Metafectene Application note

Irina Woldman, Alexandra Kattinger, Harald Reither, Christian Pifl

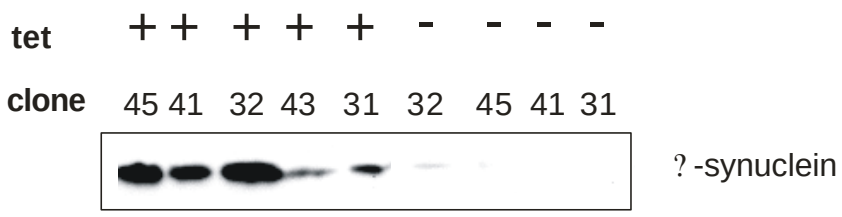
Institute of Brain Research, Spitalgasse 4, A-1090 Vienna

We used Metafectene to stably transfect SH-SY5Y cells. These cells are adherent neuroblastoma cells and in general exhibit a low transfection efficiency. SH-SY5Y cells are cultivated in MEM with Earle's salts, supplemented with glutamine, 10% FCS and gentamicine at a concentration of 50µg/ml. Cells were grown at an optimum of 30-80% confluency at 37°C and 5% CO_2.
Previously we had generated cell lines that express synuclein by use of Metafectene, but we had obtained only a few clones. We attributed this to the fact that constitutive expression of synuclein might be toxic for the cells during the selection process.
For this reason we cloned the cDNA for wildtype synuclein into a plasmid called pcDNA4/TO (T-REx expression system, Invitrogen), which is an inducible plasmid. The principle of the induction is the following: the gene of interest cloned into pcDNA4/TO is cotransfected with a plasmid called pcDNA6/TR encoding a repressor of the plasmid cDNA4/TO into the cells. In the absence of tetracycline the expression from the pcDNA4/TO plasmid is repressed, in the presence of tetracycline the expression of the gene of interest is induced. The advantage of such an inducible system is that the expression of the target gene can be induced at a given time and during the selection process the expression does not need to be on.
Furthermore we were interested to get cell clones that coexpress the human dopamine transporter (DAT) and synuclein. Therefore we actually performed triple transfections of synuclein in pcDNA4/TO (conferring resistance to Zeocin), the repressor plasmid (blasticidin) and human DAT in pRcCMV (geneticin) by use of Metafectene. The cells went through a selection with the three antibiotics alltogether, and resistant clones were obtained, which were tested for synuclein expression by induction with tetracycline: 22 positive clones had been generated, of which 12 clones also coexpressed the human DAT.
This result showed us that two factors had not been optimal during our previous transfection: first of all the constitutive expression of the synuclein and secondly the fact that we had probably used too high antibiotic concentrations. In any case this time the success shows that we were right to choose Metafectene to work with in the first row.
The following protocol contains the details of the transfection and selection procedure:
A day prior to the experiment cells were seeded at a concentration of two millions per 10cm plate. The following day eight plates were transfected with the following amounts of DNA:

1,2µg alpha-synuclein in pcDNA4/TO = 1,74µl
2,0µg human DAT in pRcCMV = 2,03µl
9,0µg repressor in pcDNA6/TR = 3,90µl

The DNA was mixed in an 1,5ml Eppendorf tube and 692µl of medium without FCS were added to yield a total volume of 700µl. In a second Eppendorf tube 40µl of Metafectene were mixed with 660µl medium without FCS. The contents of the tubes with the DNA and the Metafectene were united and incubated at room temperature for 15 minutes. Meanwhile fresh medium (14ml per 10cm plate) was added to the cells. The DNA-Metafectene complex was added to each plate with a pipet. The cells were incubated with the DNA-Metafectene complex 4h in the incubator. Thereupon the medium was exchanged. The next day the cells were split 1:2. 48 hours after transfection the selection was started by the addition of the

antibiotics: 30µg/ml Zeocin, 3µg/ml blasticidin and 600µg/ml geneticin. The selection proceeded for several weeks during which clonal outgrowth was achieved. The clones were tested for expression in the following way:
Each clone was seeded into two 35mm dishes at a concentration of 0,35 millions per dish. The following day one of the dishes was induced with tetracycline at a concentration of 1µg/ml. 24 hours later uninduced and induced cells were harvested and expression of synuclein was tested in Western blotting. Positive clones were expanded and the presence of DAT was determined by uptake experiments using Tritium labelled dopamine.



E.g.: Results from a Western blot testing the expression of alpha-synuclein.