Plant DNA Extraction Protocol

Source: Protocol modified from Keb-Llanes et al. *Plant Molecular Biology Reporter* **20**: 299a-299e. 2002.

Introduction

Plant materials are among the most difficult for high quality DNA extractions. The key is to properly prepare the tissues for extraction. In most cases this involves the use of liquid nitrogen flash freezing followed by grinding the frozen tissue with a mortar and pestle. Liquid nitrogen is difficult to handle and it is dangerous in an open laboratory environment such as a classroom. For this reason we have modified a very simple plant DNA extraction protocol to use fresh tissue. We have also used tissue prepared in advance by dessication. The protocols and results are presented here.

Reagents and Buffers

Extraction Buffer A (EBA):	**Per 100ml.**
2% (w/v) hexadecyltrimethylammonium bromide (CTAB)	2.0g
100mM Tris (pH 8.0) (Use 1M stock)	10ml
20mM EDTA (Use 0.5M stock)	1ml
1.4M NaCl	8.2g
4% (w/v) polyvinylpyrrolidone (PVP)	4.0g
0.1% (w/v) ascorbic acid	0.1g
10mM β-mercaptoethanol (BME)* (Use 14.3M stock)	70µl

Extraction Buffer B (EBB):	**Per 100ml.**
100mM Tris-HCl (pH 8.0) (Use 1M stock)	10ml
50mM EDTA (Use 0.5M stock)	2.5ml
100mM NaCl	0.6g
10mM β-mercaptoethanol (BME)* (Use 14.3M stock)	70µl

TE Buffer:	**Per 100ml.**
10mM Tris (pH 8.0) (Use 1M stock)	1.0ml
1mM EDTA (Use 0.5M stock)	50µl

Other Required Reagents:
20% (w/v) sodium dodecyl sulphate (SDS)
5M potassium acetate (Stored at –20^{o}C)
3M sodium acetate (pH 5.2)

70% ethanol (stored at -20°C)
Absolute isopropanol (stored at -20°C)

Extraction Protocol:

1. Weight out 0.3g of plant tissue

2. Place tissue on a clean glass slide. Chop the tissue into a paste using a clean single edge razor blade. (we have also modified a Dremel Roto-tool for use as a simple tissue homogenizer with good success)

3. Immediately transfer tissue to a 1.5ml microcentrifuge tube (use Kontes #749520-0090) and (optional) further grind tissue with a tube pestle (Kontes #749521-1590)

4. Once the sample is prepared add 300µl EBA, 900µl EBB, and 100µl SDS.

5. Vortex and incubate at 65 °C for 10 min.

6. Place tube on ice and add 410µl cold potassium acetate. Mix by inversion and place tube back on ice for 3 min.

7. Centrifuge at 13,200rpm for 15 min. (If possible, use a refrigerated micro-centrifuge set to 4 °C)

8. Transfer 1ml of the supernatant to a new 1.5ml microcentrifuge tube, add 540µl of ice cold absolute isopropanol, and incubate in ice for 20min.

9. Centrifuge at 10,200rpm for 10min. discard the supernatant. Wash the pellet once in 500µl 70% ethanol and let dry

10. Resuspend the dry pellet in 600µl of TE. Add 60µl 3M sodium acetate (pH 5.2) and 360µl ice cold absolute isopropanol. Incubate on ice for 20min.

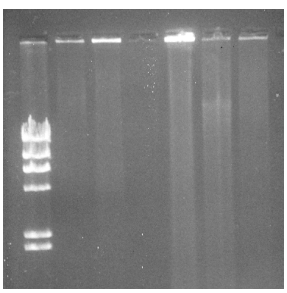
11. Repeat Steps 9-11 twice.

12. Resuspend the pellet in 50µl TE and carry out agarose gel QC.

Agarose Gel QC:

1. Cast a 1.0% (w/v) regular agarose gel in 1x TBE

2. Place 5µl of extracted DNA and 5µl sterile water in a 0.2ml microcentrifuge tube along with 2µl of gel tracking dye.



3. Run the gel for 20min. at 100v.

4. Stain gel and view result.

PCR QC:

Obtaining what appears to be good high molecular weight genomic is only the first line of QC for this protocol. The ultimate test is to see if the DNA can be used to amplify a PCR product. The test case used in developing this protocol was leaf tissue from the coleus plant (*Coleus blumei*). In order to test the DNA for PCR amplification the gene encoding tyrosine aminotransferase (GenBank #AJ458993) was submitted for PCR primer selection using the IDT SciTools software PrimerQuest. The software chose the following sequences:

Tat FOR:5'- ATA AAC CCT GGG AAC CCA TGT GGA –3'
Tat REV: 5'- AAC TTT GGG CTC ATC AAA GTG CCG –3'

These sequences were synthesized and a PCR amplification carried out using the conditions; $94^{o}C^{5:00}[94\ ^{o}C^{0:30}; 57\ ^{o}C^{0:30}; 72\ ^{o}C^{0:30}]_{35}\ 72\ ^{o}C^{7:00}$. Results of this amplification are shown below.