Optimization of Electroporation Using Gene Pulser® Electroporation Buffer and the Gene Pulser MXcell™ Electroporation System

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Introduction

The transfer of exogenous nucleic acids (such as plasmids or siRNAs) into mammalian cells is an important tool for the study of gene expression and metabolic pathways. The delivery of nucleic acids into cells may be achieved with the use of chemical (for example, lipid transfection reagents and $CaCl_2$) and physical (for example, electroporation, microinjection, or particle bombardment) methods. Importantly, not all methods work effectively for all cell types.

Electroporation is a well-established method of gene transfer, with sophisticated equipment able to deliver various types of voltage pulses to cells. Electroporation is thought to temporarily destabilize cellular membranes (Heiser 2000), causing the formation of pores (Gowrishankar et al. 2000), and thereby offering an effective means of transferring nucleic acids into cells.

The Gene Pulser MXcell electroporation system is capable of delivering square-wave or exponential-decay pulses and provides preset optimization protocols. The system works with a family of plate formats (96-, 24-, and 12-well plates) to allow rapid optimization of electroporation and provides flexibility for high throughput, sample size, and replicates.

Electroporation buffers play a critical role in the transfection protocol. Gene Pulser electroporation buffer is formulated to provide high transfection efficiency, while maintaining cell integrity and viability, and can be used with any cell type, including primary and difficult-to-transfect cells, and any type of nucleic acid.

When considering electroporation of a cell line you have not worked with, look at the protocols used in several reference papers to develop a consensus starting protocol. If no references exist for a particular cell line, references for a similar cell type (that is, epithelial, fibroblast, etc.) can be used. The first step in optimizing electroporation conditions is to choose a waveform, followed by optimization of relevant parameters: voltage, pulse duration, capacitance, and resistance. In our study we demonstrate the waveform optimization process for the electroporation of human primary fibroblasts (HPF). We optimized electroporation of plasmids and small interfering RNA (siRNA) using the Gene Pulser MXcell system and Gene Pulser electroporation buffer. The optimization criteria were simple: maximal transfection efficiency and cell viability.

Methods

Cell Culture and Transfected Materials

HPF cells (American Type Culture Collection (ATCC), #CRL-2703) were grown in medium from ATCC (Iscove's modified Dulbecco's medium with 10% fetal bovine serum). Cells were passaged 1 to 2 days prior to electroporation; they were about 75–85% confluent on the day of the experiment. The plasmid DNA used in these experiments was a luciferase expression plasmid (pCMVi-Luc).

siLentMer™ Dicer-substrate siRNA duplexes (fluorescently labeled, negative control or glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-specific) were used in these experiments. After electroporation, cells were transferred into growth medium and incubated at 37°C for 24 hr. Prior to harvesting, cell viability was assessed by comparing the percentage of cells attached (and therefore viable) under different conditions.

Electroporation

Sample Handling

Plasmid DNA or the siLentMer siRNA was added to the cell suspension at the desired final concentration and gently mixed, aliquotted into the appropriate well sets of a 96-well electroporation plate, and pulsed with the Gene Pulser MXcell system. In all cases, except the cell density study, electroporation was carried out with 1×10^6 cells/ml in Gene Pulser electroporation buffer.

Waveform Optimization

We used the Gene Pulser MXcell electroporation system preset optimization protocol Opt mini 96 well/Exp (Table 1) to identify the best waveform. We modified this protocol to fit our experience with electroporation of HPF, and to include a previously reported exponential-decay waveform with the following electroporation conditions: 250 V, 1,000 µF, and 1,000 Ω (Ray 1995).

Table 1. Preset protocol Opt mini 96 well/Exp for waveform optimization.

	Square-Wave Conditions			Exponential-Decay Conditions		
	Well Sets* 1–3			Well Sets 4–6		
Column	1	2	3	4	5	6
Rows	A–D	A–D	A–D	A–D	A–D	A–D
Voltage	200 V	250 V	300 V	250 V	250 V	250 V
Capacitance	2,000 µF	2,000 µF	2,000 µF	350 µF	500 µF	750 µF
Pulse duration	20 ms	20 ms	20 ms	—	—	—
Resistance	—	—	—	1,000 Ω	1,000 Ω	1,000 Ω

* The Gene Pulser MXcell system is programmed by well set (a set of four rows by one column in a plate; for example, rows A–D under column 1 is a well set that is displayed as ABCD1 in system programming). Each set of optimization conditions was applied to wells A–D under the corresponding column on the plate.

To illustrate how easy it is to fine-tune both square-wave and exponential-decay waveforms on the Gene Pulser MXcell system, we ran additional optimization studies. To do this, we created new protocols based on our experience with electroporation of HPF to test the following conditions:

- Square-wave voltage (200 V, 220 V, and 250 V) and pulse duration (10 ms, 15 ms, and 20 ms)
- Exponential-decay waveform voltage (200 V, 220 V, and 250 V) and capacitance (200 µF, 350 µF, 500 µF, and 1,000 µF)
- Exponential-decay waveform capacitance (350 µF and 500 µF) and resistance (350 Ω, 500 Ω, and 1,000 Ω)

The previously described optimizations are based upon changing electroporation parameters on the Gene Pulser MXcell system. However, if desired, protocols can be fine-tuned further by optimizing the electroporation volume, cell density, and nucleic acid concentration. As an example, we performed a brief optimization of cell density and plasmid concentration.

Analysis of Transfection

Cells electroporated with the pCMVi-Luc plasmid were assayed for luciferase activity. We assessed the introduction of siRNAs into HPF cells in two different ways. First, electroporation of a fluorescently labeled siRNA transfection control was used to measure transfection efficiency of siRNA by flow cytometry. Second, electroporation of the siLentMer validated 27-mer siRNA targeting GAPDH was assessed by measuring the knockdown of GAPDH using real-time PCR.

Cells electroporated with fluorescently labeled siRNA were washed with phosphate-buffered saline (PBS), trypsinized, pelleted, and suspended in PBS for analysis by flow cytometry or fluorescence microscopy. Delivery of the siLentMer siRNA was assessed by prepping total RNA from cells (Aurum™ total RNA mini prep kit), converting the mRNA into cDNA (iScript™ cDNA synthesis kit), and performing real-time PCR using specific primers and iQ™ SYBR® Green supermix on the iQ™5 real-time PCR detection system to analyze for gene silencing.

Results and Discussion

The results of preset optimization protocol Opt mini 96 well/Exp in Figure 1 show that the exponential-decay waveform is a better choice than the square-wave. We continued with the optimization process for both waveforms in parallel to illustrate how the Gene Pulser MXcell system facilitates and expedites the process.

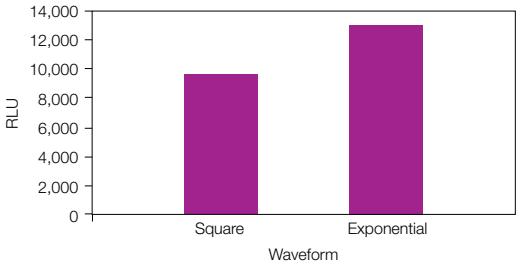
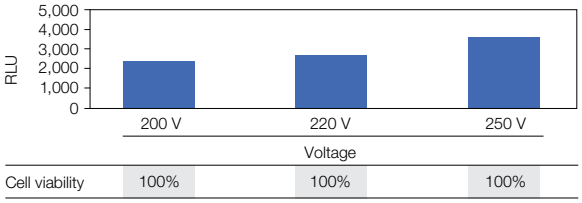


Fig. 1. Determination of electroporation waveform for HPF cells. Optimization was carried out using preset protocol Opt mini 96 well/Exp with 20 µg/ml pCMVi-Luc, assayed for luciferase activity.

The results for the optimization of square-wave electroporation shown in Figure 2 suggest that 250 V with a pulse duration of 20 ms is optimal. Although the number of pulses in this experiment did not increase transfection efficiency, we have observed that it is sometimes beneficial to divide the optimal pulse duration by two or three, and then pulse two or three times with the shorter pulse.

A. Voltage



Cell viability	100%	100%	100%

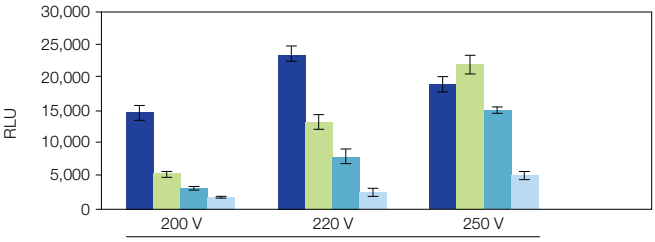
B. Pulse duration

Cell viability	95%	95%	95%

Fig. 2. Square wave optimization of voltage and pulse duration for square-wave electroporation. Luciferase activity and confluence were measured 24 hr after cells were electroporated with pCMVi-Luc using various voltages with 20 ms pulse duration (**A**), or various pulse durations at 250 V (**B**). Associated tables show resulting cell viability for each change in condition. RLU, relative light units.

Figure 3 shows the optimization of exponential-decay electroporation results. Ignoring cell viability data, the original starting conditions appear optimal. However, cell viability was significantly better at 500 µF with no reduction in gene expression, so this would be the optimal capacitance for Gene Pulser electroporation buffer.

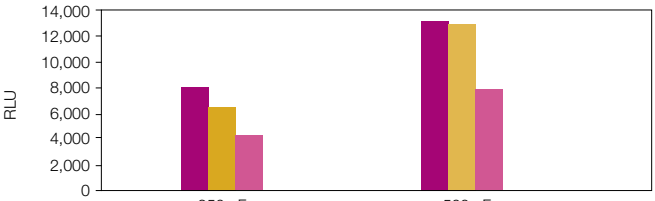
A. Capacitance



Capacitance	200 V	220 V	250 V
1,000 µF	85%	60%	20%
500 µF	95%	85%	70%
350 µF	98%	90%	85%
200 µF	100%	100%	95%

% Cell viability

B. Resistance

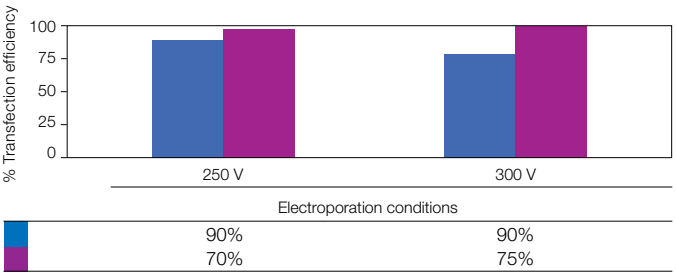


Resistance	350 µF	500 µF
1,000 Ω	90%	85%
500 Ω	88%	75%
350 Ω	88%	75%

% Cell viability

Fig. 3. Optimization of electroporation conditions using exponential pulses. Transfection efficiency and cell viability were assessed 24 hr after HPF cells were transfected with pCMVi-Luc using varying capacitance and voltage (**A**), and then varying resistance (**B**). Associated tables show resulting cell viability for each change in condition. RLU, relative light units.

In assessing the introduction of siRNAs into HPF cells, we found that electroporation efficiency, as measured by flow cytometry, was nearly 100% (Figure 4). The data for electroporation of a siLentMer validated 27-mer siRNA targeting GAPDH are presented in BioRadiations 123 (Bio-Rad Laboratories 2007).

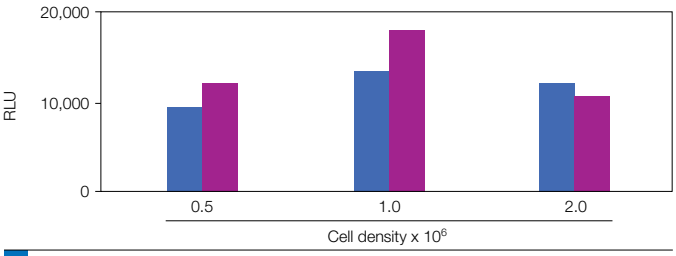


	250 V	300 V
	90%	90%
	70%	75%

% Cell viability

Fig. 4. HPF electroporation with fluorescently labeled siRNA using Gene Pulser electroporation buffer. Delivery of fluorescently labeled 27-mer siRNA transfection control by square-wave (■) and exponential-decay (■) pulses analyzed by flow cytometry. Associated table shows resulting cell viability for each change in condition.

From the cell density and plasmid concentration studies, we determined that using 1 x 10^6 cells/ml and 40 µg plasmid DNA/ml is optimal (Figure 5). Using 1 x 10^6 cells/ml not only resulted in much higher RLU, but also lower cytotoxicity than 0.5 x 10^6 cells/ml for both square-wave and exponential pulse electroporation. Conversely, using 2 x 10^6 cells/ml resulted in higher cell viability but lower RLU, suggesting that optimal conditions had been exceeded.



	0.5	1.0	2.0
	20%	70%	90%
	50%	75%	75%

% Cell viability

Fig. 5. Optimization of cell density for both square-wave (■) and exponential pulse (■) electroporation with 40 µg/ml plasmid DNA. Associated table shows resulting cell viability for each change in condition. RLU, relative light units.

Conclusions

Gene Pulser electroporation buffer can be used with the Gene Pulser MXcell system for effective electroporation of different nucleic acids (including plasmids and siRNAs) into many cell lines. It is possible to obtain both high efficiency and high cell viability in gene transfer experiments using Gene Pulser electroporation buffer. In addition, the multiwell plate format of the Gene Pulser MXcell system allowed multiple parameters to be tested simultaneously. For example, we were able to test both voltage and pulse duration in the same experiment.

Based on the results of the optimization experiments presented here, as well as experience with other cell lines, we recommend that when beginning work with Gene Pulser electroporation buffer, test previously optimized conditions alongside conditions in which the capacitance setting is decreased to one-half or one-third of the original value. Decreasing the capacitance often increases cell viability while resulting in excellent transfection efficiency.

The general optimization workflow presented here can be followed for optimizing square-wave or exponential pulses for any cell type, and with any instrument that allows some control over electroporation conditions. Many parameters can affect electroporation; however, with the Gene Pulser MXcell system, optimization of such parameters can be accomplished easily and rapidly in a single experiment.

For more information on optimization of electroporation parameters and conditions, see tech note 5622.

References

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