Optimization of Electroporation Conditions With the Gene Pulser MXcell™ Electroporation System

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Introduction

The Gene Pulser MXcell electroporation system supports the convenient plate format, allowing researchers to choose between 96-, 24-, and 12-well plates to fit experimental needs. For optimization, the 96-well plate allows testing of up to 24 different conditions. Each condition is called a well set, consisting of four individual wells that will have the same conditions applied to them. The well set allows researchers to perform high-throughput and replicate experiments. If more cells are required, 12- and 24-well plates can be used to accommodate larger sample volumes.

Electroporation Conditions

Factors that affect efficient electroporation of cells are waveform, pulse duration, field strength, cells and cell density, nucleic acid concentration and type, and electroporation buffer. These conditions can be tested and optimized rapidly using the Gene Pulser MXcell system.

Waveform

The two most common waveforms used in electroporation are the square and exponential (voltage decay) waveforms. The square wave relies on a charge being applied to the cells for a set time. The exponential waveform builds up a charge in a capacitor and when applied to the sample, the voltage delivered decays exponentially until the charge remaining is about 37% of the original pulse. The time over which voltage decay occurs is known as the time constant, τ.

Pulse Duration

The time constant is equal to (R x C), R being the resistance of the sample and system, and C the capacitance set on the instrument. Square waves are not associated with a time constant; instead, they are determined by the pulse duration (pulse length) — a time, in milliseconds, that is programmed into the instrument. It is possible to use short or long pulse durations when optimizing square-wave electroporations. Generally, small increments (±5 msec) lower and higher than the original pulse length are tested. Additionally, it has been observed that cells benefit from multiple, shorter pulses. For example, if the optimal pulse duration is 20 msec, further optimization may be possible by giving two pulses of 10 msec each instead.

The time constant in exponential waveforms is directly related to the resistance of the sample, the resistance programmed on the electroporator, and the capacitance setting on the electroporator. Resistance of the sample can be changed in several ways. The sample volume is inversely proportional to the resistance; therefore, decreasing the volume increases the resistance.

The ionic strength of the electroporation buffer can affect the resistance (see Table 1). The gap width (gap size or interelectrode distance) affects resistance; increasing the gap width increases the resistance. Changing the gap width also affects the field strength (see next section). Cell density may also play a role in sample resistance (see Cells and Cell Density section).

Table 1. Time constants (msec) of electroporation solutions with no cells at several volumes. These results were obtained using a protocol of 260 V, 500 µF, and 1,000 Ω on a Gene Pulser Xcell™ system with 0.4 cm gap width cuvettes. This can also be done on a Gene Pulser MXcell system using volumes within the recommended range.

Solution Volume (µl)	400	600	800
	Time Constant, τ (msec)		
Gene Pulser® electroporation buffer	72	49.5	40.7
Phosphate buffered saline (PBS)	15.3	10.8	8.2
Opti-MEM medium (Invitrogen)	13.2	9.4	7.4

Field Strength

Field strength, E, is equal to V/d, where d is the gap width. Field strength is inversely proportional to the gap width; in other words, when the same voltage is applied to cuvettes with 0.4 cm and 0.2 cm gap distances, the field strength for the 0.2 cm cuvette is double that of the 0.4 cm cuvette. For this reason, when converting conditions from one cuvette to another having a different gap distance, it is critical to consider the field strength and make the necessary adjustments to the voltage.

Cells and Cell Density

Cell density may play a role in sample resistance. Cells should be actively growing and healthy for use in electroporation experiments. It is important that the cells not be contaminated with *Mycoplasma*. Typical electroporations require cells at a density of about 1 x 10^6/ml to 5 x 10^6/ml for adherent cells

and 2 x 10^6/ml to 1 x 10^7/ml for suspended cells. When sample is limiting and fewer than four wells of a well set will be used, the remaining wells must contain the same volume of the same electroporation buffer.

Nucleic Acid Concentration and Type

The transfection efficiency of electroporation can be affected by the concentration, purity, and size of the molecule used. The final concentration range for plasmid DNA is typically 5–40 µg/ml. siRNA is used at final concentrations of 10–100 nM.

Electroporation Buffer

The buffer used to electroporate mammalian cells has a direct effect on the time constant, since the sample resistance, R, is mainly due to the buffer's ionic strength. The buffer components also influence transfection efficiency and cell viability. Traditionally, a buffer with high ionic strength (low resistance), such as PBS, is used in electroporation of mammalian cells at high capacitance. Serum-free growth media have also been routinely used in electroporation with the same conditions.

Gene Pulser electroporation buffer is versatile enough to use with most cell lines, including difficult-to-transfect cells and primary cells. The buffer works well with both siRNAs and plasmid DNA, and contains components that enhance transfection efficiency and maintain overall cell health and viability. Because Gene Pulser electroporation buffer is lower in ionic strength than PBS, the time constant will be different (refer to Table 1 for an example), thus minor adjustments to the time constant are required when changing from a protocol using a different buffer.

Discussion

Rapid Optimization Using Preset Protocols

The unique plate format of the Gene Pulser MXcell system allows researchers to quickly test and optimize all electroporation conditions. For example, when using Gene Pulser electroporation buffer, the capacitance can be decreased from the original protocol by the suggested 50%, while also reducing the original resistance setting by 20%. When considering electroporation of a cell line that has not been worked with, a general recommendation is to review the protocols used by several reference papers and derive a consensus starting protocol. If no references exist for a particular cell line, it is suggested that references for similar cell types be used as a starting point.

Preset protocols are available for rapid transfection optimization. They were developed based on the most commonly used cell transfection experimental parameters, and can be customized by modifying the template and saving it under a new name. For example, you can decide to optimize both exponential and square-wave pulses on the same plate, or choose to vary the exponential voltage on half of a plate and vary the capacitance on the other, as in the following example using a 96-well plate (Table 2):

Top half of plate (varying the exponential voltage):

C = 350 µF
R = 1,000 Ω
V = gradient (100–400 V)

Bottom half of plate (varying the capacitance):

V = 250 V
R = 1,000 Ω
C = gradient (200–1,000 µF)

Table 2. Plate format for sample optimization protocol.*

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	100	200	200	200	300	300	300	400	400	400
B	100	100	100	200	200	200	300	300	300	400	400	400
C	100	100	100	200	200	200	300	300	300	400	400	400
D	100	100	100	200	200	200	300	300	300	400	400	400
E	200	200	200	350	350	350	500	500	500	1,000	1,000	1,000
F	200	200	200	350	350	350	500	500	500	1,000	1,000	1,000
G	200	200	200	350	350	350	500	500	500	1,000	1,000	1,000
H	200	200	200	350	350	350	500	500	500	1,000	1,000	1,000

* Units are V (blue) and µF (red).

Once edits have been made to the preset protocol, it is simply saved under a new file name so that it can be readily accessed. There are 21 preset protocols available on the Gene Pulser MXcell system. For information on the practical application of the optimization parameters on the Gene Pulser MXcell system, see tech note 5603.

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