Application Note:

Title of Experiment:

Evaluation of transfection efficiency of plasmids of different size and origin in neural rat PC12 cells

Author, Institute and address:

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Introduction:

Neural cell lines are generally less efficient to transfect compared to common non neuronal cells. This can become a problem when plasmids with large inserts are used and high expression efficiencies are required, for example for biochemical analysis or production of viruses. Aim was to compare the efficiency of different transfection agents in the neural rat PC12 cell line, which is commonly used for studies on neuronal differentiation since the cells can be differentiated into a neuron-like phenotype. Several expression plasmids and lentiviral plasmids were used for the experiments.

Materials and methods:

Transfection agents: Metafectene Easy (Biontex) and Lipofectamine LTX (Invitrogen).

Following plasmids were used:

Name of plasmid	size (bp)	promoter	expressed protein
pEGFP-htau352wt	5824	CMV	eGFP-tau
GFL-PAGFP	10057	CMV	PAGFP-gravin
L26Sy(1.1.)GW (lentiviral plasmid)	9801	Synapsin	eGFP
L22FCK(2.4)GW (lentiviral plasmid)	9911	CaMKII	mRFP

All plasmids were prepared and purified using QIA prep spin miniprep kit (Qiagen) from an overnight liquid culture of *E. coli* DH5α cells that had been transformed with the respective vectors. DNA concentration was photometrically determined.

PC12 cells were cultured according to general lab procedures as described previously (Gauthier-Kemper et al., 2011).

Experimental procedures / transfection protocol:

$5x10^4$ PC cells were plated in a 4-well plate containing a polylysine-treated coverslip in 500 µl of cell culture medium. On the next day, medium was exchanged and the transfection mixture (2.5 µl Metafectene Easy Reagent, 1 µg of the respective DNA, 50 µl Metafectene Easy Buffer, preincubated for 15 min) or Lipofectamine LTX according to the manufacturer's instructions, was added. The medium was changed every 2 days to medium with or without NGF and cells were fixed at different times post infection using a standard formaldehyde-fixation protocol. Cells were treated with DAPI as a counterstain for nuclei and transfection efficiency was determined by fluorescence microscopy by dividing transfected cell number through total cell number. Evaluation used a 20x objective on a Nikon fluorescence microscope (Eclipse TE2000-U).

Results and discussion:

1. Transfection efficiency was highest with the smallest construct (pEGFP-htau352wt, 5824bp) and reached about 40% efficiency with Metafectene Easy (Fig. 1). Transfection of the longer constructs (GFL-

PAGFP, L26Sy(1.1.)GW, L22FCK(2.4)GW, 9800-10100 bp) was much lower with no major difference between lentiviral or non lentiviral vectors. Here, efficiencies up to ~10% could be reached.

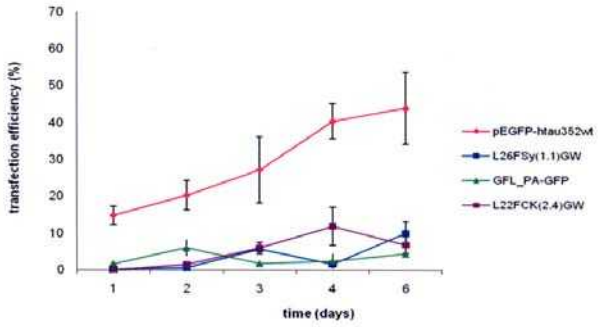

Fig. 1. Time course of transfection of PC12 cells with plasmids of different sizes. Transfection efficiency is highest with the smallest plasmid (red) and transfection efficiencies up to ~40% are reached. 5 microscopic frames per experiment were evaluated and mean and standard deviation are shown.

2. The amount of expressed protein per cell was similar for the GFP-expressing cells (transfection with pEGFP-htau352wt and f L26Sy(1.1.)GW) as judged by visual inspection of GFP fluorescence. No evidence for major neuronal degeneration as a result of the transfection procedure was observed (Fig. 2).

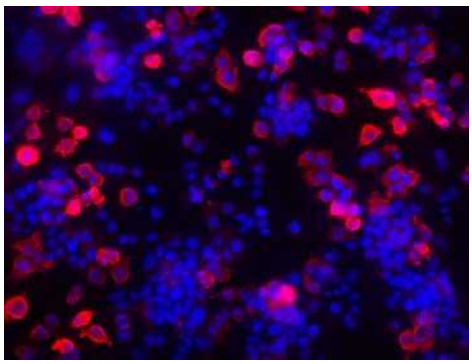

Fig. 2. Transfection of undifferentiated PC12 cells with pEGFP-htau352wt using Metafectene Easy. Cells were fixed and stained with an antibody against GFP (red) and DAPI (blue) 4 days post transfection. Note that transfected cells do not show signs of degeneration as would be for example evident by nuclear fragmentation.

3. Cells that had been transfected with Metafectene Easy could be differentiated into neuron-like cells by treatment with NGF (Fig. 3). The differentiated cells did not show signs of degeneration.

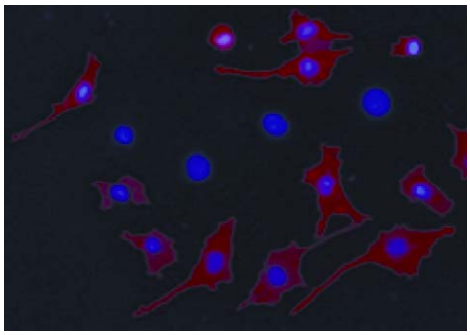

Fig. 3. Neuronal differentiation of PC12 cells that had been transfected with pEGFP-htau352wt using Metafectene Easy. Cells were differentiated for 4 days with NGF and stained with an antibody against GFP (red) and DAPI as a nuclear counterstain (blue). Formation of processes indicates neuronal differentiation.

4. In most cases, transfection efficiencies were higher with Metafectene Easy compared to Lipofectamine LTX, which was used according to the manufacturer's instructions.

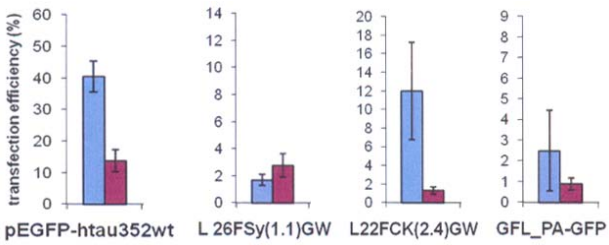

Fig. 4. Comparison of transfection efficiencies between different plasmids using Metafectene Easy (blue) and Lipofectamine LTX (red). Cells were analyzed four days post transfection and 5 microscopic frames per experiment were evaluated. Mean and standard deviation are shown.

Conclusion / summary:

Metafectene Easy provides good transfection efficiency with minimal toxicity for neural cells. Cells can be efficiently neuronally differentiated after transfection, which permits their use as a neuronal model system. Transfection efficiency drops with longer plasmids, e.g. those for lentiviral production. With most plasmids, transfection with Metafectene Easy yields a higher efficiency compared to Lipofectamine LTX.

References:

Gauthier-Kemper, A., Weissmann, C., Golovyashkina, N., Sebö-Lemke, Z., Drewes, G., Gerke, V., Heinisch, J.J., and Brandt, R. (2011) The frontotemporal dementia mutation R406W blocks tau's interaction with the membrane in an annexin A2-dependent manner. J. Cell Biol. 192:647-661.

Appendix: Tables and/or figures:

Cell code	Primary	Class	Species	Organ	Type	Identi-fication	Description	Reagent	Growth Properties	Genetic Material	Efficiency	Toxicity
PC12	no	mammalia	rat	Pheochro-mo-cytome	Neural tissue	Neural cell	Rat pheochromo-cytoma cell line	METAFEC-TENE EASY; Lipofecta mine LTX	adherent	Plasmid	Up to 40%	-