ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoBIOMICS™ Microbial Community Standard

Catalog No. D6300

Highlights

- Mock microbial community of well-defined composition
- Ideal for quality control of microbiome profiling and metagenomic analyses
- Perfect for assessing bias of DNA extraction methods since it contains a mixture of both tough- and easy-to-lyse microbes

Contents

ZYMO RESEARCH CORP.

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Notes: Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product, please call 1-888-882-9682.

Product Contents

	D6300 (10 Preps.)	Storage Temperature
ZymoBIOMICS™ Microbial Community Standard	0.75 mL	-80 °C

Specifications

- **Source:** A mixture of ten inactivated microorganisms (bacterial and fungal).
- **Storage solution**: cells were suspended in DNA/RNA Shield™ (Cat. No. R1100-50).
- **Impurity level**: < 0.01% foreign microbial DNA
- **Composition:** Table 1 shows the theoretical microbial composition of the standard. The real composition might fluctuate slightly from batch to batch. The real microbial composition of each lot was measured by shotgun metagenomics sequencing; the data can be accessed with the lot number (printed on the labels of the tubes) by the following link: http://www.zymoresearch.com/microbiomics/microbial-standards/zymobiomics-microbial-community-standards.

Table 1: Microbial Composition

Species	Theoretical Composition (%)	
	Genomic DNA	16S rRNA[1]
Pseudomonas aeruginosa	12.0	4.6
Escherichia coli	12.0	10.0
Salmonella enterica	12.0	11.3
Lactobacillus fermentum	12.0	18.8
Enterococcus faecalis	12.0	10.4
Staphylococcus aureus	12.0	13.3
Listeria monocytogenes	12.0	15.9
Bacillus subtilis	12.0	15.7
Saccharomyces cerevisiae	2.0	-
Cryptococcus neoformans	2.0	-

[1] The theoretical composition in terms of 16S rRNA gene abundance was calculated from theoretical genomic DNA composition with the following formula: 16S copy number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp) × 16S copy number per genome.

Product Description

Microbial composition profiling techniques powered by next-generation sequencing are becoming routine in microbiomics and metagenomics studies. It is well know that these analytical techniques can suffer from bias and errors in every step of the workflow, including DNA extraction, library preparation, sequencing and bioinformatics analysis. Therefore, standardization is critical for minimizing bias and quality control of the entire microbiomics workflows.

ZymoBIOMICS™ Microbial Community Standard is a mock microbial community consisting of eight bacterial and two fungal strains. It includes 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*) (Table 1). Seven of these strains are known human pathogens and have been fully inactivated with DNA/RNA Shield™ (Cat. No. R1100-50). The GC content[1] of the contained genomes covers a range from 15% to 85%. It was constructed by pooling pure cultures of the ten microbial strains. The cells and DNA content of each pure culture were quantified before pooling. Cultures were mixed to a predetermined composition (Table 1). The actual microbial composition was measured using next-generation sequencing techniques (Table 1) after extracting the genomic DNA with the ZymoBIOMICS™ DNA Mini kit (D4300).

The microbial standard is accurately characterized and contains negligible impurities (< 0.01%). This enables it to be used to expose artifacts, errors, and bias in microbiomics or metagenomics workflows. Serving as a defined input from the beginning, this standard can be used to guide construction and optimization of entire workflows and as a quality control for inter-lab studies. Benchmarking with this standard, we found that most cited DNA extraction methods are significantly biased (Figure 1).

Details regarding the ten microbial strains (including species name, genome size, ploidy, average GC content, 16S/18S copy number, phylogeny) can be found in Table 2. The 16S/18S rRNA sequences (FASTA format) and genomes (FASTA format) of these strains are available at: https://s3.amazonaws.com/zymo-files/BioPool/ZymoBIOMICS.STD.genomes.ZR160406.zip. Feel free to contact us if we can help analyze the sequencing data generated from this standard[2].

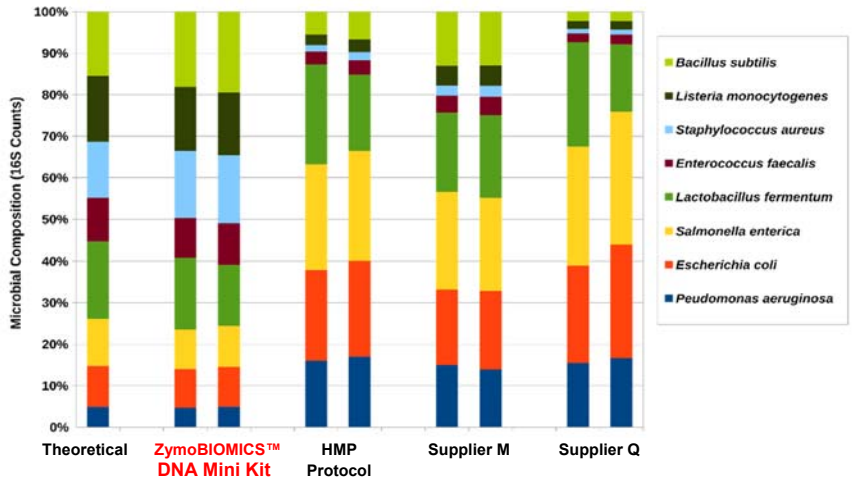

Figure 1. Benchmarking DNA extraction processes with ZymoBIOMICS™ Microbial Community Standard. DNA was extracted from ZymoBIOMICS™ Microbial Community Standard using the four different DNA extraction methods (ZymoBIOMICS™ DNA Mini Kit, Human Microbiome Project fecal DNA extraction protocol, a DNA extraction kit from Supplier M, and a fecal DNA extraction kit from 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 contained in the standard. Only ZymoBIOMICS™ DNA Mini Kit provides unbiased profiles in this comparison.

Notes:
[1] GC content can cause bias of sequencing coverage in PCR-based library preparation processes of shotgun sequencing.

[2] We can use in-house pipelines to help assess the extent of bias and artifacts in the sequencing data of this standard.

Notes:
* 18S rRNA gene copy numbers in a haploid genome of the two strains of *Saccharomyces cerevisiae* and *Cryptococcus neoformans* were estimated based on read depth information from mapping shot-gun sequencing data.

Table 2: Strain Information

Species	Genome Size (Mb)	Ploidy	GC Content (%)	16/18S Copy Number	Gram Stain
Pseudomonas aeruginosa	6.77	1	66.2	4	-
Escherichia coli	5.47	1	56.8	7	-
Salmonella enterica	4.83	1	52.2	7	-
Lactobacillus fermentum	2.08	1	52.8	5	+
Enterococcus faecalis	3.01	1	37.5	4	+
Staphylococcus aureus	2.93	1	32.7	5	+
Listeria monocytogenes	2.95	1	38.0	6	+
Bacillus subtilis	3.98	1	43.8	8	+
Saccharomyces cerevisiae	13.3	2	38.4	109*	Yeast
Cryptococcus neoformans	18.9	2	48.2	60*	Yeast

Table 2 continued

Species	NCBI Phylogeny Database
Pseudomonas aeruginosa	*Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas aeruginosa* group
Escherichia coli	*Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia*
Salmonella enterica	*Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Salmonella*
Lactobacillus fermentum	*Bacteria; Firmicutes; Bacilli; Lactobacillales; Lactobacillaceae; Lactobacillus*
Enterococcus faecalis	*Bacteria; Firmicutes; Bacilli; Lactobacillales; Enterococcaceae; Enterococcus*
Staphylococcus aureus	*Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus*
Listeria monocytogenes	*Bacteria; Firmicutes; Bacilli; Bacillales; Listeriaceae; Listeria*
Bacillus subtilis	*Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus; Bacillus subtilis* group
Saccharomyces cerevisiae	*Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycetaceae; Saccharomyces*
Cryptococcus neoformans	*Eukaryota; Opisthokonta; Fungi; Dikarya; Basidiomycota; Agaricomycotina; Tremellomycetes; Tremellales; Tremellaceae; Filobasidiella; Filobasidiella/Cryptococcus neoformans* species complex

Protocol

1. Thaw ZymoBIOMICS™ Microbial Community Standard at room temperature and gently mix by pipetting up and down before use (avoid bubbling).

2. Use 75 µL of ZymoBIOMICS™ Microbial Community Standard for each DNA extraction. For unbiased and efficient extraction we recommend using mechanical lysis as featured in Zymo Research's microbial DNA extraction kits[1]. Expected yield is approximately 2 µg DNA per preparation when using the recommended kits[3].

Note: *Unbiased results were obtained with the recommended kits after homogenizing the sample for ≥ 5 minutes using the FastPrep® -24 at max speed or ≥ 20 minutes when using the Disruptor Genie™ at max speed. The duration of homogenization (bead bashing) will vary depending on the homogenization device and needs to be optimized by the end-user.*

Ordering Information[2]

Product Description	Catalog No.	Kit Size (Preps.)
ZymoBIOMICS™ Microbial Community Standard	D6300	10

Related Products

Product Description	Catalog No.	Kit Size (Preps.)
ZymoBIOMICS™ DNA Mini Kit	D4300	50
ZymoBIOMICS™ Microbial Community DNA Standard (200 ng)	D6305	200 ng/20 µl
ZymoBIOMICS™ Microbial Community DNA Standard (2000 ng)	D6306	2000 ng/20 µl

Sample Collection	Catalog No.	Size
DNA/RNA Shield™ – Lysis Tube	R1100-1-B15	50
DNA/RNA Shield™ – Fecal Collection Tube	R1100-9-T	10
DNA/RNA Shield™ – Swab Collection Tube	R1100-1-ST-10	10
DNA/RNA Shield™	R1100-50 R1100-250	50 ml 250 ml
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml

Notes:
[1] This microbial standard contains several tough-to-lyse microbes; therefore, to extract DNA from this standard, we strongly recommend using ZymoBIOMICS™ DNA Mini kit (D4300), ZR Fungal/Bacteria DNA MiniPrep™ (Cat. No. D6005), ZR Fecal DNA MiniPrep™ (Cat. No. D6010) or ZR Soil DNA MiniPrep™ (Cat. No. D6001). These kits feature a unique lysis matrix that contains our ultra-high-density BashingBeads™, which provides unbiased lysis of bacteria and fungi for accurate microbial composition profiling.

[2] You can place your order by the following methods:

1) Online Orders: www.zymoresearch.com
2) Email Orders: orders@zymoresearch.com
3) Fax Orders: 1-949-266-9452
4) Phone Orders: 1-888-882-9682 (Toll Free USA Only)

* If you have any questions, please call Zymo Research Customer Service at: (949) 679-1190