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Total DNA extraction

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We use this protocol and it's working

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Abstract

This protocol outlines an optimized method for the extraction and purification of total DNA from woody plant samples, specifically designed for enriching native form of circular ssDNA viruses such as Grapevine Red Blotch Virus (GRBV). The process leverages a 3% CTAB extraction buffer enriched with polyvinylpyrrolidone (PVP40) and β -mercaptoethanol to efficiently handle polysaccharides and polyphenols abundant in woody plant tissues. Steps include mechanical disruption with bead beating, high-salt precipitation with sodium acetate (NaAc), RNA removal, and DNA washing to ensure high purity for downstream applications such as nanopore sequencing or molecular analysis.

Guidelines

- Ensure all equipment and materials are sterile to prevent contamination of DNase.
- Prepare fresh CTAB buffer and add β -mercaptoethanol immediately before use.
- Use personal protective equipment (PPE), including gloves and a lab coat, when handling chemicals such as β -mercaptoethanol and chloroform:isoamyl alcohol.
- Maintain the temperature of ethanol and sodium acetate (NaAc) solutions as specified to ensure optimal DNA precipitation.
- Use a Nanodrop or similar device to confirm DNA quality and concentration before proceeding to downstream applications.

Materials

CTAB Buffer Components:

- 100 mM Tris-HCl (pH 8.0)
- 2 M NaCl
- 20 mM EDTA
- 3% CTAB powder
- 2.5% Polyvinylpyrrolidone (PVP40)
- 1% β -mercaptoethanol (added fresh before use)

Other Chemicals and Solutions:

- Sodium acetate (NaAc), 3 M solution
- 100% ethanol
- Cold 75% ethanol (stored at -20°C)
- Chloroform:isoamyl alcohol (24:1)
- RNase A or RNase cocktail (containing RNase A and RNase T1)
- DNase-free water

Consumables:

- 2 ml microcentrifuge tubes
- Beads for bead beating
- Liquid nitrogen

Equipment:

- Centrifuge capable of speeds up to 15,700 g
- Heating block or water bath (set to 65°C and 50°C)
- Bead-beating equipment
- Pipettes and sterile pipette tips
- Nanodrop or equivalent device for DNA quantification

Safety Equipment:

- Lab coat
- Gloves
- Fume hood for handling hazardous chemicals

Safety warnings

- β -mercaptoethanol is toxic and should be handled in a fume hood to avoid inhalation or skin contact.
- Chloroform:isoamyl alcohol is highly volatile and carcinogenic; handle with caution in a well-ventilated area.
- Do not allow CTAB buffer to come into contact with acidic solutions, as this may release toxic cyanide gas.
- Ensure all waste is disposed of according to institutional biosafety and chemical disposal guidelines.
- Sodium acetate (NaAc) is hygroscopic; store properly and handle with care to maintain its integrity.

Before start

- Verify that all required materials, including CTAB buffer, ethanol, and RNase, are ready and prepared according to the protocol.
- Ensure the plant samples are fresh or appropriately stored to prevent DNA degradation.
- Pre-chill ethanol to -20°C and incubate the CTAB buffer at 65°C for at least 15 minutes before use.
- Check that the bead-beating equipment is functioning correctly and adjust settings if necessary.
- Prepare 3 M sodium acetate (NaAc) solution as needed for DNA precipitation.



Preparation of Materials

15m

- 1 Prepare the CTAB Buffer; autoclave for 20 minutes and add 1% β -mercaptoethanol to the CTAB buffer just before use.
 - Incubate the CTAB buffer at 65°C for at least 15 minutes before use.
- 2 Pre-chill 75% ethanol at -20°C.
Ensure sodium acetate (NaAc) solution is ready for DNA precipitation.

Sample Homogenization

15m

- 3 **Weigh Plant Material:**
 - Measure 100-150 mg of plant sample and transfer to a 2 mL microcentrifuge tube.
- 4 **Homogenize the Sample:**
 - Add 4 beads to the tube.
 - Perform bead beating for 15 seconds at 3000 rpm in the presence of liquid nitrogen.
 - Check the sample for uniform grinding; repeat if necessary.

DNA Extraction

1h 30m

- 5 **Add CTAB Buffer:**
 - Add 750 μ L of preheated CTAB buffer to the sample.
 - Vortex vigorously for 1 minute and incubate at 65°C for 30-60 minutes (longer for woody plants).
- 6 **Centrifuge the Sample:**
 - Centrifuge at 15,700 g for 5 minutes.
 - Transfer approximately 700 μ L of supernatant to a new tube.
- 7 **Perform Chloroform:Isoamyl Alcohol Extraction:**
 - Add 1 volume (700 μ L) of chloroform:isoamyl alcohol (24:1).
 - Vortex for 1 minute and centrifuge at 15,700 g for 10 minutes.
 - If necessary, repeat the step for additional washing.

Point: If your plant sample contains high amount of polysaccharides and polyphenols, another washing step by Chloroform:isoamyl alcohol (24:1) is recommended.

RNA Removal and DNA Precipitation

1h 30m

- 8 **Remove RNA:**
 - Transfer 600 μ L of the supernatant to a new tube.
 - Add 6 μ L RNase A (20 mg/mL).



- Incubate at 37°C for 30 minutes.
- Add 1 volume of chloroform:isoamyl alcohol (24:1) to the sample.
- Vortex for 1 minute and centrifuge at 15,700 g for 10 minutes.

9 **Precipitate DNA Using Sodium Acetate:**

- Transfer 500 µL of the supernatant to a clean tube.
- Add 0.1 volume of 3 M sodium acetate (NaAc) and 2.5-3 volumes of 100% ethanol.
- Invert the tube gently to mix.
- Incubate the tube at -20°C for 20 minutes.

Point: Longer incubation may precipitate smaller DNA fragments but can also increase the risk of co-precipitating contaminants or degrading nucleic acids.

- Centrifuge the tube at 15,700 g for 15 minutes at 4°C.

Wash the DNA Pellet with Cold 75% Ethanol

40m

10 **Option 1 (Rinsing the Pellet):**

- Add **1 mL of cold 75% ethanol** directly to the side of the tube where the pellet is located.
- Gently swirl the tube to rinse the pellet without dissolving it.
- Carefully discard the ethanol (repeat one more time).

Point: This method minimizes the risk of losing the DNA pellet and is recommended for shorter incubation times.

Option 2 (Dissolving and Re-Centrifuging):

- Completely dissolve the pellet in 1 mL of cold 75% ethanol by gently mixing.
- Centrifuge again at 15,700 g for 10 minutes at 4°C to re-precipitate the DNA (repeat one more time).

Point: This method is more effective at removing contaminants and is recommended after longer incubation times (e.g., overnight).

Air-Dry the Pellet:

- Carefully discard the supernatant and air-dry the tubes for **10-15 minutes**.
- Ensure the pellet is not over-dried, as it can be difficult to dissolve later.

Dissolve the DNA:

Add **51 µL of DNase-free water** to the tube.

Optional: Incubate the tube at **50°C for 5 minutes** on a preheated heating block or water bath to facilitate complete dissolution of the DNA pellet.

11 **Measure DNA Quality and Concentration:**

- Use **1 µL of the solution** with a Nanodrop or equivalent spectrophotometer to measure DNA concentration and quality.