Gene Pulser® Transfection Apparatus

Operating Instructions and Applications Guide

Version 10-90

For Technical Service Call

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BIO-RAD

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Introduction

The Gene Pulser apparatus is a pulse generator which uses capacitor discharge to produce controlled exponential pulses for cell electroporation. The unit is capable of producing pulses of up to 2,500 volts at over 125 amperes. Pulses with field strengths of up to 12,500 V/cm can be generated in the 0.2 cm cuvette (catalog number 165-2086), or pulses of up to 6,250 V/cm can be generated in the 0.4 cm cuvette (catalog number 165-2088). The unit provides digital read-out of the voltage and capacitance settings. After a pulse is delivered, the actual voltage and resistance-capacitance (RC) time constant are automatically measured and may be displayed.

The Gene Pulser apparatus contains a power supply and capacitors of 0.25, 1.0, 3.0, and 25.0 microfarads (µF). Connectors at the rear of the Gene Pulser apparatus allow the use of the optional Capacitance Extender (catalog number 165-2087). The Capacitance Extender contains capacitors of 125, 250, 500, and 960 µF. These larger capacitors have a maximum voltage limit of 450 volts. The Gene Pulser apparatus and Capacitance Extender are recommended for electroporation of most eukaryotic cells, including mammalian cells and plant protoplasts.

The Pulse Controller unit (catalog number 165-2098) is used with the Gene Pulser apparatus for electroporation of bacteria, fungi, or other applications where pulses of very high field strength are applied to samples of small volume and high resistance. The Gene Pulser apparatus should not be used to generate field strengths above ~6,000 V/cm unless connected to the Pulse Controller. If the Pulse Controller is not used under these conditions, **arcing may occur and cause serious damage to the Gene Pulser apparatus.**

The Pulse Controller greatly reduces the incidence of arcing at high voltage, and protects the Gene Pulser circuit if a high voltage, high current arc does occur. The unit also allows the Gene Pulser apparatus to fire into a high resistance sample and provides the operator with electronic control of the time constant.

The Pulse Controller should **not** be used with samples having a resistance of less than 1,000 ohms (Ω). This includes samples of buffered saline (~20 Ω), tissue culture medium (~20 Ω), and phosphate buffered sucrose (~200 Ω). The internal resistance of the Pulse Controller will cause a substantial loss in voltage if pulses are applied to low resistance samples. The voltage loss is insignificant with high resistance samples (see Section 6 and the Pulse Controller manual for a detailed discussion of this effect).

READ CAREFULLY BEFORE USING GENE PULSER APPARATUS

Section 1
Safety Precautions

1.1 Electrical Hazards

The Gene Pulser apparatus produces voltages of up to 2,500 volts and is capable of passing very high currents. The 25 µF capacitor, when charged to maximum voltage, stores about 80 joules. A certain respect is required for energy levels of this order. The safety interlocks of the system prevent accidental charging and discharge (two buttons must be depressed to deliver a pulse), and also prevent operator access to the recessed electrode contacts inside the sample chamber. This latter mechanical interlock should never be circumvented.

There is high voltage present whenever the red buttons are depressed (charging) and when the capacitors have been partially charged but not fired (for example, when the charging cycle has been interrupted before the pulse is delivered). In this condition, the charge will bleed slowly from the capacitor and **a shock hazard can exist for several minutes. During this time, do not disconnect or connect any of the accessories to the Gene Pulser apparatus** (including the Capacitance Extender, the Pulse Controller, or the sample chamber). To manually discharge the capacitor, turn the main power switch of the Gene Pulser apparatus off and on twice. This will discharge the capacitor immediately and should be done whenever there is any doubt about the status of charge in the capacitor. It is a good idea to **always** follow this procedure before connecting or disconnecting accessories to the unit, to be absolutely sure the capacitors are discharged.

If the charging cycle is aborted by releasing either of the red buttons, the charge/fire cycle can be continued by simply re-pressing the red buttons until the pulse is delivered, or the capacitor can be safety discharged by turning the power switch off and on twice.

1.2 Mechanical Hazards

The Pulse Controller greatly reduces the incidence of arcing in the cuvette when high voltage is delivered into high resistance media. However, arcing can sometimes still occur, and the cuvette can shatter. While the sample chamber is effective in containing these small explosions, it is possible for a fragment of plastic to escape the open end of the chamber. Therefore, **we recommend wearing safety glasses when using the instrument.** At the highest voltage and field strength, placing the chamber behind a plexiglass shield is a reasonable precaution.

Mechanical hazard can also occur when multiple pulses are applied to conductive media (such as saline) using the larger capacitors. Under these conditions, electrolysis produces significant quantities of H_2 and O_2 which accumulate in a capped cuvette and can be ignited by a subsequent pulse. A small explosion of this type can also shatter the cuvette and the precautions described above should be taken.

As with any instrument that can produce sparks and arcs, experiments should not be performed in the proximity of flammable solvents or fumes (including ethanol vapors).

1.3 Other Safety Precautions

Always use the Pulse Controller with the Gene Pulser apparatus when delivering voltages greater than 1.5 kV from the 25 µF capacitor into high resistance electroporation media (e.g., distilled water, sucrose, or glycerol solutions). If the Pulse Controller is not used, arcing may occur and cause severe damage to the Gene Pulser apparatus.

Do not deliver >2,000 V into low resistance electroporation media (e.g., buffered saline solutions). Make sure the 0.4 cm cuvette contains the full 0.8 ml when delivering voltages between 1,500 and 2,000 V to conductive media (see also Appendix II).

Avoid the use of multiple pulses when the voltage setting is >1,500 V.

Turn the unit off when not attended. Avoid spilling any liquids onto the apparatus. Use only water or alcohol to clean the outside surfaces of the Gene Pulser apparatus.

Warning: This equipment generates, uses, and radiates radio frequency energy. If it is not installed and used in accordance with the instructions given in this manual, it may cause interference with radio communications. It has been tested and found to comply with the limits for Class A computing devices (pursuant to Subpart J of Part 15 of FCC Rules) which provide reasonable protection against such interference, when such equipment is operated in a commercial environment. Operation of this equipment in a residential area is likely to cause interference. In this case, the user will be required, at his/her own expense, to take whatever measure may be required to correct the interference.

Section 2
Gene Pulser Apparatus Operating Instructions

1. Turn on the apparatus using the power switch on the upper rear panel. The light emitting diode (LED) display should illuminate and read ‘0.00’.
2. Press SET VOLTS. The LED above the button will illuminate. The LED display is in kilovolts (kV). Use RAISE and LOWER to adjust the voltage to the desired value in the range of 0.05 - 2.50 kV (Figure 1). If the voltage is set below 0.05 kV, ‘no’ will be displayed when the pulse buttons are pressed.
3. Select the capacitor (0.25, 1.0, 3.0, or 25 µF) using the capacitance select knob on the lower right front of the Gene Pulser panel. See Section 3 for use of the Capacitance Extender.
4. Place the cell suspension in the Gene Pulser cuvette. Use only the lower, narrow portion of the cuvette. The 0.4 cm cuvette will hold a maximum of 0.8 ml of solution; up to 0.4 ml of solution may be placed in the 0.2 cm cuvette.
5. Insert the Gene Pulser cuvette into the white slide. Push the slide into the chamber until the cuvette makes firm contact with the chamber electrodes.
6. To charge the capacitor and deliver a pulse, depress and hold both red pulse buttons *until a continuous tone sounds.* The display will flash ‘Chg’, indicating that the capacitor is being charged to the selected voltage (Figure 1). The tone signals that the pulse has been delivered and the pulse buttons may be released. For safety reasons, the Gene Pulser apparatus charges and fires only when both pulse buttons are depressed. Up to 15 seconds may be required for pulse delivery after pressing the pulse buttons, depending on the size of capacitor selected.

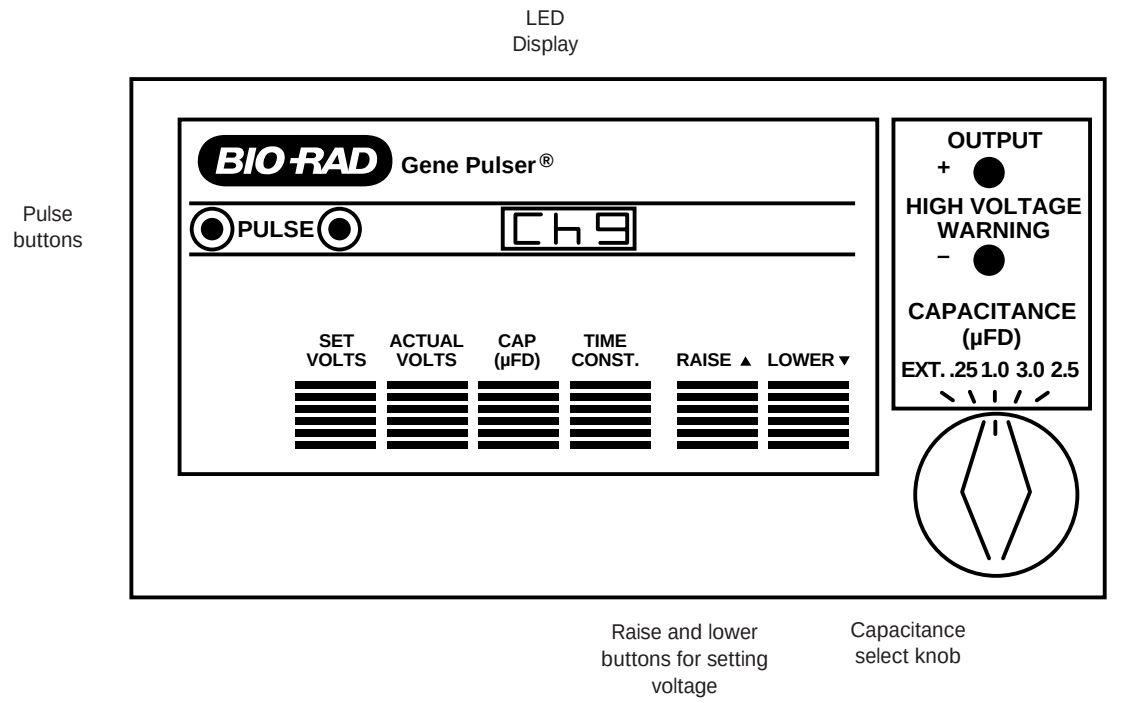

Fig. 1. The Gene Pulser apparatus.

Caution: If the pulse buttons are released before the pulse is delivered, the buttons may be re-pressed to continue the charge-fire cycle, or the capacitor may be discharged by turning the Gene Pulser apparatus off and on **twice,** and then turning it back on. The latter procedure will automatically bleed the capacitor of any residual voltage. (See Section 1 for safety precautions).

7. Withdraw the slide from the chamber, remove the cuvette, and process the sample.
8. The time constant and actual voltage can be displayed by pressing the appropriate buttons on the front panel of the Gene Pulser apparatus (see Figure 1).
9. To turn the unit off, turn the power switch off-on-off. This assures that the capacitors are completely discharged. Any accessories may now be safely disconnected.

Section 3
Capacitance Extender Operating Instructions

Note: When the Capacitance Extender is connected, the maximum voltage setting on the Gene Pulser apparatus is 0.45 kV.

1. Plug one end of the gray cable into the Capacitance Extender Readout jack on the rear panel of the Capacitance Extender. Plug the other end of the gray cable into the Capacitance Extender Readout jack on the rear panel of the Gene Pulser apparatus.
2. Plug the red cable into the red (+) jack on the rear panel of the Capacitance Extender. Plug the other end of the red cable into the red (+) jack located on the rear panel of the Gene Pulser apparatus.
3. Plug the black cable into the black (-) jack on the rear panel of the Capacitance Extender. Plug the other end of the black cable into the black (-) jack on the rear panel of the Gene Pulser apparatus.

4. Set the Gene Pulser apparatus capacitance select knob to EXT. Use the capacitance select knob on the Capacitance Extender to choose the capacitor (125, 250, 500, or 960 µF).

5. For pulse delivery and shut-down of the instrument, follow steps 6-9 in section 2.

Section 4
Instrument Readouts

The voltage and capacitor selections can be verified in the Gene Pulser display. The apparatus also automatically measures the peak voltage and resistance-capacitance (RC) time constant of each pulse. To display any of these electrical parameters, press the button associated with the parameter. The LED over the button will light and the value will be displayed.

1. SET VOLTS. The voltage setting in kilovolts (kV) is displayed.

2. ACTUAL VOLTS. The display shows the actual peak voltage (in kV) of the pulse. The electric field (kV/cm) is calculated as kV/0.2 when the 0.2 cm electrode gap cuvette is used, or kV/0.4 when the 0.4 cm electrode gap cuvette is used.

3. CAPACITOR. The size of the capacitor selected is displayed in microfarads (µF).

4. TIME CONSTANT (τ). The RC time constant is measured and displayed in milliseconds (msec). τ is equal to resistance x capacitance, and is the time required for the peak voltage to decay to approximately 37% of the initial voltage (see Figure 2). If τ is between 0.01 and 0.1 msec, it is displayed as 0.1 msec. If τ is less than 0.01 msec (due to very low resistance electroporation media, small capacitance, or for other reasons), 'no' will be displayed.

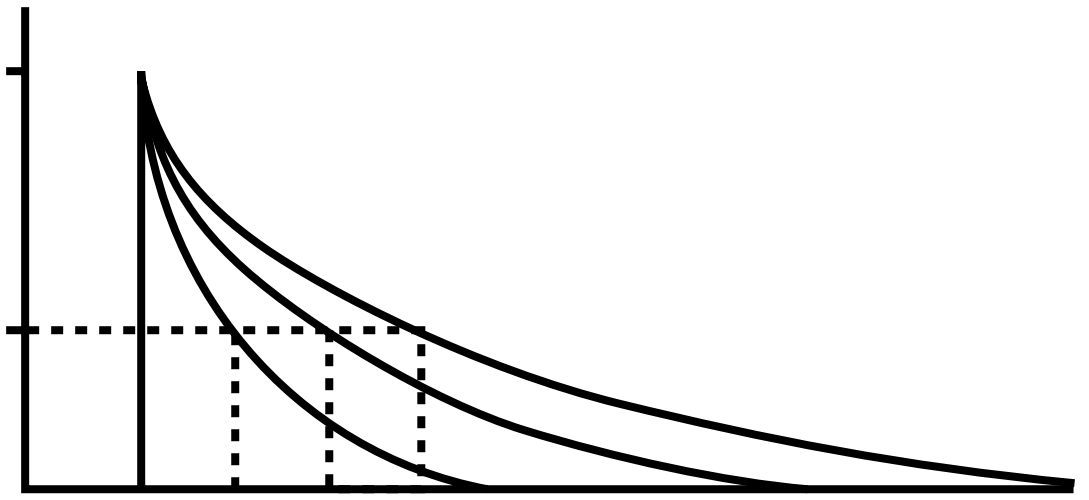

Fig. 2. Exponential decay waveform τ = R x C.

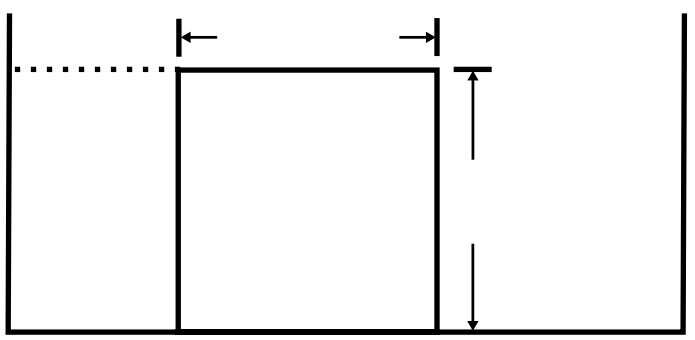

Fig. 3. Square wave.

Section 5
Measuring Output of the Gene Pulser Apparatus with an Oscilloscope

Only technically qualified persons should attempt to measure the output of the Gene Pulser apparatus with an oscilloscope. Extreme care should be taken in this procedure. In the United States, call Bio-Rad Technical Services at 1-800-4BIORAD for assistance. Outside the U.S., contact your local Technical Representative.

1. A 50 MHz storage oscilloscope and a Tektronix P6015 high voltage probe are required. **Use of an inappropriate oscilloscope or voltage probe will result in inaccurate voltage measurements.**
2. Turn the Gene Pulser apparatus off and attach the chamber. Place a cuvette containing sample electroporation medium in the cuvette holder, and slide it into position. (Pulse delivery requires a cuvette containing sample, since the Gene Pulser apparatus will not fire if no load is detected).
3. Unscrew the red and black covers on the leads which attach to the Gene Pulser apparatus. Attach the probe ground to the bottom lead; attach the probe tip to the top lead.
4. Set the oscilloscope to 5 V/cm on the x10 scale. This setting is displayed as 500 V/cm on the oscilloscope screen. Synchronize the oscilloscope.
5. Turn on the Gene Pulser apparatus, set the voltage, and deliver a pulse.

Section 6
Electrical Variables

6.1 Waveform

Two types of waveforms are usually used for electroporation: square wave and exponential decay. There is no evidence that either waveform is more effective for electroporation; however, the exponential waveform is much more commonly used. A square wave is generated by rapidly increasing the voltage to the desired amplitude, holding that voltage for a specified time (pulse width), and rapidly reducing the voltage to zero (Figure 3).

The capacitor discharge circuit of the Gene Pulser apparatus generates an electrical pulse with an exponential decay waveform (Figure 2). When the charge from the capacitor is directed to a sample placed between two electrodes, the voltage across the electrodes rises rapidly to a peak voltage (also known as the initial voltage, V_0), and declines over time as

$$V_t=V_0[e^{-(t/\tau)}] \qquad \text{Equation 1}$$

where τ is the RC time constant, a convenient expression of the pulse length. According to Equation 1, τ is the time over which the voltage declines to 1/e (~37%) of the peak value.

The voltage gradient between the electrodes is also known as the electric field (E) and is described by

$$E=V/d \qquad \text{Equation 2}$$

where d is the distance between the electrodes. The strength of the electric field and the size of the cells determine the voltage drop across each cell, and it is this voltage drop that seems to be the important manifestation of the voltage effect in electroporation.

The time constant is defined as

$$\tau=RC \qquad \text{Equation 3}$$

where τ is measured in seconds, R is resistance in ohms (Ω), and C is capacitance in farads (F). A larger capacitor requires a longer time to discharge through a given resistance; and a given capacitor discharges more slowly through a higher resistance. In designing an electroporation experiment, the time constant is adjusted by changing the size of the capacitor in the circuit, or by changing the resistance across the sample cuvette, or if the Pulse Controller is used, by selecting a different size of parallel resistor.

In a typical eukaryotic electroporation experiment (with the Gene Pulser apparatus connected **directly** to the sample chamber) **all** of the charge passes through the sample and, with any given capacitance, the resistance of the sample determines τ.

6.2 Resistance of the Electroporation Medium

The resistance of the electroporation medium is dependent on its ionic strength. As ionic strength increases, resistance of the medium decreases. A pulse delivered into a medium of higher ionic strength (lower resistance), will have a shorter time constant if all else (e.g., voltage, capacitance, cuvette geometry, and volume) is held constant.

A buffered sucrose solution (see Table 1 for more information) has a resistance about 10-fold that of phosphate buffered saline containing no Ca^{+2} or Mg^{+2} (PBS). Consequently, a pulse delivered from the same capacitor through the sucrose has a τ

which is 10 times longer than the τ for a pulse delivered into PBS. For example, discharging the 25 µF capacitor of the Gene Pulser apparatus through the sucrose solution produces a pulse with a τ of about 5 msec compared to a τ of 0.5 msec through PBS. To obtain a pulse with a τ of 5 msec in PBS, one would use the Capacitance Extender set at 250 µF.

Pulses with τ from 5 µsec to 200 msec can be obtained using these two media and the range of capacitors available with the Gene Pulser apparatus (0.25 to 25 µF) and the Capacitance Extender (125 to 960 µF). With still higher resistance media, such as HEPES solutions, pulses with τ up to 1 second can be produced.

A 5 to 10 mM HEPES solution has a resistance about 50 times that of PBS (without Ca^{+2} or Mg^{+2}). This is the highest resistance media that can be pulsed when the Gene Pulser apparatus is used without the Pulse Controller. When the Gene Pulser apparatus is used with the Pulse Controller, pulses may be delivered into higher resistance media (e.g., distilled water). Table 1 lists the pulse time constants produced by the Gene Pulser apparatus using these types of media.

Electroporation of eukaryotic cells is usually conducted in buffered saline solutions or the culture media used to grow the cells. The ionic strength of these media is usually very similar.

Table 1. The Effect of Buffer Ionic Strength on Time Constant*

Capacitor (µF)	Voltage (V)	Time Constant (msec)		
		PBS	Phosphate buffered sucrose	HEPES buffered sucrose
0.25	100	(≈0.007)[a]	(0.075)[a]	0.4
	200	“	“	0.4
	300	“	“	0.4
	400	“	“	0.4
	500	“	“	0.4
	600	“	“	0.4
	800	“	“	0.4
	1,000	“	“	0.4
	1,500	“	“	0.4
	2,000	“	“	0.4
1.0	100	(≈0.03)[a]	0.3	1.3
	200	“	0.3	1.3
	300	“	0.3	1.3
	400	“	0.3	1.1
	500	“	0.3	1.1
	600	“	0.3	1.1
	800	“	0.3	1.1
	1,000	“	0.3	1.1
	1,500	“	0.3	1.1
	2,000	“	0.3	1.1

Capacitor (µF)	Voltage (V)	Time Constant (msec) PBS	Phosphate buffered sucrose	HEPES buffered sucrose
3.0	100	0.2	0.5	(b)
	200	0.1	0.7	(b)
	300	0.1	0.7	4.0
	400	(≈0.1)[a]	0.7	3.9
	500	"	0.7	3.9
	600	"	0.7	3.7
	800	"	0.7	3.7
	1,000	"	0.7	3.6
	1,500	"	0.7	3.6
	2,000	"	0.7	3.4
25.0	100	0.6	7.1	(b)
	200	0.6	6.6	(b)
	300	0.5	5.6	37
	400	0.5	5.5	32
	500	0.5	5.3	31
	600	0.5	5.0	27
	800	0.5	4.8	26
	1,000	0.5	4.4	26
	1,500	0.5	4.0	24
	2,000	0.5	3.5	24
125	100	2.6	30	(b)
	200	2.5	25	140
	300	2.2	24	133
	400	2.1	23	133
250	100	5.6	75	(b)
	200	5.2	60	(b)
	300	4.9	55	650
	400	4.6	50	550
500	100	8.7	95	(c)
	200	8.7	95	(c)
	300	8.2	90	(c)
	400	7.2	85	(c)
960	100	16	190	(c)
	200	15	170	(c)
	300	14	160	(c)
	400	12	140	(c)

*Gene Pulser cuvettes (0.4 cm electrode gap) containing 0.8 ml of cold electroporation buffer were pulsed once with various voltage and capacitor combinations to determine the pulse time constant produced. The electroporation buffers are: 1) Dulbecco phosphate

buffered saline (no Ca^{+2} or Mg^{+2}); 2) phosphate buffered sucrose (272 mM sucrose, 7 mM potassium phosphate, pH 7.4, 1 mM $MgCl_2$); or 3) HEPES buffered sucrose (272 mM sucrose, 8 mM HEPES, pH 7.4).

a Pulses with time constants less than 0.1 msec. The pulse time constant can be estimated by extrapolation from data obtained with a larger capacitor and electroporation buffer of the same resistance.

b No pulse was delivered. Because the HEPES sucrose solution approaches the maximum resistance buffer that can be used with the Gene Pulser apparatus, pulse delivery may be prevented in some cases due to the electronic safety interlock.

c Pulses with time constants greater than 1 second. The pulse time constant can be estimated by extrapolation from data obtained with a smaller capacitor and electroporation buffer of the same resistance.

0.4 ml of these buffers in a 0.2 cm cuvette will produce a time constant of about half those listed above

Note: Do not use the 0.2 cm cuvettes for pulses greater than 1,000 V unless the Pulse Controller accessory is installed, **and** do not use the Pulse Controller when delivering pulses to PBS, tissue culture media, or phosphate buffered sucrose (see Pulse Controller manual for explanation).

6.3 Cuvette Geometry

Another variable affecting the resistance across the sample cuvette is the volume of electroporation medium in the cuvette. The resistance is proportional to the length of the path through the medium and inversely proportional to the cross-sectional area of the path. Gene Pulser cuvettes have a path length of either 0.4 or 0.2 cm in the lower, narrow portion of the cuvette. The cross-sectional area changes with the volume of the sample, and this changes the resistance and τ. The data in Table 2 demonstrate this effect. At volumes from 0.3 to 0.8 ml in the 0.4 cm cuvette, or at volumes from 0.15 to 0.4 ml in the 0.2 cm cuvette, τ can be predicted by

$$Volume_1/Volume_2 \times \tau_1 = \tau_2$$

At volumes less than 0.3 ml in the 0.4 cm cuvette, and 0.15 ml in the 0.2 cm cuvette, τ deviates somewhat from the value predicted by this equation because of the greater effect of the meniscus at these low volumes. The time constants reported in Table 1 were determined using a sample volume of 0.8 ml. If you wish to use a smaller volume to conserve cells or DNA, be sure to make the appropriate adjustment to the time constant values in Table 2. You can also use this effect to generate pulses with a variety of time constants.

Table 2: The Effect of Sample Volume on Time Constant*

Sample Volume (ml)	Time Constant (msec)
0.1	38.0
0.2	23.4
0.3	14.5
0.4	11.6
0.5	9.3
0.6	7.5
0.7	6.2
0.8	5.1

*Various volumes of cold phosphate buffered sucrose (272 mM sucrose, 7 mM potassium phosphate, pH 7.4, 1 mM MgCl2) were pulsed in a Gene Pulser cuvette (0.4 cm interelectrode distance) at 500 V and 25 µF. The resulting time constants are reported.

When the Gene Pulser apparatus is set at voltages greater than 1,500 V, the solution volume in the cuvette must be 0.8 ml.

6.4 Effect of Voltage on τ

In theory the voltage does not affect the τ, but in practice the situation is somewhat more complicated. The resistance of an aqueous solution varies with the potential applied across it. At higher voltages this effect is quite large. For example, the static resistance (at ~0 volts) of 0.8 ml of PBS in the Gene Pulser 0.4 cm cuvette is about 1,000 Ω, but at 2,000 volts the resistance is only 20 Ω. Similarly, the resistance of the buffered sucrose is 10,000 Ω at ~0 volts, but drops to 200 Ω at 2,000 volts. The 10-fold difference in the resistance of the two media is maintained at each voltage. An experiment which involves varying the voltage will show some change in the time constant. If it is necessary to hold τ to a constant value, the resistance of the sample must be adjusted.

6.5 Voltage and Field Strength

The voltage, or potential difference, between the electrodes of the sample cuvette, has a profound effect on the efficiency of electroporation and the survival of the cells. The voltage effect is better described in terms of the voltage gradient, or field strength (E), expressed in volts per centimeter of interelectrode distance.

The Gene Pulser cuvettes have an interelectrode distance of 0.2 or 0.4 cm; so, at a setting of 1,000 volts, E_0 would be 5,000 V/cm in the 0.2 cm cuvette (1,000 V/0.2 cm) or 2,500 V/cm in the 0.4 cm cuvette (1,000 V/0.4 cm). Since the cuvette electrodes are parallel flat plates, the field is nominally uniform. Because the electrode area is not large compared to the interelectrode distance, the field is not truly uniform, especially near the edges of the electrodes. For practical purposes, however, the Gene Pulser cuvette may be considered to produce a uniform field strength of E=Voltage/interelectrode distance.

6.6 Pulse Delivery Using Electrophoresis Power Supplies

Electrophoresis power supplies have been used to deliver electrical pulses for electroporation. A power supply can be discharged directly across a sample chamber, or it can be used to store a charge in a capacitor which is discharged across a sample.

In the power supply discharge method, the power supply is set at a certain voltage and the pulse is delivered through a mechanical switching device, or by touching the hot leads to the electrodes of a cuvette containing the cells. The major problem with this

method (aside from the obvious safety considerations) is that the voltage amplitude of the pulse actually delivered is very different from the voltage to which the power supply is set. For example, the power supply often used for this purpose, the ISCO 494, when set to 2,000 volts, delivers only 100 to 200 volts to the cuvette containing PBS.[94] Because the internal resistance of the power supply is large compared to the resistance of the sample in the cuvette, most of the voltage drop occurs across this internal resistance.

The second method, that of storing charge in a capacitor and discharging the capacitor through the sample, produces a pulse with a voltage that more closely approximates the voltage setting on the power supply (there is usually some loss caused by the inefficient mechanical switches used in some homemade devices).

The differences in the voltages applied by these two methods have led to some confusion in the literature. If an investigator using a capacitor-based device attempts to repeat the reported conditions of 2,000 V *discharged directly from a power supply,* the pulse from the capacitor device will be very different from that in the protocol he is trying to duplicate. When used for pulse delivery, the ISCO 494 behaves approximately like a circuit containing a capacitor of 30 to 70 µF in series with a 230 ohm resistor. Capacitors of the type found in power supplies are made to a very wide tolerance, so the pulses delivered with different ISCO units may vary significantly. Table 3 shows the actual voltages and time constants produced by one ISCO 494 power supply at various voltage settings.

Table 3. Characteristics of Pulses Produced with the ISCO 494 Power Supply*

ISCO 494 Voltage Setting (V)	Actual Peak Voltage (V)	Approximate Time Constant (msec)**
200	18	17
500	43	15
750	60	15
1,000	75	17
1,250	100	19
1,750	140	18
2,000	160	17

*Gene Pulser cuvettes (Potter type, 0.4 cm interelectrode distance) containing 0.8 ml of cold Dulbecco phosphate buffered saline (without Ca^{+2} or Mg^{+2}) were pulsed by touching the hot leads of an ISCO 494 power supply to the electrodes of the cuvette. Voltage and time constant were measured by examination of oscilloscope tracings.

**The decay curve generated by this arrangement is not actually exponential. The “time constant” is taken as the time for the voltage to drop to 37% of the initial voltage.

The pulses produced by this particular power supply and sample can be simulated with a Gene Pulser apparatus using the 960 µF capacitor of the Capacitance Extender, and a voltage setting of ~1/12 that of the ISCO. We would expect the pulses delivered by other ISCO units to have time constants from 5 to 30 msec using cuvettes of Potter’s design[52] containing 0.5 to 0.8 ml of PBS or other media of similar ionic strength.

Section 7
Other Variables

7.1 Cells

The cell type, the growth rate, and the growth phase of the population seem to be important variables in electroporation experiments. Both adherent and suspension cells have been successfully electroporated. Fast growing cells may be more efficiently transfected than slow growing cells. There is some evidence that transfection efficiency is cell cycle dependent. For example, incubation of the cells with colcemid before electroporation enhances transfection efficiency, suggesting that mitosis is a period of competency.[1]

In our experiments, cells are harvested at mid-log growth phase, and are washed with the electroporation medium. This wash is especially important when using a high resistance medium, such as a HEPES solution, because any salts contributed by the growth medium can greatly reduce τ. Most eukaryotic cells are resuspended in the electroporation medium to a concentration of 0.5 to 50 x 10^6/ml. (See the Pulse Controller manual for bacterial electroporation conditions.)

7.2 Electroporation Medium

The electroporation medium affects the cells in a number of ways: a) the resistance of the medium influences τ (refer to Section 6); b) the osmotic character of the medium affects the turgidity (hence the fragility) of the cells; c) components of the medium, such as divalent cations, may affect the stability of cell membranes during the pulse; or d) components of the medium may enter permeabilized cells, causing adverse effects.

Tissue culture medium has been used as electroporation medium for mammalian cells,[3,4] and for most types of cultured cells, the growth medium (±serum) is probably the medium of choice for electroporation.

7.3 DNA

The form of the DNA may influence the efficiency of electro-transfection. To obtain cells with integrated, stably maintained DNA it may be best to use linear DNA which may have higher recombinational activity. To obtain cells with transiently maintained or stable episomal sequences, circular DNA is usually used.

We have found that transfection frequency increases with DNA concentration in the range of 2 to 40 µg/ml. For stable transfections, 2 to 10 µg/ml is usually adequate; for some transient expression systems, 20 to 40 µg/ml may be necessary. Carrier DNA may also contribute to increased transfection efficiency.[15]

7.4 Temperature

In our experiments with mammalian cells, we follow the recommendations of Potter et al.[52] and incubate the cells with DNA on ice for about 10 minutes before and after applying the pulse. Others have reported electroporation of various cell types at room temperature to be quite efficient.[15]

Section 8
Typical Electroporation Protocol

We used the following protocol for electroporation of Chinese hamster ovary (CHO) cells and obtained a frequency of ~10^{-3} stable transfectants per cell. The following general procedure can be applied to other types of cells, although the optimum field strength, time constant, or electroporation medium may be different (see Appendix I for more information on electroporation of other cell types).

1. CHO cells were grown to 50-75% confluence in Hamm's F12 medium supplemented with 10% fetal calf serum and antibiotics.

2. The cells were harvested with trypsin. The trypsin was inactivated with fetal calf serum. Cells were washed twice in ice cold electroporation medium, then resuspended in the same medium at a concentration of 0.5 to 1.0 x 10^7 cells/ml. Three different electroporation media were tested:

   a. Dulbecco phosphate buffered saline (without Ca^{+2} or Mg^{+2})(PBS);

   b. Phosphate buffered sucrose (272 mM sucrose, 7mM sodium phosphate pH 7.4, 1 mM $MgCl_2$);

   c. Hamm's F12 medium (containing no fetal calf serum or antibiotics).

3. 2.5 to 40 µg of a linearized pSVneo vector and 0.8 ml of cell suspension were placed in a 0.4 cm Gene Pulser cuvette. The DNA and cells were thoroughly mixed by pipetting. This mixture was incubated for 10 minutes on ice prior to electroporation.*

4. The cuvette containing cells and DNA was placed in the Gene Pulser chamber, pulsed once, and then returned to ice and incubated for an additional 10 minutes at 0° C before plating.*

   a. Electroporation in PBS: 25 µF, voltage settings from 100 to 1,600 V (250 to 4,000 V/cm).

   b. Electroporation in phosphate buffered sucrose: 25 µF, voltage settings from 100 to 1,000 V (250 V/cm to 2,500 V/cm).

   c. Electroporation in Hamm's F12: 960 µF, voltage settings from 250 to 450 V (625 V/cm to 1,125 V/cm).

   Note: After electroporation, live cells remain permeable to viability dyes. This permeabilized state can persist for several hours at low temperatures. To accurately assess survival, one can determine plating efficiency or stain cells after they have been returned to culture for 6 to 12 hours.

5. The cells were diluted with an appropriate volume of equilibrated medium, then plated and allowed to grow 48 hours before applying selection. Selective medium contained 480 mg/ml (true concentration) G418. The selective medium was replaced every 48 to 72 hours. After 10 to 14 days of growth in selective media, the resistant colonies were fixed with 70% ethanol, stained with crystal violet, and counted. Figure 4 illustrates the dependence of transfection frequency on voltage and τ.

*Electroporation at room temperature has also been found to be effective.[15]

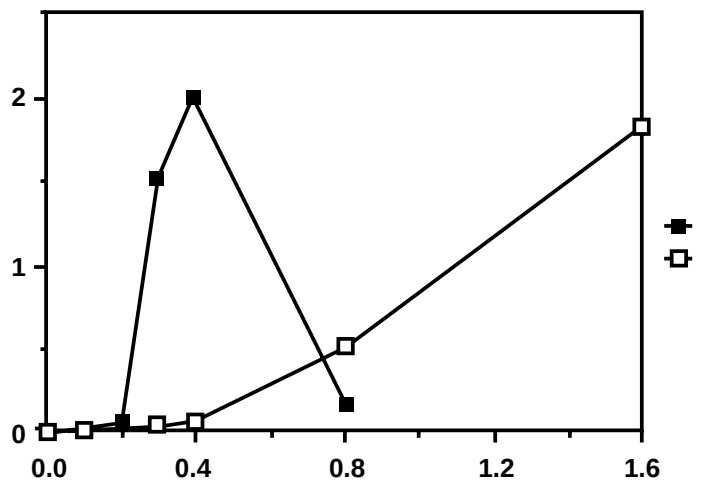

Fig. 4. Dependence of transfection frequency on voltage and time constant.

Section 9
Optimizing Electroporation Conditions

The field strength and pulse length appropriate for maximum recovery of transfectants must be determined experimentally for each cell type. If the conditions have been reported for electroporation of the cells you are interested in (or a similar cell type; size similarity is especially important), we suggest beginning with those conditions and trying several field strengths slightly above and below those reported. The conditions described for many cell types have not been optimized, and because transfection and survival are acutely dependent on the field strength, it is wise to find the flat part of the voltage-dependence curve (see Appendix I for specific conditions and references). For most cultured cells, we recommend conditions of low field strength, medium-to-long time constants, and cells suspended in a physiological salt solution Good starting conditions are pulse time constants of 5 to 20 msec, obtained by suspending the cells (10^6 to 10^7/ml) in 0.8 ml of PBS or growth medium (±serum) in a 0.4 cm cuvette, and using a 250, 500, or 960 µF capacitor. At these pulse lengths, the optimal field for animal cells and plant protoplasts is usually between 500 and 1,000 V/cm. The voltage-dependence curve can be rather narrow and it is necessary to vary the voltage in small increments of ~100 V/cm to find the peak. Cell death of from 20 to 80% will accompany effective electro-permeabilization, but the best voltage cannot be predicted by cell death alone. This is demonstrated by Chu et al.[15] where the amount of cell death at the field strength giving peak transfection varied considerably among the eight cell lines studied.

Note that after electroporation, the cells remain permeable to some viable dyes, and this permeabilized state can persist at low temperatures. To accurately assess cell survival, either determine the plating efficiency or return the cells to the culture temperature for several hours before staining with viable dyes.

Appendix I
Electroporation of Various Cell Types

This appendix contains a list of references describing electroporation conditions for a number of cell types. In many cases, the conditions described are not necessarily optimal, and improved transfection frequencies might be achieved by starting with the published conditions and following the optimization procedure described in Section 9 to fine-tune the method for the cells you are using.

The studies listed have used a variety of pulse generating devices, and determining the settings to produce a comparable pulse with the Gene Pulser apparatus sometimes requires some deduction. Here, we provide some guidance in making the necessary adjustments.

First, determine the type of waveform used. Was the pulse of exponential decay (as generated by capacitor discharge), or another waveform (square, sine, etc.)? If the latter, see Section D of this appendix. If the pulse was exponential (capacitor discharge), determine the type of device used. If it was a Gene Pulser apparatus, refer to Section A. If it was a commercial capacitor discharge device (other than a Gene Pulser apparatus), or a homemade device, see Section B. If the pulse was produced by the discharge of a power supply directly to the sample, refer to Section C.

A. Reports Using a Gene Pulser Apparatus

It is a straightforward matter to determine the appropriate instrument settings from most reports that have used the Gene Pulser apparatus. If the medium, volume, and cuvette type are described, and the settings for voltage and capacitance are given, it is a simple matter to duplicate these. If the time constant is also reported, you can verify the accuracy of your duplication by pressing TIME CONST on the face of the Gene Pulser apparatus after the pulse.

If the medium, field strength, and time constant are provided, the instrument settings are easily deduced as follows:

voltage setting (kV)=field strength (kV/cm) x electrode gap (cm).

The cuvettes with a 0.4 cm gap are usually used for eukaryotic cells, so

(0.4 cm) (field strength kV/cm)

gives the proper voltage setting. To select the appropriate capacitor:

capacitor setting=τ/R.

R, the resistance of the sample, can be estimated if the medium, volume, and cuvette dimensions are known. Usually a medium based on saline is used (PBS, DMEM, etc.), and we have defined as “standard” conditions 0.8 ml of PBS in a 0.4 cm cuvette. At greater than 200 V, this sample has a resistance of about 20 Ω. The resistance of most samples can be estimated by comparison with this standard sample. For example, halving the volume to 0.4 ml will approximately double the resistance. Tables 1 and 2 are helpful in estimating the resistance of various samples.

Once the resistance is approximated, a particular time constant can be obtained by capacitor selection. For example, if a time constant of 10 msec is desired with a standard 20 Ω sample,

$\tau/R = 10 \times 10^{-3}$ sec/20 Ω = 0.5×10^{-3}F = 500 µF

The 500 µF capacitor would be chosen. Notice that the 10 msec pulse can be obtained in several ways. One alternative would be a 0.4 ml sample (40 Ω) with the 250 µF capacitor.

B. Reports Using a Capacitor Discharge Device (Commercial or Homemade) Other than the Gene Pulser Apparatus

Determining the field strength is usually a simple matter since most authors report either the field strength or the voltage applied and the electrode gap. (Be aware that there are some electrode arrangements in use that produce non-uniform fields so that cells in different locations experience different field intensity. A wire and concentric ring electrode is an arrangement producing a non-uniform field.)

Determining the correct time constant is often more difficult because most instruments do not provide this information and, unless the author has measured it with an oscilloscope, the time constant will not be known. In this case, if the author has described the medium, sample volume, and electrode gap, then the resistance of the sample can be estimated by comparison to our "standard" 20 Ω sample in a manner similar to that described in Section A. As an example, 0.5 ml of PBS is placed between electrodes 1 cm apart. The cross-sectional area of the sample is 0.5 cm^3/1 cm = 0.5 cm^2. Our standard sample is 0.8 ml between electrodes 0.4 cm apart, and has a cross-sectional area of 0.8 cm^3/0.4 cm = 2 cm^2. Because the resistance is inversely proportional to the cross-sectional area, the resistance of the first sample is increased 4-fold over the standard sample. Additionally, because the resistance is approximately proportional to the path length between the electrodes, the resistance of the first sample is increased another 2.5-fold (1 cm/0.4 cm) because of the greater inter-electrode distance. In this example, the first sample has a resistance about 4 x 2.5 = 10-fold greater than the standard, or about 200 Ω (10 x 20 Ω).

If the authors reported using a 25 µF capacitor to discharge through this hypothetical sample, they would have produced a pulse with a time constant of

$$(200\ \Omega)\ (25 \times 10^{-6} F) = 5 \times 10^{-3} \text{ sec} = 5 \text{ msec.}$$

A comparable pulse length with our standard sample would be obtained with a 250 µF capacitor.

Note: There are some precautions required in deducing pulse conditions produced with some commercial and homemade devices.

1) Some instruments have a wave truncation feature whereby the exponential decay wave is interrupted at a selected time. This truncation time is not the time constant. Using the wave truncation feature, the shape of the waveform can be varied from a nearly square wave to almost a complete exponential decay, depending on what point the decay curve is interrupted. Since this is actually a waveform different from exponential, see Section D.

2) At least one commercial capacitor discharge device has a "time constant setting" control. This control does not set the time constant, but rather selects a resistor in parallel with the sample which, if it were the only resistance in the circuit, would give the stated time constant. The actual time constant obtained with this arrangement is dependent on the conductivity of the medium, and with the media usually used with cultured cells, the pulse length can be quite different from (less than) the setting. If you are trying to emulate the conditions from a report where this time constant setting is given, and your results are poor, try reducing the (true) time constant produced with the Gene Pulser apparatus 2- to 10-fold.

3) Some homemade units use mechanical switching devices that may degrade with use. This can introduce a higher resistance in the circuit than would be found in a commercial unit and, when used with conductive media, may cause a voltage loss. In this case, the voltage actually applied to the sample will be less than the voltage to which the capacitor is charged.

C. Reports Using Pulses Applied with a Power Supply

Emulating the pulse produced by discharging a power supply directly to a sample is complicated by the rather large effect on the voltage of changes in sample resistance. It is further complicated because the capacitance can vary significantly from one power supply to another. These effects are explained in more detail in Section 6.6 of this manual and by Fromm et al.[94] Because of these complexities, it is more useful to give some general suggestions rather than detailed step-by-step instructions for interpreting the reported pulse conditions. With the power supply most commonly used, the ISCO 494, the pulse delivered to a Potter-type cuvette containing PBS has an actual amplitude of about 10% of the voltage set on the power supply, and has a time constant of 10 to 30 msec. (Electrodes of different geometry or media of different conductivity can produce pulses of quite different voltage and time constant.)

Our general recommendation to produce pulses similar to those obtained with a power supply discharge is to try pulses of 20 msec with field strengths of 500 to 1,000 V/cm in ~100 V/cm increments (see the optimization procedure in Section 9 of this manual). With a Gene Pulser apparatus and Capacitance Extender, this may be accomplished with 0.4 ml of PBS or tissue culture medium in a 0.4 cm cuvette with a 500 µF capacitor (giving a pulse length of 20 msec), and voltage settings of 200, 250, 300, 350, and 400 V (giving fields of 500, 625, 750, 875, and 1,000 V/cm, respectively).

D. Reports Using a Waveform Other than Exponential Decay

The second most commonly used waveform is square. Square wave pulses used for electroporation can be grouped in two general categories: very high field strength, very short time constant; and low field strength, medium-to-long time constant. The first category includes pulses of 5 to > 20 kV/cm with time constants of 50 to 100 µsec; the second category includes pulses of up to ~2 kV/cm with time constants in the range of 1 to > 100 msec. The relative effectiveness of square and exponential waveforms is not understood, but some information indicates that square and exponential pulses of similar duration and amplitude are comparable in their effect on the cells.[38] The correct translation of square wave width to exponential time constant is not at all clear. A rough guide is to use an exponential time constant 2 to 3 times the square wave width. Even less is known about other waveforms. Obviously some time spent in reoptimizing the electrical conditions is necessary when changing waveforms.

E. Eukaryotic Cell Electroporation References

Refer to the previous portion of this Appendix for more information on how to interpret the electroporation conditions cited, and how to translate these into conditions for use with the Gene Pulser apparatus.

Mammalian Cells	References
3T3-L1 preadipocytes	17
FM3A	26
Hu11 (mouse erythroleukemia x human fibroblast)	67
Bovine adrenal medulla	5
Bovine endothelium	56
Chinese hamster ovary	31, 79, 87
Feline embryo fibroblast	50
Human B	7, 8, 16, 18, 24, 32, 34, 40, 41, 52, 53, 54, 57, 65, 66, 72
Human T	6, 27, 54
Human bone marrow	78
Human peripheral blood lymphocytes	13
Human carcinoma (HeLa)	15, 28, 43, 44, 86
Human carcinoma (HEp-2, HeG2)	34, 73
Human erythrocytes	64
Human erythroleukemia	33, 54, 61, 68, 79
Human fibroblast	11, 15, 69
Human macrophage	6
Human melanoma	80
Monkey kidney (COS, CV-1)	15, 44, 89
Mouse pre-B	1, 9, 52, 53, 59, 60, 84
Mouse B	52, 53, 7, 79
Mouse L	37, 47, 49, 52, 53, 70, 81
Mouse T	2, 19, 35, 42, 52, 53
Mouse bone marrow	23, 48
Mouse embryo fibroblast (NIH3T3)	15
Mouse embryo stem	20, 75
Mouse erythroleukemia	36, 55, 61, 68
Mouse fibroblast (10T 1/2)	38
Mouse hybridoma	71
Mouse keratinocytes	58
Mouse kidney	44
Mouse myeloma	3, 4, 21, 25, 39, 42, 44, 46, 62, 88
Mouse pituitary	29
Osteosarcoma	53
Rat cardiocyte	63
Rat hepatoma	53
Rat primary hepatocyte	76
Rat islet	30, 51, 82, 85
Rat macrophage	83
Rat neuronal line	14, 22, 45, 53
Rat parathyroid	53
Rat pituitary	28

Plant Protoplasts	References
Carrot	89, 93, 94, 99, 102, 103, 113
Maize	89, 94, 95, 114
Mung bean	98
Oat mesophyll	106
Panicum maximum	99
Pennisetum (several species)	99
Petunia	99
Rice	111
Saccharum officinarum	99
Sorghum	111
Soybean	90, 91, 104
Tobacco	88, 92, 94, 96, 97, 100, 101, 102, 105, 108, 109, 110, 112, 115, 116, 117
Tomato	107
Triticum monococcum	99
Wheat	111

Other Eukaryotes	
Dictyostelium	119
Sea urchin egg	121
Yeast	118, 120

Mammalian Cells

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Appendix II
Troubleshooting Guide for the Gene Pulser Apparatus and Capacitance Extender

Problem	Likely Cause and Solution
1. Display does not light when unit is turned on.	Power is not supplied to electronics. Check power cord and wall outlet power source. Check that power switch is on. Check/replace fuse.
2. Apparatus displays 'no' when pulse buttons are pressed.	Voltage setting is <0.05 kV. The unit will only deliver pulses ≥0.05 kV. Adjust voltage to 0.05 kV or higher.
	Capacitor select knob at "EXT" position, but Capacitance Extender is not properly connected to the Gene Pulse apparatus. Check red, black, and gray cables for proper connection between the Gene Pulser apparatus and the Capacitance Extender.
	Incomplete power circuit. Make sure cuvette electrodes make contact with electrical contacts inside the Gene Pulser chamber.
	Empty cuvette. Gene Pulser apparatus will not deliver a pulse if no load is detected. Place cell suspension in cuvette.
3. When the buttons are pressed, the unit continues to flash 'Chg', but the tone does not sound, or the display goes blank.	No pulse delivery. Pulse buttons are not depressed long enough. Turn power switch on and off twice to bleed any residual charge in the capacitor.
	Large capacitors require a longer charge time (~15 seconds). Keep both pulse buttons depressed while 'Chg' flashes in the display. The pulse buttons should not be released until the tone has sounded.
	Only one pulse button is depressed. Both of the red pulse buttons must be pressed for pulse delivery until the tone sounds.
	Sample resistance is too high. The Gene Pulser apparatus or Gene Pulser apparatus with the Capacitance Extender will not deliver a pulse if

Problem	Likely Cause and Solution
	sample resistance exceeds several thousand ohms. Lower the sample resistance (e.g., increase ionic strength of the electroporation medium). [Use the Gene Pulser apparatus with the Pulse Controller to deliver pulses into high resistance media.]
4. Time constant display shows 'no'.	The pulse is delivered, but the time constant is too short (<10 µsec) to be measured by the apparatus. To obtain a longer time constant, select a larger capacitor or higher resistance media.
5. Unit displays nonsense characters when time constant is displayed.	Pulse time constant was longer than 1.0 second.
6. Arcing in the cuvette.	Use caution when delivering high voltages into conductive media. Never deliver greater than 2,000 V to conductive media (PBS or tissue culture media) in the 0.4 cm cuvette. If arcing occurs, lower the voltage. **Repeated arcs will damage the instrument**. To deliver pulses between 1,500 and 2,000 V to conductive media, always use the full 0.8 ml in the 0.4 cm cuvette. Never use the 0.2 cm or 0.1 cm cuvette at greater than 1,000 V without the protection of the Pulse Controller.

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