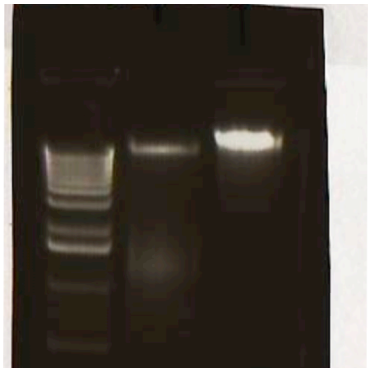
CTAB Genomic Prep

The following protocol uses small volumes in microfuge tubes, but can be scaled up without any problems.

1. Grind plant tissue really, really well in liquid nitrogen – then grind it again.
2. For 200 mg of ground tissue in a 1.5 mL tube, add 500 uL of CTAB-PVP-2ME solution.
3. Grind tissue with a green pestle (grind tissue really well)
4. Heat to 65C for 2-3 hours – grinding every 30 minutes
5. Add 15 units of RNase One (Promega – also can use 10ug/mL RNaseA) and digest at 37C for 45 minutes
6. Optional: add 3 µl 20 mg/ml Proteinase K. Mix and incubate 30 minutes at 37C (you can also digest with proteinase K at the very end, followed by P/C extraction, after you assess the quality on a gel. Proteins will get hung up in the wells of the gel.)
7. Extract with 500 uL chloroform. (Take the aqueous phase)
8. Extract with 1:1 phenol:chloroform (1:1)
9. Extract again with equal volume of chloroform to get rid of phenol traces - interphase should be clear.
10. Precipitate with 0.1 volume of cold 3M Ammonium acetate and .54 volumes of cold isopropanol. Invert to mix and incubate in -20C for ~1hour.
11. Spin at max speed for 10 minutes
12. Wash with 75% ethanol.
13. Air dry and resuspend in 100 µl TE.
14. Quantify using Nanodrop and run 1 ug on a 1% Agarose gel: Assess RNA contamination (see below) and protein/polysaccharide contamination (usually protein/DNA complexes do not exit the wells):



A: No RNase treatment
B: RNase treatment.

CTAB/NaCl solution:
0.7M NaCl, 10% CTAB
-Dissolve 4.1 g NaCl in 80 ml water. Slowly add 10 g CTAB. Stir with heat to dissolve. Bring volume to 100 ml.

CTAB-PVP-2ME solution:

CTAB/NaCl solution	PVP (Sigma: 9003-39-8)	2-mercaptoethanol
5 mL	0.2 g	25 uL
20 mL	0.8 g	100 uL

Add PVP to CTAB/NaCl solution and dissolve by heating in 65C water bath and mix occasionally. Add fresh 2ME. (Shelf life of 2ME) only ~2-3 days so make new each time.