Metafectene Technical Note

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Materials:

Plasmid pEGFP-CI (Clontech) carrying a fusion of a membrane protein C-terminally of EGFP
Sterile 12-well tissue culture plates
25 cm^2 cell culture flasks
Sterile Eppendorf tubes
Glass coverslips
Trypsin solution
DMEM (PAA) + 10 % fetal calf serum
MDCK cells (Madin Darby Canine Kidney)
GelMount Mounting Media (Biomeda Corp.)
Transfection reagents:
METAFECTENE

Optimization of the transfection for MDCK cells:

MDCK cells were grown in a 25 cm^2 cell culture flask in DMEM with 10% serum without antibiotics to near confluency, thereafter trypsinated and seeded onto glass coverslips in sterile cell culture 12-well plates. By the time of transfection (typically next day), the cells were covering 60% of the plate surface.
Optimization of the transfection with METAFECTENE was carried out as follows (all reagents at room temperature): The indicated amount of METAFECTENE transfection reagent (See Table I) was added to 50 ?l of serum-free cell culture medium (DMEM) in a 1.5 ml Eppendorf centrifuge tube. In a separate tube, 0.5 to 1.5 ?g of the plasmid DNA was mixed with 50 ?l of serum-free medium. The media containing METAFECTENE and DNA were combined and mixed by gentle tapping of the tube. The tubes were allowed to stand at room temperature for 20 min for lipid-DNA complexes to form.
During this time, the cells were supplied with fresh serum-comtaining medium, 1 ml per well. At the end of the incubation time, the lipid-DNA complex mixtures were pipetted onto the cells, mixed by tilting the plates a few times and thereafter incubated at 37°C under 5% CO_2 overnight.
48 h after transfection starting time, the cells were washed with cold PBS and fixed with cold 100% methanol at –20°C for 10 min. After repeated washing with PBS, the cells were mounted in GelMount (Biomeda corp.). The transfection rate was determined by counting the per cent of fluorescent cells expressing the EGFP-tagged protein under a Zeiss 510 confocal laser scanning microscope. At least 500 cells per sample were scored for expression. The results are shown as medium of two independent experiments (Table I).

Table 1. Transfection efficiencies of MDCK cells using the METAFECTENE reagent.

	DNA/?g	METAFECTENE/?l	Ratio DNA:METAFECTENE	Incubation time	**% cells transfected**
1	0.5	2	1:4	o/n	**3**
2	1	2	1:2	o/n	**3**
3	1.5	2	3:4	o/n	**5**
4	0.5	5	1:10	o/n	**5**
5	1	4	1:4	o/n	**6**
6	1,5	5	3:10	o/n	**6**

Conclusions:

With the METAFECTENE reagent, transfection efficiencies of about 6% can be obtained for MDCK cells. This is not very much as compared to the results for other cell lines (e.g. HeLa >90%), but using various other lipofection based transfection reagents or electroporation, we never reached more than 1% transfection efficiencies in MDCK cells. Thus, considerably higher transfection efficiencies can be reached with the METAFECTENE reagent in the case of MDCK cells as compared to other typical transfection methods.

The transfection efficiency for MDCK cells with the METACTENE reagent depends on the amount of DNA and METAFECTENE, as well as on the DNA:lipid ratio. 1 µg of DNA and 4 µl of METAFECTENE are needed for one well in a 12-well plate to reach the maximum efficiency of about 6% of expressing cells (see table 1). Lowering the DNA and METAFECTENE concentration to half those values, also reduces the transfection efficiency by 50%. By further increasing the DNA and METAFECTENE concentration, the number of transfected cells does not increase any more. However, the cells that are transfected show a higher expression level (Figure 1). The expression level is very constant within one transfected well, a result that we also observed for other cell lines (e.g. HeLa) transfected with METAFECTENE. This is a great advantage when establishing stable cell lines, because it makes the selection of subclones unnecessary.

One very important factor for efficient transfection is the confluency of the cells by the time of transfection. For MDCK cells, an optical density of 50-70% gives the best transfection results. In contrast, the incubation time of the transfection mixture with the cells seems to be of minor importance, since approximately the same transfection rates were observed when the cells were incubated either for 4 h or overnight with the transfection mixture (data not shown). METAFECTENE shows no considerable toxic effects on MDCK cells after overnight incubation.

Figure 1: MDCK cells were transfected with a plasmid encoding for a membrane-associated EGFP fusion protein using the METAFECTENE reagent according to the optimized conditions described in this study.
A: MDCK cells transfected with 1 µg DNA and 4 µl METAFECTENE reagent, B: MDCK cells transfected with 1.5 µg DNA and 10 µl METAFECTENE reagent.