I. Introduction

The original scientific observations documenting electrofusion and electroporation were conducted with experimenter-designed pulse generators. As methods for the electrical manipulation of cells became established, commercial generators appeared.
on the market. Development of improved apparati continues in both private and public laboratories. However, existing commercial equipment can be used for a wide variety of applications. The first-time buyer of electrofusion or electroporation equipment is faced with a complex buying decision. This chapter gives a brief description of many of the available units and, to the extent that the information was available, gives technical specifications.

The generators summarized in this chapter fall into two broad categories of design: equipment used for electrofusion and equipment employed for electroporation. A few generators can be useful for both applications. In general, the electroporation power supplies are designed to deliver a single discharge, which transiently permeabilizes the cell membrane. Electrofusion generators provide the additional capability of delivering an AC cell prealignment signal. A variety of approaches to prealignment are represented in the commercially available generators; the end user must decide which is optimal for the application. In electroporation procedures, low voltages and long pulse lengths are frequently applied to cells and protoplasts; bacteria and yeasts require higher field strengths. The majority of the generators deliver either decaying exponential or square-wave pulses. The potential buyer needs to decide on the intended purpose, electrofusion or electroporation, and select a generator with the appropriate electrical parameters. Frequently, the literature can serve as a guide to what capabilities are needed in a generator. Specific examples can be found in the accompanying chapters. Voltage, effective field strength, time constant or capacitance, number of repetitions, and prealignment parameters are of primary concern. Other considerations are the provision for data observation and collection, temperature control, type of controls provided, versatility, customer service, quality of construction, electrical safety, warranty, and available sample chambers. The prices charged for commercial generators vary between about $1000 to upward of $30,000 (US) for a complete system; the wide range of costs represents the widely differing capability and complexity of the apparatus.

For those qualified to undertake construction of an electronic pulse generator, a homebuilt apparatus is an economical alternative. Simple capacitive discharge generators, which deliver an exponentially decaying waveform suitable for bacterial electroporation, can be assembled with a few hundred dollars in parts. More complex apparatus, some of which use generic high-power pulse generators not specifically designed for electrical manipulation of cells, can also be used to advantage. Home-built machines also allow electrical manipulations that cannot be obtained with commercial generators. Some investigators have reported extremely satisfactory results with power supplies of their own design or construction. The would be home builder should be extremely cautious in their construction and attention to safety factors. Generators produce dangerous high-voltage, high-current discharges that are potentially lethal.
II. Commercially Available Generators Designed for Electrofusion and Electroporation

A. Bioelectronics

Bioelectronics Corporation
1852 Thunderbird Road
Troy, MI 48084
Tel. (313) 362-2727

Bioelectronics Corporation manufactures generators designed for electrofusion, injection (the company’s term for electroporation), and extraction (the removal of molecules from a cell). A unique waveform is described as an oscillating pulse with a sharp spike at the end. An optional electronically controlled centrifuge is used to bring cells together and to flatten cells prior to the electrofusion or electroporation pulse. The product line includes the model 10000 high-voltage electronic cell processor, the model 20000 electronic centrifuge accessory, and the model 30000 universal cell injector.

1. Model 10000

Type: Unique waveform with end spike
Volts output: 10–3500 V
Pulse duration: 10–40 µs in four steps
Dataout/display: Digital meters
Electrofusion: Yes
RF field: 0–400 V (rich in harmonics); 400 kHz, timer controlled
Sample chamber: 2-ml autoclavable sample chambers; multiple chambers, up to 50, can be loaded in the centrifuge for a series of experiments
Additional features: Model 20000 centrifuge offers up to 14,000 \( \times \) g.

2. Model 30000

A stand-alone cell injector centrifuge with no RF alignment capabilities and built in 3000-V, 15-µs pulse generator.

B. Bio-Rad

Bio-Rad Laboratories
3300 Regatta Blvd.

\(^{1}\)Mention of a trademark or proprietary product does not constitute a recommendation by the authors over other products which may also be suitable.
1. Gene Pulser

Microprocessor-controlled pulse generator for the electroporation of eukaryotic and prokaryotic cells. Delivers exponentially decaying pulse. Accessory units provide increased capacitance and resistive timing. Company offers sterile, individually packaged 1-, 2-, and 4-mm sample cuvettes and Electro-Competent *Escherichia coli* cells.

Type: Exponential decay
Volts output: 50–2500 V in 10-V increments
Time constant: From 5 µs to 1 s; four capacitors of 0.25, 1.0, 3.0, 25 µF are included; optional capacitance extender has four capacitors with 125, 250, 500, and 960 µF
Dataout/display: Digital readout of voltage delivered and time constant; oscilloscope output possible
Electrofusion: Yes, without dielectrophoresis
Maximum field strength: 25 kV/cm with 1-mm cuvette
Sample chamber: 1-, 2-, and 4-mm sterile disposable cuvettes with maximum working volumes of 80, 400, and 800 µl, respectively
Additional features: Capacitance extender for 125, 250, 500, and 960 µF; pulse controller contains parallel resistors of 0, 100, 200, 400, 600, 800, and 1000 Ω, which allow high-voltage pulses to be delivered. Also contains 20-Ω current-limiting resistor in series with sample.

C. Braun

B. Braun Diessel Biotech GmbH.
Postfach 110
Carl-Braun Strasse 1
3508 Melsungen, Federal Republic of Germany
Tel. 49-566-171-3943
FAX 49-566-171-3702

1. Biojet M1

High-voltage power supply generating short pulses of defined duration or long pulses of nonregulated duration.
Chapter 35 Pulse Generators

Type: Exponential
Volts output: 0–15,000 V (27,000 V/cm for 5.5-mm electrode gap)
Time constant: 0–59 s with manual control; 1 min to 59 min 59 s with automatic operation
Dataout/display: Digital display
Electrofusion: No
Sample chamber: 0.1–6.0 ml, up to 60 ml on request, electrode gap 5.5 mm
Additional features: Microprocessor controlled; stores up to 20 different user-defined programs

2. Biojet CF 100

Cell electrofusion instrument offering a high-frequency range of up to 10 MHz plus a pulse generator for square-wave pulse of controlled duration. Can store up to 50 user-defined programs in battery-buffered RAM

Type: Square wave
Volts output: 0–400 V, duration 5–100 μs, at AC off time of 10–999.9 s
Number of pulses: 1–9 at pulse interval 0.1–999.9 s
Dataout/display: Parameters available on screen; conductivity check built in
Electrofusion: Yes
Alignment parameters: 0–15 V for frequencies below 5 MHz, 0–12 V for frequencies below 8 MHz, 0–8 V for frequencies above 8 MHz; three time settings allowed with 1-s steps, 0–1000 s, 0–500 s, 0–500 s
Sample chamber: Various
Additional features: Special alternating field procedure for aligning cells which show a large difference in size, and menu-guided operation; has ramped or constant post-alignment mode of 1–999 s

3. Biojet CF 50

All-purpose-built cell electrofusion instrument. Offers a frequency range of up to 2 MHz plus a pulse generator for square wave pulses of up to 9,999 ms. Displays all relevant electrofusion parameters on one display. Electrofusion conditions are entered using the keyboard; voltages are set with two adjusting potentiometers. Operation is either manual or following preset values. Memorizes the last electrofusion condition used.

Type: Square wave
Electrofusion: Yes
Volts output: 15–250 V
Time constant: 5–9999 sec
Number of pulses: 1–99 at pulse interval of 0.1–9.9 s
Dataout/display: All operating parameters displayed on one screen
Alignment parameters: 0.5–20 V of 1–999 µs duration (0.1–2 MHz); AC off time is fixed
Sample chamber: Various including microslide and helical chamber
Additional features: Protoplast electrofusion mode provides plant biologists (and others working with large cells) with an even finer voltage control than during normal operation; has ramped postalignment mode of 1–999 s

D. BTX

BTX
3742 Jewell Street
San Diego, CA 92109
Tel. (619) 270-0861, (800) 289-2465
FAX (619) 483-3817

1. Electro Cell Manipulator 600

Single-unit design with over 1000 pulse lengths, two voltage ranges, and dual-display monitoring. The ECM 600 is designed for use with all types of cells: prokaryotes and eukaryotes. Internal monitoring measures true peak voltage and pulse length.

Type: Exponential decay
Volts output: two voltage ranges, high-voltage mode 300–2500 V, low-voltage mode 50–500 V
Time constant: Capacitance range from 25 to 3175 µF in 25-µF increments and 10 internal timing resistance settings from 13 to 720 ohms gives 1270 pulse-length options
Dataout/display: Instantaneous monitoring—set voltage, set capacitance, peak discharge voltage, pulse length, mode; pulse settings and measured parameters readily visible at all times
Electrofusion: No
Maximum field strength: >40 kV/cm with 0.56-mm Flatpacks and optional Flatpack Slider
Sample chamber: 11 designs, reusable or sterile disposable, 0.56-, 1- or 2-mm gap
Additional features: Short-circuit- and arc-proof without sample solution limitations;
BTX Electronic Genetics Database Service
Chapter 35  Pulse Generators  561

2.  **Electro Cell Manipulator 200**

Pulse power generator with both AC and DC voltage output. Equipment is designed for versatile application to plant, bacterial, and cell electroporation and electrofusion including embryo cloning. Operation in either automatic or manual mode. A built-in conductance meter monitors chamber media both before and after the pulse. Operates with all BTX electrodes including microslides, which are parallel wires adhered to glass slides for direct viewing with a microscope.

Type: Square wave  
Volts output: 0–75 V AC, 0–960 V DC  
Time constant: 0–99 µs (1-µs increments), pulses 1–99 automatic, unlimited manual  
Dataout/display: Volts, time constant, conductance  
Electrofusion: Yes  
Alignment parameters: 1 MHz RF, 75 V zero to peak, 0–99 s, special BTX sine wave, automatic and manual modes  
Sample chamber: Microslide, 1.9- and 3.5-mm cuvette, unique Meander fusion chamber sputter-coated design with 0.05-, 0.1-, and 0.2-mm gaps on slide, and molded cuvette chambers in 1- and 2-mm gap sizes; for electroporation; 11 designs, reusable or sterile disposable, 0.56, 1, or 2 mm gap  
Additional features: BTX Electronic Genetics Database Service

3.  **T 800 Electroporator**

The T800 has the same features as the ECM 200, but does not include the alignment capability or the conductance meter.

Type: Square wave  
Volts output: 0–960 V DC  
Time constant: 0–99 µs (1-µs increments), pulses 1–99 automatic, unlimited manual  
Dataout/display: Pulse amplitude (V)  
Electrofusion: No  
Sample chamber: Reusable chambers of 1.9- and 3.5-mm gap and sterile disposable cuvettes of 1- and 2-mm gaps width  
Additional features: BTX Electronic Genetics Database Service

4.  **Graphic Monitoring—Optimizor—Graphic Pulse Analyzer**

Microprocessor-controlled storage pulse analyzer, combining an oscilloscope and computer. It connects in-line between the pulse generator and the chamber. Mea-
sures, calculates, and displays multiple parameters in the cell chamber after the pulse is delivered. Displays graphical representation of pulse shape. Included with BTX ECM200 and T800 Super Systems. Optional Power Plus external voltage booster for use with ECM 200 and T 800. Employs a 2.6-fold stepup transformer to boost signal.

E. EquiBio s.a.

EquiBio s.a.
Rue Dossin, 45, B-4000
Liege, Belgium
Tel. 32 41 520009
FAX 32 41 520019

1. Cellject C2

Type: Decaying exponential waveform
Volts output: 2.5 kV at 40 μF, 450 V from 150 to 2100 μF, voltage adjustable from 100 to 2500 V with 10 V resolution
Time constant: Capacitor choice 40, 150, 300, 450, 600, 900, 1200, 1500, 1800, 2100 μF
Pulse time controller: 74, 90, 132, 192, 282, 412, 600, 1320 Ω and infinite
Dataout/display: Printer output RS 232 (300–9600 baud), single display with parameter selection
Electrofusion: No
Maximum field strength: 12,500 V/cm (C4 = 6.125 kV/cm)
Sample chamber: Disposable sterile 2- and 4-mm cuvettes with a capacity of 110–800 μl
Additional features: Continuous microprocessor control; the Cellject Twin is based on a patented double pulse process in which the electroporation pulse is followed by a second long-duration, low-amplitude pulse said to improve electroporation frequency

F. Hoefer Scientific Instruments

Hoefer Scientific Instruments
654 Minnesota Street
P.O. Box 77387
San Francisco, CA 94107
Chapter 35 Pulse Generators

PG 200 Progenitor II

Modern design features internal power supply, variable capacitance, choice of platinum reusable electrodes or disposable cuvettes. Designed for electroporation of plant, bacterial and animal cells.

Type: Exponential decay or square wave
Volts output: 0–500 V
Discharge Interval: 10 μs to 0.1 s in 10-μs intervals, from 1 to 10 s in 10-ms intervals
Maximum field strength: 25,000 V/cm depending on electrode
Time constant: Capacitance 0–2700 μF in 30 steps
Capacitor Values: (in μF) 100, 220, 490, 760, 1200, can be set in any combination
Charge time: less than 1 s to charge 1200 μF to 300 V
Dataout/display: Digital voltage meter
Electrofusion: No
Sample chamber: Two ring and multiple-plate electrodes, disposable cuvettes
Additional features: Electroporation chamber interlock; PG200 will not go through charge/discharge cycle until the lid is closed; the electrode must also be inside the chamber; automatic discharge of excess charge on capacitors

International Biotechnologies, Inc.

International Biotechnologies, Inc.
Subsidiary of Eastman Kodak Company
25 Science Park
New Haven, CT 06511
Tel. (800) 243-2555
FAX (203) 786-5694

geneZAPPER

Microprocessor-controlled pulse generator for the electroporation of plant, animal and bacterial cells. Designed for reproducible pulse delivery.

Type: Exponential RC time constant
Volts output: Settings 50–2500 V DC, in 1-V increments
Time constant: There are 34 capacitance settings between 7 and 1550 µF in 50-µF increments.
Dataout/display: 7 Segment LED displays voltage, capacitance, and time constant; BNC output connector
Electrofusion: No
Maximum field strength: 12,500 V/cm
Sample chamber: 2-, 4-, and 10-mm Reusable stainless steel plate accepts standard disposable cuvettes, clip adapter accepts presterilized, disposable, 2- or 4-mm foil-lined cuvettes
Additional features: Safety features include electrode safety guard, two discharge buttons, automatic discharge internally; protocol reference database service
Wave Controller: Selectable resistance accessory expands the capabilities of the geneZapper 450/2500 Electroporation System for bacterial cells.

H. Jouan

Jouan, Inc.
125-D Route 7, East
P.O. Box 2716
Winchester, VA 22601
Tel. (703) 665-0863
Telex 904 348

1. Cellular Electropulsator PS 10

Type: Square wave
Volts output: 0–1500V
Time constant: 5 µs to 24 ms
Dataout/display: Time, frequency
Electrofusion: Yes
Maximum field strength: 10,000 V/cm
Sample chamber: 0.15-, 0.25-, and 0.4-cm electrodes, various cuvettes, and 150 µl to 60 ml/min flow through cell
Additional features: Digital adjustment of voltage, duration, and frequency

I. Life Technologies, Inc.

Life Technologies, Inc.
8717 Grovemont Circle
P.O. Box 9418
1. BRL Cell Porator Electroporation System

Microprocessor-controlled exponential decaying pulse power supply designed for electroporation of all kinds of cells. Employs optional voltage-booster for bacterial electroporation. Features ice bath for sample cuvettes. Electrocompetent *Eschericia coli* are also available.

Type: Exponential decay  
Volts output: Two voltage ranges, high-voltage mode 50–2500 V, low-voltage mode 10–400 V  
Time constant: Capacitance in eight steps to deliver time constants for 0.1–200 ms; 3–150 ms with voltage booster  
Dataout/display: Digital readout of voltage delivered; oscilloscope output included  
Electrofusion: No  
Maximum field strength: 2.66 kV/cm; 16.6 kV/cm with voltage booster  
Sample chamber: 4- and 1.5-mm sterile disposable cuvettes with working volumes of 0.24–1 ml and 25 µl, respectively.  
Additional features: Toll-free techline; all units are safety interlocked

J. Shimadzu Corporation

Shimadzu Corporation  
1, Nishinokyo-Kuwabaracho, Nakagyo-Ku  
Kyoto 604, Japan  
Tel. 075-823-1351  
Telex 05422-166 SHMDS J  
FAX 075-822-2617

1. SSH-1

The SSH-1 (somatic cell hybridizer) is designed primarily as a convenient cell electrofusion instrument with automatic operation. The capability to deliver high-intensity DC pulses also makes it suitable for electroporation of cells.

Type: Square wave  
Volts output: 40–700 V
Duration of pulses: 10–500 μs pulse width; 10 pulses at intervals from 0.1 to 999 s
Dataout/display: Equipped with standard graphic printer for all parameters and graphs; direct readouts of parameters on status indicators
Electrofusion: Yes
Maximum field strength: 14,000 V/cm
Alignment parameters: 0–40 V AC, 0–999 s pre- and post-pulse, 0.25, 0.5, 1, or 2 MHz RF
Sample chamber: Eight chambers; 10 μl to 1.6 ml capacity with parallel electrodes of 0.5–4 mm; suitable for microscopic observation
Additional features: Sample cell temperature control system optional; remote keypad available; microprocessor-controlled and keypad programmable; 29 types of autoclavable chambers and five types of disposable chambers available.

2. GTE-10

Microprocessor-controlled exponential decaying pulse power supply designed for electroporation of all kinds of cells. Wide range of time constants and voltages.

Type: Exponential decay
Volts output: Two voltage ranges, high-voltage mode 50–2500 V, low-voltage mode 50–500 V
Time constant: Capacitance in steps of 1, 3, 10, 35 μF; Up to 2.5 s with external capacitance accessory
Dataout/display: Digital readout of pulse settings and measured parameters readily visible at all times
Electrofusion: No
Maximum field strength: Up to 54,000 V/cm with 0.5-mm chamber
Sample chamber: 29 Types of autoclavable chambers and five types of disposable chambers available.
Additional features: Optional temperature controlled cell; external capacitance enhancers of 50–200, 200–800, and 800–3200 μF and resistive time constant controller available; optional external controller and data out interface

III. Homemade and Commercial Pulse Generators

Growth in the use of electroporation and electrofusion during the 1980s was accompanied and assisted by the concurrent growth in the availability of commercial instruments specifically designed for the biotechnology market. This section is devoted to (1) a discussion of the design and use of homemade equipment and (2) the existence of other commercially available equipment that may be useable for
electroporation and electrofusion beyond that designed for the biotechnology market. We refer here to pulse generators made for industrial and engineering purposes and not intended for the electroporation and electrofusion market. Although not intended in any way as an endorsement, we mention that: Avtech (Ogdensburg NY 13669), Cober Electronics, Inc. (Stamford, CT 06902), Instrument Research Company (Columbia, MD 21045), Grass Instrument Co. (Quincy, MA), and Velonix (Santa Clara, CA 95050) all have product lines of pulse generators having enough voltage and power outputs that should be useful in many electroporation or electrofusion applications. Other manufacturers and sources can be found by consulting specialized catalogs, buying guides, research and development telephone directories, and electronic buyers handbooks. The authors have utilized a surplus property division as a source of a power supply unit used in a previously completed project. It is likely that units recovered from surplus property sites will not be accompanied by operation manuals. Hence the usefulness of such units may depend on individual resourcefulness.

All of the devices that these sources offer are likely to be much heavier, physically, and primarily designed for generally heavier use than many of those devices designed for the biotechnology market. Biotechnology applications such as electroporation and electrofusion will have to be optimized by the researcher with little input from the commercial manufacturers, since the companies will not be able to supply matching chambers to a customer. Such chambers may have to be procured from another manufacturer or be homemade. When sufficient expertise is available, homemade equipment will be cheaper, more readily modified or repaired, and more appropriate for mechanistic studies. However, the time required to build one's own equipment may exceed the investment expectations of many researchers.

Homemade devices for supplying a strong electrical pulse for electroporation and electrofusion have been described recently in considerable detail (Mischke et al., 1986; Miles and Hochmuth, 1987; Sowers, 1989; Speyer, 1990). The general construction of scientific apparatus may be found in Moore et al. (1983). It is advisable to have means for measuring and checking the electrical performance of these devices. The most practical and advisable device is a storage screen oscilloscope. As voltages that can be harmful and potentially lethal are involved, it is important to consider the risk/benefit ratio of using homemade devices. Homemade high-voltage pulse generators have been in use in the laboratory of the authors for nearly a decade without serious incident. Care in design and precautions in the use of such equipment make accidental shock exposure no more hazardous or probable than other accidental hazards in laboratory activities (fire, broken glass lacerations, other human error).

In all cases, the design of electroporation and electrofusion apparatus must involve the consideration of (1) the desired field strength, \( E = \frac{V}{d} \), where \( E \) is in volts (V) per unit distance \( d \) (e.g., mm, cm), (2) the desired duration of the pulse (decay half-time in the case of exponentially decaying waveforms and pulse width in the case of square wave pulses), and (3) the pulse power that is either available from the
generator or needed by the chamber. Most pulse generators will produce a waveform that is either qualitatively “square” or “exponentially decaying.” Although some research projects have utilized either complex waveforms or complex pulse protocols, the attributes of these waveforms may be anecdotal and are without a rigorously established validity. An apparatus that generates only low power levels will never be useable with large-volume chambers because these chambers will simply “overload” the generator. The outcome of the converse possibility—meaning the use of a strong generator with a chamber requiring only low power levels—is not predictable without technical information that is often not available from manufacturers of equipment for the biotechnology market but may be available from manufacturers of equipment for the industrial market. An example is the capacitor discharge (exponentially decaying) units. A large chamber will require a large capacitor. However, a small chamber may require a faster decay half-time than can be delivered by the lowest amount of capacitance available. Compensation for this may be possible by placing an external resistor in parallel with the chamber. The functioning of square wave generators can be based on one of many approaches which are usually complex. In some cases the fidelity of the square-waveform pulse may depend on technical details beyond the scope of this chapter.

Electrofusion requires, in addition to what is needed for electroporation, a means of bringing the membranes of cells into close contact. While many approaches can be used for this purpose, one of the most convenient methods involves the use of dielectrophoresis (Pohl, 1978). Dielectrophoresis is the name of a phenomenon in which cells in suspension become relocated (or “aligned”) into positions such that they appear as “pearl chains” as a low-strength alternating electric field is generated within the suspension. While the theory is complex, the practical result is easy and convenient to obtain under conditions useful for membrane fusion applications. Misunderstandings of what is required for dielectrophoresis are common, and the reader is referred to other chapters within this volume for further information (see Chapter 8, Sowers). The fact that both a low-strength alternating field required for dielectrophoresis and a high-strength direct-current pulse must be delivered to the chamber in electrofusion protocols suggests that both sources can be connected to the chamber. However, a means must be provided to prevent the high-strength direct-current fusion pulse from damaging the source of the low-strength alternating current. The simplest way to satisfy this requirement is with a mechanical switch or relay (Mischke et al., 1986). A more elegant way to satisfy this requirement is with an electrical network. An example is given in Sowers (1989). Many other possibilities are obvious if simple electrical circuit theory is used. The interested reader is referred to Malmstadt et al. (1963, 1981) for further information.

References