Agarose Gel Electrophoresis of DNA

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principle :

Agarose Gel Electrophoresis uses electrical field to separate macromolecules (DNA and Protein) that differ in size , charge and configuration.

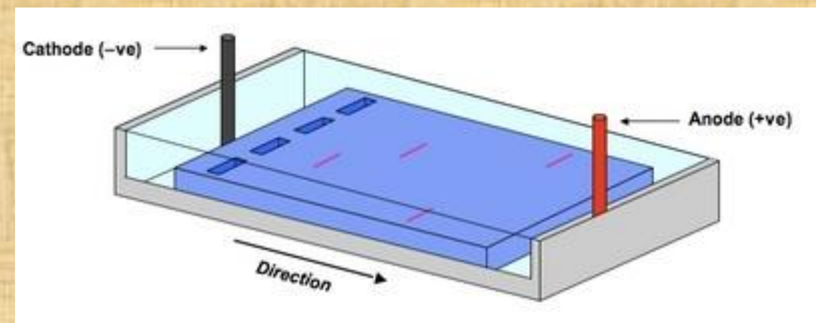
DNA :

- ❑ DNA molecules have –ve charges.
- ❑ They differ in size (bp).

Factors affect electrophoresis

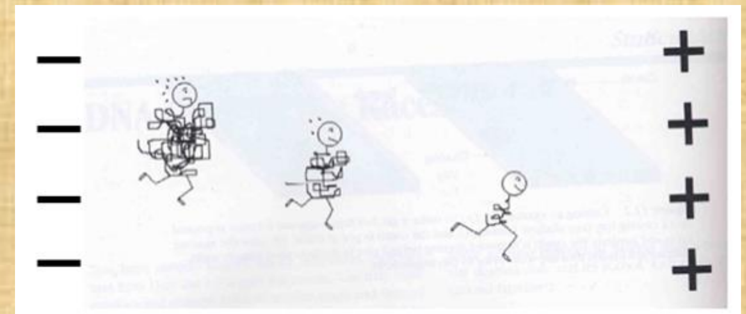
Molecule’s charge

DNA (-ve charged molecule) moves from cathod (-ve) to Anode(+ve) pole



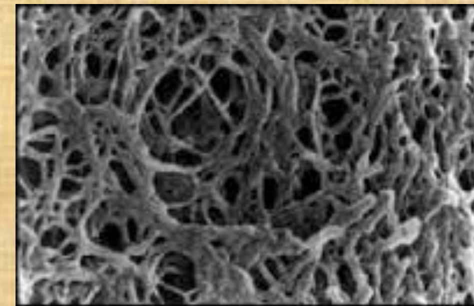
Molecule’s size

The lighter the molecule the faster it is .



The Agarose concentration:

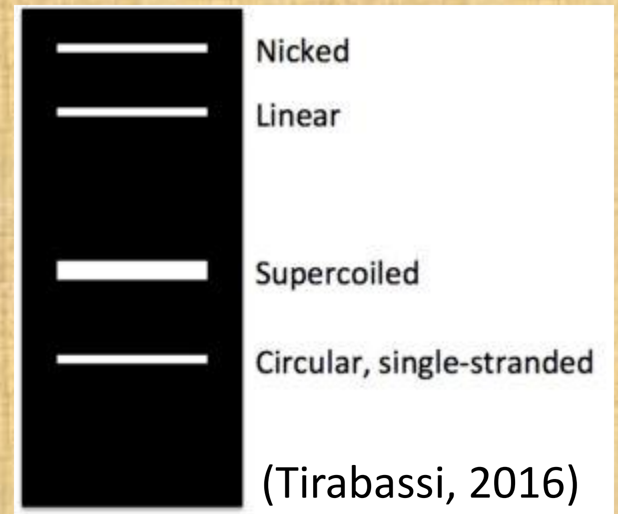
- The higher the concentration the smaller the size of molecule that can pass the matrix.
- The higher the concentration of gel the slower the movement of particles.
- 2% is the concentration of choice for separation of most of DNA fragments.



The gel matrix EM. Image

Conformation of DNA.

The liner DNA is slower than supercoiled molecule .



(Tirabassi, 2016)

Applied current

In general ,the higher the current ampere and Voltage the faster the net movement of molecules

Direction of electrical field

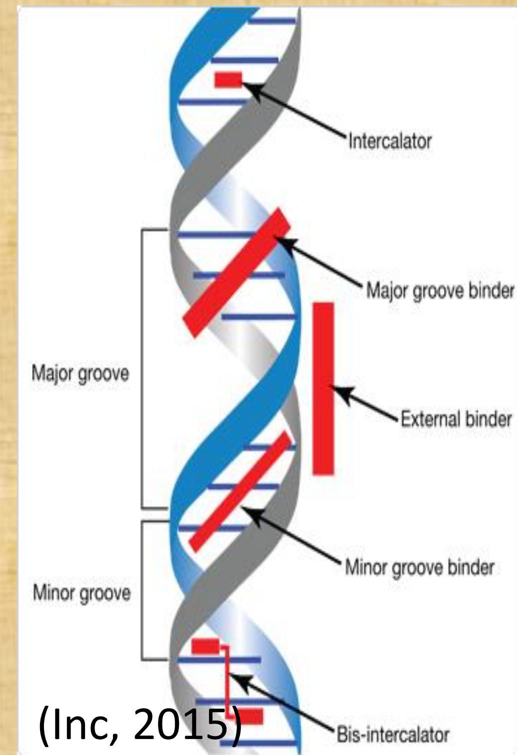
- For DNA separation the direction is from cathode to anode
- So make sure to put your sample at the right direction or no movement will result.
- Be carful to choose the right electrical connection of the device too.

electrophoresis buffer

- As it provides the ions which transfer the electrical filed within the fluid .
- Also they modify the net charges of the molecules

Intercalating dyes

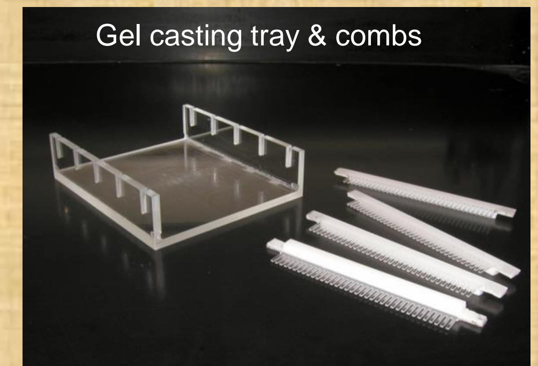
- Ethidium Bromide binds (intercalation between the base pairs) to the DNA and decrease its mobility .
- The larger the DNA molecule the more Ethidium Bromide binds to it .



(Inc, 2015)

Equipment of agarose gel electrophoresis

- ❑ Electrophoresis chamber .
- ❑ Power supply.
- ❑ Gel casting trays.
- ❑ Sample combs to form sample wells.
- ❑ Running buffer.
- ❑ Loading buffer.
- ❑ . Ethedium bromide. (mutagen)
- ❑ Transilluminator.



Running buffers:

- Choosing depends on the size of DNA.
- TAE buffer (Tris Acetate EDTA) :the most common used buffer.
- TBE buffer (Tris Borate/EDTA) is often used for smaller DNA fragment (i.e less than 500bp).

Loading buffer:

This buffer mixed with the sample, it gives the samples :

- ✓ color
- ✓ density
- ✓ makes it easy loading in the wells.

It consists of bromophenol blue(the tracing dye), sucrose and water.

Markers (DNA ladder):

- Different kinds of markers
- most of them are bacteriophage DNA cut with restriction enzymes
- Storing markers ready mixed with loading buffer at 4C.
- Choosing marker with a good resolution for fragment size in the experiment .

DNA size marker

Visualization:

Transiluminator (an ultraviolet light box) is used to visualize :

- ✓ Ethidium bromide- stained DNA in gels.
- ✓ Ethidium bromide-free techniques are available also.

Purposes :

- To look at the DNA
- To quantify it
- To isolate a particular band.

Applications :

- Estimation DNA quantity and quality
- Using a DNA ladder to approximate the size of DNA molecules.
- Analysis of PCR products.
- Separation of restriction enzyme digested DNA prior to Southern Blot transfer.
- Quantity is assessed using lambda DNA ladder .
- Quality of DNA is assessed by observing the absence of streaking or fragments (or contaminating DNA bands).

Procedure:

- Prepare a 1% agarose solution, measure 3 g agarose into a flask and add 150 ml 1X buffer. Microwave until agarose is dissolved and solution is clear.
- Allow solution to cool to about 55°C before pouring. Add 10 µl of Ethidium bromide or alternative dye
- Prepare gel tray and place comb in gel tray.
- Pour 50°C gel solution into tray and allow gel to solidify about 20 minutes at room temperature.
- To run, gently remove the comb, place tray in electrophoresis chamber, and cover (just until wells are submerged) with electrophoresis buffer (the same buffer used to prepare the agarose).
- To prepare samples for electrophoresis, add 1 µl of 6x gel loading dye for every 5 µl of DNA solution. Mix well & load. Don't forget to load the DNA size marker.
- Electrophorese at 50-150 volts until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.
- Visualize DNA under the UV transilluminator.

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