

AGAROSE GEL ELECTROPHORESIS

Like the digital micropipet, gel electrophoresis is a special technique that scientists have had to develop in order to further their research in biotechnology. The purpose of this technique is to separate molecules in a mixture by their molecular size/weight and electrical charge. Electrophoresis literally means “to carry with electricity.”

Before the development of electrophoresis, chromatography was used to separate different molecules within a mixture such as black ink and leaf pigments. In that method, differences in the molecular weight and solubility of the molecules caused them to rise at different rates eventually resulting in their separation. Separation by electrophoresis differs from chromatography in that the molecules are moved by an attraction to an electric charge and they must diffuse through a porous agarose gel.

Agarose (after it is melted) is a jelly-like material. It is a very pure form of agar, which is actually made from a kind of seaweed (algae). Samples of molecules are loaded into holes, called “wells,” in the gel and an electric current is applied. The polysaccharides in the agarose form a microscopic “web” with different sized openings. The smaller molecules of the sample can go through the openings faster than the large molecules, and will move farther down the gel. Large molecules can’t go through the web as fast and will stay close to the well.

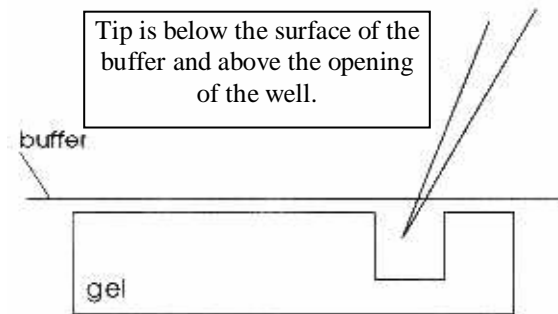
The direction of molecular movement depends on the electric charge (+ or -) of the molecules. Positively charged molecules will move toward the negative electrode (black, cathode), and negatively charged molecules will move toward the positive electrode (red, anode). DNA molecules all have the same charge, therefore, they all move in the same direction (toward the positive electrode). Therefore, when electrophoresing pieces of DNA, the pieces will separate on the basis of size while all moving in the same direction. In order to track the DNA on a gel (which is invisible), we add special dyes to the DNA sample. These dyes are markers to show us where to expect to find the DNA fragments on the gel. They tell us how fast the molecules are running, and when to turn off the electric current.

Agarose is made with the same buffer that is put into the electrophoresis gel box. The buffer serves two purposes. First, it keeps the pH of the gel box from changing radically during the process of electrophoresis. Second, it allows the flow of electricity. Electrons will not flow through air or pure water. Therefore the salts in the buffer allow the current to flow.

DYE ELECTROPHORESIS ACTIVITY

Procedure:

1. Spin microtubes in centrifuge for 5 seconds to move all dye to the bottom of the tube.
2. Place the agarose gel tray into the electrophoresis box as directed by your teacher.
 NOTE: You must load the dyes onto the gel next to the power supply—DO NOT MOVE THE GEL BOX AFTER LOADING THE DYES
3. Add enough buffer to each side of the box until the gel is barely covered. If air bubbles form the gel tray, GENTLY lift one edge of the tray to release the air. Be careful not to let the gel slide off the tray or puncture the gel with your fingers.
4. Dial 10 μ l on the micropipet. Remember to add a plastic tip, and to change tips when using different dyes. Choose dyes as directed by your teacher and load them into the wells on the gel. Draw the first dye sample into the micropipet. (**Remember: depress plunger to the FIRST STOP before lowering the tip into the dye sample**)
5. Steady the pipet over the well, using your second hand to support your pipetting arm. Position your tip just under the level of the buffer, but not deeply into the well on the gel. Be careful not to puncture the gel with the tip.



Gently depress the pipet plunger to slowly expel the dye into the well. The dye should sink to the bottom of the well if it is positioned properly.

Remember: Keep the pipet plunger depressed to the SECOND STOP until the pipet tip is out of the gel box or you will draw your sample back into the tip.

6. As you load the dyes, record which dyes you load in each well. By convention, DNA gels are read from left to right, with the wells located at the top of the gel. Your diagram should be set up in this way on the answer sheet..
7. Place the cover on the gel box.. Be sure that the wire plug-ins match black to black and red to red.
8. Check to see that the power supply is off or unplugged. Connect your gel box to the power supply, again matching red to red and black to black.
9. After all teams using that power supply are ready, check the set up, and plug the power supply into the electrical outlet. Set the power according to your teacher's instructions.

Power should be set at: _____ for _____ minutes

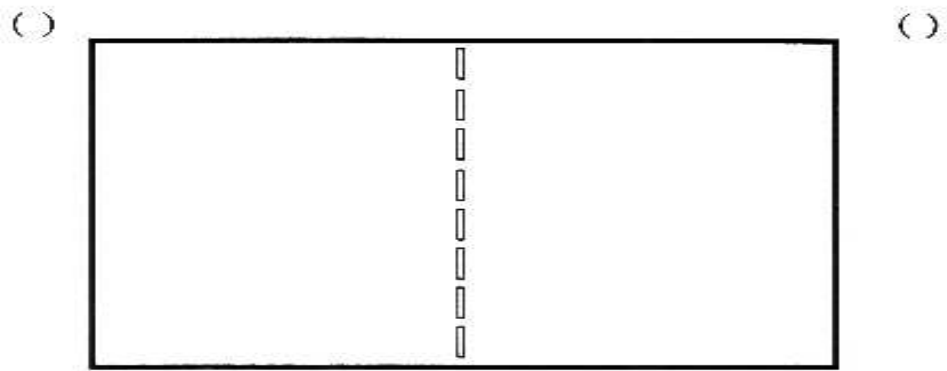
DO NOT REMOVE THE COVER WHILE THE CURRENT IS ON.

10. For 15-20 minutes, observe the movement of the dyes within the gel as the electric current passes through the buffer and gel.
11. Turn off the power supply and unplug it.
12. Remove the gel onto a piece of white paper and record your results.
13. Rinse the gel tray and place it on a piece of paper towel to dry.
14. Discard the buffer as instructed by your teacher. DO NOT throw the buffer down the sink!
15. At the end of the day, the gel boxes may be rinsed with water and turned upside down on a paper towel to dry. **DO NOT WIPE THE INSIDE OF THE GEL BOXES WITH PAPER TOWELS!!!** This can dislodge the electrodes and cause permanent damage.

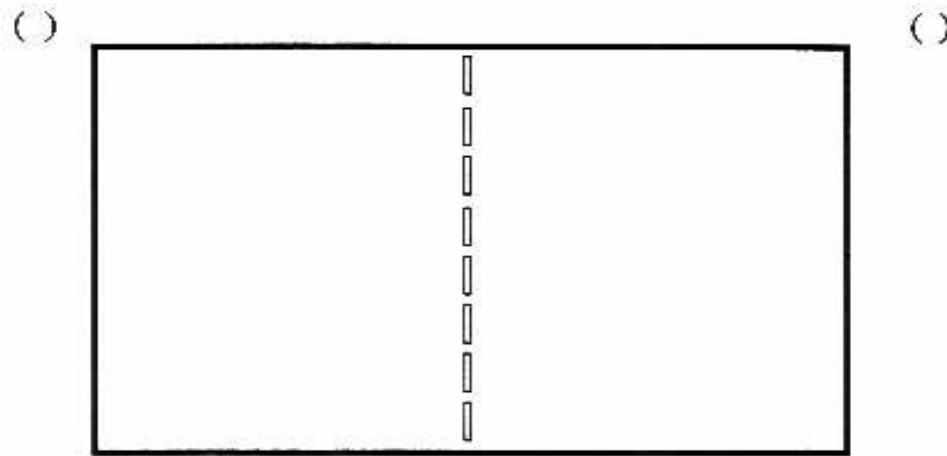
DYE ELECTROPHORESIS ACTIVITY

NAME _____

1. List the dyes you used on your gel in order from left to right. Be sure it matches how you loaded your dyes:



2. Label the diagram with the following:
- the positive and negative ends of the gel
 - Lane numbers—must correspond to the diagram above
 - Direction of current flow through the gel (the flow of electrons which are negatively charged).
 - Using colored pencils to match the colors seen on the gel, record the direction and distance moved by each dye.



- Which dyes moved to the positive end of the gel?
- What is their electrical charge?
- Which dyes moved to the negative end of the gel?

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6. What is their electrical charge?
 7. Explain how you knew which dyes from questions 3 and 5 had which electrical charge.
 8. Which dye is most likely the smallest molecule? Why?
 9. List the dyes in order of increasing molecular size.
 10. If you had a mixture of dyes, how can you tell that the dyes in the mixture are actually the same dyes found in other lanes on the gel? (color is NOT the answer)
 11. DNA is a deoxyribose sugar and phosphate polymer (this means that these units are repeated over and over hundreds of times). The phosphate units (PO_4^{3-}) give all DNA molecules a negative electrical charge. In what direction would you expect pieces of DNA to move during electrophoresis? Why?
 12. Therefore, when placing a gel into the electrophoresis box when determining the size of pieces of DNA,, the wells should be closest to which electrode? Why?