AGAROSE GEL ELECTROPHORESIS

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INTRODUCTION

- The electrophoresis refers to the movement of charged particles and macromolecular ions under the influence of an electric field.

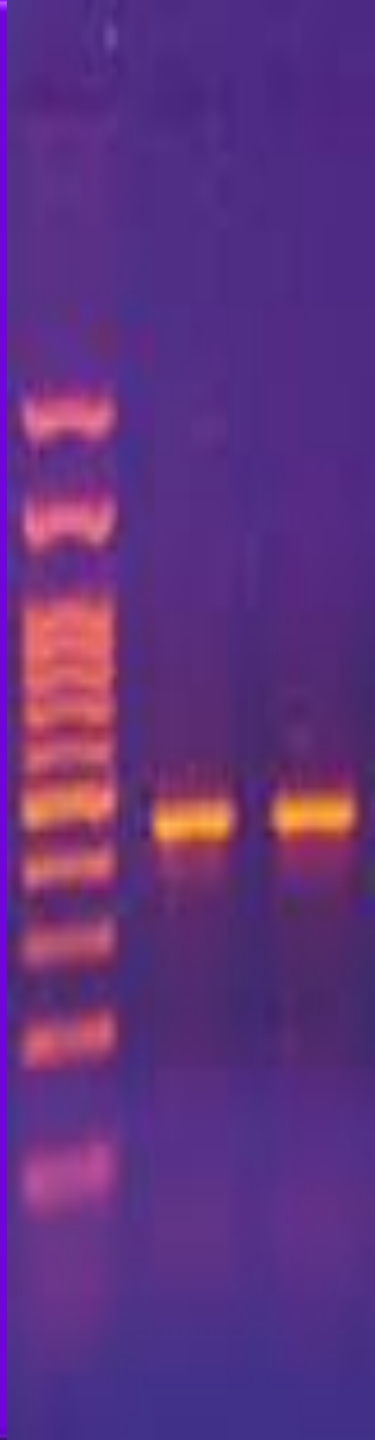
- The first recorded measurements of electrophoretic phenomenon were performed in 1861 by Quincke.

- Arne Tiselius, a Swedish biochemist won the Nobel Prize in Chemistry in 1948 for his research on electrophoresis.

Arne Tiselius

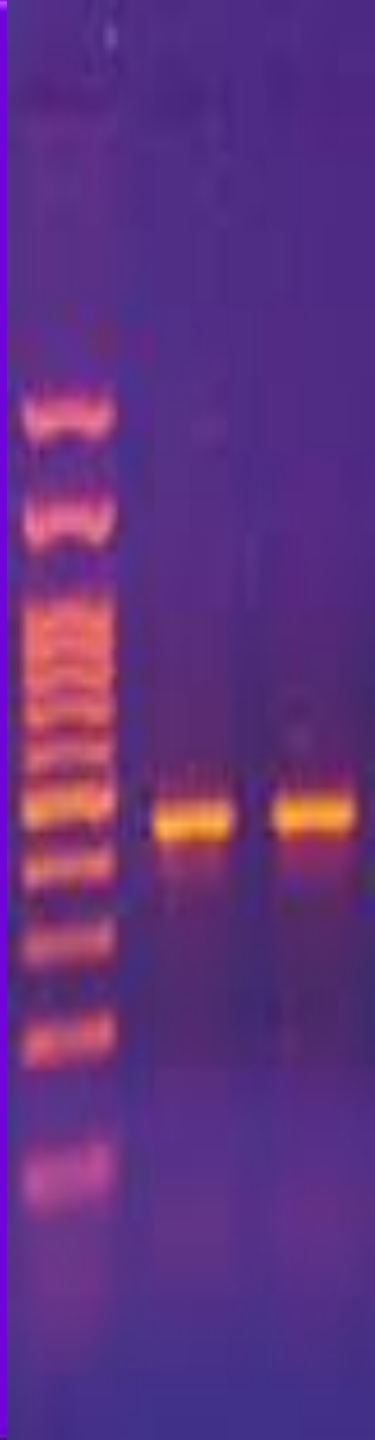
WHY AGAROSE GEL ELECTROPHORESIS

- To estimate the size of DNA molecules
- To determine DNA sequence
- Analyse PCR products, e.g.in molecular diagnosis or genotyping
- Determine the quality or quantity of DNA
- Purification of DNA



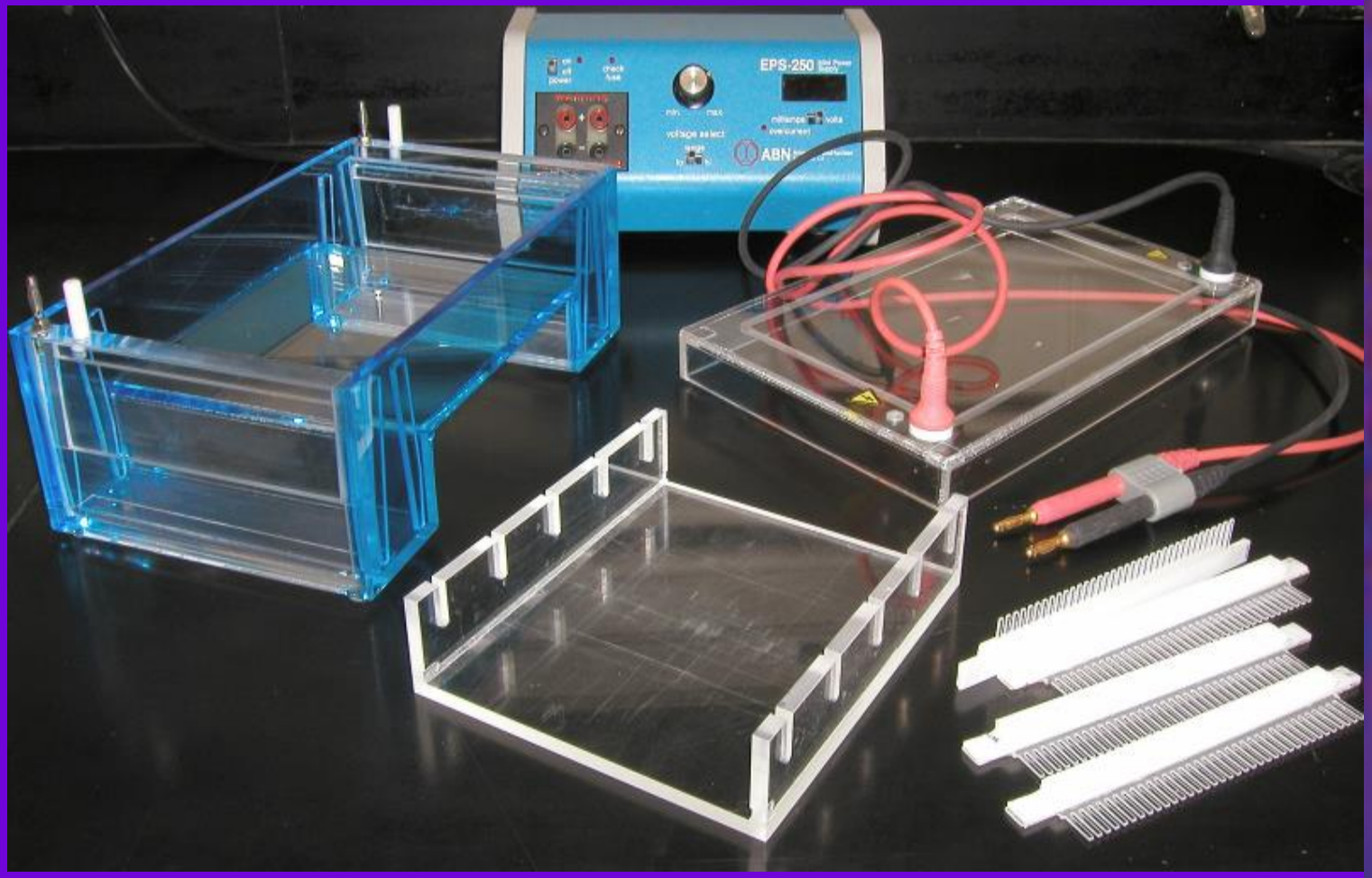
FACTORS AFFECTING ELECTROPHORETIC MOBILITY

- THE SAMPLE
  - Charge
  - Size
  - Shape
- THE MEDIUM
  - Adsorption
  - Molecular sieving
- THE BUFFER
  - Composition
  - Ionic strength
  - pH



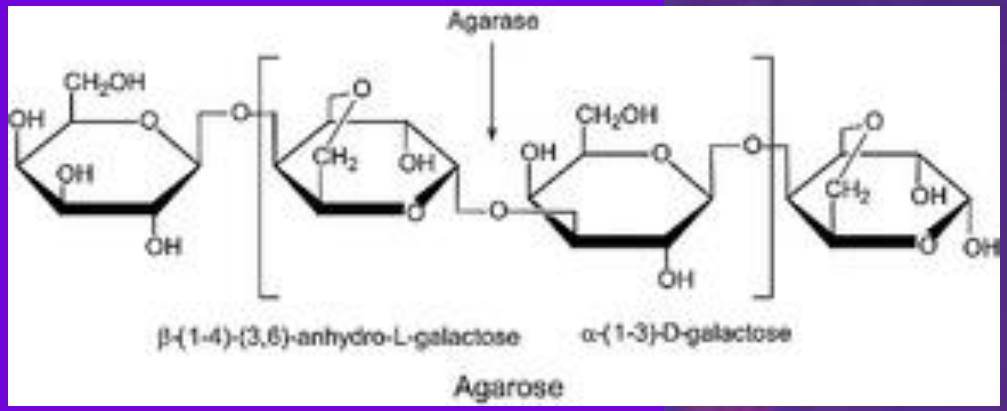
REQUIREMENTS

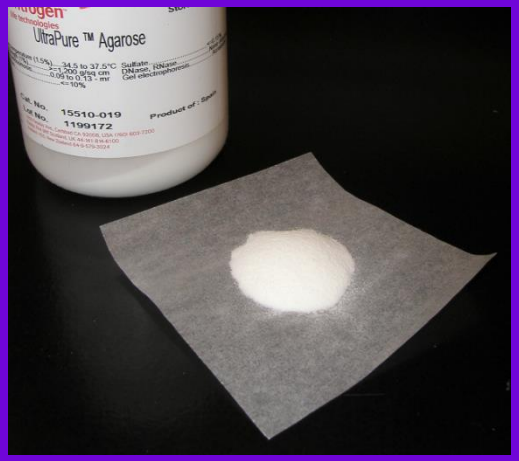
- ✓ Electrophoresis Equipments



✓ AGAROSE

Agarose is a polysaccharide, generally extracted from certain red seaweed. It is a linear polymer made up of the repeating unit of agarobiose, which is a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose





✓ DYE

- Bromophenol Blue
- Ethidium Bromide

PROCEDURE

Prepare agarose gel
Melt, cool and add Ethidium Bromide. Mix thoroughly.

⬇

Pour into casting tray with comb and allow to solidify

⬇

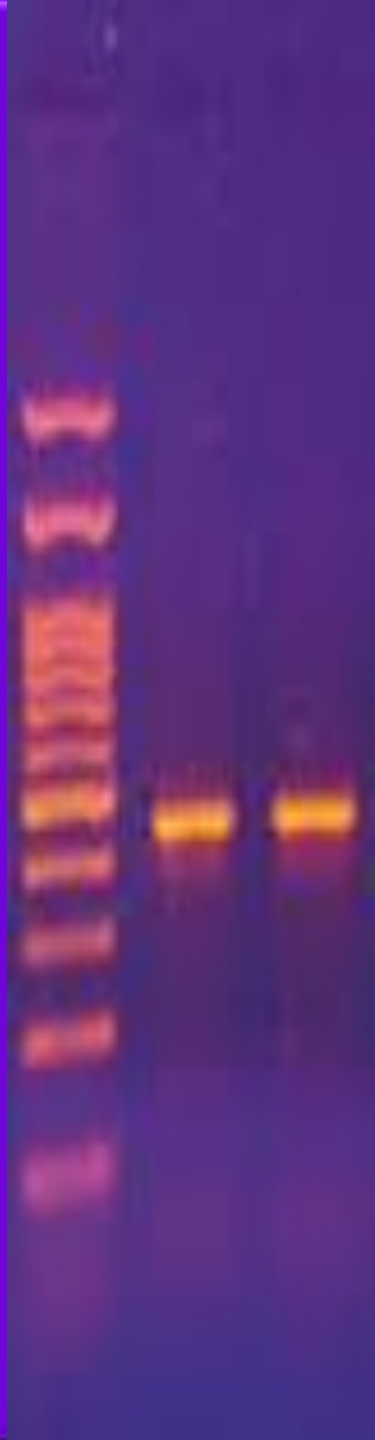
Add running buffer, load samples and marker

⬇

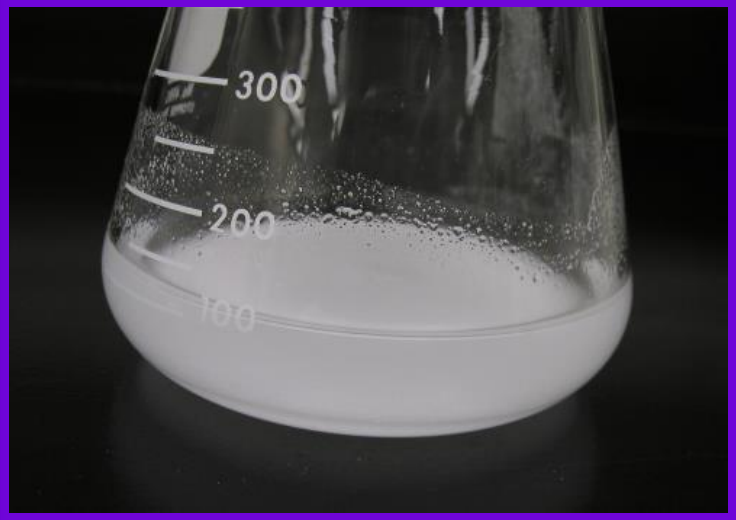
Run gel at constant voltage until band separation occurs

⬇

View DNA on UV Transilluminator

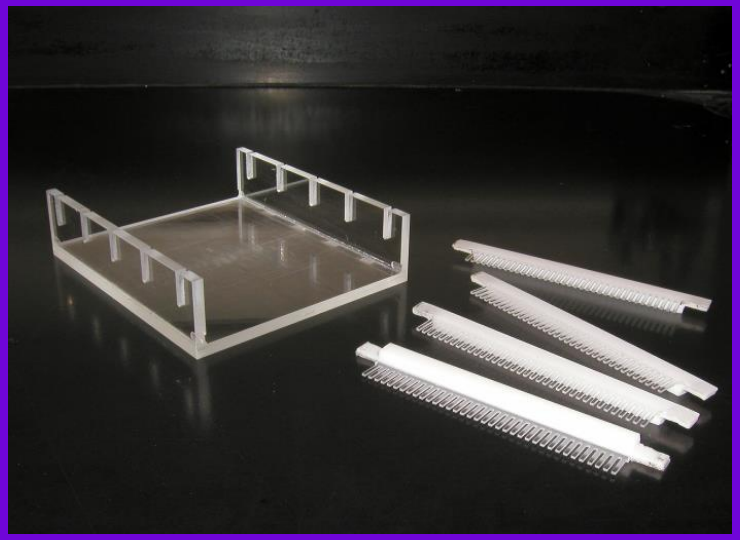


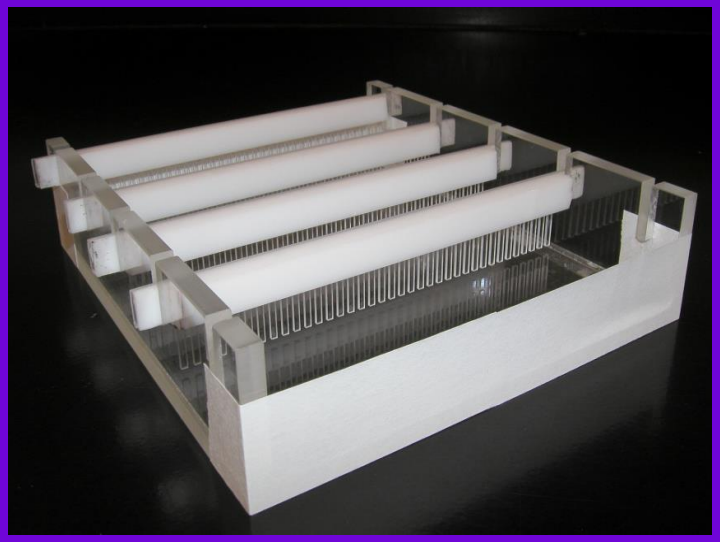
PREPARE AGAROSE GEL

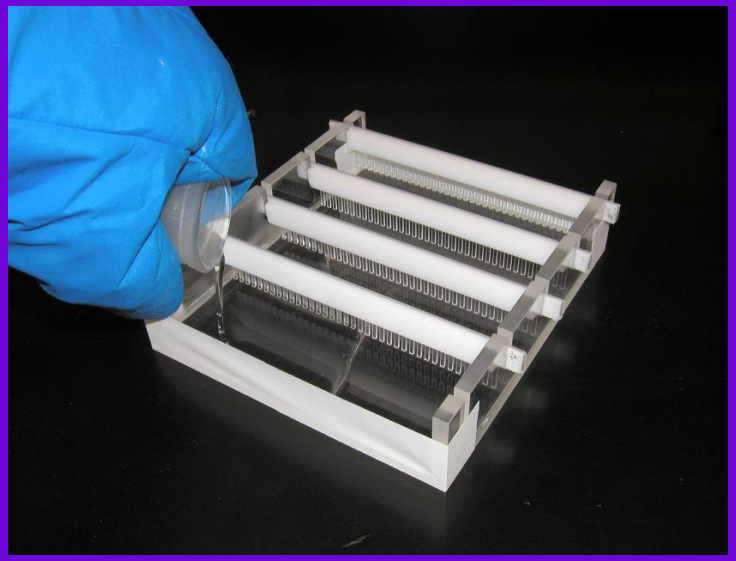


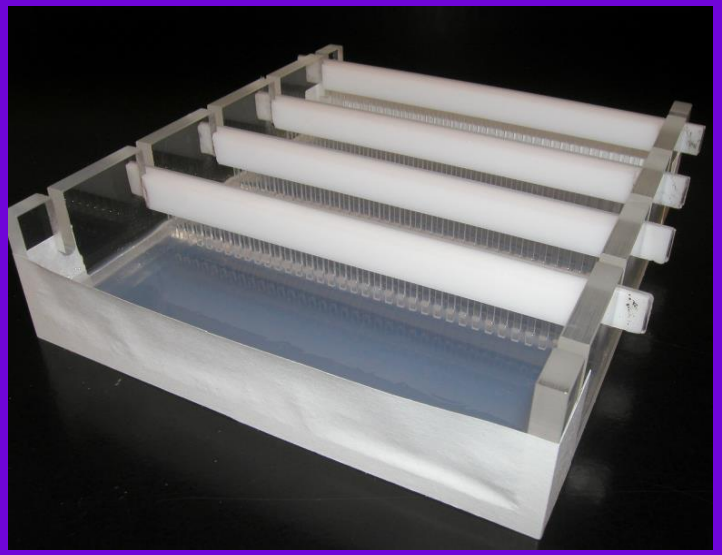
Add Ethidium Bromide

SET GEL CASTING TRAY



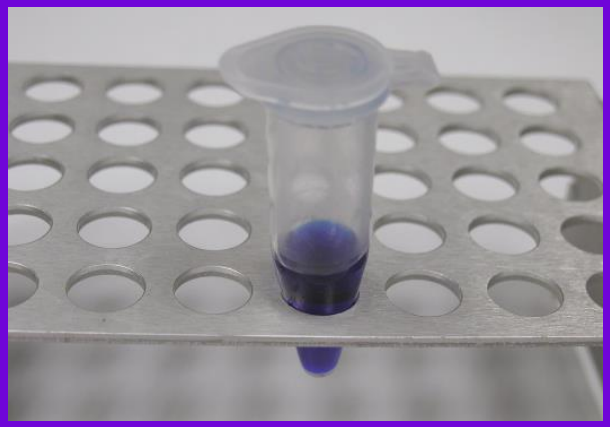






SAMPLE PREPARATION

• Mix the samples of DNA with the 6X sample loading buffer (w/ tracking dye). This allows the samples to be seen when loading onto the gel, and increases the density of the samples, causing them to sink into the gel wells.

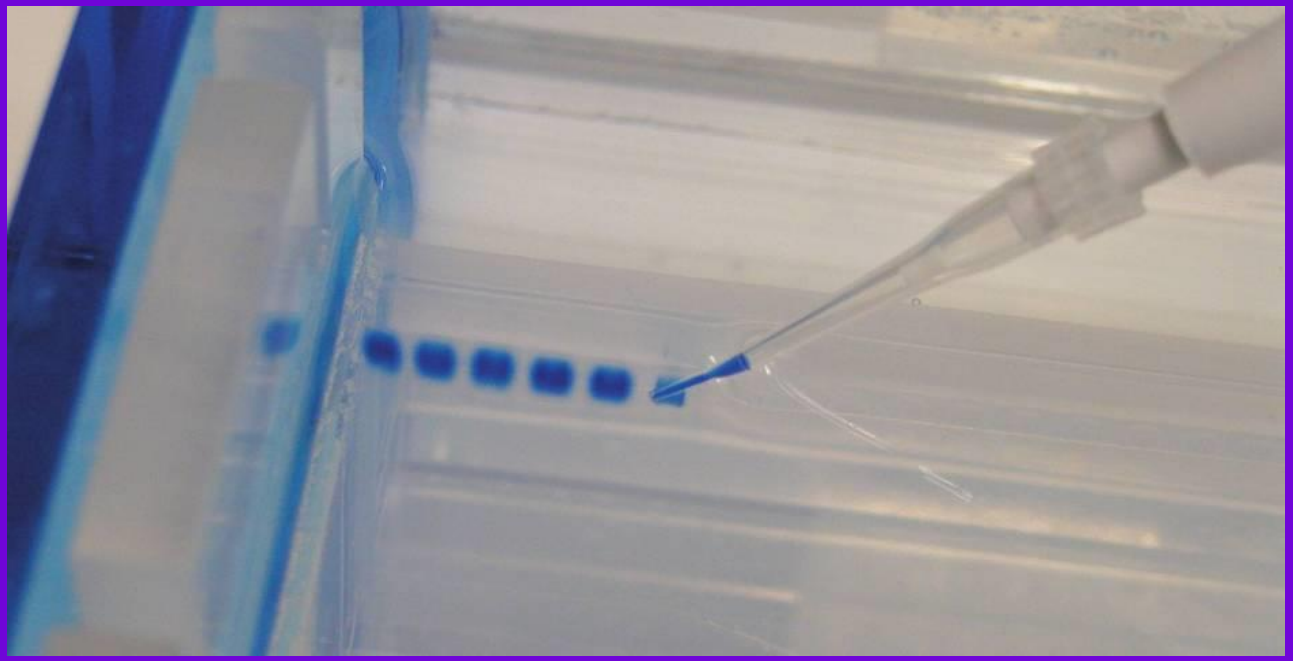


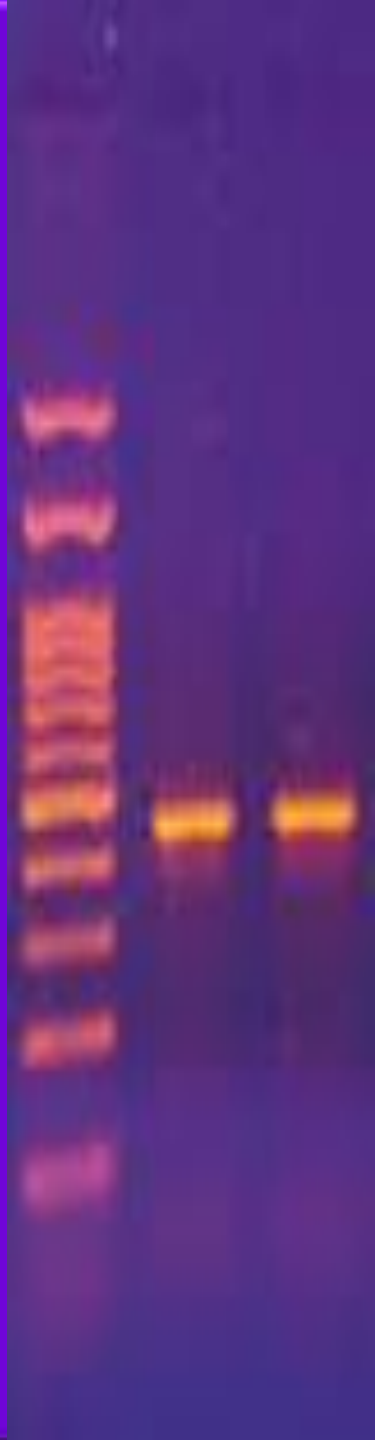
6X Loading Buffer: →

- Bromophenol Blue (for color)
- Glycerol (for weight)

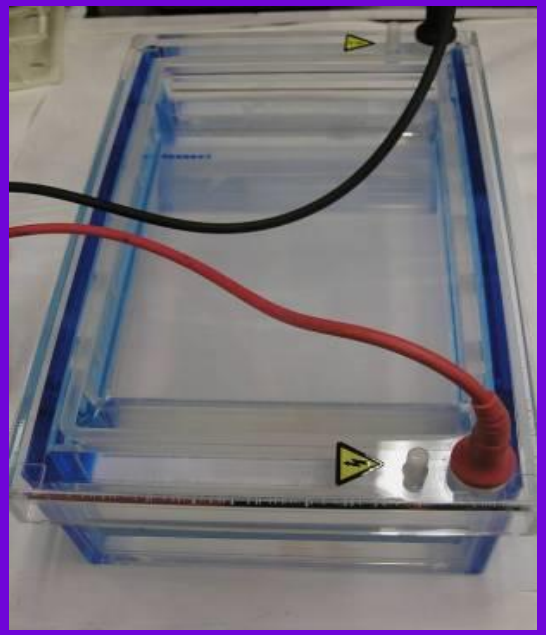
LOADING THE GEL

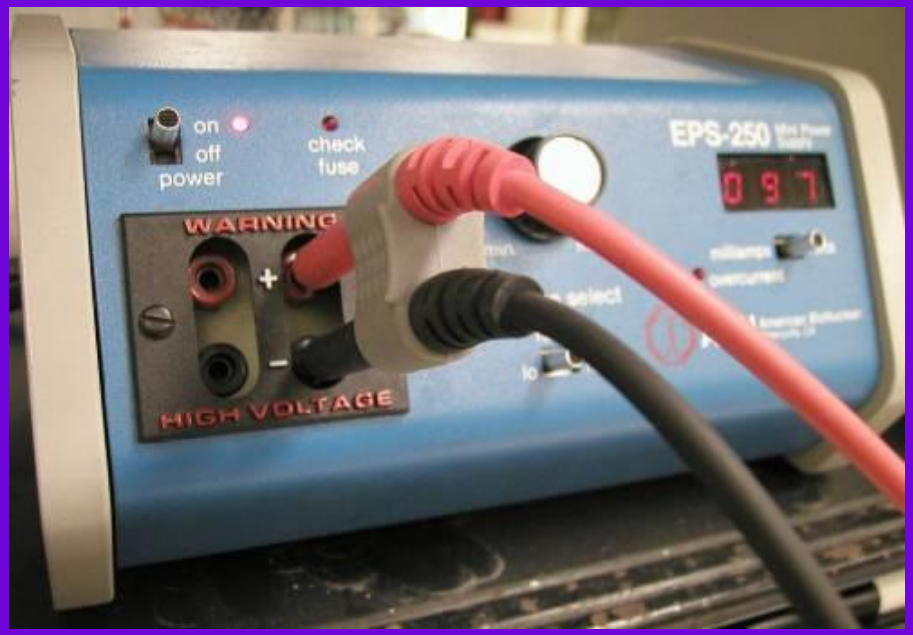
Carefully place the pipette tip over a well and gently expel the sample. The sample should sink into the well.





RUNNING THE GEL



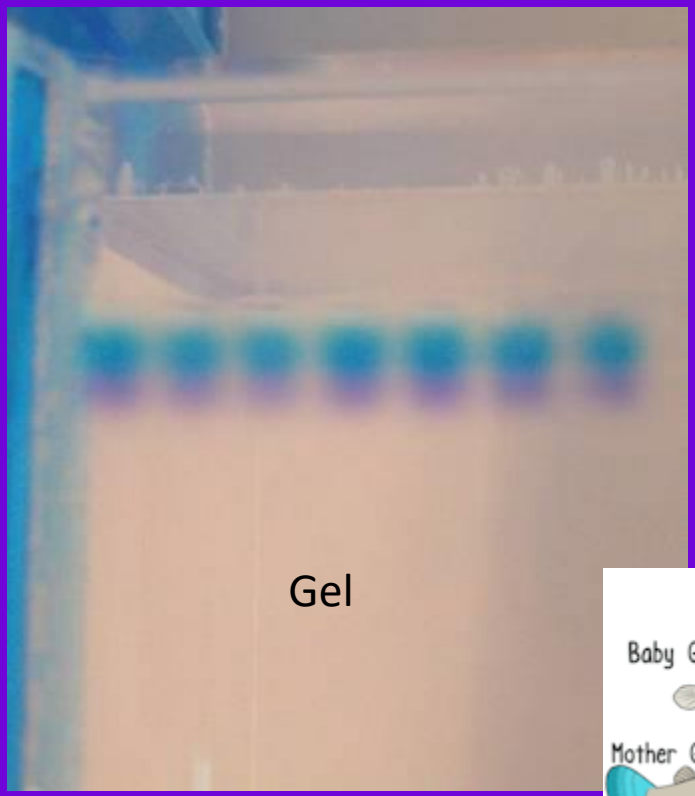


Place the cover on the electrophoresis chamber, connecting the electrical leads. Connect the electrical leads to the power supply. Be sure the leads are attached correctly - DNA migrates toward the anode (red). When the power is turned on, bubbles should form on the electrodes in the electrophoresis chamber.

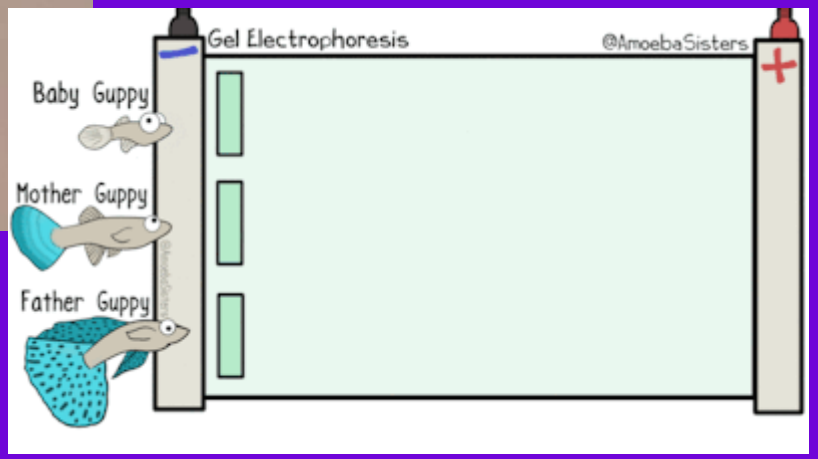
Cathode
(-)

DNA
(-)
↓

Anode
(+)

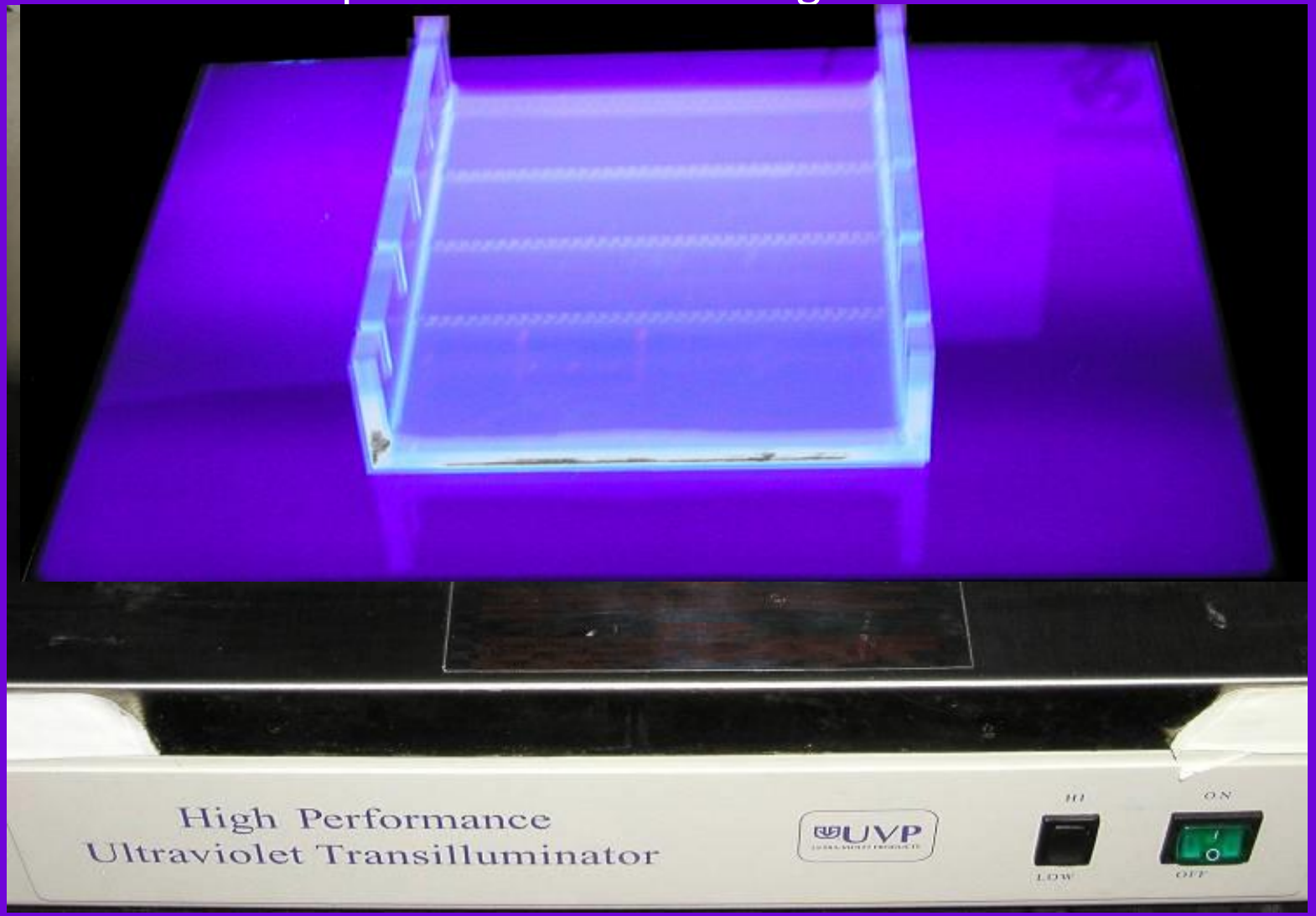


← wells
← Bromophenol Blue

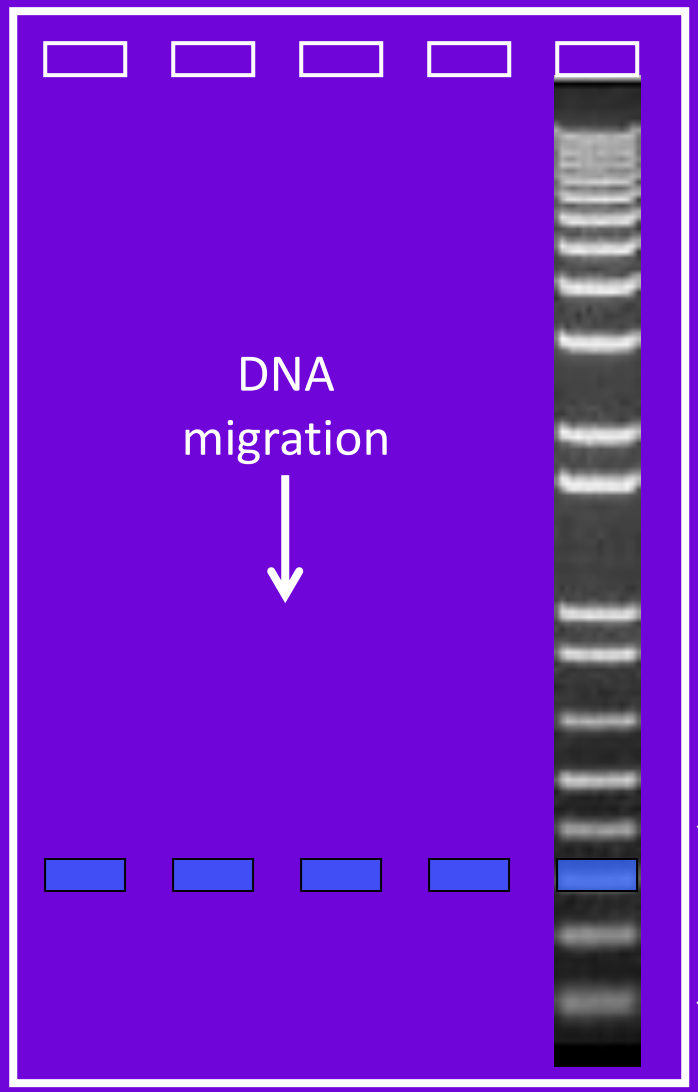


VISUALIZING THE GEL

Ethidium Bromide requires an ultraviolet light source to visualize



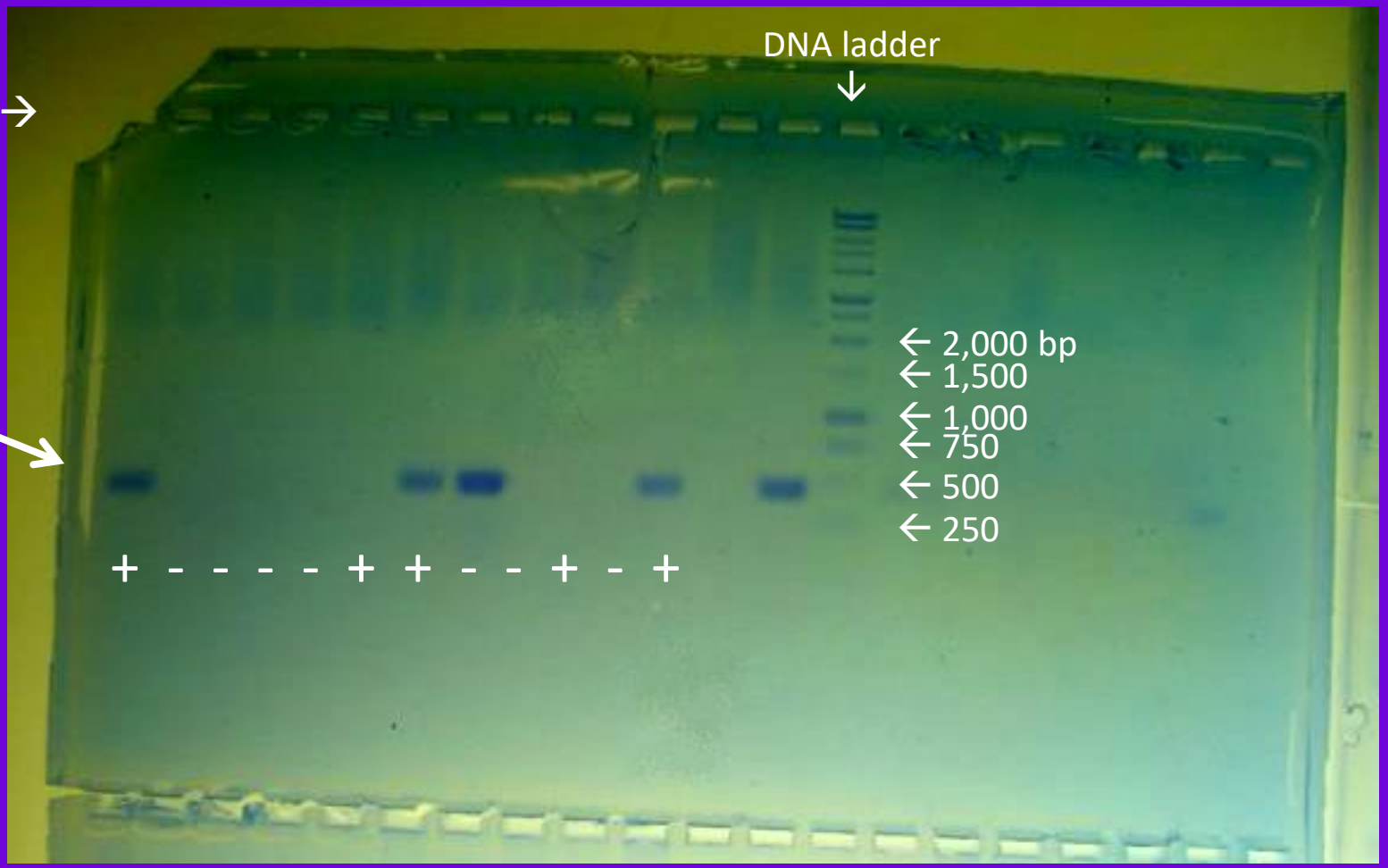
DNA Ladder Standard



Note: Bromophenol blue migrates at approximately the same rate as a 300 bp DNA molecule

Inclusion of a DNA ladder (DNAs of know sizes) on the gel makes it easy to determine the sizes of unknown DNAs.

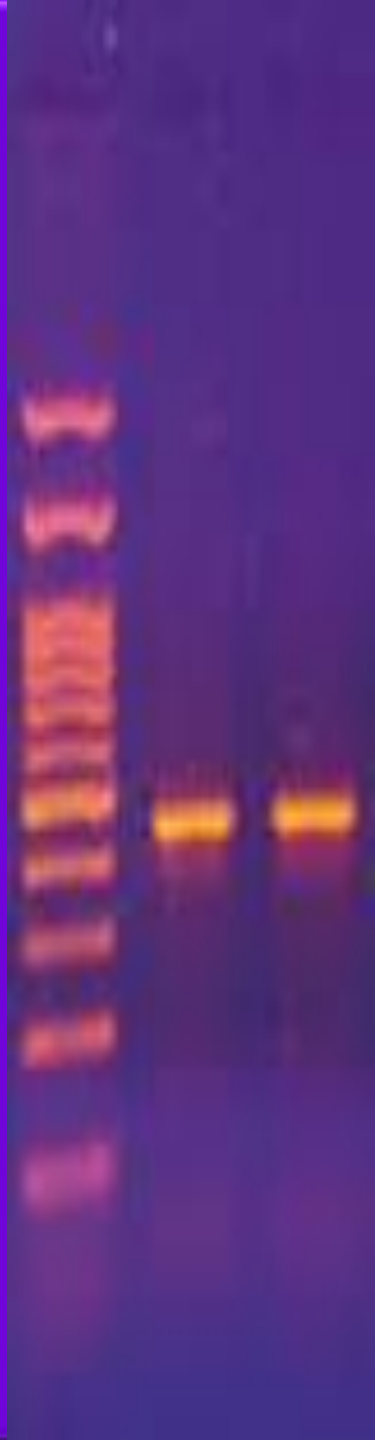
ANALYSING THE GEL



Samples # 1, 6, 7, 10 & 12 were positive for a PCR Product

PRECAUTIONS

- While placing the pipette tip over a well for expelling the sample. Be careful not to puncture the gel with the pipette tip.
- After the current is applied, make sure the Gel is running in the correct direction.
- Ethidium bromide is a powerful mutagen and is moderately toxic.
- Gloves should be worn at all times.
- Dispose of waste correctly.



Applications

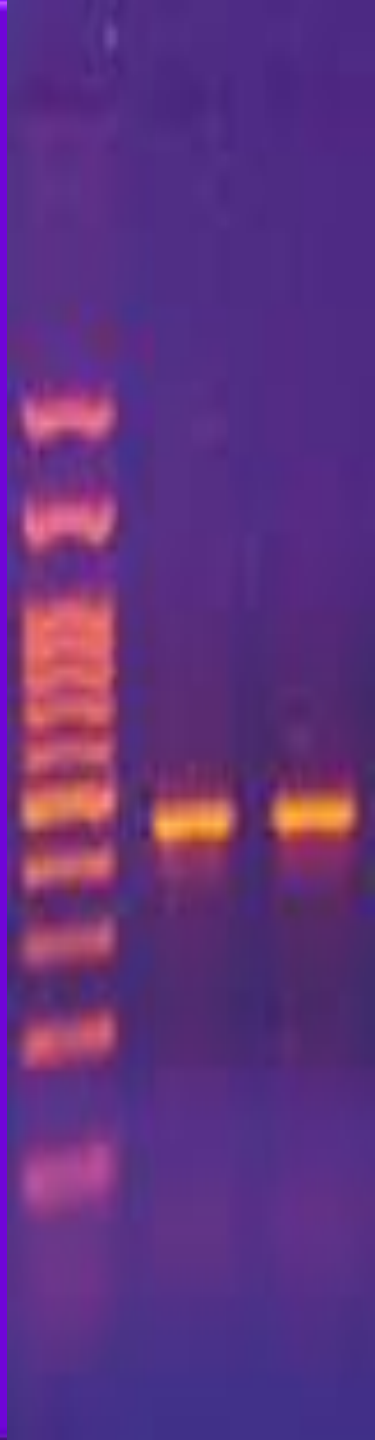
To determine the presence or amount of DNA.

To analyze PCR product.

To determine the sizes of DNA fragments.

To analyze restriction digestion products.

To determine the DNA sequence in DNA sequencing technique



REFERENCES

•Agar Archived October 16, 2007, at the Wayback Machine at lsbu.ac.uk Water Structure and Science.

•Jeppson, J. O., C. B. Laurell, and Bi Franzen (1979). "Agarose gel electrophoresis". *Clinical Chemistry*. **25** (4): 629–638. PMID 313856.

•http://hpc.ilri.cgiar.org/beca/training/IMBB/lectures/Agarose_Gels_IMBB_2013.pdf

• http://www.sas.rochester.edu/bio/labs/WerrenLab/WerrenLab-WolbachiaWorkshops_files/Gel%20Electorphoresis%20 Lecture %202006.ppt

• Upadhyay A., Upadhyay K. and Nath (2014) “Electrophoresis”, Biophysical Chemistry: Principals and Techniques, Himalaya Publishing House, 421-449.

•httpswww.google.comsearchq=dna+bands+on+agarose+gel+animation&tbm=isch&tbs=rimg CdaIy0FDelRCIjh3Vmnhnk9-Zv9GpDiD_1WhP4lBIoihZeXRR_1famcFisRlf9d7-B3pNKiRq6DVZIUd45Ei_1eqhvV7SoSCXdWaeGeT35mEYTX1x-gBxoNKhIJ_10akOIP9aE8RF8WpLv5_1mrY