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# Gene Pulser Xcell™ Electroporation System



Instruction Manual

Catalog Numbers

165-2660, 165-2661, 165-2662, 165-2666,  
165-2667, and 165-2668

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- Void the manufacturer's warranty
- Void the EN61010 safety certification
- Create a potential safety hazard

Bio-Rad Laboratories is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent.

\*EN61010 is an internationally accepted electrical safety standard for laboratory instruments.

**Model : Gene Pulser Xcell** \_\_\_\_\_

**Catalog No.: 165-xxxx** \_\_\_\_\_

Date of Delivery: \_\_\_\_\_

Serial No.: \_\_\_\_\_

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## Section 1

### The Gene Pulser Xcell™ Electroporation System

The Gene Pulser Xcell is a pulse generator that uses capacitors to produce controlled exponential or square wave electrical pulses for cell electroporation. The unit is capable of producing pulses of up to 3000 V on a high-voltage circuit, and up to 500 V on a low-voltage circuit. For generating pulses on the high voltage circuit, capacitors of 10, 15, and 25  $\mu\text{F}$  present in the Gene Pulser Xcell main unit are used and generating pulses on the low-voltage circuit requires use of capacitors in the CE Module. Exponential decay (or capacitance discharge) and square wave pulses are the most commonly used types of electrical pulse. An in-depth discussion of these two waveforms can be found in Section 4.

The Gene Pulser Xcell is a modular system, comprising of a main unit and two accessory modules, the CE module and the PC module, in addition to the shocking chamber and a cuvette with incorporated electrodes.

The CE Module is recommended for use with the Gene Pulser Xcell main unit for electroporation of most eukaryotic cells, including mammalian cells and plant protoplasts. The CE Module should only be used with low-resistance media (<1000 ohms). For exponential decay pulses, the CE Module provides a means of controlling the capacitance of the circuit by increasing the time constant of the pulse. For square wave pulses, the CE Module provides the large capacitor necessary for delivering a square wave pulse into low resistance media. This module contains a set of capacitors with a functional range between 50 and 3275  $\mu\text{F}$  and selectable in 25  $\mu\text{F}$  increments. For square wave pulses, the CE Module provides the large capacitance, 3275  $\mu\text{F}$ , necessary for delivering a square wave pulse into low resistance media.

The PC Module is recommended for the electroporation of bacteria and fungi using exponential decay, as well as in other applications where high-voltage pulses are applied to samples of small volume and high resistance. The PC Module selects resistance of 50 ohms 1000 ohms in 50-ohm increments. The unit is used to control the resistance of the circuit by placing resistors in parallel with the sample, thereby providing a means of reducing the time constant of an exponential decay pulse. This provides an effective means of controlling the time constant when using high-resistance media but has little effect on the time constant when using low-resistance media. The PC Module greatly reduces the likelihood of an arc occurring at high voltage. It is not recommended that the PC module be used for square wave pulses due to the increase in droop of the pulse that can occur (see Section 4).

Both the PC Module and CE Module have integral leads that connect to the main unit (see Section 2 for installation) and both units are controlled directly from the user interface on the front panel of the main unit.

#### 1.1 General Safety Information

This Bio-Rad instrument is designed and certified to meet the safety requirements of EN61010 and the EMC requirements of EN61326 (for Class A) and conforms to the “Class A” standards for electromagnetic emissions intended for laboratory equipment applications. This instrument is intended for laboratory application only. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or in the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

No part of the Gene Pulser Xcell system should be used if obvious external case damage has occurred or the electronic displays are not functioning as described in the manual. This instrument is only to be used with the components provided (or their authorized additions or replacements) including, but not limited to, supplied cables and ShockPod. The operating temperature range for the Gene Pulser Xcell system and its associated components is 0–35°C.

There are no user serviceable parts within the unit. The operator should make no attempt to open any case cover or defeat any safety interlock. This instrument must not be altered or modified in any way. Alteration of this instrument will

- Void the manufacturer's warranty
- Void the IEC 1010 safety certification
- Create a potential safety hazard

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended or by modification of the instrument not performed by Bio-Rad or an authorized agent.

## **1.2 Electrical Hazards**

The Gene Pulser Xcell produces voltages up to 3,000 volts and is capable of passing very high currents. When charged to maximum voltage, the instrument stores about 400 joules. A certain degree of respect is required for energy levels of this order. System safety features prevent operator access to the recessed input jacks and to the recessed electrode contacts inside the sample chamber. These mechanical interlocks should never be circumvented.

The pulse button is active whenever the character space in the lower right corner is flashing. There is high voltage present whenever the pulse button is depressed and "Pulsing" is shown on the LCD display on the front of the instrument. Because of the built-in safety interlock in the ShockPod, no pulse is delivered to the cuvette when the ShockPod lid is opened. If the capacitor has been partially charged but not fired (for example, when the charging cycle has been interrupted before the pulse is delivered), some charge may remain on the internal capacitor. This charge will dissipate over 1–2 minutes. However, the user cannot make contact with any charged electrical components due to the system safety features.

## **1.3 Mechanical Hazards**

The Gene Pulser Xcell contains a patented arc-protection circuit that dramatically reduces the incidence of arcing in the cuvette when high voltage is delivered into the sample. The unit incorporates a circuit that senses the beginning of an arc and diverts current from the sample within <10  $\mu$ sec, preventing, or greatly reducing, mechanical, visual, and auditory phenomena at the ShockPod. Should an arc occur, the sample chamber is effective in containing these small discharges, but nonetheless we strongly recommend wearing safety glasses when using the instrument.

## **1.4 Other Safety Precautions**

Avoid spilling any liquids onto the apparatus. Use only a paper towel or a cloth wet with either water or alcohol to clean the outside surfaces of the Gene Pulser Xcell.

Use only the Bio-Rad cables supplied with the Gene Pulser Xcell.

Use the ShockPod only in the assembled condition. Do not attempt to circumvent the protection of the ShockPod or use it while disassembled.

Verify the display segments periodically.

Read the instruction manual before using the Gene Pulser Xcell Electroporation System. For technical assistance contact your local Bio-Rad office or, in the US, call technical services at 1-800-4BIORAD (1-800-424-6723).



**Warning:** The Gene Pulser Xcell generates, uses, and radiates radio frequency energy. If it is not used in accordance with the instructions given in this manual, it may cause interference with radio communications. The Gene Pulser Xcell 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 their expense, to take whatever measure may be required to correct the interference.

## Section 2

### Unpacking and System Installation

The Gene Pulser XCell™ can be purchased as three systems as well as component parts:

165-2660	Gene Pulser Xcell Total System for eukaryotic and microbial cells, 100–240 V, 50/60 Hz, exponential decay and square wave delivery, includes main unit, CE Module, PC Module, ShockPod, 15 sterile cuvettes (5 each of 0.1, 0.2, and 0.4 cm gap), instruction manual
165-2661	Gene Pulser Xcell Eukaryotic System, 100/240 V, 50/60 Hz, exponential decay (25–3,275 $\mu$ F range) and square wave delivery, includes main unit, CE Module, ShockPod, 5 sterile cuvettes (0.4 cm gap), instruction manual
165-2662	Gene Pulser Xcell Microbial System, 100/240 V, 50/60 Hz, exponential decay and square wave delivery, includes main unit, PC Module, ShockPod, 10 sterile cuvettes (5 each of 0.1 and 0.2 cm gap), instruction manual
165-2666	Gene Pulser Xcell main unit, 100/240 V, 50/60 Hz
165-2667	Gene Pulser Xcell CE Module, 25–3,275 $\mu$ F range controlled by main unit, includes integral leads, 5 sterile cuvettes (0.4 cm gap), instruction manual
165-2668	Gene Pulser Xcell PC Module, 50–1,000 ohm range controlled by main unit, includes integral leads, 10 sterile cuvettes (5 each of 0.1 and 0.2 cm gap)
165-2669	Gene Pulser Xcell ShockPod shocking chamber, includes integral leads for connection to Gene Pulser Xcell, Gene Pulser II, or MicroPulser

#### 2.1 Unpacking the System Components

Remove all packing material and connect components on a flat, dry surface near an appropriate electrical outlet.

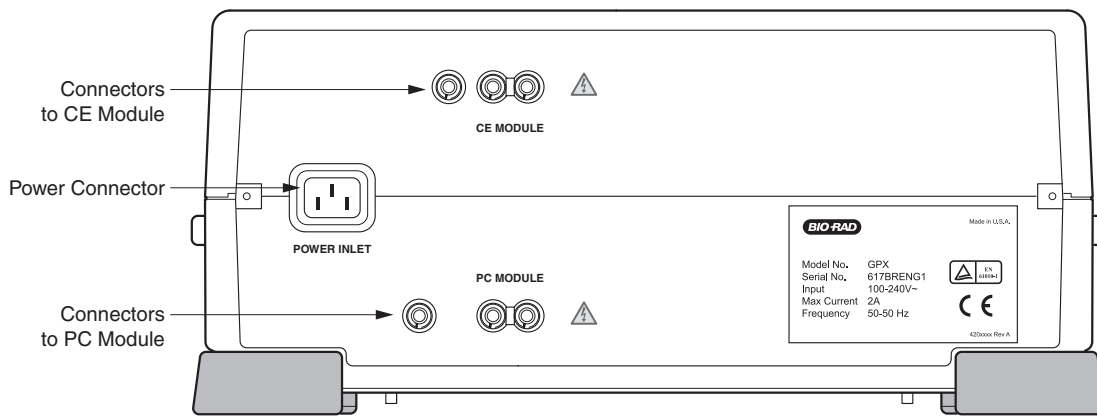
Upon receiving your instrument, please check that all items listed were shipped. If any items are missing or damaged, contact your local Bio-Rad office.

## 2.2 Setting Up the System

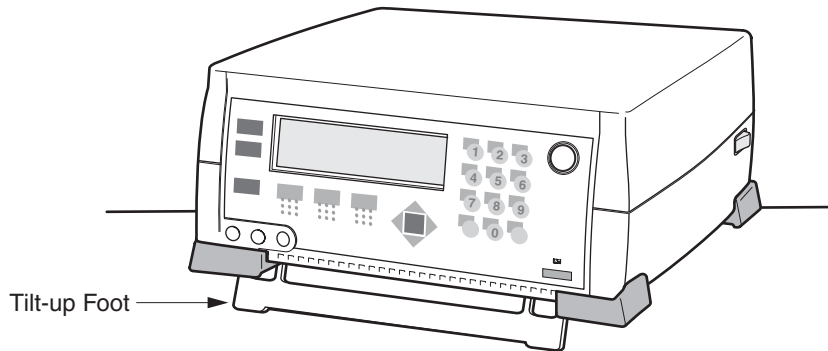
### 2.2.1 Setting Up the Gene Pulser Xcell Main Unit and Connecting the ShockPod (Cat. #s 165-2660, 165-2661, 165-2662, and 165-2666)

Follow this procedure for setting up the Gene Pulser Xcell and connecting the ShockPod. Be sure to turn off the Gene Pulser Xcell main unit before connecting or disconnecting the power cord or any cables.

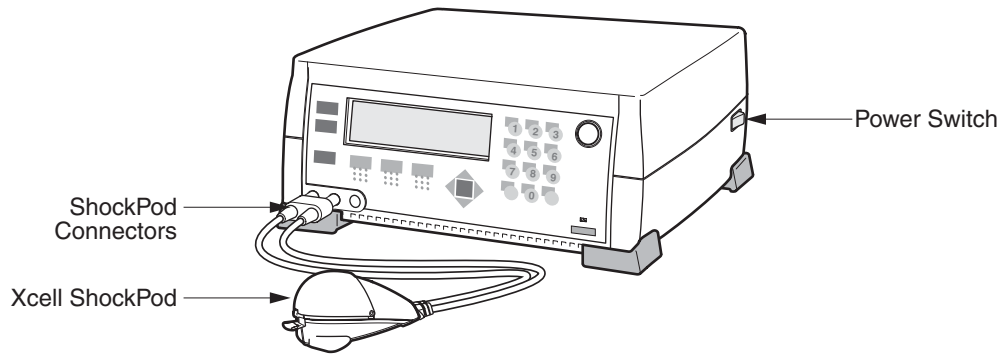
1. Attach the power cord to the three-pronged receptacle on the back of the Gene Pulser Xcell unit (Figure 2.1A). Plug the unit into an appropriate electrical outlet or power strip. There is a foot on the bottom of the Gene Pulser Xcell which may be pulled down to change the viewing angle of the LCD display (Figure 2.1B).
2. Insert the black connector of the ShockPod into the left and center output jacks on the front panel of Gene Pulser Xcell (Figure 2.2); polarity is not important to the electroporation process. The right output jack is not used with the ShockPod.
3. To turn on the Gene Pulser Xcell, press the power switch on the right rear of the instrument (Figure 2.2).



**Fig. 2.1A. Rear panel of Gene Pulser Xcell main unit showing receptacles for the power cord, CE Module, and PC Module.**



**Fig. 2.1B. Bottom panel of the Gene Pulser Xcell main unit. The foot may be pulled down to raise the front of the unit.**

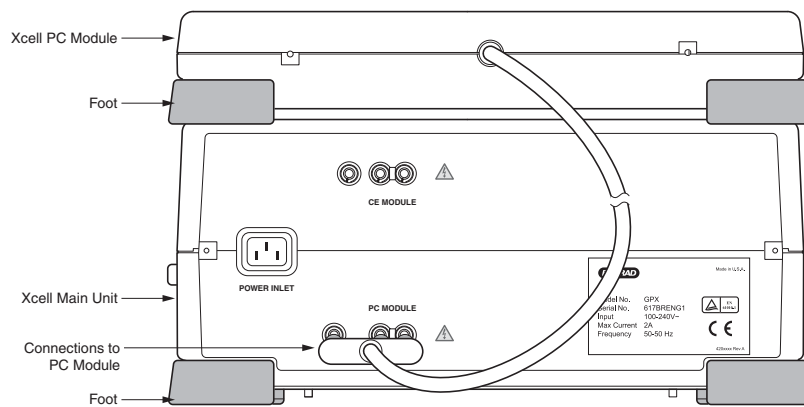


**Fig. 2.2. Connecting the ShockPod to the Gene Pulser Xcell main unit.**

**2.2.2 Connecting the PC Module to the Gene Pulser Xcell Main Unit (Cat. #s 165-2660, 165-2662, and 165-2668)**

The PC Module and CE Module may be connected to the Gene Pulser Xcell main unit in any order and both may be connected at the same time. Before connecting the PC Module to the Gene Pulser Xcell, be sure that the Gene Pulser Xcell is turned off. There is no power switch on the PC Module; it is controlled entirely by the Gene Pulser Xcell main unit.

1. Place the PC Module near the Gene Pulser Xcell main unit. The units, along with the CE Module, are stackable; either the PC Module or the Gene Pulser Xcell main unit can be placed on the bottom. Place the feet of the top unit at the corners of the bottom unit to interlock the two.
2. Insert the red/black connector that is permanently attached to the cable on the back of the PC Module into the appropriately labeled receptacle on the back of the Gene Pulser Xcell main unit (see Figure 2.3). The connector is keyed so that it will insert only into the proper receptacle and in the correct red/black orientation.

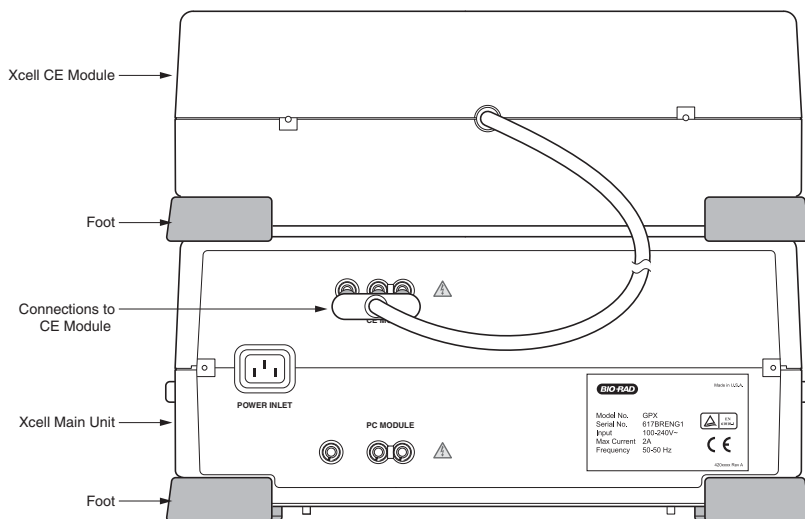


**Fig. 2.3. Rear view (back panel) of PC Module showing the connection to the Gene Pulser Xcell main unit.**

### 2.2.3 Connecting the CE Module to the Gene Pulser Xcell Unit (Cat. #s 165-2660, 165-2661, and 165-2667)

The PC Module and CE Module may be connected to the Gene Pulser Xcell main unit in any order and both may be connected at the same time. Before connecting the CE Module to the Gene Pulser Xcell, be sure that the Gene Pulser Xcell is turned off. There is no power switch on the CE Module; it is controlled entirely by the Gene Pulser Xcell unit.

1. Place the CE Module near the Gene Pulser Xcell main unit. These units, along with the PC Module are stackable in any order. Place the feet of the top unit at the corners of the bottom unit to interlock the two.
2. Insert the red/black connector that is permanently attached to the cable on the back of the CE Module (Figure 2.4) into the appropriately labeled receptacle on the back of the Gene Pulser Xcell main unit (see Figure 2.1). The connector is keyed so that it will insert only into the proper receptacle in the correct red/black orientation.

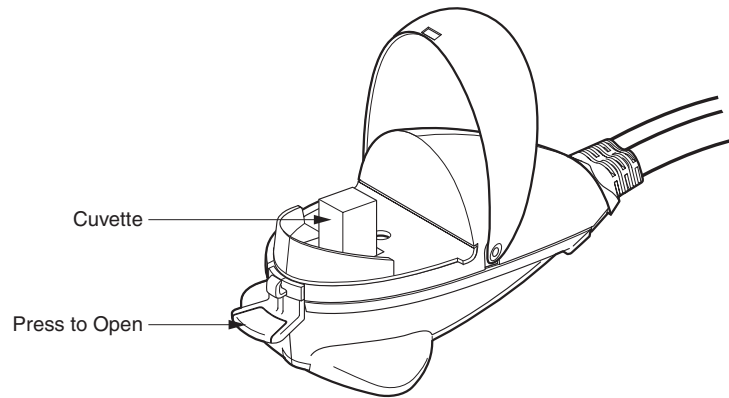


**Fig. 2.4. Rear view (back panel) of the CE Module showing the connection to the Gene Pulser Xcell main unit.**

### 2.2.4 ShockPod (Cat. #s 165-2660, 165-2661, 165-2662, and 165-2669)

The ShockPod is designed for one-hand operation. Press the tab on the front of the chamber to release the latch and to open the top. Push the top down gently to close the chamber. The safety design of the system requires that the top be closed before pulsing can occur. Additionally, the top must also be closed to use the ShockPod to measure resistance of electroporation media (see Section 3.9.1). Because of the built-in safety interlock in the ShockPod, no pulse is delivered to the cuvette when the ShockPod lid is opened.

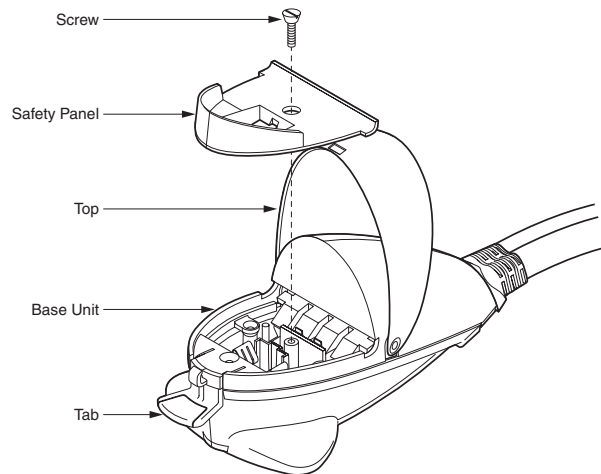
The cuvette slot will accept any commercially available cuvette. Bio-Rad cuvettes are designed with a notch on one of the non-electrode sides so they may be inserted into the cuvette slot only in the proper orientation.



**Fig. 2.5. ShockPod with cuvette.**

The plastic panel covering the electrode may be removed for cleaning should the electrode become dirty. To clean the unit:

1. Disconnect the ShockPod from the Gene Pulser Xcell main unit.
2. Press the tab to open the chamber.
3. Using a phillips screwdriver, loosen the screw holding the safety panel (Figure 2.5). Lift the screw and the safety panel out of the ShockPod by the lip on the front of the panel.
4. The area around the electrodes may be cleaned with a cotton swab and warm, soapy water. Dry the electrodes using a dry cotton swab or Kimwipe.
5. Reassemble the unit by centering the safety panel on the base of the ShockPod and tightening the screw.



**Fig. 2.6. Exploded view of the Gene Pulser Xcell ShockPod.**

## Section 3

# Gene Pulser Xcell™ Operating Instructions

### 3.1 Section Overview

This section describes the operation of the Gene Pulser Xcell. The following summarizes the organization of this section.

Section 3.2 below describes the functions of the keys on the front panel, the Home screen on the LCD display, and the Help functions built into the Gene Pulser Xcell.

- The keys on the front panel of the main unit control the Gene Pulser Xcell. Section 3.2.1 describes the uses of these keys.
- The Home screen provides easy access to programs built into the Gene Pulser Xcell as well as a direct method of manually entering pulse parameters to electroporate a sample. Section 3.2.2 describes these programs.
- On-screen help is built into the software of the Gene Pulser Xcell. This may be accessed from any screen as described in Section 3.2.3.

The Gene Pulser Xcell has three modes of operation: manual operation, pre-set protocols, and user protocols.

Section 3.3 describes the Manual mode, which may be used to rapidly program the parameters necessary for delivering either an exponential decay or a square wave pulse.

- Section 3.3.2 describes delivering an exponential decay pulse.
- Section 3.3.3 describes delivering an exponential decay pulse but specifying a time constant rather than a capacitance and resistance value.
- Section 3.3.4 describes delivering a square wave pulse.
- Section 3.3.6 explains how programmed settings may be saved as user protocols.

Section 3.4 describes the Pre-set protocols in which the pulse parameters have been optimized for a number of commonly used bacterial and fungal species and mammalian cell lines.

- Pre-set Protocols may be called up and used directly (Section 3.4.2) or may be modified prior to being used (Section 3.4.3).
- A modified Pre-set Protocol may be saved as a User Protocol (Section 3.4.4).

Section 3.5 describes a custom mode (User Protocols) in which users may store optimized pulse parameters that they use in their own work.

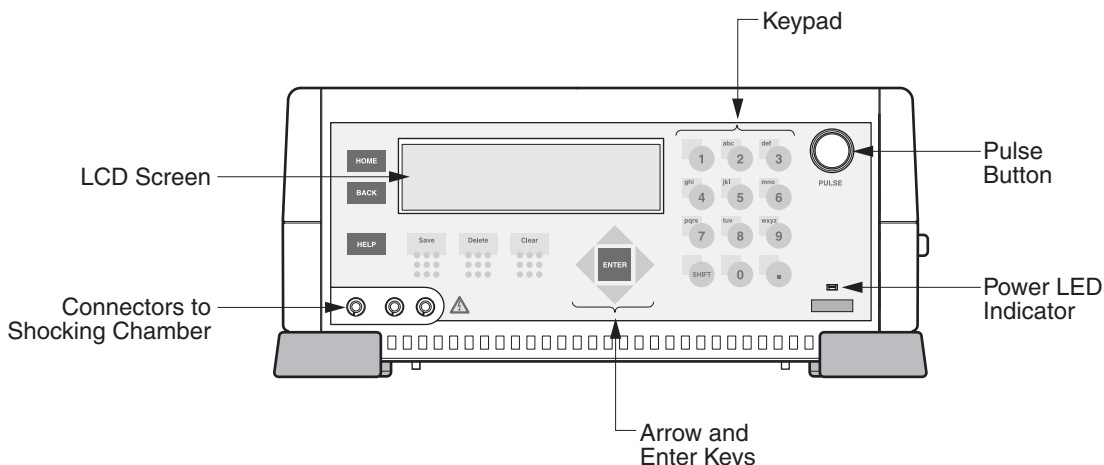
- User Protocols may be created in any of four ways:
  - In the User Protocols menu as a new protocol (Section 3.5.3).
  - In the User Protocols menu as an edited (modified) program (Section 3.5.4)
  - In the Manual menu as a new protocol (Section 3.3.4).
  - In the Pre-set Protocol Menu as a modified protocol (Section 3.4.4).
- User Protocols, once created and saved, may be called up and used directly like Pre-set Protocols (Section 3.5.1).

## 3.2 Front Panel and Home Screen

### 3.2.1 Description of the keypad

See Figure 3.1 for a view of the Gene Pulser Xcell front panel.

Alpha-numeric keys	This array of keys permits entering numbers and letters into the Gene Pulser Xcell. Pressing the Shift key toggles between alphabetic and numeric input. To type an alphabetic character, press the Shift key to enter alpha mode, then press the key with the appropriate letter. To type an a, press the 2 key once; to type a b, press the 2 key twice; to type a c, press the 2 key three times. To use the same key twice, for example to type a then b, advance the cursor using the Right Arrow Key. The firmware on Gene Pulser Xcell will automatically change between alpha and numeric input depending on the parameter being entered. In Protocol screens and Directory screens where a two-digit entry must be made, the second digit must be entered within 2 seconds of the first entry, otherwise the screen will default to the single-digit entry.
Home key	Returns the user to the Home screen from anywhere in the program.
Back key	Returns the user one level back in hierarchy toward the Home screen.
Help key	Displays on-screen help text.
Save key	Saves User Names and User Protocols.
Delete key	Removes only the last entry in the field; also used to remove User Name and User Protocol files.
Clear key	Removes the entire line of the field.
Enter key	Indicates that a choice has been made and moves the cursor to the next location.
Arrow keys	The Up and Down Arrow keys move the cursor up or down one row at a time. Depending on the screen and location of the cursor, the Right and Left Arrows may (1) move the cursor right or left one space at a time, (2) toggle forward and backward one screen when there are multiple screens for the same menu, or (3) increase or decrease numerical input values.
Pulse button:	Results in discharging a pulse. During this time “Pulsing” is shown on the LCD display. A tone sounds to indicate that the pulse has been delivered. When multiple pulses are delivered, a tone sounds after the last pulse has been delivered. The Pulse is discharged to the electrodes if the ShockPod is connected and the lid is closed. Otherwise, it is discharged safely within the instrument.



**Fig. 3-1. Gene Pulser Xcell front panel. See Section 3.2.1 for an explanation of the key functions.**

### 3.2.2 Home Screen

Upon turning on the power to Gene Pulser Xcell, the unit will run a series of test algorithms, including a check of the Pulse Trac system (see Section 3.11) and the firmware. During this time the LCD will display the Bio-Rad logo, the product name, and the version of firmware. After this instrument initialization, the Home Screen is displayed (Figure 3.2). From this screen it is easy to access the programs described below. You may return to this screen from anywhere in the program by pressing the Home key on the keypad.

Home consists of two screens. The first contains the most commonly used programs. The second contains advanced functions. The Left and Right Arrow keys can be used to toggle between them.

To select a program, when the Home screen is displayed on the LCD screen, press numbers 1–10 on the Alpha-Numeric keypad followed by the Enter key. Alternatively, use the Up and Down Arrow keys to scroll to the desired selection; press Enter to select the program.

- |                            |   |
|----------------------------|---|
| 1. Exponential protocol:   | for delivery of an exponential decay pulse: enables the user to program a set of conditions and to deliver the pulse  |
| 2. Time constant protocol: | for delivery of an exponential decay pulse by specifying a time constant: enables the user to program a set of conditions and to deliver the pulse                      |
| 3. Square wave protocol:   | for delivery of a square wave pulse: enables the user to program a set of conditions and to deliver the pulse   |
| 4. Pre-set protocols:      | pre-programmed optimized settings for electrotransformation of commonly used bacterial, fungal, and mammalian cells   |
| 5. User protocols:         | a program which allows the user to store up to 144 protocols including all instrument settings and recall these protocols to deliver a pulse                            |
| 6. Last pulse:             | allows the user to recall the electroporation parameters for the last pulse and to deliver a pulse using the same conditions  |
| 7. Optimize:               | allows the user to program electroporation parameters and an increment for voltage that may be changed after each pulse in order to optimize electroporation conditions |



- 8. Data management: allows the user to access pulse parameters and results for the last 100 pulses logged by date and time
- 9. Measurements: allows the user to measure capacitor values in the Gene Pulser Xcell or CE Module and to measure sample resistance
- 10. User Preferences: allows the user to adjust the clock, the screen light intensity, and the LCD sleep function

Right and left arrows (> and <) appear at the bottom of the first screen and at the top of the second screen, respectively. These arrows indicate that the Right and Left Arrow keys may be used to toggle between these related screens.

### Home Screen

```
HOME
1. Exponential protocol
2. Time constant protocol
3. Square wave protocol
4. Pre-set protocols
>5. User protocols
```

```
HOME
<6. Last pulse
7. Optimize
8. Data management
9. Measurements
10. User preferences
```

**Fig. 3.2. Gene Pulser Xcell Home screens. All menu functions may be readily accessed from these screens. Return to the Home screen from anywhere in the program by pressing the Home key. Press the Left and Right Arrow keys to toggle between the two screens.**

### 3.2.3 Help Screens

Help screens are accessible from all screens by pressing the Help key. Each help menu describes the keystrokes that may be entered to choose the next function or to continue the current operation. Where there is more than one help screen, use the Right and Left Arrow keys to scroll between them. To return to the program screen, press the Back key.

## 3.3 Manual Operation

### 3.3.1 Manual Operation (Quick Guide)

- From the Home screen:
  - Press Enter to select exponential decay;
  - Press 2, then Enter to select exponential decay but specifying a time constant;
  - Press 3, then Enter to select square wave.
- Use the Up and Down Arrow keys to scroll through the parameter value spaces on the screen. When a parameter value is highlighted, use the keypad to enter a value, then press Enter to accept that value.
- When the necessary parameter values have been entered, the Pulse button on the Gene Pulser Xcell is active.
- Press the Pulse button to electroporate the sample.
- Press the Back key to return to the Protocol Detail screen and to deliver another pulse.

### 3.3.2 Electroporation using Exponential Decay Pulses

See Section 4.1 for a discussion of electroporation using exponential decay pulses.

- When the Home screen (Figure 3.2) is selected, the number 1, corresponding to “Exponential protocol” is highlighted as the default choice. Press Enter to view the Protocol Detail Screen. If the number 1 on the Home screen is not highlighted, press 1 or use the Up or Down Arrow keys to highlight “Exponential protocol”, then press Enter to select. The Protocol Detail screen appears (Figure 3.3).
- The following combination of parameters may be entered:
  - Capacitance + Voltage
  - Capacitance + Voltage + Resistance

The three variables may be selected in any order, however, the set voltage will determine whether the high voltage or the low voltage circuit is to be used and will limit the range of the capacitance as indicated in Table 3.1. If a value for the capacitance is chosen that outside the range of the system, this value will default to the closest allowable value.

Specifying a resistance value requires that the PC Module be attached. This is always recommended with high resistance media (i.e., >600 ohm) such as water, sucrose, glycerol, sorbitol, or polyethylene glycol. The PC Module places a resistor in parallel with the sample to reduce the resistance of the circuit. In this way, the time constant of a high-resistance sample may be reduced and controlled.

When the Resistance value indicates infinity, no resistors from the PC Module are used. The Measurements function of Gene Pulser Xcell may be used to determine sample resistance (see Section 3.9).

### Exponential Decay Protocol Detail Screen

```

PROTOCOL DETAIL:  EXPONENTIAL

Voltage (V)           XXXX
Capacitance (uF)     XXXX
Resistance (ohm)      XXXX
Cuvette (mm)         X
  
```

**Fig. 3.3. Exponential Decay Protocol Detail Screen.** This screen shows the parameters that may be specified for exponential decay electroporation. Entering the cuvette size is optional and is for user information only.

**Table 3.1. Capacitance and voltage ranges for the high voltage / low capacitance circuit and the low voltage / high capacitance circuit of the Gene Pulser Xcell for delivering an exponential decay pulse.**

	Capacitance (uF)	Voltage (V)	
		R <sub>sample</sub> < 600	R <sub>sample</sub> > 600
HV Circuit	10 – 50	200 – 2500	200 – 3000
LV Circuit	25 – 3275	10 – 500	10 – 500

\*Use of the low voltage circuit requires that the CE Module be in place.

- Use the Up and Down Arrow keys to scroll through and highlight the parameter value spaces for capacitance, voltage, or resistance. Use the alpha-numeric keypad to input the desired value, or use the Right and Left Arrow keys to increase or decrease the parameter value by the smallest allowable increment. Use the Delete or Clear keys to change the value. When the desired value has been specified, press Enter. If a value outside the limits of the Gene Pulser Xcell is selected, the value in the field will default to the closest permitted value. Entering a value for cuvette size is optional and is for user information only.
- When the necessary parameter values have been specified, a flashing “P” appears in the character space in the lower right corner of the LCD display indicating that the pulse button on the Gene Pulser Xcell is active and that a pulse may be delivered.
- Press the Pulse button to deliver a pulse. When the Pulse button is depressed, the LCD display will blank then show “Pulsing”. Upon completion, a tone will sound and the pulse measurements will be displayed on the Protocol Results screen (see Figure 3.6, Section 3.3.5).
- Use the Left and Right Arrow keys to toggle between the Protocol Results screen and the last Protocol Detail screen.

- With the Protocol Detail screen on the LCD display another pulse can be delivered using the same pulse parameters. To change the pulse conditions, press Enter; the cursor appears in the voltage parameter value. The parameters may be changed as described above. To save the pulse parameters, see Section 3.3.6.
- To review previously delivered pulses, see Section 3.8.

### 3.3.3 Electroporation Specifying Time Constant

See Section 4.1 for a discussion of electroporation using exponential decay. Use the Gene Pulser Xcell to deliver an exponential decay pulse with a specific time constant as follows.

- From the Home screen (Figure 3.2), press 2 or use the Up or Down Arrow keys to highlight “Time constant protocol”, then press Enter. The Time Constant Protocol Detail screen appears (Figure 3.4).
- A sample must be in place to use Gene Pulser Xcell in this mode. The following parameters must be entered:

Time constant + Voltage

In time constant mode, Gene Pulser Xcell will maximize the parallel resistance (and minimize the capacitance) in order to maximize the energy delivered to the sample. Therefore, the PC Module is required to deliver pulses using the high voltage circuit, while both the CE Module and PC Module are needed to deliver pulses using the low voltage circuit. The set voltage will determine whether the high voltage or the low voltage circuit is to be used and will limit the range of the capacitance as indicated in Table 3.2. If a voltage is chosen that spans both the low voltage and high voltage range, the Gene Pulser Xcell will preferentially use the low voltage circuit. As indicated in Table 3.2, the acceptable values for the time constant are a function of the sample resistance and the voltage circuit used.

Immediately before delivering a pulse, Gene Pulser Xcell will determine sample resistance to determine whether a pulse can be delivered using the set parameters of voltage and time constant. If the set time constant is outside the range by more than 20%, the Gene Pulser Xcell will show a message indicating the approximate time constant that can be delivered, and ask that you press the pulse button again to deliver the pulse. To estimate sample resistance using Gene Pulser Xcell, see Section 3.9.

**Table 3.2. Ranges of time constants that may be achieved using Gene Pulser Xcell at several sample resistances.**

Sample resistance	Time constant range (msec)	
	LV Circuit*	HV Circuit**
20 ohms	0.5 – 65.5	0.2 – 1.0
200 ohms	5.0 – 655	2.0 – 10
>1000 ohms	25 – 3275	10 – 50
	* LV Circuit range:	10 – 500 V
	** HV Circuit range:	200 – 3000 V

### Time Constant Protocol Detail screen

```
PROTOCOL DETAIL: TIME CONSTANT

Voltage (V)                XXXX
Time constant (msec)       XXXX.X
Cuvette (mm)                X
```

**Fig. 3.4. Time Constant Protocol Detail Screen.** This screen shows the parameters that may be specified for exponential decay electroporation when the time constant is designated. Entering the cuvette size is optional and is for user information only.

- Use the Up and Down Arrow keys to highlight the parameter value space for voltage or time constant. Use the alpha-numeric keypad to input the desired value, or use the Right and Left Arrow keys to increase or decrease the parameter value by the smallest allowable increment. Use the Delete or Clear keys to correct entries. When the desired value has been specified, press Enter. If a value outside the limits of the Gene Pulser Xcell is selected, the value in the field will default to the closest permitted value. Entering a value for cuvette size is optional and is for user information only.
- When the necessary parameter values have been specified, a flashing “P” appears in the character space in the lower right corner of the LCD display indicating that the pulse button on the Gene Pulser Xcell is active and that a pulse may be delivered.
- Press the Pulse button to deliver a pulse. When the Pulse button is depressed, the LCD display will blank then show “Pulsing”. Upon completion, a tone will sound and the pulse measurements will be displayed on the Protocol Results screen (see Figure 3.7, Section 3.3.5).
- Use the Left and Right Arrow keys to toggle between the Protocol Results screen and the last Protocol Detail screen.
- With the Protocol Detail screen on the LCD display another pulse can be delivered using the same pulse parameters. To change the pulse conditions, press Enter; the cursor appears in the voltage parameter value. The parameters may be changed as described above.
- To save the pulse parameters, see Section 3.3.6.
- To review previously delivered pulses, see Section 3.8.

#### 3.3.4 Electroporation using Square Wave Pulses

See Section 4.2 for a discussion of electroporation using square wave pulses. Use Gene Pulser Xcell to deliver square wave pulses as follows.

- From the Home screen (Figure 3.2), press 3 or use the Up or Down Arrow keys to highlight “Square wave protocol”, then press Enter. The Square Wave Protocol Detail screen appears (Figure 3.5).

- The following combination of parameters may be entered:  
 Pulse length + Voltage  
 Pulse length + Voltage + Number of pulses + Pulse interval

### Square Wave Protocol Detail screen

PROTOCOL DETAIL: SQUARE WAVE	
Voltage (V)	XXXX
Pulse length (msec)	XXX.XX
Number of pulses	1
Pulse interval (sec)	00.0
Cuvette (mm)	X

**Fig. 3.5. Square Wave Protocol Detail Screen.** This screen shows the parameters that may be specified for square wave electroporation. Entering the cuvette size is optional and is for user information only.

Electroporation using square waves may be used to deliver a series of multiple pulses to the cells. This is specified by the parameter “Number of pulses.” The time between each pulse is specified as the “Pulse interval.” The default values for the number of pulses and pulse interval are 1 and 0, respectively; it is only necessary to enter other values if more than one pulse is to be delivered. See Section 4.2 for a discussion of pulse parameters.

The pulse parameters may be selected in any order. However, the set voltage will determine whether the high voltage or the low voltage circuit is to be used. Consequently, the set voltage will limit the ranges of the pulse length and pulse interval as indicated in Table 3.3. If values for the second and third parameters are chosen that are outside the range of the system, the values will default to the closest allowable values.

**Table 3.3. Pulse length and pulse interval ranges for the high voltage / low capacitance circuit and the low voltage / high capacitance circuit of the Gene Pulser Xcell for delivering a square wave pulse.**

	Voltage (V)	Pulse Length	Pulse Interval	No. Pulses (max)
LV Circuit*	10–500	0.05–100 ms	0.1–10 s	10
HV Circuit	200–3000	0.05–5 msec	5–30 s	2

\*Use of the low voltage circuit requires that the CE Module be in place.

- Use the Up and Down Arrow keys to scroll through and highlight the parameter value space for voltage, pulse length, number of pulses, or pulse interval. Use the alpha-numeric keypad to input the desired value, or use the Right and Left Arrow keys to increase or decrease the parameter value by the smallest allowable increment. Use the Delete or Clear keys to correct entries. When the desired value has been specified, press Enter. If a value outside the limits of the Gene Pulser Xcell is selected, the value in the field will default to the closest permitted value. Entering a value for cuvette size is optional and is for user information only.

- When the necessary parameter values have been specified, a flashing “P” appears in the character space in the lower right corner of the LCD display indicating that the pulse button on the Gene Pulser Xcell is active and that a pulse may be delivered.
- Press the Pulse button to deliver a pulse. When the Pulse button is depressed, the LCD display will blank then show “Pulsing”. Upon completion, a tone will sound and the pulse measurements will be displayed on the Protocol Results screen (see Figure 3.8, Section 3.3.5).
- Use the Left and Right Arrow keys to toggle between the Protocol Results screen and the last Protocol Detail screen.
- With the Protocol Detail screen on the LCD display another pulse can be delivered using the same pulse parameters. To change the pulse conditions, press Enter; the cursor appears in the voltage parameter value. The parameters may be changed as described above.
- To save the pulse parameters, see Section 3.3.6.
- To review previously delivered pulses, see Section 3.8.

### **3.3.5 Results Screens**

After delivering a pulse, the LCD displays the results on a Protocol Results screen. This screen shows the results in both graphic and tabular form. Figures 3.6, 3.7, and 3.8 show examples of the results from an exponential decay pulse, an exponential decay pulse in which the time constant was specified, and a square wave pulse, respectively.

Results of the last 100 pulses as well as of the pulse parameters are stored in Gene Pulser Xcell memory and are accessible from the Data Management program (Section 3.8).

### Exponential Decay: Results Screen

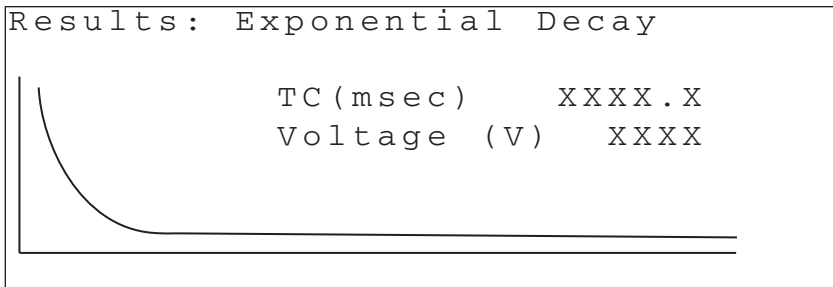


Fig. 3.6. Exponential Decay Protocol Results screen. The graph shows the exponential decay pulse. The table gives the calculated time constant (TC) and the delivered voltage (Voltage).

### Time Constant: Results screen

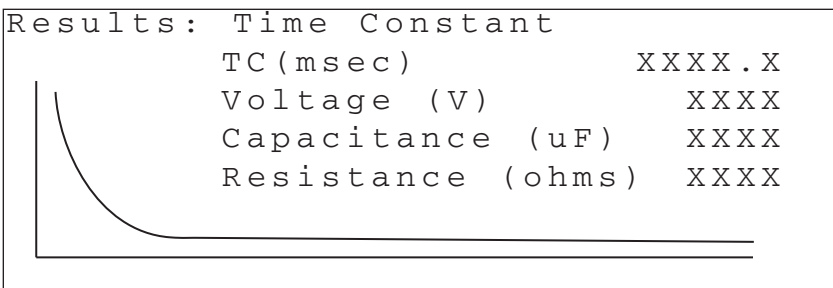


Fig. 3.7. Time Constant Protocol Results screen. The graph shows the exponential decay pulse. The table gives the delivered time constant (TC) and voltage (Voltage) as well as the selected capacitor (Capacitance) and resistor (Resistance).

### Square Wave: Results screen

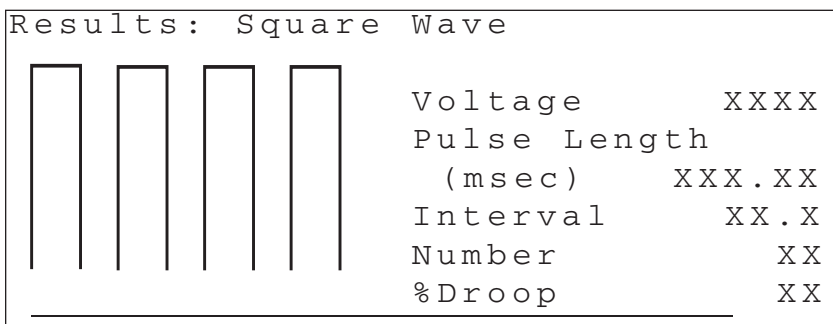


Fig. 3.8. Square Wave Protocol Results screen. The graph shows the square wave pulse; note that the x-axis may not be to scale. The table gives the actual pulse length (Pulse Length), the delivered voltage (Voltage), the calculated droop (% Droop), and, when multiple pulses are given, the pulse interval (Interval) and the number of pulses (Number).



### 3.3.6 Saving a program from Manual Operation

A protocol created in manual mode may be saved as a User Protocol as follows.

- Create a protocol as described in Section 3.3.2, 3.3.3 or 3.3.4.
- With the Protocol Detail screen open, press Save.
- The first User Directory screen will appear (Figure 3.9); the second line will read “Choose location for protocol”.
- Use the Right and Left Arrow keys to toggle between the two User Directory screens. Press 1–12 or use the Up and Down Arrow keys to highlight the User Name under which to store the protocol. Press Enter to select the User Name. The User Protocols screen will appear (Figure 3.10); the second line will read “Choose location for protocol”. If it is necessary to create a new User Name, see Section 3.5.2.
- Use the Right and Left Arrow keys to toggle between the two User Protocols screens. Press 1–12 or use the Up and Down Arrow keys to highlight a location for the new protocol. A protocol may be stored in a position without an entry (see Section 3.3.6A) or in a position with an entry (see Section 3.3.6B). If necessary, delete a User Protocol as described in Section 3.5.5.
- To use the saved protocol, press Enter to view the Protocol Detail screen. Press the Pulse button to electroporate the sample.

#### User Directory screen

```
USER DIRECTORY
Choose location for protocol
 1. User1
 2. Labguy
 3. Malcom
 4. Smith
 5. Wesson
> 6.
```

**Fig. 3.9. User Directory screen. This is an example of the first User Directory screen. Toggle between this screen and the second screen with Users 7–12 using the Left and Right Arrow keys. When purchased, the initial User Directory screen will have only numbers without any names.**

## User Protocols screen

```
USER PROTOCOLS: User1
Choose location for protocol
  1 . CHO
  2 . Monkey
  3 . Human
  4 .
  5 .
> 6 .
```

**Fig. 3.10. User Protocols screen. This is an example of the first User Protocols screen. Toggle between this screen and the second screen with Protocols 7–12 using the Left and Right Arrow keys. When purchased, the initial User Directory screen will have only numbers without any names.**

### 3.3.6A Saving a protocol in a location without a named User Protocol entry

- With a User Protocols screen displayed on the LCD, press 1–12 or use the Up, Down, Left, and Right Arrows to highlight a User Protocol without an entry (e.g., positions 4, 5, or 6 on the screen in Figure 3.10).
- Press Enter to select the position.
- Type in the name using the alpha-numeric keypad (alphabetic entries are the default; press Shift to toggle between alphabetic and numeric entries; the maximum length is 10 characters; to use the same key twice, advance the cursor using the Right Arrow Key, spaces are not allowed). Press Save or Enter. The protocol is saved in the location named on the User Protocols screen.
- To use the saved protocol, press Enter to view the Protocol Detail screen. Press the Pulse button to deliver a pulse.

### 3.3.6B Saving a protocol in a location with a named User Protocol entry

- With a User Protocols screen displayed on the LCD, press 1–12 or use the Up, Down, Left, and Right Arrows to highlight a User Protocol with a named entry (e.g., positions 1, 2, or 3 on the screen in Figure 3.10).
- Press Enter to select the position.
- A Warning screen will appear asking whether you want to overwrite the selected User Protocol (Figure 3.11).

### Warning screen: overwrite protocol

```
Do you want to overwrite the
protocol?
    YES                               NO

Press BACK or ENTER to return
to the previous screen.
Press the LEFT arrow then
ENTER to overwrite the name.
```

**Fig. 3.11. Warning screen: overwrite protocol.**

- The default selection is “No”. Press Enter if you do not want to overwrite the file. The program will return to the previous User Protocols screen.
- Press the Left Arrow key to select “Yes”, then press Enter to overwrite the file. The User Protocol will have the same name and will contain all of the parameters specified in the new User Protocol. The program will return to the User Protocols screen with the cursor highlighting the location (number) of the selected User Protocol.
- To use the saved protocol, press Enter to view the Protocol Detail screen. Press the Pulse button to deliver a pulse.

## 3.4 Pre-set Protocols

The Gene Pulser Xcell is pre-programmed with optimized settings for a number of commonly used bacterial, fungal, and mammalian cells. The settings are given in Table 3.4. The settings may also be viewed on the LCD display as described below (see Section 3.4.2). It is possible to change any of the parameters prior to pulsing a sample. While permanent changes cannot be made to any Pre-set Protocol, changes may be saved as a User Protocol (see Sections 3.4.4).

### 3.4.1 Using a Pre-set Protocol (Quick Guide)

- From the Home Screen, press 4, then Enter, to show the Pre-set Protocols screen.
- Press 1–3 to choose between bacterial, fungal, or mammalian cells; press Enter to select the organism type and to show the list of organisms. For Bacterial and Mammalian Pre-set Protocols, use the Right and Left Arrow keys to toggle between the two screens.
- Press the number next to the desired protocol to highlight it; press Enter to select and to show the Protocol Detail screen.
- Press the Pulse button to electroporate the sample.
- Press the Back key to return to the Protocol Detail screen and to deliver another pulse.

### 3.4.2 Electroporation using Pre-set Protocols

There are nine Pre-set Bacterial Protocols, six Pre-set Fungal Protocols, and 12 Pre-set Mammalian Protocols. These protocols are pre-programmed with the optimal parameters for the given organism. Use the Pre-set Protocols as follows.

- From the Home screen, press 4 or use the Up and Down Arrow keys to highlight “Pre-set Protocols”, then press Enter to select and to show the Pre-set Protocols screen (Figure 3.12).
- Press 1–3, or use the Up and Down Arrow keys, to highlight Bacterial, Fungal, or Mammalian Pre-set Protocols, then press Enter to select.
- Use the alpha-numeric keypad or the Up and Down Arrow keys to scroll through the list of names. For the Bacterial and Mammalian Pre-set Protocols, use the Right and Left Arrow keys to toggle between the two screens. When the number corresponding to the desired name is highlighted, press Enter to select and to view the Protocol Detail Screen showing the electroporation parameters for that protocol. A flashing “P” in the character space in the lower right corner of the LCD display indicates that the Pulse button is active.
  - For example, from the Pre-set Protocols screen, press 3 to highlight “Mammalian”, then press Enter to select and to bring up the first Pre-set Mammalian Protocols screen with the names of six pre-set mammalian protocols (Figure 3.13). Press the Right and Left Arrow keys to toggle between the two Mammalian Pre-set Protocols screens. Use the alpha-numeric keypad or the Up and Down Arrow keys to scroll through the list of names. When the desired name on the Mammalian Pre-set Protocols screen is highlighted, press Enter to select that protocol and to view the Protocol Detail Screen showing the electroporation parameters for that protocol. For example, from the Mammalian Pre-set Protocols screen, press 1, then Enter to bring up the Protocol Detail Screen for CHO cells in a 2 mm cuvette (Figure 3.14).
- Press the Pulse button to deliver a pulse. When the Pulse button is depressed, the LCD display will blank then show “Pulsing”. Upon completion, a tone will sound and the pulse measurements will be displayed on the Protocol Results screen (see Section 3.3.5).
- Use the Left and Right Arrow keys to toggle between the Protocol Results screen and the last Protocol Detail screen.
- With the Protocol Detail screen on the LCD display another pulse can be delivered using the same pulse parameters. To change the pulse conditions, press Enter; the cursor appears in the voltage parameter value. The parameters may be changed as described in Section 3.4.3.
- To review previously delivered pulses, see Section 3.8.

### Pre-Set Protocols Screen

```
PRE-SET PROTOCOLS

1 . B a c t e r i a l
2 . F u n g a l
3 . M a m m a l i a n
```

**Fig. 3.12. Pre-set Protocols Screen.** There are Pre-set protocols for bacteria, fungi, and mammalian cells. Use this screen to make a selection from the list.

### Mammalian Pre-set Protocols Screen (screen 1)

```
PRE-SET PROTOCOLS: MAMMALIAN

1 . C H O
2 . C o s 7
3 . M 3 T 3
4 . 2 9 3
5 . H e L a
> 6 . B H K 2 1
```

### Mammalian Pre-Set Protocols Screen (screen 2)

```
PRE-SET PROTOCOLS: MAMMALIAN

7 . A 5 4 9
8 . C V 1
9 . K 5 6 2
10 . H L 6 0
11 . J u r k a t
> 12 . H u T 7 8
```

**Fig. 3.13. Mammalian Pre-set Protocols Screens.** Protocols are provided for 12 mammalian cell lines. The names of the first six are shown on Screen 1; the names of the second six are shown on Screen 2.

### Pre-set Protocol: CHO

PROTOCOL DETAIL: CHO	
Voltage (V)	160
Pulse length (msec)	15.00
Number of pulses	1
Pulse interval (sec)	00.0
Cuvette (mm)	2
	P

**Fig. 3.14. Protocol Detail Screen for CHO cells in the Pre-set Protocols menu.**

**Table 3.4. Optimized settings found in the Pre-set protocols function of the Gene Pulser Xcell.**

Pre-set Protocol Designation	Cells	Pulse type	PL (msec)	C (μF)	PC (ohm)	V	Cuvette (cm)	Cell vol (μl)
Mammalian 1	CHO	Square wave	15			160	0.2	100
Mammalian 2	COS7	Square wave	20			110	0.2	100
Mammalian 3	3T3	Exponential decay		500	∞	160	0.2	100
Mammalian 4	293	Square wave	25			110	0.2	100
Mammalian 5	HeLa	Exponential decay		500	∞	160	0.2	100
Mammalian 6	BHK21	Square wave	25			140	0.2	100
Mammalian 7	A549	Square wave	10			150	0.2	100
Mammalian 8	CV1	Square wave	25			100	0.2	100
Mammalian 9	K562	Exponential decay		1000	∞	155	0.2	100
Mammalian 10	HL60	Square wave	25			140	0.2	100
Mammalian 11	Jurkat	Exponential decay		1000	∞	140	0.2	100
Mammalian 12	HuT78	Square wave	25			130	0.2	100
Bacterial 1	E coli	Exponential decay		25	200	1800	0.1	20
Bacterial 2				25	200	2500	0.2	20-40
Bacterial 3				25	200	3000	0.2	20-40
Bacterial 4	A tumefaciens	Exponential decay		25	200	2400	0.1	20
Bacterial 5	P aeruginosa	Exponential decay		25	200	2500	0.2	100
Bacterial 6	S aureus	Exponential decay		25	100	2900	0.2	50
Bacterial 7	B cereus	Exponential decay		50	200	1000	0.2	100
Bacterial 8	S pyogenes	Exponential decay		25	200	2100	0.2	200
Bacterial 9	L plantarum	Exponential decay		25	400	2000	0.2	40
Fungal 1	S cerevisiae	Exponential decay		25	200	1500	0.2	40
Fungal 2				25	200	2500	0.4	80
Fungal 3	S pombe	Exponential decay		25	200	2300	0.2	40
Fungal 4	C albicans	Exponential decay		25	200	1500	0.2	40
Fungal 5	P pastoris	Exponential decay		25	200	2000	0.2	40
Fungal 6	D discoideum	Square wave	1.0	10		1000	0.4	800 2 pulses

### 3.4.3 Modifying Pre-set Protocol Parameters

The parameters for a Pre-set protocol may be changed as follows.

- From the Protocol Detail screen, press the Up or Down Arrow keys to highlight the value for one of the parameter settings (voltage, capacitance, or resistance for exponential decay pulses; voltage or time constant for time constant mode; pulse length, voltage, number of pulses, or pulse interval for square wave pulses). (Note: the waveform cannot be changed in the Pre-set Protocols Mode.) When the desired parameter is selected, use the alpha-numeric keypad to input the new value. Alternatively, use the Right and Left Arrow keys to incrementally increase or decrease, respectively, the parameter value. Use the Delete or Clear keys to correct entries. When the correct value has been specified, press Enter. If a value outside the limits of the Gene Pulser Xcell is selected, the value in the field will default to the closest permitted value. Use the Up and Down Arrow keys to select other parameter values to be changed, then use the alpha-numeric keypad or the Left and Right Arrow keys to enter the desired value.
- A pulse may be delivered when appropriate parameters have been entered in the Protocol Detail screen and the character space at the lower right of the LCD display is flashing "P".
- To return to the last Protocol Detail screen, press the Back key or the Left Arrow key. Another pulse may be delivered using the same parameters shown on the LCD display. To return to the Protocol Results Screen, press the Right Arrow key. (Note: Returning to the Protocol Detail Screen returns to the modified parameters. To return to the Pre-set Protocol, press the Back key again to return to the Pre-set Protocols screen. This will remove any changes made.)
- To change the pulse conditions, with the Protocol Detail screen on the LCD display, press Enter; the cursor appears in the voltage parameter value. The parameters may be changed as described above.
- To review previously delivered pulses, see Section 3.8.

### 3.4.4 Saving Changes to Pre-set Protocols

Changes to a Pre-set Protocol may be saved as a User Protocol as follows:

- Change the Pre-set Protocol as described in Section 3.4.3.
- With the Protocol Detail screen open, press Save.
- The first User Directory screen will appear (Figure 3.9); the second line will read "Choose location for protocol".
- Use the Right and Left Arrow keys to toggle between the two User Directory screens. Press 1–12 or use the Up and Down Arrow keys to highlight the User Name under which to store the protocol. Press Enter to select the User Name. The User Protocols screen will appear (Figure 3.10); the second line will read "Choose location for protocol". If it is necessary to create a new User Name, see Section 3.5.2.
- Use the Right and Left Arrow keys to toggle between the two User Protocols screens. Press 1–12 or use the Up and Down Arrow keys to highlight a location for the new protocol. A protocol may be stored in a position without an entry (see Section 3.3.6A) or in a position with an entry (see Section 3.3.6B). If necessary, delete a User Protocol as described in Section 3.5.5.
- To use the saved protocol, press Enter to view the Protocol Detail screen. Press the Pulse button to deliver a pulse.

## 3.5 User Protocols

User Protocols enable users to store their own protocols for the Gene Pulser Xcell. Up to 144 electroporation protocols may be programmed and stored. (12 users (User Names in the User Directory) each with 12 protocols per user (User Protocols).)

A new User Protocol may be created either by:

- Creating a new protocol by entering all new parameters in manual mode (Sections 3.3.2, 3.3.3, and 3.3.4).
- Modifying (editing) a Pre-set protocol (Section 3.4.3).
- Creating a new protocol as a User Protocol (Section 3.5.3).
- Modifying (editing) an existing User Protocol (Section 3.5.4).

Protocols created or modified in the User Protocols menu may be used without being saved (Section 3.5.4).

### 3.5.1 Using a User Protocol (Quick Guide)

- From the Home screen, press 5, then Enter to open the User Protocols menu and to show the first User Directory screen. Use the Right and Left Arrow keys to toggle between the two screens.
- Press 1–12 to highlight the desired name; press Enter to select that name and to show the first User Protocols screen. Use the Right and Left Arrow keys to toggle between the two screens.
- Press 1–12 to highlight the desired protocol name; press Enter to select that protocol and to show the Protocol Detail screen. The Pulse button on the Gene Pulser Xcell is now active.
- Press the Pulse button to electroporate the sample.
- Press the Back key to return to the Protocol Detail screen and to deliver another pulse.

### 3.5.2 Creating a New User Name

A new User Name can be created when the space adjacent to that number on the User Directory screen is clear (Figure 3.15). If it is necessary to delete a User Name or a User Protocol, see Section 3.5.5.

Create a new User Name in the User Name screen as follows.

- From the Home screen (Figure 3.2), press 5 or use the Up and Down Arrow keys to highlight “User Protocols”, then press Enter to view the User Directory screen (Figure 3.15).
- Use the Right and Left Arrow keys to toggle between the two User Directory screens. Press 1–12 or use the Up or Down Arrow keys to highlight a position without an entry. Press Enter to select the position.
- Use the alpha-numeric keypad to type in the desired User Name. A name can have up to ten characters. Press Save when complete. The new User Name is saved.

### 3.5.3 Creating a New User Protocol

This section describes how to create a new User Protocol from the User Protocols screen and save the protocol. A new User Protocol can be created when the space adjacent to that number on the User Protocol screen is clear (Figure 3.16). Section 3.5.4 describes how to edit (change) an existing User Protocol and save the changes.



- To enter the User Protocols screen from the Home screen (Figure 3.2):
  - Press 5 or use the Up and Down Arrow keys to highlight “User Protocols”, then press Enter to view the first User Directory screen (Figure 3.15).
  - Use the Right and Left Arrow keys to toggle between the two User Directory screens. Press 1–12 or use the Up and Down Arrow keys to highlight the number corresponding to the desired User Name.
  - Press Enter to select the name and to view the User Protocols Screen (Figure 3.16).
- Press 1–12 or use the Up, Down, Left, and Right Arrow keys to highlight a User Protocol without an entry. If necessary, delete a User Protocol as described in Section 3.5.5. Press Enter to view the Select Method screen (Figure 3.17).
- Press 1–3 or use the Up and Down Arrow keys to highlight the number corresponding to delivering an exponential decay pulse (Exponential protocol), an exponential decay pulse specifying the time constant (Time constant protocol), or a square wave pulse (Square wave protocol). Press Enter to make a selection and to view the appropriate Protocol Detail screen.

### User Protocols – User Directory Screen (screen 1)

```
USER DIRECTORY

1 . User1
2 . Labguy
3 . Malcom
4 . Smith
5 . Wesson
>6 .
```

### User Protocols – User Directory Screen (screen 2)

```
USER DIRECTORY

<7 .
8 .
9 .
10 .
11 .
12 .
```

Fig. 3.15. User Directory screen.

### User Protocols Screen

```
USER PROTOCOLS: User1

1 . CHO
2 . Monkey
3 . Human
4 .
5 .
>6 .
```

Fig 3.16. User Protocols screen.

### Select Method Screen

```
SELECT METHOD

1.Exponential protocol
2.Time constant protocol
3.Square wave protocol
```

**Fig. 3.17. Select Method screen.**

- When “Exponential protocol” is selected, the Exponential Decay Protocol Detail screen appears (Figure 3.18) with the User Name on the first line.

### Exponential Decay Protocol Detail Screen

```
Labguy:          Protocol:

Voltage (V)             XXXX
Capacitance (uF)       XXXX
Resistance (ohm)       XXXX
Cuvette (mm)           X
```

**Fig. 3.18. Exponential Decay Protocol Detail screen.**

- When “Time constant protocol” is selected, the Time Constant Protocol Detail screen appears (Figure 3.19) with the User Name on the first line.

### Time Constant Protocol Detail Screen

```
Labguy:          Protocol

Voltage (V)             XXXX
Time constant (msec)   XXXX.X
Cuvette (mm)           X
```

**Fig. 3.19. Time Constant Protocol Detail screen.**

- When “Square wave protocol” is selected, the Square Wave Protocol Detail screen appears (Figure 3.20) with the User Name on the first line.

### Square Wave Protocol Detail Screen

Labguy :	Protocol :
Voltage (V)	XXXX
Pulse length (msec)	XXX.XX
Number of pulses	1
Pulse interval (sec)	00.0
Cuvette (mm)	X

**Fig. 3.20. Square Wave Protocol Detail screen**

- Use the Up and Down Arrow keys to scroll through the parameter value spaces on the appropriate Protocol Detail screen. Use the alpha-numeric keypad to input the desired value, or use the Right and Left Arrow keys to incrementally increase or decrease the parameter value. Use the Delete or Clear keys to correct entries. When the desired value has been specified, press Enter. If a value outside the limits of the Gene Pulser Xcell is selected, the value in the field will default to the closest permitted value (see Tables 3.1, 3.2, and 3.3). Entering a value for cuvette size is optional and is for user information only.
- When the necessary parameter values have been specified, a flashing “P” appears in the character space in the lower right corner of the LCD display indicating that the pulse button on the Gene Pulser Xcell is active and that a pulse may be delivered.
- Press the Pulse button to deliver a pulse. When the Pulse button is depressed, the LCD display will blank then show “Pulsing”. Upon completion, a tone will sound and the pulse measurements will be displayed on the Protocol Results screen (see Section 3.3.5).
- To save the pulse parameters, with the Protocol Detail screen on the LCD, press Save.
- The User Protocols screen will appear with the cursor flashing on the first character space of the selected position. Type in the name using the alpha-numeric keypad (alphabetic entries are the default; press Shift to toggle between alphabetic and numeric entries). Press Save or Enter when complete. The protocol is saved in the location named in the User Protocols screen.

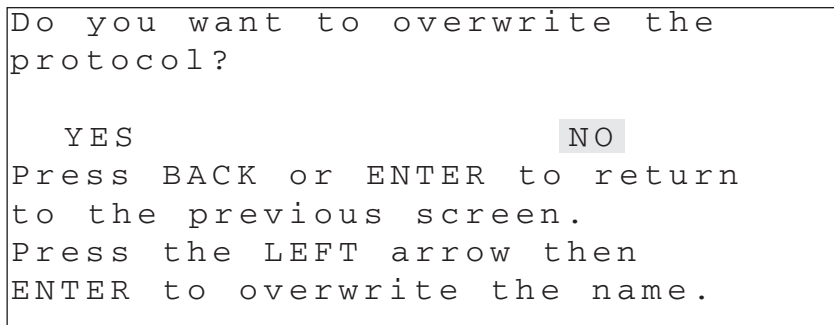
#### 3.5.4 Modifying a User Protocol

A User Protocol may be modified (changed) as follows.

- To enter the User Protocols screen from the Home screen (Figure 3.2):
  - Press 5 or use the Up and Down Arrow keys to highlight “User Protocols”, then press Enter to view the first User Directory screen (Figure 3.15).
  - Use the Right and Left Arrow keys to toggle between the two User Directory screens. Press 1–12 or use the Up and Down Arrow keys to highlight the desired User Name. Press Enter to select the name and to view the first User Protocols screen (Figure 3.16).
- Press 1–12 or use the Up, Down, Left, and Right Arrow keys to highlight the desired User Protocol to be edited. Press Enter to view the Protocol Detail screen.

- Use the Up and Down Arrow keys to highlight the value of the first parameter to be changed. Use the alpha-numeric keypad to enter a new value. Alternatively, use the Right and Left Arrow keys to incrementally increase or decrease, respectively, the parameter value. Use the Delete or Clear keys to correct entries.
- When the desired value has been specified, press Enter. If a value outside the limits of the Gene Pulser Xcell is entered, the value in the field will default to the closest permitted value.
- Use the Up and Down Arrow keys to highlight the parameter values to be changed and repeat the previous two steps. The Pulse button becomes active when the necessary parameters have been entered into the Protocol Detail screen. The edited protocol may be used without saving.
- To save the edited protocol, with the Protocol Detail screen on the LCD, press Save. A warning screen will appear asking if you want to overwrite the protocol (Figure 3.21). The default selection is “No”.
  - If you do not want to overwrite protocol name press the Back key to return to the Protocol Detail screen. Press Enter to return to the User Directory and to choose a new location (Figure 3.9). See Section 3.3.6 for instructions on selecting a location for the new protocol.
  - To overwrite the protocol, press the Left Arrow key to select “Yes”, then press Enter to overwrite the file. The User Protocol will have the same name as the old file and will contain the parameters specified in the new protocol. The LCD will display the User Protocols screen with the cursor highlighting the location (number) of the protocol.

**Warning screen: overwrite protocol**



**Fig. 3.21. Warning screen: overwrite protocol.**

**3.5.5 Deleting a User Name and a User Protocol**

Before a User Name may be deleted, it is necessary to first delete all of the User Protocols under that name.

Delete a User Protocol as follows.

- To enter the User Protocols screen from the Home screen (Fig 3.2):
  - Press 5 or use the Up and Down Arrow keys to highlight “User Protocols”, then press Enter to view the first User Directory screen (Figure 3.15). Use the Right and Left Arrow keys to toggle between the two User Directory screens.
  - Enter the number corresponding to the desired User Name or use the Up and Down Arrow keys to highlight the desired User Name.
  - Press Enter to select the name and to view the User Protocols screen.

- Use the alpha-numeric keypad or the Up and Down Arrow keys to select the position with the protocol to be deleted.
- Press the Delete key. A warning screen will appear asking whether you want to delete the selected User Protocol (Figure 3.22). The default selection is “No”.
  - If you do not want to delete the Protocol, press Back or Enter. The program will return to the previous User Protocols screen.
  - To delete the Protocol, press the Left Arrow key to select “Yes”, then press Enter. The User Protocol name as well as all of the parameters under that User Protocol is deleted. The program will return to the User Protocols screen with the selected User Protocol deleted.

### Warning screen: delete protocol

```

Do you want to delete the
protocol?

    YES                                NO
Press BACK or ENTER to return
to the previous screen.
Press the LEFT arrow then
ENTER to delete the name.
  
```

**Fig. 3.22. Warning screen: delete protocol.**

Delete a User Name as follows.

- With the User Directory screen on the LCD, press the Up or Down Arrow keys or use the alpha-numeric keypad to select the name to be deleted. Use the Left and Right Arrow keys to toggle between the two User Directory screens.
- Press the Delete key. If there are no protocols in the User Name file, a warning screen will appear asking whether you want to delete the selected User Name (Figure 3.23). The default selection is “No”.
  - If you do not want to delete the file, press Enter. The program will return to the User Directory screen.
  - To delete the User Name, press the Left Arrow key to select “Yes”, then press Enter. The program will return to the User Directory with the selected User Name deleted.
- If there are protocols in the User Name file, the following screen will appear: “All User Protocols under this name must be deleted before this user name can be deleted.” Follow the steps above for deleting the User Protocols.

### Warning screen: delete name

```
Do you want to delete the user
protocol?

      YES                               NO
Press BACK or ENTER to return
to the previous screen.
Press the LEFT arrow then
ENTER to delete the name.
```

**Fig. 3.23. Warning screen: delete name.**

### 3.5.6 Renaming a User Name or a User Protocol name

Rename a User Name or a User Protocol name as follows.

- Choose the User Name or User Protocol to rename from the User Directory screen or the User Protocols screen, respectively.
- Press the Clear key. A warning screen will appear asking whether you want to change the selected name (Figure 3.24). The default selection is “No”.
- If you do not want to change the name, press Enter. The program will return to the corresponding User Directory or User Protocols screen.
- To change the name, press the Left Arrow key to select “Yes”, then press Enter. The previous screen will appear but with the selected name cleared and with the cursor on the field for the new name. Use the alpha-numeric keypad to type in the desired new name. A name can have up to ten characters. Use the Shift key to toggle between alphabetic and numeric characters. Press Save when complete. The new name is saved.

### Warning screen: change name

```
Do you want to change the
name?

      YES                               NO
Press BACK or ENTER to return
to the previous screen.
Press the LEFT arrow then
ENTER to change the name.
```

**Fig. 3.24. Warning screen: name change.**

### 3.6 Last Pulse

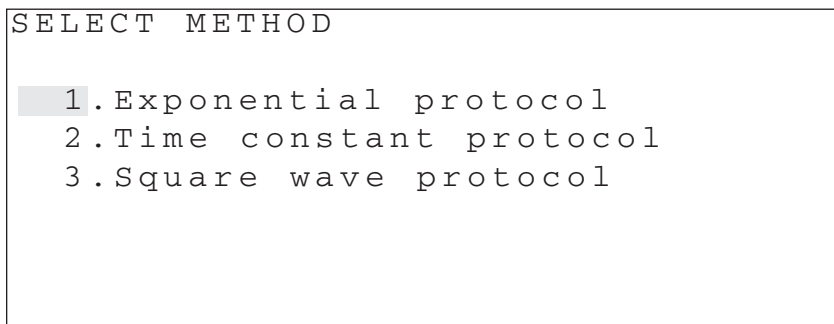
From the Home screen (Figure 3.2), press 6 or use the Up or Down Arrow keys to select “Last pulse”, then press Enter to display the Protocol Detail screen showing the last pulse delivered. This is also accessible after the instrument has been turned off and restarted.

### 3.7 Optimize Operation

The Optimize mode allows the user to program a voltage that incrementally increases or decreases when the Right and Left Arrow keys are pressed. This mode operates similarly to Manual Protocols (Section 3.3.2, 3.3.3, and 3.3.4) but requires that a voltage increment be specified.

- Access this screen from the Home screen (Figure 3.2) by pressing 7 or use the Up or Down Arrow keys to highlight “Optimize” then press Enter. The Select Method screen appears (Figure 3.17).
- Press 1–3 or use the Up and Down Arrow keys to highlight the number corresponding to optimizing voltage by delivering (i) an exponential decay pulse (“Exponential protocol”), (ii) an exponential decay pulse specifying the time constant (“Time constant protocol”), or (iii) a square wave pulse (“Square wave protocol”). Press Enter to make a selection and to view the appropriate Optimize screen.

#### Select Method screen



**Fig. 3.17. Select Method screen.**

- When “Exponential protocol” is selected, the Exponential Decay Protocol Optimize screen appears (Figure 3.25).
- When “Time constant protocol” is selected, the Time Constant Protocol Optimize screen appears (Figure 3.26).
- When “Square wave protocol” is selected, the Square Wave Protocol Optimize screen appears (Figure 3.27).



### Exponential Decay Protocol Optimize Screen

```
OPTIMIZE: EXPONENTIAL DECAY

Starting voltage (V)      XXXX
Increment (V)            XXX
Capacitance (uF)        XXXX
Resistance (OHM)         XXXX
Cuvette (mm)            X
```

Fig. 3.25. Exponential Decay Protocol Optimize screen.

### Time Constant Protocol Optimize Screen

```
OPTIMIZE: TIME CONSTANT

Starting voltage (V)      XXXX
Increment (V)            XXX
Time const (msec)        XXXX.X
Cuvette (mm)            X
```

Fig. 3.26. Time Constant Protocol Optimize screen.

### Square Wave Protocol Optimize Screen

```
OPTIMIZE: SQUARE WAVE

Starting voltage (V)      XXXX
Increment (V)            XXX
Pulse length (msec)      XXX.XX
Number of pulses         1
Pulse interval (sec)     XX.X
Cuvette (mm)            X
```

Fig. 3.27. Square Wave Protocol Optimize screen.

- Use the Up and Down Arrow keys to scroll through the parameter value spaces on the screen. When the desired parameter space is highlighted, use the alpha-numeric keypad to input the desired value (only numeric entries are permitted). Alternatively, use the Right and Left Arrow keys to incrementally increase or decrease the parameter value. Use the Delete or Clear keys to change the value. When the desired value has been specified, press Enter. If a value outside the limits of the Gene Pulser Xcell is selected, the value in the field will default to the closest permitted value.

- When the necessary parameter values have been specified, a flashing “P” appears in the character space in the lower right corner of the LCD display indicating that the pulse button on the Gene Pulser Xcell is active and that a pulse may be delivered.
- To use the program:
  - Press Pulse to deliver the pulse at the specified voltage.
  - After the pulse, the Protocol Results screen is displayed; use the Left and Right Arrow keys to toggle between the Protocol Results screen and the last Protocol Detail screen.
  - From the Protocol Detail screen, another pulse at the same voltage may be delivered by pressing Pulse. Press Enter to bring the cursor on the screen; the cursor will appear at the right character space of the first parameter (the voltage). To increase the voltage by the specified increment value, press the Right Arrow key; to decrease the voltage by the specified value, press the Left Arrow key. Press Pulse to deliver another pulse.

### 3.8 Data Management

This section of the program allows the user to view the parameter values and output of the last 100 pulses. Data for each pulse is presented on a single screen; examples are given below. Each data set includes the date and time of the pulse, an identifier for how the pulse was programmed, the set parameters, and the delivered pulse. The pulses are displayed in chronological order or in reverse chronological order and are logged by date and time. (Check that the date and time are correct under User Preferences (Section 3.10, Setting the Clock).) Selecting chronological order displays the earliest pulse in the data bank or, if a specific date was selected, the earliest pulse from the specified date. Selecting reverse chronological order displays the latest pulse in the data bank or, if a specific date was selected, the last pulse from the specific date. This is a read-only file. No changes are permitted to this file.

- From the Home screen (Figure 3.2) press 8 or use the Up and Down Arrow keys to highlight “Data Management”, then press Enter to display the Data Management screen (Figure 3.28).
- Press 1–4, then Enter to select the data to view. Selecting 3 or 4 brings up a Date screen (Figure 3.29). Use the keypad to enter a date, then press Enter to select that date and to view the data. If no data was collected on that date, a screen will appear indicating that no data is present for that date.

#### Data Management Screen

```

What data do you want to view?
All data
 1.Reverse chronological order
 2.Chronological order

Data from a specific date
 3.Chronological order
 4.Reverse chronological order

```

**Fig. 3.28. Data Management screen.**

## Date Screen

```
From what date do you want  
to view data?
```

```
Month   MM  
Date    DD  
Year    YY
```

Note: this is the default screen with the default clock preference set as MM/DD/YY; if the user changes the clock preference to DD/MM/YY, the order on the Date screen is Date, Month, Year.

### Fig. 3.29. Date screen.

- Press the Left and Right Arrow keys to view earlier and later pulse data, respectively. Press the Back key to return to the Data Management screen.

Data screens contain the following information:

Line 1: Date and Time

Line 2: Identification of how pulse was programmed (e.g., Manual, Pre-set, User)

Line 3: Type of pulse

Lines 4–8: Pulse conditions

The following are examples of data screens. It is not possible to edit a Data screen.

**Data screen (User Protocol, Square Wave)**

Data output	12-12-01	20:20
User 11	Protocol 11	
	Settings	Output
V	1230	1230
PL (msec)	123.00	123.00
#P	12	12
PI (sec)	12.3	12.3
%droop	12	12

**Data screen (Manual Mode, Exponential Decay)**

Data output	12-12-01	20:21
Manual exponential		
	Settings	Output
V	1230	1230
C(uF)	1230	1230
R(OHM)	1000	1000
TC(msec)		111.1
Cuvette(mm)	1	

**Data screen (Manual Mode, Time Constant)**

Data output	12-12-01	20:22
Manual time constant		
	Settings	Output
V	1230	1230
TC (msec)	1234.5	1234.5
Cuvette(mm)	1	

**Data screen (Standard Protocol, S. pombe setting)**

Data output	12-12-01	20:23
Std Protocol:	S.pombe	
	Settings	Output
V	2000	2047
C(uF)	25	25
R(ohm)	200	200
TC (msec)		5.1
Cuvette(mm)	2	2

**Fig. 3.30. Examples of data screens.**

## 3.9 Measurements

This program allows the user to measure the resistance of a sample prior to pulsing in the Gene Pulser Xcell and to measure the values of the capacitors in a Capacitance Extender attached to the Gene Pulser Xcell.

- From the Home screen, press 9 or use the Up and Down Arrow keys to highlight “Measurements”, then press Enter to view the Measurements Screen (Figure 3.31).

### Measurements Screen

```
Measurements
  1. Sample Resistance
  2. Capacitor
```

**Fig. 3.31. Measurements screen.**

### 3.9.1 Sample Resistance Measurements

Placing a cuvette containing a sample in the shocking chamber will allow the Gene Pulser Xcell to measure the sample resistance. The resistance determination is accurate for samples having a resistance between 5 and 1100 ohms. Samples with a resistance less than 5 ohms will show “5 ohms”. Samples with a resistance greater than 1100 ohms will show “>1100 ohms”.

- When the Measurements screen (Figure 3.31) is selected, the number 1, corresponding to “Sample Resistance” is highlighted as the default choice. Press Enter to view the Sample Resistance screen. If the number 1 on the Measurements screen is not highlighted, press 1 or use the Up or Down Arrow keys to highlight, then press Enter to select. The Sample Resistance screen appears (Figure 3.32).

### Sample Resistance Screen

```
Sample resistance
Resistance (ohms)      xxxx
Place sample between
electrodes.
```

**Fig. 3.32. Sample Resistance screen.**

- Place a sample between the electrodes to allow the Gene Pulser Xcell to monitor sample resistance. Close the top of the ShockPod and press Enter. The sample resistance is displayed on the LCD screen. The display range is 5–1100 ohms.

### 3.9.2 Calibration and Measurement of Capacitors in the CE Module

Because electrolytic capacitors change value slowly over time, to keep the instrument within its original specifications, the capacitors should be calibrated approximately every one to three months or whenever there is concern about the accuracy of the capacitors. The Capacitor Measurement function gives the user a more accurate determination of the capacitor values in the unit by making a measurement of the capacitors in the units. Capacitor calibration and measurement may be performed as follows.

- Unplug the ShockPod cables from the Gene Pulser Xcell. Connect the CE Module to the Gene Pulser Xcell.
- From the Measurements screen, press 2 or use the Up and Down Arrow keys to highlight “Capacitor”, then press Enter to display the Capacitor Measurement screen (Figure 3.33).
- To calibrate the capacitors, press the decimal point (.) on the alpha-numeric keypad. The Gene Pulser Xcell main unit will begin calibrating the capacitors in both the CE Module and the Gene Pulser Xcell main unit. Calibration will take about two minutes. At the completion of the test, 12 numbers will be displayed at the bottom of the screen. The numbers on the top row and the first three on the bottom row are the actual values of the capacitors in the CE Module. These capacitors are used in combination to set the capacitance in the exponential decay and time constant modes. When pulsing using the low voltage circuit in square wave mode, all of the capacitors from the CE Module are used together; when pulsing using the high voltage circuit in square wave mode, the 50 uF capacitor in the Gene Pulser Xcell main unit is used.
- To measure a specific capacitance, with the Capacitor Measurement screen on the LCD, use the alpha-numeric keypad to type in a capacitance value to be measured. Alternatively, use the Right and Left Arrow keys to increase or decrease, respectively, the capacitance values. Press Enter, then press Pulse. The actual capacitance value appears on the Measured Capacitance line. (No sample should be in place and no pulse occurs during this process.)

#### Capacitor Measurement Screen

```
Capacitor measurement
Capacitance (uF)          xxxx
Measured capacitance (uF)  xxxx
```

**Fig. 3.33. Capacitor Measurement screen.**

### 3.10 User Preferences

- From the Home screen, press 10 or use the Up or Down Arrow keys to highlight “User preferences”, then press Enter to display the User Preferences Screen (Figure 3.34).

#### User Preferences Screen

```
User Preferences
1.Clock
2.Screen intensity
3.Sleep function
```

**Fig. 3.34. User Preferences screen.**

#### 3.10.1 Setting the Clock

This section of the program allows the user to select how the date is presented, to enter a correct date, and to enter the correct time. The default setting for the date is Month/Date/Year; this may be changed to Date/Month/Year. The clock is a 24-hour clock.

- When the User Preferences screen (Figure 3.34) is selected, the number 1, corresponding to “Clock” is highlighted as the default choice. Press Enter to view the Set Clock screen. If the number 1 on the User Preferences screen is not highlighted, press 1 or use the Up or Down Arrow keys to highlight, then press Enter to select. The Set Clock screen appears (Figure 3.35).

#### Set Clock Screen

```
Set clock
PREFERENCE      MM / DD / YY      DD / MM / YY
DATE            XX / XX / XX      XX / XX / XX
TIME            XX : XX
```

Note: the default setting is MM/DD/YY

**Fig. 3.35. Set Clock screen.**

- Press the Left or Right Arrow keys to toggle between MM/DD/YY and DD/MM/YY. Press Enter to make a selection and to bring the cursor to the field below the choice where entries can be made.
- If no change in the date is needed, press Enter again. Use the keypad to type in the date or time (only numeric entries are permitted). “0” must be typed to specify numbers 1–9 in all fields (i.e., 01–09). Use the Delete and Clear keys to correct entries. Press Enter when complete. The cursor will move to the field for entering a new time (XX:XX).
- If no change in the time is needed, press Save. Otherwise, use the alpha-numeric keypad to enter a new time. The clock is a 24-hour clock. Press Save when complete and return to the User Preferences screen.

### 3.10.2 Adjusting the Screen Intensity

- From the User Preferences screen press 2 or use the Up and Down Arrow keys to select “Screen intensity”, then press Enter. The LCD Brightness screen appears (Figure 3.36).

#### LCD Brightness Screen

```
LCD BRIGHTNESS

Press UP/DOWN arrow keys
to change the brightness of
the LCD display.
Press ENTER to select.
```

**Fig. 3.36. LCD Brightness screen.**

- Press the Up and Down Arrow keys to increase or decrease the screen brightness, respectively.
- Press Enter to select. The LCD display returns to the User Preferences screen.

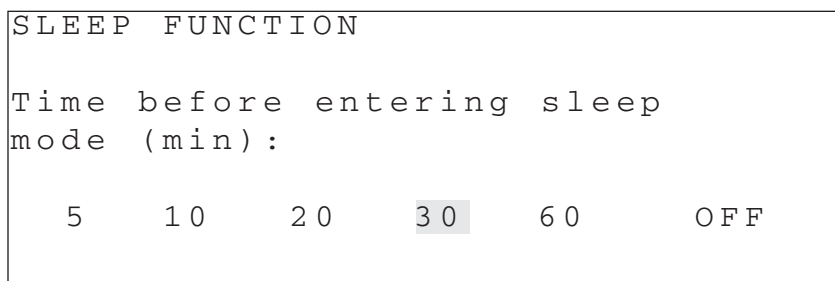
### 3.10.3 Sleep Function Setting

The sleep function is an energy-saving mode of operation, designed to maximize the life of the battery and the LCD screen. The default setting causes the LCD display to turn off after 30 min of inactivity. Pressing any alpha-numeric key will restore the last screen displayed and return the Gene Pulser Xcell to its former operating status. The user may change the time before the instrument enters sleep mode using the Sleep Function Setting.



- From the User Preferences screen press 3 or use the Up or Down Arrow keys to highlight “Sleep function”, then press Enter to select and to view the Sleep Function screen (Figure 3.37).

### Sleep Function Screen



**Fig. 3.37. Sleep Function screen.**

- Use the Right and Left Arrow keys to change the time before the LCD enters sleep mode.
- Press Enter to select. The LCD returns to the User Preferences screen.

## 3.11 The Pulse Trac™ System

The Gene Pulser Xcell uses the Pulse Trac waveform delivery system to generate the most accurate exponential decay pulses possible for optimal cell transformation. Pulse Trac accurately calculates the time constant and amplitude of each pulse based on what is actually delivered to the sample. However, more importantly, the Pulse Trac system compensates for amplitude changes caused by varying sample resistances and improves the accuracy of the low voltage capacitors. This provides:

- Sample conductivity measurement integrated with pulse output for true waveform delivery for samples with resistance in the 10–1000 ohm range;
- Internal calibration and circuit monitoring program for accurate pulse delivery throughout the lifetime of the unit;
- Time constant and voltage amplitude measurements to allow pulse delivery verification.

### 3.11.1 Pulse Trac System Description

The Pulse Trac system monitors and adjusts for the internal system resistance used to limit current and the sample resistance in the cuvette. Sample resistance depends on its conductivity, the distance between the electrodes in the cuvette, and the volume of media within the cuvette. Pulse Trac circuitry monitors the resistance of the sample and delivers the desired voltage regardless of sample volume or conductivity. When you are optimizing electroporation with the Pulse Trac system, the electrical variables are controlled with exacting precision so that your results reflect only the biological variables in your experimental design. This Pulse Trac diagnostic algorithm examines the complete electrical circuit and electronically selects the right combination of capacitors to deliver the most accurate and reproducible pulse for optimal and consistent electroporation over the lifetime of the unit.

### 3.11.2 Pulse Trac Diagnostic Algorithm

This test algorithm tests and selects the optimal capacitor circuit of the Gene Pulser Xcell unit and the CE Module module in the range of 25–3,275  $\mu\text{F}$ . This is the key bank of capacitors used in low voltage/high capacitance precision pulse delivery. The test algorithm tightens the already rigorous capacitor tolerance from  $\pm 20\%$  to  $\pm 10\%$  (other unit designs can have a capacitor variance as high as  $\pm 40\%$ ) in the 200–1075  $\mu\text{F}$  range. The high voltage capacitors in the Gene Pulser Xcell unit are not part of this system since they are pre-selected to the same 10% tolerance that this test algorithm provides.

The Pulse Trac test algorithm is activated upon startup of the Gene Pulser Xcell when the Capacitor Extender module is installed.

## Section 4 Overview of Electroporation Theory

Electroporation is a physical process in which cells are exposed to a high-voltage electric field resulting in a temporary rearrangement of the cell membrane. As a result, the cells become permeable and may take up solutes from their surrounding environment, including nucleic acids, proteins, carbohydrates, and small molecules. While much work has been done to determine how cells become permeabilized during the process of electroporation, the membrane changes that occur are still largely hypothetical (see Chang, et al., 1992).

The Gene Pulser Xcell is the only electroporation instrument capable of delivering both exponential decay and square wave pulses (Figure 4.1). The system consists of a pulse generator system (the main unit with either or both the CE Module and PC Module), a shocking chamber, and a cuvette with incorporated electrodes (see Section 2). Activating the pulse button on the Gene Pulser Xcell charges the capacitors in the unit and, if attached, in the CE Module, to a high voltage; the instrument then discharges the current in the capacitor into the sample in the cuvette. Discharging the charged capacitor generates either the exponential decay or the square wave pulse.

There are two instrument parameters that describe the changes that the cells experience upon electroporation. The first of these, the electric field strength,  $E$ , measured in volts/cm, describes the electrical environment in the electroporation chamber. Standard electrodes used in electroporation consist of two parallel plates separated by a distance,  $d$  (cm); therefore,

$$E = V / d,$$

where  $V$  is the applied voltage and  $d$  is the distance between the electrodes. In practical terms, the field strength is manipulated by altering the voltage of the instrument or by changing the distance between the electrodes. Because the electric conductance of the cell cytoplasm is much higher than that of the cell membrane, placing the cell in an electric field creates a voltage potential across the cell membrane. As the field strength increases, the transmembrane voltage experienced by the cell increases as does the likelihood that a pore will form in the membrane due to breakdown of the lipid bilayer, allowing molecules to enter the cell from the outside (Hui, 1995; Neumann, et al, 2000).

The second parameter that affects the cell is the length of time that it is exposed to the electric field. For exponential decay pulses, this is controlled by the capacitance of the instrument and the resistance within the circuit. For square wave pulses the pulse length is controlled directly by setting the time that the cells are exposed to the electric field. These are discussed for each pulse type below.

## 4.1 Exponential Decay Pulses

The exponential decay circuit of the Gene Pulser Xcell generates an electrical pulse by discharging a capacitor. When a capacitor is discharged into the sample, the voltage across the electrodes rises rapidly to the peak voltage then declines over time,  $t$ , with an exponential decay waveform (Figure 4.1A) according to the following equation:

$$V_t = V_0 [e^{-t/(RC)}],$$

where  $V_0$  is the initial voltage in the capacitor,  $V_t$  is the voltage at time =  $t$  (msec) after the pulse,  $e$  is the base of the natural logarithm,  $R$  is the resistance of the circuit (expressed in ohms), and  $C$  is the capacitance (expressed in microfarads). The time required for the initial voltage to drop to  $V_0/e$  is referred to as the time constant,  $\tau$ , a convenient expression of the pulse length (expressed in msec). When  $t = \tau = R \times C$ , the voltage has declined to  $1/e$  (~37%) of the initial value,  $V_0$  ( $V_\tau = V_0 / e$ ).

By changing the capacitor of the instrument or by changing the resistance of the circuit, the time constant may be readily changed. When high-resistance media is used (e.g., low ionic-strength media used for most bacteria and yeast), the resistance of the circuit may be controlled using the PC Module which places a resistor in parallel with the sample. For resistors connected in parallel, the total resistance of the circuit is given by the equation:

$$R_T = (R_{\text{sample}} * R_{\text{PC}}) / (R_{\text{sample}} + R_{\text{PC}}).$$

When the sample resistance is much greater than the resistor in the PC Module ( $R_{\text{sample}} \gg R_{\text{PC}}$ ), the latter is the primary determinant of the resistance of the circuit and  $R_T \sim R_{\text{PC}}$ . Therefore, the PC Module reduces the resistance of the circuit thereby reducing the time constant of the circuit.

When low-resistance media is used (e.g., high ionic-strength media such as PBS or growth media used for most mammalian cells), the time constant is most easily manipulated by selecting the proper capacitor for the circuit using the CE Module. Additionally, changing the volume of low -resistance media in the cuvette will alter the resistance of the circuit (resistance is inversely proportional to volume).

## 4.2 Square Wave Pulses

Truncating the pulse from a capacitor after discharging it into the sample generates square wave pulses. The ideal square wave pulse has the same voltage at the end as at the beginning of the pulse (Figure 4.1B). However, using a charged capacitor to produce this waveform (as is done in all commercially available electroporation instruments), the voltage at the end of the pulse,  $V_t$ , is always less than the voltage at the beginning of the pulse,  $V_0$ . This is because when the switch is closed across a charged capacitor, maximum current instantaneously flows through the circuit and gradually falls to zero. To produce a square wave, the pulse is terminated at some time,  $t$ , following discharge of the capacitor. This time ( $t$ ) is termed the pulse length. The longer the pulse length, the greater is the difference in voltage between the beginning and the end of the pulse. This voltage decay may be determined from the following equation:

$$\ln (V_0 / V_t) = t / (R C).$$

The decrease in voltage that occurs with a square wave pulse is inversely related to both the capacitance of the instrument and the resistance of the sample. The fractional decrease in voltage at the end of the pulse as a function of the initial voltage,  $V_0$ , is termed droop and is given by the following equation:

$$\text{Droop} = (V_0 - V_t) / V_0.$$

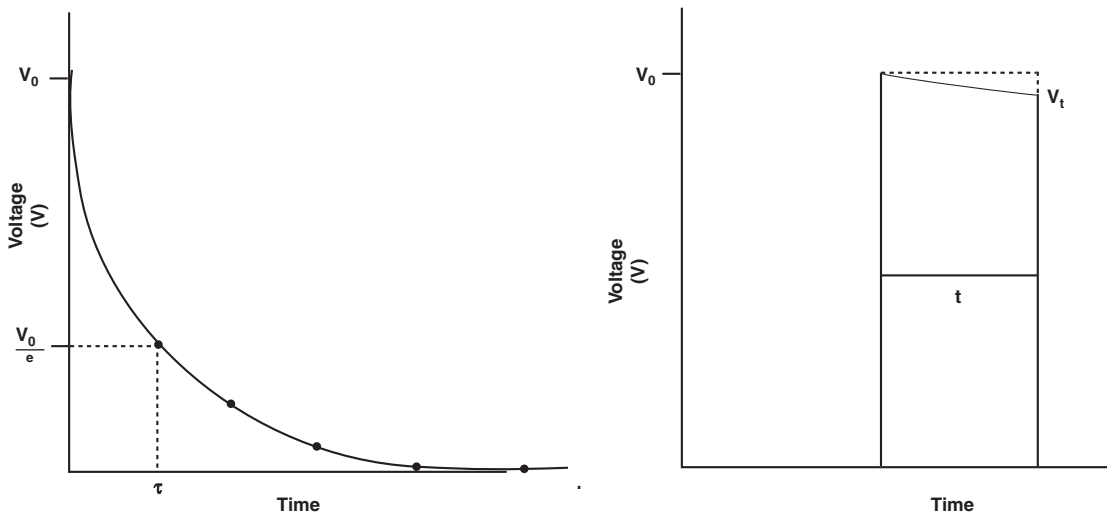
Combining the two equations:

$$\ln [1 / (1 - \text{Droop})] = t / (R C).$$

In order for the pulse to most closely approximate a true square wave, droop must be minimized (i.e.,  $V_t = V_0$  and  $V_0 - V_t = 0$ ). Experimentally, this is achieved by choosing the highest values for R and C. For any given sample, R may be considered a constant. The Gene Pulser Xcell uses a 50 uF capacitor for the high-voltage circuit and a 3275 uF capacitor for the low-voltage circuit. For each of these circuits, C may also be considered a constant. Therefore, for the same sample, as pulse length increases, droop also increases. However, increasing sample resistance reduces the droop at any given pulse length. Increasing the sample resistance may be accomplished by (1) reducing the temperature of the sample; (2) reducing the ionic concentration of the solution; and (3) reducing the volume of liquid in the electroporation cuvette in the case of low resistance media. Never use the PC Module when performing square wave pulsing because attaching a resistor from the PC Module in parallel with the sample will result in increasing the droop of the pulse. Table 4.1 gives the pulse lengths corresponding to a given droop as a function of resistance in the high voltage and low voltage ranges. For example, pulsing into a 200-ohm load, the pulse length will be 0.510 msec with a 5% droop in the high voltage range and 33.4 msec with a 5% droop in the low voltage range.

**Table 4.1 Droop associated with pulse length at various sample resistances for the high-voltage and low-voltage ranges for the Gene Pulser Xcell.**

Droop %	High voltage circuit			Low voltage circuit		
	10	20	5	10	20	
Sample Resistance (ohms)	Pulse length (msec)			Pulse length (msec)		
20	0.051	0.109	0.223	3.34	7.14	14.6
200	0.510	1.09	2.23	33.4	71.4	146
1000	2.55	5.45	11.2	167	357	730
3500	8.93	19.1	39.0	585	1249	2556



**Fig. 4.1. (A) Exponential decay pulse from a capacitance discharge system. When a capacitor, charged to an initial voltage  $V_0$ , is discharged into cells, the voltage applied to the cells decreases over time in an exponential curve such that the voltage “V” at any given time “t” is given by  $V = V_0 e^{-t/RC}$ . In the special case where  $t = CR$  then  $V_0/e$ . The value  $CR$  is known as the time constant of the voltage decay. The shorter the time constant the faster the decay. (B) Square wave pulse from a capacitance discharge system. The pulse length is the time the cells are subjected to the discharge. During the pulse the voltage again decreases in an exponential decay so that at the end of the pulse the voltage is lower than at the beginning. We call this drop in voltage the pulse droop and measure it as a percentage of the initial voltage.**

## Section 5

### Factors Affecting Electroporation: Optimizing Electroporation

The electrical conditions for the electroporation of cells have been verified through years of research (see Shigekawa & Dower, 1988, Change, et al., 1992, and Nickoloff, 1995a,b, for overviews as well as for protocols on electroporation of numerous species). For suggestions on optimizing electroporation parameters, see the following references: Calvin & Hanawalt, 1988, Dower, et al., 1988 (for bacterial cells); Shillito, et al., 1985, Fromm, et al., 1987, Dekeyser, et al., 1990, Joersbo & Brunstedt, 1991 (for plant cells); Chu, et al., 1987, Knutson & Yee, 1987, Andreason & Evans, 1989, Anderson, et al., 1989, and Heiser, 1999 (for mammalian cells). For microorganisms, optimum electrotransformation occurs using exponential decay pulses under electrical conditions similar to those used for *E. coli* and *S. cerevisiae*, two species that are most commonly used in research today. The pulse conditions consist of a low capacitance (short pulse duration) at high voltage. For *E. coli*, conditions that result in a field strength of ~18 kV/cm and a time constant of ~5 msec are optimal. For many bacterial species, including *Salmonella*, *Borrelia*, *Lactococcus*, and *Enterococcus*, the conditions for electroporation are generally identical to those used for *E. coli*. For many other bacterial species, altering the field strength will often result in higher electrotransformation. For *S. cerevisiae*, conditions that result in a field strength of ~7.5 kV/cm and a time constant of ~5 msec are near optimal. Similar optimal conditions are also found with other species of yeast.

Successful electroporation of plant protoplasts has been obtained using exponential decay pulses at both low-capacitance and high-voltage settings or high-capacitance (long pulse duration) and low-voltage settings. Typical conditions are time constants of 10–100  $\mu$ sec and field strengths of 2.5–5 kV/cm or time constants of 2–5 msec and a field strength of about 500 V/cm.

For mammalian cells, optimum electrotransformation conditions have been reported using both exponential decay and square wave pulses. Typical conditions for both types of pulses employ pulse lengths or time constants of 10–40 msec and field strengths of 400–900 V/cm. Generally there is an inverse relationship between field strength and pulse length or time constant such that, over a limited range, one variable may be increased and the other decreased in order to maintain the optimum electroporation conditions. Additionally, as cell size increases, the field strength at which optimum electrotransformation occurs generally decreases. Other conditions that are important for maximizing electroporation efficiency are discussed in the following sections.

#### 5.1 Cell growth

The optimal portion of the growth phase to harvest cells is generally dependent on the cell type. When preparing competent cells of a new species it is generally best to start with conditions employed for use within the same genus. Suggestions for factors to consider and general methods for producing electrocompetent cells are discussed in the articles by Dower, et al. (1992) and Trevors, et al. (1992). For most bacterial species, the highest transformation efficiencies are obtained when cells are harvested in early to mid-log growth. With *E. coli*, as the cells reach stationary phase, the transformation efficiency declines precipitously (Dower, 1990). In contrast, most yeast species are generally harvested in mid- to late-log growth. For *S. cerevisiae*, the transformation efficiency increases as much as 60-fold from early to late-log cultures (Becker and Guarente, 1991). For mammalian cells, the highest expression following electroporation is obtained when cells are in mid-log phase growth (Anderson, et al., 1991).

## 5.2 DNA

While the majority of electroporation applications involve delivery of plasmid DNA to cells, nearly any type of molecule can be introduced into cells by electroporation, including RNA, proteins, carbohydrates, and small molecules, such as nucleoside triphosphates and fluorescent dyes. With few exceptions, when delivering autonomously replicating plasmids, the highest transformation efficiencies are obtained when electroporating supercoiled plasmid; this is the case for microorganisms, plant, and animal cells. However, integration of electroporated plasmid into the host genome is usually most efficient using linearized plasmid, such as when isolating stable transformants of mammalian cells (Barsoum, 1995), or studying homologous recombination in *Candida* (Thompson, et al., 1998), *Pichia* (Cregg & Russell, 1998), and *Tetrahymena* (Gaertig & Gorovsky, 1995). Addition of carrier, such as salmon sperm DNA or plasmid has also been shown to increase gene expression in some cell lines (Chu, et al., 1987; Showe, et al., 1990).

Although transformation of most cell types has been accomplished using plasmid DNA isolated by a variety of methods, the plasmid purity has an effect on transformation efficiency. Significantly lower transformation efficiencies are generated with unpurified miniprep plasmid DNA than with plasmid DNA that has been purified. Plasmid produced using the Bio-Rad Aurum matrix is as efficient as CsCl-purified plasmid for transformation of both microorganisms and mammalian cells. However, as long as plasmids used for are all prepared in the same manner, changes in expression levels are due to differences in transcription or translation of the gene of interest.

Finally, when electroporating ligation mixtures into *E. coli*, components in the reactions have been shown to interfere with transformation (Dower, 1990). Heating the ligation mixture (65°C for 15 min) followed either by dilution with water (Willson and Gough, 1988), dialysis (Heery and Dunican, 1989; Jacobs et al., 1990), or ethanol precipitation (Böttger, 1988; Zabarovsky and Winberg, 1990) significantly increases the transformation efficiency of the DNA.

## 5.3 Electroporation Media

Many types of microorganisms are most efficiently electroporated in high resistance media (resistance >3000 ohms). For this reason, when preparing electrocompetent cells, it is important to wash cells thoroughly to remove all traces of ions present in the growth media. Failure to thoroughly remove the growth media from the cells may result in the sample arcing during electroporation at high field strengths. Cells should be washed at least three times with water or with non-ionic solutions, such as glucose, glycerol, sucrose, or sorbitol. For many microorganisms, 10–15% glycerol is a convenient electroporation medium, since it is recommended as a cryoprotectant for storage of cell cultures.

In some bacterial species, including low (~1 mM) amounts of  $MgCl_2$  in the electrophoresis buffer increases the transformation efficiency. At least in some bacteria,  $Mg^{++}$  ions probably maintain the structural integrity of the cell membrane. For example, *Pseudomonas aeruginosa* is very sensitive to divalent cation chelators; incubation of cells in the presence of EDTA destabilizes the outer membrane of the cells (Haque & Russell, 1974). In the absence of  $Mg^{++}$  ions, the membrane may not be able to re-form following electroporation.

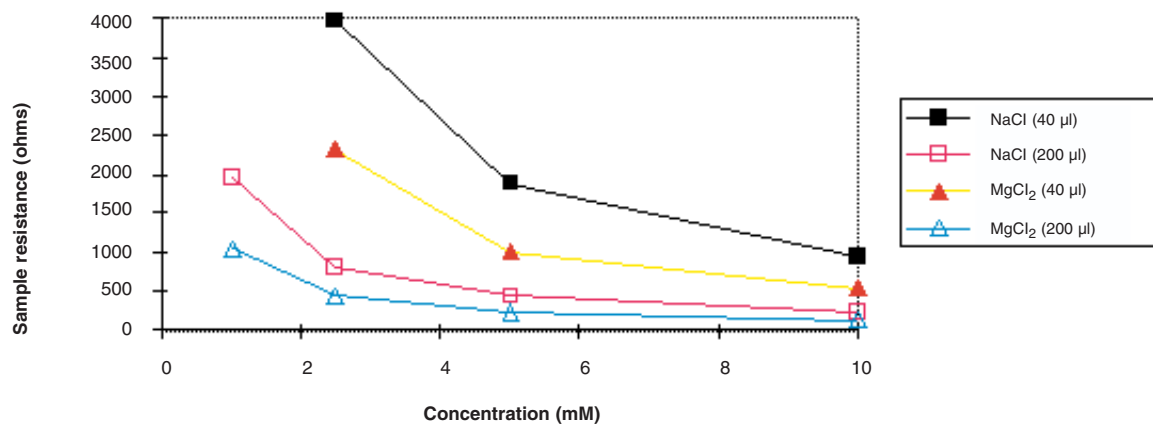
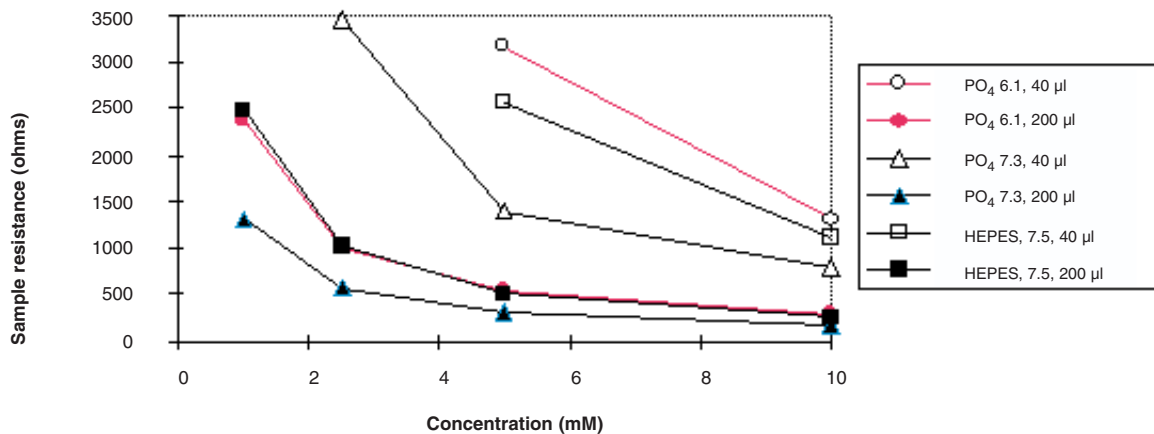
Figs 5.1A and B show the effect of concentration of several biologically important ionic solutions on sample resistance. Note that: (1) the volume of liquid in the cuvette has a significant effect on sample resistance—for ionic solutions, sample resistance is inversely proportional to the volume of solution in the cuvette; (2) the resistance of a solution containing divalent ions is lower than a solution containing the same concentration of monovalent ions; and (3) the resistance of a buffered solution is affected by its pH.

The addition of even small concentrations of ionic compounds significantly reduces the resistance of high resistance samples and may cause arcing. Residual salt from ethanol precipitation of DNA should be removed by washing the DNA pellet prior to dissolving it in either water or Tris-EDTA. Table 5.1 shows that adding a solution of plasmid in 10 mM Tris, pH 8.0 –1 mM EDTA to water does reduce the sample resistance. This should be considered when electroporating bacterial samples in high-resistance media. DNA may be used directly from enzyme reactions for transformation, but the final salt concentration in the electroporation sample should be kept below ~5 meq for high-voltage operation.

## 5.4 Temperature

The temperature at which cells are maintained during electroporation would be expected to have a role in the efficiency of the electroporation process for several reasons. First, since passing an electric pulse through the cells results in heating, keeping the cells at a low temperature during the pulse might reduce the heating and therefore increase the cell viability. Second, since electroporation involves the transient formation of pores in the cell membrane, keeping cells at a low temperature after the pulse might allow the pores to remain open longer giving the DNA in the medium more time to enter the cells. Alternatively, a higher temperature may speed pore closure and increase cell viability. Third, changing the temperature of a solution changes its conductivity. The conductivity of the media increases with increasing temperature, resulting in a decrease in the media resistance and a decrease in the time constant,  $\tau$ . Finally, diffusion rate is highly dependent on temperature, and keeping cells at a low temperature would be expected to reduce the diffusion of molecules across the cell membrane. In practice, the most efficient temperature at which to electroporate cells must be determined empirically. Most bacterial cells are most electrocompetent when maintained at  $<4^{\circ}\text{C}$  from the time of harvest until pulsing. The transformation efficiency of *E. coli* decreases by ~100-fold if pulsed at  $20^{\circ}\text{C}$  (Shigekawa & Dower, 1988); however, electrocompetent *S. aureus* should be incubated at room temperature prior to electroporation (Lee, 1995). For most mammalian cells, electroporation is most efficient when cells are maintained at room temperature both before and after the pulse (Chu, et al., 1987), although some cell types are more efficiently transformed at low temperature (Potter, et al., 1984).



**A****B**

**Fig. 5.1. Resistance of solutions (A) NaCl and MgCl<sub>2</sub> and of (B) buffers of NaPO<sub>4</sub> at pH 6.1 and 7.3 and HEPES at pH 7.5. Resistance was measured in 0.2 cm cuvettes containing either 40 µl or 200 µl of solution at room temperature.**

**Table 5.1. Resistance of Water in 0.2 cm Cuvettes to which TE has been added<sup>1</sup>**

SAMPLE	R <sub>sample</sub> (ohms) (40 µl volume)	R <sub>sample</sub> (ohms) (200 µl volume)
Water	> 600,000	> 600,000
Water + 1 µl TE	> 600,000	35,000
Water + 5 µl TE	11,200	8,700
Water + 10 µl TE	4,850	4,700

<sup>1</sup>Sample resistance in 0.2 cm cuvettes containing either 40 or 200 µl of water and the indicated volume of TE (10 mM Tris, pH 8.0, 1 mM EDTA) measured at 1000 V.

## Section 6

### Electroporation of Bacterial Cells

For most bacterial species, electroporation provides a rapid and simple method for transforming cells. Particularly for gram negative bacteria, but also for some gram positive species, this method provides higher transformation efficiencies than conjugation or chemical methods. We are interested in hearing of additional species and strains transformed by electroporation and including this information in subsequent versions in our Electroprotocols manual. Please contact your local Bio-Rad representative, access our web site at [www.bio-rad.com](http://www.bio-rad.com), or, in the U.S., call our Technical Services at (800) 424-6723 with any comments or questions.

#### 6.1 E. coli

##### 6.1.1 Preparation of Electrocompetent Cells

See Ausubel, et al. (1987) and Miller and Nickoloff (1995) for additional information.

Gene Pulser Xcell conditions: C = 25 µF; PC = 200 ohm; V = 1.8 kV (in 0.1 cm cuvettes);  
C = 25 µF; PC = 200 ohm; V = 2.5 kV, or  
C = 25 µF; PC = 200 ohm; V = 3.0 kV (in 0.2 cm cuvettes).

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate 5 ml of a fresh overnight E. coli culture into 500 ml of L-broth in a 2.8 L Fernbach flask.
2. Grow the cells at 37°C shaking at 300 rpm to an OD<sub>600</sub> 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 depends on the strain and growth conditions but should be about 4–5 × 10<sup>7</sup> cells/ml.
3. Chill the cells on ice for ~20 min. For all subsequent steps, keep the cells as close to 0°C as possible (in an ice/water bath) and chill all containers in ice before adding cells. Transfer the cells to a sterile, cold 500 ml centrifuge bottle and centrifuge at 4000 x g for 15 minutes at 4°C.
4. Carefully pour off and discard the supernatant. It is better to sacrifice yield by pouring off a few cells than to leave any supernatant behind.
5. Gently resuspend the pellet in 500 ml of ice-cold 10% glycerol. Centrifuge at 4000 x g for 15 minutes at 4°C; carefully pour off and discard the supernatant.

6. Resuspend the pellet in 250 ml of ice-cold 10% glycerol. Centrifuge at 4000 x g for 15 minutes at 4°C; carefully pour off and discard the supernatant.
7. Resuspend the pellet in ~20 ml of ice-cold 10% glycerol. Transfer to a 30 ml sterile Oakridge tube. Centrifuge at 4000 x g for 15 minutes at 4°C; carefully pour off and discard the supernatant.
8. Resuspend the cell pellet in a final volume of 1–2 ml of ice-cold 10% glycerol. The cell concentration should be about 1–3 x 10<sup>10</sup> cells/ml.
9. This suspension may be frozen in aliquots on dry ice and stored at -70°C. The cells are stable for at least 6 months under these conditions.

### **6.1.2 Electroporation**

1. Thaw the cells on ice. For each sample to be electroporated: place a 1.5 ml microfuge tube on ice, place either a 0.1 or 0.2 cm electroporation cuvette on ice, and place a 17 x 100 mm tube with 1 ml of SOC at room temperature.
2. To a cold, 1.5 ml polypropylene microfuge tube, add 20 µl of cell suspension if electroporating in 0.1 cm cuvettes, or 20–40 µl of cell suspension if electroporating in 0.2 cm cuvettes. Add 1 to 2 µl of DNA (DNA should be in a low ionic strength buffer such as water or TE). Mix well and incubate on ice for ~1 minute. (Note: it is best to mix the plasmids and cells in a microfuge tube since the narrow gap of the cuvettes prevents uniform mixing.)
3. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Bacterial Protocol screen (press 4, then Enter twice). When using the 0.1 cm cuvettes, press Enter to open E. coli, 1mm cuvette Protocol Detail screen. When using the 0.2 cm cuvettes, press 2 then Enter, or 3 then Enter, to select the Protocol Detail screens for E. coli to pulse at 2.5 or 3.0 kV, respectively. See Section 3.4 for operating instructions.
4. Transfer the mixture of cells and DNA to a cold electroporation cuvette and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the chamber lid down to close.
5. Pulse once.
6. Remove the cuvette from the chamber and immediately add 1 ml of SOC medium to the cuvette. Quickly but gently resuspend the cells with a Pasteur pipette. (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.)
7. Transfer the cell suspension to a 17 x 100 mm polypropylene tube and incubate at 37°C for 1 hour, shaking at 225 rpm.
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 LB plates with antibiotic.

### **6.1.3 Solutions and Reagents**

1. L-Broth: 10 g Tryptone peptone, 5 g Yeast extract, 5 g NaCl; dissolve in 1.0 L water. Autoclave.
2. LB agar plates with selective antibiotic: prepare L broth as above, adding 15 g of agar per liter. Autoclave. Cool to 55–60°C and add antibiotic. Pour 12–15 ml per 100 mm plate.
3. 10% (v/v) Glycerol: 12.6 g glycerol (density = 1.26 g/cc) in 90 ml of water. Autoclave or filter sterilize.
4. TE: 10 mM Tris-HCl pH 8.0, 1 mM EDTA.

5. SOB: 2.0 g Tryptone peptone, 0.5 g Yeast extract, 0.2 ml 5 M NaCl, 0.25 ml 1 M KCl; dissolve in 90 ml water. Adjust pH to 7.0. Bring volume to 100 ml. Autoclave. Add 1.0 ml sterile 1 M MgCl<sub>2</sub> and 1.0 ml sterile 1 M MgSO<sub>4</sub>.
6. SOC: to 100 ml SOB, add 2.0 ml sterile 1 M glucose (sterilize by filtration).

## 6.2 Staphylococcus aureus

### 6.2.1 Preparation of Electrocompetent Cells

See Lee (1995) for additional information.

Using this method, we have obtained transformation efficiencies of  $>1 \times 10^6$  transformants/ $\mu$ g electroporating *S. aureus* RN4220 with pLI50.

Gene Pulser Xcell conditions: C = 25  $\mu$ F; PC = 100 ohm; V = 2.9 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate a colony of *S. aureus* from a TSA plate into 3 ml of B2 broth in a 17 x 100 mm tube.
2. Incubate at 37°C overnight, shaking at 250 rpm.
3. Inoculate 1.5 ml of the overnight culture into 150 ml of B2 broth in a 1 liter flask. Incubate at 37°C, shaking at 250 rpm, to  $3\text{--}5 \times 10^8$  cells/ml (OD<sub>600</sub> ~ 0.8–0.85). The doubling time of *S. aureus* is about 30 min at 37°C; growth will take about 4 hrs.
4. Chill the cells in an ice water bath for 15 min to stop growth. Decant the cells into a sterile 500 ml centrifuge bottle. Harvest the cells by centrifugation at 12,000 x g for 15 min at 4°C.
5. Carefully pipette off the supernatant, keeping the cell pellet on ice.
6. Resuspend the cell pellet in 500 ml of sterile, ice-cold water. Pellet the cells by centrifugation at 12,000 x g for 15 min at 4°C; carefully remove the supernatant. Wash the cells 2 more times in 500 ml of sterile, ice-cold water.
7. Resuspend the cell pellet in 25 ml of sterile, ice-cold 10% glycerol. Transfer to a 30 ml sterile Oakridge tube. Pellet the cells by centrifugation at 12,000 x g for 15 min at 4°C; carefully remove the supernatant.
8. Resuspend the cells in 20 ml of 10% glycerol. Incubate at 20°C for 15 min. Pellet the cells by centrifugation at 12,000 x g for 15 min at 4°C; carefully remove the supernatant.
9. Resuspend the cell pellet in 2 ml of 10% glycerol; the final cell concentration should be  $\sim 1 \times 10^{10}$  cells/ml.
10. Dispense 250  $\mu$ l aliquots of the electrocompetent cells into sterile 1.5 ml microfuge tubes; freeze the cells in an isopropanol-dry ice bath, then store at -70°C. The cells are stable for several months under these conditions.

### 6.2.2 Electroporation

1. Pipette the DNA samples (5 ng - 2  $\mu$ g in no more than 3  $\mu$ l) to be electroporated into sterile 1.5 ml microfuge tubes.
2. Thaw the competent cells at room temperature for several minutes. Add 50  $\mu$ l of cells to each DNA sample; gently pipette up and down to mix. Incubate the samples at room temperature for 30 min.
3. For each sample to be electroporated, add 1 ml of SMMP medium containing a subinhibitory concentration of antibiotic to a 17 x 100 mm tube at room temperature and place a 0.2 cm cuvette at room temperature.

4. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Bacterial Protocol screen (press 4, then Enter twice). To select the Protocol Detail screen for *S. aureus*, press 6 or the Down Arrow key to highlight *S. aureus*, then press Enter. See Section 3.4 for operating instructions.
5. Transfer the mixture of cells and DNA to a 0.2 cm electroporation cuvette at room temperature and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the chamber lid down to close.
6. Pulse once.
7. Remove the cuvette from the chamber and immediately add 1 ml of SMMP medium containing a subinhibitory concentration of antibiotic; gently transfer the cells to a sterile 17 x 100 mm tube using a Pasteur pipette. Incubate 1 hr at 37°C, shaking at 250 rpm.
8. Check and record the pulse parameters. The time constant should be close to 2.5 milliseconds. The voltage should be approximately 2.9 kV.
9. Plate aliquots of the electroporated cells on trypticase soy agar containing selective antibiotic. Incubate plates for 36–48 hrs at 37°C.

### **6.2.3 Solutions and Reagents**

1. Trypticase soy agar (TSA): dissolve 40 g trypticase soy agar (Becton Dickinson, Sparks, MD) in 1 L of water. Autoclave.
2. B2 medium: 10 g casein hydrolysate (casamino acids), 25 g yeast extract, 5 g glucose, 25 g NaCl, 1 g K<sub>2</sub>HPO<sub>4</sub>; dissolve in 900 ml of water and adjust pH to 7.5; bring volume to 1.0 L. Autoclave.
3. 0.2 M sodium hydrogen maleate: either 11.6 g maleic anhydride and 4 g NaOH dissolved in 500 ml of water, or 13.8 g maleic acid, 4 g NaOH dissolved in 500 ml water. Autoclave.
4. 2X SMM: 25 ml of 0.2 M sodium hydrogen maleate, 40 ml of 0.1 N NaOH; adjust the pH to 6.5. Add 5 ml of 1 M MgCl<sub>2</sub>, 42.7 g sucrose; dissolve and bring volume to 125 ml. Filter sterilize.
5. 4X Penassay broth: 17.5 g Antibiotic Medium 3 (Becton Dickinson) dissolved in 250 ml of water. Autoclave.
6. SMMP: 55 ml of 2X SMM, 40 ml of 4X Penassay broth, 5 ml of 10% (w/v) bovine albumin; adjust pH to 7.0; filter sterilize. For selection of antibiotic-resistant transformants, add a sub-inhibitory concentration of antibiotic.
7. 10% (v/v) Glycerol: 12.6 g glycerol (density = 1.26 g/cc) in 90 ml of water. Autoclave or filter sterilize.

## 6.3 *Agrobacterium tumefaciens*

### 6.3.1 Preparation of Electrocompetent Cells

See Lin (1995) for additional information.

Gene Pulser Xcell conditions: C = 25  $\mu$ F; PC = 200 ohm; V = 2.4 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate an aliquot from log phase culture of *A. tumefaciens* into 1.5 L of YM broth in a 2.8 L Fernbach flask.
2. Incubate at 30°C overnight, shaking at 300 rpm to a density of 5–10  $\times 10^7$  cells/ml (OD550 ~ 1.0).
3. Decant the cells into sterile 500 ml centrifuge bottles and pellet the cells by centrifugation at 3000  $\times$  g for 10 min at 4°C.
4. Carefully pour off and discard the supernatant; place the centrifuge bottles with the cell pellets on ice.
5. Add ~50 ml of sterile, ice-cold 10% glycerol to each of the bottles and vortex to resuspend the cell pellets; bring the volume in each of the centrifuge bottles to 500 ml with sterile, ice-cold 10% glycerol. Pellet the cells by centrifugation at 3000  $\times$  g for 10 min at 4°C; pour off and discard the supernatant.
6. Combine the cell pellets and wash the cells in one bottle as in step 5.
7. Resuspend the cell pellets in 25 ml of sterile, ice-cold 10% glycerol and transfer to a chilled 30 ml Oakridge tube. Pellet the cells by centrifugation at 3000  $\times$  g for 5 min at 4°C; pour off and discard the supernatant.
8. Resuspend the cell pellet in 0.5 ml of sterile, ice-cold 10% glycerol; the final cell volume should be ~1.5 ml and the cell concentration should be ~ 5  $\times 10^{10}$  cells/ml. The cells may be used immediately or frozen. To freeze, dispense 200  $\mu$ l aliquots of the electrocompetent cells in sterile 1.5 ml microfuge tubes; freeze the cells in an isopropanol-dry ice bath, then store at -70°C. The cells are stable for about 6 months under these conditions.

### 6.3.2 Electroporation

1. Pipette the DNA samples (up to 100 ng in no more than 5  $\mu$ l of TE) to be electroporated into sterile 1.5 ml microfuge tubes. Place tubes on ice.
2. Thaw the electrocompetent *A. tumefaciens* cells on ice. For each DNA sample to be electroporated, add 20  $\mu$ l of electrocompetent cells to each DNA sample; gently tap the tubes to mix.
3. For each DNA sample to be electroporated, add 1 ml of YM broth to a 17  $\times$  100 tube at room temperature and place a 0.1 cm electroporation cuvette on ice.
4. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Bacterial Protocol screen (press 4, then Enter twice). To select the Protocol Detail screen for *A. tumefaciens*, press 4 or the Down Arrow key to highlight *A. tumefaciens*, then press Enter. See Section 3.4 for operating instructions.
5. Transfer the DNA - cell samples to the electroporation cuvettes and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.
6. Remove the cuvette from the chamber and immediately use the 1 ml of YM broth in the 17  $\times$  100 mm tube to transfer the cells from the cuvette to the tube.
7. Check and record the pulse parameters. The time constant should be about 5 milliseconds. The voltage should be about 2.4 kV.

8. Incubate the cells 3 hr at 30°C, shaking at 250 rpm. Plate aliquots of the electroporated cells on YM agar plates containing the appropriate selective media. Incubate plates for 48 hrs at 30°C.

### 6.3.3 Solutions and reagents

1. YM broth: 0.4 g yeast extract, 10 g mannitol, 0.1 g NaCl, 0.1 g MgSO<sub>4</sub>, 0.5 g K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, dissolve in 1.0 L of water and adjust to pH 7.0. Autoclave. For YM plates, add 15 g agar/1 L of YM broth.
2. 10% (v/v) Glycerol: 252 g glycerol (density = 1.26 g/cc) in 1800 ml water. Autoclave or filter sterilize.

## 6.4 Bacillus cereus

### 6.4.1 Preparation of Electrocompetent Cells

Slight modification of the method of Silo-Suh, et al. (1994).

Using this method, we have obtained transformation efficiencies of  $2 \times 10^6$  transformants/ $\mu\text{g}$  electroporating *B. cereus* UW85 with pAD123.

Gene Pulser Xcell conditions: C = 50  $\mu\text{F}$ ; PC = 200 ohm; V = 1.0 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate a colony of *B. cereus* into 10 ml of LB in a 125 ml flask; incubate overnight at 28°C shaking at 300 RPM.
2. Inoculate 2 ml of the overnight culture into 500 ml of BHI in a 2.8 L Fernbach flask; incubate at 28°C shaking at 300 RPM.
3. Monitor the culture until OD<sub>600</sub> = 0.3 (cell density is  $\sim 1 \times 10^7$  cells/ml).
4. Chill the culture on ice for 10 min.
5. Transfer the cells into 2 chilled, sterile 250 ml centrifuge bottles.
6. Pellet the cells by centrifugation at 4°C for 10 min at 10,000 x g in a chilled rotor.
7. Discard the supernatant. Resuspend each pellet in 50 ml of sterile, ice cold EP buffer. Pellet the cells as above.
8. Discard the supernatant. Resuspend each pellet in 15 ml of sterile, ice cold EP buffer. Transfer to a chilled 30 ml Oakridge tube.
9. Pellet the cells by centrifugation at 4°C for 10 min at 10,000 x g in a chilled rotor.
10. Discard the supernatant. Resuspend the cell pellet in 0.5 ml of EP buffer. Keep the cells on ice and use as soon as possible for electroporation. Cells cannot be frozen and used for electroporation.

### 6.4.2 Electroporation

1. For each sample to be electroporated, pipette the DNA (0.05 - 1  $\mu\text{g}$  in up to 10  $\mu\text{l}$  of either water or TE) into a sterile 1.5 ml microfuge tube. Place tubes on ice. Add 100  $\mu\text{l}$  electrocompetent cells to each plasmid sample; mix, and incubate on ice for 5–10 min.
2. Transfer 100  $\mu\text{l}$  of plasmid/cell suspension to chilled 0.2 cm cuvettes on ice; tap the solution to the bottom of the cuvette.
3. For each DNA sample to be electroporated, prepare a 17 x 100 tube containing 2.0 ml of LB at room temperature.

4. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Bacterial Protocol screen (press 4, then Enter twice). To select *B. cereus*, press 7 or the Down Arrow to highlight *B. cereus*, then press Enter to open the *B. cereus* Protocol Detail screen. See Section 3.4 for operating instructions.
5. Transfer the DNA - cell samples to the electroporation cuvettes and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.
6. Remove the cuvette from the chamber and immediately use a portion of the L broth in the 17 x 100 mm tube to transfer the cells from the cuvette to the tube.
7. Check and record the pulse parameters. The time constant should be about 8.6 msec and the voltage about 1.0 kV. The field strength can be calculated as actual volts (kV) / cuvette gap (cm).
8. Incubate the cells for 1–1.5 hr at 37°C, shaking at 250 rpm.
9. Plate aliquots of the electroporated cells on LB agar plates containing the appropriate selective media. Incubate plates overnight at 28°C.

### 6.4.3 Solutions and Reagents

1. EP buffer (0.5 mM K<sub>2</sub>HPO<sub>4</sub>–KH<sub>2</sub>PO<sub>4</sub>, 0.5 mM MgCl<sub>2</sub>, 272 mM Sucrose): 54.4 ml 1M Sucrose, 100 µl 1 M MgCl<sub>2</sub>, 190 µl 0.1 M KH<sub>2</sub>PO<sub>4</sub>, 810 µl 0.1 M K<sub>2</sub>HPO<sub>4</sub>. Bring volume to 200 ml with Milli-Q water (the pH should be ~7.4). Filter sterilize. Store at 4°C.
2. Brain Heart Infusion (BHI): dissolve 37 g BHI in 1000 ml water; autoclave.
3. L-Broth: 10 g Tryptone peptone, 5 g Yeast extract, 5 g NaCl; dissolve in 1.0 L water. Autoclave.

## 6.5 *Pseudomonas aeruginosa*

### 6.5.1 Preparation of Electrocompetent Cells

From Dennis & Sokol (1995) and Smith & Iglewski (1989).

Using this method, electroporating *P. aeruginosa* PAO1 with pUCP19, we have obtained transformation efficiencies >1 x 10<sup>7</sup> transformants/µg.

Gene Pulser Xcell conditions: C = 25 µF; PC = 200 ohm; V = 2.5 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate a colony of *P. aeruginosa* into 25 ml of LB in a 125 ml flask; incubate overnight at 37°C shaking at 300 RPM.
2. Inoculate 10 ml of the overnight culture into a 2.8 L Fernbach flask with 500 ml of LB; incubate at 37°C shaking at 300 RPM.
3. Monitor the culture until OD<sub>600</sub> = 0.5; the cell density should be ~3 x 10<sup>8</sup> cells/ml.
4. Chill the flask on ice for 10 min.
5. Transfer the cells into 2 chilled, sterile 250 ml centrifuge bottles.
6. Pellet the cells by centrifugation at 4°C for 10 min at 2300 x g in a chilled rotor.
7. Discard the supernatant. Resuspend each pellet in 250 ml of sterile, ice cold SMEB buffer. Pellet the cells as above.
8. Discard the supernatant. Resuspend each pellet in 125 ml of sterile, ice cold SMEB buffer. Pellet the cells as above.



9. Discard the supernatant. Resuspend each pellet in 12 ml of sterile, ice cold SMEB buffer. Transfer to a chilled 30 ml Oakridge tube.
10. Pellet the cells by centrifugation at 4°C for 10 min at 2300 x g in a chilled rotor.
11. Discard the supernatant. Resuspend the cell pellet in 2.0 ml of SMEB buffer. Keep the cells on ice and use as soon as possible for electroporation.

### 6.5.2 Electroporation

1. For each sample to be electroporated, pipette 0.05–0.5 µg of plasmid into a sterile 1.5 ml microfuge tube. Place tubes on ice. Add 100 µl electrocompetent *P. aeruginosa* cells to each plasmid sample; mix, and incubate on ice for 5–10 min.
2. Transfer 100 µl of plasmid/cell suspension to chilled 0.2 cm cuvettes on ice; tap the solution to the bottom of the cuvette.
3. For each DNA sample to be electroporated, prepare a 17 x 100 tube containing 1.0 ml of SOC at room temperature.
4. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Bacterial Protocol screen (press 4, then Enter twice). To select *P. aeruginosa*, press 5 or the Down Arrow to highlight *P. aeruginosa*, then press Enter to open the *P. aeruginosa* Protocol Detail screen. See Section 3.4 for operating instructions.
5. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.
6. Immediately after the pulse, use the 1 ml of SOC broth in the 17 x 100 mm tube to transfer the plasmid/cell suspension from the cuvette to the tube. Check and record the pulse parameters. The time constant should be about 5 msec and the voltage about 2.5 kV. The field strength can be calculated as volts (kV) / cuvette gap (cm).
7. Incubate the cells for 1 hr at 37°C shaking at 250–300 RPM.
8. Plate onto LB agar plates with selective antibiotic. Incubate overnight at 37°C.

### 6.5.3 Solutions and Reagents

1. SMEB buffer (1 mM HEPES, pH 7.0, 1 mM MgCl<sub>2</sub>, 300 mM Sucrose): 2.0 ml 0.5 M HEPES, pH 7.0, 1.0 ml 1.0 M MgCl<sub>2</sub>, 102.7 g Sucrose. Dissolve in water and bring volume to 1000 ml. Autoclave. Store at 4°C.
2. L-Broth: 10 g Tryptone peptone, 5 g Yeast extract, 5 g NaCl; dissolve in 1 L water. Autoclave.
3. LB agar plates with selective antibiotic: prepare L broth as above, adding 15 g of agar per liter. Autoclave. Cool to 55–60°C and add antibiotic. Pour 12–15 ml per 100 mm plate.
4. SOB: 2.0 g Tryptone peptone, 0.5 g Yeast extract, 0.2 ml 5 M NaCl, 0.25 ml 1 M KCl; dissolve in 90 ml water. Adjust pH to 7.0. Bring volume to 100 ml. Autoclave. Add 1.0 ml sterile 1 M MgCl<sub>2</sub> and 1.0 ml sterile 1 M MgSO<sub>4</sub>.
5. SOC: to 100 ml SOB, add 2.0 ml sterile 1 M glucose (sterilize by filtration).

## 6.6 Streptococcus pyogenes

### 6.6.1 Preparation of Electrocompetent Cells

From Simon & Ferretti (1995).

Using this method, we have obtained transformation efficiencies of  $1 \times 10^5$  transformants/ $\mu\text{g}$  electroporating *S. pyogenes* CS101 with pDC123 (Chaffin & Rubens, 1998).

Gene Pulser Xcell conditions: C = 25  $\mu\text{F}$ ; PC = 200 ohm; V = 2.1 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate a colony of *S. pyogenes* from a fresh plate into a 25 ml tube containing 20 ml of THY with 20 mM Glycine; cap the tube tightly. Incubate overnight at 37°C without shaking.
2. Inoculate 18 ml of the overnight culture into a 500 ml flask containing 250 ml of THY media with 20 mM glycine. Swirl gently to mix and continue incubation (about 2 hrs) until the OD<sub>600</sub> is ~0.2 (the cell density should be about  $3 \times 10^7$  cells/ml).
3. Chill the culture on ice for 10 min.
4. Transfer the cells into a chilled, sterile 500 ml centrifuge bottle.
5. Pellet the cells by centrifugation at 4°C for 10 min at 6000 x g in a chilled rotor.
6. Pipette off and discard the supernatant. Resuspend the cell pellet in 10 ml of ice-cold sterile Elpo medium, then bring the volume to ~225 ml. Centrifuge the cells at 8000 x g.
7. Pipette off and discard the supernatant. Resuspend the pellet in 5 ml of ice-cold sterile Elpo medium and transfer to a 30 ml Oakridge tube. Bring the volume to 30 ml, then centrifuge the cells at 12,000 x g.
8. Pipette off and discard the supernatant. Resuspend the cell pellet in about 1.5 ml of ice-cold sterile Elpo medium. Keep the cells on ice and use as soon as possible for electroporation.

### 6.6.2 Electroporation

1. Pipette the DNA samples to be electroporated (0.05–0.5  $\mu\text{g}$ ) into sterile 1.5 ml microfuge tubes. Place tubes on ice. Add 200  $\mu\text{l}$  electrocompetent *S. pyogenes* cells to each plasmid sample; mix, and incubate on ice for 5–10 min.
2. Transfer 200  $\mu\text{l}$  of plasmid/cell suspension to chilled 0.2 cm cuvettes on ice; tap the solution to the bottom of the cuvette.
3. For each sample to be electroporated, prepare a sterile 17 x 100 mm tube containing 1.0 ml of THY at room temperature.
4. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Bacterial Protocol screen (press 4, then Enter twice). To select *S. pyogenes*, press 8 or the Down Arrow to highlight *S. pyogenes*, then press Enter to open the *S. pyogenes* Protocol Detail screen. See Section 3.4 for operating instructions.
5. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.
6. Immediately after the pulse place the cuvette on ice for ~ 5 min. Check and record the pulse parameters. The time constant should be about 9.8 msec and the voltage about 2.1 kV. The field strength can be calculated as volts (kV) / cuvette gap (cm).
7. Add 1.0 ml of THY broth to the cuvette and transfer the plasmid/cell suspension to the 17 x 100 mm tube. Incubate the cells for 2 hr at 37°C without shaking.

8. Plate onto THY agar plates with selective antibiotic. Incubate 1–2 D at 37°C.

### 6.6.3 Solutions and reagents

1. THY media (Todd-Hewitt broth with 0.2% yeast extract): 30 g Todd Hewitt broth, 2 g Yeast extract; dissolve in 1 L water. Autoclave.
2. THY + Glycine: 500 ml THY media, 5 ml 2 M Glycine (15 g/100 ml).
3. THY agar with antibiotic: 30 g Todd-Hewitt broth, 2 g Yeast extract, 15 g Bacto agar; dissolve in 1 L water. Autoclave; cool to 55–60°C and add antibiotic. Pour plates.
4. Elpo medium: 122 ml 20% Glucose, 0.5 ml 1 M MgCl<sub>2</sub>; bring to 500 ml with water and pH to 6.5. Filter sterilize.

## 6.7 Lactobacillus plantarum

### 6.7.1 Preparation of Electrocompetent Cells

From Bringel & Hubert (1990). Using this method, we have obtained transformation efficiencies of  $4 \times 10^4$  transformants/ $\mu$ g electroporating *L.plantarum* NCIB7220 with pGK12.

Gene Pulser Xcell conditions: C = 25  $\mu$ F; PC = 400 ohm; V = 2.0 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate *L. plantarum* into 50 ml of MRS media in a 500 ml flask; incubate overnight at 30°C shaking at 300 RPM. The cells should be in exponential growth ( $OD_{600} < 1.5$ ).
2. Inoculate a sample of the overnight culture into 500 ml of MRS/SG in a 2.8 L Fernback flask to an  $OD_{600} = 0.05$ . Incubate at 30°C shaking at 80 RPM.
3. Monitor the culture until  $OD_{600} = 0.3$  (usually ~ 4 hrs).
4. Transfer the cells into a sterile 500 ml centrifuge bottle at room temperature.
5. Pellet the cells by centrifugation at 18°C for 10 min at 3000 x g.
6. Discard the supernatant. Resuspend the cell pellet in 250 ml of sterile water at room temperature. Centrifuge the cells as above but increase the speed of to 4000 x g.
7. Discard the supernatant. Repeat the water wash as above. Pellet the cells as above.
8. Discard the supernatant. Resuspend the cell pellet in a final volume of 2 ml of 30% PEG. Keep the cells on ice and use as soon as possible for electroporation.

### 6.7.2 Electroporation

1. For each sample to be electroporated, pipette 0.1–1.0  $\mu$ g of plasmid into a sterile 1.5 ml microfuge tube (<2  $\mu$ l). Place tubes on ice. Add 40  $\mu$ l electrocompetent cells to each plasmid sample; mix.
2. Transfer 40  $\mu$ l of plasmid/cell suspension to chilled 0.2 cm cuvettes on ice; tap the solution to the bottom of the cuvette.
3. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Bacterial Protocol screen (press 4, then Enter twice). To select *L. plantarum*, press 9 or the Down Arrow to highlight *L. plantarum*, then press Enter to open the *L. plantarum* Protocol Detail screen. See Section 3.4 for operating instructions.
4. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.

5. Immediately after the pulse place the cuvette on ice for 30 min. Add 1 ml MRS/SG to the cuvette and transfer the plasmid/cell suspension to a sterile, 17 x 100 mm tube. Incubate the cells for 3–4 hrs at 30°C without shaking.
6. Plate onto MRS agar plates containing antibiotic.
7. Incubate 2–3 D at 30°C.

### 6.7.3 Solutions and Reagents

1. 2X MRS (w/o Glucose): 10 g Proteose Peptone No 3 (Difco #211693), 10 g Beef Extract (Difco #211520), 5 g Yeast Extract (Difco #212750), 1 ml Tween 80, 11.5 ml 1 M  $K_2HPO_4$ , 12.3 ml 3 M Na Acetate, 1.9 g citric acid, 0.8 ml 1 M  $MgSO_4$ , 225  $\mu$ l 1 M  $MnSO_4$ . Dissolve in water and bring the volume to 500 ml. Autoclave
2. 1 X MRS: 500 ml 2 X MRS, 200 ml 10% Glucose (filter sterilized), 300 ml sterile water
3. 1 X MRS Agar with antibiotic: To a 1 L bottle, add 7.5 g Bacto agar and 150 ml water; autoclave. Cool to 55°C. Warm 250 ml 2 X MRS and 100 ml 10 % Glucose to 55°C; add to the agar. Add antibiotic. Pour plates.
4. MRS/SG (MRS with 0.75 M Sorbitol + 1% Glycine): 250 ml 2 X MRS, 50 ml 20 % Glucose (sterilized by filtration), 150 ml sterile 2.5 M Sorbitol, 33 ml sterile 2 M Glycine, 17 ml sterile water.
5. 2 M Glycine: 15.0 g in 100 ml water; filter sterilize.
6. 2.5 M Sorbitol: 227.8 g Sorbitol; dissolve in ~250 ml water then bring volume to 500 ml. Filter sterilize.
7. 30% PEG 1000: 150 g PEG 1000 (warm to ~50°C to melt; pour into beaker with ~200 ml water). Bring volume to 500 ml with water. Filter sterilize.

## Section 7 Electroporation of Fungal Cells

### 7.1 *Saccharomyces cerevisiae*

#### 7.1.1 Preparation of electrocompetent cells

See Becker & Guarante (1991) and Ausubel et al. (1987) for additional information. Using this procedure we have obtained  $>5 \times 10^5$  transformants/ $\mu$ g electroporating *S. cerevisiae* Sc948 with Yep352.

Gene Pulser Xcell conditions: C = 25  $\mu$ F; PC = 200 ohm; V = 1.5 kV (in 0.2 cm cuvettes), or  
C = 25  $\mu$ F; PC = 200 ohm; V = 2.5 kV (in 0.4 cm cuvettes).

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate 500 ml of YPD in a 2.8 L Fernbach flask with an aliquot from an overnight culture of *S. cerevisiae*. The doubling time of *S. cerevisiae* is approximately 2 hrs at 30°C.
2. Incubate at 30°C overnight, shaking at 250 rpm, to a density of  $\sim 1 \times 10^8$  cells/ml ( $OD_{600}$  (1:10 dilution) = 0.30–0.35).
3. Chill the cells in an ice water bath for 15 min to stop growth.
4. Decant the cells into a sterile 500 ml centrifuge bottle and pellet the cells by centrifugation at 4000 x g for 5 min at 4°C.

5. Carefully pour off and discard the supernatant; place the centrifuge bottles with the cell pellets on ice.
6. Add 80 ml of sterile water to the bottle and vortex to resuspend the cell pellet.
7. Add 10 ml of 10 X TE buffer; swirl to mix. Add 10 ml of Lithium Acetate stock solution; swirl to mix. Incubate 45 min at 30°C shaking at ~85 RPM.
8. Add 2.5 ml of 1 M DTT; swirl to mix. Incubate 15 min at 30°C shaking at ~85 RPM.
9. Bring the volume to 500 ml with sterile water. Pellet the cells by centrifugation at 4000 x g for 5 min at 4°C; pour off and discard the supernatant.
10. Add ~50 ml of sterile, ice cold water to the bottle and vortex to resuspend the cell pellet; bring the volume to 500 ml with sterile, ice-cold water. Pellet the cells by centrifugation at 4000 x g for 5 min at 4°C; pour off and discard the supernatant.
11. Resuspend the cell pellet in 25–30 ml of sterile, ice-cold 1 M sorbitol and transfer to a chilled 30 ml Oakridge tube. Pellet the cells by centrifugation at 4000 x g for 5 min at 4°C; pour off and discard the supernatant.
12. Resuspend the cell pellet in 0.5 ml of sterile, ice-cold 1 M sorbitol; the final cell volume should be 1.3–1.5 ml and the cell concentration should be  $\sim 1 \times 10^{10}$  cells/ml. Keep the cells on ice and use as soon as possible for electroporation.

### **7.1.2 Electroporation**

1. For each sample to be electroporated, prepare a 17 x 100 mm sterile tube with 1 ml of 1 M Sorbitol and place on ice; also, place a 0.2 cm or 0.4 cm cuvette on ice.
2. Pipette the DNA samples (5–100 ng in a volume of 5  $\mu$ l) to be electroporated into sterile 1.5 ml microfuge tubes. Place tubes on ice.
3. If 0.2 cm cuvettes are used, add 40  $\mu$ l of the competent cells to each DNA sample; if 0.4 cm cuvettes are used, add 80  $\mu$ l of the competent cells to each DNA sample. Mix gently and incubate on ice for ~5 min.
4. From the Home screen on Gene Pulser Xcell open the Pre-set Protocols screen, then the Fungal Protocol screen (press 4, Enter, then 2, Enter). When using 0.2 cm cuvettes, press Enter to open the *S. cerevisiae*, 2 mm cuvette Protocol Detail screen. When using 0.4 cm cuvettes, press 2 then Enter to open the *S. cerevisiae*, 4 mm Protocol Detail screen. See Section 3.4 for operating instructions.
5. Transfer the DNA - cell samples to the appropriate electroporation cuvettes that have been chilled in ice and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the lid down to close. Pulse once.
6. Remove the cuvette from the chamber, immediately add 1 ml of ice cold 1 M sorbitol to the cuvette, then gently transfer the diluted cells into a sterile 17 x 100 mm tube.
7. Check and record the pulse parameters. The time constant should be about 5 milliseconds. The voltage should be approximately 1.5 kV when pulsing the 0.2 cm cuvettes and approximately 2.5 kV when pulsing the 0.4 cm cuvettes.

8. Plate aliquots of the electroporated cells on selective agar plates containing 1 M sorbitol. Incubate plates for 48–72 hrs at 30°C.

### 7.1.3 Solutions and reagents

1. YPD: 10 g yeast extract, 20 g peptone, dissolve in 900 ml water. Autoclave. Add 100 ml sterile 20% glucose.
2. 1M sorbitol: 182.2 g sorbitol, dissolve in 800 ml water. Bring volume to 1.0 L with water. Autoclave.
3. 20% glucose: 20 g glucose, dissolve in 60 ml water. Adjust volume to 100 ml with water. Sterilize through a 0.22  $\mu$  filter.

## 7.2 Schizosaccharomyces pombe

### 7.2.1 Preparation of Electrocompetent Cells

See Prentice (1991) for additional information.

Using this method we have obtained transformation efficiencies up to  $3 \times 10^5$  transformants /  $\mu$ g electroporating *S. pombe* CHP408 with pART1 (Apolinario, et al, 1993).

Gene Pulser Xcell conditions: C = 25  $\mu$ F; PC = 200 ohm; V = 2.3 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate 200 ml of YCD in a 2.8 L Fernbach flask with an aliquot from an overnight culture of *S. pombe*. (An initial OD<sub>600</sub> of 0.1 is usually a good starting point.) The doubling time of *S. pombe* is approximately 2 hrs at 30°C.
2. Incubate at 30°C overnight, shaking at 250 rpm, to late log phase ( $\sim 2 \times 10^8$  cells/ml; OD<sub>600</sub> (1:20 dil) = 0.4–0.5).
3. Chill the cells in an ice water bath for 15 min to stop growth.
4. Decant the cells into a sterile 250 ml centrifuge bottle and pellet the cells by centrifugation at 4000 x g for 5 min at 4°C.
5. Carefully pour off and discard the supernatant; keep the cells on ice.
6. Add  $\sim 50$  ml of sterile, ice-cold 1.2 M sorbitol and vortex to resuspend the cell pellet; bring the volume in each of the centrifuge bottles to  $\sim 250$  ml. Pellet the cells by centrifugation at 4000 x g for 5 min at 4°C; pour off and discard the supernatant.
7. Wash the cells again as in step 6 with a total of  $\sim 200$  ml sterile, ice-cold 1.2 M sorbitol.
8. Resuspend the cell pellet with  $\sim 10$  ml of sterile, ice-cold 1.2 M sorbitol and transfer to a chilled 30 ml Oakridge tube; rinse the centrifuge bottle with 15–20 ml of 1.2 M ice cold 1.2 M sorbitol and transfer to the Oakridge tube. Pellet the cells by centrifugation at 4000 x g for 5 min at 4°C; pour off and discard the supernatant.
9. Resuspend the cell pellet in 3 ml of sterile, ice-cold 1.2 M sorbitol. Keep the cells on ice and use as soon as possible for electroporation.

## 7.2.2 Electroporation

1. For each sample to be electroporated, prepare a 17 x 100 mm sterile tube with 1 ml of 1.2 M Sorbitol and place on ice; also place a 0.2 cm cuvette on ice.
2. Pipette the DNA samples (up to 1 µg) to be electroporated into sterile 1.5 ml microfuge tubes. Place tubes on ice. Add 40 µl of the competent cells to each DNA sample and mix gently.
3. From the Home Screen on Gene Pulser Xcell open the Fungal Protocol Screen (press 4, Enter, 2, Enter). To select *S. pombe*, press 3 or the Down Arrow to highlight *S. pombe*, then press Enter to open the *S. pombe* Protocol Detail screen. See Section 3.4 for operating instructions.
4. Transfer the DNA - cell samples to the chilled 0.2 cm electroporation cuvettes and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.
5. Remove the cuvette from the chamber and immediately add 1 ml of ice cold 1.2 M sorbitol to the cuvette; gently transfer the diluted cells to a sterile 17 x 100 mm tube.
6. Check and record the pulse parameters. The time constant should be close to 5 milliseconds. The voltage should be approximately 2.3 kV.
7. Incubate the tubes at room temperature for 40–60 min. Plate aliquots of the electroporated cells on selective agar plates without sorbitol (e.g., EMM). Incubate plates for 6–8 days at 30°C.

## 7.2.3 Solutions and Reagents

1. YCD media: 10 g yeast extract, 2 g casamino acids, dissolve in 900 ml water. Autoclave. Add 100 ml 20% glucose.  
20% Glucose: 100 g glucose, dissolve in 350 ml water; bring volume to 500 ml with water. Filter sterilize.
2. 1.2 M sorbitol: 218.6 g sorbitol, dissolve in ~700 ml water. Add water to 1.0 L. Autoclave.
3. EMM: 12.35 g EMM – Dextrose (Q-Biogene, Catalog # 4110-122), dissolve in 900 ml water. Pour 450 ml into each of two 1 L bottles and add 10.0 g agar to each bottle. Autoclave 30 min. Cool to 55–60°C and add 50 ml 20% Glucose to each bottle. Pour plates (sufficient for ~60 plates).

## 7.3 *Pichia pastoris*

### 7.3.1 Preparation of Electrocompetent Cells

See Cregg & Russell (1998) for additional information.

Using this method we have obtained transformation efficiencies of  $\sim 6 \times 10^4$  transformants / µg electroporating *P. pastoris* X33 with Sacl-pPICZA (Invitrogen).

Gene Pulser Xcell conditions: C = 25 µF; PC = 200 ohm; V = 2.0 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate 500 ml of YPD in a 2.8 L Fernbach flask with an aliquot from a fresh overnight culture of *P. pastoris*. The doubling time of *P. pastoris* is approximately 2 hrs at 30°C.
2. Incubate at 30°C overnight, shaking at 300 rpm, to a density of  $\sim 7 \times 10^7$  cells/ml (OD<sub>600</sub> (1:10 dil) = 0.24–0.30).
3. Decant the cells into a sterile 500 ml centrifuge bottle and pellet the cells by centrifugation at 3000 x g for 5 min at 4°C.
4. Carefully pour off and discard the supernatant.

5. Add 100 ml of sterile, YPD/HEPES to each of the bottles and vortex to resuspend the cell pellets; add 2.5 ml of 1M DTT; mix gently. Incubate the cells for 15 min at 30°C without shaking.
6. Bring the volume to ~500 ml with sterile, ice-cold 1 M sorbitol. Pellet the cells by centrifugation at 3000 x g for 5 min at 4°C; pour off and discard the supernatant.
7. Add ~50 ml of sterile, ice-cold 1 M sorbitol and vortex to resuspend the cell pellets; bring the volume to 500 ml with sterile, ice-cold 1 M sorbitol. Pellet the cells by centrifugation at 3000 x g for 5 min at 4°C; pour off and discard the supernatant.
8. Resuspend the cell pellet in ~25 ml of sterile, ice-cold 1 M sorbitol and transfer to a chilled 30 ml Oakridge tube. Pellet the cells by centrifugation at 3000 x g for 5 min at 4°C; pour off and discard the supernatant.
9. Resuspend the cell pellet in 0.5 ml of sterile, ice-cold 1 M sorbitol; the final cell volume should be ~1.3 ml and the cell concentration should be  $>1 \times 10^{10}$  cells/ml. Keep the cells on ice and use as soon as possible for electroporation.

### 7.3.2 Electroporation

1. For each sample to be electroporated, prepare a 17 x 100 mm sterile tube with 1.0 ml of 1 M sorbitol and place on ice; also place a 0.2 cm cuvette on ice.
2. Pipette the DNA samples (up to 0.1 µg) to be electroporated into sterile 1.5 ml microfuge tubes. Place tubes on ice.
2. Add 40 µl of the competent cells to each DNA sample and mix gently.
3. From the Home Screen on Gene Pulser Xcell open the Fungal Protocol Screen (press 4, Enter, 2, Enter). To select *P. pastoris*, press 5 or the Down Arrow to highlight *P. pastoris*, then press Enter to open the *P. pastoris* Protocol Detail screen. See Section 3.4 for operating instructions.
4. Transfer the DNA - cell samples to the 0.2 cm electroporation cuvettes that have been chilled in ice and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.
5. Remove the cuvette from the chamber and immediately add 1.0 ml of ice cold 1.0 M sorbitol to the cuvette; gently transfer the diluted cells to a sterile 17 x 100 mm tube.
6. Check and record the pulse parameters. The time constant should be close to 5 milliseconds. The voltage should be approximately 2.0 kV.
7. When selecting for complementation of an auxotrophic mutant, the cells may be plated immediately onto minimal agar plates lacking the appropriate nutrient. When selecting for antibiotic resistance, incubate the cells at 30°C for 1 hr without shaking; add 1 ml of 1M sorbitol and continue incubating for 1 hr; plate aliquots of the electroporated cells on YPD agar plates with the appropriate antibiotic. Incubate the plates for 72–96 hrs at 30°C.

### 7.3.3 Solutions and Reagents

1. YPD: 10 g yeast extract, 20 g peptone, dissolve in 900 ml water. Autoclave. Add 100 ml sterile 20% glucose.
2. YPD/HEPES: 100 ml YPD media, 20 ml 1 M HEPES, pH 8.0.
3. 1M sorbitol: 182.2 g sorbitol, dissolve in 800 ml water. Bring volume to 1.0 L with water. Autoclave.
4. 20% glucose: 20 g glucose, dissolve in 60 ml water. Adjust volume to 100 ml with water. Sterilize through a 0.22 µ filter.



5. 1 M HEPES, pH 8.0: 23.8 g HEPES (MW = 238.3), dissolve in ~65 ml water. Adjust pH to 8.0 with 5 N NaOH. Bring volume to 100 ml with water. Sterilize through a 0.22  $\mu$  filter.
6. 1M DTT: 3.85 g DTT, dissolve in ~22 ml water. Bring volume to 25 ml with water. Sterilize through a 0.22  $\mu$  filter.

## **7.4 Candida albicans**

### **7.4.1 Preparation of Electrocompetent Cells**

See deBacker, et al. (1999) for additional information.

Using this method we have obtained transformation efficiencies of >400 transformants /  $\mu$ g electroporating *C. albicans* CAI4 with Nhel-pUX (Thompson, et al., 1998).

Gene Pulser Xcell conditions: C = 25  $\mu$ F; PC = 200 ohm; V = 1.5 kV.

This procedure requires a Gene Pulser Xcell main unit and PC Module.

1. Inoculate a colony of *C. albicans* into 5–6 ml of YPD/uridine media in a 17 x 100 mm tube; incubate overnight at 30°C shaking at 250–300 rpm.
2. Inoculate an aliquot of the overnight culture into 500 ml of YPD/uridine media in a 2.8 L Fernbach flask; incubate overnight at 30°C shaking at 250–300 rpm to a density of 5–10  $\times 10^7$  cells/ml (OD<sub>600</sub> of a 1:10 dilution = 0.3–0.45).
3. Chill the culture on ice for 10 min.
4. Transfer the cells into a sterile 500 ml centrifuge bottle.
5. Pellet the cells by centrifugation at 15–20°C for 5 min at 3000 x g.
6. Discard the supernatant. Resuspend the cell pellet in 125 ml of sterile 5 mM LiAc/10 mM DTT; incubate at 20°C for 1 hr.
7. Pellet the cells at 4°C for 5 min at 3000 x g.
8. Discard the supernatant. Resuspend the pellet in 125 ml of sterile, ice cold water. Pellet the cells as above.
9. Discard the supernatant. Resuspend the cell pellet in 125 ml of ice cold water. Pellet the cells as above.
10. Discard the supernatant. Resuspend the cell pellet in 35 ml of ice cold 1 M sorbitol. Transfer the cells to a chilled Oakridge tube. Pellet the cells at 4°C for 5 min at 3000 x g.
11. Carefully remove the supernatant. Resuspend the pellet in 0.5 ml of ice cold 1 M sorbitol.
12. Keep the cells on ice and use as soon as possible for electroporation.

### **7.4.2 Electroporation**

1. For each sample to be electroporated, prepare a 17 x 100 mm sterile tube with 1.0 ml of 1 M sorbitol and place on ice; also place a 0.2 cm cuvette on ice.
2. Pipette the linearized DNA samples (0.1–1  $\mu$ g) to be electroporated into sterile 1.5 ml microfuge tubes. Place tubes on ice.
3. Add 40  $\mu$ l of the electrocompetent cells to each DNA sample and mix gently. Incubate the cells on ice for 5 min.

4. From the Home Screen on Gene Pulser Xcell open the Fungal Protocol Screen (press 4, Enter, 2, Enter). To select *C. albicans*, press 4 or the Down Arrow to highlight *C. albicans*, then press Enter to open the *C. albicans* Protocol Detail screen. See Section 3.4 for operating instructions.
5. Transfer the DNA - cell samples to the 0.2 cm electroporation cuvettes that have been chilled in ice and tap the suspension to the bottom. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once.
6. Remove the cuvette from the chamber and immediately add 1.0 ml of ice cold 1.0 M sorbitol to the cuvette; gently transfer the diluted cells to a sterile 17 x 100 mm tube.
7. Check and record the pulse parameters. The time constant should be close to 5 milliseconds. The voltage should be approximately 1.5 kV.
8. Immediately plate an aliquot of the cells onto minimal agar plates containing 1 M sorbitol but lacking the appropriate nutrient. Incubate the plates for 4–6 days at 30°C.

### 7.4.3 Solutions and Reagents

1. YPD / Uridine: 10 g yeast extract, 20 g peptone, dissolve in 900 ml water; add 15 g agar. Autoclave. Add 100 ml sterile 20% glucose, 1 ml 25 mg/ml uridine.
2. YPD / Uridine agar: 10 g yeast extract, 20 g peptone, dissolve in 900 ml water. Autoclave. Add 100 ml sterile 20% glucose, 1 ml 25 mg/ml uridine.
3. 1M sorbitol: 182.2 g sorbitol, dissolve in 800 ml water. Bring volume to 1.0 L with water. Autoclave.
4. 20% glucose: 20 g glucose, dissolve in 60 ml water. Adjust volume to 100 ml with water. Sterilize through a 0.22  $\mu$  filter.
5. 5 mM LiAc/10 mM DTT in TE: 1 ml 1 M Lithium Acetate (10.2 g / 100 ml), 20 ml 0.1 M DTT (1.54 g / 100 ml), 2 ml 1 M Tris, pH 7.5, 0.4 ml 0.5 M EDTA, 176.6 ml water. Filter sterilize. Store at -20°C.
6. 1M sorbitol: 182.2 g sorbitol, dissolve in 800 ml water. Bring volume to 1.0 L with water. Autoclave.

## 7.5 Dictyostelium discoideum

### 7.5.1 Preparation of Electrocompetent Cells

See Howard et al. (1988) and Knecht & Pang (1995) for additional information.

Gene Pulser Xcell conditions: square wave, V = 1.0 kV, 1.0 ms Pulse length, 2 pulses, 5 sec pulse interval; V = 1.0 kV.

1. Inoculate Dictyostelium cells at a concentration of  $5-7 \times 10^5$  cells/ml into 40 ml of HL5 media in a 500 ml flask. The cells may either be scraped from a plate or transferred from liquid media. The doubling time of Dictyostelium is approximately 12 hrs at 21°C.
2. Incubate the culture at 21°C for about 24 hrs, shaking at 125 rpm. About 16–20 hrs prior to preparing the competent cells, dilute the cells to  $7 \times 10^5$  cells/ml with HL5 media. Incubate at 21°C overnight, shaking at 125 rpm.
3. Transfer 100 ml of the cells into two sterile, disposable, 50 ml centrifuge tubes and incubate on ice for 15 min to stop growth.
4. Pellet the cells by centrifugation at 400 x g for 5–7 min at room temperature.

5. Carefully pour off and discard the supernatant; place the centrifuge bottles with the cell pellets on ice.
6. Pool the cell pellets and resuspend in 50 ml of sterile, ice-cold E buffer. Pellet the cells by centrifugation at 400 x g for 5–7 min at room temperature.
7. Carefully pour off and discard the supernatant; place the centrifuge bottles with the cell pellets on ice and resuspend the cells at a concentration of  $1 \times 10^7$  cells/ml. Keep the cells on ice and use as soon as possible for electroporation.

### **7.5.2 Electroporation**

1. Pipette the DNA samples (up to 50  $\mu$ g) to be electroporated into sterile 1.5 ml microfuge tubes. Place tubes on ice.
2. Add 800  $\mu$ l of the competent cells to each DNA sample and pipette up and down to mix; incubate on ice ~1 min.
3. From the Home Screen on Gene Pulser Xcell open the Fungal Protocol Screen (press 4, Enter, 2, Enter). To select *D. discoideum*, press 6 or the Down Arrow to highlight *D. discoideum*, then press Enter to open the *D. discoideum* Protocol Detail screen. See Section 3.4 for operating instructions.
4. Transfer the DNA - cell samples to 0.4 cm electroporation cuvettes that have been chilled in ice and tap the suspension to the bottom of the tube. Place the cuvette in the ShockPod. Push the chamber lid down to close. Pulse once (the program delivers two pulses, each with a ~1 msec time constant approximately 5 sec apart).
5. Remove the cuvette from the chamber and immediately dilute the cells to 10 ml with the appropriate media.
6. Check and record the pulse parameters. The time constant should be 1 millisecond. The voltage should be approximately 1.0 kV.
7. When selecting for complementation of an auxotrophic mutant, the cells may be plated immediately into selective media lacking the appropriate nutrient. When selecting for antibiotic resistance, incubate the cells overnight at 21°C prior to adding the selective agent.

### **7.5.3 Solutions and Reagents**

1. HL5 media: 17.8 g bacteriological peptone (Oxoid, Ogdensburg, NY), 7.2 g yeast extract, 0.54 g  $\text{Na}_2\text{HPO}_4$ , 0.4 g  $\text{KH}_2\text{PO}_4$ , 130  $\mu$ l B12/Folic acid mix; bring to 1L with water and adjust to pH 6.3–6.5. Autoclave for 25 min on two successive days. Prior to use, add 20 ml of 50% glucose and 10 ml of 100 X Antibiotic-Antimycotic (Life Technologies, Gaithersburg, MD).
2. B12/Folic acid mix: 5 mg B12, 200 mg folic acid; add 95 ml water, then pH to 6.5–6.8 with 5N NaOH; bring to 100 ml with water. Filter sterilize and store at -20°C protected from light.
3. E buffer: 10 ml 100 mM  $\text{NaH}_2\text{PO}_4$ , adjusted to pH 6.1 with KOH, 10 ml 0.5 M sucrose, 80 ml water; autoclave.

## Section 8

### Electroporation of Mammalian Cells

This procedure requires a Gene Pulser Xcell main unit and CE Module.

#### 8.1 Preparation of Electrocompetent Cells

##### 8.1.1 Attached Cells

1. One – two days prior to electroporation, transfer the cells into 75cm<sup>2</sup> flasks with fresh growth medium so that they will be 50–70% confluent on the day of the experiment. For most cell lines the cell density will be 2–10 x 10<sup>6</sup> cells / flask; about 1–10 x 10<sup>5</sup> cells are needed per electroporation.
2. Aspirate the media and rinse the flasks using ~12 ml of phosphate buffered saline (PBS).
3. Aspirate the PBS and add ~0.4 ml of trypsin-EDTA; incubate the cells 2–5 min at room temperature. Tap the flask to detach the cells from the surface.
4. Neutralize the trypsin by adding 10 ml of growth media with serum.
5. Transfer the cells to a 50 ml sterile centrifuge tube; pellet the cells by centrifugation at 400 x g for 5–7 min at room temperature.
6. Aspirate the media, then resuspend the cell pellets in electroporation buffer (Optimem, growth media without serum, PBS, Hepes-buffered saline, Phosphate-buffered sucrose, or Hepes-buffered sucrose) at a density of 1–5 x 10<sup>6</sup> cells/ml. Gently pipette the cells to obtain a single-cell suspension.

##### 8.1.2 Suspension Cells

1. One – two days prior to electroporation, dilute the cells into fresh growth medium in a 75cm<sup>2</sup> flask so that they will be in mid-log phase (generally about 0.5–4 x 10<sup>6</sup> cells/ml) on the day of the experiment. About 1–10 x 10<sup>5</sup> cells are needed per electroporation.
2. Transfer the cells to a 50 ml sterile centrifuge tube; pellet the cells by centrifugation at 400 x g for 5–7 min at room temperature.
3. Aspirate the media, then resuspend the cell pellets in electroporation buffer (Opti-MEM, growth media without serum, PBS, Hepes-buffered saline, Phosphate-buffered sucrose, or Hepes-buffered sucrose) at a density of 1–5 x 10<sup>6</sup> cells/ml. Gently pipette the cells to obtain a single-cell suspension. For all Pre-set Protocols programmed in Gene Pulser Xcell, we recommend that cells be resuspended in Opti-MEM or in growth media without serum.

#### 8.2 Electroporation

1. Set the desired pulse conditions on the Gene Pulser Xcell. If a Pre-Set Protocol is used (see Table 3.4), open the Mammalian Pre-set Protocols screen as follows: from the Home screen, press 4, Enter, 3, Enter; use the alpha-numeric keypad or the Up and Down Arrow keys to select the cell line, then press Enter to open the appropriate Protocol Detail screen.

2. Add plasmid DNA(s) to the electroporation cuvettes. The amount of DNA required per sample is dependent on the cell type. Start with a concentration of 10–50 µg/ml.
3. Add cells to the cuvette and tap the side of the cuvette to mix. See Table 8.1 for suggestions on cell concentrations and volumes to use.
4. Place the cuvette in the ShockPod. Push down the lid to close. Pulse once.
5. Immediately after the pulse, transfer the cells to a plate using ~0.5 ml of media and a plugged Pasture pipette.
6. Rock the plates gently to assure even distribution of the cells over the surface of the plate. Incubate the plates at 37°C in a humidified atmosphere.
7. Assay transient gene expression 24–48 hrs following electroporation.

**Table 8.1 Suggested starting cell concentrations and volumes for electroporating mammalian cells**

<b>Cuvette (cm)</b>	<b>Cell concentration (cells/ml)</b>	<b>Cell volume (µl)</b>	<b>Growth conditions following electroporation</b>
0.2	1 x 10 <sup>6</sup>	100	48 well plate with 0.5 ml growth media
0.2	5 x 10 <sup>6</sup>	200	6 well plate with 2 ml growth media
0.4	2.5 x 10 <sup>6</sup>	400	6 well plate with 2 ml growth media

### **8.3 Reagents and Solutions for Electroporations**

1. Growth medium with 10% FBS.
2. Opti-MEM (Invitrogen, Carlsbad, CA).
3. Trypsin-EDTA: 0.05% trypsin, 0.53 mM EDTA in PBS (Invitrogen).
4. Phosphate-buffered saline: 137 mM NaCl, 2.7 mM KCl, 9.5 mM sodium phosphate, pH 7.3; autoclave.
5. Hepes-buffered saline: 10 mM Hepes, pH 7.3, 140 mM NaCl; autoclave.
6. Phosphate-buffered sucrose: 272 mM Sucrose, 10 mM sodium phosphate, pH 7.3; filter sterilize.
7. Hepes-buffered sucrose: 272 mM Sucrose, 10 mM Hepes, pH 7.3; filter sterilize.

## Section 9

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## Section 10

### Specifications and Product Information

#### 10.1 System Specifications

##### 165-2660 Gene Pulser Xcell Total System

Includes Main Unit, CE module, PC module

Outputs	Waveform:	Exponential decay or Square wave.
	Voltage:	10–3,000 Volts
Capacitance	10–500 V,	25–3275 mF in 25 mF increments.
	500–3000 V,	10, 25, 50 mF.
Resistance (Parallel)		50–1,000 $\Omega$ in 50 $\Omega$ increments plus infinity.
Sample Resistance		20 $\Omega$ minimum at 10–2,500 V
		600 $\Omega$ minimum at 2,500–3,000 V
Square Wave Timing	10–500 V:	0.05–100 msec pulse length, 1–10 pulses, 0.1–10 sec pulse interval
	500–3,000 V:	0.05–5 msec pulse length, 1–2 pulses, 5–30 sec pulse interval

##### 165-2661 Gene Pulser Xcell Eukaryotic System

Includes Main Unit, CE module

Outputs same as Total system without the parallel resistance.

##### 165-2662 Gene Pulser Xcell Microbial System

Includes main unit, PC module

Outputs	Waveform:	Exponential decay or Square wave.
	Voltage:	200–3,000 Volts
Capacitance		10, 25, 50 $\mu$ F.
Resistance (Parallel)		50–1,000 $\Omega$ in 50 $\Omega$ increments plus infinity.
Sample Resistance		20 $\Omega$ minimum at 200–2,500 V
		600 $\Omega$ minimum at 2,500–3,000 V
Square Wave Timing		0.05–5 msec pulse length, 1–2 pulses, 5–30 pulse interval

##### 165-2666 Gene Pulser Xcell Main Unit

Outputs	Waveform:	Exponential decay or Square wave.
	Voltage:	200–3,000 Volts
Discharge Capacitance		10, 25, 50 $\mu$ F.
Sample Resistance		20 $\Omega$ minimum at 200–2,500 V
		600 $\Omega$ minimum at 2,500–3,000 V
Square Wave Timing		0.05–5 msec pulse length, 1–2 pulses, 5–30 sec pulse interval

##### General

Input Voltage		100–120 VAC or 220–240 VAC, 50/60 Hz.
Power		Max 240 W (During short charging periods)
Operating environment		Temp. 0–35°C, Humidity 0–95%, (non-condensing).
Regulatory	Safety	EN 61010, EMC EN61326 Class A
Dimensions,	Main Unit:	31 x 30 x 14 cm, weight 6.62 kg.
	CE Module:	31 x 28 x 9 cm, weight 3.08 kg.
	PC Module:	31 x 28 x 5 cm, weight 1.90 kg.

## 10.2 Product Information

<b>Catalog Number</b>	<b>Product Description</b>
165-2660	Gene Pulser Xcell Total System for eukaryotic and microbial cells, 100–240 V, 50/60 Hz, exponential decay and square wave delivery, includes main unit, CE Module, PC Module, ShockPod, 15 sterile cuvettes (5 each of 0.1, 0.2, and 0.4 cm gap), Instruction Manual
165-2661	Gene Pulser Xcell Eukaryotic system, 100/240 V, 50/60 Hz, exponential decay (25–3,275 $\mu$ F range) and square wave delivery, includes main unit, CE Module, ShockPod, 5 sterile cuvettes (0.4 cm gap), Instruction Manual
165-2662	Gene Pulser Xcell Microbial system, 100/240 V, 50/60 Hz, exponential decay and square wave delivery, includes main unit, PC Module, ShockPod, 10 sterile cuvettes (5 each of 0.1 and 0.2 cm gap), Instruction Manual
165-2666	Gene Pulser Xcell main unit, 100/240 V, 50/60 Hz
165-2667	Gene Pulser Xcell CE Module, 25–3,275 $\mu$ F range controlled by main unit, includes integral leads
165-2668	Gene Pulser Xcell PC Module, 50–1,000 ohm range controlled by main unit, includes integral leads
165-2669	Gene Pulser Xcell ShockPod shocking chamber, includes integral leads for connection to Gene Pulser Xcell, Gene Pulser II, or MicroPulser.
165-2095	Gene Pulser Cuvette Rack
165-2094	Gene Pulser Electroprotocols
165-2088	Gene Pulser Cuvettes, 0.4 cm gap, 50, sterile
165-2086	Gene Pulser Cuvettes, 0.2 cm gap, 50, sterile
165-2089	Gene Pulser Cuvettes, 0.1 cm gap, 50, sterile
165-2091	Gene Pulser Cuvettes, 0.4 cm gap, 500, sterile
165-2092	Gene Pulser Cuvettes, 0.2 cm gap, 500, sterile
165-2093	Gene Pulser Cuvettes, 0.1 cm gap, 500, sterile
165-2081	Gene Pulser Cuvettes, 0.4 cm gap, 5, sterile
165-2082	Gene Pulser Cuvettes, 0.2 cm gap, 5, sterile
165-2083	Gene Pulser Cuvettes, 0.1 cm gap, 5, sterile



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