TECHNICAL MANUAL

VERSION 2.2.3

Maintenance of hPSCs in mTeSR™1 and TeSR™2

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1.0 Introduction

Undifferentiated human pluripotent stem cells (hPSCs) have the potential for unlimited expansion with the retention of normal karyotype and the ability to generate cells of all three germ layers - endoderm, mesoderm and ectoderm[1,5,19 - 20]. These germ layer cells can then further differentiate into many specific cell lineages[11]. Because of this ability, their use has been proposed in a variety of clinical applications and as a tool for the study of human cellular and developmental systems. Human cells with pluripotent characteristics were initially derived from the inner cell mass of pre-implantation blastocysts and termed human embryonic stem cells (hESCs)[11]. The discovery that human fibroblasts[15,19 - 20] can be reprogrammed by the transient overexpression of a small number of genes into induced pluripotent stem cells (iPSCs), which functionally and phenotypically resemble hESCs, raises the possibility that cellular therapies using patient-specific input cells may be a reality in the future. Collectively, hESCs and hiPSCs are known as hPSCs.

Basic techniques to culture hPSCs are well established[1], although limitations remain in many of these procedures. In particular, many existing lines have been cultured using mouse embryonic fibroblast (MEF) feeder cells and serum or other animal-sourced medium components. MEFs support self-renewal either by providing necessary factors or by removing inhibitory factors. MEF-conditioned medium, produced by harvesting spent culture medium from MEF cell cultures, also supports self-renewal. Despite this, the continued use of feeders and animal-derived components will hinder the development of clinical applications due to: a) the presence of immunogenic material; b) the risk of transmitting animal virus or prion material; and c) difficulty with quality control of these undefined components.

Therefore, an important advancement in the field is the definition of culture conditions that support the proliferation and culture of hPSCs without the need for feeders or animal-derived components. An optimal culture system would include an appropriately buffered medium that contains all metabolites, cytokines and growth factors required for self-renewal and survival of pluripotent cells as well as a culture matrix that supports cell growth.

1.1 Development of Serum-Free and Feeder-Free Culture Systems for hPSCs

Several groups have developed culture conditions for hESCs that are, to various degrees, serum- and feeder-free. Xu *et al* reported a culture system that utilized BD Matrigel™ as a culture matrix and MEF conditioned medium (consisting of animal component-containing serum replacement and basic fibroblast growth factor, bFGF) that allowed hESCs to be cultured without direct contact with feeders[2]. True feeder-independent culture has been reported using extracellular matrix and a combination of transforming growth factor β (TGFβ) and bFGF or high levels of bFGF alone[3,4], although these studies relied on an animal component-containing serum replacement. Richards *et al* reported the first xeno-free culture system for hESCs, which replaced traditionally used MEFs with human feeder cells of fetal or adult fallopian tube-epithelial cell origin. However, the culture medium used was supplemented with 20% human serum[5] and, thus, has issues with batch-to-batch variability due to its undefined nature.

Recently, a number of other publications have described defined xeno- or feeder-free media formulations for the maintenance of hESCs[6-10]. "TeSR" is a serum-free, xeno-free medium that was shown to support derivation and long-term feeder-independent culture of hPSCs, and was developed by Tenneille Ludwig and colleagues at the WiCell Research Institute (Madison, WI)[9]. The formulation of "TeSR" included high levels of fibroblast growth factor, basic (bFGF), together with transforming growth factor β (TGFβ), aminobutyric acid (GABA), pipecolic acid, and lithium chloride. The original publication described the use of cell support matrix composed of four human components (collagen IV, fibronectin, laminin, and vitronectin). In an effort to reduce the cost of this defined system for everyday research, Ludwig and colleagues continued their development

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and formulated “mTeSR”, which does include some animal-sourced proteins yet retains the advantages of being fully-defined and serum-free and supports the self-renewal of hPSCs without requiring feeder cells[10].

1.2 mTeSR™1 and TeSR™2: STEMCELL Technologies’ Serum-Free and Feeder-Independent Media for hPSCs

STEMCELL Technologies has developed mTeSR™1 (Catalog #05850) and TeSR™2 (Catalog #05860) as standardized media for feeder-independent maintenance of hPSCs in culture. They are both complete, serum-free, defined formulations based on the publications by Ludwig *et al* [9,10] made under license from the WiCell Research Institute. mTeSR™1 contains a bovine albumin source that supports the long-term, feeder-independent culture of hPSCs as well as the derivation of hiPSCs in feeder-independent conditions[15,16]. TeSR™2 is an animal protein-free formulation that similarly supports the long-term, feeder-independent culture of hPSCs. Neither media require any further addition of growth factors. They have been designed to maintain and expand undifferentiated hPSCs and can be used with BD Matrigel™ hESC-qualified Matrix (BD, Catalog #354277) as a substrate. STEMCELL Technologies has pre-qualified each batch of BD Matrigel™ to ensure consistency, reproducibility, and reliability in performance. To obtain a complete animal protein-free culture system, TeSR™2 can be used with alternate matrices as described in Section 5.6.

hPSCs maintained in mTeSR™1 and TeSR™2:

- are phenotypically homogeneous and karyotypically normal
- express high levels of multiple antigens associated with pluripotency: Oct-3/4, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81
- express genes required for pluripotency: Oct-3/4, nanog
- form teratomas containing derivatives of endo-, meso-, and ectodermal lineages
- can be differentiated into functional mature cell types *in vitro* (e.g. hematopoietic cells)
- require no adaptation period (i.e. no period of low cell yield) when transferred from feeder-based culture

Advantages of using mTeSR™1 and TeSR™2 include:

- consistent conditions for hPSC culture due to the elimination of undefined medium components and removal of the inherent variability associated with feeder cells and conditioned media
- standardization in culture methods leading to increased reproducibility of data
- time savings due to elimination of the labor involved in preparing feeder cells or conditioned media
- complete medium formulation with no additional growth factors or other supplements necessary

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2.0 Materials and Reagents

2.1 mTeSR™1 and TeSR™2 for Maintenance of hPSCs

The mTeSR™1 Medium Kit (Catalog #05850) includes:

Component	Volume	Storage Conditions
mTeSR™1 Basal Medium (Component #05851)	400 mL	2 - 8°C
mTeSR™1 5X Supplement (Component #05852)	100 mL	-20°C

The TeSR™2 Medium Kit (Catalog #05860) includes:

Component	Volume	Storage Conditions
TeSR™2 Basal Medium (Component #05861)	400 mL	2 - 8°C
TeSR™2 5X Supplement (Component #05862)	100 mL	-20°C
TeSR™2 250X Supplement (Component #05863)	2 mL	-20°C

2.2 Additional Reagents Required for hPSC Culture

Product	Catalog #
mFreSR™	05854/05855
Cryostor™CS10	07930
Dispase (1 mg/mL)	07923
Dispase (5 mg/mL)	07913
PBS (without Mg^{++} and Ca^{++})	37350
BD Matrigel™ hESC-qualified Matrix	BD, Catalog #354277
Trypan Blue	07050
70% Ethanol or Isopropanol	-
Conical tubes (15 mL)	e.g. BD, Catalog #352196
Conical tubes (50 mL)	e.g. BD, Catalog #352070
4-well tissue culture-treated plates	e.g. BD, Catalog #353654
6-well tissue culture-treated plates	e.g. BD, Catalog #353046
10 cm tissue culture-treated plates	e.g. BD, Catalog #353003
Cell scrapers	e.g. Corning, Catalog #3010
Serological pipettes (2 mL, 5 mL, 10 mL)	e.g. BD, Catalog #357507, 357543, 357551

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2.3 Equipment Required for hPSC Culture

- Vertical laminar flow hood certified for Level II handling of biological materials
- Incubator with humidity and gas control to maintain 37°C and >95% humidity in an atmosphere of 5% CO_2 in air
- Low speed centrifuge (e.g. Beckman GS-6)

  Note: All protocols described in this technical manual can be performed with the brake on.
- Tabletop centrifuge (e.g. Eppendorf 5417R)
- Pipette-aid (e.g. Drummond Scientific)
- Hemacytometer (e.g. Nebauer, Reichert)
- Inverted microscope with 2X, 4X, and 10X phase objectives (e.g. Olympus CKX31)
- Isopropanol freezing container (e.g. Nalgene, Fisher; Catalog #1535050)
- -150°C freezer or liquid nitrogen vapor tank

2.4 Preparation of Reagents

2.4.1 Preparation of mTeSR™1

1. Thaw mTeSR™1 5X Supplement (Component #05852) at room temperature (15 - 25°C) or overnight at 2 - 8°C.

   If desired, 5X Supplement can be aseptically dispensed into working aliquots and stored frozen at -20°C. Use frozen aliquots within 3 months. Thawed aliquots should be used within 1 day to prepare complete mTeSR™1 medium. Do not refreeze aliquots after thawing.
2. Aseptically add the entire 100 mL of thawed 5X Supplement to 400 mL Basal Medium for a total volume of 500 mL. Mix well. Complete mTeSR™1 is stable when stored at 2 - 8°C for up to 2 weeks or is stable when frozen at -20°C for up to 6 months. Thaw frozen medium at room temperature (15 - 25°C) or overnight at 2 - 8°C.

   If prepared aseptically, complete mTeSR™1 is ready for use. However, the medium can also be filtered using a 0.2 µm, low-protein binding filter, if desired.

2.4.2 Preparation of TeSR™2

1. Thaw TeSR™2 5X Supplement (Component #05862) and TeSR™2 250X Supplement (Component #05863) at room temperature (15 - 25°C) or overnight at 2 - 8°C

   If desired, the 5X Supplement and 250X Supplement can be aseptically dispensed into working aliquots and stored frozen at -20°C. Use frozen aliquots within 6 months. Thawed aliquots should be used within 1 day to prepare complete TeSR™2 medium. Do not refreeze aliquots after thawing.

2. Add the entire 100 mL of thawed 5X Supplement and 2 mL of thawed 250X Supplement to the 400 mL Basal Medium for a total volume of 500 mL. Mix well. Complete TeSR™2 is stable when stored at 2 - 8°C for up to 2 weeks or is stable when frozen at -20°C for up to 6 months. Thaw frozen medium at room temperature (15 - 25°C) or overnight at 2 - 8°C.

   If prepared aseptically, complete TeSR™2 is ready for use. However, the medium can also be filtered using a 0.2 µm, low-protein binding filter, if desired.

2.4.3 Preparation of Dispase

Dispase is recommended for passaging hPSCs growing in mTeSR™1 or TeSR™2. STEMCELL Technologies sells dispase at two concentrations: 1 mg/mL, specific activity 0.8 - 1.2 U/mL (Catalog #07923) and 5 mg/mL (Catalog #07913). Dispase at a concentration of 1 mg/mL (Catalog #07923) is ready to use but it is highly recommended that dispase be aliquoted into smaller working volumes and stored at -20°C. Dispase can be stored at 2 - 8°C for up to 2 weeks.

If starting with the 5 mg/mL size (Catalog #07913), dispase must be prepared as follows:

1. Aliquot a 100 mL bottle of stock dispase solution (5 mg/mL) by thawing and dispensing into smaller volumes (10 or 20 mL).
2. Store the aliquots of dispase at -20°C and thaw as required.
3. Aliquots should only be thawed once; repeated freeze/thaw is not recommended.
4. Prepare a working solution of dispase at 1 mg/mL by diluting 1 in 5 with DMEM/F-12 (e.g. 10 mL of dispase plus 40 mL of DMEM/F-12). Diluted dispase can be stored at 2 - 8°C for up to 2 weeks.

   It is highly recommended that the diluted dispase solution is aliquoted into smaller working volumes to avoid repeated warming.

2.4.4 Coating Plates with BD Matrigel™ hESC-qualified Matrix

BD Matrigel™ hESC-qualified matrix (BD, Catalog #354277) should be aliquoted and frozen down. For full instructions and recommendations on aliquot sizes, please consult the product insert supplied with BD Matrigel™. Aliquots can be stored at -70°C for up to 6 months.

Add one aliquot of BD Matrigel™ to 25 mL of DMEM/F-12 to coat four 6-well plates (1 mL/well) or three 100 mm dishes (8 mL/dish).

1. Dispense 25 mL of dilution medium (DMEM/F-12; Catalog #36254) into a 50 mL tube and keep on ice.
2. Remove an aliquot of frozen BD Matrigel™ from -70°C. Thaw on ice until liquid, then add the thawed BD Matrigel™ to the cold dilution medium (in the 50 mL tube) and mix well. The vial may be washed with cold medium if desired.
3. Immediately use the diluted BD Matrigel™ solution to coat tissue culture-treated plates. For a 6-well plate, use 1 mL of diluted BD Matrigel™ per well; for a 100 mm plate, use 8 mL of diluted BD Matrigel™ per plate. Swirl the plate to spread the BD Matrigel™ solution evenly across the surface.

   Tissue culture-treated plate(s) should be used for coating with BD Matrigel™. To coat other sizes of tissue cultureware, scale the volume of diluted BD Matrigel™ by the surface area of the vessel to be coated.

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4. Coated plate(s) should be left at room temperature (15 - 25°C) for at least 1 hour before use. Do not allow the plate(s) to dehydrate. Do not remove the BD Matrigel™ solution until the plate(s) are ready to be used.

   If not used immediately, the plate(s) must be sealed to prevent dehydration (e.g. with Parafilm®) and can be stored at 2 - 8°C for up to 7 days after coating.

   Plate(s) are not optimal for hPSC culture if the BD Matrigel™ solution does not completely cover the surface; therefore, plate(s) that have regions where the solution has evaporated are not recommended for use.

5. Gently tilt the plate(s) onto one corner and allow the excess BD Matrigel™ solution to collect in that corner. Remove the solution using a serological pipette or by aspiration. Ensure that the tip of the pipette does not scratch the coated surface. Immediately add mTeSR™1 or TeSR™2 medium and hPSCs.

   If plates have been stored at 2 - 8°C, allow the plate to come to room temperature (15 - 25°C) for 30 minutes before removing the BD Matrigel™ solution.

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3.0 Culture of hPSCs Using mTeSR™1 or TeSR™2 on BD Matrigel™

Please read the entire technical manual before beginning culture of hPSCs in mTeSR™1 or TeSR™2. Culture of hPSCs in mTeSR™1 or TeSR™2 may require different techniques than culture in other media formulations. The procedures described in this technical manual were developed using the H1 and H9 hESC lines; if using other hESC or hiPSC lines, optimal conditions may need to be established.

3.1 Thawing Cryopreserved hPSCs

hPSCs should be thawed into either 4- or 6-well plates coated with BD Matrigel™. For instructions on how to coat tissue culture plates with BD Matrigel™ see Section 2.4.4. If unsure of the number of clumps frozen down, a 4-well plate is recommended. Generally, hPSCs from 1 well of a 6-well plate cryopreserved in STEMCELL Technologies' defined, serum-free cryopreservation medium, mFreSR™ (Catalog #05854/05855) can be successfully thawed into 1 well of a 6-well plate. If the cells have been cryopreserved using other methods, this may vary.

hPSCs cultured using other maintenance protocols (e.g. with mouse embryonic feeders or their conditioned medium) should be thawed under the same media and conditions used prior to cryopreservation. Once they have recovered from the thaw, cells can be transitioned into mTeSR™1 or TeSR™2 culture.

Have all tubes, warmed medium and plates ready before starting the protocol to ensure that the thawing procedure is done as quickly as possible.

1. Quickly thaw the hPSCs in a 37°C waterbath by gently shaking the cryovial continuously until only a small frozen pellet remains. Remove the cryovial from the waterbath and wipe with 70% ethanol to sterilize.
2. Use a 2 mL pipette to transfer the contents of the cryovial to a 15 mL conical tube.

   Using a 2 mL pipette will minimize breakage of cell clumps.
3. Add 5 - 7 mL of warm mTeSR™1 or TeSR™2 dropwise to the tube, gently mixing as the medium is added.
4. Centrifuge cells at 300 *x g* for 5 minutes at room temperature (15 - 25°C).
5. Aspirate the medium, leaving the cell pellet intact. Using a 2 mL pipette, gently resuspend the cell pellet in 0.5 - 2 mL of mTeSR™1 or TeSR™2, taking care to maintain the cells as aggregates.
6. Remove the BD Matrigel™ from a coated tissue culture plate (see Section 2.4.4) by gently tilting the plate onto one corner and allowing the excess BD Matrigel™ solution to collect in that corner. Remove the solution using a serological pipette or by aspiration. Ensure that the tip of the pipette does not scratch the coated surface.

   If the plates have been stored at 2 - 8°C, allow the plates to come to room temperature (15 - 25°C) for 30 minutes before removing the BD Matrigel™ solution.
7. Transfer the appropriate amount of medium containing the cell aggregates to a BD Matrigel™-coated 4-well or 6-well plate.

   Transfer 0.5 mL per well if using a 4-well plate. Transfer 2 mL per well if using a 6-well plate. Ensure that clumps are evenly distributed between wells.

8. Place the plate into the 37°C incubator and move the plate in quick side to side, forward to back motions to evenly distribute the clumps within the wells. Culture the cells at 37°C, with 5% CO_2 and 95% humidity.
9. Perform daily medium changes. Check for undifferentiated colonies that are ready to be passaged (dense centered) approximately 5 - 7 days after thawing.

   If only a few undifferentiated colonies are observed after thawing, it may be necessary to select only these colonies for passaging and replate them in the same size well on a new BD Matrigel™-coated plate.

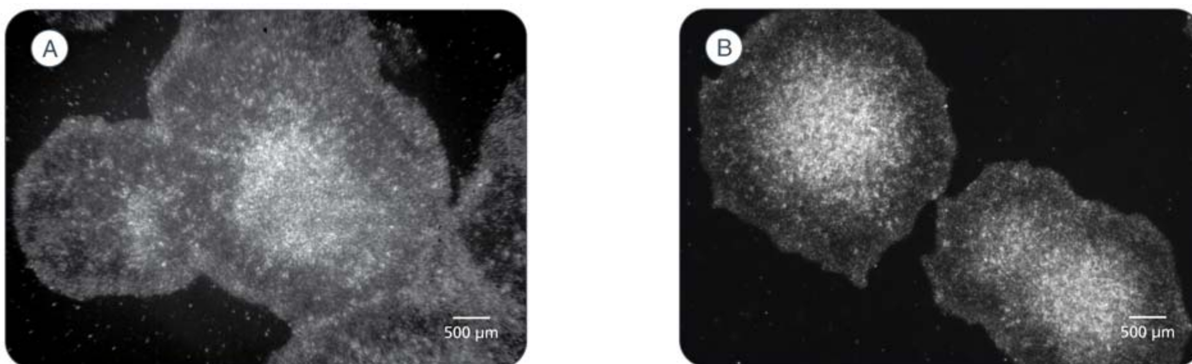
3.2 Passaging hPSCs Grown in mTeSR™1 or TeSR™2

hPSCs grown in mTeSR™1 or TeSR™2 are ready to passage when the colonies are large, beginning to merge, and have centers that are dense and phase-bright compared to their edges (see Figure 1). Depending on the size and density of seeded aggregates, cultures are usually passaged 5 - 7 days after seeding in mTeSR™1 and passaged 4 - 6 days after seeding in TeSR™2. **Thus, cells grown in TeSR™2 may need to be passaged approximately 1 day earlier than the same cells grown in mTeSR™1**.

It is to be expected that colony morphology will look different when compared to cells grown using other culture conditions. For up to 4 days after seeding in mTeSR™1, colonies may appear transparent and not very densely packed with cells. The density and robustness of the colonies increases rapidly after this time point and the morphology will change significantly in the last few days before passaging. Colonies that form in TeSR™2 medium will be more densely packed with cells earlier than colonies grown in mTeSR™1. For representative pictures of colonies passaged in mTeSR™1, see Appendix 2. For representative pictures of colonies passaged in TeSR™2, see Appendix 3.

In both media formulations, if colonies are passaged too early or too frequently, the cells may not attach well, yields will be decreased and cells may start to differentiate. If colonies are passaged too late, the culture will begin to show signs of differentiation (characterized by the emergence of cell types with different morphologies). There is an approximate 24 hour window that is optimal for passaging. If there are large colonies, with dense centers, and the colonies are beginning to merge, passage the cells within 24 hours (for further help, see Section 5.1).

Figure 1. Morphology of (A) hESC H9 colonies cultured in mTeSR™1 and (B) hiPSC iPS(IMR90)-1 colonies cultured in TeSR™2 that are ready to be passaged.
Note the dense, bright center when viewed with phase contrast or dark field under low magnification.



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Note: Cells cultured in TeSR™2 are more sensitive to enzymatic dissociation (see step 5 below). If using TeSR™2, the dispase incubation period should be decreased to 3 - 4 minutes (based on using dispase from STEMCELL Technologies). More care should also be taken when clumps are in suspension as they are more fragile than those cultured in mTeSR™1.

Note: Volumes given in this section are for 6-well culture dishes; scale accordingly for different sized tissue cultureware.

1. Aliquot sufficient mTeSR™1 or TeSR™2 (prepared according to Section 2.4.1 or 2.4.2) to passage cells. Warm aliquoted mTeSR™1 or TeSR™2, Dispase (1 mg/mL, specific activity 0.8 - 1.2 U/mL) (Catalog #07923), and DMEM/F-12 (Catalog #36254) to room temperature (15 - 25°C).
2. Use a microscope to visually identify regions of differentiation. Mark these using a felt tip or lens marker on the bottom of the plate.

   This selection should not exceed 20% of the well if the culture is of high quality.
3. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
4. Aspirate medium from the hPSC culture and rinse with DMEM/F-12 (2 mL/well).
5. Add 1 mL per well of dispase at a concentration of 1 mg/mL. Place at 37°C for 7 minutes if using mTeSR™1 or for 3 - 4 minutes if using TeSR™2.

   These time recommendations are based on STEMCELL Technologies' dispase. If using dispase from another supplier, these times may need to be adjusted due to differences in activity of the enzyme. After incubation the colony edges will appear slightly folded back but the colonies should remain attached to the plate.
6. Remove dispase, and gently rinse each well 2 - 3 times with 2 mL of DMEM/F-12 per well to dilute away any remaining dispase.
7. Add 2 mL/well of DMEM/F-12 or mTeSR™1/TeSR™2 and scrape colonies off with a cell scraper (e.g. Corning, Catalog #3010).
8. Transfer the detached cell aggregates to a 15 mL conical tube and rinse the well with an additional 2 mL of DMEM/F-12 or mTeSR™1/TeSR™2 to collect any remaining aggregates. Add the rinse to the 15 mL tube.

   If cells are scraped in mTeSR™1 or TeSR™2, Steps 9 and 10 are not necessary. Adjust volume of medium for an appropriate split and proceed to Step 11.
9. Centrifuge the 15 mL tube containing the aggregates at 300 x *g* for 5 minutes at room temperature (15 - 25°C).
10. Aspirate the supernatant. For each well of hPSC aggregates collected in the 15 mL tube, add 1 - 2 mL of mTeSR™1 or TeSR™2. Resuspend pellet by gently pipetting up and down with a P1000 micropipettor (1 - 2 times). Ensure that cells are maintained as aggregates (for more information, see Section 5.1).
11. Plate the hPSC aggregates with mTeSR™1 or TeSR™2 onto a new plate coated with BD Matrigel™.

    If the colonies are at an optimal density, the cells can be split every 4 - 7 days using 1:6 to 1:10 splits (i.e. the aggregates from 1 well of a 6-well plate can be plated in 6 - 10 wells of a 6-well plate). If the colonies are too dense or too sparse, adjust the split ratio accordingly. For an alternative method of determining split ratios, see Section 5.3. Please note that these guidelines are based on the growth characteristics of the H1 and H9 hESC lines, and may vary between different lines and laboratories.

12. Move the plate in several quick, short, back-and-forth and side-to-side motions to disperse cells across the surface of the wells. Place the plate in a 37°C incubator.

    Ensure that newly seeded colonies are evenly dispersed across the entire surface of a BD Matrigel™-coated plate. Uneven distribution of cell clumps may result in differentiation of hPSCs.

3.3 Cryopreserving hPSCs Using mFreSR™

mFreSR™ (Catalog #05854/05855) is a defined, serum-free cryopreservation medium designed specifically for hPSCs.

The following is based on hPSC cultures in 6-well plates where initial clump seeding is adjusted so that wells are 60 - 70% confluent at time of cryopreservation.

Before cryopreservation, hPSCs should be of high quality (primarily undifferentiated with less than 20% of the cells being differentiated). Cryopreservation should be done approximately 1 day before the cells are ready to passage. hPSCs will have improved survival following thawing if cryopreserved as large clumps.

1. Bring required amount of mFreSR™ to room temperature (15 - 25°C).
2. In the hPSC culture to be cryopreserved, use a microscope to visually identify regions of differentiation. Mark these using a felt tip or lens marker on the bottom of the plate.

   This selection should not exceed 20% of the well if the culture is of high quality.
3. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
4. Aspirate remaining medium from wells.
5. Rinse wells with 2 mL of DMEM/F-12 and aspirate.
6. Add 1 mL per well of dispase at a concentration of 1 mg/mL. Place at 37°C for 7 minutes if using mTeSR™1 or for 3 - 4 minutes if using TeSR™2.

   These time recommendations are based on STEMCELL Technologies' dispase. If using dispase from another supplier, these times may need to be adjusted due to differences in activity of the enzyme. After incubation the colony edges will appear slightly folded back but the colonies should remain attached to the plate.
7. Remove dispase and gently rinse each well 2 - 3 times with 2 mL of DMEM/F12 per well to dilute away any remaining dispase.
8. Add 2 mL/well of DMEM/F12 or mTeSR™1/TeSR™2 and scrape colonies off using a cell scraper or a 5 mL serological pipette.

   Take care to keep the clumps as big as possible.
9. Transfer the detached cell aggregates into a 15 mL conical tube and rinse the wells with additional 2 mL DMEM/F12 or mTeSR™1/TeSR™2 to collect any remaining aggregates. Add the rinse to the 15 mL tube containing the cell aggregates.
10. Centrifuge the 15mL tube containing the aggregates at 300 x *g* for 5 minutes at room temperature (15 - 25°C).

    Prepare and label cryotubes while cells are centrifuging.
11. Gently aspirate the supernatant taking care to keep the cell pellet intact.

12. Using a 2 mL pipette, gently resuspend the pellet in mFreSR™ medium, taking care to leave the clumps larger than would normally be done for passaging.

    1 mL of mFreSR™ should be used for every well of a 6-well plate being frozen. However, if the wells are at low density (less than 50% confluent), 1 mL of mFreSR™ may be used for every 2 wells.

13. Gently flick the tube to mix the suspension and mFreSR™. Then transfer 1 mL of clumps in mFreSR™ into each labeled cryovial using a 2 mL pipette.

    Draw up 1 mL at a time and aliquot 1 mL/tube. Mix gently before taking each aliquot. This will ensure even distribution of clumps between the vials.

14. Place vials into an isopropanol freezing container and place the container at -80°C to -150°C overnight.
15. Transfer to a liquid nitrogen vapor tank or liquid nitrogen the next day.

3.4 Cryopreservating hPSCs Using Cryostor™ CS10

CryoStor™CS10 (Catalog #07930) is an animal-component free cryopreservation medium. It is ready to use and contains cryoprotectant agents. *Note: Wipe down outside of bottle with 70% ethanol before opening.*

1. In the hPSC culture to be cryopreserved, use a microscope to visually identify regions of differentiation. Mark these using a felt tip or lens marker on the bottom of the plate.

   This selection should not exceed 20% of the well if the culture is of high quality.

2. Remove regions of differentiation by scraping with a pipette tip or by aspiration.
3. Aspirate remaining medium from wells.
4. Rinse wells with 2 mL of DMEM/F-12 and aspirate.
5. Add 1 mL per well of dispase at a concentration of 1 mg/mL. Place at 37°C for 7 minutes if using mTeSR™1 or for 3 - 4 minutes if using TeSR™2.

   These time recommendations are based on STEMCELL Technologies' dispase. If using dispase from another supplier, these times may need to be adjusted due to differences in activity of the enzyme. After incubation the colony edges will appear slightly folded back but the colonies should remain attached to the plate.

6. Remove dispase and gently rinse each well 2 - 3 times with 2 mL of DMEM/F12 per well to dilute away any remaining dispase.
7. Add 2 mL/well of DMEM/F12 or mTeSR™1/TeSR™2 and scrape colonies off using a cell scraper or a 5 mL serological pipette.

   Take care to keep the clumps as big as possible.

8. Transfer the detached cell aggregates into a 15 mL conical tube and rinse the wells with additional 2 mL DMEM/F12 or mTeSR™1/TeSR™2 to collect any remaining aggregates. Add the rinse to the 15 mL tube containing the cell aggregates.
9. Centrifuge the 15mL tube containing the aggregates at 300 x *g* for 5 minutes at room temperature (15 - 25°C).

   Prepare and label cryotubes while cells are centrifuging.

10. Gently aspirate the supernatant taking care to keep the cell pellet intact.

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11. Gently resuspend pellets with cold (2 - 8°C) CryoStor™CS10 and transfer the suspension to a cryovial.
12. Freeze cells using a standard slow rate controlled cooling protocol (approximately -1°C/min) and store at LN_2 temperature (-135°C). -80°C is not recommended for long-term storage.

    Alternatively, cells can be frozen using an isopropanol freezing vessel and a multi-step protocol: -20°C for 2 hours, followed by -80°C for 2 hours, followed by storage at LN_2 temperature (-135°C).

Warning - *Hazardous Ingredient: DMSO. Avoid contact with DMSO solutions containing toxic materials or materials with unknown toxicological properties. DMSO is readily absorbed through the skin and may carry such materials into the body. Wash exposed skin with soap and water. Flush eyes with water. For more information, refer to the MSDS.*

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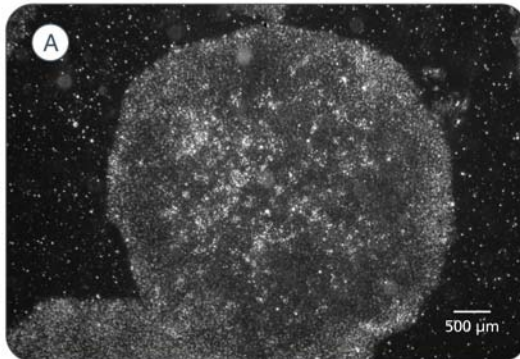
4.0 Characterization of Undifferentiated hPSCs

4.1 Morphology

Undifferentiated hPSCs grow as compact, multicellular colonies, as shown in Figure 2. They should also exhibit a high nuclear-to-cytoplasm ratio and prominent nucleoli. These colonies are characterized by a distinct border (this may be less pronounced in colonies cultured on BD Matrigel™ in mTeSR™1 or TeSR™2 compared to those grown on feeder cells). Healthy hPSC colonies will be multilayered in the center, resulting in clusters of phase-bright cells when viewed under phase contrast. Differentiation is characterized by loss of border integrity, gross non-uniformity of cell morphology within a colony, and the emergence of obvious alternate cell types.

Figure 2. Morphology of Cultured hPSCs.



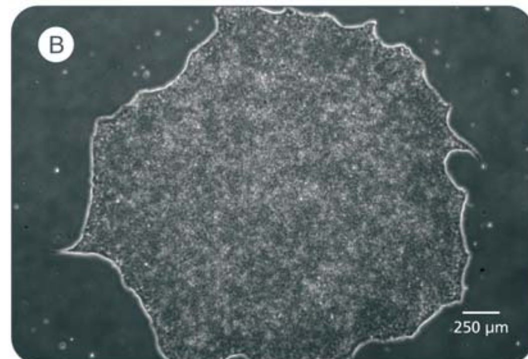
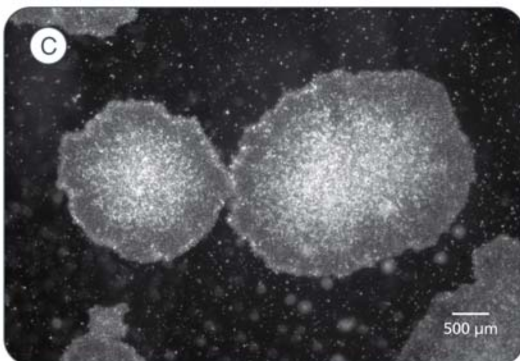
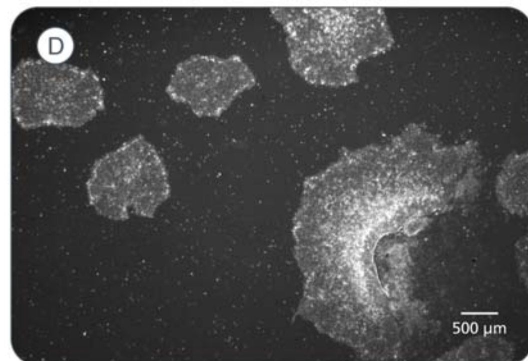


Photo courtesy of M. O'Connor and C. Eaves, the Vancouver Human Embryonic Stem Cell Core Facility

(A) An undifferentiated hESC H9 colony and (B) an undifferentiated hiPSC iPSC (IMR90)-3 colony cultured in mTeSR™1. Both cultures will be ready to passage in approximately 1 - 2 days.





(C) H9 cell line cultured in TeSR™2 ready to be passaged. (D) H9 cell line in TeSR™2 showing an area of differentiation.

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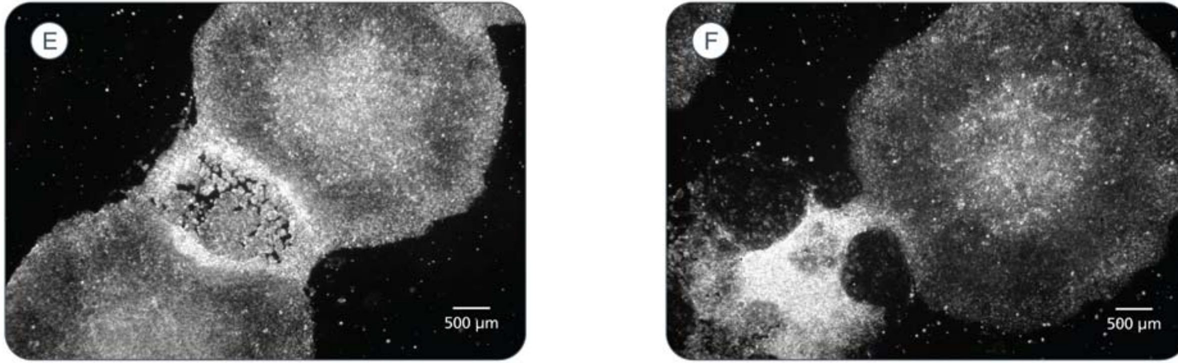

(E) Area of differentiation between 2 undifferentiated hESC H1 colonies cultured in mTeSR™1. (F) mTeSR™1 cultured hESC line H1 showing a differentiated colony adjacent to an undifferentiated colony.

4.2 Flow Cytometry Protocols

4.2.1 Reagents and Materials

Antibodies

Antibodies can be used to characterize cells by flow cytometry. The table below contains information about primary and secondary antibodies available from STEMCELL Technologies that can be used to characterize hPSCs.

For Intracellular Antigen Staining				
Primary Antibody*	**Catalog #**	**Isotype**	**Recommended Secondary Antibody***	**Catalog #**
Oct-3/4 Antibody	01550 01551	IgG1 (Mouse)	FITC-conjugated goat anti-mouse IgG	10210
For Surface Antigen Staining				
Primary Antibody*	**Catalog #**	**Isotype**	**Recommended Secondary Antibody***	**Catalog #**
SSEA-1 Antibody	01552	IgM (Mouse)	FITC-conjugated goat anti-mouse IgM	10211
SSEA-3 Antibody	01553	IgM (Rat)	APC-conjugated goat anti-rat IgM	10215
SSEA-4 Antibody	01554	IgG3 (Mouse)	FITC-conjugated goat anti-mouse IgG	10210
TRA-1-60 Antibody	01555	IgM (Mouse)	FITC-conjugated goat anti-mouse IgM	10211
TRA-1-81 Antibody	01556	IgM (Mouse)	FITC-conjugated goat anti-mouse IgM	10211
TRA-2-49 Antibody	01557	IgG1 (Mouse)	FITC-conjugated goat anti-mouse IgG	10210
TRA-2-54 Antibody	01558	IgG1 (Mouse)	FITC-conjugated goat anti-mouse IgG	10210

*Optimal working dilutions of the primary and secondary antibodies should be determined by the end user.

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General Reagents and Materials

Reagents and Materials	Catalog #
PBS (without Mg++ or Ca++)	37350
Trypsin-EDTA (0.05%)	07910
2% FBS in PBS (FBS/PBS)	07905
1.5 mL tubes	e.g. Eppendorf, Catalog #022364111
5 mL FACS tubes	e.g. BD, Catalog #352058
Conical tubes (15 mL)	e.g. BD, Catalog #352196
Nuclear stain (optional: e.g. 1 mg/mL propidium iodide diluted 1 in 1000 in 2% FBS/PBS)	e.g. Sigma, #81845

4.2.2 Preparation of a Single Cell Suspension for Flow Cytometry

1. Warm the 0.05% Trypsin-EDTA (Catalog #07910) and 2% FBS/PBS (Catalog #07905) to room temperature (15 - 25°C) before use. Calculate the total volume of Trypsin-EDTA required based on the number and size of the culture vessels, using the table below:

Culture Vessel	Trypsin-EDTA Volume
6-well plate	1 mL/well
100 mm dish	4 mL

2. Rinse the cells once with PBS, then trypsinize by adding the appropriate volume of 0.05% Trypsin/EDTA solution. Incubate at 37°C for 10 minutes.
3. Harvest by pipetting up and down with either a serological pipette or a P1000 micropipetter to ensure a single cell suspension. Transfer the cells to a 15 mL conical tube and rinse wells with an additional 2 - 4 mL of 2% FBS/PBS (Catalog #07905). Add the rinse to the tube containing the cells.
4. Centrifuge cells at 300 *x g* for 5 minutes at room temperature (15 - 25°C).
5. Resuspend cells in 2% FBS/PBS and perform a viable cell count using Trypan Blue (Catalog #07050).
6. The single cell suspension may now be used for surface antigen staining (refer to Section 4.2.3) or intracellular antigen staining (see Section 4.2.4).

4.2.3 Surface Antigen Staining Protocol

Note: Optimal concentrations of primary and secondary antibodies need to be predetermined by titration for each antibody.

1. Determine the number of samples required to perform flow cytometry including necessary staining controls.
2. Aliquot approximately 1×10^5 cells per sample into 5 mL FACS tubes (Falcon, Catalog #352058) or 1.5 mL tubes (Eppendorf, Catalog #022364111) and place on ice.
3. Make a primary antibody mix (e.g.100 µL/sample), using the primary antibody at the predetermined optimal working dilution.

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4. Centrifuge cells (aliquoted in Step 2) at 300 *x g* for 5 minutes at room temperature (15 - 25°C). Aspirate supernatant and resuspend cells in the primary antibody mix. Gently vortex. Incubate on ice for 15 - 60 minutes.
5. Add 1 mL of 2% FBS/PBS to each sample tube and gently vortex. Centrifuge all tubes at 300 *x g* for 5 minutes at room temperature (15 - 25°C).
6. While cells are in centrifuge, make a sufficient quantity of the secondary antibody mix (100 µL/sample) using the appropriate secondary antibody at the predetermined optimal working dilution.
7. Carefully remove the supernatant without disturbing the cell pellet.
8. Resuspend the cells in the secondary antibody mix. Gently vortex the tubes and incubate on ice for 15 - 60 minutes.
9. Add 1 mL of 2% FBS/PBS to each tube, gently vortex and centrifuge (300 *x g* for 5 minutes).
10. Carefully remove the supernatant without disturbing the cell pellet. Resuspend the cells in 200 - 300 µL 2% FBS/PBS (and transfer to a 5 mL FACS tube if necessary). If desired, propidium iodide (PI) can be added at a final concentration of 1 µg/mL to assess viability by flow cytometry. Place samples on ice and analyze by flow cytometry as soon as possible.

4.2.4 Intracellular Antigen Staining Protocol for Oct-3/4

Additional Reagents Required

Saponin Permeabilization Buffer (SPB)*

Component	Catalog #	Final Concentration
Saponin	e.g. Fluka Biochemika, Catalog #47036	1 mg/mL
10% BSA Solution	04915	1%
PBS (without Mg^{++} or Ca^{++})	37350	to final volume

*Mix well and store at 2 - 8°C for up to 1 month.

2% Formaldehyde Solution

Component	Catalog #	Final Concentration
Formaldehyde	e.g. AnalaR Reagents, Catalog #B10113-76	2%
PBS (without Mg^{++} or Ca^{++})	37350	to final volume

Protocol

Note: Optimal concentrations of primary and secondary antibodies need to be predetermined by titration for each antibody.

1. Determine the number of samples required to perform flow cytometry including necessary staining controls.
2. Aliquot approximately 2 - 5 x 10^5 cells per sample into 5 mL FACS tubes or 1.5 mL tubes and place on ice.
3. Centrifuge all samples at 300 x *g* for 5 minutes at room temperature (15 - 25°C).

4. Carefully remove the supernatant and add 250 µL of 2% Formaldehyde Solution to each tube, vortex briefly, and place on ice for 15 - 30 minutes.
5. Add 1 mL 2% FBS/PBS to wash. Centrifuge at 300 x *g* for 5 minutes at room temperature (15 - 25°C).
6. Remove supernatant. Add 500 µL of Saponin Permeabilization Buffer (SPB) to each tube, mix gently, and incubate for 15 minutes at room temperature (15 - 25°C).
7. Make enough primary antibody mix (e.g 100 µL/sample) using SPB as the diluent. The suggested working dilution of the Oct-3/4 primary antibody is 1:100.
8. Centrifuge cells at 300 x *g* for 5 minutes at room temperature (15 - 25°C) and remove the supernatant.

   Cells should remain in SPB until the final resuspension prior to flow cytometric analysis.
9. Add 100 µL of primary antibody mix to the appropriate sample tubes, gently mix, and incubate on ice for 15 - 60 minutes.
10. Add 1 mL of SPB to each antibody-containing tube, gently mix and centrifuge at 300 x *g* for 5 minutes at room temperature (15 - 25°C).
11. While cells are in the centrifuge, make enough FITC-conjugated goat anti-mouse IgG (Catalog #10210) secondary antibody mix (e.g. 100 µL/sample) using SPB as the diluent. The suggested working dilution for this secondary antibody is 1:100 from the reconstituted stock.
12. Carefully remove supernatant without disturbing cells.
13. Add the diluted secondary antibody to the appropriate tubes, gently vortex, and incubate on ice for 15 - 60 minutes.
14. Add 1 mL of SPB to each tube, then gently mix and centrifuge at 300 x *g* for 5 minutes at room temperature (15 - 25°C).
15. Carefully remove supernatant without disturbing cells.
16. Add 300 µL of 2% FBS/PBS to all samples, transfer each to an appropriately labeled 5 mL FACS tube and analyze by flow cytometry.

    Optional: Propidium iodide (PI) can be added to the final step to perform doublet discrimination. This ensures only single cells are assessed. In this case, add PI to 2% FBS/PBS at a final concentration of 1 µg/mL. Analyse immediately by flow cytometry. Examine a plot of FL2 area versus FL2 height in the linear range and gate out events that deviate from diagonal as in Figure 3.

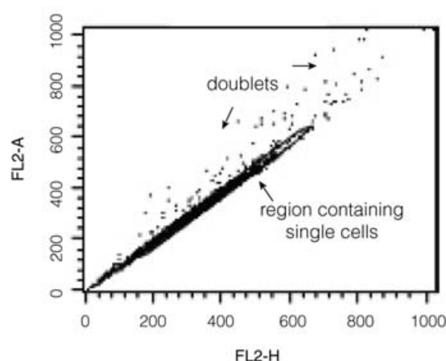

Figure 3. An example of doublet discrimination by flow cytometry.

5.0 Helpful Hints

5.1 Successful Culture of hPSCs

Culturing hPSCs is a very time-consuming process. It requires daily medium changes, use of the highest quality reagents and frequent morphologic observations of the cultures to ensure that they are maintained at the optimum density. The following tips will help ensure success with hPSC culture.

A) Preparation of hPSC Clumps for Passaging

Preparation of a uniform suspension of suitable sized hPSC clumps for passaging is very important for the successful culture of hPSCs. If the clumps are too large, an increased rate of differentiation within the colonies may occur. If the clumps are too small with many single cells present, cell survival (and resulting number and health of colonies) will be compromised. Following the dispase and washing steps, one or two gentle draws with a micropipettor and P1000 tip or a 2 mL serological pipette should be sufficient to generate appropriately-sized clumps for passaging (approximately 50 - 60 µm). If the clumps are the correct size, the majority will remain in suspension after this step. If large clumps are present that rapidly sink to the bottom of the tube, perform one or two more gentle draws with the micropipettor or pipette.

B) Density

Culture density is a critical aspect of maintaining hPSCs in mTeSR™1 or TeSR™2. Cultures that are either too sparsely or too densely populated can lead to differentiation. Many colonies in the dish should be just beginning to touch each other at the time of passaging (i.e. plate is approximately 75% confluent). Adjust plating and/or split ratios to achieve a balance between having too much space between colonies and having a confluent culture (see Figure 1, Section 3.2 and Sections 7.0 and 8.0 for examples of hPSC cultures ready for passaging). As a general guideline, an acceptable density would be approximately 150 colonies per well of a 6-well plate.

C) Differentiation in a Maintenance Culture

It is important that the starting culture is of high quality and is primarily undifferentiated. Cultures that are compromised or have large amounts of differentiated cells will continue to show these symptoms after transition into mTeSR™1 or TeSR™2. If necessary, areas of differentiation can be selectively removed by scraping off or aspirating before passaging the cultures. The morphologic hallmarks of differentiation should appear in less than 20% of colonies in a healthy culture.

D) Feeding Regime

hPSCs generally require daily medium change for optimal growth. Some hESC and hiPSC lines will tolerate occasional double feeding (adding twice the required volume of medium). For instance, it is possible to perform a double feed on a Friday, with the next medium change on Sunday. However, it is not recommended to either go longer than 1 day without a medium change, or to feed the cultures every other day continuously.

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5.2 Transitioning hPSCs to mTeSR™1 or TeSR™2

No adaptation step is required when seeding cells from feeder or conditioned medium cultures to mTeSR™1 or TeSR™2. Simply replate hPSC clumps into mTeSR™1 or TeSR™2 at the time of passaging. It is, however, recommended that a culture using the previous medium and culture system is initially maintained in parallel to ensure that the chosen plating density in mTeSR™1 or TeSR™2 is appropriate.

A) Transitioning from Feeder Culture

The following describes a collagenase method for passaging hPSCs from feeders on 6-well plates. It is also possible to use mechanical passaging or alternate methods to generate clumps for seeding cells in mTeSR™1 or TeSR™2.

1. Under a low power microscope (e.g. using a 4X or lower objective), mark any differentiated colonies on the maintenance plate of hPSCs to be removed prior to passaging (use a felt-tip or objective marker to indicate the regions that contain differentiated colonies on the bottom of the plate).
2. Remove the differentiated colonies by gently scraping marked regions with either a 2 mL plastic pipette, or a 1 mL plastic pipette tip attached to a P1000 micropipette. Aspirate the medium and scraped colonies. After aspiration, check under the microscope to ensure that the cells in the marked regions are completely removed.
3. Wash the wells with ~2 mL/well DMEM/F-12 (Catalog #36254) to remove any scraped colonies that remain loosely attached.
4. Add 1 mL/well of room temperature (15 - 25°C) collagenase solution (1 mg/mL; Catalog #07909). Incubate at 37°C for 20 minutes.
5. Using a 2 mL serological pipette or a cell scraper, scrape cells off the surface of the well. While scraping, gently pipette the collagenase up and down to wash the cells off the surface.
6. After the hPSC clumps are detached from the surface of the plate, pool the suspension into a sterile 15 mL conical tube.
7. Rinse each well with 1 mL mTeSR™1 or TeSR™2 and transfer the rinse to the 15 mL conical tube containing the colonies removed from the plate.
8. Pellet cells by centrifuging at 300 x *g* for 5 minutes at room temperature (15 - 25°C).
9. Aspirate or pour off the supernatant from the hPSC pellet. Resuspend the pellet with 1 - 2 mL mTeSR™1 or TeSR™2 using a P1000 micropipetter or a 2 mL pipette by gently pipetting up and down 1 - 2 times to break up large clumps.

   Be careful not to generate a single cell suspension with excessive pipetting.
10. Seed an appropriate volume of clumps on BD Matrigel™-coated plates with mTeSR™1 or TeSR™2.

    No adaptation is required when switching to mTeSR™1 or TeSR™2 from medium containing Knockout™ Serum Replacement. It is recommended that cultures are seeded at the same density as on feeders.

B) Transitioning from Cultures in Feeder-free Conditioned Media

Transitioning from conditioned media does not require any adaptation; cells can be plated in mTeSR™1 or TeSR™2 on BD Matrigel™ -coated plates at the time of passage.

5.3 Plating hPSCs by the Clump Count Method

An alternative to splitting clump suspensions into defined volumes is to perform clump counts on the hPSC suspension and to always plate a defined number of clumps according to the size of the well or dish that is being seeded. This can be a valuable learning tool for those new to hPSC culture because it aids in defining how much a suspension should be pipetted to achieve optimally-sized clumps. An eyepiece micrometer placed in the microscope eyepiece is required to enumerate clumps of appropriate size (~50 - 60 µm in diameter) that are likely to attach and grow. Eyepiece micrometers are available from most microscope manufacturers.

Performing a Clump Count*

1. Aliquot 40 µL of DMEM/F-12 (Catalog #36254) into 2 wells of a 96-well flat-bottom plate.
2. Draw a "+" centered on the bottom of these wells to serve as a counting grid.
3. Add 5 µL of a freshly mixed clump suspension to each well. Count clumps that are approximately 60 µm or greater in diameter (using a calibrated eyepiece micrometer). This corresponds to clumps with an area of approximately 3500 μm^2
4. Perform duplicate counts, then average the results and calculate the total number (x) of clumps.

$$\frac{\text{\# of clumps counted}}{5\ \mu L} = \frac{x\ \text{clumps}}{\text{total volume of suspension}\ (\mu L)}$$

* Protocol kindly provided by the Vancouver Human Embryonic Stem Cell Core Facility.

5. Calculate the volume of clump suspension (y) required to seed new dishes using the following guide for appropriate seeding densities:

Plate or Well Size	Target # of Clumps/Plate or Well
100 mm dish	2400 clumps
60 mm dish	1000 clumps
Wells in a 6-well dish	300 clumps

For example, to seed 1 well of a 6-well dish, the volume of clump suspension required for 300 clumps is calculated as follows:

$$\frac{\text{\# of clumps counted}}{5\ \mu L} = \frac{300\ \text{clumps}}{y\ \mu L}$$

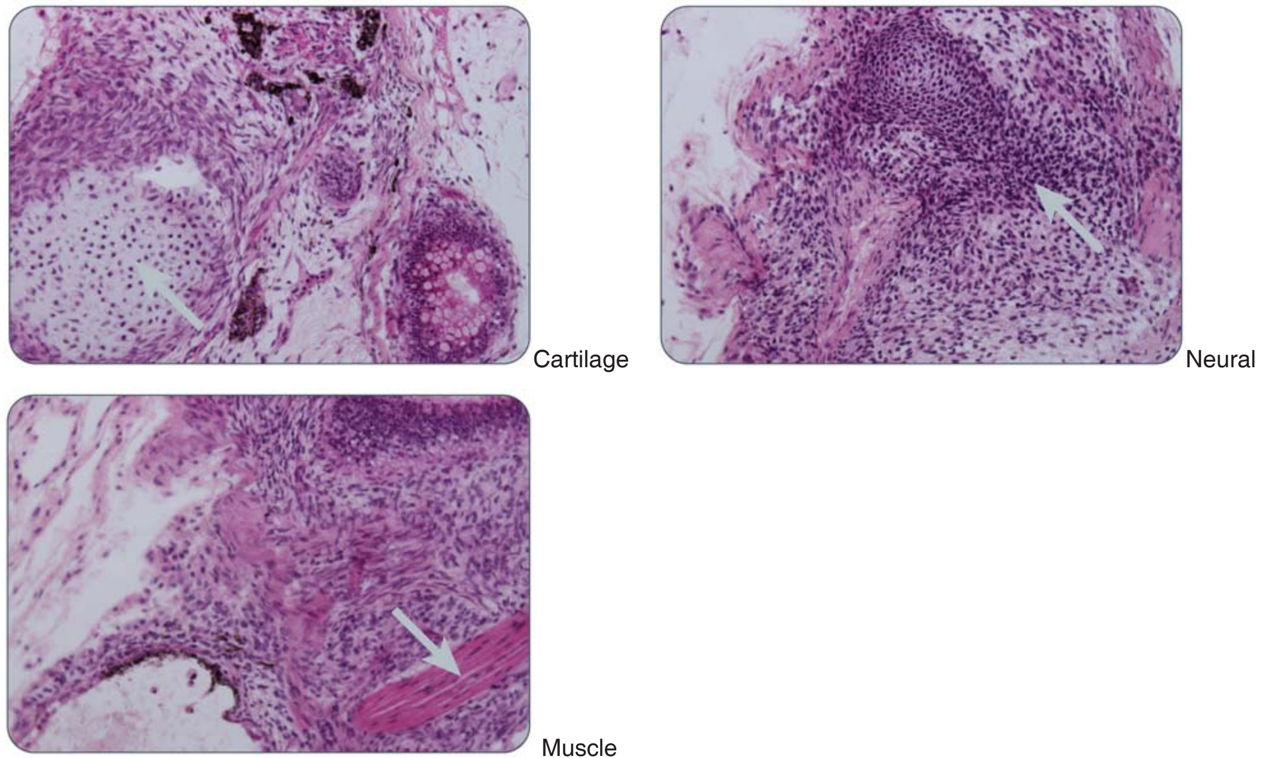
6. Completely remove excess BD Matrigel™ from a pre-coated 6-well plate (performed as described in Section 2.4.4). Add the appropriate volume of mTeSR™1 or TeSR™2 to each well.
7. Gently mix the clump suspension prior to plating to ensure a uniform suspension.
8. Add the required seeding volume of clumps to each well.
9. Move the plate in several quick, short, back-and-forth and side-to-side motions to disperse cells across the surface of the wells. Return the plate to the incubator.

   Ensure that clumps are evenly dispersed across the entire surface of the plate. Uneven distribution of clumps may result in differentiation of hPSCs.

5.4 Teratoma Assays

One of the hallmarks of hPSCs is pluripotency, the potential to differentiate into each of the three germ layers: ectoderm, mesoderm, and endoderm. This differentiation potential can either be examined *in vitro* (e.g. embryoid body formation) or *in vivo* (e.g. teratoma formation)[1,11,12]. Teratoma assay involves injection of a population of hPSCs into an immunocompromised mouse (hind limb muscle is a common injection site, but testis and subcutaneous sites have also been demonstrated). Pluripotent cells will give rise to detectable teratomas 4 - 12 weeks following injection (see Figure 4). The tumors contain tissues representative of the three germ layers and can be excised and analyzed by histology to confirm the presence of multiple tissues from each lineage. The teratoma assay is routinely used to demonstrate the pluripotency of newly established hPSC lines, long-term maintenance cultures, and genetically modified hPSCs. hPSCs maintained in mTeSR™1or TeSR™2 for multiple passages are capable of forming teratomas following injection into immunocompromised mice.

Figure 4. H9 hESCs were cultured for 6 passages in mTeSR™1 then injected subcutaneously into immunocompromised mice. The resulting teratoma contained cell types from all three germ layers. Representative tissue types are shown.



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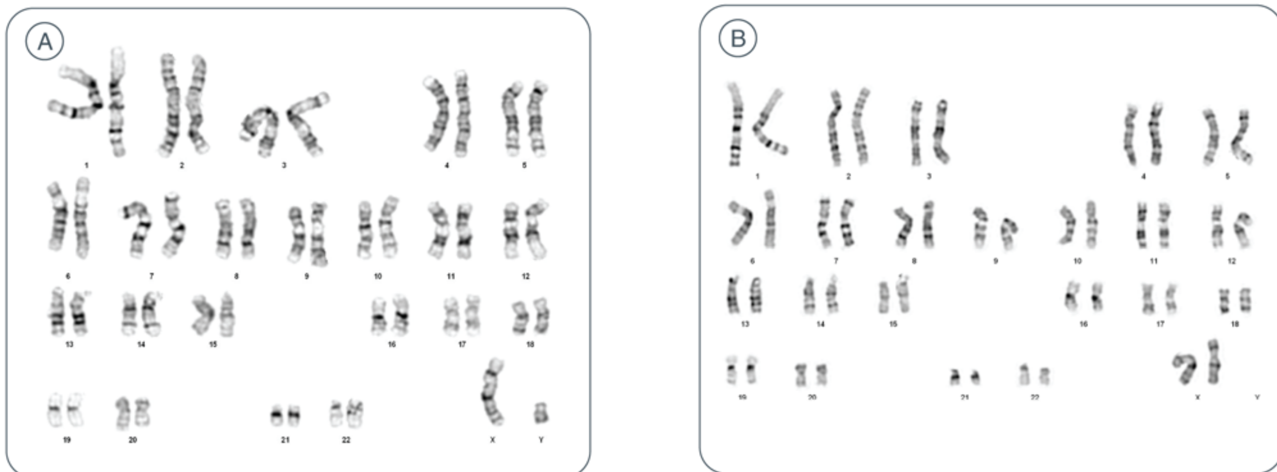
5.5 Karyotyping

hPSCs should retain a normal genetic makeup during routine culture, expansion, and manipulation. Nonetheless, chromosomal and genetic aberrations may appear during long-term passaging, presumably because they confer a proliferative advantage to a subpopulation within the culture. Specific karyotypic abnormalities have been reported to occur repeatedly *in vitro* after extended passaging, suggesting that these chromosomal regions contain genes whose overexpression is connected to self-renewal[13]. Various factors such as enzymatic passaging and high cell density may contribute towards the likelihood of karyotypic instability of hESCs[14] or hiPSCs in culture. Accordingly, it is important to periodically check hPSC cultures to exclude the possibility of an abnormal karyotype.

Cells maintained in mTeSR™1 or TeSR™2 have been shown to maintain a normal karyotype after extended (enzymatic) passaging. Figure 5 shows a G-banding preparation made from (A) the hESC line H1 after 48 passages in mTeSR™1 and (B) the hESC line H9 after 22 passages in TeSR™2. A normal karyotype was also observed for H9 cells after 32 passages in mTeSR™1 (passage 64 in total - data not shown) as well as for H1 cells after 19 passages in TeSR™2 (data not shown).

Figure 5. (A) H1 chromosomal profile (G-banding) after 48 passages in mTeSR™1* and (B) H9 chromosomal profile (G-banding) after 22 passages in TeSR™2.

*H1 cells are at passage 151 in total. (Data kindly provided by the WiCell Research Institute).



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5.6 Alternative Matrices for an Animal Protein-Free Culture System

It is a major focus in the field of pluripotent cell culture to develop animal protein-free matrices for use with animal protein-free defined medium. To date, the most robust matrix for the growth of hPSCs is BD Matrigel™. However, as it is isolated from the mouse EHS tumor, it introduces animal proteins to the system. For those who are striving towards an animal protein-free culture system, the use of BD Matrigel™ is not desirable. Furthermore, understanding the specific requirements needed to create defined and animal component-free surfaces that are capable of supporting hPSC attachment and growth has proven to be technically challenging[9]. Defined matrices often suffer from poor cell attachment, inability to support long-term passaging, or prohibitive production costs.

StemAdhere™ Defined Matrix for hPSC, developed and manufactured by Primorigen Biosciences®, is a defined, humanized matrix that supports the long-term culture of hPSCs with cells that consistently maintain puripotency[17]. It is an affordable and effective alternative to Matrigel™ and may be used with mTeSR™1 or TeSR™2 medium for a fully-defined and feeder-independent culture system.[18] This system allows for complete control over the culture environment, resulting in more consistent cell populations and more reproducible results in downstream applications. Cells easily transition from Matrigel™ to StemAdhere™ and are maintained and passaged, thereafter, using a simple, enzyme-free protocol. For complete instructions on culturing cells on StemAdhere™ Defined Matrix for hPSC, please refer to the manual "Maintenance of hPSCs on StemAdhere™ Defined Matrix for hPSC" available at www.stemcell.com.

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6.0 Appendix 1: Products for hPSC Research Available from STEMCELL Technologies

Catalog #	Product Description
Products for Maintenance of hPSCs	
05850	mTeSR™1 defined, feeder-independent maintenance medium for hPSCs
05860	TeSR™2 animal protein-free maintenance medium for hPSCs
07170	StemAdhere™ Defined Matrix for hPSC Kit
07160	StemAdhere™ Defined Matrix for hPSC
07163	StemAdhere™ Dilution Buffer
07174	Gentle Cell Dissociation Reagent
27147	Non Tissue Culture-Treated 6-well Plates
BD, Catalog #354277	BD Matrigel™ hESC-qualified Matrix (qualified for hESC culture by STEMCELL Technologies)
07909	Collagenase IV (1 mg/mL)
07913	Dispase (5 mg/mL)
07923	Dispase (1 mg/mL, specific activity 0.8 - 1.2 U/mL)
36254	DMEM/F-12
07903	Gelatin
07600	NEAA
07100	L-glutamine
07910	Trypsin-EDTA (0.05%)
07901	Trypsin-EDTA (0.25%)
Products for Characterization of hPSCs	
01550 / 01551	Oct-3/4 Antibody
01552	SSEA-1 Antibody
01553	SSEA-3 Antibody
01554	SSEA-4 Antibody
01555	TRA-1-60 Antibody
01556	TRA-1-81 Antibody
01557	TRA-2-49 Antibody
01558	TRA-2-54 Antibody
10210	FITC-conjugated goat anti-mouse IgG
10211	FITC-conjugated goat anti-mouse IgM
10215	APC-conjugated goat anti-rat IgM
Products for Cryopreservation of hPSCs	
05854 / 05855	mFreSR™ defined cryopreservation medium for hPSCs
07930	CryoStor™CS10 defined, animal protein-free cryopreservation medium for hPSCs

Products for Differentiation of hPSCs	
05210	STEMdiff™ APEL Medium
05831	STEMdiff™ Neural Induction Medium
27845 / 27945	AggreWell™400 plates(12 wells, each with approximately 940 microwells per well)
27840 / 27940	AggreWell™400Ex plates (6 wells, each with approximately 4,700 microwells per well)
27865 / 27965	AggreWell™800 plates (8 wells, each with approximately 300 microwells per well)
07010	AggreWell™ Rinsing Solution
05893	AggreWell™ Medium
07171 / 07172	Y-27632
27145	6-well ultra-low adherent plates for suspension cultures
02508	Recombinant human NT-3
02509	Recombinant human NT-4
02514	Recombinant human Activin A
02517	Recombinant human BAFF
02519	Recombinant human BDNF
02523	Recombinant human BMP2
02524	Recombinant human BMP4
02525	Recombinant human Noggin
02527	Recombinant mouse Wnt-3A
02634	Recombinant human Basic Fibroblast Growth Factor (bFGF)
02647	Recombinant human Transforming Growth Factor-1 β (TGF- β1)
Products for Reprogramming to hiPSCs	
05820	STEMcircles™-LGNSO for virus-free reprogramming
Products for Selection of hPSCs and hPSC-derived Cells	
18165	EasySep™ hESC/hiPSC SSEA-4 Positive Selection Kit
18167	EasySep™ hESC-Derived CD34 Positive Selection Kit

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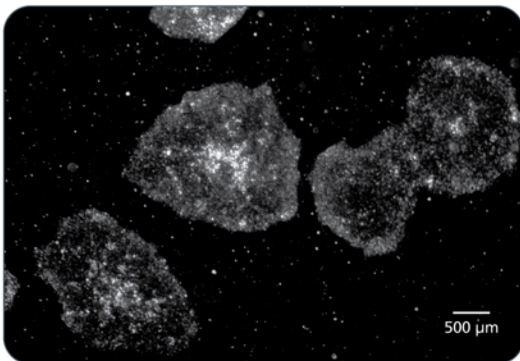
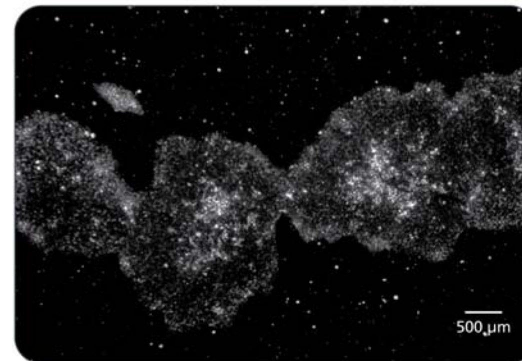
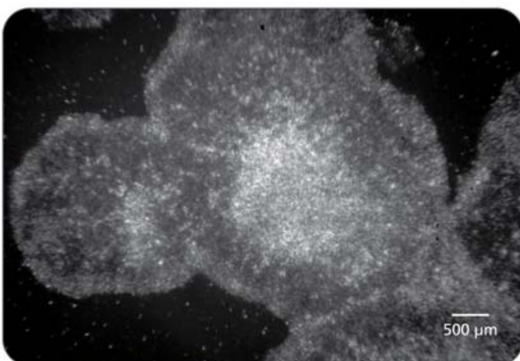
7.0 Appendix 2: Representative mTeSR™1 Cultures at Various Days After Passaging

Day 2: Colonies are small, transparent and not very densely packed with cells.





Day 4: Colonies rapidly increase in size and start to develop phase-bright centers when viewed under a phase contrast microscope. However, these colonies are not yet ready to be passaged.



Day 6: Colonies begin to merge and have phase-bright centers that are densely packed with cells. These colonies are ready to passage.

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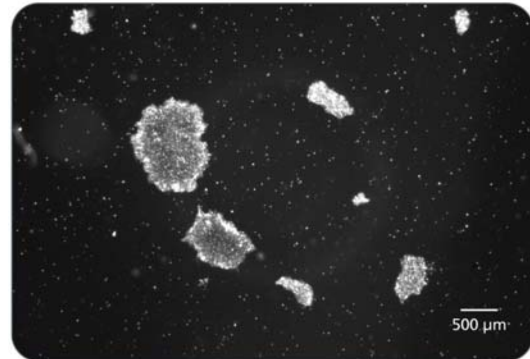
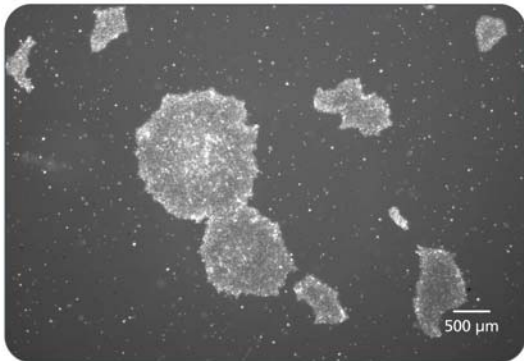
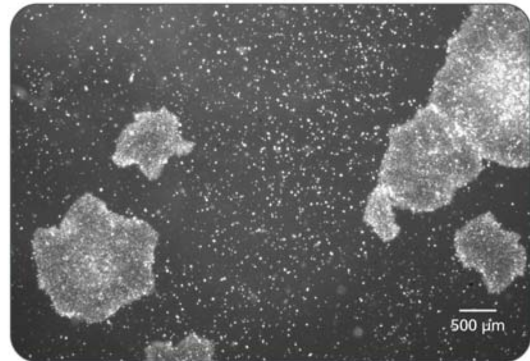
8.0 Appendix 3: Representative TeSR™2 Cultures at Various Days After Passaging

Note: cells grown in TeSR™2 may need to be passaged approximately 1 day earlier than the same cells grown in mTeSR™1. Please refer to Appendix 2 for representative mTeSR™1 cultures at various days after passaging.

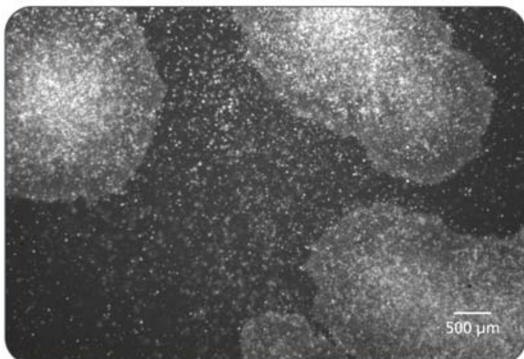


Days 1 and 2: Colonies are small, transparent and not very densely packed with cells.





Days 3 and 4: Colonies rapidly increase in size and start to develop phase-bright centers when viewed under a phase contrast microscope. However, these colonies are not yet ready to be passaged. Colonies at Day 4 should be passaged within 24 hours.



Day 5: Colonies begin to merge and have phase-bright centers that are densely packed with cells. These colonies are ready to passage.

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9.0 References

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM: Embryonic stem cell lines derived from human blastocysts. Science 282:1145-7, 1998
2. Xu C, Inokuma MS, Denham J, Golds K, Kundu P, Gold JD, Carpenter MK. Feeder-free growth of undifferentiated human embryonic stem cells. Nat Biotechnol 19(10):971-4, 2001
3. Amit M, Shariki C, Margulets V, Itskovitz-Eldor J. Feeder layer- and serum-free culture of human embryonic stem cells. Biol Reprod. 70: 837-45, 2004
4. Levenstein ME, Ludwig TE, Xu RH, Llanas RA, VanDenHeuvel-Kramer K, Manning D, Thomson JA. Basic fibroblast growth factor support of human embryonic stem cell self-renewal. Stem Cells 24: 568-74, 2006
5. Richards M, Fong CY, Chan WK, Wong PC, Bongso A. Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells. Nat Biotechnol. 20: 933-6, 2002
6. Vallier L, Alexander M, Pedersen RA. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. Cell Sci. 118: 4495-509, 2005
7. Lu J, Hou R, Booth CJ, Yang SH, Snyder M. Defined culture conditions of human embryonic stem cells. Proc Natl Acad Sci U S A. 103: 5688-93, 2006
8. Yao S, Chen S, Clark J, Hao E, Beattie GM, Hayek A, Ding S. Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. Proc Natl Acad Sci U S A. 103: 6907-12, 2006
9. Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL, Crandall LJ, Daigh CA, Conard KR, Piekarczyk MS, Llanas RA, Thomson JA.. Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol. 24: 185-7, 2006
10. Ludwig TE, Bergendahl V, Levenstein ME, Yu J, Probasco MD, Thomson JA. Feeder-independent culture of human embryonic stem cells. Nat Methods 3: 637-46, 2006
11. Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol. 18: 399-404, 2000
12. Amit M, Carpenter MK, Inokuma MS, Chiu CP, Harris CP, Waknitz MA, Itskovitz-Eldor J, Thomson JA. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev Biol. 227: 271-8, 2000
13. Draper JS, Smith K, Gokhale P, Moore HD, Maltby E, Johnson J, Meisner L, Zwaka TP, Thomson JA, Andrews PW. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat Biotechnol. 22: 53-4, 2004
14. Buzzard JJ, Gough NM, Crook JM, Colman A. Karyotype of human ES cells during extended culture. Nat Biotechnol. 22: 381-2, 2004
15. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917-1920, 2007
16. Sun N, Panetta NJ, Gupta DM, Wilson KD, Lee A, Jia F, Hu S, Cherry AM, Robbins RC, Longaker MT, Wu JC: Feeder-free derivation of induced pluripotent stem cells from adult human adipose stem cells. PNAS 106: 15720-15725, 2009

17. Nagaoka M, Si-Tayeb K, Toshihiro A, Duncan SA. Culture of human pluripotent stem cells using completely defined conditions on a recombinant E-cadherin substratum. BMC Developmental Biology. 10:60-72, 20120.
18. Couture LA. Scalable pluripotent stem cell culture. Nat Biotech. 28:562-563, 2010
19. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861-872, 2007
20. Park IH, Zhao R, West JA, Yabuuchi A, Hu H, Ince TA, Lerou PH, Lensch MW, Daley GQ. Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451:141-146, 2008

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