Simple and Rapid Optimization With Maximum Transfection Efficiency and Cell Viability in Mammalian Cell Lines Using the Screening Tools for Efficient Delivery of siRNA and Plasmid Into Mammalian Cells

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Abstract

Modulating gene expression is essential to achieve a better understanding of gene function. The transfer of exogenous nucleic acids, such as plasmids or small interfering RNAs (siRNAs), into mammalian cells remains an important technique for studying and analyzing gene function, expression, regulation, and mutation. The technique advances basic cellular research and enables drug target identification and validation. Electroporation is a well-established gene transfer method and an effective means of transferring nucleic acids into cells. The shift of today's research to newer and more scientifically relevant cell lines has created a need for efficient delivery tools. In addition, the wide adoption of RNAi technology has led to a demand for screening tools to identify the most effective siRNAs for modulating gene expression. In order to obtain consistent downstream results, it is critical that delivery conditions be highly efficient.

Here, we show data that demonstrate the benefits of using a high-throughput electroporation system to determine optimal conditions for delivery of plasmids and siRNAs into mammalian cells. We demonstrate that the use of the Gene Pulser MXcell™ electroporation system allows for rapid screening, as well as the ability to identify the most efficient delivery conditions for a difficult-to-transfect cell line. Finally, we present quantitative PCR data that validate the benefits of this technique.

Introduction

Optimal electroporation conditions result in the highest transfection efficiency and maximum cell viability. This is crucial in gene transfer experiments. Parameters that affect the efficiency of electroporation include waveform, voltage, capacitance, resistance, pulse duration, and number of pulses. The Gene Pulser MXcell electroporation system is an open platform, with the flexibility to create specific protocols by varying any of the parameters listed above. In addition, the system also provides preset protocols for all three available plate formats (96-, 24-, 12-well) to allow the end user to quickly find the optimal electroporation conditions for any cell type. A chart showing a preset protocol decision tree is shown in Figure 1.

Methods

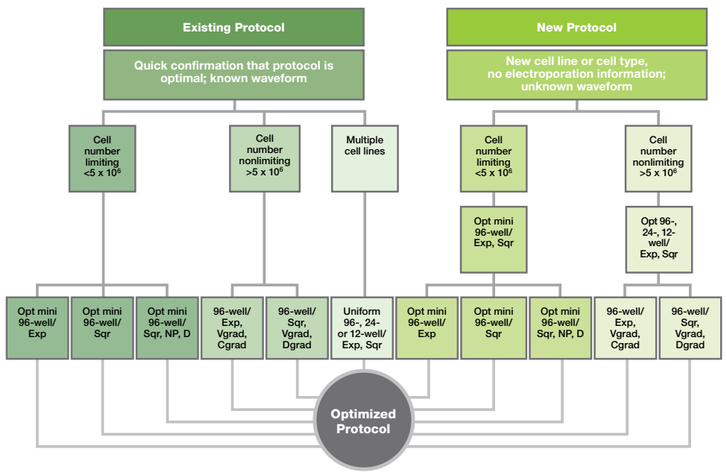


Fig. 1. Choices of available preset protocols.

Cell Lines, Plasmids, and siRNAs

HeLa cells (CCL-2) were obtained from ATCC and 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 (CCL-61) were obtained from ATCC and cultured in Ham's F12K medium supplemented with 10% FBS. MCF-7 cells (HTB-22) were obtained from ATCC and cultured in minimum essential medium (Eagle), containing 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 0.01 mg/ml bovine insulin, and 10% FBS. All media were purchased from Invitrogen Corporation and FBS was obtained from HyClone.

For the optimization of plasmid delivery, a plasmid DNA expressing the luciferase gene (pCMVi-Luc) was used. For optimization of siRNA delivery, the following siLentMer™ validated Dicer-substrate siRNA duplexes (Bio-Rad Laboratories, Inc.) were used — a negative control, a GAPDH-specific siRNA, and a fluorescently labeled negative control siRNA.

Electroporation

Cells were used at a density of 1 x 10^6 cells/ml. Cells were trypsinized, washed with PBS, and counted, and the appropriate amount of cells per experiment were aliquoted. Before electroporation, cells were resuspended in Gene Pulser® electroporation buffer, and either plasmid DNA (10 µg/ml) or a siLentMer siRNA (100 nM) was added to the suspension. Cells were transferred to electroporation plates (96-, 24- or 12-well) and pulsed with the Gene Pulser MXcell electroporation system. Electroporated cells were then transferred into growth medium and incubated at 37°C for 24 hr. Prior to harvesting the cells, cell viability was assessed by visual inspection and by comparing cell confluency between different conditions.

Analysis of Transfection

Cells electroporated with the pCMVi-Luc plasmid were assayed for luciferase activity. Cells electroporated with fluorescently labeled negative control siRNA were washed with PBS, trypsinized, pelleted, and suspended in PBS for analysis by flow cytometry. Delivery of siLentMer siRNA was assessed by reverse transcription (RT)-qPCR. Total RNA was extracted from electroporated cells using the Aurum™ total RNA mini prep kit, cDNA was synthesized using the iScript™ cDNA synthesis kit, and gene expression was analyzed by real-time PCR using gene-specific primers, iQ™ SYBR® Green supermix, and the iQ™5 real-time PCR detection system (all from Bio-Rad).

Results

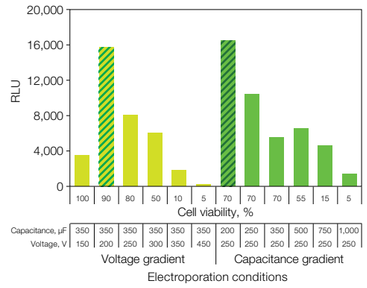
Plasmid Delivery — HeLa Cells

To find the best electroporation conditions for plasmid delivery into HeLa cells, we applied the preset protocol Opt 24-well/Exp, Sqr (Figures 2A, 2C), using a 24-well electroporation plate. Results showed higher transfection efficiency and cell viability when using exponential-decay protocols (Figure 2B) than when using square-wave protocols (Figure 2D).

A. Opt 24-well/Exp, Sqr protocol

	1	2	3	4	5	6	7	8	9	10	11	12	
A	150	200	250	300	350	450	250	250	250	250	250	250	V
	350	350	350	350	350	350	200	250	350	500	750	1,000	µF
B													
C													
D													

B. Exponential waveform



C. Opt 24-well/Exp, Sqr protocol

	1	2	3	4	5	6	7	8	9	10	11	12	
E	150	200	250	300	350	400	250	250	250	250	250	250	V
	20	20	20	20	20	20	5	10	15	20	25	30	ms
F													
G													
H													

D. Square waveform

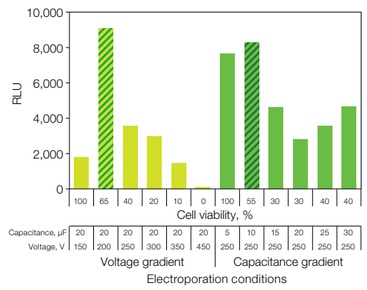


Fig. 2. Optimization of plasmid electroporation in HeLa cells. **A**, schematic of the preset protocol (Opt 24-well/Exp, Sqr) used in the experiment showing the electroporation parameters; **B**, results obtained for exponential-decay protocols. This exponential-decay protocol delivers a voltage gradient as well as a capacitance gradient. Optimal electroporation conditions are defined by the highest RLU values and the highest cell viabilities (hashed); **C**, schematic of the preset protocol; **D**, results obtained for the square-wave protocols. The square-wave protocol delivers a voltage gradient and a pulse duration gradient. The optimal electroporation conditions are defined by the highest RLU values and the highest cell densities (hashed). RLU, relative light units.

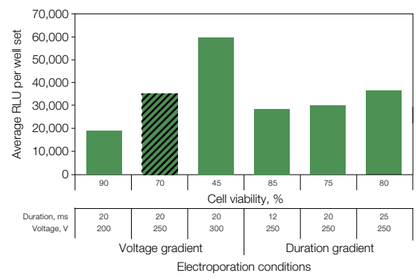
Plasmid Delivery — CHO Cells

Past experiments indicated that the highest transfection efficiency for CHO cells was obtained using square-wave protocols. To find the optimal electroporation conditions for CHO cells, different preset square-wave protocols were applied. We first applied the preset protocol Opt mini 96-well/Sqr (Figure 3A). We then used the best conditions from that square-wave protocol (Figure 3B) and improved them by varying the pulse length and the number of pulses (Figure 3C). Results showed that the highest transfection efficiency obtained with a square-wave protocol can be further improved by using two pulses of shorter duration (Figure 3D).

A. Opt mini 96-well/Sqr protocol

	1	2	3	4	5	6	
A	250	250	250	250	250	250	V
	20	20	20	15	20	25	ms
B							
C							
D							

B. Square waveform



C. Opt mini 96-well/Sqr, NP, D protocol

	1	2	3	4	
A	20	15	10	7	ms
	1	2	2	3	Number of pulses
B					
C					
D					

D. Square waveform + varied number of pulses

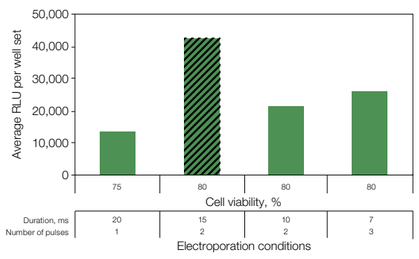


Fig. 3. Optimization of plasmid electroporation in CHO cells. **A**, schematic of the preset protocol Opt mini 96-well/Sqr; **B**, optimal electroporation conditions were defined by the highest RLU values and the highest cell viability (hashed); **C**, schematic of the preset protocol Opt mini 96-well/Sqr, NP, D; **D**, optimal conditions found in the previous experiment were further improved by using two pulses of shorter duration (15 ms). The bar showing these improved conditions is hashed.

siRNA Delivery — MCF-7 Cells

Previous experiments indicated that the best method for electroporation of MCF-7 cells is an exponential-decay pulse with low capacitance. To explore the best conditions for this cell line, three protocols were entered manually to electroporate a fluorescently labeled negative control siRNA (Table 1), and transfection efficiency was assessed by flow cytometry (Figure 4).

We found that the three protocols tested to electroporate MCF-7 cells were equally good with excellent transfection efficiencies.

Table 1. Transfection efficiency and electroporation parameters for MCF-7 cells. Electroporation parameters were manually entered in the Gene Pulser MXcell system to electroporate MCF-7 cells; transfection efficiency was determined by flow cytometry.

Voltage, V	Capacitance, µF	Transfection Efficiency, %
250	250	94.4
280	250	96.1
300	250	96.2

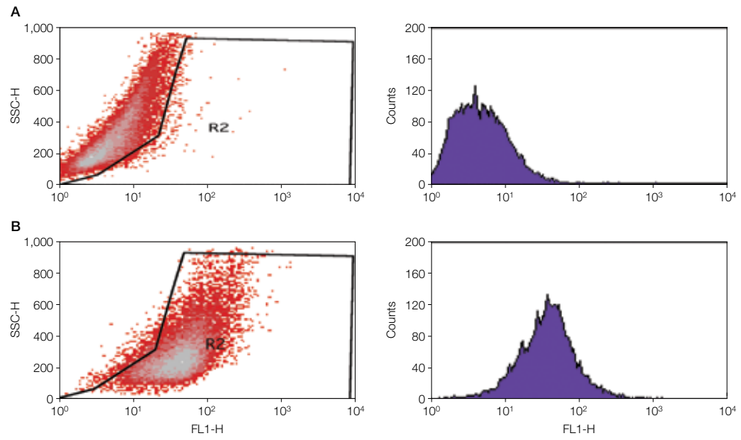


Fig. 4. Optimization of electroporation parameters for MCF-7 cells using exponential-wave conditions. **A**, flow cytometry results from electroporation of a nonfluorescent negative control siRNA; **B**, flow cytometry results after electroporation of a fluorescent negative control siLentMer siRNA using 280 V and 250 µF. Notice the shift of the fluorescent cell population and the fluorescent peak with respect to the negative control.

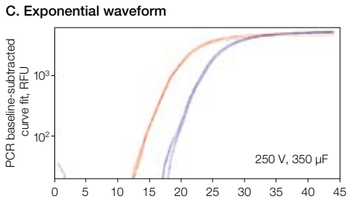
siRNA Delivery — HeLa Cells

To define the best conditions for siRNA delivery, HeLa cells were electroporated using the preset protocol Opt mini 96-well/Exp, Sqr, with a negative control siLentMer siRNA and a GAPDH-specific siLentMer siRNA. Gene silencing was used as a measure of the transfection efficiency for siRNA delivery (Figure 5).

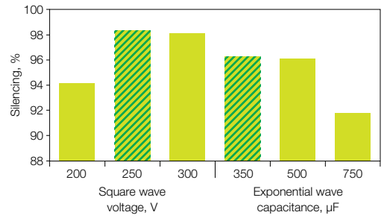
A. Opt mini 96-well/Exp, Sqr protocol

	1	2	3	4	5	6	
A	250	250	250	250	250	250	V
	2,000	2,000	2,000	850	500	750	µF
	20	20	20	15	20	25	Ω
B							
C							
D							

C. Exponential waveform



B. siRNA transfection into HeLa cells



D. Square waveform

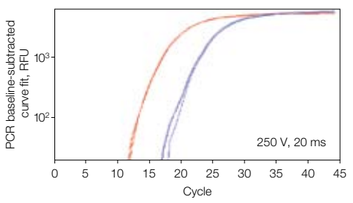


Fig. 5. Optimization of conditions for siRNA delivery into HeLa cells. **A**, schematic of the preset protocol (Opt mini 96-well/Exp, Sqr) used in the experiment, showing the electroporation parameters; **B**, percentage of GAPDH silencing after electroporation with the preset protocol. The percentage silencing is calculated by comparing GAPDH expression levels on the negative control and GAPDH siLentMer siRNA electroporations. The best conditions for the square-wave and exponential-decay protocol are marked as hashed bars; **C**, qPCR traces from the best exponential-decay protocol (250 V, 350 µF); **D**, qPCR traces from the best square-wave protocol (250 V, 20 ms). A greater than 95% reduction in transcript levels was observed in cells electroporated with the GAPDH siRNA (—) compared to electroporations with the negative control (—).

Table 2. Summary of optimal electroporation conditions for plasmid or siRNA delivery for different cell lines.

Cell Line	Molecule	Waveform	Optimal Conditions
HeLa	Plasmid	Exponential	250 V, 200 µF, or 200 V, 350 µF
HeLa	siRNA	Square	250 V, 20 ms
		Exponential	250 V, 350 µF
CHO	Plasmid	Square	250 V, 2 x 10 ms
CHO	siRNA	Square	250 V, 20 ms*
MCF-7	siRNA	Exponential	250–300 V, 250 µF

* Data not shown.

Conclusions

The Gene Pulser MXcell electroporation system allows rapid and simple optimization of electroporation conditions, resulting in high cell viability and transfection efficiency for any mammalian cell line.

- Optimization is important because cell lines behave differently. Here, we show optimization for three cell lines, each requiring distinct electroporation conditions
- Optimization is important because molecules behave differently. For the same cell line, electroporation conditions may vary depending upon the molecule to be delivered. Here we demonstrate that HeLa cells behave differently when siRNA or plasmid DNA is delivered
- Depending on the cell line and molecule to be electroporated, waveform, voltage, and number and length of pulses, all play a crucial role in delivery. Here we show that multiple pulses are critical to the optimal delivery of plasmid into CHO cells (Figure 3D)
- Optimization of transfection conditions is rapid and easy with the Gene Pulser MXcell electroporation system. Preset and gradient protocols assist in rapid optimization