CTAB genomic DNA extraction

CTAB buffer (100ml)
2.0 g CTAB (Hexadecyl trimethyl-ammonium bromide)
10.0 ml 1 M Tris pH 8.0
4.0 ml 0.5 M EDTA pH 8.0 (EthylenediaminetetraAcetic acid Di-sodium salt)
28.0 ml 5 M NaCl
40.0 ml H2O
1 g PVP 40 (polyvinyl pyrrolidone (vinylpyrrolidine homopolymer) Mw 40,000)
Adjust all to pH 5.0 with HCL and make up to 100 ml with H2O.

Procedure
- Grind 200 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer.
- Transfer CTAB/plant extract mixture to a microfuge tube.
- Incubate the CTAB/plant extract mixture for about 15 min at 55o C in a recirculating
water bath.
- After incubation, spin the CTAB/plant extract mixture at 12000 g for 5 min to spin down cell debris. Transfer the supernatant to clean microfuge tubes.
- To each tube add 250 µl of Chloroform : Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, spin the tubes at 13000 rpm for 1 min.
- Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.
- To each tube add 50 µl of 7.5 M Ammonium Acetate followed by 500 µl of ice cold absolute ethanol.
- Invert the tubes slowly several times to precipitate the DNA. Generally the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 hr at -20 o C after the addition of ethanol to precipitate the DNA.
-Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate. To wash the DNA, transfer the precipitate into a microfuge tube containing 500 µl of ice cold 70 % ethanol and slowly invert the tube. Repeat. (alternatively the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol).
- After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm for 1 min. Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min). Do not allow the DNA to over dry or it will be hard to re-dissolve.
- Resuspend the DNA in sterile DNase free water (approximately 50-400 µl H2O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 µg/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 µl RNaseA in 10ml H2O).
- After resuspension, the DNA is incubated at 65o C for 20 min to destroy any DNases that may be present and store at 4o C.
- Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness.