Metafectene Technical Note

Generation of stable cell lines: MDCK, CHO, Hela and LLC-PK1 cells

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Materials: Plasmid pEGFP-NI/-C1 (Clontech) encoding various fusion proteins with membrane-associated proteins
Sterile 6-well and 12-well tissue culture plates
Sterile 10 cm tissue culture dishes
75 ml cell culture flasks
Sterile Eppendorf tubes
Glass coverslips
Trypsin solution
DMEM (Cambrex) + 10 % fetal calf serum (FCS)
F12 with Kaighn's modifications (Gibco) + 10 % FCS
Geneticin sulphate G418
HeLa cells (Cervix carcinoma)
MDCK cells (Madine-Darby Canine Kidney)
CHO cells (Chinese Hamster Ovarian)
LLC-PK1 cells (porcine kidney epithelium)
GelMount Mounting Media (Biomeda Corp.)
METAFECTENE Transfection reagent

Optimization of transfection conditions:

Transfection conditions for various cell lines were separately optimized (see e.g. our technical notes for Hela and MDCK cells). In general, the same amount of DNA and Metafectene was found to be efficient for all cell lines tested (1 µg DNA and 4 µl Metafectene). However, some differences were found in the optimal confluency during transfections. Whereas Hela and CHO cells should be about 90% confluent (as judged by the surface area of the culture plate covered by cells = 90% optical confluency, corresponds to about 60% true confluency), MDCK and LLC-PK1 cells should be seeded out to a confluency of about 60-70%. All cell lines were grown in 75 ml cell culture flasks in DMEM (Hela, MDCK, LLC-PK1) or F12 medium (CHO cells) with 10% serum without antibiotics to near confluency, thereafter trypsinated and seeded in sterile cell culture 6-well plates. Transfection was performed next day.

Transfection with METAFECTENE was carried out as follows (all reagents at room temperature):
4 µl of METAFECTENE transfection reagent was added to 100 µl of serum-free cell culture medium in a 1.5 ml Eppendorf centrifuge tube. In a separate tube, 1 µg of the plasmid DNA was mixed with 100 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-containing medium, 2 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 overnight. A mock-transfection reaction without DNA was performed as a control for cell survival and used later as a control for antibiotic selection. No significant toxicity of Metafectene was observed with any of the cell lines used in this study.

Selection of stably expressing cells:

24 h post-transfection, the cells were washed with PBS, trypsinated and seeded onto 10 cm culture dishes. Next day, 1 mg/ml G418 (1,8 mg/ml for LLC-PK1 cells) in culture medium was applied. The media were refreshed daily for the first couple of days and thereafter every 2-3 days. Selection with 1 mg/ml was continued until the control cells were dead. Hela and CHO cells are rapidly growing cell lines, whereas MDCK and LLC-PK1 cells grow more slowly. Therefore, for the selection of single clones, different strategies were applied.

MDCK and LLC-PK1 cells: at the end of selection, clones were visible on culture plates and were marked by circles with a marking pen. Selection was removed by replacing the media with fresh culture medium without antibiotics and cells were incubated overnight. Next day, 12-well plates were prepared for picking of single clones by placing 100 ul of 2 x trypsin solution in each well. The 10 cm plates were washed with PBS, 5 ml of PBS was added to the plates and clones were picked by scratching with a 100 µl pipette ("yellow tip") and simultaneous sucking with the pipette. The cells were then placed into the 12-wells in trypsin, incubated 5 to 10 min at 37°C, and 1,5 ml of culture medium was added. The media were refreshed next day and cells were allowed to grow to confluency. Clones were checked for their expression levels by means of fluorescence microscopy (see below).

Hela and CHO cells: at the end of selection, the plates had become confluent and no single clones were detectable. Therefore, the cells were trypsinated and seeded out very sparsely onto 10 cm plates. After 4 to 5 days, clones began to emerge and were picked and selected as above. The unselected clone pools were also cultivated further and analyzed.

Fluorescence microscopy:

Cells were fixed with cold 100% methanol at –20°C for 10 min. After repeated washing with PBS and final wash in water, the cells were mounted in GelMount (Biomeda corp.). The evaluation of the clones was performed by counting the per cent of fluorescent cells expressing the EGFP-tagged protein under a Zeiss 510 Meta confocal laser scanning microscope. In all cases, 10 individual clones were screened. Even the clone pools of Hela cells were found to be relatively homogeneous and suitable for further use without subcloning, whereas in the case of the other cell lines, individual clones showed a varying degree of homogeneity, which is normal for stable clones. However, even in the case of MDCK cells, which are the most difficult to transfect of the four cell lines tested here, 20 to 30% of the clones were found to be completely homogeneous with high expression levels.

Conclusions:

We have previously shown that using the optimized transfection conditions for HeLa cells with the METAFECTENE Reagent, astonishingly high transfection efficiences of more than 90% were reached. 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 in some cases unnecessary, saving time, costs and effort. We found that the clone pools of Hela cells after selection were very well suitable for further analysis without subcloning. However, in the case of other cell lines (CHO, MDCK and LLC-PK1), subcloning was necessary, as the transient transfection rates of these cells are by far lower than those of Hela cells, although even in the case of these cell lines, METAFECTENE generally results in better transfection rates than other reagents we have tested.

General points to be considered for succesfull transfection:

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. 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% for fast-growing and appr. 60% for slower-growing cells 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.