1. DNA ISOLATION FOR PLANT TISSUES

1. Weight between 5-20 mg of dry tissue. Leave tissue usually yield high quantity of DNA with small amount of dry tissue (as small as 5mg). If you samples include other organs highly lignified, such as stems, you might want to increase the amount of dry tissue up to 20 mg.

2. Turn the hot water bath on at 65°C.

3. Add hot 600 ul of CTAB extraction buffer to each sample.

* If required, add Proteinase K (0.1 mg/mL). Add 8ul PrtK (0.1mg/mL) per sample. Proteinase K is a protease (an enzyme capable of digesting proteins). It is used to digest the cell surface proteins and to degrade histones (proteins bound with DNA). It is not usually required for plant tissue but if you have some problems having good quality DNA you might want to add it.

4. Cook tubes for at least 1h. at 65°C. After 30 min of incubation shake them, and do it again after another 30 min and let them sit at 65°C another 15 min.

* if you added PrtK incubate at 60°C for 1h and a half aprox.

5. Add 400 ul of Chloroform:isoamyl (24:1) alcohol to each tube. Invert tubes 100 times. Do not vortex them.

6. Centrifuge tubes for 10 min at maximum speed (14000xg)

7. Transfer 400 ul of the supernatant to the new tube.

* If required, add 1 µL RNase (100mg/ml DNase-free) and incubate for 30 min at 37°C.

8. Add 40 ul of 3M Sodium Acetate (pH 5.2) to each tube and mix thoroughly.
Do not vortex.

9. Allow mixture sit for 10 min.

10. Add 1 mL of 100% EtOH to the tube. Place in -20°C. freezer for 30 min or overnight.

11. Spin tubes for 10 min at 14000 RPM at 4°C.

12. Poor off ethanol.

13. Wash pellets twice with 70% ice cold EtOH (500 ul of EtOH per tube).

14. Allow the wash to continue for 5 min.

15. Spin tubes 3 min at 4000 RPM.

16. If needed, repeat washing steps (13-15).

17. Allow pellets to air dry for at least 2h.

18. Resuspend 100 ul of TE to each tube and store in -20°C freezer.

Table 1. CTAB BUFFER (final volume, 1L)		
Chemical	Stock solution	Volumen or weight
Tris-HCl	1M, pH 8.0	100 mL
EDTA (ethylenediaminetetraacetic acid)	0.5M	40 mL
NaCl	5M	280 mL
CTAB (Cetyltrimethylammonium bromide) (Fisher Cat#:0833-1Kg)		20gr
Dissolve to1L with H2Od		
Autoclave		

Table 2. WORKING SOLUTION (per sample)	
Chemical	Volume or weight per sample
CTAB buffer (see table 1)	800 ul
0.2% β-mercaptoethanol (Fisher Cat#: BP176-100)	4.5ul
PVP (polyvinylpyrrolidone, 40.000 MW) (Fisher Cat#: BP431-500)	0.036 mg

Table 3. TE buffer (final volume, 1L)		
Chemical	Stock solution	Volumen or weight
Tris-HCl	1M, pH 8.0	10 mL
EDTA (ethylenediaminetetra acetic acid)	0.5M	2 mL

Table 4. EDTA (0.5 M, pH 8, final volume,1L)		
Chemical	Stock solution	Volumen or weight
EDTA (ethylenediaminetetra acetic acid) (Fisher Cat#: BP120-1)		186.12 gr
Add 750 mL H2Odd		
Add about 20 g of NaOH pellets ; Slowly add more NaOH until pH is 8.0. Stir in a hot plate to dissolve better EDTA dissolves @ pH 8.0.		
Bring solution to 1L		

Table 5. NaCl 5 M (final volume,1L)		
Chemical	Stock solution	Volumen or weight
NaCl (Fisher Cat#: 358-10)		292.2 g
Add 700 mL H2Odd and dissolve		
Bring solution to 1L		

. Proteinase K: Quiagen Cat #19131
. Rnase (100mg/ml Dnase free) Qiagen Cat#19101

Some references

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