



Pulsed-Field Gel Electrophoresis (PFGE)

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INTRODUCTION

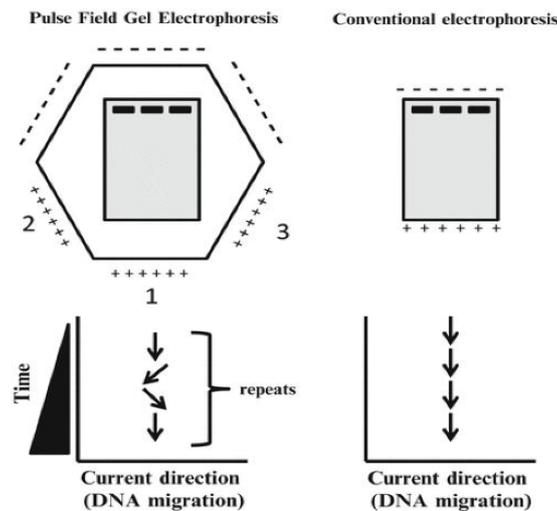
Pulsed-Field Gel Electrophoresis (PFGE) is a technique developed by Schwartz and Cantor in 1984. A more advanced electrophoretic technique known as Pulsed-Field Gel Electrophoresis (PFGE) was developed to distinguish large DNA fragments (10 kb to 10 Mb) that conventional agarose gel electrophoresis cannot effectively separate. It allowed for the separation of DNA molecule mixtures larger than 20 kb and up to 5 Mb in agarose gels, bridging the gap between the resolution of standard electrophoresis (0.1–30 kb) and cytogenetic techniques (>5 Mb). PFGE is used to separate the macro-restriction fragments of the bacterial genome in band patterns named DNA fingerprints.

Principle:

The concepts of DNA separation through PFGE rely on alternating the direction of two electric fields and the capacity of DNA molecules to realign in the new orientation of the electric field application. The duration taken by the molecules to reorient is influenced by their sizes. Subsequently, the smaller molecules adjust their positions more quickly than the larger ones, causing the movement of the larger molecules through the gel to lag behind that of the smaller, resulting in their separation from each other. Switching of Electric Fields: The direction of the field changes between various angles (typically 120°). Pulse times (switching times) are set (e.g., ranging from 1 to 60 seconds). Separation occurs due to the connection between the size of fragments and the time taken for reorientation.

Table No.01: Difference between PFGE and conventional Gel Electrophoresis

S.N.	PFGE	Gel Electrophoresis
1	Direction of current is altered at regular interval	Current is applied in single direction (for example, from top to bottom)
2	DNA from kb to over 10 Mb is separated	DNA fragment from 100-200bp up to 50kb are separated
3	Large fragment above 50kb can run and highly resolved bands are obtained	Above 50 kb because of the size of the molecules the sieving action of the gel is lost, and fragments run as a broad, unresolved band



Source: Schwartz, D. C., & Cantor, C. R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell*, 37(1), 67–75.

Method:

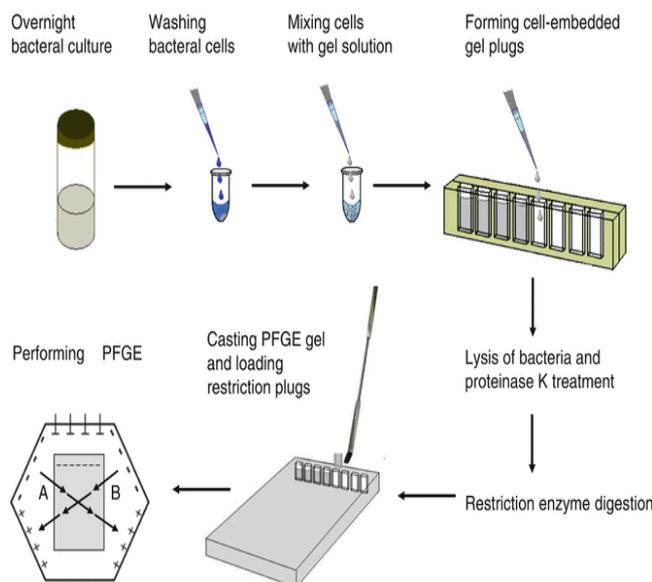
Lysis: Initially, the bacterial suspension is added to an agarose suspension. This is accomplished to safeguard the chromosomal DNA from physical harm by entrapment within agarose blocks. The bacterial cells are broken open to liberate the DNA. The suspension of agarose and DNA is referred to as a plug Mold.

Digestion of DNA: The bacterial DNA undergoes processing with unique cutting restriction enzymes, resulting in a fewer quantity of larger DNA fragments, unlike the commonly

used restriction enzymes in RFLP that generate numerous smaller fragments.

Electrophoresis: The larger DNA segments undergo pulse field gel electrophoresis by applying an electric current and periodically changing its direction (unlike traditional agarose gel electrophoresis used for separating the smaller fragments, where the current flows in one direction).

Analysis: The segments from various organisms produced by PFGE are compared to established standards either manually or through software tools such as BioNumerics.



Source: The Science Notes- Pulsed field Gel Electrophoresis (PFGE)

DIFFERENT TYPES OF PFGE

Type of PFGE	Functioning	Temperature	Advantages	Disadvantages	Source
CHEF (Clamped Homogeneous Electric Field)	Electric field direction changes periodically using hexagonal electrode arrangement; DNA migrates based on size.	14–15°C (cold buffer circulation)	High resolution for very large DNA; reproducible; widely used.	Expensive equipment; long run times; complex setup.	Chu <i>et al.</i> , 1986
C-CHEF (Contoured-CHEF)	Refined CHEF with contour-clamped electrodes for uniform field.	14–15°C	Even greater resolution; highly consistent separation	-	Vollrath and Davis (1987).
TAFE (Transverse Alternating Field Electrophoresis)	Electric field alternates transversely across gel.	4–15°C	Can separate large DNA; alternative method to CHEF.	-	Carle, and Olson (1985).
RGE (Rotating Gel Electrophoresis)	Gel rotates physically within a static field; DNA moves according to changes in field direction	4–15°C	no complex electrode arrangement.	Limited resolution; labor-intensive; outdated method.	Southern, and Cooke (1986)
OFAGE (Orthogonal Field Alternation Gel Electrophoresis)	Two perpendicular electric fields alternate at right angles.	4–15°C	Early method for large DNA separation.	less reproducible; slower runs.	Carle <i>et al.</i> (1986).

Applications

- PFGE is an efficient method for genome size estimation
- PFGE technique is useful to establish the degree of relatedness among different stains of the same species
- Quality assurance & monitoring: in food safety, healthcare facilities, public health laboratories.
- Investigation of extensive DNA damage: PFGE applied to measure DNA double-strand breaks.
- Genomic mapping: examining extensive chromosomal segments, plasmids, and studies of genome architecture.
- Epidemiological characterization of bacteria: commonly utilized to differentiate strains in outbreak studies (e.g., foodborne pathogens).

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