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ELECTRO CELL MANIPULATOR ECM® 600 ELECTROPORATION SYSTEM OPERATION MANUAL

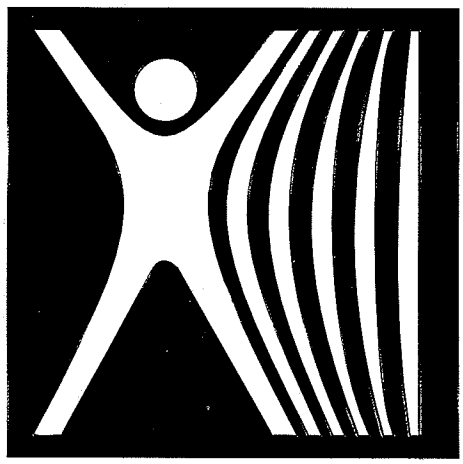
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BTX Inc.
11199-A Sorrento Valley Road
San Diego, CA 92121-1334
(619) 597-6006

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San Diego, CA 92121-1334

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Introduction

Thank you for investing in a BTX ECM 600 Electroporation System.

Since its founding in 1983, BTX's main focus has been in the area of applying controlled electric fields for genetic engineering applications. Because of this, we quickly established a reputation as the technological leader in the new field of Electronic Genetics, a BTX trademark. Our systems are installed in many prestigious institutions where they are used successfully for high efficiency transfection via electroporation of bacterial, mammalian and plant cells. We offer a variety of wave shapes and chamber options.

The ECM 600 is a general purpose Electroporation System providing the most extensive features available in a single unit design. Only ECM 600 offers such great instrument flexibility and ease of use. Now all of the necessary power and capacitance for rapid and efficient transformation of bacteria, microorganisms, plat and mammalian cells is contained in the ECM 600 without the need for any add on boxes.

Designed for ease of use, the BTX Disposable Cuvettes are injection molded with embedded 1, 2 or 4 mm gap aluminum electrodes and a round sealing cap. These cuvettes are capable of generating a wide range of field strengths up to 25 kV/cm. The BTX Flatpack Chambers can generate up to 40 kV/cm and are available for this system. Such field strengths are sufficient for the electroporation of both prokaryotic and eukaryotic cells. The chambers are individually packaged for guaranteed sterility and come in package of 50. They are designed to prevent shock to the operator when used with the BTX Safety Stand. A Cuvette Rack is available for use on the laboratory bench. The BTX Cuvettes are compatible with the complete line of BTX pulse generators as well as units from other manufacturers.

Our Technical Support Group constantly tracks published literature for any reference to electroporation or electro cell fusion. We extract and enter pertinent experimental conditions and yields from these papers into the BTX Electronic Genetics Database®. This database currently contains references of over 1600 scientific publications. As a service to our customers, we offer a complimentary literature search on any cell type of interest.

Please read this manual carefully before attempting to operate the ECM 600. If you have any questions about the unit or about particular applications, please call:

BTX Technical Support Group
U.S.A. and CANADA
1 (800) 289-2465
Fax: (619) 597-9594

For other countries call your local
BTX Dealer or call BTX directly
(619) 597-6006

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Glossary of Terms

Exponential Decay Generator: An instrument that generates a pulse from the complete discharge of a capacitor(s) resulting in an exponentially decaying waveshape.

1/e: The time duration between the steep front of the pulse and the time it takes for the maximum voltage of the pulse to decay to one over the base of the natural logarithm (e=2.718..). In practice, this is about 1/3 of the maximum value.

Specific resistivity: The inverse of specific conductivity, measured in Ohm x cm. It is determined by the concentration and type of ions in solution and is not a function of the chamber geometry.

Resistance: The property of an electrically conductive material that causes a portion of the energy of an electric current to be converted into heat. It is measured in Ohms and is a function of the chamber geometry and the specific resistivity.

Capacitor: A device that stores energy in the form of an electric field. It consists of two metal plates insulated from each other by a dielectric (insulating material, air, gas or vacuum). In an ideal capacitor, no conduction current flows between the plates. Capacitors can be fixed, variable, or adjustable.

Capacitance: The ratio of the charge on one of the plates of the capacitor to the potential difference between the plates.

Farad: The unit of capacitance (F).

Micro Farad: The unit of capacitance used by most electroporation instruments 10^{-6} Farads shown as μF.

Voltage: The difference of electric potential between two points of electrodes, expressed in volts V or kilovolts kV.

Electric field strength: The potential difference between two electrode points (in Volts) divided by the distance between the electrodes, called gap, and expressed in cm. Field strength is expressed as V/cm or kV/cm. This is true only if the electric field is homogeneous as it is in parallel plate electrodes.

Arcing: If an electric field is applied between parallel electrodes, a current will flow in an evenly distributed sheet. If the field exceeds a critical value, the sheet will be restricted to a very narrow channel with a high current density which is called an arc. The electrode material will melt at this spot and vapor will be generated explosively. As a result, the electrodes will show craters or pitting marks. Under these conditions, the cuvette can be destroyed.

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Low VM: Low Voltage Mode on the ECM 600 uses a combination of capaditors and ohmic resistors to achieve a desired pulse length.

High VM: High Voltage Mode on the ECM 600 uses a bank of parallel resistors to achieve a desired pulse length.

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1. Unpacking and Installation

The shipping carton in which your ELECTRO CELL MANIPULATOR (ECM 600) 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 for any external damage resulting from shipment.

Open the carton and carefully remove the ECM 600 and inspect the unit for any apparent damage. Save the carton and packing materials for future transportation and shipping requirements.

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 or damaged.

POWER SOURCE

As received, the instrument is ready for use with either 105-115 V AC or 220-240 V AC., 50/60 Hz. It is not possible to switch between the two options.

The power requirements are 300 watts. In the U.S.A. the power cord has a standard three prong plug. If you have the older two prong power outlet, ground the tab extending from the 3-2 wire plug adaptor and plug it into your outlet. For safety reasons, it is mandatory to ground the instrument.

INSTALLATION

Once you have determined that the component items of the ECM 600 System have not sustained any obvious damage in shipment, proceed with the installation. The location of the ECM 600 should be a dry, level surface, free from extremes in ambient temperature, dust or chemical exposures. Unpack the Safety Stand, Cuvette Rack and Disposable Cuvette Chambers.

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2. Technical Description

SIZE AND WEIGHT OF THE ECM 600 GENERATOR

	Width		Height		Depth
SIZE	13"	x	7"	x	13.5"
WEIGHT			17 lbs.		

ELECTRICAL DESCRIPTION

ELECTRICAL INPUTS AND OUTPUTS
Power : 105-125 VAC, single phase 3A or 220-240 VAC, single phase 1.5A

Low voltage mode:	LOW VM
Electroporation pulse amplitude:	50 - 500 V Peak
Capacitance Range:	25 - 3175 µF
High voltage mode	HIGH VM
Electroporation pulse amplitude:	50 - 2500 V Peak
Capacitance fixed:	50 µF
Timing resistors:	13,24,48,72,129,186, 246,360,480,720 ohms

FRONT PANEL CONTROLS

CODE	NAME	FUNCTION
P	**POWER**	Turns unit ON/OFF. Small indicator light illuminates when power is on. Push to operate.
A	**AUTOMATIC CHARGE & PULSE**	Initiates charging of the internal capacitors to the set voltage and delivers a pulse to the sample chamber in an automatic cycle that takes less than five seconds. Push button and release to operate. During charging the green indicator light glows. After the unit discharges, the green indicator light starts blinking for approximately 15 seconds. Pressing the reset button during this period will not reset the generator. After the green light stops blinking, the generator can be reset and the Automatic Charge and Pulse cycle can be repeated.

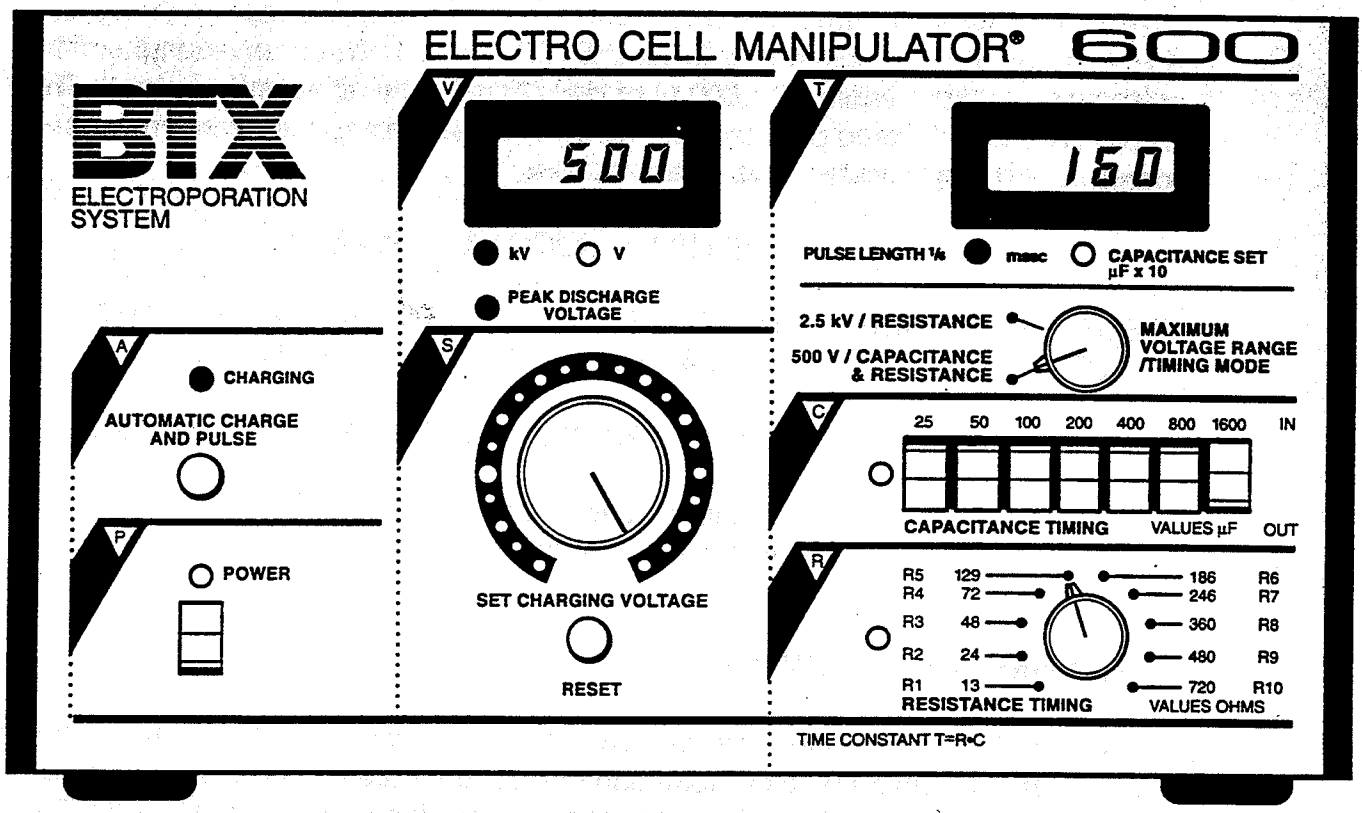
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CODE	NAME	FUNCTION
S	**SET CHARGING VOLTAGE**	This knob adjusts the amplitude of the set charging voltage applied to the internal capacitors from 50 to 500 volts in Low VM and from 0.05 to 2.5 kV in the High VM. The amplitude is shown on the display above the knob. Turn clockwise to increase, counterclockwise to decrease.
	RESET	After a pulse, Meter V displays Peak Discharge Voltage and Meter T shows Actual Pulse Length Press Reset to switch the displays to Setup Mode. Now Meter V shows Set Voltage and Meter T shows Set Capacitance in LOW VM
	MAXIMUM VOLTAGE RANGE / TIMING MODE	Use the knob to switch between High Voltage Mode (HIGH VM) 2.5 kV / RESISTANCE and Low Voltage Mode (LOW VM) 500 V / CAPACITANCE & RESISTANCE.
R	**RESISTANCE TIMING**	The pulse length of the electroporation pulse is set by selecting one of the ten timing resistors marked R1 through R10. They are active in both HIGH VM and LOW VM. The capacitance is fixed at 50 µF.

Number	Values Ohms
R1	13
R2	24
R3	48
R4	72
R5	129
R6	186
R7	246
R8	360
R9	480
R10	720

C	**CAPACITANCE**	In LOW VM only, the capacitance is set by switching in any combination of the seven additive capacitors. The total selected is displayed on Meter T (µF x 10).

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OPERATING MODES

The ECM 600 allows the user to work in two different voltage and timing modes. A clear understanding of the field strength and pulse length capabilities of the ECM 600, will substantially enhance the value of this System to the researcher. The following description is designed to show the wide range of pulse length options which are available and how to find the most convenient settings to achieve a particular pulse length.

High Voltage Mode (HIGH VM)

This mode is chosen when voltages over 500 V (field strengths >2.5 kV/cm) are required. High specific resistivity media are preferred. The maximum voltage is 2.5 kV. The pulse length is internally controlled by resistors parallel to the output. In this mode, the capacitance is fixed at 50 µF. Table 1 shows an example of the pulse lengths achievable with 600 µl of dd-H_2O using the different resistors*. The HIGH HV mode is used primarily for the transformation of bacteria and microorganisms, and some mammalian transfections.

TABLE 1 HIGH VM dd-H_2O

RESISTOR NUMBER	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
VALUE OHM	13	24	48	72	129	186	246	360	480	720
TIME (msec)	0.7	1.2	2.4	3.5	6.0	8.6	11.2	17.7	22.5	32.8

* actual values may differ slightly due to variations in capacitor performance between units

Low Voltage Mode (LOW VM)

This mode is chosen when voltages up to 500 V are required. In this mode, pulse lengths are controlled by a combination of resistors parallel to the output and a bank of seven selectable additive capacitors providing a range from 25 - 3175 µF. Tables 3,5,7, and 9 show the theoretical pulse length for any available combination of capacitors and resistors when used with various buffers.

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FACTORS AFFECTING PULSE LENGTH

Using the t=RC equation, a number of variables can affect the actual pulse length delivered as explained below:

The capacitors used in the ECM 600 are of the electrolytic type which are commonly and widely used in exponential decay pulse generators. The performance tolerances quoted by the capacitor manufacturers, however, range from -10% to +50% in capacity from the actual specified nominal value. This difference can cause the theoretical pulse length value to be different from what is actually delivered.

Specific resistivity (r) of the commonly used electroporation buffers can range as much as 2 logs. Since r is a major factor in calculating the total chamber load (resistance), this type of variance can dramatically affect the actual pulse length obtained.

Pulse length decreases with temperature and the electric field, but increases if gas bubbles are present. Varying the volume in the chamber, for low specific resistivity media such as PBS, will change the pulse length inversely; i.e. one half the volume will double the pulse length.

Taking all of these factors into account means that the actual pulse length can be significantly different than the estimated value for an individual capacitor or a series of capacitors as shown in the Tables. The ECM 600 overcomes this potentially troublesome situation by actually measuring the pulse length delivered to the chamber using a sophisticated internal monitoring circuit. Very accurate monitoring of chamber field conditions is a tremendous advantage: in rapidly establishing optimal experimental conditions, when changing between chambers with different electrode geometries, and when changing electroporation media.

DESIRED PULSE LENGTH

The following are ECM 600 setting guidelines for use with various buffers and the BTX Disposable Cuvette Chamber (2 mm gap) to achieve a desired field strength / pulse length combination. Please note that in both HIGH VM and LOW VM modes any resistance setting or resistance / capacitance combination can be selected without causing damage to the ECM 600. However, high field strengths and long pulse lengths can lead to severe arcing between the electrode gap. In addition, samples that do not arc can be subjected to a large temperature jump in the medium, in some instances as high as 50°C. This can have a deleterious effect on cell viability.

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High resistivity buffer: Mannitol

Specific Resistivity = 10,000 ohm * cm
Chamber Resistance = 1,000 ohm

In HIGH VM mode use R1 through R10 (13 - 720 ohm). The pulse length should range from 0.6 to 21 msec.

TABLE 2 MANNITOL
Nominal Pulse length in msec for different resistance / capacitance combinations

HIGH VOLTAGE MODE

RESISTOR NUMBER	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
Resistance, Ohm	13	24	48	72	129	186	246	360	480	720
Capacitance 50 µF										
Pulse length (msec)	0.6	1.2	2.3	3.4	5.7	7.8	9.9	13.2	16.2	20.9

In LOW LV mode use R1 through R10 (13 - 720 ohm). The pulse length should range from 0.3 msec to 1.3 sec. Please refer to TABLE 3 at the end of this section.

Medium High resistivity buffer: Hepes Buffered Sucrose

Specific Resistivity = 5,600 ohm * cm
Chamber Resistance = 560 ohm

In HIGH VM mode use R1 through R10 (13 - 720 ohm). The pulse length should range from 0.7 to 15.8 msec.

TABLE 4 HEPES BUFFERED SUCROSE
Nominal Pulse length in msec for different resistance / capacitance combinations

HIGH VOLTAGE MODE

RESISTOR NUMBER	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
Resistance, Ohm	13	24	48	72	129	186	246	360	480	720
Capacitance 50 µF										
Pulse length (msec)	0.6	1.2	2.2	3.2	5.2	7.0	8.6	11.0	13.0	15.8

In LOW VM mode use R1 through R10 (13 - 720 ohm). The pulse length should range from 0.3 msec to 1 sec. Please refer to table Table 5 at the end of this section.

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Medium Low resistivity buffer: Phosphate Buffered Sucrose

Specific Resistivity = 1,200 ohm * cm
Chamber Resistance = 120 ohm

In HIGH VM use R1 through R10 (13 to 720 ohm). The pulse length will range from 0.6 to 5.1 msec.

TABLE 6 PHOSPHATE BUFFERED SUCROSE
Nominal Pulse length in msec for different resistance / capacitance combinations

HIGH VOLTAGE MODE

RESISTOR NUMBER	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
Resistance, Ohm	13	24	48	72	129	186	246	360	480	720
Capacitance 50 µF										
Pulse length (msec)	0.6	1.0	1.7	2.3	3.1	3.6	4.0	4.5	4.8	5.1

In LOW VM use R1 through R10 (13 to 720 ohm). The pulse length will range from 0.3 -186 msec. Please refer to TABLE 7 at the end of this section.

Low resistivity buffer: Phosphate Buffered Saline (PBS)

Specific Resistivity = 100 ohm * cm
Chamber Resistance = 10 ohm

In HIGH VM , the pulse length is mainly determined by the chamber, independently of any resistance setting.

TABLE 8 PHOSPHATE BUFFERED SALINE
Nominal Pulse length in msec for different resistance / capacitance combinations

HIGH VOLTAGE MODE

RESISTOR NUMBER	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
Resistance, Ohm	13	24	48	72	129	186	246	360	480	720
Capacitance 50 µF										
Pulse length (msec)	0.28	0.35	0.41	0.44	0.46	0.47	0.48	0.49	0.49	0.49

In LOW VM , use R1 through R10 (13 - 720 ohm). The pulse length will range from 0.14 - 31 msec. Please refer to TABLE 9 at the end of this section.

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REAR PANEL

OUTPUT

For safety reasons the ECM 600 output is located on the rear panel of the enclosure. Connect the Safety Stand P/N 630 to the HIGH VOLTAGE OUTPUT, red to red and black to black. Push banana plugs in hard to make a good connection. Incorrect color connections will not affect instrument performance.

FUSE

A 3 amp fuse is located in a fuse holder. Check the fuse if the power light does not come on when the ECM 600 is connected to the power outlet and the power is switched on. Turn power off before removing the fuse.

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TABLE 3 MANNITOL — HIGH RESISTIVITY MEDIUM (10,000 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations in BTX Disposable Cuvette Chamber P/N 620 2.0 mm gap. Chamber Resistance 1000 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
RESISTANCE, Ohm		13	24	48	72	129	186	246	360	480	720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.32	0.59	1.15	1.68	2.86	3.92	4.94	6.62	8.11	10.47
	50	0.64	1.17	2.29	3.36	5.71	7.84	9.87	13.24	16.22	20.93
	100	1.28	2.34	4.58	6.72	11.43	15.68	19.74	26.47	32.43	41.86
	200	2.57	4.69	9.16	13.43	22.85	31.37	39.49	52.94	64.86	83.72
	300	3.85	7.03	13.74	20.15	34.28	47.05	59.23	79.41	97.30	125.58
	400	5.13	9.38	18.32	26.87	45.70	62.73	78.97	105.88	129.73	167.44
	500	6.42	11.72	22.90	33.58	57.13	78.41	98.72	132.35	162.16	209.30
	600	7.70	14.06	27.48	40.30	68.56	94.10	118.46	158.82	194.59	251.16
	700	8.98	16.41	32.06	47.01	79.98	109.78	138.20	185.29	227.03	293.02
	800	10.27	18.75	36.64	53.73	91.41	125.46	157.95	211.76	259.46	334.88
	900	11.55	21.09	41.22	60.45	102.83	141.15	177.69	238.24	291.89	376.74
	1000	12.83	23.44	45.80	67.16	114.26	156.83	197.43	264.71	324.32	418.60
	1100	14.12	25.78	50.38	73.88	125.69	172.51	217.17	291.18	356.76	460.47
	1200	15.40	28.13	54.96	80.60	137.11	188.20	236.92	317.65	389.19	502.33
	1300	16.68	30.47	59.54	87.31	148.54	203.88	256.66	344.12	421.62	544.19
	1400	17.97	32.81	64.12	94.03	159.96	219.56	276.40	370.59	454.05	586.05
	1500	19.25	35.16	68.70	100.75	171.39	235.24	296.15	397.06	486.49	627.91
	1600	20.53	37.50	73.28	107.46	182.82	250.93	315.89	423.53	518.92	669.77
	1700	21.82	39.84	77.86	114.18	194.24	266.61	335.63	450.00	551.35	711.63
	1800	23.10	42.19	82.44	120.90	205.67	282.29	355.38	476.47	583.78	753.49
	1900	24.38	44.53	87.02	127.61	217.09	297.98	375.12	502.94	616.22	795.35
	2000	25.67	46.88	91.60	134.33	228.52	313.66	394.86	529.41	648.65	837.21
	2100	26.95	49.22	96.18	141.04	239.95	329.34	414.61	555.88	681.08	879.07
	2200	28.23	51.56	100.76	147.76	251.37	345.03	434.35	582.35	713.51	920.93
	2300	29.52	53.91	105.34	154.48	262.80	360.71	454.09	608.82	745.95	962.79
	2400	30.80	56.25	109.92	161.19	274.22	376.39	473.84	635.29	778.38	1004.65
	2500	32.08	58.59	114.50	167.91	285.65	392.07	493.58	661.76	810.81	1046.51
	2600	33.37	60.94	119.08	174.63	297.08	407.76	513.32	688.24	843.24	1088.37
	2700	34.65	63.28	123.66	181.34	308.50	423.44	533.07	714.71	875.68	1130.23
	2800	35.93	65.63	128.24	188.06	319.93	439.12	552.81	741.18	908.11	1172.09
	2900	37.22	67.97	132.82	194.78	331.36	454.81	572.55	767.65	940.54	1213.95
	3000	38.50	70.31	137.40	201.49	342.78	470.49	592.30	794.12	972.97	1255.81
	3100	39.78	72.66	141.98	208.21	354.21	486.17	612.04	820.59	1005.41	1297.67
	3175	40.75	74.41	145.42	213.25	362.78	497.93	626.85	840.44	1029.73	1329.07

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TABLE 5 HEPES BUFFERED SUCROSE MEDIUM HIGH RESISTIVITY MEDIUM (5,600 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations
in BTX Disposable Cuvette Chamber P/N 620 2.0 mm gap. Chamber Resistance 560 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
RESISTANCE, Ohm		13	24	48	72	129	186	246	360	480	720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.32	0.58	1.11	1.59	2.62	3.49	4.27	5.48	6.46	7.88
	50	0.64	1.15	2.21	3.19	5.24	6.98	8.55	10.96	12.92	15.75
Capacitance	100	1.27	2.30	4.42	6.38	10.48	13.96	17.09	21.91	25.85	31.50
MicroFarad	200	2.54	4.60	8.84	12.76	20.97	27.92	34.18	43.83	51.69	63.00
	300	3.81	6.90	13.26	19.14	31.45	41.89	51.28	65.74	77.54	94.50
	400	5.08	9.21	17.68	25.52	41.94	55.85	68.37	87.65	103.38	126.00
	500	6.35	11.51	22.11	31.90	52.42	69.81	85.46	109.57	129.23	157.50
	600	7.62	13.81	26.53	38.28	62.91	83.77	102.55	131.48	155.08	189.00
	700	8.89	16.11	30.95	44.66	73.39	97.74	119.64	153.39	180.92	220.50
	800	10.16	18.41	35.37	51.04	83.88	111.70	136.73	175.30	206.77	252.00
	900	11.43	20.71	39.79	57.42	94.36	125.66	153.83	197.22	232.62	283.50
	1000	12.71	23.01	44.21	63.80	104.85	139.62	170.92	219.13	258.46	315.00
	1100	13.98	25.32	48.63	70.18	115.33	153.59	188.01	241.04	284.31	346.50
	1200	15.25	27.62	53.05	76.56	125.82	167.55	205.10	262.96	310.15	378.00
	1300	16.52	29.92	57.47	82.94	136.30	181.51	222.19	284.87	336.00	409.50
	1400	17.79	32.22	61.89	89.32	146.79	195.47	239.29	306.78	361.85	441.00
	1500	19.06	34.52	66.32	95.70	157.27	209.44	256.38	328.70	387.69	472.50
	1600	20.33	36.82	70.74	102.08	167.76	223.40	273.47	350.61	413.54	504.00
	1700	21.60	39.12	75.16	108.46	178.24	237.36	290.56	372.52	439.38	535.50
	1800	22.87	41.42	79.58	114.84	188.73	251.32	307.65	394.43	465.23	567.00
	1900	24.14	43.73	84.00	121.22	199.21	265.29	324.74	416.35	491.08	598.50
	2000	25.41	46.03	88.42	127.59	209.70	279.25	341.84	438.26	516.92	630.00
	2100	26.68	48.33	92.84	133.97	220.18	293.21	358.93	460.17	542.77	661.50
	2200	27.95	50.63	97.26	140.35	230.66	307.17	376.02	482.09	568.62	693.00
	2300	29.22	52.93	101.68	146.73	241.15	321.14	393.11	504.00	594.46	724.50
	2400	30.49	55.23	106.11	153.11	251.63	335.10	410.20	525.91	620.31	756.00
	2500	31.76	57.53	110.53	159.49	262.12	349.06	427.30	547.83	646.15	787.50
	2600	33.03	59.84	114.95	165.87	272.60	363.02	444.39	569.74	672.00	819.00
	2700	34.30	62.14	119.37	172.25	283.09	376.99	461.48	591.65	697.85	850.50
	2800	35.57	64.44	123.79	178.63	293.57	390.95	478.57	613.57	723.69	882.00
	2900	36.84	66.74	128.21	185.01	304.06	404.91	495.66	635.48	749.54	913.50
	3000	38.12	69.04	132.63	191.39	314.54	418.87	512.75	657.39	775.38	945.00
	3100	39.39	71.34	137.05	197.77	325.03	432.84	529.85	679.30	801.23	976.50
	3175	40.34	73.07	140.37	202.56	332.89	443.31	542.67	695.74	820.62	1000.13

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TABLE 7 PHOSPHATE BUFFERED SUCROSE MEDIUM LOW RESISTIVITY MEDIUM (1200 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations in BTX Disposable Cuvette Chamber P/N 620 2.0 mm gap. Chamber Resistance 120 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
RESISTANCE, Ohm		13	24	48	72	129	186	246	360	480	720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.29	0.50	0.86	1.13	1.55	1.82	2.02	2.25	2.40	2.57
	50	0.59	1.00	1.71	2.25	3.11	3.65	4.03	4.50	4.80	5.14
	100	1.17	2.00	3.43	4.50	6.22	7.29	8.07	9.00	9.60	10.29
	200	2.35	4.00	6.86	9.00	12.43	14.59	16.13	18.00	19.20	20.57
	300	3.52	6.00	10.29	13.50	18.65	21.88	24.20	27.00	28.80	30.86
	400	4.69	8.00	13.71	18.00	24.87	29.18	32.26	36.00	38.40	41.14
	500	5.86	10.00	17.14	22.50	31.08	36.47	40.33	45.00	48.00	51.43
	600	7.04	12.00	20.57	27.00	37.30	43.76	48.39	54.00	57.60	61.71
	700	8.21	14.00	24.00	31.50	43.52	51.06	56.46	63.00	67.20	72.00
	800	9.38	16.00	27.43	36.00	49.73	58.35	64.52	72.00	76.80	82.29
	900	10.56	18.00	30.86	40.50	55.95	65.65	72.59	81.00	86.40	92.57
	1000	11.73	20.00	34.29	45.00	62.17	72.94	80.66	90.00	96.00	102.86
	1100	12.90	22.00	37.71	49.50	68.39	80.24	88.72	99.00	105.60	113.14
	1200	14.08	24.00	41.14	54.00	74.60	87.53	96.79	108.00	115.20	123.43
	1300	15.25	26.00	44.57	58.50	80.82	94.82	104.85	117.00	124.80	133.71
	1400	16.42	28.00	48.00	63.00	87.04	102.12	112.92	126.00	134.40	144.00
	1500	17.59	30.00	51.43	67.50	93.25	109.41	120.98	135.00	144.00	154.29
	1600	18.77	32.00	54.86	72.00	99.47	116.71	129.05	144.00	153.60	164.57
	1700	19.94	34.00	58.29	76.50	105.69	124.00	137.11	153.00	163.20	174.86
	1800	21.11	36.00	61.71	81.00	111.90	131.29	145.18	162.00	172.80	185.14
	1900	22.29	38.00	65.14	85.50	118.12	138.59	153.25	171.00	182.40	195.43
	2000	23.46	40.00	68.57	90.00	124.34	145.88	161.31	180.00	192.00	205.71
	2100	24.63	42.00	72.00	94.50	130.55	153.18	169.38	189.00	201.60	216.00
	2200	25.80	44.00	75.43	99.00	136.77	160.47	177.44	198.00	211.20	226.29
	2300	26.98	46.00	78.86	103.50	142.99	167.76	185.51	207.00	220.80	236.57
	2400	28.15	48.00	82.29	108.00	149.20	175.06	193.57	216.00	230.40	246.86
	2500	29.32	50.00	85.71	112.50	155.42	182.35	201.64	225.00	240.00	257.14
	2600	30.50	52.00	89.14	117.00	161.64	189.65	209.70	234.00	249.60	267.43
	2700	31.67	54.00	92.57	121.50	167.86	196.94	217.77	243.00	259.20	277.71
	2800	32.84	56.00	96.00	126.00	174.07	204.24	225.84	252.00	268.80	288.00
	2900	34.02	58.00	99.43	130.50	180.29	211.53	233.90	261.00	278.40	298.29
	3000	35.19	60.00	102.86	135.00	186.51	218.82	241.97	270.00	288.00	308.57
	3100	36.36	62.00	106.29	139.50	192.72	226.12	250.03	279.00	297.60	318.86
	3175	37.24	63.50	108.86	142.88	197.39	231.59	256.08	285.75	304.80	326.57

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TABLE 9 PHOSPHATE BUFFERED SALINE (PBS) LOW RESISTIVITY MEDIUM (100 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations in BTX Disposable Cuvette Chamber P/N 620 2.0 mm gap. Chamber Resistance 10 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR RESISTANCE, Ohm		R1 13	R2 24	R3 48	R4 72	R5 129	R6 186	R7 246	R8 360	R9 480	R10 720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.14	0.18	0.21	0.22	0.23	0.24	0.24	0.24	0.24	0.25
	50	0.28	0.35	0.41	0.44	0.46	0.47	0.48	0.49	0.49	0.49
	100	0.57	0.71	0.83	0.88	0.93	0.95	0.96	0.97	0.98	0.99
	200	1.13	1.41	1.66	1.76	1.86	1.90	1.92	1.95	1.96	1.97
	300	1.70	2.12	2.48	2.63	2.78	2.85	2.88	2.92	2.94	2.96
	400	2.26	2.82	3.31	3.51	3.71	3.80	3.84	3.89	3.92	3.95
	500	2.83	3.53	4.14	4.39	4.64	4.74	4.80	4.86	4.90	4.93
	600	3.39	4.24	4.97	5.27	5.57	5.69	5.77	5.84	5.88	5.92
	700	3.96	4.94	5.79	6.15	6.50	6.64	6.73	6.81	6.86	6.90
	800	4.52	5.65	6.62	7.02	7.42	7.59	7.69	7.78	7.84	7.89
	900	5.09	6.35	7.45	7.90	8.35	8.54	8.65	8.76	8.82	8.88
	1000	5.65	7.06	8.28	8.78	9.28	9.49	9.61	9.73	9.80	9.86
	1100	6.22	7.76	9.10	9.66	10.21	10.44	10.57	10.70	10.78	10.85
	1200	6.78	8.47	9.93	10.54	11.14	11.39	11.53	11.68	11.76	11.84
	1300	7.35	9.18	10.76	11.41	12.06	12.34	12.49	12.65	12.73	12.82
	1400	7.91	9.88	11.59	12.29	12.99	13.29	13.45	13.62	13.71	13.81
	1500	8.48	10.59	12.41	13.17	13.92	14.23	14.41	14.59	14.69	14.79
	1600	9.04	11.29	13.24	14.05	14.85	15.18	15.38	15.57	15.67	15.78
	1700	9.61	12.00	14.07	14.93	15.78	16.13	16.34	16.54	16.65	16.77
	1800	10.17	12.71	14.90	15.80	16.71	17.08	17.30	17.51	17.63	17.75
	1900	10.74	13.41	15.72	16.68	17.63	18.03	18.26	18.49	18.61	18.74
	2000	11.30	14.12	16.55	17.56	18.56	18.98	19.22	19.46	19.59	19.73
	2100	11.87	14.82	17.38	18.44	19.49	19.93	20.18	20.43	20.57	20.71
	2200	12.43	15.53	18.21	19.32	20.42	20.88	21.14	21.41	21.55	21.70
	2300	13.00	16.24	19.03	20.20	21.35	21.83	22.10	22.38	22.53	22.68
	2400	13.57	16.94	19.86	21.07	22.27	22.78	23.06	23.35	23.51	23.67
	2500	14.13	17.65	20.69	21.95	23.20	23.72	24.02	24.32	24.49	24.66
	2600	14.70	18.35	21.52	22.83	24.13	24.67	24.98	25.30	25.47	25.64
	2700	15.26	19.06	22.34	23.71	25.06	25.62	25.95	26.27	26.45	26.63
	2800	15.83	19.76	23.17	24.59	25.99	26.57	26.91	27.24	27.43	27.62
	2900	16.39	20.47	24.00	25.46	26.91	27.52	27.87	28.22	28.41	28.60
	3000	16.96	21.18	24.83	26.34	27.84	28.47	28.83	29.19	29.39	29.59
	3100	17.52	21.88	25.66	27.22	28.77	29.42	29.79	30.16	30.37	30.58
	3175	17.95	22.41	26.28	27.88	29.47	30.13	30.51	30.89	31.10	31.32

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MANNITOL — HIGH RESISTIVITY MEDIUM (3,700 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations in BTX Flatpack Chamber P/N 486 0.56 mm gap. Chamber Resistance 370 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
RESISTANCE, Ohm		13	24	48	72	129	186	246	360	480	720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.31	0.56	1.06	1.51	2.39	3.09	3.69	4.56	5.22	6.11
	50	0.63	1.13	2.12	3.01	4.78	6.19	7.39	9.12	10.45	12.22
	100	1.26	2.25	4.25	6.03	9.57	12.38	14.78	18.25	20.89	24.44
	200	2.51	4.51	8.50	12.05	19.13	24.76	29.55	36.49	41.79	48.88
	300	3.77	6.76	12.75	18.08	28.70	37.13	44.33	54.74	62.68	73.32
	400	5.02	9.02	17.00	24.11	38.26	49.51	59.10	72.99	83.58	97.76
	500	6.28	11.27	21.24	30.14	47.83	61.89	73.88	91.23	104.47	122.20
	600	7.54	13.52	25.49	36.16	57.39	74.27	88.66	109.48	125.36	146.64
	700	8.79	15.78	29.74	42.19	66.96	86.64	103.43	127.73	146.26	171.08
	800	10.05	18.03	33.99	48.22	76.52	99.02	118.21	145.97	167.15	195.52
	900	11.30	20.28	38.24	54.24	86.09	111.40	132.98	164.22	188.05	219.96
	1000	12.56	22.54	42.49	60.27	95.65	123.78	147.76	182.47	208.94	244.40
	1100	13.81	24.79	46.74	66.30	105.22	136.15	162.54	200.71	229.84	268.84
	1200	15.07	27.05	50.99	72.33	114.78	148.53	177.31	218.96	250.73	293.28
	1300	16.33	29.30	55.23	78.35	124.35	160.91	192.09	237.21	271.62	317.72
	1400	17.58	31.55	59.48	84.38	133.91	173.29	206.86	255.45	292.52	342.17
	1500	18.84	33.81	63.73	90.41	143.48	185.67	221.64	273.70	313.41	366.61
	1600	20.09	36.06	67.98	96.43	153.04	198.04	236.42	291.95	334.31	391.05
	1700	21.35	38.31	72.23	102.46	162.61	210.42	251.19	310.19	355.20	415.49
	1800	22.61	40.57	76.48	108.49	172.17	222.80	265.97	328.44	376.09	439.93
	1900	23.86	42.82	80.73	114.52	181.74	235.18	280.74	346.68	396.99	464.37
	2000	25.12	45.08	84.98	120.54	191.30	247.55	295.52	364.93	417.88	488.81
	2100	26.37	47.33	89.22	126.57	200.87	259.93	310.30	383.18	438.78	513.25
	2200	27.63	49.58	93.47	132.60	210.43	272.31	325.07	401.42	459.67	537.69
	2300	28.89	51.84	97.72	138.62	220.00	284.69	339.85	419.67	480.56	562.13
	2400	30.14	54.09	101.97	144.65	229.56	297.06	354.62	437.92	501.46	586.57
	2500	31.40	56.35	106.22	150.68	239.13	309.44	369.40	456.16	522.35	611.01
	2600	32.65	58.60	110.47	156.71	248.69	321.82	384.18	474.41	543.25	635.45
	2700	33.91	60.85	114.72	162.73	258.26	334.20	398.95	492.66	564.14	659.89
	2800	35.16	63.11	118.97	168.76	267.82	346.58	413.73	510.90	585.04	684.33
	2900	36.42	65.36	123.22	174.79	277.39	358.95	428.50	529.15	605.93	708.77
	3000	37.68	67.61	127.46	180.81	286.95	371.33	443.28	547.40	626.82	733.21
	3100	38.93	69.87	131.71	186.84	296.52	383.71	458.06	565.64	647.72	757.65
	3175	39.87	71.56	134.90	191.36	303.69	392.99	469.14	579.33	663.39	775.98

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HEPES BUFFERED SUCROSE MEDIUM HIGH RESISTIVITY MEDIUM (2,070 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations in BTX Flatpack Chamber P/N 486 0.56 mm gap. Chamber Resistance 207 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
RESISTANCE, Ohm		13	24	48	72	129	186	246	360	480	720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.31	0.54	0.97	1.34	1.99	2.45	2.81	3.29	3.62	4.02
	50	0.61	1.08	1.95	2.67	3.97	4.90	5.62	6.57	7.23	8.04
	100	1.22	2.15	3.90	5.34	7.95	9.80	11.24	13.14	14.46	16.08
	200	2.45	4.30	7.79	10.68	15.89	19.59	22.48	26.29	28.93	32.16
	300	3.67	6.45	11.69	16.03	23.84	29.39	33.72	39.43	43.39	48.23
	400	4.89	8.60	15.59	21.37	31.79	39.19	44.96	52.57	57.85	64.31
	500	6.12	10.75	19.48	26.71	39.74	48.98	56.21	65.71	72.31	80.39
	600	7.34	12.90	23.38	32.05	47.68	58.78	67.45	78.86	86.78	96.47
	700	8.56	15.05	27.28	37.39	55.63	68.58	78.69	92.00	101.24	112.54
	800	9.79	17.21	31.17	42.74	63.58	78.38	89.93	105.14	115.70	128.62
	900	11.01	19.36	35.07	48.08	71.53	88.17	101.17	118.29	130.17	144.70
	1000	12.23	21.51	38.96	53.42	79.47	97.97	112.41	131.43	144.63	160.78
	1100	13.46	23.66	42.86	58.76	87.42	107.77	123.65	144.57	159.09	176.85
	1200	14.68	25.81	46.76	64.10	95.37	117.56	134.89	157.71	173.55	192.93
	1300	15.90	27.96	50.65	69.45	103.32	127.36	146.13	170.86	188.02	209.01
	1400	17.12	30.11	54.55	74.79	111.26	137.16	157.37	184.00	202.48	225.09
	1500	18.35	32.26	58.45	80.13	119.21	146.95	168.62	197.14	216.94	241.17
	1600	19.57	34.41	62.34	85.47	127.16	156.75	179.86	210.29	231.41	257.24
	1700	20.79	36.56	66.24	90.81	135.10	166.55	191.10	223.43	245.87	273.32
	1800	22.02	38.71	70.14	96.15	143.05	176.35	202.34	236.57	260.33	289.40
	1900	23.24	40.86	74.03	101.50	151.00	186.14	213.58	249.71	274.79	305.48
	2000	24.46	43.01	77.93	106.84	158.95	195.94	224.82	262.86	289.26	321.55
	2100	25.69	45.16	81.83	112.18	166.89	205.74	236.06	276.00	303.72	337.63
	2200	26.91	47.31	85.72	117.52	174.84	215.53	247.30	289.14	318.18	353.71
	2300	28.13	49.46	89.62	122.86	182.79	225.33	258.54	302.29	332.65	369.79
	2400	29.36	51.62	93.52	128.21	190.74	235.13	269.79	315.43	347.11	385.86
	2500	30.58	53.77	97.41	133.55	198.68	244.92	281.03	328.57	361.57	401.94
	2600	31.80	55.92	101.31	138.89	206.63	254.72	292.27	341.71	376.03	418.02
	2700	33.03	58.07	105.20	144.23	214.58	264.52	303.51	354.86	390.50	434.10
	2800	34.25	60.22	109.10	149.57	222.53	274.31	314.75	368.00	404.96	450.17
	2900	35.47	62.37	113.00	154.92	230.47	284.11	325.99	381.14	419.42	466.25
	3000	36.70	64.52	116.89	160.26	238.42	293.91	337.23	394.29	433.89	482.33
	3100	37.92	66.67	120.79	165.60	246.37	303.71	348.47	407.43	448.35	498.41
	3175	38.84	68.28	123.71	169.61	252.33	311.05	356.90	417.29	459.20	510.47

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PHOSPHATE BUFFERED SUCROSE — MEDIUM LOW RESISTIVITY MEDIUM (440 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations in BTX Flatpack Chamber P/N 486 0.56 mm gap. Chamber Resistance 44 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
RESISTANCE, Ohm		13	24	48	72	129	186	246	360	480	720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.25	0.39	0.57	0.68	0.82	0.89	0.93	0.98	1.01	1.04
	50	0.50	0.78	1.15	1.37	1.64	1.78	1.87	1.96	2.02	2.07
	100	1.00	1.55	2.30	2.73	3.28	3.56	3.73	3.92	4.03	4.15
	200	2.01	3.11	4.59	5.46	6.56	7.12	7.46	7.84	8.06	8.29
	300	3.01	4.66	6.89	8.19	9.84	10.67	11.20	11.76	12.09	12.44
	400	4.01	6.21	9.18	10.92	13.12	14.23	14.93	15.68	16.12	16.59
	500	5.02	7.76	11.48	13.66	16.40	17.79	18.66	19.60	20.15	20.73
	600	6.02	9.32	13.77	16.39	19.69	21.35	22.39	23.52	24.18	24.88
	700	7.02	10.87	16.07	19.12	22.97	24.91	26.13	27.45	28.21	29.03
	800	8.03	12.42	18.37	21.85	26.25	28.47	29.86	31.37	32.24	33.17
	900	9.03	13.98	20.66	24.58	29.53	32.02	33.59	35.29	36.27	37.32
	1000	10.04	15.53	22.96	27.31	32.81	35.58	37.32	39.21	40.31	41.47
	1100	11.04	17.08	25.25	30.04	36.09	39.14	41.06	43.13	44.34	45.61
	1200	12.04	18.64	27.55	32.77	39.37	42.70	44.79	47.05	48.37	49.76
	1300	13.05	20.19	29.84	35.50	42.65	46.26	48.52	50.97	52.40	53.91
	1400	14.05	21.74	32.14	38.23	45.93	49.82	52.25	54.89	56.43	58.05
	1500	15.05	23.29	34.43	40.97	49.21	53.37	55.99	58.81	60.46	62.20
	1600	16.06	24.85	36.73	43.70	52.49	56.93	59.72	62.73	64.49	66.35
	1700	17.06	26.40	39.03	46.43	55.78	60.49	63.45	66.65	68.52	70.49
	1800	18.06	27.95	41.32	49.16	59.06	64.05	67.18	70.57	72.55	74.64
	1900	19.07	29.51	43.62	51.89	62.34	67.61	70.92	74.50	76.58	78.79
	2000	20.07	31.06	45.91	54.62	65.62	71.17	74.65	78.42	80.61	82.93
	2100	21.07	32.61	48.21	57.35	68.90	74.72	78.38	82.34	84.64	87.08
	2200	22.08	34.16	50.50	60.08	72.18	78.28	82.11	86.26	88.67	91.23
	2300	23.08	35.72	52.80	62.81	75.46	81.84	85.85	90.18	92.70	95.37
	2400	24.08	37.27	55.10	65.54	78.74	85.40	89.58	94.10	96.73	99.52
	2500	25.09	38.82	57.39	68.28	82.02	88.96	93.31	98.02	100.76	103.66
	2600	26.09	40.38	59.69	71.01	85.30	92.51	97.04	101.94	104.79	107.81
	2700	27.09	41.93	61.98	73.74	88.58	96.07	100.78	105.86	108.82	111.96
	2800	28.10	43.48	64.28	76.47	91.87	99.63	104.51	109.78	112.85	116.10
	2900	29.10	45.04	66.57	79.20	95.15	103.19	108.24	113.70	116.89	120.25
	3000	30.11	46.59	68.87	81.93	98.43	106.75	111.97	117.62	120.92	124.40
	3100	31.11	48.14	71.17	84.66	101.71	110.31	115.70	121.54	124.95	128.54
	3175	31.86	49.31	72.89	86.71	104.17	112.97	118.50	124.49	127.97	131.65

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PHOSPHATE BUFFERED SALINE — LOW RESISTIVITY MEDIUM (37 Ohm*cm SPECIFIC RESISTIVITY)

Nominal pulse length in msec for different resistance / capacitance combinations in BTX Flatpack Chamber P/N 486 0.56 mm gap. Chamber Resistance 3.7 Ohms.

LOW VOLTAGE MODE

TIMING RESISTOR		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
RESISTANCE, Ohm		13	24	48	72	129	186	246	360	480	720
ALL VALUES IN msec											
CAPACITANCE (μF)	25	0.07	0.08	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
	50	0.14	0.16	0.17	0.18	0.18	0.18	0.18	0.18	0.18	0.18
	100	0.29	0.32	0.34	0.35	0.36	0.36	0.36	0.37	0.37	0.37
	200	0.58	0.64	0.69	0.70	0.72	0.73	0.73	0.73	0.73	0.74
	300	0.86	0.96	1.03	1.06	1.08	1.09	1.09	1.10	1.10	1.10
	400	1.15	1.28	1.37	1.41	1.44	1.45	1.46	1.46	1.47	1.47
	500	1.44	1.60	1.72	1.76	1.80	1.81	1.82	1.83	1.84	1.84
	600	1.73	1.92	2.06	2.11	2.16	2.18	2.19	2.20	2.20	2.21
	700	2.02	2.24	2.40	2.46	2.52	2.54	2.55	2.56	2.57	2.58
	800	2.30	2.56	2.75	2.82	2.88	2.90	2.92	2.93	2.94	2.94
	900	2.59	2.89	3.09	3.17	3.24	3.27	3.28	3.30	3.30	3.31
	1000	2.88	3.21	3.44	3.52	3.60	3.63	3.65	3.66	3.67	3.68
	1100	3.17	3.53	3.78	3.87	3.96	3.99	4.01	4.03	4.04	4.05
	1200	3.46	3.85	4.12	4.22	4.32	4.35	4.37	4.39	4.41	4.42
	1300	3.74	4.17	4.47	4.57	4.68	4.72	4.74	4.76	4.77	4.79
	1400	4.03	4.49	4.81	4.93	5.04	5.08	5.10	5.13	5.14	5.15
	1500	4.32	4.81	5.15	5.28	5.40	5.44	5.47	5.49	5.51	5.52
	1600	4.61	5.13	5.50	5.63	5.75	5.80	5.83	5.86	5.87	5.89
	1700	4.90	5.45	5.84	5.98	6.11	6.17	6.20	6.23	6.24	6.26
	1800	5.18	5.77	6.18	6.33	6.47	6.53	6.56	6.59	6.61	6.63
	1900	5.47	6.09	6.53	6.69	6.83	6.89	6.93	6.96	6.98	6.99
	2000	5.76	6.41	6.87	7.04	7.19	7.26	7.29	7.32	7.34	7.36
	2100	6.05	6.73	7.21	7.39	7.55	7.62	7.65	7.69	7.71	7.73
	2200	6.34	7.05	7.56	7.74	7.91	7.98	8.02	8.06	8.08	8.10
	2300	6.62	7.37	7.90	8.09	8.27	8.34	8.38	8.42	8.44	8.47
	2400	6.91	7.69	8.24	8.45	8.63	8.71	8.75	8.79	8.81	8.83
	2500	7.20	8.01	8.59	8.80	8.99	9.07	9.11	9.16	9.18	9.20
	2600	7.49	8.34	8.93	9.15	9.35	9.43	9.48	9.52	9.55	9.57
	2700	7.78	8.66	9.28	9.50	9.71	9.80	9.84	9.89	9.91	9.94
	2800	8.06	8.98	9.62	9.85	10.07	10.16	10.21	10.25	10.28	10.31
	2900	8.35	9.30	9.96	10.21	10.43	10.52	10.57	10.62	10.65	10.68
	3000	8.64	9.62	10.31	10.56	10.79	10.88	10.94	10.99	11.02	11.04
	3100	8.93	9.94	10.65	10.91	11.15	11.25	11.30	11.35	11.38	11.41
	3175	9.14	10.18	10.91	11.17	11.42	11.52	11.57	11.63	11.66	11.69

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3. Theory of Operation

A. The Transfection Process

Electroporation is the application of high voltage electric field pulses of short duration in order to create temporary pores or holes in the membranes of cells. This process utilizes the fact that the cell membrane is unable to pass current. Exposing the membrane to high voltage electric fields leads to a charge separation or transmembrane potential difference. Opposite electrical charges on the membrane attract each other, exerting pressure on the membrane, inducing membrane thinning. At a specific potential difference (field strength), a temporary breakdown occurs and pores are formed. These pores are generally large enough to allow macromolecules, such as DNA, as well as small molecules, such as ATP, to enter or leave the cell by diffusion. As long as the critical field strength and pulse length (duration) are not exceeded, removal of the electric field results in resealing of the pores. Following closure of the pores, the extra DNA is then free to enter the nucleus and be transcribed or to become integrated into the host genome to generate a permanently transfected line.

B. Exponential Decay Generators

There are several designs of commercial pulse generators. It is important to understand the type of system used when reviewing the scientific literature. The ECM 600 generates a pulse from the complete discharge of a capacitor(s) resulting in an exponentially decaying waveshape. This type of system is characterized by a fast rise time and an exponential tail. Exponential decay generators utilize the t=RC time constant where:

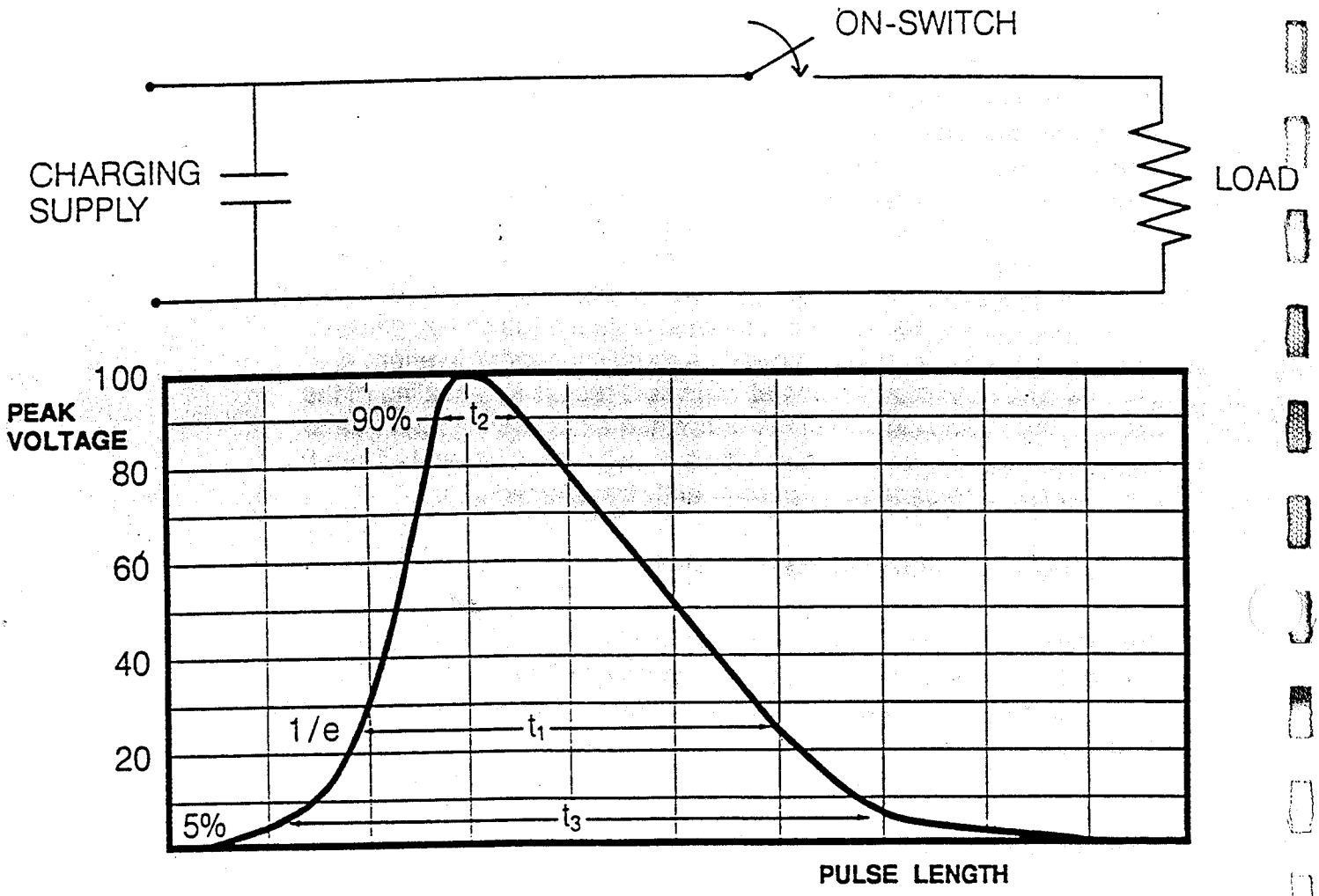
t = time constant or pulse length (in seconds)
The time duration between the steep front of the pulse and the time it takes for the maximum voltage of the pulse to decay in amplitude to 1/e, where e is the base of the natural logarithm. In practice, this is about 1/3 of the maximum value.

R = Resistance (in ohms)
The sum of all of the resistors in the circuit (mainly internal generator resistance plus chamber resistance)

C = Capacitance (in Farads)
the size of the storage capacitors in use

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In an exponential decay system, the circuit design requires only an ON switch as shown below. In this case, the capacitors are fully charged to a voltage (chosen by the user). At the initiation of the start button, the capacitors fully discharge into the sample solution.



Complete Discharge of a Capacitor

t_1 is where the ECM 600 measures the pulse length
t_2 is usually used for square wave generators
t_3 has been used by some authors to define pulse length in their experiments

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C. Pulse Length (t)

A number of variables have to be considered in achieving a desired pulse length with the ECM 600. These include the buffer type, voltage and timing mode, sample volume and chamber type used. Considering the diversity of cell types used in transformation studies, a wide working range is needed to cover the necessary pulse lengths.

The chamber resistance is determined by the type of medium as discussed earlier. It can also be altered by changing the volume in the chamber; i.e. smaller volume, higher load resistance.. Changing the volume in the chamber changes the resistance and the pulse length in an inverse relationship. If the total chamber resistance is much greater than the timing resistor used to generate the pulse, then the timing resistor remains active and gives the desired pulse length. If the chamber resistance is lower than the timing resistor chosen, then the pulse length is determined by the chamber resistance. The load resistance essentially determines the longest pulse length that can be achieved. As a general rule, when using the timing resistors, as in HIGH HV mode, the pulse length is determined by the lowest resistance in the circuit.

The exact pulse length can be calculated from this formula:

$$t = C * (R1 * Ri)/(R1 + Ri)$$

where:
- R1 = Load Resistance, in ohms
- Ri = Timing Resistance, in ohms
- t = Pulse Length, in sec
- C = Total Capacitance, in microFarads (µF)

The following table shows typical resistance values for a variety of BTX chambers when filled with typical electroporation buffers:

Resistivity:	**High**	**Medium**	**Low**
Media:	0.3 M Mannitol	Hepes	PBS
Specific Resistivity:	10,000 ohm * cm	1,000 ohm * cm	10 ohm-cm
Chamber P/N			
610	500	50	5
620	1000	100	10
640	2000	200	20
486	370	37	3.7

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(For a further discussion on this subject, please refer to Chapter 26, Hofmann,GA., Cells in Electric Fields, ELECTROPORATION AND ELECTROFUSION IN CELL BIOLOGY,389-407 (1989), attached)

D. Voltage and Field Strength

The passage of an electrical current across the cell membrane results in the creation of the 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 make-up and individual characteristics of the cell wall itself. For example, some Gram positive bacteria are quite resistant to electroporation and require very high field strengths (>17 kV/cm) before cell death or transformation occurs. Generally, field strength varies inversely to the size of the cell.

The peak field strength in the chambers can be calculated in a very simple way: take the actual peak discharge voltage value as observed on the meter (V) and multiply by a factor which is specific for the chamber type used.

Chamber P/N	Description	Gap mm	Field Strength Factor
610	Cuvette	1.0	0.010
620	Cuvette	2.0	0.005
640	Cuvette	4.0	0.0025
486	Flatpack	0.56	0.018

Example Calculation of Actual Field Strength
[E = Vpk * Field Strength Factor or E = Vpk / gap (cm)]

Cuvette Chamber P/N 620
Voltage Set 700 Volts
Peak Discharge Voltage (Actual) 680 Volts
Field Strength 680 x 0.005 = 3.4kV/cm or 680 / 0.2 = 3400 V/cm

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The formula can also be used to calculate the voltage needed to generate a particular field strength. In this case divide the field strength (kV/cm) by the factor in the table.

Example: Calculation of Actual Voltage
(Vpk = E / Field Strength Factor)

Flatpack Chamber P/N 486	
Desired Field Strength	24 kV/cm
Field Strength Factor	0.018
Amplitude Setting	24 / 0.018 = 1333 V

2. Take the actual peak disharge voltage and divide by the chamber gap in cm. $E = \frac{Vpk}{gap(cm)}$

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ELECTROPORATION AND ELECTROFUSION
IN CELL BIOLOGY
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Chapter 26

Cells in Electric Fields

Physical and Practical Electronic Aspects of Electro Cell Fusion and Electroporation

Gunter A. Hofmann

1. INTRODUCTION

Pulses of an electric field can induce cell fusion (electrofusion) or produce micropores in the cell membrane allowing materials in the media to enter (electroporation). These two techniques have wide application in science, medicine, and biotechnology (Zimmermann, 1982, 1986; Zimmermann and Pilwat, 1982; Hofmann and Evans, 1986). This chapter discusses the physical aspects and the design of instrumentation for the two techniques. It includes the electrical requirements for electrofusion and electroporation, the requirements of the electric generator, and the design of chambers for large-scale electrofusion and electroporation, hereafter referred to as electromanipulation of cells. Not all biological aspects such as characteristics of various cell lines (which are the subject of electromanipulation), genetic selection systems, optimal conditions for fusion and electroporation have been well characterized, but additional information will be found elsewhere in this volume.

An electro cell manipulation system can be divided into the following three main subsystems: (1) the electronic generator or power supply, which provides the electric signals, (2) the chamber which contains the cells and in which the electric signals from the generator create electric fields, and (3) the biological subsystem encompassing the cells and membranes and purposes of the electromanipulation. The interaction of the three subsystems can lead to two basic goals:

- Electroporation—which allows materials to be introduced into the cytoplasm following the dielectric breakdown of the cell membrane (alternatively, the contents can be induced to leak out).
- Fusion—if cells or membranes are first aligned into close contact by dielectrophoresis, then the electrical signal which induces electroporation may also induce fusion.

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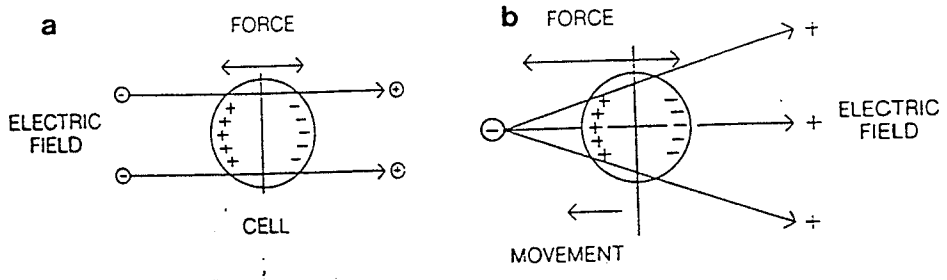


Figure 1. Charge separation and force on a cell in a homogeneous (a) and an inhomogeneous electric field (b).

2. DIELECTROPHORESIS

When cells are exposed to alternating electric fields, they experience a separation of electrical charges, forming an oscillating dipole. If the electric field is homogeneous (Fig. 1a) and the cell density low ($< 10^7/cm^3$) the cell will remain in place. If the cell density is high (10^7–$10^8/cm^3$), dipole interactions between adjacent cells occur, leading to mutual attraction. In the case of an inhomogeneous electric field, the force of the field on the side of the cell closer to the field concentration point (higher field strength) is larger than the force on the opposite side. As a result, the cell moves toward the field concentration area (Fig. 1b). The force on the cell is independent of the polarity of the electric field and always points toward the field concentration area. Therefore, an alternating (AC) electric field can be used to induce cell movement. The waveshape of the alternating field does not appear to be critical to the cell movement. We observed dielectrophoretic movement of cells with sinusoidal, square, and sawtooth-like AC waveforms. As cells approach the area of field concentration, they are attracted to each other and form pearl chains of two or more cells (alignment).

The following two derived relationships govern the movement and alignment of cells in AC electric fields (Pohl, 1978).

The force experienced by a cell is given as:

$$F = r^3 - E\ grad\ E \cdot 2\pi\epsilon_0 \frac{\epsilon-\epsilon_0}{\epsilon+2\epsilon_0}$$

where F is the force on the cell in newtons, r is the radius of the cell in meters, ϵ_0 is the dielectric constant of the liquid in farads per meter, ϵ is the dielectric constant of the cell in farads per meter, E is the electric field strength in volts per meter, and grad E is the measure for inhomogeneity of the electric field in volts per square meter. As a general rule, ϵ decreases with increasing frequency; if $\epsilon > \epsilon_0$, then cells are drawn to higher fields (positive dielectrophoresis); if $\epsilon < \epsilon_0$, then cells are drawn to lower fields (negative dielectrophoresis); if $\epsilon = \epsilon_0$, there is no force on the cells (this is typical for dead cells, where the contents leaked out. Larger cells experience a stronger force (line up faster), and smaller cells need a higher field to be collected. The lower cell radius limit for collection is 0.3 μm.

The relative length L of a pearl chain is derived as (Pohl, 1978):

$$L \sim \sqrt{t}\, V\, c\, r^4$$

where L is the pearl chain length in relative units (micrometers or number of cells), t is the collection (alignment) time, V is the voltage, c is the concentration, and r is the radius of the cells.

If the desire is to create dimers (pairs), the following guidelines should be observed: short

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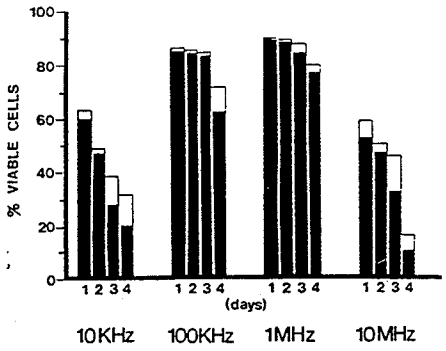


Figure 2. Effect of the alignment AC field on the viability of UC 729-6 (white bar) and PBL (black bar) at different frequencies.

alignment time, low alignment voltage, low cell concentration, moderate divergence of field. Small cells require longer time, higher voltage, higher concentration.

The viability of cells generally appears to be highest if the frequency is between 100 kHz and a few megahertz, as shown for the UC 729-6 cell line in Fig. 2.

3. DIELECTRIC BREAKDOWN—FUSION/ELECTROPORATION

Cells suspended in a liquid can be modeled as a structure consisting of a nonconducting 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 pressure on the membrane, which can induce membrane thinning. Zimmermann (1982) proposed that at a critical potential difference (field strength), localized breakdown of the membrane occurs and pores are formed, allowing a flow of medium or cytoplasm. Based on freeze-fracture electron microscopy, it appears that a large number of adjacent pores might lead to macroscopic cracks in the membrane which allow permeation of large molecules (Stenger and Hui, 1986). However, more work needs to be done before a model evolves which is sufficiently supported by experimental evidence. Removal of the field presumably leads to resealing of the pores provided that the field strength and pulse width are not excessive. If two cells touch each other during the process of pore formation, adjacent pores may form channels which permit communication between cytoplasmic compartments. This process can thus lead to the formation of a new spherical hybrid cell. Excessive field strength and/or duration of the pulses can lead to irreversible changes in the cell by damaging intracellular proteins and DNA.

Fusion yield depends on the following qualitative guidelines (Zimmermann, 1982): Smaller cells require higher fields; excessively high fields or long pulse duration may result in irreversible mechanical breakdown of the membrane; higher temperatures require lower fields; precompression of the cell membrane by pressure gradients (turgor pressure or hydrostatic pressure) leads to a decrease of the required breakdown field; rounding-off time (formation of a complete new spherical cell) may take up to 60 min and is a function of temperature; optimum time for rounding off after fusion is 3 to 5 min; rounding off is aided by adding small amounts of $CaCl_2$ or $MgCl_2$ after fusion; fusion efficiency is size dependent; pretreatment of larger cells with pronase stabilizes against high fields; for hybridoma production, it is recommended that the ratio of lymphocytes to myeloma cells in solution be 3–4 to 1; reapplying AC fields for a few minutes after the fusion pulse may aid the formation of spherical cells.

If the intensity of the electric field is excessive, the cell may lyse due to failure of membrane pores to reseal or osmotic swelling which leads to excess tension in the plasma membrane. Efficient introduction of DNA into cells by means of electroporation was found to occur at a field strength

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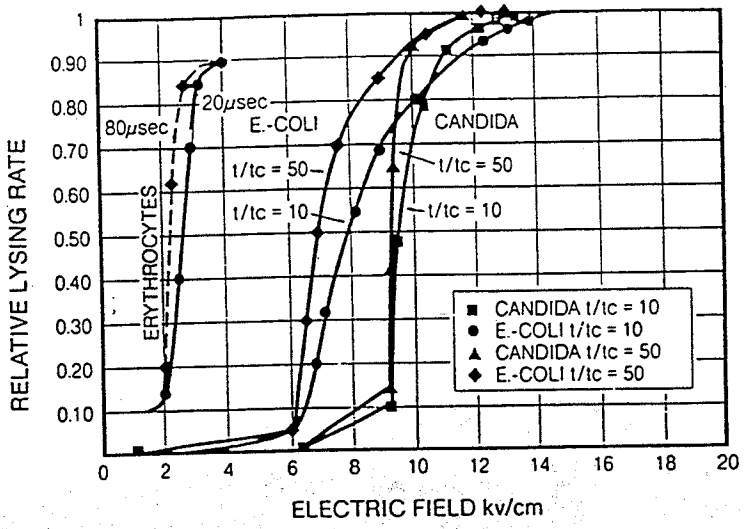


Figure 3. Lysing of cells in an electric field.

where substantial cell lysis takes place (Neumann *et al.*, 1982). The onset of lysing is quite sudden above a critical field strength. An empirical model for the lysis-induced killing of cells that shows some important relationships between experimental parameters has been developed (Hulsheger *et al.*, 1981). The relative rate of lysing can be expressed by the empirical relationship:

$$L = 1-(t/t_c)\exp[-(E-E_c)/K]$$

where L is the relative rate of lysing, t is the treatment time (this is the product of pulse width times the number of pulses, in microseconds), t_c is the threshold value for the treatment time in microseconds, E is the applied electric field strength in kilovolts per centimeter, E_c is the threshold value for the field strength in kilovolts per centimeter, and K is a model constant. t_c, E_c, and K are species-dependent constants. Typical values for bacteria are: t_c = 35 μsec, E_c = 6kV/cm, K = 6.3 cm/kV.

Figure 3 shows a typical lysing curve as a function of applied field strength. The onset of lysing is quite steep and if the field strength is selected below or above the optimum value, no lysing or complete lysing is observed, respectively. A 5 to 80% lysing rate appears to give good electroporation yields. The field strength for high-yield electroporation is critical if the chamber provides a uniform field. If the chamber provides a highly nonuniform field, some part of the chamber volume may be exposed to the optimum field strength even if the applied voltage is not optimal.

Pretreatment of cells changes the field strength at which lysing occurs (Table 1). Figure 4

Table 1. Pretreatment of Cells Changes the Critical Field Strength at Which 50% Lysing Occurs[a]

Cell line	Pretreatment	Field strength, kV/cm (pulse length 30 μsec)
UC 729-6	Trypsin	0.61
	Pepsin	1.27
	Papain	1.58
PBL	None	0.95

[a]Data from Glassy (personal communication).

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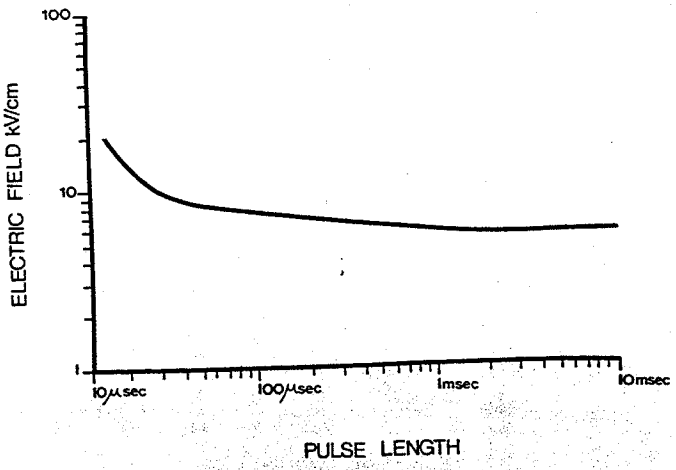


Figure 4. Fifty percent lysing curve of 16 hr *E. coli* as a function of field strength and pulse length. Below a critical field strength, no lysis is observed even with very long pulses.

shows the trade-off between field strength and pulse width to obtain a specific percentage (50%) of lysing, calculated from Eq. (3).

4. THE ELECTRIC GENERATOR

4.1. Frequency

The electric generator provides generally two electric signals: (1) continuous alternating high-frequency electric fields (AC) to align cells into pearl chains, and (2) short, unipolar pulses (often called DC pulses, signals which show only one polarity) of high amplitude. Figure 5 shows a typical sequence of electric signals for a fusion experiment. For electroporation, only the short, unipolar high electric field pulses are required. The parameters which describe the alternating fields are

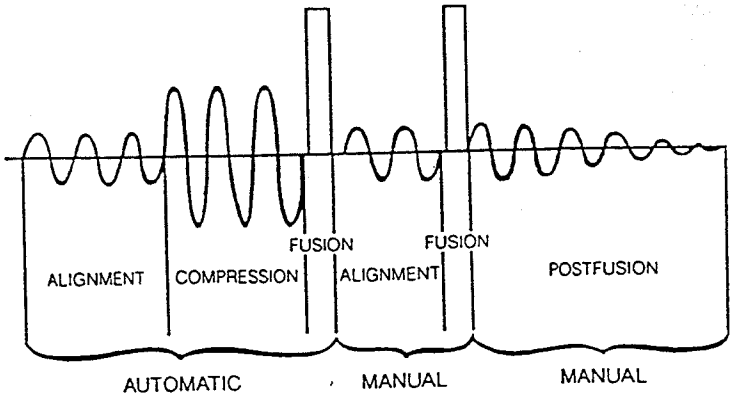


Figure 5. Typical electrical waveforms of a fusion sequence. First applied is the AC alignment field, typically for many seconds. Optional is a short (1 sec) increase in the alignment amplitude (compression) to increase cell proximity. The brief (microseconds) high-amplitude fusion pulse follows the AC signals within milliseconds. It is desirable to be able to set the sequence in an automatic mode.

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frequency, amplitude, and waveshape. Cells can be influenced by an AC field from the kilohertz to the megahertz frequency range. The viability of the cells after exposure to the AC field must remain high for successful cell manipulation. A frequency around 1 MHz appears to work well for a wide variety of cells (Fig. 2).

Cells were found to rotate at certain resonance frequencies (Holzapfel *et al.*, 1982; Zimmermann *et al.*, 1981). This is an undesirable effect for fusion because it interferes with the alignment process. This effect can be minimized by selecting an AC frequency different from the resonant frequency. If the generator has only a fixed frequency, this solution is not available. In this case it is advantageous to use a nonsinusoidal waveshape. Sharp resonance of rotation is more probable with sinusoidal waveshapes than with other waveshapes exhibiting a high content of harmonics. A basically symmetrical square wave of 1 MHz gives good alignment without measurably changing the viability as compared to a sinusoidal waveshape.

The alignment effect is even stronger with a square wave of the same amplitude as a sine wave, because the cells are exposed to the maximum amplitude for a longer period of time. The AC voltage, however, still needs to be symmetrical. A unipolar square wave was found to lead to strong turbulence in the chamber (Glassy and Hofmann, personal communication).

4.2. Power Requirements

Some definitions of typical electrical parameters encountered in the electrofusion/electroporation literature appear to be appropriate. One can choose two different ways to look at an electrical system consisting of a generator and a chamber filled with a cell solution: a macroscopic or a microscopic view.

Macroscopic view: The generator provides a potential difference, V, between the electrodes of a chamber, which is measured in volts. The chamber exhibits a certain electrical resistance, R, which is measured in ohms. The current, I, flowing through the chamber is measured in amperes and can be calculated as $I = V/R$, which is the macroscopic Ohm's law. The electrical power, P, into the chamber is $P = IV$ and is measured in watts. If the power is delivered to the chamber for t seconds, then the energy, Q, delivered into the chamber is $Q = IVt$ and is measured in joules. The cells in the solution do not experience any of these macroscopic parameters directly. If we want to understand what is happening to the cells, we need to take a microscopic view.

Microscopic view: The macroscopic potential difference between the electrodes of the chamber creates a local electric field in the solution which is measured in volts per centimeter. (For a simple parallel plate geometry, it can be calculated by dividing the voltage by the electrode gap.) The resistive properties of the solution can be described by the specific resistivity, r (ohm-cm), which is the inverse of the specific conductivity (mho/cm). Note that the specific resistivity is a medium property and does not depend on the geometry of the chamber. The local electric field, E, generates a local current density, i (A/cm^2), which is determined by the microscopic Ohm's law: $i = E/r$. Now all electrical parameters are defined as local properties to which the cells are subjected.

We can now calculate the local power density and total power requirements for the AC and DC signal from the required field strength E (V/cm) in the chamber, the medium specific resistivity r (ohm-cm), and the chamber volume W(cm^3):

$$\text{Power density (in watts/cm}^3\text{): } p = E^2/r$$

$$\text{Total power (in watts): } P = E^2 \cdot W/r$$

which is equal to VI from the generator. A typical range for the required power is shown in Table 2. For larger volumes the power requirements are quite substantial, especially for the high electric field pulse required for fusion or poration. It becomes a nontrivial engineering task to economically provide high-frequency AC power and DC pulses.

The DC pulse needs to be of much higher amplitude than the AC signal because the necessary

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Table 2. Power Requirements for AC and DC Signals in Typical Small and Large Chambers for a High- and a Low-Resistivity Medium

Chamber type and volume	R (ohms)	Field (V/cm)	Specific resistivity (ohm-cm)	Power (W)
Microslide, 20 μl	850	200	10,000[a]	0.08 (AC)
	8.5	200	100[b]	8.0 (AC)
	850	8000	10,000	3.2 (DC)
	8.5	8000	100	320 (DC)
Production, 50 ml	11	200	10,000	200 (AC)
	0.1	200	100	20,000 (AC)
	11	8000	10,000	8,000 (DC)
	0.1	8000	100	800,000 (DC)

[a]0.3 M mannitol.
[b]Electrolytes such as PBS.

field strength for electroporation is typically between 0.2 kV/cm for plant protoplasts and 10 kV/cm for bacteria. The momentary power during the pulse can be as high as 800,000 watts or more. However, the average power can be much lower (80 watts) because the electroporation pulse (typically 100 μsec in length) may be applied only once per second. In practice a storage device for electrical energy (a capacitor) is charged slowly with low power and then discharged very rapidly, delivering the required high-power pulse.

4.3. Circuit Configurations

The circuit configurations which are generally being used to generate pulses of high power can be grouped into two different types: (1) circuits which require ON–OFF switches (closing and opening a circuit when current is still flowing), resulting in a partial discharge of the energy storage device (mostly a capacitor), and (2) circuits which require only ON switches (i.e., discharging the energy storage device completely: both the current and the voltage go to zero). This distinction is important because it is rather difficult to switch off large currents against the high voltages which might still remain in the capacitor and this difficulty expresses itself in higher costs.

An example of a circuit which requires ON–OFF switches is the partial discharge of a capacitor shown in Fig. 6. The resulting waveshape resembles a square wave. Typical switching elements are vacuum tubes, high-power transistors, or force-commutated (injection of countercurrent to create an artificial zero current) thyristors, also called silicon-controlled rectifiers.

The following circuits require only ON switches: the complete discharge of a capacitor, resulting in an exponentially decaying waveshape (Fig. 7) with a time constant (the time of the maximum voltage to drop to $1/e = 1/2.7$) equal to the product of RC where C is the size of the storage capacitors and R is the sum of all resistances in the circuit (mainly internal generator resistance plus chamber resistance). Another circuit consists of the discharge of a pulse-forming network (an array of capacitors and inductors, or a long coaxial cable, Fig. 8), resulting in a more or less square pulse with some ripple. Useful switches for these circuits are triggered vacuum gaps, triggered spark gaps, thyratrons, thyristors, or even mechanical switches (relays) for some special applications. Fruengel (1965) will be helpful for further study.

The AC circuit needs to be electrically separated from the pulse circuit so there is no uncontrolled addition of the amplitudes nor any destructive interaction between the circuit components. A convenient method is the use of a fast, mechanical, high-voltage relay. This allows switch-over times between typically 30 msec for high-power versions and as short as 1 msec for lower-power requirements. After being aligned and in contact, it appears that cells can tolerate such a pause before the DC pulse is applied. If shorter pauses are desired, electronic switching needs to be

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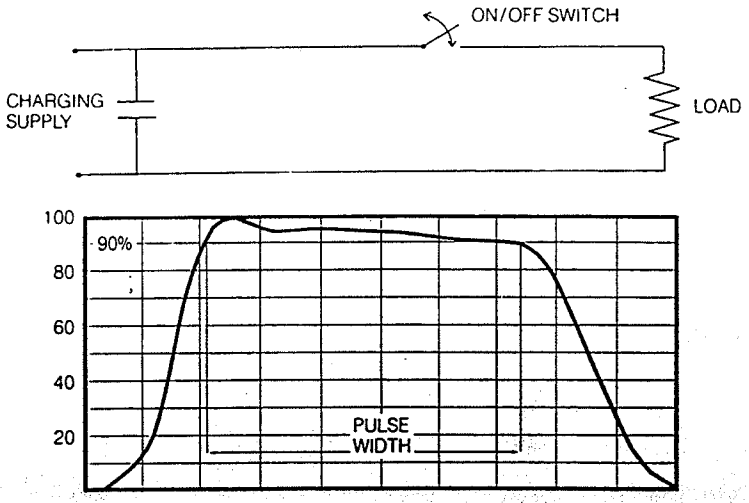


Figure 6. Waveform of a partial capacitor discharge. A convenient way of defining width is the time between the 90% points of maximum amplitude.

employed, but this is not an inexpensive task at high power levels. AC can also be reapplied after the fusion pulse because the membrane stays in a fusogenic state for quite a long time, on the order of many seconds at lower temperatures (Sowers, 1985, 1986).

4.4. Load Matching

The chambers, in which the cell manipulation takes place, are filled with a liquid medium in which the cells are suspended. This medium can exhibit a wide range of specific ohmic resistivities

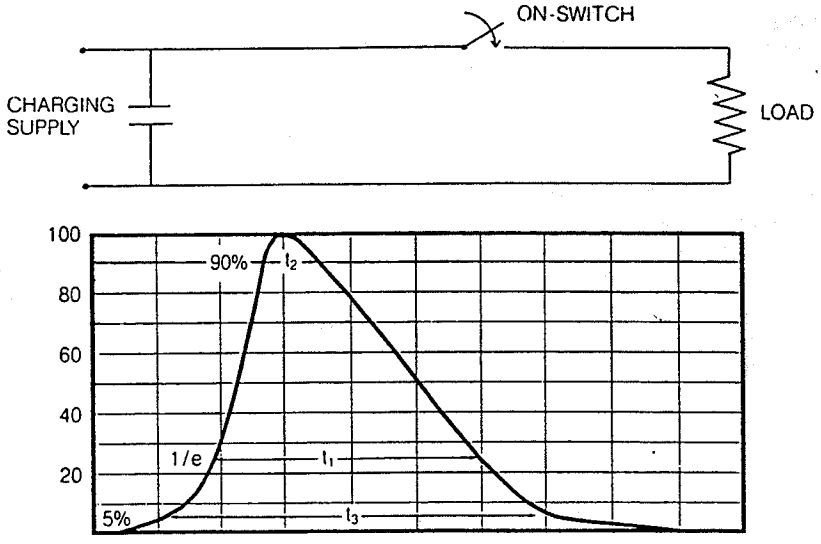


Figure 7. Waveform of a complete capacitor discharge with various definitions of pulse lengths. Most convenient is the $1/e$ time constant which is the product of the storage capacity C and the circuit resistance R (sum of generator and chamber resistance).

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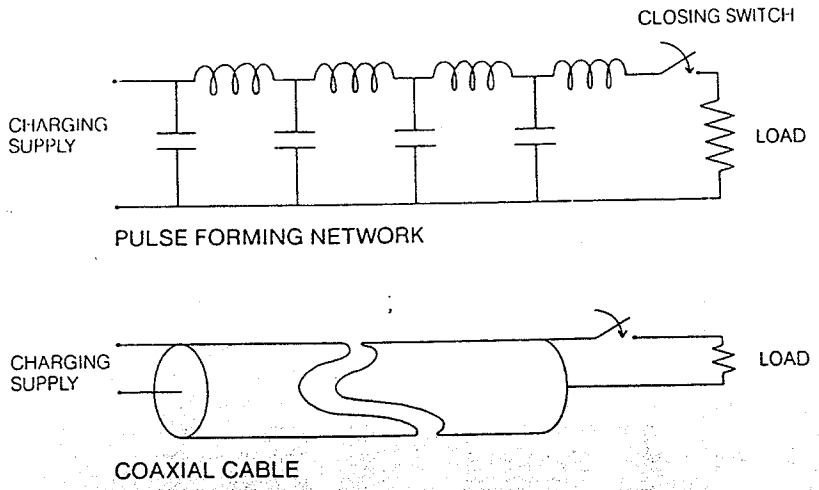


Figure 8. Generation of square pulses with a pulse-forming network (top) or a coaxial cable (bottom). An approximate square pulse will only result if the impedance of the generator (the charged-up pulse-forming network or the coaxial cable) is the same as the impedance of the load (the chamber). In practice, this is unachievable most of the time because of the widely varying medium conductivity. In order to get pulses of microsecond length, the coaxial cable needs to be thousands of feet long.

(ohm-cm), depending on the type of medium and type and amount of additives. Values can range from 10,000 ohm-cm for 0.3 M mannitol to 100 ohm-cm for phosphate-buffered saline solution (PBS) which results in a widely varying chamber resistance (ohm). In either case, medium-filled chambers exhibit a resistive behavior up to frequencies of about 10 MHz. At higher frequencies, capacitive effects start to play a role. Here, we will be concerned with frequencies below 10 MHz and therefore will treat the medium-filled chambers as ohmic resistors. It is important to understand how power is transferred from a generator to a resistive load. If the "match" between generator and load is poor, then the actual voltage on the chamber is substantially lower than what is normally assumed to be the generator output voltage.

Every generator has a certain internal resistance, R_i (measured in ohms), which is defined as the open circuit voltage V_O (no load connected) divided by the short circuit current (the maximum current the generator is capable of delivering). The voltage V on the load is expressed as:

$$V = V_O \cdot R_L/(R_L + R_i)$$

Figure 9 shows the relative voltage on the load for different ratios between load and generator resistance. If the resistance of the chamber, R_L, is much larger than the internal resistance, R_i, of the power supply, then the full open circuit voltage, V_O, appears across the load. This situation is typical for small-volume chambers such as microslides. If both resistances are equal, then half of the open circuit power supply voltage appears across the load. If the load resistance is much smaller than the internal resistance, then only a fraction of the open circuit power supply voltage appears across the load:

$$V = V_O \cdot R_L/R_i$$

This situation represents a poor match between power supply and chamber and is often encountered when working with a highly conductive medium such as PBS in larger volumes. The following is a good example for the importance of understanding the interaction between generator and chamber. Many early experiments in electroporation utilized continuous DC electrophoresis power supplies. Such supplies can be used as a pulse source because most have internal capacitors of capacitance C

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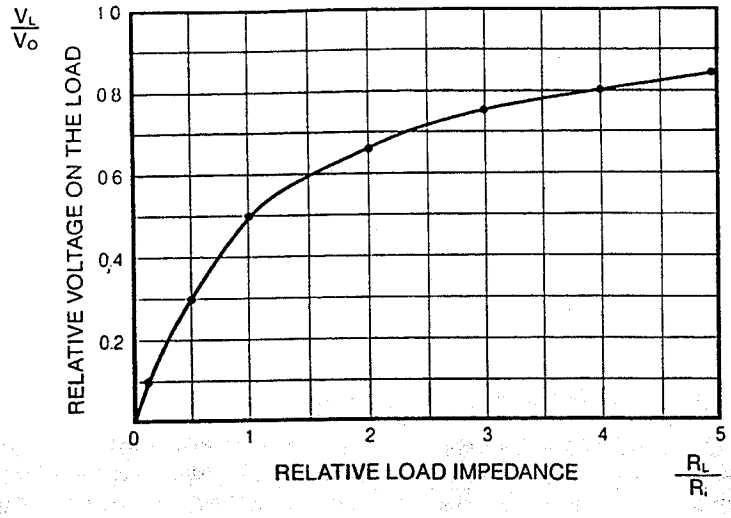


Figure 9. Variation of the load voltage (V_L) with the ratio between loan (chamber) resistance (R_L) and the internal generator resistance (R_i). V_0 is the open circuit (no load) voltage of the generator. This is the maximum generator output voltage.

across the output in order to generate a smooth DC output voltage. If a closing switch is put in series with the output, these capacitors will discharge and provide a pulse depending on R_i, R_L, and C. However, this is a very marginal setup as a pulse generator because the power supply most likely exhibits a high internal impedance. One specific power supply had an internal resistance of about 230 ohms. As a result, the voltage across production-size chambers of low resistance was much lower than the voltage set on the power supply dial. Figure 10 shows the actual voltage on a load for a full power supply setting of 2000 V. If the chamber used with the power supply is a cuvette with a 4-mm gap and the cuvette is filled with PBS or a similar medium, then a pulse of only about 90 V

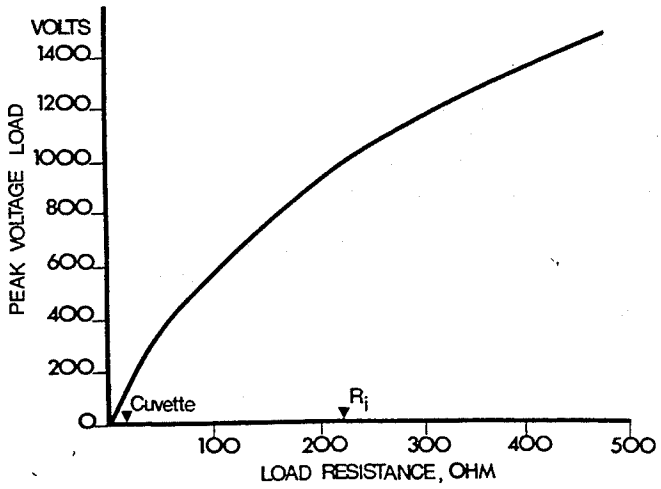


Figure 10. Output voltage of a typical electrophoresis power supply for different load resistances. If the power supply is used with a cuvette filled with PBS, then only a very small fraction of the open circuit voltage appears across the cuvette. This is a result of the high internal impedance (230 ohms) of the power supply.

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will appear across the electrodes. The resulting field strength is only about 0.24 kV/cm. The pulse decay time constant is about 21 msec. If the cuvette is filled with mannitol, 1700 V will be across the cuvette and the field strength is 4.26 kV/cm. The pulse decay time constant is about 60 msec. In either case there is a follow-on DC output after the pulse of typically one-third the pulse amplitude. This will lead to a substantial, uncontrolled heating of the medium (25°C, if left on for 1 sec). Potter *et al.* (1984) have reported success with such a power supply. However, it is not designed specifically for electroporation purposes, and does not work well with PBS or other high-conductivity media. The values for field strength cited in the literature are by more than one order of magnitude too high, making it very difficult to exactly reproduce experiments with other systems. The actual field strength in the chamber is dependent on the medium conductivity and reproducible results are difficult to achieve. There is no choice of pulse length unless some easily controlled variable is changed. In practice, this is not available.

4.5. Practical Design and Safety Considerations

The output voltages of low-internal-resistance electro cell manipulation systems are of dangerous amplitude. Improper handling and carelessness with high-voltage systems can lead to serious injuries or even fatalities. We caution biological researchers attempting to assemble their own power supply. Besides the electrical danger, it will be difficult to duplicate exact conditions as cited in reliable literature unless the biological researcher is prepared to establish a quite sophisticated and expensive electronics laboratory with oscilloscope, wide-band high-voltage probes, and current transformers or shunts. Systems for small chamber volumes such as microslides (20 µl typically) can be assembled from high-impedance (50 ohm), low-voltage commercial pulse and AC signal generators. For most biologists without training in physics and electronics, it is impractical to custom design and build the necessary equipment for large-volume applications.

5. CHAMBERS

5.1. General Requirements

Chambers for fusion and transfection need to create the required field strength and geometry, contain the desired volume, and be easily sterilizable, filled, emptied, and cleaned.

The electric field between electrodes is caused by a potential difference (voltage) and is expressed in volts or kilovolts per centimeter. If the field is homogeneous, as it is between infinite parallel plane electrodes, the field strength can be calculated by dividing the voltage by the electrode gap (cm). The electric field between wire electrodes is higher at the electrode surface than farther away. This increase in field strength over a homogeneous field is called the field enhancement factor and is a function of the specific electrode geometry. It can have values of 5 or more, if the electrodes have sharp edges (Alston, 1968).

Dielectrophoresis (alignment, pearl-chaining) is a consequence of cells being exposed to an inhomogeneous field. The intensity of this effect depends on the electric field strength and gradient and not directly on the voltage between the electrodes. A certain electric field can be achieved either by a high voltage across a large gap or a lower voltage across a smaller gap. For safety reasons, it is preferable to operate with lower voltages and small gaps.

The ohmic resistance of a chamber is a function of the geometry and the specific resistivity, r (ohm-cm), of the solution in the chamber. For the simple case of a parallel plate electrode chamber with a gap between the electrodes of d centimeters and a cross section (electrode area) of q square centimeters, the resistance is:

$$R = r \cdot d/q \quad \text{(ohms)}$$

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The resistance can also be expressed as:

$$R = r \cdot d^2/W$$

where W is the chamber volume in cubic centimeters. The specific resistivity of chamber solutions ranges from 10,000 ohm-cm (0.3 M mannitol) to 100 ohm-cm (salt solutions such as PBS), gaps typically vary from 0.05 cm to 0.4 cm, and the volume from 10 µl to more than 50 ml. The chamber resistance varies accordingly over a very wide range typically from 0.1 ohm or less to 1000 ohms or more. This wide range poses a considerable design challenge for power supplies which are generally efficient only over a narrow range of load resistance (see Section 4.4).

The resistance of the load as it appears to the generator, can be changed by inserting a transformer between the generator and the load. The apparent resistance of the load changes with the square of the turn ratio of the transformer secondary to primary windings. In this way a better match between generator and load can be achieved. However, care needs to be taken in the transformer design so that the high-frequency alignment signal can pass through.

The unipolar (the voltage does not change polarity) fusion/electroporation pulse presents another transformer design requirement, which can be characterized by the product of peak pulse voltage times the pulse duration. If the actual pulse exceeds the design value, the magnetic material of the transformer saturates before the full pulse has passed and cuts the pulse off. This transformer behavior, on the other hand, can be used to advantage to intentionally shorten long exponential pulses from a complete capacitor discharge (Fig. 7). It makes it look like a short square pulse by using a pulse transformer with a volt-second design value substantially below the exponential pulse volt-second value. The magnetic material of the transformer acts in this case like a magnetic off switch.

5.2. Uniform Fields

Chambers with uniform electric fields subject all cells to the same electric field. This is advantageous for electroporation where no pearl chaining is required. High transformation yields are possible provided that the field strength is optimal. The optimum value is not always known, and transformation yields can drop substantially above or below the optimum field strength (Fig. 11).

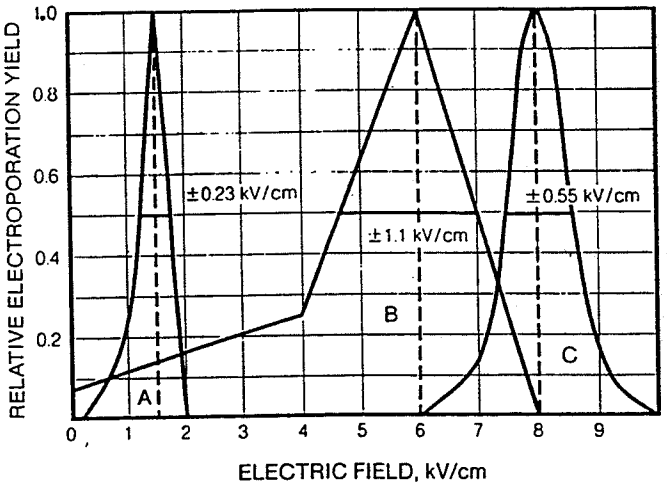


Figure 11. Relative electroporation yield as a function of applied electric field. (A) *Nicotiana tabacum* (Shillito *et al.*, 1985). (B) TK mammalian cells (Zerbib *et al.*, 1985). (C) Mouse L cells (Neumann *et al.*, 1982).

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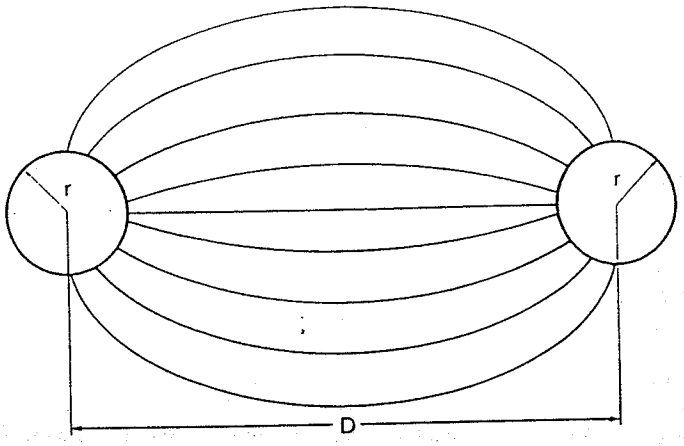


Figure 12. Field between parallel wires, a typical configuration for microslide chambers.

Practical realizations of uniform field chambers are chambers with flat parallel electrodes or to some degree coaxial chambers with a diameter many times larger than the electrode gap (see Fig. 13B). Alignment and fusion are still possible in uniform AC fields provided the cell density is sufficiently high for mutual dielectrophoresis (attraction) to take place. Without an AC field, fusion can be achieved using the avidin/biotin method (Lo *et al.*, 1984), by using high cell density and allowing the cells to settle into a multilayer on the chamber bottom, or by using PEG or other chemicals for cell agglutination.

5.3. Chambers with Nonuniform Fields

Chambers with nonuniform fields allow alignment at low cell densities with an AC field, and reduce the need to have exactly the right field strength in order to get high transfection yields. Generally, any electrode configuration besides an infinite parallel plate configuration provides a nonuniform field. It is desirable to use configurations which are practical to manufacture and have well-defined field shapes.

For small volumes (10 to 100 μl), two simple well-proven geometries are available:

1. Microslides with thin metal sheets separated by a small gap. The field inhomogeneity stems from the effect of adjacent electrode edges and depends on the fine structure of the edges which cannot be easily controlled.
2. A better geometry for small volumes is one which utilizes parallel wires (Fig. 12). The maximum electric field is well defined for this case: $E = V/2r \ln(D/r)$ if $D >> 2r$, where D is the distance between wire centers and r is the wire diameter.

For medium-size volumes of a few milliliters, coaxial configurations can be easily manufactured and give well-defined field configurations. They can be built with high or low divergence or field inhomogeneity (Fig. 13). For a highly divergent field the center electrode can be a wire or thin tubing; for a low-divergence field both electrodes can be tubes with a diameter much larger than the gap between them. The field strength between coaxial electrodes is: $E = V/r \ln(R_2/R_1)$. The chamber volume varies linearly with the chamber length.

Large volumes of 10 ml and more with inhomogeneous fields require a different design approach in order to provide multiple field enhancement points throughout the volume. One chamber design makes use of the field enhancement properties of layers of wire mesh, kept apart by insulating spacers. This "sandwich" construction method can be extrapolated to very large vol-

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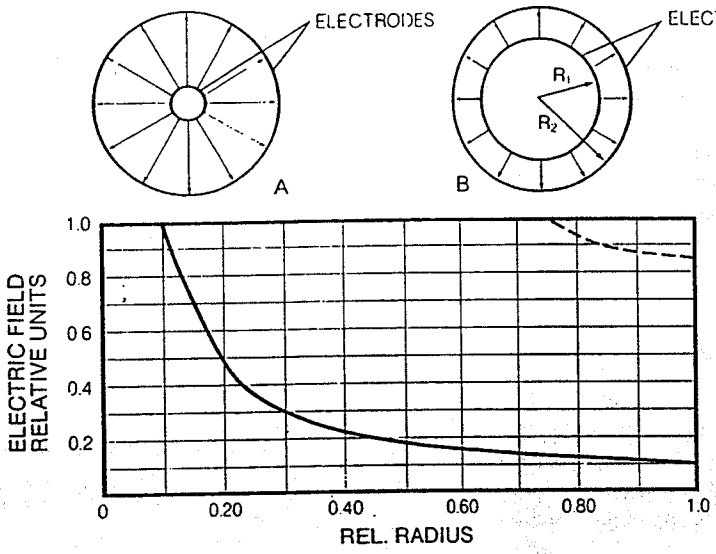


Figure 13. Cross section of coaxial chambers with high-divergence (A) and low-divergence (B) field.

umes. Figure 14 shows the sandwich structure of a chamber with a maximum volume of 75 ml which can be autoclaved and used as a flowthrough chamber. The power supply has to be matched to a chamber of such a large size so that the desired fields for cell manipulation can be achieved.

5.4. Chamber Materials

Preferred isolating materials with good mechanical, electrical, and thermal (for possible autoclaving) properties are polysulfone and nylon. The electrodes need not be made out of noble

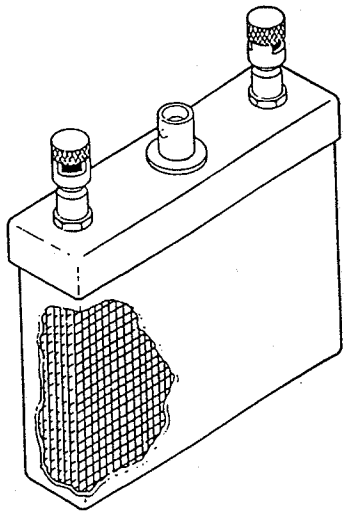


Figure 14. Large-volume (75 ml) chamber with inhomogenous fields. The electrodes consist of alternating layers of wire mesh held apart by insulating spacers. This design lends itself to extrapolation to very large volumes.

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materials such as gold or platinum. We found no detrimental effect on cell viability when stainless steel 316 or 304 was used. Aluminum foil is not recommended because the oxide layer which forms rapidly on an aluminum surface in air is an electrical insulator. Applying a voltage between oxidized aluminum electrodes in a cell suspension liquid might lead to local breakdown of this oxide layer in the form of microscopic arcs which pit the electrode surfaces and eject electrode material into the liquid. This effect is known as the "Malter effect" (Malter, 1936a,b) and can be looked upon as "electroporating the electrodes." Presently available on the market are disposable (sterile) cuvette chambers with aluminum foil electrodes and reusable noble metal or stainless steel electrodes using low-cost disposable plastic cuvettes as the container. Sterilization of the electrodes with alcohol and/or brief flaming was found to be sufficient to prevent cross-contamination (Evans, personal communication).

5.5. Chamber Medium

The medium, in which the cells are suspended, is subjected to the electric field generated by the voltage across the chamber electrodes. The medium will carry an electric current according to its specific resistivity, following Ohm's law. For frequencies of up to several megahertz, the chamber/medium system behaves like a purely ohmic resistor; for higher frequencies, capacitive effects begin to play a role and voltage and current are out of phase with each other. Because the optimum frequencies for alignment with high cell viability in electro cell fusion were found to be around 1 MHz, we can treat the chamber/medium system as a purely ohmic resistance. The specific resistivity of the medium is determined by the concentration of charge carriers (ions). Sugar (e.g., mannitol or sucrose) solutions have a low charge carrier concentration and exhibit a high specific resistivity of typically 10,000 ohm-cm. They are also relatively compatible with cells at least for a limited amount of time if the sugar solution is isosmotic or hyperosmotic with respect to the cytosol of the cell. They are, therefore, a preferred medium for electrofusion and electroporation in cases where heating effects need to be minimized. Solutions with high ionic concentration such as PBS exhibit a low specific resistivity of typically 60 to 150 ohm-cm. For most electroporation experiments, PBS appears to be the preferred medium; however, the electrical power which is required to generate an electric field is about 100 times higher in PBS than in a typical sugar solution. An electrolyte such as PBS exhibits nonlinear electrical characteristics. Its specific electrical resistivity decreases with temperature and with increasing applied field. Fields of up to 18 kV/cm can be generated in an electrolyte (Bishop and Edmonds, 1985). Local inhomogeneities such as air or gas bubbles in a liquid cause a local field enhancement which can lead to arcing, where the current flow restricts to a thin filament, and a possible catastrophic failure or destruction of the chamber.

5.6. Chamber Medium Temperature Rise

The temperature rise of the medium as a consequence of the electric field application needs to be considered as one important system parameter. The temperature influences the critical field strength for membrane poration: it is lower at higher temperatures (Zimmermann, 1982). It should be pointed out that a heat shock might be beneficial to the electroporation process as was demonstrated with a separately applied heat shock (Shillito *et al.*, 1985). We can speculate that a yield improvement might occur if the heat shock is applied directly through the electric field pulse. For this reason and to calculate excessive temperature rise of the medium, we derive general formulas for the temperature rise. Earlier we derived the local power input into the medium as $p = Ei$ or $p = E^2/r$ (where p is in watts/cm^3, E is in V/cm, i is in A/cm^2, and r is in ohm-cm). Integrating the power over time gives the energy e deposited in a volume element (energy density). For a square wave this means multiplying the power by the pulse length t: $e = pt$ (watt-sec/cm^3 or joule/cm^3). This energy is being transformed into thermal energy leading to a rise in temperature T (K). For a square pulse with pulse length t (sec), $T = E^2\, t/4.2\, r$, r being the specific resistivity (ohm-cm). The

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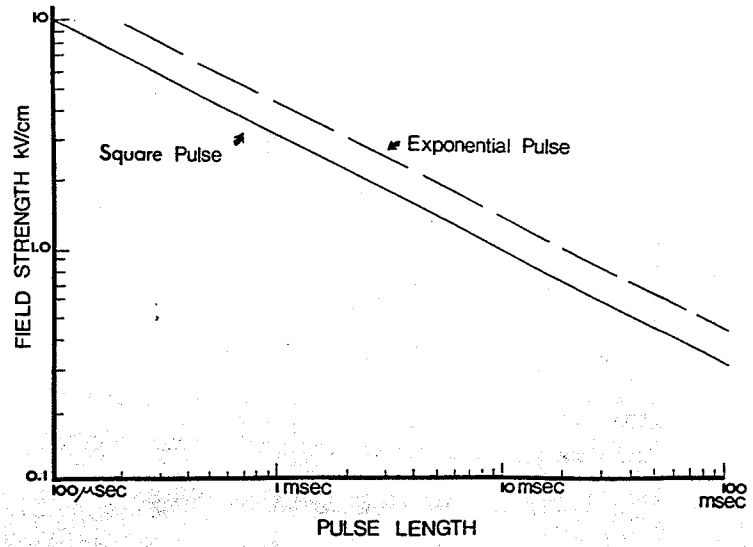


Figure 15. 25 K temperature rise in PBS as a function of field strength and pulse length for square and exponential pulses. For a given field strength the graph shows the pulse length which will result in a 25 K temperature rise of the medium; for a given pulse length, it allows determination of the field strength resulting in such a temperature rise. Pulse length for the exponential pulse is defined as the $1/e$ time constant.

factor 4.2 expresses the fact that 4.2 joules = 1 cal. We assumed that the density (1 g/cm^3) and the specific heat (1 cal/g per K = 4.2 J/g per K) of the medium are close to those of water.

A similar calculation can be performed for exponentially decaying pulses by integrating the momentary power input over the full duration of the pulse: $T = E^2 t/8.4\, r$, where $t = RC$, the $1/e$ decay time constant.

Figure 15 shows a graph of a 25 K temperature rise of the medium as a function of field strength and pulse length for square and exponentially decaying pulses.

6. SYSTEM MONITORING

6.1. Electro Cell Fusion

The most convenient approach is the direct observation of the alignment, fusion, and rounding-off process under a microscope in a microslide chamber of typically 10–20 μl volume. Once good electrical parameters are established by direct observation, similar conditions in larger or different chambers can be achieved by adjusting the generator output voltages so that identical electric fields are realized (see Section 6.3). Optimum yield of viable cell hybrids can be achieved by optimizing the fusion pulse amplitude (Fig. 16).

6.2. Electroporation

The electroporation process cannot be observed under a microscope. However, good electroporation yields are usually achieved with electric field/pulse length conditions which result in lysis of some of the cells (5–80%). This can be utilized to establish good electrical operating parameters by constructing a lysis/viability curve: percentage of cell survival versus field strength. Lysis/viability can be observed either visually, by trypan blue exclusion, methylene blue, or plating and observation of growth. Scale up to different or larger chambers can be done by maintaining the electric field amplitude and pulse length.

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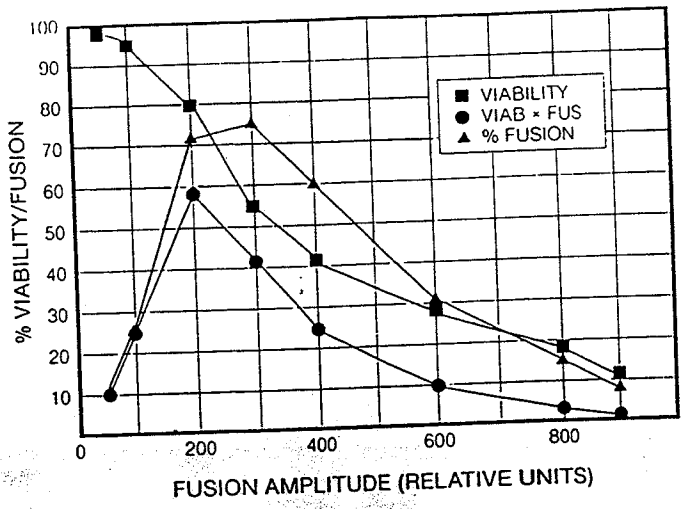


Figure 16. Viability and fusion yield of tobacco protoplasts as a function of applied electric field (Saunders, personal communication).

6.3. Determining the Actual Electrical Parameters in a Chamber

6.3.1. Calculations

The following steps will determine the chamber electrical field strength:

1. Measure the specific resistivity of the medium with a laboratory conductivity meter.
2. Calculate chamber resistance from known chamber geometry and specific resistivity.
3. Calculate the actual voltage on the chamber from the generator output voltage setting, internal resistance of the generator, and chamber resistance (see Section 4.4).
4. Calculate the electric field in the chamber from the actual voltage on the chamber and the geometry.

This process is quite tedious and it still does not yield the shape of the pulse.

6.3.2. Oscilloscope

An electronic oscilloscope and a high-voltage probe can be used to measure the actual voltage on the chamber, eliminating steps 1 to 3 above. The field can then be calculated from the voltage and the chamber geometry.

The pulse shape can be observed with the oscilloscope if it has a wide enough bandwidth. Such oscilloscopes are expensive and require operational knowledge. A commercial instrument is on the market which combines the functions of a digital storage scope with a computer to measure and calculate peak voltage and chamber field and resistance of the chamber as well as the medium specific resistivity.

7. RESULTS AND DISCUSSION

Electro cell manipulation is rapidly becoming an accepted technique in genetic engineering. The literature is growing steadily with hundreds of papers describing experiments in electrofusion or

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Table 3. Comparison of the Techniques of Electromanipulation of Cells

Parameter	Exponentially decaying pulse	Square pulse
Heating of medium	More severe. Beneficial heat shock possible	Minimized
Electric field amplitude	Varies widely during pulse decay	Well defined
Available pulse length	Long	Short
Generator impedance	Lower	Higher
Acquisition costs	Lower	Higher
Efficacy	Can be high	Can be high
Required electric field strength	Lower	Higher
Size of stable pores	Smaller	Larger
Number of stable pores	Larger	Smaller
Possible size of transfectant	Smaller	Larger

electroporation. In some instances the values given for the electric field strength might not represent the actual field strength in the chamber because no electrical measurements were performed. However, it is important to realize that many different cell lines have already been fused or transfected with good yields. Most fusion experiments have been performed in nonionic media. The AC alignment signal, which is applied for a relatively long time (seconds), would lead to substantial heating otherwise. The unipolar fusion pulse is in most cases of short duration (microseconds) and high amplitude (many kV/cm). The electroporation literature falls into two distinctive categories: high fields (many kV/cm)–short pulses (microseconds, often square waveshape) and low fields (few hundred V/cm)–long pulses (milliseconds, exponentially decaying waveshape). Good transfection yields have been reported with both techniques. A systematic side-by-side comparison of the two regimes would be desirable. Some general trade-offs are suggested in Table 3. Biological methods and results are the subject of other chapters in this volume.

ACKNOWLEDGMENTS. I thank Eve Morris for her continuous encouragement and support, and John Fewster for valuable suggestions.

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4. Operation of the ECM 600 System

PROCEDURE

Familiarize yourself with all the front panel controls and read this manual completely before attempting to use the ECM 600.

SAFETY INFORMATION

WARNING HIGH VOLTAGE

The ECM 600 capacitive energy source contains a substantial amount of energy: 400 Joules at full charging voltage. When this energy is rapidly discharged into a low resistance load, a very high power pulse on the order of several million watts can result. Generating high electric fields in the electroporation chambers requires the delivery of such large amounts of electrical energy and power. This energy is transmitted via high voltage cables.

EXTREME CARE NEEDS TO BE TAKEN TO ENSURE SAFE OPERATION

The two main hazards are electric shock to the operator or chamber explosion. The chambers are made of plastic and can disintegrate if used at the maximum settings. An internal short circuit in a connecting cable can cause an explosion. In both instances flying debris can be generated.

The ECM 600 is engineered and manufactured to meet strict, safety standards. This includes being short circuit proof as a further safety measure for the operator.

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PRECAUTIONS

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

DO NOT CHARGE THE ECM 600 TO FULL VOLTAGE WHEN USING SMALL GAP CUVETTE OR FLAT ELECTRODE CHAMBERS.

DO NOT OPEN THE ECM 600 ENCLOSURE.

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

DO NOT ALLOW UNAUTHORIZED OR UNINFORMED PERSONNEL TO OPERATE THE ECM 600.

WEAR SAFETY GLASSES AT ALL TIMES.

READ THE OPERATING MANUAL CAREFULLY.

CONNECT THE SAFETY STAND CABLES TO THE OUTPUT ON THE REAR PANEL SO THAT A GOOD ELECTRICAL CONTACT IS MADE.

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

NOTIFY BTX CUSTOMER SUPPORT - (619) 597-6006 or 1 (800) 289 2465 IF YOU NOTICE ANYTHING UNUSUAL OR HAVE ANY QUESTIONS.

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1. **SET UP**
Connect BTX Safety Stand to unit output in rear using cables supplied. Plug power cord into correct voltage outlet.

2. **SAMPLE PREPARATION**
Load sample into BTX Disposable Chamber, insert Chamber between the Safety Stand contacts. Make sure there is a good contact between the cuvette and the Safety Stand contact.

3. **POWER UP / RESET** **P**
Turn Unit on with **POWER** Switch **P** or press **RESET** if already on. Before next pulse, press **RESET** and adjust settings as desired.

4. **CHOOSE MODE** **T**
MAX VOLTAGE RANGE / TIMING MODE
a) HIGH VOLTAGE (HIGH VM) :
For field strengths > 2.5 kV/cm select
2.5 kV / RESISTANCE
b) LOW VOLTAGE (LOW VM) :
For field strengths < 2.5 kV/cm select
500 V / CAPACITANCE & RESISTANCE

5. **SET CAPACITANCE** **C**
LOW VOLTAGE MODE ONLY
For Capacitance Timing **C** - amber indicator on, switch on individual capacitor values to give total required. All values are additive.
Range is 25 µF to 3175 µF in 25 µF increments.
Total selected (x 10) is displayed on meter **T.**

6. **SET RESISTANCE** **R**
HIGH & LOW VOLTAGE MODES
For Resistance Timing **R** - amber indicator on, turn knob to desired setting. In HIGH VM mode capacitance is fixed internally at @ 50 µF, display **T** reads zero.

7. **SET CHARGING VOLTAGE** **S**
Use knob to adjust charging voltage, value is displayed on meter **V.**

8. **AUTOMATIC CHARGE & PULSE** **A**
Verify that all settings and connections are correct. Press **A** to activate Automatic Charge & Pulse sequence. Charging Light ON. An exponential decay pulse is delivered to the chamber when the Charging light goes out.

9. **MONITORING**
Meter **V** displays Peak Discharge Voltage.
Meter **T** displays Actual Pulse Length in msec.
Record both values. Press **RESET** and repeat.

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5. Experimental Methods

A. Electroporation Parameters

A large number of variables influence the efficiency of gene transfer by electroporation. These include:

* the particular characteristics of the cell line
* the selection system used
* the gene to be introduced
* the use of linear or supercoiled DNA
* the media in which electroporation is done
* the gap width of the chamber used
* the electrical parameters of the instrument
* the strength of the electric field

B. Cell Selection

1. Mammalian

Electroporation has been shown to be an easy means for obtaining a permanently transfected cell line or transient gene expression in mammalian cells. Results show high efficiencies and yields and none of the toxicity associated with traditional chemical methods. Actively growing adherent or cell suspensions may be used. Work by Chu et.al, has shown that best results were obtained when the cells were 50% confluent[1].

2. Bacteria

Electroporation is now used to transform a wide variety of species including the previously recalcitrant gram positives. Intact cells as well as protoplasts may be used. Selection during different stages of the growth cycle is critical to their ability to be transformed. For cell lines where optimal transformation parameters are unknown, it may be necessary to harvest samples at: early-log, mid-log, late-log and stationary phases[2]. Generally, the best conditions are rapid growth and high densities of cells.

Harvest the cells by centrifugation and wash in cold buffer. It is important to wash the cells sufficiently to remove any residual ions from the growth medium. Some cell types can be frozen by resuspending in sterile cold glycerol and pipetted into aliquots for storage at -70°C.

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Additionally, the growth media may make a substantial difference in the electroporation yields and should be investigated. Some cells grown in broth cultures yield better results while others do much better if initially grown on plates.

C. Electroporation Medium or Buffer

The electroporation chambers are filled with a liquid medium in which the cells are suspended. The medium will be subjected to the voltage across the chamber electrodes. This medium will act like an ohmic resistor following Ohm's law due to its specific resistivity. Specific resistivity (in ohm*cm) is determined by the concentration and type of ions in solution. As mentioned previously, different specific resistivities result in different pulse lengths. Low specific resistivity solutions undergo electrolysis at the electrodes resulting in pH changes as well as causing a heating effect, both of which will impact cell viability. For these reasons, it is generally recommended that the lowest possible ionic solution that the cells will tolerate should be used. Mammalian cells are commonly electroporated in PBS or HBS[3,4] plants in Mannitol[5] and bacteria in double-distilled H_2O[2] or 10% glycerol.

D. Pulse Width or Length (t)

See Section 3 page 24

E. Voltage and Field Strength

See Section 3 page 25

F. Plasmid DNA (microorganisms and mammalian cells)

Purified plasmid DNA should be prepared in which the gene of interest and the selectable marker are present in the same vector. Alternatively, a single plasmid may be constructed that contains several desired genes, or a mixture of several separate plasmids may be used. This process is called co-transfection. While co-transfection has been shown to be successful, optimal conditions have not yet been established[6]. Digest the plasmid DNA with a restriction enzyme to linearize the DNA. It is possible to use supercoiled DNA,

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however, efficiencies will be 5-100x higher with linearized DNA[3]. This appears to be the result of the ease with which it is integrated into the cellular genome. Linear DNA is generally used for permanent transfection, while circular DNA, which is more easily packaged into chromatin is usually used for transient gene expression[6].

The frequency of transformation is dependent on the concentration of DNA used. Generally, transformation efficiencies increase correspondingly to increasing amounts of DNA up to about 50-100µg/ml[4].

Plasmid preparation procedures are outlined in Current Protocols[6]. Further purification should be done by using a CsCl/ethidium bromide density gradient. For mammalian cell transfection work performed more than 3-4 days after DNA preparation, sterilize the DNA by ethanol precipitation prior to use.

G. Viral RNA (plants)

Purified viral RNA should be prepared by the procedure described by Saunders et.al[5].

H. Optimization

All of the different variables contribute to establishing the optimum yield for a specific cell type or species. Because slight variations can cause great differences, it is necessary to fine tune these parameters in a process called optimization. The process consists of finding the best buffer system, optimum field strength (voltage) and pulse length.

For those cells that have been cited in the literature, the amount of tests required are minimal. Due to differences in manufacturers equipment, only fine tuning adjustments of pulse length and peak voltage may be required. It is recommended that the voltage be changed in increments of 20-50 V (using 2 mm gap chamber).

For those cells that have not been previously cited, a series of investigative tests must first be run. A good starting point is for those cells that have not been previously cited, a series of investigative tests must first be run. A good starting point is to see if there have been citations of like (similar) cells or of any cells of equal or similar size. As stated previously, there is a good correlation between cell size and field strength. A viability curve will establish the range of voltages to be

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applied. This should be followed by variations in buffer, pulse length and peak voltage. Once the optimum conditions have been determined, the actual efficiency tests may be completed. These are outlined in the next section.

I. Dielectric Breakdown

If the critical field strength is exceeded or the pulse length is too long, the cell may lyse. Above a critical field strength, lysis occurs quite suddenly. Below the optimum field strength,even very long pulse lengths will not result in lysis. This phenomena may be due to either failure of the pores to reseal or by osmotic swelling leading to excess tension in the plasma membrane[6].

Not all lysis is bad, however. Most cells are optimally electroporated at a field strength where substantial cell lysis occurs. A generally accepted lysis rate is 50% (this value should be determined for each cell and for each individual situation).

J. Lysis Curves

In order to establish the optimal electrical parameters for a particular line of cells, a lysis/viability curve should be constructed. In this type of experiment, the percent of cell survival is plotted against field strength. Lysis/viability can be observed visually, by trypan blue exclusion or methylene blue, or by plating and observing growth. Once the optimal field strength is determined, the effects of varying pulse lengths and media may then be investigated.

K. Lysis Curve Procedures

The following procedures are intended to be guidelines in establishing a viability curve. BTX provides a free ELECTRONIC GENETICS Database service to the scientific community. This Database should be consulted for reference literature and protocols prior toperforming the electroporation procedure.

1) Follow set-up procedures in Section 4 and the approporiate attached protocol.
2) Pre-chill the cuvette chamber by placing in an ice bath. (keep caps on and use aseptic technique)
3) If the cells are adherent, collect cells by trypsinization; if the cells grow in suspension, collect by cetrifugation.

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4) Wash the cells with appropriate buffer. Resuspend cells to the recommended concentration in ice-cold buffer.
5) Add the cell suspension to the cuvette electrode, insert into the BTX slider and push into BTX Safety Stand.
6) Plot % viability (abscissa) against field strength (ordinate) on attached graph.
7) Determine the field strength that yields a 50% kill rate. This will be the starting point for subsequent optimization.
8) Continue with optimization procedure.

M. MICROORGANISM PROTOCOLS

Electroporation became a widely used tool when it became apparent that it was possible to transform both protoplasts and intact cells. Ongoing experimentation has shown that most microorganisms are now able to be transformed by this process. For the gram positives, this was made possible by the high field strengths achievable with BTX systems. Efficiencies vary with strain and species and, for E. coli, have exceeded 10^{10} transformants/µg DNA[8] which is useful for constructing cDNA libraries as well as performing shotgun cloning experiments.

A selection of key papers using primarily BTX Systems can be found in Appendix C. Further interesting papers and their respective subjects follow:

Application of electroporation to the industrially important gram positive species Lactococcus lactis (using intact cells):
McIntyre D.A.,et al, Appl. & Enviro. Micro. **55(3)**, 604-610,1989

Plasmid characterization of Salmonella typhimurium from a salmonellosis outbreak:
Schuman J.D., et al, Appl. & Enviro. Micro., **55(9)**, 2344-2348,1989

Successful electroporation of the difficult to transform species Bordetella using high field strengths:
Zealey G., et al, FEMS Microbiology Letters, **56**: 123-126, 1988

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N. MAMMALIAN PROTOCOLS

The ability to transfect DNA into a wide range of eukaryotic cells is fundamental to the science of molecular biology. Because of the complex, physical diversity of these cells, chemical manipulation has not always been successful or highly efficient. Electroporation is not dependent upon any special characteristics of the cell and, therefore, can be used with virtually any cell type. Electroporation can be adjusted to result in one to many copies of inserted DNA per recipient cell. For gene expression studies this allows for observation of the responsible copy. In general, cell types that can be efficiently transfected into a permanent line may also be transfected for transient expression. Increasing the concentrations of cells and DNA used in transient studies usually circumvents low efficiency problems and low promoter/enhancer efficiencies[6].

A selection of key papers using primarily BTX Systems can be found in Appendix C. Further interesting papers and their respective subjects follow:

Function of normal and mutated gene promoters in electroporated K562 cells:
Ley T., Ulrich J.M., Blood, **75**: 990-999, 1990

Optimization studies for four human hematopoeitic cell lines:
McNally M.A.,et al., BioTechniques Vol **6**.,No.9,882-885, 1988

Single site, single copy insertion of DNA into mouse erythroleukemia cells:
Boggs S.S, et. al., Exp. Hematol., **14**: 988-994,1986

Transfection procedure for CV-1, HeLa, IMR-90, NIH3T3, VA13, XP12RO:
Chu G., et al., Nucleic Acids Research, **15(3)**, 1311-1325,1987

Homologous recombination in embryonic stem cells resulting in transmission of mutant allele to offspring:
Zijlstra M., et al, Nature, **342(6248)**, 435-438, 1989

Transfection of DNA into SP2/0-Ag14 cells to produce a chimeric antibody with specificity to HBV:
Li Y.W., et al, Molecular Immunology, **27(3)**, 303-311, 1990

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PLANT PROTOCOLS

Electroporation and electro cell fusion have allowed the production and modification of hybrid somatic gene pools from protoplasts. For some applications, however, protoplast fusion transfers unwanted, unnecessary or deleterious genes into the recipient. Additionally, propagating plants from protoplasts is a difficult and time consuming task. For these reasons, electroporation is the method of choice for specific and controlled genetic engineering of plants[9]. For plant protoplasts within the size range of 40 µ (35-55µ), use the attached protocol.

A selection of key papers using primarily BTX Systems can be found in Appendix C. Further in-depth discussions of the techniques are outlined by Saunders, et.al., in the following papers:

Saunders J.A., et al, "A comparison of electrofusion and electroporation in plant gene transfer": Electroporation and Electrofusion in Cell Biology, Plenum Press, 1989

Saunders J.A., et al., A model system using tobacco protoplasts for the comparison between square wave and exponential decay wave electroporation pulses on the uptake and expression of CMV: BioTechniques, **7(10)**, 1124-1131 1989

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Troubleshooting guide for the ECM 600

Problem, probable cause and solution.

1. No power to the unit.
Check to see if the power cable is plugged into the wall outlet and verify that the outlet is energized by trying out another electrical device for instance, a lamp. Check the fuse on the back of the unit and, if blown, replace it with another 3 A, 250 V slow blow fuse. If fuse blows again, contact BTX.

2. Charge/Discharge cycle cannot be executed.
Internal failure. Contact BTX.

3. Displays show erratic readings.
The pulse length display can show an erratic reading (negative numbers or a 1), if the pulse length exceeds 200 msec. This can occur in the low voltage mode with a large capacitor setting. Disregard the reading. The peak voltage display can show erratic readings if the charging voltage is set below 50 V (0.05 kV) in the LOW VM. Increase the charging voltage. If an arc occurs, the peak voltage and pulse length readings might be erratic. Disregard and proceed with experiments.

4. No pulse delivered to the chamber.
Either the unit is malfunctioning or the connections from the unit to the chamber are interrupted. Check the unit for proper functioning without the chamber. If the unit appears to function, check the banana plugs for proper contact (pull slightly). Verify a visibly good connection to the contact pieces in the stand from the underside of the stand. If there are any loose connections contact BTX for further assistance.

5. Arcing in the chamber.
This can occur with high conductivity media (low specific resistivity), such as PBS, combined with high field strengths, incomplete washing procedures and long pulse lengths. Arcing is a natural event and can occur for a number of reasons. If arcing occurs:

a) Reduce voltage (corresponding field strength) and/or decrease pulse length time.
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) For electroporation done at high field strengths, use a non-ionic medium to reduce excessive heating.

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High voltage arcs can occur at field strengths exceeding 12 kV/cm. Proceed with experiments.

Low voltage arcs can occur due to excessive energy delivered into the chamber medium, especially with PBS. This situation can occur if a large capacitance setting is selected together with voltages close to 500 V. The cuvette can explode violently. Clean up debris and proceed with experiments at a lower capacitance and/or voltage settings. The unit should not experience any failure.

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SERVICE

WARRANTY

The BTX ECM 600 Generator and 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 BTX 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 NEGLIGENCY) 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 and labor by BTX.

All service under the warranty will be made at the BTX San Diego, CA facilities. 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 ECM 600 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 electroporation chambers not manufactured by BTX are used in the ECM 600.

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OBTAINING SERVICE

SERVICE DURING WARRANTY

1. Write or call the BTX, Inc. Customer 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 in the continental United States.

Customers outside of the continental United States who have purchased BTX equipment from distributors, should contact their distributor. Customers who have purchased directly from BTX should contact contact BTX. 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 without prior approval from BTX.

OUT OF WARRANTY SERVICE

Proceed exactly as for Warranty Service, 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 a 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. The output signal of this equipment is dangerous and needs to be tested only by qualified personnel. Please call (619) 597-6006 OR 1 (800) 289- 2465 and ask for Technical Support.

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ECM 600
Generator Test

1. Disconnect the Safety Stand 630 or 630A from the ECM 600 generator.
2. Choose the LV mode or the HV mode.
3. Set up the system to deliver the voltages listed in the HV mode or LV mode.

LV mode

4. Set the Capacitance and the Resistance in the LV mode as indicated in the following page.
5. Press A to activate the Automatic Charge and Pulse Sequence.
6. Write down the reading form the voltage and pulse length monitors in the spaces provided.
7. Press the Reset button.
8. Repeat the above procedure for all the 10 Resistance settings.

HV Mode

9. In the HV mode, set the Resistance and the Voltage.
10. Press A to activate the Automatic Charge and Pulse Sequence.
11. Write down the reading from the voltage and pulse length monitors in the space provided.
12. Change the Resistance.
13. Press the Reset button.
14. Repeat the above procedure for all the 10 Resistance settings.

FAX BOTH THE LV MODE AND HV MODE MONITOR DATA SHEETS TO
BTX TECHNICAL SUPPORT
(619) 597-9594.

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