FRED HUTCHINSON CANCER RESEARCH CENTER
SCIENCE EDUCATION PARTNERSHIP

TEACHER'S GUIDE
ELECTROPHORESIS EXPLORATION

PURPOSE

To introduce students to the gel electrophoresis equipment and the principles of electrophoresis. The procedure was modified especially for use with the SEP Gel Electrophoresis kit equipment. Possible expanded purposes:

- nature of electrical flow: current, voltage, resistance, Ohm's Law
- chemical half-reactions
- pH
- buffer activity

This is an engaging activity designed as an inquiry lab for students to learn about electrophoresis. Allow your students to explore the concepts of conductivity and chemistry by instructing them to follow the procedure as written. Students should use the Record Sheet to fill in information as they work through the procedures. Refrain from giving your students too much information so that they may explore on their own.

DURATION

One 50-minute period.

SEQUENCE OF LABS

This version was written as the students' INTRODUCTION to the electrophoresis equipment as well as to help them understand how it works. You can teach this exploration AFTER the students have worked through the dye/indicator electrophoresis lab, to give students background about the principles of electrophoresis. However, there are some things that will seem out of sequence if you use it after students have already met and used the gel box.

PREPARATION BEFORE LAB

1. Please read through this guide and the lab before you get started.
2. The procedure assumes you have the SEP Gel electrophoresis kit. You will need to supply the following items which are not included in the kit:
   - ❑ red pens, 8
   - ❑ overheads of Student Record Sheet pages 1 & 2
   - ❑ distilled water, about 2,000 ml/class
3. Dilute the 50X TAE in the kit to 1X TAE.
   20 ml 50X TAE + 980 ml distilled water = 1000 ml 1X TAE

4. Set out and/or aliquot the following (amounts are for 1 class, 8 groups/class):
   - ❑ distilled water,~1000 ml
   - ❑ 5M NaCl, ~3 ml
   - ❑ 1X TAE, 1000 ml
   - ❑ phenol red solution (saturated) 16 ml
   - ❑ pH paper, ~50 strips
   - ❑ gel boxes, 8 boxes
   - ❑ power supplies, 4 supplies
   - ❑ 150 ml beakers, 8 beakers
   - ❑ P1000 micropipets and tips, 4 sets
   - ❑ plastic transfer pipets (to measure phenol red solution), 8 pipets
   - ❑ plastic transfer pipets (to use as stirrers), 8 stirrers
   - ❑ protocol cards (*Gel Box/Power Supply)*, 8 cards
   - ❑ containers for waste, 8
   - ❑ red pens, 8
   - ❑ black pens, 8

WHAT TO DEMONSTRATE/EXPLAIN

- This lab embodies discovery learning, so resist the urge to say too much at the outset!

- Identify the parts of an electrophoresis apparatus as students label their Electrophoresis Exploration Record Sheet. Discuss relevant safety concerns. Have students refer to the blue protocol card, which has the labeled diagrams of the power supply and gel boxes.

- To demonstrate the properties of phenol red, you can set two Petri dishes of phenol red solution on the overhead projector. Squeeze some lemon juice into one and put a couple drops of ammonia in the other to illustrate the pH induced color change.

TECH TIPS

- In Step 11, 12 and 15, be sure students **stir** their gel box solution **thoroughly**. This means at least 10-12 complete stirs. Stirring with the end of a plastic pipet is not as effective as stirring with a scoop or more "spoon-like" object. It may be helpful if you do step 12 (mixing the phenol red indicator into the salt solution) for yourself ahead of time, to see how much mixing is required. It takes a lot! Removing the gel casting deck out of the gel box helps, but keep close track of these essential and expensive (over $30 each) parts.

- If well mixed after Step 12, Step 13 will show a dramatic color change.
  At the black cathode, (OH^- produced, basic pH) the phenol red turns deep pink.
  At the red anode, (H^+ produced, acidic pH) the phenol red turns yellow.

- Although lab calls for distilled water, deionized water works too.

- If a fuse should go out on a power supply, replace it with a new one. It's as easy as changing the batteries in a flashlight. The fuse (1 amp SloBlo) is at the back of the power supply. Extra fuses are in the "small box" in crate #3.

- After Step 9, the chemical reactions (half-reactions) for the electrolysis of water are given. This is to explain what is happening as a result of running electric current through water. You can use these equations to explain the bubbles and pH at each electrode.

- Using buffer stabilizes the pH, and (at Step 16) you will not see the dramatic color changes witnessed at Step 13. Eventually, however, changes in color *do* develop if you let the box run for 5-10 minutes because the capacity of the buffer is exceeded. You might experiment with buffer capacity by having some teams add more or less buffer to their boxes.

- Students often have a problem graphing their results from steps 8 – 10. The graph in their handout does not have quantities on the x-axis. It may be necessary to help your students plan what to put along the x-axis.

- This kit is designed for a class divided into eight groups. **SEP supplies you with stocks of all the reagents you need; however you will need to aliquot these for your classroom use.** Remember to arrange your students so that two groups can comfortably share one power supply (there are eight gel boxes and four power supplies per kit).

- For more information on the aqueous solutions used in electrophoresis, refer to the Electrophoresis Reagents document in the DNA Lab section of the Gel Kit Notebook.

BACKGROUND

Ionic solutions and buffers

Electrophoresis requires a conducting solution to work.
The solution can be

- **an ionic** solution, such as sodium chloride solution, or a
- **a buffer**, an ionic solution that both conducts electricity and maintains a specific pH. Buffer solutions are used in molecular biology applications because the wrong pH can have a strong effect on the shape and activity of molecules. (DNA can be denatured, proteins can unfold, etc.) Buffers are selected to maintain a good pH environment for the molecules you are studying or using.

Phenol Red pH indicator

Phenol red transition interval: pH 6.8 yellow to 8.2 red. The initial color observed in your class will depend upon the pH of your water source.

DC Circuits and Ohm's Law

Georg Simon Ohm (1787-1854), a German physicist, discovered that the ratio of the voltage to the current in a closed circuit is a constant. This constant is the resistance of the circuit. This is known as Ohm's Law of resistance. Voltage= (current) (resistance)
or
$V=I*R$ where
V= potential in volts DC
I= current in amperes
R= resistance in ohms

The data you get from the Electrophoresis Exploration allow you and your students to derive and prove Ohm's Law for yourselves. Use the data and graph to illustrate the direct, linear relationship between voltage and current (given the same resistance). See the note above in Tech Tips about student's graphs.

Current change and two gel boxes

SEP power supplies provide constant voltage at whatever setting you have chosen (usually 100 V in our protocols). What varies as you add gel boxes (assuming they contain a conducting ionic solution) is the current provided by the power supply.

If you have one gel box hooked up to the power supply, with resistance R, voltage of 100, and (say) 44 milliamps:
100=0.44(R) or R =100/0.044= 2273 ohms.

But, if you have two identical gel boxes (therefore two resistances) hooked up in parallel, the total resistance (R_T) seen by the power supply becomes 0.5R when the following formula is applied: $R_T = 1/(1/ R_1 + 1/ R_2)$.

100= I(0.5)(2273)
or 100=I(1136)= 0.088 Amps
which is 88 milliamps total. The current supplied per gel box is still 44 milliamps.

REFERENCES

Gene Connection v1.5: 1994 San Mateo County Biotechnology Curriculum (No longer available-out of print.)

How Things Work: The Physics of Everyday Life, 1997, Louis A. Bloomfield, John Wiley & Sons, Inc. Chapter 11

Basic Laboratory Methods for Biotechnology, ,2000 Lisa A. Seidman & Cynthia J. Moore, Prentice-Hall, Inc. Pages 261-265