NEUROSCIENCE

TRANSFECTING A HUMAN NEUROBLASTOMA CELL LINE WITH MONSTER GREEN™ FLUORESCENT PROTEIN

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We expressed an improved green fluorescent protein, Monster Green™ Fluorescent Protein, in a human neuroblastoma cell line, SH-SY5Y. Optimized transfection was accomplished using TransFast™ Transfection Reagent. Expression of the Monster Green™ Fluorescent Protein was bright and did not appear to interfere with the development of normal neuronal cell morphology.

Introduction

Green Fluorescent Protein (GFP) is a commonly used reporter molecule that can be visualized without cell lysis using standard fluorescent microscopy. It is most often used to monitor gene expression but can also be used to monitor intracellular protein trafficking by creating C- and N-terminal protein fusions.

Monster Green™ Fluorescent Protein[a,b,c,d] is an improved synthetic version of the green fluorescent protein gene from the great star coral *Montastrea cavernosa* (hMGFP; 1). The fluorescence intensity is increased compared to other commercially available green fluorescent proteins. Peak excitation occurs at 505nm and peak emission occurs at 515nm. Standard FITC filters may be used to visualize hMGFP fluorescence.

This article demonstrates transfection of Monster Green™ Fluorescent Protein in a human neuroblastoma cell line, SH-SY5Y. The SH-SY5Y cell line is commonly used to study neuritogenesis, differentiation, and tumorigenesis (2–6). Neuronal cells and cell lines have been transfected using many different techniques with highly variable levels of success (7). Successful expression of Monster Green™ Fluorescent Protein in the SH-SY5Y cells was accomplished by transfecting the hMGFP reporter vector using TransFast™ Transfection Reagent[e] and optimized conditions.

Methods

We cultured the human neuroblastoma cell line SH-SY5Y in 45% DMEM/45% F12-K medium supplemented with 10% fetal bovine serum at 37°C in 5% CO_2. For all transfection experiments, we plated the cells in clear 24-well tissue culture plates (between $3–12 \times 10^4$ cells/well). Transfection was investigated using various reagents, (TransFast™, Transfectam®, Lipofectamine™ 2000, and *Trans*IT®-Neural™ Reagents), and initial experiments demonstrated that TransFast™ Reagent produced the highest level of transfection (Table 1). We performed all transfection reagent comparisons and TransFast™ transfection optimization experiments using the *Renilla* luciferase reporter vector phRL-CMV[a,b,c,d,g] and the *Renilla* Luciferase Assay System[b,c]. All transfection reagents were used according to the manufacturer's instructions.

We optimized TransFast™ transfection for cell density, lipid:DNA ratio and amount, DNA amount, and incubation time with the lipid:DNA mixture following Technical Bulletin #TB260 (Table 2). The optimal conditions were: ~20–30% cell confluency at time of transfection, 0.75µg plasmid DNA and 4.6µl TransFast™ Reagent (2:1 charge ratio of TransFast™ Reagent:DNA) in 200µl Opti-MEM medium per well, with a 1-hour exposure time before adding complete medium.

These transfection conditions were used to introduce the Monster Green™ Fluorescent Protein vector (phMGFP) into the SH-SY5Y cells. Forty-eight hours following transfection the cells were viewed under a Zeiss Axiovert® S100 fluorescent microscope using 470/40nm excitation filter and a 515nm long-pass emission filter. The imaging data was collected using Spot Diagnostic Imaging equipment.

Results and Discussion

We optimized transfection of the human neuroblastoma SH-SY5Y cells for various transfection parameters, including transfection reagent, DNA and lipid amount, cell confluency and incubation time with the DNA:lipid mixture using *Renilla* luciferase vectors. All of the reagents tested recommended transfection at cell densities of at least 50%, so approximately 50% confluency and 75% confluency were tested. The results of this initial experiment are shown in Table 1 and demonstrate that TransFast™ Reagent showed maximal *Renilla* luciferase reporter activity compared to the other transfection reagents tested.

The use of the TransFast™ Transfection Reagent with SH-SY5Y cells was further optimized for cell density and incubation time with the DNA:lipid mixture when using 0.75µg of plasmid at a 2:1 charge ratio of lipid:DNA per well. The results are shown in Table 2 and demonstrate that lower cell density and an 1-hour incubation period with the DNA:lipid mixture were optimal for SH-SY5Y cells. The TransFast™ Transfection Reagent has also been shown to be successful for transfecting human dendritic cells and primary neurons with GFP (8,9).

Introducing the Monster Green™ Fluorescent Protein phMGFP vector using the TransFast™ Transfection Reagent and the optimized transfection conditions resulted in the

Table 1. Comparison of Transfection Reagent Efficacy in SH-SY5Y Cells

Transfection Reagent	*Renilla* Luciferase Reporter Activity (Relative Light Units)	Activity Relative to TransFast™ Reagent (set to 100%)	Optimized Conditions
TransFast™ Reagent	125277 (±5484)	100%	0.75µg plasmid 4.6µg reagent 44–55% confluency
Transfectam® Reagent	36629 (±2942)	29%	1.0µg plasmid 2µl reagent 70–80% confluency
Lipofectamine™ 2000	90080 (±3518)	72%	1µg plasmid 1µl reagent 70–80% confluency
TransIT® Neural™ Reagent	39286 (±3116)	31%	1µg plasmid 1µl reagent 70–80% confluency

Results are the average of triplicates. DNA amounts varied from 0.25–1.0µg/well in a 24-well plate, while reagent amounts varied from 0.5–8.0µl/well. Cell density was either ~45–55% or 70–80%.

Table 2. *Renilla* Luciferase Reporter Activity for SH-SY5Y Cells Transfected Using TransFast™ Transfection Reagent at Different Cell Densities and Incubation Times with the DNA/Lipid Mixture.

Cell Density (cells plated/well; approximate confluency of well at time of transfection)	Incubation Time (hours)	, Luciferase Reporter Activity (Relative Light Units)
3×10^4 (~20–30%)	1.0	540526 ±28026 (*100%)
3×10^4 (~20–30%)	2.5	228881 ±12444 (*42%)
6×10^4 (~40–50%)	1.0	341381 ±15069 (*63%)
6×10^4 (~40–50%)	2.5	388493 ±19424(*72%)
12×10^4 (~75–85%)	1.0	51322 ±3113 (*10%)
12×10^4 (~75–85%)	2.5	27977 ±2263 (*5%)

*percent relative to best
Results are the average of quadruplicates. The DNA amount was 0.75µg/well, and the lipid amount was 4.6µl/well in a 24-well plate.

expression of detectable green fluorescent protein in the SH-SY5Y cells (Figure 1A). In addition, the cells expressing Monster Green Fluorescent Protein were still able to maintain and/or develop neuron-like morphology, suggesting the nontoxic nature of this reporter molecule (Figure 1B). Notice the branching neurites forming in the center cell in Figure 1B. This supports earlier results in which Monster Green™ Fluorescent Protein exhibited persistent expression in NIH3T3 cells over a 7-day period, again suggesting the nontoxic nature of the Monster Green™ Fluorescent Protein compared to other commercially available green fluorescent reporter proteins (1).

Conclusion

We transfected human neuroblastoma SH-SY5Y cells using an optimized protocol and TransFast™ Transfection Reagent. The transfection efficiency was confirmed using the Monster Green™ Fluorescent Protein phMGFP Vector, which was shown to express bright green fluorescent protein that did not appear to interfere with normal cell morphology (i.e., neurite formation). This transfection reagent and reporter vector may be useful for other neural cell types as well. ■

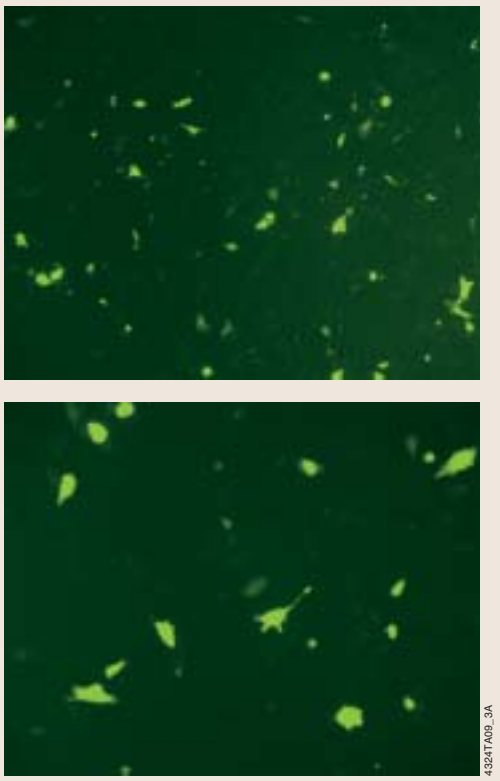

Figure 1. Expression of Monster Green™ Fluorescent Protein in transfected human neuroblastoma SH-SY5Y cells forty-eight hours following transfection. Image B was taken using a higher power objective than image A.

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Neuroblastoma Transfection

References

1. Almond, B.D. (2003) *Promega Notes* **84**, 12–15.
2. Vu, D. *et al.* (2003) *Brain Res. Mol. Brain Res.* **115**, 93–103.
3. Simpson, P.B. *et al.* (2001) *Anal. Biochem.* **298**, 163–9.
4. Yu, E., Choi, E.K. and Kim, C.J. (2003) *Virchows Arch.* **442**, 278–83.
5. van Golen, C.M. *et al.* (2003) *Oncogene* **22**, 2664–73.
6. Singleton, J.R., Randolph, A.E. and Feldman, E.L. (1996) *Cancer Res.* **56**, 4522–29.
7. Washbourne, P. (2002) *Curr. Opin. Neurobiol.* **12**, 566–73.
8. Strobel, I. *et al.* (2000) *Gene Therapy* **7**, 2028–35.
9. Ango, F. *et al.* (1999) *Neuropharmacology* **38**, 793–803.

Protocols

Monster Green™ Fluorescent Protein phMGFP Vector Technical Bulletin #TB320
(http://www.promega.com/tbs/tb320/tb320.html)

TransFast™ Transfection Reagent Technical Bulletin #TB260
(http://www.promega.com/tbs/tb260/tb260.html)

Synthetic Renilla *Luciferase Reporter Vectors Technical Manual* #TM237
(http://www.promega.com/tbs/tm237/tm237.html)

Renilla *Luciferase Assay System Technical Manual* #TM055
(http://www.promega.com/tbs/tm055/tm055.html)

Ordering Information

Product	Size	Cat.#
Monster Green™ Fluorescent Protein phMGFP Vector[a–d]	20µg	E6421
TransFast™ Transfection Reagent[e]	1.2mg	E2431
phRL-CMV Vector[a,b,c,d,g]	20µg	E6271
Renilla Luciferase Assay System[b,c]	100 assays	E2810
	1,000 assays	E2820

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[e]The cationic lipid component of the TransFast™ Transfection Reagent is covered by U.S. Pat. Nos. 5,824,812, 5,869,715 and 5,925,623, Australian Pat. No. 713093 and pending foreign patents.

[f]Transfectam is a registered trademark of Promega Corporation, which sells the Transfectam® product for research purposes only under a license from Polyplus-Transfection. The Transfectam® product was developed by J.P. Behr and J.P. Loeffler and is covered by U.S. Pat. Nos. 5,171,678, 5,476,962, 5,616,745 and corresponding foreign patents.

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