# DNA extraction protocol for anemone

## Labeling your tube

1. Each student will obtain a ZR BashingBead™ Lysis tube.
2. Write Genet name, number and treatment on the cap of the tube. For example: ARB 50, 32$^{o}$C. or ARB 50, Menthol (*Use the latter format only if you treated with menthol*)
3. Write student name, section number, table number and date. For example: Josh S12 T1, 170618

## Preparing anemone for DNA extraction

1. Grab the Falcon tube containing your stressed anemone.

*If you incubated your control anemone at 25$^{o}$C, then consider step 2. Otherwise, skip to step 3. Do not consider this step if you treated with Menthol.*

2. Cut a portion of the cling wrap in front of the lab and transfer your anemone from the Falcon tube onto the cling wrap using the plastic transfer pipette. Thereafter, grab a scalpel and slice your anemone into two as directed by your TA. Return one ramet to the cup and use the second ramet for DNA extraction.

3. Transfer your anemone into the labeled ZR BashingBead™ Lysis Tube (2.0 mm). Try to avoid as much sea water as possible as you transfer it.

4. Add 750 µl **Lysis Solution** to the tube.

3. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at 6.0 speed for 40 seconds. (**No more than 8 tubes at a time**). *Do not use Eppendorf tube please*. *If you cannot open the beater, pull the rope vertically downwards. Let your TA operate this device.*

4. Remove your sample from the bead beater and transfer to a centrifuge. Centrifuge the ZR BashingBead™ Lysis Tube (2.0 mm) in a microcentrifuge at 10,000 x g for 1 minute.

5. Transfer up to 400 µl supernatant to a **Zymo-Spin™ IV Spin Filter** (*orange top*) in a **Collection Tube** and centrifuge at 7,000 x g for 1 minute.

6. Add 1,200 µl of **Genomic Lysis Buffer** to the filtrate in the Collection Tube from Step 5 and mix.

7. Transfer 800 µl of the mixture from Step 6 to a **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge at 10,000 x g for 1 minute.

8. Discard the flow through from the **Collection Tube** and repeat Step 7

9. Add 200 µl **DNA Pre-Wash Buffer** to the **Zymo-Spin™ IIC Column** in a new **Collection Tube** and centrifuge at 10,000 x g for 1 minute.

10. Add 500 µl **g-DNA Wash Buffer** to the **Zymo-Spin™ IIC Column** and centrifuge at 10,000 x g for 1 minute.

11. Transfer the **Zymo-Spin™ IIC Column** to a clean 1.5 ml microcentrifuge tube and add 40 µl **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.

12. Store your DNA at -20°C or proceed to PCR. Ensure your samples are labeled.