

## **DIY Gel Electrophoresis**

### Introduction

A gel electrophoresis is a tool utilized by molecular geneticists to separate and view different parts of macromolecules such as DNA, RNA, or proteins. This technique works because most macromolecules are negatively charged. When placed in the gel's wells on the negative end of the chamber and an electric field is applied, the molecules will migrate to the positive end through the gel's microscopic holes. The smaller and more negative the molecules are, the further they will migrate to the positive end. This results in separation of the macromolecules based on mass and charge. If the molecules are dyed, one can observe the distinct bands and interpret the results. The results of a gel electrophoresis have many applications, including forensic investigations, paternity tests, and measuring protein levels.

### Materials

Plastic box – 1 per group

Stainless Steel or copper wire – to cut 2 pieces per group

9 Volt Batteries (5 per group)

Agar Powder – 1/4 teaspoon (1 gram) per group

Baking Soda– 1/2 teaspoon (2 grams) per group

Distilled Water– 200mL per group

2 Alligator Clips with Leads per group

Food Coloring Dyes – Red, Blue, and Green

Craft foam

### Tools

Wire cutters

Scissors

Measuring spoons – size: 1/4 and 1/2 teaspoons

Small mixing bowl (1 per group)

Microwave-Safe Bowl (1 per group)

Microwave

Pipettes

Ruler

1. Make a buffer

The buffer solution will need to be a 1% solution of baking soda.

Combine 1/4 teaspoon of baking soda with 100 mL of distilled water in your mixing bowl. Stir the solution well with a spoon.

2. Make the Agarose Gel

Place 1/8 teaspoon of agar powder in the microwave safe bowl.

Add 50 mL of the buffer solution made in the previous steps to the agar powder in the microwave safe bowl.

In order to dissolve the agar powder, place the bowl in the microwave and turn it on for 1-2 minutes.

**Watch out. It get's hot.**

Every 15 seconds, stop the microwave and stir the solution. Keep doing this process until the solution begins to **bubble**.

Once this happens, quickly and carefully remove the bowl from the microwave to avoid having the solution bubble over. The solution should be translucent.

**Next steps:**

1. Cut two equal pieces of the stainless steel or copper wire using the wire cutters.

Both of these pieces should be about 2 cm longer than the width of your rectangular, plastic box.

This box will serve as the gel chamber. The wires need to be longer than the width of the gel chamber.

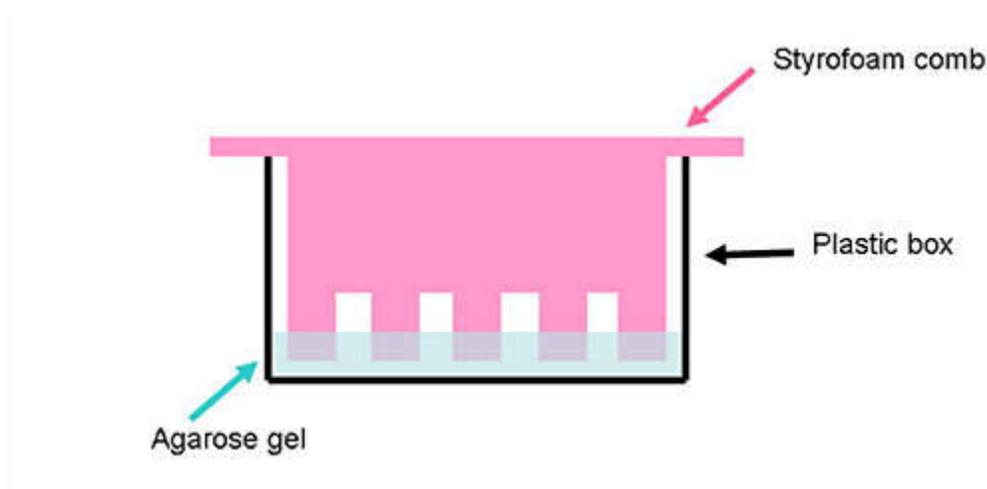
Bend the extra 2 cm of wire into a hook. This will allow the wire to hook over the side of the chamber.

2. Assemble the batteries.

A positive terminal of one battery will be snapped into a negative terminal of another battery. You need to continue this process with all the batteries until one positive and one negative terminal is not snapped to another battery.

3. Make the Foam comb

Trace out the gel comb from craft foam using the image below as an example. Measure the width of the plastic container. Cut out with scissors.



The comb will need to be wider at the top so it can rest on the edges of the chamber. Slits can be cut into the outer edges of the comb. This will allow it to stay upright easier.

The bottom of the comb needs to have one tooth for each color of food dye.

Each tooth should not be touching the bottom of the gel chamber. There needs to be around 5mm of clearance between the bottom of the teeth and the chamber.

The teeth also need to be evenly spaced away from each other.

The size and spacing of the teeth will depend on the dimensions of the chamber you are using.

4. Place the comb into the gel chamber. The comb should be about  $\frac{1}{2}$  cm from one of the ends of the gel chamber.
5. Slowly pour the 1% agarose gel into the chamber. Stop pouring when the agarose gel has covered approximately 5mm of the comb's teeth.

Place the gel chamber in a safe spot and wait for the agarose gel to solidify. The gel will have the consistency of jello when it has reached room temperature and is ready.

6. Gently pour the rest of the buffer solution over the gel in the chamber. The buffer solution should completely cover and submerge the gel.

Depending on the size of the chamber, you may not need to pour all of the remaining buffer solution.

7. Firmly grab onto the top of the gel comb and carefully pull it straight up and out of the gel.

The wells formed by the comb in the gel will hold the food coloring for this experiment.

8. Make room for the wire electrodes. Take a plastic knife and cut 2 lines (1cm away from each end) across the width of the chamber on both ends. Cut all the way down to the bottom of the chamber.

Place the wire electrodes back in the chamber. It is highly important the entire length of the electrodes are under the surface of the buffer solution. The hook of the electrode does not have to be submerged.

#### 9. Add food coloring

One at a time, each of the different colors of food coloring will need to be placed in its own well with the pipette. Only a couple drops of each dye are needed.

The less dye placed in the well, the clearer the results of the experiment will be in the end. Rinse out the pipette after each colour is deposited.

**Tip:** Place the tip of the pipette loaded with food coloring beneath the surface of the buffer solution and slightly inside the well before you push the plunger to release the dye.

#### 10. Run the electrophoresis

Place one clip of one of the alligator leads on the exposed positive battery terminal. Place the other end of this same lead onto the positive electrode. The positive electrode should be the wire farthest away from the wells.

Take the other alligator clip lead, one clips should be clipped onto the exposed negative battery terminal, the other end on the negative electrode. The negative electrode should be the wire closest to the wells.

**Tip:** You should be seeing bubbles bubbling up from the electrodes in the buffer solution because the current is passing through them.

#### 11. The food coloring will migrate from the side of the chamber with the negative electrode to the end with the positive electrode.

Keep the gel running until food coloring bands have clearly separated away from each other. Check every 5 to 10 minutes. This process takes around 15 to 20 minutes.

### **Interpretation of results**

The secondary color of food dye separated into different colored bands. This is because the secondary colored dye contains different sized macromolecules. Different sized molecules will separate at different speeds, resulting in bands at certain locations down the length of the gel. The same process occurs with DNA and other macromolecules. The phosphate backbone of the DNA and RNA molecule is negatively charged, therefore when placed in an electric field, DNA fragments will migrate to the positively charged electrode.

### **Resources:**

<https://www.exploratorium.edu/snacks/gel-electrophoresis>

<https://www.yourgenome.org/facts/what-is-gel-electrophoresis>