METAFECTENE Application Note

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Transient transfection of primary rat hepatocytes:

***Cell type*:**

Being the central organ in collecting, distributing and storing nutrient components, the liver plays a major role in the intermediate metabolism. The liver does not only participates in the anabolic and catabolic metabolism of proteins, carbohydrates and lipids, in the synthesis and storage of hormones and vitamins, and in the synthesis of urea and bile acids, more than any other organ the liver plays an major role in the detoxification and elimination of toxic endogenous and exogenous compounds. Most of the complex liver functions are carried out by parenchymal liver cells, also called hepatocytes, which account for approximately 80 % of total liver weight. Primary hepatocytes, isolated by *in situ* collagenase perfusion, are an important *in vitro* system for biochemical, pharmacological and toxicological research. As a model, primary hepatocyte cultures (Figure 1) allow to study uptake, metabolism and toxicity of endo- and xenobiotics. It is also possible to examine processes involved in chemical carcinogenesis as to shed light onto regulatory mechanisms. Depending on suitable culture conditions, adherent hepatocyte cultures exhibit differentiated liver specific functions and are able to react to growth stimuli. The sensitivity of primary hepatocyte cultures on the other hand puts limitations to applications such as transient transfection since the cells undergo a limited number of cell division cycles only. An additional drawback lies in the loss of differentiated functions after a few days in conventional culture.

Optimisation of the transfection protocol for primary rat hepatocytes:

Primary rat hepatocytes were isolated from adult male Wistar rats (180-200g) by *in situ* collagenase perfusion as described previously (1). Cells were resuspended in MX-82 medium (2) containing 10% foetal calf serum at a cell density of 0,5 x 10^6 cells/ml. 2ml of the cell suspension were plated per well of a sixwell culture plate. Following an initial attachment period of 4h at 37°C in a humidified atmosphere of 10% CO_2 and 90% air cells were washed once with PBS and were subsequently cultured in 2 ml of serum-free MX-83 medium (2) per well. 16h later medium was replaced by 2ml of fresh MX-83 medium per well and transfection was performed.

For the optimised transfection of primary rat hepatocyte cultures with METAFECTENE (all reagents were used at room temperature) 1µg of plasmid-DNA per well was added to 100µl serum-free MX-83 medium per well and the resulting solution was gently vortexed. In another cup 100µl MX-83 were mixed with 4µl of METAFECTENE. The two solutions were subsequently combined by carefully pipetting several times. To form the lipid-DNA complex the combined solutions (DNA:lipid range of 1:4) were incubated for 20min at room temperature. The DNA-lipid complexes were added dropwise to the culture plates and were mixed gently with the culture medium. Cells were subsequently incubated with the lipid-DNA complexes for 4-6h at 37°C in an 10% CO_2 incubator. Medium containing the lipid-DNA complexes was then replaced by 2ml of fresh serum-free MX-83 medium. Cell culture was stopped 24-48h later.

For transfection of primary rat hepatocytes, reporter gene constructs were utilised, harbouring the firefly-luciferase gene under control of an ABC transporter gene promoter or the promoter of a phase I enzyme of the xenobiotic metabolism. As a control, reporter gene constructs were co-transfected, which contained the renilla-luciferase gene under control of a constitutively active promoter (SV40). The firefly-luciferase activities were subsequently corrected by renilla-luciferase activities. In addition, a reporter gene construct was used containing the GFP-gene under control of the CMV-promoter.

Cells from experiments with luciferase reporter gene constructs were lysed 48h after transfection and luciferase activities were measured luminometrically in the cell lysates, using the Dual-Luciferase-Kit of Promega and a Berthold Lumat LB 9501 luminometer. With the GFP-construct, GFP gene expression of the cells was determined microscopically 24h after transfection (Figure 1).

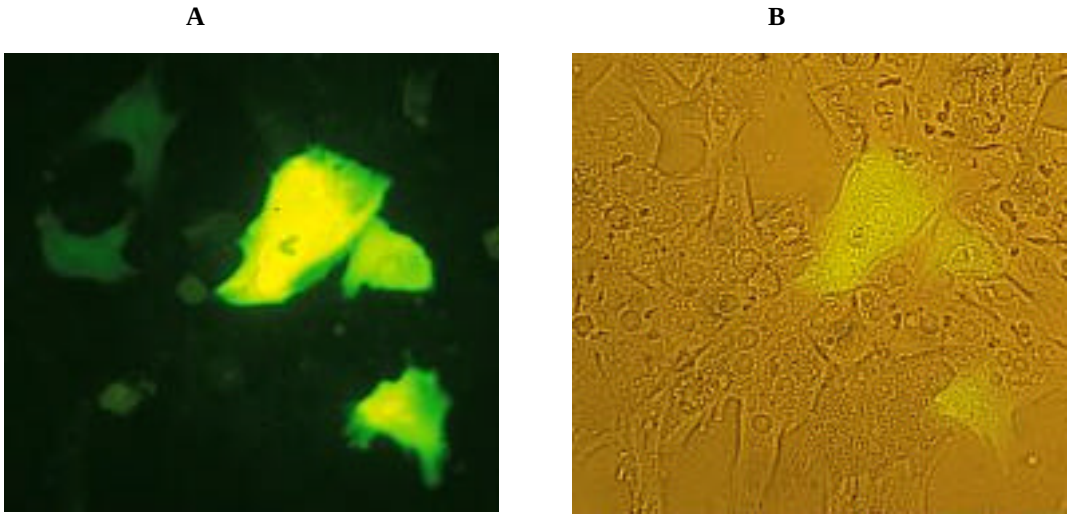

Figure 1: Primary rat hepatocyte cultures were transfected with a reporter gene construct harbouring the GFP gene under control of the CMV-promoter. Transfection was performed using the optimised METAFECTENE transfection protocol for rat hepatocyte cultures. **A** Fluorescence image of transfected cells. **B** Combination of transillumination and fluorescence imaging.

As a first approach we started optimisation of the METAFECTENE transfection protocol for primary rat hepatocytes using a DNA-lipid range of 1:13 optimised for another commercially available transfection reagent Lipid E (subsequently called TRX). Employing constant DNA concentrations (luciferase constructs) of 0,75µg/well METAFECTENE volumes were varied between 5-15µl/well and transfection efficiency was compared to TRX as transfection reagent. Highest luciferase activities were achieved using a DNA:METAFECTENE ratio of approximately 1:7, but luciferase activities were significantly lower as compared to TRX (Figure 2).

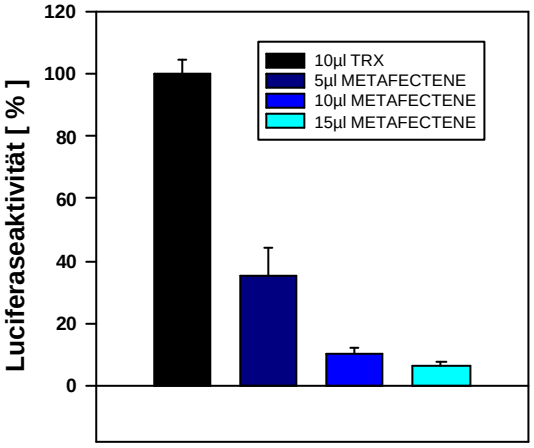

Figure 2: Primary rat hepatocyte cultures were transfected with a reporter gene construct harbouring the firefly-luciferase gene under control of 1224 bp of the ABC transporter gene promoter (0.75µg of total plasmid-DNA per well) using different amounts of METAFECTENE (5-15µl/well of a sixwell plate) or TRX under optimised conditions (10µl/well).

Subsequently a higher constant plasmid-DNA concentration of 1µg/well was used and the DNA-lipid ratio was varied between 1:2 and 1:6. Optimum luciferase activities could be demonstrated at a DNA-lipid ratio of 1:4 (Figure 3A). In the present experiment transfection efficiency was comparable with TRX (Figure 3B) at a DNA-lipid ratio of 1:4 and could not further be improved at a DNA-lipid ratio of 1:6 (Figure 3A).

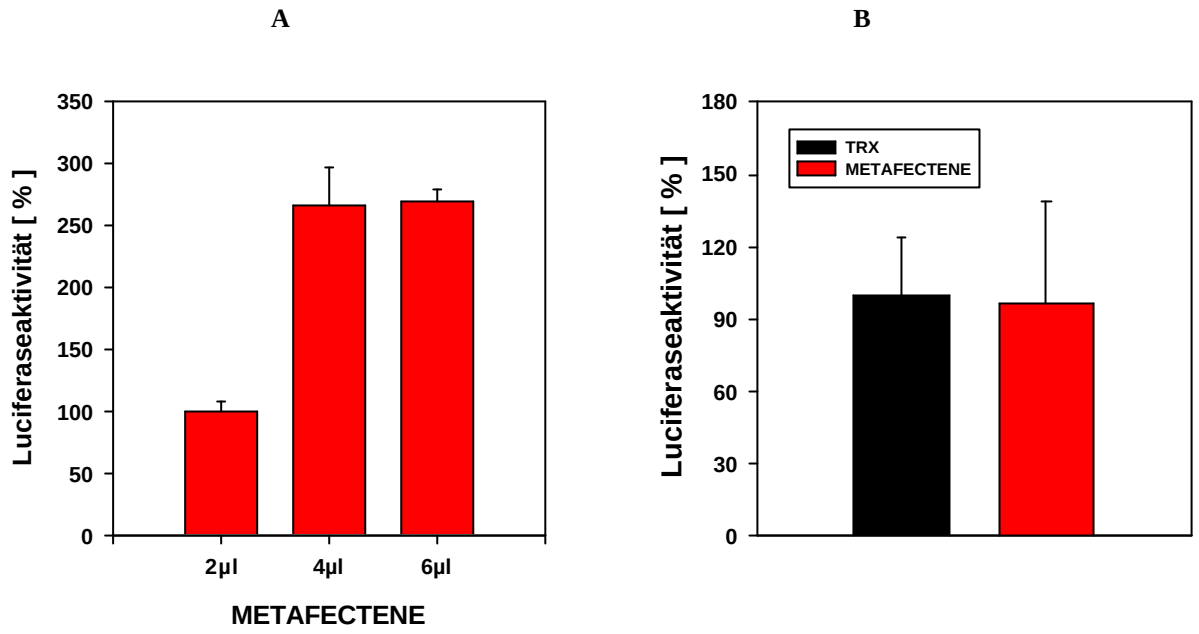

Figure 3: Using METAFECTENE or TRX, primary rat hepatocyte cultures were transfected with plasmids, harbouring the firefly-luciferase gene under control of parts of a transporter gene promoter. **A** Standard DNA concentration of 1µg plasmid-DNA per well was used and different amounts of METAFECTENE reagent were tested. **B** Primary rat hepatocytes were transfected via the optimised METAFECTENE:DNA ratio of 1:4 and compared to TRX transfected cells.

Discussion:

Using the optimised transfection protocol for METAFECTENE, primary rat hepatocytes could be transfected successfully with different reporter gene constructs. Regulation of reporter gene activity by known modulators of the gene promoter used could be demonstrated (not shown).

As compared to TRX, cytotoxicity of METAFECTENE was negligible, even at concentrations of 15µg/well of a sixwell plate. Consequently METAFECTENE led to better transfection efficiencies in cell preparations with lower viability. On the other hand, cell preparations with high viability often showed superior transfection rates using TRX.

Concerning primary rat hepatocyte cultures, fairly high transfection rates could be achieved with METAFECTENE using DNA-lipid ratios between 1:3 and 1:4. Transfection rates of about 90 %, observed for some cell lines using METAFECTENE, could not be realised in cultured rat hepatocytes due to limited proliferation rates, even in suitable media. To ensure some proliferation of cultured rat hepatocytes, cell number should not be less than 1×10^6 cells per well of a sixwell plate and, if compatible with the experimental protocol, the culture medium should contain mitogens like insulin or epidermal growth factor (EGF).

Primary rat hepatocytes, successfully transfected with the GFP-expression vector, demonstrated high GFP-expression even after 24h of incubation (Figure 1). In the top left corner of the image, there are areas of the cell monolayer showing low green fluorescence, i.e. cells with beginning GFP-expression.

1. Hirsch-Ernst KI, Ziemann C, Schmitz-Salue C, Foth H, Kahl GF. Modulation of P-glycoprotein and mdr1b mRNA expression by growth factors in primary rat hepatocyte culture. Biochem Biophys Res Commun 1995;215:179-185.

2. Hoffmann B, Piasecki A, Paul D. Proliferation of fetal rat hepatocytes in response to growth factors and hormones in primary culture. J Cell Physiol 1989;139:654-662.