CTAB extraction protocol (Adult coral samples)

Materials and Reagents:

- 2% CTAB for 50 ml: (store at room temperature)

Stock conc.	**Final conc.**	**For 50ml sol.**
CTAB	2%	2g
1M Tris pH8.0	100mM	5ml
0.5M EDTA	20mM	2ml
NaCl**	1.4M	4.1g
ddH2O		43ml

**Note:
CTAB is Hexadecyltrimethylammonium bromide. Dissolve it before adding NaCl, with stirring and a little warmth, if necessary.
When NaCl is dissolved, lots of tiny bubbles come of solution; they rise to the surface very slowly, stimulation undissolved material.

- Add right before use:

Take amount needed for number of samples
_____samples x 800ul CTAB=____
_____samples x 1ul Proteinase K (Thermo Fisher #EO0491)=_____
_____samples x 1.6ul BME=_____
Add appropriate amount of BME and Proteinase K to CTAB

Stock conc.	**Final conc.**	**Per Sample (800ul CTAB)**
20mg/ml Proteinase K	20ug/ul	1ul
B-mercaptoethanol	0.2%	1.6ul

- Ice cold 100% Isopropanol
- Preheat elution buffer to 65C
- Beads
- Phase lock tube
- PCA (chloroform/isoamyl alcohol (24:1))
- Warm Elution buffer
- 80% ethanol
- Make 1mg/ml RNase A diluted in elution buffer (10mg/ml stock), then store in Freezer. Add RNase A to samples to final concentration of 0.1mg/ml. So, for every 10ul, add 1ul of RNase A (1mg/ml)

Method:

Day 1

1. Add beads (appropriate amount) to 2 ml Bead tube (blue).
2. Aliquot 800ul CTAB mixture (BME and Proteinse K) to the 2ml bead tube (blue).
3. Transfer coral fragments onto clean kimwipe using forceps to Blot away excess ethanol.
4. Add sample to the tube with bead tube. Be sure to wipe off forceps with 80% ethanol between samples.
5. Macerate sample in the bead beater for 45 seconds (on 6th floor)
6. Incubate in 42°C overnight

Day2

1. Pre-warm Elution buffer to 65°C
2. Spin phase lock tube for 2 min at 15,000 rcf before use
3. Centrifuge samples 15 min at 16,000 rcf at RT
4. Transfer aqueous phase to phase lock tube
5. Add 800ul (1 volume) of PCA (make sure to pipet the bottom layer) to the sample. Vortex for few seconds. Leave on ice for 1 minute. Vortex again for few seconds.
6. Centrifuge at 4°C for 20 minutes at 16,000 rcf
7. Transfer the aqueous phase (top layer) into a new clean tube.
8. Add 550ul (2/3 volume) of ice cold 100% isopropanol and gently mix by inverting tubes (20-30 times)
9. Incubate for 20 minutes at -20°C (freezer)
10. Centrifuge at 4°C for 20 minutes at 16,000 rcf
11. Discard the supernatant by decanting
12. Add 1000ul of 80% ethanol.
13. Centrifuge at 4°C for 5 minutes at 16,000 rcf
14. Discard supernatant by decanting. Let the pellet completely air dry (~15 minutes)
15. Resuspend the DNA in 50ul of warm (65°C) elution buffer
16. Add 50ul of (prediluted 1mg/ml) RNase A and incubate at 37°C for 10 minutes. Proceed with genomic clean-up kit
17. Store DNA at 4°C (fridge).