

## Agarose gel and PCR cleanup

## Genetics 362

Last week you set up a PCR reaction to amplify a section of the plant genome called ITS. Today you will run your reaction on an agarose gel to determine whether your PCR reaction was successful. That is, you will carry out agarose gel electrophoresis to determine whether you have obtained a DNA fragment of the expected size.

### **Objectives:**

Learn how to run an agarose gel

Learn how agarose gel electrophoresis works and how to determine the size of a PCR fragment.

Find out if PCR was successful, clean fragment to prepare for sequencing.

### ***What is agarose gel electrophoresis***

*Agarose gel electrophoresis* is a technique that allows us to separate fragments of DNA by size. DNA is negatively charged (because of the phosphate backbone). As you run the gel, the electrical current pulls negatively charged ions towards the positive pole, and pulls positively charged ions towards the negative pole. DNA will run towards the positive pole. The pores in the agarose gel impede the large DNA fragments more than the small fragments, so small fragments migrate faster than large ones.

*What is an agarose gel?* Agarose is a chain of sugar molecules extracted from seaweed. When mixed with water, heated, and then cooled, it forms a solid, jello-like substance. Rather than mixing with water, we mix the agarose with a water-based buffer. The buffer contains salts that conduct electricity.

*Size standard:* In order to determine the size of your PCR fragment, you need a size standard. The size standard contains DNA fragments of known size. To determine the size of your PCR product, you can compare its position on the gel to the size standard. We often refer to the size standard as a "ladder."

*DNA stain:* You will not be able to see your DNA unless you stain it. The most common DNA stain is ethidium bromide. Ethidium bromide inserts itself between the DNA nucleotides and becomes visible when exposed to UV light. Your gel contains ethidium bromide, so we will be able to see the DNA from your PCR reaction when we put the gel on the UV light box.

**Ethidium bromide may cause mutations and cancer**, so please be careful and **wear gloves** when you are loading your sample onto the gel. **UV light can damage your eyes**, so don't look at the light box unless the protective plastic cover is over the light.

### ***Pouring agarose gel***

Your TA will pour your gel so you don't need to sit around while it sets. You will need to share a gel with other students.

### ***Load and Electrophorese PCR Products***

- Put on a pair of gloves
- Obtain:
  - Your PCR reaction
  - a tube of loading dye
  - a 100-200 $\mu$ l pipettor and tips
- Add:
  - 7 $\mu$ l loading dye** to your PCR reaction. The loading dye is heavier than the buffer that your gel is sitting in. It will make your sample sink into the well.
- Mix by **gently** pipetting up and down gently until the entire solution is uniform in color. Release the plunger of the pipettor gently, otherwise, you may contaminate the pipettor with your PCR product. Pipettor contamination is the bane of the genetics lab.
- The gels should be submerged under some liquid (running buffer – a salt solution that can conduct electricity). There is only 1 gel for the whole class, but you just need one lane for your sample. Make sure your DNA will run the right direction - DNA runs black to red, so you should load the gel at end near the black terminals.
- Use the pipettor to slowly and gently add **7ul of your PCR product to an empty well** in one the gel. Push out the liquid **slowly**. If you push the liquid out of the pipettor too fast, your sample will shoot out of the well. You want your sample to sink into the well.
- Count the wells from the left and write down which has your sample**

There should be a tube of DNA ladder (a size standard) sitting near the gels. Your TA will add 6ul of ladder should get added to one of the wells. If ladder hasn't already been loaded on your gel, go ahead and add it to an empty well.

When all the samples have been have been added to the gels, turn on the electricity (100-120 Volts) and your DNA should start moving towards the other end of the gel. Since Ethidium Bromide is NOT visible to the naked eye, you should NOT actually see your DNA. The orange, purple and blue bands are colored chemicals in the loading dye. If you come back in 5 minutes, you should see that the loading dye has moved.

Ethidium Bromide is positively charged. As you run your gel, the ethidium bromide that is not attached to DNA migrates in the opposite direction as the DNA.

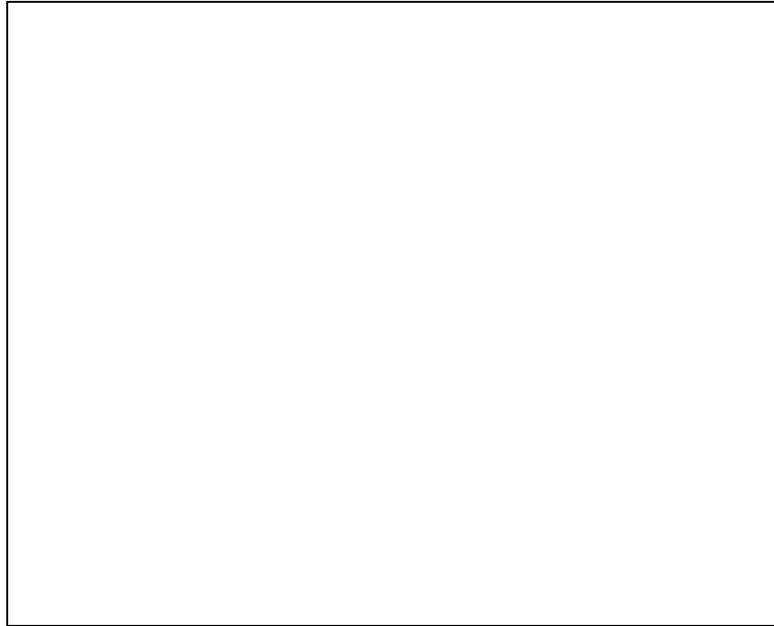
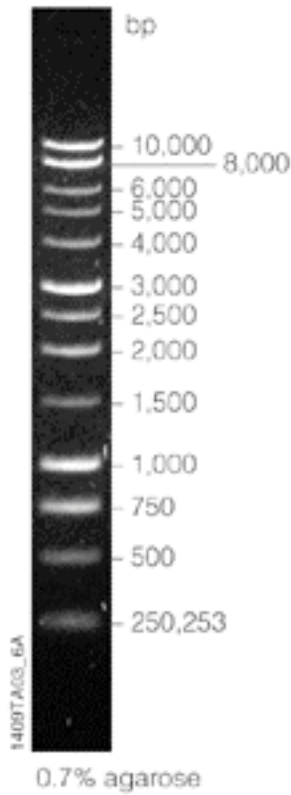
Let your gel run for about 20 minutes.

- When the gel is finished running, turn off the electricity, and see if your PCR reaction worked.

Draw what you see on your gel. Label the sizes of the fragments on the gel.

size standard

your gel



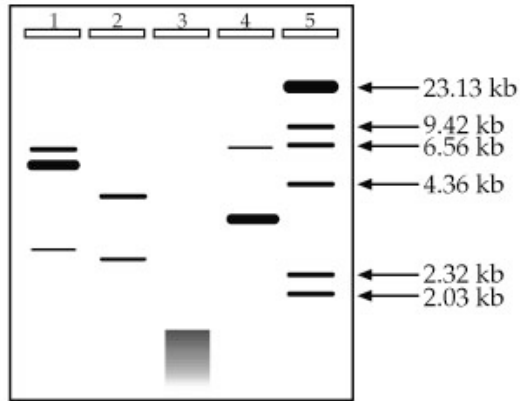
What size are the PCR products? \_\_\_\_\_

Did your PCR reaction work? \_\_\_\_\_

If not, you won't be able to use your sample for sequencing. Next week, use your lab partner's sample (or someone else's) and be sure to record what sample you are using!

**Gel Electrophoresis and PCR cleanup questions:**

1) Identify the approximate sizes of each fragment on this gel.



2) Why does agarose gel electrophoresis separate molecules?

3) Why is loading dye added to the PCR product before loading it into the wells?

4) Ethidium bromide is positively charged, while DNA is negatively charged. If you add too much ethidium bromide to your sample before loading it into the gel, what do you think might happen?