**MCB 525, Fall 2016**

**1.1: DNA extraction from sea anemones**

**Objective:** Obtain high-quality genomic DNA from animal tissue using a spin-column based DNA extraction kit (Qiagen DNeasy Blood & Tissue Kit).

**Protocol:**

1. Remove one live *Aiptasia* sea anemone from culture by gently sliding a metal spatula under the pedal disc. Place into labeled 1.5 mL microcentrifuge tube.
2. Use a pipet to remove extra seawater from the microcentrifuge tube.
3. Add 180 μL Buffer ATL.
4. Homogenize tissue with pestle.
5. Add 20 μL proteinase K.
6. Mix by vortexing and incubate 1 hr at 56°C, vortexing occasionally during incubation.
7. Add 200 μL Buffer AL.
8. Add 200 μL 100% ethanol; mix by vortexing.
9. Pipet mixture to a DNeasy spin column.
10. Centrifuge 1 min. at 8,000 rpm. Discard flow-through and collection tube.
11. Transfer spin column to new 2 mL collection tube. Add 500 μL Buffer AW1. Centrifuge 1 min. at 8,000 rpm. Discard flow-through and collection tube.
12. Transfer spin column to new 2 mL collection tube. Add 500 μL Buffer AW2. Centrifuge 3 min. at 13,000 rpm. Discard flow-through and collection tube.
13. Transfer spin column to new 1.5 mL microcentrifuge tube.
14. Elute the DNA by adding 50 μL Buffer AE to the center of the spin column membrane. Incubate 1 min at room temperature and centrifuge 1 min. at 8,000 rpm.

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1. *After the last wash step, the protocol includes an extra long spin step because it is crucial that no ethanol comes into contact with the spin column. Why might this be?*