Lab# 8

Agarose gel electrophoresis

BCH 333 [practical]

Agarose gel electrophoresis:

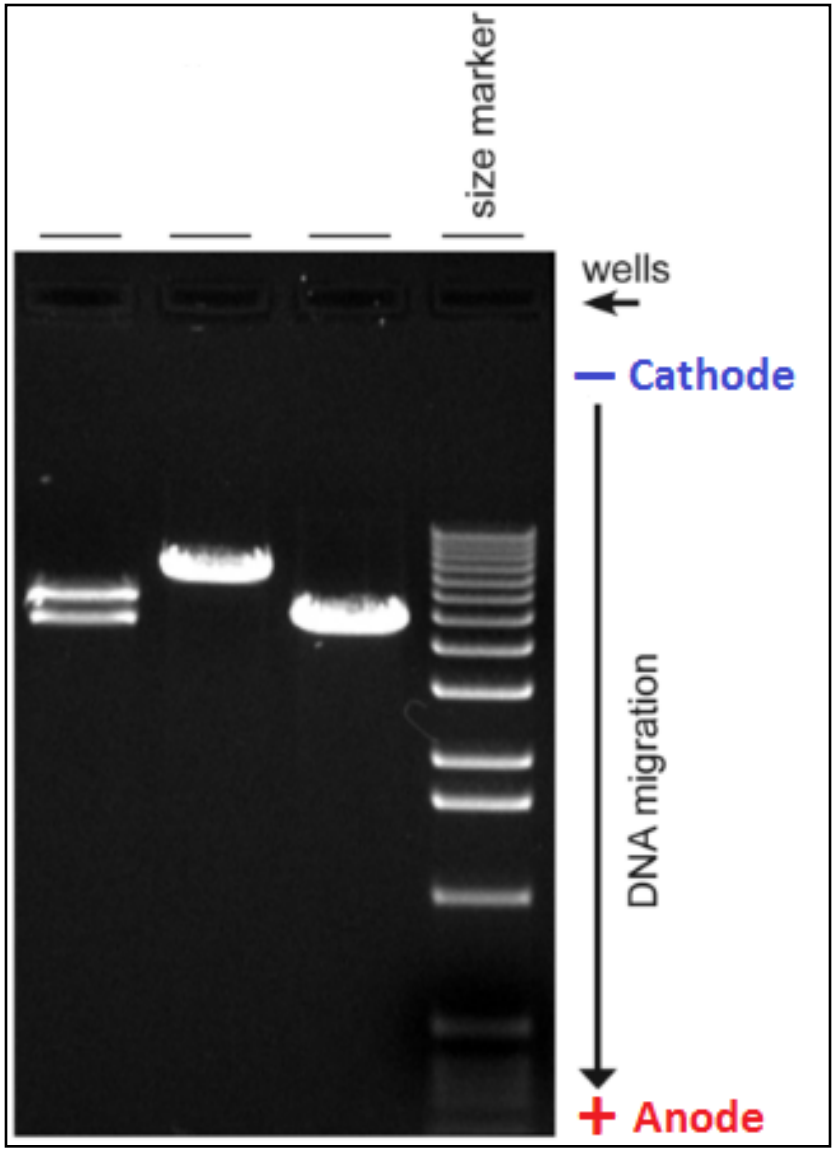
is a method of gel electrophoresis used in biochemistry and molecular biology to separate and analyze DNA or RNA molecules by size.

-It is used mostly to determine the size and checking the purity of isolated DNA.

Principle:

-Biomolecules [DNA or RNA] are separated by applying an electric field to move the negatively charged molecules [-] through an agarose matrix towards the anode [+] , and the biomolecules are separated by size in the agarose gel matrix.

-The largest molecules will have the most difficulty passing through the gel pores, whereas the smallest molecules will move faster.



Separation of DNA molecules using agarose gel electrophoresis

3- Agarose gel:

-Is a linear polymer of D-galactose and 3,6-anhydro-1-galactose and forms a gel that is held together by hydrogen bonds.

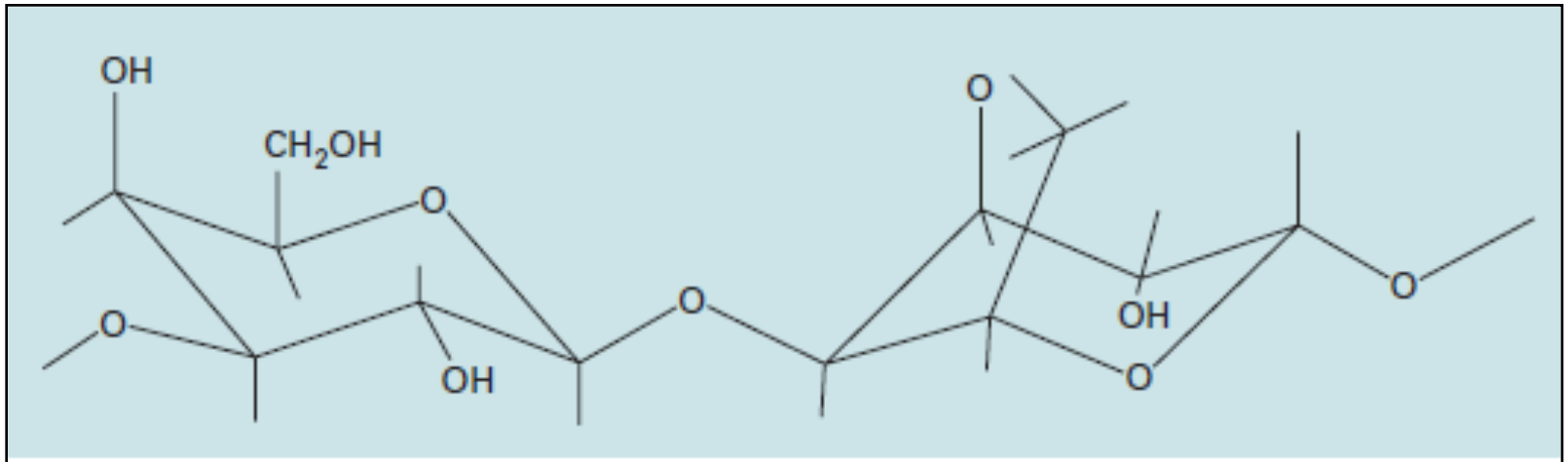


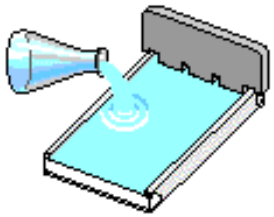
Fig. 10.4 Agarobiose, the repeating unit of agarose.

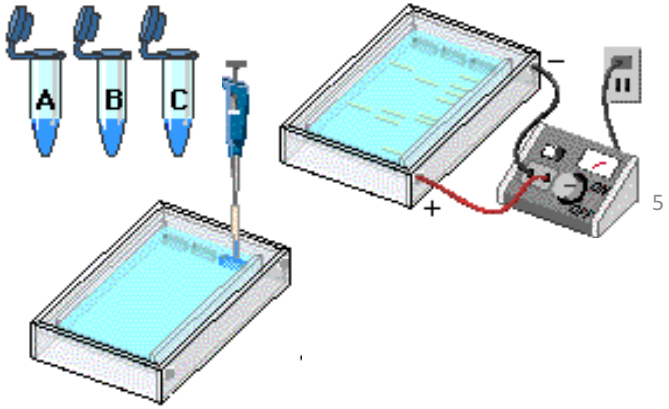
Performing Agarose gel electrophoresis

(1) Gel preparation

(2) Load the sample and start the run

(3) Visualizing the sample





- Agarose is present in powder forms that are dissolved in buffer [TBE or TAE] close to boiling temperatures , then cooled down to around 40 degrees it sets and forms a gel . [polymerized]

-The concentration of the material in the gel determines the size of the pores. [high concentration of the gel → small pore size]

- Agarose gels have larger pore sizes compared to Dextran and polyacrylamide gels. This makes it useful for the analysis or separation of large globular proteins or long, linear molecules such as DNA.

Prepare agarose gel with 0.8%?

Buffer used:

-helps is deliver the electric current through the gel.

Buffer used is either TBE or TAE.

- TBE buffer: is made with Tris/Boric acid/ EDTA.

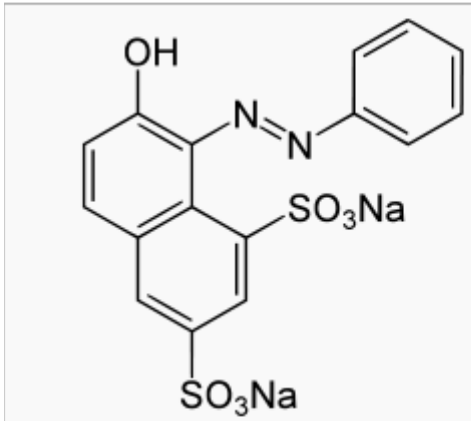
- TAE buffer: is made with Tris/Acetic acid/ EDTA.

Tracking Dye:

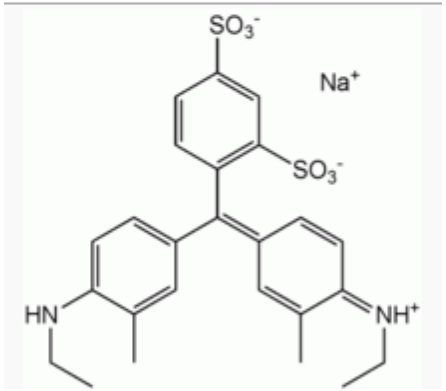
A dye such as bromophenol blue, Xylene cyanol or orange dye, is also included in the sample; it makes it easier to see the sample that is being loaded and also acts as a marker of the electrophoresis front.

Note: a glycerol or sucrose is included with the tracking dye , to render the samples denser than the running buffer (so that the samples sink in the well).

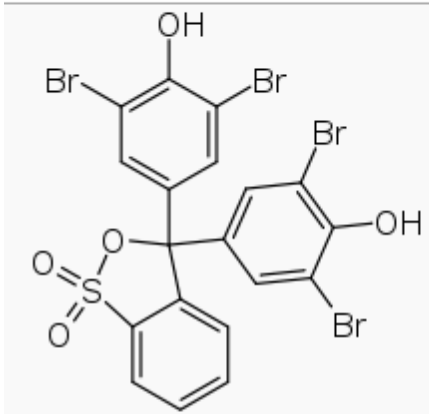
Orange G

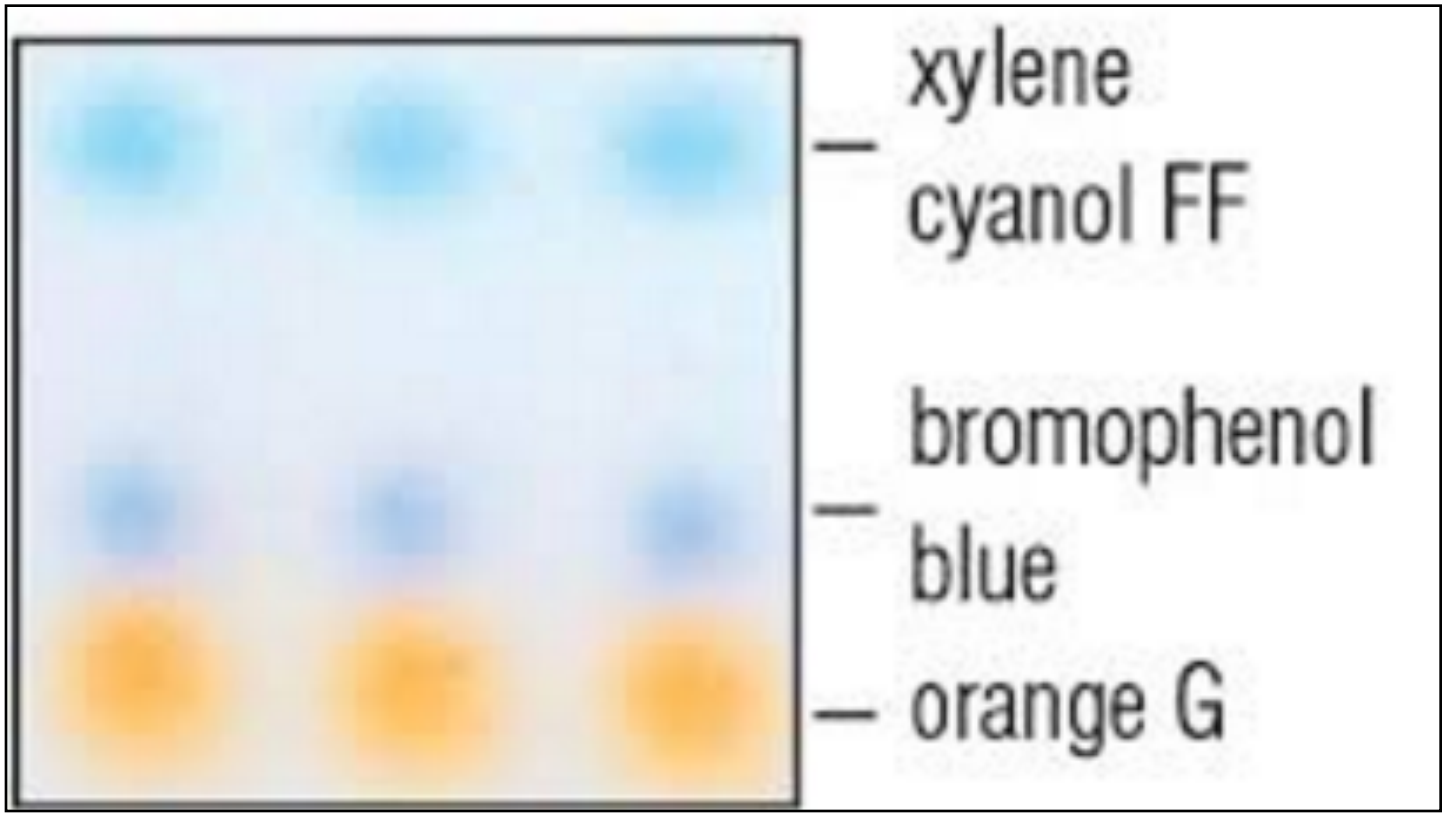


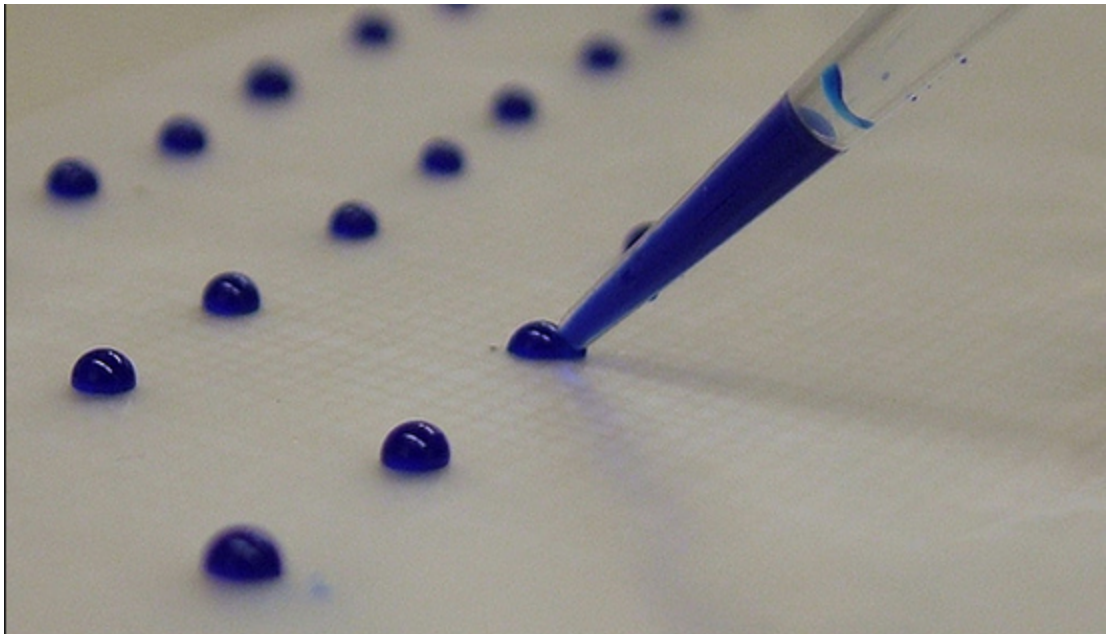
Xylene cyanol



Bromophenol blue




xylene
cyanol FF
bromophenol
blue
orange G



To load the sample, we should take for example: 7 µl from the DNA sample + 2 µl from the dye

-After the samples of DNA are applied to the wells of agarose gel along with the tracking dye.

↓

-The power is turned on, after filling the buffer[?] in the tank to allows current to pass.

↓

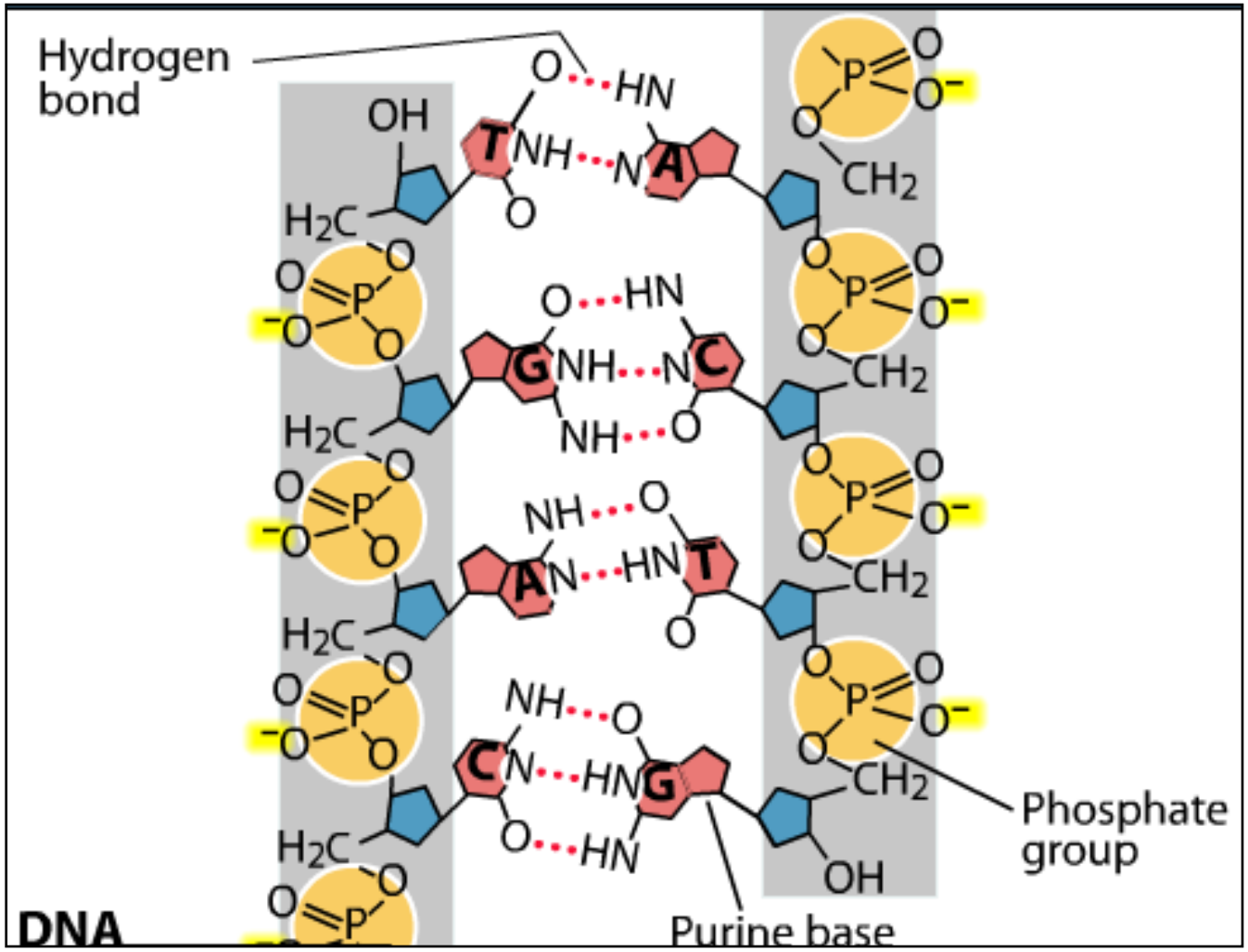
-The tracking dye and the DNA samples, are negatively charged [-] and migrate towards the positive electrode [+].

↓

-The DNA backbones contain negatively charged phosphate groups, which are attracted to the positive electrode.

↓

-Large DNA fragments retard earlier, while the smallest fragments move the fastest.



DNA: note the phosphate groups in the backbone are negatively charged.

Gel staining:

-The DNA in the gel needs to be stained and visualized, The reagent most widely used is the fluorescent dye ethidium bromide **"EtBr“**, that emits orange light after binding to DNA.

Note: That the gel will be viewed under ultraviolet light.

[Ethidium bromide is a cyclic planar molecule that binds between the stacked base-pairs of DNA.].

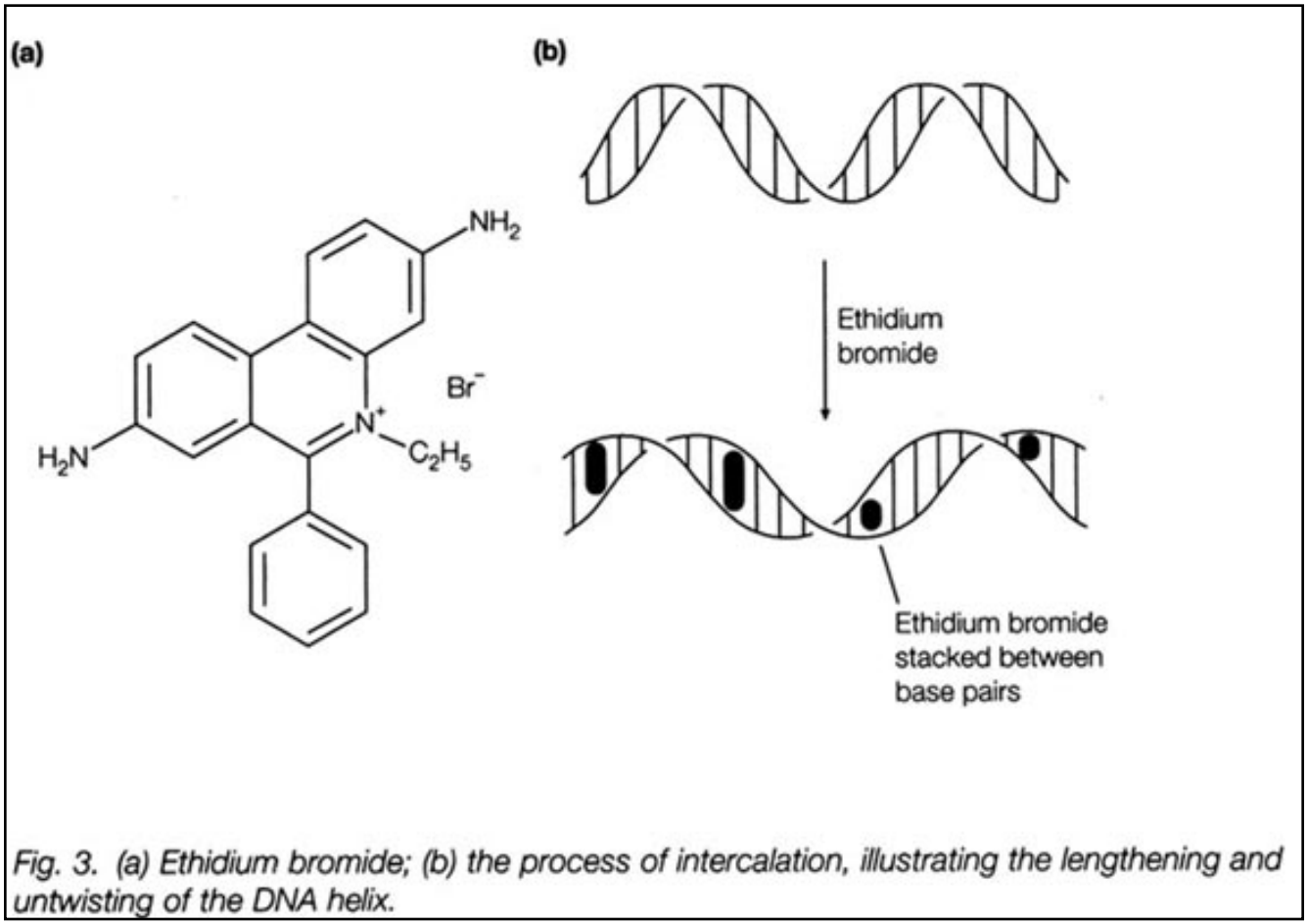


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

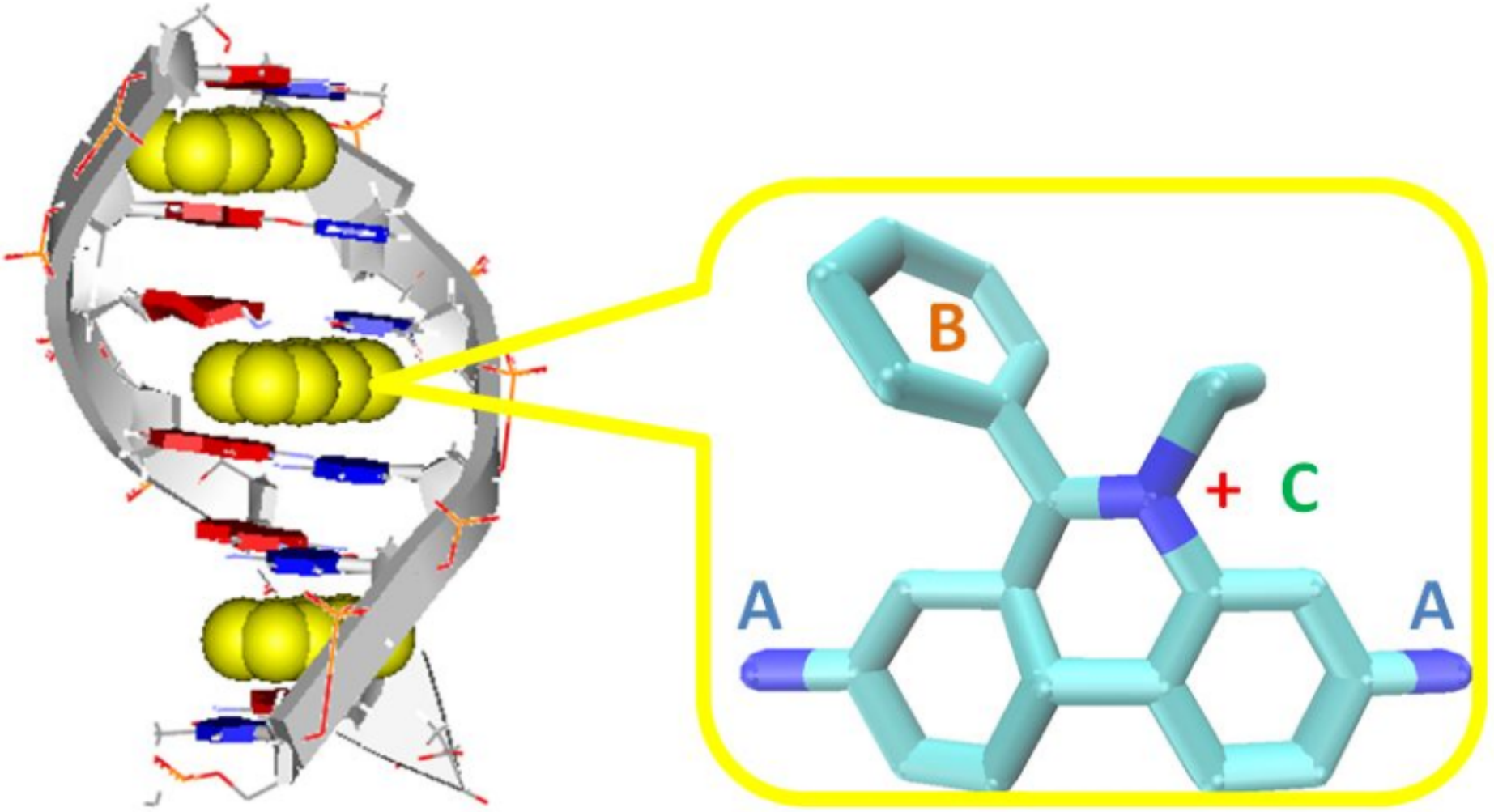
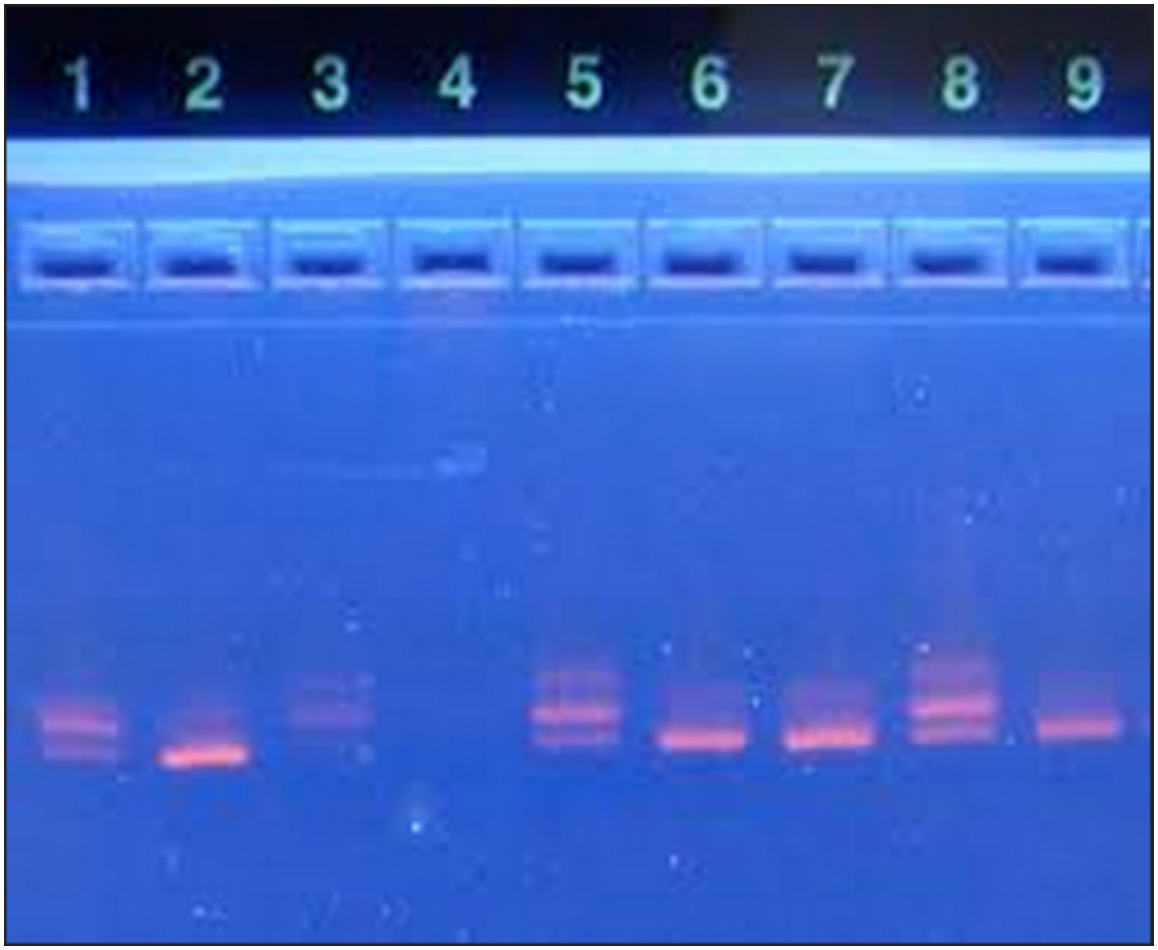
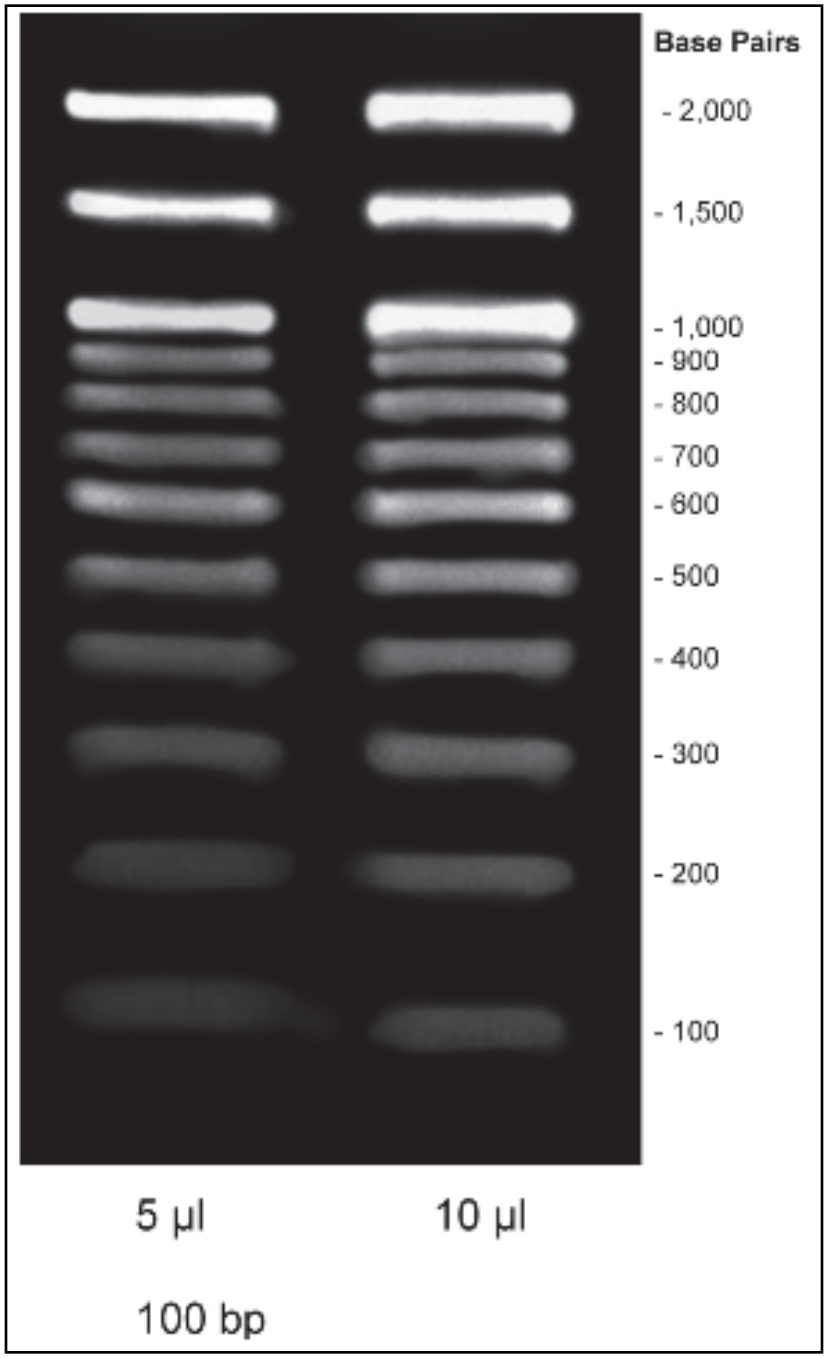


Figure 1: Schematic presentation of the intercalative binding mode by the neighbour exclusion principle and important structural features of ethidium bromide: A) amino substituents responsible for fluorescence increase upon DNA intercalation; B) phenyl substituent for steric control and also impact on fluorimetric properties; C) permanent positive charge for aqueous solubility and electrostatic attraction to the DNA or RNA phosphate backbone.

http://www.beilstein-journals.org/bjoc/single/articleFullText.htm?publicId=1860-5397-10-312



"EtBr"orange light after binding to DNA.

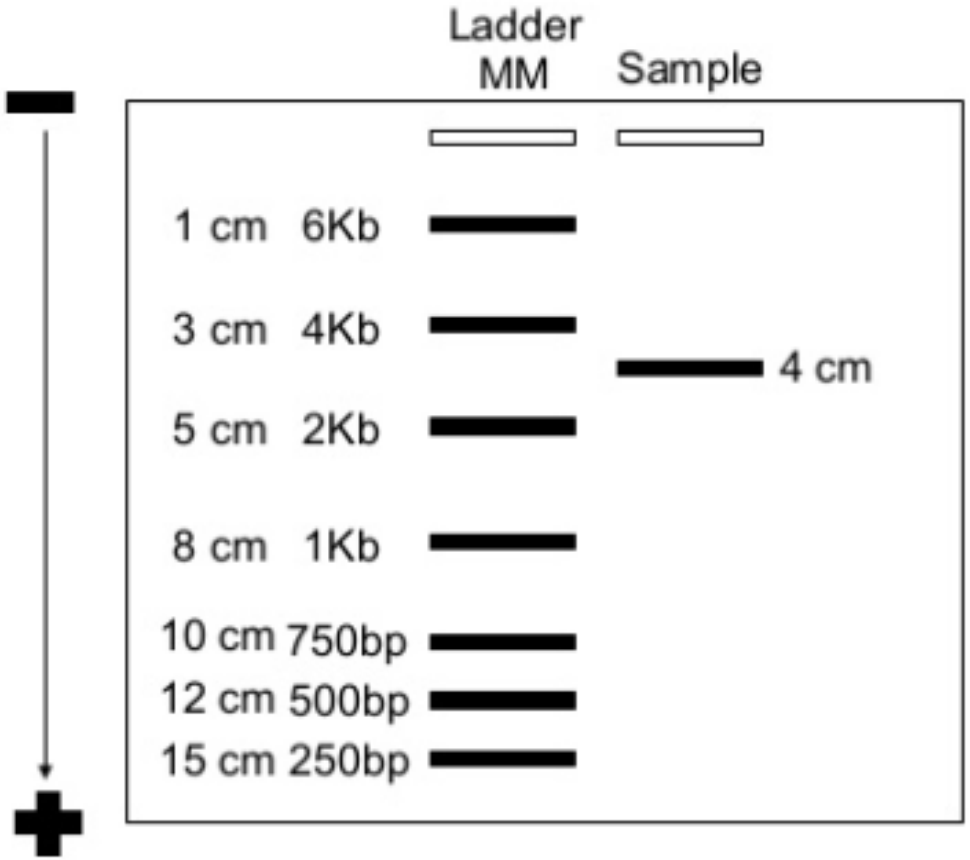


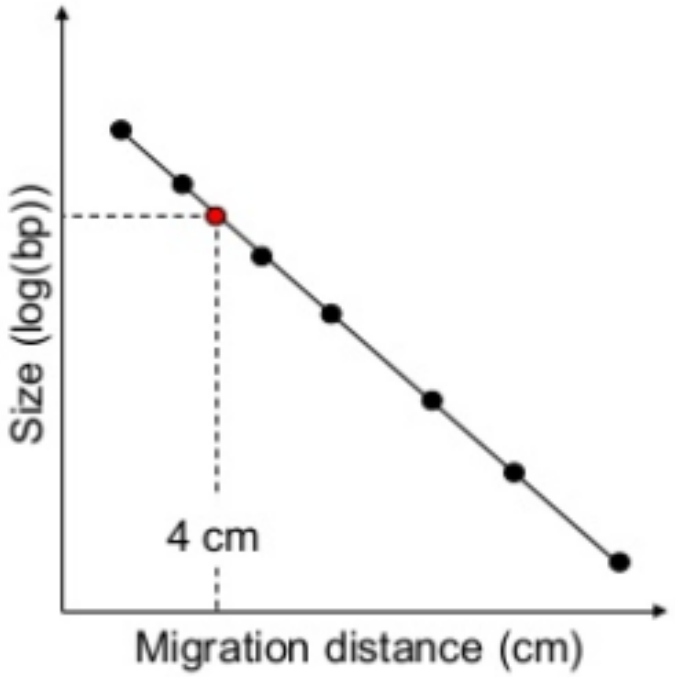
Determine the size of the DNA fragment:

-Since agarose gels separate DNA according to size, the size of a DNA fragment may be determined from its electrophoretic mobility by running a number of standard DNA markers of known sizes on the same gel.

Figure: The 100 bp DNA Ladder is suitable for sizing double-stranded DNA fragments from 100-2000 bp.

DNA (band) size estimation





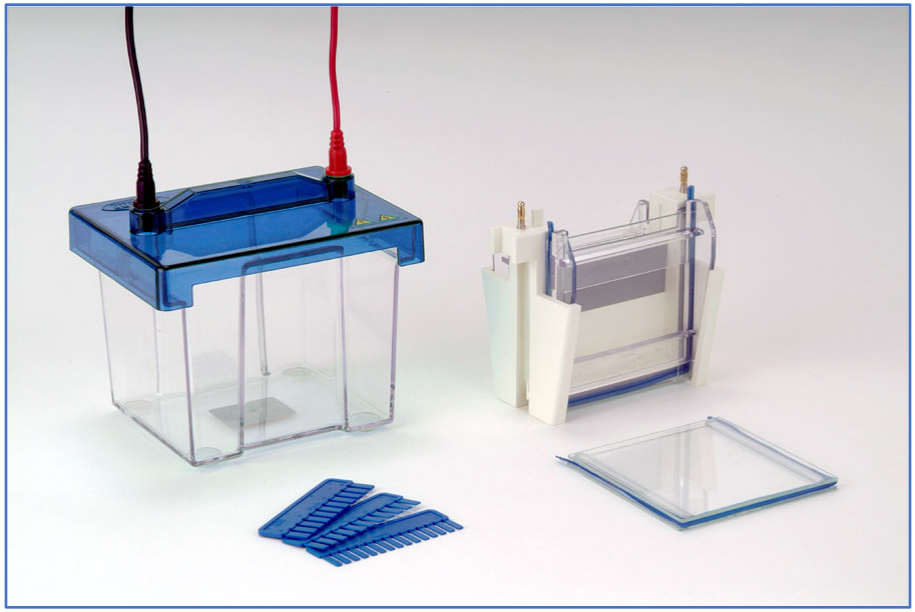
http://www.slideshare.net/hhalhaddad/forensic-lecture-6

Note that:

-The higher the voltage → the more quickly the gel runs.

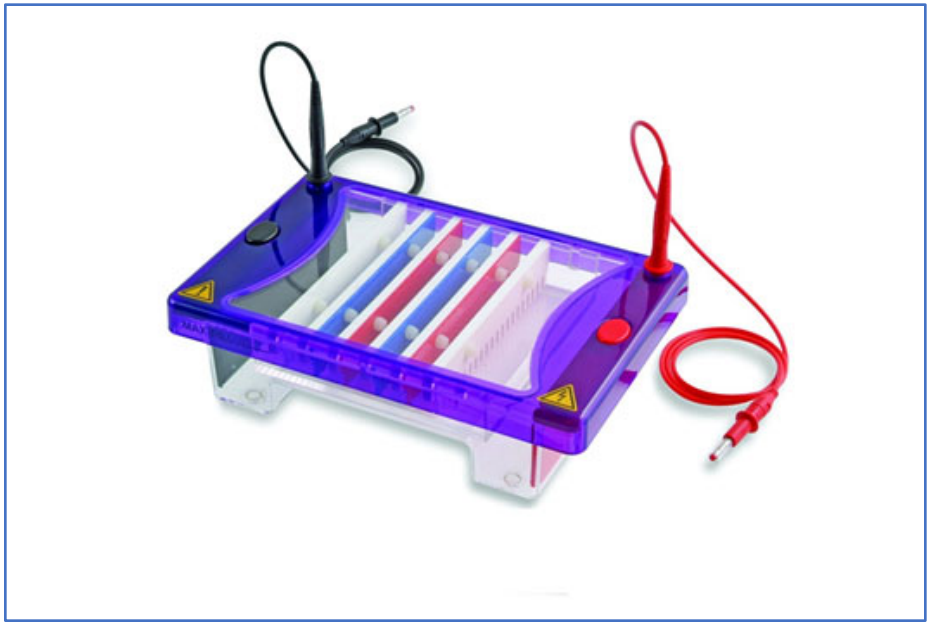
[↑ Voltage → ↑ rate of migration].

-Gel concentrations must be chosen to suit the size range of the molecules to be separated.



SDS-PAGE
Vertical pace devices

Running buffer
Staining buffer [lamp]
De-staining buffer
Acrylamide gel
[stacking, separation gel]
Rf value



Agarose gel electrophoresis
Horizontal agarose devices

Running buffer
Stained and view using UV light
Agarose gel
Rf value

http://learn.genetics.utah.edu/content/labs/gel/

https://www.youtube.com/watch?v=2UQIoYhOowM