

## **Transfection of human HepG2 cells with pEGFP-N1 plasmid**

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### **Introduction:**

The goal of the current experiment was to establish optimal conditions for the transfection of HepG2 cells. HepG2 is cell line derived from hepatocellular carcinoma. We use HepG2 cells in our work routinely for investigation of platelet-derived growth factor (PDGF) signal transduction.

### **Materials and methods:**

#### Materials

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biont Laboratories GmbH (Munich, Germany). The plasmid, pEGFP-N1 encoding green fluorescent protein was used for evaluating transfection efficiency.

The HepG2 cells were cultured in Dulbecco's modified Eagle's MEM (DMEM) (Hyclone) supplemented with 10% FBS (Hyclone), penicillin (100 U/ml), streptomycin (100 µg/ml) and L-glutamine (4 mM) (DMEM/10)

### **Experimental procedures / transfection protocol:**

For transfection, HepG2 cells were seeded in 1.5 ml of DMEM/10 in 24-well culture plates one day before transfection, and transfected at approximately 50 % confluence. Metafectene PRO was complexed with the pEGFP-N1 plasmid at reagent and DNA ratios:

2 µg of DNA with 0.25, 0.5, 0.75, 1, 1.5 and 2 µl of Metafectene PRO;

0.5 µl of Metafectene PRO with 0.5, 1, 1.5, 3, 4, 6 µg DNA.

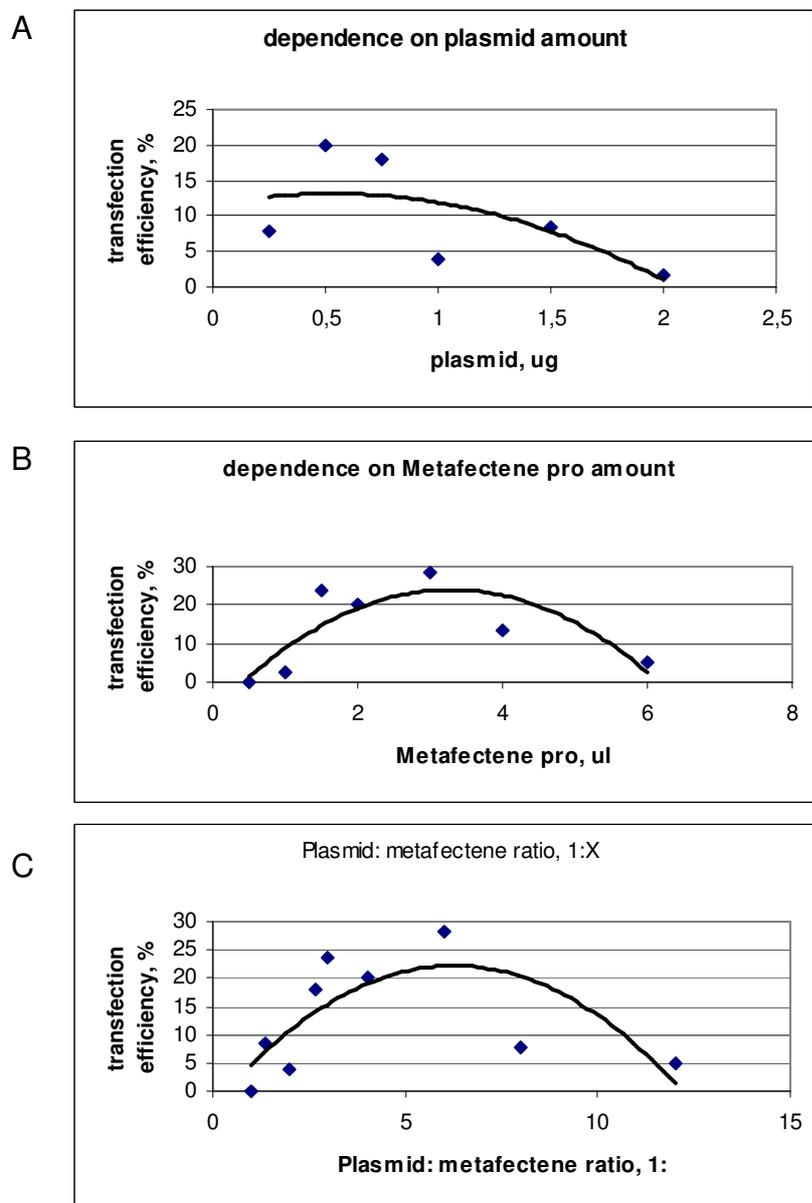
The solutions DNA or Metafectene PRO were prepared with 50 µl of serum-free DMEM medium. Complexes were formed by the addition of Metafectene PRO solution into plasmid solution and incubating for 20 min at room temperature. Changes in transfection protocol, such as prolonged plasmid incubation with Metafectene pro, dropwise mixing, special transfection media (Optimem from

Invitrogen), etc. did not affected or compromised the transfection. Metafectene PRO complexes with DNA were added to the cell propagation media DMEM/10.

Transfection efficacy was evaluated after 24 hours by calculating total/fluorescent cells ratio.

## Results and discussion:

Metafectene PRO was complexed with the pEGFP-N1 plasmid at ratios mentioned above (Fig. 1).



**Figure 1.** Optimization of transfection efficiency in HepG2 cells. A. Fluorescent cells percentage transfecting with 2  $\mu$ g of DNA and 0.25, 0.5, 0.75, 1, 1.5 and 2  $\mu$ l of Metafectene PRO; B. Fluorescent cells percentage transfecting with 0.5  $\mu$ l of Metafectene PRO and 0.5, 1, 1.5, 3, 4, 6  $\mu$ g DNA. C. Fluorescent cells percentage depending on the plasmid:metafectene ratio.

## Conclusion / summary:

The transfection was most efficient using 0.5 ul Metafectene pro with 1.5-3 ug plasmid. The ratio between transfection reagent and plasmid DNA was 1:3 – 1:6 (ul:ug). However very efficient transfection sometimes was associated with slightly toxic effect, regardless of the plasmid used. Changes in transfection protocol, such as prolonged plasmid incubation with Metafectene pro, dropwise mixing, special transfection media (Optimem from Invitrogen), etc. did not affected or compromised the transfection.

We have compared Metafectene pro with others transfection reagents used in our laboratory. The transfection results with Metafectene pro was more or less the same as of Lipofectamine 2000 (Invitrogen) (data not shown). However, the default amount of Metafectene pro used in standart transfection is lower than that of Lipofectamine 2000, so the Metafectene pro is more efficient. We have also compared Metafectene pro with usual calcium chloride transfection (data not shown). The transfection with Metafectene pro was slightly more efficient and less dependent on the purity of plasmid which is a great advantage when transfecting several different plasmids simultaneously.