INTEGRATED DNA TECHNOLOGIES

Plant DNA Extraction Protocol

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

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 100 mL**
2% (w/v) hexadecyltrimethylammonium bromide (CTAB)	2.0 g
100 mM Tris (pH 8.0) (Use 1 M stock)	10 mL
20 mM EDTA (Use 0.5 M stock)	1 mL
1.4 M NaCl	8.2 g
4% (w/v) polyvinylpyrrolidone (PVP)	4.0 g
0.1% (w/v) ascorbic acid	0.1 g
10 mM β-mercaptoethanol (BME)* (Use 14.3 M stock)	70 µL

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

TE Buffer	**Per 100 mL**
10 mM Tris (pH 8.0) (Use 1 M stock)	1.0 mL
1 mM EDTA (Use 0.5 M stock)	50 µL

Other Required Reagents

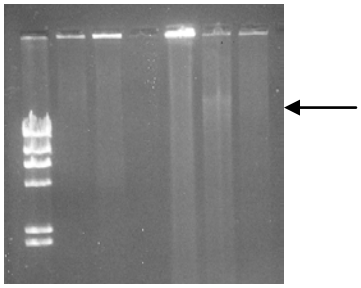
20% (w/v) sodium dodecyl sulphate (SDS)
5 M potassium acetate (Stored at –20°C)
3 M sodium acetate (pH 5.2)
70% ethanol (stored at -20°C)
Absolute isopropanol (stored at -20°C)

Extraction Protocol

1. Weight out 0.3 g 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 1.5 mL 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 300 μL EBA, 900μl EBB, and 100 μl SDS.
5. Vortex and incubate at 65^{o}C for 10 min.
6. Place tube on ice and add 410 μL cold potassium acetate. Mix by inversion and place tube back on ice for 3 min.
7. Centrifuge at 13,200 rpm for 15 min. (If possible, use a refrigerated micro-centrifuge set to 4^{o}C)
8. Transfer 1 mL of the supernatant to a new 1.5 mL microcentrifuge tube, add 540 μL of ice cold absolute isopropanol, and incubate in ice for 20 min.
9. Centrifuge at 10,200 rpm for 10 min. discard the supernatant. Wash the pellet once in 500 μL 70% ethanol and let dry
10. Resuspend the dry pellet in 600 μL of TE. Add 60 μL 3M sodium acetate (pH 5.2) and 360 μL ice cold absolute isopropanol. Incubate on ice for 20 min.
11. Repeat Steps 9–11 twice.
12. Resuspend the pellet in 50 μL 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 5 μL of extracted DNA and 5 μL sterile water in a 0.2 mL microcentrifuge tube along with 2 μL of gel tracking dye.
3. Run the gel for 20 min. 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; 5 min. 94^{o}C; 35 x (30 sec. 94^{o}C, 30 sec. 57^{o}C; 30 sec 72^{o}C); 7 min. 72^{o}C. Results of this amplification are shown below.