RNA Extraction from Wheat Grains

1. Grind 5-10 grains of seeds in a mortar and pestle and transfer the ground material into a 1.5 mL eppendorf. Depending on the grain size, you probably wouldn't need all the ground material, ¼ volume of the eppendorf will do.
2. Add the 2-mercaptoethanol to the RNA extraction buffer.
3. Add 500 µL of RE (RNA Extraction Buffer) and mix.
4. A. Add 100-200 µL of Ambion Plant RNA Isolation Aid and mix
   B. Centrifuge for 10 mins at 13,000rpm and transfer supernatant to a fresh 1.5 mL tube.
5. Add 300 µL of 1:1 acidic Phenol (PH4.3)/ Chloroform.
6. Mix every few minutes for 10 mins.
7. Centrifuge for 15 mins.
8. Carefully remove supernatants to a fresh 1.5 mL tube and add 240 µL and 30 µL of Isopropanol and 3M Na Acetate(PH=5.2) respectively. Precipitate nucleic acid at -80oC for 15 mins.
9. Centrifuge for 30 mins at 13,000 rpm and discard supernatant. Be careful not to lose the RNA pellet while decanting supernatant.
10. Wash twice with 750 µL of 70% ethanol and centrifuge btw washes at 13,000 rpm for 5 mins
11. Discard supernatant and remove residual ethanol
12. Re-suspend pellets in 100 µL of RNAse Free water Reagents
13. Remove genomic DNA contamination using DNAse treatment of choice (there can be quite a lot of genomic DNA remaining so be sure to check removal is complete).

Reagents required

- RNA Extraction Buffer (RE buffer; 0.1 M Tris pH 8.0, 5 mM EDTA pH 8.0, 0.1 M NaCl, 0.5% SDS). AUTOCLAVE.
- Add 1% 2-mercaptoethanol to RNA extraction buffer before use.
- Acidified phenol pH 4.3 ± 0.2 (Sigma-Aldrich, Cat.#P4682).
- Chloroform.
- Isopropanol (2-propanol).
- 3 M sodium acetate (pH 5.2).
- 70% ethanol.
- Nuclease-free water.