DNA Extraction

Tuesday, February 05, 2013
3:01 PM

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Materials:
Microfuge tubes
Small forceps
LN_2
Ball Mill, and 4 mm dia ball bearings
Shorty extraction buffer
Ice bucket
100% Isopropanol
70% Ethanol
Sterile dH_2O

Method:
1. Using small forceps take a small young leaf at the center of the rosette if possible, place in microfuge tube
2. Add a small, 4 mm dia, stainless steel ball bearing to tube, close and store at -20°C until ready to continue with DNA extraction
3. Grind leaf material frozen (LN_2) in ball mill for 30 seconds at a frequency of 30 (max).
4. Add 500 µl of shorty buffer, vortex to mix place on ice
5. Centrifuge tube at maximum speed for 5 minutes
6. Wear gloves for all subsequent steps to avoid contamination, pipette off 350 µl of supernatant and transfer it to a new tube, discard old tube with pellet
7. Add 350 µl of isopropanol to transferred supernatant
8. Invert tube ≈ 20 times to mix
9. Centrifuge tube at maximum speed for 10 minutes
10. Don't worry if you can't see the pellet, its there, pour off supernatant, add 500 µl 70% EtOH
11. Flick tubes or use up and down pipetting to resuspend pellet, only vortex if you have to
12. Centrifuge tubes at maximum speed at room temperature for 2 minutes
13. Discard supernatant, and allow pellet to air dry for 10 – 15 minutes, don't allow them to dry too long
14. Resuspend pellet in 50 µl sterile dH_2O, and freeze tubes

DNA Quick Extraction Reagents

Shorty extraction buffer pH 9.0 250 ml
In 225 ml dH_2O
+ 6.055 g Tris, 200 mM (FW 121.1)
+ 12.5 ml 8M LiCl, 400 mM (Sigma L-7026)
+ 2.6 g EDTA, 25 mM (FW 416.2; Sigma ED4SS)
pH to 9.0 using HCl or NaOH
+ 12.5 ml 20 % SDS, 1% (Fluka 05030)

70% EtOH 500 ml
In 150 ml dH_2O
+ 350 ml 100% EtOH