ZYMO RESEARCH

Microbiomics
Made Simple™

ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution)

Assess the performance and limits of detection in microbiome NGS workflows

Highlights

- **Log abundance distribution**: used to assess detection limits of as little as the DNA of three microbes.
- **Accurate composition**: allows for benchmarking and validation of NGS microbiome workflows.
- **Negligible impurity:** guaranteed to contain <0.01% foreign microbial DNA.

Catalog Number:
D6311

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

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Product Contents

ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution)	D6311 (220 ng)	Storage Temperature
ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution)	20 µl	-20°C

Specifications

- **Source –** eight bacteria (3 Gram-negative and 5 Gram-positive) and 2 yeasts.
- **Reference Genomes and 16S & 18S rRNA Genes** https://s3.amazonaws.com/zymo-files/BioPool/ZymoBIOMICS.STD.refseq.v3.zip
- **Storage Solution –** 10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0
- **DNA Concentration –** 11 ng/µl
- **Impurity Level –** <0.01% foreign microbial DNA
- **Relative Abundance Deviation in Average –** <30%
- **Microbial Composition –** Table 1 shows the theoretical microbial composition of the standard.

The microbial composition of each lot was measured by shotgun metagenomic sequencing post mixing. The results (including the composition, impurities and abundance deviation) can be accessed through the Certificate of Analysis based on the lot number (printed on tube level) by the following link: https://www.zymoresearch.com/pages/certificate-of-analysis.

Table 1. Microbial Composition

Species	Theoretical Composition (%)				
	Genomic DNA	16S Only[1]	16S & 18S[1]	Genome Copy[2]	Cell Number[3]
Listeria monocytogenes	89.1	95.9	91.9	94.8	94.9
Pseudomonas aeruginosa	8.9	2.8	2.7	4.2	4.2
Bacillus subtilis	0.89	1.2	1.1	0.7	0.7
Saccharomyces cerevisiae	0.89	NA	4.1	0.23	0.12
Escherichia coli	0.089	0.069	0.066	0.058	0.058
Salmonella enterica	0.089	0.07	0.067	0.059	0.059
Lactobacillus fermentum	0.0089	0.012	0.012	0.015	0.015
Enterococcus faecalis	0.00089	0.00067	0.00064	0.001	0.001
Cryptococcus neoformans	0.00089	NA	0.0014	0.00015	0.00007
Staphylococcus aureus	0.000089	0.0001	0.0001	0.0001	0.0001

[1] The theoretical composition in terms of 16S (or 16S & 18S) rRNA gene abundance was calculated from theoretical genomic DNA composition with the following formula: 16S/18S copy number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp) × 16S/18S copy number per genome. Use this as reference when performing 16S targeted sequencing.

[2] The theoretical composition in terms of genome copy number was calculated from theoretical genomic DNA composition with the following formula: genome copy number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp). Use this as reference when inferring microbial abundance from shotgun sequencing data based on read depth/coverage.

[3] The theoretical composition in terms of cell number was calculated from theoretical genomic DNA composition with the following formula: cell number = total genomic DNA (g) × unit conversion constant (bp/g) / genome size (bp)/ploidy.

Product Description

ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution) is a mixture of genomic DNA of eight bacterial and two fungal strains. The microbial standard is accurately characterized and contains negligible impurity (<0.01%). It was constructed by pooling DNA extracted from pure cultures of the ten microbial strains[1]. The DNA from each pure culture was quantified before pooling. After mixing, the microbial composition was confirmed using NGS-based sequencing (Figure 1). This microbial standard can be used to assess the performance of microbiomics workflows and can also be used as a positive control for the routine QC purpose.

DNA samples were mixed to create log-distributed abundance (Table 1), which allows the user to easily assess the detection limit of a microbiomics workflow. 1 µl of the standard (11 ng DNA) can be used to assess the detection limit of as low as the abundance of *Staphylococcus aureus contained in the standard*, which is 0.000089% by relative abundance or is equivalent to the amount of DNA from 3 cells. If desired, the standard can also be used to mix with human genomic DNA, e.g. Human HCT116 DKO DNA (#D5014-1), to mimic a human microbiome sample.

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 from the link below. Feel free to contact us if you need help analyzing sequencing data generated from this standard[2].

Reference Genome Download:
https://zymo-files.s3.amazonaws.com/BioPool/ZymoBIOMICS.STD.refseq.v3.zip

Background on the Need for Microbiome Standards: Microbial composition profiling techniques powered by next-generation sequencing are becoming routine in microbiomics and metagenomics studies. It is well known 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. To assess the performance of different microbiomics workflows, there is an urgent need in the field for reliable reference materials, *e.g.* a mock microbial community with defined composition.

1 Genomic DNA from each culture was extracted and quantified before mixing; this DNA standard is not a direct derivative and manufactured independently of the ZymoBIOMICS® Microbial Community Standard II (#D6310).
2 We can use in-house pipelines to help assess the extent of bias in the sequencing data of this standard.

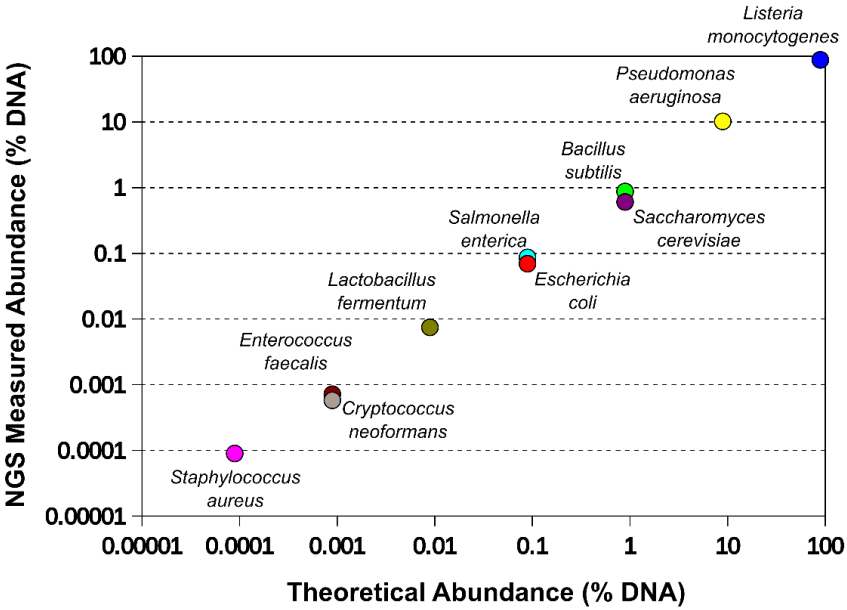

Figure 1. The microbial composition of the standard measured by NGS shotgun sequencing as compared to the defined composition. After mixing, the microbial composition of the standard was confirmed using deep Illumina shotgun sequencing. Briefly, library preparation was performed using an in-house protocol. Shotgun sequencing was performed using Illumina HiSeq™ or MiSeq™. Microbial abundance was estimated based on the number of reads that were mapped to references genomes of the organisms.

Table 2. Strain Information

Species	NRRL Accession	Genome Size (Mb)	Ploidy	G/C (%)	16/18S Copy No.	Gram Stain
Pseudomonas aeruginosa	B-3509	6.792	1	66.2	4	-
Escherichia coli	B-1109	4.875	1	46.7	7	-
Salmonella enterica	B-4212	4.760	1	52.2	7	-
Lactobacillus fermentum	B-1840	1.905	1	52.4	5	+
Enterococcus faecalis	B-537	2.845	1	37.5	4	+
Staphylococcus aureus	B-41012	2.730	1	32.9	6	+
Listeria monocytogenes	B-33116	2.992	1	38.0	6	+
Bacillus subtilis	B-354	4.045	1	43.9	10	+
Saccharomyces cerevisiae	Y-567	12.1	2	38.3	109[1]	Yeast
Cryptococcus neoformans	Y-2534	18.9	2	48.3	60[2]	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

1 18S rRNA gene copy numbers in a haploid genome of the two strains of *S. cerevisiae* and *C. neoformans* were estimated based on read depth information from mapping shotgun sequencing data.

Protocol

1. Thaw the standard on ice. Once thawed, pulse vortex the standard, then centrifuge briefly to settle the liquid.

2. The amount of DNA used depends on the library preparation process being evaluated. Example quantities are shown below:

Table 3. Typical DNA Input for Different Library Prep Processes

Library Prep Method	DNA Input (ng)
16S Amplicon Libraries	10
Illumina DNA Prep	1
Illumina TruSeq® Nano	>200
Illumina TruSeq® PCR-free	2000
KAPA® HyperPlus	1 - 2000

Appendices

Appendix A: Bioinformatics Analysis Recommendations

Assessing accuracy of taxonomy identification

A fundamental goal in microbiome studies is to identify what microbes are present in a sample. After analyzing this microbiome standard using a workflow that includes wet-lab processing and dry-lab interpretation, the taxa identified can be compared with the taxonomy information of the ten strains included in the standard (Table 2). This allows a performance assessment of a workflow regarding the limit of the taxonomy resolution, false positives, and false negatives. False positives can be caused by contaminations from wet-lab processes, chimeric sequences during library prep, sequencing errors, demultiplexing errors and defects in bioinformatics analysis. We certify that the impurity level of the standard is <0.01% (by DNA abundance). Therefore, it can be concluded that any alien taxa present at >0.01% (by DNA abundance) in the standard is introduced artificially by the user's workflow. The detection limit of a workflow can be easily determined by checking what strains are detected in the microbiome standard as their abundance follows log distribution.

Assessing bias in composition profiling

To assess composition bias, compare the composition profile determined by the user's workflow to the defined composition shown in Table 1. Both wet-lab and dry-lab processes can introduce bias. To determine the quality of a wet-lab process, an accurate/unbiased dry-lab analysis method is needed to interpret the sequencing data from the standard. A straightforward and accurate method to infer the microbial composition from sequencing data of our microbiome standard is through direct read-mapping against reference genomes (or against reference 16S & 18S sequences in the case of targeted sequencing). The reference sequences of this microbiome standards can be found in the Specifications.

Note: *Bacterial strains that are phylogenetically distant can potentially share highly similar sequences in their genomes, e.g. ribosomal RNA sequences and conserved single-copy genes. In the process of direct read mapping, the presence of these highly homologous regions can cause reads that are derived from high-abundance microbes to be assigned to low-abundance microbes, resulting in the overestimation of low-abundance microbes in the standard. One way to overcome this issue is to use a mapping tool that can choose to ignore reads that map to more than one genome. Another way to address this problem is to filter these highly conserved sequences from the reference genomes. Please contact us if you need assistance.*

Appendix B: Additional Strain Information

Species	NRRL Accession NO.	NCBI Reference Accession No.	Strain Name[1]
Bacillus subtilis	B-354	CP118021 CP118022	*Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 ATCC 6633=NRRL B-209=NRS-231=PCI 219
Cryptococcus neoformans	Y-2534	JAQZRY000000000	*Cryptococcus deneoformans* T. Boekout & F. Hagen (2014) 32045=ATCC 32719=CBS 132=CCRC 20528=CCY 17-1-2=DBVPG 6010=IFO 0608=IGC 3957=NRRL Y-8347=PYCC 3957
Enterococcus faecalis	B-537	CP117970	*Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 ATCC 7080
Escherichia coli	B-1109	CP117971 CP117972	Castellani and Chalmers 1919, 01485cm
Lactobacillus fermentum	B-1840	CP132481	*Lactobacillus fermentum* Beijerinck 1901 19lc3=ATCC 14931=BCRC 12190=CCUG 30138=CECT 4007=CIP 102980=DSM 20052=IFO 15885=JCM 1173=KCTC 3112=LMG 6902=NBRC 15885=NCDO 1750=NCIMB 11840=NRIC 1752=NRRL B-4524.
Listeria monocytogenes	B-33116	CP117973	*Listeria monocytogenes* (Murray et al. 1926) Pirie 1940 2847=ATCC 19117
Pseudomonas aeruginosa	B-3509	CP117974 CP117975	*Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900 ATCC 15442=NCIB 10421=Pdd-10
Saccharomyces cerevisiae	Y-567	JAQZRZ000000000	*Saccharomyces cerevisiae* Meyen ex E. C. Hansen (1883) ATCC 9763=CBS 2978=CBS 5900=CCY 21-4-48=CCY 21-4-54=NCTC 10716=NCTC 7239=NCYC 87=Pattee 6=PCI M-50
Salmonella enterica	B-4212	CP117976 CP117977 CP117978	*Salmonella enterica* subspecies *enterica*, Castellani and Chalmers 1919, TA1536
Staphylococcus aureus	B-41012	CP117979 CP117981	*Staphylococcus aureus* Rosenbach 1884

1 The strain information was extracted from the website of the Agricultural Research Service Culture Collection (NRRL, https://nrrl.ncaur.usda.gov/).

Ordering Information

Product Description	Catalog No.	Size
ZymoBIOMICS® Microbial Community DNA Standard II (Log Distribution)	D6311	220 ng

Related Products	Catalog No.	Amount
ZymoBIOMICS® Microbial Community Standard	D6300	10 preps.
ZymoBIOMICS® Microbial Community DNA Standard (200ng)	D6305	200 ng
ZymoBIOMICS® Microbial Community DNA Standard (2000ng)	D6306	2000 ng
ZymoBIOMICS® Microbial Community Standard II (Log Distribution)	D6310	10 preps.
ZymoBIOMICS® Spike-in Control I (High Microbial Load)	D6320 D6320-10	25 preps. 250 preps.
ZymoBIOMICS® Spike-in Control II (Low Microbial Load)	D6321 D6321-10	25 preps. 250 preps.
ZymoBIOMICS® HMW DNA Standard	D6322	5000 ng
ZymoBIOMICS® Gut Microbiome Standard	D6331	10 preps.

Complete Your Workflow

✓ To collect and transport microbiome samples at ambient temperatures:

DNA/RNA Shield™ and Collection Devices		
R1100	DNA/RNA Shield™ Reagent	50 ml, 250 ml
R1200	DNA/RNA Shield™ Reagent (2x Concentrate)	25 ml, 125 ml
R1101	DNA/RNA Shield™ Fecal Collection Tube	10 pack
R1150	DNA/RNA Shield™ Blood Collection Tube	50 pack
R1160	DNA/RNA Shield™ SafeCollect Swab Collection Kit	1 ml, 2 ml
R1211	DNA/RNA Shield™ SafeCollect Saliva Collection Kit	2 ml

✓ Unbiased and inhibitor-free DNA and RNA extraction for microbiome profiling, available in a variety of formats to suit your needs:

ZymoBIOMICS® DNA and RNA Kits		
D4300	ZymoBIOMICS® DNA Miniprep Kit	50 preps
D4301	ZymoBIOMICS® DNA Microprep Kit	50 preps
D4302	ZymoBIOMICS® 96 MagBead DNA Kit	2 x 96 preps
R2001	ZymoBIOMICS® RNA Miniprep Kit	50 preps
R2137	ZymoBIOMICS® MagBead RNA Kit	96 preps
R2002	ZymoBIOMICS® DNA/RNA Miniprep Kit	50 preps
R2135	ZymoBIOMICS® MagBead DNA/RNA Kit	96 preps

✓ Ultra-streamlined workflows offering the fastest targeted 16S library prep available, with only 30 minutes of hands-on time and no tedious normalization required:

Quick-16S/ITS™ Plus NGS Library Prep Kits		
D6421	*Quick*-16S® Plus NGS Library Prep Kit (V3-V4)	768 Indexes
D6424	*Quick*-ITS® Plus NGS Library Prep Kit (ITS2)	384 Indexes
D6430	*Quick*-16S® Plus NGS Library Prep Kit (V4)	384 Indexes
D6434	*Quick*-16S® Plus NGS Library Prep Kit (V1-V2)	96 Indexes
D6440	*Quick*-16S® Plus NGS Library Prep Kit (V1-V3)	96 Indexes

Notes

Notes

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