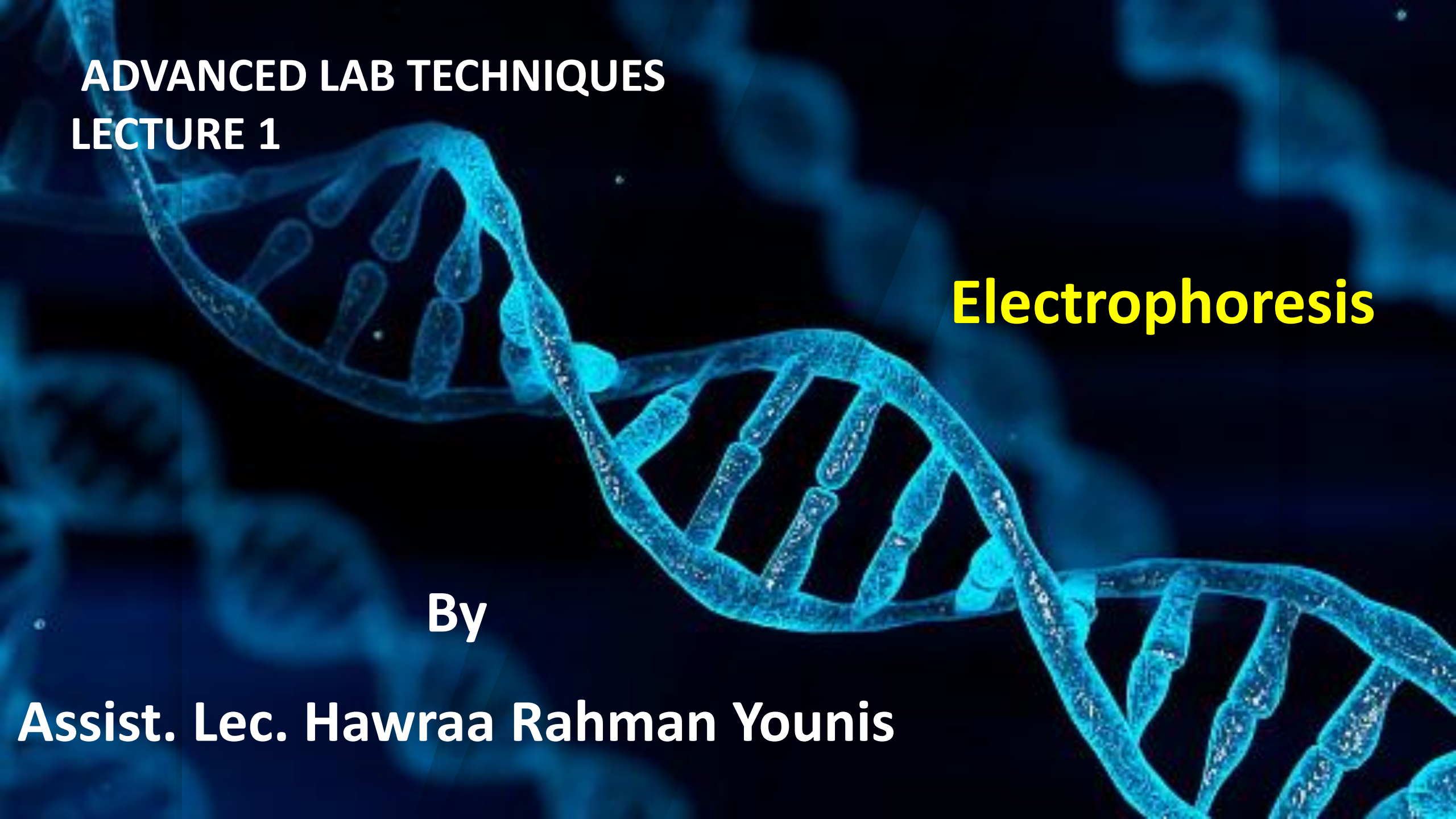
ADVANCED LAB TECHNIQUES
LECTURE 1

Electrophoresis



By

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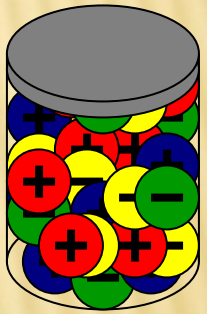
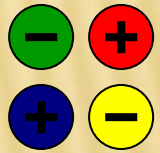
WHAT IS ELECTROPHORESIS ?

- **Electrophoresis is a laboratory technique for separating molecules based on their charge**





ELECTROPHORESIS

- **Electro=Electric; phoresis Migration;Carry accross .**
- **A kind of separation technique based on the differential migration features of charged molecules in an electric field.**
- **An analytical method frequently used in molecular biology, biochemistry and medicine.**

PRINCIPLE:

- In an electrical field charged molecules and particles migrate to the opposite charge.
- Usually in aqueous solution(Buffer).
- Due to their varying charges and masses, different molecules and particles in the mixture are migrate at different speeds.
- As a result; separated into single fractions(bands).

Migration Depends on

- Strength of electric fields.•
- Temperature•
- Features of the molecule
  - Net charge of molecule
  - Size of molecule
  - Shape of molecule•
- Features of the Gel
  - Gel type
  - Gel concentration•
- Buffer Type/ pH.

Electrophoresis Separates :

- Nucleic acids
- Proteins
- Peptides
- Amino acids
- Organic acids/bases
- Drugs
- Pesticides
- Inorganic anions/ cations .
- Everything that can carry a charge.

Electrophoresis Types

- Gel electrophoresis
  - Agarose gel
  - Polyacrylamide gel
- Others
- Pulsed Field Gel Electrophoresis
- Capillary Electrophoresis
- Isoelectric focusing
- 2 D electrophoresis

GEL ELECTROPHORESIS

- Use of a gelatinous material.
- The gel acts as a support medium
- Used to separate proteins or nucleic
- acids.

GEL TYPES

- Starch Rarely used
- Polyacrylamide Protein, small nucleic acid fragments
- Agarose Nucleic acids, large proteins
- Cellulose acetate Proteins

GEL ELECTROPHORESIS

- **Agarose** gel electrophoresis Easy, fast, well established method for separating DNA fragments, or RNA molecules by size.
  - Agarose , a polysaccharide derived from seaweed.
  - Dissolves in boiling water, and hardens, becomes gel when cooling.
  - Bigger pore size than polyacrylamide
- This is achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field (electrophoresis).
- Shorter molecules move faster and migrate farther than longer ones .

At any given P^H, exist in a solution as electrically charged species either as a cation (+) or anion(-).

Under the influence of an electric field these charged particles will migrate either to cathode or anode, depending on the nature of their **net** charged

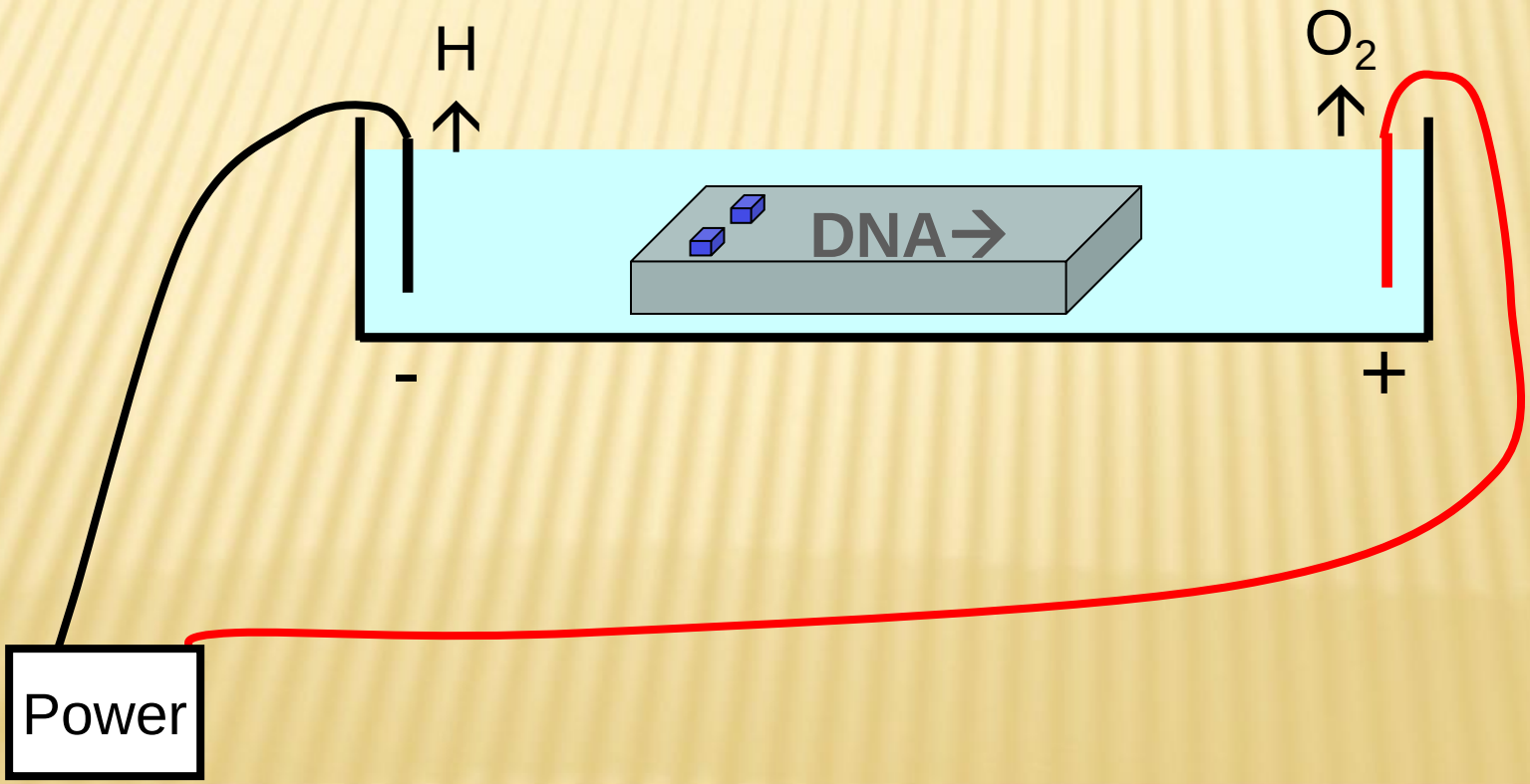
- Electrophoresis is the movement of molecules by an electric current.

- Nucleic acid moves from a negative to a positive pole.

- DNA is negatively charged.

- When placed in an electrical field, DNA will migrate toward the positive pole (Cathode).

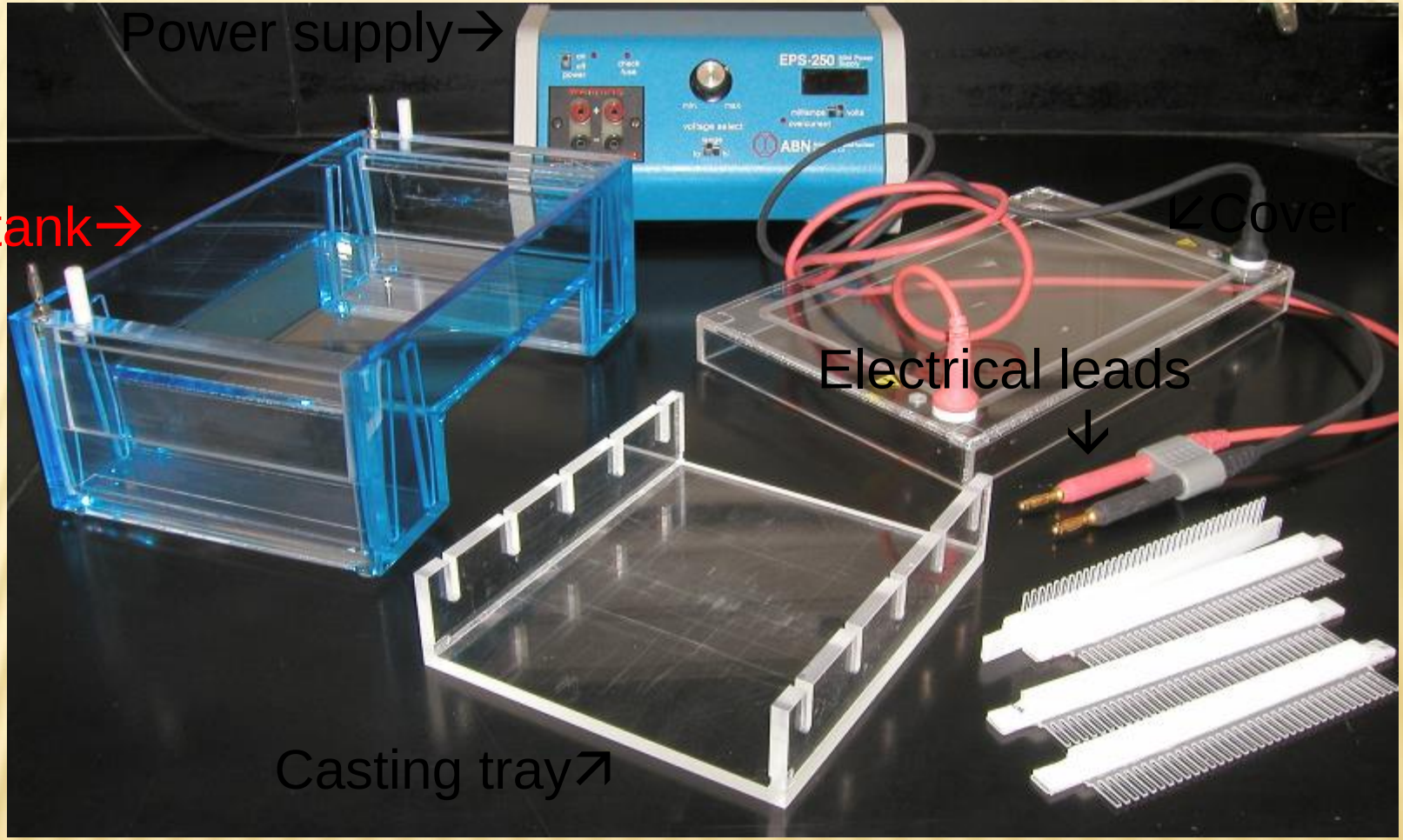
- An agarose gel is used to slow the movement of DNA and separate by size.



COMPONENTS OF AN ELECTROPHORESIS SYSTEM

- Power supply and chamber, a source of negatively charged particles with a cathode and anode
- Buffer, a fluid mixture of water and ions
- Agarose gel, a porous material that DNA migrates through
- Gel casting materials
- DNA ladder, mixture of DNA fragments of known lengths
- Loading dye, contains a dense material and allows visualization of DNA migration
- DNA Stain, allows visualizations of DNA fragments after electrophoresis

Electrophoresis Equipment



Agarose Gel

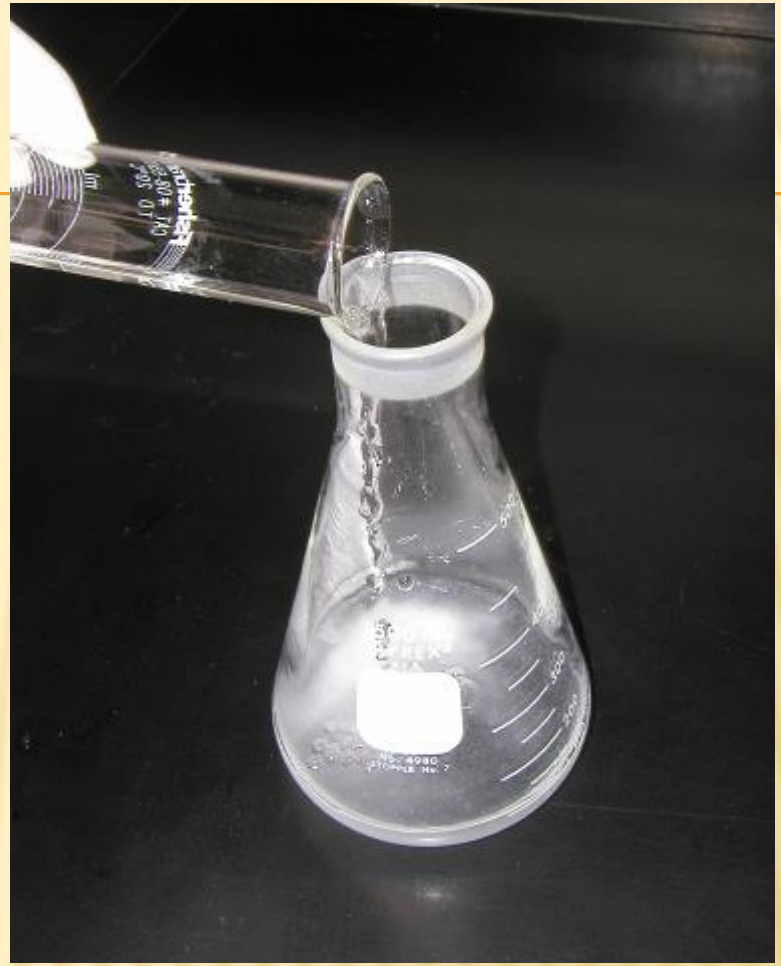
- A porous material derived from red seaweed
- Acts as a sieve for separating DNA fragments; smaller fragments travel faster than large fragments
- Concentration affects DNA migration
  - Low conc. = larger pores→ better resolution of larger DNA fragments

1% agarose

2% agarose

High conc. = smaller pores→ better resolution of smaller DNA fragments

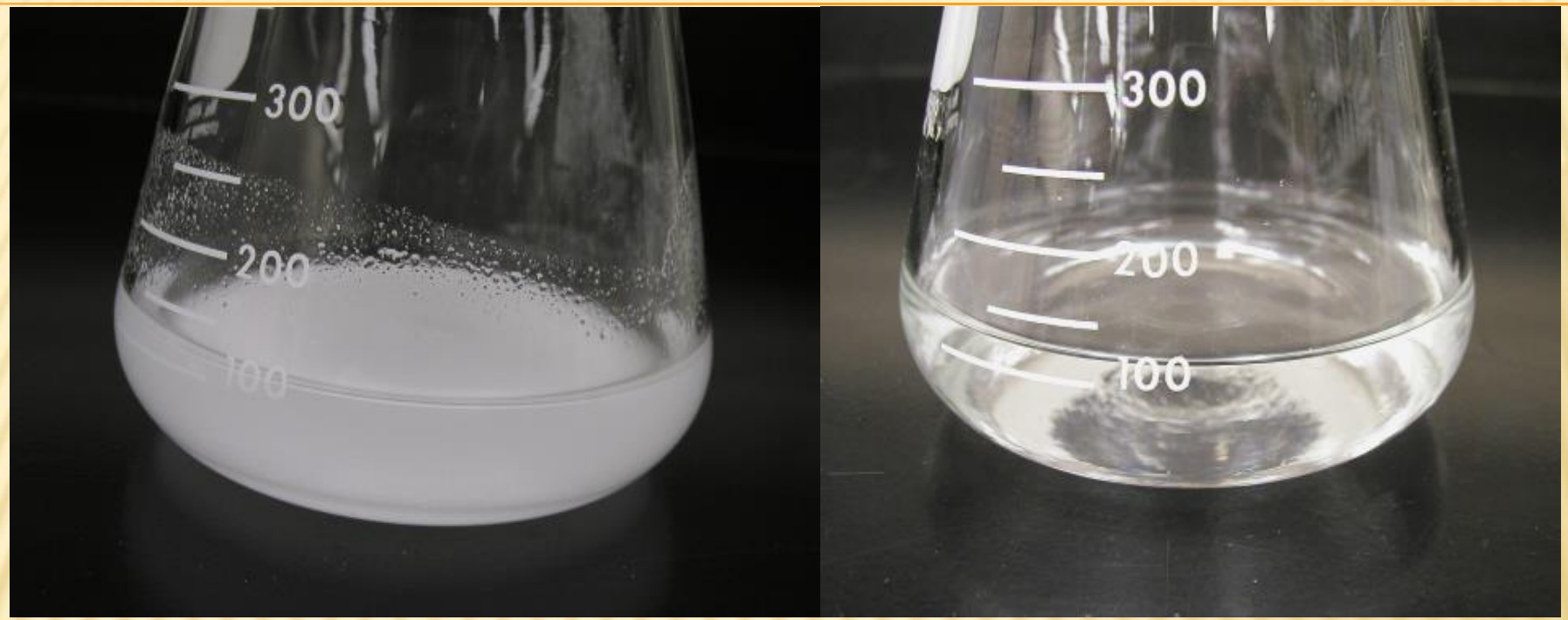
Agarose



Buffer Solution

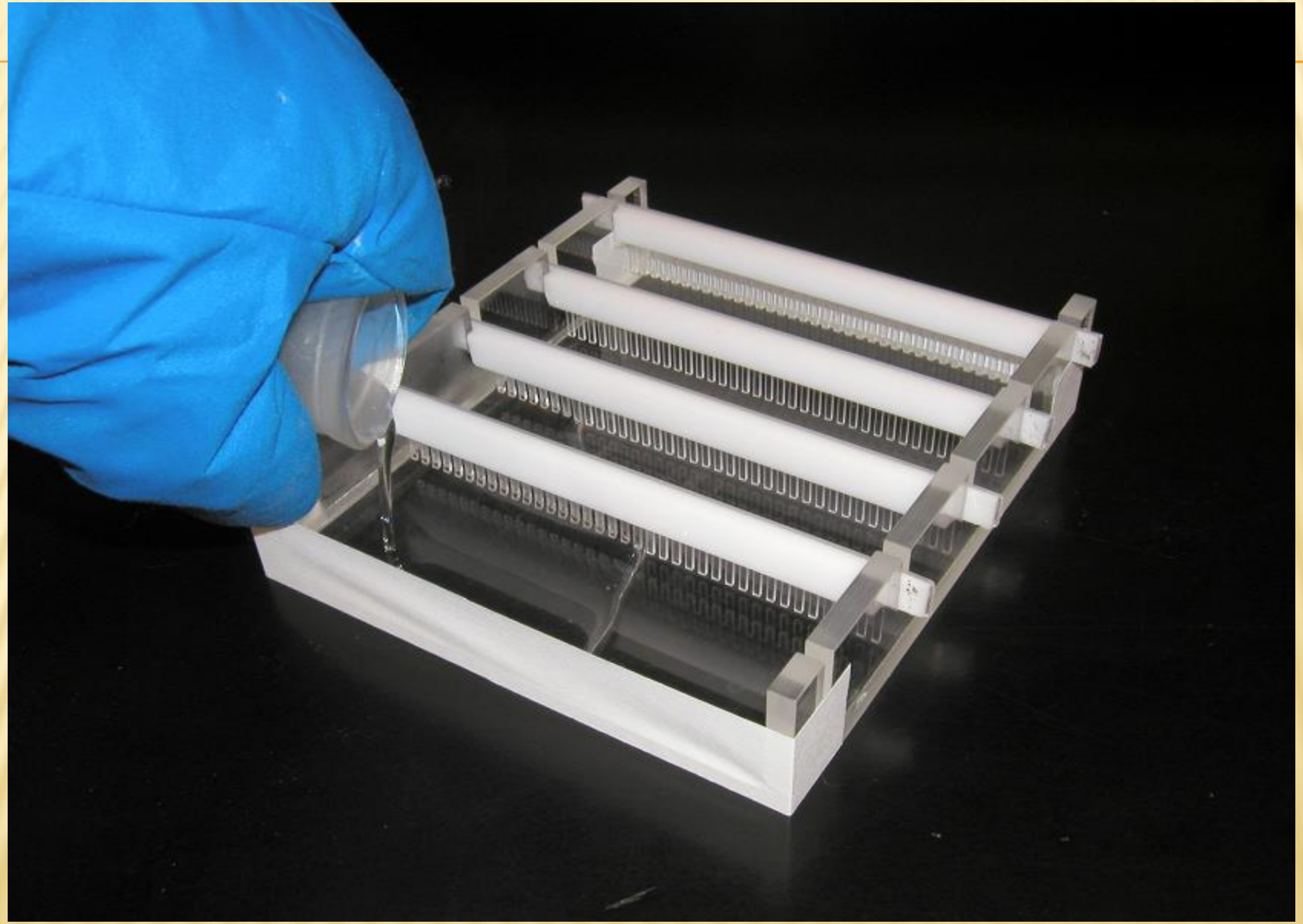
Combine the agarose powder and buffer solution. Use a flask that is several times larger than the volume of buffer.

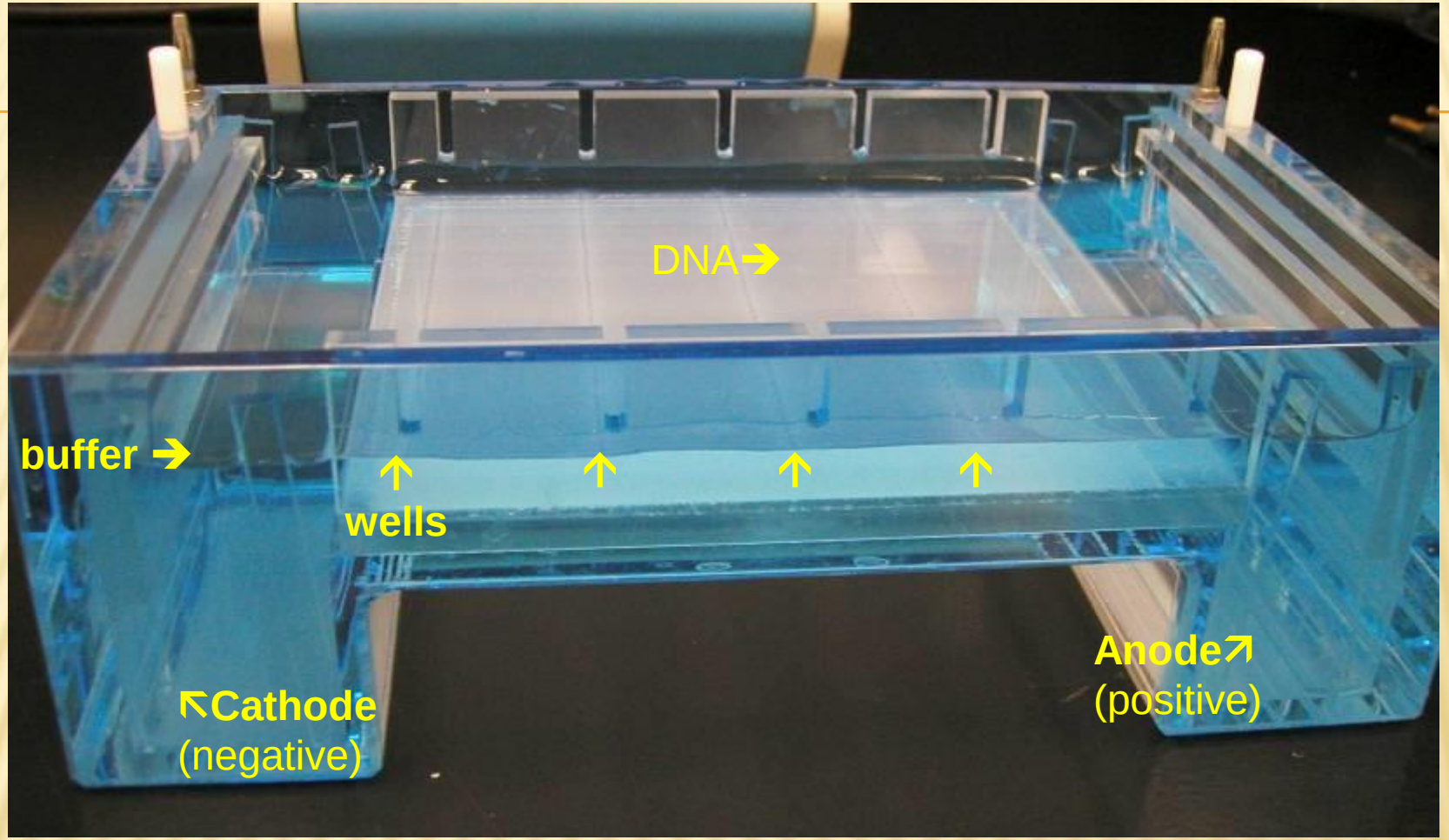
Melting the Agarose



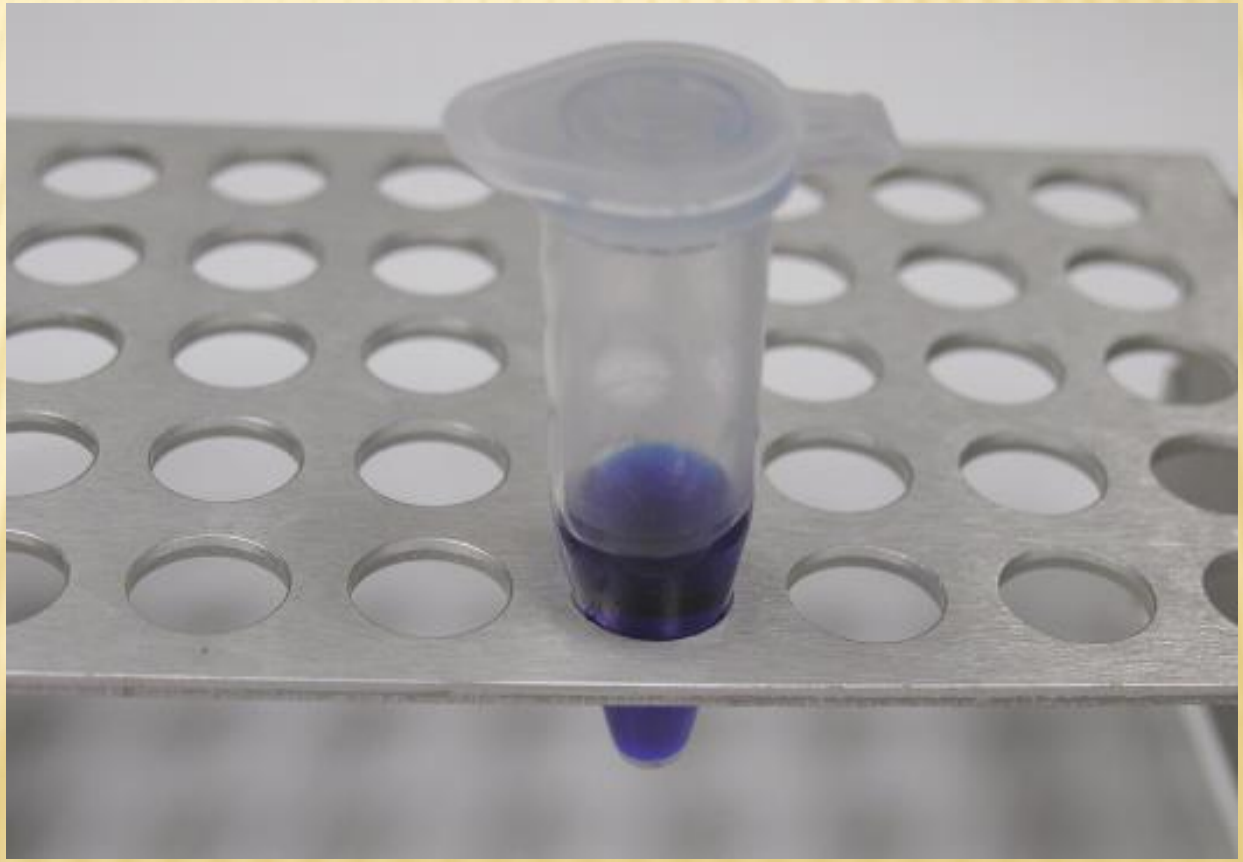
Agarose is insoluble at room temperature (left).
The agarose solution is boiled until clear (right).

Pouring the gel

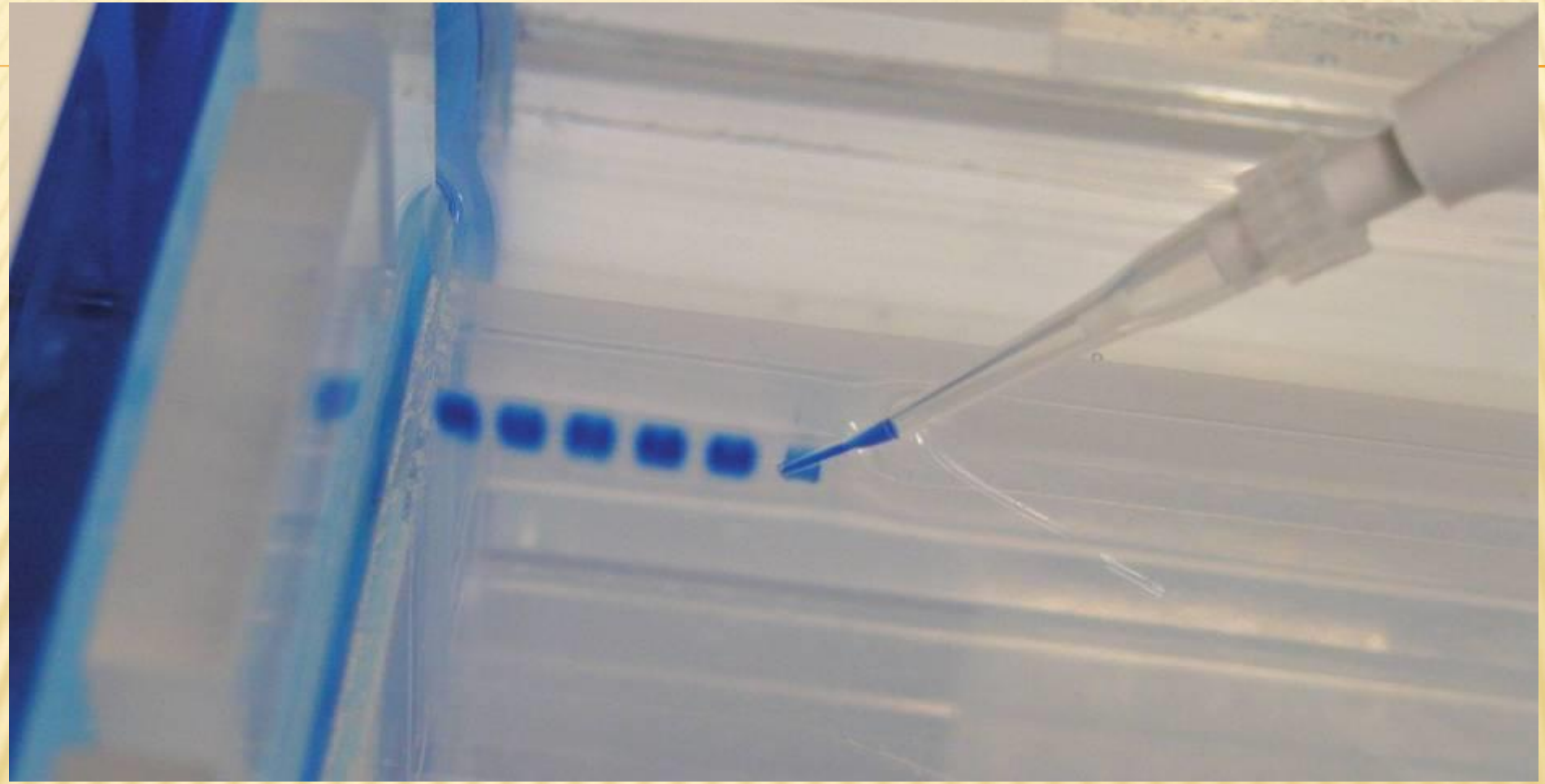



DNA➔
buffer ➔
wells
↖Cathode
(negative)
Anode↗
(positive)

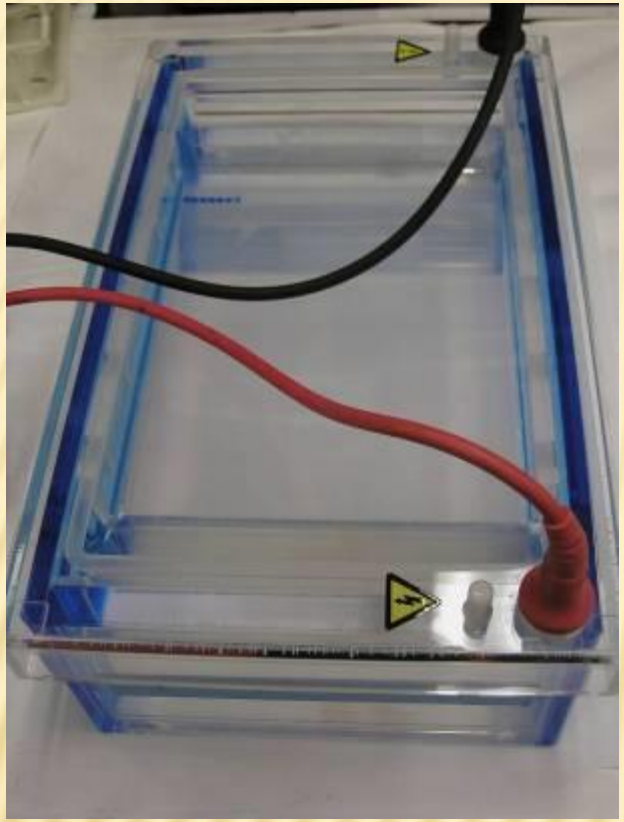
Sample Preparation

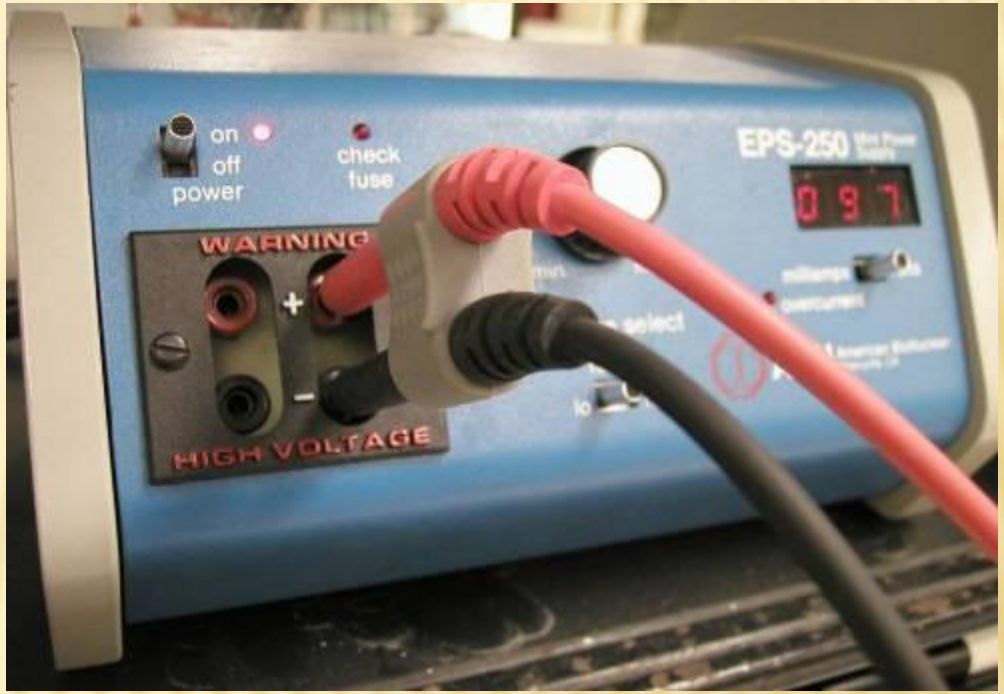


Loading the Gel



Running the Gel





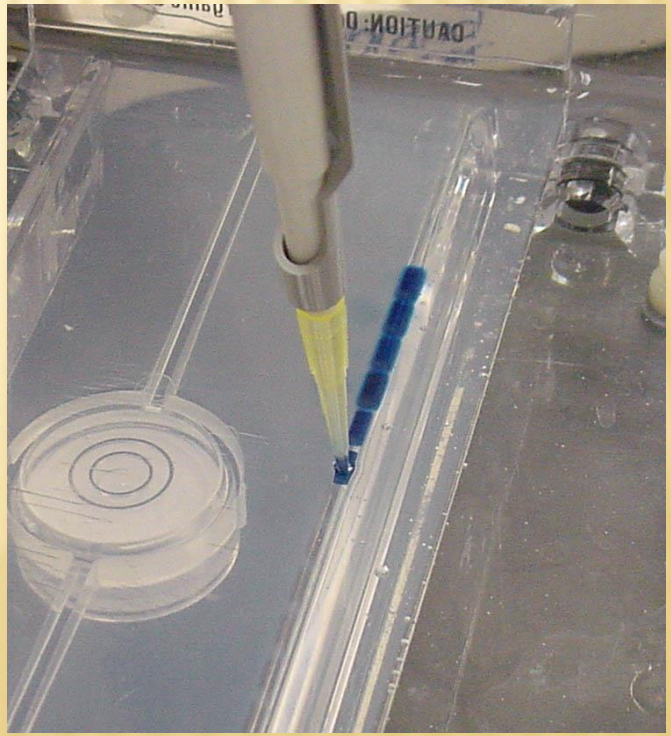
Electrophoresis Buffer

- TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA) are the most common buffers for duplex DNA
- Establish pH and provide ions to support conductivity
- Concentration affects DNA migration
  - Use of water will produce no migraton
  - High buffer conc. could melt the agarose gel
- New Sodium Borate (SB) buffer allows gels to be run at higher voltages in less time than traditional buffers

Loading Dye

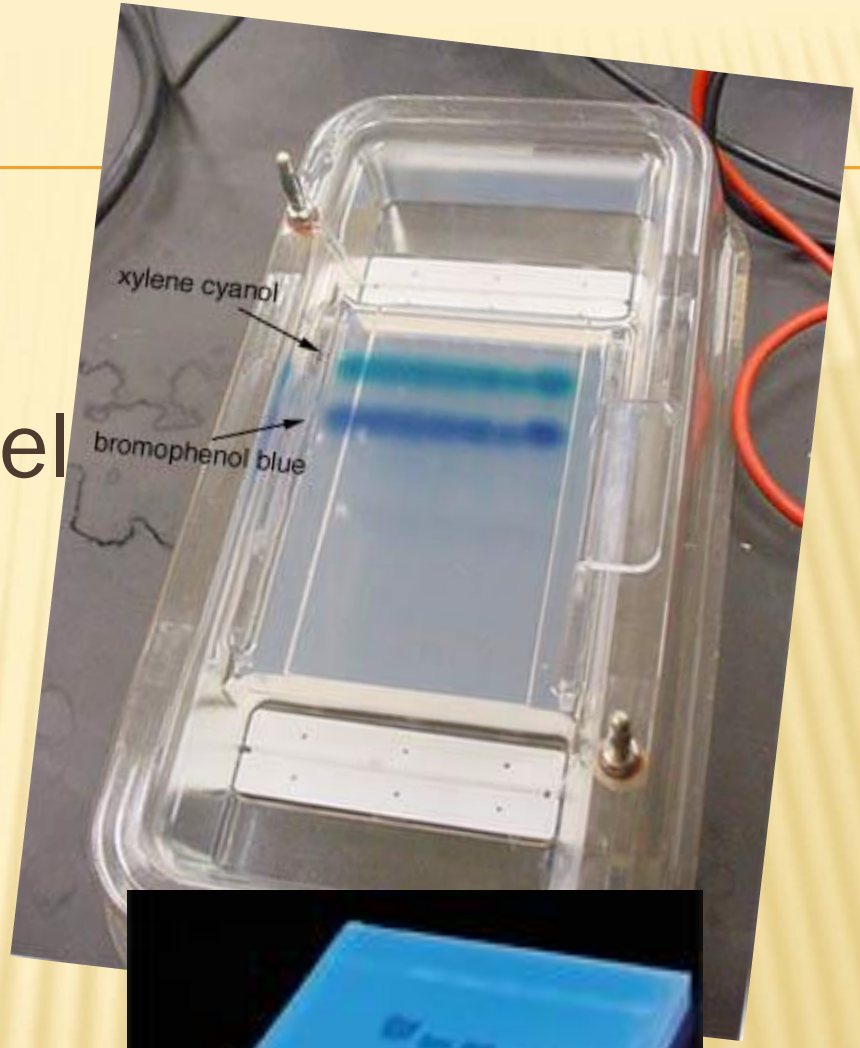
DNA samples are loaded into a gel AFTER the tank has been filled with buffer, covering the gel

- Contains a dense substance, such as glycerol, to allow the sample to "fall" into the sample wells
- Contains one or two tracking dyes, which migrate in the gel and allow monitoring of how far the electrophoresis has proceeded.



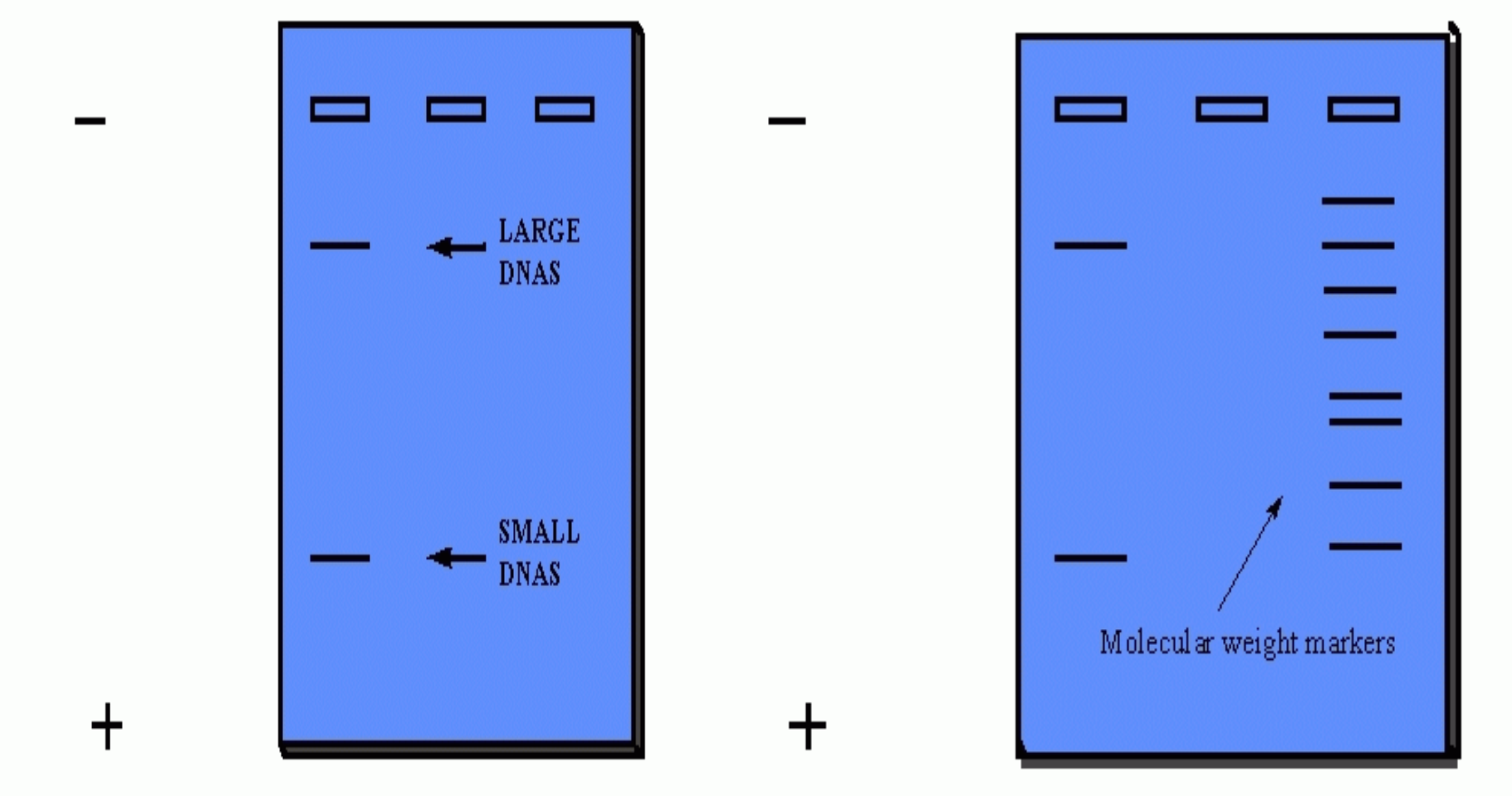
DNA Staining

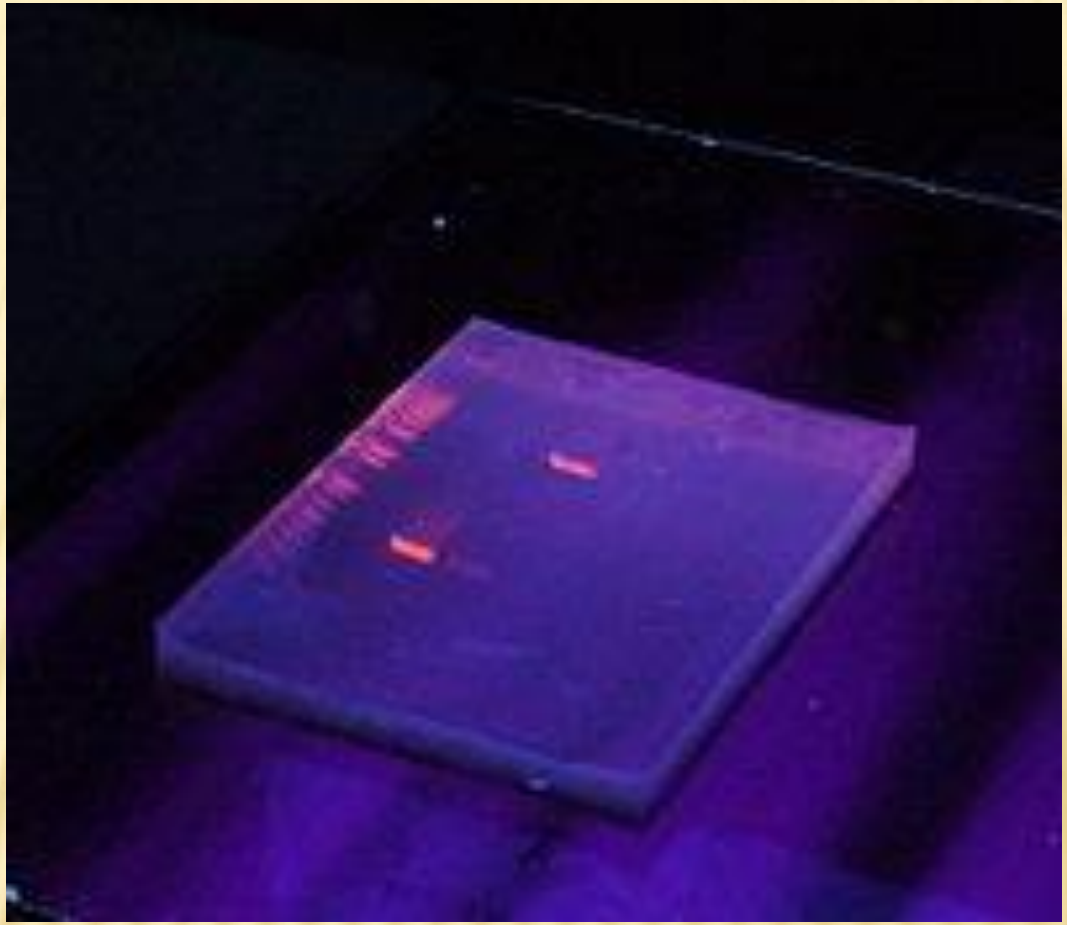
- Allows DNA visualization after gel electrophoresis
- Ethidium Bromide
  - In gel staining



Analysis

- After electrophoresis the gel is illuminated with an ultraviolet lamp to view the DNA bands. The ethidium bromide fluoresces reddish-orange in the presence of DNA.
- photograph it with a digital camera.


-
LARGE
DNAS
SMALL
DNAS
+
-
Molecular weight markers
+



Applications

Estimation of the size of DNA molecules following restriction enzyme digestion, e.g. in restriction mapping of cloned DNA.

Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting

Separation of DNA fragments for extraction and purification.

Separation of restricted genomic DNA prior to Southern transfer, or of RNA prior to Northern transfer.

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

- Synthetic polymer
- Formed from acrylamide subunits.
- Acrylamide with a cross linker, methylene bis acrylamide .
- Polymerization catalysts:
  - Ammonium persulfate (APS)
  - Tetramethylethylenediamine (TEMED)
- Light
- 3.5 20 % concentration.
- High resolution.
- Acrylamide is a dangerous neurotoxin

BUFFER

- Provides ions in solution for electrical conductivity.
- Prevents the pH changing.
- Common using buffers:
  - Tris Borate EDTA (TBE) Stable, expensive,PAGE , long separation time.
  - Tris Acetate EDTA (TAE) Inexpensive,short separation time.
  - Tris Phosphate EDTA (TPE)

DO NOT FORGET!

- DNA molecule is an organic acid.
- Negatively charged.
- Migrate toward the positive electrode(Cathod) in an electromagnetic field.
- Do not forget " Running of the gel "
- Cut off electricity before taking gel from
- apparatus.

PROTEIN ELECTROPHORESIS

- Simple to use and highly reproducible technique.
- Provide information of the molecular weight,charged, subunits, purity of protein mixture.

OTHER PROTEIN ELECTROPHORESIS TECHNIQUES

- **IEF(Isoelectric focusing)** : Separates proteins by their isoelectric points (pI) by using pH gradient of the gel.
- **2D PAGE (Two dimensional gel electrophoresis):** Separates proteins by two properties (eg : pI and size) in a mixture.
- **Western blotting**: Separating proteins first by size then staining with specific antibody antigen reactions. technique gives molecular weight and identifies specific protein.

PULSED FIELD GEL ELECTROPHORESIS (PFGE)

- Used for separating very large DNA molecules. based on the periodically changes of directions in the electric field. used for genotyping.

CAPILLARY ELECTROPHORESIS APPLICATIONS

- Analyzing proteins in physiological matrices eg.(Serum , urine)
- DNA analysis
- Drug screening.
- Analysis of pesticides, food content,
- pollutants.