piPS Cells
Cat. #SC801A-1 & SC802A-1

User Manual

Store cells in liquid nitrogen

ver. 1-021111

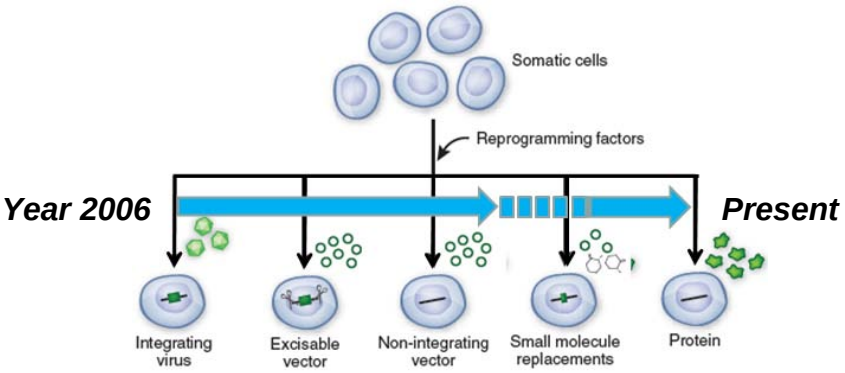
Contents

I. Introduction and Background

A. Evolution of Reprogramming Technology

The concept of reprogramming was initially demonstrated by Gurdon et al. in 1958, where they generated adult Xenopus from somatic cells by nuclear reprogramming. After the discovery of induced pluripotent stem cells, iPSC technique using defined transcription factors has been a basis and standard for generation of pluripotent stem cells. In comparison to somatic cell nuclear transfer, the iPSC technology offers an unprecedented technical simplicity and enables generation of patient-specific pluripotent stem cells with less ethical concerns.

Although iPSC technology provides unprecedented opportunities in biomedical research and regenerative medicine, there remains a great deal to learn about iPSC safety, the reprogramming mechanisms, the quality of iPSCs from different source cells and the variations using different reprogramming technology. Since 2006, iPSC technology has evolved from integrated virus (Retrovirus and lentivirus) to non-integrated virus (Adenovirus) viral methods, to non-viral methods (plasmids), genetic methods (DNA or RNA vectors) to non-genetic methods (proteins). These technical advances provide safer iPSCs for more meaningful mechanistic studies, iPSC-based disease modeling, and drug screening.

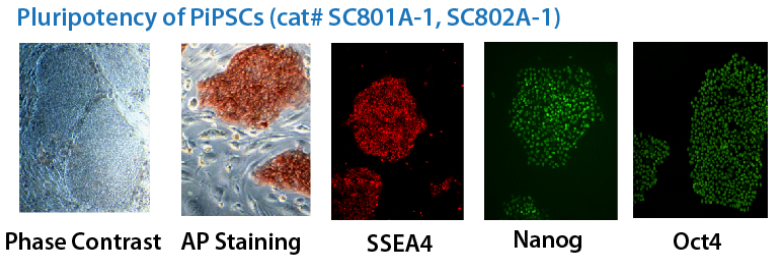


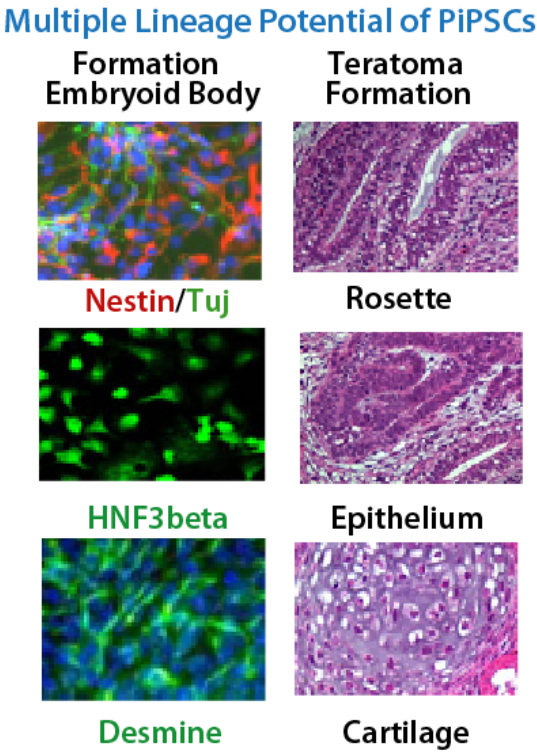
B. Protein derived iPS cell line

SBI offers the human protein iPS cell lines highlighted in *Cell Stem Cell*, Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins. Kim D, et al., 2009, 4:472-476. The piPS cell lines were derived from the most commonly used source cells, newborn human fibroblasts.

To date, all methods to generate iPSCs require the use of genetic materials and/or potentially mutagenic chemicals. Using protein engineering technology, stable piPSCs were induced from human fibroblasts by directly delivering four reprogramming proteins (Oct4, Sox2, Klf4, and c-Myc) fused with a cell-penetrating peptide. These piPSCs exhibited similarities to human embryonic stem cells in morphology, proliferation, global gene expression, DNA methylation patterns, and expression of characteristic pluripotency markers.

PiPSC lines produced with recombinant proteins were successfully maintained for more than 35 passages and differentiated into derivatives of all three embryonic germ layers during the formation of embryoid body (*in vitro*) and teratomas (*in vivo*), the most stringent tests for the quality of human iPS cells. This protein reprogramming system eliminates the potential risks associated with the viruses, DNA transfection, and potentially harmful chemicals. Therefore, it provides a promising safe source of patient-specific cells for the future regenerative medicine.




Multiple Lineage Potential of PiPSCs
Formation Embryoid Body
Teratoma Formation
Nestin/Tuj
Rosette
HNF3beta
Epithelium
Desmine
Cartilage

II. Protocols

A. Materials

Human ESC medium

Component	Cat. #	Final concentration	Source
Knockout serum replacement	10828028	20%	Invitrogen
Glutamax-1	35050061	2 mM	Invitrogen
Nonessential amino acid	11140050	1 x 10^{-4} M	Invitrogen
2-mercaptoethanol	M7522	1 x 10^{-4} M	Sigma
penicillin and streptomycin	15140122	50 U and 50 µg /ml	Invitrogen
bFGF	233-FB-025	10 ng/ml	R&D
KO DMEM/F12	12660012		Invitrogen

MEF medium

Component	Cat. #	Final concentration	Source
FBS	16000077	10%	Invitrogen
Glutamax-1	35050061	2 mM	Invitrogen
penicillin and streptomycin	15140122	50 U and 50 µg /ml	Invitrogen
DMEM	11995065		Invitrogen

Other Required Reagents

Component	Cat. #	Final concentration	Source
Rock Inhibitor Y-27632	Y0503	10 µM/ml	Sigma
0.1% (w/v) Gelatin	G1890	Dissolve 0.5 g of gelatin from porcine skin in 500 ml DPBS and autoclave. Stable for 1 yr at room temperature	Sigma
Accutase	SCR005	Aliquot in 10 ml and store in -20°C. Dilute 1:1 with DPBS before use.	Millipore

Human ES freezing medium

90% FBS plus 10% DMSO, with 10 µM ROCK inhibitor Y-27632

NOTE: This protocol is for growing piPS cells on MEF feeder cells. These should already be growing before you plate your piPS cells.

B. Protocol for Human iPS Cell Culture

Growth condition for mouse fibroblasts (for feeder layers)

Gelatin treatment of plates

1. Add enough sterile/autoclaved 0.1% gelatin to cover the bottom of the wells.
   Approximate amounts: 10cm - 5ml

   6 well - 1.5ml/well

   24 well - 0.5 ml/well

   96 well - 200 µl/well
2. Incubate the gelatin-coated dishes for at least 15 min at 37 °C.
3. Aspirate excess gelatin solution before using.

Thawing MEF cells

To insure the highest level of viability, be sure to warm medium to 37 °C before using it on the cells. Cells should be plated at a minimum cell density of 1 x 10^4 cells/cm^2.

1. Remove the vial from liquid nitrogen and thaw quickly in 37 °C water bath.
2. Remove the vial from the water bath as soon as the cells are half way thawed, and sterilize by spraying with 70% ethanol.
3. Transfer the cells with 10 ml of MEF medium to a 15-cm conical tube and pellet the cells by centrifugation at 200x g for 5 min.
4. Discard the supernatant and resuspend the cells with 10 ml fresh MEF medium and plate the cells at seed density of 1 x 10^4 cells/cm^2.

5. Incubate at 37 °C with 5% CO_2 in air atmosphere, until the cells reach 80-90% confluency.
6. Change medium twice a week or when pH decreases.

Passage of MEF cells

Cells should be split when they reach confluency. A split based on seed density of 0.5 x 10^4 cells /cm^2 is recommended.

1. Discard the medium and wash the cells twice with PBS.
2. Aspirate PBS, and add 1 ml per T75 flask of 0.25% trypsin-EDTA, and incubate for 1 min.
3. Add 5 ml of MEF medium, and break up the cell clumps by gently pipetting up and down several times.
4. Transfer cells into a conical tube and centrifuge at 200 g for 5 min.
5. Discard the supernatant, and resuspend the cell pellet in 10 ml MEF medium.
6. Count the number of cells, plate cells at 0.5 x 10^4 cells/cm^2, and incubate at 37 °C with 5% CO_2.

Freezing MEF cells

1. Follow steps 1-4 from the Passage of Cells above.
2. Discard the supernatant, and resuspend the pellet in MEF medium. Add approximately 1 ml for each T75 flask.
3. Count the number of cells and dilute the cell suspension to 1 x 10^7 cells/ml.
4. Add an equal volume of cold 2X Freezing Media (containing 20% DMSO and 80% FBS) to the cell suspension.
5. Aliquot 1 ml of suspension into each cryovial (5 x 10^6 cells/vial).

6. Place the vials in a cell-freezing container and keep it at -80 °C overnight.
7. Transfer the vials to a liquid nitrogen tank for long-term storage.

Mitomycin C treatment of MEF

At confluence, MEF cells are treated with mitomycin C to halt the division of the cells when they are still able to condition the medium as the feeder layers for human iPS cells.

1. Add 6 mL of fresh MEF medium containing 50 µl of mitomycin C solution (1mg/ml) to one T75 flask of confluent MEF cells, and swirl it briefly.
2. Incubate at 37 °C for at least 3 h.
3. After incubation, aspirate the mitomycin C-containing medium off the cells, and wash the cells twice with 10 ml of PBS.
4. Aspirate off PBS, add 1 ml of 0.25% trypsin-EDTA, swirl to cover the entire surface, and incubate for 1 min at room temperature.
5. Add 5 ml of MEF medium, and break up the cells to a single cell suspension by pipetting up and down. Count the number of cells. Seed the cells on gelatin-coated dishes (1x 10^6 cells per 100-mm dish, or 1.5 x 10^5 cells per well of 6-well plate).
6. Cells should be ready to use by the next day.

Growth condition for human iPS cells

Thawing human iPS cells

To insure the highest level of viability, be sure to warm medium to 37 °C before using it on the cells. Due to the low survival rate of cryopreserved human iPS cells, the recovery is expected to take at least one week.

1. Remove the vial from liquid nitrogen and thaw quickly in 37 °C water bath.
2. Remove the vial from the water bath as soon as the cells are half way thawed, and sterilize by spraying with 70% ethanol.
3. Transfer the cells with 10 ml of human ES medium to a 15-cm conical tube and pellet the cells by centrifugation at 200x g for 5 min.
4. While centrifuging, remove MEF medium from the 6-well plate with MEF feeder cells, wash the well twice with 1 ml of KO DMEM/F12, and add 1 ml of human ES medium supplemented with ROCK inhibitor Y-27632 (10 μM).
5. Discard the supernatant of the tube containing human iPS cells, resuspend the cells with 1 ml of fresh human ES medium with ROCK inhibitor Y-27632, and plate the cells in the wells of 6-well plate with MEF feeder cells.
6. Incubate at 37 °C with 5% CO_2 in air atmosphere, until the cells reach 80% confluency.
7. Change the medium everyday.

Note: Y-27632 is not necessary for regular human iPS cell culture.

Maintenance of human iPS cells

It is important to note that do NOT keep human iPS cells in culture for long periods in order to maintain the pluripotency.

1. Aspirate the medium, and wash the cells twice with 1 ml of PBS.
2. Remove PBS completely, add 0.5 ml of 1:1 Accutase to each well of a 6-well plate, and incubate at room temperature for 1 min.
3. While incubating, remove a 6-well plate with MEF feeder cells from the incubator. Aspirate MEF medium, wash with 1 ml of KO DMEM/F12 twice for each well, and add 1 ml of human ES medium with 10 μM ROCK inhibitor to each well.

4. When the edge of the human iPS cell colonies have fold up, aspirate the Accutase solution and wash the cells three times with DMEM/F12 medium.
5. Add 1 ml per well of human ES medium, and dislodge the cell colonies by using cell scraper.
6. Transfer the contents into a 15 ml conical tube with 5 ml of pre-warmed human ES medium. Use another 1 ml of medium to wash the well one more time and combine it to the same tube.
7. Centrifuge at 200x g for 5 minutes at room temperature.
8. Carefully aspirate the overlaying medium, then gently finger tap the tube bottom to dislodge the cell pellet.
9. Gently add 3 to 6 ml of fresh human ES medium with 10 µM ROCK inhibitor, and resuspend the cells by gently pipetting up and down.
10. Add 1 ml of the human iPS cell suspension to each well of the 6-well plate. Right after plating iPS cells, gently swirl the plate back-and-forth and side-to-side to evenly distribute the cells, and incubate at 37°C.
11. The ES media must be changed every day and human iPS cells sub-cultured every 5-7 days when the undifferentiated colonies are big enough. Track passage number of iPS cells.

Note: human iPS cells could also be grown on HFF feeders.

Freezing human iPS cells

1. Grow human iPS cells to the exponential phase in a 6-well plate, and pre-treat cells with 10 µM Y-27632 for one hour prior to freeze.
2. Remove PBS completely, add 0.5 ml of 1:1 Accutase, and incubate at room temperature for 1 min.

3. While incubating, remove a 6-well plate with MEF feeder cells from the incubator. Aspirate MEF medium, wash with 1 ml of KO DMEM/F12 twice for each well, and add 1 ml of human ES medium to each well.
4. When the edge of the human iPS cell colonies fold up, aspirate Accutase solution and wash the cells three times with KO DMEM/F12 medium.
5. Add 1 ml per well of human ES medium, and dislodge the cell colonies using cell scraper.
6. Transfer the contents into a 15 ml conical tube with 5 ml of pre-warmed human ES medium. Use another 1 ml of medium to wash the well one more time and combine it to the same tube.
7. Centrifuge at 200x g for 5 minutes at room temperature.
8. Carefully aspirate the overlaying supernatant, then gently finger tap the tube bottom to dislodge the cell pellet.
9. Gently add 2 ml of human ES freezing medium supplemented with 10 µM Y-27632, resuspend the cells by gently pipetting up and down, and aliquot it at 1 ml per vial.
10. Put the vials in a cell-freezing container, and store the vials at –80°C overnight.
11. Transfer the vials to liquid nitrogen for long-term storage.

III. References

Kim D et al., Generation of Human Induced Pluripotent Stem Cells by Direct Delivery of Reprogramming Proteins. Cell Stem Cell. 2009; 4:472-476.

Fangjun Jia et al. A nonviral minicircle vector for deriving human iPS cells. Nature Methods 2010;7(3):197-9.

IV. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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Mountain View, CA 94043

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