

Section 1 General Information

1.1 Introduction

Pulsed field electrophoresis is a technique for resolving chromosome size DNAs. Conventional electrophoresis does not permit resolution of DNA fragments larger than 50,000 base pairs. By alternating the electric field between spatially distinct pairs of electrodes, DNAs on the order of 10 megabases are able to reorient and move differentially through the pores in an agarose gel.¹⁻³

The CHEF-DR II system (Figure 1) is an advanced pulsed field system based on the CHEF (clamped homogeneous electric fields) technique.³ The key to high resolution, sharp bands, straight lanes, and reproducible separations is a uniform electric field at all points of a gel, and an optimal 120 degree angle of alternating pulses. The CHEF-DR II system accomplishes both of these by establishing the electric field along the contour of a hexagonal array of 24 electrodes. Electrodes along the sides of the hexagon are powered in such a way as to generate two different alternating electric field vectors. Each electrode "clamps" the voltage of its individual region of space as necessary to maintain field homogeneity.



Fig. 1. The CHEF-DR II electrophoresis system.

The CHEF-DR II system features *dynamic regulation* of the individual electrode voltages. Proprietary circuitry in the drive module continually senses the voltages at individual electrodes, and actively regulates them to the proper level. Variations in gel thickness, buffer conductivity, or temperature are compensated so that uniform fields are achieved at all times, and at all points of the gel.

1.2 Specifications

The specifications below pertain to the complete system. The CHEF-DR II system is also available as a basic unit, which includes the gel chamber, drive module, pump, and accessories. Detailed specifications for the switcher and power supply are given in the Pulsewave 760 Switcher and Model 200/2.0 power supply instruction manuals, respectively.

CHEF-DR II Specifications

Gel chamber	
Construction	acrylic
Dimensions	11 x 43 x 44 cm
Weight	9 kg
Buffer capacity	2 l
Electrodes	platinum, 0.010 inch diameter, replaceable
Drive module	
Chassis	aluminum
Dimensions	14 x 26 x 43 cm
Weight	7.8 kg
Maximum voltage	300 V DC
Minimum voltage	25 v
Operating temperature range	0-35 °C, humidity 0-95% without condensation
Pulsewave 760 Switcher	
Maximum voltage	760 V DC
Maximum current	500 mA
Weight	5.0 kg
Power supply	
Maximum Voltage	200 V DC
Maximum current	2.0 A
Weight	5.0 kg
Pump	
Voltage	120 V AC, ground isolated, variable speed
Flow rate	1 liter/minute, typical
Weight	2 kg
Chemical compatibility	The CHEF-DR II chamber is not compatible with chlorinated hydrocarbons (<i>eg.</i> , chloroform), aromatic hydrocarbons (<i>eg.</i> , toluene, benzene), or acetone. Use of organic solvents voids all warranties.
Cooling recommendations	The Model 1000 Mini Chiller (170-3654) or a refrigerated circulating water bath is recommended to keep the buffer temperature at 14 °C. for optimal results.
Fuses	1 A slow blow for 120/100 V 0.5 A slow blow for 220/240 V 0.5 A fast blow
Total system weight	28 kg

Section 2

Description of Major Paris

2.1 Electrophoresis Chamber

The CHEF-DR II electrophoresis chamber consists of a 43 x 44 cm (17" x 17.5") acrylic box with 24 horizontal electrodes arranged in a hexagon. (See Figure 1.) Gels are electrophoresed horizontally, submerged under recirculated buffer. A 14 x 12.7 cm (5.5" x 5") gel is cast in a separate casting stand, removed, and placed in the center of the hexagon. It is held in place by two gel stops, which are inserted into the two innermost holes on the chamber floor. DNA migration is in the direction of the arrow on the lid. Other holes accommodate other gel sizes.

The individual electrodes are replaceable for easy maintenance (see Section 10). The electrode units are each sealed with an O-ring and silicone sealant to provide double protection against leakage.

There are two small chambers below the level of the main chamber floor, at the front and rear of the main chamber. These chambers allow buffer to drain and permit easy priming of the pump. Buffer enters these lower chambers through six holes at the front of the box.

There are two ports for buffer circulation. Buffer enters the main electrophoresis chamber from the rear and flows in the direction of the arrow on the lid. The plastic block at the entry point baffles the flow so the gel is not disturbed. Buffer exits the chamber at the front through the T fitting. The base of the box has four leveling screws for even gel submersion.

The lid contains two handles to allow easy lid removal, and an interlock for safety. Current from the external power supply entering the drive module passes through a short path in the lid interlock before it reaches the drive module circuitry. If the lid is removed, the current flow is broken. There is a second interlock circuit from the Pulsewave 760 Switcher to the drive module. If the Pulsewave 760 Switcher is off, there will be no current to the chamber. A third interlock requires a power supply voltage of 25 V or more for the drive module to operate.

Warning: There are high voltages and currents within the chamber, which can be harmful. Do not attempt to circumvent these safety interlocks. Always turn off the external power supply when working within the gel box.

2.2 Drive Module

The drive module is a separate electronic device which distributes and maintains the individual electrode voltages in the gel chamber. It consists of proprietary circuitry for "clamping" the individual electrode potentials so that uniform fields are obtained at all points of the gel and at all times. That is, it provides dynamic regulation of the potentials so that the proper voltages are obtained regardless of gel size, or fluctuations in buffer conductivity or temperature. If a given electrode potential begins to change due to a change in the buffer conductivity, it is automatically readjusted to the proper level.

Figure 2A shows the relative potentials of each electrode pair. When the A channel from the Pulsewave 760 Switcher is activated, the net current vector is from NW to SE. The highest potentials are found along the SE segment of the hexagon. The potentials gradually decline along the adjacent segments. The segment directly opposite the SE has zero potential, represented in the diagram as the negative terminals. Figure 2B shows the

relative potentials when the B channel is activated. The net vector is from NE to SW. The highest potential is found along the SW segment of the hexagon, with declining potentials along the adjacent segments, until there is zero potential along the NE segment, as shown in Figure 2B. The relative orientation of the electric field vectors with the A and B channels activated, constitutes the optimum 120° reorientation angle.

Note: The CHEF-DR II does not perform FIGE (Field Inversion Gel Electrophoresis). For this and other variable angle functions use the CHEF Mapper™ electrophoresis system.³²

The drive module allows a maximum of 300 volts DC from the power supply. If the incoming voltage exceeds this, the fuse will blow. The minimum operating voltage is 25 V.

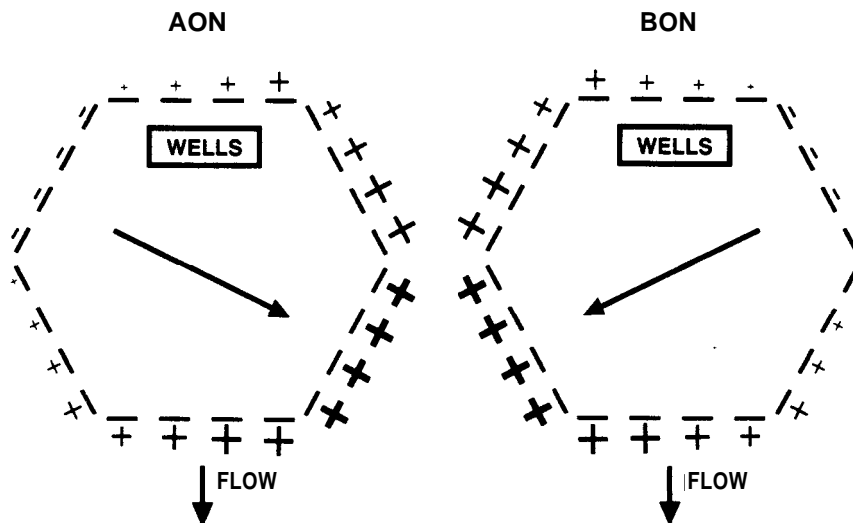


Fig. 2. Voltage clamping by the CHEF-DR II system. A. Relative electrode potentials and field vector when A channel of the Pulsewave 760 Switcher is on. **B.** Relative electrode potentials and field vector when B channel is on. The average vector during the run is in the direction of the arrow marked FLOW

The front panel of the drive module contains switches, jacks, and a fuse as shown in Figure 3.

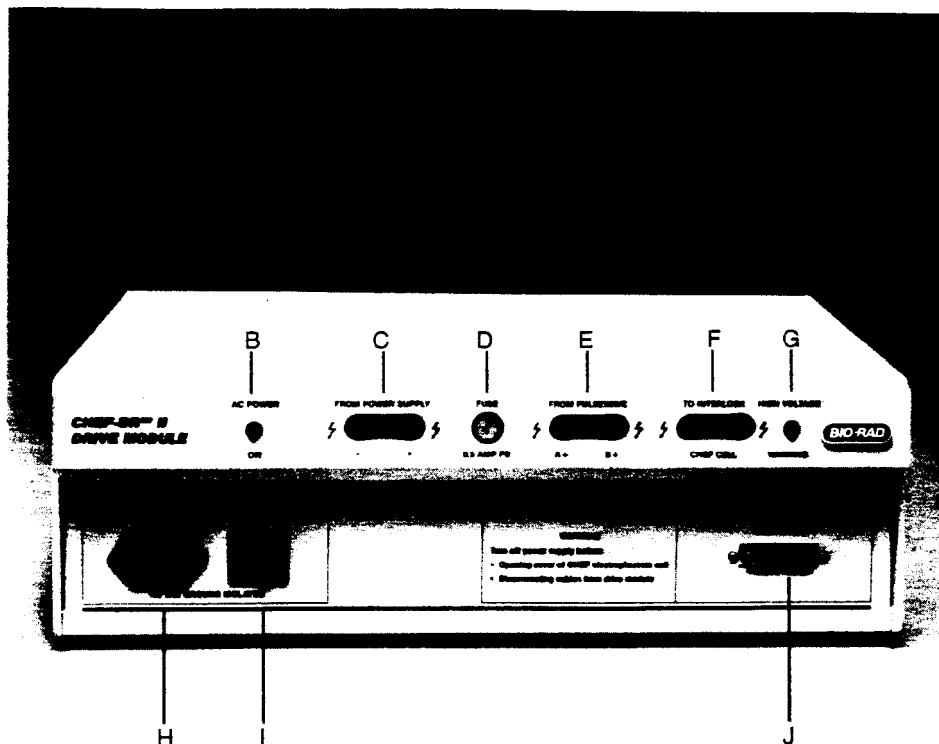


Fig. 3. CHEF-DR II drive module. **A.** Drive module power switch (rear of module). **B.** AC power indicator light; lit when module is turned on. **C.** Leads from power supply. **D.** 0.5 Amp fuse, fast blow: protects circuitry in drive module if power supply exceeds 300 V. **E.** Leads from Pulsewave 760 Switcher The A+ and B+ jacks of Pulsewave 760 Switcher are used (red leads). **F.** Interlock to CHEF-DR II electrophoresis cell. The coiled wire from the cell connects here as a safety precaution. When disconnected, the unit will not operate. **G.** High voltage indicator light; light glows in proportion to the voltage applied. THE LID TO THE CHAMBER SHOULD NOT BE OPENED WHILE THIS LIGHT IS ON. TURN OFF THE POWER SUPPLY FIRST. **H.** Power cord for pump. **I.** Pump switch. **J.** Output cable to CHEF-DR II electrophoresis cell.

2.3 Pump and Accessories

Each basic unit or system includes a variable speed pump. The pump provides a suitable flow rate of buffer through the chamber. Substitution of other pumps could pose a safety hazard and **cause** improper flow, and therefore lower resolution. The pump's power supply is electrically isolated within the drive module for safety. Its voltage requirement is independent of the line voltage supplied to the drive module (e.g. 120, 100, 220, or 240 volts). This pump should not be plugged into any equipment other than the CHEF-DR II drive module.

The pump is connected to Tygon® or plastic tubing. This tubing circulates buffer in and **out** of the chamber. The tubing may also pass through a water chiller. In this case, the pump should be located after the chiller, so that buffer flows through the chiller and then to the pump. Typically, the dial is set at 70, for about 1 l/min.

Caution: The buffer flowing in the tubing is electrically active. Care should be taken not to handle the tubing or exposed liquid while the power supply is on. Tube connections should be made with the power supply off.

A casting stand, 10 well comb, and comb holder are included for casting the gel. A 10 sample plug mold is provided for preparing **agarose** blocks. Details are given in Section 4. A sample of yeast **Chromosomal** DNA is included with catalog numbers 170-3612 through 170-3615 to help you calibrate the system. A sample of **Chromosomal** Grade **agarose** is included for megabase (> 1 mb) samples.

2.4. Pulsewave 760 Switcher

The Pulsewave 760 Switcher alternates the fields for the **CHEF-DR II** chamber, sets the length of the switching intervals, and sets the **electrophoresis** run time. The Pulsewave 760 Switcher permits ramping the switch time from the beginning of the run to the end of the run. Special modes allow pause, countdown, linking to a computer, recovery from power failure, and automatic conversion from one set of switch conditions to another after a specified run time. These and other major features of the Pulsewave 760 Switcher are described in the Pulsewave 760 Switcher instruction manual.

2.5 Model 200/2.0 Power Supply

The Model 200/2.0 power supply provides up to 200 V for **CHEF electrophoresis**. If 200-300 volts are required, the Model 1000/500 power supply may be substituted. The Model 200/2.0 power supply regulates the voltage to $\pm 2\%$, sufficient for uniform fields in the **CHEF** chamber. Other power supplies may have up to $\pm 10\%$ fluctuation of voltage, which can lead to noticeable distortion in the fields.

For details of operation of the Model 200/2.0 power supply, see the power supply instruction manual.

Section 13

Product Information

Catalog Number	Description
170-3670	CHEF Mapper XA Chiller System, 120 V , includes CHEF Mapper XA power module with embedded auto algorithm for protocol optimization, interactive algorithm program disc, bar code reader, electrophoresis cell, Model 1000 Mini Chiller, Variable Speed Pump, temperature probe, 12 feet Tygon® tubing, 14 cm wide x 12.7 cm long casting stand, 10 well comb and comb holder, 10 samole plug mold, leveling bubble, cables, <i>S. cerevisiae</i> standards, 5 grams Chromosomal Grade A&rose, instruction manual
170-3671	CHEF Mapper XA Chiller System, 100 V
170-3672	CHEF Mapper XA Chiller System, 220 V
170-3673	CHEF Mapper XA Chiller System, 240 V
170-3686	Algorithm Upgrade Kit , includes EPROM with embedded algorithm to convert CHEF mapper to XA, Interactive algorithm disc, bar code reader, cable: PC to CHEF Mapper
170-3612	Complete CHEF-DR II System, 120 V , includes gel box, drive module, Pulsewave 760 Switcher, Model 200/2.0 power supply, pump, 5.5" x 5" casting stand and comb holder, 10 well comb and 10 sample plug mold, 12' Tygon tubing, chromosomal DNA size standard.
170-3613	Complete CHEF-DR II System, 100 V
170-3614	Complete CHEF-DR II System, 220 V
170-3615	Complete CHEF-DR II System, 240 V
170-3616	CHEF-DR II Basic Unit, 120 V , includes gel box, drive module, pump, 5.5" x 5" casting stand and comb holder, 10 well comb and 10 sample plug mold, 12' Tygon tubing.
170-3617	CHEF-DR II Basic Unit, 100 V
170-3618	CHEF-DR II Basic Unit, 220 V
170-3619	CHEF-DR II Basic Unit, 240 V
170-3600	Pulsewave 760 Switcher, 120 V
170-3601	Pulsewave 760 Switcher, 100 V
170-3602	Pulsewave 760 Switcher, 220 V
170-3603	Pulsewave 760 Switcher, 240 V
165-4761	Model 200/2.0 Constant Voltage Power Supply, 100/120 V
165-4762	Model 200/2.0 Constant Voltage Power Supply, 220/240 V
165-4710	Model 1000/500 Power Supply, 100/120 V
165-4711	Model 1000/500 Power Supply, 220/240 V

Accessories and Reagents

Catalog Number	Description
170-3654	Model 1000 Mini Chiller, 120 V
170-3688	Model 1000 Mini Chiller, 100 V
170-3655	Model 1000 Mini Chiller, 220/240 V
170-3668	External. Temperature Probe
170-3644	Variable Speed Pump, 120 V
170-4046	Leveling Table
170-3625	Gel Stops (4)
170-3643	Gel Scoop
170-3646	Standard Electrodes (0.01"),6
170-3648	Thick Electrodes(0.02"),6
165-5031	GS Gene Linker, UV crosslinking chamber, 120 V
165-5034	GS Gene Linker, UV crosslinking chamber, 100 V
165-5032	GS Gene Linker, UV crosslinking chamber, 220 V
165-5033	GS Gene Linker, UV crosslinking chamber, 240 V
170-3604	Pulsewave 760 Extender Block
170-3630	Extender Cables
170-3620	Casting Stand, 5.5" x 5"
170-4326	10 Well Comb, 5.5" wide, 1.5 mm thick
170-4325	10 Well Comb, 5.5" wide, 0.75 mm thick
170-4324	15 Well Comb, 5.5" wide, 1.5 mm thick
170-4323	15 Well Comb, 5.5" wide, 0.75 mm thick
170-4322	20 Well Comb, 5.5" wide, 1.5 mm thick
170-4321	20 Well Comb, 5.5" wide, 0.75 mm thick
170-4344	30 Well Comb, 5.5" wide, 1.5 mm thick
170-3626	Casting Stand, 8.25" x 5"
170-3664	Casting Stand, 14 cm wide x 21 cm long, and frame
170-3627	15 Well Comb, 8.25" wide, 1.5 mm thick
170-3628	30 Well Comb, 8.25" wide, 1.5 mm thick
170-3645	45 Well Comb, 8.25" wide, 1.5 mm thick
170-3629	Comb Holder, 8.25"
170-3622	10 Sample Plug Mold
170-3623	Prep Comb, 2 well (plus 2 outer wells for size standards), 5.5" wide, 1.5 mm thick
170-3605	DNA Size Standards, <i>S. cerevisiae</i>
170-3633	DNA Size Standards, <i>S. pombe</i>
170-3635	DNA Size Standards, lambda ladder

Catalog Number	Description
170-3624	DNA Size Standards, 5 kb ladder
162-0017	Low Melt Preparative Grade Agarose, 25 g
162-0135	Chromosomal Grade Agarose, 25 g
162-0133	Molecular Biology Certified Agarose, 100 g
162-0159	Zeta-Probe Roll, 33 cm x 3 m
162-0115	Nitrocellulose Roll, 33 cm x 3 m