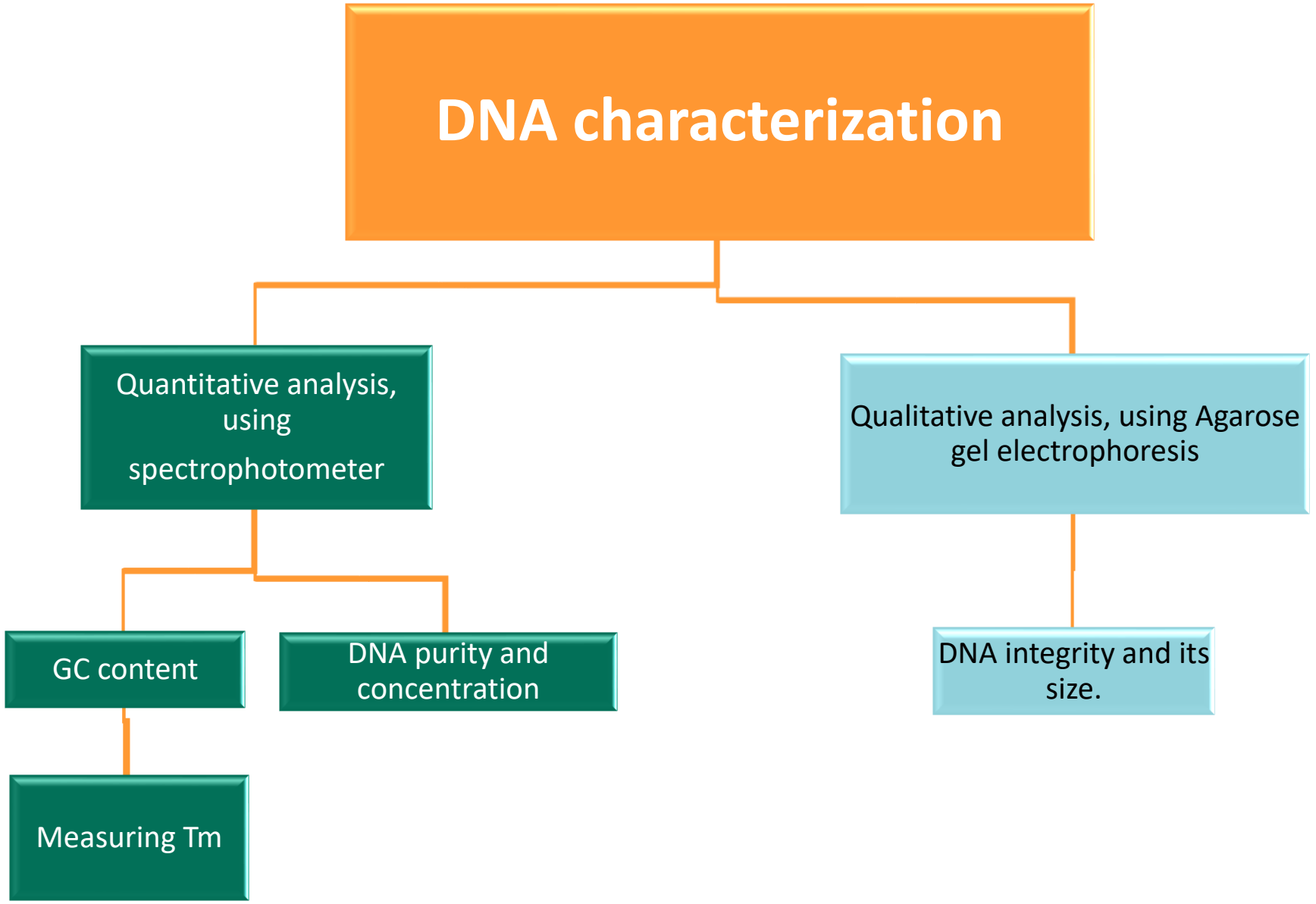
Agarose Gel Electrophoresis (AGE)

What NEXT ….. After DNA Extraction?



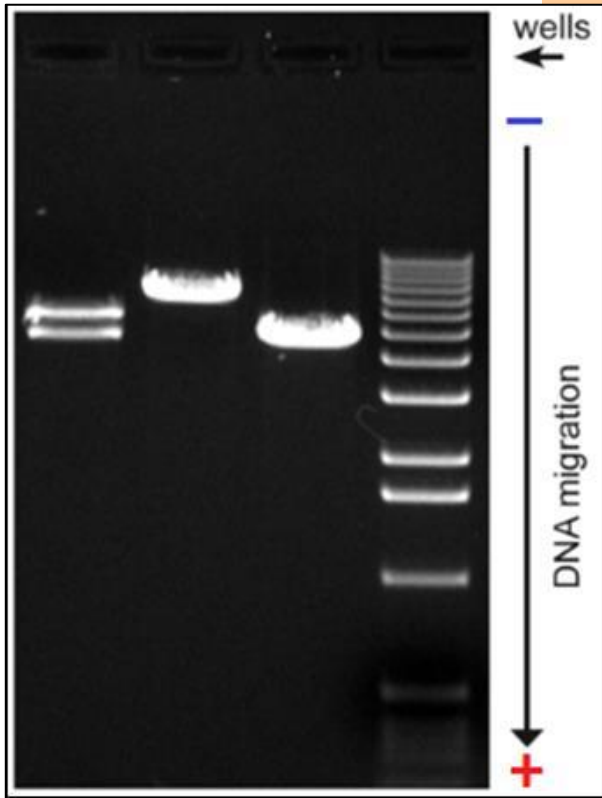
AGAROSE GEL:

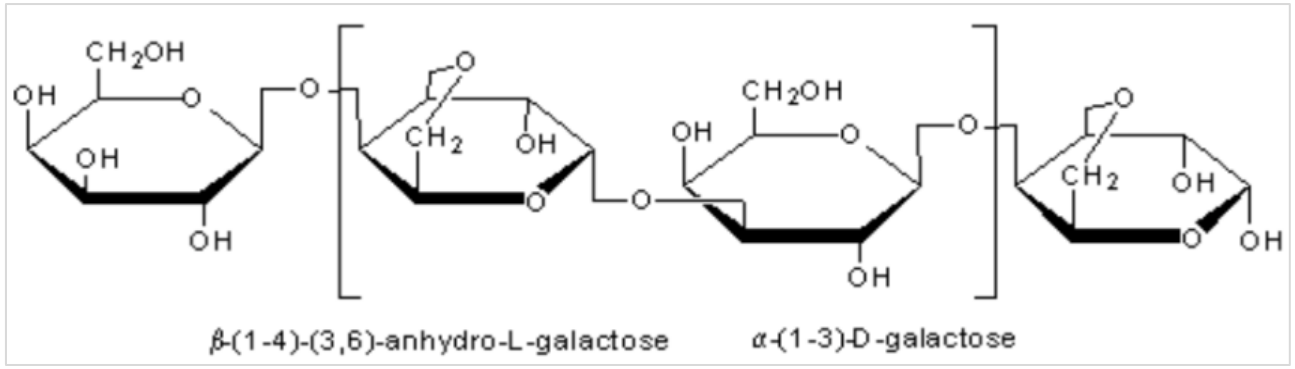
- Is a liner polymer composed of alternative residues of D-galactose and 3,6-anhydro-L-galactopyranose joined by α (1→3) and β (1→4) glycosidic linkages.

- Agarose, is present in powder 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]

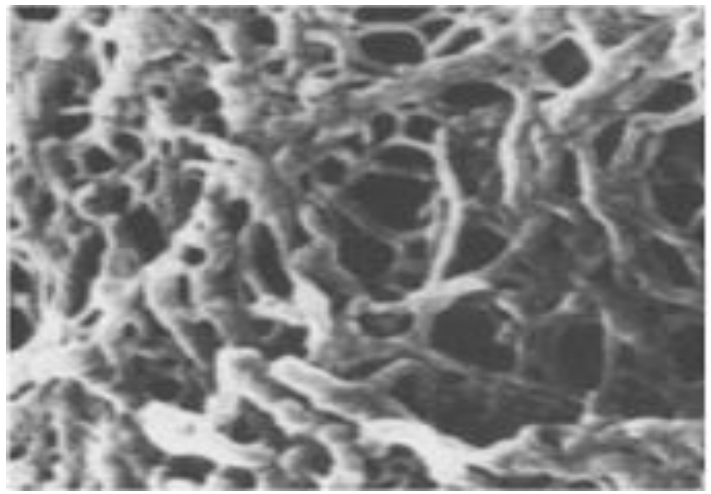
- **Prepare agarose gel with 0.8%?**

Gel staining, with Ethidium bromide to visualize the [DNA or RNA] samples under the UV. light





Agarose



Polymerized agarose

ELECTROPHORESIS "IN GENERAL":

- "Techniques involve the movement of charged particles (e.g., DNA) under the influence of an electric field."

Agarose gel electrophoresis:

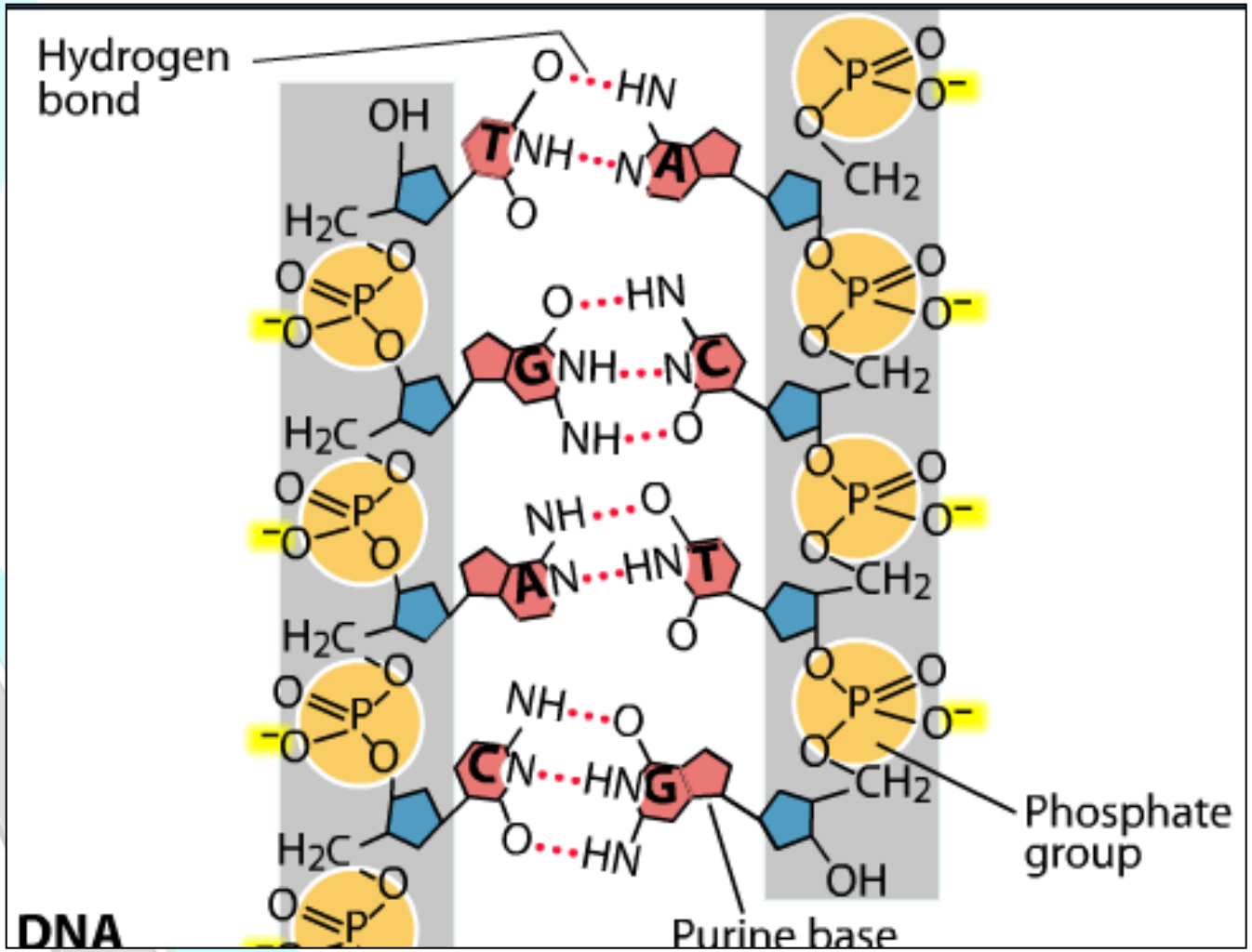
- Is a method of gel (made of agarose) electrophoresis, used to separate and analyse DNA or RNA molecules by **size.**

Principle:

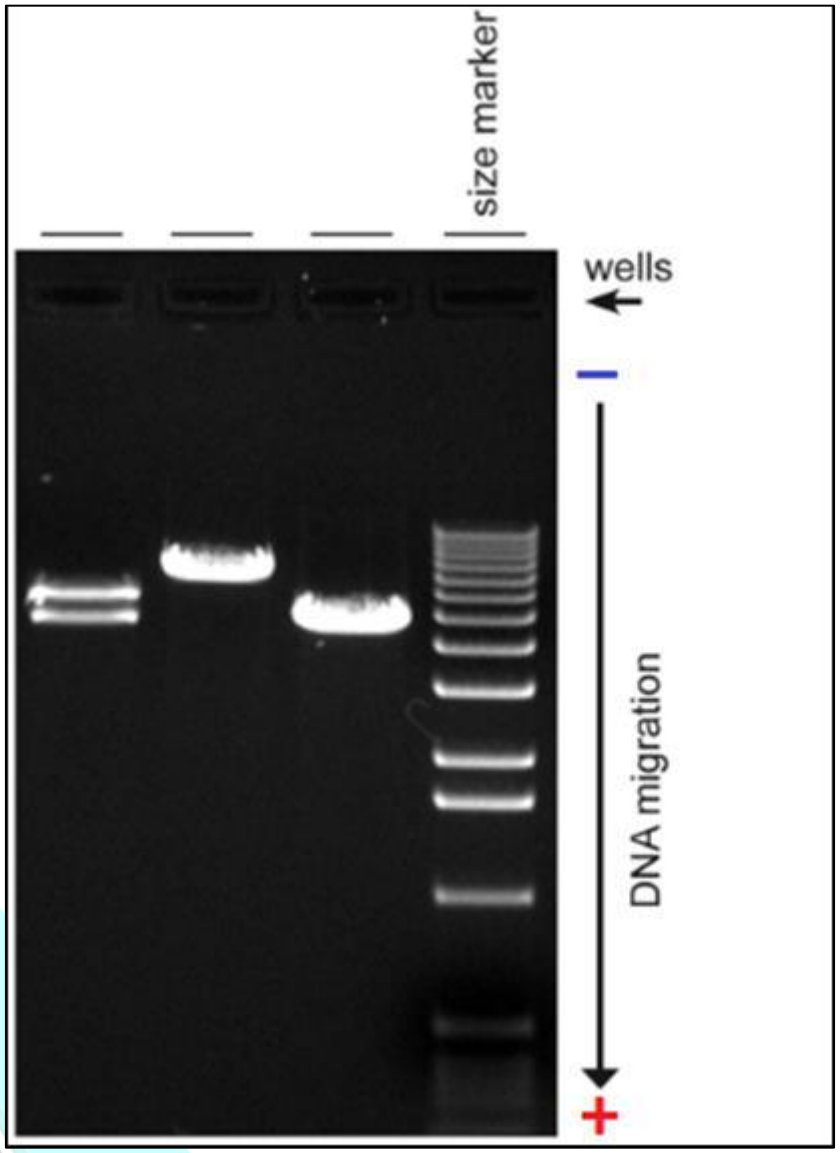
-Biomolecules [DNA or RNA] are separated by applying an electric field to move the negatively charged molecules [-] through an agarose matrix towards [+] , and the biomolecules are separated by size in the agarose gel matrix.

-The largest molecules will have the most difficulty passing through the gel pores,

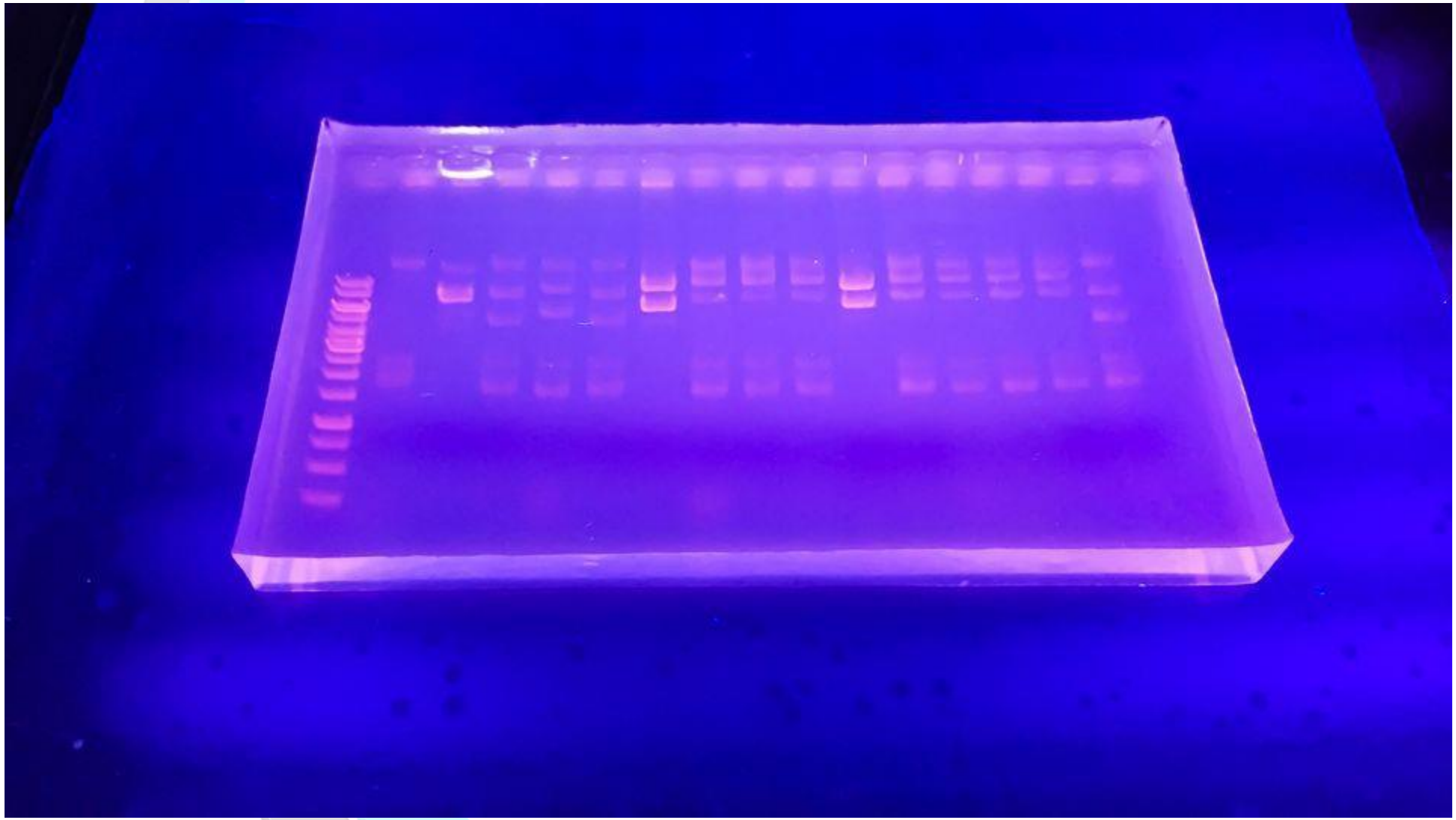
whereas the smallest molecules will move faster.



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



Separation of DNA molecules using agarose gel electrophoresis



"EtBr"orange light after binding to DNA.

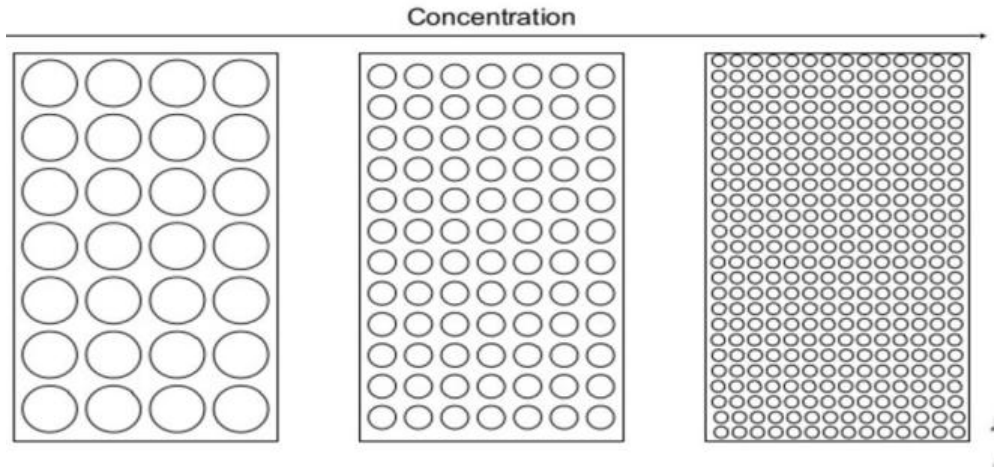
https://www.technologynetworks.com/analysis/articles/agarose-gel-electrophoresis-how-it-works-and-its-uses-٣٥٨١٦١

Agarose gel electrophoresis:

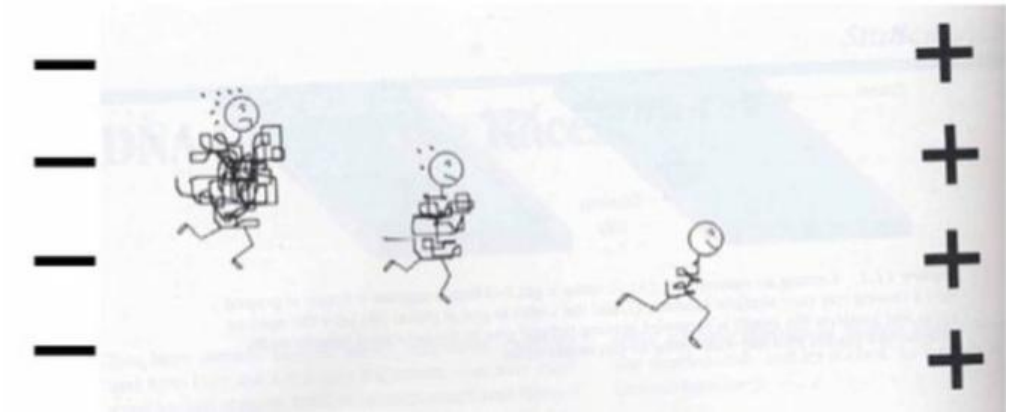
- **The electrophoretic migration rate of nucleic acids depends on:**
  - Size of DNA molecules.
  - Concentration of agarose gel.
  - Voltage applied.
  - Conformation of DNA.
  - Buffer used for electrophoresis.

How to control the pores size?

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



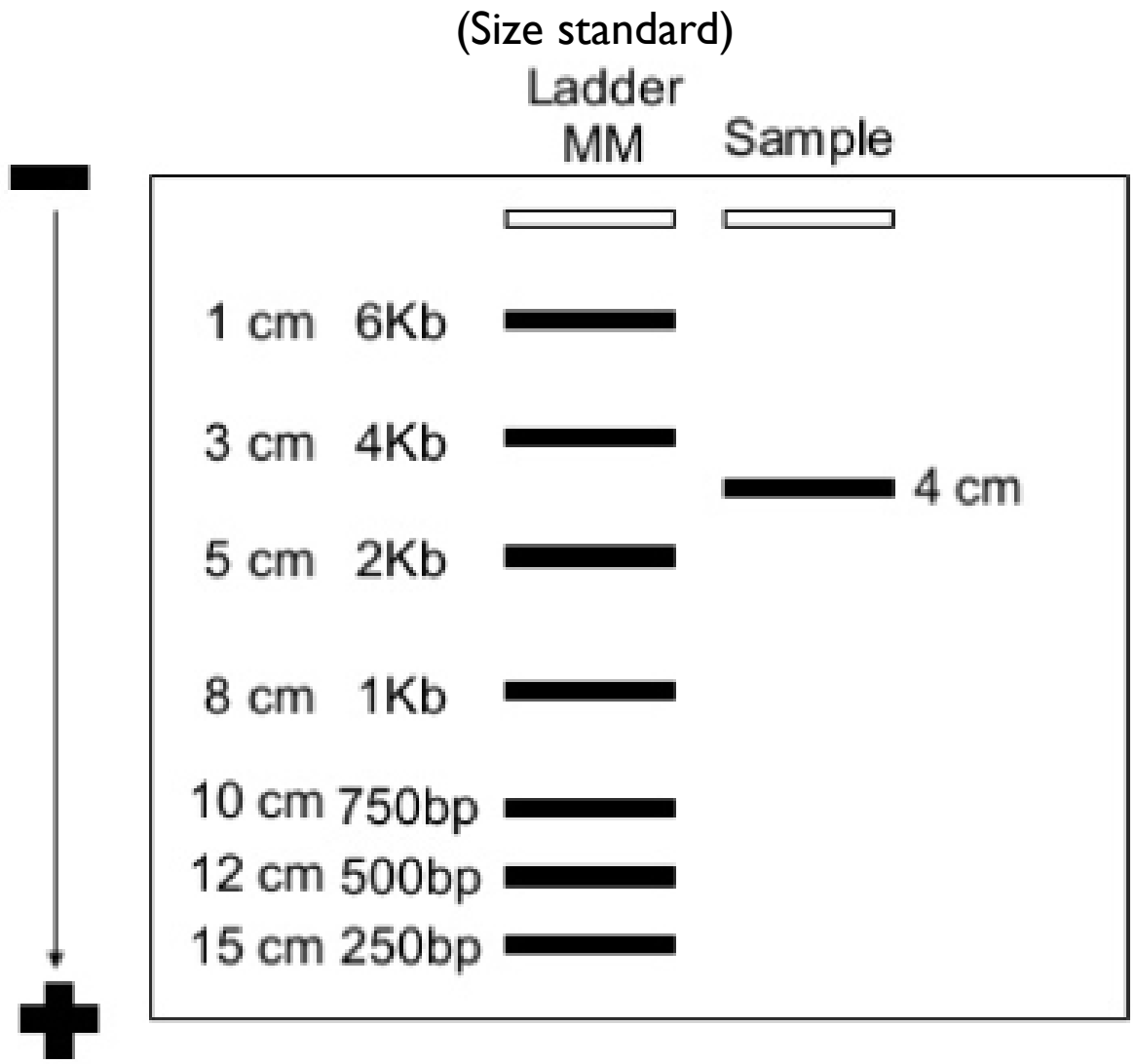
- The largest molecules will have the most difficulty passing through the gel pores.



When should you use agarose gel electrophoresis ?

- Analyse the integrity of DNA samples.
- Calculate the size of DNA ➔ **by the use of appropriate size markers.**
- To see if your DNA fragments is pure and there is no contamination (?).
- Purification of nucleic acids fragments mixture

DNA (band) size estimation



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

Practical Part

Aim:

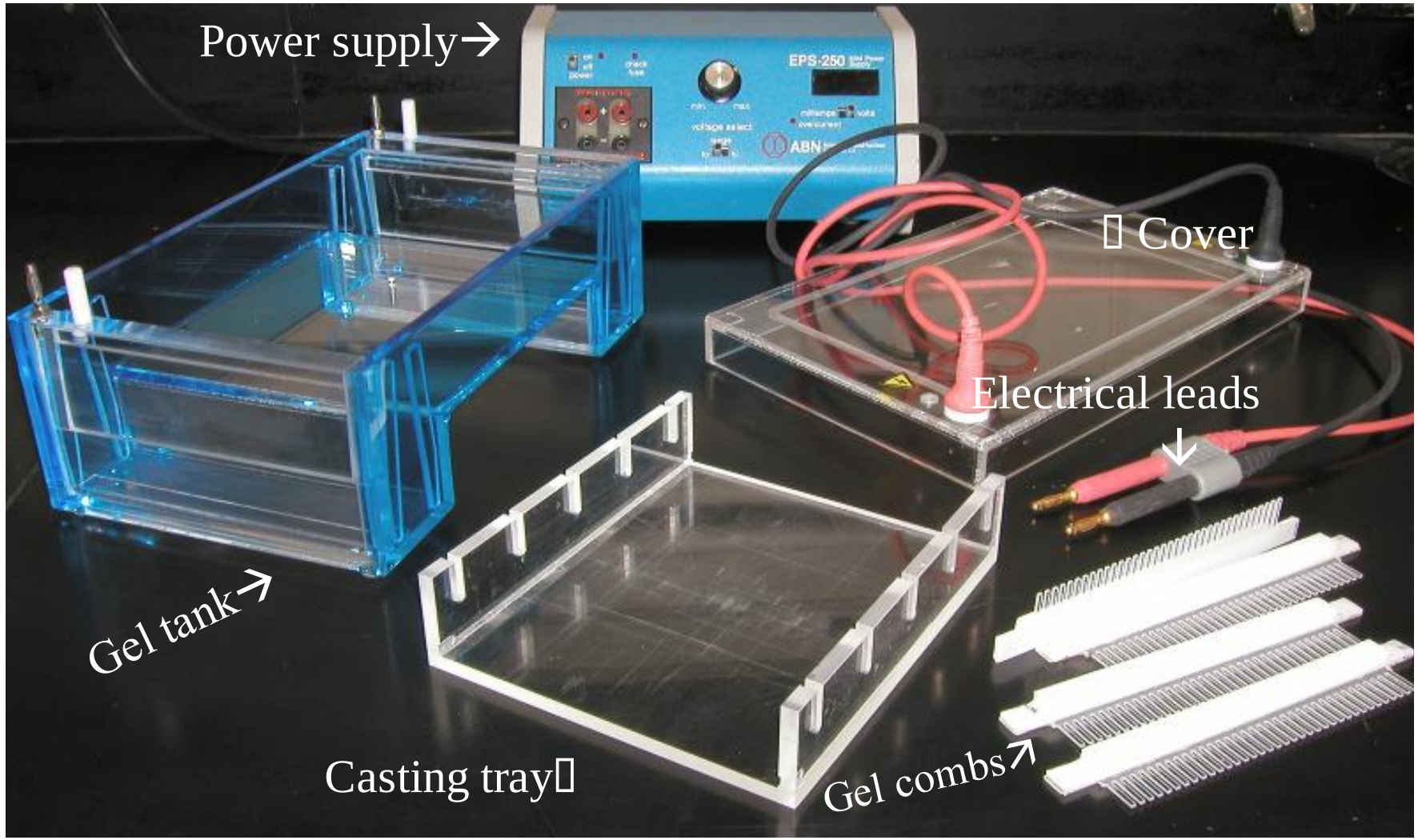
- Examination of extracted DNA by agarose gel electrophoresis.
- To separate and calculate the molecular size of DNA fragment by comparing the separated bands with known standard molecular weight marker.

Principle:

- AS MENTIONED BEFORE

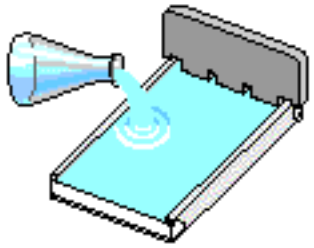
- **Note: the distance travelled by a DNA molecule is inversely correlated with its size.**

Electrophoresis glassware:

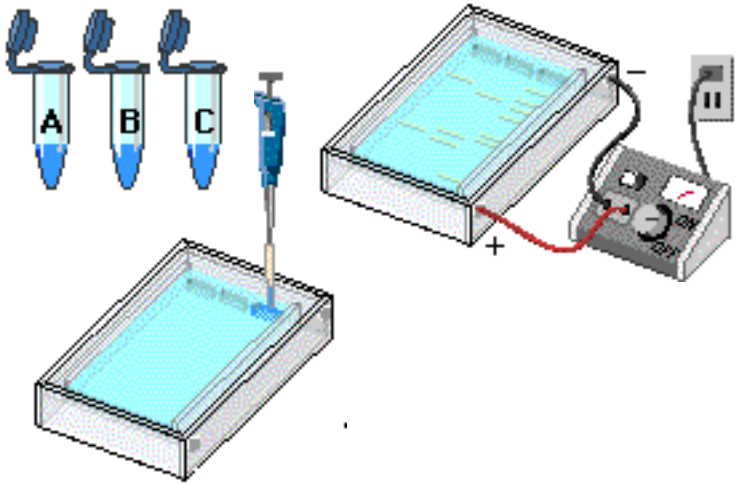


Performing Agarose gel electrophoresis:

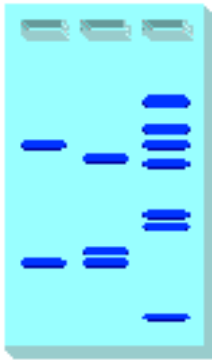
(1) Gel preparation



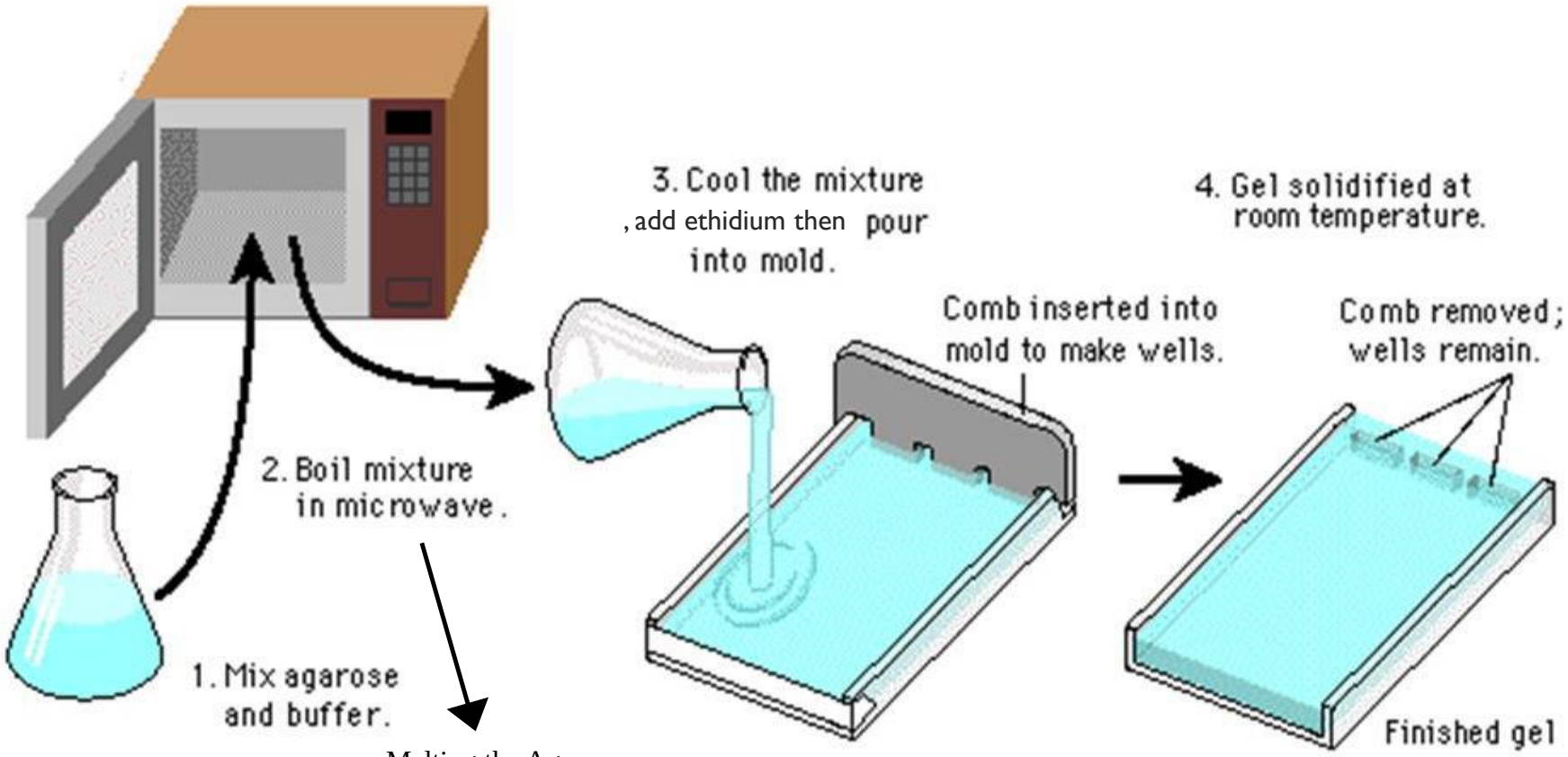
(2) Load the sample and start the run



(3) Visualizing the sample



(1) Agarose Gel Preparation

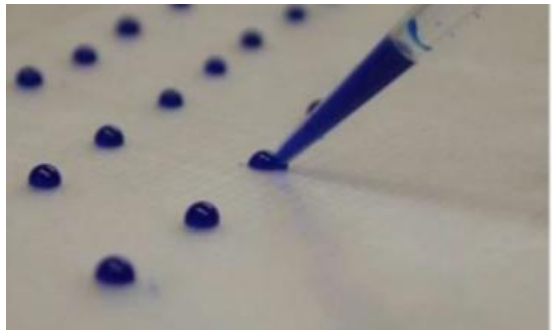


Melting the Agarose

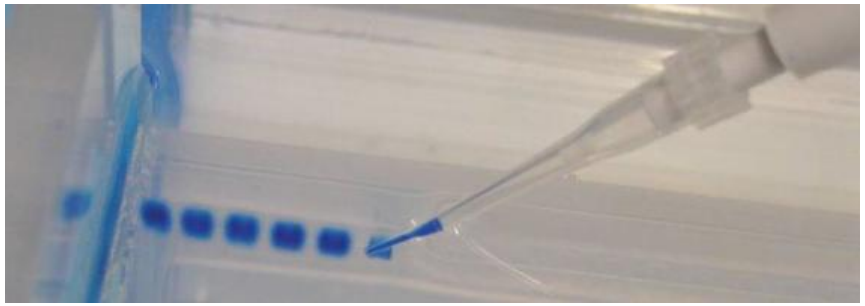
(2) Load the sample and start the run

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.

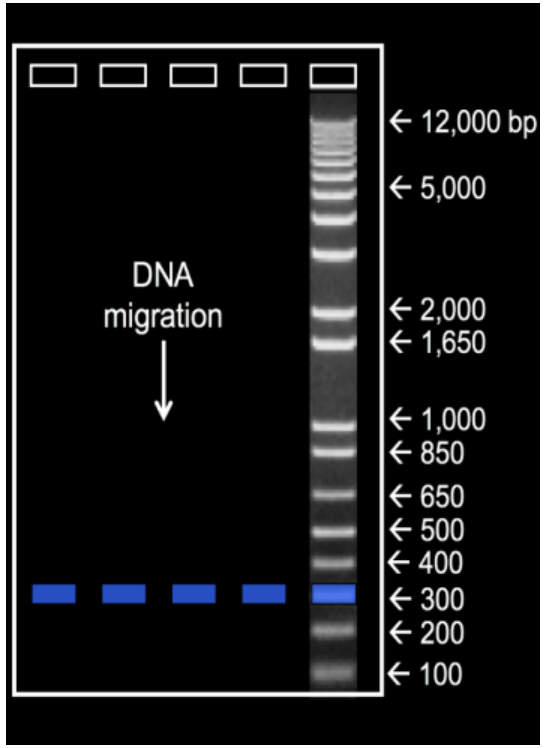
1)



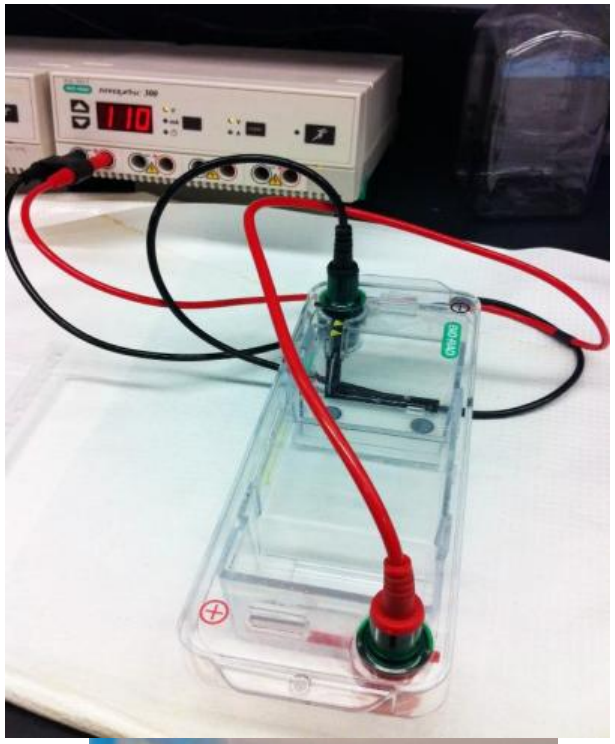
2)

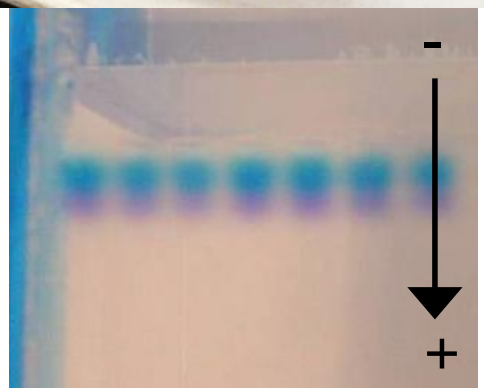


3)



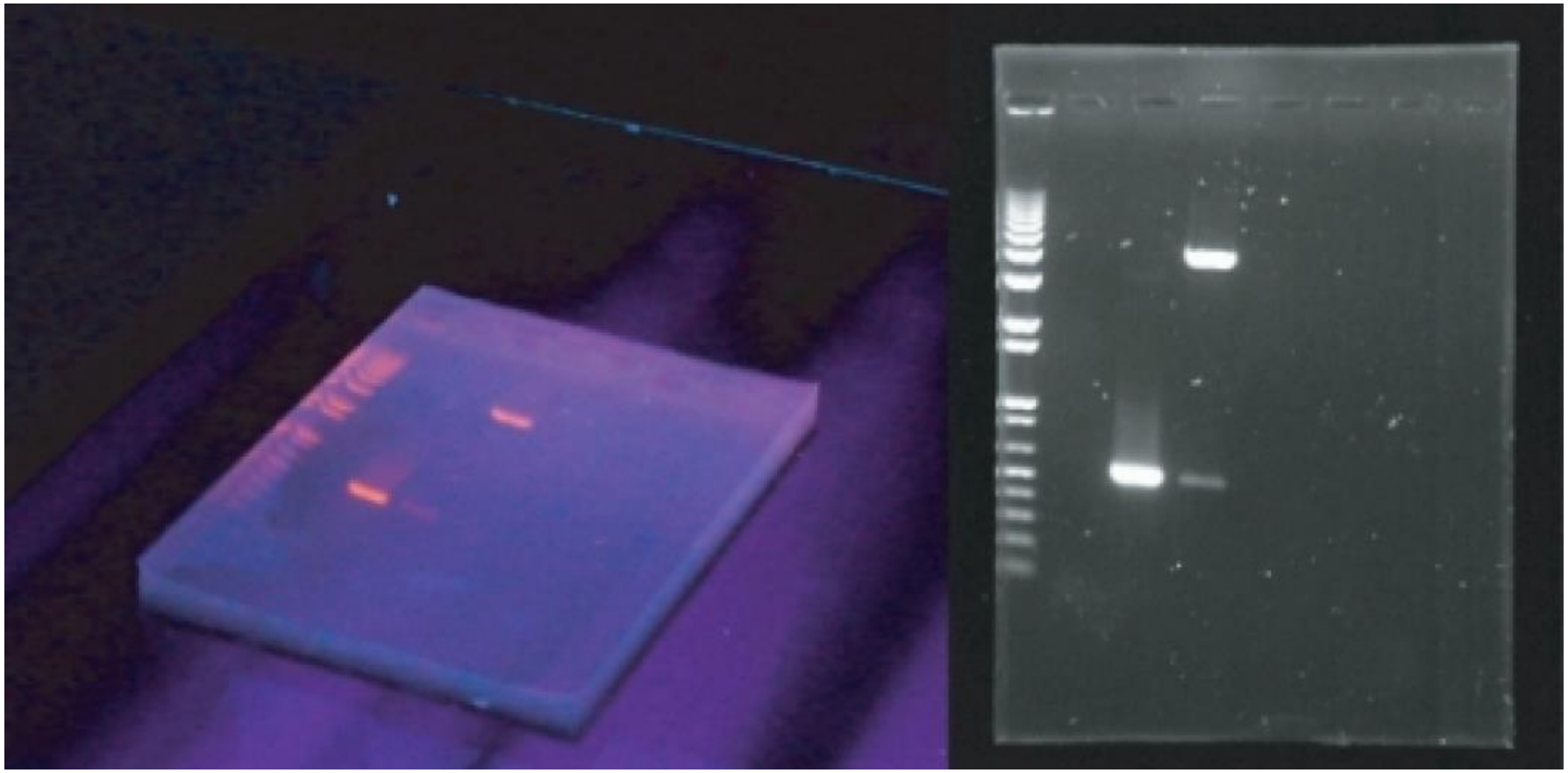
4)





(3) Visualizing the sample

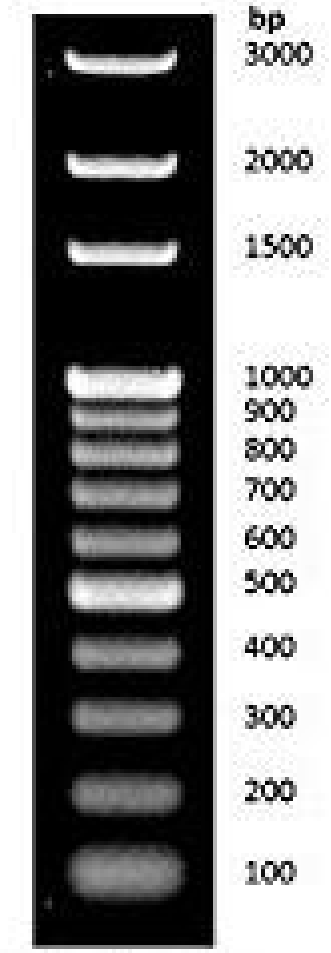
- Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a gel.



DNA Marker (Ladder) :

- 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.

- **Ladder can come in different ranges of fragments! You must choose your ladder carefully!**



Virtual Lab

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

Watch the following videos:

https://www.youtube.com/watch?v=wXiiTW3pflM

https://www.youtube.com/watch?v=U2-5ukpKg_Q