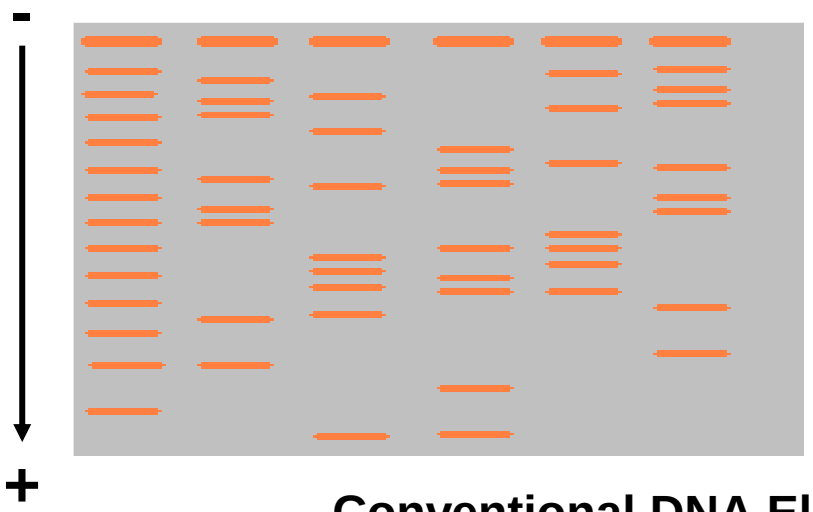
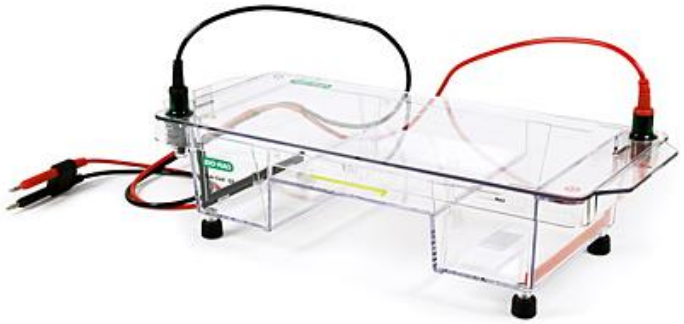
Pulse Field Gel Electrophoresis

BIO-RAD

Electrophoresis of DNA

- DNA molecules are negatively charged.
- DNA migrates in an electric field. As DNA migrates along the electric field, it is retarded by agarose gel.
- The rate at which DNA molecules migrates is dictated by their size: the larger the fragments, the slower they migrate.



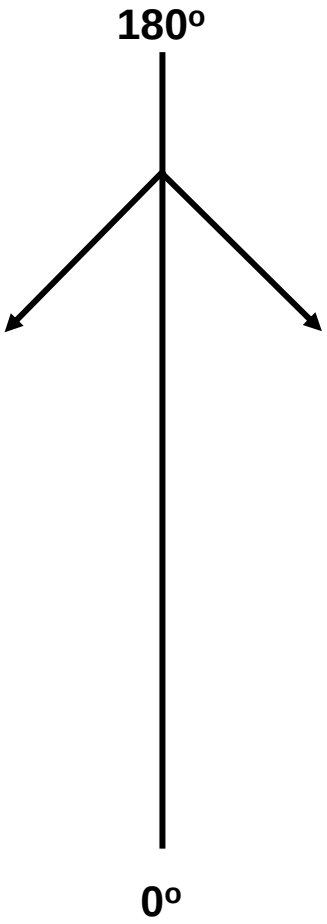


Conventional DNA Electrophoresis

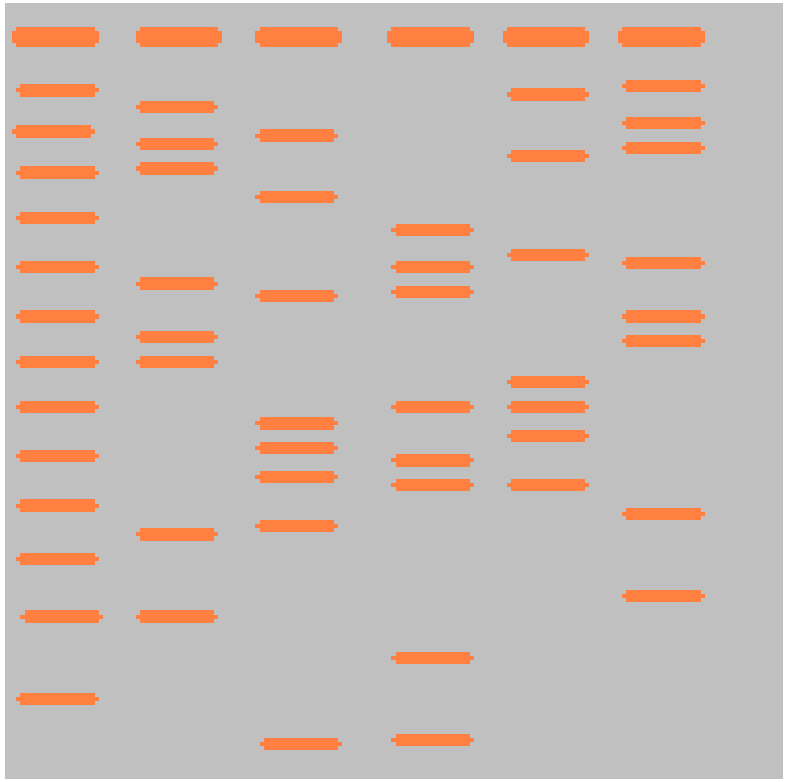
DNA molecules in the size range of 30 Kb and larger will no longer migrate in a conventional agarose gel electrophoresis setting.

Pulse Field Gel Electrophoresis

Directions of pulsing electric field:



The end result:



Factors Affecting DNA Separation

Conventional

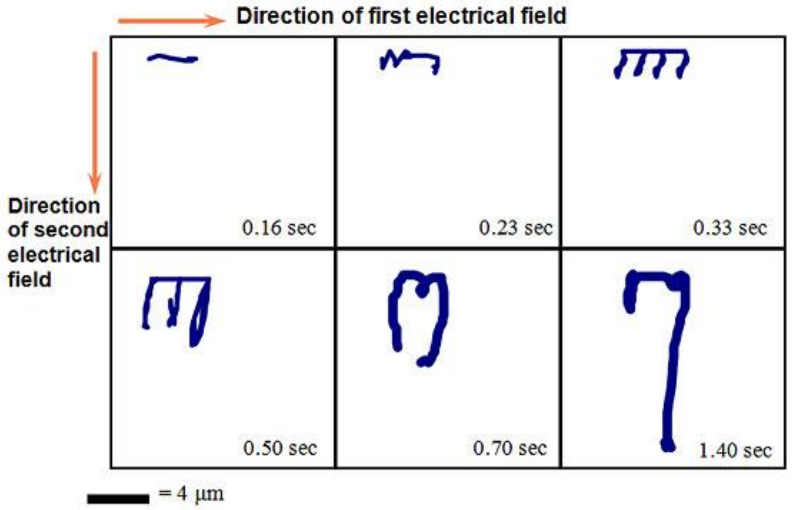
- DNA size
- Agarose – type and concentration
- Buffer – type, concentration and temperature
- Electrophoresis parameters:
  - Voltage
  - Run time

PFGE

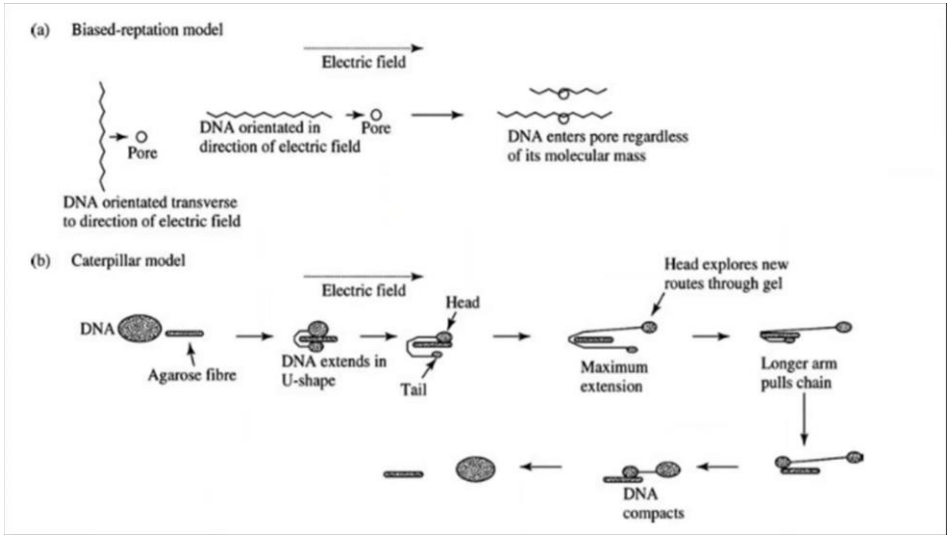
- DNA size
- Agarose – type and concentration
- Buffer – type, concentration and temperature
- Electrophoresis parameters:
  - Voltage gradient
  - Run time
  - Switch time
  - Switch time ramping
  - Pulse angle

How Does PFGE Work?

- PFGE stemmed from the observation that DNA molecules elongate upon application of an electric field and return to an unelongated state upon removal of the electric field; this relaxation rate is dependent on the size of the DNA.

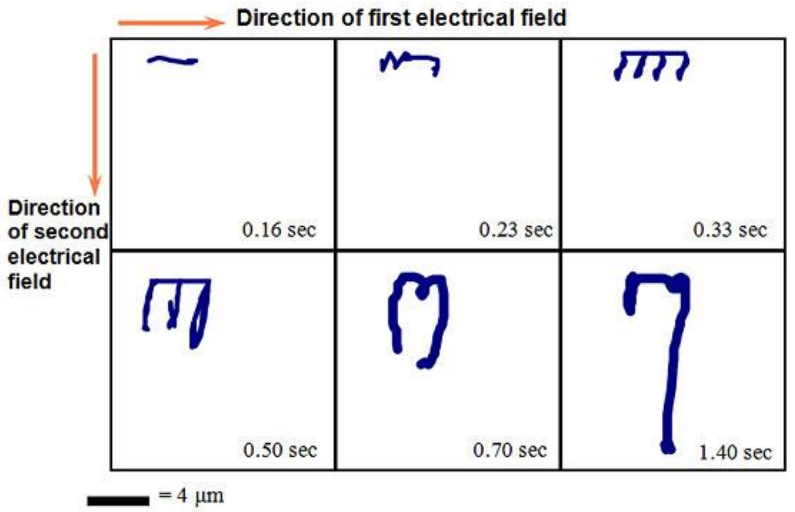


- When the orientation of the electric field is changed during electrophoresis, the DNA molecules must return to their elongated form prior to reorientation, thus affecting the migration rate. This effect can be used to greatly extend the size range over which electrophoretic DNA separations are possible.

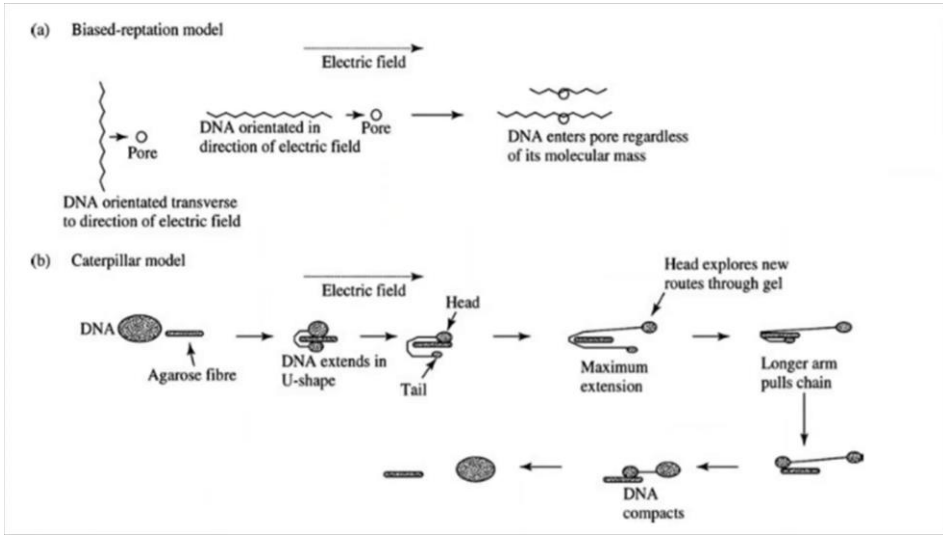


How Does PFGE Work?

- When the electrical field is applied to the gel, the DNA molecules elongate in the direction of the electrical field. The first electrical field is then switched to the second field according to the run specifications. The DNA must change conformation and reorient before it can migrate in the direction of this field.



- As long as the alternating fields are equal with respect to the voltage and pulse duration, the DNA will migrate in a straight path down the gel

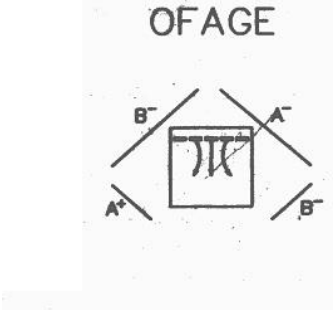


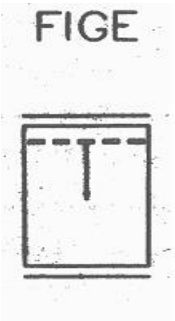
PFGE Techniques

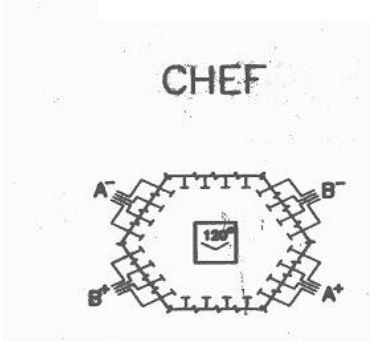
- **OFAGE (1984) - Orthogonal field alternation gel electrophoresis, uses two sets of electrodes. Separation of large DNA but outside lanes are skewed from non-homogenous electrical fields.**

- **FIGE (1986)- Field inversion gel electrophoresis, utilizes polarity reversal of the electrical field. The polarity in the forward direction is three times longer than that in the reverse direction. Lanes are straight but total resolution is limited to approximately 200 Kb.**

- **CHEF (1986)- Contour-clamped homogeneous electric field, uses a homogenous electrical field to produce a uniform electrical environment over the full range of the gel. Resolution extends to at least 3 Mb.**



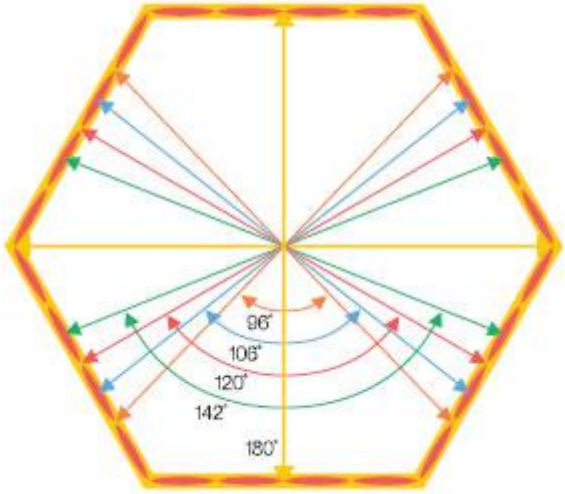


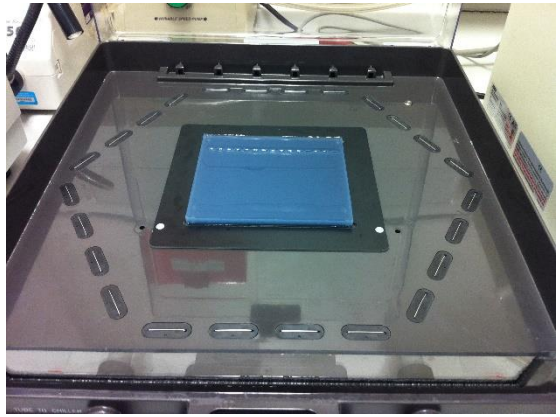


PFGE Techniques

- **PACE (1988)- Programmable autonomously controlled electrophoresis, Improved on CHEF, with the ability to use any angle of separation (0 - 360˚) to within 1˚ and further improves resolution up to 10 Mb.**

- **DR (1988)- Dynamic Regulation is a further improvement on CHEF. Introduces independent circuitry for each electrode and ensures field continuity.**





Bio-Rad PFGE Systems

- **CHEF Mapper XA – 1703672, 1703670**

Electrophoresis Chamber

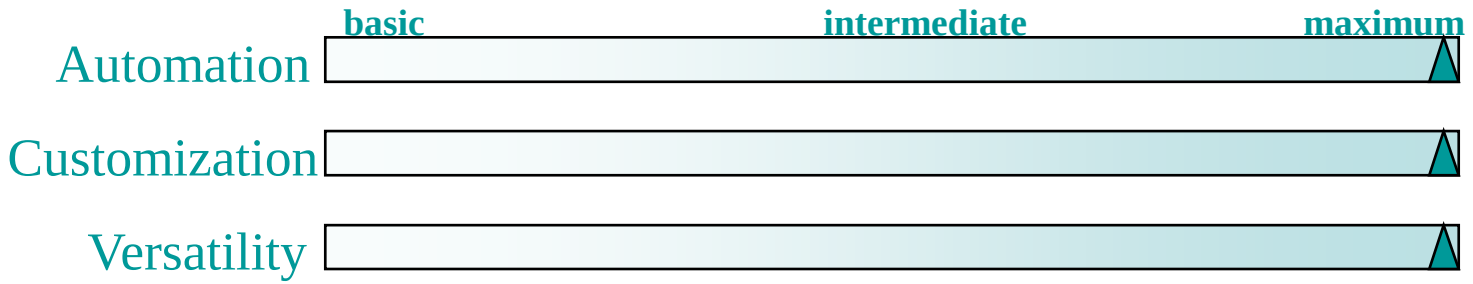
Power Module

Variable Speed Pump

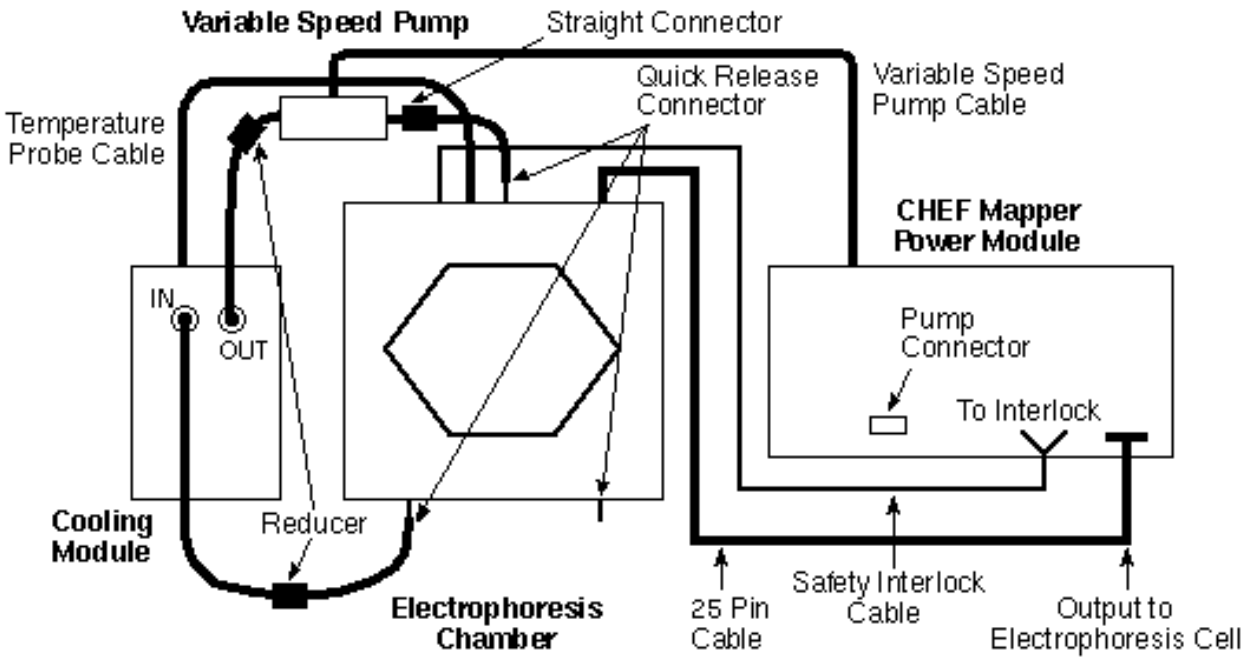
Cooling Module

Features of the CHEF Mapper XA

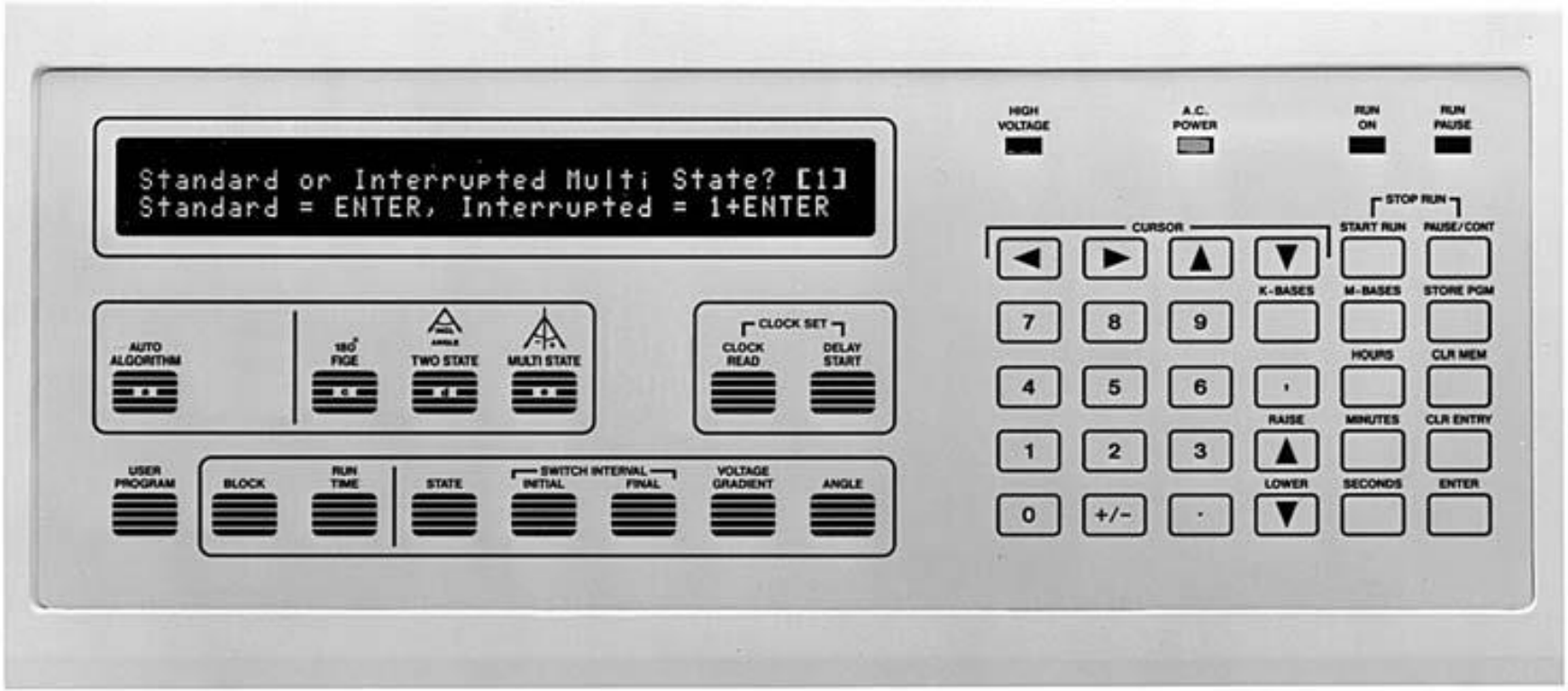
- Resolves DNA fragments in the size range of 100 bp – 10 Mb
- Eight contiguous blocks of programming are possible
- User can specify any angle of electrophoresis from 0° - 360° as well as specify runs in which the angles are asymmetrical
- Stores 99 simple programs or 20 complex programs with up to 8 blocks of programming each
- True nonlinear switch time ramping for maximum flexibility in achieving resolution
- Set clock delay for runs as well as battery backed up RAM for runs that are interrupted
- Secondary pulsing, and multistate flexibility to enhance separations



- **Instrument set up**



- **Instrument parameters**



Auto algorithms used to Determine Run Conditions

Chef Mapper XA can optimize your separations.

The unique built-in algorithm, called the AutoAlgorithm, can automatically select conditions for your separation.

- You supply only the size of the smallest and largest fragments to be separated
- The AutoAlgorithm derives conditions assuming the most common parameters for the gel, for DNA < 2.5 mb, 0.5x TBE at 14ºC, in a 1.0% PFC agarose gel be used. For DNA > 2.5 mb the buffer must be 1.0x TAE at 14ºC, with a gel of 0.8% PFC agarose.

PFGE Sample Preparation

Cultured Cells
Unicellular Organisms

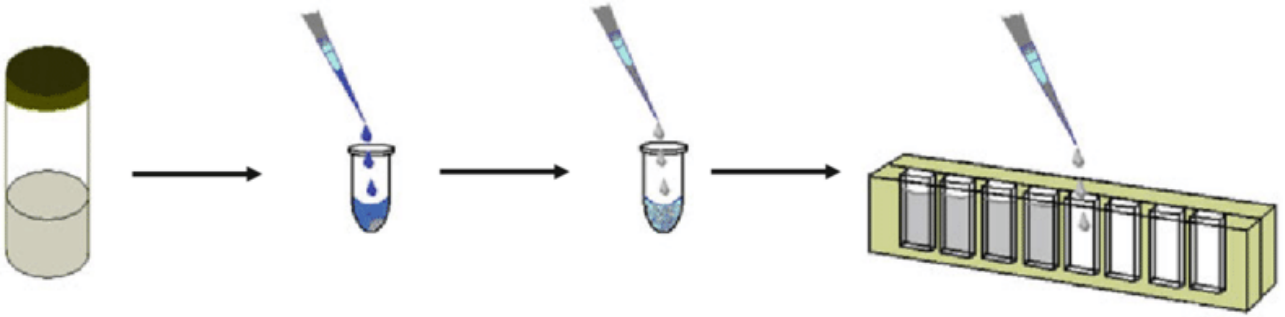
Tissues

Blood

Harvest Cells

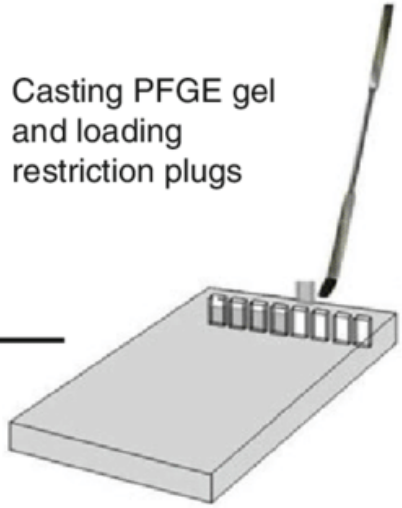
Dissociate

Concentrate White Cells

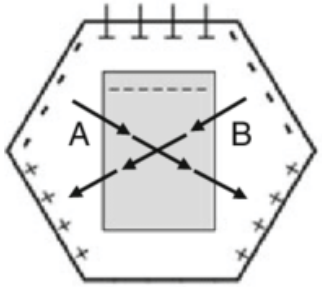


Lysis of bacteria and proteinase K treatment

Restriction enzyme diges



Casting PFGE gel and loading restriction plugs

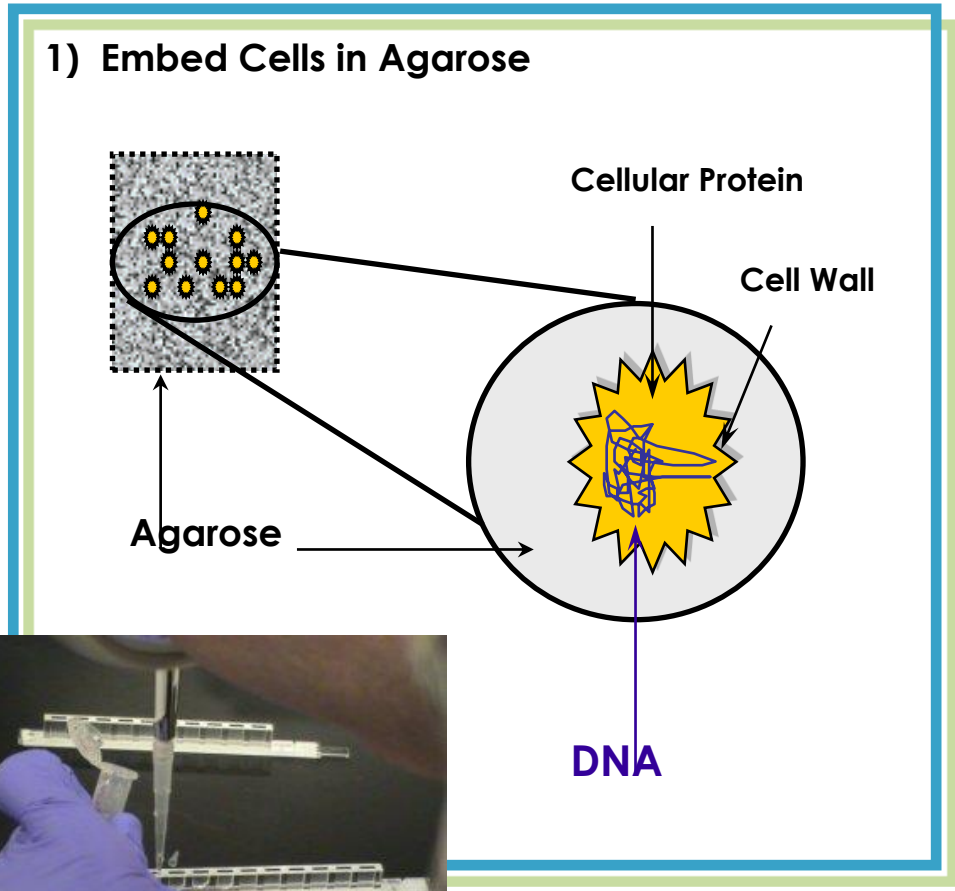


Performing PFGE

PFGE Sample Preparation

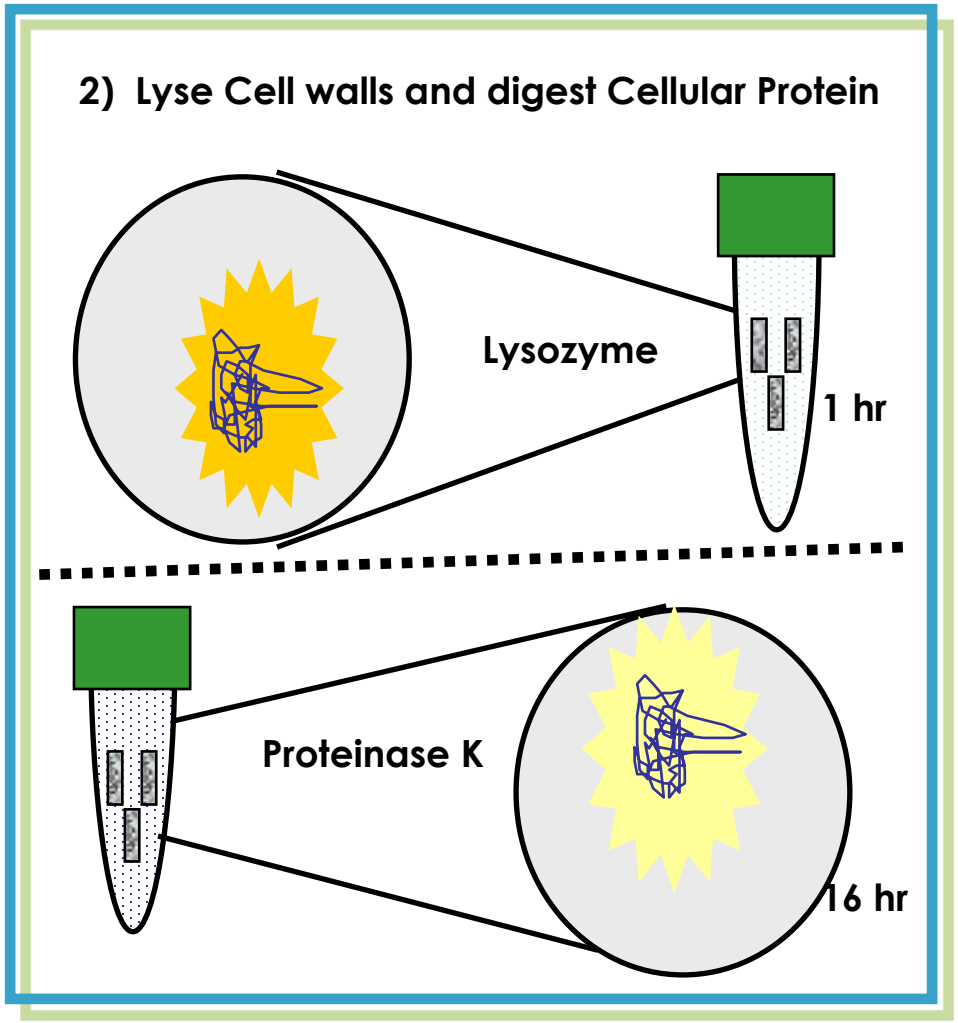
- **Intact cells are embedded in agarose. This prevents shearing of the genomic DNA during the lysis and digestion steps.**

- **The agarose/cell mixture is poured into a small mold (1.5mm x 10mm x 5mm). Once solidified, the "plug" is extruded from the mold.**



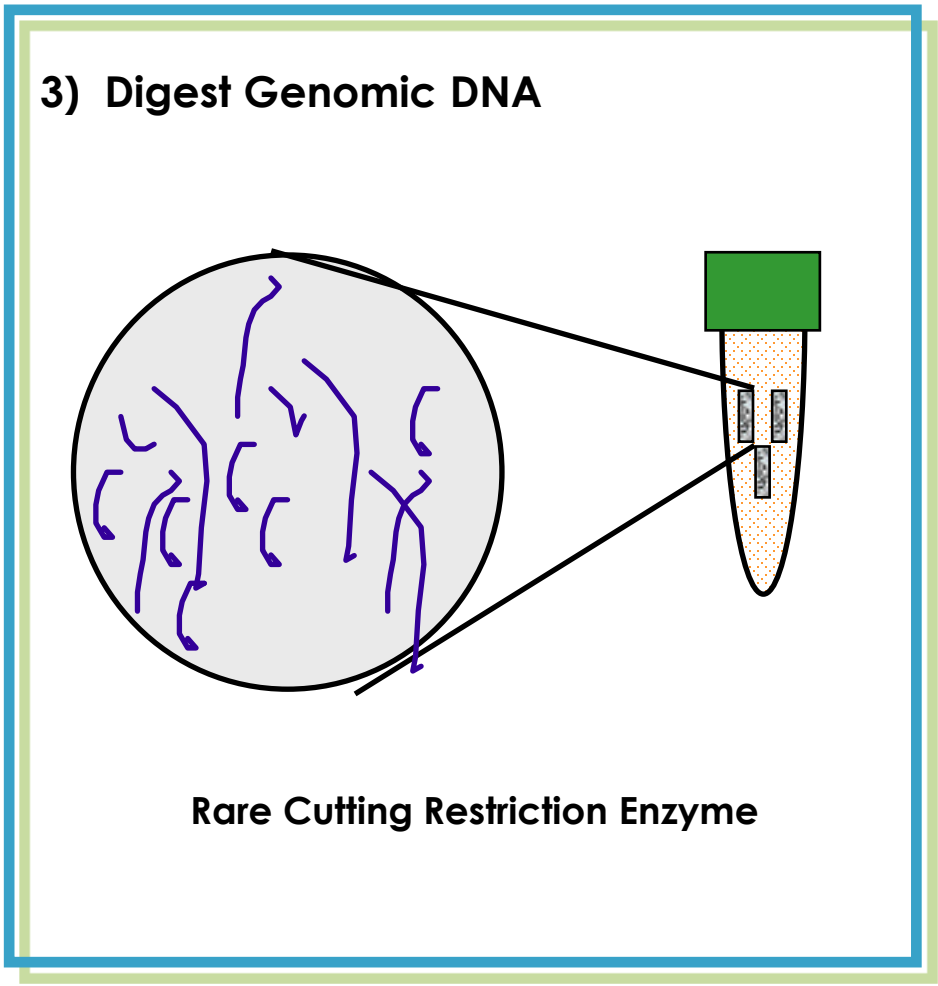
PFGE Sample Preparation

- **The plug preparation and gel electrophoresis can be done in as little as 24 hours or as long as 4 days.**
- **The agarose "plugs" are incubated first in a lysozyme (or appropriate) solution for 1hr. Then they are incubated in Proteinase K solution overnight.**



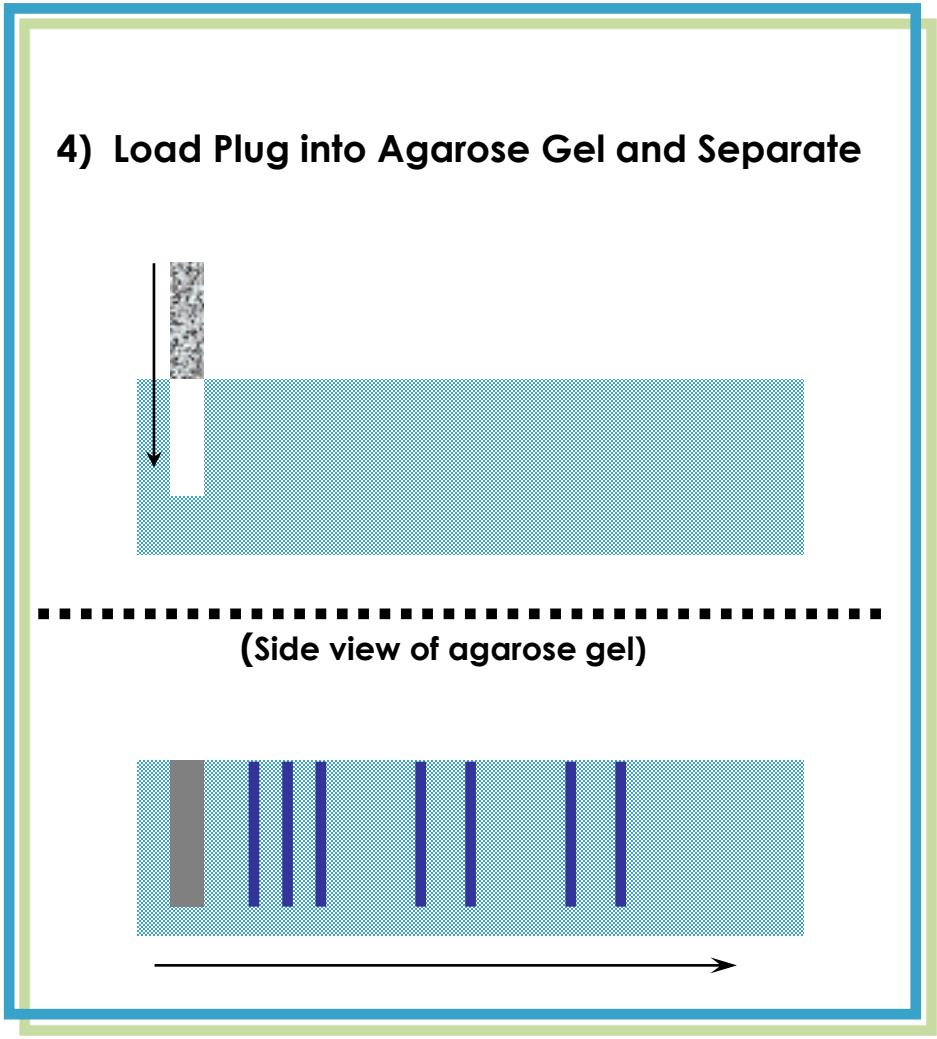
PFGE Sample Preparation

- **The rare cutting enzymes are used to digest the genomic DNA and produce about 15-20 bands that can identify distinct subtypes.**



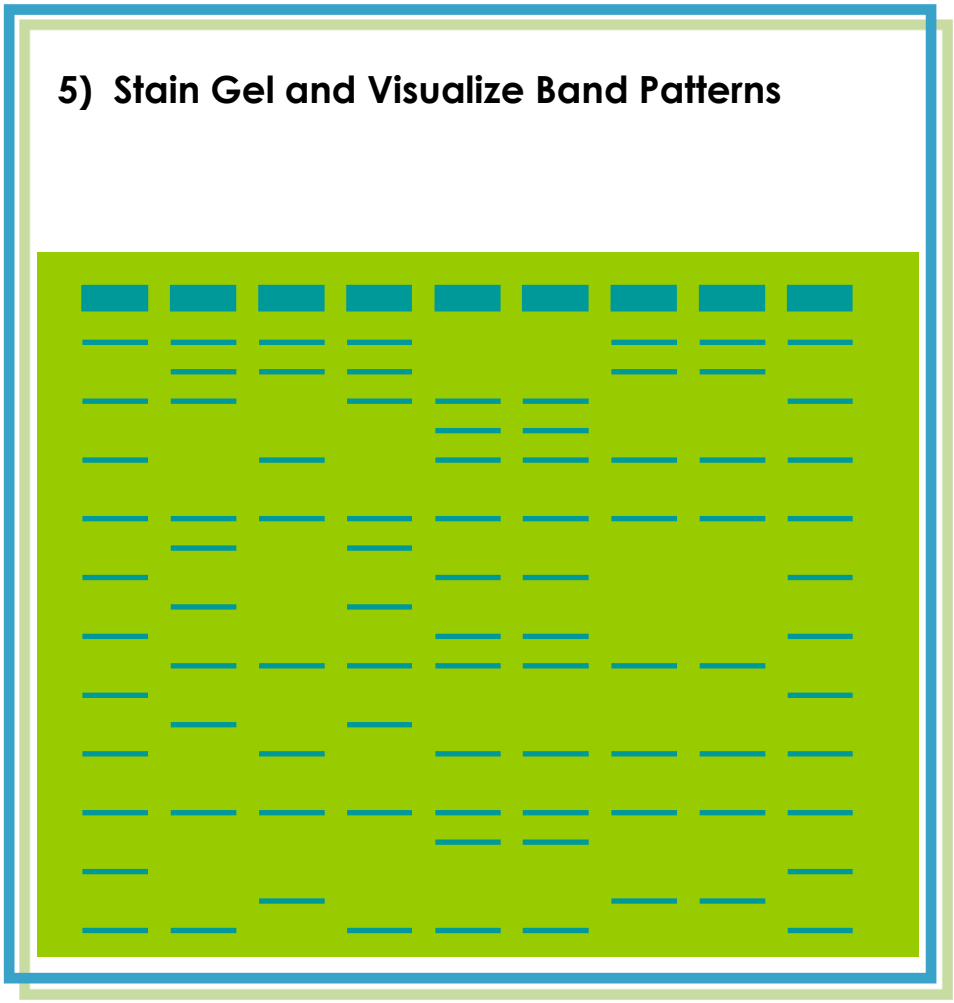
Electrophoresis

- **Samples are loaded to agarose gel and electrophoresis is performed to separate DNA fragments. This is achieved by delivering the electric current in short pulses and alternating the direction of the electrical field. Separations in PFGE usually take 14 - 24 hours.**



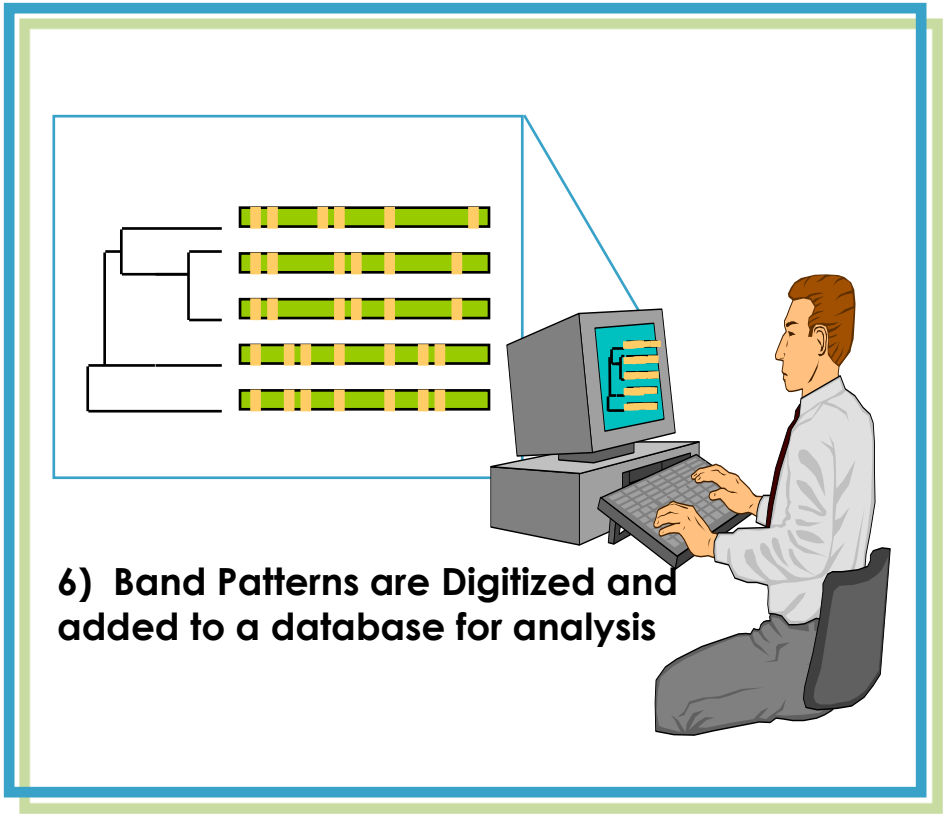
Data Analysis

- **The DNA is visualized by staining the gel with ethidium bromide or fluorescence stain and illuminating the stained gel under a UV or fluorescence light source.**



Data Analysis

- **The gel image can be captured by CCD camera or scanner and the digitized gel is ready for comparison in a band pattern database. These comparisons can be easily shared between labs electronically.**



Important Run Parameters

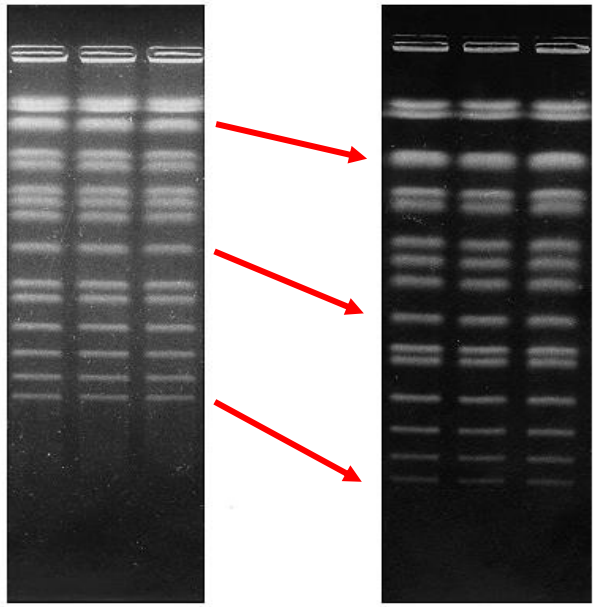
- Buffer
- Agarose
- Switch time
- Switch time ramping
- Voltage gradient (electric field strength)
- Pulse angle
- Run time

Buffer

- **The two buffers most frequently used for PFGE of DNA are 0.5 x TBE (Tris Borate EDTA) and 1.0 x TAE (Tris Acetate EDTA).**
- **In general, the lower the ionic strength of the buffer, the faster the run.**
- **The run time advantage of low ionic strength buffer must be weighed against the limited buffering capacities of dilute buffers and the loss of resolution due to buffer break down.**
- **1X TAE is very useful when separating large DNA fragments (>3 Mb).**
- **0.5X TBE is useful for most separations up to Mb sized DNA and does not need to be changed each time. This is the most commonly used buffer in CHEF gels.**

Buffer

- **The TAE buffer is lower in ionic strength (40 mM buffering agent) vs. 90 mM in the TBE. Fragments of all sizes have a greater velocity, and therefore, migrate farther, in the TAE gel than that of the TBE gel.**

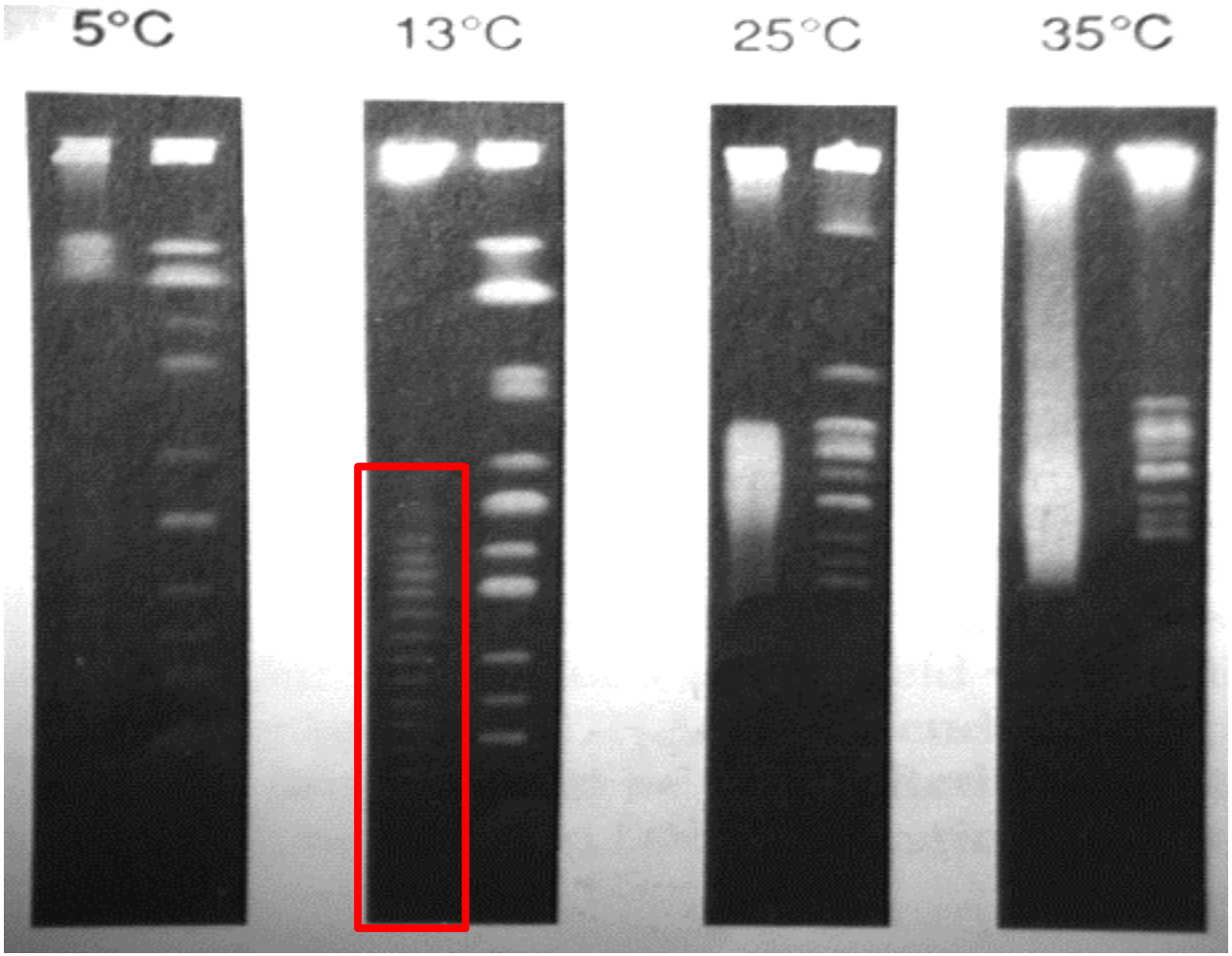


0.5X TBE 1.0X TAE

Buffer

- **Cooling the buffer to between 12ºC and 15ºC is most common and the best compromise between run time and resolution.**
- **The warmer the buffer temperature, the faster the run. The resolution decreases with the temperature increase.**
- **Buffer temperatures at 4ºC will provide the sharpest resolutions but at a greatly increased run time.**
- **Buffer temperatures at room temperature will decrease the run time by about 50% over chilling to 14ºC but the resolution loss will be so great, it might not be worth the time savings.**

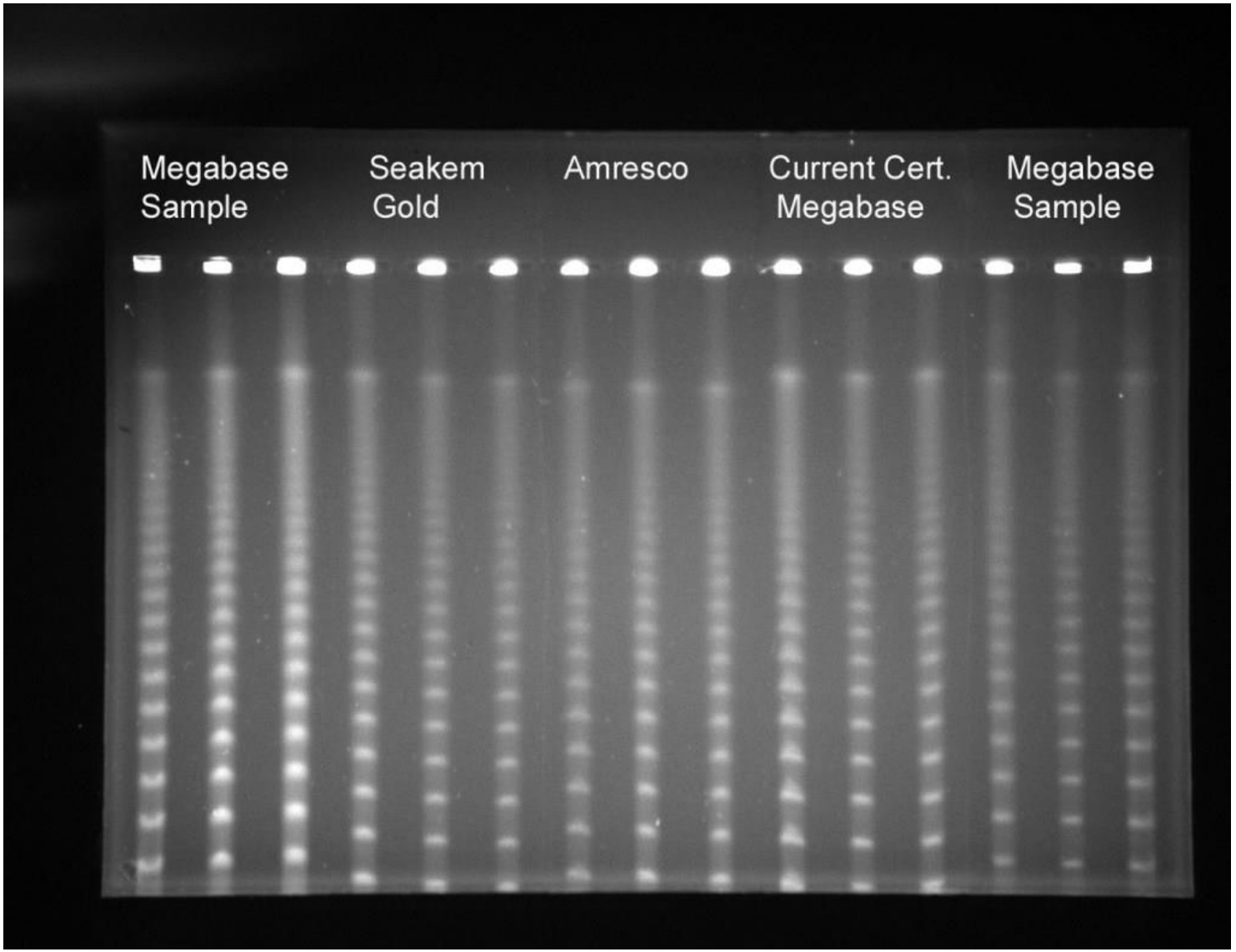
Buffer



Agarose

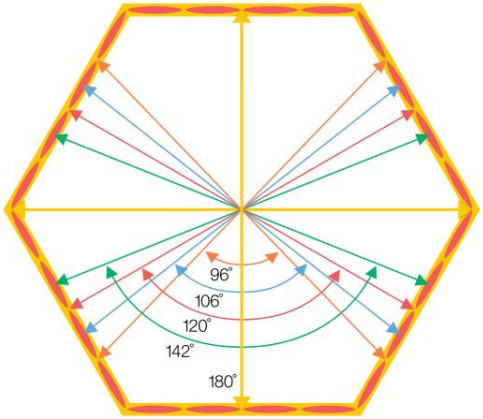
- **Agaroses for PFGE must be very pure, have a high tensile strength, and have a minimum of charged contaminants.**
- **Agaroses are available specifically for use with PFGE that provide superior resolution (Bio-Rad's PFGE Certified) as well as agaoroses designed for decreasing run time while maintaining good resolution (Bio-Rad's Molecular Biology Certified and Chromosomal Grade.)**
- **Generally 0.8% - 1% agarose is ideal for PFGE separations of up to 3 Mb and 0.5% - 0.9% is useful for the range above 3 Mb.**
- **As the agarose concentration decreases, the DNA migrates faster and a larger range of DNA can be resolved on one gel.**
- **However, as gel concentration decreases, the DNA is less well resolved and the gel becomes more difficult to handle.**

Agarose


Megabase Sample
Seakem Gold
Amresco
Current Cert. Megabase
Megabase Sample

Pulse Angle

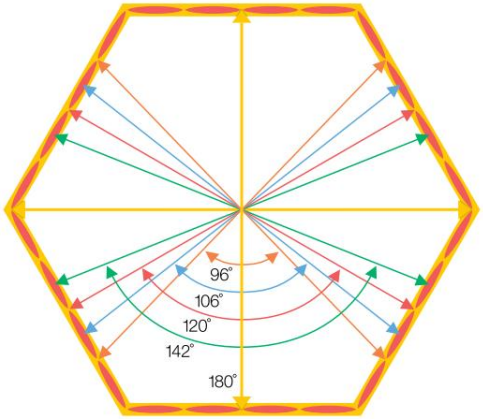
- **CHEF Mapper allows DNA separation with electric field in any direction in the plane of the gel, 0 – 360º.**
- **Most CHEF protocols are optimized for the 120º angle**
- **It has been shown that decreasing the included angle from 120º to 94º increases the velocity of the DNA and saves run time.**



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

Pulse Angle

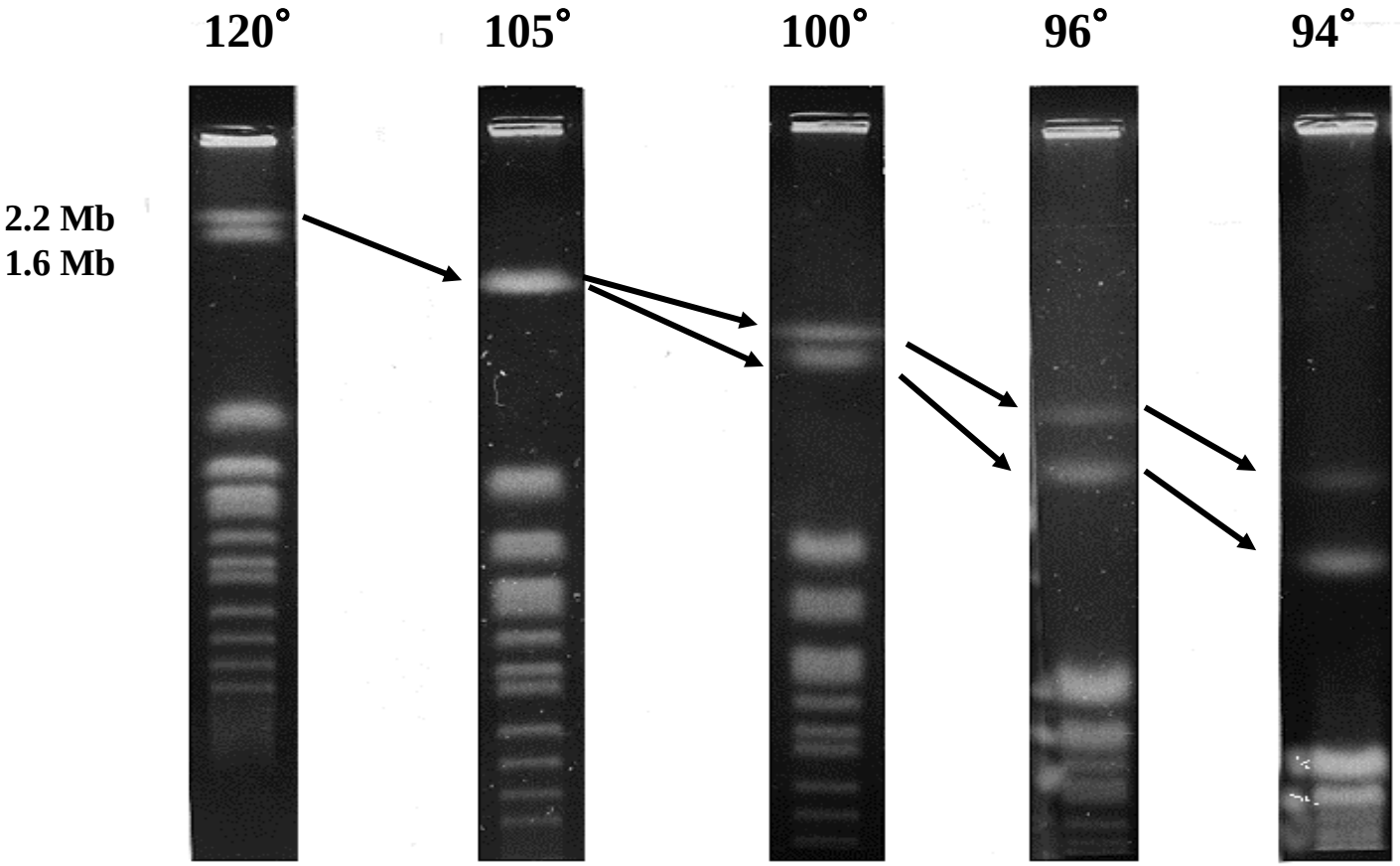
- **Separations of large DNA fragments, i.e. chromosomal DNA, are improved by the use of a smaller included angle (106º).**
- **As the angle is decreased, the resolution of the largest fragments generally improves and the smaller fragments become compressed.**



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

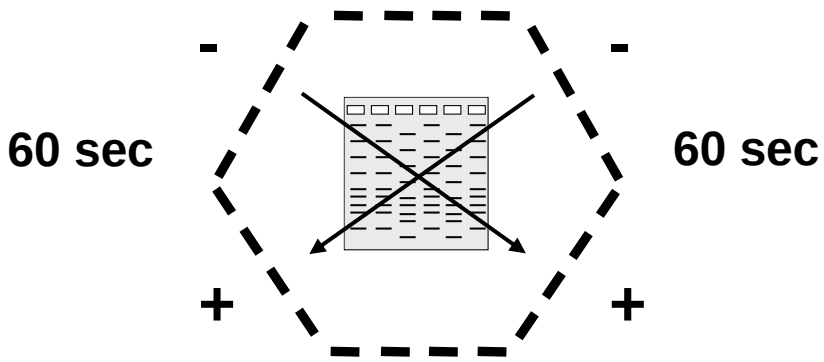
Pulse Angle on Sample Resolution

- **The smaller the angle, the faster the DNA migrates, the better the resolution of large DNA fragments.**



Switch Time

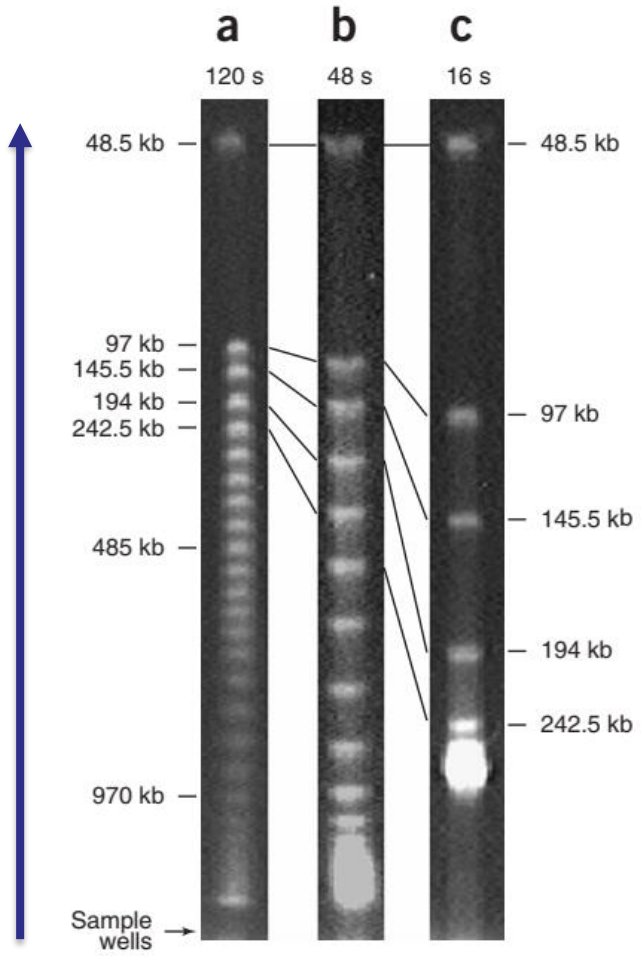
- **The switch time is the length of time the electrical field is pulsed in a single direction. A 60s switch time means that the electrical field will be pulsed in one direction for 60s and then switched to the other direction for 60s.**



- **The switch time is generally shorter for samples with smaller DNA fragments and longer for samples with larger DNA fragments.**

Switch Time on Sample Resolution

- **Longer switch times provides better resolution of large DNA fragments**



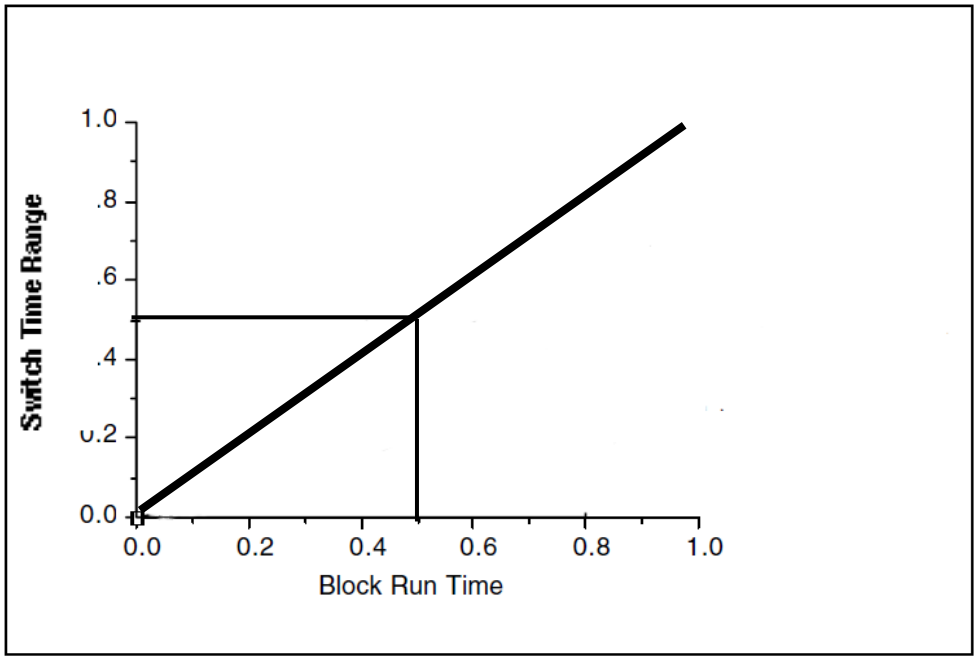
Pulsed-field gel electrophoresis
Jill Herschleb, Gene Ananiev & David C Schwartz

Switch Time Ramping

- **Samples with a wide range of DNA fragments sizes can be resolved by changing the switch time over the course of the run.**
- **This is referred to as Switch Time Ramping because the switching time will "ramp up" from a short switch time to a longer switch time. This increase can be from just a few seconds up to several minutes, depending on the size of the fragments.**
- **The "ramp" of the switch time**
  - **linear, that is increasing in exact increments over the course of the run**
  - **non-linear, so that you can concentrate the switch times on the largest or smallest regions as is necessary to achieve better resolution.**

Linear Switch Time Ramping

- **In linear ramping, the switch times are increasing in exact increments over the course of the run. It means that 50% of total run time has passed when the run reaches the mid-point of the switch time range.**



Non-Linear Switch Time Ramping

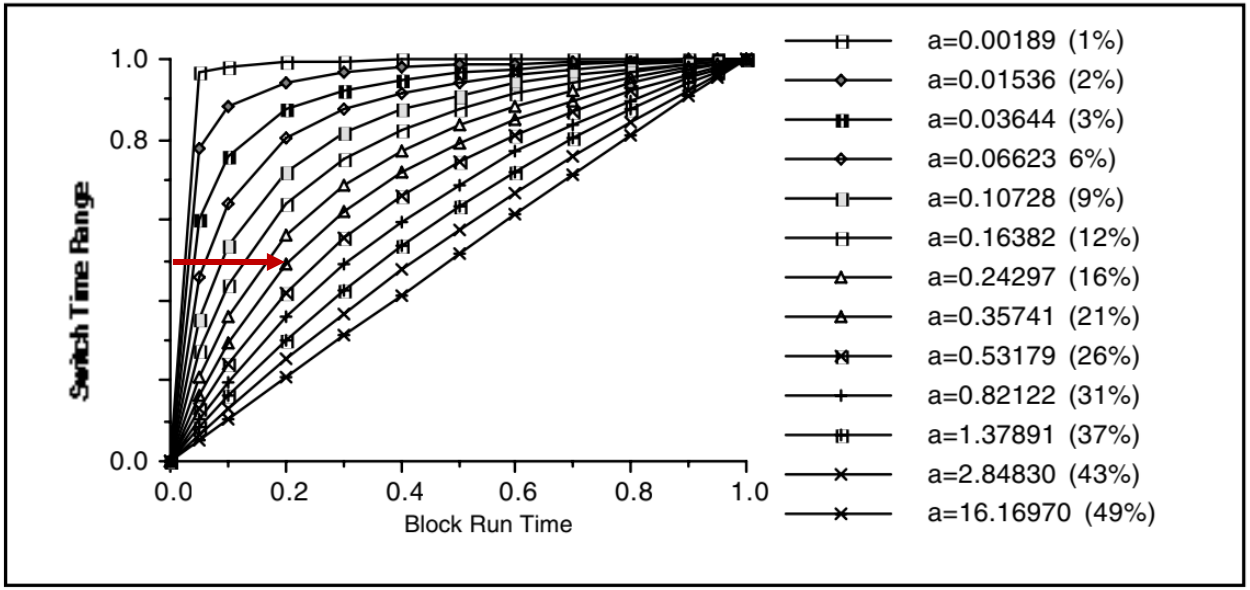
- **The non-linear ramps are described by the amount of the total run time passed (%) when a run reaches the mid-point (50%) of the switch time range.**

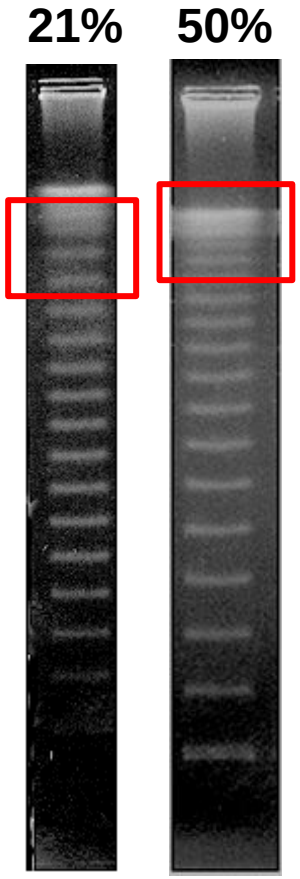
- **A non-linear ramp of less than 50%, results in greater separation of the large range of DNA fragments with a compression of smaller DNA fragments.**

- **A non-linear ramp of greater than 50%, results in greater separation of smaller DNA fragments with a compression of larger DNA fragments.**

Non-Linear Switch Time Ramping

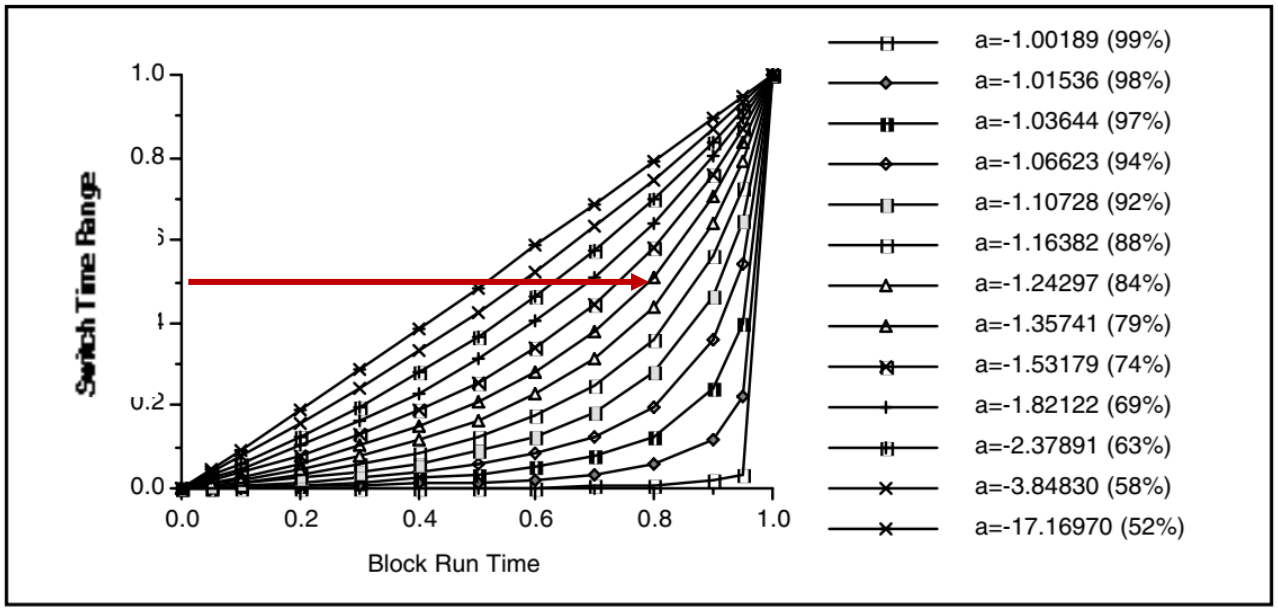
21% non-linear ramping-greater separation of the large range of DNA fragments with a compression of smaller DNA fragments.

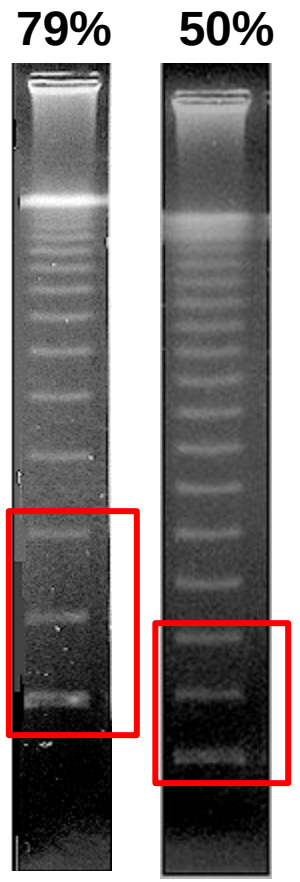




Non-Linear Switch Time Ramping

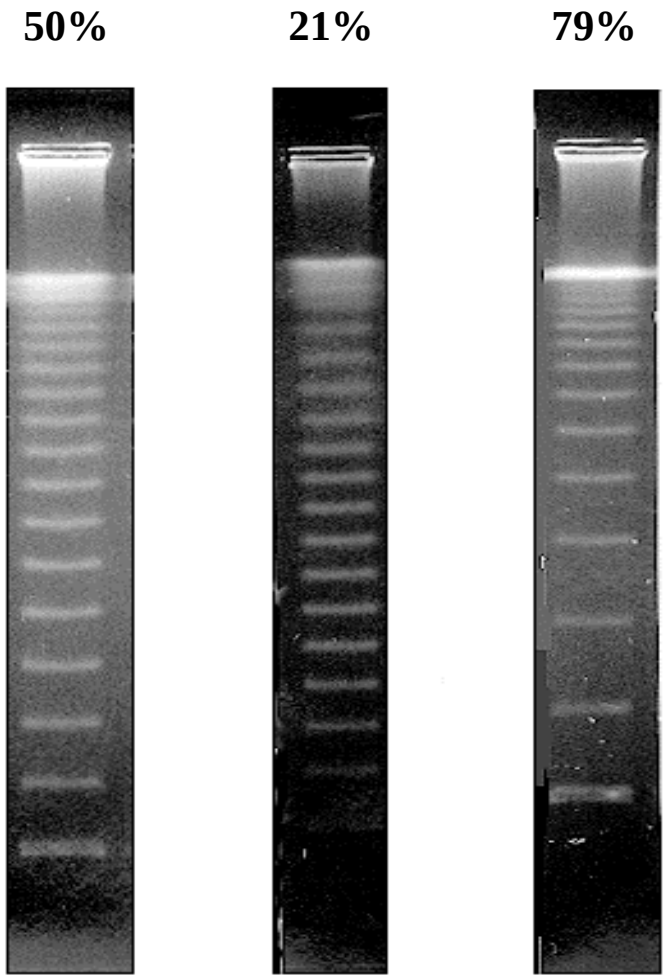
79% non-linear ramping- greater separation of smaller DNA fragments with a compression of larger DNA fragments.





Switch Time Ramping

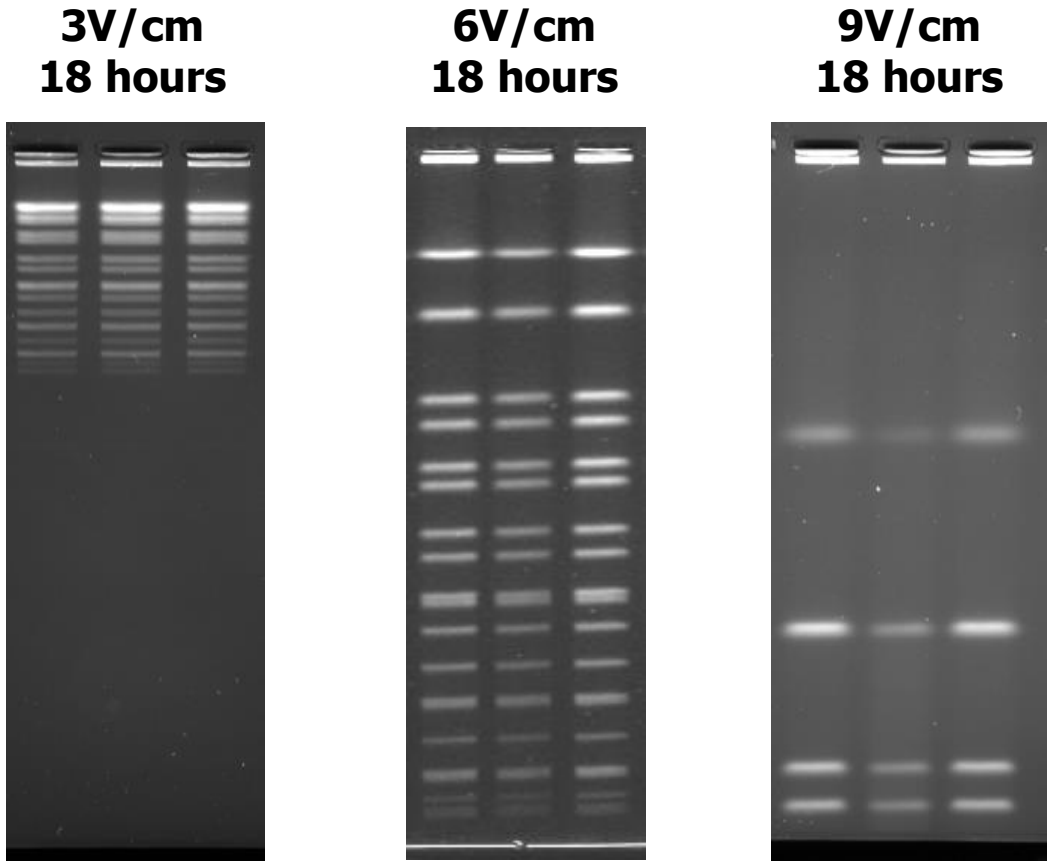
- **A 50% ramping is a linear ramping.**
- **A 21% ramping**
  - **The run takes 21% of the total run time to complete the shorter half of the switch times**
  - **79% of run time to complete the longer half of the switch times**
  - **focuses on resolving larger DNA fragments.**
- **A 79% ramping**
  - **The run takes 79% of the total run time to complete the shorter half of the switch times**
  - **focuses on resolving smaller DNA fragments.**



Voltage Gradient

- **The voltage gradient**
  - **the strength of the electrical field and is represented as V/cm**
  - **total voltage is divided over the distance between opposite electrodes.**
  - **DNA migration increases with increase of voltage and decreased band resolution.**
  - **Distance in a CHEF gel box is approximately 33cm, a 6V /cm run is really about 200V.**
- **Most CHEF protocols are optimized at 6V/cm.**
- **The optimal voltage gradient is dependent on the size of DNA.**
  - **Typically, the best resolution of chromosomal-sized DNA fragments (>3 Mb) is chieved when using low-voltage gradients, 1.5–3 V/cm, combined with a narrow angle of electrophoresis.**
  - **High resolution of DNA fragments up to 250 kb can be achieved by using high-voltage gradients at 9 V/cm. This voltage can be combined with a narrow angle of electrophoresis to resolve samples in very short run times, 4 hr or less.**

Voltage Gradient on Sample Resolution



- **REMEMBER: Voltage alone will not improve resolution: switch times and pulse angle must be changed to produce the best results.**

Run Time

- **Run time is determined by the migration rates of DNA fragments.**
  - **migration rates are affected by switch time, voltage gradient, and pulse angle.**
  - **as the migration rate of the DNA fragments decreases, the run time must be increased to resolve the DNA fragments of interest.**

- **Optimize for the shortest run time that allows clear sample resolution. This will maximize the efficiency of PFGE related experiments.**

PulseNet

- **PulseNet is a national network of public health and food regulatory agency laboratories coordinated by the Centers for Disease Control and Prevention (CDC).**
- **The network consists of: state health departments, local health departments, and federal agencies (CDC, USDA/FSIS, FDA).**
- **PulseNet participants perform standardized molecular subtyping (or “fingerprinting”) of food borne disease-causing bacteria by pulsed-field gel electrophoresis (PFGE).**
- **DNA “fingerprints,” or patterns, are submitted electronically to a dynamic database at the CDC, which allows for rapid comparison of the patterns.**

PulseNet - Role

- **Detect foodborne disease case clusters by PFGE.**
- **Facilitate early identification of common source outbreaks.**
- **Assist epidemiologists in investigating outbreaks:**
  - **Separate outbreak-associated cases from other sporadic cases.**
  - **Assist in rapidly identifying the source of outbreaks.**
  - **Act as a rapid and effective means of communication between public health laboratories.**

PulseNet - Process

Patient Specimen Collection

Specimen

Grow isolated colony

Cell Lysis and Plug Washing

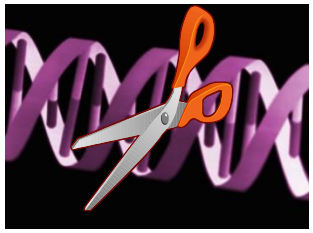
Cells Trapped in Plug

=

Agarose

+

Cell Suspension

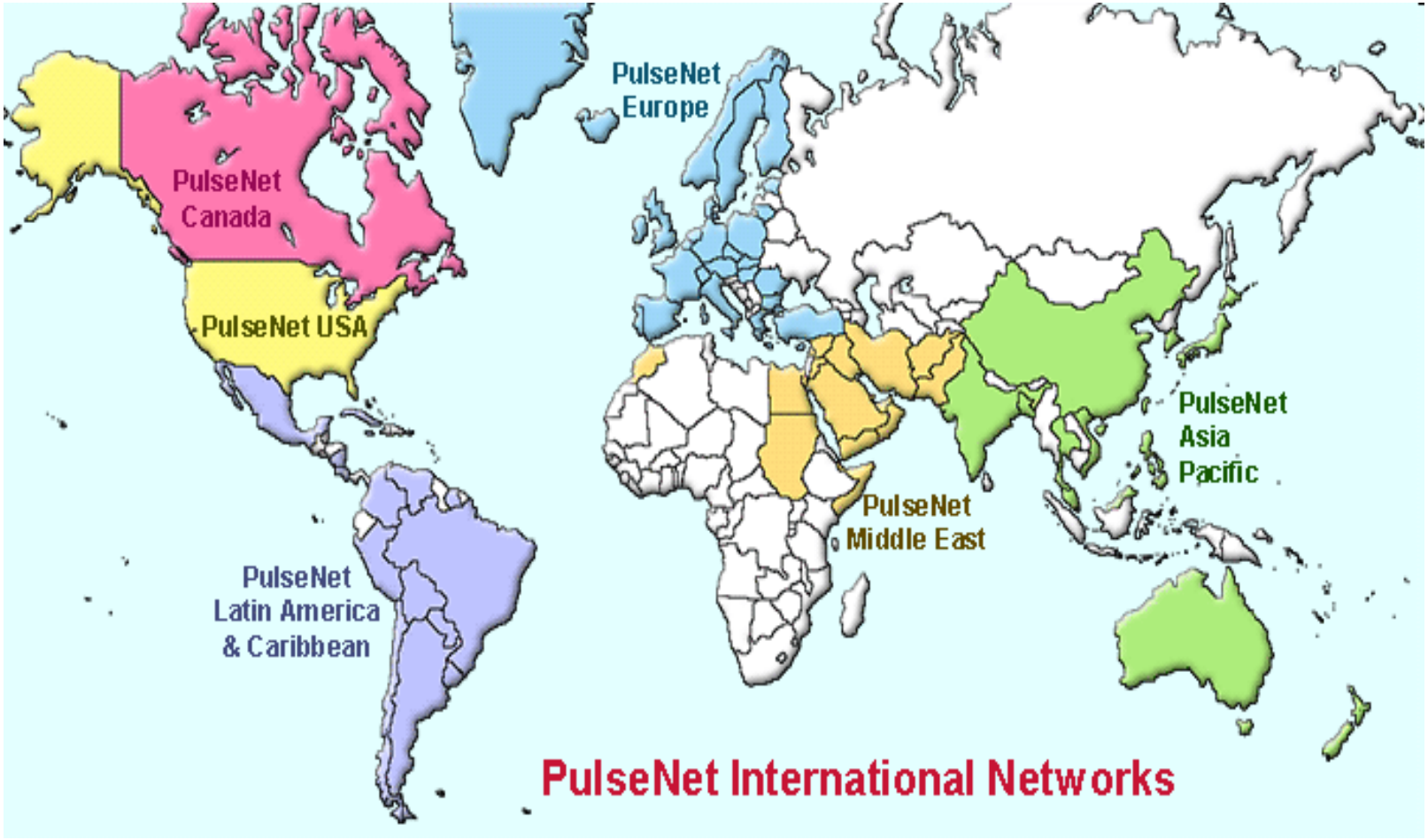


Restriction

Electrophoresis

Imaging

PulseNet - Participants



Preparing and Running a Gel

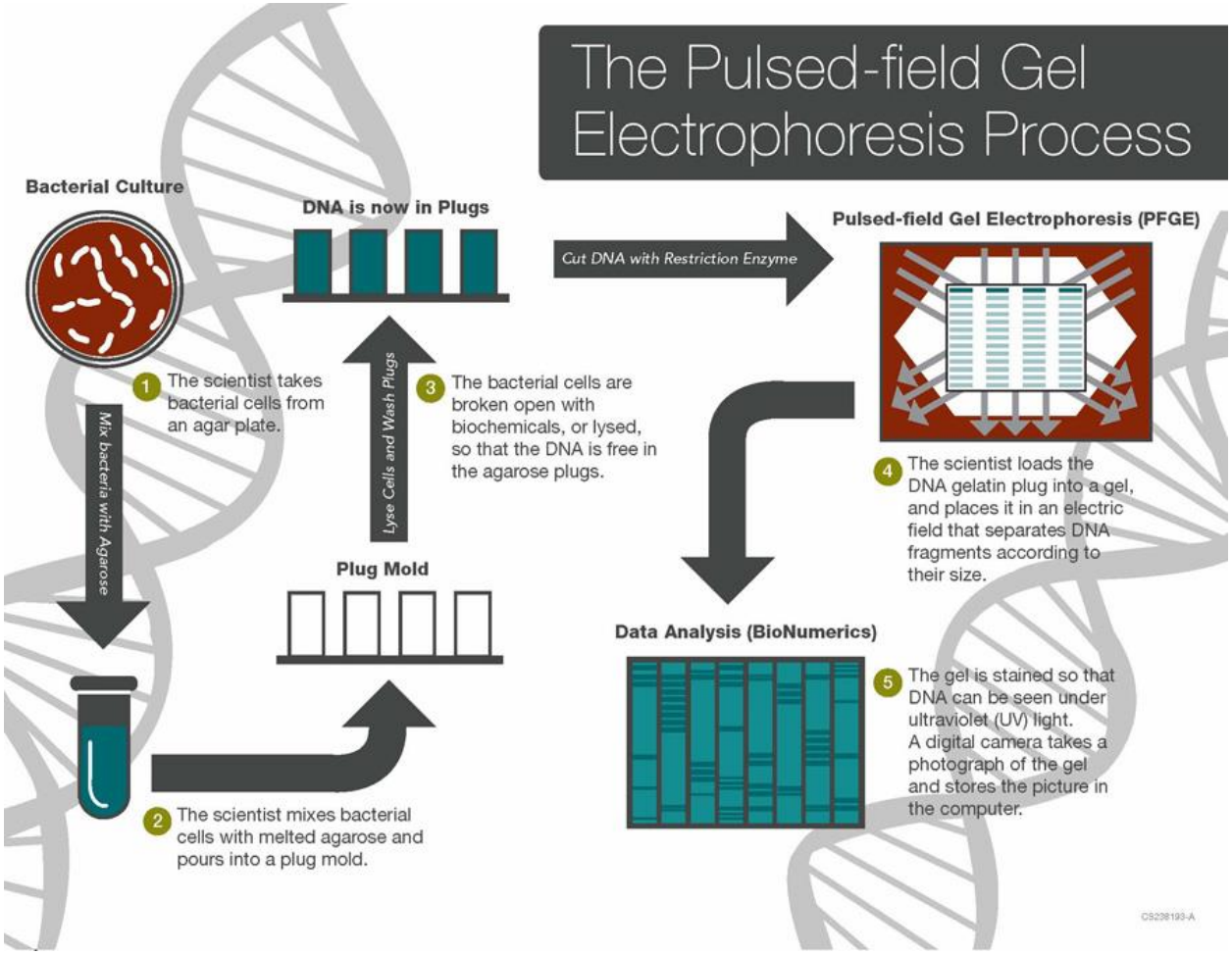
Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive, Hercules, CA 94547

Maintenance and Troubleshooting

Bio-Rad Laboratories, Inc.
2000 Alfred Nobel Drive, Hercules, CA 94547

Recommended Reading

- **Bulletin 1753** — Preparation and Restriction Digestion of *Escherichia coli* Chromosomal DNA in Agarose Plugs for Use in PFGE
- **Bulletin 1589** — Preparation and Separation of Mammalian DNA by Pulsed Field Gel Electrophoresis
- **Bulletin 1659** — Extending the Upper Limits of Pulsed Field DNA Electrophoresis Using Programmed Voltage Gradients
- **Bulletin 1648** — Application of Two-Dimensional Pulsed Field Electrophoresis for Determining Molecular Karyotypes
- **Tutorial** — www.bio-rad.com -> Life Science Research > Electrophoresis > Nucleic Acid Electrophoresis > Pulsed Field Gel Electrophoresis > CHEF Mapper XA System


The Pulsed-field Gel Electrophoresis Process
Bacterial Culture
DNA is now in Plugs
Cut DNA with Restriction Enzyme
Pulsed-field Gel Electrophoresis (PFGE)
1 The scientist takes bacterial cells from an agar plate.
Mix bacteria with Agarose
Lyse Cells and Wash Plugs
3 The bacterial cells are broken open with biochemicals, or lysed, so that the DNA is free in the agarose plugs.
4 The scientist loads the DNA gelatin plug into a gel, and places it in an electric field that separates DNA fragments according to their size.
Plug Mold
Data Analysis (BioNumerics)
5 The gel is stained so that DNA can be seen under ultraviolet (UV) light. A digital camera takes a photograph of the gel and stores the picture in the computer.
2 The scientist mixes bacterial cells with melted agarose and pours into a plug mold.
CS238193-A