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 50µg/ml. Cells were grown at an optimum of 30-80% confluency at 37°C and 5% CO_2.
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. 5µg plasmid coding for human synuclein wt + 5µg plasmid coding for human dopamine transporter (DAT)
2. 5µg plasmid coding for human synuclein wt + 5µg empty plasmid (no DAT)
3. 5µg plasmid coding for human synuclein mutant A53T + 5µg plasmid coding for human DAT
4. 5µg plasmid coding for human synuclein mutant A53T + 5µg empty plasmid (no DAT)
5. 5µg plasmid coding for human synuclein mutant A30P + 5µg plasmid coding for human DAT
6. 5µg plasmid coding for human synuclein mutant A30P + 5µg empty plasmid (no DAT)

The transfection was performed in the following way:
10µg total DNA were mixed with 692µl medium without FCS in tube 1 (resulting in a total volume of 700µl). 32µl of Metafectene were mixed with 668µl of medium without FCS in tube 2 (resulting in a total volume of 700µl). 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 300µg/ml G418 and 150µg/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.