Bandit™ Building Activity

From Circuits to Molecules

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Student's Guide

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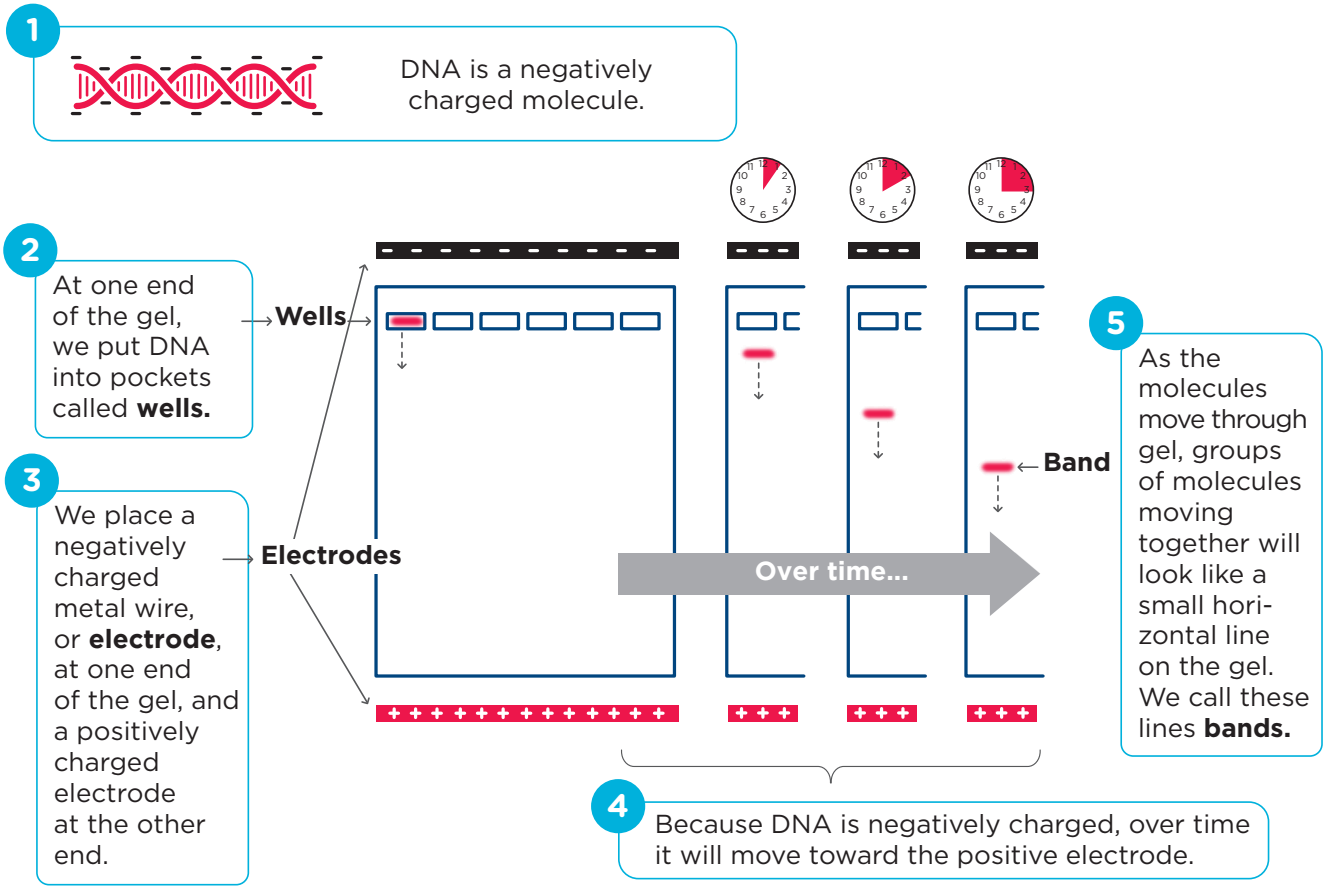
Student's Guide Contents

Background: What is electrophoresis?

Electrophoresis is one of the most common ways of studying molecules in a biology lab. Electrophoresis separates molecules, which can help us identify them. It can be used to identify criminals, to figure out whether people are related, and to diagnose diseases. Electrophoresis can be used to study many different kinds of molecules, including a couple you may have heard of: DNA and proteins. Because it is one of the most common molecules to study with electrophoresis, we will use DNA as an example in this activity.

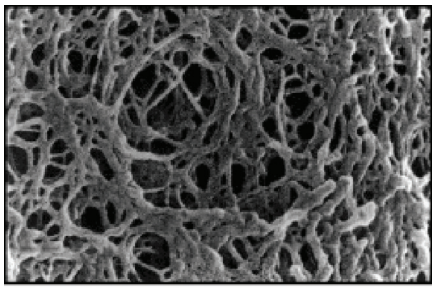
The word electrophoresis means carried by electricity. During DNA gel electrophoresis, we use electricity to make pieces of DNA move through a gel. But how does it work?

In gel electrophoresis, electrical forces make charged molecules move through a gel

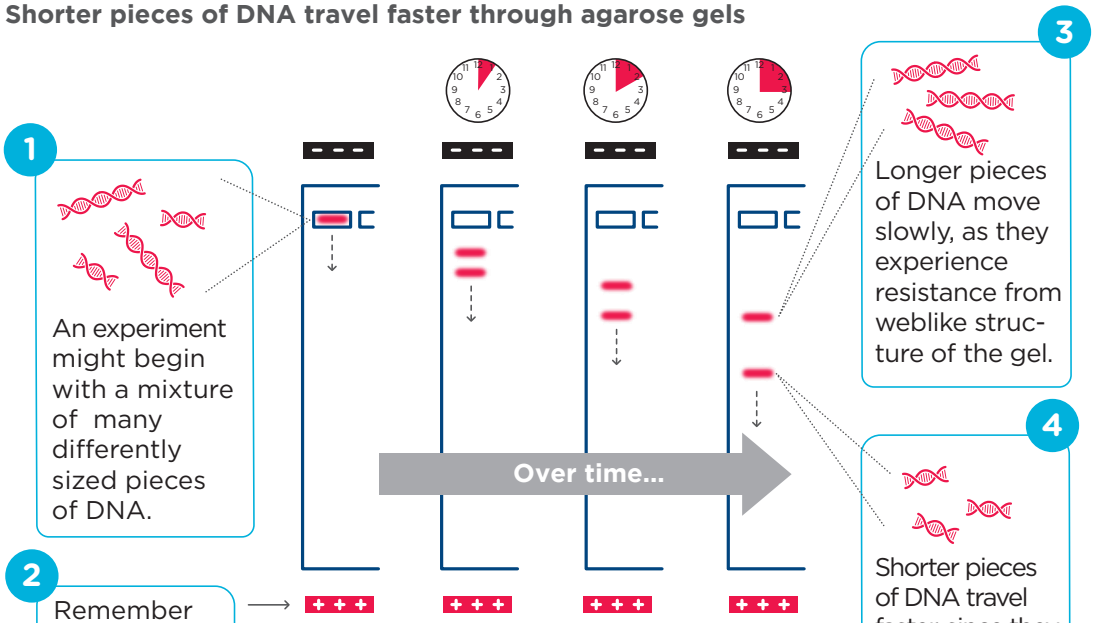


Agarose gels separate charged molecules by size

We'll again use DNA as an example. The picture to the right is a microscope image of the inside of an agarose gel. You can see that it looks like a web. This weblike structure is what allows us to separate DNA fragments of different sizes: small molecules can move through the web pretty easily, while larger molecules move more slowly.



Shorter pieces of DNA travel faster through agarose gels



Identifying DNA through gel electrophoresis helps scientists answer all kinds of scientific questions. For example, it might help scientists figure out whether a patient is carrying a mutation that causes disease or determine whether two individuals carry the same version of a gene.

Background: Stop and think

Circle the word(s) that complete each sentence:

Q1. In an electrophoresis system, (negatively/positively) charged DNA is attracted to the (negatively/positively) charged electrode.

Q2. Larger pieces of DNA move through the gel (faster/slower) than small pieces.

Q3. Gel electrophoresis starts when you load DNA into the wells. Over time, pieces of DNA will travel away from the wells. The pieces that travel the farthest will be (smaller/larger) than the pieces that stay closer to the wells.

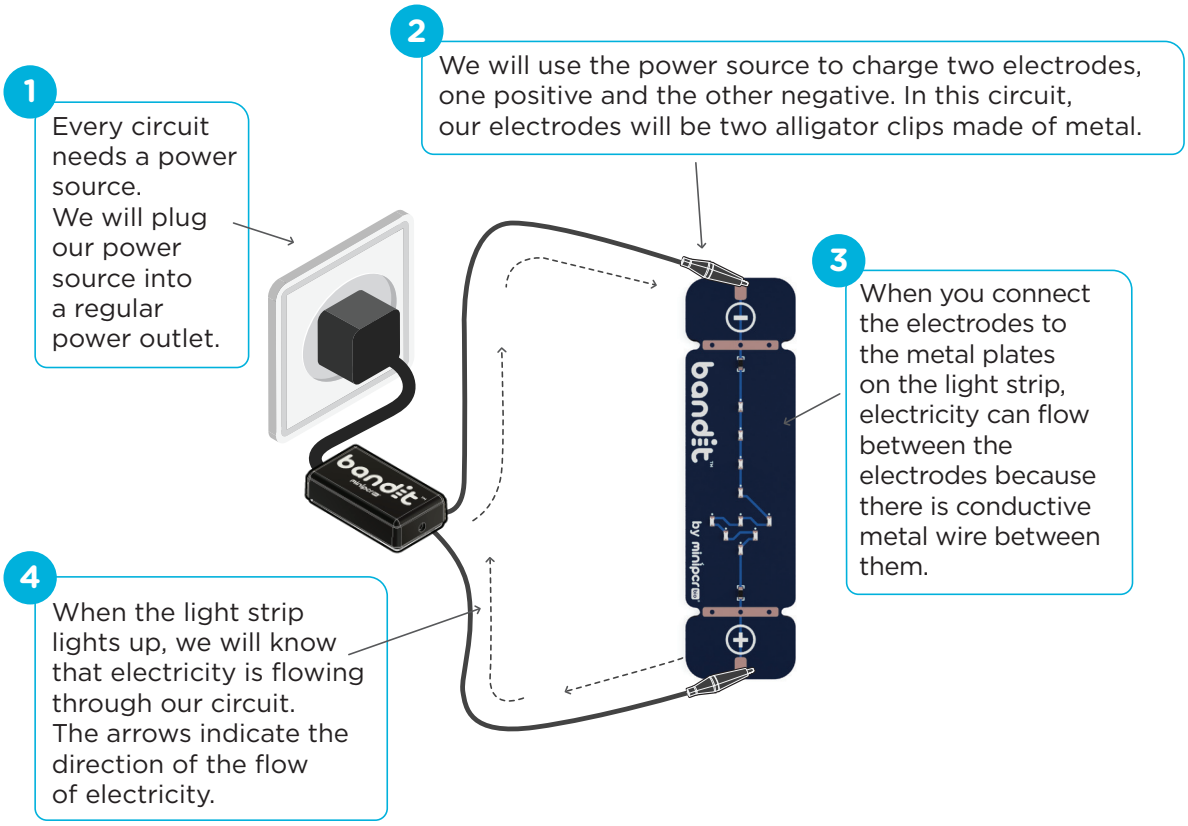
Challenge 1: Build a circuit

Materials needed:

- Bandit™ STEM Electrophoresis Kit
- USB-C power source

We use electric force to move DNA through the gel. To do an electrophoresis experiment, we need to provide a path or circuit for electricity to follow. An electrical circuit forms when two oppositely charged electrodes are connected by a material that conducts electricity. In gel electrophoresis, electricity is conducted through the agarose gel and the liquid buffer that surrounds it. In this challenge, you will build an electrical circuit that conducts electricity through a light strip.

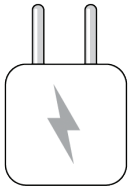
How we will make our circuit:



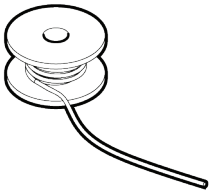
Now we will build a circuit like the one pictured in the previous page

1. Gather your materials

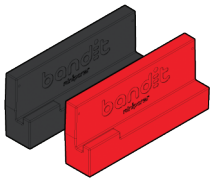
Supplied by user: USB-C power source (*e.g.*, phone charger)



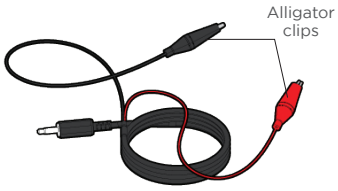
USB-C cable



Electrode wire

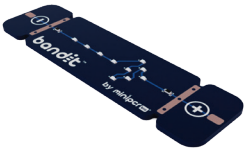


Electrodams™



Electrode cable

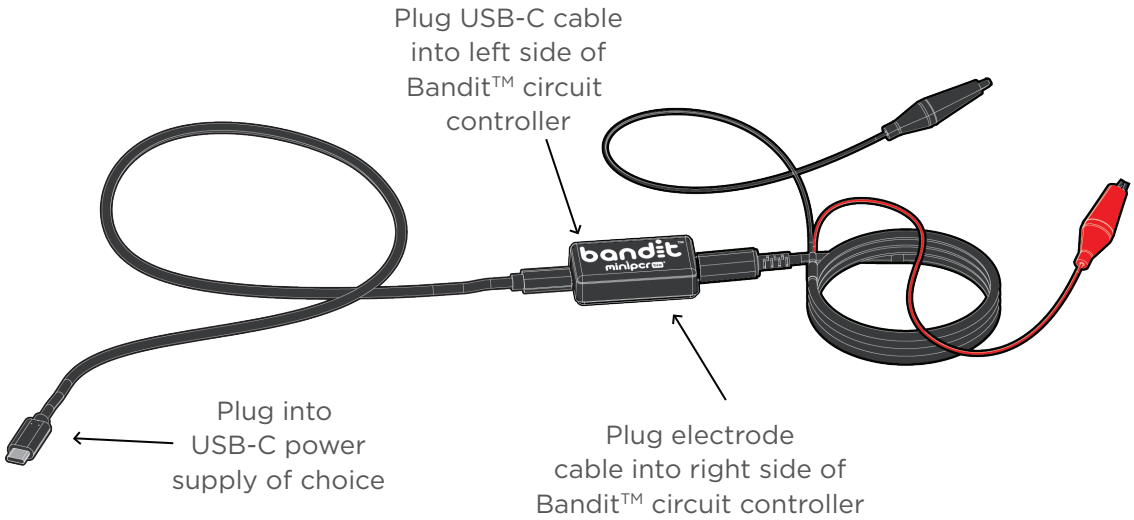
Bandit™ circuit controller



Light strip

2. Connect the power cords

- Plug the electrode cable with the alligator clips into the round port on the right side of Bandit™ circuit controller.
- Plug the USB-C cord into the USB-C port on the left side of the Bandit™ circuit controller.
- Plug the other end of your USB-C cord into your power source of choice.
  Note: USB-C power source is provided by the user.
- Plug the power source into a power outlet.



Notice that one alligator clip is black, and the other is red. Remember that we are using these clips as our electrodes in this circuit. The clip color tells you about its charge (positive or negative). In an electrical circuit, electricity flows from the negative electrode to the positive electrode. In this activity, you will form a circuit using the light strip to figure out which electrode is positive and which one is negative.

The light strip we are using will only light up if electricity flows through it in the direction of the arrow. That is, if the arrow lights up, you know that the negative electrode has been matched correctly to the negative end of the light strip (labeled -), and the positive electrode has been matched to the positive end (labeled +).

3. Follow the directions to run two tests and record your results in the table below
Note: when illuminated, the light strip is very bright!

Test	Follow these directions	Does the arrow light up? (Circle your answer below)
#1	1 Attach **red alligator clip** to metal plate at **negative end** of light strip. It may help to press the clip against the plate on the light strip to ensure good contact.  2 Attach **black alligator clip** to metal plate at **positive end** of light strip. Press the clip against the plate on the light strip to ensure good contact.	Yes / No
#2	Repeat the procedure described for test #1, but reverse the connections. 1 Attach **black alligator clip** to metal plate at **negative end** of light strip. It may help to press the clip against the plate on the light strip to ensure good contact.  2 Attach **red alligator clip** to metal plate at **positive end** of light strip. Press the clip against the plate on the light strip to ensure good contact.	Yes / No

4. When you have completed your tests, unplug your power cord from the power outlet

Challenge 1: Stop and think

Based on the results of your tests, circle the answer that completes each sentence below:

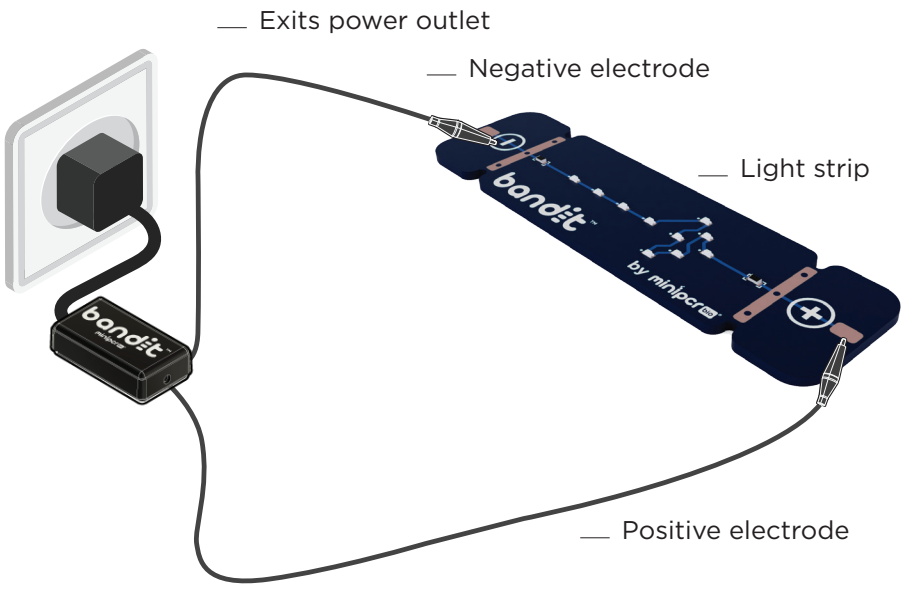
Q1. The (black/red) lead is the negative electrode.

Q2. The (black/red) lead is the positive electrode.

Q3. Later, we will use these electrodes to carry electricity into our electrophoresis system. Because DNA is negatively charged, it will be (attracted to/repelled from) the negative electrode.

Q4. DNA will be (attracted to/repelled from) the positive electrode.

Q5. On the diagram below, fill in the blanks with numbers 1, 2, 3, and 4 to indicate the order in which electricity flows through your circuit.



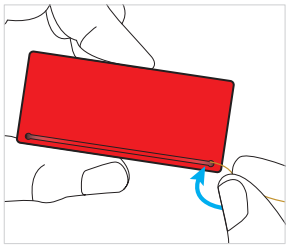
Now, we need to prepare our circuit for electrophoresis. During electrophoresis, we will extend the electrodes so they can more easily conduct electricity through the gel. If your electrodes have not yet been prepared, follow the steps below to set them up.

5. Cut the electrodes

- Cut two approximately 8-inch pieces of electrode wire from the included spool.

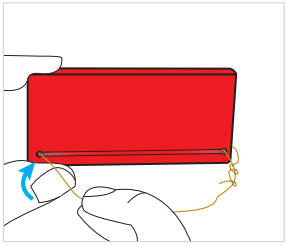
6. Prepare the Electrodams™

- Wrap one piece of electrode wire around the red Electrodam™ by following the steps below.

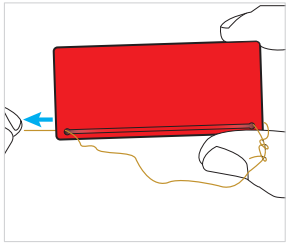


- Starting from the tall side of the Electrodam™ with a channel present, thread the wire through one of the small holes.
- You only need about 2 cm of wire to come through on the other side.

- Flip the Electrodam™ over.
- Twist the short free end of the wire that you just threaded through the hole around the longer piece of the wire to prevent it from slipping back through the hole.

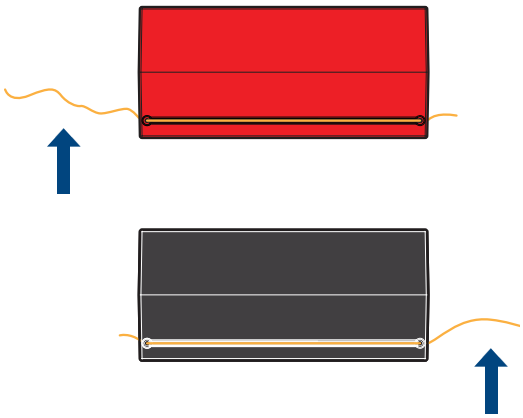


- Flip the Electrodam™ back over so you are looking at the side with the channel again.
- Thread the loose end of the wire through the small hole on the other side of the Electrodam™.



- Slowly pull the long free end of the wire until is it taut and the electrode wire sits flush in the channel.

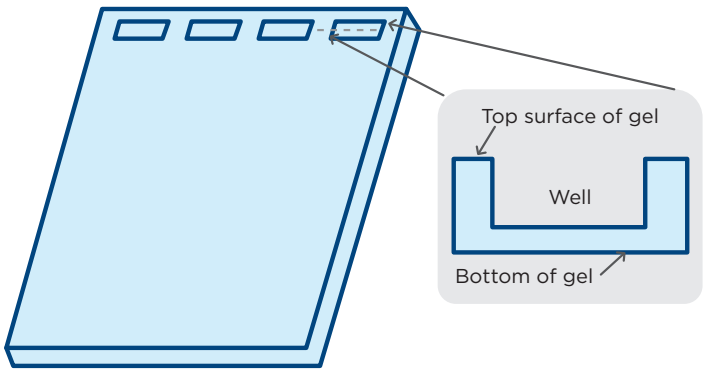
- Repeat for the black Electrodam™, but thread the electrode wire from the opposite side so that the long free ends of the electrode wires extend in opposite directions.
- Note: The electrode wire can remain attached to the Electrodam™ for several uses.



Congratulations, you've built a circuit and prepared it for electrophoresis!

If you are ready to make your electrophoresis gel, continue on to *Challenge 2* on the next page.

Challenge 2: Make your gel



Materials needed:

- Bandit™ STEM Electrophoresis Kit
- Agarose Tab™
- 1X TBE buffer

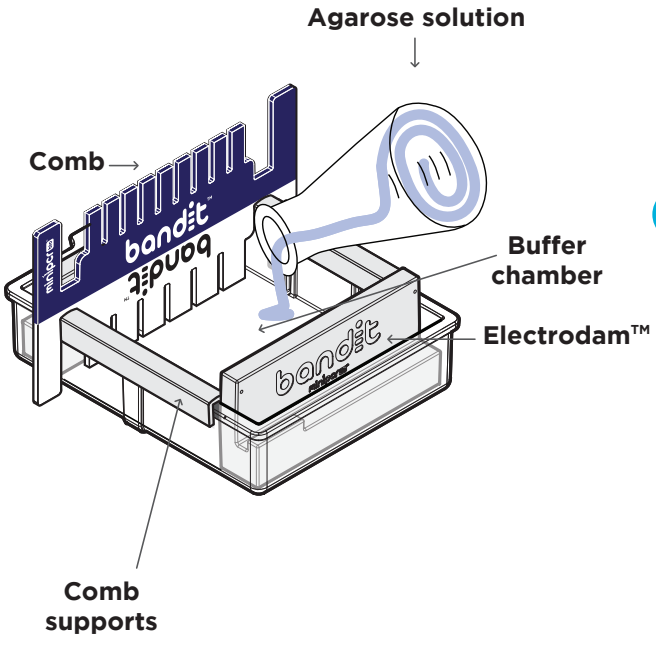
Remember that we will use an electrical circuit to move charged molecules through a gel. This gel is like a filter that separates molecules based on their size. In this challenge, you will make the gel that we will later connect to our circuit.

A typical gel is square or rectangular, about the size of a pack of playing cards, and has the consistency of firm Jello. A gel has a row of wells where we will later load our DNA samples. When we look down at a gel from above, wells might look like holes, but they don't go all the way through the gel. Instead, they are more like pockets in the gel that can hold small amounts of liquid (see image at right). This is where you will add your DNA or other molecules to the gel.

How we will make our gel

1. Electrophoresis gels are made from agarose. **Agarose** is a sugar that comes from seaweed. Agarose gels have the texture of firm Jell-O. To make a gel, agarose powder is melted down in a liquid, then poured into a mold where it will cool and harden.

2. A **comb** is placed in the mold. When the gel hardens and the comb is removed, small pockets called wells are left behind.

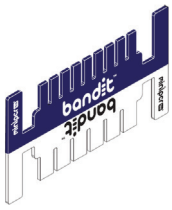


3. **Comb supports** keep the teeth of the comb from touching the bottom of the chamber. This makes it so the comb will be surrounded by *agarose* on the sides and the bottom. When the comb is removed, wells will be left in the gel.

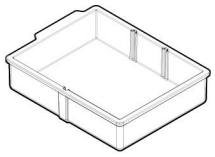
4. You will use the **buffer chamber** as the mold for your gel. The **Electrodams™** will create space at either end of your gel, since we want our gels to be a bit smaller than the buffer chamber.

First, assemble your mold:

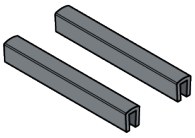
1. Gather your materials



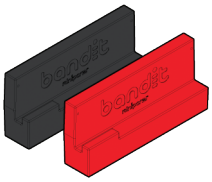
Comb



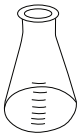
Buffer chamber



Comb supports



Electrodams™



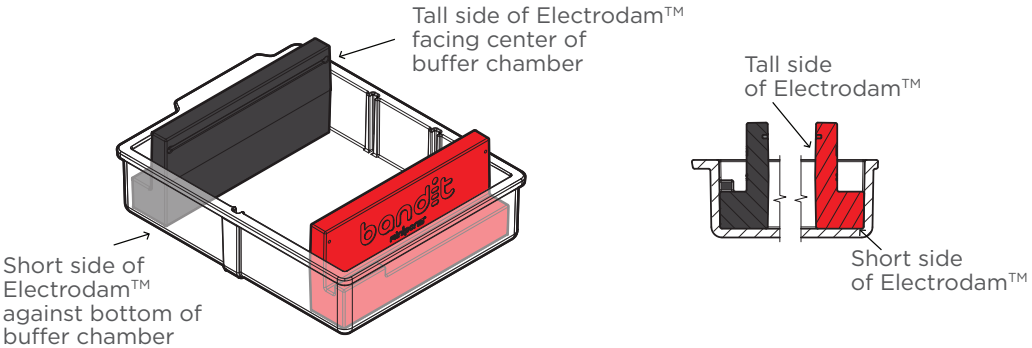
Heatproof container

Agarose Tab™

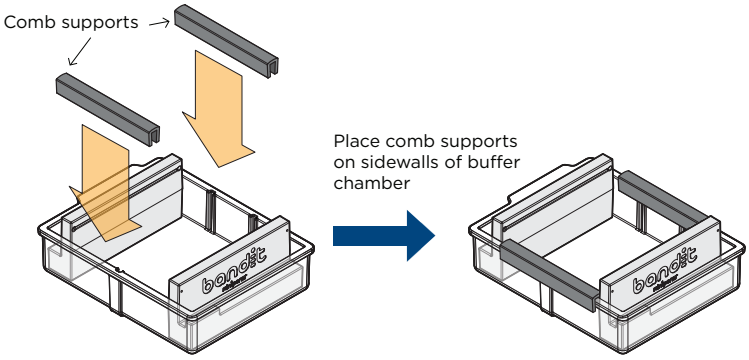
30 ml 1X TBE buffer

2. Set up the Electrodams™ inside the buffer chamber, as shown in the picture below

- One Electrodam™ should go at each end of the buffer chamber.
  Note: Electrodams™ may be used as described here with or without electrode wire attached.
- Make sure the Electrodams™ are set up so the tallest side (see diagram) faces the center of the buffer chamber.
- Each Electrodam™ should be pushed all the way down so there are no gaps between the Electrodam™ and the bottom of the buffer chamber.

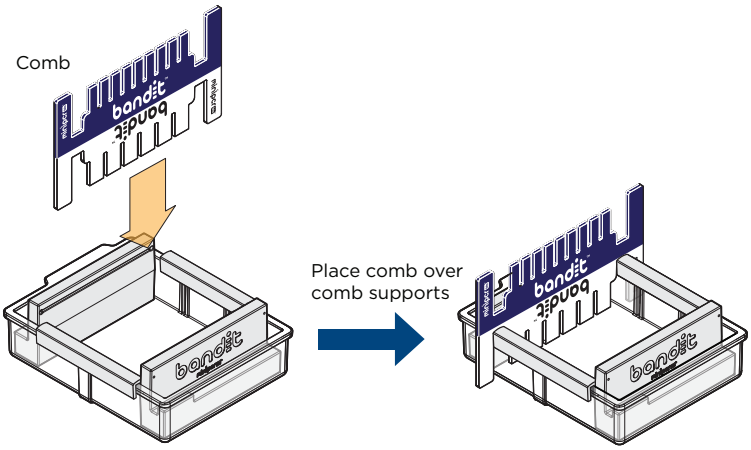


3. Place the comb supports over each side of the buffer chamber

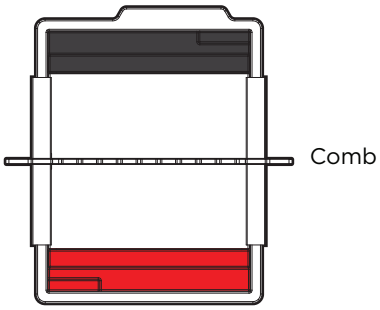


4. Place the comb in the buffer chamber, resting over the comb supports

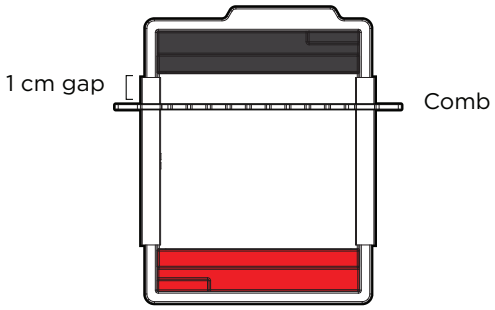
- The white side of the comb with 6 teeth should be facing down.
- The comb should be straight (parallel with the back of the buffer chamber).



If the samples in your gel may run in both directions:
Position the comb in the center of the buffer chamber, halfway between the two Electrodams™.



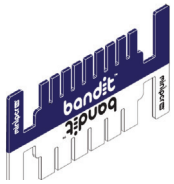
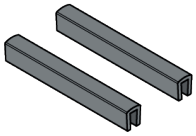
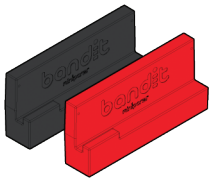
For most labs, especially dye labs that simulate DNA:
Leave approximately the width of one pinky finger (1 cm or ~1/2 inch) between the Electrodam™ and the comb.



Challenge 2: Stop and think

You have just created the mold for your agarose gel. Before we move on, remember the job each part of your mold will have in creating your final product: a rectangular gel with a row of wells.

Q1. In the table below, explain the function of each part of your gel mold. Why do we need to include each of these parts in our mold?

Part	Function
 Comb	
 Comb supports	
 Electrodams™	

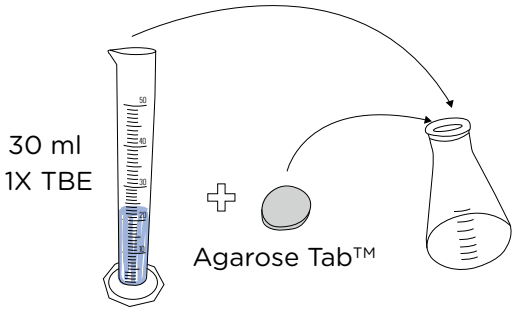
Q2. What do we call the "pockets" formed by the teeth in the comb?

Continue on to make your agarose solution and pour your gel:

Protective gloves and eyewear should be worn for the entirety of this experiment.

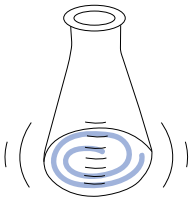
5. Prepare an agarose solution

- Use a heatproof container that is at least three times larger than the volume you wish to add.
- For ***each gel*** you plan to make, mix 30 ml of room temperature 1X TBE buffer and one Agarose Tab™ in your heatproof container.
- Allow the tabs to soak until they fully disintegrate (this could take a few minutes).
- Swirl the flask or beaker to ensure the tabs have fully disintegrated before heating.



6. Heat the agarose solution

- Heat until the solution boils and continue until the solution becomes fully transparent.
- If you're using a standard microwave, expect to heat for about 60 seconds per 30 ml of agarose.



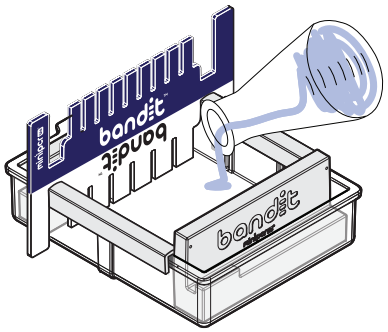
Caution: The solution may boil over the top of some containers. Watch your container to ensure that the liquid does not boil over. Wear a heatproof mitt when handling the container.

7. Pour the agarose solution into the prepared Bandit™ mold

- The agarose solution should cover the bottom of the buffer chamber and the bottom portion of the comb's teeth.

8. Allow gel to solidify completely

- Gels will typically be ready in about 10 minutes.
- Gel is ready when cool and firm to the touch.

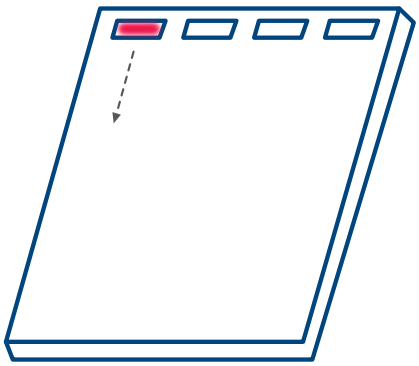


Congratulations, you've made an electrophoresis gel!

You are almost ready to run your experiment. If you are ready to continue, move on to *Challenge 3* on the next page. Otherwise, store your gel for up to five days. You can either:

- Store your gel in the assembled Bandit™: Cover your assembled Bandit™ system with plastic wrap or put it in an airtight container, then move it to a location where it will not be disturbed.
- Remove the gel so the Bandit™ can be used by other students: Follow the instructions on page 23 to remove the comb and Electrodams™. Remove the gel from the buffer chamber and store in an airtight container.

Challenge 3: Run your gel



Materials needed:

- Bandit™ STEM Electrophoresis Kit
- USB-C power source
- 1X TBE buffer
- Dye electrophoresis Learning Lab™ samples

In this challenge, we will connect our gel to our circuit, and use electrical force to move our samples through the gel. The gel will act as a filter for DNA fragments in our sample: The web in the gel will slow down the movement of larger molecules, while smaller molecules will travel farther faster. The result will be separation of the molecules based on their size.

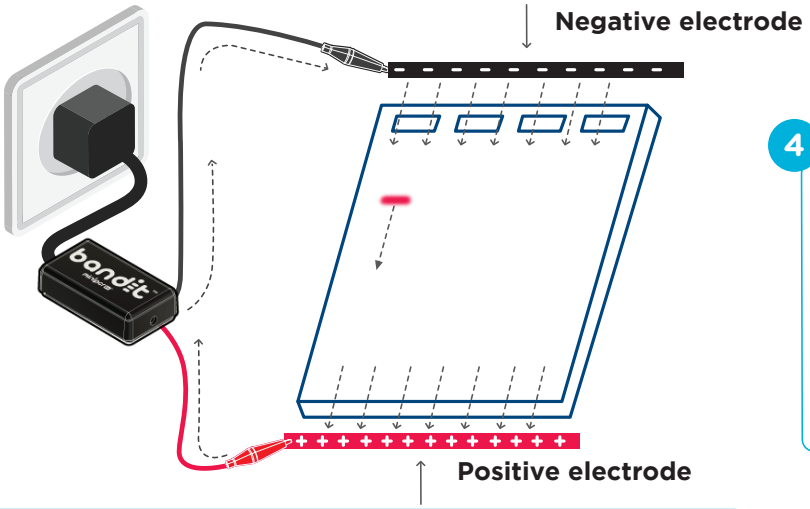
How we will run our gel:

1. We will add electrodes to our circuit to generate electric force throughout our gel. Electric force is the attraction between molecules of different charges or the repulsion between molecules of the same charge.

Particles with opposite charges are attracted to each other

Particles with the same charge are repelled from each other

2. The electrode we place at the end of the gel near the wells will be negatively charged, and will repel negatively charged molecules inside the gel, including DNA...



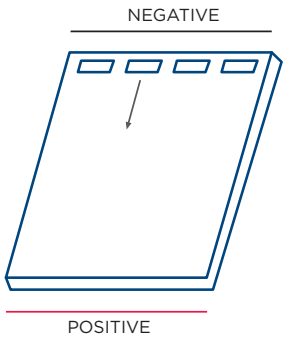
3. ... while the electrode we place at the opposite end of the gel will be positively charged, and will attract negatively charged molecules, including DNA.

4. We will pour a liquid called electrophoresis buffer over the gel and the electrodes. Together, the buffer and gel take the place of the light strip we used in Challenge 1, conducting electricity between the electrodes and completing our circuit.

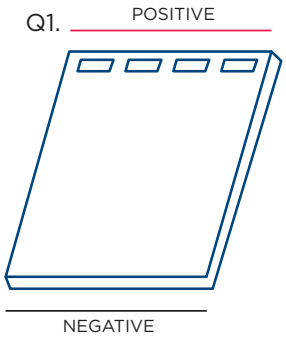
Challenge 3: Stop and think

In an electrophoresis gel, the electrodes are almost always arranged in the same way. But let's think what would happen if you set things up differently. On each of the gel diagrams below, draw an arrow showing the direction DNA will travel if the electrodes are arranged as pictured. Remember the DNA will start off in the wells at the top of the gel. An example has been completed for you to the right.

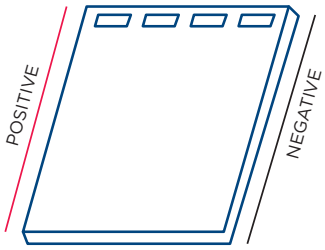
Example:



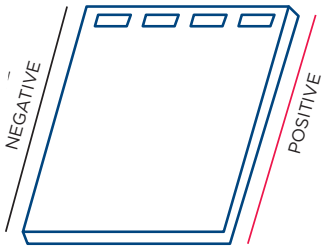
Q1.



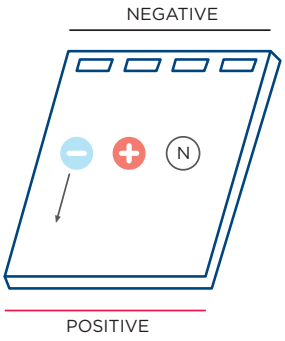
Q2.



Q3.



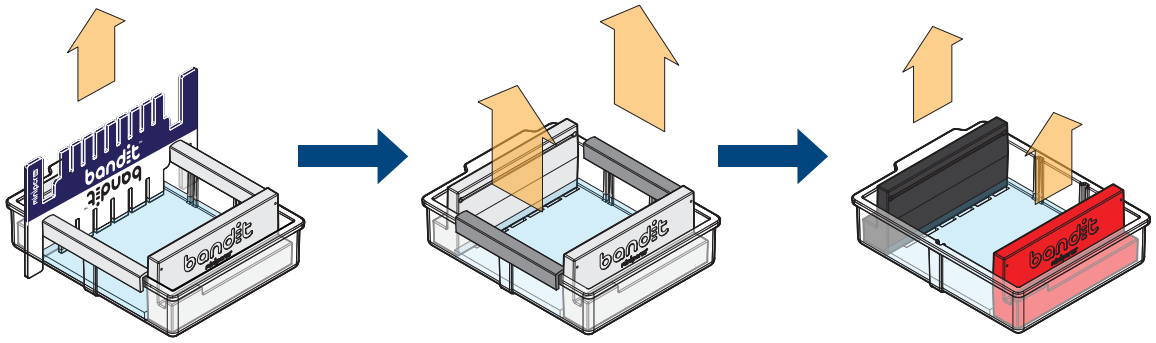
Q4. There are three molecules in the gel below: one negatively charged (-), one positively charged (+), and one with no charge ("N" for neutral). Draw an arrow showing the direction that each molecule will travel when the electrodes are powered. The first one has been completed for you.



Steps to run your gel:

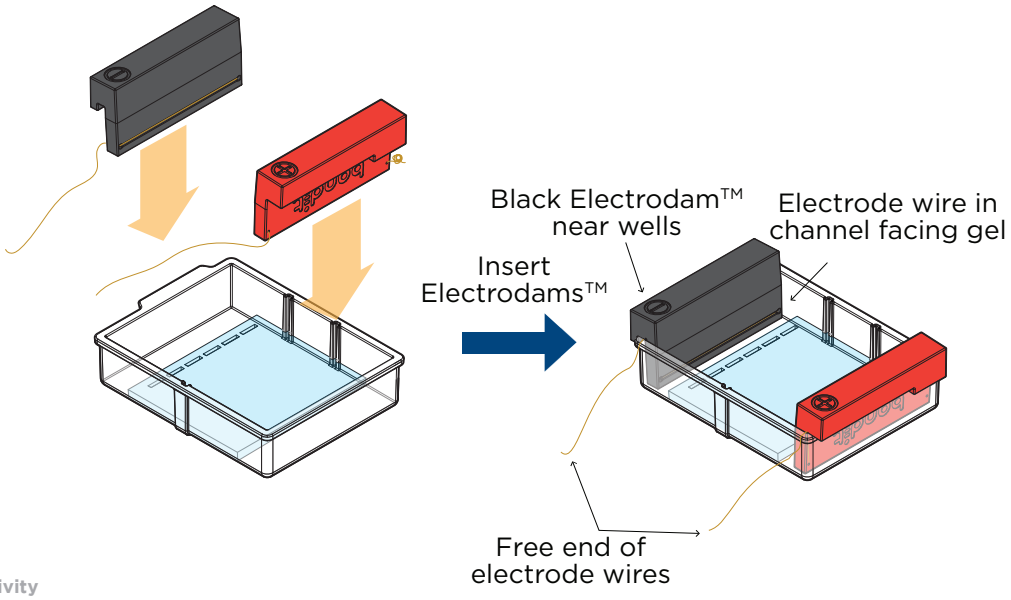
Protective gloves and eyewear should be worn for the entirety of this experiment.

1. Remove the comb, comb supports, and Electrodams™ by pulling firmly upwards



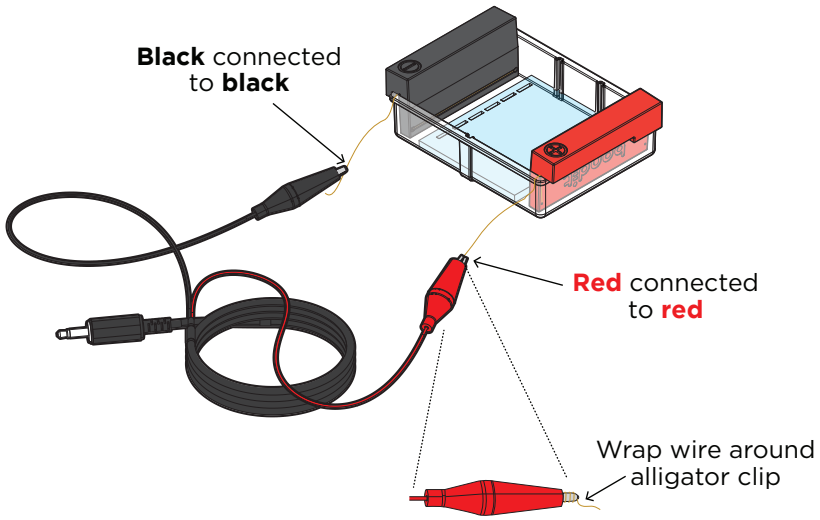
2. Insert the Electrodams™

- The Electrodams™ should have the electrode wires threaded (refer to page 15 for detailed instructions).
- The Electrodams™ should be inserted with the electrode wire near the bottom of the buffer chamber, facing the gel. They will be upside down compared to when you used them to make your gel.
- Place the **black Electrodam™** in the buffer chamber at the end of the gel closest to the wells of the gel.
- Place the **red Electrodam™** at the other end of the buffer chamber, away from the wells of the gel.
- Make sure you leave a ~1 cm gap between the Electrodams™ and the edge of the gel.
- Make sure that the long free ends of the electrodes are accessible.



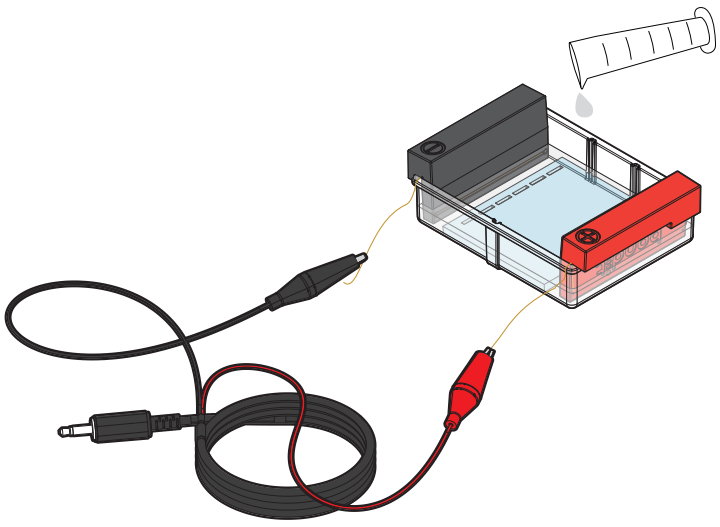
3. Connect the electrodes

- Connect the **black alligator clip** to the free electrode wire coming out of the **black Electrodam™**.
- Connect the **red alligator clip** to the free electrode wire coming out of the **red Electrodam™**.
- It may help to wrap the electrode wires around the alligator clips to ensure good contact.

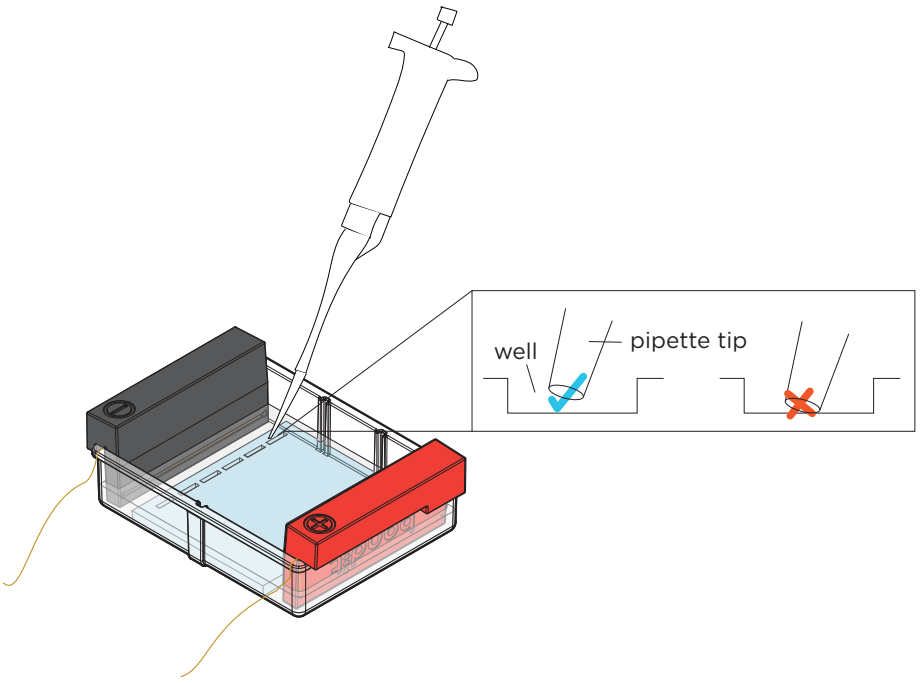


4. Add 30 ml of 1X TBE electrophoresis buffer

- Remember that this buffer conducts electricity between the electrodes, completing the circuit through our gel.
- The buffer should just cover the gel and fill the wells, in addition to the spaces between the Electrodams™ and the gel.
- Ensure that there are no air bubbles in the wells (shake the gel gently if bubbles need to be dislodged).

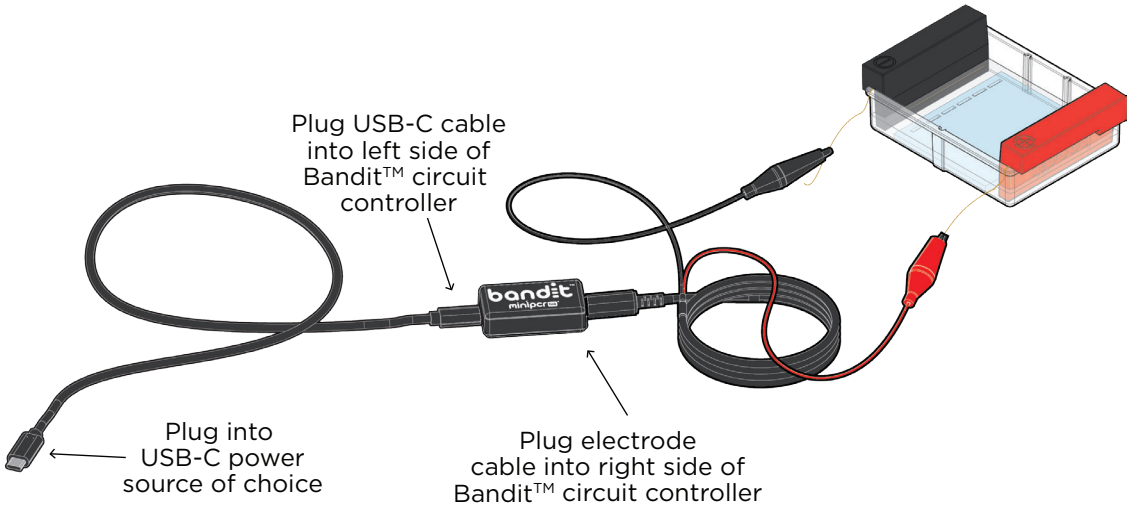


5. **Use a micropipette to load samples into the wells of your gel according the specific instructions in the laboratory guide for the specific dye electrophoresis Learning Lab™ you are using**
   - You will load your gel while it is submerged in TBE buffer. Your samples are heavier than the buffer and will sink.
   - When loading your gel, place the micropipette tip just inside the opening of the well. You do not need to get your tip near the bottom of the well (see image below).
   - When dispensing your sample, press the micropipette plunger slowly until you reach the first stop. Hold it there (but don't release!)—pressing further will add bubbles to the well, and may displace your sample.
   - Carefully lift the micropipette while still holding the plunger at first stop. Once the micropipette tip is raised above the buffer, you may release the plunger.



6. Connect the power cords

- Plug the electrode cable into the round port on the right side of Bandit™ circuit controller.
- Plug the USB-C cord into the USB-C port on the left side of the Bandit™ circuit controller.
- Plug the other end of your USB-C cord into your power source of choice.
  Note: USB-C power source is provided by the user
- Plug the power source into a power outlet.
- A small light on the right side of the Bandit™ circuit controller will illuminate to indicate that the power is on.
- Look for bubbles on the electrodes to verify that all the wires are connected and current is flowing.



7. Conduct electrophoresis for 15-25 minutes

Study questions

Use words from the word bank to fill in the blanks in the statements below. Each word will only be used once.

Word Bank
electrophoresis buffer
DNA
agarose
web
electricity
wells

1. The word "electrophoresis" means "carried by _____."

2. In an electrophoresis experiment, we load samples into _____ in a gel made of _____.

3. If you looked at it with a microscope, the inside of an electrophoresis gel would look like a _____.

4. Electrophoresis allows us to separate molecules like _____ based on their size.

5. _____ is a liquid that conducts electricity throughout the gel.

One way to understand how all the different parts of the Bandit™ work is to imagine what would happen if we left them out of the system. For each row of the table below, explain how leaving out each part would affect your experiment.

Test	Part	How would your experiment be affected if you left this part out?
6.	Comb	
7.	Electrodes	
8.	Buffer	
9.	Agarose gel	

10. Imagine DNA were positively charged instead of negatively charged, but were otherwise the same. How would you want to design your electrophoresis system differently?

11. When you're doing an electrophoresis experiment, sometimes you will be working with mostly small pieces of DNA. Other times, you will be working with mostly very large pieces of DNA. Which experiment do you think would take longer to run? Explain your answer.