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A Rapid and High Yielding DNA Miniprep for Cotton (*Gossypium* spp.)

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Abstract. A rapid DNA minipreparation method was developed for cotton and yields 500-600 µg DNA from 1.0 g fresh leaf tissue. Cotton DNA extracted using this method is completely digested with restriction enzymes, supports PCR and Southern DNA analyses and was used successfully in these applications.

Key words: DNA extraction, *Gossypium*, minipreparation

Introduction

Isolation of DNA from cotton and other plants high in secondary compounds can be problematic; the yield of DNA isolated is often low and the quality can be poor. Commercial kits have been useful for DNA extraction from cotton tissues but the yield of DNA is often low and the quality of DNA is variable. Time-tested DNA mini-preparations that work well with other plant species (Dellaporta et al., 1983; Doyle and Doyle, 1991) do not give satisfactory results with cotton. DNA solutions turn brown, yields are low and often the DNA cannot be digested or used in PCR amplification. More recent procedures for cotton DNA extraction (Paterson et al., 1993; Permingeat et al., 1998; Zhang and Stewart, 2000) result in low yields as well.

We report a rapid and high yielding two-step mini-preparation method for cotton DNA from a small amount of fresh leaf tissue (50-100 mg). The procedure involves nuclei isolation and lysis steps (Patterson et al., 1993). The preparation is enriched in genomic DNA, supports enzymatic digestion, PCR amplification (Mullis et al., 1986) and is produced in sufficient quantity for Southern DNA analysis (Southern, 1975). Fifty to sixty µg DNA is routinely recovered from 100 mg of cotton leaf tissue. The high DNA yield of the procedure is achieved through use of two vortex-mixing steps.

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Table 1. The effect of vortex mixing and adding sugars in the extraction buffer on the cotton DNA yield.^{1,2}

Sample Preparation	Average Yield $\mu\text{g DNA/g FW}$
Mixing Step	
Tube Inversion	114.75 \pm 18.84
Vortex - 20 s	523.43 \pm 49.51
Vortex - 40 s	570.83 \pm 98.04
Vortex - 60 s	594.53 \pm 44.97
Sugars in Buffer	
Control - no sugar	428.0 \pm 51.1
Sorbitol	503.3 \pm 96.0
Glucose	318.0 \pm 41.0
Mannitol	394.7 \pm 54.5
Sucrose	283.3 \pm 96.0
Method Comparison³	
Tube Inversion	132.16 \pm 25.8
Vortex - 40 s	641.8 \pm 129.0
Kit	192.0 \pm 37.4

¹Leaf size: 2.0-2.5 cm diameter

²Leaf source: Growth Chamber

³Leaf source: Greenhouse

Materials and Methods

Cotton leaf DNA minipreparation

- DNA extraction buffer (EB): 100 μM Tris-base and 5 μM EDTA (Fulton et al., 1995). Before using, add sodium bisulfite, 0.4 g/100 mL buffer. We also routinely include 0.35 M sorbitol.
- Lysis buffer (LB): 0.2 M Tris-base (pH 8.0), 50 μM EDTA (pH 8.0) (Fulton et al., 1995), 2 M NaCl and 55 μM CTAB (Murray and Thompson, 1980).
- Five percent Sarkosyl (N-Lauroylsarcosine, sodium salt).

1. Leaf tissue preparation

Harvest about 0.05-0.1 g young leaves (2.0-2.5 cm in diameter). Fold leaves and place in a 1.5 mL microcentrifuge tube. Keep tissue samples on ice. Freeze tissue with liquefied N_2 and grind to a fine powder in a 1.5 mL microcentrifuge tube with a pellet pestle (pestle can be mounted on a drill for grinding).

2. DNA extraction

Add 600 μL extraction buffer (EB) and vortex 40-60 s until thoroughly mixed. Centrifuge 6500-7000 g-force for 15 min at room temperature. Discard supernatant.

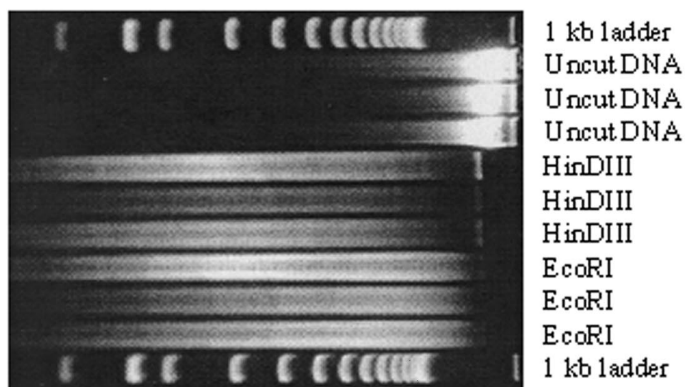
Add 250 μL EB to pellet and vortex 40-60 s.

Add 5 μL RNase (pancreatic RNase A) 10 mg/mL (optional)

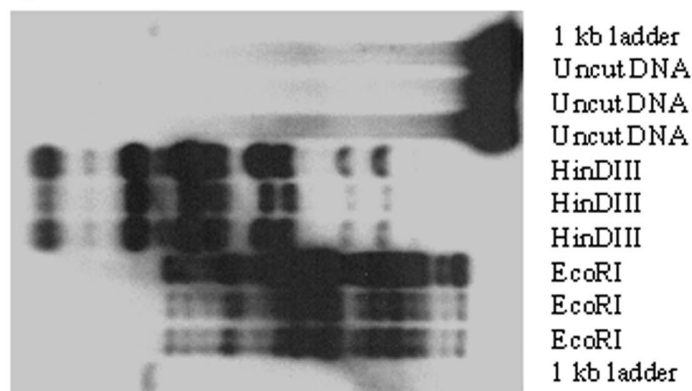
Add 600 μL lysis buffer (LB) and 60 μL 5% Sarkosyl. Invert tube 20-40 times until thoroughly mixed.

Incubate at 65°C for 15 min.

A DNA Digestion



B Southern Analysis



C PCR Amplification



Figure 1. (A) Digestion of cotton DNA isolated from cotton leaves using the described procedure (10 μ g DNA was loaded into each lane): (lanes 1 and 11) 1 kb ladder; (lanes 2-4) uncut DNA; (lanes 5-7) DNA digested with *Hind* III; (lanes 8-10) DNA digested with *Eco*R I; (B) Southern blot of gel seen in Figure 1A, probed using the *G. arboreum* delta-cadinene (CAD) gene (Meng et al., 1999); (lanes as in A); (C) Agarose gel of PCR amplified 600 bp fragment of the delta-cadinene (CAD) gene from cotton leaf DNA, isolated using the described procedure: (lane 1) 1 kb ladder; (lanes 2,3) amplified 600 bp fragment of the native CAD gene. Primer sequences used: forward 5'-ATAAGGATGAAATGCG-3' and reverse 3'-GAAGCTTGGTAAAGTTCA-5'.

Add 500 μL of chloroform:isoamyl alcohol (CIA, 24:1, v/v) and vortex samples 40-60 s to mix contents.

Centrifuge 6500-7000 *g*-force for 5-10 min at room temperature.

Pipette aqueous supernatant into a new clean 1.5 mL microcentrifuge tube.

Repeat CIA extraction.

3. DNA precipitation

Add 1 volume of cold isopropanol (-20°C).

Gently mix by tube inversion until DNA precipitates.

Centrifuge 6500-7000 *g*-force for 10-15 min. Discard the supernatant.

Wash DNA pellet with 70% EtOH.

Dry the pellet 30 min and dissolve DNA into 30-60 μL H_2O or TE buffer.

Results and Discussion

A vortex-mixing step significantly improved the yield of cotton DNA over tube inversion (Table 1). Although not significantly different among 3 vortex-mixing treatments, the yield of DNA appeared to increase with vortex time and we recommend 40 s vortex during DNA extraction. In addition, efficiency of the vortex step appeared to improve with addition of sorbitol in the extraction buffer, and appeared to be reduced when mannitol, glucose (Patterson et al., 1993) or sucrose (Permingeat et al., 1998) were used (Table 1). Using sorbitol in the extraction buffer (EB), the yield of DNA obtained with vortex mixing (40 s) exceeded the yield of DNA from the same extraction procedure without vortex mixing and also exceeded the yield of DNA obtained by using a kit (Table 1). The yield of DNA obtained using this procedure (500-600 μg DNA/g fresh weight) is unusually high for cotton and is comparable to the yield of DNA extracted from other plant species using improved minipreparation methods (Chen and Ronald, 1999). Cotton DNA from these preparations supports enzymatic digestion (Figure 1A), is produced in sufficient quantity and quality for Southern DNA analysis (Figure 1B) and also supports PCR amplification (Figure 1C).

This rapid general-purpose cotton DNA extraction procedure employs several small 1.5 mL microcentrifuge tubes, relatively dilute buffers and includes several vortex-mixing steps. The yield of cotton DNA obtained from leaves using this procedure is higher than the DNA obtained from the same amount of leaf tissue with a good DNA extraction kit.

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