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Metafectene Technical note

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Optimization of COS-7 cell transfection

Thawed COS-7 cells were cultured in a T25 flask containing DMEM with 10% serum and 1% ABAM (Antibioticum and Antimytoticum, Gibco/BRL). At confluency they were trypsinized and seeded in 12 well plates. The seeding density was chosen to reach about 90 % confluency the next day, in order to be suitable for transfection.

Experimental Procedure

The solutions for transfection were prepared according to the supplier instructions. Different concenration series were tested, which led to the experiments A1-A6, B1-B6 and so on.

	A	B	C	D	E
DNA	0.5 µg	1 µg	1.5 µg	5 µg	10 µg
Metafectene 1	1 µl	1 µl	1 µl	1 µl	1 µl
Metafectene 2	2 µl	2 µl	2 µl	2 µl	2 µl
Metafectene 3	3 µl	3 µl	3 µl	3 µl	3 µl
Metafectene 4	4 µl	4 µl	4 µl	4 µl	4 µl
Metafectene 5	5 µl	5 µl	5 µl	5 µl	5 µl
Metafectene 6	6 µl	6 µl	6 µl	6 µl	6 µl

The transfection mixes were pipetted carefully on top of the cells and spreaded over the cells by slightly agitating the cell culture plates. The well plates were incubated over night at 37 °C and 5% CO_2. With half of the plates the transfection solution was replaced by fresh media after 4 hours.

The transfection efficiency was compared with the DEAE-Dextran method performed as dercribed by Ausubel et al (1995).

Results and Diskussion

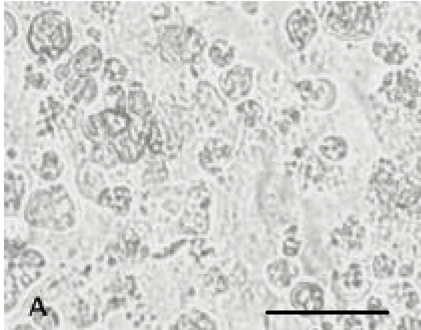
By using the optimated transfection conditions for METAFECTENE on COS-7 cells, good transfection efficiencies were obtained, reaching maximal 80%. These findings are comparable to the well-characterized DEAE-Dextran method. We found that not necessarly the used amount of DNA was the limiting factor, but the ratio of DNA to METAFECTENE reagent.
If enough METAFECTENE reagent was used in relation to DNA high transfection efficiencies were obtained. Probably due to the optimal komplex formation under those conditions. Efficiency was decreased drastically when the METAFECTENE to the DNA ratio was too little. Best results, with efficiencies up to 80% were obtained by using the amounts shown in the table in the yellow boxes. Explicitely 2 to 5 µl METAFECTENE combined with 0.5 µg DNA, 4 to 5 µl METAFECTENE with 1 µg DNA and 5 to 6 µl METAFECTENE with 1.5 µg DNA.
The tested ratios in the series D and E showed clearly less transfection efficiencies, probably because the used amount of METAFECTENE reagent was not enough to form stable condensates with the DNA.
Furthermore, for a good transfection efficiency it was very important that the cells were in a high proliferative stadium and had a confluency of about 90%. Small variantions in confluency were possible without decreasing transfection rate.
The incubation time of the DNA-lipide-mixture doesn't seem to make a difference for transfection efficiency. No differencies in expression efficiencies were seen when incubating 4 or 24 hours. Neither were any toxic effects of METAFECTENE reagent noticed, since even after long incubation with METAFECTENE no morphologic differencies could be seen and cells remained vital.
Inside one cell population the expression niveaus of the single cells were very high and uniform. The high transfection efficiencies and the easy handling make METAFECTENE an attractive transfection tool!

Figures

Figure1: COS 7 cells were tranfected with a plasmid coding for the enhanced green fluorescent protein fusion protein (EGFP) by using METAFECTENE. One of the optimized transfection conditions (A3) were used.

A) Phase contrast picture of the cells.
B) Immunofluorescence of the transfected cells. It shows, that a large percentage of the cells express the EGFP fusion Protein.
C) Superposition of A and B.

The scale represents 50 µm.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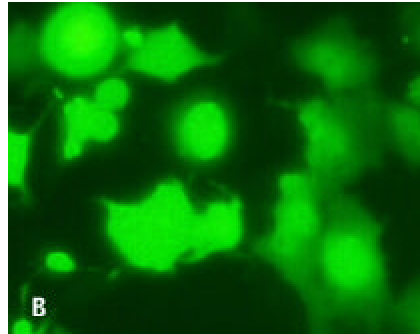
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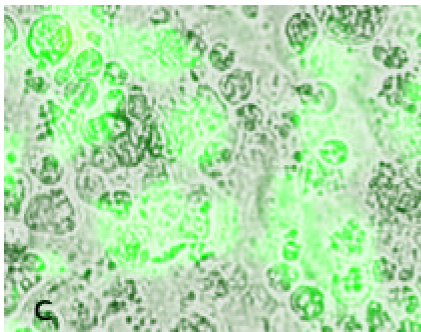
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