Total RNA extraction from Arabidopsis and tobacco

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RNA extraction buffer:

- 0.1M NaCl
- 2% SDS
- 50mM Tris/HCl (pH9)
- 10mM EDTA
- 20mM ß-mercaptoethanol

1. Grind leaf tissue on liquid N in mortar and pestle.
2. Transfer the ground tissue to a 10ml conical bottom centrifuge tube (don't let the tissue thaw) that contains 5vol/g of extraction buffer and an equal volume of phenol (pH 8.0). Vortex the sample 1min. to make sure mixed well. Leave sample at room temp. while grinding the remaining samples
3. Once all the samples are in extraction buffer/phenol add an equal volume chloroform and vortex briefly.. Vortex each sample for 1 minute.
4. Spin samples at 4C for 10 minutes at 8K.
5. Remove the aqueous layer to new tube.
6. Do an additional phenol/chloroform extraction, vortex for at least 1 minute and spin as in step 4.
7. Remove the aqueous phase to a new tube.
8. Add 2x the volume of chloroform and vortex for 1 minute.
9. Spin samples at 4C for 10 minutes at 8K.
10. Remove aqueous layer to new tube.
11. Add 1/10vol 3M NaOAc (pH 5.2) and 3 volumes of cold 100% EtOH to samples. Mix well and place at –80C for 1hour for precipitation.
12. Spin pellet down at 8K for 30 minutes.
13. Remove the liquid and wash pellet with 70% EtOH. Re-spin for 5-10 minutes.
14. Remove the EtOH and allow the pellet to dry (5-10 min. on bench is sufficient).
15. Dissolve pellet in RNase-free H_2O.
16. Quantitate RNA and use for Northern

I use between 5-30ug of total RNA for small RNA detection (depending on how abundant your small RNA of interest is).