Automated Genomic DNA Purification Using the Wizard® Magnetic 96 DNA Plant System

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Abstract

The use of molecular biology techniques is rapidly expanding in basic and applied plant and agricultural research. Molecular analysis has become the method of choice for large-scale marker-assisted breeding, seed-quality testing, SNP discovery and scoring, and analysis of transgenic plants. Automated purification of high-quality DNA from plant seed and leaf is essential to these emerging methods. Here we introduce the highly consistent and automated Wizard® Magnetic 96 DNA Plant System.

The use of MagneSil™ Paramagnetic Particles(a) (PMPs) in this system offers highly consistent yield and ease of handling in either manual or automated formats.

Introduction

The purification of plant genomic DNA is important for many applications in plant molecular biology. As large-scale plant genomics and plant breeding projects are undertaken, there is an increasing need for automated high-throughput purification of genomic DNA from a wide variety of plant species. Analysis of plants during marker-assisted breeding or the insertion of transgenic elements to make genetically modified plants may require the use of robotic platforms to facilitate the purification of hundreds of thousands of samples per year.

Plant scientists often use "homebrew" methods to purify genomic DNA from plant material. These methods can be labor- and time-intensive, often involving several centrifugation and incubation steps. For example, CTAB (hexadecyltrimethylammonium bromide) extraction is an accepted methodology with many described variations for different seed and leaf materials (1). This method generally involves lysis and detergent solubilization of plant material in a CTAB-containing buffer followed by organic extraction and alcohol/salt precipitation of DNA. Other techniques such as differential lysis, salting out, and silica-filter plates or columns are not widely used. None of these methods are readily adapted to automated robotic protocols.

We designed the Wizard® Magnetic 96 DNA Plant System(a) (Cat.# FF3760, FF3761) to provide high-quality DNA for downstream applications such as PCR(b), random amplification of polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP; 2,3) and simple sequence repeat/short tandem repeat analysis (SSR/STR; 4). The use of MagneSil™ Paramagnetic Particles(a) (PMPs) in this system offers highly consistent yield and ease of handling in either manual or automated formats. Protocols are available for the Biomek® 2000 Robotic Workstation (Beckman Coulter) as well as other robotic platforms. Send request for information about automated protocols to: *plantbio@promega.com*.

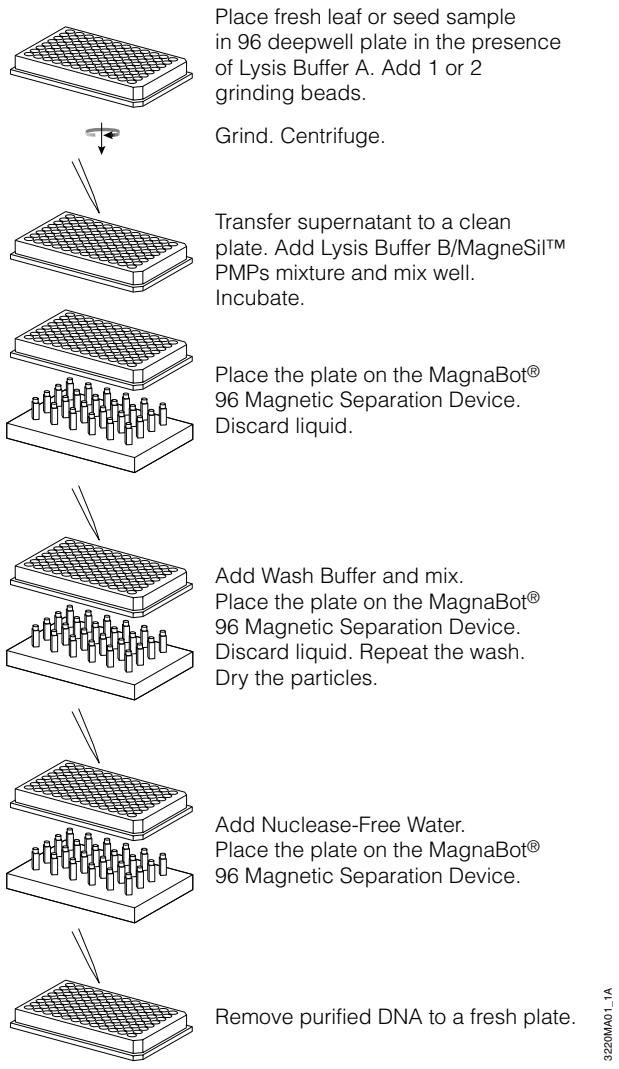


Figure 1. Schematic of DNA isolation using the Wizard® Magnetic 96 DNA Plant System.

Results of DNA Isolation from Plants

The Wizard® Magnetic 96 DNA Plant System protocol is outlined in Figure 1. This flexible procedure allows the purification of genomic DNA from a wide variety of seed and leaf material with only minor modifications to the protocol. When performed manually, the single centrifugation step combined with the ease of handling of the MagneSil™ Paramagnetic Particles saves time over other methods (Table 1).

Table 1. Times Required to Isolate DNA from Plant Tissue Using Three Listed Procedures.

Method	Steps	Time
Wizard® Magnetic 96 DNA Plant System	11	40 minutes
Commercial membrane plate	17	55 minutes
CTAB homebrew method	16	135 minutes

The key initial step in this procedure is grinding the plant samples in a 96 well format. Plant tissue may be fresh, lyophilized or frozen in liquid nitrogen. Our data is from fresh or frozen plant material ground at room temperature using a Geno/Grinder® mill (SPEX CertiPrep, Metuchen, NJ). A Retsch Grinding Mill (Haan, Germany) provides comparable results under similar conditions. Table 2 shows suggested grinding conditions for a variety of plant materials.

Table 2. Milling of Leaf and Seed Samples Using a SPEX Certiprep Geno/Grinder® Mill.

Sample Type	Conditions
Tender leaf or seedling	1 minute at 400 (1,200rpm)
Mature leaf	1–1.5 minutes at 500 (1,500rpm)
Fibrous leaf	1.5–2 minutes at 500 (1,500rpm)
Seeds	2–3 minutes at 500 (1,500rpm), depending on size and seed coat. May be repeated.

As illustrated in Figure 2, we isolated DNA from multiple plant materials using the Wizard® Magnetic 96 DNA Plant System and the automated protocol for the Biomek® 2000 robotic platform. The Wizard® Magnetic 96 DNA Plant System consistently delivered high-molecular weight DNA that was largely free of contaminating RNA.

DNA isolated using the Wizard® Magnetic 96 DNA Plant System can be used for many downstream applications. Figure 3 shows PCR amplification of an intron from the *TrnL* chloroplast gene from a number of plant seed and leaf tissue DNA samples isolated using the method described in Figure 1. For some plant samples such as corn leaf, DNA yield is sufficient to perform at least 100 standard PCR amplifications. All samples showed strong amplification of the *TrnL* sequence. The

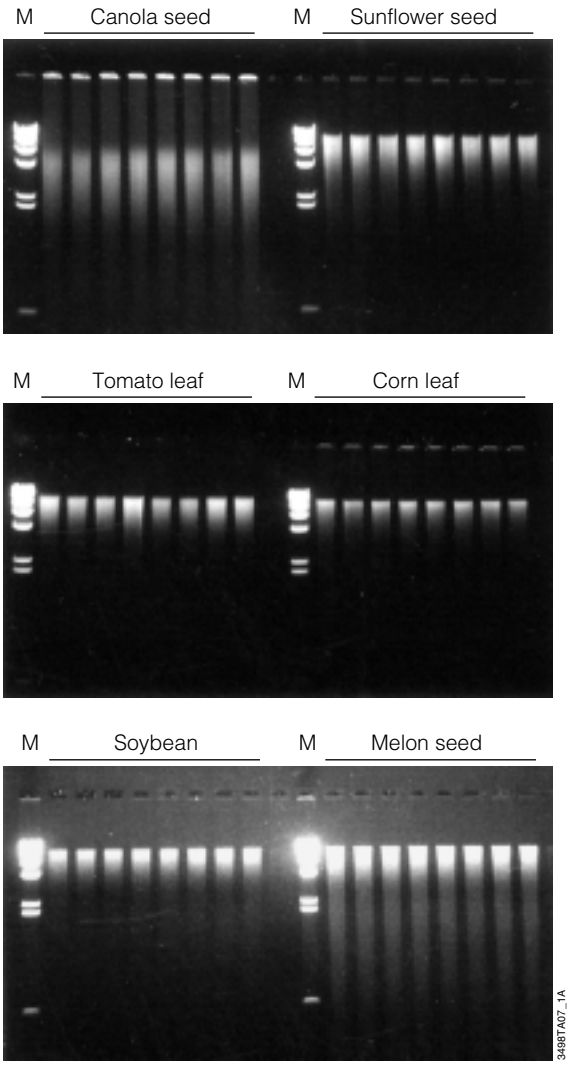
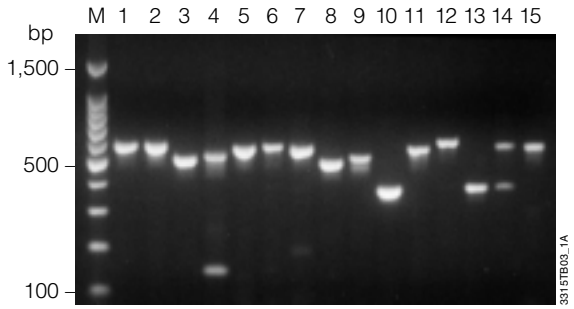


Figure 2. DNA isolated from different plant species using the Wizard® Magnetic 96 DNA Plant System. DNA was isolated using the automated protocol available for the Biomek® 2000 instrument. The starting material for each lane was either 5 canola seeds, 1 sunflower seed, 8 tomato leaf punches (8mm in diameter), 8 corn leaf punches (8mm in diameter), 20mg of soybean or 1 watermelon seed. Samples were eluted in 50µl Nuclease-Free Water, and 15µl of eluate were run on a Latitude® (BioWhittaker) 1% agarose TBE gel stained with ethidium bromide. Eight samples were shown for each plant tissue to indicate reproducibility of the method. DNA from the canola seed appears partially sheared due to milling. Lanes M, Lambda DNA/*Hin*d III Markers (Cat.# G1711).

diversity of amplification products is due to polymorphism within these loci between plant species.

We have purified DNA from many different plant samples using the Wizard® Magnetic 96 DNA Plant System and demonstrated that the DNA could be used for PCR or RAPD analysis (Table 3). Typical yields from several plant



1 Tobacco seedling
2 Soybean
3 Lettuce leaf
4 Corn leaf
5 Potato
6 *Arabidopsis* leaf
7 Cabbage seed
8 Green pepper seed
9 Prairie grass, seed head
10 Chives
11 Tomato leaf
12 Rice
13 Canola seed
14 Sunflower seed
15 Carrot seed

Figure 3. The Wizard® Magnetic 96 DNA Plant System produces PCR-quality DNA from a variety of plant species. Shown are PCR products for chloroplast DNA using a universal primer pair that amplifies the intron of the *Trn*L chloroplast gene across many plant species (5). The template was 1µl of eluate. One or more bands are produced depending on the plant species.

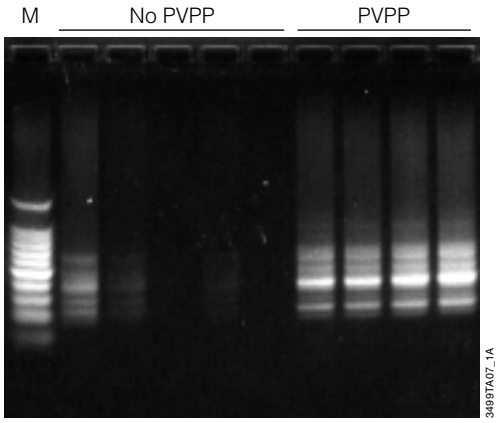


Figure 4. RAPD analysis of cotton leaf DNA isolated using the Wizard® Magnetic 96 DNA Plant System in the presence or absence of PVPP. A cotton leaf was homogenized using a SPEX CertiPrep Geno/Grinder® mill in the presence or absence of 10mg PVPP/300µl Lysis Buffer A. DNA was isolated manually from each leaf as described in Technical Bulletin #TB289. RAPD analysis was performed on cotton leaf DNA using the Pharmacia Ready-To-Go™ RAPD Analysis Kit. Samples were diluted 1:10 in water, and 1, 2, 3 or 4µl were used in a RAPD reaction with primer #3. Five microliters of each sample were analyzed on a 1.2% E-Gel™ agarose gel (Invitrogen).

Table 3. Plant Sample Types Processed Using the Wizard® Magnetic 96 DNA Plant System.

Arabidopsis	Cotton seed	Soybean
Cabbage seed	Grass seed	Squash
Canola leaf	Green pepper seed	Squash seed
Canola seed	Lettuce	Strawberry leaf*
Carrot seed	Milkweed leaf	Sunflower seed
Chicory leaf	Potato tuber	Tobacco seedling
Chives	Radish leaf	Tomato leaf
Corn leaf	Rice leaf	Tomato seed
Cotton leaf*	Sorghum	Watermelon seed

*These samples need the addition of PVPP to the lysis buffer to remove phenolic compounds that inhibit PCR.

Table 4. Typical DNA Yield From Different Plant Species Using the Wizard® Magnetic 96 DNA Plant System.

Sample	Yield
Arabidopsis tissue (50mg)	10ng/mg tissue
Canola leaf punches* (twelve)	26ng/leaf punch
Canola seeds (five)	343ng/seed
Corn leaf punches* (twelve)	98ng/leaf punch
Cotton seed (one)	29ng/seed
Lettuce leaf punches* (eight)	13ng/leaf punch
Melon seed (one)	166ng/seed
Radish leaf punches* (twelve)	89ng/leaf punch
Soybean (10mg)	10ng/mg bean
Squash seed (one)	279ng/seed
Sunflower seed (one)	405ng/seed
Tomato leaf punches* (twelve)	111ng/leaf punch

*Leaf punches are 6mm in diameter.

species and tissues are shown in Table 4. DNA was quantitated using PicoGreen® fluorescence (Molecular Probes, Eugene, OR).

PCR Amplification of Purified DNA and RAPD Analysis of Cotton Leaf

To further illustrate the importance of high-quality DNA for amplification, we analyzed DNA purified from cotton leaf. Cotton leaf contains high amounts of polyphenolics (6), which irreversibly bind to DNA during purification and can lower yield as well as inhibit downstream analysis of purified DNA (7). Polyvinylpolypyrrolidone (PVPP) is thought to reduce copurification of phenolic compounds with DNA that occurs during the initial crushing or grinding steps of the protocol (8).

Figure 4 shows RAPD analysis using DNA isolated from cotton leaf with the Wizard® Magnetic 96 DNA Plant System in the presence or absence of PVPP (e.g., Cat.# P6755, Sigma, St. Louis). Cotton leaf DNA purified without PVPP showed limited amplification. Inhibition of the RAPD reaction increased as increasing amounts of DNA sample were used, suggesting the presence of inhibitors in the purified DNA. In contrast, the addition of PVPP to the Wizard® Magnetic 96 DNA Plant System resulted in strong amplification of the expected diverse amplification products. The use

of PVPP with the Wizard® Magnetic 96 DNA Plant System is a simple, cost-effective method that can be used in an automated system or manually to isolate high-quality DNA from tissue containing high levels of phenolic compounds.

Conclusions

The Wizard® Magnetic 96 DNA Plant System can be used manually or with an automated workstation to consistently isolate high-quality DNA from plant tissue. The flexible design of this system allows the reliable purification of DNA from widely diverse plant materials and sample types. PVPP can be used with the Wizard® Magnetic 96 DNA Plant System to isolate DNA from highly phenolic plant samples.

References

1. Doyle, J.J. and Doyle, J.L. (1987) *Phytochem. Bull.* **19**, 11–15.
2. Vos, P. *et al.* (1995) *Nucl. Acids Res.* **23**, 4407–4414.
3. Zambeau, M. and Vos, P. (1993) European Patent Application No. 92402629.7. Publication No. 0534858A1.
4. Edwards, A. *et al.* (1992) *Genomics* **12**, 241.
5. Taberlet, P. *et al. (1991) Plant Mol. Biol.* **17**, 1105–1109.
6. Zhang, J. and Stewart, J. McD. (2000) *J. Cotton Sci.* **4**, 193–201.
7. Schneiderbauer, A., Sandermann, Jr., H. and Ernst, D. (1991) *Anal. Biochem.* **197**, 91–95.
8. Lodhi, M.A. *et al.* (1994) *Plant Mol. Biol. Reporter* **12**, 6–13.

Protocol

- *Wizard® Magnetic 96 DNA Plant System Technical Bulletin* #TB289, Promega Corporation. (www.promega.com/tbs/tb289/tb289.html)

lab fact

The Biomek® 2000 instrument can be used to automate DNA isolation from plant tissue and seeds. For more information, please see the Bioworks® methods available online at: www.promega.com/plantbio.

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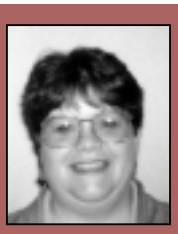

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Ordering Information

Product	Size	Cat.#
Wizard® Magnetic 96 DNA Plant System[a]	2 × 96	FF3760
	4 × 96	FF3761
Wash Buffer, Plant	40ml	A3811

Related Products

Product	Size	Cat.#
MagnaBot® Magnetic Separation Stand	each	V8151
MagnaBot® Spacer	each	V8381

[a]U.S. Pat. No. 6,027,945 and other patents pending.

[b]The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

Technical Questions?
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