**DNA Extraction Quality Control**

**Gel Electrophoresis (DNA size, quantity, fragmentation):**

**Materials:**

* Agarose
* 1X Sodium Borate Buffer (200mM NaOH, pH ~8.2)
  1. Add 8g NaOH (MW=40), 47g boric acid (MW=61.83) to 900ml distilled water, make sure all powers dissolve completely.
  2. Make final volume to 1L by adding water.
  3. **\*optional\*** Use 0.2 micron filter membrane to filter. pH should be around 8.2.
  4. Dilute to 1X as needed
* SYBRSafe or SYBRGreen gel fluorescent dye (3.5 µL per gel)
* Gel mold, electrophoresis rig
* 1 Kb+ DNA ladder
* P20 and tips

**Procedure:**

1. Make a 0.8% Sodium Borate Gel
   1. Measure out 0.15 g Agarose, add to small Erlenmeyer flask
   2. Use graduated cylinder to measure 15ml 1X Sodium Borate, add to the flask
   3. Swirl flask, place in science microwave for ~30 seconds
   4. When the flask starts boiling, use hot pad to remove flask
   5. Swirl flask, if particles are visible, return to microwave and repeat until solution is clear
   6. Once solution is clear, add 3.5µl SyberSafe/SyberGree/Bluelight dye directly to solution
   7. Use hot pad to slowly pour gel into gel cast (gel combs need to already be present), avoiding the introduction of air bubbles
   8. Use a pipet tip to manually pop any bubbles
   9. Let sit for 15-20 minutes, until cloudy and firm
2. Remove gel combs, immerse gel in 1X Sodium Borate buffer, making sure that no air bubbles remain in wells and that liquid completely covers gel
3. Prepare samples:
   1. Using parafilm, create wells over a pcr tube plate
   2. Add 2 µL loading dye to each well
   3. Add 50-100 ng DNA to the loading dye bubble – **determine µL to add using qubit-based concentration**
4. Add 3.5 µL 1 Kb+ ladder to first well
5. Add samples to wells from left to right, skipping the first and last lanes, write down sample order in lab notebook
6. Run gel at 300 volts for 15 minutes **OR** 150 volts for 30 minutes **OR** 100 volts for 45 minutes

**Qubit fluorometer (DNA quantity):**

**Materials:**

* Sample(s)
* hsDNA Qubit buffer, reagent
* hsDNA concentration standards (on ice)
* Vortex
* P1000, P200, P20 Pipets, tips
* Qubit tubes
* 1.5 mL microcentrifuge tubes

**Procedure:**

1. Label a 1.5 mL tube with the letter M to denote “Master Mix”
2. Add 199\*(#samples + 2) µL HS dsDNA Buffer to master mix tube
   * Example: For 6 samples, add 199\*8 = 1592µL Buffer to the master mix tube
3. Add 1\*(#samples + 2) µL HS dsDNA Reagent to master mix tube
   * Example: For 6 samples, add 1\*8 = 8µL Reagent to the master mix tube
4. Vortex the tube to mix
5. Label qubit tubes, include two extra tubes for standards (for 6 samples, you need 8 tubes)
6. Add 190µL master mix to each standard tube
7. Add 199µL master mix to each sample tube
8. Add 10µL HS dsDNA standard #1 DNA to standard #1 qubit tube
9. Add 10µL HS dsDNA standard #2 DNA to standard #2 qubit tube
10. Add 1µL sample to each of your sample tubes
11. Vortex qubit tubes, let incubate for two minutes
12. Standardize qubit using low standard (#1) and high standard (#2)
13. Obtain qubit values for each of your samples, record in your lab notebook
14. Discard used qubit tubes and master mix tube, return reagent and buffer to dark, return standards to 4ºC fridge

**Nanodrop spectrophotometer (DNA purity):**

**Materials:**

* Sample
* ddH2O
* P20 (or smaller) and tips
* kimwipes

**Procedure:**

1. Raise nanodrop arm, pipet 1µL ddH2O onto platform, lower arm, raise arm, dab with kimwipe **(DO NOT RUB)**
2. Add 1µL of H2O, lower arm, press blank
3. Once blanking completes, raise arm, dab platform with kimwipe **(DO NOT RUB)**, add 1µL of your sample to platform, lower arm
4. Run the sample, record concentration, 260/280, and 260/230 ratios in your lab notebook
5. Raise arm, dab with kimwipe, add 1µL of ddH2O, lower arm, raise arm, dab with kimwipe **(DO NOT RUB)**
6. Repeat steps 4-5 with remaining samples.
7. Once data collection is complete, clean the platform with ddH2O once more