Conditioning of transfection protocols using commercially available liposome based reagents

Graham Diering, Masayuki Numata. University of British Columbia. Dept. Of Biochemistry and Molecular Biology, 2350 Health Sciences Mall, Life Sciences Centre, Vancouver, BC, Canada

Introduction:

We wished to determine the best reagent and condition which could be used to deliver plasmid vectors into the difficult to transfect neuroendocrine PC12 cell line with high transfection efficiency and reproducibility.

Materials and methods:

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). Fugene6 trasfection reagent was obtained from Roche. Lipofectamine 2000 was obtained from Invitrogen

Experimental procedures / transfection protocol:

PC12 cells growing in log phase were seeded into standard 12-well tissue culture plates at a density of 200,000 cells/well. A sterile 1cm round glass coverslip was included into each well. Cells were transfected with a mammalian expression vector pcDNA3 encoding a cDNA of enhanced GFP either immediatly upon plating, while the cells were still in suspension or 24 hours following plating, at which time the cells were completely attached to the glass coverslips. 0.8µg of plasmid DNA was introduced to cells using 1.5µL , 2µL, or 3µL of Metafectine Pro, or 1.0µg of DNA was introduced using 2µL, 4µL, or 8µL of Metafectene Pro to PC12 cells either newly plated (in suspension) or following 24 hours of attachment (adherrant). Similar transfections were performed using Lipofectamin 2000 (Invitrogen) or Fugene6 (Roche) according to the manufacturor's instructions.

48 hours post transfection, coverslips with attached cells were fixed with 4% para formaldehyde (PFA) in PBS for 10min at 4oC. Nuclei of fixed cells were staind with Hoechst DNA stain (500ng/mL) for 10 min at room temp. Coverslips were then

mounted onto glass slides and transfection efficiency was dtermined from the fluorescence of GFP.

Results and discussion:

Under all conditions Metafectene Pro (MFP) was able to deliver GFP plasmid DNA into PC12 cells. The lowest MFP:DNA ratios yielded lower transfection efficiencies in all cases, approximately 5%. For cells in suspension, the highest MFP:DNA ratios gave the best results, approximately 20% transfection efficiency was achieved. When cells were allowed to adhere, intermediate MFP:DNA ratios yielded 15-20% transfection efficiency. Increasing the amount of MFP used for transfection of Addherrent cells did not improve the trasfection efficiency over that observed for the intermediate ratio. The MFP reagent appeared to produce a large amount of precipitate which was highly fluorescent when transfected cells were viewed under a fluorescence microscope. Transfection efficiency obtained was adequate for many applications. Further cell toxicity with MFP appeared to be minimal. However the fluorescent precipitate resulting from the use of MFP limits the use of this reagent in experiments involving fluorescence microscopy. MFP reagent was much more effective than Fugen6 reagent which gave <1% transfection efficiency under most conditions, and gave approximately 10% transfection efficiency at its best. Lipofectamine 2000 yielded the highest transfection efficiency of GFP into PC12 cells, >50%, but this reagent also appeared to cause the greatest amount of cell death as compared to the other reagents tested.

Conclusion / summary:

Using MFP reagent, 20% transfection efficiency was obtained in PC12 cells. Transfection of these cells 24hours after plating gave good transfection efficiency with less reagent, suspension cells required the maximum amount of reagent for high transfection efficiency. MFP was more effective than Fugene6 (Roche) and had less visble toxicity than Lipofectamine 2000 (Invitrogen). However the fluorescent precipitate which results from the use of MFP limits the usefulness of this reagent for some applications.