Virginia Tech Small Grains Breeding
DNA Extraction Protocol for Wheat or Barley

For single microcentrifuge tubes

1. Collect leaf tissue in 2 mL microcentrifuge tubes.

2. Store the tissue in the -70°C degree freezer for at least twelve hours.

3. Grind leaf tissue with liquid nitrogen using a mortar and pestle. OR place one stainless steel bead in each tube and grind the tissue using the genogrinder.

4. Add 600 ul CTAB extraction buffer to each tube and mix thoroughly.

5. Incubate at 60 to 65°C for 20 to 40 minutes. This can be done using the water bath or the Eppendorf thermomixer. Set the thermomixer for 30 minutes, 400 rpm, and 65°C.

6. Add 800 ul of chloroform/octanol (24:1) to each tube. Mix each sample by inverting several times, then place on the rotating shaker for 30 minutes to 1 hour. This step can be up to 1.5 hours if needed for lunch. Instead of the shaker you may anternatively use the Eppendorf thermomixer set for 22°C and 1400 rpm.

7. Centrifuge the samples at 12,000 rpm for 10 minutes. Be sure and balance the tubes when loading the centrifuge.

8. Transfer the upper phase (around 600 ul) into a new 1.5 mL tube. Add 600 ul of cold isopropanol to each sample.

9. Mix the tubes gently by inverting several times. The DNA should precipitate. You may continue on to the next step if you see visible DNA or let the samples sit overnight at 4°C.

10. Centrifuge samples at 12,000 rpm for 10 minutes. Pour out the isopropanol carefully. The DNA pellet should remain at the bottom of the tube.

11. Add 800 ul of 75% ethanol to the tube with pellet. Invert the tubes several times.

12. Centrifuge at 12,000 rpm for 10 minutes. Pour out the ethanol carefully. The pellet should remain in the tube.

13. Repeat steps 11 and 12.

14. Air-dry the pellet. Once all ethanol has evaporated from the tubes add 50 to 100 ul of molecular grade water or 1/10x TE buffer to resuspend the DNA. Add 50 ul if the DNA pellet was small and add 100 ul if the pellet was large.

15. Allow the samples to sit at room temperature for approximately 1 hour to allow the DNA to resuspend.

16. Add 1 ul of Rnase A (10 mg/ml) to each sample. Heat the samples to 37°C for 30 minutes. Samples are now ready to use and should be stored at 4°C for short periods (a few months) or -70°C for longer periods (years).

17. Analyze each sample with the Nanodrop Spectrophotomer to quantify DNA concentration.

CTAB Extraction buffer recipe for 1L

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| 1M Tris | 100 mL |
| 5 M NaCl | 280 mL |
| 0.25 M EDTA | 80 mL |
| CTAB | 20 grams |