Transfection of Chinese Hamster Ovary-Derived DG44 Cells Using the Gene Pulser MXcell™ Electroporation System

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Introduction

Large-scale production of recombinant proteins requires the effective delivery of plasmid DNA into mammalian cell lines that can be easily cultivated in vitro. Chinese hamster ovary (CHO)-derived DG44 cells are widely used as a model system for large-scale production of recombinant proteins. These cells exhibit the dihydrofolate reductase (DHFR) deficiency selection and amplification system and are grown in suspension (Wurm 2004). However, transfection of these cells is difficult.

Electroporation is becoming a preferred method for delivering plasmid DNA and small interfering RNA (siRNA) into difficult-to-transfect cell lines. The high-throughput Gene Pulser MXcell electroporation system is designed to rapidly optimize experimental conditions for transfection of molecules into mammalian cells. The Gene Pulser MXcell system conveniently allows testing of a range of electroporation conditions in a single experiment. It can deliver square-wave or exponential-decay pulses while varying voltage, capacitance, and pulse duration. By altering each of these parameters in the initial electroporation experiment, it is possible to quickly determine desirable conditions for transfecting any cell type. The MXcell system offers a number of preset protocols that provide good starting points for optimizing transfection of any cell type.

In this paper we report successful electroporation of DG44 cells using the MXcell system's preset protocols and Gene Pulser® electroporation buffer. We describe the steps for selecting electroporation conditions by applying preset protocols using both square and exponential waveforms to CHO DG44 cells at different cell densities.

Methods

Cell Culture and Sample Handling

CHO DG44 cells were cultured in CD DG44 medium with 1.8% Pluronic F-68 and 8 mM L-glutamine (Invitrogen Corporation). The cells were passaged one day prior to electroporation, rinsed in PBS, counted, and divided into two 15 ml centrifuge tubes at 2 x 10^6 and 5 x 10^6 cells/ml, respectively.

Electroporation and Transfection Material

Prior to electroporation, the cells were centrifuged and resuspended in Gene Pulser electroporation buffer (Bio-Rad Laboratories, Inc.). A DNA plasmid expressing the luciferase gene, pCMViLuc, was added to each cell suspension at a final concentration of 10 µg/ml. Cells were gently mixed, and 125 µl aliquots were transferred into the wells of a 96-well electroporation plate.

The Gene Pulser MXcell electroporation system was programmed to run the Opt mini 96-well/Sqr, Exp preset protocols (Figure 1). Each transfection condition was applied to four wells (A–D). Control cells did not receive a pulse or plasmid DNA.

Analysis of Transfection

After electroporation, 100 µl of transfected cells were transferred from each electroporation plate well to 24-well tissue culture plates containing 0.5 ml of medium per well. After incubation for 24 hours at 37ºC with 5% CO_2, the cells were centrifuged and washed once with PBS.

To measure luciferase activity in transfected cells, PBS was replaced with 100 µl of luciferase lysis buffer (0.1 M phosphate buffer, pH 7.8, 1% Triton X-100, 2 mM EDTA, and 1 mM DTT), and 10 µl of the resulting cell lysate were transferred to a 96-well plate for analysis. Luciferase activity was measured using a luminometer (Dynex Technologies, Inc.).

	Square waveform			Exponential waveform		
	1	2	3	4	5	6
A	200 V 2,000 µF 20 ms	250 V 2,000 µF 20 ms	300 V 2,000 µF 20 ms	250 V 350 µF 1,000 Ω	250 V 500 µF 1,000 Ω	250 V 750 µF 1,000 Ω
B						
C						
D						

Fig. 1. Schematic of the Opt mini 96-well/Sqr, Exp preset protocols. Conditions were applied to each well set (four wells in each column).

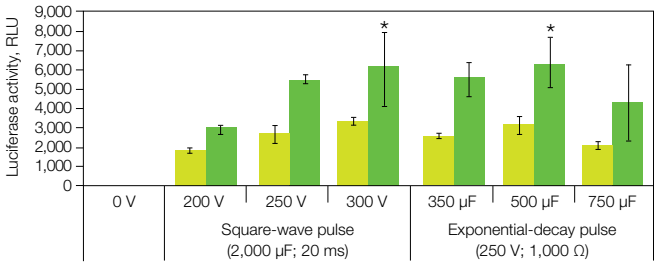

Fig. 2. Transfection efficiencies of CHO DG44 cells under different electroporation conditions. Transfection efficiency was assessed by measuring luciferase reporter gene expression 24 hours after electroporation. Cells were electroporated at a density of 2 x 10^6 cells/ml (■) and 5 x 10^6 cells/ml (■). The best conditions for the square-wave and exponential-decay protocols are indicated by asterisks. Each bar represents the mean value of 3 replicate wells. Error bars represent standard deviations. RLU, relative luminescence units.

Results and Discussion

Transfection Efficiency

The Opt mini 96-well/Sqr, Exp preset protocols illustrated in Figure 1 were used to identify conditions for transfection of CHO DG44 cells. These protocols apply a square-wave pulse at varying voltages (200, 250, or 300 V) while maintaining capacitance at 2,000 µF and pulse duration at 20 ms and an exponential-decay pulse at varying capacitances (350, 500, or 750 µF) while maintaining voltage at 250 V and resistance at 1,000 Ω. These conditions were applied to cell suspensions at two different cell densities (2 x 10^6 and 5 x 10^6).

Uptake of plasmid by DG44 cells was evaluated by measuring luciferase activity in cell lysate samples from each well. Results are shown in Figure 2. Control nonelectroporated cells (0 V) did not show any luciferase activity (Figure 2).

At either cell density there is little difference in the highest transfection efficiency between square-wave and exponential-decay treatments (Figure 2). With square-wave pulse conditions, as the voltage increased the transfection efficiency also increased, resulting in highest transfection efficiency at 300 V. With exponential-decay pulse conditions, as the capacitance increased the transfection efficiency increased and then decreased, resulting in peak transfection efficiency at 500 µF.

Overall, when the difference in cell density is taken into account, the transfection efficiencies were similar following square-wave and exponential-decay treatments (Figure 2), and the best electroporation conditions for both cell densities were reached using the same parameters.

Conclusions

Preset protocols, such as the Opt mini 96-well/Sqr, Exp used in this experiment, provide convenient initial starting conditions for developing a transfection protocol for any cell line. In this single experiment, we were able to identify parameters that delivered plasmid to the cells, and further demonstrated that these conditions could be scaled-up for a higher cell density.

This study did not measure the rate of cell death, an important parameter to consider when choosing transfection conditions. Increased voltage or capacitance can lead to increased transfection; however, it is also likely to increase cell permeability. For example, the apparent decrease in transfection efficiency at 750 µF for both cell densities could be due to increased cell death.

Electroporation conditions for the CHO DG44 cell line could now be further optimized by (1) testing voltage conditions slightly above and below the conditions that resulted in peak transfection in this study (square-wave pulse, 300 V; exponential-decay pulse, 500 µF), (2) testing a wider range of cell densities, and (3) varying plasmid concentration.

References

Wurm FM (2004). Production of recombinant protein therapeutics in cultivated mammalian cells. Nat Biotechnol 22, 1393-1398.

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