Gel Electrophoresis Protocol (modified Addgene protocol) Version 1.1, 2017

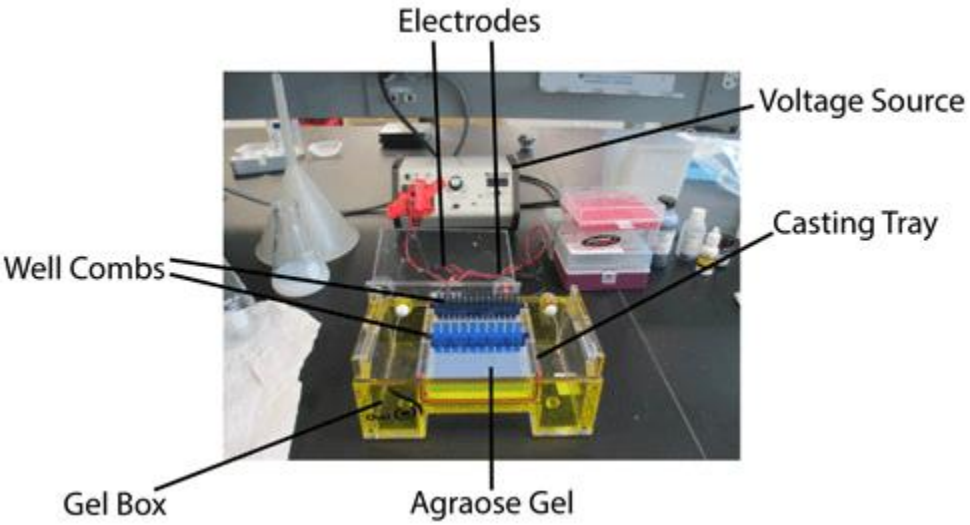
Background Information: Gel electrophoresis is the standard lab procedure for separating DNA by size (e.g., length in base pairs) for visualization and purification. Electrophoresis uses an electrical field to move the negatively charged DNA through an agarose gel matrix toward a positive electrode. Shorter DNA fragments migrate through the gel more quickly than longer ones. Thus, you can determine the approximate length of a DNA fragment by running it on an agarose gel alongside a DNA ladder (a collection of DNA fragments of known lengths).

Pouring a Standard 1% Agarose Gel:

1. Measure 1 g of agarose.

   Note: *Agarose gels are commonly used in concentrations of 0.7% to 2% depending on the size of bands needed to be separated. Simply adjust the mass of agarose in a given volume to make gels of other agarose concentrations (e.g., 2 g of agarose in 100 mL of TAE will make a 2% gel).*

2. Mix agarose powder with 100 mL 1xTAE or TBE in a microwavable flask.



   Note: *TBE can be used instead of TAE, labs usually use one or the other, but there is very little difference between the two.*

   Note: *Make sure to use the same buffer as the one in the gel box (do not mix different buffers and do not use water).*

3. Microwave for 1-3 min until the agarose is completely dissolved (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel. Many people prefer to microwave in pulses, swirling the flask occasionally as the solution heats up.).

   Note: *Caution HOT! Be careful stirring, eruptive boiling can occur.*

   Note: *It is a good idea to microwave for 30-45 sec, stop and swirl, and then continue towards a boil. Keep an eye on it as the initial boil has a tendency to boil over. Placing saran wrap over the top of the flask can help with this, but is not necessary if you pay close attention.*

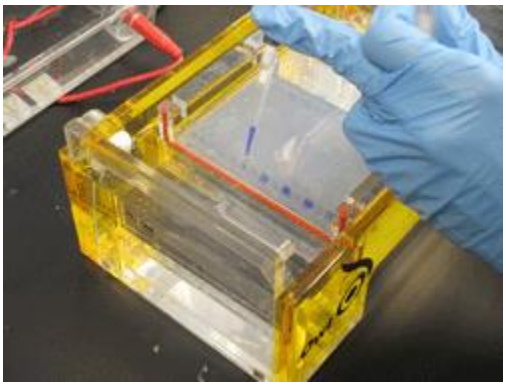
4. Let agarose solution cool down to about 55°C (about when you can comfortably keep your hand on the flask), about 5 mins.

5. Pour the agarose into a gel tray with the well comb in place.

   Note: *Pour slowly to avoid bubbles which will disrupt the gel. Any bubbles can be pushed away from the well comb or towards the sides/edges of the gel with a pipette tip.*

6. Place newly poured gel at 4°C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

   Note: *If you are in a hurry the gel can also be set more quickly if you place the gel tray at 4°C earlier so that it is already cold when the gel is poured into it.*

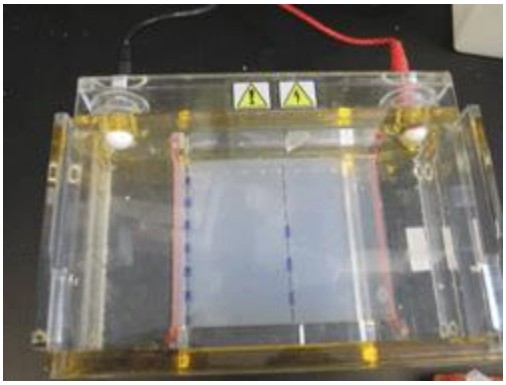


Loading Samples and Running an Agarose Gel:

1. Add loading buffer to each of your digest samples. You may skip this step if running PCR product already containing coral loading dye.

   Note: *Loading buffer serves two purposes: 1) it provides a visible dye that helps with gel loading and will also allows you to gauge how far the gel has run while you are running your gel; and 2) it contains a high percentage of glycerol, so it increases the density of your DNA sample causing it settle to the bottom of the gel well, instead of diffusing in the buffer.*

2. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
3. Fill gel box with 1xTAE (or TBE) until the gel is covered.
4. Carefully load a molecular weight ladder into the first lane of the gel. We use several different ladders, so please read the label on the ladder to ensure you are adding the correct amount.



   Note: *When loading the sample in the well, maintain positive pressure on the sample to prevent bubbles or buffer from entering the tip. Place the very top of the tip of the pipette into the buffer just above the well. Very slowly and steadily, push the sample out and watch as the sample fills the well. After all of the sample is unloaded, push the pipettor to the second stop and carefully raising the pipette straight out of the buffer.*

5. Carefully load your samples into the additional wells of the gel.
6. Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel.

   Note: *Black is negative, red is positive. (The DNA is negatively charged and will run towards the positive electrode.)* ***Always Run to Red.***

Note: *A typical run time is about 1-1.5 hours, depending on the gel concentration and voltage.*

7. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box. Rinse the chamber and set it in our designated drying location.
8. Place the gel into a staining container with ~100 mL of 1X Fast Blast and rock for several hours or overnight.
9. Use the Gel Doc EZ System and a computer to visualize your DNA fragments.

    Note: *If you will be purifying the DNA for later use, use long-wavelength UV and expose for as little time as possible to minimize damage to the DNA.*

    Note: *The fragments of DNA are usually referred to as 'bands' or amplicons (when using PCR) due to their appearance on the gel.*

Analyzing Your Gel:

Always save your gel image with the date, name of the run, and your last name. **Be sure to always save as a .SCN file.**

Purifying DNA from Your Gel:

If you are conducting certain procedures, such as molecular cloning, you will need to purify the DNA away from the agarose gel. For instructions on how to do this, visit the Gel Purification page.

Tips and FAQ

- **How do you get better resolution of bands?**

  A few simple ways to increase the resolution (crispness) of your DNA bands include: a) running the gel at a lower voltage for a longer period of time; b) using a wider/thinner gel comb; or c) loading less DNA into well. Another method for visualizing very short DNA fragments is polyacrylamide gel electrophoresis (PAGE), which is typically used to separate 5 - 500 bp fragments.

- **How do you get better separation of bands?**

  If you have similarly sized bands that are running too close together you can adjust the agarose percentage of the gel to get better separation. A higher percentage agarose gel will help resolve smaller bands from each other, and a lower percentage gel will help separate larger bands.

- **10% Rule:**

  For each sample you want to load on a gel, make 10% more volume than needed because several microliters can be lost in pipetting. For example, if you want to load 1.0 µg in 10 µL, make 1.1 µg in 11 µL.