DNA extraction procedure

Contributed by Jim Anderson

Sample preparation:

1. Harvest an approximately 2 cm sized leaf from a seedling (1-2 leaf stage) into a 1.5 mL tube and keeps them in liquid nitrogen.
2. Grind leaf in the 1.5 mL tube using a cold glass rod (kept in liquid nitrogen) over the vortex.
3. Extract DNA immediately or store samples in -80°C

Extraction buffer:

Buffer, total volume = 50 mL.		
Component	**Concentration**	**Volume, mL**
Dd H_2O	-	32.0
EDTA	0.5 M	5.0
Tris HCL	1.0 M	5.0
NaCl	5.0 M	5.0
SDS	20%	3.1
NaOH	10 M	152 µL
Na-bisulfite	0.19 g	-

Extraction procedure :

1. Heat solution 30 sec. in 1000W microwave (to 65°C).
2. Place on heat plate to maintain temp.
3. Dispense 700 µL warm buffer into tube, invert to mix thoroughly.
4. Incubate in water bath at 65°C for 30 min.
5. Invert every 5 min.
6. Dispense 700 µL 24:1 Chloroform/Isoamylic alcohol into tube, mix vigorously.
7. Spin in microfuge 10 min @10,000 rpm.
8. Carefully remove from tubes and keep at slant in a rack.
9. Pipette about 500 µL of aqueous layer from the top of the tube. Transfer to new tube.
10. Add 1 mL of cold 95% ethanol, invert to precipitate the DNA.
11. Spin DNA to bottom of tube (10 minute at 10,000 rpm).
12. Decant, and wash pellet in 1 mL of 70% ethanol.
13. Spin the tubes (1 min, 10,000 rpm) and pour out 70% ethanol and blot tube on Kimwipe.
14. Incubate at 50°C for 30 min. to dry pellets.
15. Resuspend in 150 µL TE buffer.
16. Dissolve in water bath at 65°C for about 6 hrs.
17. Prepare 1:5 dilutions for PCR.