# Automated microbiome extraction: bias-free, high-throughput, quality results

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## ABSTRACT

Microbiomics is a burgeoning field with implications for a wide range of topics in human health. Modern sequencing techniques can deliver rich and novel data but are constrained by DNA extraction methods that are difficult, time consuming, and, especially in microbiomics samples, prone to bias. Automated systems can resolve some of these challenges but must be designed and calibrated with care and need to be paired with appropriate reagents to deliver quality results that are free of bias. In the present set of tests, the Opentrons OT-2 Nucleic Acid Extraction Workstation was used along with the ZymoBIOMICS™ 96 Magbead DNA Kit to extract DNA from typical microbiomics samples. Performance of this system and kit was assessed with yield and purity metrics. Extraction bias and cross-contamination were also assessed. Results indicated that the OT-2 together with the ZymoBIOMICS kit produced high-yield and high-purity DNA samples free of bias and cross contamination. Several adjustments were identified to further improve performance. Together, these tests exemplify the OT-2's accuracy and precision during implementation of diverse applications, delivering optimal performance with an industry leading microbiomics DNA extraction kit.

## INTRODUCTION

The human microbiome no longer stands in the background of biological research. Diverse, large, and active, the microbiome has moved into the spotlight, and it is increasingly appreciated for its broad role in human health, including infectious disease, circadian rhythms, and psychological conditions. Automation can support this burgeoning field with bias-free performance at the critical nucleic acid extraction step.

Quality DNA extraction relies on effective lysis, which is especially challenging with microbiome samples given the diverse organisms they contain. Some organisms are hardier and more resistant to lysis, while others are more susceptible and easier to lyse. If a lysis method fails to overcome these differences, hardy species will yield less DNA, while more susceptible species will yield more, leading to biased representation in down-stream analysis.

Two major lysis methods are commonly used: enzymatic or reagent-based lysis, and mechanical lysis. This application note used mechanical lysis performed with a bead-based technique in the ZymoBIOMICS 96 Magbead DNA Kit. The ZymoBIOMICS kit uses ultra-high density BashingBeads™ to deliver uniform lysis of microbial species and works well with automation, as the beads are preloaded in sample-ready tubes.

Automation offers further improvement to DNA extraction. Aside from lysis challenges, manual DNA extraction protocols are time consuming and prone to variability caused by errors and differences between lab technician performance. Automated procedures can eliminate variability and increase throughput but must be calibrated with care to ensure that DNA is isolated with good yield and purity.

The present study sought to assess the performance of the OT-2 Nucleic Acid Extraction Automated Workstation in concert with the ZymoBIOMICS 96 MagBead DNA Kit for microbiomics. Yield and purity were assessed in typical microbiomics fecal samples using standard extraction performance metrics. These metrics were compared between the automated workstation and manual procedures performed by highly trained technicians. The automated workstation was also tested for cross-contamination between plate wells, and several

tests were conducted to optimize the protocol for this kit and system.

The OT-2 avoided extraction bias and delivered samples with yield and purity comparable to manual procedures performed by highly trained technicians. Cross-well contamination was also comparable between the two extraction methods. Further, several protocol adjustments were identified to improve yield and reduce turnaround time using the OT-2 automated workflow.

## RESULTS

### Automated DNA extraction with the OT-2 Workstation and ZymoBIOMICS MagBead Kit successfully purified DNA without introducing bias

Purification bias can result from uneven lysis among microbiome species in a sample. Some species are less amenable to lysis and thus can be underrepresented in downstream sequencing data. The BashingBead technology used in the ZymoBIOMICS kit is a system used to perform mechanical lysis via the bead mill or bead beating technique. Such techniques involve combining samples with small glass, steel, or ceramic beads and mixing the two vigorously.[1] During mixing, the beads collide against cells and break cell membranes and walls, releasing DNA. BashingBeads reagents use ultra high-density beads to uniformly lyse microbiomics samples in this way, preventing bias.

To assess this performance, DNA extraction with the OT-2 workstation and the ZymoBIOMICS 96 MagBead DNA Kit was conducted on samples from the ZymoBIOMICS Microbial Community Standard (N=8). Extracted DNA was then analyzed with 16S rRNA gene targeted sequencing using primers targeting the V3-V4 region followed by sequencing on the Illumina® MiSeq™ instrument. The microbial community standard is a well-characterized reference sample with species including Gram-positive and Gram-negative bacteria as well as yeasts of varying sizes and cell wall compositions. The observed composition generated by biased lysis methods would not reflect the theoretical composition of the standard. Sequencing data revealed that automated extraction with the OT-2 Nucleic Acid Extraction Workstation and the ZymoBIOMICS 96 MagBead DNA Kit delivered and observed microbial profile that matched the microbial community standard with high fidelity **(Figure 1)**.

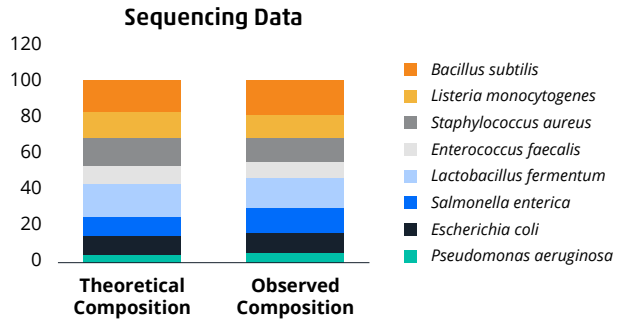


**Figure 1: Automated extraction did not introduce purification bias.** Observed composition of extracted DNA compared to the theoretical composition of the ZymoBIOMICS Microbial Community Standard shown in relative abundance of sequenced species at species level. The community standard sample was analyzed with 16S rRNA targeted sequencing using primers targeting the V3-V4 region followed by sequencing on the Illumina MiSeq sequencer. Observed composition of the extracted sample closely matched the theoretical composition of the known standard.

### Kit automation delivered yield and purity matching manual extraction

Performance of the OT-2 Nucleic Acid Extraction Workstation with the ZymoBIOMICS 96 MagBead DNA Kit in DNA extraction from typical microbiomics fecal samples was assessed for DNA yield and purity and compared to manual procedures performed by a highly trained technician (N=12). A NanoDrop 2000 UV-Vis Spectrophotometer was used to measure DNA concentration and absorbance ratios (A260/230, A260/280).

Manual and automated procedures delivered near identical results **(Figure 2)**. Automated and manual procedures delivered an average yield of 57.88 ng/µL and 57.85 ng/µL, with average 260/280 absorbance values of 1.94 and 1.90, and average 260/230 absorbance values of 1.85 and 1.87, respectively.

### Automated procedures avoided cross-contamination

Cross-contamination between plate wells presents a significant problem for DNA extraction procedures, as contaminated samples compromise data integrity. However, contamination can be hard to prevent with manual procedures. In contrast, automation can reduce contamination and facilitate auditing procedures that will identify contamination if it does occur. To assess the OT-2 Nucleic Acid Extraction Workstation for cross-contamination, *Cryptococcus neoformans* and ZymoBIOMICS DNase/RNAse-Free Water were added to a

96-well plate in an alternating, checkerboard fashion. The plate was than processed with the automated workstation and eluates from each well were tested via qPCR with the Femto™ Bacterial DNA Quantification Kit on the CFX96 Touch Real-Time PCR Detection System. No *C. neoformans* was detected in any of the water-filled control wells, indicating that cross contamination had not occurred **(Figure 3)**.

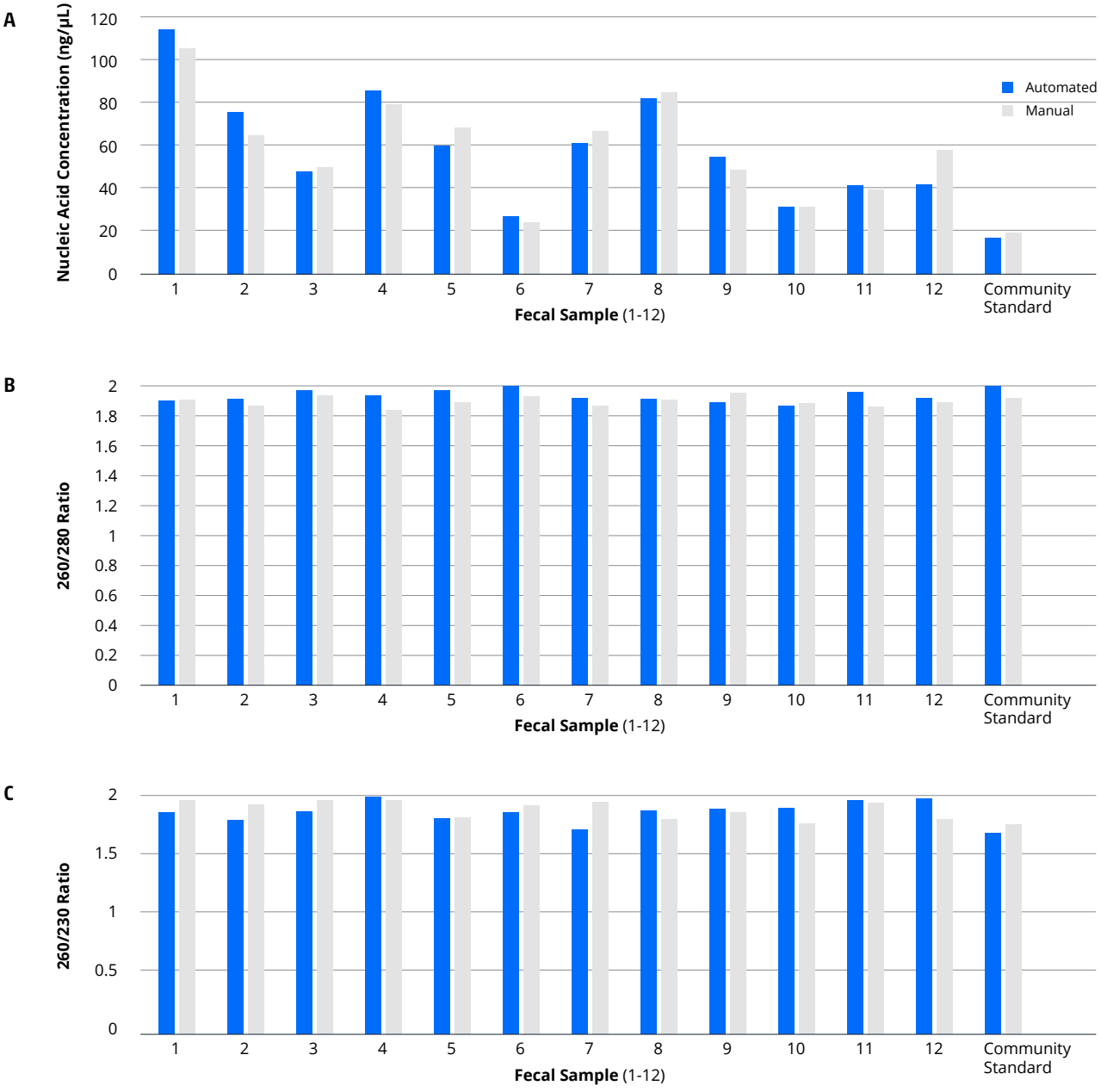


**Figure 2: Automation and manual procedures delivered comparable DNA yield and purity.** DNA from fecal samples was extracted with the OT-2 Nucleic Acid Extraction Workstation and the ZymoBIOMICS 96 MagBead DNA kit. Purified DNA was assessed for yield and purity in terms of ng/µL, and 260/280 and 260/230 absorbance values using a NanoDrop 2000 UV-Vis Spectrophotometer. Near identical results were obtained in terms of yield (A), and purity (B and C).

**Cross Contamination**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | 11.28 | 39.62 | 11.47 | >45 | 11.45 | >45 | 11.30 | 42.40 | 11.33 | >45 | 11.47 | >45 |
| **B** | 40.05 | 11.71 | >45 | 11.42 | 38.66 | 11.32 | >45 | 11.28 | 39.04 | 11.36 | >45 | 12.02 |
| **C** | 11.28 | 39.78 | 11.07 | >45 | 11.13 | 39.84 | 11.10 | 39.04 | 10.75 | >45 | 10.55 | >45 |
| **D** | 41.02 | 11.56 | 40.80 | 11.34 | >45 | 10.91 | >45 | 11.27 | 38.33 | 11.43 | >45 | 11.29 |
| **E** | 11.37 | 39.49 | 11.53 | 40.52 | 11.37 | 40.54 | 11.32 | 39.49 | 11.19 | >45 | 10.62 | 40.75 |
| **F** | 40.07 | 11.74 | >45 | 11.68 | >45 | 11.23 | 38.74 | 11.40 | 37.52 | 11.33 | 38.61 | 11.34 |
| **G** | 11.56 | 38.70 | 11.46 | 39.69 | 11.35 | 40.00 | 11.25 | 39.29 | 11.32 | 40.17 | 11.49 | 38.66 |
| **H** | >45 | 11.77 | 40.96 | 11.93 | 42.91 | 11.71 | >45 | 11.77 | 39.88 | 11.77 | >45 | 11.89 |

**Figure 3: Automation did not introduce cross contamination between wells.** *C. neoformans* and ZymoBIOMICS DNAse/RNAse-Free Water were added to a 96-well plate in alternating checkerboard fashion before processing using the OT-2 Nucleic Acid Extraction Workstation and qPCR analysis with the Femto Bacterial DNA Quantification Kit on the CFX96 Touch Real-Time PCR Detection System. C. *neoformans* was not detected in water-filled control wells, indicated by Ct values of >38 (grey cells). C. *neoformans* was detected in sample wells, indicated by Ct values between approximately 10.5 and 12 (blue cells).

## CONCLUSION

Evaluation of DNA extraction performed by the OT-2 Nucleic Acid Extraction Workstation with the ZymoBIOMICS 96 MagBead DNA Kit revealed no purification bias. Automated extraction delivered yield and purity comparable to manual extraction by highly trained technicians while avoiding cross contamination between wells. Further, several protocol adjustments were found to improve performance and speed of the extraction workflow.

DNA extraction using the OT-2 Nucleic Acid Extraction Workstation and the ZymoBIOMICS 96 MagBead DNA Kit delivered high-purity DNA, with good yield and no purification bias or cross contamination. Excellent performance without contamination or purification bias is necessary for DNA extraction protocols to keep up with ever advancing microbiomics research. These high-performance results indicate that the OT-2 can provide a reliable purification solution for microbial workflows. The data also demonstrates the OT-2's flexibility for use with kits that support a wide array of applications. The OT-2 can provide the increased throughput and reduced turn-around-times to facilitate more ambitious experiments and save valuable time while maintaining high standards for quality data.

## REFERENCES

1. Shehadul Islam, M., Aryasomayajula, A., & Selvaganapathy, P. R. (2017). A Review on Macroscale and Microscale Cell Lysis Methods. *Micromachines*, 8(3), 83. https://doi.org/10.3390/mi8030083