GEL ELECTROPHORESIS BACKGROUND

Gel Electrophoresis

We want you to understand how gel electrophoresis works. Electrophoresis is an important general technique, used in many different circumstances (separation of DNA molecules is only one application), and we want you to get a sense of the circumstances when it would be a useful technique to use.

Gel electrophoresis is a technique used to separate various types of molecules based on size and charge. Today, you will use gel electrophoresis to separate pieces (commonly called "fragments") of DNA based on their size, which we'll refer to in terms of the number of base pairs.

The Gel

The "gel" part of gel electrophoresis is a gelatinous matrix (it might help to think of it as a mesh or a complex system of tunnels) which molecules can move through at different rates depending on their physical characteristics. In this lab, you'll be using 1% or 2% agarose to make your gel.

Agarose (Fig. 1) is a polysaccharide (for you inquisitive folks, agarose is a linear polymer of alternating D-galactose and 3,6-anhydro-L-galactose.). When solidified, agarose provides an ideal matrix for separating DNA molecules on the basis of their length.

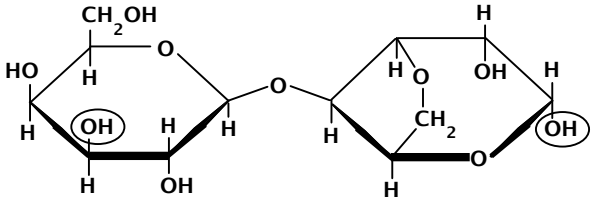

Figure 1. Repeating unit of agarose. Long chains are formed by linking units at the circled groups.

Agarose comes from seaweed, where it helps prevent desiccation. It consists of long chains of sugars which pair up and wind around each other in a double helix. Generally, we make up a gel by boiling agarose in water to dissolve it (a lot like JELL-O®); as it cools, different chains of sugars crosslink, forming a network of sugar molecules in the gel. The gels we'll be making are flat and rectangular, or slab-like, in shape.

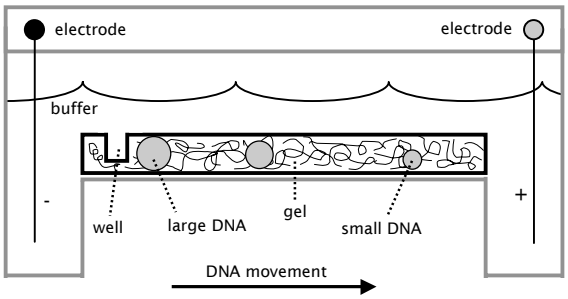

Figure 2. Gel box, side view. DNA (represented by circles) is loaded into the well of the gel at the end near the negative electrode. The DNA is pulled toward the positive electrode at the other end of the gel; how fast it moves depends on how big it is (bigger pieces of DNA make their way through the gel more slowly).

Getting DNA Into the Gel

There are wells at one end of the gel (Figs. 2 and 3); these small, rectangular depressions provide a place to introduce a sample. Glycerol is mixed with the DNA sample before adding it to the gel, which causes the sample to sink into the well (glycerol is about the same texture as thick honey).

Moving DNA Through the Gel

The wells in a gel are located near a negative electrode, and there is a positive electrode at the far end of the gel (Fig. 2). All DNA is negatively charged (remember all those oxygen atoms in the phosphate groups of the backbone?), so when a charge is applied to the system, the DNA moves towards the positive electrode.

In order to apply a current to move the DNA in a gel, the gel is placed in a gel box under a liquid (1XTBE buffer in this exercise) that supplies salts (for example, Na^+ and Cl^-) that help to provide electrical current that will move the DNA through the gel. This process of applying a current to a gel is typically referred to as "running a gel."

Why DNA Fragments Separate Based on Size

In the absence of a supporting medium (like a gel), all DNA molecules move in an electric field at essentially the same rate. However, longer DNA fragments (that is, larger DNA molecules) become tangled in the agarose matrix and as a result move much more slowly than the shorter (smaller)

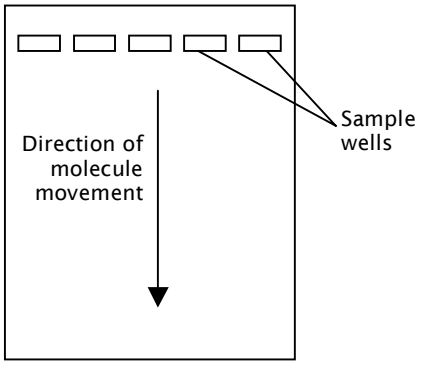

Figure 3. View of agarose gel from above, showing multiple wells. Once DNA is stained, bright bands will appear like ladder rungs, parallel to each well. Each rung will represent DNA of a certain size: rungs near the top represent large pieces of DNA and rungs near the bottom represent shorter bands of DNA.

fragments that are able to slither easily through the matrix. A 1.0% agarose gel can be used to separate DNA fragments that range in length from 300 bp (base pairs) to approximately 10 kb (10,000 base pairs). As a reference point, you have 3 billion base pairs of DNA divided amongst 46 chromosomes in each of your cells, and an *E. coli* bacterium contains 4.6 million base pairs.

Knowing When to Stop Running a Gel

How long you run a gel depends on many things, including what the gel is made of (and at what concentration) and the sizes of the DNA molecules you are interested in. Since very small DNA molecules separate from each other fairly quickly, it will not take as long to run a gel with them as it will if your DNA fragments are quite large.

The DNA is too small to see, so we can't monitor how fast it is moving directly. To prevent us from running all of the DNA off the end of the gel (and there's really nothing to stop it), we will add a mixture of two blue dyes to the DNA sample. (The dye mixture also contains glycerol, mentioned above.) One of these dyes (bromophenol blue) moves through a 1% agarose gel with the same speed as a DNA molecule 5,000 base pairs long, and the other dye (xylene cyanol) moves with the same speed as a 500 base pair DNA molecule. Since the DNA fragments we're expecting are somewhere between these two sizes, we know we'll be fine as long as we turn off the current before the smaller dye molecule reaches the end of the gel.

Keep in mind that these two dyes are *not* binding to the DNA--they are merely useful markers while we run the gel.

Seeing the DNA

In order to visualize the DNA after the completion of the electrophoresis, we will need to stain it. we will use a fluorescent stain called **GelStar** to stain our gels. GelStar fluoresces when exposed to ultraviolet light. We will add this stain to our agarose solution before we pour our gel. After we run the gel, we'll then view it under ultraviolet light and photograph the stained gel. GelStar, because it binds to DNA, is treated as a carcinogen. Be sure to wear gloves when working with the gels once the GelStar has been added.

In Fig. 3, note that the wells in a gel are positioned at the top; this is a standard orientation for reporting results. You may have seen photos of gels like this, with dark bands parallel to the wells.

Bands representing larger DNA molecules will appear near the wells, and bands representing smaller DNA molecules will appear closer to the bottom of the gel.

Knowing How Big Your DNA Is

Since you are separating DNA based on size, and all pieces of DNA that are the same size migrate through the DNA at the same rate, it is useful to include a marker lane on your gel. A **marker lane**, or **ladder**, contains a set of DNA fragments which are of known sizes. You can actually graph the relationship between the distance a band of DNA moves from the well and the number of base pairs in that band of DNA. If you do this for each of the bands in the marker lane, you will see a relationship emerging. The relationship between size and distance, as you will see, is not linear; as the length of the DNA decreases, the distance traveled increases exponentially.

How does this relationship in the marker lane help us determine the size of DNA in other lanes? It should be easy for you to believe that if we have a band of DNA in one of our "unknown" lanes that is the same distance from its well as, say, the 8000 bp band in our marker lane is from its well, then the band of unknown DNA is also 8000 bp in length. Having a graphical relationship between the marker lane bands and distances allows us to determine the

size of unknown bands which fall between two of the marker lane bands (for example, at 7500 bp).

Experimental Procedures

Gel Electrophoresis I: Preparing the Gel

1. Making an agarose solution:
   a. Calculate the amount of agarose you should weigh out to make your solution (you can do this before lab for both concentrations):

      40 ml 2% agarose:

      weigh out _____ g of agarose

      dilute in _____ ml of TBE buffer

      Use the fact that 1 g is equal to 1 ml to do these calculations. Measuring the volume of TBE buffer to the nearest milliliter is fine when you are making agarose for a gel.

   b. Check your calculations with your lab instructor or your TA before proceeding.

   c. Using one of the balances in lab, carefully weigh out the appropriate amount of agarose, and mix it in the proper amount of TBE buffer (not water). Use the glass flask at your bench.

      It will not go into solution until the next step. The TBE buffer is in a large plastic container with a spigot on the side bench.

   d. Microwave your agarose solution, checking the flask often to prevent boiling over. Use a red rubber pot holder (these are called "hot hands") to handle the flasks, since the glass will get very hot.

      The agarose should be completely dissolved and the solution should be uniformly clear when you swirl it around.

   e. Take your flask back to your bench and let it cool until you can comfortably touch the glass flask.

      You can do step 3a while you are waiting.

2. Adding GelStar DNA stain to your agarose:
   a. After your flask of agarose has cooled enough that you can touch it near the top comfortably, it is time to PUT ON GLOVES.

      Any time you work with your agarose or gel for the remainder of the lab, you will need to be wearing gloves.

   b. Ask your TA to add 4 µl of GelStar stain to your flask. Wearing gloves, swirl the flask gently to mix, and proceed to the next step.

3. Casting a gel:
   a. Wearing gloves, raise the sliding gates of your gel-casting tray and fasten them in place by *gently* tightening the plastic screws at either end (Figure 4). Place the plastic comb into the slots nearest the end of the gel casting tray.

      The gel-casting tray is a small, clear plastic rectangular tray with short sides. Place the prepared tray on a level, undisturbed location on your bench so that when you pour the liquid agarose solution into the gel-casting tray it can solidify unperturbed.

   b. Wearing gloves, pour the agarose solution into the prepared casting tray, watching to make sure the agarose does not overflow. You may not need to use all the agarose you have prepared.

      If you pour the agarose while it is too hot, you can warp the casting tray, which may cause leaking.

   c. After pouring, check to make sure that there are no large bubbles in the molten agarose. If there are bubbles, use a clean pipette tip to move the bubbles to the side of the tray.

   d. Leave the gel alone to harden for several minutes while you go on to the next section.

Gel Electrophoresis II: Loading and Running the Gel

4. Practicing loading a gel:
   a. Find the round, glass finger bowl at your lab bench, and fill it half full (or so) with distilled tap water (the tap with the black handle at any

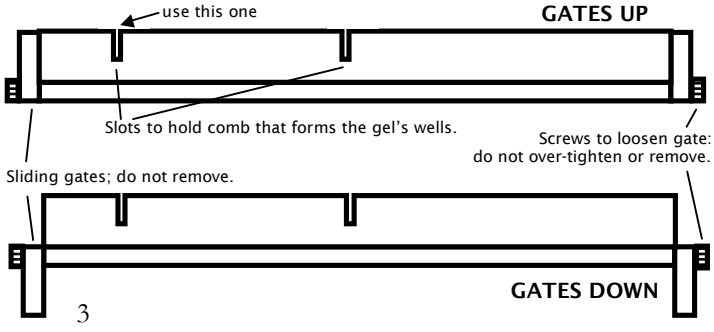

Figure 4. Gel casting tray, side view. Top: gates up. Bottom: gates down.

of the sinks).

b. Carry the finger bowl over to the practice gels (floating in a plastic box on a side or back bench). Using a spatula, carefully move one of the practice gels from the large container of water (on the side or back bench) to the finger bowl. Make sure the wells are right-side up: try not to flip the practice gel over while moving it.

c. The water in your finger bowl should cover the top of your practice gel; add more water if needed.

d. Make up a practice loading solution:

- find a clean microfuge tube
- add 60 µl of water from your glass bottle to the tube
- add 9 µl of loading dye to the tube
- mix

The loading dye, also called "loading buffer" or "blue juice" is what contains the two blue dyes (for visualization of the sample) and the glycerol (to help the solution sink into the well).

e. Take turns at your bench loading 11 µl of the practice loading solution into each well of your practice gel. Each person should load at least two lanes.

Dip the pipette tip through the surface of the water, centering it over the well. The tip should just barely enter the well. Do not jam the tip down into the bottom of the well (you might make a hole in the bottom of the well). Anchor your elbows on the table top (never mind what your mother told you) and use your free hand to steady your pipetting hand. Slowly and steadily depress the exhaust plunger. It may (or may not) be necessary to depress the plunger to the second stop to exhaust the entire sample. You should see the blue sample sink neatly into the well. Do not release the plunger until you have removed the tip from the well. Congratulations! You may throw away the practice gel after all the members of your group have practiced: gel in the trash, water in the sink.

5. Loading a real gel:
   a. Make sure the gates on your tray are down, and place the tray, still containing the two gel halves, into the gel rig (the bigger plastic box). Place the tray into the gel box with the proper orientation: the comb end of the gel should be closest to the black (negative) electrode.

   If there is already buffer in the rig, you can leave it there and re-use it (as long as the rig has been covered to prevent evaporation).

   b. Add enough TBE buffer to completely cover the gel.

   Do not fill the gel rig completely (it will run too slowly); just make sure the gel is completely submerged.

   c. Locate the two tubes you prepared earlier. Tap the bottom of each tube gently on the bench top to collect any droplets of sample.

   You will be loading a marker lane and an unknown lane into each half of the gel; this will allow you to compare how the same samples look in different concentrations of agarose.

   d. Load 11 µl from each tube into each half of the gel, making sure you have a marker lane and an unknown lane loaded into each agarose concentration. Be careful not to let any bubbles into the pipette tip when you are drawing the sample into the tip. Use the same technique you used with the practice gel.

   It doesn't matter which wells you use, unless you believe the center lanes (3 from your gel and 4 from the other lab groups' gel) were damaged in the transfer. In that case, use the outside two wells for each gel half (1, 2, 5, and 6). In your notebook, make a clear record of which sample is loaded into which well.

6. Running the gel:
   a. When all of the samples have been loaded, re-check the orientation of the gel with respect to the electrical leads. Remember that DNA, having a net negative charge, will migrate toward the positive (red) electrode.

   b. Placing the lid on the box connects the leads to the gel box. Connect the other ends of the leads to the power supply. Check that the power supply is set at the voltage specified by your lab instructor, and then turn on the "juice".

7. Viewing the gel:
   a. First, turn off the power to the rig. Then, unplug the cords from the power supply and remove the lid from the gel rig. If the other group at your lab bench needs to run their gel longer, be sure to turn the power back on for them.

b. Carefully, wearing gloves, remove your casting tray (still containing the gel) from the gel rig and set it on your benchtop.

c. Remove the gel from the casting try and transfer it to the plastic sandwich container at your bench. Do not allow the gel to flip over as you make the transfer. Carry the gel over to the sink, and rinse it gently with distilled tap water to remove any unbound stain from the gel. When this destaining has been completed, you will view your gel on the transilluminator (UV light box). Your TA or lab instructor will help you with this.