

Optimizing Electroporation Conditions for High-Efficiency mRNA Transfection of CD8⁺ T Cells with the Gene Pulser Xcell Electroporation System

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Abstract

In this study, we optimized electroporation conditions for transfection of CD8⁺ T cells with GFP mRNA using the Gene Pulser Xcell Electroporation System and Gene Pulser Electroporation Buffer. Preliminary experiments focused on Jurkat cells to reduce overall cost and waste of precious sample cells. The optimal electroporation conditions for Jurkat cells were square wave pulses in the range of 160–220 V with a duration of 2 ms. We used the parameters optimized for Jurkat cells to identify the starting test conditions for transfecting CD8⁺ T cells. We determined that a single 200 V square wave pulse for 2 ms was optimal for CD8⁺ T cells, resulting in over 81% transfection efficiency while maintaining greater than 91% viability. This work is intended to provide a starting place for researchers interested in electroporation of mRNA in CD8⁺ T cells.

Introduction

For efficient gene transfer into a mammalian cell line or type of interest, it is important to find optimal conditions that balance maximum transfection efficiency with maximum cell viability (Shi et al. 2018). Electroporation optimization can be broken into discrete steps, starting with determination of the optimal waveform. The two most common waveforms for mammalian cell electroporation are exponential decay and square wave. Exponential decay waveform electroporation of mammalian cells is typically done using high capacitance and low voltage. Electroporation using square wave pulses is performed with a relatively low voltage, but unlike exponential waveforms, the duration of square wave pulses can be specified. The Gene Pulser Xcell Electroporation System is capable of generating both types of pulses. Once the most appropriate waveform is selected, one can optimize the voltage (V) and capacitance (μ F) settings for exponential decay or the voltage and pulse duration (ms) for square wave pulses.

Investing time up front in a small optimization study can yield benefits in the long run, especially when reproducibility over different samples is desired. This is particularly true in situations where the effectiveness of a therapy is dependent on the efficiency with which the target receptor is expressed and the number of viable cells expressing the receptor, for example, in the generation of CAR-T cells. Traditionally, CAR-T cells have

been produced using viral transfection methods. However, these methods can be problematic due to their cost, safety concerns, and time requirements. Additionally, cancer cells are known to lose or switch expression of the target antigen (Han et al. 2019), which may cause CAR-T cells stably transformed using viral transfection to have off-target effects in the long term. Electroporation of mRNA into CD8⁺ T cells has been proposed as an alternative due to the low cost, ease of large-scale production, and ephemeral nature of CAR expression. Here we present an optimized protocol for electroporation of T cells using the Gene Pulser Xcell Electroporation System and Gene Pulser Electroporation Buffer to achieve maximum transfection efficiency while maintaining high cell viability. A series of experiments were performed with different electroporation conditions and constant concentration of cell and transfection material. Cells were electroporated with GFP mRNA to permit easy readout of transfection efficiency using a ZE5 Cell Analyzer.

Materials and Methods

Human Peripheral Blood CD8⁺ T cells (STEMCELL Technologies) were thawed from cryogenic storage and grown in a 6-well plate in ImmunoCult-XF T Cell Expansion Medium (STEMCELL Technologies) supplemented with 10 ng/ml of Human Recombinant IL-2 (CHO-expressed) (STEMCELL Technologies) in the presence of ImmunoCult Human CD3/CD28 T Cell Activator (STEMCELL Technologies) per the

manufacturer's instructions. In all experiments, cells were no older than 14 days post-thaw. Jurkat cells (ATCC) were grown in RPMI 1640 Medium (Thermo Fisher Scientific Inc.) containing 10% fetal bovine serum (FBS). Cells were seeded at a cell density between 5×10^6 and 1×10^6 cells/ml the day before electroporation to ensure cells were in the exponential growth phase with high viability before the experiment.

Prior to electroporation with a Gene Pulser Xcell Eukaryotic System (Bio-Rad, #1652661), cells were counted, assessed for viability with Trypan Blue (Bio-Rad, #1450021), and washed twice with Dulbecco's Phosphate-Buffered Saline (1x, without calcium and magnesium, Corning). Cells were then resuspended at a density of 5×10^6 cells/ml in Gene Pulser Electroporation Buffer (Bio-Rad, #1652666). Aliquots of the cell/electroporation buffer mix were transferred into 1.5 ml microcentrifuge tubes (150 μ l per condition). DasherGFP mRNA (20 μ g/ml, Aldevron) was added to each microcentrifuge tube containing the cell/electroporation buffer mix. The mixture was homogenized by pipetting up and down, then transferred into a sterile Gene Pulser/MicroPulser Electroporation Cuvette with 0.2 cm gap (Bio-Rad, #1652086) and pulsed with voltage varying in 20 V increments from 100 to 220 V and pulse lengths of 2, 5, or 10 ms using the square waveform pulse.

After pulsing, cells were immediately transferred into a 24-well plate containing 850 μ l prewarmed ImmunoCult-XF T Cell Expansion Medium (T cells) or RPMI 1640 Medium (Jurkat cells).

Viability and GFP expression were determined after 24 hr using a ZE5 Cell Analyzer (Bio-Rad, #12004279) with a VivaFix 353/442 Cell Viability Assay (Bio-Rad, #1351111) and a ZOE Fluorescent Cell Imager (Bio-Rad, #1450031).

Results

Under our experimental conditions, we found that the parameters that resulted in the highest viability with the best transfection efficiency for transfection of Jurkat cells were two 2 ms square wave pulses at 200 V each. Under these conditions we were able to transfect Jurkat cells with GFP mRNA with 96% efficiency and 94% cell viability. Alternatively, we found that pulse length could be increased to 5 ms and combined with a lower voltage (140–160 V) with similar results (Table 1).

The optimal electroporation conditions for Jurkat cells were used as a starting point when determining the best parameters for CD8+ T cells. We found that the optimal electroporation conditions for transfection of a GFP mRNA construct into CD8+ T cells was a single square wave pulse of 2 ms at 200–220 V (Table 2, Figures 1 and 2).

Table 1. Conditions tested for optimal electroporation of GFP mRNA in Jurkat cells using square wave pulses of varying number, voltage, and duration. Electroporation using an exponential decay wave pulse is shown for comparison.

Square Wave Voltage, V	Number of Pulses	Pulse Duration, ms	Viability, %	Transfection Efficiency, %
0	1	No Pulse	97.2	0.4
100	1	1	99.5	1.2
120	1	1	99.4	16.6
140	1	1	98.7	48.4
160	1	1	97.7	72.4
180	1	1	97.4	89.4
200	1	1	96.5	78.3
220	1	1	95.4	93.7
100	2	2	98.2	9.6
120	2	2	97.9	51.3
140	2	2	96.6	82.6
160	2	2	93.8	94.0
180	2	2	92.3	94.5
200	2	2	94.3	95.7
220	2	2	93.4	85.1
100	5	5	98.2	23.2
120	5	5	97.8	70.8
140	5	5	96.9	94.0
160	5	5	91.4	95.1
180	5	5	83.8	94.4
200	5	5	54.2	90.7
220	5	5	25.0	76.0
100	10	10	98.4	41.5
120	10	10	97.2	85.7
140	10	10	89.3	95.2
160	10	10	62.8	72.2
180	10	10	26.5	60.1
200	10	10	17.7	11.8
220	10	10	32.0	20.8
Exponential Decay Wave Voltage, V	Resistance, Ω	Capacitance, μF	Viability, %	Transfection Efficiency, %
140	1,000	250	71.3	64.0

Table 2. Conditions tested for optimal electroporation of GFP mRNA in CD8+ T cells.

Square Wave Voltage, V	Number of Pulses	Pulse Duration, ms	Viability, %	Transfection Efficiency, %
160	1	2	91.0	49.9
180	1	2	91.4	77.9
200	1	2	91.1	80.7
220	1	2	90.9	81.3
160	2	2	90.9	62.8
180	2	2	87.8	78.9
200	2	2	88.3	74.1
220	2	2	86.6	78.3
160	3	2	86.8	63.3
180	3	2	80.9	74.0
200	3	2	80.1	71.0
220	3	2	81.5	68.5
160	4	2	85.4	64.0
180	4	2	80.3	65.2
200	4	2	81.2	65.6
220	4	2	76.8	57.4

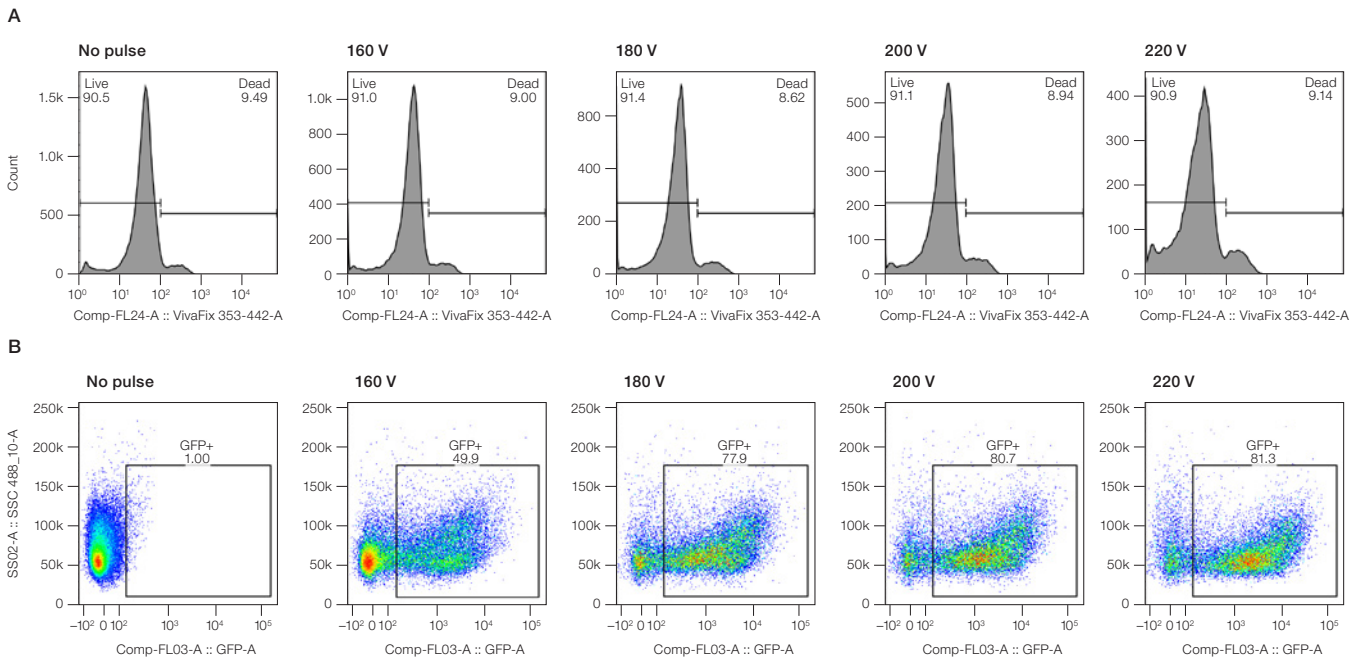


Fig. 1. Viability and transfection efficiency for CD8+ T cells. Cells were subjected to electroporation with a single square wave pulse between 160 and 220 V with a duration of 2 ms. Transfection viability and efficiency of cells treated with no pulse are shown for comparison. **A**, cell viability was measured using a VivaFix 353/442 Cell Viability Assay. Live/dead cutoff ranges for VivaFix fluorescence intensity (x-axis) are indicated by inclusion gates. The percentages of live and dead cells in each group are given in the upper corners. **B**, to assess transfection efficiency, GFP fluorescence (x-axis) and side scatter (y-axis) were measured using a ZE5 Cell Analyzer. The fraction of cells successfully transfected (expressing GFP) are gated and the percentage of the total population is shown.

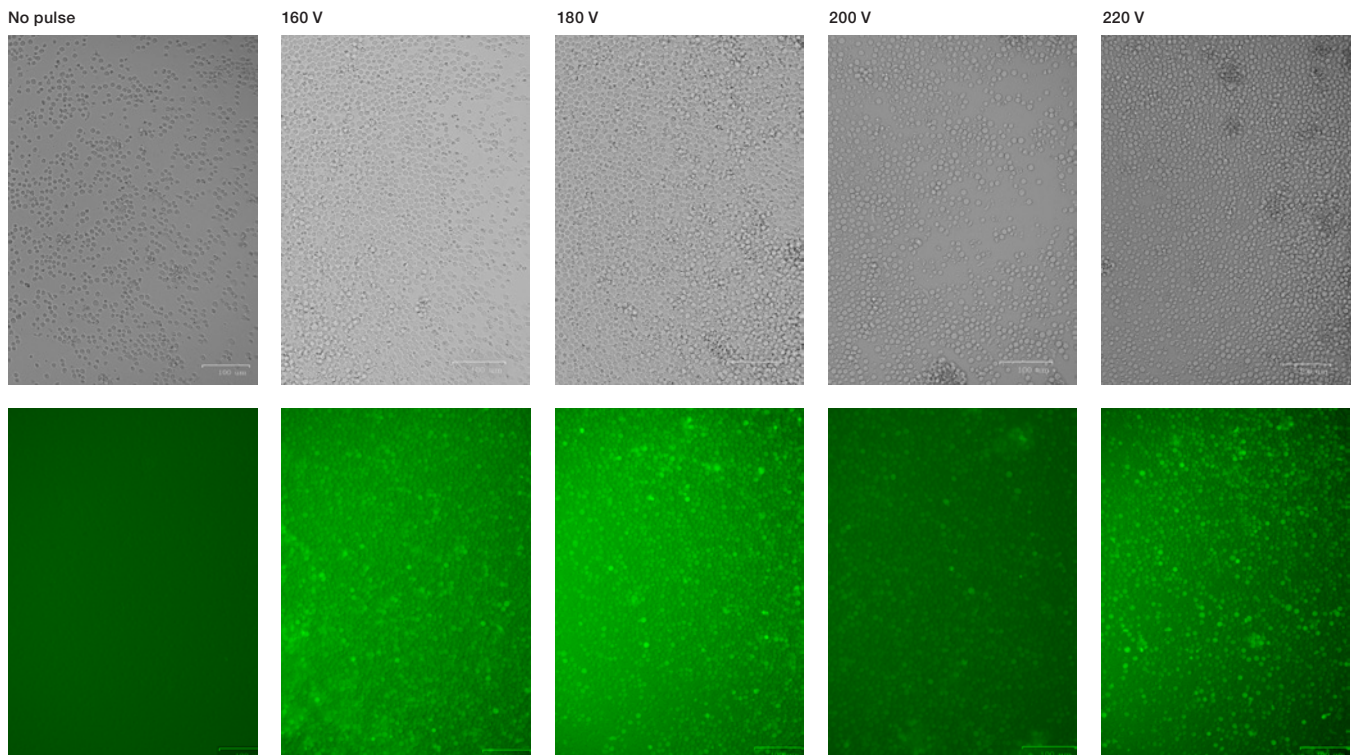


Fig. 2. Imaging of electroporated T cells. Representative brightfield (top) and GFP (bottom) images of pulsed CD8+ T cells electroporated with no pulse or a single square wave pulse using given voltage. Images were captured using a ZOE Fluorescent Cell Imager. Scale bars = 100 μ m.

Conclusions

T cells are sensitive and can be difficult to transfect; however, by optimizing electroporation conditions with the Gene Pulser Xcell Electroporation System, we were able to transfect T cells with 91% viability and over 81% efficiency. This is in agreement with a recent paper showing successful electroporation of a CAR construct into T cells under GMP conditions with 74.1% viability and 88.0% efficiency (Wiesinger et al. 2019). The high transfection efficiency and survival rates are critical, as large numbers of CAR-T cells are needed for clinical applications.

The Gene Pulser Xcell System is highly flexible and has been used for many cell types, including those that are sensitive or difficult to transfect. The instrument arrives with preset protocols for electroporation into common cell types, and an electroporation protocol library is available at biorad-ads.com/transfection_protocols. Additionally, the conditions found in this publication may be used as a starting point for further optimization, as the precise ideal conditions may vary depending on the cell line and electroporation buffer used.

References

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