Benchmarks

protocol, we found that we could omit the use of Fairbanks B and Fairbanks C solutions. This elimination speeds up the processing time to about 15 min with only a twofold sacrifice of sensitivity. It also reduces the number of solutions that need to be prepared from four to only two. Bringing the solution containing the gel to the boiling point appears to be a prerequisite for the improved sensitivity and speed of our method. Our method is quite forgiving, however, and all steps can be performed for longer periods. As long as the gels are not boiled to near dryness, the actual boiling times are not important. Evaporation of the isopropanol presumably occurs early in the boiling process, so for consistent results, we avoid reusing the solutions. Though longer boiling or rinsing times do not reduce sensitivity, they also do not improve it. The times listed have been optimized for speed.

Silver staining is another commonly used technique with extremely high sensitivity. However, unlike our process, it involves the use of toxic chemicals, its process is lengthy, complicated and unforgiving. Moreover, it stains different proteins to different degrees, depending on the protein's silver-binding property, making silver less suited for protein quantification. Fluorescent reagents such as fluorescamine and *o*-phthalaldehyde are also sensitive. However, their fluorescent intensity diminishes with time, and UV radiation must be used to observe the staining pattern. Coomassie blue remains the most popular protein stain. In conclusion, our rapid Fairbanks method combines all the features of a useful protein staining technique: it is fast, highly sensitive and inexpensive.

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- ## Modified CTAB Protocol Using a Silica Matrix for Isolation of Plant Genomic DNA
- BioTechniques* 28:432-434 (March 2000)
- Of the DNA extraction methods described in the literature, the cetyltrimethylammonium bromide (CTAB) method (1) is widely used because it is fast, efficient and can yield high-quality DNA from a variety of starting materials. However, with this method, it is difficult to obtain high-quality DNA

from many woody plant species that contain high levels of polysaccharides and/or secondary metabolites. Such polysaccharides have solubility properties similar to those of DNA and interfere with the isolation of pure DNA when ethanol or isopropanol is used to precipitate the DNA.

Complex or time-consuming procedures such as the cesium chloride density gradient technique are frequently used to surmount this problem. But this procedure is not suitable for genetic analyses in population studies, where large numbers of samples are often used. Glass or silica can specifically bind nucleic acids in the presence of a chaotropic salt (5). These materials may be alternatives to ethanol or isopropanol for DNA precipitation. They have been widely used for DNA fragment binding, as in cleaning up PCR products. The purity of the recovered DNA is high and adequate for most molecular analyses.

In this study, we describe a simple, efficient miniprep procedure for genomic DNA extraction from recalcitrant plant species, such as some mangrove species and sweet potato. The procedure combines the advantages of the high DNA yield using the CTAB method with the high DNA purity from cleaning up silica. The plant species *Amaranthus tricolor* L. was used to optimize conditions for high quantitative yield of DNA. The recalcitrant mangrove plant *Bruguiera gymnorrhiza* (L.) Lamk. (Rhizophoraceae) and several other plant species, including sweet potato *Ipomoea batatas* (L.) Lam. (Convolvulaceae), *Sonneratia caseolaris* (L.) Engl. (Sonneratiaceae), *Sonneratia alba* J. Smith and *Alpinia formosana* K. Schum. (Zingiberaceae) were used to test the quality of extracted DNA. Reagents used in the procedure include the following: homogenization buffer (HB) containing 2% (wt/vol) CTAB, 100 mM Tris-HCl, pH 8.0, 1.4 M NaCl and 50 mM EDTA; extraction buffer (EB): chloroform: isoamyl alcohol (24:1, vol/vol); Sephaglas BP (Amersham Pharmacia Biotech, Piscataway, NJ, USA); 4 M guanidine thiocyanate (GuSCN); and TE buffer, pH 8.5.

PCR amplifications of inter-simple sequence repeat (ISSR), using UBC primer no. 807 (5'-AGAGAGAGAGA-

Protocol 1. DNA Extraction Procedure

1. Harvest about 0.1 g fresh leaves, transfer into a 1.5 mL Microcentrifuge tube (Treff AG, Degersheim, Switzerland). Place the tube on ice.
2. Aliquot 200 μ L of HB (including 0.2% mercaptoethanol) into each tube, mix with a bit of sand. As an alternative, liquid nitrogen can be used for hard tissue.
3. Homogenize leaf tissue with a tissue homogenizer.
4. Add 500 μ L of HB to each tube, mix and incubate at 65°C for 20 min with occasional mixing.
5. Add 700 μ L of EB to each tube and mix by inversion for 1 min.
6. Centrifuge at 10 000 rpm (Model No. 5417C, Eppendorf® Centrifuge, Hamburg, Germany) for 5 min.
7. Transfer the top (aqueous) layer into a fresh tube.
8. Add 1.0 volume of 4 M GuSCN and adjust to pH 6.5 with HCl.
9. Add 15 μ L silica suspension and incubate the mixture at room temperature for 5 min with occasional mixing.
10. Centrifuge at 8000 rpm for 30 s and discard the supernatant.
11. Resuspend the matrix containing DNA with 500 μ L of 70% ethanol. Centrifuge at 8000 rpm for 30 s and discard supernatant. Repeat the step.
12. Remove all the liquid and let the pellet dry at room temperature for 15 min.
13. Add 100 μ L of TE buffer to the dried pellet, tap the tube to disperse the tissue and incubate at 60°C for 10 min.
14. After centrifugation at 12 000–14 000 rpm for 30 s, transfer the solution containing DNA to a new tube for further use.
15. The yield of DNA was quantified by DyNA Quant™ 200 (Amersham Pharmacia Biotech).

GAGAGT-3'; the Nucleic Acid-Protein Service Unit, Biotechnology Laboratory University of British Columbia, Vancouver, BC, Canada) and template DNA of *B. gymnorrhiza*, were carried

out to test the DNA samples extracted with our protocol and with some other methods, such as CTAB (1), modified CTAB (4) and modified SDS (6). The amplification conditions adopted in this

Figure 1. Electrophoretic analysis (0.8% agarose gel in 0.5x TBE buffer) of isolated DNA. Lane 3, *B. gymnorrhiza*; lane 4, *I. batatas*; lane 5, *S. caseolaris*; lane 6, *S. alba*; lane 7, *A. formosana*; and lane 8, *A. tricolor*. Lane 1, λ DNA; lane 2, λ DNA digested with *Hind*III.

Figure 2. Electrophoretic analysis (1.5% agarose gel in 0.5x TBE buffer) of PCR-amplified ISSR products using UBC primer no. 807 (5'-AGAGAGAGAGAGAGAGT-3') and template DNA of *B. gymnorrhiza*. Lanes 1–4, isolated with CTAB (1); lanes 5–8, modified CTAB (4); lanes 9–12, modified SDS (6); lanes 13–16, our CTAB with silica method; lane 17, molecular weight markers (100 bp ladder of DNA).

Benchmarks

study were as described in Reference 3. Amplifications were carried out in 1.5 mM MgCl₂, 2% formamide, 200 nM primer, 1 U *Taq* DNA polymerase and 10 ng of genomic DNA per 20 µL reaction. The following PCR cycle profile was used: 1 cycle at 94°C for 5 min followed by 45 cycles at 94°C for 45 s, 50.5°C for 45 s, 72°C for 1.5 min and a final 7 min extension at 72°C. The amplified products were electrophoresed on 1.5% agarose gels and detected by staining with ethidium bromide.

The yield of DNA with our protocol depends on the rate of binding and elution of DNA to and from silica matrix. Three chaotropic salt solutions (guanidine thiocyanate, sodium iodide and sodium perchlorate) were tested for optimal yield of DNA. The highest DNA yield (6.4 µg DNA per 100 mg fresh leaves) was obtained with 2.0 M guanidine thiocyanate, pH 6.5.

The pH is the most critical factor for a high yield of DNA. If the pH is higher than 7.5 or lower than 4.5, the yield of

DNA will be low. A pH of 6.5 is optimal for DNA binding. Chloroform extraction is necessary in the procedure because chloroform can extract CTAB, which reacts with the chaotropic salt in the supernatant. The pH of elution buffer also affects the yield of DNA, and a pH of 8.0–8.5 is adequate for a high elution rate.

With optimal binding conditions, typical yield was 3–4 µg DNA per 100 mg fresh leaves of *B. gymnorrhiza*. This is enough DNA for at least 200 RAPD PCRs. Absorbance ratios of DNA at A₂₆₀/A₂₈₀ were between 1.9 and 2.0. The length of isolated DNA was around 50 kb in the species tested (Figure 1). The DNA was free of contaminants interfering with digestion by restriction endonucleases (we tested *Rsa*I, *Hind*III, *Eco*RI and *Bam*HI, all requiring different reaction buffer systems). PCR amplifications of ISSR were used to test DNA samples extracted by our protocol and the CTAB (1), modified CTAB (4) and modified SDS (6) methods. DNA extracted by our protocol was as good as that from the more complicated and time-consuming SDS method (Figure 2). No amplifications occurred with DNA extracted by the CTAB method. Larger DNA fragments were not amplified or amplified in low quantity from the modified CTAB method, leading to weak or missing bands in the gel. This indicated that DNA isolated by the two CTAB methods might contain substances that interfere with the PCR.

Our method has advantages over other silica-based isolation methods (2) because of its rapidity (DNA can be obtained within 1.5 h) and high DNA yield. Based on our protocol, we have succeeded in isolating DNA from 35 recalcitrant species belonging to six plant families: Sonneratiaceae: *S. alba*, *S. apetala*, *S. caseolaris*, *S. hainanensis*, *S. ovata*, *S. paracaseolaris*, *Duabanga grandiflora*; Rhizophoraceae: *B. gymnorrhiza*, *Ceriops tagal*; Myrsinaceae: *Aegiceras corniculatum*; Veronaceae: *Avicennia marina*; Convolvulaceae: *I. batatas*, *I. cynanchifolia*, *I. cordatotriloba*, *I. grandifolia*, *I. leucantha*, *I. ramossissima*, *I. tiliacea*, *I. triloba*, *I. trifida*, *I. umbraticola*, *I. lacunosa*, *I. tabascana*, *I. tenuississima*, *I. alba*, *I. cairica*, *I. aristolochiifolia*; Zingiberaceae: *A. for-*

mosana, *A. zerumbet*, *Boesenbergia fal-lax*, *Hedychium flarum*, *Globba bathei*, *Curcuma kwangsiensis*, *Ethingera yunnanensis* and *Amomum villosum*.

In summary, our minipreparation procedure combines the advantages of CTAB and silica-cleaning methods. It is rapid (150 samples per day), phenol-free and yields high-purity DNA that is suitable for most plant molecular genetic studies, including cloning experiments and DNA sequencing.

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