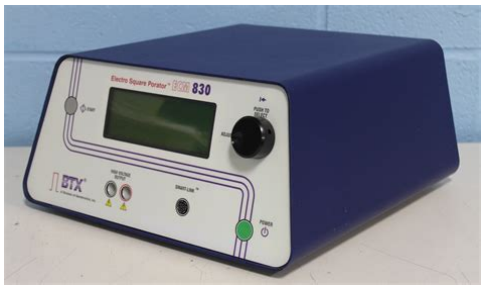
btx electroporator manual



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ECM 830 Electroporation System ECM 830 Electroporator only 110 V INT ECM 830 Electroporator on.The European Union EU has enacted two Directives, the first on product recycling Waste Electrical and Electronic Equipment, WEEE and the second limiting the use of certain substances Restriction on the use of Hazardous Substances, RoHS. Over time, these Directives will be implemented in the national laws of each EU Member State. Once the final national regulations have been put into place, recycling will be offered for our products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a “wheelie bin” symbol. Most of our products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. All inquiries concerning these products should refer to the serial numbers on the units. 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 BTX HARVARD APPARATUS without BTX HARVARD APPARATUS’ express and prior approval, c used in violation of instructions furnished by BTX HARVARD APPARATUS. This warranty extends only to the original customer purchaser. Failure to use the Enhancer 3000 High Voltage probe to connect a BTX Generator to an external digital oscilloscope for monitoring will result in voiding your warranty; connecting directly to the external monitoring equipment or modified monitoring setup will damage the Generator.Without limiting the generality of the foregoing, BTX HARVARD APPARATUS shall not be liable for any claims of any kind whatsoever, as to the equipment delivered or for nondelivery of equipment, and whether or not based on negligence. Goods will not be accepted for return unless an RMA Returned Materials Authorization number has been issued by our customer service department.http://www.sport-foods.ru/userfiles/dcf-manual-nj.xml

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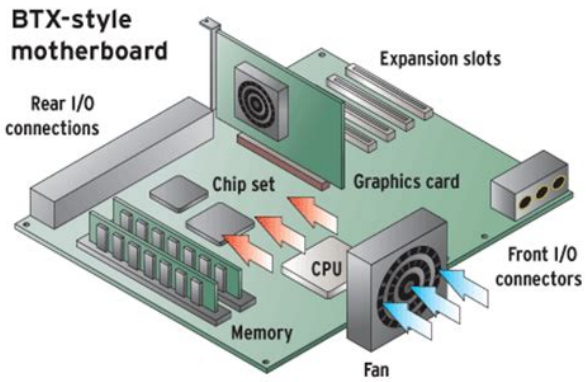
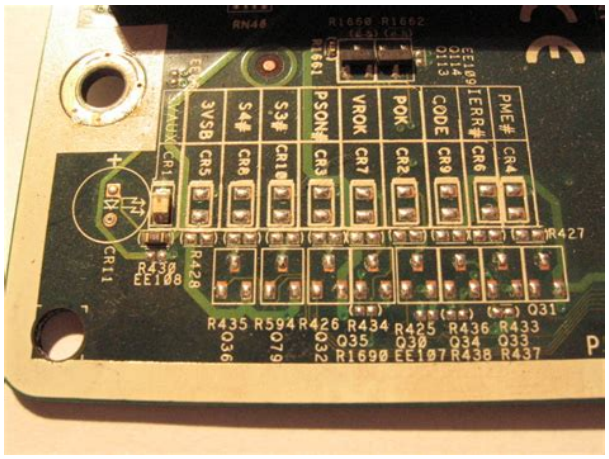


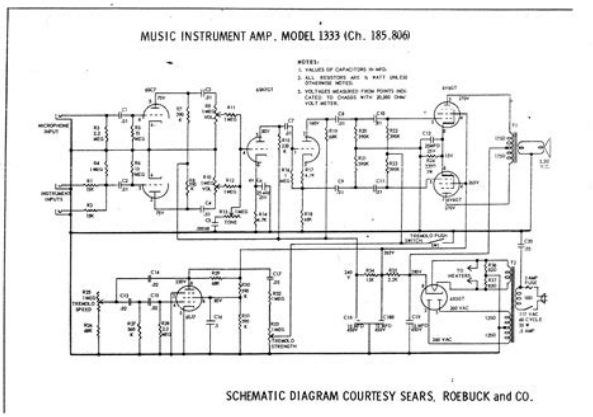
The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device. This warranty gives you specific rights, and you may also have other rights, which vary from state to state. Service All service under the warranty will be made at the BTX HARVARD APPARATUS, Holliston, Massachusetts facilities or an authorized service site. Owner will ship instrument prepaid to Holliston, Massachusetts, USA or the service site. BTX HARVARD APPARATUS will return the instrument after servicing, freight prepaid to owner's address. Obtaining Service Service During Warranty 1. Write or call the BTX HARVARD APPARATUS Customer Support Group and describe the nature of the problem. 2. Carry out minor adjustments or tests as suggested by BTX HARVARD APPARATUS. 3. If proper performance is not obtained, BTX HARVARD APPARATUS 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 in the continental United States. Customers outside of the continental United States who have purchased our equipment from distributors should contact the distributor. If you have purchased your equipment from us, you should contact us directly. We will repair at no charge, but will not pay for shipment, documentation, etc. These charges will be billed at cost. Note Under no condition should the instrument or accessories be returned without prior approval from BTX HARVARD APPARATUS. If our Service Department can assist you by phone or correspondence, we will be glad to, at no charge. Repair service will be billed on the basis of labor and materials. A complete statement of time spent and materials used will be supplied. Shipment to BTX HARVARD APPARATUS should be prepaid.http://www.bmsk.ru/images/static/dcf-florida-medicaid-manual.xml



Your bill will include return shipment freight charges. Disassembly by the user is prohibited. Service should only be carried out by experienced BTX HARVARD APPARATUS technicians. Repair Facilities and Parts BTX Harvard Apparatus stocks replacement and repair parts. When ordering, please

describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample or drawing. To avoid potential hazards, use this product only as specified. Only qualified personnel should perform service procedures. To Avoid Fire or Personal Injury USE PROPER POWER CORD Use only the power cord specified for this product and certified for the country of use. CONNECT AND DISCONNECT PROPERLY Do not connect or disconnect probes or test leads while they are connected to a power source. GROUND THE PRODUCT This product is grounded through the grounding conductor of the power cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making connections to the output terminals of the product, ensure that the product is properly grounded. OBSERVE ALL TERMINAL RATINGS To avoid fire or shock hazard, observe all ratings and markings on the product. Consult the product manual for further ratings information before making connections to the product. DO NOT OPERATE WITHOUT COVERS Do not operate this product with covers or panels removed. Use Proper Fuse. Use only the fuse type and rating specified for this product. AVOID EXPOSURE TO CIRCUITRY Do not touch exposed connections and components when power is present. DO NOT OPERATE IN LOW IMPEDANCE Sample Load or Sample If the electroporation samples have an impedance of less than 20.DO NOT OPERATE WITH SUSPECTED FAILURES If you suspect there is damage to this product, have it inspected by qualified BTX service personnel. Warning statements identify conditions or practices that could result in injury or loss of life. CAUTION.

Caution statements identify conditions or practices that could result in damage to these products or other property. BTX square wave technology offers researchers the ability to transfect cells efficiently and with higher cell viabilites. It is normal for the instrument to be slightly warmer than its’ operating environment. 3. Choose an outlet that is readily accessible. Note A stabilization period of 3 seconds is required after connecting to an outlet. Start Switch 1. The electronic Start Switch on the upper left front panel is activated in the “ready” mode when the parameter set screen is displayed. 2. Once the start switch is activated, the generator will charge the capacitor bank, then “settle” by bleeding off the capacitors to the preset voltage, prior to delivering the pulse. 3. The maximum setting time is 3 seconds. A pulse sequence may be aborted by pressing the start switch a second time before the delivery of the pulse. 4. Following the delivery of a pulse, press the start switch once to deliver another pulse, or press the parameter control knob to return to “ready” mode. The arrow will move to the right of the value displayed for that parameter. 2. To adjust the value of a parameter under control, rotate the knob clockwise to increase the value and counter clockwise to decrease it. After the values are set under control, push the knob to lock set value and arrow will return to left of parameter under control. 3. In order to move between screens, move the cursor to the bottom of the screen and rotate the knob clockwise to move to the next screen. 4. In order to move to the previous screen, move the cursor to the top of the screen and rotate the knob counterclockwise. Mode The Mode indicates “LV” for low voltage mode or “HV” for high voltage mode. Adjusting the voltage as appropriate automatically controls the mode. There is no manual mode control.



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The low voltage mode range is 5 V to 500 V in 1 V increments and the high voltage mode range is 505 V to 3000 V in 5 V increments. To estimate approximate pulse lengths at various voltages in the high voltage mode, refer to Table 1 below. 4. Please note that the voltage determines the maximum pulse length in the HV mode. 5. If the voltage is increased and the preset pulse length is longer than the maximum pulse length allowed, the pulse length will automatically adjust to that level. Interval The Interval indicates the time duration between pulses. The interval range is 100 ms to 10.0 seconds, switching from units of ms to seconds after 999 ms, with 0.1 sec resolution. Advanced Features The final Set Parameters page enables the user to save, view, and load up to three programs. Please note that parameters can be changed inadvertently once a program is loaded. A default program is used to initialize the system. Program 1 is used to automatically store changes in parameters set each time the system is pulsed. Push the parameter control knob again to return to the setup screen. Push the parameter control knob again to return to the setup screen. Load 1. To load a saved program, push the parameter control knob to move the arrow to the left of the program number. Please note that in addition to three available programs, there is also the default program as outlined in the “Initializing” section. 2. Rotate the knob to change the program number. 3. Push and hold the knob in, releasing after a new screen is displayed. The new screen will read “Loaded Set N to Current Parameters”. 4. Push the parameter control knob again to return to the setup screen. OnLine Help In the Set Parameter Mode, an OnLine Help function is enabled. OnLine Help provides a definition for all set parameters and advanced functions. 1. To use OnLine Help, rotate the parameter control knob so that the arrow is to the left of the parameter or feature of interest not the value of the parameter. 2.

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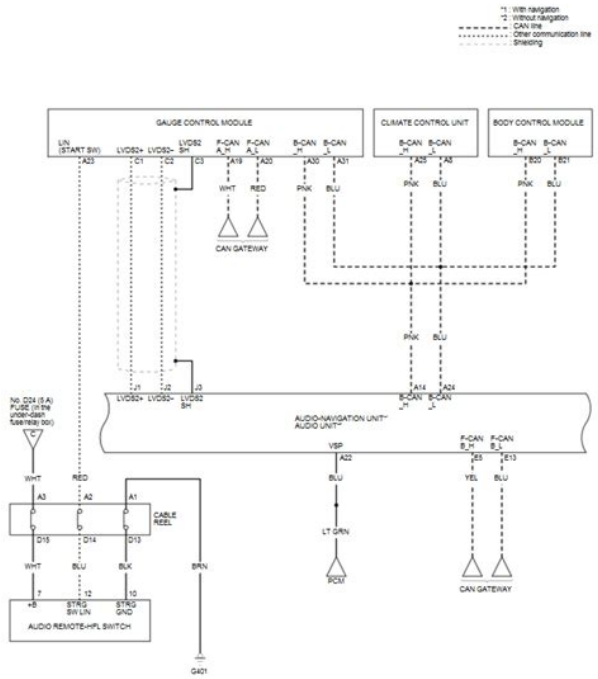

Push the parameter control knob in and hold until the definition is displayed. 3. Push a second time to return to the Set Parameter Screen. The following status messages may be observed Charging, Pulsing, Pulse Aborted, and Temperature Failure. Charging As soon as the start switch is pressed, the “Charging” status message is displayed. Pulsing Once the capacitors have reached the preset voltage level, they are discharged and the “Pulsing” status message is displayed for the duration of the pulse or preset number of pulses. A click or a beep will be heard upon the delivery of each pulse. The sound that is heard is a function of the pulse length. At pulse lengths less than 1 to 2 ms, clicks will likely be heard. At pulse lengths at or above 1 to 2 ms beeps should be heard. A final, more extended beep will be heard upon completion of the pulsing sequence. Following the pulsing status message, the Pulsing Completed Screen is displayed. Pulsing Aborted During Charging If the start switch is activated a second time prior to delivery of a pulse, the pulsing sequence is aborted and the “Pulsing Aborted During Charging” status message is displayed. In this event, the status message observed is “Unit Cooling” “Please Wait”. When the unit has cooled down to a safe level, two beeps will sound and the message will change to “Ready”. Pushing the start button, or rotating the parameter control knob will shift user back to Parameter Set Screen. Pulsing Completed Screen The Pulsing Completed Screen is displayed following the delivery of a pulse or train of pulses and is indicated by the display “Pulsing Completed” on the top line of each of two screen pages. 1. Page 1 comes up automatically; rotate the parameter control knob clockwise to view page 2 and counter clockwise to go back to page 1. 2. Press the knob to return to the Set Parameters Screen or press the start switch to deliver another pulse.

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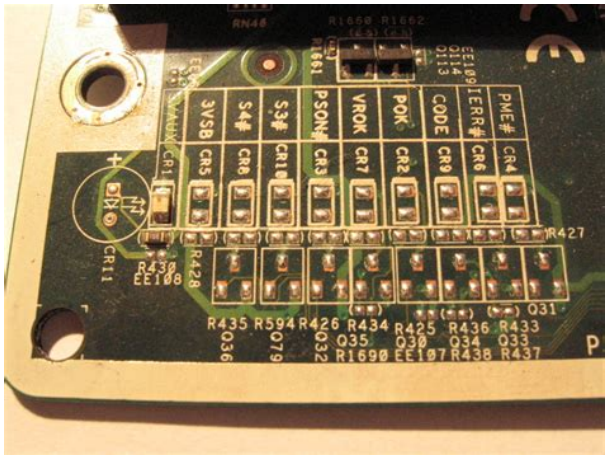

The pulsing completed screen displays monitored parameters including Mode, Voltage, Pulse Length, Number of Pulses, Pulse Interval and Polarity. Please note that parameters will be displayed, even if Safety Stand or other output is not connected. Always verify that your sample is connected with the HV output. Mode Mode indicates the voltage mode used in the delivery of the last pulse LV or HV. Voltage Voltage indicates the output voltage of the last pulse, in volts. Polarity The Polarity used in the last sequence of pulses is monitored and displayed as Unipolar. High Voltage Output The High Voltage Output is located in the lower center portion of the front panel. Plug the high voltage cables into this output following the colorcoded polarity. If your instrument falls in this category and you wish to use the footswitch, please see appendix K on page 44. Rotate knob to adjust voltage. Push again to select that voltage. 4. Rotate parameter control knob to move arrow to P 630B 450207 Length and push to select. Rotate knob to adjust pulse length. Rotate knob to adjust the number of pulses. Push again to select that number of pulses. 6. Rotate parameter control knob to move arrow to Interval if you have designated more than one pulse in 5 and push to select. Rotate knob to adjust the pulse interval. Push again to select that pulse interval. 7. Prepare sample, pipette into the appropriate BTX Disposable Cuvettes Plus, place the cuvette in the 630B Safety Stand and secure the safety cover. 8. Press the Start button. Follow the above instructions in conjunction with instructions provided for the specific electrode. Use with Footswitch. Please see Appendix K on page 44. Now rotate to select which program number the chosen settings see “Preset Parameters” above should be saved under. Now rotate to select the appropriate program number. 8. Push and hold knob in, releasing only after a new screen. The pores formed are of the order of 40 to 120 nm.

Most pores reseal within a few seconds, after allowing the transfer of materials into and out of the cells. During a typical electroporation process, target cells and molecules are mixed together. When an electroporation pulse is delivered, the result is the formation of temporal pores. Before the pores reseal, the target molecules are observed to enter the cells. Upon resealing of the pores, the molecules become incorporated within the cell. The eventual target site depends on the application; for example, molecules can remain in the cytoplasm, interact with the membrane, and move into the nucleus. Applications for electroporation include permeabilization of virtually all cells to a wide variety of molecules and ions. The most common applications for electroporation are the transformation or transfection of cells with DNA or RNA. Other applications for electroporation include electroactivation, electroinsertion of proteins into cell membranes and electroextraction of molecules from cells. Although electroporation has mainly been used as a research tool, recent work has demonstrated its potential for clinical applications. After delivery of the direct current DC pulse, pores that have been formed in close juxtaposition may reseal upon one another. If the process results in an intact hybrid, electrofusion has occurred. Commonly, a second round of AC alignment is employed following resealing, in an attempt to “compress” or stabilize the hybrid, and increasing the efficiency of the process. Applications for electrofusion include animal cloning, animal nuclear transfer, animal embryo manipulation, hybridoma formation, and transgenic plant production. Electroporation and electrofusion generators can be characterized by waveform. To achieve a

desired pulse length, appropriate resistance and capacitance must be selected on the instrument. Voltage may be directly set on the instrument.

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The square wave pulse is produced by a partial discharge of a large capacitor, which requires the interruption of high currents against high voltages. Typically, voltage, pulse length, and number of pulses are all directly set on the instrument. Square wave pulses have well defined electric field amplitude, are both effective and also relatively mild to cells to yield higher viability. Parameters, which typically may be set include amplitude, duration, and frequency. Field strength and pulse length are critical parameters for reporting, optimization and troubleshooting bacterial and yeast applications. Mammalian Cell Electroporation Electroporation has been used successfully to introduce many different molecule types into cells. Most commonly, electroporation is used for the processes of transfection, in which nucleic acid DNA and RNA, is introduced into cells. Electroporation can be used to deliver oligonucleotides into cells for siRNA or Gene Silencing antisense applications. It can be used to deliver proteins into cells, even large enzymes such as restriction enzymes and antibodies, for various purposes. Peptides have also been electroincorporated. Electroporation is also used to electroinsert proteins into the cell membrane. Finally, electroporation has been used to introduce drugs, such as the chemotherapeutic agent bleomycin, into cancer cells, in vitro and in vivo. The use of low impedance buffers such as PBS may result in a voltage drop so that the actual peak voltage delivered to samples may be less than the set voltage. With exponential decay generators, monitoring is necessary to identify the pulse length, or time constant, since this parameter may be very much dependent on the impedance of the sample sample load. When using complex and custom electroporation applicators and chambers, the electroporation waveform may be altered and monitoring is again strongly recommended.

High GFP expression in Mouse PE501 Plant Protoplast Electroporation Electroporation has been used to introduce molecules into plant protoplasts, pollen and most recently, direct transfer into plant tissue in vivo. Other Electroporation Applications 1. Transgene incorporation, in which simple transfection of fish embryos has resulted in transgenic zebrafish. Plant Protoplast 2. Utilization of sperm as biological DNA carriers, in which pulsed fields cause the complexing of DNA to sperm, which then act as carriers upon fertilization. Electrofusion Embryo Manipulation Embryo Manipulation includes nuclear transplantation experiments, in which blastomeres are inserted by micromanipulation through the zona pellucida into the perivitelline space of a surrogate enucleated egg. After application of an AC wave to facilitate compression and alignment, a single DC square wave fuses the pair and subsequent pulses activate the resulting nuclear transplant embryo. Hybridoma Formation Electrofusion has been used by researchers as a replacement for polyethylene glycol PEG, in the production of monoclonal antibodies by hybridomas. Microelectrofusion of hybridomas is used to generate hybrid hybridomas, or quadromas, secreting bifunctional antibodies, without the use of drug selection markers, resulting in higher yields than PEG without the delays of drug selection. Plant Protoplast Fusion The process of electrofusion has been used extensively to fuse plant protoplasts, resulting in viable hybrid offspring. Other Electrofusion Applications Electrofusion has been used for the fusion of yeast and bacteria cells. The area beneath each line represents parameters that will lead to the delivery of a full pulse. One, or several pulses of the appropriate field strength, pulse length, and wave shape may be required for this purpose. The key to success with electroporationbased technologies involves a proper combination of biological, physical, chemical, and pulse parameters.

In general, cells must be in midlogarithmic growth for optimal electroporation. Various temperature regimens have been described. It has been shown that a variety of chemical techniques may increase electroporation and electrofusion efficiencies, including addition of EDTA, DMSO, intracellular salts,

and serum before or after the pulse. Optimizing protocols abound. Analysis of these optimization regimens has lead to proposals of universal protocols, involving very limited optimization over a narrow range. Electroporation 1. Vary the voltage in order to vary the field strength, keeping other parameters constant. Assay sample for both viability and endpoint. Directly vary square wave instrument pulse length. Plot the number of pulses versus both viability and endpoint, and extrapolate the optimal number of pulses. Assay sample for both viability and fusion efficiency. Plot the field strength versus both viability and endpoint and extrapolate the optimal field strength voltage divided by gap size and voltage. 2. Vary the square wave instrument pulse length. Verify that the fuse is not blown. Disconnect power cord from the instrument before removing the fuse holder. Replace the fuse, if necessary, with same rated fuse as indicated on back panel. In the case of a malfunction, one of the following messages will appear on the display. Note the instructions on the following page. EEPROM Failure The unit has detected a malfunction in its internal memory system. The validity of the data might be compromised. Turning or pressing the knob will bring the Set Parameters screen. Verify carefully every setpoint before pulsing. This verification is performed during power up and every time that data is loaded from memory. Contact BTX Technical Support if this error message is displayed again, after a power up sequence. Pulsing Aborted Charging Timed Out A charging time limit of 20 seconds is provided for circuit safety.

If the capacitors are not charged to the preset voltage level after 20 seconds, the “PULSING ABORTED CHARGING TIMED OUT” message is displayed. For assistance with this situation, please contact BTX Technical Support. Press the encoder or the pulse switch once to get back to the Set Parameters screen. Verify properties of cell sample do cells need to be washed. Low or no transfection efficiency, or incorporation Verify physical, biological, and chemical parameters. Verify delivery of the pulse and pulse parameters. Is the voltage correct. Chamber gap Pulse length or appropriate instrument settings. Number of pulses If so, follow Optimization Guidelines outlined in Appendix A. Low viability Verify physical, biological, and chemical parameters. Are the voltage, chamber gap, pulse length time constant, pulse number and other instrument settings correct. If so, reduce voltage, pulse length, or number of pulses and reoptimize protocol to improve viability as outlined in Appendix A. Low fusion yield Verify physical, biological, and chemical parameters. Troubleshoot alignment as outlined in above. Verify delivery of DC fusion pulse, voltage, field strength, and pulse length. For additional information, please contact BTX Technical Support. Voltage Drop A drop in output voltage accompanies pulse delivery into highly conductive samples for example, PBS. Thus the displayed voltage may in these situations be less than that expected, given 5% full scale accuracy and the monitoring accuracy of 5%. Thus for nonrectangular waveforms, a difference between the set and actual voltages may be observed. Used to induce a divergent electric field, allowing for dielectrophoresis. Amplitude The instantaneous value of current or voltage in amperes or volts. Capacitor A device that stores electric energy in the form of an internal electric field. Energy is delivered when a current flows out of a capacitor. The current normally follows an exponential curve.

Dielectric A material that has a high resistivity and can store energy in the form of an electric field. Direct Current DC Current whose amplitude is constant with time. Direct currents are used to form temporary pores in bilipid membranes. Cells may fuse when pores in the membranes of two juxtaposed cells reseal after a DC application. Divergence The deviation of electric field lines from a parallel homogeneous condition. A highly divergent field has field lines that rapidly change amplitude or strength and direction in the area of interest. Electric Field The electric potential difference between two points divided by the distance separating those points. Electric Field Force The mechanical force acting on any electric charge when placed in an electric field. Exponential Decay Non linear waveform typical of capacitor charge and discharge currents and voltages. Homogenous Electric Field An electric field where the direction and strength of the field lines are constant. A consequence of cells being exposed to an inhomogeneous or divergent electric field,

resulting in their movement toward the electrodes, and subsequent alignment or pearl chain formation. Cloning In terms of applications for electro cell fusion, cloning refers to the ability to generate identical, viable animals, through processes such as nuclear transplantation. The result of an AC alignment. Dielectric Breakdown The reversible breakdown of lipid bilayer membranes as a result of the application of a DC electroporation pulse. Sufficiently high field strength may increase the membrane potential past a critical point leading to the breakdown of the membrane. Dielectrophoresis See Alignment. Dimer Formation The bringing together of 2 cells, through the process of dielectrophoresis, so they may be fused, resulting in a hybrid. Refer also to Pearl Chain. Electrofusion EF or Electro Cell Fusion ECF Electric field induced cell fusion.

A novel physical means to bond two cells together by applying a high intensity electric field pulse. The use of electroporation to incorporate molecules, or ions, into cells, protoplasts, or liposomes. Dimer Formation Electroinsertion The use of electroporation to insert molecules into lipid bilayer membranes. Electropermeabilization The use of electroporation to make cells, protoplasts, or liposomes permeable to ions and small molecules in their extracellular environment. Embryo Manipulation The cloning of animals can be accomplished through embryo manipulation techniques, such as nuclear transfer and electrofusion. Hybrid A viable daughter cell resulting from the fusion of two parent cells. Hybridoma The fusion of an antibodyproducing cell with an immortalized cell, resulting in an immortalized hybrid cell capable of generating antibodies. Hydrostatic Pressure The pressure in liquids at rest. Lipid Bilayer An assembly of lipid and protein molecules held together by noncovalent interactions. All biological membranes share this common structure. Osmotic Pressure The applied pressure required to prevent the flow of solvents of different concentration across a semipermeable membrane. Pearl Chains See Alignment and Dielectrophoresis. Chains of cells or vesicles brought into alignment during electro cell fusion, prior to electroporation. Pore A small, mostly transient, opening in a cell wall caused by the application of a brief high electric field pulse. Pressure Gradient The difference in pressure between two points in a medium. Protoplasts The plant cell proper, with the cellulose cell wall removed. Relaxation Time The time a system requires to reach equilibrium. Transfection The introduction of nucleic acids into animal cells. Stable transfections result in integration of nucleic acids into host chromosomes and the inheritance of associated traits in progeny cells. Transient transfections result in temporary expression of exogenous nucleic acids.

Transformation The introduction of nucleic acids into microorganisms and plant cells. Turgor Pressure The pressure in capillaries. CAUTION To avoid damage to the instrument, do not expose to sprays, liquids, or solvents. Cleaning Inspect the instrument, as often as operating conditions require. To clean the instrument exterior, perform the following steps 1. Remove loose dust on the outside of the instrument with a lintfree cloth. Use care to avoid scratching the clear plastic display filter. 2. Use a soft cloth dampened with water to clean the instrument. Use an aqueous solution of 75% isopropyl alcohol for more efficient cleaning. CAUTION To avoid damage to the surface of the instrument, do not use any abrasive or chemical cleaning agents. Use caution not to drop or cause any unwarranted physical harm to the instrument during any cleaning operations. If the serial number is 8310249 or sequentially higher than this number, the unit is compatible with a footswitch. If the serial number is 8310248 or sequential lower than this number, than the unit is not compatible with a footswitch. BTX Generator Footswitch Model 1250 FS Instruction Sheet Footswitch 450211 Background Information The BTX Generator Footswitch designed for used with BTX ECM 830 Square Wave Generator. Versions 1.08 and beyond have software that support the footswitch operation, so no further modification of the generator is required. Warning Make sure the Generator is turned off prior to connecting any cables to it. If using the Enhancer 3000 to monitor output, connect the banana plugs into the output ports of the High Voltage Probe. Use the black and red high voltage cables to connect the BTX generator to the input ports of the Enhancer 3000. 5. Open the Safety Stand cover. 6. Use the thumb wheel to slide the electrodes open. 7. Place a BTX cuvette

in between the electrodes with the aluminum of the cuvette coming in contact with the electrodes 8.

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