The transfection of the highly-transfectable 293T/17 and a high-passaged Vero 76 cell line with metafectene

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Introduction

Several methods for the transfection of eukaryotic cells have been established, including calcium phosphate transfection, electroporation or lipid mediated transfers. The efficiency of transfection depends on multiple factors such as cell type and age and the cytopathogenicity of the reagents used.
This study describes the convenience of metafectene for the transient transfection of 293T/17 cells (pass. 5). The subclone 17 (ATCC, CRL-11286) of the human embryonic kidney derived cell line includes an additional neomycine resistence. These cells generally allow a high efficiency of transfection.
Additionally, the Vero 76 cell line (passage >320) was applied for transfection. This African green monkey derived kidney cell line was tested for the application of the metafectene reagent in a high-passaged cell line.
The transfection of eukaryotic cells by the calcium phosphate as well as the electroporation method and the metafectene reagent, have been compared using a GFP expression plasmid. Additionally, a plasmid encoding the gene sequence of one of the two Rubella virus envelope proteins, the E1 protein, has been transfected. The E1 protein expression was demonstrated by an indirect immunofluorescence assay.

Establishing transfection parameters

Briefly, 3x 10^6 293T/17 cells were seeded in a 6 well tissue culture plate the day prior to transfection. For the maintenance of 293T/17 cells, Dulbecco's modified Eagle's high glucose medium with Glutamax (Gibco, cat. no. 61965-026), supplemented with penicillin/streptomycin, was used. The 293T/17 cells were transfected at a confluency of about 70-80% with 0.5 µg pEGFP-C1 (Clontech, cat. no. 6084-1). Different conditions were chosen to establish and optimize the metafectene transfection protocol for this cell line (table 1). Different ratios of metafectene to DNA, a variation of the incubation time of the cells with the transfection complex, and an increase in the quantity of DNA applied for transfection were tested (figure 1 and table 1). Transfected cells were analyzed 48 hours posttransfection by FACS to determine GFP-expression. Within the range of a 1:4 to 1:20 ratio of metafectene to DNA, the highest transfection efficiency of about 82% was achieved with the 1:10 ratio. A saturation effect was observed with ratios of metafectene to DNA higher than the 1:10. Hence, this ratio was applied for subsequent transfection experiments.
An exposure of the 293T/17 cells to the transfection complex for 4h, 6h or 8h had no observable influence on the transfection efficiency. Hence, the incubation time of the 293T/17 cells with the transfection complex did not influence the outcome of the experiment applying the above mentioned parameters. An exposure time of 6h was chosen for subsequent experiments.
Varying amounts of DNA as the third optimization parameter were tested using the 1:10 ratio and an incubation time of 6h.
The application of a DNA concentration of 0.5, 1.0 and 1.5 µg revealed that changing the quantity of the DNA complex did not alter transfection efficiency. Nevertheless, an increasing degree of cytotoxicity could be observed with expanded DNA concentrations.
Using a concentration of 1.5 µg pEGFP-C1 the viable cell number was reduced by 70% compared to the

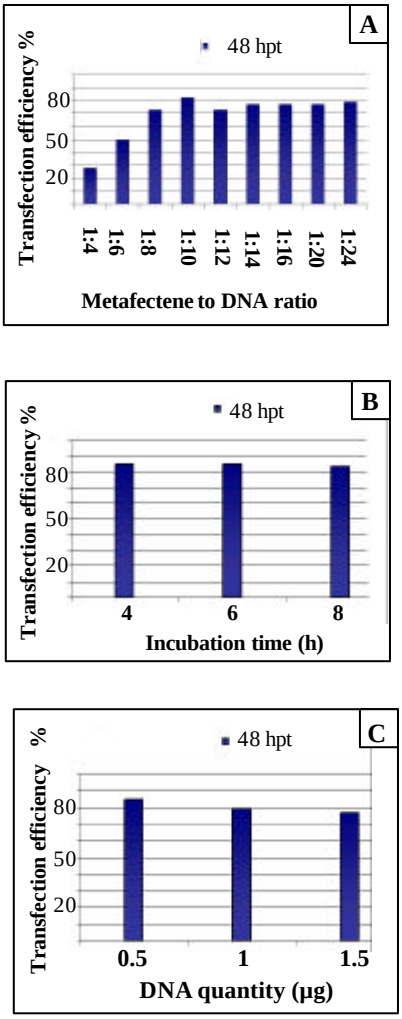


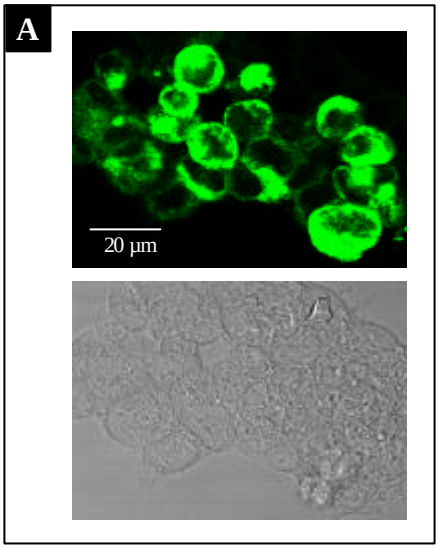
Abb. 1: Optimization of transfection parameters: A) Application of different metafectene to DNA ratios using 0.5 µg GFP. The 1:10 ratio yields the highest transfection efficiency by using the lowest possible quantity of the transfection complex. Continuing with the 1:10 ratio B) varying incubation periods with 0.5 µg pEGFP-C1 and C) different amounts of DNA (with 6h incubation time) were applied. The two last-named parameters seem to have no influence on the transfection outcome within the settings applied for these experiments.

48 hpt: 48 hours post transfection

control population of non-transfected cells.

Application for immunofluorescence analysis

With the above mentioned optimized parameters (0.5µg DNA, a metafectene to DNA ratio of 1:10 and an incubation time of 6h) the eukaryotic expression plasmid pcDNA3 was transfected into 293T/17 cells using the metafectene transfection reagent. The gene sequence of one of the two envelope proteins of the rubella virus, the E1 protein, had been cloned into this plasmid.
Analysis of the transfection efficiency of this plasmid was performed by immunofluorescence analysis with a monoclonal anti-E1 antibody (M19, Roche Diagnostics). Due to the mainly intracellular localization of the Rubella E1 protein FACS assay was not available for the analysis of the transfection of the plasmid encoding this protein.
Figure 2A shows the high efficiency of metafectene transfection for plasmids other than pEGFP-C1, which is a highly-transfectable plas-mid.
Furthermore, metafectene is a reagent that is easy to handle with a good application for immunofluorescence analysis. A low quantity of DNA is sufficient to transfect a great amount of cells without changing their morphology (figure 2B).



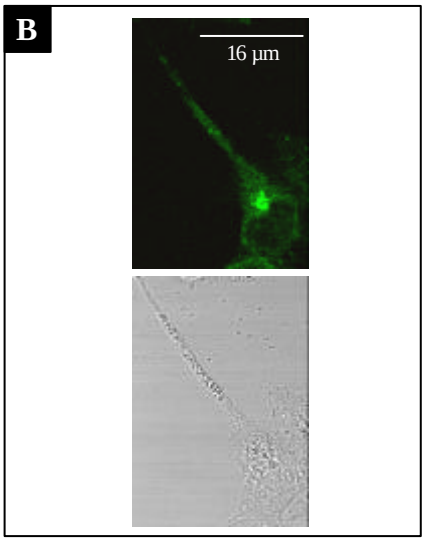


Figure 2: Immunofluorescence analysis of the transfection of 293T/17 cells with the plasmid pcDNA3, encoding the rubella E1 envelope protein. The rubella protein was stained with monoclonal anti-E1 antibody and FITC-conjugated secondary antibody.
Figure 2A: Immunofluorescence analysis confirms the high transfection efficiency with metafectene for a plasmid other than pEGFP-C1.
Figure 2B: No morphological changes occur after the metafectene transfection of 293T/17 cells.

The transfection of a high-passaged cell line

Apart from the highly-transfectable 293T/17 cell line, a high-passaged Vero 76 cell line (passage >320) was also tested for the application of metafectene transfection. Vero 76 cells were maintained in DMEM with 25 mM Hepes (Gibco, cat. no. 42430-025), supplemented with Glutamin, 5% FCS and streptomycin/penicillin.
Vero 76 cells were seeded at a density of 1.5x 10^6 cells in a 6 well cell culture dish 24h prior to transfection. The optimized parameters for the metafectene transfected 293T/17 cells were adopted for the Vero 76 cell line (0.5µg pEGFP-C1 and 6h incubation time). Only the ratio of metafectene to DNA was optimized for this cell line (figure 3). The highest transfection efficiency of 48% was achieved with the 1:20 ratio.

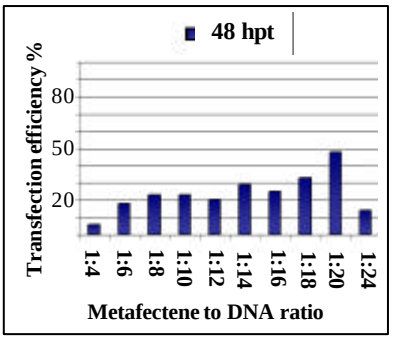


Figure 3: Transfection optimization: Different metafectene to DNA ratios were used for 0.5 µg pEGFP-C1. The highest transfection efficiency was achieved with the 1:20 ratio. The incubation time was 6h.

48 hpt: 48 hours post transfection

Conclusion

Metafectene represents an efficient transfection reagent. The metafectene to DNA ratio is of main importance and dependent upon the cell line and the cell passage applied for metafectene transfection. Furthermore, the usage of a small amount of DNA is recommended. Additionally, this is a favorable aspect, as a high quantity of DNA is not required to get a satisfying transfection result. The calciumphosphate transfection requires a higher amount of DNA to achieve a result similar to that by the metafectene transfection.
The comparison of the metafectene agent with other methods appliable for transfection reveals its good application for transient transfection experiments.

The calciumphosphate transfection of pEGFP-C1 gives efficiencies of about 80-90%, but the transfection of pcDNA3 plasmids with different inserts results in lower efficiencies of about 50%. Admittedly, the application of the calciumphosphate method is limited for immunofluorescence analysis, as morphological changes are observable within the transfected cells.

There are also high transfection efficiencies achievable via electroporation, but a general survival rate of 30-40% for the transfected cells is insufficient for some experimental approaches. Even a high-passaged Vero 76 cell line is susceptible for metafectene transfection giving satisfying results of up to 50% transfected cells. The calciumphosphate transfection of this cell line using the same conditions resulted only in about 30% efficiency.

In conclusion, the metafectene transfection reagent offers good application in experimental approaches that require culturing of cells for a longer period of time while excluding morphological changes.

µg DNA	metafectene (µl)	ratio	incubation time (h)	% transfected cells
0.5	2	1:4	6	28
0.5	3	1:6	6	50
0.5	4	1:8	6	73
0.5	5	1:10	6	82
0.5	6	1:12	6	73
0.5	7	1:14	6	77
0.5	8	1:16	6	77
0.5	10	1:20	6	77
0.5	12	1:24	6	79
0.5	5	1:10	4	85
0.5	5	1:10	8	83
1.0	10	1:10	6	79
1.5	15	1:10	6	77

Table 1: Efficiencies for the metafectene transfection of 293T/17 cells. The experiment was performed in a 6 well tissue culture plate, whereby each reaction mixture given in the table was applied for just one well. 3x 10^6 cells were seeded 24h before transfection. A 100 µl dilution volume of DNA as well as of metafectene was used. Dilution was done in non-serumfree DMEM with Glutamax, but no antibiotics or FCS were added.