Electroporation Conditions for Chinese Hamster Ovary Cells Using the Gene Pulser MXcell™ Electroporation System

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Introduction

The modulation of gene expression is a valuable molecular biology tool for analysis of gene function and for production of recombinant proteins. In mammalian cells, gene expression can be modified using RNA interference by introducing synthetic short interfering RNAs that result in post-transcriptional gene silencing and by introducing plasmids that carry recombinant genes coding for specific proteins of interest.

Chinese hamster ovary (CHO) cells are a common mammalian cell line used in laboratories to overexpress proteins, and they are useful for a wide range of studies, including genetic manipulations, toxicity and nutrition, and protein production. A range of genetic variants of these cells is available, from the original proline auxotrophs to UV-sensitive mutants.

Introduction of plasmids into mammalian cell lines for stable or transient expression can be effectively achieved by electroporation. CHO cell lines have been used as model systems for many investigations involving electroporation. However, cell line variants or cells grown under different conditions may require adjusted electroporation parameters in order to obtain both maximum plasmid delivery and cell viability.

In this paper we report results of experiments using the Gene Pulser MXcell electroporation system, performed to rapidly optimize the electroporation conditions for efficient transfection of a plasmid into the CHO-K1 strain.

Methods

Cell Culture and Plasmids

CHO-K1 cells (ATCC, #CCL-61) were cultured in Ham's F12K medium supplemented with 10% fetal bovine serum. Cells were passaged 1 to 2 days prior to electroporation, resulting in a cell density of about 75–85% confluency on the day of the experiment.

A DNA plasmid expressing the luciferase gene (pCMViLuc) was used as a reporter for determining the optimal plasmid delivery conditions.

Electroporation

Before electroporation, the cells were harvested by trypsinization, washed with PBS, counted, and resuspended in Gene Pulser® electroporation buffer at a density of $1x10^6$ cells/ml. Plasmid DNA was added to the cell suspensions at a concentration of 10 µg/ml, and the cells were then transferred to a 96-well electroporation plate and pulsed with the Gene Pulser MXcell electroporation system. Three replicates were performed for each condition.

After electroporation, the cells were transferred into growth medium and incubated at 37°C for 24 hours. Cell survival was assessed by examining cell density. Cell viability was determined by visual inspection and by assessing cell density under different electroporation conditions. Transfection efficiency was then determined by harvesting the cells and assaying for luciferase activity using a luminometer (Dynex Technologies, Inc.). Luciferase activity was reported in relative luminescence units (RLU).

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Results and Discussion

Plasmid Delivery

Current literature indicates that the highest transfection efficiency for CHO cells is obtained by electroporation using a square-wave pulse (as opposed to an exponential-decay pulse); therefore, we used a square-wave pulse to optimize plasmid delivery conditions into CHO cells with the Gene Pulser MXcell system. The Opt mini 96-well/Sqr preset protocol (Figure 1A) was used to determine the initial electroporation conditions. This protocol provides a square-wave pulse and varies the voltage and pulse duration in 6-well sets. To identify the best electroporation conditions, we assessed post-transfection cell viability and transfection efficiency. Initial results indicated that the best reporter gene expression levels are obtained with a 20 ms pulse at 300 V (Figure 1B). However, with these conditions, cell viability is the lowest (only 45% of pre-electroporation density). One set of parameters, 20 ms and 250 V, resulted in good transfection efficiency with acceptable cell viability (Figure 1B).

We further optimized electroporation conditions by testing a wider range of voltages (100–400 V) and pulse durations (10–30 ms) using the 96-well/Sqr, Vgrad, Dgrad preset protocol (Figure 2A). Across this range of conditions we found that a 30 ms pulse of 250 V resulted in the highest reporter gene expression with acceptable cell loss (60% survival) (Figure 2B). Higher cell viability could be obtained, but with a loss of transfection efficiency.

Finally, we tested the effect of varying the number of pulses applied to the cells by selecting the preset protocol Opt 96-well/Sqr, NP, D (Figure 3A). While maintaining a square waveform at 250 V, this protocol applies 1–3 pulses of varying durations (7–20 ms) to the cells. Results show that both the highest cell viability (80%) and the best transfection efficiency (~45,000 RLU) are obtained with 2 pulses of 15 ms (Figure 3B).

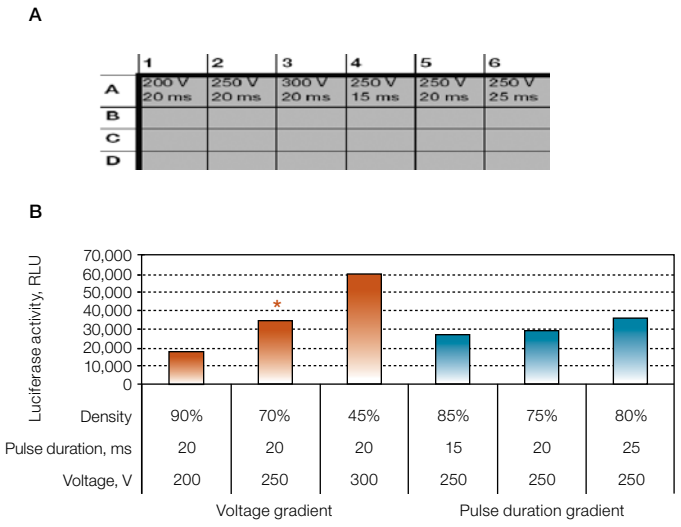


Fig. 1. Optimization of electroporation conditions for plasmid delivery into CHO cells. A, schematic of the preset protocol Opt mini 96-well/Sqr; **B**, reporter gene expression and cell survival under different conditions of voltage and pulse duration. Numbers are mean values of 3 replicate wells. The best set of parameters is indicated by an asterik. RLU, relative luminescence units.

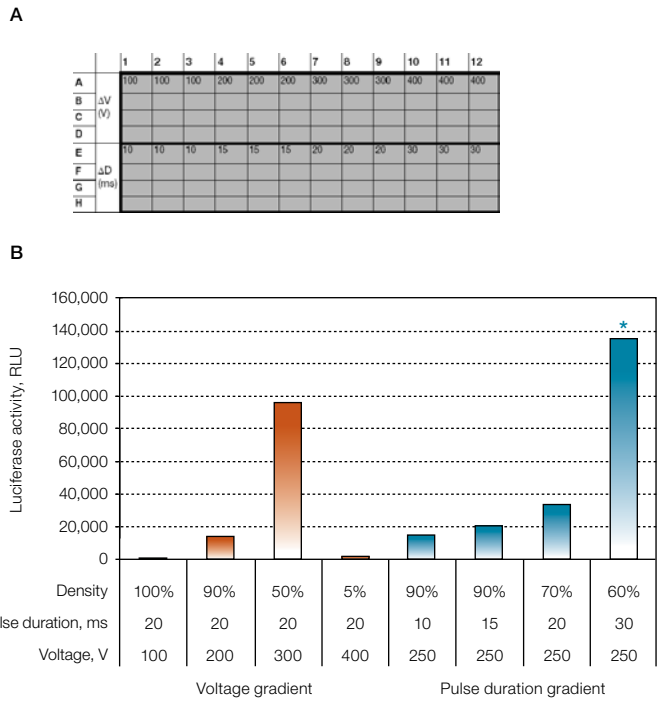
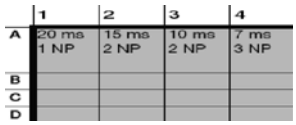


Fig. 2. Optimization of electroporation conditions for plasmid delivery into CHO cells. A, schematic of the preset protocol 96-Well/Sqr, Vgrad, Dgrad; **B**, reporter gene expression and cell survival under a wide range of voltage conditions and pulse durations. Numbers are mean values of 3 replicate wells. The best set of parameters is indicated by an asterik. RLU, relative luminescence units.

A



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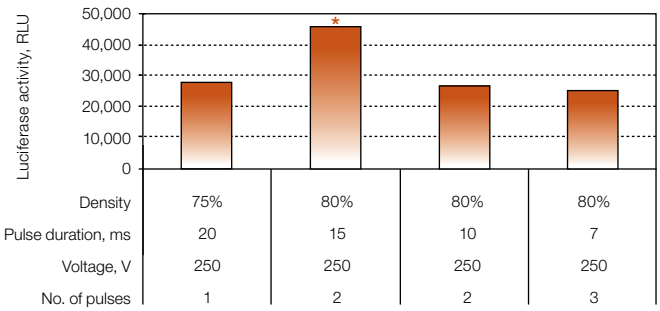

Fig. 3. Optimization for pulse length and number. A, schematic of the preset protocol Opt 96-well/Sqr, NP, D; **B**, reporter gene expression and cell survival under different conditions of varying pulse numbers and durations. Numbers are mean values of 3 replicate wells. The best set of parameters is indicated by an asterik. RLU, relative luminescence units.

Conclusions

Using the Gene Pulser MXcell electroporation system, we were able to rapidly optimize electroporation conditions to obtain high transfection efficiency and cell viability for this CHO-K1 cell line. Our results show, that for a given set of parameters, transfection efficiency can be improved by applying 2 consecutive pulses to the cells. Other CHO cell line variants or delivery of different molecules may require different parameter settings.

This work also demonstrates the power of the Gene Pulser MXcell electroporation system and its ease of use for optimizing electroporation conditions with preset protocols. By providing the ability to test multiple conditions in a single experiment, these protocols provide a critical tool to quickly find the best electroporation parameters for transfecting any molecule into any mammalian cell line.

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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