**Gel Electrophoresis Protocol**

*Updated: 2.7.18 SMB*

**Purpose:**

To get a rough estimate of the size and concentration of DNA (or protein) in your DNA extraction or PCR product (or protein purification, etc.). This is s good initial quality control step, but more precise measurements are needed to know specific quantities and fragment lengths.

**Health and Safety Warnings**

* Wear nitrile gloves and lab coat.
* The stain we are using is not considered a carcinogen but as a precaution, it should be treated as a carcinogen, gel work should only be done on the designated gel bench
* Use heat resistant gloves when handling hot agarose bottles
* Loosen the cap first when microwaving agarose bottles

**Quality Control:**

* Wear nitrile gloves, goggles, closed-toe shoes and a lab coat.
* The electrophoresis buffer should be reasonably fresh and at the desired level (should entirely cover gel trays) in gel rigs; complete or refresh rigs if necessary.
* The agarose medium should be free of bubbles when pouring into gel tray.
* If running DNA in the gel, make sure to aliquot a small amount of it before loading into gel to avoid contaminating the entire sample.

**Equipment needed:**

* TBE buffer 10x concentrate
* milliQ water
* 500 ml orange cap glass bottles
* Agarose powder
* Scale
* Plastic weigh boat
* Spatula
* 10,000x sybr safe gel stain
* 1-10ul pipette + tips
* Gel tray
* Gel combs
* Lab tape
* Parafilm or dilution plate
* Orange G
* DNA ladder
* Gel rig and power source
* UV transilluminator

**Protocol**

1. Acquire or prepare 1x TBE Buffer:
   * To make 500 ml combine 50ml of 10x TBE concentrate with 450ml milliQ water in an orange cap 1L bottle
2. Acquire or prepare 2% agarose gel; if it has hardened, loosen the bottle cap and microwave in 30 second increments until liquid again
   * To make 100ml combine 2g Agarose powder and 100ml 1X TBE in a 500ml bottle
   * Swirl to mix, loosen cap, and microwave in 30 second increments until the powder is dissolved. Do not allow bottle to boil.
   * Add 10ul of 10,000X SybrSafe Stain and swirl bottle to mix
3. Arrange gel combs and in gel trays to accommodate the number of samples and ladder. (Note: you need one well of ladder per row of samples)
4. Use lab tape to seal open ends of gel trays
5. Carefully pour agarose gel into taped gel trays
6. Let gel solidify for ~20 minutes
7. While waiting for the gel to set, prepare your samples: pipette 3 μl of Orange G loading dye per sample onto parafilm or into a plastic dilution plate.
8. Separate from your product pipette 3μl of DNA ladder (blue dye) onto parafilm or into dilution plate (usually one ladder per comb).
9. Pipette 5ul of your product into the Orange G on your parafilm or dilution plate. (Note: If you plan to run DNA on a gel, use 1ul of DNA or for certain applications (NGS) you may want to standardize the amount in ng, ie.100ng)
10. Once the agarose is completely solidified remove combs from gel then remove gel tray from tray holder.
11. Add ladder to the first well of each row. Then add 8ul of your DNA/Product and Orange G to the remaining wells in the gel.
12. After all samples and ladders are loaded into the gel, place the gel into the gel rig, making sure the gel is oriented correctly (samples will be traveling from negative (black) to positive (red) charge, since DNA is negatively charged) and make sure the gel is placed on straight, not crooked.
13. Make sure there is enough 1X TBE buffer in the rigs to cover the gels completely. Add more MQ water if necessary.
14. Cover the gel rigs, again, making sure orientation is correct (black wires, should be on top and the red wires, should be on the bottom)
15. Set the voltage generators to 85 V (and 400 Amp) and set time for 20 minutes. Keep in mind that the samples will travel slower in higher percent agarose gels, thus requiring longer running time, and less voltage. Smaller fragments will migrate faster than larger fragments, so voltage and running time may need adjustment depending on the size of the fragment you targeted in the PCR.
16. After setting the voltage generators to run, watch for small bubbles flowing through buffer in the rig as they indicate that the electric current is active.
17. After your gel is finished, remove it from the gel rig and place on the UV transilluminator.
18. Make sure to put down the UV (plastic) shield over the gel before turning the UV transilluminator on.
19. Use the switch on the left side of the transilluminator to view the gel. If you take any pictures make sure to remove your gloves before touching your phone. Turn off the transilluminator when you are done.
20. Throw your used gel, and other waste into the sybr safe solid waste bucket

**Waste Management:**

Throw your used gel, and other waste into the sybr safe solid waste bucket.