2X CTAB DNA Isolation Protocol to separate Host and Symbiont Tissue
(Modified from Coffroth et al 1992)

PREPARATION:

1. Make sure 65° C waterbath is turned on and water level adequate on day of extraction. Put 2X CTAB in H2O bath to thaw.
2. **If samples are frozen**:
   a. Label eppies with sample numbers, if necessary (label one set as "Z' and one as "A"
   b. Place eppies in a rack and add 300 ul of 2x CTAB buffer
   c. Chill two pair of forceps, two razor blades and a metal "cutting board" wrapped in aluminum foil.
   d. Keeping samples on dry ice, remove coral piece from its cryovial with a pair of well-chilled forcebs. BE VERY CAREFUL TO PREVENT THE SAMPLE FROM THAWING AT THIS TIME. Switch to the other pair of forcebs if necessary. Scrap off the of sample and **place it in appropriate "Z" eppie.** Wipe each instrument used and the cutting surface with a Kimwipe in between samples to prevent cross-contamination. Keep tissue on dry ice at all times; store at -70°C
3. **If extracting samples stored in ETOH**, transfer a portion of the tissue to 1.5ml eppie with 300 ul 2X CTAB buffer and proceed with Protocol below.

PROCEDURE:

1) Add 300µl 2X CTAB to the eppie labeled "Z" prior to adding the tissue and grind sample with blue dounce, rinse dounce w/ 300µl 2X CTAB into eppie (total CTAB volume 600µl.)
2) Place the "Z" eppie in a 15 ml round bottom Sardarst tube and spin in the floor (low speed) centrifuge at 500 rpm for 5 min.
3) Remove the supernatant from the "Z" and place in the appropriate "A" tube;
4) Add 300 ul of 2X CTAB to the "Z" tube, vortex briefly and repeat step (2)
5) Discard the supernatant and repeat step (4)
6) Discard the supernatant and add 300 ul of 2X CTAB to the "Z" tube.
7) Repeat step (5) and (6)
8) After above spin, resuspend the pellet in 300 ul 2X CTAB
9) Add one layer of glass beads to the cells and rupture cells by placing on Turbo-mixer (vortex mixer) for 5 minutes. .
10) Add 3.0µl of Proteinase K (stock=20mg/ml) to the tubes labeled "A" and 1.5 µl of Proteinase K to the tubes labeled "Z. Mix well by pipeting up and down, **don't vortex**. Incubate at 65°C for 30 min. Record time in and out of water bath. Invert tubes intermittently if possible.
11) Add equal or greater volume of chloroform-isoamylalcohol (CIA - 24:1), (~600ul for the "A" tubes and ~300ul for the "Z"). Buzz each sample on vortex machine

for approximately 15 seconds. Centrifuge for 5 minutes at 12K (high speed in microfuge).

12) Transfer the aqueous phase (top) with P200 to labeled 1.5 ml clear eppies.
13) Add an equal or greater volume of buffered phenol: chloroform: isoamylalcohol (ϕ: CIA - 25:24:1) (~600ul for the "A" tubes and ~300ul for the "Z"). Buzz each sample on vortex machine for approximately 15 seconds and centrifuge for 5 minutes at 12K.
14) Transfer aqueous phase (top) to final tubes. Repeat the phenol/chloroform step if the interphase is large or the aqueous layer is not clear.
15) Add 2 volumes (1000µl for the "A" tubes and ~500ul for the "Z") of cold 95% ETOH, shake it very well by inverting and place at -20ºC to precipitate the DNA over night or at -70ºC in hall for ½ hour.
16) Collect precipitate by centrifugation (high speed for 30 minutes). NOTE: In the case of zoox from polyps, often you cannot see a pellet. It is essential that you always orient the tubes in the same way so that you know where the DNA is. Discard supernatant, but be careful not to lose pellet – pipet or pour out supernatant from opposite side from where DNA should be.
17) Add 500µl 70% ETOH, mix and spin at 10k for 5 minutes. Remove the ETOH being carefully not to lose the pellet.
18) Repeat the 70% ETOH wash and spin for 5 minutes. Again remove ETOH, spin the samples for 30 sec and remove the rest of the ETOH with a pipet. Use a greater volume of ETOH if pellet is large or initial ETOH ppt. volume greater.
19) Dry the pellet in freeze drier or speed-vac for 15 minutes.
20) Resuspend in 5 to 15µl TE, depending on size of pellet. To do this, carefully wash the side of the tube where the DNA should be repeatedly with the 5 to 15ul of TE that you are resuspending in. The idea is to dissolve all DNA adhering to the side of the tube in a minimum volume. (If there is no pellet, then 10ul. If there is pellet, then 15ul). If it doesn't dissolve, incubate at 65ºC for 5 minutes, vortex briefly and spin down.
21) Store samples at 4ºC freezer (short-term, i.e., overnight in well-labeled red bullet boxes) or -20º C long-term in white boxes.