DNA extraction: Comparison of methodologies

Ambika B Gaikwad
ambika@nbpgr.ernet.in

Principle

Good quality DNA is a prerequisite for all experiments of DNA manipulation. All plant DNA extraction protocols comprise of the basic steps of disruption of the cell wall, cell membrane and nuclear membrane to release the DNA into solution followed by precipitation of DNA while ensuring removal of the contaminating biomolecules such as the proteins, polysaccharides, lipids, phenols and other secondary metabolites. This is brought about by disruption of the tissue in a mortar and pestle aided by liquid nitrogen and the various components of the homogenization or extraction buffer followed the precipitating and purification methods employed. Since DNA can be extracted from various types of tissues such as seedlings, leaves, cotyledons, seeds, endosperm, tissue culture callus, roots etc., the tissue type along with the concentration of DNA finally required determine the methodology of DNA extraction to be followed by the experimenter. The most commonly used basic plant DNA extraction protocols are those of Dellaporta et al. 1983 and Saghai Maroof et al.,1984 along with many others that are modifications of the components of these protocols to suit a particular tissue type or downscaling them for miniprep. In addition to these basic protocols, a panorama of DNA isolation kits based on either anion exchange chromatography or silica gel membranes are available commercially.

Components

The role various components of DNA extraction protocol is as follows:

A. The extraction buffer
   This includes a detergent such as cetyl trimethyl ammonium bromide(CTAB) or SDS which disrupts the membranes, a reducing agent such as B mercaptoethanol which helps in denaturing proteins by breaking the disulfide bonds between the cysteine residues and for removing the tanins and polyphenols present in the crude extract, a chelating agent such as EDTA which chelates the magnesium ions required for DNase activity , a buffer which is almost always Tris at pH 8 and a salt such as sodium chloride which aids in precipitation by neutralizing the negative charges on the DNA so that the molecules can come together.
B. Phenol chloroform extraction
   Nucleic acid solutions commonly contain undesirable contaminants that are chiefly made of proteins. A classic method of purifying is phenol –chloroform extraction by which the the nucleic acid solution is extracted by successively washing with a volume of phenol(pH 8.0); a volume of phenol: chloroform: isoamyl alcohol (25: 24:

1) and chloroform: isoamyl alcohol (24:1). Centrifugation is performed intermittently and the upper aqueous phase is transferred to a new tube while avoiding the interphase. The contaminants are denatured and and accumulate in the organic phase or in the marginal layer between the two phases and the nucleic acids are preserved in the aqueous phase. Another way of removing proteins is by using the enzyme proteinase K which however again is denatured by phenol via phenol chloroform extraction.

C. Precipitation of nucleic acids
Alcohol precipitation is the most commonly used method for nucleic acid precipitation. This requires diluting the nucleic acid with a monovalent salt , adding alcohol to it and mixing gently. The nucleic acid precipitated spontaneously and can be pelleted by centrifugation. The salts and alcohol remnants are removed by washing with 70% alcohol. The most commonly used salts include sodium acetate pH 5.2(final volume 0.3M), sodium chloride (final concentration 0.2M), ammonium acetate (2- 2.5M), lithium chloride (0.8M) and potassium chloride. Ethanol (twice the volume) or isopropanol (two thirds volume) are the standard alcohols used for nucleic acid precipitation.

D. Resuspending DNA
The nucleic acid pellet can be resuspended in either sterile distilled water or TE(10 mM Tris:1mM EDTA)

E. Purification of DNA
The DNA is purified by incubating the nucleic acid solution with RNase A (10mg/ml) at 37°C and reprecipitation following phenol: chloroform extraction to remove the RNase.

Conventional methods of DNA extraction

The protocol detailed here is a modification from Saghai maroof et al.

Reagents

1. Extraction(CTAB) Buffer
   1.4 M Na Cl
   100 mM Tris (pH 8.0)
   20 mM EDTA (pH 8.0)
   2% β-Mercaptoethanol
   2% CTAB
2. Isopropanol
3. Saturated phenol pH 8.0
4. Chloroform : isoamylalcohol (24:1) mixture
5. Tris:EDTA (10mM:1mM) pH 8.0
   10 mM Tris

6. RNase A (10mg / ml):
Dissolve RNase A in 10mM Tris-Cl, pH 7.5, 15 mM NaCl. Heat at 100^0C for 15 min. Cool to room temperature. Store as aliquots at -20°C.
7. 70% ethanol

Miscellaneous

Morter and pestle
Pipettes and sterile tips
Sterile centrifuge and microcentrifuge tubes
Sterile glassware

Procedure

- Weigh 2 g of clean young leaf tissue and grind to fine powder with a pestle and mortar after freezing in liquid nitrogen.
- Transfer to 50 ml centrifuge tube with 10 ml extraction buffer maintained at 65°C in a water bath. Mix vigorously or vortex.
- Incubate at 65°C for one hour. Mix intermittently. Allow to come to room temperature.
- Add 10 ml of chloroform : isoamyl alcohol. Mix gently by makinf a figure of '8".
- Centrifuge at 10,000 rpm for 10 min at 25°C.
- Transfer aqueous phase to a fresh centrifuge tube. Add 0.6 volume of chilled isopropanol and let the DNA to precipitate for 30 min. by keeping it in – 20°C deep freezer.
- Spool out the DNA. Drain out the excess chemicals with a pipette.
- Add 0.5 ml of 70% ethanol. Mix gently and keep it at room temperature for 15 min. Decant and repeat the 70% ethanol treatment. Decant off and dry the pellet under vacuum or air dry.
- Dissolve DNA in 1 ml of 10 : 1 TE.
- Add RNAse A (10 µl) and incubate at 37°C for one hour.
- Add equal volume of phenol : chloroform : isoamyl alcohol (25:24:1), mix properly for at least 5 min and centrifuge at 10000 rpm for 10 minutes. Extract twice with chloroform : isoamyl alcohol.
- Precipitate DNA by adding 1/10 volume of 3M NaOAc and 2.0 times of the total volume chilled ethanol. Mix gently and spool out the DNA or alternatively precipitate by centrifugation at 12,000 rpm for 15 minutes. Remove extra salts by two washings with 70% ethanol. Dry under vacuum or air dry.
- Add minimum volume of TE (10:1). Resuspend the pellet at room temperature. Store at – 20°C.

Precautions

- Material finely ground in liquid nitrogen should be immediately transferred into the extraction buffer and must not be allowed to 'sweat'..
- In chloroform : isoamyl alcohol extraction, the aqueous phase should be carefully removed and organic phase re-extracted to ensure full recovery of DNA. If no separation is observed between the two phases, may be due to high concentration of DNA and /or cell debris in aqueous phase, dilution with more digestion buffer and re-extraction is the solution.
- Care should be taken to do the operations as gently as possible. Vortexing, pipetting using fine tips etc. should be avoided to prevent the shearing of DNA.
- DNA should not be over dried as resuspesion in TE become difficult.
- All the glassware, plastic ware, pestles and mortars etc. should be decontaminated properly. Care should be taken to prevent cross-contamination.
- Blank extraction controls are carried out along with normal extractions to check for any contamination.

DNA Isolation using kits

These include isolation of nucleic acids using anion-exchange chromatography or silica gel membrane technology. These kits are available commercially.

Isolation of nucleic acids using anion-exchange chromatography
Nucleic acids are highly charged, linear poly-anions and can therefore be separated from other components by anion-exchange chromatography. The QIAGEN resin (separation range: 0.1 m to 1.6 M) is a macro-porous anion-exchanger with a particle size of 100µm, and a hydrophilic surface coating that allows dense coupling of diethylaminoethyl groups. The large pore size, together with high density of anion-exchange groups, provides a broad separation range that allows selective separation of nucleic acids from proteins, polysaccharides and metabolites.

Materials

1. Liquid nitrogen
2. Mortar and pestle
3. Buffer QP (Lysis buffer): 100mM Tris/HCL pH 9.5, 2% CTAB, 1.4 M NaCl, 1% PEG 6000 (or PEG 8000), 20mM EDTA
4. β-mercaptoethanol
5. RNase A
6. Chloroform: Isoamylalcohol 24:1
7. QIAGEN Genomic-tip (gravity flow columns) 500/G (binding capacity; 500µg DNA) including tip holders
8. Buffer QBT for equilibration; Buffer QC for wash and Buffer QF for elution

Procedure

QIAGEN-tip 500/G is designed for the isolation of DNA from up to 0.4-1 g of plant tissue. DNA can be isolated from very difficult species also such as Quercus, Abies, Pinus and Ulmus and ranges in size from 20-150 kb with an average length of 50-100 kb.
The procedures have the following main steps:

- Preparation of the lysate
- Selective binding of the desired nucleic acid to QIAGEN-tip under low salt conditions
- Washing of the tip with buffers of the moderate salt concentration to remove impurities
- Elution of the nucleic acid with a high salt buffer
- Isopropanol precipitation for desalting

Isolation of nucleic acids using silica-gel based membranes
The DNeasy membrane from QIAGEN combines the binding properties of a silica-gel based membrane with microspin technology. DNA adsorbs to the DNeasy membrane in the presence of high salt concentrations of chaotropic salt, which remove water from hydrated molecules in solution. Buffer conditions in DNeasy plant procedure are designed to allow specific adsorption of DNA to the silica-gel membrane and optimal removal of carbohydrates, polyphenolics and other plant metabolites.

Material

- DNeasy Plant kit including
- DNeasy spin colums

- QIAshredder
- Collection tubes
- BufferAP1 (Lysis buffer)
- Buffer AP2 (Precipitation buffer)
- Buffer AP3 (Binding buffer)
- Buffer AW concentrate (Wash buffer)
- Buffer AE (Elution Buffer)
- RNase A
- Liquid nitrogen
- Mortar and pestle

Procedure

- Lysis of the plant cell
- Removal of cell debris and precipitates in a single step by a brief spin through QIA shredder, a unique filtration and homogenization unit.
- The cleared lysate is transferred to a new tube and binding buffer and ethanol are added to promote binding of DNA to the DNeasy membrane.
- The sample is then applied to a DNeasy spin-column and spun briefly in a centrifuge
- DNA binds to the membrane while contaminants such as proteins and polysaccharides are efficiently removed by two wash steps.
- Pure DNA is eluted in a small volume of low salt buffer or water

The detailed protocols of QIAGEN Anion-exchange chromatography and silica-gel membrane technology are available along with their respective kits.

AuPreP™ DNA easy Plant Mini Kit

AuPreP™ DNA easy Plant Mini Kit is specially designed for rapid isolation of genomic DNA (including virus, chloroplast or mitochondria) from a wide variety of plant and fungal species. The system provides shearing tubes for simple and fast homogenization as well as filtration of tissues. The simple spin-column method can isolate genomic DNA of predominantly 20-30 kb free of protein and salt contaminants without need of performing time-consuming phenol/chloroform extraction and ethanol precipitation.

Material

1. Freeze-drier
2. Bench top centrifuge
3. Liquid Nitrogen
4. 98-100% ethanol
5. TE (pH 9.0)
6. PX1 Buffer
7. PX2 Buffer
8. PX3 Buffer
9. WS Buffer
10. Rnase A
11. Plant Genomic DNA Mini Column
12. Collection Tube
13. Shearing Tube (For Mini column)

Procedure

- Grind up to 100 mg plant sample under liquid nitrogen to a fine powder and quickly transfer to a sterile 1.5-ml or 2-ml eppendorf tube.
- Add 400 µl PX1 Buffer and 4 µl RNase A stock solution (100 mg/ml) to the tissue powder and vortex vigorously. Incubate the mixture at 65°C for 10 minutes. Invert mix every 2 minutes during incubation.
- Meanwhile, preheat ddH_2O (pH 9.0), 10 mM Tris-HCl (pH 9.0), or TE buffer (500 µl /prep) at 65°C for DNA elution.
- Add 130 µl PX2 Buffer to the lysate, and vortex the mixture. Incubate it on ice for 5 minutes.
- Place a Shearing tube onto a Collection tube. Apply lysate (or lysate supernatant) to the Shearing tube and centrifuge for 2 minutes. Transfer the flow-through lysate from the Collection tube to a new sterile tube.
- Determine the volume of flow-through lysate obtained. Add 0.5 volume of PX3 Buffer and mix by pipetting. Add 1 volume of 98-100% ethanol to the mixture and mix by pipetting. (E.g., for 450 µl lysate, add 225 µl PX3 Buffer and 450 µl ethanol).
- Place a DNA easy Plant Mini column onto a Collection tube. Apply 650 µl of the ethanol-added sample (including any precipitate) from step 6 to the column, close the cap, centrifuge at 8,000 x *g* (10,000 rpm) for 1 minute. Discard the flow-through.
- Repeat step 6 for the rest of the sample.
- Transfer the column onto a new 1.5-ml tube. Discard the collection tube and flow-through.
- Elute DNA with 200 µl (or 100 µl x 2) (or at least 50 µl) elution buffer such as ddH_2O (pH 9), 10 mM Tris-HCl (pH 9.0), or TE buffer preheated at 65°C. Stand for 5 minutes, and centrifuge for 1-2 minutes.
- Store eluted DNA at 4°C for frequent use or -20°C for long-term storage.

References

1. Dellaporta, S.L., Wood, J. and Hicks, J.B. 1983. A plant DNA mini preparation: Version II. *Plant Molecular Biology Reporter* 1: 19-21.
2. Saghai-Maroof, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W. 1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Proc. Natl. Acad. Sci.* USA 81: 8014-8018.