



BIOTECH PROJECT

BIO5 Institute & UA Science,
Molecular & Cellular Biology

This lab activity was created by The BIOTECH Project at the University of Arizona. More information on the BIOTECH Project can be [found here](#).

Lab Activity: Build an Electrophoresis Box

The purpose of this lab activity is to build an electrophoresis box. Electrophoresis means *to move using electricity*. Molecules will move through an agarose gel matrix (Figure 1) in which an electric field is created, such that the molecules will separate based on size and charge. All charged molecules will move in the electric field, the negatively charged molecules will move towards the positive side (anode) and the positively charged molecules will move toward the negative side (cathode). As the molecules move through the gel matrix, smaller molecules will move farther than larger molecules in a given amount of time (Figure 2). Research scientists use this technique to separate different DNA fragments or protein molecules from each other.

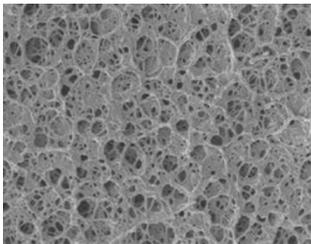


Figure 1: Image of Agarose Gel

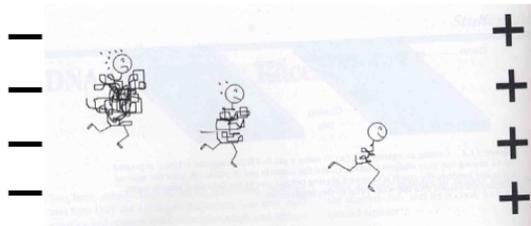


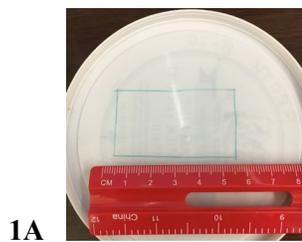
Figure 2: Molecules of different sizes moving through gel matrix

Materials for this activity (everything **bolded** is provided in the kit—**Save all materials, including paperclips and tips, in case you need to rerun the activity or for future experiments**):

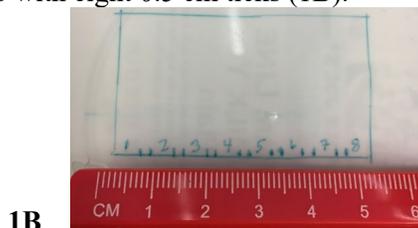
- a source of electricity (**9V Battery**),
- a tray to cast and run the gel (**medium sized weight boat**),
- electrodes to create the anode and cathode poles (**paperclips**),
- connection from the poles to the power (**alligator clips and wire**),
- agarose gel (0.8% **agarose** in TBE),
- an electrically conductive buffered solution (**TBE**),
- a way to create wells in the agarose for your charged molecules samples (comb)
- charged molecules (**dyes**)
- **pipet and tips** for adding dyes to the wells of the gel

Step 1: Making the comb

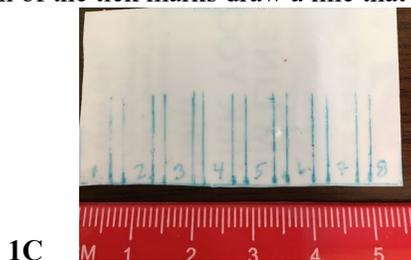
Using some kind of flexible plastic, such as the lid to a margarine or yogurt container, draw a rectangle that is about 5.5 cm by 3 cm (1A), and cut out the rectangle.



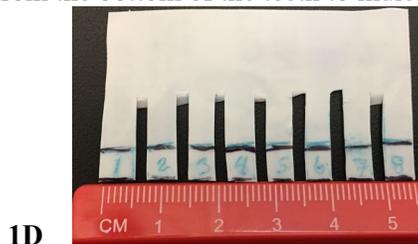
Then make tick marks beginning at 0.5 cm, then 0.2 cm, along the 5.5 cm side of the plastic, so that you wind up with eight 0.5 cm ticks (1B).



For each of the tick marks draw a line that is 1.5 cm long (1C).



The eight 0.5 cm segments will be the teeth of the comb, and the 0.2 cm segments cut out and to make the spacers between the teeth of the comb (1D). Cut and remove each of the spacers. Draw a line about 0.5cm from the bottom of the teeth to indicate how deep your wells need to be.



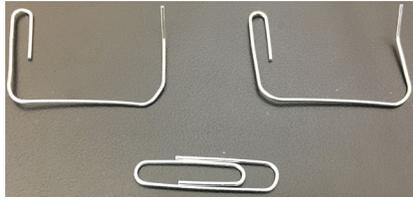
You now have the comb to create your wells in the agarose gel. You may need to trim a little off the sides if the comb is wider than the weigh boat, but you will see when you construct the electrophoresis box. You may also need to trim a little of the top if it is too long. Check to see if all the teeth are aligned and straighten them if any are crooked.

Step 2: Making the Electrophoresis Box

The electrophoresis unit generally has a tray with electrodes for creating the electric field to run the gel, and a separate smaller gel casting tray that fits inside the gel running tray. For simplicity and ease, you will cast the gel and run it in the same tray.

1. Open two paperclips by bending one side to a right angle then bend the other side to a right angle, then put a small out of plane bend on the top of the straight side (2A).

2A



2. Attach the electrodes (paperclips) to either end of the tray (weigh boat). Use a binder clip to attach the paperclip, on the small out of plane bend, to the weigh boat. The long side of the paperclip will sit at the bottom of each side of the tray; **make sure the bottom pieces are straight, see red arrow below (2B).**

2B



2. Using two paperclips at either end of the comb, attach the comb to the tray, take care that the **teeth of the comb do not touch the bottom** of the tray (2C). The teeth of the comb will make wells (holes) in the gel for your sample. The electrophoresis box is now complete.

2C



Step 3: Mix the TBE Buffer and make the Agarose gel

Making TBE solution

Tris base, Borate, and EDTA are combined to make TBE solution (Ziplock bag labeled TBE). Empty a 500 mL (16.9 oz) water bottle and clearly label it **TBE solution/DO NOT DRINK**. Add the contents of the TBE ziplock bag to the 500 ml bottle, fill the bottle to the top with water* (500 mL), cap and mix gently until completely dissolved. *If you have deionized water, use that to make the solution (this is the kind of water you would use in your iron or humidifier); otherwise tap water will work.

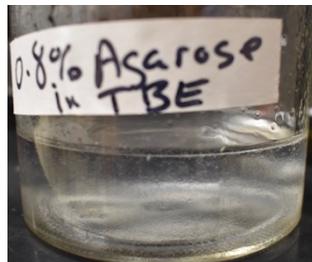
Making agarose gel

1. Obtain a clean clear glass jar (1 cup volume or larger) for mixing and melting your agarose gel. Label with a sharpie (if available): 0.8% Agarose in TBE
2. Add all of the agarose solid (smaller ziplock baggie labeled “agarose”) to the jar
3. Use a measuring cup and add ½ cup of TBE solution to the agarose (3A) Notice the agarose does not dissolve in the TBE. The mixture needs to be heated and melted to go into solution.



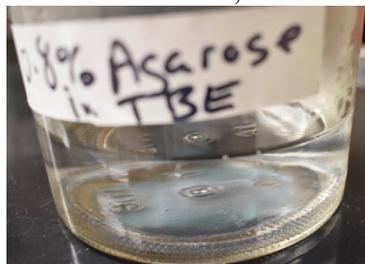
3A

4. Microwave the jar with agarose + TBE for 1 minute. **DO NOT PUT A LID ON THE JAR!**
5. **CAUTION the contents will be very hot! Let jar with hot agarose sit in microwave for 30 seconds before you pick it up.** Use a potholder (ovenmitt) to carefully remove the hot agarose from the microwave.
6. Remove the agarose and gently swirl the jar, being careful not to cause the contents to overflow. There will likely be some agarose that has not completely dissolved (3B zoom in to see the undissolved agarose). Microwave the contents for another 30 seconds and let it sit in the microwave for another 30 seconds before removing it.



3B

7. After letting the jar sit, carefully remove the jar using a potholder (ovenmitt) and swirl gently. The agarose should look clear, like water when it is 100% dissolved. Keep microwaving in 30 second intervals until the agarose is as clear as water, with no undissolved agarose.



3C

Step 4: Casting the gel

1. Make sure the comb is **not touching** the bottom of the electrophoresis tray (2C).

2. Let the microwaved agarose cool for a few minutes and then pour the agarose steadily into the tray, so that the agarose covers about 1/3 of the bottom of the comb, approximately 0.5 cm.



4A

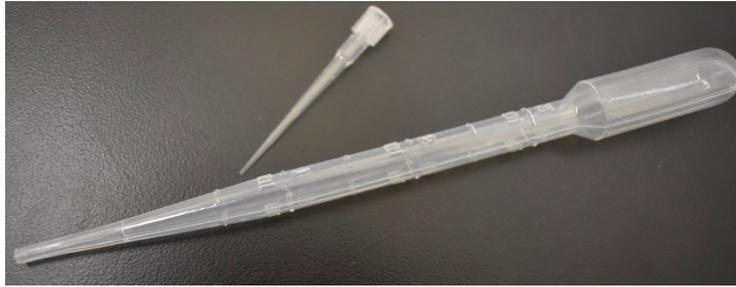
3. Let agarose solidify about 15 minutes and then gently lift the comb out of the gel, being careful not to disturb the gel. The agarose will appear translucent (harder to see through than when it was transparent like water) Note the little wells (holes) in the gel where the teeth of the comb were. These wells are where you will place your charged molecule samples (4B—wells are circled in red).



4B

Step 5: Loading samples into the gel

1. To add small amounts of samples to a gel one usually uses a precision instrument called a pipettor. These are usually adjustable from less than a microliter (μl) to 1000 μl or 1 ml. You will use the disposable tips used with these instruments attached to a disposable transfer pipet (5A).



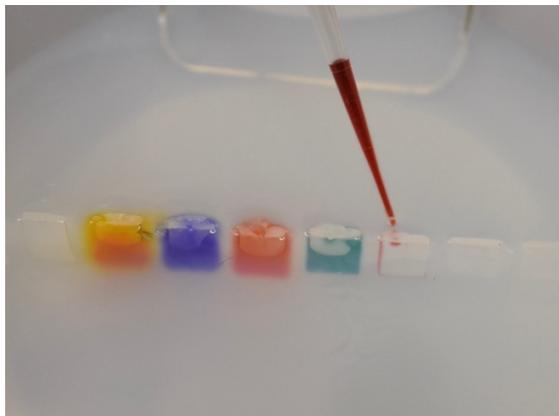
5A

2. Practice pipetting with a disposable tip with a water first to see how much pressure you need to add sample to the tip. If you look at the tip there are gradations, the first is 2 μl , the next is 10 μl . You will want to use the second gradation as to the amount of sample to add to the gel. Twist the disposable tip onto the end of the transfer pipet so that it is tightly attached (pull on the attached tip to confirm that it is tightly attached). Practice pipetting with water to learn how much pressure you need to fill the tip to the 10 μl mark (5B). You should barely need to push on the bulb.



5B

3. BE EXTREMELY CAREFUL to not over pipet your samples (overflow the wells). Pipette 10 μl of sample #1 to one of the wells, be sure to write down which well it is. Do the same with the remaining samples keeping track of which sample is in which well. Make sure your tip is just inside the well but not touching the bottom of the well when you release the sample (5C), you do not want to tear the gel.



5C

4. Gently squeeze the transfer pipette to release the sample; do not release the bulb until the tip is out of the gel.

Step 6: Run the gel

1. Once the five dye samples have been added to five different wells, carefully pour TBE to cover the gel. Do not pour directly over the wells, as the samples could be displaced out of the wells (6A).



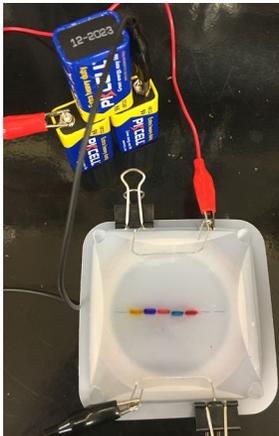
6A

2. Attach three 9V batteries to each other by connecting the negative of one battery to the positive of another, and then the positive of the first battery to the negative of a third battery. This will create a 27V power source (6B). Attach one of the colored wires with alligator clips to the exposed negative terminal and the other colored wire to the exposed positive terminal. Write down which wire is attached to which terminal. The charge of each terminal is written on the side of the battery.



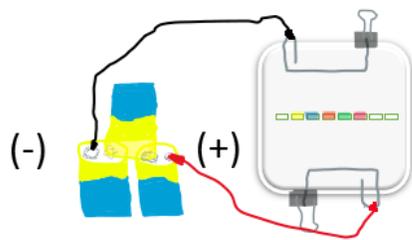
6B

3. Attach one of the colored wires with an alligator clip to one of the binder clip/paperclip electrodes. Attach the other colored wire to the other binder clip/paperclip electrode (6C).



6C

4. Draw a schematic of your set up (6D) and be sure to designate which electrode is positive charge and which is negative (you will find this on the side of the battery). The voltage from the battery will create the electric field and allow the charged molecules to move. Since each battery is only 9V, the total voltage is 27V, this will not move as quickly as it would in a lab where a 100-120V charge is used.



6D

Step 7: Observations

Make observations every 5-10 minutes while the gel is running.

1. Record the time of your observation and describe what you observe happening at the electrodes and with the various dyes.
2. The dyes are molecules of different size and charges. Once the dye molecules are moving out of the wells and through the gel, you will be able to determine what their charges and relative sizes are.
3. After running the gel for 20-30 minutes, fill out the data table below
 - A. Identify which dyes have a positive (+) and which have a negative (-) charge
 - B. Rank the relative size of the positively charged molecules, #1 being the smallest dye molecule, #2 the next largest and so on. Do the same with the negatively charge molecules, #1 being the smallest of these molecules, #2 the next largest and so on.

Dye	+ or - charge	Rank/size
#1 Yellow		
#2 Blue		
#3 Light Red		
#4 Green		
#5 Dark Red		

4. Explain your thinking for how you assigned those size rankings for each of the dyes.
5. Describe two concepts that you found most interesting about constructing and using your own gel electrophoresis apparatus.
6. This technology is used in many research labs. Generally, researchers are not running different charged dye samples. Conduct a search on the Internet and describe three uses for this technology.
7. **Save all of your electrophoresis box materials and use them in another activity.**