



viPS™ Vector Kit



Brought to you in conjunction with Aruna Biomedical, Inc.

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IMPORTANT SAFETY NOTE

Follow NIH guidelines regarding lentiviral production and transduction; follow Biosafety Level 2 (BL2) or BL2+ laboratory criteria.

NIH Agent Summary Statement: <http://bmbf.od.nih.gov/viral2.htm#retro>

NIH Biosafety Level 2 Description: <http://bmbf.od.nih.gov/sect3bsl2.htm>

NIH/RAC “Guidance on Biosafety Considerations for Research with Lentiviral Vectors”:

http://www4.od.nih.gov/oba/RAC/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf

PRODUCT DESCRIPTION

The viPS™ Vector Kit for reprogramming fibroblasts into induced pluripotent stem cells was developed by Thermo Fisher Scientific, Inc. in collaboration with ArunA Biomedical, Inc. Six transcription factors (Lin28, c-Myc, Klf4, Nanog, Sox2 and Oct4 (Pou5f1)) have been cloned into a lentiviral vector system to create a resource for producing induced pluripotent stem (iPS) cells, allowing for the generation of patient- and disease-specific cells. Ectopic expression of these factors has been shown to create pluripotent cells which resemble human embryonic stem (hES) cells (Takahashi *et al.*; Yu *et al.*). The set of six factors is available in high-titer virus, ready for transduction.

INTRODUCTION

Pluripotent stem cells show compelling potential as a tool for research given their capacity to grow indefinitely while maintaining pluripotency. The ability to produce induced pluripotent stem (iPS) cells from differentiated somatic cells is highly desirable (Takahashi *et al.*; Yu *et al.*), due to their potential in providing patient- or disease- specific pluripotent stem cell populations. iPS cells are generated by reprogramming somatic cell types to an embryonic-like state. The most common method for reprogramming somatic cell types involves the ectopic expression of transcription factors which regulate pluripotency via lentiviral vector gene transfer. Lentiviral gene transfer is an efficient technology for stably introducing genetic material into the genome of the target cell. By reprogramming adult somatic cells into iPS cells, large quantities of patient- and disease- specific cells can potentially be produced to serve as models for disease mechanisms and development, drug screening and toxicology studies.

DESIGN INFORMATION

The viPS™ Vector Kit lentiviral vectors express human Oct4, Sox2, Nanog, Klf4, c-Myc, and Lin28 under control of the human elongation factor-1 alpha promoter (EF1 α). Vectors were generated by PCR amplifying each pluripotency factor open reading frame (ORF) followed by direct cloning downstream of the EF1 α promoter. Unique endonuclease restriction sites placed within the PCR primers were used to facilitate cloning. A Kozak consensus sequence was also included to ensure efficient translation.

The cDNAs used for PCR were obtained from the Human Pluripotency Tool Kit™ (Catalogue number PPK4919), which consists of:

1. Oct4 (Pou5f1) (NM_002701, Incyte clone ID:LIFESEQ259583);
2. Sox2 (NM_003106. IMAGE clone ID:2823424);
3. Nanog (NM_024865. IMAGE clone ID: 40004920);

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4. Klf4 (NM_004235, IMAGE clone ID: 5111134);
5. c-Myc (NM_002467, IMAGE clone ID: 2985844);
6. Lin28 (NM_024674, IMAGE clone ID: 841184).

Each of the pluripotency factor ORFs has been sequenced verified. For efficient expression, the lentiviral vector also contains a woodchuck hepatitis virus post-transcriptional regulatory element (WPRE) and the pput/cPPT (CTS, DNA flap) sequence. The pVIPS lentiviral vector is self-inactivating (SIN). Additionally, a control lentiviral vector with TurboGFP was generated in a similar fashion.

VECTOR INFORMATION

Versatile vector design

Features of the pVIPS lentiviral vector (Figure 1, Figure 2, and Table 1) that make it a versatile tool for generating induced pluripotent stem cells include:

- The ability to perform transductions using the replication incompetent lentivirus (Shimada *et al.*) including difficult to transduce cell lines
- Expression driven by a RNA Polymerase II EF1 α promoter
- Self inactivating (SIN) LTR to ensure minimal secondary recombination to form infectious particles

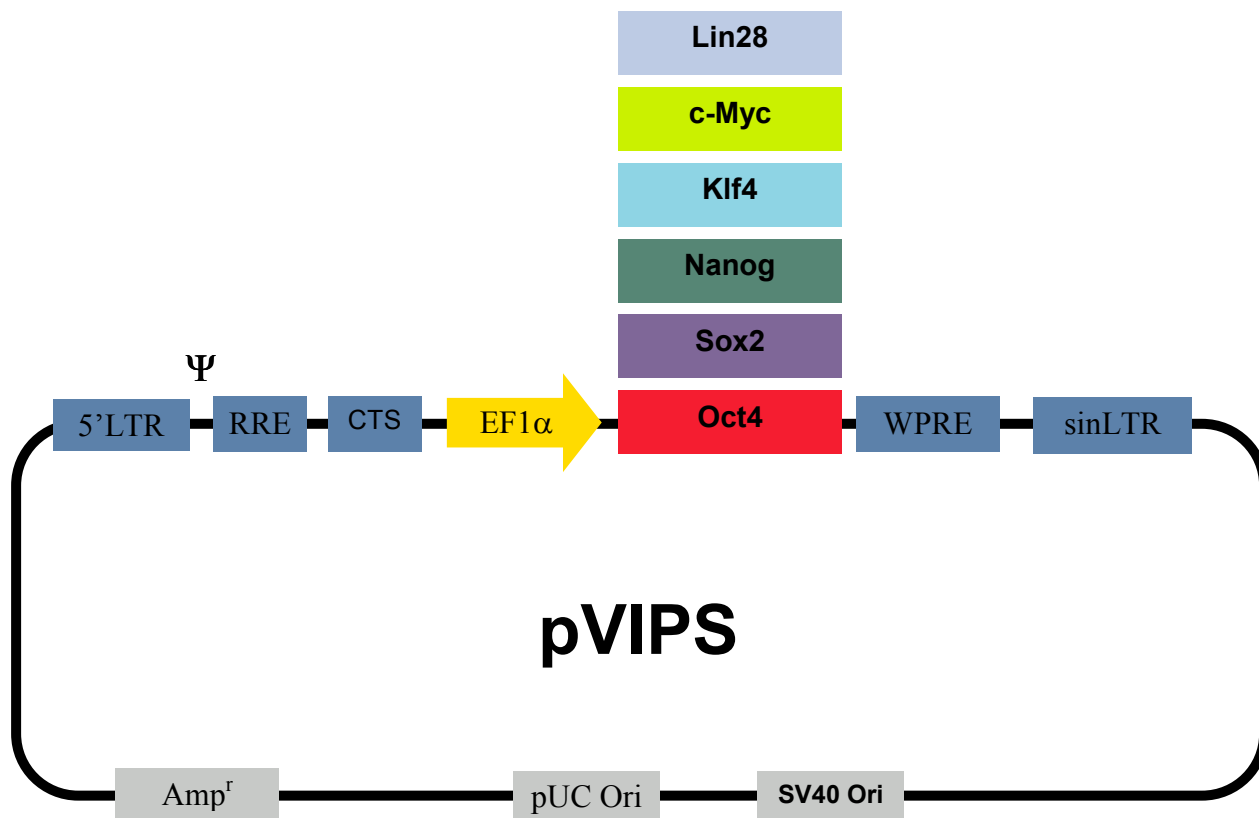


Figure 1: pVIPS lentiviral vector

Table 1: Features of the pVIPS vector

Vector Element	Utility
5'LTR	5' long terminal repeat
RRE	Rev response element
CTS	Central polypurine tract helps translocation into the nucleus of non-dividing cells
EF1 α Promoter	RNA polymerase II promoter
WPRE	Enhances the stability and translation of transcripts
SIN-LTR	3' Self inactivating long terminal repeat (Shimada, <i>et al.</i>)
SV40 Ori	SV40 Ori Allows for episomal replication of plasmid in eukaryotic cells
pUC Ori	High copy replication and maintenance of plasmid in <i>E. coli</i>
Amp resistance	Ampicillin (carbenicillin) bacterial selectable marker

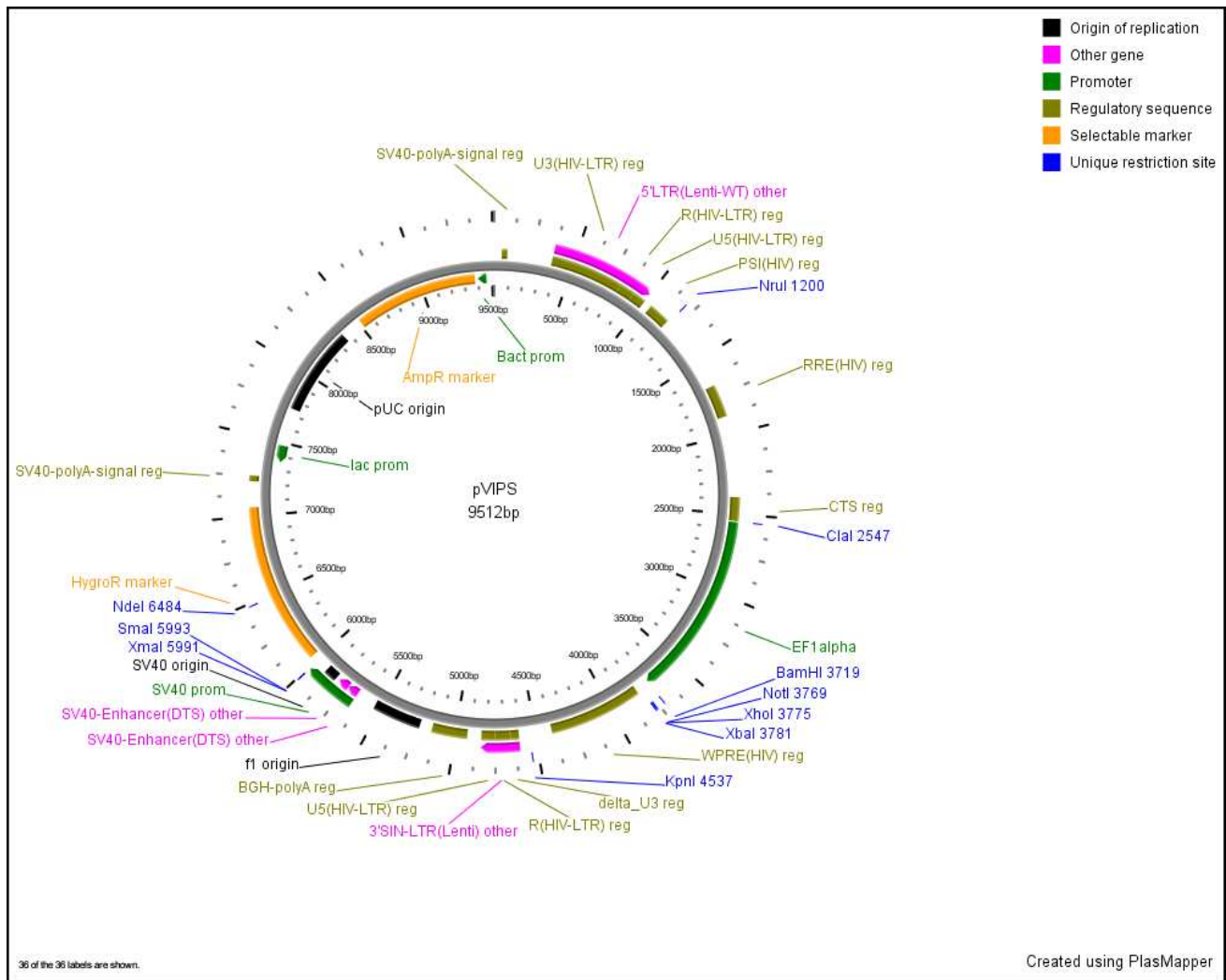


Figure 2: Detailed map of pVIPS lentiviral vector.

QUALITY CONTROL

1. All constructs included in this kit were sequence-verified to confirm the identity of the inserts.
2. All virus preparations included with this kit have passed through internal quality control (Q/C) process to ensure high quality and performance
 - a. Each vector has been titered in HEK293T cells and verified to contain at least 5×10^8 TU/ml (for exact titer information see the Certificate of Analysis included with each viPS™ Vector Kit);
 - b. All components of this kit have been tested to demonstrate the ability to reprogram the control cell line IMR-90 (ATCC Number: CCL-186).
3. The Q/C tests include:
 - a. Verifying protein production in HEK293T cells following transduction by Western blot;

- b. Evaluating by quantitative PCR (qPCR) the level of transcript in the control cell line (IMR-90) is sufficient for reprogramming;
- c. Appearance of iPS cell colonies following the reprogramming protocol outlined below.

PROTOCOL

The following protocol is for use with our viPS™ Vector Kit (catalogue number IPS5449) and describes a method for transducing human somatic cell types, like IMR-90 human lung fibroblasts, with lentiviral vectors encoding six transcription factors (Oct4, Nanog, Sox2, Lin28, Klf4, and c-Myc) to initiate reprogramming via ectopic expression and generate iPS colonies.

The following components are included with the kit:

viPS-EF1 α -Oct4 lentiviral particles	3 vials (20 μ l/vial)
viPS-EF1 α -Nanog lentiviral particles	3 vials (20 μ l/vial)
viPS-EF1 α -Sox2 lentiviral particles	3 vials (20 μ l/vial)
viPS-EF1 α -Lin28 lentiviral particles	3 vials (20 μ l/vial)
viPS-EF1 α -Klf4 lentiviral particles	3 vials (20 μ l/vial)
viPS-EF1 α -c-Myc lentiviral particles	3 vials (20 μ l/vial)
viPS-EF1 α -TurboGFP control lentiviral particles	4 vials (20 μ l/vial)

Important Notes

1. The following protocols provide a general method for generating human iPS cells from adherent cells, such as IMR-90 human lung fibroblasts. The utility of this protocol is as a starting point for determining the optimal conditions for generating iPS cells from your human target cells. We strongly recommend carrying out the transduction test first (described below) before initiating your work with your cell line of interest.
2. The lentiviral vector stocks will remain stable for at least 1 year without appreciable loss of titer. Lentivirus stocks must be stored at -80°C. When ready to use, thaw out vector by placing the tube in a 37°C water bath and quickly place the tube on ice after it is thawed. There is only a small decrease in titer after the first freeze/thaw; however, subsequent freeze/thaws result in a decrease in titer of 2 to 5-fold. Therefore, after the first freeze/thaw, any remaining virus should be frozen back for future use in workable aliquots to avoid unnecessary freeze/thaws. All Q/C studies have been carried out after one freeze/thaw.
3. MOI is indicative of the multiplicity of infection. The supplied virus has been titered in HEK293T cells. Other cell lines may have lower transduction efficiency. Thus, to ensure a reasonable chance of all six factors getting into a target cell, an MOI of 10 based on titering in HEK293T cells is recommended. When working with your cell line of interest, you might have to adjust the protocol accordingly (i.e. increase the MOI) based on the results of a transduction test. A control lentivirus (viPS-EF1 α -TurboGFP) is provided to assess general transduction efficiency of the target cell line.

Additional Materials Required

I. Cell lines:

- Human target cell line: IMR-90 human lung fibroblasts: American Type Culture Collection (ATCC No. CCL-186). In order to obtain high-quality iPS cell colonies, using IMR-90 cells as the target cell line is recommended.
- Inactivated mouse embryonic fibroblasts (MEFs): It is very important that the MEFs used to generate iPS cells following the protocol below are pre-validated to support human iPS cell growth. Using viPS™ mouse feeders (Catalogue number IPS5445) is highly recommended. Note: MEFs demonstrated to maintain pluripotency in mouse cells are not sufficient.

II. Cell Culture Media for growing MEFs (MEF medium) and iPS cell colonies (iPS medium):

MEF medium should be based on the formulation commonly used for MEFs intended for human embryonic stem cell culture (West *et al.*). iPS medium should be based on the formulation commonly used for human embryonic stem cell culture (West *et al.*), with the exception that the basic fibroblast growth factor (bFGF) concentration be increased to 10 ng/ml.

III. Cell Culture Supplies:

Table 2: Cell culture requirements and supplies

Cell Culture Supplies	Supplier	Catalogue Number
6-well plates	Fisher Scientific	08-772-1B
24-well plates	Fisher Scientific	08-772-1H
15 ml conical tubes	Fisher Scientific	14-959-49B
PBS ⁺⁺	Fisher Scientific	SH30264.02
10 cm dishes	Fisher Scientific	353003
0.05% trypsin/0.53 mM EDTA	Fisher Scientific	ICN1689149
35 mm dishes	Fisher Scientific	08-772A
Glass Pasteur pipettes	Fisher Scientific	13-678-20D

EXPERIMENTAL PLAN: TRANSDUCTION TEST

Materials:

One vial viPS-EF1 α -TurboGFP control lentiviral particles (average titer: 5 x 10⁸ TU/ml, for exact titer see the Certificate of Analysis provided with your lentiviral particles) at 20 μ l/vial.

A transduction test is suggested here to provide information regarding the efficiency of the lentiviral vector particles to transduce the target cells of interest that are to express the pluripotency factors and be reprogrammed.

The following protocol describes the transduction of your target cell with viPS-EF1 α -TurboGFP control lentiviral particles using five different MOIs, ranging from 1 to 50 (MOI = 1, 5, 10, 25, and 50). We suggest that the transductions be performed in a 24-well plate so as to reduce the number of target cells transduced and accommodate the range of MOIs that can be tested using one vial of the viPS-EF1 α -TurboGFP control lentiviral particles.

Day 1:

The day before transduction, plate target cells into 7 wells of a 24-well tissue culture plate. Five wells will be used for the five different MOIs; one well to be used as a non-transduced control, and one well for counting the number of cells in the well at the time of transduction. Seed the appropriate number of cells so that the confluency of the culture is between 30-40% at the time of transduction on Day 2. The number of cells to be seeded will vary depending on the cell type, as there are differences in cell size and morphology. (The user may need to determine the number of cells to seed for their target cell prior to initiating the transduction test). Use the same culture media that is needed to maintain the target cell of interest.

Day 2:

Thaw 1 vial of viPS-EF1 α -TurboGFP control lentiviral particles by placing the tube in a 37°C water bath and quickly placing the tube on ice after it is thawed. Keep virus on ice and work quickly. Before removing the cap, quick spin the vial in a microfuge.

Prior to transduction, count the number of cells in one well of the 24-well plate. The number of cells will be used in calculating the volume of virus needed for a given MOI. Please refer to the equation below (Equation 1) for calculating the MOI. For example, if the number of cells in the well at the time of transduction is 5×10^4 , and the titer of viPS-EF1 α -TurboGFP control lentiviral particles is 5×10^8 TU/ml, then the volume of virus for the following range of MOI is as follows:

MOI	Volume of viral particles (μ l)
1	0.1
5	0.5
10	1.0
25	2.5
50	5.0

Note: For small volumes (<2.5 μ l), it is suggested that 5.0 μ l of the viPS-EF1 α -TurboGFP control lentiviral particles be transferred to another tube and diluted 1:9 with 45 μ l DMEM (containing no serum). Place the tube containing the diluted lentiviral particles back on ice. Remember, that when using the diluted virus, the volume of viral particles added to the cells would have to be increased 10-fold from the volumes indicated on the above chart.

Transduction:

1. Label five 1.5 ml microfuge tubes with the MOI = 1, 5, 10, 25, and 50. Label one tube for the non-transduction control.
2. Pipette into each tube 200 μ l of fresh culture medium.
3. Pipette the appropriate volume (for the desired MOI) of lentiviral particles into the corresponding tube.
4. Remove the culture medium from the plated cells by aspiration.
5. Slowly pipette the medium and virus directly into the corresponding well. (For increased transduction efficiency, the volume of medium is reduced to a level that is sufficient to just cover the cells).
6. Incubate the cells with virus for 18-24 hours at 37°C, 5% CO₂, or conditions particular to your target cell.

Day 3:

1. 18-24 hours post transduction, aspirate the culture medium.
2. Slowly pipette 1.0 ml of fresh culture medium to each well
3. Place plate back in incubator and culture the cells at 37°C, 5% CO₂, or conditions particular to your target cell.

Days 4 – 6:

Monitor GFP fluorescence over the next 72 hours and analyze transduction efficiency as desired (i.e. by visual observation or flow cytometry).

Anticipated results:

This protocol will test the transduction efficiency of your cell line of interest before proceeding with the reprogramming protocol. **The protocol for reprogramming described below assumes that you are able to achieve a transduction efficiency of at least 70%.**

In case the transduction efficiency of your cell line of interest based on the experiment above is less than 70% at an MOI = 10, you still can proceed with the reprogramming protocol outlined below but expect to find fewer iPS colonies following reprogramming. Alternatively, you might want to consider switching to a different cell line that allows for higher transduction efficiencies. Lastly, you can also modify the reprogramming protocol outlined below and use a different MOI (for example, in case the transduction experiment indicated a 70% transduction efficiency at an MOI=25, you can modify the reprogramming protocol for an MOI=25 per factor); use Equation 1 for your calculations.

EXPERIMENTAL PLAN: REPROGRAMMING

A. Transduction of target cell lines

Day 1:

1. The day before transduction, plate IMR-90 human lung fibroblasts (or other target cell line) at 1.25×10^5 cells per well in two 6-well plates, as diagramed below in Figure 3. Seeding

each 6-well plate in triplicate may be desired. Use 2 ml of MEF medium per well for the seeded IMR-90 cells. **Plate 1** will be used for transductions with lentivirus stocks encoding the six pluripotency factors to generate iPS cells. **Plate 2** will be used for transductions with viPS-EF1 α -TurboGFP control lentivirus to assess transduction efficiency (labeled GF in plate 2). Allow at least one well to be used as a “non-transduced” control (labeled CNT in plate 2).

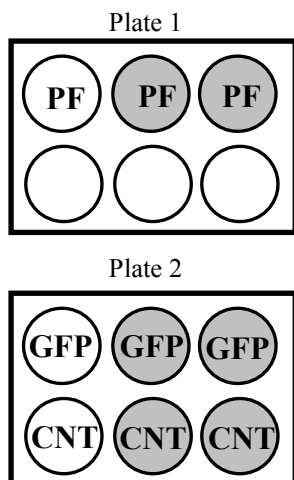


Plate 1: IMR-90 cells plated in triplicate (shown here as an example; plate in single well, duplicate or triplicate, as desired) for transduction at 1.25×10^5 cells/well (see Day 2) with lentivirus encoding 6 pluripotency factors (each delivered at an MOI of 10). PF = Pluripotency Factor

Plate 2: IMR-90 cells plated in triplicate for transduction at 1.25×10^5 cells/well (see Day 2) with pVIPS-EF1 α -TurboGFP control lentivirus at an MOI of 10 plus non-transduced control in triplicate. GFP = Green Fluorescent Protein, CNT = Control

Figure 3: Plating of target cells Day 1.

Day 2 (prepare to perform transduction 18 hours after plating target cells):

1. Prepare Virus Transduction Medium (MOI of 10 per virus); see Equation 1.

1.1. Pluripotency Factors (Plate 1):

- 1.1.1. Calculate the volume of each lentivirus stock (6 stocks for each of the 6 pluripotency factors) needed to deliver each pluripotency factor at a multiplicity of infection (MOI) of 10 per well for 1.25×10^5 cells/well (see Equation 1 below). Target cells should be transduced with lentivirus for all six pluripotency factors simultaneously (Oct4, Nanog, Sox2, Klf4, Lin28, c-Myc) for a total MOI of 60 delivered to target cells.
- 1.1.2. Label a 15 ml tube “PF” for the virus transduction medium. If transduction is to be conducted in triplicate, three 15 ml tubes will be required, one 15 ml tube per well of target cells.
- 1.1.3. Add 1.0 ml of MEF medium to each 15 ml tube labeled “PF” for the IMR-90 cells.
- 1.1.4. For each pluripotency factor (Oct4, Nanog, Sox2, Klf4, Lin28, c-Myc), thaw 1 vial of lentivirus stock and place on ice. Keep virus on ice and work quickly. Before removing the cap, quickly spin the vial in a microfuge.

- 1.1.5. Add the appropriate volume of each virus stock for a MOI of 10 per virus (6 stocks for each of the 6 pluripotency factors) to each 15 ml tube labeled “PF” for the IMR-90 cells (see Figure 4 below).
- 1.1.6. Mix virus transduction medium in the 15 ml tubes by pipetting.
- 1.1.7. Remove the medium on the plated cells by aspiration. Wash with 1 ml per well of PBS⁺⁺.
- 1.1.8. Aspirate PBS⁺⁺. Add the virus transduction medium in the 15 ml tubes labeled “PF” to the IMR-90 cells on **Plate 1** (1.0 ml of virus transduction medium per well of target cells).

1.2. viPS-EF1 α -TurboGFP control lentivirus (Plate 2):

Calculate the volume of GFP control lentivirus stock needed for a multiplicity of infection (MOI) of 10 per well for 1.25×10^5 cells/well (see Equation 1 below).

- 1.2.1. Label a 15 ml tube “GFP” for the GFP control virus transduction medium. If transduction is to be conducted in triplicate, three 15 ml tubes will be required, one 15 ml tube per well of target cells.
- 1.2.2. Add 1.0 ml of MEF medium to each 15 ml tube labeled “GFP” for the IMR-90 cells.
- 1.2.3. Thaw 1 vial of viPS-EF1 α -TurboGFP control lentivirus and place on ice. Keep virus on ice and work quickly. Before removing the cap, quickly spin the vial in a microfuge.
- 1.2.4. Add the appropriate volume of viPS-EF1 α -TurboGFP control lentivirus stock for an MOI of 10 to the 15 ml tube labeled “GFP” for the IMR-90 cells (see Figure 2 below).
- 1.2.5. Mix virus transduction medium in the 15 ml tubes by pipetting.
- 1.2.6. Remove the medium on the plated cells by aspiration.
- 1.2.7. Add the virus transduction medium in the 15 ml tube labeled “GFP” to the IMR-90 cells on Plate 2 (1.0 ml of virus transduction medium per well of target cells).
- 1.2.8. Incubate the cells with virus for 24 hours at 37°C, 5% CO₂.

Notes:

- Calculating MOI is based on the number of target cells at the time of transduction, as well as titer of the virus (TU/ml). The above example is given for IMR-90 cells (with an approximate doubling time of 36 hours) to be transduced 18 hours after plating. For other target cell lines with a faster proliferation rate, it may be necessary to do cell counts in parallel to determine if a significant change in cell number from the initial plating density should be used to calculate
- Approximately enough virus stock is supplied per aliquot (20 μ l/vial) to conduct experiments in triplicate at an MOI of 10 for 2 cell lines at 1.25×10^5 cells/well; 3 vials will allow for 3 independent experiments. See notes above regarding freezing aliquots of leftover virus.

- The MOI calculation is carried out using an average virus titer of 5×10^8 TU/ml (Equation 1). Please see the Certificate of Analysis for the actual titers. For carrying out the reprogramming experiment, you can follow the instructions using the average titer of 5×10^8 TU/ml or recalculate using the actual titers based on Equation 1.

Day 3:

1. 24 hours post transduction, aspirate the culture medium and wash with 1 ml of PBS⁺⁺.
 - 1.1. For the Plate 1 cultures transduced with the pluripotency vectors (Oct4, Nanog, Sox2, Klf4, Lin28, c-Myc), proceed with Day 3, step 2 of the protocol.
 - 1.2. For viPS-EF1 α -TurboGFP control transduction Plate 2, aspirate PBS⁺⁺ and add 3 ml per well of MEF medium to the IMR-90 cells and place in 37°C, 5% CO₂ incubator. Monitor GFP fluorescence over the next 72 hours and analyze transduction efficiency as desired (i.e. by visual observation or flow cytometry).
2. Aspirate PBS⁺⁺, add 2 ml per well of MEF medium to the transduced IMR-90 cells.

B. Transfer of transduced target cells onto mouse embryonic fibroblast cells (MEFs)

Day 4:

1. Thaw inactivated MEFs into a 10 cm dish (7.2×10^6 MEFs/10cm dish), or alternatively into six 35 mm dishes (1.2×10^6 MEFs/35 mm dish). Use 10 ml of MEF medium per 10 cm dish or 2 ml of MEF medium per 35 mm dish.

Day 5:

1. Change MEF medium on MEFs. Use 10 ml of MEF medium per 10 cm dish or 2 ml of MEF medium per 35 mm dish.
2. Change MEF medium on transduced IMR-90 cells (2 ml per well).

Day 6:

1. Aspirate MEF medium from transduced IMR-90 cells and wash with 1 ml of PBS⁺⁺.
2. Aspirate PBS⁺⁺, add 1 ml per well of 0.05% trypsin/0.53 mM EDTA, and incubate at 37°C for 4 min.
3. Add 3 ml of the MEF medium, gently pipette cells from the well and transfer to individual 15 ml tubes (1 per cell type).
4. Centrifuge cells (200 x g), remove medium by careful aspiration and resuspend each tube to a single cell suspension in 3 ml of fresh iPS medium.
5. Count the number of cells and adjust the concentration to 12,500 cells per ml for IMR-90 cells.

Note: The concentration of transduced IMR-90 cells seeded onto MEFs is very significant. Seeding too high a density of transduced IMR-90 cells will result in overgrowth of granular non-iPS cell colonies and will obstruct the ability to distinguish high quality iPS cell colonies with morphologies more closely resembling human embryonic stem (hES) cells. Alternatively, a range of concentrations of transduced IMR-90 cells can be seeded onto the

six 35 mm dishes to determine the optimum concentration for generating iPS cell colonies most closely resembling hES cell morphology.

6. Transfer 10 ml of cell suspension from each tube to a single 10 cm dish of inactivated MEFs (7.2×10^6 MEFs/10cm dish; prepared on Day 4 above). Alternatively, seed a range of concentrations of transduced IMR-90 cells onto the six 35mm dishes of inactivated MEFs previously prepared.

Day 7:

1. **IMPORTANT:** do not change the medium on dishes the day after seeding the transduced IMR-90 cells on MEFs.

Day 8 – Day 30 (Daily Maintenance):

1. Grow the transduced IMR-90 cells on MEFs in a 37°C, 5% CO₂ incubator, changing the medium every day with fresh iPS medium until the colonies are big enough to be picked up and passaged. Feed the cells at the same time every day. Watch for colony formation 7-30 days post seeding transduced IMR-90 cells on MEFs. They should become large enough to be picked up around day 20.

Note: Observe your cultures daily. Colonies may become large enough to passage before or after day 20.

C. Picking initial colonies

Note: Pasteur pipettes must be autoclaved prior to use.

1. Pasteur Pipette Preparation (be sure to follow all standard laboratory safety precautions).
Hook for sectioning:
 - 1.1. Prepare hook by heating glass Pasteur pipette on a low flame using a Bunsen burner and pulling it out to stretch the glass by 3 inches.
 - 1.2. Place the thinned area of the pipette above the flame, and pull glass apart slowly. This should form a bend in the glass that resembles a hook (Note: only touch portions of glass not in contact with the flame).
 - 1.3. Place pipette under sterile biological safety cabinet until ready to use. At no point should the center of the pulled glass be touched by anything. After flaming and pulling, the pipette is considered sterile.

Day 1:

1. Once the colonies begin to form, thaw inactivated MEFs into 6 x 35 mm dishes in MEF medium (1.2×10^6 MEFs/35mm dish).

Day 3:

1. Observe dishes daily. When the colonies are large enough to isolate (see Figure 9 “Controls and Validation” section), feed the colonies in the 10 cm dishes with 10 ml fresh iPS medium 1-2 hours before passaging.
2. Using 35 mm inactivated MEF dishes aged 2 days (from Day 1, Step 1), aspirate MEF medium, wash once with PBS⁺⁺, and replace with 2 ml of iPS medium 1-2 hours before passaging.
3. Under a dissection microscope, carefully cut colonies on the original 10 cm dishes into smaller pieces (approximately 5-10 pieces per colony) using the hook on the Pasteur pipette. It is better to cut colonies into small pieces rather than large pieces. Overly large pieces may roll up, ball or clump too much and form more differentiated colonies on the new dish. Avoid any “bad” areas of differentiated cells, leaving them attached to the dish. Good colonies will be spherical in shape with a more defined outer border and a translucent, light-refractive appearance compared to the surrounding MEFs. From the 10 cm dishes, passage as many colonies as possible that resemble human embryonic stem (hES) cell colonies in morphology (see Tips for Selecting Reprogrammed Colonies). Once the colonies have been cut into the desired pieces, use a micropipette to transfer the pieces to the pre-equilibrated 35 mm MEF dishes. (If possible, aim to have approximately 30-60 pieces per 35 mm dish to generate new colonies.)
4. To ensure even distribution of iPS cells, move dish from side to side and up and down on a flat surface. Avoid swirling as cell clusters are sticky and will clump together.
5. Place new iPS cell dish in 37°C in 5% CO₂. Once on shelf, repeat side to side and up and down motion.

D. Manual passaging and propagation**Day 1 after plating on 35 mm dishes:**

1. Colonies which were broken up and plated onto new 35 mm dishes as described in Section C (picking initial colonies) should plate down and start to form new colonies. Observe to make sure that this is true. Do not change the medium the day after passaging.

Day 2 after plating on 35 mm dishes:

1. Change medium (2 ml per 35 mm dish) on the 35 mm dishes which contain the iPS cell colonies with fresh iPS cell medium.
2. Thaw sufficient MEFs for the next passage into 6 x 35 mm dishes in MEF medium (1.2 x 10⁶ MEFs/35mm dish).

Day 3-4 after plating on 35 mm dishes:

1. Change the medium on the colonies daily with fresh iPS medium. Change the medium on the iPS cell colonies at the same time each day. When the colonies are large enough to isolate (see Figure 9 in the “Controls and Validation” section) feed the colonies on the 35 mm dishes 1-2 hours before passaging.
2. Using 35 mm MEF dishes aged 2-4 days, aspirate MEF medium, wash once with PBS⁺⁺, and replace with 2 ml of iPS medium 1-2 hours before passaging.
3. Prepare a Pasteur pipette as described in section C.
4. Under a dissection microscope, carefully cut iPS cell colonies into approximately 5-10 pieces per colony using the hook on the Pasteur pipette. Avoid any “bad” areas of differentiated cells leaving them attached to the dish. Good colonies will be spherical in shape with a more defined outer border and a translucent, light-refractive appearance compared to the surrounding MEFs. It is better to cut colonies into small pieces rather than large pieces. Overly large pieces may roll up, ball or clump too much and form more differentiated colonies on the new dish. Passage 10-20 colonies for each new 35 mm dish.
5. Once the iPS cell colonies have been cut into the desired pieces, use a micropipette to transfer the pieces to the pre-equilibrated MEF dish. (Aim to have approximately 30-60 pieces per 35 mm dish to generate new colonies.)
6. To ensure even distribution of iPS cells, move dish from side to side and up and down on a flat surface. Avoid swirling as cell clusters are sticky and will clump together.
7. Place new iPS cell dish in 37°C in 5% CO₂. Once on shelf, repeat side to side and up and down motion.

Note: Repeat section D for additional propagation.

Equation 1: To deliver a multiplicity of infection (MOI) of 10 per virus stock per well.

MOI = Number of transducing units (infectious virus particles) per target cell.

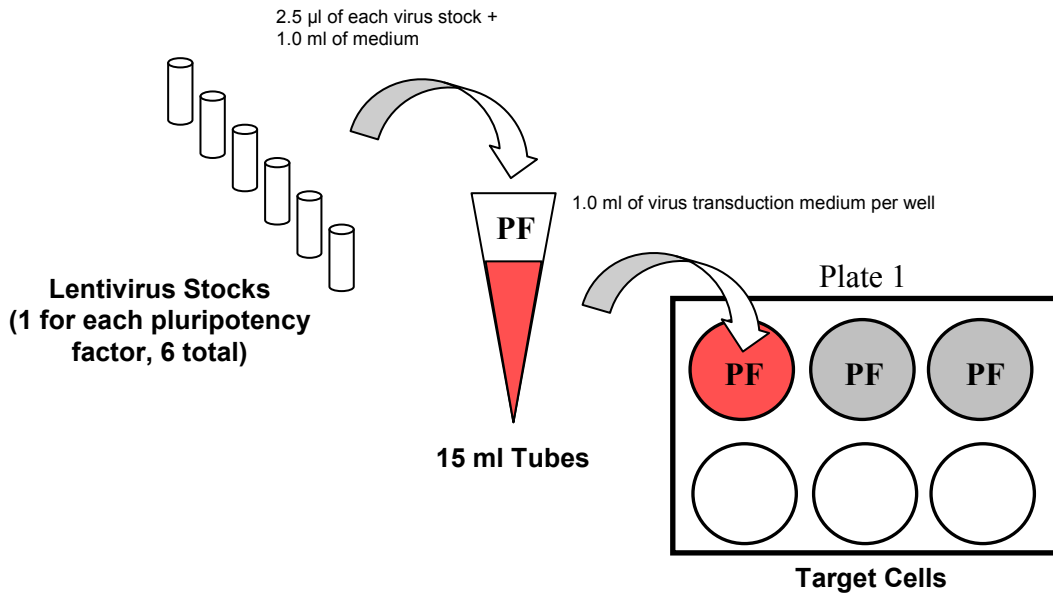
Example Titer of Virus Stock: 5×10^8 TU/ml = 5×10^5 TU/ μ l

Number of target cells (IMR-90) per well (6-well plate) at the time of transduction = 1.25×10^5 cells

1.25×10^5 cells x MOI of 10 = 1.25×10^6 total TU required.

1.25×10^6 total TU required / 5×10^5 TU/ μ l virus stock = 2.5 μ l of each virus stock required per well.

Example Single Well Transduction with Pluripotency Factors (please adjust your experiment accordingly for carrying out transductions in duplicate or triplicate)



Example Single Well Transduction with viPS -EF1 α -TurboGFP Control Virus

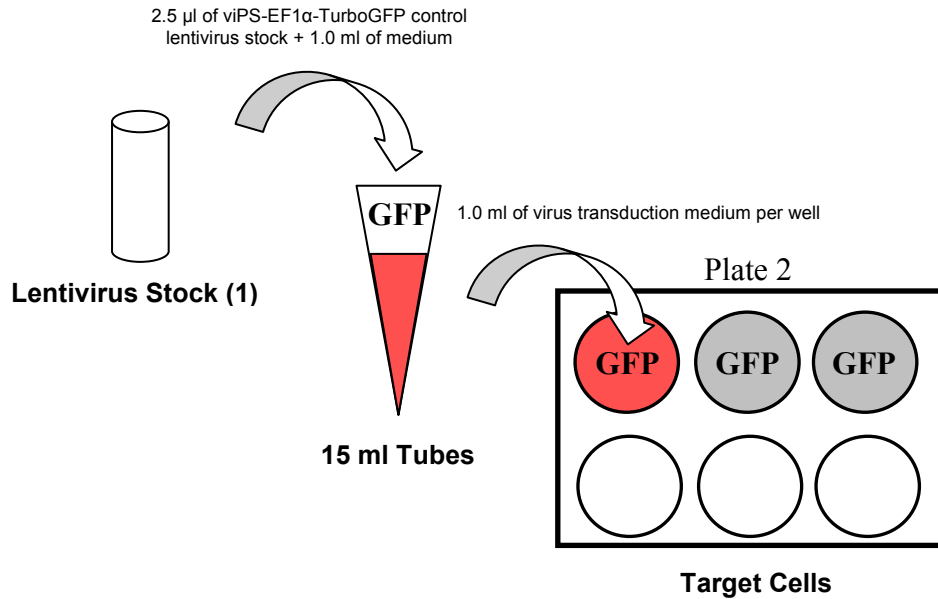


Figure 4: Transduction of target cells Day 2.

TIPS FOR SELECTING REPROGRAMMED COLONIES

- 1) Observe your colonies closely and be very selective about choosing reprogrammed, high-quality iPS cell colonies to expand from the original MEF dish and analyze. Reprogramming efficiencies are expected to be low, less than 1%. Reprogramming efficiency (%) can be calculated as the number of iPS cell colonies initially generated divided by the number of transduced cells seeded onto MEFs x 100.
- 2) Morphological characteristics to look for in fully reprogrammed iPS cell colonies include:
 - a. Shape of colonies: truly pluripotent colonies will be very spherical in shape, not irregular. The rounder the better.
 - b. Colony definition: pluripotent colonies will be immediately distinguishable from the surrounding MEFs with well-defined borders.
 - c. Individual cell properties: individual cells within a pluripotent colony will be irregular in shape with well-defined borders. Individual cells will also have a high nuclear to cytoplasmic ratio.
 - d. Architecture: pluripotent colonies will have more of a 3D multi-layer architecture and will not be a single monolayer of cells.
 - e. Color: pluripotent colonies will appear “whitish” in color against the surrounding MEFs, with a very translucent, light-refractive appearance.
- 3) iPS cell colonies generated from transduced IMR-90 cells should proliferate at a very similar rate to hES cell colonies (approximately 24 hours doubling time). Thus iPS cell colonies will need to be passaged every 3-4 days.
- 4) Growth of irregular and granular colony-like structures may emerge first after transferring transduced IMR-90 cells to inactivated MEFs. These colonies are not iPS cells.
- 5) When in doubt about selecting initial iPS cell colonies after transduction and transfer to MEFs, it is better to select and passage a potential colony earlier rather than waiting. Waiting to passage may possibly lead to non-iPS cell colonies overgrowing and obstructing the ability to distinguish high quality iPS cell colonies most resembling hES cell colonies.
- 6) When passaging iPS cell colonies, they should come apart in “sticky” pieces. Colonies that are granular and easily shred into single cells when passaging are not iPS cells, even though these colonies may be very spherical in shape.
- 7) Avoid flat, monolayer colonies grayish in color. These are not iPS cells.

REFERENCE FIGURES

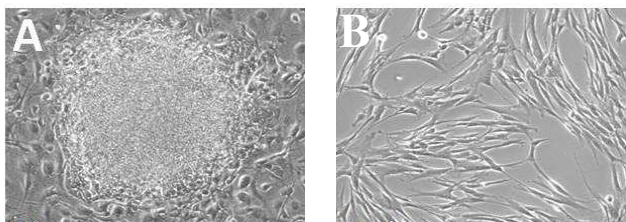


Figure 5: Morphology of non iPS cells (phase contrast images, 10X).

- A: A colony of human fibroblasts on feeders.
- B: IMR-90 cells prior to transduction.

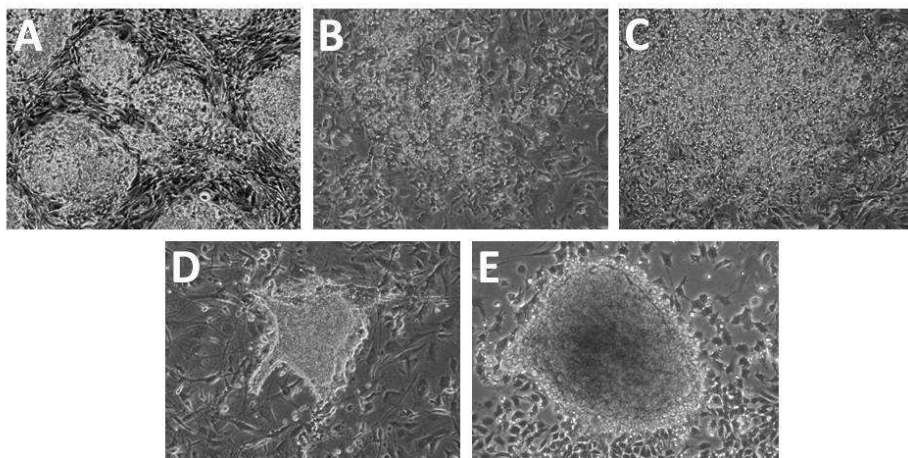


Figure 6: Undesirable morphologies to avoid (phase contrast images, 10X).

- A: Overgrowth and overcrowding of colony-like structures.
- B-C: Loose growth of cells on feeders, non colony-like structures.
- D: Irregularly-shaped colony-like structure.
- E: Colony-like structure with beginnings of a necrotic core and resemblance to a differentiating embryoid body.

ANTICIPATED RESULTS

By following this protocol, one can expect iPS cell colonies begin to appear as early as 10 – 13 days post seeding the transduced IMR-90 cells onto inactivated MEFs. By 13-21 days post seeding on MEFs, the iPS cell colonies should be large enough to passage. These iPS colonies then have to be appropriately maintained as outlined in the protocol above. The isolated colonies will appear morphologically very similar to hES cells. In addition, one can check for the upregulated expression of all six pluripotency factors using qPCR (see Table 3 for a list of recommended ABI TaqMan assays) in comparison to non-transduced IMR-90 cells. We also

recommend immunostaining, alkaline phosphatase staining (Vector Red Alkaline Phosphatase Substrate Kit I from Vector Laboratories, Inc (Burlingame, CA); Catalogue Number: SK-5100) and assessing the developmental potential of your cells using standard protocols (West *et al.*). Anticipated results for these additional tests to assess pluripotency can be found in the next section.

Table 3: ABI Gene Expression Assay number

Gene Symbol	ABI assay number
c-Myc	Hs99999003_A1
Lin28	In-house design
Nanog	Hs02387400_g1
Klf4	Hs00358836_A1
Oct4	Hs03005111_g1
Sox2	In-house design

CONTROLS AND VALIDATION

Controls

The control vector viPS-EF1 α -TurboGFP is provided as a tool to assess general transduction efficiency of the target cell line.

Validation

1. Transduction efficiency of IMR-90 human lung fibroblasts (Figure 7)

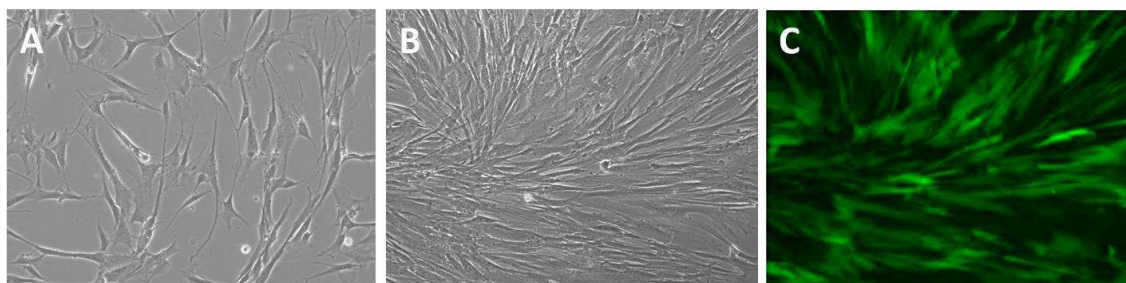


Figure 7: IMR-90 human lung fibroblasts (A) before lentiviral transduction and (B) 72 hours post transduction with lentiviral vectors encoding 6 pluripotency factors – Oct4, Nanog, Sox2, Lin28, Klf4, and c-Myc (phase contrast images, 10X). IMR-90 cells show greater than 70% transduction efficiency (C) when co-transduced with Thermo Fisher's control viPS-EF1 α -GFP lentiviral vector encoding green fluorescent protein (GFP), fluorescent image (10X).

2. Expression levels of all six pluripotency factors following transduction and comparison to control expression (Figure 8)

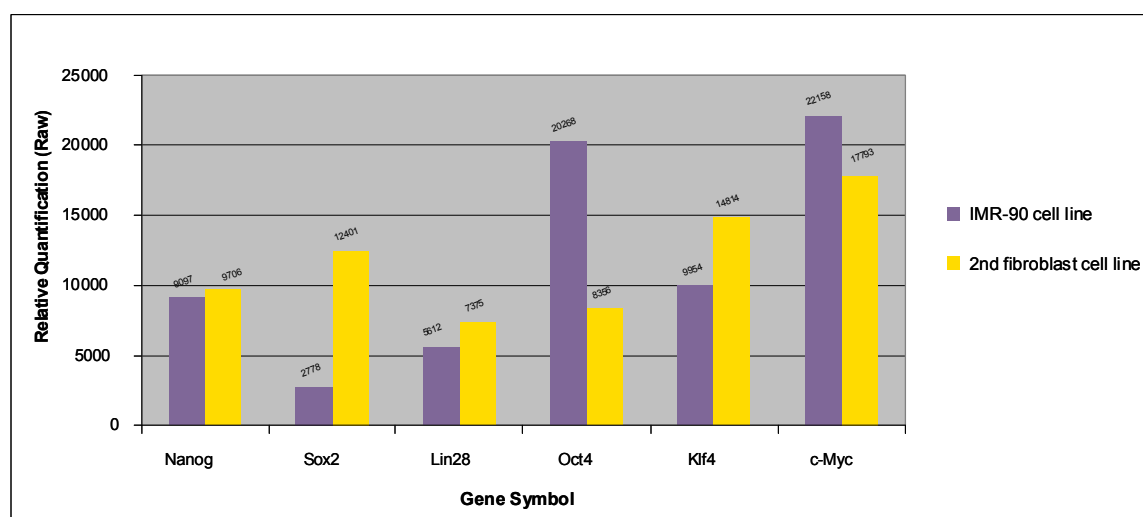


Figure 8: Relative gene expression in transduced IMR-90 and a second fibroblast cell line; relative gene expression levels in non-transduced controls for all six genes are low or undetectable (data not shown)

3. IMR-90-derived induced pluripotent stem cell colonies resemble human embryonic stem cell colonies and maintain GFP expression (Figure 9).

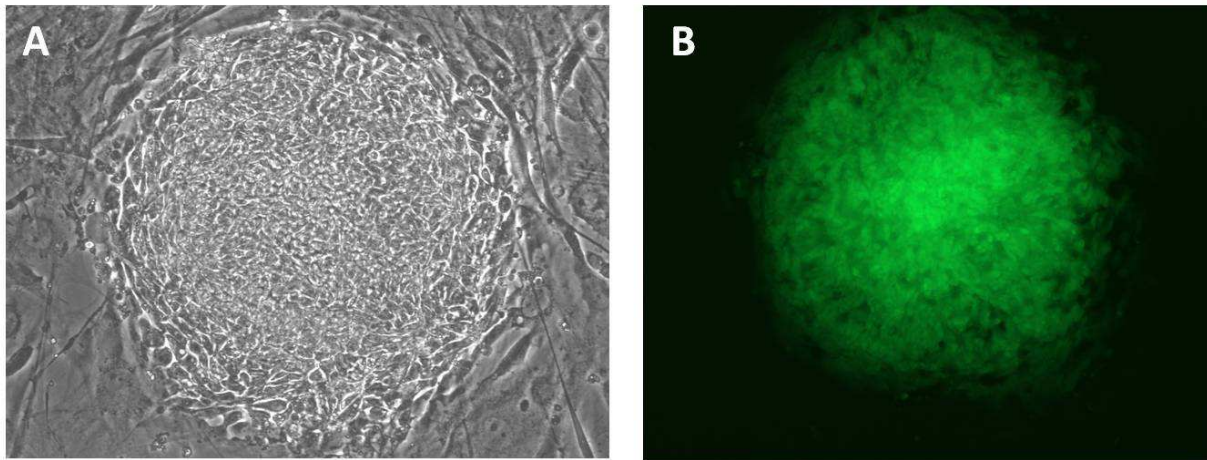


Figure 9: Transduced IMR-90 cells cultured on mouse embryonic fibroblasts (MEFs) form colony-like structures that (A) resemble hES cell colonies. iPS cell colonies maintain GFP expression (B) for at least 10 passages (phase contrast image 20X, fluorescent image 10X).

4. iPS cell colonies resemble hES cell colonies in morphology (Figure 10).

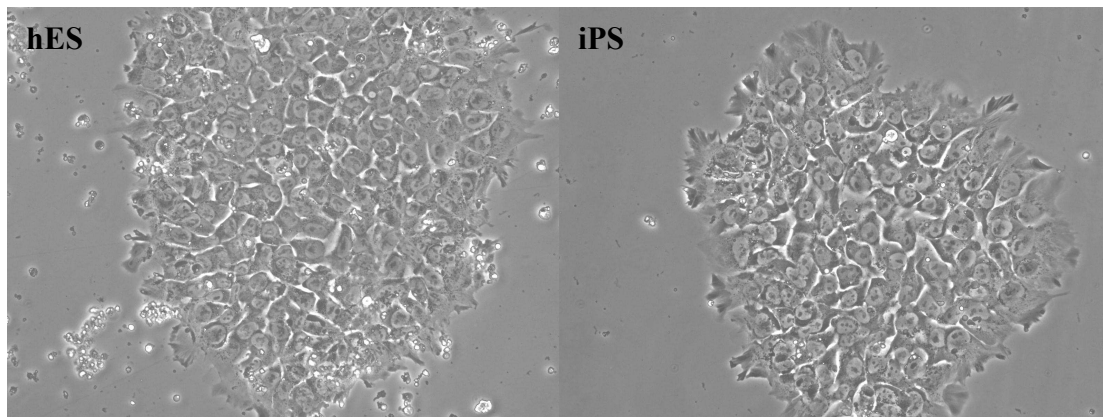


Figure 10: iPS cells resemble hES cell in morphology (phase contract, 20X). Cultures grown on Matrigel.

5. Alkaline phosphatase staining (Figure 11).

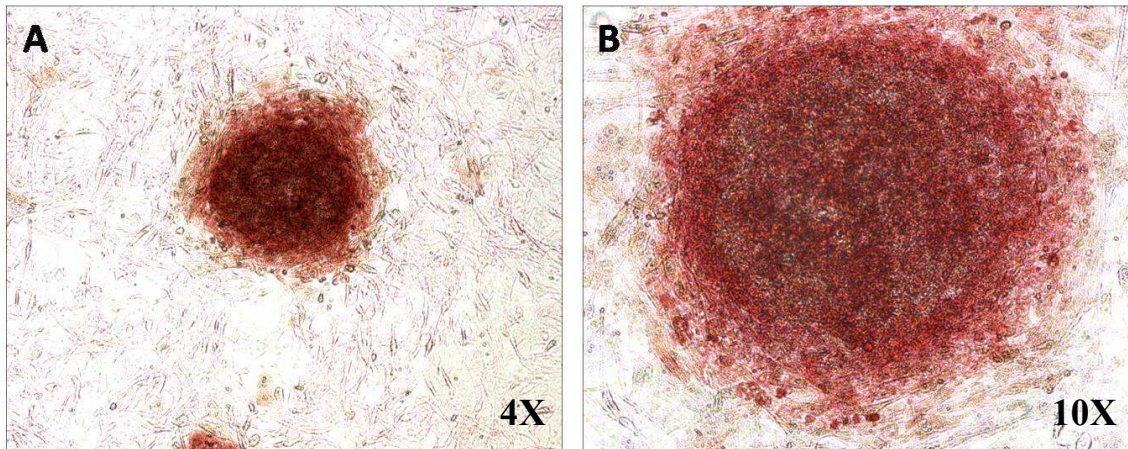


Figure 11: iPS cell colonies cultured on MEFs stain positive for alkaline phosphatase, an indicator of pluripotency (brightfield; 4X, 10X).

6. Immunostaining for pluripotency factors (Figure 12).

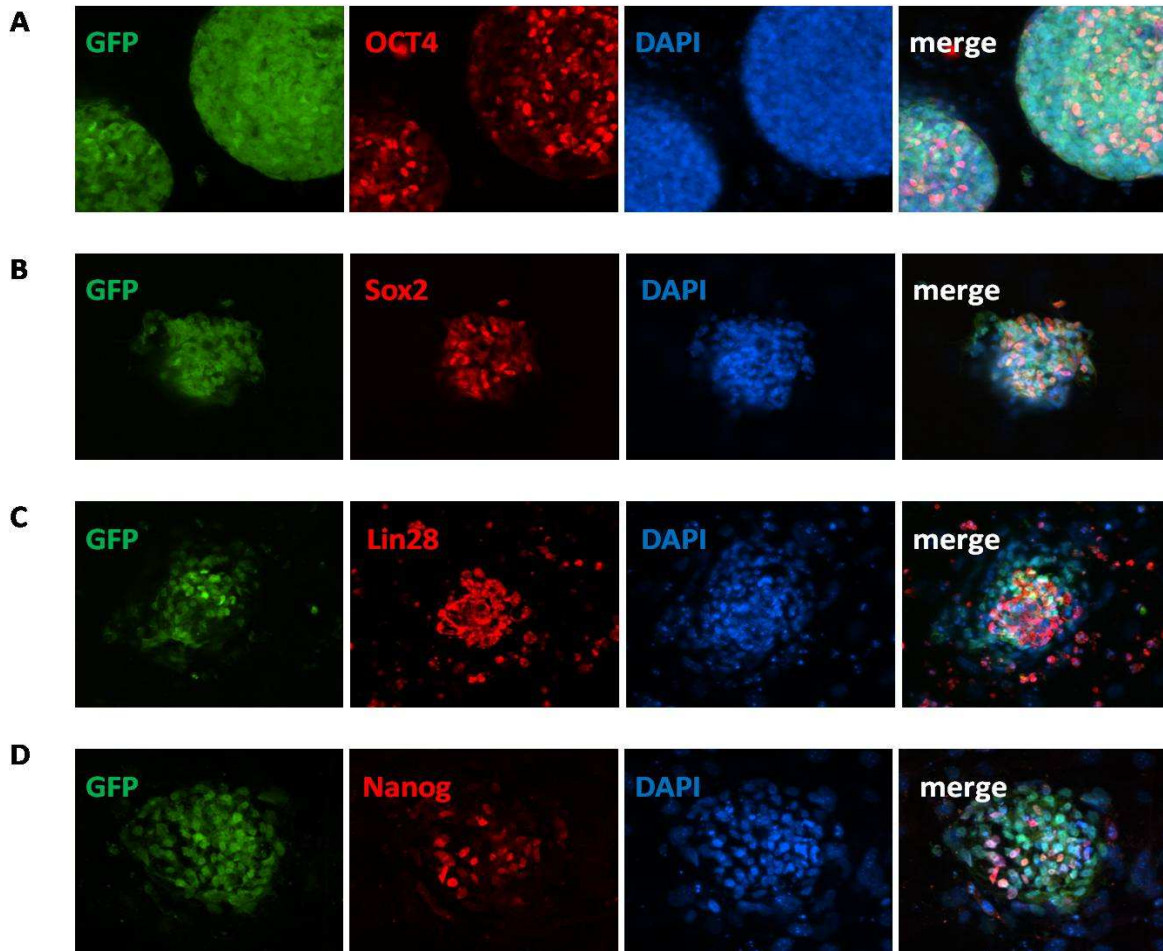


Figure 12: Nonclonal iPS cells demonstrate protein expression of the pluripotency factors following lentiviral transduction. Immunocytochemistry for (A) Oct4, (B) Sox2, (C) Lin28, and (D) Nanog (20X). Nuclei were stained with DAPI.

7. iPS cells stain positive for SSEA4 (Figure 13).

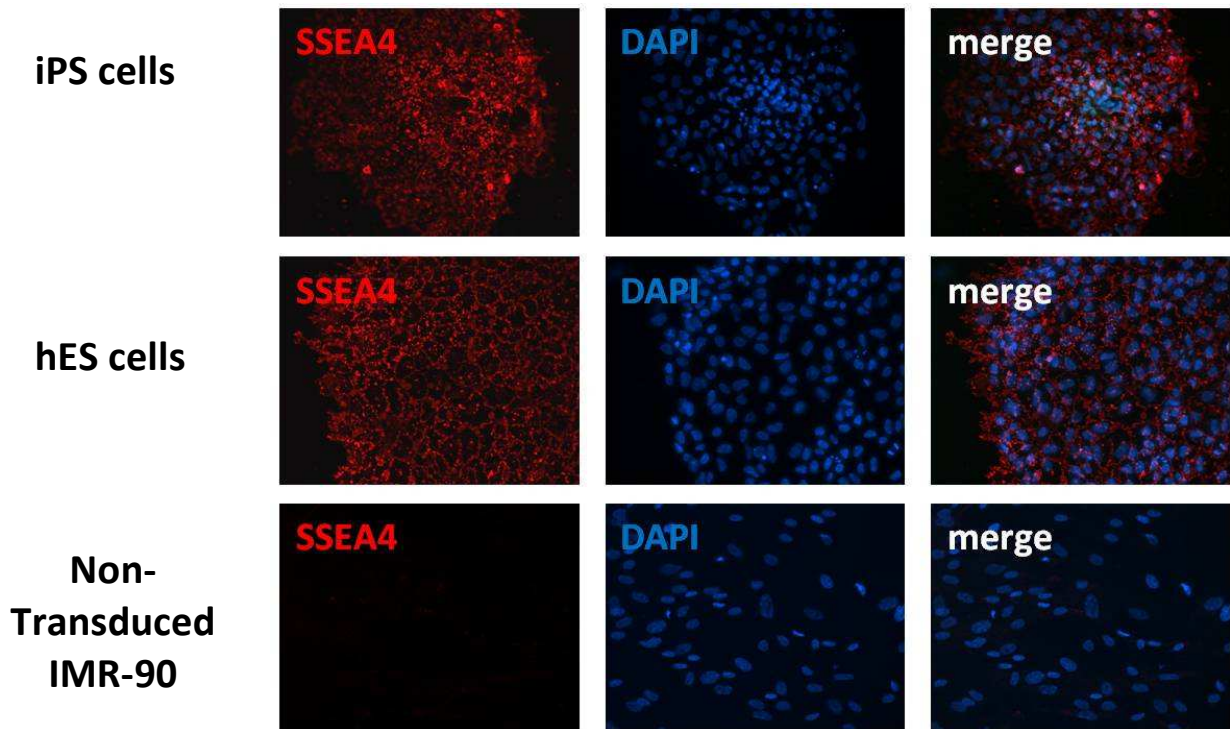


Figure 13: Nonclonal iPS cells demonstrate protein expression of the pluripotency marker SSEA4 similar to hES cells. Nuclei were stained with DAPI (fluorescent images, 20X).

8. Differentiation of iPS cell colonies (Figure 14).

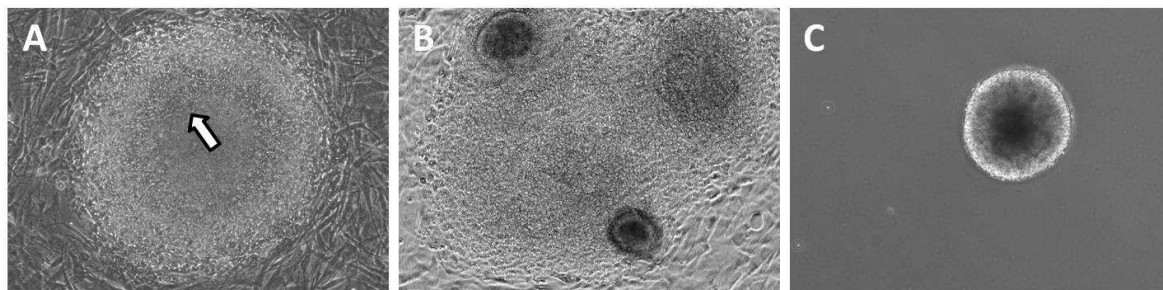


Figure 14: iPS cell colonies cultured on MEFs show spontaneous differentiation either (A) in the center of the colony or (B) in close proximity with other colonies (phase contrast, 10X). iPS cells can also form (C) suspension embryoid bodies (embryoid body, day 7, phase contrast, 10X).

9. iPS cell differentiation (Figure 15).

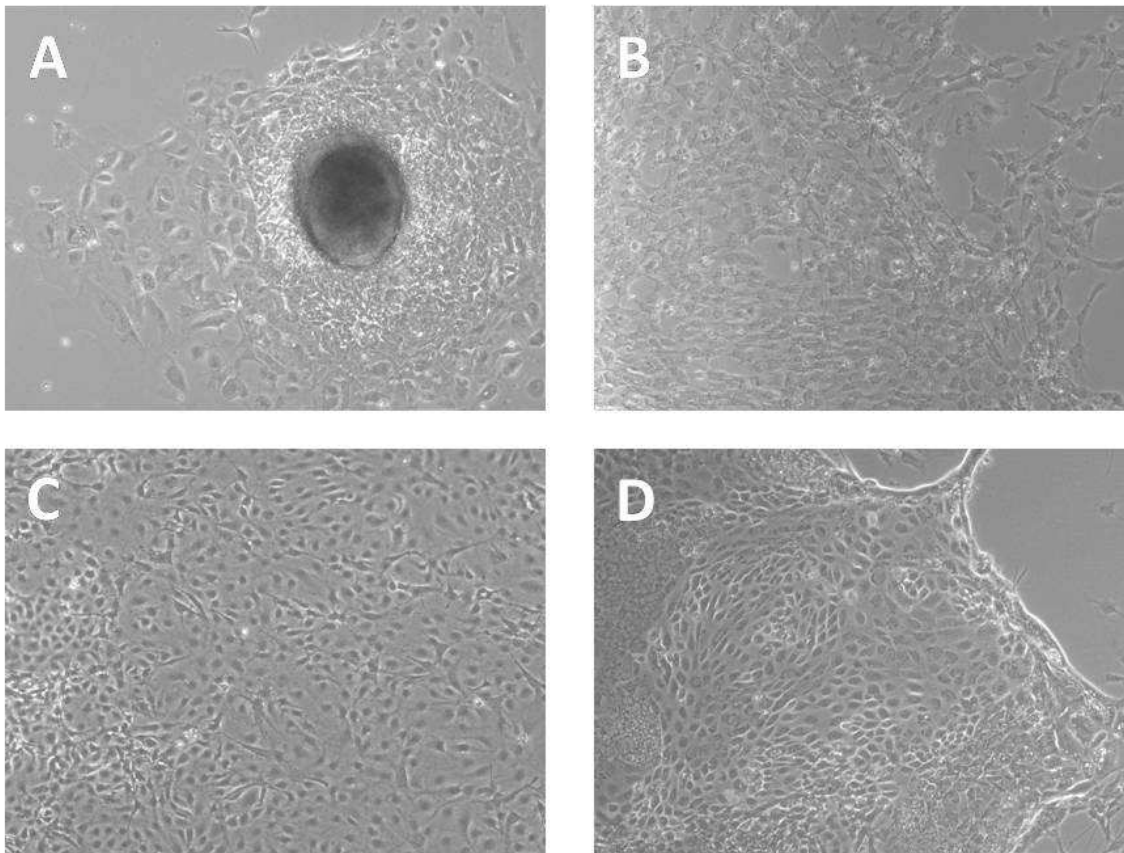


Figure 15: iPS cells randomly differentiate into various cell types when embryoid bodies are plated on gelatin. Differentiated iPS cells grow out from the plated embryoid body (A) and show (B) neuron-like, (C) epithelial-like, and (D) cobblestone-like morphologies (phase contrast, 10X).

10. GFP expression decreases in iPS cell differentiation (Figure 16).

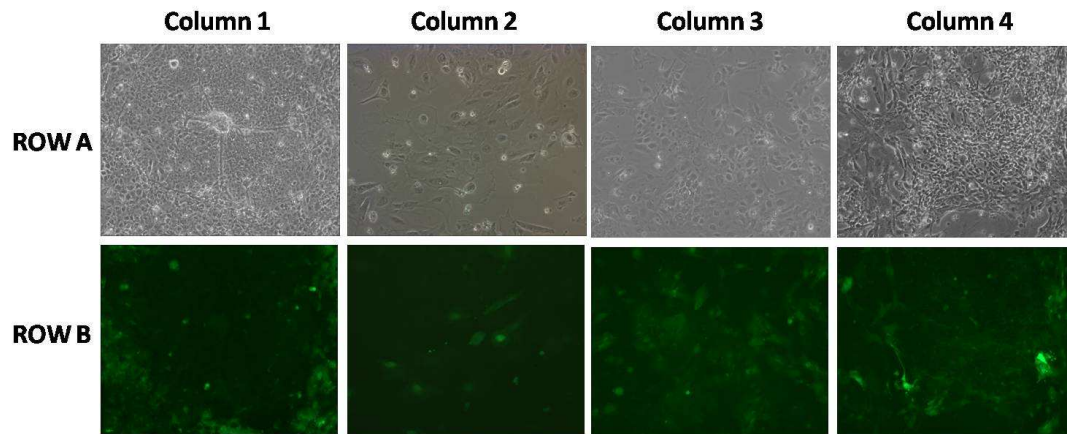


Figure 16: GFP expression decreases during differentiation of iPS cells (Row A phase contrast, Row B corresponding fluorescent images, 10X). Differentiated iPS cells in Column 1 show a neural-like central region with almost no GFP expression surrounded by epithelial-like cells expressing some GFP. Columns 2-4 show differentiated iPS cells expressing low levels of GFP.

11. Differentiated iPS cells express markers from all three germ layers (Figure 17).

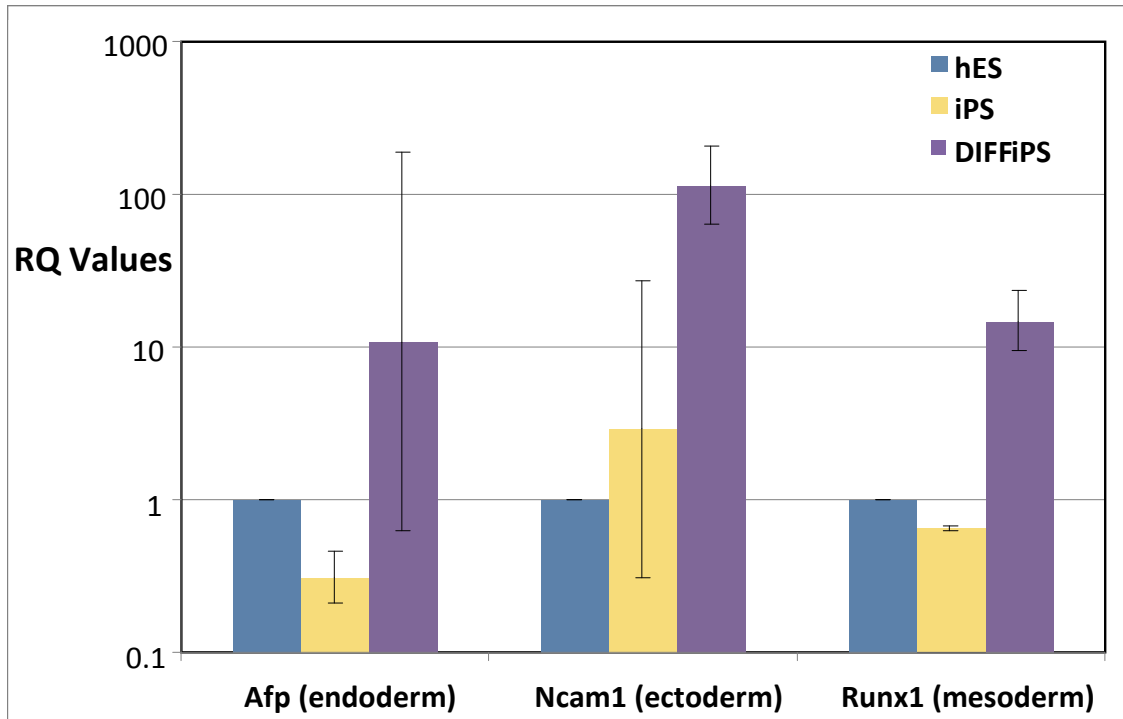


Figure 17: qPCR demonstrated that iPS cells differentiated (DIFFiPS) by embryoid body formation and culture on gelatin expressed higher levels of germ layer markers (Afp, Ncam1, Runx1) than non-differentiated hES cells (hES) and iPS cell colonies (iPS).

FAQS

For answers to questions that are not addressed here, please email technical support at info@openbiosystems.com with your question, your sales order or purchase order number and the catalog number of the product with which you are having trouble.

1. I do not detect upregulation of the pluripotency factors following transduction.

You may be experiencing poor transduction efficiency. You may need to optimize your transduction protocol if you are not using IMR-90 cells.

2. My cells show signs of toxicity following transduction of the pluripotency vectors.

Target cell viability may be poor for some cell types because the MOI for transductions is too high. High concentrations of viral particles can be toxic to certain cell lines, possibly due to the VSV-G envelope present on the surface of the viral particles. Overexpression of one or more of the pluripotency factors may also lead to toxicity. Though we have not observed toxicity using IMR-90 cells at the MOI suggested in the protocol, other cell types may be more efficiently transduced and the EF1 α promoter more active, leading to higher levels of factor expression. If toxicity is seen:

- a. Try reducing the MOI for transductions
 - b. Decrease the time of transduction to 6-10 hours
3. I have managed to make colonies, but am not sure which are truly pluripotent-like and which I can discard. What should I do?

Growth of irregular and granular colony-like structures may emerge first about 1 week after transferring transduced IMR-90 cells to inactivated MEFs. These colonies should not be selected as iPS cell colonies. Also avoid flat, monolayer colonies grayish in color. Colonies with these characteristics are not iPS cells. IMR-90-derived iPS colonies that are truly pluripotent-like will closely resemble human embryonic stem (hES) cell colonies. Please see details in the product manual for descriptions of characteristics demonstrated by pluripotent cells.

4. What else do I do besides look at morphology to determine if the colonies are pluripotent?

Measures to ascertain pluripotency in iPS cells are the same that have been well-documented for human embryonic stem cells which include:

- a. *In vitro*:
 - i. Self-renewal - Proliferation rates similar to hES cells
 - ii. Alkaline phosphatase activity
 - iii. SSEA4, Tra-1-60 and Tra-1-81 surface protein expression
 - iv. Endogenous Oct4, Nanog and Sox2 expression
 - v. Ability to spontaneously differentiate
 - vi. Ability to form embryoid bodies (EBs)

- vii. Once randomly differentiated, demonstrate expression of markers for all 3 germ layers
 - b. *In vivo*:
 - i. Form teratomas following implantation in mice
 - ii. Generate chimeric mice
- 5. My iPS cells do not stain positive for alkaline phosphatase activity or SSEA4 expression.

You may need to select alternative colonies to propagate and analyze, as the ones chosen are not pluripotent. If the original dish of transduced cells transferred to MEFs is not available, transductions may need to be repeated.

- 6. My transduction efficiency with the viPS-EF1 α -TurboGFP is about 60% - what should I do?

This is borderline as we recommend a 70 % transduction efficiency. You can go ahead but have to be aware you will have fewer colonies to choose from eventually and this may lead to a failure to retrieve reprogrammed colonies. As an alternative you may want to try another cell line.

- 7. I am experiencing poor transduction efficiency with the control viPS-EF1 α -TurboGFP virus.

Reasons for poor transduction efficiency may include:

- a. Improper storage of lentivirus stocks leading to their inactivity: Check to ensure all virus stocks are stored at -80°C.
- b. If not using IMR-90 cells, transduction protocol may not be optimal.
 - i. Try increasing MOI
 - ii. Try keeping the same MOI, but reducing the total volume of virus transduction medium added to cells to 0.5 ml/well of 6-well plate
 - iii. Try adding polybrene to the virus transduction medium (2-12 μ g/ml)
 - iv. Try centrifuging transduced cells at 1100 x g for 10 min
 - v. Try using serum-free medium for the transductions
- 8. I only got 50% recovery from the frozen feeder cells (catalogue # IPS5445), what should I do?

Feeder density used for culturing pluripotent stem cells varies from lab to lab. Feeders at a density as low as 1×10^6 cells per 10 cm dish have been successfully used to culture human embryonic stem (hES) cells (this is the density used by the Harvard Stem Cell Institute (HSCI): www.hsci.harvard.edu). So, potentially, you could still use the feeder cells even if you got 50% recovery. However, we cannot assure the generation of iPS cells using feeders at a lower density.

The other option would be to reevaluate your thawing technique and purchase more feeder cells to replace those thawed (see catalogue # IPS5445, 3 vials).

9. If I am worried about lentivirus, especially with the factors this kit carries, how safe am I?

The SinLTR is one of the fail-safe's we use. The viral particles also do not carry all the elements needed to be infectious outside of the experimental cell line. Having said this, all precautions should be taken including the use of a BSL2 facility (see also recommendations in product manual).

10. I want to reprogram cells other than fibroblasts. Do you have protocols available?

At this point we only have specific protocols available for reprogramming the lung fibroblast cell line IMR-90.

11. I know that my cell line does not allow for expression from the EF1 α promoter. What can I do?

We currently offer our viPSTM Vector Kit only in one configuration utilizing the EF1 α promoter. EF1 α expresses in a wide variety of cell lines. You might want to carry out your own test using the viPS-EF1 α -TurboGFP control plasmid to determine the expression level in your cell line of interest based on GFP. Alternatively, you might have to consider working with a different cell line.

12. Do your factors/protocols work for mouse?

This kit has not been validated for use with mouse target cell lines. However, Woltjen *et al.* showed that transfecting human fibroblast cells with a transposon plasmid encoding mouse c-Myc, Klf4, Oct4, and Sox2 and driven by a doxycycline inducible promoter was a successful protocol for generating human iPS cells. This study demonstrates functional conservation of these transcription factors between mouse and human cells. Woltjen, K. *et al.* 2009. Nature, 458 (7239), 766-70.

13. Can you make available the TaqMan primer/probe information for your in-house design?

We have designed our TaqMan primer/probes using Applied Biosystems' File Builder software: (https://www2.appliedbiosystems.com/support/software/assaysbydesign/installs.cfm?prod_id=1542). You can use the same software to design your own TaqMan primer/probes.

14. I see a lot of growth on my dishes, but no iPS-like colonies. What's happening?

Depending on where you are in the iPS cell generation process, this growth can be normal. About one week following transfer of transduced IMR-90 cells onto MEFs, the first growth to appear is often of irregular and granular colony-like structures. Other growth also includes flat, monolayer colonies grayish in color. Colonies with these characteristics are not iPS cell colonies.

If using IMR-90 cells as the target cell line, you will most likely detect true iPS cell colonies about 2 weeks following transfer of transduced IMR-90 cells onto MEFs.

15. Approximately how many colonies should I get? What is reprogramming efficiency?

Reprogramming efficiency for iPS cells is usually defined as the number of pluripotent reprogrammed colonies generated divided by the number of transduced cells plated. If using IMR-90 cells as the target cell line, we anticipate that you can achieve a reprogramming efficiency of 0.03-0.04%. This translates to about 37-50 iPS cell colonies per 125,000 transduced IMR-90 cells transferred to a 10 cm dish of MEFs. Alternatively, this is about 6-8 iPS cell colonies per 20,000 transduced IMR-90 cells transferred to a 35 mm dish of MEFs.

16. I only got 2 colonies after carrying out the experiment, should I proceed to the next step?

Reprogramming efficiencies for generating iPS cells have been consistently low across published studies. It is highly possible you may only generate a few iPS colonies, especially if you are unfamiliar with culturing pluripotent stem cells. The decision to keep colonies should be based on their pluripotent properties rather than how many were generated. If these iPS cells are of high quality and demonstrate pluripotent properties, they can be expanded from a single colony. Given the laborious process required to generate them, discarding the colonies is not recommended. If, however, you desire more than 2 clonal iPS cell lines for study, you will have to repeat the iPS cells process outlined in the product manual to generate more iPS cell colonies.

17. What do differentiated colonies look like?

It is important to distinguish that there are 2 types of differentiation that you may encounter. The first type of differentiated growth may come about one week following transfer of transduced IMR-90 cells onto MEFs to generate iPS cells. Following one week after transfer, irregular, loose, grayish or granular colonies may appear that are differentiated and not iPS cells.

The second type of differentiation that may occur is spontaneous differentiation of a previously pluripotent iPS cell colony. Differentiation that occurs in this situation is usually marked by differentiation in the center of the colony that expands outward (doughnut-shape). The differentiated cells in the center will form rosette-like structures or flatten out to a monolayer and turn gray and unrefractive. Alternatively, the cells in the center of the colony may overgrow 3-dimensionally into embryoid body-like structures. It is normal for some colonies to spontaneously differentiate in an iPS cell culture. It can be interpreted as a healthy sign that the culture has the capacity to differentiate into multiple cell types. However, less than 20% of the culture should be spontaneously differentiating. If the majority of the colonies spontaneously differentiate, culture technique and conditions need to be reevaluated to determine what is causing the differentiation.

18. Initially my iPS cell colonies closely resembled human embryonic stem (hES) cell colonies, but now they appear differentiated.

You will need to be very diligent in monitoring the pluripotent state of your iPS cell colonies, just as you would with hES cell colonies. Refreshing the medium daily, keeping medium formulations consistent, passaging every 3-4 days before colonies start spontaneously differentiating, picking specific colonies with the most hES cell-like morphology for continued propagation are all very important in iPS cell culture.

19. If I wish to, how do I clonally expand colonies?

Briefly, colonies can be clonally expanded by picking a single colony with a pipetman and transferring it to a single well on a 96-well plate containing PBS. The colony is trypsinized in the well and then iPS cell medium is added. The trypsinized cells are then added to 1 well of a 24-well plate to be further expanded once new colonies grow. To further ensure clonality, expanded iPS cell colonies should be carefully analyzed for transgene copy number and insert location.

20. What happens if I realize I didn't store the virus at -80°C? Should I still use it?

As RNA viruses, lentiviruses should always be stored at -80°C to preserve the integrity of their nucleic acid and ensure delivery of the genes of interest.

However, if you have stored the virus at -20°C, you may see a reduction in virus titer. You will need to conduct a control transduction experiment with the viPS-EF1 α -TurboGFP control lentivirus (described in the product manual) to determine the transduction efficiency of the virus following improper storage and make decisions on how to proceed with iPS cell generation.

If you have stored your virus at 4°C, you will need to discard the now inactive virus and purchase a new kit.

21. What percent of transduced cells will receive all 6 factors?

A low percentage of cells will receive all 6 factors. Assuming no other variables except that each virus stock at MOI 10 has a transduction efficiency of ~70% in IMR-90 cells, a simple calculation reveals that only about 12% of transduced cells will receive all 6 factors.

When the low percentage of cells receiving all the factors is coupled with the unknown probability that a cell receiving all the factors will properly reprogram, iPS reprogramming efficiency has been found to be 1% or lower across published studies of iPS cell generation.

22. How many copies per cell do you get integrated for each of the six pluripotency factors?

We have not determined the exact copy number that gets integrated for each of the six pluripotency factors. However, we know that we get sufficient integration events when using an MOI=10 in IMR-90 cells for reprogramming.

23. If I only want to use 4 of the 6 pluripotency factors you provide in the viral pool, how can I go about doing this?

You can use as many of the six factors as you wish. We cannot, however, assure the result or the effectiveness of reprogramming using our protocol with fewer than all six factors.

24. Can I add more of 1 factor than another?

How you choose to use the kit is ultimately up to your discretion. If you wish to add more of 1 factor than another, you should choose what new MOI you would like to explore and calculate the new volume of virus stock as outlined in the manual.

However, the kit has only been validated to generate iPS colonies when a MOI of 10 per factor is used on IMR-90 cells and all 6 factors are used together at a 1:1:1:1:1:1 ratio to each other.

25. How do I freeze down my iPS cells?

Briefly, iPS cells can be frozen down by cutting the MEF layer with iPS cell colonies into pieces with a heat-sharpened Pasteur pipette. The large cut aggregates should be pooled in a 15 ml tube and centrifuged. iPS cells should then be frozen in iPS cryopreservation medium consisting of 60% iPS cell medium, 30% FBS, and 10% DMSO. One 35mm dish should be frozen per single cryovial.

Basically, iPS cells can be cultured in the same manner as human embryonic stem (hES) cells. Details on hES cell culture are included in the following references:

- a. Amit, M. *et al.* 2000. *Developmental Biology*, 227, 271-8.
- b. Mitalipova, M. and Palmarini, G. 2006. *Methods Molecular Biology*, 33, 55-76
- c. National Stem Cell Bank (NSCB): www.nationalstemcellbank.org – under “NSCB Protocols”.

26. If I'm not able to do the manual passaging, how else can I passage iPS cells?

Passaging colonies could also be done by using a P20 pipettor and 5 µl pipet tips to break the colonies into small pieces. Other alternative means of passaging iPS cells include trypsin (0.05% trypsin/0.53mM EDTA) or collagenase passage. (Amit, M. *et al.* 2000. *Developmental Biology*, 227, 271-8.).

However, caution should be exercised when using enzymatic means to passage pluripotent stem cells. Enzymatic passaging can lead to karyotype abnormalities in human embryonic stem (hES) cells and may also in iPS cells. Regularly checking karyotype status and limiting enzymatic passaging to less than 15 passages is highly recommended.

27. If the transduction test reveals that I need a MOI = 25 to achieve 60-70% transduction efficiency, then don't I need to worry about toxicity to my cells if transducing each of the six factors at such a high MOI?

The viability of some cell types may be affected if transduced at a total MOI of 150 (6 factors X 25 MOI), possibly reducing the number of iPS cell colonies formed during the reprogramming process. If you suspect that cell viability is compromised following transduction of each pluripotency factor at an MOI of 25, then you should reduce the MOI of each factor to 10, but plate and transduce twice the number of wells.

28. Turning off expression of the pluripotency factors is required for differentiation of the stem cells. How do you ensure that expression of the pluripotency factors from the EF1a promoter in your lentiviral vector is shut down?

The iPS cell colonies generated using the viPS™ Vector Kit clearly demonstrate differentiation into cells of all three germ layers. iPS cell colonies that were transduced with the viPS-EF1α-TurboGFP control in addition to the six pluripotency factors, also showed loss of GFP expression upon differentiation, providing further evidence that vector expression is shut down.

TROUBLESHOOTING

For help with transduction of your target cells with the viPS™ Vector Kit's lentiviral constructs, please email technical support at info@openbiosystems.com with your questions, your sales order or purchase order number and the catalog number of the product with which you are having trouble.

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