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iBIO STEM Kits: Electrophoresis

iBIO STEM Kits welcomes you to a SCIENTIFIC JOURNEY!

Today we will do an investigation using electrophoresis, a laboratory technique used to separate molecules using their size and electrical charge. The purpose of this investigation is to explore how to build an electrophoresis chamber to create a complete circuit through an electrophoresis gel. You will add three dye samples to the gel in order to separate the molecules using the electricity from the power source. We challenge you to explore this investigation as a scientist would. What does this mean?

Scientific exploration is different than just playing around because it asks you to think about HOW you investigate. This means you need to do your investigation by observing what happens when you change a variable you have carefully chosen. This helps you to understand WHY something happens. Scientific exploration also means that you record WHAT you see or measure and that you record WHY you think it happens. The STEM Kit Notebook that you are holding will help to guide your investigation and give you a place to record your observations, measurements and conclusions.

Follow the QR code at the top of the page for additional resources on this activity. There are many resources for you to use on our website. This type of investigation is associated with some very exciting careers! We hope that you will explore these resources while you are doing your investigation!

Let's Get Started!

FIRST, you will need to prepare your workspace. This can be a messy investigation, so make sure that you are using a space that will not be easily damaged. A kitchen table will work nicely. To make your clean up easier, you should protect your surface by laying out some used newspaper or opening up a paper grocery bag.

SECOND, you want to unpack your materials. Use the list below to identify which materials are used in each part and organize them in your workspace.

Materials for Part A:
- Ziploc Small Square One Press Container
- 2 Jumbo Stainless Steel Paper Clips
- 3"x3" craft foam square
- Paper Comb Pattern
- 15 cm ruler
- A ballpoint pen

General Supplies:
- Paper to cover your workspace
- Scissors

Materials for Part B:
- Five 9-volt batteries

Materials for Part C:
- The empty plastic tube from Part B
- ⅛ tsp of baking soda
- 30 ml of water
- Plastic knife
- 3 pipettes

Materials for Part D & E:
- Two alligator clip test leads
- Plastic Sandwich Bag

General Supplies:
- Colored pencils or crayons

LAST, you need to be prepared for experimenting safely. You will be heating the gel before pouring it into your chamber. You may want to have an adult help you handle the hot container safely. You will also be using a power source to make a circuit in your electrophoresis chamber. Once the circuit is in place, you should not touch any metal pieces on your chamber.

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iBIO STEM Kits: Electrophoresis

Gel electrophoresis *is a technique used to separate mixtures of molecules (like DNA and proteins) so that you can see what the mixture is made of. The word electrophoresis comes from –**electro**, because an electric field is used, and –**phoresis**, which means movement. Gel electrophoresis uses an electric charge to move molecules through a gel (like gelatin).*

*The electrophoresis chamber that you will be building is a big circuit. A **circuit** is a path for electricity to move through. It's sort of like a big loop. As electricity moves, or flows, the electricity might light a bulb, turn a fan, or in our case, it will move the molecules in your mixture. The molecules in the mixture will separate as they move because they are positively or negatively charged. The molecules will also separate because they have different sizes.*

The first component (part) for electrophoresis is the electrophoresis chamber. In this investigation, we will be building our own electrophoresis chamber. We will use a plastic storage container as the gel chamber. We will bend stainless steel paper clips so that they can act as electrodes. We will use 9-volt batteries to create the electric field power to the system. Lastly, we will cut a piece of craft foam to make openings in the gel, called wells. This is where we will put the mixtures that we will separate.

Let's get started!!!

Part A: ENGINEER - Build the Electrophoresis Chamber

Here's what you will need from your kit:

- Ziploc Small Square One Press Container
- 2 Jumbo Stainless Steel Paper Clips
- 3"x3" craft foam square
- A ballpoint pen
- Paper Comb Pattern

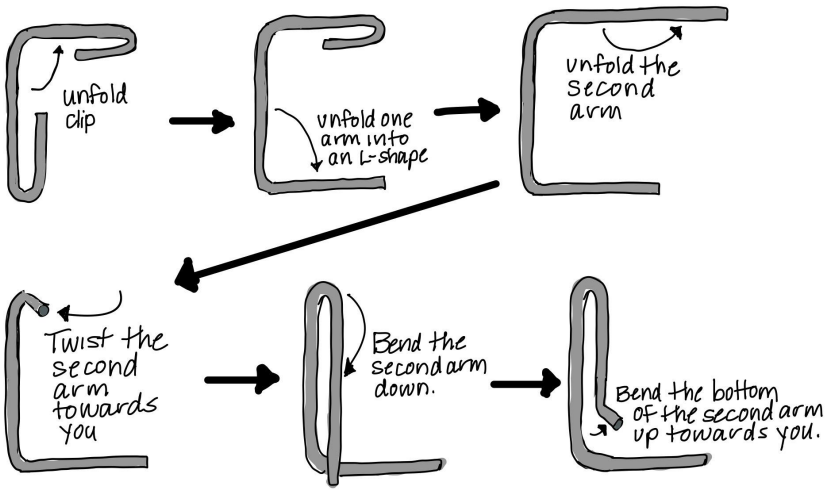
General Supplies:

- Scissors

Directions for making the Electrophoresis Chamber:

1. You will be using a plastic ziplock container to make the chamber. Remove the top and put it aside. The top will not be used in our electrophoresis chamber.

2. Follow the directions in the diagram (and video) below to unfold and manipulate the paperclip. Repeat this with the second paperclip.



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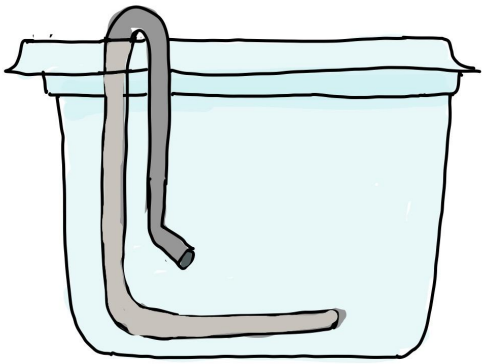
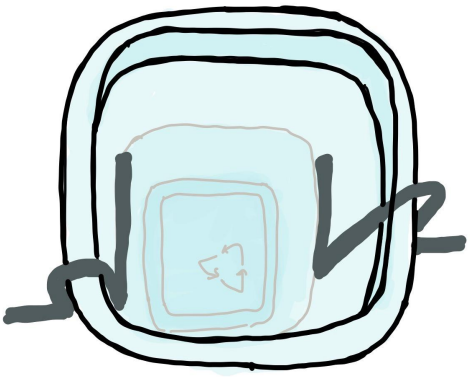
4. Hook one bent paperclip over one side of the plastic container so that the bottom “L” runs along the bottom width of the container. This paperclip will be the negative electrode. You may find that your electrode does not sit in the box well. You may need to take it out and manipulate it further so that it better fits in your container. Squeeze the two sides together so that the clip does not wiggle.



5. Hook the other bent paperclip at the opposite side of your container in the same way. This will be your positive electrode. You may find that your electrode does not sit in the box well. You may need to bend it further so that it fits better in your container. Squeeze the two sides together so that the clip does not wiggle.

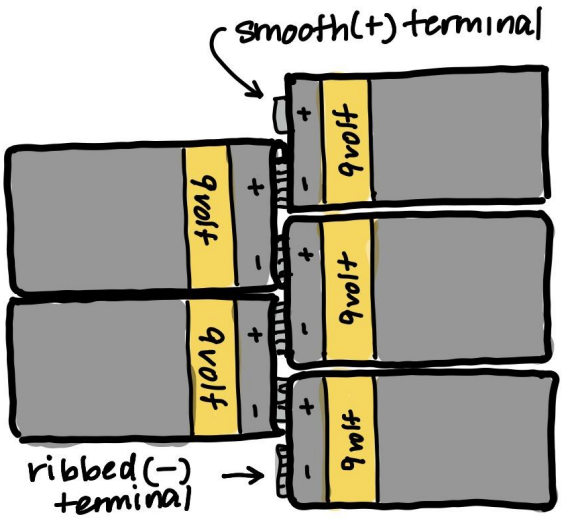


6. Using a pair of scissors, cut out the paper tracing pattern for the comb. Using a black ball point pen, trace the pattern on the piece of craft foam. Now cut the comb shape out of the craft foam. You will need to trim the comb so that it fits easily into the chamber and hangs without touching the sides of the chamber. The bottom of the comb should NOT touch the bottom of the plastic container. The comb will be placed vertically into the plastic box and needs to stand upright, so its shape is wider at the top so that the comb can rest on the edges of the plastic container.

*The second component in our electrophoresis chamber is the **power source**. The power source is what pushes the electricity through the circuit. We are going to build our power source out of 9-volt batteries.*

Directions for setting up the Battery Power Source:

7. Connect the five 9-volt batteries together in series by snapping the positive (+) terminal of one into the negative (-) terminal of another until you've formed a battery pack with all five batteries. There should be one positive and one negative terminal left exposed.



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Part B: Pouring the Gel

Here's what you will need from your kit:

- The Plastic Tube with 30 ml of electrophoresis gel
- Three micropipettes
- Three microcentrifuge tubes (A, B, C) containing the samples

General Supplies:

- A microwave and a drinking glass OR
- A stovetop, a small saucepan and a drinking glass.

CAUTION: YOU MAY NEED TO HAVE AN ADULT HELP YOU WITH THIS STEP.
You will be heating the plastic tube to melt the gel. The hot tube and the hot gel can burn you.

*Another component is the **load**. This is the thing being powered by the electricity in a circuit. In our electrophoresis chamber, the load is the gel. The gel we are using is a protein mesh that has many small holes and passageways. The molecules in your sample mixtures will be moving through the passageways in the gel. Since each molecule moves at a different speed, this will help us to see the different molecules in the mixture.*

We will need to put the mixture into the gel for this to work! So how do we do this?

***Easy!** When the gel is melted into a liquid, we can pour it into the chamber and it will take on the shape of the chamber. If we put a comb into the gel while it is still a liquid, it will create small holes, called wells. We can put our mixture into the wells so that the molecules are right next to the passageways in the gel.*

1. **CAUTION: YOU MAY NEED TO HAVE AN ADULT HELP YOU WITH THIS STEP.**

   First, you need to melt the gel. There are two ways to melt the gel in the plastic tube.

   The first way is in the **microwave**.
   a. Unscrew the top of the plastic tube. Place the cap on top of the tube so that the tube is covered, but DO NOT screw the cap on the tube.
   b. Put the plastic tube (with the loose cap) in a drinking glass to hold it upright.
   c. Place the drinking glass (with the tube in it) into the microwave.
   d. Heat the tube for 30 seconds. It is possible that the steam may pop the top off of the tube. Do not be alarmed.
   e. Allow the tube to sit for one minute in the microwave. Once the gel is completely melted, place the tube into the drinking glass so that it stands upright while you get your electrophoresis chamber ready.

   The second way is by using your **stovetop**.
   a. Fill a small saucepan halfway with water. Bring the water to a boil.
   b. Make sure the cap is securely tightened on the plastic tube.
   c. Put the closed plastic tube into the boiling water.
   d. Boil the tube for 10 minutes. Use a spoon to take the tube out of the water. Check to see if the gel is completely melted. If it is not, return it to the boiling water. It may take up to 15 minutes to melt the gel completely.
   e. Once the gel is completely melted, place the tube into the drinking glass so that it stands upright while you get your electrophoresis chamber ready.

2. Make sure your electrophoresis chamber is on a flat surface. Remove the paperclip electrodes and put them to the side.

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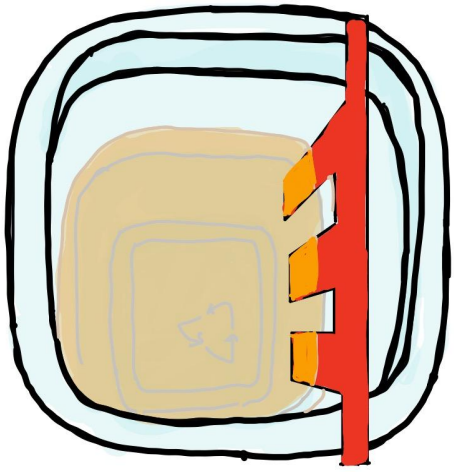
3. You will be pouring the melted gel into the plastic container of the electrophoresis chamber. Remove the cap from the plastic tube of gel. The tube will be hot, so you will need to use an oven mitt or towel to hold the tube.



4. Carefully pour the liquid gel into the plastic container of your electrophoresis chamber. You will be using the plastic tube to make your buffer solution in Part C, so DO NOT THROW IT AWAY. Rinse and dry the plastic tube and cap so that you can use it for Part C.

5. Now insert the comb that you cut in Part A into the plastic container close to one of the sides. You will need to let the gel sit quietly so that it can solidify into a solid gel. This will take 20-30 minutes. *Tip:* When the gel is set, it should be firm to the touch and wiggle like solid jello.

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Part C: Adding the Buffer and Sample

You should now have an electrophoresis chamber with a solid gel and a power source made of 9-volt batteries.

Remember that in our electrophoresis chamber, the load is the gel. The gel breaks the circuit. We needed something to carry the electricity and move the molecules through the gel. That is why we use a buffer!

A buffer is a liquid solution that has ions that carry electricity. When it is put into the electrophoresis chamber, the circuit is complete because the buffer carries electricity across the gel. The electricity provides the force to move the different molecules in our samples.

Here's what you need to make the Buffer:

Materials:

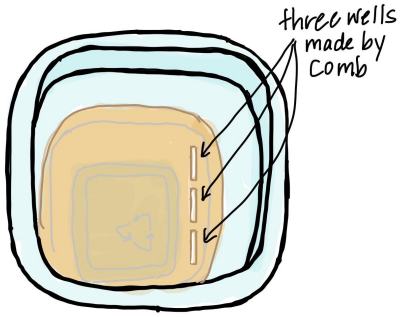
- The empty plastic tube from Part B
- ⅛ tsp of baking soda
- 30 ml of water

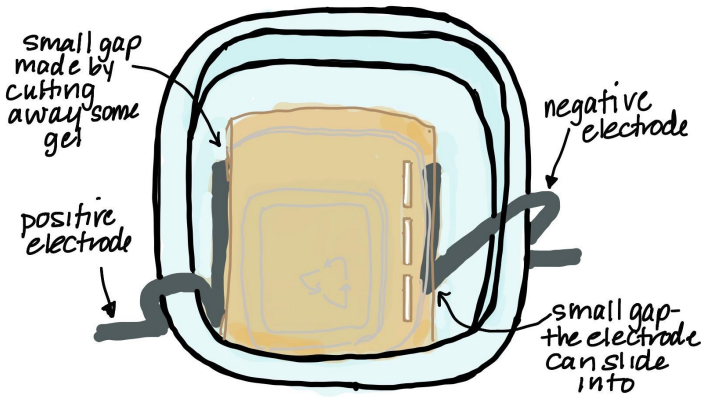
Directions:

1. Measure 30 ml of water into the plastic tube.
2. Add 1/8 tsp of baking soda to the plastic tube.
3. Put the cap on the tube and make sure that it is twisted closed. Shake well to dissolve the baking soda.
4. Now the buffer is ready to be used!

Directions for Setting up the Chamber:

1. Your gel should now be solid. Pour the buffer solution (that you made in the plastic tube) over the solid gel. The gel should be under the liquid buffer.
2. Gently pull the comb out of the gel. This will leave small holes in the gel, called wells. The wells will be the location for the samples.





3. **CAUTION: YOU MAY NEED TO HAVE AN ADULT HELP YOU WITH THIS STEP.**
   Using the plastic knife, carefully cut a thin slice of the gel from the top (by the wells) and the opposite side to make room for the electrodes. Be careful not to cut into the wells. This will leave a small gap between the plastic and the gel for the paperclip electrodes to slip in.

NOTE: When you cut the ends of the gel, you need to make sure that you do not cut the wells.

If you cut into the wells by mistake, you will need to re-melt your gel. Pour the buffer into a cup and scoop out the gel. Break the gel into pieces and put the pieces back into the plastic tube. Follow the directions in Part B to re-melt and re-pour the gel.

4. Now re-attach the paperclip electrodes to the electrophoresis chambers so that the electrodes are in the small gap that was just made when the gel was cut.

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Adding Samples:

5. The samples are in microcentrifuge tubes. There should be three microcentrifuge tubes and three small plastic pipettes for each team of two campers.

   Sample A, Sample B, Sample C

6. Flip open the top of the microcentrifuge tube with Sample A. Be careful! The sample will sometimes fly out of the container and the samples can stain your clothing!
7. Squeeze the top of one small plastic pipette. While still squeezing, put the tip of the pipette into the sample in the microcentrifuge tube. Then stop squeezing to take up some of the sample into the pipette.
8. Place the tip of the dropper carefully into the well on the left and place one drop of the sample into the well. If the well does not look filled, place the tip of the dropper into the well again and place another drop of the sample into the well. You do not want the sample to come out of the gel.

9. With a fresh pipette, move on to Sample B. Take up some of the sample into a small, plastic dropper. Place the tip of the dropper carefully into the well in the middle and place one drop of the sample into the well.
10. With a fresh pipette, move on to Sample C. Take up some of the sample into a small, plastic dropper. Place the tip of the dropper carefully into the well on the right and place one drop of the sample into the well.

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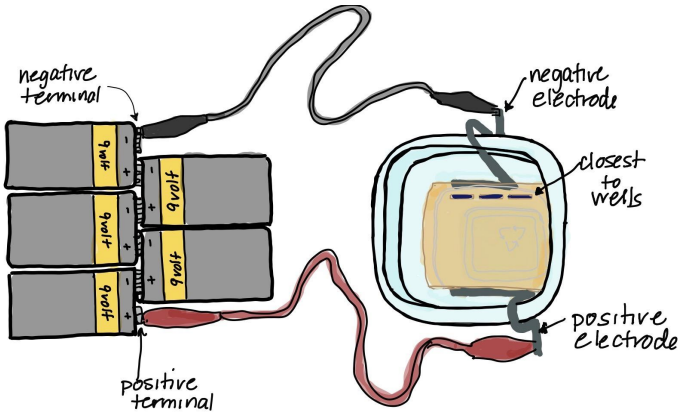
Part D: Turning on the Power and "Running the Gel"

Here's what you will need from your kit:

- Two alligator clip test leads

Caution: As you are setting up your power source, be careful when you complete your circuit with the alligator clips. Make sure you are only touching the parts that are covered with plastic so that you do not get shocked by the current.

1. Now that your gel is all set up with your three samples, you will need to hook up the power. With one alligator clip, connect the negative (ribbed) terminal of the battery pack to the electrode nearest the wells.
2. With the second alligator clip, complete the circuit by connecting the positive (smooth) terminal of the battery to the electrode furthest away from the wells. As soon as you do this, you should see bubbles forming around the electrodes in the buffer as the current passes through them. If you don't see bubbles, recheck the electrical connections. Make sure the batteries are properly placed in *series,* and that the batteries are fresh and fully charged.



3. Your samples will now be starting to move. Because they are so small, they will move slowly, so you will need to be patient and give them some time to run. But you should check the progress of the gels every 10 minutes so that you can see what is happening.

   What do you see the electrophoresis chamber doing? What are the samples doing? Are the samples still in the wells? What do the samples look like right now? Do all of the samples look the same?

4. Run the gel until you see good migration and separation of the food coloring dyes. You will need to wait about 30 minutes.

So…What is happening in the electrophoresis chamber?

Gel electrophoresis *is a technique used to separate mixtures like DNA and proteins. The separation is based on how positively or how negatively charged a molecule is, and its size. Gel electrophoresis uses a gel (like gelatin) and an electric field is put through the gel.*

*The word electrophoresis comes from –**electro**, because an electric field is used, and –**phoresis**, which means movement.*

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*Our electrophoresis chamber is a big circuit. A **circuit** is a path for electricity to move through. It's sort of like a big loop. As electricity moves, or flows, the electricity might light a bulb, turn a fan, or in our case, it will move molecules. Let's take a look at the parts of the circuit in our electrophoresis chamber to better understand how this works.*

*All circuits have some basic components. One component is the **power source**, also called a **voltage** source. The power source is what pushes the electricity through the circuit. This is the battery pack we have built.*

*Next, circuits need **connectors**. Connectors connect all the parts of the circuit and create the path or loop that the electricity travels through. Connectors are often made of wire or other metal. We have two sets of connectors in our electrophoresis chamber. First, we have our paper clip connectors. They extend into our plastic chamber, hook over the edges and extend out. Our second connectors are the alligator clip leads. You clipped the first alligator clip to the paperclip on one end and connected the other side to the negative terminal of the power source. You clipped the second alligator clip to the positive terminal of the power source and clipped the other end to the paperclip on the other side of the chamber. Can you see the circle through which the electricity can flow?*

*A third component is the **load**. This is the thing being powered by the electricity in a circuit. In our electrophoresis chamber, the load is the gel. The gel breaks the circuit. We needed something to carry the electricity and move the molecules through the gel. That is why we used the buffer!*

When we put a buffer into the chamber, the circuit is complete because the buffer can carry electricity. This is what will move the molecules in our samples.

In this experiment, negatively charged sample 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. This separates the different color molecules.

If a large molecule has a big charge, its attraction to the opposite charge is also large. But, since the molecule is large, it will have a difficult time moving through the thick gel. In gel electrophoresis, large molecules are going to move slower.

A small charged molecule will move through the gel more easily. Shorter molecules move faster and move further than longer ones because shorter molecules get through the pores of the gel more easily. This phenomenon is called sieving.

If the molecule does not have any charge, it will not move."

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Part E: Compare Molecule Bands

Here's what each camper will need:

- A plastic sandwich bag

General Supplies:

- Set of colored pencils or crayons

Directions:

1. Once the colors have separated, you should disconnect the alligator clips from the electrodes. This will "turn off" the electrophoresis chamber.
2. Carefully remove the paper clip electrodes from the chamber.
3. You will have an easier time seeing all of the colors if you remove the gel from the plastic container. The gel will be a bit soft because the electrical field generates some heat.
4. With your fingers, you should slide the gel out of the plastic container. Allow the buffer solution to drip off of the gel and then slide the gel into the plastic bag. From the plastic bag, you should be able to see the results clearly.
5. The gels will not "keep" because over time, the colors will continue to move by diffusing into the gel and will no longer be reliable results. You will need to record your results by drawing the bands you see, in color, in the gel in this packet on page 11.
6. Now do some analysis!
    a. How do the bands from Sample A compare to Samples B and C? Do they have any bands that are the same?
    b. How do the bands from Sample B compare to Sample C?
    c. Which sample has molecules that were the biggest?
    d. Which sample has molecules that are the smallest?
7. Clean and rinse your electrophoresis chambers.

Suggestion: If you want to run your electrophoresis chamber again with other chemicals (like Kool-aid or Soda or any liquid that has coloring) you can make new gels by making Knox Original Unflavored Gelatin. Follow the directions on the package and use 30 ml of the gelatin to make a new gel.

If you run the chamber again, you will eventually need new batteries. The five batteries that came with your kit will be about to run your chamber 2-3 times before needing to be replaced.

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Electrophoresis Results

Color the bands you see on your gel in the picture below.