ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoBIOMICS™ DNA Mini Kit

Catalog No. D4300

Highlights

- Rapid, robust, and simple purification of high quality, inhibitor-free DNA from any sample including feces, soil, water, biofilms, swabs, saliva, body fluids, etc.
- ZymoBIOMICS™ innovative lysis system enables efficient and unbiased lysis of microbes including Gram-positive and negative bacteria, fungus, protozoans, algae, and viruses.
- Unbiased extraction of ultra-pure DNA makes the ZymoBIOMICS™ DNA Mini Kit ideal for 16S rRNA gene sequencing, shotgun metagenomic sequencing, arrays, PCR and other sensitive applications.

Contents

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 10^9 bacterial cells, 10^8 yeast cells and 10^7 mammalian 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 unbiased 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.

Product Contents

ZymoBIOMICS™ DNA Mini Kit (Kit Size)	**D4300** (50 Preps.)	**Storage Temperature**
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)	50	Room Temp.
ZymoBIOMICS™ Lysis Solution	40 ml	Room Temp.
ZymoBIOMICS™ DNA Binding Buffer[1]	100 ml	Room Temp.
ZymoBIOMICS™ DNA Wash Buffer 1	50 ml	Room Temp.
ZymoBIOMICS™ DNA Wash Buffer 2	60 ml	Room Temp.
ZymoBIOMICS™ DNase/RNase Free Water	3 x 10 ml	Room Temp.
Zymo-Spin™ IV Spin Filters (Orange Tops)	50	Room Temp.
Zymo-Spin™ IV- HRC Spin Filters (Green Tops)	50	Room Temp.
Zymo-Spin™ IIIC- Z Columns	50	Room Temp.
Collection Tubes	200	Room Temp.
Instruction Manual	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, algae, viral, mitochondrial, and host DNA is efficiently isolated from ≤ 200 mg of mammalian feces, ≤ 250 mg soil, and 50 – 100 mg (wet weight) of fungal bacterial cells[2], biofilms and water[3].
- **Bead Beating System** – ZymoBIOMICS™ innovative lysis system enables complete homogenization/disruption of the microbial cells walls and accurate microbial DNA analysis, free of bias. To ensure unbiased lysis it is recommend that the ZymoBIOMICS™ Microbial Community Standard is used for calibration of each bead beating device (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.
- **DNA Integrity** – Generally, post bead beating, genomic DNA has an average size of 15-20 kb depending on the initial quality of the sample making it amenable to Next-Gen sequencing platforms requiring high molecular weight DNA. For optimal DNA integrity, collect samples in DNA/RNA Shield™[4].
- **DNA Recovery** – Up to 25 µg total DNA can be eluted into 100 µl (50 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** – Microcentrifuge, vortex/Disruptor Genie®, high speed cell disrupter (recommended).

Product Description

The ZymoBIOMICS™ DNA Mini Kit is designed for purifying DNA from a wide array of sample inputs (*e.g.* feces, soil, water, and biofilms), that is 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, fungus, protozoans, and algae)[1] making it ideal for microbial community profiling. Unbiased mechanical lysis of tough microbes is achieved by bead beating with the innovative ZymoBIOMICS™ ultra-high density BashingBeads™ and validated using the ZymoBIOMICS™ Microbial Community Standard[2], as shown in Figure 3. In addition, the ZymoBIOMICS™ DNA Mini kit is equipped with Zymo'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™ purification technology results in superior yields of ultra-pure DNA ideal for all downstream applications including PCR, arrays, 16S rRNA gene sequencing, and shotgun sequencing.

A schematic of the ZymoBIOMICS™ DNA Mini Kit workflow is shown below.

Innovation. Pure & Simple.™

Workflow:

Bias free lysis
Using ZymoBIOMICS™ Innovative Lysis System.[1]

Filter debris from lysate using Zymo-Spin™ IV.

Superior yields.
Simply Bind, Wash, and Elute from the Zymo-Spin™ IIIC-Z.

Complete PCR Inhibitor Removal by simply filtering the eluate with a Zymo-Spin™ HRC.

DNA is ready for all downstream applications.

Superior Yields

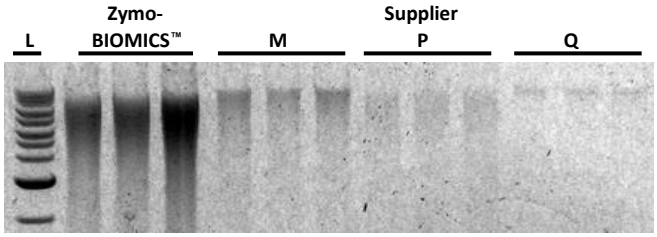


Figure 1. The **ZymoBIOMICS™ DNA Mini Kit** provides superior yields when compared to Suppliers M, P, and Q. 80 mg of feces was processed using each kit according to the manufactures' recommended protocol. DNA was eluted using 100 µl. 6 µl of each sample was analyzed in a 1.0% (w/v) agarose/ethidium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

Ultra-pure DNA from Inhibitor Rich Samples

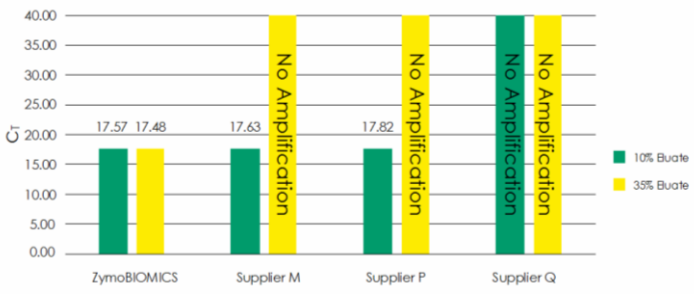


Figure 2. The **ZymoBIOMICS™ DNA Mini Kit** provides inhibitor-free DNA even when challenged with extremely inhibitor rich samples. Real-time PCR was used to evaluate eluates recovered using the **ZymoBIOMICS™ DNA Mini Kit**, or Suppliers M, P, and Q. Reaction volumes consisted of either 10% or 35% of 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.

[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 3.

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

[3] DNA is predominately 15-20 kb and amenable to next-generation sequencing techniques requiring high molecular weight DNA.

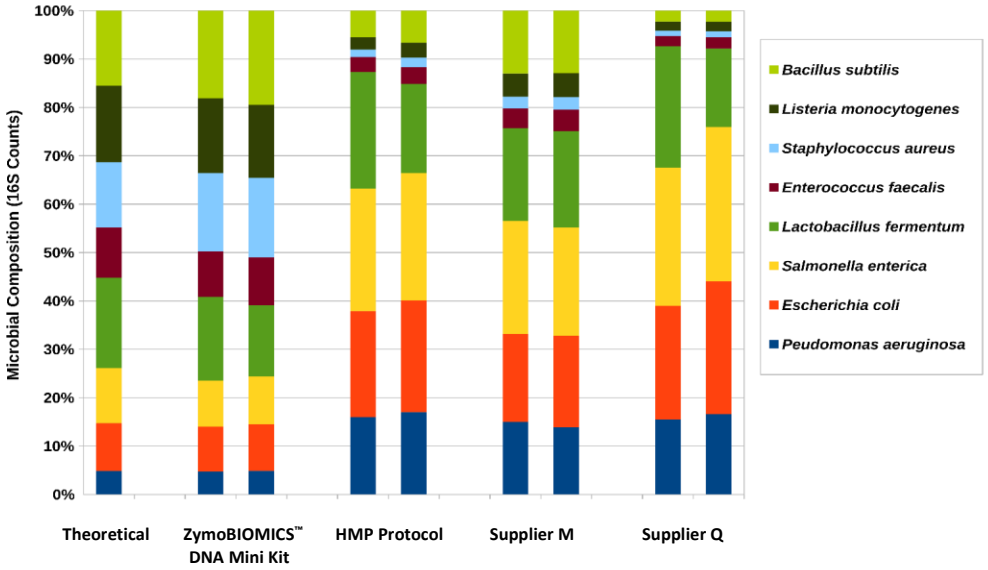
Zymo Research offers a full suite of **ZymoBIOMICS™ Services** for reliable, accurate microbial and metagenomic analyses.

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

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



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

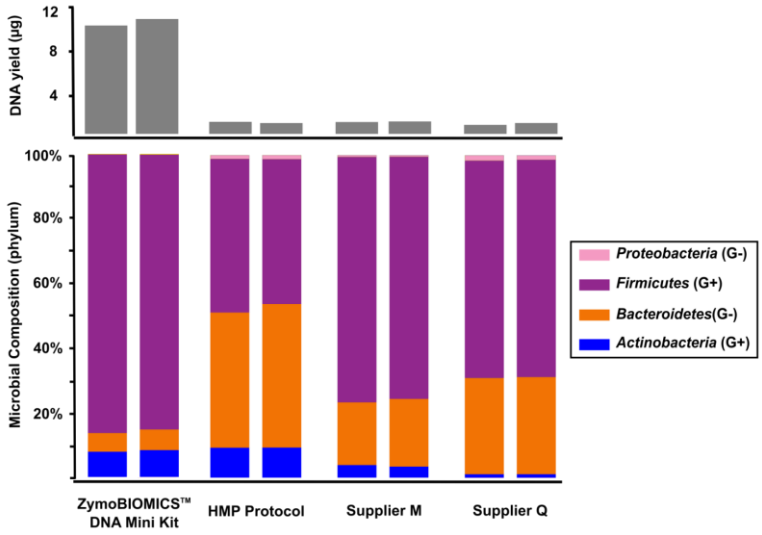


Figure 3. A) The ZymoBIOMICS™ DNA Mini Kit provides unbiased representation of the organisms extracted from the ZymoBIOMICS™ Microbial Community Standard. DNA was extracted from ZymoBIOMICS™ Microbial Community Standard using four different DNA extraction methods (ZymoBIOMICS™ DNA Mini Kit, Human Microbiome 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 Mini 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 abundance when DNA was isolated using the ZymoBIOMICS™ DNA Mini 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 Mini 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).

Protocol

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

1. Add sample to a **ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)**. Add 750 µl **ZymoBIOMICS™ Lysis Solution** to the tube. (Cap tightly to assure no leakage during bead beating.)

   If lysing samples stored in DNA/RNA Shield™ Lysis Tubes, samples can be bead beat directly inside the DNA/RNA Shield™ Lysis Tube. Do not add ZymoBIOMICS Lysis Solution and proceed to Step 2.

Sample Type	Maximum Input
Feces	200 mg
Soil	250 mg
Liquid Samples and Water[1]	250 µl
Cells (Suspended in DNA/RNA Shield™ or isotonic buffer, *e.g.* PBS)	50-100 mg (wet weight) (10^9 bacterial, 10^8 yeast cells, 10^7 mammalian cells)
Samples in DNA/RNA Shield™ (10% v/v Sample)[2]	250 µl
Swabs can be broken or cut and bead beat directly or vortexed in DNA/RNA Shield™ or isotonic buffer to be processed as a liquid sample.	

2. Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for ≥ 5 minutes.[3]

   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 Tubes (0.1 & 0.5 mm)** in a microcentrifuge at ≥ 10,000 x *g* for 1 minute.

4. Transfer up to 400 µl supernatant to a **Zymo-Spin™ IV Spin Filter (Orange Top)** (snap of base before use) in a **Collection Tube** and centrifuge at 7,000 x *g* for 1 minute.

5. Add 1,200 µl of **ZymoBIOMICS™ DNA Binding Buffer** to the filtrate in the Collection Tube from Step 4.

6. Transfer 800 µl of the mixture from Step 5 to a **Zymo-Spin™ IIIC-Z Column** in a Collection Tube and centrifuge at 10,000 x *g* for 1 minute.

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

8. Add 400 µl **ZymoBIOMICS™ DNA Wash Buffer 1** to the Zymo-Spin™ IIIC-Z Column in a new Collection Tube and centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through.

9. Add 700 µl **ZymoBIOMICS™ DNA Wash Buffer 2** to the Zymo-Spin™ IIIC-Z Column in a Collection Tube and centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through.

10. Add 200 µl **ZymoBIOMICS™ DNA Wash Buffer 2** to the Zymo-Spin™ IIIC-Z Column and centrifuge at 10,000 x g for 1 minute.

11. Transfer the Zymo-Spin™ IIIC-Z Column to a clean 1.5 ml microcentrifuge tube and add 100 µl[4] **ZymoBIOMICS™ DNase/RNase Free Water** directly to the column matrix and incubate for 1 minute. Centrifuge at 10,000 x *g* for 1 minute to elute the DNA[5, 6].

12. Preparing **Zymo-Spin™ IV-HRC Spin Filter (Green Top).**[7]
    a. Snap off the base of the Zymo-Spin™ IV-HRC Spin Filter (Green Top) and place into a clean Collection Tube. Centrifuge at 8,000 x *g* for 3 mins. Discard the flow-through.
    b. Remove the cap and add 400 µl ZymoBIOMICS™ DNase/RNase Free Water to the Zymo-Spin™ IV-HRC Spin Filter. Loosely cap Zymo-Spin™ IV-HRC Spin Filter and centrifuge at 8,000 x *g* for 2 minute.

13. Transfer the eluted DNA (Step 11) to a prepared Zymo-Spin™ IV-HRC Spin Filter in a clean 1.5 ml microcentrifuge tube. Loosely cap the Zymo-Spin™ IV-HRC Spin Filter and centrifuge at exactly 8,000 x *g* for 1 minute.

For **Technical Assistance**: 1-888-882-9682 or E-mail 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] See Appendix A for additional information on sample collection in DNA/RNA Shield™.

[3] 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.

[4] See Appendix D for additional elution information.

[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.

[7] For time savings, skip Step 12b and proceed to step 13. This alternative method does not affect downstream performance, but can affect accurate quantification using spectrophotometry.

Appendix A

Sample Collection

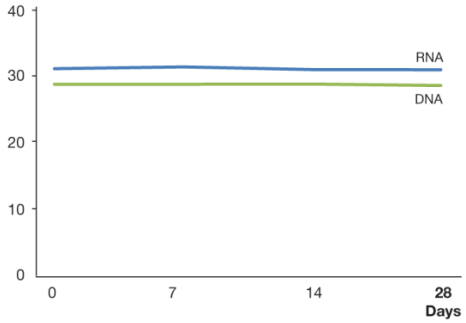
For high quality reproducible microbiomics data it is recommended that samples be collected in **DNA/RNA Shield™** to avoid bias or erroneous results due to compositional changes from nucleic acid degradation or microbial growth. DNA/RNA Shield™ provides an unbiased molecular snapshot of the sample at 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 – Simply add sample, seal and store at ambient temperature. The lysis tube is immediately ready for bead beating thereby streamlining collection to extraction transition. (Catalog No. **R1100-1-B15**)

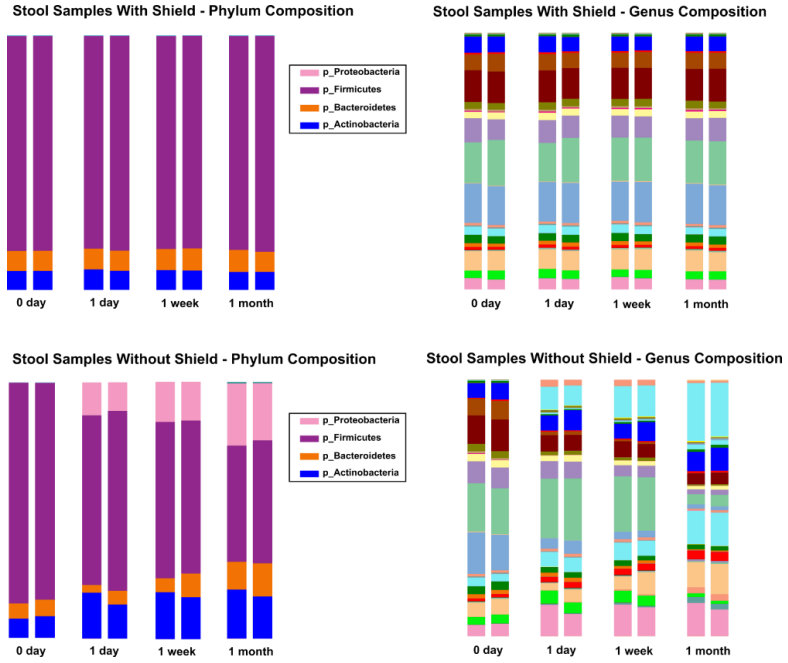
DNA/RNA Shield™ – Fecal Collection Tube – The collection device is specifically designed for easy collection and stabilization of feces. Includes a scoop built for collecting 1 gram of feces (or any other sample such as saliva or soil). (Catalog No. **R1100-9-T)**

DNA/RNA Shield™ (reagent) can be purchased separately to fill into any collection device (Catalog No. **R1100** or **R1200**). Samples should be suspended in DNA/RNA Shield™ at ≤ 10% v/v or w/v.

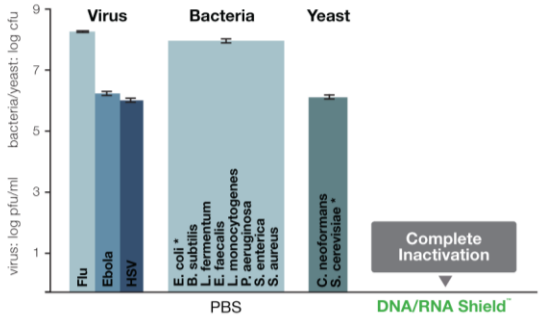
A) DNA/RNA Shield™ Preserves Nucleic Acids at Room Temperature



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



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



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 Mini 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 (Catalog No. **R1100-1-B15**)

When utilizing samples stored in DNA/RNA Shield™ Lysis Tubes, samples can be bead beat directly within the DNA/RNA Shield™ Lysis Tube with no need for addition of ZymoBIOMICS™ Lysis Solution. Simply move to Step 2 of the protocol (page 4) and bead beat according to instructions provided. The rest of the steps of the protocol (page 4) can be completed as normal.

Viruses

For unbiased metagenomic analysis of viruses it is recommended to incorporate a Proteinase K digestion following bead beating. Add 5% v/v of Proteinase K (D3001-2-5) to the lysate after Step 2 (page 4) and incubate for 30 minutes at 55˚C. Continue to Step 3 (page 4).

Cheese & Milk

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

1. Add 0.3-0.4 g of cheese or 250 µl of milk to the ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm). Add 400 µl of ZymoBIOMICS™ Lysis Solution and 350 µl of DNA/RNA Shield™ (R1100). Process cheese by bead beating until cheese is homogenized, for milk skip initial homogenization.

2. Next, add 5% v/v of Proteinase K (D3001-2-5) to the ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm) and incubate for 1 hour at 55˚C.

3. Continue on to Step 2 of the ZymoBIOMICS™ DNA Mini Kit 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 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. All other steps can be followed normally via ZymoBIOMICS™ DNA Mini Kit protocol (page 4).

Water/Air Samples

Filter samples using desired filter (not provided) prior to Step 1 (page 4). Cut the filter into small pieces, place into ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm), and Continue to Step 2. Similarly air samples that are collected on a filter can be processed by the same method.

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 Tubes (0.1 & 0.5 mm) and sealed and left inside for bead beating. Alternatively, the swab can be mixed around inside the ZymoBIOMICS™ Lysis Buffer to remove microbes from the swab before removing the swab from the tube and continuing to the bead beating step (Step 2).

Appendix C

Standardize Sample Preparation with ZymoBIOMICS™ Microbial Standards

The **ZymoBIOMICS™ Microbial Community Standard (D6300)** is a mock microbial community of defined and well characterized composition making it the perfect control for all microbiome profiling and metagenomics analyses.

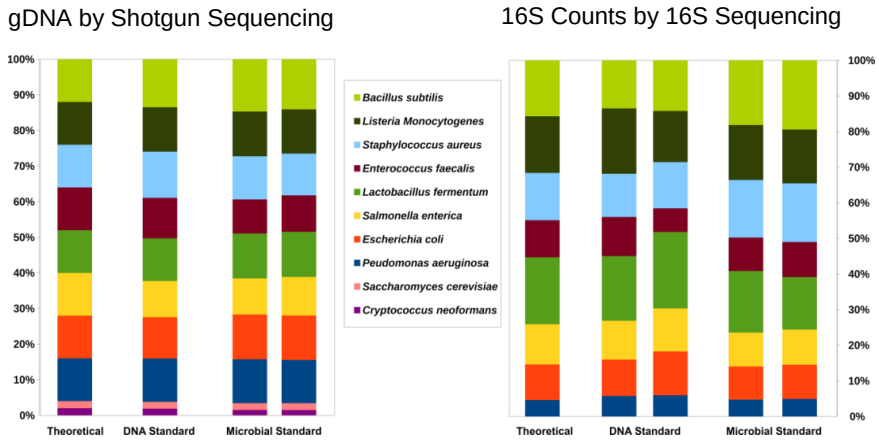
It is ideal for assessing bias of DNA extraction methods since it contains three easy-to-lyse Gram-negative bacteria (*e.g. Escherichia coli*), five tough-to-lyse Gram-positive bacteria (*e.g. Listeria monocytogenes*), and two tough-to-lyse yeasts (*e.g. Saccharomyces cerevisiae*).

Bead Beating Device Calibration Protocol:
Zymo 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 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 (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.

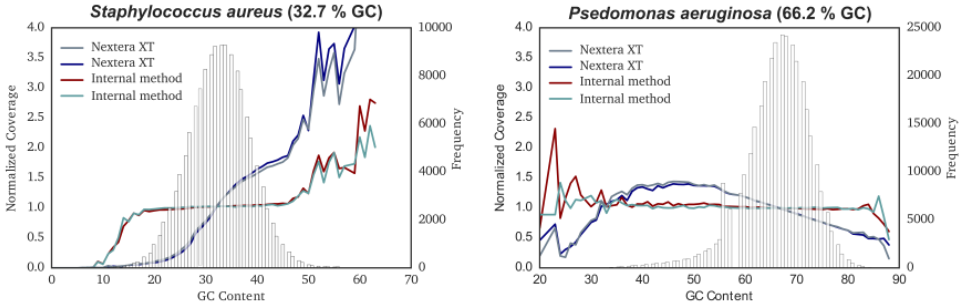


Species	GC %	Gram Stain	gDNA Abun. (%)
Pseudomonas aeruginosa	66.2	-	12
Escherichia coli	56.8	-	12
Salmonella enterica	52.2	-	12
Lactobacillus fermentum	52.8	+	12
Enterococcus faecalis	37.5	+	12
Staphylococcus aureus	32.7	+	12
Listeria monocytogenes	38.0	+	12
Bacillus subtilis	43.8	+	12
Saccharomyces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

Figure 1. 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.

Figure 2. A) Use ZymoBIOMICS™ Microbial Standards for assessing GC-Bias in Shotgun Metagenomics

Figure 2. B) Perfect for tracking PCR Chimera in 16S rRNA Gene Sequencing



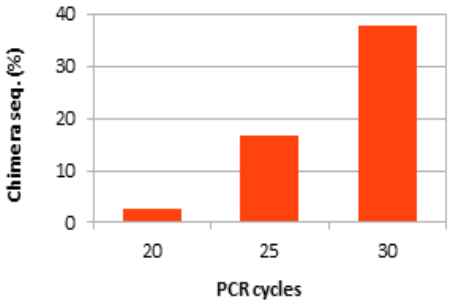


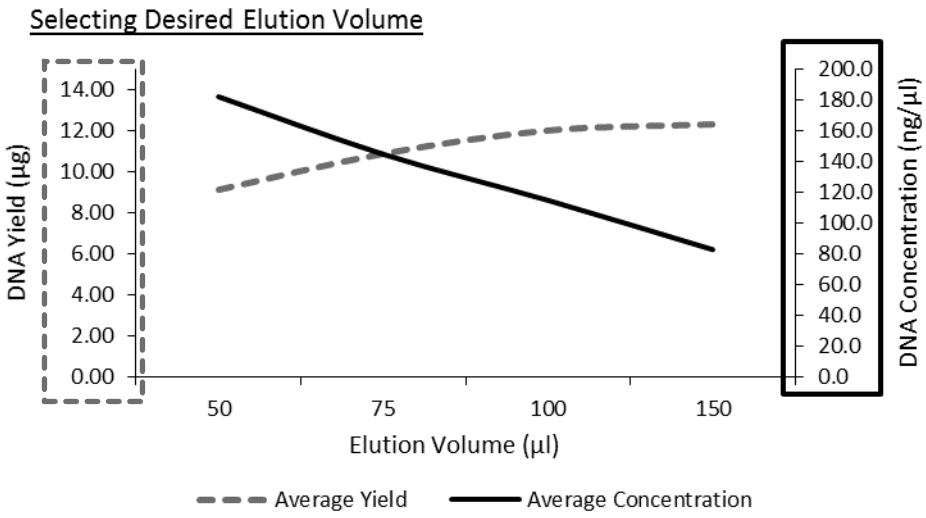
Figure 2.
A) Library preparation for shotgun metagenomic sequencing was performed in two different ways: one by Illumina® Nextera® XT kit and one by an in-house method. Shotgun sequencing was performed on Illumina® MiSeq™ with paired-end sequencing (2 x 150 bp). Raw reads were mapped to the 10 microbial 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 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

Troubleshooting:

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

DNA Elution Guide



The Relationship between Elution Volume, DNA Yield, and DNA Concentration Using *Bacillus Subtilus* Culture. Using a smaller elution volume results in higher concentrations of DNA samples, but with reduced yields. Using a larger elution volume results in higher DNA yields, but at a reduced concentration. Choose an elution volume that best fits your individual application. Suggested elution volume is 100 µl.

Troubleshooting (Continued):

Problem	Possible Causes and Suggested Solutions
Background Contamination	• Clean workspace, centrifuge, and pipets with 10% bleach routinely to avoid contamination. • Use of kit in exposed environment without proper filtration. Check pipets, pipet 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. 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.) See Appendix C for details. • When using FastPrep™-24 or similar devices run max speed for 5 minutes to ensure unbiased lysis. (6.5m/s on FastPrep®-24).
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 column in the next step. Input • If the lysate does not pass through the column or is extremely viscous, use less input material. Too much input could cause cellular debris to overload the column and not pass through properly. • Consult the Sample Type table on Page 4 for information on your particular input limit based on sample.

Low DNA Yield (Continued)

Binding Step

- Ensure that binding buffer is completely mixed with lysate before proceeding with loading step. This could cause loss of DNA if not properly mixed.

Elution Procedure

- Ensure the **ZymoBIOMICS™ DNase/RNase Free Water** hydrates the matrix for at least 1 minute before centrifugation.
- Make sure **Zymo-Spin™ IV–HRC** column cap is loosely tightened during the “wash” step. This can create a vacuum and cause lower elution volume.
- To increase yields, heat the **ZymoBIOMICS™ DNase/RNase Free Water** to 60ºC before use. You can also reload the eluate a second time, incubate at room temperature for 3 minutes, and centrifuge again to increase yield without further dilution.

Ordering Information

Product Description	Catalog No.	Kit Size (Preps.)
ZymoBIOMICS™ DNA Mini Kit	D4300	50
ZymoBIOMICS™ DNA Mini Kit *(BashingBead Lysis Tubes Not Included)*	D4301	50

For Individual Sale	Catalog No.	Size
ZR BashingBead™ Lysis Tubes (0.1 & 0.5 mm)[1]	S6012-50	50
ZymoBIOMICS™ Lysis Solution	D4300-1-40	40 ml
ZymoBIOMICS™ DNA Binding Buffer	D4300-2-100	100 ml
ZymoBIOMICS™ DNA Wash Buffer 1	D4300-3-50	50 ml
ZymoBIOMICS™ DNA Wash Buffer 2	D4300-4-10	50 ml
ZymoBIOMICS™ DNase/RNase Free Water	D4302-5-10	10 ml
Zymo-Spin™ IV Spin Filters (Orange Tops)	C1007-50	50
Zymo-Spin™ IV- HRC Spin Filters (Green Tops)	C1010-50	50
Zymo-Spin™ IIIC- Z Columns	C1006-50-G-ZB	50
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1,000

Sample Collection	Catalog No.	Size
DNA/RNA Shield™ - Lysis Tube	R1100-1-B15	50 tubes
DNA/RNA Shield™ – Fecal Collection Tube	R1100-9-T	10 tubes
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™ DNA Mini Kit is for research use only. ZymoBIOMICS™ DNA Mini 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.