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CHEF-DR® II Pulsed Field Electrophoresis Systems

Instruction Manual and Applications Guide

Catalog Numbers
170-3612
through
170-3729

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The CHEF-DR II control module, drive module, chamber, variable speed pump, and accessories are warranted against defects in materials and workmanship for 1 year. If any defects occur in the instruments or accessories during this warranty period, Bio-Rad Laboratories will repair or replace the defective parts at its discretion without charge. The following defects, however, are specifically excluded:

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2. Repair or modification done by anyone other than Bio-Rad Laboratories or an authorized agent.
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4. Use of fittings or spare parts supplied by anyone other than Bio-Rad Laboratories
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7. Corrosion caused by improper solvent* or sample.

This warranty does not apply to parts listed below:

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2. Tubing
3. Electrodes

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Void the IEC1010-1 safety certification

Create a potential safety hazard

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** IEC1010-1 is an internationally accepted electrical safety standard for laboratory instruments.

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1st Revision

* The CHEF-DR II chamber is not compatible with chlorinated hydrocarbons (*e.g.*, chloroform), aromatic hydrocarbons (*e.g.*, toluene, benzene), or acetone. Use of organic solvents voids all warranties.

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Section 1
General Information

1.1 Safety 

The CHEF-DR II system uses high voltage and current and should be operated with care at all times. The safety interlocks are for your protection and should not be circumvented. To avoid shock, set up the CHEF-DR II components in a dry area. Immediately wipe up any spilled buffers or salt solutions.

When pausing or aborting a run, always check that the current display goes to zero or displays **OFF**. This can take 2-5 seconds while the power supply discharges. It is then safe to remove the lid from the chamber.

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 power to the chamber before working within the electrophoresis cell.

The Model 1000 Mini Chiller is ground isolated. Although there is virtually no current flowing through the Tygon® tubing into the chiller, you should avoid assembling or disassembling the tubing while the CHEF-DR II system is operating during a run.

When inserting or removing the external temperature probe, be sure that the control module is turned off. Do not handle the probe with wet hands.

Definition of Symbols



Caution, risk of electric shock



Caution (refer to accompanying documents)

1.2 Overview

Pulsed field electrophoresis is a technique for resolving chromosome-sized DNA molecules. By alternating the electric field between spatially distinct pairs of electrodes, megabase (mb) sized molecules are able to reorient and move at different speeds through the pores in an agarose gel. Overview articles and specific applications are listed in the references in Section 8.

The CHEF-DR II system is based on CHEF[31,215] (Clamped Homogeneous Electric Fields) technology. CHEF exhibits two unique properties which have made it the most commonly used PFGE technique. First, CHEF provides highly uniform, or homogeneous, electric fields within the gel. This is accomplished using an array of 24 electrodes, which are “clamped” or held to intermediate potentials to eliminate lane distortion. Thus, lanes are straight. Second, CHEF generates a 120° reorientation (field) angle, due the hexagonal geometry of the electrode array, which is the optimal angle for separating DNA molecules ranging from 100 kb to 6 mb in size. In addition, the CHEF-DR II system maintains homogeneous electric fields using patented Dynamic Regulation[216] (DR). With DR, the electrodes not only generate the electric field, but also sense changes in local buffer conductivity due to buffer breakdown, change in buffer type, gel thickness, or temperature. Voltage potentials are readjusted immediately to maintain uniform fields, thus insuring high resolution separations during runs and between runs. These properties make the CHEF-DR II system a cost-effective instrument for separating everything from Yeast Artificial Chromosomes (YACs) to cosmid inserts.

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1.3 Specifications

These specifications pertain to the complete CHEF-DR II system. A system including the Model 1000 Mini Chiller is also available.

CHEF-DR II Specifications

Drive Module

Dimensions	26 (depth) x 43 (width) x 14 (height) cm
Construction	Aluminum chassis
Weight	7.8 kg
Maximum voltage	250 VDC
Maximum current	500 mA
Electrode potentials	Dynamically regulated (feedback adjustment) +/- 0.5%
Input voltage range	90-132 VAC/47-63 Hz/0.7 amps (maximum) 198-264 VAC/47-63 Hz/0.35 amps (maximum)
Fuses	0.5 amp Fast Blow for high voltage output (front panel) 1.0 amp Slow-Blow (100/120 V) or 0.5 amp Slow-Blow (220/240 V)
Environmental	
Operating	50 °F (10 °C) to 90 °F (32 °C) temperature 30-80% humidity
Storage	32 °F (0 °C) to 140 °F (60 °C) temperature 10-90% humidity

Control Module

Dimensions	13.5 (depth) x 31 (width) x 17 (height) cm
Maximum current	500 mA
Allowable voltage gradients	0.6 - 6 V/cm, in 0.1 V/cm increments
Data entry	Keyboard
Power supply	250 V maximum, to allow maximum gradient of 6 V/cm,continuously adjustable
Functional	
Switching range	0.1 sec to 65,000 sec (all electronic switching)
Maximum program blocks	2, with automatic execution
Maximum run time	999 hours per block
Input voltage range	90-132 VAC/47-63 Hz/1.7 amps (maximum) 198-264 VAC/47-63 Hz/0.85 amps (maximum)
Fuses	2.0 amp Slow-Blow (100/120 V) or 1.0 amp Slow-Blow (220/240 V)
Environmental	
Operating	50 °F (10 °C) to 90 °F (32 °C) temperature 30-80% humidity
Storage	32 °F (0 °C) to 140 °F (60 °C) temperature 10-90% humidity
Electrophoresis cell	
Dimensions	43 (depth) x 44 (width) x 11 (height) cm, horizontal format
Construction	Acrylic
Lid	Safety interlocked
Weight	8.5 kg
Electrodes	24, platinum (0.01 inch diameter)
Temperature monitoring	Via precision temperature probe mounted through lid (optional)

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Accessories included

Variable speed pump	120 V, ground isolated. Flow rate 0.75 liter/min, typical
Casting stand	14 cm x 13 cm
Comb	10 well comb and comb holder
Tygon tubing	365 cm
Sample plug mold	10 slot
Yeast DNA Standard	*S. cerevisiae* YNN295, 2 plugs
Chromosomal Grade Agarose	5 grams
Pulsed Field Certified Agarose	5 grams
Leveling bubble	1
Fuses	0.5 Amp Fast Blow, 2
Manual	1

Model 1000 Mini Chiller (Optional)

Weight	14 kg
Construction	Aluminum
Dimensions	42 cm long x 23 cm wide x 24 cm high
Cooling capacity	75 watts of input power at 14 °C
Operating range	5 °C - 25 °C
Fuses	3.0 Amp Slow-Blow (100/120 V) or 1.5 Amp Slow-Blow (220/240 V)

Note: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

1.4 Description of Major Components

Drive Module

The drive module is a separate electronic device which distributes and maintains the individual voltages for the 24 electrodes in the electrophoresis cell. It contains the electrode drivers which provide and maintain the clamped homogeneous electric fields in the electrophoresis cell. This feature, dynamic regulation (US Patent 4,878,008 issued to Bio-Rad Laboratories), modulates the potentials so that the proper voltages are maintained regardless of gel size, or fluctuations in buffer conductivity or temperature.

Control Module

The control module contains the both the power supply function, which provides the voltage for the 24 drivers in the drive module, and the switcher function, which alternates the electric field in the electrophoresis cell. All run conditions, including initial and final switch time (ramp), voltage gradient, and run time, are programmed directly into the control module. In addition, up to 2 consecutively executing blocks of run conditions can be programmed, increasing the variety of possible separations. The power supply outputs a maximum voltage gradient of 6 V/cm, or 200 V. The lowest gradient is 0.6 V/cm, or 20 V.

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Figure 1.1 A shows the relative potentials of each electrode pair when the + 60° vector (indicated by the arrow) is activated. Net field 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 NW segment, directly opposite the SE, has 0 potential, represented in the diagram as negative terminals. When the - 60° angle is activated, the pattern of electric charges is as shown in Figure 1.1 B. Together, the two pulses result in a 120° included field angle. Other angles will result in values for the relative electrode potentials, according to predetermined values.

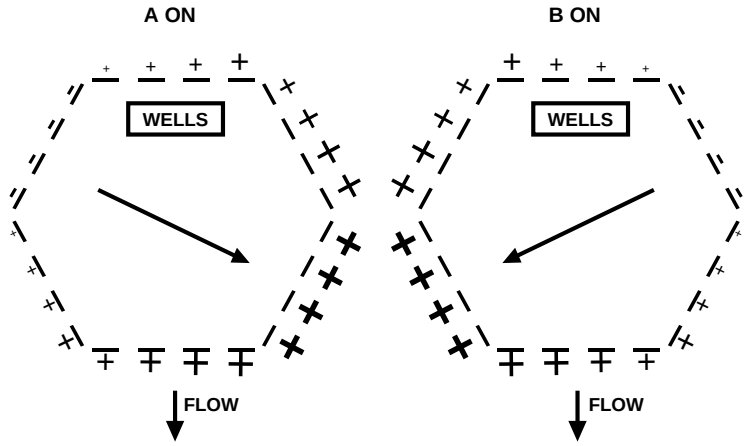


Fig. 1.1. Voltage Clamping by the CHEF-DR II system. **A.** Relative electrode potentials when the + 60° field vector is activated. **B.** Relative electrode potentials when the - 60° field vector is activated.

Electrophoresis Cell

The CHEF-DR II electrophoresis cell consists of a 43 x 44 cm (17” x 17.5”) acrylic box with 24 horizontal electrodes arranged in a hexagon. Gels are electrophoresed horizontally, submerged under recirculated buffer. A 14 x 13 cm (5.5” x 5”) gel is cast on a platform in a separate casting stand. The platform (along with the gel) is removed from the casting stand, and placed in the center of the hexagon. The platform is held in place by a frame positioned on the chamber floor. A combination wide/long format is available as an accessory (catalog number 170-3704). DNA migration and buffer flow is in the direction of the arrow mounted on the lid.

The electrodes are individually wired to the 24 pin computer cable, which in turn connects to the drive module. The individual electrodes are replaceable for easy maintenance (see Section 6). Electrodes are 0.01” diameter platinum wire. They are each sealed with an O-ring and silicone sealant to provide double protection against leakage. The electrodes will wear out more rapidly when switch times below 1 second are used, and/or when 6 V/cm gradients are employed.

There are two small chambers below the level of the main chamber floor at the front and rear of the main chamber. These chambers are used for buffer circulation and priming the pump. Buffer enters the main chamber through 6 holes in the floor near the top. A flow baffle is located just in front of these holes to prevent gel movement. Buffer exits the chamber at the front through the T fitting. One arm is for draining, the other for circulation. The base of the chamber has four leveling screws for even gel submersion in buffer.

The lid contains an interlock for safety. The voltage directly passes from the drive module through a short-path in the lid interlock. If the lid is removed, the current flow is broken

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and high voltage to the electrophoresis cell is disrupted. The lid also contains a mount at the upper right for an external temperature probe, which monitors buffer temperature in the chamber, and regulates cooling by the optional Model 1000 Mini Chiller.

Pump and Accessories

The CHEF-DR II system includes a variable speed pump, which 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 0.75 l/min.

The complete CHEF-DR II system is shown in Figure 1.2.



Fig. 1.2. The complete CHEF-DR II system, with electrophoresis cell, control module, drive module, variable speed pump, and optional Model 1000 Mini Chiller.

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Model 1000 Mini Chiller

The Model 1000 Mini Chiller is a stand alone, portable refrigerated apparatus specifically designed for use with the CHEF-DR II system. Electrophoresis buffer is circulated by the variable speed pump directly through the unique heat exchanger, which is a tube within a tube. Buffer circulates through the inner stainless steel tube, while liquid refrigerant circulates through the outer copper tube, resulting in rapid and efficient cooling at a rate of 0.75 °C/minute (from ambient temperature to 14 °C). The external temperature probe regulates cooling by the Model 1000 Mini Chiller, resulting in precise maintenance of buffer temperature in the electrophoresis cell.

Section 2
Operation

2.1 Instrument Setup

Place the CHEF-DR II electrophoresis cell on a level surface, with the control module on top of the drive module and to the right or on a shelf above. The electrophoresis cell should be positioned with the T connector facing you and the safety interlock to your right. If the system includes a Model 1000 Mini Chiller, place it to the left of the cell. Place the variable speed pump at the rear of the cell and connect the plug from the pump to the port labeled PUMP CONNECTOR in the front of the drive module. Level the electrophoresis cell with the four leveling feet at each corner of the electrophoresis cell by placing the leveling bubble (provided) in the center of the base of the cell.

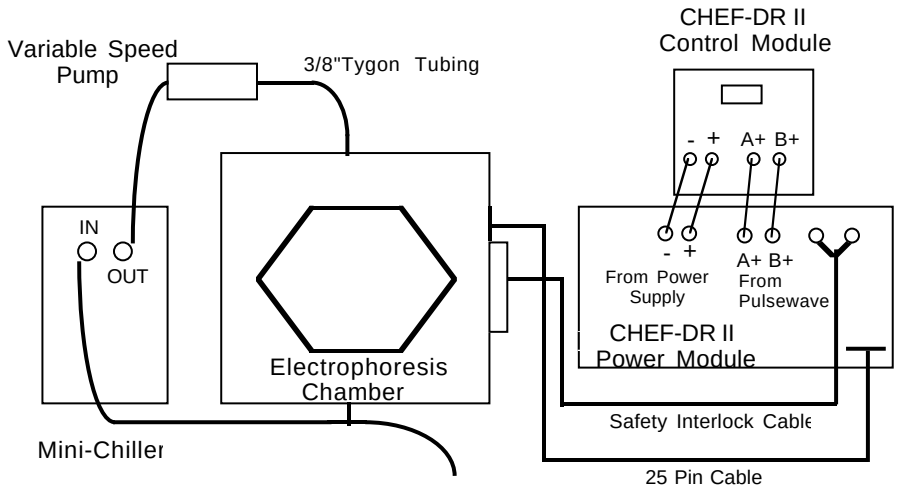


Fig. 2.1. Interconnections between components of the CHEF-DR II system.

Attach the power cords for the control module, drive module, and Model 1000 Mini Chiller in the back of each instrument. Be sure that both the control module and drive module are powered off. Connect the 25-pin cable from the electrophoresis cell to the port labeled OUTPUT TO ELECTROPHORESIS CELL on the front panel of the drive module. Connect the coiled interlock cable from the electrophoresis cell to the jacks labeled TO INTERLOCK on the drive module.

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Connect the set of black and red cables from the left set of jacks on the control module labeled POWER SUPPLY (- and +) to the left set of jacks on the drive module labeled FROM POWER SUPPLY. Connect - to - with the black cable, and + to + with the red cable. **Note:** the metal plugs at the ends of these cables have smaller diameters.

Connect the set of red cables from the right set of jacks on the control module labeled A+ and B+ to the right set of jacks on the drive module labeled FROM PULSEWAVE. Connect A+ to A+ and B+ to B+.

2.2 Electrophoresis Cell Operation

When using the Model 1000 Mini Chiller, attach approximately 1-2 feet of 1⁄4 inch internal diameter Tygon tubing to both the FLOW IN and FLOW OUT ports on the Model 1000 Mini Chiller, and secure the tubing with plastic clamps (provided). Connect 2 feet of 3⁄8 inch internal diameter Tygon tubing directly from the left port of the T connector to the 1⁄4 inch tubing from the FLOW IN of the Model 1000 Mini Chiller using a 3⁄8 to 1⁄4 inch reducer (provided). The pump should be positioned between the outlet of the Model 1000 Mini Chiller and the inlet of the electrophoresis cell. Connect the 1⁄4 inch tubing from the FLOW OUT of the Model 1000 Mini Chiller to the inlet of the CHEF-DR II pump using another 3⁄8 to 1⁄4 inch reducer. Connect approximately one to two feet of Tygon tubing from the outlet of the pump to the single connector at the rear of the electrophoresis cell using a 3⁄8 inch connector (provided). When using a general purpose, recirculating water bath as a chiller, attach about 9.5 feet of 3⁄8 inch internal diameter Tygon tubing to the left outlet port of the T connector on the electrophoresis cell. Connect the other end of the Tygon tubing to the inlet of the CHEF-DR II pump. Connect two feet of Tygon tubing from the outlet of the pump to the single connector at the rear of the electrophoresis cell. Coil approximately 4-5 feet of the longer tubing within the bath of the chiller.

Finally, connect a six inch piece of Tygon tubing to the right outlet port of the T connector and clamp the tubing securely closed; this will be used to drain the buffer in the electrophoresis cell.

Insert the gel frame into the positioning holes in the electrophoresis cell. There are two sets of three positioning holes. Place the frame only in the holes at the bottom corners of the gel, opposite the wells, so that the frame is centered in the cell. The outermost holes are for the optional 21 x 14 cm (8.25 x 5 inch) gel format (See casting stand Instruction Manual for more details).

If the system includes the Model 1000 Mini Chiller, connect the temperature probe to the port labeled REMOTE SENSOR on the front panel of the Model 1000 Mini Chiller. Insert the temperature probe into the lid of the electrophoresis cell. Remove the temperature probe when opening the lid to prevent breakage and for easy access to the electrophoresis cell.

It is desirable to establish the correct buffer flow prior to attempting any electrophoresis runs. Once the desired flow rate has been achieved, subsequent electrophoresis runs should be with the same setting on the variable speed pump. Fill the chamber with 2 liters of the buffer of choice. The desired flow rate of buffer through the electrophoresis cell is approximately 0.75 liter per minute (approximately 70 on the variable speed pump). Turn on the pump and measure the flow of buffer at the drain port by removing the clamp from the 6” piece of tubing. Make adjustments to the buffer flow with the variable speed pump.

It is also very beneficial to fine tune the chiller before attempting any electrophoresis runs. Turn on the chiller and pump approximately 1⁄2 hour before adjusting the temperature. Initially, it will be necessary to fine tune the temperature setting to achieve a consistent running temperature.

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2.3 CHEF-DR II Operation

This section describes general operation. See Sections 3 and 4 for sample preparation, gel casting, gel running, and staining. In the following sections capitalized words refer to keys, modes, or indicator lights on the CHEF-DR II control module (*e.g.* RUN TIME) while boldface words refer to messages on the display (*e.g.* **End**).

Power Up

The CHEF-DR II front panel display is shown in Figure 2.2. The front panel consists of a three segment display, BLOCK, VOLTS/CM, RUN TIME, START/PAUSE, INITIAL SWITCH TIME, FINAL SWITCH TIME, CURRENT, and RAISE and LOWER keys. On power up, the unit will beep once and display **b-1** with the BLOCK indicator light illuminated, indicating Block 1 is active. The program parameters will be at the default settings.



Fig. 2.2. Front Panel display of the CHEF-DR II system.

Parameter Entry

The CHEF-DR II system has the flexibility of using up to two separate, consecutively executing Blocks. Each Block has the run parameters of Initial Switch Time, Final Switch Time, Run Time, and Volts/cm. During a run, Block 1 is run first and then Block 2. To start entering the run parameters, turn on the control module. The unit will beep once and display **b-1** with the BLOCK indicator light illuminated, indicating Block 1. If not, press LOWER until **b-1** is displayed. Press VOLTS/CM (the VOLTS/CM indicator light will be illuminated), and enter the desired value with RAISE and LOWER. Press RUN TIME (the RUN TIME indicator light will be illuminated), and enter the desired value with RAISE and LOWER. Press BLOCK and VOLTS/CM simultaneously (both the BLOCK and VOLTS/CM indicator lights will be illuminated), and set Initial Switch Time with RAISE and LOWER. Press VOLTS/CM and RUN TIME simultaneously (both the VOLTS/CM and RUN TIME indicator lights will be illuminated), and set Final Switch Time with RAISE and LOWER. If more than 1 Block is desired, press BLOCK and go to Block 2 by pressing RAISE. The unit will display **b-2** with the BLOCK indicator light illuminated, indicating Block 2. Continue entering the run parameters as in Block 1. Below are the limits for each of the run parameters.

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Block

Adjust from 1-2 Blocks. Block 1 is run first, then Block 2. A run time of 0 will disable a Block.

Initial Sw. Time

Press BLOCK and VOLTS/CM simultaneously. Adjust from 0.1 second-65,000 seconds (displayed at 65 t).

Final Sw. Time

Press VOLTS/CM and RUN TIME simultaneously. Adjust from 0.1 second-65,000 seconds (displayed at 65 t).

Run Time

Adjust from 0.1 hours-999 hours. A run time of 0 will disable a Block.

Volts/cm

Adjust from 0.6 volts to 6.0 volts in 0.1 volt increments.

Actual Current

Press BLOCK and RUN TIME simultaneously. This will display the current in milliamps provided by the power supply. This parameter is not adjustable.

Run Program

When the parameters are set, the program may be initiated by pressing START/PAUSE. Once initiated, the actual current (in mA) will be displayed for 5 seconds, with the BLOCK, RUN TIME, and START/PAUSE indicator lights illuminated. When the program is in progress the unit will display the time remaining (hours) in the current Block with the RUN TIME and START/PAUSE indicator lights illuminated. The current (milliamps) can be displayed by pressing BLOCK and RUN TIME simultaneously, with the BLOCK, RUN TIME and START/PAUSE indicator lights illuminated. Once the program is initiated, it is not possible to edit any of the run parameters. During a run, the RAISE and LOWER keys are disabled and the remaining keys will operate as follows:

Block

This will display the current Block.

Initial Sw. Time

This will display the set initial switch time for 3 seconds after which the remaining time for the current Block in progress will be displayed.

Final Sw. Time

This will display the set final switch time for 3 seconds after which the remaining time for the current Block in progress will be displayed.

Current Switch

This will display the current switch time for 3 seconds after which the Time remaining time for the current Block in progress will be displayed. This display is activated by pressing BLOCK, VOLTS/CM, and RUN TIME simultaneously.

Run Time

This will display the set run time for the current Block for 3 seconds after which the remaining time for the current Block in progress will be displayed.

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Volts/cm

This will display the set voltage gradient for 3 seconds after which the remaining time for the current Block in progress will be displayed.

Actual Current

This will display the actual current for the Block in progress. The BLOCK and RUN TIME indicator lights will be illuminated. If the power supply is at current limit (500 mA maximum), the BLOCK and RUN TIME indicator lights will flash.

Pause/Start Run

Initially, this will initiate the program, and the actual current (in mA) will be displayed with the BLOCK, RUN TIME, and START/PAUSE indicator lights illuminated. After 5 seconds, the remaining run time (hours) will be displayed with the RUN TIME and START/PAUSE inidicator lights illuminated. While the program is running, pressing this key will put the program into PAUSE and the actual current will be displayed with the BLOCK and RUN TIME indicator lights illuminated, while the START/PAUSE indicator light will flash. Pressing this key again will resume the program, and the actual current will be displayed for 5 seconds.

In a muti-block program it is possible to examine the run parameters of any Block that is not currently being displayed by pausing the CHEF-DR II system. While in PAUSE, the Raise and Lower keys are active to scroll through the two Blocks. Any parameter in either Block may be displayed by pressing the appropriate key. Editing of run parameters is not possible once the program has started.

Pausing the Program

The program in progress can be paused to examine set run parameters by pressing START/PAUSE while the unit is running. When START/PAUSE is pressed, the actual current will be displayed, with the BLOCK and RUN TIME indicator lights illuminated, while the START/PAUSE indicator light will flash. **Warning:** There are high voltages and currents within the chamber, which can be harmful. Wait for the displayed current to reach zero before removing the lid of the electrophoresis cell. To resume the program, press START/PAUSE, and actual current will be displayed for 5 seconds, after which the remaining time for the current Block in progress will be displayed.

Clearing the Program

It is possible to completely clear every parameter in Blocks 1 and 2 of a current program to the default settings. This can be done turning off the control module. Individual parameters may be reset to defaults by pressing RAISE and LOWER simultaneously for 6 seconds (it will beep once per second).

Program Termination

The program in progress may be manually terminated by holding down START/PAUSE for three to four seconds. A program can only be terminated while it is in the RUN mode, it can not be terminated in the PAUSE mode. When the program is terminated it will beep twice and the unit will display **OFF**. Pressing START/PAUSE again will start the program from the beginning.

When the program terminates under the timer control, the RUN TIME and START/PAUSE indicator lights will go off, it will sound 2 beeps per second for 10 seconds, and the unit will display **End**. The run timers will be reset and all parameters will be retained. The run parameters may be used again as is, or further modified, and the program may be started again by pressing START/PAUSE.

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Section 3
Sample Preparation

3.1 Agarose Blocks

Standard procedures for DNA preparation do not yield intact, high molecular weight DNA molecules. Large DNA molecules (chromosome-sized) are so fragile that they are sheared by mechanical forces, such as pipetting, during isolation. To prevent breakage of large DNA molecules, intact cells embedded in agarose are lysed and deproteinized *in situ*.[219] The agarose matrix protects the embedded DNA from shear forces and provides an easy way to manipulate samples. Processed genomic DNA-embedded agarose plugs are loaded directly into sample wells of agarose gels.

The most important and difficult task in preparing cells for embedding in agarose is to obtain the proper cell concentration. Although optical density is frequently used, it is not reliable. Different strains, plasmid content and growth media all contribute to the actual cell number achieved for a particular optical density. Variation in cell number will cause the amount of DNA per agarose plug to vary leading to over and/or under loading of the sample. To eliminate the need to generate a growth curve for each individual strain, a hemocytometer provides the most reproducible method for achieving the proper cell concentration for different types of cells, bacteria, yeast, and fungi. Detailed instructions for the use of a hemocytometer can be found in Section 3.7.

Sample inserts are cast in Bio-Rad's disposable plug mold, catalog number 170-3713. Each sample mold produces up to fifty 10 x 5 x 1.5 mm agarose plugs. The block thickness allows rapid and efficient diffusion of enzymes during sample preparation and permits samples to be loaded into wells formed with Bio-Rad's standard well-forming combs without excessive trimming.

3.2 Liquid Samples

High molecular weight DNA can be prepared by standard procedures. DNA fragments of up to several hundred kilobases do not require preparation in agarose blocks, but can be added to the wells in liquid form. When working with DNA in the range of 50-200 kb, it may be necessary to use pipette tips with large openings. When running only liquid samples, the best resolution and sharpness of bands can be achieved by using a thin well comb (0.75 mm).

3.3 Preparation of Agarose Embedded Mammalian DNA

The buffers, enzymes, and agarose found in the following procedure are provided in the CHEF Mammalian Genomic DNA Plug Kit (catalog number 170-3591; see Section 9 for more information).

1. Prepare a cell suspension in isotonic saline or tissue culture medium without fetal bovine serum. Count the cells and remove 5 x 10^7 cells for each ml of agarose plugs to be made and place on ice. See Section 3.7 for hemocytometer usage. The 50 well plug mold makes 5 ml of agarose plugs we recommend making slightly more than 5 ml if all fifty wells are to be utilized.

2. Prepare a 2% CleanCut™ agarose (Bio-Rad) solution in sterile water and melt using a microwave. Equilibrate the solution to 50 °C in a water bath.

3. Centrifuge the cell suspension at 1,000 x g for 5 minutes at 4 °C. Resuspend the cells in one-half the final volume of plugs to made using Cell Suspension Buffer (10 mM Tris, pH 7.2, 20 mM NaCl, 50 mM EDTA) and equilibrate the cell suspension to 50 °C.

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4. Combine the cell suspension with an equal volume of 2% CleanCut agarose and mix gently but thoroughly. Keeping the cell/agarose mixture at 50 °C, transfer the mixture to plug molds using sterile transfer pipettes (Bio-Rad's disposable transfer pipettes catalog 223-9524 are recommended). Allow the agarose to solidify. This step can be expedited by placing the molds at 4 °C for 10-15 minutes, and it also adds strength to the agarose for removal from the mold.

6. Using a 50 ml conical centrifuge tube, add 5 ml of Proteinase K Reaction Buffer (100 mM EDTA, pH 8.0, 0.2% sodium deoxycholate, 1% sodium lauryl sarcosine, 1 mg/ml Proteinase K) for each ml of agarose plugs (*e.g.* use 25 ml of Proteinase K Reaction Buffer for 5 ml of agarose plugs). Push the solidified agarose plugs, using the snap off tool provided on the plug mold, into the 50 ml centrifuge tube containing the Proteinase K solution. Incubate the plugs overnight at 50 °C without agitation.

   NOTE: various cell lines have been incubated up to 4 days in Proteinase K without detrimental effects to the quality of DNAs.

7. Wash the plugs four times in 50 ml of wash buffer (20 mM Tris, pH 8.0, 50 mM EDTA), 30 minutes to 1 hour each at room temperature with gentle agitation. If the plugs are to be used in subsequent enzymatic reactions, it is advisable to wash the plugs in 1 mM PMSF during the second or third wash to inactivate any residual Proteinase K activity.

8. Store the plugs at 4 °C. The plugs are stable for 3 months to one year.

9. Maintain the plugs in 1x Wash Buffer for long term storage. However , for subsequent restriction digestion, the EDTA concentration must be lowered. Wash the plugs to be restricted for 30 minutes in 0.1x wash buffer or TE. See Section 3.7 for more information on restriction digestion of plugs.

3.4 Preparation of Agarose Embedded Bacterial DNA

The buffers, enzymes, and agarose found in the following procedure are provided in the CHEF Bacterial Genomic DNA Plug Kit (catalog number 170-3592; see Section 9 for more information).

1. Inoculate a bacterial culture into 50 ml of LB Broth or appropriate media and grow with agitation to an OD_{600} of 0.8 - 1.0 at the appropriate temperature.

2. When the desired OD_{600} is reached, add chloramphenicol to a final concentration of 180 µg/ml and continue incubation up to one hour while performing step 3.

   Note: Chloramphenicol is used to synchronize ongoing rounds of chromosomal replication and inhibit further rounds of replication. This step is optional, but regions near the replication terminus might be under represented. In addition, chloramphenicol will alter the morphology of the cells over time causing the appearance of a mixed culture, therefore proceed as quickly as possible with step 3.

3. Make a twenty-fold dilution of the above bacterial suspension using 10 µl bacteria, 20 µl Gram Crystal Violet, and 170 µl saline or PBS. Place a small amount of the bacterial suspension on a hemocytometer and count at 400x power. See Section 3.7 for hemocytometer usage.

4. Prepare a 2% CleanCut agarose solution using sterile water and melt using a microwave. Equilibrate the solution to 50 °C in a water bath.

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5. Remove 5 x 10^8 cells for each ml of agarose plugs to be made. Centrifuge for three minutes in a microcentrifuge. If the volume is too large, spin at 10,000 x g for 5 minutes at 4 °C in an appropriate size tube. Resuspend the cells in one-half the final volume of plugs to be made using Cell Suspension Buffer (10 mM Tris, pH 7.2, 20 mM NaCl, 50 mM EDTA) and equilibrate the cell suspension to 50 °C.

   Caution: Some bacteria may be sensitive to the concentration of EDTA or the osmotic strength of cell suspension buffer resulting in premature lysis of the bacteria. This premature lysis will result in DNA that is unacceptable for PFGE. Bacteria such as *Enterococci* require 1M NaCl in the buffer to prevent osmotic imbalance resulting in lysis. *Pseudomonas* is sensitive to EDTA concentration, and dilution of the buffer may be necessary. Most bacteria require no alteration of the buffer, but as stated in the above procedure, mixing and imbedding of the bacteria should proceed as quickly as possible.

6. Combine the cell suspension with an equal volume of 2% CleanCut agarose and mix gently but thoroughly. Keeping the cell/agarose mixture at 50 °C, transfer the mixture to plug molds using sterile transfer pipettes (Bio-Rad's disposable transfer pipettes catalog number 223-9524 are recommended). Allow the agarose to solidify. This step can be expedited by placing the molds at 4 °C for 10-15 minutes, and it also adds strength to the agarose for removal from the mold.

7. Using a 50 ml conical centrifuge tube, add 5 ml of lysozyme buffer (10 mM Tris, pH 7.2, 50 mM NaCl, 0.2% sodium deoxycholate, 0.5% sodium lauryl sarcosine, 1 mg/ml lysozyme) for each ml of agarose plugs, (*e.g.* use 25 ml of lysozyme buffer for 5 ml of agarose plugs). Push the solidified agarose plugs, using the snap off tool provided on the plug mold, into the 50 ml centrifuge tube containing the lysozyme buffer. Incubate the plugs 30 minutes to 1 hour at 37° C without agitation.

   Note: Bacteria such as *Staphylococcus aureus* and some others are insensitive to lysozyme, therefore lysostaphin must be substituted in place of lysozyme buffer. Additionally, adding lysostaphin to the cell suspension immediately prior to imbedding with agarose produces high quality *S. aureus* plugs. See Section 3.5, step 6 for a more detailed description of the procedure.

8. Remove the lysozyme buffer and rinse the plugs with 25 ml of 1x wash buffer (see step 9 for wash buffer recipe). Add 5 ml of Proteinase K Reaction Buffer (100 mM EDTA, pH 8.0, 0.2% sodium deoxycholate, 1% sodium lauryl sarcosine, 1 mg/ml Proteinase K) for each ml of agarose plugs. Incubate the plugs overnight at 50 °C without agitation. **NOTE:** various cell lines have been incubated up to 4 days in Proteinase K without detrimental effects to the quality of DNAs.

9. Wash the plugs four times in 50 ml of wash buffer (20 mM Tris, pH 8.0, 50 mM EDTA), 30 minutes to 1 hour each at room temperature with gentle agitation. If the plugs are to be used in subsequent enzymatic reactions, it is advisable to wash the plugs in 1 mM PMSF during the second or third wash to inactivate any residual Proteinase K activity.

10. Store the plugs at 4 °C. The plugs are stable for 3 months to one year.

11. Maintain the plugs in 1x Wash Buffer for long term storage. However , for subsequent restriction digestion, the EDTA concentration must be lowered. Wash the plugs to be restricted for 30 minutes in 0.1x wash buffer or TE. See Section 3.7 for more information on restriction digestion of plugs.

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3.5 Preparation of Agarose Embedded Yeast DNA

The buffers, enzymes, and agarose found in the following procedure are provided in the CHEF Yeast Genomic DNA Plug Kit (catalog number 170-3593; see Section 9 for more information).

1. Inoculate a single colony into 50 to 100 ml YPG broth or appropriate media. Grow with aeration to an OD_{600} of >1.0 at the appropriate temperature for your strain.
2. When the desired OD_{600} is reached, centrifuge the cells at 5,000 x g, 10 minutes at 4 °C. Decant the supernatant and resuspend in 10 ml cold 50 mM EDTA, pH 8.
3. Determine the cell concentration by adding 10 µl of cells to 990 µl of water. Place the yeast suspension on a hemocytometer and count at 400x power. See Section 3.7 for hemocytometer usage.
4. Prepare a 2% CleanCut agarose solution using sterile water and melt using a microwave. Equilibrate the solution to 50 °C in a water bath.
5. Remove 6 x 10^8 cells for each ml of plugs to be made. Centrifuge in a microfuge for 3 minutes if volumes are small, otherwise centrifuge the cells at 5,000 x g, for 10 minutes at 4 °C. Resuspend the cells in one-half the final volume of plugs to be made using Cell Suspension Buffer (10 mM Tris, pH 7.2, 20 mM NaCl, 50 mM EDTA) and equilibrate the cell suspension to 50 °C.
6. Just prior to mixing the cells with agarose, add Lyticase to a final concentration of 1 mg/ml for each ml of plugs to be made, to the cell suspension and immediately proceed with step 7.

   NOTE: It is recommended that Lyticase be added immediately prior to imbedding the cells in agarose. It has been found that certain strains of yeast do not give acceptable DNA when Lyticase is added after the cells have been imbedded into agarose.
7. Immediately combine the cell suspension with an equal volume of 2% CleanCut agarose and mix gently but thoroughly. Keeping the cell/agarose mixture at 50 °C, transfer the mixture to plug molds using sterile transfer pipettes (Bio-Rad's disposable transfer pipettes catalog 223-9524 are recommended). Allow the agarose to solidify. This step can be expedited by placing the molds at 4 °C for 10-15 minutes, and it also adds strength to the agarose for removal from the mold.
8. Using a 50 ml conical centrifuge tube, add 5 ml of lyticase buffer (10 mM Tris, pH 7.2, 50 mM EDTA, 1 mg/ml lyticase) for each one ml of plugs. Push the solidified agarose plugs, using the snap off tool provided on the plug mold, into the 50 ml centrifuge tube containing the lyticase buffer. Incubate the plugs 30 minutes to 1 hour at 37 °C without agitation.
9. Remove the lyticase buffer and rinse the plugs with 25 ml of 1x wash buffer (see step 10 for wash buffer recipe). Add 5 ml of Proteinase K Reaction Buffer (100 mM EDTA, pH 8.0, 0.2% sodium deoxycholate, 1% sodium lauryl sarcosine, 1 mg/ml Proteinase K) for each ml of agarose plugs. Incubate the plugs overnight at 50 °C without agitation. **NOTE**: various cell lines have been incubated up to 4 days in Proteinase K without detrimental effects to the quality of DNA's.
10. Wash the plugs four times in 50 ml of wash buffer (20 mM Tris, pH 8.0, 50 mM EDTA), 30 minutes to 1 hour each at room temperature with gentle agitation. If the plugs are to be used in subsequent enzymatic reactions, it is advisable to wash the plugs in 1 mM PMSF during the second or third wash to inactivate any residual Proteinase K activity.
11. Store the plugs at 4 °C. The plugs are stable for 3 months to 1 year.

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12. Maintain the plugs in 1x Wash Buffer for long term storage. However , for subsequent restriction digestion, the EDTA concentration must be lowered. Wash the plugs to be restricted for 30 minutes in 0.1x wash buffer or TE. See Section 3.7 for more information on restriction digestion of plugs.

3.6 Accelerated Sample Preparation and Multiple Sample Preparation

In many cases the above procedures can be shortened by reducing or eliminating some of the incubation steps. For example, bacteria and yeast can be mixed with the cell wall lysing enzyme immediately prior to casting the plug. By allowing the plug to solidify for 15 to 20 minutes at room temperature, the cell walls are sufficiently degraded to allow complete lysis of the cells. The cell walls do not have to be completely degraded for PFGE, only enough to allow release of the DNA is required. The plugs can be incubated in the Proteinase K buffer after solidification, and the procedure can be shortened by 1 to 2 hours. The Proteinase K incubations typically begin at the end of the day, and are usually allowed to proceed overnight. This step can be reduced to 1-2 hours. Alternatively, the Proteinase K digestion can be eliminated by incubating the lysed cells in low salt buffers (such as TE) at elevated temperatures (55 °C).[218] The drawback is that the DNA degrades after storage for more than a week or two.

3.7 Restriction Enzyme Digestion of Plugs

1. Place one to three plugs per digest in a sterile 1.5 ml microcentrifuge tube, or if multiple digests are to be done place the plugs in a 24 well microtiter plate. Incubate the plug with 1 ml of the appropriate 1x restriction enzyme buffer for about 1 hour with gentle agitation at room temperature. Aspirate off the buffer and add 0.3 ml of fresh 1x enzyme buffer. Add the restriction enzyme (50 U per 100 µl plug) and incubate 2 hours at the appropriate temperature for the restriction enzyme.

   NOTE: Digestion with multiple restriction enzymes may require very different optimal reaction temperatures.

2. After digestion, remove the buffer and incubate in 1 ml of wash buffer or electrophoresis buffer for approximately thirty minutes with gentle agitation.

   NOTE: If the plugs are to be stored for more than one day, remove the running buffer from the tube and store at 4 °C. This will prevent possible diffusion of small (<100 kb) DNA fragments out of the agarose plug.

3. Load 1/2 of a plug per well and adjust the volume if necessary on subsequent gels. In addition, always load appropriate size standards. The concentration of cells recommended in the above procedures is optimized for a 10 mm wide well (the size of the standard-comb provided with the CHEF-DR II system). For a smaller width well (*e.g.* 5 mm), the plug should be cut in half along the long (10 mm) dimension, then trimmed appropriately to fit into the well.

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3.8 Hemocytometer Usage

A hemocytometer is usually divided into nine large squares (Figure 3.1). Each large square is 1 x 10^{-4} cm^2 or 0.1 mm^3, one such square (**A**) is shown in the figure with darkened borders. The large circle around the center square (**B**) represents your field of view at 100x power (10x objective lens, 10x eye piece). The center square (**C**) is subdivided into 25 smaller squares. The smaller circle in the center square represents your field of view at 400x power (40x objective lens, 10x eye piece). These 25 center squares are further divided into 16 squares.

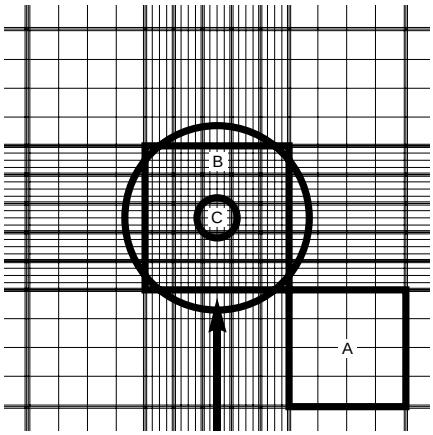


Fig. 3.1. Hemocytometer grid.

A. Mammalian or Tissue culture cells:

Because of the large size, tissue culture cells are able to be counted at 100x power. Count 10 of the large squares, five on each side of the hemocytometer. Determine the average cells per square using the equations below:

Hemocytometer Equations:

$$\frac{\text{Cells Counted}}{\text{Number of Squares}} = \text{Average Cells per Square}$$

$$\frac{\text{Desired Cell Concentration}}{\text{Actual Cell Concentration}} \text{ (ml of plugs to be made)} = \text{ml of cell suspension needed}$$

(Average Cells per Square)(Dilution Factor)(10^4) = Cells per ml.

For Example: 230 cells in 10 squares = average of 23 cells /square x 5 (dilution factor) x 10^4 = 1.2 x 10^8 cells per ml. So for 5 ml of plugs you need 5 ml x 5 x 10^7 cells final concentration divided by 1.2 x 10^8 cells/ml concentration = 0.41 ml of cell suspension is required to make 5 ml of agarose plugs.

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B. Bacteria and Yeast Cells:

Count five to ten of the 25 center squares, at 400x power, to get a representative sample of your cell suspension. You should have approximately 25 to 75 cells per square. The cells should be relatively free of clumps. Bacteria which naturally chain or grow in clusters are relatively easy to count and do not have to be dispersed by chemical or enzymatic methods. The Grams Crystal Violet aids in the visualization of bacteria.

For example: 300 bacteria in 5 squares = average of 60 bacteria/square x 25 (squares) x 20 (dilution factor, yeast use 100 for dilution factor, or whatever dilution factor you prefer to use) x 10^4 = 3 x 10^8 bacteria per ml. So for 5 ml of plugs you need 5 ml x 5 x 10^8 cells final concentration ÷ 3 x 10^8 cells/ml concentration = 0.83 ml of cell suspension is required.

3.9 Estimation of Agarose Plug DNA Concentration:

Two pieces of information are needed to determine DNA concentration:

1. The size in base pairs of the genome. This information is readily available for most organisms, otherwise a best guess is necessary. We use 6 x 10^9 for mammalian, 4.5 x 10^6 for *Escherichia coli* and 1.5 x 10^7 for *Saccharomyces cerevisiae* in the following examples.
2. You need to determine the number of genomes per cell. For example, for stationary growth phase in yeast or bacterial cells or confluent growth in tissue culture cells, assume one genome per cell. However, for exponential phase growing cells there is more than one genome per cell. Make a best guess or assume one per cell which will give the minimum concentration of DNA. In the below examples we use a value of 1.2 genome equivalents (20%) for mammalian cells, 2.5 genome equivalents for bacteria, and 2 genome equivalents for yeast.[217]

Equations for Estimation of DNA Concentration in Agarose Plugs

(**A**)

$$\frac{(\text{Genome Size bp})(660\ \text{g/mole})}{6.02 \times 10^{23}\ \text{bp/mole}} = \text{grams DNA/cell}$$

(**B**)

(grams DNA/cell)(cell/ml) = (grams DNA/ml)(1x10^6 µg/g) = µg DNA/ml

(**C**)

(µg DNA/ml)(genome equivalents (Section 3.9.2)) ≅ (µg DNA/ml)

(**D**)

$$\frac{\text{µg DNA/ml}}{\text{10 plugs/ml}} \cong \frac{\text{µg DNA/plug}}{\text{2 lanes/plug (Section 3.7.3)}} \cong \text{µg DNA/lane}$$

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Example Calculations:

Mammalian:

(**A**)

$$\frac{(6 \times 10^{9} \text{ bp})(660 \text{ g/mole})}{6.02 \times 10^{23} \text{ bp/mole}} = 6.547 \times 10^{-12} \text{ g DNA/cell}$$

(**B**)

$(6.578 \times 10^{-12}$ g/cell$)(5 \times 10^{7}$ cells/ml) =
$(3.289 \times 10^{-4}$ g DNA/ml$)(1 \times 10^{6}$ µg/g) - 329 mg DNA/ml

(**C**)

(329 µg DNA/ml)(1.2 genome equivalents) ≅ (394 µg DNA/ml)

(**D**)

$$\frac{394 \text{ µg DNA/ml}}{10 \text{ plugs/ml}} \cong \frac{40 \text{ µg DNA/plug}}{2 \text{ lanes/plug}} \cong 20 \text{ µg DNA/lanes}$$

Bacterial:

(**A**)

$$\frac{(4.5 \times 10^{6} \text{ bp})(660 \text{ g/mole})}{6.02 \times 10^{23} \text{ bp/mole}} = 4.933 \times 10^{-15} \text{ g DNA/cell}$$

(**B**)

$(4.933 \times 10^{-15}$ g DNA/cell$)(5 \times 10^{8}$ cells/ml) =
$(2.467 \times 10^{-6}$ g DNA/ml$)(1 \times 10^{6}$ µg/g) = 2.5 µg DNA/ml

(**C**)

(2.5 µg DNA/ml)(2.5 genome equivalents) ≅ (6.25 µg DNA/ml)

(**D**)

$$\frac{6.25 \text{ µg DNA/ml}}{10 \text{ plugs/ml}} \cong \frac{0.625 \text{ µg DNA/plug}}{2 \text{ lanes/plug}} \cong 0.3 \text{ µg DNA/lane}$$

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Yeast:

(**A**)

$$\frac{(1.5 \times 10^{7}\ \text{bp})(660\ \text{g/mole})}{6.02 \times 10^{23}\ \text{bp/mole}} = 1.644 \times 10^{-14}\ \text{g DNA/cell}$$

(**B**)

$(1.644 \times 10^{-14}\ \text{g DNA/cell})(6 \times 10^{8}\ \text{cells/ml}) =$
$(9.876 \times 10^{-6}\ \text{g DNA/ml})(1\text{x}10^{6}\ \mu\text{g/g}) = 9.876\ \mu\text{g DNA/ml}$

(**C**)

$(9.876\ \mu\text{g DNA/ml})(2\ \text{genome equivalents}) \cong (20\ \mu\text{g DNA/ml})$

(**D**)

$$\frac{20\ \mu\text{g DNA/ml}}{10\ \text{plugs/ml}} \cong \frac{2\ \mu\text{g DNA/plug}}{2\ \text{lanes/plug}} \cong 1.0\ \mu\text{g DNA/lane}$$

Section 4
Gel Electrophoresis

4.1 Casting the Gel

Casting the gel consists of using the following components: a casting stand with removable end plates; a platform on which the gel is cast, a comb and comb holder, and a frame which positions the gel and platform in the electrophoresis cell. The casting stand provided with the CHEF-DR II system is 14 cm (5.5”) wide x 13 cm (5”) long. An optional wide/long combination casting stand, 21 cm (8.5”) x 14 cm (5.5”), is are available for greater throughput or increased seapration.

The gel should be cast on a level surface. Bio-Rad’s Leveling Table (catalog number 170-4046) is recommended for this purpose. For more detailed instructions, refer to the manual provided with the casting stand.

1. Slide the platform into the casting stand. There is no sidedness to the platform. Position one end gate over the screws protruding from the casting stand, insuring that the horizontal slot is facing towards the platform. Slide the edge of the platform into the slot, press down on the end gate, and gently tighten the screws.
2. Position the other end gate over the screws, and slide it towards the platform until the edge of the platform is inserted into the slot. Press down on the end gate, and gently tighten the screws. The slots force the platform against the bottom of the casting stand.
3. To attach the desired comb to the comb holder, place the comb over the 2 metal pins, and turn the screw clockwise. This causes one pin to move towards the screw, holding the comb in place. Adjust the height of the comb to 2 mm above the surface of the platform by loosening the screw (counterclockwise), then tightening when the comb is properly positioned. A thin plastic ruler makes a good height gauge.

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4. Place the comb holder (with attached comb) into one of the two positioning slots on each side of the casting stand. Check that the bottom of the comb is at least 2 mm above the surface of the platform. Pour approximately 100 ml of the desired agarose solution (<60 °C) into the casting stand for a thickness of approximately 5-6 mm. Allow the gel to solidify for 30 minutes at room temperature.

5. Carefully remove the comb holder and comb; it is sometimes helpful to rock the holder back and forth slightly during its removal. Sample plugs can be added to the wells while the gel remains in the casting stand.

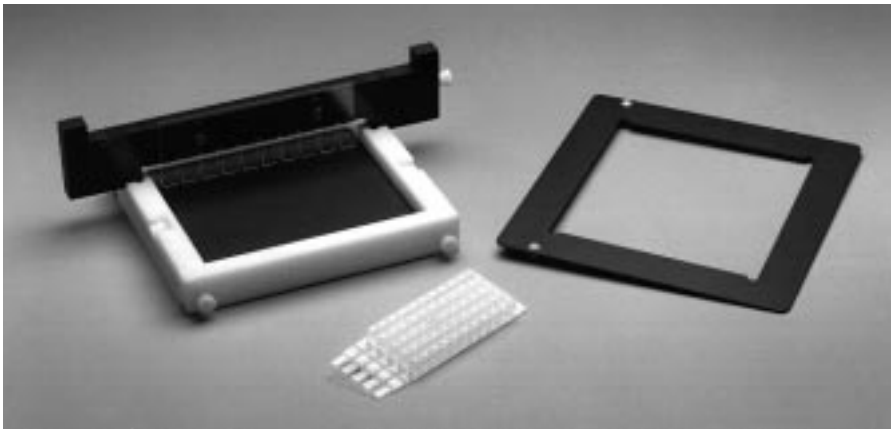


Fig. 4.1. The CHEF-DR II casting stand, frame, and comb holder.

4.2 Buffer Circulation

Level the CHEF-DR II electrophoresis cell by adjusting the leveling screws at the corners. Place the leveling bubble in the center of the base of the cell. Position the frame in the electrophoresis cell by placing the 2 plastic pins into the bottom set of holes (towards the "T" connector) in the floor of the cell so that the frame is centered (center hole in each group of 3 holes). Pour 2 liters of buffer (appropriate concentration of TBE or TAE) into the cell. Switch on the CHEF-DR II power, then switch on the variable speed pump. Circulate at ~ 0.75 L/min (a setting of ~ 70 on the pump regulator). Maintain the flow rate at the maximum setting that does not disturb the gel. Allow the buffer to equilibrate to the desired temperature. We recommend 14 ˚C buffer temperature in cell. The electrophoresis buffer can be chilled by the following methods:

1. Attach the Model 1000 Mini Chiller (see Model 1000 Mini Chiller manual for set-up and operation).

2. Coil pump tubing into a temperature-controlled water bath with the temperature set so that the buffer temperature in the electrophoresis cell is 14 ˚C.

Prior to beginning the electrophoresis run, the current output displayed on the CHEF-DR II control module should be checked to insure that the correct buffer concentration is used. The following values are for 2 liters of buffer at 14 °C circulating through the electrophoresis cell.

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Buffer Concentration	Voltage Gradient	Current Range
0.5x TBE (at 14 ˚C)	2 V/cm	30-40 mA
0.5x TBE (at 14 ˚C)	3 V/cm	50-60 mA
0.5x TBE (at 14 ˚C)	6 V/cm	110-120 mA
1.0x TAE (at 14 ˚C)	2 V/cm	80-90 mA
1.0x TAE (at 14 ˚C)	3 V/cm	120-130 mA
1.0x TAE (at 14 ˚C)	6 V/cm	260-270 mA

If the current output is significantly different from the values listed above, the buffer should be drained, and new buffer should be added. Premixed 10x TBE is available from Bio-Rad (catalog number 161-0733).

Concentrations of Buffers

Different final concentrations of electrophoresis buffer have been employed in pulsed field electrophoresis. Bio-Rad's recommended final buffer concentrations are:

0.5x TBE Buffer:	45 mM Tris 45 mM borate 1.0 mM EDTA pH 8.3	**10x TBE Buffer: (per liter)**	108 g Tris base 55 g boric acid 40 ml 0.5M EDTA, pH 8.0
1.0x TAE Buffer:	40 mM Tris 40 mM acetate 2.0 mM EDTA pH 8.0	**50x TAE Buffer: (per liter)**	242 g Tris base 57.1 ml glacial acetic acid 100 ml 0.5M EDTA pH 8.0

4.3 Loading the Samples

One of the following methods should be used to load the sample.

1. DNA in a sample plug should be placed on a smooth clean surface, and cut to size using a razor blade or spatula. Samples should be less than 90% of the height of the wells. Place agarose plugs onto the front walls of the sample wells using a spatula and gently press them to the bottoms of the wells. Press the plugs firmly against the front walls of the wells. Fill each sample well with Low Melt Preparative Grade Agarose (catalog number 162-0017) at an agarose concentration equal to that of the gel, and allow the agarose to harden at room temperature for 10 to 15 minutes.
2. Alternatively, the sample plug can be cut into blocks and placed on each tooth of the comb. Cast around the comb.The plug will remain in place when the comb is removed.
3. Liquid samples can be added to the sample wells with the gel positioned under the electrophoresis buffer in the cell. Turn the pump off when adding liquid samples to prevent samples from washing out of the wells. Run the samples into the gel for approximately 5 minutes before turning the pump back on.

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4.4 DNA Size Standards

Bio-Rad recommends running standards in each gel to allow the sizes of unknown samples to be determine and to verify the electrophoresis conditions. Figure 4.2 shows four Bio-Rad standards for pulsed field electrophoresis. These come as blocks of 1.0% Low Melt agarose. Recommended running conditions are given in the figure legend.

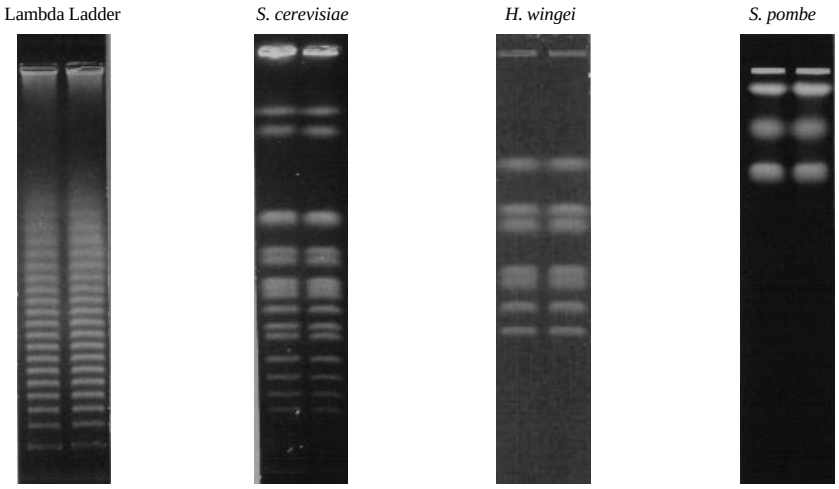


Fig. 4.2. A. Lambda ladder (catalog number 170-3635) was separated on a 1.0% Molecular Biology Certified Agarose (catalog number 162-0133) gel in 0.5x TBE, recirculated at 14 °C. The run time was 22 hours at 6 V/cm with a 50 to 90 second switch time ramp.

B. *Saccharomyces cerevisiae Strain YNN295.* (Catalog number 170-3605). Chromosomes were separated on a 1.0% Pulsed Field Certified Agarose (catalog number 162-0137) gel in 0.5x TBE, recirculated at 14 °C. The run time was 24 hours at 6 V/cm with a 60 to 120 second switch time ramp.

C. *Hansenula wingei Strain YB-4662-VIA.* (Catalog number 170-3667). Chromosomes were separated on a 0.8% Molecular Biology Certified Agarose gel in 1.0x TAE, recirculated at 14 °C. The run time was 50 hours at 3 V/cm with a 250 to 900 second switch time ramp.

D. *Schizosaccharomyces pombe Strain 972 h-.* (Catalog number 170-3633). Chromosomes were separated on a 0.6% Chromosomal Grade Agarose (catalog number 162-0135) gel in 1.0x TAE, recirculated at 14 °C. The run time was 72 hours at 2 V/cm with a 20 to 30 minute switch time ramp.

4.5 Electrophoresis

Remove both end gates by loosening the screws. Push the end gates off the edge of the platform for removal, and slide the platform out of the casting stand. Place the gel and platform assembly into the frame so that the bottom of the platform rests on the floor of the cell. Do not remove the gel from the platform. Check the buffer level to insure that the gel is covered by about 2 mm of buffer. Adjust the buffer flow, if necessary, by using the flow adjustment knob on the Variable Speed Pump. Enter your run parameters (refer to Section 2 for complete operating instructions).

Prior to the first separation of experimental samples, we recommend an initial separation of one or more of the four DNA size standards illustrated above (Figure 4.2), using the run conditions described in the legend. Obtaining separations similar to those in Figure 4.2 will indicate that the CHEF-DR II system is functioning properly.

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4.6 Separations at Room Temperature

Electrophoresis may be conducted at room temperature, without a chiller, but the buffer should not be allowed to exceed 30 °C. It is important to maintain the temperature at a steady value. To facilitate heat transfer, it is recommended that 4-5 feet of the Tygon tubing be coiled into a bucket of water. Recirculation of the buffer is required. It is recommended that the buffer be changed every 24 hours.

Since heat generation is proportional to the square of the voltage, it is essential to lower the field strength to 4.5 V/cm or less, depending on the size of DNA to be resolved. *S. cerevisiae* chromosomes should be electrophoresed at 3.8-4.5 V/cm. Gel strength and buffer concentration do not need to be changed, although switch times and run times may be increased 10 to 20% at the lower field strength. The conditions for resolution of *S. cerevisiae* chromosomes are the same as those given in Table 2, Section 5.3, except that the voltage should be reduced to 4.5 V/cm when the temperature is 29 °C.

Alternatively, the ionic strength of the buffer may be decreased to 0.25x TBE. In this case, voltage must be decreased even more than above or some DNA may not enter the gel. In some cases, DNA bands may be slightly more diffuse at room temperature than when resolved at 14 °C.

4.7 Removing and Staining the Gel

Before removing the gel make sure the run is completed (The unit will display **End**). To stain the gel after a run, remove the frame from the electrophoresis cell, then remove the gel (on the platform) from the cell. Slide the gel off the platform into a 0.5 µg/ml ethidium bromide solution in water and let the gel stain for 20-30 minutes. (**Caution:** Ethidium bromide is a mutagen. Always wear gloves while handling gels or solutions containing the dye.) Destain the gel in distilled water for 1-3 hours. The DNA can be visualized by placing the gel on a UV transilluminator (254-360 nm). Remove the buffer from the electrophoresis cell by unclamping the drain tube and allowing the buffer to drain into a 2 liter container with the pump turned off. Discard used buffer and reclamp the drain tube.

Note: Leaving electrophoresis buffer in the cell with the lid on, when not in use, may lead to warpage of the lid. Leave the lid ajar without buffer in the cell when not in use to minimize potential warpage.

Section 5
Applications

5.1 Strategies for Electrophoretic Separations

There are several parameters that must be considered before performing an electrophoretic separation of very high molecular weight DNA. The separations of large DNA molecules in agarose gels are affected by agarose concentration, buffer concentration, buffer temperature, initial and final switch times, voltage, total electrophoresis run time, and field angle.

The agarose concentration affects the size range of DNA molecules separated, and the sharpness, or tightness, of the bands. Agarose concentrations of 1.0% are useful in separating DNA molecules up to 3 mb in size. Agarose concentrations in the range of 1.2-1.5% are typically used for improved band tightness, however run times will increase proportionately. Gel concentrations below 1.0% (0.5-0.9%) are useful in separations of extremely high molecular weight DNA, greater than 3 mb, though the bands are a bit more diffuse.

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There are several agarose types that allow easy handling of low concentration gels. These agaroses, in the concentration range of 0.5-0.8%, can be used to decrease the run time on separation of large DNA (> 2 mb). An example of this type of agarose is Bio-Rad's Chromosomal Grade Agarose (162-0135).

Buffer Concentration and Temperature

In pulsed field electrophoresis, the mobility of the DNA is sensitive to changes in buffer temperature. As the buffer temperature increases, the mobility of the DNA increases, but the band sharpness and resolution decrease. It is recommended that the buffer be chilled to 14 ˚C to maintain band sharpness and to dissipate heat generated during prolonged runs. Also, buffer recirculation is required to prevent temperature gradients from occurring. High voltage runs (200 V) exceeding 1 day require buffer changes after each 48 hour period, to prevent any possible buffer degradation.Standard Tris-borate or TBE, at a concentration of 0.5x, is the most commonly used buffer in pulsed field electrophoresis. Tris-acetate buffer, or TAE, at a concentration of 1.0x, can be used in place of TBE. Other buffer concentrations used are in the range of 0.25 - 1.0x. In Figure 5.1 two different gels, one using 0.5x TBE and the other using 1.0x TAE, were run to show the difference in mobility of DNA in the two buffers.

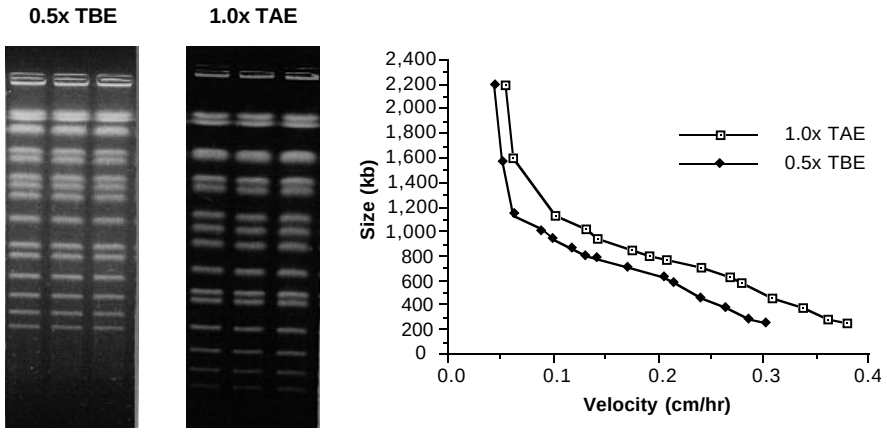


Fig. 5.1. Two gels, one in 0.5x TBE and the other in 1.0x TAE, were run to show the difference in mobility of DNA in the two buffers. *S. cerevisiae* was separated on a 1.0% Pulsed Field Certified Agarose (catalog number 162-0137) gel with a 60 second switch time for 15 hours, followed by a 90 second switch time for 9 hours, at 6 V/cm. Notice the increased migration of the DNA molecules in the TAE gel when compared with the TBE gel.

Switch Times

The migration rate of DNA molecules through an agarose gel is dependent on switch time, voltage (field strength), field angle and run time. In pulsed field electrophoresis, DNA molecules are subjected to alternating electric fields imposed for a period called the switch time. Each time the field is switched, the DNA molecules must change direction or reorient in the gel matrix. Larger molecules take longer to reorient and therefore have less time to move during each pulse, so they migrate slower than smaller molecules. Resolution will be optimal for DNA molecules with reorientation times comparable to the switch time. So, as the DNA size increases, the switch time needs to increase to resolve the molecules. Under some conditions, larger molecules sometimes run ahead of smaller ones.[50]

Voltage (Field Strength)

DNA migration will increase with increases in voltage or field strength. However, greater migration is accompanied by decreased band sharpness. In general, as the size of the DNA molecules increases, the field strength should decrease. At high field strengths (6 V/cm) some

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very large DNA (>3 mb) cannot be resolved on the gel and the field strength needs to be reduced. Moreover, some large DNA molecules will not enter the gels at high field strengths. Therefore, in selecting the field strength for an experiment, a compromise between run time and resolution has to be made.

Electrophoresis Run Time

The electrophoresis run time is determined by the migration rates of the DNA molecules under investigation. The migration rates, in turn, are affected by the switch time, field strength, and field angle. As the migration rate of the DNA molecules decreases, the electrophoresis run time needs to increase to adequately resolve the DNA molecules of interest.

5.2 Pulsed Field Conditions by DNA Size

In the table below are suggested run parameters that can be used for the various DNA size ranges listed.

	DNA 1-100kb	DNA 0.1 - 2.0 mb	DNA 2 - 4 mb	DNA > 4 mb
% Agarose	1.0-1.2%	0.8-1.2 %	0.6-1 %	0.5-0.8 %
Buffer	0.5x TBE	0.5x TBE	1.0x TAE	1.0x TAE
Temperature	14° C	14° C	14° C	14° C
Voltage	6 V/cm	4.5-6 V/cm	2-3 V/cm	1.5-2.5 V/cm
Pulse Parameters	0.05-10 sec	10-200 sec	200-1800 sec	10-60 min
Run Times	2-15 hr	15-30 hr	24-72 hr	72-144 hr

5.3 Pulsed Field Conditions by Organism

In the table below are the run parameters for various types of DNA samples.

DNA	DNA size (kb)	Agarose Conc.	Switch time (seconds)	Run Time (hours)	Voltage	Angle	[Buffer]
Restriction Fragments	0.2-23	1.2%	0.01a	4	6 V/cm	120°	0.5x TBE
5 kb Ladder	5-75	1.0%	1-6 b	11	6 V/cm	120°	0.5x TBE
Lambda Ladder	50-1,000	1.0%	50-90 c	22	6 V/cm	120°	0.5x TBE
Saccharomyces cerevisiae	200-2,200	1.0%	60-120 d	24	6 V/cm	120°	0.5x TBE
Candida albicans	1,000-4,000	0.8%	120 e 240	24 36	3.5 V/cm	106°	1.0x TAE
Schizo-saccharomyces pombe	3,500-5,700	0.8%	1,800 f	72	2 V/cm	106°	1.0x TAE
Dictostelium discodium	3,600-9,000	0.8%	2,000-7,000 g 7,000-9,600	158 82	1.8 V/cm 1.5 V/cm	120° 120°	0.25x TBE

(a) 0.09 second single switch time for 3 hours.

(b) Ramped switch time from 1 to 6 seconds over 11 hours.

(c) Ramped switch time from 50 to 90 seconds over 22 hours.

(d) Ramped switch time from 60 to 120 seconds over 24 hours.

(e) 120 secondswitch time for 24 hours followed by 240 second switch time for 36 hours.

(f) 30 minute single switch time for 72 hours.

(g) Two blocks, with voltage change in the second block. Buffer temperature is 10 °C. Cox *et al., Proc. Natl. Acad. Sci. USA,* **87**, 8247-8251 (1990).

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5.4 Blotting Megabase DNAs†

Southern Blot Transfer

Pulsed field electrophoresis has become a powerful technique for physical mapping of genes in various organisms. In order to determine the chromosomal location of a gene in a microorganism or the size of the restriction fragment containing a gene in mammalian systems, large DNA fragments separated by CHEF are transferred onto membranes and detected by Southern hybridization analysis. The procedures described for Southern transfer of DNA from standard agarose gels onto membranes are applicable to large DNA fragments separated by CHEF, with the addition of the gel pretreatment step listed below.

Gel Pretreatment

Since DNA fragments larger than 20 kb cannot be transferred efficiently, DNA fragments separated by pulsed field gels must be cleaved before transfer onto membranes. DNA can be cleaved by using either acid (depurination) or UV irradiation. The depurination reaction is harder to control and is extremely sensitive to temperature. Exposure to shortwave UV light is a reliable method for nicking DNA in pulsed field gels before transfer.

Procedure

The following procedure was developed for use with the GS Gene Linker® UV chamber. For optimal results, this protocol must be followed rigorously.

1. Stain the gel with 1.0 µg/ml ethidium bromide (EtBr) for exactly 30 minutes with constant agitation. Use a fresh dilution of the EtBr stock for each gel. Do not destain the gel prior to nicking.
2. Immediately UV irradiate the gel, using the GS Gene Linker chamber, with 60 mJoules of energy. The gel should be photographed using very short exposures (<1 second) to minimize exposure to UV radiation. The gel can also be destained if desired. Transfer the nicked DNA to nylon membrane using alkali or neutral conditions (see discussion).
3. Soak the gel in 0.4N NaOH, 1.5M NaCl for 15 minutes. Transfer the DNA onto Zeta-Probe® GT nylon membrane (162-0196) using 2 liters of 0.4N NaOH, 1.5M NaCl as the transfer solvent.
4. Set up the capillary transfer as follows, from bottom to top:
   A. Corning Pyrex glass dish (28 x 18 x 4 cm).
   B. A plexiglass or plastic box for support, about 3 cm high and small enough to fit in the glass dish (*e.g.*, Eppendorf yellow pipette tip rack).
   C. Glass plate (16 x 20 cm).
   D. Two sheets of blotting paper as a wick (18 x 30 cm; S&S, GB002).
   E. Agarose gel (top side down).
   F. Zeta-Probe GT membrane cut to the same size as the gel and prewetted with distilled water.
   G. Two sheets of blotting paper (18 x 15 cm; S&S, GB002).
   H. A stack of paper towels 10 cm high.
5. Transfer the DNA 24-48 hours.

† Contributed by Dr. Eric Lai, University of North Carolina

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6. Carefully remove the paper towel and blotting papers. Remove the membrane together with the gel, turn over the membrane and gel, lay them gel side up, and mark the location of the wells and the orientation marker on the top of the gel. The position of the wells can be accurately marked on the membrane by using a fine point permanent marker pen, cutting through the bottoms of the wells.

7. Neutralize the membrane in 0.5M Tris, pH 7.0 (neutralization buffer) for 5 minutes, followed by rinsing briefly in 2x SSC. Transferred DNA can be visualized on the membrane by placing the damp blot on a transilluminator.

8. Dry the membrane by blotting onto 3MM or other adsorbent paper and proceed with hybridization. UV crosslinking of the DNA to the membrane is not recommended with this alkaline transfer method.

Discussion

1. The procedure is based on gels approximately 6 mm thick. If thicker gels are used, the staining period may be prolonged to allow diffusion of EtBr into the middle of the gels. DNA that is not stained with EtBr will not be nicked by the UV light and thus will not be transferred from the gel.

2. If the output of the UV light source is not known and no UV meter is available, you can titrate your UV light source as follows. Run a CHEF gel with eight lanes of *S. cerevisiae* chromosomes as markers using a switch time that will provide resolution from 200-1,000 kb. Stain the gel with EtBr, and photograph with medium-wave 302 nm UV light and fast film (Polaroid type 667) to minimize nicking of DNA. Note the exposure time of the photo. Cut the gel into eight strips, each containing a lane of separated yeast chromosomes. UV irradiate the strips with a 254 nm light source for time intervals of 5, 10, 15, 30, 45, 60, 90, and 300 seconds. If a 254 nm light source is not available, 302 nm light can be used, but exposure times have to be lengthened approximately five-fold. Alkaline transfer the gel strips as described, and stain the gels after transfer. Take a photograph of the gel strips using the same UV light source, film, and exposure time as before transfer, and compare it with the photograph before transfer. Choose the time period that results in 80-90% transfer of DNA. Do not choose the time intervals with complete transfer because most of the transferred DNA fragments will be too short for effective hybridization. If less than 10 second short-wave UV irradiation is required, you may need to use a 302 nm light source for taking the picture of the gel and cutting away excess gel area. As a general rule, 10 seconds or less exposure time is needed with a new UV transilluminator. The UV output will decrease with time, to as little as 30% of its initial rating after 7 years.

3. Presoaking the gel in NaOH prior to transfer decreases background and increases transfer efficiency.

4. Pulsed field gels can also be blotted onto membranes using 20x SSC as the transfer buffer solvent with standard alkaline denaturation followed by neutralization. Alkaline transfer onto nylon membranes gives as good or better sensitivity as standard transfers onto nitrocellulose filters. The alkaline procedure is much simpler and faster. In addition, nylon membranes can be reused many more times than nitrocellulose filters. Some blots may be reused as many as 20 times.

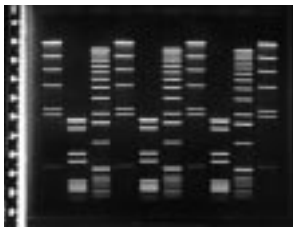
5. DNAs separated on the CHEF-DR II, CHEF-DR III or CHEF Mapper® system can also be vacuum transferred onto nylon membranes in 4 hours using a commercial vacuum blotter, such as the Model 785 Vacuum Blotter (165-5000), and NaOH as buffer.

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6. The DNA is transferred from the back of the gel (the side opposite the wells) onto the membrane because irregularities in the surface of the gel frequently occur during solidification of these high percentage gels (1%). These surface artifacts will interfere with the transfer of the DNAs from the gel. Transfer from the other side of the gel insures smooth surface contact between the gel and the membrane.
7. It is essential to neutralize the membrane after transfer to prevent changing the pH of the hybridization buffer during hybridization.
8. It is not absolutely necessary to bake nylon membranes after alkaline transfer since the DNA should be fixed onto the membrane by NaOH.
9. To monitor the efficiency of the transfer, stain the gel in neutralization buffer for 30 minutes with 1 µg/ml EtBr. Take a photograph of the post-transferred gel, and compare with the original picture.

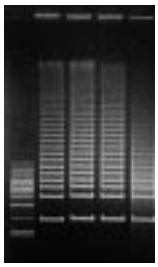
5.5 Separations of DNA Size Standards

1. Restriction fragments



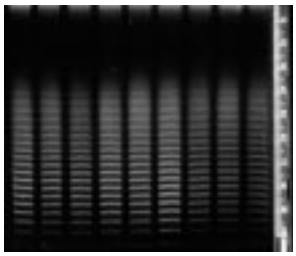
Size Range:	0.2-23 kb
Agarose:	1.0% Molecular Biology Certified
Buffer:	0.5x TBE
Temperature:	14 °C
Switch Time:	0.1 second
Run Time:	4 hours
Voltage Gradient:	6 V/cm

2. 5 kb Ladder



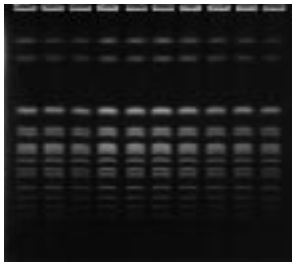
Size Range:	5-75 kb
Agarose:	1.0% Molecular Biology Certified
Buffer:	0.5x TBE
Temperature:	14 °C
Switch Time:	1-6 seconds
Run Time:	11 hours
Voltage Gradient:	6 V/cm

3. Lambda Ladder



Size Range:	50-1000 kb
Agarose:	1.0% Molecular Biology Certified
Buffer:	0.5x TBE
Temperature:	14 °C
Switch Time:	50-90 seconds
Run Time:	22 hours
Voltage Gradient:	6 V/cm

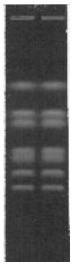
4. *Saccharomyces cerevisiae*



Size Range:	240-2200 kb
Agarose:	1.0% Pulsed Field Certified
Buffer:	0.5x TBE
Temperature:	14 °C
Switch Time:	60-120 seconds
Run Time:	24 hours
Voltage Gradient:	6 V/cm

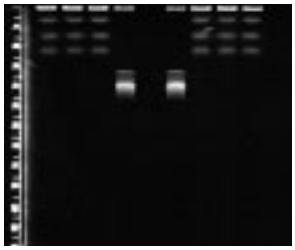
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5. ***Hansenula wingei***



Size Range:	1-3.1 mb
Agarose:	0.8% Molecular Biology Certified
Buffer:	1.0x TAE
Temperature:	14 °C
Switch Time:	250-900 seconds
Run Time:	50 hours
Voltage Gradient:	3 V/cm

6. ***Schizosaccharomyces pombe***



Size Range:	3.5-5.7 mb
Agarose:	0.8% Chromosomal Grade
Buffer:	1.0x TAE
Temperature:	14 °C
Switch Time:	1800 seconds
Run Time:	72 hours
Voltage Gradient:	2 V/cm

Section 6
Maintenance of Equipment

6.1 Replacing Electrodes

The electrophoresis cell requires little maintenance except for rinsing after every run. Dirt and other build-up can be removed with laboratory detergent and a fine cloth. Care should be taken not to bend or break the electrodes.

Fast switch times (<2 seconds) with high voltage gradients (6 V/cm) may lead to increased electrode failure If one of the electrodes should break, or leak at the O-ring, it may be replaced. Additional electrodes (Catalog number 170-3646) are available from Bio-Rad Laboratories. Thick electrodes (0.02”; Catalog number 170-4648) which are very resistant to the breakage associated with high voltage, short switch time run conditions, are also available.

To replace an electrode, turn the electrophoresis cell upside down and remove the six screws. Lift off the base plate. Remove the hexagonal nut on the wire of the broken electrode, then remove the hexagonal nut on the electrode to be replaced. Push down firmly on the post to remove the old electrode. Turn the box over, insert the new electrode, pack with self-leveling silicone sealant (RTV-type silicone sealer available at most hardware stores), and replace the nut. Replace the wire and base plate.

If one of the pins to the serial cable bends, use tweezers to carefully straighten it. Replacement cables may be ordered from Bio-Rad.

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6.2 Fuses

If the DC current during a run exceeds 500 mA entering the electrophoresis cell, the 0.5 ampere FB (fast blow) fuse will blow on the front panel of the drive module. Replace the fuse by unscrewing the cartridge at the front of the drive module. Replace with 0.5 ampere FB fuse (2 replacement fuses are provided). Make sure the drive module is off when replacing a fuse.

A power surge may cause a SB (slow blow) line fuse to blow. The LED lights on the front panel of either the drive module or the control module will go out. The line fuse is located at the rear of each module. **Control module**: Replace the fuse with a 2.0 A SB if your line voltage is 100 or 120 VAC, or a 1.0 A SB if your line voltage is 220 or 240 VAC. **Drive module:** Replace the fuse with a 1.0 A SB if your line voltage is 100 or 120 VAC, or a 0.5 A SB if your line voltage is 220 or 240 VAC. A 10 Amp fuse is used on the neutral side for 100 and 120 VAC.

Replacement fuses are available from a variety of sources, including Radio Shack® (Tandy Corporation). The 0.5 A FB fuse (front panel of drive module) can be obtained as catalog number 270-1241, the 1.0 A SB fuse (line fuse of drive module) as catalog number 270-1283, and the 2.0 A SB fuse (line fuse of control module) as catalog number 270-1172 from Radio Shack.

If the unit still does not operate, contact Bio-Rad Laboratories. Do not attempt to open and repair the power module, or the warranty may be voided.

6.3 Maintenance of the Electrophoresis Cell

When the cell is not in use, even for short periods, all buffer should be removed to prevent damage to the plastics. In addition, the lid should be left ajar to minimize possible warpage.

Section 7
Troubleshooting Guide

Problem	Solution
Equipment	
No power to displays or indicator lights	1. Check line fuse at back of control module 2. Check line fuse at back of drive module 3. Check source of A/C power 4. Contact Bio-Rad Laboratories
No voltage across electrodes, with AC power light on (left side of drive module)	1. Check that lid is on 2. Check that 24-pin serial cable is fully inserted at both ends 3. Check that coiled interlock cable is firmly attached 4. Confirm that all leads are properly attached between Control Module and Drive Module 5. Check lid interlock connection at electrophoresis cell 6. Check HV fuse on front panel of drive module (0.5 A FB) and replace if necessary 7. Check that the unit is not set in PAUSE mode

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Problem	Solution
Gel floats away	1. Pump flow rate is too high. Adjust with Variable Speed pump
No or low buffer flow	1. Look for kink in tubing 2. Check Model 1000 Mini Chiller; buffer in the heat exchanger can freeze if the chiller is cooling, but the pump is not on 3. Check pump plug connection and if the pump is on
Gel band patterns appear very distorted; lanes very curved*, bands sharp, but slanting	1. Foreign object in chamber (remove thermometer, etc.) 2. Insufficient or non-uniform cooling due to low pump flow 3. Check that buffer is level with surface of gel. Use leveling feet 4. Check that the current is equal for both switch directions 5. Replace damaged electrode 6. Control or drive module fault; contact Bio-Rad Laboratories

* Slight distortion of the outermost lane is normal

Problem	Solution
Applications	
Bands smeary or fuzzy	1. Excessive heating. Lower the voltage or ionic strength of buffer 2. Improper switch times (see Section 5.1) 3. Gel percentage too low. Increase 4. Sample degraded; Prepare new samples (see Section 3) 5. Agarose impurities. Use recommended agaroses 6. Sample overload. Lower sample concentration (see Section 3)
Larger DNAs not resolved	1. Increase switch time or use switch time ramp 2. Agarose impurities. Use recommended agaroses 3. Lower the voltage to below 2 V/cm
High background in lanes	1. Insufficient washing of samples (see Section 3) 2. Sample may be contaminated with RNA or other material 3. DNA concentration too high; reduce cells/plug (see Section 3)
Distorted bands	1. Sample contains too high salt or detergent concen tration (see Section 3) 2. Wells were distorted. Recast gel 3. Buffer breakdown. Change every 48 hours 4. Sample plugs were crushed when placed in wells 5. Pump flow rate too low. Check for kink along tubing
Thick bands	1. Use thinner wells 2. Load less sample

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Section 8
References

8.1 Applications in Pulsed Field Electrophoresis

The following are references in pulsed field electrophoresis, primarily from 1987-1989. The list surveys a wide area of applications and organisms, but is not exhaustive. Underlined references use the CHEF-DR II pulsed field electrophoresis system.

Organism	**Reference numbers**
Aspergillus	20
Bacteria	8, 49, 55, 80, 90, 93, 120, 128, 149, 152, 190, 195
Candida albicans	92, 110, 111, 112, 150, 170
Caulobacter	52
Dictyostelium	36
Drosophila	64, 204
Epstein-Barr virus	75
Giardia	2
Histoplasma	176
Human	9, 14, 27, 33, 37, 43, 50, 60, 66, 67, 82, 83, 88, 102, 122, 132, 133, 136, 137, 142, 144, 147, 183, 186, 189, 202
Leischmania	61, 69, 103, 135, 156, 163
Mouse	18, 19, 29, 89, 97, 179, 207
Mycoplasma	6, 25, 114, 139
Neurospora	96, 130
Paramecium	68
Plants	72, 187
Plasmodium	10, 42, 86, 138, 161, 171
Pseudomonas	7
S. cerevisiae	3, 23, 31, 54, 87, 91, 123, 124, 151,162, 166, 182, 194, 205, 206
S. pombe	28, 53, 73, 75, 119, 169, 184
Tetrahymena	38
Trypanosoma	113, 192
Ureaplasma	34

Application	**Reference Numbers**
Alkaline blotting (Zeta-Probe membrane)	120 , 131
Blotting	1, 9, 26, 31, 66, 72, 120, 125, 130, 145, 202

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CHEF	13, 20, 26, 28, 30, 31, 36, 37, 39, 42, 45, 56, 57, 58, 61, 85, 91, 93, 94, 96, 107, 121, 123, 136, 139, 143, 145, 151, 156, 162, 163, 168, 171, 173, 197, 198, 201, 202

Application	**Reference Numbers**
Chromosome rearrangements	127, 141, 147, 166
Circular DNA	8, 75, 78, 79, 104, 118, 159, 160, 163, 164, 190, 207
Cosmid mapping	14, 46, 70, 98, 173, 193
Diagnostics (*e.g.*, cancer)	148
DNA over 5 megabases	20, 72, 130, 197
DNA under 200,000 bases	12, 34, 39, 77, 93
Epstein-Barr virus	75
FIGE	7, 12, 14, 15, 17, 24, 25, 33, 47, 65, 68, 77, 146, 173, 182, 187
FIGE with *S. pombe*	184
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Mapping	1, 18, 59, 66, 87, 134, 140, 199
Minute chromosomes	26, 113
OFAGE	23, 52, 54, 79, 82, 87, 147
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Sample preparation - bacteria	58, 167, 172, 195
Sample preparation - cell lines	167
Sample preparation - general	145, 155, 167, 188
Sample preparation - tissues	106, 167
Sample preparation-YACs	45
Secondary structure analysis	30, 118, 128
Single strand DNA	181
Size standards	39, 74, 116, 200
Strain characterization	35, 69, 71, 121

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TAFE	51, 62, 63, 132, 148, 179
Theory	15, 22, 44, 48, 76, 81, 100, 101, 117, 126, 129, 130, 158, 174, 175, 177, 178, 185, 196

Application	**Reference Numbers**
Two-D PFG	10, 203
Virus	15, 145
Visualization of DNA (microscope)	154, 168
Yeast artificial chromosomes (YACs)	10, 16, 41, 45, 93, 94, 95, 107, 115, 144, 183, 198

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Section 9
Systems, Accessories, and Reagents for Pulsed Field Electrophoresis

Catalog Number	Product Description
170-3725	**CHEF-DR II Chiller System,** 120 VAC, includes CHEF-DR II control module; drive module; electrophoresis cell; Model 1000 Mini Chiller; vari able speed pump; Tygon tubing, 12 feet; 14 cm wide x 13 cm long cast ing stand and frame; 10 well comb and comb holder; 50 well sample plug mold; leveling bubble; cables; 3/8 inch straight tubing connectors, 2; 0.5 A FB fuses, 2; *S. cerevisiae* DNA size standards; Pulsed Field Certified Agarose sample, 5 g; Chromosomal Grade Agarose sample, 5 g; manual
170-3728	**CHEF-DR II Chiller System,** 100 VAC
170-3726	**CHEF-DR II Chiller System,** 220 VAC
170-3727	**CHEF-DR II Chiller System,** 240 VAC
170-3612	**CHEF-DR II System,** 120 VAC, includes CHEF-DR II control module; drive module; electrophoresis cell; variable speed pump; Tygon tubing, 12 feet; 14 cm wide x 13 cm long casting stand and frame; 10 well comb and comb holder; 50 well sample plug mold; leveling bubble; cables; 3/8 inch straight tubing connectors, 2; 0.5 A FB fuses, 2; *S. cerevisiae* DNA size standards; Pulsed Field Certified Agarose sample, 5 g; Chromosomal Grade Agarose sample, 5 g; manual
170-3613	**CHEF-DR II System,** 100 VAC
170-3614	**CHEF-DR II System,** 220 VAC
170-3615	**CHEF-DR II System,** 240 VAC
170-3654	**Model 1000 Mini Chiller,** 120 V
170-3655	**Model 1000 Mini Chiller,** 220/240 V
170-3688	**Model 1000 Mini Chiller,** 100 V
170-3665	**External Temperature Probe,** for Model 1000 Mini Chiller
170-3644	**Variable Speed Pump**
170-3646	**Electrodes, standard gauge (0.01"),** 6
170-3648	**Electrodes, thick gauge (0.02"),** 6
170-3711	**CHEF Screened Caps,** for preparing plugs; 5
170-3713	**CHEF Disposable Plug Mold,** 50 wells, for casting plugs; 5
170-3689	**Standard Casting Stand,** includes 14 x 13 cm frame and platform
170-3699	**Combination Comb Holder**
170-3704	**Wide/Long Combination Casting Stand,** includes 21 x 14 cm frame and platform
170-4326	**10 Well Comb,** 14 cm wide, 1.5 mm thick
170-4325	**10 Well Comb,** 14 cm wide, 0.75 mm thick
170-4324	**15 Well Comb,** 14 cm wide, 1.5 mm thick
170-4323	**15 Well Comb,** 14 cm wide, 0.75 mm thick
170-4322	**20 Well Comb,** 14 cm wide, 1.5 mm thick
170-4344	**30 Well Comb,** 14 cm wide, 1.5 mm thick
170-3623	**Preparative Comb, 14 cm wide,** 1.5 mm thick, plus 2 outer sample wells for size standards
170-3627	**15 Well Comb,** 21 cm wide, 1.5 mm thick
170-3628	**30 Well Comb,** 21 cm wide, 1.5 mm thick
170-3645	**45 Well Comb,** 21 cm wide, 1.5 mm thick
170-4046	**Leveling Table,** 20 cm x 30 cm for casting gels
170-3643	**Gel Scoop,** for removing gels from chamber

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Catalog Number	Product Description
162-0017	**Low Melt Preparative Grade Agarose,** 25 g
162-0019	**Low Melt Preparative Grade Agarose,** 100 g
162-0133	**Molecular Bology Certified Agarose,** 100 g
162-0134	**Molecular Bology Certified Agarose,** 500 g
162-0135	**Chromosomal Grade Agarose,** 25 g
162-0136	**Chromosomal Grade Agarose,** 100 g
162-0137	**Pulsed Field Certified Agarose,** 100 g
162-0138	**Pulsed Field Certified Agarose,** 500 g
170-3605	**CHEF DNA Size Standards,** *S. cerevisiae*, 5 blocks
170-3624	**CHEF DNA Size Standards,** 5 kb ladder, 20 µg
170-3633	**CHEF DNA Size Standards,** *S. pombe*, 5 blocks
170-3635	**CHEF DNA Size Standards,** lambda ladder, 5 blocks
170-3667	**CHEF DNA Size Markers,** *H. wingei*, 5 blocks
170-3707	**CHEF DNA Size Standards,** 8-48 kb, 25 µg
170-3591	**CHEF Mammalian Genomic DNA Plug Kit**
170-3592	**CHEF Bacterial Genomic DNA Plug Kit**
170-3593	**CHEF Yeast Genomic DNA Plug Kit**
165-5031	**GS Gene Linker® UV Chamber,** 120 V
165-5032	**GS Gene Linker UV Chamber,** 220 V
165-5033	**GS Gene Linker UV Chamber,** 240 V
165-5034	**GS Gene Linker UV Chamber,** 100 V
161-0196	**Zeta-Probe® GT Charged Nylon Membrane,** 30 cm x 3.3 m roll
161-0197	**Zeta-Probe GT Charged Nylon Membrane,** 20 cm x 3.3 m roll
170-3590	**Gene-Lite™ Chemiluminescent Detection Kit**

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