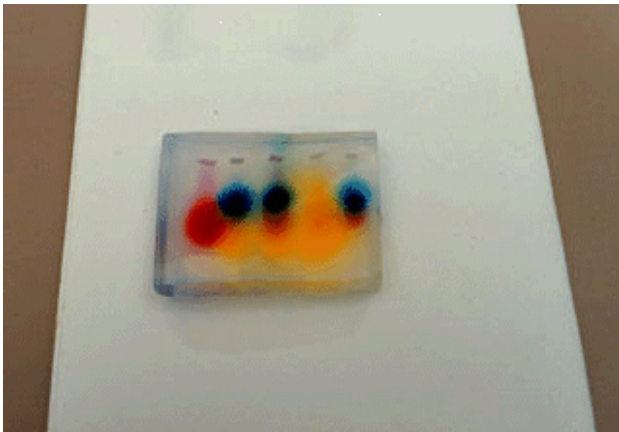




Developing Troubleshooting Skills: ImProvElectrophoresis

A Step-by-Step Guide with Color Illustrations



Gel electrophoresis is a key technique in molecular biology. It uses an electric field to move charged molecules through a gel. Researchers use electrophoresis to detect a compound, to separate one compound from a mixture, to characterize a mixture, and to purify a compound. The compounds most commonly used in electrophoresis are proteins and nucleic acids.

The most common gelling agents are agarose for nucleic acids and polyacrylamide for proteins.

ImProvElectrophoresis

DNA electrophoresis is exciting but expensive. Starting with this version has several advantages. It's cheaper because you make your own rig and you use a small gel piece and you race food dyes instead of DNA samples. It's faster: the electrophoresis takes only 15 minutes to get an answer, and no staining and destaining is needed. It's smaller, so you can do it with less space, less mess and less cost. Overall, these advantages mean your students can practice and learn from their mistakes and try their own variations.

Characterizing a compound: The Power of Electrophoresis

Here's a puzzle: is green food coloring dye really green?

What else could it be?

Could it be a mixture of blue and yellow?

Science Savvy: we start with an observation about a dye, ask a question, inject some doubt, generate some alternatives.

Now we get to design ways to discern by testing experimentally the two competing ideas.

Uff, Da Thinking

Uff, Da can stand for "If, Then." If, Then thinking can be amazingly powerful.

Let's imagine a 100 meter dash. During this race, 8 racers in 8 lanes are separated by their speed. All racers start at the same starting line at the same time, but during the race down the track they separate on the basis of their footspeed.

Electrophoresis is like an electric 100 meter dash. But instead of 1 person running in each lane, think of 10,000 molecules running in each lane. Instead of running on a track, electrophoresis races are run in agarose gels. And instead of being driven by legs, the dyes are driven by electricity.

In this analogy, if the green dye really is a true green, then we would expect there to be a whole bunch of green molecules, and in an "electric race" we'd expect all of them to run the same speed, all in one group.

But if the green dye is really a mix of blue dye and of yellow dye, and if the blue dye molecules runs slower than the yellow dye, then we'd expect the green to separate into two groups, one yellow and one blue, during an electric race.

Should the green dye be the only runner? Designing Appropriate Controls.

Let's imagine that green is the only runner, and when we run the electrophoresis, it moves as a single band. A skeptical student could ask: how do you know the electrophoresis is even capable of separating the yellow and blue dyes? Maybe the two dyes move at the same speed under the conditions we're using. Maybe the two dyes when mixed together and exposed to electric current chemically react to form a single compound.

To address these possibilities, let's run some yellow dye, some blue dye, and some yellow mixed with blue (it looks green). By thinking ahead, by welcoming skepticism, you can design a better experiment. By running a yellow sample next to blue sample, we can test if they move at different speeds or at about the same speed. By running the mixed or "reconstructed" sample of yellow and blue, we can test if electrophoresis can separate yellow and blue, even after they've been mixed together and subjected to electricity.

So we have a logic fork: If the green dye is a true green molecule, then we would expect it to "run" as one green band. On the other hand, if the green dye is formed by a mix of blue dyes and yellow dyes, and if the two dyes move at different speeds during the "electric race," then we expect the green to separate into at least two bands, one yellow and the other blue.

Objectives:

Develop troubleshooting skills by planning, assembling and using your own electrophoresis equipment.
Grasp the importance including appropriate controls when running an assay.

Demonstrate the separation of components of a mixture;

Demonstrate the use of "reconstruction" experiments;

Introduce electrochemistry and electricity, molarity and buffers.

Cost: Under \$30 for the gel rig, agar and dyes.

Items:

Set of food colors; this activity is designed for a set with green, yellow and blue(\$2)

Rubbermaid-type small container with snap-on lid (\$2)(4" x 6", 10cm x 15 cm)

AC/DC converter, 120 V AC in, 12 V 300mAmps DC out (\$10)

agarose or agar (science supply house) or agar-agar (buy at health food store)

Powders to make the electrolyte/buffer: Tris base (science supply house) and boric acid. (0.05M NaCl or phosphate buffer are inferior substitutes as electrolyte.)

forceps or tweezers or spring-loaded hairclips

paring knife or jackknife

filter paper (3 MM or other--needs to absorb the food colors)

paper binder clips made of metal



Note: there are many technical challenges in doing this the first time. If your students enjoy such challenges, give them a chance to know the problem and suggest and test their own solutions. You may want to make this a week-long project. This gives the students time to practice, to test their ideas, to learn from their mistakes, to gain confidence, to achieve mastery.

Overview:

- 1 Chart out the question, the competing hypotheses, and their predictions.
- 2 Make the electrolyte/buffer.
- 3 Make the agarose gel.
- 4 Put the electrophoresis rig together.
- 5 Prepare the green sample, yellow sample, blue sample, and yellow plus blue mixed sample .
- 6 Load the samples into the gel
- 7 Race the dyes. Expect about 15 minutes to get an answer.
- 8 Ask and observe: Did the green move as a single green band?
Or did the green gradually separate into a yellow band and a blue band?

Make the Electrolyte/Buffer.

I have used a Tris-borate buffer (TB). This is what researchers commonly use. It requires Tris base (a powder from a chemical supply company), boric acid from a pharmacy or discount store.

The 10X TB stock solution is made by putting

54 g Tris base and

27.5 g boric acid

in water, dissolving it, and

bringing the volume to 1.0L.

NOTE: Dilute this 1:10 for use both in making the gel and as the running buffer. For example, 100 ml 10X TB plus 900 ml water to make 1000 ml 1X TB.

Alternate: 0.05M sodium chloride solution. Put 58 g of salt (NaCl) into 500 ml of water and make the volume to 1 liter. The concentration is 1 Molar. Dilute this stock solution 1:20 in tap water to get 0.05 Molar; use the 0.05 M to make gels and for the electrolyte.

Make the Gel.

Try 1% agar in 1X TB.

Place 1g agarose in a 250 ml erlenmeyer flask, add 100 ml 1X TB, and heat to near boiling.

Problem: how to heat the solution?

A microwave oven works well for heating such liquids.

A hot plate is an alternative.

Cool the hot gel solution to about 55C.

Pour it into the plastic container to make a layer about 4-5mm deep.

Let the gel cool and harden.

The gel can be saved for many weeks as long as you don't let it dry out.

Alternative to agarose:

Agar as the type used to make agar plates, or agar-agar from a health-food store can be used at 1.0-1.3%.



Make the Gel Rig.

Make sure the adapter is not plugged in.

The AC/DC adapter will have several different adapters at the end of the wire. With a heavy-duty scissors, cut off the adapters, leaving as much of the connecting wires as possible.

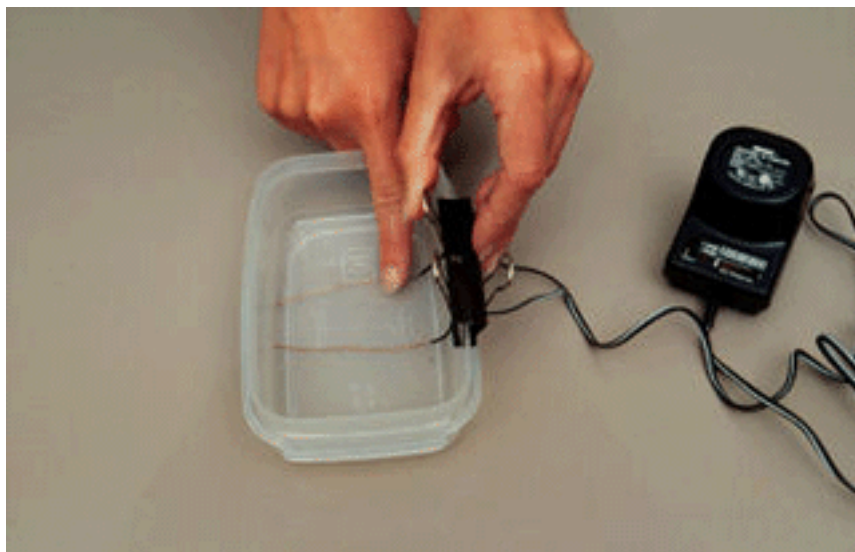
Separate the two wires for about 15cm. This will make a kind of wire fork at the end.

Strip the insulation off the end of each wire, exposing about 8 cm of wire.

Use the metal binding clips to attach the insulated portion of the wire to the side wall of a second plastic vessel.

Leave the exposed 8 cm on the floor of the vessel.

The distance between the wires is adjustable. Later when you've cut your large gel into smaller pieces, you can adjust the distance between the wires so that it is just longer than the length of the piece of gel.



Putting the Electrolyte/Buffer in the Rig.

Make sure the adapter is not plugged in.

Pour 50-100 ml 1X TB in the plastic vessel.

Make sure the two bare wires (electrodes) are immersed.

Make sure the two bare wires are not touching each other.

Plug in the adapter and test if current is running through the solution between the electrodes.

What happens at the electrodes?

If the wires are copper, then expect bubbles to form at one wire, and a blue precipitate to form at the other.

UNPLUG the adapter.

Now we have a gel, an electrolyte/buffer and a gel rig.

How do we load the dye in the gel?

There are many possible solutions to the loading problem. You may want to let your students generate and test their own solutions.



This is the simplest way I know of to load the dyes.

Cut filter paper into long strips 3 mm wide.

Dip a filter paper strip into the green dye.

Let the dye saturate the lower part of the strip.

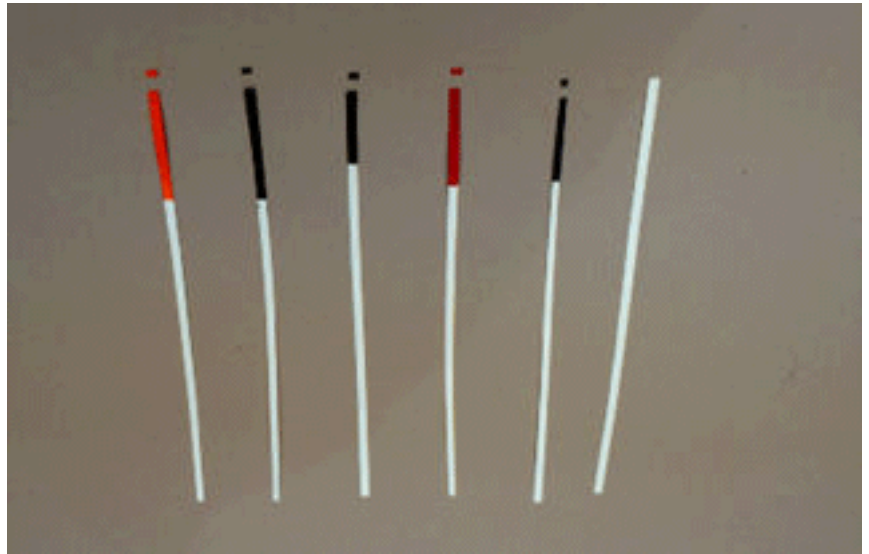
Hang the strip and let the dye dry for 10 minutes or so.

Cut the strip into small rectangles of the same size, about 3x3 mm.

Repeat with clean strips, one for each of the three remaining samples (yellow, blue, and yellow and blue mixed to make a "reconstructed" green). These 3x3mm pieces can be saved indefinitely.

Problem: what is the precision of each of these steps?

Can the precision be estimated?



Cutting the Gel.

The cooled gel covers to a depth of about 5 mm the bottom of the first plastic vessel.

How can you use the gel? One way is to consider it one big gel.

Another option is to cut it like brownies into many smaller gels.

With a paring knife, cut the gel once down the middle along its long axis.

Then cut four times crosswise to get 10 gel pieces.

Use the knife to lift the first piece out.

Let the students practice handling the gel pieces. Expect these pieces not to survive the handling. The pieces make good practice pieces for loading.



Loading the Gel.

Put one of the gel pieces on a desktop.

With a knife make a slit in the gel about 7 mm from one edge.

With a tweezers place the dyed filter paper rectangle in the slit.

Make similar slits for the other samples: yellow alone, blue alone, and yellow and blue mixed (it will look green).

When completely loaded, the gel should have four "racers" at the starting line.

Running the Gel.

Make sure the AC/DC adaptor is turned off.

1. Place the gel piece containing the dyed paper pieces in the gel rig.
2. Use the metal binder clips to adjust the wires/electrodes so that the wires are just barely wide enough apart so the gel piece can fit between them.
3. Add enough electrolyte/buffer to cover the gel.

(Note: use the same solution the gel is made of)

4. Cover the gel rig with clear plastic wrap so the students can see the dyes move but can't put their fingers in the electrolyte.

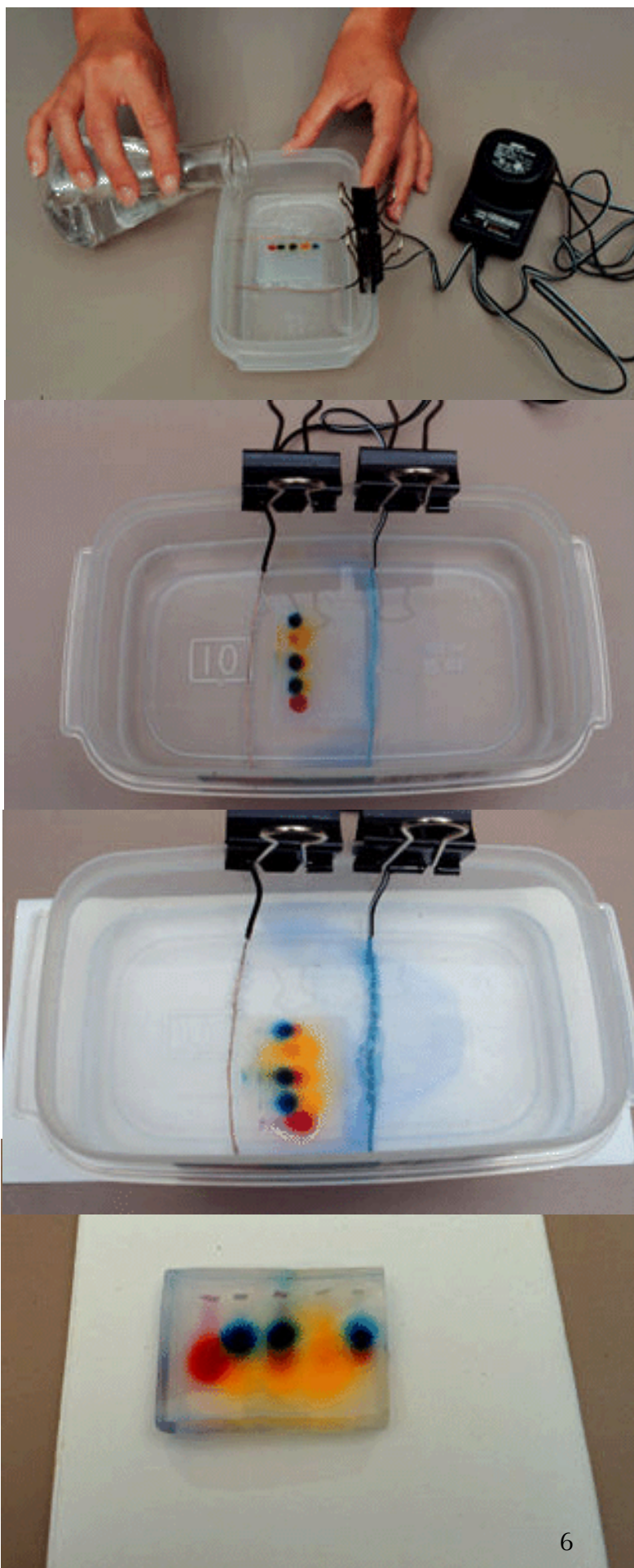
5. Plug in the AC/DC adapter.

6. Again observe the electrodes. Bubbles should form at one, and a blue precipitate at the other (if you're using copper electrodes.)

7. Which way will the dyes move?

8. In my experience, using an adaptor with a maximum rating of 12 Volts and 300 Amps DC, the dyes move perceptibly within 10 minutes.

9. Let them run until the colors are separated sufficiently to tell whether the green is a true green or a mixture of blue and yellow. The run time depends on the power supply and electrolyte/buffer you use.



Teaching Analogy:

Teach as a dye race. Imagine you're in a blimp over the Olympic track stadium, and below the 100 meter dash is just about to begin. But instead of only one person per lane, imagine 100 people in a lane. In lane one there are 100 people who all run the same speed and who all have on blue shirts; 100 other people in yellow shirts are in the second lane who run twice as fast as the blue-shirts; and 50 people in blue shirts and 50 people in yellow shirts are in the third lane; and 100 green-shirted people in the fourth lane who all run the same speed. The race is a sprint, and you take a snapshot when the first runners cross the finish line. What does the picture look like?

Troubleshooting Things That Can Go Wrong Along the Way

Most problems are traced to these components:

- the buffer
- the AC adaptor
- the arrangement of the dyes samples relative to the electrodes.

1. Bubbles don't form in the electrolyte/buffer at either electrode. Is the AC/DC adapter plugged in? Is the outlet working? Does the electrolyte have the correct amount of salts/buffers called for? Has the adaptor been "shorted out" as a result of the two bare wires touching each other while the adaptor is plugged in?
2. Dyes run slowly. Did you make the gel with plain water instead of electrolyte/buffer? If it is adjustable, is the AC/DC adaptor set at 1.5V or at 12V? How far apart are the two wires/electrodes: the shorter the distance the faster the movement of the dyes.
3. Dyes run akimbo. Are the samples lined up right? The two wires should be parallel to each other. The four samples should make a "starting line" also make a line parallel to the two wires, and between them. Also, as a hint, in my experience the dyes at pH 8 move "from bubbles to blue"--that is, away from the wire that makes bubbles and toward the wire that makes the blue precipitate.

For Further Sleuthing

Let the students make their own mixtures of the concentrated dyes .

Use small test tubes or any clean vial.

Note that the student can vary the colors, and the relative amount of each color, in the mixture.

Problem: how do they remember and record how they made their mixture?

How can other students figure out what colors were in a particular mixture made by another student?

Write us about your experience using these instructions in the classroom

