Lab C-1: Electrophoresis

(Casting and Loading an Agarose Gel)

Background:

In Lab B you investigated the components of **electrolysis**. Now you will use what you have learned and apply it to **electrophoresis. Electrophoresis** is a technique for electrically separating and analyzing mixtures of charged molecules. The mixture to be separated is "loaded" into slots or "wells" of a slab of jellylike material called **agarose**. To prepare or "cast" an agarose gel, agarose powder is mixed with buffer, heated, and poured into a **casting** or **gel tray** containing a **comb**. When the gel has cooled and solidified, the entire casting tray is lowered into the gel box and covered with buffer that allows the electricity to flow and prevents changes in pH. The comb is removed, creating empty **wells**. A micropipette is used to place a small amount – usually just a few microliters -- of the mixture to be separated into each well. In order to track where the "invisible" mixture runs on a gel, we add two dyes to the sample. One dye runs slightly faster and the other dye runs slower than the substances we are separating in the mixture. The dyes act like book ends that mark the approximate location of the unknowns in the mixture.

Purpose:

To practice the steps required in the casting and loading of an agarose gel and to observe the migration of four dyes commonly used to track DNA in agarose gels.

Materials per team:

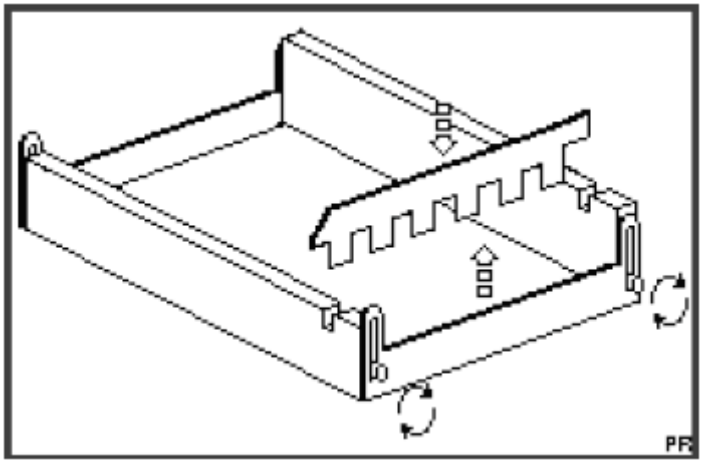
power supply
50 mL glass beaker for agarose
Loading dye beaker
P-20 micropipet & tips
liquid soap
buffer [1X TAE or TBE]
gel box with gel tray
500 mL beaker for buffer
container for waste
paper towels/Kimwipes microfuge
agarose, [0.8%], melted and kept hot in a 65°C bath or incubator

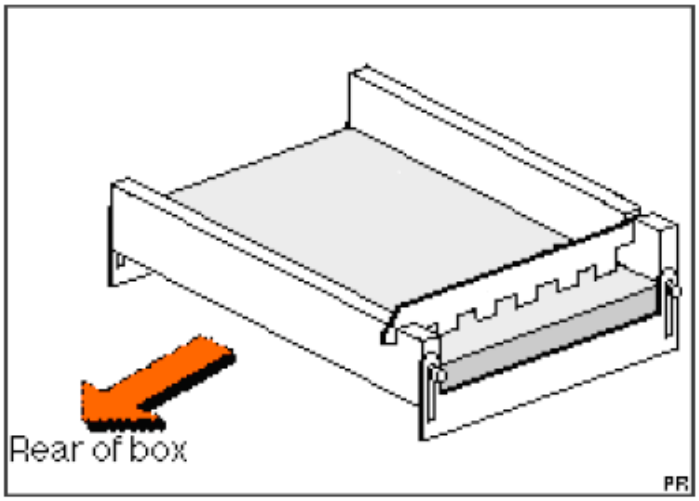
CAUTION:

If two teams are connecting their gel boxes to one power supply, be sure to communicate with each other whenever the power supply is turned ON or OFF. **The power supply must be OFF every time anyone needs to touch or open a gel box.**

Procedure: Casting a Gel

1. Obtain the casting tray with agarose gel from your teacher.
2. ORIENT THE GEL BOX SO THAT THE WIRES ARE FACING YOU. Fill the gel box with 300 mL of 1X TAE electrophoresis buffer. (A previous class may have done this.)
3. USING THE HIGHER SIDE OF THE CASTING TRAY AS A HANDLE, TIP THE TRAY SLIGHTLY AND INSERT THE LOWER SIDE OF THE TRAY TO THE REAR OF THE BOX. Submerge the tray onto its platform in the gel box. The comb should be located on the **left** (cathode or negative) side with the black lead The wells should be on the
4. Gently remove the comb from the gel by pulling it straight out. Notice that the six little empty "slots" or wells in the gel.

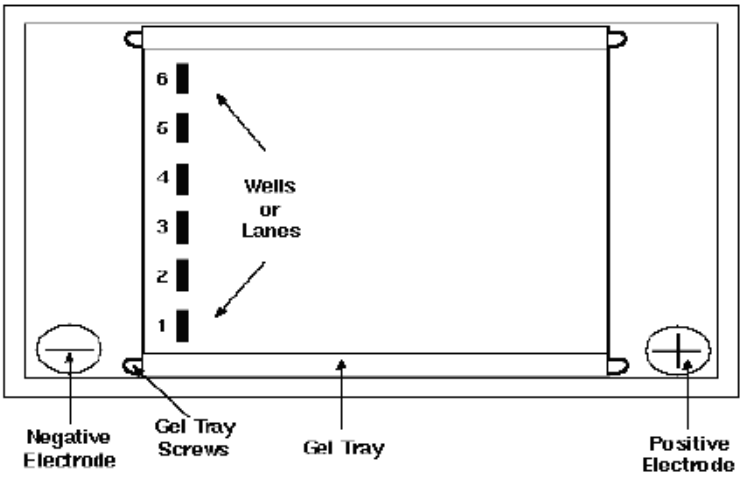




Procedure: Loading the Gel

5. Be sure the gel box is oriented with the wells to the left and the leads facing you (see the diagram below).

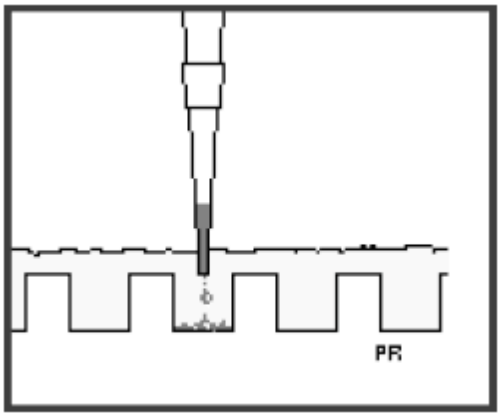
BACK SIDE



FRONT SIDE

6. Use a **P-20 micropipette** to practice the technique of loading a well (there are six wells, so every member of your team can practice this):
   - Draw 2 L of loading dye into the micropipette. (**Remember to depress the plunger to the FIRST STOP before lowering the tip into the dye sample.**)
   - Steady the pipet over the well, using your second hand to support your pipetting hand or arm and elbows on the lab table.
   - Lower the tip of the pipet under the surface of the buffer directly over the well -- but **DO NOT** lower the tip into the well itself, or you risk puncturing the bottom of the gel.
   - Gently depress the pipet plunger to slowly expel the loading dye into the well. If the tip of the micropipette is centered over the well, the dye will sink to the bottom of the well.
   - **REMEMBER** - keep the pipette plunger depressed to the SECOND STOP until the pipet tip is out of the gel box or you'll draw your sample back into the tip!

Procedure: Electrophoresis of the Dye

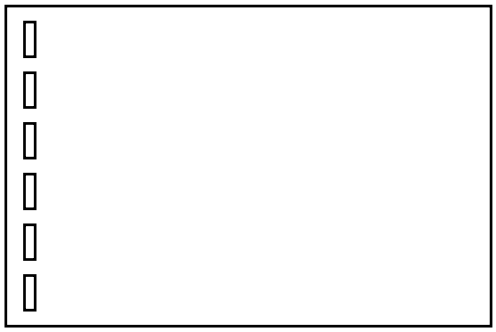


7. Apply ONE drop of liquid soap to a piece of paper towel and spread evenly around the inside of the gel box LID. If previous periods already did this, do not add more soap. DO NOT GET THE SOAP IN THE BUFFER. Close the top of the gel box and connect electrical leads anode to anode (red to red) and cathode to cathode (black to black). Both electrodes should be connected to one power supply channel.
8. Set the power supply to approximately 100 V, and turn it ON. To double-check this, switch the display to look at the current: if one gel is running, it should read about 40 milliamps; two gels should read about 80 milliamps. (As a check to see that electricity is flowing, look for bubbles at the wire at either end of the gel box.)
9. Shortly after the voltage is turned on, you should see the dyes slowly moving through the gel toward the positive side of the gel box.
10. Run the electrophoreses test for about 10 minutes. Draw the position of the dyes on your in the data section of the lab.
11. Turn off the power and disconnect the leads.
12. Save the buffer for reuse by the next class. Return gels to teacher for reuse after melting.

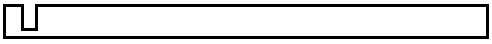
Data and Analysis

1. Cut out and glue the two gel diagrams into your lab notebook. Record the location of the loading dye(s)* on the diagrams below (BOTH the top and side views)
2. Label the positive and negative ends of the gel in Figures A and B.

Fig. A. Gel: immediately after loading

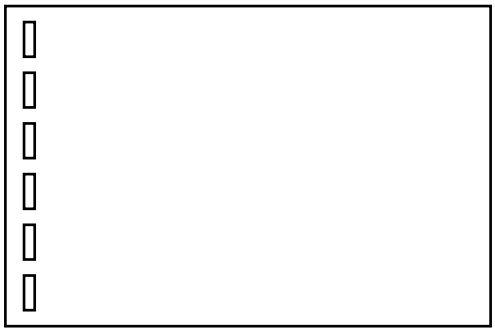


Top view of gel

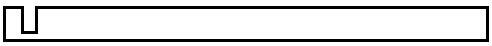


Side view of gel

Fig. B.Gel: after it has been run.



Top view of gel



Side view of gel

Questions for your lab notebook.

1. Explain why the dyes move toward the positive (anode) end of the gel.
2. Why would you want the end of the gel with the wells to be closer to the negative (cathode) end of the gel box?

Check: *In this lab two dyes were combined to form "loading dye". One of these dyes moves faster than the small pieces of DNA, the other moves more slowly than the largest pieces of DNA.*

In this activity, DNA was not mixed with this loading dye. However, it will be when we need to separate fragments of DNA.