

# Lentiviral Reprogramming System

For direct reprogramming of somatic cells into  
human induced pluripotent stem cells (iPSC)  
using lentiviral particles

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# Contents

Contents and Storage .....	2
<b>Introduction .....</b>	<b>3</b>
Description of the System .....	3
Biosafety of the System .....	6
Experiment Outline .....	7
<b>Methods .....</b>	<b>8</b>
Required Materials .....	8
Guidelines for Lentiviral Reprogramming .....	10
Determining the Amount of Lentivirus Particles to Use .....	13
Identifying iPSC Colonies .....	17
Picking iPSC Colonies .....	20
Freezing iPSCs .....	21
Troubleshooting .....	22
<b>Appendix .....</b>	<b>23</b>
Preparing Reagents and Media .....	23
Blasticidin .....	26
Preparing MEF Culture Dishes .....	27
Generating a Stable Oct4-GFP Reporter Cell Line .....	29
Generating an Inducible Reprogramming System .....	31
iPSCs Derived from Different Species and Somatic Cell Types .....	32
Accessory Products .....	34
Technical Support .....	36
Purchaser Notification .....	37
References .....	40

# Contents and Storage

## Contents

Each vial contains 100  $\mu$ L of lentivirus particles at a concentration of  $5 \times 10^7$  to  $5 \times 10^9$  transducing units/mL.

**Note:** The lentiviral titer is lot dependent. For the specific titer of your lentivirus particles, refer to the Certificate of Analysis supplied with the product. Certificates of Analysis are also available on our website. Go to [www.lifetechnologies.com/support](http://www.lifetechnologies.com/support) and search for the Certificate of Analysis by product lot number, which is printed on the vial.

Product	Catalog number
Lentivirus hOct4	A1357101
Lentivirus hSox2	A1357201
Lentivirus hKlf4	A1357301
Lentivirus hc-Myc	A1357401
Lentivirus hNanog	A1357501
Lentivirus hLin28	A1357601
Lentivirus CMV-GFP Transduction Reporter	A1357701
Lentivirus Oct4-GFP Reprogramming Reporter	A1357801

## Shipping/Storage

Lentivirus particles are shipped on dry ice. Place lentivirus particles at  $-80^{\circ}\text{C}$  for long-term storage.

**Note:** Repeated freezing and thawing of lentivirus particles is not recommended because it may result in loss of viral titer. After long-term storage, we recommend re-titering your viral stocks before transducing your cells.

## Intended use

For research use only. Not for human or animal therapeutic or diagnostic use.

## Purpose of this manual

This manual provides instructions for the direct reprogramming of somatic cells to human induced Pluripotent Stem Cells (iPSC) using lentivirus particles. Instructions for continual maintenance of iPSCs and their characterization are also provided.

# Introduction

## Description of the System

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### Induced pluripotent stem cells (iPSC)

Induced pluripotent stem cells (iPSCs) are genetically reprogrammed adult cells which exhibit a pluripotent stem cell-like state similar to embryonic stem cells. (Meissner *et al.*, 2007; Park *et al.*, 2008; Takahashi & Yamanaka, 2006; Wernig *et al.*, 2007; Yu *et al.*, 2007). While these artificially generated cells are not known to exist in the human body, they show qualities remarkably similar to those of embryonic stem cells (ESC); thus, they are an invaluable new source of pluripotent cells for drug discovery, cell therapy, and basic research.

There are multiple methods to generate iPSCs, including lentivirus-mediated gene transduction and chemical induction. Lentiviral systems are highly-efficient and offer reproducible delivery methods for many expression constructs, and they remain a popular method of generating iPSCs by reprogramming somatic cells.

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### Lentiviral Reprogramming System

The Lentiviral Reprogramming System uses replication-incompetent lentivirus particles to safely and effectively deliver and express key genetic factors necessary for reprogramming somatic cells to iPSCs. In contrast to many available protocols that rely on multiple media systems and viral vectors, the Lentiviral Reprogramming System combines Invitrogen's ViraPower™ HiPerform™ platform with Gibco® media and reagents to offer an efficient and complete workflow. In addition, the system includes two control lentivirus particles, one as a control for transduction and the other for reprogramming. The table below lists the lentivirus particles available as part of the Lentiviral Reprogramming System.

**Note:** The lentivirus particles are available individually from Invitrogen (see page 34 for ordering information).

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### How lentivirus works

After the lentivirus enters the target cell, the viral RNA is reverse-transcribed, actively imported into the nucleus (Lewis & Emerman, 1994; Naldini, 1999), and stably integrated into the host genome (Buchsacher & Wong-Staal, 2000; Luciw, 1996). After the lentiviral construct integrates into the genome, you may assay for transient expression of your recombinant protein or use antibiotic selection to generate a stable cell line for long-term expression studies.

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## Description of the System, continued

**Lentivirus particles** The table below lists the lentivirus particles offered as part of the Lentiviral Reprogramming System. Researchers have successfully used different combinations of reprogramming genes to generate iPSCs and have achieved efficient reprogramming by co-expressing four gene products: Oct, Sox2, Klf4, and c-Myc (Takahashi *et al.*, 2007), or Oct4, Sox2, Lin28, and Nanog (Yu *et al.*, 2007). The minimum requirement for reprogramming human somatic cells to human iPSCs appears to be expression of Oct4 and Sox2 (Stadtfield & Hochedlinger, 2010). See page 32 for a more comprehensive list of reprogramming genes used to derive iPSCs from different species and somatic cell types.

**Note:** The lentivirus particles containing the various genetic factors necessary for reprogramming are available individually and not as a set. See page 34 for ordering information.

Lentivirus particle	Factor	GenBank ID	Promoter	Use
Lentivirus hOct4	human Oct4	BC117435	CMV/TO	reprogramming
Lentivirus hSox2	human Sox2	NM_003106.2	CMV/TO	reprogramming
Lentivirus hKlf4	human Klf4	NM_004235	CMV/TO	reprogramming
Lentivirus hc-Myc	human c-Myc	BC000917	CMV/TO	reprogramming
Lentivirus hNanog	human Nanog	NM_024865	CMV/TO	reprogramming
Lentivirus hLin28	human Lin28	NM_024674	CMV/TO	reprogramming
Lentivirus CMV-GFP Transduction Reporter	EmGFP*	–	CMV/TO	control for optimizing transduction
Lentivirus Oct4-GFP Reprogramming Reporter	EmGFP*	–	Oct4	reprogramming control

\*The reporter lentivirus particles carry the gene for Emerald Green Fluorescent Protein (EmGFP).

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## Description of the System, continued

### Key features of the lentivirus particles

The backbone of the lentivirus particles in the Lentiviral Reprogramming System is based on the ViraPower™ HiPerform™ pLenti6.3/TO/V5-DEST™ vector, which contains the elements listed below.

- Hybrid CMV/TO promoter consisting of the human CMV promoter (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987) and tetracycline operator 2 (TetO<sub>2</sub>) sites (Hillen & Berens, 1994) for high-level, tetracycline-regulated expression of the gene of interest  
**Note:** Lentivirus Oct4-GFP Reporter (Cat. no. A1357801) contains the human Oct4 promoter to drive the expression of the GFP reporter gene.
- The Woodchuck Posttranscriptional Regulatory Element (WPRE) from the woodchuck hepatitis virus for enhanced transgene expression (Zufferey *et al.*, 1998)
- The central Polypurine Tract (cPPT) sequence from the HIV-1 integrase gene for increased copy number of lentivirus integrating into the host genome and enhancing viral titer (Park, 2001)  
**Note:** WPRE and cPPT together produce at least a 4-fold increase in protein expression in most cell types, compared to other vectors that do not contain these elements.
- Blasticidin selection marker for stable selection under control of SV40 promoter (Kimura *et al.*, 1994)

### Advantages of the Lentiviral Reprogramming System

- Efficiently delivers the key factors necessary for reprogramming mammalian somatic cells in culture to iPSCs.
- Facilitates enhanced expression of reprogramming factors, up to 4-fold or greater, compared to traditional lentiviral expression systems.
- Includes multiple features designed to enhance the biosafety of the system.
- *Optional:* Allows the expression of the reprogramming factors to be regulated by tetracycline, which diminishes the risk of continued transgene expression and enables the selection of fully reprogrammed iPSCs.  
**Note:** Tetracycline-regulated expression of the reprogramming factors requires the co-transduction of the target cells with the reprogramming particles and the lentiviral stocks generated using the pLenti3.3/TR plasmid expressing the tetracycline (Tet) repressor. pLenti3.3/TR plasmid is included in the ViraPower™ HiPerform™ T-REx™ Gateway® Expression System (Cat. no. A11141), which is available separately from Invitrogen (for ordering information, see page 34).

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# Biosafety of the System

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## Biosafety features of the Lentiviral Reprogramming System

The backbone of the lentivirus particles in Lentiviral Reprogramming System is based on lentiviral vectors developed by Dull and coworkers includes the following features designed to enhance their biosafety and minimize their relation to the wild-type, human HIV-1 virus:

- A deletion in the 3' LTR ( $\Delta$ U3) results in “self-inactivation” of the lentivirus after transduction of the target cell (Yee *et al.*, 1987; Yu *et al.*, 1986; Zufferey *et al.*, 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
  - The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994).
  - The lentiviral particles are replication-incompetent and only carry the gene of interest.
- 

## Biosafety Level 2



Despite the inclusion of the safety features discussed above, the lentivirus particles still pose some biohazardous risk because they can transduce primary human cells. For this reason, **we highly recommend that you treat lentiviral stocks generated using this System as Biosafety Level 2 (BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.**

For more information about the BL-2 guidelines and lentivirus handling, refer to the document, “Biosafety in Microbiological and Biomedical Laboratories”, 5<sup>th</sup> Edition, published by the Centers for Disease Control (CDC). This document may be downloaded at <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>.

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## Important

Handle all lentiviruses in compliance with established institutional guidelines. Because safety requirements for use and handling of lentiviruses may vary at individual institutions, we recommend consulting the health and safety guide-lines and/or officers at your institution before using of the Lentiviral Reprogramming System.

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# Experiment Outline

## Workflow

The table below describes the major steps required for reprogramming somatic cells to generate iPSCs using lentivirus particles that express the key reprogramming genes.

Step	Action	Page
1	Transduce your cells of interest with Lentivirus CMV-GFP Reporter to assess the lentiviral transduction efficiency and toxicity for your cell type.	13
2	Co-transduce your cells of interest with reprogramming lentivirus particles.	14
3	Perform live staining with anti-Tra1-60 and anti-Tra1-81 antibodies to identify iPSC colonies.	18
4	Manually pick iPSC colonies and expand until you have sufficient cells (two 60-mm plates) for cryopreservation.	20
5	<i>Optional:</i> Generate a stable cell line with Lentivirus Oct4-GFP Reporter.	29
6	<i>Optional:</i> Create an inducible system where the expression of reprogramming genes is controlled by the Tet repressor.	30



## Methods

### Required Materials

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#### Lentivirus particles and cells

- Lentivirus particles at  $>1 \times 10^7$  transducing units/mL
  - Mammalian cells to reprogram
  - Gibco® Mouse embryonic Fibroblasts (Irradiated) (Cat. no. S1520-100)
- 

#### Media and reagents

- D-MEM with GlutaMAX™-I (high glucose) (Cat. no. 10569-010)
  - D-MEM/F-12 (Cat. no. 10565-018)
  - Fetal Bovine Serum (FBS), ES Cell-Qualified (Cat. no. 16141-061)
  - Knockout™ Serum Replacement (KSR) (Cat. no. 10828-028)
  - MEM Non-essential Amino Acids (NEAA) (Cat. no. 11140-050)
  - Basic FGF, recombinant human (Cat. no. PHG0261)
  - $\beta$ -mercaptoethanol (Cat. no. 21985-023)
  - Antibiotic-Antimycotic (Cat. no. 15240-062)
  - Attachment Factor (Cat. no. S-006-100)
  - TrypLE™ Select Cell Dissociation Reagent (Cat. no. 12563-029) or 0.05% Trypsin/EDTA (Cat. no. 25300-054)
  - Collagenase Type IV (Cat. no. 17104-019)
  - D-PBS without  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  (Cat. no. 14190-144)
  - Blasticidin (Cat. no. A11139-02)
  - Polybrene® (Hexadimethrine bromide) (Sigma, Cat. no. H9268)
  - Valproic acid (Sigma, Cat. no. P4543)
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#### Antibodies for characterizing iPSCs

- Mouse anti-Tra1-60 antibody (Cat. no. 41-1000)
  - Mouse anti-Tra1-81 antibody (Cat. no. 41-1100)
  - Mouse anti-SSEA4 (Cat. no. 41-4000)
  - Alexa Fluor® 594 goat anti-mouse IgG antibody (Cat. no. A11032)
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## Required Materials, continued

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### Equipment

- Sterile cell culture hood (i.e., biosafety cabinet) equipped with a stereomicroscope
  - Inverted microscope
  - Incubator set at 37°C
  - Water bath set at 37°C
  - Sterile serological pipettes (5-mL, 10-mL)
  - Centrifuge
  - 15-mL centrifuge tubes
  - 60-mm and 100-mm tissue culture-treated dishes
  - 6-well and 96-well tissue culture-treated plates
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# Guidelines for Lentiviral Reprogramming

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## General guidelines for lentiviral reprogramming

- To maintain sterile culture conditions, carry out all of the procedures in this manual using sterile laboratory practices in a laminar flow hood.
- Viral titers can decrease as much as 10% with each freeze/thaw cycle. Avoid repeated freezing and thawing of your reprogramming lentivirus particles.
- Prior to starting, ensure that the media are equilibrated to 37°C and appropriately gassed.
- Depending on your cell type, a high viral load may result in cell toxicity while a low viral load will compromise reprogramming efficiency. Before starting your reprogramming experiments, transduce your cell line of interest with the Lentivirus CMV-GFP Reporter. Based on the transduction efficiency of Lentivirus CMV-GFP Reporter that yields the least toxicity and highest GFP expression levels, determine the volume of reprogramming lentivirus particles to use (see page 13).
- It is possible to concentrate VSV-G pseudotyped lentiviruses using a variety of methods without significantly affecting their transducibility. If your experiment requires that you use a large volume of viral supernatant (e.g., a relatively high MOI), concentrate your virus before proceeding to transduction. For details and guidelines for concentrating your virus, refer to published reference sources (Yee, 1999).

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## Minimum requirement for reprogramming

Researchers have successfully used different combinations of transcription factors (i.e., reprogramming factors) to generate iPSCs and achieved high reprogramming efficiencies by co-expressing a combination of four gene products: Oct, Sox2, Klf4, and c-Myc (Takahashi *et al.*, 2007), or Oct4, Sox2, Lin28, and Nanog (Yu *et al.*, 2007). The minimum requirement for reprogramming human somatic cells to human iPSCs appears to be expression of Oct4 and Sox2 (Stadtfeld & Hochedlinger, 2010). However, when only Oct4 and Sox2 are used, the reprogramming efficiencies are usually lower compared to those achieved with the full complement of four reprogramming factors. See page 32 for a more comprehensive list of reprogramming genes used to derive iPSCs from different species and somatic cell types.

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## Guidelines for Lentiviral Reprogramming, continued

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### Using the Lentivirus CMV-GFP Reporter

Lentivirus CMV-GFP Reporter (Cat. no. A1357701) is used for determining the amount of reprogramming lentivirus needed to efficiently transduce your cells. The Lentivirus CMV-GFP Reporter is identical to the reprogramming lentivirus particles, but contains the Emerald Green Fluorescent Protein (EmGFP) gene instead of a reprogramming factor. See page 13 for instructions on using the Lentivirus CMV-GFP Reporter.

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### Using the Lentivirus Oct4-GFP Reporter

Lentivirus Oct4-GFP Reporter (Cat. no. A1357801) is a control lentivirus for verifying the successful reprogramming of somatic cells into iPSCs. Cells transduced with the Lentivirus Oct4-GFP Reporter express EmGFP only if they have been successfully reprogrammed into iPSCs (i.e., have become pluripotent). Although hOct4-GFP expression is observed only in pluripotent cells, a small amount of basal EmGFP expression may be detected in non-pluripotent cells as a background (usually less than 10%).

- Lentivirus Oct4-GFP Reporter is used in co-transformation experiments with the reprogramming lentivirus particles (page 14), where the successfully reprogrammed cells are picked and expanded based on transient Oct4-GFP expression.
  - Alternatively, Lentivirus Oct4-GFP Reporter can be used for generating a stable Oct4-GFP reporter cell line by first transducing the target cells with the Lentivirus Oct4-GFP Reporter and then performing Blasticidin selection to isolate stable transductants. The Oct4-GFP reporter cell line can then be transduced with reprogramming lentivirus particles and successfully reprogrammed cells identified based on Oct-GFP expression (page 29).
- 

### Creating a Tet-inducible system

The reprogramming lentivirus particles in the Lentiviral Reprogramming System are based on Invitrogen's ViraPower™ HiPerform™ platform and can be used to create a tetracycline-inducible system.

To create an inducible system where the reprogramming genes are under the control of the Tet repressor, use the pLenti3.3/TR plasmid from the ViraPower™ HiPerform™ T-REx™ Gateway® Expression System (available separately, Cat. no. A11141) to generate a Tet repressor virus and co-transduce your cell line with the repressor virus and reprogramming particles. For more information, refer to the ViraPower™ HiPerform™ T-REx™ Gateway® Expression System manual, which is available for downloading at [www.lifetechnologies.com](http://www.lifetechnologies.com).

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## Guidelines for Lentiviral Reprogramming, continued

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### Using Polybrene® during transduction

Lentivirus transduction may be enhanced if cells are transduced in the presence of hexadimethrine bromide (Polybrene®, Sigma Cat. no. H9268). For best results, we recommend performing transduction in the presence of Polybrene®. However, some cells are sensitive to Polybrene® (e.g., primary neurons). Before performing any transduction experiments, test your cell line for sensitivity to Polybrene® at a range of 0–10 µg/mL. If your cells are sensitive to Polybrene® (e.g., exhibit toxicity or phenotypic changes), do not add Polybrene® during transduction. In this case, cells can still be successfully transduced with your lentivirus.

See page 23 for instructions on preparing and storing Polybrene® stock solution.

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### Recommended reading

The Lentiviral Reprogramming System is designed to help you to deliver and express key reprogramming factors in somatic mammalian cells to generate iPSCs. Although the system has been designed to help you express the reprogramming factors in the simplest, most direct fashion, use of the system is geared towards those users who are familiar with the principles of retrovirus biology and tissue culture techniques. For more information about these topics, refer to the following published reviews:

- Retrovirus biology and the retroviral replication cycle: see Buchschacher and Wong-Staal (2000) and Luciw (1996) .
  - Retroviral and lentiviral vectors: see Naldini (1998), Naldini (1999), Yee (1999) and Pandya et al., 2001 (Naldini, 1998; Naldini, 1999; Pandya *et al.*, 2001; Yee, 1999).
- 



Remember that you are working with media containing infectious virus. Follow the recommended Federal and institutional guidelines for working with BL-2 organisms.

- Perform all manipulations within a certified biosafety cabinet.
  - Treat media containing virus with bleach.
  - Treat used pipettes, pipette tips, and other tissue culture supplies with bleach and dispose of as biohazardous waste.
  - Wear gloves, a laboratory coat, and safety glasses or goggles when handling viral stocks and media containing virus.
-

# Determining the Amount of Lentivirus Particles to Use

## Overview of the procedure

To determine the amount of reprogramming lentivirus particles to use for reprogramming, transduce your cells with varying amounts of Lentivirus CMV-GFP Reporter and determine the amount that yields the least toxicity and highest EmGFP expression in transduced cells.

**Note:** You can qualitatively determine toxicity due to high viral load by observing cell death, which is manifested by cells curling up and detaching from the culture plate. Alternatively, you can perform Trypan Blue staining using a small aliquot of cells.

## Transduction procedure using the Lentivirus CMV-GFP Reporter

1. Maintain your cells to be transduced under the recommended culture conditions using the appropriate culture medium.
2. 24 hours before transduction, determine the viable cell count using your method of choice.
3. Plate the cells on a tissue culture-treated 96-well plate in the appropriate cell-specific culture medium so that they are 70–80% confluent on the day of transduction. Incubate the cells overnight at 37°C and 5% CO<sub>2</sub>.
4. On the day of transduction, thaw the vial of Lentivirus CMV-GFP Reporter in a 37°C water bath until only a small pellet of ice is left in the vial. Place the vial on ice to complete thawing.
5. Make a serial dilution of the Lentivirus CMV-GFP Reporter in lentivirus transduction medium (see page 24) as described in the table below. Mix the medium containing the virus gently by pipetting up and down.

Lentivirus transduction medium	49 µL	48 µL	45 µL	40 µL	30 µL
Lentivirus CMV-GFP Reporter	1 µL	2 µL	5 µL	10 µL	20 µL
Total volume	50 µL	50 µL	50 µL	50 µL	50 µL

6. Remove the culture medium from the cells and add 50 µL of Lentivirus CMV-GFP Reporter particles (diluted in lentivirus transduction medium) into each well containing the cells.
7. Add Polybrene® (if desired) to a final concentration of 6 µg/mL.
8. Swirl the plate gently to mix and incubate it overnight in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.
9. The next day, replace the lentivirus transduction medium with 100 µL of culture medium appropriate for your cell type.
10. 48 hours after transduction, evaluate the GFP expression using fluorescence microscopy or flow cytometry.
11. Choose the volume of the Lentivirus CMV-GFP Reporter that yields the least toxicity and highest GFP expression level to determine the optimal volume of reprogramming lentivirus particles to use for transduction.

# Reprogramming Somatic Cells to Generate iPSCs

## Minimal requirements for reprogramming

Researchers have successfully used different combinations of reprogramming genes to generate iPSCs. The minimum requirement for reprogramming human somatic cells to human iPSCs appears to be the co-expression of 4 gene products: Oct, Sox2, Klf4, and c-Myc (Takahashi *et al.*, 2007), or Oct, Sox2, Lin28, and Nanog (Yu *et al.*, 2007). See page 32 for a more comprehensive list of reprogramming genes used to derive iPSCs from different species and somatic cell types.

## Overview of the reprogramming protocol

### Basic Reprogramming

The basic reprogramming protocol uses a reprogramming cocktail consisting of only reprogramming lentivirus particles. The target cells are transduced with the reprogramming cocktail and passaged onto MEF culture dishes using human iPSC medium after they have recovered. The cells are fed with fresh medium daily and observed under the microscope for the emergence of iPSCs. Reprogramming is confirmed using antibodies against the pluripotency markers Tra1-60 and Tra1-81. See page 15 for the basic reprogramming protocol.

### Co-transduction with the Lentivirus Oct4-GFP Reporter

The workflow for the co-transduction reprogramming experiments is identical to basic reprogramming, but the reprogramming cocktail includes the Lentivirus Oct4-GFP Reporter in addition to the combination reprogramming particles. The amount of Lentivirus Oct4-GFP Reporter used in co-transduction is determined in the same manner as the reprogramming lentivirus particles (i.e., using Lentivirus CMV-GFP Reporter to optimize MOI and calculating the volume of lentivirus to use based on its titer; see page 13 for more information). After the transductants are passaged onto MEF culture dishes and allowed to proliferate, successfully reprogrammed cells are selected on the basis of uniform EmGFP expression.

## Preparing the cells for transduction

1. Maintain your cells to be transduced under the recommended culture conditions using the appropriate culture medium.
2. 24 hours before transduction, determine the viable cell count using your method of choice.
3. Plate the required number of cells in each well of a tissue culture-treated 6-well plate using the appropriate cell-specific culture medium. Incubate the cells overnight in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.

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# Reprogramming Somatic Cells to Generate iPSCs, continued

## Preparing the reprogramming cocktail

1. On the day of transduction, thaw the reprogramming lentivirus particles (and the Lentivirus Oct4-GFP Reporter, if applicable) in a 37°C water bath until only a small pellet of ice is left in the vials. Place the vials on ice to complete thawing.
2. Determine the required transduction volume for each reprogramming lentivirus based on the transduction with the Lentivirus CMV-GFP Reporter (see page 13).  
**Note:** For additional reprogramming protocols, refer to the literature cited in this manual.
3. Prepare the reprogramming cocktail consisting of the desired lentivirus combination (and the Lentivirus Oct4-GFP Reporter, if applicable) by diluting the lentivirus particles in lentivirus transduction medium (see page 24) to a combined volume of 1 mL per well (i.e., if transducing cells in each well of a 6-well plate, prepare 6 mL of transduction mixture).
4. Gently pipet the transduction mixture up and down to mix. Do **not** vortex the lentivirus particles.

## Basic reprogramming protocol

### Day 0: Transduce cell line of interest

1. Aspirate the culture medium from the wells of the 6-well plate containing the cells to be transduced.
2. Add 1 mL of the reprogramming transduction cocktail in each well of a 6-well plate. If desired, add Polybrene® to a final concentration of 6 µg/mL (see page 23).
3. Move the culture plate in several quick back-and-forth and side-to-side motions to disperse the reprogramming cocktail across the surface of the wells and incubate the plate overnight in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.

### Day 1: Replace transduction medium with human iPSC medium

4. On Day 1 post-transduction, replace the transduction medium with 2 mL of human iPSC medium (see page 24).

### Day 3 or 4: Prepare MEF culture dishes

5. On Day 3 or 4 post-transduction, prepare MEF culture dishes by plating inactivated or Mitomycin C-treated MEFs on gelatin-coated 100-mm culture dishes. See page 27 for detailed instructions on preparing MEF culture dishes.

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# Reprogramming Somatic Cells to Generate iPSCs, continued

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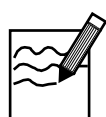
## Reprogramming protocol, continued

### Day 5: Plate transduced cells on MEF culture dishes

6. On Day 5 post-transduction, carefully remove the cells from the 6-well plate using TrypLE™ Select reagent or 0.5% trypsin following the procedure recommended by the manufacturer and collect the cells in a 15-mL conical centrifuge tube.  
**Note:** At this stage, the cells in the 6-well plate (i.e., the transduction plate) will be confluent and occasional clusters of transformed cells will be visible as small colonies.
7. Centrifuge the cells at  $200 \times g$  for 2 minutes. Remove the supernatant and resuspend the cells in an appropriate volume of human iPSC medium.
8. Determine the viable cell count using your method of choice (e.g., Countess® Automated Cell Counter).
9. Plate the cells on 2–3 MEF culture dishes (from step 5) at varying seeding densities of between 50,000–200,000 cells per 100-mm using human iPSC medium.
10. Incubate the cells in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.

### Day 7–21: Feed and monitor the cells

11. 48 hours after seeding (Day 7 post-transduction), replace the spent medium with fresh human iPSC medium. Change the medium daily until you are ready to manually pick the iPSC colonies.
12. Starting on Day 9 post-transduction, observe the plates every other day under a microscope for the emergence of cell clumps indicative of transformed cells (see **Note** below).
13. On Day 21 post-transduction, perform live staining using anti-Tra1-60 and anti-Tra1-81 antibodies to identify iPSC colonies (see page 16).
14. Manually pick iPSC colonies to expand for further characterization (see page 18).



## Note

Although colonies of “transformed” cells may emerge as early as 7 days after transduction, most of these colonies will not be correctly “reprogrammed” cells. iPSCs usually emerge a little later (around day 14 post-transduction) and resemble embryonic stem cells in morphology. If you have co-transduced your cells with the Lentivirus Oct4-GFP Reporter, successfully reprogrammed cells will exhibit uniform GFP expression. For more information on identifying iPSCs, see page 17.

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# Identifying iPSC Colonies

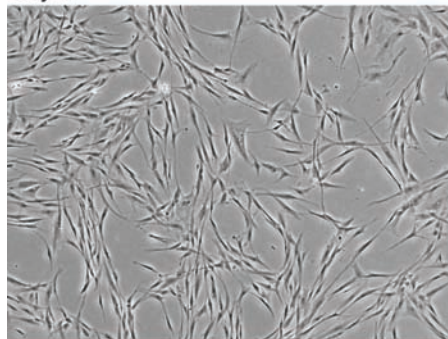
**Visual identification** By Day 21 post-transduction, the cell colonies on the MEF culture dishes will have become large and compact, covering the majority of the surface area of the culture dish. However, only a fraction of these colonies will consist of iPSCs, which exhibit a hESC-like morphology characterized by a flatter cobblestone-like appearance with individual cells clearly demarcated from each other in the colonies (see Figure 1, below). Therefore, we recommend that you perform live staining with Tra1-60 or Tra1-81 antibodies that recognize undifferentiated hESCs (see page 18).

## Morphology of reprogrammed cells

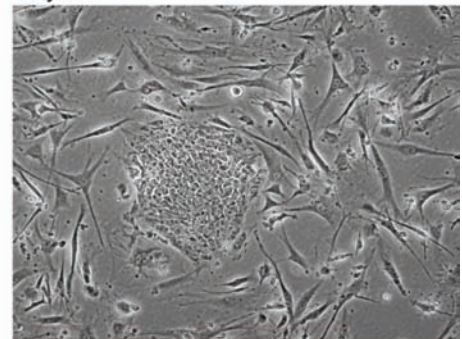
The images below show the morphology of human dermal fibroblasts that were reprogrammed into iPSCs using the Lentiviral Reprogramming System.

**Figure 1** Human dermal fibroblasts were transduced with a reprogramming cocktail consisting of Lentivirus hOct4, Lentivirus hSox2, Lentivirus hKlf4, and Lentivirus hc-Myc. On Day 5 post-transduction, the transformed cells were passaged onto MEF culture dishes and allowed to proliferate on MEF feeder layers in human iPSC medium. The images were obtained using a 10X objective on Day 1, Day 20, Day 25 after passaging the transduced cells onto MEF feeder plates, and after picking and further expanding the reprogrammed cells.

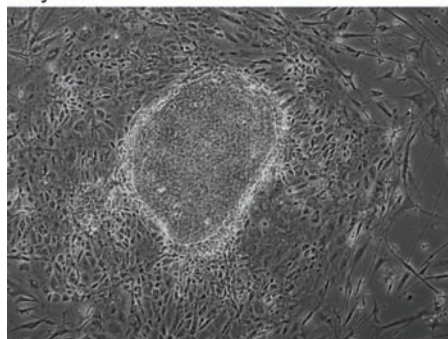
Day 1



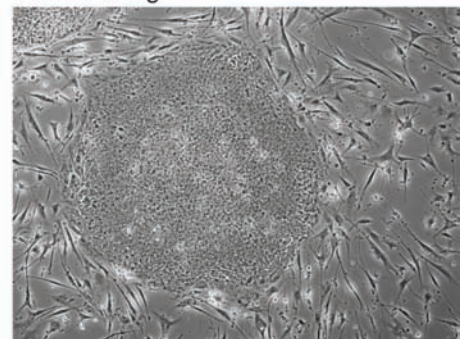
Day 20



Day 25



Human iPSC colony after picking and culturing



*Continued on next page*

## Identifying iPSC Colonies, continued

### Live staining with antibodies

One of the fastest and most reliable methods for selecting a reprogrammed colony is live staining with Tra1-60 or Tra1-81 antibodies that recognize undifferentiated hESCs and enable the identification of reprogrammed cells from a variety of human cell types (see page 34 for ordering information). Live staining with these antibodies has little adverse effects on the growth of the cell colonies even after multiple rounds of staining, regardless of whether the cells stain positive or negative for these surface markers indicating pluripotency.

### Required antibodies

#### Primary antibody:

- Mouse anti-Tra1-60 antibody (Cat. no. 41-1000) and/or mouse anti-Tra1-81 antibody (Cat. no. 41-1100), diluted 1:100 in D-MEM/F-12

#### Secondary antibody:

- Alexa Fluor® 594 goat anti-mouse IgG antibody (Cat. no. A11032), diluted 1:500 in D-MEM/F-12

### Live staining protocol

1. Aspirate the human iPSC medium from the reprogramming dish.
2. Wash the cells once with D-MEM/F-12.
3. Add the primary antibody, diluted 1:100 in DMEM/F-12, to the cells (1 mL per one well of a 6-well plate or 6 mL per 100-mm dish).
4. Incubate the primary antibody and the cells at 37°C for 60 minutes.
5. Remove the primary antibody solution from the dish.  
**Note:** The primary antibody solution can be stored at 4°C for 1 week and re-used up to 2 times.
6. Wash cells three times with D-MEM/F-12.
7. Add the secondary antibody, diluted 1:500 in D-MEM/F-12, to the cells (1 mL per one well of a 6-well plate or 6 mL per 100-mm dish).
8. Incubate the secondary antibody and the cells at 37°C for 60 minutes.
9. Remove the secondary antibody solution from the dish.  
**Note:** The secondary antibody solution can be stored at 4°C for 1 week and re-used up to 2 times.
10. Wash cells three times with D-MEM/F-12 and add fresh D-MEM/F-12 to cover the surface of the cells (1 mL per well of a 6-well plate or 6 mL per 100-mm dish).
11. Visualize the cells under a standard fluorescent microscope. Successful antibody staining can very specifically distinguish reprogrammed colonies from just plain transformed counterparts (see Figure 2, page 19), and can be detected for up to 24–36 hours. This is particularly useful because it helps identifying and tracking of candidate iPS colonies before picking and the day after they are transferred into a new culture dish for expansion.

*Continued on next page*

## Identifying iPSC Colonies, continued

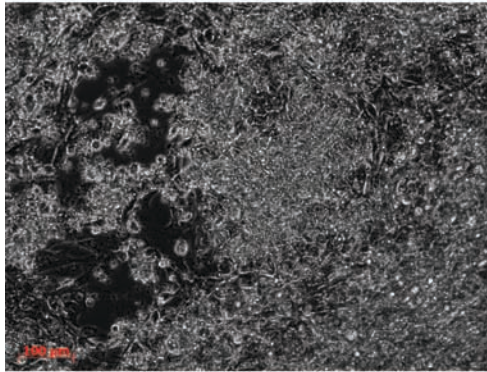
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### Reprogrammed cells stained with anti-Tra1-81 antibody

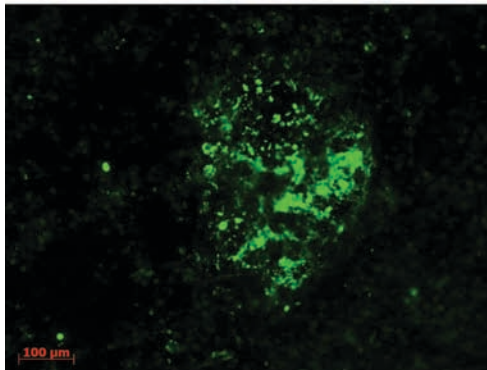
The images below show successfully reprogrammed cells that were analyzed by live staining for the presence of cell surface marker Tra1-81, which indicates pluripotency.

**Figure 2** Human dermal fibroblasts were transduced with a reprogramming cocktail consisting of Lentivirus hOct4, Lentivirus hSox2, Lentivirus hKlf4, and Lentivirus hc-Myc. On Day 5 post-transduction, the transformed cells were passaged onto MEF culture dishes and allowed to proliferate on MEF feeder layers in human iPSC medium. On Day 21, the cells were analyzed by live staining using the antibody against the cell surface marker Tra1-81. The images were obtained using a 10X objective.

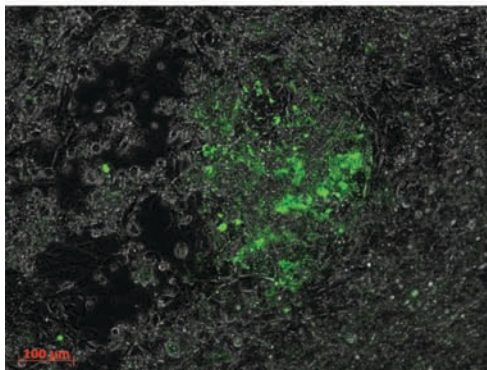
Phase contrast



FITC filter



Overlap of phase contrast and FITC filter



# Picking iPSC Colonies

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## Protocol for picking iPSCs

1. Place the culture dish containing the reprogrammed cells under an inverted microscope and examine the colonies under 10X magnification.
  2. Mark the colony to be picked on the bottom of the culture dish.  
**Note:** We recommend picking at least 10 distinct colonies by the end of each reprogramming experiment and expanding them in separate 24-well MEF culture plates (see below).
  3. Transfer the culture dish to a sterile cell culture hood (i.e., biosafety cabinet) equipped with a stereomicroscope.
  4. Using a 25 gauge 1½ inch needle, cut the colony to be picked into 5–6 pieces in a grid-like pattern.
  5. Using a 200 µL pipette, transfer the cut pieces to a freshly prepared 24-well MEF culture plate (see page 26) containing human ESC medium.  
**Note:** Human ESC medium does not contain valproic acid, but otherwise is identical to human iPSC medium (see page 24).
  6. Incubate the MEF culture plate containing the picked colonies in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.
  7. Allow the colonies to attach to the culture plate for 48 hours before replacing the spent medium with fresh human ESC medium. After that, change the medium every other day.
  8. Treat the reprogrammed colonies like normal human ESC colonies and passage, expand, and maintain them using standard culture procedures until you have frozen cells from two 60-mm plates (see page 21).
-

# Freezing iPSCs

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## Freezing protocol

1. Prepare the required volume of fresh freezing medium and place it on ice (see page 25).
  2. Aspirate the culture medium and rinse the dishes twice with D-PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (2 mL for 35-mm or 4 mL for 60-mm culture dishes).
  3. Gently add Collagenase IV solution to the culture dish (1 mL per 35-mm culture dish or 2 mL per 60-mm culture dish).
  4. Incubate the dish with cells for 5–20 minutes in a 37°C incubator with a humidified atmosphere of 5%  $\text{CO}_2$ .  
**Note:** Incubation times may vary among different batches of collagenase. We recommend that you optimize the appropriate incubation time by examining the colonies periodically under microscope during incubation.
  5. Stop the incubation when the edges of the colonies are starting to pull away from the plate.
  6. Remove the culture dish from the incubator, aspirate the Collagenase IV solution, and gently rinse the dish with D-PBS without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ .
  7. Add 2 mL of iPSC culture medium or D-MEM/F-12 and gently dislodge the cells off the surface of the culture dish using a sterile pipette or a cell scraper. Transfer the cells to a sterile 15-mL centrifuge tube. Rinse the dish with additional iPSC medium or D-MEM/F-12 to collect any leftover colonies.
  8. Centrifuge the cells at  $200 \times g$  for 2–4 minutes at room temperature.
  9. Discard the supernatant, gently tap the tube to dislodge the cell pellet from the tube bottom, and resuspend the cells in **freezing medium A** (see page 25). After the cell clumps have been uniformly suspended, add an equal volume of **freezing medium B** to the cell suspension in a drop-wise manner while gently swirling the cell suspension to mix.  
**Note:** At this point, the cells are in contact with DMSO, and work must be performed efficiently with no or minimum delays. After the cells come into contact with DMSO, they should be aliquoted and frozen within 2–3 minutes.
  10. Aliquot 1 mL of the cell suspension into each cryovial.
  11. Quickly place the cryovials containing the cells in a cryo freezing container (e.g., Mr. Frosty) to freeze the cells at 1°C per minute and transfer them to –80°C overnight.
  12. After overnight storage at –80°C, transfer the cells to a liquid nitrogen tank vapor phase for long term storage.
-

# Troubleshooting

The table below lists some potential problems and possible solutions that may help you troubleshoot your reprogramming experiments.

Symptom	Cause	Solution
Low transduction efficiency	Viral stocks stored incorrectly	Aliquot and store stocks at $-80^{\circ}\text{C}$ . Do not freeze/thaw the lentivirus particles more than 3 times.
	Low viral titer	Increase the volume of the lentivirus.
	Polybrene <sup>®</sup> not included transduction	Add Polybrene <sup>®</sup> to transduction mix.
Cytotoxic effects observed after transduction	Viral load too high	Determine the correct volume of lentivirus to use for your cell type by performing viral dilution for each individual viral particle (page 13) and repeat the transduction procedure with a fresh a transduction cocktail (page 15).
	Volume of reprogramming cocktail too high	<ul style="list-style-type: none"> <li>• Replace spent medium with fresh medium.</li> <li>• Decrease the volume of reprogramming cocktail by concentrating the virus.</li> </ul>
	Polybrene <sup>®</sup> used during transduction	Verify the sensitivity of your cell type to Polybrene <sup>®</sup> . If your cells are sensitive, omit the Polybrene <sup>®</sup> during transduction.
	Transduction carried out for too long	Transduction should be carried out for 18–24 hours, after which the transduction medium should be replaced with fresh human iPSC medium.
No iPSC colony formation	Insufficient viral load	<ul style="list-style-type: none"> <li>• Recalculate correct amount of virus for each viral titer given.</li> <li>• Determine the correct volume to use by performing viral dilution for each individual viral particle and repeat transduction on your cell type to determine the best volume to use for each virus.</li> </ul>
	Viral stocks stored incorrectly	Aliquot your virus stocks to prevent repeated freeze/thaw cycles. Repeatedly freezing and thawing will decrease viral titers 5 to 10-fold.
	Incorrect virus used for optimization	Make sure that you use the correct viruses for reprogramming (hOct4 promoter GFP virus is a reprogramming reporter and it is expressed in pluripotent cells; however, it is not a transduction reporter virus. It will not be expressed in fibroblasts and it should not be used to determine MOI).
Very few colonies are present after reprogramming	Starting cell number too low	Plate greater number of cells per experiment (50,000–100,000 cells per 100-mm dish).
	Not enough virus in cells	Increase the volume of lentivirus, and decrease the starting cell number.

## Appendix

### Preparing Reagents and Media

- 
- Basic FGF solution**
- To prepare 1000  $\mu\text{L}$  of 10- $\mu\text{g}/\text{mL}$  Basic FGF solution, aseptically mix the following components:
 

Basic FGF	10 $\mu\text{g}$
D-PBS without $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$	980 $\mu\text{L}$
10% BSA	10 $\mu\text{L}$
  - Aliquot and store the Basic FGF solution at  $-20^{\circ}\text{C}$  for up to 6 months.
- 

- Polybrene<sup>®</sup> solution**
- Prepare a 6-mg/mL Polybrene<sup>®</sup> stock solution in deionized, sterile water.
  - Filter-sterilize the stock solution and dispense 1 mL aliquots into sterile microcentrifuge tubes.
  - You may store the working stock at  $4^{\circ}\text{C}$  for up to 2 weeks. Store at  $-20^{\circ}\text{C}$  for long-term storage (up to 1 year). Do not freeze/thaw the stock solution more than 3 times as this may result in loss of activity.
- 

- Collagenase IV solution**
- Prepare a 10-mg/mL Collagenase Type IV stock solution in D-MEM/F-12. The stock solution can be aliquoted and frozen at  $-20^{\circ}\text{C}$  until use.
  - Prepare a 1-mg/mL Collagenase Type IV working solution in D-MEM/F-12. Always prepare fresh working solution.
- 

- Complete MEF medium**
- To prepare 100 mL of complete MEF medium, aseptically mix the components listed in the table below. Complete MEF medium can be stored at  $2-8^{\circ}\text{C}$  for up to 1 week.

Component	Stock concentration	Final concentration	Volume
D-MEM with GlutaMAX <sup>™</sup> -I	–	1X	89 mL
FBS, ESC Qualified	–	10%	10 mL
MEM Non-Essential Amino Acids Solution	10 mM	0.1 mM	1 mL
$\beta$ -mercaptoethanol	55 mM	0.1 mM	182 $\mu\text{L}$

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*Continued on next page*



## Preparing Reagents and Media, continued

### Lentivirus transduction medium

To prepare 100 mL of lentivirus transduction medium, aseptically mix the components listed in the table below. Lentivirus transduction medium can be stored at 2–8°C for up to 1 week.

Component	Stock concentration	Final concentration	Volume
D-MEM with GlutaMAX™ -I	1X	1X	89 mL
Penicillin-Streptomycin	100X	1X	1 mL
FBS, ES Cell-Qualified	–	10%	10 mL
Polybrene	–	6 µg/mL	–

### Human iPSC medium

To prepare 100 mL of human iPSC medium, aseptically mix the components listed in the table below. Human iPSC medium can be stored at 2–8°C for up to 1 week.

**Note:** Supplement human iPSC medium daily with fresh FGF and valproic acid.

Component	Stock concentration	Final concentration	Volume
D-MEM/F-12	1X	1X	78 mL
Knockout™ Serum Replacement	–	1X	20 mL
MEM Non-Essential Amino Acids Solution	10 mM	100 µM	1 mL
β-mercaptoethanol	55 mM	55 µM	100 µL
Antibiotic-Antimycotic Solution	100X	1X	1 mL
Basic FGF	10 µg/mL	12 ng/mL	120 µL
Valproic acid	–	0.5 mM	–

### Human ESC medium

To prepare 100 mL of human ESC medium, aseptically mix the components listed in the table below. Human ESC medium can be stored at 2–8°C for up to 1 week.

Component	Stock concentration	Final concentration	Volume
D-MEM/F-12	1X	1X	78 mL
Knockout™ Serum Replacement	–	1X	20 mL
MEM Non-Essential Amino Acids Solution	10 mM	100 µM	1 mL
β-mercaptoethanol	55 mM	55 µM	100 µL
Antibiotic-Antimycotic Solution	100X	1X	1 mL
Basic FGF	10 µg/mL	12 ng/mL	120 µL

*Continued on next page*

## Preparing Reagents and Media, continued

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### iPSC freezing medium

Prepare the Freezing Media A and B immediately before use.

1. In a sterile 15-mL tube, mix together the following reagents for every 1 mL of **freezing medium A** needed:

Human ESC medium	0.5 mL
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KSR	0.5 mL
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2. In another sterile 15-mL tube, mix together the following reagents for every 1 mL of **freezing medium B** needed:

Human ESC medium	0.8 mL
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DMSO	0.2 mL
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3. Place the tube with freezing medium B on ice until use (you can keep freezing medium A at room temperature). Discard any remaining freezing medium after use.
-

# Blasticidin

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## Description

Blasticidin S HCl is a nucleoside antibiotic isolated from *Streptomyces griseochromogenes* which inhibits protein synthesis in both prokaryotic and eukaryotic cells. Resistance is conferred by expression of either one of two Blasticidin S deaminase genes: *BSD* from *Aspergillus terreus* (Kimura *et al.*, 1994) or *bsr* from *Bacillus cereus* (Izumi *et al.*, 1991). These deaminases convert Blasticidin S to a non-toxic deaminohydroxy derivative (Izumi *et al.*, 1991).

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## Handling Blasticidin

Always wear gloves, mask, goggles, and a laboratory coat when handling Blasticidin. Weigh out Blasticidin and prepare solutions in a hood.

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## Preparing and storing stock solutions

- Blasticidin is soluble in water and acetic acid.
  - Prepare a stock solution of 5 to 10 mg/mL Blasticidin in sterile water and filter-sterilize the solution.
  - Aliquot in small volumes suitable for one time use and freeze at  $-20^{\circ}\text{C}$  for long-term storage or store at  $4^{\circ}\text{C}$  for short term storage.
  - Aqueous stock solutions are stable for 1 week at  $4^{\circ}\text{C}$  and 6–8 weeks at  $-20^{\circ}\text{C}$ .
  - pH of the aqueous solution should not exceed 7.0 to prevent inactivation of Blasticidin.
  - Do not subject stock solutions to freeze/thaw cycles (**do not store in a frost-free freezer**).
  - Upon thawing, use what you need and discard the unused portion.
  - Medium containing Blasticidin may be stored at  $4^{\circ}\text{C}$  for up to 2 weeks.
- 

## Determining Blasticidin sensitivity

To select for stably transduced cells using Blasticidin, you must first determine the minimum concentration of Blasticidin required to kill your untransduced mammalian cell line (i.e., perform a kill curve experiment). Typically, concentrations ranging from 2–10  $\mu\text{g/mL}$  Blasticidin are sufficient to kill most untransduced mammalian cell lines. We recommend that you test a range of concentrations (see protocol below) to ensure that you determine the minimum concentration necessary for your cell line.

1. Plate cells at approximately 25% confluence. Prepare a set of 7 plates. Allow the cells to adhere overnight.
  2. The next day, substitute culture medium with medium containing varying concentrations of Blasticidin, as appropriate.
  3. Replenish the selective media every 3–4 days and observe the percentage of surviving cells.
  4. Determine the appropriate concentration of Blasticidin that kills the cells within 10–14 days after addition of antibiotic.
-

# Preparing MEF Culture Dishes

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## Coating culture vessels with gelatin

1. Cover the whole surface of each culture vessel with Attachment Factor (AF) solution and incubate the vessels for 30 minutes at 37°C or for 2 hours at room temperature.  
**Note:** AF is a sterile 1X solution containing 0.1 % gelatin available from Invitrogen (see page 34 for ordering information).
  2. Using sterile technique in a laminar flow culture hood, completely remove the AF solution from the culture vessel by aspiration.  
**Note:** It is not necessary to wash the culture surface before adding cells or medium. Coated vessels may be used immediately or stored at room temperature for up to 24 hours.
- 

## Thawing MEFs

1. Remove the cryovial containing inactivated MEFs from the liquid nitrogen storage tank.
  2. Briefly roll the vial between hands to remove frost, and swirl it gently in a 37°C water bath.
  3. When only a small ice crystal remains in the vial, remove it from water bath. Spray the outside of the vial with 70% ethanol before placing it in the cell culture hood.
  4. Pipet the thawed cells gently into a 15-mL conical tube.
  5. Rinse the cryovial with 1 mL of pre-warmed MEF medium. Transfer the medium to the same 15-mL tube containing the cells.
  6. Add 4 mL of pre-warmed MEF medium **dropwise** to the cells. Gently mix by pipetting up and down.  
**Note:** Adding the medium slowly helps the cells to avoid osmotic shock.
  7. Centrifuge the cells at  $200 \times g$  for 5 minutes.
  8. Aspirate the supernatant and resuspend the cell pellet in 5 mL of pre-warmed MEF medium.
  9. Remove 20  $\mu$ L of the cell suspension and determine the viable cell count using your method of choice.
-

## Preparing MEF Culture Dishes, continued

### Plating MEFs

1. Centrifuge the remaining cell suspension (step 9, page 27) at  $200 \times g$  for 5 minutes at room temperature.
2. Aspirate the supernatant. Resuspend the cell pellet in MEF medium to a density of  $2.5 \times 10^6$  cells/mL.
3. Aspirate the gelatin solution from the gelatin coated culture vessel (step 4, page 27), and wash the vessels once with PBS.
4. Add the appropriate amount of MEF medium into each culture vessel (refer to the table below).
5. Into each of these culture vessels, add the appropriate amount of MEF suspension (refer to the table below).  
**Note:** The recommended plating density for Gibco® Mouse Embryonic Fibroblasts (Irradiated) is  $2.5 \times 10^4$  cells/cm<sup>2</sup>.
6. Move the culture vessels in several quick back-and-forth and side-to-side motions to disperse the cells across the surface of the vessels.
7. Incubate the cells in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.
8. Use the MEF culture vessels within 3–4 days after plating.

Vessel size	Growth area	Volume of medium	Number of MEFs*	Volume of MEF suspension*
96-well plate	0.32 cm <sup>2</sup> /well	0.1 mL	$1.0 \times 10^4$ /well	4 µL
24-well plate	2 cm <sup>2</sup> /well	0.5 mL	$5.0 \times 10^4$ /well	20 µL
12-well plate	3.8 cm <sup>2</sup> /well	1 mL	$1.0 \times 10^5$ /well	40 µL
6-well plate	9.6 cm <sup>2</sup> /well	2 mL	$2.5 \times 10^5$ /well	0.1 mL
60-mm dish	19.5 cm <sup>2</sup>	5 mL	$5.0 \times 10^5$	0.2 mL
100-mm dish	58.95 cm <sup>2</sup>	10 mL	$1.5 \times 10^6$	0.6 mL
25-cm <sup>2</sup> flask	25 cm <sup>2</sup>	5 mL	$6.3 \times 10^5$	0.25 mL
75-cm <sup>2</sup> flask	75 cm <sup>2</sup>	15 mL	$1.9 \times 10^6$	0.75 mL

\* The number of MEFs and the volume of MEF suspension in these columns are based on a seeding density of  $2.5 \times 10^4$  cells/cm<sup>2</sup>, which is the recommended seeding density for Gibco® Mouse Embryonic Fibroblasts (Irradiated) (Cat. no. A11560). For other seeding densities, adjust the amounts accordingly.



### Important

The number of cells and the volume of cell suspension given in the table above are optimized **for MEFs only**. For plating other cell types, calculate the number of cells to be plated using the growth area and the recommended plating density for the specific cell type you are using.

# Generating a Stable Oct4-GFP Reporter Cell Line

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## Stable Oct4-GFP Reporter cell line

Lentivirus Oct4-GFP Reporter can be used for generating a stable Oct4-GFP reporter cell line that expresses Oct4-GFP upon successful reprogramming into iPSCs. Although hOct4-GFP expression is observed only in pluripotent cells, a small amount of basal GFP expression may be detected in non-pluripotent cells as a background (usually less than 10%).

To create a stable Oct4-GFP reporter cell line, transduce your cell target line with the Lentivirus Oct4-GFP Reporter and then perform Blasticidin selection to select for the stable transductants. This requires a minimum of 10–12 days after transduction, but allows the generation of clonal cell lines that stably express hOct4-GFP.

---

## Protocol for generating stable Oct4-GFP Reporter cell line

1. Maintain your cells to be transduced under the recommended culture conditions using the appropriate culture medium.
  2. Determine the appropriate amount of Lentivirus Oct4-GFP Reporter to use for transduction based on Lentivirus CMV-GFP expression (see page 13).
  3. Prepare the transduction cocktail by diluting the Lentivirus Oct4-GFP Reporter in lentivirus transduction medium (see page 24) to a combined volume of 1 mL per well of a 6-well plate.
  4. Add 1 mL of the reprogramming transduction cocktail in each well of a 6-well plate. If desired, add Polybrene<sup>®</sup> to a final concentration of 6 µg/mL (see page 23).
  5. Swirl the plate gently to mix and incubate it overnight in a 37°C incubator with a humidified atmosphere of 5% CO<sub>2</sub>.
  6. 24 hours following transduction, remove the medium from the transduction plate and replace with fresh, complete culture medium containing the appropriate amount of Blasticidin (see page 26).
  7. Incubate the cells at 37°C for 24 hours, then trypsinize and replate them into a larger-sized tissue culture format in fresh, complete culture medium containing Blasticidin.
  8. Replace medium with fresh medium containing Blasticidin every 2–3 days until you can identify Blasticidin-resistant colonies (generally 10–14 days after starting selection).
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*Continued on next page*

## Generating a Stable Oct4-GFP Reporter Cell Line, continued

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### Protocol for generating stable Oct4-GFP Reporter cell line, continued

9. Pick at least 10 Blasticidin-resistant colonies and expand each clone.  
Alternatively, you may pool the heterogeneous population of Blasticidin-resistant cells.  
**Note:** Integration of the lentivirus into the genome is random. Depending upon the influence of the surrounding genomic sequences at the integration site, you may see varying levels of gene expression from different Blasticidin-resistant clones. We recommend testing at least 10 Blasticidin-resistant clones, and selecting the clone that provides the lowest level of basal GFP expression.
  10. Once you have sufficient amount of Blasticidin-resistant colonies (i.e., stable Oct4-GFP reporter cells), aliquot to cryopreserve seeding stocks.
  11. To reprogram Oct4-GFP reporter cells, follow the basic reprogramming protocol (see page 15).
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# Generating an Inducible Reprogramming System

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## pLenti3.3/TR plasmid

The Lentivirus Reprogramming System enables the tetracycline-regulated expression of reprogramming genes. To generate an inducible system, you need to produce a Tet repressor virus using the pLenti3.3/TR plasmid, which is included in the ViraPower™ HiPerform™ T-REx™ Gateway® Expression System (Cat. no. A11141).

The pLenti3.3/TR plasmid contains the *TetR* gene and the Neomycin resistance marker to allow stable expression of the Tet repressor in any mammalian cell line. To use pLenti3.3/TR:

1. Co-transfect the vector and the ViraPower™ Packaging Mix into 293FT cells to generate a lentiviral stock.
2. Transfect the Lenti3.3/TR lentiviral construct into the mammalian cell line of choice.
3. Use Neomycin selection to generate a stable “ViraPower™ T-REx™” cell line expressing the Tet repressor. The ViraPower™ T-REx™ cell line becomes the host for reprogramming lentivirus particles.

For more information, refer to the ViraPower™ HiPerform™ T-REx™ Gateway® Expression System manual, which is available at [www.lifetechnologies.com](http://www.lifetechnologies.com).

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# iPSCs Derived from Different Species and Somatic Cell Types

## Introduction

iPSCs have been derived from a number of different species and somatic cell populations, demonstrating that the fundamental features of the transcriptional network governing pluripotency is universally conserved (Stadtfield & Hochedlinger, 2010). The table below, adapted from Stadtfield and Hochedlinger's review of induced pluripotency, lists the first demonstration of the reprogramming of a given cell type, the genetic factors used in reprogramming, and the smallest combination of reprogramming genes reported.

Species	Germ layer	Cell type	Factors*	Reference
Human	Mesoderm	Fibroblasts	OKSM	(Takahashi <i>et al.</i> , 2007)
			OSLN	(Yu <i>et al.</i> , 2007)
			OKS	(Nakagawa <i>et al.</i> , 2008)
		Mobilized peripheral blood	OKSM	(Loh <i>et al.</i> , 2009)
		Cord blood endothelial cells	OSLN	(Haase <i>et al.</i> , 2009)
		Cord blood stem cells	OKSM	(Eminli <i>et al.</i> , 2009)
			OS	(Giorgetti <i>et al.</i> , 2009)
	Adipose-derived stem cells	OKSM	(Sugii <i>et al.</i> , 2010)	
		OKS	(Aoki <i>et al.</i> , 2010)	
	Endoderm	Hepatocytes	OKSM	(Liu <i>et al.</i> , 2010)
	Ectoderm	Keratinocytes	OKSM	(Aasen <i>et al.</i> , 2008)
			OKS	(Aasen <i>et al.</i> , 2008)
Neural stem cells		O	(Kim <i>et al.</i> , 2009)	
Extraembryonic	Amniotic cells	OKSM	(Li <i>et al.</i> , 2009)	
		OSN	(Zhao <i>et al.</i> , 2010)	
* O: Oct4, K: Klf4, S: Sox2, M: c-Myc, N: Nanog, L: Lin28				

\* O: Oct4, K: Klf4, S: Sox2, M: c-Myc, N: Nanog, L: Lin28

*Continued on next page*

## iPSCs Derived from Different Species and Somatic Cell Types, continued

Species	Germ layer	Cell type	Factors*	Reference
Mouse	Mesoderm	Fibroblasts	OKSM OKS	(Takahashi & Yamanaka, 2006) (Nakagawa <i>et al.</i> , 2008; Wernig <i>et al.</i> , 2008)
		Mature B and T cells	OKSM	(Eminli <i>et al.</i> , 2009)
		Myeloid progenitors	OKSM	(Eminli <i>et al.</i> , 2009)
		Hematopoietic stem cells	OKSM	(Eminli <i>et al.</i> , 2009)
		Adipose-derived stem cells	OKSM	(Sugii <i>et al.</i> , 2010)
		Dermal papilla	OKM OK	(Tsai <i>et al.</i> , 2010) (Tsai <i>et al.</i> , 2010)
	Endoderm	Pancreatic $\beta$ cells	OKSM	(Stadtfield <i>et al.</i> , 2008)
		Hepatic endoderm	OKS	(Aoi <i>et al.</i> , 2008)
	Ectoderm	Neural stem cells	OK O	(Kim <i>et al.</i> , 2008) (Kim <i>et al.</i> , 2009)
		Melanocytes	OKM	(Utikal <i>et al.</i> , 2009)
Rat	Mesoderm	Fibroblasts	OKSM OKS	(Liao <i>et al.</i> , 2009) (Chang <i>et al.</i> , 2010)
	Endoderm	Liver progenitor cells	OKS	(Li <i>et al.</i> , 2009)
	Ectoderm	Neural progenitor cells	OKS	(Chang <i>et al.</i> , 2010)
Pig	Mesoderm	Embryonic fibroblasts	OKSM	(Esteban <i>et al.</i> , 2009)
Rhesus monkey	Mesoderm	Ear skin fibroblasts	OKSM	(Liu <i>et al.</i> , 2008)
Marmoset	Mesoderm	Skin fibroblasts	OKSM	(Wu <i>et al.</i> , 2010)
* O: Oct4, K: Klf4, S: Sox2, M: c-Myc, N: Nanog, L: Lin28				

## Accessory Products

**Lentivirus particles** The lentivirus particles containing the various genetic factors necessary for reprogramming are available individually and not as a set. Each vial contains 1 mL of lentivirus particles at a concentration of  $5 \times 10^7$  to  $5 \times 10^9$  transducing units/mL.

Product	Quantity	Cat. no.
Lentivirus hOct4	1 mL	A1357101
Lentivirus hSox2	1 mL	A1357201
Lentivirus hKlf4	1 mL	A1357301
Lentivirus hc-Myc	1 mL	A1357401
Lentivirus hNanog	1 mL	A1357501
Lentivirus hLin28	1 mL	A1357601
Lentivirus CMV-GFP Reporter	1 mL	A1357701
Lentivirus Oct4-GFP Reporter	1 mL	A1357801

**Media and reagents** The following media and reagents are suitable for use with the Lentiviral Reprogramming system. Ordering information is provided below. For more information, refer to [www.lifetechnologies.com](http://www.lifetechnologies.com) or contact Technical Support (page 36).

Product	Quantity	Cat. no.
D-MEM with GlutaMAX™-I (high glucose)	500 mL	10569-010
D-MEM/F-12	500 mL	10565-018
Fetal Bovine Serum (FBS), ES Cell-Qualified	100 mL	16141-061
Knockout™ Serum Replacement (KSR)	500 mL	10828-028
MEM Non-essential Amino Acids (NEAA)	100 mL	11140-050
Basic FGF, recombinant human	100 µg	PHG0261
β-mercaptoethanol	50 mL	21985-023
Antibiotic-Antimycotic	100 mL	15240-062
Attachment Factor	100 mL	S-006-100
TrypLE™ Select Cell Dissociation Reagent	500 mL	12563-029
Trypsin, 0.05% with EDTA 4Na	100 mL	25300-054
Collagenase Type IV	1 g	17104-019
D-PBS without Ca <sup>2+</sup> or Mg <sup>2+</sup>	500 mL	14190-144
Blasticidin	20 mL	A11139-02

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## Accessory Products, continued

### Antibodies for characterizing iPSCs

The following primary and secondary antibodies are used for characterizing iPSCs. Ordering information is provided below. For more information, refer to [www.lifetechnologies.com](http://www.lifetechnologies.com) or contact Technical Support (page 36).

Product	Quantity	Cat. no.
Mouse anti-Tra1-60 antibody	100 µg	41-1000
Mouse anti-Tra1-81 antibody	100 µg	41-1100
Mouse anti-SSEA4	100 µg	41-4000
Alexa Fluor® 594 goat anti-mouse IgG (H+L) antibody	0.5 mL	A11032

### Additional products

The following products are suitable for use with the Lentiviral Reprogramming system. Ordering information is provided below. For more information, refer to [www.lifetechnologies.com](http://www.lifetechnologies.com) or contact Technical Support (page 36).

Product	Quantity	Cat. no.
Gibco® Mouse Embryonic Fibroblasts (Irradiated)	1 mL	S1520-100
ViraPower™ HiPerform™ T-REx™ Gateway® Expression System	20 reactions	A11141

# Technical Support

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## Obtaining support

For the latest services and support information for all locations, go to [www.lifetechnologies.com](http://www.lifetechnologies.com).

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
  - Search through frequently asked questions (FAQs)
  - Submit a question directly to Technical Support ([techsupport@lifetech.com](mailto:techsupport@lifetech.com))
  - Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
  - Obtain information about customer training
  - Download software updates and patches
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## Safety Data Sheets (SDS)

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## Certificate of Analysis

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## References

---

- Aasen, T., Raya, A., Barrero, M. J., Garreta, E., Consiglio, A., Gonzalez, F., Vassena, R., Bilic, J., Pekarik, V., Tiscornia, G., Edel, M., Boue, S., and Izpisua Belmonte, J. C. (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 26, 1276-1284
- Andersson, S., Davis, D. L., Dahlbäck, H., Jörnvall, H., and Russell, D. W. (1989) Cloning, Structure, and Expression of the Mitochondrial Cytochrome P-450 Sterol 26-Hydroxylase, a Bile Acid Biosynthetic Enzyme. *J. Biol. Chem.* 264, 8222-8229
- Aoi, T., Yae, K., Nakagawa, M., Ichisaka, T., Okita, K., Takahashi, K., Chiba, T., and Yamanaka, S. (2008) Generation of pluripotent stem cells from adult mouse liver and stomach cells. *Science* 321, 699-702
- Aoki, T., Ohnishi, H., Oda, Y., Tadokoro, M., Sasao, M., Kato, H., Hattori, K., and Ohgushi, H. (2010) Generation of induced pluripotent stem cells from human adipose-derived stem cells without c-MYC. *Tissue Eng Part A* 16, 2197-2206
- Boshart, M., Weber, F., Jahn, G., Dorsch-Häsler, K., Fleckenstein, B., and Schaffner, W. (1985) A Very Strong Enhancer is Located Upstream of an Immediate Early Gene of Human Cytomegalovirus. *Cell* 41, 521-530
- Buchschacher, G. L., Jr., and Wong-Staal, F. (2000) Development of Lentiviral Vectors for Gene Therapy for Human Diseases. *Blood* 95, 2499-2504
- Burns, J. C., Friedmann, T., Driever, W., Burrascano, M., and Yee, J.-K. (1993) Vesicular Stomatitis Virus G Glycoprotein Pseudotyped Retroviral Vectors: Concentration to a Very High Titer and Efficient Gene Transfer into Mammalian and Nonmammalian Cells. *Proc. Natl. Acad. Sci. USA* 90, 8033-8037
- Chang, M. Y., Kim, D., Kim, C. H., Kang, H. C., Yang, E., Moon, J. I., Ko, S., Park, J., Park, K. S., Lee, K. A., Hwang, D. Y., Chung, Y., Lanza, R., and Kim, K. S. (2010) Direct reprogramming of rat neural precursor cells and fibroblasts into pluripotent stem cells. *PLoS One* 5, e9838
- Dull, T., Zufferey, R., Kelly, M., Mandel, R. J., Nguyen, M., Trono, D., and Naldini, L. (1998) A Third-Generation Lentivirus Vector with a Conditional Packaging System. *J. Virol.* 72, 8463-8471
- Emi, N., Friedmann, T., and Yee, J.-K. (1991) Pseudotype Formation of Murine Leukemia Virus with the G Protein of Vesicular Stomatitis Virus. *J. Virol.* 65, 1202-1207
- Eminli, S., Foudi, A., Stadtfeld, M., Maherali, N., Ahfeldt, T., Mostoslavsky, G., Hock, H., and Hochedlinger, K. (2009) Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. *Nat Genet* 41, 968-976
- Esteban, M. A., Xu, J., Yang, J., Peng, M., Qin, D., Li, W., Jiang, Z., Chen, J., Deng, K., Zhong, M., Cai, J., Lai, L., and Pei, D. (2009) Generation of induced pluripotent stem cell lines from Tibetan miniature pig. *J Biol Chem* 284, 17634-17640
- Giorgetti, A., Montserrat, N., Aasen, T., Gonzalez, F., Rodriguez-Piza, I., Vassena, R., Raya, A., Boue, S., Barrero, M. J., Corbella, B. A., Torrabadella, M., Veiga, A., and Izpisua Belmonte, J. C. (2009) Generation of induced pluripotent stem cells from human cord blood using OCT4 and SOX2. *Cell Stem Cell* 5, 353-357
- Haase, A., Olmer, R., Schwanke, K., Wunderlich, S., Merkert, S., Hess, C., Zweigerdt, R., Gruh, I., Meyer, J., Wagner, S., Maier, L. S., Han, D. W., Glage, S., Miller, K., Fischer, P., Scholer, H. R., and Martin, U. (2009) Generation of induced pluripotent stem cells from human cord blood. *Cell Stem Cell* 5, 434-441
- Hillen, W., and Berens, C. (1994) Mechanisms Underlying Expression of Tn10 Encoded Tetracycline Resistance. *Annu. Rev. Microbiol.* 48, 345-369

---

*Continued on next page*

## References, continued

---

- Izumi, M., Miyazawa, H., Kamakura, T., Yamaguchi, I., Endo, T., and Hanaoka, F. (1991) Blasticidin S-Resistance Gene (*bsr*): A Novel Selectable Marker for Mammalian Cells. *Exp. Cell Res.* 197, 229-233
- Kim, J. B., Greber, B., Arauzo-Bravo, M. J., Meyer, J., Park, K. I., Zaehres, H., and Scholer, H. R. (2009) Direct reprogramming of human neural stem cells by OCT4. *Nature* 461, 649-643
- Kim, J. B., Sebastiano, V., Wu, G., Arauzo-Bravo, M. J., Sasse, P., Gentile, L., Ko, K., Ruau, D., Ehrich, M., van den Boom, D., Meyer, J., Hubner, K., Bernemann, C., Ortmeier, C., Zenke, M., Fleischmann, B. K., Zaehres, H., and Scholer, H. R. (2009) Oct4-induced pluripotency in adult neural stem cells. *Cell* 136, 411-419
- Kim, J. B., Zaehres, H., Wu, G., Gentile, L., Ko, K., Sebastiano, V., Arauzo-Bravo, M. J., Ruau, D., Han, D. W., Zenke, M., and Scholer, H. R. (2008) Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. *Nature* 454, 646-650
- Kimura, M., Takatsuki, A., and Yamaguchi, I. (1994) Blasticidin S Deaminase Gene from *Aspergillus terreus* (*BSD*): A New Drug Resistance Gene for Transfection of Mammalian Cells. *Biochim. Biophys. ACTA* 1219, 653-659
- Lewis, P. F., and Emerman, M. (1994) Passage Through Mitosis is Required for Oncoretroviruses but not for the Human Immunodeficiency Virus. *J. Virol.* 68, 510-516
- Li, C., Zhou, J., Shi, G., Ma, Y., Yang, Y., Gu, J., Yu, H., Jin, S., Wei, Z., Chen, F., and Jin, Y. (2009) Pluripotency can be rapidly and efficiently induced in human amniotic fluid-derived cells. *Hum Mol Genet* 18, 4340-4349
- Li, W., Wei, W., Zhu, S., Zhu, J., Shi, Y., Lin, T., Hao, E., Hayek, A., Deng, H., and Ding, S. (2009) Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. *Cell Stem Cell* 4, 16-19
- Liao, J., Cui, C., Chen, S., Ren, J., Chen, J., Gao, Y., Li, H., Jia, N., Cheng, L., Xiao, H., and Xiao, L. (2009) Generation of induced pluripotent stem cell lines from adult rat cells. *Cell Stem Cell* 4, 11-15
- Liu, H., Ye, Z., Kim, Y., Sharkis, S., and Jang, Y. Y. (2010) Generation of endoderm-derived human induced pluripotent stem cells from primary hepatocytes. *Hepatology* 51, 1810-1819
- 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., and Deng, H. (2008) Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell* 3, 587-590
- Loh, Y. H., Agarwal, S., Park, I. H., Urbach, A., Huo, H., Heffner, G. C., Kim, K., Miller, J. D., Ng, K., and Daley, G. Q. (2009) Generation of induced pluripotent stem cells from human blood. *Blood* 113, 5476-5479
- Luciw, P. A. (1996) in *Fields Virology* (Fields, B. N., Knipe, D. M., Howley, P. M., Chanock, R. M., Melnick, J. L., Monath, T. P., Roizman, B., and Straus, S. E., eds), 3rd Ed., pp. 1881-1975, Lippincott-Raven Publishers, Philadelphia, PA
- Meissner, A., Wernig, M., and Jaenisch, R. (2007) Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat Biotechnol* 25, 1177-1181
- Nakagawa, M., Koyanagi, M., Tanabe, K., Takahashi, K., Ichisaka, T., Aoi, T., Okita, K., Mochiduki, Y., Takizawa, N., and Yamanaka, S. (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 26, 101-106
- Naldini, L. (1998) Lentiviruses as Gene Transfer Agents for Delivery to Non-dividing Cells. *Curr. Opin. Biotechnol.* 9, 457-463
- 

*Continued on next page*

## References, continued

---

- Naldini, L. (1999) in *The Development of Human Gene Therapy* (Friedmann, T., ed), pp. 47-60, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Nelson, J. A., Reynolds-Kohler, C., and Smith, B. A. (1987) Negative and Positive Regulation by a Short Segment in the 5'-Flanking Region of the Human Cytomegalovirus Major Immediate-Early Gene. *Molec. Cell. Biol.* 7, 4125-4129
- Pandya, S., Klimatcheva, E., and Planelles, V. (2001) Lentivirus and foamy virus vectors: novel gene therapy tools. *Expert Opinion on Biological Therapy* 1, 17-40
- Park, F., and Kay, MA. (2001) Modified HIV-1 based lentiviral vectors have an effect on viral transduction efficiency and gene expression *in vitro* and *in vivo*. *Mol Ther.* 4(3). 164-173
- Park, I. H., Lerou, P. H., Zhao, R., Huo, H., and Daley, G. Q. (2008) Generation of human-induced pluripotent stem cells. *Nat Protoc* 3, 1180-1186
- Stadtfield, M., Brennand, K., and Hochedlinger, K. (2008) Reprogramming of pancreatic beta cells into induced pluripotent stem cells. *Curr Biol* 18, 890-894
- Stadtfield, M., and Hochedlinger, K. (2010) Induced pluripotency: history, mechanisms, and applications. *Genes Dev* 24, 2239-2263
- Sugii, S., Kida, Y., Kawamura, T., Suzuki, J., Vassena, R., Yin, Y. Q., Lutz, M. K., Berggren, W. T., Izpisua Belmonte, J. C., and Evans, R. M. (2010) Human and mouse adipose-derived cells support feeder-independent induction of pluripotent stem cells. *Proc Natl Acad Sci U S A* 107, 3558-3563
- Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861-872
- Takahashi, K., and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-676
- Tsai, S. Y., Clavel, C., Kim, S., Ang, Y. S., Grisanti, L., Lee, D. F., Kelley, K., and Rendl, M. (2010) Oct4 and klf4 reprogram dermal papilla cells into induced pluripotent stem cells. *Stem Cells* 28, 221-228
- Utikal, J., Maherali, N., Kulalert, W., and Hochedlinger, K. (2009) Sox2 is dispensable for the reprogramming of melanocytes and melanoma cells into induced pluripotent stem cells. *J Cell Sci* 122, 3502-3510
- Wernig, M., Meissner, A., Cassady, J. P., and Jaenisch, R. (2008) c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* 2, 10-12
- Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B. E., and Jaenisch, R. (2007) In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* 448, 318-324
- Wu, Y., Zhang, Y., Mishra, A., Tardif, S. D., and Hornsby, P. J. (2010) Generation of induced pluripotent stem cells from newborn marmoset skin fibroblasts. *Stem Cell Res* 4, 180-188
- Yee, J.-K., Miyanohara, A., LaPorte, P., Bouic, K., Burns, J. C., and Friedmann, T. (1994) A General Method for the Generation of High-Titer, Pantropic Retroviral Vectors: Highly Efficient Infection of Primary Hepatocytes. *Proc. Natl. Acad. Sci. USA* 91, 9564-9568
- Yee, J. K. (1999) in *The Development of Human Gene Therapy* (Friedmann, T., ed), pp. 21-45, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- Yee, J. K., Moores, J. C., Jolly, D. J., Wolff, J. A., Respass, J. G., and Friedmann, T. (1987) Gene Expression from Transcriptionally Disabled Retroviral Vectors. *Proc. Natl. Acad. Sci. USA* 84, 5197-5201

---

*Continued on next page*

## References, continued

---

- Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., Slukvin, II, and Thomson, J. A. (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917-1920
- Yu, S. F., Ruden, T. v., Kantoff, P. W., Garber, C., Seiberg, M., Ruther, U., Anderson, W. F., Wagner, E. F., and Gilboa, E. (1986) Self-Inactivating Retroviral Vectors Designed for Transfer of Whole Genes into Mammalian Cells. *Proc. Natl. Acad. Sci. USA* 83, 3194-3198
- Zhao, H. X., Li, Y., Jin, H. F., Xie, L., Liu, C., Jiang, F., Luo, Y. N., Yin, G. W., Li, Y., Wang, J., Li, L. S., Yao, Y. Q., and Wang, X. H. (2010) Rapid and efficient reprogramming of human amnion-derived cells into pluripotency by three factors OCT4/SOX2/NANOG. *Differentiation* 80, 123-129
- Zufferey, R., Dull, T., Mandel, R. J., Bukovsky, A., Quiroz, D., Naldini, L., and Trono, D. (1998) Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery. *J. Virol.* 72. 9873-9880
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**Headquarters**

5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

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