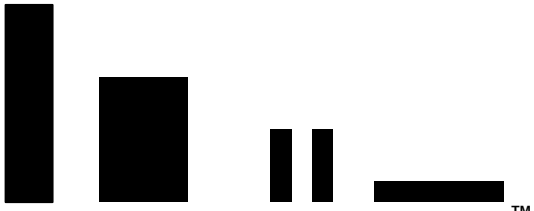

Cyto Pulse Sciences, Inc.™

Model PA-301

Pulse Booster™

User Manual

Cyto Pulse Sciences, Inc.
P. O. Box 609
Columbia, MD 21045

1-410-787-1890
1-410-787-1891 FAX
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PA-301 Pulse Booster

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The equipment is not approved by the FDA for use with in vitro or in vivo diagnostics or therapy.

The information in this manual is subject to change without notice.

PA301UMANrev.1-11/04 Price $100.00

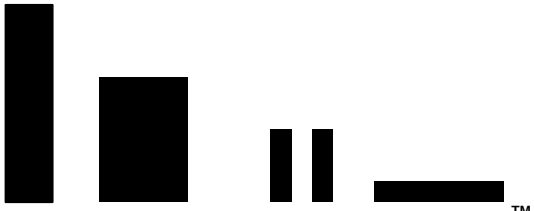

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Table of Contents

List of Figures

Caution Notice

The PA-4000 to be connected to this instrument contains a high voltage power supply that can be adjusted to beyond 1,000 volts. Additionally, the PA-301 can output a pulse of 3,000 volts. Such voltages can be lethal.

The user must read this manual carefully before the instrument is placed into operation.

Removing the cover may void the warranty.

Do not connect or disconnect the high voltage cable with the high voltage enabled. To connect or disconnect the cable, turn line/mains power off and unplug line cord.

Do not open the cuvette holder while the high voltage is on. If a problem occurs during a run, push the **STOP/RESET** button on the front panel.

If there are any questions about the operation of this instrument, call Cyto Pulse Customer service.

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1. Introduction

The PA-301 Pulse Booster™ add-on to the PA-4000 computer-controlled electroporator provides the ability to establish higher electric fields required for smaller prokaryotic cells. All pulse parameters are tunable to give the operator maximum flexibility in performing protocols.

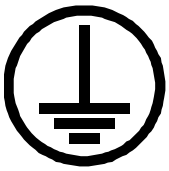
Summary of PA-4000/PA-301 Pulse Booster™ System Pulse Specifications

Pulse Amplitude	300 to 3000 V, in 15 V steps
Pulse Width	0.001 to 0.600 ms, in 0.001 ms steps 0.100 ms maximum at 3000 V
Minimum Load	200 ohms

This manual has been designed to help you realize the maximum benefit from using the PA-4000/PA-301 ***PulseAgile***® Pulse Booster™ system. It contains information on how to operate the electroporator, safety tips, and applications.

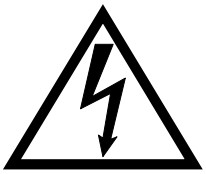
Note: The PA-4000 with the PA-301 contains a high voltage power supply and was designed with safety features to protect the user and the equipment. If used properly, the PA-4000 with the PA-301 is a safe and reliable instrument. Chapter 2 explains some important concepts related to operator safety in addition to concepts needed for accurate use of the instrument. Chapter 2 must be read before setting up this instrument. Our goal is the safe and productive use of the PA-4000 with the PA-301.

Back Panel Symbols

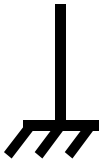


Protective Terminal Conductor

Caution: Refer to documentation



Caution: Risk of electric shock



Chassis Ground

The PA-4000, with the optional PA-301, is rated for operation with line/mains voltage of 100-240 VAC, maximum current of 2 amps, at 50-60 Hz. The AC mains power supply cord is the disconnect device for this product. The power supply cord shall be a Type SJT, rated 300 Volts AC, 18 AWG, 105° C, 3-conductor including ground.

This unit is rated for environmental conditions of 5-40°C, 80% relative humidity to 31°C, decreasing linearly to 50% relative humidity at 40°C, altitude to 2000 meters.

There are *no* operator replaceable parts inside the system; Cyto Pulse recommends that the user *not* remove the cabinet covers.

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2. PA-4000/PA301 Set-Up

2.1 Introduction

The PA-301 Pulse Booster™ is an optional attachment to the PA-4000 ***PulseAgile***® Electroporator. It is a transformer that boosts the pulsed voltage output of the PA-4000 by a factor of three. Subsequently, the pulsed electric field produced in a cuvette is 3-times that produced by the PA-4000 alone. For example, electric field strengths of up to 7500 V/cm are capable in a standard 4 mm cuvette. The complete Pulse Booster™ system consists of:

PA-4000	Electroporator
PA-301	Pulse Booster™
PA4-SW	***PulseAgile***® Software
CE-20	Cuvette Holder
CS-LN-__	Line Cable set
CS-OPT	Interface Cable Set
CUV-M	Cuvette Multi-Pack
PA4-UMAN	PA-4000 User Manual
PA301-UMAN	PA-301User Manual

Laptop shown is not included.

Figure 2-1: The PA-4000/PA-301 Pulse Booster™ Electroporation System

2.2 Connecting the PA-301 to the PA-4000

The PA-301 should be placed on top of the PA-4000. The Mains/Line Power Switch must be off and the Mains/Line Power Cord must be unplugged from the PA-4000. Referring to Figure 2-2, make the following connections:

Figure 2-2: Back Panel Connections PA-4000/PA-301

- Connect the Option Interconnect cable between the DB25 jacks on the back of the PA-301 and PA-4000.

!DO NOT ATTACH TO A COMPUTER PARALLEL PORT OR A PRINTER!

- Connect the high voltage cable between the MHV jack on the back of the PA-4000 and the MHV jack labeled *Pulse In* on the back of the PA-301. This cord delivers the pulses generated by the PA-4000 to the PA-301.

!DO NOT USE A CABLE WITH BNC PLUGS!

- Connect the HV cable with the MHV plug from the CE-20 to the MHV jack labeled *Pulse Out* on the back of the PA-301.

!DO NOT CONNECT A PA-96W TO THIS JACK!

- Connect the Cuvette Interlock cable from the CE-20 to the RCA-type phono jack on the back panel of the PA-4000.

2.3 PA-301 Front Panel Display

The PA-301 front panel display consists of two light-emitting diodes (LED). Both of the LEDs will light when the PA-301 is properly connected to the PA-4000 and line/mains power is turned on. The *Interface* LED indicates that the DB25 interface cable is installed and communication between the PA-4000 and the PA-301 has been established. The *Power* LED indicates that the PA-301 line/mains cord is plugged into the back of the PA-4000 and that the PA-301 is receiving power.

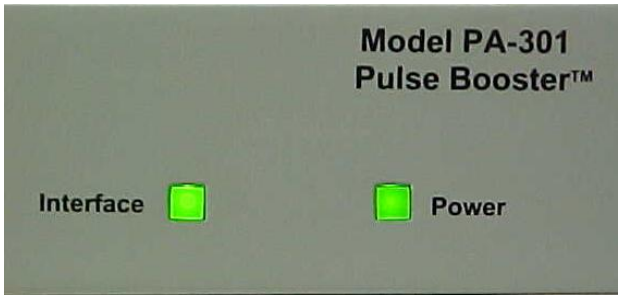


Figure 2-3: PA-301 Front Panel Display

2.4 Power Up

With all cables connected, including the serial communication cable from the host computer, turn on line/mains power and start the ***PulseAgile***® software. The set-up of the system is now complete. The *Getting Started* chapter will give instruction on the proper operation of the Pulse Booster™ System.

3. Getting Started with the PA-301

3.1 Introduction

The PA-301 Pulse Booster™ System expands the high voltage pulse amplitude range available to researchers. It is capable of producing rectangular wave pulses up to 3000. A summary of the specifications is provided below. This Chapter will provide details regarding the proper operation of the PA-301 using the PA-4000 ***PulseAgile***® interface software.

3.2 Application Software

The PA-301 application software is a part of the PA-4000 ***PulseAgile***® interface software. It is accessible only when a PA-301 is connected using the DB25 interface cable as described in Chapter 3. After installing the PA-301, close the Cuvette Holder handle, turn on the PA-4000, and start the ***PulseAgile***® software. The screen should appear as shown in Figure 3-1. In the *Options Connected* area, look for a check mark in the box labeled 3:1 Transformer. If there is no check, then the DB25 interface cable is not connected properly. Power down the system and check the cable. If the problem persists, then call Cyto Pulse Sciences Customer Service.

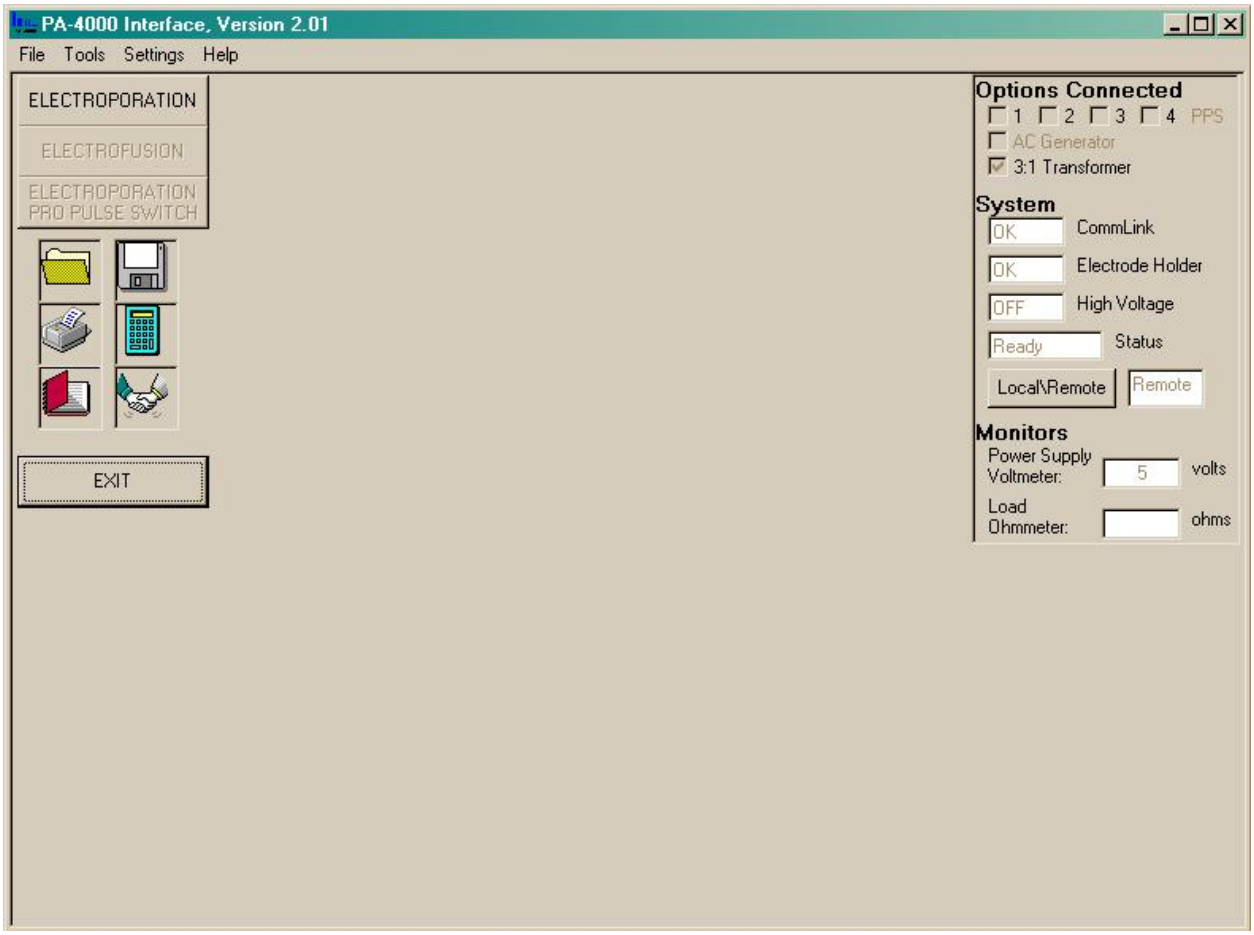


Figure 3-1: *PulseAgile*® Opening Screen showing PA-301 Pulse Booster™ attached

3.2.1 Electroporation Protocol Screen

Click *Electroporation* and the screen should now appear as shown in Figure 3-2. The default pulse parameters are shown in the various boxes. Details regarding programming protocols were given in Chapter 4 of the PA-4000 User Manual, so this chapter will only cover the software functions specific to the use of the PA-301.

3.2.2 Protocol programming

There are several differences between programming a Pulse Booster™ protocol and a standard PA-4000 protocol. Specifically, the minimum and maximum allowable pulse amplitudes are different, and the maximum allowable pulse width varies with pulse amplitude range. Additionally, there is no *Low Range/High Range* select; the PA-4000 reservoir capacitor is fixed to the *High Range* value by default.

3.2.2.1 Programming Pulse Amplitude

The minimum pulse amplitude for the Pulse Booster™ system is 300 volts. As an exercise try this:

- Click on the Pulse Amplitude parameter box.
- Change the value to 100 volts.
- Click *Replace*
- Note how the Pulse Amplitude defaults back to 300 volts

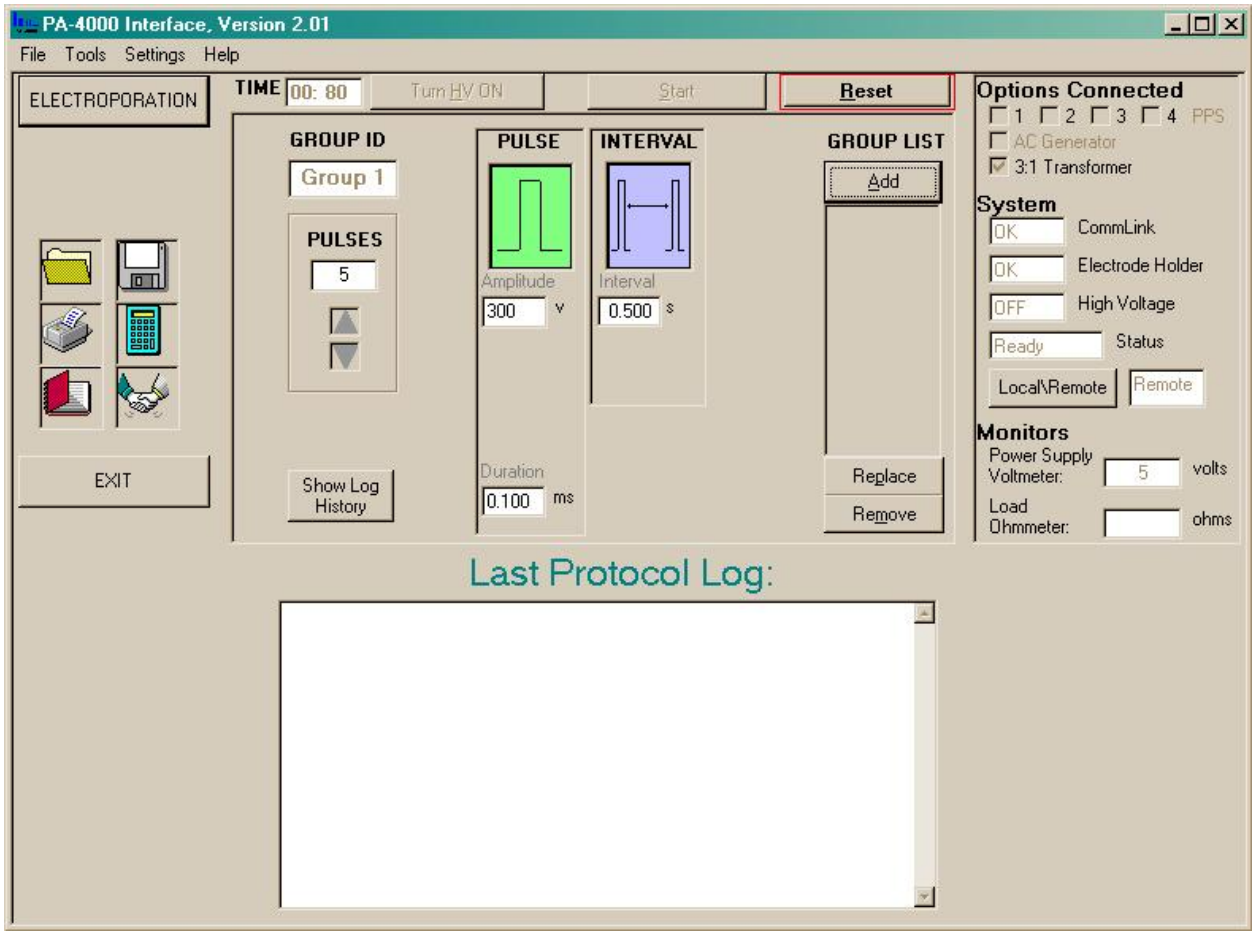


Figure 3-2: *PulseAgile*® Electroporation Protocol Programming Screen

The maximum pulse amplitude for the Pulse Booster™ system is 3000 volts. So now try this:

- Click on the Pulse Amplitude parameter box.
- Change the value to 3100 volts.
- Click *Replace*
- Note how the Pulse Amplitude defaults to 3000 volts

The minimum voltage step achievable is 15 volts (for a standard PA-4000 it is 2 volts in *Low Range* and 5 volts in *High Range*). Here is an exercise to demonstrate this:

- Click on the Pulse Amplitude parameter box.
- Change the value to 310 volts.
- Click *Replace*
- Note how the Pulse Amplitude defaults to 300 volts
- Change the value to 320 volts.
- Click *Replace*
- Note how the Pulse Amplitude defaults to 315 volts

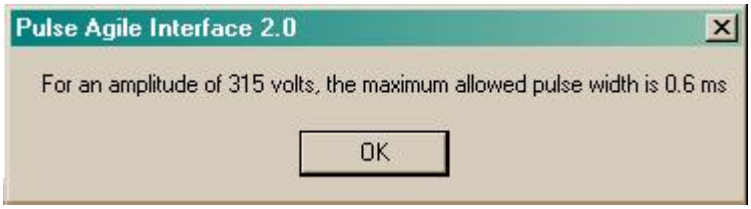
3.2.2.2 Programming Pulse Width

The maximum allowable pulse width in the PA-301 Pulse Booster™ System varies according to the desired pulse amplitude. This insures that all pulses delivered meet Cyto Pulse Sciences standard of pulse droop of ≤5%. The table below gives the maximum pulse width allowed for ranges of pulse amplitude settings.

Pulse Amplitude volts	Maximum Pulse Width ms
300 - 585	0.600
600 - 795	0.500
810 - 1095	0.300
1110 - 1590	0.200
1605 - 2190	0.150
2205 - 3000	0.100

If values outside the above ranges are entered, the software will display an error message window, and then set the pulse width to the maximum allowed value as demonstrated here:

- Click on the Pulse Width parameter box.
- Change the value to 1.000 ms
- Click *Replace*
- The following Message Box should display:



- Click OK and note how the Pulse Width is now set to 0.600 ms.

3.2.3 Run a protocol, PA-301Test.pro

Before running this protocol, check that there is a cuvette installed in the cuvette holder and the handle is closed. For this test, an empty 4mm or greater cuvette is recommended.

- Open the protocol file *PA-301Test.pro* located in the /protocol folder.
- Click *Turn HV On*.
- After the system charges, note that the Power Supply Voltmeter reads 1000 volts, **not** 3000 volts. This is the case because the PA-4000 is going to deliver a 1000 volt pulse to the PA-301, which in turn will boost the pulse voltage by a factor of three, subsequently delivering a 3000 volt pulse to the cuvette.
- Click *Start*.
- The log report should look something like that shown in Figure 3-3.

```
Protocol File: PA-301Test.pro
12-7-2004-3 13:59:40
Mode: Electroporation w/ 3:1 Transformer
Monitor Voltage x 3

>GRP    NUM     WIDTH    INTVL    SetV     MonV
> 1       1     0.100     0.50     1000     1000

>GRP    NUM     WIDTH    INTVL    SetV     MonV
> 2       1     0.200     0.50      500      500

>GRP    NUM     WIDTH    INTVL    SetV     MonV
> 3       1     0.400     0.50      250      255

>GRP    NUM     WIDTH    INTVL    SetV     MonV
> 4       1     0.600     0.50      125      130

>Estimated load > 100 ohms
>Estimated conductance <   0.001 siemens
$
```

Figure 3-3: *PA-301Test.pro* Protocol Log Report

It is important to note that when programming a protocol, the user will enter the pulse amplitude of the waveform delivered to the sample. That is, the pulse out of the PA-301 and to the test sample. The ***PulseAgile***® software, however, is monitoring the voltage set inside the PA-4000, which will be one-third of the output pulse. This concept is illustrated in Figure 3-4.

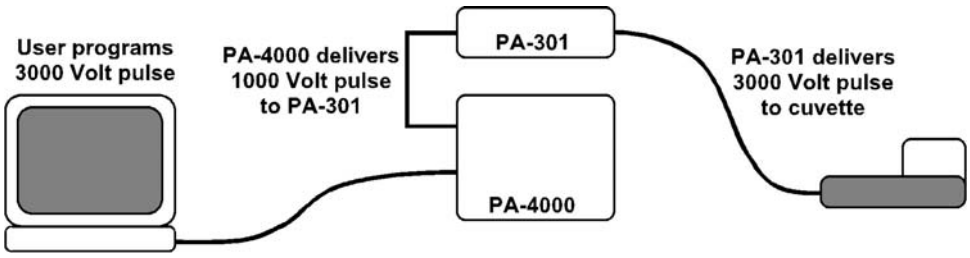


Figure 3-4: Relationship among User-programmed volts, PA-4000 volts, and PA-301 volts

4. Applications

4.1 Introduction

The general principles for electroporation of prokaryotic organisms are the same as those described for eukaryotic organisms in the PA-4000 User Manual. However, the smaller size of the prokaryotic cells and the presence of a cell wall around many prokaryotic cells, present challenges to efficient transfection through electroporation.

4.2 Electric Field Intensity

Electric field intensity is the most important pulse parameter determining electroporation efficacy. Since the required electric field is inversely proportional to cell diameter, smaller cells require higher electric fields. There is a wide range of electric field intensity (3-30 KV/cm) reported in the literature for electroporation of small prokaryotic cells (most are for *E. coli*). The majority of protocols use electric fields around 12 KV/cm. In contrast, the electroporation of eukaryotic cells is generally performed at electric field intensities on the order of 1 kV/cm to 2.5 kV/cm.

4.2.1 Rectangular vs. Exponential Decay Waveforms

Most published methods for electroporation of *E. coli* describe exponential decay pulses. The PA-4000 with PA-301 delivers a rectangular wave pulse. There is no direct way to convert an exponential decay pulse protocol to a rectangular wave pulse protocol. For best results, a range of parameters should be tried initially

Here are guidelines that can be followed to assist in making that conversion.

1. Rectangular wave pulses are more precisely controlled than exponential decay pulses. For instance, the pulse width can be fine tuned to avoid overheating.

2. Since rectangular wave pulses are shorter in duration than exponential decay pulses, a higher pulsed electric field may be required. For example, a protocol designed around a 12 KV/cm field delivered by a exponential decay pulser might require that a 20 KV/cm field be used if redesigned for a rectangular wave pulser. The Pulse Booster™ system can produce a field intensity of up to 30 KV/cm in a 1mm cuvette.

3. Multiple rectangular wave pulses may be better than single pulses.

4. Since less heat is generated using rectangular wave pulses, the cells should not be pre-cooled. Electroporation should be done at room temperature.

5. More cells will be viable after using rectangular wave electroporation.

Rectangular and exponential decay waveform electroporation do have some common requirements, however.

- Low conductivity medium should be used to reduce arching and heat
- Electroporation competent *E. coli* (or other bacteria) should be used

4.3 Preparation of Electrocompetent Cells

Bacterial cells need to be prepared for electroporation by making them electrocompetent. Electrocompetent cells can be purchased from companies such as Invitrogen. Alternatively, one of the following procedures can be used.

4.3.1 E. coli[1] and Gluconobacter[2]

1. Grow 1 Liter culture to A_{600} of 0.5. This usually requires growing a small culture overnight from a colony then inoculating two 500 ml cultures on a shaker at 37° C.
2. Add culture to 500 ml centrifuge tubes and cool to 4° C.
3. Centrifuge at 3000 X g for 10 minutes.
4. Discard supernatant and add 250 ml ice cold water or 1 mM HEPES pH 7.0.
5. Centrifuge at 3000 X g for 10 minutes.
6. Discard supernatant and add 40 ml ice cold water or 1 mM HEPES pH 7.0 and transfer to 50 ml conical tube.
7. Centrifuge at 3000 X g for 10 minutes.
8. Discard supernatant and add 20 ml ice cold10% glycerol in water.
9. Centrifuge at 3000 X g for 10 minutes.
10. Aspirate supernatant and combine all pellets in 1.5 ml cold 10% glycerol in water.
11. Aliquot in small amounts (40 to 80 μl).
12. Freeze in liquid nitrogen or an ethanol/dry ice bath.
13. Store at -80° C.

4.3.2 Lactococcus[3]

1. Grow *L. lactis* in GM17, 30° C to A600 of 0.5
2. Dilute cells and grow in SGM17 containing 0.2 to 4% glycine (strain dependent) to A600 of 0.5
3. Centrifuge 5000 X g, 4 °C
4. Discard supernatant and add ice cold10% glycerol in 0.5 M sucrose
5. Centrifuge 5000 X g, 4 °C
6. Discard supernatant and add ice cold10% glycerol in 0.5 M sucrose
7. Centrifuge 5000 X g, 4 °C.
8. Discard supernatant, add 1/100 culture volume of ice cold 10% glycerol in 0.5M sucrose.
9. Use immediately or store at -80 °C.

4.3.3 E. Coli Transformation Example.

The following procedure was performed to transform *E. coli* with a plasmid containing a Kanamycin resistance gene:

1. Prepared culture plates in advance:
   Mix LB agar and water.
   Microwave to boiling.
   Microwave on low 1 minute.
   Cool to 70^{o} C.
   Add Kanamycin if desired (10 μl/ml of 100 μg/ml Kanamycin).
   Pour 13 mL into 100 mm petri dishes and cool to room temperature.

2. Thawed 1 vial of Invitrogen Electromax® DH5α-E cells.

3. Added 2 μL plasmid (2.5 μg/mL to 100 μL cells. Note, as little as 20 μL cells can be used.

4. Added cells to a 1 mm gap cuvette.

5. Applied three pulses: 2000 volts amplitude
   100 μs pulse width
   0.2 second interval
   using a Cyto Pulse Sciences PA-4000/PA-301 Pulse Booster™ system.

6. Added 1 ml SOC medium, mix well and moved to Falcon™ tube.

7. Prepared 10 fold dilutions.

8. Plated dilutions by adding 5 μl cell suspension to culture plate with Kanamycin and to an identical plate without Kanamycin.

9. Incubated 18 hours 37^{o}C.

10. Counted colonies and analyzed selected colonies using an Invitrogen Clone Checker® System.

Results: 130,000 transformed colonies were obtained (65 colonies counted at 1:100 dilution assaying 1/20 of the cell suspension)

References:

1. Nickoloff (editor), Methods in Molecular Biology Vol 47, Miller, EM, Nickoloff, JA, Chapter 8, Echerichia coli Electrotransformation,1995, 105-113
2. Mostofa, HE, Heller, KJ, Geis, A, Cloning of Escherichia coli lacZ and lacY genes and their expression in Gluconobacter oxydans and Acetobacter liquifaciens, Applied and Environmentqal Microbiology, 2002, 68(5):2619-2623
3. Nickoloff (editor), Methods in Molecular Biology Vol 47, Chapter 19, Transformation of Lactococcus by electroporation, 1995, 195-199

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5. Customer Service

5.1 Limited Warranty

CYTO PULSE products are warranted against defect in materials and workmanship. If the customer provides notice of such a defect during warranty period, CYTO PULSE, at its option, will either repair or replace the products, which were found to be defective. The limited warranty set forth above is exclusive and no other warranty whether written or oral, is expressed or implied. CYTO PULSE specifically disclaims implied warranties of merchantability and fitness for a particular purpose.

EXCEPT AS SET FORTH ABOVE, CYTO PULSE MAKES NO WARRANTY WITH RESPECT TO THE PRODUCT, AND IN NO EVENT, REGARDLESS OF CAUSE, SHALL CYTO PULSE BE LIABLE FOR INDIRECT, SPECIAL, OR CONSEQUENTIAL DAMAGES OR OTHER LOSSES OF ANY KIND ARISING FROM BREACH OF WARRANTY OR OTHER USES OF THIS PRODUCT. CYTO PULSE'S OBLIGATION TO REPAIR OR TO REPLACE TO THE EXTENT SET FORTH ABOVE CONSTITUTES THE EXCLUSIVE REMEDIES OF THE CUSTOMER FOR ANY BREACH OF WARRANTY.

This warranty shall not apply to products, which after inspection by CYTO PULSE, were found to be improperly used or to have been modified in any manner. CYTO PULSE recommends that the user not open the product cabinet. This limited warranty is valid for two years from the date of shipment.

5.2 Customer Service

If the user believes that there is a defect in the CYTO PULSE product, the customer should contact CYTO PULSE Customer Service by e-mail at customer@cytopulse.com or phone 410-787-1890, or contact the local CYTO PULSE representative. A determination if the product is still in warranty will be made. If the warranty period is still in effect, the user will be given an authorization number (RMA) to return the product. If after receipt and inspection the product is found to be defective, it will be replaced or repaired and returned to the customer. If the product is found to have been modified or misused, the user will be given a quote for repair. If the warranty period has expired and the user requests repair, CYTO PULSE will inspect the product and provide a written quote for repair. The user must provide a purchase order number before the product will be repaired. If the unit is damaged in shipment, the user must recover the insured value to replace or repair from the carrier.

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