**Protocol: Extracting DNA/RNA suitable for PCR amplification/Reverse Transcription from coral samples (S. Sunagawa from the Medina Laboratory, U CA Merced)**

Part I: Preparation of coral powder
Part IIa: Extraction of DNA

Materials:

Part I:

- DNAse/RNAse free mortar, pestle, chisel, hammer, tweezers, kimwipes, spatula
- Styrofoam box with dry ice
- 15 mL Falcon tubes
- EtOH

Part IIa:

- PowerPlant kit (MoBio) cat #: 13200-100
- 1.5 mL centrifuge tubes
- water bath or hybridization oven (65°C)
- Bead beater (BioSpec) with 0.1 mm and 0.5 mm Zirconia/Silica beads (cat #s:11079101z, 11079105z)
- Ready-Lyse Lysozyme solution (Epicentre) cat #: R1802M
- Proteinase K – 20mg/ml (Invitrogen)

## Methods - Part I: Preparation of coral powder

1. Place DNAse/RNAse free Mortar in center of Styrofoam box filled with dry ice. Make sure it is cooled down to dry ice temperature.

2. Place DNAse/RNAse free pestle, chisel, spatula, and tweezers on dry ice, leave them for about a minute until they are cold.

3. Label and place a 15mL Falcon tube in the dry ice so that it is almost completely covered.

4. Take coral samples from freezer and quickly place them in mortar. Briefly examine coral with tweezers to see if there is any calcium carbonate or unwanted foreign bodies that can be removed with the chisel.

5. After removing as much calcium carbonate and foreign bodies as possible, proceed to grind up the coral sample in mortar with pestle.

6. Grinding difficulty varies with coral species, the best way in general is to hit the coral with the pestle until it breaks into smaller pieces, then grind the pestle in a circular motion until a homogeneous powder is obtained.

7. Scrape the powder together with spatula and prepare to transfer coral to 15mL Falcon tube. Remove lid from tube, while being careful not to touch the tube itself if possible. Once removed, let the tube sit for 15 seconds to ensure that its temperature is not altered by the lid removal.

8. Pick up the mortar being careful not to freeze your fingers, and quickly scrape the coral powder into the tube with the spatula. When finished knock any excess coral from around the rim of the tube and replace the lid. Store powder at -80°C.

9. Thoroughly clean mortar with EtOH, let it dry and repeat steps 1-8 with next sample.

## Methods - Part II: Extraction of DNA

1. Pulverize coral fragment using a mortar and pestle on dry ice. Keep amount of calcium carbonate to a minimum. Powder can be kept at -80°C.

2. Transfer approximately 50 mg ("less is more") to a PowerPlant Bead Tube (PowerPlant DNA Isolation Kit; MoBio, Carlsbad, CA) and add 550 µL of PowerPlant Bead Solution.

3. Add 0.19 µL of Ready-Lyse Lysozyme Solution (final: 10U/µL; Epicentre, Madison, WI), vortex briefly and incubate for 10 min at room temperature (mix occasionally).

4. Add 60 µL of Solution PB1, and 25 µL of Proteinase K (20 mg/mL; Invitrogen, Carlsbad, CA, USA) and vortex briefly.

5. Incubate for 60-90 min at 65°C in hybridization oven or water bath.

6. Add 400 mg of each 0.1 and 0.5 mm zirconia/silica beads and bead-beat for 30 s using a Mini-BeadBeater-8 (Biospec Products, Inc., Bartlesville, OK, USA).

7. Centrifuge for 2 min at 10,000 x g at room temperature and transfer supernatant to new 1.5 mL collection tube by decanting.

8. Centrifuge for 2 min at max. speed at room temperature and transfer 250 µL of the supernatant to a 2 mL collection tube.

9. Add 250 µL of Solution PB2, mix, and incubate for 5 min at 4°C.

10. Centrifuge for 5 min at max. speed at 4°C and quickly transfer supernatant to new 1.5 mL collection tube (work in order of sample numbers, e.g. sample 1 → sample 5).

11. Centrifuge for 5 min at max. speed at 4°C and quickly transfer supernatant to new 1.5 mL collection tube (work in reverse order of sample numbers, e.g. sample 5 → sample 1).

12. Add 500 µL of Solution PB3, mix and incubate for 10 min at room temperature.

13. Centrifuge for 20 min at max. speed at 4°C, then remove supernatant and resuspend pellet in 100 µL of Solution PB6.

15. Add 500 µL of Solution PB4, mix, and transfer mix to Spin Filter.

16. Centrifuge for 1 min at 10,000 x g at room temperature.

17. Discard the flow through, add 500 µL of Solution PB5.

18. Centrifuge for 30 s at 10,000 x g at room temperature.

19. Transfer Spin Filter to new 1.5 mL collection tube.

20. Centrifuge for 1 min at max. speed at room temperature.

21. Transfer Spin Filter to new 1.5 mL collection tube.

22. Add 30 µL of Solution PB6 to center of Spin Filter, let sit for 1 min.

23. Elute DNA by centrifugation (1 min at max. speed at room temperature).