**MCB 525, Fall 2016**

**1.2: DNA concentration**

**Objective:** To create 2bRAD libraries, we need a large amount of DNA in a small volume of water. Our genomic DNA will be concentrated using a quick bead-based protocol. Cheaper methods can be substituted (e.g. ethanol precipitation, drying under spin-vac), but it is advised to resuspend the DNA pellet O/N if using methods in which the DNA pellet is completely dried.

**Protocol:**

1. Determine concentration of DNA extracted in Section 1.1 via PicoDrop, using Buffer AE as a blank.
2. Note concentration, A260/A280 and A260/A230 values. Absorbance values should be close to 1.8 (260/280) and 2.0-2.2 (260/230) for DNA.
3. Based on the concentration measured via PicoDrop, determine the volume of sample needed to obtain 2.5 μg, and pipet this volume into a new (PCR-sized) tube.
4. Add 10 μL of diluted bead solution (Turbo ChargeSwitch Kit, ThermoFisher, Waltham, MA) to sample.
5. Add a volume of 1.5M NaCl SPRI Hybridization Buffer equal to the sample volume and mix by pipetting up and down.
6. Allow to equilibrate 5 min.
7. Place on magnet until a visible pellet is observed (~2 min).
8. On magnet, remove supernatant and discard, being careful not to disturb pellet.
9. On magnet, wash pellet with 150 μL 70% EtOH solution.
10. On magnet, repeat wash step.
11. On magnet, remove as much of the EtOH as possible, avoiding disturbance of the pellet, and then dry in hood ~20 min.
12. Off magnet, resuspend pellet in 11.0 μL of nuclease-free water (NFW) to release DNA from magnetic beads.
13. Return to magnet until pellet forms (~2 min) and remove 8 μL sample to new tube, keeping pipet tip to opposite side of the tube as the pellet and leaving behind the beads.
14. Save tube with remaining ~2μL for later comparison with digested DNA (store at -20°C)

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1. *Where can the DNA be found during each of these steps? What is the purpose of each step in the protocol?*
2. *Though we quantified the DNA by PicoDrop (a UV-Vis spectophotometer-based method), more accurate determinations of concentration are obtained through use of fluorescent dyes that bind to dsDNA. Why might the NanoDrop overestimate DNA concentration.*