Protocol

Plant DNA Extraction Protocol

Introduction

DNA extraction is usually the first step required for many molecular biology investigations, including genotype identification, correlation of genes with economic traits, and gene diversity. However, the total DNA extracted from different plant species may vary according to the content of common metabolites such as polysaccharides and polyphenols. Finding a universal DNA protocol can be challenging. However, there is a trend in plant biology in using CTAB-based methods for DNA extraction from complex plants.

We selected the protocol reported by Aboul-Maaty and Oraby, 2019 as our choice plant DNA extraction protocol. This protocol was tested in seven different plant orders, covering 19 species, which makes it valuable for starting plant DNA extraction. The protocol is based on the CTAB-method. CTAB is the acronym for hexadecyltrimethyl-ammonium bromide. It is a powerful detergent that captures lipids from cell membranes during DNA extraction forming micelles, which break the cell membranes and favor the nuclear content release. Furthermore, CTAB and some other chemicals like PVP are used to minimize contaminants such as polysaccharides and polyphenols. For additional information, go to our GoldBio article, Overview of Plant DNA extraction.

Materials

Plant material

The plant material tested with this protocol includes plant species belonging to the following orders: Poales, Solanales, Cucurbitales, Fabales, Cruciferas, Cyperales, and Malvales. Some of the plant species tested include *Zea mays*, *Oryza sativa*, *Solanum tuberosum* and *Phaselus vulgaris*. To see the entire list of species tested in this protocol, please refer to Aboul-Maaty and Oraby, 2019.

Buffer and Stock solutions

0.1% DEPC-treated water per 1000 mL:

- 1 mL DEPC (diethyl pyrocarbonate) reagent
- 1000 mL of Milli-Q water
- Mix on a magnetic stir plate to dissolve the DEPC. Leave the solution mixing overnight.
- Autoclave at 121°C for 15 minutes to inactivate the DEPC.

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15M NaCl per 100 mL:

- 87.6 g of NaCl
- Fill to 100 mL with 0.1% DEPC-treated water
- Store at room temperature

6M NaCl per 100 mL:

- 40 mL of 15M NaCl previously prepared
- Fill to 100 mL with 0.1% DEPC-treated water
- Store at room temperature

1M Tris-HCl per 1000 mL:

- 121.14 g Tris-HCl
- 800 mL of 0.1% DEPC water
- Adjust to pH 8.0 with 1M HCl
- Fill to 1000 mL with 0.1% DEPC-treated water
- Store at room temperature

5M EDTA Disodium per 100 mL:

- 186 g of EDTA Disodium
- 80 mL of 0.1% DEPC-treated water
- Adjust to pH 8.0 with 5M NaOH
- Fill to 100 mL with 0.1% DEPC-treated water
- Store at room temperature

3X CTAB extraction buffer per 100 mL:

- 3 g CTAB (w/v)
- 9.33 mL of 15M NaCl
- 80 mL of 1M Tris-HCl, pH 8.0 (previously prepared)
- 10 mL of 5M EDTA, pH 8.0 (previously prepared)
- Autoclave at 121°C for 15 minutes

1X TE buffer per 100 mL:

- 1 mL of 1M Tris- HCl, pH 8.0 (previously prepared **)**
- 0.02 mL of 5M EDTA Disodium, pH 8.0 (previously prepared)
- 98.98 mL of 0.1% DEPC-treated water
- Autoclave at 121°C for 15 minutes
- Store at room temperature

3M Sodium acetate per 100 mL:

- 24.6 g of sodium acetate

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- 50 mL of 0.1% DEPC-treated water
- Adjust to pH 4.0 with 1M HCl
- Fill to 100 mL with 0.1% DEPC-treated water
- Store at room temperature

Chloroform: Isoamyl alcohol (24:1) per 25 mL:

- 24 mL of chloroform
- 1 mL of isoamyl alcohol
- Prepare just before start the DNA extraction

70% ethanol per 100 mL:

- 70 mL of absolute ethanol
- 30 mL of autoclaved 0.1% DEPC-treated water
- Mix well and store at 4°C.

Individual Reagents

- β-mercaptoethanol
- Isopropyl alcohol

Supplies and Equipment

- Ceramic mortar and pestle
- Glassware
- Micro-spoon or spatula
- DNase/RNase free microcentrifuge tubes
- DNase/RNase free pipet tips
- Tube racks
- Fume hood chamber
- Magnetic stirrer/ with heat
- pH meter
- Vortex
- Autoclave
- Centrifuge
- Gel electrophoresis
- Nanodrop

Method

1. Pretreat all the plastic, glassware, mortar, and pestle with 0.1% DEPC-treated, Milli-Q water and autoclave. Similarly, prepare all the solutions with autoclaved DEPC-treated water.

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2. On the extraction day, heat the 3X CTAB extraction buffer at 65°C in a water bath. Add fresh 0.3% (0.3 mL) β-mercaptoethanol to the buffer immediately before use.

Lysis

3. Using liquid nitrogen, grind 50 mg of plant tissue until powder with the help of a mortar and pestle.

1. Add 800 µL of the warm 3X CTAB extraction buffer to the plant powder still on the mortar and proceed to mix gently with the pestle.

2. Transfer the sample mixture to microcentrifuge tubes and incubate at 65°C for 1 hour in a water bath. Invert the tubes several times every 20 minutes. Remove from the bath and let cool to room temperature (~25°C).

Isolation

3. Add an equal volume of chloroform:isoamyl alcohol (24:1 v/v) and mix by inversion.

4. Centrifuge at 26,500 x g for 15 minutes at room temperature.

5. Carefully transfer the upper aqueous phase to a new micocentrifuge tube. If the upper aqueous phase is not clear, repeat steps 6-8.

6. Add half of the volume of the aqueous phase with 6M NaCl. For instance, if the aqueous phase is approximately 700 µL, add 350 µL of 6M NaCl and mix well.

7. Add 1/10 the initial volume (about 70 µL) of 3M potassium acetate.

8. Add 500 µL ice-cold 100% isopropyl alcohol (⅔ of the aqueous phase volume). Mix gently by inversion to precipitate DNA.

9. Incubate under cold conditions at -20°C for 30 minutes.

10. Centrifuge at 26,500 x g for 5 minutes and remove supernatant.

11. Place the tube upside down on tissue paper to drain off any remaining supernatant.

Precipitation

12. Add 500 µL 70% ethanol to wash the DNA pellet. Invert once to remove any residual salts.

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13. Centrifuge again at 26,500 x g for 5 minutes.

14. Remove the 70% ethanol and allow the pellet to dry briefly at room temperature. Be careful not to over dry the pellet.

15. Add 50 µL of 1X TE buffer. Incubate the samples at 50°C for 1-2 hours to ensure complete re-suspension.

16. Store at -20°C until further use.

Measurement of the extracted DNA quality and quantity

Nanodrop

1. Take out the tubes containing the extracted DNA from the freezer and let thaw slowly on ice.

2. Gently mix the extracted DNA via 10 µL pipet.

3. Add 1 µL of the solution on the Nanodrop.

4. Measure the absorbance for A260/A230 and A260/A280. Ratios of ≥2 (A260/A230) and 1.8 (A260/A280) for each absorbance would suggest high quality DNA. The Nanodrop will also calculate the amount of extracted DNA in µg/µL.

Gel electrophoresis running conditions

1. Clean a 250 mL beaker by adding 20 mL of deionized water. Heat for 90 seconds in a microwave, then discard any remaining water.

2. Weight 1 g agarose and add 100 mL of 1X TAE to the 250 mL beaker.

3. Heat in a microwave up to 90 seconds (for higher volumes, heat for up to 99 seconds), or until agarose boils. Do not let the agarose solution boil over.

4. Allow the agarose to cool to about 50°C (10-15 minutes) and add 3 µL of Gel Red.

5. Pour the agarose slowly on the electrophoresis gel box and let the agarose solidify. If case bubbles form, they can be pushed away using a small pipet tip.

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6. In a small tube, add 2 µL of loading buffer to 4 µL of each DNA sample and briefly mix by pipet. Load the DNA samples into the gel.

7. Run for 20-30 minutes at 137 volts.

8. Read in the trans-illuminator.

9. You should be able to visualize one clear band per sample. Smeared bands are an indication that the DNA may be degraded.

Associated Products

- Diethylpyrocarbonate (DEPC) (GoldBio Catalog # D-340)
- Tris HCl (GoldBio Catalog # T-095
- EDTA Disodium, dihydrate (GoldBio Catalog # E-210)
- Agarose LE (GoldBio Catalog # A-201)
- 1 kb PLUS™ DNA Ladder (GoldBio Catalog # D011)
- 100 bp PLUS™ DNA Ladder (GoldBio Catalog # D003)

References

Aboul-Maaty, N.AF., Oraby, H.AS. Extraction of high-quality genomic DNA from different plant orders applying a modified CTAB-based method. Bull Natl Res Cent 43, 25 (2019). https://doi.org/10.1186/s42269-019-0066-1.