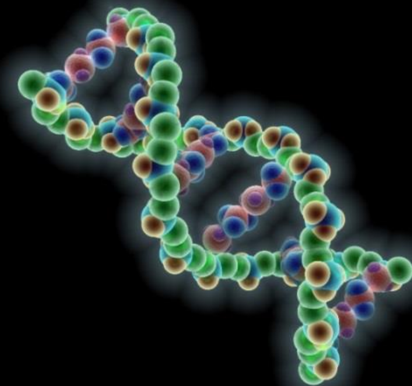
Gel Electrophoresis of DNA

Azam Safary
Assistant Professor of Medical Biotechnology

Agarose Gel Electrophoresis

- Agarose gel electrophoresis is routinely used for the preparation and analysis of DNA.

- Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field.

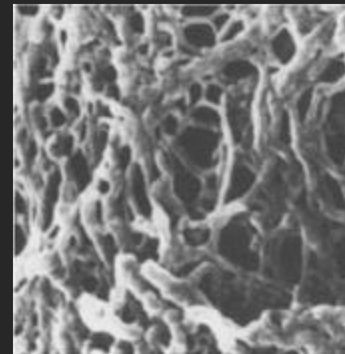


DNA is negatively charged

- When placed in an electrical field, DNA will migrate toward the positive pole (anode).
- An agarose gel is used to slow the movement of DNA and separate by size.

• Polymerized agarose is porous, allowing for the movement of DNA

Scanning Electron Micrograph of Agarose Gel (1 × 1 μm) →



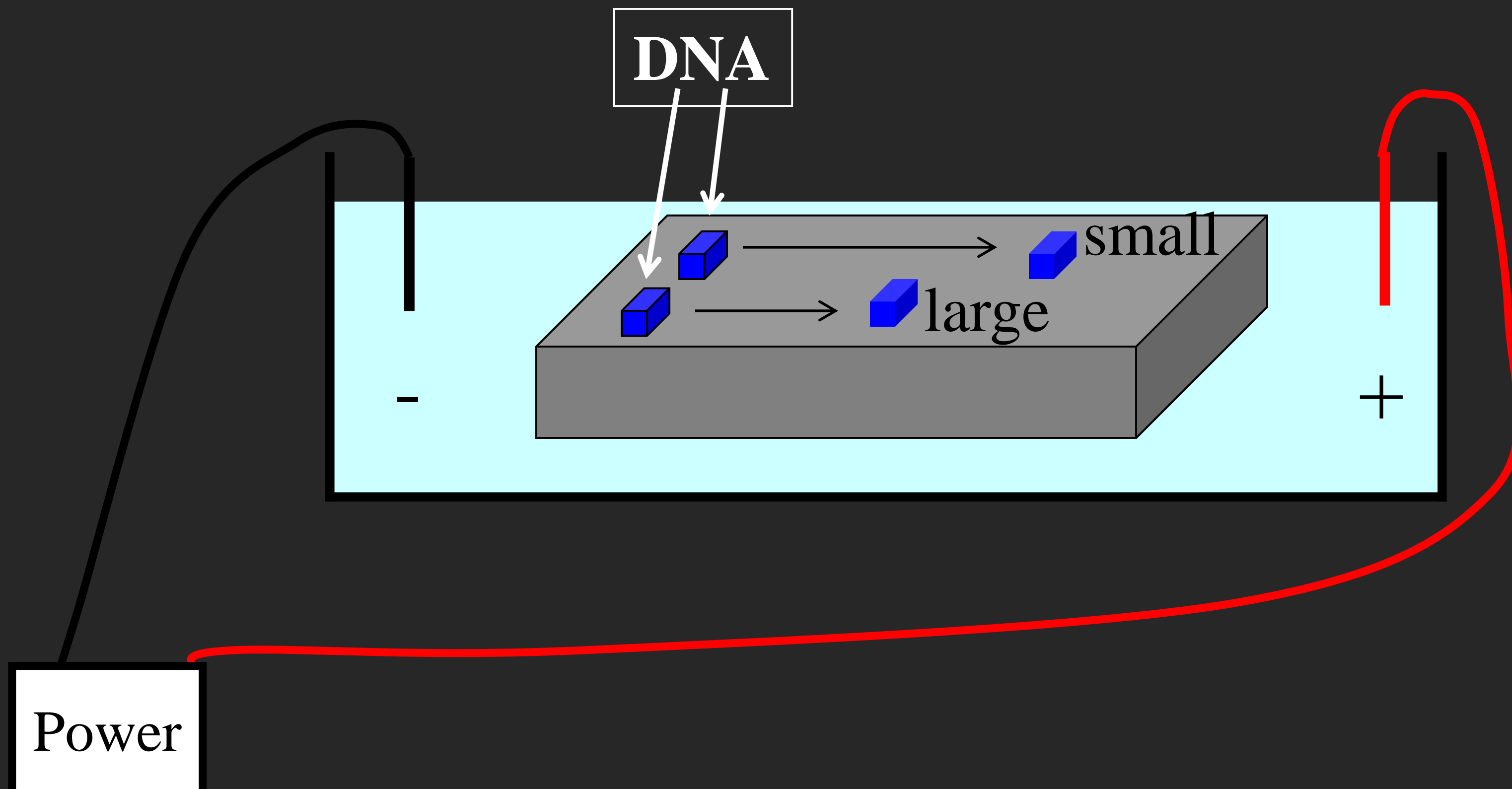
How fast will the DNA migrate?

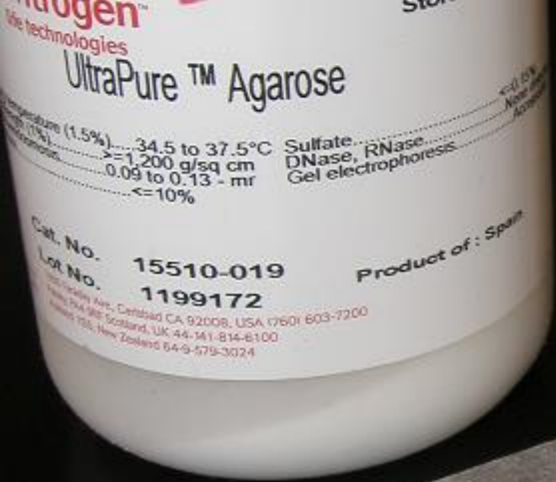
strength of the electrical field, buffer, density of agarose gel…

Size of the DNA!

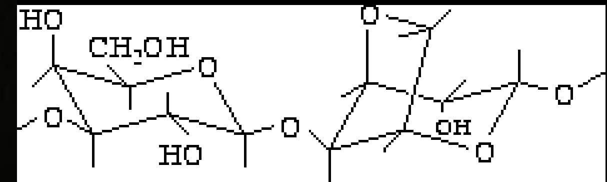
* Small DNA move faster than large DNA

…gel electrophoresis separates DNA according to **size**





Agarose



D-galactose

3,6-anhydro L-galactose

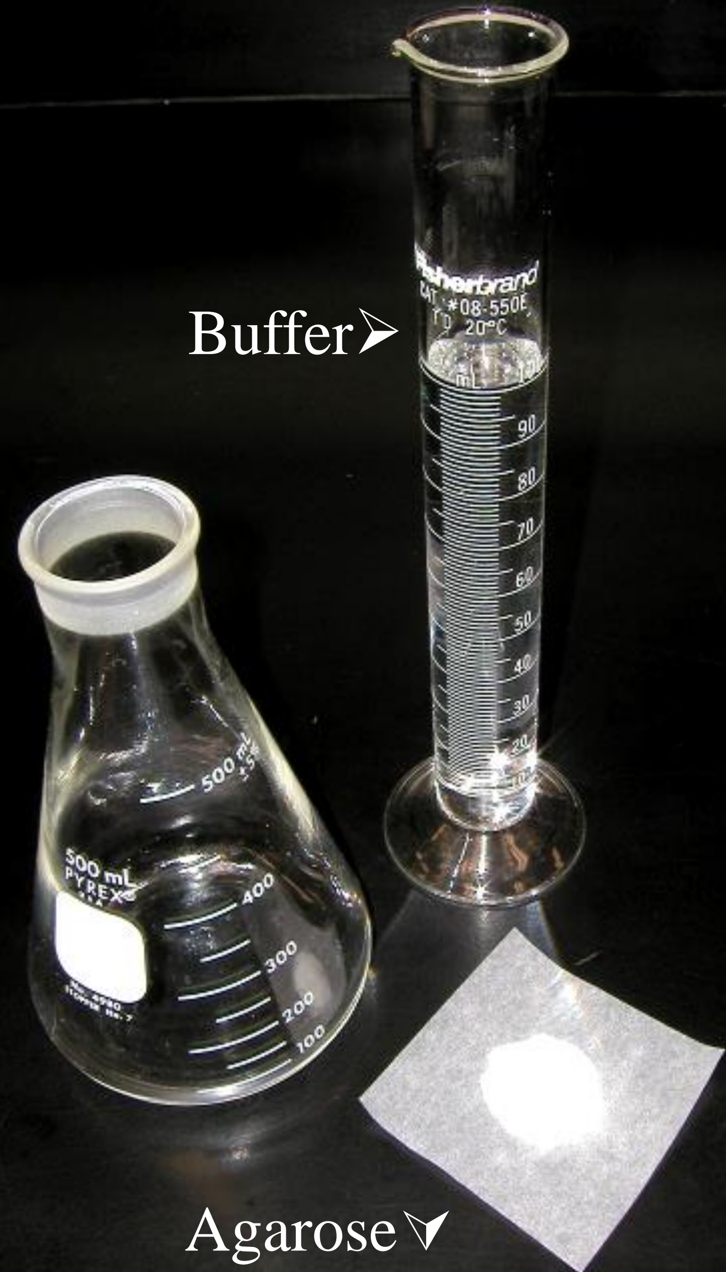
- Agarose was first used in biology when Robert Koch* used it as a culture medium for Tuberculosis bacteria in 1882

***Lina Hesse**, technician and illustrator for a colleague of Koch was the first to suggest agar for use in culturing bacteria

Agarose is a linear polymer extracted from seaweed.

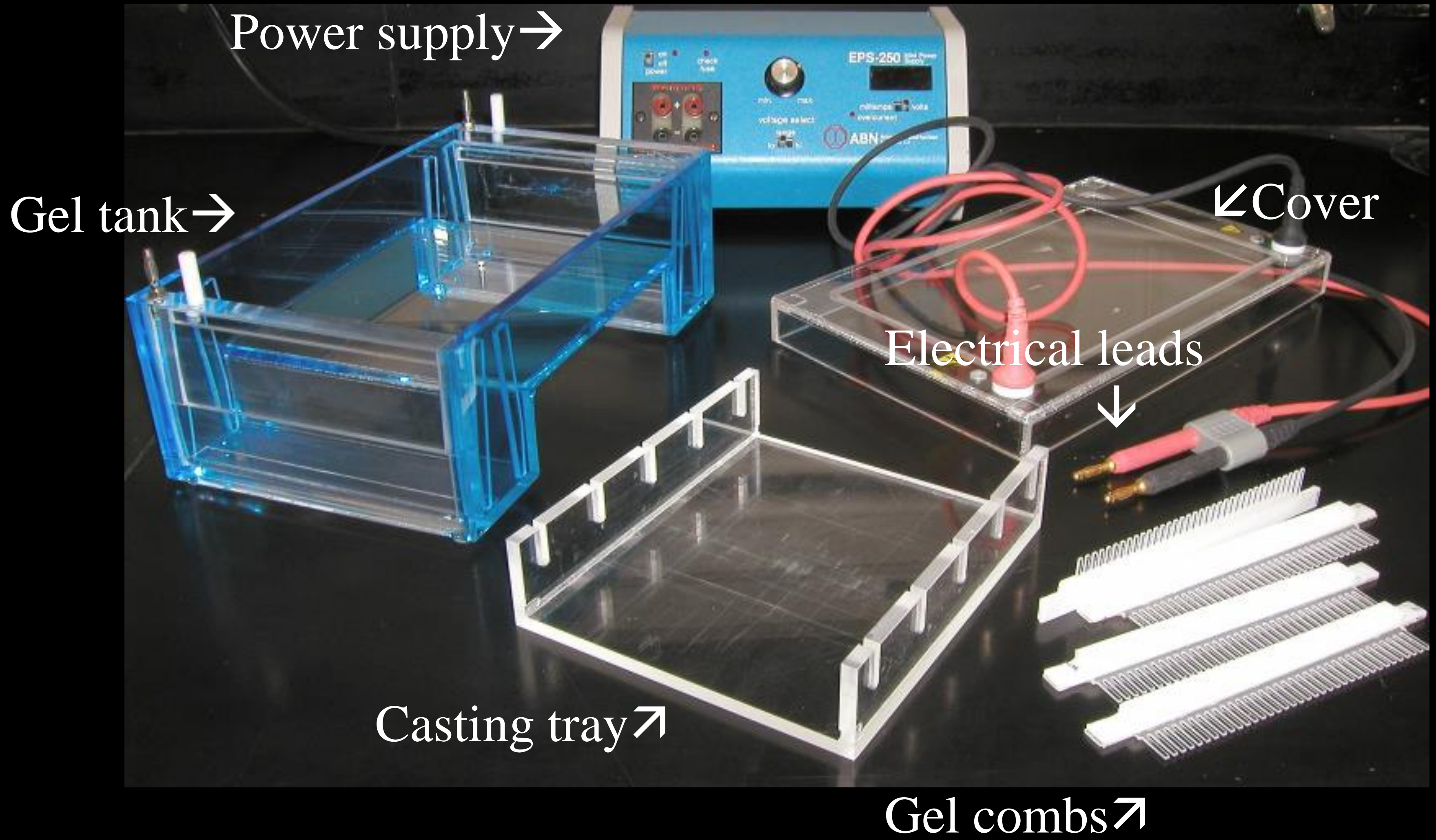
Making an Agarose Gel

- An agarose gel is prepared by combining agarose powder and a buffer solution.

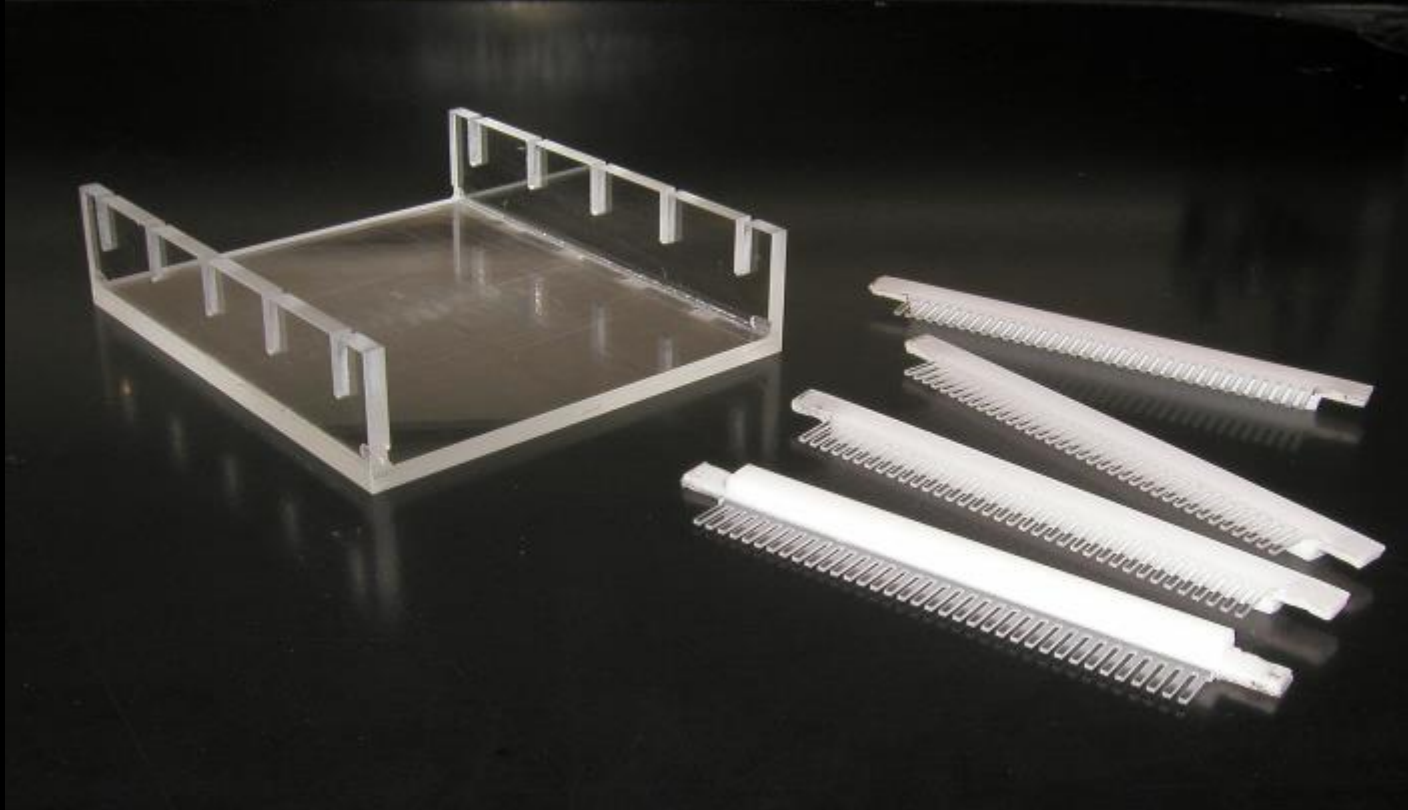


Flask for boiling

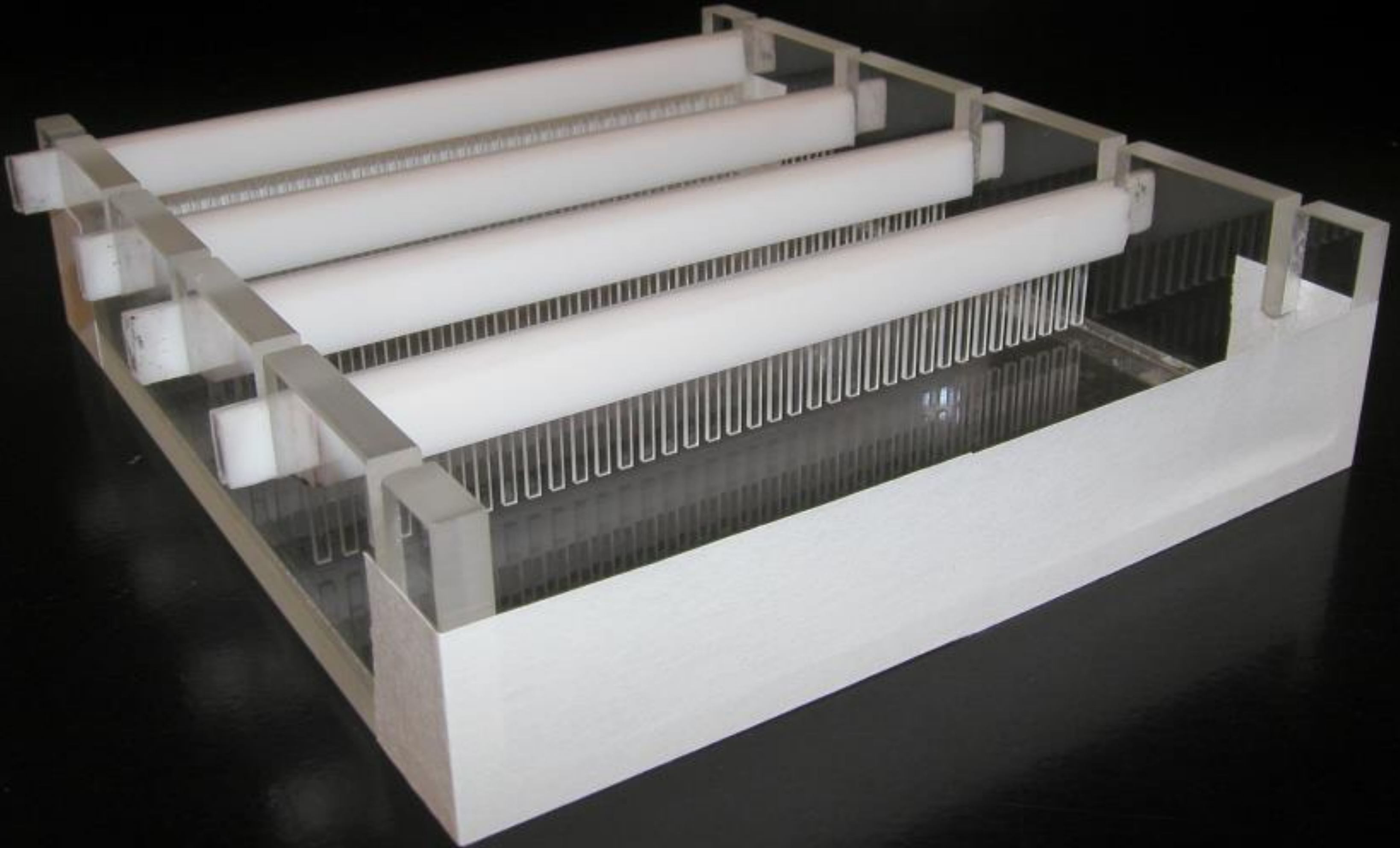
Electrophoresis Equipment



Gel casting tray and combs



Preparing the Casting Tray



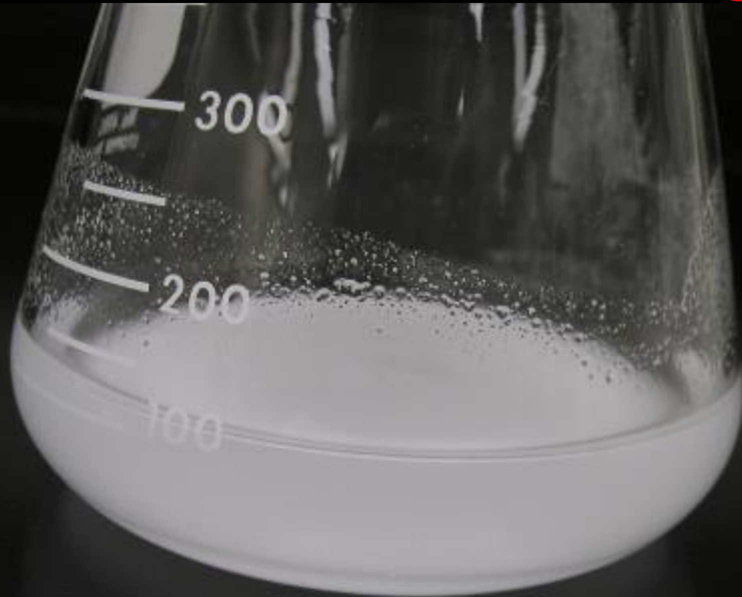
Seal the edges of the casting tray and put in the combs. Place the casting tray on a level surface. None of the gel combs should be touching the surface of the casting tray.

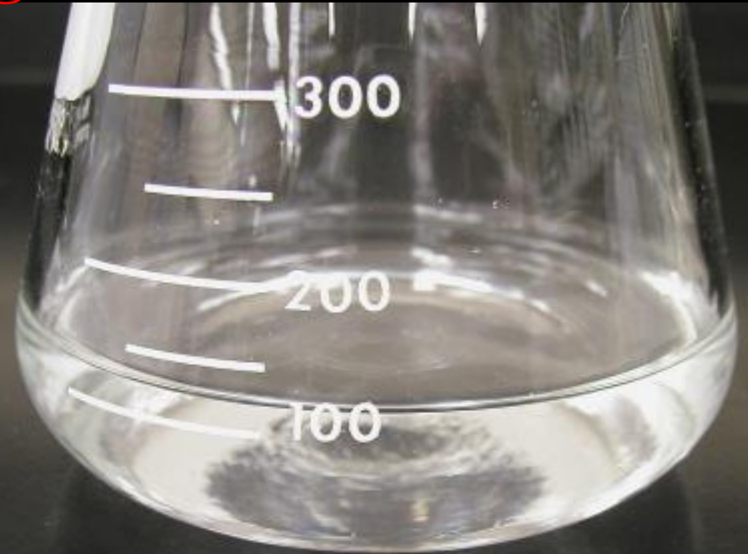
Agarose

Buffer Solution

- Combine the agarose powder and buffer solution. Use a flask that is several times larger than the volume of buffer.

Melting the Agarose

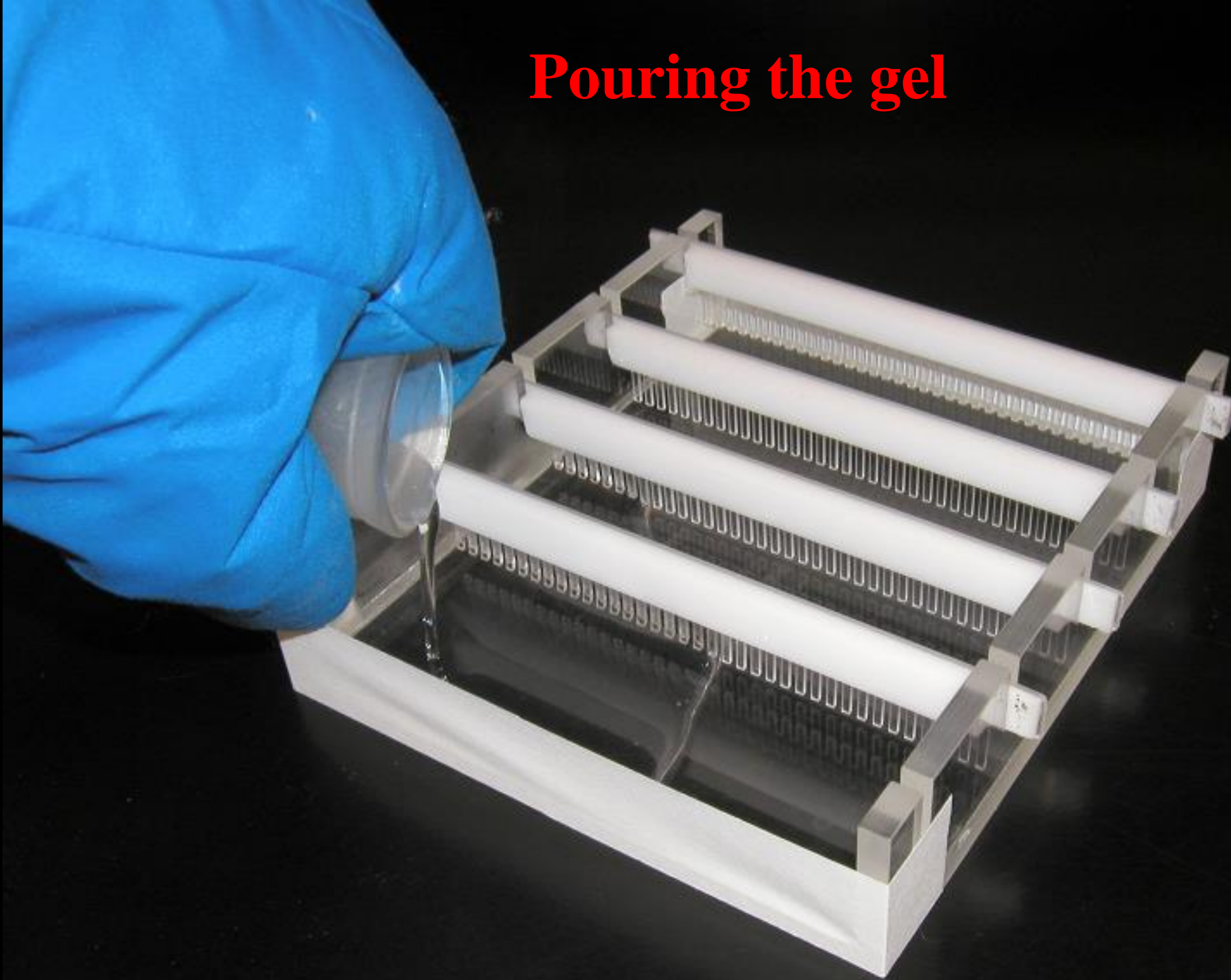




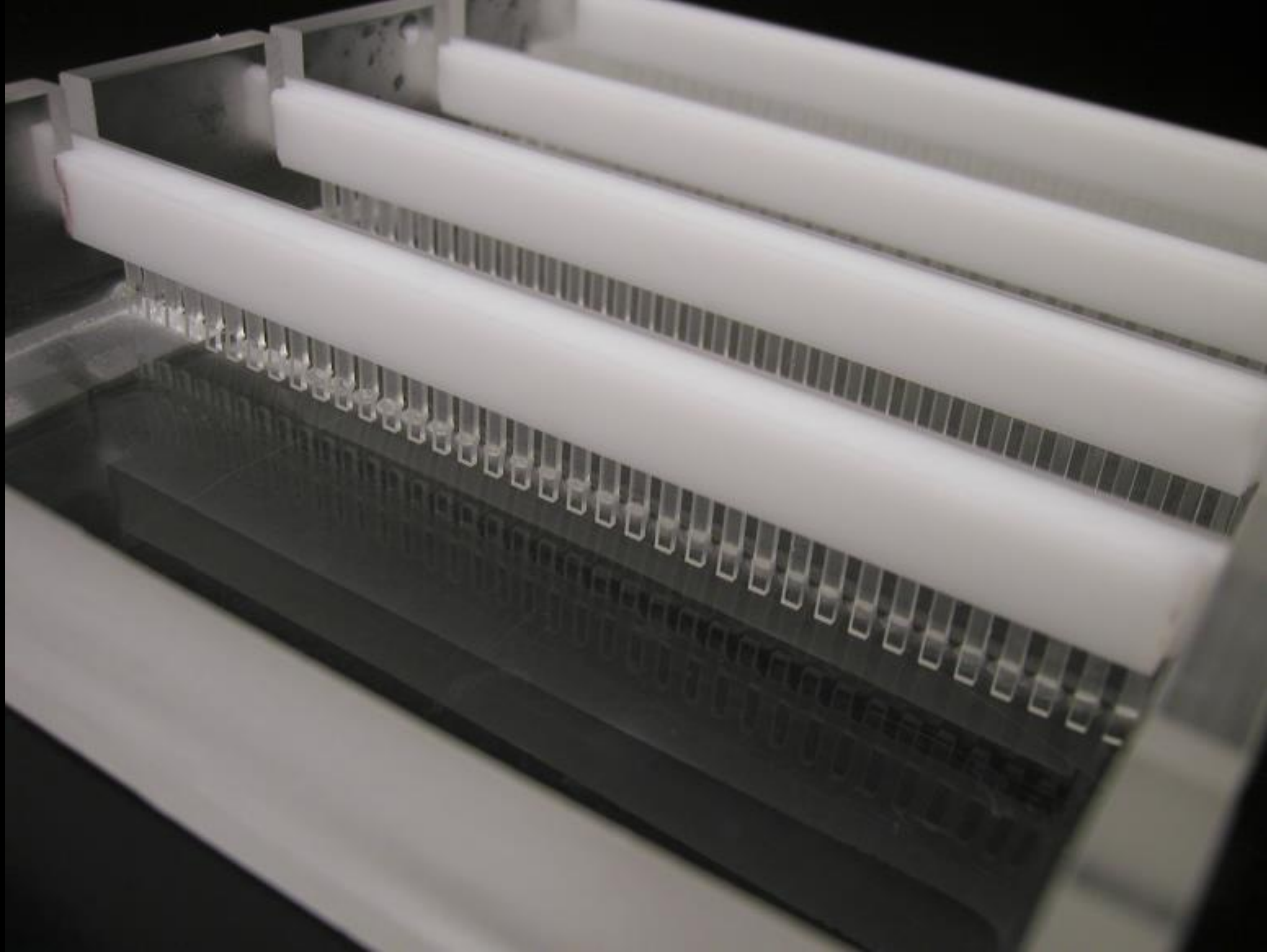
Agarose is insoluble at room temperature (left).
The agarose solution is boiled until clear (right).

Gently swirl the solution periodically when heating to allow all the grains of agarose to dissolve.
***Be careful when boiling - the agarose solution may become superheated and may boil violently if it has been heated too long in a microwave oven.

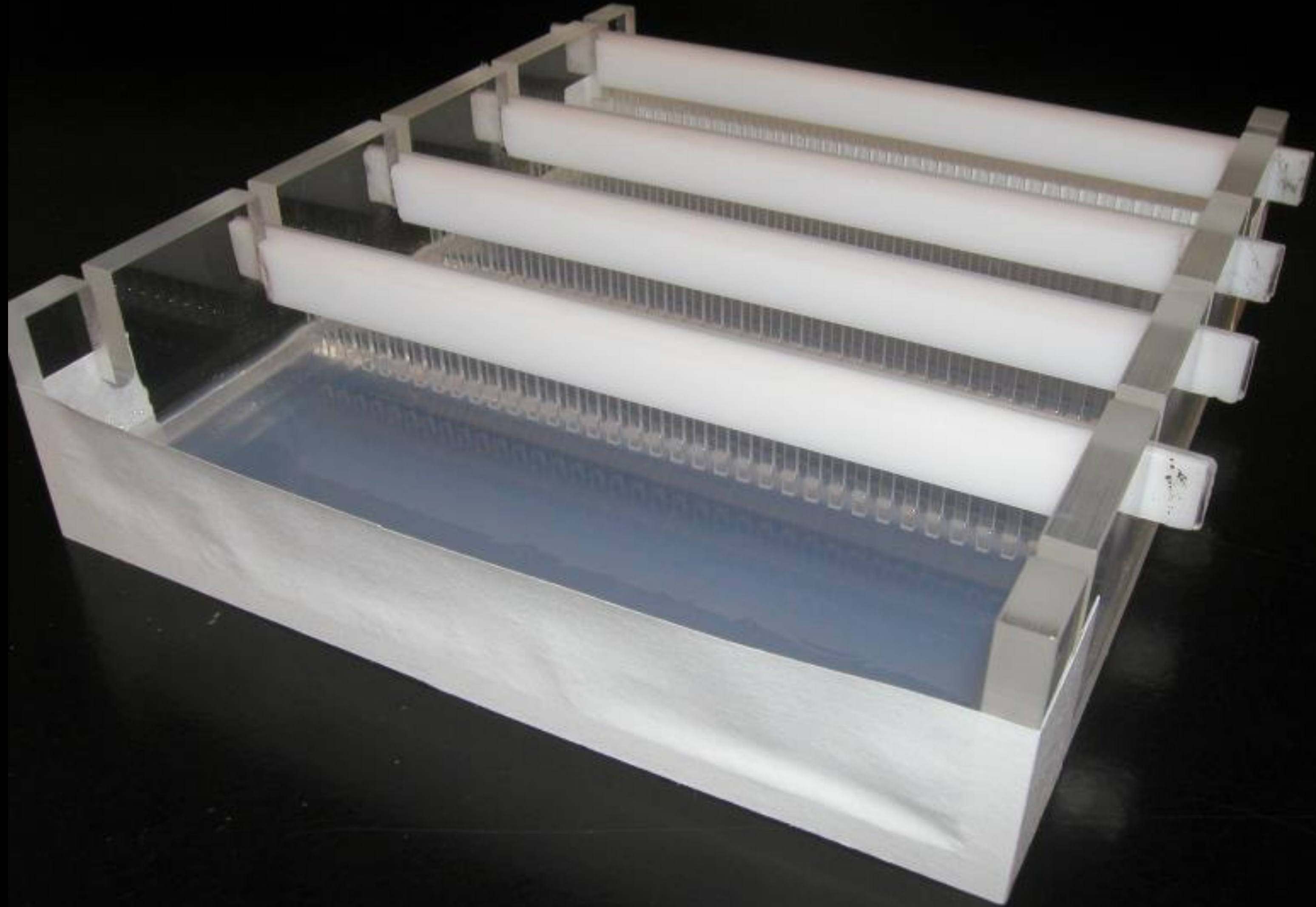
Pouring the gel



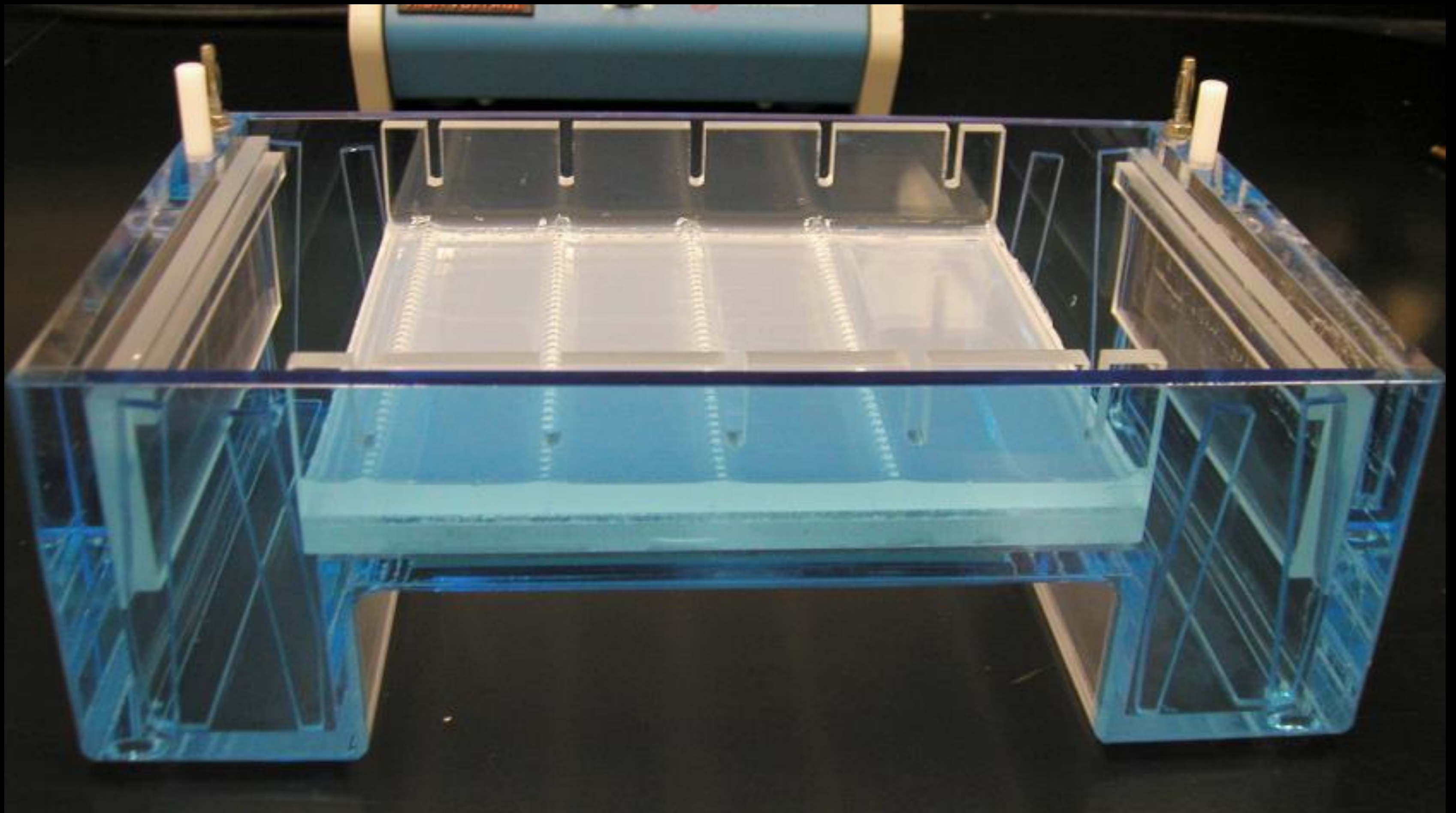
- Allow the agarose solution to cool slightly (~60ºC) and then carefully pour the melted agarose solution into the casting tray. Avoid air bubbles.



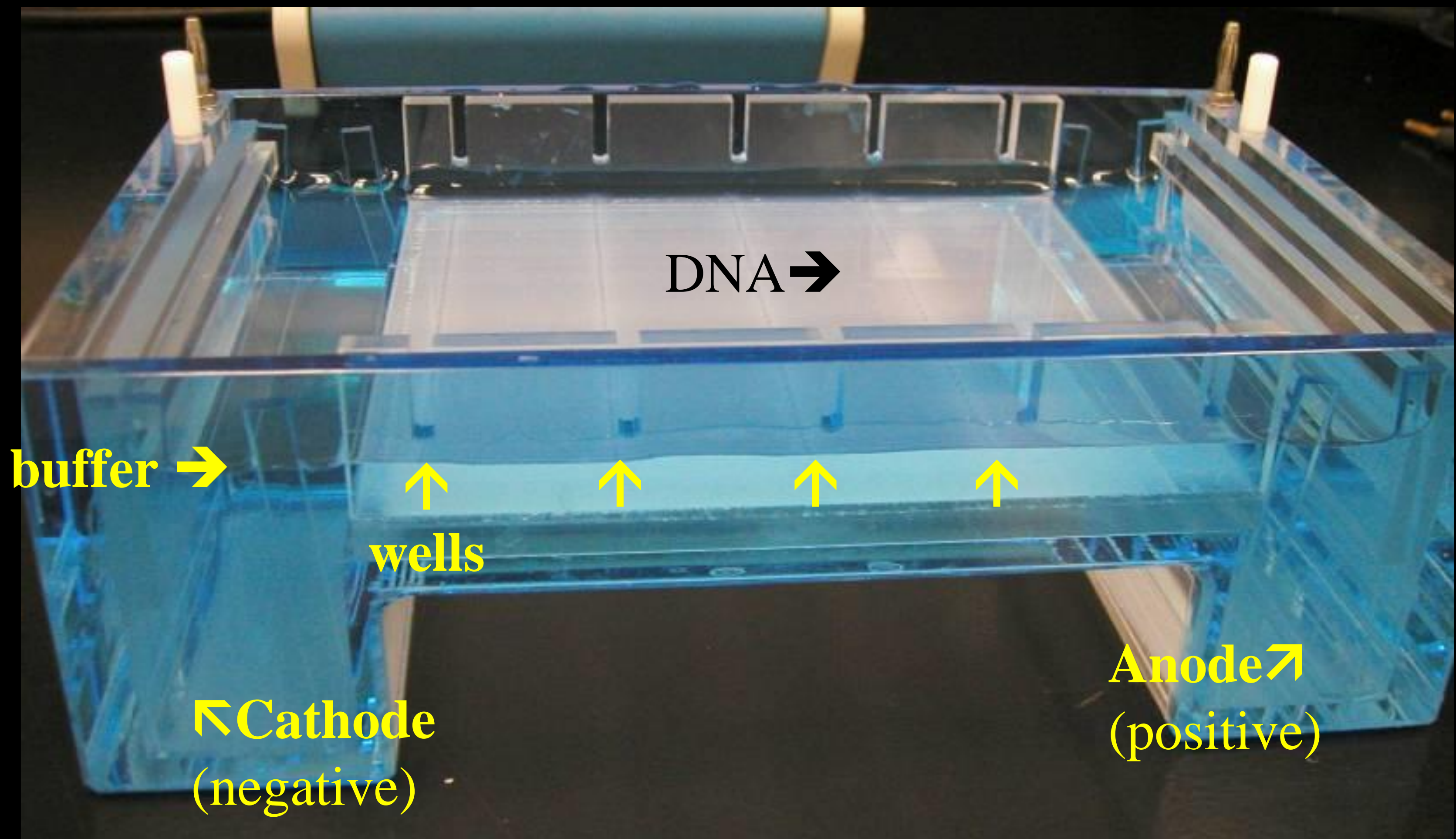
Each of the gel combs should be submerged in the melted agarose solution.



- ✓ When cooled, the agarose polymerizes, forming a flexible gel. It should appear lighter in color when completely cooled (30-45 minutes). Carefully remove the combs and tape.



Place the gel in the electrophoresis chamber.



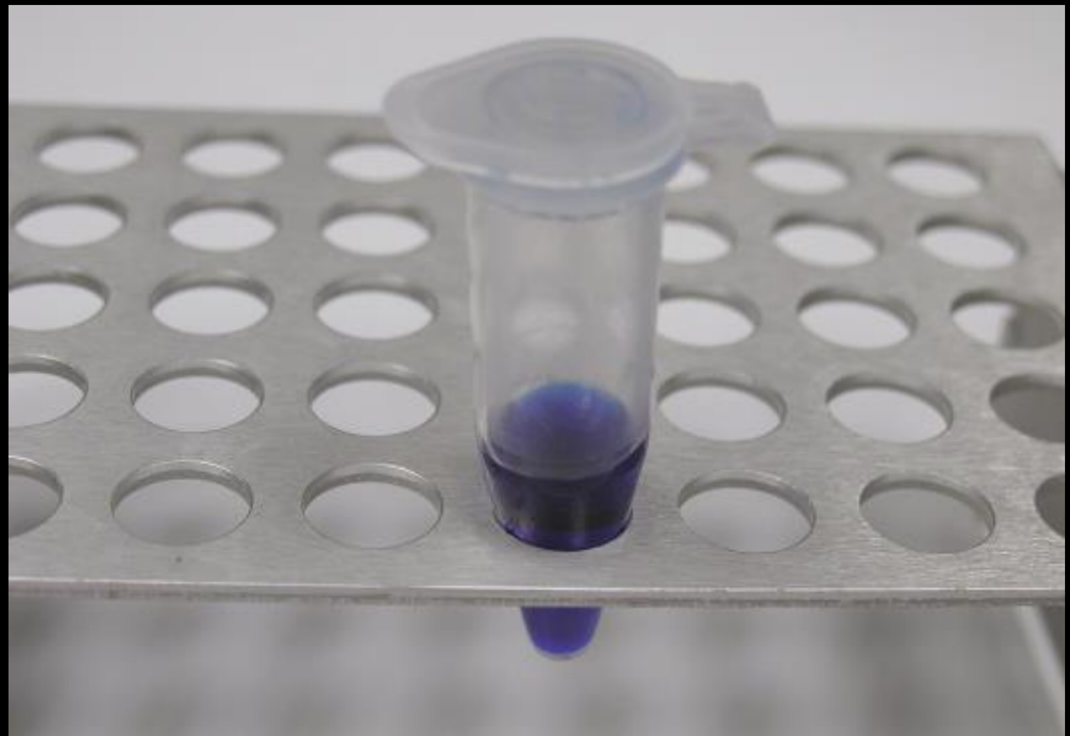
Add enough electrophoresis buffer to cover the gel to a depth of at least 1 mm. Make sure each well is filled with buffer.

Sample Preparation

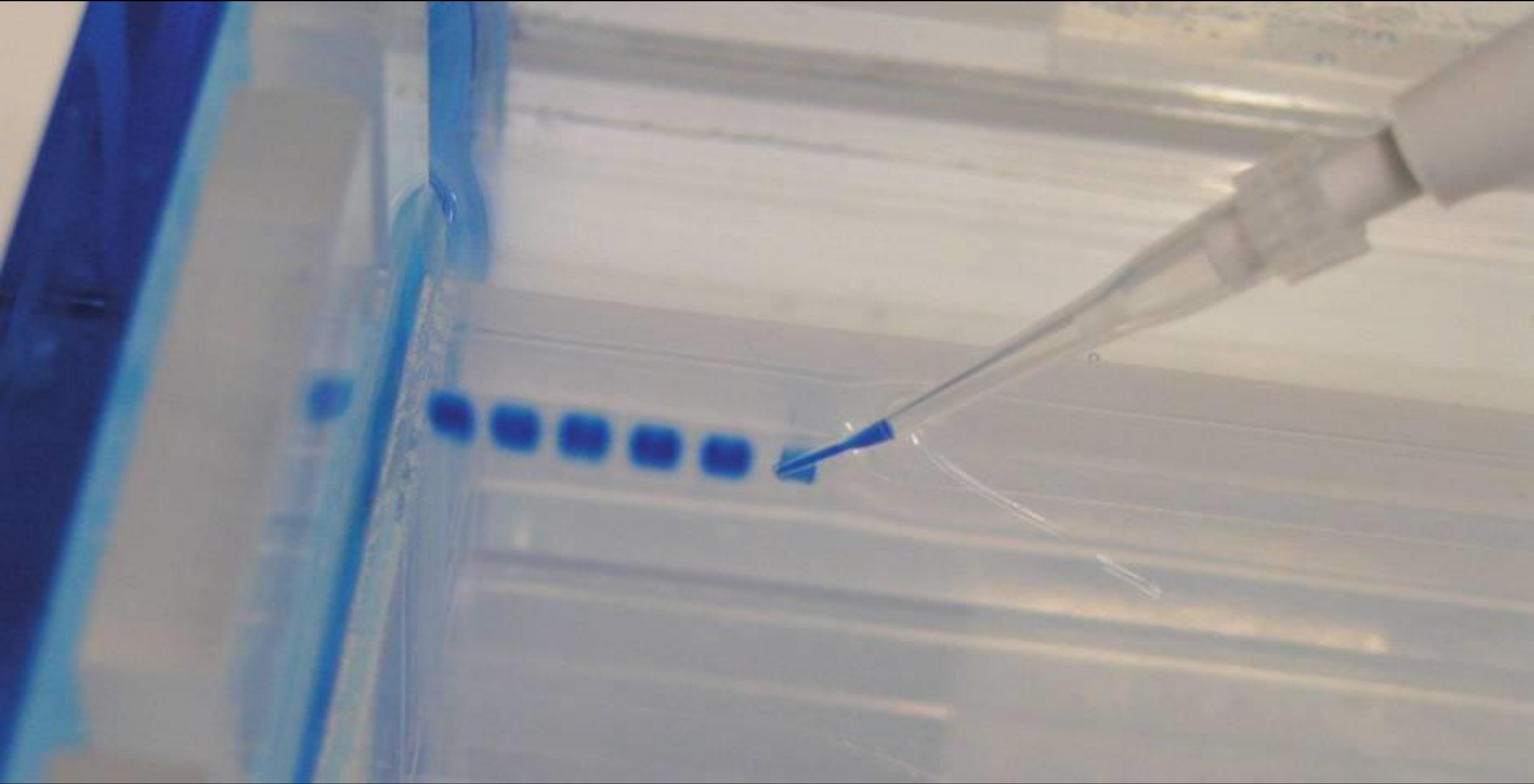
- Mix the samples of DNA with the 6X sample loading buffer.
- This allows the samples to be seen when loading onto the gel.
- Increases the density of the samples, causing them to sink into the gel wells.

6X Loading Buffer: →

- Bromophenol Blue (for color)
- Glycerol (for weight)

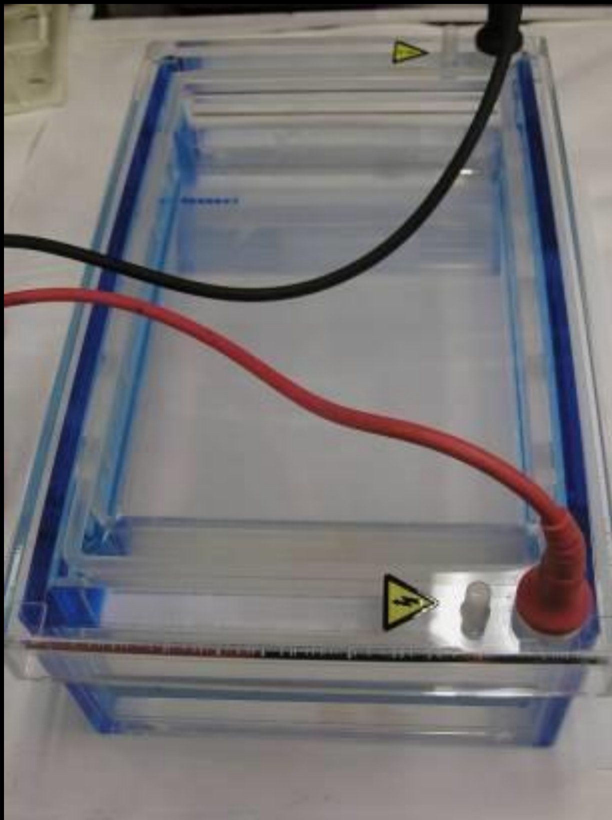


Loading in the Gel



Carefully place the pipette tip over a well and gently expel the sample. The sample should sink into the well. Be careful not to puncture the gel with the pipette tip.

Running the Gel

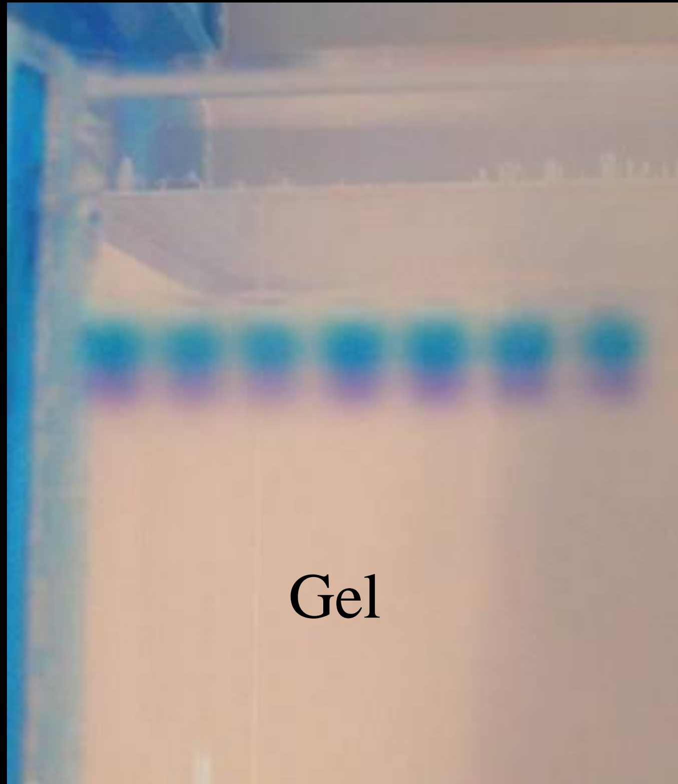


Place the cover on the electrophoresis chamber, connecting the electrical leads. Connect the electrical leads to the power supply. Be sure the leads are attached correctly - DNA migrates toward the anode (red). When the power is turned on, bubbles should form on the electrodes in the electrophoresis chamber.

Cathode
(-)

DNA
(-)

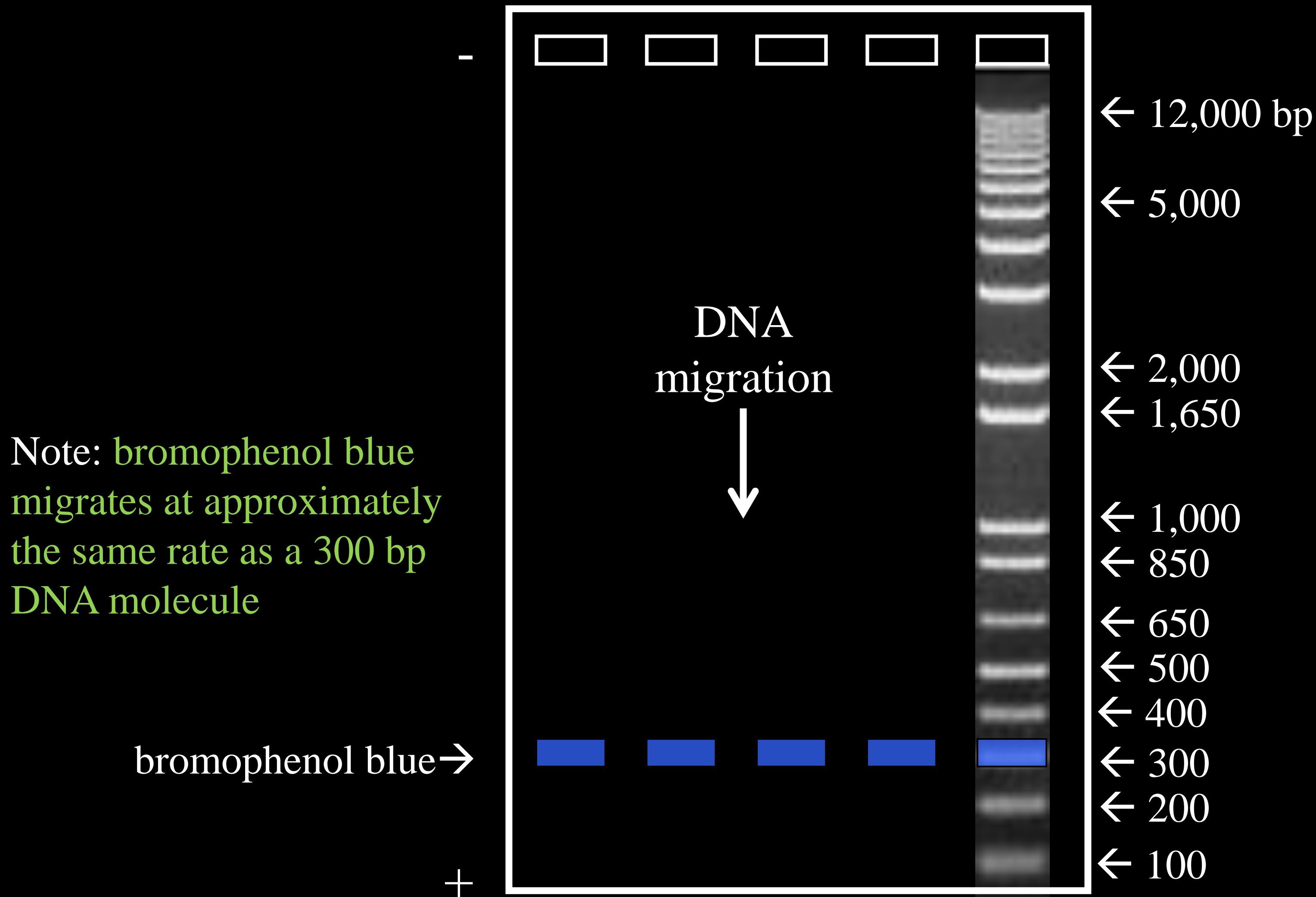
Anode
(+)



← wells
← Bromophenol Blue

After the current is applied, make sure the Gel is running in the correct direction. Bromophenol blue will run in the same direction as the DNA.

DNA Ladder Standard

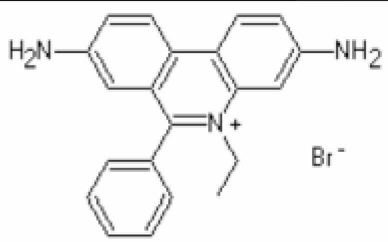


Inclusion of a DNA ladder (DNAs of know sizes) on the gel makes it easy to determine the sizes of unknown DNAs.

Staining the Gel

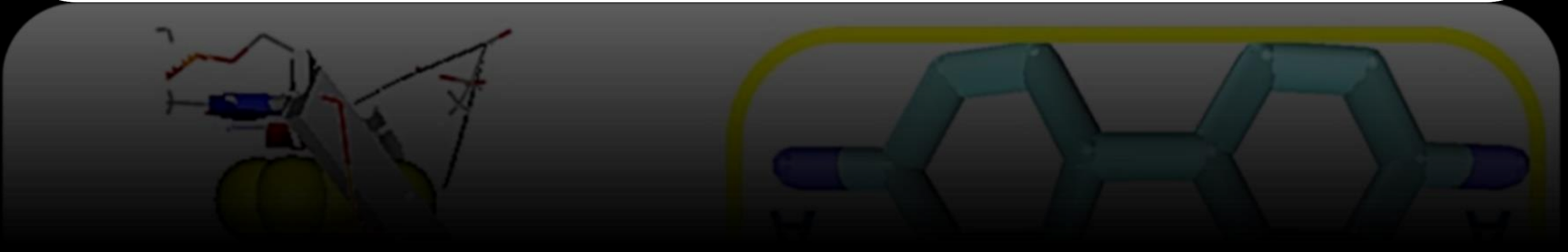
• Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a Gel.

• Ethidium bromide can be added to the **gel** and/or **running buffer** before the gel is run or the gel can be stained after it has run.



***CAUTION! Ethidium bromide is a powerful mutagen and is moderately toxic. **Gloves should be worn at all times**.

- **Ethidium bromide intercalated between two adenine-thymine base pairs.**



Safer alternatives to Ethidium Bromide

- Methylene Blue
- BioRAD - Bio-Safe DNA Stain
- Ward's - QUIKView DNA Stain
- Carolina BLU Stain

…others

advantages

Inexpensive
Less toxic
No UV light required
No hazardous waste disposal

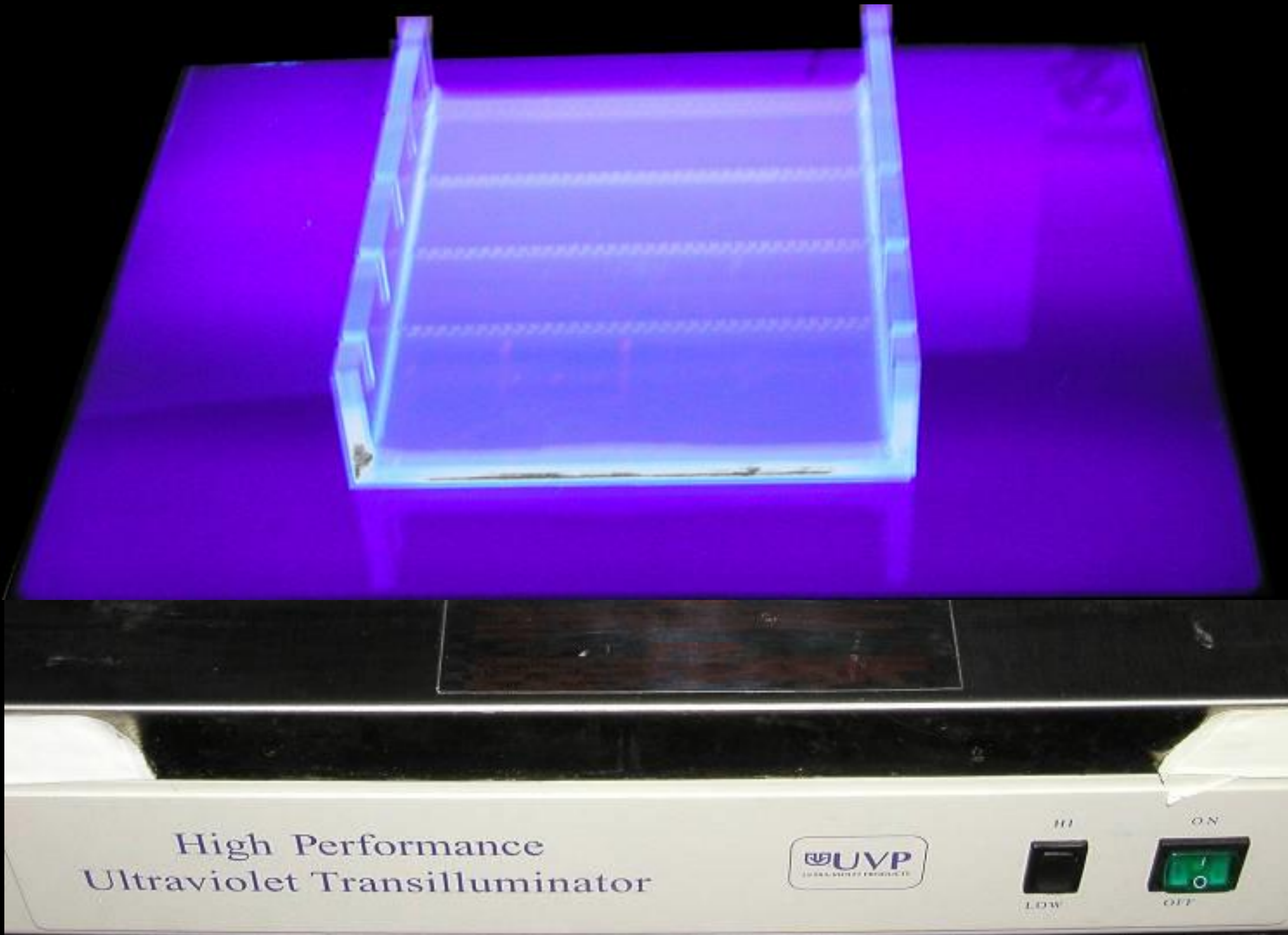
disadvantages

Less sensitive
More DNA needed on gel
Longer staining/destaining time

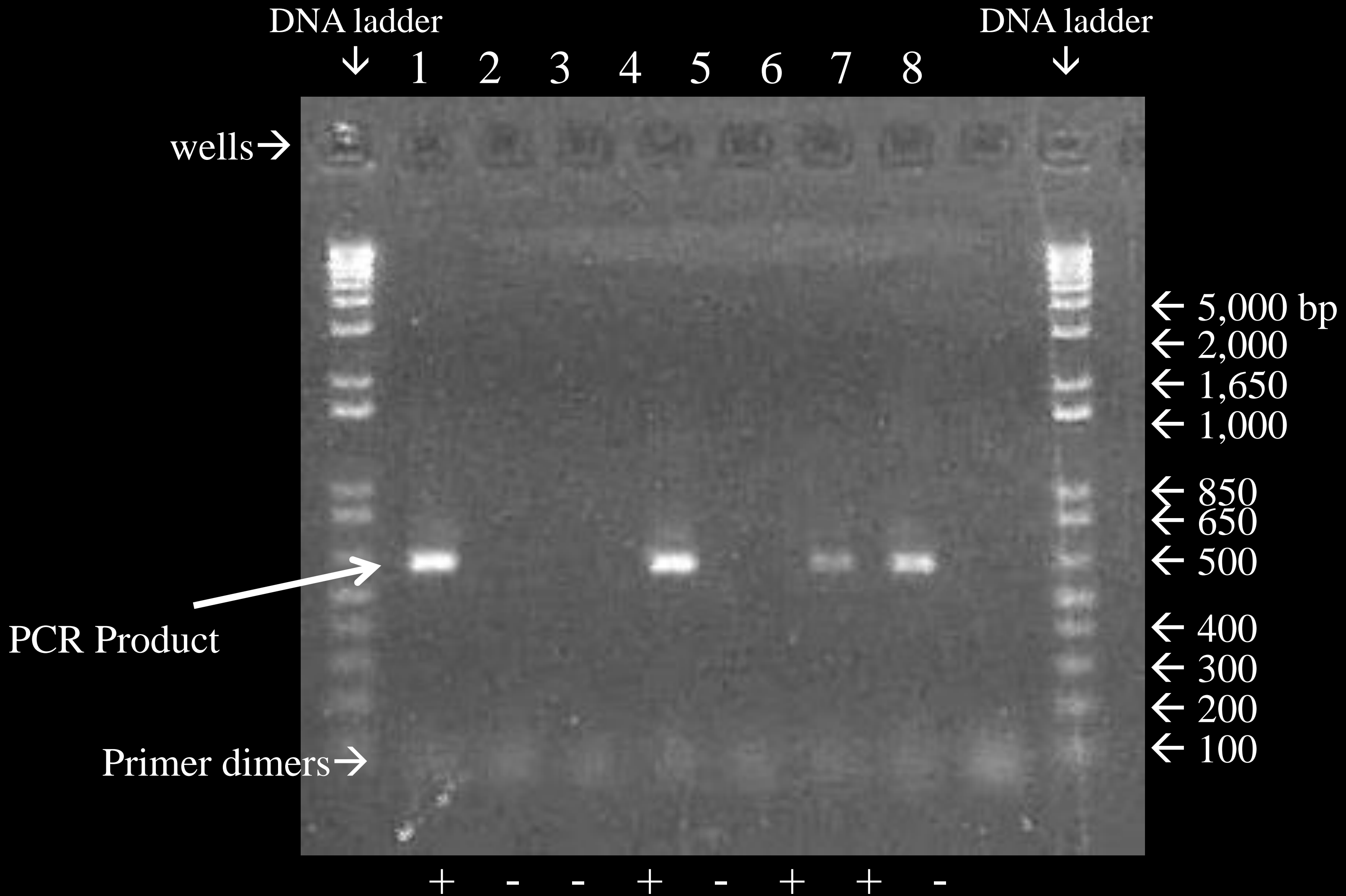
Staining the Gel

- ✓ Place the gel in the staining tray containing diluted stain.
- ✓ Allow the gel to stain for 10-15 minutes.
- ✓ To remove excess stain, allow the gel to destain in water.
- ✓ Replace water several times for efficient destain.

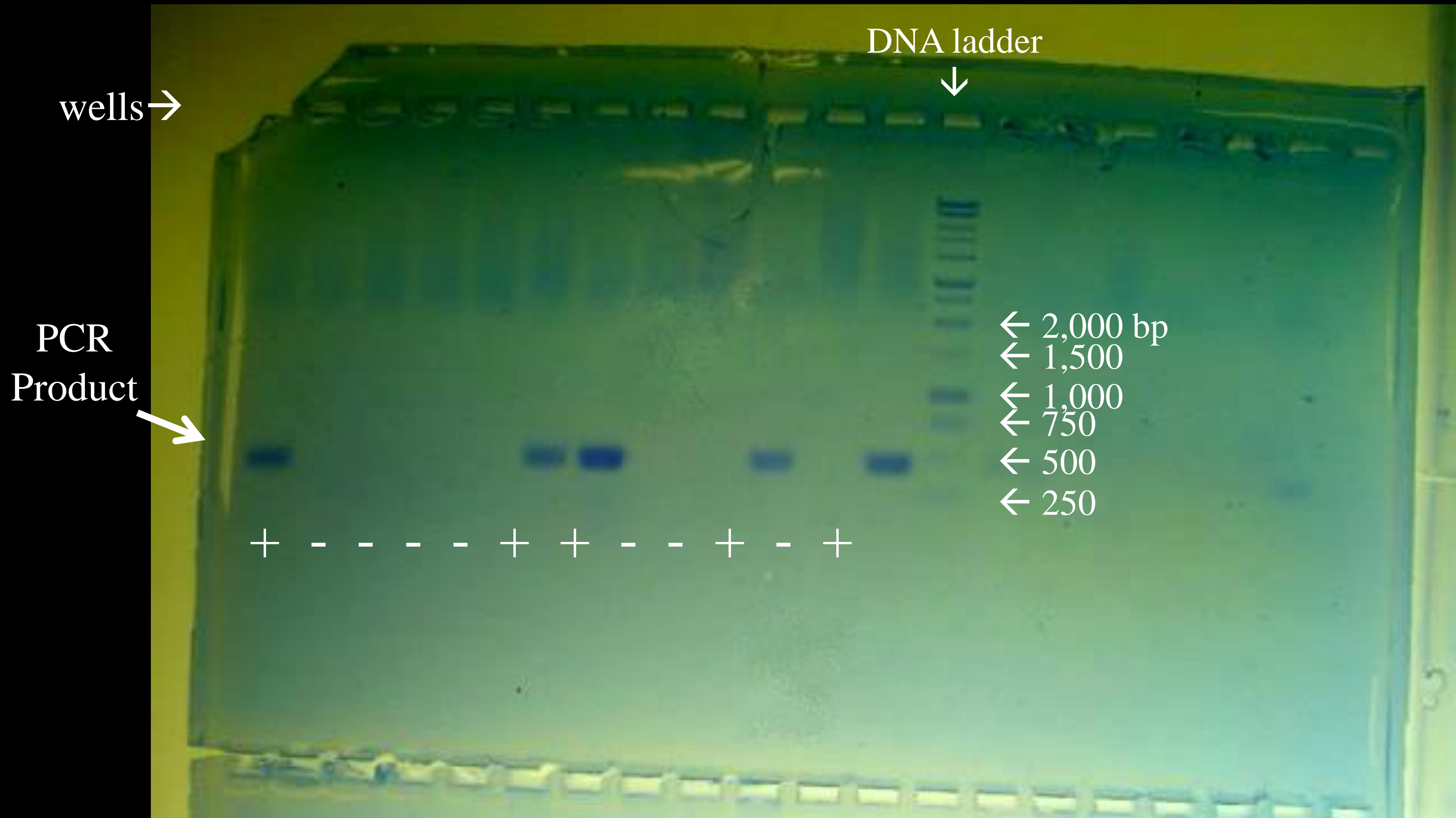
- Ethidium Bromide requires an ultraviolet light source to visualize



Visualizing the DNA (ethidium bromide)

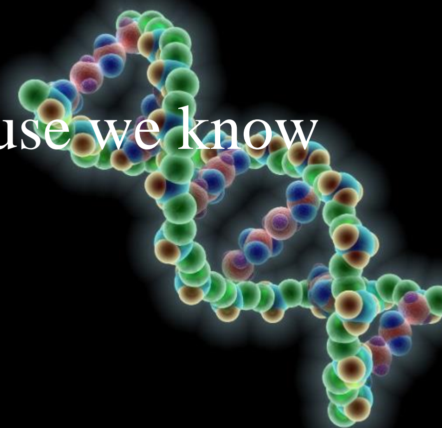


Visualizing the DNA (QuikVIEW stain)



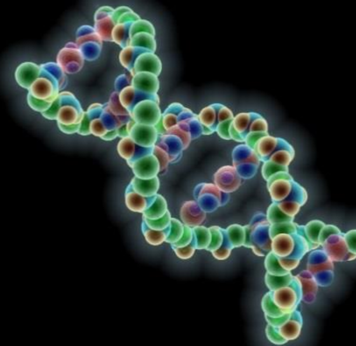
Abstract

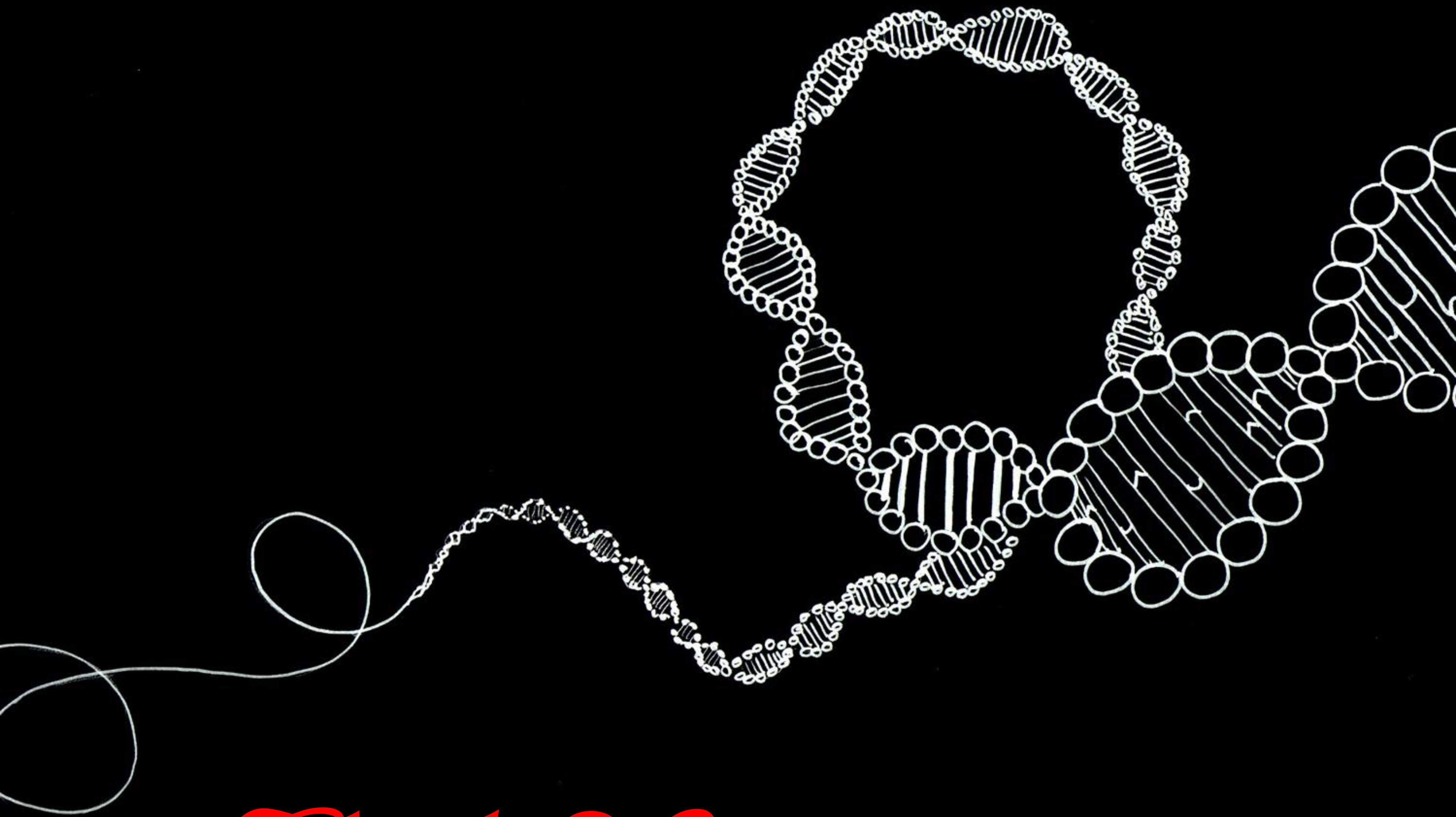
- Gel electrophoresis detects the presence of DNA in a sample
- Gel electrophoresis detects the number of nucleotides in a fragment of DNA
  - e.g., the number of nucleotides in a DNA region which was amplified by PCR
  - Is a rough estimate, is not exact, need more sophisticated sequencing techniques to get an exact number of nucleotides
  - Can be used to tentatively identify a gene because we know the number of nucleotides in many genes



- A sample which contains fragments of DNA is forced by an electrical current through a firm gel which is really a sieve with small holes of a fixed size
  - Phosphate group in DNA is negatively charged so it is moved towards a positive electrode by the current
  - Longer fragments have more nucleotides
    - So have a larger molecular weight
    - So are bigger in size
    - So aren't able to pass through the small holes in the gel and get hung up at the beginning of the gel
  - Shorter fragments are able to pass through and move farther along the gel
  - Fragments of intermediate length travel to about the middle of the gel

- DNA fragments are then visualized in the gel with a special dye
- The number of nucleotides are then estimated by comparing it to a known sample of DNA fragments which is run through the gel at the same time





Thank You