*DNA/RNA protocols*

HMW DNA extraction with CTAB buffer

Yi Jin Liew, updated 25 Oct 2015

1. Grow the Symbiodinium culture to exponential phase, harvest, pellet the cells, and snap-freeze the cells.
2. Pre-grinding ritual: fill up LN2 carrier with liquid N2, pre-chill mortar and pestle with liquid N2 before even taking cells out of -80 °C freezer.
3. Add 10 ml CTAB buffer (Teknova C2190 - CTAB Extraction Solution) for each litre of culture you used and resuspend the pellet.
4. Pipette the resuspended cells into the chilled mortar. It should freeze instantly.
5. Add in ~0.3 g silica/zirconia/whatever beads. From experience, the bead material isn’t crucial. Just add the same amount to each sample if you’re grinding multiple samples (no need to measure, just mark the side of an Eppendorf and use the same tube to measure beads out). Helps with the grinding.
6. GRIND. DAT. SHIT. Experience is your best teacher. Grind it to a point where everything looks like whitish powder, with no brownish chunks anywhere. Feel free to add some liquid N2 when things look like they’re thawing, you don’t want shit to stick to the walls of the mortar.
7. Scrape everything into one (or multiple) Eppendorfs, or Falcon tube, depending on scale. Remember to chill the scraper (i.e. put the thing into the mortar and then pour liquid N2 over it), else sadly shit sticks to scraper.

**NOTE:** Protocol assumes Falcon tube is used. If not, use Eppendorf tubes and scale down appropriately – use tabletop centrifuge instead of swinging bucket centrifuge etc.

1. Add 100 ul RNAse A (100 mg/ml) per 10 ml CTAB buffer and incubate at 60 °C for 30 min.
2. Add 60 ul Proteinase K (20 mg/ml) per 10 ml CTAB buffer and incubate at 37 °C or RT for 4 hours to overnight on a slow shaker (orbital or wheel).
3. Add 1 volume PCI (Phenol-Chloroform-Isoamyl 25:24:1) and mix carefully by inverting the Falcon tube several times. Gently shake on shaker for 5 min.
4. Centrifuge tube in Eppendorf swinging bucket centrifuge at 3000 rcf for 5 min.
5. Transfer the upper phase to a new purple 50 ml Falcon tube and add 1 volume CIA (Chloroform Isoamyl Alcohol, can also be substituted with pure chloroform). Mix gently by inverting the tube several times and shake on shaker for 10 min.
6. Centrifuge tube in eppendorf swinging bucket centrifuge at 3000 rcf for 5 min.
7. Transfer the upper phase to a new purple 50 ml Falcon tube and add 0.7 volumes cold isopropanol. Mix gently by inverting the tube several times and cool at -20 °C for 30 min.
8. Centrifuge tube in Beckmann centrifuge with 50 ml Falcon tube rotor at 15,000 rcf for 30 min (before centrifugation mark the outer side of the tube to know where the pellet will end up).
9. Decant the supernatant into a new tube without disturbing the pellet. Keep the tube as backup until the end.
10. Take up the pellet in the now empty tube in 10 ml CTAB buffer and carefully resuspend it until no clumps are visible.
11. Add one volume CIA, mix gently by inverting the tube several times and shake on shaker for 10 min.
12. Centrifuge tube in Eppendorf swinging bucket centrifuge at 3000 rcf for 5 min.
13. Transfer the upper phase to a new purple 50 ml Falcon tube and add 0.7 volumes cold isopropanol. Mix gently by inverting the tube several times and cool at -20 °C for 30 min.
14. Centrifuge tube in Beckmann centrifuge with 50 Falcon tube rotor at 15,000 rcf for 30 min (before centrifugation mark the outer side of the tube to know where the pellet will end up).
15. Decant the supernatant into a new tube without disturbing the pellet. Keep the tube as backup until the end.
16. Wash with 1 volume, ice cold 70% ethanol, let stand for 5 min and centrifuge at 15,000 rcf for 20 min.
17. Decant the supernatant into a new tube without disturbing the pellet. Keep the tube as backup until the end.
18. Spin the Falcon tube in the centrifuge for just some seconds to collect residual drops of ethanol in the bottom. Be careful to position the tube in the same position in the rotor as it was before so the pellet won’t move (easy thanks to the mark you made earlier).
19. Carefully pipette the remaining ethanol from the bottom of the tube and lay horizontal on the lab bench to dry. Do not overdry the pellet, as soon as it gets glassy add 150 ul of TE buffer per litre of starting culture and resuspend it. It might take some time to dissolve but be gentle.
20. Check the concentration on the Qubit and run 2 ul on the Nanodrop to get the quality specs and RNA contamination. Run an appropriate amount on the gel using the 1kb plus ladder (or equivalent up to 40 kb ladder) to check integrity and purity.
21. If you got your DNA you can now toss all the Falcon tubes with the supernatants you collected along the way. If not, test the different fractions you kept to find out when you lost it and to recover the DNA from that fraction.

## Reagents required

* CTAB (ordered from Teknova)
* RNase A
* Proteinase K
* Chloroform
* Isopropanol
* Ethanol

## Source

Copied + slight editing from Mani’s protocol.

Profanities are mine.