Plant Genomic DNA Extraction using CTAB

Introduction

The search for a more efficient means of extracting DNA of both higher quality and yield has lead to the development of a variety of protocols, however the fundamentals of DNA extraction remains the same. DNA must be purified from cellular material in a manner that prevents degradation. Because of this, even crude extraction procedures can still be adopted to prepare a sufficient amount of DNA to allow for multiple end uses.

DNA extraction from plant tissue can vary depending on the material used. Essentially any mechanical means of breaking down the cell wall and membranes to allow access to nuclear material, without its degradation is required. For this, usually an initial grinding stage with liquid nitrogen is employed to break down cell wall material and allow access to DNA while harmful cellular enzymes and chemicals remain inactivated. Once the tissue has been sufficiently ground, it can then be resuspended in a suitable buffer, such as CTAB. In order to purify DNA, insoluble particulates are removed through centrifugation while soluble proteins and other material are separated through mixing with chloroform and centrifugation. DNA must then be precipitated from the aqueous phase and washed thoroughly to remove contaminating salts. The purified DNA is then resuspended and stored in TE buffer or sterile distilled water. This method has been shown to give intact genomic DNA from plant tissue. To check the quality of the extracted DNA, a sample is run on an agarose gel, stained with ethidium bromide, and visualised under UV light.

Materials

CTAB buffer
Microfuge tubes
Mortar and Pestle
Liquid Nitrogen
Microfuge
Absolute Ethanol (ice cold)
70 % Ethanol (ice cold)
7.5 M Ammonium Acetate
55^{o} C water bath
Chloroform : Iso Amyl Alcohol (24:1)
Water (sterile)
Agarose
6x Loading Buffer
1x TBE solution
Agarose gel electrophoresis system
Ethidium Bromide solution

CTAB buffer 100ml

2.0 g	CTAB (Hexadecyl trimethyl-ammonium bromide)
10.0 ml	1 M Tris pH 8.0
4.0 ml	0.5 M EDTA pH 8.0 (EthylenediaminetetraAcetic acid Di-sodium salt)
28.0 ml	5 M NaCl
40.0 ml	H_2O
1 g	PVP 40 (polyvinyl pyrrolidone (vinylpyrrolidine homopolymer) Mw 40,000)

Adjust all to pH 5.0 with HCL and make up to 100 ml with H_2O.

1 M Tris pH 8.0

Dissolve 121.1 g of Tris base in 800 ml of H_2O. Adjust pH to 8.0 by adding 42 ml of concentrated HCL. Allow the solution to cool to room temperature before making the final adjustments to the pH. Adjust the volume to 1 L with H_2O. Sterilize using an autoclave.

5x TBE buffer

54 g Tris base
27.5 g boric acid
20 ml of 0.5M EDTA (pH 8.0)
Make up to 1L with water.
To make a 0.5x working solution, do a 1:10 dilution of the concentrated stock.

1% Agarose gel

1 g Agarose dissolved in 100 ml TBE

Procedure

- Grind 200 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer.
- Transfer CTAB/plant extract mixture to a microfuge tube.
- Incubate the CTAB/plant extract mixture for about 15 min at 55^{o} C in a recirculating water bath.
- After incubation, spin the CTAB/plant extract mixture at 12000 g for 5 min to spin down cell debris. Transfer the supernatant to clean microfuge tubes.
- To each tube add 250 µl of Chloroform : Iso Amyl Alcohol (24:1) and mix the solution by inversion. After mixing, spin the tubes at 13000 rpm for 1 min.
- Transfer the upper aqueous phase only (contains the DNA) to a clean microfuge tube.
- To each tube add 50 µl of 7.5 M Ammonium Acetate followed by 500 µl of ice cold absolute ethanol.
- Invert the tubes slowly several times to precipitate the DNA. Generally the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 hr at -20 ^{o} C after the addition of ethanol to precipitate the DNA.

- Following precipitation, the DNA can be pipetted off by slowly rotating/spinning a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate. To wash the DNA, transfer the precipitate into a microfuge tube containing 500 µl of ice cold 70 % ethanol and slowly invert the tube. Repeat. ((alternatively the precipitate can be isolated by spinning the tube at 13000 rpm for a minute to form a pellet. Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol)).
- After the wash, spin the DNA into a pellet by centrifuging at 13000 rpm for 1 min. Remove all the supernatant and allow the DNA pellet to dry (approximately 15 min). Do not allow the DNA to over dry or it will be hard to re-dissolve.
- Resuspend the DNA in sterile DNase free water (approximately 50-400 µl H_2O; the amount of water needed to dissolve the DNA can vary, depending on how much is isolated). RNaseA (10 µg/ml) can be added to the water prior to dissolving the DNA to remove any RNA in the preparation (10 µl RNaseA in 10ml H_2O).
- After resuspension, the DNA is incubated at 65^o C for 20 min to destroy any DNases that may be present and store at 4^o C.
- Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness.

DNA quality confirmation

- Prepare a 1 % solution of agarose by melting 1 g of agarose in 100 mL of 0.5x TBE buffer in a microwave for approximately 2 min. Allow to cool for a couple of minutes then add 2.5 µl of ethidium bromide, stir to mix.
- Cast a gel using a supplied tray and comb. Allow the gel to set for a minimum of 20 min at room temperature on a flat surface.
- Load the following into separate wells
    - 10 µL 1kb ladder
    - 5 µL sample + 5 µL water + 2 µL 6x Loading Buffer
- Run the gel for 30 min at 100 V
- Expose the gel to UV light and photograph (demonstration)
- Confirm DNA quality, presence of a highly resolved high molecular weight band indicates good quality DNA, presence of a smeared band indicates DNA degredation.

Plant Genomic DNA Extraction using Qiagen plant mini kit

The advantages of using DNA isolation kits over crude methods (described above), is they are fast, simple, do not contain harmful chemicals such as phenol or chloroform and involves minimal handling. The technology makes use of spin columns, which contain a silica-gel-based membrane that binds the DNA. The DNA while bound to the membrane can be washed and cleaned from contaminants and then eluted from the column (membrane) using water. The DNA obtained is usually more pure and clean than DNA isolated from the crude method described above. One disadvantage of the kits is the cost, with kits ranging in price from $250 to $300+ for 50 reactions.

Below is an exert from the QIAGEN DNeasy Plant Mini Kit Handbook, which can be viewed on the QIAGEN web site at:-
http://www1.qiagen.com/literature/handbooks/PDF/GenomicDNAStabilizationAndPurification/FromAnimalAndPlantIssues/DNY_MinMax/1026510HB_DNY_012004WW_LR.pdf

Protocol: Isolation of Total DNA from Plant Tissue Using the DNeasy Plant Mini Kit

Important points before starting

■ If using the DNeasy Plant Mini Kit for the first time please read “Important Notes” (page 12).
■ Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
■ All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.

Things to do before starting

■ Buffers AP1 and AP3/E concentrate may form precipitates upon storage. If necessary, warm to 65°C to redissolve (before adding ethanol to Buffer AP3/E). Do not heat Buffer AP3/E after ethanol has been added.
■ Buffers AW and AP3/E are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
■ Preheat a water bath or heating block to 65°C.

Manual disruption

Grind plant or fungal tissue under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Proceed immediately to the DNA preparation protocol.

DNA Preparation

1. Add 5 ml of Buffer AP1 (preheated to 65°C) and 10 µl of RNase A stock solution (100 mg/ml) to a maximum of 1 g of ground tissue and vortex vigorously.
No tissue clumps should be visible. Vortex or pipet further to remove any clumps. Clumped tissue will not lyse properly and will therefore result in lower DNA yields.
Note: Do not premix Buffer AP1 and RNase A prior to use.
2. Incubate the mixture for 10 min at 65°C. Mix 2–3 times during incubation by inverting the tube.
This step lyses the cells.
3. Add 1.8 ml of Buffer AP2 to the lysate, mix, and incubate for 10 min on ice.
This step precipitates detergent, proteins, and polysaccharides.
4. Centrifuge lysate at 3000–5000 x *g* for 5 min at room temperature.
A pellet will form, but some particles will also float.

5. Decant supernatant into the QIAshredder Maxi Spin Column (lilac) placed in a 50 ml collection tube and spin at 3000–5000 x *g* for 5 min at room temperature (15–25°C) in a swing-out rotor. Transfer flow-through, without disturbing the pellet in the collection tube, to a new 50 ml tube (not supplied), and record the volume.
Typically, 5–6 ml of lysate is recovered. After centrifugation of the sample, most of the debris and precipitates will be retained in the filter but there will also be a pellet in the collection tube. Avoid disturbing the pellet when transferring the supernatant.
6. Add 1.5 volumes of Buffer AP3/E (see "Things to do before starting") directly to the cleared lysate and mix immediately by vortexing.
For example, to 5 ml of cleared lysate add 7.5 ml of Buffer AP3/E. Reduce the amount of Buffer AP3/E accordingly if the volume of lysate is smaller. A precipitate may form after the addition of Buffer AP3/E but this does not affect the DNeasy procedure.
Note: Ensure ethanol has been added to Buffer AP3/E (see "Things to do before starting").
Note: It is important to pipet the Buffer AP3/E mixture directly into the cleared lysate and to mix immediately.
7. Apply sample to the DNeasy Maxi Spin Column (colorless spin column) including any precipitate which may have formed (maximum loading volume 15 ml). Centrifuge at 3000–5000 x *g* for 5 min. Discard flow-through and reuse collection tube.
8. Add 12 ml Buffer AW to the DNeasy Maxi Spin Ccolumn and centrifuge for 10 min at 3000–5000 x *g* to dry the membrane. Discard flow-through and collection tube.
It is important to dry the membrane of the DNeasy Maxi Spin Column since residual ethanol may interfere with subsequent reactions. This spin ensures that no residual ethanol will be carried over during elution.
After washing with Buffer AW, the DNeasy Maxi Spin Column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer AW, refer to "Darkly colored membrane" in the Troubleshooting Guide on page 25.
9. Transfer the DNeasy Maxi Spin Column to a new 50 ml tube (supplied). Pipet 0.75–1 ml of Buffer AE directly onto the DNeasy Maxi Spin Column membrane and leave for 5 min at room temperature (15–25°C). Centrifuge for 5 min at 3000–5000 x *g* to elute.
Note: Elution may also be performed with 0.5 ml of Buffer AE (instead of 0.75–1 ml). This increases the final DNA concentration in the eluate, but also reduces overall DNA yield. See "Elution", page 13.
10. Add another 0.75–1 ml of Buffer AE and repeat the elution step as described in step 9.
The first and second eluates may be combined or collected separately. For separate collection of the eluates, see "Elution" on page 13.