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# CHEF-DR® III Pulsed Field Electrophoresis Systems

# Instruction Manual and Applications Guide

**Catalog Numbers**
**170-3690**
**through**
**170-3703**

BIO-RAD

# Warranty

The CHEF-DR III power 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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6. Damage caused by disaster.
7. Corrosion caused by improper solvent* or sample.

This warranty does not apply to parts listed below:

1. Fuses
2. Tubing
3. Electrodes

For any inquiry or request for repair service, contact Bio-Rad Laboratories. Inform Bio-Rad of the model and serial number of your instrument.

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2nd Revision

* The CHEF-DR III 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 III 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 III 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 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 the safety interlocks. Always turn off the power to the chamber before working within the gel box.

The Cooling Module is ground isolated. Although there is virtually no current flowing through the Tygon® tubing into the chiller, avoid assembling or disassembling the tubing while the CHEF-DR III system is operating.

### 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 DNAs. By alternating the electric field between spatially distinct pairs of electrodes, megabase (mb) sized DNAs are able to reorient and move at different speeds through the pores in an agarose gel. Overview and applications articles are listed Section 8.

The CHEF-DR III system separates large and small DNA fragments with better resolution, speed, and accuracy, than initial pulsed field methods. DNAs from 100 bases to over 10 megabases (mb) may be effectively resolved. For example, the chromosomal DNA of *Schizosaccharomyces pombe* can be resolved in 1 day using a 106° pulse angle, compared to 2 days at 120°. Everything from Yeast Artificial Chromosomes (YACs) to M13 inserts can be separated with a single instrument. Applications include top down and bottom up mapping (*Not* I and cosmid cloning, respectively), electrophoretic karyotyping, analysis of tumor cell DNA rearrangements, mammalian DNA analysis, and testing for bacterial, yeast, and parasite strain homogeneity.

The CHEF-DR III system uses two leading technologies, CHEF (Clamped Homogeneous Electric Fields)[215] and PACE (Programmable Autonomously Controlled Electrodes).[216] The system provides highly uniform, or homogeneous, electric fields within the gel, using an array of 24 electrodes, which are “clamped” or held to intermediate potentials to eliminate lane distortion. Thus, lanes are straight. The system maintains uniform fields using patented Dynamic Regulation (US Patent 4,878,008). The electrodes sense changes in local buffer conductivity due to buffer breakdown, change in buffer type, gel thickness, or temperature, and poten-

tials are readjusted immediately to maintain uniform fields, thus insuring high resolution. In PACE, the voltage potential of each of the 24 electrodes is regulated independently. Unlike the CHEF-DR II system, which has a fixed reorientation (field) angle of 120° due the hexagonal geometry of the electrode array, the CHEF-DR III system can generate field angles from 90–120°. In addition, the CHEF-DR III system permits up to three consecutively executing blocks of run conditions with battery backed-up RAM and automatic restart after power interruption.

## 1.3 Specifications

These specifications pertain to the complete CHEF-DR III system. A system including the Cooling Module is also available.

## CHEF-DR III Specifications

### Power Module:

| | |
|---|---|
| **Dimensions** | 43 (depth) x 48 (width) x 17.5 (height) cm |
| Construction | Aluminum chassis |
| Weight | 10 kg |
| Power supply | 350 V maximum, to allow maximum gradient of 9 V/cm, continuously adjustable; built in |
| **Electrical** | |
| Maximum amps | 0.5 amperes |
| Allowable voltage gradients | 0.6–9 V/cm, in 0.1 V/cm increments |
| Battery back-up | All parameters in memory |
| Electrode potentials | Dynamically regulated (feedback adjustment) +/- 0.5% |
| Data entry | Keyboard |
| **Functional** | |
| Switching range | 0.1 sec to 65K sec |
| Switch angle variable | 90–120 degrees (all electronic switching) in 1° increments |
| Maximum program blocks | 3, with automatic execution |
| Maximum run time | 999 hours per block |
| Input voltage range | 100–120 VAC/50–60 Hz/4 amps<br>220–240 VAC/50–60 Hz/2 amps |
| **Fuses** | 0.5 amp Fast Blow for high voltage output<br>3.15 amp Slow-Blow (100/120 V) or<br>1.60 amp Slow-Blow (220/240 V) |
| **Environmental** | |
| Operating | 50 °F (10 °C) to 90 °F (32 °C) temperature<br>30–80% humidity |
| Storage | 32 °F (0 °C) to 140 °F (60 °C) temperature<br>10-90% humidity |

### Electrophoresis cell:

| | |
|---|---|
| Dimensions | 11.4 x 44.2 x 50.3 cm, horizontal format |
| Construction | Cover: Vacuum formed polycarbonate<br>Base: Injection molded polycarbonate |
| Lid | Safety interlocked |
| Weight | 10.2 kg |
| Electrodes | 24, platinum (0.02 inch diameter) |
| Temperature monitoring | Via precision temperature probe mounted in base of cell |

**Accessories included:**

| | |
|---|---|
| Variable speed pump | 120 V, ground isolated. Flow rate 1 liter/min, typical |
| Casting stand | 14 cm x 13 cm |
| Comb | 10 well comb and comb holder |
| Tygon tubing | 365 cm |
| Disposable sample plug mold | 50 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 spares |
| Manual | 1 |
| Screened cap | 1 |

**Cooling Module (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 |
| **Total System Weight** | 41.7 kg |

**Note:** This equipment complies with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits 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, and the user will be required to correct the interference at his own expense.

## 1.4 Description of Major Components

### Power Module

The Power Module contains the electronics for pulsed field electrophoresis, including a 350 V power supply, the switching functions, and drivers for the 24 electrodes. The drivers provide clamped homogeneous electric fields in the electrophoresis cell, and maintain them regardless of the field angle selected. This dynamic regulation feature modulates the potentials so that the proper voltages are maintained regardless of gel size, or fluctuations in buffer conductivity or temperature. The fused power supply operates with a maximum voltage gradient of 9 V/cm, or 300 V. The lowest gradient is 0.6 V/cm, or 20 V.

Figure 1.1A 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 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 1.1B. 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.

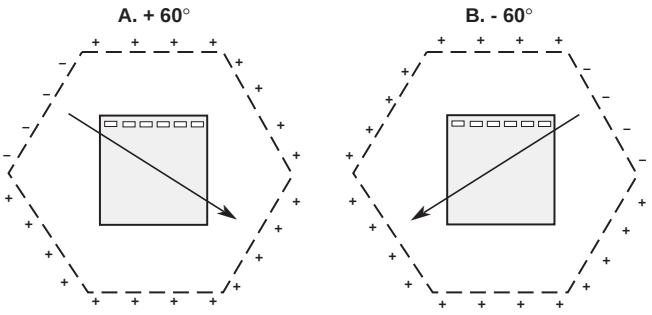



**Figure 1.1. Voltage clamping by the CHEF-DR III system. A.** Relative electrode potentials when the + 60° field vector is activated. **B.** Relative electrode potentials when the - 60° field vector is activated.

## Electrophoresis Chamber

The CHEF-DR III electrophoresis cell consists of a 44.2 x 50.3cm (17.4" x 19.8") 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, removed, 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. DNA migration and buffer flow is in the direction of the arrow on the lid.

The heavy duty 0.02" diameter platinum wire electrodes, replaceable for easy maintenance (see Section 6), are individually connected to the 24 pin computer cable,which connects to the power module. 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 9 V/cm gradients are employed.

The two small chambers below the level of the main chamber floor at the front and rear of the main chamber are used for buffer circulation and priming the pump. Buffer enters the main chamber through six holes in the floor near the top. A flow baffle just in front of the holes prevents gel movement. Buffer exits the chamber at the front through the two ports. The right is for draining, the left for circulation. The base of the chamber has four leveling screws for even gel submersion in buffer.

The hinged lid contains a safety interlock. The voltage passes directly from the Power Module through a short-path in the lid interlock. If the lid is opened, the current flow is broken and voltage to the gel chamber is disrupted. The cell also includes an internal temperature probe, which monitors buffer temperature in the chamber and regulates cooling by the Cooling Module.

## Pump and Accessories

Each 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 power module for safety. Its voltage requirement is independent of the line voltage supplied to the drive module (*e.g.* 100, 120, 220, or 240 volts). This pump should not be plugged into any equipment other than the CHEF-DR III power 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 placed 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.

### Cooling Module

The Cooling Module is a stand alone, portable refrigerated apparatus specifically for use with the CHEF-DR III system. The variable speed pump circulates electrophoresis buffer 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 temperature probe in the cell regulates cooling by the Cooling Module, resulting in precise maintenance of buffer temperature.

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



**Fig. 1.2. The complete CHEF-DR III chiller system, with chamber, power module, variable speed pump, and Cooling Module.**

# Section 2
# Operation

## 2.1 Instrument Setup

Place the CHEF-DR III electrophoresis chamber on a level surface, with the power module to the right or on a shelf above. Position the electrophoresis chamber with the two ports facing you and the lid safety interlock to the rear. If the system includes a Cooling Module, place it to the left of the chamber. Place the variable speed pump at the rear of the chamber and connect the plug from the pump to the port labeled PUMP CONNECTOR on the back of the power module. Level the electrophoresis cell with the leveling feet at each corner by placing the casting platform in the center of the cell, then placing the leveling bubble (provided) on the casting platform. Putting the casting platform in the center of the cell will level the gel with respect to the electrophoresis cell.

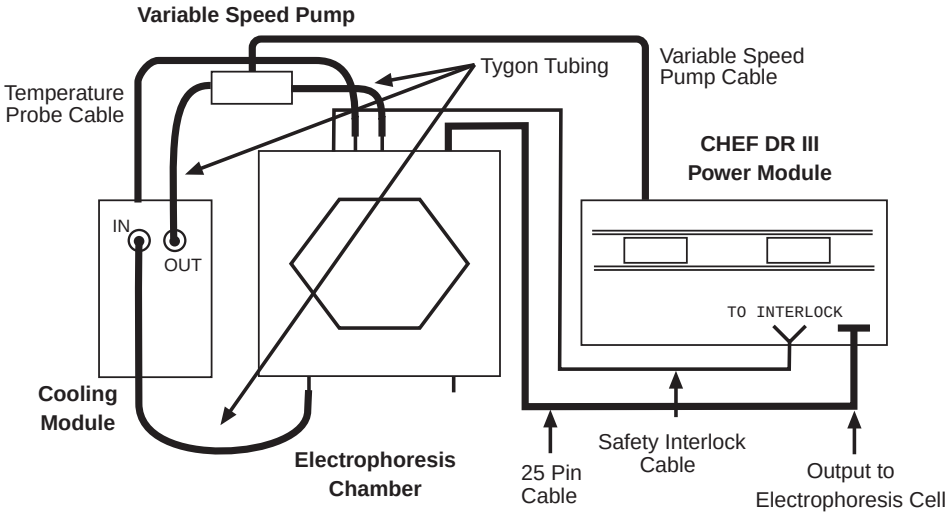



**Fig. 2.1. Interconnections between components of the CHEF-DR III system.**

Attach the power cords for the power module and Cooling Module to the back of each instrument. Be sure the power module is off. Connect the 25-pin cable from the electrophoresis chamber to the port labeled OUTPUT TO ELECTROPHORESIS CELL on the front of the power module. The 25-pin cable has an safety interlock on the end which is attached to the power module. Connect the coiled interlock cable from the electrophoresis chamber to the jacks labeled TO INTERLOCK on the power module.

## 2.2 Electrophoresis Chamber Operation

To connect the cell to the Cooling Module, attach approximately 1–2 feet of ¼ inch ID Tygon tubing to both the Flow In and Flow Out ports on the Cooling Module, and secure the tubing with the plastic clamps. Connect the quick release connector to 2 feet of ⅜ inch ID Tygon tubing. Attach the quick release connector to the left front port of the cell. Attach the other end of the ⅜ inch tubing to the ¼ inch tubing from the Flow In of the Cooling Module using the ⅜ to ¼ inch reducer. Place the pump between the outlet of the Cooling Module and the inlet (rear) of the Electrophoresis Cell. Connect the ¼ inch tubing from the Flow Out of the Cooling Module to the inlet of the pump using a ⅜ to ¼ inch reducer. Connect approximately 2 feet of ⅜ inch Tygon tubing to the outlet of the pump using the ⅜ to ⅜ straight connector. Connect a quick release connector to the other end of the ⅜ inch tubing. Connect the quick release connector to the inlet of the cell.

Connect a quick release connector to a 6 inch piece of ⅜ inch Tygon tubing, and connect it to the right front port of the cell. This tube will drain the buffer in the cell.

Connect the 9 pin gray temperature probe cable from the back of the cell to the Remote Sensor port on the back of the Cooling Module.

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).

If the system includes the Cooling Module, connect the temperature probe cable to the REMOTE SENSOR port on the rear panel of the Cooling Module. Insert the other end of the temperature probe cable into the rear of the electrophoresis chamber.

Establish the correct buffer flow before attempting any electrophoresis runs. The optimal flow rate of buffer through the electrophoresis chamber is approximately 0.8–1 liter per minute (approximately 70 on the pump). When the correct flow rate has been achieved, use that pump setting for all subsequent electrophoresis runs. Fill the chamber with 2.2 liters of buffer. 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 pump.

It is beneficial to fine tune the chiller before attempting any electrophoresis runs. Turn on the chiller and pump approximately ½ hour before adjusting the temperature. Initially, it will be necessary to fine tune the temperature setting to achieve a consistent running temperature.

## 2.3 CHEF-DR III Operation

This section describes general operation. See Sections 3 and 4 for sample preparation, gel casting, gel running, and staining.

### Power Up

The CHEF-DR III front panel display is divided into two sections (see Figure 2.3.). On the left are, BLOCK, INITIAL SWITCH TIME, FINAL SWITCH TIME, RUN TIME, and RAISE and LOWER keys. On the right are VOLTS/CM, INCLUDED ANGLE, ACTUAL CURRENT, PAUSE/START RUN, and RAISE and LOWER keys. At power up, the left display will show 1 with BLOCK lit, indicating Block 1 is active. The right display will show OFF. The program parameters will be at the default setting or those last set before the power was turned off, since the battery back-up RAM stores the last program entered. If a program was in progress or in PAUSE, the run parameters will be retained and the PAUSE mode will be active (flashing PAUSE light).




**Fig. 2.2. Front panel display of the CHEF-DR III system.**

### Parameter Entry

The CHEF-DR III system has the flexibility of using up to three separate, consecutively executing Blocks. Each Block has the run parameters of Initial Switch Time, Final Switch Time, Run Time, Volts/cm, and Included Angle. During a run, Block 1 is run first, then Block 2, then Block 3. To enter run parameters into Block 1, press BLOCK. The left display should show 1, indicating Block 1. If not, press RAISE or LOWER on the left side of the display panel until 1 is shown. Enter the Initial Switch Time, Final Switch Time, and Run Time with the RAISE and LOWER keys on the left side of the display panel. Enter the Volts/cm, and Included Angle with the RAISE and LOWER keys on the right side of the display panel. If more than 1 Block is needed, then press BLOCK and go to Block 2 by pressing RAISE. The left display will show the number 2, indicating Block 2. Continue entering the run parameters as in Block 1. If a third Block is needed, press BLOCK, go to Block 3 by pressing RAISE, then continue entering the run parameters as in Block 1. Below are the limits for each of the run parameters.

| | |
|---|---|
| **Block** | Program from 1–3 Blocks. Block 1 is run first, then Block 2, then Block 3. A run time of 0 disables a Block. |
| **Initial Switch Time** | Adjust from 0.1–65K seconds. |
| **Final Switch Time** | Adjust from 0.1–65K seconds. |
| **Run Time** | Adjust from 0.1–999 hours. A run time of 0 disables a Block. |
| **Volts/cm** | Adjust from 0.6–9.0 volts in 0.1 volt increments. |
| **Included Angle** | Adjust from 90–120° in 1° increments. |
| **Actual Current** | Displays the current, in mA, provided by the power supply. This parameter is not adjustable. |

### Run Program

When the parameters are set, start the program by pressing PAUSE/START RUN. When the program is in progress, the left panel display will show the time remaining (hours) in the current Block with RUN TIME lit, and the right panel display will show the actual current (milliamps) with ACTUAL CURRENT and PAUSE/START RUN lit. After the program is started, 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** | Displays the current Block. |
| **Initial Switch Time** | Displays the set initial switch time for 3 seconds, then displays the remaining time for the current Block. |
| **Final Switch Time** | Displays the set final switch time for 3 seconds, then displays the remaining time for the current Block. |
| **Current Switch Time** | Displays the current switch time for 3 seconds, then displays the remaining time for the current Block. This display is activated by pressing INITIAL SWITCH TIME and FINAL SWITCH TIME simultaneously. |
| **Run Time** | Displays the set run time for the current Block for 3 seconds then displays the remaining time for the current Block. |
| **Volts/cm** | Displays the set voltage gradient for the current Block for 3 seconds then displays the actual current (in mA) for the current Block. |
| **Included Angle** | Displays the set included angle for the current Block for 3 seconds, then displays the actual current (in mA) for the current Block. |
| **Actual Current** | Displays the actual current for the Block in progress. The indicator light should be on during a run. If the power supply is at current limit (500 mA maximum), the light will flash. |
| **Pause/Start Run** | Initially, this starts the program and the indicator light will be lit. While the program is running, pressing this key will put the program into PAUSE and the light will flash. Pressing the key again will restart the program. |

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

### Program Termination

The program in progress may be manually terminated by holding down PAUSE/START RUN for 3– 4 seconds. A program can be terminated only while it is in the run mode; it can not be terminated in PAUSE. When the program is terminated two beeps will sound, and the right display will show OFF. Pressing PAUSE/START RUN again will start the program from the beginning.

When the program terminates under the timer control, the PAUSE/START RUN light will go off, it will sound two beeps per second for 5 seconds, and the right display will show OFF. 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 PAUSE/START RUN.

### Clearing the Program

All parameters in Blocks 1, 2, and 3, can be cleared simultaneously to the default settings when the program is stopped or off. Press RAISE and LOWER on the right side of the panel for 5 seconds (it will sound 2 beeps per second).

### Power Disruption

The CHEF-DR III system has a battery backed-up memory RAM that retains the current program if the power is interrupted. If the program was in progress (not in PAUSE) when the power went down, the program will automatically resume after 2 minutes in PAUSE mode after power is restored. The PAUSE/START RUN light will flash during this 2 minutes.

# 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 during isolation. To prevent breakage of large DNA molecules, intact cells embedded in agarose are lysed and deproteinized *in situ.* The agarose matrix protects the embedded DNA from shear forces and provides an easy way to manipulate samples. Processed agarose plug-DNA inserts are loaded directly into sample wells of agarose electrophoresis gels.

The most important and difficult task in preparing cells for imbedding in agarose is to obtain the proper cell concentration. Although optical density is frequently used, it is not reliable. Different cell lines or 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 greatly leading to over and/or under loading of the sample. To eliminate the need to generate a growth curve for each strain, a hemocytometer provides the most reproducible method for achieving the proper cell concentration for different types of mammalian, bacterial, yeast, or fungal cells. 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, and 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 is achieved using a thin well comb (0.75 mm).

## 3.3 Preparation of Agarose Embedded Mammalian DNA

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

2. Prepare a 2% low melt agarose (2% CleanCut™ agarose is recommended, catalog number 170-3594) 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.

4. Combine the cell suspension with an equal volume of 2% CleanCut agarose and mix gently but thoroughly. This results in a final concentration of 1% agarose. 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. This also adds strength to the agarose for removal from the mold.

5. 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 DNA.

6. 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.

7. Store the plugs at 4 °C. The plugs are stable for 3 months to 1 year.

8. 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 3.6 for information on restriction digestion of plugs.

## 3.4 Preparation of Agarose Embedded Bacterial DNA

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

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

2. When the desired $O.D._{600}$ is reached, add chloramphenicol to a final concentration of 180 µg/ml and continue incubation up to 1 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 use.

4. Prepare a 2% low melt agarose (2% CleanCut agarose is recommended, catalog number 170-3594) solution using sterile water and melt using a microwave. Equilibrate the solution to 50 °C in a water bath.

5. Remove 5 x $10^8$ cells for each ml of agarose plugs to be made. Centrifuge for 3 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 1 M 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. This results in a final concentration of 1% agarose. 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. 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 for lysozyme buffer. Additionally, adding lysostaphin to the cell suspension immediately prior to embedding with agarose produces high quality *S. aureus* plugs.

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 DNA.

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 1 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 3.6 for more information on restriction digestion of plugs.

## 3.5 Preparation of Agarose Embedded Yeast DNA

The buffers, enzymes, and agarose 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 $O.D._{600}$ of >1.0 at the appropriate temperature for your strain.

2. When the desired $O.D._{600}$ 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 use.

4. Prepare a 2% low melt agarose (2% CleanCut agarose is recommended, catalog number 170-3594) solution using sterile water and melt using a microwave. Equilibrate the solution to 50 °C in a water bath.

5. Remove $6 \times 10^8$ 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:** Add Lyticase immediately prior to embedding the cells in agarose. Certain strains of yeast do not give acceptable DNA when Lyticase is added after the cells have been embedded into agarose.

7. Immediately combine the cell suspension with an equal volume of 2% CleanCut agarose and mix gently but thoroughly. This results in a final concentration of 1% agarose. 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.

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 1 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.

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, 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.

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 3.6 for more information on restriction digestion of plugs.

## 3.6 Restriction Enzyme Digestion of Plugs

1. Place one plug per digest in a sterile 1.5 ml microcentrifuge tube. 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 restriction enzyme buffer. Add the restriction enzyme (30-50 U per 100 µl plug) and incubate overnight at the appropriate temperature.

   **Note**: Some restriction enzymes require shorter incubation times for complete digestion (2-4 hours). This should be determined empirically.

2. After overnight digestion, remove the buffer and add 1 ml of wash buffer.

   **Note:** If the plugs are to be stored for more than 1 day, remove the wash 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 ⅓ to ½ of a plug (approximately 2 mm) per well and adjust the volume if necessary on subsequent gels. In addition, always load appropriate size standards.

   **Note:** For a 10 mm wide well use ½ of the plug (10 mm x 2 mm). For a 5 mm wide well use ⅓ of the plug (5 mm x 2 mm).

## 3.7 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 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.

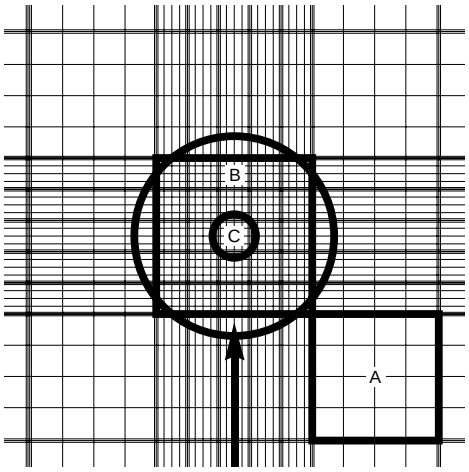



**Fig. 3.1. Hemocytometer grid.**

### Mammalian or tissue culture cells

Because of the large size, tissue culture cells can 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:

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

$$\text{Average Cells per Square} \times \text{Dilution Factor} \times 10^4 = \text{Cells per ml}$$

Use the following ratio to determine how many ml of cell suspension to use to achieve the desired cell concentration for the plugs.

$$\frac{5 \times 10^7 \text{ Cells Desired}}{\text{Actual Cell Concentration}} \times \text{ml of plugs to be made} = \text{ml of cell suspension to use}$$

**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 $10^8$ actual cells concentration = 2.1 ml of cell suspension is required to make 5 ml of agarose plugs.

### 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.

Use the equations below to determine the cell concentration:

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

$$\text{Average Cells per Square} \times 25 \text{ Squares} \times \text{Dilution Factor} \times 10^4 = \text{Cells per ml}$$

Use the following ratio to determine how many ml of cell suspension to use to achieve the desired cell concentration for the plugs.

$$\frac{\text{Desired Cell Concentration}}{\text{Actual Cell Concentration}} \times \text{ml of plugs to be made} = \text{ml of cell suspension to use}$$

**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) 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 $10^8$ actual cells concentration = 8.33 ml of cell suspension is required.

## 3.8 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:

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

$$(\text{grams DNA/cell})(\text{cell/ml}) = (\text{grams DNA/ml})(1 \times 10^6\ \mu\text{g/ml}) = \mu\text{g DNA/ml} \quad \textbf{(B)}$$

$$(\mu\text{g DNA/ml})(\text{genome equivalents}) \cong (\mu\text{g DNA/ml}) \quad \textbf{(C)}$$

$$\frac{(\mu\text{g DNA/ml})}{10\ \text{plugs/ml}} \cong \frac{(\mu\text{g DNA/plug})}{2\ \text{lanes/plug}} \cong \mu\text{g DNA/lane} \quad \textbf{(D)}$$

### Example Calculations:

**Mammalian:**

$$\frac{(6 \times 10^9\ \text{bp})(660\ \text{g/mole})}{6.02 \times 10^{23}\ \text{bp/mole}} = 6.578 \times 10^{-12}\ \text{g DNA/cell} \quad \textbf{(A)}$$

$$(6.578 \times 10^{-12}\ \text{g/cell})(5 \times 10^7\ \text{cell/ml}) = \quad \textbf{(B)}$$
$$(3.289 \times 10^{-4}\ \text{g/DNA/ml})(1 \times 10^6\ \mu\text{g/ml}) = 329\ \mu\text{g DNA/ml}$$

$$(3.29\ \mu\text{g DNA/ml})(1.2\ \text{genome equivalents}) \cong 394\ \mu\text{g DNA/ml} \quad \textbf{(C)}$$

$$\frac{(394\ \mu\text{g DNA/ml})}{10\ \text{plugs/ml}} \cong \frac{(40\ \mu\text{g DNA/plug})}{2\ \text{lanes/plug}} \cong 20\ \mu\text{g DNA/lanes} \quad \textbf{(D)}$$

**Bacterial:**

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

$$(4.933 \times 10^{-15}\ \text{g DNA/cell})(5 \times 10^{8}\ \text{cell/ml}) = \quad \textbf{(B)}$$
$$(2.467 \times 10^{-6}\ \text{g/DNA/ml})(1 \times 10^{6}\ \mu\text{g/ml}) = 2.5\ \mu\text{g DNA/ml}$$

$$(2.5\ \mu\text{g DNA/ml})(2.5\ \text{genome equivalents}) \cong 6.25\ \mu\text{g DNA/ml} \quad \textbf{(C)}$$

$$\frac{(6.25\ \mu\text{g DNA/ml})}{10\ \text{plugs/ml}} \cong \frac{(0.625\ \mu\text{g DNA/plug})}{2\ \text{lanes/plug}} \cong 0.3\ \mu\text{g DNA/lane} \quad \textbf{(D)}$$

**Yeast:**

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

$$(1.644 \times 10^{-14}\ \text{DNA/cell})(6 \times 10^{8}\ \text{cell/ml}) = \quad \textbf{(B)}$$
$$(9.864 \times 10^{-6}\ \text{g/DNA/ml})(1 \times 10^{6}\ \mu\text{g/ml}) = 9.864\ \mu\text{g DNA/ml}$$

$$(9.864\ \mu\text{g DNA/ml})(2\ \text{genome equivalents}) \cong (20\ \mu\text{g DNA/ml}) \quad \textbf{(C)}$$

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

# Section 4
# Gel Electrophoresis

## 4.1 Casting the Gel

Casting the gel requires the use of the following components: casting stand with removable end plates, the casting platform, a comb and comb holder, and the frame which positions the gel and platform in the electrophoresis cell. The casting stand provided with the CHEF-DR III system is 14 cm (5.5") wide x 13 cm (5") long. Optional stands are 21 cm (8.5") wide x 14 cm (5.5") long, and 14 cm (5.5") wide x 21 cm (8.5") long. The gel should be cast on a level surface. Bio-Rad's Leveling Table (catalog number 170-4046) is useful for this purpose. For detailed instructions, refer to the Casting Stand manual.

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, with the horizontal slot facing 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 toward the platform until the edge of the platform is in 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.

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 with the gel in the casting stand.

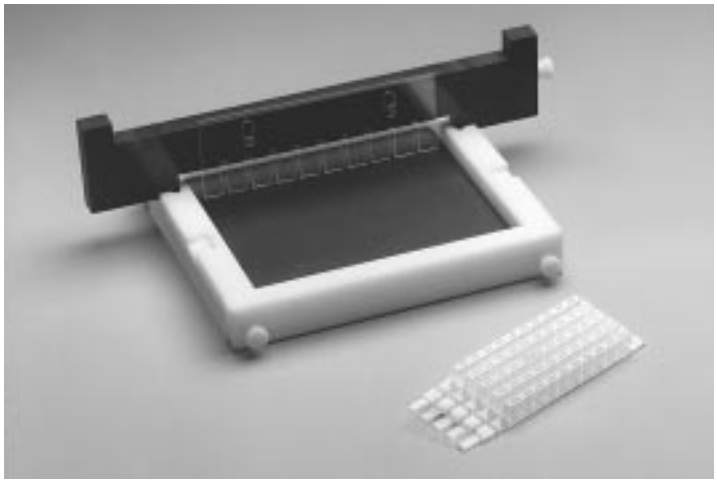


**Fig. 4.1. The CHEF-DR III casting stand and comb holder.**

## 4.2 Buffer Circulation and Temperature

Level the electrophoresis cell with the leveling feet at each corner by placing the casting platform in the center of the cell, then placing the leveling bubble (provided) on the casting platform. Putting the casting platform in the center of the cell will level the gel with respect to the electrophoresis cell. Remove the casting platform after leveling. Position the frame in the electrophoresis cell by placing the 2 plastic pins into the bottom set of holes (toward the front ports) in the floor of the cell so that the frame is centered (center hole in each group of 3 holes). Pour 2.0–2.2 liters of buffer (appropriate concentration of TBE or TAE) into the cell. Switch on the CHEF-DR III 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

**Note:** If the buffer circulation appears slower that normal (*i.e.* less than 500 ml / min at a setting of 70 on the pump regulator, it is possible that the outlet ports on the electrophoresis chamber are clogged with agarose. Reverse the recirculation flow to unclog the ports. Remove any agarose debris from the chamber and restore the flow to original condition.

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 Cooling Module (see Cooling Module 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 gel chamber is 14 ˚C.

Before beginning the electrophoresis run, check the current output displayed on the CHEF-DR III power module 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.

| Buffer Concentration | Voltage Gradient | Current Range |
|---|---|---|
| 0.5x TBE (at 14 ˚C) | 2 V/cm | 30–45 mA |
| 0.5x TBE (at 14 ˚C) | 3 V/cm | 50–65 mA |
| 0.5x TBE (at 14 ˚C) | 6 V/cm | 115–135 mA |
| 0.5x TBE (at 14 ˚C) | 9 V/cm | 190–210 mA |
| 1.0x TAE (at 14 ˚C) | 2 V/cm | 75–90 mA |
| 1.0x TAE (at 14 ˚C) | 3 V/cm | 115–130 mA |
| 1.0x TAE (at 14 ˚C) | 6 V/cm | 260–275 mA |
| 1.0x TAE (at 14 ˚C) | 9 V/cm | 380–410 mA |

If the current output is significantly different from the values listed above, drain the buffer, and add new buffer. 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. Recommended final buffer concentrations are:

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

## 4.3 Loading the Samples

Use one of the following methods to load the sample.

1. Place DNA in a sample plug 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 at an agarose concentration equal to that of the gel, and allow the agarose to harden at room temperature for 10–15 minutes.
2. Cut the sample plug into blocks and place on each tooth of the comb. Cast around the comb.The plug will remain in place when the comb is removed.
3. Add liquid samples to the sample wells with the gel positioned under the electrophoresis buffer in the chamber. 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.

## 4.4 DNA Size Standards

Bio-Rad recommends running standards in each gel to allow the sizes of unknown samples to be determined 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.

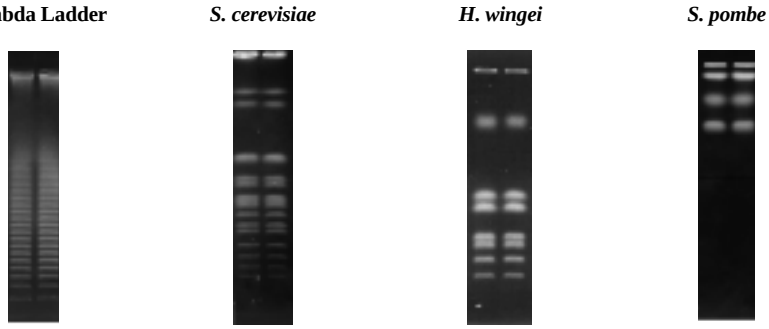



**Fig. 4.2. Lambda ladder** was separated on a 1.0% Molecular Biology Certified Agarose (catalog number 162-0133) gel in 0.5x TBE, recirculated at 14 °C. The run time was 22 hours at 6 V/cm with a 50 to 90 second switch time ramp at an included angle of 120°. ***Saccharomyces cerevisiae*** **Strain YNN295.** 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 at an included angle of 120°. ***Hansenula wingei*** **Strain YB-4662-VIA.** Chromosomes were separated on a 0.8% Pulsed Field Certified Agarose gel in 1.0x TAE, recirculated at 14 °C. The run time was 48 hours at 3 V/cm with a 500 second switch time at a included angle of 106°. ***Schizosaccharomyces pombe*** **Strain 972 h-.** 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 1.5 V/cm with a 30 minute switch time at a included angle of 106°.

## 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 in Figure 4.2, using the conditions described in the legend. Obtaining separations similar to those in Figure 4.2 will indicate that the CHEF-DR III system is functioning properly.

## 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, coil 4-5 feet of the Tygon tubing into a bucket of water. Recirculation of the buffer is required. Change the buffer 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. Electrophorese *S. cerevisiae* chromosomes 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, decrease voltage 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 during a run, push PAUSE/START RUN on the CHEF-DR III system. Remove the gel (on the platform) from the cell, slide it 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. Visualize the DNA by placing the gel on a UV transilluminator (254–360 nm). Remove the buffer from the gel box by attaching a 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 closed, when not in use, may lead to warpage of the lid. Leave the lid slightly opened 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.

### Agarose Concentration

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.

There are several agarose types that allow easy handling of low concentration gels. These agaroses, in concentrations 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 (catalog number 162-0135).

### Buffer Concentration and Temperature

In pulsed field electrophoresis, DNA mobility is sensitive to changes in buffer temperature. As the buffer temperature increases, the mobility of the DNA increases, but band sharpness and resolution decrease. Chill the buffer 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 (300 V) exceeding 1 day require buffer changes after each 48 hour period, to prevent 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 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.

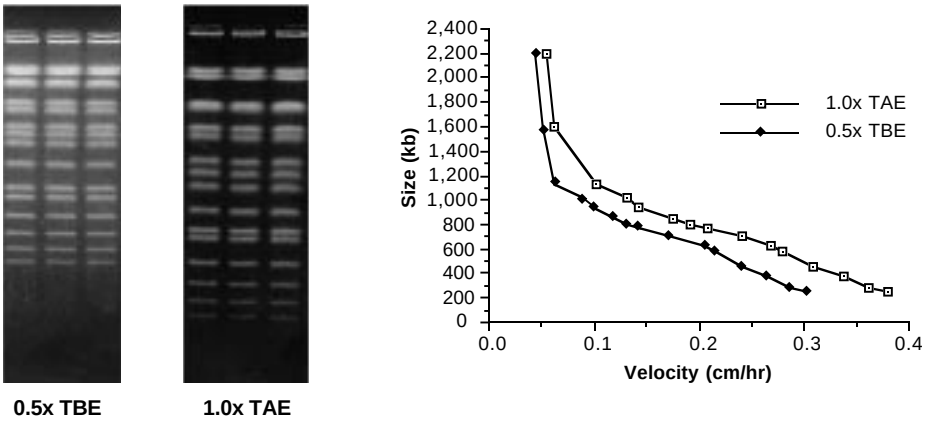



**Fig. 5.1. Two gels, one in 0.5x TBE and the other in 1.0x TAE, were run to show the difference in mobility of DNA in the two buffers.** *S. cerevisiae* was separated on a 1.0% Pulsed Field Certified Agarose (catalog number 162-0137) gel with a 60 second switch time for 15 hours, followed by a 90 second 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 is optimal for DNA molecules with reorientation times comparable to the switch time. So as the DNA size increases, increase the switch time to resolve the molecules. Under some conditions, larger molecules may run ahead of smaller ones.[50]

### Voltage (Field Strength)

DNA migration increases 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 very large DNA (>3 mb) cannot be resolved on the gel and the field strength must 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.

### Field Angle

The CHEF-DR III system allows separations to be carried out with electric field vectors oriented in any direction in the plane of the gel (90°–120°). With two field vectors, resolution of DNA molecules up to 1 mb is independent of the angle between them (Birren, Lai, Clark, Hood, *Science,* 1203-1205, 1988). It has been shown that decreasing the included angle from 120° to 94° increases the velocity of the DNA, with the mobilities of large DNAs (>1 mb) affected to a greater degree by the change in angle than are smaller DNAs (<1 mb). Figure 5.2 shows the effect of the included angle on the separation of yeast chromosomes. Decreasing the included angle will decrease the resolution of smaller DNAs by causing them to pile up on each other. This same effect on small DNA can be seen with long switch times. It is recommended that the included angle be decreased (<120°) when separating large DNA molecules greater than 2 mb.

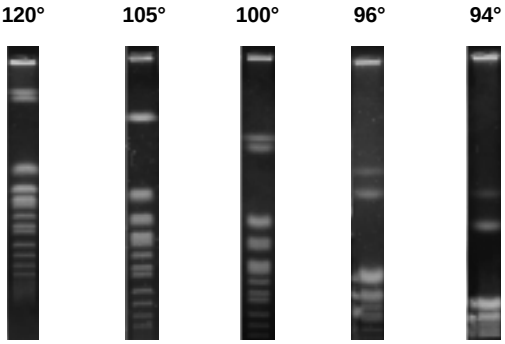



**Figure 5.2. Separation of *S. cerevisiae* chromosomes using angles from 120° to 94°.**

### 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 must increase to adequately resolve the DNA molecules of interest.

## 5.2 Pulsed Field Conditions by DNA Size

The table below gives suggested run parameters for the various DNA size ranges.

| | DNA 1-100 kb | 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 | 6–9 V/cm | 4.5–6 V/cm | 2–3 V/cm | 1.0–2.5 V/cm |
| Pulse Parameters | 0.05–10 sec | 10–200 sec | 200–1,800 sec | 10–60 min |
| Run Times | 2–15 hr | 15–30 hr | 24–72 hr | 72–144 hr |
| Angle | 120° | 120° | 120°, 106° | 106° |

## 5.3 Pulsed Field Conditions by Organism

This table gives 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% | 0.09 a | 3 | 9 V/cm | 120° | 0.5x TAE |
| 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<br>240 | 24<br>36 | 3.5 V/cm | 106° | 1.0x TAE |
| *Schizo-saccharomyces pombe* | 3,500–5,700 | 0.8% | 1,800 f | 72 | 1.5 V/cm | 106° | 1.0x TAE |
| *Dictostelium discodium* | 3,600–9,000 | 0.8% | 2,000–7,000 g<br>7,000–9,600 | 158<br>82 | 1.8 V/cm<br>1.5 V/cm | 120°<br>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 second switch 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).

## 5.4 Blotting Megabase DNAs†

### Southern Blot Transfer

Pulsed field electrophoresis is a powerful technique for physical mapping of genes in various organisms. 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 given 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. Photograph the gel 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 0.4 N NaOH, 1.5 M NaCl for 15 minutes. Transfer the DNA onto Zeta-Probe® GT nylon membrane (catalog number 162-0196) using 2 liters of 0.4 N NaOH, 1.5 M 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.
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 0.5 M 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.

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

### Discussion

1. The procedure is for 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 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 (catalog number 165-5000), and NaOH as buffer.

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.

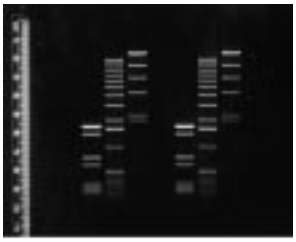
7. It is essential to neutralize the membrane after transfer to prevent changing the pH of the hybridization buffer during hybridization.

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: | 1.2% Molecular Biology Certified |
| Buffer: | 0.5x TBE |
| Temperature: | 14 °C |
| Switch Time: | 0.1 second |
| Run Time: | 3 hours |
| Angle: | 120° |
| Voltage Gradient: | 9 V/cm |

**2. 5 kb Ladder**



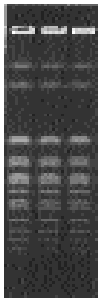
| | |
|---|---|
| Size Range: | 5–75 kb |
| Agarose: | 1.0% Molecular Biology Certified |
| Buffer: | 0.5x TBE |
| Temperature: | 14 °C |
| Switch Time: | 1-6 seconds |
| Run Time: | 11 hours |
| Angle: | 120° |
| Voltage Gradient: | 6 V/cm |

**3. Lambda Ladder**



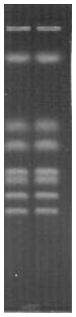
| | |
|---|---|
| Size Range: | 50–1,000 kb |
| Agarose: | 1.0% Molecular Biology Certified |
| Buffer: | 0.5x TBE |
| Temperature: | 14 °C |
| Switch Time: | 50-90 seconds |
| Run Time: | 22 hours |
| Angle: | 120° |
| Voltage Gradient: | 6 V/cm |

**4. *Saccharomyces cerevisiae***



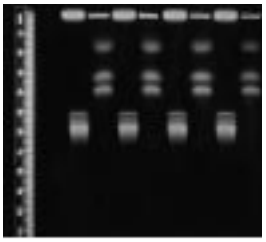
| | |
|---|---|
| Size Range: | 240–2,200 kb |
| Agarose: | 1.0% Pulsed Field Certified |
| Buffer: | 0.5x TBE |
| Temperature: | 14 °C |
| Switch Time: | 60–120 seconds |
| Run Time: | 24 hours |
| Angle: | 120° |
| Voltage Gradient: | 6 V/cm |

**5.** ***Hansenula wingei***



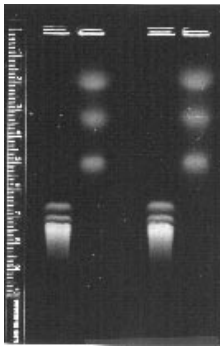
| | |
|---|---|
| Size Range: | 1–3.1 mb |
| Agarose: | 0.8% Molecular Biology Certified |
| Buffer: | 1.0x TAE |
| Temperature: | 14 °C |
| Switch Time: | 500 seconds |
| Run Time: | 48 hours |
| Angle: | 106° |
| Voltage Gradient: | 3 V/cm |

**6.** ***Schizosaccharomyces pombe***



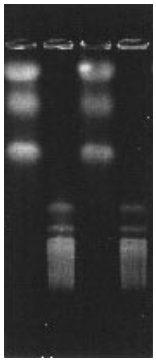
| | |
|---|---|
| Size Range: | 3.5–5.7 mb |
| Agarose: | 0.8% Chromosomal Grade |
| Buffer: | 1.0x TAE |
| Temperature: | 14 °C |
| Switch Time: | 1,800 seconds |
| Run Time: | 72 hours |
| Angle: | 106° |
| Voltage Gradient: | 2 V/cm |

**7.** **Angle Ramp:** ***S. pombe***



| | | | |
|---|---|---|---|
| Size Range: | 3.5–5.7 mb | | |
| Agarose: | 0.8% Chromosomal Grade | | |
| Buffer: | 1.0x TAE | | |
| Temperature: | 14 °C | | |
| | **Block 1** | **Block 2** | **Block 3** |
| Switch Time: | 1,200 sec. | 1,500 sec. | 1,800 sec. |
| Run Time: | 24 hours | 24 hours | 24 hours |
| Angle: | 96° | 100° | 106° |
| Voltage Gradient: | 2 V/cm | 2 V/cm | 2 V/cm |

**8.** **Voltage Ramp:** ***S. pombe***



| | | | |
|---|---|---|---|
| Size Range: | 3.5–5.7 mb | | |
| Agarose: | 0.8% Chromosomal Grade | | |
| Buffer: | 1.0x TAE | | |
| Temperature: | 14 °C | | |
| | **Block 1** | **Block 2** | **Block 3** |
| Switch Time: | 1,800 sec. | 1,500 sec. | 1,200 sec. |
| Run Time: | 24 hours | 24 hours | 24 hours |
| Angle: | 106° | 100° | 96° |
| Voltage Gradient: | 1.5 V/cm | 2 V/cm | 2.5 V/cm |

# Section 6
# Maintenance of Equipment

## 6.1 Replacing Electrodes

The gel chamber requires little maintenance except rinsing after every run. Dirt and other build-up can be removed with laboratory detergent and a fine cloth. Do not bend or break the electrodes.

Fast switch times (<2 seconds) with high voltage gradients (6-9 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 170-3648) are available from Bio-Rad Laboratories.

To replace an electrode, turn the gel chamber 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.

## 6.2 Fuses

If the DC current during a run exceeds 500 mA entering the gel chamber, the 0.5 ampere FB (fast blow) fuse will blow, and error code F2 will be displayed. 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 external power supply is off when replacing a fuse.

A power surge may cause the SB (slow blow) line fuse to blow. The LED lights on the power module will go off. The fuse is at the rear of the drive module. Replace the fuse with a 3.15 A SB if your line voltage is 100 or 120 VAC, or a 1.6 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 can be obtained as catalog number 270-1241, and the 3.15 A SB as catalog number 270-1173 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 slightly opened to minimize possible warpage.

# Section 7
# Troubleshooting Guide

| Problem | Solution |
|---|---|
| **Equipment** | |
| No power | 1. Check fuse at back of power module<br>2. Check source of A/C power<br>3. Contact Bio-Rad Laboratories |
| No voltage across electrodes, with AC power light on | 1. Check that lid is on<br>2. Check that serial cable is fully inserted at both ends<br>3. Check that coiled interlock cable is firmly attached<br>4. Confirm that all leads are properly attached<br>5. Check HV fuse on front panel (0.5 A FB) and replace if necessary<br>6. Check that the unit is not set in pause mode |
| 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<br>2. Check Cooling Module; buffer in the heat exchanger can freeze if the chiller is cooling, but the pump is not on<br>3. Check pump connection and if the pump is on<br>4. Electrophoresis chamber outlet port may be clogged. Reverse buffer recirculation flow to unclog the ports. Remove agarose debris from the chamber and restore the flow to original condition. |
| Gel band patterns appear very distorted; lanes very curved,* bands sharp, but slanting | 1. Foreign object in chamber (remove thermometer, etc.)<br>2. Insufficient or non-uniform cooling due to low pump flow<br>3. Check that buffer is level with surface of gel. Use leveling feet.<br>4. Check that the current is equal for both switch directions<br>5. Replace damaged electrode<br>6. Power module fault; contact Bio-Rad Laboratories<br>7. Not enough buffer in cell (total should be 2.2 liters)<br>8. Check to insure the electrophoresis chamber is level (Section 4.2) |

* Slight distortion of the outermost lane is normal

| | |
|---|---|
| **Applications** | |
| Bands smeary or fuzzy | 1. Excessive heating. Lower the voltage or ionic strength of the buffer.<br>2. Improper switch interval. See Section 5.1.<br>3. Gel percentage too low. Increase.<br>4. Sample degraded. Impure enzymes, or wash cycles too short (agarose blocks).<br>5. Agarose impurities. Consult Bio-Rad.<br>6. Sample overload. Adjust sample concentration. |

| Problem | Solution |
|---|---|
| Large DNAs not resolved | 1. Lower the voltage to below 2 V/cm<br>2. Increase switch time or use switch time ramp<br>3. Agarose impurities |
| High background in lanes | 1. Insufficient washing of samples<br>2. Sample may be contaminated with RNA or other material<br>3. DNA concentration too high |
| Distorted bands | 1. Sample contains too high salt or detergent concentration<br>2. Buffer breakdown. Change every 48 hours.<br>3. Wells were distorted. Recast gel.<br>4. Sample plugs were crushed when placed in wells<br>5. Pump flow rate too low. Check for kink along tubing.<br>6. Not enough buffer in cell (total should be 2.2 liters) |
| Thick bands | 1. Use thinner wells<br>2. Load less sample |

### Failure Codes

| | |
|---|---|
| F-0 | 1. Error detected in the PS processor code during power up. Contact Bio-Rad Laboratories |
| F-1 | 1. Error detected in the DAC processor code during power up. Contact Bio-Rad Laboratories |
| F-2 | 1. The power supply control processor is not getting adequate feedback from the high voltage power supply during a run. Normal operation will continue and the display may be changed by pressing a display mode key.<br>Check fuses and safety interlock<br>2. Contact Bio-Rad Laboratories |
| F-3 | 1. The DAC external RAM has been corrupted (all parameters will reset to default values). Normal operation can be resumed by pressing any key on the right control panel. The battery RAM on the DAC board did not maintain its data when the power went down. If this occurs consistently, there may be a hardware problem on the board, or the battery may be bad. Contact Bio-Rad Laboratories |
| F-4 | 1. If a gross overcurrent is detected (>500 mA), the system is put into pause automatically and F-4 is displayed. When the problem is corrected, the run may be resumed by pressing START.<br>2. Buffer concentration is too high. Remake the buffer at the appropriate concentration. |

# Section 8
# References

## 8.1 Applications in Pulsed Field Electrophoresis

The following references in pulsed field electrophoresis are 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 |
| 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 |
| Chromosome rearrangements | 127, 141, 147, 166 |

| Application | Reference Numbers |
|---|---|
| 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 |
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# Section 9
# Systems, Accessories, and Reagents for Pulsed Field Electrophoresis

| Catalog Number | Product Description |
|---|---|
| 170-3700 | **CHEF-DR III Chiller System**, 120 VAC, includes CHEF-DR III power module; electrophoresis cell; Cooling Module; variable speed pump; Tygon tubing, 12 feet; 14 cm wide x 13 cm long casting stand and frame; 10 well comb and comb holder; screened cap; 50 well disposable 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 |
| 170-3702 | **CHEF-DR III Chiller System**, 220/240 VAC |
| 170-3703 | **CHEF-DR III Chiller System**, 100 VAC |
| 170-3695 | **CHEF-DR III System**, 100/120 VAC, includes CHEF-DR III power 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 disposable plug mold; screened cap; 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 |
| 170-3697 | **CHEF-DR III System**, 220/240 VAC |
| 170-3654 | **Cooling Module**, 120 V |
| 170-3655 | **Cooling Module**, 220/240 V |
| 170-3688 | **Cooling Module**, 100 V |
| 170-3644 | **Variable Speed Pump** |
| 170-3648 | **Electrodes**, thick gauge (0.02”), 6 |
| 170-3711 | **Screened Caps,** 5 |
| 170-3622 | **50 Well Disposable Plug Mold**, for casting plugs |
| 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 |

| Catalog Number | Product Description |
|---|---|
| 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 |
| 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 |
| 170-3591 | **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 |

| Catalog Number | Product Description |
|---|---|
| 170-3590 | **Gene-Lite Chemiluminescent Detection Kit** |
| 170-3742 | **Standard Documentation System,** 120 VAC, includes Mini-Transilluminator, 100 V, DS-34 camera, standard hood, deep yellow DS-34 camera filter |
| 170-3746 | **Standard Documentation System,** 100 VAC |
| 170-3747 | **Standard Documentation System,** 220/240 VAC |
| 170-3743 | **Wide/Long Documentation System,** 120 VAC, includes Mini-Transilluminator, 100 V, DS-34 camera, wide/long hood, deep yellow DS-34 camera filter |
| 170-3748 | **Wide/Long Documentation System,** 100 VAC |
| 170-3749 | **Wide/Long Documentation System,** 220 /240 VAC |
| 170-3745 | **Mini-Transilluminator,** 100 VAC |
| 170-3737 | **Mini-Transilluminator,** 120 VAC |
| 170-3738 | **Mini-Transilluminator,** 220/240 VAC |
| 170-3739 | **Standard Camera Hood,** 5" x 7" (12.7 x 17.8 cm) |
| 170-3740 | **Wide/Long Camera Hood,** 6" x 9" (15.2 x 22.9 cm) |
| 170-3741 | **DS-34 Camera** |
| 170-3744 | **Deep Yellow DS-34 Camera Filter** |

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