Application Notes: Metafectene

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Efficient transfection of cell lines is a prerequisite for most molecular biology applications and systems nowadays. Transfection of cells, especially adherent cells, with lipophillic reagents has several advantages:

1. easy handling
2. less cellular stress, resulting in very low cell death as compared to electroporation and calcium phosphate techniques.
3. higher transfection efficiency rates

Thus, the improvement of lipofection reagents with respect to their efficiency and easiness of handling is of great importance. **Metafectene** was tested using the human embryonic kidney cell line HEK293 and the reporter vector peGFP-C1 (Clontech). Therefore, the peGFP-C1 expression plasmid was transiently transfected in the HEK293 cell line using **Metafectene**, Lipid L and Lipid F (both commercially available transfection reagents). Green fluorescence protein distribution was quantified by fluorescence microscopy 16h following transient transfection (see materials and methods).

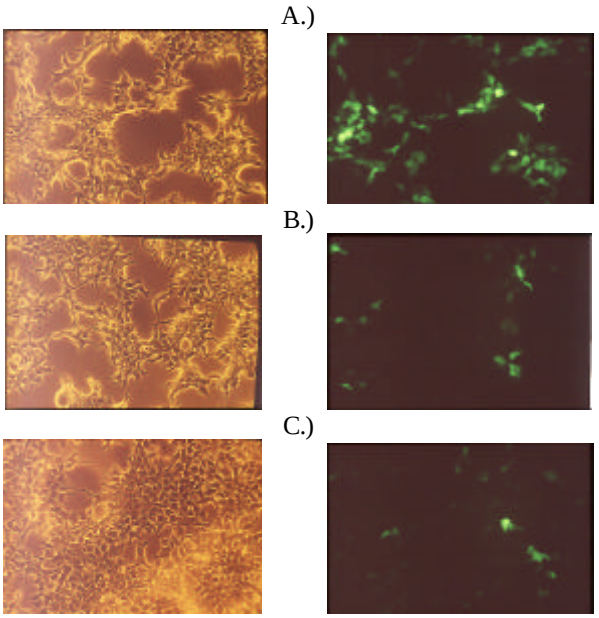

Figure 1: GFP expression in HEK293 cells following transient transfection of peGFP-C1. HEK293 cells ($5x10^5$ cells per well in 6-well plates) were transfected with 0.5µg of peGFP-C1 expression plasmid using A.) Metafectene, B.) Lipid F, and C.) Lipid Le in comparable dosages. Left side: visible light; right side: UV-light. Magnification 40x.

Transfection efficiency in HEK293 cells using **Metafectene** was largely supperior to both Lipid F and Lipid L (Figure 1 and 2). A quantitative approach to determine GFP fluorescence revealed a transfection efficiency of

>40% using **Metafectene**, which was consistently 2-4 fold superior to either transfection reagent tested. Additionally, both transfection reagents, **Metafectene** and Lipid F, can be used in the presence of fetal calf serum in the medium, thus avoiding a time-consuming and error-prone change of medium before transfection, as is necessary for Lipid L. One further advantage of **Metafectene** observed in the present set of experiments is its rather high insensitivity towards cell confluency: even at high confluency of approx. 90%, transfection efficiency remained robust (Figure 2).

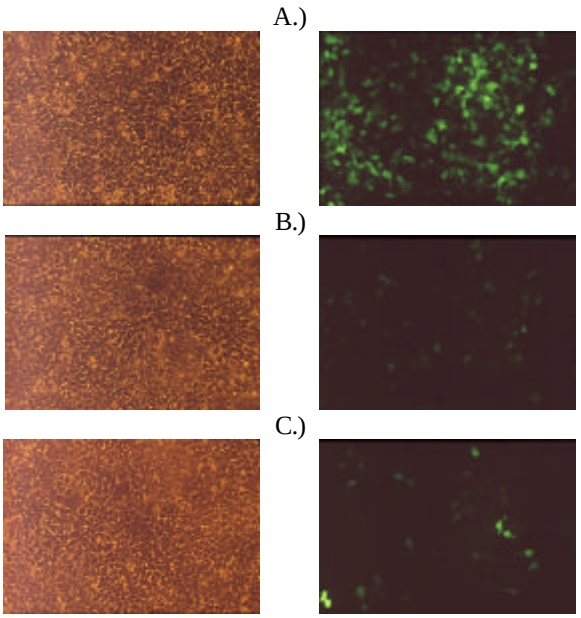

Figure 2: See legend figure 1. Same experiment as in figure 1 except for the cell confluency, which was higher. Magnification 20x.

Materials and Methods

HEK293 cells (Graham et al., 1977) were cultured over night in six-well plates ($5x10^5$ cells/well). 24h later or at a cell density of 90%, 1.0 µg peGFP-C1 plasmid DNA (Clontech) was transiently transfected per well. To do so, Metafectene (5µl in 45µl serum- and antibiotics-free medium) was incubated 15min with the peGFP-C1 DNA (1.0µ µg in 50µl serum- and antibiotics-free medium) and thereafter supplemented dropwise to the HEK293 cells. Incubation of cells with the DNA-Metafectene complex was carried out over night at 37°C and 5% CO2 saturation. Transfection efficiency was assessed upon GFP fluorescence using an Axiovert 135 Microscop (Zeiss) 16h following transfection.

Advantages of Metafectene:

1. Easy usage
2. High transfection efficiency
3. High reproducibility
4. Compatible with serum-containing culture medium
5. Insensitivity towards cell confluency

References

Graham, F.L., Smiley, J., Russell, W.C. and Nairn, R. (1977) Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *J Gen Virol*, **36**, 59-74.