The Versatility of the Maxwell® 16 System for Genomic DNA Extraction

ABSTRACT The Maxwell® 16 Integrated System combines compact instrumentation, optimized automated methods, prefilled reagent cartridges, service and support to save time, enhance productivity and improve consistency of results. In this article we demonstrate the versatility of the Maxwell® 16 System and the Maxwell® 16 DNA Purification Kits is demonstrated for isolating genomic DNA from model organisms, plant tissue, bacteria, fungal and food samples.

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The Maxwell® 16 Integrated System offers an automated low- to moderate-throughput option for isolating genomic DNA from plant, animal, fungal and bacterial samples for use in PCR.

INTRODUCTION

Purification and PCR analysis of DNA are critical techniques used to determine genetic strains of plants, to detect mutations or genetic modifications of an organism, or to identify microorganisms present in a sample. DNA purifications are performed repeatedly and routinely for these applications, and purification techniques are often tailored to meet the demands of the sample type. The Maxwell® 16 System was developed to meet the needs of low- to moderate-throughput users by providing automated purification at a scale appropriate to their workload without considerable capital investment, training or maintenance (1). The prefilled cartridge design and mechanical plunger action of the Maxwell® 16 System make it ideal for simple, rapid purification of a wide variety of sample types that might otherwise require time-consuming or labor-intensive preprocessing steps (e.g., overnight protease digest, bead beating or grinding in the presence of liquid nitrogen). The Maxwell® 16 System kits and automated methods are optimized for isolation of genomic DNA from some of the most frequently used samples, such as blood, cells and tissue. This article details the use of these cartridges and methods for isolation of genomic DNA from a broad range of basic research- and applied testing-related sample types.

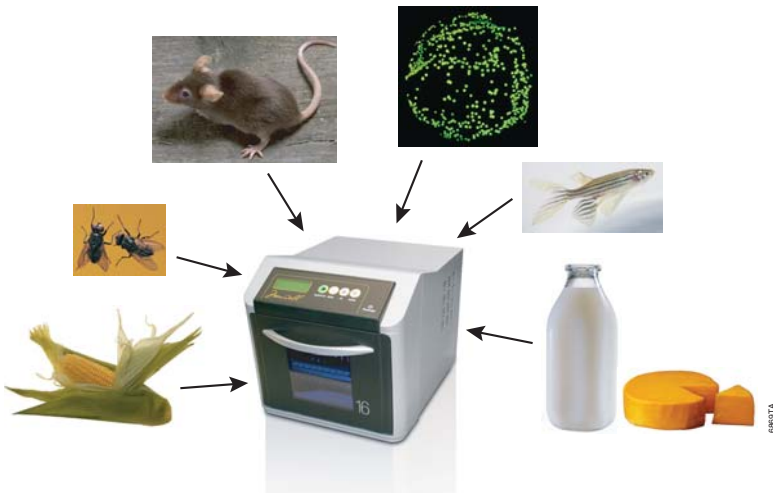

Figure 1. Maxwell® 16 System extracts DNA from a wide variety of sample types.

ORGANISMS

Genomic DNA is frequently isolated from insects and model organisms, such as fruit flies and *Caenorhabditis elegans*. The purified DNA is then used in a variety of downstream applications, including cloning, amplification and mutational analysis. Isolation of DNA typically begins with disruption of the organism by grinding frozen tissue or conducting lengthy protease digests. Here we demonstrate the utility of the Maxwell® 16 Instrument for isolating DNA directly from organisms without the need for preprocessing. Whole organisms or tissue samples were placed directly into the Maxwell® 16 Tissue DNA Purification Kit[(a)] cartridge. Using the Maxwell® 16 tissue method, the indicated amount

Table 1. Average Yield of Genomic DNA from a Variety of Organisms. The indicated quantity of sample was added directly to well #1 of the Maxwell® 16 Tissue DNA Purification cartridge. DNA was then tested using real-time PCR (Plexor® qPCR System).

Organism	Sample Size	Yield	Real-Time PCR Analysis
Ant	1 (4–5 mm)	3.1 µg	nt
Brine Shrimp: *Artemia franciscana*	1 (3–4 mm)	1.7 µg	+
Fruit fly: *Drosophila melanogaster*	1 fly	324 ng	+
	5 flies	1.5 µg	+
Nematode: *Caenorhabditis elegans*	~50,000 worms	78 ng	+
Mosquito	1 (5–6 mm)	3.6 µg	nt
Ticks or Nymphs: *Ixodes ricinus*	as few as 1	nt	*
Zebrafish: *Danio rerio*	50 mg tissue	2.5 µg	+

+ = successful detection; nt = not tested; *endpoint PCR.

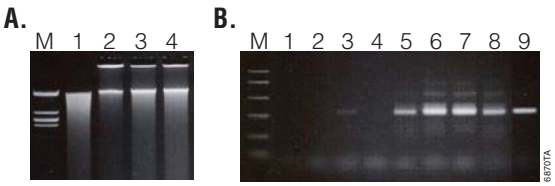

Figure 2. Genomic DNA isolated from feces by the Maxwell® 16 System is high molecular weight and can be amplified using eubacterial 16S rRNA primers. Panel A. Each lane represents 5 µl of genomic DNA purified from mouse fecal pellets. Lane M, Lambda DNA/EcoRI Markers (Cat.# G1721); lane 1, DNA isolated by a fecal DNA extraction kit using bead beating; lanes 2–4, DNA isolated using Maxwell® 16 Tissue DNA Purification Kit with no preprocessing. **Panel B.** Each lane represents 10% of the 50 µl amplification reaction containing 1 µl of template. The amplicon size is 350 bp. Lane M, BenchTop PCR Markers (Cat.# G7531); lane 1, no template control; lanes 2–4, undiluted fecal DNA; lanes 5–7, DNA diluted 1:100 in water; lane 8, DNA processed over a Zymo-Spin™ IV-HRC spin filter; lane 9, JM109 DNA control.

of DNA was isolated (Table 1). The quality of the purified genomic DNA was tested in real-time PCR using the Plexor® qPCR System (Cat.# A4011). Organism-specific primers were designed and used for each sample type. All purified DNA samples tested in PCR were successfully amplified. See sample-specific *Application Notes* for details, available at: **www.promega.com/maxwell16/appnotes.htm**

FECES

Detection of bacteria in fecal samples is critical for screening for potentially harmful pathogens. However, isolation of amplifiable DNA can be a challenge not only because the DNA can be difficult to release but also because PCR inhibitors are often copurified. Isolation typically involves preprocessing steps such as bead beating or lengthy incubations to break apart samples. We found the mechanical action of the Maxwell® 16 Instrument plungers was sufficient to break apart fecal samples with minimal DNA shearing (Figure 2, Panel A). Using single mouse fecal pellets (average weight = 38 mg) with the Maxwell® 16 Tissue DNA Purification cartridge and method, we obtained an average of 48 µg purified DNA per sample. (data not shown).

Often DNA isolated from fecal samples will contain significant amounts of inhibitors, such as phenolics and polysaccharides, which may prevent DNA amplification. To detect microbial flora in the fecal material, DNA was isolated by the Maxwell® 16 System and tested using conserved eubacterial 16S ribosomal RNA primers (2; Figure 2, Panel B). One out of eight samples amplified without dilution or further treatment of the purified DNA. When DNA was either diluted 1:100 in water or passed over a crosslinked-polyvinylpyrrolidone-filled Zymo-Spin™ IV-HRC spin filter (Zymo Research) and centrifuged for one minute, the DNA from all samples could be used directly in PCR.

Table 2. Average Yield of Genomic DNA from a Number of Plant Samples. One 8 mm punch per purification was used for leaf samples, except *Arabidopsis* (1 leaf), rice (5 mm punch) and wheat (5 mm slice). Seed sample size was one seed per purification, except canola (5 seeds). DNA was tested using real-time PCR (Plexor® qPCR System).

	Yield		Detected by Real-Time PCR
Plant Sample	Leaf	Seed	
Alfalfa: *Medicago sativa*	1.4 µg	nt	+
Arabidopsis thaliana	139 ng	nt	+
Canola: *Brassica napus*	512 ng	2.3 µg	+
Corn: *Zea mays*	655 ng	nt	+
Cotton: *Gossypium hirsutum*	854 ng	2.6 µg*	+
Grape: *Vitis vinifera*	20 ng**	nt	+
Rice: *Oryza sativa*	238 ng*	216 ng*	+
Soy: *Glycine max*	587 ng	3.1 µg*	+
Strawberry: *Fragaria ananassa*	193 ng**	nt	+
Tomato: *Solanum lycopersicum*	734 ng	nt	+
Wheat: *Triticum aestivum*	5.6 µg*	1.4 µg*	+

nt = not tested;
*samples digested with CelluACE™ XG System prior to isolation; **samples digested with CelluACE™ XG System in the presence of polyvinylpyrrolidone prior to isolation.

PLANT

DNA is routinely isolated from plants to determine the genetic strain or to screen for genetic modifications. We tested the ability of the Maxwell® 16 System to extract DNA from a range of plant leaves and seeds. Many plant samples tested gave significant quantities of amplifiable DNA using the Maxwell® 16 Tissue DNA Purification Kit without the need for preprocessing (Table 2). However, some plant samples, such as tough leaves and seeds, can be difficult to break apart. These sample types were pretreated with CelluACE™ XG System (Cat.# FF3800), a blend of cellulose and other carbohydratases that digest plant tissue.

Some plant tissues are high in phenolics, which can reduce DNA isolation efficiency and inhibit subsequent PCR. For those samples known to be high in phenolics, polyvinylpolypyrrolidone (PVPP; Sigma) was included in the CelluACE™ XG System digest step. DNA was then tested in real-time PCR using the Plexor® qPCR System. All purified DNA samples tested were successfully amplified. See sample-specific *Application Notes* for details, available at:
www.promega.com/maxwell16/appnotes.htm

BACTERIA AND FUNGI

DNA is isolated from environmental and clinical samples to test for the presence of microbes such as bacteria or fungi. Table 3 gives examples of bacteria and fungi that have been used successfully with the Maxwell® 16 Cell DNA Purification Kit[a]. For DNA isolation from bacteria, as much as 400 µl of liquid overnight cultures

Microbial DNA isolated from fecal samples can be diluted 1:100 for successful PCR analysis.

were used (up to 2 × 10^9 cells); for fungal samples, single colonies were used. To maximize the release of DNA, Gram-positive bacteria were pretreated with lysozyme (treated in 400 µl of TE buffer with 2.5 mg lysozyme for 2 hours at 37 °C) and fungi were pretreated with lyticase (single colonies were treated with 200 units of lyticase in 100 µl TE buffer for 3 hours at 37 °C).

Table 3. Genomic DNA Successfully Purified from a Variety of Bacteria and Fungi. DNA was isolated from liquid cultures using the Maxwell® 16 Cell DNA Purification Kit. The Gram-positive samples were treated with lysozyme, and the fungal samples were digested with lyticase prior to isolation.

Gram-negative bacteria	Gram-positive bacteria	Fungi
Escherichia coli	*Arthrobacter luteus*	*Candida albicans*
Proteus vulgaris	*Bacillus cereus*	*Saccharomyces cereviesiae*
Pseudomonas aeruginosa	*Bacillus subtilis*	
Salmonella typhimurium	*Enterococcus faecalis*	
Yersinia enterocolitica	*Staphylococcus aureus*	

FOOD

Food sample DNA can be purified using the Maxwell® 16 Integrated System and tested by PCR for the presence of microbial DNA or GMOs.

Isolation and detection of microbial DNA is a critical safety step in the food industry. DNA is also routinely isolated from food for detection of genetically modified organisms (GMOs). The Maxwell® 16 Tissue DNA Purification Kit was used to extract DNA from milk or cheese for subsequent PCR analysis. Milk or cheese (400 µl or ~50 mg) was added directly to well #1 of the Maxwell® 16 Tissue DNA Purification cartridge. DNA yield from milk was influenced by the quantity of somatic cells present, which varied among samples (Figure 3, Panel A). A range of cheese consistencies was tested (Figure 3, Panel A; soft, aged, hard, curds, aged frozen). All cheese samples had detectable DNA.

To examine the quality of DNA, all the samples were tested by PCR for β-casein, and all were successfully amplified (data not shown). The DNA then was used in multiplex PCR for microbial gene detection (3). Most samples were positive for *S. aureus* (23S rRNA amplimer), but only sample 9 and the positive control were positive for Toxin D (Figure 3, Panel B).

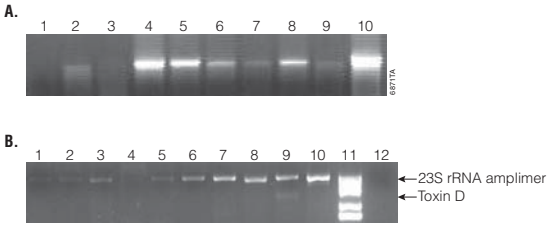

Figure 3. DNA isolated from milk and cheese can be amplified to detect bacteria and toxins. DNA was isolated using the Maxwell® 16 Tissue DNA Purification Kit without preprocessing and eluted in 400 µl. **Panel A.** Ten microliters of each eluted DNA was analyzed on a 1% agarose gel. Lanes 1–5, 400 µl of milk samples; lanes 6–9, ~50 mg or 400 µl of cheese samples; lane 10, 400 µl of milk spiked with *S. aureus*. **Panel B.** Purified DNA samples shown in Panel A were used in multiplex PCR for detection of *Staphylococcus aureus* and eventual enterotoxins (3). Sample 9 is positive for Toxin D. Lane 11, positive control (ATCC# 700699 strain Mu50, positive for the endotoxins I, G, A, C and L); lane 12, no-template control.

SUMMARY

The Maxwell® 16 System offers rapid automated low- to moderate-throughput isolation of genomic DNA from a wide range of sample types. The mechanical action of the plunger is an ideal design for breaking apart many types of solid samples including feces, plant tissues and food. For samples with tough cell walls, such as fungi or wheat leaves, enzymatic pretreatment may be necessary to maximize yield. The simple design and robust chemistries of the prefilled cartridges in the Maxwell® 16 DNA Purification Kits mean diverse solid and liquid sample types can easily be accommodated to yield amplifiable DNA.

REFERENCES

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2. Brow, M.A. *et al.* (1996) *J. Clin. Microbiol.* **34**, 3129–37.
3. Cremonesi, P. *et al.* (2005) *Mol. Cell. Probes* **19**, 299–305.

PROTOCOL

- *Maxwell® 16 DNA Purification Kits Technical Manual*, #TM284, Promega Corporation
  www.promega.com/tbs/tm284/tm284.html

ADDITIONAL RESOURCES

For additional details and other sample types tested, visit:
www.promega.com/PN97Maxwell16

ORDERING INFORMATION

Product	Size	Cat.#
Maxwell® 16 Instrument	1 each	AS2000
Maxwell® 16 Blood DNA Purification Kit	48 preps	AS1010
Maxwell® 16 Cell DNA Purification Kit	48 preps	AS1020
Maxwell® 16 Tissue DNA Purification Kit	48 preps	AS1030

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