Optimization of Electroporation Conditions for Jurkat Cells Using the Gene Pulser MXcell™ Electroporation System

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Efficient delivery of exogenous molecules into mammalian cells is an important aspect of cellular genomics. Electroporation is a commonly used method for delivery of molecules such as plasmid DNA and siRNA. However, optimization of electroporation conditions prove to be challenging and time consuming. With the introduction of the Gene Pulser MXcell electroporation system, optimal electroporation conditions can be determined quickly, allowing scientists to perform experiments with minimal delay. In this note, we describe optimal electroporation conditions developed for Jurkat cells, a difficult-to-transfect cell line, using the Gene Pulser MXcell electroporation system and Gene Pulser® electroporation buffer. These results were then used to compare transfection efficiency to a competitor's instrument, in which optimization can be performed only by varying proprietary buffers or using preset protocols. Finally, a time-course experiment was conducted to assess successful delivery of both siRNA and plasmid DNA into the cells. Results demonstrate that gene silencing was achieved using the Gene Pulser MXcell system after 4 hr, and that this silencing persisted for 48 hr. In addition, results from the 4 hr time point suggest that the plasmid DNA was successfully delivered directly into the nucleus.

Methods

Jurkat cells (ATCC #TIB-152) were grown in RPMI-1640 media (Invitrogen Corporation) with 10% fetal bovine serum (FBS), and subcultured 24 hr prior to electroporation. Cells were washed in PBS and then suspended in Gene Pulser electroporation buffer at a density of 5 x 10^6/ml for electroporations. Electroporations on the Gene Pulser MXcell system were carried out using 96-well electroporation plates, with 150 µl cells/well. Negative control, GAPDH, and β-actin siLentMer™ validated Dicer-substrate siRNA duplexes (Bio-Rad Laboratories, Inc.) were used at a final concentration of 100 nM. Plasmid DNAs (pCMVi Luc) were electroporated at a final concentration of 20 µg/ml. Luciferase activity or RT-qPCR analysis using the iQ™5 real-time PCR detection system were used to measure electroporation.

Results

Optimization of Electroporation Conditions

The initial optimization experiment tested both square-wave and exponential-decay protocols. Four voltages were tested with square-wave conditions in the initial optimization experiment, followed by optimization of pulse duration (msec). A total of seven exponential-decay pulses were tested at constant voltage and resistance (250 µF, 1,000 Ω), but varied capacitance (150–600 µF). Four additional exponential-decay pulses were delivered at constant capacitance and resistance (300 µF, 1,000 Ω), but varied voltage (220–310 V). The exponential-decay protocols resulted in much higher luciferase activity than the square-wave protocols. Optimal conditions using exponential waveform are shown in Table 1. Conditions are based on results obtained from luciferase activity assay (pCMViLuc).

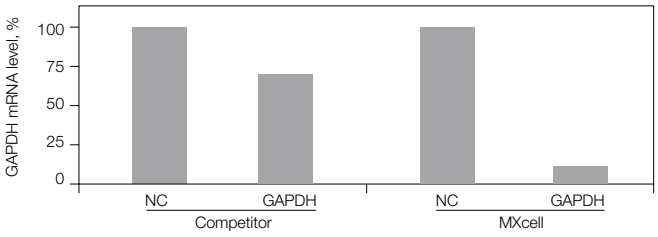
Table 1. Optimized electroporation conditions for Jurkat cells in Gene Pulser electroporation buffer using exponential waveform.

Parameter	Optimal Condition
Voltage	250–300 V
Capacitance	300–350 µF
Resistance	1,000 Ω
Volume	150 µl

Comparison of Time Course of Gene Silencing Between Different Instruments

A second experiment was performed to compare the results obtained using the optimized exponential-decay protocol with those obtained using a competitor's instrument and their recommended buffer. In this experiment, a GAPDH or negative control siLentMer siRNA was delivered to Jurkat cells, and the cells were assayed either 4 or 24 hr postelectroporation. Transcript levels were measured by RT-qPCR to assess the level of gene silencing. After 4 hr, 88% of GAPDH mRNA was silenced using the Gene Pulser MXcell system and Gene Pulser electroporation buffer, compared to only 31% using the competitor's system and buffer (Figure 1). Gene silencing persisted 24 hr postelectroporation using the Gene Pulser MXcell system, while it declined appreciably (~17%) using the competitor's system and buffer.

A. 4 hr postelectroporation



B. 24 hr postelectroporation

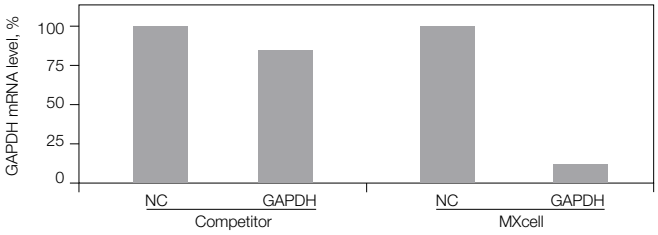


Fig. 1. GAPDH mRNA levels in Jurkat cells postelectroporation. **A**, GAPDH mRNA levels 4 hr postelectroporation; **B**, GAPDH mRNA levels 24 hr postelectroporation. After 4 hr, >88% knockdown was obtained using the Gene Pulser MXcell system and Gene Pulser electroporation buffer, compared to only 31% knockdown using competitor's system. NC, negative control.

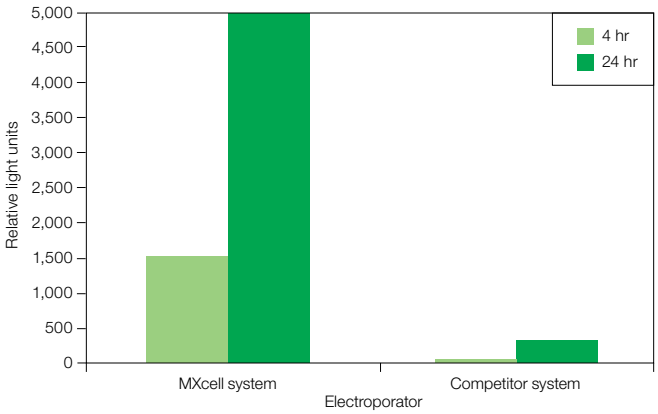


Fig. 2. Results of luciferase activity assays after delivery of plasmid into Jurkat cells using optimized electroporation conditions.

Assessment of Plasmid Delivery and Expression

Luciferase activity could be detected shortly after electroporation, indicating that delivery of plasmid into the nucleus was effective. Luciferase activity was measured 4 hr after electroporation using either the Gene Pulser MXcell system or a competitor's electroporation system. High amounts of luciferase activity were observed in cells electroporated with the MXcell system, while minimal activity was observed in cells electroporated with the competitor's system and buffer (Figure 2).

Conclusions

The Gene Pulser MXcell electroporation system can be used to quickly optimize electroporation conditions, which can then be used in the overall scientific design. In this study, the Gene Pulser MXcell system was used for screening and determining optimal electroporation conditions for Jurkat cells. Results from RT-qPCR and luciferase assays demonstrate highly efficient transfection. Studies comparing performance of the MXcell system to a competitor's system indicate superior results using the MXcell system. Time-course experiments using the same conditions demonstrate expression of protein and sustained silencing using the Gene Pulser MXcell electroporation system. High luciferase expression and efficient gene knockdown were observed only 4 hr postelectroporation.

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