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

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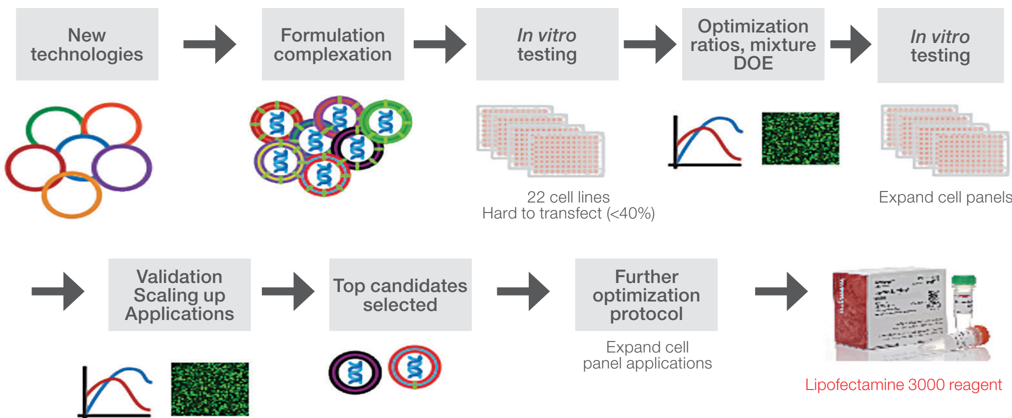
Introduction

With the increase of research in more biologically relevant cell models, existing molecular and cellular techniques need to be improved. Invitrogen™ Lipofectamine™ 3000 Transfection Reagent, a reagent developed to improve delivery and enable use of new technologies, can be used in more relevant systems and enables 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 reagent demonstrates significant improvement in a broader spectrum of cell lines when compared to current commercially available lipid-mediated transfection reagents. More importantly, Lipofectamine 3000 reagent has the potential to help propel many of these novel and exciting technologies forward.

Methods

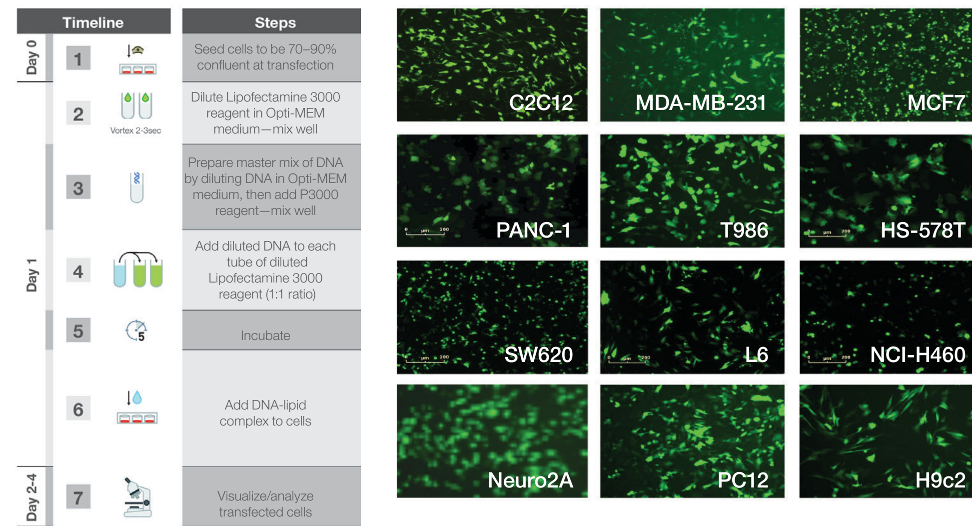
Figure 1. Strategy to develop Lipofectamine 3000 reagent.



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 reagent.

Protocol

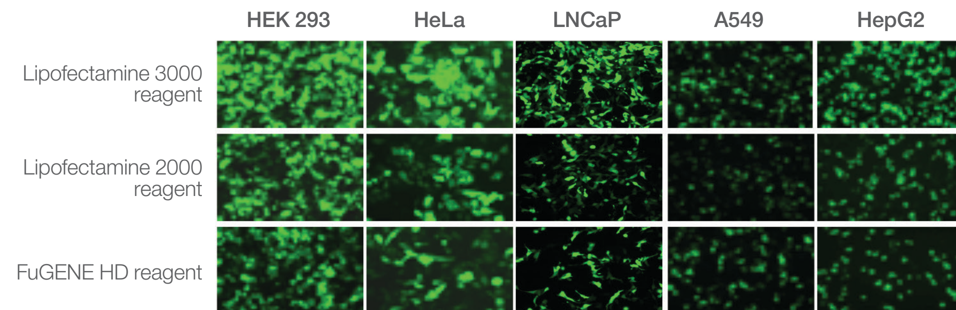
Figure 2. Enhanced and simple transfection protocol for a variety of cell lines.



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

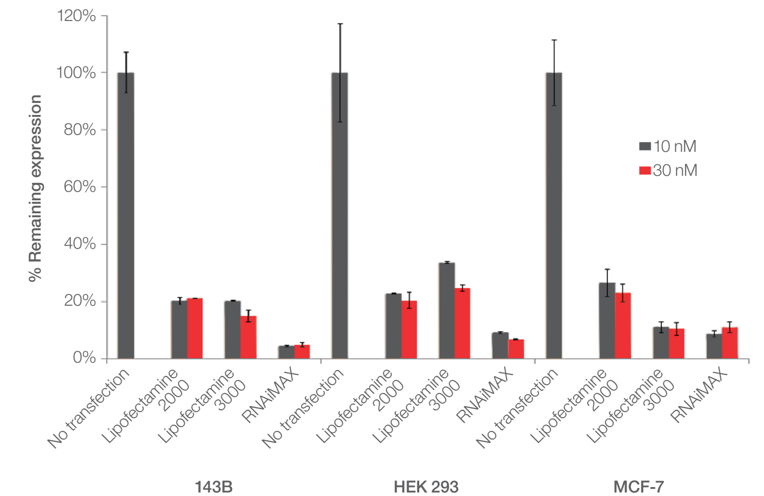
Results

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



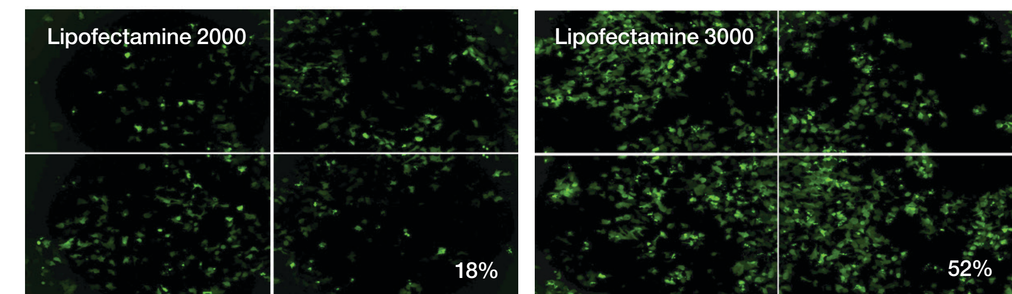
Each reagent was used to transfect HEK 293, HeLa, LNCaP, A549, and HepG2 cells in 96-well format, and GFP expression was analyzed 48 hours posttransfection. Lipofectamine 3000 reagent provided higher GFP transfection efficiency than Invitrogen™ Lipofectamine™ 2000 Transfection Reagent and FuGENE™ HD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

Figure 4. Delivery of siRNA for gene knockdown.



Lipofectamine 3000 reagent 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 reagent, Lipofectamine 2000 reagent, and Invitrogen™ Lipofectamine™ RNAiMAX Transfection Reagent. Reagents were complexed with Invitrogen™ Ambion™ Silencer™ Select siRNA targeting luciferase at the specified siRNA dosages.

Figure 5. Transfection in H9 embryonic stem cells.



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

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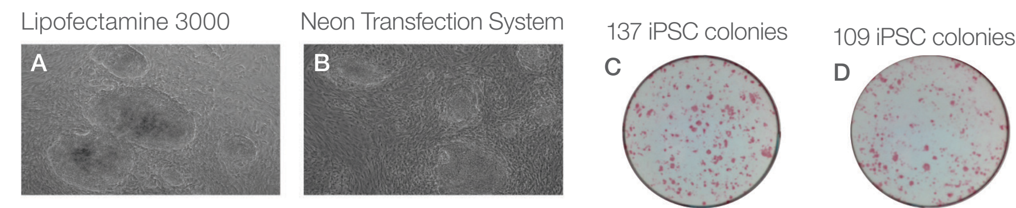
Lipofectamine 3000 reagent for iPSC reprogramming.

Table 1. Culture and transfection conditions.

Delivery method	Cell density	Media change posttransfection
Neon Transfection System	1.0 x 10^5 per 6-well plate	Day 1–day 14: use N-2™/B-27™ media with 100 ng/mL FGF Day 15–day 16: use StemPro SFM Day 17: stain with alkaline phosphatase
Lipofectamine 3000 reagent	3.0 x 10^5 per 6-well plate	

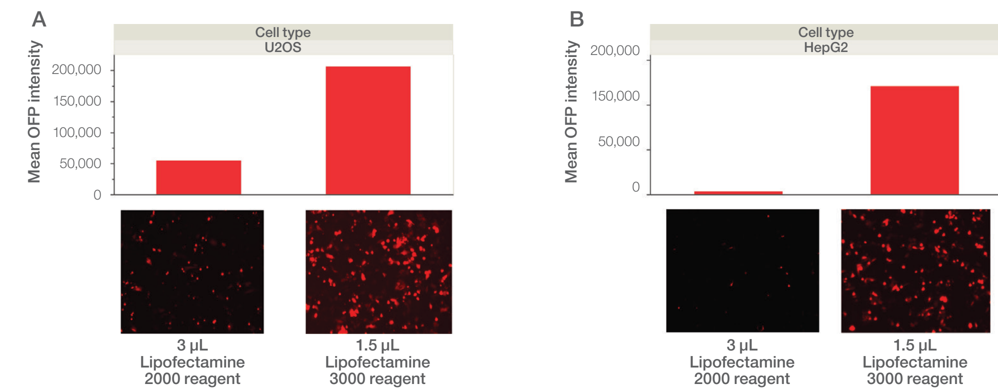
Transfection was performed in BJ fibroblasts using the Invitrogen™ Neon™ Transfection System at the recommended conditions and Lipofectamine 3000 reagent at 3.6 µL per well. Invitrogen™ Epi5™ Episomal iPSC Reprogramming Vectors were used (Cat. No. A14703). Media changes were performed daily according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

Figure 6. Reprogramming results.



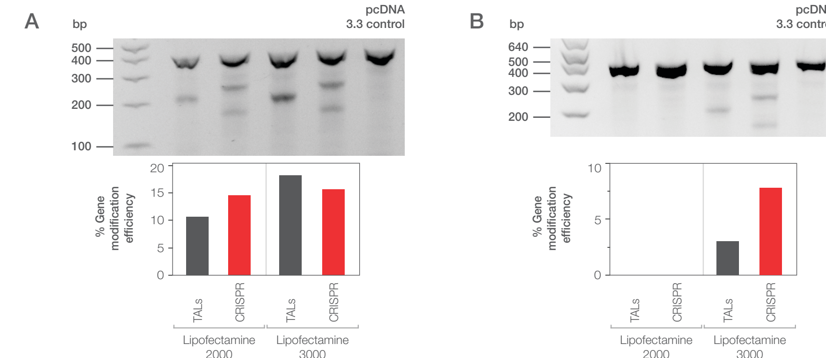
Results obtained, via brightfield microscopy, for **(A)** Lipofectamine 3000 reagent and **(B)** the Neon Transfection System indicate that reprogramming was successful in generating iPSC colonies. A terminal stain was performed with red alkaline phosphatase, and colony counts are indicated in **(C)**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

Figure 7. Genomic modification using Invitrogen™ 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 reagents were used to transfect U2OS and HepG2 cells in 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, respectively, with Lipofectamine 3000 reagent.

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



Cleavage efficiency was determined with the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit. Lipofectamine 2000 reagent and Lipofectamine 3000 reagent were used to deliver Invitrogen™ GeneArt™ Precision TALs and CRISPR nucleases targeting the *AAVS1* safe harbor locus in U2OS and HepG2 cell lines in 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 reagent. **(A)** U2OS cells transfected with Lipofectamine 3000 reagent 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	Protein expression improvement (x-fold), Lipofectamine 3000 vs. Lipofectamine 2000 reagents
3T3	51–79%	4
4T1	51–79%	2
A431	<30%	2
A549	51–79%	3
ACHN	30–50%	2
bEnd.3	<30%	9
BJ	<30%	3
BT-549	51–79%	4
C2C12	51–79%	3
C6	30–50%	5
Caco-2	51–79%	2
Caki-1	<30%	4
CHO-K1	51–79%	1
CHO-S	<30%	1
COLO 205	<30%	4
COS-7	51–79%	4
DU 145	30–50%	2
H460	51–79%	3
H9c2	51–79%	3
HCC1937	<30%	5
HCT116	>80%	1

Cell type	Lipofectamine 3000 reagent transfection efficiency	Protein expression improvement (x-fold), Lipofectamine 3000 vs. Lipofectamine 2000 reagents
HEK 293	>80%	2
HeLa	>80%	3
Hep-3B	51–79%	2
Hepa 1-6	51–79%	1
HepG2	>80%	9
Hs 578T	>80%	3
cHT29	<30%	1
Huh-7	51–79%	4
Jurkat	<30%	1
K-562	30–50%	1
L6	30–50%	8
L929	<30%	2
LNCaP	>80%	6
MCF 10A	30–50%	5
MCF7	30–50%	2
MDA-MB-231	51–79%	3
MDA-MB-435	51–79%	1
MDA-MB-468	<30%	9
MDCK	<30%	1
Neuro-2a	>80%	1
NCI-H23	51–79%	2
NCI-H460	<30%	17
P19	30–50%	1
PANC-1	51–79%	3
PC12	51–79%	2
RAW264.7	<30%	4
RBL-2H3	<30%	2
RD	51–79%	4
Saos-2	51–79%	4
SH-SY5Y	<30%	1
SK-BR-3	51–79%	4
SK-MEL-28	51–79%	2
SK-N-SH	30–50%	6
SK-OV-3	30–50%	3
SW480	51–79%	2
SW620	<30%	5
T98G	51–79%	4
U2OS	>80%	3
U937	<30%	2
Vero	30–50%	1

Transfection efficiency <30% ■□□□ 30–50% ■■□□ 51–79% ■■■□ >80% ■■■■

Find out more at **thermofisher.com/3000**

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