

# TRANSFECTION OF HUMAN NEUROBLASTOMA SH-SY5Y CELLS AND MONKEY COS-7 CELLS WITH METAFECTENE PRO

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## Materials

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biont Laboratories GmbH (Munich, Germany). The cell growth culture media (RPMI-1640 and Minimum Essential Medium Eagle), penicillin and streptomycin solutions were obtained from Sigma.

The plasmid, pEGFP-C1 (PT3027-5, Clontech Laboratories), encoding green fluorescent protein was used for evaluating transfection efficiency.

## Cells

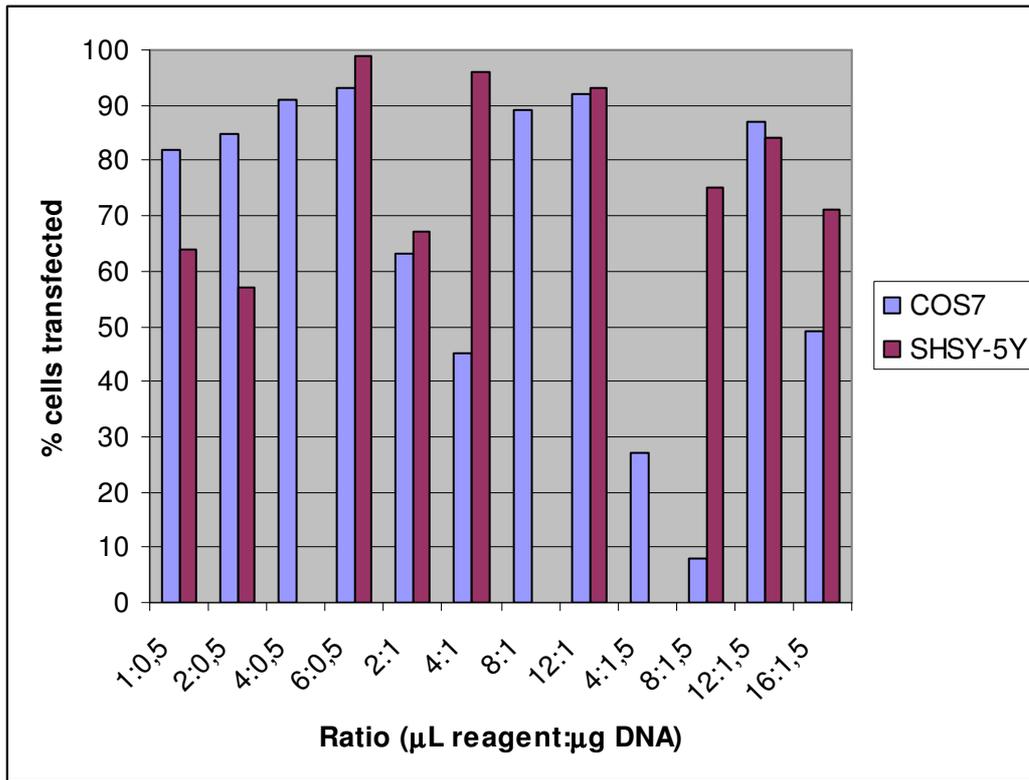
The human neuroblastoma cells SHSY-5Y were cultured in Minimum Essential Medium Eagle supplemented with 10% Fetal Bovine Serum (Sigma), anphotericin (1.25 mg/mL) penicillin (100U/mL) and streptomycin (100U/mL). The COS-7 cells were cultured in RPMI medium with the same supplements. The growth conditions were 37°C and 5% of CO<sub>2</sub>.

## Transfection protocol

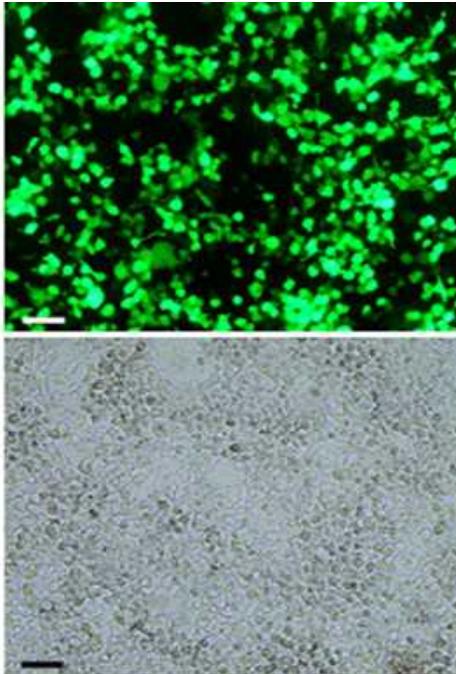
For transfection, SHSY-5Y ( $1.5 \times 10^5$  cells/well) and COS 7 cells ( $2 \times 10^5$  cells/well) were seeded in 1 mL of serum free medium (OPTI-MEM<sup>®</sup>, Gibco) in a 12-well microtiter plate, and then incubated at 37°C in a 5% CO<sub>2</sub> incubator overnight to obtain 80-90% confluence. Cells were pre-washed with serum-free OPTI-MEM<sup>®</sup> medium and covered with 1 mL of the same medium. Metafectene PRO was complexed with the pEGFP-C1 plasmid at reagent:DNA ( $\mu\text{L}:\mu\text{g}$ ) ratios of 1:0.5, 2:0.5, 4:0.5, 6:0.5, 2:1, 4:1, 8:1, 12:1, 4:1.5, 8:1.5, 12:1.5 or 16:1.5. Complexes were prepared by mixing Metafectene PRO with 0.1 mL of serum-free OPTI-MEM<sup>®</sup> medium, followed by the addition of plasmid DNA. The mixture was incubated for 15 min at room temperature after addition of transfection reagent, and another 15 min after addition of DNA. Metafectene PRO complexes with DNA were added in a volume of 0.1 mL per well and cells were incubated for 24 h at 37°C in a 5% CO<sub>2</sub> incubator. Then cells were washed three times with sterile PBS, added 1 mL of fresh PBS and analyzed under UV light in a Nikon microscope (Eclipse E 800).

## Results

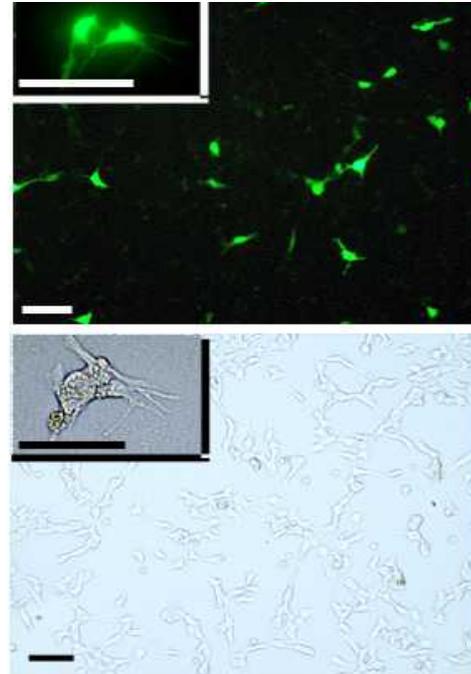
Metafectene PRO was complexed with the pEGFP-C1 plasmid at reagent:DNA ( $\mu\text{L}:\mu\text{g}$ ) ratios of 1:0.5, 2:0.5, 4:0.5, 6:0.5, 2:1, 4:1, 8:1, 12:1, 4:1.5, 8:1.5, 12:1.5 or 16:1.5. In case of COS-7 cells, all ratios showed transfection, but the best ratios were 2:0.5, 4:0.5, 8:1, 12:1 and 12:1.5, obtaining 80-95% of transfection level. Neuroblastoma cells were not transfected with ratios 4:0.5, 8:1 and 4:1.5. The transfection percentages are shown in Figure 1. Figure 2 and 3 show transfected COS7 and SHSY-5Y cells.



**Figure 1.** Percentage of cells transfected with pEGFP-C1 using Metafecten-PRO. Two different cell lines, COS7 and neuroblastoma cells (SHSY-5Y) were transfected with different ratios of Metafecten-PRO/DNA. Approximately in 70% of the experiments we observed a transfection level higher than 50%.



**Figure 2.** The above image shows UV microscopy of COS7 cells transfected with pEGFP-C1 using Metafecten-PRO. The ratio used was 4 ml reagent/0.5 µg DNA. Image shows optical microscopy of the same cells. Scale bars are equivalent to 100 µm.



**Figure 3.** Neuroblastoma cells transfected with pEGFP-C1 using Metafecten-PRO. The ratio was 2 ml reagent/0.5 µg DNA. Top: UV microscopy of neuroblastoma cells. Bottom: optical microscopy of the same cells. The boxes show some cells detailed. Scale bars are equivalent to 100 µm.