Efficient Transfection of the hepatocarcinoma cell line Hep 3B using METAFECTENE

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Studies on the effects of transiently (over-)expressed (heterologous) genes in mammalian cells rely on high transfection efficiency. Classical methods of transfection, utilizing calcium phosphate or DEAE-dextran, show acceptable results with a limited number of cells lines only. On the other hand, lipofection protocols often need to be optimized for a given cell line.

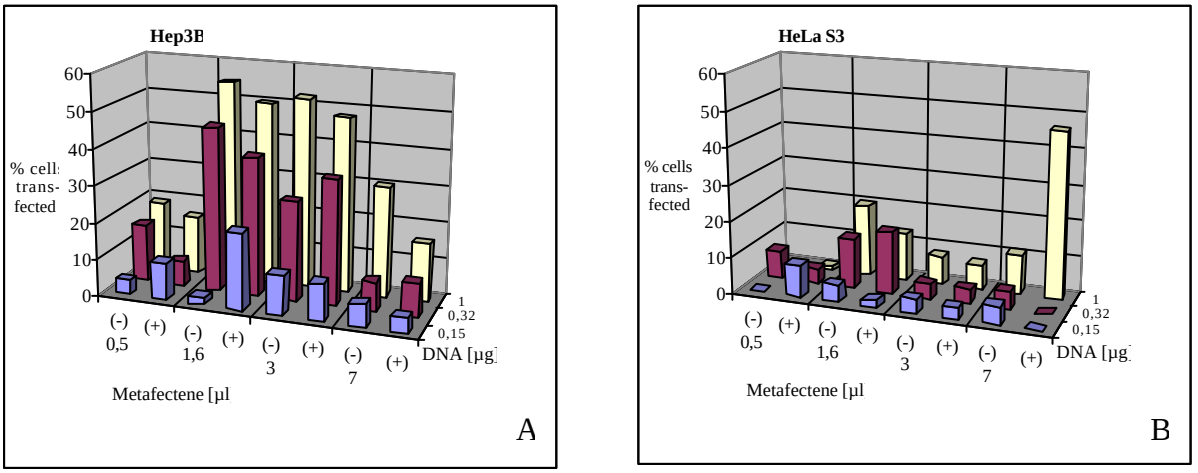
Here we use the human carcinoma cell line **Hep 3B** (ATCC HB-8064) which has been isolated from a liver tumor biopsy of an 8-year old boy in 1976. These adherently growing cells display an epithelial morphology and contain an integrated hepatitis B virus genome. A partial deletion in the p53 gene locus results in a total loss of activity of this central tumor suppressor. The cells, e.g. after transfection of specific p53 constructs, are therefore well suited to studying cellular processes connected to p53 which so far are poorly understood.

In order to compare transfection efficiencies, two more cell lines were tested under identical conditions: **HeLa S3** (ATCC CCL-2.2) - like Hep 3B - is a human virus-transformed carcinoma cell line showing epithelial morphology and adherent growth, and is well known for its poor transfectability. In addition, **COS-1** cells (ATCC CRL-1650) were included in the study, a cell line commonly used for transfection experiments.

Initially, the METAFECTENE protocol for 24 well plates was used for all three cell lines in order to test a maximum of different conditions simultaneously. Optimum transfection conditions for each cell line were verified afterwards in a scale-up experiment using 10 cm-tissue culture dishes. Cells were cultivated in DMEM + 10 % FCS without antibiotics. 8x 10^4 cells per well were inoculated for small-scale transfections and incubated under optimum growth conditions for several hours to allow for attachment of the cells to the surface of the dish. The plasmid pCMVß (BD Clontech) was used as a reporter vector, as expressed ß-galactosidase is readily and specifically quantitated in transfected mammalian cells. DNA and METAFECTENE were diluted separately in DMEM free of serum and antibiotics, and thereafter combined for complex formation for 20 minutes at ambient temperature.

One fraction of cells was first incubated in the presence of various concentrations of DNA*METAFECTENE complexes in serum-free DMEM for 5 hours, followed by cultivation in DMEM + 10 % FCS for another 60 hours. A second group of cells was incubated with DNA*METAFECTENE complexes in DMEM + 10 % FCS for 65 hours. The ß-galactosidase activity assay (staining) was carried out according to standard protocol [1] and the efficiency of transfection determined by counting stained and unstained cells.

Fig. 1



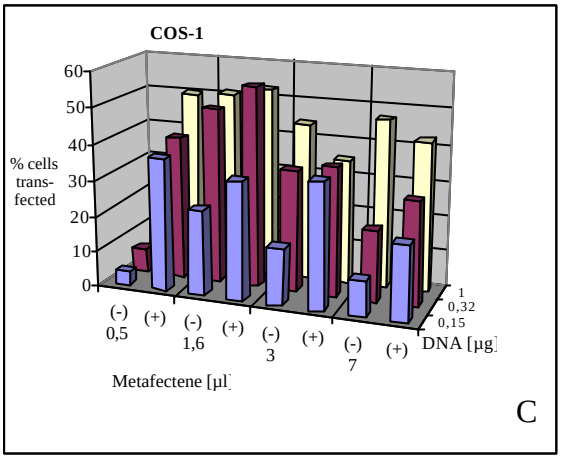


Fig. 1: Comparison of transfection efficiencies of Hep 3B (A), HeLa S3 (B) and COS-1 cells (C) using METAFECTENE.
Cells were incubated in 24 well plates in the presence of DNA*METAFECTENE complexes, which consisted of the indicated amounts of pCMVß and METAFECTENE, either for 5 h in DMEM without FCS (-) or for 65 h in DMEM + 10 % FCS (+).
Transfected cells were histochemically stained and counted.

The highest transfection efficiency of **Hep 3B** cells (about 50 %) was detected using 1.6 or 3 µl METAFECTENE and 1 µg DNA in the 24 well scale (Fig. 1A). Incubation with the DNA*METAFECTENE complexes under serum-free conditions resulted in slightly higher transfection rates.

In contrast, under the conditions tested **HeLa S3** cells displayed a comparable transfection efficiency only in the presence of FCS with the highest concentrations of METAFECTENE (7 µl) and DNA (1 µg) used (Fig. 1B), so that further optimization would be necessary for this cell line. In addition, optimum transfection conditions with METAFECTENE cannot be simply transferred from one cell line to another even if the latter appears similar to the tested one.

COS-1 cells (Fig. 1C) showed a broader transfection optimum then Hep 3B cells and the maximum efficiency achieved here is again about 50 %. Thereby, METAFECTENE facilitates a two- to threefold higher transfection efficiency of COS cells as compared with the DEAE-dextran method.

In addition, we checked whether Hep 3B cells could be transfected with an efficiency similar to METAFECTENE using other techniques as well. These studies were performed in terms of a cost benefit analysis. In the following, the characteristics of the methods used in parallel experiments are shortly described:

DEAE-dextran mediated transfection uses low amounts of DNA, but works efficiently with a limited number of cell lines only. DEAE-dextran has cytotoxic effects and is used for transient transfection only. The underlying mechanism of this method is not known in detail, it is thought that the polymer binds DNA and thereby inhibits nucleases and/or binds to the cell surface and facilitates endocytosis of DNA. Various strategies to improve the method include prolonged incubation of the cells with lower DEAE-dextran concentrations or usage of 'helper' substances such as DMSO, chloroquine or glycerol.

Many cell lines are satisfactorily transfected using the **calcium phosphate*DNA coprecipitation** method (depending on the cell type, efficiencies of up to 50 % have been achieved), but the technique relies on relatively high concentrations of DNA. Also, the efficiency of the method is extremely pH-dependent. DNA is incorporated into the cells as a coprecipitate with calcium phosphate. Some calcium phosphate*DNA coprecipitates after endocytosis can be released from endosomes or lysosomes into the cytoplasm, from where they are transported into the nucleus.

Dendrimer-mediated transfection: The transfection reagent consists of dendrimer-activated molecules, which have a defined size and spherical shape. From a central nucleus, branches protrude and end in charged amino groups, which in turn interact with the negatively charged phosphate groups of the DNA. The uniform structure of the dendrimer-activated molecules warrants formation of uniform transfection complexes and thereby reproducible transfection results. The reagent builds the DNA into compact structures, which attach to the cell surface and are incorporated into the cell by unspecific endocytosis. The reagent buffers the pH of the endosome, which leads to a pH-inhibition of the endosomal nucleases and assures the stability of the reagent*DNA complexes.

In the process of **lipofection,** cationic, amphiphilic lipids (liposomes) interact electrostatically with the negatively charged phosphate backbone of the DNA. These stable hydrophobic complexes are DNase I resistant, attach to the negatively charged surface of the cell membrane, and thus mediate the transport of the DNA into the inside of the cell.

A particularly positive factor of the two latter methods are the short incubation times (mostly 1 to 4 hours), which for many cell lines drastically reduce potential cytotoxic effects and, in addition, the time needed per experiment. The costs per transfection, however, differ considerably (Table 1).

Table 1: Costs of transfection reagents per 10 cm-tissue culture dish

Transfection method	Supplier	Costs per transfection [€]
(1) DEAE-dextran	many; used here: protocol cf. [1]	 << 1 ¤
(2) Calcium precipitation	many; used here: protocol cf. [1]	 << 1 ¤
(3) Dendrimer-mediated	Supplier Q	minimum 11.20 ¤
(4) Lipofection / Lipid C	Supplier C	3.10 ¤
(5) Lipofection / METAFECTENE	Biontex	on average 7.50 ¤

Experiments were performed in 10 cm-tissue culture dishes. Hep 3B cells were cultivated in DMEM + 10 % FCS without antibiotics as described above. On the day before transfection, confluent cells were trypsinized and 10^6 cells per dish inoculated in fresh medium, so that the cells were attached to the surface of the dish and were exponentially growing at the time of transfection.

Plasmid pCMVß was used as the reporter vector as before. As a control for potential unspecific blue staining of cells, vector pCMV5 was employed, which lacks the ß-galactosidase gene. In each transfection 10 µg plasmid was used (this corresponds to an upscale of 0.32 µg DNA in the 24 well format; see above) as this DNA concentration is within the recommended range for all methods studied.

(1) The standard protocol for the **DEAE-dextran method** [1] was modified as follows: Just before transfection, cells were washed twice using DMEM without FCS and then DMEM without FCS was applied to the cells. 10µg of plasmid DNA and the DEAE-dextran solution (final concentration 250µg/ml) were added per dish. After 4 hours of incubation under optimum growth conditions, the medium was removed from the cells. Per dish, 4 ml of a mixture consisting of HBS and DMSO (1+9) was applied for 2 minutes in order to improve the efficiency of endocytosis and hence, of transfection. The medium was then removed and the cells washed twice with DMEM (lacking FCS), followed by incubation of the cells in DMEM + 10 % FCS under optimum conditions for another 72 hours.

(2) For **calcium precipitation** [1], the optimum pH (7.12) of the HBS solution had been determined in previous experiments. DNA dilutions (100ng/µl in 0,1x TE pH 8,0) were prepared directly before transfection. Formation of the calcium phosphate*DNA coprecipitate was carried out in a total volume of 1 ml per dish that contained 100µl diluted DNA, 2x HBS, 0,1x TE pH 8.0. After mixing, 124 mM $CaCl_2$ was added and the solution was incubated at ambient temperature for 30 minutes. Meanwhile the cells were provided with fresh DMEM + 10 % FCS. After carefully resuspending the calcium phosphate*DNA pellet formed, the suspension evenly dispensed in the cell culture dish and the cells incubated under optimum conditions for 24 hours. The cells were then washed once with PBS and cultivated for another 48 hours.

(3) The reagent for **dendrimer-mediated transfection**, provided by supplier Q, was added to the DNA solution, mixed and incubated at room temperature for 10 minutes in order to form DNA*dendrimer complexes. The complexes were then mixed with DMEM + 10 % FCS and directly applied to the cells. After 3 hours of cultivation under optimum growth conditions, the medium was exchanged and cells were incubated for another 72 hours.

(4) For **lipofection** according to the protocol of supplier C, respective dilutions of the reagent and the DNA were prepared, mixed and incubated at ambient temperature for 20 minutes. The complexes were added to the cells in serum-free DMEM and then 2 % FCS added. After 3 hours of incubation under suitable growth conditions the cells were washed with PBS and cultivated for another 72 hours.

(5) METAFECTENE was used according to the Biontex protocol for 10 cm-cell culture dishes. The DNA*METAFECTENE complexes were directly added to the cells in DMEM + 10 % FCS followed by incubation of the cells under optimum conditions for 72 hours.

The ß-galactosidase assay was performed as described above and showed no blue staining of cells transfected with the control vector pCMV5 with either transfection method which proves the specificity of this assay for the presence of ß-galactosidase.

Transfection efficiencies of Hep 3B cells achieved with the different methods tested are presented in Fig. 2. Transfection using DEAE-dextran (method 1) apparently is not a suitable technique for Hep 3B cells. Based on calcium precipitation (method 2), 15 to 20 % of the cells were transfected. This is a mediocre result that does not comply with the aim of a high transfection rate (see above). In contrast, dendrimer-mediated transfection (method 3) as well as METAFECTENE (method 5) provide about twice as much transfected cells as compared with the calcium phosphate method, whereby METAFECTENE is even slightly more efficient than the

dendrimer reagent. Surprisingly, lipofection with the reagent of supplier C (method 4) resulted in a very rare occurrence of ß-galactosidase expressing (blue) cells with a high background of non-transfected cells. Obviously, differences in reagent composition play a crucial role in transfection in spite of the underlying identical mechanism of lipofection so that transfection results based on this principle may differ considerably.

The conditions used here in the 10 cm-dish format correspond to a combination of 1.6 µl METAFECTENE and 0.32 µg DNA in the 24 well format (Fig. 1A). In both test series, which were performed independently, about 40 % of the Hep 3B cells were transfected. This is a remarkable result with respect to the reliability and reproducibility of METAFECTENE-mediated lipofection.

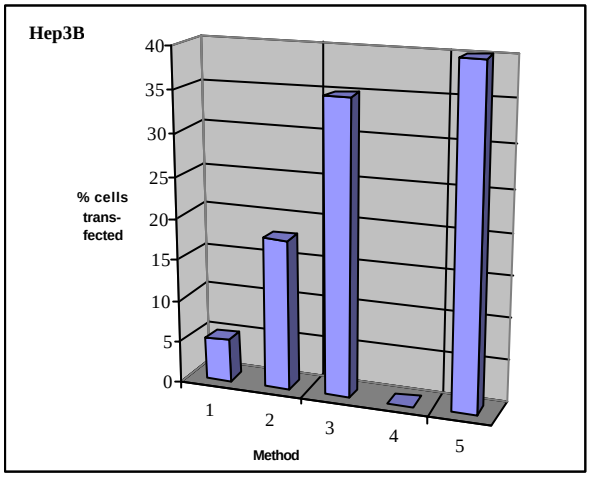


Fig. 2: Transfection efficiencies of different methods with cell line Hep 3B
The cells were transfected in tissue culture dishes (d = 10 cm) using five different protocols. Method 1: DEAE-dextran, method 2: calcium phosphate precipitation, method 3: Dendrimer-mediated transfection (supplier Q), method 4: lipid-mediated transfection (supplier C), method 5: METAFECTENE-mediated lipofection. After 72 hours, transfected cells were specifically stained and counted.

Transfection of mammalian cells with METAFECTENE is a very handy, timesaving method, which can successfully be applied to cells that are hard to transfect with other methods. Transfection efficiencies of about 50 % achieved with METAFECTENE are significantly higher than those obtained with most other methods or reagents. Thus, METAFECTENE is of great advantage, particularly in studies relying on high transfection rates. Moreover, as compared with the costs of the similarly efficient dendrimer-mediated transfection method, METAFECTENE is markedly less expensive.

References

[1] Sambrook J and Russell DW (2001) Molecular Cloning. A Laboratory Manual. 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY