E. coli Pulser™ Transformation Apparatus

Operating Instructions and Applications Guide

Catalog Numbers
165-2101, 165-2102,
165-2103, 165-2104

BIO-RAD

Model _____

Catalog Number _____

Date of Delivery _____

Warranty Period _____

Serial Number _____

Invoice Number _____

Purchase Order Number _____

Warranty

Bio-Rad Laboratories warrants the E. coli Pulser apparatus against defects in materials and workmanship for 1 year. If any defects occur in the instrument during this warranty period, Bio-Rad Laboratories will at Bio-Rad's option repair or replace the defective parts free. The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
3. Use of fittings or other spare parts supplied by anyone other than Bio-Rad Laboratories.
4. Damage caused by accident or misuse.
5. Damage caused by disaster.
6. Corrosion due to use of improper solvent or sample.

For any inquiry or request for repair service, contact Bio-Rad Laboratories after confirming the model, serial number, invoice number, and purchase order number of your instrument.

Table of Contents

Introduction

The E. coli Pulser apparatus is used for the electroporation of *Escherichia coli* where pulses of very high field strength are applied to samples of small volume and high resistance.

The internal circuitry of the E. coli Pulser apparatus greatly reduces the incidence of arcing at high voltage, and is protected if a high voltage, high current arc does occur. The unit is designed to fire into a high resistance sample.

The internal resistance of the E. coli Pulser apparatus will cause a substantial loss in voltage when pulses are applied to low resistance samples (less than 1000 Ω). This includes samples in which the growth medium was not completely removed from the *E. coli* cells, and DNA samples containing salt contributed by residual cesium chloride, or ligation mixtures. The voltage loss is insignificant with high resistance samples (see Section 3 for a detailed discussion of this effect). If you have a question or doubt concerning the resistance of your sample, contact your Technical Representative, or, in the U. S., call Technical Services at 1-800-4BIORAD.

Section 1
Safety Precautions

READ THIS INFORMATION CAREFULLY
BEFORE USING THE E. COLI PULSER APPARATUS.

1.1 Electrical Hazards

The E. coli Pulser apparatus produces voltages up to 2,500 volts and is capable of passing very high currents. When charged to maximum voltage, the instrument stores about 32 joules. A certain degree of 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 input jacks, and to the recessed electrode contacts inside the sample chamber. The latter mechanical interlocks should never be circumvented.

There is high voltage present whenever the rectangular red buttons are depressed (charging) and when the capacitor has 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 the sample chamber.*** To manually discharge the capacitor, turn the main power switch of the 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 ***always*** to follow this procedure before connecting or disconnecting the sample chamber to the unit, to be absolutely sure the capacitor is discharged.

If the charging cycle is aborted by releasing either of the rectangular red buttons, the charge/fire cycle can be continued simply by re-pressing the red pulse 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 circuitry of the E. coli Pulser apparatus greatly reduces the incidence of arcing in the cuvette when high voltage is delivered into high resistance media. However, arcing can some-

times still occur. The sample chamber is effective in containing these small explosions, but nonetheless ***we strongly recommend wearing safety glasses when using the instrument.***

Do not use the E. coli Pulser apparatus with samples suspended in conductive media (such as saline). Refer to Section 5 for sample preparation.

1.3 Other Safety Precautions

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 E. coli Pulser apparatus.

This instrument is intended for laboratory use only.

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 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
E. coli Pulser Apparatus Operating Instructions

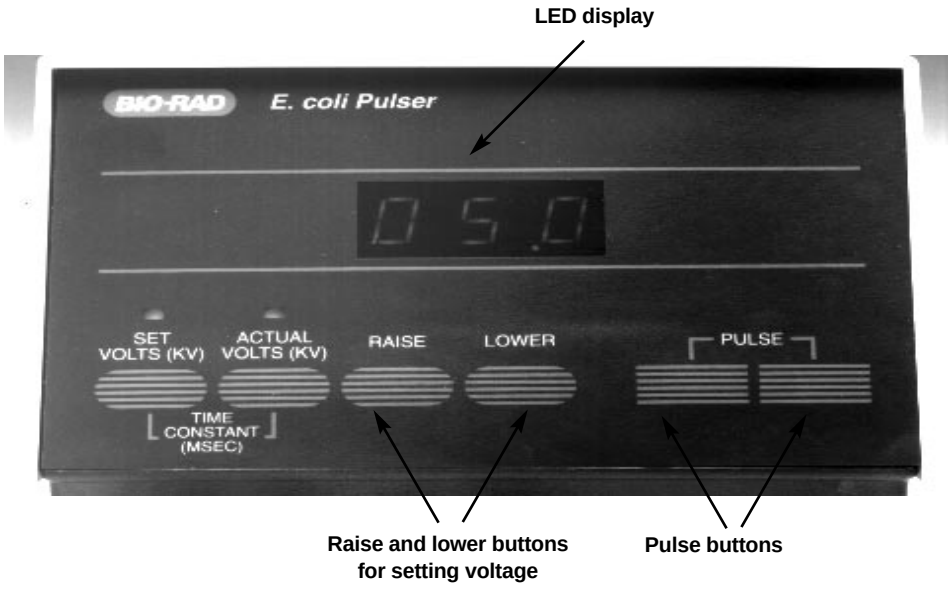

Fig 1. The front panel of the E. coli Pulser apparatus. Notice that the lights above both the SET VOLTS (KV) and ACTUAL VOLTS (KV) buttons are illuminated, indicating that the TIME CONSTANT (MSEC) is displayed in the LED>

1. Connect the black power cord to the rear panel of the E. coli Pulser apparatus. Plug the cord into a wall outlet or power strip.

Fig. 2. 0.2 cm and 0.1 cm electrode gap cuvettes.

Fig. 3. 400 µl in a 0.2 cm cuvette (the maximum volume for pulse delivery).

2. Connect the leads from the sample chamber to the output jacks marked TO SHOCKING CHAMBER on the front panel of the E. coli Pulser apparatus. Be sure to observe polarity (red to red, black to black).

3. Turn on the apparatus using the power switch on the right-hand panel. The light emitting diode (LED) display should illuminate and read “0.00”.

4. The LED above the oval SET VOLTS (KV) button will be illuminated. The LED display is in kilovolts (kV). Use the oval RAISE and LOWER buttons to adjust the voltage to the desired value in the range of 0.20 - 2.50 kV (Figure 1). If the voltage is set at 0.00 kV, ‘no’ will be displayed when the pulse buttons are pressed. To quickly set the voltage to 1.80 kV (recommended when using 0.1 cm cuvettes, Figure 2), press the RAISE and LOWER buttons simultaneously. “1.80” will be displayed in the LED. To quickly set the voltage to 2.50 kV (recommended when using the 0.2 cm cuvettes, Figure 2), press the RAISE and LOWER buttons simultaneously twice. “2.50” will be displayed. Pressing both buttons a third time will reset the instrument to 0.00 kV.

5. Place the cell suspension in the chilled electroporation cuvette. Use only the lower, narrow portion of the cuvette between the aluminum plates. Up to 0.4 ml (400 µl) of solution may be placed in the 0.2 cm cuvette, and the 0.1 cm cuvette can hold 0.08 ml (80 µl) during pulse delivery. See Figure 3.

Fig. 4. The slide with a cuvette at the back of the shocking chamber.

Fig. 5. A display of "Chg" indicates that the capacitor is being charged.

6. Insert the cuvette into the white slide. Push the slide into the chamber until the cuvette makes firm contact with the chamber electrodes (Figure 4).

7. To charge the capacitor and deliver a pulse, depress and hold **both** rectangular 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 5). The tone signals that the pulse has been delivered and the pulse buttons may be released. For safety reasons, the E. coli 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 voltage selected. At higher voltages, a small-volume (40 µl) sample will "twitch," indicating a pulse has passed through the sample.

   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 E. coli Pulser apparatus off and on* ***twice****. The latter procedure will automatically bleed the capacitor of any residual voltage (see Section 1 for safety precautions). The voltage setting will be erased when the E. coli Pulser apparatus is turned off. It must be re-entered before continuing.*

8. Withdraw the slide from the chamber, remove the cuvette, and process the sample.

9. The actual voltage delivered to the sample can be displayed by pressing the ACTUAL VOLTS (KV) button on the front panel of the apparatus (see Figure 1). The LED above the button will illuminate. The TIME CONSTANT (MSEC) can be displayed by simultaneously pressing the SET VOLTS (KV) and ACTUAL VOLTS (KV) buttons. Both lights above the buttons will illuminate (Figure 1). The time constant is displayed in milliseconds.

10. To turn the unit off, turn the power switch off-on-off. This assures that the capacitor is completely discharged. The sample chamber may now be safely disconnected, if desired.

11. The E. coli Pulser apparatus is provided with output jacks marked WAVEFORM TEST that can be used to visualize the exponential waveform generated by the instrument. A probe of 500 V maximum voltage and a 50 MHz (or higher) oscilloscope are required. The WAVEFORM TEST jacks provide an output that is approximately 1/10 the voltage supplied to the shocking chamber. Hence, the need for a high-voltage probe is obviated. Simply attach the oscilloscope through the probe to the rightmost jacks of the E. coli Pulser apparatus. Set the oscilloscope to trigger on a positive waveform, the amplitude for full, and the time base for 1 msec/division. If 2500 V are selected on the E. coli Pulser apparatus, a pulse of approximately 250 V will be produced at the rightmost jacks, the shape of which may be observed on the oscilloscope.

Section 3
Electrical Variables

The electrical conditions for the electroporation of *E. coli* have been verified through years of research (Böttger, 1988; Dower, W. J., 1990; Dower, *et al.*, 1988*;* Heery *et al.*, 1989*;* Jacobs *et al.*, 1990*;* Kilbane and Bielaga, 1991; Leonardo and Sedivy, 1990; Marcus *et al.*, 1990*;* Summers and Withers, 1990; Taketo, 1989; Willson and Gough, 1988). The E. coli Pulser apparatus is designed to deliver precisely those pulse parameters needed for the highest transformation efficiencies. The time constant has been set at 5 milliseconds when working with high-resistance samples. The voltage setting depends on the cuvette used (1.80 kV are recommended for 0.1 cm cuvettes, for a field strength of 18.0 kV/cm, and 2.50 kV are recommended for 0.2 cm cuvettes, for a field strength of 12.5 kV/cm).

The E. coli Pulser apparatus is not meant for use with samples of low resistance (less than 1000 Ω; e.g. buffered saline solutions or buffered sucrose solutions). 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 $[R_{20}/(R_{20} + R_{sample})]$. 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%.

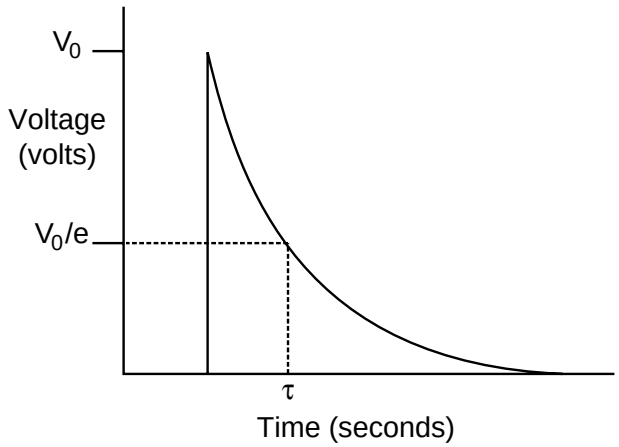

Fig. 6. Exponential decay waveform. τ = R x C.

The capacitor discharge circuit of the E. coli Pulser apparatus generates an electrical pulse with an exponential decay waveform (Figure 6). 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 follows,

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

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 time constant has been fixed at 5 milliseconds, the optimum for the electroporation of *E. coli.*

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

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

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.

An additional function of the E. coli Pulser apparatus circuit is to place a 20 Ω resistor in series with the sample. This protects the instrument by limiting the current should an arc occur. With an *E. coli* sample prepared as described in Sections 4 and 5, the resistance is about 5000 Ω; therefore, during normal operation (no arc) the voltage drop across the 20 Ω protective resistor will be less than 1%. Notice, however, that 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, the E. coli Pulser apparatus is not used with samples of less than 1,000 Ω.***

Section 4
Bacterial Electroporation (Electro-transformation)

4.1 DNA

The effect of size and topology of the plasmid DNA on electro-transformation of *E. coli.* has been examined. Plasmids of 21 kb transform *E. coli* with the same molar efficiency as plasmids of 3 to 7 kb (Dower, W. J., 1990). Supercoiled and relaxed circular forms of plasmids up to at least 20 kb transform with the same efficiency. 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.

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 (Dower *et al.,* 1988). 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 x 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 cotransformations). In each case, a small sample volume (20-50 µl) allows economical use of DNA and cells. (See Dower *et al.,* 1988 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 Tris-HCl pH 8.0, 1 mM EDTA) 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. Transformation with ligation mixture, for example, is successful when the ionic strength is reduced by dilution (Willson and Gough, 1988), dialysis (Heery and Dunican, 1989; Jacobs *et al.*, 1990), or ethanol precipitation (Böttger, 1988; Zabarovsky and Winberg, 1990).

4.2 Electroporation Media

Components contributing to the conductivity of the electroporation medium include the solution used to resuspend the cells, the cells themselves, and the DNA solution. For best results (highest efficiencies) it is very important to keep the resistance of the medium as high as possible, by making sure that all salts have been removed from the cell and DNA preparations. Glycerol is a convenient electroporation medium, since it is recommended as a cryoprotectant for storage of *E. coli*.

4.3 Electroporation Conditions

For electroporation of *E. coli*, small volumes are generally best. With the 0.2 cm or 0.1 cm cuvette, our standard volume is 40 µl (up to 400 µl may be used in the 0.2 cm cuvette; up to 80 µl may be used in the 0.1 cm 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* 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 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 (Figure 7).

Fig. 7. White cuvette slide and cuvettes equilibrating to 0 °C before the pulse.

Incubating *E. coli* cells with DNA for more than a minute before pulsing does not improve transformation efficiency (Dower *et al.*, 1988). For this species, binding of the DNA to the cells is probably not necessary. 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 narrow gap of the cuvettes prevents uniform mixing).

The period between applying the pulse and transferring the cells to outgrowth medium is ***crucial*** for recovering *E. coli* transformants (Dower *et al.*, 1988). 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.

Appendix I lists references of several *E. coli* strains that have been transformed by electroporation. Appendix II provides information on the transformation efficiencies and survivabilities of several *E. coli* strains pulsed at various field strengths with the E. coli Pulser apparatus. We are interested in hearing of additional strains 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 5
High Efficiency Electro-transformation of *E. coli*

Electroporation provides a method of transforming *E. coli* to efficiencies greater than are possible 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 is related to the intensity of the field (field strength = voltage/distance between electrodes) and to the length of the pulse (RC time constant).

Protocols for preparing and electro-transforming *E. coli* to high efficiencies are described in Table 1.

Table 1. 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 approximately 0.5-0.7 (the best results are obtained with cells that are harvested at early- to mid-log phase; the appropriate cell density therefore depends on the strain and growth conditions).

*3. To harvest, centrifuge cells in cold centrifuge bottles in a cold rotor at 4000 x g_{max} for 15 minutes.

*4. Remove as much of the supernatant (medium) as possible. It is better to sacrifice the yield by pouring off a few cells than to leave any supernatant behind.

*5. Gently resuspend the pellets in a total of 1 liter of ice-cold 10% glycerol[b] taking care not to lyse them. Centrifuge as in step 3.

*6. Resuspend in 0.5 liter of ice-cold 10% glycerol. Centrifuge as in step 3.

*7. Resuspend in ~250 ml of ice-cold 10% glycerol. Centrifuge as in step 3.

* Keep the cells as close to 0 °C as possible (in an ice/water bath) throughout their preparation.

8. Resuspend to a **final** volume of 3 to 4 ml in ice-cold 10% glycerol. The cell concentration should be about 1 - 3 x 10^{10} cells/ml.

9. 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 and Plating

1. Gently thaw the cells at room temperature and then immediately place them on ice. Remove the sterile cuvettes from their pouches and place them on ice. Place the white chamber slide on ice (Figure 7).

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[c]). Mix well and let sit on ice ~0.5 - 1 minute.

3. Set the E. coli Pulser apparatus to 2.50 kV when using the 0.2 cm cuvettes. Set it to 1.80 kV when using the 0.1 cm cuvettes. See Section 2 for operating instructions.

4. Transfer the mixture of cells and DNA to a cold electroporation cuvette, and shake the suspension to the bottom. 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 (Figure 4).

5. Pulse once.

6. Remove the cuvette from the chamber and **immediately** add 1 ml of SOC[d] medium to the cuvette and quickly but gently 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. Check and record the pulse parameters. The time constant should be close to 5 milliseconds. The field strength can be calculated as actual volts (kV) / cuvette gap (cm).

9. Plate on selective medium.

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

b 10% Glycerol: Prepare fresh weekly with sterilized water. Do not autoclave or filter-sterilize the glycerol solution.

c 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.

d 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
E. coli Electro-transformation References

The following literature references report the transformation of *Escherichia coli* by electroporation. Asterisks denote work done with the Gene Pulser® system.

	Escherichia coli	References
	spheroplasts	Cymbalyuk *et al.* (1988)
*	(strain not mentioned)	Dahlman and Harlander (1988) Dower *et al*. (1992) Fikes *et al*. (1990) Hofmann and Evans (1986) Hosoda *et al*. (1990) Larimer and Mural (1990) O'Callaghan and Charbit (1990) Sale and Hamilton (1967) Sale and Hamilton (1968) Speyer (1990b)
*	wild type	Fiedler *et al*. (1989) Wirth *et al*. (1989)
*	strain 1106	Cocconcelli *et al*. (1991)
	strain 300 V	Hamilton and Sale (1967)
*	strain 3132 (wild-type)	Fiedler *et al*. (1989) Wirth *et al*. (1989)
*	strain AB1157	Binotto *et al*. (1991) Hartke and Schulte-Frohlinde (1991) Sixou *et al*. (1991)
*	strain BB	Taketo (1989)
*	strain BHB2600	Gaier *et al*. (1992) Knauf *et al*. (1992)
*	strain BW 313-1	Hsieh *et al*. (1991)
*	strain C	Taketo (1989) Taylor and Burke (1990)
*	strain CAI^{Q1}	Zink *et al*. (1991)
*	strain C-600	Binotto *et al*. (1991) Kilbane and Bielaga (1991) Marcus *et al*. (1990) Yuan *et al*. (1984)
*	strain C600Sm	Metzger *et al*. (1992)
*	strain C600-2Sm	Metzger *et al*. (1992)
*	strain CU9276	Batt *et al*. (1990)
*	strain DB11	Mermelstein *et al*. (1992)

Escherichia coli	References
* strain DH1	Craig *et al*. (1989) Flannagan and Clewell (1991) Fujimoto *et al*. (1991) Heery *et al*. (1989) Taketo (1989)
* strain DH10B	Gruber (1992) Hanahan *et al*. (1991) Hsieh *et al*. (1991) Li *et al*. (1990) Pacholczyk *et al*. (1991) Rice *et al*. (1992) Rodriguez-Palenzuela *et al*. (1991) Smith *et al*. (1990)
* strain DH5	Hsieh *et al*. (1991) Inoue *et al*. (1990)
* strain DH5α	Allen and Blaschek (1990) Cirillo *et al*. (1991) Dower *et al*. (1988) Fujimoto *et al*. (1991) Gilchrist and Smit (1991) Kallio *et al*. (1991) Lyra *et al*. (1991) Smith *et al*. (1990) Sung *et al*. (1990)
strain DH5αF´	Marcil and Higgins (1992)
strain DH5αF´IQ	Smith *et al*. (1990)
* strain DS941	Summers and Withers (1990)
* strain EM24	Thomson and Flint (1989)
strain ER1451	Sixou *et al*. (1991)
* strain GM1829	Joerger and Klaenhammer (1990)
* strain HB101	Calvin (1988) De Rossi *et al*. (1991) Dower (1987) Fiedler *et al*. (1989) Fiedler and Wirth (1988) Haider *et al*. (1991) Haynes and Britz (1990) Keller and Maniatis (1991) Kilbane and Bielaga (1991) Kim and Blaschek (1989) Regué *et al*. (1992) Smith *et al*. (1990) Speyer (1990a) Sung *et al*. (1990) Wirth *et al*. (1989)

*	strain JC8679	Summers and Withers (1990)
*	strain JM101	Halling and Zehr (1990)
*	strain JM105	Fiedler *et al*. (1989) Fiedler and Wirth (1988) Jacobs *et al*. (1990) Wirth *et al*. (1989)
*	strain JM107	Dower (1987)
*	strain JM109	Boivin and Bellemare (1991) Calvin (1988) Gerischer and Dürre (1990) Kovalic *et al*. (1991) Smith *et al*. (1990) Taketo (1988) Tobin *et al*. (1991) Ward and Jarvis (1991)
*	strain JM110	Raya and Klaenhammer (1992)
*	strain JPN15.96	Jerse *et al*. (1990)
*	strain JS4	Leonardo and Sedivy (1990)
	strain K12	Hülsheger and Niemann (1980) Hülsheger *et al*. (1981)
*	strain K12A	Taketo (1989)
*	strain K2-1-4	Schendel *et al*. (1992)
*	strain K802	Gilchrist and Smit (1991)
*	strain K803	Jacobs *et al*. (1990) Ware *et al*. (1992)
*	strain LE392	Binotto *et al*. (1991) Dower (1987) Dower (1990) Dower and Cwirla (1992) Dower *et al*. (1988) Fujimoto *et al*. (1991) Taketo (1988) Zabarovsky and Winberg (1990)
*	strain M5361	Takahashi and Kobayashi (1990)
*	strain MC1061	Calvin (1988) Calvin and Hanawalt (1988) Cwirla *et al*. (1990) Dower (1990) Dower and Cwirla (1992) Dunican and Shivnan (1989) Hanahan *et al*. (1991) Rubenstein *et al*. (1990) Smith *et al*. (1990)

* strain MC1061 (cont.) ..... Willson and Gough (1988)
Zabarovsky and Winberg (1990)

* strain MC1061/P3..... Kieffer (1991)
Sheen (1989)
Ymer (1991)

* strain MG1655..... Pfau and Youderian (1990)

* strain MM294 ..... Zink *et al.* (1991)

* strain MV1190..... Dower (1987)

strain NCTC 8196 ..... Hamilton and Sale (1967)

* strain NK5898 ..... Mahillon and Kleckner (1992)

* strain NM522..... Boivin and Bellemare (1991)
Willson and Gough (1988)

* strain p678-54 ..... Marcus *et al.* (1990)

* strain QC774..... Van Camp *et al.* (1990)

* strain SCS-1 ..... King and Goodbourn (1992)
Pfau and Youderian (1990)

* strain SDM..... Batt *et al.* (1990)

* strain SM10..... Rodriguez-Palenzuela *et al.* (1991)

* strain SR101 ..... Garrard *et al.* (1991)

* strain SURE ..... Clarke *et al.* (1992)
Wang *et al.* (1992)

* strain TG-1..... Heery and Dunican (1989)
Zabarovsky and Winberg (1990)

* strain UM2..... Knauf *et al.* (1992)

* strain WA321..... Fiedler *et al.* (1989)
Fiedler and Wirth (1988)
Wirth *et al.* (1989)

* strain WM1100..... Dower (1990)
Elliot *et al.* (1990)
Hsieh *et al.* (1991)

* strain XL-1 Blue ..... Barbas *et al.* (1991)
Hermanson *et al.* (1991)
O'Neill and Söll (1990)
Ostrander *et al.* (1992)
Patel *et al.* (1991)
Petzel and McKay (1992)
Starr and Huse (1990)
Swaroop *et al.* (1991)
Taketo (1988)
Zabarovsky and Winberg (1990)

* strain XS127 ..... Böttger (1988)

Special Applications	References
* Ligation mixtures	Barbas *et al*. (1991) Böttger (1988) Cirillo *et al*. (1991) Clarke *et al*. (1992) Elliot *et al*. (1990) Flannagan and Clewell (1991) Gaier *et al*. (1992) Gerischer and Dürre (1990) Gilchrist and Smit (1991) Gruber (1992) Halling and Zehr (1990) Hanahan *et al*. (1991) Heery and Dunican (1989) Jacobs *et al*. (1990) Keller and Maniatis (1991) Kieffer (1991), Kieffer (1992) King and Goodbourn (1992) Knauf *et al*. (1992) Kovalic *et al*. (1991) O'Neill and Söll (1990) Ostrander *et al*. (1992) Pacholczyk *et al*. (1991) Patel *et al*. (1991) Petzel and McKay (1992) Wang *et al*. (1992) Willson and Gough (1988) Ymer (1991) Zabarovsky and Winberg (1990) Zink *et al*. (1991)
* Library construction	Böttger (1988) Elliot *et al.* (1990) Gerischer and Dürre (1990) Kieffer (1991), Kieffer (1992) Ostrander *et al*. (1992) Pacholczyk *et al*. (1991) Wang *et al*. (1992)
* Single-stranded DNA	Rubenstein *et al*. (1990) Swaroop *et al*. (1991)
* M13 DNA	Hartke and Schulte-Frohlinde (1991) Heery and Dunican (1989) Starr and Huse (1990)
* Other bacteriophage DNA	Lyra *et al*. (1991)
* Large DNA fragments	Larimer and Mural (1990) Leonardo and Sedivy (1990)

Special Applications	References
* Influence of DNA size on efficiency	Allen and Blaschek (1990) Dower (1990) Leonardo and Sedivy (1990) Marcus *et al*. (1990) Regué *et al*. (1992) Smith *et al*. (1990)
* Influence of DNA conformation on efficiency	Dower (1990) Hartke and Schulte-Frohlinde (1991) Leonardo and Sedivy (1990)
* RNA	Taketo (1989)
* Release of cell components	Calvin (1988) Calvin and Hanawalt (1988) Hamilton and Sale (1967) Heery *et al*. (1989) Li *et al*. (1990) Sixou *et al*. (1991)
* Direct transfer (donor to recipient)	Gilchrist and Smit (1991) Kilbane and Bielaga (1991) Marcil and Higgins (1992) Pfau and Youderian (1990) Summers and Withers (1990) Ward and Jarvis (1991)
* Use of more than one electroporation unit	Li *et al*. (1990)

Review Articles	
	Dower (1990) Dower *et al*. (1992) Hanahan *et al*. (1991) Hofmann and Evans (1986)

Key to Electroporation Units Used

GP Gene Pulser apparatus
PC Pulse Controller
CE Capacitance Extender
ED Exponential Decay (other than a Gene Pulser apparatus)
SW Square Wave
OT Other
UK Unknown

* Work done with the Gene Pulser system

References

GP	Allen, S. P., and Blaschek, H. P., Factors involved in the electroporation-induced transformation of *Clostridium perfringens*, *FEMS Microbiol. Lett.*, **70**, 217 (1990).
GP + PC	Barbas, III, C. F., Kang, A. S., Lerner, R. A., and Benkovic, S. J., Assembly of combinatorial antibody libraries on phage surfaces: the gene III site, *Proc. Natl. Acad. Sci. USA*, **88**, 7978 (1991).
GP	Batt, C. A., Oren, P., Webb, J., Flicke, P., An improved method for oligonucleotide-mediated site-directed mutagenesis, *BioTechniques*, **9**, 554 (1990).
GP + PC	Binotto, J., MacLachlan, P. R., and Sanderson, K. E., Electrotransformation in *Salmonella typhimurium* LT2, *Can. J. Microbiol.*, **37**, 474 (1991).
GP + PC	Boivin, R., and Bellemare, G., A novel approach to the rapid isolation and nucleotide sequencing of genomic clones, *Genet. Analysis - Techn. Applic. (GATA)*, **8**, 181 (1991).
GP + PC	Böttger, E. C., High-efficiency generation of plasmid cDNA libraries using electro-transformation, *BioTechniques*, **6**, 878 (1988).
OT	Calvin, N., I. Quantitation and analysis of furocoumarin: DNA adducts II. Introduction and recovery of DNA in bacterial cells by electroporation, Ph.D. Dissertation, Stanford University, Ch. 5, pp. 90 - 120 (1988).
OT	Calvin, N. M., and Hanawalt, P. C., High-efficiency transformation of bacterial cells by electroporation, *J. Bacteriol.*, **170**, 2796 (1988).
GP + PC	Cirillo, J. D., Barletta, R. G., Bloom, B. R., and Jacobs, Jr., W. R., A novel transposon trap for mycobacteria: isolation and characterization of IS*1096*, *J. Bacteriol.*, **173**, 7772 (1991).
GP + PC	Clarke, B., Stancombe, P., Money, T., Foote, T., and Moore, G., Targeting deletion (homoeologous chromosome pairing locus) or addition line single copy sequences from cereal genomes, *Nucl. Acids Res.*, **20**, 1289 (1992).
GP + PC	Cocconcelli, P. S., Gasson, M. J., Morelli, L., and Bottazzi, V., Single-stranded DNA plasmid, vector construction and cloning of *Bacillus stearothermophilus* α-amilase in *Lactobacillus*, *Res. Microbiol.*, **142**, 643 (1991).
GP + PC + CE	Craig, F. F., Coote, J. G., Parton, R., Freer, J. H., and Gilmour, N. J. L., A plasmid which can be transferred between *Escherichia coli* and *Pasteurella haemolytica* by electroporation and conjugation, *J. Gen. Microbiol.*,**135**, 2885 (1989).
GP + PC	Cwirla, S. E., Peters, E. A., Barrett, R. W., and Dower, W. J., Peptides on phage: a vast library of peptides for identifying ligands, *Proc. Natl. Acad. Sci. USA*, **87**, 6378 (1990).
ED	Cymbalyuk, E. S., Chernomordik, L. V., Broude, N. E., and Chizmadzhev, Y. A., Electro-stimulated transformation of *E. coli* cells pretreated by EDTA solution, *FEBS Lett.*, **234**, 203 (1988).
ED	Dahlman, D., and Harlander, S., Electroporation of intact microbial cells, *Abstr. Annual Meeting Amer. Soc. Microbiol.*, **88**, 155 (1988).
GP + PC	De Rossi, E., Brigidi, P., Rossi, M., Matteuzzi, D., and Riccardi, G., Characterization of gram-positive broad host-range plasmids carrying a thermophilic replicon, *Res. Microbiol.*, **142**, 389 (1991).
GP (no PC)	Dower, B., Electro-transformation of intact bacterial cells, *Molecular Biology Reports* (Bio-Rad Laboratories), **1**, 5 (1987).
GP + PC	Dower, W. J., "Electroporation of bacteria: a general approach to genetic transformation," in *Genetic Engineering—Principles and Methods*, 1990, vol. 12, pp. 275-296, Plenum Publishing Corp., New York.
GP + PC	Dower, W. J., and Cwirla, S. E., Creating vast peptide expression libraries: electroporation as a tool to construct plasmid libraries of greater than 10^9 recombinants, in *Guide to Electroporation and Electrofusion* (D. C. Chang, B. M. Chassy, J. A. Saunders, and A. E. Sowers, eds.), pp. 291 -301, 1992, Academic Press Inc., San Diego.

GP + PC	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).
GP + PC	Dunican, L. K., and Shivnan, E., High frequency transformation of whole cells of amino acid producing coryneform bacteria using high voltage electroporation, *Bio/Technol.*, **7**, 1067 (1989).
GP + PC	Elliott, J. F., Albrecht, G. R., Gilladoga, A., Handunnetti, S. M., Neequaye, J., Lallinger, G., Minjas, J. N., and Howard, R. J., Genes for *Plasmodium falciparum* surface antigens cloned by expression in COS cells, *Proc. Natl. Acad. Sci. USA*, **87**, 6363 (1990).
GP	Fiedler, S., Friesenegger, A., and Wirth, R., "Electroporation: a general method for transformation of gram-negative bacteria," in *Genetic Transformation and Expression*, chapter 7, pp. 65 - 69, 1989, L. O. Butler, C. Harwood, and B. E. B. Moseley, eds., Intercept Ltd, Andover, Hants, United Kingdom.
GP (no PC)	Fiedler, S., and Wirth, R., Transformation of bacteria with plasmid DNA by electroporation, *Anal. Biochem.*, **170**, 38 (1988).
GP + PC	Fikes, J. D., Becker, D. M., Winston, F., and Guarente, L., Striking conservation of TFIID in S*chizosaccharomyces pombe* and *Saccharomyces cerevisiae*, *Nature*, **346**, 291 (1990).
GP + PC	Flannagan, S. E., and Clewell, D. B., Conjugative transfer of Tn*916* in *Enterococcus faecalis*: *trans* activation of homologous transposons, *J. Bacteriol.*, **173**, 7136 (1991).
ED	Fujimoto, S., Hashimoto, H., and Ike, Y., Low cost device for electrotransformation and its application to the highly efficient transformation of *Escherichia coli* and *Enterococcus faecalis*, *Plasmid*, **26**, 131 (1991).
GP + PC	Gaier, W., Vogel, R. F., and Hammes, W. P., Cloning and expression of the lysostaphin gene in *Bacillus subtilis* and *Lactobacillus casei*, *Lett. Appl. Microbiol.*, **14**, 72 (1992).
GP + PC	Garrard, L. J., Yang, M., O'Connell, M. P., Kelley, R. F., and Henner, D. J., FAB assembly and enrichment in a monovalent phage display system, *Bio/Technol.*, **9**, 1373 (1991).
GP + PC	Gerischer, U., and Dürre, P., Cloning, sequencing, and molecular analysis of the acetoacetate decarboxylase gene region from *Clostridium acetobutylicum*, *J. Bacteriol.*, **172**, 6907 (1990).
GP + PC + CE	Gilchrist, A., and Smit, J., Transformation of freshwater and marine caulobacters by electroporation, *J. Bacteriol.*, **173**, 921 (1991).
ED	Gruber, C. E., High-efficiency cDNA cloning: a comparison of electroporation and *in vitro* packaging, *BioTechniques*, **12**, 804 (1992).
GP + PC	Haider, M. Z., Al-Taho, N., Al-Salameen, F., Kadri, M. H., Al-Amad, S., and Spanier, E., Efficient transformation of thermotolerant methanol-utilizing strains of *Methylophilus* spp. via electroporation, *Acta Biotechnol.*, **11**, 295 (1991).
GP + PC	Halling, S. M., and Zehr, E. S., Polymorphism in *Brucella* spp. due to highly repeated DNA, *J. Bacteriol.*, **172**, 6637 (1990).
SW	Hamilton, W. A., and Sale, A. J. H., Effects of high electric fields on microorganisms, II. Mechanism of action of the lethal effect, *Biochim. Biophys. Acta*, **148**, 789 (1967).
GP + PC, ED	Hanahan, D., Jessee, J., and Bloom, F. R., Plasmid transformation of *Escherichia coli* and other bacteria, *Meth. Enzymol.*, **204**, 63 (1991).
GP + PC	Hartke, A., and Schulte-Frohlinde, D., Survival of M13mp18 gapped duplex DNA as a function of gap length, *Mutat. Res.*, **255**, 39 (1991).
GP + PC	Haynes, J. A., and Britz, M. L., The effect of growth conditions of *Corynebacterium glutamicum* on the transformation frequency obtained by electroporation, *J. Gen. Microbiol.*, **136**, 255 (1990).
GP + PC	Heery, D. M., and Dunican, L. K., Improved efficiency M13 cloning using electroporation, *Nucl. Acids Res.*, **17**, 8006 (1989).

GP + PC	Heery, D. M., Powell, R., Gannon, F., and Dunican, L. K., Curing of a plasmid from *E. coli* using high-voltage electroporation, *Nucl. Acids Res.*, **17**, 10131 (1989).
GP + PC	Hermanson, G. G., Hoekstra, M. F., McElligott, D. L., and Evans, G. A., Rescue of end fragments of yeast artificial chromosomes by homologous recombination in yeast, *Nucl. Acids Res.*, **19**, 4943 (1991).
UK	Hofmann, G. A., and Evans, G. A., Electronic genetic - physical and biological aspects of cellular electromanipulation, *IEEE Engineering in Medicine and Biology Magazine*, **5**, 6 (1986).
UK	Hosoda, F., Nishimura, S., Uchida, H., and Ohki, M., An F factor based cloning system for large DNA fragments, *Nucl. Acids Res.*, **18**, 3863 (1990).
GP + PC	Hsieh, C.-L., McCloskey, R. P., Radany, E., and Lieber, M. R., V(D)J recombination: evidence that a replicative mechanism is not required, *Molec. Cell. Biol.*, **11**, 3972 (1991).
ED	Hülsheger, H., and Niemann, E.-G., Lethal effects of high-voltage pulses on *E. coli* K12, *Radiat. Environ. Biophys.*, **18**, 281 (1980).
ED	Hülsheger, H., Potel, J., and Niemann, E.-G., Killing of bacteria with electric pulses of high field strength, *Radiat. Environ. Biophys.*, **20**, 53 (1981).
GP + PC	Inoue, H., Nojima, H., and Okayama, H., High efficiency transformation of *Escherichia coli* with plasmids, *Gene*, **96**, 23 (1990).
GP + PC	Jacobs, M., Wnendt, S., and Stahl, U., High-efficiency electro-transformation of *Escherichia coli* with DNA from ligation mixtures, *Nucl. Acids Res.*, **18**, 1653 (1990).
GP	Jerse, A. E., Yu, J., Tall, B. D., and Kaper, J. B., A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells, *Proc. Natl. Acad. Sci. USA*, **87**, 7839 (1990).
GP + PC	Joerger, M. C., and Klaenhammer, T. R., Cloning, expression, and nucleotide sequence of the *Lactobacillus helveticus* 481 gene encoding the bacteriocin helveticin J, *J. Bacteriol.*, **172**, 6339 (1990).
GP	Kallio, P. T., Fagelson, J. E., Hoch, J. A., and Strauch, M. A., The transition state regulator Hpr of *Bacillus subtilis* is a DNA-binding protein, *J. Biol. Chem.*, **266**, 13411 (1991).
GP + PC	Keller, A. D., and Maniatis, T., Selection of sequences recognized by a DNA binding protein using a preparative southwestern blot, *Nucl. Acids Res.*, **19**, 4675 (1991).
GP + PC	Kieffer, B. L., Optimised cDNA size selection and cloning procedure for the construction of representative plasmid cDNA libraries, *Gene*, **109**, 115 (1991).
GP + PC	Kieffer, B. L., Optimised cDNA size selection and cloning procedure for the construction of representative plasmid cDNA libraries, *Gene*, **116**, 117 (1992). [Correction]
GP + PC	Kilbane, II, J. J., and Bielaga, B. A., Instantaneous gene transfer from donor to recipient microorganisms *via* electroporation, *BioTechniques*, **10**, 354 (1991).
GP (no PC)	Kim, A. Y., and Blaschek, H. P., Construction of an *Escherichia coli-Clostridium perfringens* shuttle vector and plasmid transformation of *Clostridium perfringens*, *Appl. Environ. Microbiol.*, **55**, 360 (1989).
GP + PC	King, P., and Goodbourn, S., A method for sequence-specific deletion mutagenesis, *Nucl. Acids Res.*, **20**, 1039 (1992).
GP + PC	Knauf, H. J., Vogel, R. F., and Hammes, W. P., Cloning, sequence, and phenotypic expression of *katA*, which encodes the catalase of *Lactobacillus sake* LTH677, *Appl. Environ. Microbiol.*, **58**, 832 (1992).
ED	Kovalic, D., Kwak, J.-H., and Weisblum, B., General method for direct cloning of DNA fragments generated by the polymerase chain reaction, *Nucl. Acids Res.*, **19**, 4560 (1991).

GP + PC	Larimer, F. W., and Mural, R. J., "Megabase cloning in *E. coli*," in *Abstracts of papers presented at the 1990 meeting on Genome Mapping and Sequencing*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, p. 99 (1990).
GP + PC	Leonardo, E. D., and Sedivy, J. M., A new vector for cloning large eukaryotic DNA segments in *Escherichia coli*, *Bio/Technol.*, **8**, 841 (1990).
GP + PC, ED	Li, S. J., Landers, T. A., and Smith, M. D., Electroporation of plasmids into plasmid-containing *Escherichia coli*, *Focus*, **12**, 72 (1990).
GP + PC	Lyra, C., Savilahti, H., and Bamford, D. H., High-frequency transfer of linear DNA containing 5´-covalently linked terminal proteins: electroporation of bacteriophage PRD1 genome into *Escherichia coli*, *Molec. Gen. Genet.*, **228**, 65 (1991).
GP + PC	Mahillon, J., and Kleckner, N., New IS*10* transposition vectors based on a gram-positive replication origin, *Gene*, **116**, 69 (1992).
ED	Marcil, R., and Higgins, D. R., Direct transfer of plasmid DNA from yeast to *E. coli* by electroporation, *Nucl. Acids Res.*, **20**, 917 (1992).
GP + PC	Marcus, H., Ketley, J. M., Kaper, J. B., and Holmes, R. K., Effects of DNase production, plasmid size, and restriction barriers on transformation of *Vibrio cholerae* by electroporation and osmotic shock, *FEMS Microbiol. Lett.*, **68**, 149 (1990).
GP + PC	Mermelstein, L. D., Welker, N. E., Bennett, G. N., and Papoutsakis, E. T., Expression of cloned homologous fermentative genes in *Clostridium acetobutylicum* ATCC 824, *Bio/Technol.*, **10**, 190 (1992).
GP + PC	Metzger, M., Bellemann, P., Schwartz, T., and Geider, K., Site-directed and transposon-mediated mutagenesis with pfd-plasmids by electroporation of *Erwinia amylovora* and *Escherichia coli* cells, *Nucl. Acids Res.*, **20**, 2265 (1992).
GP + PC	O'Callaghan, D., and Charbit, A., High efficiency transformation of *Salmonella typhimurium* and *Salmonella typhi* by electroporation, *Mol. Gen. Genet.*, **223**, 156 (1990).
GP + PC	O'Neill, G. P., and Söll, D., Expression of the *Synechocystis* sp. strain PCC 6803 $tRNA^{Glu}$ gene provides tRNA for protein and chlorophyll biosynthesis, *J. Bacteriol.*, **172**, 6363 (1990).
GP	Ostrander, E. A., Jong, P. M., Rine, J., and Duyk, G., Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences, *Proc. Natl. Acad. Sci. USA*, **89**, 3419 (1992).
ED	Pacholczyk, T., Blakely, R. D., and Amara, S. G., Expression cloning of a cocaine- and antidepressant-sensitive human noradrenaline transporter, *Nature*, **350**, 350 (1991).
GP + PC	Patel, K., Cox, R., Shipley, J., Kiely, F., Frazer, K., Cox, D. R., Lehrach, H., and Sheer, D., A novel and rapid method for isolating sequences adjacent to rare cutting sites and their use in physical mapping, *Nucl. Acids Res.*, **19**, 4371 (1991).
GP + PC	Petzel, J. P., and McKay, L. L., Molecular characterization of the integration of the lactose plasmid from *Lactococcus lactis* subsp. *cremoris* SK11 into the chromosome of *L. lactis* subsp. *lactis*, *Appl. Environ. Microbiol.*, **58**, 125 (1992).
GP + PC	Pfau, J., and Youderian, P., Transferring plasmid DNA between different bacterial species with electroporation, *Nucl. Acids Res.*, **18**, 6165 (1990).
GP + PC	Raya, R. R., and Klaenhammer, T. R., High-frequency plasmid transduction by *Lactobacillus gasseri* bacteriophage øadh, *Appl. Environ. Microbiol.*, **58**, 187 (1992).
ED	Regué, M., Enfedaque, J., Camprubí, S., and Tomás, J. M., The O-antigen lipopolysaccharide is the major barrier to plasmid DNA uptake by *Klebsiella pneumoniae* during transformation by electroporation and osmotic shock, *J. Microbiol. Meth.*, **15**, 129 (1992).
GP + PC	Rice, G. C., Goeddel, D. V., Cachianes, G., Woronicz, J., Chen, E. Y., Williams, S. R., and Leung, D. W., Random PCR mutagenesis screening of secreted proteins by direct expression in mammalian cells, *Proc. Natl. Acad. Sci. USA*, **89**, 5467 (1992).

GP + PC	Rodriguez-Palenzuela, P., Burr, T. J., and Collmer, A., Polygalacturonase is a virulence factor in *Agrobacterium tumefaciens* biovar 3, *J. Bacteriol.*, **173**, 6547 (1991).
GP + PC	Rubenstein, J. L. R., Brice, A. E. J., Ciaranello, R. D., Denney, D., Porteus, M. H., and Usdin, T. B., Subtractive hybridization system using single-stranded phagemids with directional inserts, *Nucl. Acids Res.*, **18**, 4833 (1990).
SW	Sale, A. J. H., and Hamilton, W. A., Effects of high electric fields on microorganisms, I. Killing of bacteria and yeasts, *Biochim. Biophys. Acta*, **148**, 781 (1967).
UK	Sale, A. J. H., and Hamilton, W. A., Effects of high electric fields on micro-organisms, III. Lysis of erythrocytes and protoplasts, *Biochim. Biophys. Acta*, **163**, 37 (1968).
GP + PC	Schendel, F. J., August, P. R., Anderson, C. R., Hanson, R. S., and Flickinger, M. C., Cloning and nucleotide sequence of the gene coding for citrate synthase from a thermotolerant *Bacillus* sp., *Appl. Environ. Microbiol.*, **58**, 335 (1992).
GP + PC	Sheen, J., "High-efficiency transformation by electroporation," in *Current Protocols in Molecular Biology*, supplement 5, pp. 1.8.4 - 1.8.7, 1989, F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, John A. Smith, K. Struhl, eds., John Wiley & Sons, Inc., New York.
SW	Sixou, S., Eynard, N., Escoubas, J. M., Werner, E., and Teissié, J., Optimized conditions for electrotransformation of bacteria are related to the extent of electropermeabilization, *Biochim. Biophys. Acta*, **1088**, 135 (1991).
ED	Smith, M., Jessee, J., Landers, T., and Jordan, J., High efficiency bacterial electroporation: 1 x 10^{10} *E. coli* transformants/µg, *Focus*, **12**, 38 (1990).
ED	Speyer, J. F., A simple and effective electroporation apparatus, *BioTechniques*, **8**, 28 (1990a).
ED	Speyer, J. F., Multi-sample electroporation, *BioTechniques*, **8**, 508 (1990b).
GP + PC	Starr, L., and Huse, W. D., Electro-transformation of *E. coli* with M13 DNA, Bio-Rad Laboratories Bulletin 1353 (1990).
GP + PC	Summers, D. K., and Withers, H. L., Electrotransfer: direct transfer of bacterial plasmid DNA by electroporation, *Nucl. Acids Res.*, **18**, 2192 (1990).
GP + PC	Sung, L. M., Jackson, M. P., O'Brien, A. D., and Holmes, R. K., Transcription of the Shiga-like toxin type II and Shiga-like toxin type II variant operons of *Escherichia coli*, *J. Bacteriol.*, **172**, 6386 (1990).
GP + PC	Swaroop, A., Xu, J., Agarwal, N., and Weissman, S. M., A simple and efficient cDNA library subtraction procedure: isolation of human retina-specific cDNA clones, *Nucl. Acids Res.*, **19**, 1954 (1991).
GP + PC	Takahashi, N., and Kobayashi, I., Evidence for the double-strand break repair model of bacteriophage λ recombination, *Proc. Natl. Acad. Sci. USA*, **87**, 2790 (1990).
GP (no PC)	Taketo, A., DNA transfection of *Escherichia coli* by electroporation, *Biochim. Biophys. Acta*, **949**, 318 (1988).
GP (no PC)	Taketo, A., RNA transfection of *Escherichia coli* by electroporation, *Biochim. Biophys. Acta*, **1007**, 127 (1989).
GP + PC	Taylor, L. D., and Burke, Jr., W. F., Transformation of an entomopathic strain of *Bacillus sphaericus* by high voltage electroporation, *FEMS Microbiol. Lett.*, **66,** 125 (1990).
GP + PC	Thomson, A. M., and Flint, H. J., Electroporation induced transformation of *Bacteroides ruminicola* and *Bacteroides uniformis* by plasmid DNA, *FEMS Microbiol. Lett.*, **61**, 101 (1989).
GP + PC	Tobin, J. F., Laban, A., and Wirth, D. F., Homologous recombination in *Leishmania enriettii*, *Proc. Natl. Acad. Sci. USA*, **88**, 864 (1991).
GP + PC	Van Camp, W., Bowler, C., Villarroel, R., Tsang, E. W. T., Van Montagu, M., and Inzé, D., Characterization of iron superoxide dismutase cDNAs from plants obtained by genetic complementation in *Escherichia coli*, *Proc. Natl. Acad. Sci. USA*, **87**, 9903 (1990).

GP + PC	Wang, M. L., Leitch, A. R., Schwarzacher, T., Heslop-Harrison, J. S., and Moore, G., Construction of a chromosome-enriched HpaII library from flow-sorted wheat chromosomes, *Nucl. Acids Res.*, **20**, 1897 (1992).
GP + PC	Ward, L. J. H., and Jarvis, A. W., Rapid electroporation-mediated plasmid transfer between *Lactococcus lactis* and *Escherichia coli* without the need for plasmid preparation, *Lett. Appl. Microbiol.*, **13**, 278 (1991).
GP	Ware, C. E., Bauchop, T., Hudman, J. F., and Gregg, K., Cryptic plasmid pBf1 from *Butyrivibrio fibrisolvens* AR10: its use as a replicon for recombinant plasmids, *Curr. Microbiol.*, **24**, 193 (1992).
GP	Willson, T. A., and Gough, N. M., High voltage *E. coli* electro-transformation with DNA following ligation, *Nucl. Acids Res.*, **16**, 11820 (1988).
GP (no PC)	Wirth, R., Friesenegger, A., and Fiedler, S., Transformation of various species of gram-negative bacteria belonging to 11 different genera by electroporation, *Molec. Gen. Genet.*, **216**, 175 (1989).
GP + PC	Ymer, S., Heat inactivation of DNA ligase prior to electroporation increases transformation efficiency, *Nucl. Acids Res.*, **19**, 6960 (1991).
SW	Yuan, L., Lian-ying, S. and Wong, T., Study on transfer plasmid PBCI by electric-pulse fusion in Butirosin producing strain *Bacillus circulans* 3342 and *E. coli* C600, *Chinese J. Antibiotics*, **9**, 450 (1984). [Chinese]
GP + PC	Zabarovsky, E. R., and Winberg, G., High efficiency electroporation of ligated DNA into bacteria, *Nucl. Acids Res.*, **18**, 5912 (1990).
GP + PC	Zink, A., Klein, J. R., and Plapp, R., Transformation of *Lactobacillus delbrückii* ssp. *lactis* by electroporation and cloning of origins of replication by use of a positive selector, *FEMS Microbiol. Lett.*, **78**, 207 (1991).

Appendix II
Field Strength and Survivability Studies of 12 *E. coli* Strains

Electroporation is a method by which *E. coli* can be transformed to high efficiencies. Using the E. coli Pulser apparatus, transformation efficiencies of 10^9 to 10^{10} transformants/µg can be obtained with various *E. coli* strains with pTZ18U DNA (Table 2). The transformability and viability of *E. coli* are sensitive to the initial field strength of the pulse. With the time constant fixed at 5 milliseconds, we looked at the effect of field strength on transformation efficiency for twelve different *E. coli* strains.

The *E. coli* strains were grown to an ABS_{600} of 0.7 - 1.0 and prepared as in Section 5. The plasmid pTZ18U was prepared by double banding in CsCl and resuspending in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA). The preparation contained about 90% supercoiled DNA and 10% relaxed circular DNA. The DNA concentration was determined by measuring the absorbance at 260 nm. In addition, agarose gel electrophoresis revealed no contaminating material. Each strain was tested using DNA from the same dilution tube. The cells (40 µl) were electroporated with 10 pg pTZ18U (1 µl) in a chilled 0.1 cm gap cuvette. The detailed electroporation conditions are described in Section 5. The pulse parameters used in these studies were a field strength range from 10 kV/cm to 22 kV/cm, and a time constant of 5 milliseconds.

Field strengths from 16 to 19 kV/cm were required to obtain maximum transformation as shown in Figures 8 and 9. If one is interested in achieving the maximum transformation efficiency, it is best to determine the optimum field strength for the *E. coli* strain of choice. Cell survival declines steadily with increasing field strength, as shown in Figures 10 and 11. The maximum transformation efficiency is reached when 40 to 50% of the cells survive the pulse.

Table 2. Transformation Efficiencies of 12 *E. coli* Strains

The optimum field strength was determined for each of the strains with pTZ18U DNA.

E. coli strain	Optimum Field Strength (kV/cm)	Transformation Efficiencies (transformants/µg)
WM1100	16.0	6.3×10^9
K12	17.0	2.2×10^{10}
MC1061	17.0	1.7×10^{10}
NM522	17.0	5.2×10^9
JS4	18.0	1.2×10^{10}
LE392	18.0	1.0×10^{10}
JM109	18.0	5.7×10^9
HB101	18.0	5.1×10^9
C600	19.0	1.2×10^{10}
CJ236	19.0	1.1×10^{10}
MV1190	19.0	7.7×10^9
JS5	19.0	5.9×10^9

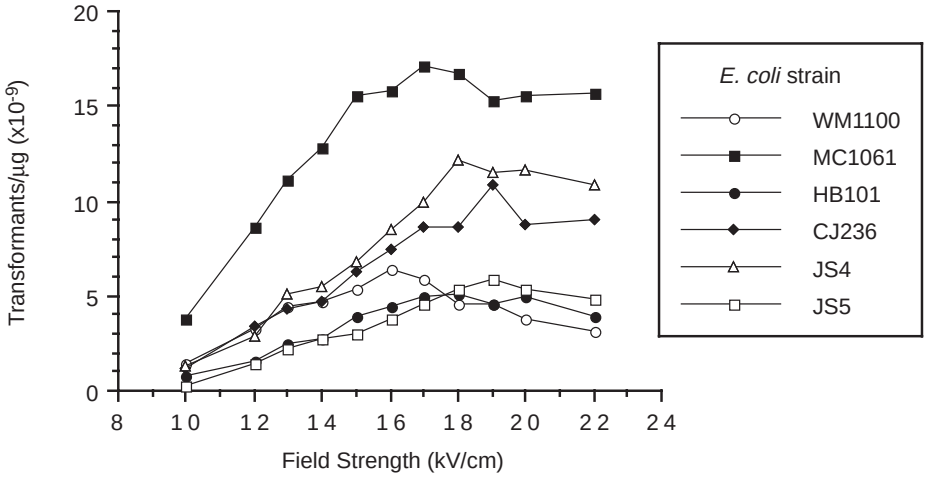

Fig. 8. Effect of field strength on transformation of 6 *E. coli* strains. Experiments were performed with the E. coli Pulser apparatus and 0.1 cm cuvettes as described above.

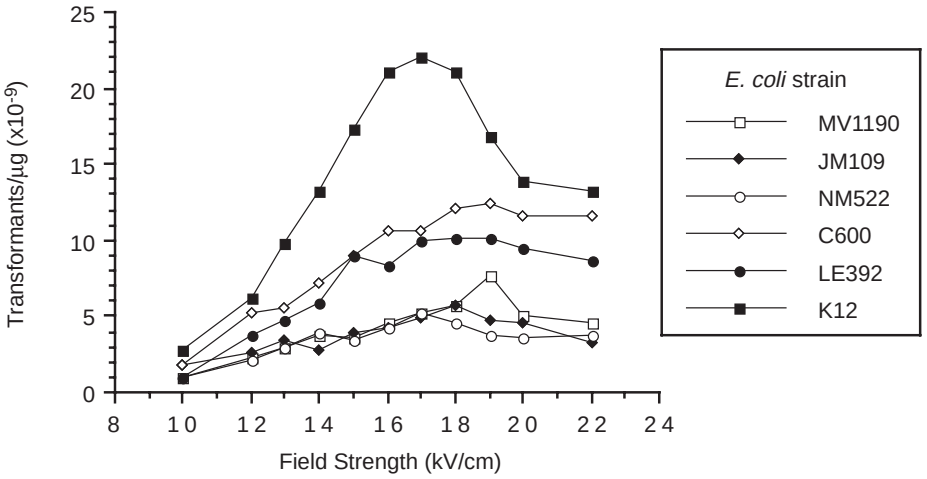

Fig. 9. Effect of field strength on transformation of 6 *E. coli* strains. Experiments were performed with the E. coli Pulser apparatus and 0.1 cm cuvettes as described above.

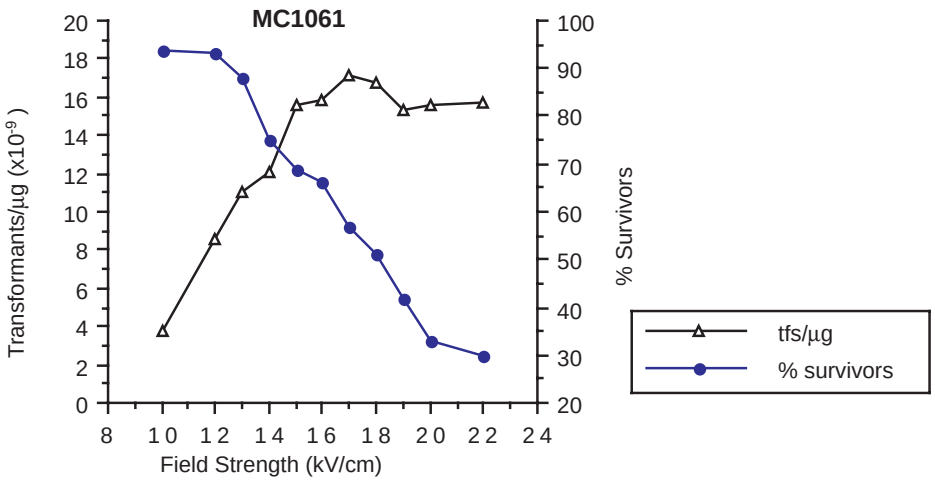

Fig. 10. The effect of field strength on the transformation efficiency (Δ) and on cell survival (•) is displayed for *E. coli* strain MC1061.

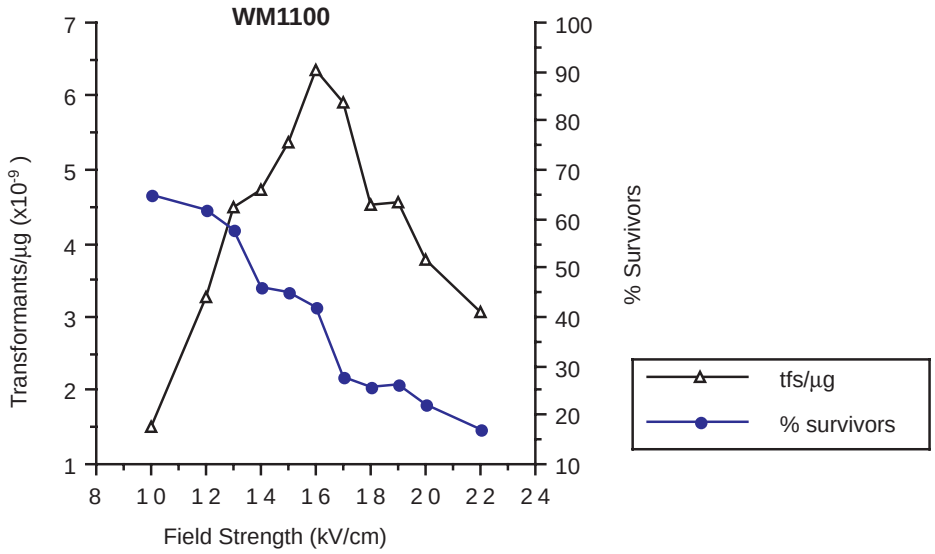

Fig. 11. The effect of field strength on the transformation efficiency (Δ) and on cell survival (•) is displayed for *E. coli* strain WM1100.

Appendix III
Troubleshooting Guide
for the E. coli Pulser Apparatus

Operational

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. When the buttons are pressed, the unit does not indicate “Chg”, or the unit continues to flash “Chg” and the tone does not sound, or the display goes blank.	No pulse delivery. Pulse buttons are not depressed long enough. Turn power switch off and on twice to bleed any residual charge in the capacitor, or re-press both buttons to continue. If problem persists, contact Bio-Rad. Only one pulse button is depressed. Both of the red pulse buttons must be pressed for pulse delivery until the tone sounds.

Electrical

Problem	Likely cause and solution
1. Arcing in the cuvette.	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 preparation, or residual salts from ethanol precipitation or ligation. Electroporation with cuvettes above 0 °C.

Problem	Likely cause and solution
2. Wrong time constant.	When using the E. coli Pulser apparatus, the time constant should be close to 5 msec. 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". Correct the problem of high conductivity by additional washing of the cells, or removal of salts from the DNA preparation.
3. Sample does not "twitch".	This may mean that the pulse is not reaching the sample. Check the connections between the E. coli Pulser apparatus and sample chamber. Check to see that the contacts in the base of the sample chamber are not broken.
4. Instrument displays "no" on front panel.	The absence of a twitch does not always mean an error. At voltages below 1.50 kV the pulse may not be strong enough to cause the sample to twitch. Sometimes the effect is simply difficult to see. Check connections. SET VOLTS (KV) not set to 0.20 kV or higher.

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 with a small-volume (40 µl) sample 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. Also make sure that the cuvette is making contact with the electrodes at the back of the sample chamber. Replace electrodes (catalog number 165-2099) if broken or corroded. Are the amplitude and length of the pulse sufficient? *E. coli* require pulses of approximately 5 milliseconds with field strengths of 12 to 18 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

Problem	Likely cause and solution
	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, use a pulse length of ~5 msec and test for transformation over a range of field strengths.
2. The DNA.	Check the quantity and quality of the DNA on a gel. Often, mini-preps contain less DNA than expected. DNA stored improperly 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 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 transfomation.
	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 *E. coli* this is very important.
	Is the correct selection applied after the recovery period?
4. The 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)?

Appendix IV
Product Information

Catalog Number	Product Description
165-2101	**E. coli Pulser Apparatus,** 100 V (for use in Japan) includes chamber with power leads, 6 sterile sample cuvettes (3 0.2 cm gap and 3 0.1 cm gap), cuvette rack
165-2102	**E. coli Pulser Apparatus,** 120 V (for use in North America and Taiwan)
165-2103	**E. coli Pulser Apparatus,** 220 V (for use in Europe, Hong Kong, and the Middle East)
165-2104	**E. coli Pulser Apparatus,** 240 V (for use in Asia, Australia, and the U.K.)
165-2086	**E. coli Pulser/Gene Pulser Cuvettes,** 0.2 cm electrode gap, 50, sterile
165-2089	**E. coli Pulser/Gene Pulser Cuvettes,** 0.1 cm electrode gap, 50, sterile
165-2095	**E. coli Pulser/Gene Pulser Cuvette Rack**
165-2097	**E. coli Pulser/Gene Pulser Chamber**
165-2099	**E. coli Pulser/Gene Pulser Chamber Electrode Contacts,** 1 pair
170-3105	**Electro-Competent *E. coli* Strain WM1100,** includes 0.5 ml *E. coli* cells, 50 µl (10 pg/µl) control plasmid DNA, instructions
170-3106	**Electro-Competent *E. coli* Strain MC1061,** 0.5 ml
170-3113	**Electro-Competent *E. coli* Strain HB101,** 0.5 ml
170-3114	**Electro-Competent *E. coli* Strain CJ236,** 0.5 ml
170-3115	**Electro-Competent *E. coli* Strain MV1190,** 0.5 ml
170-3116	**Electro-Competent *E. coli* Strain JS4,** 0.5 ml
170-3117	**Electro-Competent *E. coli* Strain JS5,** 0.5 ml

Specifications

Input voltage	100 V RMS, 50/60 Hz 120 V RMS, 50/60 Hz 220 V RMS, 50/60 Hz 240 V RMS, 50/60 Hz
Input current	2 amp RMS
Maximum output voltage and current	2,500 V peak into ≥3.3 kΩ load limited to 125 amp peak max.
Output waveform	Decaying exponential waveform with RC time constant of 5 msec, assuming loads of ≥3.3 kΩ
Output voltage adjustment	Voltage adjustable in 200-2,500 V range with 10 V resolution, 2 preprogrammed voltage steps
Ambient operating temperature	0 to 35 °C
Dimensions (L x W x H)	29 x 17 x 19 cm
Weight	7.0 kg

Bio-Rad Laboratories

Life Science Group

2000 Alfred Nobel Drive
Hercules, California 94547
Telephone (510) 741-1000
Fax: (510) 741-5800
www.bio-rad.com

Australia, *Bio-Rad Laboratories Pty. Ltd., Block Y, Unit 1, Regents Park Industrial Estate, 391 Park Road, Regents Park, NSW 2143 Phone 02 9914 2800 • Fax 02 9914 2889*
Austria, *Bio-Rad Laboratories Ges.m.b.H., Auhofstraße 78D, A-1130 Wien • Phone (01)-877 89 01 • Fax (01)-876 56 29*
Belgium, *Bio-Rad Laboratories S.A.-N.V., Begoniastraat 5, B-9810 Nazareth • Phone 09-385 55 11 • Free Phone 0800/97032 • Fax 09-385 65 54*
Brazil, *Bio-Rad Laboratories (Brazil), Rua dos Invalidos 212 - 5 andar, Lapa - Rio de Janeiro - RJ, CEP 20331-020 • Phone 55 21 507 6191*
Canada, *Bio-Rad Laboratories (Canada) Ltd., 5671 McAdam Road, Mississauga, Ontario L4Z 1N9 • Phone (905) 712-2771 • Fax (905) 712-2990*
China, *Bio-Rad China (Beijing), Rm 615, Shang Fang Plaza, No. 27, North Third Round Center Road, West District, Beijing 100029 Phone 86-10-8201-1366/68 • Fax 86-10-8201-1367*
Denmark, *Bio-Rad Laboratories, Generatorvej 8 C, 2730 Herlev • Phone 45 44 52-1000 • Fax 45 44 52-1001*
Finland, *Bio-Rad Laboratories, Pihatörmä 1A, FIN-02240 Espoo • Phone 358 (0)9 804 2200 • Fax 358 (0)9 804 1110*
France, *Bio-Rad Laboratories, 3, Boulevard Raymond Poincaré, 92430 Marnes-la-Coquette • Phone 01 47 95 69 65 • Fax 01 47 41 9133*
Germany, *Bio-Rad Laboratories GmbH, Heidemannstraße 164, D-80939 München, Postfach 45 01 33, D-80901 München Phone 089 318 84-177 • Fax 089 318 84-123*
Hong Kong, *Bio-Rad Pacific Ltd., Unit 1111, 11/F, New Kowloon Plaza, 38 Tai Kok Tsui Road, Tai Kok Tsui, Kowloon Phone 852-2789-3300 • Fax 852-2789-1257*
India, *Bio-Rad Laboratories (India) Pvt. Ltd., B&B1, Enkay Towers Vanijyanikunj, Udhyog Vihar Phase V, Gurgaon, Haryana 122016 Phone (91-124)-6398112/113/114 • Fax (91-124)-6398115*
Israel, *Bio-Rad Laboratories, Ltd., 14 Homa Street, P.O. Box 5044, Rishon Le Zion 75150 • Phone 03 951 4124 • Fax 03 951 4129*
Italy, *Bio-Rad Laboratories S.r.l., Via M. Peroglio 23, 00144 Rome • Phone 34 91 590 5200 • Fax 34 91 590 5211*
Japan, *Nippon Bio-Rad Laboratories KK, 7-18 Higashi-Nippori 5-chome, Arakawa-ku Tokyo 116-0014 • Phone 03-5811-6270 • Fax 03-5811-6272*
Korea, *Bio-Rad Korea Ltd., Cambridge Building, 1461-15 Seocho-Dong Seocho-Ku, Seoul 137-070 • Phone 82-2-3473-4460 • Fax 82-2-3472-7003*
Latin America, *Bio-Rad Latin America, 14100 Palmetto Frontage Road, Suite 101, Miami Lakes, Florida USA 33016 • Phone 305-894-5950 • Fax 305-894-5960*
Mexico, *Bio-Rad Laboratorios Mexico, Adolfo Prieto No. 1653, Col. De Valle, CP. 03100, Mexico D.F. • Phone 52 5 534 2552 to 54 • Fax 52 5 524 5971*
The Netherlands, *Bio-Rad Laboratories B.V., Fokkerstraat 10, 3905 KV Veenendaal • Phone 0318-540666 • Fax 0318-542216*
New Zealand, *Bio-Rad Laboratories Pty Ltd., PO Box 300-571, Albany, Auckland • Phone 64-9-4152280 • Fax 64-9-443 3097*
Norway, *Bio-Rad Laboratories, Johan Scharffenbergs vei 91, N-0694 Oslo • Phone 47-23-38-41-30 • Fax 47-23-38-41-39*
Russia, *Bio-Rad Laboratorii, ul. Butirskaya 79 "B", office 156 RF-125015 Moscow • Phone 7 095 979 98 00 • Fax 7 095 979 98 56*
Singapore, *Bio-Rad Laboratories, Singapore, 211 Henderson Rd. #03-02, Henderson Industrial Park, 159552 • Phone 65-2729877 • Fax 65-2734835*
Spain, *Bio-Rad Laboratories, S.A., Lopez de Hoyos, 245-247, 28043 Madrid • Phone 34-91-590-5200 • Fax 34-91-590-5211*
Sweden, *Bio-Rad Laboratories AB, Vintergatan 1, Box 1097, S-172 22 Sundbyberg • Phone 46 (0)8-55 51 27 00 • Fax 46 (0)8-55 51 27 80*
Switzerland, *Bio-Rad Laboratories AG, Nenzlingerweg 2, CH-4153 Reinach • Phone 061-717-9555 • Fax 061-717-9550*
United Kingdom, *Bio-Rad Laboratories Ltd., Bio-Rad House, Maylands Avenue, Hemel Hempstead, Hertfordshire HP2 7TD Phone 0181 328 2000 • Free Phone 0800-181134 • Fax 01442-259118*