



**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.

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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

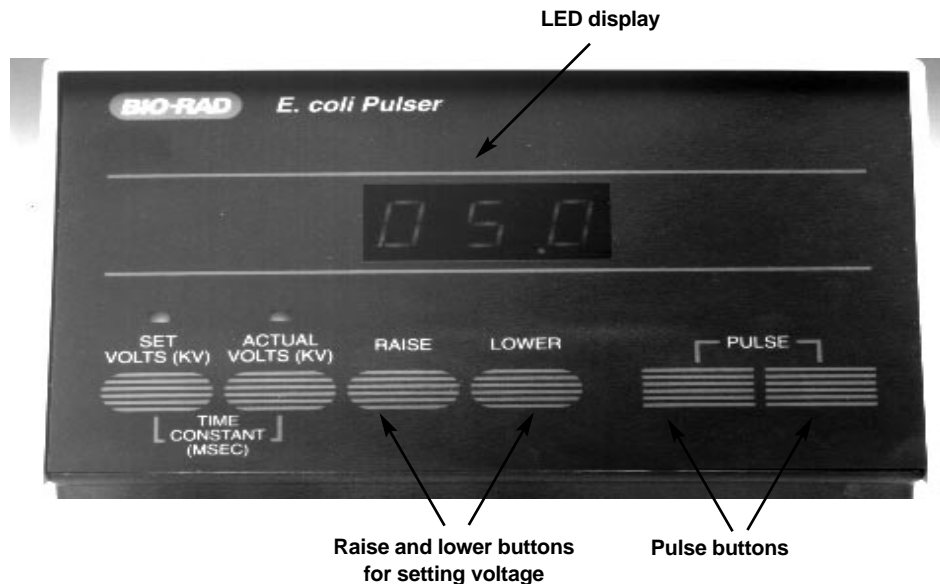


Fig 1. The front panel of the E. coli Pulser apparatus. Notice that the lights above both the SET VOLTS (KV) and ACTUAL VOLTS (KV) buttons are illuminated, indicating that the TIME CONSTANT (MSEC) is displayed in the LED>

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.

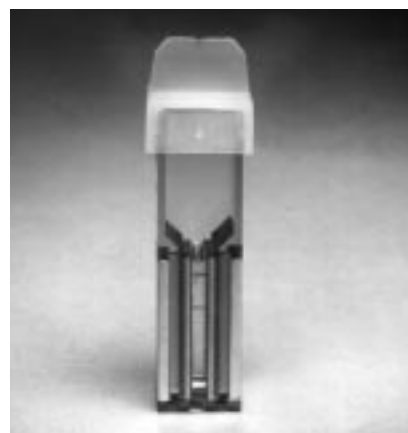


Fig. 3. 400 μ l in a 0.2 cm cuvette (the maximum volume for pulse delivery).

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 μ l) of solution may be placed in the 0.2 cm cuvette, and the 0.1 cm cuvette can hold 0.08 ml (80 μ l) 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 μ l) 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 $[R_{20}/(R_{20} + R_{\text{sample}})]$. 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%.

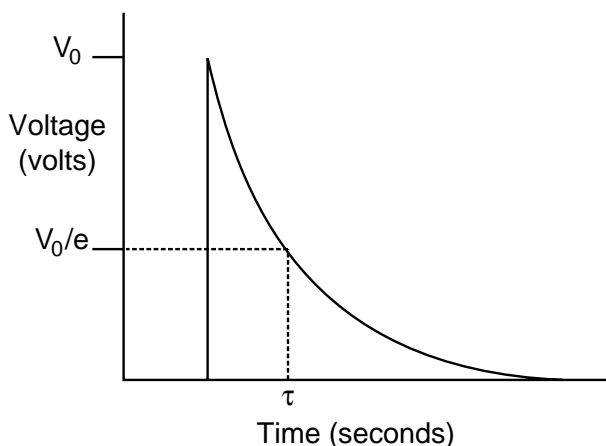


Fig. 6. Exponential decay waveform. $\tau = R \times C$.

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 *recBC*⁺ 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 μ g/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/ μ g 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 cotransformations). In each case, a small sample volume (20-50 μ l) 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 μ l (up to 400 μ l may be used in the 0.2 cm cuvette; up to 80 μ l 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 μ l 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 $4000 \times g_{\text{max}}$ 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 $1 - 3 \times 10^{10}$ cells/ml.
9. This suspension may be frozen in aliquots on dry ice, and stored at $-70\text{ }^{\circ}\text{C}$. 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 $40\text{ }\mu\text{l}$ of the cell suspension with 1 to $2\text{ }\mu\text{l}$ of DNA (DNA should be in a low ionic strength buffer such as TE^c). Mix well and let sit on ice $\sim 0.5 - 1$ 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 $37\text{ }^{\circ}\text{C}$ 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 MgCl₂, 10 mM MgSO₄, 20 mM glucose.

Appendix I

E. coli Electro-transformation References

The following literature references report the transformation of *Escherichia coli* by electro-
poration. Asterisks denote work done with the Gene Pulser[®] system.

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

- * 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 DH5 αAllen 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 DH5 α F'Marcil and Higgins (1992)
- strain DH5 α F'IQ.....Smith *et al.* (1990)

- * strain DS941Summers and Withers (1990)

- * strain EM24Thomson and Flint (1989)

- strain ER1451Sixou *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)
Regu e *et al.* (1992)
Smith *et al.* (1990)
Speyer (1990a)
Sung *et al.* (1990)
Wirth *et al.* (1989)

Escherichia coli**References**

- * strain JC8679Summers and Withers (1990)
- * strain JM101Halling and Zehr (1990)
- * strain JM105Fiedler *et al.* (1989)
Fiedler and Wirth (1988)
Jacobs *et al.* (1990)
Wirth *et al.* (1989)
- * strain JM107Dower (1987)
- * strain JM109Boivin 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 JM110Raya 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 K802Gilchrist and Smit (1991)
- * strain K803Jacobs *et al.* (1990)
Ware *et al.* (1992)
- * strain LE392Binotto *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 M5361Takahashi and Kobayashi (1990)
- * strain MC1061Calvin (1988)
Calvin and Hanawalt (1988)
Cwirla *et al.* (1990)
Dower (1990)
Dower and Cwirla (1992)
Duncan and Shivan (1989)
Hanahan *et al.* (1991)
Rubenstein *et al.* (1990)
Smith *et al.* (1990)

Escherichia coli**References**

- * 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 MM294Zink *et al.* (1991)
- * strain MV1190.....Dower (1987)
- strain NCTC 8196Hamilton and Sale (1967)
- * strain NK5898Mahillon 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-1King and Goodbourn (1992)
Pfau and Youderian (1990)
- * strain SDM.....Batt *et al.* (1990)
- * strain SM10.....Rodriguez-Palenzuela *et al.* (1991)
- * strain SR101Garrard *et al.* (1991)
- * strain SURE.....Clarke *et al.* (1992)
Wang *et al.* (1992)
- * strain TG-1.....Heery and Dunican (1989)
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- * Large DNA fragmentsLarimer and Mural (1990)
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Special Applications

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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

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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.

<i>E. coli</i> 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

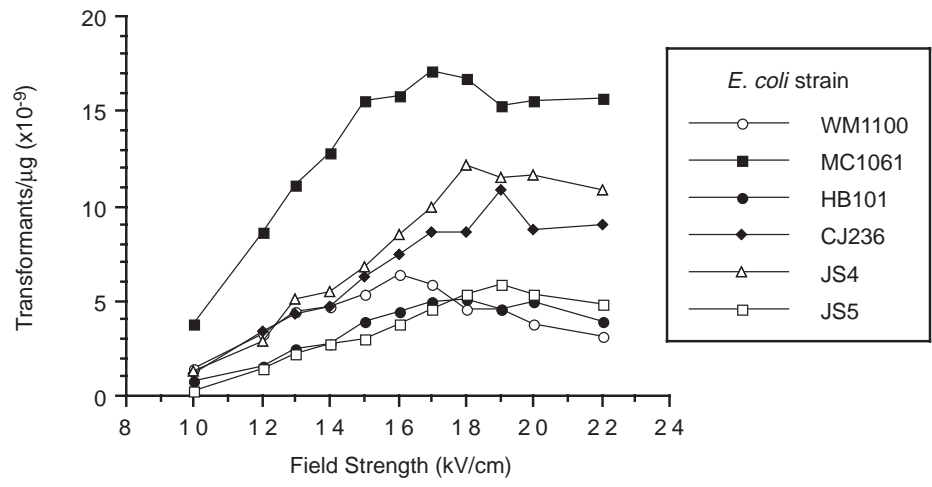


Fig. 8. Effect of field strength on transformation of 6 *E. coli* strains. Experiments were performed with the *E. coli* Pulser apparatus and 0.1 cm cuvettes as described above.

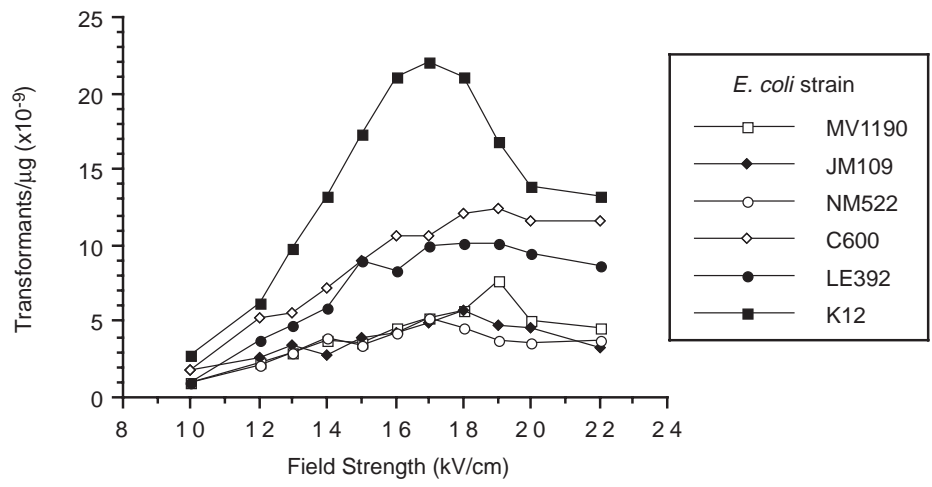


Fig. 9. Effect of field strength on transformation of 6 *E. coli* strains. Experiments were performed with the *E. coli* Pulser apparatus and 0.1 cm cuvettes as described above.

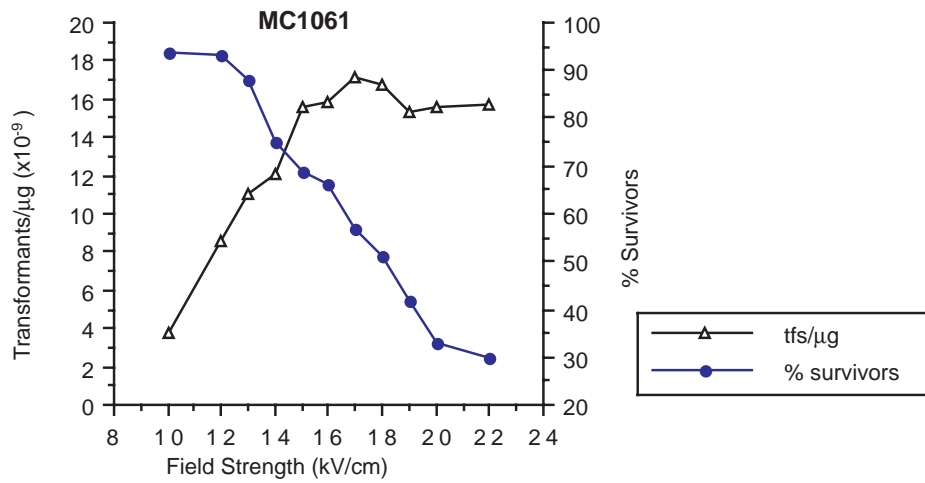


Fig. 10. The effect of field strength on the transformation efficiency (Δ) and on cell survival (\bullet) is displayed for *E. coli* strain MC1061.

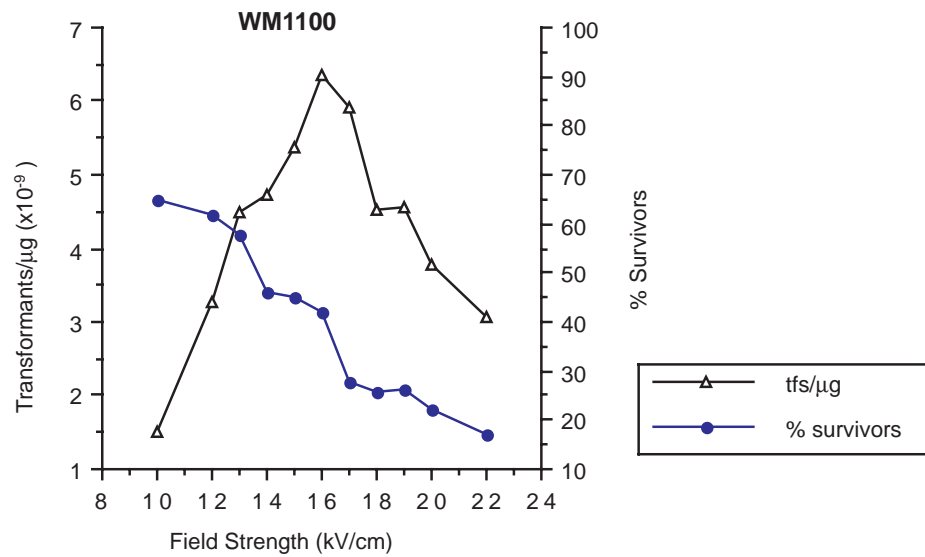


Fig. 11. The effect of field strength on the transformation efficiency (Δ) and on cell survival (\bullet) is displayed for *E. coli* strain WM1100.

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 <i>E. coli</i> , 10 meq or higher will certainly arc. There are several causes of excessive conductivity: <ol style="list-style-type: none"> 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 <i>E. coli</i> 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 <i>E. coli</i> 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 μ l) 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? <i>E. coli</i> 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 transformation.
- 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 <i>E. coli</i> Strain WM1100 , includes 0.5 ml <i>E. coli</i> cells, 50 µl (10 pg/µl) control plasmid DNA, instructions
170-3106	Electro-Competent <i>E. coli</i> Strain MC1061 , 0.5 ml
170-3113	Electro-Competent <i>E. coli</i> Strain HB101 , 0.5 ml
170-3114	Electro-Competent <i>E. coli</i> Strain CJ236 , 0.5 ml
170-3115	Electro-Competent <i>E. coli</i> Strain MV1190 , 0.5 ml
170-3116	Electro-Competent <i>E. coli</i> Strain JS4 , 0.5 ml
170-3117	Electro-Competent <i>E. coli</i> 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 k Ω load limited to 125 amp peak max.
Output waveform	Decaying exponential waveform with RC time constant of 5 msec, assuming loads of ≥ 3.3 k Ω
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

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