Gel electrophoresis

To check if the DNA extraction worked or if the PCR steps worked and actually amplified a sequence of DNA, we can use gel electrophoresis (or "run a gel" - genetics jargon) to show us the DNA. We run a gel to see if there are any bands (DNA) at all, and what size the fluorescent bands are.

Before you begin:
1) Make sure you are starting with a tidy, clean space, and that you have your lab safety attire on. Wipe down your workspace and tools with bleach wipes.
2) Read through the protocol. If something doesn't make sense, ask for clarification!
3) Write down key information in your lab notebook (Date, title of experiment for the day, lab partner, sample ID/#s you will be working on).
*Make space in your notebook to write down which well in the gel each sample went in.
4) Make sure you have everything you need for the lab.

Materials you should have:
Gloves
Notebook
Your DNA extraction or PCR product (check the label!)
Access to a microcentrifuge

Materials:
Agarose
1xTAE
Erlenmeyer flask
Microwave + oven mitts
EtBr (Ethidium Bromide)* *- be VERY careful with this. It binds to DNA, which is why we use it, but that quality also makes it a mutagen.*
Pipetters and pipette tips

Agarose recipe and protocol for 1% agarose gels:
1g agarose
100ml 1xTAE

STEP 1: Measure out 1g of agarose into weighboat (or tare the scale with the flask and add the agarose).
STEP 2: Add 100ml of 1xTAE buffer
STEP 3: Swirl to mix and then heat in microwave at 30 sec intervals. Between each interval, wear a protective mitt, take out, swirl check for unmelted crystals and reheat until all crystals disappear.
***While some of the group is working on making the agarose solution, others can set up the gel mold with the appropriate combs.

STEP 4: Let the agarose solution cool until it's hot to the touch but doesn't burn you. Then add 1μl of **Ethidium Bromide**, swirl until well mixed and pour into the gel mold. Let sit ~20 minutes or until gel is set.

Meanwhile: Fill out notebook with any notes that should be added (how long does it take to get the agarose to melt? The gel to solidify? Start setting up to load the gel rig with DNA while you wait for the gel.

Gel loading prep:
Cut a small strip of parafilm (~3cm x 4 cm) and put 1μl drop of loading gel onto it.
Have your PCR product ready and on ice.

STEP 5: When the gel has solidified, carefully remove the comb, set the gel so the wells are at the cathode (-/black) end and the rest of the gel is on the cathode (+/red). Fill the rig with 1xTAE buffer so that it covers the gel and fills the wells.
STEP 6: Add 3μl of **EtBr** to the 1xTAE buffer at each end of the rig.
STEP 7: To load the gel with your PCR product, pipet 3μl of DNA onto your drop of loading dye on the parafilm. Mix it gently by pipetting in and out a few times. Then, pipet 3μl of the PCR product + dye and carefully pipet into your designated well. Make sure you are not loading over another persons sample and make sure you write down which gel, and which well, your sample went into.
STEP 8: Once all the samples in your gel group have been loaded, put on the lid to the rig, set the voltage to 80-100V and start. Check that there is bubbling in the TAE buffer of the rig to know that it is working properly.
STEP 9: Check on the gel regularly to make sure that the loading dye is moving (and moving in the right direction!) When the band is about ⅓ of the way down the gel, turn off the power and remove the gel.