

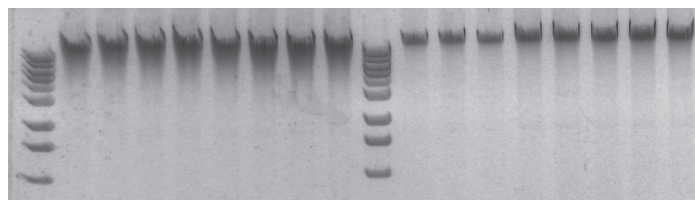
Automation of the ZymoBIOMICS™-96 MagBead DNA Kit

High-throughput, magnetic bead-based purification of DNA, directly from any environmental sample or biological fluid for unbiased analysis of microbial communities on the MicroLab® STAR™.

Features and Highlights

High-throughput, magnetic bead-based purification

of high-quality total DNA directly from microbial communities in feces, soil, water, biofilms, swabs, saliva, body fluids, etc.



ZymoBIOMICS

Supplier A

Figure 1: The ZymoBIOMICS-96 MagBead DNA Kit provides superior yields when compared to Supplier A. 80 mg of feces was processed according to each manufacturer's recommended protocol. 6 µl of each sample was analyzed in a 1.0% (w/v) agarose/ethidium bromide gel.

Built-in inhibitor removal technology allows for streamlined purification without the need for precipitation or centrifugation steps.

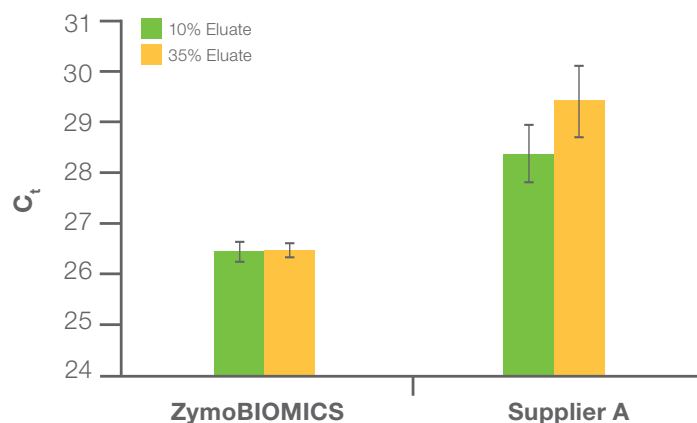


Figure 3: The ZymoBIOMICS-96 MagBead DNA Kit provides inhibitor-free DNA using real-time PCR. Reaction volumes consisted of either 10% or 35% of the eluate to detect the presence of PCR inhibitors. Each reaction contained 10 ng of *Brettanomyces* DNA. Delayed amplification indicates PCR inhibition from inefficient inhibitor removal (n=8).

Linear Recovery with unparalleled sensitivity

of DNA isolated from trace or dilute sample sources.

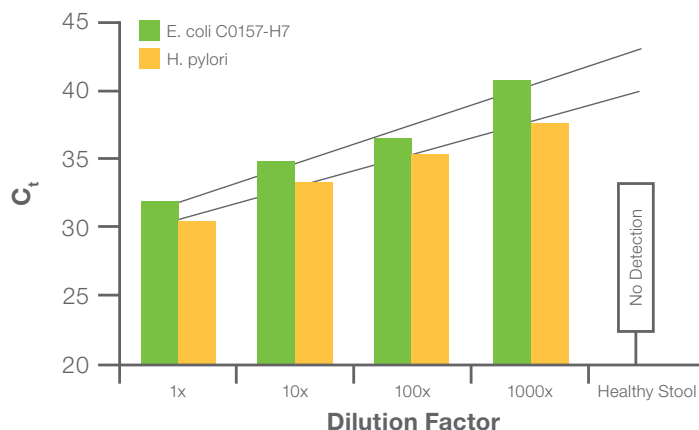


Figure 2: The ZymoBIOMICS-96 MagBead DNA Kit produces linear recovery of DNA for sensitive applications. A dilution series was created using stool infected with 1×10^9 Shiga toxin-producing *E. coli* cells and stool infected with *H. pylori* cells and shows effective purification and qPCR amplification even at 1000:1 dilution (n=8).

Non-biased recovery of DNA for accurate microbial community profiling using patented ZR BashingBead™ technology.

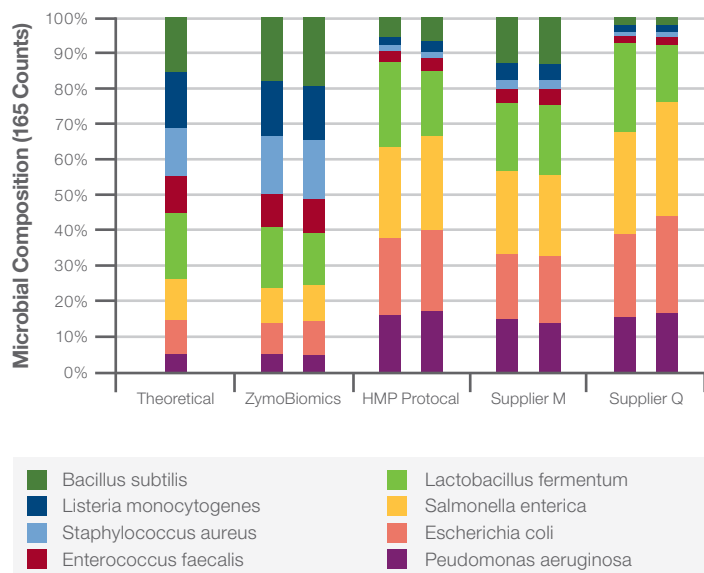


Figure 4: The ZymoBIOMICS system provides an unbiased representation of the organisms extracted from the ZymoBIOMICS Microbial Community Standard. DNA was extracted from ZymoBIOMICS Microbial Community Standard using four different DNA extraction methods (ZymoBIOMICS, Human Microbiome Project Protocol, Supplier M, and Supplier Q) and analyzed using 16S rRNA gene sequencing. 16S rRNA genes were amplified with primers targeting v3-4 region and the amplicons were sequenced on Illumina MiSeq (2x250bp). Overlapping paired-end reads were assembled into complete amplicon sequences. The composition profile was determined based on sequence counts after mapping amplicon sequences to the known 16S rRNA genes of the eight different bacterial species.

Introduction

The ZymoBIOMICS-96 MagBead DNA Kit (Zymo Research Corporation, Irvine, CA) is designed for purifying DNA from a wide array of sample inputs (e.g. feces, soil, water, and biofilms) that are immediately ready for microbiome or metagenome analyses. The ZymoBIOMICS innovative lysis system eliminates bias associated with unequal lysis efficiencies of different organisms (e.g. Gram negative/positive bacteria, fungi, protozoans, and algae), making it ideal for microbiomics studies. Unbiased mechanical lysis of tough microbes is achieved by bead beating with Zymo Research's proprietary, ultra-high density BashingBeads and validated using the ZymoBIOMICS Microbial Community Standard. The automation friendly workflow integrates the PCR inhibitor removal technology directly into the purification system, removing the need for complex precipitation steps commonly used in other methodologies. The ZymoBIOMICS-96 MagBead DNA Kit allows for a simple bind, wash, elute procedure that is unmatched in providing ultra-pure DNA that is free of PCR inhibitors



(e.g. polyphenols, humic acids) in as little as 90 minutes for 96 samples, making it ideal for all downstream applications including PCR, arrays, 16s rRNA gene sequencing, and shotgun sequencing.

Materials and Methods

The Microlab STAR used was configured with 8 x 5 mL independent pipetting channels, autoloader, CO-RE® 96 Multi-Probe Head (MPH), CO-RE Grippers, Hamilton Heater Shaker (HHS), Zymo magnetic rack, as well as required tips and reagent carriers.

The DNA concentration was analyzed using Thermo Scientific NanoDrop® 2000 UV-Vis Spectrophotometer and gel electrophoresis using a 1% agarose gel.

Seventy-two samples of various origins detailed in Table 1 (below) were homogenized using a ZR BashingBead Lysis Rack placed on an MP-Biomedicals FastPrep-96 bead mill. DNA was then extracted from the samples with ZymoBIOMICS-96 MagBead DNA Kit (Cat# D4302) using the extraction workflow shown in Figure 5 (next page). All samples were processed using the automated Hamilton Microlab STAR liquid handler. In a separate plate, ninety-six samples with 20 mg feces each were processed on the Hamilton Microlab STAR in tandem with sixteen manually processed samples with same input amount.

Table 1: Sample Input Types and Amounts Used for Validation of the ZymoBIOMICS-96 MagBead DNA Kit Workflow, n=8 per Sample Type

| Sample Type | Input Amount |
|--------------------------------|--|
| Soil | 200 mg |
| Fecal | 80 mg |
| Blood | 200 µl |
| Listeria Monocytogenes Culture | 2x10 ⁸ Cells |
| Saccharomyces Cerevisiae | 2x10 ⁷ Cells |
| Plant | 50 mg |
| Filtered Water | 200 µl Stream Water w/ 2x10 ⁶ E. coli cells added |
| Saliva | 200 µl |

Automation Equipment

- Microlab STAR with 8 independent pipette channels, autoloader, CO-RE 96 MPH, and CO-RE Gripper
- Hamilton Heater Shaker (HHS)
- 96-well magnetic stand
- All required tip and reagent carriers



Figure 5: Workflow for the ZymoBIOMICS-96 MagBead DNA Kit

Results and Discussion

Consistent Yields and High Quality

DNA concentration and total DNA yields from replicate samples were compared between 8 samples processed on the Hamilton Microlab STAR of a variety of sample types. Results indicate that the kit is capable of purifying DNA from a variety of sample types reliably and consistently (Figure 6).

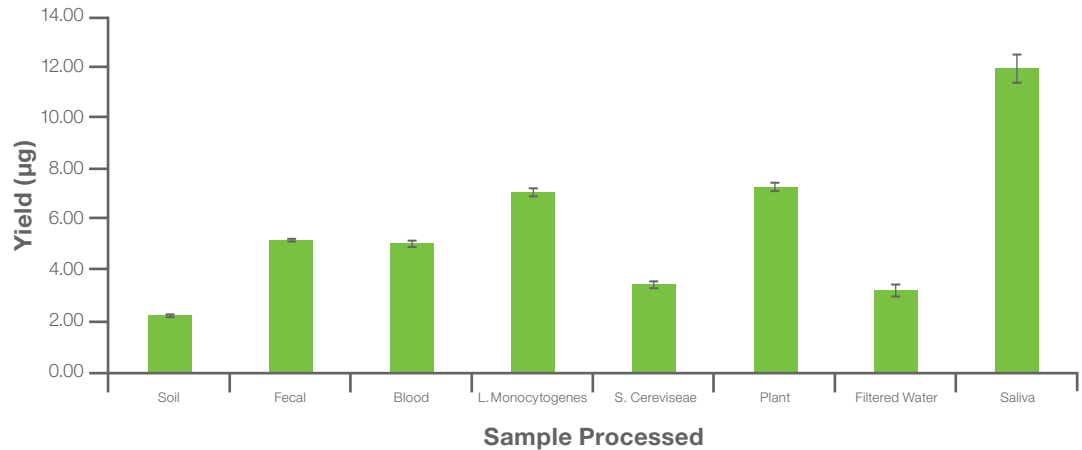


Figure 6: DNA yield recovered after processing each replicate sample type on the Hamilton Microlab STAR liquid handling system with the ZymoBIOMICS-96 MagBead DNA Kit.

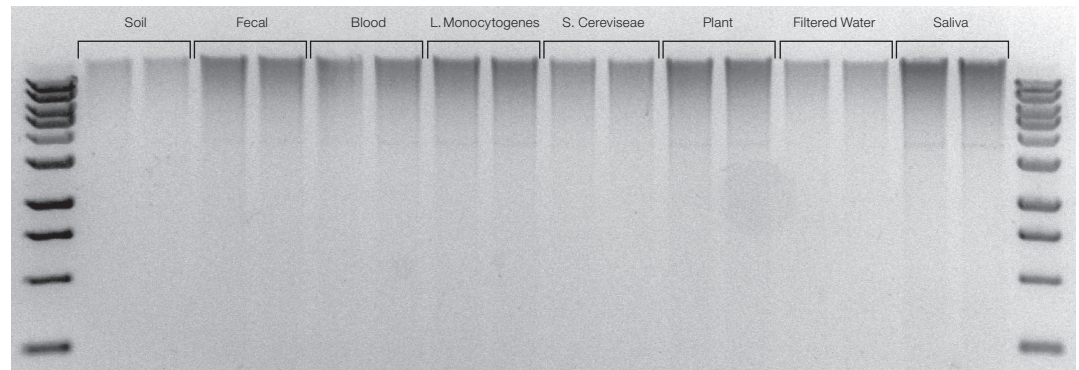


Figure 7: Agarose gel electrophoresis images recovered after processing samples on the Hamilton Microlab STAR liquid handling system with the ZymoBIOMICS-96 MagBead DNA Kit. Samples include soil; fecal; blood; L. Monocytogenes; S. Cerevisiae; Plant; Filtered Water; Saliva.

Conclusions

Samples processed using the ZymoBIOMICS-96 MagBead DNA Kit procedure with the Hamilton Microlab STAR are capable of being purified with consistency and reliability. This is shown by the successful recovery and excellent reproducibility and consistency in concentration and yield. This innovative method yields high-quality total DNA from microbial communities from a wide array of sample sources providing an efficient solution for reliable high-throughput hands-free DNA purification.





Table 2: Zymo Products

| Product Description | Catalog No. | Kit Size |
|--|-------------|---------------|
| ZymoBIOMICS-96 MagBead DNA Kit (Includes ZR BashingBead Lysis Rack) | D4302 | 2 x 96 preps. |
| ZymoBIOMICS-96 MagBead DNA Kit (Lysis Matrix Not Included) | D4306 | 2 x 96 preps. |
| ZymoBIOMICS-96 MagBead DNA Kit (Includes ZR BashingBead Lysis Tubes) | D4308 | 2 x 96 preps. |

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 Lit. No. L50164 v1.0 — 04/2017



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