Paramagnetic Cellulose DNA Isolation Improves DNA Yield and Quality Among Diverse Plant Taxa

Authors: Moeller, Jackson R., Moehn, Nicholas R., Waller, Donald M., and Givnish, Thomas J.

Source: Applications in Plant Sciences, 2(10)

Published By: Botanical Society of America

URL: https://doi.org/10.3732/apps.1400048
**PARAMAGNETIC CELLULOSE DNA ISOLATION IMPROVES DNA YIELD AND QUALITY AMONG DIVERSE PLANT TAXA**

**JACKSON R. MOELLER**, **NICHOLAS R. MOEHN**, **DONALD M. WALLER**, AND **THOMAS J. GIVNISH**

**2Department of Botany, University of Wisconsin–Madison, 430 Lincoln Drive, Madison, Wisconsin 53706 USA**

**Key words:** cetyltrimethylammonium bromide (CTAB); DNA isolation; DNeasy; paramagnetic cellulose particles (PMC); silica columns.

Obtaining good yields of DNA at high molecular weight and purity is an essential first step for all subsequent analyses of DNA genetic variation from biological samples. Many plant groups, however, contain tannins, tough fibrous material, and/or secondary compounds that may bind or otherwise interfere with DNA isolation. The diversity of these compounds has made it difficult to identify any single technique that is uniformly effective for extracting DNA from diverse plant samples. As a consequence, dozens of methods for isolating DNA from plants have been published over the past 30 years (Murray and Thompson, 1980; Hoopes and McLure, 1981; Tracy, 1981; Zimmer and Newton, 1982; Doyle and Doyle, 1987; Rogers and Bendich, 1988; Gaillard and Strauss, 1990; Smith et al., 1992; Sytsma, 1994; Williams and Ronald, 1994; Vorwerk, 2001; Michiels et al., 2003; QIAGEN, 2006; Varma et al., 2007; Mandrekar et al., 2010; Sahu et al., 2012; Telfer et al., 2013; Gillings, 2014).

Although silica-based column methods have enjoyed great commercial success and have been scaled up to a 96-well format, they may perform poorly on some plant species and tissues. The use of cetyltrimethylammonium bromide (CTAB) as an extraction buffer, followed commonly by phase separation by chloroform and later alcohol precipitation is another common method (Doyle and Doyle, 1987). CTAB methods are often employed when silica-based methods are inadequate or too expensive. While CTAB methods can also be scaled up to 96-well format, they are more toxic and laborious than silica-based methods. Furthermore, CTAB methods require more training and access to a fume hood, while kit-based methods are relatively easy to perform and troubleshoot. Ideally, a single DNA isolation method could be developed to produce consistently high DNA yields across a diverse set of plants with minimal effort.

Here we test a new approach to plant DNA extraction using MagnaCel paramagnetic cellulose particles (PMC) integrated with the Maxwell 16 robotic instrument (Promega Corporation, Madison, Wisconsin, USA) (adapted from Mandrekar et al., 2010). These particular cellulose particles have a high DNA-binding capacity (Su and Comeau, 1999), which Promega asserts is greater than silica. We compared the DNA yield and purity across a wide range of flowering plants among PMC, the silica-based DNeasy Plant Mini kit (QIAGEN, Dusseldorf, Germany), and a CTAB-based method (adapted from Doyle and Doyle, 1987). PMC averaged twice the DNA yield per unit sample mass across the taxa surveyed relative to DNeasy and CTAB, and provided samples of comparable purity and generally higher concentrations of amplifiable DNA. PMC is also automated, making DNA isolation quick, labor-efficient, and less prone to cross-contamination.

**METHODS AND RESULTS**

**Tissue collection and preservation**—We collected leaf tissue samples from four individuals of each of 25 herbaceous angiosperm species growing in Wisconsin forests in summer 2013 (Table 1). We selected young expanding leaves free of damage whenever possible. However, as plants matured, we also sampled older tissue. Individual tissue samples were placed in tea filters (Five Mountains,
were sampled from each plant. According to the DNeasy Plant Handbook 4-mm silica beads (silicagelpackets.com, Charlotte, North Carolina, USA).

**Order**

- **Liliales Liliaceae**
- **Ranunculales Berberidaceae**
- **Poales Cyperaceae**
- **Brassicales Brassicaceae**
- **Asparagales Asparagaceae**
- **Apiales Apiaceae**
- **Lamiales Phrymaceae**
- **Saxifragales Saxifragaceae**
- **Rosales Rhamnaceae**
- **Lamiales Liliaceae**
- **Geraniales Geraniaceae**
- **Brassicales Brassicaceae**
- **Rosales Urticaceae**

**TABLE 1. Vouchers and locality information for species included in the study; all vouchers are deposited in WIS.**

| Order          | Family         | Genus   | Species          | Abbr. | Voucher | Latitude (°) | Longitude (°) |
|----------------|----------------|---------|------------------|-------|---------|--------------|---------------|
| Asterales      | Asteraceae     | Ageratina | altissima        | agal  | DOB-0053 | 43.0232      | 89.4440       |
| Brassicaceae   | Alliaria       | petiolaris | petiolaris       | alpe  | DOB-0004 | 42.7302      | 89.3553       |
| Fabales        | Fabaceae       | Amphiarcuca | bracteata       | ambr  | DOB-0124 | 43.0526      | 89.8593       |
| Alismatales    | Aracea         | Arisema  | tripliybium      | artr  | DOB-0003 | 43.0517      | 89.3725       |
| Poales         | Cyperaceous    | Carex   | albursinas       | caal  | DOB-0653 | 42.5271      | 89.4595       |
| Ranunculales   | Berberidaceae  | Caulophyllum | thalictroides   | cath  | DOB-0362 | 42.5460      | 89.4079       |
| Myrtales       | Orceae         | Cinerea  | lutetiana        | cilia | DOB-0018 | 42.6862      | 89.4831       |
| Liliales       | Liliaceae      | Clinaiontia | borealis   | clbo  | DOB-0356 | 46.0147      | 89.6576       |
| Asterales      | Asteraceae     | Eurybia  | macrophylla      | euma  | DOB-0093 | 46.0807      | 89.7162       |
| Lamiaceae      | Lamiaceae      | Galeopsis | tetrait        | gate  | DOB-0446 | 45.5434      | 88.5046       |
| Geraniaceae    | Geranium       | maculatum | maculatum       | gema  | DOB-0218 | 42.6854      | 89.4825       |
| Boraginaceae   | Hydrophyllaceae | Hydrophyllum | virginianum   | hyvi  | DOB-0003 | 42.5407      | 89.4079       |
| Fabales        | Fabaceae       | Hylodesmum | glutinosum     | hygl  | DOB-0283 | 42.9466      | 89.5883       |
| Ericales       | Balsaminaceae  | Impatiens | pallida        | impa  | DOB-0277 | 42.7532      | 89.4488       |
| Asparagales    | Asparagaceae   | Maianthemum | canadense     | mara  | DOB-0005 | 42.6857      | 89.4833       |
| Asparagales    | Asparagaceae   | Maianthemum | racemosum     | mara  | DOB-0005 | 42.6857      | 89.4833       |
| Saxifragales   | Saxifragaceae  | Metella  | dilphylla       | menl  | DOB-0029 | 42.6852      | 89.4845       |
| Apiaceae       | Apiaceae       | Omorhiza | chatonoiy       | oscl  | DOB-0029 | 42.6852      | 89.4845       |
| Lamiaceae      | Phrymaceae     | Phryma   | leptostachysa    | phle  | DOB-0256 | 43.0924      | 89.4322       |
| Rosales        | Urticaceae     | Pilea    | pumila           | pipu  | DOB-0426 | 43.0718      | 89.4827       |
| Rosales        | Rhamnaceae     | Rhamnus  | cathartica       | rhca  | DOB-0075 | 43.0519      | 89.3699       |
| Saxifragales   | Saxifragaceae  | Ribes    | missouriense     | rimi  | DOB-0671 | 42.9823      | 89.2363       |
| Ranunculales   | Papaveraceae   | Sanguinaria | canadensis   | saca  | DOB-0270 | 42.6458      | 89.4872       |
| Liliales       | Liliaceae      | Streptopus | lanceolatus var. | sana  | DOB-0682 | 46.0387      | 89.6170       |
| Liliales       | Liliaceae      | Trillium  | grandiflorum    | trg  | DOB-0715 | 43.6446      | 89.1903       |

DNA isolation—We used electrophoresis on 1% agarose gels to visualize the relative quantity and quality of DNA isolations. A Nanodrop 2000 (Thermo Scientific, Wilmington, Delaware, USA) machine was used to assess A 260 : A 280 .

**TABLE 2. Mean ± SE yield by Quant-iT PicoGreen ddDNA (Life Technologies) in nanograms per microliter of double-stranded DNA for PMC, DNeasy, and CTAB for the 25 species used in this study. Species are indicated by abbreviations in Table 1; those used in qPCR analyses are indicated by an asterisk. Differences in superscripts indicate significant differences in yield under repeated-measures ANOVA with Holm-Bonferroni post hoc tests.**

| Species                  | PMC  | DNeasy | CTAB |
|--------------------------|------|--------|------|
| All 25 taxa              |      |        |      |
| agal*                    | 38.2 ± 10.8 | 16.8 ± 8.2 | 17.9 ± 5.4 |
| alpe*                    | 25.0 ± 8.8  | 3.5 ± 1.3  | 25.3 ± 4.9  |
| cath*                    | 57.5 ± 7.9  | 23.3 ± 4.4  | 10.6 ± 1.9  |
| cat*                     | 65.1 ± 2.7  | 53.4 ± 4.6  | 3.8 ± 2.8   |
| cili*                    | 34.3 ± 6.3  | 10.3 ± 3.8  | 5.1 ± 0.2   |
| elbo*                    | 73.0 ± 4.5  | 61.2 ± 3.8  | 0.5 ± 0.1   |
| euma*                    | 29.1 ± 1.9  | 5.9 ± 0.7   | 1.0 ± 0.2   |
| eury*                    | 28.7 ± 5.2  | 5.9 ± 0.3   | 14.3 ± 6.2  |
| hyvi*                    | 39.8 ± 6.8  | 9.1 ± 0.9   | 14.3 ± 3.4  |
| hygl*                    | 33.9 ± 7.3  | 9.0 ± 1.0   | 26.6 ± 5.7  |
| mene*                    | 18.0 ± 4.5  | 5.6 ± 0.3   | 20.5 ± 3.0  |
| mace*                    | 35.5 ± 8.8  | 20.2 ± 5.2  | 28.4 ± 5.3  |
| marea*                   | 103.1 ± 11.1 | 25.7 ± 6.1  | 27.5 ± 8.4  |
| mida*                    | 11.4 ± 1.4  | 2.9 ± 0.3   | 14.2 ± 8.4  |
| oscl*                    | 56.5 ± 5.3  | 16.0 ± 1.3  | 11.1 ± 2.9  |
| phle*                    | 12.9 ± 6.5  | 5.8 ± 2.2   | 18.3 ± 2.3  |
| pipu*                    | 15.9 ± 4.0  | 4.8 ± 0.8   | 16.5 ± 3.5  |
| rhca*                    | 58.5 ± 4.7  | 29.9 ± 6.4  | 35.5 ± 4.3  |
| rimi*                    | 49.7 ± 8.1  | 19.4 ± 3.1  | 25.7 ± 6.1  |
| sana*                    | 23.3 ± 8.3  | 14.3 ± 5.1  | 11.4 ± 5.6  |
| stla*                    | 15.5 ± 2.9  | 9.4 ± 1.6   | 2.2 ± 2.3   |
| trig*                    | 40.8 ± 1.7  | 11.7 ± 0.3  | 45.8 ± 7.5  |

DNA analysis—We used electrophoresis on 1% agarose gels to visualize the relative quantity and quality of DNA isolations. A Nanodrop 2000 (Thermo Scientific, Wilmington, Delaware, USA) machine was used to assess A 260 : A 280 .

http://www.bioone.org/loi/apps
and A_{260}/A_{230} absorbance ratios. For absolute double-stranded DNA (dsDNA) quantification, we used Quant-IT PicoGreen dsDNA Reagent (Life Technologies, Carlsbad, California, USA) with a BioTek Synergy 2 Microplate Reader (BioTek, Winooski, Vermont, USA). Twelve species were chosen for additional analyses of amplifiable DNA yield using quantitative PCR (qPCR). CTAB gave the highest average PicoGreen dsDNA concentrations for five of these species, while PMC gave the highest initial yield in the remaining seven species (Table 2). The qPCR reaction mix was: 10 μL of 2× GoTaq qPCR Master Mix (Promega Corporation), 8.9 μL of nuclease-free water, 1 μL of template genomic DNA, and 0.9 μL of [10 mmol each] forward and reverse ATP1-1 (ATPase SUBUNIT J) primer. The universal primer pair used was ATP1-1 forward 5′-TGAAY-GAGATTYAAGYTGGGGAAATGGT-3′ ATP1-1 reverse 5′-CCCTCCTCTC-CATCAATRRACTCTCCA-3′ (Wang et al., 2011). A Stratagene Mx3000P thermocycler was used with these conditions: initial melt at 94°C for 2 min, then 40 cycles of 94°C for 5 s and 60°C anneal and extension for 35 s, followed by melting curve analysis.

Statistical analysis—To test for significant differences in PicoGreen dsDNA values among species and extraction techniques, we used repeated-measures ANOVA. To compare individual pairs of these values in post hoc tests, we corrected for multiple comparisons by applying the Holm sequential method in R (R Core Team, 2013). Repeated-measures ANOVA essentially is the analogue of paired t tests for comparisons of mean scores of a dependent variable across identifiable individuals at three or more times, or (as in this case) across three or more treatments (Gleason, 1999). It provides greater statistical power for comparisons of means when values can be matched by individuals across times or treatments.

We used the same approach (rANOVA, Holm sequential method for post hoc tests) to compare the A_{260}/A_{280} and A_{260}/A_{230} absorbance ratios. For each species and extraction protocol, we calculated the mean ± SE dsDNA concentrations. We also regressed concentrations estimated from samples using the PMC technique against those obtained using the DNeasy and CTAB methods. We estimated PCR efficiency from the qPCR data from a standard curve prepared for each species tested. Each sample was amplified in duplicate. The quantification cycle (Cq) was then averaged for each isolation method for each species. We then compared these amplification results to those obtained via the DNeasy method (mean ± SE; DNeasy method = 1) using the same approach as outlined above for DNA yields and absorbance ratios.

Results—Averaged across species, PMC yields were 38.2 ± 10.8 ng/μL, more than twice those for DNeasy (16.8 ± 8.2 ng/μL) and CTAB (17.9 ± 5.4 ng/μL) and significantly greater than both (P < 0.0006 and P < 0.0000005, respectively, based on repeated-measures ANOVA and post hoc tests) (Table 2). We found no significant difference between the DNeasy and CTAB yields. Agarose gel electrophoresis mirrored these results (data not shown). Furthermore, the PMC average yield was higher than the DNeasy kit for all 25 species, and significantly higher in eight species (Table 2, Fig. 1A). Comparison of PMC to CTAB showed higher average yields for PMC in 17 species, with nine of those being significantly higher. Of the eight species for which CTAB had a higher average yield, none were significantly higher (Fig. 2, 1B).

Regressing PMC yield on DNeasy yield showed that PMC yield was elevated by essentially a constant amount relative to the y = x line, indicating that PMC provided roughly a constant advantage in absolute yield, and an often substantial advantage in relative yield in species that produced low absolute yields under both methods (Fig. 1A). This result is consistent with Promega’s claim that the cellulose-based particle has higher binding capacity than silica. The slope of the regression of PMC yield on CTAB yield did not differ significantly from zero, with PMC often producing a dramatic relative advantage at low absolute CTAB yields, with a 5.25-fold median edge for 14 species (Table 2, Fig. 1B). This large difference between PMC and CTAB among low-yielding samples is likely because the isopropanol precipitation employed in CTAB is highly inefficient for low amounts of DNA.

We assayed DNA purity using A_{260}/A_{280} and A_{260}/A_{230} absorbance ratios. Pure DNA has an A_{260}/A_{280} ratio of ~1.8 (Thermo Fisher Scientific, 2011); samples contaminated with proteins have values below this. Based on repeated-measures ANOVA, PMC produced a significantly greater A_{260}/A_{230} ratio across species than did DNeasy (P < 5 × 10^{-8}), but there were no significant differences in these ratios between PMC and CTAB and CTAB and DNeasy (Table 3). CTAB produced the highest average value for the A_{260}/A_{230} ratio.
species indicated that a higher proportion of purified DNA was across the 25 species tested. Additionally, qPCR analysis of 12 demonstrating sample purity showed PMC had less protein contamination than DNeasy, and was overall more consistent in absorbance

**CONCLUSIONS**

PMC dsDNA yield was double that of DNeasy and CTAB across the 25 species tested. Additionally, qPCR analysis of 12 species indicated that a higher proportion of purified DNA was amenable from PMC isolations, an important consideration for downstream applications. Further, absorbance ratios demonstrating sample purity showed PMC had less protein contamination than DNeasy, and was overall more consistent in absorbance ratios across the 25 species. In terms of price per sample, CTAB is currently <$1.00/sample, DNeasy is $3.55/sample (with University of Wisconsin–Madison pricing), and PMC, a product newly released to the plant community, is $4.79/sample (now offered in the Maxwell 16 LEV Plant DNA Kit, AS1420). However, CTAB is more laborious and time-consuming, and involves the use of hazardous chemicals, imposing greater

**Table 3.** Mean ± SE of A$_{260}$/A$_{280}$ and A$_{260}$/A$_{230}$ ratios across all 25 species, and for individual species. Differences in superscripts between protocols indicate statistically significant differences in purity absorbance ratio under repeated-measures ANOVA with Holm-Bonferroni post hoc tests, excluding the extreme outlier (middi). See Table 1 for nomenclature; species used in qPCR analyses are indicated by an asterisk.

| Species | A$_{260}$/A$_{280}$ | A$_{260}$/A$_{230}$ | A$_{260}$/A$_{280}$ | A$_{260}$/A$_{230}$ |
|---------|-------------------|-------------------|-------------------|-------------------|
|         | Ratio             | Ratio             | Ratio             | Ratio             |
| All 25  | 1.52 ± 0.02       | 1.32 ± 0.05       | 1.66 ± 0.09       | 1.48 ± 0.17       |
| agal*   | 1.52 ± 0.08       | 1.13 ± 0.01       | 1.71 ± 0.05       | 1.63 ± 0.11       |
| alpe*   | 1.75 ± 0.11       | 1.51 ± 0.08       | 1.36 ± 0.24       | 1.03 ± 0.01       |
| anbr*   | 1.31 ± 0.09       | 1.14 ± 0.04       | 1.64 ± 0.07       | 1.13 ± 0.02       |
| arrr*   | 1.43 ± 0.06       | 1.61 ± 0.01       | 1.35 ± 0.06       | 1.58 ± 0.11       |
| caal    | 1.75 ± 0.04       | 1.49 ± 0.08       | 1.49 ± 0.11       | 1.08 ± 0.03       |
| cathy*  | 1.32 ± 0.1        | 1.59 ± 0.09       | 1.22 ± 0.12       | 1.08 ± 0.13       |
| cila    | 1.44 ± 0.09       | 1.19 ± 0.0       | 1.13 ± 0.04       | 1.31 ± 0.08       |
| cibio   | 1.49 ± 0.08       | 1.27 ± 0.04       | 1.53 ± 0.14       | 1.15 ± 0.11       |
| euma    | 1.37 ± 0.02       | 1.31 ± 0.06       | 1.54 ± 0.09       | 1.28 ± 0.08       |
| gate    | 1.6 ± 0.07        | 1.27 ± 0.05       | 2.21 ± 0.26       | 1.57 ± 0.07       |
| gema    | 1.54 ± 0.16       | 1.45 ± 0.1        | 1.01 ± 0.03       | 1.4 ± 0.04       |
| hyvi    | 1.68 ± 0.05       | 1.38 ± 0.04       | 1.83 ± 0.07       | 1.19 ± 0.05       |
| hygl*   | 1.59 ± 0.06       | 1.21 ± 0.05       | 1.52 ± 0.06       | 1.09 ± 0.08       |
| impa    | 1.43 ± 0.06       | 1.16 ± 0.03       | 1.78 ± 0.01       | 1.39 ± 0.03       |
| maca*   | 1.62 ± 0.04       | 1.51 ± 0.04       | 2.19 ± 0.06       | 1.23 ± 0.06       |
| mala    | 1.48 ± 0.03       | 1.65 ± 0.02       | 1.99 ± 0.05       | 1.11 ± 0.05       |
| mida*   | 1.44 ± 0.05       | 1.19 ± 0.04       | 30.3 ± 12.0a      | 1.58 ± 0.05       |
| osci*   | 1.67 ± 0.02       | 1.43 ± 0.04       | 1.49 ± 0.06       | 1.77 ± 0.28       |
| phle    | 1.36 ± 0.09       | 0.32 ± 0.11       | 1.82 ± 0.05       | 1.17 ± 0.05       |
| pipu*   | 1.58 ± 0.10       | 1.14 ± 0.04       | 1.92 ± 0.03       | 1.29 ± 0.12       |
| rhca*   | 1.7 ± 0.04        | 1.53 ± 0.03       | 1.96 ± 0.08       | 1.36 ± 0.08       |
| rini*   | 1.59 ± 0.05       | 1.71 ± 0.03       | 1.97 ± 0.05       | 0.99 ± 0.01       |
| saca    | 1.34 ± 0.03       | 1.54 ± 0.07       | 1.71 ± 0.2        | 1.1 ± 0.04       |
| stla    | 1.52 ± 0.03       | 1.25 ± 0.04       | 2.59 ± 1.6a       | 1.17 ± 0.02       |
| trgr*   | 1.56 ± 0.03       | 1.29 ± 0.03       | 1.55 ± 1.17       | 1.31 ± 0.04       |

**Fig. 2.** Mean ± SE of yields of amplifiable DNA based on qPCR of matched DNA extractions obtained using PMC, DNeasy, and CTAB protocols. Species are indicated by four-letter codes determined by the first two letters of the generic name and specific epithet, respectively (refer to Table 1). Each protocol is marked with letters (a, b, c) indicating statistically significant differences in amplifiable yield under repeated-measures ANOVA with Holm-Bonferroni post hoc tests (see Table 2).
risks and requiring added, sometimes expensive, precautionary measures like the use of a fume hood. DNeasy-based systems are offered in an automated format as 96-well systems such as in the QIAcube HT system (QIAGEN), which could increase sample throughput. The Maxwell 16 robot automates PMC isolation, yielding purified DNA from extracts for up to 16 samples in less than an hour, and was designed to reduce the likelihood of contamination for forensic use. The robot, however, has a substantial purchase price ($24,995 at the time this article was published, with 1–3-yr lease-to-own options). Given the advantages of PMC in terms of DNA yield and effective isolation of amplifiable DNA, especially for samples with low concentrations of genomic DNA, as well as its absence of hazardous chemicals and the considerable labor savings inherent in the robotic system, it seems likely that the new Promega PMC approach will offer advantages to many plant investigators, especially as costs decline with increasing production.

LITERATURE CITED

**DOYLE, J. J., AND J. L. DOYLE.** 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

**GAILLARD, C., AND F. STRAUSS.** 1990. Ethanol precipitation of DNA with linear polyacrylamide as carrier. *Nucleic Acids Research* 18: 378.

**GILLINGS, M. R.** 2014. Rapid extraction of PCR-competent DNA from recalcitrant environmental samples. In I. T. Paulsen and A. J. Holmes [eds.], *Environmental microbiology: Methods and protocols*, 17–23. Humana Press, New York, New York, USA.

**GLEASON, J. R.** 1999. Within subjects (repeated measures) ANOVA, including between subjects factors. *Stata Technical Bulletin* 47: 40–45.

**HOOPES, B., AND W. R. MCLURE.** 1981. Studies on the selectivity of DNA precipitation by spermine. *Nucleic Acids Research* 9: 5493–5504.

**MANDREKAR, P. V., Z. MA, S. KRUEGER, AND C. COWAN.** 2010. High-concentration (>100ng/μl) genomic DNA from whole blood using Maxwell® 16. Website http://www.promega.com/resources/pubhub/high-concentration-genomic-dna-from-whole-blood-using-the-maxwell-16-low-elution-volume-instrument/ [accessed 10 September 2014].

**MICHELS, A., W. VAN DEN ENDE, M. TUCKER, L. VAN RIET, AND A. VAN LAERE.** 2003. Extraction of high-quality genomic DNA from latex-containing plants. *Analytical Biochemistry* 315: 85–89.

**MURRAY, M. G., AND W. F. THOMPSON.** 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* 8: 4321–4325.

**QIAGEN.** 2006. DNeasy Plant Handbook. Website http://dna.uga.edu/wp-content/uploads/2013/12/DNeasy-Plant-Handbook-for-DNA-purification-Qiagen.pdf [accessed 10 September 2014].

**R CORE TEAM.** 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Website http://www.R-project.org/ [accessed 10 September 2014].

**ROGERS, S. O., AND A. J. BENDEICH.** 1988. Extraction of DNA from plant tissues. In S. B. Gelvin and R. A. Schilperoort [eds.], *Plant molecular biology manual*, A6. Kluwer Academic Publishers, Boston, Massachusetts, USA.

**SU, X., AND A. M. COMEAU.** 1999. Cellulose as a matrix for nucleic acid purification. *Analytical Chemistry* 267: 415–418.

**SYSMA, K. J.** 1994. DNA extraction from recalcitrant plants: Long, pure, and simple? In R. P. Adams, J. S. Miller, E. M. Golenberg, and J. E. Adams [eds.], *Conservation of plant genes II: Intellectual property rights and DNA utilization*, 69–81. Missouri Botanical Garden, St. Louis, Missouri, USA.

**TELFER, E., N. GRAHAM, L. STANBRA, S. T. MANLEY, AND P. WILCOX.** 2013. Extraction of high purity genomic DNA from pine for use with a high-throughput genotyping platform. *New Zealand Journal of Forestry Science* 43: 3.

**WANG, J., C. WANG, Y. LONG, C. HOPKINS, S. KURUP, K. LIU, G. J. KING, AND J. MENG.** 2011. Universal endogenous gene controls for bisulphite conversion in analysis of plant DNA methylation. *Plant Methods* 7: 39.

**ZIMMER, E. A., AND K. J. NEWTON.** 1982. A simple method for the isolation of high molecular weight DNA from individual maize seedlings and tissues. In W. F. Sheridan [ed.], *Maize for biological research*. University of North Dakota University Press, Grand Forks, North Dakota, USA.