Transfection of HeLa and HEK293-cells with METAFECTENE

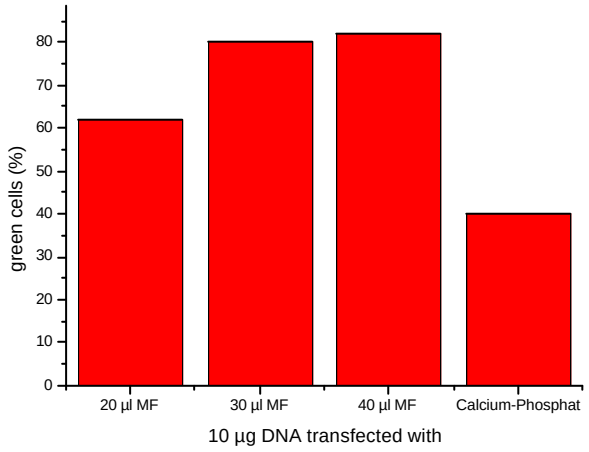
Gudula Schmidt

Institut für Experimentelle und Klinische Pharmakologie und Toxikologie
Universität Freiburg, Albertstr. 25, D-79104 Freiburg

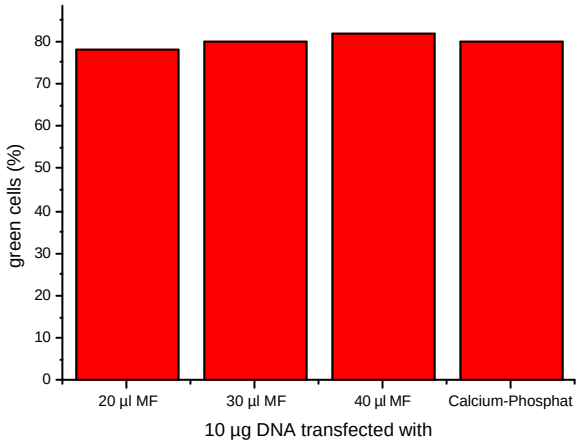
Transfection of mammalian cells is widely used for example to express protein mutants or tagged proteins to analyze structure function connections of the respective proteins in the living cell or to precipitate the proteins with interaction partners. For both applications a high transfection rate is needed.

HEK293-cells (human embryonic kidney cells; ATCC CRL-1573) are easy to transfect. With these cells one can obtain transfection rates up to 80% using common transfection methods like transfection with calcium-phosphate. To study cell morphology HeLa-cells (human cervix-carcinoma cells; ATCC CCL-2) are often used, because they have a more constant morphology than HEK cells. These cells can not be transfected easily with calcium-phosphate (Abb. 1).

We compared transfection rates of HeLa- and HEK293-cells using METAFACTENE and calcium-phosphate. Cells were grown in Dulbeccos modified Eagles Medium (DMEM) containing penicillin/streptomycin und 5% foetal bovine serum. A day before transfection cells were seeded subconfluently in 10 cm – plastic-petridishes. For transfection with METAFECTENE 10 µg pEGFP per dish (constitutive promoter coding for the green fluorescent protein (GFP)) was solved in 100 µl culture medium (solution A) and 30 µl METAFECTENE were mixed with 70 µl culture medium (solution B). the solutions (A and B) were mixed and incubated for 20 minutes at room temperature.
In the meantime the culture medium of the cells was exchanged against 4 ml fresh medium and the DNA/METAFECTENE- mixture added under constant shaking of the culture dishes. The cells were then incubated at 37 °C. 24 hours after adding the mixture expression of GFP was checked by means of fluorescence microscopy. Fluorescent cells were counted. For comparison cells were transfected using a calcium-phosphate method optimised in our lab.



HeLa



HEK293

Using METAFECTENE we could obtain high transfection rates for HeLa, as well as for HEK 293-cells. Comparable results were achieved for COS7- and NIH3T3-cells.