**Agarose Gel Electrophoresis** In order to analyze PCR results, the products are run on an agarose gel and the resulting gel is observed in UV light. Run the products on a 2% agarose gel. Load all 10 µl of each sample (adding an appropriate amount of loa ding buffer to them).

First, the gel has to be made. A standard 1% agarose gel uses 1g of agarose for every 100 ml of TBE buffer. One may use percentages between 0.7% to 3%, depending on the size differences between fragments and the sizes of those fragments. The prep room will handle creating the molten 3% agarose solution with GelRed, which allows us to visualize DNA using UV light (see image). In the lab, you will need to assemble a gel rig and find a comb with an appropriate number of wells, then place the comb into the rig. Pour the warm agarose solution from the flask into the rig and wait about 20 minutes for it to solidify.

GelRed staining of DNA in an agarose gel. Image credit: Biotium.

Once the gel has solidified, make sure it is oriented correctly relative to the red and black leads in the chamber lid. Pour buffer over the gel, filling the gel chamber to 1 cm from the top but no higher. Gather all samples products that are to be run, an appropriately sized ladder (usually a 100-bp ladder), and 6x loading dye. First, load 5 µl of DNA ladder into the first well.

> Promega 100 bp DNA ladder contains DNA fragments of specific lengths, from 100 bp to 1000 bp in 100 bp increments and an 11$^{th}$ fragment that is 1500 bp in length.

Next, add a ~1-2µl drop of loading dye directly to each sample tube. Once all samples are combined with dye, load the 10 µl of sample into the gel, making note of what sample goes into what lane.

> **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 4x4 sheet of parafilm, make a drop of ~1 µl of dye onto the parafilm for each sample to be run. Add 5–10 µl of PCR product to the dye and pipette up and down to homogenize. Immediately add the sample to the appropriate gel 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 150V and run the gel for about 30 minutes. It is advisable to check up on the gel from time to time to make sure that it is proceeding normally. You do this by monitoring the bubbles from the platinum wire lead in the buffer chamber and the migration of the different dye fronts in the gel itself.

> The blue **loading dye** used in this lab contains three dyes that migrate as follows:
> xylene cyanol FF at 4 kb
> bromophenol blue at 300 bp
> orange G at 50 bp

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. Inform your TA that you are ready to visualize your gel. Your TA will take the gel to the imaging machine and provide you with a gel image. Clean up your gel station and lab bench during this time.