Pulsed Field Gel Electrophoresis
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).

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

Bio-Rad is the leader in PFGE, offering the exclusive clamped homogenous electrical field (CHEF) technology 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 InfoQuestFP 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. wingel marker; F. S. pombe marker.
Choose the System You Need for Your Specific Application

**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 rapid separations of DNA in the 100 bp–10 Mb range using CHEF and PACE technologies.

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 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

- 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
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- 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 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 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.

- 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.
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.

**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

**Asymmetrical angles:** further optimizes separation of both chromosomal and plasmid DNA with one system.

**Nonlinear switch-time ramping:** expands linear range of fragment separation to 50–700 kb, thus providing accurate fragment size measurements.

**Multistate separation:** optimizes separation of subsets of fragments for enhanced resolution in selected fragment size ranges and faster separation.

**Secondary pulses (voltage interrupts):** releases large DNA caught in the gel matrix and enhances separation and resolution of very large DNA molecules.

**FIGE and AFIGE:** superior resolution of small fragments in 100 bp–250 kb range.

**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

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**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

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>170-3670</td>
<td>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</td>
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<tr>
<td>170-3671</td>
<td>CHEF Mapper XA Chiller System, 100 V, for Japan</td>
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<tr>
<td>170-3672</td>
<td>CHEF Mapper XA Chiller System, 220 V, for Asia Pacific/Europe</td>
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<tr>
<td>170-3673</td>
<td>CHEF Mapper XA Chiller System, 240 V, for Asia Pacific/Europe</td>
</tr>
<tr>
<td>170-3700</td>
<td>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</td>
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<tr>
<td>170-3702</td>
<td>CHEF-DR III Variable Angle Chiller System, 220/240 V, for Asia Pacific/Europe</td>
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<tr>
<td>170-3703</td>
<td>CHEF-DR III Variable Angle Chiller System, 100 V, for Japan</td>
</tr>
<tr>
<td>170-3725</td>
<td>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</td>
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<td>170-3727</td>
<td>CHEF-DR II Chiller System, 220/240 V, for Asia Pacific/Europe</td>
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<td>170-3728</td>
<td>CHEF-DR II Chiller System, 100 V, for Japan</td>
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<td>170-8190</td>
<td>Molecular Imager Gel Doc XR+ System, PC and Mac</td>
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<tr>
<td>170-9300</td>
<td>FQQuest Basic Software</td>
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<tr>
<td>170-9301</td>
<td>FQQuest Cluster Analysis</td>
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<tr>
<td>170-9302</td>
<td>FQQuest Identification and Library Manager</td>
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<tr>
<td>170-9310</td>
<td>InfoQuestFP Basic Fingerprint Types</td>
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<td>170-9314</td>
<td>InfoQuestFP Cluster Analysis</td>
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<td>170-9315</td>
<td>InfoQuestFP Identification and Library Manager</td>
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<tr>
<td>170-3591</td>
<td>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</td>
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<tr>
<td>170-3592</td>
<td>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</td>
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<tr>
<td>170-3593</td>
<td>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</td>
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Agaroses and Size Standards for PFGE

<table>
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<tr>
<th>Catalog #</th>
<th>Description</th>
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<tbody>
<tr>
<td>161-3108</td>
<td>Certified Megabase Agarose, 25 g</td>
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<tr>
<td>161-3109</td>
<td>Certified Megabase Agarose, 125 g</td>
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<tr>
<td>161-3110</td>
<td>Certified Megabase Agarose, 500 g</td>
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<tr>
<td>161-3100</td>
<td>Certified Molecular Biology Agarose, 25 g</td>
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<tr>
<td>161-3101</td>
<td>Certified Molecular Biology Agarose, 125 g</td>
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<tr>
<td>161-3102</td>
<td>Certified Molecular Biology Agarose, 500 g</td>
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<tr>
<td>162-0137</td>
<td>Pulsed Field Certified Agarose, 100 g</td>
</tr>
<tr>
<td>162-0138</td>
<td>Pulsed Field Certified Agarose, 500 g</td>
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<tr>
<td>170-3594</td>
<td>CleanCut Agarose, 2%, 12 ml; makes 24 ml of sample mixture or 100 plugs</td>
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<tr>
<td>170-3605</td>
<td>CHEF DNA Size Marker, S. cerevisiae, 0.2–2.2 Mb, 5 agarose blocks, sufficient for 25–40 plugs</td>
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<tr>
<td>170-3687</td>
<td>CHEF DNA Size Marker, H. wingei, 1–3.1 Mb, 5 agarose blocks, sufficient for 25–40 plugs</td>
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<tr>
<td>170-3633</td>
<td>CHEF DNA Size Marker, S. pombe, 3.5–5.7 Mb, 5 agarose blocks, sufficient for 25–40 plugs</td>
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<tr>
<td>170-3624</td>
<td>CHEF DNA Size Standard, 5 kb ladder, 4.9–120 kb, 20–25 lanes</td>
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<tr>
<td>170-3707</td>
<td>CHEF DNA Size Standard, 8.3–48.5 kb, 125 lanes</td>
</tr>
<tr>
<td>170-3635</td>
<td>CHEF DNA Size Standard, lambda ladder, 0.05–1 Mb, 5 agarose blocks, sufficient for 25–40 plugs</td>
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Premixed Nucleic Acid Electrophoresis Buffers and Stains

<table>
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<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>161-0733</td>
<td>10x Tris/Boric Acid/EDTA (TBE), 1 L bottle</td>
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<tr>
<td>161-0770</td>
<td>10x Tris/Boric Acid/EDTA (TBE), 5 L cube</td>
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<tr>
<td>161-0743</td>
<td>50x Tris/Acetic Acid/EDTA (TAE), 1 L bottle</td>
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<tr>
<td>161-0773</td>
<td>50x Tris/Acetic Acid/EDTA (TAE), 5 L cube</td>
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<tr>
<td>161-0433</td>
<td>Ethidium Bromide Solution, 10 mg/ml, 10 ml</td>
</tr>
</tbody>
</table>

References

1. Chu G et al. (1986). Science 234, 1582-1585. CHEF (U.S. patent 5,549,786 issued to Stanford University) is exclusively licensed to Bio-Rad Laboratories, Inc.
3. AFIGE (U.S. patent 5,178,737 issued to University of North Carolina) is co-exclusively licensed to Bio-Rad Laboratories, Inc.
4. CHEF DNA Size Marker, 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
5. CHEF DNA Size Marker, S. pombe, lambda ladder, 0.05–1 Mb, 5 agarose blocks, sufficient for 25–40 plugs
6. CHEF DNA Size Marker, H. wingei, 1–3.1 Mb, 5 agarose blocks, sufficient for 25–40 plugs
7. CHEF DNA Size Marker, S. pombe, 3.5–5.7 Mb, 5 agarose blocks, sufficient for 25–40 plugs
8. CHEF DNA Size Standard, 5 kb ladder, 4.9–120 kb, 20–25 lanes
9. CHEF DNA Size Standard, 8.3–48.5 kb, 125 lanes
10. CHEF DNA Size Standard, lambda ladder, 0.05–1 Mb, 5 agarose blocks, sufficient for 25–40 plugs
11. 10x Tris/Boric Acid/EDTA (TBE), 1 L bottle
12. 10x Tris/Boric Acid/EDTA (TBE), 5 L cube
13. 50x Tris/Acetic Acid/EDTA (TAE), 1 L bottle
14. 50x Tris/Acetic Acid/EDTA (TAE), 5 L cube
15. Ethidium Bromide Solution, 10 mg/ml, 10 ml

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