ZYMO RESEARCH

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Validated High-throughput Microbiome Extraction:
Demonstrated on Human Fecal Samples & Skin Swabs

ABSTRACT

The rapid growth of microbiomics necessitates a high-throughput solution for the extraction of microbiome-grade DNA from various samples that is unbiased and PCR inhibitor-free. The ZymoBIOMICS® 96 Magbead DNA Kit chemistry was incorporated onto the DreamPrep NAP in a collaboration between Zymo Research and Tecan. The workflow was evaluated with human fecal samples and skin swabs to evaluate the DNA yield and purity. This was done in the context of next-generation sequencing applications as demonstrated with 16s rRNA gene targeted sequencing. The workflow was also analyzed for cross-contamination between samples. The plug-and-play solution delivered consistent and reproducible DNA recovery and purity that was unbiased when sequenced. This system provides a complete walk-away extraction for microbiome-grade DNA.

INTRODUCTION

The microbiomics field has grown exponentially in recent years, but the reproducibility and standardization of data remains a problem. Obtaining complete lysis of all types of microbes in a sample presents a challenge, and can introduce extraction bias by underrepresenting species that are hard to lyse, such as Gram-positive bacteria. To accommodate larger throughputs and reduce any bias associated with manual processing, microbiomics workflows require high throughput, unbiased and PCR inhibitor-free solutions for the extraction of sequencing-grade DNA from various sample types.

The DreamPrep NAP workstation featuring Zymo Research offers an automated extraction workflow that eliminates bias and produces DNA that is ready for microbiomic or metagenomic analysis. It combines the capabilities of the Fluent® Automation Workstation and the ZymoBIOMICS® 96 MagBead DNA Kit to generate high purity DNA from a wide variety of sample types, including feces, soil, water and biofilms.

This application note describes an automated workflow delivering ultra-pure, inhibitor-free, sequencing-grade DNA ready for downstream applications – including PCR, arrays, targeted and shotgun sequencing – in just 90 minutes. DNA was extracted from human fecal and skin swab samples, and analyzed using 16S rRNA gene targeted sequencing on a MiSeq® System (Illumina).

MATERIALS AND METHODS

The microbiome extraction workflow was automated on the DreamPrep NAP workstation featuring Zymo Research, a system based on the Fluent 480 Automation Workstation in combination with FluentControl™ GX Assurance Software. The system is configured for nucleic acid extraction workflows using magnetic bead-based procedures. An integrated Infinite® 200 PRO reader in M Nano+ configuration allows quantification and normalization following nucleic acid extraction.

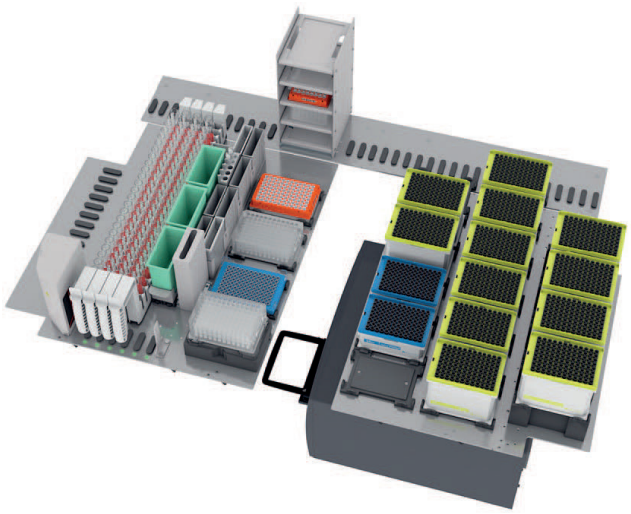

Figure 1: Configuration of the DreamPrep NAP featuring Zymo Research worktable.

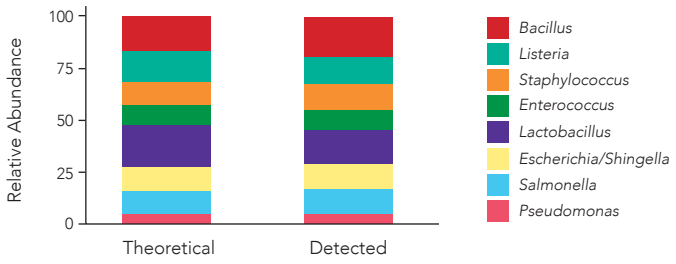

Figure 2: No Purification Bias Introduced. The ZymoBIOMICS® Microbial Community Standard was purified using the high-throughput, automated workflow. Duplicate samples of purified DNA were analyzed using 16S rRNA gene targeted sequencing using primers targeting the V3-V4 region. The resulting amplicons were sequenced on the Illumina MiSeq®. Displayed are the relative abundance at genus-level resolution. The composition of the ZymoBIOMICS® Microbial Standard was compared to the theoretical composition per the manufacturer's specifications.

The DreamPrep NAP workstation featuring Zymo Research is equipped with an Air Flexible Channel Arm™ (Air FCA), a Robotic Gripper Arm™ (RGA), Fluent ID™ and handheld (Honeywell) barcode scanners for sample and reagent identification, a BioShake™ D30-T elm (QInstruments) for heating and shaking, and a Magnum FLX® Enhanced Universal Magnet Plate (Alpaqua).

The automated workflow was tested using the following general protocol. Samples were homogenized using ZR BashingBead Lysis tubes, mixed on a Vortex-Genie® 2 (Scientific Industries) at maximum speed for 40 minutes. The homogenized samples were added to a 2 ml Nunc™ 96-Well DeepWell™ Plate (Cat. No. 278743) and processed using the ZymoBIOMICS® 96 MagBead DNA Kit workflow script in FluentControl. DNA was eluted in 50 µl of

DNA Elution Buffer and analyzed using a NanoDrop™ 2000 UV-Vis Spectrophotometer (ThermoFisher Scientific), followed by qPCR with the Femto™ Bacterial DNA Quantification Kit on a CFX96 Touch™ Real-Time PCR Detection System (BioRad Laboratories). The resultant DNA was analyzed using 16s rRNA gene targeted sequencing with primers targeting the V3-V4 region, sequencing the amplicons on a MiSeq System.

RESULTS AND DATA ANALYSIS

Evaluation of lysis bias using the ZymoBIOMICS® Mock Microbial Community Standard

The automated extraction system was evaluated using the ZymoBIOMICS® Mock Microbial Community Standard (Figure 2, n = 4), showing no purification bias was introduced.

Evaluation of human fecal samples

Frozen human fecal samples (50 mg) from nine individual donors were used to evaluate the robustness and reproducibility of the workflow (n = 8/donor). The DNA concentration and purity absorbance ratios (A260/230, A260/280) were determined using a NanoDrop 2000 UV-Vis Spectrophotometer, showing consistent recovery rates and high purity (Figure 3).

The extraction and quantification process was repeated for quality control (n = 16/donor total), showing very consistent recovery across the nine donors (Figure 4A), and a subset of samples was processed manually using the ZymoBIOMICS® 96 MagBead DNA Kit, showing comparable results to automated extraction (Figure 4B/C).

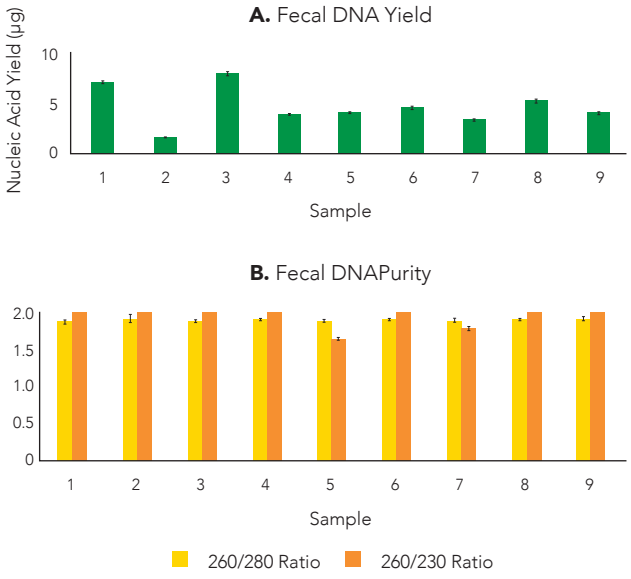

Figure 3: Consistent Sample Processing. DNA was purified from 50 mg human feces (n=16) from 9 individual donors. Samples were processed using the ZymoBIOMICS® 96 Magbead DNA Kit workflow scripted on the DreamPrep NAP. (A) Total DNA recovery across the 9 donors was very consistent (B) Purity absorbance ratios were consistently high (A260/230; A260/280: >1.8). Absorbance A260/230, and total DNA recovery (µg) were quantified by NanoDrop™ 2000.

Fecal sample inhibitor tests and ZymoBIOMICS® Mock Microbial Community Standard

Eluates from nine different fecal donors and the ZymoBIOMICS® Mock Microbial Standard were tested for the presence of inhibitors via qPCR on the CFX96 Touch Real-Time PCR Detection System. Inhibition was tested by spiking 10 % of the eluate from each sample into a qPCR reaction amplifying 25 ng of Brettanomyces DNA. Delayed and/or no amplification compared to the positive control would indicate inefficient inhibitor removal. None of the samples delayed the amplification, indicating effective removal of PCR inhibitors (Figure 5).

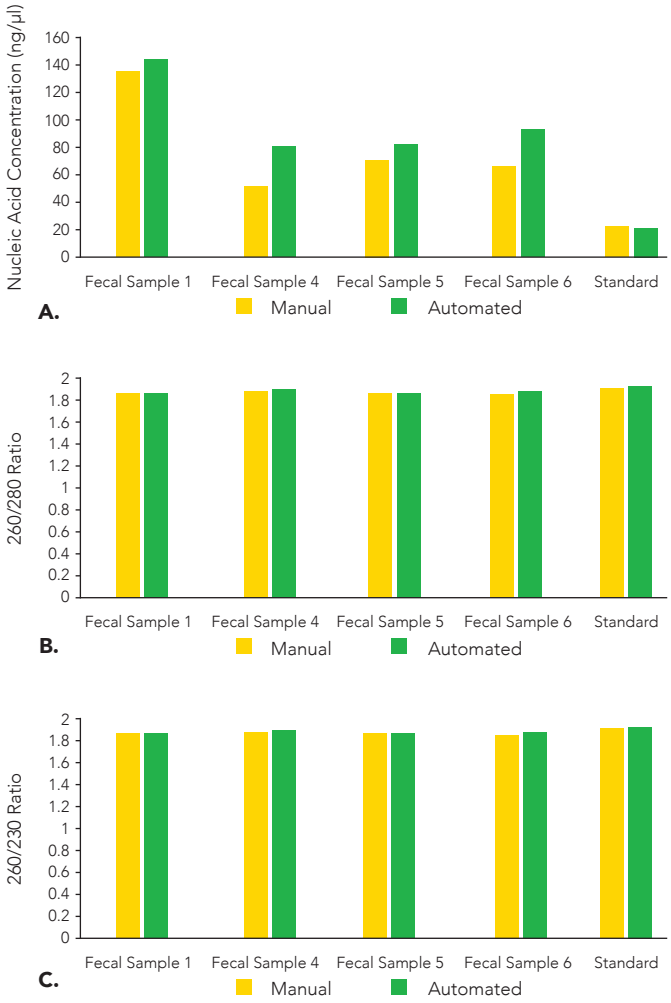

Figure 4: Comparison with Manual Extraction. DNA was purified from 50 mg human feces from 9 individual donors using the ZymoBIOMICS® 96 Magbead DNA Kit either manually or using the scripted workflow on the DreamPrep NAP. Absorbance A260/280, A260/230, and total DNA recovery (µg) were quantified by NanoDrop™ 2000. (A) Total DNA recovery across the 9 donors was very consistent. Purity absorbance ratios were consistently high for (B) A260/230 ratios and (C) A260/280 ratios with both above 1.8.

Low biomass detection

DNA from human skin swabs was processed to evaluate the system for use with low biomass samples. Samples were collected from two individual donors using DNA/RNA Shield™ Collection tubes with swabs (n = 4). Following processing, the eluates were quantified by real-time PCR, using the Femto Bacterial DNA Quantification Kit on the CFX96 Touch Real-Time PCR Detection System (Figure 6).

Multiple sample testing

Purified human skin and feces DNA samples from two donors (n = 4/donor) were analyzed using 16S rRNA gene targeted sequencing with primers targeting the V3-V4 region. The resulting amplicons were sequenced on a MiSeq System (Figure 7).

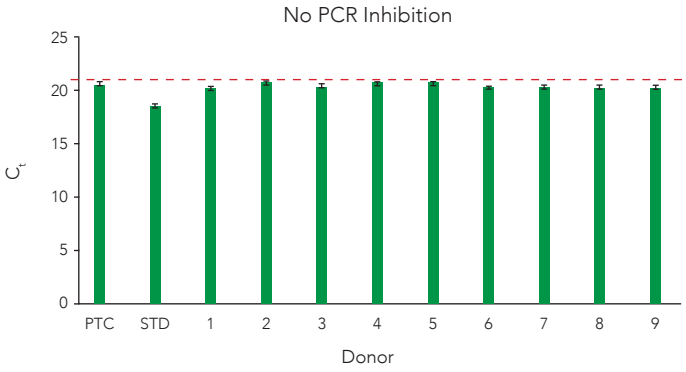

Figure 5: No PCR Inhibition. Real-time PCR was used to evaluate eluates from 9 different fecal donors and the ZymoBIOMICS® Mock Microbial Standard (STD) recovered using the ZymoBIOMICS® 96 MagBead DNA Kit workflow scripted on the DreamPrep NAP. A reaction of 25 ng of Brettanomyces DNA was used as a positive template control (PTC). This reaction was repeated with 25 ng of *Brettanomyces* DNA and 10% of eluates from each fecal donor spiked into each reaction. This was performed to detect the presence of PCR inhibitors. Delayed and/or no amplification compared to the positive control indicates PCR inhibition from inefficient inhibitor removal. No template control resulted in no amplification. None of the samples delayed the amplification indicating that there was no PCR inhibition.

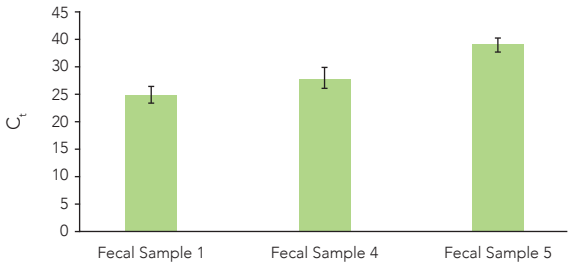

Figure 6: Low Biomass Detection. DNA was purified from skin swabs collected using the DNA/RNA Shield™ Collection tubes with swabs (n=4) from 2 separate donors using the ZymoBIOMICS® 96 Magbead DNA Kit scripted on the DreamPrep NAP. Real-time PCR was used to quantify these eluates with the Femto Bacterial DNA Quantification Kit. Shown here are the detection for these low biomass samples.

Cross-contamination

Cross-contamination between wells was analyzed by adding *Cryptococcus neoformans* and ZymoBIOMICS® DNase/RNase-Free Water to every other well across a 96-well plate in a checkerboard pattern. The plate was then processed, and eluates from each well were amplified by qPCR using the Femto Bacterial DNA Quantification Kit on the CFX96 Touch Real-Time PCR Detection System.

The plate was analyzed to check for the presence of C. neoformans DNA in the water-filled control wells, demonstrating that no cross-contamination occurred (Figure 8).

SUMMARY

The DreamPrep NAP workstation featuring Zymo Research offers complete walkaway extraction of high quality, sequencing-grade DNA from a wide variety of microbiome sample types, providing

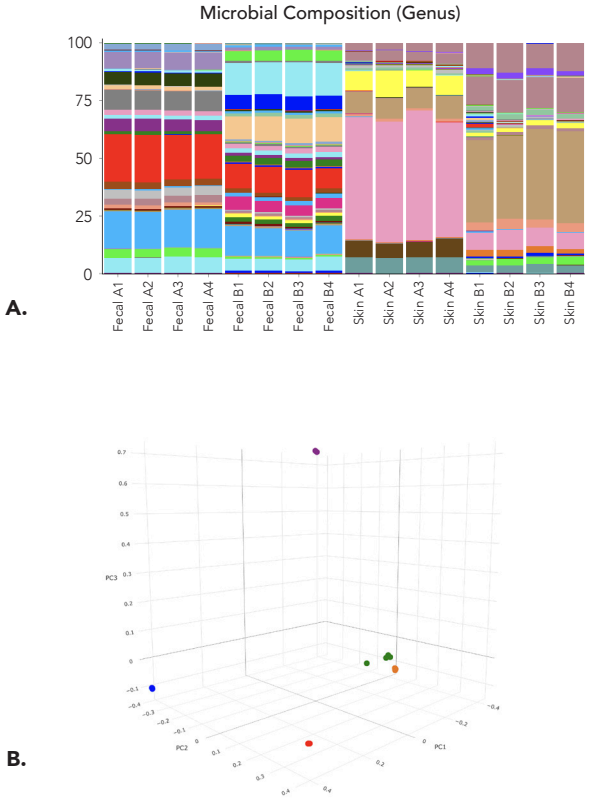

Figure 7: Similar Microbial Profiles Across Samples. DNA from various sample types was purified using the ZymoBIOMICS®-96 Magbead DNA Kit scripted on the DreamPrep NAP: 50 mg human feces (N=4) from 2 separate donors, skin swabs collected using the DNA/RNA Shield™ Collection tubes with swabs (n=4) from 2 separate donors. These DNA samples were then analyzed using 16S rRNA gene targeted sequencing with primers targeting the V3-V4 region. The resulting amplicons were sequenced on the Illumina MiSeq®. (A) Displayed are the relative abundance at genus-level resolution. (B) Displayed are the beta diversity plot matrix of paired-wise distance between samples calculated by the Bray-Curtis dissimilarity. Each dot on the figure represents the whole microbial composition profile. Samples with similar microbial composition profiles are closer to each other.

an effective solution for reliable, high throughput and hands-free DNA purification. This plug-and-play solution delivers unbiased, consistent and reproducible DNA yields and purities, with no cross-contamination observed.

The data presented in this application note demonstrates the successful recovery, excellent reproducibility and consistency between sample preparations when evaluating the yield, concentration, and purity of the extracted DNA.

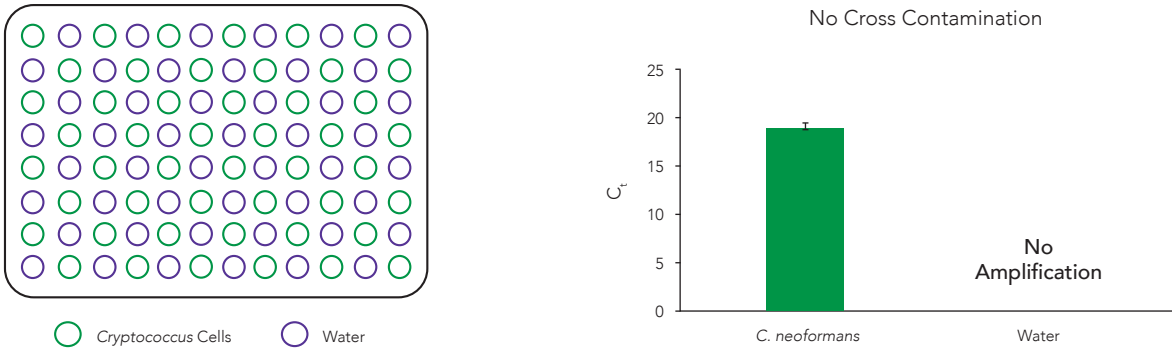

Figure 8: No Cross Contamination. *Cryptococcus neoformans* and DNase/RNase free water was added to every other well across a 96 well plate in a checkerboard pattern. All 96 wells were extracted using the ZymoBIOMICS® 96 Magbead DNA Kit scripted on the DreamPrep NAP. Eluates from each well were amplified via qPCR using the Femto DNA Quantification kit. Wells containing both *C. neoformans* and water were analyzed to check for cross contamination of *C. neoformans* into the wells filled with water.

About the Authors

Shaun Veran is an Application Specialist at Zymo Research with a degree in Microbiology, Immunology, and Molecular Genetics from the University of California - Los Angeles. He specializes in high-throughput DNA automation, research and design, and product development.

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Acknowledgments
This protocol was developed by in collaboration with Tecan (www.tecan.com) and is intended for research use only. Users are responsible for determining the suitability of the protocol for their application. For further information, visit www.zymoresearch.com.