DNA Extraction Protocol

2 x CTAB buffer: 20 g

CTAB powder: 2%(m/v)

Tris-HCL (ph. = 8.0): 100 mmol/L

EDTA: 20 mmol/L (Prepare 70% Ethanol)

NaCl: 1.4 mol/L

Autoclave above solution for 15 min

- Add 20 ul RNase stock (50mg/ml) into 40 ml pre-warmed 2x CTAB buffer before use. Frozen Medicago leaf tissue was grounded by ----- and (then the tubes were transferred into the liquid nitrogen. The tubes were taken out from the liquid Nitrogen and) 700 ul of the 2x CTAB solution was added to each tube. After mixing the tubes vigorously, they were kept in water bath at 65^{o} C for 30 min. Shake the extract mixture for every 10 min.
- Add equal volume of 700 ul Chloroform, vigorously mix and centrifuge the tubes at 13,000 rpm, for 15 min at room temp.
- Take out the up layer (about 400ul) into the new tubes and add 2/3 rd volume of isopropanol. Mix them and leave them for 15 min at -20^{o} C and centrifuge 13,000 rpm 15 min at 4^{o}C. Throw up the up layer and then add 1 ml of 70% Ethanol.
- Centrifuge again for 5 min at 4 C, (13,000 rpm) and throw away above ethanol and use 200 ul pipette take out the left out Ethanol residue and then air dry/vacuum and add 100 ul autoclaved water to dissolve the pellet. Add more 100 ul of water if it is not dissolved.
- use 1 ul above solution as PCR templates.