Neuro2A Trasfection efficiencies using Metafectene PRO

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INTRODUCTION

The aim of this report is to find the best reagent and conditions in order to transfect Neuro 2A cell line (mouse neuroblastoma). In our lab we have tested various reagents in order to establish the best ones to obtain high transfection efficiency in Neuro-2a cells.

MATERIALS AND METHODS

Neuro 2A cells were grown in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 U/ml penicillin and 10 µg/ml streptomycin, in a 5% CO_2 incubator at 37°C.
The transfection reagents used in this study were: Metafectene PRO (Biontex Laboratories GmbH). FuGENE6 (Roche), Lipofectamine and Lipofectamine 2000 (Invitrogen) and DOTAP (Roche).

EXPERIMENTAL TRANSFECTION PROTOCOL

Neuro2A cells were plated one day before transfection into 24-well tissue culture plates at a density of either 8.0 x 10^4 or 8.0 x 10^5 onto sterile glass coverslip that were placed into each well. Cells were transfected with the mammalian expression vector pEGFP-C1 encoding GFP.
We have tested several DNA:transfection reagent ratio following manufacturer's protocols, as reported in the table below. For each reagents we follow trasfection procedures indicated by manufacturers.
After 20 hours of transfection, coverslips were fixed with 3% paraformaldehyde (PFA) in PBS 1X for 10' at 4°C and then were mounted with Mowiol onto glass slide. Transfection efficiency was determined by counting cells expressing GFP.

RESULTS AND DISCUSSION

The best results were obtained by plating 8.0 x 10^4 cells/well. In these conditions, using Lipofectamine, Lipofectamine 2000, DOTAP or Fugene6 reagents we obtained few transfected cells (maximun transfection was efficiency 5%). In contrast, we obtained a very good transfection efficiency (about 60% of GFP-expressing cells) using Metafectene PRO reagent with DNA:reagent ratio of 0.5:2. After transfection cells were differentiated successfully by serum withdrawal.

Cells density (cells/well)	DNA : transfection reagent ratio (µg/µl)	Transfection efficiency (percentage of transfected cells)	Toxicity (percentage of dead cells)
8.0 x 10^5	0.25 : 4 Lipofectamine 0.5 : 1.5 Fugene 0.5 : 2 Metafectene PRO 0.5 : 6 DOTAP	0 0 20 0	5 2 2 2
8.0 x 10^4	0.4 : 4 Lipofectamine 0.4 : 1 Lipofectamine 1 : 3 Lipofectamine 2000 1 : 4 Metafectene PRO 0.5 : 2 Metafectene PRO 0.5 : 6 DOTAP	3 5 3 40 60 3	10 5 5 3 2 2

CONCLUSION

Neuro 2A cells were best transfected using Metafectene PRO reagent with the conditions reported below:

- Cell plating density => 8.0 x 10^4
- DNA : Metafectene PRO reagent ratio (µg/µl) => 0.5 : 2

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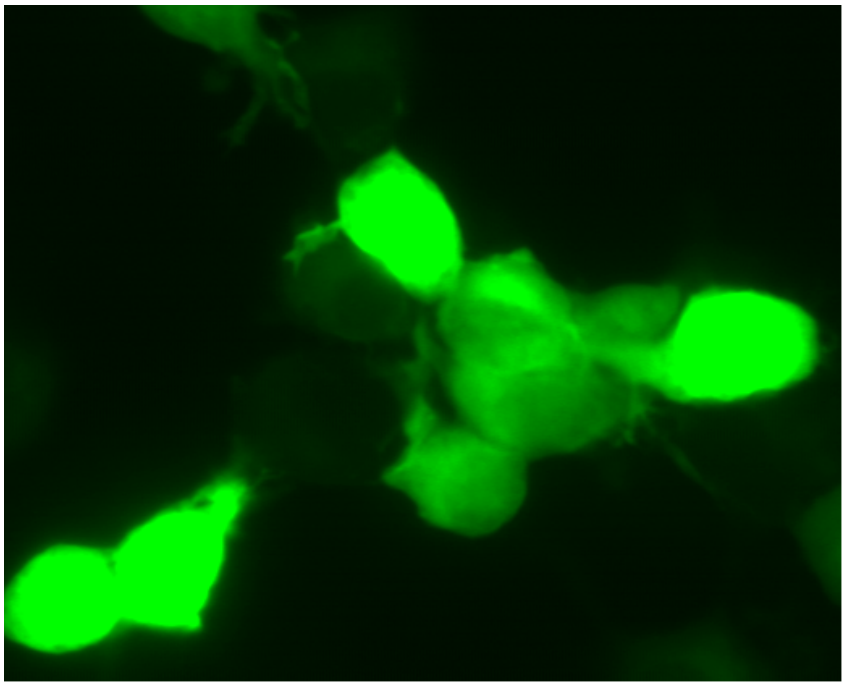

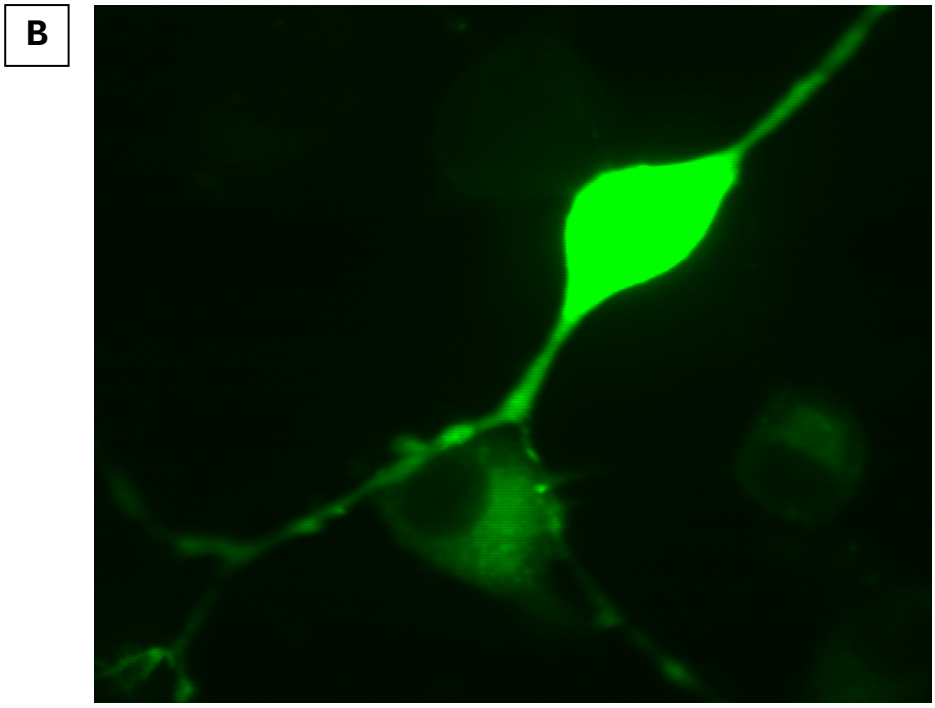

Neuro 2A cells transfected with Metafectene Pro, before differentiation (A) and after differentiation (B) by serum withdrawal for two days.