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Prospective of Gel Electrophoresis in Biotechnology

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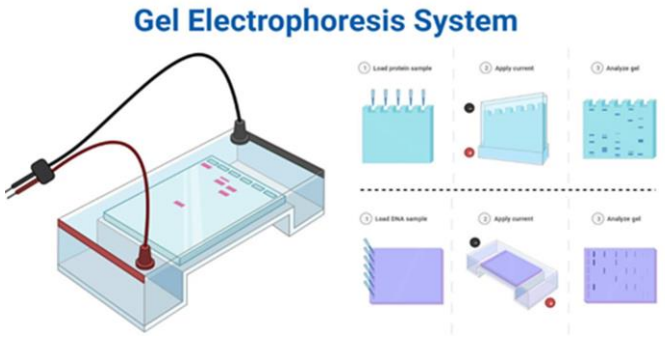
INTRODUCTION

Gel Electrophoresis is a laboratory technique used to separate DNA, RNA or protein molecules based on their size and electrical charge. An electric current is used to move the molecules through a gel or other matrix. Pores in the gel or matrix work like a sieve, allowing smaller molecules to move faster than larger molecules. To determine the size of the molecules in a sample, standards of known sizes are separated on the same gel and then compared to the sample
The relative mobility of individual molecule depends on several factors.

- Net charge
- Charge / mass ratio
- Molecular Shape

Principle:

The flow of charged particles in an electric field is the basis of the gel electrophoresis principle. Negatively charged molecules like DNA and RNA migrate towards the positive electrode (anode) when an electric current is applied, whereas positively charged molecules migrate towards the negative electrode (cathode).
Depending on the size and structure of the molecules, the gel functions as a molecular sieve, slowing their flow. Larger molecules flow more slowly through the gel pores than smaller molecules. As a result, molecules are separated based on their size.



Source: Agarose gel electrophoresis, how it Works and Its Uses." *Technology Networks*, 2024, www.technologynetworks.com/…

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Gel Electrophoresis Types

i. Based on Equipment Orientation

a) Horizontal Gel Electrophoresis

The gel, which is typically agarose, is positioned horizontally in a tank filled with buffer. The sample wells are at one end, and the electric field is applied horizontally across the gel. Negatively charged DNA/RNA fragments move toward the positive (anode) side. The gel remains submerged in buffer during the run to prevent drying and maintain conductivity. Used for: Nucleic acids (DNA and RNA)

b) Vertical Gel Electrophoresis

The gel (usually polyacrylamide**)** is cast between two glass plates and positioned vertically. The buffer chambers are above and below the gel, creating a vertical electric field**.** Protein samples move through the gel matrix vertically, from top to bottom. Used for: Protein (SDS PAGE, native PAGE) and small nucleic acids

ii Based on Purpose and sample Type

a) Agarose Gel Electrophoresis

Agarose gel electrophoresis is a technique used to separate nucleic acids primarily by size. and Agarose gel is a polysaccharide extracted from seaweed. It forms a porous matrix when solidified, allowing molecules to migrate through. Depends on agarose concentration (0.7–2% typical). Lower concentrations allow larger fragments to pass; higher concentrations are used for smaller fragments. Agarose gel electrophoresis is the most effective way of separating DNA fragments of varying sizes ranging from 100 bp to 25 kb (Sambrook and Russell , 2001) . Agarose is isolated from the seaweed genera *Gelidium* and *Gracilaria*, and consists of repeated agarobiose (L- and D-galactose) subunits (Kirkpatrick, 1991). During gelation, agarose polymers associate non-covalently and form a network of bundles whose pore sizes determine a gel's molecular sieving properties. The use of agarose gel electrophoresis revolutionized the separation of DNA. Prior to the adoption of agarose gels, DNA was primarily separated using sucrose density gradient centrifugation, which only provided an approximation of size.

b) Polyacrylamide Gel Electrophoresis (PAGE)

It is used at a concentration of up to 3-30% (pH range: 4-9.0): protein separation requires a higher concentration than DNA separation, and vice-versa.

Its run vertical gel electrophoresis

I. SDS-PAGE Electrophoresis

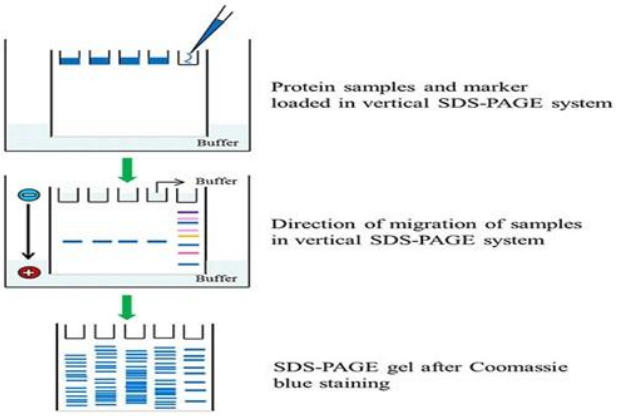
Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis is used for the separation of proteins on the basis of their mass It involves the use of vertical gel apparatus to separate protein

II. Native PAGE

Separates proteins according to their mass and charge in their native, non-denatured state (preserving their structure and shape).

III. soelectric Focusing (IEF)

The isoelectric point (pI), or the pH at which a protein has no net charge, is used to separate proteins. Proteins move along a pH gradient until they hit their pI, at which



{Source: Singh,R.,& Kumar, A (2020)}

2D Gel Electrophoresis

Two- dimensional gel electrophoresis was developed by Patrick O' Farrell in 1975 .It is used to Fractionate complex mixture of protein by using two different techniques The best high-resolution method for separating complicated protein mixtures

1st Dimension: Proteins are separated according to charge tolerance using isoelectric focussing (IEF).

2nd Dimension: The proteins are separated according to size (molecular mass) using the focused IPG strip on top of an SDS-PAGE gel.

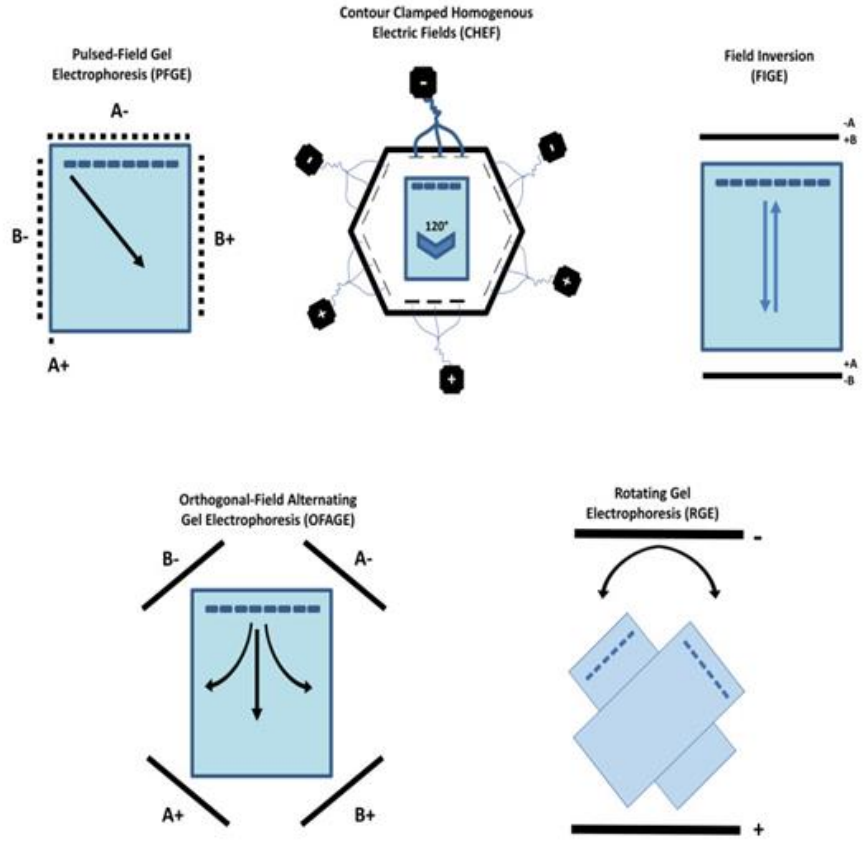
C)Pulsed -field Gel Electrophoresis

In 1984, David C. Schwartz and Charles Cantor introduced this method

Pulsed field gel electrophoresis is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction.

separate extremely large DNA molecules (up to 1 megabases), including whole bacterial chromosomes. By periodically shifting the electric field's direction, it gets around the size restriction of conventional agarose gel. Effective separation is made possible by forcing the big DNA molecules to realign themselves, which takes time directly proportional to their size. It is considered the "gold standard" for DNA fingerprinting in epidemiology.

PFGE is commonly used for bacterial typing and chromosomal mapping



Source: ScienceDirect Topics. (n.d.). *Pulsed-field gel electrophoresis overview diagram*

d) Capillary Gel Electrophoresis (CGE)

Narrow capillary tubes loaded with gel or polymer matrix are used in capillary gel electrophoresis (CGE), a contemporary automated electrophoresis technique. An electric field propels molecules through the capillary, where they are automatically detected, typically through laser-induced fluorescence. For DNA sequencing, genotyping, and forensic analysis, CGE is useful because it provides high precision, speed, and automation.

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