CTAB DNA Extraction for high quality/molecular weight DNA (Yuan Lab)

1. Grind plant tissue in a mortar cooled with liquid nitrogen.
2. Add 750ul of CTAB DNA Extraction buffer (see protocol below).
3. Wait until it warms up and becomes a green paste and transfer to an eppie tube.
4. Incubate the CTAB/plant extract mixture for 15 minutes at 55°C in the heat block and invert to mix throughout the 15 minutes.
5. Add 500ul of Chloroform: IsoAmyl Alcohol (24:1) in the hood and mix the solution by inverting the tubes (do not vortex).
6. Centrifuge at 13000 rpm for 10 minutes.
7. Transfer the upper aqueous phase only to a new eppie tube (~500ul).
8. Add RNase A (10ug/ml) – 5ul of 1mg/ml stock if you have 500ul of sample.
9. Incubate at 37°C for 30 minutes.
10. Add 50ul of 7.5M Ammonium acetate followed by 500ul of ice cold 100% ethanol and invert to mix.
11. Put tubes in -20°C freezer for 1 hour (or longer) to precipitate the DNA.
12. 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).
13. Remove the supernatant and wash the DNA pellet by adding 500ul of ice cold 70% ethanol and centrifuging at 13000 rpm for 5 minutes.
14. Repeat the wash.
15. 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.
16. Resuspend the DNA in 50ul of dH20.
17. NanoDrop the sample to estimate the concentration.
18. Alternatively the DNA can be run on a gel to estimate the concentration or size of the DNA. Running a 0.4% gel overnight with the lambda DNA mono-cut ladder can give you an estimate of the size (it still doesn't separate the large bands very well)