Highly Efficient Transfection of Mouse ES Cells With TransFectin™ Lipid Reagent

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Introduction

Embryonic stem (ES) cells have become invaluable tools in the generation of animal models with single gene mutations, which are used in research areas as diverse as developmental biology, genomics, or cancer research. In addition, ES cells provide a powerful resource to study early differentiation processes because of their unlimited capacity for self-renewal and their potential to differentiate into a number of different cell types and tissues.

The introduction of genetic material into these cells is not only required for the generation of mutated alleles, but also can be used to study the function of specific proteins or RNAs in the differentiation programs mentioned above. This can be achieved by overexpression of the respective genes or the reduction of endogenous transcript levels by expressing small hairpin RNAs inducing RNA interference (Kunath et al. 2003).

There are several established methods for the introduction of DNA into ES cells. Electroporation (Doetschman et al. 1988), on one hand, is fast, but requires large numbers of cells and DNA and subjects the cells to extremely harsh experimental conditions, leading to low survival rates. Retroviral gene transfer (Blesch 2004), on the other hand, provides very effective means for the introduction of foreign genetic material. However, it is time consuming, as it involves the production of infectious retroviral particles in a packaging cell line before the final target cell can be infected. In addition, there is a size limit to the retroviral genome that can be packaged into a virion, sometimes providing a practical obstacle when large DNA segments have to be transferred into a recipient cell. Also, the use of retroviruses invariably leads to stable integration of the provirus in the host genome, which, due to the insertional mutagenesis event associated with it, is not always desired, for example, in situations where only transient expression of a gene is necessary.

These days, a number of differently formulated transfection reagents are available that have been used successfully in many cultured mammalian cell lines. Most of them are simple to use and exhibit low toxicity. Here we compare three such reagents (based on different classes of chemical compounds) with respect to their efficiency in the transfection of a mouse ES cell line using an enhanced Green Fluorescent Protein (EGFP) expression vector to estimate the number of transfected cells.

Methods

Transfection

For transfection of E14 mouse ES cells (Hooper et al. 1987) in the absence of a feeder layer, standard 24-well tissue culture plates were coated with gelatin by covering the culture surface for 5 min with a 0.1% (w/v) aqueous gelatin solution. Directly after aspiration of the gelatin, 10^5 cells per well were seeded in regular growth medium (DMEM, 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 µM nonessential amino acids, 1,500 U/ml leukemia inhibitory factor, 10^{-6} M β-mercaptoethanol) and allowed to attach to the surface overnight. The next day, the cells were transfected with one of three commercially available transfection reagents: Bio-Rad's TransFectin transfection reagent, another supplier's linear polyethyleneimine, which acts as a "proton sponge" (reagent A), or a third supplier's nonliposomal blend of lipids (reagent B). For transfection with TransFectin, 50 µl growth medium without serum was mixed with 0.8 µg plasmid DNA and another 50 µl of the same medium with 2 µl TransFectin. Both mixtures were combined and incubated for 20 min at room temperature. During this time, the cells were re-fed with 500 µl complete growth medium containing serum. Afterwards, the transfection solution was added dropwise to the wells. The TransFectin-containing medium was removed 4 hr later, and the cells were cultivated with fresh growth medium until analysis. Transfections with other reagents were performed according to the manufacturers' specifications.

Analysis of Transfection Efficiency

A derivative of the reporter plasmid pEGFP-C (Clontech) was used to quantitate transfection efficiencies. In this derivative, the EGFP coding sequence was placed under transcriptional control of the human elongation factor 1α (EF-1α) gene promoter. Control transfections were performed with plasmid pcDNA3.1 (Invitrogen).

ES cells were harvested by trypsinization 2 days after transfection, collected by centrifugation for 5 min at 180 x g, washed once with 2 ml PBS, resuspended in 500 µl 2% (v/v) paraformaldehyde in PBS, and analyzed using a FACScan system (Beckton-Dickinson).

Results

Whereas at most 3% of the cells could be transfected with the two other commercially available reagents, the use of TransFectin resulted in about 20% of cells expressing the plasmid-coded EGFP gene, indicating a roughly 7-fold higher transfection efficiency (Figure 1). This efficiency was obtained with only a 4 hr TransFectin treatment, after which no obvious damage of the cells was visible by microscopic observation.

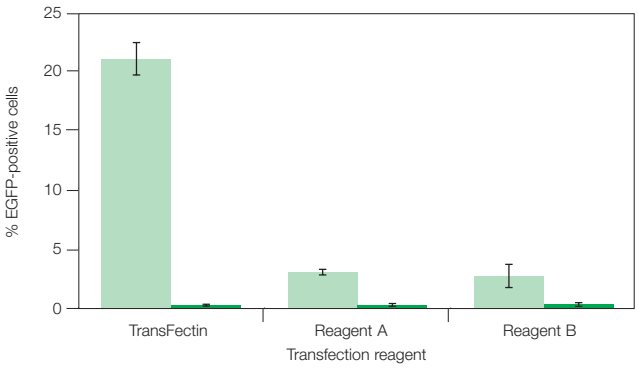

Fig. 1. Transfection efficiencies of E14 mouse ES cells obtained using three commercially available transfection reagents. Shown is the percentage of EGFP-positive cells (mean ± SEM of three independent transfections) 2 days after transfection with a reporter plasmid, pEGFP-C (■), or a control plasmid, pcDNA3.1 (■). Control expression was <0.1% for all three reagents.

Conclusions

Especially in situations where only short-term transient expression of foreign genes in mouse ES cells is required, such as when recombinases like Cre or Flp are used for genomic engineering purposes and selective pressure cannot be applied, it is mandatory to transfect a large proportion of the cells. Efficient transfection is also advantageous when the derivation of stably transfected cells is desired. We have shown that a fairly short treatment of mouse E14 ES cells with TransFectin is sufficient to obtain a large proportion of transfected cells. It is possible that even higher transfection rates could be achieved by longer incubation with the reagent. However, it seems advisable to keep treatments of ES cells, which are generally used for differentiation studies or generation of whole animals, as short as possible to avoid any adverse effects on pluripotency and differentiation capacity.

References

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