Gel Electrophoresis

Biotechnology on a budget to dye for

Simulate the process of DNA fingerprinting by using electricity to separate colored dyes.

Materials

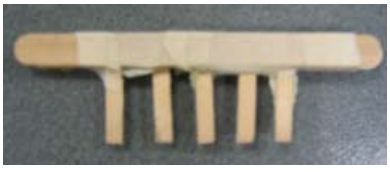
For the electrophoresis chamber:
small plastic box approximately 8x12 cm
(empty micropipet tip boxes are perfect – available at RAFT)
2 regular popsicle sticks
2 narrow popsicle sticks (coffee stirrer kind)
scissors
masking tape
two 5" pieces of stainless steel wire
2 electrical leads with alligator clips
five 9V batteries ("super heavy duty" kind work fine)

For the gel and buffer:
water
baking soda
agar-agar powder (available from Asian grocery stores) or agarose (available from chemical supply companies)
mat knife or razor blade

For the samples:
water
food coloring
glycerin (available from pharmacies)
needle-tip disposable pipet (Flinn #FB1260; $29.95 for 400)
(optional) beaker of water for rinsing tips between samples

Assembly

1. Make a comb to create wells in the gel that will eventually hold the samples. Cut the narrow popsicle sticks so that they sit just above the bottom of the base when hung from a regular popsicle stick (~3-4 cm depending on the depth of your box). Cut 5 teeth and tape them to a regular popsicle stick so that they are evenly spaced and hang down to the same level. Tape the other regular popsicle stick on the other side to secure the teeth, and check to see that they hang evenly when placed on the box without touching the bottom. Place the comb vertically near the top of your box.

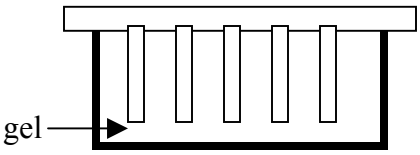


here's a cross-section

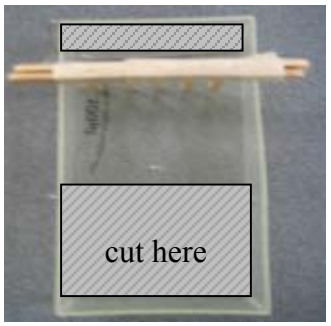
2. Make a 0.2% sodium bicarbonate buffer by dissolving 2 g of baking soda in 1 L of water. You will need ~100 ml per set up.

3. Make a 1% gel solution by adding 1 g of agar-agar powder to 100 ml of sodium bicarbonate buffer. You will need 40-50 ml of gel solution per set up. To dissolve the powder, heat the solution in the microwave, stopping every so often to vigorously stir the solution. Watch the solution carefully, as it will quickly boil over when hot enough. When you see bubbles, stop the microwave, and swirl the solution until the agar-agar particles completely dissolve. The solution should be translucent and will solidify at room temperature.

4. Once the solution is cool enough to pour, add just enough into the box so that ~0.5 cm of the comb teeth are submerged. Adjust the comb by sliding it so that it is ~1.5 cm from the top of the box. Thinner gels will yield better separations.

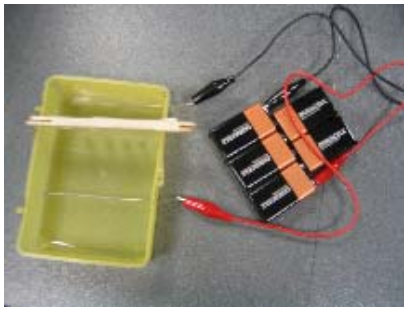


5. The actual gel only needs to be half the size of the box. In addition, you need to make space to place the electrodes. Once the gel sets (~5-10 min), use a knife to cut off the bottom half of the gel. Also, without disturbing the comb, cut out a thin strip from the top of the gel to make room for a wire electrode. Your gel should now be around 6 cm long and 8 cm wide (still the full width of your box). You can recycle the pieces of gel that you cut out by reheating them in the microwave.



6. Bend each piece of stainless steel wire to run along the width of the box and hook over the side. Place one on either side of the gel. Use tape to secure them to the box if you need to. These will be the positive and negative electrodes.

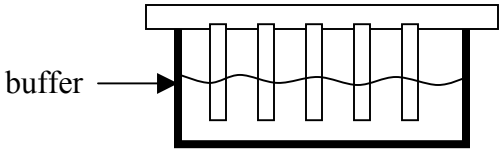
7. Make a high voltage power supply by connecting the five 9V batteries. Clip two batteries together by inserting the positive terminal of one into the negative terminal of another. Attach the remaining batteries one by one in this way until you have a five-battery pack. Clip an electrical lead to each of the exposed terminals of the pack. You should now be able to use the battery pack to power something by attaching the other ends of the electrical leads.



8. Prepare 5 different samples by mixing 1-2 drops of food coloring with 1 ml glycerin and 1 ml water in a small tube. We used blue, red, green, yellow, and purple (made by mixing blue and red food coloring).

To do and notice

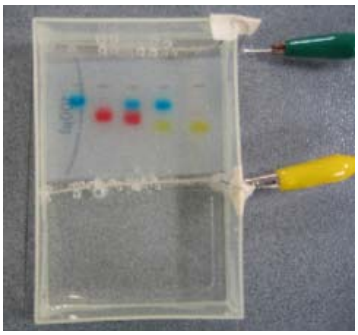
1. When your gel set-up is ready, pour just enough buffer to cover the solidified gel. Make sure you fill up the space left from the cut gels and that the gel is completely submerged.



2. Gently remove the comb by pulling straight up without tearing the gel. The wells should fill with buffer.

3. Use the needle tip pipet to transfer ~10 µl of each sample to an empty well. The volume of the thin tip of the pipet is about 10 µl. Submerge the tip in the buffer directly above the well and gently squeeze the sample into the well. It should fall into the well since it is denser than the surrounding buffer. You should use a new pipet for each sample to prevent contamination between samples. If you only have a few pipets, rinse out the tip well in a large beaker of water before re-using.

4. Once all the samples are loaded, connect the leads from the power supply to the stainless steel wire electrodes attached to the box. Connect the negative terminal to the electrode at the top of the gel (near the combs) and the positive terminal to the electrode at the bottom of the gel. You should see bubbles forming along the electrodes when a complete circuit is made.



5. Allow the samples to run for 15-20 minutes and observe what happens to each sample.

What's going on?

Gel electrophoresis is one of the most important tools used in molecular biology and genetic engineering. By conducting an electric current through an electrolyte buffer (such as the sodium bicarbonate buffer used in this activity), charged molecules will migrate towards the oppositely charged terminal. When suspended within a polymer matrix (such as the agar gel), the molecules will move at different speeds based on their size. In this activity, negatively charged dye molecules are loaded into the gel. When a current is passed through the gel, the molecules migrate towards the positive terminal, with smaller molecules moving faster than larger ones. Look at the ingredients list of the food coloring you used to see what molecules are inside. We used Smart and Final brand dyes that have Blue #1, Red #40, and Yellow #5. Using gel electrophoresis, we discovered that McCormick brand "blue" food coloring is made of a blue dye plus a small amount of red. McCormick "red" dye is actually made of two different red colored molecules. Test different brands to discover the true composition of each color.

In molecular biology, this method is used to separate biological compounds such as DNA or proteins based on size. Like the colored dyes, DNA and proteins are negatively charged, so will migrate towards the positive electrode at different speeds depending on their size. Scientists can use special enzymes to cut a large strand of DNA into many smaller pieces. The size of the pieces will depend on the specific base sequence of the large DNA strand. This idea is used in DNA fingerprinting to identify people based on the genetic code of their DNA.

Additional Information

Molecular Cell Biology by Lodish et. al., W. H. Freeman (2000)
A classic molecular biology text available FREE online at:
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mcb

http://en.wikipedia.org/wiki/Gel_electrophoresis

http://en.wikipedia.org/wiki/Food_dye
An article with links to the chemical structures of different dyes.