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 26. 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.
   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:** Do not puncture the gel or it will become unusable.

   **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.