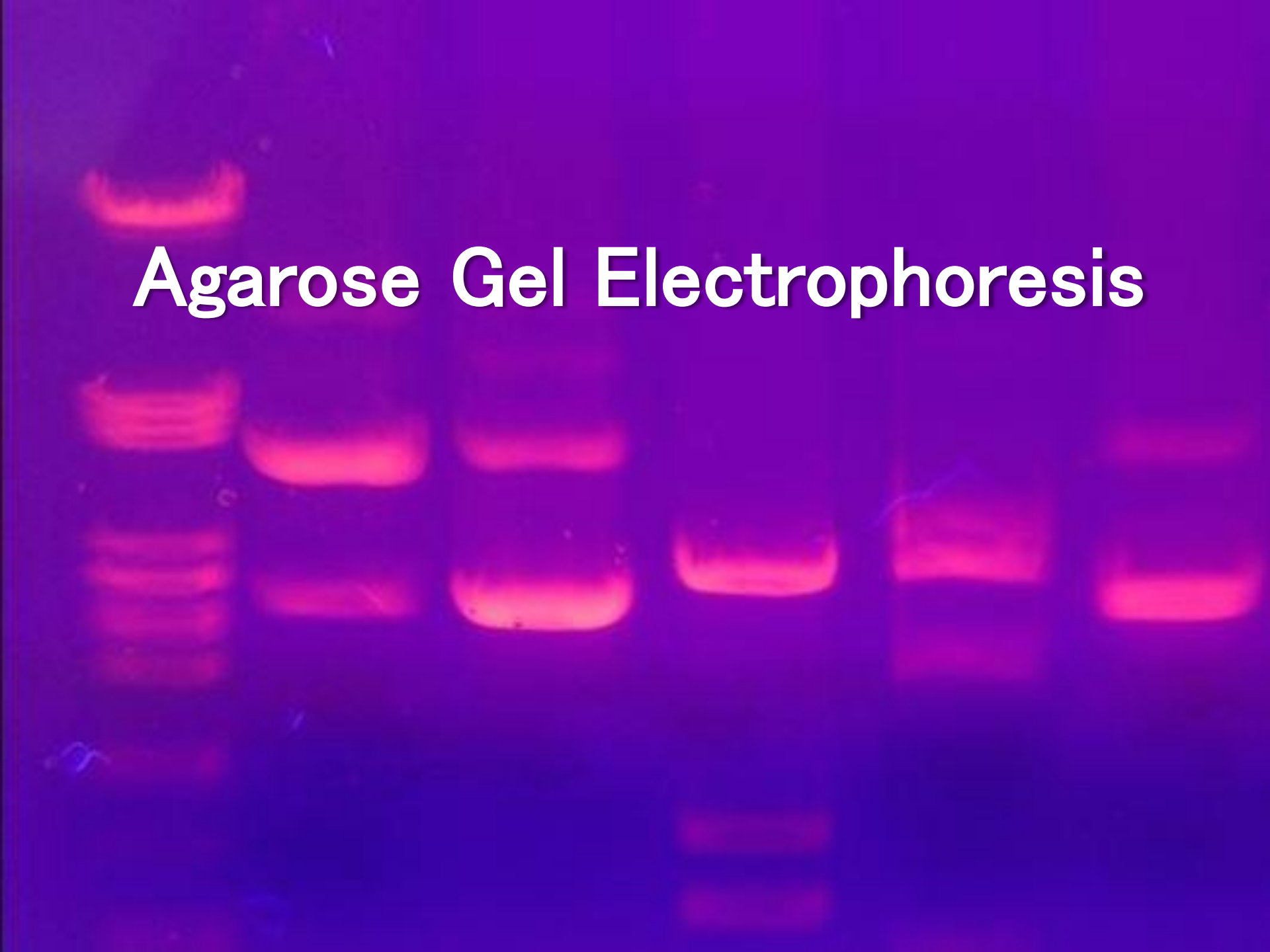
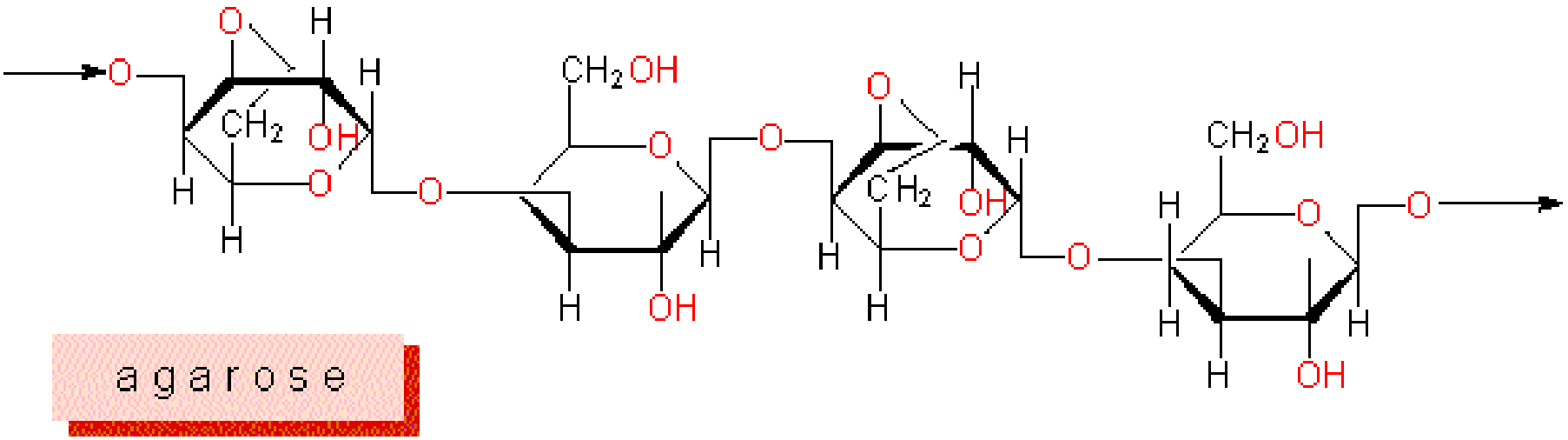

Agarose Gel Electrophoresis

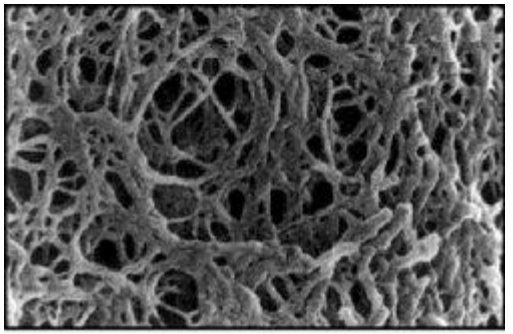
What is agarose?



•Basic unit of agar which is a cell wall and intercellular component of some red marine algae, usually *Gelidium* and *Gracillaria*.

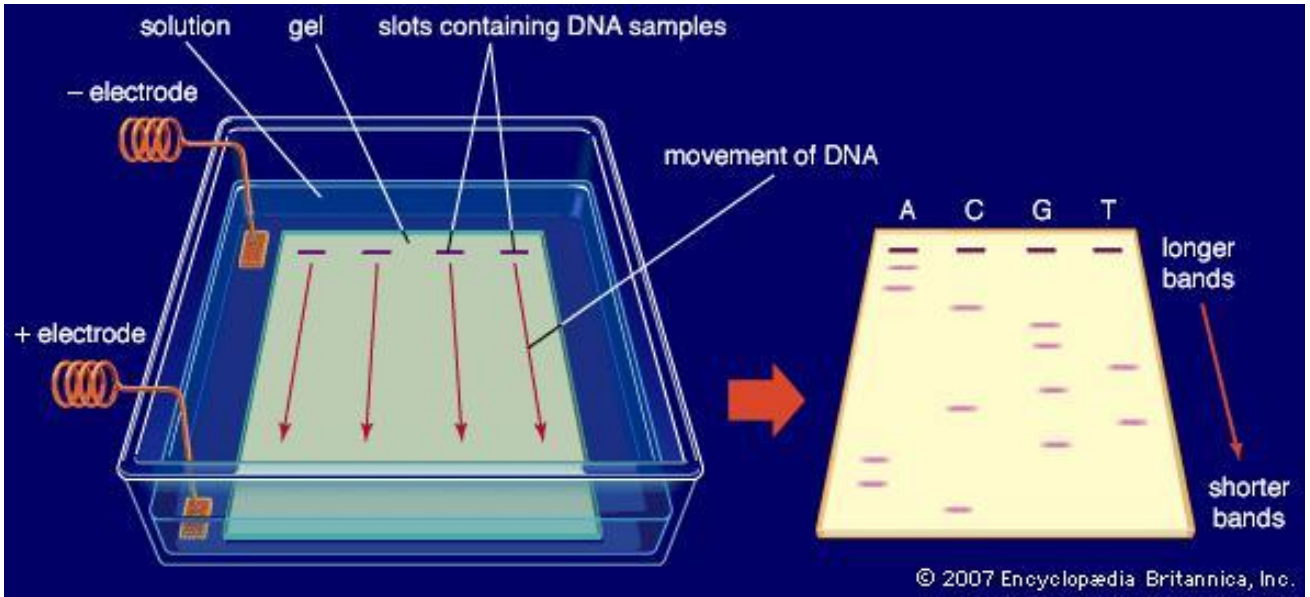
•Linear polysaccharide that contains double helices stabilized by water molecules.

•Exterior hydroxyl groups allow helices to aggregate into suprafibers that branch off to form a matrix.



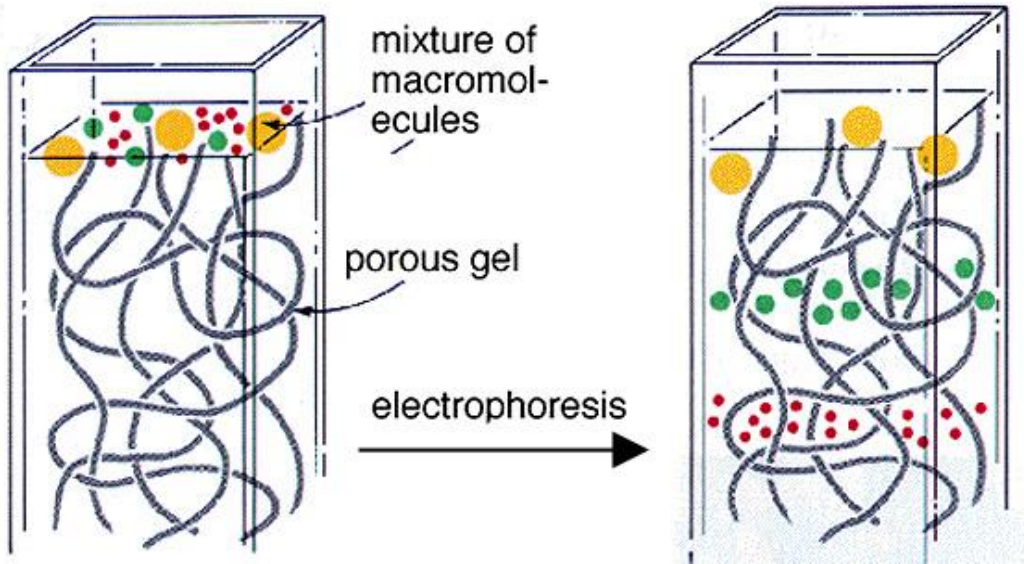
What is gel electrophoresis?

- Method used to separate DNA fragments generated by restriction endonucleases.
- Separates molecules on the basis of size, charge, and/or shape.
- Electromotive force moves molecules through the matrix at different speeds based on size and/or charge.
- Agarose gel electrophoresis can resolve DNA fragments that range roughly from 500 to 30,000 base pairs.



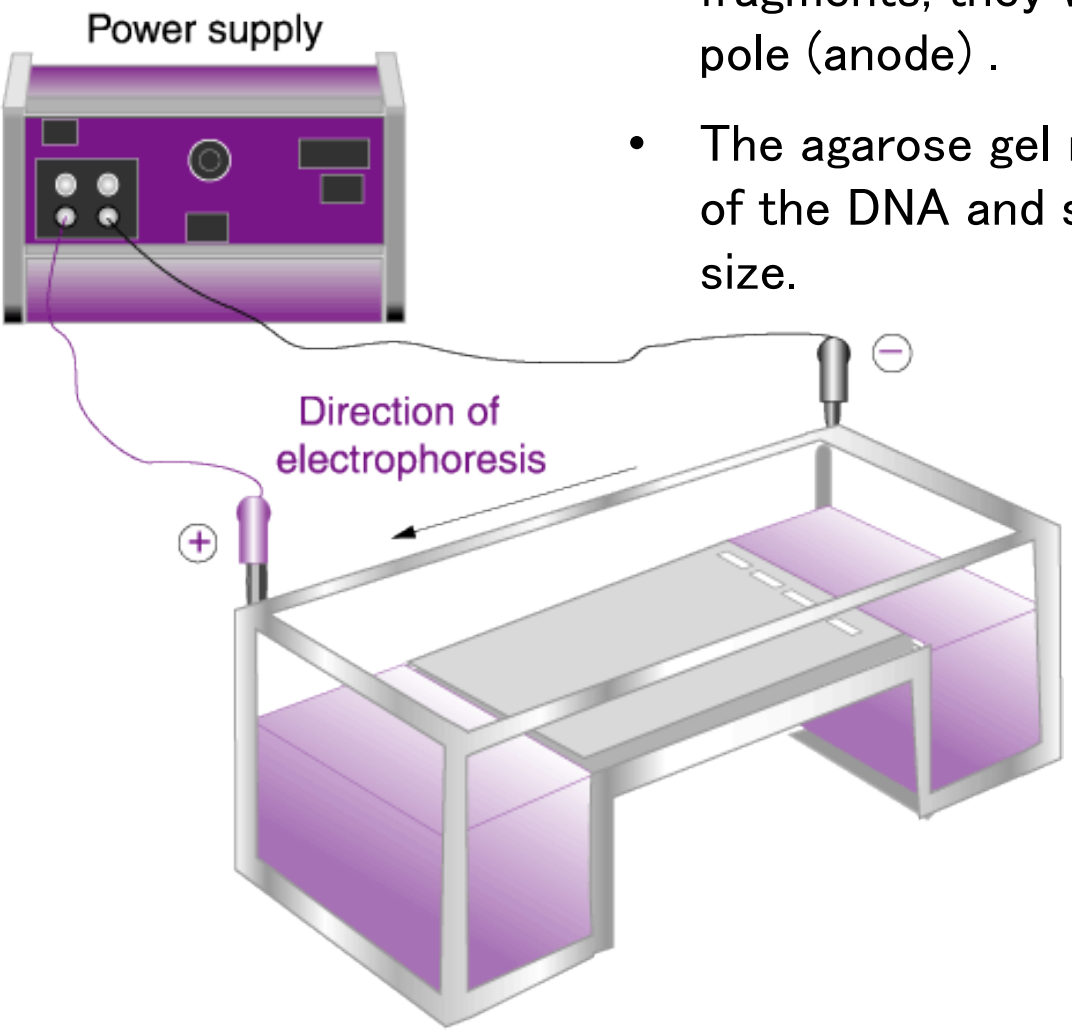
How does electrophoresis work?

- Linear double stranded DNA fragments basically have the same rod shape, so shape is not a factor in the separation.
- All DNA has identical charge to mass ratio, one negatively charged phosphate per nucleotide.
- As DNA fragments increase in length, charge increases preserving the charge to mass ratio.
- When an electric field is applied, negatively charged dsDNA of all lengths will migrate toward the positive pole at an equal rate.
- Forcing DNA to travel through a porous matrix will allow the smaller fragments to run faster while slowing the rate of larger molecules.



Electrophoresis Theory Summarized

- When an electric field is applied to dsDNA fragments, they will migrate toward the positive pole (anode) .
- The agarose gel matrix restricts the movement of the DNA and separates the fragments by size.



Resolving DNA Fragments

Percent Agarose Gel (w/v)	DNA Size Resolution(kb = 1000)
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

Table 1: Correct Agarose Gel Concentration for Resolving DNA Fragments

how well you can resolve your DNA fragments.

Will you use a higher concentration agarose or a lower concentration agarose?

Explain your reasoning.

Gel Electrophoresis: Running Buffer

- **Tris- acetate- EDTA (TAE) running buffer** is typically used in DNA gel electrophoresis because it does not interfere with subsequent enzyme activity.
- Prepare1 L 10x stock solution by adding 48.5 g Tris-HCl, 11.4 mL glacial acetic acid, and 20 mL 0.5 M EDTA pH to 8.0 with NaOH to 800 mL distilled deionized (dd) H_2O. Mix well, and bring up to 1L with ddH_2O.
- Dilute stock 1:10 with ddH_2O to make a 1x working solution of 40mM Tris-HCl, 20 mM acetic acid, and 1 mM EDTA.
- Running buffer mitigates pH changes during electrophoresis while water hydrolysis is occurring:

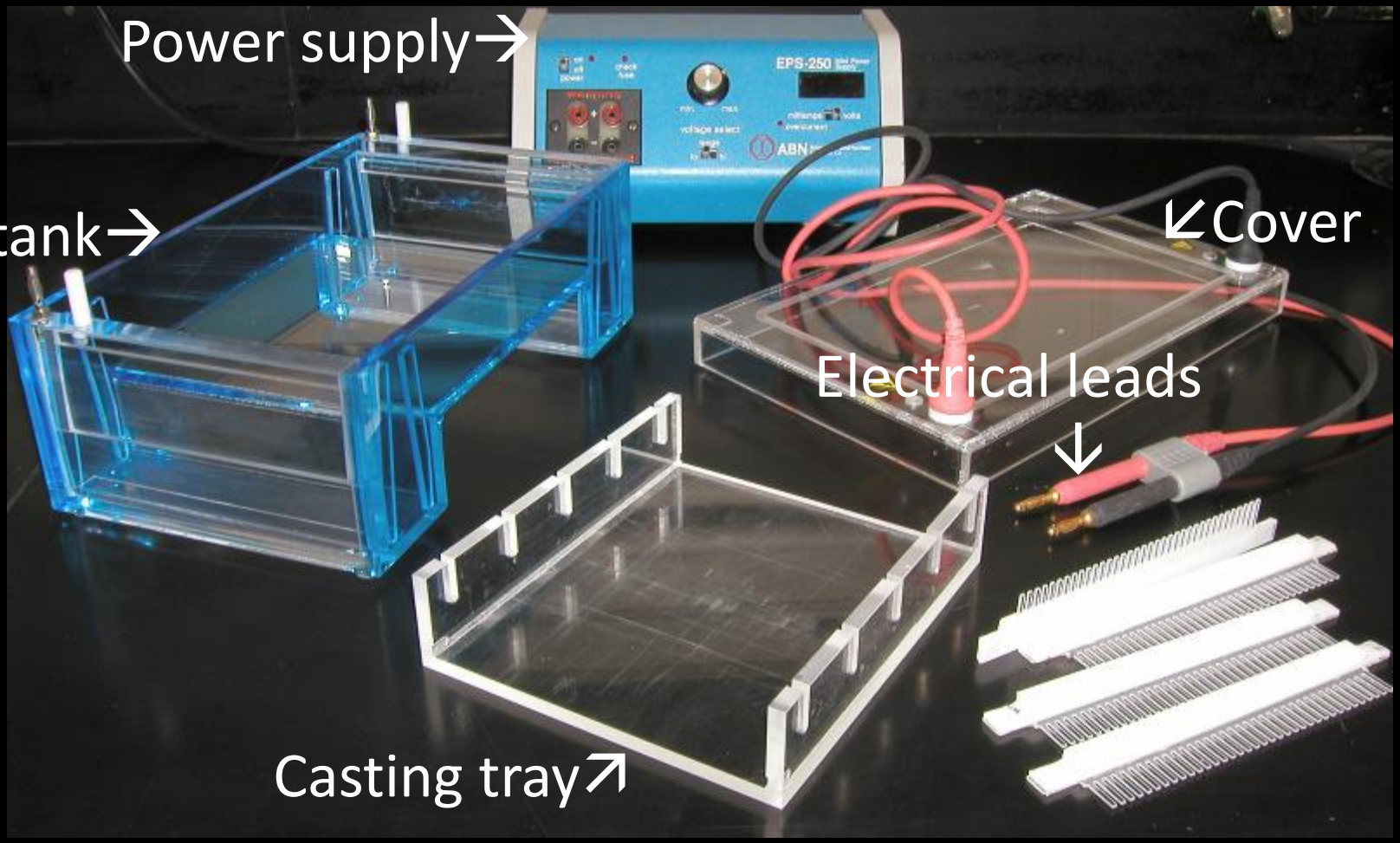
$$4H_20 \rightarrow 4H^+ + 4OH^-$$

$$4H^+ + 4e^- \rightarrow 2H_2 \text{ at the cathode}$$

$$4OH^- \rightarrow O_2 + 2H_2O + 4e^- \text{ at the anode}$$

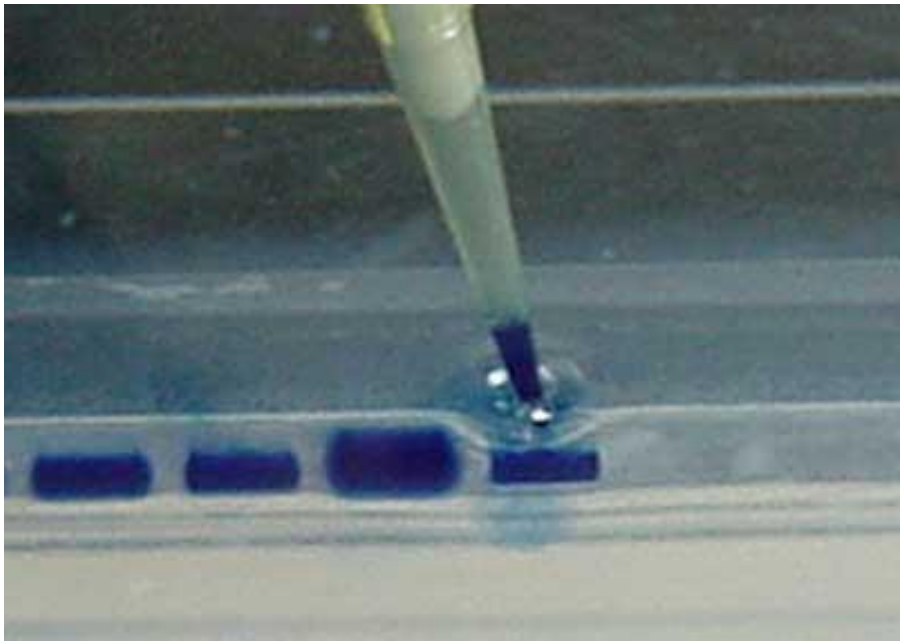
- *At which pole will you see more bubbles form when voltage is applied? Explain.*

Gel Electrophoresis Materials: Hardware



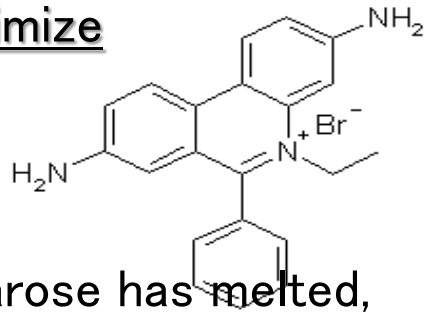
Gel Electrophoresis: Loading Buffer

- DNA must be mixed with 6x loading buffer before adding to gel wells.
- Stock 6x loading buffer:
  - 0.2 g bromothymol blue dye for visualization as gel is running
  - 6 mL 50% glycerol to weight DNA sample to bottom of well
  - 4 mL sterile ddH_2O
- Keep refrigerated



Making an Agarose Gel

- To make a 1% agarose gel, mass out 0.5 g of agarose, and add to 50 mL 1x TAE running buffer in a small (250 mL) Erlenmeyer flask.
- Microwave for 40–60 seconds or until low boil. Try to minimize evaporation.



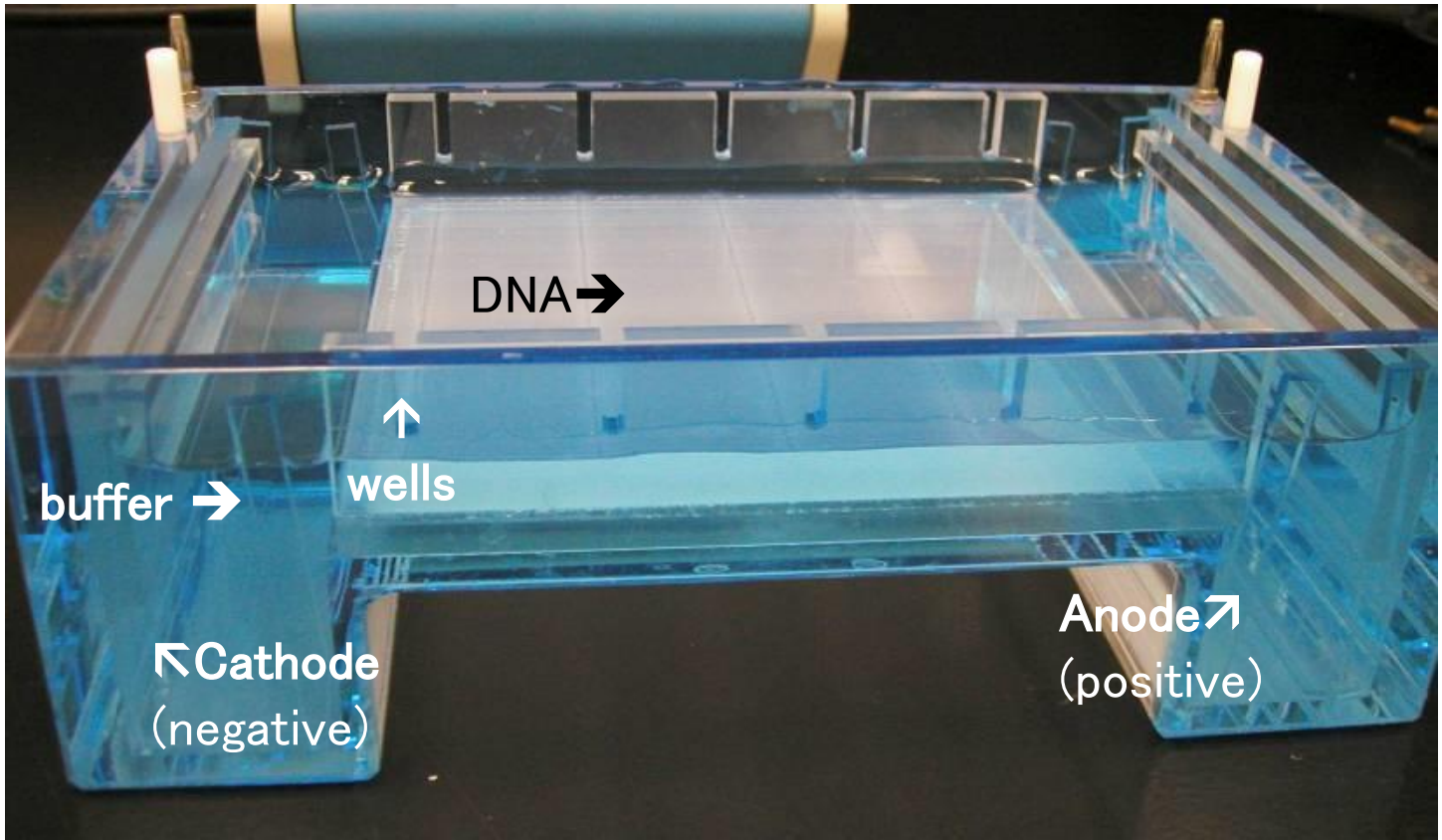
- Swirl gently to ensure all agarose has melted, and allow to cool somewhat so that vapor is not being emitted from the mouth of the flask.
- Add 1 μL of ethidium bromide which binds perpendicularly with DNA helices via Van der Waals forces and fluoresces under UV light with wavelength about 590 nm. [CAUTION: EtBr is a potent mutagen and must be handled with extreme caution and disposed properly.]

Making an Agarose Gel

- Set up the gel caster with a well comb placed about 1–2 cm from the top of the casting tray.
- Make sure the ends of the casting tray are sealed off with rubber clamps or sealing tape.
- Make sure tray is level.
- Pour the warm agarose into the tray slowly to prevent bubbles.
- Allow to cool for 20–30 minutes, remove the seals and comb.
- Cooled gel will be translucent in appearance.

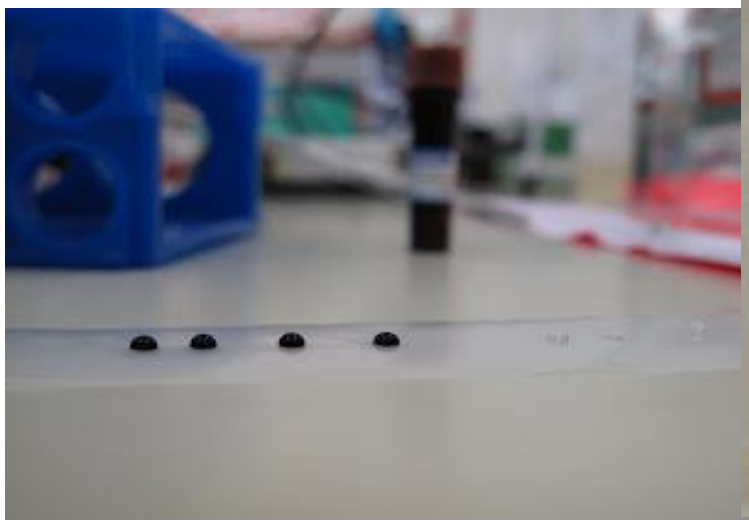
Setting Up the Gel Rig

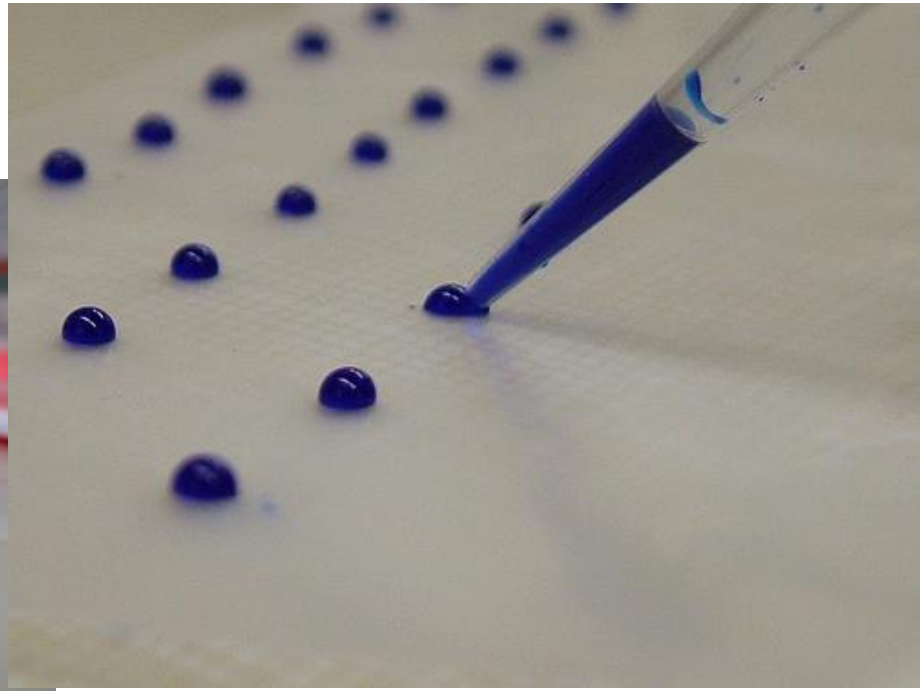
- Place the casting tray containing the gel into the electrophoresis rig.
- Add enough 1x TAE to the gel electrophoresis rig to cover the gel 3–5 mm.



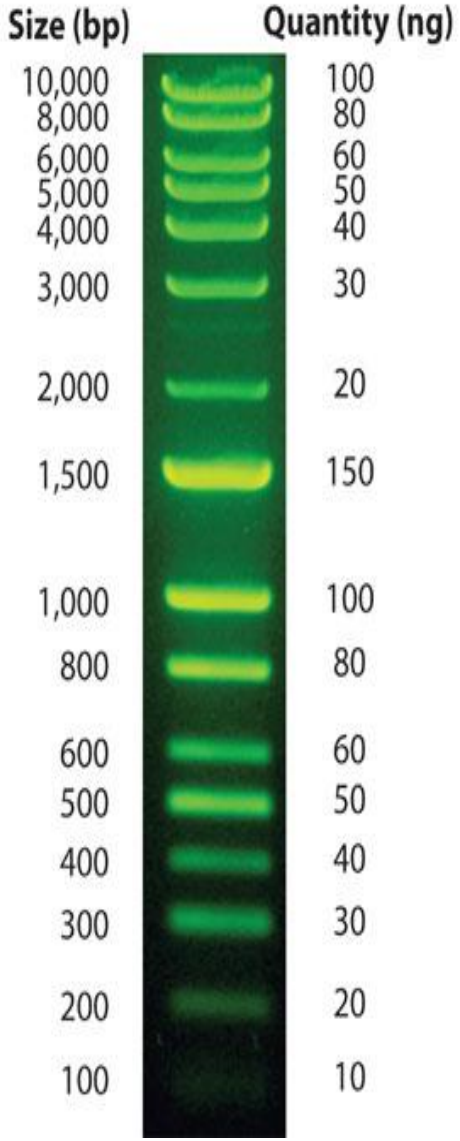
Preparing DNA Samples

- Pipet approximately 2-5 uL of 6x loading buffer for each DNA sample including positive and negative controls onto a clean piece of Parafilm.
- Carefully pipet 10-15 uL of each DNA sample , including positive and negative controls, into dots of loading buffer.

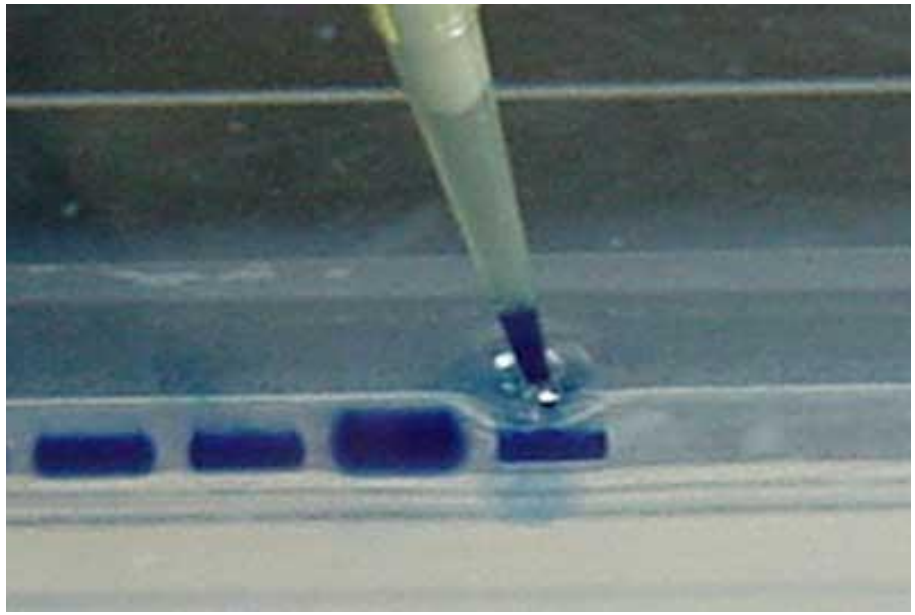




Loading the Gel

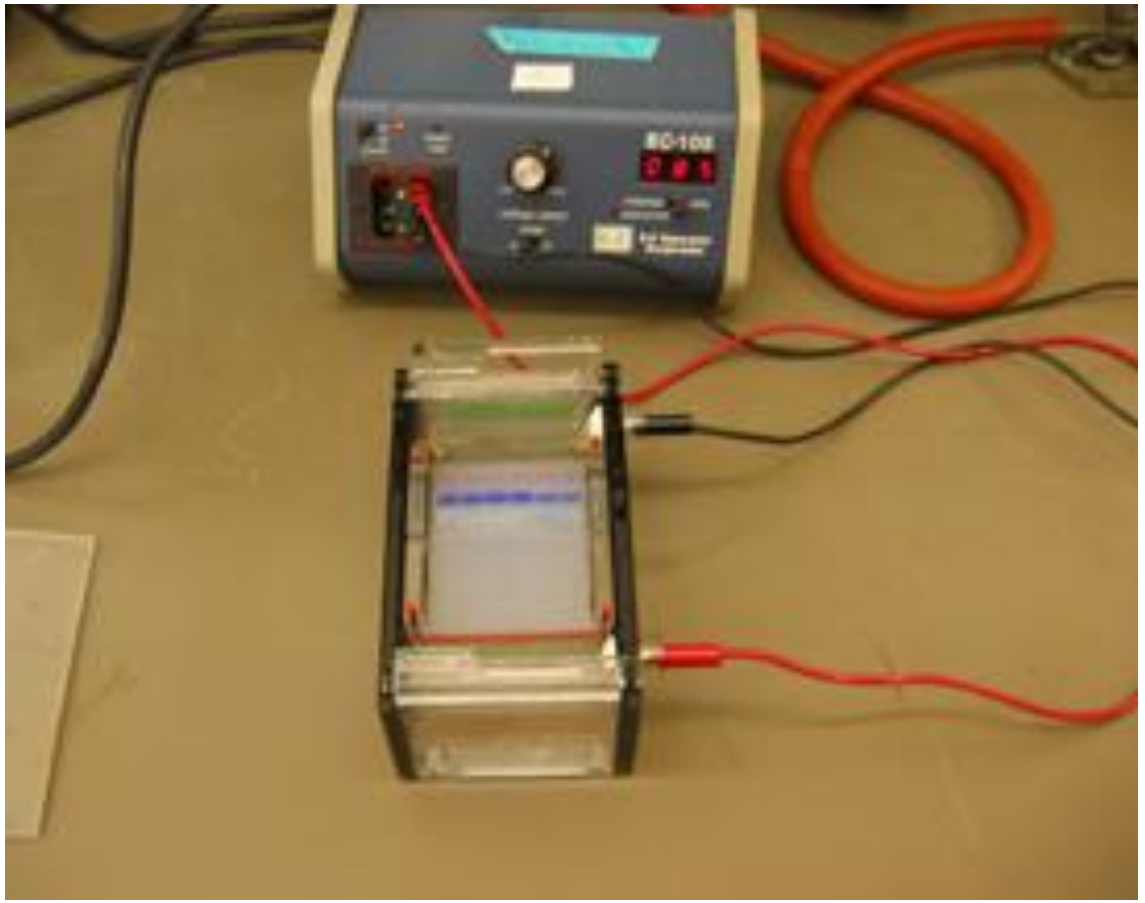


- The first well is normally loaded with a DNA ladder which is a specifically manufactured solution of DNA molecules of different lengths.
- The ladder consists of DNA fragments of known sizes and is used to determine the size of unknown DNA fragments.



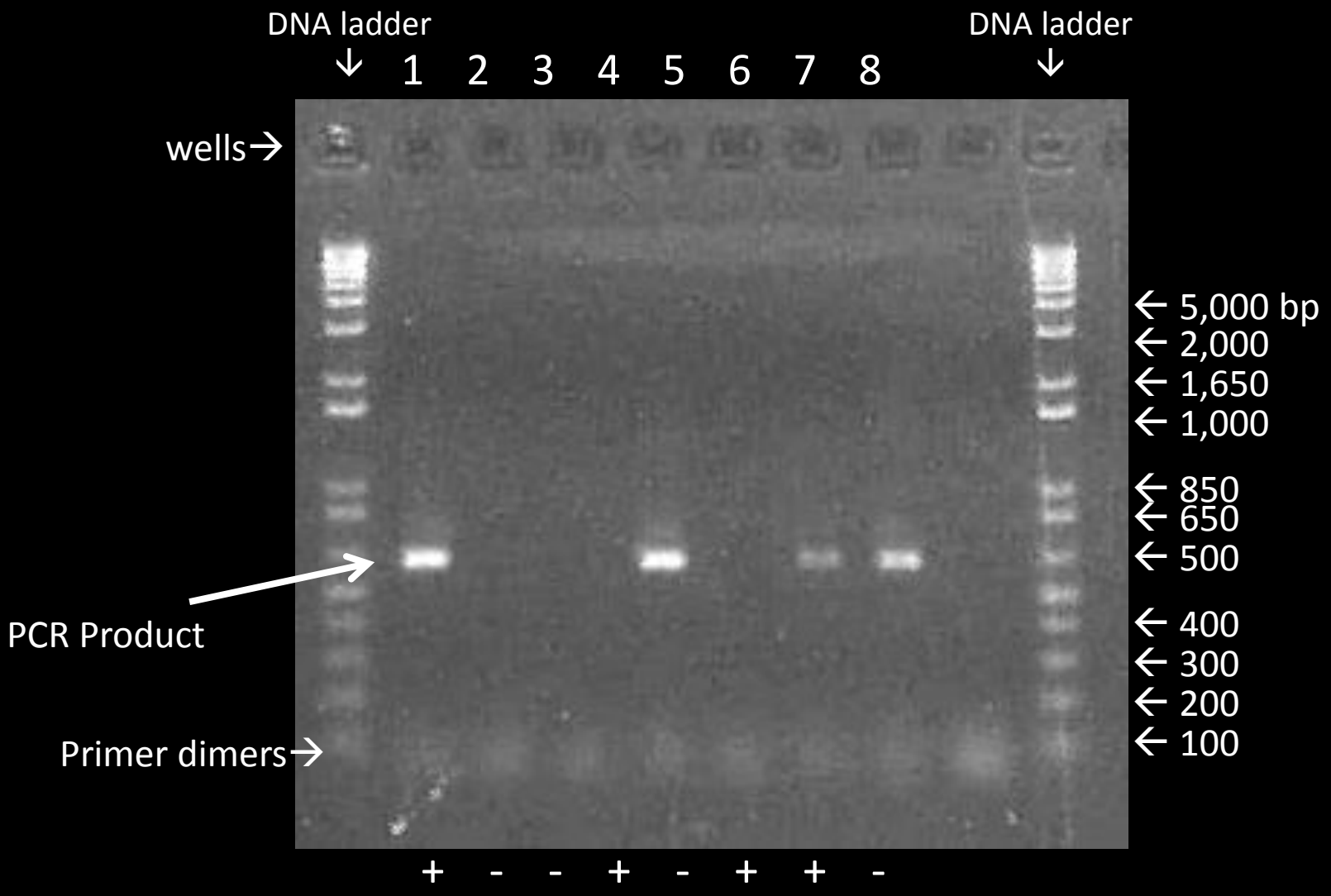
Running the Gel

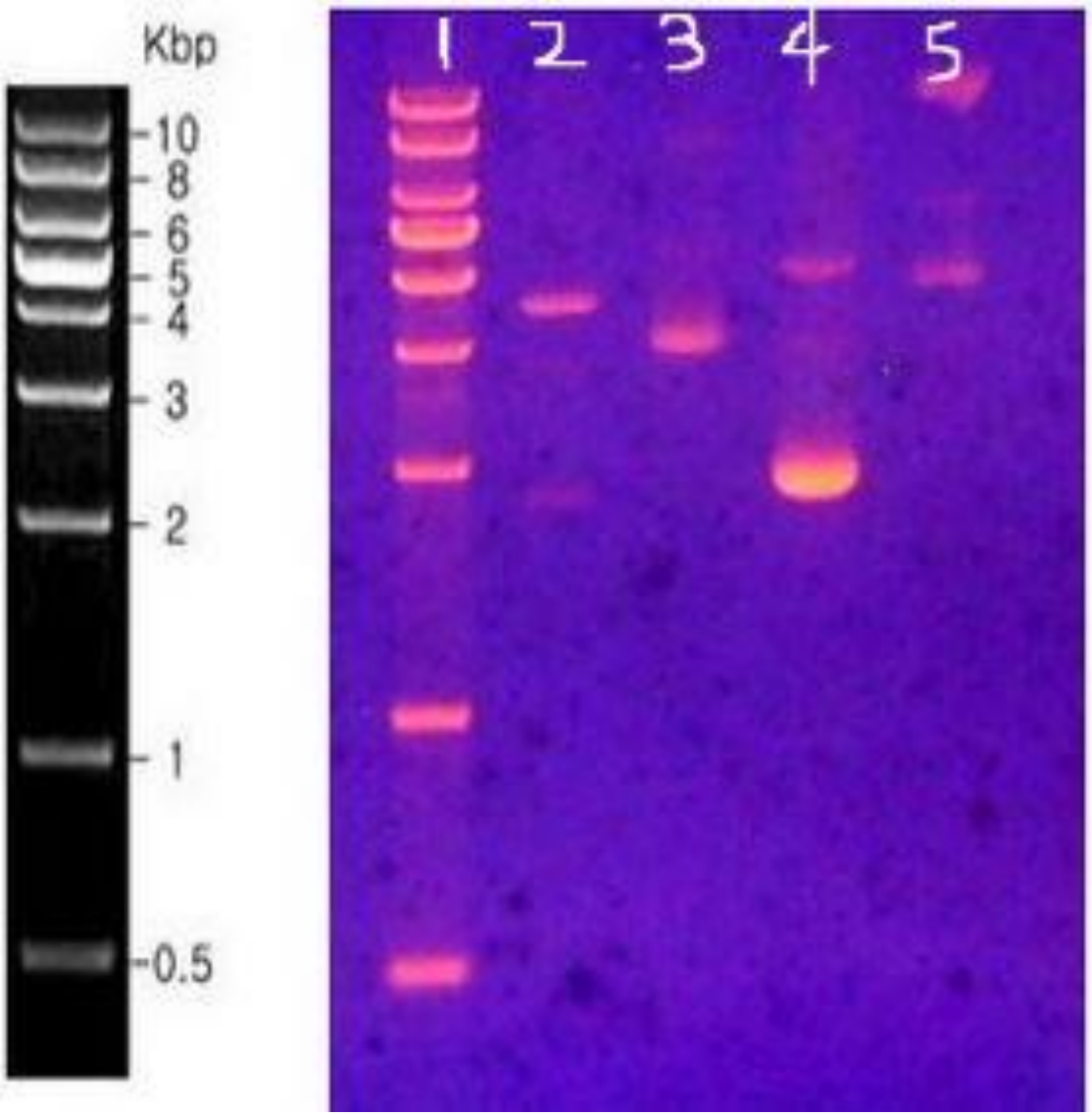
- Secure the cover on the gel rig.
- Connect the leads to the rig and to the power supply. Remember DNA migrates toward the anode which is red.
- Run the gel at 5 V per cm between leads, normally 50 V is adequate, and 60–100 mA.
- Stop the run when the dye marker is approximately ¾ of the way down the gel. (This tracking dye runs similarly to a 200–400 bp DNA fragment.)



Protecting Self and Others

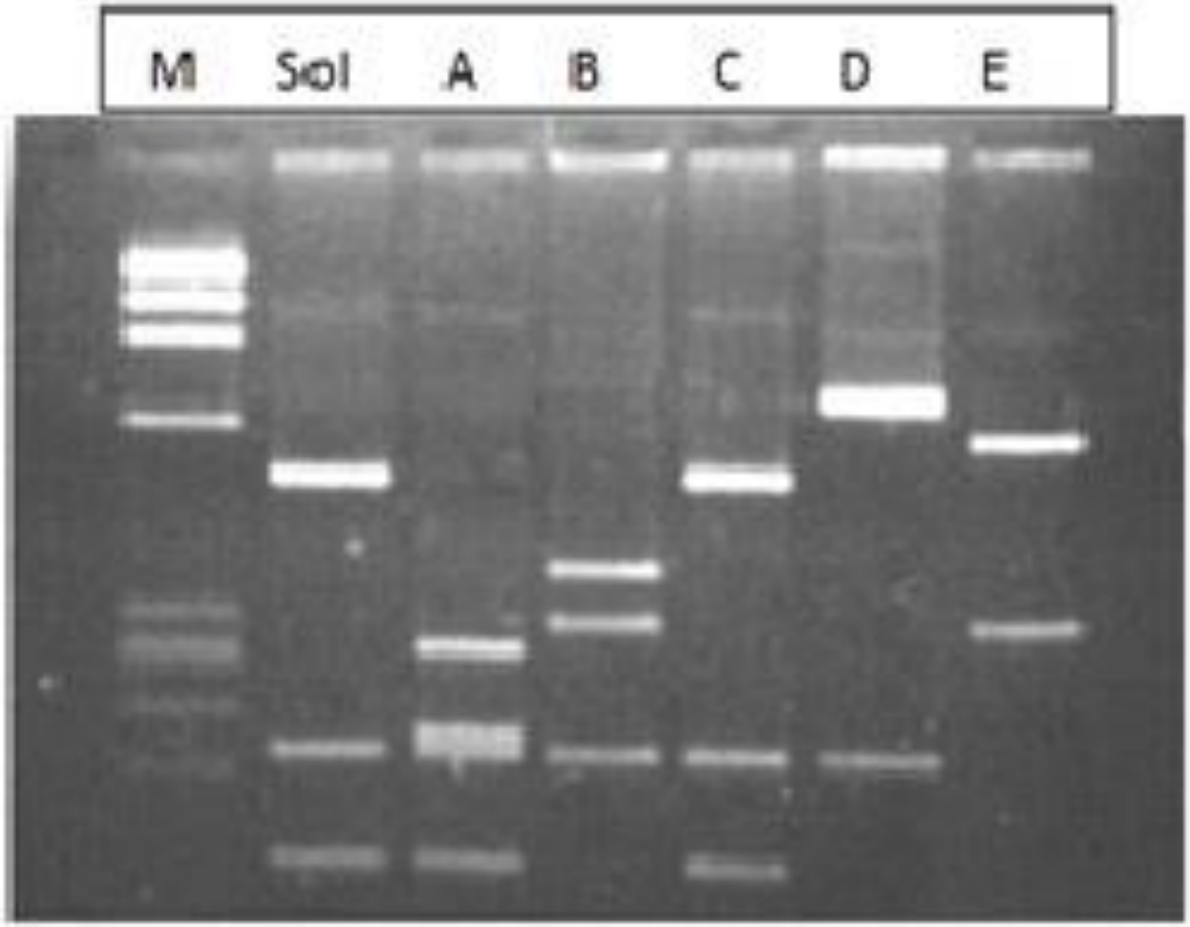
Visualizing the DNA





Lane 1: 1 kb DNA ladder
Lanes 2–5: Test samples

You are trying to isolate a test sample that produced an excised fragment of 3,000 bp. Which sample is your most likely candidate?

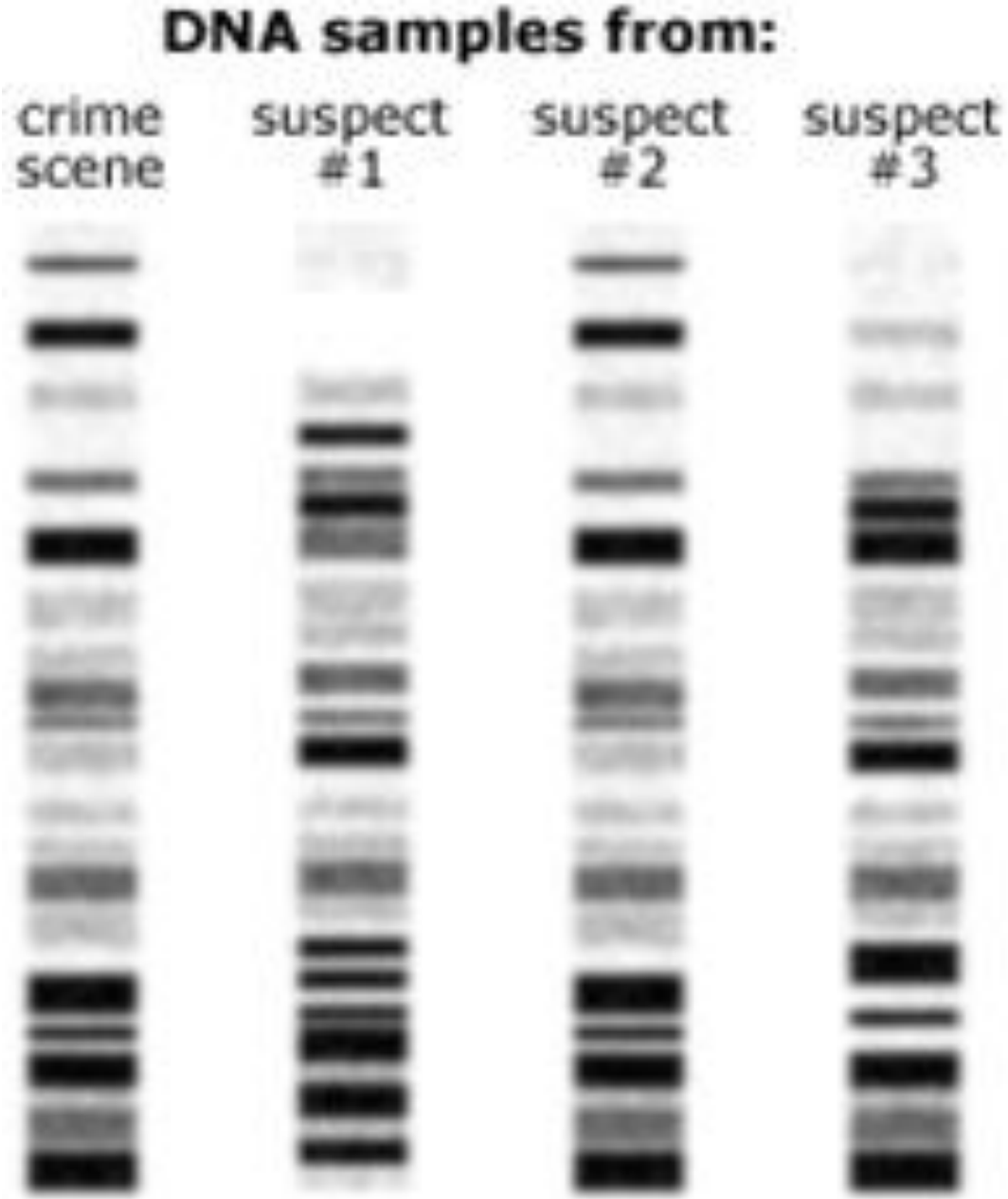


M: DNA marker

Sol: Sample taken from crime scene

Which suspect is most likely to have committed the crime?

- *Which suspect would you exclude from this crime?*



- *Analyze this photo of actual DNA fingerprinting.*