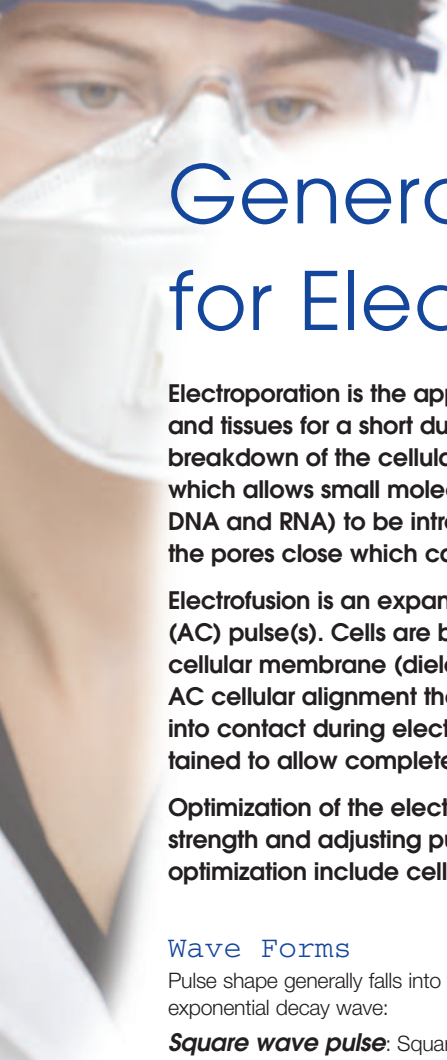

General Optimization Guide for Electroporation

Electroporation is the application of controlled direct current (DC) electrical pulses which are applied to living cells and tissues for a short duration of time. The pulse induces a transmembrane potential which causes the reversible breakdown of the cellular membrane. This action results in the permeation or "pore formation" of the cell membrane which allows small molecules (such as dye, oligonucleotides or peptides) and large molecules (such as proteins, DNA and RNA) to be introduced into the cell. During this process the cellular uptake of the molecules continue until the pores close which can take milliseconds to minutes.

Electrofusion is an expansion of electroporation using different buffers and one or more proprietory alternating current (AC) pulse(s). Cells are brought together or "aligned" by the use of an AC pulse which causes charges to form on the cellular membrane (dielectrophoresis) resulting in alignment of cells or pearl-chain (dimer) formation. Following the AC cellular alignment the DC pulse is applied to induce permeation of the cell membrane. When cells are brought into contact during electroporation, these cells are induced to fuse. Following this DC pulse the AC pulse is maintained to allow complete cell membrane fusion during the recovery period.

Optimization of the electroporation process involves several factors. Choosing the wave form, determining field strength and adjusting pulse length are just a few critical variables. Other parameters which play a crucial role in optimization include cell diameter, DNA concentrations, temperature and electroporation buffer.

Wave Forms

Pulse shape generally falls into two categories, square wave or exponential decay wave:

Square wave pulse: Square wave pulses rise quickly to a set voltage level, maintains this level during the duration of the set pulse length and quickly turns off. This square wave system yields higher efficiencies and viabilities in mammalian cells. Square wave EP in in vivo and ex vivo tissues, embryo's, cell fusions and plant protoplast applications yield better results in comparison to an exponential decay wave system.

Exponential decay wave pulse: Exponential decay waves generate an electrical pulse by allowing a capacitor to completely discharge. A pulse is discharged into a sample the voltage rises rapidly to the peak voltage set then declines over time. The powerful exponential decay wave pulse is routinely used for transformation of gram-negative and gram-positive, bacterial, yeast, plant tissues, insect cells and some mammalian cells.

Field Strength

The field strength is measured as the voltage delivered across an electrode gap and is expressed as kV/cm. Field strength is critical to surpassing the electrical potential of the cell membrane to allow the temporary reversible permeation or "pore formation" to occur in the cell membrane. Three factors should be considered for optimizing field strength:

1. Cuvette Gap Size
2. Cell Diameter
3. Temperature

Cell Types	Field Strength Ranges
Bacteria/Yeast	3-24 kV/cm
Mammalian	0.25-3 kV/cm
Plant	3-12 kV/cm

1. Cuvette Gap Size

The distance between electrodes, or "gap size" is important when optimizing your electroporation experiment. Field strength is calculated using voltage divided by gap size. For example, using a 4mm gap cuvette with 500V would provide a field strength of 1.25kV/cm. If instead of a 4mm gap cuvette, a 2mm gap cuvette was used, the voltage would have to be reduced by half or 250V in order to maintain the same field strength of 1.25kV/cm. It is possible to derive the voltage needed to accomplish electroporation if the desired field strength and gap size are known. The calculation for this is Field strength (kV) multiplied by gap size (cm) equals voltage. For example, if a user was certain that a 1.25 kV/cm field strength was required in a 1mm gap cuvette the calculation would be: 1.25kV x 0.1cm= 0.125kV or 125volts.

Example: A field strength of 1.25 kV/cm
4mm gap cuvette = 500 volts
2mm gap cuvette = 250 volts
1mm gap cuvette = 125 volts

2. Cell Diameter

Generally, smaller cell sizes require higher voltages while larger cell diameters require lower voltages for successful cell membrane permeation.

Cell Diameter	Cuvette 4mm Room Temp. (Volt)	Cuvette 4mm 4°C
10	500 Volts	1000 V
15	350 Volts	700 V
20	250 Volts	500 V
30	180 Volts	360 V
40	130 Volts	250 V
50	100 Volts	200 V

General Optimization Guide for Electroporation (continued)

3. Temperature

The temperature at which cells are maintained during electroporation effects the efficiency of the electroporation for several reasons. For a majority of mammalian cell lines are effectively electroporated at room temperature. Samples which are pulsed at high voltage or exposed to multiple pulses and long pulse durations can cause the sample to heat up. These conditions cause increased cell death and lowers the transfection efficiency. Maintaining the sample at lower temperatures can diminish the heating effects on cell viability and efficiency. Since electroporation causes the transient formation of pores, keeping the cells at lower temperature following the pulse may allow the pores to remain open longer to allow more uptake of the exogenous molecule. Yet lower temperatures on other cell lines can be damaging and cause high cell mortality. This effect is specific to each cell line and should be considered during optimization studies. The standard pulse voltage used for cells at room temperature will need to be approximately doubled for electroporation at 4°C in order to effectively permeate the cell membrane.

Pulse Length

The pulse length is the duration of time the sample is exposed to the pulse. This is measured as time in micro to milliseconds ranges. Adjusting this parameter is dependent on the pulse generator in use square wave or exponential decay wave. The pulse length in a square wave system can be inputted directly. The pulse length in an exponential decay wave system is called the "time constant" which is characterized by the rate at which the pulsed energy (e) or voltage is decayed to 1/3 the original set voltage. This time constant is modified by adjusting the resistance and capacitance (RC) values in an exponential decay. Time constant calculation T=RC, where T is time and R is resistance and C is capacitance.

The pulse length works indirectly with the field strength to increase pore formation and therefore the uptake of target molecules. Generally, during optimization of parameters an increase in voltage should be followed by an incremental decrease in pulse length. Decreasing the voltage, the reverse is true. Pulse length is a key variable that works hand in hand along with voltage and needs to be considered when optimizing electrical parameters to maximize the results for a given cell type.

Number of Pulses

Electroporation is typically carried out as a single pulse for most cell types. However, other cell lines may require multiple pulses to achieve maximum transfection efficiencies. Usually lower voltages are used when applying multiple pulses in order to gradually permeate the cell membranes. This allows the transfer of molecules while avoiding damage to delicate or whole tissue samples. This method of multiple pulsing is critical for maximum gene delivery without causing tissue damage to in vivo, in utero and ex-plant tissue environments. The use of multiple pulsing will require the optimization of key electrical parameters including voltage and pulse length. Typically, for in vivo applications the use of lower voltages between 10-100 volts with pulse lengths ranging 30-50msec provides efficient transfection. The optimal voltage, pulse length and number of pulses will vary depending on the cell type and molecule (DNA or RNA) transfected.

Electroporation Buffer

The buffers used for electroporation can vary depending on the cell type. Many applications use highly conductive buffers such as PBS (Phosphate Buffered Saline <30 ohms) and HBSS (Hepes Buffer <30 ohms) or standard culture media which may contain serum. Other recommended buffers are hypoosmolar buffers in which cells absorbs water shortly before pulse. This swelling of the cells results in lowering the optimal permeation voltage while ensuring the membrane is more easily permeable for many cells but can be damaging to others. Prokaryotic cells such as bacteria require the use of high resistance buffers (>3000 ohms) for this reason proper preparation and washing of the cells is essential to remove excess salt ions to reduce the chance of arcing. Ionic strength of an electroporation buffer has a direct affect on the resistance of the sample which in turn will affect the pulse length or time constant of the pulse. The volume of liquid in a cuvette has significant effect on sample resistance for ionic solutions, the resistance of the sample is inversely proportional to the volume of solution and pH. As the volumes are increased resistance decreases which increases the chance of arcing, while lowering the volume will increase the resistance and decrease the arc potential.

BTX now offers BTXpress™ High Performance Electroporation Solution, a low conductance buffer that achieves higher transfection efficiencies with minimal cell toxicity. The BTXpress buffer is a single buffer developed to facilitate high efficiency gene delivery into mammalian cells.

DNA/RNA Concentrations

Electroporation is typically thought of as a nucleic acid (DNA, mRNA, siRNA and miRNA) transfer method into prokaryotic and eukaryotic cells. Electroporation is not limited to just nucleic acid delivery, it can introduce proteins, antibodies, small molecules and fluorescent dyes. The standard range of DNA used for transfections is 5-20 g/ml for most cell types; however in some instances increasing the DNA concentration as high as 50 g/ml improves transfection efficiency without changing other parameters. Determining the optimal DNA concentration through a DNA titration can be beneficial. The size of a molecule will have an effect on the electrical parameters used to transfect the cell. Smaller molecules (siRNA or miRNA) may need higher voltage with microsecond pulse lengths and larger molecules (DNA) may need lower voltages with longer pulse lengths. Buffers such as EDTA or Tris can drastically reduce the transfection efficiency. Therefore, we recommend resuspending DNA in distilled water. Finally, electroporating ligation mixtures into E.coli can cause arcing and reduced transformations. Diluting the ligation mixture a minimum of 1:5 with diH_2O, dialysis, or ethanol precipitation can significantly improve transformation efficiencies and reduce the potential for arcing.