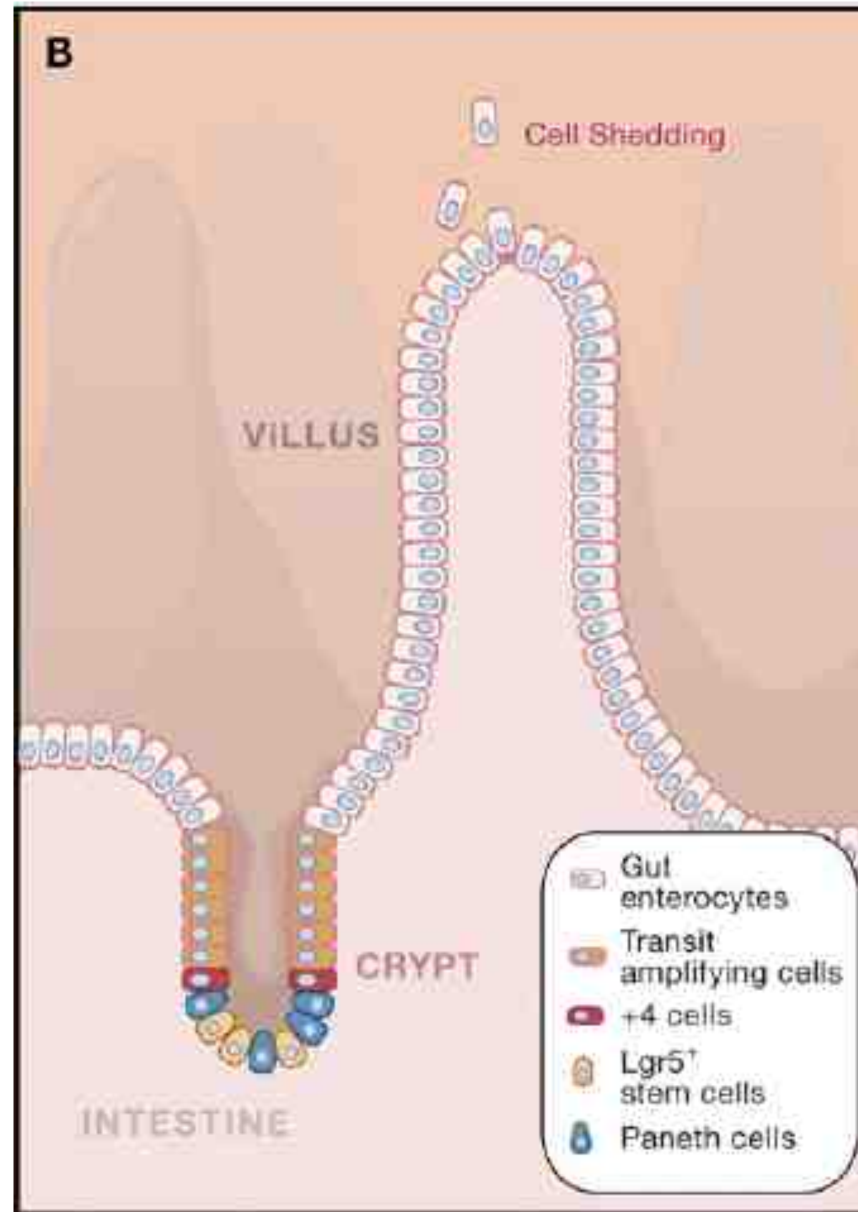
Lgr5: Leucine-rich repeat-containing G-protein coupled receptor 5

+4: cells hypothized to be the stem cells

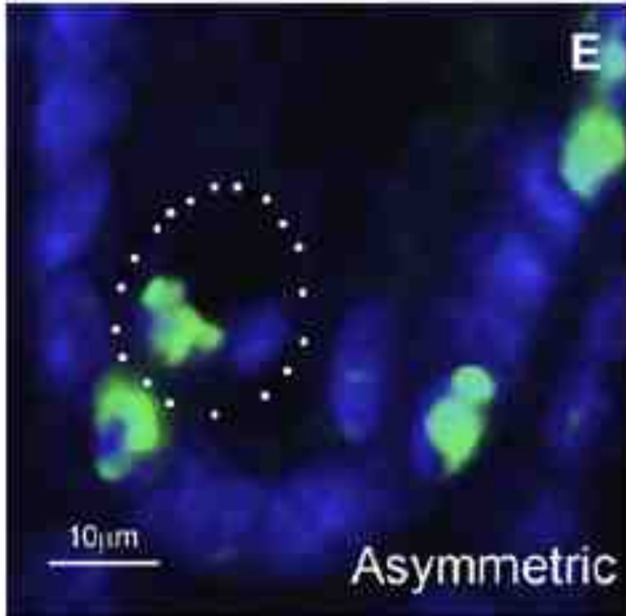


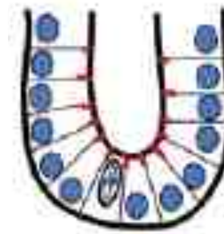
11_bcma_2011

Spindle Orientation Bias in Gut Epithelial Stem Cell Compartments Is Lost in Precancerous Tissue

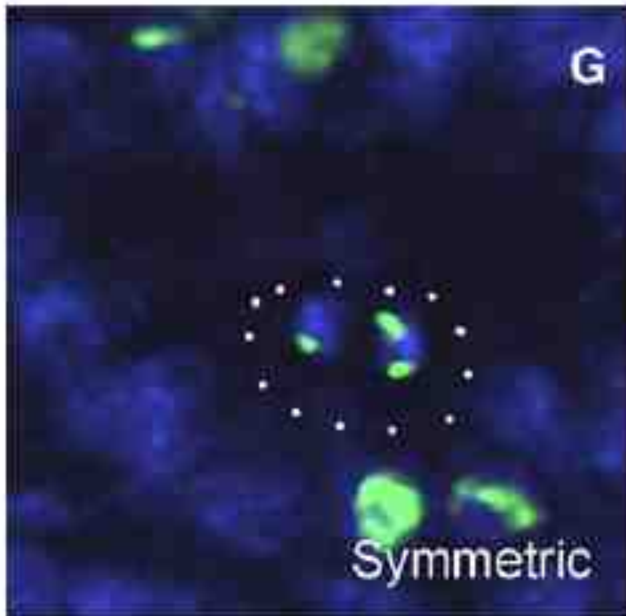
Aaron J. Quyn,[1,2] Paul L. Appleton,[1] Francis A. Carey,[3] Robert J.C. Steele,[2] Nick Barker,[4] Hans Clevers,[4] Rachel A. Ridgway,[5] Owen J. Sansom,[5] and Inke S. Näthke[1,*]
[1]Cell and Developmental Biology, University of Dundee, Dundee, DD1 5EH, UK
[2]Surgery and Molecular Oncology
[3]Pathology and Neuroscience
Ninewells Hospital and Medical School, University of Dundee, Dundee, DD1 9SY, UK
[4]Hubrecht Laboratory, KNAW Postbus 85164, Utrecht AD 3508, Netherlands
[5]Beatson Institute for Cancer Research, Glasgow, G611BD, UK
*Correspondence: i.s.nathke@dundee.ac.uk
DOI 10.1016/j.stem.2009.12.007

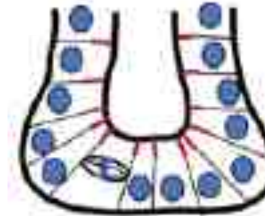
Cell Stem Cell *6*, 175–181, February 5, 2010 ©2010





Normal





Pre-cancerous

 Cell Stem Cell *6*, 175–181, February 5, 2010 ©2010

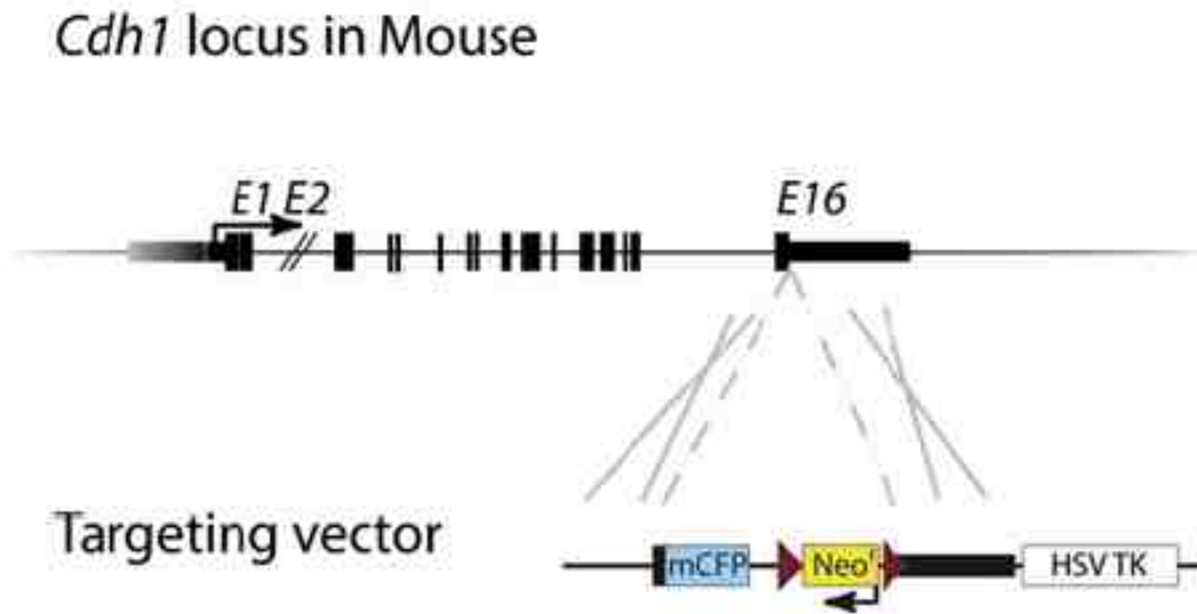
Intestinal Crypt Homeostasis Results from Neutral Competition between Symmetrically Dividing Lgr5 Stem Cells

Hugo J. Snippert,[1] Laurens G. van der Flier,[1] Toshiro Sato,[1] Johan H. van Es,[1] Maaike van den Born,[1] Carla Kroon-Veenboer,[1] Nick Barker,[1] Allon M. Klein,[2,3] Jacco van Rheenen,[1] Benjamin D. Simons,[3] and Hans Clevers[1,*]
[1]Hubrecht Institute, KNAW and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT Utrecht, The Netherlands
[2]Department of Systems Biology, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA
[3]Department of Physics, Cavendish Laboratory, J.J. Thomson Avenue, Cambridge CB3 0HE, UK
*Correspondence: h.clevers@hubrecht.eu
DOI 10.1016/j.cell.2010.09.016

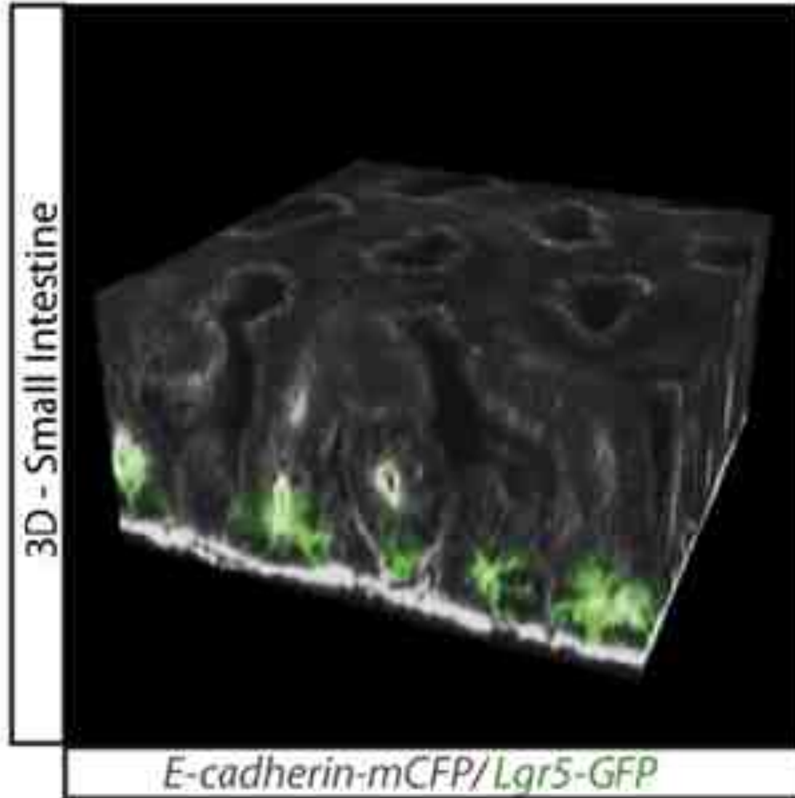
134 Cell 143, 134–144, October 1, 2010 ©2010 Elsevier Inc.

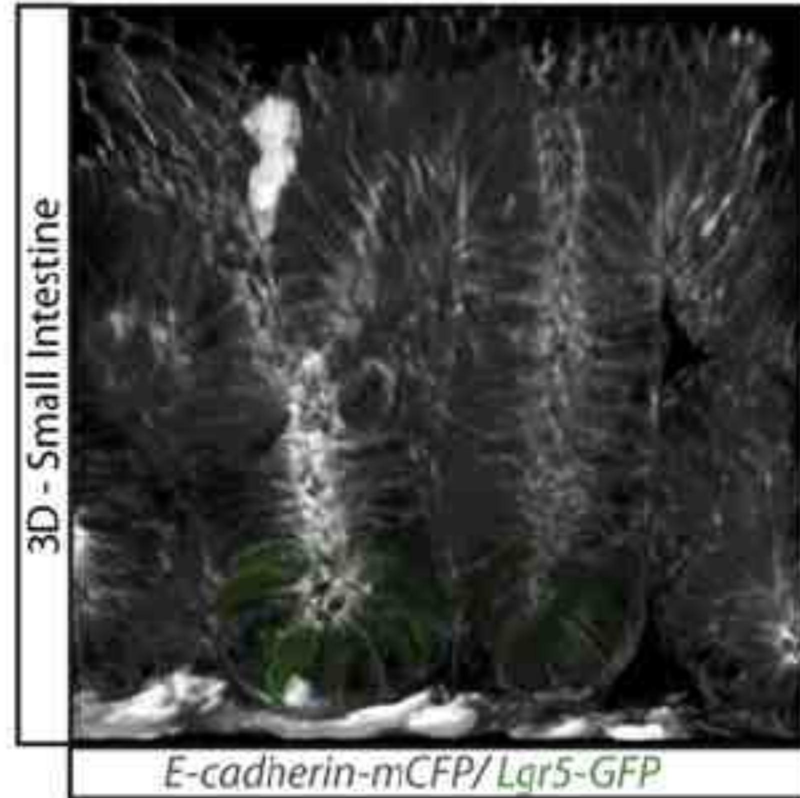
Location and Number of Lgr5hi Cells per Crypt

(A) E-cadherin knock-in strategy in which the fluorescent protein monomer Cyan (mCFP) is fused to the C terminus of Cdh1.

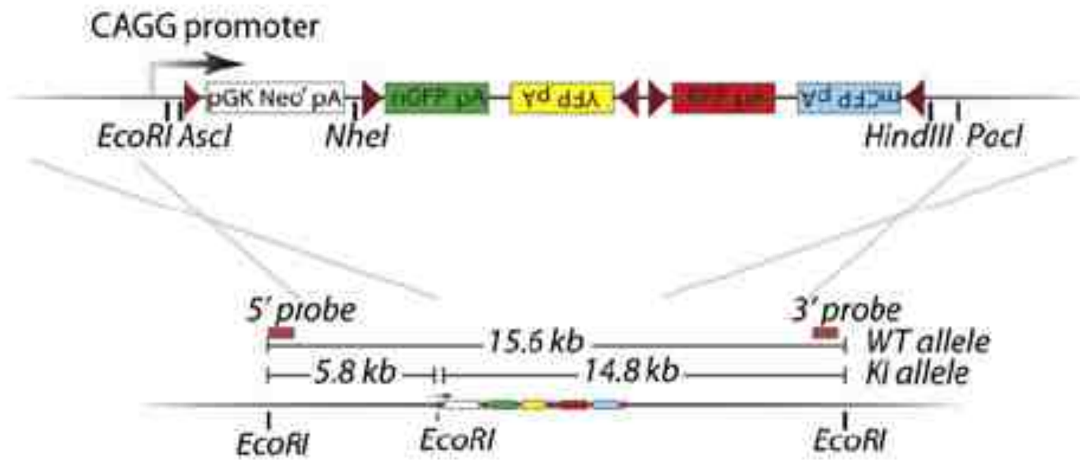


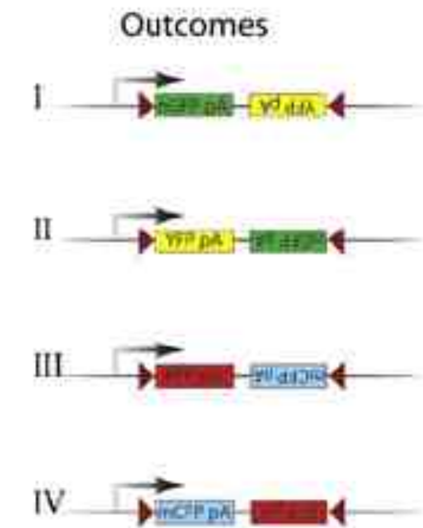
C

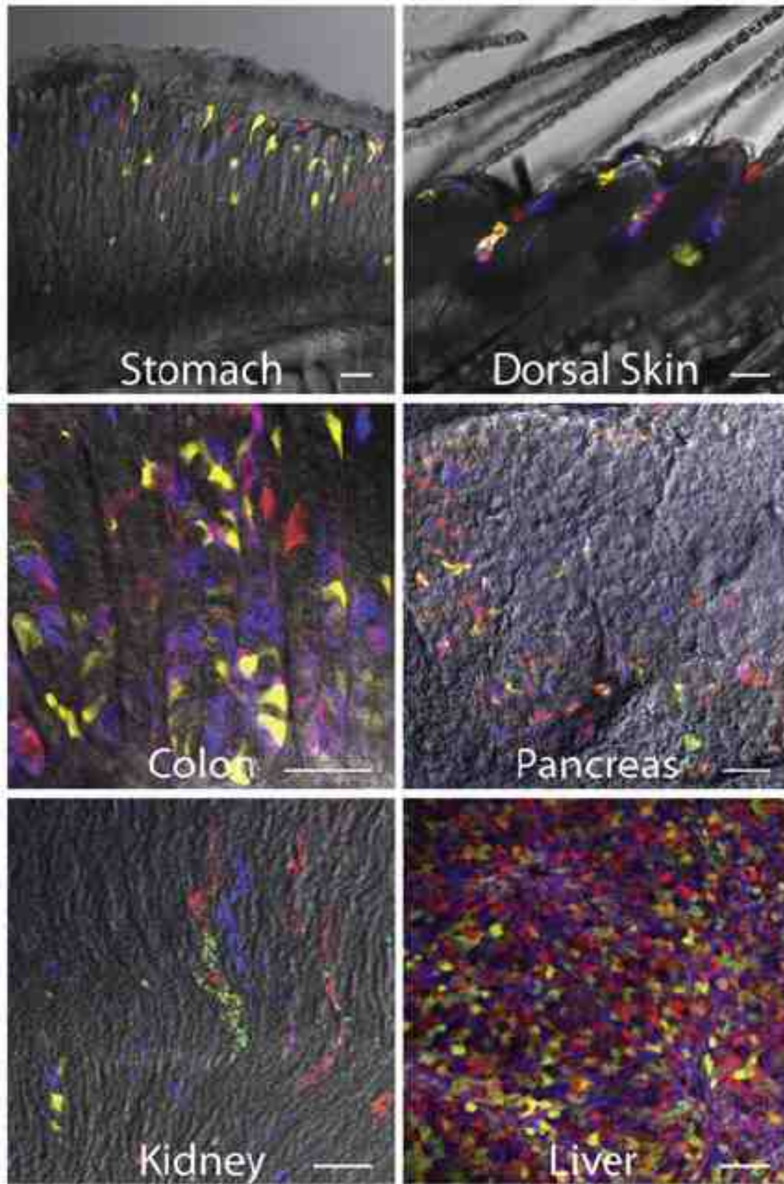




Confetti knock-in strategy

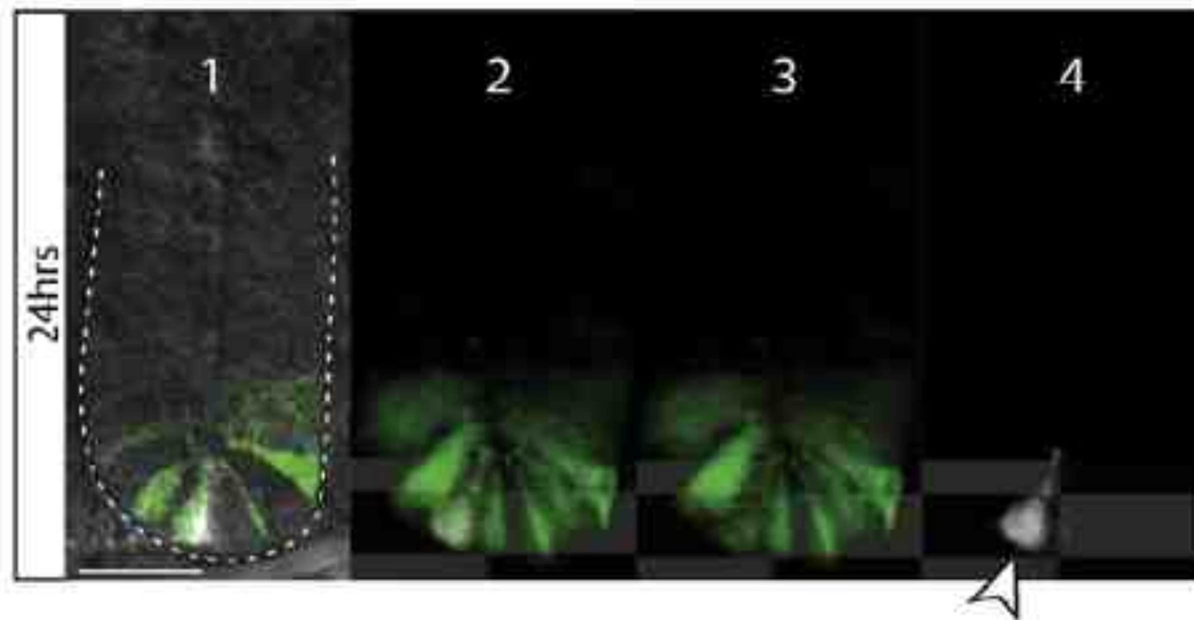






Confetti knock-in strategy

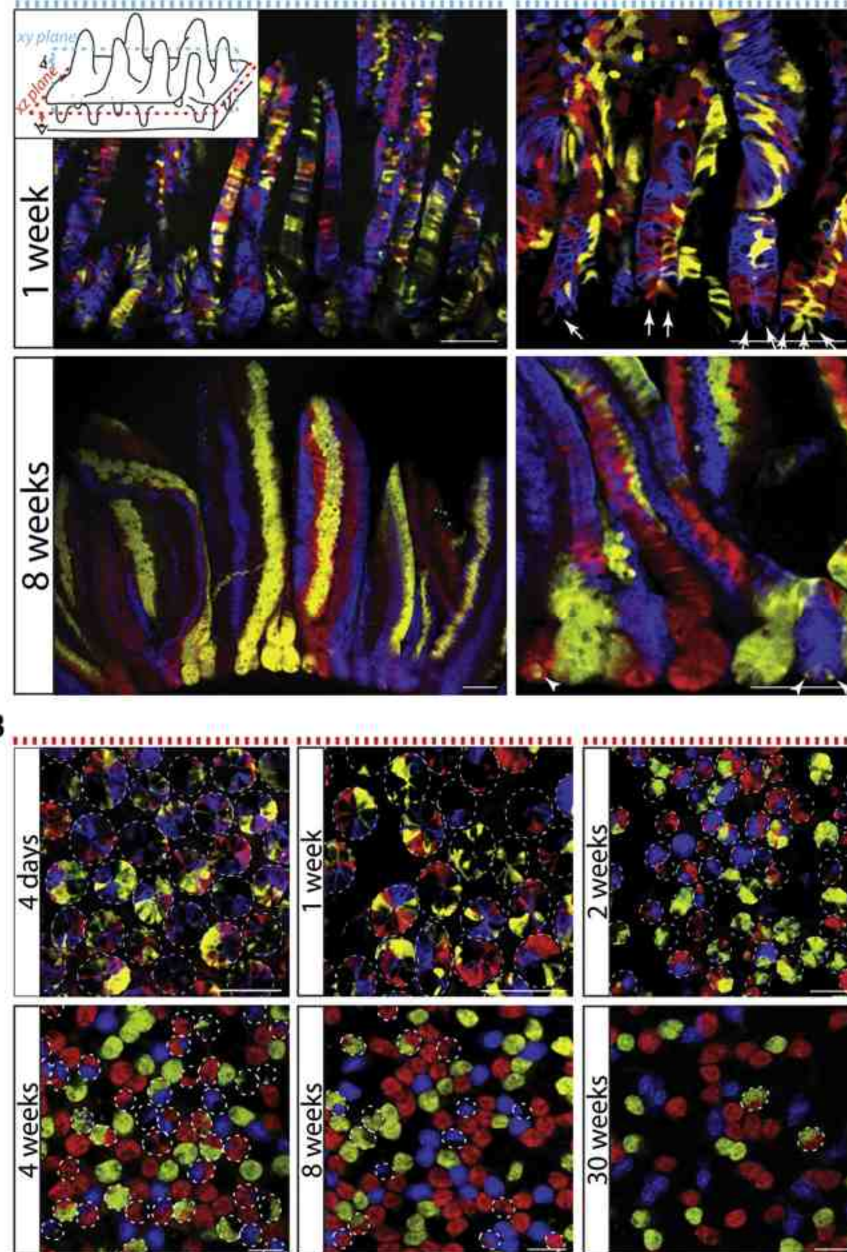
R26R-Confetti mice were crossed with Lgr5-EGFPIres- CreERT2 mice. Tracing was sporadically induced in
single Lgr5hi cells (1 Confetti color in 6 crypts). Cytosolic GFP marks the Lgr5hi stem cell population. Panels from left to right: (1) single plane-2D image of crypt with one YFP (white, false color) labeled Lgr5hi cell. Background is DIC image; (2) 3D reconstruction of the same crypt showing Lgr5hi cells (green) and the traced cell (white); (3) same, but GFP only; (4) same but YFP only. Arrowheads point to Lgr5hi cells within a clone, arrows point to TA cells within clone that lost Lgr5hi activity.



B

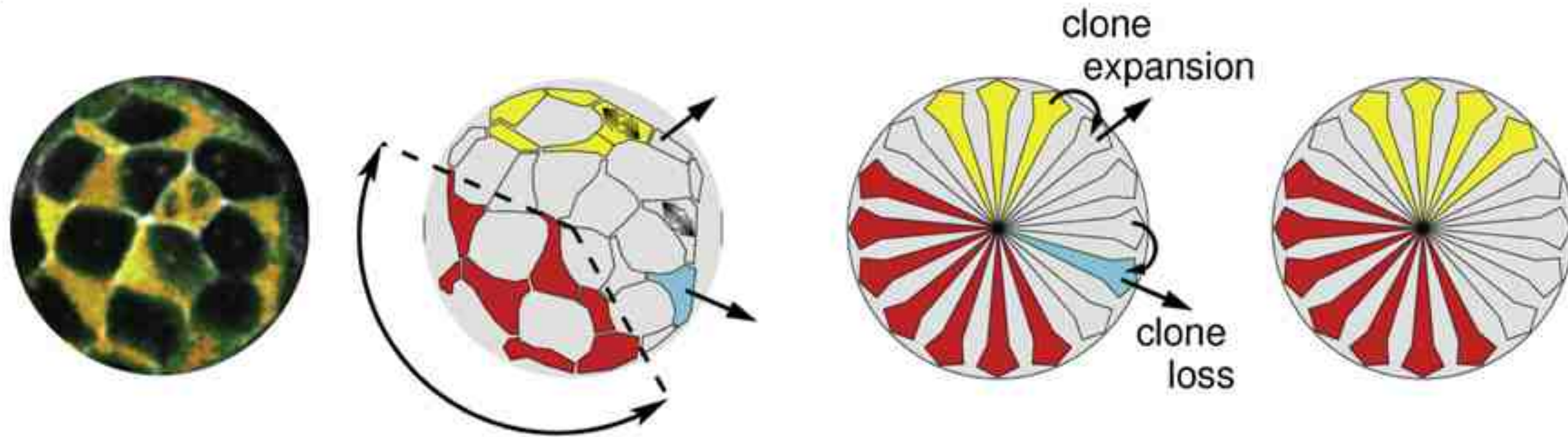
24 hrs

Clone size (number of cells) ↓ / Number of $Lgr5^{hi}$ cells within clones →	0	1	2	3	4	5	6	7	8	9	10
1	4	34									
2	1	2	2								
3	0	0	0	0							
4	0	0	0	0	0						
5	0	0	0	0	0	0					
6	0	0	0	0	0	0	0				
7	0	0	0	0	0	0	0	0			
8	0	0	0	0	0	0	0	0	0		
9	0	0	0	0	0	0	0	0	0	0	
10	0	0	0	0	0	0	0	0	0	0	0



Only one type of Lgr5hi cell exists, 14 per crypt, all endowed with the potential for long-term stemness. Cell fate is determined after division of the Lgr5hi stem cell, potentially by competition for available niche space at the crypt base. Thus, homeostasis is obtained by neutral competition between equal stem cells and occurs at the population level. To evaluate the possibility that the stochastic model indeed underlies the homeostatic self-renewal in crypts, we subjected our quantitative short- and long-term tracing data to a theoretical analysis.

A



B

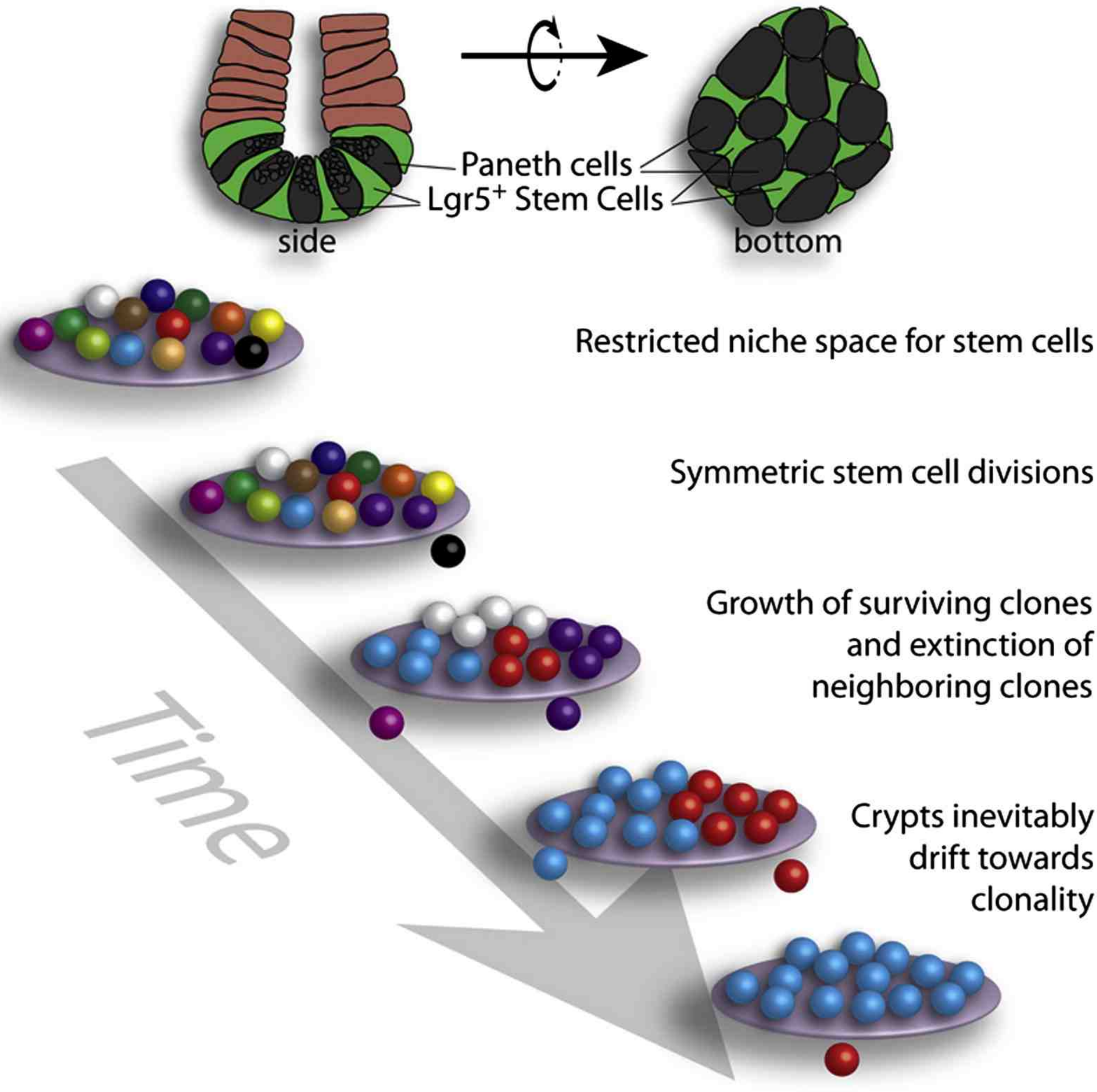
Domain size in circumference (in "sextadecals")

Time post-induction (weeks)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0.5	131	117	80	36	14	12	8	4	2	0	1	0	1	0	1	1
1	21	50	58	58	37	29	23	15	10	6	7	5	3	2	0	3
2	66	132	137	135	86	78	74	73	55	38	28	40	15	21	12	51
4	6	23	23	31	22	19	36	43	36	22	18	28	9	18	7	280
8	3	7	5	9	13	10	11	11	4	7	1	3	0	7	3	256
18	0	0	0	0	1	0	3	2	5	0	0	0		0	0	148
30	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	150

C

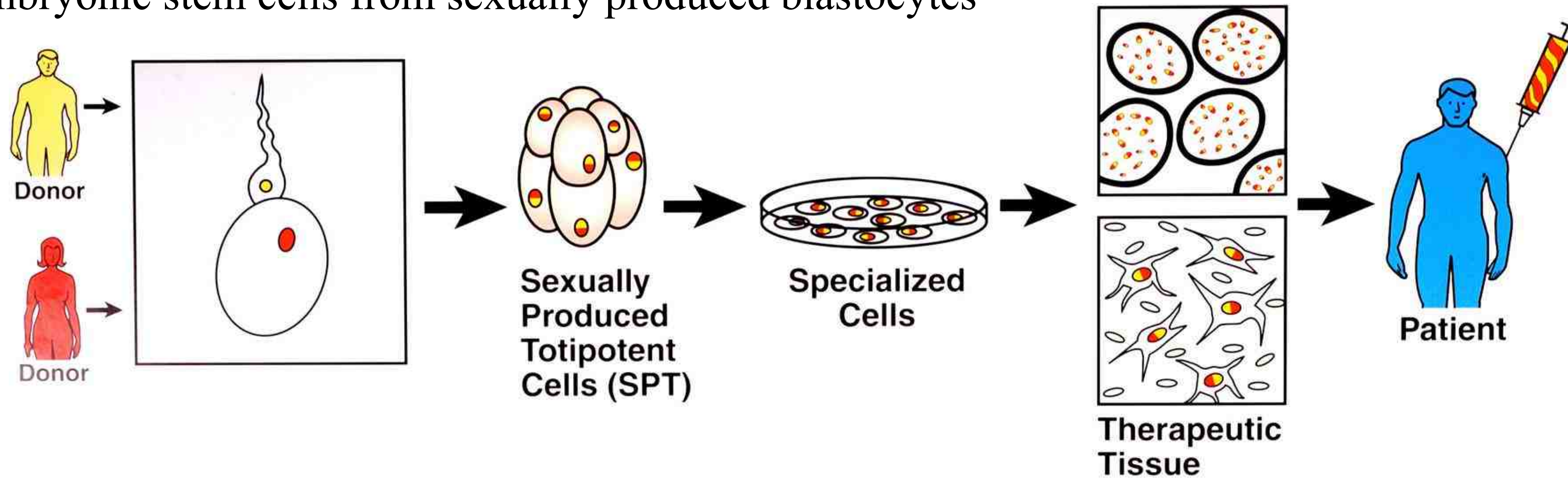
Time post-induction (weeks)	Frequency (%) monochromatic crypts
0.5	0
1	1
2	5
4	45
8	73
18	93
30	98

11_bcma_2011

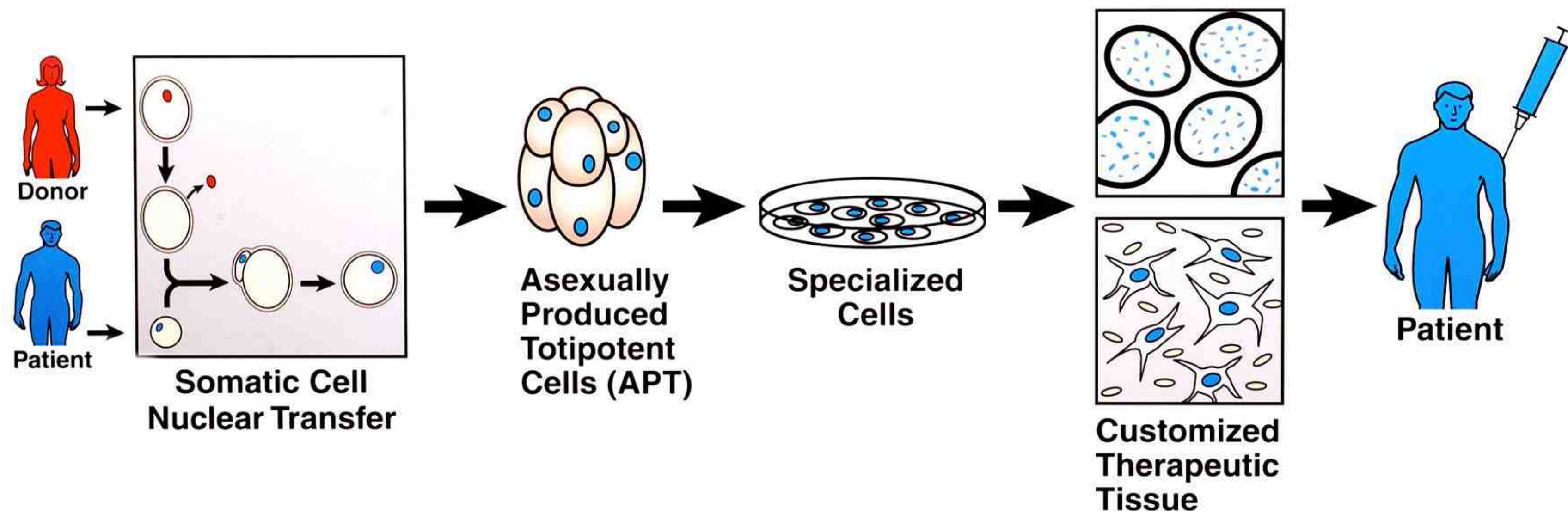


11_bcma_2011

Embryonic stem cells from sexually produced blastocytes



Embryonic stem cells from asexually produced blastocyte (somatic cell nuclear transfer)



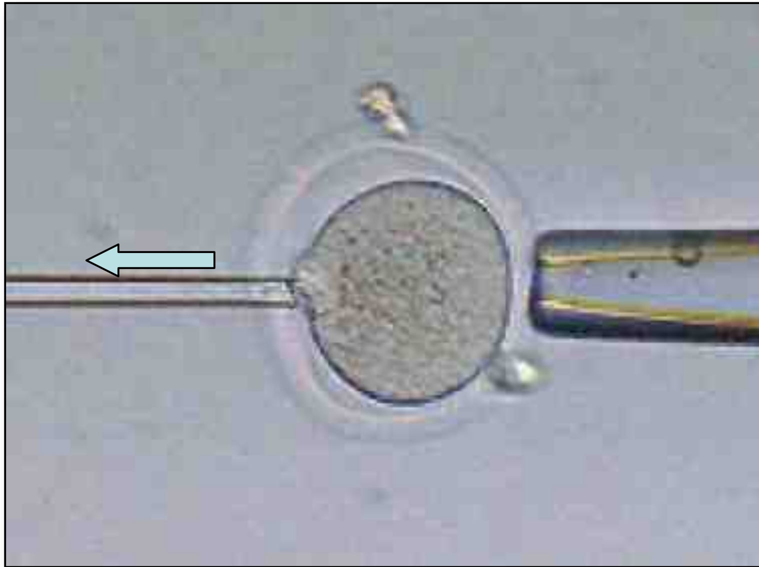
Process of generating embryonic stem cells

The first step in the process of generating embryonic stem cells is to remove the nucleus from an unfertilized egg cell

(A). We use a suction pipette (B) to hold the egg cell steady and a glass needle (C) to remove the cellís nucleus.

Because the egg cell is only 100 micrometers, or one-tenth of a millimeter, wide, we monitor this fine surgical extraction with a microscope (see previous image).

Remove the nucleus from an unfertilized egg cell



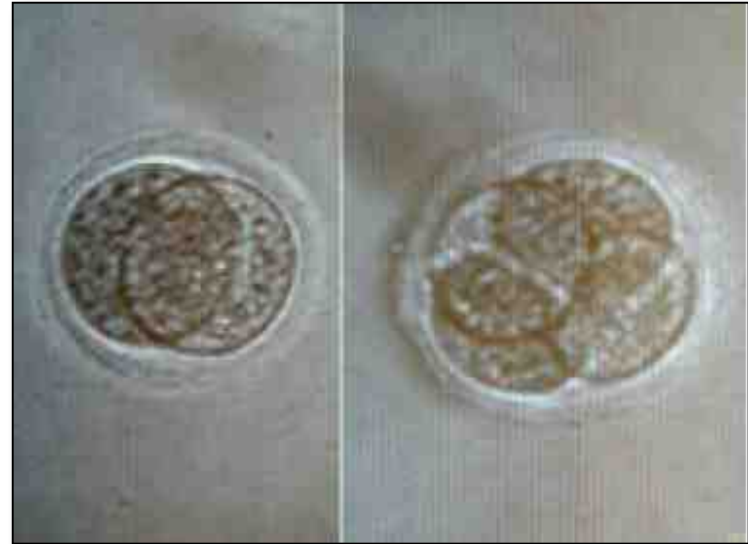
We have gently pushed the glass needle through the tough shell that surrounds the egg cell. In nature, the zona pellucida, as this shell is known, protects the egg as it travels down the fallopian tube on its way to the uterus; it also regulates fertilization so that only a single sperm may enter the egg. Here, the glass needle is in the process of removing the nucleus from within the egg. If you look closely at the tip of the needle, you can just make out the genetic material being drawn out.

We have released the egg cell's nucleus (A) outside of the egg. This nuclear material will no longer be needed. What remains is an "enucleated" egg (B) that still contains protein, RNA molecules, and other important factors that will ultimately help to establish embryonicstem cells.

Inject the nucleus (at arrow) from a donor cell into the enucleated egg cell

In the future, such a donor cell might be a skin cell from a disease sufferer whom doctors hope to treat using the patient's own stem cells grown in culture; the procedure would be essentially the same as we're showing here. Once again we ease the tip of the glass needle through the zona pellucida and deep into the enucleated egg cell, where we then deposit the donor nucleus.

”Activate" the unfertilized egg cell using a chemical or electrical treatment that stimulates cellular division.



The first division results in two cells (left image), the next makes four cells, and so on. This structure is now termed an embryo.

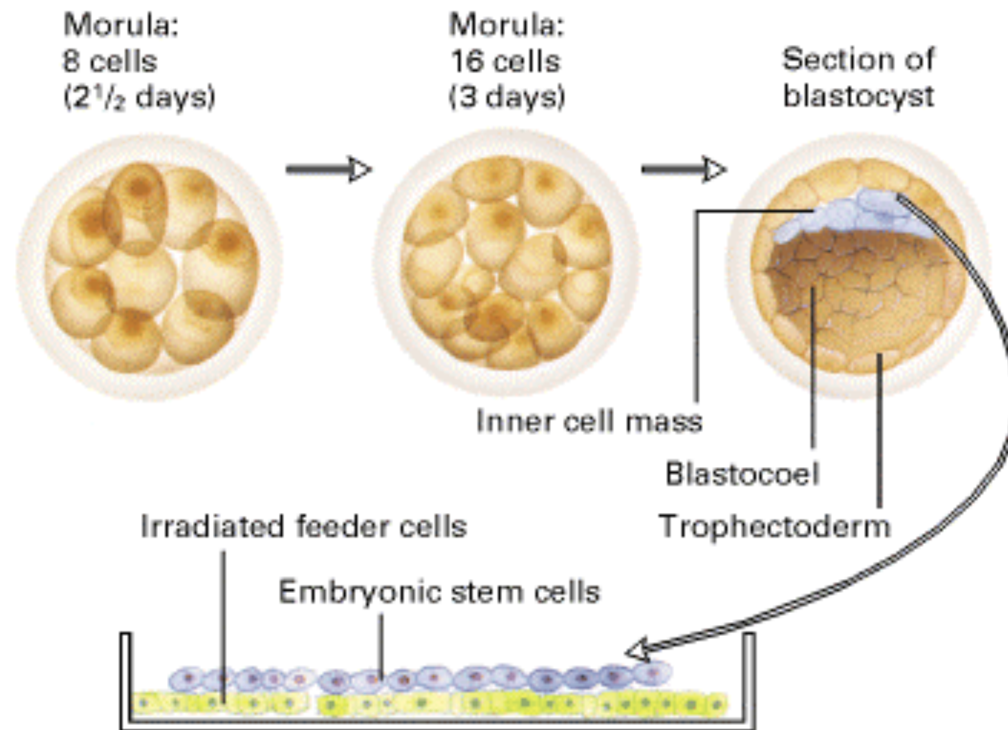
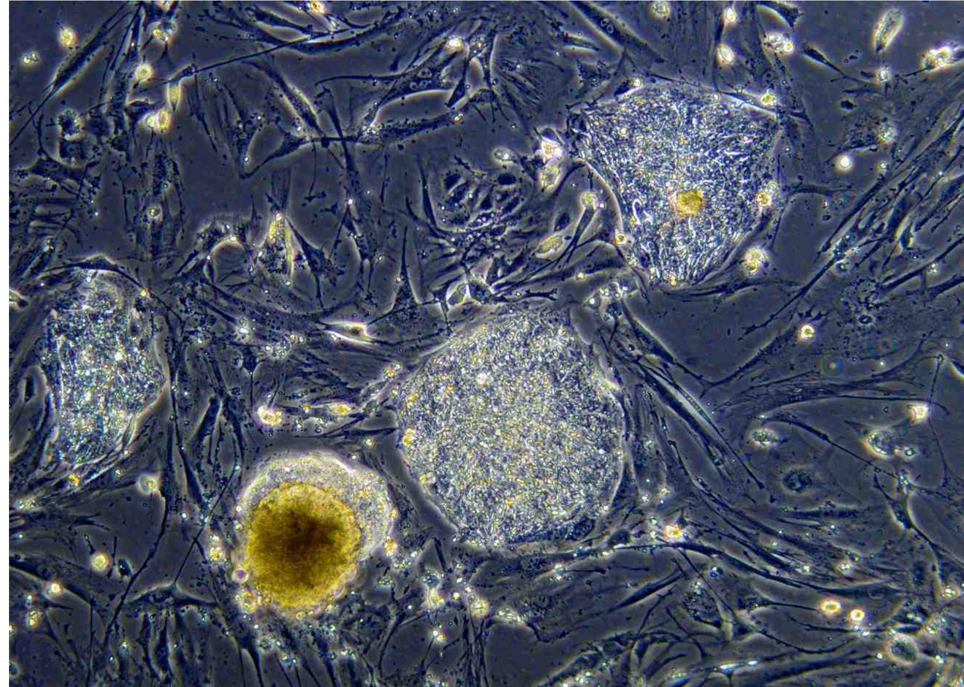


Figure 8-32. Preparation of embryonic stem (ES) cells. Fertilized mouse eggs divide slowly at first; after 41/2 days, they form the blastocyst, a hollow structure composed of about 100 cells surrounding an inner cavity called the blastocoel. Only ES cells, which constitute the inner cell mass, actually form the embryo. Other cells form the trophectoderm, which gives rise to the membranes (amnion and placenta) by which the embryo is attached to the uterine wall. Embryonic stem cells can be removed from the blastocyst and grown on lethally irradiated “feeder cells.” [See E. Robertson et al., 1986, Nature 323:445.]



Microscopic 10x view of a colony of undifferentiated human embryonic stems cells being studied in developmental biologist James Thomson's research lab. The embryonic stem cell colonies are the rounded, dense masses of cells. The flat, elongated cells in between the embryonic stem cell colonies are fibroblasts that are used as a "feeder layer" on which the embryonic stem cells are grown. (Source: University of Wisconsin-Madison.)

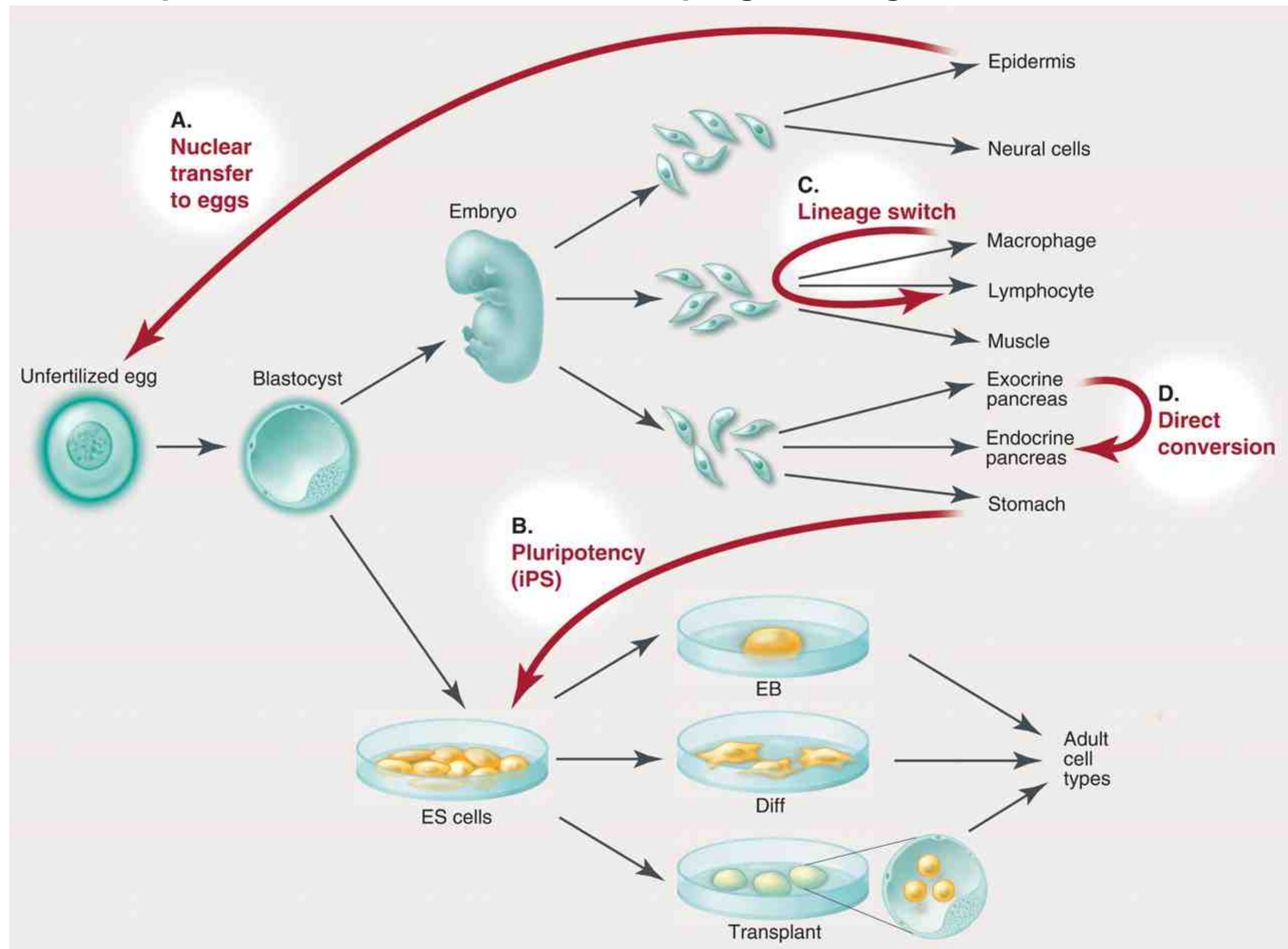
Fig. 5. Four experimental routes for nuclear reprogramming

(A) by nuclear transfer to eggs

(B) by induced pluripotency iPS

(C) by lineage switching back to a branch point and out again in a different direction

(D) by direct conversion



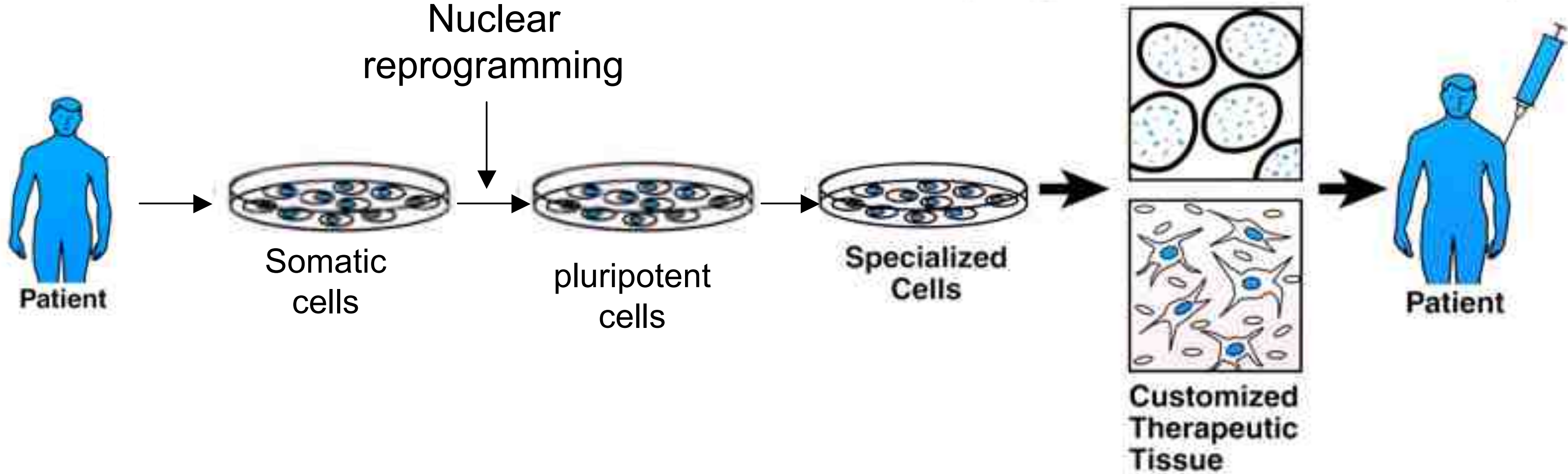
The lower part of the figure shows reprogramming by the generation of ES cells; these can be aggregated into an embryoid body (EB), made to differentiate in culture (diff), or transplanted to a blastocyst. In each case, various types of adult cells can be formed.

Published by AAAS

J. B. Gurdon et al., Science 322, 1811 -1815 (2008)

Science AAAS

Nuclear Reprogramming in cells



Cell *126*, 663–676, August 25, 2006

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

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]
[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan
[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Contact: yamanaka@frontier.kyoto-u.ac.jp
DOI 10.1016/j.cell.2006.07.024

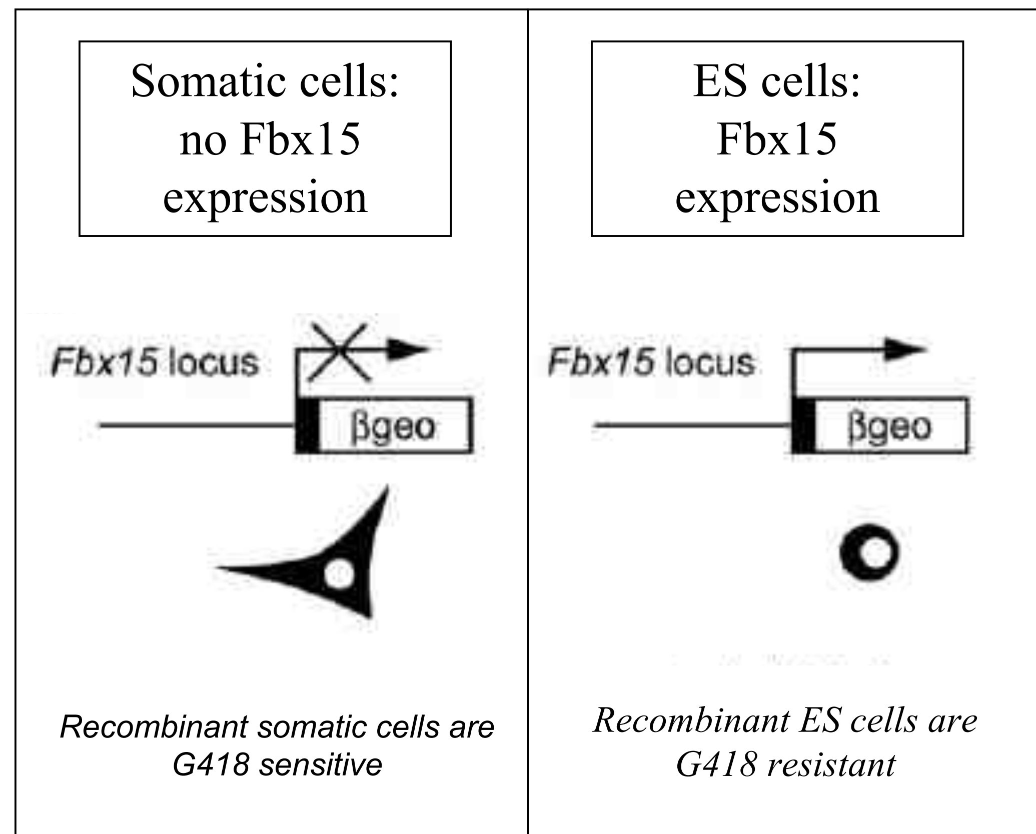
"We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells".

We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity. For b-catenin, c-Myc, and Stat3, we used active forms, S33Y-b-catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively.
Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2DSH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.

Assay system in which the induction of the pluripotent state could be detected as resistance to G418.

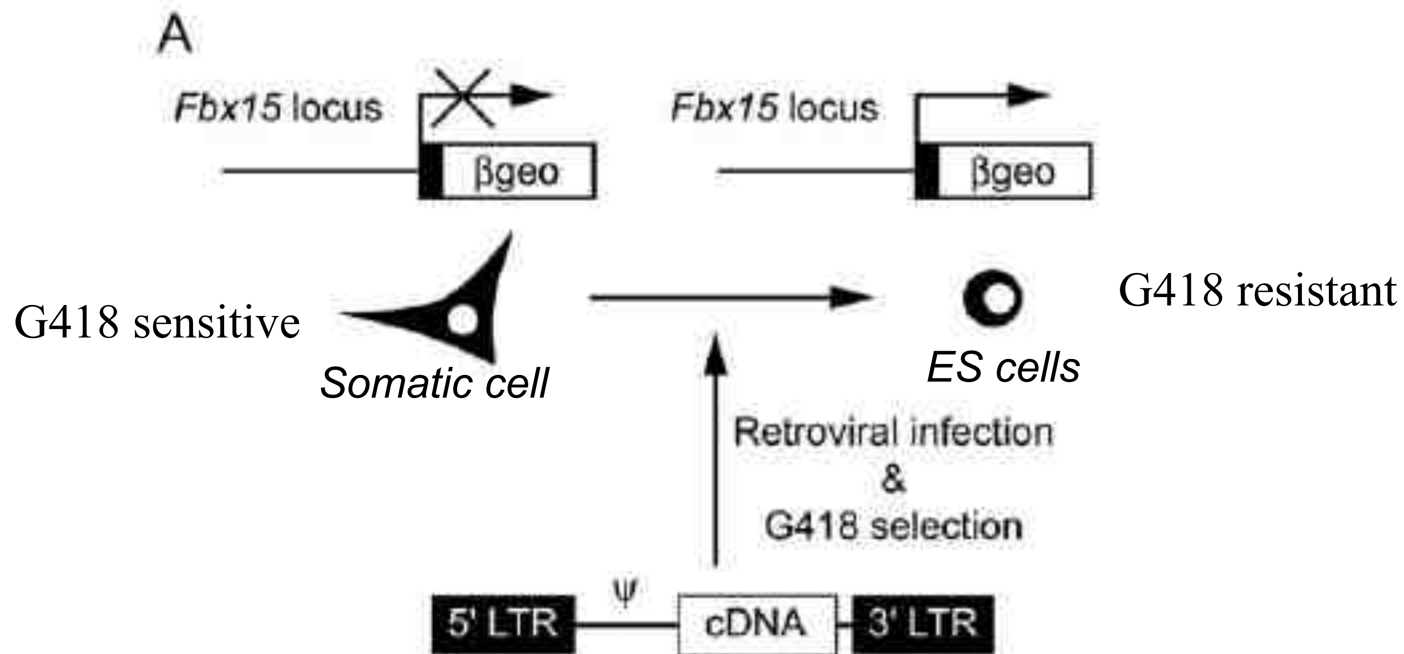
We inserted a βgeo cassette (a fusion of the β -galactosidase and neomycin resistance genes) into the mouse Fbx15 gene by homologous recombination (Tokuzawa et al., 2003). Although specifically expressed in mouse ES cells and early embryos, Fbx15 is dispensable for the maintenance of pluripotency and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

ES cells homozygous for the β geo knockin construct (Fbx15 β geo/ β geo) were resistant to extremely high concentrations of G418 (up to 12 mg/ml), whereas **somatic cells** derived from Fbx15bgeo/bgeo mice were sensitive to a normal concentration of G418 (0.3 mg/ml). We expected that even partial activation of the Fbx15 locus would result in resistance to normal concentrations of G418.



Recombinant somatic cells are G418 sensitive

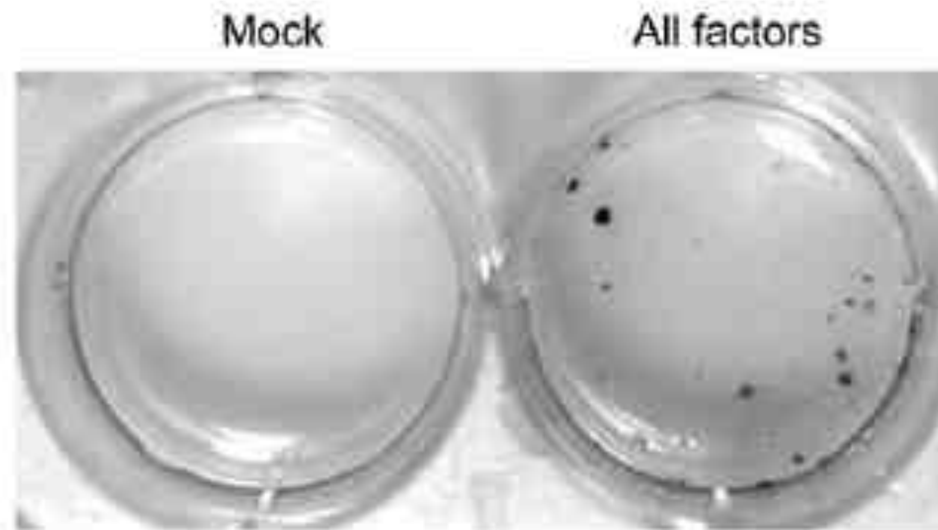
Recombinant ES cells are G418 resistant



Retroviral infection with constructs for each of those factors known to be important for plutipotency

Ecat1	Klf4
Dppa5	β-catenin
Fbxo15	c-Myc
Nanog	Stat3
ERas	Grb2
Dnmt3l	Ecat8
Oct3/4	Gdf3
Sox2	Sox15
Rex1	Dppa4
Utf1	Dppa2
Tcl1	Fthl17
Dppa3	Sall4

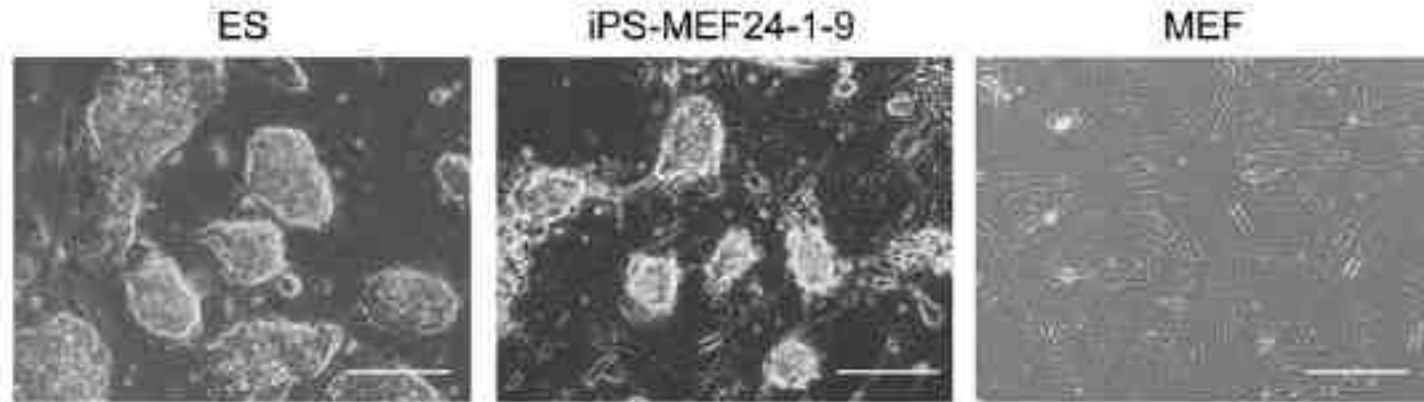
We introduced each of the 24 candidate genes into mouse embryonic fibroblasts (MEFs) from Fbx15 b geo/ b geo embryos by retroviral transduction. Transduced cells were then cultured on STO feeder cells in ES cell medium containing G418 (0.3 mg/ml).



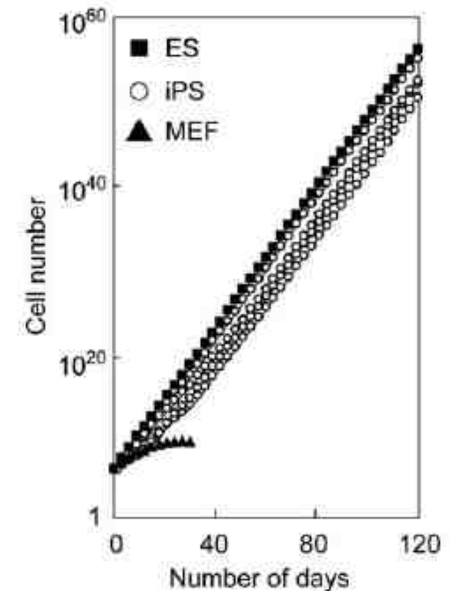
G418-resistant colonies were observed 16 days after transduction with a **combination of 24 factors**. Cells were stained with crystal violet.

no drug-resistant colonies with any single factor, indicating that no single candidate gene was sufficient to activate the Fbx15 locus

Of the 12 clones for which we continued cultivating under selection, 5 clones exhibited morphology similar to ES cells, including a round shape, large nucleoli, and scant cytoplasm



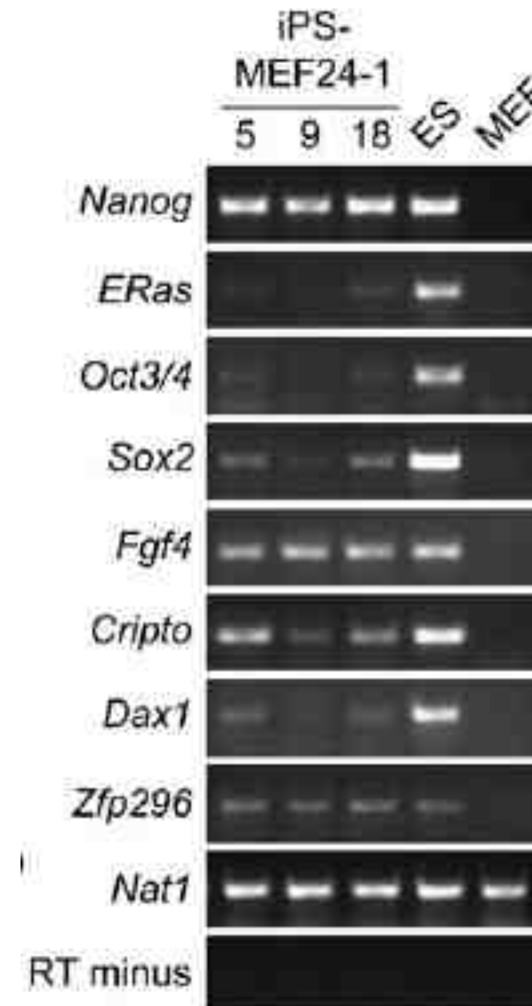
iPS-MEF24 = ‘‘pluripotent stem cells induced from MEFs by 24 factors



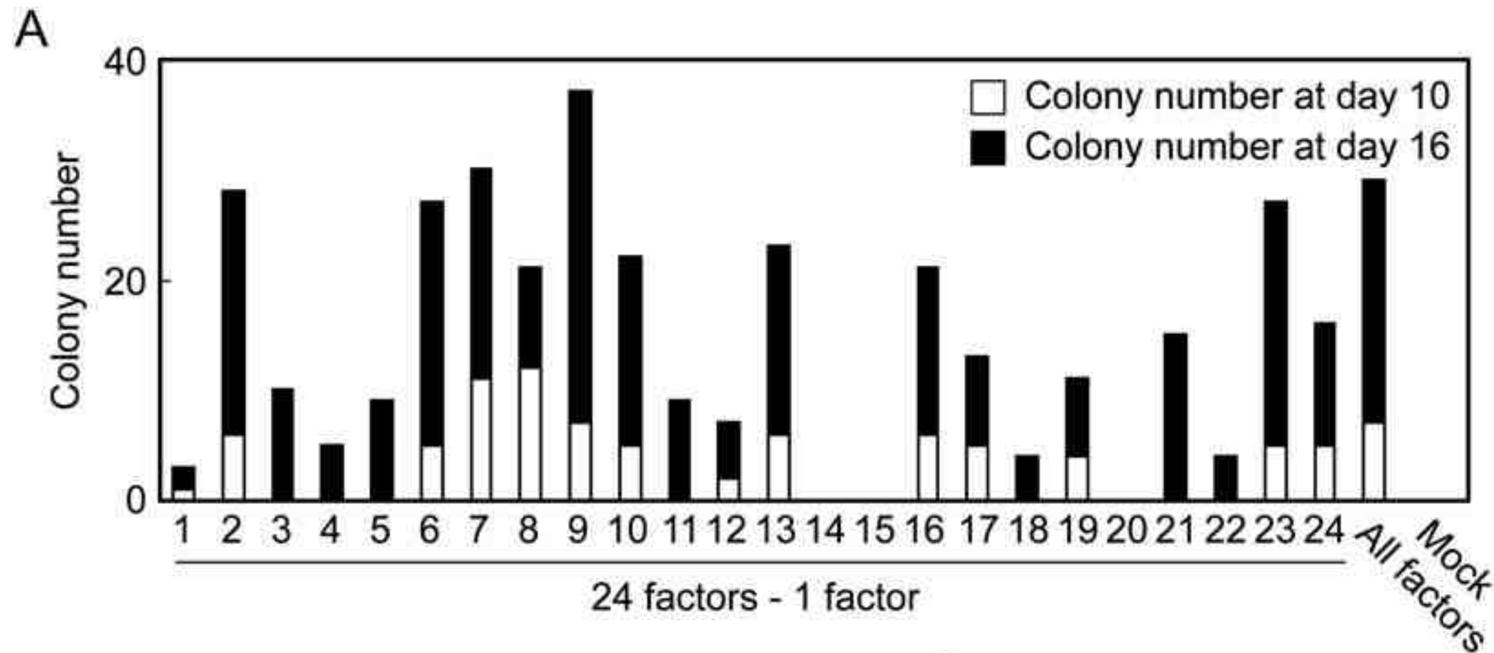
4 of these clones possessed ES cell-like morphology and proliferation properties

Reverse transcription PCR (RT-PCR) analysis revealed that the iPS-MEF24 clones expressed ES cell markers, including Oct3/4, Nanog, E-Ras, Cripto, Dax1, and Zfp296 and Fgf4

These data indicate that some combination of these 24 candidate factors induced the expression of ES cell marker genes in MEF culture.

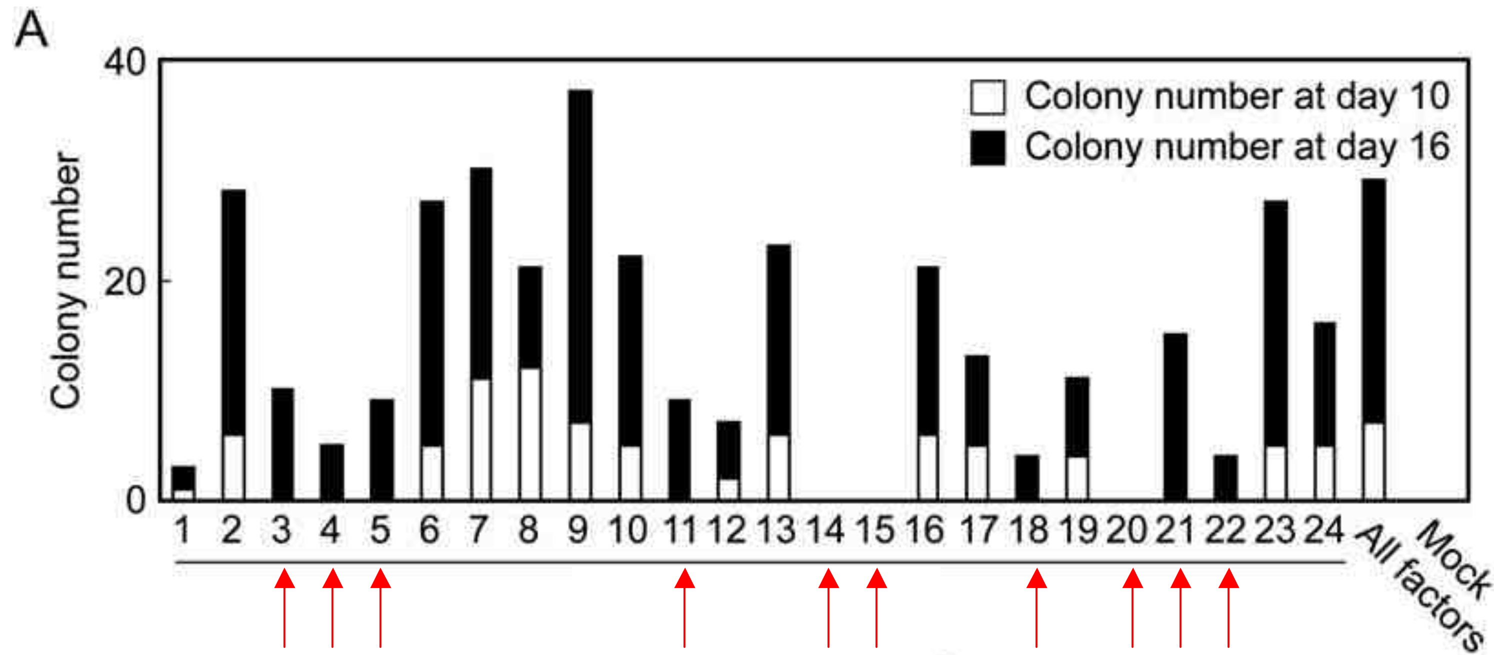


we examined the effect of withdrawal of individual factors from the pool of transduced candidate genes on the formation of G418-resistant colonies

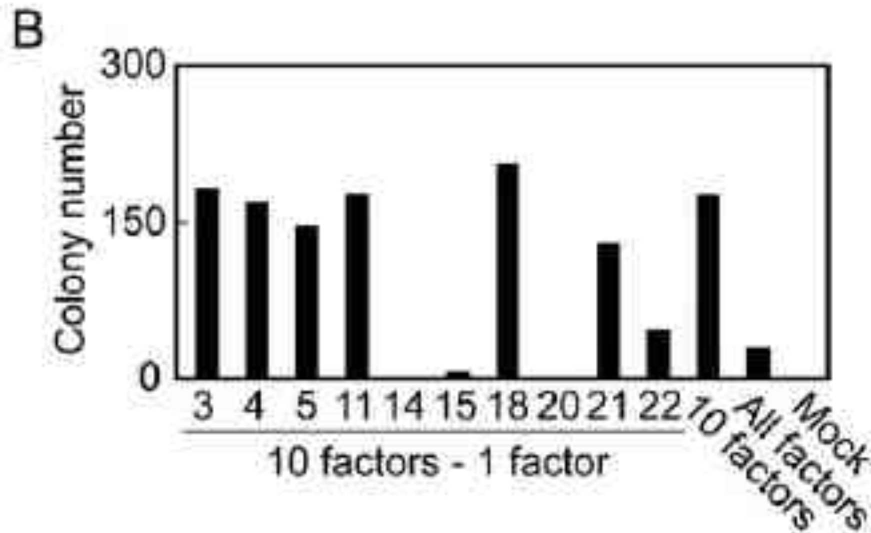


Effect of the removal of individual factors from the pool of 24 transduced factors on the formation of G418-resistant colonies. Fbx15bgeo/bgeo MEFs were transduced with the indicated factors and selected with G418 for 10 days (white columns) or 16 days (black columns).

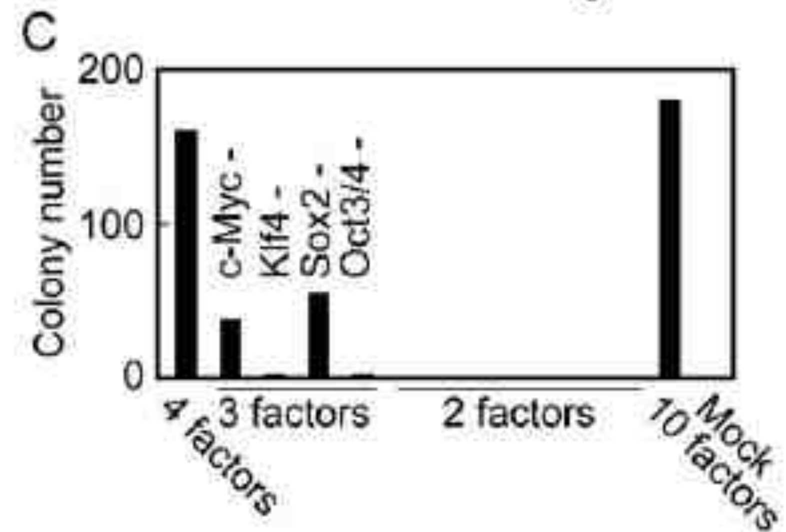
we examined the effect of withdrawal of individual factors from the pool of transduced candidate genes on the formation of G418-resistant colonies



Effect of the removal of individual factors from the pool of 24 transduced factors on the formation of G418-resistant colonies. Fbx15bgeo/bgeo MEFs were transduced with the indicated factors and selected with G418 for 10 days (white columns) or 16 days (black columns).



Effect of the removal of individual factors from the selected 10 factors on the formation of G418-resistant colonies 16 days after transduction

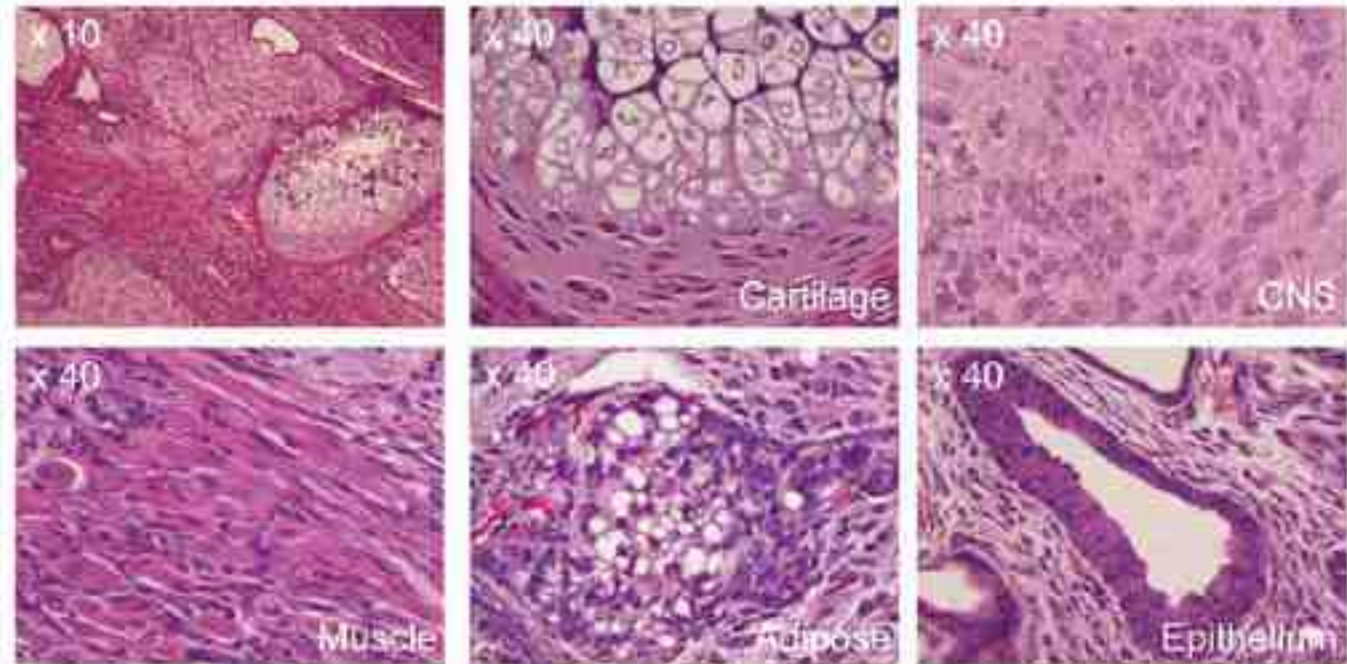


Effect of the transduction of pools of four, three, and two factors on the formation of G418-resistant colonies 16 days after transduction

These data demonstrate that iPS cells can be induced from MEF culture by the introduction of four transcription factors, Oct3/4, Sox2, c-Myc, and Klf4.

Pluripotency of iPS Cells Derived from MEFs

(A) Various tissues present in teratomas derived from iPS-MEF4-7 cells.

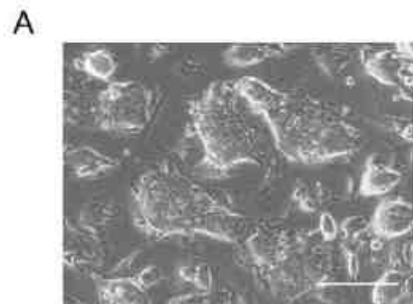


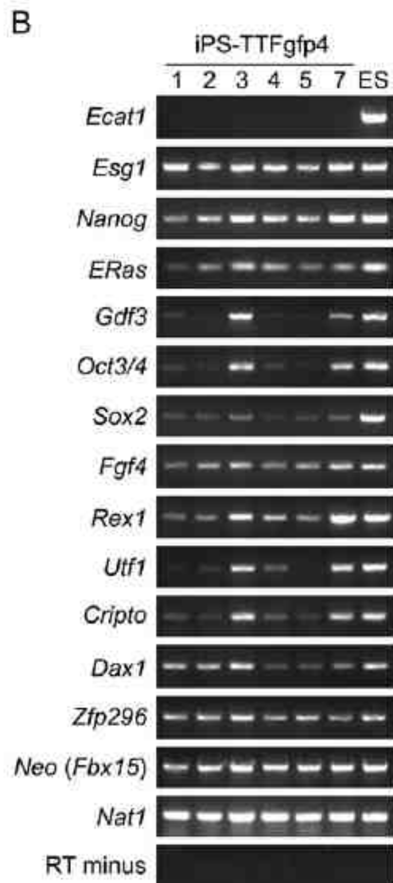
(B) Immunostaining confirming differentiation into neural tissues and muscles in teratomas derived from iPS-MEF4-7.

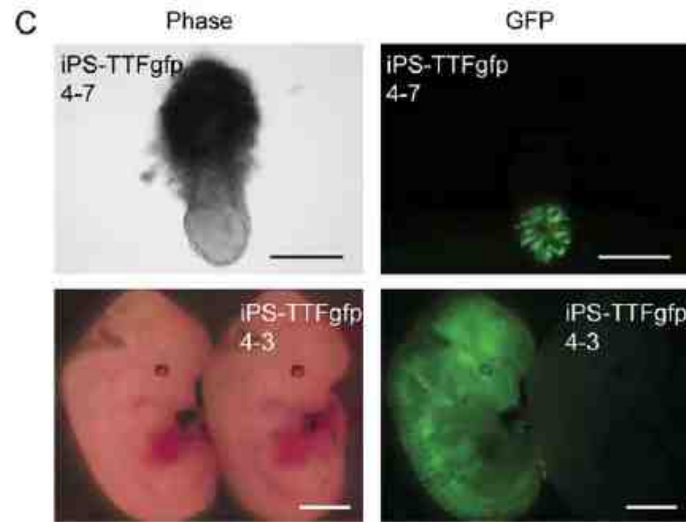
From adult fibroblasts?

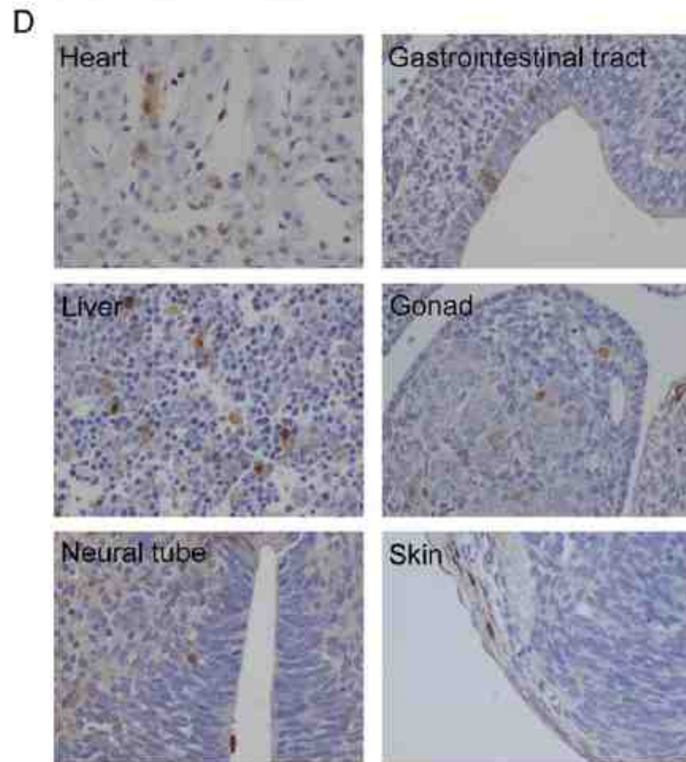
We next introduced the four selected factors into tail-tip fibroblasts (TTFs) of four 7-week-old male Fbx15bgeo/bgeo mice on a C57/BL6-129 hybrid background. We obtained 3 G418-resistant colonies, from each of which we could establish iPS cells (iPS-TTF4). We also introduced the four factors into TTFs from a 12-week-old female Fbx15bgeo/bgeo mouse, which also constitutively expressed green fluorescent protein (GFP) from the CAG promoter and had a C57/BL6-129-ICR hybrid background.

Characterization of iPS Cells Derived from Adult Mouse Tail-Tip Fibroblasts









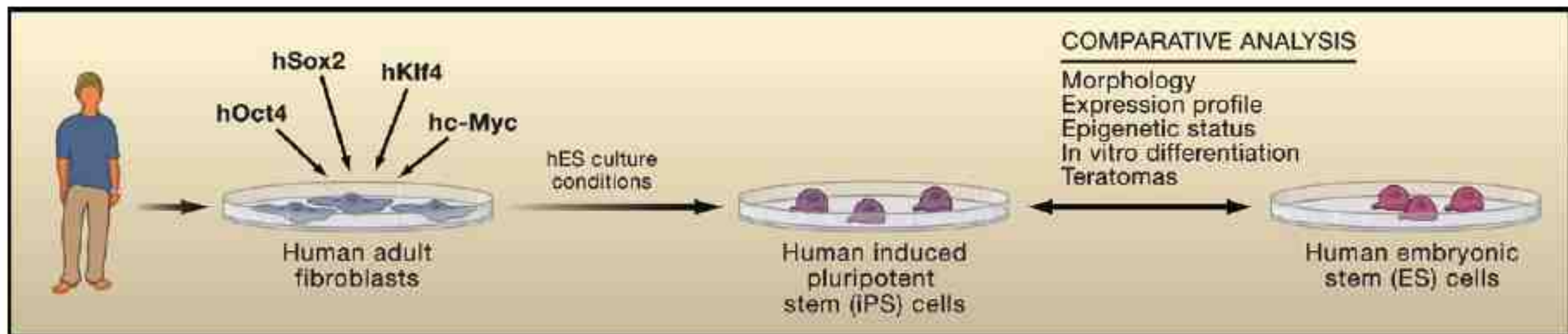
(C) Contribution of iPS-TTFgfp4-7 and iPS-TTFgfp4-3 cells to mouse embryonic development. iPS cells weremicroinjected into C57/BL6 blastocysts.

Embryos were analyzed with a fluorescence microscope at E7.5 (upper panels, iPS-TTFgfp4-7) or E13.5 (lower panels, iPS-TTFgfp4-3). Scale bars = 200 mm (upper panels) and 2 mm (lower panels).

(D) The E13.5 chimeric embryo was sectioned and stained with anti-GFP antibody (brown). Cells were counterstained with eosin (blue).

Induction of Pluripotency: From Mouse to Human

?

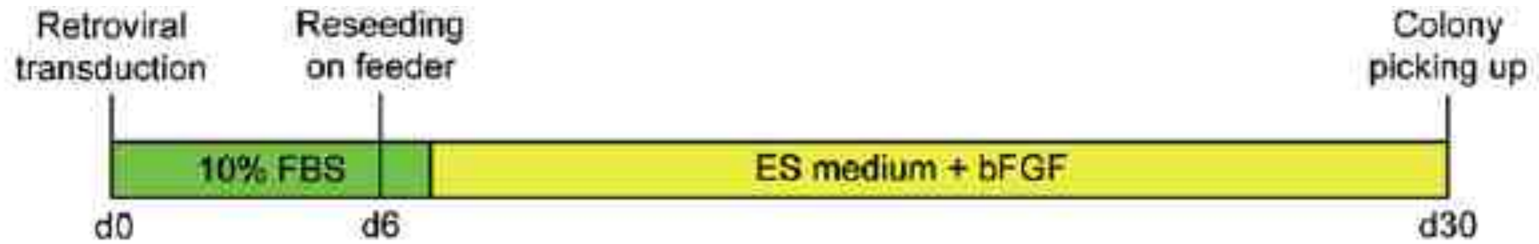


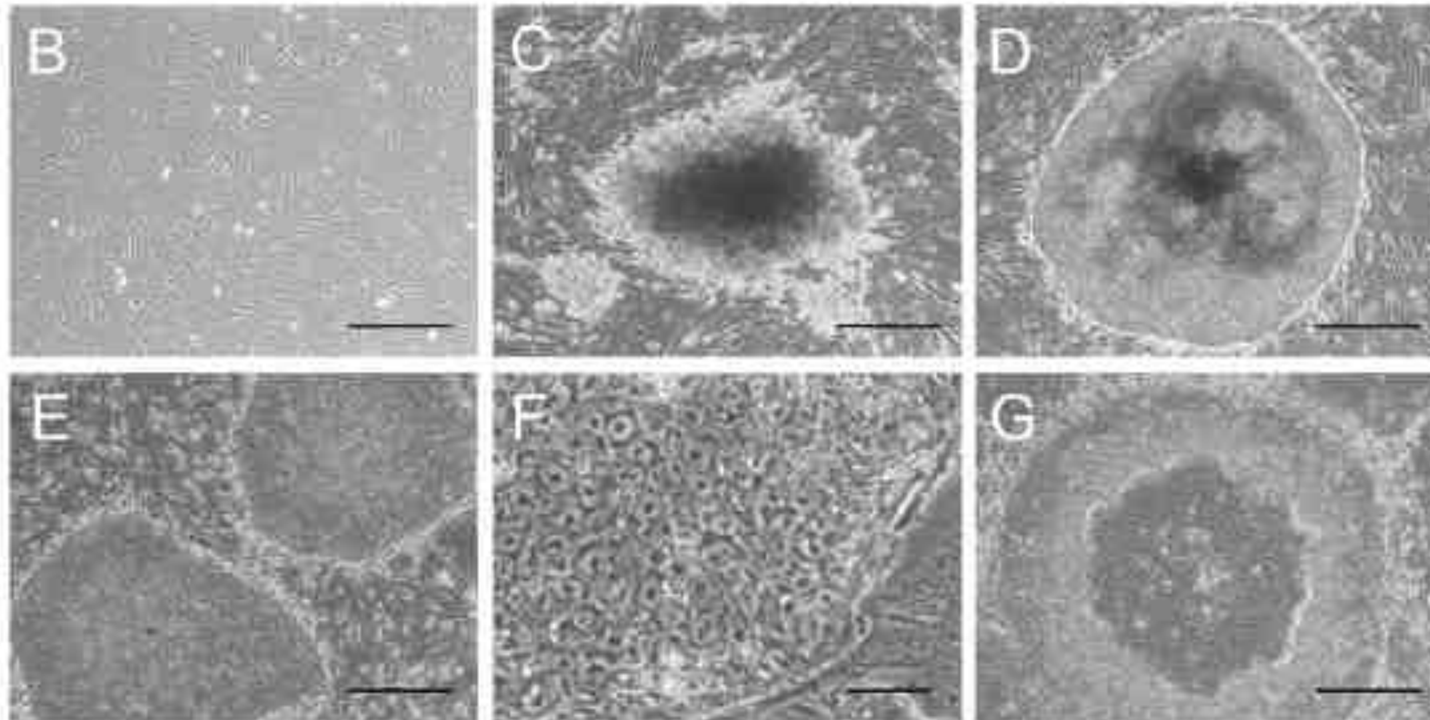
Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

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,*]

Cell *131*, 861–872, November 30, 2007

(A) Time schedule of iPS cell generation from adult Human Dermal Fibroblasts (HDF).





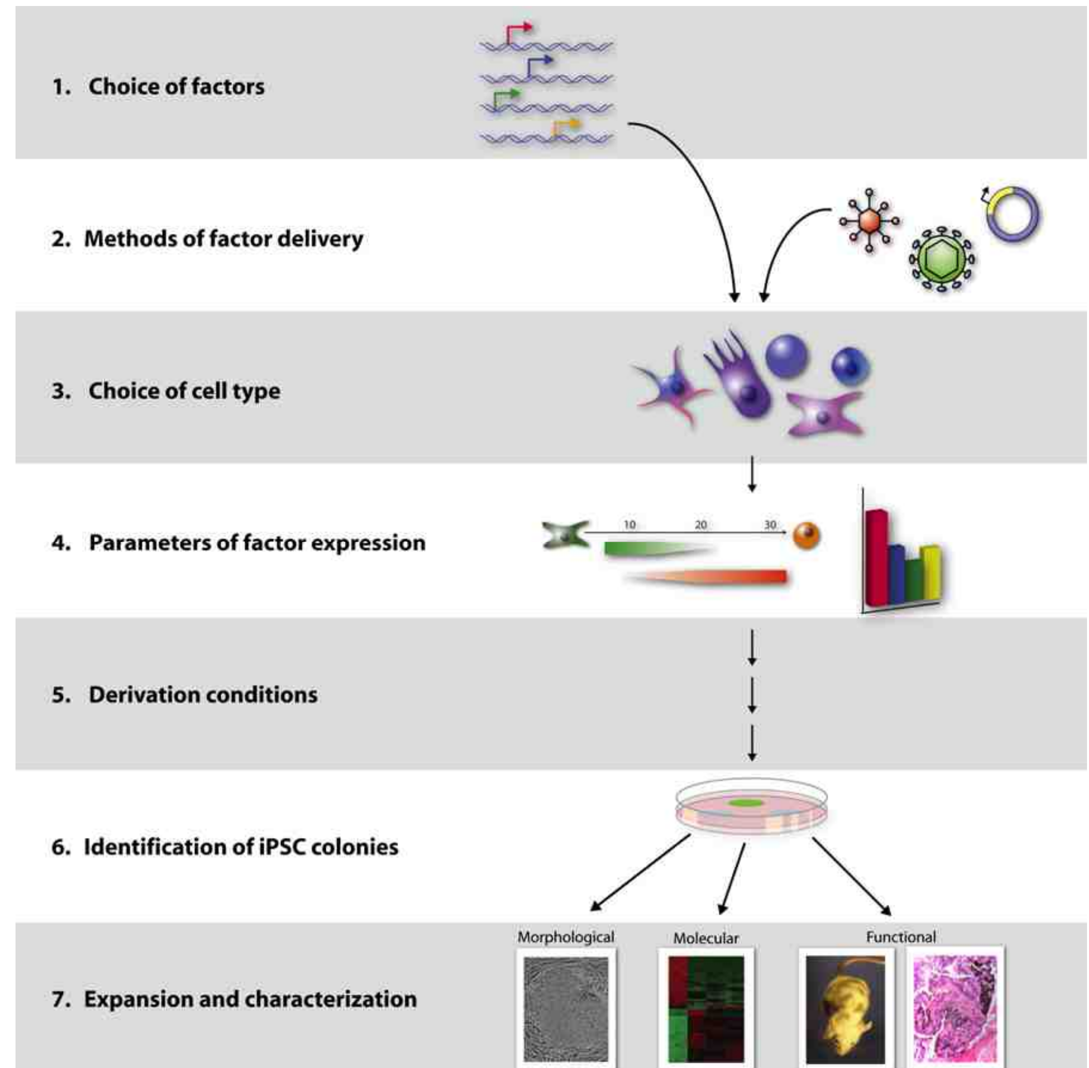
(B) Morphology of HDF.
(C) Typical image of non-ES cell-like colony.
(D) Typical image of hES cell-like colony.
(E) Morphology of established iPS cell line at passage number 6 (clone 201B7).
(F) Image of iPS cells with high magnification.
(G) Spontaneously differentiated cells in the center part of human iPS cell colonies.

Conclusion:

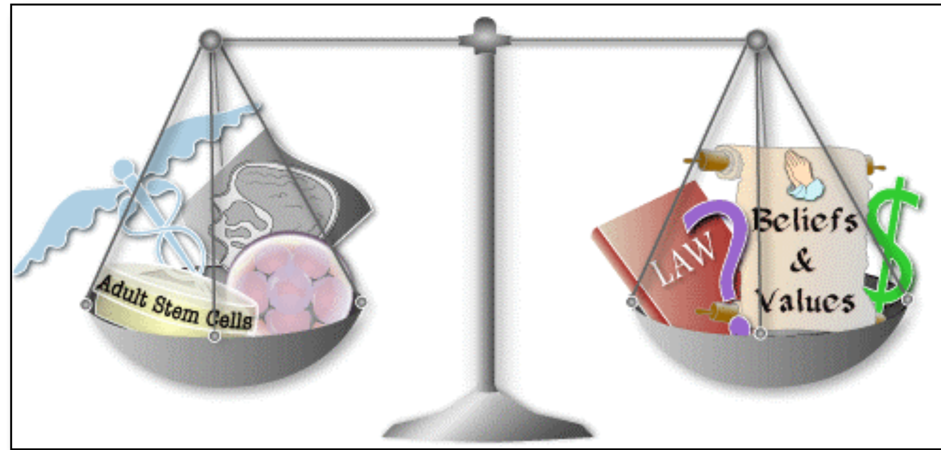
Our study has opened an avenue to generate patient and disease-specific pluripotent stem cells.
Even with the presence of retroviral integration, **human iPS cells are useful for understanding disease mechanisms, drug screening, and toxicology**. For example, hepatocytes derived from iPS cells with various genetic and disease backgrounds can be utilized in predicting liver toxicity of drug candidates.
Once the safety issue is overcome, human iPS cells should also be applicable in **regenerative medicine**. Human iPS cells, however, are not identical to hES cells: DNA microarray analyses detected differences between the two pluripotent stem cell lines. Further studies are essential to determine whether human iPS cells can replace hES in medical applications.

Cell Stem Cell 3, December 4, 2008 ©2008 Elsevier Inc. 597

Guidelines and Techniques for the Generation of Induced Pluripotent Stem Cells



Ethical, legal and social issues.



There are several types of issues to consider as we think about stem cell research.

Ethical issues are those that ask us to consider the potential moral outcomes of stem cell technologies.

Legal issues require researchers and the public to help policymakers decide whether and how stem cell technologies should be regulated by the government.

Social issues involve the impact of stem cell technologies on society as a whole.

STEM CELLS and CANCER

Gastric Cancer Originating from Bone Marrow–Derived Cells

JeanMarie Houghton,[1*] Calin Stoicov,[1] Sachiyo Nomura,[2,3]
Arlin B. Rogers,[4] Jane Carlson,[1] Hanchen ...
James G. Fox,[4] ...

Bone Marrow Contribution to Gastric Cancers?

Although the cellular origin of epithelial cance... ...ch as gastric cancer induced by
Helicobacter pylori infection, remains to be ... a prevailing assumption is
that they derive from resident epithelial ... ...ntrast to this theory,
Houghton *et al*. (p. 1568; see the news ... ...hat gastric cancers
caused by experimental *Helicobacter* inf... ...marrow, rather
than epithelial cell, origin. Bone ma... ...mice were
tracked in chronically infected recipients ... ...ucosa
where they displayed features of neoplastic pr...
lial cancers. If an equivalent contribution of bone m...
cancers could be established in humans, this finding w...
understanding of the origin and progression of malignancy.

MEDICINE

Bone Marrow Cells: The Source of Gastric Cancer?

Stomach cancer is a major cause of cancer deaths, especially in developing countries; it claims roughly 600 000 lives worldwide ...

began engrafting in the stoma... they started to differenti... the characteristics...