**MATERIALS**

* Qiagen DNeasy kit:
  1. buffer ATL
  2. buffer AL
  3. buffer AW1
  4. buffer AW2
  5. proteinase k (if not in kit, may be stored in freezer)
* 100% ethanol
* Nuclease free H20
* Autoclaved sterile beads
* 1.5 mL and 2mL tubes
* Paper towels or kimwipes
* Forceps
* Flame

**PREP**

1. Set incubator to 56˚C
2. Check that you have aliquots of all necessary reagents (to make aliquots: # reactions + 10%)
   1. buffer ATL (120ul per reaction)
   2. proteinase k (20ul per reaction)
   3. buffer AL (200ul per reaction)
   4. 100% ethanol
   5. buffer AW1 (500ul per reaction)
   6. buffer AW2 (500ul per reaction)
   7. Nuclease free H20
3. Decide which samples you’ll be extracting DNA from
   1. Record in your lab notebook
4. Label 2ml Tissuelyser tubes
   1. Place 1 autoclaved sterile bead in each tube
5. Prep tubes with:
   1. 120 μl buffer ATL
   2. 20 μl of proteinase k
   3. Vortex tubes
6. Get samples from freezer and let thaw
   1. Record any info on tube in your lab notebook. (e.g., color of coral, color of writing on tube)
7. Prepare materials:
   1. Clean stack of paper towels or kimwipes
   2. Forceps and spatula/scoopula
   3. Bowl with ethanol
   4. Flame
   5. 70% ethanol
   6. Mortar and pestle
8. Rinse mortar and pestle with ethanol, flame sterilize tweezers and scoop
9. Cut a large tic-tac sized piece of coral fragment (~ 2 x 4 mm)
   1. Can cut by placing coral fragment in mortar and chiseling using scoop
   2. Place coral fragment on paper towel or kimwipe and dab to dry off RNAlater, which can interfere with the reaction
   3. Place into correct tube
   4. Vortex
10. Flame sterilize instruments between samples and redo step 9 for each sample
11. Place all tubes in Tissuelyser for 5 min at 50 hz
12. Place samples in incubator for 1 hour at 56°C
13. Clean up materials, put corals back into freezer

**PART 1: DNA Extraction**

1) After samples have incubated at 56° for 1 hour:

2) Add 200 μl buffer AL, vortex

3) Incubate at 56° for 10 minutes

4) Centrifuge at 8000 rpm for 30 seconds

5) Transfer supernatant to new tubes

* Add 200 μl 100% ethanol, vortex
* Centrifuge at 8000 rpm for 30 seconds

6) Pipette into the spin column with collection tube (be careful to not pipette remaining pellet)

* Centrifuge at 8000 rpm for 1 minute

7) Discard flow through, can re-use collection tube

* Add 500 μl buffer AW1
* Centrifuge at 8000 rpm for 1 minute

8) Discard flow through, can re-use collection

tube

* Add 500 μl buffer AW2
* Centrifuge at 14000 rpm for 3 minutes

9) Discard flow through, get a new 1.5mL elution tube

**PART 2: Elution**

1) Add 50 μl ultra-pure H20 to spin column

* Centrifuge at 14000 rpm for 2 minutes
* Pipette into labeled PCR tubes (labeled with sample # and date)