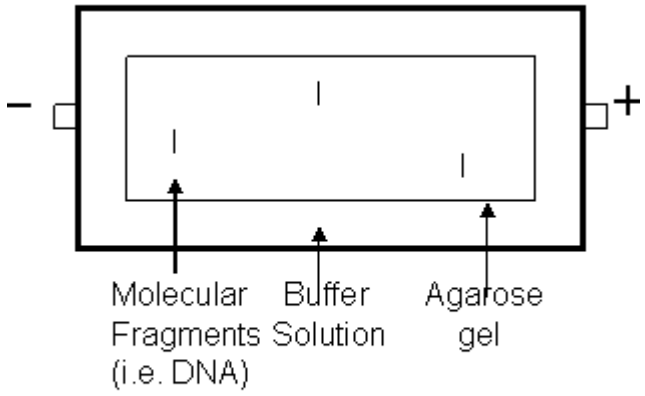
GEL ELECTROPHORESIS OF DYES

Protocol for Students

In this experiment you will be using electrophoresis to separate dye samples which have different sizes and charges.



Electrophoresis unit with gel in place as seen from above

Precautions

STUDENTS: Check with your teacher to be sure that you understand all of the safety instructions for using this equipment.

Objectives

1. **Understand how gel electrophoresis is able to separate molecules.**
2. **Learn how to use a pipetman to load a gel.**
3. **Review safety considerations when working with an electric current.**
4. **Determine the components of an unknown dye mixture.**

Procedure

1. Put on gloves. You may be sharing dye samples and the agarose gel with another lab group.

2. Seal each end of the gel tray with laboratory tape. Place the plastic comb into the middle of the tray. Go to the hot water bath and get the bottle of melted agarose.

3. Carefully pour the agarose into the gel tray until it is approximately 1/3 of the way up the teeth of the comb. This should use about 30ml of agarose solution. Make sure that there a no bubbles in the gel.

4. Let the gel harden without disturbing it for about 10 minutes.

5. Carefully remove the comb from the gel by pulling straight out of the solidified gel. Remove the tape from the ends of the gel tray.

6. Place gel into electrophoresis unit. Add 150 ml 1X TBE buffer to completely fill the box and to cover the top gel surface with about 2 mm of buffer.

NOTE: At this point the gel box can be covered and left until the next day if necessary

7. On the gel load 5-10 µl of each dye into a well. Keep track of which dye goes into which well on a notebook sheet. Use a new tip for each dye and be careful not to puncture the bottom of the well.

   Dyes to be used:

   - Bromophenol blue
   - Janus green
   - Orange G
   - Safranin O
   - Xylene cyanol
   - Mixtures of any of the above dyes as provided by the teacher

8. Clean up any spilled buffer or any other liquid surrounding the gel box thoroughly.

9. Make sure that the power supply is unplugged and switched off before proceeding.

10. Carefully place the lid on the gel box and connect the terminals correctly.

11. Plug in the power supply, select the 100V option and turn on the machine. The run light will illuminate, signifying that power is running to the cell.

12. Observe the tiny bubbles that form along the platinum electrodes.

13. Let the gel run undisturbed for about 30 minutes but check frequently to determine that the dyes will not run off the end of the gel into the buffer compartment.

14. When the dyes reach approximately 1 cm from the end of the gel, turn off the power supply. Then disconnect the patch cords from the power supply and unplug the unit.
15. Make sure that your gloves are on. The gel will be removed from the box for you to record your observations on the activity sheet.
16. Complete the activity sheet.

Clean up

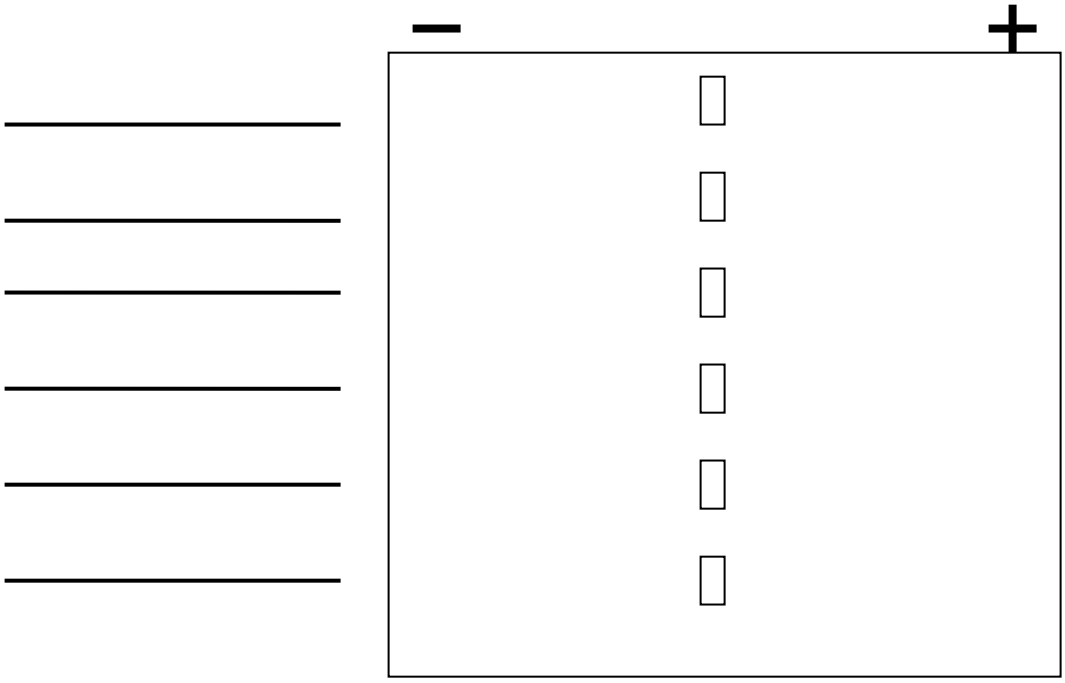
Discard all pipette tips and gels into plastic bags that can then be placed in the regular trash. The gel box, tray and comb should be rinsed in distilled water as outline above and then allowed to air dry. Be very careful not to damage the tiny electrode wires at each end of the gel box.

Wash your hands thoroughly and clean the area where you were working.

STUDENT ACTIVITY

1. Why is the comb placed in the center for this electrophoresis experiment?

2. The diagram below represents your gel. Using colored pencils or markers draw in the results of your dye separation. Be sure to label each lane with name of the dye that was in the well.



3. Which dye likely contained the smallest molecule?

4. How do we know this?

5. List the dyes that have a negative charge.

6. Explain why you chose these dyes.

7. If you were pouring a gel to run DNA through, where would you place the comb? Explain your answer.