Used the PMC4435465 protocol modified with Dan’s CTAB recipe

**CTAB recipe**  
2% CTAB

1.4M NaCl

100mM Tris- pH 8.0

20 mM EDTA

Protocol needs 2 mL tubes, or reduce all volumes by 1/3

1. Dissolved 0.03g of PVP (water bath ~50) into **3mL** of CTAB solution, added 6 uL of beta-mercap before use.
2. Ground tissue by hand with mortar and pestle in liquid nitrogen bath for about 5 minutes keeping tissue frozen the entire time, removed a scoopula worth of frozen ground tissue and added 900 uL of CTAB mixture.
3. Vortex and incubate at 60C for 7 minutes. This step can be left out, but DNA concentration is significantly lower.
4. Clean up lysis buffer/extract nucleic acids: Add 1 mL **chloroform:IAA**, invert to mix, spin 10 min at 14,000 × *g* at RT.
5. Carefully pull the aqueous supernatant off and place into a new 2-mL tube (about 950 μL). Leave the water layer that lays directly on the debris at the separation layer
6. Repeat extraction, add 1 mL of **phenol:chloroform:IAA** solution to each tube, invert to mix.
7. Centrifuge the samples at 14,000 × *g* for 10 min at RT.
8. Carefully pull the aqueous supernatant off and place into a new 2-mL tube. Be careful not to pull the milky white protein that sits between the layers.
9. Clean 1x-2x times with a chloroform:IAA step. Add 1 mL **chloroform:IAA**, invert to mix, spin 10 min at 14,000 × *g* at RT. Carefully pull the aqueous supernatant off and place into a new 2-mL.

You can precipitate now (.1 V 3M sodium acetate, 2.5 V 100% EtOH, 1 hour at -20C, spin max speed 15 min), but RNA contamination is very high.

Step 9 to go 🡪

RNase treatment

1. Add 5 µl of RNAse A solution and incubate at 32C for 20 min.
2. Add equal volume **phenol:chloroform:IAA**, gentle shake to mix, 14,000G 10 min
3. Pull aqueous, add equal vol. chloroform:IAA, gentle shake to mix, 14,0000G 10 min
4. Repeat, add equal vol. chloroform:IAA, gentle shake to mix, 14,0000G 10 min.
5. Pull aqueous, precipitate with .1 V 3M sodium acetate, 2.5 V 100% EtOH, 1 hour at -20C, spin max speed 15 min. Dump EtOH, add 750 µL 75% EtOH, light vortex. Spin at 8600G for 6 min. Remove EtOH, let dry and resuspend in TE.