Transfection of epithelial and fibroblast cell lines using Metafectene

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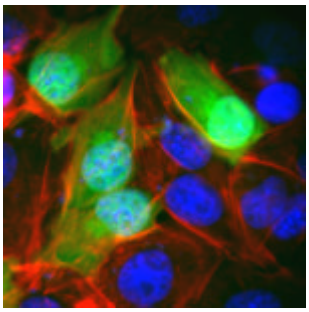
Heterologous expression of cDNAs is a common technique in molecular cell physiology. Optimal transfection strategies should combine high transfection efficiency with the need of only small amounts of the respective cDNA and low cell toxicity.

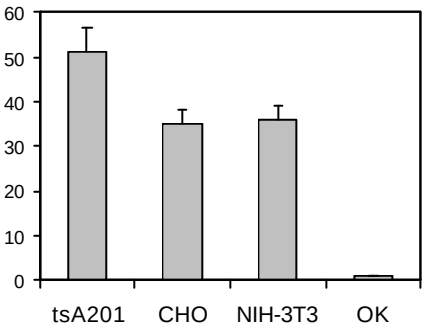
Here, we evaluated the transfection efficiency of Metafectene in the following adherent cell lines: (**1**) a SV40 transformed variant of human embryonic kidney HEK293 cells (*tsA201*), which is commonly chosen as a high-level expression system, (**2**) chinese hamster ovary cells deficient for dehydrofolate reductase (*CHO-DHFR*$^-$) that are well suited for electrophysiological experiments because of the low background of endogenous ion channel activity, (**3**) opossum kidney cells (*OK*), a cell line that polarizes into apical and basolateral membrane domains and is therefore used in subcellular targeting studies, and (**4**) *NIH/3T3* fibroblasts that are best suited for transformation assays.

Cells were grown in DMEM (1,4), DMEM-F12 (3), and alpha-MEM (2) supplemented with 10 % FCS and 1 % penicillin/streptomycin (Invitrogen) at 37 °C and 5 % CO_2. For transfection, cells were seeded on coverslips in 24-well plates. At ~ 80 % confluence, cells were transfected with pEGFP-C1 (Clontech Laboratories) using Metafectene reagent following the supplier's directions without optimization (standard protocol: 0.5 µg DNA/2.5 µl Metafectene). 24 hrs after transfection, cells were fixed and transfection rates were calculated. The results show, that further optimization is necessary for OK cells, which was performed following.

A



B **Unoptimized results**



C **Optimized results for OK cells**

DNA [µg]	Metafectene [µl]	TR [%]
0.25	0.5	< 1
0.5	2	5
1	2.5	4
1	6	11

Transfection efficiency of Metafectene in indicated epithelial and fibroblast cell lines. (**A**) Representative image of CHO-*DHFR*$^-$ cells expressing EGFP, counterstained for the cell nuclei (*blue*) and the actin cytoskeleton (*red*). (**B**) Transfection rates were calculated by dividing the number of green fluorescent cells by the number of DAPI stained nuclei in three areas of 10,000 µm^2 each per well (n = 9 independent transfections). Data are given as unoptimized mean transfection rates [%] ± SEM. Standard protocol: 0.5 µg DNA/2.5 µl Metafectene. (**C**) Variation of the DNA to Metafectene ratio for optimizing OK cell transfection rates (TR).

Besides holding the strong advantage of easy and straight-forward application, Metafectene allows transfection of the tested non-polarizing epithelial and fibroblast cell lines at rates of high efficiency. If used in polarized epithelial cells such as OK cells, the DNA to Metafectene ratio needs to be optimized in order to give best results.