SCHNABLE LAB
Plant Genomics

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Dellaporta DNA Extraction

Citation: Stephen L. Dellaporta,Jonathan Wood , James B. Hicks. A plant DNA minipreparation: Version II. Plant Molecular Biology Reporter, 1983, Volume 1, Issue 4, pp 19-21.

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Edited by: Tsui-Jung Wen / Alina Ott
Updated: 08/20/2014

Reagents and recipes:

EB1 (Extraction Buffer 1)	[Addition]	[Final]
per liter:		
0.5 M EDTA pH 8.0	100 ml	50 mM
1 M Tris pH 8.0	100 ml	100 mM
4 M NaCl	125 ml	500 mM
dH2O	up to 1 L	
BME (mercaptoethanol)	700 ul (add after autoclave)	

For RNase treatment: Add 1 uL RNase A (20 ug/mL) (Invitrogen Cat#12091-021) per mL of EB1 (e.g. 1,000 uL to 1,000 mL) before use.

EB2 (Extraction Buffer 2)	[Addition]	[Final]
per liter:		
0.5 M EDTA pH 8.0	20 ml	10 mM
1 M Tris pH 8.0	50 ml	50 mM
dH2O	up to 1 L	

20 % SDS (do not autoclave) - prepare in hood!

per 100 ml:	
SDS	20 g
dH2O	80 ml

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5M KOAC (Potassium acetate)	[Addition]
per 100 ml:	
KOAC	49.1 g
dH2O	up to 100 ml

3M NaOAC (Sodium acetate)	[Addition]
per 100 ml:	
NaOAC	24.6 g
dH2O	up to 100 ml

Isopropanol
80% EtOH
Liquid N2
Mortar and pestle
30 ml Oak Ridge Tube (Thermo Scientific, Cat# 3119-0030) - 2 per sample
1.7 mL Eppendorf tube - 2 per sample

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Protocol:
Prep: Make sure a water bath is set to 65°C.

1. Weigh 1 g of tissue (young leaf or baby ear preferred), quick freeze in liquid N2 and grind to fine powder in a mortar and pestle. Tissue can be stored in -80 either before or after grinding if needed. Tissue should not thaw before EB1 is added.

2. Transfer the fine powder into a 30 mL Oak Ridge Tube and add 15 mL EB1.

3. Add 1mL of 20 % SDS to each tube.

4. Make sure libs are screwed on tightly. Mix thoroughly by vigorous shaking, and then incubate tubes in a 65°C water bath for 10 min.

5. Add 5 mL of 5M KOAC.

6. Mix thoroughly by vigorous shaking, then incubate tubes at 0°C (on ice) for 20 min.

7. Spin tubes at 13K rpm for 20 min in a Sorval centrifuge with the SA-600 rotor (~25,000 x g).

8. Pour supernatant through Miracloth (cheesecloth) into a 30 ml Oak Ridge Tube containing 10 mM isopropanol.

9. Invert 20 times to mix well and incubate at -20°C for 30 min.

10. Spin tubes at 12K rpm for 15 min in a Sorval centrifuge with the SA-600 rotor (~20,000 x g).

11. Gently pour off supernatant and lightly dry DNA pellets by inverting the tubes on paper towels for 10 min.

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12. Redissolve each DNA pellet with 0.7 mL EB2. May need to let sit overnight at 4°C if having trouble dissolving. Transfer into a 1.7 mL Eppendorf tube.

13. Spin the Eppendorf tubes in a microcentrifuge for 10 min at max speed to remove insoluble debris

14. Transfer the supernatant to a new Eppendorf tube and add 75 uL 3M NaOAC and 500 uL Isopropanol.

15. Mix well by inverting 20 times and pellet the DNA for 30 sec in a microfuge

16. Wash pellet with 500 mL 80% EtOH, dry thoroughly and redissolve in 100 uL TE, EB or H20.

Comments and suggestions:

1. If tissue is less than 1 g, still use the volumes of buffer listed in the protocol.

2. The procedure can be paused at step 9 and stored at -20°C overnight. Step 12 can also sit overnight.

3. If you have many samples, store the ground powder at -20 C temporarily after grinding with liquid nitrogen, then add buffer to all samples at the same time when grinding has been completed.

4. The most efficient number of samples for Dellaporta DNA Isolation is 12 each time due to centrifuge rotor capacity.