Genomic DNA Isolation

1. Pre-heat water bath to 65°C, pre-chill mortar and pestle in liquid nitrogen
2. Prepare extraction buffer (EXB), 1 liter as an example:
   a. Mix

5.0 M NaCl	100 mL
1.0 M Tris-HCl (pH 8.0)	100 mL
0.5 MEDTA (pH 8.0)	100 mL
20% SDS	62.5 mL

   b. Bring to 900 mL with ddiH_2O
   c. Heat to 65°C in water bath
   d. Add 3.8g $NaSHO_4$
   e. Adjust to pH 8.0
   f. Bring to 1,000 mL with ddiH_2O
   g. Keep in water bath at 65°C until use
3. Grind leaf tissue in liquid nitrogen (approx. 5 mL^3 ground tissue per 50 mL falcon tube)
4. Add 20 mL EXB
5. Vortex to mix
6. Incubate at 65°C for 30-60 min, inverting tubes every 10 min
7. Remove from incubation and allow to cool slightly
8. Add 20 mL 24:1 chloroform-isoamyl alcohol (work in the hood)
9. Vortex to mix
10. Spin at room temperature at 4,750 rpm for 15 min
11. Transfer supernatant to a new tube with 30 mL 100% pre-chilled EtOH
12. Incubate at room temperature for 15 min
13. Mix by gently inversion
14. Loop DNA out of tube with a disposable pipette, and place in a tube with 30 mL 70% EtOH
15. Wash 1 hour or overnight (at 4°C) if necessary
16. Spin at 4,750 rpm for 1 min
17. Pour off supernatant, transfer DNA pellet to a 2.0 mL tube
18. Invert onto Kimwipe until dry
19. Suspend pellet in 1.0 mL ddiH_2O