Purification of Genomic DNA from Mouse Feces Using the Maxwell® 16 System

ABSTRACT

The Maxwell® 16 System combines compact instrumentation, optimized automated methods, prefilled reagent cartridges, service and support. The system provides time savings, enhanced productivity and improved consistency of results. We were able to amplify mouse fecal DNA purified using the Maxwell® 16 Tissue DNA Purification Kit.

Doug Wieczorek and Trista Schagat
Promega Corporation

Publication Date: July 2007

Introduction

Genomic DNA is frequently isolated from animal fecal samples to detect bacterial pathogens. Often DNA isolated from these sample types will contain significant amounts of inhibitors, such as phenolics and polysaccharides, which may prevent DNA amplification. In many cases, it may be necessary to dilute the purified DNA or treat with Chelex® resin or PVPP (polyvinylpolypyrrolydone) for successful amplification. Using the Maxwell® 16 Tissue DNA Purification Kit, bacterial DNA from mouse fecal samples was successfully isolated and the ability to amplify the purified DNA was achieved.

Methods

Genomic DNA Isolation: Fecal pellets collected from three-day old mouse bedding were stored at –20 °C until use. A single fecal pellet (average weight = 38mg) was placed directly into the Maxwell® 16 Tissue DNA Purification Kit (Cat.# AS1030) sample cartridge. Genomic DNA was purified using the Maxwell® 16 Instrument (Cat.# AS1000; firmware version 3.2, *NOTE:* Cat.# AS2000 now replaces Cat.# AS1000) as described in the *Maxwell® 16 DNA Purification Kits Technical Manual* #TM284. For some experiments, the eluted DNA was transferred to a prepared Zymo-Spin™ IV-HRC Spin Filter (Zymo Research Cat.# C1010-50) and centrifuged at 8,000 × *g* for 1 minute per the manufacturer's instructions. DNA yield and purity were determined using the NanoDrop® spectrophotometer.

PCR Analysis: PCR was performed on aliquots of DNA from a minimum of six separate purifications using conserved eubacterial 16S ribosomal RNA primers (1) . *E. coli* JM109 genomic DNA [isolated from 0.4ml overnight culture using the Maxwell® 16 Cell DNA Purification Kit (Cat.# AS1020) was used as a positive control. One microliter of eluted DNA or DNA diluted 1:100 in water was used per 50µl amplification reaction.

Results

Using single fecal pellets, we obtained an average of 48µg DNA per sample (n = 8). The DNA had an average concentration of 308ng/µl with a 260nm/280nm absorbance purity ratio of 1.62.

As seen in Figure 1, the Maxwell® 16 Tissue DNA Purification Kit consistently isolated high-molecular-weight DNA from the fecal pellets. The mechanical action of the Maxwell® 16 Instrument plungers was sufficient to break apart the samples with minimal DNA shearing; so the conventional preprocessing method of bead beating was not necessary.

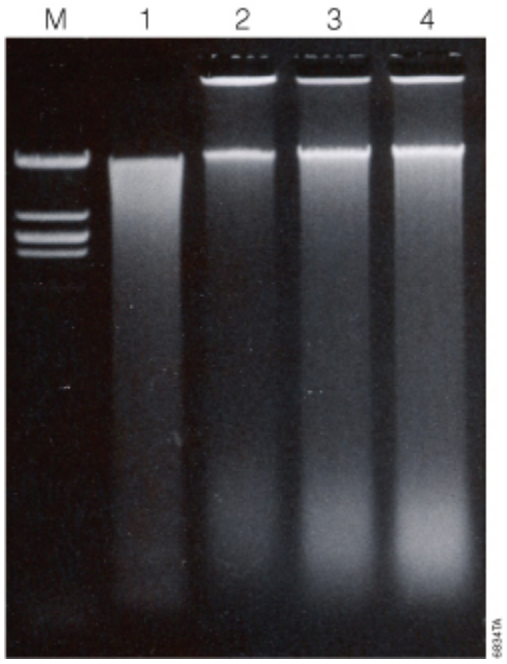

Figure 1. Genomic DNA isolated from mouse feces. Each lane represents 5µl of purified genomic DNA. Lane M, Lambda DNA/EcoRI Markers (Cat.# G1721); lane 1, DNA isolated by a fecal DNA extraction kit using bead beating; lanes 2–4, DNA isolated using Maxwell® 16 Tissue DNA Purification Kit with no preprocessing.

The ability to detect microbial flora in the fecal DNA was then tested using conserved eubacterial 16S ribosomal RNA primers (1; Figure 2). One out of 8 samples gave amplifiable product without dilution or further treatment of the purified DNA. When the DNA was diluted 1:100 in water prior to amplification, we were able to amplify DNA from all samples.

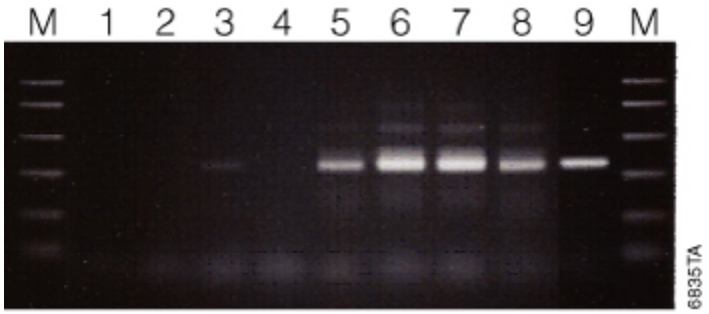

Figure 2. Amplification of eubacterial 16S rRNA from fecal DNA extracted using the Maxwell® 16 Tissue DNA Purification Kit. Each lane represents 10% of the 50µl amplification reaction containing 1µl of template. The amplicon size is 350 bp. Lane M, BenchTop PCR Markers (Cat.# G7531); lane 1, no template 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.

To improve amplification from undiluted DNA, we also tested a subsequent treatment of the Maxwell® 16 purified DNA that removes PCR inhibitors. After pipetting the eluate onto a Zymo-Spin™ IV-HRC Spin Filter (filled with crosslinked polyvinylpyrrolidone) and centrifuging for 1 minute, the DNA could be used directly in PCR (Figure 2, lane 8; example is representative of six replicates).

Conclusion

The Maxwell® 16 System, which includes the Maxwell® 16 Tissue DNA Purification Kit and the Maxwell® 16 Instrument, can be used to isolate genomic DNA from mouse fecal pellets without any initial preprocessing steps. For subsequent PCR amplification, dilution of the purified DNA or removal of inhibitors over a Zymo-Spin™ IV-HRC Spin Filter is recommended for greatest success. To learn more about the Maxwell® 16 System, visit www.promega.com/maxwell16.

REFERENCES

1. Brown, M.A. *et al.* (1996) Differentiation of bacterial 16S rRNA genes and intergenic regions and *Mycobacterium tuberculosis* katG genes by structure-specific endonuclease cleavage. *J. Clin. Microbiol.* **34**, 3129–37.

HOW TO CITE THIS ARTICLE

Scientific Style and Format, 7th edition, 2006

Wierczorek, D. and Schagat, T. Purification of Genomic DNA from Mouse Feces Using the Maxwell® 16 System. [Internet] July 2007 . [cited: year, month, date]. Available from: http://www.promega.com/resources/pubhub/enotes/purification-of-genomic-dna-from-mouse-feces-using-the-maxwell-16-system/

American Medical Association, Manual of Style, 10th edition, 2007

Wierczorek, D. and Schagat, T. Purification of Genomic DNA from Mouse Feces Using the Maxwell® 16 System. Promega Corporation Web site. http://www.promega.com/resources/pubhub/enotes/purification-of-genomic-dna-from-mouse-feces-using-the-maxwell-16-system/ Updated July 2007 . Accessed Month Day, Year.