Transfection of HeLa cells with METAFECTENE

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For applications in human gene therapy it is important to develop efficient nonviral gene transfer. The safety of the gene vector, the ability to target specific cells, and ability to provide a high, but controlled, expression are important issues for the gene delivery system. In vitro experiments are usually the first assays to test the function of a new gene vector. To conduct experiments with cell culture, we need a transfection method that allows a high expression of the reporter gene in a short time after transfection. An easy protocol and high reproducibility are important to obtain valuable results.

In some single pre-experiments we tested the transfection reagent METAFECTENE. In order to estimate the improvement of a new gene vector we needed a transfection reagent that releases the DNA in the cytoplasm.

The experiments were performed with HeLa cells (DSM ACC 57) in 12-well plates. The cells were seeded 24 h before transfection ($2*10^5$cells/well) and incubated overnight in DMEM with 10 % serum and antibiotics. The next morning we changed the medium and incubated the cells for 6 h in 800 µl serum-free DMEM. We transfected the cells with three different vector constructs containing the reporter gene for luciferase. The transfection procedure was carried out like described in the manufacturers protocol for 12-well plates. We used 0,5 µg, 1 µg, and 2 µg of DNA. The ratio of DNA:METAFECTENE was 1:5. The cells were 80 – 90 % confluent. For transfection we incubated the cells in 800 µl of serum-containing DMEM. We removed the medium 90 min after transfection, washed the cells in PBS, and then added new DMEM containing 10 % serum. The reporter gene assay was performed 7 h after transfection (Fig. 1).

In the assay (Fig.1), we compared luciferase expression after transfection with Dendrimer S and METAFECTENE. The transfection procedure with Dendrimer S was established as recommended by the manufacturers.

METAFECTENE transfection showed a higher expression of luciferase than Dendrimer S. We noticed that the different DNA amounts do not have such a large influence on expression level. In further experiments, we are planing to compare the influence of different ratios of METAFECTENE to DNA.

pNok-Luc (CMV-Promoter)

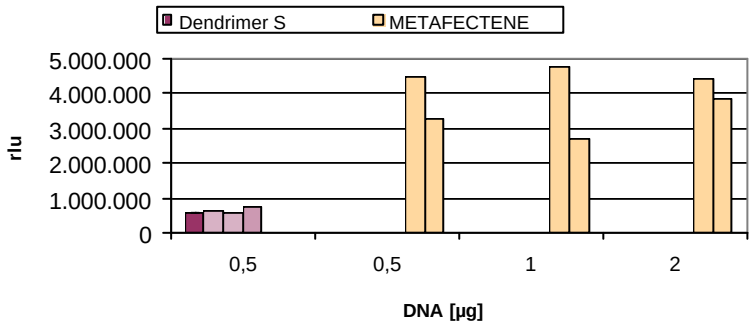

Fig. 1 Transfection of HeLa cells with Dendrimer S; 0,5 µg DNA, four samples and METAFECTENE; 0,5 µg, 1 µg, and 2 µg DNA, two samples each; rlu: relative light units