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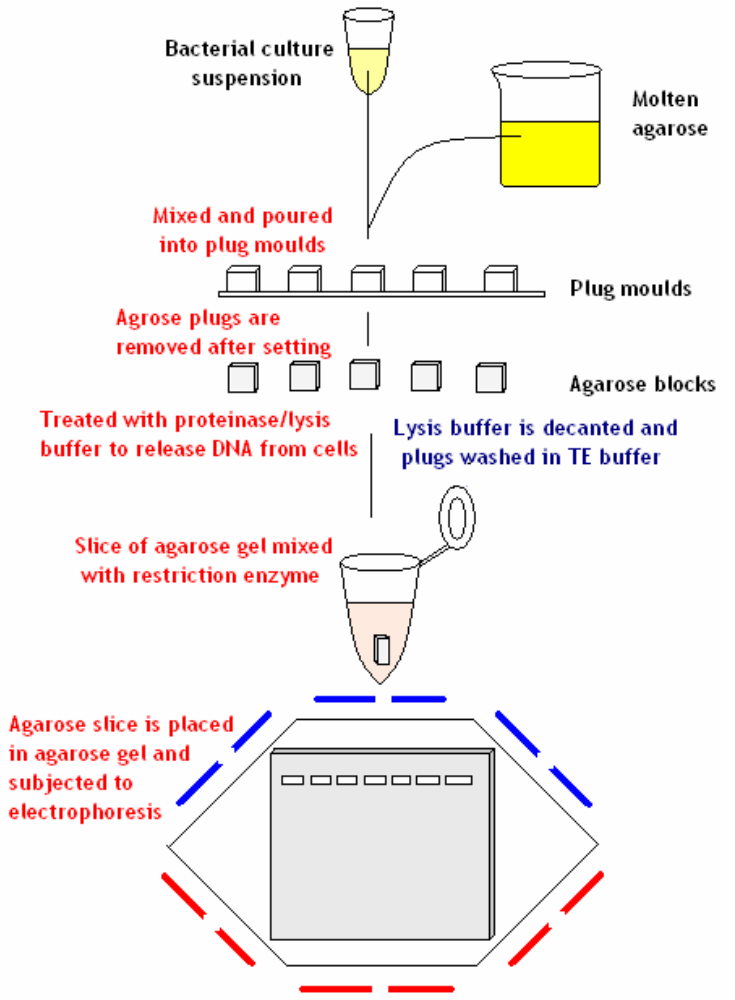
Pulsed field gel electrophoresis (PFGE)

This technique was developed by Shwartz and Cantor in 1984.



DNA, being negatively charged moves towards anode in an electric field during electrophoresis. Since DNA is a large molecule, it would end up migrating to a single band. Hence, DNA is cut using specific restriction endonucleases.

This results in formation of several DNA fragments. Under the electric field, DNA fragments migrate to the other end relative to their molecular size. The smallest fragment migrates furthest and the largest fragment migrates least. Conventional electrophoresis can conveniently separate fragments up to 20Kb in size.

Sometimes, large fragments (>20 Kb) or fragments with little difference in molecular size tend to coalesce and form a single fuzzy band. By alternating the electrical field between spatially distinct pairs of electrodes, these fragments are separated into discrete bands. Instead of constantly running the voltage in one direction as in regular electrophoresis, the voltage is periodically switched among three directions; one that runs through the central axis of the gel and two that run at an angle of 120 degrees either side.

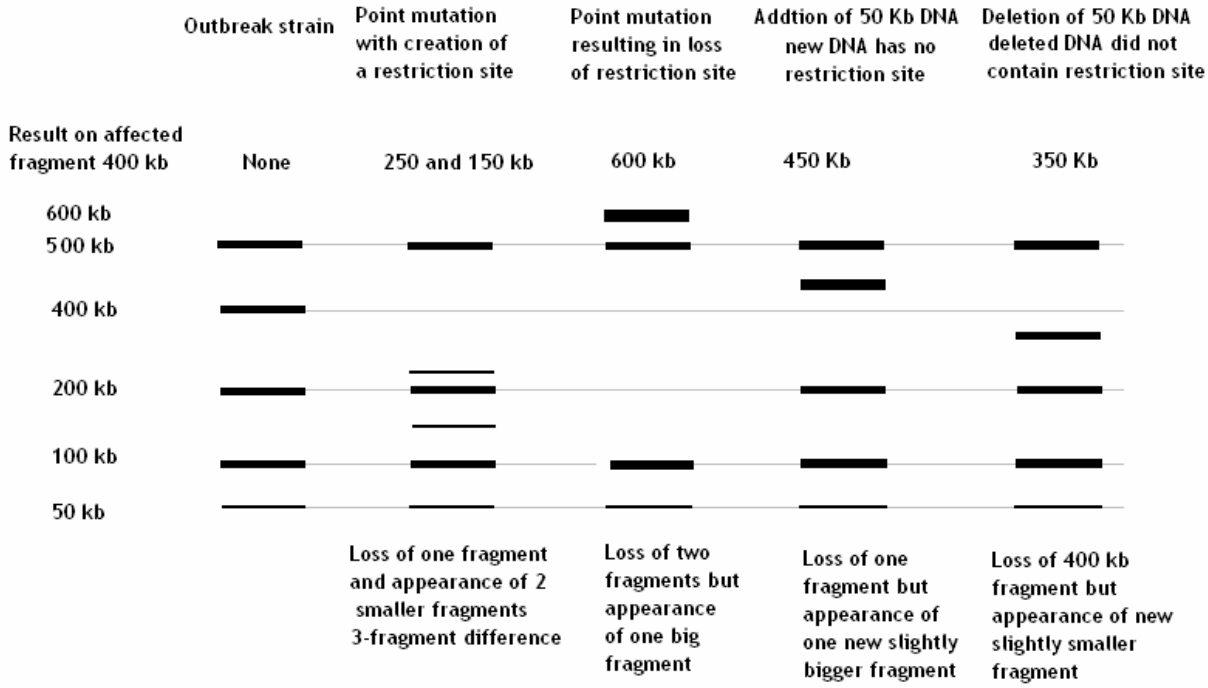
Procedure:

In PFGE, the organisms are embedded in agarose, lysed in situ to extract the DNA and followed by digesting the DNA by using a restriction enzyme that cleaves infrequently. Slices of agarose are then inserted into wells of an agarose gel and electricity applied at three different angles to resolve the DNA into discrete bands. Multiple strategies have been developed to produce homogenous electrical field by different companies. For rapid separation of fragments 100bp to 250 kb in size, electrical field is fixed at 180^{o} angle and is inverted in the forward and reverse directions. In another approach different voltages are applied to forward and reverse directions.

The organism is cultured on non-selective agar medium to obtain isolated colonies. The colonies are suspended in SE buffer (75mM NaCl, 25mM EDTA, pH 7.5) and adjusted to turbidity matching 0.12 abs at 620 nm. The cell suspension is mixed with molten 1% agarose solution (54^{o}C) and the mixture is dispensed into plug moulds. After the agarose is set, it removed from the plug moulds and transferred to tubes containing lysis buffer/proteinase K and incubated in water bath for 2 hours at 54^{o}C. During this process, any DNA'se present in the agarose gets destroyed and the agar plug contains only embedded DNA. The lysis buffer is decanted and the agarose plug is washed four times in warm TE buffer. A 2 mm slice of the agarose gel is cut using scalpel blade and mixed with Xba I restriction enzyme mixture in an eppendorf tube and is incubated for 2 hours at 37^{o}C. 1% agrose gel is cast with comb in place to create wells. The plug slice is removed from the tube and carefully pushed into the well of agarose gel. The wells may be sealed with 1% agarose solution. The gels were electrophoresed for ~18 h, depending on the equipment used. After electrophoresis, the entire gel is transferred to a staining bath containing 1µg/ml ethidium bromide. The gel is de-stained and washed few times in distilled water and placed on UV transilluminator or a GelDoc system. The bands are observed and photographed for documentation. In case the DNA has been autolysed by the organism, a smear in the lane would be seen instead of discrete bands. This can be avoided by using Thiourea in the electrolysis buffer.

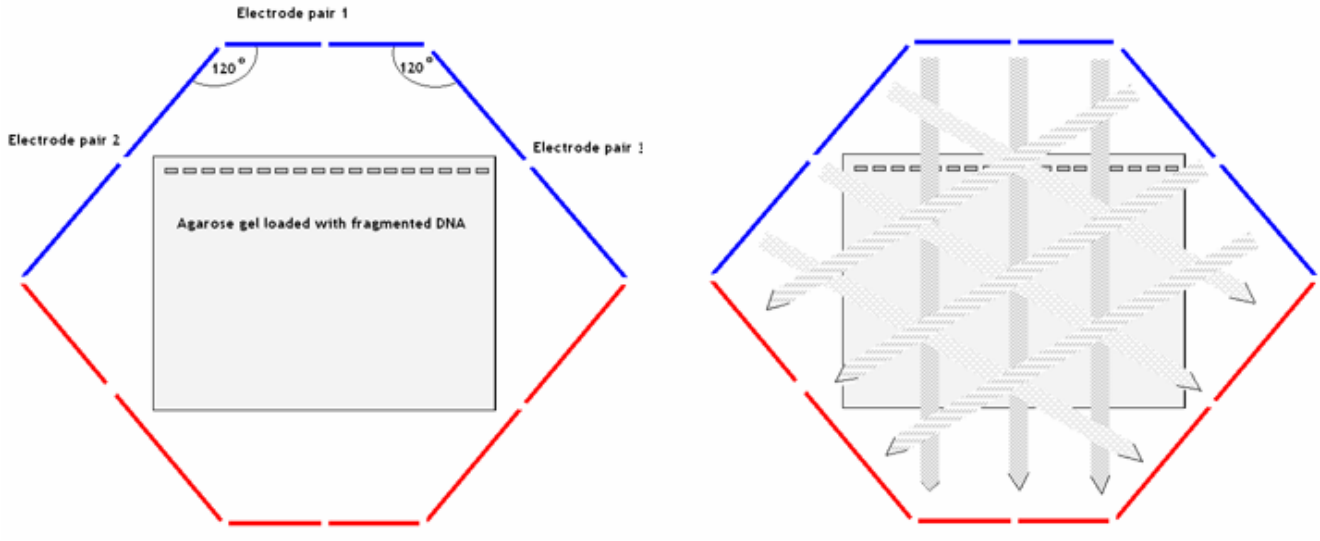
Reading and interpretation of bands:

Effect of genetic events on band formation is depicted on a 400 Kb fragment



Interpretation of PFGE pattern

Category	No. of genetic difference wrt to outbreak strain	No. of fragment differences wrt to outbreak strain	Epidemiological interpretations
Indistinguishable	0	0	Isolate is part of outbreak
Closely related	1	2-3	Isolate is probably part of outbreak
Possibly related	2	4-6	Isolate is possibly part of outbreak
Different	≥3	≥7	Isolate is not part of outbreak



A well characterized control strain should be processed along with the unknown samples. A molecular size standard (ladder) should also be run to provide size orientations of the fragment. Agarose plugs with restriction enzyme digested DNA of known organisms are also commercially available. The molecular size of the unknown fragment can be determined by plotting distance of migration from the bottom of well in gel vs log10 of molecular size of fragments. When typing a set of isolates that are suspected of being a part of an outbreak, it is prudent to include few epidemiologically unrelated isolates so that outbreak-associated strains can be differentiated from strains that are not. This is useful in analyzing outbreaks caused by *S. aureus* as number of PFGE patterns produced in *S. aureus* is limited.

Applications:

PFGE is accurate and results are reproducible with good efficiency, it is used in several areas.

- Molecular studies of food borne pathogenic organisms such as Salmonella, *E. coli* O157:H7, Shigella, Listeria, Campylobacter etc.
- The wine industry use PFGE to monitor the genetic stability of organisms involved in the fermentation processes.
- PFGE is the first step in cutting and separating large DNA fragments before cloning in vectors.
- It is very useful in mapping applications such as mapping specific disease loci, identifying chromosome rearrangements, RFLP and DNA fingerprinting.
- Detecting related strains in case of hospital outbreaks

Limitations of PFGE:

- PFGE is suitable for epidemiological studies of potential outbreaks in hospitals or communities spanning relatively short periods of 1-3 months. It is not suitable for analyzing isolates collected over extended periods (>1 year).
- Expensive instrumentation, time consuming (~24 hours), requires high level skill
- Some strains are untypeable; complex patterns are difficult to interpret
- Bands of same size don't guarantee same DNA; relatedness is only relative and not absolute

Further reading: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel J. Clin. Microbiol. 1995, 33(9):2233.

Last edited on 1st May, 2012