ZYMO RESEARCH

Microbiomics
Made Simple™

ZymoBIOMICS® Oral Microbiome Standard

Assess bias and validate NGS-based microbial composition profiling workflows

Highlights

- **Accurate representation:** Comprised of 12 different strains to mimic the human oral microbiome.
- **Precise composition:** allows for benchmarking and validation of NGS microbiome workflows.
- **Assess bias in DNA isolation:** contains both Gram-positive and negative bacteria sorted in a staggered abundance to assess profiling bias and detection limits.

Catalog Number:
D6332

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

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Product Contents

ZymoBIOMICS® Oral Microbiome Standard	D6332 (10 preps.)	Storage Temperature[1]
ZymoBIOMICS® Oral Microbiome Standard	0.75 ml	-80°C

Specifications

- **Source –** Twelve bacteria (six Gram-positive and six Gram-negative) found in the human oral cavity.
- **Reference Genomes and 16S rRNA Genes –** https://s3.amazonaws.com/zymo-files/BioPool/D6332.refseq.zip
- **Storage Solution –** DNA/RNA Shield™
- **Biosafety –** this product is not biohazardous as microbes have been fully inactivated.
- **Total Cell Concentration –** ~1.30 x 10^9 cells/ml
- **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.

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

Table 1. Microbial Composition

Species	Theoretical Composition (%)			
	Genomic DNA	16S Copy[1]	Genome Copy[2]	Cell Number[3]
Streptococcus parasanguinis	16	16.9	17.1	17.1
Neisseria subflava	16	15.8	16.1	16.1
Veillonella parvula	16	16.7	17.0	17.0
Prevotella nigrescens	16	12.6	12.8	12.8
Haemophilus parainfluenzae	8	12.9	8.8	8.8
Streptococcus mitis	8	9.0	9.1	9.1
Schaalia odontolytica	8	5.6	7.6	7.6
Rothia dentocariosa	8	5.4	7.3	7.3
Fusobacterium nucleatum	1	1.2	1.0	1.0
Streptococcus mutans	1	1.4	1.2	1.2
Streptococcus salivarius	1	1.5	1.0	1.0
Porphyromonas gingivalis	1	1.0	1.0	1.0

[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. 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® Oral Microbiome Standard is a mixture of 12 bacterial strains in staggered abundances to mimic a true oral microbiome. The standard presents multiple challenges for NGS pipelines, such as tough-to-lyse Gram-positive bacteria (e.g., *Streptococcus mitis*) to test lysis efficiency, genomes with a wide range of GC content to test sequencing coverage bias, low-abundance pathogenic organisms for detection limit assessment, and 4 different species of *Streptococcus* to test taxonomic resolution. These challenge points can be used to expose artifacts, errors, and biases in microbiomics or metagenomics workflows. Serving as a defined input, this standard can be used to guide construction and optimization of 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 12 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 12 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 we can help to analyze sequencing data generated from this standard.

Reference Genome Download:
https://s3.amazonaws.com/zymo-files/BioPool/D6332.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.

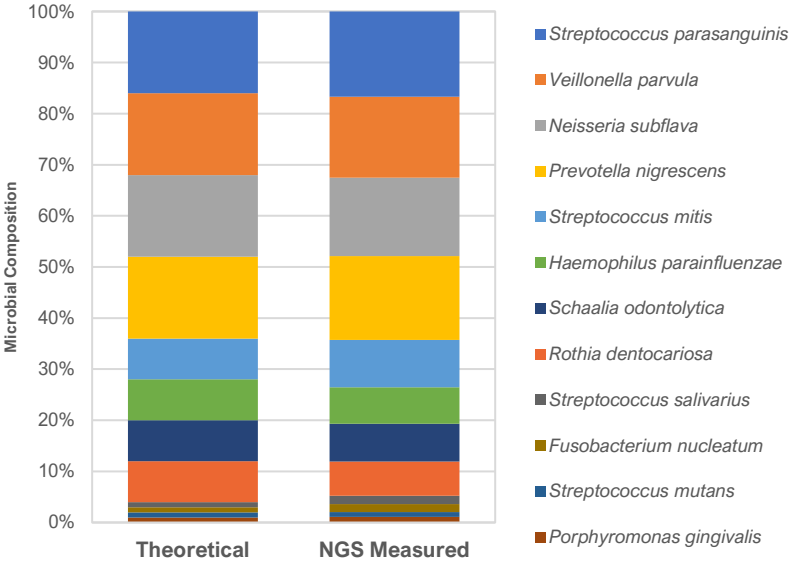

Figure 1. The microbial composition of the standard measured by NGS shotgun sequencing as compared to the defined composition. The microbial composition of the standard was confirmed using Illumina® shotgun sequencing. Genomic DNA was extracted using the ZymoBIOMICS® DNA Miniprep Kit. Library preparation was performed using an in-house protocol. Shotgun sequencing was performed using Illumina NextSeq™. Microbial abundance was estimated based on the number of reads that were mapped to references genomes of the organisms.

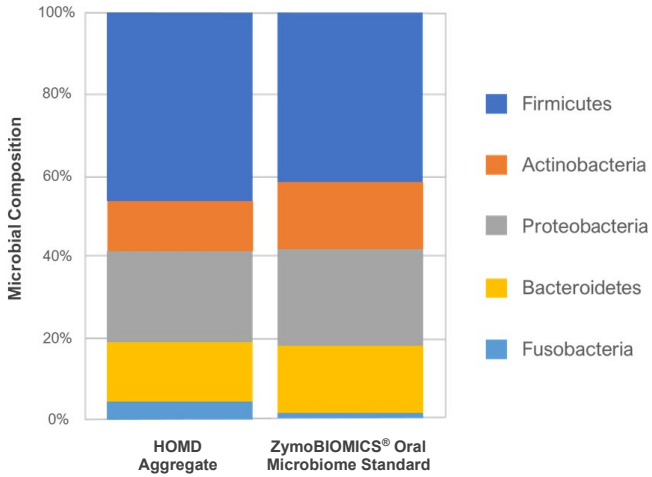

Figure 2. The microbial composition of the standard as compared to the Human Oral Microbiome Database (HOMD). The microbial composition of the standard was designed to reflect a real, human oral microbiome based on aggregate data found on the Human Oral Microbiome Database (HOMD), coupled with internally generated data. Phyla-level comparison shows the five most abundant taxa present in HOMD data are found in similar proportion in the ZymoBIOMICS® Oral Microbiome Standard.

Table 2. Strain Information

Species	Strain ID	Genome Size (Mb)	GC Content (%)	16S Copy Number	Gram Stain
Streptococcus parasanguinis	B-1756	2.126	41.9	4	+
Neisseria subflava	LMG 5313	2.262	49.1	4	-
Veillonella parvula	DSM 2007	2.144	38.6	4	-
Prevotella nigrescens	DSM 13386	2.838	42.7	4	-
Haemophilus parainfluenzae	DSM 8978	2.079	39.4	6	-
Streptococcus mitis	LMG 14557	2.002	40.0	4	+
Schaalia odontolytica	LMG 18080	2.394	65.4	3	+
Rothia dentocariosa	B-8017	2.493	53.7	3	+
Fusobacterium nucleatum	LMG 13131	2.392	27.0	5	-
Streptococcus mutans	LMG 14558	1.964	36.8	5	+
Streptococcus salivarius	LMG 11489	2.187	39.8	6	+
Porphyromonas gingivalis	DSM 20709	2.332	48.4	4	-

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. We recommend using mechanical lysis featured in Zymo Research's microbial DNA isolation kits[1,2] for unbiased and efficient isolation. Expected yield is approximately 500 ng[3] of 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 optimization 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, refer to Appendix C for workaround.
2 This 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 500 ng. Yields significantly lower than 500 ng may suggest inefficient lysis during DNA extraction.

Appendices

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® Oral 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 less than 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 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.

Appendix B: Additional Strain Information

Species	Strain ID	NCBI Reference Accession	Strain Name[1]
Streptococcus parasanguinius	B-14574	CP133988	*Streptococcus parasanguinis* Whiley et al., 1990; ATCC 903= SK 132
Neisseria subflava	LMG 5313	CP133462	*Neisseria subflava* (Flugge, 1886) Trevisan,1889, *biovar subflava*; ATCC 49275=CDN-17
Veillonella parvula	DSM 2007	CP133463	*Veillonella parvula* (Veillon & Zuber, 1898) Mays et al., 1982; ATCC 17745=259=VPI 11224
Prevotella nigrescens	DSM 13386	CP133464-CP133469	*Prevotella nigrescens* Shah & Gharbia, 1992; ATCC 33563=VPI 8944 =NCTC 9336
Haemophilus parainfluenzae	DSM 8978	CP133470	*Haemophilus parainfluenzae* Rivers, 1922; ATCC 33392= NCTC 7857
Streptococcus mitis	LMG 14557	CP133471	*Streptococcus mitis* (Andrewes & Horder,1906) (Judicial Opinion 66,1993) ATCC 49456=NCTC 12261=NS 51
Schaalia odontolytica	LMG 18080	CP133472	*Schaalia odontolytica* (Batty, 1958) Nouioui et al., 2018; ATCC 17929=CDC X363=NCTC 9935
Rothia dentocariosa	B-8017	CP133473	*Rothia dentocariosa* (Onishi, 1949) Georg & Brown, 1967; ATCC 17931=CDC X599
Fusobacterium nucleatum	LMG 13131	CP133474	*Fusobacterium nucleatum* subsp. *nucleatum* Knorr, 1922; ATCC 25586=VPI 4355
Streptococcus mutans	LMG 14558	CP133475	*Streptococcus mutans* Clarke, 1924; ATCC 25175=NCTC 10449 =IFO 13955
Streptococcus salivarius	LMG 11489	CP133476	*Streptococcus salivarius* subsp. *salivarius* Andrewes & Horder, 1906; ATCC 7073=NCTC 8618
Porphyromonas gingivalis	DSM 20709	JAVIVL000000000	*Porphyromonas gingivalis* (Coykendall et al., 1980) Shah & Collins,1988; ATCC 33277=2561

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

Appendix C: Workaround for Incompatible Extraction Kits

Some suppliers offer DNA extraction kits that provide PCR inhibitor removal technology at the start of the protocol, rather than the end, making them incompatible with most nucleic acid and/or sample preservation reagents. The **ZymoBIOMICS® Oral Microbiome Standard** (D6332) is provided in **DNA/RNA Shield™**, one such sample preservation reagent. Thus, attempting to process the standard with these kits, following the protocol as written, will result in poor DNA recovery and purity.

As the **ZymoBIOMICS® Microbial Community Standard** (D6332) does not contain PCR inhibitors like humic/fulvic acid or polyphenolics, it is generally safe to forego the PCR inhibitor removal steps in these kits, thus circumventing the incompatibility. See below for an example:

Modified Protocol:

Processing ZymoBIOMICS® Oral Microbiome Standard with Qiagen DNeasy® PowerSoil® Pro Kit

For reference, the manufacturer's provided protocol can be found here:
DNeasy PowerSoil Pro Kit Handbook: "Protocol: Experience User"

1. Perform Steps 1 – 4 as stated in the protocol (also stated below).

> 1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to 250 mg of soil and 800 µl of Solution CD1. Vortex briefly to mix.
> 2. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.
>
>   **Note:** If using the Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5–10 min.
>
>   **Note:** For alternative ways to homogenize samples, see the detailed protocol on page 13–14.
> 3. Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.
> 4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).
>
>   **Note:** Expect 500–600 µl. The supernatant may still contain some soil particles.

(*continued on next page*)

2. Do NOT perform Steps 5 – 6 as stated in the DNeasy PowerSoil Pro Kit Handbook protocol. **Proceed directly to Step 7**.

3. Perform steps 7 – 17 as stated in the protocol (also stated below).

7. Add 600 µl of Solution CD3 and vortex for 5 s.

8. Load 650 µl of lysate to an MB Spin Column. Centrifuge at 15,000 x g for 1 min.

9. Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB Spin Column.

10. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.

11. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.

12. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

13. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.

14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).

15. Centrifuge at up to 16,000 x g for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).

16. Add 50–100 µl of Solution C6 to the center of the white filter membrane.

17. Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.

    Note: We recommend storing the DNA frozen (–30 to –15°C or –90 to –65°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide.

Ordering Information

Product Description	Catalog No.	Size
ZymoBIOMICS® Oral Microbiome Standard	D6332	10 preps.

Related Products	Catalog No.	Amount
ZymoBIOMICS® Microbial Community Standard	D6300	10 preps.
ZymoBIOMICS® Microbial Community DNA Standard (200 ng)	D6305	200 ng
ZymoBIOMICS® Microbial Community DNA Standard (2000 ng)	D6306	2000 ng
ZymoBIOMICS® Microbial Community Standard II	D6310	10 preps.
ZymoBIOMICS® Microbial Community DNA Standard II	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
ZymoBIOMICS® Fecal Reference w/ TruMatrix™ Technology	D6323	10 preps.
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

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This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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