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Bacterial Electro-transformation

and

Pulse Controller Instruction Manual

Catalog Number 165-2098

Version 8-90

Table of Contents

Introduction

The Pulse Controller unit is used with the Gene Pulser apparatus for the electroporation of bacteria, or for 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 it is 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 voltages, 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 of less than 1,000 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 when pulses are applied to low resistance samples. The voltage loss is insignificant with high resistance samples (see Section 3 for a detailed discussion of this effect).

Section 1
Safety Precautions

READ THIS INFORMATION CAREFULLY BEFORE USING THE PULSE CONTROLLER WITH THE GENE PULSER APPARATUS

1.1 Electrical Hazards

The Gene Pulser apparatus produces voltages up to 2,500 volts, and is capable of passing very high currents. The 25 microfarad (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 discharging (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 safely 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. The sample chamber is effective in containing these small explosions, but 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). Operation in anaerobic atmospheres containing H_2 is also a potential explosion hazard.

1.3 Biological Hazards

Consideration of biological hazards is especially important for bacterial electroporation, where high voltage is used and arcing is more likely to occur. Electroporation of pathogenic bacteria should **always** be performed under containment appropriate for the organism (such as a biohazard hood). If an arc occurs, even if the cuvette remains intact, the sample may be aerosolized and may cause contamination.

In general, use of the Pulse Controller greatly reduces the incidence of arcing, but arcing can occur (it is somewhat dependent on the resistance of the sample) and the precautions described above should always be observed.

1.4 Other Safety Precautions

Always use the Pulse Controller with the Gene Pulser apparatus to produce fields greater than 5 kV/cm across high resistance media. If the Pulse Controller is not used, arcing may occur and may cause severe damage to the Gene Pulser apparatus.

Do not apply fields greater than 4 kV/cm across low resistance electroporation media (e.g. buffered saline solutions).

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 of the Gene Pulser manual 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.

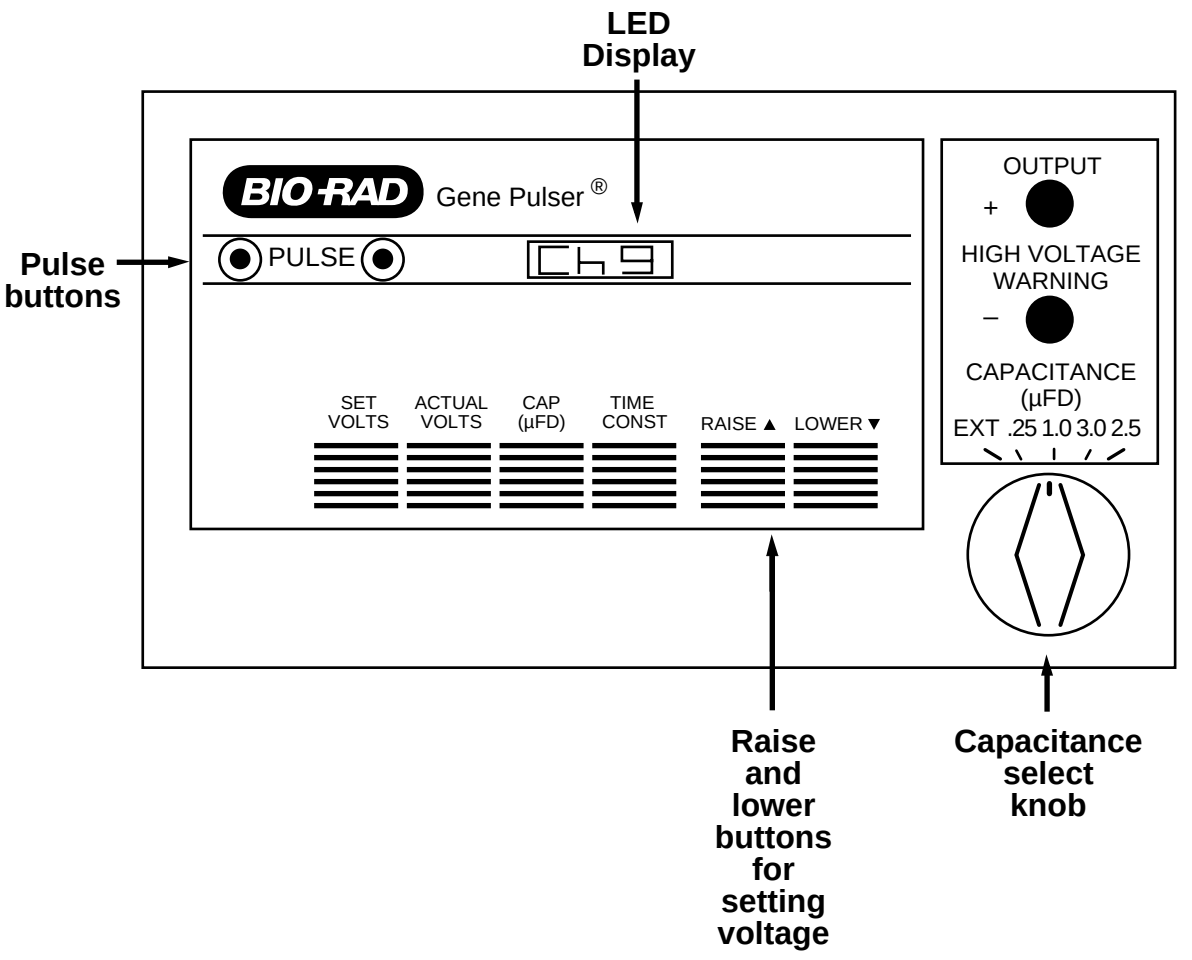

Fig. 1. The Gene Pulser apparatus.

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.

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,** 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
Pulse Controller

3.1 Description

The Pulse Controller is installed between the output of the Gene Pulser apparatus and the sample chamber, as shown in Figure 2. The multi-position switch on the front of the Pulse Controller determines which of six resistors (100, 200, 400, 600, 800, or 1,000 Ω) is placed in parallel with the sample chamber. When this resistor is much smaller than the resistance of the sample, it is the primary determinant of the resistance of the circuit. This resistance and the size of the capacitor determine the length of the pulse (see Table 1).

For example, selecting the 25 µF capacitor and the 200 Ω parallel resistor (with a high resistance sample as described in Section 6 for bacterial electroporation), the RC time constant = R x C = (200 ohms) x (25 x 10^{-6} farads) = 5 x 10^{-3} sec = 5 msec (see Section 5 for a detailed description of the electrical variables). With the Pulse Controller, each capacitor can be used to produce time constants over a 10-fold range. The ∞ resistance setting means that an open circuit exists in parallel with the sample and all the charge will pass through the sample. This setting is not recommended for most purposes, because arcing is much more likely to occur.

The Pulse Controller is not meant for use with samples of low resistance (less than 1,000 Ω; e.g. buffered saline solutions, buffered sucrose solutions, or tissue culture media). This is because the unit contains a 20 Ω resistor, R_{20}, in series with the sample, and this resistor will decrease the voltage applied to the sample by $[R_{20}/(R_{20} + R_{sample})]$. The resistance of a sample consisting of 0.8 ml of PBS in a 0.4 cm cuvette is about 20 Ω. The error in the applied voltage that would result from using the Pulse Controller with this sample is

$$\frac{20\ \Omega\ (\text{resistor})}{20\ \Omega_{(\text{sample})} + 20\ \Omega\ (\text{resistor})} = 0.5 = 50\%$$

In this example, half of the voltage drop would occur at the resistor in series, and only half would be applied across the sample. No significant error in voltage occurs if the sample resistance is large compared to the 20 Ω resistor. For example, the high resistance samples used for electroporation of bacteria are ~2,000 to 5,000 Ω. The error in voltage applied to these samples would be less than 1%.

3.2 Operating Instructions

1. First connect the double male leads to the input jacks on the front panel of the Pulse Controller, and then to the output jacks on the front panel of the Gene Pulser apparatus. **Always connect the two units in this order. It is a safety hazard to leave double male leads attached to the Gene Pulser apparatus with the output plugs exposed** (i.e., unattached to an accessory). Be sure to observe polarity (red to red, black to black). Finally, connect the leads from the sample chamber to the output jacks on the front panel of the Pulse Controller.

2. Turn on the power switch on the upper left of the back panel of the Gene Pulser apparatus.

3. Select a capacitor and set the desired voltage on the front panel of the Gene Pulser apparatus.

4. Select the appropriate parallel resistor with the multi-position switch on the front of the Pulse Controller.

5. Follow steps 6-9 in Section 2 for pulse delivery and shut-down of the instrument. At higher voltages, the sample will "twitch," indicating a pulse has passed through the sample (and assuring you that the connections between the Gene Pulser and Pulse Controller are correct).

6. Disconnect in reverse order after fully discharging the capacitors.

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 is displayed in kilovolts (kV).

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 (t).The RC time constant is measured and displayed in milliseconds (msec). Equal to resistance x capacitance, t is the time required for the peak voltage to decay to approximately 37% of the initial voltage (see Figure 3). If t is between 0.01 and 0.1 msec, it is displayed as 0.1 msec. If a pulse less than ~0.01 msec is delivered (due to very low resistance electroporation media, small capacitance, or for other reasons), 'no' will be displayed.

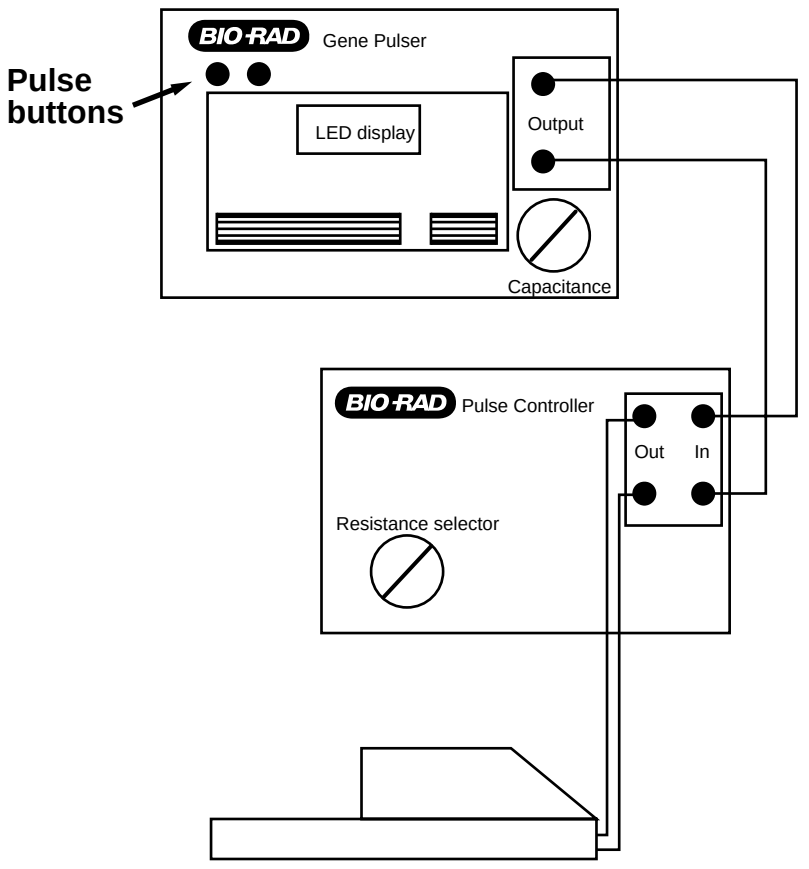

Fig. 2. Connections between the Gene Pulser apparatus, the Pulse Controller, and the Shocking Chamber.

Section 5
Electrical Variables

The capacitor discharge circuit of the Gene Pulser apparatus generates an electrical pulse with an exponential decay waveform (Figure 3). 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, V0), 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.

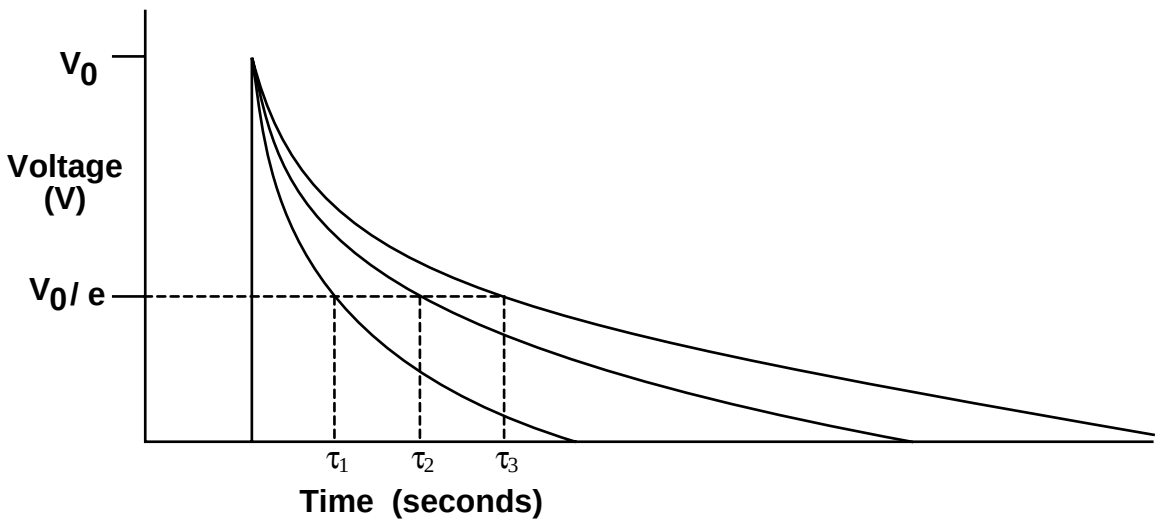

Fig. 3. Exponential decay waveform.

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). 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.
For bacterial electroporation, a different electrical arrangement is required to prevent arcing at the extremely high field strengths used. In this configuration, the Pulse Controller shunts part of the energy around the sample and through one of six resistors placed in parallel with the sample (Figure 4). With samples prepared as described in Sections 6 and 7 of this manual, the parallel resistance is much smaller than the resistance of the sample and determines the total resistance of the circuit. This allows control of the time constant by the choice of the parallel resistor (see Equation 3). Table 1 shows the approximate time constants obtained with various combinations of capacitors and resistors.

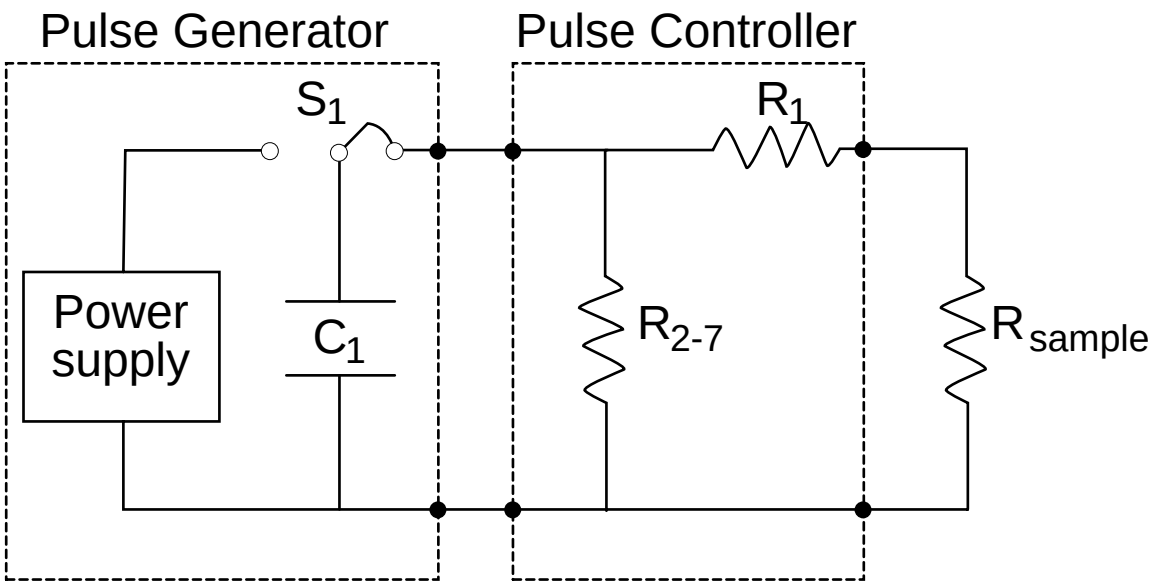

Fig. 4. Electrical circuit for bacterial electroporation. The pulse discharged from the capacitor (C_1) is directed through the small current-limiting resistor (R_1) placed in series with the sample (R_{sample}), and one of six resistors (R_{2-7}) placed in parallel with the sample.

An additional function of the Pulse Controller circuit is to place a 20 Ω resistor in series with the sample. This protects the Gene Pulser circuit by limiting the current should an arc occur. With a bacterial sample prepared as described in Sections 6 and 7, the resistance is about 5,000 Ω; therefore, during normal operation (no arc) the voltage drop across the 20 Ω protective resistor will be less than 1%. However, if this circuit is used with samples of the much lower resistance typical of eukaryotic electroporation (20-200 Ω), the voltage lost across the protective resistor becomes highly significant. **For this reason, do not use the Pulse Controller with samples of less than 1,000 Ω.**

Table 1. Approximate Time Constants Produced with Various Combinations of Capacitors and Resistors[c]

	Time Constants (msec) with Various Capacitors			
	0.25 µF	1.0 µF	3.0 µF	25 µF
Resistor (ohms)				
100	0.025[a]	0.1	0.3	2.5
200	0.05[a]	0.2	0.6	5
400	0.1	0.4	1.2	10
600	0.15	0.6	1.8	15
800[b]	0.2	0.8	2.4	20
1,000[b]	0.25	1.0	3.0	25

a) Pulses of less than 0.1 msec are displayed as 0.1 on the Gene Pulser apparatus LED.
b) With the larger resistors, the pulse length may be more greatly affected by the sample resistance. The actual pulse lengths may be about 20% lower than those shown in this Table.
c) A high resistance sample was prepared as described in Table 2.

Section 6
Bacterial Electroporation (Electro-transformation)

The Pulse Controller is used with the Gene Pulser apparatus for the electro-transformation of prokaryotes. In particular, the Pulse Controller is required when high voltages, narrow electrode gaps, and small-volume samples with high resistance are used. (The Pulse Controller may also be used for electroporation of eukaryotes, but certain limits on sample conductivity apply.)

This section discusses practical aspects of electroporation of prokaryotes (see Appendix I for a list of representative electrical conditions for electroporation of a variety of bacterial species). Section 7 discusses the transformation of *E. coli* to extremely high frequencies and efficiencies, and provides detailed protocols for preparing and transforming the cells.

A common limitation in studies of the molecular genetics of many prokaryotes is the lack of convenient and reliable gene transfer systems. For many bacteria, electroporation may provide such a system. Recently, intact bacteria of many species have been transformed by electroporation.[1-11] Two species in particular have served as models for the analysis of some of the practical aspects of electroporation: *Campylobacter jejuni* [8] and *E. coli*.[2] From data we have gathered on these, we can make some general comments that may be applicable to many bacterial species.

6.1 Growing and Harvesting Cells

With some species, the growth phase at which the cells are harvested is related to their competence in electro-transformation. The period of greatest competence for *E. coli* is early- to mid-logarithmic phase, with a significant reduction as the cells move into late log. The particular growth conditions may also influence the competence of some bacteria. For example, *Campylobacter* grown on plates are electro-transformed much more efficiently than those from broth cultures.[8] Therefore, if first attempts at electroporation of a particular species are unsuccessful, alternative growth conditions may be required. In general, we use conditions where the cells are still growing rapidly at densities (and volumes) high enough to recover very large numbers of cells.

Most species are probably best harvested by chilling and centrifuging. For electrical purposes, it is very important to reduce the ionic strength of the cell suspension. Washing several times with very low salt buffer accomplishes this. If the cells you are using do not pellet easily (such as some filamentous types), or are damaged by centrifugation, filtration may be a useful alternative. Processing the cells in the cold may not be necessary. Some species of cold labile prokaryotes have been successfully electro-transformed after preparation at room temperature. Many cell types can be prepared in bulk and stored frozen in glycerol at -70° C. Recovery from freezing varies with cell type and the initial experiments are probably best done with fresh cells.

With *E. coli* and some other species we have found that increasing the cell concentration increases the yield of transformants; therefore we recommend resuspending the cells to a very high concentration. Note that for cells that secrete nucleases, high cell concentration may exacerbate any problem with DNA degradation.

The structures enveloping the cells may have a profound effect on their susceptibility to electroporation. In some cases it may be necessary to reduce or remove such structures. This might be done by altering growth conditions, or by chemical or enzymatic treatment of the cells.

One technique that may be generally useful in enhancing transformation of gram positive organisms is a very gentle digestion of the cell wall. This approach, which renders the cells osmotically fragile, but does not require the lengthy regeneration typical of protoplasts, has been used to improve the electroporation of some species of *Streptococcus.*[9]

6.2 DNA

DNA introduced into bacteria by electroporation is more vulnerable to restriction nucleases than that transferred by conjugation or natural transformation systems. [In fact, once conditions have been established, electroporation of unmodified DNA can be used as an indicator of the presence of restriction systems.[8]] Because of the likelihood of restriction barriers, we strongly recommend that initial attempts at electroporation of a species be done with DNA isolated from that species. If such DNA is not available, then high concentrations (> 10 µg/ml) of unmodified DNA should be used to increase the chances of detection of transformation. A 10^3- to 10^4- fold decrement in efficiency is likely with unmodified DNA. For many purposes (subsequent to optimizing conditions) the use of DNA cycled through the recipient strain is not feasible. Unfortunately we do not yet have a good general strategy for circumventing restriction barriers.

We have examined the effect of size and topology of the plasmid DNA on electro-transformation of *E. coli.* Supercoiled and relaxed circular forms of plasmids up to at least 20 kb transform with the same efficiency. Plasmids of 20 kb transform with the same molar efficiency as plasmids of 3 to 5 kb (Dower, W. J., unpublished data). Linear DNA is about 10^4- fold less active than the corresponding circular plasmid in the *rec* BC+ strain of *E. coli* that we use. We have not yet tested the activity of linear DNA with *rec* BC sbc hosts. Some species of bacteria have been transformed at high efficiencies with larger plasmids of up to 40 to 50 kb.

The concentration of DNA greatly affects the recovery of transformants. With *E. coli*, the frequency of transformation (transformants/survivor) is strictly dependent on DNA concentration over at least six orders of magnitude (10 pg/ml to 7.5 µg/ml). At the higher DNA concentrations, up to 80% of the survivors are transformed.[2] Because the number of transformants recovered is the product of the frequency and the number of cells present, the efficiency (transformants/µg DNA) increases with cell concentration over the range of 10^9 to at least 3×10^{10} cells/ml. To obtain high **frequencies,** we use high DNA concentration. To obtain high **efficiencies,** we use high cell concentration (and low DNA concentration to avoid co-transformations). In each case, a small sample volume (20-50 µl) allows economical use of DNA and cells. (See reference 2 for a detailed discussion of these factors.)

An important technical consideration in preparing DNA for use in high voltage electroporation is the ionic strength of the solution. Cesium chloride in a plasmid preparation or residual ammonium acetate from ethanol precipitation, for example, can cause arcing and should be reduced to 10 mM or less. DNA dissolved in TE (10 mM, 1 mM) is fine as long as it is diluted about 10-fold with the cell suspension. DNA used directly from various enzyme reactions also works, but the final salt concentration in the electroporation sample should be kept below ~5 meq for high voltage operation.

6.3 Electroporation Medium

The electroporation medium will usually be primarily composed of the solution used to resuspend the cells, with some contribution from the cells themselves and the DNA solution. The best medium for most cells is probably one of the lowest ionic strength compatible with the well-being of the cells. The need for buffering has not been studied, but will probably vary among species.

For cells that can be stored frozen, the use of glycerol as a cryoprotectant is perfectly compatible with electroporation of some species. The effects of divalent cations differ among species. Electro-transformation of *Campylobacter* is strongly inhibited by Ca^{2+}, Mg^{2+}, or Mn^{2+} at less than 1 mM,[8] while *E. coli* transformation seems unaffected by a concentration of several millimolar of these cations.

Many prokaryotes have been electro-transformed to high efficiencies in the presence of either 1 mM $MgCl_2$ or no added divalent cations. It is not yet known if any species **require** divalent cations for electroporation. As with any ionic constituent, addition should be limited to a few meq so as not to exceed the conductivity threshold, where arcing becomes a problem.

6.4 Electroporation Conditions

A major difference between the electroporation of eukaryotes and prokaryotes is the high electrical field required for the latter. In our experience, only fields greater than 3 kV/cm with pulse lengths of ~5 to 10 msec are effective with bacteria. For some species, fields greater than 10 kV/cm are most effective. This requirement for high field strength is primarily due to the small size of most bacteria. For some of the larger species, optimal fields are intermediate between those effective for eukaryotes and for most prokaryotes. The Gene Pulser apparatus will produce field strengths up to 12.5 kV/cm when used with the narrow gap, 0.2 cm electroporation cuvettes.

Pulse lengths from 2 to 20 msec are quite effective for many cell types, and the pulse length seems not to be as critical as field strength in its effect on transformation. Our recommendation for initial attempts to electroporate a species is to begin with a time constant of ~5 or 10 msec and to vary the voltage to find the appropriate field strength. A 5 msec pulse is obtained with the Gene Pulser apparatus set at 25 µF and the Pulse Controller set at 200 ohms (with a sample of high resistance). See Table 1 for the approximate time constants available with other combinations of capacitors and resistors.

For bacterial electroporation, small volumes are generally best. With the 0.2 cm cuvette, our standard volume is 40 µl (up to 400 µl may be used in this cuvette). This permits economical use of cells and DNA. An additional benefit of the small sample volume is that with a smaller cross-section, it provides the higher resistance required for high voltage operation.

With a 40 µl sample, shake the sample to the bottom of the cuvette before pulsing, and recover the sample by washing with 1 ml of outgrowth medium from a Pasteur pipette.

Electroporation at low temperature (0-4° C) seems to work best. With *E. coli* and *C. jejun*i, using our electrical conditions, efficiencies drop ~100-fold when the cells are pulsed at room temperature. Our general recommendation is to mix the DNA with a cold cell suspension and to transfer to a cold cuvette (the 0.2 cm cuvette equilibrates quickly because of the solid aluminum electrodes). The slide that holds the cuvette may also be chilled by holding it in ice between pulses. Cells that do not tolerate the cold may be pulsed at room temperature.

Incubating *E. coli* cells with DNA for more than a minute before pulsing does not improve transformation efficiency. For this species, binding of the DNA to the cells is probably not necessary. Since the lack of binding would seem to be a fundamental feature of the mechanism of electro-transformation, it is likely that pre-incubation of cells and DNA is not necessary for other species either. In some cases, pre-incubation of the cells and DNA is clearly detrimental, probably because of secretion of nucleases by the cells.[1] Therefore, we recommend adding the DNA to the cells in a cold polypropylene tube, mixing well, transferring to a cold cuvette, and applying the pulse.

The period between applying the pulse and transferring the cells to outgrowth medium is **crucial** for recovering *E.coli* transformants. Delaying this transfer by even 1 minute causes a 3-fold drop in transformation. This decline continues to a 20-fold drop by 10 minutes. With *E. coli*, we transfer the cells to medium as soon as possible after the pulse. Until data for other cell types is available, we recommend the same for all other species. As a rough rule of thumb, an outgrowth (or expression) period of one or two doubling periods is probably adequate, although some species may require an even longer recovery.

Appendix I lists conditions that have been used for the electro-transformation of several bacterial species. These conditions have not necessarily been optimized. For organisms as yet untried, use the procedures for *E.coli* transformations described in the next section and modify according to the principles discussed in this section.

We are interested in hearing of additional species transformed by electroporation, and including this information in subsequent versions of this manual. Please contact your local Bio-Rad representative, or in the U.S., call our Technical Services at (800) 424-6723 with any comments or questions.

Section 7
High Efficiency Electro-transformation of E. coli

Electroporation provides a method of transforming *E. coli* to efficiencies greater than available with the best chemical methods. By subjecting mixtures of cells and DNA to exponentially decaying fields of very high initial amplitude, we routinely obtain 10^9 to 10^{10} transformants/µg of DNA with various strains and several plasmids. The survival and transformation of cells are related to the intensity of the field (field strength = voltage/distance between electrodes) and to the length of the pulse (RC time constant).

We have found several combinations of field strength and pulse length that produce extremely high efficiencies of transformation. For example, with pulses of 20 msec we see a steep increase in transformation as we increase the field from 1 to 7 kV/cm, and then a sharp decline with further increases in field strength as cell survival drops. With pulses of 5 msec, transformation rises to a maximum at a field strength of about 11 kV/cm. In each case, transformation efficiency is in the range of 10^9 to 10^{10}, depending on the host strain and plasmid, and is reached when 30 to 40% of the cells survive. Thus, we can compensate for shorter pulses by increasing the amplitude of the field. The range of this compensation is limited, however. We have not obtained high efficiency transformation with fields of less than 3 kV/cm, even with very long pulses.

The details of these experiments are presented in reference 2. Protocols for preparing and electro-transforming *E. coli* to high efficiencies are described in Table 2.

Table 2. Procedure for High Efficiency Electro-transformation of *E. coli*

A. Preparation of Cells

1. Inoculate 1 liter of L-broth[a] with 1/100 volume of a fresh overnight culture.

2. Grow cells at 37° C with vigorous shaking to an ABS_{600} of 0.5 to 1.0 (the best results are obtained with cells that are growing rapidly; the appropriate cell density, therefore, depends on the strain and growth conditions).

3. To harvest, chill the flask on ice for 15 to 30 minutes, and centrifuge in a cold rotor at 4000 x g_{max} for 15 minutes.

4. Remove as much of the supernatant (medium) as possible. Resuspend pellets in a total of 1 liter of cold water. Centrifuge as in step 3.

5. Resuspend in 0.5 liter of cold water. Centrifuge as in step 3.

6. Resuspend in ~20 ml of cold 10% glycerol. Centrifuge as in step 3.

7. Resuspend to a final volume of 2 to 3 ml in cold 10% glycerol. The cell concentration should be about 1 - 3 x 10^{10} cells/ml.

8. This suspension may be frozen in aliquots on dry ice, and stored at -70° C. The cells are good for at least 6 months under these conditions.

B. Electro-transformation

1. Gently thaw the cells at room temperature and place them on ice.

2. In a cold, 1.5 ml polypropylene tube, mix 40 µl of the cell suspension with 1 to 2 µl of DNA (DNA should be in a low ionic strength buffer such as TE[b]). Mix well and let sit on ice ~0.5 - 1 minute.

3. Set the Gene Pulser apparatus at 25 µF and 2.5 kV. Set the Pulse Controller to 200 Ω.

4. Transfer the mixture of cells and DNA to a cold, 0.2 cm electroporation cuvette, and shake the suspension to the bottom of the cuvette. Place the cuvette in a chilled safety chamber slide. Push the slide into the chamber until the cuvette is seated between the contacts in the base of the chamber.

5. Pulse once at the above settings. This should produce a pulse with a time constant of 4 to 5 msec. (The field strength will be 12.5 kV/cm.)

6. Remove the cuvette from the chamber and immediately add 1 ml of SOC[c] medium to the cuvette and quickly resuspend the cells with a Pasteur pipette. (This rapid addition of SOC after the pulse is very important in maximizing the recovery of transformants.)

7. Transfer the cell suspension to a 17 x 100 mm polypropylene tube and incubate at 37° C for 1 hour. (Shaking the tubes at 225 RPM during this incubation may improve the recovery of transformants.)

8. Plate on selective medium.

[a]L-Broth: 1% Bacto tryptone, 0.5% Bacto yeast extract, 0.5% NaCl.

[b]DNA containing too much salt will make the sample too conductive and cause arcing at high voltage. TE: 10 mM Tris-HCl, pH 8.0, 1 mM EDTA.

[c]SOC: 2% Bacto tryptone, 0.5% Bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose.

Appendix I
Electro-transformation Conditions

The following conditions have been taken from reports using the Gene Pulser apparatus. In some cases, the authors have supplied updated information on conditions giving better transformation.

Many of these studies were done before the Pulse Controller high voltage system was available. In some cases, we have given what we consider to be the equivalent electrical conditions with the Pulse Controller unit.

Species	***Actinobacillus pleuropneumoniae***
Reference	Lalonde, G., Miller, J. F. and O'Hanley, P., *Am. J. Vet. Res.*, in press.
Medium	272 mM sucrose, 3 mM potassium phosphate, pH 7.4, 15% glycerol
Cell concentration	OD_{600} = 6.0
Sample volume	0.6 ml
Temperature	0-4° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	6.25 kV/cm
Time constant	12 to 22 msec

Authors have found higher field strength to yield better transformation:

Medium	272 mM sucrose, 10% glycerol
Volume	40 µl
Electrode gap	0.15 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	400 ohms
Actual electrical conditions	
Field strength	16.7 kV/cm
Time constant	11 msec

Species	***Bordetella pertussus***
Reference	Miller, J. F. (unpublished data).
Medium	272 mM sucrose, 3 mM potassium phosphate, pH 7.4, 15% glycerol
Cell concentration	OD_{600} = 12
Sample volume	0.6 ml
Temperature	0-4° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	6.25 kV/cm
Time constant	13.4 msec

Similar electrical conditions are available with the Pulse Controller as follows:

Medium	272 mM sucrose, 15 % glycerol
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	1.25 to 2.5 kV (6.25 to 12.5 kV/cm)
Capacitor	25 µF
Pulse Controller setting	400 ohms (10 msec)

Species	***Campylobacter jejuni***
Reference	Miller J. F., Dower, W. J. and Tompkins, L.S., *Proc. Natl. Acad. Sci. USA*, **85**, 856 (1988).
Medium	272 mM sucrose, 3 mM potassium phosphate, pH 7.4, 15% glycerol
Cell concentration	5 x 10^9/ml
Sample volume	600 µl
Temperature	0-4° C
Electrode gap	0.15 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	16.7 kV/cm
Time constant	2 msec (longer time constants also effective)

Similar electrical conditions are available with the Pulse Controller as follows:

Medium	272 mM sucrose, 15% glycerol
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	2.5 kV (12.5 kV/cm)
Capacitor	25 µF
Pulse Controller setting	100 to 400 ohms (2.5 to 10 msec)

Species	***Enterococcus faecalis* (protoplasts)**
Reference	Fiedler, S. and Wirth, R., *Anal. Biochem.*, **170**, 38-44 (1987).
Medium	300 mM sucrose, 10 mM Tris-Cl, pH 8.0, 1 zmM EDTA
Cell concentration	10^8/ml
Sample volume	0.8 ml
Temperature	18-25° C
Electrode gap	0.4 cm
Gene Pulser settings	1 or 3 pulses (1 min cooling between pulses)
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	6.25 kV/cm
Time constant	5-7 msec

Species	***Escherichia coli***
strains	**LE392** **DH5α** **MC1061** **WM1100** **and many others**
References	1) Dower, W. J., *Mol. Biol. Rep.*, **1,** 5 (1987). 2) Dower, W. J., Miller, J. F. and Ragsdale, C. W., *Nucl. Acids Res.*, **16**, 6127 (1988).
Medium*	10% glycerol
Cell concentration	$>10^{10}$/ml
Sample volume	40 µl
Temperature	0-4° C
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	200 ohms
Actual electrical conditions	
Field strength	12.5 kV/cm
Time constant	4.5 to 5 msec

* Original conditions[1] called for an electroporation medium of
272 mM sucrose
7 mM potassium phosphate, pH 7
1 mM $MgCl_2$
± glycerol

To reduce the ionic strength for higher voltage operation with the Pulse Controller, a 10% glycerol solution is now recommended.

Note: A detailed protocol for high efficiency electroporation of *E. coli* is presented in Section 7 of this manual.

Species	***Lactobacillus casei***
Reference	Chassy, B. M. and Flickinger, J. L., *FEMS Microbiol. Lett.*, **44,** 173 (1987).
Medium	272 mM sucrose, 7 mM potassium phosphate, pH 7, 1 mM $MgCl_2$
Cell concentration	Not given
Sample volume	0.8 ml
Temperature	0-4° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.0 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	5.0 kV/cm
Time constant	~ 5 msec

Species	**Lactobacillus acidophilus** **L. fermentum** **L. casei** **Staphyloccocus aureus** **Bacillus cereus**
Reference	Luchansky, J. B., Muriana, P. M. and Klaenhammer, T. R., Molec. Microbiol., **2,** 637 (1988).
Medium	"HEPES-electroporation buffer"
Cell concentration	6 x 10^9/ ml
Sample volume	0.8 ml
Temperature	0-4° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	6.25 kV/cm
Time constant	Not reported

Similar electrical conditions are available with the Pulse Controller as follows:

Medium	(very low ionic strength)
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	1.25 to 2.5 kV (6.25 to 12.5 kV/cm)
Capacitor	25 µF
Pulse Controller setting	200 ohms (~5 msec)

Species	***Psuedomonas putida***
Reference	Fiedler, S. and Wirth, R., *Anal. Biochem.*, **170,** 38 (1988).
Medium	300 mM sucrose, 7 mM sodium phosphate, pH 7.4, 1 mM $MgCl_2$
Cell concentration	1 x 10^9/ml
Sample volume	0.8 ml
Temperature	room temperature
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller	Pulse Controller not used*
Actual electrical conditions	
Field strength	6.25 kV/cm
Time constant	~5 msec

Note: Field strengths higher than 6.25 kV/cm may improve transformation of this species.

*To attain these fields, a 0.2 cm cuvette, the Pulse Controller unit, and a reduction in buffer conductivity are required. The following conditions may be useful:

Medium	300 mM sucrose, no buffer or cations
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	1.25 to 2.5 kV (produces field strengths of 6.25 to 12.5 kV/cm)
Capacitor	25 µF
Pulse Controller setting	200 ohms (5 msec)

Species	***Salmonella typhimurium*** ***Escherichia coli,* strains** **BB** **C** **DH1** **JM109** **LE392**
Reference	Taketo, A., *Biochim. Biophys. Acta*, **949**, 318 (1988).
Medium	10% sucrose, 10 mM Tris-Cl, pH 7.5, 2 mM $MgCl_2$
Cell concentration	4 to 5 x 10^9/ml
Sample volume	0.8 ml
Temperature	0° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	6.25 kV/cm
Time constant	Not reported

Species	***Salmonella typhimurium***
Reference	G. Lalonde (unpublished data)
Medium	272 mM sucrose, 3 mM potassium phosphate, pH 7.4, 15% glycerol
Cell concentration	ABS_{600} =10
Sample volume	0.5 ml
Temperature	0-4° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	6.25 kV/cm
Time constant	18 to 20 msec

Similar electrical conditions are available with the Pulse Controller as follows:

Medium	272 mM sucrose, 15% glycerol
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	1.25(+) kV (field strength = 6.25(+) kV/cm)
Capacitor	25 µF
Pulse Controller setting	800 to 1,000 ohms (15 to 20 msec)

Species	***Streptococcus lactis*** **strains LM0230, C2, C6, C8, C10, DR4** ***S. cremoris*** **strains M41, EB4, HP, C11**
Reference	Powell, I. B., Achen, M. G., Hillier, A. J. and Davidson, B. E., *Appl. Environ. Microbiol.*,**54,** 655 (1988).
Medium	0.5 M sucrose, 7 mM potassium phosphate, pH 7.4, 1 mM $MgCl_2$
Cell concentration	2 to 5 x 10^9/ml
Sample volume	0.8 ml
Temperature	0-4° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.5 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	6.25 kV/ml
Time constant	~5 msec

Note: The authors reported a significant enhancement of electro-transformation efficiency by very gentle digestion of the cell walls with lysozyme. This technique may be generally useful with gram positive organisms. Similar electrical conditions (and higher field strengths) are available with the Pulse Controller as follows:

Medium	0.5 M sucrose
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	1.25 to 2.5 kV (6.25 to 12.5 kV/cm)
Capacitor	25 µF
Pulse Controller setting	200 ohms (5 msec)

Species	***Streptococcus thermophilus*** ***S. faecalis***
Reference	Somkuti, G. A. and Steinberg, D. H., *Proc. 4th Eur. Cong. Biotechnol.*, **1**, 412 (1987).
Medium	300 mM raffinose, 7 mM potassium phosphate, pH 7.4, 1 mM $MgCl_2$
Cell concentration	Not given
Sample volume	0.8 ml
Temperature	0-4° C
Electrode gap	0.4 cm
Gene Pulser settings	
Voltage	2.0 kV
Capacitor	25 µF
Pulse Controller setting	Pulse Controller not used
Actual electrical conditions	
Field strength	5.0 kV/cm
Time constant	4.5 msec

Similar electrical conditions are available with the Pulse Controller as follows:

Medium	300 mM raffinose
Electrode gap	0.2 cm
Gene Pulser settings	
Voltage	1.25(+) kV (6.25(+) kV/cm)
Capacitor	25 µF
Pulse Controller setting	200 ohms (~5 msec)

Prokaryotic Cell Electroporation References

Bacteria	**References**
Actinobacillus	5
Bacillus	6
Campylobacter	8
Enterococcus	3, 6
Escherichia	2, 11
Lactobacillus	1, 6
Lactococcus	6
Leuconostoc	6
Pediococcus	6
Propionibacterium	6
Pseudomonas	3
Salmonella	11
Staphylococcus	6
Streptococcus	3, 4, 9, 10
Streptomyces	7

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2. Dower, W. J., Miller, J. F. and Ragsdale, C. W., High efficiency transformation of *E. coli* by high voltage electroporation. *Nucl. Acids Res.*, **16**, 6127 (1988).

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5. Lalonde, G., Miller, J. F. and O'Hanley, P., Transformation of *Actinobacillus pleuropneumoniae* and analysis of R factors by electroporation, *Am. J. Vet. Res.*, in press.

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10. Somkuti, G. A. and Steinberg, D. H., Introduction of DNA into lactic bacteria by electroporation, *Society for Industrial Microbiology News*, **37**, 78 (1987).

11. Taketo, A., DNA transfection of *Escherichia coli* by electroporation, *Biochim. Biophys. Acta,* **949**, 318 (1988).

Appendix II
Troubleshooting Guide for the Gene Pulser Apparatus and Pulse Controller

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 $\leq$ 0.05 kV. The unit will only deliver pulses $\geq$ 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 Pulser apparatus. Check that the knob on the front of the Gene Pulser unit is set to the capacitor desired. Incomplete power circuit. Make sure cuvette electrodes make contact with electrical contacts inside the Gene Pulser chamber.
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, or re-press both buttons to continue. 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. Make sure that the Pulse Controller is set to one of the parallel resistors (100 Ω to 1,000 Ω).
4. Unit displays 'no' when time constant is displayed.	The pulse was delivered, but the time constant is too short (<10 µsec) to be measured by the apparatus. If a longer pulse is desired, select a larger capacitor or a larger parallel resistor. If the problem persists (>30 seconds), an SCR is burned out. Have the instrument serviced.

Electrical

Problem	**Likely Cause and Solution**
1. Arcing in the cuvette.	When the Pulse Controller is properly placed in the circuit, arcing in the cuvette is usually caused by a medium that is too conductive. The limit of conductivity depends on the voltage, electrode gap, and sample volume, but with our standard conditions for *E. coli,* 10 meq or higher will certainly arc. There are several causes of excessive conductivity: 1. Washing and resuspending cells in a buffer too high in ionic strength. 2. Insufficient washing of the cells–salts from the growth medium are not completely removed. 3. Lysed cells in the preparation–cell contents contribute to conductivity. 4. DNA solution is too high in salt; for example, CsCl carried over from plasmid prep, or residual ammonium acetate from ethanol precipitation. 5. Electroporation with cuvettes above 0° C.
2. Wrong time constant.	When using the Pulse Controller, the time constant should be close to that predicted by Table 1. If it is much shorter than the expected value (e.g., 3 msec instead of 5 msec), the sample is too conductive. The probable reasons for this are listed above under “arcing”. There are two solutions: (1) correct the problem of high conductivity by additional washing of the cells, or (2) use the cell preparation as it is and increase the time constant to the desired length with a larger resistor or capacitor. However, the second approach may result in arcing, and a reduction of the ionic strength may be the only solution. If the time constant is significantly **longer** than predicted by Table 1, it is likely that the resistor or capacitor setting is wrong.
3. Sample does not “twitch”.	This may mean that the pulse is not reaching the sample. Check the connections between the Gene Pulser unit, Pulse Controller, and sample chamber. Be sure they are arranged as shown in Figure 2. Also check to see that the contacts in the base of the sample chamber are not broken. The absence of a twitch does not always mean an error. At voltages below 1.5 kV, the pulse may not be strong enough to cause the sample to twitch. Sometimes the effect is simply difficult to see.

Problem	Likely Cause and Solution
4. Instrument charges, but screen blanks and instrument will not fire.	This often means an open circuit. In this condition the capacitor is charged and a shock hazard is present at all connections. **Discharge by turning the Gene Pulser power switch off-on-off.** Then check all connections. Also check the resistor setting on the Pulse Controller. If the Gene Pulser apparatus is used without the Pulse Controller, or if the Pulse Controller is set at "∞", **and** the sample in the cuvette is greater than 1,000 Ω (as for example with a bacterial sample), the instrument will not fire. To correct this, either set the Pulse Controller to one of the resistors from 100-1,000 Ω, or add a small amount of salt (1-2 mM) to the sample.
5. Instrument displays "no" on front panel.	Check capacitor setting. Check connections.

Biological

The general symptom addressed in this section is transformation efficiencies that are too low to detect or too low to be useful. The following is a list of the areas of possible problems and some suggested solutions.

Problem	Likely Cause and Solution
1. The pulse.	Is the pulse actually applied to the sample? At high voltage this is easy to check. The sample will "twitch" when pulsed. If you don't see a twitch, refer to the electrical troubleshooting section for information on electrical problems. Are the amplitude and length of the pulse sufficient? Bacteria of average size (1-2 µm) require pulses of at least a few milliseconds with field strengths of 5 to 15 kV/cm. There should (usually) be some cell death with electrical conditions producing transformation. Survival rates of 20 to 80% are to be expected. If no cell death occurs, the pulse is probably too weak. Conversely if too many cells are killed (>80%), the pulse is too intense and transformation will probably be poor. To find the optimum pulse characteristics, select a pulse length of ~5 to 10 msec and test for transformation over a range of field strengths in 1 or 2 kV/cm increments.

Problem	Likely Cause and Solution
2. The DNA.	The most common problem in the category of DNA is the presence of restriction barriers. Initial experiments should be done with DNA modified in the target strain. If such modified DNA is not available, use a high concentration of unmodified DNA (10-100 µg/ml) to increase the chances of detecting transformation. Check the quantity and quality of the DNA on a gel. Often, mini-preps contain less DNA than expected. DNA improperly stored for long periods may be degraded and lack transforming activity. Some preparations of DNA may contain substances that inhibit transformation or are toxic to the cells. Try to use DNA free of SDS, phenol, etc. Is the DNA replicated and expressed in the target host? Is the selection appropriate for the marker (and its level of expression)?
3. The cells.	Were the cells harvested in early- to mid-log growth? Rapidly growing cells seem to electro-transform best. Different growth conditions may improve transformation. Is the size of the cells unusual? Large cells require lower field strength (and are killed in high fields), and small cells require very high field strength for transformation. Does the cell have a capsule? Some capsules may impede the entry of DNA. If available, try methods for removing the capsule (different growth conditions, nutrient deprivation, or a chemical or enzymatic treatment). Some cell types may require an extended recovery period after the pulse and before selection (longer than the two doubling periods recommended). Do the cells secrete nucleases? If so, the vector may be degraded before transformation occurs. Adding DNA to cells **immediately** before the pulse, adding large amounts of carrier DNA, or using specific or non-specific DNase inhibitors may be helpful.

Problem	Likely Cause and Solution
	Are too many cells killed? The pulse is too intense, toxic substances are present in DNA or cell preparations, wrong temperature of electroporation are all possibilities. Are the cells transferred to outgrowth medium **immediately** after the pulse? For some cells this is very important. Is the correct selection applied after the recovery period?
4. Temperature.	Are the cuvettes cold? Is the cuvette holder (slide) prechilled? If frozen, have the cells been stored properly (usually 10-15% glycerol, -70° C)? Are these cells cold labile? If so, the pulses may be applied at room temperature.