Metafectene Technical Note

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Materials:
Plasmid pEGFP-NI (Clontech) carrying a fusion of a membrane protein N-terminally of EGFP
Sterile 12-well tissue culture plates
75 ml cell culture flasks
Sterile Eppendorf tubes
Glass coverslips
Trypsin solution
DMEM (PAA) + 10 % fetal calf serum
HeLa cells (Cervix carcinoma)
GelMount Mounting Media (Biomeda Corp.)
Transfection reagents:
METAFECTENE
Lipid E4

Optimization of the transfection for HeLa cells:

Hela cells were grown in a 75 ml 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 about 90% of the plate surface (90% optical confluency, corresponds to about 60% true confluency).

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 µl of serum-free cell culture medium (DMEM) in a 1.5 ml Eppendorf centrifuge tube. In a separate tube, 0.5 or 1.5 µg of the plasmid DNA was mixed with 50 µl 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 incubator under 5% CO2. One batch was incubated with the DNA-Lipid mixture for 4h and thereafter supplied with fresh medium, whereas the other batch was directly incubated overnight with the transfection mixture.

The transfection efficiency with Metafectene was compared to that obtained using another commercial lipid-based transfection reagent E4. The transfection with E4 was performed as recommended by the manufacturer using preoptimized conditions for HeLa cells.

24 h post 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 300 cells per sample were scored for expression. The results are shown as medium of two independent experiments (Table I).

Tabel I. Transfection efficiencies of HeLa cells using the METAFECTENE reagent.

DNA/µg	METAFECTENE/µl	Ratio DNA:METAFECTENE	Incubation time	**% cells transfected**
0.5	2	1:4	4h	**50**
1	2	1:2	4h	**40**
1.5	2	3:4	4h	**10**
0.5	5	1:10	4h	**50**
1	4	1:4	4h	**>90**
1.5	5	3:10	4h	**>90**
0.5	2	1:4	o/n	**40**
1	2	1:2	o/n	**40**
1.5	2	3:4	o/n	**10**
0.5	5	1:10	o/n	**40**
1	4	1:4	o/n	**>90**
1,5	5	3:10	o/n	**>90**

Conclusions:

Using the optimized transfection conditions for HeLa cells with the METAFECTENE Reagent, astonishingly high transfection efficiences of more than 90% were reached. In comparison, using the lipid-based transfection reagent E4, the maximal transfection efficiency obtained for HeLa cells was between 50 and 60 %.

The following points seem to be of major importance for obtaining the highest possible transfection efficiencies with the METAFECTENE reagent. The ratio DNA to METAFECTENE should be low enough, that is: enough transfection reagent should be present for efficient formation of the complex. Very high efficiencies were reached using 1 µg DNA and 4 µl METAFECTENE reagent, whereas 1.5 µg DNA and 2 µl METAFECTENE resulted in sharply decreased transfection efficiencies, probably due to inefficient complex formation. Another very important variant to be considered is the confluency of the cells by the time of transfection. The cells should be in an active proliferative phase and show an optical confluency of about 90% by the time of transfection. Though some variation of confluency is tolerated without significantly affecting the transfection efficiencies, we observed highly decreased transfection efficiences when the cells were seeded either too dense or too sparse for transfection. In contrast, the incubation time of the transfection mixture with the cells seems to be of minor importance, since approximately same transfection rates were observed when the cells were incubated either for 4 h or overnight with the transfection mixture. This also indicates that the METAFECTENE transfection reagent shows no considerable toxic effects on Hela cells even in higher amounts after prolonged incubation (5 µl per well in a 12-well plate).

The cells transfected with the lipid E4 showed considerably lower transfection efficiencies than those transfected with METAFECTENE. Moreover, individual cells showed a high variation in their expression levels after transfection with E4. Figure 1 shows that extremely high transfection efficiencies are obtained using the METAFECTENE transfection reagent. In addition, already 24 h posttransfection, the expression levels of individual cells are relatively high and even, resulting in a population of cells in which individual cells are comparable with each other. Using other transfection methods, we have in most cases observed a high variation of expression levels between individual clones of cells. Thus, the high transfection efficiencies and even expression after METAFECTENE transfection are of great advantage when establishing stable cell lines, because it makes the selection of subclones unnecessary, saving time, costs and effort.

Figure Legend: HeLa 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) Fluorescent image of the transfected cells shows that almost all cells express the GFP fusion protein. B) Phase contrast image of the cells C) Overlay of A and B.

Figure 1