Preparation of retrovirus for transfection

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Introduction:

It is known that disturbed iron metabolism is a cause of the neurodegenerative disorders like Parkinson's disease. DMT1 is one of the most important iron uptake proteins. To examine the role of DMT1 in brain iron uptake, we would like to transfect the Psuper plasmid into Phi-NX cell in order to produce retrovirus. We can investigate the change of iron level in cells with decreased DMT1 expression. Also, interactions between iron transport proteins can be studied.

Materials and methods:

Metafectene PRO, DMEM, Psuper plasmid, Phi-NX cell line

Experimental procedures / transfection protocol:

1. Plate $1X10^6$ Phi-NX cells into 6-well plate with 2 ml of 10% FBS DMEM and plate at 37^oC incubator for 24 hrs prior to transfection.
2. 4 hrs prior to transfection, remove medium and replace with plain medium (2 ml per well)
3. 25 min prior to transfection, prepare transfection cocktail by adding

- 20ul Psuper plasmid in 100ul PBS in tube A
- 20ul Metafectene PRO in 100ul PBS in tube B
- Mix the solutions gently by pipetting 1 time
- Combine the solutions without mixing and incubate at room temperature for 20 min.

Add this solution into the cells, then gently swirl the plate to ensure uniform mixing.

4. Incubate at 37^oC incubator for 24 hrs and replace with fresh medium
5. Select the cells with puromycin

Results and discussion:

Comparing the viability of cells after selecting the cells (48 hr after transfection) with puromycin, over 80% of cells are viable using METAFECTENE PRO which is higher than other transfection reagents (~30%). It may be due to the decrease in toxicity effect. Moreover, the transfection efficiency is high compared with other transfection reagents which are not successful.

Conclusion / summary:

METAFECTENE PRO is a transfection reagent with a high efficiency and has an advantage of low toxicity effect.

Appendix: Tables and/or figures: