The Electroporator II

INSTRUCTION MANUAL

Version A
151128

25-0028

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TABLE OF CONTENTS

WARRANTY INFORMATION

Invitrogen Corporation will repair or replace this unit due to any defect or malfunction under normal use. This warranty does not cover damage caused by carelessness, misuse, or neglect, or as a result of conditions beyond our control. *This warranty does not cover power supplies used with the Electroporator II.* If during this time a unit proves defective in material or workmanship, Invitrogen will repair or replace the unit. Invitrogen Corporation is not liable for bodily harm, damage to power supplies, or for the loss of any biological sample(s) caused by use of this unit. See separate warranty card for complete information. Certain power supplies may not be compatible with this unit (see Appendix A, page 36, for details). Please call for authorization before returning the unit.

ELECTROPORATOR II SPECIFICATIONS

Power supply	Accepts common laboratory power supplies See Appendix A for details
Voltage range	100-1800 volts direct current (DC) depending on selected capacitance
Input	1-180 milliamps (mA) maximum while charging
Capacitance settings	50 microfarads (µF), 1800 V DC maximum 71 µF 1500 V DC maximum 250 µF 660 V DC maximum 500 µF 330 V DC maximum 1000 µF 330 V DC maximum
Load resistance settings	70 ohms (Ω), 100 Ω, 150 Ω, 200 Ω, 500 Ω, ∞ Ω
Waveform	Direct capacitance discharge, exponential RC time constant (τ)
Pulse lengths (t=RC)	3.5-25 milliseconds (ms) using non-conductive buffers (adjustable). <1-20 ms using conductive buffers (i.e., HBS buffered saline (adjustable)
Contacts	Internal, stainless steel contacts, accepts standard 0.1 to 0.4 cm gap width cuvettes (Potter type)
Internal resistance	5 Ω at 50 µF, less than 0.1 Ω at all other settings
Safety features	Disarming switch, cuvette cover switch, insulated case, recessed electrical contacts, protected by fuse
Charge time	Approximately 20 seconds (average) at 50 µF, 2-3 minutes at 1000 µF
Size	21 centimeters (cm) x 21 cm x 12 cm
Weight	1.5 kilograms (kg)

ELECTROPORATOR II PRECAUTIONS

Never reduce the voltage or change capacitance settings while the unit is charging. Doing so may cause backvoltage which can damage your power supply or cause some capacitors in the Electroporator II to exceed their maximum voltage ratings. Carefully read this instruction manual before operating the Electroporator II.

HIGH VOLTAGE PRECAUTIONS

Electroporation is becoming the method of choice for introducing DNA and other macromolecules into a wide variety of cells. When used properly, electroporation equipment is reliable and safe. However, the operator must be aware of the limitations and hazards of such devices. The charged capacitors of the Electroporator II and the high voltage power supplies required for use with the unit can deliver a potentially lethal shock.

The unit itself can be damaged by misuse, carelessness or operator error. Keep the unit away from water and flammable materials. Do not chill the unit on ice. Never short-circuit the output contacts or terminals. Never place your finger in the chamber or touch the cuvette while delivering a pulse.

POWER SUPPLY PRECAUTIONS

This unit is designed to work with most DC constant voltage electrophoretic and sequencing power supplies used in molecular biology laboratories. Voltage settings and measurements are taken from your existing voltmeter; the voltage input meter on the Electroporator II is for input voltage approximation (+/- 100 V accuracy) only. Before turning off your power supply, discharge the Electroporator II by setting the charge/pulse switch to *pulse*. The unit can be discharged safely without a cuvette in the cuvette chamber. Once the charge/pulse switch is in the *pulse* position, the charge will dissipate internally across the selected load resistor and will cause no damage.

Calibration: Be sure your power supply is accurately calibrated. If in doubt, use a separate, dependable voltmeter to take the voltage measurements. Electroporation results from unit are dependent on accurate voltage delivery. The peak voltage delivered will be 96–99% of the charging voltage when fully charged.

Voltage Fluctuation: Some power supplies may experience voltage fluctuations while charging. Many power supplies will drop in voltage momentarily when the Electroporator II is charging then return to the original voltage setting. This fluctuation will not damage either the power supply or the Electroporator II and will still allow for the unit to be charged completely.

The voltage output of some power supplies, however, may increase as much as 25-50% while charging the unit. This increased voltage reading on the power supply reflects the actual voltage being delivered to the Electroporator II and may be detrimental to the capacitors of the unit. For this reason, test the unit in conjunction with the power supply before electroporating samples (Chapter 1, Testing Unit Before Use, page 11). If your power supply behaves incorrectly, it is recommended that an alternate power supply be used with the Electroporator II.

POWER SUPPLY PRECAUTIONS
(continued)

Maximum Voltage: Set the power supply to voltages no higher than 1800 V. Exceeding the maximum input voltage ratings of the capacitors may cause a short circuit which can damage your power supply. The voltage input meter has been added to the Electroporator II for verification of the power supply voltage setting. Never exceed the maximum input voltage recommended for *any* capacitance selection. Doing so may short circuit your power supply and possibly damage the electrolytic capacitors in the Electroporator II. Check the capacitor/voltage rating marked on the Electroporator II before applying power to the unit. For example, at the 1000 µF capacitance setting, set the power supply no higher than 330 V. At the 50 µF setting, set the power supply to no higher than 1800 V.

Power Supply Compatibility: Due to low internal resistance, the Electroporator II is compatible with most common power supplies. However, if your power supply fails to charge the Electroporator II or exhibits unexpected behavior, please contact Invitrogen Technical Services at 1-800-955-6288.

ARCING PRECAUTIONS

The cuvette may occasionally arc at high voltages, causing the cuvette cap and contents to be displaced upwards. Tightly cap the cuvette and close the cuvette cover before delivering a pulse. The use of safety glasses is recommended. See Chapter 5, Troubleshooting, for more information on arcing.

FUSE ASSEMBLY

The fuse assembly protects against possible damage to your power supply. Fuses are available from most electronic supply stores: Use only 1/32 amp, 250 V, time-delay safety fuses. The 1/32 amp fuse in the Electroporator II does not guarantee protection when the safety guidelines are not followed. See Chapter 5, Troubleshooting (page 35), for details on replacing the fuse.

Failure to follow these guidelines negates the product warranty and may result in damage to your power supply.

INTRODUCTION TO THE ELECTROPORATOR II

Utilizing the diagrams below, please familiarize yourself with the Electroporator II. Before the unit is connected to a power supply, become accustomed to setting the dials and operating the switches.

Figure 1: The Electroporator II

- **A** Capacitance Dial
- **B** Arm/Disarm Dial
- **C** Armed Light
- **D** Charging Light
- **E** Voltage Input Meter
- **F** Cuvette Chamber
- **G** Pulse Indicator Light
- **H** Charge/Pulse Switch
- **I** Resistance Dial

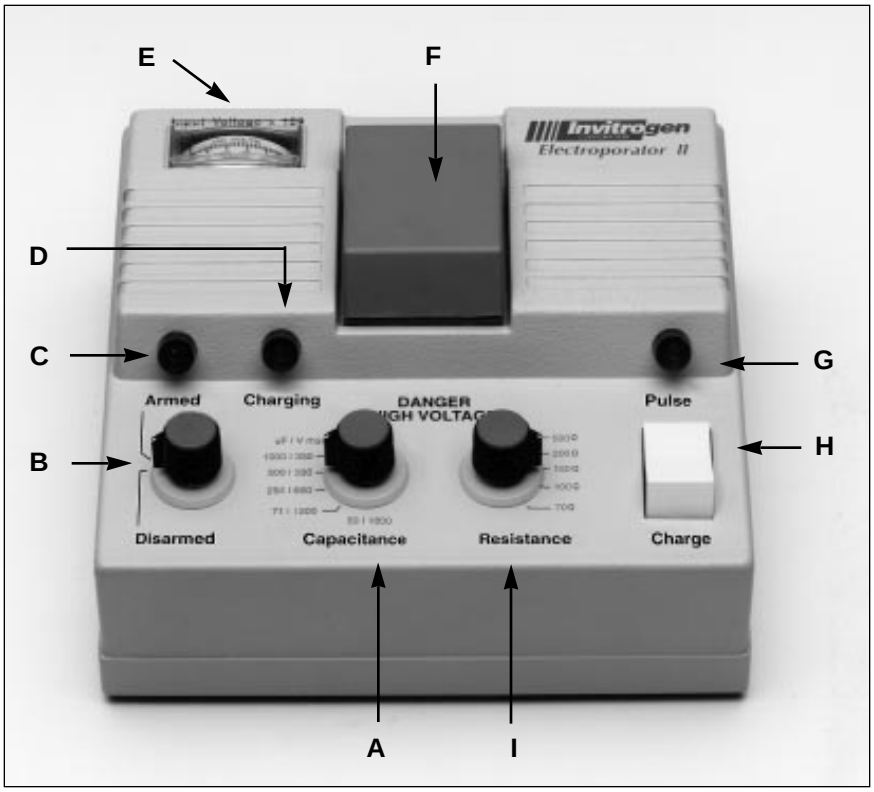
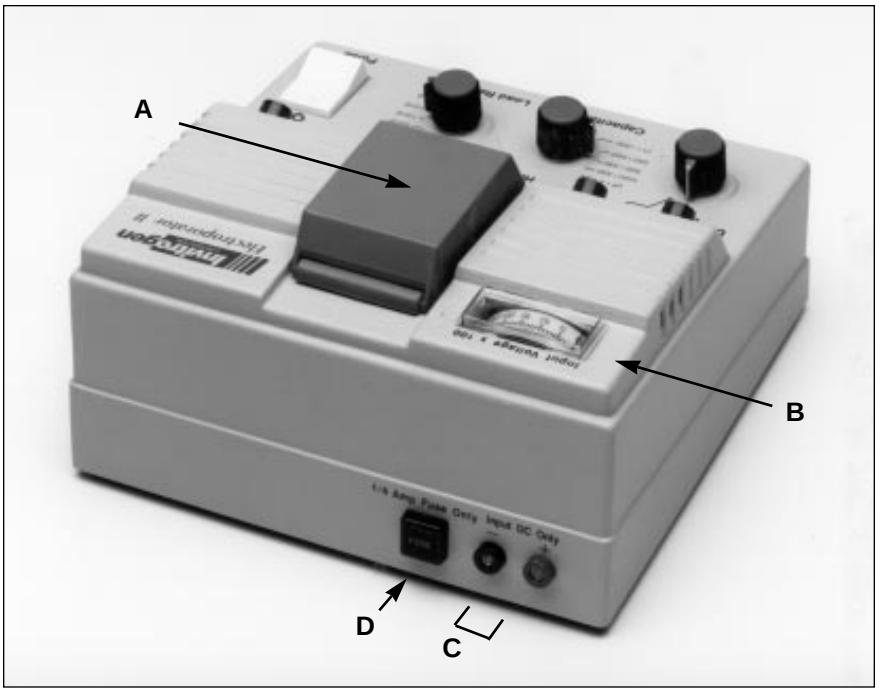


Figure 2: Back view of the Electroporator II

- **A** Cuvette Chamber
- **B** Voltage Input Meter
- **C** Voltage Input Terminals
- **D** Fuse Assembly

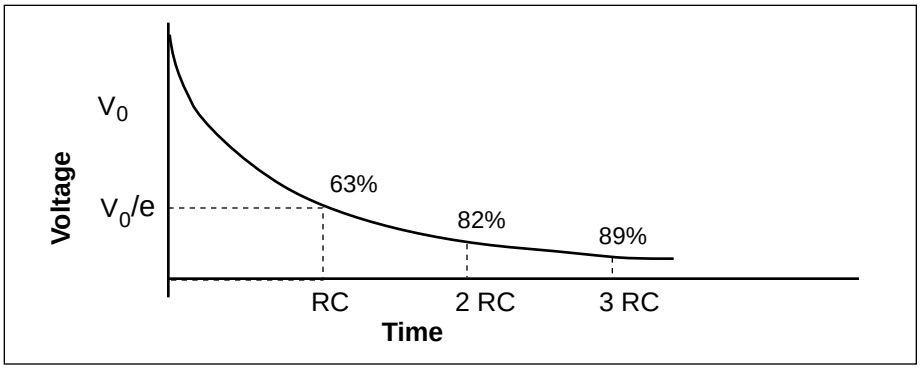


THE BASICS OF ELECTROPORATION

First described in 1982, electroporation is a method of introducing macromolecules such as DNA into cells by using a brief electrical pulse to cause transient membrane pore formation. This method usually produces better results than chemical transformation, and is successful with many cell types resistant to other methods of transformation. Large molecules are also electroporated at higher efficiencies. Maximum efficiency of electroporation occurs when the pulse is strong enough to yield 40-80% cell death. Each cell type requires an optimal set of parameters to produce a maximum number of transformants; these values are usually determined experimentally and are widely published in biological journals.

Electrical pulses are generated by discharging a capacitor through a cell suspension and parallel load resistor. The characteristics of a simple capacitance discharge are shown in the diagram below.

Figure 3: Simple capacitance discharge (exponential decay).



The voltage of such a pulse starts at the initial (peak) value and decays exponentially according to the equation $V_t = V_0 e^{-t/RC}$ where R is equal to the resistance and C is equal to the capacitance of the circuit. The pulse length, t, is defined as the time required to drop to 1/e (37%) of its initial value.

Therefore, the pulse length τ can be expressed as τ **= RC** where τ is in seconds, **R** is in ohms (Ω) and **C** is in farads (**F**). The pulse length is also called the "RC time constant." The resistance **R** of the circuit is the net effective impedance of the discharge pathway (the sum of the resistance of the cell suspension and the load resistor in parallel).

When the cell buffer has a very high resistance (such as 10% glycerol) the pulse length is determined almost solely by the capacitance selection (μF) and the parallel load resistance (Ω). When the cell buffer has a very low resistance (such as HBS), the pulse length is determined by the capacitance selection and the volume of the cell suspension. Because of slight differences in conductivity or cuvette geometry, pulse lengths may vary slightly from sample to sample.

The two most important electrical parameters for electroporation consideration are pulse length and field strength. Field strength is defined as volts/centimeter (V/cm) where V is equal to the initial peak voltage, and cm is equal to the measurement of the gap between the electrodes of the

cuvette in centimeters. Other important considerations are buffer composition and temperature of the electroporation buffer and cuvette. The pulse discharge will generate heat within the sample which can affect cell survival.

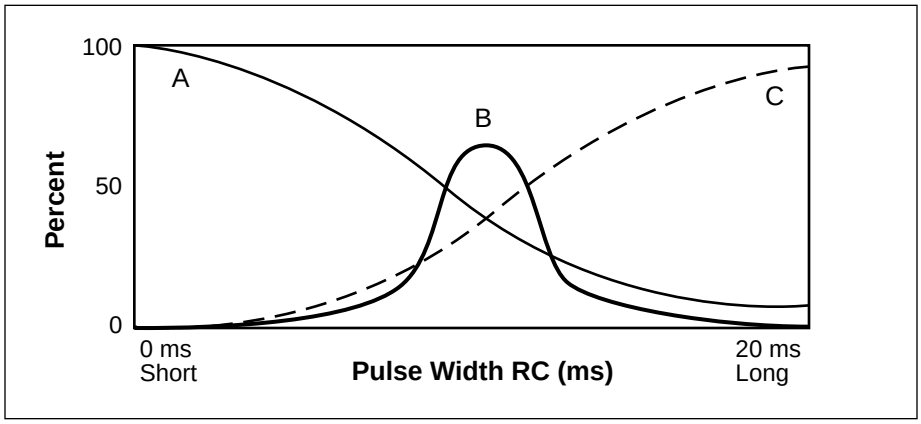
Within limits, field strength and pulse length are inversely related. A shorter pulse length can usually be compensated for by a higher field strength. Multiple pulses do not increase transformation efficiencies. Bacteria require much higher field strengths (6-25 kV/cm) than most eukaryotic cells. Mammalian cells are transformed more reliably at lower field strengths (350-750V/cm) with longer pulse lengths (5-25 milliseconds). Cells with exposed membranes should not be electroporated at cell densities greater than 1-2 x 10^6 cells/ml. Higher cell densities may give rise to a significant proportion of cell fusions (electrofusion).

The capacitors used in this unit have internal resistances which can affect the pulse length slightly. Some cell types require that parameters must be reproduced exactly. Conditions can be optimized by trial. The conductance of a cell suspension can be measured by using an AC impedance meter at approximately 200 Hz.

Do not use the 50 µF capacitance setting when HBS or other conductive electroporation buffer is used (any mammalian cell electroporation buffer). The 50 µF capacitor has a 5 Ω resistor in series and under these conditions, a 10-20% drop in the peak output voltage will occur. There is no significant voltage drop when using 10% glycerol, 1 M sorbitol, or buffered sucrose. The 5 Ω resistor is essential and helps protect the unit from damage caused by arcing at high voltages when electroporating bacteria.

Figure 4 below shows the relationship between cell viability after electroporation, electroporation efficiency and cell membrane pore formation as pulse width is varied and the voltage gradient is constant.

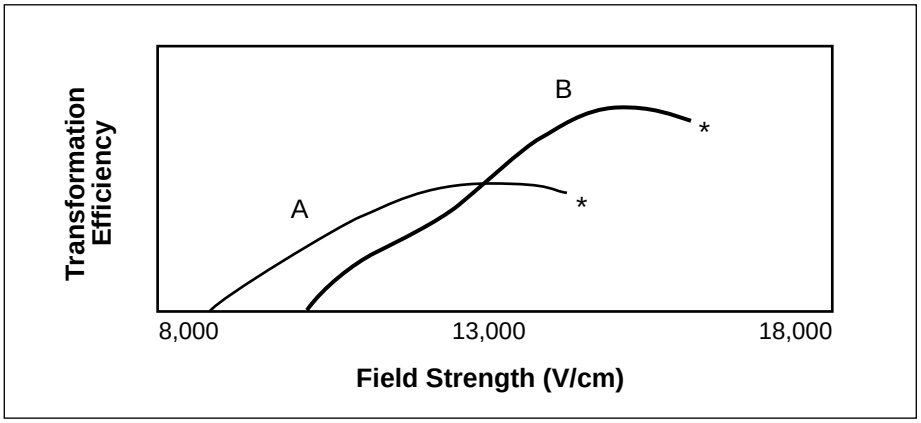
Figure 4: Diagram of expected results using a fixed voltage gradient



Curve A: percent viable cells
Curve B: resulting number of transformants (*E. coli*)
Curve C: transient pore formation

Figure 5 shows results from electroporation of INVαF' *E. coli* with 10 pg of supercoiled pUC18 plasmid. The Electroporator was set to 50 µF capacitance and 150 Ω resistance. The input voltage was varied to demonstrate that an optimal voltage gradient (or field strength) is achieved with a 0.1 cm gap width cuvette at 1500 V.

Figure 5: Diagram of expected results using a fixed pulse length



Curve A: 0.2 cm cuvette
Curve B: 0.1 cm cuvette
* Denotes high occurrance of arcing (field strengths above this point are not recommended)

TESTING UNIT BEFORE USE

The Electroporator II may cause some power supplies to perform differently than during normal electrophoresis or sequencing operation. The voltage output of some commonly used power supplies may rise or behave abnormally during use. The following steps must be taken to insure accurate and safe voltage settings. It is especially important to familiarize yourself with the operation of the unit before attempting electroporation at moderate to high voltages.

1. Place the unit on a dry benchtop near your direct current electrophoresis or sequencing power supply. *Be sure your power supply is off*.

Figure 6. Connecting Electroporator II unit to power supply

2. With the Electroporator II charge/pulse switch in the *pulse* position, connect the output of your power supply to the input jacks on the back side of the unit using the supplied leads. Connect red leads to the red terminals (+) and the black leads to black terminals (-).

3. If your power supply has a constant voltage or constant current switch, choose the constant voltage position. If the voltage meter on the power supply has overlapping scales, select the proper scale (i.e. for this test, a scale reading of 0 – 500 V is sufficient).

4. Set the arm/disarm dial to *disarmed,* the capacitance selector switch to 500 µF and the load resistance switch to 500 Ω. No cuvette is needed.

5 Turn on your power supply. If your power supply has a “DC ON” or similar button, activate it. If your power supply has power or current-limiting capability, set the maximum power to 25 W and the current to 25 mA. Set the power supply to deliver 150 volts. Allow the power supply to stabilize while the charge/pulse switch on the Electroporator II is still in the *pulse* (discharge) position.

6. Now press the charge/pulse switch to the *charge* position. The charging lamp should start to glow after 15–20 seconds. If it does not, set the charge/pulse switch to *pulse*, then turn off your power supply and see Section 5, Troubleshooting, page 32. The voltage output of most power

TESTING UNIT BEFORE USE *(continued)*

supplies will drop momentarily while the capacitors charge, then rise again as the capacitors become stabilized to the set voltage. *Important: Do not change the capacitance selector switch on the Electroporator II or turn off your power supply while charging or both may be damaged.* Check the voltage meter again to see that it reads 150 V.

6. Set the arm/disarm dial to the *armed* position. The armed light will glow. Note: the armed light will blink when the Electroporator II is charged at voltages below 150 V for better visibility.

7. Discharge the capacitors through the load resistor by switching the charge/pulse switch to the *pulse* position. The pulse light should glow briefly and both the charging light and the armed light should go out.

8. When finished testing, leave the switch in the *pulse* (discharge) position.

If any part of the test fails, refer to Section 5, Troubleshooting (page 32). This test may be repeated at different settings and voltages until you are comfortable with the operation and voltage changes of your power supply. **Caution:** The Electroporator II will not discharge internally at $\infty\ \Omega$. Do not use that setting for this test.

DNA PREPARATION FOR BACTERIAL ELECTROPORATION

Contaminants: Residual salts in DNA preparations can raise the conductivity of the cell suspension significantly. This will greatly increase the possibility of arcing and affect the pulse length. Never electroporate all of your valuable DNA at one time. Occasionally the sample may arc, causing complete loss of your sample. Arcing is most likely at higher voltage gradients (>12 kV/cm) and when the sample preparation has a high conductivity (ionic strength) due to excess NH_4^+, Na^+, Mg^{2+}, etc.

Contaminants such as proteins in the DNA preparation can inhibit electrotransformation. The presence of high amounts of T4 DNA ligase and other DNA binding proteins can significantly reduce efficiencies. Extract the DNA sample with phenol/chloroform (or heat inactivate at 65°C for 10 minutes) prior to an ethanol precipitation with sodium acetate. Wash with 70% ethanol to remove salt. When precipitating <2 µg of DNA, consider adding a carrier such as 3-5 µg of mussel glycogen (2 mg/ml), yeast tRNA, or inert polyacrylamide.

Concentration: Resuspend the DNA pellet in a small volume of low ionic strength buffer such as TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) or water. For *E. coli,* highest efficiencies are obtained using only 1-2 µl of DNA per 40 µl of cells (5% of total volume or less). Figure 7 describes concentration and amounts of supercoiled and ligated DNA recommended for bacterial electroporation.

If the DNA solution is dilute and is difficult to concentrate, the cell volume can be increased to keep the total amount of DNA solution added to 5% or less. Increasing the amount of cells used can increase the number of satellite colonies or background due to high plating densities (especially on ampicillin plates). Follow the recommendations in Figure 7 to avoid excessive background or satellite colonies.

When electroporating supercoiled DNA such as the pUC18 plasmid, always use a fresh dilution of the stock DNA for highest efficiency. Supercoiled DNA will transform 10-100 fold more efficiently than nicked (relaxed) or linear DNA. Recombination in some cells may possibly be stimulated by electroporation. Linear DNA will recombine *in vivo* more readily than circular DNA. No differences in recombination frequencies in *E. coli* using electroporation over chemical transformation have been observed.

Figure 7: Concentration and amount of DNA recommended for bacterial electroporation

DNA Type	40 µl cells in 0.1 cm gap cuvette	80 µl cells in 0.1 cm gap cuvette
Ligated	1 ng – 200 ng in 1 – 2 µl	200 ng – 500 ng in 2 – 4 µl
Supercoiled	10 pg – 10 ng in 1 – 2 µl	10 pg – 10 ng in 2 – 4µl

PREPARATION OF DNA FOR BACTERIAL ELECTROPORATION

(continued)

Linearity of Electroporation: The number of colonies resulting from electroporation is linear with respect to the number of molecules of plasmid DNA electroporated over several orders of magnitude. The level of saturation exceeds traditional heat shock methods. Although saturation curves vary with respect to plasmid size and form, in general, it is not recommended to exceed 200 ng DNA per 40 µl of cells, or 500 ng DNA per 80 µl of *E. coli.* Saturating conditions should be avoided when optimum representation is required, such as in the construction of a cDNA library.

ELECTROCOMPETENT BACTERIAL CELLS

Invitrogen offers electrocompetent *E. coli* at efficiencies as high as 1 x 10^{10} transformants per µg of supercoiled DNA, ideal for any transformation task including cDNA library construction. These Electrocomp™ Transformation Kits include 5 or 10 convenient 80 µl aliquots of electrocompetent *E. coli.* Six strains are available; see Appendix E for details. To produce electrocompetent cells in your lab, please refer to the protocol .

Preparation of Electrocompetent *E. coli*

Transformation efficiencies will vary between different electrocompetent cell preparations and between different strains. Thorough washing and gentle centrifugation are recommended to ensure high transformation efficiencies. Follow published protocols if applicable to a specific strain of bacteria. Efficiencies should range from 1 x 10^8 to 1 x 10^{10} transformants/µg DNA.

Equipment Preparation

1. Autoclave one 1 liter centrifuge bottle for each liter of cultured cells and pre-chill in a -20°C freezer. Note: Avoid washing bottles to be used for competent cell production with detergent.
2. Pre-chill sterile 50 ml cell culture tubes at -20°C. You will need one for each liter of culture.
3. Pre-chill a swinging bucket rotor in the slow-speed centrifuge at 0°C.
4. Pre-chill an SS34-type rotor at 0°C.
5. Pre-chill autoclaved, nanopure water on ice. You will need 1.5 liters for each liter of cell culture.
6. Pre-chill autoclaved 10% glycerol (made with nanopure water) on ice. You will need 42 ml for each liter of cell culture.
7. Pre-chill five sterile 25 ml pipettes at 0°C.

Electrocompetent Cell Production Protocol

Important: Cells must be kept cold at all times.

1. Grow 50 ml culture overnight in LB broth containing the appropriate antibiotic for each liter of cells to be prepared.
2. Inoculate 1 liter of LB broth (with antibiotic if appropriate) with the 50 ml of overnight culture in a 2 liter or larger flask.
3. Grow to an OD_{550} of 0.5-0.6. The cells should be in mid-log phase.
4. Transfer the culture to a chilled, 1 liter centrifuge bottle and keep on ice for 30 minutes.
5. Spin at 3500 rpm (approximately 4000 x g) in a low speed centrifuge with swinging bucket rotor for 15 minutes at 0°C.
6. Decant the broth and shake out bottle quickly to remove most of the liquid. Place on ice.

Electrocompetent Cell Production Protocol

(continued)

7. Add 50-100 ml of water in each bottle, close and place on ice. Shake to resuspend cells, then return to ice and add another 50-100 ml water. It will take several rounds of shaking to resuspend all the cells. Continue to shake and add water until cells are resuspended in 1 liter of water.

   Make sure to resuspend the entire pellet; residual salts trapped in an undissolved cell pellet will render a cell prep with high conductivity. High conductivity may result in arcing during electroporation.

8. Centrifuge at 3500 rpm (approximately 4000 x g) in low speed centrifuge for 15 minutes at 0°C. Keep the rotor cold between spins.

9. Decant water and place bottles on ice. Resuspend cells in a total of 500 ml of sterile, cold water as described above. At this point the cells should resuspend much quicker than at Step 7.

10. Centrifuge at 3500 rpm (approximately 4000 x g) in a low speed centrifuge for 15 minutes at 0°C.

11. Gently remove bottles from centrifuge; the pellet is easily disturbed at this point. Remove water and place bottles on ice. Resuspend cells in a total volume of 40 ml of ice-cold, sterile water with 10% glycerol. Remove the 50 ml tubes from the freezer and place on ice. With a pre-chilled, sterile 25 ml pipette, quickly transfer cells from bottles to 50 ml tubes.

12. Using an SS34 or similar rotor at 0°C, centrifuge tubes at 5800 rpm (4000 x g) for 15 minutes.

13. Decant glycerol and quickly shake tubes to remove most of the glycerol. Place tubes on ice immediately. Resuspend each pellet in 2 ml of sterile 10% glycerol in water by vortexing at highest setting. Pool resuspended cells into one tube. The final volume (including the volume of the pellets) will be approximately 3 ml. Keep the tube on ice at all times.

14. Aliquot into sterile microfuge tubes that have been pre-chilled on dry ice. Aliquot 80 µl per tube and store at -70°C until use. There will be approximately 36 vials per liter of cultured cells.

BACTERIAL ELECTROPORATION

The following protocol is a general reference for electroporating bacteria. During bacterial electroporation, the electroporator delivers a pulse of short duration. High field strength is necessary for efficient electroporation, therefore high input voltage and use of 0.1 cm gap cuvettes is required.

Electroporation results in efficiencies 10- to 100-fold greater than those achieved via chemical transformation methods. Efficiencies of 10^9 to 10^{10} colonies per µg supercoiled DNA are common, making electroporation ideal for all transformation needs, especially for protocols which demand high efficiency (such as cDNA library construction).

The following protocol utilizes voltage, capacitance and resistance settings sufficient for a broad range of bacteria. Protocols specific for certain strains may require a precise pulse length and field strength. The tables below aid in calculating Electroporator II settings from specific pulse length or field strength values. They may also be used to determine optimal electroporation conditions for a specific bacterial strain.

When following a protocol developed for a different electroporation device for the first time, perform a number of electroporations at the specified voltage gradient with slightly different pulse lengths (above and below the specified value). This will ensure optimal conversion of parameters.

Figure 8 describes the relationship of pulse length to resistance setting when capacitance is held constant at 50 µF. Figure 9 reflects the direct relationship of input voltage to field strength using 0.1 cm gap cuvettes.

Figure 8: Relationship between resistance and pulse length

50 µF	**RC (ms)**	—	25.0	10.0	7.5	5.0	3.5
	Ω	∞	500	200	150	100	70

Figure 9: Relationship between voltage and field strength

0.1 cm cuvette	**kV/cm**	18	15	12	9	6
	Voltage (kV)	1.8	1.5	1.2	0.9	0.6

Required Materials

Electrocompetent cells (store at -70°C, thaw on ice immediately before use)
DNA resuspended in water or TE (chilled on ice)
Ice bucket for cells and DNA
SOC media, 1-2 ml per sample, (room temperature)
15 ml culture tubes
Sterile Pasteur pipettes and pipette tips
Electroporation cuvettes, 0.1 cm (prechilled to 0°C). *Note that 10 cuvettes of this size are included with the Electroporator II.*
Electroporator II
Power supply capable of delivering 1500 V
LB Plates containing appropriate antibiotic

Protocol for Bacterial Electroporation

1. Place the unit on a dry benchtop near your DC electrophoresis power supply. Place the charge/pulse switch in the *pulse* position. Turn your power supply *off.* Plug the supplied input leads to the input jacks on the back of the unit—observe correct polarity (red +, black -). Connect the leads to your power supply, maintaining correct polarity.
   Caution: Incorrect connections may overheat the unit or cause the fuse to blow.

2. Select a new sterile cuvette and have ready all pipettes and recovery media (i.e., SOC).

3. With the Electroporator II in the *pulse* position, set the capacitance to 50 µF and the resistance selector to 150 Ω.

4. Set the voltage output of your power supply to 1500 V. If the power supply has current limiting capability, set the limits to 25 mA and 25 watts. Allow 10 seconds for the current to stabilize. Figure 10 displays standard Electroporator II and power supply settings for bacterial electroporation and the corresponding pulse length and field strength which will result from these settings in non-conductive media.

   Figure 10: Bacterial electroporation settings

Power supply settings		Electroporator II settings	
Voltage:	1500 V	Capacitance:	50 µF
Current:	25 mA	Resistance:	150 Ω
Power:	25 W		
Pulse Length (using 0.1 cm cuvette) =		7.5 ms	
Field Strength using above settings =		15,000 V/cm	

   If you prefer to calculate settings according to specific voltage gradient and pulse length requirements for the strain being electroporated, set the capacitance, resistance and input voltage according to the formula $\tau = RC$.

5. Set the charge/pulse switch to *charge* and allow the unit to develop a full charge. The current meter on the power supply will rise briefly, drop,

Bacterial Electroporation Protocol
(continued)

and stabilize at a low steady level. Verify that the voltage input meter on the Electroporator II indicates 1500 V (+/- 100 V). The 50 µF capacitors will require 20-30 seconds to charge. The larger capacitors (250-1000 µF) will require 2 minutes. (Longer charging times will not affect performance.)

Caution: Do not rotate the capacitance switch, reduce the voltage, or turn off your power supply while charging the unit (see Chapter 1, Power Supply Precautions section, page 5).

6. Charge and discharge the unit once or twice without a cuvette in place, leaving the arm/disarm dial in the *armed* position. Be sure the armed, charging and pulse lights are working properly. Verify that your power supply is operating normally and is delivering the proper voltage. This step should be performed once at the beginning of a series of electroporations and whenever the capacitance setting has been changed.

7. In a microfuge tube, prepare a fresh mixture of cells and DNA to be electroporated as described in Section 1, page 13. The cell mixture(s) and cuvette(s) must be completely chilled on ice. Mix gently. For *E. coli,* no incubation period is necessary. Some bacterial strains exude a hardy nuclease which can degrade exogenous DNA in a short time. *Deliver the pulse within one minute after mixing DNA with the cells.*

8. Transfer the DNA/cell mixture into the cuvette (on ice), avoiding the formation of air bubbles. Tap or shake the cuvette so that no air bubbles are visible and the solution reaches the cuvette bottom. Replace cap.

9. Set the arm/disarm dial to the *disarmed* position (armed light should be off). Dry the outside surfaces of the cuvette with tissue paper. With the notch facing toward you, insert the cuvette smoothly and carefully into the cuvette chamber.

   To facilitate removal of the cuvette after pulsing, position the cuvette so that the bottom of the cap protrudes 5 mm to 1cm from the surface of the cuvette chamber. This will not affect delivery of the pulse to the cuvette.

10. Close the cuvette chamber lid. Distance your hands and face from the chamber area. Use of protective eyewear is recommended at this time. Arm the unit by setting the arm/disarm dial to the *armed* position (armed light is on). While observing the pulse light, flip the charge/pulse switch to the *pulse* position. The pulse light will blink briefly and then go out. Do not touch the cuvette until the unit has fully discharged (pulse light is off).

    Caution: Arcing will be recognized as an audible pop. If the cuvette arcs, proceed as usual; some transformation may still have occurred. Test the unit again (Step 3-6) before electroporating another sample. See Section 5, Troubleshooting, if this occurs—excessive arcing can damage the charge/pulse switch and may cause the unit to overheat and malfunction.

11. Remove the cuvette from the chamber. Immediately add 10-20 volumes of SOC media (1-2 ml) to the cuvette and mix well (by pipetting up and

Bacterial Electroporation Protocol
(continued)

down). Transfer the cells to a sterile 15 ml tube using a sterile tip or Pasteur pipette, and shake at 37°C for exactly one hour to allow expression of antibiotic resistance genes. Specific strains may require different recovery treatments.

12. If additional samples are to be electroporated, repeat steps 5-11. To avoid cross-contamination of cells and/or DNA, use a new sterile cuvette for each sample.

13. When finished using the Electroporator II, check to see that the unit is in the *pulse* position and the pulse light is off. Turn off the power supply and disconnect leads to the Electroporator II.

14. Plate bacteria using agar plates containing appropriate antibiotic (i.e. 40 µg/ml Tetracycline or 75 µg/ml Ampicillin) and incubate overnight at 37°C. Count colonies after 16 to 24 hours and calculate electroporation efficiency according to the formula below.

$$\text{Colony forming units (CFU) per µg DNA} = \frac{\left(\frac{\text{colonies on plate}}{\text{pg of DNA transformed}}\right) \times \left(\frac{1 \times 10^6 \text{ pg}}{\text{µg}}\right)}{\left(\text{percent of total transformation plated}\right)}$$

Example: 10 pg of pUC18 plasmid was electroporated into 40 µl of electrocompetent TOP10αF' *E. coli*. The cells were recovered in 1 ml SOC. After a one hour incubation at 37°C, 50 µl of the transformation mix were plated on LB plates containing 75 µg/ml Ampicillin. Plates were incubated overnight. After 24 hours, 600 colonies were observed, yielding an electroporation efficiency of 1.2×10^9 transformants per µg of supercoiled DNA.

$$\text{1.2×10^9 colony forming units (CFU) per µg DNA} = \frac{\left(\frac{600 \text{ colonies}}{10 \text{ picograms}}\right) \times \left(\frac{1 \times 10^6 \text{ pg}}{\text{µg}}\right)}{\left(\frac{0.05}{\text{(50 µl plated of 1 ml transformation mix)}}\right)}$$

ELECTROPORATION OF YEAST

Yeast electroporation conditions are very similar to bacterial conditions. Recommendations on pages 13 and 14 concerning preparation of DNA for electroporation also apply to yeast. The following protocol is designed for *S. cerevisiae*. This protocol is adapted from a method described by Becker and Guarente (Guide to Electroporation and Electrofusion, D. Chang, editor, 1992, pg 501). This process is ideal for transfection of low levels of DNA, being most appropriate for introduction of plasmid constructs into yeast strains. Efficiencies reach 5 x 10^4 transformants/100 ng plasmid DNA.

Required Materials

YPD Media
1 M HEPES, pH 8.0, sterile, ice-cold
1 M dithiothreitol, sterile, ice-cold
Sterile, double distilled, H_2O, ice-cold
1 M sorbitol, sterile, ice-cold
TE (10 mM Tris-HCl, pH 8, 1 mM EDTA), sterile, ice-cold
Selective plates containing 1 M sorbitol
Electroporator II
Power supply capable of delivering 1500 V
0.2 cm gap cuvettes, chilled on ice

Preparation of Yeast for Electroporation

1. Inoculate 500 ml YPD media in a 2 liter flask and grow *S. cerevisiae* with vigorous shaking at 30°C to OD_{600} 1.3–1.5 (approximately 1 x 10^8 cells/ml).

2. Harvest the cells by centrifugation and resuspend in 100 ml YPD broth. Add 2.0 ml sterile 1 M HEPES, pH 8.0, (20 mM final concentration) and then add 2.5 ml sterile 1 M dithiothreitol (DTT; 25 mM final concentration) while swirling gently. Incubate 15 minutes at 30°C with gentle shaking.

3. Bring to 500 ml with ice-cold double distilled H_2O.

4. Concentrate the cells approximately 1000-fold with several centrifugations, resuspending the successive pellets as follows:
   1st pellet: 500 ml ice-cold double distilled H_2O
   2nd pellet: 250 ml ice-cold double distilled H_2O
   3rd pellet: 20 ml ice-cold 1 M sorbitol
   4th pellet: 0.5 ml ice-cold 1 M sorbitol
   Resuspension should be vigorous. The rotor, speed and exact duration of the spins are not critical. All solutions should be ice-cold. Total volume of resuspended yeast should be about 1.0 to 1.5 ml. Yeast cells prepared in this manner will not retain high electroporation efficiency if stored; proceed directly with the following electroporation steps.

Protocol for Electroporation of Yeast

5. Resuspend up to 100 ng DNA (per electroporation) in ≤ 5 µl of a low ionic strength buffer such as TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

6. Mix 40 µl of yeast suspension with the 5 µl of DNA. Transfer the mixture into a pre-chilled 0.2 cm gap cuvette, avoiding the formation of air bubbles. Tap or shake the cuvette so that no bubbles are visible and the solution reaches the bottom of the cuvette. Replace cap. Incubate on ice for 5 minutes.

Protocol for Electroporation of Yeast

(continued)

7. Place the Electroporator II on a dry benchtop near your DC electrophoresis power supply. Place the charge/pulse switch in the *pulse* (discharge) position. Make sure the power supply is off. Set the capacitance dial to 50 µF. Set the resistance dial to 100 Ω. The arm/disarm dial should be in the *disarm* position.

   Figure 11 displays Electroporator II and power supply settings for this protocol and the corresponding pulse length and field strength which will result from these settings in non-conductive media.

Figure 11: Yeast Electroporation Conditions

Power supply settings		Electroporator II settings	
Voltage:	1500 V	Capacitance:	50 µF
Current:	25 mA	Resistance:	100 Ω
Power:	25 W		
Pulse Length (using 0.2 cm cuvette) =		5.0 ms	
Field Strength using above settings =		7,500 V/cm	

8. Connect to power supply by plugging the supplied input leads to the input jacks on the back of the Electroporator II. Connect the leads to your power supply. Observe correct polarity (red +, black -) in all cases. *Caution:* Incorrect connections may overheat the unit or blow the fuse.

9. Turn on power supply. Set voltage to 1500 V. The voltage input meter on the Electroporator II should indicate a similar voltage (+/- 100 V).

10. Set the charge/pulse switch on *charge.* Charge at 1500 volts for a minimum of 20 seconds.

11. Set the Electroporator II to the *disarmed* position (armed light should be off). Holding the cuvette by the clear (non-metal) sides, dry the outside surfaces of the cuvette with tissue paper. With the notch facing forward, insert the cuvette smoothly into the cuvette chamber.

    To facilitate removal of the cuvette after pulsing, position the cuvette so that the bottom of the cap protrudes 5 mm to 1 cm from the surface of the cuvette chamber. This will not affect delivery of the pulse to the cuvette.

12. Set arm/disarm dial to the *armed* position. Distance your hands and face from the cuvette chamber area. Use of protective eyewear is recommended at this time. While observing the pulse light, deliver charge to cuvette by snapping the pulse/charge switch to the *pulse* position. The pulse light will blink on briefly during the pulse.

13. Immediately add 0.5 to 1 ml ice-cold 1 M sorbitol to the cuvette and transfer the yeast (with gentle mixing) to a culture tube.

14. A growth period is not required; spread aliquots of the transformation directly on selective plates containing 1 M sorbitol.

MAMMALIAN CELL ELECTROPORATION

Electroporation involves transiently exposing cells to a strong electrical field in the presence of DNA. It is thought that this results in the opening of ion channels in the cell membrane, allowing the uptake of DNA. Transfections are carried out in 0.4 cm cuvettes. Electroporation of mammalian cells should be carried out in either tissue culture media without fetal bovine serum (FBS), phosphate buffered saline (PBS), or HEPES buffered saline (HBS). The voltage, resistance, and capacitance settings for each of these electroporation media will differ. Maximum efficiency of electroporation generally occurs at levels of 40-80% cell death.

Electroporation can be used for all types of expression systems. It is generally the method of choice for transfection of cells cultured in suspension. A major advantage of electroporation is that it enables the introduction of significantly larger amounts of DNA than other methods (between 10-100 µg). The length of the DNA has less effect on transformation efficiency than many other transfection methods. The main disadvantage to electroporation is the requirement for large numbers of cells per transfection.

DNA may be prepared with a standard alkaline lysis technique followed by ethanol precipitation. Alternatively, CsCl/ethidium bromide gradient centrifugation may be used, followed by phenol/chloroform extraction and ethanol precipitation. DNA should be resuspended in TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) or sterile, nanopure (18 megaohm) H_2O at a concentration of 1–2 µg/µl. DNA which is to be used for mammalian cell electroporations should be handled under sterile conditions whenever possible.

Both stable and transient transfections may be accomplished using electroporation. For stable transfections, linearizing the DNA by cleavage at a unique restriction site (in a nonessential region of the vector) will improve integration frequency. DNA electroporated for transient expression may be transformed without cleavage.

All eukaryotic cell protocols utilize conductive electroporation buffers. The conductivity of this buffer is primarily responsible for the resistance of the sample. It is therefore not possible to determine an exact pulse length without knowing the conductivity of the buffer and cells in the sample.

Figure 12 provides a reference for determining what pulse length is achieved according to the capacitance setting for high salt electroporation buffers.

Figure 12: Approximate pulse lengths for HBS electroporation buffer using 0.4 cm gap cuvettes.

500 µl Cells at 0°C		500 µl Cells at 22°C	
Capacitance (µF)	**Pulse Length (ms)**	**Capacitance (µF)**	**Pulse Length (ms)**
71	4.0	71	3.0
250	12.0	250	7.5
500	14.0	500	9.0
1000	20.0	1000	14.0

MAMMALIAN CELL ELECTROPORATION

Parameters for Optimization

The following parameters can be adjusted to determine the optimal protocol for transfection of your specific cell lines. All mammalian cell electroporations are performed using 0.4 cm gap cuvettes. The voltage and capacitance used must be optimized for each cell line used. The resistance is determined by the electroporation buffer, the volume in which the cells are suspended, and the gap width of the cuvette. Therefore, the infinite resistance ($\infty\ \Omega$) setting is typically used for all eukaryotic cells.

Figure 13 recommends consecutive settings for optimization. Proceed with parameters listed top to bottom and left to right when testing individual settings.

Electroporation buffer
1. High salt [e.g. phosphate buffered saline (PBS)]
2. Low salt (e.g. growth media without serum supplements)

Voltage
1. High salt buffer: Range 200-1200 V.
2. Low salt buffer: Range 100-400 V.

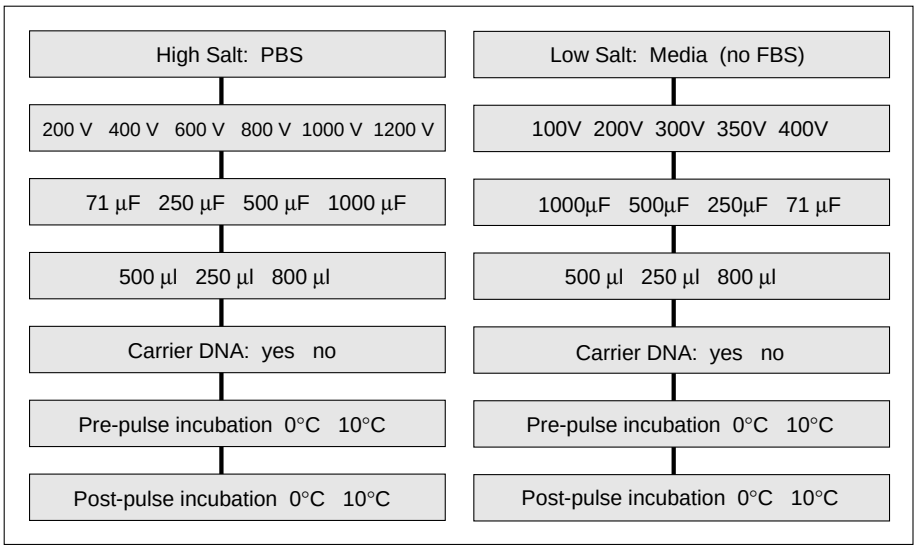
Capacitance
1. High salt buffer: Starting at 71 µF, increase to lengthen the pulse.
2. Low salt buffer: Starting at 1000 µF, reduce to shorten the pulse.

Volume
1. Starting at 500 µl, decrease to 250 µl to increase the sample resistance.
2. Alternatively, increase volume to 800 µl to reduce the resistance.

Carrier DNA
The inclusion of 20 to 100 µg of carrier DNA may result in increased efficiency of electroporation for some cell lines. Sonicated herring or salmon sperm DNA are commonly used as carrier DNA.

Pre/post incubation temperature
The 10 minute incubation of the cell-DNA mixture prior to and following the pulse may be varied. Start at 0°C and then try room temperature.

Figure 13: Optimization parameters for mammalian cell electroporation



General Procedure for Electroporation of Mammalian Cells

1. Grow cells to a confluency of 80%. Harvest the cells from flasks by standard trypsinization or by immersing cells in phosphate buffered saline (PBS) for 5 minutes. Detach cells from flask by pipetting PBS over the cells. Transfer resuspended cells to a centrifuge tube and pellet by centrifugation at 1500 rpm for 5 minutes.

2. Wash cells by resuspending in ice cold PBS. At this point, remove 100 µl of cells and count with hemocytometer. Spin down remaining cells at 1500 rpm for 5 minutes.

3. Resuspend cells in a low salt electroporation buffer (media without FBS) or high salt electroporation buffer (HBS or PBS) at a density of 3×10^6 to 1×10^7/500 µl.

4. Add 20 µg of DNA (at a concentration of 1–2 µg/µl) to a 0.4 cm cuvette on ice.

5. Add 500 µl cells to the cuvette and chill on ice for 10 minutes.

6. Place Electroporator II on a dry benchtop near your DC electrophoresis power supply. Place the charge/pulse switch in the pulse position. Turn your power supply *off.* Plug the supplied input leads to the input jacks on the back of the unit, observe correct polarity (red +, black -). Connect the leads to your power supply. Note: incorrect connections may overheat the unit or cause the fuse to blow.

   Caution: Do not rotate the capacitance selector switch, reduce the voltage, or turn off your power supply while charging the unit (see Safety Precautions, page 5).

   Note: At the beginning of a series of electroporations, or if the capacitance setting has been changed, charge and discharge the unit once or twice without a cuvette in place. Set the resistance dial to 500 Ω. (If the unit is set to ∞ Ω, the unit will not discharge internally and the pulse light will remain on.) With the arm/disarm dial in the *armed* position, set the charge/pulse switch to *pulse*. Verify that the pulse light blinks on, that your power supply is operating normally and is delivering the proper voltage. *Important: reset the resistance to ∞ Ω before continuing.*

7. With the Electroporator II in the *pulse* position, set the desired capacitance. If optimizing for a new cell line, begin with 1000 µF when using a low salt buffer, or 71 µF if using high salt buffer. Set resistance to ∞ Ω.

8. Set the voltage output of your power supply to the desired setting (See Chapter 4, Parameters for Optimization section, page 24). If the power supply has current limiting capability, set the limits to 25 mA and 25 watts. Allow 10 seconds for the current to stabilize.

9. Set the charge/pulse switch to *charge* and allow the unit to develop a full charge. The current will rise briefly, drop and stabilize at a low steady level. Verify that the voltage input meter on the Electroporator II indicates the voltage set on the power supply (+/- 100 V). The Electroporator II will require 3 minutes to develop a full charge. Longer charging times will not affect performance. The charging light should be on at this point and will remain on until the unit is pulsed (discharged).

10. Set the Electroporator II to the *disarmed* position (armed light should be off). Charge the unit for at least 3 minutes.

11. Remove cuvette from ice and tap gently to resuspend the cells. Holding the cuvette by the clear (non-metal) sides, dry the outside surfaces with tissue paper. With the notch facing forward, insert the cuvette smoothly and carefully into the cuvette chamber.

    To facilitate removal of the cuvette after pulsing, position the cuvette so that the bottom of the cap protrudes 5 mm to 1 cm from the surface of the cuvette chamber. This will not affect delivery of the pulse to the cuvette.

12. Set arm/disarm dial to the *armed* setting. Distance your hands and face from the cuvette chamber area. Use of protective eyewear is recommended at this time. While observing the pulse light, deliver charge to cuvette by setting the charge/pulse switch to the *pulse* position. The pulse light will glow during the pulse.

13. Place the cuvette on ice for an additional 10 minutes.

14. Add 1 ml of pre-warmed, complete media to the cuvette and transfer cells to a 100 mm dish (already wet with media) containing 9 ml of complete media.

15. Incubate in a humidified, 37°C, 5% CO_2 incubator for 16–24 hours. The next day, change the media and continue incubation for another 24–48 hours. The total post-transfection incubation time will be 48 to 72 hours.

16. To harvest cells, remove media and wash once with PBS. Using a rubber policeman, scrape cells gently off the bottom of the dish. Transfer to a 15 ml culture tube and spin 1500 rpm for 5 minutes. Resuspend the cell pellet in 1 ml of PBS and transfer to a sterile microfuge tube. Spin 5 minutes in an microfuge centrifuge. Remove supernatant and freeze pellets at -70°C until needed.

    If establishing stable cell lines, switch to the appropriate selective media after 48 hours to select for stable integration of electroporated plasmid.

COS Cells

OPTIMIZED ELECTROPORATION PROTOCOL

Note: All of the steps in this procedure, with the exception of the application of the pulse, should be carried out under sterile tissue culture conditions. See the General Procedure on page 25 for details.

1. Grow COS cells to a density of 80% confluency. The cells can then be harvested by trypsinization and the cell number determined using a hemocytometer. Pellet cells at 1500 rpm in a benchtop centrifuge. Wash cells once with phosphate buffered saline (PBS) and pellet by centrifugation.

2. Resuspend cells at a density of 3–4 x 10^6 cells / 500 µl in ice cold PBS.

3. Under a tissue culture laminar flow hood, remove the required number of 0.4 cm cuvettes from the individual, sealed packages. Remove the caps and add 500 µl of resuspended cells to each cuvette.

4. Resuspend 20 µg of DNA at a concentration of 1 to 2 µg/µl in sterile water or TE (10 mM Tris, pH 7.5, 1 mM EDTA) for each electroporation.

5. Add DNA to cuvette and gently tap several times to mix. Replace the caps on the cuvettes and incubate on ice for 10 minutes.

6. While cells are incubating, begin charging the Electroporator II. Set the unit and the power supply according to Figure 14. **Note:** Charge the Electroporator II for at least 3 minutes before pulsing the sample.

Figure 14: Electroporation conditions for COS cells

Power supply settings		Electroporator II settings	
Voltage:	330 V	Capacitance:	500 µF
Current:	25 mA	Resistance:	∞ Ω
Power:	25 W		

7. Following the 10 minute incubation on ice, begin electroporating. Gently flick the cuvette a few times to resuspend any cells that may have settled, then apply the pulse. Following pulse, place cuvette on ice for an additional 10 minutes.

8. Cells are plated following the second 10 minute incubation period. Under a tissue culture laminar flow hood, remove the cap from the cuvette and add 1 ml of complete media (DMEM with 10% FBS). Gently aspirate the cells from the cuvette using a sterile Pasteur pipette and add to a 100 mm dish containing 9 ml of complete media. Incubate overnight at 37°C with 5% CO_2.

9. Change the media after 16–24 hours.

10. Harvest cells 48-72 hours after electroporation and/or begin selection for stable transformants.

CHO Cells

OPTIMIZED ELECTROPORATION PROTOCOL

Note: All of the steps in this procedure, with the exception of the application of the pulse, should be carried out under sterile tissue culture conditions. See the General Procedure on page 25 for details.

1. Grow CHO cells to a density of 80% confluency. The cells can then be harvested by trypsinization and the cell number determined using a hemocytometer. Pellet cells by centrifugation at 1500 rpm in a benchtop centrifuge. Wash cells once with phosphate buffered saline (PBS) and pellet by centrifugation.

2. Resuspend cells at a density of 3–4 x 10^6 cells/250 µl in ice cold HAMS F12 media without fetal bovine serum (FBS).

3. Under a tissue culture laminar flow hood, remove the required number of 0.4 cm cuvettes from the individual, sealed packages. Remove the caps and add 250 µl of resuspended cells to each cuvette.

4. Resuspend 20 µg of DNA at a concentration of 1 to 2 µg/µl in sterile water or TE (10 mM Tris pH 7.5, 1 mM EDTA) for each electroporation.

5. Add DNA to cuvette and gently tap several times to mix. Replace the caps on the cuvettes and incubate on ice for 10 minutes.

6. While cells are incubating, begin charging the Electroporator II. Set the unit and the power supply according to Figure 15. **Note:** Charge the Electroporator II for at least 3 minutes before pulsing the sample.

Figure 15: Electroporation conditions for CHO cells

Power supply settings		Electroporator II settings	
Voltage:	330 V	Capacitance:	1000 µF
Current:	25 mA	Resistance:	∞ Ω
Power:	25 W		

7. Following the 10 minute incubation, begin electroporating. Gently flick the cuvette a few times to resuspend any cells that may have settled, then apply the pulse. Following pulse, place cuvette on ice for an additional 10 minutes.

8. Cells are plated following the second 10 minute incubation period. Under a tissue culture laminar flow hood, remove the cap from the cuvette and add 1 ml of complete media (HAMS F12 with 10% FBS). Gently aspirate the cells from the cuvette using a sterile Pasteur pipette and add to a 100 mm dish containing 9 ml of complete media. Incubate overnight at 37°C with 5% CO_2.

9. Change the media after 16–24 hours.

10. Harvest cells 48-72 hours after electroporation and/or begin selection for stable transformants.

HeLa Cells

OPTIMIZED ELECTROPORATION PROTOCOL

Note: All of the steps in this procedure, with the exception of the application of the pulse, should be carried out under sterile tissue culture conditions. See the General Procedure on page 25 for details.

1. Grow HeLa cells to a density of 80% confluency. The cells can then be harvested by trypsinization and the cell number determined using a hemocytometer. Pellet cells by centrifugation at 1500 rpm in a benchtop centrifuge. Wash cells once with phosphate buffered saline (PBS) and pellet by centrifugation.

2. Resuspend cells at a density of 3–4 x 10^6 cells/250 µl of Earl's Modified Eagle Media (EMEM) without fetal bovine serum (FBS) or L-glutamine.

3. Under a tissue culture laminar flow hood, remove the required number of 0.4 cm cuvettes from the individual, sealed packages. Remove the caps and add 250 µl of resuspended cells to the cuvette.

4. Resuspend 20 µg of DNA at a concentration of 1 to 2 µg/µl in sterile water or TE (10 mM Tris pH 7.5, 1 mM EDTA) for each electroporation.

5. Add DNA to cuvette and gently tap several times to mix. Replace the caps on the cuvettes and incubate on ice for 10 minutes.

6. While cells are incubating, begin charging the Electroporator II. Set the unit and the power supply according to Figure 16. **Note:** Charge the Electroporator II for at least 3 minutes before pulsing the sample.

Figure 16: Electroporation conditions for HeLa cells

Power supply settings		Electroporator II settings	
Voltage:	300 V	Capacitance:	1000 µF
Current:	25 mA	Resistance:	∞ Ω
Power:	25 W		

7. Following the 10 minute incubation, begin electroporating. Gently flick the cuvette a few times to resuspend any cells that may have settled, then apply the pulse. Following pulse, place cuvette at room temperature for an additional 10 minutes.

8. Cells are plated following the second 10 minute incubation period. Under a tissue culture laminar flow hood, remove the cap from the cuvette and add 1 ml of complete media (EMEM with 10% FBS and 4 mM L-glutamine). Gently aspirate the cells from the cuvette using a sterile Pasteur pipette and add to a 100 mm dish containing 9 ml of complete media. Incubate overnight at 37°C with 5% CO_2.

9. Change the media after 16–24 hours.

10. Harvest cells 48-72 hours after electroporation and/or begin selection for stable transformants.

293 EBNA Cells

OPTIMIZED ELECTROPORATION PROTOCOL

Note: All of the steps in this procedure, with the exception of the application of the pulse, should be carried out under sterile tissue culture conditions. See the General Procedure on page 25 for details.

1. Grow 293 EBNA cells to a density of 80% confluency. Harvest by immersing cells in phosphate buffered saline (PBS) for 5 minutes. Detach from flask by pipetting PBS over the cells. Determine the cell number using a hemocytometer. Cells are pelleted by centrifugation at 1500 rpm in a benchtop centrifuge. Wash cells once with PBS and pellet by centrifugation.

2. Resuspend cells at a density of 4 x 10^6 cells/500 µl Dulbecco's Modified Eagle Media (DMEM) without fetal bovine serum (FBS).

3. Under a tissue culture laminar flow hood remove the required number of 0.4 cm cuvette from the individual sealed packages. Remove the caps and add 500 µl of resuspended cells to the cuvette.

4. Resuspend 20 µg of DNA at a concentration of 1 to 2 µg/µl in sterile water or TE (10 mM Tris pH 7.5, 1 mM EDTA) for each electroporation.

5. Add DNA to cuvette and gently tap several times to mix. Replace the caps on the cuvettes and incubate on ice for 10 minutes.

6. While cells are incubating, begin charging the Electroporator II. Set the unit and the power supply according to Figure 17. **Note:** Charge the Electroporator II for at least 3 minutes before pulsing the sample.

Figure 17: Electroporation conditions for 293 EBNA cells

Power supply settings		Electroporator II settings	
Voltage:	330 V	Capacitance:	1000 µF
Current:	25 mA	Resistance:	∞ Ω
Power:	25 W		

7. Following the 10 minute incubation, begin electroporation. Gently flick the cuvette a few times to resuspend any cells that may have settled, then apply the pulse. Following pulse, incubate cuvette at room temperature for an additional 10 minutes.

8. Cells are plated following the second 10 minute incubation period. Remove the caps from the cuvette, under a tissue culture laminar flow hood, and add 1 ml of complete media (DMEM with 10% FBS). Gently aspirate the cells from the cuvette using a sterile Pasteur pipette and add to a 100 mm dish containing 9 mls of complete media. Incubate overnight at 37°C with 5% CO_2.

9. Change media after 16–24 hours.

10. Harvest cells 48-72 hours after electroporation and/or begin selection for stable transformants.

NIH 3T3 Cells

OPTIMIZED ELECTROPORATION PROTOCOL

Note: All of the steps in this procedure, with the exception of the application of the pulse, should be carried out under sterile tissue culture conditions. See the General Procedure on page 25 for details.

1. Grow NIH 3T3 cells to a density of 80% confluency. Harvest by immersing cells in phosphate buffered saline (PBS) for 5 minutes. Detach from flask by pipetting PBS over the cells. Determine the cell number using a hemocytometer. Cells are pelleted by centrifugation at 1500 rpm in a benchtop centrifuge.
2. Resuspend cells at a density of 6 x 10^6 cells/500 µl PBS.
3. Under a tissue culture laminar flow hood, remove the required number of 0.4 cm cuvettes from the individual sealed packages. Remove the caps and add 500µl of resuspended cells to the cuvette.
4. Resuspend 20 µg of DNA at a concentration of 1 to 2 µg/µl in sterile water or TE (10 mM Tris pH 7.5, 1 mM EDTA) for each electroporation. Add to this 20 µg of carrier DNA (i.e., sonicated salmon sperm DNA).
5. Add plasmid DNA and herring sperm carrier to cuvette and gently tap several times to mix. Replace the caps on the cuvettes and incubate at room temperature for 10 minutes. **Note:** Transfection will occur without carrier DNA but at a much lower efficiency.
6. While cells are incubating, begin charging the Electroporator II. Set the unit and the power supply according to Figure 18. **Note:** Charge the Electroporator II for at least 3 minutes before pulsing the sample.

Figure 18: Electroporation conditions for NIH3T3 cells

Power supply settings		Electroporator II settings	
Voltage:	330 V	Capacitance:	1000 µF
Current:	25 mA	Resistance:	∞ Ω
Power:	25 W		

7. Following the 10 minute incubation, begin electroporating. Gently flick the cuvette a few times to resuspend any cells that may have settled, then apply the pulse. See General Protocol for Mammalian Cell electroporation for details. Following pulse, incubate cuvette at room temperature for an additional 10 minutes.
8. Cells are plated following the second 10 minute incubation period. Remove the caps from the cuvette, under a tissue culture laminar flow hood, and add 1 ml of complete media [Dulbecco's Modified Eagle Media (DMEM) with 10% FBS]. Gently aspirate the cells from the cuvette using a sterile Pasteur pipette and add to a 100 mm dish containing 9 mls of complete media. Incubate overnight at 37°C.
9. Change media after 16–24 hours.
10. Harvest cells 48-72 hours after electroporation and/or begin selection for stable transformants.

COMMON PROBLEMS AND SOLUTIONS

If a malfunction occurs, it can usually be solved by trial and error attempts. Below is a list of situations (in bold) you may encounter with the Electroporator II and the most probable solutions (numbered) for that problem. In any situation, if you are concerned about a problem or attempting a recommended solution, please contact the Invitrogen Technical Service Department at 1-800-955-6288. If the unit continues to malfunction and no solution can be found, contact Invitrogen before returning the unit.

A. Power supply will not start or "cuts out" while charging the Electroporator II.

1. Make sure power supply is set to constant voltage mode (if available).
2. Power supply may be delivering incorrect voltage. Power supplies occasionally require recalibration of the load detector. Have the power supply inspected by an authorized electronics technician.
3. This power supply may be partially incompatible with the Electroporator II (see Appendix A, page 36, and Chapter 1, page 6, for details). Discharge Electroporator II by placing the charge/pulse switch in the *pulse* position. Turn power supply *off.* This power supply may be used with the Electroporator II using the following steps. With the charge/pulse switch in the *charge* position, turn power supply *on* again to see if it successfully charges the unit. If this still does not charge the unit, test another power supply or call Invitrogen for assistance.
4. Fuse may be shorted out. Inspect and replace if necessary according to instructions on page 35.
5. Power supply is incompatible with Electroporator II. Contact Invitrogen.

B. The charge light does not come on when connected to the power supply. The power supply is on and functioning properly.

1. Make sure the charge/pulse switch is in the *charge* position.
2. Check to see if the input leads are connected with correct polarity.
3. If your power supply has both "set" and "on" buttons, make sure that the "on" button is selected.
4. Make sure voltage is set above 80 volts.
5. Fuse may be shorted out. Inspect and replace if necessary according to instructions on page 35.
6. Power supply may not be compatible with the Electroporator II. See Appendix A, page 36 for details. Test another power supply if available.

C. When pulsing (discharging) the Electroporator II, the pulse light does not come on.

1. Make sure the arm/disarm dial is in the *armed* position. Pulsing in the *disarmed* position occurs internally and the pulse light will not blink.

COMMON PROBLEMS AND SOLUTIONS
(continued)

2. Pulse light not visible. Charge unit. Pulse while carefully monitoring the pulse light. You may wish to shield the pulse light from overhead light to improve visibility.
3. Short duration of pulse light: the length of time during which the pulse light is visible will vary with the pulse length. In general, bacterial electroporation settings will yield very brief durations of the pulse light.
4. Charge/pulse switch damaged or short circuited. Call Invitrogen for authorization before returning unit for repair.
5. Make sure safety lid is shut.

D. Arcing: a loud "snap" is heard while pulsing or cuvette cap popped off during pulse. Sample may be splattered inside of cuvette.

1. This can be caused by excessive conductivity in the sample. There may be an excess amount or volume of DNA, contaminants (salt) or protein in the sample. Make sure DNA has been desalted by precipitation and occupies no more than 5% of the total sample volume for bacterial electroporation (see Chapter 2, Bacterial Electroporation, page 13). For mammalian cell electroporation, make sure DNA has been prepared according to recommendations (see Chapter 4, page 23) and that there is no protein contamination (FBS) present in the media.
2. Arcing can also be caused by air bubble(s) in the sample. Before inserting cuvette into chamber, inspect carefully for air bubbles. Tap or flick the bottom of the cuvette to release trapped air before inserting cuvette.
3. Cuvettes should be chilled prior to electroporation. This may lead to condensation on the cuvette surface. Wipe cuvettes dry before inserting into the Electroporator II.
4. Defective cuvette. Note: we recommend use of Invitrogen 0.1, 0.2 and 0.4 cm cuvettes for use with the Electroporator II.
5. Voltage gradient or pulse length too high. Check settings and adjust if necessary before electroporating another sample.

E. The Electroporator draws a high, steady current from the power supply while charging; the pulse light may not blink while pulsing.

1. The charge/discharge switch may be damaged or short-circuited: avoid further use the Electroporator II as more damage may occur. Please call Invitrogen Technical Services for assistance.

F. Low transformation efficiency or no transformation results.

1. Cuvette should maintain contact with electrodes when inserted into the cuvette chamber. Insert cuvette completely into cuvette chamber (cuvette can be positioned slightly higher—so that bottom of cap protrudes 5 mm to 1 cm—to facilitate removal).

COMMON PROBLEMS AND SOLUTIONS
(continued)

2. Voltage gradient or pulse width too high, killing 100% of the cells (cell death is typically between 40 and 80%). Check capacitance, resistance setting and voltage before electroporating another sample.

3. Pulse length and voltage gradient may not sufficient for electroporation of this particular sample type. Check references and literature for published electroporation conditions. Adjust capacitance and resistance settings if necessary before electroporating another sample.

4. Make sure proper cuvette size is being used. As a general rule, use of 0.1 cm cuvettes is appropriate for bacterial electroporation, 0.2 cm gap cuvettes for yeast, and 0.4 cm cuvettes for mammalian cells. Use of an incorrect gap size will significantly affect the voltage gradient and electroporation efficiency.

5. Cuvette may have arced. See **Situation D** for additional information.

6. Poor efficiency electrocompetent bacteria. Run controls using fresh competent cells and pUC supercoiled DNA. Improperly prepared yeast or eukaryotic cells. See pages 15, 16, 21, and 23-26 for details on preparing cells for electroporation.

7. DNA is degraded or contaminated with protein or high salt concentration.

8. Power supply may be delivering incorrect voltage. Power supplies occasionally require recalibration. Use a standard voltmeter to verify the voltage being delivered or have the power supply inspected by an authorized electronics technician.

G. A constant "clicking" sound emanates from the Electroporator II while charging.

1. This indicates that a safety circuit in the Electroporator II is protecting the capacitor from excessive voltage. Pulse (discharge) the Electroporator II immediately. Verify voltage and capacitance setting and/or have power supply recalibrated. This will sometimes cause the fuse to be shorted out. Inspect fuse as directed on page 35 before continuing.

H. Please contact Invitrogen if the Electroporator reaches high temperatures while in use, or behaves in any other unexpected manner. Always pulse the unit before turning off the power supply and disconnecting.

The Invitrogen Technical Service Department is available to answer questions relating to use of the Electroporator II from 7:00 AM to 5:00 PM Pacific Standard Time. To reach by phone, dial 1-800-955-6288. To fax questions or comments, dial 1-619-597-6201.

FUSE INSPECTION AND REPLACEMENT

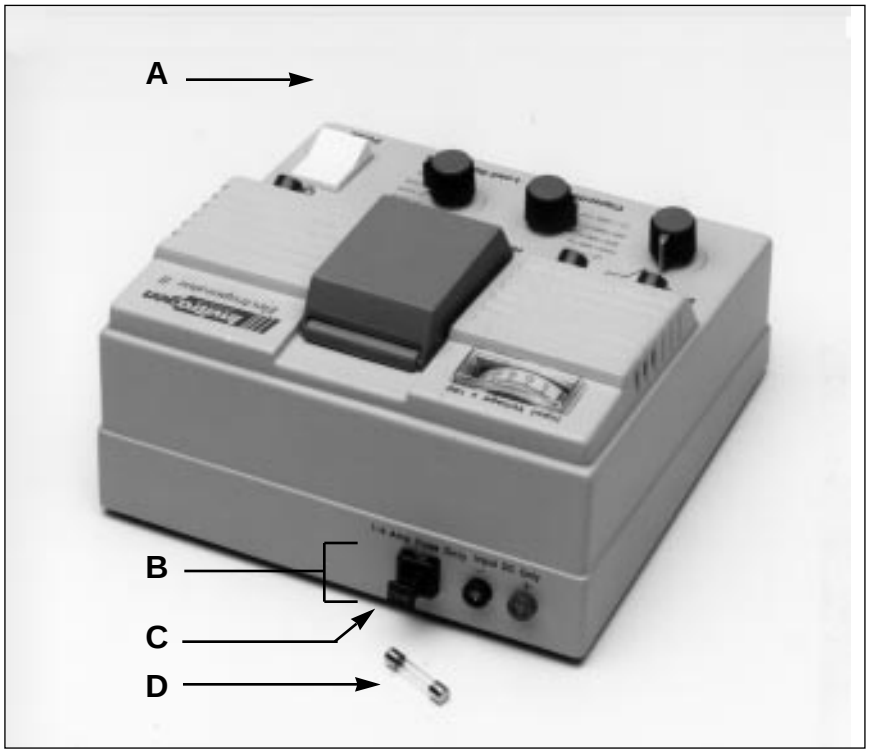
A fuse assembly has been incorporated into the Electroporator II design to protect the unit and connected power supplies. If the Electroporator II is not charging, the fuse may have shorted out.

To inspect the fuse, follow the instructions below.

1. Make sure the Electroporator II is in the *pulse* position (discharged).
2. Verify that all lights are off on the Electroporator II.
3. Turn off power supply and disconnect from Electroporator II.
4. Locate the fuse assembly on back of the Electroporator II (see Chapter 1, page 7, and the diagram below).
5. Insert the tip of a small pointed object (such as a paper clip or a small knife) into the slot on the top of the fuse assembly. The fuse holder should pop out and may be removed from the unit.
6. Examine the fuse. If the metal strip inside the fuse is intact, replace fuse holder. If the metal strip inside appears damaged or burned through, replace fuse with a new 1/32 amp, 250 V, time-delay fuse (available from Invitrogen Technical Services).
7. Verify the correct settings on the Electroporator II and the power supply. Calibrate power supply before performing any further electroporations. Please call Invitrogen with any further questions.

Figure 19: Fuse replacement for the Electroporator II.

A Charge/pulse switch
B Fuse Assembly
C Fuse Holder
D Fuse (1/32 amp, time-delay)



POWER SUPPLY COMPATIBILITY WITH THE ELECTROPORATOR II

The following power supplies have been used successfully with the Electroporator. Due to alignment drift of the load detector circuitry, older models may function inconsistently.

If your power supply is not on this list, test it using the protocol on page 11. Contact Invitrogen with any problems you encounter. See also the Power Supply Precautions discussed on page 5. Please include the manufacturer and model of compatible power supplies when you return the Electroporator II Warranty Registration card.

E-C Apparatus models 500, 600, 600-90, 703, 4000P
LKB model 2197
BRL models 500, 4000
BioRad models 1420A, 1420B
Fisher Biotech model FB600
Haake Buchler model 3000
Consort model E654
Dan-Kar model 32-1
Isco models 494, 495
Novex model 3450
Ephortec model 3000
Photodyne model 4200

Some power supplies may operate poorly in conjunction with the Electroporator II. They may have difficulty "sensing" the unit or it will "cut out" at higher voltages because they are designed to work with a larger load. The following power supplies are not able to successfully charge the Electroporator II:

E-C Apparatus models 3000, 3000P, 2000P, 500-90
Pharmacia model ECPS 3000
BioRad model 3000xi

If you do not have access to a power supply which functions properly with the Electroprator II, contact the Invitrogen Technical Services Department at 1-800-955-6288 for assistance.

PLASMID MINIPREP PROCEDURE

Reference: Zhou et al., *Biotechniques* V.8., No.2: 172 (1990).

1. Grow 2 mls of bacterial culture (L-Broth with appropriate antibiotic) at 37°C overnight.

2. Spin 1.5 ml of the culture in microcentrifuge tubes for 10 seconds, decant supernatant leaving 50-100 µl of medium in the tube. Vortex to resuspend cells completely.

3. Add 300 µl of TENS solution (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 N NaOH, 0.5% SDS). Vortex for 2-5 seconds or until the mixture becomes viscous.

4. Add 150 µl of 3.0 M sodium acetate, pH 5.2, vortex for 2-5 seconds to mix completely.

5. Spin for 2 minutes in microcentrifuge to pellet cell debris and chromosomal DNA. Transfer supernatant to a fresh tube, add 900 µl of cold 100% ethanol and mix well.

6. Spin 5 minutes to pellet plasmid DNA and RNA. The pellet should have a white appearance. Discard supernatant and rinse the pellet twice with 1ml of 70% ethanol. Remove residual ethanol after another quick spin.

7. Resuspend pellet for further analysis in 20-50 µl of TE buffer or sterile water containing RNase A at a concentration of 100 µg/ml.

PLASMID PREPARATION PROTOCOL

1. Inoculate 0.5 liter or less of L-broth + antibiotic with a single colony and incubate with shaking for 12-16 hours at 37°C. Use 5 mg/ml of tetracycline for growing pCDM8 or pcDNA I, 50µg/ml of ampicillin for pcDNA II. Alternatively, grow a 5 ml culture overnight with antibiotic then dilute this culture into 0.5-1 liter of L-broth without antibiotic. Grow overnight.

2. Spin the culture down in 500 ml Sorvall GS-3 centrifuge bottles in GS-3 rotor at 4,000 rpm (or larger bottles in appropriate rotor) for 10 minutes at 4°C. If you use a different rotor, spin at 3,000 x g for 10 minutes.

3. Remove supernatant. Resuspend cells with 30 ml per 1 liter culture of cold Solution I (50 mM glucose, 25 mM Tris-HCl, pH 8.0, 10 mM EDTA) containing 5 mg/ml lysozyme. If necessary, resuspend cells by stirring with spatula.

4. Transfer resuspended cells to clean 500 ml centrifuge bottle using a 10 or 25 ml plastic pipette. Note: Do not use glass pipette or container with DNA preparation unless siliconized. Let stand at room temperature for 5 minutes.

5. Add 60 ml of freshly prepared Solution II (0.2 N NaOH, 1% SDS). Mix gently by inverting. Leave on ice for 10 minutes.

6. Add 45 ml of cold Solution III (3 M sodium acetate, adjusted to pH 5.3 with acetic acid). Mix thoroughly by shaking. Chill on ice for 10 minutes.

7. Spin at maximum rotor speed in GS-3 rotor for 20 minutes at 4°C.

8. Filter into clean 500 ml centrifuge bottles through Dacron wool.

10. Precipitate by adding 270 ml of cold 200 proof ethanol. Place at -20°C for 1 hour or until very cold.

11. Spin in cold GS-3 at maximum rotor speed for 15 minutes. Decant supernatant and drain ethanol by placing bottle upside down on paper towel for 5 minutes. Invert bottle and remove remaining ethanol with lab wipe. Be careful not to disturb the DNA/RNA pellet.

    Note: We recommend cleaning the supercoiled plasmid by centrifugation through a CsCl density gradient containing ethidium bromide. Be careful when handling ethidium bromide. Ethidium bromide is a hazardous chemical and a carcinogen.

12. Resuspend pellet in 5 ml of TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). Check volume of sample and add 1 g of solid cesium chloride per every ml of sample plus one additional gram.

13. Pipette sample into polyallomer or similar ultracentrifuge tube using a plastic transfer pipette, add 500µl of ethidium bromide stock (5 mg/ml) and fill tube with prepared CsCl solution.

PLASMID PREPARATION PROTOCOL
(continued)

14. Seal tube, mix, and centrifuge in ultracentrifuge over night at appropriate speed and temperature.

15. Remove tube from rotor. Examine using UV light. Wear eye protection such as goggles. Locate RNA pellet at bottom of tube and two DNA bands approximately in the middle of the tube. The upper band is chromosomal DNA and nicked plasmid, the lower band is supercoiled plasmid.

16. Insert another 18 gauge needle in to the very top of the tube for venting. Use an 18 gauge needle to insert into the side of the tube just underneath the supercoiled plasmid band and attach a plastic syringe.

17. Extract the supercoiled plasmid band from the gradient and transfer to sterile plastic tube (i.e. 50 ml conical cell culture tube). Protect tube and DNA from light until ethidium bromide is removed (step 18).

18. Add an equal volume isobutyl or isoamyl alcohol to remove the ethidium bromide. Mix well, centrifuge briefly to separate phases. Remove the top organic phase which now contains most of the ethidium bromide. Repeat extraction until the bottom aqueous phase containing the DNA is completely colorless.

19. Remove CsCl by dialysis against TE for several hours.

20. Recover the DNA from dialysis bag and precipitate with 1/10X volume of 5 M NaCl and 2X volumes of 200 proof ethanol. Store at -20°C until further use.

GENE TRANSFER PRODUCTS FROM INVITROGEN

INVITROGEN ELECTROPORATION CUVETTES

Maximum efficiency and reproducibility of your electroporation results are important. We recommend use of Invitrogen Electroporation Cuvettes with your Electroporator II due to their consistency in gap width, sterility, and high quality construction. Invitrogen cuvettes are available in 0.1, 0.2 and 0.4 cm gap widths and are compatible with all electroporation devices which accept "Potter-type" cuvettes. Included with the Electroporator II are ten 0.1 cm gap cuvettes for bacterial electroporation. Listed below are some of the benefits of our superior quality assurance process.

Features

- All cuvettes are sterilized by gamma-irradiation
- Each cuvette comes with a snap tight, color-coded cap and is individually packaged
- Tight tolerance in gap width settings
- Cuvettes conform to common industry size standards

Benefits

- Sterility ensured
- Maintains sterility until use
- Eliminates variability in field strength and ensures reproducible results
- Compatibility with virtually all common electroporation devices

Figure 20: Invitrogen Cuvette Specifications

Cuvette Specifications			
Catalog Number	P410-50	P450-50	P460-50
Cuvettes per Bag	50	50	50
Gap Size	0.1 cm	0.2 cm	0.4 cm
Common Cell Use	Bacteria	Yeast	Mammalian
Minimum Volume	20 µl	40 µl	80 µl
Maximum Volume	80 µl	400 µl	800 µl
Cap Color	white	blue	red

GENE TRANSFER PRODUCTS FROM INVITROGEN

(continued)

ELECTROCOMPETENT *E. coli*

The size of cDNA libraries constructed in plasmid vectors is limited by the efficiency of the competent *E. coli* used for transformation. Invitrogen offers the largest selection of electrocompetent *E. coli* , with cloning efficiencies greater than 1 x 10^9 colony forming units/µg of supercoiled plasmid plus the only complete transformation kits. Due to the importance of high-efficiency cells for cDNA library construction, Invitrogen offers the most widely used strains in convenient library size transformation kits.

Electrocomp™ Transformation Kits offer:

- The only complete kits available that include SOC media
- Electrocomp™ kits containing five or ten 80 µl aliquots of electrocompetent cells
- Library size electroporation kits containing five 300 µl aliquots of chemically competent cells (for small-scale ligations) plus ten 160 µl aliquots of electrocompetent cells (for full-scale ligations)
- Largest selection of electrocompetent *E. coli* strains

MC1061/P3 is a high-efficiency strain that is defective in the restriction systems which recognize *Alu* I–methylated DNA sites. MC1061/P3 can be used only with plasmids carrying a tyrosine tRNA amber suppressor (synthetic *sup*F gene). The tRNA suppressor will provide genetic selection by suppressing the ampicillin and tetracycline amber mutations present on the P3 plasmid.

Genotype: F^- *hsd*R (r_k^-, m_k^+) *ara*D139 Δ(*ara*ABC-*leu*)7679 *gal*U *gal*K Δ*lac*X74 *rps*L *thi mcr*B {P3: Kan^R Amp^R (am) Tet^R (am)}

TOP10 is a recombination negative strain designed for stable replication of high copy number plasmid. The strain is *rec*A1 for increased stability of inserts and *end*A1 for improved quality of minipreps. The strain is also hsdRMS to eliminate cleavage by endogenous restriction enzymes.

Genotype: F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nupG*

TOP10F' is identical to TOP10 with the addition of a selectable F' to allow rescue of single-strand DNA from phagemid vectors.

Genotype: F´ {*lac*I^q *Tn10*(Tet^R)} *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *deo*R *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

HB101 is recombination deficient and *mcr*B^- and is commonly used for large-scale plasmid production. The strain was derived by mating *E. coli* K12 with *E. coli* B.

Genotype: F^- *hsd*S20 (r_B^-, m_B^+) *thi*-1 *sup*E44 *ara*14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20 (Str^R) *xyl*-5 *mtl*-1 *rec*A13 *mcr*B

GENE TRANSFER PRODUCTS FROM INVITROGEN
(continued)

NM522 is a recombination positive and restriction negative strain that offers high titers with M13 and high single-strand DNA yields from phagemid rescue. This strain has the inducible *lac*I^{q}ZΔM15 repressor and can be used for blue/white screening.

Genotype**:** F´ {*pro*AB^{+}, *lac*I^{q} *lac*ZΔM15} *sup*E *thi*-1 Δ(*lac-pro*AB) Δ*hsd*5 (r_k^-, m_k^-)

Figure 21: Electrocompetent *E. coli* from Invitrogen

Electrocomp™ Transformation Kits			
E. coli Strain	400 µl Size	800 µl Size	Library Size
MC1061/P3	C663-55	C663-11	L1103-55
TOP10	C664-55	C664-11	—
TOP10F'	C665-55	C665-11	L1165-55
NM522	C659-55	C659-11	—
HB101	C661-55	C661-11	—

SOLUTIONS & SELECTION OF TRANSFORMANTS

BACTERIA:

LB Medium (1 L)

Deionized water	950 ml
Bacto-tryptone	10 g
Bacto-yeast extract	5 g
NaCl	10 g

Once dissolved, adjust pH to 7.0 with 5 N NaOH. Adjust volume to 1 liter with deionized water. Autoclave 20 minutes at 15 psi on liquid cycle.

SOB Medium (1 L)

Deionized water	950 ml
Bacto-tryptone	20 g
Bacto-yeast extract	5 g
NaCl	0.5 g

Once dissolved, add 10 ml of a 250 mM KCl solution. Adjust pH to 7.0 with 5 N NaOH. Adjust volume to 1 liter. Autoclave 15 minutes at 15 psi on liquid cycle. [For **SOC Medium**, add 20 ml of 1 M Glucose after the solution cools to 60°C.] Before use of either SOB or SOC, add 5 ml sterile 2 m $MgCl_2$.

YEAST:

YEPD medium liquid (1 L)

yeast extract	10 g
peptone	20 g
dextrose	20 g
water	1000 ml
autoclave	15 psi 121°C 20'

1 M Sorbitol: 182.2 g/l in sterile water. Sterilize by ultrafiltration.

MAMMALIAN CELLS:

Neomycin: Geneticin (G418) is available from Gibco/BRL. Use 100 to 800µg/ml of G418 in complete medium. Prepare in a highly buffered solution (e.g. 100mM HEPES, pH 7.3) so the addition of the drug does not alter the pH of the medium. Varying concentrations of G418 should be tested (cells differ in susceptibility to G418). Concentration should be calculated using the amount of active drug so that variance is controlled. Cells will divide once or twice in the presence of lethal doses of G418; the effects of the drug take several days to become apparent. Complete selection may take 3 weeks. Reference: Southern, P.J. and Berg, P (1982) "Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter." J. Mol. Appl. Gen. **1**:327-341.

Hygromycin: Prepare mock-transfected cells. Seed cells at low density confluence (2.5% for fast growing cell lines and up to 10% for slow growing cell lines) in 6 well dishes. Add complete media with hygromycin concentration ranging from 0–600µg/ml at 50–100 µg increments. Change the media every 4 days. After 10-14 days, assess cell viability. Visual inspection will usually suffice (trypan blue may be used). Choose the lowest concentration of hygromycin which resulted in complete killing.

Histidinol: Selection can be carried out in histidine-free DMEM/10% (v/v) fetal calf serum at a final concentration of 0.125mM L-histidinol (Sigma). Histidinol is added from a sterile 50mM stock solution in 0.1M Hepes (pH 7.4). Solution can be filter sterilized and stored at -20°C. For some cell lines or different serum lots, the concentration may have to be increased to 0.150mM L-histidinol. Selection media should be replaced every 3 days. Selection takes 10-20 days. Control-treated cells should be dead within a week. Reference: Hartman and Mulligan,Two Dominant-Acting Selectable Markers for Gene Transfer Studies in Mammalian Cells. PNAS. (1988) **85:**8047.

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