ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoBIOMICS™ 96 MagBead DNA Kit

Catalog Nos. **D4302, D4306 & D4308**

Highlights

- High throughput purification of high quality, inhibitor-free DNA from any sample including feces, soil, water, biofilms, swabs, saliva, and body fluids.
- The ZymoBIOMICS™ innovative lysis system enables efficient and unbiased lysis of microbes including Gram positive/negative bacteria, fungi, protozoans, algae, etc.
- The automation friendly workflow enables nearly any sample to be processed in as little as 90 minutes for 96 preps.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you should be dissatisfied with this product please call 1-888-882-9682.

[1] For optimal performance, add beta-mercaptoethanol to 0.5% (v/v) i.e., 500 µl per 100 ml.

[2]This equates to approximately 2 x 10^8 bacterial cells, 2 x 10^7 yeast cells and 2 x 10^6 mammalian cells.

[3] For water samples, filter using desired filter (not provided). Cut the filter into small pieces and place into the BashingBead Module. Alternatively up to 250 µl water can be processed directly.

[4] DNA/RNA Shield™ (R1100) 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 for more information.

[5]96-Well Blocks (P1001-2) can be purchased separately at www.zymoresearch.com

Product Contents

ZymoBIOMICS™ 96 MagBead DNA Kit (Kit Size)	**D4302** (2x 96 preps.)	**D4306** (2x 96 preps.)	**D4308** (2x 96 preps.)	**Storage Temperature**
ZymoBIOMICS™ Lysis Solution	150 ml	-	150 ml	Room Temp.
ZymoBIOMICS™ MagBinding Buffer[1]	150 ml	150 ml	150 ml	Room Temp.
ZymoBIOMICS™ MagWash 1	100 ml x 2	100 ml x 2	100 ml x 2	Room Temp.
ZymoBIOMICS™ MagWash 2	200 ml x 2	200 ml x 2	200 ml x 2	Room Temp.
ZymoBIOMICS™ DNase/RNase Free Water	50 ml	50 ml	50 ml	Room Temp.
ZymoBIOMICS™ MagBinding Beads	12 ml	12 ml	12 ml	Room Temp.
ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)	2	-	-	Room Temp.
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	-	-	200	Room Temp.
Instruction Manual	1	1	1	-

Note - 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 maximal performance and reliability.

Specifications

- **Sample Sources** – Bacterial, fungal, protozoan, algal, viral, mitochondrial, and host DNA is efficiently isolated from ≤ 100 mg of mammalian feces, ≤ 200 mg soil, and 5 – 20 mg (wet weight) of fungal bacterial cells[2], biofilms, and water[3].
- **Bead Beating System** – The ZymoBIOMICS™ innovative lysis system enables complete homogenization/disruption of the microbial cell 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 for details).
- **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 (NGS).
- **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 10 µg total DNA can be eluted into 50 µl (37.5 µl minimum) **ZymoBIOMICS™ DNase/RNase Free Water**.
- **Bioburden** – A single preparation is guaranteed to contain less than 3 bacterial genomic copies per 1 µl of eluate, as determined by quantitative amplification of the 16S rRNA gene when eluted using 100 µl water.
- **Equipment** – Centrifuge fitted with a 96 well microplate carrier, 96 Well Magnetic Stand, Liquid handler or other robotic sample processor, 96 well plate heat block, 2 mL 96 well plates and reagent carriers[5] (user supplied).

Product Description

The **ZymoBIOMICS™ 96 MagBead DNA Kit** 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)[1], making it ideal for microbiomic 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[2] as shown in Figure 4. 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 unique system allows for a simple Bind, Wash, Elute procedure that is unmatched in providing ultra-pure DNA[3] 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.

Innovation Pure and Simple™

Ultra-pure DNA from Inhibitor Rich Samples

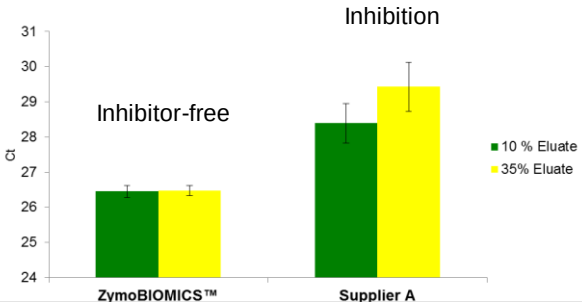

Figure 1. The **ZymoBIOMICS™ 96 MagBead DNA Kit** provides inhibitor-free DNA even when challenged with extremely inhibitor rich samples. Real-time PCR was used to evaluate eluates recovered using the **ZymoBIOMICS™ 96 MagBead DNA Kit**, or Supplier A. Reaction volumes consisted of either 10% or 35% of the eluate from each kit 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

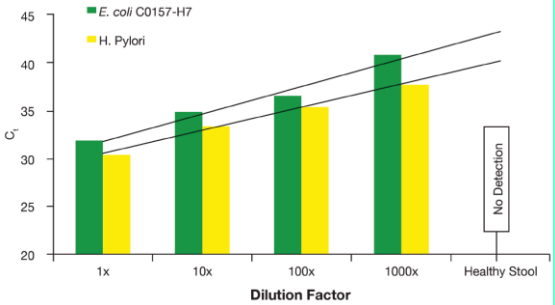

Figure 2. The **ZymoBIOMICS™ 96 Magbead DNA Kit** produces linear recovery of DNA for sensitive applications, detecting pathogenic organisms such as *E. coli* and *H. pylori* in assays with up to a 1000x dilution factor. A dilution series was created using stool infected with 1 x 10^6 shiga-toxin producing *E. coli* O157:H7 cells and stool infected with *H. pylori* cells. These stool samples were then extracted using the **ZymoBIOMICS™ 96 Magbead DNA Kit,** showing effective purification and qPCR amplification, even at 1000:1 dilution. N=8

Superior Yields

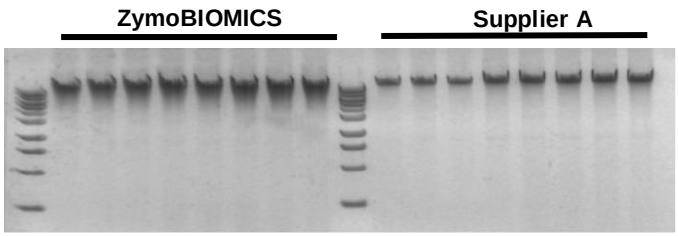

Figure 3. The **ZymoBIOMICS™ 96 Magbead DNA Kit** provides superior yields when compared to Suppliers M, P, and Q. 80 mg of feces was processed using each kit according to the manufactures' recommended protocol. DNA was eluted using 100 µl **ZymoBIOMICS™ DNase/RNase Free Water.** 6 µl of each sample was analyzed in a 1.0% (w/v) agarose/ethidium bromide gel. Samples were processed in triplicate. L is a 1Kb ladder.

Notes:

[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. To learn more about this topic see Figure 5.

[2] ZymoBIOMICS™ Microbial Community Standard (D6300) & ZymoBIOMICS™ Microbial Community DNA Standard (D6305), for more information see Appendix C.

[3] DNA is predominately 15-20 kb and amenable to Next-Generation Sequencing techniques requiring high molecular weight DNA.

Zymo Research offers a full suite of **ZymoBIOMICS™ Services** for reliable, accurate microbial and metagenomic analyses.

Services include: Microbial Composition profiling, Novel Microbe Identification, and Customizable Bioinformatics.

For details, visit us at: http://www.zymoresearch.com/services/metagenomics

Or Contact us at: services@zymoresearch.com

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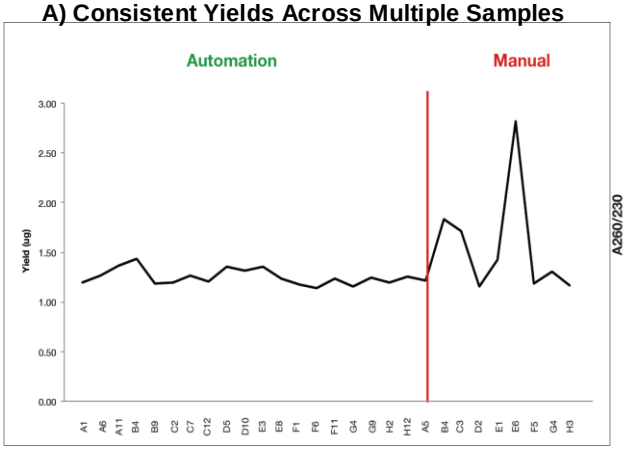
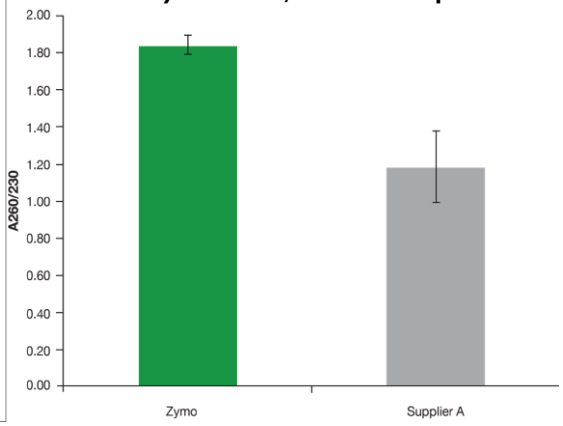
[1] ZymoBIOMICS™ Microbial Community Standard (D6300), for more information see Appendix C.

Reliability and Consistency

A) Consistent Yields Across Multiple Samples



B) Reliable, Clean Samples



C) No Cross Contamination Between Sample Wells

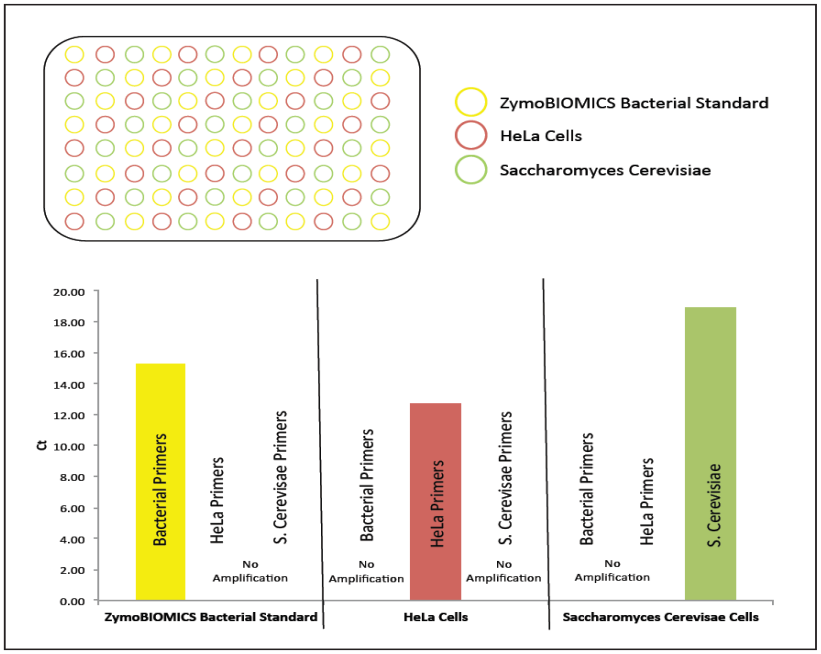
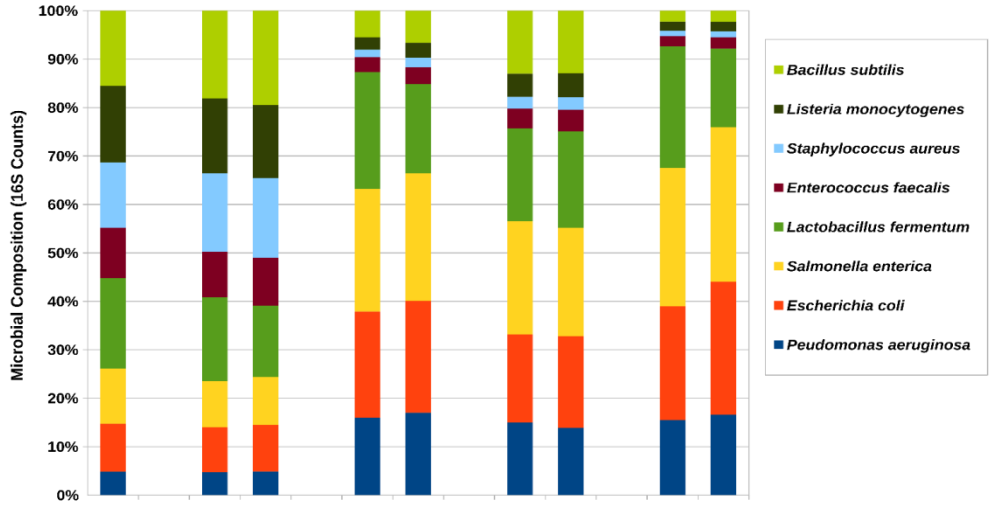


Figure 4A). The **ZymoBIOMICS™ 96 MagBead DNA Kit** provides consistent yields when DNA is purified from 20 mg of human fecal sample using an automated liquid handling system. Consistent and replicable yields were observed when DNA isolation was performed on an automated liquid handler. Samples were evaluated using spectrophotometric analysis in technical duplicates.
B). The **ZymoBIOMICS™ 96 MagBead DNA Kit** reliably purifies clean DNA from 20 mg of fecal sample, providing DNA with A260/230 values ≥1.80 and more consistent values compared to Supplier A. DNA was quantified using spectrophotometric analysis in technical duplicates. N=8.
C). Coupling the **ZymoBIOMICS™ 96 MagBead DNA Kit** with a liquid handler, isolated DNA is free from cross contamination when purified across a standard 96-well plate. Plate was setup with alternating rows of ZymoBIOMICS™ Microbial Community Standards[1], HeLa cells, and *S. Cerevisiae* cells, and DNA was purified simultaneously from these samples on a liquid handling platform. Samples were evaluated using quantitative PCR with primer sets targeted at the bacterial 16S gene, the human LINE gene, and the fungal ITS gene. PCR was performed in technical duplicates.

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



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

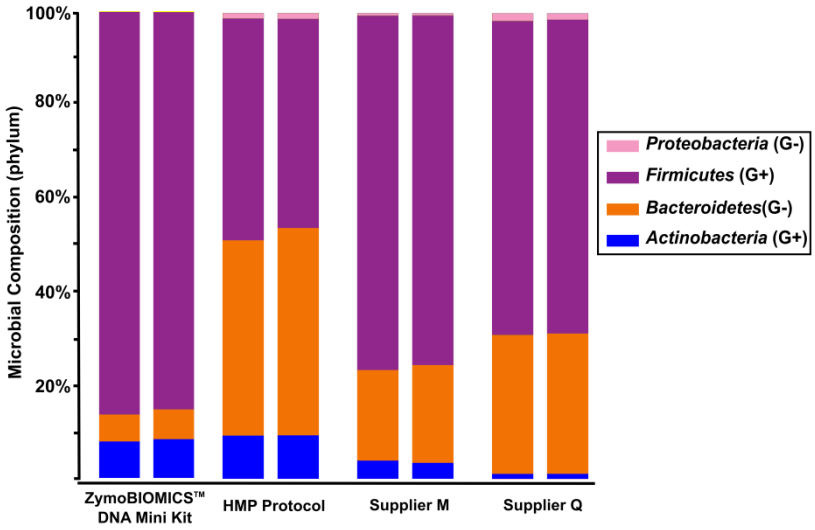


Figure 5. A) The ZymoBIOMICS™ DNA Miniprep Kit provides unbiased representation of the organisms extracted from the ZymoBIOMICS™ Microbial Community Standard. DNA was extracted from ZymoBIOMICS™ Microbial Community Standard using four different DNA extraction methods (ZymoBIOMICS™ DNA Miniprep Kit, Human 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.

B) The ZymoBIOMICS™ DNA Miniprep 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 Miniprep 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 Miniprep Kit, 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. Amplicon sequences were profiled with Qiime using Greengenes 16S rRNA gene database (gg_13_8).

For automated scripts and **Technical Assistance** regarding generation of scripts for automated platforms, contact **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

[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 and placed directly in bead beating tube. For more information on processing swab samples, see Appendix B.

[3] See Appendix A for additional information on sample collection in DNA/RNA Shield™.

[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.

Protocol

For optimal performance:
Add beta-mercaptoethanol (user supplied) to the **ZymoBIOMICS™ MagBinding Buffer** to a final dilution of 0.5% (v/v) *i.e.*, 500 µl per 100 ml.

Sample Lysis

For all mixing steps: pipette mix or shake at 1200 rpm.

1. Add sample to the **ZR BashingBead™ Module** using the table below.
   a. If using **ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)**, add 650 µl **ZymoBIOMICS™ Lysis Solution.**
   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	200 mg
Liquid Samples[1] and Swab Collections[2]	250 µl
Cells (Suspended in DNA/RNA Shield™ or isotonic buffer, *e.g.* PBS)	5-20 mg (wet weight) (2×10^8 bacterial, 2×10^7 yeast cells, 2×10^6 mammalian cells)
Samples in DNA/RNA Shield™ (10% v/v Sample)[3]	250 µl

2. Secure in a bead beater fitted with the appropriate holder assembly for your bead beating module and process at maximum speed for 5 minutes[4].

   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. Centrifuge the ZR BashingBead™ Lysis Module:
   a. If using **ZR 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.

Sample Purification

4. Transfer 200 µl supernatant to the deep-well block (not provided). Add 600 µl **ZymoBIOMICS™ MagBinding Buffer.**

   For samples with excessive amounts of solid particulate, centrifuge at 4,000 x g for 5 minutes to reduce clogging.

5. Dispense 25 µl of **ZymoBIOMICS™ MagBinding Beads** to each well. Mix well by pipette or shaker plate for 10 minutes.

6. Transfer the 96-well block to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.

7. Dispense 900 µl of **ZymoBIOMICS™ MagWash 1** and mix well by pipette or shaker plate for 5 minutes.

8. Transfer the 96-well block to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.

9. Dispense 900 µl **ZymoBIOMICS™ MagWash 2** and mix well by pipette or shaker plate for 5 minutes.

10. Transfer the deep-well block to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.

11. Repeat the wash (Steps 9-10).

12. Transfer the 96-Well Block onto a heating element (65°C) until beads dry (approximately 10 minutes). If no heating element is available, air dry for approximately 20-30 minutes.

    Note: *Alternatively, to avoid heating step, keep the pelleted beads on the magnetic stand, add 900 µl of* ***ZymoBIOMICS™ DNase/RNase Free Water*** *to the wells,* ***DO NOT MIX****, and incubate for 1 minute. Remove the supernatant and proceed with Step 13.*[5]

13. Dispense 50 µl of **ZymoBIOMICS™ DNase/RNase Free Water** to each well and re-suspend beads. Mix the beads well for 10 minutes and then transfer the plate onto the magnetic stand for 2-3 minutes until the beads pellet[6].

14. Transfer the supernatant (containing the eluted DNA) to a clean **Elution Plate**[7,8].

The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

[5]This alternative step may result in a slight (≤5%) loss of genomic DNA and complete loss of nucleic acids less than 100 bp in size. For ordering information on ZymoBIOMICS™ DNase/RNase Free Water, see page 16.

[6]See Appendix D for additional elution information.

[7] The DNA is now suitable for all downstream applications.

[8] For optimal spectrophotometric quantification, eluate may be centrifuged at 4,000 x g for 5 minutes and transfer eluate to a new plate while avoiding the pellet.

For all buffer additions, mix well by pipetting up and down several times and/or (if available) by vortexing at ~1,200 rpm.

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[1]If a shaker module is not installed, pipette mix for the duration of the shaking step

Automation Setup Guide

For optimal performance, add beta-mercaptoethanol (user supplied) to the **ZymoBIOMICS™ MagBinding Buffer** to a final dilution of 0.5% (v/v) *i.e.*, 500 µl per 100 ml.

1. Add 75 ml of **ZymoBIOMICS™ MagBinding Buffer** to a 96 well reagent trough.
2. Add 100 ml (one bottle) of **ZymoBIOMICS™ MagWash Buffer 1** to a 96 well reagent trough.
3. Add 200 ml (one bottle) of **ZymoBIOMICS™ MagWash Buffer 2** to a 96 well reagent trough.
4. Add 25 ml of **ZymoBIOMICS™ DNase/RNase Free Water** to a 96 well reagent trough.
5. Vortex the **ZymoBIOMICS™ MagBinding Beads** vigorously for 30 seconds, then add 6 ml to a 96 well low dead volume reagent trough.

Automation Protocol

1. Place the following components on the deck prior to initialization of the protocol:
   a. Place five 96-well racks of 300 µl standard volume tips on the deck.
   b. Place five 96-well racks of 1000 µl high volume tips on the deck.
   c. Place three 96-well racks of 50 µl low volume tips on the deck.
   d. Place a magnetic stand on the deck.
   e. Place a 96-Well Block on the deck.
   f. Place the pre-homogenized bead beating module on the deck in the appropriate carrier and remove the lids.
   g. Place two empty 96-well reagent troughs for waste disposal.
2. If a Heater/Shaker module is installed, begin preheating to 65° C before proceeding to the next step.
3. Aspirate 200 µl lysate from the bead beating module 20 mm from the container bottom.
4. Dispense 200 µl lysate into the empty 96-Well Block and discard the pipette tips.
5. Aspirate 600 µl **ZymoBIOMICS™ MagBinding Buffer** from the appropriate reagent trough.
6. Dispense 600 µl **ZymoBIOMICS™ MagBinding Buffer** into the 96-Well Block 2mm from the container bottom. After dispensing, pipette mix (400 µl for 15 cycles) 2 mm from the container bottom.
7. Premix the **ZymoBIOMICS™ MagBinding Beads** (50 µl for 10 cycles). Aspirate 25 µl ZymoBIOMICS™ MagBinding Beads.
8. Dispense 25 µl ZymoBIOMICS™ MagBinding Beads to the 96-Well Block 2 mm from the container bottom.
9. Using 1000 µl high volume tips, mix the lysate (600 µl for 25 cycles).
10. Transfer the 96-Well Block to a shaking device and shake at 1200 rpm for 10 minutes[1].
11. Transfer the 96-Well Block to a 96-well magnetic stand; allow it to stand for 2 minutes.
12. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 830 µl supernatant and discard.
13. Transfer the 96-Well Block from the magnetic stand to a normal plate carrier.
14. Aspirate 900 µl **ZymoBIOMICS™ MagWash Buffer 1**.
15. Dispense 900 µl **ZymoBIOMICS™ MagWash Buffer 1** into the 96-Well Block 2 mm from the container bottom. After dispensing, pipette mix (400 µl for 25 cycles).
16. Transfer the 96-Well Block to a shaking device and shake at 1200 rpm for 2 minutes.

17. Transfer the 96-Well Block to a 96-well magnetic stand; allow it to stand for 2 minutes.
18. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 900 µl supernatant and discard.
19. Transfer the 96-Well Block from the magnetic stand to a normal plate carrier.
20. Aspirate 900 µl **ZymoBIOMICS™ MagWash Buffer 2**.
21. Dispense 900 µl ZymoBIOMICS™ MagWash Buffer 2 into the 96-Well Block 2 mm from the container bottom. After dispensing, pipette mix (400 µl for 25 cycles).
22. Transfer the 96-Well Block to a shaking device and shake at 1200 rpm for 2 minutes.
23. Transfer the 96-Well Block to a 96-well magnetic stand; allow it to stand for 2 minutes.
24. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 900 µl supernatant and discard.
25. Transfer the 96-well 96-Well Block from the magnetic stand to a normal plate carrier.
26. Aspirate 900 µl **ZymoBIOMICS™ MagWash Buffer 2**.
27. Dispense 900 µl ZymoBIOMICS™ MagWash Buffer 2 into the 96-Well Block 2 mm from the container bottom. After dispensing, pipette mix (400 µl for 25 cycles).
28. Transfer the 96-Well Block to a shaking device and shake at 1200 rpm for 2 minutes.
29. Transfer the 96-Well Block to a 96-well magnetic stand; allow it to stand for 2 minutes.
30. Using a slow aspirate mode (≤ 50 µl/s flow rate) remove 900 µl supernatant and discard.
1. Proceed with one of the following methods depending on accessibility to a heating device:

    For platforms with a heating element:

    a. Transfer the 96-well block to the heating element preheated to 65° C.
    b. Let the 96-well block stand for 10 minutes.

    For platforms without a heating element:

    a. Let the 96-well block stand at room temperature for 20-30 minutes.

    Alternatively:

    a. While still on the 96-well magnetic stand, dispense 900 µl **ZymoBIOMICS™ DNase/RNase Free Water** to the 96-well block. Let it stand for 1 minute. Do not mix[1].
    b. Remove 900 µl **ZymoBIOMICS™ DNase/RNase Free Water** from the 96-well block. Transfer to the heating element preheated to 65° C.
31. Aspirate 50 µl **ZymoBIOMICS™ DNase/RNase Free Water.**
32. Dispense 50 µl ZymoBIOMICS™ DNase/RNase Free Water into the 96-Well Block 2mm from the container bottom. After dispensing, pipette mix (40 µl for 25 cycles).
33. Transfer the 96-Well Block to a shaking device and shake at 800 rpm for 3 minutes.
34. Transfer the 96-Well Block to a 96-well magnetic stand; allow it to stand for 2 minutes.
35. Aspirate 40 µl **ZymoBIOMICS™ DNase/RNase Free Water** from the 96-Well Block.
36. Dispense 40 µl **ZymoBIOMICS™ DNase/RNase Free Water** containing the eluted DNA to the elution plate[2,3,4].

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[1]This alternative step may result in a slight (≤5%) loss of genomic DNA and complete loss of nucleic acids less than 100 bp in size.

[2] See Appendix D for additional elution information.

[3] The DNA is now suitable for all downstream applications.

[4] For optimal spectrophotometric quantification, eluate may be centrifuged at 4,000 x g for 5 minutes and transfer eluate to a new plate while avoiding the pellet.

Appendix A

Sample Collection

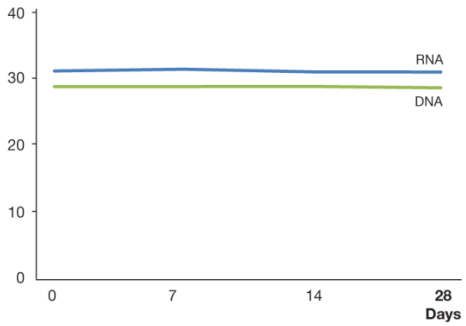
For high quality reproducible microbiomics data , **DNA/RNA Shield™** is recommended for sample collection to avoid bias or erroneous results due to compositional changes from nucleic acid degradation or microbial growth. DNA/RNA Shield™ provides an unbiased molecular snapshot of the sample at the time 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**)

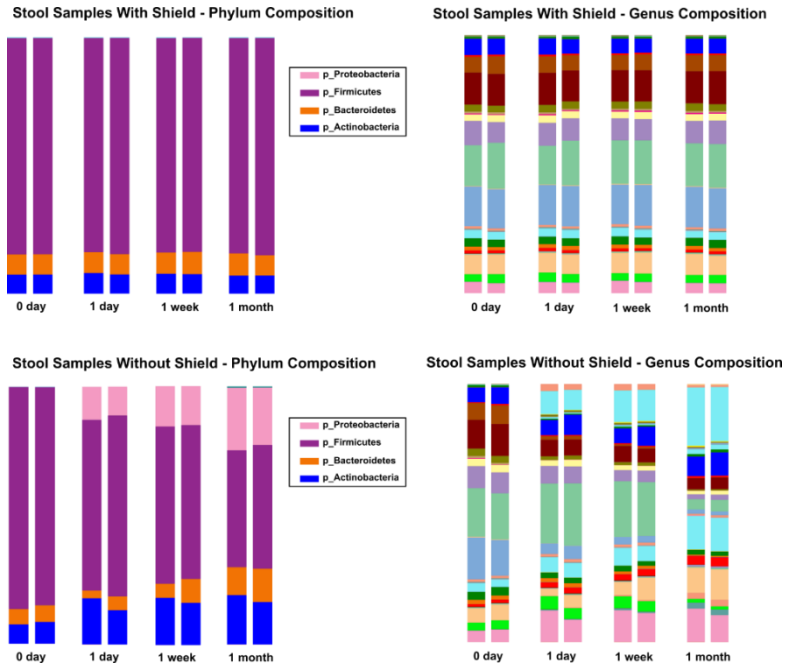
DNA/RNA Shield™ – Fecal Collection Tube – The collection device is specifically designed for easy collection and stabilization of feces. Includes a scoop built for collecting 1 gram of feces (or any other sample such as saliva or soil). (Cat. No. **R1101)**

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

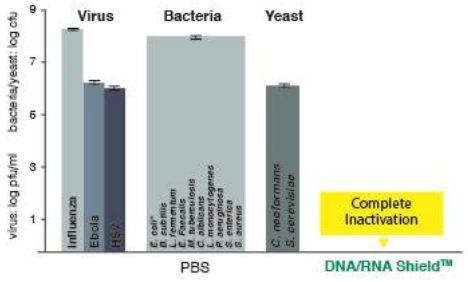


Figure 6. A) Nucleic acids in stool are effectively stabilized in DNA/RNA Shield™ at room temperature. Graph shows spike-in DNA and RNA controls from stool purified at the indicated time points and analyzed by (RT)qPCR. Controls: HSV-1 and HIV (AcroMetrix™, Life Technologies)

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 Miniprep 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 (virus, 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

Appendix B

Application Notes

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

Addition of ZymoBIOMICS™ Lysis Solution is not necessary for samples stored in DNA/RNA Shield™ Lysis Tubes (Microbe) and samples can be immediately processed via bead beating. Simply move to Step 2 of the protocol (page 5) and bead beat according to instructions provided. Proceed with the remaining protocol as written (page 5).

DNA Viruses

For unbiased metagenomic analysis of viruses, incorporating a Proteinase K digestion prior to bead beating is recommended. Add 5% v/v of Proteinase K (Cat. No. D3001-2-5) to the lysate after Step 2 (page 5) and incubate for 30 minutes at 55˚C. Continue to Step 3 (page 5).

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

Substitute the following for Step 1 (page 4) in ZymoBIOMICS™ 96 Magbead DNA Kit protocol section:

1. Add 0.3-0.4 g of cheese or 200 µl of biofluid to the ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm). Add 650 µl of ZymoBIOMICS™ Lysis Solution.
2. Add 2% v/v of Proteinase K (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 on to Step 2 (page 4) in ZymoBIOMICS™ protocol for further lysis.

Plant Tissue (Leaves and other plant material)

Plant tissue such as leaves 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 material to exclude host tissue from the bead beating process.

Prior to Step 1 (Page 4), suspend plant tissue in PBS and gently sonicate with sonication bath for effective removal of microbes. Alternatively, place plant tissue in a submerging volume of PBS inside of a conical tube and vortex briefly. The plant tissue can then be removed and the microbes can be centrifuged at high speeds to concentrate. Alternatively, a filter can also be used to concentrate the microbes and water removal. The filter can subsequently be cut and placed directly into the ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) for bead beating (Step 2).

Plant Root

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 as stated in Step 2 (page 4). Lysis should be performed with a lower speed bead beating device (e.g. vortex adapter) to avoid the host tissue contamination. Proceed with the remaining protocol as written (page 4).

Water/Air Samples

Filter samples using desired filter (not provided) prior to Step 1 (page 5). Cut the filter into small pieces, place into ZymoBIOMICS™ Lysis Module, and continue to Step 2.

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 or deep-well block, then add a solution of 95 µl water, 95 µl **Solid Tissue Buffer** (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. Process the lysate by proceeding to Step 1, Page 4 (Liquid Sample).

(B) Bead beating -*Tissue and Insect*
1. Place up to 15 mg of tissue/insect sample in a ZR BashingBead Module:
    a. If using **ZR BashingBead™ Lysis Tube (2.0 mm)** (Cat. No. S6003-50), add 750 µl of **ZymoBIOMICS™ Lysis Solution**.
    b. If using **ZR BashingBead™ Lysis Rack (2.0 mm)** (Cat. No. S6002-96-2), add 650 µl of **ZymoBIOMICS™ Lysis Solution**.

2. Secure in a bead beater fitted with the appropriate holder assembly for your bead beating module and process at maximum speed for 5 minutes[4].

    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) and proceed to Step 2, Page 4.

(C) Mortar & Pestle - *Tissue and Insect*
1. Pre-homogenize up to 15 mg tissue/insect sample with a pestle and mortar while submersed in liquid nitrogen.
2. Proceed to Step 1, Page 4 and process the entire sample.

Samples Collected with Swabs

Place swab directly into the ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) with 750 µl of ZymoBIOMICS™ Lysis Buffer. The swab can be cut at the height of the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) and left inside for bead beating in Step 2 (page 5). Alternatively, vortex the swab in the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) with the ZymoBIOMICS™ Lysis Solution for 30 seconds to transfer the microbes into solution. Remove the swab and proceed to bead beating in Step 2 (page 5).

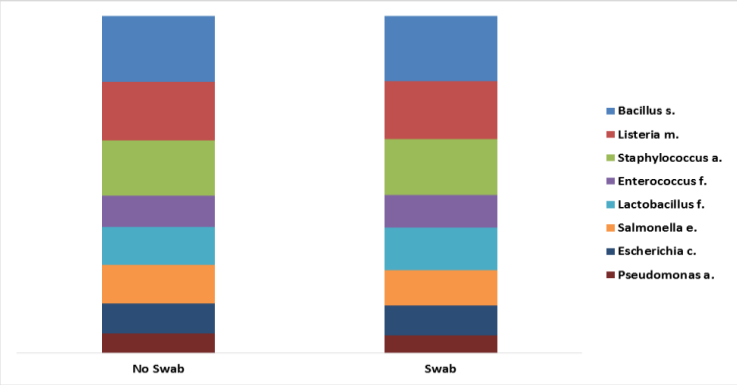


Figure 7. Phylum composition of a simulated microbial community when bead beating was performed with and without the presence of a Puritan HydraFlock® sterile flocked collection device placed in a BeadBashing tube and processed at maximum speed (6.5 m/s) for 5 minutes. The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Experiment was performed in technical duplicates

Urine

Pelleting of Cells by Centrifugation

1. Pellet the bacterial cells by centrifuging the urine at 15,000 x g for 10 minutes and then proceed to Step 4 below.

Alternatively

Separation of Cells by Centrifugation

2. Add 70 µl Urine Conditioning Buffer (Cat. No. D3061-1-140) for every 1 ml of urine and mix well by vortexing. 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 3.
3. Centrifuge at 3,000 x g for 15 minutes.
4. Without disturbing the pellet, slowly decant or pipette out the supernatant, leaving behind 100 – 400 µl of pellet.
5. 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 with the remaining protocol as written (page 5), starting at Step 2

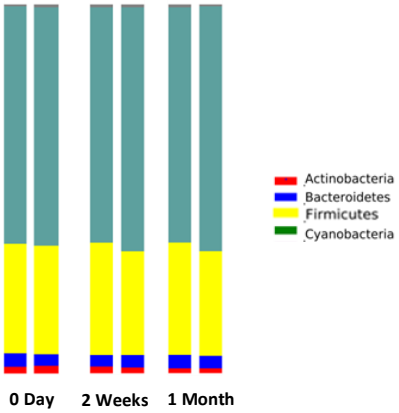


Figure 8. Phylum composition of urine preserved in Urine Conditioning buffer™ (UCB™), which preserves the microbial composition of urine with simulated stool contamination for a month at room temperature. Urine with UCB™ added (Zymo Research, D3061-1-160) was stored at room temperature and analyzed over a month period. At the indicated time points (0 Days, 2 weeks, and 1 month), DNA was extracted using the ZymoBIOMICS™ DNA Mini 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.

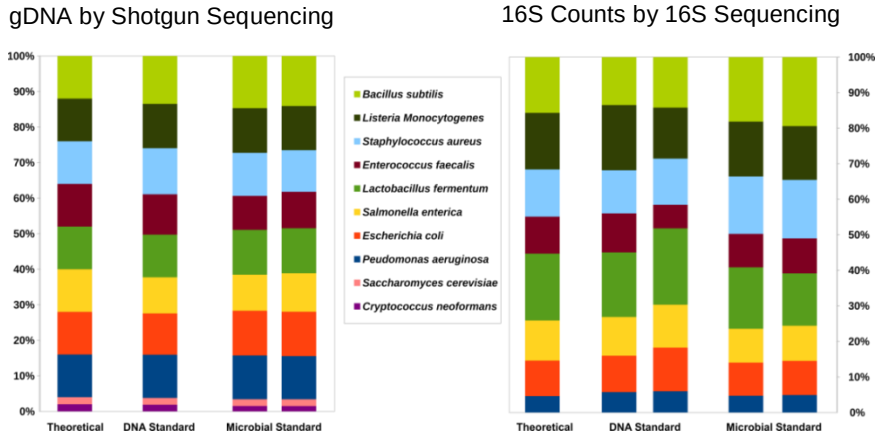
It is perfect for assessing bias of DNA extraction methods since it contains three easy-to-lyse Gram-negative bacteria (*e.g. Escherichia coli*), five tough-to-lyse Gram-positive bacteria (*e.g. Listeria monocytogenes*), and two tough-to-lyse yeasts (*e.g. Saccharomyces cerevisiae*).

Zymo Research suggests calibrating your own bead beating methods with ZymoBIOMICS™ Microbial Community Standard in order to ensure complete bias free microbial extraction. Several devices have been validated at Zymo with varying sample inputs but bead beating times will vary depending on your unique sample type. For Disruptor Genie®, vortex adapters, and vortex lysis we suggest a time course ranging 5, 10, 20, & 30 minutes with vortex at maximum speed. For high speed cell disruptors such as the MP FastPrep -24® we suggest a time course at maximum speed (6.5m/s for MP) with a range of 3, 5, 6, and 7 minutes.

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.

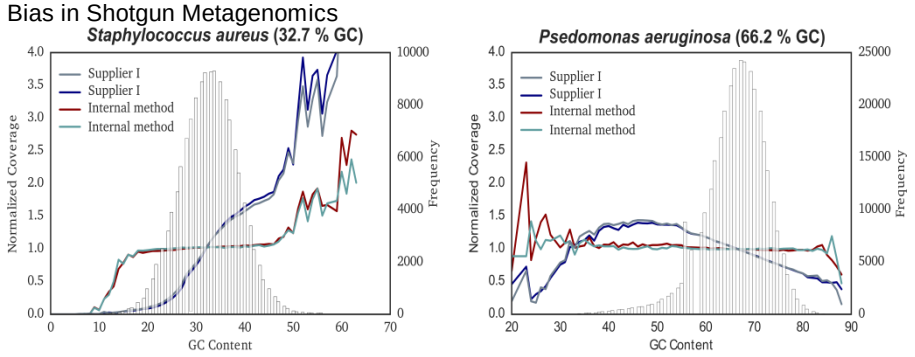
Figure 1. Accurate composition for reliable use to evaluate shotgun seq. and 16S rRNA seq.



Species	GC %	Gram Stain	gDNA Abun. (%)
Pseudomonas aeruginosa	66.2	-	12
Escherichia coli	56.8	-	12
Salmonella enterica	52.2	-	12
Lactobacillus fermentum	52.8	+	12
Enterococcus faecalis	37.5	+	12
Staphylococcus 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 9. 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

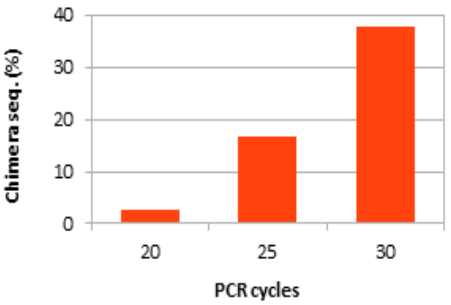


Figure 10.
A) Library preparation for shotgun metagenomic sequencing was performed in two different ways: one by Supplier I kit and one by an in-house method. Shotgun sequencing was performed on Illumina® MiSeq™ with paired-end sequencing (2 x 150 bp). Raw reads were mapped to the 10 microbial genomes 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 ZymoTaq Master Mix and with primers that target v34 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

Troubleshooting:

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

Troubleshooting (Continued):

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. • **ZymoBIOMICS™ DNase/RNase Free Water** when opened and used frequently could be a source of contamination. The entire solution can be autoclaved. Simply loosely fasten the cap and autoclave for 80 minutes at 121˚C. • 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.

Lysis Methods

- When using a Disruptor Genie®, vortex adapter, vortex, or similar processing times will vary. Suggested time is anywhere from 5-20 minutes. Calibrate bead beating times to your particular device and application by testing several different time points before using precious samples. (Suggested times to test: 10, 20, and 30 minutes.)
- When using FastPrep™-96 or similar devices run max speed for 5 minutes to ensure complete lysis. (1800 rpm on FastPrep®-96).

Low DNA Yield

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™ IV Spin Filter in the next step.

Input

- Consult the Sample Type table on Page 5 for information on your particular input limit based on sample.

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.

Note

ZymoBIOMICS™ -96 MagBead DNA Kit is for research use only. ZymoBIOMICS™ -96 MagBead DNA Kit is not sold for use in diagnostic procedures. Reagents included with this kit are irritants. Follow the safety guidelines and rules enacted by your research institution or facility including the wearing of protective gloves and eye protection when using this kit.

Ordering Information

Product Description	Catalog No.	Kit Size
ZymoBIOMICS™ DNA Microprep Kit	D4301	50 preps.
ZymoBIOMICS™ DNA Microprep Kit (Lysis Matrix Not Included)	D4305	50 preps.
ZymoBIOMICS™ DNA Miniprep Kit	D4300	50 preps.
ZymoBIOMICS™ DNA Miniprep Kit (Lysis Matrix Not Included)	D4304	50 preps.
ZymoBIOMICS™ 96 DNA Kit (includes ZR BashingBead™ Lysis Rack)	D4303	2x96 preps.
ZymoBIOMICS™ 96 DNA Kit (includes ZR BashingBead™ Lysis Tubes)	D4309	2x96 preps.
ZymoBIOMICS™ 96 DNA Kit (Lysis Matrix Not Included)	D4307	2x96 preps.
ZymoBIOMICS™ 96 Magbead DNA Kit (includes ZR BashingBead™ Lysis Rack)	D4302	2x96 preps.
ZymoBIOMICS™ 96 Magbead DNA Kit (includes ZR BashingBead™ Lysis Tubes)	D4308	2x96 preps.
ZymoBIOMICS™ 96 Magbead DNA Kit (Lysis Matrix Not Included)	D4306	2x96 preps.

For Individual Sale	Catalog No.	Amount
ZymoBIOMICS™ MagBinding Buffer	D4302-2-250	250 ml
ZymoBIOMICS™ MagWash 1	D4302-3-100	100 ml
ZymoBIOMICS™ MagWash 2	D4302-4-200	200 ml
ZymoBIOMICS™ DNase/RNase Free Water	D4302-5-50	50 ml
ZymoBIOMICS™ MagBinding Beads	D4302-6-12	12 ml
ZymoBIOMICS™ Lysis Solution	D4300-1-150	150 ml
ZR BashingBead™ Lysis Rack (0.1 & 0.5 mm)	S6002-96-3	2 Racks
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	S6012-50	50 Tubes

Sample Collection	Catalog No.	Amount
DNA/RNA Shield™ - Lysis Tube (Microbe)	R1103	50
DNA/RNA Shield™ – Fecal Collection Tube	R1101	10
DNA/RNA Shield™ – Swab and Collection Tube	R1106 R1107	10 50
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml

ZymoBIOMICS™-96 DNA Kit is for research use only. ZymoBIOMICS™-96 DNA Kit is not sold for use in diagnostic procedures. Reagents included with this kit are irritants. Follow the safety guidelines and rules enacted by your research institution or facility including the wearing of protective gloves and eye protection when using this kit.