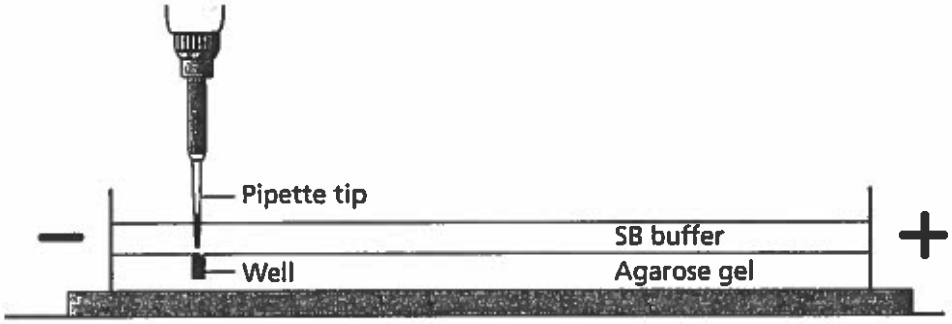
LABORATORY 1.2: GEL ELECTROPHORESIS

The purpose of this laboratory is to give you experience with gel electrophoresis, which is used to separate and identify a mixture of biomolecules including DNA; the components of each mixture can then be identified by their location in the gel. Gel electrophoresis works based on the fact that biomolecules have a negative charge, which means that they will move in response to an electric charge. The biomolecules move through a gel, and their speed varies primarily according to their weight, although molecular shape and degree of charge also influence their movement. In the genetic engineering process, gel electrophoresis is used to separate and identify plasmids and short linear pieces of DNA.

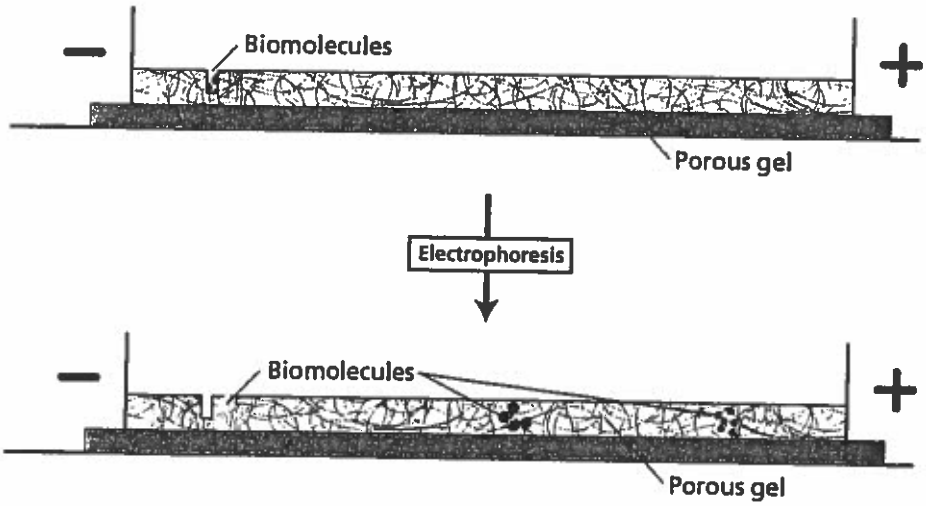
The electrophoresis setup consists of a box containing an agarose gel and two electrodes that create an electric field across the gel when the box is attached to a power supply. The negative electrode is black, and the positive electrode is red. Samples of biomolecules are pipetted into wells near the negative (black) electrode. The samples move through the gel toward the positive (red) electrode, as shown in **Figure 1.4.**

Figure 1.4: The gel electrophoresis unit



The gel that the biomolecules move through is composed of *agarose*, a polysaccharide (complex sugar) found in seaweed. Its structure is a porous matrix (like a sponge) with lots of holes through which the solution and biomolecules flow. See **Figure 1.5.**

Figure 1.5: How biomolecules, including DNA, move through the agarose gel matrix in gel electrophoresis



BEFORE THE LAB

Respond to the following questions with your group, and be prepared to share your answers with the class.

1. In what circumstances might it be important to use gel electrophoresis to separate and identify plasmids and short linear pieces of DNA.
2. Read through the Methods section on pages A-26 through A-29 and briefly outline the steps for *Part A* and for *Part B*, using words and a flowchart.

MATERIALS

Reagents

- A plastic microfuge tube rack with the following:
  - Microfuge tube of red dye solution (RD)
  - Microfuge tube of dye solution 1 (S1)
  - Microfuge tube of dye solution 2 (S2)
  - Microfuge tube of dye solution 3 (S3)
- 50-mL flask containing 1x sodium borate buffer (1x SB) (shared with another group)

Equipment and Supplies

- P-20 micropipette (measures 2.0–20.0 µL)
- Tip box of disposable pipette tips

- 2 pipetting practice plates loaded with 0.8% agarose gel
- Electrophoresis box loaded with 0.8% agarose gel (will be shared among groups)
- Microcentrifuge (will be shared among all groups)
- Waste container for used tips and microfuge tubes (will be shared among groups)

SAFETY: Wear chemical-resistant goggles and aprons.

- **All appropriate safety precautions and attire required for a science laboratory should be used, including safety goggles. Please refer to your teacher's instructions.**
- **Wash your hands well with soap after completing the lab.**

METHODS

PART A: PIPETTING INTO WELLS

You will practice pipetting RD into preformed wells in an agarose gel.

1. Check your rack to make sure that you have the RD tube.
2. Fill the two pipetting practice plates with 1x SB to a level that just covers the entire surface of the gel. If you see any "dimples" over the wells, add more buffer.

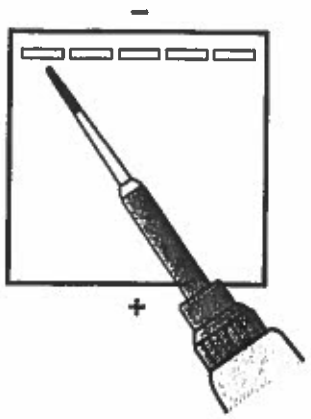
3. Set the P-20 micropipette to 10.0 µL and put on a pipette tip.
4. Load the RD into the pipette with 10.0 µL of RD.

LAB TECHNIQUE: Do not lay down a micropipette with fluid in the tip or hold it with the tip pointed upward.

5. Dispense RD into a well in one of the practice plates by doing the following:
   a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
   b. Lower the pipette tip until it is under the buffer but just above the well.

LAB TECHNIQUE: Be careful not to place your pipette tip into the well or you might puncture the gel, which will make the well unusable.

   c. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.



6. Repeat steps 4 and 5 until all the practice plate wells have been filled. Everyone in your group should get an opportunity to practice pipetting into the wells.

7. Eject the pipette tip.

PART B: SEPARATING DYES WITH GEL ELECTROPHORESIS

Now you will use gel electrophoresis to separate different dyes. First you will add dyes into wells in the gel electrophoresis unit. You will then turn the unit on in order to move the negatively charged dyes through the gel. (You will share the electrophoresis boxes with one other group; your teacher will tell you which wells your group should use.)

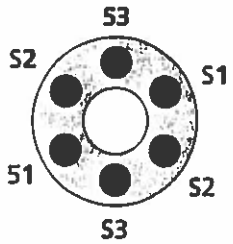
1. Check your rack to make sure that you have the three dye solutions (S1, S2, and S3).

2. Review Figure 1.4 on page A-24. Check to make sure that the wells in the gel are located near the negative (black) electrode.

3. Fill the box with 1x SB to a level that just covers the entire surface of the gel. If you see any "dimples" over the wells, add more buffer.

4. Centrifuge the S1, S2, and S3 tubes.

   LAB TECHNIQUE: Distribute the tubes evenly in the microcentrifuge so that their weight is balanced.



5. Make a drawing in your notebook that shows the location of the wells in the electrophoresis box. Record which solution you will place in each well.
6. Set the P-20 micropipette to 10.0 µL and put on a pipette tip.
7. Load 10.0 µL of S1 into the pipette.
8. Dispense the S1 into the well you've designated for that solution by doing the following:
   a. Place your elbow on the table to steady your pipette hand. If needed, also use your other hand to support your pipette hand.
   b. Lower the pipette tip until it is under the buffer but just above the well.

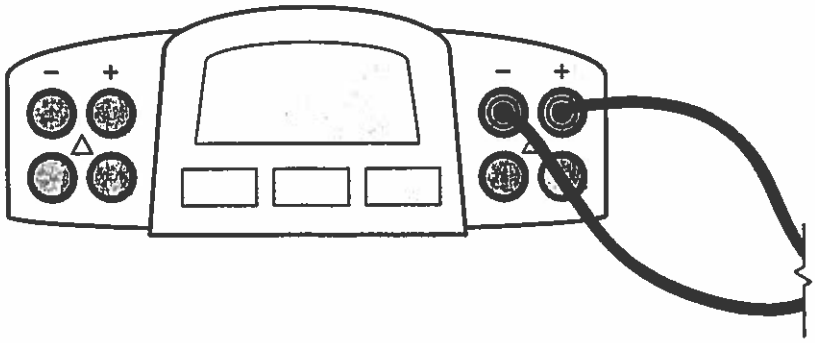
      LAB TECHNIQUE: Do not puncture the gel or it will become unusable.

   c. Gently press the plunger to slowly dispense the sample. To avoid getting air into the buffer, do not go past the first stop. The sample will sink into the well.

      LAB TECHNIQUE:

      - **While the plunger is still depressed, pull the tip out of the buffer so that you don't aspirate the solution or buffer.**
      - **Use a fresh pipette tip for each sample.**
9. Repeat steps 7 and 8 for S2 and S3, using a new pipette tip with each solution.
10. When all the samples have been loaded, close the cover tightly over the electrophoresis box. (Carefully close the cover in a horizontal motion, so that samples don't spill.)
11. Connect the electrical leads to the power supply. Connect both leads to the same channel, with cathode (–) to cathode (black to black) and anode (+) to anode (red to red). See Figure 1.6.

Figure 1.6: Leads from electrophoresis box connected to correct channel in power supply



12. Turn on the power supply and set the voltage to 130–135 V. (You will see bubbles form in the buffer at the red (+) end of the electrophoresis unit.)

13. After two or three minutes, check to see if the dyes are moving toward the positive (red) electrode. You should begin to see the purple dye (bromophenol blue) beginning to separate from the blue dye (xylene cyanole).

STOP AND THINK:

- **Study your gel electrophoresis results. Which solution sample contained a single dye: S1, S2, or S3? How do you know?**
- **What electrical charge do the dyes have? Explain your reasoning.**
- **The dyes that you are separating are orange G (yellow), bromophenol blue (purple), and xylene cyanole (blue). If the molecular shape and electric charge of all three dyes are similar, what is the order of the dyes from heaviest to lightest molecules, based on your initial results? Why do you think this is the correct order?**

14. In approximately 10 minutes, or when you can distinguish all three dyes, turn off the power switch and unplug the electrodes from the power supply. Do this by grasping the electrode at the plastic plug, NOT the cord.

15. Carefully remove the cover from the gel box and observe the dyes in the gel.

16. In your notebook, draw the relative location of the bands and their colors in each of the lanes containing your samples.

17. Leave the gels in the gel box.

CHAPTER 1 QUESTIONS

1. What is the importance of micropipettes and gel electrophoresis in genetic engineering?

2. During the labs, you were often reminded to avoid contact with the pipette tips—for example, you were asked to put the pipette tip on without using your hands, to avoid setting down the micropipette, to use the ejector button to remove the tip, and to keep the tip box closed. If you were working with plasmids and bacterial cells, why would these precautions be important?

3. Study your gel electrophoresis results:

   a. Which solution sample contained a single dye: S1, S2, or S3? How do you know?

   b. The molecular weights for the dyes are 452.38 atomic units (au) for orange G, 669.98 au for bromophenol blue, and 538.62 au for xylene cyanole. How do these weights compare with your original conclusions about the weights of the dyes?

4. Do you think there was a difference in the amount of charge on one of the dye molecules? Explain the reasoning for your response.

DID YOU KNOW?

Gel Electrophoresis in DNA Fingerprinting

DNA fingerprinting uses gel electrophoresis to distinguish between samples of genetic material. In DNA fingerprinting, human DNA molecules are treated with enzymes that chop them at certain characteristic points, thereby reducing the DNA to a collection of smaller and more manageable pieces. The DNA fragments are loaded into a gel and placed in an electrical field, which electrophoretically sorts the DNA fragments into various bands. These bands can be colored with a radioactive dye to make them visible to imaging techniques. Methods of DNA identification have been applied to many branches of science and technology, including medicine (prenatal tests, genetic screening), conservation biology (guiding captive breeding programs for endangered species), and forensic science. In the latter discipline, analysis of the pattern of DNA fragments that results from the action of restriction enzymes enables us to discriminate between suspects accused of a crime, or potential fathers in a paternity suit.