2XCTAB Protocol for Initial DNA isolation of *Symbiodinium*

PRELIMINARY STEPS:

a) Fill out data sheet and choose samples.
b) Turn on 65° water bath, with adequate water level.
c) Put 2XCTAB in warming water bath.

SAMPLE PREPARATION:

A. *For whole tissue samples in EtOH:* 1) Label eppies. 2) Put 600 µl of 2XCTAB in each eppie. 3) Using forceps, microscope slide, and razor blade; remove sample from EtOH tube and slice off a small piece (<1/2 cm). Working quickly, place small piece in eppie, and put sample back in tube. 4) Grind up small tissue in eppie with blue dounce, until there are no big chunks left. 5) Add a small amount of glass beads to each tube. 6) Turbo-mix for 5-7 minutes.
B. *For cultures:* 1) Spin for 5 minutes at 13K to pellet the sample. 2) Pour/aspirate off supernatant. 3) Add 600 µl 2XCTAB. 4) Add a small amount of glass beads (Sigma #G8772, 425-600 um) to each tube. 5) Turbo-mix for 5-7 minutes, until pellet is broken up.
C. *For frozen samples:* 1) Chill, on dry ice; forceps, metal plate wrapped in tinfoil, razor blade, and labeled tubes. 2) Obtain samples from freezer; put in cooler with dry ice and tools. 3) Using forceps, quickly remove frozen tissue from tube, place on metal plate, and cut a small (<1/2 cm) piece. Place in tubes, and put back on dry ice. 4) Put frozen samples back in –70 degree freezer. 5) Add 600 µl 2XCTAB to tissue in tube, and return to dry ice. 6) Grind up tissue in CTAB until there are no big chunks left. 7) Add a small amount of glass beads to each tube. 8) Turbo-mix for 5-7 minutes.

WITH SAMPLE IN 600 µL 2XCTAB:

1) Add 3.6 µl of Proteinase K (20mg/ml). Mix by inverting several times.
2) Incubate at 65° for 30- 60 minutes. Record time in and out of waterbath. Invert occasionally while incubating.
3) Add 600 µl of CIA (Chloroform-isoamyl alcohol). Shake on orbital shaker for 5 minutes or buzz on vortex machine for 10 sec.
4) Centrifuge for 5-15 minutes at 13K. Label second set of 1.5 ml tubes.
5) Remove aqueous phase (top), and transfer to new labeled tube. Discard bottom organic layer in CIA waste container.
6) **OPTIONAL:** Add 600 µl of Phenol-CIA. Make sure to draw from bottom organic layer, not top TE buffer. Shake on orbital shaker for 5 minutes or buzz on vortex machine. 7) Centrifuge for 5-15 minutes at 13K. Label third set of 1.5 ml tubes. (this step can often be omitted without problems).
8) Remove aqueous phase and transfer to final tube. Discard bottom organic layer again in waste container. (NOTE: If concerned about purity of the DNA, add second CIA step here, adding 600 µl CIA, to aqueous layer, shaking on orbital shaker for 5 minutes, and centrifuging for 5-15 minutes at 13K. Draw off aqueous layer again, and transfer to a final set of labeled tubes.)
9) Add 1 ml of cold 95% EtOH and shake well by hand.
10) Place in –20 degree freezer if precipitating overnight. Place in –70 degree freezer if precipitating for 30 minutes.
11) After precipitation, turn on 65° (if needed for resuspension).
12) Centrifuge for 30 minutes at 13K. Orient tubes so hinge is up, and DNA pellet will be easy to find.
13) Discard supernatant, being very careful not to lose the pellet. Add .5 ml 70% EtOH, invert to mix and spin at 13K for five minutes.
14) Discard supernatant, again being careful not to lose pellet. Add .5 ml 70% cold EtOH, invert to mix and spin at 13K for five minutes.
15) Discard supernatant, spin for 15-30 seconds and remove the rest of the cold EtOH with a pipet.
16) Dry the pellet in the speed-vac for 15 minutes, with caps open. Do not open speed –vac if still spinning-dry pellets will be suctioned out.
17) Resuspend pellet in 10-25 µl TE or water, depending on the size of the pellet. If pellet doesn't dissolve immediately, incubate at 65° for five minutes. Can store at –20° at this point.
18) Add .5 µl of .0l µg/µl stock Rnase to each tube. Mix by vortexing and spin 15-30 seconds to get sample to bottom of tube. (**step 18 and 19 can often be omitted without problems**)
19) Incubate at 56° for 30 minutes.

20) Store samples at –20°, or run out 1% TAE agarose gel.

TO RE-EXTRACT (if DNA is contaminated or not amplifying)
1) Start with sample suspended in TE or ddH_2O. Add ddH_2O to bring volume to 100-200 µl.
2) If desired, add 200 µl of CIA, spin for 15 minutes, and draw off about 180 µL of supernatant. Put in another, labeled tube.
3) Add 3 M NaOAc to bring to a final concentration of .3M NaOAc (10 µL if using a 100 µL volume, 18 if 180 µl volume, etc.).
4) Add 200 µL (or more) 95% EtOH. SHAKE.
5) Put in –70 degree freezer for 30 minutes.
6) Continue with normal extraction (Step 11 above), with one possible exception: dry for 30 minutes rather than 15 in speed vac, or as long as it takes to completely dry the EtOH pellet.

RECIPES:

2XCTAB Buffer (500 ml) (please reference Coffroth et al. (1992) Marine Biology 114: 317-325 for extraction methodology)

To a 1000 ml beaker in a hood and on a stir plate w/ bar, add the following:

5.0 M NaCl	140 ml
0.5M EDTA (pH = 7.8)	20 ml
1.0 M Tris-HCl (pH = 8.0)	50 ml
CTAB powder	10 g
2-betamercaptoethanol	1.0 ml

Add ddH2O to just under 500 ml. Gentle heat to 65C while stirring to have the CTAB go into solution. Once dissolved, bring final volume to 500 ml using a graduated cylinder. Filter sterilize (0.2 um), aliquot to sterile 50 ml Falcon tubes and store at -20C. Heat to 65C before using since freezing leads to the CTAB coming out of solution.

Chloroform-isoamyl alcohol (CIA)*

Chloroform	24 ml
isoamyl alcohol	1.0 ml

* Can be made up to any volume as long as it conforms to the above ratios.

Phenol-Chloroform-isoamyl alcohol (Phenol-CIA)*

Phenol	25 ml
Chloroform	24 ml
isoamyl alcohol	1.0 ml

* Can be made up to any volume as long as it conforms to the above ratios.