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Agarose Gel Electrophoresis

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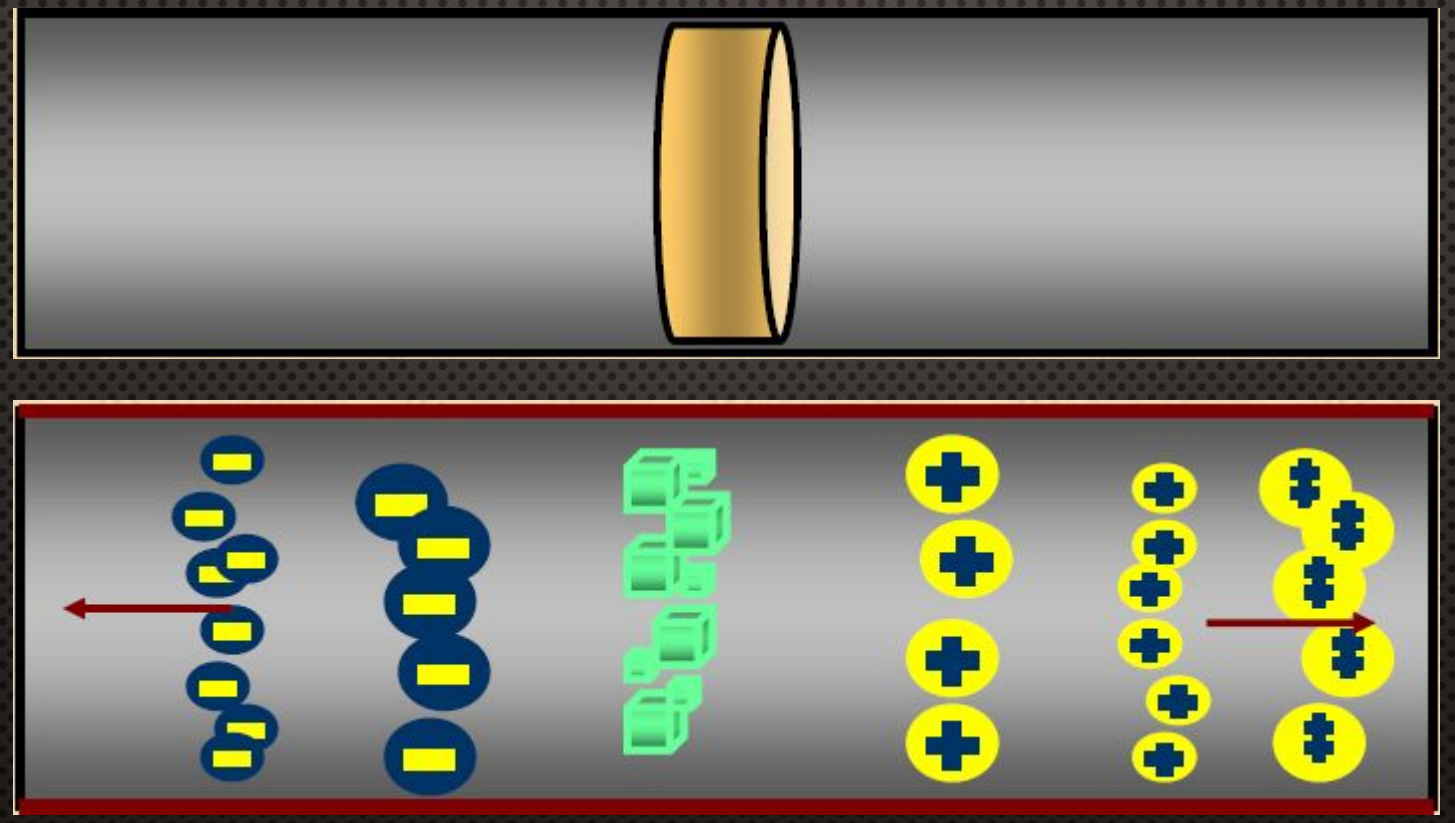
Electrophoresis:- Introduction

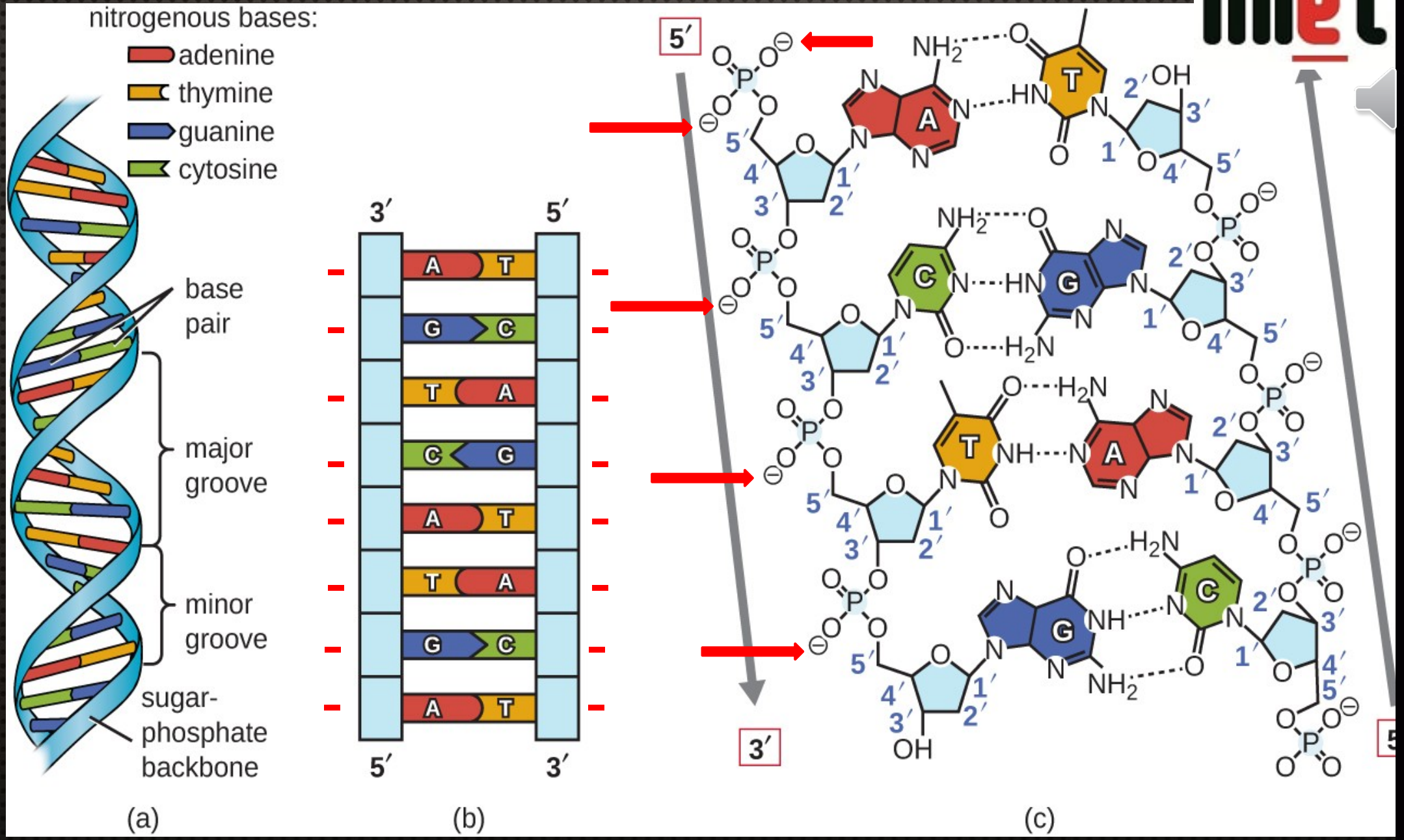
- Electrophoresis (greek term: "to bear electrons“) discovered by **Arne Tiselius**, a Swedish biochemist in 1931.
- It describes the migration of a charged particle under the influence of an electric field
- Biological molecules: **amino acids (also peptides & proteins), nucleotides and nucleic acids.**
- Both these biomolecules possess **ionisable groups**, therefore, at any given pH, exist in solution as **electrically charged (+ or -).**
- These charged particles will migrate either to the cathode or to the anode.

Fundamentals:

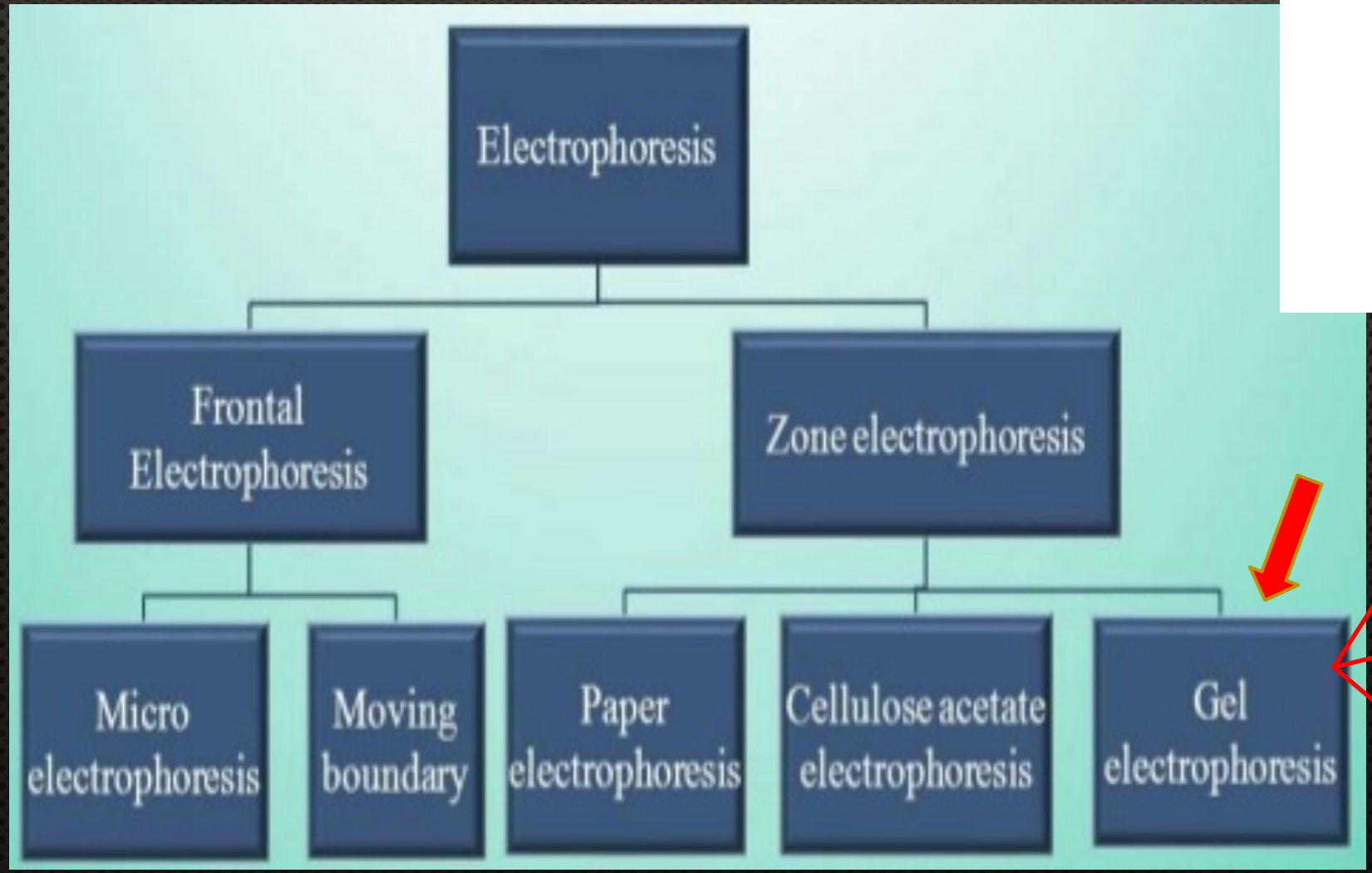


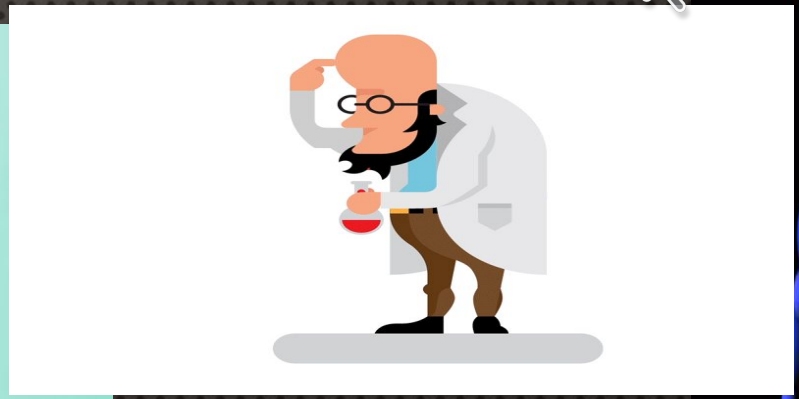
The migration of electrically charged particles or ions in solutions due to an applied electric field.

- Electrophoresis is used extensively in **DNA, RNA and Protein Analysis**


nitrogenous bases:
adenine
thymine
guanine
cytosine
base pair
major groove
minor groove
sugar-phosphate backbone
3′
5′
A T
G C
T A
C G
A T
T A
G C
A T
5′
3′
5′
3′
(a)
(b)
(c)

TYPES OF ELECTROPHORESIS





a. **Agarose Gel Electrophoresis**

b. **Starch Gel Electrophoresis**

c. **Poly-acrylamide Gel Electrophoresis**

Agarose Gel Electrophoresis:

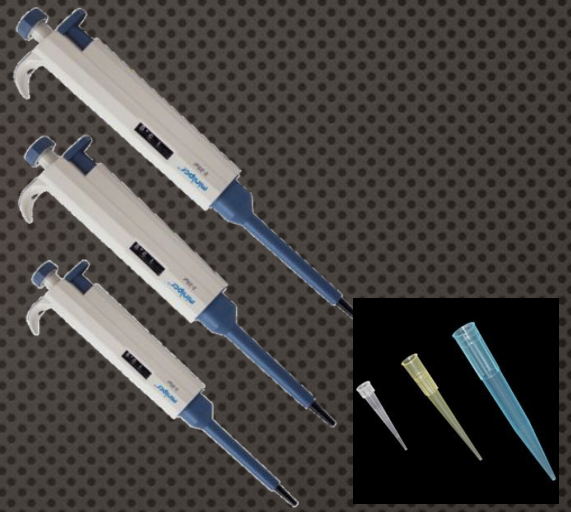
Agarose gel electrophoresis is the easiest and most popular way of separating and analysing DNA. Here DNA molecules are separated on the basis of charge by applying an electric field to the electrophoretic apparatus. Shorter molecules migrate more easily and move faster than longer molecules through the pores of the gel and this process is called sieving. The gel might be used to look at the DNA in order to quantify it or to isolate a particular band. The DNA can be visualized in the gel by the addition of Ethidium bromide.

Principle:

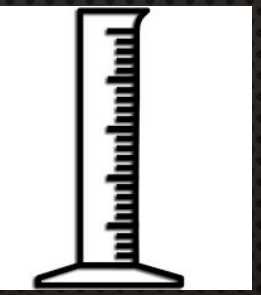
Agarose gel electrophoresis separates the biomolecules based on the movement of charged particles under the influence of uniform electric field. Thus particles get separated under sieved matrix based on variation in their charges and sizes.

Materials Required:

Glasswares:
Conical flask, Measuring cylinder, Beaker



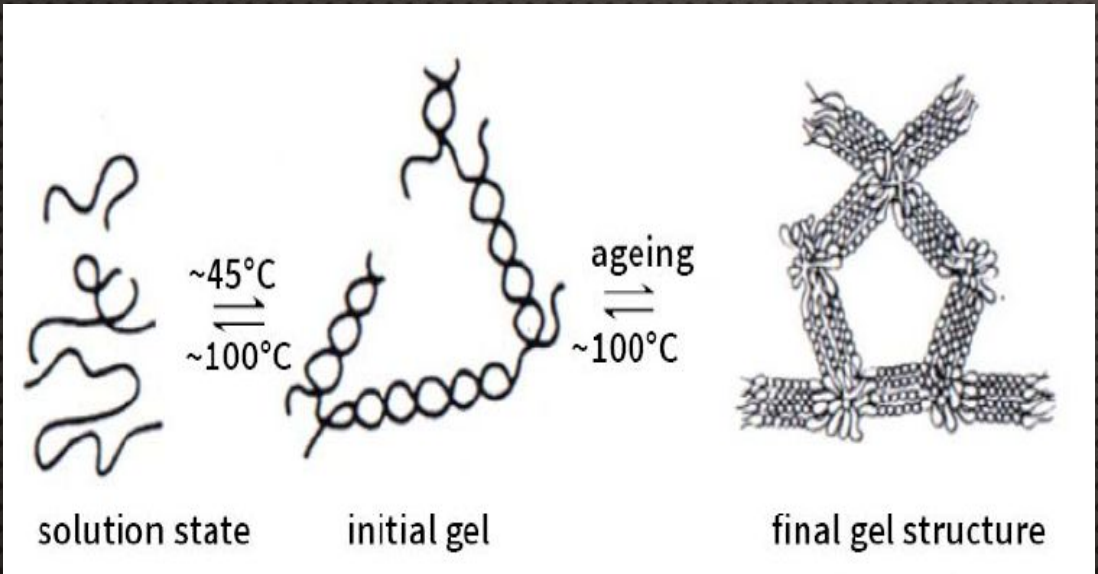
Reagents:
Distilled water, Agarose, TAE Buffer (50X), Ethidium bromide (10 mg/ml), Loading dye



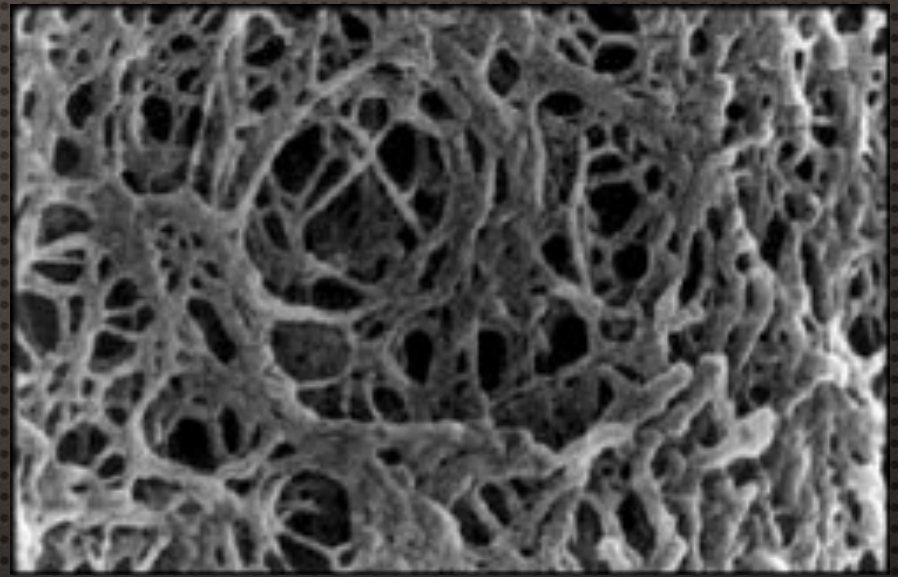
Other Essential Requirements:
Electrophoresis apparatus, UV Transilluminator, Micropipettes with Tips, Adhesive tape, Microwave/Burner/Hotplate

Sample:
DNA Sample obtained after DNA isolation technique

Agarose Gel: A CROSS LINKING POLYMER MATRIX



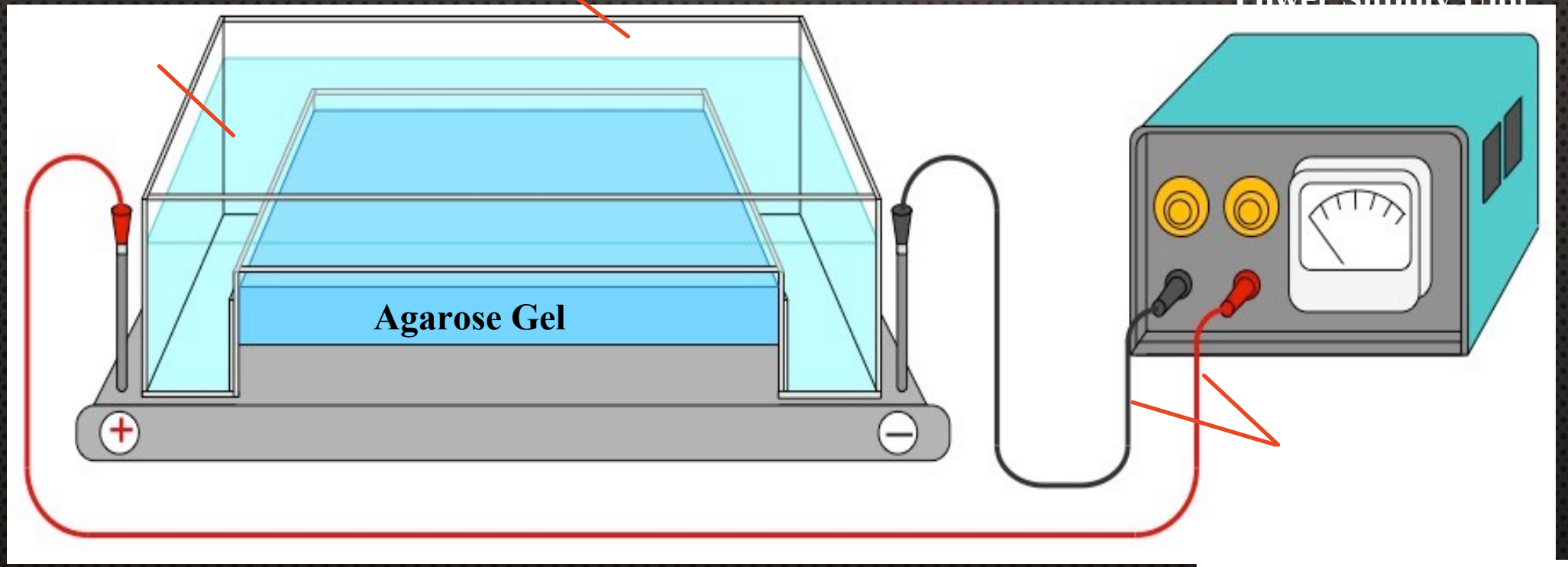
Different states of agarose gel formation depending upon the temperature



Highly magnified view of Agarose gel matrix

- Separation molecular sieving technique, based on the molecular size of substances.
- Acts as a "molecular sieve" for separating molecules.
- is electrically neutral.
- acts as a sieve by retarding /obstructing the movement of macro-molecules

ELECTROPHORESIS APPARATUS

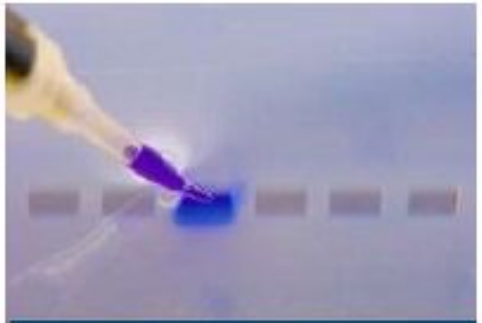


Procedure

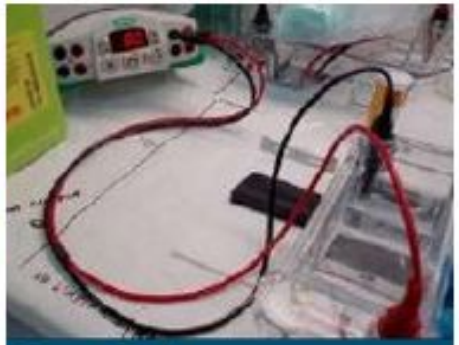
Set up gel

Load Buffer

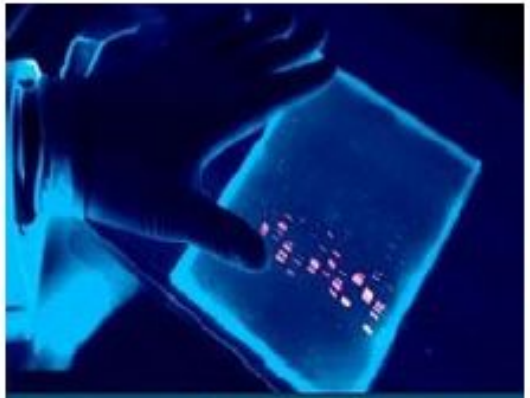
Get your sample obtained from previous purifying technique



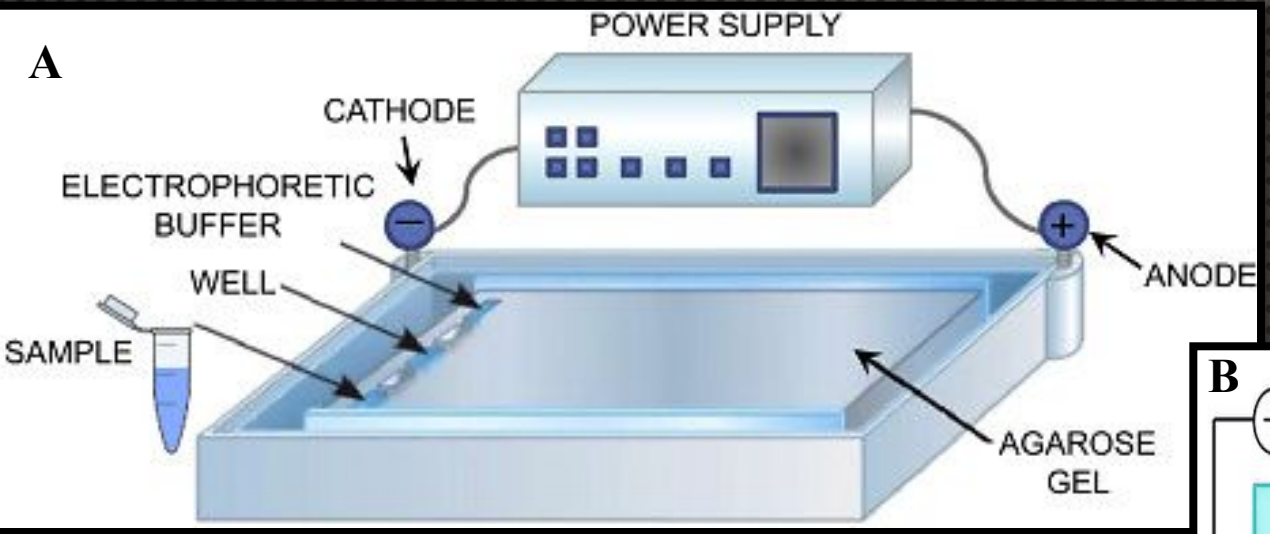
Load Sample

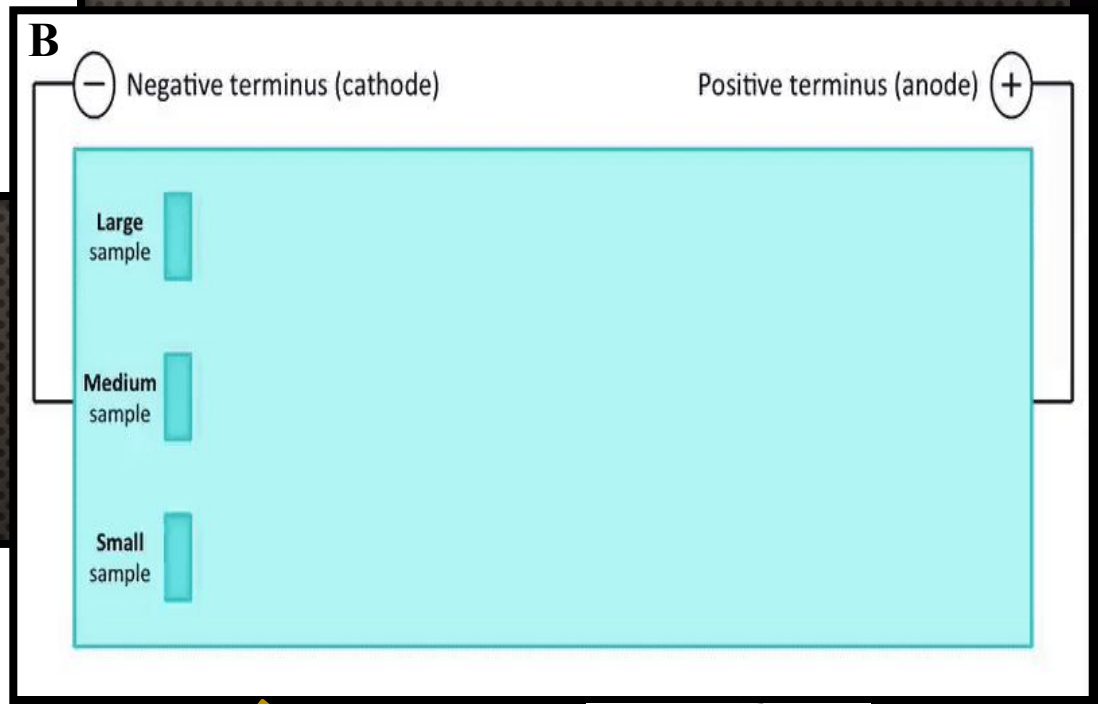


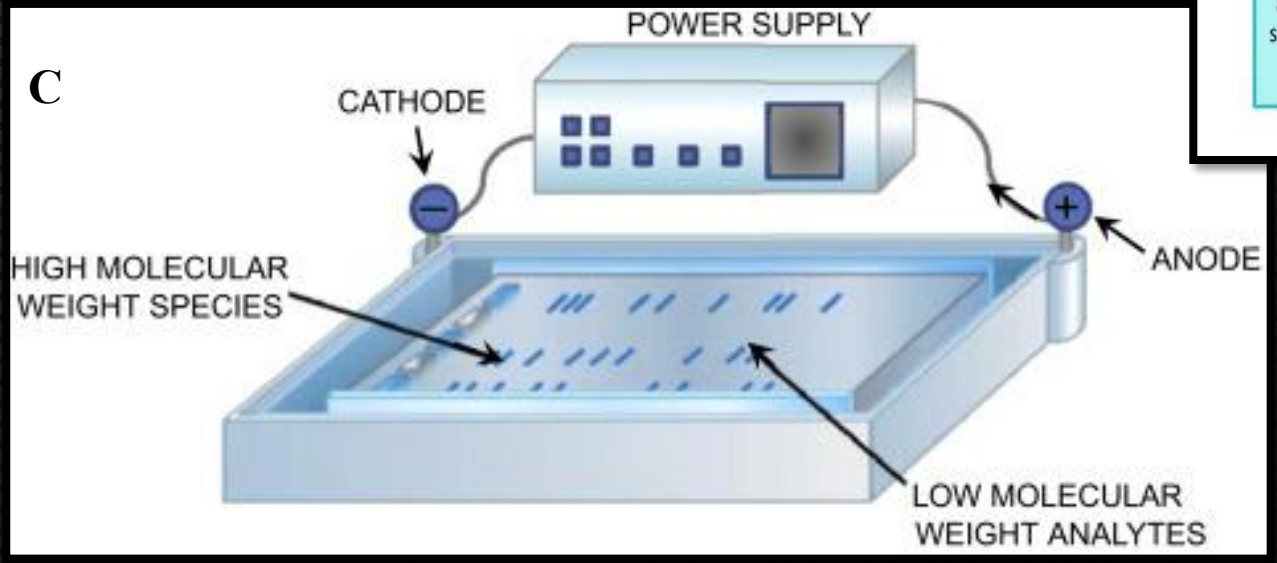
Run Gel



Stain and look at with UV light


A
POWER SUPPLY
CATHODE
ELECTROPHORETIC BUFFER
WELL
SAMPLE
ANODE
AGAROSE GEL


B
Negative terminus (cathode)
Positive terminus (anode)
Large sample
Medium sample
Small sample


C
POWER SUPPLY
CATHODE
ANODE
HIGH MOLECULAR WEIGHT SPECIES
LOW MOLECULAR WEIGHT ANALYTES

Let's do

MOLECULAR BIOLOGY LAB

miet

Agarose Gel Electrophoresis

ADVANTAGES:

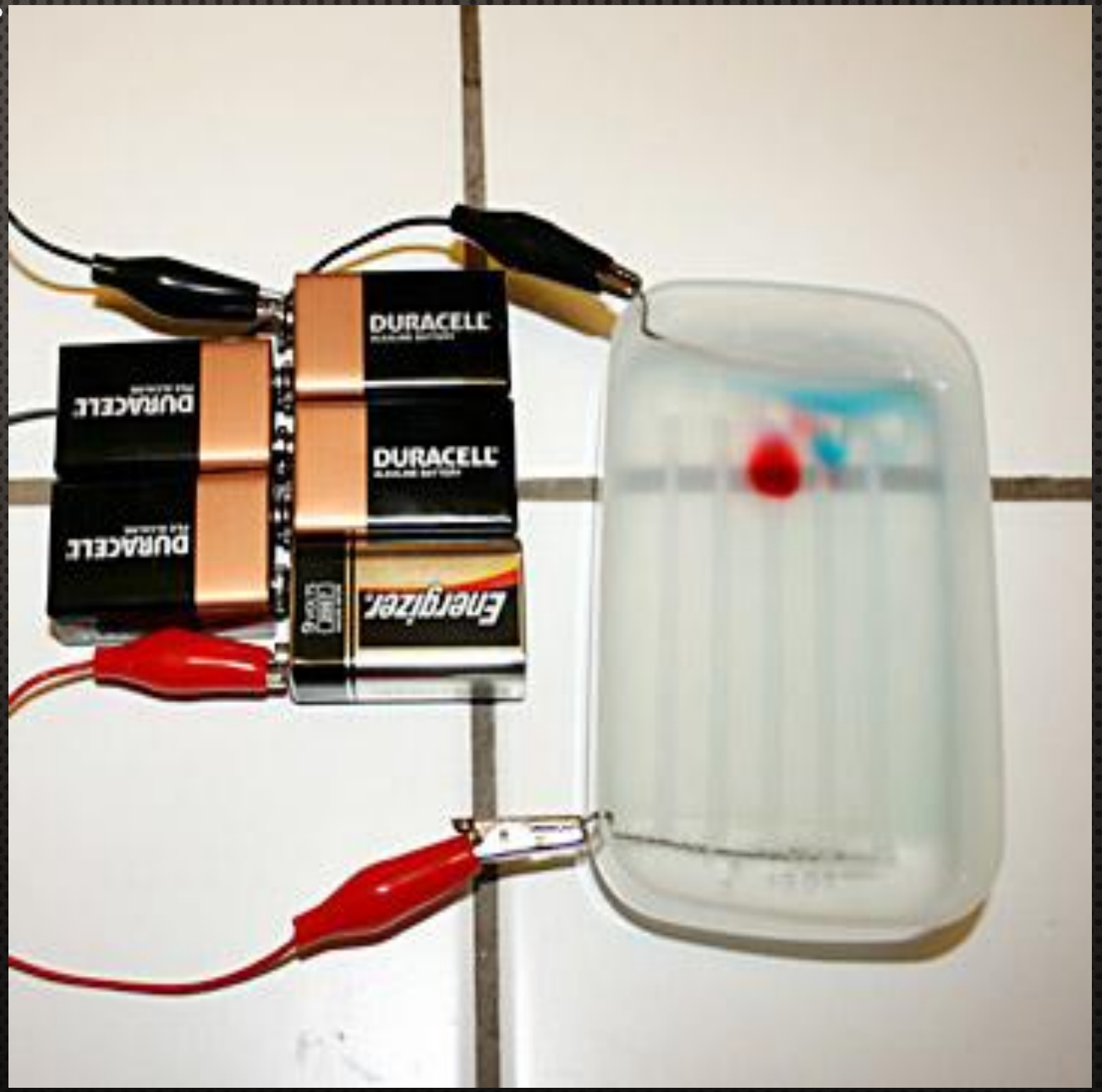
- Easy to prepare and small concentration of agarose is required.
- Resolution is good enough.
- Small sample quantities can be separated and recovered.
- Adsorption of negatively charged protein molecule is negligible.
- Sharp bands are obtained thus easy to differentiate.
- Recovery of specific segment is possible.
- good method for preparative purpose

A Bonus DIY tip:

Create your own Electrophoresis Unit-

Materials Required:

1. A rectangular Plastic box
2. Batteries (preferably couple of 9v's)
3. Connecting wires
4. Metal wires (Bare/sleeveless)
5. Gelling agent (ex: Gelatin)
6. Samples (try with ink mixtures)



THANK YOU

ANY QUESTION