This protocol is for the extraction of genomic DNA from brown algal samples, modified to purify kelp sporophyte DNA. The highlighted portions are modifications which are optional, in that they are meant to enhance extraction from sporophyte tissue.

PPE: gloves and lab coat, fumehood for emulsification steps (i.e. when handling chloroform and phenol)

Disposal: CTAB is toxic, be sure to dispose in appropriate chemical waste. Chloroform and phenol require their own disposal container, and consumables in contact with chloroform and phenol (i.e. tubes and tips) must also be disposed into a solid waste container, and left in the fumehood until collected for disposal.

Safety: Be sure to establish Risk Assessments and Standard Operating Procedures when bringing this procedure into the lab.

CTAB buffer recipe (100 ml)

1. g CTAB = 1%

28 ml 5 M NaCl (8.18g dry weight)

4 ml 0.5 M EDTA

1% PVP (1 g dry weight)

10 ml Tris-HCl (10 mM, pH 8)

Top up to 100 ml with MilliQ or DNAse free water water. Note, you will need to mix on a heated plate, preferably with a magnetic stirrer, to dissolve the CTAB.

prepare

* make mixture of 500 µl CTAB isolation buffer and 10 µl of Proteinase-K (20mg/ml) per sample
* Depending on the amount of material, you may need to increase CTAB buffer and ProK amount (make extra, up to 250ul extra of master mix/specimen, and increase proportions of ProK and RNAseA accordingly)
* Sterilize mortar and pestle (I use 10% bleach for 15 mins, then 90oC washer)
* get silica gel (a fine grain gel is best, eg. Fontainebleau has been recommended; I grind silica beads into a fine dust in mortar prior to processing samples)

sample disruption

* set up 2.0 ml tubes and tissue. Approximately 8-12 cm2 should be sufficient (3 pieces about the size of my thumbnail).
* grind your sample material in a mortar with a small amount of ground silica gel (this is to keep the sample dry, i.e. absorption from ambient humidity will degrade the DNA).
* Don’t overgrind, just enough until there are no notable pieces of the specimen remaining. The sample will look like a fine sand in the end.
* add sample to 2.0 ml tube and **immediately** add 500 µl CTAB buffer, more if there is a lot of material.
* Add 5ul of RNAse, scale up if needed

incubate

* incubate at **55oC** for 30-40 mins, invert every 5–10 minutes (no vortex)
* spin at full speed for 10 minutes (13.3G)

extraction iteration 1 **\*\*to be conducted in the fumehood\*\***

* transfer aqueous (upper) layer to a clean tube and add an equal volume of 25:24:1 Phenol:chloroform:isoamyl alcohol (don’t take liquid from the upper layer of phenol, should be clear; don’t use the phenol mixture if solution is yellow or discoloured, shelf life is approximately 6 months) and invert a few times (no vortex) to emulsify
* spin at full speed for 10 minutes

Alternatively, you can use 24:1 chloroform:isoamyl alcohol for this step (seems to work fine in cultured species). Phenol is very toxic, so you may want to avoid using it if possible, however, it may be necessary to inactivate secondary compounds depending on the species being extracted. In my experience, phenol is necessary for brown sporophytes, but for small unicellular heterokonts and filamentous reds/green algae, we have had good success with just chloroform for this step.

extraction iteration 2 **\*\*to be conducted in the fumehood\*\***

* transfer aqueous (upper) layer to a clean tube. If using phenol there should be an obvious white solid layer in between bottom (dirty) solution and upper (mostly clear, CTAB) solution.
* when transferring aqueous layer, be sure to pipette gently hovering above the upper/lower boundary, and leave a bit of the aqueous layer behind to avoid phenol contamination (if you hit the phenol or mix the layers re-spin; phenol can inhibit downstream protocols such as library prep)
* Add 0.2-0.3 vol of 100% ethanol. Add this *slowly, drop by drop*, and agitate the tube while doing so (either by flicking or shaking, this can be a bit tricky). This will precipitate polysaccharides while ensuring the ethanol is not locally concentrated enough to precipitate the DNA (because there are salts present in the CTAB). You may see a stringy white precipitate form, this is the polyssacharides. DNA takes longer and requires higher relative volumes of ethanol to precipitate.
* add an equal volume of 24:1 Chloroform:isoamyl alcohol (to volume of aqueous solution moved over) and invert a few times (no vortex) to emulsify
* spin for 5 minutes (12000G)
* transfer aqueous (upper) layer to a clean tube
* As before, when transferring aqueous layer, be sure to pipette gently, hovering above the upper/lower boundary, and leave a bit of the aqueous layer behind. Respin if layers get mixed again.

DNA precipitation and washing

* Add 1 vol of isopropanol
* Do not incubate, move strait to spin step. Longer incubation times at lower temperatures will only help precipitate contaminant molecules, longer spin times are best way to ensure high yield.
* Spin at full speed for 30 mins, room temp.
* Pipette supernatant, careful to avoid taking up the pellet. It may be clear or white.
* Wash the pellet with 250 ul of 70% ethanol, spinning at 12G for 15 mins.
* Repeat above two steps, for two total ethanol washes
* Discard supernatant and allow pellet to dry (cap open) for 30 mins or until dry. Do not over dry. Pellet may become translucent or flake off. Carefully monitor and ready your TE sol.
* Elute the pellet in 50ul of 0.1x TE. No need to agitate while pellet dissolves.
* Store at -20oC

Post extraction cleanup

Clean up of the DNA extract may be necessary with a magnetic bead-based protocol. The above protocol does not consistently remove compounds that inhibit library prep, and QC (nanodrop, Qubit), does not pick up on this contamination. You can create low cost magnetic bead solutions using serapure beads, or you can pay for high quality beads (AMPure). My impression is that post extraction clean up is less of an issue depending on life history stage; for instance, does not appear to be necessary for cultured kelp gametophyte tissue, but needed for the sporophyte).

Recently with phenol extractions we get good sequencing results.

Notes

1. It is critical to remove polysaccharides during the extraction process. Polysaccharide contamination may appear as odd or inconsistent nanodrop QC readings (260/230 ratios differ depending on the nanodrop iteration used, sometimes they look good [1.8-2.0] sometimes they are very high, sometimes they are very low [-2.25], point being, do not rely on nanodrop to pick up polysaccharide contamination)
2. Best evidence for polysaccharide contamination is sticky or viscous extract that draws up and down slowly. DNA also won't run on a 2% agarose gel, or will exhibit "lane constriction".
3. Typical yield for the kelp Alaria is ~30-300ng/ul using this protocol. This protocol is optimized for extraction in this genus but may need refining depending on the taxa of interest.