Transfection of Mammalian Cells Using Preset Protocols on the Gene Pulser MXcell™ Electroporation System

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Introduction

The ability to modulate gene expression is essential for achieving a better understanding of gene function. The transfer of exogenous nucleic acids, such as plasmids or siRNAs, into mammalian cells is an important tool for the study and analysis of gene function and regulation of expression, and has advanced basic cellular research, drug target identification, and validation. Electroporation is a well-established method for transferring nucleic acids into cells. Finding optimal transfection conditions in a gene transfer experiment is crucial for obtaining the highest transfection efficiency with maximum cell viability. There are many parameters that affect the efficiency of electroporation, including waveform (exponential or square-wave), voltage, capacitance, resistance, pulse duration, and number of pulses.

The Gene Pulser MXcell electroporation system and Gene Pulser® electroporation buffer were designed to address the need for attaining the highest transfection efficiency and cell viability in mammalian cells. The Gene Pulser MXcell system is an open platform that provides the flexibility for creating specific protocols and varying parameters, including the unique option of providing both square and exponential waveforms in the same instrument. Preset and gradient protocols allow easy optimization of all parameters. Preset protocols are defined for whole or partial (mini protocol) plates to accommodate cell availability. A preset protocol decision tree is shown in Figure 1.

Here we demonstrate the use of Gene Pulser electroporation buffer with preset protocols to achieve maximum transfection efficiency and cell viability.

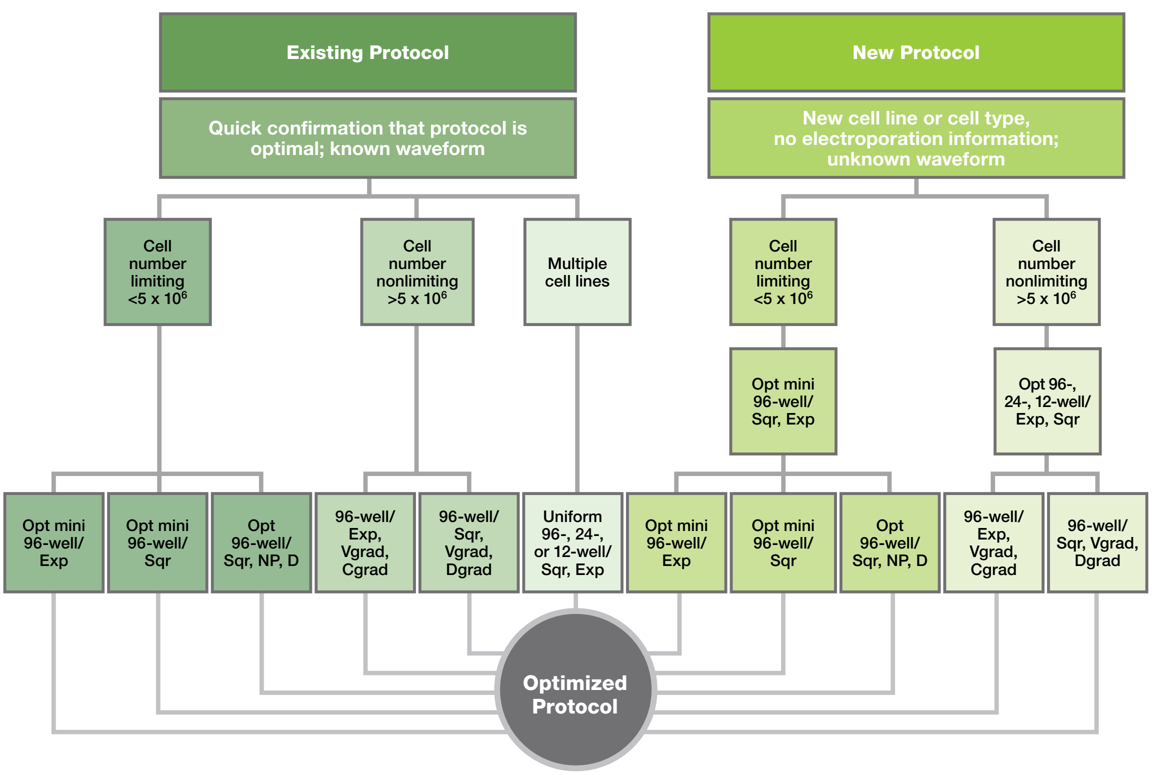

Fig. 1. Gene Pulser MXcell system preset protocol decision tree.

Methods

Cell Lines, Plasmids, and siRNAs

Cells were obtained from the American Type Culture Collection (ATCC). HeLa cells (ATCC, #CCL-2) were cultured in Dulbecco's modified Eagle's medium containing 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 10% fetal bovine serum (FBS). CHO-K1 cells (ATCC, #CCL-61) were cultured in Ham's F-12K medium supplemented with 10% FBS.

For optimization of siRNA delivery, fluorescently labeled siLentMer™ Dicer-substrate siRNA duplexes (Bio-Rad Laboratories, Inc.) targeting the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) or negative controls were used. Negative control and luciferase-specific siRNAs were also used. For the optimization of plasmid delivery, a DNA plasmid expressing the luciferase gene (pCMViLuc) was used.

Electroporation

Cells were used at a density of 1 x 10^6 cells/ml, unless indicated otherwise. Electroporation was performed in either 96- or 24-well electroporation plates. After trypsinization, harvested cells were washed with phosphate buffered saline (PBS), counted, and the appropriate number of cells per experiment was aliquoted. Before electroporation, the cells were resuspended in Gene Pulser electroporation buffer, and plasmid DNA (10 µg/ml) or siLentMer siRNA (100 nM) was added to the mix. The cells were then transferred to electroporation plates (96- or 24-well) and pulsed with the Gene Pulser MXcell electroporation system (Bio-Rad). Electroporated cells were transferred to tissue culture plates containing the appropriate growth medium and incubated at

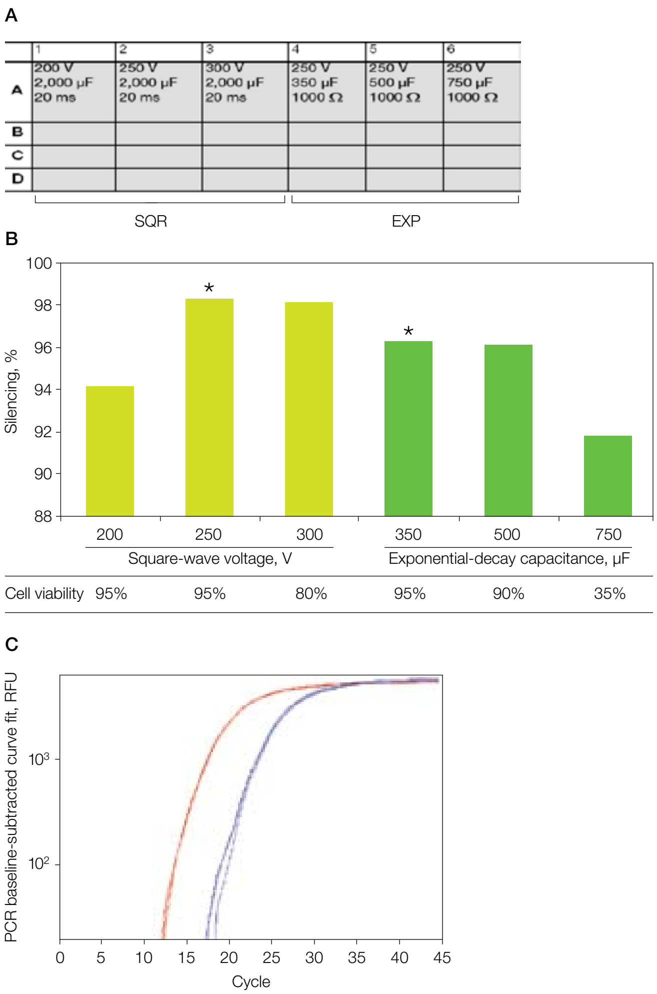

Fig. 2. Preset protocol for siRNA delivery into HeLa cells. A, schematic of the partial-plate preset protocol Opt mini 96-well/Sqr, Exp used in the experiment showing the electroporation parameters and well sets used. Conditions shown in row A are applied to all 4 wells in the column; **B**, percentage of *GAPDH* silencing, which is calculated by comparing *GAPDH* expression levels in cells electroporated with the negative control to that of cells electroporated with siRNA targeting *GAPDH*. The best conditions for the square-wave and exponential-decay protocols are indicated by asterisks. Associated table shows resulting cell viability for each condition; **C**, qPCR traces from knockdown experiments. *GAPDH* (—) and negative control (—) qPCR traces from the best square-wave protocol (250 V, 2,000 µF, 20 ms). RFU, relative fluorescence units.

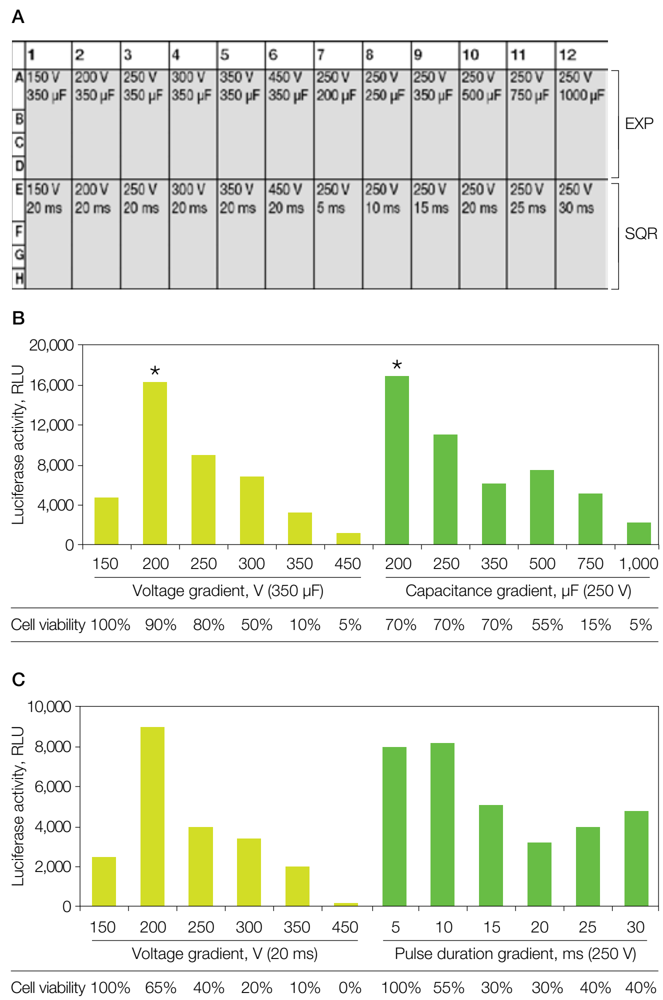

Fig. 3. Optimization of plasmid electroporation in HeLa cells in a 24-well format. A, schematic of the preset protocol Opt 24-well/Exp, Sqr used in the experiment showing the exponential-decay electroporation parameters for each column in rows A–D and square-wave parameters for each column in rows E–H; **B**, results from the preset exponential-decay protocol, which allows for a voltage gradient (■) and a capacitance gradient (■); **C**, results for the square-wave protocol, which allows for a voltage gradient (■) and a pulse duration gradient (■). Optimal electroporation conditions (**B**) are marked by asterisks. Associated tables show resulting cell viability for each condition. RLU, relative luminescence units.

37°C for 24 hr. Prior to harvesting, cell viability was assessed by visual inspection and by comparing cell confluencies achieved under different transfection conditions.

Analysis of Transfection

Cells electroporated with the pCMViLuc plasmid were assayed for luciferase activity. Cells electroporated with fluorescently labeled siRNA were washed with PBS, trypsinized, pelleted, and resuspended in PBS for analysis. Delivery of the *GAPDH* siLentMer siRNA was assessed by real-time quantitative PCR (rt-qPCR). To analyze for gene silencing, total RNA was extracted from electroporated cells using the Aurum™ total RNA mini kit (Bio-Rad) and used for cDNA synthesis (iScript™ cDNA synthesis kit, Bio-Rad). The synthesized DNA was subjected to rt-PCR using gene-specific primers and iQ™ SYBR® Green supermix on the iQ™5 real-time PCR detection system (all from Bio-Rad).

Results and Discussion

siRNA Delivery Into HeLa Cells

To define the best conditions for siRNA delivery, HeLa cells were electroporated using the Gene Pulser MXcell system with a negative control or *GAPDH*-specific siLentMer siRNA using the preset protocol Opt mini 96-well/Sqr, Exp in a 96-well format. This protocol uses 3 square-wave and 3 exponential-decay conditions in 6-well sets, as shown in Figure 2A. Gene silencing was used as a measure of the transfection efficiency of siRNA (Figure 2B, C). With this protocol, conditions in well set 2 (250 V, 2,000 µF, 20 ms) were found to be optimal. Cell viability was high as measured by cell confluency, and a greater than 95% reduction in transcript levels was observed in cells electroporated with siRNA targeting *GAPDH* compared to those electroporated with the negative control.

Plasmid Delivery Into HeLa Cells

To find the best electroporation conditions for plasmid delivery into HeLa cells, the preset protocol Opt 24-well/Exp, Sqr (Figure 3A) was applied using a 24-well electroporation plate. This protocol delivers either a voltage or capacitance gradient with an exponential waveform to the top half of the plate, and either a voltage or pulse duration gradient to the bottom half of the plate using a square-wave protocol. Transfection efficiency, assessed by reporter gene expression, was double with the exponential-decay protocol compared to the square-wave protocol (Figure 3B, C). Cell density was also higher for the exponential-decay than for the square-wave protocols 24 hr after electroporation. Together, these results indicate that the better protocol for electroporating HeLa cells with this plasmid DNA is an exponential-decay waveform at 200 V and 350 µF or 250 V and 200 µF (Figure 3B).

Plasmid Delivery in CHO Cells

Previous electroporation conditions in the Gene Pulser Xcell™ single cuvette system, indicated that the highest transfection efficiency for CHO cells is obtained using square-wave protocols. In the following experiments, different preset square-wave protocols were applied to CHO cells to determine the optimal electroporation conditions for plasmid delivery into CHO cells. The preset protocol Opt mini 96-well/Sqr (Figure 4A) was applied first. This protocol applies a square wave and generates either a voltage or pulse duration gradient for 6-well sets. Although 300 V yielded the highest luciferase activity, cell viability was only 45%. Lower voltage conditions (250 V) resulted in greater cell viability, but lower luciferase activity (Figure 4B).

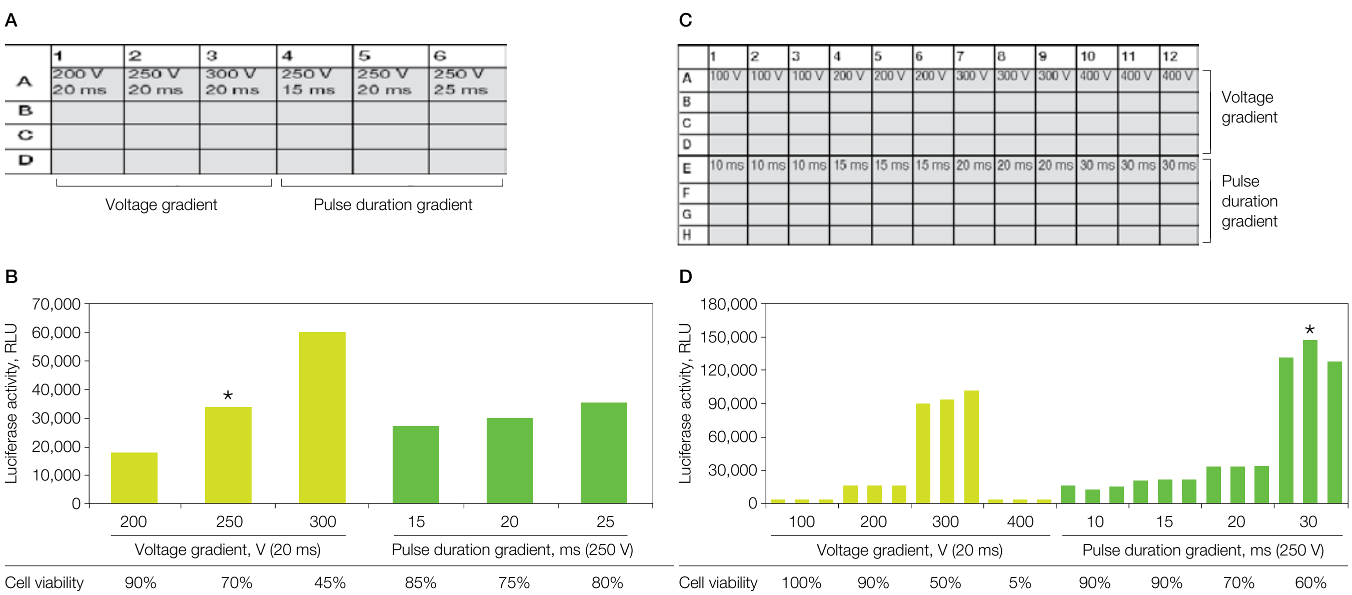

Fig. 4. Optimization of plasmid electroporation in CHO cells. A schematic of the preset protocol used in each experiment is shown above the results chart. The partial-plate preset protocol Opt mini 96-well/Sqr (**A**) and whole-plate protocol Opt 96-well/Sqr, NP, D (**C**) were performed in 96-well electroporation plates. The optimal electroporation conditions are defined by the highest luciferase activity and the highest cell density (marked by an asterisk) (**B**, **D**). Associated tables show resulting cell viability for each change in condition. RLU, relative luminescence units.

A final experiment in which voltage and pulse duration were varied was performed in a 96-well plate (Figure 4C). The results from this experiment further verified those already obtained. The optimal voltage was 250 V and pulse duration was 30 ms (Figure 4D).

Conclusions

Preset protocols on the Gene Pulser MXcell electroporation system allow rapid, thorough optimization of electroporation parameters to improve transfection efficiency of siRNA and plasmid DNA in mammalian cells. Preset protocols were created to allow many factors that affect electroporation to be tested simultaneously. The data shown exemplify how preset protocols can be used for optimizing electroporation conditions for the mammalian cell line of interest. Both mini- and whole-plate preset protocols utilizing 96- or 24-well electroporation plate formats were used to electroporate siRNA targeting human *GAPDH* into HeLa, or plasmid DNA (pCMViLuc) into CHO cells, respectively, using exponential-decay or square-wave pulses. The data also demonstrate the benefits of fine-tuning or optimizing transfection experiments, which lead to significantly greater transfection efficiency and cell viability.

Information in this tech note was current as of the date of writing (2008) and not necessarily the date this version (rev A, 2008) was published.

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