TEACHER'S GUIDE
THE DYE/INDICATOR LAB
SEPARATION OF MOLECULES USING AGAROSE GEL ELECTROPHORESIS

Overview

1. This lab was written for SEP participants using the Gel Electrophoresis Kit and supplies.

2. Please read through this guide and the lab before you get started.

3. This lab includes colored samples; some are biological dyes and others are pH indicators. Dyes are able to bind to other molecules to change their colors (for example, indigo dye gives jeans their color). Indicators undergo a color change depending on the pH of the solution. The attached chemical descriptions tell you which samples are dyes and which are pH indicators.

4. This lab is a straightforward and visual way to demonstrate the concepts of agarose gel electrophoresis. You can design lab extensions and have students test several samples, mixtures of samples, and you can encourage students to test samples that are not included in this kit.

5. Please, go through the kits and equipment to make certain that you have everything that is included in this kit. You may need to make some solutions and aliquot other solutions for classroom use. Further, some critical items are not supplied in the kit; **e.g. microwave oven or hot plate, balances, and distilled or deionized water for diluting the 50X TAE buffer. You will need to use your own resources for these items.**

Concepts and Objectives

The main concept of this lab is that complex mixtures of molecules can be separated by electric charge, size and shape using gel electrophoresis.

Objectives: Upon completion of this laboratory series students will be able to do the following activities.

- Accurately use a micropipet and measure volumes in microliter units.
- Cast an agarose gel of correct agarose percentage.
- Load an agarose gel with specific amounts of material in the wells.
- Analyze dye/indicator samples by gel electrophoresis and interpret the results.
- Identify and manipulate variables involved in separating biological molecules.
- Explain the theory behind molecular separation of molecules by electrophoresis.

Lab Equipment Tips and Notes

- The electrophoresis gel box lids crack easily and they are expensive to replace. Please encourage your students to be careful when using and cleaning the boxes. Store and repack carefully. When repacking, don't put anything on top of the gel boxes and make sure they are not crushed by the green crates' lids.

- For more information about aqueous solutions and agarose gel electrophoresis, please refer to the DNA Lab section of the Gel Kit Notebook.

- We have provided pH paper to use in some of the gel box labs. This paper is very expensive. Please use the pH paper for the Electrophoresis Exploration Lab and/or once in either the Dye/Indicator or DNA Labs. It is not useful to measure the pH in every lab performed.

- For best results in pouring gels, let the hot agarose solution cool until it is hot, yet comfortable enough to hold the container. Students can either measure out 25 ml to pour, or pour slowly and directly in between the dams to the height of the gel deck—which is 25 ml.

- The kit contains enough precut acetate sheets for each student in each class for which you requested supplies. If you need more, a template and additional full size sheets are included for you or the students to cut.

- The kit contains equipment for making practice gels. Use the combs from the gel boxes and make comb holders from the two pencils held together with rubber bands. Use the square plastic tissue culture dishes and place the comb holders over the square tissue culture dish so that the comb sits about 2 mm off the bottom. Pour about 20 ml of **cooled** agarose into the dish to form wells for practice loading. (This plastic isn't as strong as the gel box plastic; make certain the agarose is cool or the dish will warp.)

- If you have difficulty with any of the equipment, please refer to the equipment manuals in the notebook, or call us at SEP 206.667.4487. We're happy to help!

Classroom Management

- In order for this lab to run smoothly, predict and prevent any bottlenecks that may occur. If space allows, set up a few stations for weighing agarose, pouring buffer, and making solutions. Agarose gel solutions can be made days ahead of the actual lab day. Have your students label their solutions and put them aside at room temperature.

- **SEP supplies you with stocks of all of the reagents you need; however, you need to aliquot these stocks for your classroom use**. Aliquot dyes/indicators so that students can obtain what they need for their lab group. Depending on your class size, you can prevent bottlenecks by having these items accessible to as many groups who need them.

- Make practice gels a few days ahead of the lab. Review basic micropipetting skills and have students practice loading gels ahead of time.

- ❑ Before the lab day, have students read and flowchart the lab. While gels are running, model how to trace the gel and make the blot. Explain that students will need to be careful with the gels and that each student in a group will need a trace and a blot to measure and record data.

- ❑ You can save raw data for future reference by having groups wrap their gels in plastic wrap and store in the freezer.

Suggested Sequence and Schedule

This lab activity will take three class periods (assuming earlier practice with micropipets), including time for students to manipulate variables with their own experiments. Teacher preparation time ahead of class, 1-2 hours. If the teacher prepares practice gels (recommended), add an additional hour.

For 50-minute class periods, this is a suggested sequence:

- **Day 1.** Students will make the agarose solution, pour a gel in the gel casting tray, practice pipetting, and prepare samples.

- **Day 2.** Students will set up the gel box, add buffer, load the wells, run the electrophoresis, draw a transparency of the results directly off the gel, and make a blot onto filter paper to record their results.

- **Day 3.** Students will determine what knowledge has been generated with the experiment and answer analysis questions. To make the lab activity more open ended and as a part of assessment, have the students generate their own questions and design an experiment. For example, students could design an experiment to test either another set of substances or test some of the variables used in the electrophoresis experiment.

Extensions

You could have your student groups prepare a simple mix of dyes/indicators and give it to another group to determine the components. Note that in most cases, when negative- and positive-charged molecules are mixed, salt precipitates are formed rapidly. Mixes may have to be all negatively or positively charged dyes/indicators.

Things to try: mercurochrome, food colors, inks, Easter egg dyes, tie dyes, flower extracts, cabbage juice, beet juice, berry juice, lily pollen extract, iodine... use your imagination.

The pre-test “Stuff to Know Before You Go”, “To Dye For: A Catalog Experience” worksheet and post-assessment “Electrophoresis Test” are included for your use.

Stock Solutions and Recipes for Solutions in the Kit. (THESE ARE ALL SUPPLIED IN THE KIT---THE RECIPES ARE FOR YOUR INFORMATION.)

1X TAE: also known as Tris Acetate, gel buffer, electrophoresis buffer, running buffer. **The buffer may be reused several times if mixed well between runs.**

Prepare from 50X TAE stock by diluting 40ml of 50X stock with water to a final volume of 2 liters. The gel boxes each use about 125ml of 1X buffer. (125ml X 8 gel boxes = 1 liter).

Recipe for 50X TAE:
242g Tris base
57.1ml glacial acetic acid
100ml 0.5M EDTA, pH 8.0 (this stock is made from disodium EDTA)
make volume to 1 liter with deionized or distilled H_20.

Dyes/Indicators:
Most of the samples are supplied as 0.25% solutions in water plus a tenth volume of glycerol to make the solution dense for easy loading in the wells of the gel. As dry powders, pH indicators and these dyes require careful handling-both to avoid a colorful mess and to avoid inhaling any of the powders. If you prepare your own solutions, you should wear gloves and place your balance in a ventilating fume hood for weighing, mixing, and dissolving the powders.

DYE/pH INDICATOR LIST

D=dye; I=indicator	Charge (at this pH)	Travels to which pole
bromocresol green (I)	–	+ (red pole, anode)
bromocresol purple (I)	–	+
bromophenol blue (I)	–	+
crystal violet = gentian violet (D)	+	– (black pole, cathode)
eosin Y (D)	–	+
m-cresol purple (I)	–	+
methyl green (D)	+	–
methylene blue (D)	+	–
o-cresol red (I)	–	+
orange G (D)	–	+
phenol red (I)	–	+
safranin O (D)	+	–
xylene cyanol (D)	–	+
(13 total)		

Mixture **XXX:** xylene cyanol & methylene blue (with long storage, this one precipitates so mix fresh for each day's use.)
Mixture **ZZZ:** orange G, xylene cyanol, & bromophenol blue

References

DNA SCIENCE: A First Course in Recombinant DNA Technology, 1990, Dave Micklos & Greg Freyor, Cold Spring Harbor Press.

Gene Connection: San Mateo County Biotechnology Curriculum, 1994. (No longer available-out of print.)

Recombinant DNA and Biotechnology:A Guide for Teachers, 1996, Kreuzer&Massey,ASM Press