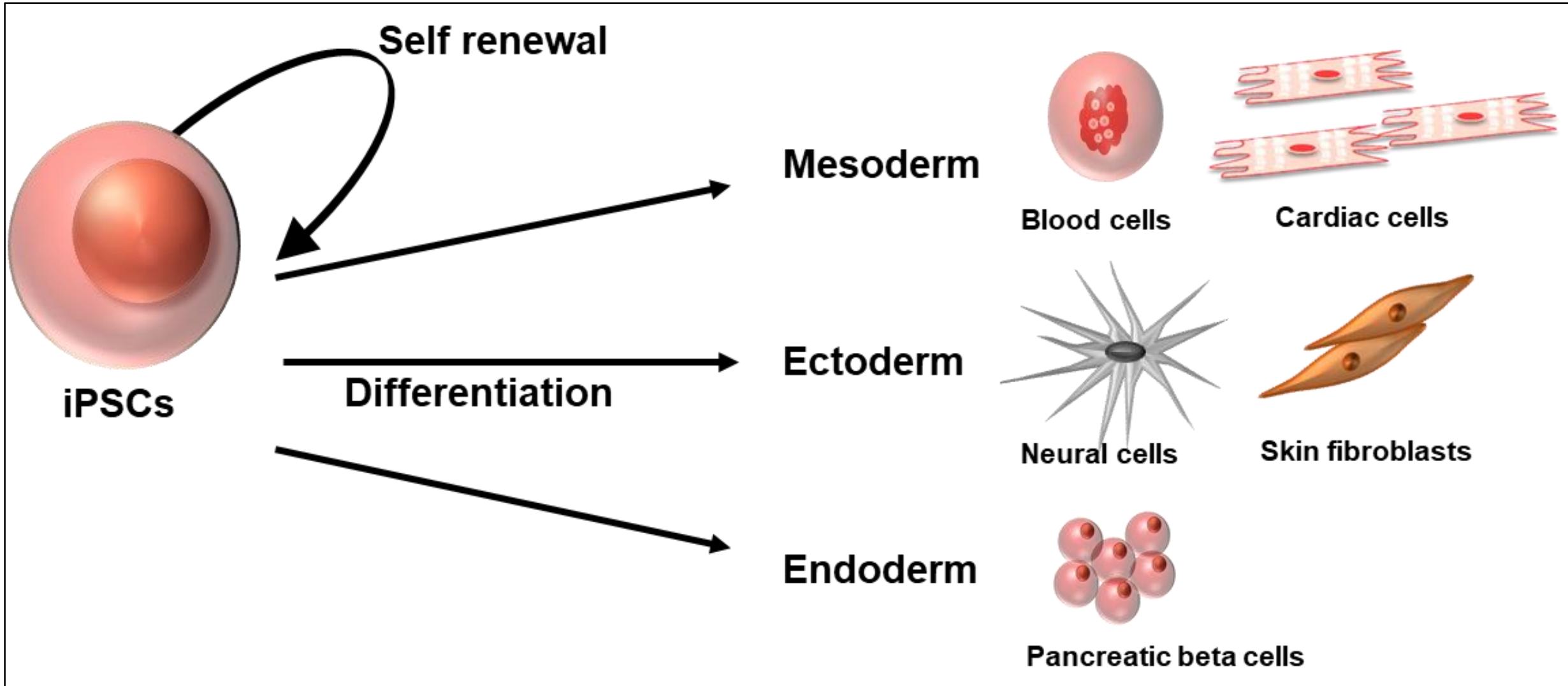
- Unlimited self-renewal ability (maintaining normal karyotype)



Advantages of iPSCs

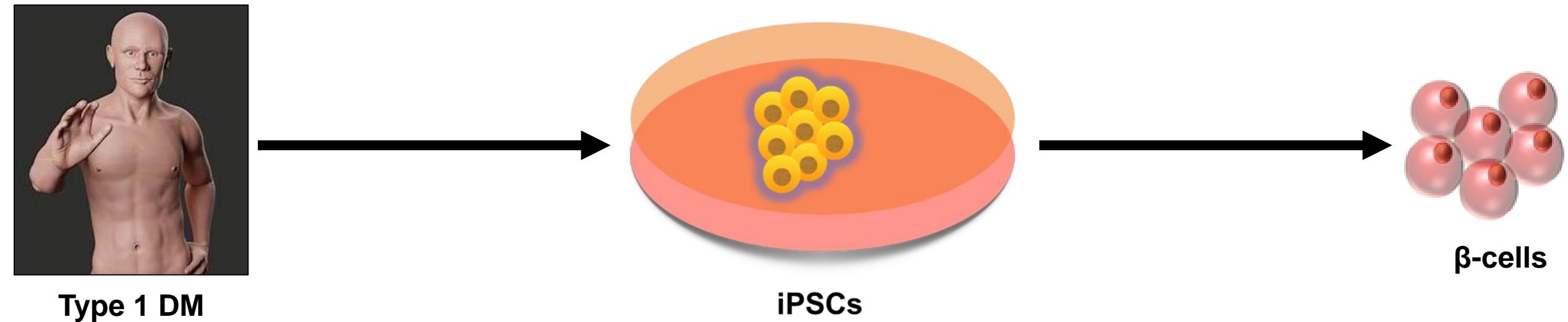
➤ Source of pluripotent cells

200-220 cell types

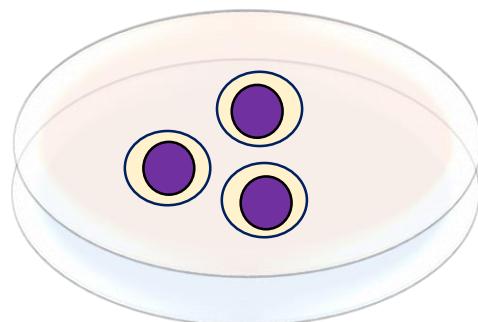
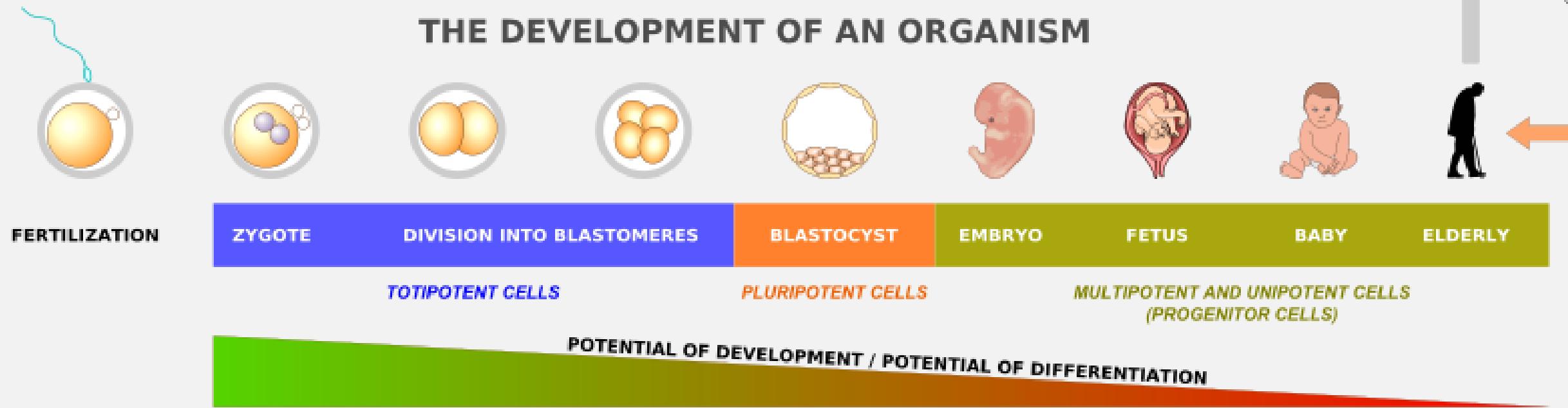


Advantages of iPSCs

- Generates autologous cells (patient-specific iPSCs)

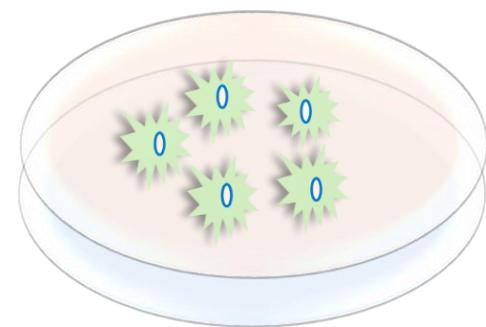


THE DEVELOPMENT OF AN ORGANISM



iPSCs

Reprogramming



Somatic cells

Advantages of iPSCs

- **No destruction of embryo** (non-invasive source)
- **Unlimited self-renewal ability** (maintaining normal karyotype)
- **Source of pluripotent cells** (form desired cell type)
- **Generates autologous cells** (patient-specific iPSCs)

Applications of Stem Cells

Direct delivery

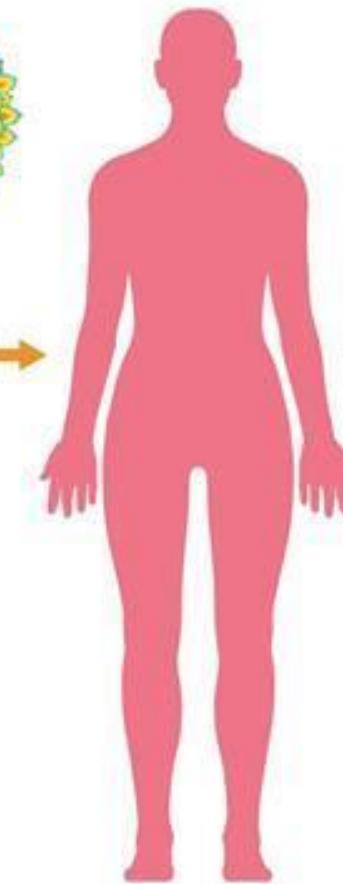
Treatment or missing gene.



The treatment gene is added to a vector, such as an adeno-assisted virus...



...which is delivered directly to the patient by injection.

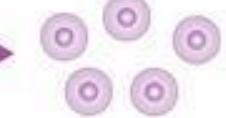


Cell-based delivery

Treatment or missing gene.



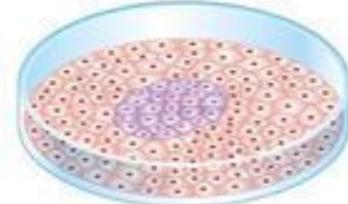
The patient's own stem cells are removed from the body and cultured.



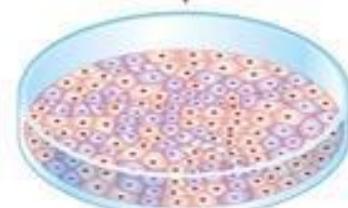
The treatment gene is added to a harmless retrovirus or lentivirus...



...which, in turn, introduces it to the isolated stem cells.

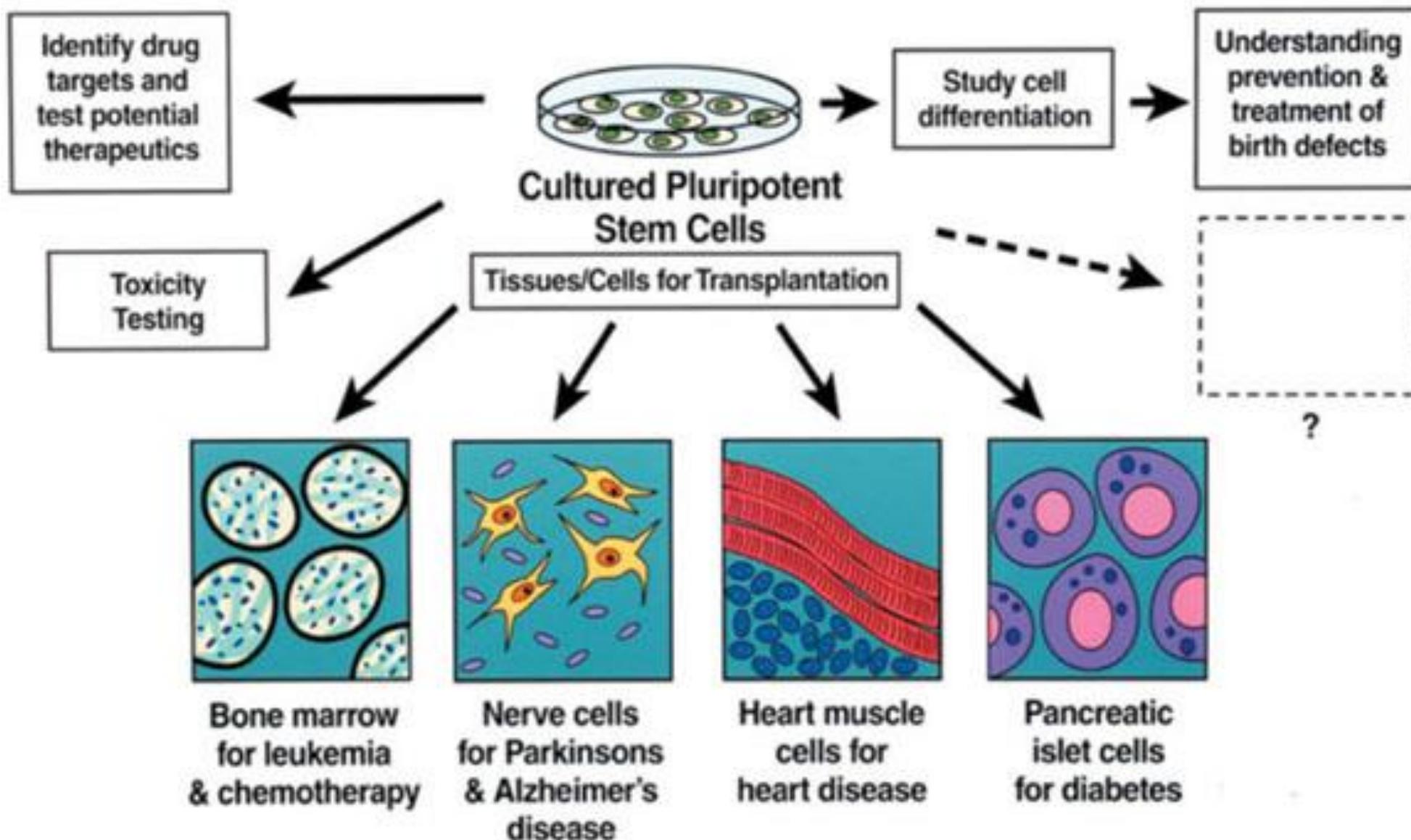


The stems cells, now containing the treatment gene, are returned to the patient.

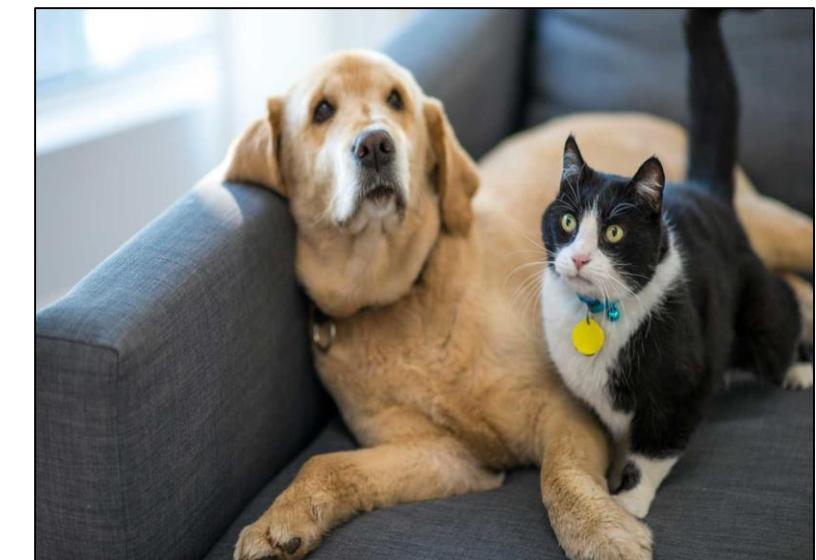


Applications of Stem Cells

The Promise of Stem Cell Research



iPSCs generated from variety of species



Induced Pluripotent Stem Cells (iPSCs)



Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors



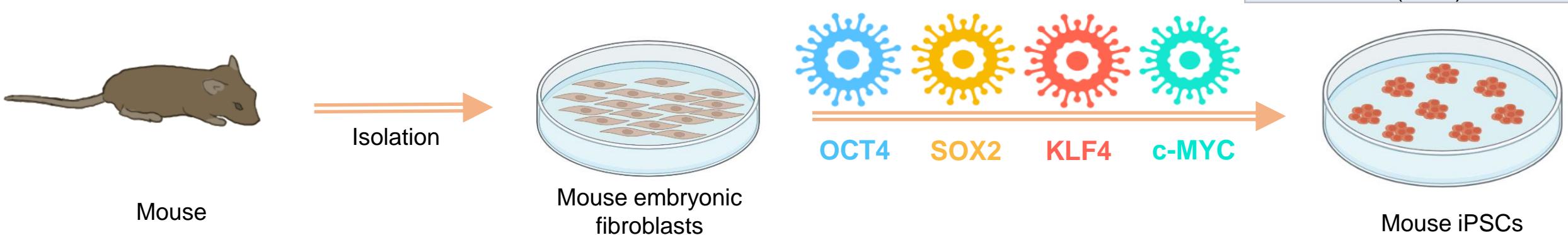
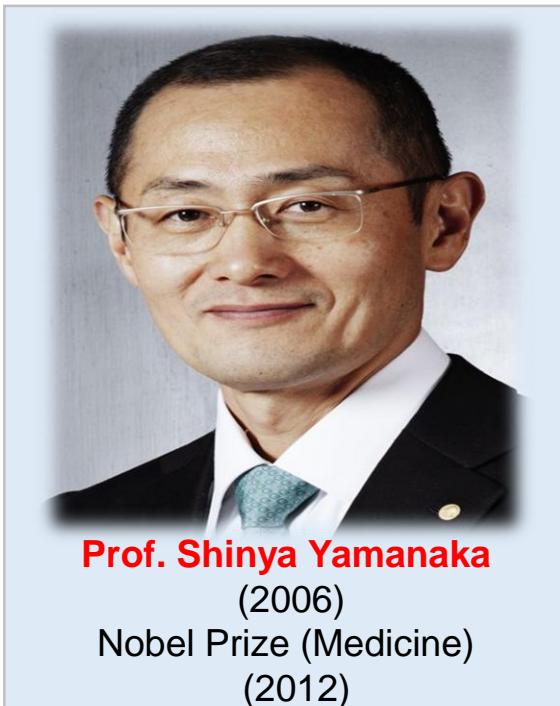
Kazutoshi Takahashi¹ and Shinya Yamanaka^{1,2,*}

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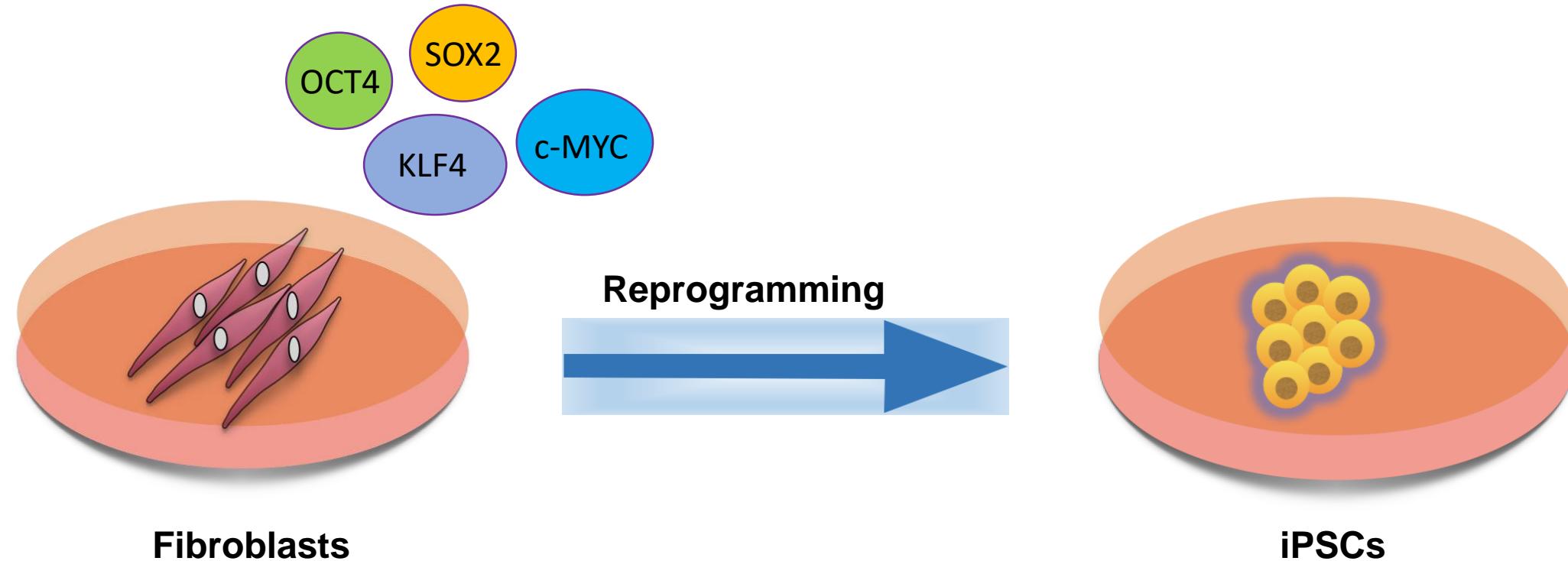
*Contact: yamanaka@frontier.kyoto-u.ac.jp

DOI 10.1016/j.cell.2006.07.024



- Derived by reprogramming somatic cells to pluripotent state

Induced Pluripotent Stem Cells



24 genes

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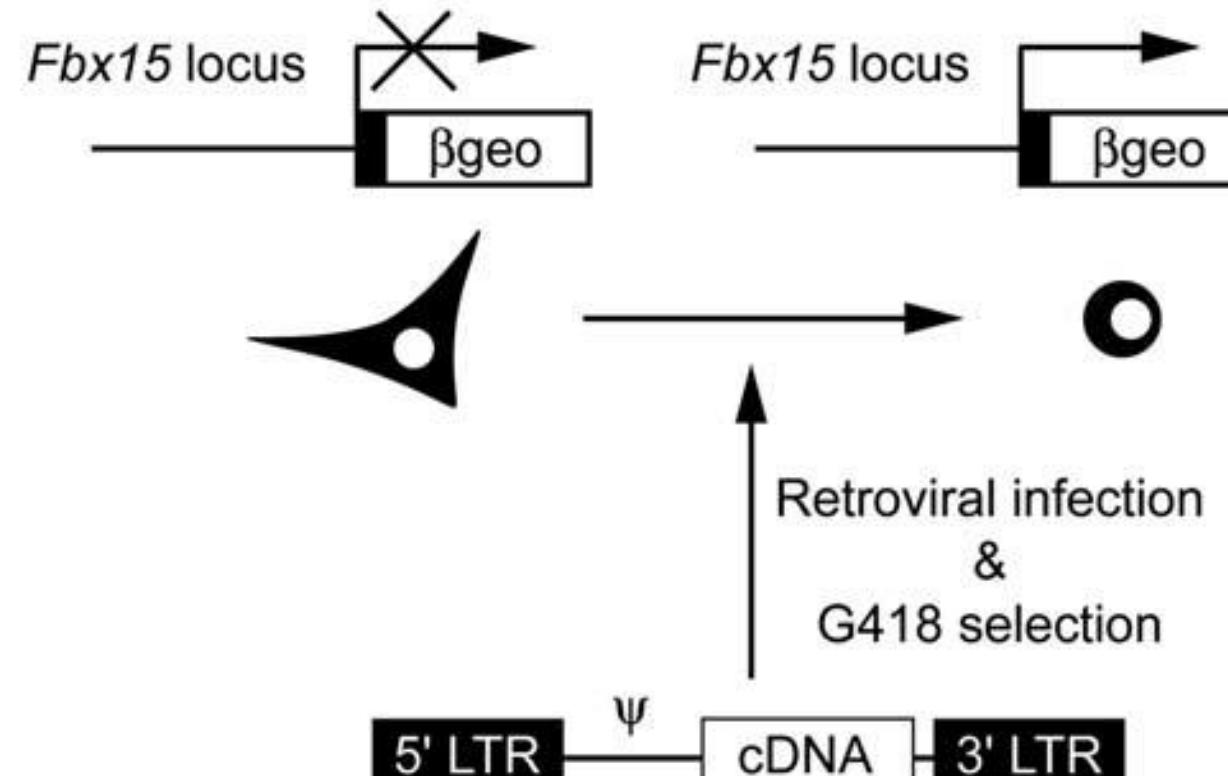
Photograph by Atsushi Mizutani, Center for iPS Cell Research and Application, Kyoto University

Shinya Yamanaka

No	Genes	References
1	Ecat1	Mitsui et al., 2003
2	Dppa5 (Esg1)	Mitsui et al., 2003
3	Fbxo15	Mitsui et al., 2003
4	Nanog	Chambers et al., 2003; Mitsui et al., 2003
5	ERas	Takahashi et al., 2003
6	Dnmt3l	Mitsui et al., 2003
7	Ecat8	Mitsui et al., 2003
8	Gdf3	Mitsui et al., 2003
9	Sox15	Maruyama et al., 2005
10	Dppa4	Mitsui et al., 2003
11	Dppa2	Mitsui et al., 2003
12	Fthl17	Mitsui et al., 2003
13	Sall4	Mitsui et al., 2003
14	Oct3/4 (Pou5f1)	Nichols et al., 1998; Niwa et al., 2000
15	Sox2	Avilion et al., 2003; Maruyama et al., 2005
16	Rex1 (Zfp42)	Rogers et al., 1991
17	Utf1	Okuda et al., 1998
18	Tcl1	Mitsui et al., 2003
19	Dppa3 (Stella)	Mitsui et al., 2003
20	Klf4	Li et al., 2005
21	β-catenin	Kielman et al., 2002; Sato et al., 2004
22	c-Myc	Cartwright et al., 2005
23	Stat3	Matsuda et al., 1999; Niwa et al., 1998
24	Grb2	Burdon et al., 1999; Cheng et al., 1998; Miyamoto et al., 2004

Strategy to test candidate factors

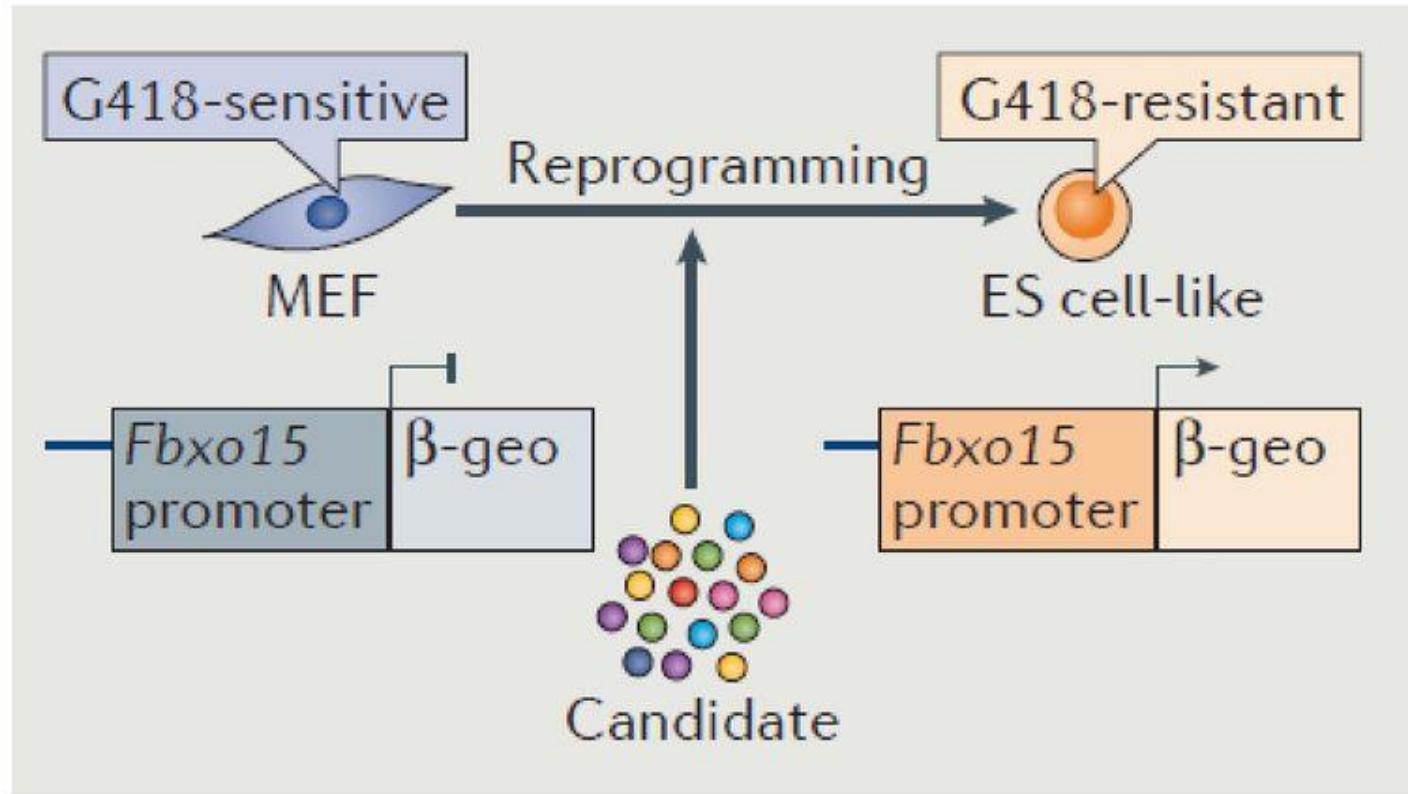
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G418, also known as G418 sulfate and Geneticin, is an aminoglycoside antibiotic similar in structure to gentamicin B1, produced by *Micromonospora rhodorangea*.

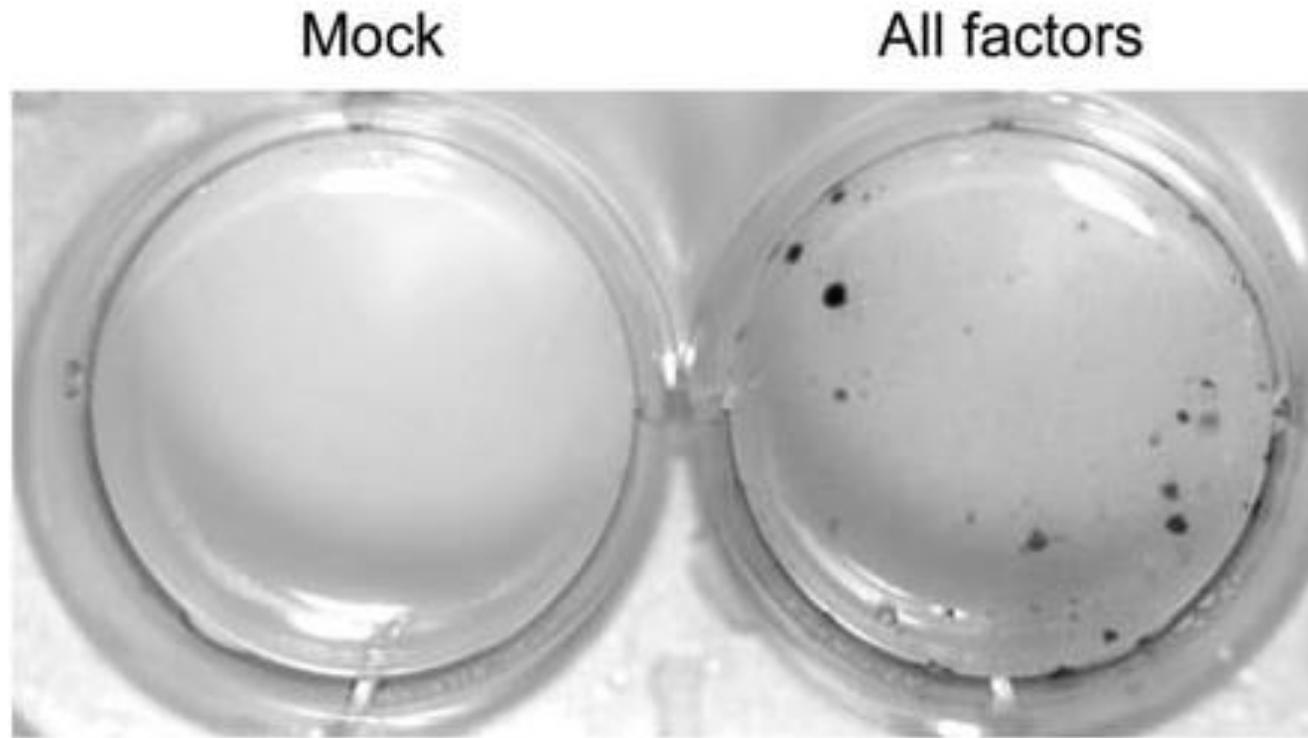
G418 blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. Resistance to G418 is conferred by the Neomycin resistance gene (*neo*) from Tn5 encoding an aminoglycoside 3'-phosphotransferase, APH 3' II.

β -geo cassette (a fusion of the β -galactosidase and neomycin resistance genes) into the mouse *Fbx15* gene by homologous recombination (Tokuzawa et al., 2003). Although specifically expressed in mouse ES cells and early embryos, *Fbx15* is dispensable for the maintenance of pluripotency and mouse development.



Transgenic fibroblasts with a knock-in gene at the *Fbx15* locus. The knock-in was β geo: a fusion of LacZ gene and neomycin-R gene. Normally, ***Fbx15* is highly expressed in ES cells, but not expressed in fibroblasts**

Strategy to test candidate factors



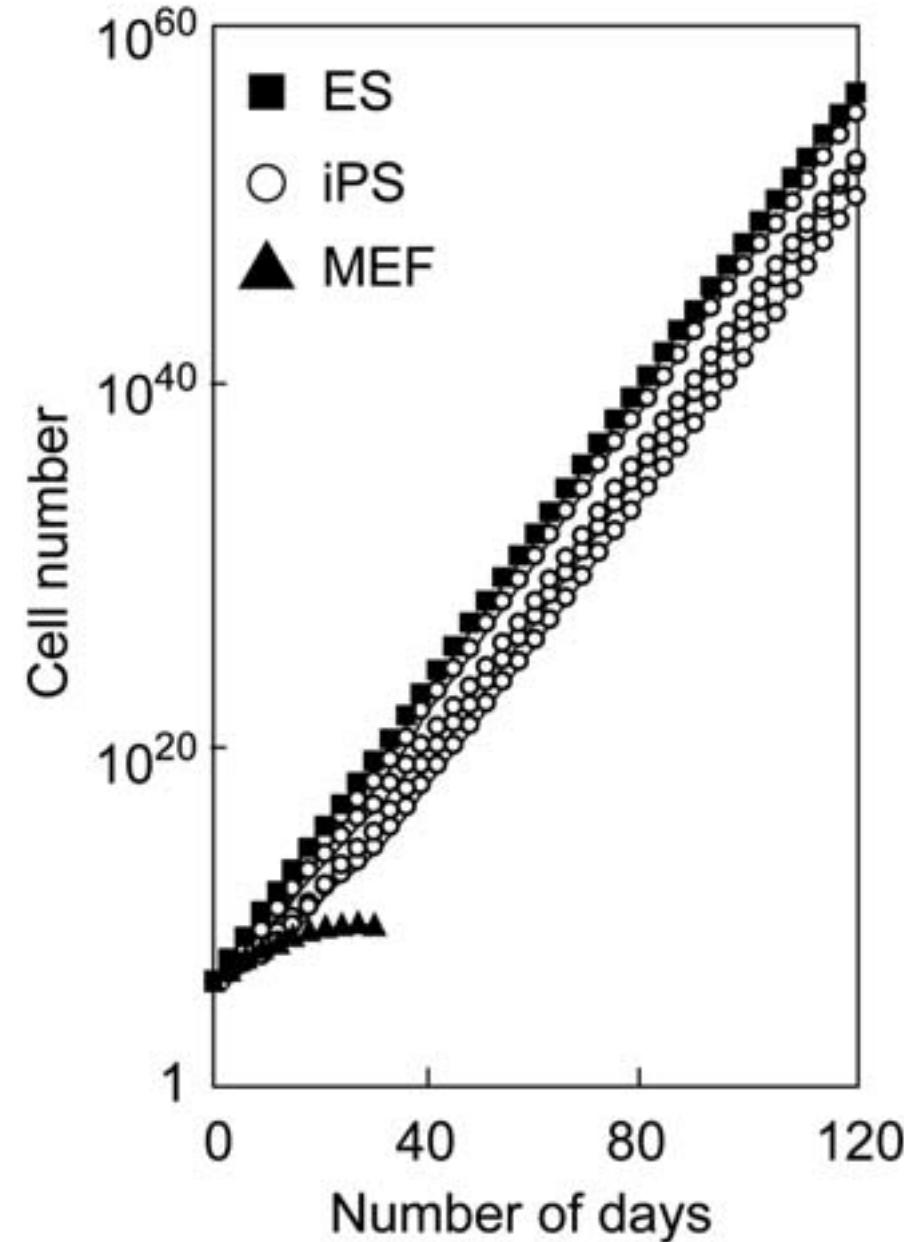
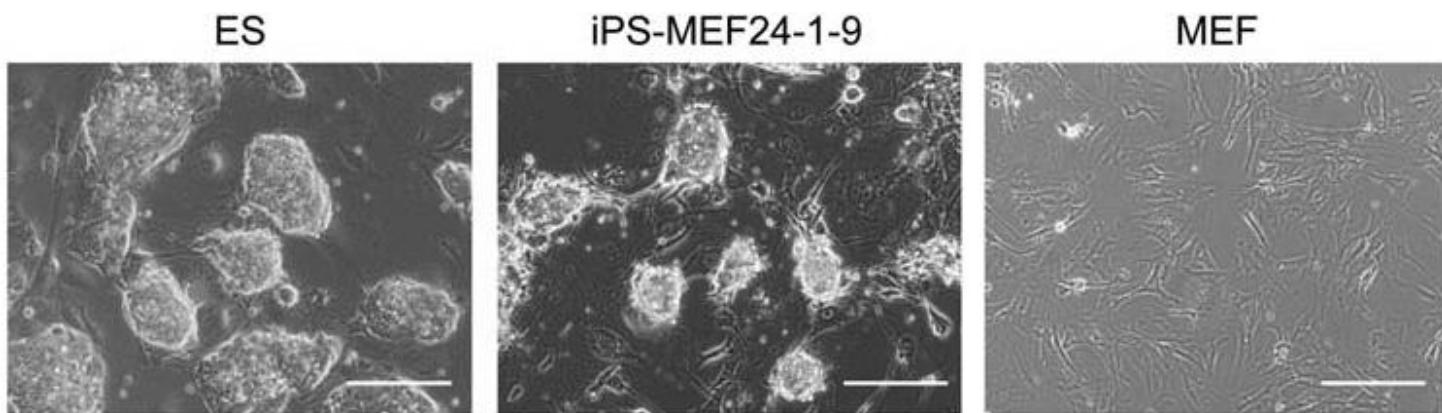
1. 22 G418 resistant colonies – 12 clones tested – 5 clones exhibited ES cell morphology
2. 29 G418 resistant colonies – 06 clones tested – 4 clones exhibited ES cell morphology

iPS-MEF24

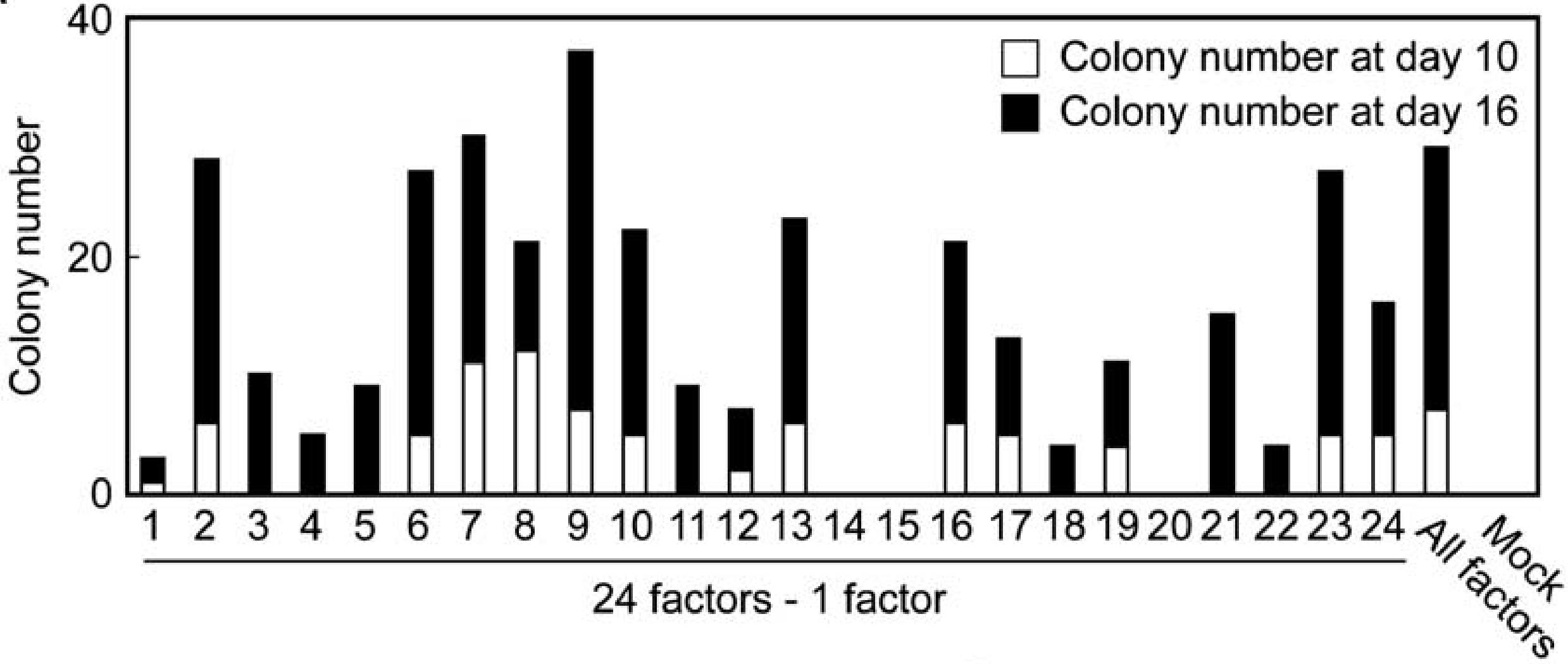
Date	Cells	Transgenes	G418-Resistant Colonies	Colonies Picked up	iPS Clones Established
7/19/2005	MEF 1×10^5	Mock	0		
		Ecat1	0		
		Esg1	0		
		Fbx15	0		
		Nanog	0		
		ERas	0		
		Dnmt31	0		
		Ecat8	0		
		Gdf3	0		
		Sox15	0		
		Dppa4	0		
		Dppa2	0		
		Fthl17	0		
		Sall4	0		
		Oct3/4	0		
		Sox2	0		
		Rex1	0		
		Utf1	0		
		Tcl1	0		
		Dppa3	0		
		Klf4	0		
		β -catenin S33Y	0		
		Myc T58A	0		
		Stat3-C	0		
		Grb2 Δ SH2	0		
		24 factors	22	12	5

Date	Cells	Transgenes	G418-Resistant Colonies	Colonies Picked up	iPS Clones Established
7/19/2005	MEF 1×10^5	Mock	0		
		Ecat1	0		
		Esg1	0		
		Fbx15	0		
		Nanog	0		
		ERas	0		
		Dnmt31	0		
		Ecat8	0		
		Gdf3	0		
		Sox15	0		
		Dppa4	0		
		Dppa2	0		
		Fthl17	0		
		Sall4	0		
		Oct3/4	0		
		Sox2	0		
		Rex1	0		
		Utf1	0		
		Tcl1	0		
		Dppa3	0		
		Klf4	0		
		β -catenin S33Y	0		
		Myc T58A	0		
		Stat3-C	0		
		Grb2 Δ SH2	0		
		24 factors	22	12	5
8/15/2005	MEF 1×10^5	Mock	0		
		24 factors	29	6	4 MEF24-2-1~4

iPS-MEF24 clones

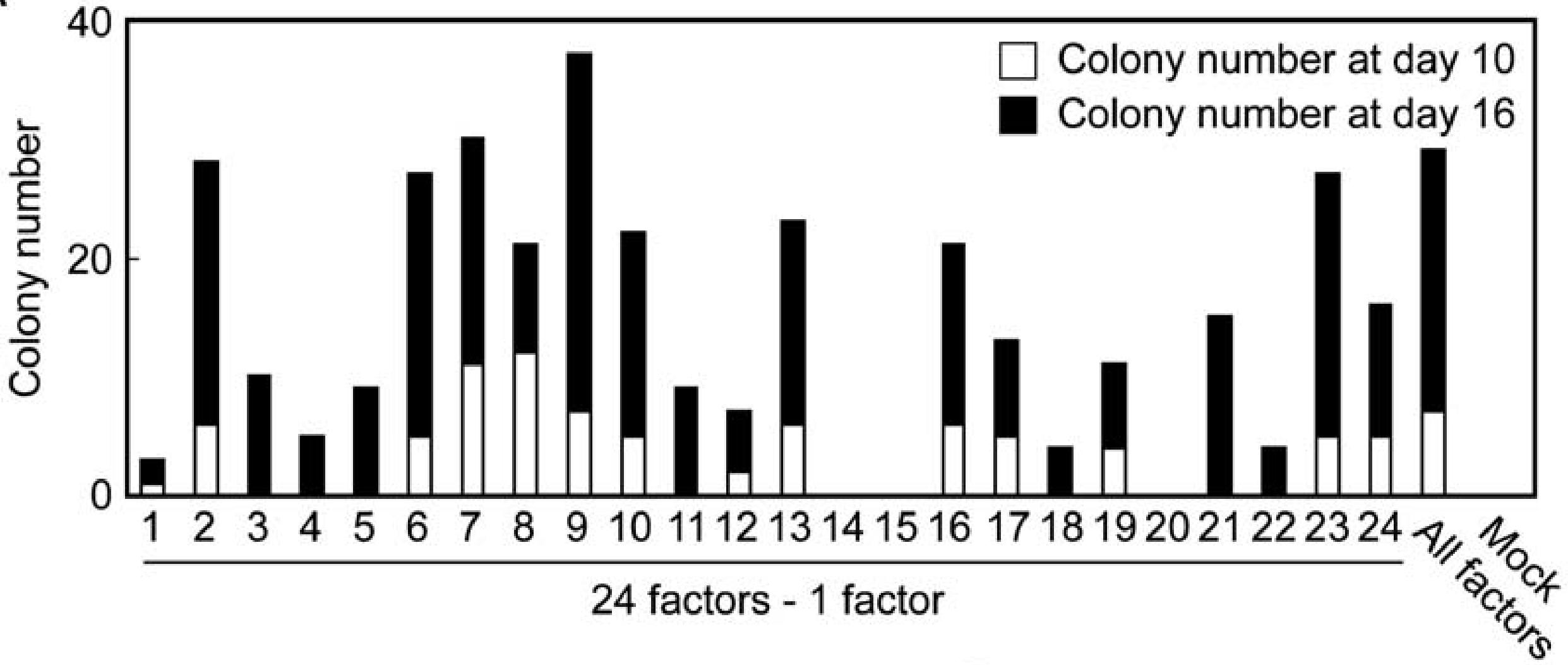


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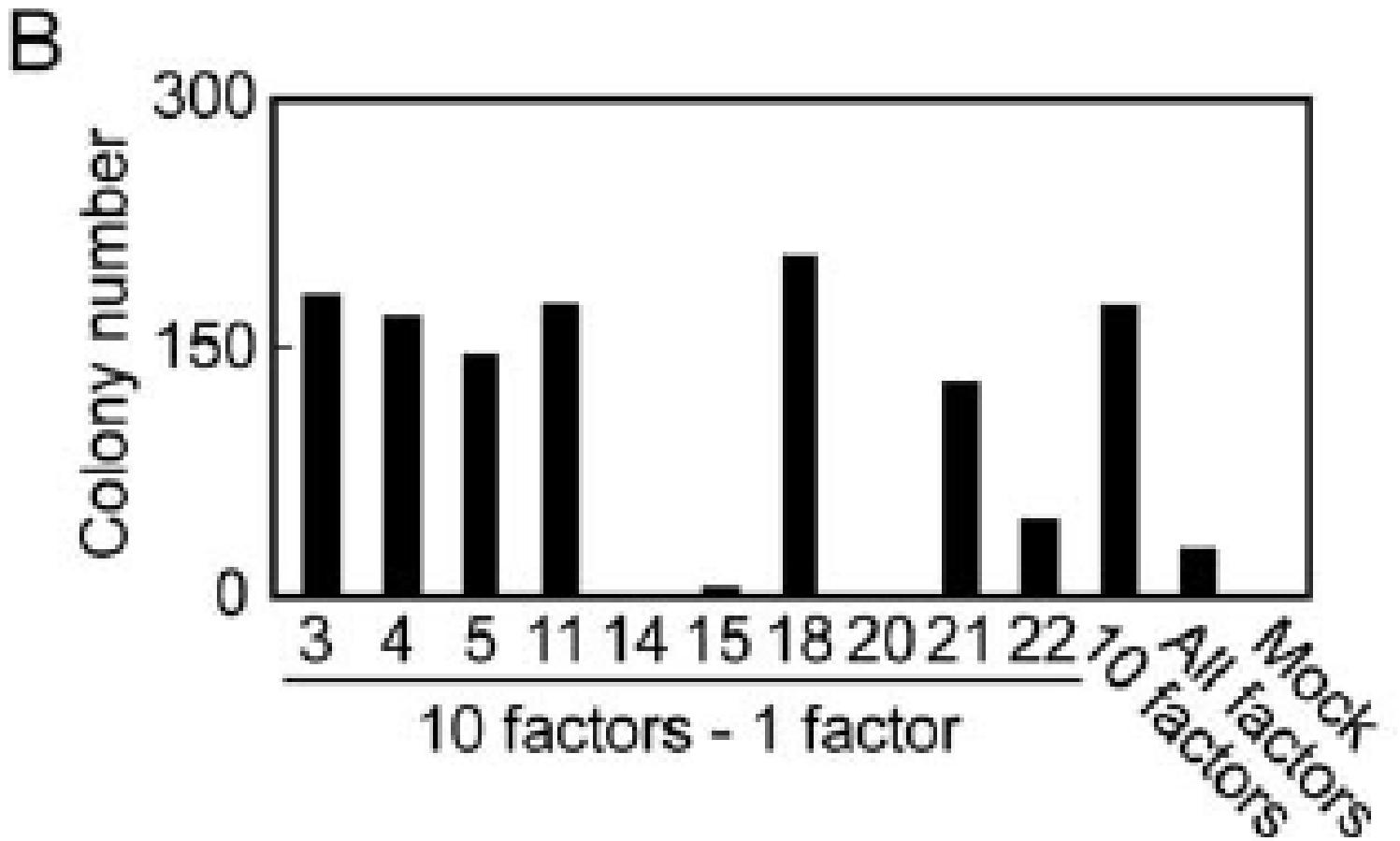
No	Genes	References
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21	β-catenin	Kielman et al., 2002; Sato et al., 2004
22	c-Myc	Cartwright et al., 2005
23	Stat3	Matsuda et al., 1999; Niwa et al., 1998
24	Grb2	Burdon et al., 1999; Cheng et al., 1998; Miyamoto et al., 2004

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Date	Cells	Transgenes	G418-Resistant Colonies	Colonies Picked up	iPS Clones Established
8/29/2005	MEF 1×10^5	Mock	0		
		24 factors - Ecat1	2	1	frozen in 24-well plate
		24 factors - Esg1	22	1	
		24 factors - Fbx15	10	1	
		24 factors - Nanog	5	1	
		24 factors - ERas	9	1	
		24 factors - Dnmt31	22	1	
		24 factors - Ecat8	19	1	
		24 factors - Gdf3	9	1	
		24 factors - Sox15	30	1	
		24 factors - Dppa4	17	1	
		24 factors - Dppa2	9	1	
		24 factors - Fthl17	5	1	
		24 factors - Sall4	17	1	
		24 factors - Oct3/4	0		
		24 factors - Sox2	0		
		24 factors - Rex1	15	1	frozen in 24-well plate
		24 factors - Utf1	8	1	
		24 factors - Tcf1	4	1	
		24 factors - Dppa3	7	1	
		24 factors - Klf4	0		
		24 factors - β -catenin S33Y	15	1	frozen in 24-well plate
		24 factors - Myc T58A	4	1	
		24 factors - Stat3-C	22	1	
		24 factors - Grb2 Δ SH2	11	1	
		24 factors	22	1	

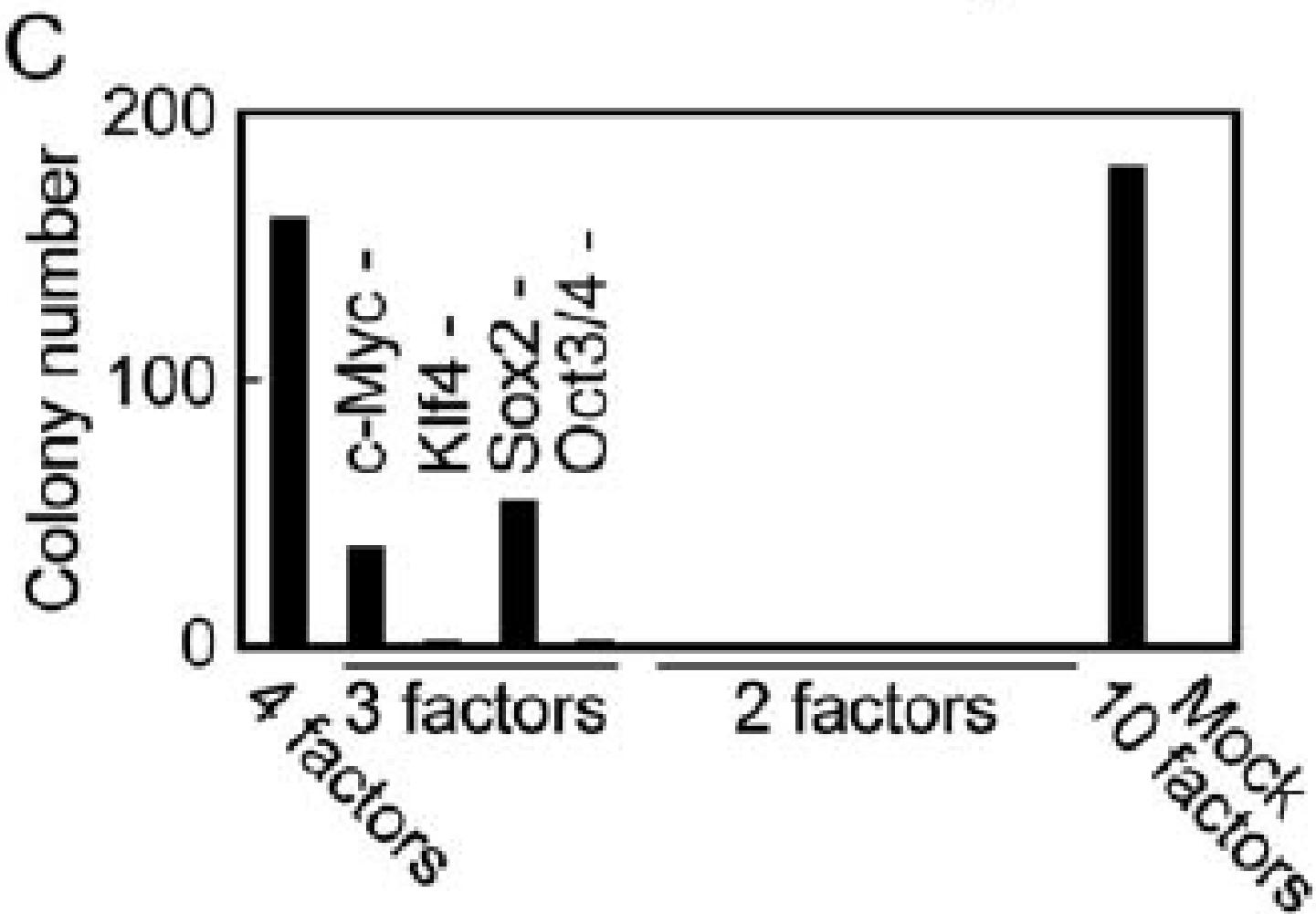
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20	Klf4	Li et al., 2005
21	β-catenin	Kielman et al., 2002; Sato et al., 2004
22	c-Myc	Cartwright et al., 2005
23	Stat3	Matsuda et al., 1999; Niwa et al., 1998
24	Grb2	Burdon et al., 1999; Cheng et al., 1999; Miyamoto et al., 2004



Date	Cells	Transgenes	G418-Resistant Colonies	Colonies Picked up	iPS Clones Established
9/12/2005	MEF 8 x 10 ⁵	Mock	0		
		10 factors – Fbx15	183	2	frozen in 24-well plate
		10 factors – Nanog	170	2	
		10 factors – ERas	146	2	
		10 factors – Dppa2	177	2	
		10 factors – Oct3/4	0		
		10 factors – Sox2	5	2	frozen in 24-well plate
		10 factors – Tcf11	206	2	
		10 factors – Klf4	0		
		10 factors – β -catenin S33Y	129	2	frozen in 24-well plate
		10 factors – Myc T58A	46	2	
		10 factors	176	2	
		24 factors	28	2	

No	Genes	References
1	Ecat1	Mitsui et al., 2003
2	Dppa5 (Esg1)	Mitsui et al., 2003
3	Fbxo15	Mitsui et al., 2003
4	Nanog	Chambers et al., 2003; Mitsui et al., 2003
5	ERas	Takahashi et al., 2003
6	Dnmt3l	Mitsui et al., 2003
7	Ecat8	Mitsui et al., 2003
8	Gdf3	Mitsui et al., 2003
9	Sox15	Maruyama et al., 2005
10	Dppa4	Mitsui et al., 2003
11	Dppa2	Mitsui et al., 2003
12	Fth17	Mitsui et al., 2003
13	Sall4	Mitsui et al., 2003
14	Oct3/4 (Pou5f1)	Nichols et al., 1998; Niwa et al., 2000
15	Sox2	Avilion et al., 2003; Maruyama et al., 2005
16	Rex1 (Zfp42)	Rogers et al., 1991
17	Utf1	Okuda et al., 1998
18	Tcl1	Mitsui et al., 2003
19	Dppa3 (Stella)	Mitsui et al., 2003
20	Klf4	Li et al., 2005
21	β-catenin	Kielman et al., 2002; Sato et al., 2004
22	c-Myc	Cartwright et al., 2005
23	Stat3	Matsuda et al., 1999; Niwa et al., 1998
24	Grb2	Burdon et al., 1999; Cheng et al., 1999; Miyamoto et al., 2004

c



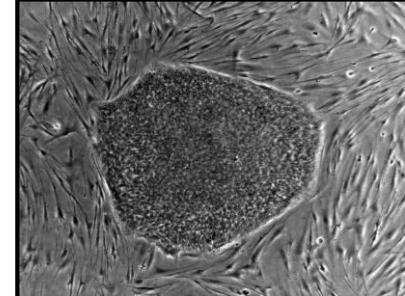
Date	Cells	Transgenes	G418-Resistant Colonies	Colonies Picked up	iPS Clones Established
9/26/2005	MEF 8×10^5	Mock	0		
		4 factors	160	12	3 MEF4-2, MEF4-3, MEF4-7, MEF4-10
		4 factors – Myc T58A	36	6	
		4 factors – Klf4	1	1	
		4 factors – Sox2	54	6	6 MEF3-1~6
		4 factors – Oct3/4	1	1	
		Oct3/4 + Sox2	0		
		Oct3/4 + Klf4	0		
		Oct3/4 + Myc T58A	0		
		Sox2 + Klf4	0		
		Sox2 + Myc T58A	0		
		Klf4 + Myc T58A	0		
		10 factors	179	12	5 MEF10-1, MEF10-3, MEF10-6, MEF10-7, MEF10-10

O+S+K = 36 G418 resistant colonies
O+K+M = 54 G418 resistant colonies

How will you identify whether a cell is a pluripotent stem cell?



Mouse ES cells



Human ES cells

Normal Karyotype (46, XX or 46, XY)

- *in vitro*

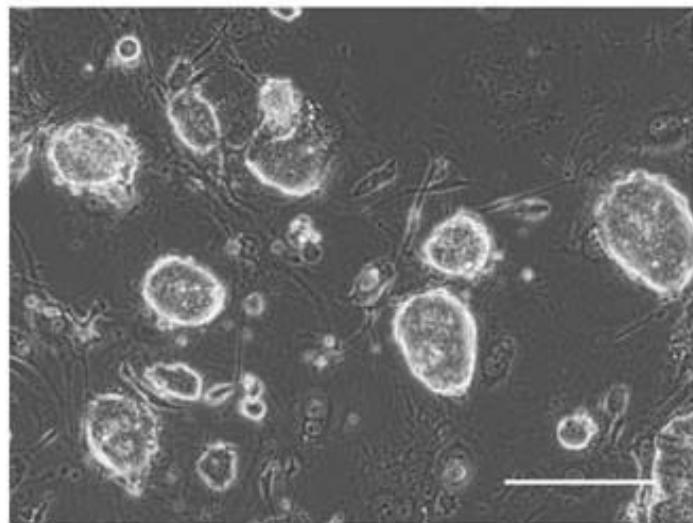
- Colony Morphology analysis
- AP staining
- Telomerase expression
- Expression of stem cell markers (RNA and protein levels)
- Normal *in vitro* differentiation to all germ layers

- *in vivo*

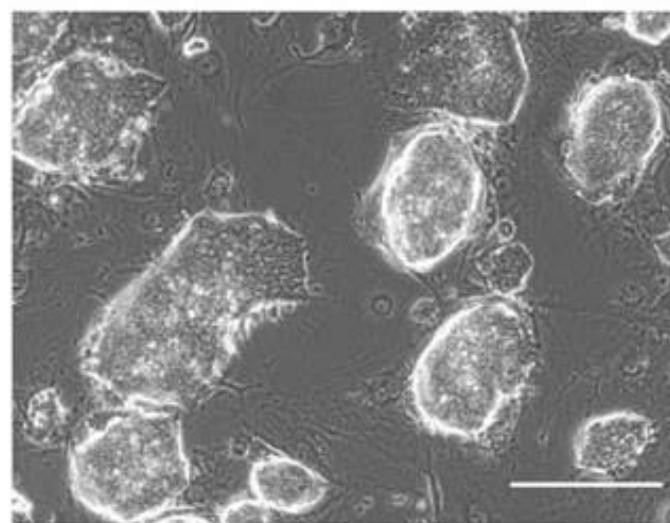
- Teratoma formation after injection into immunocompromised mice
- Chimera formation through blastocyst injection or morula aggregation
- Tetraploid complementation

D

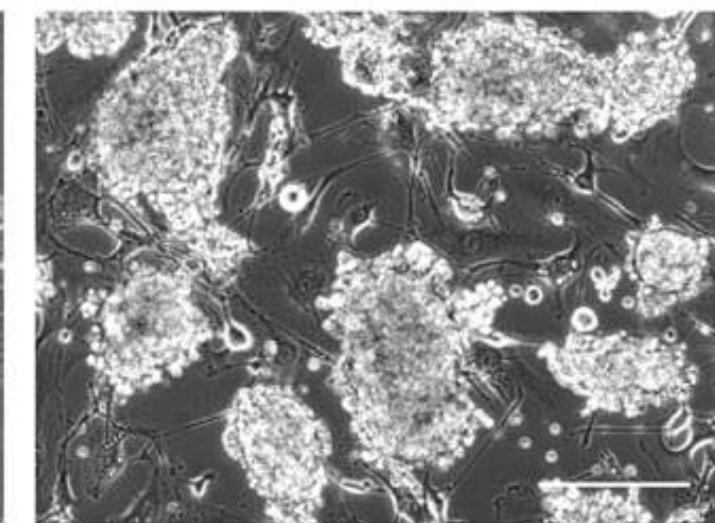
iPS-MEF4-7



iPS-MEF10-6



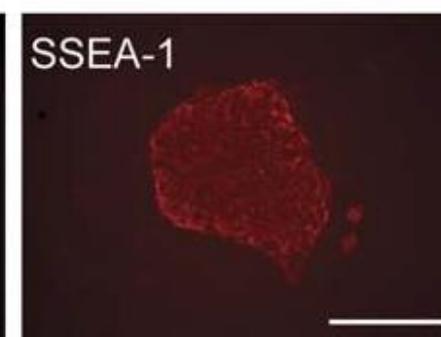
iPS-MEF3-3

**D**

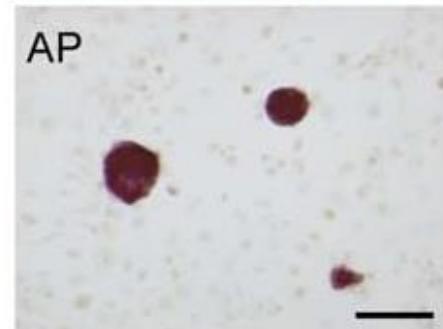
iPS-MEF4-7



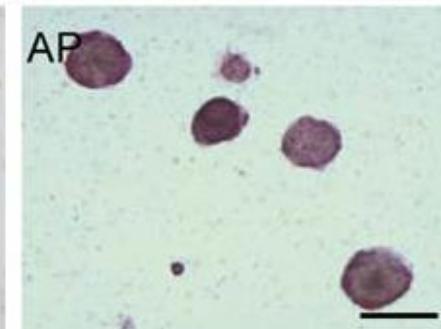
iPS-MEF10-6



AP



AP



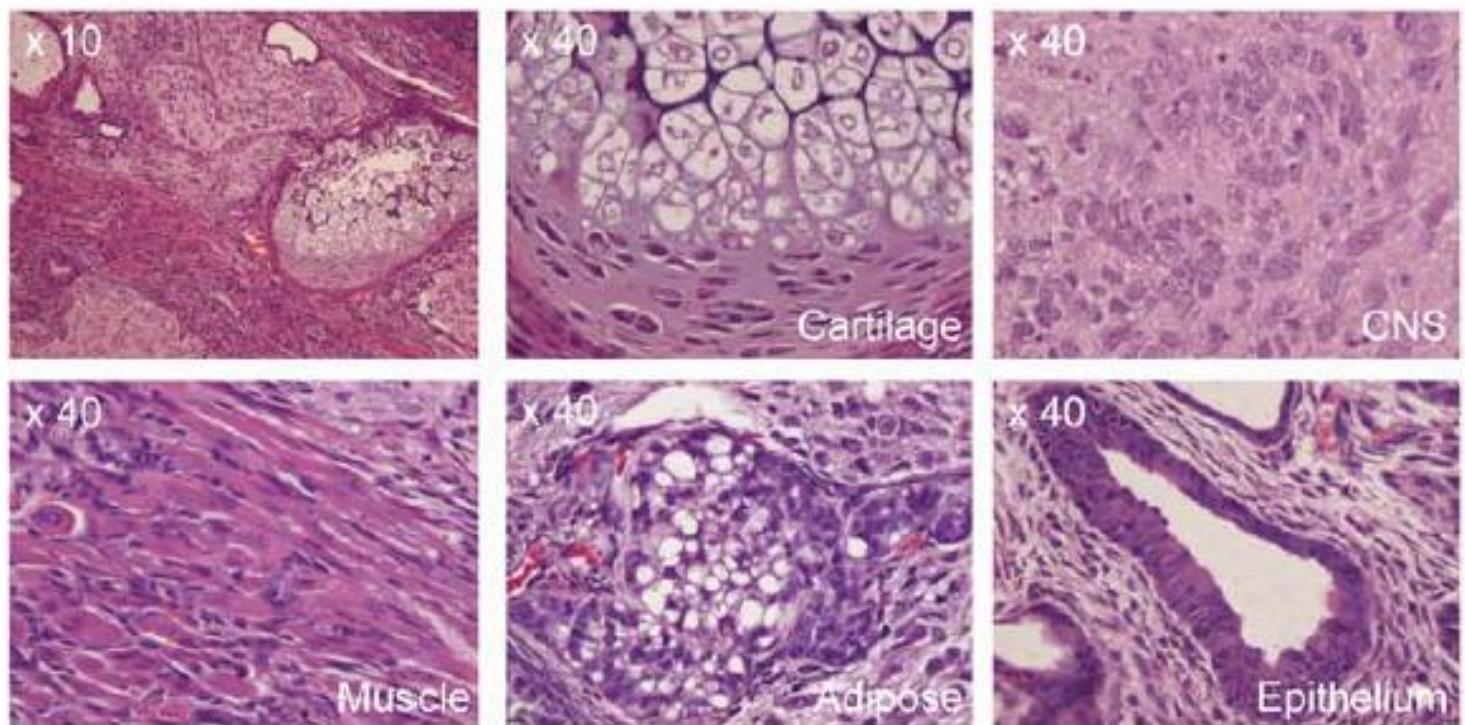
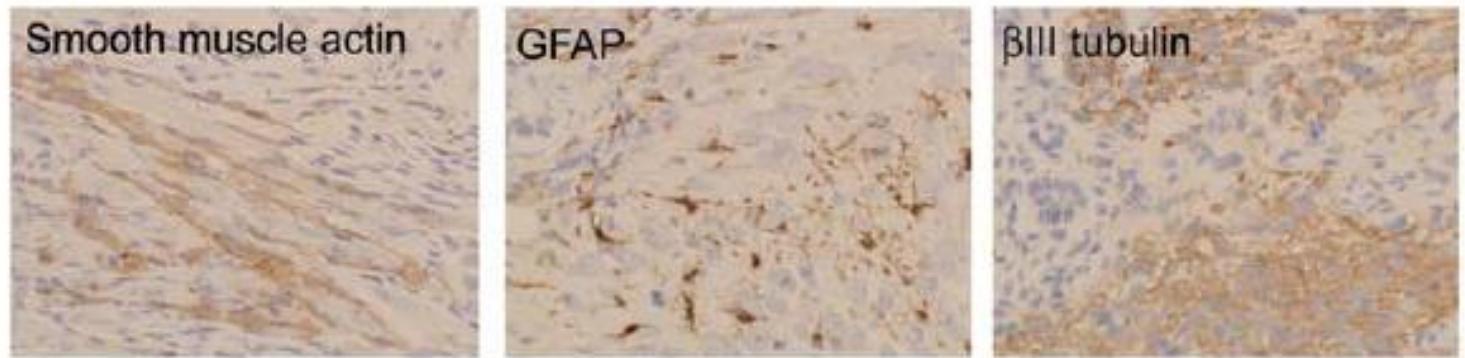
A**B**

Figure 5. Pluripotency of iPS Cells Derived from MEFs

(A) Various tissues present in teratomas derived from iPS-MEF4-7 cells. Histology of other teratomas is shown in Figure S3 and Table S6.

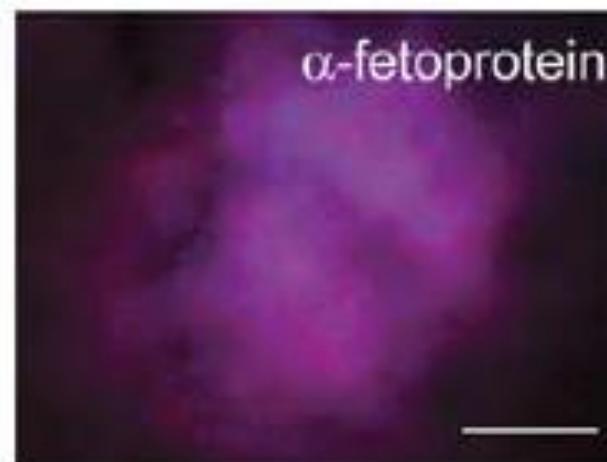
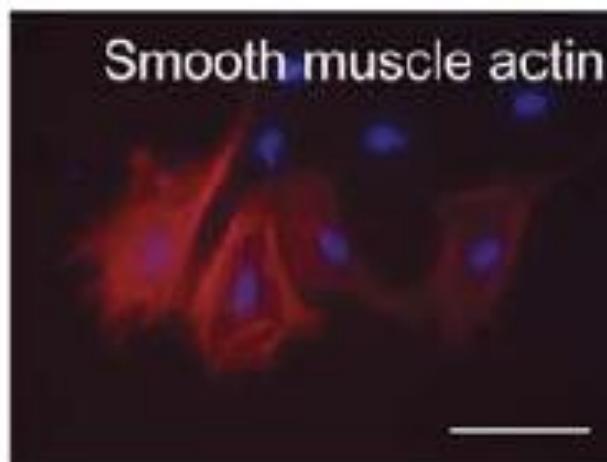
(B) Immunostaining confirming differentiation into neural tissues and muscles in teratomas derived from iPS-MEF4-7.

(C) In vitro embryoid body formation (upper row) and differentiation (lower row). Scale bars = 200 μ m.

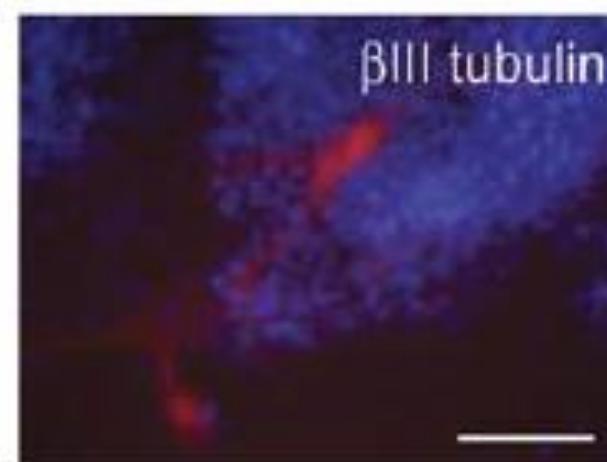
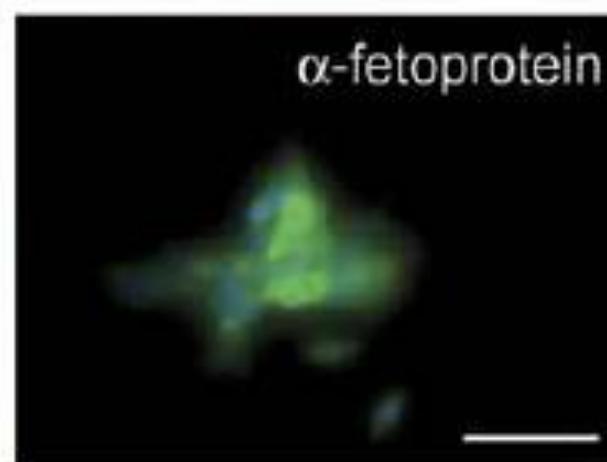
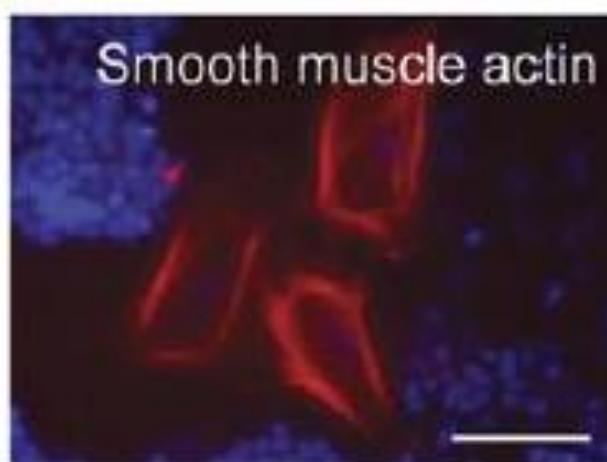
(D) Immunostaining confirming in vitro differentiation into all three germ layers. Scale bars = 100 μ m. Secondary antibodies were labeled with Cy3 (red), except for α -fetoprotein in iPS-MEF10-6, with which Alexa 488 (green) was used.

D

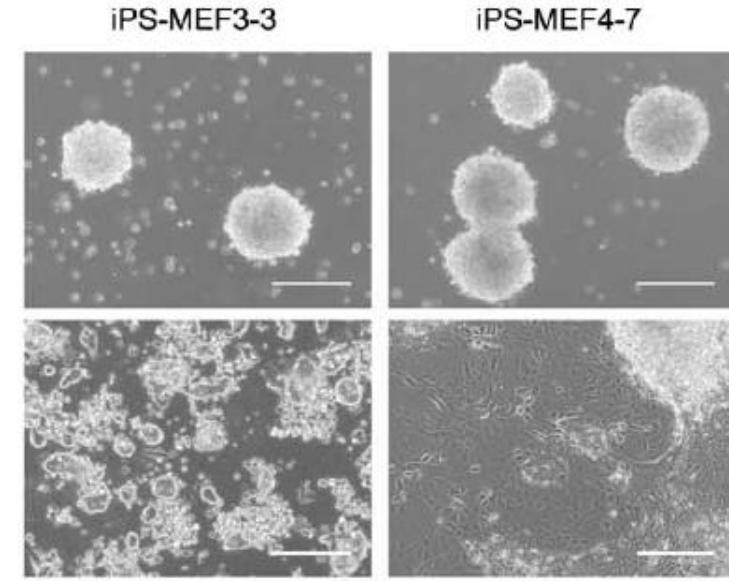
iPS-
MEF4-7



iPS-
MEF10-6

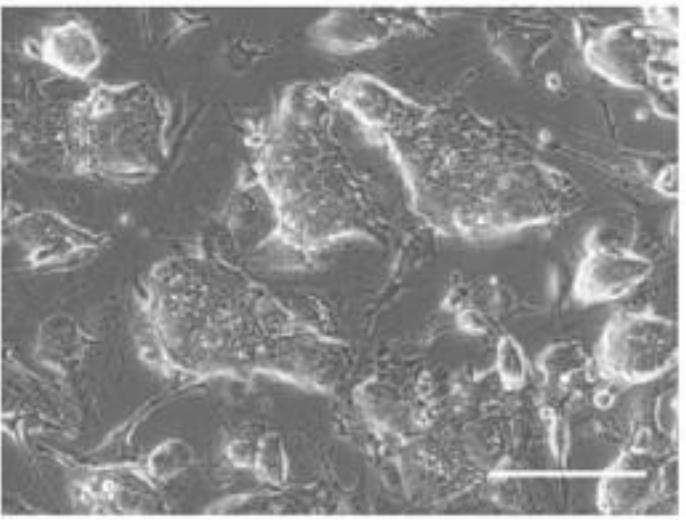


Date	Cells	Transgenes	G418-Resistant Colonies	Colonies Picked up	iPS Clones Established
9/26/2005	MEF 8×10^5	Mock	0		
		4 factors	160	12	3 MEF4-2, MEF4-3, MEF4-7, MEF4-10
		4 factors – Myc T58A	36	6	
		4 factors – Klf4	1	1	
		4 factors – Sox2	54	6	6 MEF3-1~6
		4 factors – Oct3/4	1	1	
		Oct3/4 + Sox2	0		
		Oct3/4 + Klf4	0		
		Oct3/4 + Myc T58A	0		
		Sox2 + Klf4	0		
		Sox2 + Myc T58A	0		
		Klf4 + Myc T58A	0		
		10 factors	179	12	5 MEF10-1, MEF10-3, MEF10-6, MEF10-7, MEF10-10

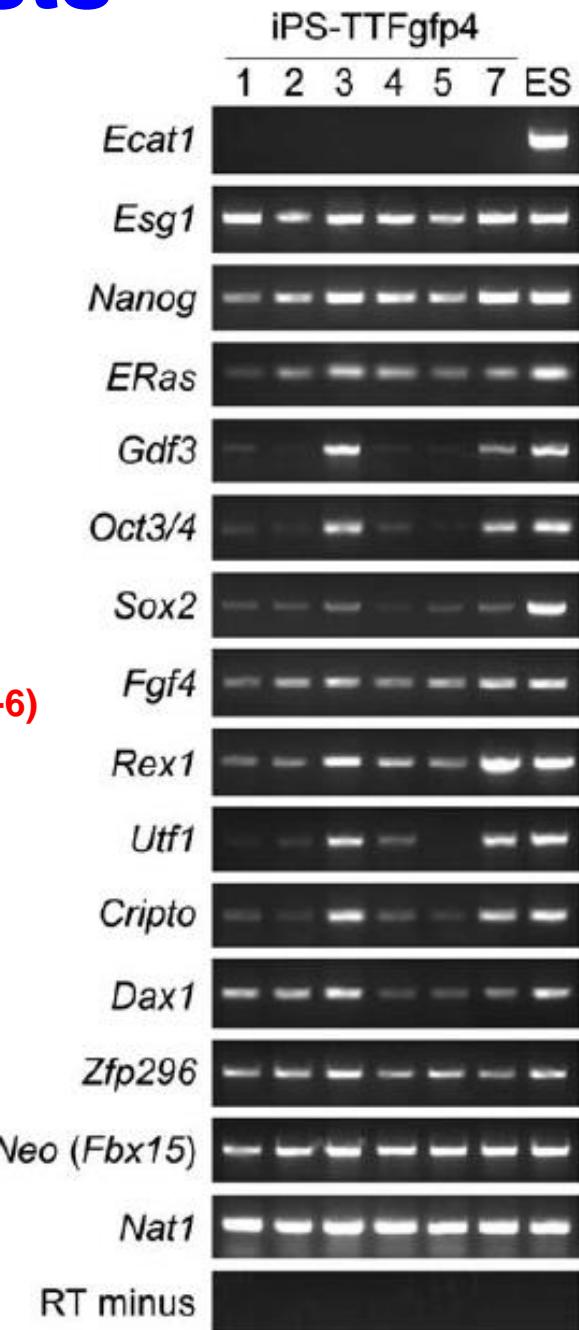


In contrast, all tumors derived from iPSC-ME3 clones were composed entirely of undifferentiated cells

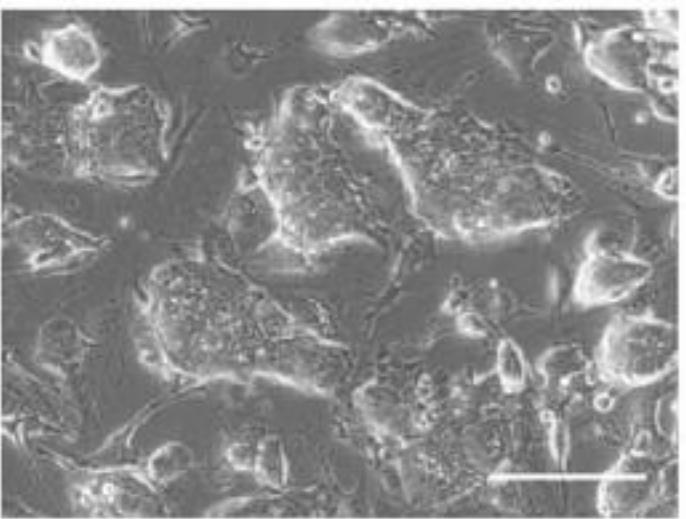
Reprogramming Tail-tip fibroblasts



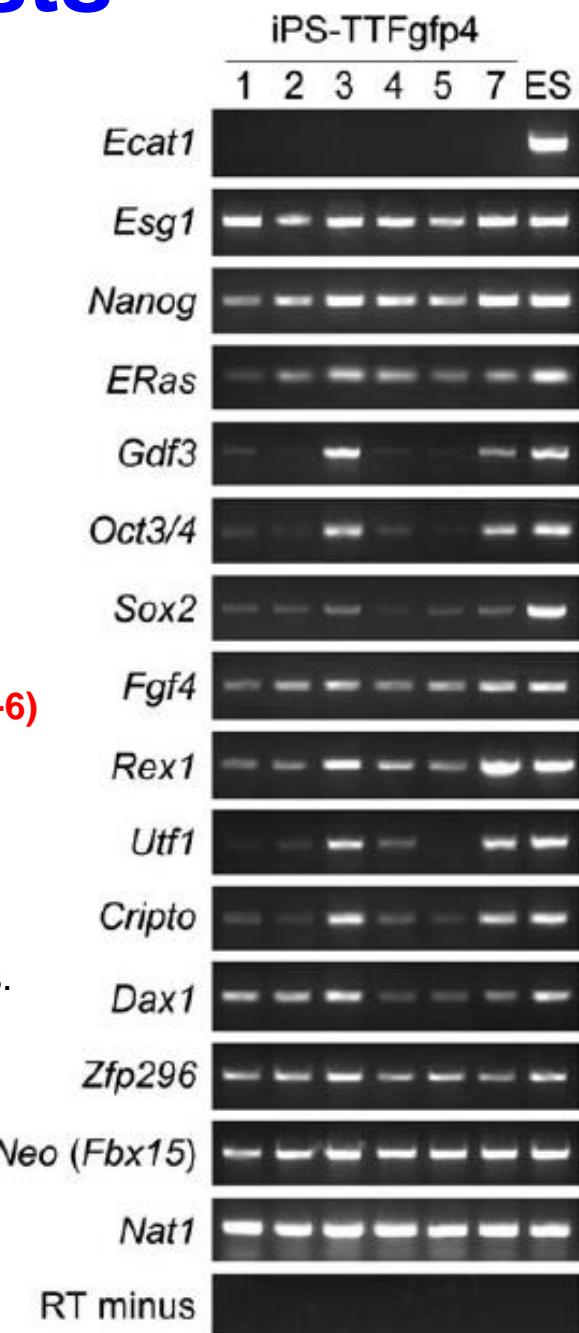
1. 7-week old male Fbx15 β geo/geo mice – **3 G418 resistant colonies (iPS-TTF4)**
 2. 12-week-old female Fbx15 β geo/geo mice with constitutive GFP – **13 G418 resistant colonies (iPS-TTGF4P4; 1-6)**



Reprogramming Tail-tip fibroblasts



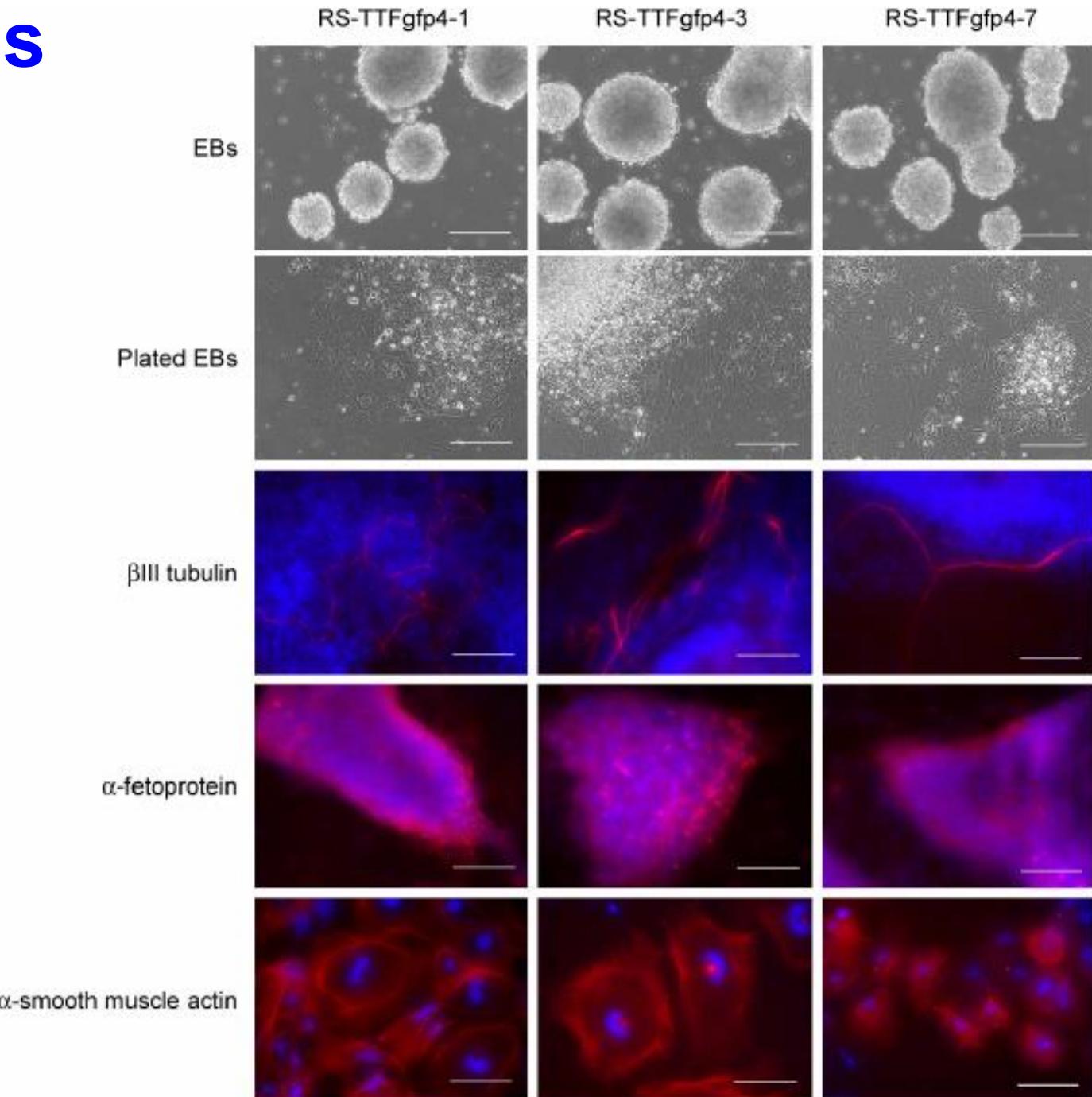
1. 7-week old male *Fbx15* β geo/geo mice – **3 G418 resistant colonies (iPS-TTF4)**
2. 12-week-old female *Fbx15* β geo/geo mice with constitutive GFP – **13 G418 resistant colonies (iPS-TTGF4P4; 1-6)**



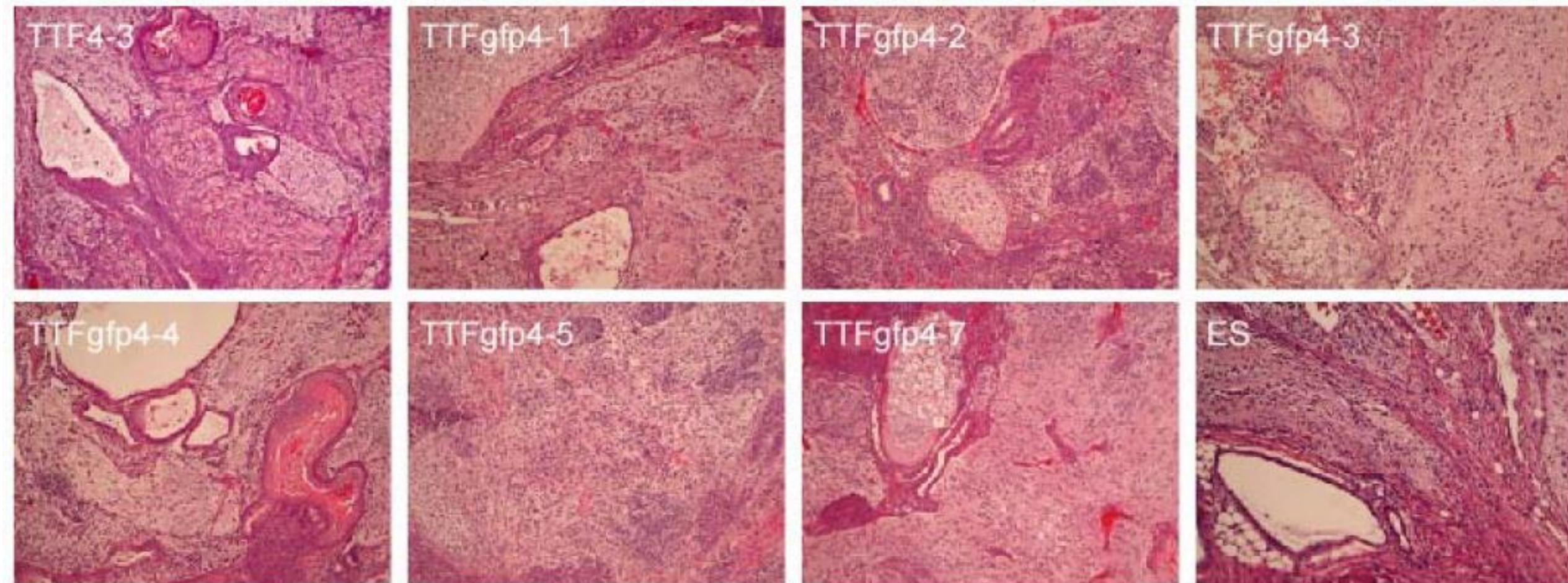
Differentiation of iPS cells from tail-tip fibroblasts

iPS-TTGF4-3/7

Clones 3 and 7 of iPS-TTGF4 expressed the majority of ES cell markers at high levels and others at low levels.



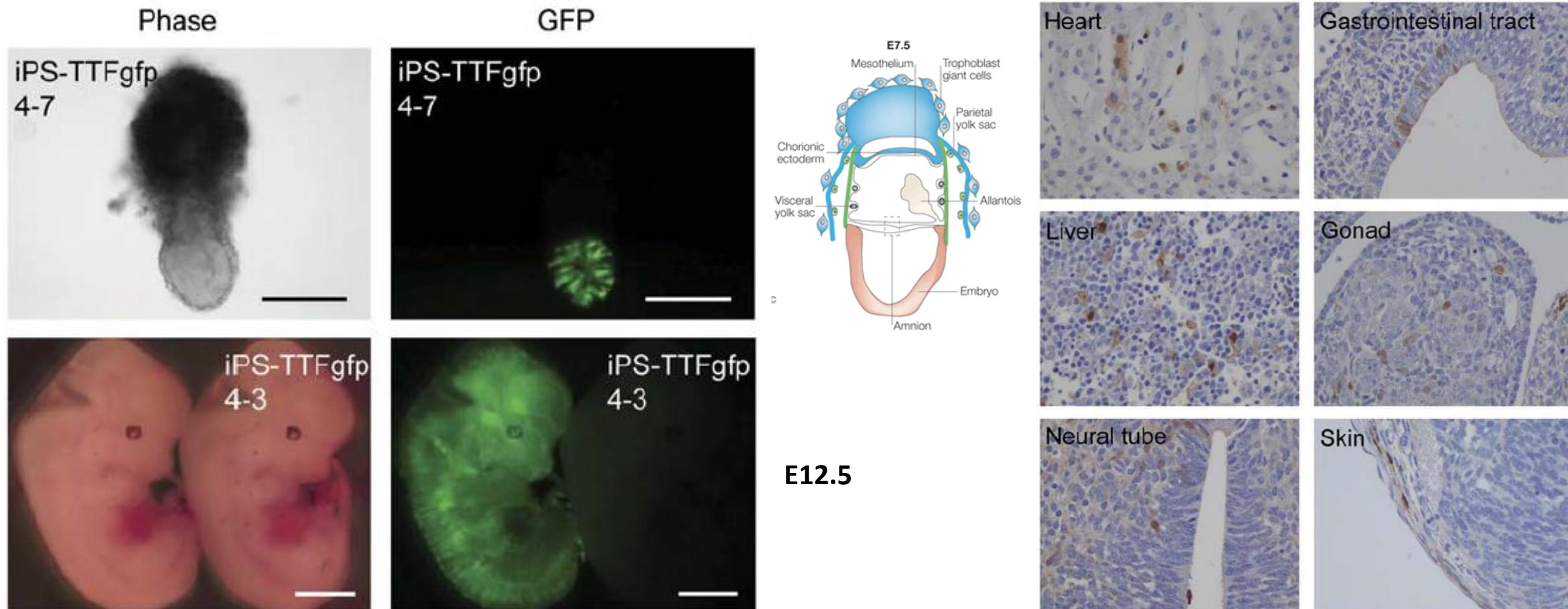
Pluripotency determination of iPS cells using teratoma assay



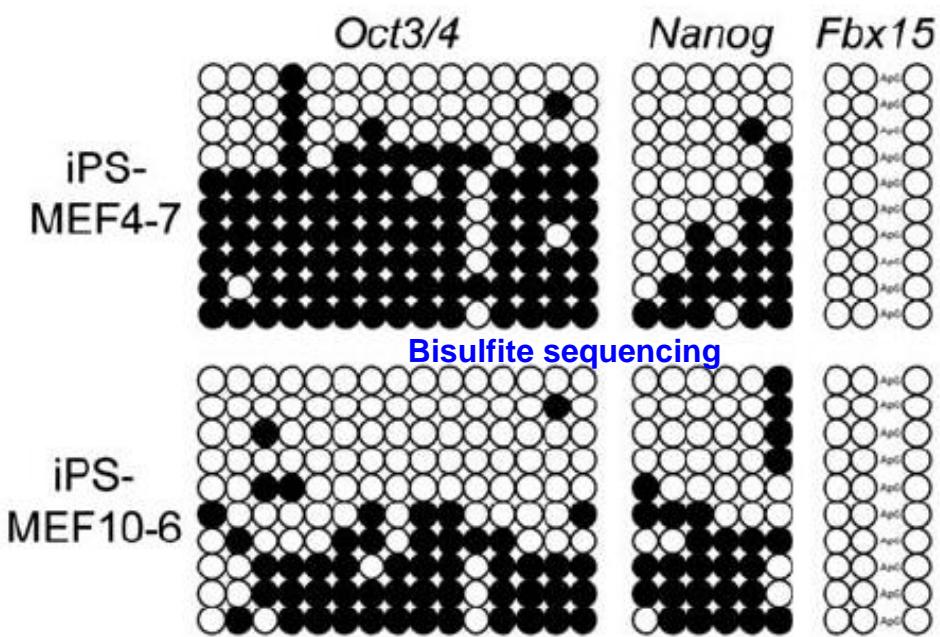
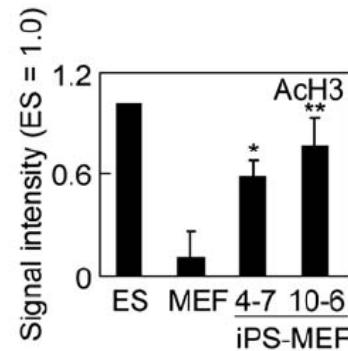
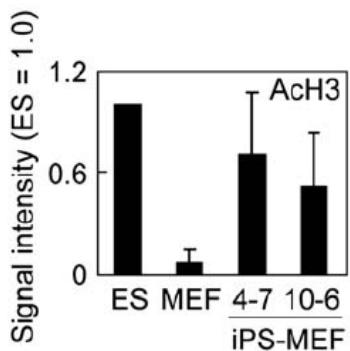
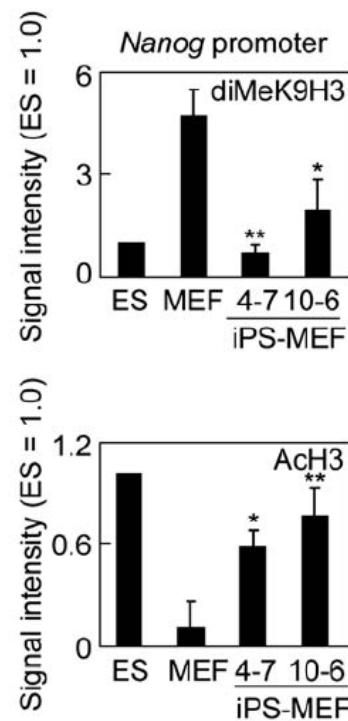
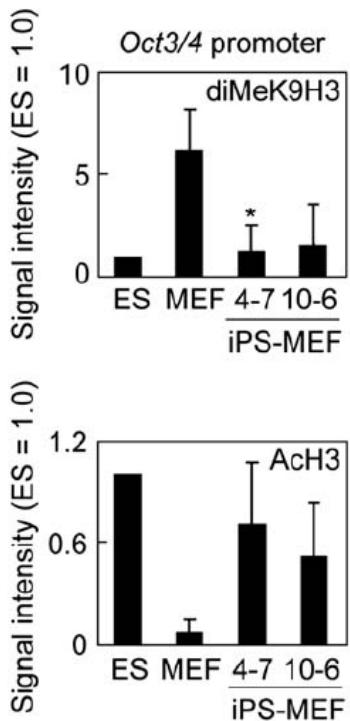
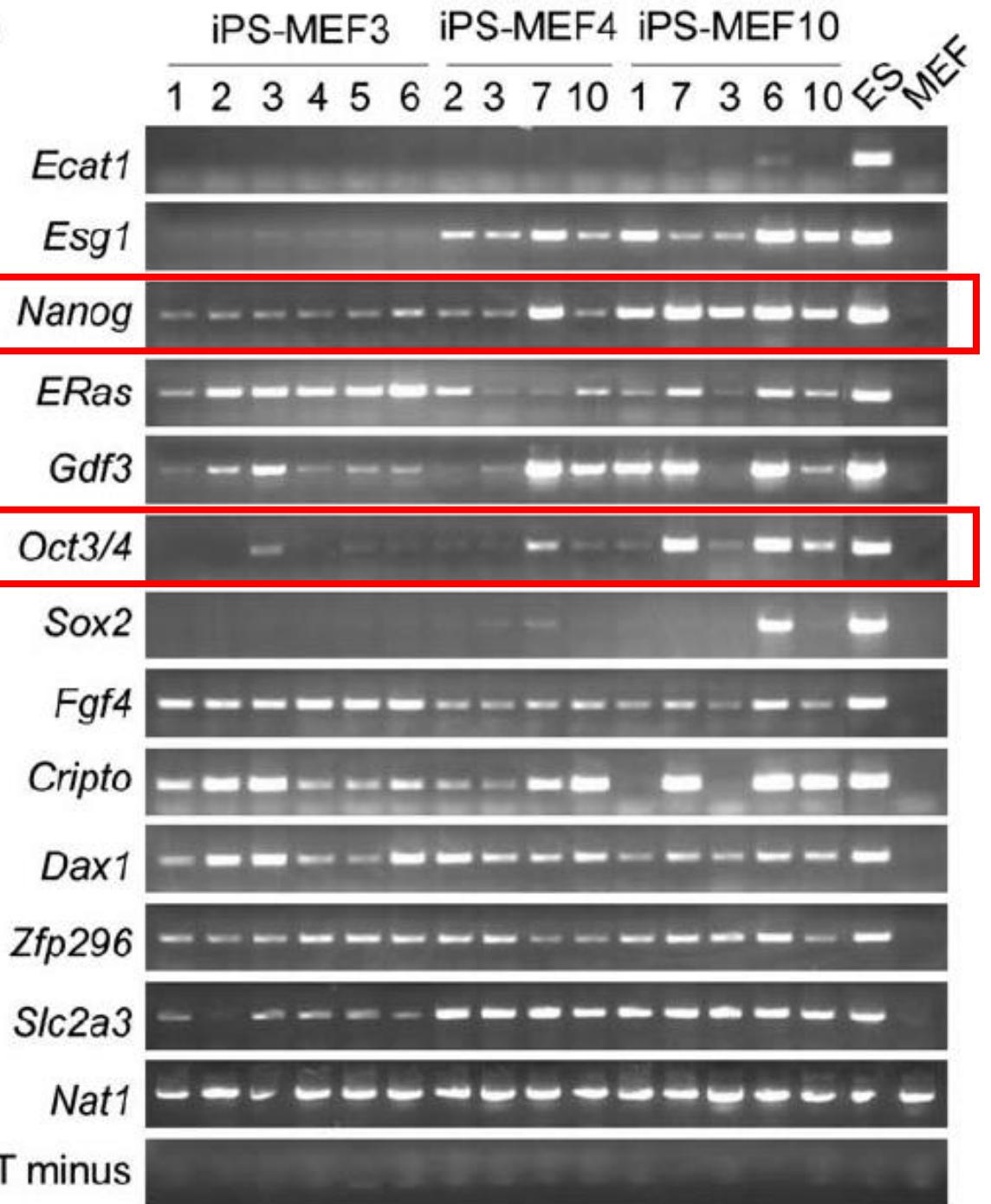
Pluripotency determination of iPS cells – iPSTTFGFP4-3

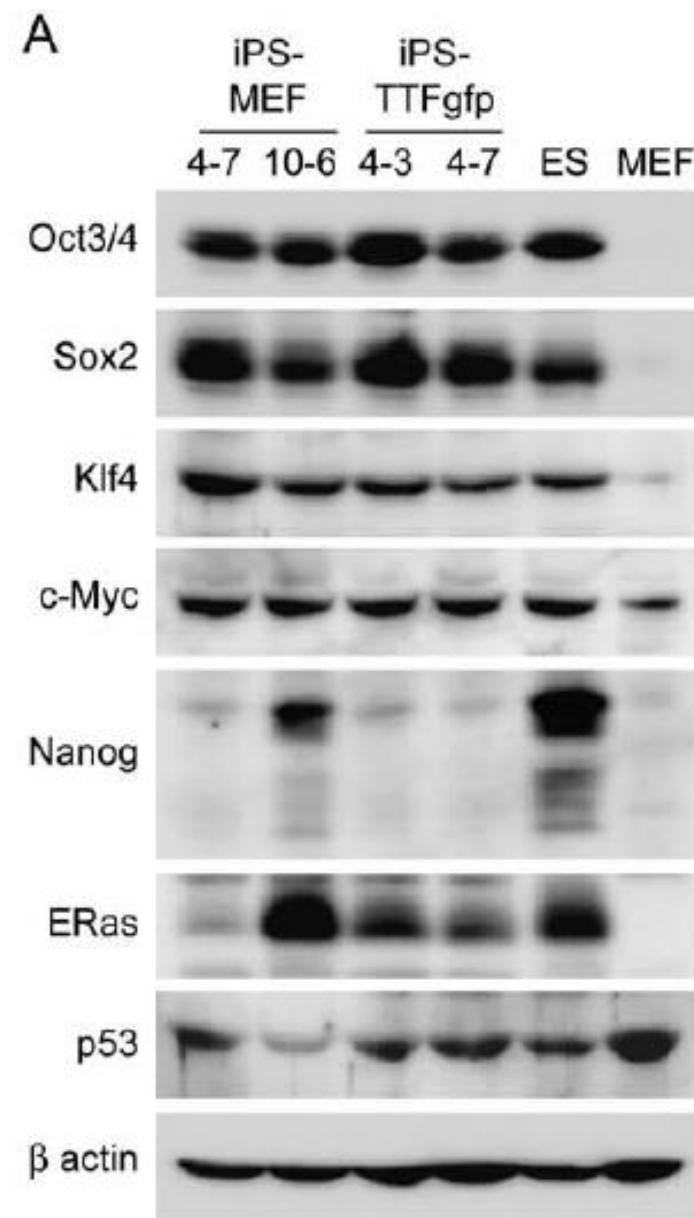
constitutively expressed green fluorescent protein (GFP) from the CAG promoter

Introduction of 2 clones of iPS-TTFgfp cells (clones 3 and 7) into C57/BL6 blastocysts by microinjection. With iPS-TTFgfp4-3, they obtained 18 embryos at E13.5, 2 of which showed contribution of GFP-positive iPS cells



A



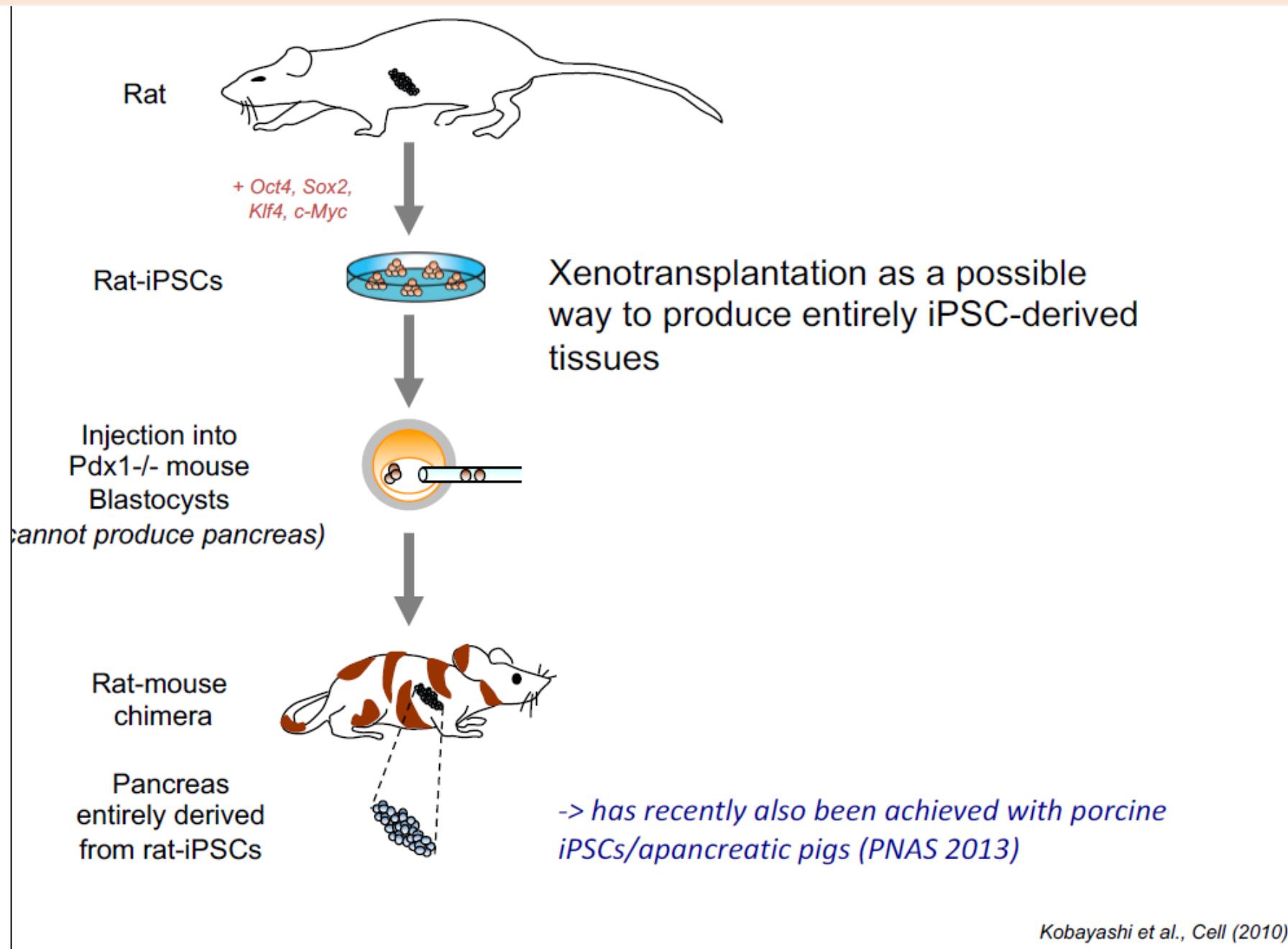


Western blot analyses of the four factors and other proteins in iPS cells (MEF4-7, MEF10-6, TTFgfp4-3, and TTFgfp4-7), ES cells, and MEFs.

Conclusions

- Among the three (O/S/N), Oct3/4 and Sox2 are essential for the generation of iPS cells. Surprisingly, Nanog is dispensable.
- c-Myc and Klf4 as essential factors. These two tumor-related factors could not be replaced by other oncogenes including E-Ras, Tcf1, b-catenin, and Stat3.
- The c-Myc protein has many downstream targets that enhance proliferation and transformation (Adhikary and Eilers, 2005), many of which may have roles in the generation of iPS cells.
- Within the mammalian genome, there may be up to 25,000 c-Myc binding sites (Cawley et al., 2004), many more than the predicted number of Oct3/4 and Sox2 binding sites (Boyer et al., 2005; Loh et al., 2006). c-Myc protein may induce global histone acetylation (Fernandez et al., 2003), thus allowing Oct3/4 and Sox2 to bind to their specific target loci.
- Klf4 might contribute to activation of Nanog and other ES cell-specific genes through p53 repression. Alternatively, Klf4 might function as an inhibitor of Myc-induced apoptosis through the repression of p53 in our system.
- On the other hand, Klf4 activates p21CIP1, thereby suppressing cell proliferation (Zhang et al., 2000). This antiproliferation function of Klf4 might be inhibited by c-Myc, which suppresses the expression of p21CIP1 (Seoane et al., 2002).
- The balance between c-Myc and Klf4 may be important for the generation of iPS cells.
- **Low reprogramming efficiency** - the levels of the four factors required for generation of pluripotent cells may have narrow ranges, and only a small portion of cells expressing all four of the factors at the right levels can acquire ES cell-like properties.
- **No multipotent stem cells contamination**

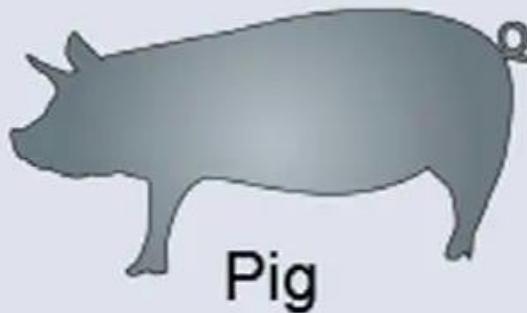
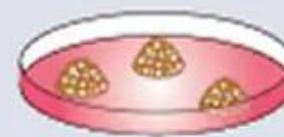
iPSC as a tool to generate entire organs



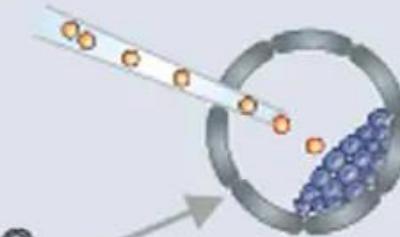


Human

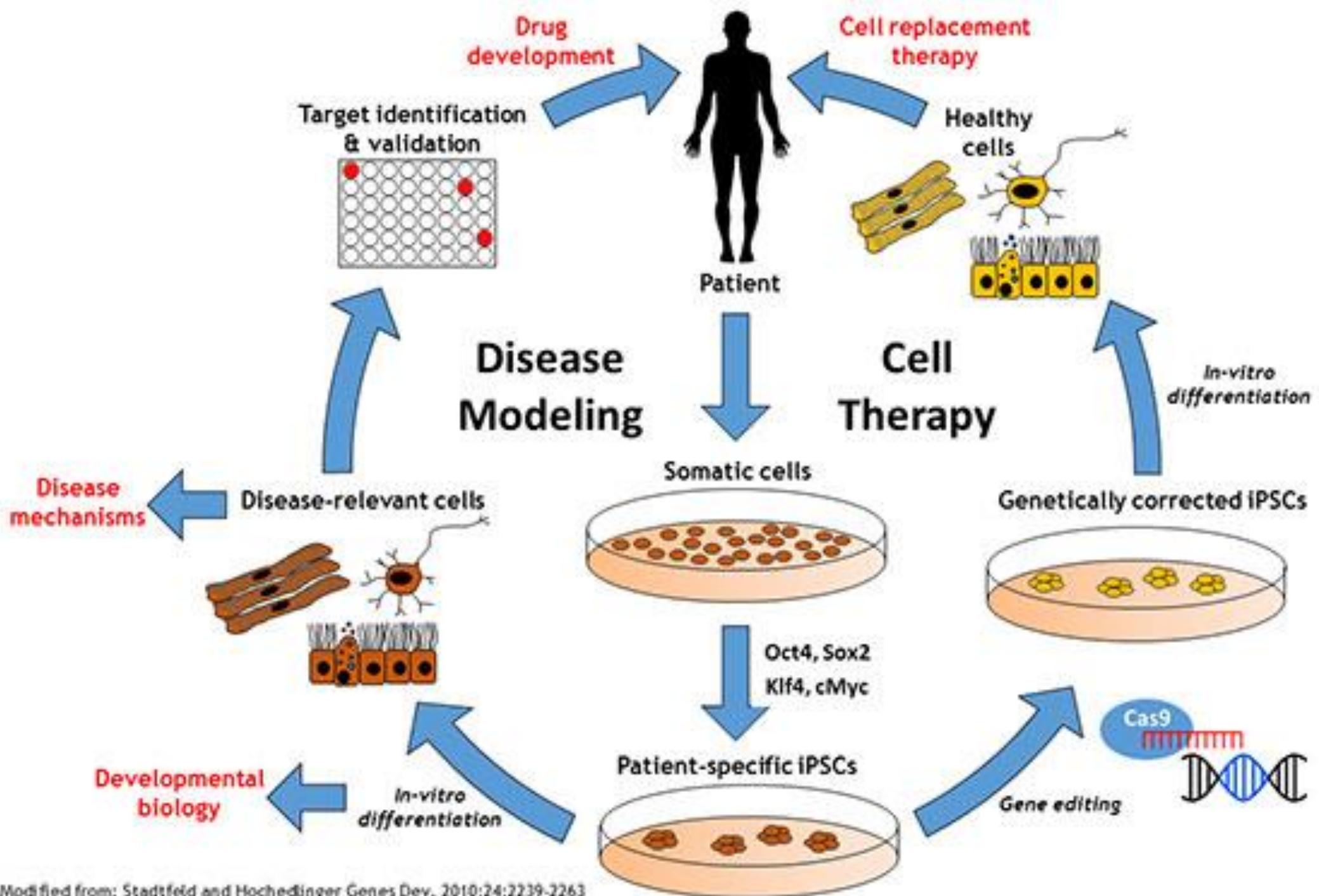
Human iPSCs

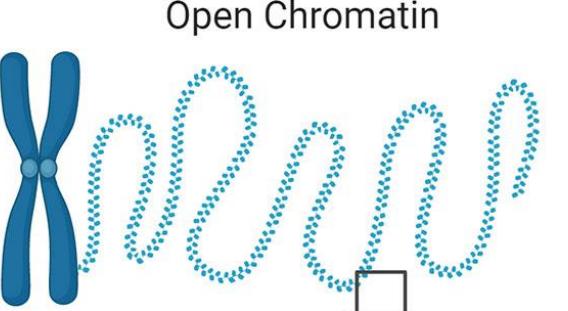


Pig

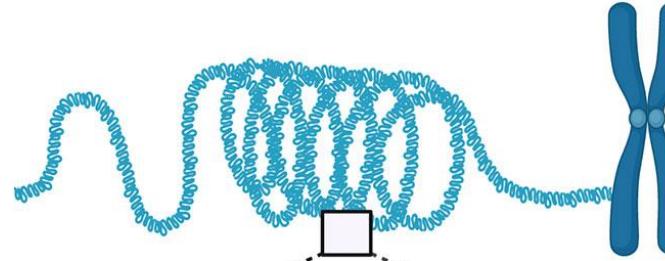


Chimerism





Condensed Chromatin



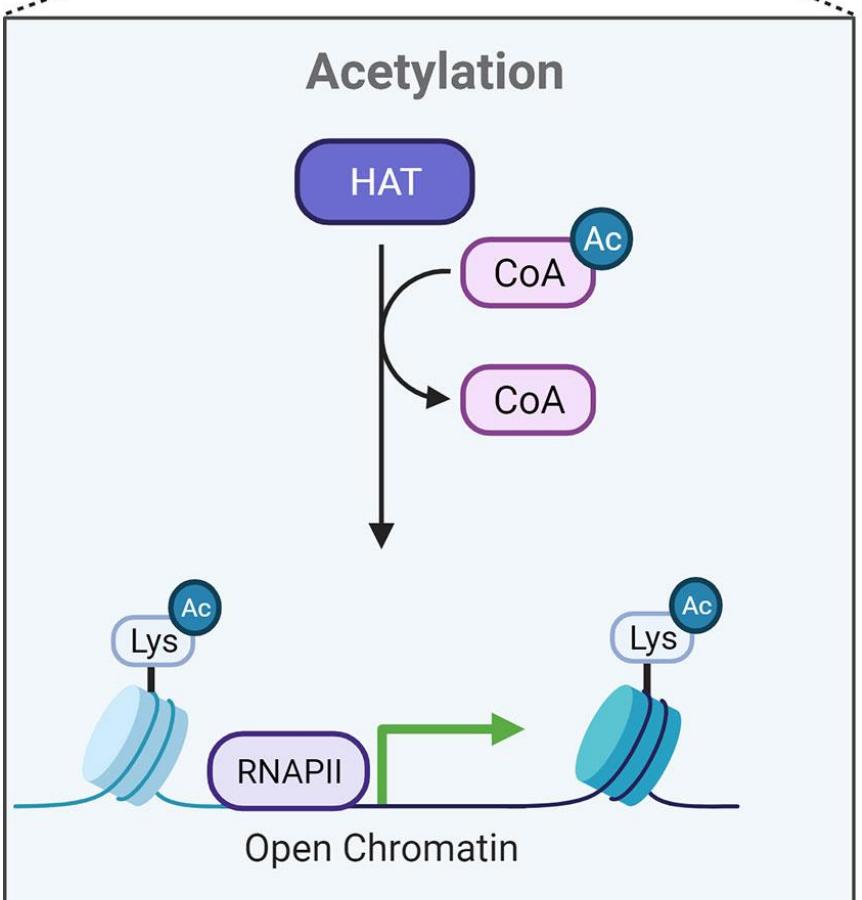
Acetylation

Acetylation

HAT



CoA



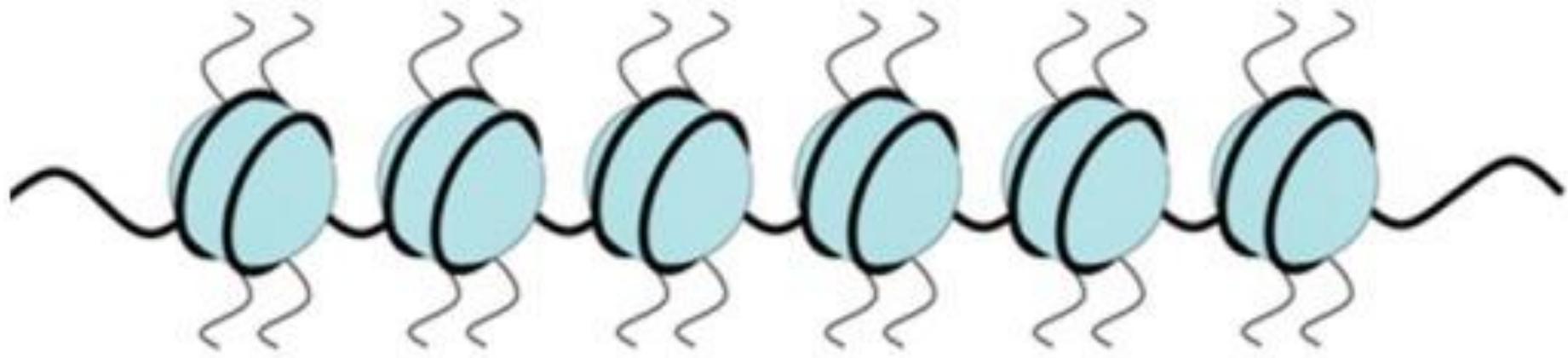
Transcription ON

Deacetylation

HDAC



Transcription OFF

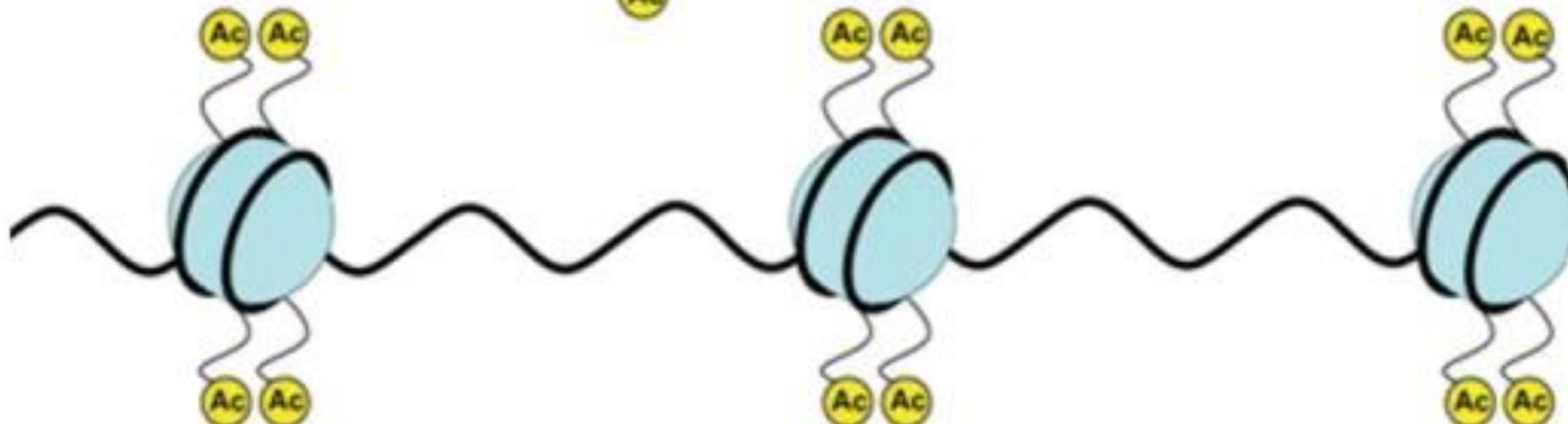


Deacetylation
(HDAC)

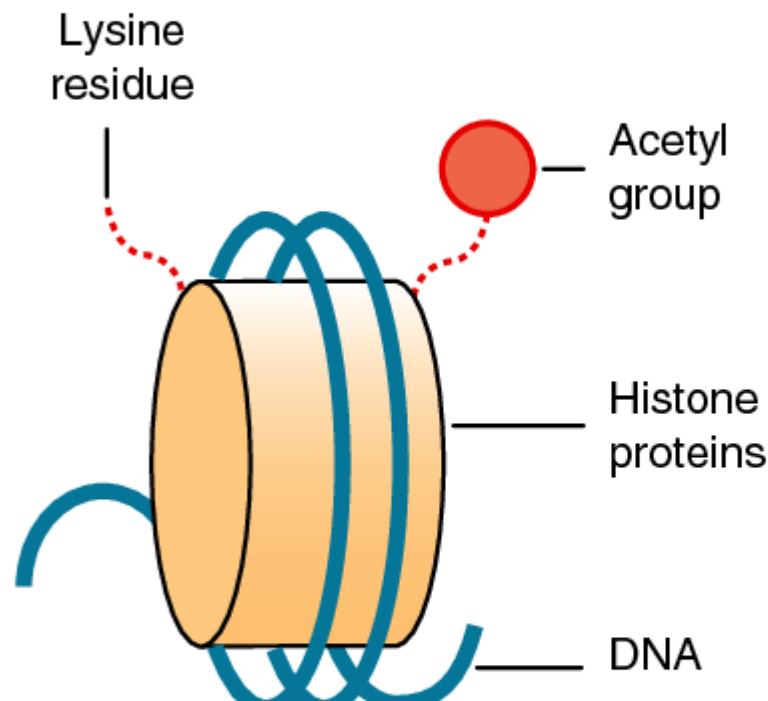
Acetyltransferase (HAT)

Acetyl groups (Ac) are shown as yellow circles.

A grey double-headed arrow connects the two processes, indicating they are opposing reactions.



(a)



(b)

