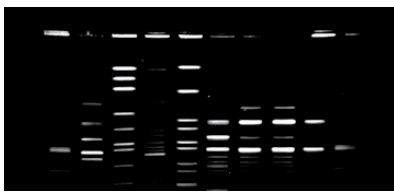


Pulsed Field Gel Electrophoresis

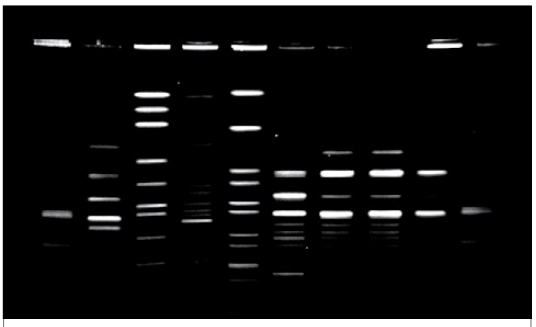
Map It Out

BIO-RAD

The Direct Route to Your Large Molecule Applications

PFGE Is Used in Many Research Areas

Pulsed field gel electrophoresis (PFGE) has enabled progress in cancer research, food safety, public health, quality control, and genome mapping. It is widely used in molecular epidemiology for strain typing and it has been adopted by PulseNet, a global network of health and food regulatory agency laboratories coordinated by the Centers for Disease Control and Prevention (CDC).



Electrophoretic profile of *Salmonella enteritidis*. Courtesy of Kara L Cooper, Centers for Disease Control and Prevention, Atlanta, Georgia.

Molecular Epidemiology

Strain Typing in Public Health and Food Safety

PFGE is used for epidemiological studies of pathogenic organisms such as *Escherichia coli* O157:H7, *Salmonella, Shigella, Listeria, Campylobacter,* or *Vibrio cholerae.* When epidemiologists need to precisely identify the strain variants from a sample, genetic fingerprinting is the method of choice. Rare cutting restriction enzymes yield large DNA fragments which are analyzed using the CHEF system; the variant-specific electrophoretic gel pattern is then compared to the PulseNet database. The PulseNet database, which contains thousands of patterns, is used by member laboratories to identify and track foodborne infections worldwide.

Food Quality Control

PFGE is in widespread use as a quality control method in the food industry. For example, the beer and wine industries use it to monitor the genetic stability of organisms in fermentation processes.

Cancer Research

DNA Damage and Repair Studies

Research efforts using PFGE are focused on better understanding the factors mediating the damage to DNA caused by ionizing radiation and chemical treatment. It is important to quantitatively measure the dsDNA breaks due to these treatments. DNA from treated cells is subjected to PFGE and the density of the DNA in different molecular weight regions indicates the integrity of DNA and the extent of its repair.

Apoptosis Assays

Apoptotic DNA fragmentation is a key characteristic of programmed cell death. Analysis of the fragmentation that occurs in the apoptosis process demonstrates either a “ladder” pattern at ~200 bp intervals (200–600 bp) or the formation of larger fragments (50–300 kb). Both of these size ranges can be visualized on one gel using field inversion gel electrophoresis (FIGE), available on the CHEF Mapper® XA system, enabling easier assessment of the fragmentation process.

Genomics Applications

Generation of Artificial Chromosome Libraries

Cloning large DNA (100 kb–1 Mb) is the first step in sequencing complex genomes. FIGE is often used to separate and isolate the large digested DNA fragments, which are then cloned into artificial chromosomes to generate yeast, bacterial, human, and mammalian libraries.

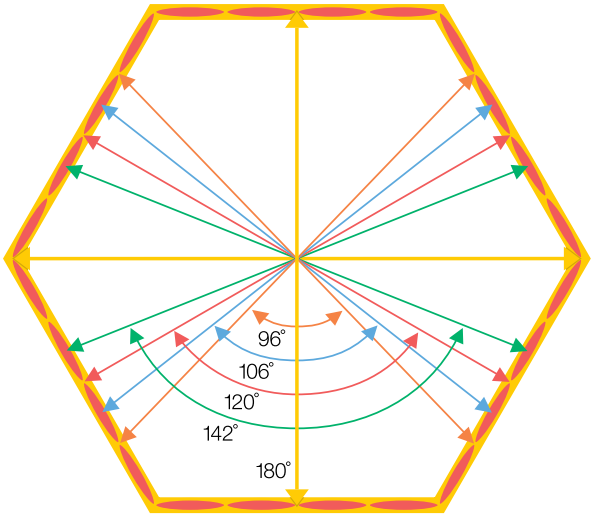
Genome Mapping

PFGE is still the benchmark for mapping applications. The libraries constructed using FIGE can also be used for mapping applications and specific assays used in research areas, including mapping specific disease loci, identifying chromosome rearrangements, and RFLP and DNA fingerprinting.

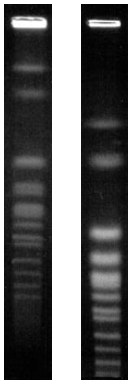
DNase I Hypersensitivity Assay

PFGE is used in mapping the genome for DNase I hypersensitive sites, which involves identifying different types of regulatory domains, such as active promoters and enhancers, where DNA-binding proteins are bound within nuclear chromatin. Conformation of chromatin at these sites causes them to be sensitive to DNase I cleavage. These sites are often located near active genes and play a role in eukaryotic gene regulation.

The Leader in PFGE Technology



Multistate switching capability. Use this feature of the CHEF Mapper XA system to select vectors to dramatically speed up your separations and improve resolution.



0 sec 3 sec

Multistate mode, 20 hr run,
120° included angle
60–120 sec switch-time ramp
6 V/cm (200 V), 0.5x TBE, 14°C
1.0% Certified™ molecular biology agarose

Secondary pulses
6 V/cm (200 V), 0° angle
3 sec switch time
4 pulses/min

Increased separation with secondary pulsed field electrophoresis. *Saccharomyces cerevisiae* chromosomes separated under two-state conditions (left) and under two-state conditions with secondary pulses (right).

Bio-Rad is the leader in PFGE, offering the exclusive clamped homogenous electrical field (CHEF) technology (Chu et al. 1986) for pulsed field electrophoresis that has been used in mapping genomes since 1988.

Conventional electrophoresis can effectively separate fragments up to 20 kb; DNA fragments larger than 20 kb in a sample comigrate and when imaged appear as a large fuzzy band at the top of the gel. In 1984, Schwartz and Cantor invented PFGE to overcome this problem. PFGE resolves DNA by alternating the electrical field between spatially distinct pairs of electrodes, causing DNA molecules as large as several megabases to reorient and move at different speeds through the pores in an agarose gel.

Technologies Used in Bio-Rad's PFGE Systems

To achieve straight runs and good resolution in PFGE it is necessary to create homogenous electrical fields. There have been multiple approaches to PFGE but the combination of CHEF, PACE, and DR technologies used in Bio-Rad PFGE systems works best in creating the homogenous electrical fields that ensure consistency and run-to-run reproducibility.

- CHEF (clamped homogeneous electrical field) technology resolves DNA over a wide range of molecular weights in a straight lane; it employs the principles of contour-clamped electrophoresis to generate homogenous electrical fields
- PACE (programmable autonomously controlled electrodes) technology allows users to select the angle of electrophoretic pulsing optimal for the desired size range
- DR (dynamic regulation) is the electronics design by which each of the 24 electrodes is regulated; CHEF-DR® systems are capable of compensating for changes in buffer conductivity or gel size, preventing these changes from affecting the reproducibility of results
- FIGE (field inversion gel electrophoresis) is used for rapid sample resolution in the 100 bp–250 kb size range; in FIGE the electrical field is fixed at 1 angle (180°) and is inverted in the forward and reverse directions
- AFIGE (asymmetric field inversion gel electrophoresis) is a further refinement of the FIGE technology; AFIGE applies a different voltage to the forward direction electrical field than to the reverse direction electrical field, which optimizes the sample resolution in the FIGE size range

Complete Solutions for PFGE

Molecular Imager® Gel Doc™ XR+ System

The Molecular Imager Gel Doc XR+ instrument is an easy-to-use gel documentation system that documents and analyzes fluorescent gels, and produces publication-quality output at a fraction of the cost of film. It combines a compact darkroom, UV transilluminator workstation, high-resolution CCD camera, and powerful, user-friendly software for unsurpassed flexibility.

FPQuest™ and InfoQuest™FP Software

FPQuest and InfoQuestFP modular software packages offer customizable applications to meet a variety of laboratory informatics requirements.

- FPQuest software offers advanced analysis and statistical tools for analyzing banding patterns and multiple fingerprints in gels
- InfoQuestFP software includes all the functionality of FPQuest software, with the ability to analyze many other data types for more comprehensive studies of biological relationships

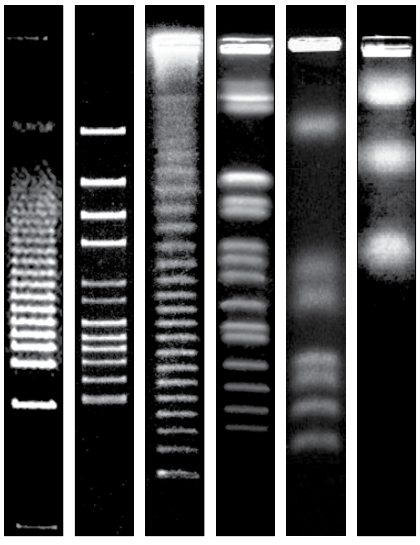
CHEF Genomic DNA Plug Kits

Three DNA plug kits are available for preparing bacterial (lysozyme-sensitive) or mammalian genomic DNA and yeast chromosomes (YACs). Each kit contains sufficient enzymes, reaction buffers, and restriction digest–qualified CleanCut™ agarose for 100 plugs. Disposable molds and screened caps simplify plug preparation.

Pulsed Field Quality Agarose

The type of agarose and the amount (percentage) used in an experiment play a crucial role in achieving optimal fragment resolution. Bio-Rad's Certified agaroses for PFGE are 100% pure and GQT grade (genetic quality tested).

- Pulsed field Certified agarose has an optimal separation range of 1 kb–2 Mb; its running conditions are a preset selectable method of the CHEF Mapper XA system auto-algorithm
- Certified megabase agarose has an optimal separation range of 1 kb–5 Mb and has high gel strength, a high exclusion limit, and high electrophoretic mobility; gels are easy to handle, even at 0.3%, allowing shorter run times

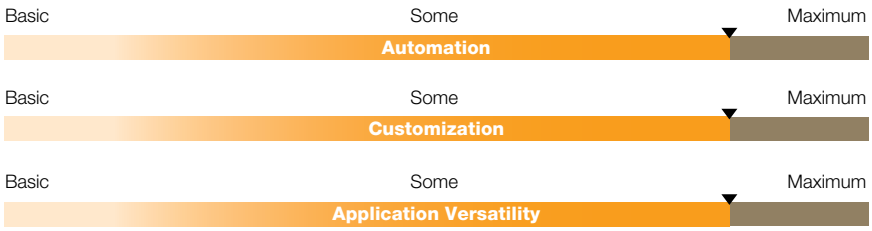


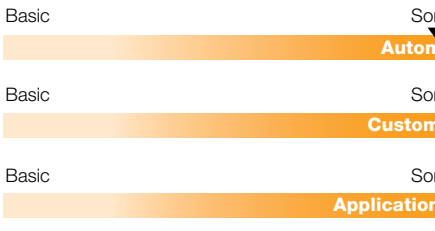
Standards and Markers

Bio-Rad offers standards for all PFGE applications, from FIGE separation of cosmid inserts to the largest chromosomal separations. Higher molecular weight standards are prepared in low-melt agarose blocks that can be cut to fit any well dimensions.

A, 5 kb ladder standard;
B, 8.3–48.5 kb standard;
C, lambda ladder standard;
D, *S. cerevisiae* marker;
E, *H. wingei* marker;
F, *S. pombe* marker.

Choose the System You Need for Your Specific





CHEF Mapper XA System

The CHEF Mapper XA system is the ultimate tool, ideal for all PFGE applications. It offers multistate, secondary pulse, and a combination of CHEF, PACE, DR, FIGE, and AFIGE technologies, making optimal resolution in all size ranges possible.

- Users can achieve optimal resolution of both megabase- and kilobase-sized DNA fragments in 1 lane by selecting any pulse angle (0–360°) and applying asymmetrical angles
- Secondary pulses can be applied to release DNA caught in the gel matrix to further enhance the separation and resolution of very large DNA molecules
- FIGE and AFIGE functions enable enhanced and rapid resolution of small fragments (100 bp–250 kb)

The system is ideal for both the PFGE novice and the expert because it offers two ways to optimize DNA separations:

- Built-in auto-algorithm automatically selects and executes optimal separation conditions from only the entered fragment sizes
- Protocols can be refined using the Windows-based interactive algorithm, which allows users to simultaneously specify several run variables to derive optimal separation protocols
- System can store 99 simple programs or 20 complex programs with up to 8 blocks of programming each

CHEF-DR III Variable Angle System

The CHEF-DR III system is optimized for r
100 bp–10 Mb range using CHEF and PA

- Users enter run conditions, and can optimize fragment resolution by selecting optimal voltage gradient, switch time, and pulse angle (90–120°) for the specific DNA size range
- Users can vary run conditions to obtain accurate size estimations; fine resolution in a complicated digest can be achieved with minimal programming and sample preparation

Application

me Maximum
nation

me Maximum
nization

me Maximum
n Versatility

Basic Some Maximum
Automation

Basic Some Maximum
Customization

Basic Some Maximum
Application Versatility

apid separations of DNA in the
CE technologies.

- System will recall the last conditions and use them as the default protocol; it also has a battery-operated backup RAM to recall current run conditions and resume the run without user intervention in the event of a power failure

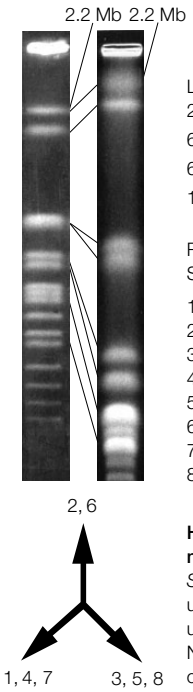
CHEF-DR II System

The CHEF-DR II system effectively and reliably resolves DNA fragments in the 5 kb–6 Mb range by adjusting the running conditions for low voltage and extended run times.

- System is easy to program and cost effective, allowing enhanced resolution by executing two blocks of running conditions successively; users can input optimal run time, voltage gradient, and switch intervals for each run
- System employs the most common pulse angle for PFGE, 120°; the instrument manual provides examples of run conditions for a variety of size separation ranges for easy startup



Feature	CHEF Mapper XA System	CHEF-DR III Variable Angle System	CHEF-DR II System
Fragment size	100 bp–10 Mb	100 bp–10 Mb	5 kb–6 Mb
Optimal separation size range	100 bp–10 Mb	100 bp–10 Mb	100 kb–2 Mb
Auto-algorithm and interactive algorithm: algorithmic derivation of optimal run conditions	Yes	No	No
Program storage: storage and easy access of run conditions	20 complex programs	Last program run	No
Programming blocks of run conditions: optimized separation of fragments	8 blocks	3 blocks	2 blocks
Battery-operated backup RAM: recalls current run conditions and run progress in the event of power failure	Yes	Yes	No
Pulse angle: selection of different pulse angles optimizes resolution of both chromosomal and plasmid DNA with one system	Any angle from 0 to 360°	Any angle from 90 to 120° (in 1° increments)	Fixed angle of 120°
Asymmetrical angles: further optimizes separation of both chromosomal and plasmid DNA with one system; necessary for difficult samples	Yes	No	No
Nonlinear switch-time ramping: expands linear range of fragment separation to 50–700 kb, thus providing accurate fragment size measurements	Yes	No	No
Multistate separation: optimizes separation of subsets of fragments for enhanced resolution in selected fragment size ranges and faster separation	Yes	No	No
Secondary pulses (voltage interrupts): releases large DNA caught in the gel matrix and enhances separation and resolution of very large DNA molecules	Yes	No	No
FIGE and AFIGE: superior resolution of small fragments in 100 bp–250 kb range	Yes	No	No
Recommended use	• Ideal for all PFGE applications • Best resolution in all size ranges • Most accurate results • Most reproducible results • Fastest runs	• Better suited for more advanced separations than CHEF-DR II system • Better separation of DNA fragments >2 Mb	• Suitable for routine separations with the same organism • Separation of DNA fragments <2 Mb

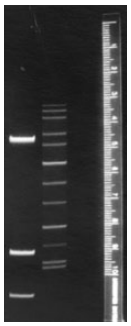


Left, two-state mode,
24 hr run, 120° included angle
60–120 sec switch-time ramp
6 V/cm, 0.5x TBE, 14°C
1.0% pulsed field Certified agarose

Right, multistate mode, 60 hr run
State (pulse angle):

1. 90 sec switch time, –60° angle
2. 45 sec switch time, 180° angle
3. 90 sec switch time, 60° angle
4. 90 sec switch time, –60° angle
5. 90 sec switch time, 60° angle
6. 45 sec switch time, 180° angle
7. 90 sec switch time, –60° angle
8. 90 sec switch time, 60° angle

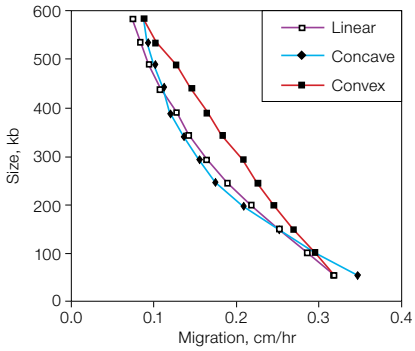
High-resolution separation with multiple states (pulse angles). *S. cerevisiae* chromosomes separated under two-state conditions (left) and under multistate conditions (right). Notice separation of the comigrating chromosomes under multistate conditions.



FIGE mode,
180° angle
200–800 ms
switch-time ramp
9 V/cm forward,
6 V/cm reverse
1x TAE, 14°C,
18 hr run
Forward
switch time =
reverse time

Lane 1: Bio-Rad λ HindIII standard (6.6, 9.4, 23.1 kb)
Lane 2: Bio-Rad 8.3–48.5 kb size standard (8.3, 8.6, 10.0, 12.2, 15.0, 17.1, 19.4, 22.6, 24.8, 29.9, 33.5, 38.4, 48.5 kb)

High resolution of 8.3–48.5 kb size standard on the CHEF Mapper XA system with AFIGE.



Mobility effects of nonlinear switch-time ramps on the CHEF Mapper XA system. Molecular size vs. migration for linear, concave, and convex ramps. The convex ramp results in the widest linear range.

Ordering Information

Catalog #	Description
170-3670	**CHEF Mapper XA Chiller System**, 120 V, includes CHEF Mapper XA power module, embedded auto-algorithm for protocol optimization, interactive algorithm program disk, electrophoresis cell, cooling module, variable-speed pump, Tygon tubing (12'), 14 x 13 cm (W x L) casting stand, 15-well 1.5 mm comb and comb holder, screened cap, disposable plug molds, leveling bubble, cables, *S. cerevisiae* DNA size standards, two 0.5 A FB fuses, 5 g pulsed field Certified agarose, 5 g Certified megabase agarose, instructions, for North America
170-3671	**CHEF Mapper XA Chiller System**, 100 V, for Japan
170-3672	**CHEF Mapper XA Chiller System**, 220 V, for Asia Pacific/Europe
170-3673	**CHEF Mapper XA Chiller System**, 240 V, for Asia Pacific/Europe
170-3700	**CHEF-DR III Variable Angle Chiller System**, 120 V, includes power module, electrophoresis cell, cooling module, variable-speed pump, 14 x 13 cm casting stand with frame and platform, comb holder, 15-well 1.5 mm thick comb, screened cap, disposable plug molds, 12' Tygon tubing, 2 plugs *S. cerevisiae* DNA size standards, two 0.5 A FB fuses, 5 g pulsed field Certified agarose, 5 g Certified megabase agarose, instructions, for North America
170-3702	**CHEF-DR III Variable Angle Chiller System**, 220/240 V, for Asia Pacific/Europe
170-3703	**CHEF-DR III Variable Angle Chiller System**, 100 V, for Japan
170-3725	**CHEF-DR II Chiller System**, 120 V, includes electrophoresis cell, drive module, cooling module, control module, variable-speed pump, 14 x 13 cm casting stand with frame and platform, comb holder, 15-well 1.5 mm thick comb, screened cap, disposable plug molds, 12' Tygon tubing, 2 plugs *S. cerevisiae* DNA size standards, two 0.5 A FB fuses, 5 g pulsed field Certified agarose, 5 g Certified megabase agarose, instructions, for North America
170-3727	**CHEF-DR II Chiller System**, 220/240 V, for Asia Pacific/Europe
170-3728	**CHEF-DR II Chiller System**, 100 V, for Japan
170-8190	**Molecular Imager Gel Doc XR+ System**, PC and Mac
170-9300	**FPQuest Basic Software**
170-9301	**FPQuest Cluster Analysis**
170-9302	**FPQuest Identification and Library Manager**
170-9310	**InfoQuestFP Basic Fingerprint Types**
170-9314	**InfoQuestFP Cluster Analysis**
170-9315	**InfoQuestFP Identification and Library Manager**
170-3591	**CHEF Mammalian Genomic DNA Plug Kit**, contains 12 ml cell suspension buffer, 1.3 ml proteinase K, 30 ml proteinase K reaction buffer, 12 ml 2% CleanCut agarose, 60 ml 10x wash buffer, screened cap, 2 disposable plug molds, instructions; makes 100 plugs
170-3592	**CHEF Bacterial Genomic DNA Plug Kit**, contains 12 ml cell suspension buffer, 1.3 ml proteinase K, 30 ml proteinase K reaction buffer, 12 ml 2% CleanCut agarose, 60 ml 10x wash buffer, 1.6 ml lysozyme (25 mg/ml), 30 ml lysozyme buffer, screened cap, 2 disposable plug molds, instructions; makes 100 plugs
170-3593	**CHEF Yeast Genomic DNA Plug Kit**, contains 12 ml cell suspension buffer, 1.3 ml proteinase K, 30 ml proteinase K reaction buffer, 12 ml 2% CleanCut agarose, 60 ml 10x wash buffer, 1.6 ml lyticase, 25 ml lyticase buffer, screened cap, 2 disposable plug molds, instructions; makes 100 plugs

Agaroses and Size Standards for PFGE

161-3108	**Certified Megabase Agarose**, 25 g
161-3109	**Certified Megabase Agarose**, 125 g
161-3110	**Certified Megabase Agarose**, 500 g
161-3100	**Certified Molecular Biology Agarose**, 25 g
161-3101	**Certified Molecular Biology Agarose**, 125 g
161-3102	**Certified Molecular Biology Agarose**, 500 g
162-0137	**Pulsed Field Certified Agarose**, 100 g
162-0138	**Pulsed Field Certified Agarose**, 500 g
170-3594	**CleanCut Agarose**, 2%, 12 ml; makes 24 ml of sample mixture or 100 plugs
170-3605	**CHEF DNA Size Marker**, *S. cerevisiae*, 0.2–2.2 Mb, 5 agarose blocks, sufficient for 25–40 plugs
170-3667	**CHEF DNA Size Marker**, *H. wingei*, 1–3.1 Mb, 5 agarose blocks, sufficient for 25–40 plugs
170-3633	**CHEF DNA Size Marker**, *S. pombe*, 3.5–5.7 Mb, 5 agarose blocks, sufficient for 25–40 plugs
170-3624	**CHEF DNA Size Standard**, 5 kb ladder, 4.9–120 kb, 20–25 lanes
170-3707	**CHEF DNA Size Standard**, 8.3–48.5 kb, 125 lanes
170-3635	**CHEF DNA Size Standard**, lambda ladder, 0.05–1 Mb, 5 agarose blocks, sufficient for 25–40 plugs

Premixed Nucleic Acid Electrophoresis Buffers and Stains

161-0733	**10x Tris/Boric Acid/EDTA (TBE)**, 1 L bottle
161-0770	**10x Tris/Boric Acid/EDTA (TBE)**, 5 L cube
161-0743	**50x Tris/Acetic Acid/EDTA (TAE)**, 1 L bottle
161-0773	**50x Tris/Acetic Acid/EDTA (TAE)**, 5 L cube
161-0433	**Ethidium Bromide Solution**, 10 mg/ml, 10 ml

Reference

Chu G et al. (1986). Science 234, 1582–1585. CHEF (U.S. patent 5,549,796 issued to Stanford University) is exclusively licensed to Bio-Rad Laboratories, Inc.

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