Optimization of Electroporation Conditions for Two Different Burkitt Lymphoma Cell Lines Using the Gene Pulser MXcell™ System

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Introduction

Burkitt lymphoma (BL)–derived cell lines are commonly used as model systems to study the molecular basis of Burkitt lymphoma. Electroporation is the main nonviral method used for gene transfer into difficult-to-transfect cells. However, electroporation can be challenging, since several parameters must be optimized to achieve good transfection efficiencies, including waveform, voltage, capacitance, and duration. The Gene Pulser MXcell electroporation system is designed to deliver plasmid DNA, siRNA, and other molecules into mammalian cells, especially difficult-to-transfect cells. The enhanced user interface contains optimized preset protocols to easily identify the best possible transfection conditions. Gene Pulser® electroporation buffer is formulated to improve electroporation by minimizing cell death while ensuring highly efficient delivery of nucleic acids.

In this technical report, we describe the optimization of electroporation conditions for the Burkitt lymphoma cell lines Ramos (EBV negative) and Namalwa (EBV positive) using the Gene Pulser MXcell system along with Gene Pulser electroporation buffer. We achieved transfection efficiencies of up to 50% for Ramos and up to 30% for Namalwa cells, while the viability was 50 and 80%, respectively. Our data demonstrate that the Gene Pulser MXcell system is an excellent tool to electroporate difficult-to-transfect cell lines.

Methods

Ramos and Namalwa cells were purchased from the American Type Culture Collection (ATCC CRL-1596, ATCC CRL-1432). For electroporation, cells were washed in PBS and resuspended in Gene Pulser electroporation buffer at a density of 5 x 10^6 cells/ml. Reporter gene plasmid DNA (gWiz luciferase or gWiz GFP, both from Aldevron) was added at 20 µg/ml final DNA concentration. The suspension was mixed and transferred to the appropriate wells of a 96-well electroporation plate (150 µl of suspension/well). All transfection reactions were performed in duplicate. Electroporation was carried out on the Gene Pulser MXcell system as indicated. After electroporation, cell suspensions were immediately transferred to growth media (7.5 x 10^5 cells/ml final cell concentration) and incubated for 24 hr in a cell culture incubator. Lactate dehydrogenase (LDH) levels in the cell culture supernatants were determined using the CytoScan LDH cytotoxicity assay (G Biosciences). Luciferase activity of cell lysates was determined using standard procedures. To determine percentages of viable GFP-positive cells, cells were stained with propidium iodide and analyzed on the BD FACSCalibur instrument using BD CellQuest Pro software (BD Biosciences).

Results

The first set of experiments was devoted to determining the optimal waveform and starting conditions for voltage, capacitance, and pulse duration. The Gene Pulser MXcell system preset 96-well plate protocols (preset protocols 13, Opt 96-well/Exp, Sqr) were chosen. These are designed to test 12 different conditions for both exponential and square waveforms (Figure 1A). These preset protocols are composed of four identical wells per condition, which allow testing of up to four different cell lines on one electroporation plate. We used the luciferase reporter gene for the first set of experiments because luciferase assays are ideal for high-throughput experiments.

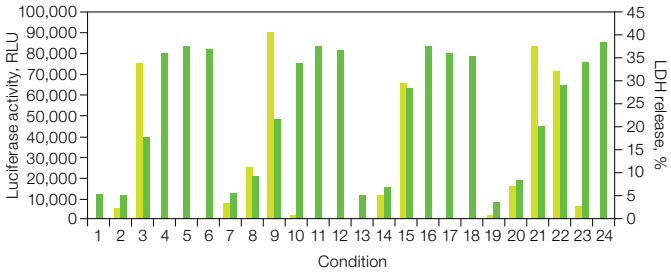
Twenty-four hours after transfection, luciferase activity was determined in cell lysates for quantitation of reporter gene expression. In addition, LDH levels in cell culture supernatants (a biomarker of plasma membrane damage or rupture) were measured to evaluate cytotoxicity.

As shown in Figure 1B, Ramos cells expressed the highest luciferase levels (light green bars) when they were transfected using exponential-decay conditions at 250 V/350 µF (Figure 1A, conditions 3 and 9). Similar values were obtained with square-wave conditions at 250 V/15 ms (Figure 1A, condition 21). LDH levels (dark green bars) were comparable for all of these conditions, indicating that Ramos cells are amenable to electroporation with both waveforms.

A. Electroporation Conditions of Preset Protocols Opt 96-Well/Exp, Sqr

Rows A–D, exponential waveform												
Column	1	2	3	4	5	6	7	8	9	10	11	12
Voltage (V)	150	200	250	300	350	400	250	250	250	250	250	250
Capacitance (µF)	350	350	350	350	350	350	200	250	350	500	750	1,000
Rows E–H, square waveform												
Column	13	14	15	16	17	18	19	20	21	22	23	24
Voltage (V)	150	200	250	300	350	400	250	250	250	250	250	250
Pulse length (ms)	20	20	20	20	20	20	5	10	15	20	25	30

B. Transfection of Ramos Cells (Opt 96-well/Exp, Sqr)



C. Transfection of Namalwa Cells (Opt 96-well/Exp, Sqr)

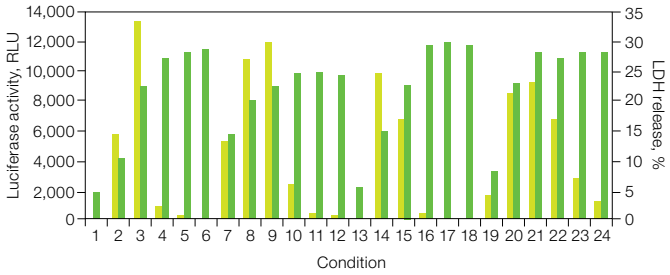


Fig. 1. Optimization of transfection conditions for Ramos and Namalwa cells using preset protocols Opt 96-well/Exp, Sqr. A, the 96-well plate was divided into two sets of conditions; the top four rows were used to deliver exponential-decay pulses and the bottom four rows were used to deliver square-wave pulses. Twenty-four different conditions of voltage/capacitance or voltage/pulse duration were applied to four wells each. Identical conditions are boxed. **B** and **C**, cell viability and luciferase activity of Ramos and Namalwa cells transfected with a luciferase construct using 12 conditions each of exponential and square waveforms as indicated in panel A. Each condition was applied in duplicate. LDH release in cell culture supernatants and luciferase activities in cell lysates were determined 24 hr after transfection. Values represent the average of duplicate samples. Luciferase activity (■), LDH level (■). RLU, relative luminescence units.

Namalwa cells displayed the highest luciferase activity with the exponential-decay condition at 250 V/350 µF (Figure 1A, conditions 3 and 9). Slightly lower luciferase levels were obtained when the square-wave condition was used (200 V/20 ms; Figure 1A, condition 14), while cytotoxicity was considerably lower than in samples transfected with conditions 3 and 9, indicating that condition 14 may be used for applications when low cytotoxicity is desired. We decided to further optimize the exponential-decay conditions for maximal expression.

Although the setting of 250 V/350 µF was the best exponential-decay condition out of the preset protocols for both cell lines, perhaps better condition sets could be identified in the range between the tested values. Therefore, we tried to further fine-tune these conditions by testing additional capacitance values in the range of 250–500 µF at constant voltage (250 V). We obtained maximal luciferase expression in Ramos cells when the capacitance was set at 400 µF (Figure 2A). Under these conditions, 70% of Ramos cells were viable and 40% of viable cells were actually transfected, as shown by GFP expression (Figure 2B). Maximal transfection efficiency (50% GFP-positive cells of viable cells) was obtained with 450 µF (Figure 2B). However, only 50% of cells were viable when this setting was used. Depending on whether maximal protein expression, maximal transfection efficiency, or low cytotoxicity is desired, a capacitance setting between 400 and 450 µF can be chosen for Ramos cells.

In Namalwa cells, the previously identified condition of 350 µF was still the best setting for maximal luciferase expression (Figure 2C) and maximal transfection efficiency of 30% (Figure 2D), while about 80% of cells were viable. Transfection efficiency could not be further improved by varying the capacitance. We also tried to improve the transfection efficiency by testing voltage settings between 200 and 300 V in increments of 25 V at constant capacitance (350 µF). However, the initial setting of 250 V was still the best condition (data not shown).

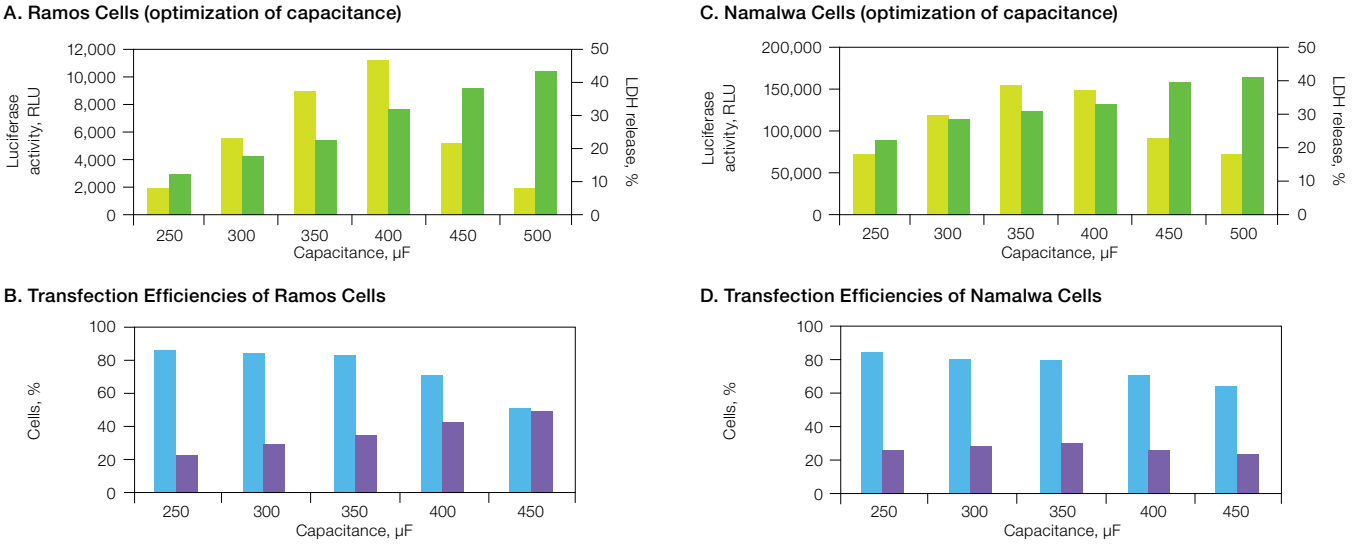


Fig. 2. Fine-tuning of capacitance for maximal transfection efficiencies of Ramos and Namalwa cells. Ramos and Namalwa cells were transfected with luciferase (**A**, **C**) and GFP (**B**, **D**) expression vectors. On the electroporation plate, two rows were used for each plasmid. The settings were an exponential-decay condition of 250 V/1,000 Ω, and capacitance as indicated. Luciferase activity, LDH release, % cell viability, and GFP expression were determined 24 hr posttransfection. Values represent the average of duplicate samples. Luciferase activity (■), LDH level (■), cell viability (■), GFP transfection (■). RLU, relative luminescence units.

Conclusions

We optimized transfection conditions for the Ramos and Namalwa cell lines, two difficult-to-transfect Burkitt lymphoma cell lines, using the Gene Pulser MXcell electroporation system. Our results show that both cell lines can be successfully transfected using the exponential waveform. We achieved 30% transfection efficiency for Namalwa cells with a setting of 250 V/350 µF. Ramos cells showed maximal protein expression when they were transfected with a setting of 250 V/400 µF, resulting in 70% viability and 40% transfection efficiency. Maximal transfection efficiency (50% of viable cells) was obtained at 450 µF, with decreased viability at 50%.

Our data show that for some cell lines, optimal conditions can be rapidly identified by screening with the preset 96-well plate protocols. Fine-tuning of the settings can lead to a considerable improvement in transfection efficiencies.

Information in this tech note was current as of the date of writing (2009) and not necessarily the date this version (rev B, 2010) was published.

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