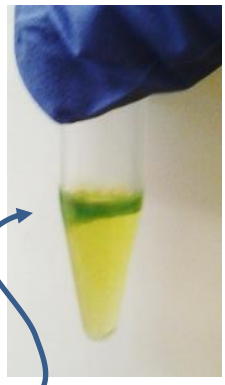
DNA extraction,
Nymphaea thermarum
(young leaves and young floral buds)

Becky Povilus, Friedman Lab, 2016

Notes:

- The tendency of macerated tissues to produce mucilage renders most spin-column based extraction protocols ineffective.
- Roots, leaves, and floral buds have been tested. Young leaves and young floral buds proved to be the most effective tissues to sample from.
- This protocol was modified for a large volume of tissue, and can be scaled down to reduce the number of tubes being processed in parallel for each sample.
- Is it really necessary to extract the same sample three times with CTAB? Some have noted that a second or third extraction is more clean than the first extraction (keep this in mind if you combine your tubes at the end).
- For many samples, a green viscous layer is present on top of the CTAB buffer, after centrifugation. Try to pipette around this layer as carefully as possible.



Reagents:

- CTAB buffer (100 mL 1M TRIS HCL pH 8.0, 280 mL 5 M NaCl, 40 mL 0.5 M EDTA, 20 g CTAB, then fill up to 1 L with Nanopure water)
- TE buffer (10 mL 1 M Tris HCl pH 8.0, 2 mL 0.5 M EDTA, full up to 1 L with Nanopure water)
- 7.5 M ammonium acetate (28.905 g ammonium acetate, fill up to 50 mL with Nanopure water)
- 100% ethanol, chilled (-20 C)
- 70% ethanol, chilled (-20 C)
- PVP (polyvinylpyrrolidone)
- 2-mercaptoethanol
- RNase A
- Chloroform
- Isopropanol

Equipment:

- mortar and pestle, spatula
- liquid nitrogen
- 15 or 50 mL centrifuge tubes (1 per sample)
- 1.7 mL microcentrifuge tubes (up to 24 per sample)
- centrifuge that can fit 15 or 50 mL tubes
- refrigerated microcentrifuge
- hot water baths, heat blocks, or incubators (set to 65 C and 37 C)
- pipettes and pipette tips

1) Weigh 0.5 g tissue (note: can be scaled down, to eliminate use of 15 or 50 mL centrifuge tubes and the need for 8 parallel 1.7 tubes for each sample in steps 2a-2c).

2a) Freeze and grind tissue in liquid nitrogen, with a pre-cooled ceramic mortar and pestle. Transfer to 15 or 50 mL centrifuge tube. Add 4 mL CTAB+ (20 mL CTAB *plus* freshly added 0.2 g PVP and 100 ul 2-mercaptoethanol), incubate 1 hour at 65 C, vortexing every 15 min. Centrifuge at 5000 rpm for 15 min. Pipette 500 ul of supernatant to each of eight individual 1.7 mL tubes (# 1-8).

2b) Add 4 mL CTAB+ to cell debris from 2a. Incubate 1 hour at 65 C, vortexing every 15 min. Centrifuge at 5000 rpm for 15 min. Pipette 500 ul of supernatant to each of eight individual 1.7 mL tubes (# 9-16).

2c) Add 4 mL CTAB+ to cell debris from 2b. Incubate 1 hour at 65 C, vortexing every 15 min. Centrifuge at 5000 rpm for 15 min. Pipette 500 ul of supernatant to each of eight individual 1.7 mL tubes (#17-24).

3) Add 1.5 ul RNase A to each tube. Incubate 15 min at 37 C.

4a) Add 500 ul of chloroform to each tube and vortex. Centrifuge at 13k rpm for 3 min. Transfer aqueous (upper) layer to new tube.

4b) Repeat step 4a.

5) Add 0.66 volumes of isopropanol. Incubate for at least 40 min at -20 C. Centrifuge at 13k rpm at 4 C for 15 min. Discard supernatant (without disturbing pellet).

6) Resuspend pellet in 100 ul of TE buffer. Incubate 15 min. Add 0.5 volumes (50 ul) of 7.5 M Ammonium acetate and mix. Add 2 volumes (200 ul) of cold 100% ethanol and mix. Incubate at least 40 min at -20 C. Centrifuge at 13k rpm for 15 min. Discard supernatant (without disturbing pellet).

7) Wash with 600 ul of cold 70% Ethanol, centrifuge at 13k rpm for 5 min. Discard supernatant (without disturbing pellet).

8) Resuspend in 200 ul (or other volume) Nanopure water. Incubate at room temperature overnight. If desired, vacufuge to reduce liquid volume and combine liquid from the appropriate tubes.

9) Use and Qbit or Nanodrop to assess concentration and quality of extraction.