Capture and storage of plant genomic DNA on a readily available cellulose matrix

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Whatman FTA® Cards are a fast and efficient method for capturing and storing nucleic acids but can be cost-prohibitive for large numbers of samples. We developed a method that substitutes a readily-available cellulose matrix and homemade washing buffer for commercial FTA Cards and FTA Purification Reagent. This method is suitable for long-term storage of DNA from many plant species prior to PCR.

In protocols where large numbers of DNA samples are collected, stored and later analyzed by PCR, decreasing the time and/or cost associated with preparing the DNA templates is desirable. For *Arabidopsis thaliana* leaf samples, the method of Klimyuk *et al.* [1] is quick and low cost, samples become less reliable as PCR templates after several weeks of storage at 4°C or –20°C. As an alternative, Whatman FTA® Cards (GE Healthcare Life Sciences, PA, USA), which are impregnated with proprietary chemicals, can be used to collect and store DNA samples [2–5]. Samples (body fluids or plant tissue) are pipetted or imprinted onto FTA® Cards, then dried completely for storage. When DNA is needed for PCR, small disks are removed from the card, washed twice with commercial FTA® Purification Reagent and then twice with TE_{0.1} (10-mM Tris-Cl, pH 8.0, 0.1-mM EDTA). Disks are dried completely at room temperature for at least 1h before adding PCR reagents. While this method is relatively fast and efficient, the FTA® Purification Reagent and FTA® Cards are costly. We investigated reliable and cost-effective substitutions for these materials.

As a replacement for commercial FTA® Purification Reagent to wash FTA® paper disks, we tested five other solutions: homemade TENT buffer (10-mM Tris-Cl, pH 8.0, 1-mM EDTA, 12-mM NaCl, and 2.5% Triton X-100), sterilized deionized

water, TE_{0.1}, 20-mM NaOH, or 1% SDS. Both 20-mM NaOH and 1% SDS have been previously reported as alternative washing solutions for FTA® paper disks [6]. Testing was performed using leaves from 4-week-old *A. thaliana* ecotype Columbia-0 and the amplicon was a region from an *Arabidopsis* protein phosphatase gene. FTA® paper disks washed with water, TE_{0.1}, NaOH, or SDS yielded inconsistent results (data not shown). Products were consistently generated from disks washed with either TENT buffer or commercial FTA® Purification Reagent (data not shown); thus, homemade TENT buffer was used for the initial two washes in all subsequent experiments.

Next, we investigated alternatives to FTA® Cards for sample storage and to TE_{0.1} for the final wash solution. Since DNA tightly associates with cellulose fibers [7], we tested three cellulose-based matrices: untreated Grade 1 filter paper (Whatman) and either untreated or treated grade 238 chromatography paper (VWR International, Radnor, PA, USA). Treated paper was prepared by soaking in Paper Washing Buffer (400 mM Tris-Cl [pH 8.0], 5% sodium dodecyl sulfate [SDS], 25-mM EDTA) for 2 h, followed by air-drying at room temperature overnight. Treated paper can be stored at room temperature for at least 6 months prior to use. The treated paper was sandwiched between pieces

of parchment paper to prevent contamination of tools while performing tissue printing and to avoid physical contact between samples during storage. Plant leaf tissue was pressed into the paper by rubbing the overlaid parchment paper using moderate pressure from a ceramic pestle. The cellulose matrix was dried for at least 1h before extracting 1.5-mm disks using a Miltex biopsy punch (Ted Pella, Inc., Redding, CA, USA). We tested three alternatives to TE_{0.1} for the final two washes: 100% isopropanol, 100% ethanol, or sterilized, deionized water. After the final washes, samples were dried for 20 min in a vacuum centrifuge (Vacufuge Concentrator 5301, Eppendorf, Hauppauge, NY, USA) at 45°C. PCR Master Mix 1 (see Supplementary Protocol) was added directly to the disks immediately prior to thermal cycling. To streamline the procedure, multiple disks from the same DNA sample can be washed together using 100 µl of each wash solution per disk. PCR products from treated chromatography paper were indistinguishable from products from FTA® Cards (Figure 1A & B). This experiment also indicated that the initial washes were essential for robust amplification, while the final washes were not as important. Untreated chromatography paper was not suitable for DNA storage (Figure 1C). Untreated filter paper tended to warp when plant material was applied, which made subsequent manip-

METHOD SUMMARY

Here we report a low-cost method for capture and long-term storage of plant genomic DNA on chromatography paper. Stored DNA can be prepared for use as a PCR template in less than 1h.

Figure 1. Comparison of four cellulose matrices and five washing conditions for isolation of DNA for PCR. *Arabidopsis thaliana* ecotype Columbia-0 leaf tissue was imprinted onto (A) commercial FTA® Card, (B) treated grade 238 chromatography paper, (C) untreated grade 238 chromatography paper, or (D) untreated grade 1 filter paper. Initial wash treatments were: (1) unwashed; (2–6) washed twice with TENT buffer; (7) washed with commercial FTA® Purification Reagent. Final wash treatments were: (1–2) no wash (3) 100% isopropanol, (4) 100% ethanol, (5) water or (6–7) TE_{0.1}. PCR products (491 bp) were visualized on a 1.5% agarose gel using ethidium bromide.

ulations difficult (Figure 1D); as a result, this matrix was eliminated from further consideration.

To test whether *Arabidopsis* DNA was stable for 1 month before amplification, PCR was performed 1 day, 1 week or 4 weeks after application of leaf tissue to commercial FTA® Cards or treated chromatography paper. Disks were washed twice with TENT buffer and twice with TE_{0.1} before thermocycling. Samples

degenerate primer pair, followed by a second reaction with a nested primer pair (see Supplementary Protocol). Identity of the products as *GAPDH* genes was confirmed by sequencing since the amplicons contained multiple introns and ranged from 600 to 1600 bp, depending on the species. Products were detected from all matrices imprinted with *Crocus sativus*, *Solanum lycopersicum*, and *Cucurbita pepo* (Figure 2).

Lactuca sativa DNA was not amplified when imprinted on any of the matrices but did yield product if purified genomic DNA was used as template with the *GAPDH* primers (data not shown). *B. vulgaris* DNA was amplified only from the treated chromatography paper and not from FTA® Cards, while both matrices worked for *Allium* DNA. These results indicate that some plant species may contain compounds that interfere with storage or amplification on certain matrices.

Our results show that: i) grade 238 chromatography

paper pretreated with homemade Paper Washing Buffer is a reasonable substitute for Whatman FTA® Cards for many plant species; ii) homemade TENT buffer is a reliable replacement for FTA® Purification Reagent; and iii) the final disk washes can be performed with a variety of reagents. Sample processing time can be reduced by processing multiple disks from the same sample together and by using a vacuum centrifuge to dry the disks after the final wash. A detailed protocol is available online.

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Competing & financial interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at www.future-science.com/doi/full/10.2144/btn-2018-0109

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Figure 2. Comparison of cellulose matrices for amplification of *GAPDH* genes from a variety of plants. Cellulose matrices tested were: (A) FTA® Card, (B) treated grade 238 chromatography paper, or (C) untreated grade 238 chromatography paper. Young leaves imprinted on each cellulose matrix were from: (1) *Cucumis sativus* cv. Diva (cucumber), (2) *Solanum lycopersicum* cv. Ceylon (tomato), (3) *Lactuca sativa* cv. Outredgeous (lettuce), (4) *Cucurbita pepo* cv. Super Pik (summer squash), (5) *Allium fistulosum* x *A. cepa* cv. Guardsman (onion), (6) *Beta vulgaris* subsp. *vulgaris* cv. Bright Lights (Swiss chard). All disks were washed twice with TENT buffer and twice with TE_{0.1}. PCR products were separated on a 1.5% agarose gel and detected with ethidium bromide.

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