The Gene Pulser MXcell™ Electroporation System Delivers Consistent Results Required for Optimizing Delivery Protocols

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Introduction

Delivery of exogenous material into cells is an important component of life science research. Commonly used techniques for gene delivery include viral transfer, lipid transfection, and electroporation. Each method has its own limitations due to the complex physical and biochemical nature of the cell membrane. Viral transfer is dependent on the interaction of viruses with host membrane molecules, such as glycoproteins, to gain entry into the cell, as exemplified by entry of the herpes simplex virion into mammalian cells (Garner 2003). Lipids enter the cell through the formation of unilamellar vesicles, which bind to exogenous DNA and facilitate fusion with the lipid bilayer of the cell membrane (Felgner et al. 1987). Lipids can be quite toxic and are somewhat cell line dependent. Electroporation physically shocks the outer membrane and temporarily disrupts the lipid bilayer to allow molecules to enter the cell (Heiser 2000). Since lipid bilayers can be quite different between different cell lines, parameters for efficient delivery with minimal cell death require optimization.

A wide variety of molecules are introduced inside cells, including small molecules such as RNA and dyes, and larger molecules such as plasmid DNA, chromosomal DNA, and antibodies. The biophysical properties of these molecules play a role in efficiency of delivery, emphasizing the need to optimize delivery protocols.

The Gene Pulser MXcell electroporation system was developed to address the need for optimization in electroporation. The system was designed to program up to 24 different protocols in a 96- or 24-well plate, or 12 protocols in a 12-well plate, and will deliver pulses within two minutes of setting up the protocol. Within each protocol, any electroporation parameter — waveform, voltage, capacitance, resistance, or duration and number of pulses — can be altered. Use of 96-well plates enables screening and faster replication of experiments, while use of 12- or 24-well plates increases the number of cells that can be electroporated for optimal laboratory-scale experiments.

This paper describes results of transfection experiments conducted to investigate uniform delivery of plasmid DNA or siRNA within a plate and consistent delivery between plates and plate formats. Results reveal that optimized electroporation conditions can be obtained regardless of plate format or molecule used in the experiment.

Methods

Three different cell lines were used for the optimization experiments. HeLa cells were plated in DMEM with 1% sodium pyruvate/1% nonessential amino acid (NEAA) and 10% fetal bovine serum (FBS). CHO and 5F2C (CHO cells stably expressing the luciferase gene) cells were maintained in nutrient mixture F-12 Ham (Sigma-Aldrich), supplemented with 10% FBS. 5F2C cells received 200 µg/ml of G418 in the medium.

HeLa and CHO cells were passaged 1–15 times and 5F2C cells were passaged 1–10 times. Prior to electroporation, cells were trypsinized, resuspended in media, pelleted, washed with 1x PBS, and counted. Cells were then aliquoted, pelleted, and suspended in Gene Pulser® electroporation buffer at a concentration of 1 x 10^6 cells/ml.

For plasmid delivery experiments, 10 µg/ml of pCMVi-Luc, a plasmid encoding the luciferase gene, was used. Transfection efficiency was determined by measuring activity of the luciferase reporter gene and reported in relative light units (RLUs). For siRNA delivery, 5F2C cells were transfected with 100 nM of either a scramble siLentMer™ Dicer-substrate siRNA duplex (Scm, the negative control) or luciferase (Luc) siRNA to silence the luciferase gene. Percentage silencing was used as a measure of transfection efficiency. RLUs from the scramble siRNA transfections were set at 100% activity, and the percentage activity of luciferase was calculated in reference to the scramble transfections. Luciferase activity was measured in cell extracts using a luminometer.

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HeLa cells were electroporated using an exponential-decay protocol (voltage, 205 V; capacitance, 200 µF; resistance, 1,000 Ω). CHO and 5F2C cells were electroporated using a square-wave protocol (voltage, 250 V; capacitance, 2,000 µF; resistance, 1,000 Ω; duration, 20 msec).

Following electroporation, 100 µl aliquots of cells were transferred to 24-well tissue culture plates containing 500 µl of growth medium. Aliquots from 24- and 12-well electroporation plates were transferred into 4 wells of a 24-well tissue culture plate, while aliquots from a 96-well electroporation plate were transferred into one well of a 24-well plate. Cells were incubated for 24 hr at 37°C with 5% CO_2 and analyzed for luciferase activity 24 hr posttransfection.

Details of transfection conditions for each plate format are represented in Table 1. Exceptions are noted in the text.

Table 1. Experimental conditions used in validation experiments.

Cell Line	Plate Format	Buffer/Well Volume, µl	Volume (µl) Transferred to Culture Plate
HeLa/CHO/5F2C	96-well	125	100
HeLa/CHO	24-well	500	100
HeLa/CHO	12-well	1,000	100

Results

Well-to-Well Uniformity

A plasmid encoding luciferase was delivered to HeLa cells in a 96-well plate, and luciferase activity was measured for the whole plate to ascertain uniformity in delivery. A 100 µl aliquot from each well in a well set (a set of four individual wells that have the same condition applied to them) was assessed for transfection efficiency. The average activity value was 3,671 with a variation of less than 12% (Figure 1).

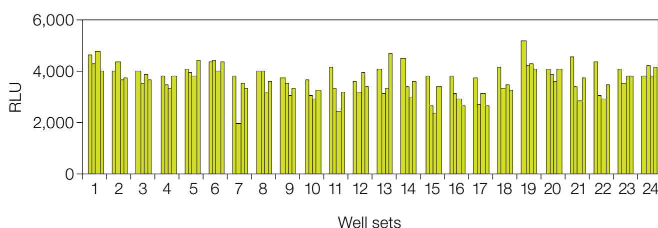

Fig. 1. Uniformity of plasmid transfection within a well set of a 96-well electroporation plate.

Well Set-to-Well Set and Plate-to-Plate Consistency

HeLa cells were tested for plasmid delivery in all three plate formats (96-, 24-, and 12-wells; n = 3 for each plate format) to examine consistency from well set to well set and from plate to plate. The RLU values for each well set were averaged and compared to the total average value of the whole plate for each plate format. Variation in average RLU between well sets was less than 20% (Figure 2).

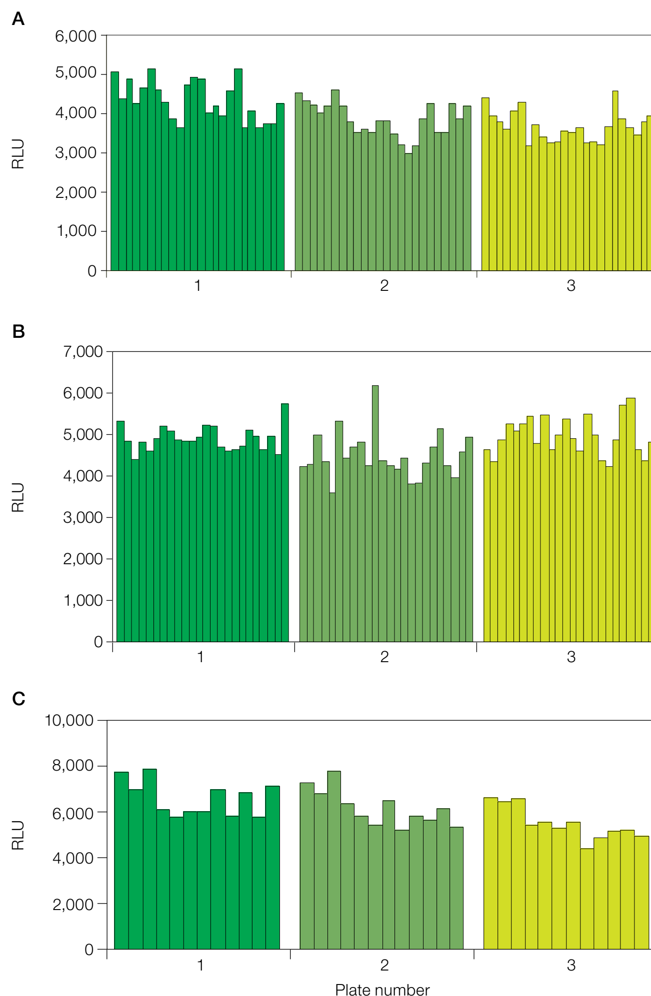

Fig. 2. Consistency of transfection between well sets and between plates. HeLa cells were transfected with a plasmid encoding luciferase in 96-, 24-, or 12-well electroporation plates. **A**, 96-well plate; **B**, 24-well plate; **C**, 12-well plate.

5F2C cells were transfected with siRNA, and consistency in delivery was assessed between well sets and plates using 96-well plates. The percentage of luciferase silencing was calculated for every luciferase siRNA transfection with reference to the scrambled siRNA transfections (Figure 3).

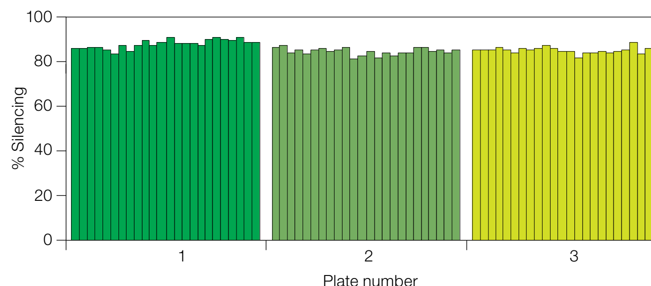

Fig. 3. Consistency in siLentMer siRNA delivery into 5F2C cells between well sets of a 96-well electroporation plate. Measurements were carried out on 96-well plates.

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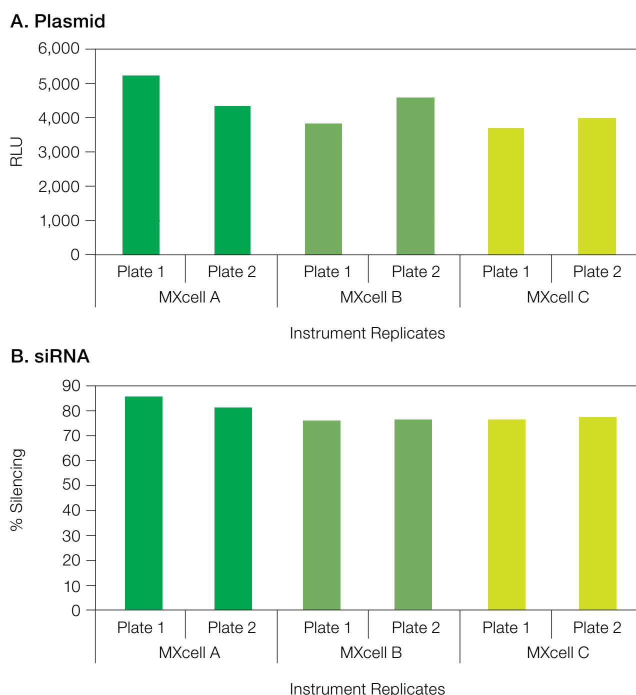

Fig. 4. Plate-to-plate consistency measured between 96-well plates in three different Gene Pulser MXcell electroporation systems (MXcell A–C). A, exponential waveform conditions were used to deliver plasmid DNA into HeLa cells; **B**, square waveform conditions were used to deliver siRNA into 5F2C cells.

Consistency Between Instruments

Consistency in delivery of plasmids and siRNA was determined between plates and instruments. HeLa and 5F2C cells were used for delivery of plasmids and siRNA, respectively. Consistency was tested in all three plate formats (96-, 24- and 12-wells; n = 2 for each format) in three different Gene Pulser MXcell systems. Values were averaged from duplicates and compared within an individual plate. Results for the 96-well plate are shown in Figure 4. Similar results were obtained for the 24- and 12-well plates.

Consistency Between Plate Formats

A 96-well plate, a 24-well plate, and a 12-well plate were used in this experiment. A 150 µl aliquot of cells at a concentration of 1 x 10^6 cells/ml was used for electroporation of a luciferase expression plasmid into 96-well plates. The plates were divided into 3 sections in groups of four columns per instrument (Figure 5).

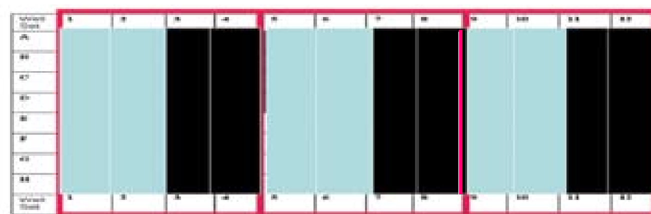

Fig. 5. Experimental design for measuring plate-to-plate consistency. Two out of four well sets were used to pulse a luciferase expression plasmid into HeLa cells (blue columns) using an exponential-decay waveform. The other two well sets were used to pulse 5F2C cells (black columns) using a square waveform.

Electroporations were performed using the same parameters for all plate formats. The average RLUs after electroporation were compared between plate formats within the same instrument, and between instruments. All values showed a variation of less than 20% (Figure 6).

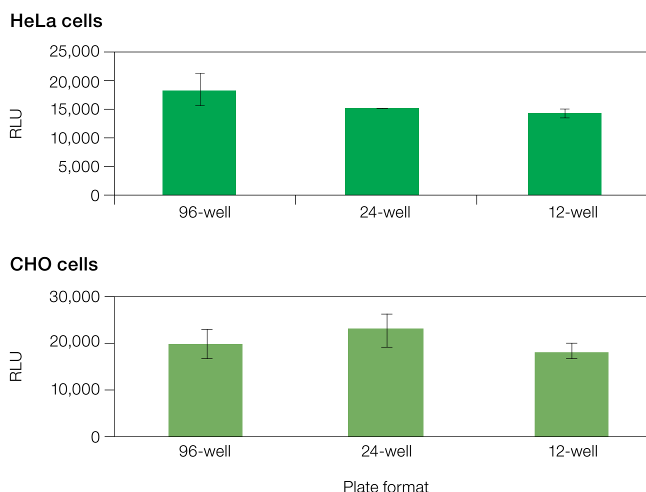

Fig. 6. Consistency of plasmid transfection between different plate formats.

Conclusions

The Gene Pulser MXcell electroporation system and Gene Pulser electroporation buffer provide highly consistent results within wells of a plate, between plates, and between plate formats. Consistency was also observed between three different Gene Pulser MXcell electroporation instruments. These results are independent of the type of molecule delivered or the mammalian cell line used. In all cases, the variation was less than 20%. This type of consistency is critical for optimizing conditions for electroporation. These results demonstrate that once an optimized protocol has been identified in a 96-well plate, it can easily be adapted to a larger-scale transfection by using a 24- or 12-well plate.

References

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