ZYMO RESEARCH

Microbiomics
Made Simple™

ZymoBIOMICS™ 96 DNA Kit

DNA for microbiome or metagenome analyses

Highlights

- **Validated Unbiased for Microbiome Measurements:** Unbiased cellular lysis validated using the ZymoBIOMICS Microbial Community Standard.
- **Inhibitor-Free DNA from Any Sample:** Isolate ultra-pure DNA ready for any downstream application.
- **Certified Low Bioburden:** Boost your detection limit for low abundance microbes.
- **Simple Workflow:** Simply bead-beat sample, purify via spin-plate, and filter to remove PCR inhibitors. No precipitation or lengthy incubations!

Catalog Numbers:
D4303, D4307, D4309

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

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Product Contents

ZymoBIOMICS™ 96 DNA Kit	D4303 (2 x 96 Preps.)	D4307 (2 x 96 Preps.)	D4309 (2 x 96 Preps.)
ZymoBIOMICS™ Lysis Solution	150 ml	-	150 ml
ZymoBIOMICS™ DNA Binding Buffer	250 ml	250 ml	250 ml
ZymoBIOMICS™ DNA Wash Buffer 1	100 ml	100 ml	100 ml
ZymoBIOMICS™ DNA Wash Buffer 2	200 ml	200 ml	200 ml
ZymoBIOMICS™ DNase/RNase Free Water	10 ml	10 ml	10 ml
ZymoBIOMICS™ HRC Prep Solution	30 ml	30 ml	30 ml
96-Well Block	2	2	2
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Zymo-Spin™ I-96-Z Plate	2	2	2
Collection Plate	2	2	2
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Instruction Manual	1	1	1

Storage Temperature - Store all kit components (*i.e.*, buffers, columns) at room temperature.

Specifications

- **Sample Sources –** Bacterial (including endospores)[1], fungal, protozoan, algal, viral, mitochondrial, and host DNA is efficiently isolated from ≤ 50 mg of mammalian feces, ≤ 100 mg soil, and 5 – 20 mg (wet weight) of bacterial/fungal cells[2], biofilms, and water[3].

- **Bead Beating System –** The innovative ZymoBIOMICS™ lysis system enables complete homogenization/disruption of the microbial cells walls and accurate microbial DNA analysis, free of bias. To ensure unbiased lysis, calibration of each bead-beating device is recommended by using the ZymoBIOMICS™ Microbial Community Standard (see Appendix C).

- **DNA Purity –** High quality, inhibitor-free DNA is eluted with ZymoBIOMICS™ DNase/RNase Free Water and is suitable for all downstream applications including PCR and Next-Generation Sequencing.

- **DNA Integrity –** On average, post bead beating, genomic DNA is between 15-20 kb depending on the initial quality of the sample, making it amenable to Next-Generation Sequencing platforms requiring high molecular weight DNA. For optimal DNA integrity, collect samples in DNA/RNA Shield™[4].

- **DNA Recovery –** Up to 5 µg total DNA can be eluted into 20 µl.

- **Bioburden –** A single preparation is guaranteed to contain less than 3 bacterial genomic copies per µl of eluate as determined by quantitative amplification of the 16S rRNA gene when eluted using 100 µl water.

- **Equipment –** Centrifuge w/ microplate carriers and 96-well plate/block disruptor or pulverizer.

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1 See endospore lysis efficiency data in Appendix B.
2 This equates to approximately 2 x 10^8 bacterial cells and 2 x 10^7 yeast cells.
3 For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm). Alternatively, up to 250 µl water can be processed directly.
4 DNA/RNA Shield™ provides an accurate molecular signature of the sample at the time of collection by preserving nucleic acids at ambient temperature and inactivating organisms including infectious agents (see Appendix A).

Product Description

The **ZymoBIOMICS™ 96 DNA Kit** is designed for purifying DNA from a wide array of sample inputs (*e.g.* feces, soil, water, biofilms, etc.), that is immediately ready for microbiome or metagenome analyses. The ZymoBIOMICS™ innovative lysis system eliminates bias associated with unequal lysis efficiencies[1] of different organisms (*e.g.* Gram-negative/positive bacteria including endospores[2], fungi, protozoans, algae, etc.) making it ideal for microbial community profiling. Unbiased mechanical lysis of tough microbes is achieved by bead beating with the innovative ultra-high density BashingBeads™ and validated using the ZymoBIOMICS™ Microbial Community Standard[3], as shown in Figure 3. In addition, the **ZymoBIOMICS™ 96 DNA Kit** is equipped with Zymo Research's proprietary OneStep™ PCR Inhibitor Removal technology, enabling PCR from the most PCR prohibitive environmental samples rich in humic and fulvic acids, tannins, melanin, and other polyphenolic compounds. Coupling state-of-the-art lysis technology with Zymo-Spin™ Technology results in superior yields of ultra-pure DNA ideal for all downstream applications including PCR, arrays, 16S rRNA gene sequencing, and shotgun sequencing[4].

Innovation. Pure & Simple.™

[1] Chemical, enzymatic, and inferior lysis matrices (beads) lead to unrealistic representation of organisms in downstream metagenomic analyses that is not reflective of actual abundance.
[2] See endospore lysis efficiency data in Appendix B.
[3] For more information on the ZymoBIOMICS™ Microbial Community Standard (D6300) & ZymoBIOMICS™ Microbial Community DNA Standard (D6305), see Appendix C.
[4] DNA is predominately 15-20 kb and amenable to Next-Generation Sequencing techniques requiring high molecular weight DNA.

Ultra-pure DNA from Inhibitor Rich Samples

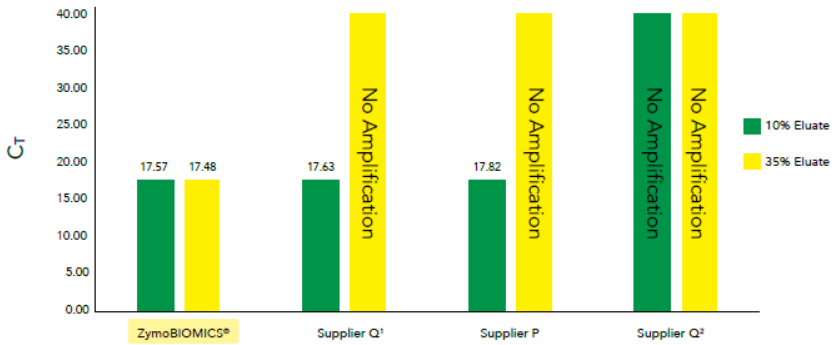


Figure 1. The **ZymoBIOMICS™ DNA Kit** provides inhibitor-free DNA even when challenged with extremely inhibitor rich samples. Real-time PCR was used to evaluate eluates recovered using the **ZymoBIOMICS™ DNA Kit**, and kits from Suppliers Q[1], P, and Q[2]. Reaction volumes consisted of either 10% or 35% of the eluate from each kit to detect the presence of PCR inhibitors. Each reaction contained 25 ng of *Brettanomyces* DNA. Delayed and/or no amplification indicates PCR inhibition from inefficient inhibitor removal.

Superior Yields

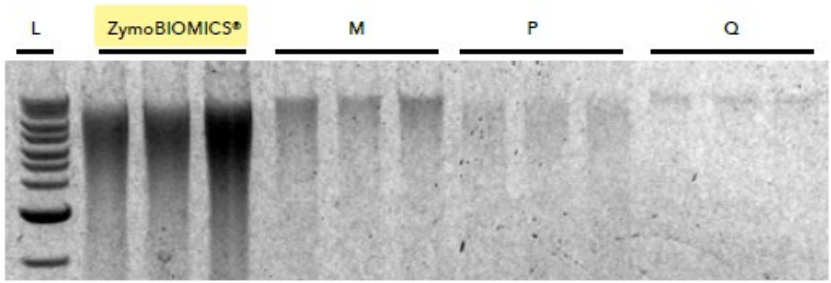
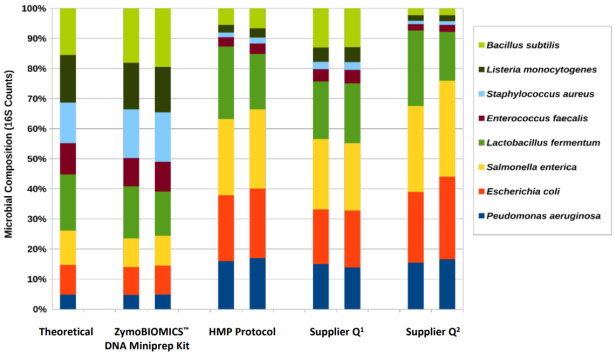


Figure 2. The **ZymoBIOMICS™ DNA Kit** provides superior yields when compared to Suppliers M, P, and Q. 80 mg of feces was processed using each kit according to the manufacturers' recommended protocol. DNA was eluted using 100 µl ZymoBIOMICS™ DNase/RNase Free Water. 6 µl of each sample was visualized in a 1.0% (w/v) agarose/ethidium bromide gel. Samples were processed in triplicate. L is a 1Kb ladder.

A) Bias Free Microbial DNA Extraction Using ZymoBIOMICS™ DNA Kit Validated with the ZymoBIOMICS™ Microbial Community Standard



B) Bias Free Microbial DNA Extraction Using ZymoBIOMICS™ DNA Kit From Human Stool

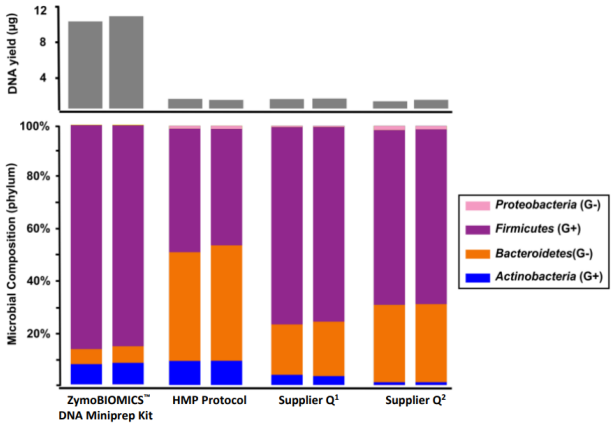


Figure 3. A) The ZymoBIOMICS™ DNA Kit provides unbiased representation of the organisms extracted from the ZymoBIOMICS™ Microbial Community Standard. DNA was extracted from ZymoBIOMICS™ Microbial Community Standard using four different DNA extraction methods (ZymoBIOMICS™ DNA Kit, Human Microbiome Project Protocol, Supplier Q[1], and Supplier Q[2]) 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™ (2 x 250 bp). 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.
B) The ZymoBIOMICS™ DNA Kit reliably isolates DNA from even the toughest to lyse Gram positive organisms, enabling unbiased analyses of microbial community compositions. There is a significant increase in yield and Gram-positive bacterial abundance when DNA was isolated using the ZymoBIOMICS™ DNA Kit. Correlated with the results in Figure 3A, it can be concluded that unbiased DNA isolation was achieved. DNA was extracted from 200 µl of human feces suspended in PBS (10 % m/v) using four different DNA extraction methods (ZymoBIOMICS™ DNA Kit, Human Microbiome Project Protocol, Supplier Q[1], and Supplier Q[2]) 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™ (2 x 250 bp). Overlapping paired-end reads were assembled into complete amplicon sequences. Amplicon sequences were profiled with Qiime using Greengenes 16S rRNA gene database (gg_13_8).

Protocol

Before Starting: (Fecal/Soil samples only) The wells of a **Silicon-A™-HRC Plate** need to be prepared prior to use:

(1) Mount the **Silicon-A™-HRC Plate** onto a **Collection Plate**.
(2) Pierce the middle of the cover foil and add 150 µl **ZymoBIOMICS™ HRC Prep Solution** to the wells. Incubate for 5 minutes at room temperature.
(3) Centrifuge the plate at exactly 3,500 x *g* for 5 minutes. Empty the Collection Plate and save for later use.
(4) The Silicon-A-HRC Plate is now ready for use.

1. Add sample to the **BashingBead™ Lysis Module** using the table below:
   a. If using **ZymoBIOMICS™ BashingBead™ Lysis Rack (0.1 & 0.5 mm),** add 550 µl **ZymoBIOMICS™ Lysis Solution**. Remove cover before bead beating, secure clamp directly to the lysis tube caps.
   b. If using **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)**, add 750 µl **ZymoBIOMICS™ Lysis Solution**.

Sample Type	Maximum Input
Feces	100 mg
Soil	100 mg
Liquid Samples[1] and Swab Collections[2]	250 µl
Cells (Suspended in PBS)	5-20 mg (wet weight) (2×10^8 bacterial and 2×10^7 yeast cells)
Samples in DNA/RNA Shield™[,3]	≤ 800 µl

Note: For samples stored and lysed in **DNA/RNA Shield™ Lysis Tubes**, do not add ZymoBIOMICS™ Lysis Solution and proceed to Step 2.

2. Secure in a bead beater fitted with the appropriate holder assembly for your bead beating module and process using optimized beat beating conditions (speed and time) for your device (see Appendix D)[4].

Optional Stopping Point: *Following Step 2 is the best stopping point if breaking up the work is needed. Samples post lysis can be stored for several hours at room temperature or can be stored at – 80 °C for long term storage.*

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[1] For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm).
[2] Swabs can also be cut or broken, then placed directly in bead beating tube. For more information on processing swab samples, see Appendix B.
[3] Up to 800 µl of sample in DNA/RNA Shield can be processed directly in ZR BashingBead™ Lysis Tube. Adjust final volume to 800 µl with ZymoBIOMICS™ Lysis Solution or DNA/RNA Shield, if necessary.
[4] For optimal lysis efficiency and unbiased profiling all bead beater devices beyond those validated by Zymo Research should be calibrated using the ZymoBIOMICS™ Microbial Community Standard. See Appendix C.

3. Centrifuge the **BashingBead™ Lysis Module**:
    a. If using **ZymoBIOMICS™ BashingBead™ Lysis Rack (0.1 & 0.5 mm)**, centrifuge at ≥ 4,000 x *g* for 5 minutes.
    b. If using **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)**, centrifuge at ≥ 10,000 x *g* for 1 minute.

4. Transfer up to 400 µl of the supernatant to each well of a **96-Well Block**.

5. Add 1,200 µl of **ZymoBIOMICS™ DNA Binding Buffer** to the supernatant in the 96-Well Block from Step 4. Cover completely with Cover Foil and mix thoroughly by vortexing for 2 minutes.

6. Transfer 800 µl of the mixture from Step 5 to a **Zymo-Spin™ I-96-Z Plate** in a **Collection Plate** and centrifuge at ≥ 3,500 x *g* for 5 minutes.

7. Discard the flow through from the Collection Plate and repeat Step 6.

8. Discard the flow through from the Collection Plate.

9. Add 400 µl **ZymoBIOMICS™ DNA Wash Buffer 1** to the Zymo-Spin™ I-96-Z Plate in the **Collection Plate** and centrifuge at ≥ 3,500 x *g* for 5 minutes. Discard the flow-through.

10. Add 700 µl **ZymoBIOMICS™ DNA Wash Buffer 2** to the Zymo-Spin™ I-96-Z Plate in a Collection Plate and centrifuge at ≥ 3,500 x *g* for 5 minutes. Discard the flow-through.

11. Add 200 µl **ZymoBIOMICS™ DNA Wash Buffer 2** to the Zymo-Spin™ I-96-Z Plate and centrifuge at ≥ 3,500 x *g* for 5 minutes.

12. Transfer the I-96-Z Plate to an **Elution Plate** and add 20 µl **ZymoBIOMICS™ DNase/RNase Free Water** directly to the column matrix and incubate for 1 minute. Centrifuge at exactly 3,500 x *g* for 5 minutes to elute the DNA[5, 6].

13. Transfer the eluted DNA from Step 12 to a prepared **Silicon-A™-HRC Plate** mounted onto a new **Elution Plate**. Centrifuge the assembly at exactly 3,500 x *g* for 3 minutes.

    The filtered DNA is now suitable for PCR and other downstream applications.

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[5] In some cases a brown-colored pellet may form at the bottom of the tube after centrifugation. Avoid this pellet when collecting the eluted DNA.
[6] If fungi or bacterial cultures were processed; the DNA is now suitable for all downstream applications.

Appendices

Appendix A

Sample Collection

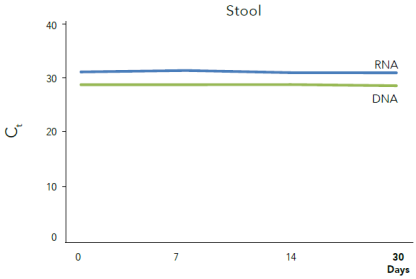
For high quality reproducible microbiomics data, **DNA/RNA Shield™** is recommended for sample collection to avoid bias or erroneous results due to compositional changes from nucleic acid degradation or microbial growth. DNA/RNA Shield™ provides an unbiased molecular snapshot of the sample at the time of collection by preserving nucleic acids at ambient temperature and inactivating organisms including infectious agents. Samples can be stored and transported easily and safely with DNA/RNA Shield™ and is ideal for applications such as PCR, 16S rRNA gene sequencing, and shotgun metagenomic sequencing. DNA/RNA Shield™ can preserve nucleic acids in nearly any sample including feces, soil, saliva, blood, and tissues.

DNA/RNA Shield™ - Lysis Tube (Microbe) – Simply add sample, seal, and store at ambient temperature. The Lysis Tube is immediately ready for bead beating, thereby streamlining the collection to extraction transition. (Cat. No. **R1103**)

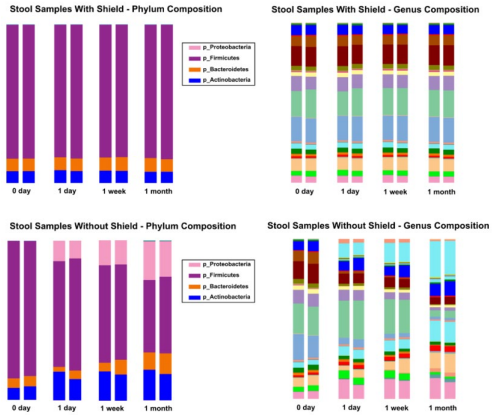
DNA/RNA Shield™ – Fecal Collection Tube – The collection device is specifically designed for easy collection and stabilization of feces. Includes a scoop built for collecting 1 gram of feces (or any other sample such as saliva or soil). (Cat. No. **R1101**)

DNA/RNA Shield™ – Swab Collection Tube – Easy collection of biological samples; swab has breakable tip to allow for easy sample collection and removes the need to dispose of a potentially biohazardous swab material. (Cat. No. **R1106** & **R1107**).

A) DNA/RNA Shield™ Preserves Nucleic Acids at Room Temperature



B) DNA/RNA Shield™ Preserves Microbial Composition at Room Temperature



C) DNA/RNA Shield™ Inactivates Pathogens for Safe Transport and Storage

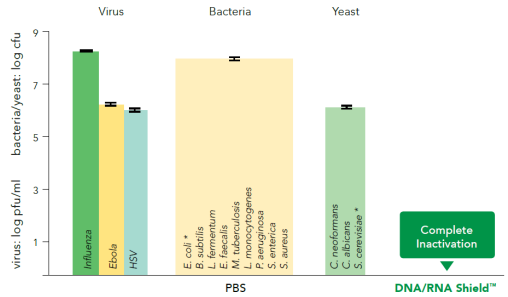


Figure 4.

A) Nucleic acids in stool are effectively stabilized in DNA/RNA Shield™ at room temperature. Graph shows spike-in DNA and RNA controls from stool purified at the indicated time points and analyzed by (RT)qPCR. Controls: HSV-1 and HIV (AcroMetrix™, Life Technologies).

B) Microbial composition of stool is unchanged after one month at ambient temperature with DNA/RNA Shield™. Stool samples suspended in DNA/RNA Shield™ and stored at room temperature were compared to stool without preservative for one month. They were sampled at the indicated time points and processed with ZymoBIOMICS™ DNA Kit. The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Graphs show both phylum composition (left) and genus composition (right). Samples stored with DNA/RNA Shield™ had a constant microbial composition while the samples stored without shifted dramatically.

C) Viruses, bacteria and yeast are effectively inactivated by DNA/RNA Shield™. Samples containing the infectious agent (viruses, bacteria, yeast) were treated with DNA/RNA Shield™ or mock (PBS) treated for 5 minutes. Titer (PFU) was subsequently determined by plaque assay. Validated by: Influenza A - D. Poole and Prof. A. Mehle, Department of Medical Microbiology and Immunology, University of Wisconsin, Madison; Ebola (Kikwit) - L. Avena and Dr. A. Griffiths, Department of Virology and Immunology, Texas Biomedical Research Institute; HSV-1/2 - H. Oh, F. Diaz and Prof. D. Knipe, Virology Program, Harvard Medical School; E. coli, L. fermentum, B. subtilis, S. cerevisiae – Zymo Research Corporation).

Appendix B

Application Notes

DNA/RNA Shield™ Lysis Tubes (Microbe) (Cat. No. **R1103**)

1. Collect sample directly into the **DNA/RNA Shield™ Lysis Tube (Microbe)**.
2. Directly proceed to Step 2 of the protocol (page 6) and bead beat in the DNA/RNA Shield™ Lysis Tube (Microbe) according to provided recommendations.
3. Proceed with the remaining protocol as written.

DNA Viruses

For unbiased metagenomics analysis of viruses, incorporating a Proteinase K digestion prior to bead beating is recommended.

1. Following Step 2 (page 6) add 5% (v/v) of **Proteinase K** (Cat. No. D3001-2-5) to the lysate within the **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)** and incubate for 30 minutes at 55˚C.
2. Proceed to Step 3 (page 6) and continue with the remaining protocol as written.

Cheese and Protein Rich Biofluids (*e.g.* Milk, Sputum, Saliva, Spinal Fluid, Blood, and Serum)

1. Add ≤ 0.4 g of cheese or ≤ 200 µl of biofluid to the **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)**. Add 750 µl of **ZymoBIOMICS™ Lysis Solution**.
2. Add 20 µl of **Proteinase K** (20 µg/µl) (cat. no. D3001-2-5) to the ZymoBIOMICS™ Lysis Tubes (0.1 & 0.5 mm) and incubate for 30 minutes at 55˚C.
3. Continue to Step 2 (page 6) and proceed with the protocol as written.

Plant Tissue (Leaves and other plant material)

Plant tissues such as leaves and roots contain DNA sources within the host tissue that can overwhelm 16S rRNA gene targeted sequencing (from both mitochondria & chloroplast). Microbes must be removed from the plant surface to exclude host tissue from the bead beating process.

(A) *Plant tissue – Centrifugation of cells*

1. Suspend plant tissue in isotonic solution (*e.g.* PBS) and gently sonicate or vortex briefly.
2. Remove plant tissue from solution and centrifuge at 15,000 x *g* for 10 minutes to pellet the cells.
3. Without disturbing the pellet, slowly decant or pipette out the supernatant, leaving behind 100 – 300 µl of pellet.
4. Add **ZymoBIOMICS™ Lysis Solution** to the cells to a final volume of 1 ml and mix to resuspend. Transfer the mixture to the **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)** and proceed to Step 2 (page 6).

(B) *Plant tissue – Filtration of cells*

1. Place plant tissue in a submerging volume of PBS inside of a conical tube and gently sonicate or vortex briefly. Remove plant tissue from liquid volume.
2. Filter liquid using a 0.22 µm filter (not provided).
3. Cut the filter and place directly into the **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)** and proceed to Step 1 (page 6).

(C) *Plant root – Lysis of surface microbes*

1. Cut root into small pieces and place directly into **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)** with 750 µl of **ZymoBIOMICS™ Lysis Buffer.**
2. Lysis should be performed with a lower speed bead beating device (*e.g.* vortex adapter for 20 minutes) to avoid the host tissue contamination.
3. Continue to Step 3 (page 6) and proceed with the remaining protocol as written.

Water/Air Samples

1. Filter samples using desired filter (not provided) prior to Step 1 (page 6).
2. Cut the filter into small pieces and place them inside the **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)** and add 750 µl of **ZymoBIOMICS Lysis Solution.**
3. Continue to Step 2 (page 6) and proceed with the remaining protocol as written.

Lytic Enzymes

Lytic enzymes, such as Lysozyme, Lysostaphin, MetaPolyzyme, etc. can be used with this kit using the following:

(A) *Enzymatic lysis followed by bead beating:*

1. Perform enzymatic digestion under manufacturer's recommended conditions (temperature/time/concentration).

   Note: *If sample is stored in* ***DNA/RNA Shield****, perform the following:*

   a. *Centrifuge sample at ≥ 10,000 x g for 1 minute.*
   b. *Transfer supernatant to a* ***ZR BashingBead Lysis Tube (0.1 & 0.5 mm)****, to be used in Step 2, below.*
   c. *Re-suspend pellet in a buffer suitable for enzymatic treatment (ex. PBS or other isotonic solution).*

2. Transfer the digestion mixture to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**.
3. Add 750 µl **ZymoBIOMICS™ Lysis Solution**.

   Note*: For samples in DNA/RNA Shield, raise to a final volume of 1 ml with DNA/RNA Shield.*

4. Proceed to Step 2 (page 6) and continue with the remaining protocol as written.

(B) *Enzymatic lysis only (no bead beating):*

1. Perform enzymatic digestion under manufacturer's recommended conditions (temperature/time/concentration).

   Note: *If sample is stored in* ***DNA/RNA Shield****, perform the following:*

   a. *Centrifuge sample at ≥ 10,000 x g for 1 minute.*
   b. *Transfer supernatant to a clean microcentrifuge tube, to be used in Step 2.*
   c. *Re-suspend pellet in a buffer suitable for enzymatic treatment (ex. PBS or other isotonic solution).*

2. Raise the volume of sample to 400 µl with **ZymoBIOMICS™ Lysis Solution**.
3. Continue to Step 4 (page 6) and proceed with the remaining proceed as written.

Hair, Feather, and Nail Samples:

1. To ≤ 25 mg sample, add 90 µl Water, 90 µl **Solid Tissue Buffer (Blue)** (Cat. No. D4068-2-6), 10 µl 1M DTT, and 10 µl **Proteinase K** (Cat. No. D3001-2-5) in a microcentrifuge tube.
2. Mix thoroughly or vortex 10-15 seconds and then incubate the tube at 55˚C overnight.
3. Transfer lysate to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)** and then add 750 µl **ZymoBIOMICS™ Lysis Solution**.
4. Continue to Step 2 (page 6) and proceed with the remaining protocol as written.

Tissue and Insect Samples

Tissue and Insect samples can be processed three different ways, depending on the sample type and the equipment available. The recommendations are listed next to the options below:

(A) *Proteinase K - Tissue*

1. Add up to 15 mg of tissue to a 1.5 ml microcentrifuge tube, then add a solution of 95 µl water, 95 µl **Solid Tissue Buffer (Blue)** (Cat. No. D4068-2-6) and 10 µl **Proteinase K** (Cat. No. D3001-2-5). Incubate for at least 1 hour at 55° C or until tissue clarifies (samples can be incubated overnight without affecting DNA quality).
2. Transfer digestion to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)** and add 750 µl of **ZymoBIOMICS™ Lysis Solution.**
3. Proceed to Step 2 (page 6) and continue with the protocol as written.

(B) *Bead beating - Tissue and Insect*

1. Add up to 15 mg of tissue/insect sample in a **ZR BashingBead™ Lysis Tube (2.0 mm)** (Cat. No. S6003-50) with 750 µl of **ZymoBIOMICS™ Lysis Solution**.
2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.

   Note: *Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep® - 24) or as long as 20 minutes when using lower speeds (e.g., Disruptor Genie®).*

3. Transfer the entire lysate to the **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**, proceed to Step 2 (page 6), and continue with protocol as written.

(C) *Mortar & Pestle - Tissue and Insect*

1. Homogenize up to 15 mg tissue/insect sample with a mortar and pestle while submersed in liquid nitrogen.
2. Transfer the entire sample into the **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)** and add 750 µl of **ZymoBIOMICS™ Lysis Solution.**
3. Proceed to Step 2 (page 6) and continue with the protocol as written.

Samples Collected with Swabs

(A) *Directly process swab*

1. Directly break swab at breakpoint or cut the swab into a **ZR BashingBead Lysis Tube (0.1 & 0.5 mm)**.
2. Proceed to Step 1 (page 6) and continue with the protocol as written.

(B) *Indirectly process swab*

1. Vortex the swab in the **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)** with 750 µl of **ZymoBIOMICS™ Lysis Solution** for 30 seconds to transfer the microbes into solution.
2. Remove the swab and proceed to bead beating in Step 2 (page 6).

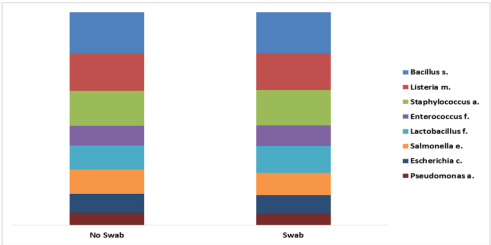


Figure 5. Phylum composition of a simulated microbial community when bead beating was performed with and without the presence of a Puritan HydraFlock® sterile flocked collection device placed in a ZR BashingBead Lysis Tube and processed at maximum speed (6.5 m/s) for 5 minutes. The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Experiment was performed in technical duplicates.

Bacterial Endospore Lysis

ZymoBIOMICS DNA Kit is capable of effectively lysing bacterial endospores, and also achieves higher yield when compared to competition.

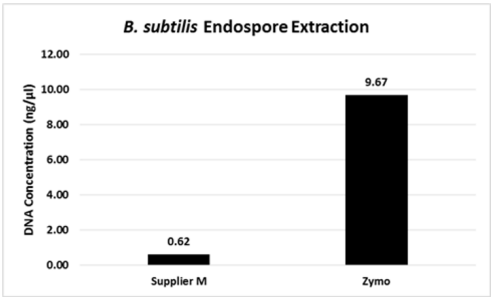


Figure 6. DNA Extractions were performed using the ZymoBIOMICS® DNA Kit and DNeasy PowerSoil with 6 x 10^8 *B. subtilis* CFU. DNeasy PowerSoil recovered 0.62 ng/µl DNA, while the ZymoBIOMICS® DNA Kit was capable of recovering 9.67 ng/µl in a 50 µl elution volume. Extractions were performed in triplicate and quantified via Qubit.

Urine

(A) Pelleting cells from fresh/frozen urine

1. Pellet the bacterial cells by centrifuging the urine at 15,000 x *g* for 10 minutes.
2. Without disturbing the pellet, slowly decant or pipette out the supernatant, leaving behind 100 – 400 µl of pellet.
3. Add **ZymoBIOMICS™ Lysis Solution** to a final volume of 800 µl and then transfer the mixture to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**. Proceed to Step 2 (page 6) and continue with the protocol as written.

(B) *Pelleting cells from stabilized urine*

1. Add 70 µl **Urine Conditioning Buffer** (Cat. No. D3061-1-140) for every 1 ml of urine and mix well by vortexing.

   Note: *Urine stabilized by the Urine Conditioning Buffer can be stored for up to 1 month at ambient temperature. When samples are ready to be processed, mix well by vortexing, and proceed to Step 2.*

2. Centrifuge at 3,000 x *g* for 15 minutes.
3. Without disturbing the pellet, slowly decant or pipette out the supernatant, leaving behind 100 – 400 µl of pellet.
4. Add **ZymoBIOMICS™ Lysis Solution** to a final volume of 800 µl and then transfer the mixture to a **ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm)**. Proceed to Step 2 (page 6) and continue with the protocol as written.

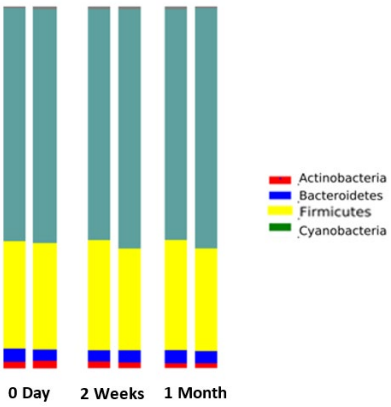


Figure 7. Phylum composition of urine preserved in Urine Conditioning Buffer™ (UCB™), which preserves the microbial composition of urine with simulated stool contamination for a month at room temperature. Urine with UCB™ added (Zymo Research, D3061-1-160) was stored at room temperature and analyzed over a month period. At the indicated time points (0 Days, 2 weeks, and 1 month), DNA was extracted using the ZymoBIOMICS™ DNA Kit. The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Experiment was performed in technical duplicates.

Appendix C

Standardize Sample Preparation with ZymoBIOMICS™ Microbial Community Standards

The **ZymoBIOMICS™ Microbial Community Standard (Cat. No. D6300)** is a mock microbial community of defined and well characterized composition making it the perfect control for all microbiome profiling and metagenomics analyses.

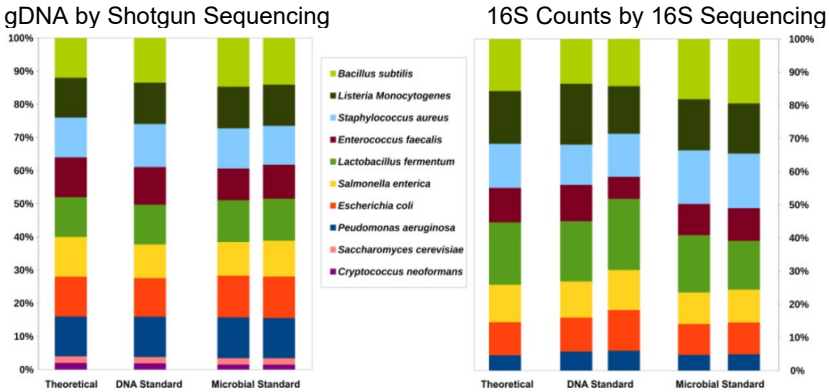
It is ideal for assessing bias of DNA extraction methods since it contains three easy-to-lyse Gram-negative bacteria (*e.g. Escherichia coli*), five tough-to-lyse Gram-positive bacteria (*e.g. Listeria monocytogenes*), and two tough-to-lyse yeasts (*e.g. Saccharomyces cerevisiae*).

Bead Beating Device Calibration Protocol:
Zymo Research suggests calibrating bead beating devices with the ZymoBIOMICS™ Microbial Community Standard in order to ensure bias free microbial extraction. For Disruptor Genie®, vortex adapters, and vortex lysis we suggest a time course ranging from 10-45 minutes with the vortex at maximum speed. For high speed cell disruptors such as the MP FastPrep® -24 we suggest a time course at maximum speed with a range of 3-10 minutes. The resulting DNA should be evaluated by quantifying DNA yield and changes in microbial profile at each time point. The bead beating time that yields a profile that closely matches the theoretical composition should become standard operating procedure for the bead beating device.

ZymoBIOMICS™ Microbial Community DNA Standard (Cat. No. D6305) is a mixture of genomic DNA extracted from pure cultures of eight bacterial and two fungal strains. Genomic DNA from each culture was quantified before mixing. The ZymoBIOMICS™ Microbial Community Standard allows for assessment of bias from library preparation, sequencing, and bioinformatics analysis.

It serves perfectly as a microbial standard for benchmarking the performance of microbiomics or metagenomics analyses, including those provided by a 3rd party.

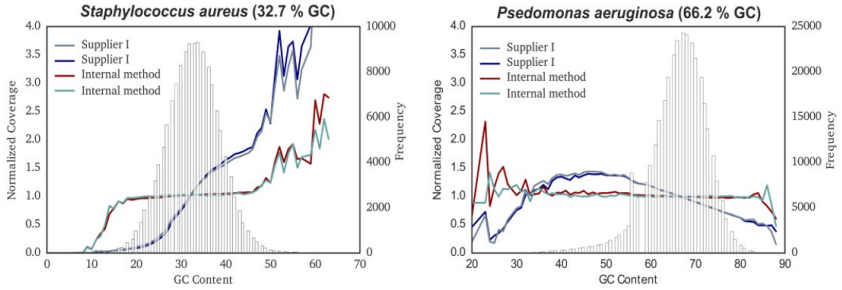
Accurate composition for reliable use to evaluate shotgun seq. and 16S rRNA seq.



Species	Avg. GC (%)	Gram Stain	gDNA Abun. (%)
Pseudomonas aeruinosa	66.2	-	12
Escherichia coli	56.8	-	12
Salmonella enterica	52.2	-	12
Lactobacillus fermentum	52.8	+	12
Enterococcus faecalis	37.5	+	12
Syaphylococcus aureus	32.7	+	12
Listeria monocytogenes	38.0	+	12
Bacillus subtilis	43.8	+	12
Saccharomyces cerevisiae	38.4	Yeast	2
Cryptococcus neoformans	48.2	Yeast	2

Figure 8. Characterization of the microbial composition of the two ZymoBIOMICS™ standards with shotgun metagenomic sequencing (left panel) and 16S rRNA gene targeted sequencing (right panel). The measured composition of the two standards agrees with the theoretical/designed composition. "DNA Standard" represents ZymoBIOMICS™ Microbial Community DNA Standard (DNA version) and "Microbial Standard" represents ZymoBIOMICS™ Microbial Community Standard (cellular version). Genomic DNA composition by shotgun sequencing was calculated based on counting the amounts of raw reads mapped to each genome. 16S composition by 16S rRNA gene targeted sequencing was calculated based on counting the amount of 16S raw reads mapped to each genomes.

A) Use ZymoBIOMICS™ Microbial Standards for assessing GC-Bias in Shotgun Metagenomics



B) Perfect for tracking PCR Chimera in 16S rRNA Gene Sequencing

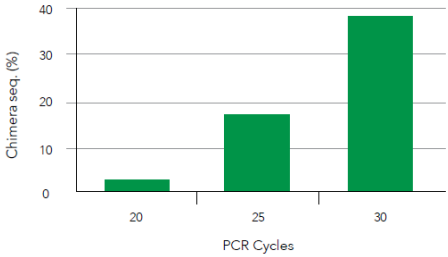


Figure 9.

A) Library preparation for shotgun metagenomic sequencing was performed in two different ways: one by supplier I and one by an in-house method. Shotgun sequencing was performed on Illumina® MiSeq™ with paired-end sequencing (2 x 150 bp). Raw reads were mapped to the 10 microbial genomes to evaluate the potential effect of GC content on sequencing coverage. Normalized coverage was calculated by normalization by the average sequencing coverage of each genome.

B) PCR chimera increases with PCR cycle number in the library preparation process of 16S rRNA gene targeted sequencing. 20 ng ZymoBIOMICS™ Microbial Community Standard was used a template. The PCR reaction was performed with ZymoBIOMICS™ PCR Premix and with primers that target v3-4 region of 16S rRNA gene. Chimera rate in percentage was determined with Uchime and using the 16S rRNA gene of the 8 bacterial strains in the standard as reference PCR.

Appendix D

Optimized Lysis Protocols for Bead-Beating

The following conditions with different mechanical lysis machines were validated with minimum bias using the **ZymoBIOMICS™ Microbial Community Standard**.

1 Vortex Genie with 2ml BashingBead™ Tubes

Recommended for ease of use and accessibility

Use Microtube Adaptor (Scientific Industries, Inc. Cat. No. S5001-7)

1. 40 minutes of continuous bead beating (max of 18 tubes per adaptor)

2 Bertin Precellys Evolution with 2 ml BashingBead™ Tubes

Recommended for ease of use and ultra-high speed.

1. 1 minute on at 9,000 RPM
2. 2 minutes rest
3. Repeat cycle 4 times for a total of 4 minutes of bead beating

3 MP Fastprep-24™ (Classic & 5G) with 2 ml BashingBead™ Tubes

Maximum of 20 tubes. The weight of > 20 tubes may cause a system error.

1. 1 minute on at 6.5 m/s
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

4 Omni Bead Ruptor Elite with 2 ml BashingBead™ Tubes

1. 1 minute on at 6 m/s
2. 5 minutes rest
3. Repeat cycle 3 times for a total of 3 minutes of bead beating

5 Biospec Mini-BeadBeater-16 with 2 ml BashingBead™ Tubes

1. 1 minute at maximum speed
2. 5 minutes rest
3. Repeat cycle 5 times for a total of 5 minutes of bead beating

6 Biospec Mini-BeadBeater-96 with 2 ml BashingBead™ Tubes

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 4 times for a total of 20 minutes of bead beating

7 Biospec Mini-BeadBeater-96 with 96 well lysis rack

1. 5 minutes on at Max RPM
2. 5 minutes rest
3. Repeat cycle 8 times for a total of 40 minutes of bead beating

✕ TissueLyser II

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ TissueLyser LT

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

✕ Retsch Mixer Mill MM 400

No tested conditions yielded accurate profiles. This device is not validated by Zymo Research for microbiome research.

Troubleshooting

For **Technical Assistance**, please contact 1-888-882-9682 or E-mail tech@zymoresearch.com.

Problem	Possible Causes and Suggested Solutions
Background Contamination	• Clean workspace, centrifuge, and pipettes with 10% bleach to routinely to avoid contamination. • Use of kit in exposed environment without proper filtration. Check pipettes, pipette tips, microcentrifuge tubes, workspace, etc. for contamination. • Make sure bags of columns and buffer bottles are properly sealed for storage. Use of these outside a clean room or hood can result in contamination.
Low DNA Yield	Lysis Methods • Refer to Appendix D for validated bead beating devices and parameters. • Bead beating devices that oscillate in a single dimension (only vertically or only horizontally) have been observed to inefficiently lyse very recalcitrant species. Devices that oscillate three-dimensionally or in a figure-8 motion often lyse microbes efficiently. Incomplete Debris Removal • For high density samples, ensure lysate is centrifuged properly to pellet insoluble debris following bead beating. Ensure that none of the debris is transferred to the **Zymo-Spin™ III-F Filter** in the next step. Input • If the lysate does not pass through the column or is extremely viscous, use less input material. Too much sample input can cause cellular debris to overload the column and insufficient flow. • Consult the Sample Input Table on Page 6 for information on your particular input limit based on sample.

Low DNA Yield (cont.)

Binding Step

- Ensure that the **ZymoBIOMICS™ DNA Binding Buffer** is completely mixed with lysate before loading onto the column. Improperly mixed samples can lead to poor DNA recovery.

Elution Procedure

- Ensure the **ZymoBIOMICS™ DNase/RNase Free Water** hydrates the matrix for at least 1 minute before centrifugation.

- To increase yields, heat the **ZymoBIOMICS™ DNase/RNase Free Water** to 60ºC before use. Additionally, users can reload the eluate onto the column matrix, incubate at room temperature for 3 minutes, and centrifuge again to increase yield without further dilution.

Ordering Information

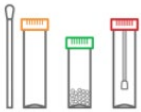
Product Description	Catalog No.	Size
ZymoBIOMICS™ DNA Microprep Kit	D4301	50 Preps.
ZymoBIOMICS™ DNA Microprep Kit (Lysis Matrix Not Included)	D4305	50 Preps.
ZymoBIOMICS™ DNA Miniprep Kit	D4300T	5 Preps.
ZymoBIOMICS™ DNA Miniprep Kit	D4300	50 Preps.
ZymoBIOMICS™ DNA Miniprep Kit (Lysis Matrix Not Included)	D4304	50 Preps.
ZymoBIOMICS™-96 DNA Kit (Includes BashingBead™ Lysis Rack)	D4303	2 x 96 Preps.
ZymoBIOMICS™-96 DNA Kit (Includes BashingBead™ Lysis Tubes)	D4309	2 x 96 Preps.
ZymoBIOMICS™-96 DNA Kit (Lysis Matrix Not Included)	D4307	2 x 96 Preps.
ZymoBIOMICS™-96 Magbead DNA Kit (Includes BashingBead™ Lysis Rack)	D4302	2 x 96 Preps.
ZymoBIOMICS™-96 Magbead DNA Kit (Includes BashingBead™ Lysis Tubes)	D4308	2 x 96 Preps.
ZymoBIOMICS™-96 Magbead DNA Kit (Lysis Matrix Not Included)	D4306	2 x 96 Preps.

Individual Kit Components	Catalog No.	Amount
ZymoBIOMICS™ DNA Binding Buffer	D4300-2-250	250 ml
ZymoBIOMICS™ DNA Wash Buffer 1	D4300-3-100	100 ml
ZymoBIOMICS™ DNA Wash Buffer 2	D4300-4-200	200 ml
ZymoBIOMICS™ DNase/RNase Free Water	D4302-5-10	10 ml
Collection Plate	C2002	2
Elution Plate	C2003	2
96-Well Block	P1001-2	2
ZymoBIOMICS™ Lysis Solution	D4300-1-150	150 ml
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50 Tubes
OneStep-96™ PCR Inhibitor Removal Kit	D6035	2 x 96 Preps.

Sample Collection	Catalog No.	Amount
DNA/RNA Shield™ - Lysis Tube (Microbe)	R1103	50 Pack
DNA/RNA Shield™ – Fecal Collection Tube	R1101	10 Pack
DNA/RNA Shield™ – Swab and Collection Tube	R1106 R1107	10 Pack 50 Pack
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml

Explore Other Microbiome Products

✓ To collect and transport samples at ambient temperatures:

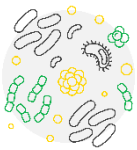


DNA/RNA Shield™ and Collection Devices	
1X Reagent #R1100	For sample lysis and stabilization of DNA/RNA
2X Concentrate #R1200	Reagent concentrate (2X) for use with liquids at 1:1 ratio
Fecal Collection Tube #R1101	15 mL container (prefilled with 9 mL DNA/RNA Shield™). Direct collection of up to 1g or 1 mL stool
Collection Tube w/ Swab #R1106	12 x 80 mm screwcap container filled with 1 mL DNA/RNA Shield™ and sterile swab for specimen collection

✓ Streamlined workflows with comprehensive bioinformatics analysis and publication-ready plots and figures:

ZymoBIOMICS™ Services	
Targeted Sequencing Service 16S #Q2001	With DNA Extraction
Targeted Sequencing Service 16S #Q2012	Without DNA Extraction
Targeted Sequencing Service ITS #Q2003	With DNA Extraction
Targeted Sequencing Service ITS #Q2003	Without DNA Extraction

✓ Microbial standards and references for profiling quality control, benchmarking, positive controls, and to assess performance of entire microbiomic/metagenomic workflows:



ZymoBIOMICS™ Standards and Reference Materials	
Microbial Community Standard #D6300	Contains 8 bacteria and 2 yeasts for QC and method optimization
Microbial Community DNA Standard #D6305	Contains 8 bacteria and 2 yeasts DNA for bioinformatics optimization
Gut Microbiome Standard #D6331	Contains 21 different human gut strains for method benchmarking
Fecal Reference with TruMatrix™ Technology #D6323	Contains real human fecal material for benchmarking and improved data reproducibility

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