CTAB DNA Extraction for genotyping (Yuan Lab)

1. Grind fresh plant tissue with liquid nitrogen or silica-gel dried tissue (a little silica gel grains in the tube actually helps the grinding) in a 1.5 ml Eppie tube.
2. Add 750ul of CTAB DNA Extraction buffer (see protocol below).
3. Incubate the CTAB/plant extract mixture for 15 minutes at 55°C in the heat block and invert to mix throughout the 15 minutes.
4. Add 500ul of Chloroform: IsoAmyl Alcohol (24:1) in the hood and mix the solution by inverting the tubes (do not vortex).
5. Centrifuge at 13000 rpm for 10 minutes.
6. Transfer the upper aqueous phase only to a new eppie tube (~500ul).
7. Add 50ul of 7.5M Ammonium acetate followed by 500ul of ice cold 100% ethanol and invert to mix.
8. Put tubes in -20°C freezer for 30 minutes (or longer) to precipitate the DNA.
9. Centrifuge at 13000 rpm for 15 minutes – you should see a pellet at the bottom (align the tubes so that you know where the pellet is in case you can't see it very well).
10. Remove the supernatant and wash the DNA pellet by adding 500ul of ice cold 70% ethanol and centrifuging at 13000 rpm for 5 minutes.
11. Repeat the wash.
12. Remove all the supernatant and allow the DNA pellet to dry in the hood (approx. 20 minutes) – do not over dry the pellet since it will be hard to re-dissolve.
13. Resuspend the DNA in 100ul of dH20.
14. Run the DNA on a 1.0% agarose gel to check the quality of the DNA.