Stem cell transfection guide

Introduction

Stem cells continue to show immense promise for the future of regenerative medicine and personalized therapeutic treatments. Since the discovery of induced pluripotent stem cells (iPSCs) in 2006, researchers have continued to manipulate and develop their potential for drug discovery, cell therapy, and disease modeling. In addition, differentiation strategies have evolved to allow researchers to direct pluripotent cells to specific progenitor lineages for more specialized research. More recently, genetic manipulation of stem cells has been achieved utilizing genome editing tools such as the CRISPR-Cas9 system and TAL effector nucleases. However, the lack of advanced technologies is hindering the pace of research. Improved delivery of DNA, RNA, or protein using validated solutions can help expedite the progress of research leading to new therapies. We have created the following guide as a starting point for the transfection of various types of stem cells. Our goal is to provide a holistic view of the options, trade-offs, and delivery recommendations for a range of stem cell models (Figures 1 and 2).

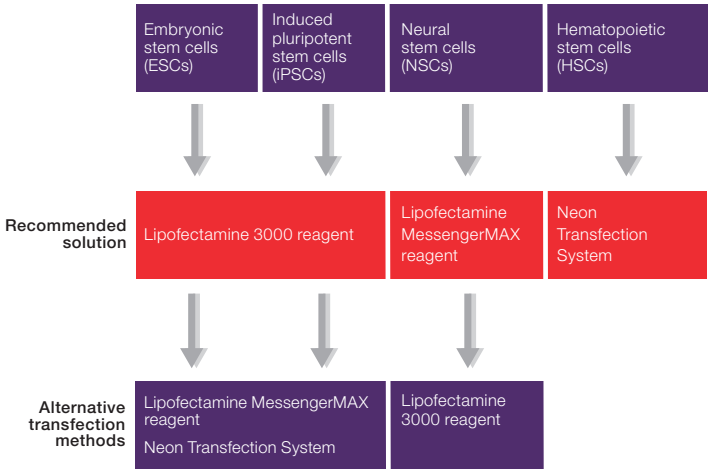

Figure 1. Recommended transfection methods, by cell type. High transfection efficiency, ease of use, and low toxicity make Invitrogen™ Lipofectamine™ 3000 Transfection Reagent the preferred choice for transfection of two types of stem cells. For researchers interested in other delivery methods, we also offer additional Invitrogen™ Lipofectamine™ reagents for mRNA transfection and lentiviral production as well as the Invitrogen™ Neon™ Transfection System for electroporation.

	Transfection products	ESCs	iPSCs	NSCs	HSCs
Performance					
	Lipofectamine 3000 reagent	+++	+++	++	NA
	Lipofectamine MessengerMAX reagent	++++	++++	+++	NA
	Neon Transfection System	++++	++++	+++	+++
Cell viability					
	Lipofectamine 3000 reagent	+++	+++	+++	NA
	Lipofectamine MessengerMAX reagent	++++	+++	+++	NA
	Neon Transfection System	++	++	++	++
Ease of use					
	Lipofectamine 3000 reagent	++++	++++	++++	NA
	Lipofectamine MessengerMAX reagent	+++	+++	+++	NA
	Neon Transfection System	++	++	++	++
Cost per reaction					
	Lipofectamine 3000 reagent	$	$	$	NA
	Lipofectamine MessengerMAX reagent	$$	$$	$$	NA
	Neon Transfection System	$$$	$$$	$$$	$$$

Figure 2. Trade-offs of delivery technologies by cell type. Options for gene expression and gene editing techniques are shown, along with the various factors that can influence experiments.

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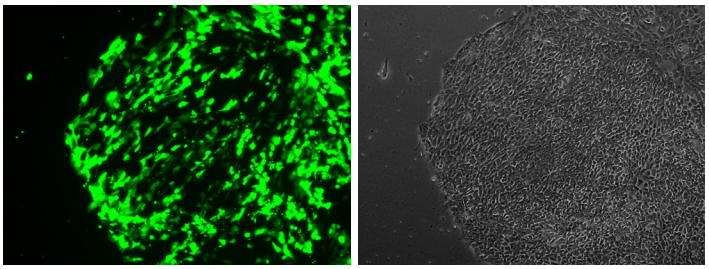
Results

Transfection results for our recommended delivery solutions are shown in Figure 3. High transfection efficiencies were observed with Lipofectamine 3000 reagent for the iPSCs and ESCs tested using a GFP reporter plasmid. For optimal results with more difficult-to-transfect cell lines such as NSCs and HSCs, we recommend using mRNA transfection or electroporation.

A Human Episomal iPSC Line

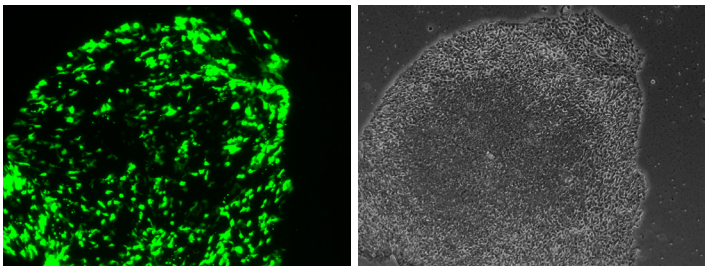
Experimental conditions	Recommendation
Delivery platform	Lipofectamine 3000 reagent: 1.5 µL/well
Plate format	12-well plate
DNA	GFP plasmid, 1.0 µg/well
Medium	MEF-conditioned medium with FGF-basic
Extracellular matrix	Gibco™ Geltrex™ matrix
Cell density	3.5 x 10^5 cells/well



Lipofectamine 3000 reagent
SSEA4$^+$/GFP$^+$: 69%

B H9 human ESCs

Experimental conditions	Recommendation
Delivery platform	Lipofectamine 3000 reagent: 1.5 µL/well
Plate format	12-well plate
DNA	GFP plasmid, 1.3 µg/well
Medium	MEF-conditioned medium with FGF-basic
Extracellular matrix	Gibco™ Geltrex™ matrix
Cell density	3.5 x 10^5 cells/well



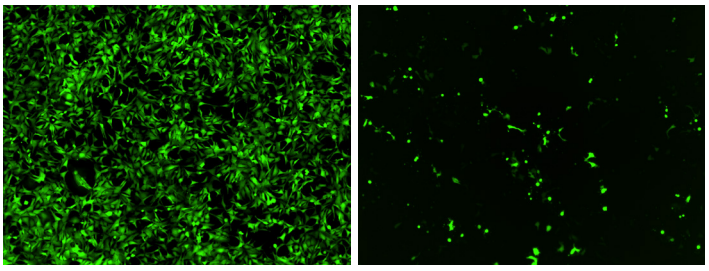
Lipofectamine 3000 reagent
SSEA4$^+$/GFP$^+$: 42%

Advantages of mRNA vs. DNA

The unique properties of mRNA make it more effective than DNA and a great alternative for transfection when working with difficult-to-transfect stem cell or progenitor models, such as NSCs. Since nuclear entry is not necessary with mRNA, transfection efficiency is generally much higher. Additional benefits include a transient transfection and faster time to protein expression than with DNA. Lipofectamine MessengerMAX reagent also allows mRNA transfection to be utilized for novel applications such as genome editing where genomic integration is a concern.

C Human Neural Stem Cells (H9-Derived)

Experimental conditions	Recommendation
Delivery platform	Lipofectamine MessengerMAX reagent: 1.5 µL per well
Plate format	24-well plate
mRNA	GFP mRNA, 0.5 µg/well
Medium	Gibco™ StemPro™ NSC SFM
Extracellular matrix	Gibco™ CTS™ CELLstart™ Substrate
Cell density	1x10^5 cells/well



Lipofectamine MessengerMAX reagent, mRNA
GFP$^+$: 92%

Leading reagent, DNA
GFP$^+$: 8%

Figure 3. Transfection results using various stem cell lines. (A) Gibco™ Human Episomal iPSC Line transfected using Lipofectamine 3000 reagent. **(B)** H9 human ESCs transfected using Lipofectamine 3000 reagent. **(C)** Gibco™ Human Neural Stem Cells (H9-Derived) transfected with mRNA using Lipofectamine MessengerMAX reagent or DNA using a leading DNA delivery reagent.

Transfection of HSCs

Transfection of HSCs and circulating blood cells is generally difficult, time consuming, and expensive. As a result, reagent solutions are not a viable option. Therefore, we recommend electroporation using the Neon Transfection System.

The experimental conditions below are specified for reprogramming of $CD34^+$ HSCs with the Invitrogen™ Epi5™ Episomal iPSC Reprogramming Kit. However, the Neon Transfection System with these conditions can also be used for standard delivery of DNA into PBMCs.

Experimental conditions	Recommendation
Delivery platform	Neon Transfection System (1,650 V/10 ms/3 pulses)
Neon tip and buffer	10 µL with Buffer T
DNA	2 µg
Medium	Gibco™ StemPro™-34 SFM with cytokines (SCF, IL-3, and GM-CSF)
Cell density	1.0–1.4 x 10^7 cells/mL

Other factors to consider for improved transfection of stem cells

In order to achieve optimal transfection efficiency, there are other key factors that need to be considered in addition to healthy cells and an optimized delivery protocol. Listed below are a few key factors that can influence an experiment in order of importance.

Ranking	Factors
Most important	Cell health
	Reagent dose
Important	Culture medium
	Medium supplements
	Extracellular matrices
Less important	Feeder vs. feeder-free culture
	Source of cells

Conclusions

There are many options for the transfection of stem cells, each with trade-offs in regards to performance, cell viability, ease of use, and cost. Many cell types require unique protocols for optimal transfection efficiency. For human iPSCs and ESCs, we recommend using Lipofectamine 3000 reagent because of its superior performance, ease of use, and versatility. It was developed as an optimized transfection solution for stem cells by providing a highly efficient, cost-effective alternative to electroporation. The advanced lipid nanoparticle technology minimizes the stress on cells compared to electroporation and enables advanced gene editing technologies.

When working with some progenitor stem cell models, such as NSCs or HSCs, alternative delivery solutions are needed. NSCs are difficult to transfect with a DNA delivery system, so Lipofectamine MessengerMAX reagent with mRNA is recommended for superior performance. Even though mRNA transfection does require some additional upfront work to prepare mRNA, the significantly improved efficiency outweighs this extra step. In addition, mRNA is significantly more cost-effective and easier to use than electroporation. Reagent options are ineffective for HSCs, so we recommend electroporation with the Neon Transfection System for superior efficiency. Thus, each stem cell type is unique and should be approached differently using the recommendations in this guide.

Transfection protocol

Human iPSCs and ESCs

The following protocol enables efficient transfection of human iPSCs or ESCs in MEF-conditioned medium (from feeder-free culture) using Lipofectamine 3000 reagent with Geltrex matrix–coated 12-well plates (Figure 4).

Materials

- Gibco™ DMEM/F-12, GlutaMAX™ supplement (Cat. No. 10565-018)
- Gibco™ FGF-Basic (AA 1-155) Recombinant Human Protein (Cat. No. PHG0264)
- Gibco™ Collagenase, Type IV (Cat. No. 17104019)
- Gibco™ Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix (Cat. No. A1413302)
- Gibco™ TrypLE™ Select Enzyme (Cat. No. 12563011)
- Gibco™ StemPro™ EZPassage™ Disposable Stem Cell Passaging Tool (Cat. No. 23181010)
- MEF-conditioned medium (harvested from MEF culture and filtered; add fresh FGF-basic at 4 ng/mL at time of use)
- Invitrogen™ Lipofectamine 3000™ Transfection Reagent (Cat. No. L3000015)
- Gibco™ Opti-MEM™ I Reduced Serum Medium (Cat. No. 31985062)

Preparation of collagenase IV solution (1 mg/mL, for 50 mL)

1. Add 50 mg Collagenase, Type IV to 50 mL DMEM/F-12.
2. Sterilize through a 0.22 µm filter and store at 4°C for up to 14 days.

Preparation of Geltrex matrix–coated 12-well plates

1. Thaw a 5 mL vial of Geltrex matrix at 2–8°C overnight.
2. Dilute the Geltrex matrix 1:1 with 5 mL of ice-cold D-MEM/F-12. Dispense the diluted stock as 1 mL aliquots into prechilled microcentrifuge tubes. These aliquots can be stored at –20°C or used immediately.
3. Prior to use, further dilute the Geltrex matrix stocks 1:50 with ice-cold DMEM/F-12 (for a final 1:100 dilution). Note: An optimal dilution of Geltrex matrix may need to be determined for each cell line. Try various final dilutions from 1:30 to 1:100.
4. Cover the entire surface of each well of a 12-well plate (4 cm^2 surface area) with 750 µL diluted Geltrex matrix solution.
5. Incubate in a 37°C, 5% CO_2 incubator for 1 hour. Note: Plates can now be used immediately or stored at 2–8°C for up to a week. Do not allow plates to dry out. If stored at 4°C, pre-equilibrate the plates at 37°C for at least 30 minutes prior to use.
6. Prior to use, aspirate the diluted Geltrex matrix solution from the culture plate and discard. Cells in MEF-conditioned medium can now be passaged directly onto the plates.

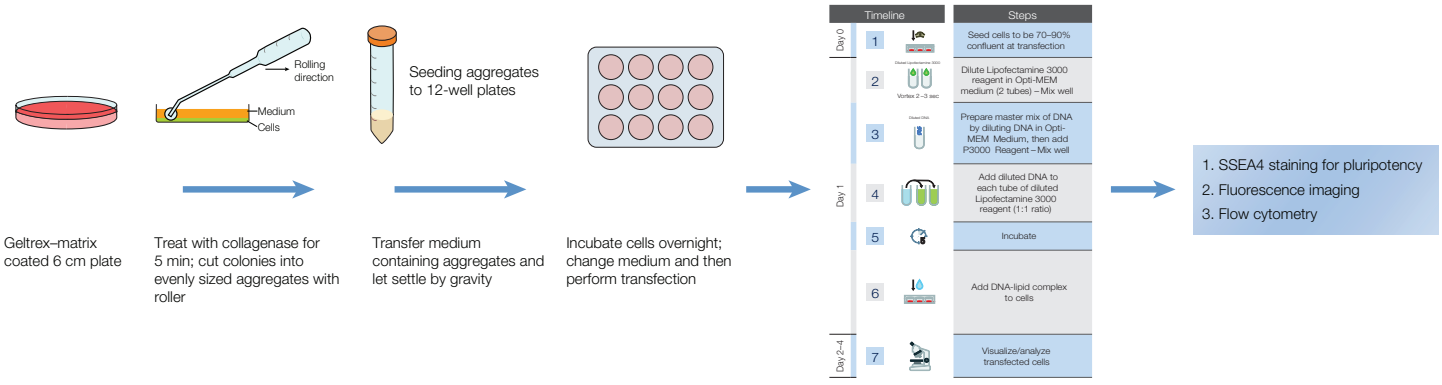


Figure 4. Protocol outline for transfection of pluripotent stem cells with Lipofectamine 3000 reagent. Cells are transferred into multiwell plates from Geltrex matrix–coated plates and transfected with Lipofectamine 3000 reagent. Transfection efficiency can be assessed using flow cytometry or fluorescence imaging.

Plating human iPSCs or ESCs on Geltrex matrix–coated 12-well plates

1. Assumed stem cell starting point: Geltrex matrix–coated 60 mm culture dishes with 70–80% confluent iPSCs or ESCs. Remove differentiated colonies with a Pasteur pipette by scraping them off and removing the spent medium.
2. An accurate cell number is required for seeding of cells and efficient transfection. Using approved laboratory procedures, determine cell count number and viability with either a hemocytometer or the Invitrogen™ Countess™ Automated Cell Counter. Pluripotency staining can also be performed using various markers if desired.
3. For dissociation of cells, roll the StemPro EZPassage Disposable Stem Cell Passaging Tool across the entire dish from left to right, then rotate the dish 90 degrees and roll the tool across the entire dish again. This will generate relatively uniformly sized cell clumps.
4. Use a cell scraper to gently detach the cells off the dish surface.
5. Transfer cell clumps using a 5 mL pipette into a 15 mL conical tube, taking care not to break up the clumps too much.
6. Add an additional 2 mL prewarmed conditioned medium to the dish to collect the residual clumps.
7. From the determined cell number at step 2, resuspend the cell clumps from step 6 to achieve 3.5 x 10^5 cells in 1 mL conditioned medium (with FGF-basic) for each well of the 12-well plate. Place plates in a 37°C, 5% CO_2 incubator overnight.

Transfection with Lipofectamine 3000 reagent

1. Replace the spent medium with fresh conditioned medium (with FGF-basic) right before transfection.
2. Prepare plasmid DNA–lipid complexes according to the Lipofectamine 3000 reagent protocol (Pub. No. MAN0009872). Example protocol for one well of a 12-well plate:
   a. Dilute 1.5 µL of Lipofectamine 3000 reagent in 50 µL of Opti-MEM medium and mix well.
   b. Dilute 1 µg of DNA in 50 µL of Opti-MEM medium, then add 2 µL P3000™ Reagent and mix well.
   c. Add diluted DNA–P3000 mix to diluted Lipofectamine 3000 reagent (1:1 ratio).
   d. Incubate for 5 minutes at room temperature.
3. Add lipid-DNA complexes to cells.
4. Incubate cells for 1–2 days at 37°C (change conditioned medium daily), then analyze transfected cells.

For additional protocols, please go to:
NSCs: **thermofisher.com/messengermax**
HSCs: **thermofisher.com/epi5**—Please see Epi5™ Episomal iPSC Reprogramming Kit User Guide pages 13–18

For additional CRISPR information, please go to:
CRISPR details: **thermofisher.com/CRISPR**
Stem cell editing: **thermofisher.com/genome-editing-stem-cells-app-note**

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Find out more at **thermofisher.com/transfection**

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