Agarose Gel Electrophoresis

To analyze PCR results, we run the PCR products on an agarose gel and visualize the separated DNA or RNA using UV light.

A standard 1% agarose gel contains 1g of agarose for every 100 ml of TBE buffer. Lab protocols may require percentages between 0.7% to 3%, depending on the size differences between the DNA fragments and the absolute sizes of those fragments. We add GelRed to the liquid agarose solution. Once the gel is solidified, the GelRed is used to visualize DNA fragments in the gel under UV light.

Place the tray into a gel box so the DNA will 'run to red' that is, travel towards the red lead, when the lid is in place. Pour buffer over the gel, so hat it just covers the gel, and no higher to 1 cm from the chamber top. Gather all samples that are to be run, an appropriately sized marker or ladder, and 6x loading dye.

Make a gel map to record in which lanes your group plans to put each sample and the DNA marker.

> **Research Tip**: If you want to use your PCR product later for cloning or another assay, don't add loading dye into your PCR tubes. Instead, combine dye and DNA on parafilm as you load the gel. To do this, cut a 2x2 sheet of parafilm, use a P20 to make a drop of ~2-3 ul of dye onto the parafilm for each sample to be run. Add 10-20 ul of PCR product to the dye and pipette up and down to homogenize. Do not allow the different samples to mix on the parafilm. Immediately add the sample to the appropriate gel well.

Load the sample/dye mix into each well. Once all samples have been loaded, attach a lid to the rig, and attach the lid to the power supply (NOTE: always make sure that the current is off or paused before inserting or removing a cords from the power supply). Set the voltage to 100V and run the gel for about 60 minutes. If current is flowing through the gel, then tiny bubbles will be visible rising from one end of the box. It is advisable to check up on the gel from time to time to make sure that it is proceeding in the correct direction and to see how far the samples have migrated.

> The blue **loading dye** used in this lab contains 2–3 dyes that migrate as follows:
> xylene cyanol FF at 4 kb
> bromophenol blue at 300 bp
> orange G at 50 bp (not always included)

When the gel the DNA has migrated the distance you desire, pause/stop the voltage, disconnect and remove the lid, and carefully slide your gel into a Ziploc sandwich bag. Label the bag up near the zipper. Obtain a picture of your gel under UV light using the Bio-Rad Gel Documentation system in the back of room 123. Clean up your gel station and lab bench during this time.