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Chapter 24

CTAB-Based extraction of total plant genomic DNA: Principles, protocols, and applications

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1. Introduction:

DNA isolation is a fundamental technique in molecular biology, essential for various applications such as DNA fingerprinting, restriction fragment length polymorphism (RFLP), construction of genomic libraries, and PCR analysis (Sambrook, and Russell, 2001;). It serves as the initial step in studying specific DNA sequences within a complex genome, as well as in analyzing genome structure and gene expression. In prokaryotic cells, DNA is contained within the nucleoid, not separated by a membrane from the cell sap. In contrast, eukaryotic DNA resides predominantly in the nucleus, separated by a membrane from the cytoplasm. The goal of DNA isolation is to effectively separate DNA from other cellular components, yielding a homogeneous DNA preparation that represents the entire genetic information of the cell (Doyle, and Doyle 1990; Reddy et al., 2018; Srivastava et al., 2022).

While DNA isolation from small molecules is straightforward due to DNA's large molecular weight, challenges in plant DNA isolation include DNase activity that degrades DNA and the co-precipitation of other macromolecules such as polysaccharides, polyphenols, and proteins during the isolation process. Three primary techniques or combinations thereof—differential solubility, adsorption methods, and density gradient centrifugation—are employed for nucleic acid isolation, chosen based on the type of DNA and its intended application (Brown, 2010l; Tripathi et al., 2021). The primary objective of nucleic acid isolation is to remove proteins, achieved through their distinct chemical properties.

2. Principle:

High-quality DNA is imperative for all DNA manipulation experiments. Plant DNA extraction protocols universally involve cell wall, cell membrane, and nuclear membrane disruption to release DNA into solution, followed by DNA precipitation while ensuring

the removal of contaminating biomolecules such as proteins, polysaccharides, lipids, phenols, and secondary metabolites. Tissue disruption using liquid nitrogen in a mortar and pestle, aided by components of the homogenization or extraction buffer, facilitates this process. The choice of DNA extraction methodology depends on the tissue type—from seedlings, leaves, cotyledons, and seeds to tissue culture callus and roots—and the required DNA concentration.

3. Materials Required:

1. Leaves.
2. Saturated Phenol Ph 8.0
3. Chloroform:Isoamyl Alcohol (In 24:1 Ratio)
4. Te Buffer (Tris: Edta, 10mm:1mm, Ph 8.0)
5. 70% Ethanol, Rnase A (10 Mg/Ml).
6. Water Bath, Micropipettes
7. Centrifuge
8. Electrophoresis Chamber
9. UV Transilluminator.
10. Sterile Tips, Sterile Microcentrifuge Tubes
11. Tube Racks
12. Mortar and Pestle.

4. Procedure:

1. Grind 1.0 g of tissue in liquid nitrogen.
2. Transfer the powdered tissue into a 50 mL centrifuge tube containing 10 mL of 2% CTAB buffer and add 7 µL of β-mercaptoethanol. Incubate at 60°C for approximately 30 minutes.
3. Perform chloroform:isoamyl alcohol (24:1) extraction once or twice, then centrifuge at 6000 rpm for 15 minutes at room temperature.
4. Transfer the upper aqueous phase to a fresh centrifuge tube, add half the volume of 5 M NaCl and 1/10th volume of 3 M sodium acetate.
5. Precipitate DNA by adding chilled isopropanol (5 mL).
6. Mark the centrifuge tubes to predict the pellet's location.
7. Centrifuge at 3000 rpm for 3 minutes, followed by an additional 3 minutes at 5000 rpm.
8. Wash the DNA pellet with chilled 70% ethanol.
9. After drying, resuspend the DNA pellet in 1X TE buffer using a wide-bore pipette tip and transfer to a microcentrifuge tube.
10. Centrifuge again at 3000 rpm at 4°C for 5 minutes to remove any remaining debris.

11. Add 1 µL of RNase A (from 10 mg/mL stock) to 100 µL of DNA suspension and incubate at 37°C for one hour.
12. Adjust the volume to 500 µL with MilliQ water (double distilled water).
13. Perform Chloroform:Isoamyl Alcohol (24:1) Extraction Twice and Remove Upper Phase Using Wide Bore Tips
14. Perform chloroform:isoamyl alcohol (24:1) extraction twice using an equal volume (500 µL each time) and carefully remove the upper aqueous phase using wide bore tips.
15. Precipitate DNA in 1/10th Sodium Acetate and 95% Ethanol (Add 5.0 M NaCl if Necessary)
16. Precipitate DNA by adding 1/10th volume of sodium acetate and an equal volume of chilled 95% ethanol. Add 5.0 M NaCl if needed to facilitate precipitation.
17. Centrifuge at 10,000 rpm for 10 Minutes at 4 ºC and Wash Pellet Thrice with 95% EtOH
18. Centrifuge the sample at 10,000 rpm for 10 minutes at 4 ºC. Wash the DNA pellet three times with chilled 95% ethanol.
19. Dry Pellet and Elute in 0.1X TE, Then Quantify on Gel
20. Dry the DNA pellet and resuspend it in 0.1X TE buffer. Quantify the DNA by loading it onto an agarose gel for analysis.

5. Observation:

The gel was examined under UV radiation to detect the presence or absence of DNA bands. A DNA marker was loaded in lane 1 to determine the size of the DNA fragments.

6. Result:

The bands observed at the top of the gel indicate high molecular weight DNA, larger than all the DNA fragments in the marker lane. The intensity and clarity of these bands suggest a high concentration of intact genomic DNA.

7. Precautions:

1. Handle the centrifuge with care during operation.
2. Maintain a clean laboratory environment throughout the experiment.
3. Use DNase-free plastic ware and reagents. Ensure tubes and tips are clean, dry, and autoclaved.
4. Exercise caution when handling ethanol, as it is highly flammable.
5. Repeat phenol chloroform extraction as needed to obtain pure DNA depending on the DNA source.
6. Take precautions when working with ethidium bromide, as it is carcinogenic. Wear gloves when handling ethidium bromide-stained agarose gels.

7. Check reagents for precipitation before use. If any reagent forms a precipitate (excluding enzymes), warm it at 55-65 °C until dissolved, then cool to room temperature (15-25 °C) before use.

8. References:

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