Pulsed Field Gel Electrophoresis (PFGE)
Kingdom of Saudi Arabia - 2010
Principle of bacteria typing

Goal
- Clonality determination of a strain (population coming from the same precursor by non sexual multiplication, H.J. Webber, 1903)
- 70% genome homology minimum

CRITERIA
- Typing
  - Percentage of strains that can be typed by the method
- Discriminating
  - Good differentiation between related organisms and non related organisms
- Reproducibility
  - Same result by the same technique on the same strain
- Reading
  - Ease of interpretation

METHOD
- Phenotyping
  - Gene expression analysis
- Genotyping
  - Genome molecular analysis
Phenotyping Methods

BIOTYPE
- Highlight metabolic activity

ANTIBIOTYPE
- Antibiotic resistance profile

SEROTYPE
- Highlight cell wall antigen activity

LYSOTYPE
- Presence of bacteriophages receptors

IMMUNOBLOTTING
- Phenotyping variation related to protein expression

ISO-ENZYMES
- Various structurally related form of an enzyme transforming the same substrate but with differing properties or immunologically characteristics
<table>
<thead>
<tr>
<th>Technique</th>
<th>Typing</th>
<th>Discriminating</th>
<th>Reproducibility</th>
<th>Legibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotype</td>
<td>Excellent</td>
<td>Variable</td>
<td>Variable</td>
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</tr>
<tr>
<td>Antibiogram</td>
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<td>Variable</td>
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<tr>
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<td>Good</td>
<td>Excellent</td>
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<tr>
<td>Lysotype</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Acceptable</td>
<td>Weak</td>
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<tr>
<td>Immunoblotting</td>
<td>Excellent</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Iso-enzymes</td>
<td>Excellent</td>
<td>Good</td>
<td>Excellent</td>
<td>Excellent</td>
</tr>
</tbody>
</table>
Genotyping Methods

**REA (Restriction Enzyme Analysis)**
- Chromosomal DNA digestion – Frequent cuts
- Classical electrophoresis

**Plasmid profile**
- Plasmid DNA migration on classical electrophoresis

**Plasmid restriction**
- Plasmid DNA digestion – Frequent cuts
- Classical electrophoresis

**RFLP (Restriction Fragment Length Polymorphism)**
- Chromosomal DNA digestion – Frequent cuts
- Classical electrophoresis
- Hybridisation with probes (Southern Blot)

**RAPD (Random Amplified Polymorphic DNA) & AP-PCR (Arbitrarily Primed-PCR)**
- PCR amplification with random primers

**Pulsed Field Gel Electrophoresis (PFGE)**
- Chromosomal DNA digestion – Rare cuts (Macrorestriction)
- Pulsed field Gel Electrophoresis
# Genotyping methods

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Typing</th>
<th>Discriminating</th>
<th>Reproducibility</th>
<th>Legibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid Profile</td>
<td>Acceptable</td>
<td>Variable</td>
<td>Acceptable</td>
<td>Acceptable</td>
</tr>
<tr>
<td>Plasmid Restriction</td>
<td>Acceptable</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Genomic Restriction</td>
<td>Good</td>
<td>Good</td>
<td>Weak</td>
<td>Weak</td>
</tr>
<tr>
<td>Pulsotype PFGE</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Good</td>
</tr>
<tr>
<td>RAPD</td>
<td>Excellent</td>
<td>Medium</td>
<td>Medium</td>
<td>Good</td>
</tr>
<tr>
<td>RFLP Southern Blot</td>
<td>Good</td>
<td>Excellent</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>PCR + Restriction</td>
<td>Excellent</td>
<td>Acceptable</td>
<td>Excellent</td>
<td>Excellent</td>
</tr>
</tbody>
</table>
Pulsed Field Gel Electrophoresis (PFGE)

DNA molecules higher than 20-30 kb could not migrate under classical electrophoresis conditions.

DNA fragments of this size are too big to be separated under those conditions.

In 1984, Schwartz and Cantor established a technique with a reorientation of the electrical field to separate big fragments.

By moving the electrical field, during few moments (Electrical pulses), the migration of fragments > 30 kb become possible.

- That is why this technique is called Pulsed Field Gel Electrophoresis (PFGE)
CLASSICAL ELECTROPHORESIS

- Big DNA fragments are block in the upper part of the gel
- Limit
  - Variation of agarose percentage
    - Most agarose gels are made with between 0.7% (good separation or resolution of large 5–10kb DNA fragments) and 2% (good resolution for small 0.2–1kb fragments) agarose dissolved in electrophoresis buffer
    - Big fragments can not be separated

PULSED FIELD GEL ELECTROPHORESIS

- Constant agarose percentage – In general 1 % agarose gel
- Periodical changing of field direction, the various lengths of DNA react to the change at differing rates.
  - That is, larger pieces of DNA will be slower to realign their charge when field direction is changed, while smaller pieces will be quicker
You can see here the “kinking” of the DNA in the gel during the electrophoresis process. As the fields are switched, several kinks emerge. Eventually one kink “wins out” and pulls the whole molecule through the gel.
How does PFGE work?

Conventional electrophoresis uses a single electrical field to cause biomolecules to migrate through a matrix according to the mass:charge ratio present in the biomolecule. Thus the migration distance of this biomolecule is indicative of its mass.

- **DNA in a Native State**: When an electrical field is applied, the DNA stretches out to align with the field.
- **DNA that is less than 25kb**: Will migrate in a conventional agarose gel according to its size.
- **Larger DNA, 25kb - 10+MB in size**: Will migrate according to its size in PFGE by pulsing the DNA through the agarose gel at alternating angles.
Many techniques have evolved to accomplish PFGE

**OFAGE (1984)**
- Only two sets of electrodes - establishes separation of large DNA but outside lanes are skewed from non-homogenous electrical fields.

**FIGE (1986)** - Sequential inversion of the electrical field.
- Polarity is the sense of the migration is 3 time higher than the opposite direction.
- Lanes are straight but resolution is limited to 200Kbp fragments

**TAFE (1986)** - Electrodes are placed on opposite sides of a vertical gel
- The lanes are straight but resolution is limited because the angle of electrophoresis is changing
- This results in compression of the bands

**CHEF (1986)** - Uses a homogenous electrical field to produce a uniform electrical
- Resolution extends to at least 3Mbp

**PACE (1988)** - Improvement on CHEF, includes ability to use any angle (0 - 360°)
- improves resolution upto 10Mbp

**DR (1988)** - Dynamic Regulation is a further improvement on CHEF.
- Introduces independant circuitry for each electrode and ensures field continuity.
Schematics of various PFGE strategies
PFGE is a widely used technique

This method of electrophoresis enabled progress in a whole new group of researchers to make progress in analyzing DNA fragments.

- Cancer Research, the effectiveness of radiation and chemical therapies is measured and evidence of programmed cell death is monitored using PFGE
- Food Safety and Public Health, spread of various bacteria and fungi are tracked using PFGE
- Quality control in the industries like beer and wine, in which yeasts are used for fermentation
- Genome Mapping of many organisms, including human and the tracking of chromosomal rearrangements.
PFGE Analysis step summary

1. **Bacteria suspension**
2. **Melted agarose**
3. **Plug**
4. **Mold**
5. **Lysozyme & PK**
   - Bacteria lysis and PK treatment in the plug itself
6. **Restriction**
   - DNA digestion by enzymes
7. **Plugs insertion into the gel**
8. **Migration & Results interpretation**
<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agarose into the plugs</td>
<td>Cell Suspension / Agarose / Plugs</td>
</tr>
<tr>
<td>Cell Wall destruction</td>
<td>Lysozyme * (or other) / 1Hr / 37°C</td>
</tr>
<tr>
<td>Protein digestion</td>
<td>Proteinase K / Over Night / 50°C / Several washes before restriction</td>
</tr>
<tr>
<td>DNA Restriction</td>
<td>Restriction enzyme / Over Night / 25 or 37°C / 20 - 50 U per insert</td>
</tr>
<tr>
<td>Migration</td>
<td>TBE 0.5X or TAE 1X / 18 – 24 Hrs / 14°C</td>
</tr>
</tbody>
</table>
Bacteria culture (After an over night growth)

- Method standardisation
  - Turbidimetry – depends on the type of spectrophotometer and bacteria
  - Pellet size estimation after centrifugation (10 – 12000 rpm 2 min)

- Cell suspension
  - Cold cell suspension buffer is recommended (+ 4°C)

Plug preparation

- Time limited to avoid agarose polymerisation (Maintain around 50 – 55°C)
  - Possible solidification of the agarose
- Do not forgot to identify the plug molds
- Fill more than the plug capacity
  - Size reduction after solidification
Cell wall lysis depends on the bacteria type

- In general Lysozyme (Gram + bacteria)
  - 1 mg/ml (Final concentration)
  - Adding of lysostaphine (2mg/ml)
- 1h / 37°C
- Washing steps (TE pH 8.0)

Protein digestion
- Proteinase K (PK)
- 16 to 20 h / 50°C
- Plugs, after PK treatment, can be stored in wash buffer at +4°C up to 6 months
Enzymatic Restriction

- Restriction enzymes depend on the genome sequence
- Several washes after PK treatment
- Restriction enzyme storage is a key point (-20°C)
  - Some of enzymes have an activity loss above 0°C
- Be careful to the temperature – In general 37°C
  - 25°C for SMA I
- 16 h à 37°C (or different temperature if required)
  - A 4 hour digestion step gives in general good results
- Plugs, after enzymatic restriction, can be stored in wash buffer at +4°C up to 1 month
The choice of the restriction enzyme is based on the GC% – The goal is to have a limited number of bands (10 – 20 bands).

<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sma I</td>
<td>Enterococcus ■ Staphylococcus ■ Listeria ■ Streptococcus ■ Clostridium ■ Campylobacter</td>
</tr>
<tr>
<td>Not I</td>
<td>Escherichia coli ■ Helicobacter pylori ■ Neisseria meningitidis ■ Bacteroides ■ Vibrio cholerae</td>
</tr>
<tr>
<td>Spe I</td>
<td>Pseudomonas ■ Neisseria gonorrhoeae ■ Burkholderia cepacia ■ Serratia marcescens ■ Enterobacter</td>
</tr>
<tr>
<td>None</td>
<td>Candida</td>
</tr>
<tr>
<td>Sfi I</td>
<td>Legionella pneumophila ■ Proteus mirabilis ■ Corynebacterium diphtheriae</td>
</tr>
<tr>
<td>Xba I</td>
<td>E. coli O157: H7 ■ Salmonella ■ Klebsiella ■ Shigella ■ Yersinia pestis ■ Mycobacterium</td>
</tr>
</tbody>
</table>
### PFGE – Program examples

<table>
<thead>
<tr>
<th>Strain</th>
<th>Switch time (sec)</th>
<th>ADN (kb)</th>
<th>Angle (°)</th>
<th>Gradient (V/cm)</th>
<th>Ramping</th>
<th>Duration (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>5,3</td>
<td>34,9</td>
<td>50-500</td>
<td>120</td>
<td>6,0</td>
<td>1 ■ Linéaire</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>5,3</td>
<td>49,9</td>
<td>50-700</td>
<td>120</td>
<td>6,0</td>
<td>1 ■ Linéaire</td>
</tr>
<tr>
<td><strong>Enterobacter</strong></td>
<td>5,3</td>
<td>34,9</td>
<td>25-300</td>
<td>120</td>
<td>6,0</td>
<td>1 ■ Linéaire</td>
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<tr>
<td><strong>Candida</strong></td>
<td>90</td>
<td>325</td>
<td>1000-3500</td>
<td>120</td>
<td>6,0</td>
<td>1 ■ Linéaire</td>
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<tr>
<td><strong>Staphylococcus</strong></td>
<td>5,3</td>
<td>66</td>
<td>50-500</td>
<td>120</td>
<td>6,0</td>
<td>0,357 ■ Non linéaire</td>
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<tr>
<td><strong>E. coli O157:H7</strong></td>
<td>2,2</td>
<td>54,2</td>
<td>50-500</td>
<td>120</td>
<td>6,0</td>
<td>1 ■ Linéaire</td>
</tr>
<tr>
<td><strong>Screening</strong></td>
<td>1,0</td>
<td>25,0</td>
<td>/</td>
<td>120</td>
<td>6,0</td>
<td>1 ■ Linéaire</td>
</tr>
<tr>
<td>/</td>
<td>0,1</td>
<td>2,0</td>
<td>1-50</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
</tr>
<tr>
<td>/</td>
<td>1,0</td>
<td>3,0</td>
<td>1-100</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
</tr>
<tr>
<td>/</td>
<td>1,0</td>
<td>8,0</td>
<td>1-200</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
</tr>
<tr>
<td>/</td>
<td>1,0</td>
<td>12,0</td>
<td>25-250</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
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<tr>
<td><strong>C. diphtheriae</strong></td>
<td>1,0</td>
<td>17,0</td>
<td>25-300</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
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<tr>
<td><strong>Mycobacterium</strong></td>
<td>1,0</td>
<td>23,0</td>
<td>25-400</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
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<tr>
<td><strong>Bordetella pertussis</strong></td>
<td>5,3</td>
<td>34,9</td>
<td>50-500</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
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<tr>
<td><strong>Salmonella KS</strong></td>
<td>5,3</td>
<td>42,0</td>
<td>50-600</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
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<tr>
<td><strong>Helicobacter pylori</strong></td>
<td>5,3</td>
<td>60,0</td>
<td>50-900</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
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<tr>
<td><strong>Salmonella &amp; H. pylori</strong></td>
<td>5,3</td>
<td>66,0</td>
<td>50-1000</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
</tr>
<tr>
<td><strong>Candida glabrata</strong></td>
<td>5,3</td>
<td>120,0</td>
<td>50-2000</td>
<td>120</td>
<td>6,0</td>
<td>21% ■ Non linéaire</td>
</tr>
<tr>
<td><strong>Candida glabrata</strong></td>
<td>120,0</td>
<td>600,0</td>
<td>1000-3000</td>
<td>106</td>
<td>3,5</td>
<td>1 ■ Linéaire</td>
</tr>
<tr>
<td><strong>Candida krusei</strong></td>
<td>600,0</td>
<td>1800,0</td>
<td>1000-4500</td>
<td>106</td>
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<tr>
<td><strong>E. coli O157:H7 CDC</strong></td>
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<td>54,2</td>
<td>/</td>
<td>120</td>
<td>6,0</td>
<td>1 ■ Linéaire</td>
</tr>
</tbody>
</table>
PFGE Result Analysis

Strain clonality determination

- **Result under statistical form**
  - No international consensus
  - Clonality higher or equal to 70-80% between the strains

- **Results under graphical form**
  - Dendograms creation (Phylogenetic trees)

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of genetic differences compared with outbreak strain</th>
<th>Epidemiologic interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genetic event</td>
<td>Fragments</td>
</tr>
<tr>
<td>Indistinguishable</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Closely related</td>
<td>1</td>
<td>2 - 3</td>
</tr>
<tr>
<td>Possibly related</td>
<td>2</td>
<td>4 - 6</td>
</tr>
<tr>
<td>Different</td>
<td>≥ 3</td>
<td>≥ 7</td>
</tr>
</tbody>
</table>
Guidelines to interpreting PFGE results

GUEST COMMENTARY

Interpreting Chromosomal DNA Restriction Patterns Produced by Pulsed-Field Gel Electrophoresis: Criteria for Bacterial Strain Typing

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Vol. 33, No. 9

JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1995, p. 2233–2239
0095-1137/95/$04.00+0
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PFGE Key parameters

- Pulse / Reorientation Angle
- Switch Time and Switch Time Ramping
- Voltage Gradient
- Buffer Type, Concentration, and Temperature
- Agarose Type and Concentration
- Run Time
Angle is an important factor in achieving optimal resolution.

Separations of larger sizes of DNA, i.e., chromosomal, are greatly improved by the use of a smaller included angle (106°).

Using a smaller included angle for large fragments of DNA (over 1Mb in size) results in a 50% savings in run time.

As the angle is decreased, the resolution of the largest fragments generally improves and the smaller fragments become crowded.

Most CHEF protocols are optimized for the 120° angle.
Effects of switch time on sample resolution

As the switch time increases, the larger DNA fragments move farther into the gel during the same time period.

The brackets indicate the same region of the lane for each gel but the sizes of the fragments in this region get larger as the switch time increases.

As you can see Switch Time modification is the most powerful parameter in PFGE.
Switch Time Ramping and Sample Resolution

Samples with a large range of DNA fragments sizes, for example the *S.cerevisiae* chromosomes, 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 one.

The “ramp” of the switch time can be:

- **Linear**, that is increasing in exact increments over the course of the run; or

- **Non-linear**, so that you can concentrate the switch times on the largest or smallest regions as is necessary to achieve better resolution.
Ramping: Linear or Non Linear

**Linear**
- 50% of the migration time, *Switch time* is equal to 50%

**Non Linear**
- Below 50% for a better resolution of high molecular weight fragments and with a compression of low molecular weight fragments
- Above 50% for a better resolution of low molecular weight fragments and with a compression of high molecular weight fragments
Ramping: Linear vs Non-Linear

The non-linear ramps are described by the amount of the total run time passed (%) when you hit the mid-point of the range of switch times.

This means that a 50% ramp is Linear. A 21% ramp means that only 21% of the total run time has elapsed by the time you are halfway through the switch times. In practice, this means you are focused on the larger fragments.

A 79% ramp would have the opposite effect, focusing time on the smallest fragments.
The voltage gradient describes the strength of the electrical field and is represented as V/cm where the total voltage is divided over the distance between two electrodes.

Since that distance in a CHEF gel box is approximately 33cm, a 200V run is really about 6V/cm.

Typically the best resolution of chromosomal sized DNA fragments (> 3 Mbp) is achieved when using low voltage gradients, 1.5-3 V/cm combined with a narrow angle of electrophoresis.

High resolution of DNA fragments up to 250kb can be achieved by using high voltage gradients, 9V/cm. This voltage can be combined with a narrow angle of electrophoresis to resolve samples in very short run times, 4 hours or less.

Most CHEF protocols are optimized at 6V/cm
Buffer concentration

- In general, the lower the ionic strength of the buffer …
  - Faster is the migration

Buffer Type

- **TBE 0,5X (Tris Borate EDTA)**
  - [90 nM]
  - To separate fragments up to megabases order
  - No need to change buffer after every run
  - *This is the most commonly used buffer in CHEF gels.*

- **TAE 1,0X (Tris Acetate EDTA)**
  - [40 nM] Faster migration
  - very useful when separating MB sized DNA fragments (>3Mbp).bp
Effects of Buffer Temperature on sample resolution

Electrophoresis Buffer can be chilled
- Up to 4°C
- Or not at all

Temperature effects on resolution:
- Higher the temperature is, faster is the migration
- The resolution decreases with the temperature increase

A Room Temperature migration decrease by half the migration time
- But with a loss of resolution – No time saving

Buffer chilled at + 4°C gives the best results
- Significant increase of the migration time

The most common is to chilled the buffer between 12 to 15°C
- Best compromise between resolution and migration time
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Advises & Troubleshooting

PFGE is a reliable technique but needs some optimization at several steps

- Plug preparation
- Lysis step
- Restriction step
- Running conditions

To understand and optimize a result issue
It is important to « standardize » the steps of this technique
Advises & Troubleshooting

It is advise to run on the gel a reference strain of the same species of the studied bacteria.

Control Strain only submitted to the restriction step
- Helps to troubleshoot – If needed

3 Possibles scenarios

- No band at all on all the tracks of the gel (Samples /Control Strain / MW)
- No band for all the samples and the control strain but good result for the MW
- No band for all the samples but good result for the control strain and the MW
### Advises & Troubleshooting

<table>
<thead>
<tr>
<th>Results</th>
<th>No band at all – All the tracks (Sample + Control Strain+ MW)</th>
<th>No band for Samples &amp; Control strain</th>
<th>No band for Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Good results for MW</td>
<td>Good Results for Control strain and MW</td>
<td></td>
</tr>
<tr>
<td>Conclusion</td>
<td>Sample preparation</td>
<td>Sample preparation</td>
<td>Sample preparation</td>
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<tr>
<td></td>
<td>Lysis</td>
<td>Lysis</td>
<td>Lysis</td>
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<tr>
<td></td>
<td>Restriction</td>
<td>Restriction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Running conditions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Conclusion

PFGE is a reliable technique that needs some attention on the following steps:

Regarding the type of bacteria
Reagent change (Eg Restriction Enzyme)
Adding a Control Strain
Work with sterile material (For all the steps)
Avoid powdered gloves
The quality of water for the buffer preparation (Ultrapure sterile water)
PFGE – Bio-Rad Offer

- Instruments
- Reagents
- Softwares
- Expertise

Pulsed Field Gel Electrophoresis

Map It Out
## CHEF Systems Selection Guide

<table>
<thead>
<tr>
<th>Feature</th>
<th>CHEF Mapper XA</th>
<th>CHEF-DR® III</th>
<th>CHEF-DR II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragment size</td>
<td>100 bp–10 Mb</td>
<td>100 bp–10 Mb</td>
<td>5 kb–6 Mb</td>
</tr>
<tr>
<td>Optimal separation size range</td>
<td>100 bp–10 Mb</td>
<td>100 bp–6 Mb</td>
<td>100 kb–2 Mb</td>
</tr>
<tr>
<td>Auto-algorithm and interactive algorithm</td>
<td>•</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Program storage</td>
<td>20 complex programs</td>
<td>Last program run</td>
<td>–</td>
</tr>
<tr>
<td>Programming blocks of run conditions</td>
<td>8 blocks</td>
<td>3 blocks</td>
<td>2 blocks</td>
</tr>
<tr>
<td>Battery-operated backup RAM</td>
<td>•</td>
<td>•</td>
<td>–</td>
</tr>
<tr>
<td>Pulse angle</td>
<td>Any angle from 0 to 360°</td>
<td>Any angle from 90 to 120° in 1° increments</td>
<td>Fixed angle of 120°</td>
</tr>
<tr>
<td>Asymmetrical angles</td>
<td>•</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nonlinear switch-time ramping (expands linear range of fragment separation to 50–700 kb)</td>
<td>•</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Multistate separation</td>
<td>•</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Secondary pulses (voltage interrupts)</td>
<td>•</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fige and asymmetric FIGE</td>
<td>•</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(resolution of fragments in the 100 bp–250 kb range)</td>
<td>•</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
PFGE - Reagents

- Bio-Rad CHEF DNA Genomic Plug kit optimized for
  - Genomic mammalian DNA
  - Genomic Bacterial DNA
  - Yeast Chromosomes
### FPQuest & InfoQuest SW

<table>
<thead>
<tr>
<th>Software Analysis Features</th>
<th>FPQuest</th>
<th>InfoQuestFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Application Modules (Type of Data Handled)</td>
<td>Basic software (170-9300)*</td>
<td>Basic fingerprint types (170-9310)*</td>
</tr>
<tr>
<td>1-D gels, chromatograms, density curves, and comparative quantitation of bands between groups of patterns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microarrays, profiles, test panels, dot blots, etc.</td>
<td></td>
<td>Character types (170-9311)</td>
</tr>
<tr>
<td>Sequence data; direct import of EMBL, GenBank, Flat A, and FASTA formats</td>
<td></td>
<td>Sequence types (170-9312)</td>
</tr>
<tr>
<td>Similarity and distance matrices; partial (DNA homology) matrices accepted</td>
<td></td>
<td>Matrix types (170-9313)</td>
</tr>
<tr>
<td>Analysis Tools (Type of Analysis Performed)</td>
<td>Cluster analysis (170-9301)</td>
<td>Cluster analysis (170-9314)</td>
</tr>
<tr>
<td>Dendrogram generation and cluster analysis</td>
<td>Identification and library manager (170-9302)</td>
<td>Identification and library manager (170-9315)</td>
</tr>
<tr>
<td>Database screening and library generation</td>
<td>Basic fingerprint types (170-9310)</td>
<td>Basic fingerprint types (170-9310)</td>
</tr>
<tr>
<td>Principal component analysis and nonhierarchical grouping by MDS</td>
<td>Dimensioning techniques (170-9304)</td>
<td>Cluster analysis (170-9314)</td>
</tr>
<tr>
<td>Data sharing over the Internet</td>
<td>Database sharing tools (170-9305)</td>
<td>Dimensioning techniques (170-9316)</td>
</tr>
<tr>
<td>Analysis of series of readings as a function of changing factors that define a trend</td>
<td>Database sharing tools (170-9317)</td>
<td>Trend data (170-9318)</td>
</tr>
</tbody>
</table>
# Comparison of Pulsed Field Standards

<table>
<thead>
<tr>
<th>Range</th>
<th>Contents</th>
<th>Amount</th>
<th>Number of Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 kb ladder</td>
<td>Concatemers of pBR328</td>
<td>20 µg in 200 µl</td>
<td>20–25</td>
</tr>
<tr>
<td>8–48 kb ladder</td>
<td>Mixed digest of phage λ</td>
<td>25 µg in 125 µl</td>
<td>125</td>
</tr>
<tr>
<td>Lambda (λ) ladder</td>
<td>Concatemers of phage λcl857Sam7</td>
<td>5 agarose blocks</td>
<td>25–40</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>Saccharomyces cerevisiae chromosomal DNA</td>
<td>5 agarose blocks</td>
<td>25–40</td>
</tr>
<tr>
<td>H. wingei</td>
<td>Hansenula wingei chromosomal DNA</td>
<td>5 agarose blocks</td>
<td>25–40</td>
</tr>
<tr>
<td>S. pombe</td>
<td>Schizosaccharomyces pombe chromosomal DNA</td>
<td>5 agarose blocks</td>
<td>25–40</td>
</tr>
</tbody>
</table>

![PFGE gel images](image)
Wishing you good results as shown… with Bio-Rad equipment

Bio-Rad / CHEF DR III / S.Aureus (Data from Jordan University of Science and Technology)
Thanks for your attention