Dr. Chen's Lab

# CTAB DNA Extraction Protocol

(3/29/00)

1. Sample leaves and two grinding beads in 2 ml tube.
2. Add 600µL CTAB and 12µL β-mercaptoethanol in 2mL tube in hood.
   (If you have CTAB mixed with β-mercaptoethanol, add only 600µL)
3. Grind sample in the grinding machine (3-5 min; 13.3K rpm).
4. Place in 65°C for 30-40 minutes.
5. Add equal volume (600µL) 24:1 chloroform / isoami mix to each tube and mix. Solution should be cloudy. Shake the tubes well for mixing.
6. Centrifuge for 10 minutes @13K.
7. Transfer upper phase (400ul) into new 1.5mL tube containing 280µL cold isopropanol (found in freezer). Do not transfer any chloroform into new tube. Shake the tubes well for mixing.
8. Place tubes on ice for 5 minutes.
9. Centrifuge for 15 minutes @13K.
10. Dump supernatant.
11. Add 400µL cold 70% ethanol (in freezer) to wash DNA pellet.
12. Centrifuge for 5 minutes @ 13K.
13. Decant supernatant and dry the pellet in vacuum dryer 20-30 minutes, until dry.
14. Resuspend DNA pellet in 200µL dd$H_2O$. Quantify with nanodrop and dilute to desire concentration.
    >If doing PCR same day, leave tubes at room temperature for 1 hour.
    >Otherwise, store DNA solutions at -20°C /4°C.