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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

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- Trockeneiszuschlag
- Gefahrgutzuschlag
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ZYMO RESEARCH

Microbiomics
Made Simple™

ZymoBIOMICS® Gut Microbiome Standard

Assess bias and errors in NGS-based microbial composition profiling workflows

Highlights

- **True to Life:** comprised of 21 different strains, designed to mimic the human gut microbiome.
- **Accurate composition:** allows for benchmarking and validation of NGS-based microbiome workflows.
- **Cross kingdom representation:** includes bacteria, archaea, and fungi.

Catalog Number:
D6331

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

tech@zymoresearch.com www.zymoresearch.com Toll Free: (888) 882-9682

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Product Contents

ZymoBIOMICS® Gut Microbiome Standard	D6331 (10 preps.)	Storage Temperature[1]
ZymoBIOMICS® Gut Microbiome Standard	750 µl	-80°C

Specifications

- **Biosafety –** this product is not biohazardous as microbes have been fully inactivated.
- **Reference Genomes and 16S & 18S rRNA Genes –** https://s3.amazonaws.com/zymo-files/BioPool/D6331.refseq.zip
- **Storage Solution –** 2X DNA/RNA Shield™ (R1200-125)
- **Total Cell Concentration –** ~3.94 x 10^9 cells/ml
- **Impurity Level –** <0.01% foreign microbial DNA
- **Relative Abundance Deviation in Average –** <15%
- **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.

1 For short-term storage or regular use, -20°C may be used.

Table 1. Microbial Composition

Species	Theoretical Composition[1] (%)				
	Genomic DNA	16S Only[1]	16S & 18S	Genome Copy	Cell Number
Faecalibacterium prausnitzii	14	17.63	15.96	14.77	14.82
Veillonella rogosae	14	15.87	14.37	19.94	20.01
Roseburia hominis	14	9.89	8.95	12.43	12.47
Bacteroides fragilis	14	9.94	9.00	8.33	8.36
Prevotella corporis	6	4.98	4.51	6.26	6.28
Bifidobacterium adolescentis	6	8.78	7.95	8.83	8.86
Fusobacterium nucleatum	6	7.49	6.79	7.53	7.56
Lactobacillus fermentum	6	9.63	8.72	9.68	9.71
Clostridioides difficile	1.5	2.62	2.37	1.10	1.10
Akkermansia muciniphila	1.5	0.97	0.87	1.62	1.62
Methanobrevibacter smithii	0.1	0.066	0.060	0.17	0.17
Salmonella enterica	0.01	0.009	0.008	0.007	0.0065
Enterococcus faecalis	0.001	0.0009	0.0008	0.0011	0.0011
Clostridium perfringens	0.0001	0.0002	0.0002	0.00009	0.00009
Escherichia coli (JM109)	2.8	2.53	2.29	1.82	1.83
Escherichia coli (B-3008)	2.8	2.53	2.29	1.82	1.82
Escherichia coli (B-2207)	2.8	2.29	2.07	1.64	1.65
Escherichia coli (B-766)	2.8	2.31	2.09	1.66	1.66
Escherichia coli (B-1109)	2.8	2.46	2.23	1.77	1.77
Candida albicans	1.5	N/A	3.11	0.31	0.16
Saccharomyces cerevisiae	1.4	N/A	6.35	0.32	0.16

[1] The reference composition will depend on the sequencing method. For 16S targeted sequencing, use the 16S copy percentage as a reference. For shotgun sequencing data based on read depth/coverage, use the genome copy percentage as a reference.

Product Description

ZymoBIOMICS® Gut Microbiome Standard is a mixture of 18 bacterial strains, 2 fungal strains, and 1 archaeal strain in staggered abundances to mimic a true gut microbiome. The standard presents multiple challenges for NGS pipelines, such as tough-to-lyse Gram-positive bacteria (e.g. Roseburia hominis) to test lysis efficiency, genomes with a wide range of GC content[1] to test sequencing coverage bias, low-abundance pathogenic organisms for detection limit assessment and 5 different strains of *E. coli* to test taxonomic resolution. These challenge points can be used to expose artifacts, errors, and bias in microbiomics or metagenomics workflows. Serving as a defined input, this standard can be used to guide construction and optimization of entire workflows or as a quality control tool for inter-lab studies.

The microbial standard is accurately characterized and contains negligible impurity (< 0.01%). It was constructed by pooling cells from pure cultures of 21 microbial strains. The cells from each pure culture were quantified before pooling. After mixing, the microbial composition was confirmed using NGS-based sequencing (Figure 1).

Details regarding the microbial strains (including species name, genome size, average GC content, 16S/18S copy number) 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 we can help to analyze sequencing data generated from this standard.

Reference Genome Download:
https://s3.amazonaws.com/zymo-files/BioPool/D6331.refseq.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 GC content can cause bias of sequencing coverage in PCR-based library prep workflows of shotgun sequencing.

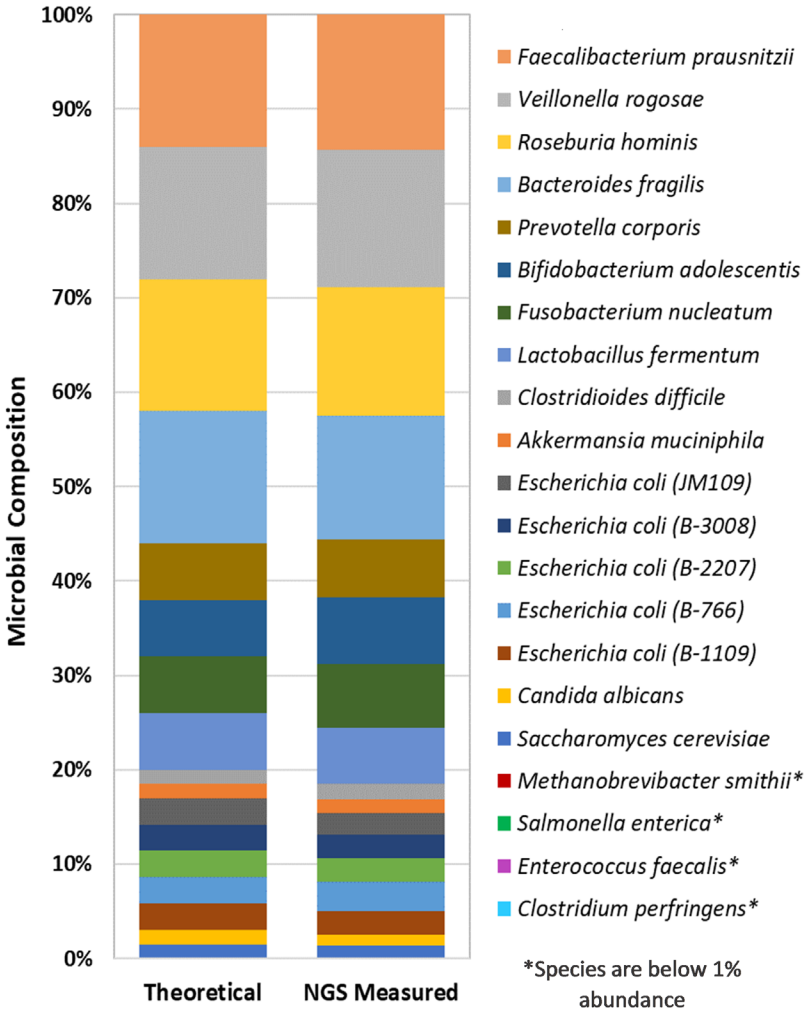

Figure 1. The microbial composition of the standard measured by NGS shotgun sequencing as compared to the defined composition. The microbial composition of the standard was confirmed using Illumina® shotgun sequencing. Genomic DNA was extracted using the ZymoBIOMIC® DNA Miniprep Kit. Library preparation was performed using an in-house protocol. Shotgun sequencing was performed using Illumina 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	Strain ID	Genome Size (Mb)	GC Content (%)	16/18S Copy Number	Gram Stain
Faecalibacterium prausnitzii	AP34BHI	2.914	57.8	6	+
Veillonella rogosae	AC2811 AN NA 2	2.158	39.0	4	-
Roseburia hominis	OB EAV1 11 DCM	3.463	48.7	4	+/-
Bacteroides fragilis	OB EAV1 11 D6 FAA	5.167	43.3	6	-
Prevotella corporis	OB21 FMU 4	2.947	44.4	4	-
Bifidobacterium adolescentis	LMG 10502	2.090	59.2	5	-
Fusobacterium nucleatum	2/1/50A	2.448	27.0	5	-
Lactobacillus fermentum	B-1840	1.905	52.3	5	+
Clostridioides difficile	P4D3A1-1	4.209	28.8	12	+
Akkermansia muciniphila	OB21 FAA NB 28	2.851	55.5	3	-
Methanobrevibacter smithii	DSM 861	1.853	31.0	2	+
Salmonella enterica	B-4212	4.760	52.2	7	-
Enterococcus faecalis	IP101412 AER FAA 2	2.845	37.5	4	+
Clostridium perfringens	OB21 TSA 19	3.436	28.3	10	+
Escherichia coli	JM109	4.729	50.9	7	-
Escherichia coli	B-3008	4.739	50.9	7	-
Escherichia coli	B-2207	5.234	50.7	7	-
Escherichia coli	B-766	5.191	50.8	7	-
Escherichia coli	B-1109	4.875	50.5	7	-
Candida albicans	IHEM 3108	14.68	33.6	55[2]	Yeast
Saccharomyces cerevisiae	Y-567	13.30	38.3	109[2]	Yeast

2 18S rRNA gene copy numbers in a haploid genome of the two strains of *Saccharomyces cerevisiae* and *Candida albicans* were estimated based on read depth information from mapping shotgun sequencing data.

Protocol

1. Thaw the standard on ice. Once thawed, vortex the standard for at least 30 seconds, then spin down briefly.

***Note**: Cells might aggregate due to freeze-thaw cycling; therefore, it is critical to mix the cellular standard thoroughly before use.*

2. For DNA extraction of the standard, use 75 µl per prep[1]. We recommend using mechanical lysis featured in Zymo Research's microbial DNA isolation kits[2] for unbiased and efficient isolation. Expected yield is approximately 1 µg[3] DNA per prep when using **ZymoBIOMICS® DNA Miniprep Kit** (D4300).

***Note**: The duration of homogenization (bead beating) will vary depending on the homogenization device, and may require optimized by the end-user. Zymo Research has validated optimized lysis parameters for many common homogenization devices, which can be found here:* https://files.zymoresearch.com/documents/bead_beating_short_protocol_tables.pdf

1. For use with kits that are incompatible with DNA/RNA Shield and other sample preservation reagents, such as Qiagen's PowerFecal and PowerSoil kits, reducing the input volume can improve compatibility. For kits such as these, it is recommended to reduce the input of this standard to ≤ 20 µl.
2 This microbial standard contains several tough-to-lyse microbes; therefore, to extract DNA from this standard, we strongly recommend using ZymoBIOMICS® DNA Miniprep Kit (D4300), Quick-DNA™ Fungal/Bacteria DNA Miniprep Kit (D6005), Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (D6010). 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.
3 The expected yield for one prep (75 µl) of the standard is approximately 1 µg. Yields significantly lower than 1 µg may suggest inefficient lysis during DNA extraction.

Appendix

Appendix A: Bioinformatics Analysis Recommendations

Assessing accuracy of taxonomy identification

The value of sequencing-based microbiome studies lies in their ability to identify microbial organisms without the need for culturing. Therefore, the accuracy of taxonomic identification is critical. The user can use the ZymoBIOMICS® Gut Microbiome Standard to compare workflow results with the theoretical composition (Table 1) to assess taxonomic identification accuracy. This will allow for the assessment of taxonomic resolution limits, and false positive and false negative rates of the workflow. False positives may be introduced by contaminations during wet-lab processing, chimeric sequences during library preparation, sequencing errors, demultiplexing errors and defects during bioinformatics analysis. The standard is certified to contain <0.01% of foreign contaminants. Therefore, any alien taxa present at >0.01% in the analysis can be attributed to contaminants introduced by the processing workflow.

Assessing bias in composition profiling

Accurately determining microbial composition of a sample is critical for conducting microbiome studies. Both wet-lab and dry-lab processes can introduce bias into the composition results samples. To determine biases introduced during wet-lab procedures, an accurate and unbiased method of bioinformatical analysis is needed. We have found that direct read-mapping against reference genomes (or against reference 16S &18S sequences, rather than assigning sequences to taxonomies, is a straightforward and accurate way to infer microbial composition of the standard from sequencing data. The reference sequences of this standard can be found in the Specifications.

Ordering Information

Product Description	Catalog No.	Size
ZymoBIOMICS® Gut Microbiome Standard	D6331	10 preps.

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® Microbial Community DNA Standard II (Log Distribution)	D6311	220 ng
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

Complete Your Workflow

✓ To collect and transport microbiome samples at ambient temperatures:

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

✓ Unbiased and inhibitor-free DNA and RNA extraction (high-throughput and automatable) for microbiome profiling:

ZymoBIOMICS® DNA and RNA Kits	
DNA Miniprep (D4300)	Up to 25 µg DNA
DNA Microprep (D4301)	Up to 5 µg DNA
MagBead DNA (D4302)	Automatable (Tecan, Hamilton, Kingfisher, etc.)
96-Well DNA (D4309)	Spin-plate
DNA/RNA Miniprep Kit (R2002)	Up to 100 µg DNA/RNA

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

ZymoBIOMICS® Services	
Targeted Sequencing Service 16S (Q2001)	With DNA Extraction
Targeted Sequencing Service 16S (Q2012)	Without DNA Extraction
Targeted Sequencing Service ITS (Q2003)	With DNA Extraction
Targeted Sequencing Service ITS (Q2003)	Without DNA Extraction

Notes

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