Lab 7: Gel Electrophoresis

Gel Electrophoresis

The term literally means migration with electricity. It involves the separation of components of a sample by the differential rate of migration of ions by attraction or repulsion in an applied dc electric field. Gel electrophoresis is a widely used technique for the analysis of nucleic acids and proteins. As an analytical tool, electrophoresis is simple, rapid and highly sensitive.

Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field. Electrophoresis is usually done with gels formed in tubes, slabs, or on a flat bed.

The Net Charge is Determined by the pH of the Medium

Proteins are amphoteric compounds, that is, they contain both acidic and basic residues. Each protein has its own characteristic charge properties depending on the number and kinds of amino acids carrying amino or carboxyl groups. Nucleic acids, unlike proteins, are not amphoteric. They remain negative at any pH used for electrophoresis.

Example: DNA is negatively charged. When placed in an electrical field, DNA will migrate toward the positive pole (anode). An agarose gel is used to slow the movement of DNA and separate by size.

Types of Gels used in Electrophoresis

There are different types (but are relatively electrically neutral) of gels which can be used

- Agar and Agarose gel
- Starch
- Sephadex
- Polyacrylamide gels.

Agarose and Polyacrylamide

Although agarose and polyacrylamide differ greatly in their physical and chemical structures, they both make porous gels. A porous gel acts as a sieve by slowing down

or, in some cases, by completely blocking the movement of macromolecules while allowing smaller molecules to migrate freely. By preparing a gel with a restrictive pore size, the operator can take advantage of molecular size differences among proteins. Because the pores of an agarose gel are large, agarose is used to separate macromolecules such as nucleic acids, large proteins and protein complexes. Polyacrylamide, which makes a small pore gel, is used to separate most proteins and small oligonucleotides.

How fast will the molecules (macro or micro) migrate?

The speed of the molecule movement depends on

- Strength of the electrical field
- Buffer
- Density of agarose gel
- Size of the DNA
- Viscosity
- Sometimes the temperature

Agarose Gel

Agarose is a highly purified uncharged polysaccharide derived from agar. Agarose dissolves when added to boiling liquid. It remains in a liquid state until the temperature is lowered to about 40° C at which point it gels. The pore size may be predetermined by adjusting the concentration of agarose in the gel. Agarose gels are fragile, however; they are actually hydrocolloids, and they are held together by the formation of weak hydrogen and hydrophobic bonds.

Agarose Gel	**Polyacrylamide**
• Polysaccharide extracted from sea weed. • Gel casted horizontally • Non-toxic. • Separate large molecules • Commonly used for DNA separations.	• Cross-linked polymer of acrylamide. • Gel casted vertically. • Potent neuro-toxic. • Separate small molecules. • Used for DNA or protein separations.

Staining Gel

Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a Gel.

Ethidium bromide can be added to the gel and/or running buffer before the gel is run or the gel can be stained after it has run.

Uses of Gel Electrophoresis

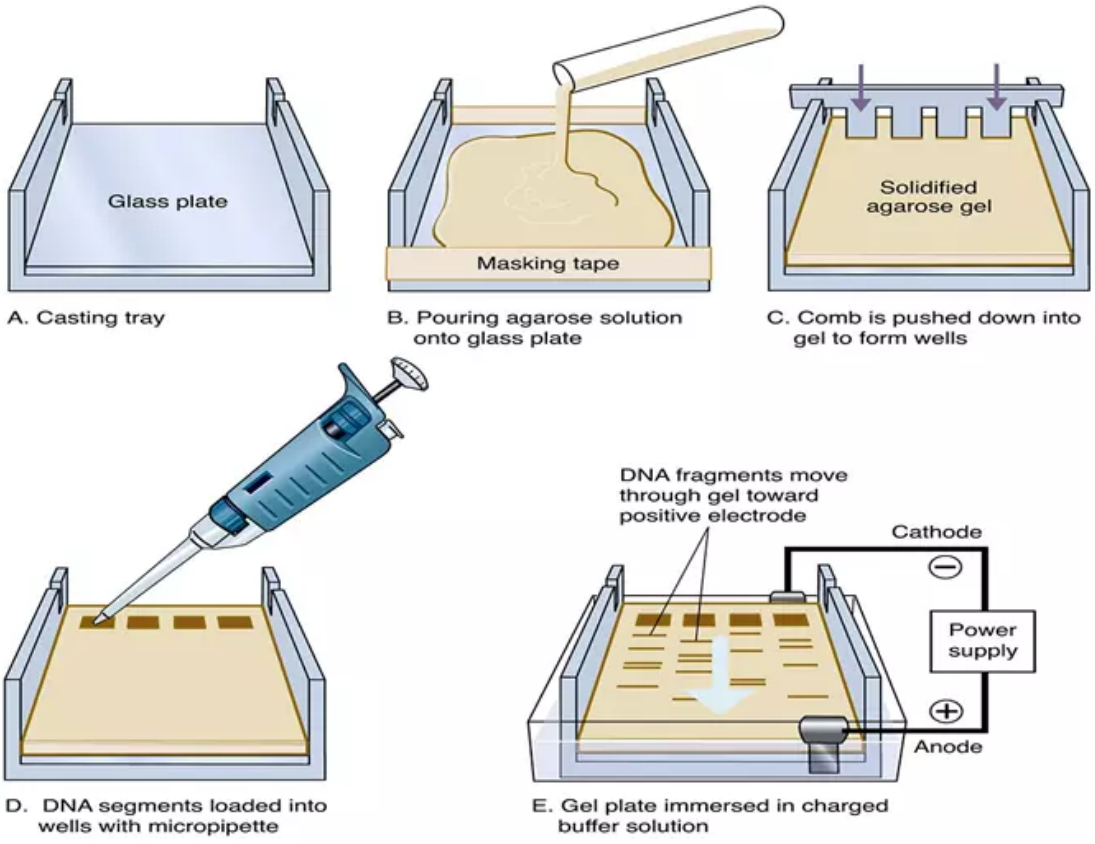
1. Solve Criminal cases
2. Solve Paternity cases
3. Diagnose Genetic disorders
4. Determine genetic kindship among species

Advantages (Pros)

1. Easy to do
2. DNA does not get ruined in the process
3. You only need small amount of DNA to start with
4. DNA can be detected no matter what size it is

Disadvantages (cons)

1. Expensive
2. Time consuming
3. Use Hazardous material



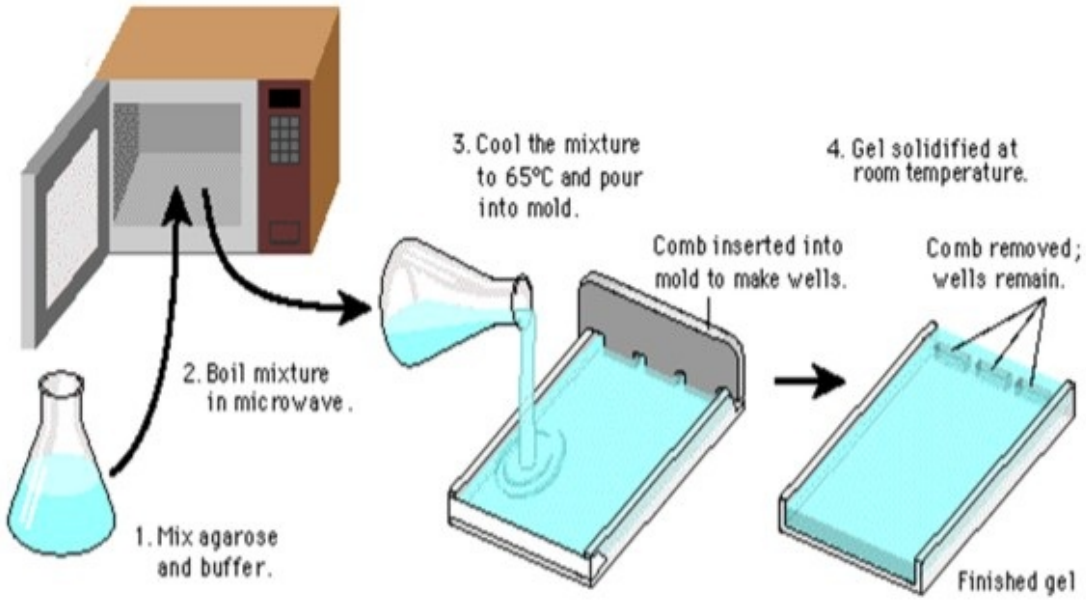
A. Casting tray

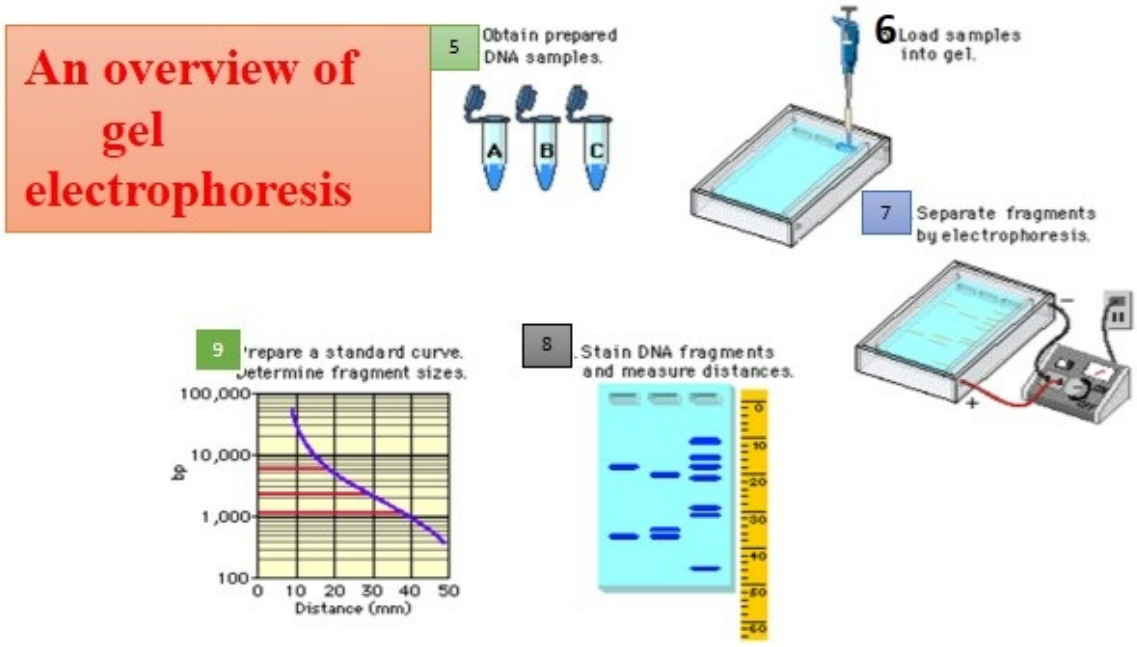
B. Pouring agarose solution onto glass plate

C. Comb is pushed down into gel to form wells

D. DNA segments loaded into wells with micropipette

E. Gel plate immersed in charged buffer solution


1. Mix agarose and buffer.
2. Boil mixture in microwave.
3. Cool the mixture to 65°C and pour into mold.
Comb inserted into mold to make wells.
4. Gel solidified at room temperature.
Comb removed; wells remain.
Finished gel


An overview of gel electrophoresis
5 Obtain prepared DNA samples.
A
B
C
6 Load samples into gel.
7 Separate fragments by electrophoresis.
9 Prepare a standard curve. Determine fragment sizes.
100,000
10,000
1,000
100
bp
0 10 20 30 40 50
Distance (mm)
8 Stain DNA fragments and measure distances.