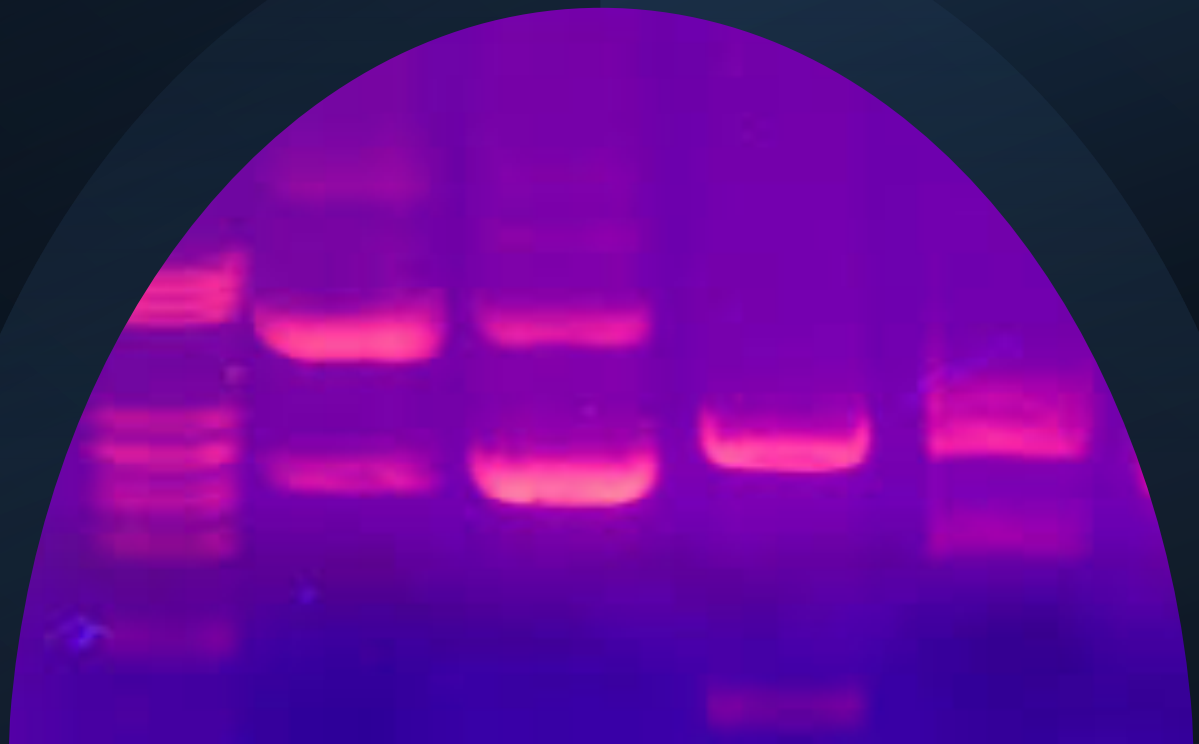
Agarose Gel Electrophoresis



What is the use of Gel Electrophoresis

- To separate different nucleic acid molecules on the basis of size.
- To monitor PCR products
- To monitor cutting and joining of DNA molecules (initially done by velocity sedimentation in sucrose gradients)

Gel electrophoresis

- **Gel Material**: polyacrylamide or agarose.
- Agarose for separating DNA fragments size between a few hundred base pairs to about 20 kb.
- Polyacrylamide is preferred for smaller DNA fragments.

The protocol can be divided into four stages

- preparation of agarose/polyacrylamide gel
- Sample and marker loading
- Running the gel at optimum voltage and optimal time for effective separation
- Staining the gel for viewing DNA

Concentrations for Separating DNA Fragments of Various Sizes

Agarose (%)	Effective range of resolution of linear DNA fragments (kb)
0.5	30 to 1
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.4
1.5	3 to 0.2

Mechanism of separation

- DNA molecules have negatively charged phosphates along the DNA backbone therefore migrate towards the anode in an electric field
- strength of electric field depends on
  - the length of the gel
  - potential difference at the ends (V/cm).
- The migration velocity is affected by
  - frictional force imposed by the gel matrix.
  - Charge
  - size
- **Charge to mass ratio is the same for DNA molecules of different lengths hence, size of the DNA, effectively determines the rate at which it passes through the gel**

Mechanism of separation

- DNA fragments travel through Agarose at a rate proportional to the applied voltage
- With increasing voltages, large DNA molecules migrate at a rate proportionately faster than small DNA molecules.
  - Therefore higher voltages are less effective in resolving large DNA fragments
- For large DNA molecules, gels are run at low agarose concentrations and low voltages (~1 V/cm, 0.5% agarose).

DNA movement and molecular weight

- Agarose is complex network of polymeric molecules with pores
  - average pore size depends on buffer composition, type and concentration of agarose used
- The mobility of DNA through these pores depends on molecular weight of DNA.
- The electrophoretic mobility of DNA in free solution is independent of molecular weight.

How DNA moves through gel?

- Earlier Hypothesis: DNA movement through the gel is like motion of a snake (reptation).
- Current view: DNA shows elastic behavior by stretching in the direction of the applied field and then contracting into dense balls.

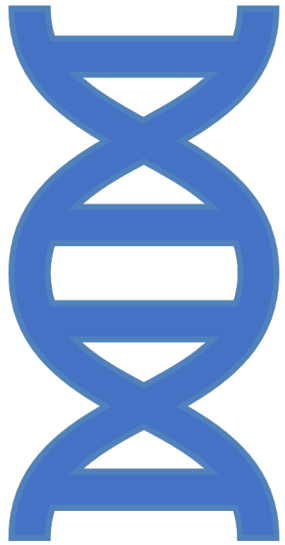
How DNA moves through gel?

- The bigger the pore size of the gel, the greater the ball of DNA which can pass through and hence the larger the molecules which can be separated
- If DNA ball is larger than the pore, it can only pass through by reptation.

- This occurs with molecules about 20 kb in size and it is difficult to separate molecules larger than this.
  - For larger molecules PFGE is done. This method can separate molecules as large as 10 Mb.

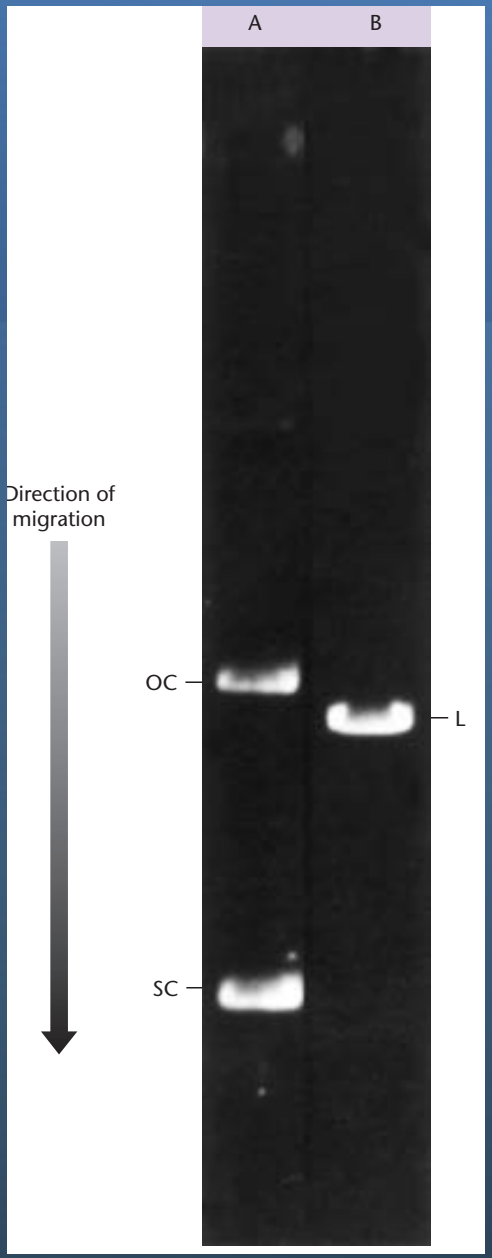
DNA conformations

- Covalently Closed circular (intact double stranded circular DNA), CCC
- Nicked circular (OC)
- Linear duplex DNA
  - DNA of same molecular weight in different conformations migrate at different rates through agarose gels



Q.Why In the absence of Etbr, CCC supercoiled plasmid DNAs migrate faster than their linear counterparts?

- Supercoiling winds the molecules up, giving them a smaller radius and allowing them to pass more readily through the gel matrix.
  - Therefore Nicked or relaxed circular molecules that have lost all of their superhelicity migrate appreciably slower than either supercoils or linear molecules

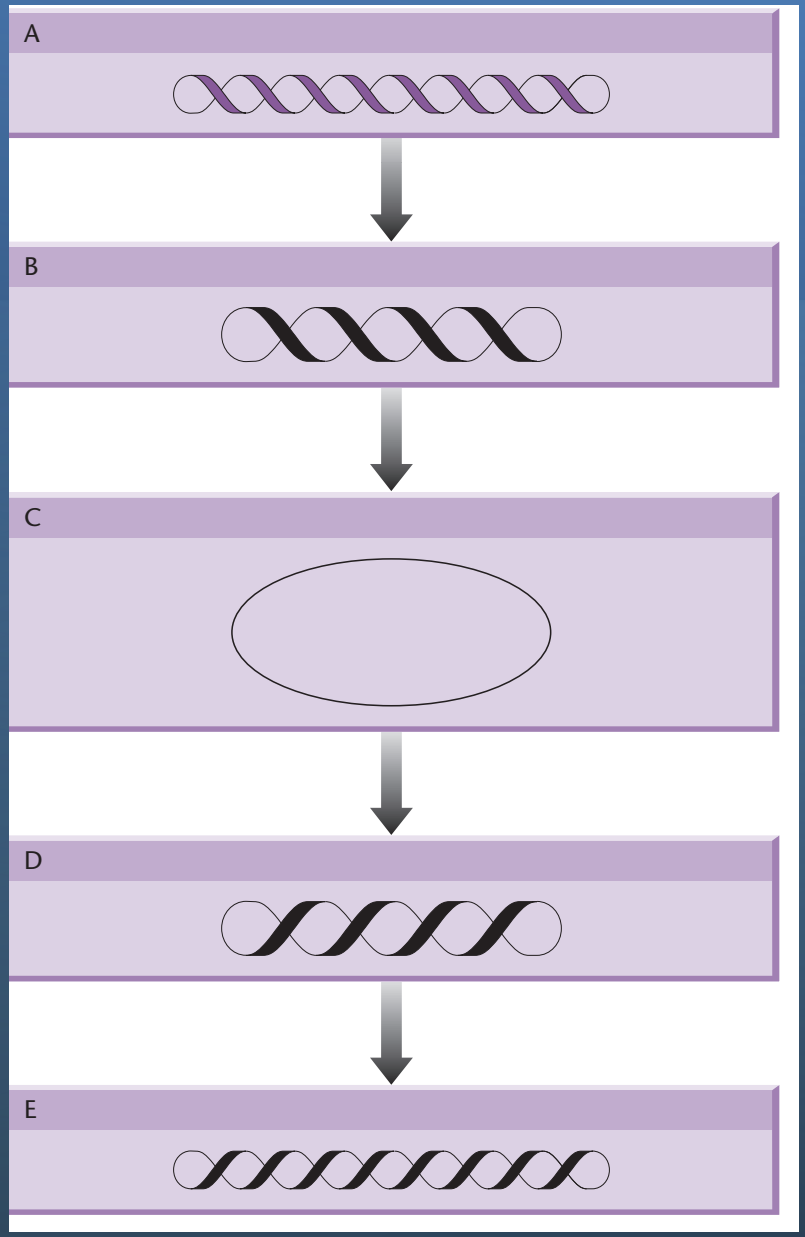

A
B
Direction of migration
OC
L
SC

Role of EtBr

- Etbr reduces the mobility of linear duplexes and also affects mobility of cccDNA. It changes the superhelical density of ccc molecules by inducing positive supercoils.

Role of EtBr

- With increasing conc of etbr, negative supercoils are gradually removed, causing a decrease in the mobility.
- This occurs until a critical free dye concentration is reached where no more superhelical turns remain (usually between 0.1 to 0.5 µg/ml).
- As more etbr is bound, positive superhelical turns are generated which, like negative supercoils, cause an increase in the electrophoretic mobility of the molecules


A
B
C
D
E

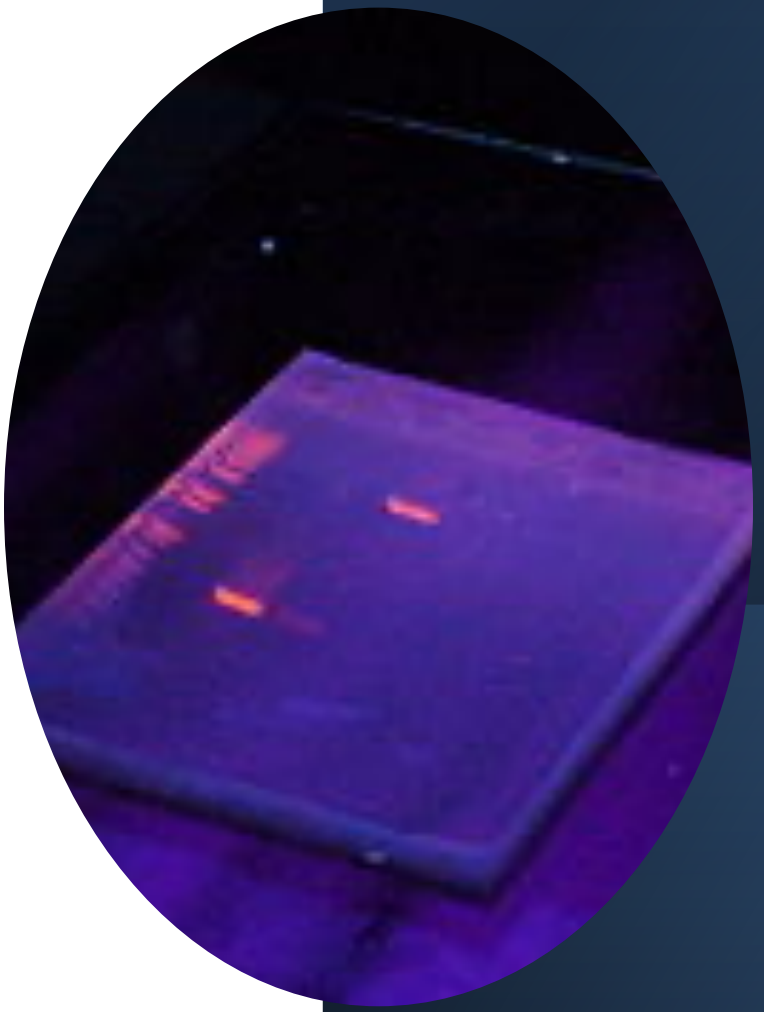
FUNCTION of Tracking dyes: added to loading buffer

- Monitors the progress of an electrophoretic separation.
- e.g. bromphenol blue and xylene cyanol.

Observing DNA

- Etbr used for viewing DNA. It intercalates between the stacked bases and fluoresces red-orange (560 nm) with UV light (260 to 360 nm). This allows very small amounts of DNA detection
- Etbr is either added to the gel prior to electrophoresis. Or the gel is stained post running.

Observing DNA



Observing DNA

- EtBr can detect as little as 0.05 ug of DNA when the gel is illuminated with ultraviolet light.

- It is mutagenic and a potential carcinogen. Hence a fluorescent DNA stain called SYBR Safe is preferred