PFGE - Pulse Field Gel Electrophoresis

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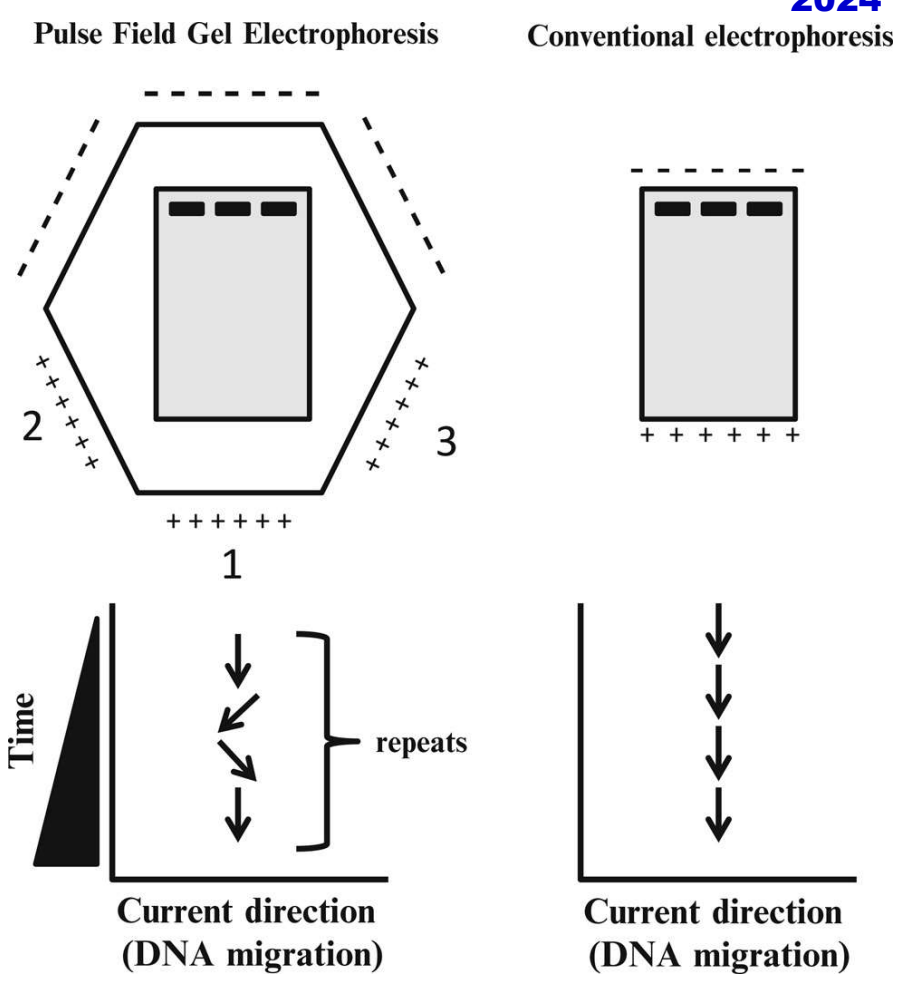
2024

PFGE

Pulse Field Gel Electrophoresis



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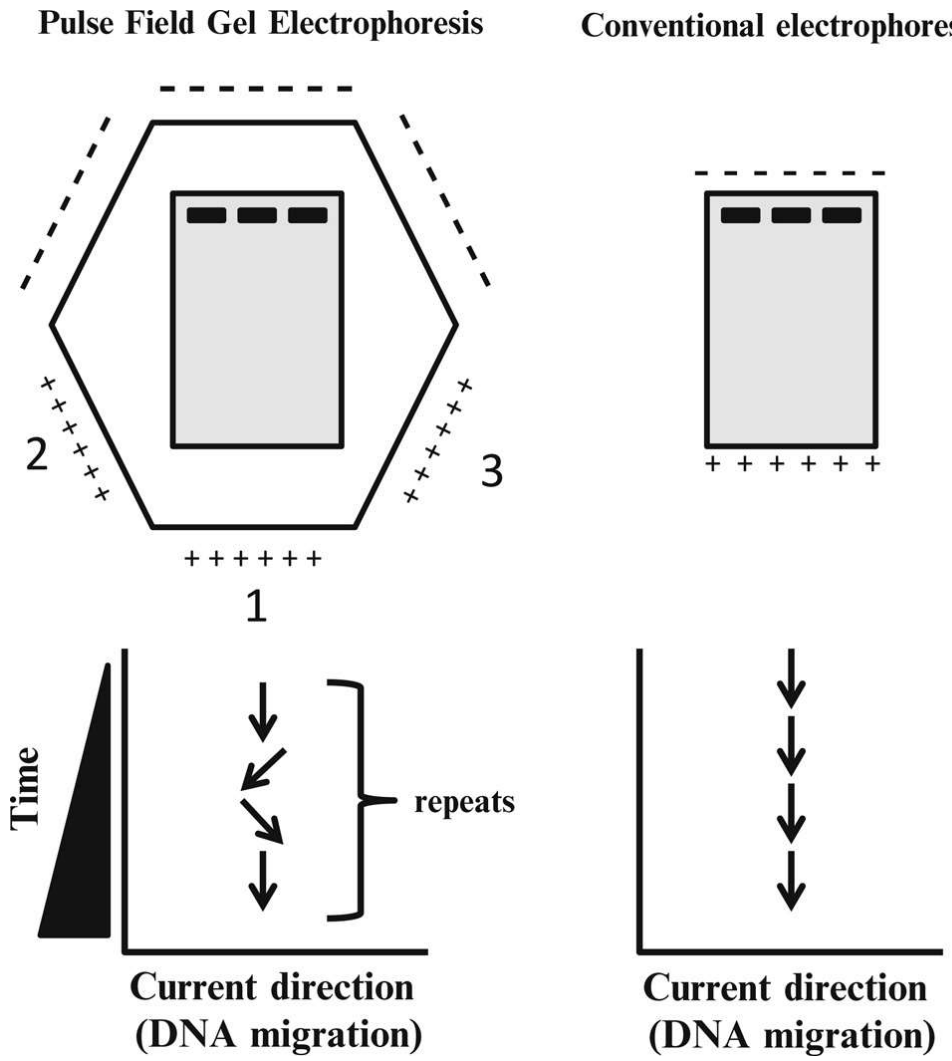

PFGE - Pulse Field Gel Electrophoresis

▪ Pulse Field Gel Electrophoresis

Pulse Field Gel Electrophoresis (PFGE) is a powerful **genotyping technique** used for the **separation of large DNA molecules** (entire genomic DNA) ***after*** digesting it with unique restriction enzymes and applying to a gel matrix under the **electric field that periodically changes direction**.

PFGE - Pulse Field Gel Electrophoresis

▪ Pulse Field Gel Electrophoresis

• **Conventional electrophoresis** uses a **single electrical field** to cause biomolecules to migrate through a matrix according to its **mass-to-charge ratio**; the **migration distance** of the biomolecule is **indicative of** its **mass** or **size** (Klotz and Zimm 1972). Conventional electrophoresis can effectively separate DNA fragments **up to ~20 kb**.

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- However, **larger fragments** will **comigrate** and appear as a **large band** at the top of the gel when imaged.
- In 1984, Schwartz and Cantor invented pulsed field gel electrophoresis (PFGE) **to overcome this problem**.

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PFGE - Pulse Field Gel Electrophoresis

▪ Pulse Field Gel Electrophoresis

- PFGE **resolves** DNA by **alternating the electrical field** between spatially distinct pairs of electrodes.
- This technique results in the separation of DNA fragments of **up to ~10 Mb** by their **reorientation and movement at different speeds** through the pores of an agarose gel.

PFGE - Pulse Field Gel Electrophoresis

▪ 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.

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▪ Pulse Field Gel Electrophoresis

• 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.

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▪ Pulse Field Gel Electrophoresis

• 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.

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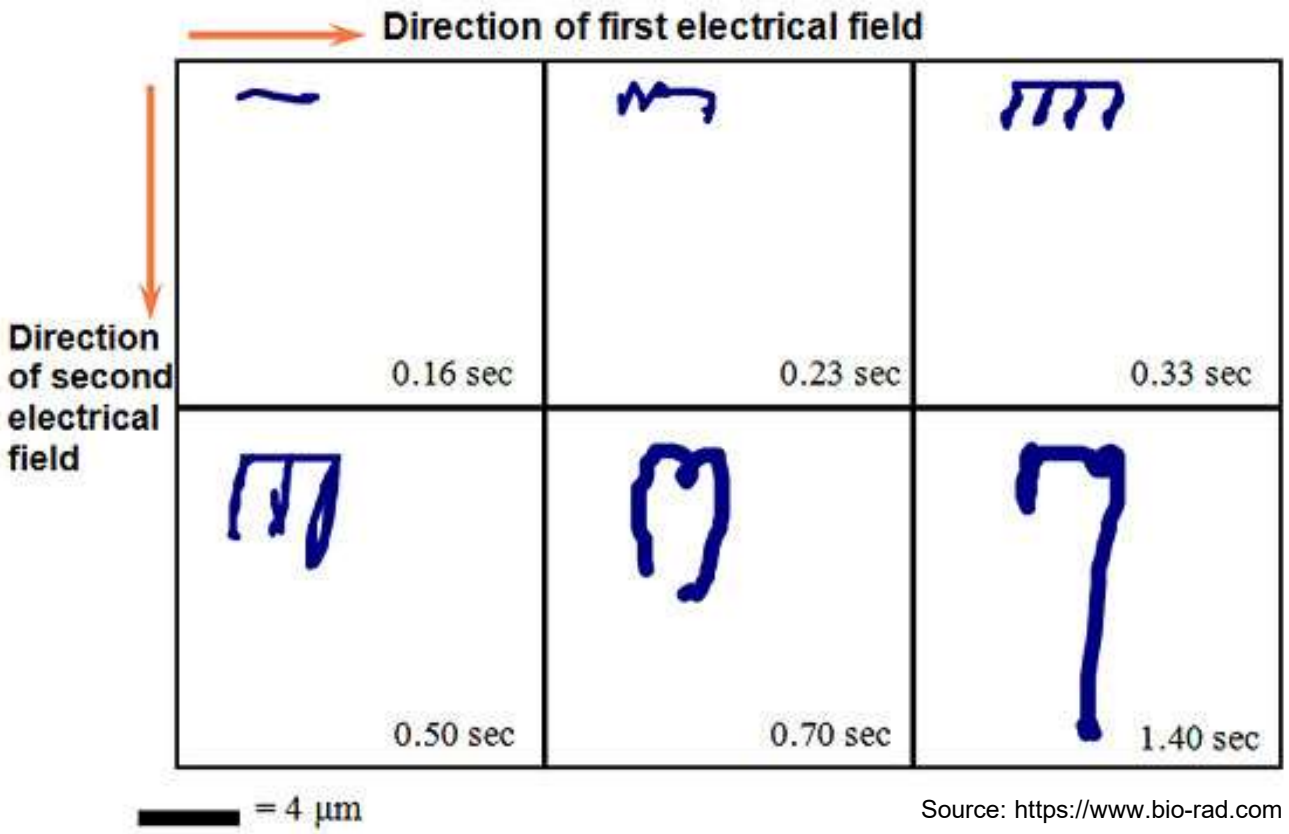
• As long as the alternating **fields are equal** with respect to the **voltage** and **pulse duration**, the DNA will migrate in a straight path down the gel (see below).

PFGE - Pulse Field Gel Electrophoresis



Source: https://www.bio-rad.com

Time-lapse representation of DNA molecules undergoing PFGE.

PFGE - Pulse Field Gel Electrophoresis

▪ PFGE Sample Preparation

- The large size of DNA molecules to be separated by PFGE imposes certain constraints on sample preparation and handling.
- High molecular weight DNA is easily cleaved through shearing and imparts very high solution viscosity.
- For these reasons, DNA samples for PFGE are generally prepared by embedding in gel medium.

PFGE - Pulse Field Gel Electrophoresis

▪ PFGE Sample Preparation

• Cellular **source material** is suspended in low gelling agarose and the gelled suspension is poured into molds.

• All subsequent manipulations (for example, cell lysis, protein removal, and restriction digestion) are performed by **diffusing reagents** into the resultant gel plugs.

• The processed gel plugs are then carefully loaded into wells of an agarose gel used for PFGE.

PFGE - Pulse Field Gel Electrophoresis

▪ PFGE *... till now*

- A powerful **molecular typing technique**
- By which genomic **DNA is isolated** from the organism of interest, followed by **restriction enzyme analysis**.
- The digestion products are then analyzed on an **agarose gel** by applying an **electric field** that periodically changes direction

PFGE - Pulse Field Gel Electrophoresis

- **PFGE** *... till now*

• Allowing for separation of the larger DNA fragments (entire genomic DNA) and **approximate measurement** of fragment length.

• PFGE can separate **large DNA molecules** (**up to 10 Mb**), whereas standard DNA gel electrophoresis commonly resolves fragments up to ~50 kb.

• **PFGE takes 2–3 days**, excluding sample preparation.

PFGE - Pulse Field Gel Electrophoresis

- **PFGE** *... till now*

• The largest molecule so far resolved by Pulse-Field Gel Electrophoresis is estimated at **14 Mb** (Barry and Pollard, 1993)

PFGE - Pulse Field Gel Electrophoresis

PFGE is a variation of Agarose Gel Electrophoresis. Some of the major differences are:

Property	**Conventional gel electrophoresis**	**Pulsed-field gel electrophoresis**
Resolving power (Separating power)	Separates DNA fragments up to ~50 kb in size.	can separate large DNA molecules(up to 10 Mb)
Direction of current	Current only runs in a single direction	The direction of the current switches from the primary to the secondary electrodes located at a 60° angle from the centre of the agarose gel.

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Steps of pulsed-field gel electrophoresis

1. **Preparation of Bacterial cells** - Streak bacterial isolates (e.g. Staphylococcus aureus) onto TSA plates (Tryptic Soy Agar Plate) and incubate at 37 °C for 18–24 h.
2. **Mix bacterial cells with melted agarose and pour into a plug mold** - Bacterial cells are embedded into an agarose gel to prevent shearing of chromosomal DNA, and DNA digestion is performed in situ.

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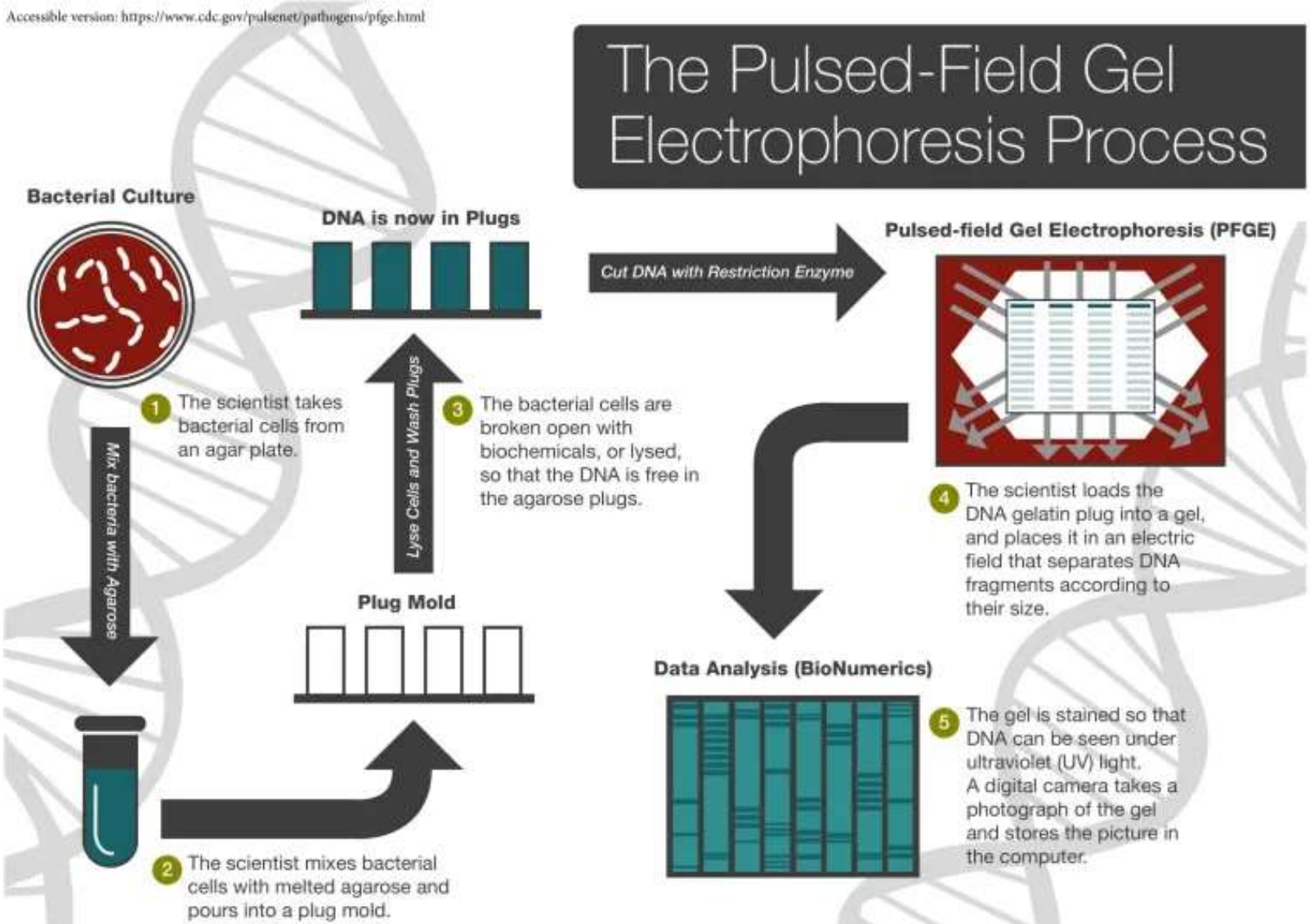

Steps of Pulsed Field Gel Electrophoresis (Source: CDC)

PFGE - Pulse Field Gel Electrophoresis

▪ Steps of pulsed-field gel electrophoresis

3. **Lysis of Bacterial cells** - The bacterial cells are broken open with biochemicals, or lysed, so that the DNA is free in the agarose plugs. The bacterial chromosome is digested using a rare cutting enzyme that recognizes specific DNA sequences ranging from 6 to 8 nucleotides, resulting in a limited number of DNA fragments of varying lengths. After DNA restriction, slices of the agarose blocks are inserted into wells of the agarose gel matrix.

PFGE - Pulse Field Gel Electrophoresis

- **Steps of pulsed-field gel electrophoresis**

4. **Electrophoresis** - Digested DNA samples are loaded into the DNA gelatin plug into a gel, and are subjected to separation by alternating the electric field between spatially distinct pairs of electrodes

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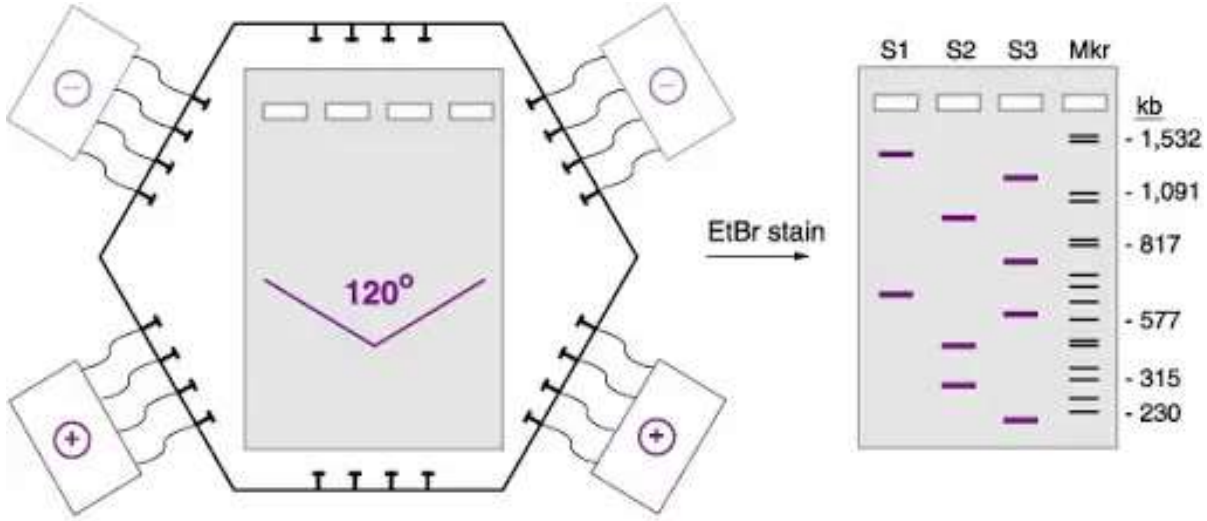
▪ PFGE – the Basic concept

Application of an **electric field** that is periodically **reorienting** causes **long DNA** molecules to **reorient** during electrophoresis. The **time required** for **reorientation** is also **inversely proportional to the size** of the DNA fragment. As **smaller molecules reorient** more **quickly** than larger molecules, separation of various DNA fragment sizes ranging from kilobases (Kb) to megabases (Mb) ensues if the gel is **run for a sufficient time**. This process generates a **fingerprint profile** that can be digitally imagined.

PFGE - Pulse Field Gel Electrophoresis

This **system** consists of **24 electrodes** in an **octagon arrangement** producing a **constant electrophoresis gradient** that **switches** from the **primary to the secondary** electrodes located at a **60° angle** from the centre of the agarose gel. Consequently, this configuration causes the **DNA molecules to re-orient** in the agarose gel matrix over a **120° angle**.



Electric field alternates 120° every 90 seconds for 18 to 24 hours at 14° C

PFGE - Pulse Field Gel Electrophoresis

- **Steps of pulsed-field gel electrophoresis**

5. **Staining the gel** - The gel is stained so that DNA can be seen under ultraviolet (UV) light.

6. Taking photograph and processing the Image

PFGE - Pulse Field Gel Electrophoresis

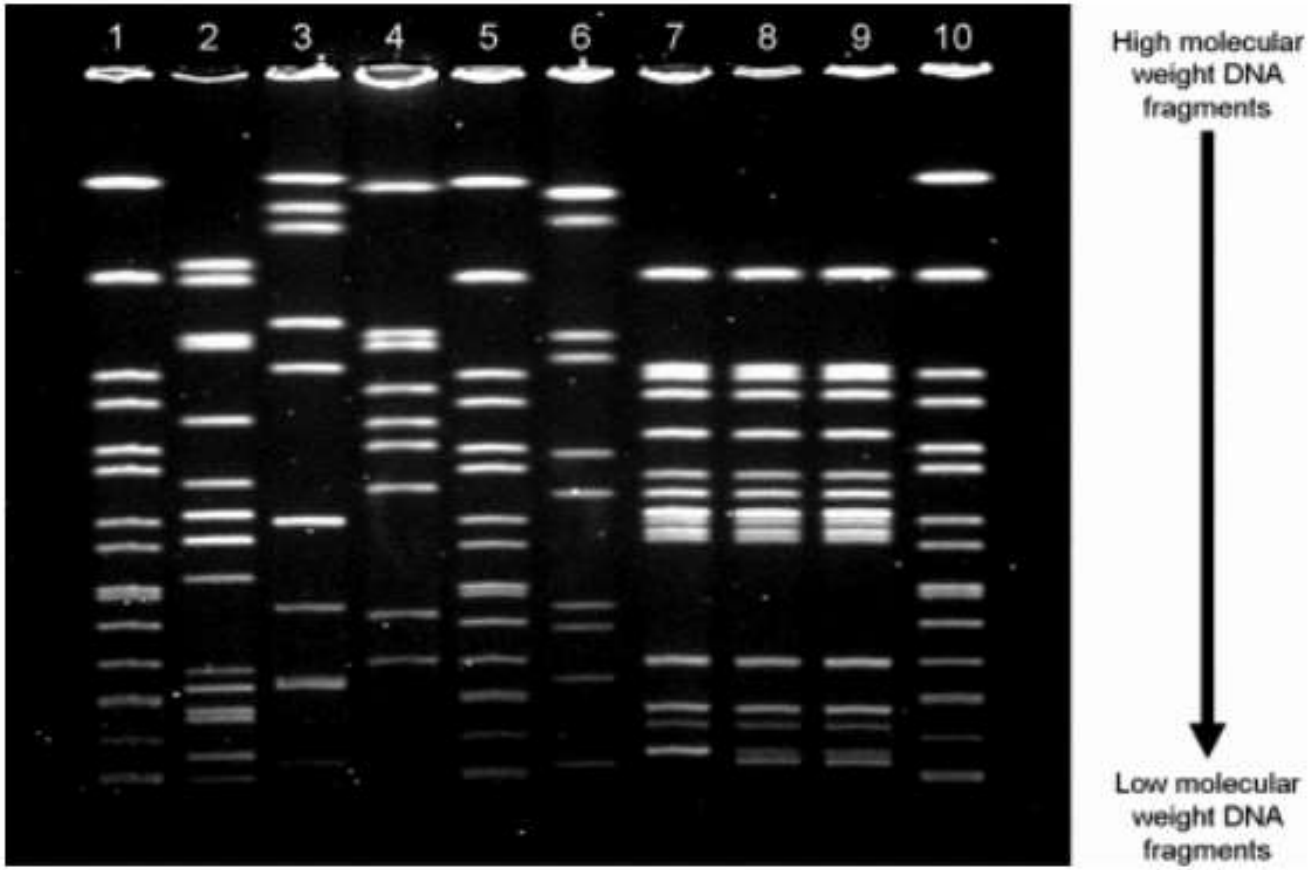
- **Steps of pulsed-field gel electrophoresis**

Taking photograph and processing the Image

A digital camera takes a photograph of the gel and stores the picture in the computer. The obtained gel images are normalized and patterns of the DNA fragments are analyzed using various software. These software and similar other softwares help for storing, sharing and analyzing PFGE data.

PFGE - Pulse Field Gel Electrophoresis

Taking photograph and processing the Image



DNA Fingerprint profile generated by PFGE

PFGE - Pulse Field Gel Electrophoresis

Different Types of PFGE

- **CHEF** (Clamped Homogeneous Electrical Field)
- **PACE** (Programmable Autonomously Controlled Electrodes)
- **DR** (Dynamic Regulation)
- **FIGE** (Field Inversion Gel Electrophoresis)
- **AFIGE** (Asymmetric Field Inversion Gel Electrophoresis)

Main Sources / Reference

- https://europepmc.org
- https://www.bio-rad.com
- https://microbeonline.com

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