PC12 transfection 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 PC12 cell line (derived from a pheochromocytoma of the rat adrenal medulla). In our lab we have tested various reagents in order to establish the best ones to obtain high transfection efficiency for this cell line.

MATERIALS AND METHODS

PC12 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 2000 (Invitrogen).

EXPERIMENTAL TRANSFECTION PROTOCOL

PC12 cells were plated one day before transfection in DMEM supplemented with 10% FBS, 2 mM glutamine, without antibiotics, in a 5% CO_2 incubator at 37°C. 1.0 x 10^4, 7.0 x 10^4 or 2.0 x 10^5 cells/well were seeded onto sterile glass coverslips that were placed into the wells of 24 wells plates. 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 reagent we followed transfection 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

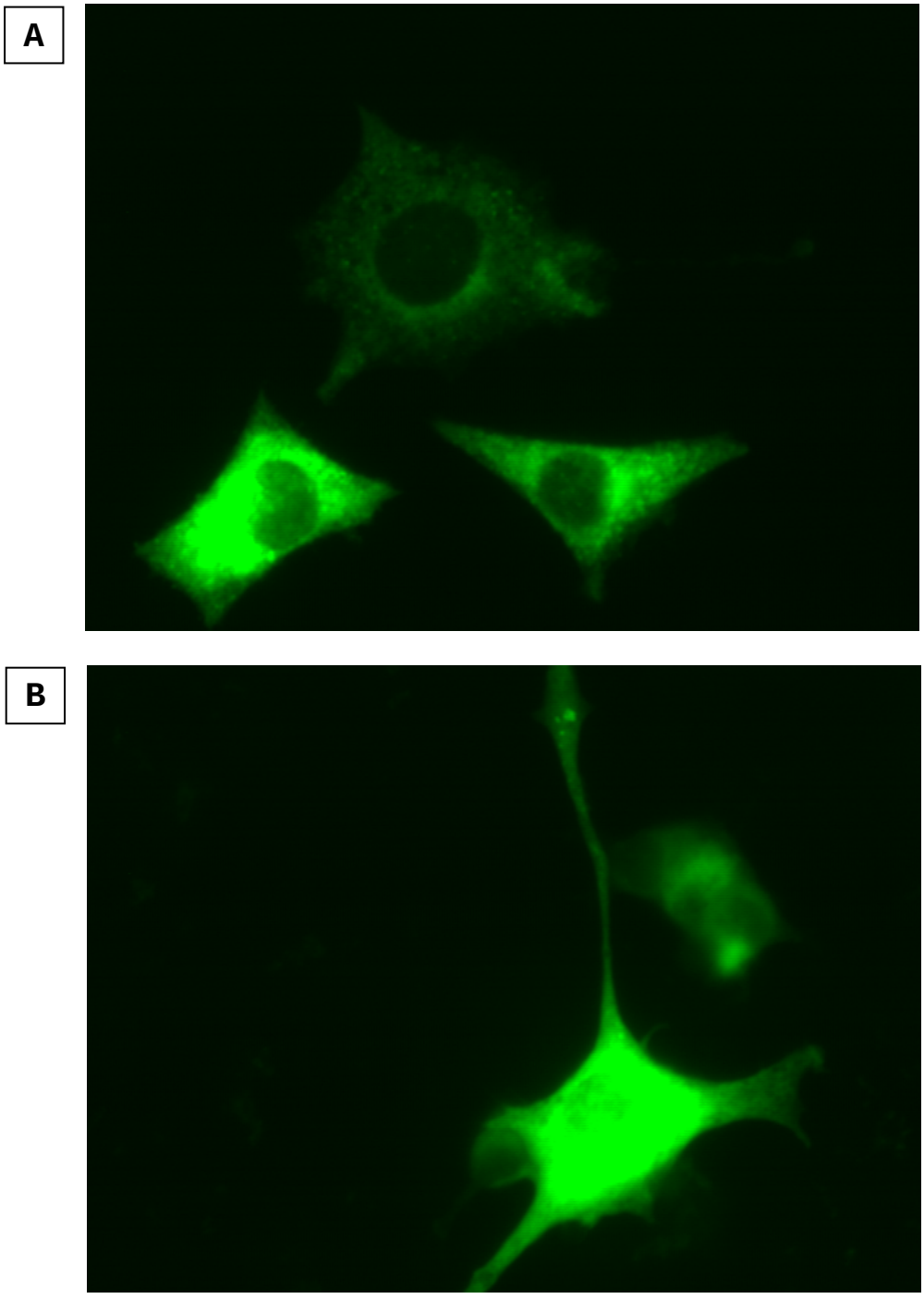
The best results were obtained by plating 2.0 x 10^5 cells/well. In these conditions, with FuGENE6 reagent we could not obtain transfected cells, while we had a good transfection using Lipofectamine 2000 reagent with intermediate DNA:reagent ratio and using Metafectene PRO reagent with the highest DNA:reagent ratio. In both, the transfection efficiency was about 30%, but with metafectene PRO we obtained a stronger fluorescence signal than that obtained with Lipofectamine 2000.
After transfection cells were differentiated successfully by adding NGF for three days.

Cells density (cells/well)	DNA : transfection reagent ratio (µg/µl)	Transfection efficiency (Percentage of transfected cells)	Toxicity (percentage of dead cells)
2.0 x 10^5	0.18 : 0.6 Lipofectamine 2000	3	5
	0.8 : 2 Lipofectamine 2000	30	8
	1 : 4 Lipofectamine 2000	10	10
	0.5 : 1 FuGENE6	0	2
	0.8 : 2 Metafectene PRO	10	3
	1 : 4 Metafectene PRO	30	3
6.7 x 10^4	0.18 : 0.6 Lipofectamine 2000	3	5
	0.8 : 2 Lipofectamine 2000	10	8
	1 : 4 Lipofectamine 2000	0	10
	0.5 : 1 FuGENE6	0	2
	0.8 : 2 Metafectene PRO	5	2
	1 : 4 Metafectene PRO	20	1
1.0 x 10^4	0.18 : 0.6 Lipofectamine 2000	1	1
	0.8 : 2 Lipofectamine 2000	1	2
	1 : 4 Lipofectamine 2000	0	8
	0.5 : 1 FuGENE6	0	2
	0.8 : 2 Metafectene PRO	0	3
	1 : 4 Metafectene PRO	0	3

CONCLUSION

PC12 cells were best transfected using Metafectene PRO reagent with the conditions reported below:

- Cells density => 2.0 x 10^5
- DNA : Metafectene PRO reagent ratio (µg/µl) => 1 : 4



PC12 cells transfected with Metafectene Pro, before differentiation (A) and after differentiation (B) by adding NGF for three days.