Published in final edited form as:

Methods Mol Biol. 2016 ; 1357: 183–193. doi:10.1007/7651_2014_159.

Induced pluripotent stem cells from nonhuman primates

Anuja Mishra, Zhifang Qiu, Steven Farnsworth, Jacob Hemmi, Miao Li, Alex Pickering, and Peter Hornsby

South Texas Veterans Health Care System, San Antonio, Texas and Department of Physiology/Barshop Institute, University of Texas Health Science Center at San Antonio

Summary

Induced pluripotent stem cells from nonhuman primates (NHPs) have unique roles in cell biology and regenerative medicine. Because of the relatedness of NHPs to humans, NHP iPS cells can serve as a source of differentiated derivatives that can be used to address important questions in the comparative biology of primates. Additionally, when used as a source of cells for regenerative medicine, NHP iPS cells serve an invaluable role in translational experiments in cell therapy. Reprogramming of NHP somatic cells requires the same conditions as previously established for human cells. However, throughout the process, a variety of modifications to the human cell protocols must be made to accommodate significant species differences.

Keywords

Induced pluripotent stem cells; nonhuman primates; marmoset; reprogramming; retroviruses; teratoma

1. Introduction

Following the generation of human induced pluripotent stem cells by Yamanaka's group (1, 2) the first nonhuman primate species to be reprogrammed was the rhesus macaque (*Macaca mulatta*) (3). Following this, the second nonhuman primate was the common marmoset (*Callithrix jacchus*) by our group, published in 2010 (4). Another group subsequently published a somewhat different method for the generation of marmoset iPS cells (5). This chapter focuses on the marmoset, as the species with which we are most familiar. We have used essentially the same techniques to generate and grow iPS cells from the chimpanzee (*Pan troglodytes*) and other primates.

Based on the genetic relatedness of humans and these various NHP species, methods developed for human cells can generally be used for NHPs. However, throughout the process of generation, growth and differentiation of NHP iPS cells, we have found it necessary to adjust the conditions previously found suitable for human cells to these other species. Some advances described for human cells, such as the use of TeSR1 medium – the first defined medium described for human pluripotent cells (6) – have not been found

suitable for marmoset iPS cells. On the other hand, E8 medium, essentially a simpler version of TeSR1 (7), has worked well in our hands for NHP iPS cells. The necessity of adapting each method developed for human pluripotent cells to each NHP species may give the impression that the cells are difficult to derive and maintain. However, when optimized conditions are used, we find that NHP iPS cells are no more difficult to handle than human iPS cells, reflecting the fact that most attention has been paid to human cells and therefore, unsurprisingly, protocols have been fine-tuned for the latter species.

2. Materials

Maintain sterility for all components. Antibiotics (100 µg/ml penicillin and 50 µg/ml gentamicin) are added to all components that come into contact with cells to prevent bacterial contamination (Note 1).

2.1. Cell culture medium

1. Dulbecco's Modified Eagle's Medium (DMEM) with 10% Cosmic Calf Serum (CCS; Thermo Hyclone catalog number SH30087.03HI): used for skin fibroblast culture and for the first stage of reprogramming of fibroblasts to iPS cells (Note 2).
2. E8 medium (7): used for all routine growth of iPS cells, as well as expansion of newly derived clones of cells; made up from components as described (7) or available from commercial sources. We prepare it from a basal medium comprising DMEM/F12 (Sigma catalog number D8437). Many NHP iPS cell clones require the addition of 10% fetal bovine serum (FBS) to E8 (Note 3). A batch of the medium can be divided into 50 ml tubes to be stored at -20°C ; thaw out just before use.
3. DMEM/F12 with Knockout Serum Replacement (KSR): a medium comprising DMEM/F12, 20% KSR (Life Technologies catalog number 10828-028) and 20 ng/ml FGF-2, with the addition of sodium bicarbonate (25 ml of 18 mg/ml NaHCO_3 added to 100 ml medium) to avoid excessive acidity. We use this medium in the early expansion of reprogrammed cells (see section 3.2).
4. mFreSR (Stemcell Technologies Inc., Vancouver, Canada): used for cell cryopreservation. Aliquot and store at -20°C until needed.
5. ROCK inhibitor Y-27632: add to the medium for iPS cells. The compound is made up at 10 mM in water and stored frozen in aliquots.

¹The combination of penicillin and gentamicin is very effective against bacterial contamination, and is helpful when initiating cultures from potentially nonsterile skin samples. We have tested several antimycotics for prevention of yeast contamination, and have not found any that do not interfere with the growth of fibroblasts or iPS cells. Contamination by yeast is an occasional problem with primary cultures, and if it occurs contaminated cultures should be discarded as soon as the growth of yeast is noted.

²Cosmic Calf Serum is a commercially available substitute for fetal bovine serum. It supports good growth of fibroblasts and many other cells.

³Some marmoset iPS cell clones can be grown in E8 with ROCK inhibitor without further additions. Other clones grow much better with the addition of 10% FBS. We use "ES-qualified" FBS (GlobalStem). In addition to E8, some other defined media such as Pluriton (Stemgent) may be used for marmoset iPS cells (16). E8 is preferred, because it can readily be made up from defined components, enabling it to be modified as needed.

2.2. Enzymes

1. Crude collagenase (Sigma Type I) for preparation of fibroblasts from skin.
2. Accutase (a mix of enzymes with proteolytic and collagenolytic activities) available from several commercial sources as a premade solution: use for subculture of pluripotent cells. Store at -20°C in 15 ml aliquots and thaw just before use.

2.3. Materials for reprogramming

1. Use the reprogramming factors (Oct4, Sox2, Klf4, c-Myc) expressed by retroviruses in the pMXs vector (2). A suitable preparation of the mix of the four viruses can be prepared in-house (8) by transfection of the retroviral plasmids into a suitable packaging cells line such as Plat-A cells (9) or can be purchased as a ready to use mix (Salk Institute Gene Transfer Targeting and Therapeutics Core) (Note 4).
2. The supernatant from the packaging cells should be filtered through a $45\text{ }\mu\text{m}$ syringe filter and then can be aliquoted and stored at -80°C . Refreezing aliquots is not advisable.
3. Valproic acid (VPA) is used as an epigenetic modifier to enhance the rate of reprogramming. It is made up as a 100 mM stock in water and added to cells at 1 mM (10).

2.4. Extracellular matrix components

1. For routine feeder-free culture, culture dishes can be coated with Matrigel (BD Biosciences catalog number 354234). When received, Matrigel should be thawed once to 4°C and aliquoted in 300 μl aliquots. Store at -20°C .
2. Dishes, pipettes, medium and other items should be pre-cooled to 4°C before starting the protocol.
3. Mix 25 ml DMEM/F12 with 300 μl Matrigel using a polythene transfer pipette (Fisher Scientific, catalog number 13-711-20).
4. Pipette 2 ml of the mix into each 35 mm dish. At this point, the dishes and contents can be allowed to warm to room temperature.
5. Place the dishes on a rotating shaker for 1 hour.
6. Store dishes at 4°C and use within 1 week.

⁴Although many protocols for reprogramming have been published, in our hands the original pMXs-based retroviral vectors have been the most reliable. We have also used a polycistronic retroviral vector successfully for marmoset cells (16) but this has proven to be much less efficient than the mix of four retroviruses. We also found that a Sendai virus-based kit ("Cytotune", developed by DNASIS Corporation and available from Life Technologies) can be used successfully for chimpanzee cells, but currently we have not been able to use this method to reprogram marmoset cells. Interestingly, this kit has been reported to be able to generate induced neural progenitor cells, but not iPS cells, using rhesus macaque fibroblasts (17). Another study showed that cells that produced dysgerminomas were generated by the introduction of Oct4/Sox2/Klf4/c-Myc reprogramming factors in marmoset cells using lentiviral vectors (18). Clearly it is desirable to develop robust protocols that use nonintegrating vectors or purely chemical methods for reliable reprogramming of NHP cells.

2.5. Cells for preparing feeder layers

1. Mitotically inactivated mouse embryo fibroblasts (Note 5): cells can be prepared from mouse embryos in house, or can be conveniently obtained commercially (e.g. mitomycin-inactivated, GlobalStem Inc., catalog number GSC-6001M) Cryopreserved cells are stored in liquid nitrogen until needed.
2. Cells are thawed at 37 °C and then plated on Matrigel-coated dishes in DME/CCS. Dishes are incubated at 37 °C. They can be used the following day or up to 3–4 days after plating.

3. Methods

All procedures should be carried out under BSL2 conditions (Note 6).

3.1. Preparation of NHP skin fibroblasts

1. Obtain a skin sample from the animal. Include the subcutaneous tissue whenever possible. Once excised from the animal, the tissue should be kept on ice in a suitable container, on ice, but it is not necessary to add medium or buffer. Small samples such as punch biopsies from living animals can be successfully used. Animals that have died of natural causes can also yield skin for successful culture, if the skin is obtained within a few hours of death. Skin samples can be shipped on ice and/or stored at 4 °C for several days and still yield a large number of viable cells.
2. Dissolve collagenase at 5 mg/ml in DMEM/CCS. Add the collagenase solution to the skin sample in a dish. Using scalpel and forceps, cut the skin into small fragments (1–2 mm). Incubate the fragments in the collagenase at 37 °C in an incubator. After one hour, flush the fragments through a polythene transfer pipette. Repeat every hour, until the tissue has dissociated to single cells and small clumps of cells. This may take several hours.
3. When the tissue has dissociated, centrifuge the single cells and clumps and resuspend them in DME/CCS. Plate the cells in 35-mm, 6-cm or larger dishes as needed. Change the medium the next day and every 2 days thereafter.
4. Cells can be infected with reprogramming viruses as soon as they start dividing rapidly. Some cells should be continued in standard fibroblast culture and then cryopreserved in 5% DMSO for optional later repeat of reprogramming.

3.2. Reprogramming using retroviruses encoding Oct4, Sox2, Klf4 and c-Myc

1. Grow skin fibroblasts as described above and ensure that the cells are dividing at a maximal rate (Note 7).

⁵The use of E8 medium, with the addition of FBS when needed, and Matrigel-coated plates makes the routine mass culture of NHP iPS cells feasible without the use of traditional feeder layers. However, when early reprogrammed clones are being expanded, we find that mouse fibroblast feeder layers are required for successful expansion of clones.

⁶Refer to your institution's specific guidelines for the handling of materials from NHPs. Generally, work with NHP materials, as well as retroviruses, should be under BSL2 conditions (19).

2. Subculture the cells into wells of a 6-well culture plate. The optimal cell density should be determined for each population of fibroblasts, but typically about 60–70% cell confluency is optimal.
3. Thaw a suitable aliquot of frozen viral supernatant, containing all four viruses, with warm water, not allowing to the supernatant to exceed room temperature. For each well use 400 μ l of virus mix plus 2 ml fibroblast culture medium. Add 8 μ g/ml Polybrene to the mix (8). Replace the culture medium with the virus mix (Note 8).
4. Allow the cells to equilibrate in the incubator for 5 minutes. Wrap the plate in Parafilm and then transfer to the centrifuge.
5. Using a suitable plate carrier (e.g. Beckman Coulter Inc., SX4750 rotor), centrifuge the plate at 1,200 g for 1 hour at 15 °C (8).
6. Remove the Parafilm and place the plate in the incubator. After 3 hours, add extra DME/CCS to the virus mix to fill the well.
7. After 24 hours, repeat the process of infection (steps 3 to 6).
8. After a further 24 hours, replace the medium with DME/CCS plus 1 mM VPA. If the cells become confluent, subculture them at a ratio typical for fibroblasts (e.g. 1:4).
9. After 6 days in these conditions, subculture the cells using trypsin at a ratio of 1:4. Plate the cells onto a mouse embryo fibroblast feeder layer in DMEM/F12 with 20% KSR (Note 9).
10. Over the next 1–2 weeks, colonies of cells of altered appearance should become apparent (Fig. 1). The change in morphology is initially the result of a mesenchymal to epithelial transition (11). As the colonies develop, they adopt an appearance characteristic of iPS cells (small, rapidly dividing cells with prominent nuclei). Colonies of NHP iPS cells are generally flat and do not adopt the domed appearance of mouse pluripotent cells.
11. Once the colonies have the appearance of typical iPS cells, change the medium to E8 with 10% FBS and 10 μ M ROCK inhibitor.
12. When colonies have developed to a size of several hundred cells, they are isolated by detaching them with Accutase. Flood the dish with Accutase. Wait until cells in

⁷The process of infecting cells with retroviruses is simple, but can be of low efficiency. Cells must be dividing to be infected, and the use of a positively charged polymer with low speed centrifugation of the culture plate (“spinoculation”) can greatly increase infection rates (8). A high infection rate is critical because a cell must be infected with all four retroviruses to undergo reprogramming.

⁸The amount of virus that is required for successful reprogramming is difficult to predict in advance of the experiment. If possible, prepare batches of virus that are large enough to permit multiple reprogramming experiments. As necessary, adjust the amount of virus used per experiment to permit reprogramming for that particular somatic cell culture. In a successful experiment, large numbers of iPS cell clones will be obtained, more than is typically needed. If the number of clones obtained is very low, or zero, attempt to increase the virus as much as possible so that some successful reprogramming occurs.

⁹We use this medium at this stage of the reprogramming because it is permissive for the growth of pluripotent cells, while not encouraging the growth of fibroblasts. Therefore, the culture can be maintained for 7 – 14 days without subculturing, as the iPS cell colonies appear and the fibroblasts slowly degenerate. Although E8 is better for the long term maintenance of NHP iPS cells, it does also permit growth of fibroblasts.

the colonies round up. Under a phase contrast microscope, aspirate the colony using a fine-tip polyethylene transfer pipette (Sarstedt, catalog number 86.1180).

13. Plate the cells in E8 with 10% FBS on a mouse embryo fibroblast feeder layer in a small well (e.g. 48-well plate).
14. As the clone grows, transfer to successively larger wells and dishes.
15. Cryopreserve the clone as soon as it comprises sufficient cells.

3.3. Routine maintenance of iPS cells

1. NHP iPS cells can be grown as mass cultures (12), using E8 and Matrigel-coated dishes (Fig. 2). This is a much simpler protocol than the traditional colony-based growth on feeder layers. Use E8 medium and 10 μ M ROCK inhibitor, with the addition of 10% FBS as necessary (Note 3).
2. Change the medium every day. The pH of the medium gives a good indication of when to subculture; do not let the medium become too acid, although high cell density with some accompanying acidity is beneficial for cell growth.
3. For subculturing, aspirate the medium, wash the monolayer of cells once with Accutase, add 1 ml more Accutase to the cells, and place the dish in the incubator. When the cells begin to round up, but are not yet detaching, remove the Accutase from the monolayer and flush the cells off the dish into fresh medium using a polythene transfer pipette (Note 10).
4. Plate 1.5 ml cell suspension per 35 mm dish and then change the next day with 2 ml medium. Generally, a split ratio of 1:2 is suitable. Using this split ratio, NHP iPS cells can often be grown with a subculture every 2 days.
5. Clones of iPS cells should be cryopreserved when the clone is first isolated and again at early passages (Note 11). Remove the cells from the plate using Accutase, as for subculturing. Centrifuge the cells at 1000 r.p.m. for 3 minutes and then resuspend in 1.8 ml mFreSR per vial. Place the vials in a Coolcell (BioCision) at -80°C overnight, and then transfer to liquid nitrogen for long term storage.
6. At early passages, perform basic characterization of the clone, including expression of pluripotency genes (particularly *NANOG* and *OCT4/POU5F1*), proper silencing of the retroviral genes, and karyotyping (4).
7. To recover iPS cells from liquid nitrogen storage, place the vial in 37°C water and thaw as rapidly as possible (13). Add 1 ml warm medium and transfer to a 15 ml tube. After 2 minutes, add 2 ml more medium; after a further 2 minutes add 4 ml medium; and then after 2 more minutes centrifuge the cells at 1000 r.p.m. for 3

¹⁰Although conventionally cells are resuspended in fresh medium and centrifuged before replating, this step is unnecessary for efficient subculturing of pluripotent cells, as noted previously for an EDTA-based protocol (20). Timing of Accutase treatment is critical and must be customized for each cell line. Under these conditions, it is simple to aspirate the Accutase without disturbing the cell monolayer and then to flush the cells off into fresh medium, which also serves to neutralize the enzymes in Accutase.

¹¹We have not extensively determined the importance of passage number on the properties of NHP iPS cells. However, it is always advisable to use cells at a known passage, and usually at as low a passage number as practical.

minutes. Add 1.5 ml medium to the cell pellet for a 35 mm dish and transfer to the incubator.

3.4. Investigation of pluripotency by teratoma formation

This section describes a protocol for assessment of the differentiation potential of pluripotent cells by teratoma formation (Note 12).

1. Various types of immunodeficient mice have been used successfully for the formation of teratomas. Following earlier demonstrations that more profoundly immunodeficient mice are superior for efficiency and rapidity of teratoma formation (14), we use *Rag2*^{-/-}, *Il2rg*^{-/-} mice (available from Taconic, model 4111). These animals must be housed under conditions suitable for immunodeficient mice, the most important of which is the use of microisolator cages that provide a cage-level barrier against exposure to pathogens. With care not to expose these mice to pathogens derived from conventional mice, this strain can be housed without evidence of bacterial/viral disease under relatively convenient housing conditions.
2. Prepare cells that are being tested for teratoma formation as for routine subculture as described above. Produce a pellet of at least 10⁶ cells. Cover the pellet with culture medium in a 1.6 ml tube and transport on ice for injection into the mouse.
3. Remove the medium from the pellet and resuspend in 20 µl of a mix of 50% Matrigel and 50% DMEM/F12 (see section 2.4) kept on ice until used.
4. Anesthetize the mouse with Avertin (15) or a suitable alternative.
5. After the cells are mixed into the 50% Matrigel, draw the cell suspension up into a cold 50 µl glass syringe (Hamilton) fitted with a suitable needle for subcutaneous injection (Note 13).
6. Inject the cell suspension subcutaneously; an ideal location is the skin on the head just caudal to the external ear. This location provides nearby blood vessels for growth support and is in a location that cannot be reached by the mouse during development of the teratoma.
7. After teratomas can be felt under the skin, euthanize the mice and excise the tumors for histological or other processing (Fig. 3).

Acknowledgments

This work was supported by VA grant I01BX001454, NIH grants R21 AG033286 and R03 AG045481, and by grants from the Owens Medical Research Foundation and the Ted Nash Long Life Foundation.

¹²The teratoma assay, as an assay for pluripotency, has been criticized on several grounds (21), yet remains an important test for NHP iPS cells. For human cells, patterns of gene expression under relatively simple differentiation conditions (e.g. embryoid body formation) have been defined adequately such that a newly derived cell line can be verified to be a genuine iPS cell line without the teratoma assay. However, for many other species, similar assays have not yet been developed. In those species the teratoma assay remains the “gold standard” for pluripotency determination.

¹³The needle should be carefully chosen to permit the injection of a gel (Matrigel plus cells) efficiently under the skin. We use a custom 22 gauge needle (Hamilton Company) that is short (0.5 inches) and has a 45° beveled tip.

References

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–76. [PubMed: 16904174]
2. 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*. 2007; 131:861–72. [PubMed: 18035408]
3. Liu H, Zhu F, Yong J, Zhang P, Hou P, Li H, Jiang W, Cai J, Liu M, Cui K, Qu X, Xiang T, Lu D, Chi X, Gao G, Ji W, Ding M, Deng H. Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell*. 2008; 3:587–90. [PubMed: 19041774]
4. Wu Y, Zhang Y, Mishra A, Tardif SD, Hornsby PJ. Generation of induced pluripotent stem cells from newborn marmoset skin fibroblasts. *Stem Cell Res*. 2010; 4:180–188. [PubMed: 20363201]
5. Tomioka I, Maeda T, Shimada H, Kawai K, Okada Y, Igarashi H, Oiwa R, Iwasaki T, Aoki M, Kimura T, Shiozawa S, Shinohara H, Suemizu H, Sasaki E, Okano H. Generating induced pluripotent stem cells from common marmoset (*Callithrix jacchus*) fetal liver cells using defined factors, including Lin28. *Genes Cells*. 2010; 15:959–69. [PubMed: 20670273]
6. 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*. 2006; 24:185–7. [PubMed: 16388305]
7. Chen G, Gulbranson DR, Hou Z, Bolin JM, Ruotti V, Probasco MD, Smuga-Otto K, Howden SE, Diol NR, Propson NE, Wagner R, Lee GO, Antosiewicz-Bourget J, Teng JM, Thomson JA. Chemically defined conditions for human iPSC derivation and culture. *Nat Methods*. 2011; 8:424–9. [PubMed: 21478862]
8. Wu Y, Melton DW, Zhang Y, Hornsby PJ. Improved coinfection with amphotropic pseudotyped retroviral vectors. *J Biomed Biotechnol*. 2009; 2009:901079. [PubMed: 19478961]
9. Morita S, Kojima T, Kitamura T. Plat-E: an efficient and stable system for transient packaging of retroviruses. *Gene Ther*. 2000; 7:1063–6. [PubMed: 10871756]
10. Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S, Muhlestein W, Melton DA. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol*. 2008; 26:1269–75. [PubMed: 18849973]
11. Polo JM, Hochedlinger K. When fibroblasts MET iPSCs. *Cell Stem Cell*. 2010; 7:5–6. [PubMed: 20621040]
12. Chen KG, Mallon BS, Hamilton RS, Kozhich OA, Park K, Hoepfner DJ, Robey PG, McKay RD. Non-colony type monolayer culture of human embryonic stem cells. *Stem Cell Res*. 2012; 9:237–48. [PubMed: 22910561]
13. Valbuena D, Sanchez-Luengo S, Galan A, Sanchez E, Gomez E, Poo ME, Ruiz V, Genbacev O, Krtolica A, Pellicer A, Moreno R, Simon C. Efficient method for slow cryopreservation of human embryonic stem cells in xeno-free conditions. *Reprod Biomed Online*. 2008; 17:127–35. [PubMed: 18616900]
14. Koch CA, Jordan CE, Platt JL. Complement-dependent control of teratoma formation by embryonic stem cells. *J Immunol*. 2006; 177:4803–9. [PubMed: 16982921]
15. Papaioannou VE, Fox JG. Efficacy of tribromoethanol anesthesia in mice. *Lab Anim Sci*. 1993; 43:189–92. [PubMed: 8320967]
16. Wu Y, Mishra A, Qiu Z, Farnsworth S, Tardif SD, Hornsby PJ. Nonhuman primate induced pluripotent stem cells in regenerative medicine. *Stem Cells Int*. 2012; 2012:767195. [PubMed: 22577396]
17. Lu J, Liu H, Huang CT, Chen H, Du Z, Liu Y, Sherafat MA, Zhang SC. Generation of integration-free and region-specific neural progenitors from primate fibroblasts. *Cell Rep*. 2013; 3:1580–91. [PubMed: 23643533]
18. Yamaguchi S, Marumoto T, Nii T, Kawano H, Liao J, Nagai Y, Okada M, Takahashi A, Inoue H, Sasaki E, Fujii H, Okano S, Ebise H, Sato T, Suyama M, Okano H, Miura Y, Tani K. Characterization of common marmoset dysgerminoma-like tumor induced by the lentiviral expression of reprogramming factors. *Cancer Sci*. 2014; 105:402–8. [PubMed: 24521492]

19. Adams, SR.; Muchmore, E.; Richardson, JH. Biosafety. In: Bennett, BT.; Abee, CR.; Henrickson, RV., editors. *Nonhuman Primates in Biomedical Research: Biology and Management*. Academic Press; San Diego: 1995. p. 377-420.
20. Beers J, Gulbranson DR, George N, Siniscalchi LI, Jones J, Thomson JA, Chen G. Passaging and colony expansion of human pluripotent stem cells by enzyme-free dissociation in chemically defined culture conditions. *Nat Protoc*. 2012; 7:2029–40. [PubMed: 23099485]
21. Dolgin E. Putting stem cells to the test. *Nat Med*. 2010; 16:1354–1357. [PubMed: 21135828]

Fig. 1.

Derivation of marmoset iPS cells from skin fibroblasts. (A) Skin fibroblasts (adult marmoset) growing in culture before infection with reprogramming retroviruses. (B) About 8 days after infection, small numbers of cells with an altered morphology (epithelial rather than fibroblastic) are noted, if reprogramming is proceeding successfully. (C) About 11 days after infection, distinct colonies of cells that have the appearance of iPS cells are noted; cells are small, rapidly dividing, with prominent nuclei.

Fig. 2.

Growth of marmoset iPS cells under feeder-free mass culture conditions. Cells of clone #15 (4) were plated on Matrigel-coated dishes in E8 medium with 10% FBS. The figure shows the appearance of the cells at 1 to 3 days after plating as they achieve a high density again. As the cells grow to high density, it is typical to observe rounded cells, some of which detach or adhere to the surface of the monolayer. The medium also becomes acidic, even though it is changed every 24 hours. The level of acidity is a useful indication that the cells should be passaged again.

Fig. 3. Histological appearance of teratomas formed from a marmoset iPS cell line (B8; see ref. 4). A and B show examples of various tissue structures within teratomas. Staining with tissue-specific antibodies embryos shows that tissues within teratomas originate from all three germ layers (4). Hematoxylin and eosin stained sections from conventionally fixed and paraffin-embedded tissue samples.