The "Auto"matic Choice for Plant DNA Purification

Automating the Wizard® Magnetic 96 DNA Plant System on Thermo Labsystems' Kingfisher®

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Abstract

The Wizard® Magnetic 96 DNA Plant System is designed for manual or automated 96-well purification of DNA from plant leaf and seed tissue. We developed an automated protocol using this system and Thermo Labsystems' Kingfisher® Magnetic Particle Processor. Here we describe the results of DNA purification from leaf tissue of the five crops mandated by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Andhra Pradesh, India.

The purification of DNA from plant tissue is often a bottleneck in the genotyping process. The Wizard® Magnetic 96 DNA Plant System is an automated solution for DNA purification from a broad range of plants.

Introduction

Marker-assisted plant breeding is the primary aim of the MS Swaminanthan Applied Genomics Lab (AGL) at ICRISAT. This breeding strategy requires the genotyping of a large number of samples. The starting point for genotype analysis is the extraction of DNA from plants, which frequently represents a serious bottleneck for the entire genotyping process. For example, Dilworth and Frey (1) estimated that 60% of the time required for sample processing, from leaf collection to PCR results, was used for DNA extraction. Moreover, tropical plant species often represent a particular challenge to obtaining high-quality DNA using rapid methods.

In this report, we evaluate the Wizard® Magnetic 96 DNA Plant System[a] (Cat.# FF3760) used with Thermo Labsystems' Kingfisher® Magnetic Particle Processor. The Wizard® Magnetic 96 DNA Plant System is designed for purification of DNA from plant tissues using MagneSil™ Paramagnetic Particles[a] as a mobile solid phase. The particles are completely resuspended during the wash steps, assisting with the removal of contaminants and increasing nucleic acid purity. Over the past few years, automated systems have emerged that increase the speed, efficiency and reproducibility of DNA purifications (2).

Materials & Methods

The Thermo Labsystems Kingfisher® Instrument: The Kingfisher® instrument eliminates the aspiration and other liquid handling steps that can lead to waste or contamination in systems requiring vacuum extraction and manual liquid transfer methods. Purification is straightforward; samples, beads and reagents are placed in a multiwell plate that is contoured at the bottom to eliminate crevices in which materials and beads can collect. To mix the MagneSil™ Particles, the mechanical arm lowers a row of plastic "sleeves", like a plunger, mixing the liquids and beads. To bind the MagneSil™ Particles, a second arm lowers magnetic probes into the sleeves collecting the beads on the outer surface of the sleeve. The Kingfisher® instrument moves bound particles from well to adjacent well instead of moving liquids. DNA is eluted from the MagneSil™ particles in a small volume. Up to 24 samples may be processed per instrument run, 12 samples per plate, 2 plates per run. A standard run using the protocol we developed will take 15 minutes.

We tested this method using ICRISAT's five mandated crops: *Sorghum bicolor* (L.) Moench (sorghum), *Pennisetum glaucum* (L.) R. Br. (pearl millet), *Cicer arietinum* L. (chickpea), *Arachis hypogaea* L. (groundnut) and *Cajanus cajan* (L.) Millspaugh (pigeon pea). We purified the DNA in two sets. For each crop, we purified DNA from two strains (Table 1) for a total of ten genotypes. Leaf material was taken from 6-day-old seedlings; the amount of starting material used is listed in Table 2.

Table 1. List of Accessions Used for DNA Extraction.

Crop	Accession Numbers
Sorghum	IS21807 IS18551
Pearl Millet	IP22291 IP22303
Chickpea	JG62 ICCV2
Groundnut	TMV2 ICGV91278
Pigeon Pea	ICP2376 ICP14772

Table 2. Approximate Weight of Leaf Starting Material.

Crop	Set 1	Set 2
Sorghum	30mg	20mg
Pearl Millet	30mg	20mg
Chickpea	20mg	10mg
Groundnut	20mg	10mg
Pigeon Pea	20mg	14mg

DNA Purification: The leaf tissue was placed in a sealed 96-deep-well plate (Marsh Biomarket) together with two 4mm stainless steel balls and 300ml Lysis Buffer A (Wizard® Magnetic 96 DNA Plant System). The samples were processed in a Geno/Grinder® 2000 (Spex CertiPrep), following the manufacturer's instructions, at 500 strokes per minute for 2 minutes. The plates were then centrifuged at 1,700 × *g* for 10 minutes to collect the cell debris. A 125µl aliquot of each sample was transferred to the Kingfisher® strip tubes (Table 3) and 60µl of MagneSil™/Lysis Buffer B (Wizard® Magnetic 96 DNA Plant System) was added to each well. The Kingfisher® plate preparation was then processed using a protocol we created using the Kingfisher® software v 1.0. (Table 4).

Table 3. Reagents Added to the 96-Well Kingfisher® Plate.

Row ID	Tube Reagents
A	125µl sample lysate in Lysis Buffer A 60µl MagneSil™/Lysis Buffer B
B	150µl Wash Solution
C	100µl Wash Solution
D	50µl Nuclease-Free Water

Table 4. Kingfisher® Protocol.

1. Bind Magnetic Beads (row A) Bind parameters: Bind time: 1 minute; Speed: Slow Collection parameters: Collect time: 30 seconds
2. Wash Beads (row B) Release parameters: Release time: 10 seconds; Speed: Fast Wash parameters: Wash time: 20 seconds; Speed: Medium Collection parameters: Collect time: 30 seconds
3. Wash Beads (row C) Release parameters: Release time: 10 seconds; Speed: Fast Wash parameters: Wash time: 20 seconds; Speed: Medium Collection parameters: Collect time: 30 seconds
4. Dry Beads (row C) Dry time: 5 minutes Tip position: Inside well/tube
5. Elute Beads (row D) Release parameters: Release time: 30 seconds; Speed: Fast Elution parameters: Elute time: 5 minutes; Speed: Very slow Remove Beads

Results

The sample and plate preparation took one hour, and the protocol itself lasted 15 minutes; therefore, the time required for 24 samples to be purified with this process is 1 hour and 15 minutes.

We evaluated the quality of the extracted DNA through two procedures: agarose gel electrophoresis and SSR-PCR. Figure 1 shows the result of the extracted DNA run on a 0.8% agarose gel, stained with ethidium bromide and visualized with UV light.

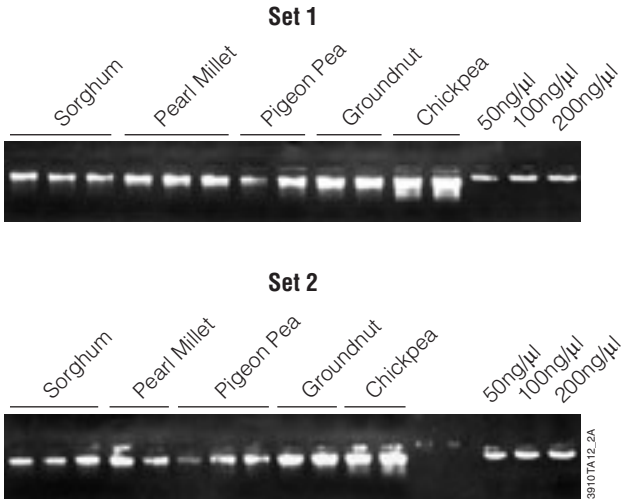

Figure 1. DNA extracted from five ICRISAT-mandated crops. DNA was purified from two sets of samples using the method described in Materials and Methods. The amount of starting material is shown in Table 2. Five microliters of the purified DNA was separated on a 0.8% agarose gel, stained with ethidium bromide and visualized with UV light. Aliquots of 50ng/µl, 100ng/µl and 200ng/µl of DNA purified with an optimized CTAB method were used as DNA standards.

We performed SSR-PCR amplification tests on all samples using primers and protocols previously optimized in the AGL. Figure 2 shows amplification products from pearl millet and chickpea samples. In addition, we included samples extracted using an optimized CTAB miniprep method as a control. Samples from each crop purified with the Wizard® Magnetic 96 DNA Plant System amplified well compared to those purified with the CTAB method.

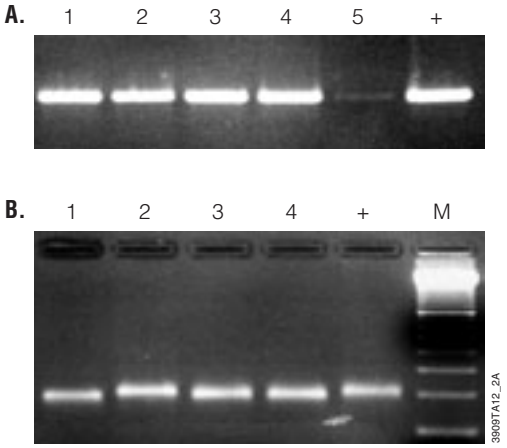

Figure 2. Amplification of purified DNA with SSR-PCR. DNA was purified using the method described. The purified DNA was amplified using SSR-PCR, and the amplification products were separated on a 1.5% agarose gel, stained with ethidium bromide and visualized with UV light. For reference, a positive control (+) was included. **Panel A.** Five pearl millet samples amplified using SSR primer Psm2086 (3). **Panel B.** Four chickpea samples amplified using SSR primer Tr1 (4). Lane M contains a 100bp DNA size marker.

Conclusions

DNA purification from plant samples has become the bottleneck in sample processing from plant tissue to PCR result. The combination of the Wizard® Magnetic 96 DNA Plant System and the Kingfisher® instrument we described here was quick and easy to use. This procedure can be used to purify high-quality DNA from plant material using a 15-minute walkaway protocol, once the plant lysate is prepared. Purified DNA performed well in SSR-PCR and gave good yield. This allows plant molecular biologists to achieve increased productivity when purifying plant genomic DNA in low- to moderate-throughput systems.

References

1. Dilworth, E. and Frey, J.E. (2000) *Plant Mol. Biol. Report.* **18**, 61–64.
2. Constans, A. (2000). *The Scientist* **14[13]**, 23.
3. Winter P. *et al.* (1999) *Mol. Gen. Genet.* **262**, 90–101.
4. Qi, X. (2002) personal communication.

Protocols

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

Not Pictured

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Ordering Information

Product	Size	Cat.#
Wizard® Magnetic 96 DNA Plant System[a]	2 × 96 preps	FF3760
	4 × 96 preps	FF3761

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