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**Appendix A:** ELECTRONIC GENETICS® DATABASE service
**Appendix B:** Electroporation Protocols
**Appendix C:** Technical Literature

# 1. UNPACKING AND INSTALLATION

## 1.1 UNPACKING

The shipping carton in which your *ElectroSquarePorator* (T820) is packed has been specifically designed to provide maximum protection to the instrument during transportation and normal handling conditions. Upon receipt, the carton should be examined and if necessary, inform BTX in writing of any damage resulting from shipping.

Open the carton, carefully remove the T820 and inspect the unit for any apparent damage. (Save the carton and packing materials for future transportation and shipping requirements.)

## 1.2 PACKING DATA

Check the packing slips to ensure that all items ordered and listed are included in the shipment. Inform BTX immediately if any parts are missing.

## 1.3 POWER SOURCE

As received, the instrument is ready for use with either a 105-125, 60 Hz or a 220-240, 50 Hz VAC power source. For conversion from one to the other contact BTX.

**For safety reasons, it is mandatory that the instrument is grounded.**

## 1.4 INSTALLATION

Once you have determined that the components of the T820 have not sustained any damage in shipment, proceed with the installation. The location of the T820 should be on a dry, level surface, free from extremes in ambient temperature, dust or chemical exposures. Unpack cables, any chambers and accessories and refer to "OPERATION" for further instructions.

# 2. TECHNICAL DESCRIPTION

## 2.1 SIZE AND WEIGHT OF T820 GENERATOR

| SIZE | Width | | Height | | Depth |
|---|---|---|---|---|---|
| | 13" | x | 7" | x | 13.5" |

WEIGHT 27 lbs.

## 2.2 ELECTRICAL DESCRIPTION

### 2.2.1 ELECTRICAL INPUTS AND OUTPUTS

Power: 105-125 VAC, 2.5 Amp slow-blow
220-240 VAC, 1.25 Amp slow-blow

| | |
|---|---|
| Low voltage mode: | LOW VM |
| Electroporation pulse amplitude: | 50-500 V peak |
| Pulse length: | 0.3-99 msec |
| | |
| High voltage mode: | HIGH VM |
| Electroporation pulse amplitude: | 100-3000 V peak |
| Pulse length: | 5-99 µsec |
| | |
| Number of Pulses: | 1-99 |
| Interval Between Pulses: | 1 sec |

## 2.3 FRONT PANEL CONTROLS

| NAME | FUNCTION |
|---|---|
| CONNECT TO SELECTED MODE | This connects the safety stand to the HV or LV mode output. |
| MODE | Use the switch to choose between High Voltage mode (HV) 100-3000 volts and Low Voltage mode (LV) 50-500 volts |
| SET CHARGING VOLTAGE | Sets the desired amplitude 50-3000 volts depending on the mode |
| PULSE LENGTH | 1-99 msec in LV mode, 5-99 µsec in HV mode<br>* In LV mode when setting the pulse length at 0, the generator delivers a 0.3 msec pulse. |

| | |
|---|---|
| NUMBER OF PULSES | 1-99 |
| PUSH TO START | Initiates charging to the set voltage and delivers a pulse to the sample chamber in an automatic cycle. It takes less than eight seconds to charge and pulse. |

## 2.4 REAR PANEL

The fuse is located in the power entry module. Check the fuse if the front panel lights do not come on when the T820 is connected to the power outlet and the power switch is in the ON (I) position.

The rear panel contains an input module with the Power On switch on the top, the plug for the power cord in the middle and the fuse holder on the bottom.

The BNC coaxial output connector is used for the monitoring of the output voltage in the HV mode. The voltage ratio is 1:100. The output is electrically isolated (floating) from the pulse output terminals.

### 2.4.1 Remote Start Input

Connect the 820 Remote Start Button to the T820 Remote Start Button Input, located in the back panel, using the phone jack cables provided.

# 3. THEORY OF OPERATION FOR THE ELECTROPORATION OF CELLS

## 3.1 THE TRANSFECTION PROCESS

The process of transfecting cells with electric fields (electroporation) is, generally, done in one step. Electroporation is the application of high voltage electric field pulses to a cell sample over a short duration of time in order to create temporary pores or holes in the membranes of cells. This is initiated by one or several unidirectional pulses of high voltage. Each cell type requires individual parameters for optimal transfection yields. The cells are suspended with the DNA fragments or plasmids. During the electroporation pulses, pores open in the cells and the DNA migrates inside the cell.

## 3.2 DIELECTRIC BREAKDOWN - ELECTROPORATION

Cells suspended in liquids can be regarded for our purposes as a structure consisting of a non-conducting membrane with aqueous solutions on both sides. Exposure to an electric field leads to charge separation in the membrane similar to the charge separation in the dielectric layers of an electrical capacitor. This results in a transmembrane potential difference. Opposite electrical charges on the membrane attract each other, exerting a pressure on the membrane, which can cause thinning of the membrane. At a critical potential difference, field strength localized breakdowns occur and pores are formed, allowing a flow of medium or cytoplasm. These pores are generally large enough to allow macromolecules, such as DNA, and small molecules, such as ATP, to enter or leave the cell. As long as the critical field strength and pulse length duration are not exceeded, removal of the field can lead to healing (closing) of the pores. Following closure of the pores, the exogenous material (DNA or other) is then free to enter the nucleus and be transcribed or to become integrated into the host genome and generate a transiently, or permanently, transfected line.

Excessive field strength and/or duration of the pulses can lead to irreversible changes in the cell by damaging intracellular proteins and DNA. Another effect is that the pores generated by the excessive field strength are too large to reseal and the cell lyses.

## 3.3 FUSION

If two cells touch each other during the process of pore formation, there is a good chance that there will be adjacent pores and that channels will form, allowing intracellular exchange of the cytoplasm. The cytoplasm continuity favors the formation of bridges between the membranes of two adjacent cells. This process can lead to the formation of a new spherical hybrid cell. The T820 can be used for cell fusion using the avidin-biotin technique to bring the cells together prior to the electroporation pulse.

# 4. OPERATION OF THE T820 SYSTEM

## 4.1 PROCEDURE

Familiarize yourself with all the front panel controls and read this manual completely before attempting to use the T820.

### 4.1.1 SAFETY INFORMATION

**WARNING HIGH VOLTAGE**

For practical and safety reasons, do not touch any of the cables or electrode connections while the T820 is in operation. The generation of high electric fields in the electroporation chambers require the delivery of large amounts of electrical energy and power. This energy is transmitted via a coaxial cable. Some of the chambers MICROSLIDE P/N 453 (3.2 mm gap) have exposed terminals and use micrograbber terminal clips. Use of a cover slip is recommended with chambers of this type. Check that the unit is not operating and is in standby when connecting and disconnecting cables.

**EXTREME CARE NEEDS TO BE TAKEN TO ENSURE SAFE OPERATION.**

The two main hazards are electric shock to the operator or explosion. The chambers made of plastic or glass can disintegrate if used at the maximum settings. An internal short circuit in the connecting cable can cause an explosion. In both instances flying debris can be generated. For this reason, it is recommended that all electroporation experiments be performed inside a hood. The included coaxial cable is generally long enough the allow the safety stand apparatus to be placed in the hood while leaving the T820 on the lab bench top.

The T820 is engineered and manufactured to strict safety standards and is short circuit proof as a further safety measure for the operator.

The HV mode output is floating. The pulse is delivered through a pulse transformer. The LV mode output is connected directly to the capacitor banks. The potential of both terminals follow the capacitor charging voltage; during the pulse, one terminal is pulled to ground.

While this operating mode is completely safe when performing experiments in chambers in a safety stand, it is not advisable to connect these outputs directly to an oscilloscope.

### 4.1.2 PRECAUTIONS

**DO NOT TOUCH THE OUTPUT OR ANY PART OF THE CHAMBERS WHILE PUSHING THE START BUTTON.**

**DO NOT OPEN THE T820 ENCLOSURE.**

**DO NOT VIEW THE CHAMBERS FROM A CLOSE DISTANCE DURING A DISCHARGE.**

**DO NOT ALLOW UNAUTHORIZED OR UNINFORMED PERSONNEL TO OPERATE THE T820.**

**WEAR SAFETY GLASSES AT ALL TIMES.**

**READ THE OPERATING MANUAL CAREFULLY.**

**CONNECT THE SAFETY STAND CABLE OR COAXIAL CABLE TO THE OUT PUT SO THAT A GOOD ELECTRICAL CONTACT IS MADE.**

**TURN OFF THE POWER SWITCH WHEN THE GENERATOR IS NOT IN USE.**

**NOTIFY BTX CUSTOMER SUPPORT AT (619) 597-6006 OR (800) 289-2465 IF YOU NOTICE ANYTHING UNUSUAL OR HAVE ANY QUESTIONS.**

## 4.2 T820 OPERATING SUMMARY

**1. SET UP**
Plug power cable into a 105-125 VOLTS AC 60 Hz or 220-240 V AC 50/60 Hz outlet.

**2. POWER UP**
Switch T820 POWER to ON (The power switch is located in the rear panel).

**3. CHOOSE MODE**
Choose between HV mode (100-3000 V, 5-99 µsec) and LV mode (50-500 V, 1-99 msec).

**4. SET PULSE LENGTH**
Use the dial to set appropriate PULSE LENGTH.

**5. SET VOLTAGE**
Use SET CHARGING VOLTAGE dial to adjust charging voltage; value is read on meter.

**6. SET NUMBER OF PULSES**
Use the dial to set desired NUMBER OF PULSES.

**7. CONNECT CHAMBER**
Connect appropriate BTX chamber cable to the SELECT MODE OUTPUT Choose between the HV mode (100-3000 V; 5-99 µsec) and LV mode (50-500 V; 1-99 msec). The yellow light indicates the selected output.

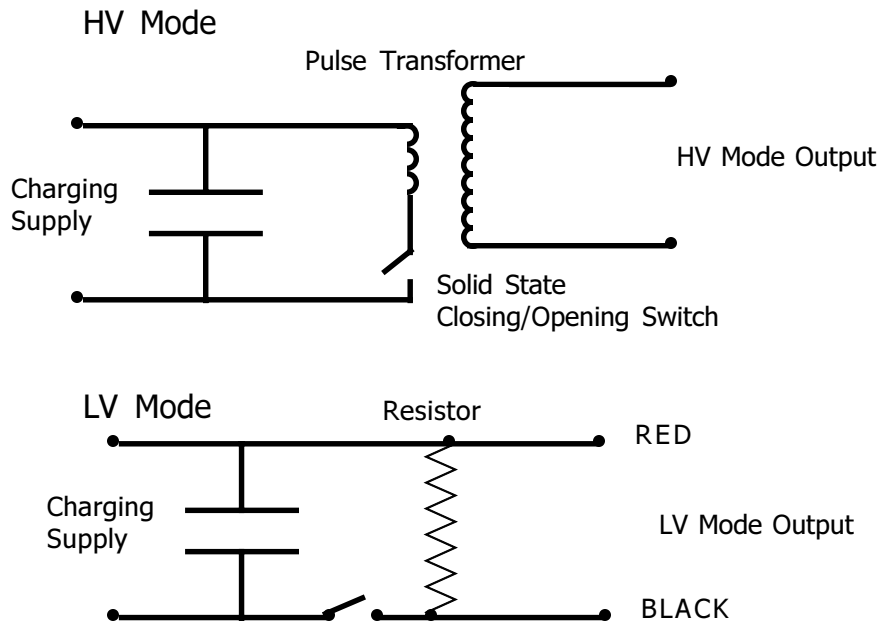
**8. LOAD SAMPLE**
Following protocol, load BTX chamber with cell suspension/reagents. Properly insert chamber into Safety Stand and place in hood.

**9. VERIFY**
Check that all settings and connections are correct .

**10. PULSE**
Press PUSH TO START: T820 charges within 8 seconds and delivers all preset parameters to the chamber. The green light comes on during charging and a beep will be heard when the pulse is delivered. The red light indicates that the capacitor bank is being discharged and the generator reverts to the stand by mode.

## 4.3 OUTPUT CIRCUITS

The T820 delivers pulses with a square pulse shape. This is achieved with an **ON/OFF** solid state switch, partially discharging a large capacitor.




The resulting wave shape is square wave, which generates a well defined field strength over a well defined pulse length.

## 4.4 ELECTRIC FIELD STRENGTH IN THE CHAMBERS

The passage of an electrical current across the cell membrane results in the creation of transient pores which are critical to the transformation process. The generator provides the voltage (in kV) that travels across the chamber gap (in cm) between the two parallel plates of the electrodes. This potential difference defines what is called the electric field strength where E=kV/cm. Each cell species has its own critical field strength for optimum transformation. This appears to be due to cell size, membrane makeup and individual characteristics of the wall itself. Generally, the required field strength varies inversely to the size of the cell.

The internal impedance (Ri) of the T820 generator is very low, about 2 Ohm in the HV Mode and 0.5 Ohm in the LV Mode. The field strength in the chambers can be calculated in a very simple way: Take the voltage value as observed on the pulse amplitude and divide by the chamber electrode gap in cm.

## 4.5 FIELD STRENGTH CALCULATION FACTORS

**Example 1: Disposable Cuvette P/N 620 2.0 mm gap**

Set Electroporation Voltage: 700 Volts
Expected Field Strength: 700 V / 0.2 cm = **3500 V/cm or 3.5 kV/cm**

Alternatively, the formula can also be used to calculate the voltage needed to achieve a particular field strength.

**Example 2: Disposable Cuvette P/N 620 2.0 mm gap**

Maximum field strength desired: 2500 V/cm or 2.5 kV/cm
Voltage Setting: 2500 V/cm x 0.2 cm = **500 V or 0.500 kV**

## 4.6 ESTIMATION OF ACTUAL VOLTAGE DELIVERED

To calculate the precise actual voltage, estimate the chamber resistance (R chamber) and multiply the set voltage by following correction factor

$$\text{Correction Factor} = R_{\text{chamber}} / Ri + R_{\text{chamber}}$$

For chamber resistances above 20 Ohms, the correction factor is close to one and can be neglected.

# 5. EXPERIMENTAL METHODS

If you are new to the field of gene transfer by electroporation, we advise that you refer to the following publications listed in the bibliography prior to performing any experiments. The papers describe experimental methods for all types of cells.

Suggested Reading:


1) Guide to Electroporation and Electrofusion, edited by Chang, Chassy, Saunders and Sowers, Academic Press, 1991.
2) Fromm *et all*, Expression of genes transferred into monocot and dicot plant cells by electroporation, PNAS, Vol 82: 5824-5828,1985.
3) Hofmann *et al*, Electronic genetic-physical and biological aspects of cellular electromanipulation, IEEE Engineering in Med. and Biol. Mag. Vol 5(4):6-25,1986.
4) Jonak *et al*, Gene transfer into mammalian cells by electroporation, Internal Report 1987.
5) Karube *et al*, Transformation of Saccharomyces cerevisiae spheroplasts by high electric pulse, FEBS Vol 182: 90-94, 1985.
6) Meilhoc et al, High efficiency transformation of intact yeast cells by electric field pulses, Biotechnology, Vol 8(3): 223-227, 1990.
7) Neumann *et al*, Gene transfer into mouse myeloma cells by electroporation in high electric fields, EMBO Journal, Vol 1(7): 841-845,1982.
8) Potter *et al*, Enhancer-dependent expression of human kappa immunoglobulin genes introduced into mouse pre-B lymphocytes by electroporation, PNAS, Vol 81: 7181-7165, 1984.
9) Saunders *et al*, Plant gene transfer using electrofusion and electroporation, In: Electroporation & Electrofusion in Cell Biology, Plenum Press, 1988.
10) Sukharev, et. al., Electrically-Induced DNA Transfer Into Cells. Electrotransfection In Vivo, In: Gene Therapeutics: Methods and Applications of Direct Gene Transfer, Berkhauser Boston, 210-232, 1994.
11) Takahashi et al, Gene transfer into human leukemia cell lines by electroporation. Experience with exponentially decaying and square wave pulses. Leukemia Research Vol 15(6): 507-513, 1991.
12) Xie, Ting-Dong, and Tsong, Tian Y., Study of Mechanisms of Electric Field-Induced DNA Transfection. V. Effects of DNA Topology on Surface Binding, Cell Uptake, Expression, and Integration into Host Chromosomes of DNA in the Mammalian Cell, Biophysical Journal, Vol 65: 1684-1689, 1993.


To establish experimental conditions for your applications it is essential to decide the magnitude of the field strength applied to the chamber. Field strengths are measured in kilovolts/cm (kV/cm). It is not possible to compare scientific results if only the voltage output of the generator is listed. The voltage applied to a chamber is modified depending on the chamber geometry, conductivity of the medium and the impedance of the generator.

A survey of the literature shows that typical electroporation field strengths required for successful results follows:

| | |
|---|---|
| Plant Cells | 1 - 3 kV/cm |
| Mammalian Cells | 4 - 8 kV/cm |
| Bacteria | 9 - 25 kV/cm |

## 5.1 ELECTROPORATION TRANSFECTION PROTOCOLS

The introduction of cloned genes into eukaryotic and prokaryotic cell types via traditional transfection procedures is unfortunately not applicable to many cell types. Electroporation offers an alternative method to chemically sensitive or recalcitrant cell types and allows efficient introduction of DNA into a broad spectrum of cell types.

Transfection of DNA occurs during the electroporation pulse when pores, created within the cell wall via a DC pulse, become channels through which the transfer of genetic material becomes possible. Additional electroporation pulses may be implemented to further increase DNA expression up to the point where cell viability might become threatened.

Because of the diversity of cell types, it might be advantageous to couple protocols with other techniques to obtain the proper amount of transfected DNA. Too much or too little DNA appears to be deleterious to the expression of the gene. The following list is given both as an aid and also to illustrate to the researcher the almost unlimited freedom available to procure maximize results.

1. Linear DNA is more easily transfected than supercoiled DNA.

2. Gene transfer is more effective at 0°C than room temperature.

3. Treatment of cells with sublethal concentrations of colcimide (0.1 µg / ml) for 18 hours prior to electroporation can increase transfection.

4. DNA (8-16 µg/ml) complexed to DEAE - dextran (MW 500,000 from Pharmacia) when added to cells and $CO_2$ incubation for 5 to 20 hours before electroporation might increase transfectants.

5. To overcome the negative charges that inhibit cell wall and DNA interaction, certain positively charged polymers (Poly-l-lysine), enzymes (Neuraminidase), dyes (Carbo-cyanine), chemicals ($Ca^{2+}$, $Mg^{2+}$) might be used to coat either the cell or the DNA to increase contact between transfectants before electroporation.

6. Positively charged histones commonly associated with some types of DNA might be used as coupling agents to increase interaction before electroporation.

7. Liposomal encapsulation of DNA can be utilized in conjunction with electrofusion techniquesfor DNA introduction.

8. The addition of 40% PEG has been used to enhance plasmid DNA (50 µg/ml) transformation into bacterium protoplasts ($4 \times 10^8$ cells/ml).

9. Magnesium chloride has been used successfully to absorb DNA onto the cell surface and after the addition of electric pulses shown to increase the yield over traditional methods.

10. Trypsin, DMSO, pronase, dispase, etc. might facilitate DNA entry by increasing cell wall permeability.

11. Osmotic differentiation between cells and solution can induce DNA uptake during electroporation.

12. Prokaryotic cells lend themselves toward genetic recombination via bacteria previously transformed with plasmid DNA by the addition of 40% PEG and electroporation.

13. Results of studies of transfection and expression of transfected DNA in mammalian cells indicates that DNA uptake into the cell does not depend on DNA topology, while both transient and stable expression are observed at higher levels using supercoiled and closed circular plasmids in comparison to linear plasmid DNA.

## 5.2 METHODS FOR THE STABLE TRANSFORMATION OF MAMMALIAN CELLS BY ELECTROPORATION

Electroporation is a simple and efficient method for the introduction of exogenous DNA into many mammalian cell lines. If the gene of interest is linked to a gene for a selectable function, such as kanamycin/neomycin phosphotransferase ($neo^r$), guanine phosphoribosyl transferase (Ecogpt), dihydrofolate reductase (DHFR), thymidine kinase (tk), or hypoxanthine-guanine phosphoribosyl transferase (HGPRT), stable permanently transformed cells can be obtained by selection in the appropriate media. The following methods describe procedures for the determination of optimal pulse voltages and pulse lengths for electroporation in mammalian cells, and the transfection of cell lines using the $neo^r$ gene and G418 (geneticin) selection.

Electrical and biological conditions for electroporation and transfection differ from cell type to cell type. The following steps should be taken to assure successful gene transfer:

1) Determine the optimal electrical parameters by measuring the sensitivity of the cell to high voltage electric fields. This is best done by assessing cell viability after pulsing.

2) Determine the sensitivity of the cell to the selection system.

3) Prepare linear plasmid DNA free from contaminating toxic materials.

4) Electroporation and selection using optimal conditions.

### 5.2.1 DETERMINATION OF CELL VIABILITY AFTER EXPOSURE TO HIGH VOLTAGE ELECTRIC FIELDS

A. Cells are grown in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal calf serum. Collect cells for electroporation by removing them from the tissue culture plate with trypsin-EDTA. Collect the cells by centrifugation at 500 x *g*. Wash the cells once in cold phosphate buffered saline solution (PBS). If suspension cells rather than adherent cells are to be used, collect them by centrifugation and wash once in cold PBS. Resuspend cells in PBS at a concentration of $2 \times 10^7$/ml.

B. Add an equal volume of 10 µg/ml propidium iodide in PBS.

C. Add cells to the electroporation chamber at the concentration to be used for gene transfer, usually $10^7$ cells/ml. Set the pulse number to three and pulse length to 10 µsec.

D. Beginning at a low voltage (0.5 kV/cm), pulse the cells, incubate for 10 minutes on ice, and examine the cells under the fluorescent microscope set for red fluorescence (rhodamine). Dead cells will appear bright red, live cells will be dark.

(If a fluorescent microscope is not available, viability may be determined in a similar manner using trypan blue exclusion).

E. Repeat the above procedure using increased field strengths until the cell viability is 90-95%.

Electroporation appears to be most efficient when 5 to 10% of the cells are killed.

F. After determining optimal peak voltage, calibrate the pulse length. The optimal peak voltage appears to decrease as the pulse width is increased. Avoid very long pulse lengths; the heat generated may damage the cells. Vary the pulse length from 10 to 300 µs and determine the optimal conditions for electroporation.

### 5.2.2 DETERMINATION OF OPTIMAL DRUG CONCENTRATION FOR SELECTION

A. A major variable is the ability of the kanamycin derivative G418 to kill the cells to be transformed. It is important to optimize the drug concentration for each cell line. The sensitivity of different cell lines to G418 may vary from 50 µg/ml to over 1 mg/ml. If the drug concentration is too high, a low transformation efficiency may be obtained because the enzyme produced by the linked resistance gene is unable to rescue the transfected cell. If the drug concentration is too low, clones may be obtained which are G418 resistant without incorporating the selectable gene.

B. Determine the optimal G418 concentration by plating cells at $5 \times 10^4$ cells/100 $mm^2$ tissue culture dish in DMEM with 10% fetal calf serum, gradually increasing the amount of G418 from 20 µg/ml to 80 µg/ml with each successive plate.

C. Replace the media with fresh media containing the appropriate amount of G418 every three days.

D. After 7 to 10 days, examine plates under the microscope and determine the cell viability in each concentration of drug. Choose the lowest G418 concentration in which all of the cells are killed. Typical G418 concentrations for mouse lymphocyte cell lines are 400 to 800 µg/m

E. Note also that G418 purchased from scientific supply houses is only about 30-50% pure. Therefore, a solution of 800 µg/ml drug may be only about 320 µg/ml G418.

## 5.3 PREPARATION OF PLASMID DNA FOR ELECTROPORATION

A. Prepare plasmid DNA in which the gene of interest and the $neo^r$ (SV2-$neo^r$) gene are present in the same vector. Alternatively, a plasmid containing the $neo^r$ gene may be mixed with a plasmid containing the gene of interest, and introduced by co-transformation. (While co-transformation has been shown to be successful, optimal conditions have not yet been established). Plasmids may be prepared by double banding on CsCl gradients and extensively extracted with isoamyl alcohol to remove the ethidium bromide. Ethidium bromide is highly toxic to mammalian cells and may decrease transfection efficiencies. Dialyse the plasmid preparations against water, ethanol precipitate and resuspend in sterile distilled water. Alterna tive methods of plasmid preparation may be used, however, transfection efficiency is dependent on the purity of the trasnfectant, thus methods not leading to high purity of the resulting DNA are discouraged.

B. Digest the plasmid DNA with a restriction enzyme to linearize the DNA. Destroy the enzyme by extraction with phenol, chloroform and purify by ethanol precipitation. Resuspend in sterile distilled water at 1-2 µg/ul. Alternatively, one may transfect with supercoiled plasmid DNA. However, the transformation efficiency will be 2 to 20X higher with linearized DNA.

## 5.4 GENERAL ELECTROPORATION PROCEDURE

A. Disposable Cuvettes (P/N 610, 620 and 640) and Disposable Flatpack Chambers (P/N 486) are pre-sterilized. No further sterilization is necessary. Handle aseptically. If using reusable chamber/electrodes, sterilize by rinsing with 70% ethanol. Rinse the chamber well with sterile PBS or electroporation buffer to remove the alcohol.

B. Collect log growth cells by trypsinization if the cells are adherent, or by centrifugation if they grow in suspension. Wash the cells in PBS. Resuspend cells at a concentration of $1 \times 10^7$ cells/ml in PBS without calcium or magnesium. Add the DNA to the cell suspension at 50 µg/ml. The transformation efficiency increases with increasing DNA concentration up to about 50 to 100 µg/ml.

C. Incubate the cells on ice for 10 minutes.

D. Add the cell/DNA suspension to the electroporation chamber. The cell viability should be calibrated for the chamber which will be used for transfection.

E. Program the instrument to apply the optimal voltage (field strength) and pulse length. After pulsing, incubate the cell/DNA suspension for 10 minutes on ice.

F. Dilute the cells with 10 ml of DMEM with 10% fetal calf serum and plate in 2 to 10/100 $mm^2$ tissue culture plates. Alternatively, for suspension cells, plate in 96-well microtiter plates.

G. After 24 hours, remove the medium and replace with DME supplemented with 10% fetal calf serum and G418 at the appropriate concentration for the cell line (usually 400 to 600 µg/ml).

H. Replace medium with fresh selective medium (DME with fetal calf serum and with G418) every 2 to 4 days. Since G418 may take up to 10 days to kill non-transfected cells, the media may initially turn acidic (orange) and require more frequent changes.

I. Clones should be visible after 2 to 3 weeks and may be isolated for further characterization after 3 to 4 weeks.

J. Determine the transformation efficiency by counting G418 resistant clones. Transformation efficiencies are most often expressed in terms of the resulting number of transformants per ug of DNA. Efficiencies are quite reproducible when the same plasmid, cells and electrical parameters are used. However, when cell line, selectable gene, or electroporation parameters are varied, efficiencies can range over several orders of magnitude. Transformation frequencies can also be determined, and are generally expressed as the number of transformants per total number of cells in the transformation.

## 5.5 SPECIFIC CONDITIONS

Using the following conditions, plasmid DNA containing exogenous genes may be introduced into the T-lymphoma cell line BW5147 at efficiencies of $1/10^3$ to $1/10^4$:

* 3 pulses of 4 kV/cm (amplitude 400 volts)
* 10 to 50 µsec pulse length
* temperature $0^o$ C
* Disposable Cuvette Chamber P/N 610 (70 µl volume, 1.0 mm gap)
* 50 µg/ml linearized DNA containing the $neo^r$ gene expressed from the M-MLV promoter
* cell concentration $10^7$ cells/ml in $PBS^-$ without $Ca^{++}$ or $Mg^{++}$ (with specific resistivity of 13.7 Ohm*cm)
* selection in 600 µg/ml G418

Optimal conditions may vary for other cell lines, see protocols or call Technical Support Group at 1(800) 289-2465.

# 6. SERVICE

## 6.1 WARRANTY

The BTX T820 Square Wave Pulse Generator and its accessories, excluding chambers are guaranteed to be free of defects for a period of 2 years from the time of delivery. Chambers, are under warranty for a period of 90 days. If any defects covered by this warranty appear within the above period, BTX shall have the option of repairing or replacing the equipment at BTX's expense. Such repair or replacement shall be the customer's exclusive remedy for breach of warranty or for negligence. This warranty does not extend to any instrumentation which has been (a) subjected to misuse, neglect, accident or abuse, (b) repaired or altered by anyone other than a BTX representative without BTX's expressed and prior approval, (c) used in violation of instructions furnished by BTX.

BTX SHALL NOT BE LIABLE FOR ANY SPECIAL OR CONSEQUENTIAL DAMAGES OR FOR LOSS, DAMAGE OR EXPENSE (WHETHER OR NOT CAUSED BY, OR RESULTING FROM, BTX'S INTELLIGENCE) DIRECTLY OR INDIRECTLY ARISING FROM USE OF THE INSTRUMENTATION SOLD HEREUNDER EITHER SEPARATELY OR IN COMBINATION WITH ANY OTHER EQUIPMENT OR FROM ANY OTHER CAUSE. THE ABOVE WARRANTY SHALL BE IN LIEU OF, AND EXCLUDES, ALL OTHER EXPRESSED OR IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS, OR OTHERWISE.

Without limiting the generality of the foregoing, BTX shall not be liable for any claims of any kind whatsoever, as to the equipment delivered or for non-delivery of equipment, and whether or not based on negligence.

Any malfunction not caused by operator abuse will be corrected, at no charge for parts or labor, by BTX.

All service under this warranty will be made at the BTX facilities in San Diego, CA. Owner will ship instrument prepaid to San Diego, CA. BTX will return the instrument after servicing, freight prepaid to owner's address. Warranty is void if the T820 is changed in any way from its original factory design or if repairs are attempted without written authorization by BTX Inc.

Warranty is void if parts, connections or chambers not manufactured by BTX are used with the T820.

## 6.2 OBTAINING SERVICE

### 6.2.1 SERVICE DURING WARRANTY

1. Write or call the BTX Inc. Technical Support Group and describe the nature of the problem.
2. Carry out minor adjustments or tests as suggested by BTX.
3. If proper performance is not obtained, BTX will notify you to ship the instrument, prepaid, to its Service Department. The instrument will be repaired and returned at no charge for all customers within the continental United States.

> Customers outside of the continental United States, who have purchased our equipment from distributors, should contact their distributor. If you have purchased directly, contact us. We will repair at no charge, but will not pay for shipment, documentation, etc. These charges will be billed at cost.

NOTE! Under no conditions should the instrument or accessories be returned with out prior approval from BTX.

### 6.2.2 OUT-OF-WARRANTY SERVICE

Proceed exactly as for service during warranty, above. If our Service Department can assist you by phone or correspondence, we will be glad to do so at no charge.

Repair service will be billed on the basis of time and materials. A complete statement of time spent and materials used will be supplied. Shipment to BTX Inc. should be prepaid. Your bill will include return shipment freight charges.

Disassembly by the user is not recommended. Service should be carried out by experienced BTX technicians only.

## 6.3 TROUBLE SHOOTING

### 6.3.1 POWER FAILURE

If the front panel lights do not illuminate, check 110/220 V power source. Check fuse located on the rear panel of the generator cabinet and, if blown, replace with another 3 A, fast blow fuse. If fuse blows again, contact BTX.

### 6.3.2 PRESENCE OF GENERATOR OUTPUT PULSES

To determine if pulses are present, use cells in a Microslide. Cell lysing can be observed if the generator output is present. If the cells do not lyse at high amplitudes, the pulse was not delivered to the chamber. Verify with an Ohmmeter that the cables show continuity. Refer to Section 6.2 if pulses are absent.

### 6.3.3 ARCING IN THE CHAMBER

Arcing is a natural statistical event and can occur for a number of reasons. Sometimes an arc needs to be tolerated in order to obtain transformants using high voltage and pulse length. It can occur with high conductivity media (low specific resistivity), such as PBS, combined with high field strengths, incomplete washing procedures and long pulse lengths. If arcing occurs:

A) Reduce voltage (field strength) and/or decrease pulse length.

B) Add an additional washing step to reduce ionic strength of the cell suspension.

C) Clean and polish the reusable electrodes so that no rough spots are visible or replace electrodes.

D) Use non-ionic media at high field strengths to reduce excessive heating.

High voltage arcs can occur at field strengths > 12 kV/cm.

Low voltage arcs can occur due to excessive energy delivered into the chamber medium, especially with PBS.

During an arc, the cuvette can explode violently. Clean up debris and proceed with experiments at a lower voltage or shorter pulse length. The T820 generator should not experience any failure as it is arc and short circuit proof.

### 6.3.4 MISCELLANEOUS

Any other type of trouble shooting must be discussed with BTX. The output signal of this equipment is dangerous and needs to be tested only by qualified personnel. Please call (619) 597-6006 or (800) 289-2465 and ask for Technical Support.

# *ElectroSquarePorator*

# T820

## ELECTROPORATION SYSTEM

## OPERATING MANUAL

For research purpose only

BTX INC.
11199-A Sorrento Valley Rd.
SAN DIEGO, CA 92121-1334

1(800) 289-2465
1(619) 597-6006
1(619) 597-9594 FAX

**All the following protocols are applicable to T820.**