Production of Lentiviruses Using HEKFectin™ Cell-Specific Lipid Reagent for Multiplasmid Transformation

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Introduction

Transfection of DNA into mammalian cells is commonly used to study the regulation and function of genes, as well as to express proteins and produce viruses. A number of transfection reagents with unique chemistries are available for such applications. HEKFectin cell line-specific lipid reagent is a mixture of a cationic compound and a colipid specifically formulated for transfecting HEK 293 cells and HEK 293-derived cell lines with maximum efficiency and minimal toxicity.

A popular system for studying insulin signalling, glucose homeostasis, and lipid loading is the 3T3-L1 preadipocyte differentiation model. Our laboratory uses a lentivirus vector system derived from the human immunodeficiency virus (HIV-1) to generate virus particles for the introduction of transgenes into 3T3-L1 fibroblasts and differentiated, nondividing 3T3-L1 adipocytes (Dull et al. 1998, Carlotti et al. 2004). This third-generation lentivirus system uses only a subset of HIV-1 genes (*gag/pol* for capsid proteins, and *rev* for reverse transcriptase), and the lentiviruses are pseudotyped with the glycoprotein from the vesicular stomatitis virus (VSV-G) to infect a variety of cell types. The *gag/pol*, *rev*, and *VSV-G* genes (collectively referred to as the packaging mixture) are encoded on three separate vectors, while a fourth plasmid carries the lentiviral expression cassette containing the gene of interest. To produce the lentivirus, all four constructs are simultaneously transfected into HEK 293T host cells, and the culture supernatant containing infectious viral particles is subsequently harvested.

In this article, we demonstrate the effectiveness of HEKFectin lipid reagent as compared to traditional calcium phsphate-based transfection for virus production in HEK 293T cells .

Methods

Plasmid Mix

HEK 293T cells were transfected with the packaging mixture and a lentiviral vector with the green fluorescent protein (GFP) gene cloned downstream of the human phosphoglycerate kinase (PGK) promoter (pRRL-PGK-GFP).

Transfection With HEKFectin

The day before transfection, HEK 293T cells were plated to a density that would yield 70–80% confluence the following day. HEKFectin lipid was added to a serum-free medium (Ham's F12, PAA Laboratories GmbH, Germany) in a polystyrene container, and the DNA was separately diluted in the same serum-free medium to the final concentration. We found that 16 µl HEKFectin lipid and approximately 5 µg total DNA were sufficient to transfect adherent HEK 293T cells in a 25 cm^2 dish.

For transfection, 1 µg DNA was mixed with 3 µl HEKFectin solution and the mixture was incubated for 20 min at room temperature to form DNA-lipid complexes. This mixture was added directly to the cells in serum-containing medium (Hams' F12 with 10% fetal calf serum, PAA Laboratories GmbH). Fresh medium was added 24 hr posttransfection. Supernatant was harvested 48 and 72 hr posttransfection and passed through a 0.45 µm filter. The amount of virus was measured using an antigen-capture ELISA for HIV-1 p24 protein (Gentaur).

To infect 3T3-L1 fibroblasts, the recovered virus-containing supernatant was added to the cells at a concentration of 40 ng p24 per 10^5 cells, corresponding to a multiplicity of infection (MOI) of 1. To infect differentiated 3T3-L1 adipocytes, 120 ng p24 was added per 10^5 cells (an MOI of 3) in the presence of 8 µg/ml hexadimethrine bromide (Sigma-Aldrich). Cells were incubated with virus overnight, and the medium was changed the following day.

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Transfection with Calcium Phosphate

The day before transfection, HEK 293T cells were seeded in a T25 flask. The medium was refreshed 2 hr prior to transfection, and for transfection, 10.9 µg total DNA (the plasmid mix) was used. Cells were transfected in a 25 cm^2 dish, and the medium was refreshed 24 hr posttransfection to remove precipitates. Virus-containing supernatant was collected 48 hr and 72 hr posttransfection.

Analysis of Transfection Efficiency

Transfection efficiency was analyzed 3–7 days posttransduction. Cells were fixed with paraformaldehyde, nuclei were stained with TO-PRO-3 iodide (Invitrogen Corp.), and cells were analyzed for GFP expression using confocal fluorescence microscopy.

Results

Viral production in HEK 293T cells using HEKFectin lipid was rapid, efficient, and reliable. At 24 hr posttransfection, we detected GFP-positive HEK 293T cells, and the fluorescence intensity increased to a maximum after 48 hr. As judged by GFP fluorescence (Figure 1), the transfection efficiency of HEK 293T cells was approximately 80%. We observed no cytotoxicity, and the transfected cells grew similarly to untransfected controls.

HEK 293T cells transfected with the packaging mixture and GFP construct produced GFP-encoding virus, which was secreted into the supernatant. Supernatants harvested 48 hr and 72 hr posttransfection were pooled, and the viral titer as determined by p24 ELISA, reached ~300 ng/ml, corresponding to 7.5×10^5 infectious particles/ml.

GFP-encoding lentiviruses were used to infect 3T3-L1 fibroblasts and differentiated 3T3-L1 adipocytes. After 7 days of cell culture, GFP fluorescence was analyzed by confocal microscopy. As shown in Figure 2, nearly all cells were GFP positive, indicating that they were effectively infected. The infected cells remained viable for at least 2 weeks (data not shown).

By comparison, the transfection efficiency of HEK 293T cells using the calcium phosphate method was lower than 50% (Figure 3).

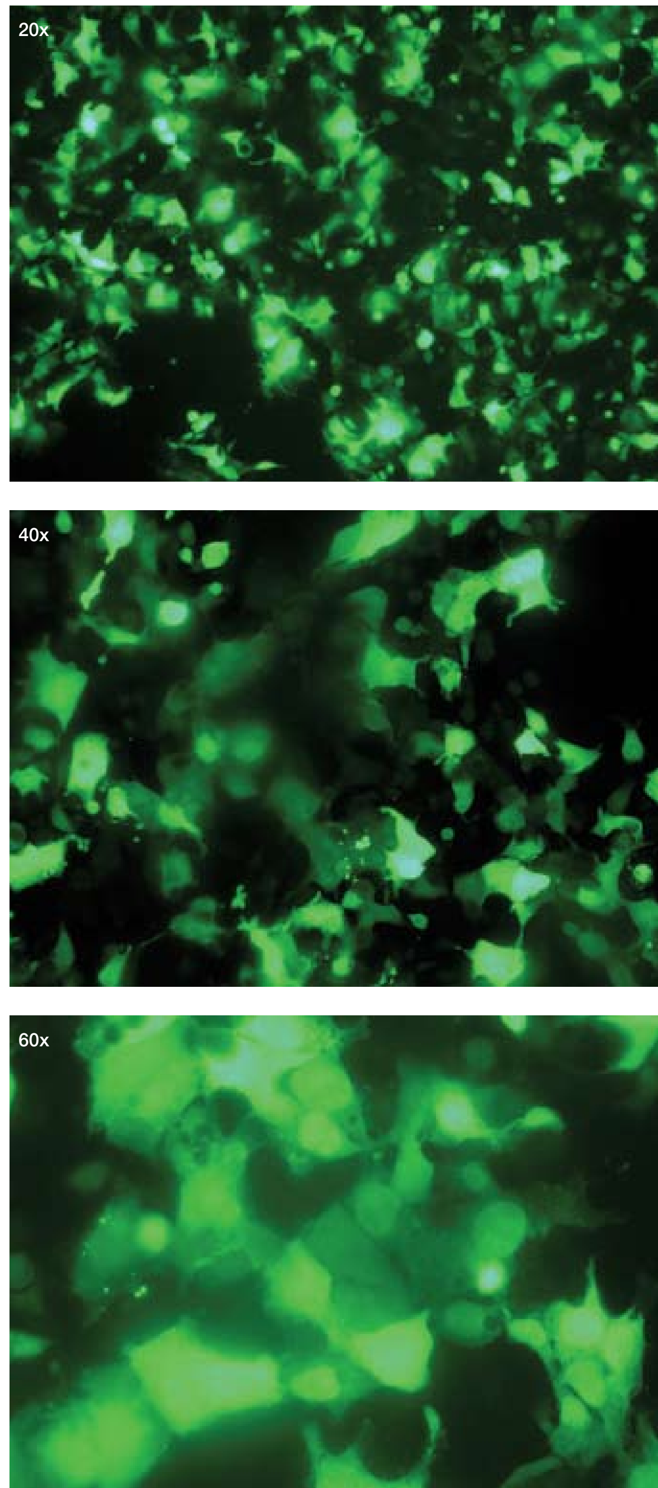

Fig. 1. Fluorescence microscopy of HEK 293T cells transfected with HEKFectin reagent. Cells were transfected with four plasmids and HEKFectin lipid reagent to produce a GFP-encoding lentivirus. Images were taken 48 hr posttransfection at different magnifications.

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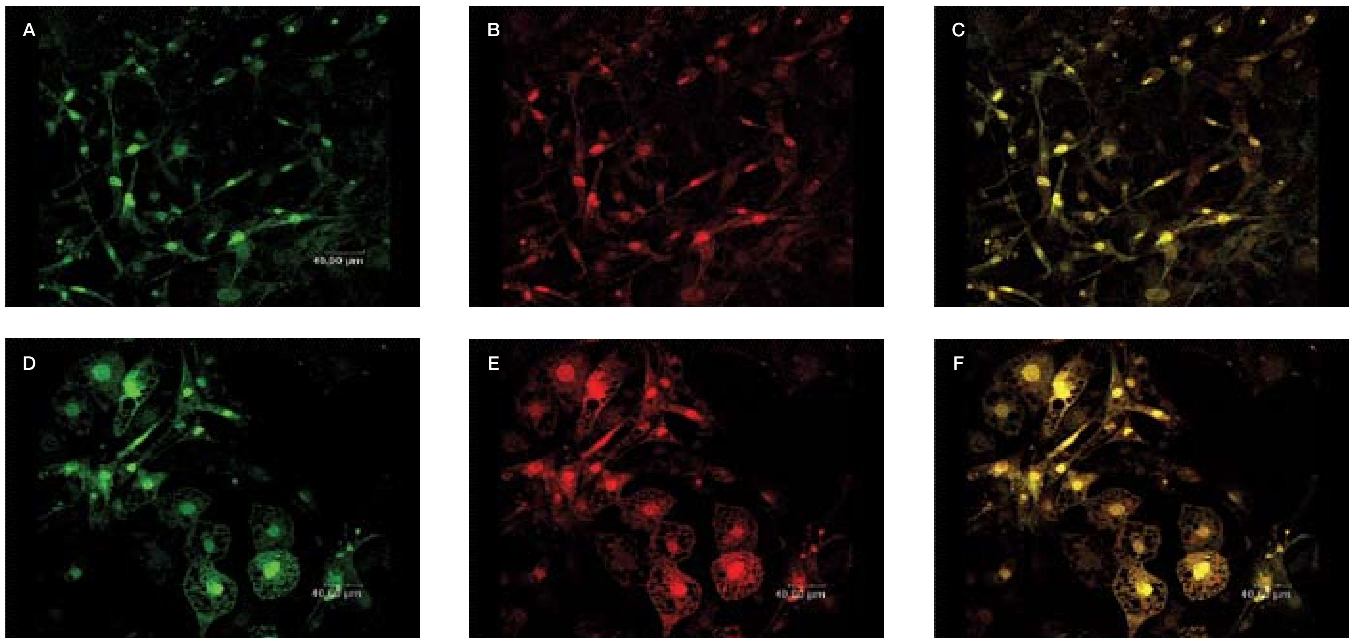

Fig. 2. Lentiviral transduction of 3T3-L1 cells. 3T3-L1 fibroblasts (A–C) and adipocytes (D–F) were infected with a GFP-encoding virus with an MOI of 1 and 3, respectively. After 7 days of cell culture, the nuclei were stained with TO-PRO-3 iodide (B, E), and GFP fluorescence (A, D) was analyzed by confocal microscopy. In both cell types, nearly all cells expressed GFP, as shown by overlay of the nuclear staining and GFP images (C, F).

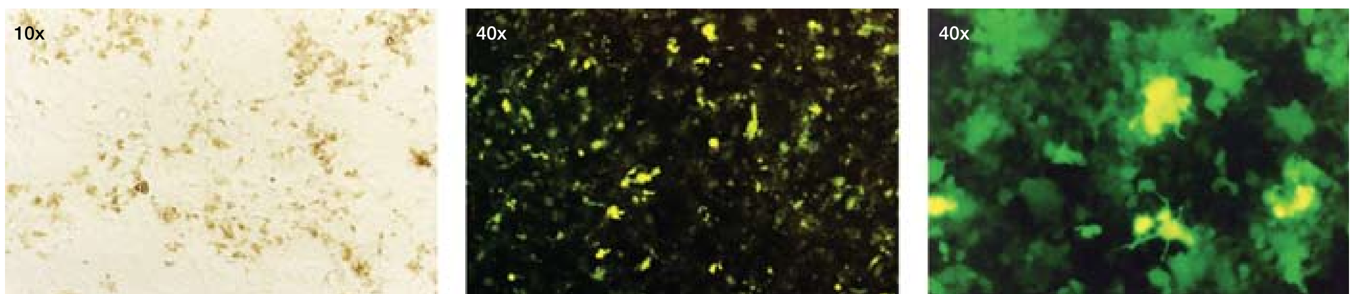

Fig. 3. Microscopy of HEK 293T cells transfected with calcium phosphate. Cells were transfected with four plasmids and calcium phosphate to produce a GFP-encoding lentivirus. Images were taken 48 hr posttransfection at different magnifications. Note the precipitates, which are clearly visible in the brightfield image at left.

Conclusions

HEKFectin lipid is a simple-to-use and potent cell line-specific transfection reagent. It permitted highly efficient transfection of four different plasmids into HEK 293T cells in a single reaction0 to generate recombinant lentiviruses. HEKFectin gave a consistently high yield of intact, biologically active lentivirus particles and required no serum-free incubation step. Application of HEKFectin lipid improved both the throughput and quality of virus production compared to conventional calcium phosphate transfection (Jordan et al. 1996).

References

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