Gene Pulser® II
RF Module

Instruction Manual

Catalog Numbers
165-2112
165-2113

BIO-RAD

Warranty

The Gene Pulser II RF module is warranted against defects in materials and workmanship for 1 year. If any defects occur in the instruments or accessories during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

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

This warranty does not apply to parts listed below:

Fuses

For any inquiry or request for repair service, contact Bio-Rad Laboratories. Inform Bio-Rad of the model and serial number of your instrument

IMPORTANT: This Bio-Rad instrument is designed and certified to meet EN 61010* safety standards. Certified products are safe to use when operated in accordance with the instruction manual. This instrument should not be modified or altered in any way. Alteration of this instrument will:

Void the manufacturer's warranty

Void the EN 61010 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.

*EN 61010 is an internationally accepted electrical safety standard for laboratory instruments.

Table of Contents

Section 1
Introduction

1.1 Advantages of RF Electroporation

The RF Gene Pulser module, the latest addition to the Gene Pulser II electroporation family, permits the user to electroporate samples using either square or sinusoidal waves. Electroporation is a process whereby the membrane of a cell is temporarily destabilized in specific regions by exposure to high intensity electric field pulses. During this destabilization period, the cell membrane is highly permeable to exogenous molecules present in the surrounding media. Electroporation can thus be regarded as a massive micro-injection technique that is used to inject anywhere from one cell to millions of cells with specific biological molecules (typically DNA or proteins). These transformed molecules are then used as tools to unravel key issues concerning the control and expression of cell growth and development.

The RF module is used in conjunction with the Gene Pulser II main module to deliver an entirely different waveform for the transfer of biological molecules (DNA or protein) into cells. This new waveform uses RF (radio-frequency) output, in combination with the advanced circuitry of the Gene Pulser II system, to create innovative FlexWave™ technology that delivers many advantages over conventional capacitor discharge and square wave electroporation.

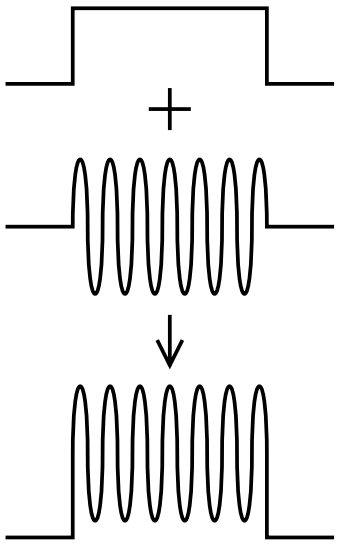


Fig. 1. The FlexWare technology of the Gene Pulser II RF module delivers the unique combination of a pulse of radio-frequency (RF) field and a DC pulse with the same pulse width for improved transformation.

Advantages of FlexWave technology

- Increased cell viability for better transformation efficiency, especially with mammalian cells
- Achieve more transformation with less DNA (reduce DNA concentrations at least 100 fold)
- Multiple pulse capability (up to 25 pulses)
- Programmable pulse settings (store up to 30 programs)
- Capable of square wave delivery
- Modular design for maximum flexibility, quality, and value

1.2 Principle of Operation

Figure 2 is an overview of the parameters which can be varied and which should be optimized to obtain maximum transformation efficiency using RF electroporation.

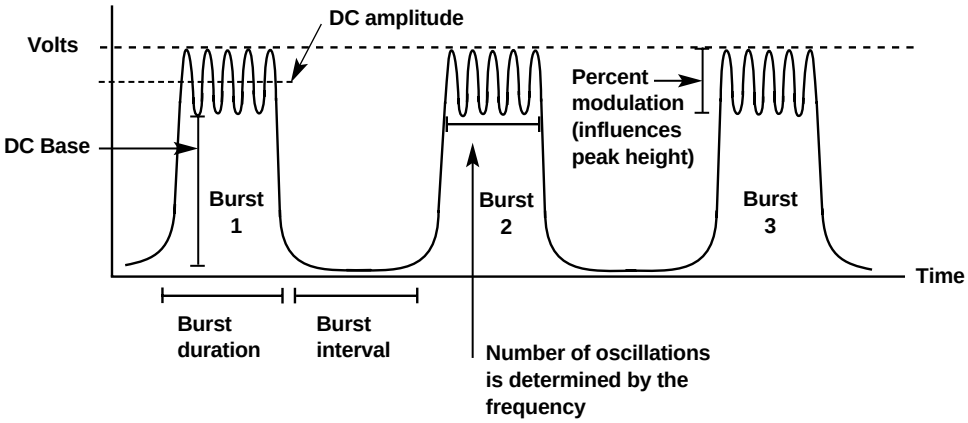


Fig. 2. Relationship between the system variables: voltage, percent modulation, burst duration, frequency, burst interval, and number of bursts.

Section 2
Unpacking the System Components

Read this before you use the Gene Pulser II RF module with the Gene Pulser II main unit.

2.1 Safety

This instrument is intended for laboratory use only.

This product conforms to the Class A standards for electromagnetic emissions intended for laboratory equipment applications. It is possible that the emissions from this product may interfere with some sensitive appliances when placed nearby or on the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

This Bio-Rad instrument is designed and certified to meet EN 61010* safety standards. In addition, the system meets the requirements of the Federal Communications Commission (FCC), Class A. Certified products are safe to use when operated in accordance with the instruction manual.

No part of the Gene Pulser II system and RF module 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 shocking chamber. The operating temperature range for the RF module and Gene Pulser II system and its associated components is 0–35 °C.

*EN 61010 is an internationally accepted electrical safety standard for laboratory instruments.

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 EN 61010 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.

This label indicates hazardous voltages. The RF Module and Gene Pulser II system are designed to prevent contact by means of long insulation paths and other interlock means. Do not attempt to contact any points showing the indicated symbol. Do not add to or modify any cabling.

2.2 Unpacking the System Components

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

The Gene Pulser II RF module contains the following components:

1 RF Module

1 9-wire cable (plugs into 9-pin port on Gene Pulser II main unit and 9 pin port on RF Module)

1 power cord

1 instruction manual

1 warranty card (please complete and return)

2 cable mounts

2.3 Setting up the System

Setting up the RF Module

The RF Module unit is designed to be used exclusively with the Gene Pulser II apparatus. The coiled wire on the back of the RF module plugs into the Capacitance Extender port on the back of the Gene Pulser II main unit; therefore, only one or the other unit may be connected to the Gene Pulser II unit at one time. The 9-wire cable connects the 9-pin port on the RF Module and Gene Pulser II main unit; this cable may remain attached while using the Capacitance Extender II or PLUS modules.

To use the Gene Pulser II RF module, follow this procedure.

1. Verify Gene Pulser II RF module voltage compatibility with local electrical output by checking that the red voltage switches in the back of the unit are in the proper position (Figure 3).
   - Units operating in the 100–120 V range must have the red voltage switches positioned so 115 V is visible on both switches.
   - Units operating in the 220–240 V range must have the red voltage switches positioned so 230 V is visible on both switches.
2. Attach the power cord to the three pronged receptacle on the back of the Gene Pulser II unit (Figure 3) and plug the unit into an appropriate electrical outlet or power strip.

Connecting the RF Module to the Gene Pulser II Unit

1. Place the RF module on top of or near the Gene Pulser II unit. If the Pulse Controller II (or PLUS) module is to be connected to the Gene Pulser II unit, we recommend that you first place the Pulse Controller II (or PLUS) module on top of the Gene Pulser II unit and then place the RF module atop the Pulse Controller module. The Capacitance Extender II (or PLUS) module should be placed near by the Gene Pulser II main unit
2. Make certain that the power switch is off on the Gene Pulser II unit. Attach the power cord to the three pronged receptacle on the back of the RF module and plug into the appropriate electrical outlet or power strip.
3. Insert the red/black leads that are permanently attached to the back of the RF module into the Capacitance Extender receptacle on the back of the Gene Pulser II unit. The leads are keyed so that they will insert only into the proper output jack in the correct red/black orientation.
4. Insert one end of the nine wire cable into the 9 pin port on the back panel of Gene Pulser II unit. Secure cable to port with the two cable attachment screws-finger tighten with a clockwise motion.

   Attach the other end of the 9 wire cable into the 9 pin port on the back of the RF module. Secure cable to port with the two cable attachment screws-finger tighten with a clockwise motion.

   Optional: The supplied cable is 6 feet long. To minimize cable tangle, use the supplied self-adhesive cable mounts.

   Peel off the adhesive protection film on each of the cable mounts and firmly press in place at the upper corners of the RF module back (Figure 3).

   Capture the un-used portion of the cable by inserting the excess cable between the two mounts, A and B (Figure 3).
5. When using the RF module, connect the leads from the shocking chamber to the output jacks on the front of the RF module.

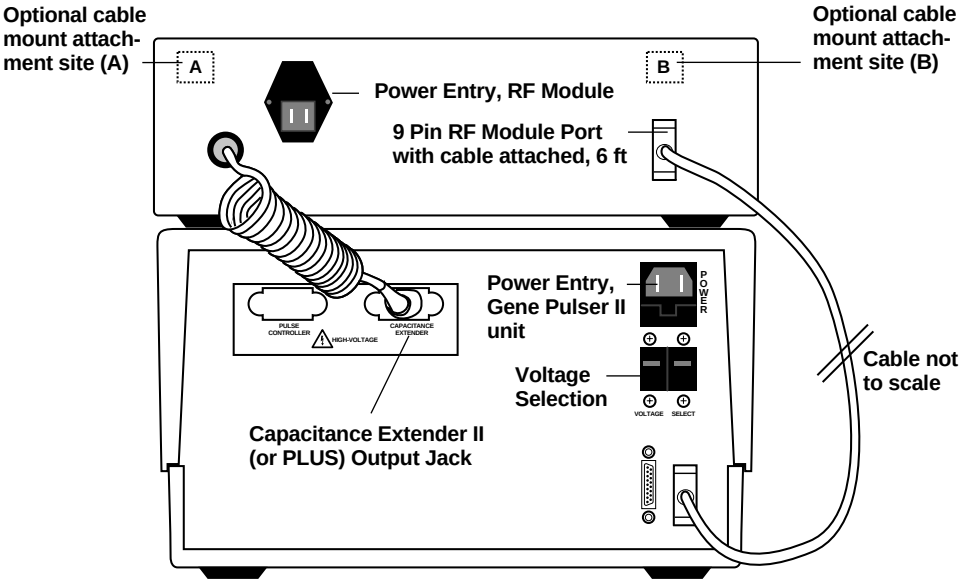


Fig. 3. Red/black leads and the 9 pin cable of the RF module (back panel) connected to the appropriate output jacks and port on the back panel of the Gene Pulser II unit.

Section 3
Guide to Switches and Keypads

3.1 Gene Pulser II Main Unit Control Panel

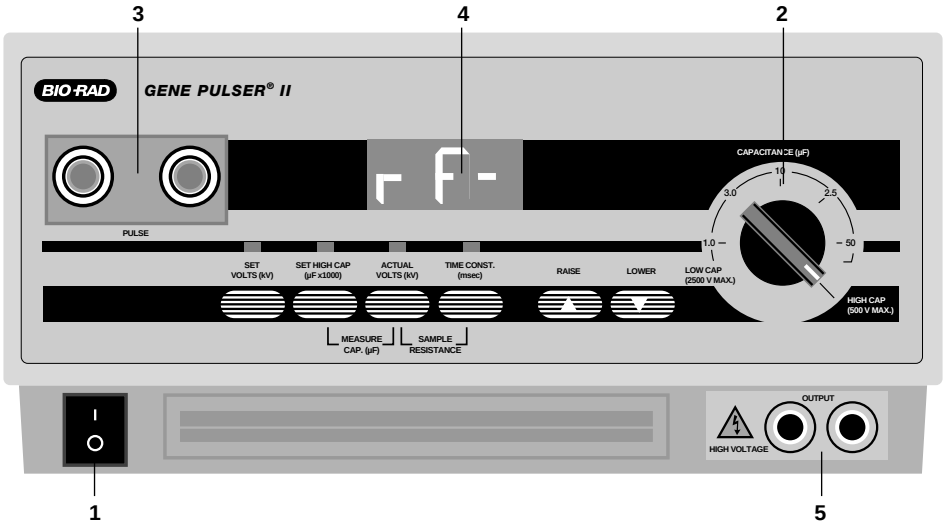
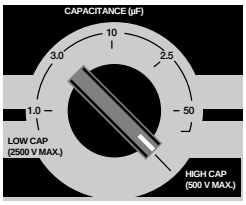


Fig. 4. The control panel of the Gene Pulser II apparatus. Front panel identification of switches and keypads for the Gene Pulser II unit. Note that the keypad(s) are active when the light(s) above them are illuminated.

1. POWER

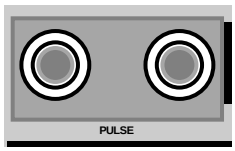
Function	Result/Display
Turns unit on and off.	With the power on, the system initializes in the Set Volts mode (the light above SET VOLTS will be illuminated).

2. CAPACITANCE (µF) rotary switch



Function	Result/Display
LOW CAP. (2,500 V max.). Positions 1.0, 3.0, 10, 25, or 50 µF select the specific high voltage/low value capacitor inside the Gene Pulser II unit.	Selecting any of the Low Cap. capacitors (used with the Pulse Controller II (or PLUS) module—high voltage) allows the Set Volts adjustment to range from 0.2 to 2.5 kV in 10 V (0.01 kV) increments. Not used with the RF module.
HIGH CAP. (500 V max.) position activates RF module.	Selecting the High Cap. position on Capacitance rotary switch activates the RF module and allows the pulse parameters to be set on the RF module (after wait light disappears).

3. PULSE

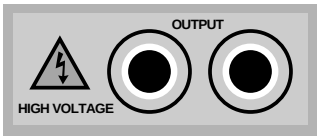


Function	Result/Display
Pressing both Pulse buttons simultaneously activates the RF module if the module is properly connected to the Gene Pulser II main unit and power outlet.	rF- is displayed when the Gene Pulser II main unit is active in the RF mode.

4. LED

Function	Result/Display
LED (Light Emitting Diode) display.	Displays RF module function indicators (3 digit) (rF-).

5. Shocking Chamber Output Jack



Function	Result/Display
Shocking chamber output jack.	Not used for RF electroporation.

3.2 Gene Pulser II RF Module Control Panel

The following is a brief description of the control panel on the front of the RF module:

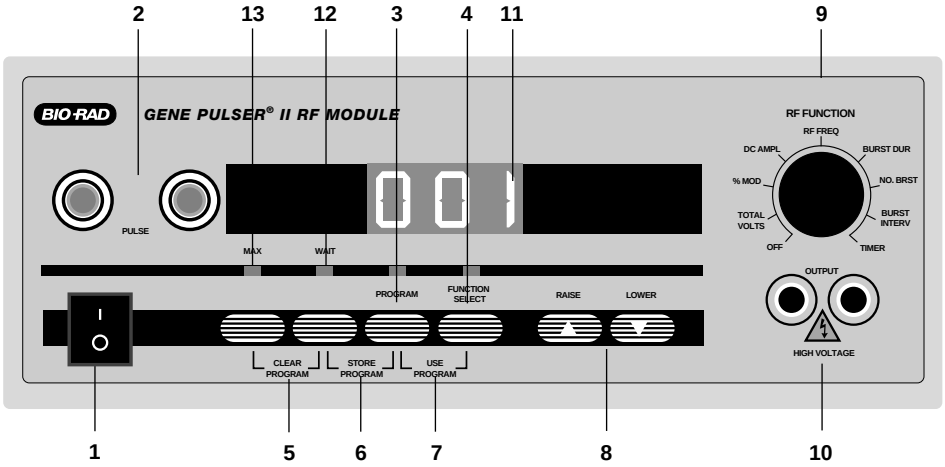
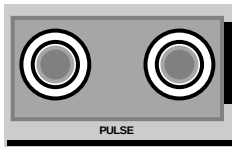


Fig. 5. The control panel of the RF module. Front panel identification of switches and keypads for the RF module. Note that the keypad(s) are active when the light(s) above them are illuminated.

1. POWER

Function	Result/Display
Turns unit on and off.	With the power on, the system initializes in the Program mode (the light above Program will be illuminated).

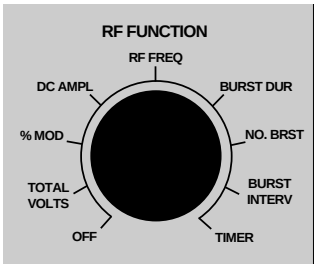
2. PULSE



Function	Result/Display
Pressing both Pulse buttons continuously activates the RF module to deliver the pulse parameters to the cuvette.	Pulse settings selected are activated. Pulse is delivered to cuvette as tone sounds. Release the pulse buttons after the tone sounds.

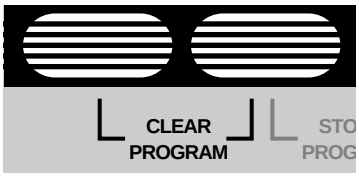
3. PROGRAM

Function	Result/Display
Activates program function.	Active program number displayed. LED display over Program key should light. Program function is not available when wait light is lit.



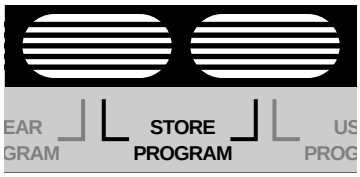
4. RF FUNCTION Select

Function	Result/Display
Control knob to select specific pulse parameter.	Active pulse parameter setting displayed.



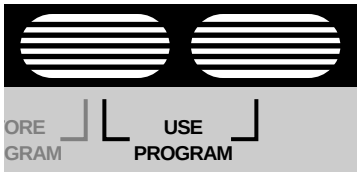
5. CLEAR PROGRAM

Function	Result/Display
Clears program parameters.	Active program number selected is cleared.



6. STORE PROGRAM

Function	Result/Display
Stores program parameters.	Active program number selected is stored.

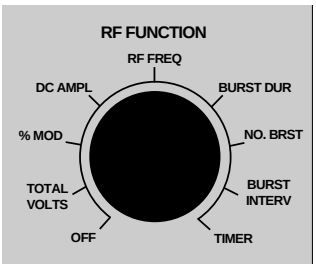


7. USE PROGRAM

Function	Result/Display
Retrieves program parameters.	Active program number selected is called up for use.

8. RAISE and LOWER

Function	Result/Display
Changes value of selected pulse parameter.	Active pulse parameter value is raised or lowered.



9. RF FUNCTION Dial

The RF Function dial allows the user to toggle between the various parameters which can be set for RF electroporation. Rotating the knob toggles between the following settings

Function	Result/Display
9A. OFF	RF Module is turned off. Display on RF module is OFF and Gene Pulser II main unit display is .000 if Capacitance rotary switch is in High Cap. position.
9B. TOTAL VOLTS	Range = 5–400 V. The selectable voltage range.
9C. % MODULATION	Range = 0–100%. The percentage of the wave which will equal the peak-peak amplitude of the sine wave (see Figure 6).
9D. DC AMPLITUDE	Range = 5–400 V. Either total volts or % modulation must be set for value to be displayed. This value cannot be set but is for information only. It reflects the changes in % modulation and total volts settings. The value is the DC amplitude (DC component) upon which the AC component is based. The value is calculated from the following equation: $\text{DC Amplitude} = \text{Total V} \times \left(1 - \frac{\%\ \text{modulation}}{200}\right)$ When % modulation = 0, the negative term becomes 0; the result is a square wave (see Figure 6) and the DC Amplitude = AC voltage. When % modulation = 100, the negative term becomes one-half of the first term; the result is a sine wave positioned between 0 and the set (AC) voltage; the DC Amplitude = 0.5 x AC voltage (see Figure 4C).
9E. PROGRAM NUMBER.	For programming up to 30 combinations of parameters (see the following section).
9F. FREQUENCY	Range = 5–50 kHz. The frequency (cycles/sec, or Hz) of oscillations within a pulse.
9G. BURST DURATION	Range = 0.1–100 msec. The length of a burst.
9H. NUMBER OF BURSTS	Range = 1–25. The number of RF bursts that occur when the two pulse buttons are pressed.
9I. BURST INTERVAL	Range = 0.1–10 sec. The time between RF bursts.
9J. TIMER	Range = 1–999 sec. A repeating and auto-resetting timer for queuing. Set using Raise and Lower keys. Displayed when Function Select key is pressed and RF Function dial set to Timer.

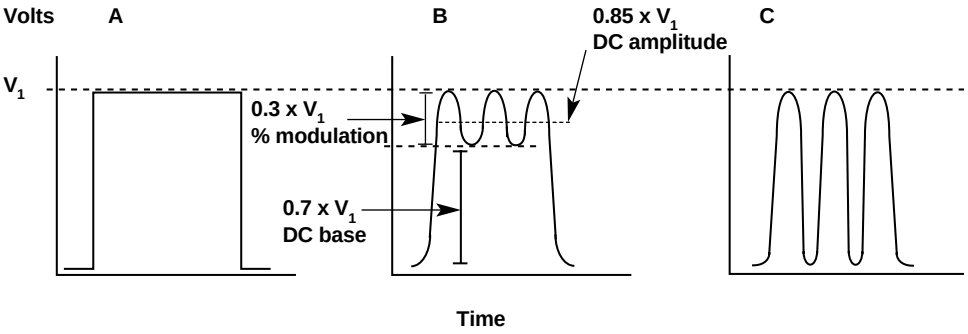
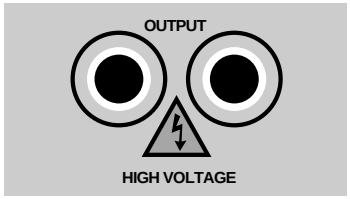


Fig. 6. Effect of changing percent modulation on the output waveform. A. 0% modulation produces a square wave. **B.** 30% modulation produces a sine wave with a peak-peak voltage during the pulse, 30% lower than the selected voltage and a DC base that is 70% of the selected voltage. The DC amplitude is the midpoint of the selected modulation over the DC base (0.7 + .15 = .85 in this example). **C.** 100% modulation produces a sine wave with a maximum voltage at the selected voltage and a minimum voltage of 0 V during the pulse (the DC base is 0 V).

10. Shocking Chamber Output Jack



Function	Result/Display
Shocking chamber output jack.	Used as receptacle for red/black leads of shocking chamber when the RF module is used for electroporation.

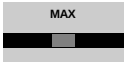
11. LED Displays

Function	Result/Display
LED (Light Emitting Diode) display.	Displays RF module values and function indicators (3 digit). Defaults to 001 when RF Function Select is set to any position other than off and unit is turned on.

12. WAIT Indicator Light

Function	Result/Display
Indicates wait in circuitry.	Part of circuitry protection system. Activated when voltage changed or unit initially turned on. Pulse not delivered when active.

13. MAX Indicator Light



Function	Result/Display
Future function.	Not active.

Section 4
Operation

4.1 Activating the RF Module

1. Turn on the Gene Pulser II unit and set the Capacitance rotary switch to HIGH CAP. The LED display should read .000.
2. Turn on the RF module. The instrument should beep; the LED display should momentarily flash OL-, then OFF if the RF Function dial is set to Off, or 001 if the RF Function dial is set to any other position. Set the RF Function dial to any position except Off.
3. Press the Output buttons on the Gene Pulser II unit; the LED display should indicate rF-; the RF module is now functional.

4.2 Instructions for Using the RF Module

1. Press the Function Select keypad. The LED display over the Function Select key should light. Rotating the RF Function dial will display the settings which have been entered for each of the parameters.
2. Rotate the RF Function dial to set each of the parameters. Change the chosen parameter by using the Raise and Lower keypads. For example, with the RF Function dial set on Total Volts, use the Raise and Lower keypads to select any voltage between 5 and 400 V. Rotate the RF Function dial to % MOD and set this value using the Raise and Lower keypads. In a similar manner, rotate the RF Function dial to RF Freq, Burst Dur, No. Brst, and Burst Interv, setting each parameter using the Raise and Lower keypads.

Parameter	Range
Total Volts	5–400 V
% Mod (Percent modulation)	0–100%
RF Freq (RF frequency)	5–50 kHz
Burst Dur (Burst duration)	0.1–100 msec
No. Brst (Number of bursts)	1–25
Burst Interv (Burst interval)	0.1–10 sec

3. Insert the leads of the shocking chamber into the output jacks on the front of the RF module.
4. Place the cell/DNA suspension at the bottom of the electroporation cuvette between the aluminum plates.
5. Insert the cuvette into the cuvette slide. Push the slide into the shocking chamber until the cuvette makes firm contact with the chamber electrodes.
6. Press both Pulse buttons simultaneously and hold them until the tone sounds. Release both Pulse buttons when the tone sounds.
7. Remove the cuvette from the chamber and immediately add the appropriate outgrowth media to the cuvette. Resuspend the cells with a transfer or Pasteur pipette and transfer to an appropriate tissue culture dish.

4.3 Instructions for Using the RF Module Programming Feature

Setting up and storing a program

Up to 30 combinations of parameters can be pre-programmed into the RF module for future use. To program the parameters, activate the RF module.

1. Press the Function Select key. The LED display over the Function Select key should light. The display should indicate the value of the parameter selected as shown by the position of the RF Function dial.
2. Rotate the RF Function dial to set each of the parameters. Change the chosen parameter by using the Raise and Lower keypads. For example, with the RF Function dial set on Total Volts, use the Raise and Lower keypads to select any voltage between 5 and 400 V. Rotate the RF Function dial to % MOD and set this value using the Raise and Lower keypads. Likewise, set the RF Freq, Burst Dur, No. Brst, and Burst Interv.
3. Verify that all parameters are as desired for the program to be stored by rotating the dial to display each of the parameters.
4. Press the Program key. The LED over the Program key should light and the Program number should be displayed. The Wait LED may light for a few seconds then go out. Program functions are not available when the Wait LED is on. The Wait LED will go out when the capacitors finish charging. If the Wait LED remains lit more than a few seconds, make sure that rF- is displayed on the Gene Pulser II display LED.
5. Use the Raise and Lower keys to select the desired program number (1–30) where the program verified in Step 3 is to be stored. Storing a program will overwrite all existing parameters previously stored at the selected location.
6. Press the two Store Program keys. The RF Module should beep; an S and the program number should be displayed on the LED. For example, when the parameters are stored in Program 1, the LED will display S01. The program has now been stored and can be verified or retrieved later with the Use Program function.

Using a stored a program

1. Press the Program key. The LED over the Program key should light. The default Program Number displayed in the LED display is always 001.
2. Use the Raise and Lower keys to select the desired program number (1–30). The active program number is shown in the LED display.
3. Press and hold down the two Use Program keys. (Note: the left key should be pressed at the same time or slightly before the right key to avoid activating the Program function.) This activates the Use Program function. A "U" will be displayed in front of the program number and the RF Module will beep. For example, when Program 1 is activated, the LED will display U01. The Wait LED may light for a few seconds then go out. Program functions are not available when the Wait LED is on. The Wait LED will go out when the capacitors finish charging. If the Wait LED remains lit more than a few seconds, make sure that rF- is displayed on the Gene Pulser II display LED.

4. To check the functions programmed, press the Function Select key. The Function Select LED should light. The display should indicate the value of the parameter selected, which is indicated by the position of the RF Function dial. Rotate the dial to verify each parameter in the program.
5. Place the cell/DNA suspension at the bottom of the electroporation cuvette between the aluminum plates.
6. Insert the cuvette into the cuvette slide. Push the slide into the shocking chamber until the cuvette makes firm contact with the chamber electrodes.
7. Press both Pulse buttons simultaneously and hold them until the tone sounds. Release both Pulse buttons when the tone sounds.
8. Remove the cuvette from the chamber and immediately add the appropriate outgrowth media to the cuvette. Resuspend the cells with a transfer or Pasteur pipette and transfer to an appropriate tissue culture dish.

Clearing a stored program

Use the Clear Program function to simultaneously remove all parameters from a program. After clearing a program, the LED display indicates 000 for all parameters in that program. Therefore, this feature is useful when re-programming the RF module to be sure that none of the parameters used in the previous program are inadvertently retained.

1. Press the Program key. The LED over the Program key should light. The current Program Number selected is displayed. The Wait LED may light for a few seconds then go out. Program functions are not available when the Wait LED is on. If the Wait LED remains lit more than a few seconds, make sure that rF- is displayed on the Gene Pulser II display LED.
2. Use the Raise and Lower keys to select the desired program number (1–30). The active program number is shown in the LED display.
3. Press the two Clear Program keys on the front panel. A "C" will be displayed in front of the program number and the RF module will beep. For example, when Program 1 is activated, the LED will display C01. Program 1 has now been cleared. The next time Program 1 is used (by pressing the two Use Program keypads after selecting Program 1) and the Function Select keypad is pressed, upon rotating the RF Function dial all of the parameters should be 000 on the LED display.

Section 5
Setting Up an Experiment

The RF Gene Pulser module must typically be used with high-resistance buffers (□80 □). However, for low voltage applications, PBS and similar high ionic strength buffers may be used at voltages between 5 and 100 V. For mammalian cells, the most commonly used high resistance buffers are phosphate buffered sucrose and HEPES buffered sucrose. Suggested formulations for these buffers are given in Section 8.

5.1 Parameters to Optimize

The parameters to optimize for electroporation using the RF Gene Pulser module are voltage, burst duration, percent modulation, radio frequency, number of bursts, and burst interval. Of these, the voltage and burst duration are the principal parameters to optimize. Conditions for electroporation of mammalian cells are generally in the range of 200 V, 2 msec burst duration, 100% modulation, 40 kHz, 5 bursts, 1 sec burst interval. In initial experiments to optimize electroporation, keep the percent modulation, radio frequency, number of bursts, and burst interval constant as above and determine the optimum voltage at several burst durations above and below 2 msec; as burst duration is increased, the voltage resulting in optimum electroporation will decrease. In a similar manner, optimize percent modulation, radio frequency, number of bursts, and burst interval.

Initial experiments estimating cell viability (or cell killing) 24 hr post-electroporation may be performed to identify the range in which to test the parameters. Optimum RF electroporation generally results in 40–80% cell killing.

Finally, conditions identified above for RF electroporation were performed in 0.2 cm cuvettes with 0.1–0.2 ml of PB Sucrose.

Optimization Strategy with the RF Module

Function (Settings)	Range	Optimization Strategy
Total volts	5–400 V	Vary voltage in 0.2 cm cuvette for field strength of 0.7–1.4 kV/cm
% Mod (Percent modulation)	0–100%	100%
RF Freq. (Radio frequency)	5–50 kHz	40–50 kHz
Burst Dur. (Burst duration)	0.1–100 msec	1 msec
No. Brsts (Number of bursts)	1–25	5–10 (repeat 1–2 times)
Interv. (Burst interval)	0.1–10 sec	100 msec

Example Cell Types

Reported by Dr. Donald Chang, Hong Kong University, 8/96 developed with the Gene Pulser II/RF module system.

Section 6
Instrument Diagnostics and Troubleshooting

1. Err is displayed on the LED display when the power is turned on.

   Turn off power, turn on power. If Err is still displayed, contact Bio-Rad,

2. Continuous, rapid beeping.

Check that the LED display on Gene Pulser II panel indicates rF-. If not, press both pulse buttons on the Gene Pulser II unit. If Gene Pulser II display does indicate rF-, press both pulse buttons on the RF module. If beeping resumes, contact Bio-Rad.

Section 7
Specifications and Product Information

7.1 System Specifications

Gene Pulser II RF Module

Input voltage	100/120 V RMS, 50/60 Hz
	220/240 V RMS, 50/60 Hz
Max input current (inrush)	15 amp RMS
Maximum output voltage and current	400 V at 6 amp peak (normal load); limited to 6 A during arc.
Output waveform	Combination of sine wave w/DC level and square wave user adjusted
Ambient operating environment	Temperature 0–35 °C
	Humidity 0–95% without condensation
Regulatory	Passes requirements of EN 61010. In addition, the system passes requirements for FCC, Class A.
Dimensions	24 x 31 x 9.7 cm
Weight	3.7 kg

7.2 Product Information

Catalog Number	Product Description
165-2105	**Gene Pulser II Apparatus,** 100/120 V, 50/60 Hz; includes 1 shocking chamber, 15 sterile sample cuvettes (five 0.1 cm gap, five 0.2 cm gap, and five 0.4 cm gap), 1 cuvette rack
165-2106	**Gene Pulser II Apparatus,** 220/240 V, 50/60Hz; includes 1 shocking chamber, 15 sterile sample cuvettes (five 0.1 cm gap, five 0.2 cm gap, and five 0.4 cm gap), 1 cuvette rack
165-2107	**Capacitance Extender II,** 25–1,075 µF range measured by Gene Pulser II apparatus; includes integrated leads
165-2108	**Capacitance Extender PLUS,** 25–3,275 µF range measured by Gene Pulser II apparatus; includes integrated leads
165-2109	**Pulse Controller II,** 100-1,000 ohm range, seven settings (100, 200, 400, 600, 800, 1,000, infinity ohms); includes integrated leads
165-2110	**Pulse Controller PLUS,** 50–1,000 ohm range, twelve settings (50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, infinity ohms); includes integrated leads
165-2111	**Gene Pulser II /E.coli Pulser™ Shocking Chamber**
165-2112	**Gene Pulser II RF Module,** 100/120 V
165-2113	**Gene Pulser II RF Module,** 220/240 V
165-2095	**Gene Pulser Cuvette Rack**
165-2094	**Gene Pulser Electroprotocols**
165-2088	**Gene Pulser Cuvettes,** 0.4 cm electrode gap, package of 50, sterile
165-2086	**Gene Pulser/E. coli Pulser Cuvettes,** 0.2 cm electrode gap, package of 50, sterile
165-2089	**Gene Pulser /E. coli Pulser Cuvettes,** 0.1 cm electrode gap, package of 50, sterile

Section 8
Buffer Preparation

Samples to be electroporated using the RF module must be in high resistance media. Sugars such as sucrose and mannitol are usually used to maintain the correct ionic strength for mammalian cells. The following buffers have been previously used for RF electroporation.

HEPES buffered media (Zheng and Chang, 1991)

2 mM HEPES

15 mM K phosphate buffer

250 mM mannitol

1 mM $MgCl_2$

Final pH = 7.2

Phosphate buffered sucrose media (Chang, 1989)

27 mM Na phosphate, pH 7.5

150 mM sucrose

PB-Sucrose

272 mM sucrose

7 mM Na phosphate, pH 7.4

1 mM $MgCl_2$

Section 9
References

Chang, D. C., Cell poration and cell fusion using an oscillating electric field, *Biophys. J.*, **56**, 641-652 (1989).

Zheng, Q. and Chang, D. C., High efficiency transfection by *in situ* electroporation of cultured cells, *Biochim. Biophys. Acta,* **1088**, 104-110 (1991).

Arnold, W. M., Gessner, A. G., & Zimmermann, U., Dielectric measurements on electro-manipulation media, *Biochim Biophys Acta*, **1157**, 32–44 (1993).

Chang, D. C., Gao, P. Q., & Maxwell, B. L., High effiency gene transfection by electroporation using a radiofrequency electric field, *Biochem Biophys Acta*, **1092**, 153–160 (1991).

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