

Metafectene Application note

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We used Metafectene to stably transfect SH-SY5Y cells. SH-SY5Y are an adherent neuroblastome cell line and in general exhibit low transfection efficiency. SH-SY5Y were cultivated in EMEM, supplemented with Glutamine, 10% FCS and Gentamycin at a concentration of 50μ g/ml. Cells were grown at an optimum of 30-80% confluency at 37° C and 5% CO₂.

A day prior to transfection cells were seeded at a concentration of either 0,84 or 1,12 millions per 10cm plate. Six different transfections with each cell density were performed:

- 1. $5\mu g$ plasmid coding for human synuclein wt + $5\mu g$ plasmid coding for human dopamine transporter (DAT)
- 2. $5\mu g$ plasmid coding for human synuclein wt + $5\mu g$ empty plasmid (no DAT)
- 3. $5\mu g$ plasmid coding for human synuclein mutant $A53T + 5\mu g$ plasmid coding for human DAT
- 4. $5\mu g$ plasmid coding for human synuclein mutant A53T + $5\mu g$ empty plasmid (no DAT)
- 5. $5\mu g$ plasmid coding for human synuclein mutant A30P + $5\mu g$ plasmid coding for human DAT
- 6. $5\mu g$ plasmid coding for human synuclein mutant A30P + $5\mu g$ empty plasmid (no DAT)

The transfection was performed in the following way:

 $10\mu g$ total DNA were mixed with $692\mu l$ medium without FCS in tube 1 (resulting in a total volume of $700\mu l$). $32\mu l$ of Metafectene were mixed with $668\mu l$ of medium without FCS in tube 2 (resulting in a total volume of $700\mu l$). The contents of tube 1 and tube 2 were united and incubated at room temperature for 15min. Meanwhile the cells were prepared by replenishing fresh medium (14ml per 10cm plate). The DNA-Metafectene complex was added to the plate with a pipet. After 2h cells the medium containing the Metafectene-DNA complex was removed and fresh medium was added. 48h hours after transfection the selection was started by the addition of $300\mu g/ml$ G418 and $150\mu g/ml$ Zeocin. We supplemented the medium with 20% conditioned medium during the selection period of four weeks to promote clonal outgrowth.

At the very end (after testing the clones for expression of the proteins encoded by the transfected plasmids) we obtained a total of nine clones that were positive for either synuclein form. In comparison: with a different polykationic lipid transfection reagent with obtained only one positive clone. We suppose that the constitutive expression of synuclein is toxic to the cells and therefore only a minority of clones survive the selection procedure.