Procedure & Checklist - Using the BIO-RAD® CHEF Mapper® XA Pulsed Field Electrophoresis System

Before You Begin

This procedure can be used to evaluate genomic DNA (gDNA) to ensure that it is of high quality and high molecular weight prior to being used in library construction. Quality analysis of the library (during construction) is also important when targeting larger insert sizes (>20 kb) and determining the effectiveness of shearing strategy. Finally, it can help determine the appropriate size selection protocol to use.

Overview

Conventional electrophoresis uses single electrical fields to cause DNA fragments to migrate through a matrix, which effectively separates DNA fragments up ~20 kb. However, when imaged, larger fragments co-migrate and appear as large bands at the top of the gel. Pulsed field gel electrophoresis (PFGE) overcomes this problem by alternating the electrical field between spatially distinct pairs of electrodes. This technique results in the separation of DNA fragments up to 10 Mb by their reorientation and movement at different speeds through the pores of an agarose gel. The BIO-RAD® CHEF Mapper® XA is based on the principles of clamped homogeneous electric fields (CHEF) and programmed autonomously controlled electrodes (PACE). For more detailed information, please refer to the BIO-RAD® CHEF Mapper® XA Pulsed Field Electrophoresis System Instruction Manual and Application Guide.

Materials Needed

Item	Vendor	Part Number
Electrophoresis Reagents		
10X TBE Electrophoresis Buffer	Bio-Rad	161-07
Pulsed Field Certified Agarose	Bio-Rad	161-0137
DNA Markers		
CHEF DNA Size Standard 8-48 kb	Bio-Rad	170 - 3707
CHEF DNA Size Standard 5 kb	Bio-Rad	170 - 3624
Staining/Imaging		
DNA Loading Buffer, 6X	Lonza	50655
Ethidium bromide	Teknova	53050
1X Elution Buffer	Pacific Biosciences	100-159-800
Equipment		
Pulsed Field Gel Electrophoresis System: CHEF Mapper XA	Bio-Rad	170-3670
Standard Casting Stand with 14 x 13 cm frame and platform	Bio-Rad	170-3689
Combination Comb Holder	Bio-Rad	170-3699
15 Well Comb	Bio-Rad	170-3627
KS 260 Basic Shaker	IKA	2980201
Kimble 500 ml Bottle	Cole-Palmer	EW-34523-04

Prepare and Run the Gel

The following procedure is for the Standard Casting Stand 14 cm x 13 cm frame for use with any BIO-RAD CHEF System.

1. Prepare 3 liters of 0.5X TBE Electrophoresis Buffer.
   a. In a 1 Liter graduated cylinder, add 50 mL of 10X TBE Electrophoresis Buffer to 950 mL of deionized water. Repeat two more times for a total of 3 liters.
2. Prepare a 1.0% Agarose Gel.
   a. On an analytical balance, carefully measure out 1.0 g of Bio-Rad Pulsed Field Certified Agarose.
   b. Pour 100 mL of 0.5X TBE Electrophoresis Buffer into a 500 mL Kimble bottle.
   c. Pour the 1.0 g of Bio-Rad Pulsed Field Certified Agarose to the 100 mL of 0.5X TBE Electrophoresis Buffer. Mix the solution while adding the agarose to prevent clumping.
   d. Place bottle in a microwave and heat on high for 1 – 2 minutes or until powder is fully dissolved. **Caution:** Bottle and content will be hot. Handle with care.
   e. Set the IKA Shaker to 50 rpm. Place the bottle on the shaker and shake gently for 10 minutes to allow solution to cool off.
      Note: After 10 minutes of shaking, the bottle should be cool enough to touch. However, further cooling may be required. Do not let the solution cool too long because it will solidify in the bottle.
   f. Ensure the CHEF Mapper casting stand with platform and comb holder is assembled based on the manufacturer recommendations as shown in figure 1 below. Refer to the manual provided with the casting stand.
   g. Cast the gel on a level surface.
   h. Pour the solution into the casting stand with the 15-well comb in place (see figure 1). Note: Pour slowly to avoid bubbles which will disrupt the gel. Bubbles can be managed by pushing them toward the edges with a pipette tip.

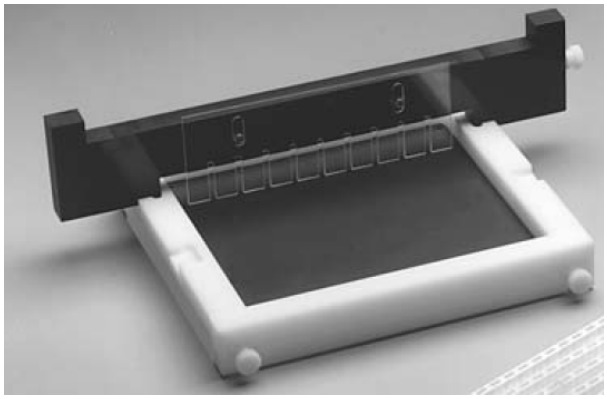


Figure 1. CHEF Mapper casting stand and comb holder

   i. Let the gel sit at room temperature for 25 minutes, or until it is fully solidified.
   j. When ready to use, disassemble the casting tray and place gel in the chamber and pour 2.2 liters of 0.5X TBE buffer into the electrophoresis chamber (see figure 2). The buffer level inside the frame should be approximately 1-2 mm above the surface of the gel.

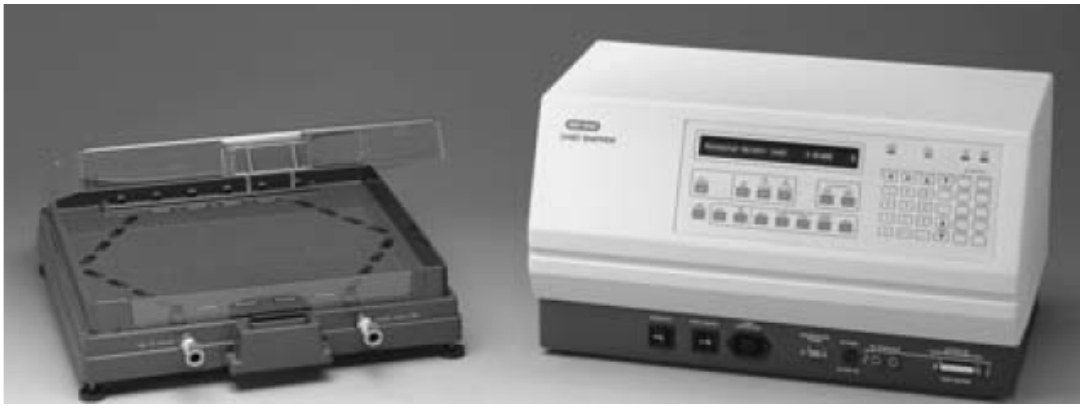


Figure 2. Bio-Rad CHEF Mapper chamber and power module CHEF Mapper XA

3. Prepare samples:
   a. Prepare up to 13 samples at 100 - 150 ng per well.

Component	Volume
Sample	*X* µL of 100 - 150 ng
6X Lonza DNA Loading Buffer	2 µL
1X Elution Buffer	Add to bring up to total volume
Total	**12 µL**

4. Prepare DNA Markers. We highly recommend using the following gel markers loaded on adjacent wells.

CHEF DNA Size Standard 8 - 48 kb

a. Prepare a 1:3 dilution of the CHEF DNA Size Standard. Add 1 µL of the CHEF DNA Size Standard + 2 µL 1X Elution Buffer.
b. Add 1 µL of the diluted CHEF DNA Size Standard + 9 µL 1X Elution Buffer+ 2 µl Lonza 6X Loading Dye for a total of 12 µL.

CHEF DNA Size Standard 5 kb

a. Prepare a 1:3 dilution of the CHEF DNA Size Standard. Add 1 µL of the CHEF DNA Size Standard + 2 µL 1X Elution Buffer.
b. Add 1 µL of the diluted CHEF DNA Size Standard + 9 µL 1X Elution Buffer + 2 µl Lonza 6X Loading Dye for a total of 12 µL.

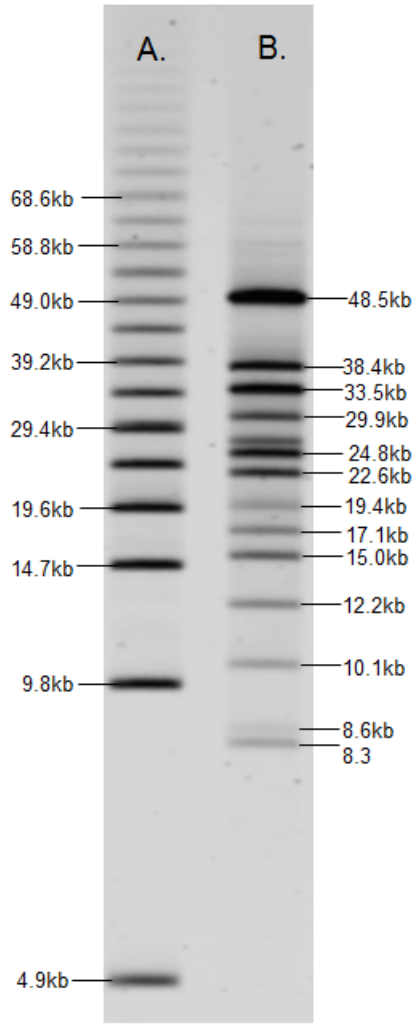


Figure 3. DNA Markers

A. Bio-Rad CHEF DNA Size Standard 5 kb
B. Bio-Rad CHEF DNA Size Standard 8-48 kb

5. Run the CHEF Mapper XA.
   a. Turn on the Bio-Rad CHEF Mapper XA Pulsed Field Electrophoresis System in the order below:
      I. CHEF Mapper Power
      II. CHEF Mapper Pump
      III. CHEF Mapper Chiller – set to 14℃
   b. Allow the electrophoresis buffer and gel to equilibrate to 14℃ before the run is started (approximately 20 minutes).
   c. Load DNA samples and markers onto gel (12 µL per well).
   d. Press **AUTO ALGORITHM.** The following screen will appear:

   e. After **Molecular Weight: Low,** key in **5**, press **K-BASES**, then **ENTER**.
   f. After **Molecular Weight: High,** key in **100**, press **K-BASES**, then **ENTER**.
   g. Press **ENTER** to accept all default parameters.

h. The final display is **“A program is in memory**. **Please enter another command.”** Start the run by pressing **START RUN.**

i. The run time for this program is approximately 15.5 hours.
j. When the run is completed the screen displays the message “**Run is Completed**”.
k. Turn off the CHEF Mapper XA in the following order:
    I. CHEF Mapper Chiller
    II. CHEF Mapper Pump
    III. CHEF Mapper Power

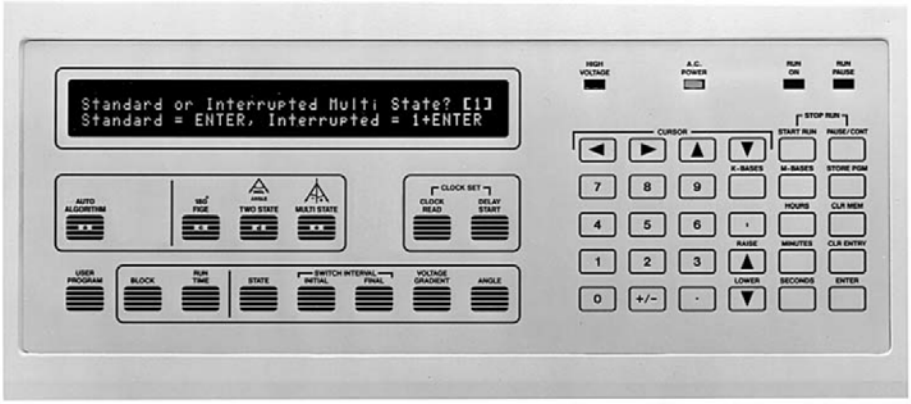
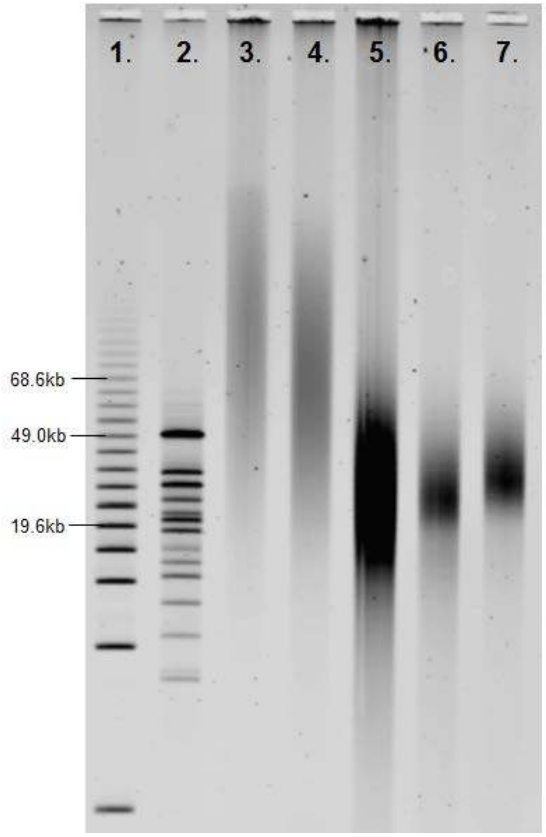


Figure 4. CHEF Mapper XA Front Panel Power Module

6. Stain the gel.
    a. Remove the gel from the gel chamber.
    b. Place the gel into a 4.5 L Pyrex dish (400 mm x 270 mm).
    c. Add 300 mL of deionized water + 7.5 µl Ethidium bromide into the Pyrex dish.
    d. Cover the Pyrex dish with aluminum foil.
    e. Shake the gel and stain gently for 30 minutes. Place the covered Pyrex dish on an IKA shaker and set shaker to 50 rpm.
    f. De-stain the gel in 300 mL of deionized water for 30 minutes.

7. Image the gel.
    a. Image gel according to manufacturer’s user guide.



1. Bio-Rad CHEF 5kb Ladder
2. Bio-Rad CHEF 8-48kb Ladder
3. Genomic DNA
4. Genomic DNA
5. 50 kb Shear on Megaruptor
6. 50 kb Shear – Size Selected at 20 kb using Blue Pippin system
7. 50 kb Shear – Size Selected at 30 kb using Blue Pippin system

Figure 5. Evaluation of gDNA quality by PFGE using a CHEF Mapper XA System

Revision History (Description)	Version	Date
Initial release. Converted from “Unsupported” to “Supported”. No other changes.	01	June 2018