Expanding the Capabilities of Plant Genomic DNA Purification

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We describe modifications of two current genomic DNA purification systems to address researchers' needs for automated plant genomic DNA purification. We have developed methods in a 96-well format to obtain greater than 1µg of genomic DNA from leaf or seed as well as methods to obtain a specific yield of genomic DNA from plant tissue. These methods improve productivity by providing sufficient DNA for multiple assays from a single purification or by obtaining a fixed yield of DNA, eliminating the need to quantitate and normalize before an assay.

The use of MagneSil™ PMPs offers scalability for increased sample amounts and increased DNA yield, circumventing the limits of extensive centrifugation or vacuum filtration.

Introduction

One of the major concerns of plant molecular biologists is the time required to extract genomic DNA from plant tissue. Studies have shown that sample processing is the major bottleneck for genotyping processes, accounting for over 60% of the time required from sample collection to genotype results (1). Whether the goal is large-scale plant genomics research, commercially focused seed quality control, or marker-assisted breeding, the need to do more work with limited resources drives the choice of methods. In some cases, researchers need to maximize yield per extraction to have sufficient DNA for large numbers of assays from a single purification. In other cases, researchers need to eliminate time-consuming quantitation and normalization downstream of DNA purification to maximize productivity. Both cases benefit from consistent DNA purity that minimizes failed analyses due to poor-quality extracted DNA.

The Wizard® Magnetic 96 DNA Plant System[a] (Cat.# FF3760) was designed for purification of DNA from commercially important agricultural crops. Promega scientists and customers have focused their efforts on DNA purification from "high-value" agricultural crops such as corn, soy, cotton, tomato, canola, and several other vegetable leaves and seeds (2). A typical sample size for the Wizard® Magnetic 96 DNA Plant System is one 6–8mm leaf punch or 1–5 seeds. Plant samples are typically ground in a 96-well grinder and then extracted either manually or by an automated method, using off-the-shelf automated workstations such as the Beckman Coulter Biomek® 2000 or the Biomek® FX liquid-handling workstations or the Tecan Genesis® workstation. Genomic DNA is eluted in 100µl of water at a concentration sufficient so that 1µl can be used in a PCR reaction for most targets.

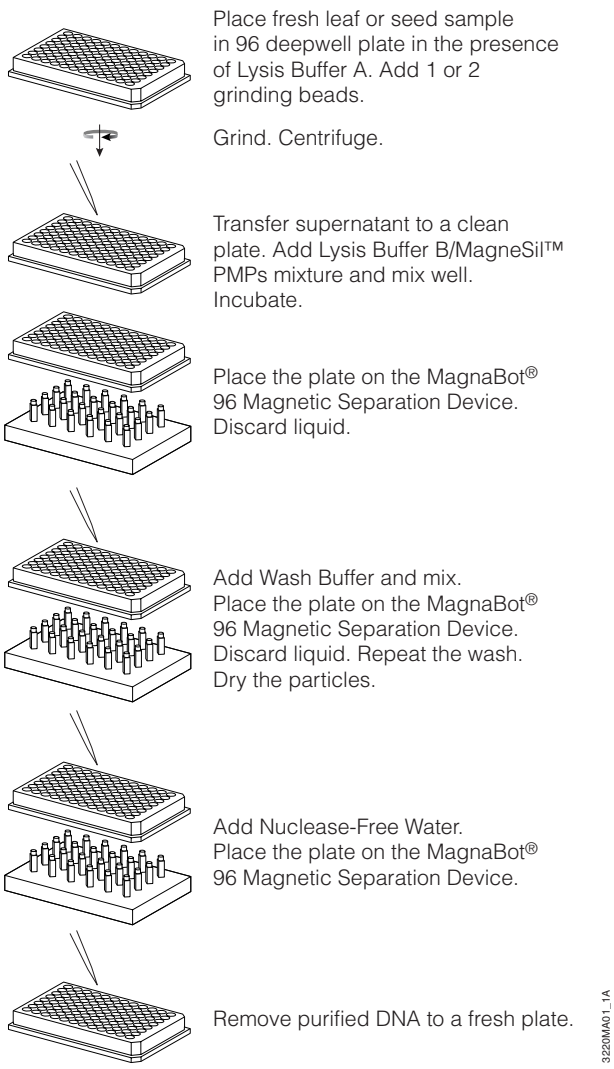

Figure 1. The Wizard® Magnetic 96 DNA Plant System Protocol.

The ability to extract plant genomic DNA in a 96-well manual or automated format is a step forward, given the historical dependence on manual or semi-automated CTAB (organic solvent-based) methods. However, some plant molecular biologists require larger amounts of DNA for large-scale marker analysis or for archiving. The Wizard® Magnetic 96 DNA Plant System, which relies on patented MagneSil™ paramagnetic particle (PMPs) technology[a], offers scalability for increased sample amounts and increased DNA yield, circumventing the limits of extensive centrifugation or vacuum filtration methods.

Often plant molecular biologists will need to quantitate and normalize genomic DNA yields before performing amplification or other analyses. The MagneSil™ ONE, Fixed Yield Blood Genomic System[a] (Cat.# MD1370) allows the purification of specific yields of genomic DNA. This system can be modified for purifying genomic DNA from plant material and provides the researcher with high-quality, fixed-yield plant genomic DNA suitable for amplification and other analyses.

Scaling Plant Genomic DNA Preps for Large Yield

The Wizard® Magnetic 96 DNA Plant System protocol is outlined in Figure 1. The procedure allows the purification of genomic DNA from a wide variety of seed and leaf material with only minor modifications to the protocol. To increase genomic DNA yield, we developed two general strategies that may be followed based on how much of an increased yield is required. The simplest modification is to add 5 or more leaf punches with the same protocol and reagent amounts as described in the "standard protocol" in the *Wizard® Magnetic 96 DNA Plant System* Technical Bulletin #TB289. With this simple modification, we have obtained two- to fourfold increases in total yield depending on the starting leaf material (Figure 2).

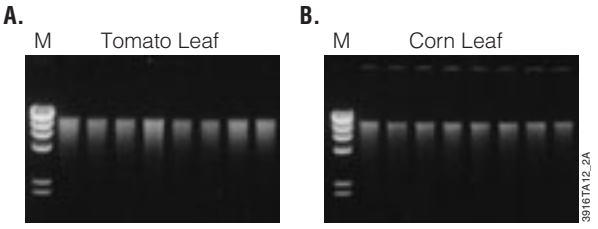

Figure 2. Yield and quality of plant genomic DNA isolated using a scale-up of the Wizard® Magnetic 96 DNA Plant System. Ten microliters of a 50µl preparation was run on a 1% agarose gel. The method was modified to use an increased amount of plant material added and ground (see Table 1 and Box 1). Lambda DNA/*Hin*d III Markers (Cat.# G1711) was used as a molecular size standard (Lane M).

A second method that we developed to increase yield involves proportionally scaling the amount of sample and reagents. In order to use more plant lysate in the purification process, the protocol must be carried out using deep-well plates. We developed a deep-well plate method for the Biomek® FX workstation (Figure 3) to ease sample handling. This method requires a magnetic separation stand that provides optimal magnetic binding of particles when using higher volumes of reagents in deep-well plates. The Deep Well MagnaBot® 96 Magnetic Separation Stand (Cat.# V3031) and MagnaBot® Spacer, 1/8 inch (Cat.# V8581) are designed to work optimally with deep-well plates when purifying DNA with MagneSil® PMPs.

Figure 3. Deck layout of the Biomek® FX workstation for the deep-well plate method using the Wizard® Magnetic 96 DNA Plant System protocol.

Table 1. Typical Yields Using Modified Wizard® Magnetic 96 DNA Plant System Protocol.

Sample	Increased Sample*	Deep Well**	Yield (ng)
Canola seedling	Yes		541
Canola seedling		Yes	1,271
Canola seed	Yes		370
Canola seed		Yes	614
Tomato seed	Yes		311
Corn leaf		Yes	1,100
Canola leaf (1)		Yes	1,292
Canola leaf (1/2)		Yes	1,104
Canola leaf (1/4)		Yes	708

Scale-up of plant genomic DNA preparations
*Increased sample = 5 leaf punches versus 1; other volumes kept the same.
**Deep Well = 10 leaf punches; increased lysate; increased PMPs and washes.

Purified DNA is eluted in 100µl of Nuclease-Free Water. Typical yields from seeds are shown in Table 1. Users can obtain yields of 1µg or greater, depending on the plant material tested. For plant tissue containing phenolics (e.g., cotton or strawberry leaf), conditions need to be optimized with the use of PVPP (insoluble polyvinyl poly-pyrrolidone; 2) to obtain amplifiable DNA.

With both of these scale-up procedures, complete homogenization of the plant material and centrifugation to separate the debris from the lysate is critical. Suspended plant material can easily clog robotic pipette tips and reduce yields. You may need to extend grinding time or centrifuge at higher speeds or for a longer time to prepare a properly cleared lysate. In addition to these steps prior to liquid handling, the aspiration height of the liquid handler 96-well head needs to be above plant debris when removing lysate from the sample grinding plate to the working plate.

PCR Amplification of Purified DNA with RAPD Analysis

To illustrate the quality of plant genomic DNA isolated with the Wizard® System, we analyzed DNA purified from canola leaf. Figure 4 shows multi-locus PCR amplification[b] of random sequences from canola genomic DNA. DNA samples were isolated using the method described in Box 1. All samples showed varying band patterns in this RAPD analysis (Random Amplified Polymorphic DNA) analysis.

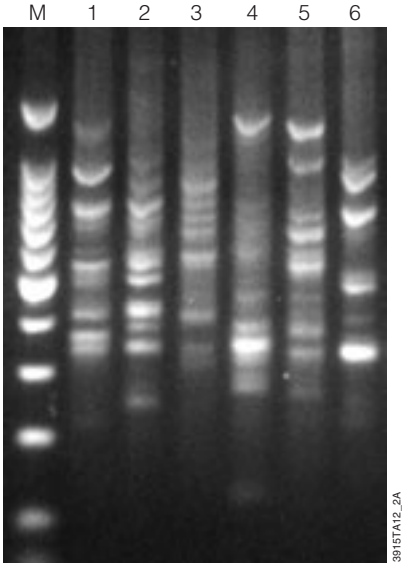

Figure 4. DNA was isolated from canola leaf using the deep-well scale up method and the Wizard® Magnetic 96 DNA Plant Systems. Samples were analyzed using 6 different RAPD primers and the Ready-To-Go™ RAPD Analysis Kit (Amersham Biosciences). One microliter of a 1:10 dilution of DNA was used as a template. M = 100bp DNA ladder (Cat.# G2101). Lanes 1–6: Canola leaf DNA amplified with RAPD primers 1–6, respectively.

Application of the MagneSil™ ONE System for Isolation of Fixed-Yield DNA from Plant Material

The MagneSil™ ONE, Fixed Yield Blood Genomic System (Cat.# MD1370) was designed for purification of DNA from small volumes of blood. Reliable defined yields can be obtained and may eliminate the need for measuring of DNA before downstream applications. This reagent system is also useful for purifying DNA from plant material (method described in Box 2). Depending on the plant material tested, yield is run-to-run consistent using the system (Table 2). Yield varies from plant to plant and with sample size. Small sample sizes consisting of 1–2 leaf punches or 1–2 small seeds can be used.

Box 1. Protocol for Large-Yield Plant Genomic DNA Isolation.

Sample Size and Preparation	**Leaf Punches:** Begin with 6–20 leaf punches homogenized with 400µl of Lysis Buffer A until no large pieces remain. Add an additional 600µl of Lysis Buffer A and centrifuge the plate at 1800 × *g* for 20 minutes to pellet debris. **Seeds:** Sample size will vary greatly, depending on seed dimensions and coatings.
Automated Protocol	The liquid handler transfers 500µl of lysate to the deep-well plate containing 250µl Lysis Buffer B and 30µl MagneSil™ particles. Mixing is interspersed with short incubations at room temperature before the deep-well plate is transferred to the Deep-Well MagnaBot® device. The supernatant is transferred to the waste plate. The plate is moved back to the work area, and two washes with 300µl Lysis Buffer B are performed, using 4-corner mixing to resuspend the particles. The liquid handler performs three 500µl Wash Buffer washes and aspirates all wash solution before drying for 10 minutes. The plate is moved to the heat transfer block and 100–200µl water or TE is added for elution. DNA is eluted by pipette mixing the particles with elution solution and incubating on the heat block. **Note:** A heat block is used during the elution step to increase recovery. The plate is transferred back to the Deep Well MagnaBot® device, and the eluate is removed to a 96-well plate.

Materials	• two 96-well polypropylene deep-well plates (1–1.2ml; Corning or Marsh)
	• four 2ml deep, square-well, 96-well plates to contain reagents,waste and tip wash
	• 1 Greiner 96 or polypropylene plate
	• Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031)
	• Deep Well MagnaBot® Spacer, 1/8 inch (Cat.# V8581)
	• heat transfer plate
	• ALPs (Automated Lab Positioner) for the Biomek® FX station:
	  • heating-cooling unit
	  • tip-wash station (optional, but useful)

Table 2. Consistent Yields from the MagneSil™ ONE System.

Sample	Resin Used	Yield (ng)
Canola leaf punch, 8mm	6µl	149
Canola seedling	6µl	273
Canola seed (1)	6µl	204

This technique has obvious advantages for the high-throughput plant genomics laboratory testing multiple samples of the same plant species. One isolation will provide sufficient DNA for several PCR reactions as well as for stringent applications such as RAPD analysis. For this reason the Biomek® FX workstation, with the capability of pipetting 96 wells at a time, was chosen as the optimal instrument platform for this application (Figure 5).

Figure 5. Deck layout for the MagneSil™ ONE protocol on the Biomek® FX workstation, modified for plant genomic DNA isolation.

Yield

The MagneSil™ ONE System uses a novel mechanism for DNA purification. The paramagnetic particles used in the MagneSil™ ONE System are used to capture a consistent amount of DNA from the lysate. The resin has a defined DNA binding capacity in the presence of excess DNA, so by varying the amount of resin you can "choose your yield" (Figures 6 and 7). As a result, you can eliminate the need to quantitate and normalize purified DNA prior to PCR analysis.

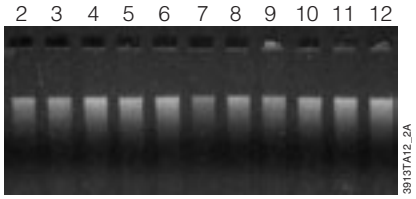

Figure 6. Fixed yield isolation of plant genomic DNA. DNA was isolated from 100µl plant lysate after grinding 2 canola seeds per well using the MagneSil™ ONE Lysis Buffer. Eight microliters of resin was used in an automated protocol on the Beckman Biomek® FX workstation. Ten microliters of the 100µl of eluted DNA was analyzed on a 1% agarose gel. This represents a total yield of approximately 200ng.

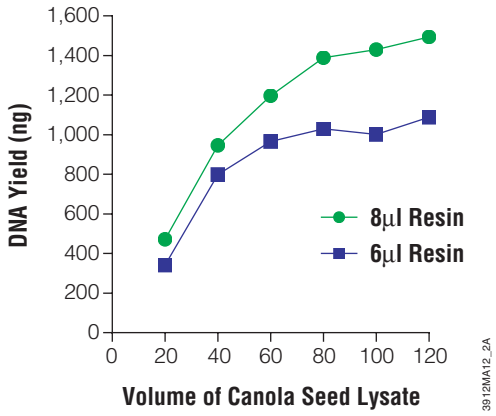

Figure 7. The MagneSil™ ONE System allows fixed-yield genomic DNA isolation from plant material. Plant genomic DNA was isolated using the method described in Box 2.

Box 2. Protocol for Fixed-Yield Plant Genomic DNA Isolation.

Sample Size and Preparation	**Leaf Punches:** Homogenize 1–2 leaf punches with 325µl Lysis Buffer, Blood, until no large pieces remain. Centrifuge the plate at 1800 × *g* for 20 minutes to pellet debris. **Note:** Foaming will occur because of detergent present in the lysis buffer. This will disperse after centrifuging. **Seeds:** Sample size will vary greatly, depending on seed dimensions and coatings. Grinding with metal balls on the Geno/Grinder® works best in a small amount of Lysis Buffer. If too much Lysis Buffer is used, the seed will not be broken up by the shaker action. Add an additional 300µl Lysis Buffer after grinding.
Automated Protocol	The liquid handler will transfer 100µl lysate to a deep-well plate containing 7µl of MagneSil™ PMPs fixed-yield. Mixing by pipetting will be interspersed with short incubations before the plate is transferred to the Deep Well MagnaBot® Device. The supernatant is transferred to the waste plate. The plate is moved back to the work area, and two 300µl Lysis Buffer, Blood, washes are done using 4-corner mixing to resuspend particles. The liquid handler performs three 500µl Alcohol Wash, Blood, washes and aspirates all wash solution before drying for 10 minutes. The plate is moved to the heat transfer block, and 100–200µl of water or TE is added for elution. The DNA is eluted from the particles by mixing the particles with elution solution and incubating them on the heat transfer block. The robot transfers the plate back to the Deep Well MagnaBot® device, and the eluate is removed to a 96-well plate.

Materials	• one 96-well polypropylene deep-well plate (1–1.2ml; Corning) for sample grinding.
	• four 2ml deep, square-well, 96-well plates to contain reagents, waste and tip wash.
	• one Griener 96 or polypropylene plate
	• Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031)
	• ALPs for the Biomek® workstation:
	  •heating-cooling unit, tip wash
	  •tip wash station (optional, but helpful)

Conclusion

The Wizard® Magnetic 96 DNA Plant System provides consistently high-quality DNA from plant tissue in a manual or automated 96-well format. The MagneSil™ ONE System, modified for plant DNA extractions, allows you to choose your desired yield. Both of these systems help plant molecular biologists save time by providing more efficient extraction methods by allowing larger yields per prep, minimizing the need for repeated extractions, or by eliminating the need to quantitate and normalize DNA concentration prior to PCR amplification. In this article, we have described methods to increase DNA yield or achieve a fixed DNA yield from plants. These applications of the Wizard® Magnetic 96 DNA Plant System and the MagneSil™ ONE System provide the tools plant scientists need to improve productivity.

Reference

1. Dilworth, E., and Frey, J.E. (2000) A rapid method for high-throughput DNA extraction from plant material for PCR amplification. *Plant Mol. Biol. Reporter* **18**, 61–4.
2. Koller, S. *et al.* (2001) Automated genomic DNA purification using the Wizard® Magnetic 96 DNA Plant System *Promega Notes* **79**, 25–8.

Protocols

- *Wizard® Magnetic 96 DNA Plant System Technical Bulletin* #TB289, Promega Corporation. (www.promega.com/tbs/tb289/tb289.htm)
- *MagneSil™ ONE Fixed Yield Blood Genomic System Technical Bulletin* #TB313, Promega Corporation. (www.promega.com/tbs/tb313/tb313.htm)

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Ordering Information

Product	Size	Cat.#
Wizard® Magnetic 96 DNA Plant System[a]	2 × 96 preps	FF3760
	4 × 96 preps	FF3761
MagneSil™ ONE, Fixed Yield Blood Genomic System*[a]	1 × 96 preps	MD1370
Deep Well MagnaBot® Magnetic Separation Device*	1 each	V3031
MagnaBot® Spacer, 1/8 inch	1 each	V8581

*For Laboratory Use.

[a] U.S. Pat. Nos. 6,027,945 and 6,368,800, Australian Pat. No. 732756 and other patents and 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.