





# ZymoBIOMICS<sup>™</sup> Fecal Reference with TruMatrix<sup>™</sup> Technology

Enable run-to-run consistency and cross study comparison for microbiome research

#### **Highlights**

- Real-Life Representation: a microbiome quality control made from real human fecal material
- Consistent: two million uniform preps available to act as a reliable, single point of reference for all microbiome researchers
- Well-Characterized: deeply sequenced using targeted, metagenomic, and metatranscriptomic sequencing

Catalog Numbers: D6323



Scan with your smart-phone camera to view the online protocol/video.







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### **Product Contents**



# **Specifications**

- **Biosafety** This product contains no biohazard as potential pathogens have been fully inactivated.
- **Stability** Stored in DNA/RNA Shield, which preserves the microbial profile and protects against degradation from freeze-thaw cycles.
- Source The fecal material used in this product was derived from healthy adult donors.
- **Consistency** All vials are derived from a single, homogenized batch of fecal material to minimize composition variation.
- **Concentration** 1mL of the product contains about 10 mg feces (wet weight).
- Total DNA Content 1mL of the product contains about 6 µg DNA.<sup>1</sup>
- Total RNA Content 1mL of the product contains about 11 µg RNA.<sup>2</sup>

Storage Temperature – Store the fecal reference material at -20C or -80C.

1 Yield estimated based on extraction with the ZymoBIOMICS™ DNA Miniprep Protocol

2 Yield estimated based on extraction with the ZymoBIOMICS™ DNA/RNA Miniprep Protocol

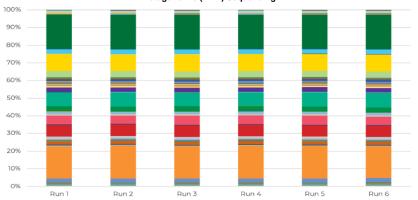
### **Product Description**

Metagenomic and metatranscriptomic profiling based on Next-Generation Sequencing (NGS) are becoming more and more popular. Such NGS workflows are complicated and are prone to bias or errors without proper quality control. The lack of controls combined with different methodology have led to conflicting study results, calling the validity of many datasets into question and hindering progress in the field. In order to benchmark and improve these measurements, there is an urgent need for reliable, widely used reference that replicates the major challenges encountered in microbiome workflows.

The **ZymoBIOMICS™ Fecal Reference with TruMatrix™ Technology** serves as a gold standard that is used across labs and studies to facilitate data comparison in the microbiomics field. It is made from genuine human fecal material collected from healthy adult donors and homogenized in one large batch using the TruMatrix™ Technology from The BioCollective. The product is derived from one large homogenized suspension, and all aliquots have been demonstrated to have consistent metagenomic and metatranscriptomic profiles (Figure 1). With millions of preps generated from a single source, this fecal reference will be the most characterized fecal sample in the world, acting as a constant, single point of reference for all microbiome researchers.

The product is intended to be processed in your microbiome workflow as a positive control and as an individual sample in parallel to actual samples. The metagenomic and metatranscriptomic (Figure 2) profiles of this product have been deeply sequenced and thoroughly characterized. Links to the full profiles and raw sequencing data are available to download in Appendix A.

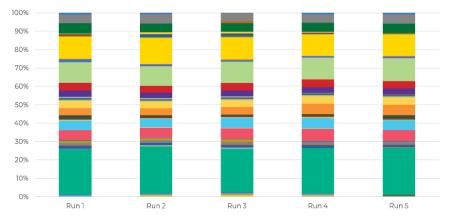
To facilitate method comparison and validation, a platform for users to anonymously share and download sequencing results of this product is under development. We encourage all users to submit sequencing data, analysis results and descriptions of applied methodology to <u>microbiomics@zymoresearch.com</u>. The platform will also provide comparison analyses of profiles resulting from different methods, enabling users to determine cross-compatibility of data.



#### **Consistent Profiles Across Runs**

Metagenomic (DNA) Sequencing

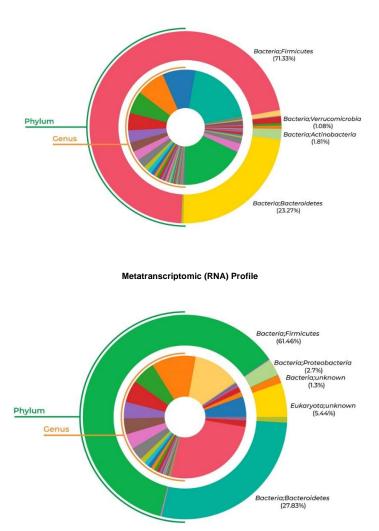
Metatranscriptomic (RNA) Sequencing



**Figure 1**: Stability and consistency of taxonomy profiles at the genus-level of the ZymoBIOMICS<sup>™</sup> Fecal Reference with TruMatrix<sup>™</sup> Technology across different runs of metagenomic and metatranscriptomic sequencing. Workflow description, downloadable raw data, and bioinformatics analyses can be found in Appendix A.

#### Characterization

Metagenomic (DNA) Profile



**Figure 2**: The taxonomy profiles (phylum and genus level) determined by metagenomic sequencing and metatranscriptomic sequencing. Workflow description, downloadable raw data, and bioinformatics analyses can be found in Appendix A.

### Protocol

#### To use:

- 1. Thaw the microbial standard completely on ice.
- Mix thoroughly by vortex to ensure sample is homogenous and the material is evenly resuspended.<sup>1</sup>
- Process 100 µL of the product as an individual sample in parallel with other samples (e.g. feces). Please refer to the maximum input recommendations of the extraction method used if higher yields are required.

To minimize bias during DNA or RNA extraction, we recommend using the ZymoBIOMICS<sup>™</sup> DNA Miniprep kit (Cat# D4300) or the ZymoBIOMICS<sup>™</sup> RNA Miniprep kit (Cat# R2001).

We encourage users to submit their data and contribute to the effort of building a platform for method comparison. (For more information, refer to the Product Description on page 2.) Contact <u>microbiomics@zymoresearch.com</u> to find out how. All information will remain anonymous.

<sup>1</sup> Small particles within the product are normal. Ensure everything is evenly resuspended and that pipetting is performed smoothly.

<sup>2</sup> The expected yield for one prep (100 μl) of reference material is approximately 0.6 μg. Yields lower than 0.35 μg may suggest inefficient lysis during DNA extraction.

# **Appendix A: NGS Characterization**

| Projects                               | Brief Workflow Description  | Bioinformatics<br>Report |
|--|---|--------------------------|
| Metagenomic<br>profiling               | 100 µl of the product was extracted using the<br>ZymoBIOMICS <sup>™</sup> DNA Miniprep (D4300). The<br>shotgun library was prepared using the Illumina <sup>®</sup><br>DNA Prep kit. DNA sequencing was performed<br>on Illumina <sup>®</sup> NovaSeq <sup>®</sup> . Bioinformatics analysis<br>was performed using the ZymoBIOMICS <sup>™</sup><br>Metagenomics Service Pipeline, which uses<br><u>Centrifuge</u> (v1.0.4) for taxonomy identification.      | <u>Download</u>          |
| Metagenomic<br>Consistency<br>Testing  | Same as above.  | <u>Download</u>          |
| Metatranscriptomic<br>Profiling        | 100 µl of the product was extracted using the<br>ZymoBIOMICS™ RNA Miniprep (R2001). The<br>RNA library was prepared using the Zymo-Seq<br>RiboFree <sup>®</sup> Total RNA Library Kit (R3003).<br>Sequencing was performed on Illumina <sup>®</sup><br>HiSeq™. Bioinformatics analysis was performed<br>using the ZymoBIOMICS™ Metatranscriptomics<br>Service Pipeline, which uses <u>Centrifuge</u> (v1.0.4)<br>for taxonomy identification.                 | <u>Download</u>          |
| Metatranscriptomic<br>consistency test | Same as above.  | <u>Download</u>          |
| 16S profiling                          | 100 µl of the product was extracted using the<br>ZymoBIOMICS <sup>™</sup> DNA Miniprep (D4300). 16S<br>sequencing library (V3-V4) was prepared using<br>the Quick-16S <sup>™</sup> NGS Library Prep Kit (D6400).<br>Sequencing was performed on Illumina <sup>®</sup><br>MiSeq <sup>™</sup> (2x300bp). Bioinformatics analysis was<br>performed using the ZymoBIOMICS <sup>™</sup> 16S<br>Service Pipeline, which uses <u>Dada2</u> for<br>taxonomy analysis. | <u>Download</u>          |

Links to download raw sequence data and more detailed method description can be found from this link.

# **Ordering Information**

| Product Description                                       | Catalog No. | Size     |
|---|-------------|----------|
| ZymoBIOMICS™ Fecal Reference with TruMatrix<br>Technology | D6323       | 10 preps |

# **Related Products**

| Related Products  | Catalog No.       | Amount                  |
|---|-------------------|-------------------------|
| ZymoBIOMICS™ Microbial Community Standard                                     | D6300             | 10 preps.               |
| ZymoBIOMICS™ Microbial Community<br><u>DNA</u> Standard (200ng)               | D6305             | 200 ng                  |
| ZymoBIOMICS™ Microbial Community<br><u>DNA</u> Standard (2000ng)              | D6306             | 2000 ng                 |
| ZymoBIOMICS™ Microbial Community<br>Standard II (Log Distribution)            | D6310             | 10 preps.               |
| ZymoBIOMICS™ Microbial Community<br><u>DNA</u> Standard II (Log Distribution) | D6311             | 220 ng                  |
| ZymoBIOMICS™ Spike-in Control I<br>(High Microbial Load)                      | D6320<br>D6320-10 | 25 preps.<br>250 preps. |
| ZymoBIOMICS™ Spike-in Control II<br>(Low Microbial Load)                      | D6321<br>D6321-10 | 25 preps.<br>250 preps. |
| ZymoBIOMICS™ HMW DNA Standard   | D6322             | 5000 ng                 |

## **Complete Your Workflow**

✓ Collect and transport microbiome samples at ambient temperatures:

| 1X Reagent #R1100 For sample lysis and stabilization of   |
|---|
| DNA/RNA   |
| 2X Concentrate #R1200 Reagent concentrate (2X) for use wi liquids at 1:1 ratio  |
| Fecal Collection Tube #R1101<br>Fecal Collection Tube #R1101<br>DNA/RNA Shield <sup>™</sup> ). Direct collection of<br>up to 1g or 1 mL stool |
| Collection Tube w/ Swab #R1106<br>with 1 mL DNA/RNA Shield <sup>™</sup> and steri<br>swab for specimen collection                             |

✓ Unbiased and inhibitor-free DNA and RNA extraction (high-throughput and automatable) for microbiome profiling:

| ZymoBIOMICS™ DNA and RNA Kits |  |
|-------------------------------|--|
| DNA Miniprep #D4300           | Up to 25 µg DNA                                    |
| DNA Microprep #D4301          | Up to 5 µg DNA                                     |
| MagBead DNA #D4302            | Automatable (Tecan, Hamilton,<br>Kingfisher, etc.) |
| 96-Well DNA #D4309            | Spin-plate   |
| DNA/RNA Miniprep Kit #R2002   | Up to 100 µg DNA/RNA                               |

✓ Streamlined workflows with comprehensive bioinformatics analysis and publicationready plots and figures:

| $\frown$ | ZymoBIOMICS™ Serv                                | ices                |
|----------|--|---------------------|
|          | 16S Targeted Sequencing Service #Q2001           | With DNA Extraction |
|          | ITS Targeted Sequencing Service #Q2003           | With DNA Extraction |
|          | Shotgun Metagenomic Sequencing Service<br>#Q2003 | With DNA Extraction |
|          | Metatranscriptomic Sequencing Service            | COMING SOON         |

# Notes

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Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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