The isolation of high-quality DNA from plants, especially woody plants, is often difficult. Although there are many different methods for DNA extraction in use, it is not unusual to find plant species for which commonly used extraction methods do not produce good-quality DNA. The main reason for the inability to obtain high-quality genomic DNA from Australian native plants is the high levels of cytosolic compounds such as polysaccharides, polyphenolics, and tannins.

A reliable DNA extraction procedure for eucalypts (5) was developed by modification of a method of Wagner et al. (9) and has been successfully used to extract high-quality DNA from other Australian native species such as *Lambertia orbifolia* (4) and *Macrozamia riedlei* (6). The procedure is based on a crude organelle isolation from which the DNA is extracted using a cetyltrimethylammonium bromide (CTAB) method. Modifications to this protocol have been used to obtain high-quality DNA from other species. The addition of high levels of salt to the extraction protocol, followed by a caspase treatment, resulted in the removal of polysaccharide contamination from *Acacia mangium* DNA (2). In *Melaleuca alternifolia*, degradation of DNA during restriction enzyme digestion was prevented by the incorporation of a differential solvent precipitation into the extraction procedure (3).

The use of the modified Wagner method (5) to isolate DNA from a number of Acacia species (*A. acuminata*, *A. anfractuosa*, *A. lobulata*, *A. sciophanes*, and *A. verricula*) resulted in degradation of DNA immediately after extraction, as determined by agarose gel electrophoresis (Figure 1). Several modifications to the protocol were tested, including the ones noted above, but the best results were obtained by the addition of 0.1 M sodium sulfite to the two buffers used in the extraction procedure (the initial extraction buffer and the buffer used to resuspend the organelle pellet). No other modifications to the modified Wagner extraction procedure were necessary when sodium sulfite was used, and no other modifications were successful when sodium sulfite was not used. The addition of sodium sulfite resulted in extraction of high-quality DNA that was not degraded, while the standard protocol produced DNA that was degraded (Figure 1). The DNA extracted with sodium sulfite was suitable for use in restriction fragment length polymorphism (RFLP) analyses. Figure 2 shows the effect on RFLP analysis of the DNA extracted with and without the addition of sodium sulfite to the buffers. The high molecular weight DNA extracted with the addition of sodium sulfite to the extraction buffers gives a clean hybridization profile, while the degraded DNA obtained from the standard method gives a poor hybridization profile with smeared lanes and a weak hybridization signal.

The direct action of sodium sulfite in the extraction procedure is unclear. Sodium sulfite is a reducing agent for polyphenol oxidase, and its presence will prevent the production of polyphenolic compounds. It seems likely that there is a nuclease present in the extraction solution that becomes bound with a polyphenolic compound and is not removed by the organic extraction. Prevention of polyphenolic formation by the addition of sodium sulfite would leave the nuclease free to be removed during the organic extraction. Alternatively, there may be a compound in the polyphenol pathway that has nuclease properties.

Other studies of *Acacia* species using RFLP techniques have also encountered problems with the quality of DNA. Polysaccharide contamination was problematic in *A. mangium* (2) and a number of other *Acacia* species (1), and another study required purification of DNA through cesium chloride gradi-

\[ -\text{Na}_2\text{SO}_3 \quad +\text{Na}_2\text{SO}_3 \]

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Figure 1. *A. verricula* DNA extracted with and without addition of sodium sulfite to the buffers in the modified Wagner extraction method. Lanes 1–5, DNA extracted from the Merredin population without using sodium sulfite in the extraction buffers. Lanes 6–10, DNA extracted from the Merredin population with the addition of sodium sulfite to the extraction buffers.
ents, although the authors did not indicate why such purification was necessary (7). Degradation of DNA was not a problem in DNA extractions from A. mangium (2), and the problem of polysaccharide contamination that was encountered in A. mangium did not occur in the Acacia species discussed here. These species are from the southwest of Western Australia and are taxonomically quite distinct from the tropical A. mangium (Bruce Maslin, personal communication), and it is not surprising that they would contain different cytosolic compounds. These results highlight that it is not valid to make assumptions about DNA isolation for previously untested plant species, even when they are members of the same genus.

The addition of sodium sulfite in buffers during DNA extraction is a small modification to the method, yet it had a significant effect on the quality of the DNA extracted, such that it was suitable for RFLP analysis and could be stored without degradation. The use of sodium sulfite during DNA extraction is recommended in plant species that contain high levels of polyphenolics and tannins and exhibit isolation of poor-quality DNA.

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Figure 2. Hybridization profiles of A. verricula DNA extracted with and without addition of sodium sulfite to the buffers in the modified Wagner extraction method. DNA was digested with EcoRV, electrophoresed for 16 h at 30 V, transferred to nylon membrane, and hybridized with a petunia chloroplast probe P6 (8). Lanes 1–5, DNA extracted with sodium sulfite added to the extraction buffers. Lanes 6–10, DNA extracted without adding sodium sulfite to the buffers.
Use of Inexpensive Dyes to Calibrate and Adjust Your Microarray Printer

BioTechniques 30:748 (April 2001)

A critical factor in the preparation of cDNA microarrays is the calibration and adjustment of the array printer to optimize spotting. Here are a couple techniques to help you detect the quality of your print, the proper calibration, and the condition of your printing pins. A simple way to determine the quality of the array spots is to examine the salt deposits remaining after the droplet of printer material has dried. However, this method does not necessarily reflect the morphology of the nucleic acid on the array because of the drying effects. Another common method includes the use of free Cy-3-dCTP/dUTP or Cy-5-dCTP/dUTP dyes (Amersham Pharmacia Biotech, Piscataway, NJ, USA) or dyes containing oligonucleotides, followed by fluorescence scanning (1). Here, we describe an inexpensive alternative to using expensive fluorescent probes for the calibration and adjustment of pin-type array printers.

Using a Stanford cDNA microarray printer (http://cmgm.stanford.edu/pbrown/mguide/index.html) with ChipMaker™ 3 spotting pins (TeleChem International, Sunnyvale, CA, USA), we compared the results of printing under two conditions, with and without DNA. The goal of the calibration and adjustment steps is to obtain uniform and consistent spots. We used plain red and blue food coloring dye in 1:1000 dilution of 3x standard saline citrate (SSC) with sheared salmon sperm DNA at 0.25 mg/mL to mimic the actual printing conditions. All fluorescent scans were performed on a Packard/GSI Lumonics 3000 scanner (GSI Lumonics, Northville, MI, USA), with 85% laser and 90% PMT settings (Figure 1). The quality of the DNA spots was further examined with DNA binding dyes (data not shown).

In the future, with the help of food coloring dye, we are going to compare different types of printing buffers such as 50% dimethyl sulfoxide (DMSO) with and without DNA, formamide, and a new printing buffer that recently became available from Mosaic Technologies (Waltham, MA, USA) and Clontech Laboratories (Palo Alto, CA, USA).

REFERENCES


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Received 17 October 2000; accepted 27 November 2000.

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