

DNA Molecular Size Determination

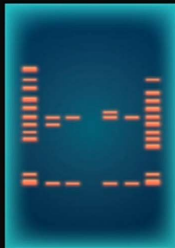
Gel Electrophoresis



Separation of DNA Fragments on a Piece of Gel

Gel Electrophoresis

Technique to separate the different DNA fragments based on their sizes

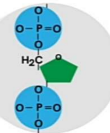


Gel Medium

Movement of charged molecules $\xrightarrow{\text{Occurring in}}$ Electrical field



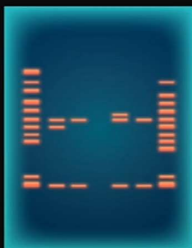
DNA \rightarrow Negatively charged
(Due to Phosphate groups)



Agarose gel
(Obtained from Sea weeds)

Gel Electrophoresis

Technique to separate the different DNA fragments based on their sizes



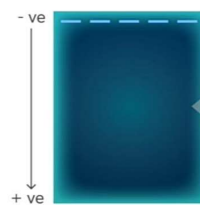
Gel Medium

Movement of charged molecules $\xrightarrow{\text{Occurring in}}$ Electrical field

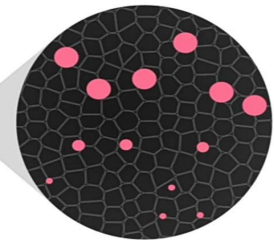


Sample of DNA

Principle of Separation of DNA Fragments



Agarose Gel



Mesh in Gel

Gel Electrophoresis

Requirements



Casting Tray



Gel



Comb

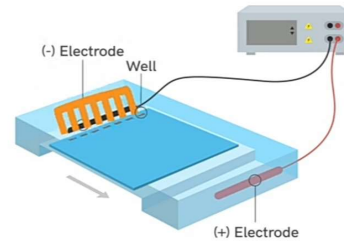


Electrical supply

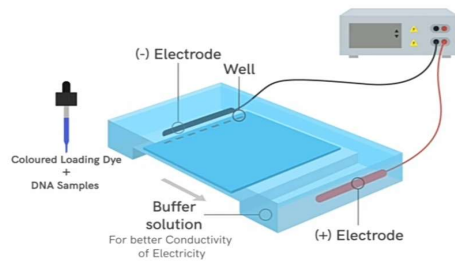


DNA Sample

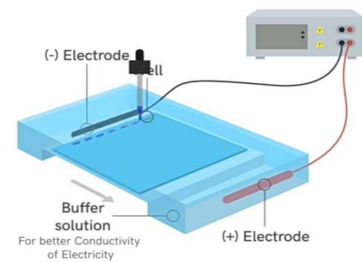
Gel Electrophoresis



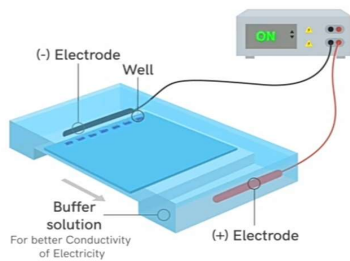
Gel Electrophoresis



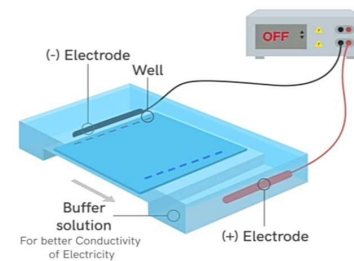
Gel Electrophoresis

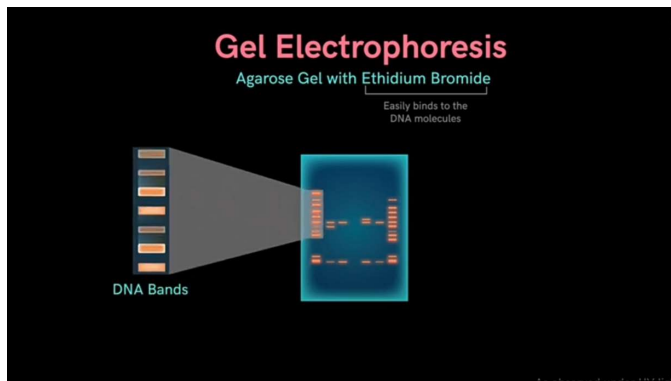


Gel Electrophoresis



Gel Electrophoresis





Aim: To determine the molecular size of double stranded DNA fragment

• **Introduction:** Agarose gel electrophoresis method is used to measure the molecular size of the DNA.

• **Principle:** Negative charged DNA migrates towards the positive charge anode according to their molecular size.

Requirements

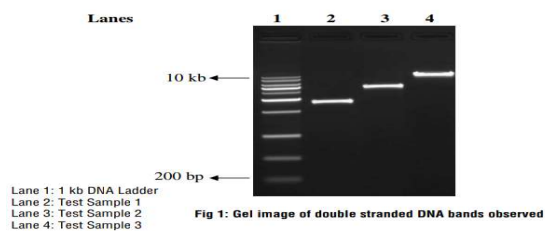
- Agarose.
- 50x TAE
- 1kb DNA ladder
- Glasswares
- Reagents (D/w, EtBr.)
- Other requirements (Electrophoresis apparatus, UV transilluminator, etc.)

Procedure

1. Preparation of gel.
2. Pour the gel in the tray, let it solidify.
3. Place the tray in electrophoretic chamber filled with 1X TAE buffer.
4. Load the DNA ladder and DNA sample into the wells.
5. Switch on the electric supply, and carry out the run.
6. Monitor the temp. periodically.
7. Switch off the power supply by tracking the dye.
8. Observe the gel on a UV transilluminator.

Observation and Result

Perform Agarose Gel Electrophoresis. Visualize the DNA bands using UV transilluminator.



- Calculate the distance travelled by the DNA by using the Rf value.

$$Rf = \frac{\text{Distance travelled by DNA molecule}}{\text{Distance travelled by the dye}}$$