Quick CTAB Extraction

1. Turn on heat block to 60°C.
2. Grind 1-2 cubic millimeters of tissue in a 1.5 ml Eppendorf tube with 300 µl of 2X CTAB buffer at 60°C (you can add a pinch of white quartz sand, 50 to 70 mesh, from Sigma or some ground glass to help grind tough tissues). You can use a blue tip.
3. Thoroughly mix contents of the tubes.
4. Place samples in the heat block (60°C) for 30 minutes or until the tissue is completely dissolved. Shake samples to dislodge any solid pieces. Any large pieces can be mashed using a blue tip.
5. Add 300:1 of chloroform-isoamyl alcohol (96:4) and shake for 2 min.
6. Centrifuge samples at full speed (14K) for 10 minutes.
7. Label new tubes.
8. Collect aqueous (top) phase – be conservative by leaving what can not be cleanly collected. Place the pipette tip on the side away from the pellet. Place the aqueous phase into the newly labeled tubes.
9. Repeat the addition of 300 µl of chloroform-isoamyl alcohol (96:4) and shake for 2 min.
10. Centrifuge samples at full speed (14K) for 10 minutes.
11. Label new tubes.
12. Collect aqueous (top) phase – be conservative by leaving what can not be cleanly collected. Place the pipette tip on the side away from the pellet. Place the aqueous phase into the newly labeled tubes.
13. To the tubes with the aqueous phase add 600:1 of cold 70% EtOH, 25:1 3M NaOAc. Mix by inversion.
14. Centrifuge samples at full speed (14K) for 10 minutes.
15. Pour off alcohol, leaving the pellet of DNA behind.
16. Wash the pellet by adding ~500:1 of 70% alcohol. The same tip can be used if the alcohol is dribbled in from above the tube.
17. Repeat steps 15 and 16. If the DNA pellet becomes loose, spin for 2 minutes at 14K.
18. Remove all alcohol, leaving the pellet of DNA behind.
19. Let the pellets either air dry or dry in the speedwac.
20. When the tubes are dry re-suspend the pellet in 50 1 of dH_2O and let dissolve.
21. Store suspended DNA at -20°C freezer.

2X CTAB Isolation Buffer
100 mM Trisma, pH 8.6
1.4 M NaCl
20 mM EDTA
2% Hexadecyltrimethylammonium bromide (CTAB)
2% polyvinylpyrolidone (40,000 MW) **or** alternately use 20 mg / ml Proteinase K - **add only immediately prior to use!**
0.2% 2-mercaptoethanol -- **add only immediately prior to use!**

(We actually make the buffer from solids and do not adjust pH. For a 100 ml batch this takes 1.21 gm Tris, 8.182 gm NaCl, 0.74 gm EDTA, 2.0 gm CTAB, 2 ml polyvinylpyrolidone, 0.2 ml 2-mercaptoethanol, and water to volume. Take proper precautions when handling 2-mercaptoethanol. We have not examined the shelf-life of the CTAB buffer but have used a single batch for 2-3 months without problems.)