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.

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Protocol Note

Paramagnetic cellulose DNA isolation improves DNA yield and quality among diverse plant taxa[1]

Jackson R. Moeller[2], Nicholas R. Moehn[2], Donald M. Waller[2], and Thomas J. Givnish[2,3]

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

- *Premise of the study:* The chemical diversity of land plants ensures that no single DNA isolation method results in high yield and purity with little effort for all species. Here we evaluate a new technique originally developed for forensic science, based on MagnaCel paramagnetic cellulose particles (PMC), to determine its efficacy in extracting DNA from 25 plant species representing 21 families and 15 orders.
- *Methods and Results:* Yield and purity of DNA isolated by PMC, DNeasy Plant Mini Kit (silica column), and cetyltrimethylammonium bromide (CTAB) methods were compared among four individuals for each of 25 plant species. PMC gave a twofold advantage in average yield, and the relative advantage of the PMC method was greatest for samples with the lowest DNA yields. PMC also produced more consistent sample purity based on absorbance ratios at 260 : 280 and 260 : 230 nm.
- *Conclusions:* PMC technology is a promising alternative for plant DNA isolation.

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,

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[3]Author for correspondence: givnish@wisc.edu

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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 (°N)	Longitude (°W)
Asterales	Asteraceae	*Ageratina*	*altissima*	*agal*	DOB-0053	43.0323	89.4440
Brassicales	Brassicaceae	*Alliaria*	*petiolata*	*alpe*	DOB-0004	42.7302	89.3553
Fabales	Fabaceae	*Amphicarpaea*	*bracteata*	*ambr*	DOB-0124	43.0256	89.8593
Alismatales	Araceae	*Arisaema*	*triphyllum*	*artr*	DOB-0032	43.0517	89.3725
Poales	Cyperaceae	*Carex*	*albursina*	*caal*	DOB-0653	42.5271	89.4595
Ranunculales	Berberidaceae	*Caulophyllum*	*thalictroides*	*cath*	DOB-0362	42.5460	89.4079
Myrtales	Onagraceae	*Circaea*	*lutetiana*	*cilu*	DOB-0018	42.6862	89.4831
Liliales	Liliaceae	*Clintonia*	*borealis*	*clbo*	DOB-0356	46.0147	89.6576
Asterales	Asteraceae	*Eurybia*	*macrophylla*	*euma*	DOB-0093	46.0807	89.7162
Lamiales	Lamiaceae	*Galeopsis*	*tetrahit*	*gate*	DOB-0446	45.5434	88.5046
Geraniales	Geraniaceae	*Geranium*	*maculatum*	*gema*	DOB-0218	42.6854	89.4825
Boraginales	Hydrophyllaceae	*Hydrophyllum*	*virginianum*	*hyvi*	DOB-0003	42.5460	89.4079
Fabales	Fabaceae	*Hylodesmum*	*glutinosum*	*hygl*	DOB-0283	42.9466	89.5883
Ericales	Balsaminaceae	*Impatiens*	*pallida*	*impa*	DOB-0277	42.5732	89.4488
Asparagales	Asparagaceae	*Maianthemum*	*canadense*	*maca*	DOB-0202	46.0477	89.6573
Asparagales	Asparagaceae	*Maianthemum*	*racemosum*	*mara*	DOB-0005	42.6857	89.4833
Saxifragales	Saxifragaceae	*Mitella*	*diphylla*	*midi*	DOB-0463	45.5229	88.4969
Apiales	Apiaceae	*Osmorhiza*	*claytonii*	*oscl*	DOB-0029	42.6852	89.4845
Lamiales	Phrymaceae	*Phryma*	*leptostachya*	*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	Grossulariaceae	*Ribes*	*missouriense*	*rimi*	DOB-0671	42.9823	89.2363
Ranunculales	Papaveraceae	*Sanguinaria*	*canadensis*	*saca*	DOB-0470	42.6458	89.4172
Liliales	Liliaceae	*Streptopus*	*lanceolatus* var. *longipes*	*stla*	DOB-0682	46.0387	89.6170
Liliales	Melanthiaceae	*Trillium*	*grandiflorum*	*trgr*	DOB-0715	43.6446	88.1903

San Francisco, California, USA) and stored in Ziploc bags with orange indicating 4-mm silica beads (silicagelpackets.com, Charlotte, North Carolina, USA).

***DNA isolation*—**For each DNA isolation, three 9-mm punches (1.9 cm^2) were sampled from each plant. According to the DNeasy Plant Handbook (QIAGEN, 2006), 1.9 cm^2 should have a dry weight of 5.4–16.2 mg and a fresh weight of 27–81 mg. Each plant produced three replicate tissue samples, one each for the three DNA isolation methods; replicate samples were obtained for each of four individuals per species. For replicates, tissue samples were obtained from the same leaf or split evenly among multiple leaves to reduce variation among samples. Each tissue sample was powdered and homogenized using a 3-mm tungsten bead (QIAGEN catalog no. 69997) in a 2-mL safe-lock tube (no. 0030 120.094, Eppendorf, Hauppauge, New York, USA) with a TissueLyser (QIAGEN) for two rounds of 1 min at 30 Hz without buffer. We then immediately added extraction buffers for each method to the respective samples.

We performed DNeasy extraction according to kit instructions, with two elutions with buffer AE (10 mM Tris, 0.5 mM EDTA, pH 9.0) of 50 µL each (100 µL total volume). CTAB DNA extraction followed the Soltis Laboratory protocol (http://www.flmnh.ufl.edu/museum-voices/soltis-lab/files/2014/02/CTAB-DNA-Extraction.pdf) adapted from Doyle and Doyle (1987). We incubated samples at 55°C for 1 h for extraction followed by overnight (15–20 h) isopropanol precipitation at −20°C. We then rehydrated the pellet in 100 µL TE (10 mM Tris, 1 mM EDTA, pH 8.0) for 4 h at 4°C.

For MagnaCel PMC DNA isolation, we added 300 µL of Tail Lysis Buffer A (A509, Promega Corporation), 10 µL of RNase A (4 mg/mL), and 30 µL of 20 mg/mL Proteinase K (MC500C, Promega Corporation) to each sample. After incubating these samples for 20 min at 56°C, we added these volumes to LEV Blood DNA Kit cartridges (AS1290, Promega Corporation) and placed these into the Maxwell 16 robot unit (AS1000, Promega Corporation) along with 290 µL of water to reach the ideal volume of 630 µL. The robot can purify DNA from one to 16 samples simultaneously (one sample per cartridge); we ran samples at the "Blood DNA" setting, which runs to completion in 43 min. Each cartridge has a disposable plunger, which binds the beads, then binds and washes the DNA before being eluted in 50 µL of TE-4 (10 mM Tris, 0.1 mM EDTA, pH 8.0). After elution, 50 µL more of TE-4 was added so each method had equal elution volumes (100 µL). Since this work was completed, Promega has begun offering the Maxwell 16 LEV Plant DNA Kit (AS1420), differing primarily from the protocol here in that the kit does not contain Proteinase K or require any incubation time. In addition, Maxwell 16 robot units also have a "Plant DNA" setting on the machine, which is equivalent to the former "Blood DNA" setting. We performed all three extraction methods on the four individuals of each plant species on the same day.

***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 $A_{260}:A_{280}$

TABLE 2. Mean ± SE yield by Quant-iT PicoGreen dsDNA (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	38.2 ± 10.8^{a}	16.8 ± 8.2^{b}	17.9 ± 5.4^{b}
*agal**	25.0 ± 8.8^{ab}	3.5 ± 1.3^{b}	25.3 ± 4.9^{a}
*alpe**	57.5 ± 7.9^{a}	22.3 ± 4.4^{a}	10.6 ± 1.9^{b}
*ambr**	15.4 ± 2.8	3.7 ± 1.1	22.6 ± 8.2
*artr**	65.1 ± 2.7^{a}	63.4 ± 4.6^{a}	3.8 ± 2.8^{b}
caal	34.3 ± 6.3	10.3 ± 3.8	5.1 ± 0.2
*cath**	73.0 ± 4.5^{a}	61.2 ± 3.8^{a}	0.5 ± 0.1^{b}
cilu	29.1 ± 1.9^{a}	5.9 ± 0.7^{b}	1.0 ± 0.2^{c}
clbo	28.7 ± 5.2	5.9 ± 0.3	14.1 ± 6.2
euma	39.8 ± 7.1^{a}	12.5 ± 4.1^{b}	23.4 ± 4.9^{a}
gate	27.2 ± 2.1^{a}	9.6 ± 2.4^{b}	40.5 ± 13.7^{ab}
gema	48.9 ± 5.2^{a}	41.8 ± 8.5^{ab}	15.0 ± 10.0^{b}
hyvi	50.9 ± 6.8^{a}	9.1 ± 0.9^{b}	14.3 ± 3.4^{b}
*hygl**	33.9 ± 7.3	9.0 ± 1.0	26.6 ± 5.7
impa	18.0 ± 4.5^{ab}	5.6 ± 0.3^{b}	20.5 ± 3.0^{a}
*maca**	35.5 ± 8.8	20.2 ± 5.2	28.4 ± 5.3
mara	101.3 ± 11.1^{a}	25.7 ± 6.1^{b}	20.7 ± 8.4^{b}
*midi**	11.4 ± 1.4^{a}	2.9 ± 0.3^{b}	14.2 ± 8.4^{ab}
*oscl**	56.5 ± 5.3^{a}	16.0 ± 1.3^{b}	11.1 ± 2.9^{b}
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**	58.3 ± 4.7^{a}	29.9 ± 6.4^{ab}	35.5 ± 4.3^{b}
rimi	49.7 ± 8.1	19.4 ± 3.1	25.7 ± 6.1
saca	23.3 ± 3.3	14.3 ± 3.4	11.2 ± 5.6
stla	15.5 ± 2.9	9.4 ± 1.6	2.2 ± 2.3
*trgr**	40.8 ± 1.7^{a}	11.7 ± 0.3^{b}	45.8 ± 7.5^{a}

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 1*) primer. The universal primer pair used was ATP1-1 forward 5′-TGAAYGAGATTYAAGYTGGGGAAATGGT-3′ ATP1-1 reverse 5′-CCCTCTTCCATCAATARRTACTCCCA-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 (Table 2, Fig. 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 $A_{260}:A_{280}$ ratio across species than did DNeasy ($P < 3 \times 10^{-8}$), 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 $A_{260}:A_{280}$ ratio

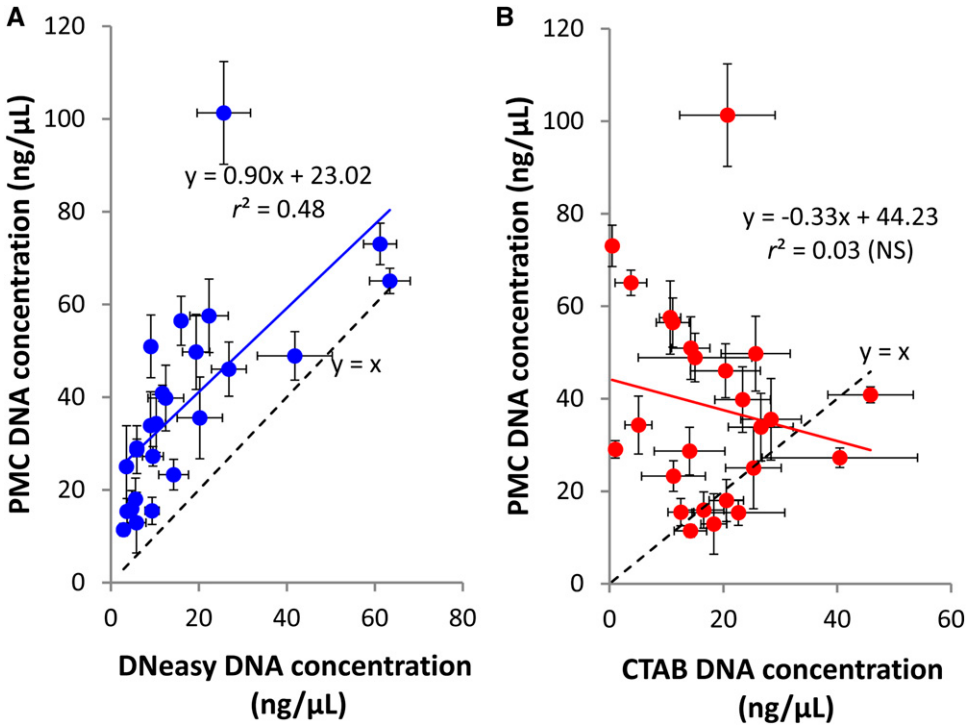

Fig. 1. (A) DNA yields (ng/µL) obtained via PMC isolation plotted against those obtained via DNeasy isolation. Each point represents the mean yields via PMC vs. DNeasy for an individual species. Error bars indicate standard errors associated with replicate isolations via PMC (vertical bars) and DNeasy (horizontal bars). Solid line indicates linear regression between PMC and DNeasy DNA yields; dashed line indicates line y = x. (B) DNA yields via PMC isolation vs. CTAB isolation. Points, error bars, and solid and dashed lines are as in part A, but representing yields via PMC vs. CTAB.

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 (*midi*). See Table 1 for nomenclature; species used in qPCR analyses are indicated by an asterisk.

	$A_{260}:A_{280}$ ratio			$A_{260}:A_{230}$ ratio		
Species	PMC	DNeasy	CTAB	PMC	DNeasy	CTAB
All 25	1.52 ± 0.02[a]	1.32 ± 0.05[b]	1.66 ± 0.09[ab]	1.27 ± 0.04	1.43 ± 0.18	1.36 ± 0.14
*agal**	1.52 ± 0.08[b]	1.13 ± 0.01[c]	1.71 ± 0.05[a]	1.48 ± 0.17	0.06 ± 1.55	1 ± 0.09
*alpe**	1.75 ± 0.11	1.51 ± 0.08	1.36 ± 0.24	1.63 ± 0.08	1.31 ± 0.49	1.36 ± 0.23
*ambr**	1.31 ± 0.09	1.14 ± 0.04	1.64 ± 0.07	1.03 ± 0.01	1.8 ± 0.48	1.15 ± 0.03
*artr**	1.43 ± 0[b]	1.61 ± 0.01[a]	1.35 ± 0.06[b]	1.13 ± 0.02[b]	2.12 ± 0.09[a]	0.49 ± 0.12[c]
caal	1.75 ± 0.04	1.49 ± 0.08	1.49 ± 0.11	1.58 ± 0.1[a]	0.7 ± 0.02[b]	0.91 ± 0.09[ab]
*cath**	1.32 ± 0.1	1.5 ± 0.09	1.22 ± 0.12	1.08 ± 0.03[a]	1.65 ± 0.34[ab]	0.35 ± 0.09[b]
cilu	1.44 ± 0.09	1.19 ± 0	1.13 ± 0.04	1.08 ± 0.13[a]	1.2 ± 0.04[a]	0.41 ± 0.05[b]
clbo	1.49 ± 0.08	1.27 ± 0.04	1.53 ± 0.14	1.31 ± 0.08	4.65 ± 2.93	1.65 ± 0.55
euma	1.37 ± 0.02	1.31 ± 0.06	1.54 ± 0.09	1.15 ± 0.11	0.86 ± 0.21	1.1 ± 0.04
gate	1.6 ± 0.07[a]	1.27 ± 0.05[b]	2.21 ± 0.26[a]	1.28 ± 0.08	1.11 ± 0.11	1.84 ± 0.43
gema	1.54 ± 0.16[ab]	1.45 ± 0.1[a]	1.01 ± 0.03[b]	1.57 ± 0.07	1.38 ± 0.46	1.17 ± 0.11
hyvi	1.68 ± 0.05[a*]	1.38 ± 0.04[b]	1.83 ± 0.07[a]	1.4 ± 0.04[a]	0.52 ± 0.13[b]	1.39 ± 0.21[a]
*hygl**	1.59 ± 0.06[a]	1.21 ± 0.05[b]	1.52 ± 0.06[ab]	1.19 ± 0.05	1.06 ± 1.84	1.13 ± 0.05
impa	1.43 ± 0.06[b]	1.16 ± 0.03[c]	1.78 ± 0.01[a]	0.9 ± 0.08[b]	1.25 ± 1.48[ab]	1.18 ± 0.06[a]
*maca**	1.62 ± 0.01[b]	1.51 ± 0.04[b]	2.19 ± 0.06[a]	1.39 ± 0.03	1.59 ± 0.22	2.39 ± 0.94
mara	1.48 ± 0.03[c]	1.65 ± 0.02[b]	1.99 ± 0.05[a]	1.23 ± 0.06[c]	3.14 ± 0.31[a]	1.59 ± 0.08[b]
*midi**	1.44 ± 0.05[a]	1.19 ± 0.04[b]	30.3 ± 15.12[ab]	1.11 ± 0.05	0.76 ± 0.07	2.35 ± 1.53
*oscl**	1.67 ± 0.02[a]	1.43 ± 0.04[b]	1.49 ± 0.06[ab]	1.58 ± 0.05	0.43 ± 0.89	1.14 ± 0.16
phle	1.36 ± 0.09[b]	0.32 ± 0.11[c]	1.82 ± 0.05[a]	1.77 ± 0.28	1.17 ± 0.05	0.92 ± 0.07
*pipu**	1.58 ± 0.11[b]	1.14 ± 0.04[b]	1.92 ± 0.03[a]	1.29 ± 0.12[b]	2.53 ± 0.28[a]	1.14 ± 0.09[b]
*rhca**	1.7 ± 0.04	1.53 ± 0.03	1.96 ± 0.08	1.36 ± 0.08[b]	1.53 ± 0.32[ab]	1.86 ± 0.18[ab]
rimi	1.59 ± 0.05[b]	1.71 ± 0.03[ab]	1.97 ± 0.05[a]	0.99 ± 0.01	0.65 ± 0.29	1.55 ± 0.21
saca	1.34 ± 0.03	1.54 ± 0.07	1.71 ± 0.2	1.1 ± 0.04	0.47 ± 0.17	0.97 ± 0.19
stla	1.52 ± 0.03[b]	1.25 ± 0.04[c]	2.59 ± 0.16[a]	1.17 ± 0.02	1.15 ± 0.08	3.92 ± 0.93
*trgr**	1.56 ± 0.03[a]	1.29 ± 0.03[b]	1.55 ± 0.17[ab]	1.31 ± 0.04	3.15 ± 0.8	1.75 ± 0.45

(Table 3). However, CTAB DNA extracts in eight species had $A_{260}:A_{280}$ ratios ≥ 1.9, suggesting contamination by RNA or other substances capable of elevating that ratio (Table 3). In contrast, all $A_{260}:A_{280}$ ratios for PMC and DNeasy were less than 1.8 (Table 3). Across species, all three extraction methods produced DNA of similar quality by measure of the $A_{260}:A_{230}$ ratio (Table 3). Pure DNA has an $A_{260}:A_{230}$ ratio from 2.0 to 2.2 (Thermo Fisher Scientific, 2011). $A_{260}:A_{230}$ ratios < 1.8 usually reflect contamination by organic compounds like phenol and other aromatics. Only two species had average $A_{260}:A_{230}$ ratios < 1.0 when using the PMC method, vs. four species for CTAB and seven for DNeasy. PMC showed the greatest consistency in both purity ratios, with standard errors of 0.026 and 0.045, respectively, vs. values of 0.054 and 0.157 for DNeasy, and 0.091 and 0.147 for CTAB (Table 3). Thus, although the PMC isolations often did not differ significantly from those based on DNeasy or CTAB in mean purity absorbance ratios, they did show greater consistency.

Quantitative PCR showed that PMC yielded more amplifiable DNA than DNeasy or CTAB in all 12 species tested (Fig. 2). PMC produced significantly more amplifiable DNA in five and nine species relative to the DNeasy and CTAB methods, respectively, based on paired *t* tests. PMC yielded at least twice as much amplifiable DNA as DNeasy in eight of the 12 species. DNA isolated using CTAB in some samples of *Hylodesmum glutinosum* and *Caulophyllum thalictroides* showed no amplification after 40 cycles of PCR (Fig. 2). This suggests that additional post-isolation cleanup and/or modification to the CTAB procedure may be needed to amplify DNA in these species.

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 amplifiable 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

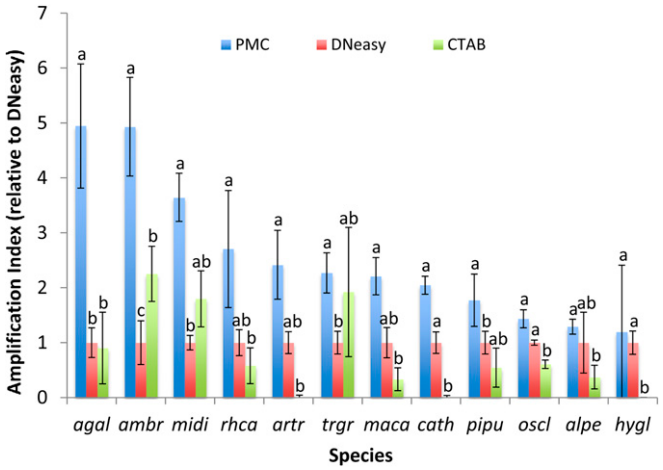

Fig. 2. Mean ± SE of yields of amplifiable DNA based on qPCR of matched DNA extractions obtained using PMC, DNeasy, and CTAB protocols. Species are indicated by four-letter codes determined by the first two letters of the generic name and specific epithet, respectively (refer to Table 1). Each protocol 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].

Michiels, 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. Bendich. 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.

Sahu, S. K., M. Thangaraj, and K. Kathiresan. 2012. DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. *ISRN Molecular Biology* 2012: 205049.

Smith, J. F., K. J. Sytsma, J. S. Shoemaker, and R. L. Smith. 1992. A qualitative comparison of total cellular DNA extraction protocols. *Phytochemical Bulletin* 23: 2–9.

Su, X., and A. M. Comeau. 1999. Cellulose as a matrix for nucleic acid purification. *Analytical Chemistry* 267: 415–418.

Sytsma, 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.

Thermo Fisher Scientific. 2011. Assessment of nucleic acid purity. T042 Technical Bulletin, NanoDrop spectrophotometers. Website http://www.nanodrop.com/Library/T042-NanoDrop-Spectrophotometers-Nucleic-Acid-Purity-Ratios.pdf [accessed 10 September 2014].

Tracy, S. 1981. Improved rapid methodology for the isolation of nucleic acids from agarose gels. *Preparative Biochemistry* 11: 251–268.

Varma, A., H. Padh, and N. Shrivastava. 2007. Plant genomic DNA isolation: An art or a science? *Biotechnology Journal* 2: 386–392.

Vorwerk, S. 2001. Wizard® genomic DNA purification kit and the isolation of plant genomic DNA. Website http://www.promega.com/resources/pubhub/enotes/wizard-genomic-dna-purification-kit-and-the-isolation-of-plant-genomic-dna/ [accessed 10 September 2014].

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.

Williams, C. E., and P. C. Ronald. 1994. PCR template-DNA isolated quickly from monocot and dicot leaves without tissue homogenization. *Nucleic Acids Research* 22: 1917–1918.

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.