AGAROSE GEL ELECTROPHORESIS (AGE)

Lab#7

BCH 333

Agarose Gel Electrophoresis (AGE):

- Is a method of gel (made of agarose) electrophoresis used in biochemistry and molecular biology to separate and analyse DNA or RNA molecules by **size.**

- **Agarose** is a heteropolysaccharide, generally extracted from certain seaweeds.
- **Electrophoresis** is the movement of charged particles under the influence of an electric field.

(phoresis means "migration").

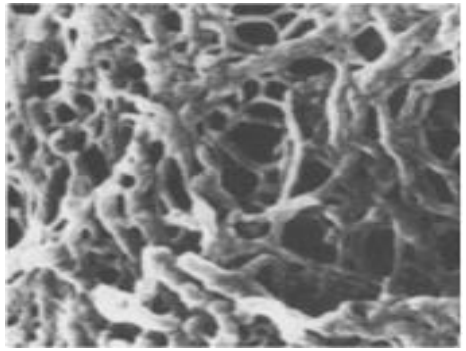
➔**Agarose gel electrophoresis** is a method for separating nucleic acids by size.

Principle of Agarose Gel Electrophoresis (AGE):

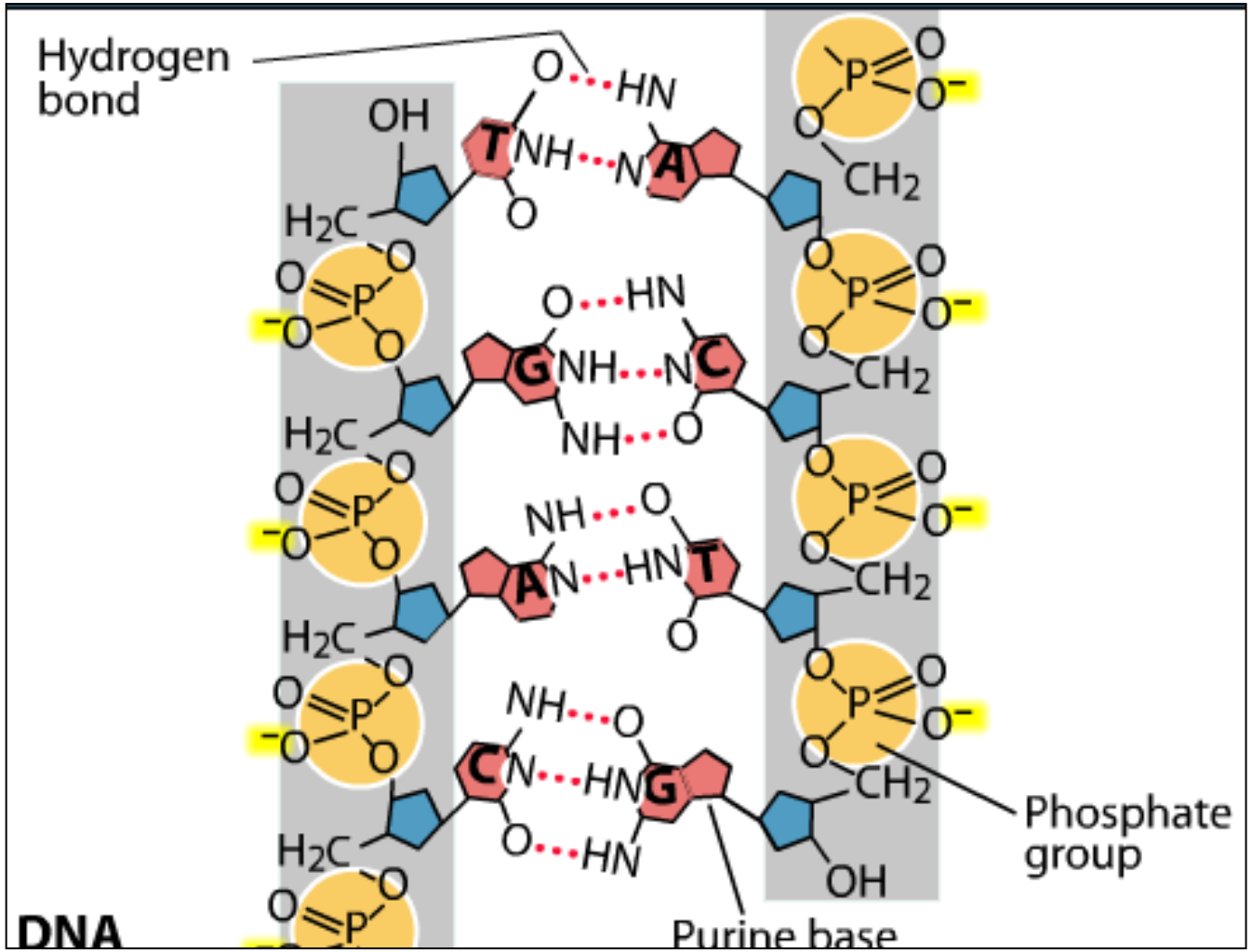
- Nucleic acids (DNA or RNA) are separated by applying an electric field to move the negatively charged molecules [-] through an agarose matrix towards [+] , and the biomolecules are separated by size in the agarose gel matrix.

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

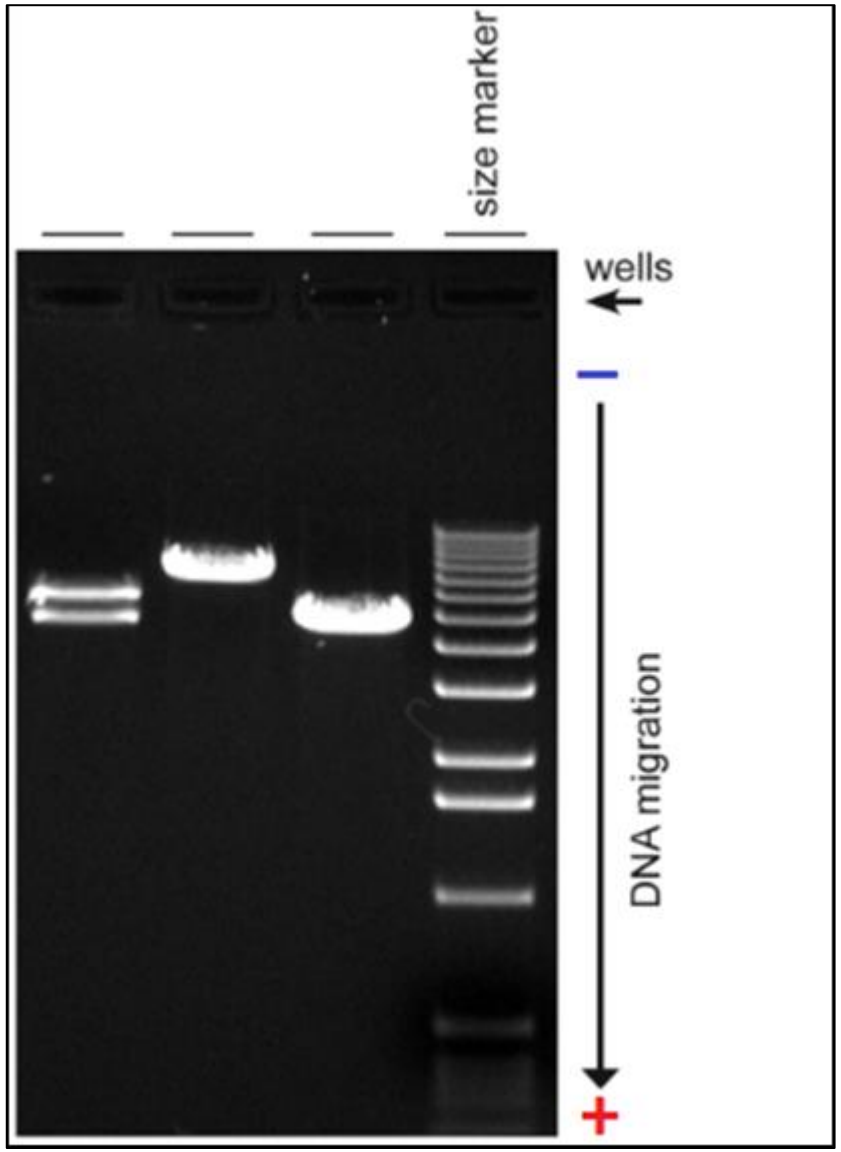
➔the distance travelled by a DNA molecule is inversely correlated with its size.



Polymerized agarose



➔ Note the phosphate groups in the backbone are **negatively** charged.



Separation of DNA molecules using agarose gel electrophoresis

Agarose Gel:

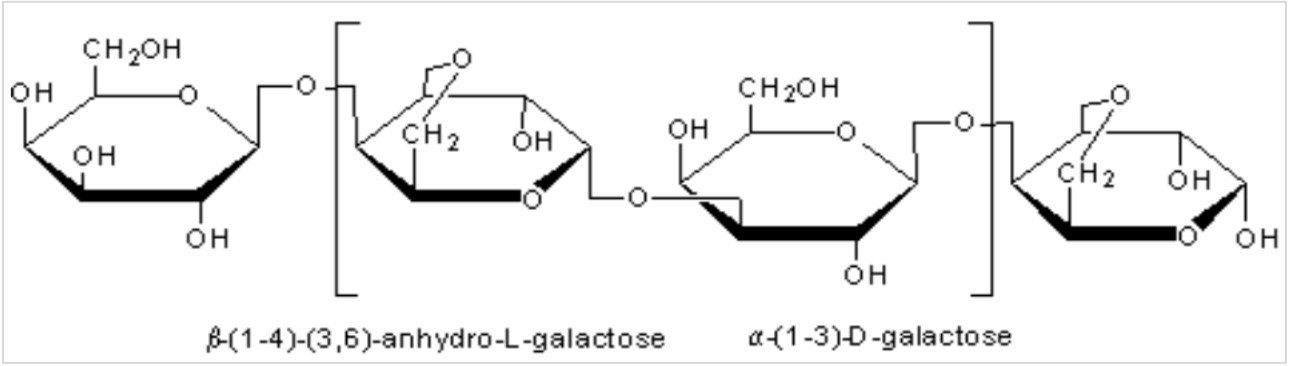
- Is a liner polymer composed of alternative residues of D-galactose and 3,6-anhydro-L-galactopyranose joined by α (1→3) and β (1→4) glycosidic linkages.

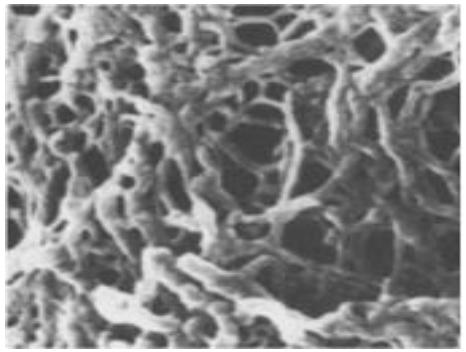
- Agarose is present in powder form that is dissolved in buffer (TBE or TAE) close to boiling temperatures, then cooled down to around 40 ℃, it sets and forms a gel (polymerized).

- The concentration of the agarose in the gel determines the size of the pores.

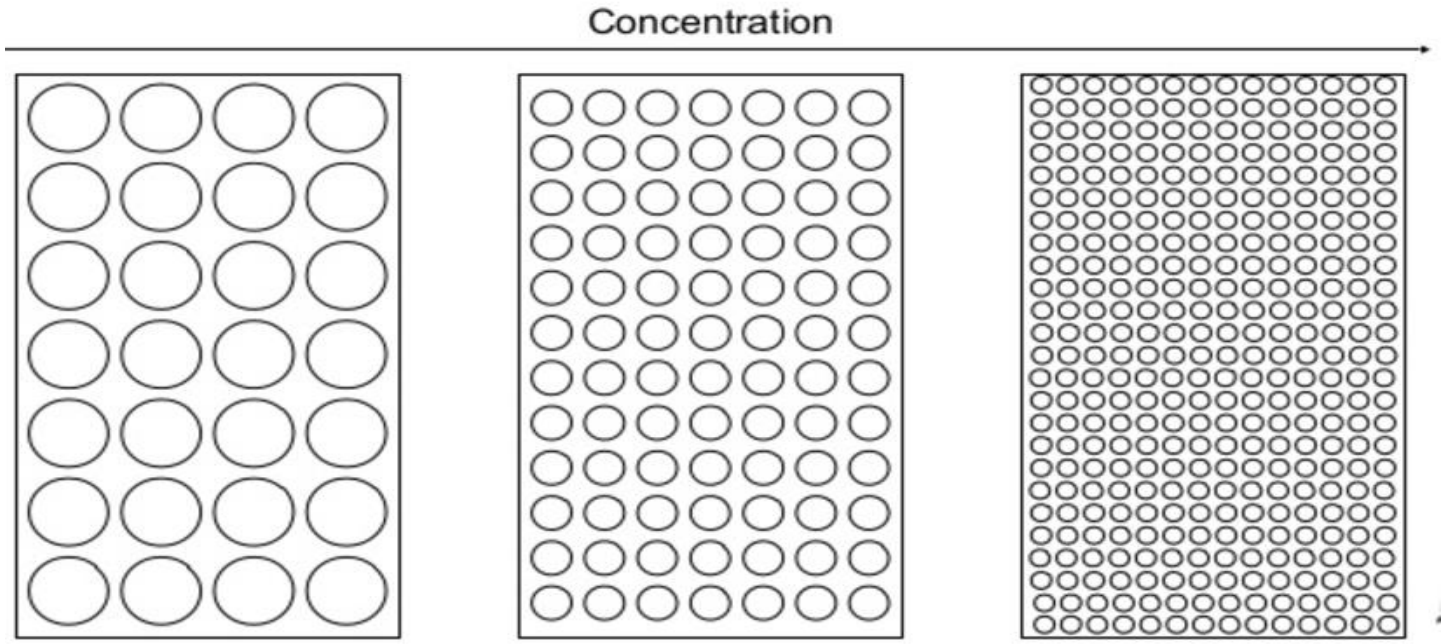
  High concentration of the agarose ➔ Smallest the pore size

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





Polymerized agarose



The pore size in the gel is controlled by the initial concentration of agarose.

Agarose Gel Concentration:

- The choice of gel matrices and gel concentration depends on the size of nucleic acid molecules, as the concentration of the agarose determines the pores size:

Gel concentration ↓

w/v% Gel	Size of DNA fragments (Kb = 1000 bp)
0.5 %	1 kb to 30 kb
0.7 %	800 bp to 12 kb
1.0 %	500 bp to 10 kb
1.2 %	400 bp to 7 kb
1.5 %	200 bp to 3 kb
2.0 %	50 bp to 2 kb

↑ DNA size

*PAUSE AND THINK ➔ What is the relation between the concentration of the gel and the pore size?

Buffer Types Used in Preparing The Gel:

- Helps in deliver the electric current through the gel.

- Buffer used is either TBE or TAE :
  - TBE: is made with Tris/Boric acid/ EDTA.
  - TAE: is made with Tris/Acetic acid/ EDTA.

TAE vs. TBE:

TAE (The Quick Runner)	TBE (The Precision Enhancer)
Higher conductivity ➔makes DNA move quickly	Lower conductivity ➔means less heat during the process, resulting in sharper bands and better resolution
Great when you need results fast or are just doing routine DNA separations	Good when you need to separate DNA fragments that are close in size
For routine DNA analysis, PCR product checks, or when you‘re not too concerned about super sharp bands	For more demanding tasks like separating DNA fragments that are similar in size, analyzing restriction enzyme digests, or when you‘re doing DNA sequencing and need every detail to be crystal clear.
Conclusion: Higher conductivity, faster migration, lower resolution, suitable for larger fragments and recovery.	**Conclusion**: Lower conductivity, slower migration, better for small fragments, higher buffering capacity, great for everyday use.

TAE vs. TBE:

Speed vs precision

Tracking Dye and Loading Solution:

- Since nucleic acid is colourless, the loaded sample need to be tracked ➔ This is achieved by using a loading dye solution.

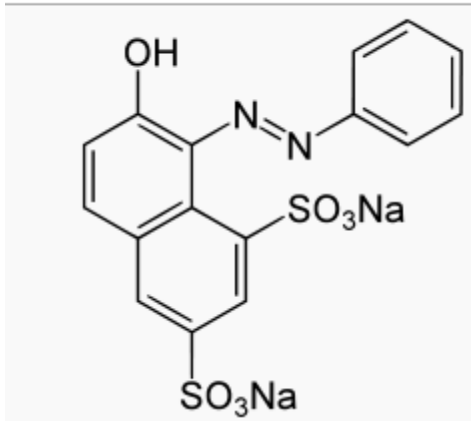
- There are several tracking dyes used such as: **bromophenol blue, Xylene cyanol or orange dye.**

➔it makes it easier to see the sample that is being loaded and also acts as a marker of the electrophoresis front (electrophoretic color marker).

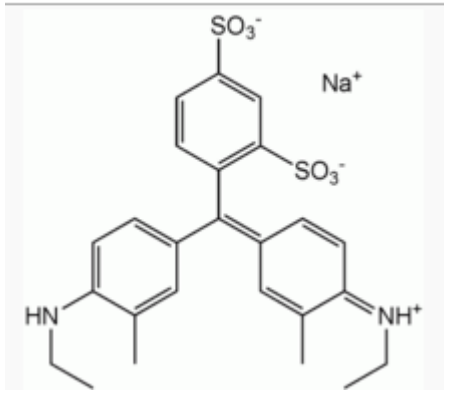
- A glycerol or sucrose is included with the tracking dye, to render the samples denser than the running buffer (so that the samples sink in the well).

Loading solution = Tracking dye + Glycerol/Sucrose

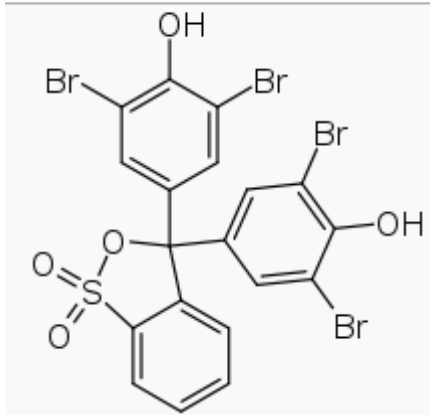
Orange G

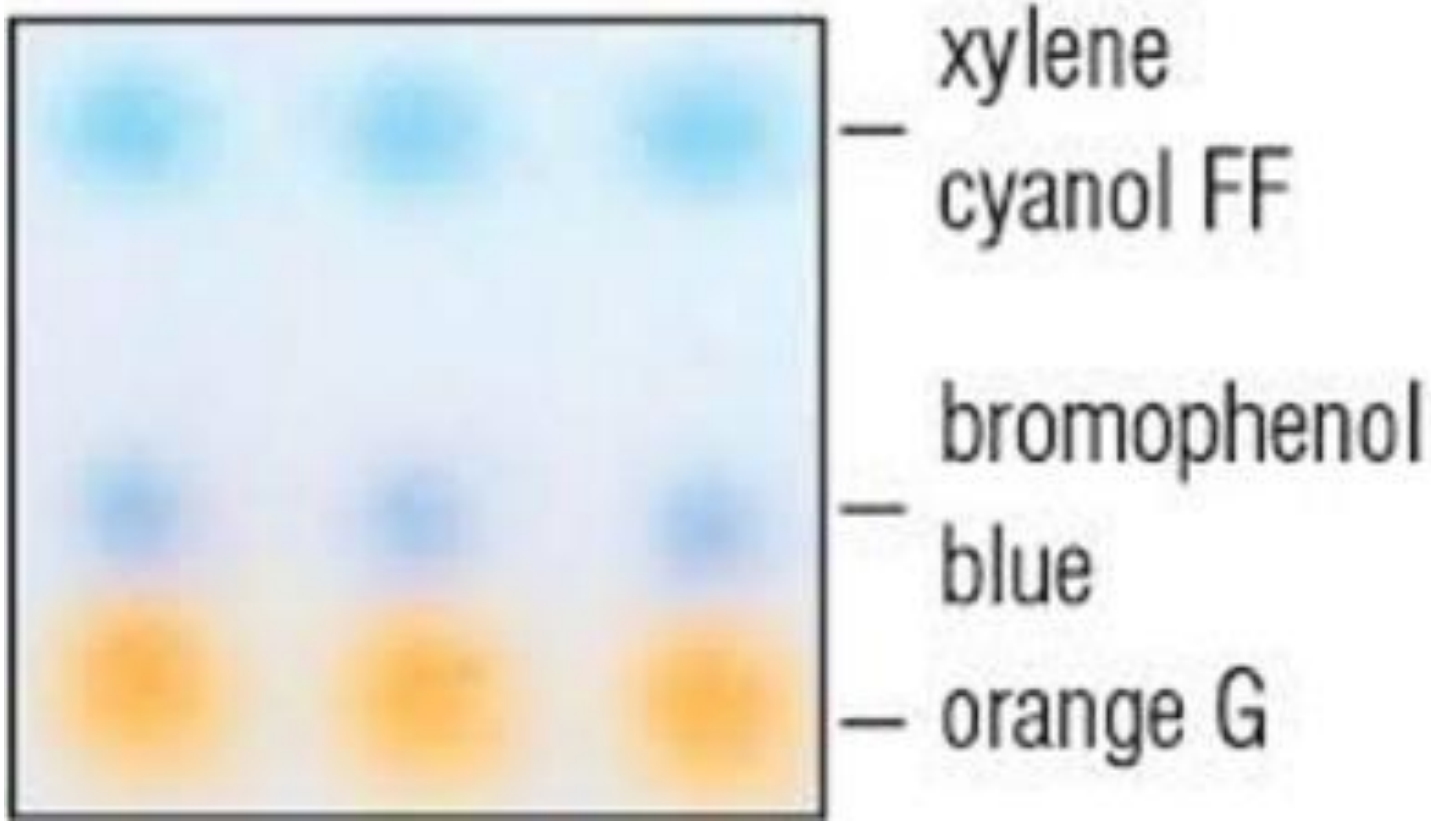


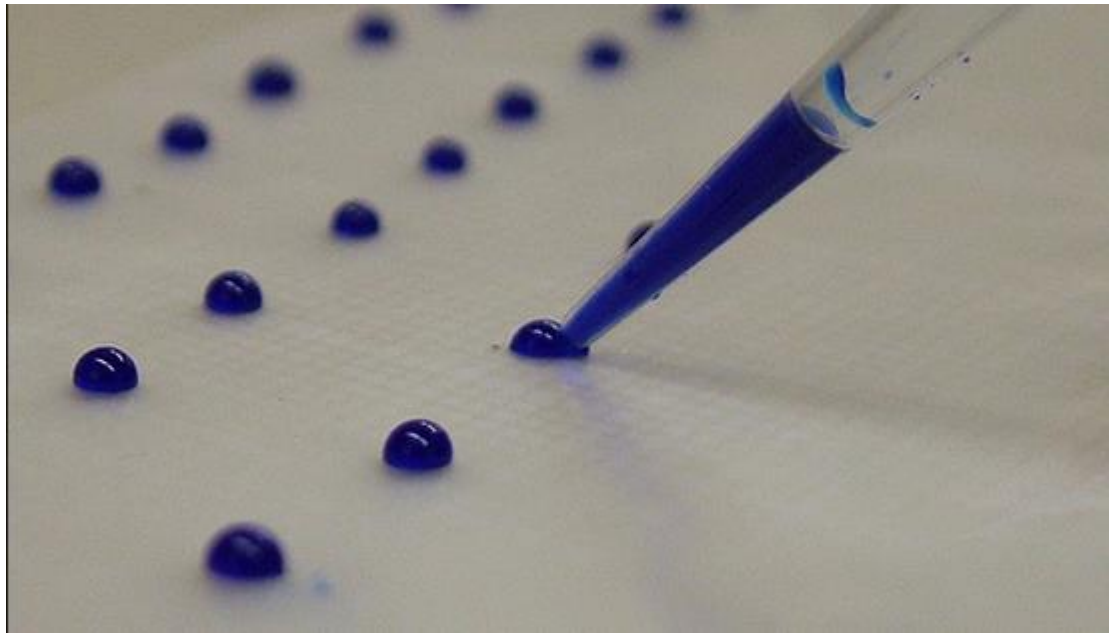
Xylene cyanol



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 loading solution.

After the samples of DNA are applied to the wells of the agarose gel along with the loading dye solution.

↓

The power is turned on after filling the buffer in the tank to allow current to pass.

↓

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

↓

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

Visualizing The Sample:

1. Ethidium bromide:

- The DNA in the gel needs to be stained and visualized; the reagent most widely used is the fluorescent dye ethidium bromide "EtBr".
- Ethidium bromide binds to DNA and fluoresces under UV light (emits orange light), allowing the visualization of DNA on a gel.
- Ethidium bromide is a cyclic planar molecule that binds between the stacked base-pairs of DNA.

2. Midori Green:

- Is a safe alternative to the traditional nucleic acid stain ethidium bromide.
- It is a non-carcinogenic and less mutagenic dye for detecting dsDNA, ssDNA and RNA in agarose gels with a very high sensitivity.

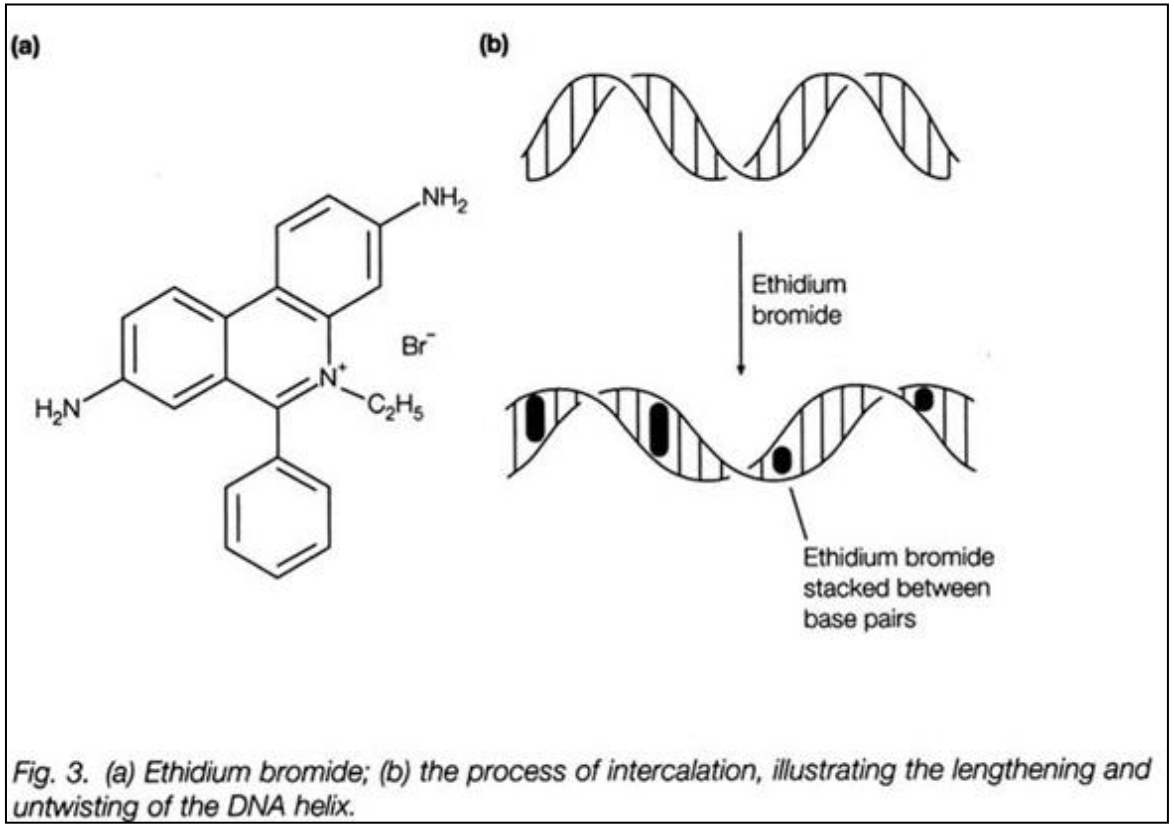
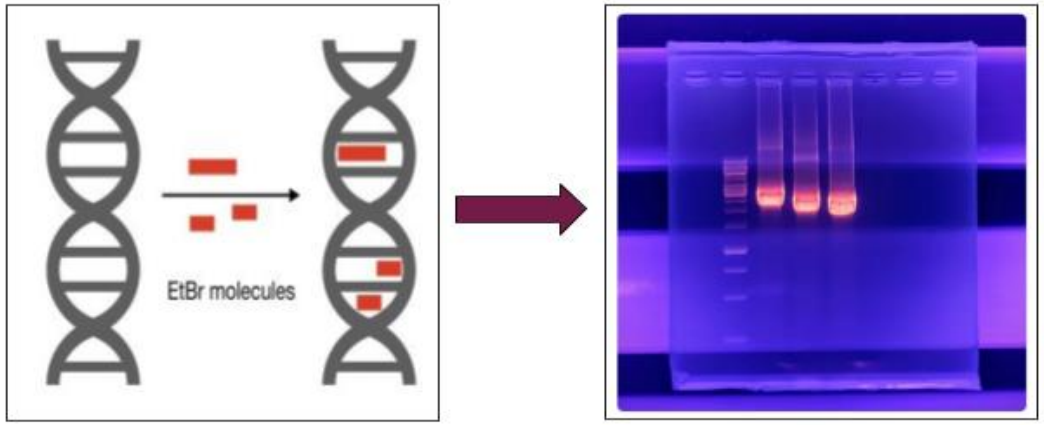
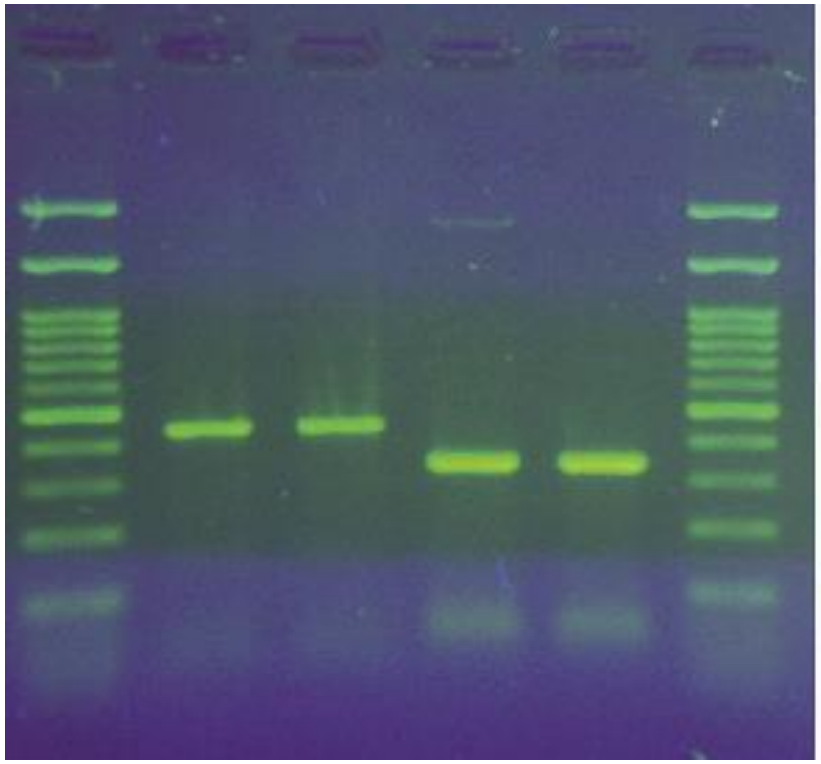


Fig. 3. (a) Ethidium bromide; (b) the process of intercalation, illustrating the lengthening and untwisting of the DNA helix.

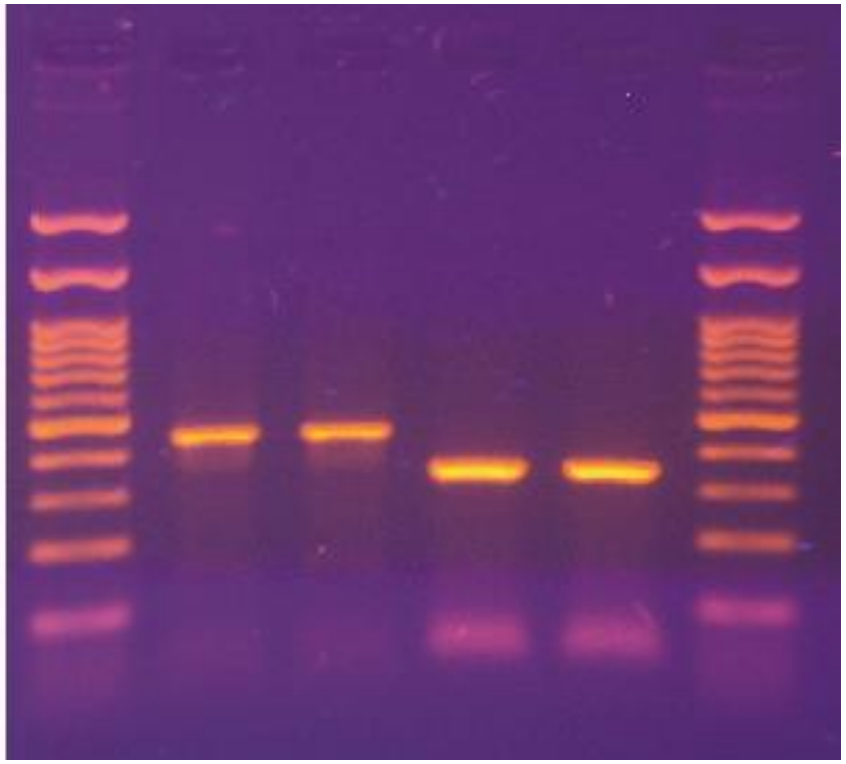


(a)

(b)



Midori Green emits green light under UV after binding to DNA



EtBr emits orange light under UV after binding to DNA

Agarose Gel Electrophoresis Application:

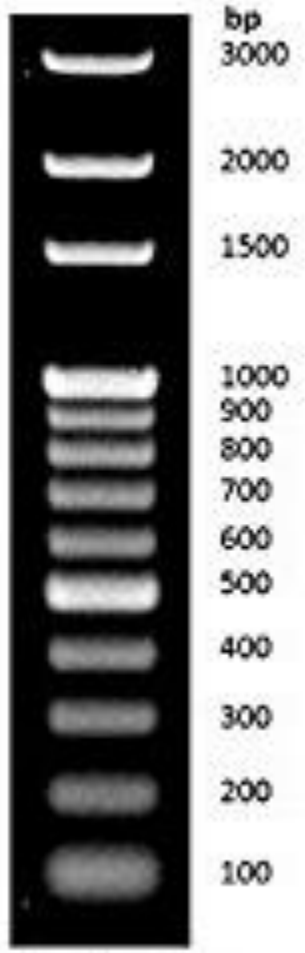
- Analyse the integrity of nucleic acids samples.
- To see if your DNA fragments is pure and there is no contamination (?).
- Purification of nucleic acids fragments mixture.
- PCR products.
- Calculate the size of DNA ➔ HOW?

1. Determination the size of a 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 (size marker)of known sizes on the same gel.
- These size markers are called Ladder.

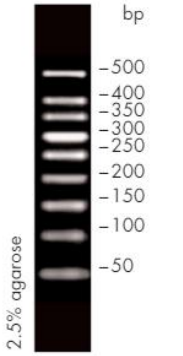
- Ladder is a DNA and RNA size markers contain a mixture of DNA (or RNA) fragments of known length, making them suitable for estimating the fragment length of concurrently run samples.

*Note: The ladder can come in different ranges of fragments; you must choose your ladder carefully!

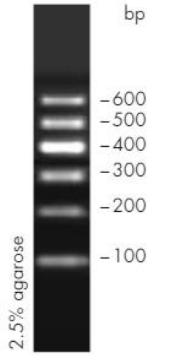


DNA Molecular Weight Markers (Ladder)

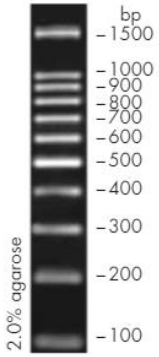
For easy and accurate sizing of DNA fragments



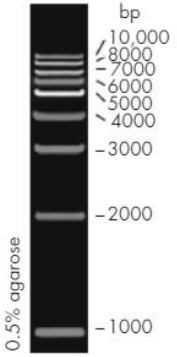
GelPilot 50 bp
Ladder (100)
(cat. no. 239025)



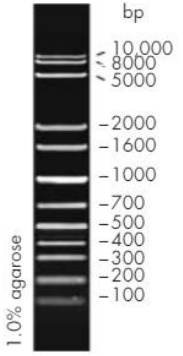
GelPilot 100 bp
Ladder (100)
(cat. no. 239035)



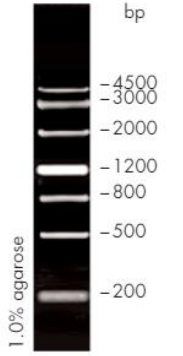
GelPilot 100 bp Plus
Ladder (100)
(cat. no. 239045)



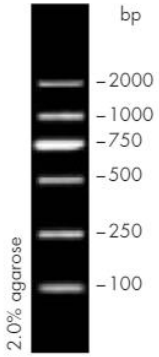
GelPilot 1 kb
Ladder (100)
(cat. no. 239085)



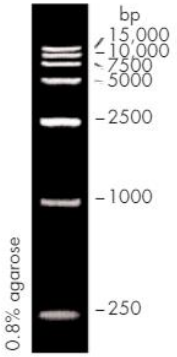
GelPilot 1 kb
Plus Ladder (100)
(cat. no. 239095)



GelPilot Wide Range
Ladder (100)
(cat. no. 239125)

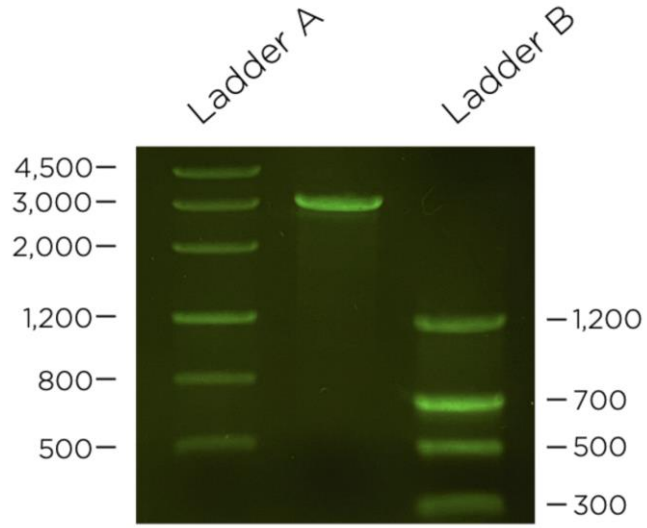


GelPilot Mid Range
Ladder (100)
(cat. no. 239135)

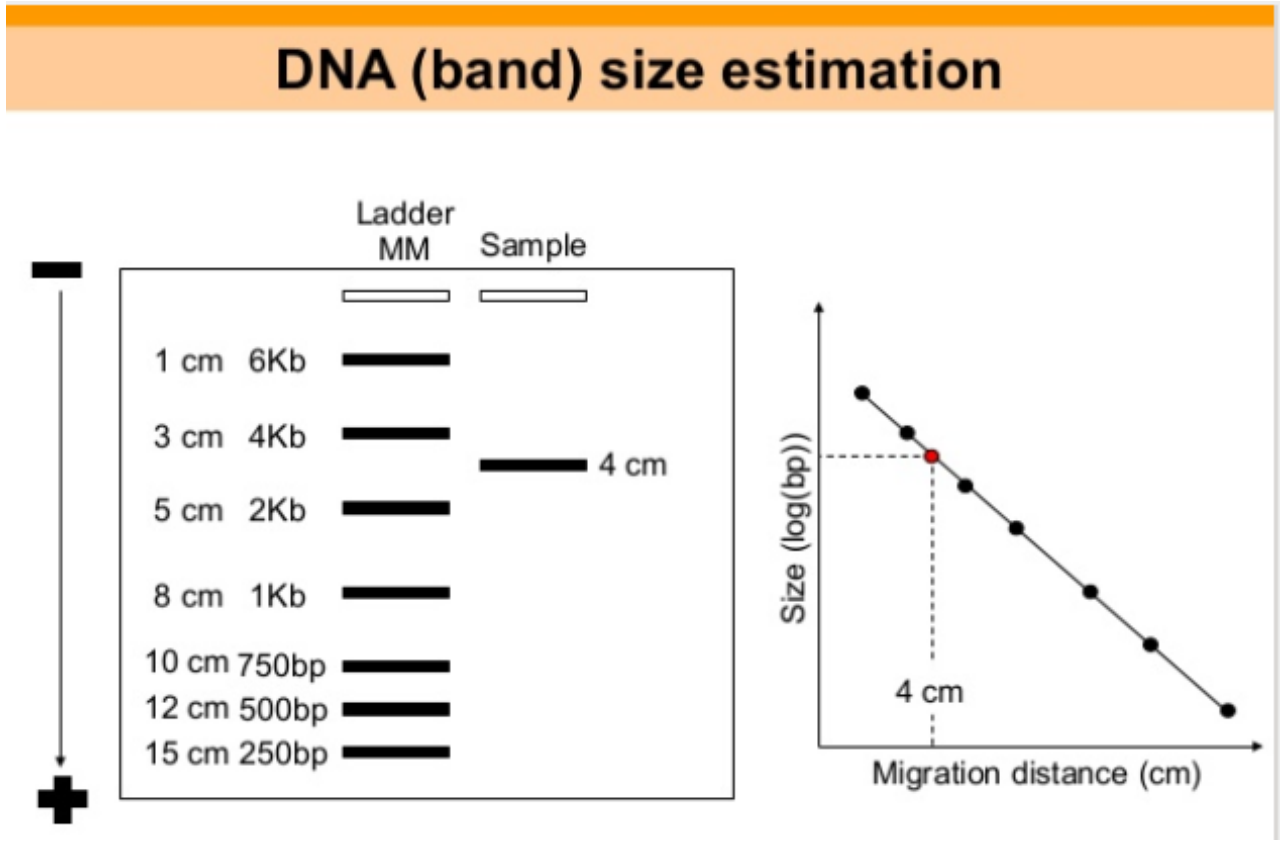


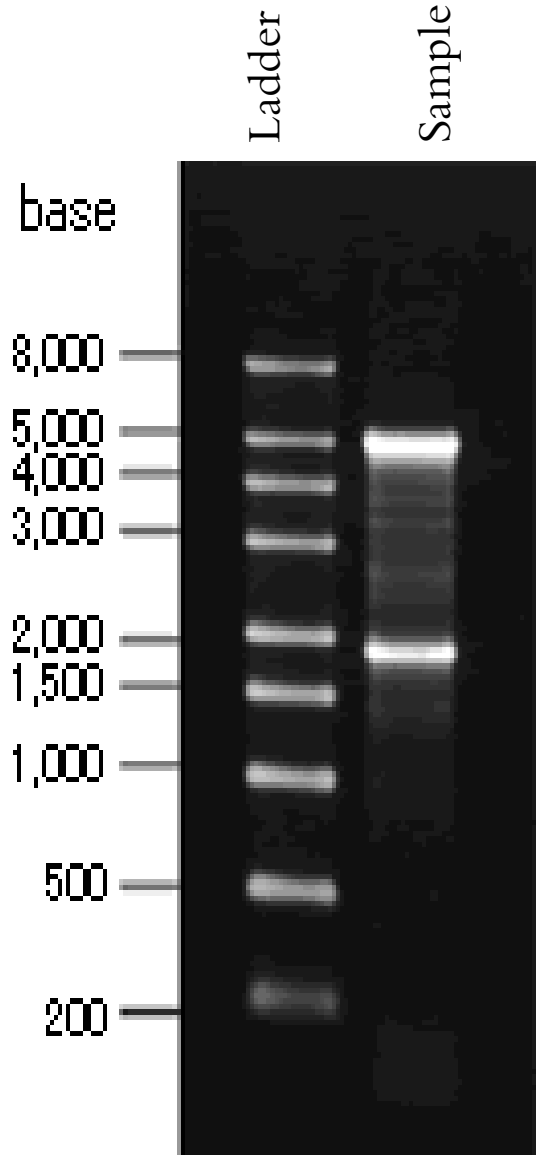
GelPilot High Range
Ladder (100)
(cat. no. 239145)

How to choose the right ladder for your sample?

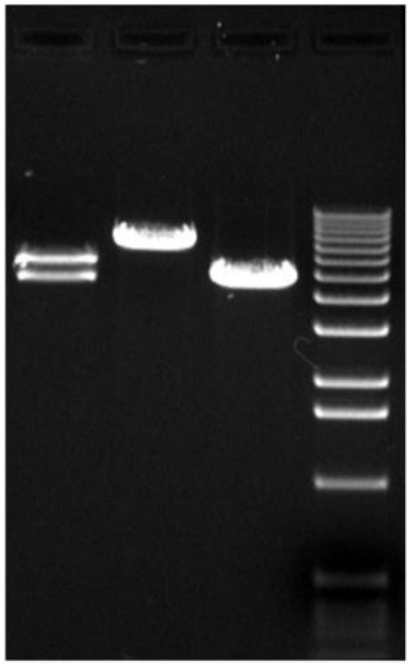


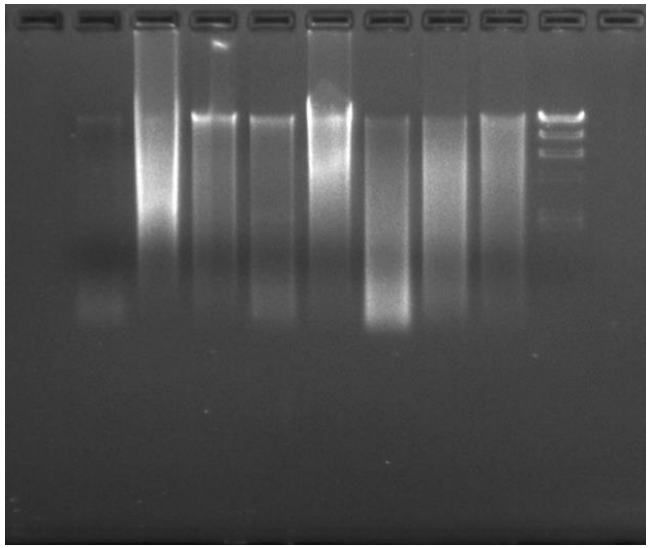
- Calculate the size of DNA.



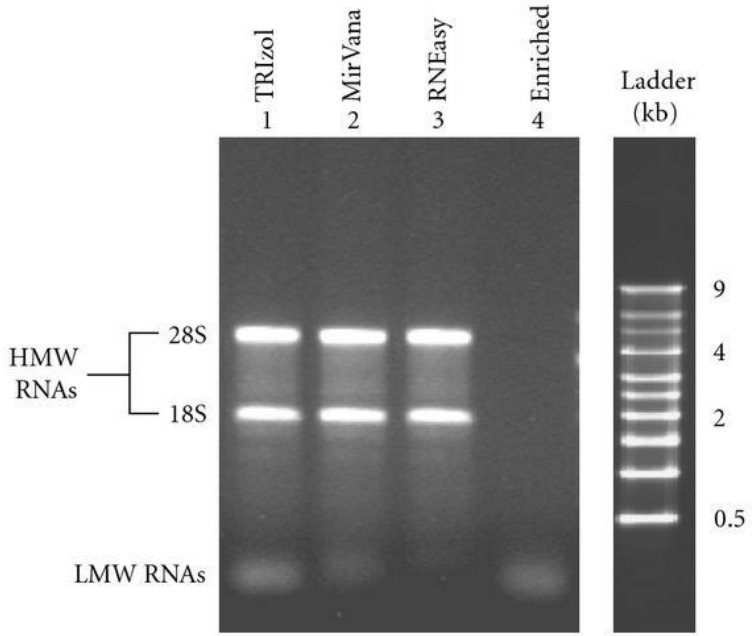


2.Analyse the integrity of nucleic acids samples:

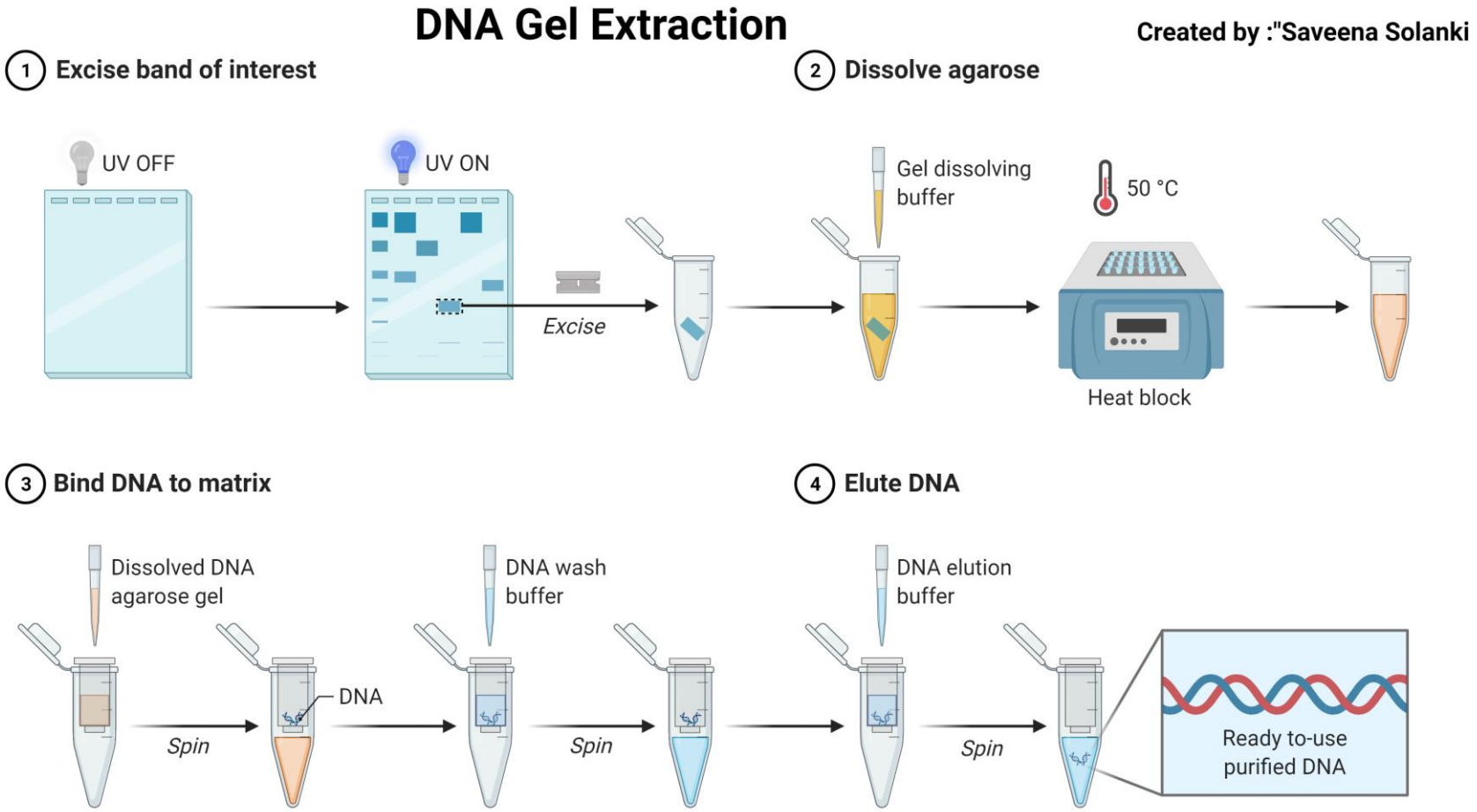




Smear



3. Purification of nucleic acids fragments mixture:



Factors Affecting the migration rate:

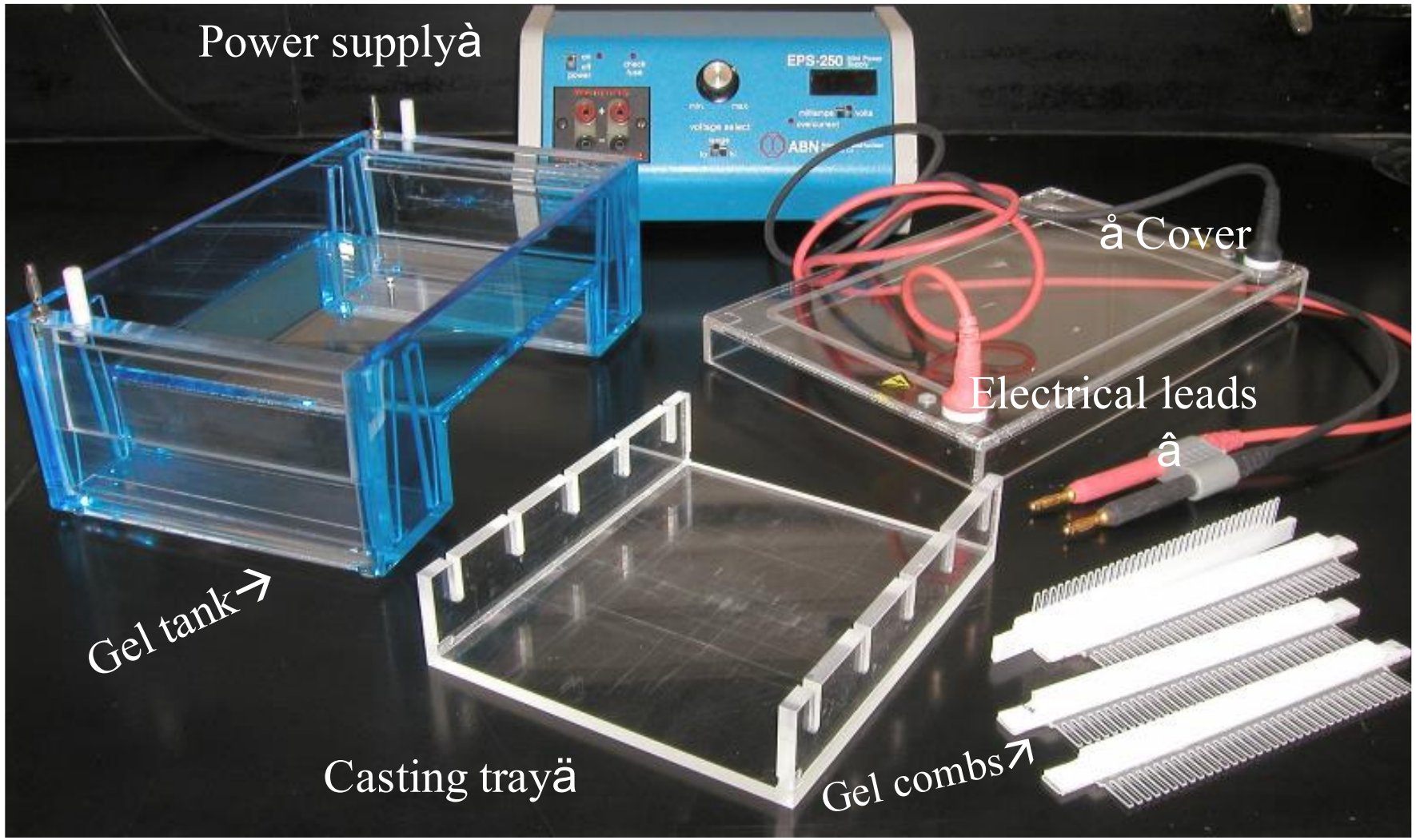
- Size of DNA molecules (inversely correlated).
- Conformation of DNA.
- Concentration of agarose gel (must be chosen to suit the size range of the molecules to be separated).
- Buffer used for electrophoresis (the DNA mobility in TBE buffer is approximately two times slower than in TAE buffer).
- Voltage applied (the higher the voltage ➔ , the more quickly the gel runs) (positively correlated).

Practical part

OBJECTIVES:

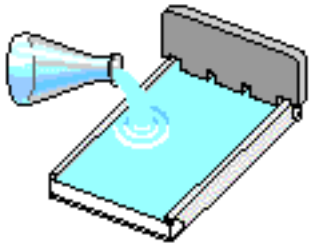
- To separate and calculate the molecular size of DNA fragment by comparing the separated bands with known standard molecular weight marker.

Agarose Gel Electrophoresis Glassware:

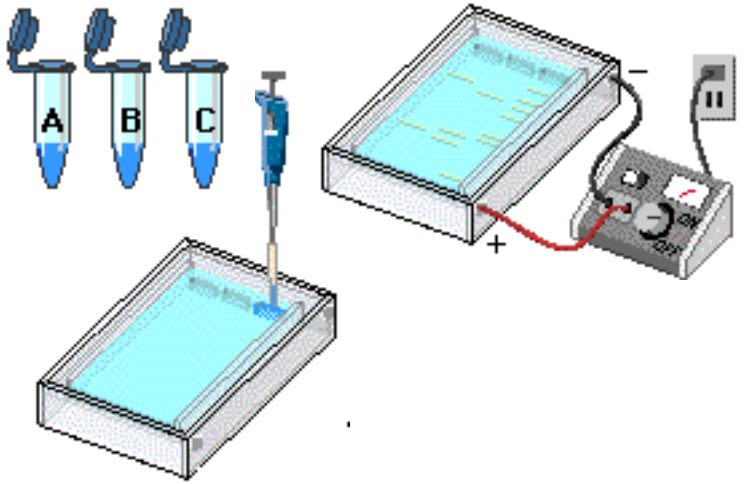


Performing Agarose gel electrophoresis:

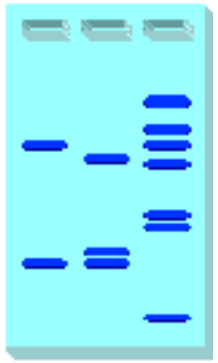
(1) Gel preparation



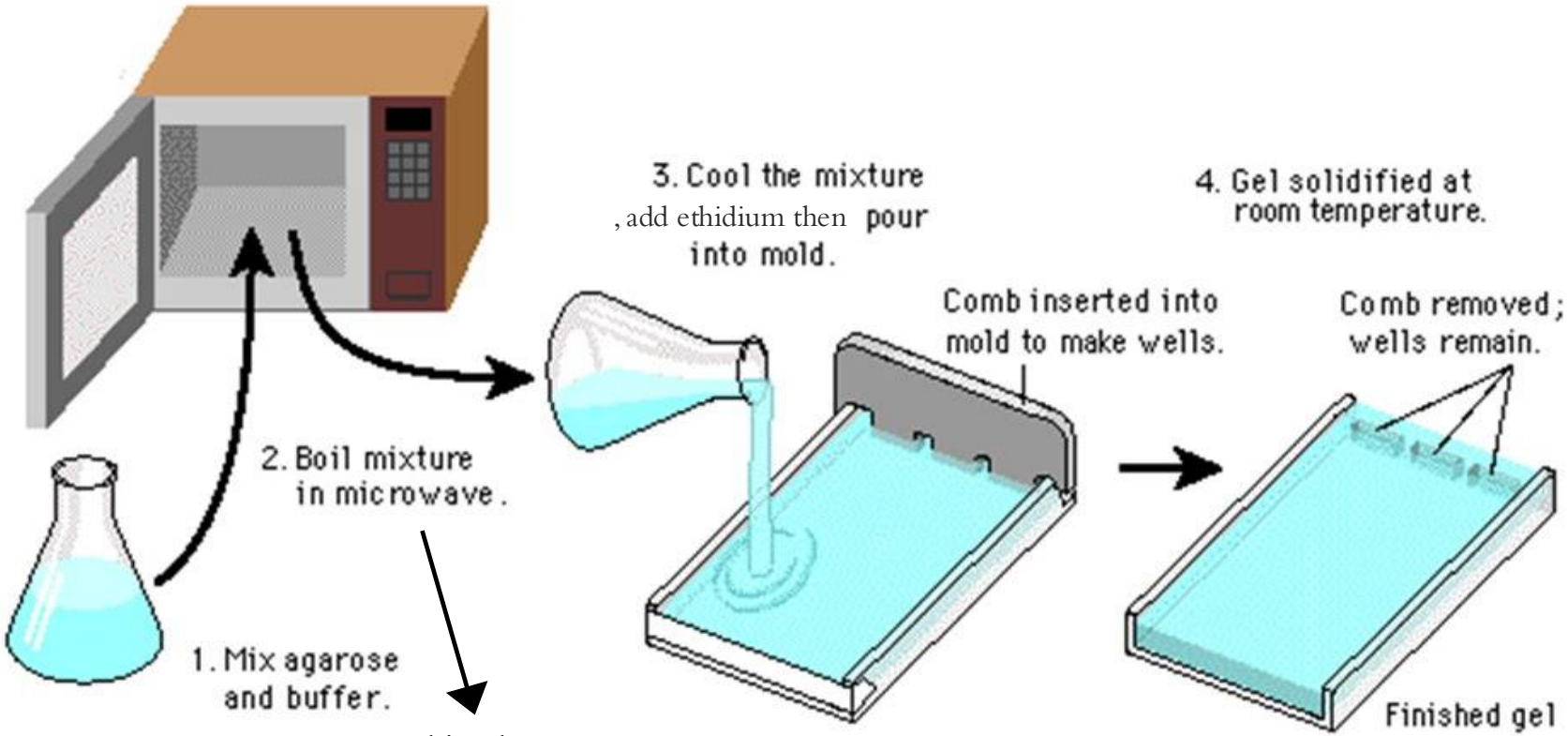
(2) Load the sample and start the run



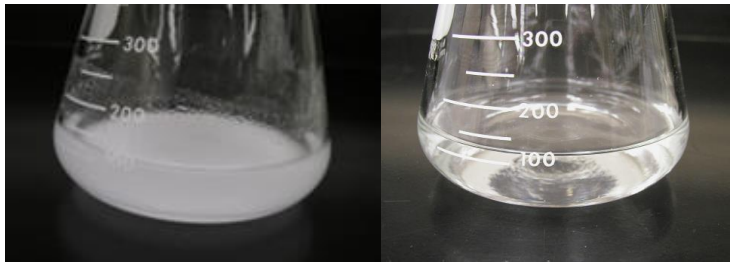
(3) Visualizing the sample



(1) Agarose Gel Preparation:



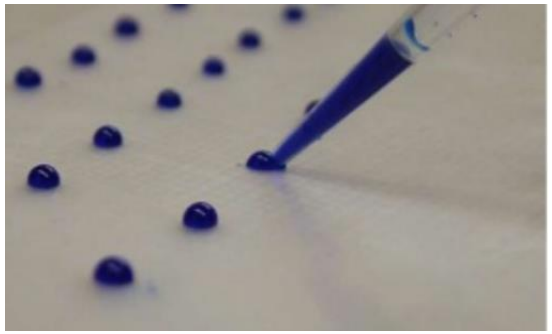
Melting the Agarose



(2) Sample Loading and Running The Gel:

1. Mix the DNA samples with the loading dye … **why?**
2. Load the sample into the well using pipette tip.
3. Load the DNA marker (Ladder).
4. Run the gel and track the sample.

1)



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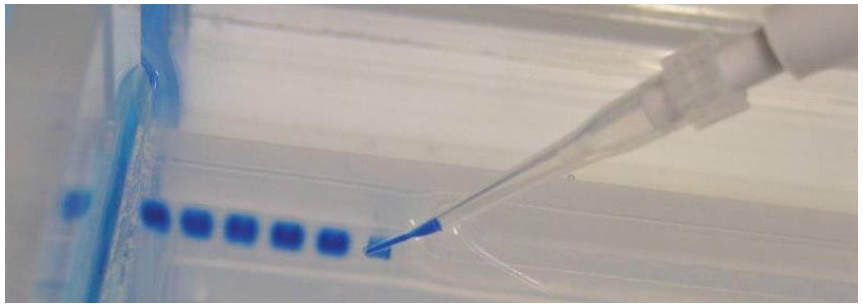

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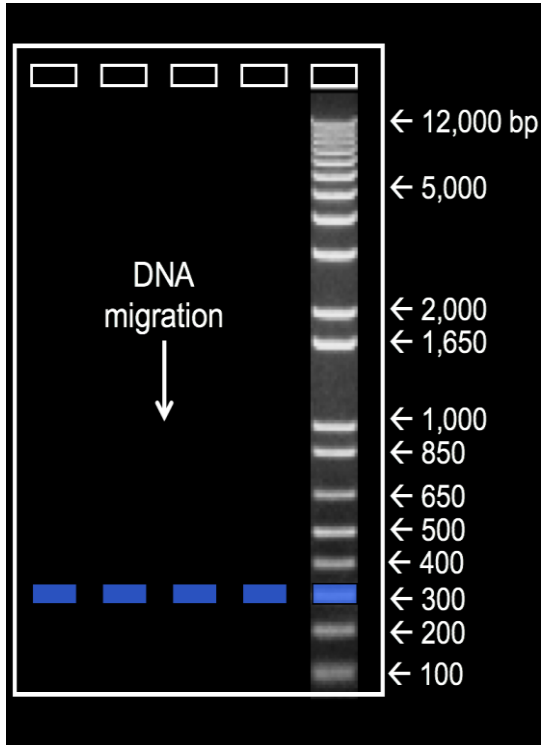

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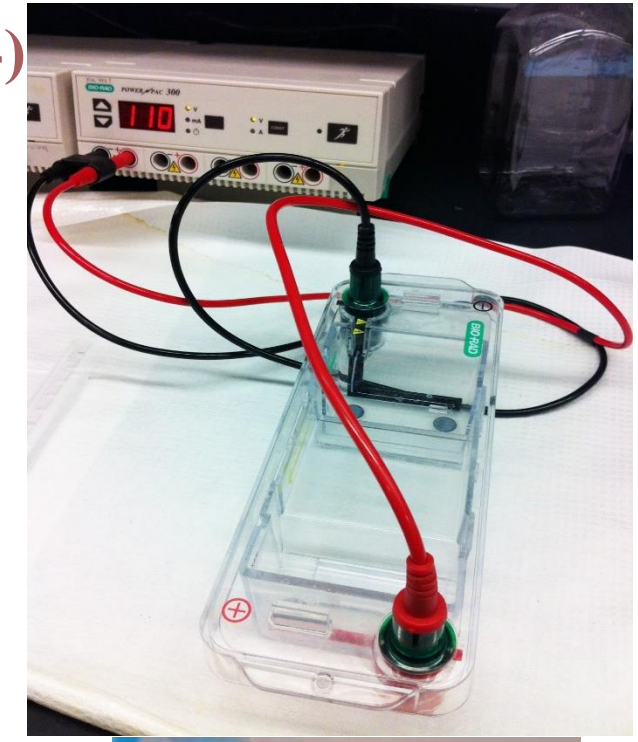
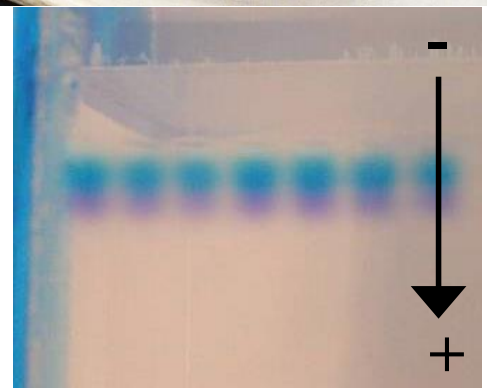
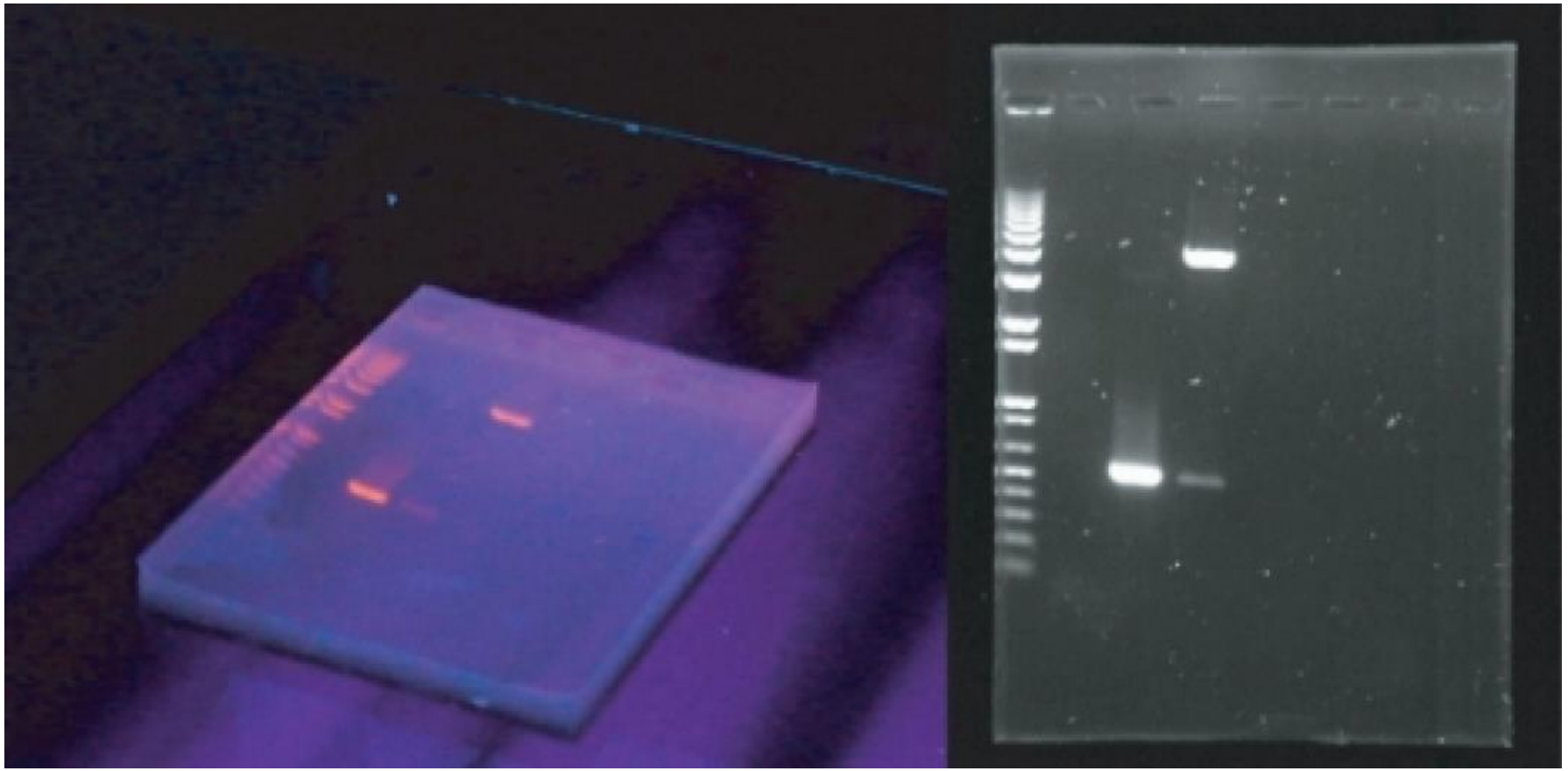




(3) Visualizing The sample Under UV:



Agarose Gel Electrophoresis - Animated Video

https://www.youtube.com/watch?v=hdmQaAycafc