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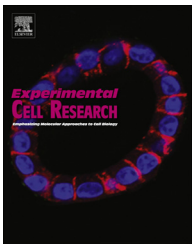

Research Article

A simple and efficient method for transfecting mouse embryonic stem cells using polyethylenimine

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ABSTRACT

Mouse embryonic stem cells (ESCs) can be transfected by electroporation, liposomal reagents, and viral transduction methods. The cationic polymer polyethylenimine (PEI) has been shown to transfect a variety of differentiated mammalian cell types, including mouse ESCs, but existing methods require the use of additional equipment that is not readily accessible to most labs. Here we describe conditions that permit for the efficient transfection of mouse ESCs with low cytotoxicity and without the need for specialized equipment. Our goal was to devise a protocol for the PEI-mediated transfection of mouse ESCs that was comparable in ease to commercial transfection reagents. For these studies, we compared PEI transfection efficiency and cytotoxicity to a well-known liposomal transfection reagent, Lipofectamine2000™ (LF2K), using fluorescence microscopy, flow cytometry, cell viability assays, and Western blotting. We provide evidence that PEI transfection of mouse ESCs compares favorably to LF2K. Our optimized protocol for efficient transfection of mouse ESCs with PEI is detailed in this report.

Introduction

Gene delivery systems are commonly used to transfect mammalian cells both transiently and stably for downstream analyses [1]. Transfection efficiency is cell-type specific and generally depends on the reagent. The most common techniques utilize liposomes, viruses, chemicals, and electroporation. Regardless of the gene delivery system, two critical goals are achieving high transfection efficiency and minimizing cytotoxicity. For mouse embryonic stem cells (ESCs), lipid-based reagents, such as Lipofectamine2000™ (LF2K), are often used because they meet both of these criteria. Despite their effectiveness, the cost of commercial lipid-based reagents can be a limiting factor for labs that work with mouse ESCs.

Linear 25 kDa polyethylenimine (PEI) is a relatively inexpensive cationic polymer that has been shown to be a suitable transfection reagent for several types of differentiated mammalian cells used in many labs, including but not limited to human embryonic kidney cells (HEK293) [2–6], African green monkey kidney cells (Cos-7), human cervical epithelial carcinoma cells (HeLa) [7], and human embryonal carcinoma cells (NCCIT) [2]. Comparative studies have been performed to assay PEI transfection efficiency

Abbreviations: ESCs, embryonic stem cells; PEI, polyethylenimine; LF2K, Lipofectamine 2000™; GFP, green fluorescent protein; HEK-293, Thuman embryonic kidney 293T cells

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compared to liposomal gene delivery, electroporation, and other chemical reagents [2,3,7]. While PEI has been used in many cell types, its utility has only begun to be demonstrated for mouse ESCs [8,9].

In this study, we set out to determine if PEI could be used for transfection of mouse ESCs in a manner similar to LF2K, and if so, to optimize the transfection conditions. By using LF2K transfection conditions, and then adjusting a number of the parameters, such as the amount of plasmid DNA, the concentration of PEI, the serum concentration, the final volume, and the order of component addition, we were able to determine the optimal transfection efficiency combined with low cytotoxicity. Flow cytometry was used to quantify the percentage of ESCs transfected with green fluorescent protein (GFP) using both LF2K and PEI. We also used Western blotting to show the efficient transfection and expression of unrelated plasmids. To ensure that the pluripotency of the ESCs remained unaffected by transfection, we measured the expression of both *Oct4* (*Pou5f1*) and *Nanog* by RT-qPCR. While LF2K transfects ESCs with high efficiency and low cytotoxicity, PEI also transfects ESCs with high efficiency, maintains the expression of *Oct4* and *Nanog*, while having the added benefit being slightly less toxic to ESCs. The protocol we developed for using cost-effective PEI results in approximately 34% of mouse ESCs being transfected, making PEI an alternative transfection reagent for mouse ESCs that performs comparably to commercial reagents such as LF2K.

Materials and methods

Cell culture

Feeder-free wild-type mouse ESCs (E14K) were grown on 0.1% gelatin-coated plates with high glucose DMEM (Invitrogen) supplemented with 15% fetal bovine serum (HyClone), 1% non-essential amino acids, 1% sodium pyruvate, 1% L-glutamine, 1% penicillin/streptomycin (Gibco) 55 μM 2-mercaptoethanol, and 1000 units/mL recombinant leukemia inhibitory factor (LIF) [10] or ESGRO (Millipore). Media were replenished every other day.

Microscopy

ESCs were visualized 24 h post-transfection using an Advanced Microscopy Group (AMG) Evos fluorescent microscope. Images presented in this manuscript are under 10-fold magnification using either transmitted light or a green fluorescent protein filter.

Flow cytometry

Twenty-four hours post-transfection, ESCs were washed once with PBS, trypsinized, and centrifuged at 1500 rpm for 2 min. ESCs were resuspended in PBS supplemented with 0.1 mM EDTA to prevent clumping. A BD Accuri C6 flow cytometer was used to detect 2 μg pMaxGFP transfected in WT mESCs. The detection threshold was set using a ddH_2O blank run to eliminate spurious events. 200,000 events were collected per sample. Gating was based on untransfected ESCs and percent transfected ESCs were calculated after the gating was set. Each sample was assayed in triplicate.

Transfection reagents

Linear polyethylenimine was obtained from Polysciences, Inc. (cat. no. 23966). A stock solution of 40 μM PEI was prepared in 25 mM HEPES buffer [140 mM NaCl, 1.5 mM Na_2HPO_4, and pH adjusted to 7.05 using 5 N NaOH]. The solution was sterilized through a 0.2 μM filter and stored at −20 °C. Lipofectamine2000™ was purchased from Life Technologies (cat. no. 11668027).

Lipofectamine2000™ transfection protocol

The ESC transfection protocol, provided by Dr. Brad Doble (McMaster University), was adapted from [11]. 2 μg DNA was diluted in 50 μL Opti-MEM and 4 μL LF2K was diluted in 46 μL Opti-MEM per transfection reaction for a 6-well plate. LF2K:Opti-MEM was incubated at room temperature for 5 min. DNA:Opti-MEM was combined with LF2K:Opti-MEM and incubated at room temperature for 20 min. During the DNA:LF2K:Opti-MEM incubation, 10 cm confluent dishes of wild-type mouse ESCs were trypsinized and centrifuged at 1500 rpm for 2 min. Cells were resuspended in complete ESC growth media and live cells were counted using 0.4% Trypan Blue and a Countess Automated Cell Counter (Invitrogen). 1×10^6 cells per 6-well transfection reaction were aliquoted and centrifuged at 1500 rpm for 2 min. ESCs were resuspended in the DNA:LF2K:Opti-MEM mixture. The transfection reaction was plated in a 6-well plate with complete ESC media.

Modified PEI transfection protocol

1×10^6 cells per 6-well transfection reaction were aliquoted and centrifuged at 1500 rpm for 2 min. ESCs were immediately resuspended in 300 μL Opti-MEM and mixed by pipetting. 2 μg plasmid DNA then added to cells and gently mixed. 100 μL PEI added to ESC:DNA:Opti-MEM mixture and mixed by gentle pipetting, then incubated at room temperature for 30 min. ESC:DNA:Opti-MEM mixture was then added to single well of 6-well plate.

Protein isolation and Western blot

Protein expression in LF2K and PEI-transfected ESCs was compared by Western blotting. ESCs were transfected with 200 ng pMaxGFP and 1800 ng Gsk-3β pDEST40. ESCs were resuspended in lysis buffer (137 mM NaCl, 10 mM Tris, pH 7.4, 1% Nonidet P-40) containing protease inhibitor cocktail (1:100 Sigma) and lysed by vigorous vortexing. Lysates were electrophoresed (25 μg/lane) through 7.5% Tris/Tricine gels and transferred onto nitrocellulose membranes at 100 V for 1 h. Blots were blocked for 1 h with either 5% milk (V5 antibody)/TBST (150 mM NaCl, 50 mM Tris, pH 7.4, 0.1% Tween) or 5% BSA (tubulin)/TBST. Blots were incubated in primary antibody diluted in 5% milk/TBST or 5% BSA/TBST for 22 h at 4 °C. Anti-V5 mouse mAb (Invitrogen cat. no. R960-25) was diluted 1:5000 and anti-tubulin rabbit mAb (Cell Signaling Technologies cat. no. 2125) was diluted 1:1000. Blots were incubated in anti-mouse or anti-rabbit IgG HRP secondary antibody (GE Healthcare) diluted 1:5000 in 5% milk/TBS or 5% BSA/TBST for 30 min. Proteins were visualized using ECL Plus detection reagents (GE Healthcare). Blots were stripped in a buffer consisting of 2% SDS, 62.5 mM Tris–HCl. pH 6.7, and 100 mM

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