

## Transfection of Neuroblastoma Cell Lines Using the Gene Pulser MXcell™ Electroporation System

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### Introduction

The ability to transfer exogenous nucleic acids into mammalian cells has allowed scientists to study gene expression and molecular pathways. Gene transfer can be mediated by one of several commonly used techniques, including lipid transfection, viral infection, and electroporation. Not all transfection techniques work equally well in all cell types.

Neuroblastoma is a childhood cancer that accounts for approximately 7% of all cancers in children under 15 years of age (Gurney et al. 1995). Because some neuroblastomas (stage 4S) spontaneously regress in infants (Haas 1998), the identification of gene expression differences between nonlethal and lethal forms of the disease (Voth et al. 2007) may lead to a greater understanding of tumor biology, apoptosis, and therapies for the disease.

Neuroblastoma cell lines are used as model systems for the study of neural cell development because stimulation by commonly used differentiation agents such as phorbol esters, N-(4-hydroxyphenyl) retinamide, and cytosine arabinoside results in different responses by different cell lines (Thiele 1988). In addition, one study on the metastatic properties of neuroblastoma revealed two subpopulations within a cultured neuroblastoma cell line: one with high- and the other with low-invasive properties (Xie et al. 2007).

This report describes how we optimized conditions for two neuroblastoma cell lines for which electroporation conditions had not yet been defined.

### Methods

Neuroblastoma cell lines IMR-32 (American Type Culture Collection, ATCC #CCL-127) and SK-N-SH (ATCC #HTB-11) were grown in RPMI-1640 medium containing 25 mM HEPES and L-glutamine (Invitrogen Corporation) supplemented with 12.5% fetal bovine serum, 1% penicillin-streptomycin, and 0.85% sodium pyruvate.

Cells were trypsinized, washed with PBS, and resuspended in Gene Pulser® electroporation buffer at a final cell density of either  $1 \times 10^6$  cells/ml (IMR-32 and SK-N-SH cells) or  $3 \times 10^6$  cells/ml (SK-N-SH cells). A luciferase expression plasmid, pCMVi-Luc (Bio-Rad Laboratories, Inc.), was added to IMR-32 cells and SK-N-SH cells ( $1 \times 10^6$  and  $3 \times 10^6$ /ml) at a final concentration of 20  $\mu$ g/ml. Alternatively, siLentMer™ Dicer-substrate siRNA duplexes (negative control and *GAPDH*) were added to  $3 \times 10^6$ /ml SK-N-SH cells at a final concentration of 50 and 100 nM, respectively. Once plasmid DNA or siRNA was added to cells, the suspension was mixed gently, and 150  $\mu$ l of the mixture was aliquotted into the appropriate well sets of a 96-well electroporation plate. Cells were electroporated using the Gene Pulser MXcell electroporation system.

Following electroporation, 100  $\mu$ l of the cell suspension was removed from each well and added to 0.5 ml of medium in each well of a 24-well culture plate. The medium volume was increased to 1 ml for cells electroporated at a density of  $3 \times 10^6$ /ml. Since we did not have prior experience electroporating IMR-32 and SK-N-SH cells, we initially used preset protocols 1 (Opt mini 96-well/Sqr, Exp) on the Gene Pulser MXcell system. These protocols are designed to test both square and exponential waveforms, with three different voltages applied for square and three different capacitance settings applied for exponential waveforms (Figure 1). Cells were washed with PBS 24 hr following electroporation and used for either the luciferase activity assay or real-time reverse transcription polymerase chain reaction (RT-PCR) analysis. For gene expression analysis, total RNA was extracted from cells using the Aurum™ total RNA mini kit and used as a template to prepare cDNA using the iScript™ cDNA synthesis kit. To assess gene silencing, real-time PCR reactions were performed with iQ™ SYBR® Green supermix and the iQ™5 real-time PCR detection system.

## Results and Discussion

When IMR-32 and SK-N-SH cells were electroporated with a luciferase expression plasmid, the highest luciferase activity was obtained with a square-wave protocol (Figures 2 and 3), suggesting that square waveforms, and not exponential waveforms, may be optimal for these cells. During our initial testing, we observed that the maximal luciferase activity for square-wave electroporations was obtained using the lowest voltage (200 V); without additional testing, we cannot be sure that optimal conditions were achieved.

Since we wanted to test a voltage lower than 200 V, we selected 150 V for the next electroporation experiment with IMR-32 and SK-N-SH cells (data not shown). Much lower luciferase activity was observed than at 200 V (data not shown).

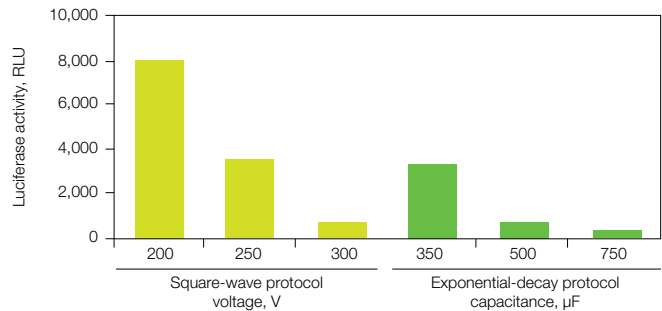
Neuroblastoma cells tend to be small, which may affect transfection efficiency, so we also tested cell density in this study. Increasing the cell density 3-fold (from  $1 \times 10^6$  to  $3 \times 10^6$ /ml) resulted in a commensurate amount of activity: RLU values obtained were at least 3-fold higher in the higher-cell-density electroporations as expected.

According to our study, the optimal conditions for electroporating a luciferase expression plasmid into  $3 \times 10^6$ /ml SK-N-SH cells are a square-wave pulse of 200 V and 2,000  $\mu\text{F}$ , with a pulse duration of 20 ms. At both cell densities the square-wave protocols resulted in greater cell viability than the exponential-decay protocols (data not shown).

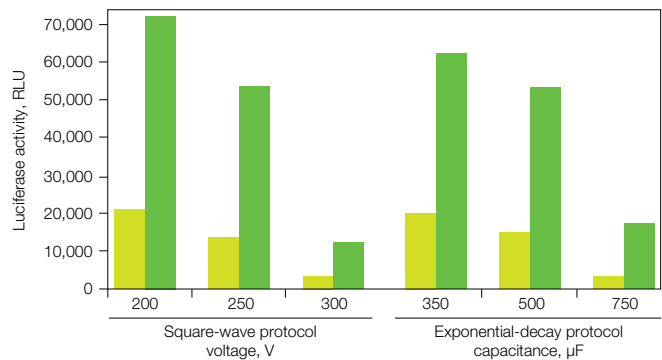
Under conditions determined in the plasmid experiments,  $3 \times 10^6$ /ml SK-N-SH cells were electroporated with either the negative control or *GAPDH*-specific siLentMer siRNAs, and gene silencing was assessed. We observed over 90% *GAPDH* gene silencing after 50 or 100 nM *GAPDH* siLentMer electroporations compared to corresponding negative control siLentMer siRNAs (Figure 4).

|          | 1                                     | 2                                     | 3                                     | 4                          | 5                          | 6                          |
|----------|---------------------------------------|---------------------------------------|---------------------------------------|----------------------------|----------------------------|----------------------------|
| <b>A</b> | 200 V<br>2,000 $\mu\text{F}$<br>20 ms | 250 V<br>2,000 $\mu\text{F}$<br>20 ms | 300 V<br>2,000 $\mu\text{F}$<br>20 ms | 250 V<br>350 $\mu\text{F}$ | 250 V<br>500 $\mu\text{F}$ | 250 V<br>750 $\mu\text{F}$ |
| <b>B</b> |                                       |                                       |                                       |                            |                            |                            |
| <b>C</b> |                                       |                                       |                                       |                            |                            |                            |
| <b>D</b> |                                       |                                       |                                       |                            |                            |                            |
|          | Square waveform                       |                                       |                                       | Exponential waveform       |                            |                            |

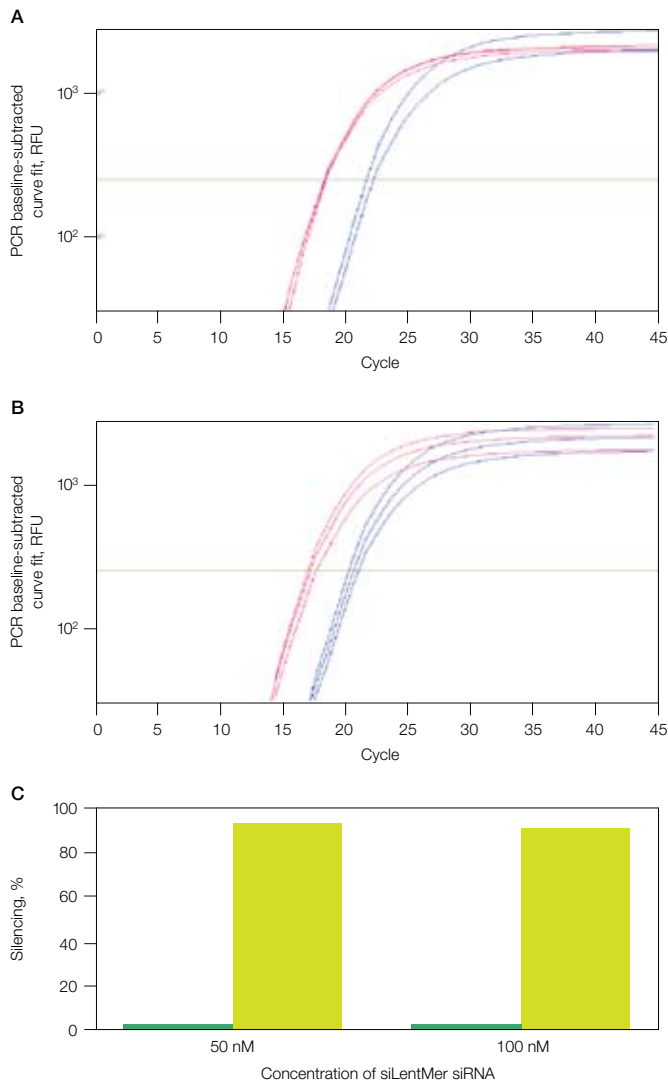
**Fig. 1. Gene Pulser MXcell preset protocols 1 (Opt mini 96-well/Sqr, Exp).** Conditions for each well set (a set of four rows in one plate column, as shown) are indicated in the appropriate column.



**Fig. 2. Luciferase activity of IMR-32 cells ( $1 \times 10^6$ /ml) electroporated with a luciferase expression plasmid using preset protocols 1 (Opt mini 96-well/Sqr, Exp; see Figure 1) on the Gene Pulser MXcell system.**



**Fig. 3. Luciferase activity of SK-N-SH cells electroporated with a luciferase expression plasmid using protocols 1 (Opt mini 96-well/Sqr, Exp; see Figure 1) on the Gene Pulser MXcell system.** Two cell densities were tested,  $1 \times 10^6$ /ml (■) and  $3 \times 10^6$ /ml (■). RLU, relative luminescence units.



**Fig. 4. Gene silencing in SK-N-SH cells electroporated with either a siLentMer *GAPDH* siRNA or a siLentMer nonspecific siRNA assessed using real-time RT-PCR.** Cells ( $3 \times 10^6$ /ml) were electroporated (20 ms square-wave pulse of 200 V and 2,000  $\mu$ F) with 50 nM (**A**) or 100 nM (**B**) siRNA. Nonspecific siRNA (—); *GAPDH* siRNA (—). **C**, over 90% gene silencing was obtained in cells treated with 50 or 100 nM *GAPDH* siRNA (■) compared to cells treated with the corresponding concentration of nonspecific siRNA (■) (negative control). RFU, relative fluorescence units.

## Conclusions

In this study, we identified optimal waveform and voltage conditions for electroporating IMR-32 and SK-N-SH neuroblastoma cell lines with plasmid DNA or siRNA. The data indicate that the best conditions for electroporating these cells are a 20 ms square-wave pulse of 200 V and 2,000  $\mu$ F. These conditions may be a good starting point when working with other neuroblastoma cell lines, which may or may not require subsequent optimization of the final parameters.

We also observed that using a higher cell density yielded results that were at least as good as those obtained with cells at lower densities. This demonstrates that cell concentration is another important variable to consider when performing electroporations.

The amount of material delivered is also crucial. We tested plasmid DNA at a final concentration of 20  $\mu$ g/ml, while siRNA was tested at final concentrations of 50 and 100 nM. Further optimization of cell density and plasmid DNA and siRNA concentrations, accomplished quickly and easily with the Gene Pulser MXcell system, may provide even better results.

## References

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