FRED HUTCHINSON CANCER RESEARCH CENTER
SCIENCE EDUCATION PARTNERSHIP

THE DYE/INDICATOR LAB
SEPARATION OF MOLECULES USING AGAROSE GEL ELECTROPHORESIS

OBJECTIVES: YOU WILL BE ABLE TO PERFORM THE FOLLOWING ACTIVITIES.

- Make and load the wells of an agarose gel.
- Electrophorese samples in the gel and interpret the results.
- Identify and manipulate variables involved in separating biological dyes & pH indicators.
- Design and carry out a simple electrophoresis experiment.
- Explain the theory behind separation of molecules by electrophoresis.

MATERIALS: **FOR LAB GROUP OF 4**

electrophoresis apparatus (BRL Horizon 58 gel box)	Power Supply (shared by two groups)	beaker for used tips
1% agarose gel See How to Make a Gel	1X TAE buffer (gel buffer or running buffer)	micropipet tips
micropipets	blot paper (1 per person)	ziplock bag
acetate sheets (1 per person)	plastic wrap	marker pen
ruler (mm)	microtube rack	microtubes

A. SAMPLES TO RUN

1. bromophenol blue
2. methylene blue
3. orange G
4. xylene cyanol
5. mixture XXX
6. mixture ZZZ
7. choose from B list
8. choose from B list

B. EXTRA DYES/ pH INDICATORS

crystal violet
eosin Y
methyl green
o-cresol red
phenol red
safranin O
bromocresol green
bromocresol purple
m-cresol purple

PROCEDURE: (SEE HOW TO MAKE A GEL PINK CARD OR HANDOUT PAGES 5 AND 6.)

1. To prepare the electrophoresis apparatus: place the gel into the gel box. Add 1X TAE buffer to just cover the gel about 1-2mm (~125ml). Measure the pH of the buffer at each end of the gel box and note on record sheet.

2. To prepare your samples, number tubes and label tubes with the names of the samples you will use.

3. Obtain samples by pipetting 20μl (this is 15μl to load in the well plus a little extra) of each sample from the class supply into the appropriately labeled microtube.

4. Before loading samples, be sure your gel box is near the power supply. Load 15μl of the sample into the appropriate gel well. Record which sample is loaded into each well on your record sheet.

5. When all 8 samples are loaded, close the lid on the gel box. Student groups will share the power supplies. Check your power supply first: the main power switch on the power supply must be turned off when gel boxes are being connected to the power supply. Connect the electrodes to the gel box and the power supply connecting red to + (anode) and black to — (cathode). Turn on power supply and set it to 100 volts. Check the milliamp output. Electrophorese for a total of 10 min. If power supply beeps, turn down the voltage.

6. Observe and record the changes that you see happening after about 5 min on your record sheet. Toward which electrode did the greater number of samples run?

7. After the 10 min run, turn off the power. Unplug the electrodes and open gel box. Measure the pH of the buffer at each end of the box. Lift out the gel deck and gel and tip slightly to pour off excess buffer. Place them on the lab table on a paper towel and wipe off excess buffer. Record your results on your record sheet.

8. Place a piece of acetate sheet on top of the gel. Using a permanent marker pen, mark the wells and each colored spot. Note the + and — ends. After the blotting step, you can trace copies for other group members.

9. To make a blot, wear gloves and place a piece of plastic wrap in the palm of one hand. Pick up the gel deck with gel, turn it over into the palm of the hand with the plastic wrap and slide the gel onto the plastic wrap. Set a piece of blot paper on the bottom side of the gel (which is now facing up) and wrap the piece of plastic wrap over the blot paper. Flip it over on the lab table and use the gel deck to apply steady even pressure on the gel for about 5 seconds. Color will transfer from the gel to the blot paper, making a record of your results. Flip the plastic wrapped gel+blot over, unwrap, and carefully lift the blot paper off the gel to dry. Add a fresh piece of blot paper and repeat the blotting procedure so that each lab group member has a copy.

10. Wrap up the gel in plastic wrap, label, and freeze for future reference if needed.

11. Make copies of the acetate trace so that each group member has a copy.

12. Measure the distance that each colored spot traveled on the gel. You can use the gel or the acetate sheet for this. Measure in mm from the lower edge of the well to the center of the spot. Write your data on your record sheet.

BACKGROUND INFORMATION: AGAROSE GEL ELECTROPHORESIS*

Agarose gel electrophoresis is a powerful and widely used analytical method that separates molecules on the basis of charge, size and, shape. The ionic strength, viscosity, and temperature of the medium also affect the mobility or migration rate of molecules in the electric field. The method is particularly useful in separating charged biomolecules such as DNA, RNA, and proteins.

Agarose gel electrophoresis possesses great resolving power, yet is relatively simple and straightforward to perform. The gel is made by dissolving agarose powder in a buffer solution by boiling the solution. The solution is then cooled and poured into a mold where it solidifies. The gel is then submerged in a buffer-filled chamber which contains electrodes.

Samples are prepared for electrophoresis by mixing them with a dense solution such as glycerol or Ficoll™. This makes the samples denser than the electrophoresis buffer. These samples can then be loaded with a micropipet or transfer pipet into the wells that were created in the gel by a template called a comb. The dense samples sink through the buffer and settle in the wells.

A direct current power supply is connected to the electrophoresis apparatus and current applied. The buffer in the gel chamber completes an electric circuit between the electrodes. Charged molecules in the sample enter the porous gel through the walls of the wells. Molecules having a net negative charge (anions) migrate toward the + electrode (anode, red) while positively charged molecules (cations) migrate towards the negative electrode (cathode, black). The higher the applied voltage, the faster the molecules travel. The buffer salts serve to make the water a better conductor of electricity and to control the pH. The pH is important to the charge and stability of many types of molecules.

Agarose is a polysaccharide derivative of purified agar. The agarose gel contains microscopic pores which act as molecular sieves. The sieving properties of the gel influence the rate at which molecules migrate. Smaller molecules move through the pores more easily than larger ones. Molecules can have the same molecular weight and charge but different shapes. Molecules having a more compact shape (sphere is more compact than rod) can move more easily through the pores. Given two molecules of the same molecular weight and shape, the one with the greater charge will migrate faster. The factors of charge, size and shape interact with one another to various extents depending on the molecule's structure and composition, buffer conditions, gel concentrations and voltage.

*[From **Edvotec Lab #101**, with permission]

STUFF TO KNOW BEFORE YOU GO

1. On what basis does electrophoresis separate molecules?

2. What are three types of charged biomolecules that electrophoresis is particularly useful in separating?

3. What is the gel made of?

4. In what is the gel submerged?

5. What substance is used to make the samples denser?

6. Why do the samples need to be made more dense than the buffer?

7. What is the purpose of the buffer in the gel chamber?

8. Which way will negatively charged molecules migrate?

9. What is the purpose of the buffer salts?

10. Why is it important for the buffer to maintain pH?

11. What characteristic of agarose makes it useful for electrophoresis?

12. What happens to molecules having the same charge and weight when run through an agarose gel?

13. List variables that influence the way the charge, weight and shape of molecules interact with each other during electrophoresis.

14. DNA in solution is negatively charged; if you ran a sample of DNA, what would you predict about its migration?

HOW TO MAKE A GEL
1.0% agarose for Dye/Indicator Gels

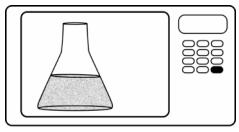
Wear goggles and hot gloves when handling hot agarose*

Materials:

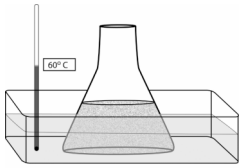
centigram balance
weigh boats or paper
bottle or flask, 3X volume of gel solution
graduated cylinder for agarose solution
hot gloves and goggles
microwave oven or hot plate
gel electrophoresis box & power supply
150 ml plastic beaker to hold buffer
agarose powder
1X TAE buffer

Consult chart on back for amounts of agarose and buffer to use, and for useful hints

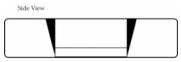
1. **Add:** ____ grams agarose to ____ milliliters buffer in large Erlenmeyer flask or bottle. (Lid **MUST** be loose before heating!)



2. **Heat:** Until all particles are dissolved, ~30 sec to 1 min <u>after</u> solution boils. Mix by swirling flask or bottle several times during heating.

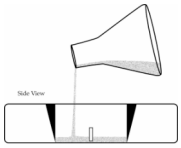


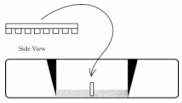
3. **To Cool or Store:** Keep flask in a 60°C water bath**.**

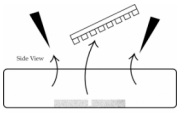


4. **Set up:** Place dams in gel box at each end of gel tray

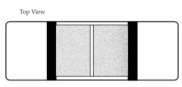
5. **Pour:** 25 ml of agarose into the gel tray. Insert comb in the center position for the Dye/Indicator lab.

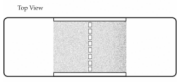






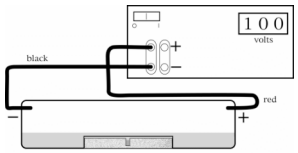
6. **Cool:** Let gel harden 10 min. Pour: some buffer over top of gel. Remove comb & dams gently.





7. If you choose to **store the gel before running:** Write your name on an acetate sheet and slide it under the gel. Store them in a Tupperware container or Ziploc bag with buffer covering all of the gels.

8. **Run:** Add 125 ml 1X buffer, load 15 µl samples, and run the gel at 100 volts.



MAKING GELS

Make the agarose solution

1. Wear goggles. Obtain a bottle with a **loosened cap** or an Erlenmeyer flask. The container's volume should be about 3X the volume of the solution to prevent boiling over.
2. Use Table 1 or 2 to calculate the amount of agarose and buffer you will need. Add the agarose powder to the buffer and mix.
3. Note: Wear hot gloves. Agarose will boil over quite easily! **Beware of steaming hot agarose!**
4. To dissolve the agarose, heat the mixture to boiling in microwave or on a hot plate for ~30 seconds to 1 min after the mixture begins to boil. Swirl the bottle occasionally as it heats.
5. Swirl the bottle to see if agarose is dissolved. If any clear floating particles are visible, heat it for another 30 seconds and check again.
6. Place the hot container in a 60°C water bath or oven to hold the melted agarose at the right temperature for pouring gels throughout the day.

Table 1
1% agarose gel for Dye/Indicator Lab
Place comb in center.

Number of gels (25 ml each)	agarose	1X TAE
2	0.50 g	50 ml
4	1.0 g	100 ml
10	2.5 g	250 ml

Table 2
0.7% agarose gel for DNA Labs
Place comb at negative (black) end.

Number of gels (25 ml each)	agarose	1X TAE
2	0.35 g	50 ml
4	0.70 g	100 ml
10	1.75 g	250 ml

Pour the gel

7. You can measure 25 ml or just fill the tray, with dams in, to the edge with agarose. You do **not** have to cool the agarose to pour the gel in the Horizon 58 gel boxes. They can take the heat. You do, however, need to pour the agarose *slowly* so that it does not leak under the dams.

Store the gel

8. The gel can be covered in plastic wrap, placed in a storage bag, or left in the staining tray covered in plastic wrap and stored in the refrigerator. Add a small volume of 1X TAE buffer to prevent the gel from drying out.

RECORD SHEET FOR GEL ELECTROPHORESIS

Sample #	Name of the Dye or pH Indicator	Distance traveled (in mm)	To which pole +/-	Comments & Observations
1				
2				
3				
4				
5				
6				
7				
8				

pH DATA	Black end (-)	Red end (+)
pH before gel run		
pH after gel run		

1. Record observations of the gel after 5 minutes of electrophoresis.

2. Draw an accurate illustration of your gel results on the sheet provided.

3. What dyes/indicators are in sample XXX? What evidence do you have to support your answer?

4. What dyes/indicators are in sample ZZZ? What evidence do you have to support your answer?

5. By what properties does gel electrophoresis separate molecules?

6. If you were asked to improve the separation of these molecules, what are some of the variables you could modify in your own experiments?

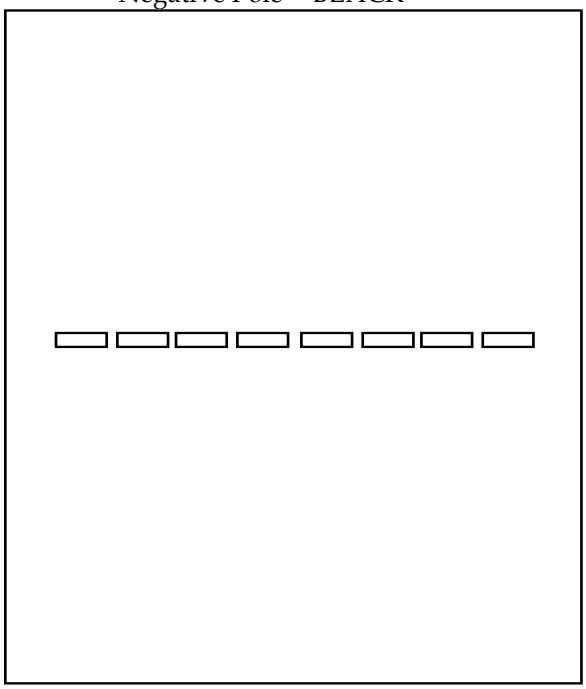
7. Write two questions you have about this process and the results. Indicate how you might generate answers to these questions through experimentation.

THE DYE/INDICATOR LAB

Gel Diagrams

Before running gel

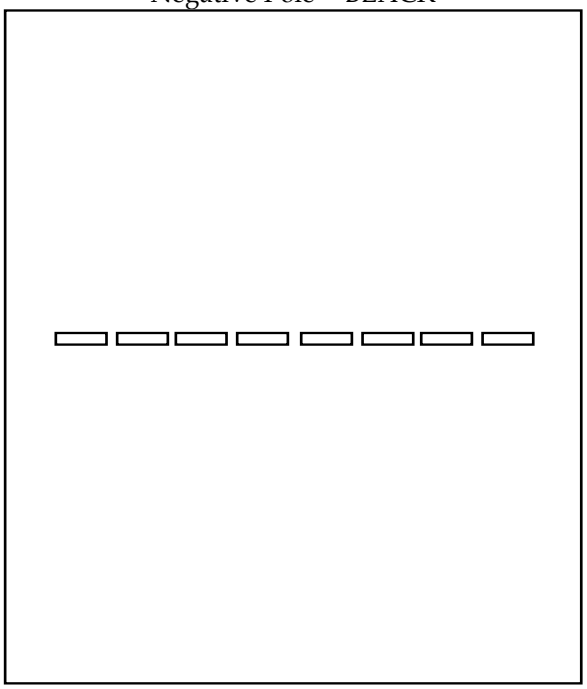
Negative Pole = BLACK



Positive Pole = RED

After running gel

Negative Pole = BLACK



Positive Pole = RED

STEP 1
- prepare gel
- add 125 ml buffer

- load 15 μl into each well
- record the sample names above

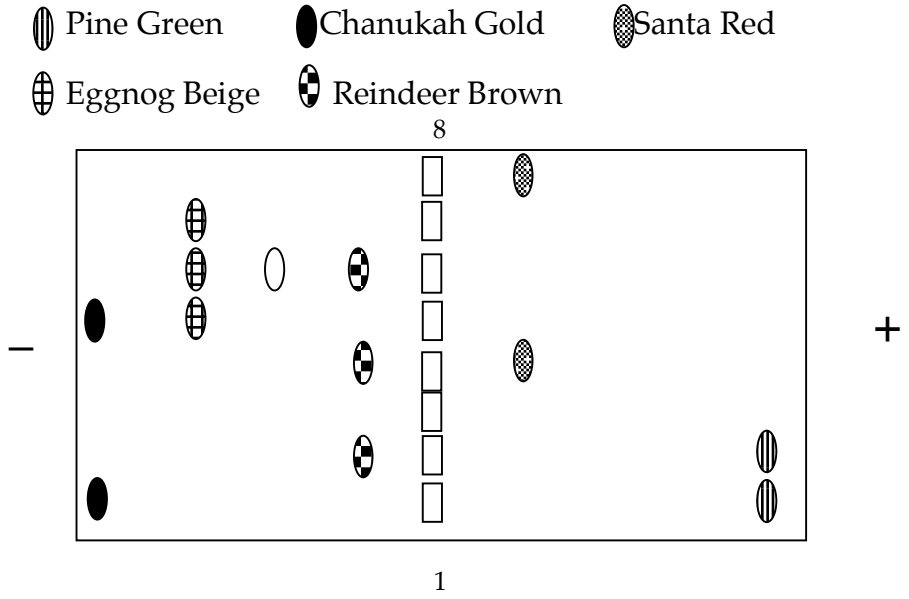
STEP 2
- run gel 10 min at 100 V
- record pH
- make a tracing on acetate sheet
- make a blot onto paper
- measure distances in mm
- record bands above

STEP 3
- tape acetate and blot in lab notebook

**CLEAN UP

ELECTROPHORESIS TEST

Below is a picture of a gel. The lanes are labeled 1-8. A different sample was loaded into each lane. Use the key to identify the dyes.



1. Which of the lanes contain mixtures of molecules? (Write the number(s) of the correct answers.)

2. Which of the dyes/indicators is the smallest, negatively charged molecule?

3. What are the possible reasons that reindeer brown runs the way that it does?

4. Which of the dyes/indicators is the largest positively charged molecule?

5. Which of the lanes contains an unknown dye/indicator? What can you tell about this unknown?

6. Order the negatively charged molecules from largest to smallest.

Largest _____ Smallest

7. Order the positively charged molecules from largest to smallest.

Largest _____ Smallest

TO DYE FOR: A CATALOG EXPERIENCE*

Objectives:

- Students will learn more about the chemistry of the dyes & pH indicators.
- Students will gain experience in analyzing information presented in a chemical catalog.

Use the pages from the Kodak Laboratory Chemical Catalog No. 54, December 1990* to answer the following questions.

1. What does the hazard rating for phenol red say?

2. What does CAS# refer to?

3. Which dye/indicator has the lowest molecular weight?

4. Which of these dyes/indicators do not have a sulfur atom in them?

5. Which dye/indicator has a structure most like phenol red?

6. Which dye/indicator is most similar in structure to crystal violet?

7. One of these dyes/indicators is available for research and development only. Suggest a possible reason using the information given.

8. Which of these dyes/indicators can change color with changes in pH? These are called acid-base indicators.

9. What is the difference between the two methylene blue dyes listed?

   What is the advantage of methylene blue (cert)?

   What is the advantage of methylene blue (pract)?

10. Which of the structural diagrams indicate that the dye/indicator has charged atoms?