Transfection of Human Embryonic Kidney Cell-line 293T (HEK293T) Cells with METAFECTENE PRO

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Introduction:

Human embryonic kidney cell-line 293T (HEK293T) cells are frequently used to exogenously express target proteins for analyses of protein-protein interactions by co-immunoprecipitation or to produce biologically active proteins that are poorly produced in non-mammalian cells. Higher transfection efficiency is required to gain reproducible experimental results. In the present study we investigated whether HEK293T cells are good recipient cells for transfection of mammalian expression plasmid DNAs with Metafectene Pro. To evaluate the transfection efficiency, we expressed enhanced green fluorescent protein (EGFP) and analyzed by fluorescent microscopy.

Materials and methods:

Materials

A mammalian expression plasmid of enhanced green fluorescent protein (EGFP), pEGFP-C2, was obtained from Clontech and site-directed mutagenesis was performed to construct a vector for monomeric EGFP (pmGFP-C2)).

Cell culture

HEK293T (human embryonic kidney 293T) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% heat-inactivated FBS (fetal bovine serum), 100 units/ml penicillin and 100 µg/ml streptomycin at 37 °C under humidified air containing 5% CO_2.

Transfection protocol

After HEK293T cells ($2x10^5$ cells) were seeded in a 35-mm petri-dish (Greiner Bio-One Bioscience, Cat. No. 627 160) and cultured for one day, medium was changed. A solution A (100 µl PBS plus 1.5 µg of pmGFP-C2) was added drop-wisely to mix with a solution B (100 µl PBS plus either 3 µl or 6 µl of Metafectene Pro). After the two solutions were mixed by pipetting and allowed to stand for 20 min at room temperature, they were added to the seeded cells drop-wisely and evenly throughout the dish. Cells were cultured for 26 hours and were subjected to fluorescent microscopic analysis.

Results and discussion:

Under fluorescent microscopic observation, strong fluorescent signals derived from GFP were detected in 60 - 70% cells that were transfected with pmGFP-C2 using Metafectene Pro at both DNA-lipid ratios of 1:2 and 1:4. Although transfection efficiency seems slightly better at the DNA-lipid ratio of 1:4, some cells were detached from the dish (rounded cells in Figure) and replacement of the culture medium might have been needed several hours after transfection to reduce cytotoxicity of the reagent.

Conclusion / summary:

We investigated whether HEK293T cells are good recipient cells for transfection of mammalian expression plasmid DNAs with Metafectene Pro. To evaluate the transfection efficiency, we expressed enhanced green fluorescent protein (EGFP) and analyzed by fluorescent microscopy. Metafectene Pro was found to be useful to introduce expression vectors into HEK293T cells as comparable to other commercially available transfection reagents.

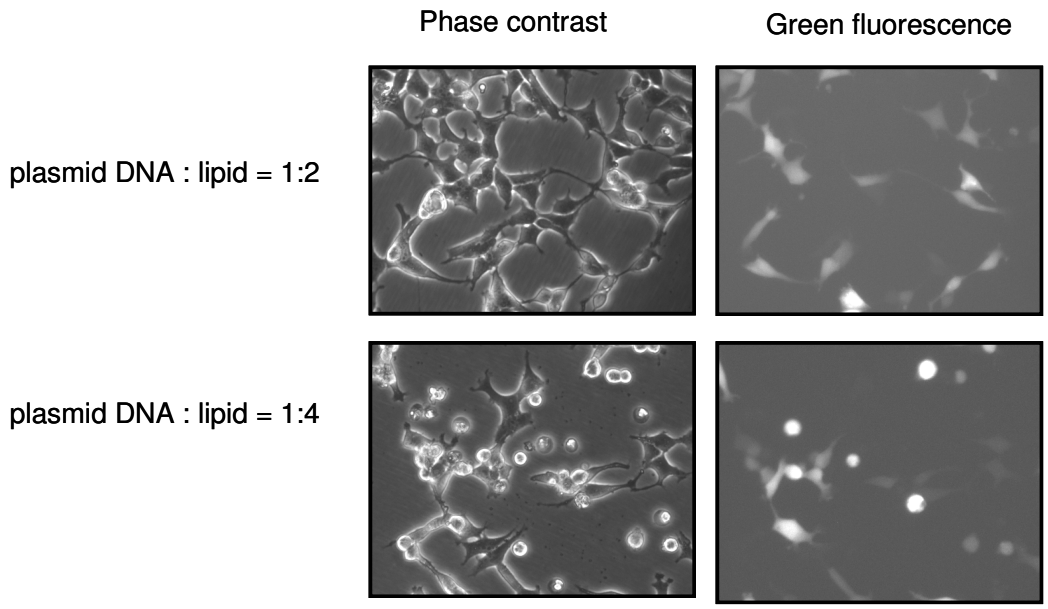

Figure Transfection of HEK293T cells with Metafectene Pro
HEK293T cells were transfected with pmGFP-C2 using Metafectene Pro and expression of GFP was detected with fluorescent microscopy.