Lipofectamine® 3000 Reagent Protocol

Protocol Outline

A. Plate cells so they will be 70–90% confluent at the time of transfection.
B. Prepare plasmid DNA-lipid complexes (recommend 2 doses of lipid).
C. Add DNA-lipid complexes to cells.

Transfection Amounts

Component	96-well	24-well	6-well
DNA per well	100 ng	500 ng	2500 ng
P3000™ Reagent per well	0.2 µL	1 µL	5 µL
Lipofectamine® 3000 Reagent per well	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL

Transfection of siRNA

To transfect cells with siRNA, follow the protocol as described for DNA but **do not** add P3000™ Reagent when diluting the siRNA (step 3).

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Lipofectamine® 3000 Reagent Protocol

invitrogen by life technologies™

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Package Contents

Catalog Numbers	Size:
L3000001	0.1 mL
L3000008	0.75 mL
L3000015	1.5 mL
L3000075	5 × 1.5 mL
L3000150	15 mL

Storage Conditions

- Store at 4°C (do not freeze).

Required Materials

- Plasmid DNA (0.5–5 µg/µL stock)
- Opti-MEM® Reduced Serum Medium
- Microcentrifuge tubes

Timing

Preparation: 10 minutes
Incubation: 5 minutes
Final Incubation: 1–3 days

Selection Guide

Lipofectamine® Reagents
Go online to view related products.

Product Description

- Lipofectamine® 3000 Reagent is a proprietary formulation for transfecting nucleic acids into a wide range of eukaryotic cells and especially designed for hard to transfect cells

Important Guidelines

- Make DNA-Lipofectamine® 3000 complexes in serum-free medium such as Opti-MEM® Reduced Serum Medium and add directly to cells in culture medium, in the presence or absence of serum/antibiotic.
- It is not necessary to remove complexes or change/add medium after transfection.
- The amount of Lipofectamine® 3000 Reagent for successful transfection varies. Start any new transfection by testing the recommended two concentrations of Lipofectamine® 3000 Reagent to determine an optimum amount.

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

For Research Use Only. Not for use in diagnostic procedures.

Lipofectamine® 3000 Transfection Reagent Protocol

Transfect cells according to the following table. Use the indicated volume of DNA and P3000™ Reagent with each of the two volumes of Lipofectamine® 3000 (when performing optimization). **Each reaction mix volume is for one well** and accounts for pipetting variations. **Scale volumes proportionally for additional wells**.

Timeline			Steps
Day 0	1		**Seed cells to be 70–90% confluent at transfection**
	2	 Vortex 2–3 sec	**Dilute Lipofectamine® 3000 Reagent in Opti-MEM® Medium (2 tubes) – Mix well**
Day 1	3		**Prepare master mix of DNA by diluting DNA in Opti-MEM® Medium, then add P3000™ Reagent – Mix well**
	4		**Add Diluted DNA to each tube of Diluted Lipofectamine® 3000 Reagent (1:1 ratio)**
	5		**Incubate**
	6		**Add DNA-lipid complex to cells**
Day 2–4	7		**Visualize/analyze transfected cells**

Procedure Details (Two Reaction Optimization)

Component	96-well	24-well	6-well
Adherent cells	1–4 × 10^4	0.5–2 × 10^5	0.25–1 × 10^6
Opti-MEM® Medium	5 µL × 2	25 µL × 2	125 µL × 2
Lipofectamine® 3000 Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
Opti-MEM® Medium	10 µL	50 µL	250 µL
DNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 µg
P3000™ Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL
Diluted DNA (with P3000™ Reagent)	5 µL	25 µL	125 µL
Diluted Lipofectamine® 3000 Reagent	5 µL	25 µL	125 µL
Incubate for 5 minutes at room temperature.			

Component (per well)	96-well	24-well	6-well
DNA-lipid complex	10 µL	50 µL	250 µL
DNA amount	100 ng	500 ng	2500 ng
P3000™ Reagent	0.2 µL	1 µL	5 µL
Lipofectamine® 3000 Reagent used	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.			

Lipofectamine® 3000—efficient, reproducible transfection for biologically-relevant cell models

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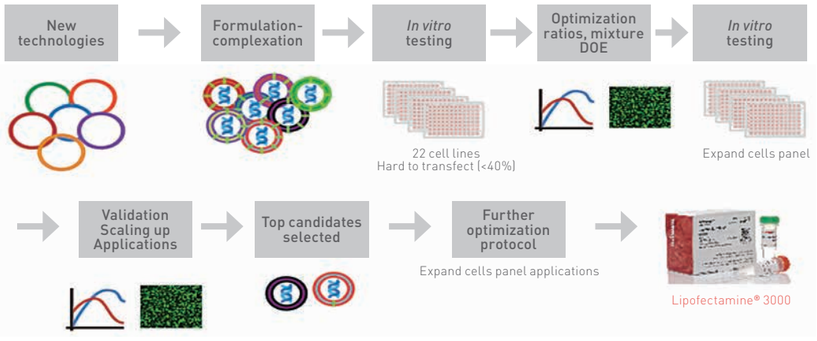
Introduction

With the increase of research in more biologically-relevant cell models, existing molecular and cellular techniques need to be improved. Lipofectamine® 3000, a new transfection reagent developed to improve delivery and enable use of new technologies, can be used in more relevant systems enabling faster and more reliable outcomes. Genome editing, stem cell manipulation, and immunotherapy are a few of the many rapidly growing areas that require more advanced techniques to maximize their potential applications. Lipofectamine® 3000 demonstrates significant improvement in a broader spectrum of cell lines when compared to current commercially available lipid-mediated transfection reagents. More importantly, Lipofectamine® 3000 has the potential to help propel many of these novel and exciting technologies forward.

Methods

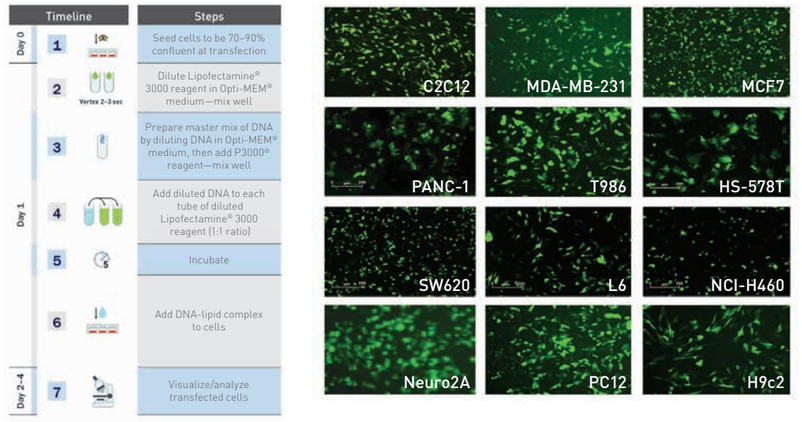
Figure 1. Strategy to identify Lipofectamine® 3000.



New technologies were screened in 22 hard-to-transfect cell lines using an EmGFP construct and analyzed by flow cytometry. Hard-to-transfect cell lines were defined as having less than 40% transfection efficiency. Hits were optimized and retested in expanded cell panels. Further optimization was performed to ensure reproducible and reliable results with the newly developed Lipofectamine® 3000.

Protocol

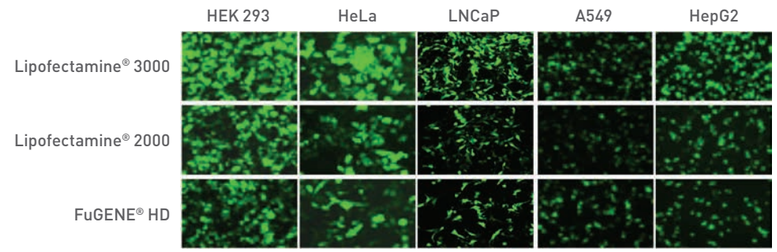
Figure 2. Enhanced and simple transfection protocol for a variety of cell lines.



The transfection protocol for Lipofectamine® 3000 was developed to be easy to use while still ensuring optimum performance and reliability in a wide panel of cell lines.

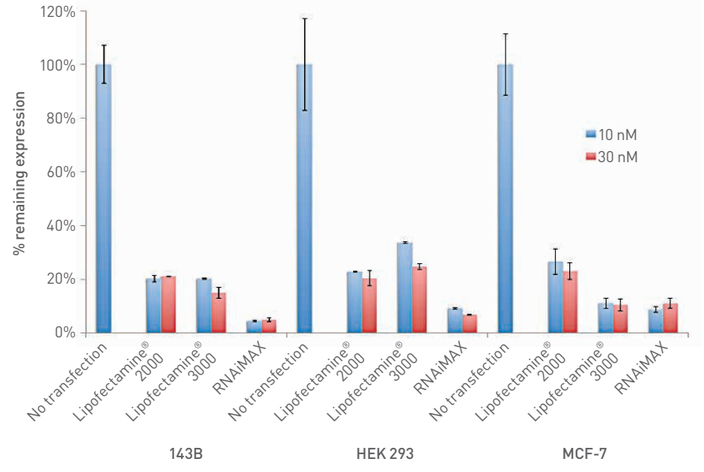
Results

Figure 3. Transfection efficiency and protein expression in various cell lines.



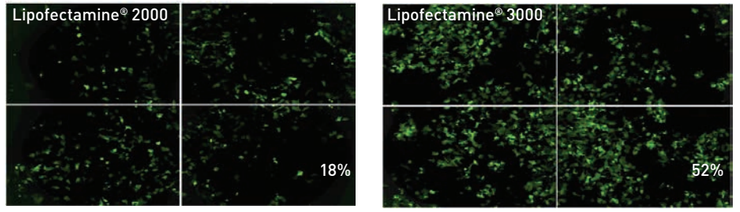
Each reagent was used to transfect HEK 293, HeLa, LNCaP, A549, and HepG2 cells in a 96-well format, and GFP expression was analyzed 48 hours posttransfection. Lipofectamine® 3000 reagent provided higher GFP transfection efficiency than Lipofectamine® 2000 and FuGENE® HD reagents for all five cell lines.

Figure 4. Delivery of siRNA for gene knockdown.



Lipofectamine® 3000 is a versatile reagent that can also be used to deliver siRNA using the same transfection protocol. Simply substitute siRNA for DNA. For this experiment, knockdown of endogenous luciferase was achieved in three engineered luciferase cell lines using Lipofectamine® 3000, Lipofectamine® 2000, and Lipofectamine® RNAiMax. Reagents were complexed with Silencer® Select siRNA targeting luciferase at the specified siRNA dosages.

Figure 5. Transfection in H9 embryonic stem cells.



Transfection performed in H9 human embryonic stem cells with Lipofectamine® 2000 and Lipofectamine® 3000 in a 96-well format. GFP expression analysis was performed 24 hours posttransfection.

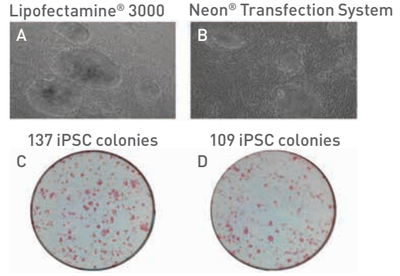
Lipofectamine® 3000 reagent for iPSC reprogramming.

Table 1. Culture and transfection conditions

Delivery method	Cell density	Media change posttransfection
Neon® Transfection System	1.0 x 10^4 per 6-well plate	Day 1–Day 14: use N2B27 media with 100 ng/mL FGF Day 15–Day 16: use StemPro® SFM Day 17: stain with alkaline phosphatase
Lipofectamine® 3000	3.0 x 10^4 per 6-well plate	

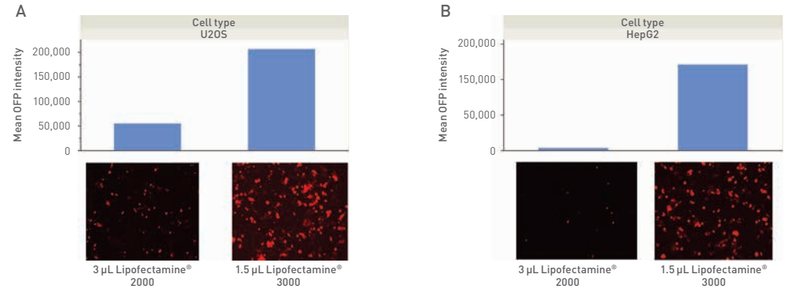
Cell culture and transfection conditions. Transfection was performed in BJ fibroblasts using the Neon® Transfection System at the recommended conditions and Lipofectamine® 3000 at 3.6 µL per well. Epi5™ Episomal iPSC Reprogramming Vectors were used (Cat. No. A14703). Media changes were performed daily according to the following protocol: Generation of human induced pluripotent stem cells (hiPSCs) from fibroblasts using episomal vectors (see Table 1).

Figure 6. Reprogramming results



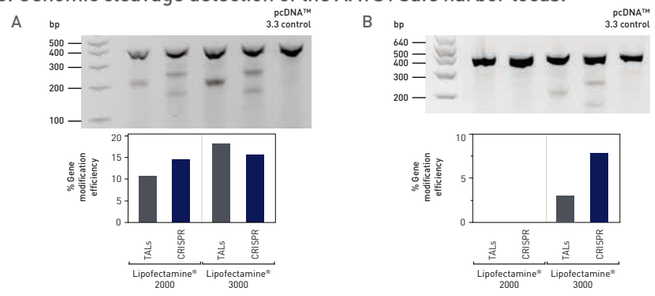
Results obtained, via brightfield microscopy, for (A) Lipofectamine® 3000 and (B) the Neon® Transfection System indicate that reprogramming was successful in generating iPSC colonies. A terminal stain was performed with red alkaline phosphatase and colony counts are indicated in (C) and (D). Detailed culture protocols can be found at **lifetechnoloiges.com**

Figure 7. Genomic modification using GeneArt® CRISPR Nuclease Vector Kits.



The all-in-one GeneArt® CRISPR vector system contains a Cas9 nuclease expression cassette and a guide RNA cloning cassette that was used to target the AAVS1 safe harbor locus; a downstream orange fluorescent protein (OFP) gene helps determine delivery efficiency and can also be used for enrichment. Lipofectamine® 2000 and Lipofectamine® 3000 were used to transfect U2OS and HepG2 cells in a 12-well format. Efficiency and OFP expression were analyzed 72 hours posttransfection and (A) U2OS and (B) HepG2 cells showed 4-fold and 80-fold improvement with Lipofectamine® 3000, respectively.

Figure 8. Genomic cleavage detection of the AAVS1 safe harbor locus.



Cleavage efficiency determined with the GeneArt® Genomic Cleavage Detection Kit. Lipofectamine® 2000 and Lipofectamine® 3000 were used to deliver GeneArt® Precision TALs and CRISPR nucleases targeting the AAVS1 safe harbor locus in U2OS and HepG2 cell lines in a 12-well format. Cell lysates were collected and processed to determine cleavage efficiency. Increased TALEN- and CRISPR-mediated cleavage were observed in both cell lines transfected with Lipofectamine® 3000. (A) U2OS cells transfected with Lipofectamine® 3000 showed 1.5-fold improved TALEN cleavage efficiency and slightly improved CRISPR cleavage. (B) HepG2 cells had 3-fold and 8-fold improved efficiency for TALEN- and CRISPR-mediated cleavage, respectively.

Cell type	Lipofectamine® 3000 reagent transfection efficiency	Fold protein expression improvement, Lipofectamine® 3000 vs. 2000 reagent
3T3		4
4T1		2
A431		2
A549		3
ACHN		2
bEnd.3		9
BJ		3
BT-549		4
C2C12		3
C6		5
Caco-2		2
Caki-1		4
CHO-K1		1
CHO-S		1
COLO 205		4
COS-7		4
DU 145		2
H460		3
H9c2		3
HCC1937		5
HCT116		1
HEK 293		2
HeLa		3
Hep-3B		2
Hepa 1-6		1
HepG2		9
Hs 578T		3
cHT29		1
Huh-7		4
Jurkat		1
K-562		1

Cell type	Lipofectamine® 3000 reagent transfection efficiency	Fold protein expression improvement, Lipofectamine® 3000 vs. 2000 reagent
L6		8
L929		2
LNCaP		6
MCF 10A		5
MCF7		2
MDA-MB-231		3
MDA-MB-435		1
MDA-MB-468		9
MDCK		1
Neuro-2a		1
NCI-H23		2
NCI-H460		17
P19		1
PANC-1		3
PC12		2
RAW264.7		4
RBL-2H3		2
RD		4
Saos-2		4
SH-SY5Y		1
SK-BR-3		4
SK-MEL-28		2
SK-N-SH		6
SK-OV-3		3
SW480		2
SW620		5
T98G		4
U2OS		3
U937		2
Vero		1

Transfection efficiency (%): <30% 30–50% 51–79% >80%

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