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Commentary

Ready-to-Use DNA Extracted with a CTAB Method Adapted for Herbarium Specimens and Mucilaginous Plant Tissue

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Abstract. This report summarizes major changes in previously published protocols for DNA extraction to improve the quality of DNA extracted from plants. Here, we highlight the critical modifications in the original protocols. The efficiency of these changes results in high-quality DNA ready to use in a variety of phylogenetically distant plant families, in particular species with mucopolysaccharides. The DNA obtained can be used without further purification in various molecular biology assays, including direct sequencing and AFLP and RAPD (random-amplified polymorphic DNA) analyses. The effectiveness of this method is proven by the amplification and sequencing of PCR products of up to 1 kb with DNA extracted from herbarium tissue ≥60 years old. This versatility is not usually found in DNA extraction protocols. In addition, this method is quick, adaptable to standard laboratories, and most important, safer and more cost-effective.

Key words: CTAB, DNA isolation, herbarium specimens, mucopolysaccharides

Abbreviations: CsCl, cesium chloride; NaCl, sodium chloride; NaOAc, sodium oxaloacetate; RAPD, random amplified polymorphic DNA; SEVAG, chloroform:isoamyl alcohol.

Introduction

Studies investigating phylogenetic relationships and levels of genetic diversity use various molecular methods, for example, direct sequencing, random-amplified polymorphic DNA (RAPD) analysis, and AFLP analysis, for which the availability of a safe, quick, and cost-effective DNA extraction method is desirable. To generate reproducible results, these techniques require clean, relatively pure DNA, which must be digestible with restriction endonucleases and amplifiable via PCR (Lodhi et al., 1994).

Fresh or frozen plant tissue is ideal; however, quality DNA has been obtained from specimens preserved in herbaria for >100 years (Soltis and Soltis, 1993; Taylor and Swann, 1994; Golenberg, 1999). In fact, in recent years, the use of botanical collections has increased significantly in phylogenetic and evolutionary

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studies of plants (Drábková et al., 2002; DeCastro and Mendel, 2004; Jankowiak et al., 2005). This is in part due to the vast taxonomic diversity and relative ease of accessibility of endangered or understudied species in herbaria. Unfortunately, several factors hinder the extraction of high-quality DNA from historical specimens, including inappropriate preservation, exposure to chemicals, and the natural degenerative process of DNA (Biss et al., 2003). The isolation of DNA from plants and its subsequent use in molecular techniques may also be problematic because of the presence of polysaccharides, phenols, and other organic compounds (Porebski et al., 1997). Mucopolysaccharides are common in cacti and other succulent species (Griffith and Porter, 2003) and are easily detected in the extraction process by a viscous consistency in the final elution with tris-EDTA (Lodhi et al., 1994) or during the isopropanol precipitation (Cota-Sánchez, personal observation). These complex molecules interfere with DNA quality, lead to low yields (Tel-Zur et al., 1999), and inhibit *Taq* polymerase (Fang et al., 1992). Hence, their removal is crucial for successful use of DNA in molecular techniques.

A number of protocols (e.g., Porebski et al., 1997; Li et al., 2002; Cheng et al., 2003) have been proposed to extract high-quality DNA free of chemicals and impurities, in particular polysaccharides and contaminants in herbarium tissue. Protocols using a CTAB solution combined with lengthy precipitation are effective in extracting DNA from herbarium specimens (Drábková et al., 2004). Early approaches to DNA extraction used CsCl gradients (Paterson et al., 1993) but have lost popularity because of the expense, time, and use of excessive amounts of ethidium bromide. Newer methods use NaCl, a more efficient, cost-effective, and safe alternative (Lodhi et al., 1994).

Despite the improvements in the extraction of DNA, numerous protocols are limited to specific plant groups. In this commentary, we present a modified and versatile protocol to extract DNA from fresh, frozen, silica gel–dried, and herbarium material from a variety of plant families, including some with mucopolysaccharides. We present a simple, quick, and low-cost method adaptable to standard laboratory conditions to isolate high-quality DNA that can be used in various techniques without further purification. Our method, originally modified to extract DNA from cacti, is based on previous protocols using CTAB (Murray and Thompson, 1980; Saghai-Maroof et al., 1986). We have made significant modifications (Table 1), making this method a practical alternative to more difficult or expensive protocols.

Materials and Methods

Reagents and solutions

- CTAB extraction buffer (2× CTAB: 50 mL of 1.0 tris-HCl, pH 8, 140 mL of 5 M NaCl, 50 mL of 0.25 M EDTA pH 8, 10 g of CTAB, distilled water to 500 mL)
- 2-mercaptoethanol
- Chloroform:isoamyl alcohol (SEVAG), 24:1
- Isopropanol

Table 1. A comparison of the Murray and Thompson (1980) and Saghai-Maroof et al. (1986) methods relative to modifications discussed herein.

Step in Extraction Protocol	Murray and Thompson (1980)	Saghai-Maroof et al. (1986)	Cota-Sánchez et al. (current study)
CTAB concentration	1×	1×	2× or 3×
Organic extraction	Repeated organic extraction (chloroform: octanol) with CTAB buffer and Tris-HCl	Single organic extraction (chloroform: octanol)	Repeated organic extraction (chloroform: isoamyl alcohol)
DNA precipitation	CsCl gradients	In isopropanol for 2–4 inversions	In isopropanol overnight
Resuspension of DNA	Not applicable	Immediately after isopropanol precipitation in EDTA	Resuspension in EDTA and RNase; incubation at 37°C for 30 min
Second DNA precipitation and resuspension in TE	None	None	Overnight precipitation in 95% ethanol; 70% ethanol wash; drying period; resuspension in TE

- Tris-EDTA (TE)
- RNase, 10 mg/mL
- Sodium oxaloacetate (NaOAc), 2.5 M
- Ethanol, 70% and 95%

DNA extraction protocol

1) Add 750 µL of 2× CTAB buffer and 3.0 µL of 2-mercaptoethanol to Eppendorf tubes.
2) Grind 0.5–1.0 g of tissue with liquid nitrogen and sterilized sand until finely powdered.
3) Add a spatula-tip of powdered tissue to each tube and mix well.
4) Incubate in a water bath at 55-60°C for 1–5 h, mixing every 15 min.
5) Add 700 µL of SEVAG to each tube and mix thoroughly. Centrifuge at 9240 *g* for 10–15 min. Transfer the aqueous phase to a new Eppendorf tube.
6) Add 0.33 vol of ice-cold isopropanol and store at -30°C for at least 1 h.
7) Spin at 9,240–13,305*g* for 10 min at room temperature. Discard supernatant without disturbing the pellet. Vacuum dry. Repeat steps 6 and 7 two to four times if the aqueous phase is viscous.
8) Resuspend pellet in 100–200 µL of TE. Add 1-2 µL of 10 mg/mL RNase. Mix well and incubate for 30 min at 37°C.
9) Add 20 µL (0.1 vol) of 2.5 M NaOAc and 500 µL (2-2.5 vol) ice-cold 95% ethanol and store at -20°C for ≥30 min. Spin at 9,240–13,305 *g* for 5 min. Discard supernatant.
10) Wash pellet with 1 mL of 70% ethanol. Do not disturb the pellet. Spin at 9,204 *g* for 4 min and pour off ethanol. Vacuum-dry pellet. Do not over-dry.
11) Resuspend pellet in ~100-200 µL of TE. Store at -20°C.
12) Run DNA in 1% agarose gel.

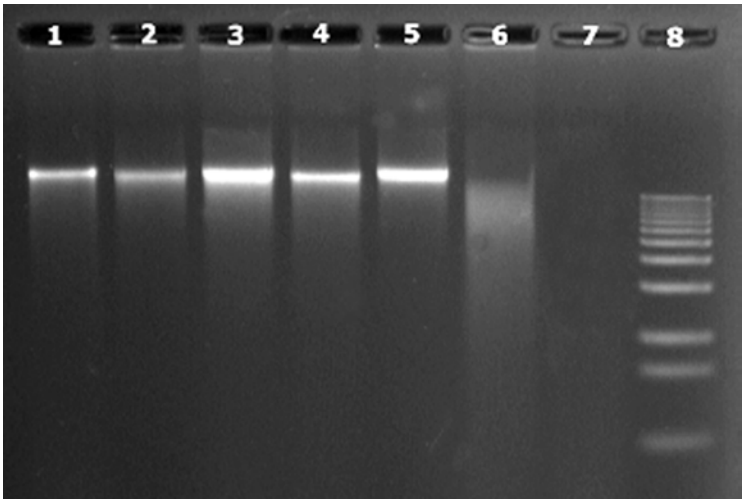

Figure 1. Total DNA extracted visualized on a 1% agarose gel. Lane 1, *Disocactus biformis*; lane 2, *Vanilla fragrans*; lane 3, *Oncidium sphacelatum*; lane 4, *Poa glauca*; lane 5, *Tradescantia virginiana*; lane 6, *Tradescantia occidentalis*; lane 7, *Tradescantia occidentalis*; lane 8, 1KB+ marker. Lanes 1–5 are from fresh tissue. Lanes 6 and 7 are from herbarium-preserved material dated from 1953 and 1944, respectively.

PCR amplification and DNA analysis

After extraction, total DNA was quantified in a Biophotometer UV Spectrophotometer (Eppendorf AG), run on a 1% agarose gel against a marker of known molecular weight, and stained with ethidium bromide for visualization (Figure 1). A series of PCR techniques was used to test the quality and performance of the DNA extracted, including sequencing of the *rcb*L gene and non-coding *trn*L-F regions of the chloroplast genome, RAPD, and AFLP. The PCR amplification of the *trn*L-F region involved primer set E/F, which targets a fragment of 400–600 bp, and primer combinations C/F and Ci/Fdowny, both targeting a ~1-kb fragment (Figure 2). Primers Z1 and IntR were used to amplify a ~1000-bp fragment of the *rbc*L gene (Figure 2). Oligonucleotide sequences are given in Table 2.

Results

The concentration of DNA obtained from herbarium specimens ranged from 20 to >1050 ng/μL. The average concentration of DNA extracted from fresh tissue was 300 ng/μL and ranged from 100 to 2500 ng/μL.

Amplification of regions of the chloroplast genome was successful in all samples tested, including the 50 and ≥60-year-old herbarium specimens (Figure 2, lanes 10, 11, 22 and 23). PCR fragments of 1 kb (Figure 2) can be amplified for different chloroplast markers with DNA extracted from herbarium and fresh tissue using this method. The results with RAPD and AFLP assays (not shown) were equally effective as those from the amplification of plastid markers with DNA from preserved and fresh tissue.

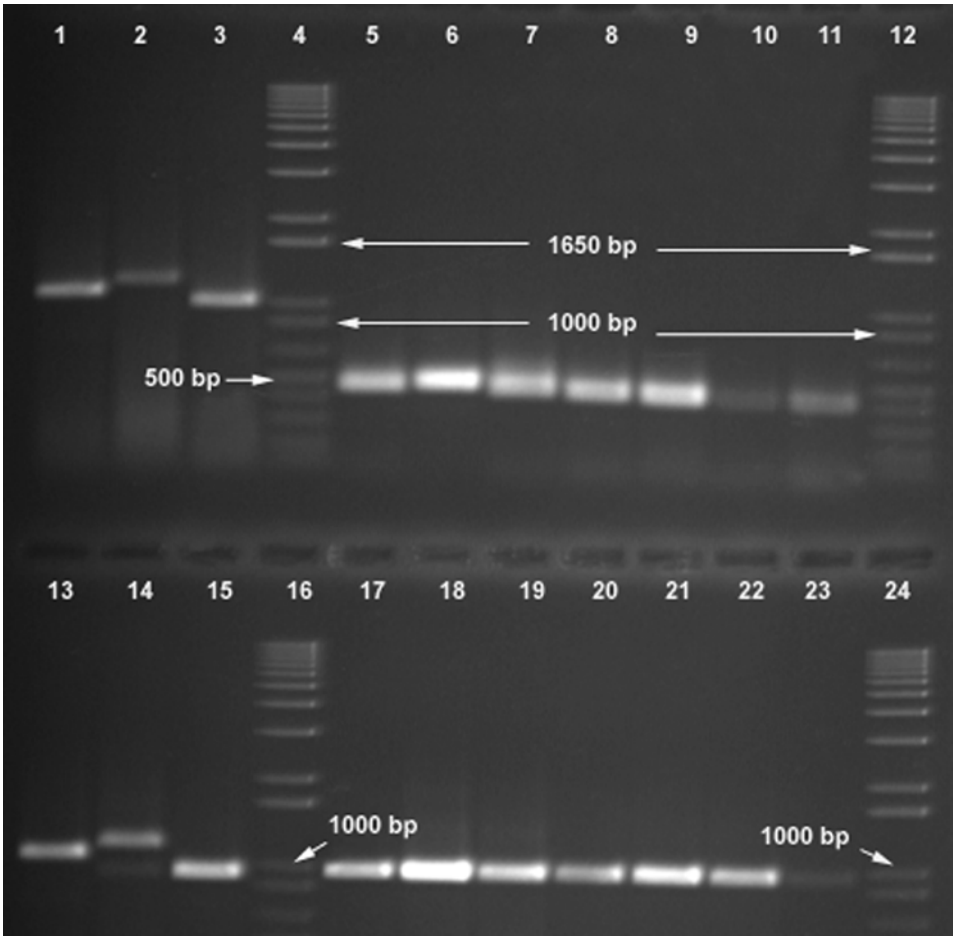

Figure 2. PCR products of selected chloroplast regions. Lanes 1–3, primers C to F (*trn*L-F); lanes 5–11, primers E to F (*trn*L-F); lanes 13–15, primers Ci to Fdowny (*trn*L-F); and lanes 17–23, primers Z1 to INTR (*rbc*L). Lanes 1, 6, 13, and 18, *Vanilla fragrans*; lanes 2, 7, 14, and 19, *Oncidium sphacelatum*; lanes 3, 8, 15, and 20, *Poa glauca*; lanes 4, 12, 16, and 24, 1KB+ marker; lanes 5 and 17, *Disocactus biformis*; lanes 9 and 21, *Tradescantia virginiana*; lanes 10 and 22, *Tradescantia occidentalis* (from 1953); lanes 11 and 23, *T. occidentalis* (from 1944).

Discussion

The DNA extracted with this protocol is of sufficient quality to amplify PCR fragments of up to 1 kb (Figure 2). These results are superior that those reported by Soltis and Soltis (1993) and Jankowiak et al. (2005), who indicate that it is more likely to amplify fragments of <500 bp with DNA from herbarium specimens. Furthermore, the DNA from fresh and preserved specimens was of suitable quality to screen levels of genetic diversity using AFLP, proving that the DNA can be cut with restriction endonucleases and amplified via PCR.

In summary, the efficient extraction of total DNA from plant families with different chemical properties is easy using the modifications proposed here. The quantity and quality of total DNA extracted from fresh and preserved specimens

Table 2. Primer sequences (from 5′ to 3′) used in the current study.

Technique	Primer Name and Sequence	Source
cpDNA sequencing	*rbc*L Z1: atg tca cca caa aca gaa act aaa gca agt	Wolf et al (1994)
	*rbc*L IntR: cca cca gac ata cgt aac cg	Wolf et al (1994)
	*trn*L-F C: cga aat cgg tag acg cta cg	Taberlet et al (1991)
	*trn*L-F Ci: tcg gta gac gct acg gac tt	This study
	*trn*L-F E: ggt tca agt ccc tct atc cc	Taberlet et al (1991)
	*trn*L-F F: att tga act ggc gac acg ag	Taberlet et al (1991)
	*trn*L-F Fdowny: cag tcc tct gct cta cca gc	This study
RAPD	Primer No. 2: cct ggg ctt g	University of British Columbia
	Primer No. 6: cct ggg ctt a	University of British Columbia
	Primer No. 8: cct ggc ggt a	University of British Columbia
AFLP (selective primers)	Primer 1: E-gac tgc cca att cac t	Applied Biosystems
	M-gat gag tga gta act g	
	Primer 2: E-gac tgc gta cca att cac a	Applied Biosystems
	M-gat gag tcc tga gta aga g	

are adequate for its immediate application in molecular biology assays. In our laboratory, this method has been used to extract DNA from leaves, stems, and petals of plants from the Aizoaceae, Araceae, Asteraceae, Bignoniaceae, Caryophyllaceae, Commelinaceae, Malvaceae, Nyctaginaceae, Poaceae, Portulacaceae, and Rubiaceae. In addition, this procedure is affordable and does not require sophisticated equipment, making it a superior choice relative to expensive commercial kits for DNA extraction.

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References

Biss P, Freeland J, Silvertown J, McConway K, and Lutman P (2003) Successful amplification of rice chloroplast microsatellites from century-old grass samples from the Park Grass Experiment. Plant Mol Biol Rep 21: 249-257.

Cheng YJ, Guo WW, Yi HL, Pang XM, and Deng X (2003) An efficient protocol for genomic DNA extraction from *Citrus* species. Plant Mol Biol Rep 21: 177a-177g.

DeCastro O and Mendle B (2004) PCR amplification of Michele Teniere's historical specimens and facility to utilize an alternative approach to resolve taxonomic problems. Taxon 53: 147-151.

Drábková L, Kirschner J, and Vlcek C (2002) Comparison of seven DNA extractions in amplification protocols in historical herbarium specimens of Juncaceae. Plant Mol Biol Rep 20: 161-175.

Fang G, Hammar S, and Grumet R (1992) A quick and inexpensive method for removing polysaccharides from plant genomic DNA. Biofeedback 13: 52-54.

Golenberg EM (1999) Isolation, identification, and authentication of DNA sequences derived from fossil material. In: Jones TP and Rowe NP (eds), Fossil Plants and Spores: Modern Techniques, pp 156-160, The Geological Society, London.

Griffith MP and Porter JM (2003) Back to basics: A simple method of DNA extraction for mucilaginous cacti. Bradleya 21: 126-128.

Jankowiak K, Buczkowska K, and Szweykowka-Kulinska S (2005) Successful extraction of DNA from 100-year-old herbarium specimens of the liverwort *Bazzania trilobata*. Taxon 54: 335-336.

Li YX, Su ZX, and Chen F (2002) Rapid extraction of genomic DNA from leaves and bracts of dove tree (*Davidia involucrata*). Plant Mol Biol Rep 20: 185a-185e.

Lodhi MA, Ye G, Weeden NF, and Reisch BI (1994) A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep 12: 6-13.

Murray MG and Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8: 4321-4325.

Paterson AH, Brubaker CL and Wendel JF (1993) A rapid method for extraction of cotton (*Gossypium* spp.) genomic DNA suiPorebski S, Bailey LG, and Baum BR (1997) Modification of a CTAB DNA extraction protocols for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep 15: 8-15.

Porebski S, Bailey LG, and Baum BR (1997) Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol Rep 15: 8-15.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, and Allard RW (1986) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81: 8014-8018.

Soltis PS and Soltis PE (1993) Ancient DNA: Prospects and limitations. New Zealand J Bot 31: 203-209.

Taberlet P, Gielly L, Pautou G, and Bouvet J (1991) Universal primers for amplification of three non-coding regions of cpDNA. Plant Mol Biol 17: 1105-1109.

Taylor JW and Swann EC (1994) DNA from herbarium specimens. In: Hermann B and Hummel S (eds), Ancient DNA, pp 166-181, Springer-Verlag, New York.

Tel-Zur NS, Abbo S, Myslabodksi D, and Mizrahi Y (1999) Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *Hylocereus* and *Selenicereus* (Cactaceae). Plant Mol Biol Rep 17: 249-254.

Wolf PG, Soltis PS, and Soltis DE (1994) Phylogenetic relationships of Dennstaedtioid ferns: Evidence from *rbc*L. Mol Phylogenet Evol 3: 383-392.