ZYMO RESEARCH

*The Beauty of Science is to Make Things Simple*

# INSTRUCTION MANUAL

## ZymoBIOMICS™ Microbial Community Standard I (Even, DNA Mix)

Catalog Nos. D6305 (200 ng) and D6306 (2000 ng)

## Highlights

- **Accurate composition:** composition cross-validated with multiple types of measurements.
- **Negligible impurity:** Guaranteed to contain <0.01% foreign microbial DNA.
- **Wide range of GC content:** 15%-85%, for assessing bias cause by GC content variation.

**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

| Product Name | D6305 (200 ng) | D6306 (2000 ng) | Storage Temp. |
|---|---|---|---|
| **ZymoBIOMICS™ Microbial Community Standard I (Even, DNA Mix)** | 200 ng / 20 µl | 2000 ng / 20 µl | -20 °C |

## Specifications

**Source:** eight bacteria (3 gram-negative and 5 gram-positive) and 2 yeasts.

**Reference Genomes:** ZymoBIOMICS_STD_v2.refseq.ZR170924.zip.

**Storage solution:** 10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0.

**DNA concentration:** 10 ng/µl (D6305) and 100 ng/µl (D6306).

**Impurity level**: < 0.01% foreign microbial DNA.

**Microbial composition:** Table 1 shows the theoretical microbial composition of the standard. The real composition might vary slightly from batch to batch. The real microbial composition of each lot was measured by shotgun metagenomic sequencing; the data can be accessed based on 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 Only[1] | 16S & 18S[1] | Genome Copy[2] | Cell Number[3] |
| *Pseudomonas aeruginosa* | 12 | 4.2 | 3.6 | 6.1 | 6.1 |
| *Escherichia coli* | 12 | 10.1 | 8.9 | 8.5 | 8.5 |
| *Salmonella enterica* | 12 | 10.4 | 9.1 | 8.7 | 8.7 |
| *Lactobacillus fermentum* | 12 | 18.4 | 16.1 | 21.6 | 21.4 |
| *Enterococcus faecalis* | 12 | 9.9 | 8.7 | 14.6 | 14.5 |
| *Staphylococcus aureus* | 12 | 15.5 | 13.6 | 15.2 | 15.1 |
| *Listeria monocytogenes* | 12 | 14.1 | 12.4 | 13.9 | 13.8 |
| *Bacillus subtilis* | 12 | 17.4 | 15.3 | 10.3 | 10.2 |
| *Saccharomyces cerevisiae* | 2 | NA | 9.3 | 0.57 | 1.13 |
| *Cryptococcus neoformans* | 2 | NA | 3.3 | 0.37 | 0.73 |

[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.

[3] The theoretical composition in terms of cell 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)/ploidy.

## Product Description

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.

**ZymoBIOMICS™ Microbial Community Standard I (Even, DNA Mix)** is a mixture of genomic DNA isolated from pure cultures of eight bacterial and two fungal strains. Genomic DNA from each pure culture was isolated and quantified before mixing[1]. The GC content[2] of the containing genomes covers a range from 15% to 85%. 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. This product is ideal for assessing biases and errors associated with library preparation, sequencing and bioinformatic analyses. It serves perfectly as a microbial standard for benchmarking the performance of microbiomics or metagenomics analyses or as a quality control tool for inter-lab studies. This standard is also ideal to help users construct and optimize workflows, e.g. assessing PCR chimera rate (Figure 1) and removing false positives (Figure 2) in 16S rRNA gene targeted sequencing, and assessing GC bias in sequencing coverage of shotgun metagenomic sequencing (Figure 3).

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[3] are available at: https://s3.amazonaws.com/zymo-files/BioPool/ZymoBIOMICS_STD_v2.refseq.ZR170924.zip. Feel free to contact us if you need analyzing the sequencing data generated from this standard.

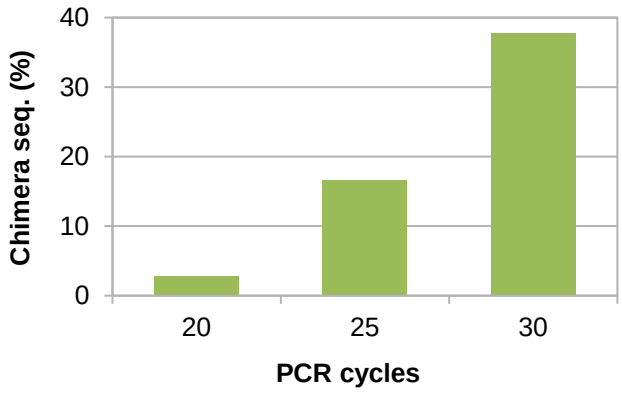


**Figure 1. PCR chimera increases with increasing PCR cycle number in the library preparation process of 16S rRNA gene targeted sequencing.** 20 ng ZymoBIOMICS™ Microbial Community Standard (Even, DNA Mix) was used as a template. The PCR reaction was performed with primers that target v3-4 region of 16S rRNA gene. Chimera sequences were identified with Uchime (http://drive5.com/usearch) and using the 16S rRNA gene of the 8 bacterial strains contained in the standard as reference.

**Notes:**

[1] Genomic DNA from each culture was extracted and quantified before mixing so this DNA standard was independent and not a direct derivative of the microbial version, ZymoBIOMICS™ Microbial Community Standard I (Even Cellular Mix).

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

[3] Several strains within the standard were replaced with similar strains in version 1.10. This update will not affect the species composition of the standard. For analyses that require reference genomes of the standard, please update the reference genomes accordingly, which can be found in Page 2 of this manual.

**Notes:**

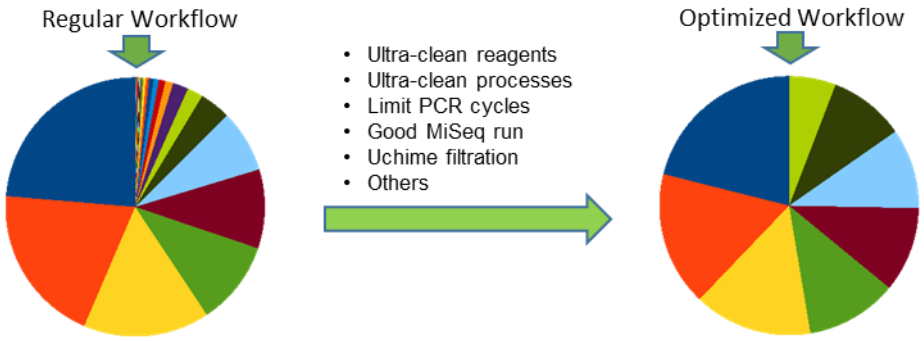


**Figure 2. Eliminating noise/false positives in 16S rRNA gene targeted sequencing guided with ZymoBIOMICS™ Microbial Community Standard I (Even, DNA Mix).** The pie chart on the left is microbial composition profile of the standard determined by a regular workflow of 16S sequencing using primers targeting 16S v3-4 region. The pie chart on the right is the profile of the same standard determined using the same primer sets, but with an optimized in-house 16S sequencing workflow. Noise observed on the left panel were mainly caused by PCR chimera, process contamination and reagent contamination, which were controlled in the optimized workflow. The accuracy of the standard's microbial composition is critical for revealing the presence of composition bias and false positives when optimizing a workflow.

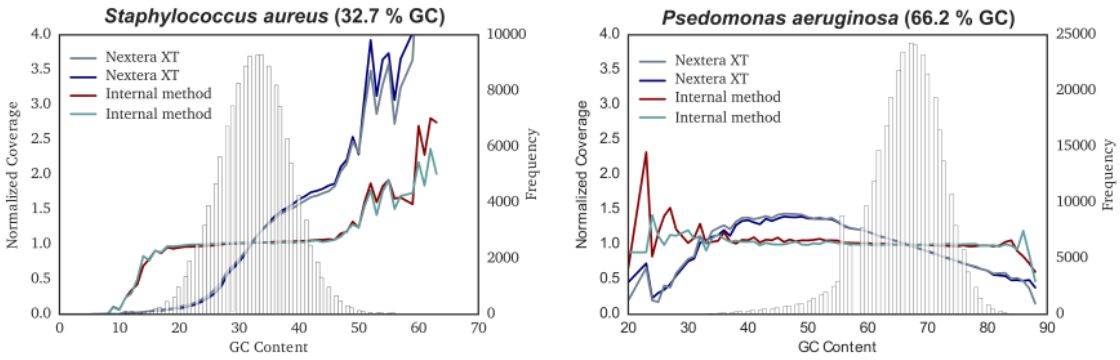


**Figure 3. Assessing GC bias of two different library preparation methods in shotgun metagenomic sequencing.** Library preparation for shotgun metagenomic sequencing was performed in two different ways: one by Illumina Nextera® XT kit and one by an in-house method. Shotgun sequencing was performed on MiSeq™ with paired-end sequencing (2x150 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 with the average sequencing coverage of each genome. The coverage profiles of two selected genomes, *Staphylococcus aureus and Pseudomonas aeruginosa*, were picked to demonstrate as they cover a wide range of GC content, 15%-85%. While the in-house method shows little/no GC-bias, the Nextera® XT kit has reduced representation for both low GC and high GC regions.

## Table 2: Strain Information

| Species | NRRL Accession NO.[1] | Genome Size (Mb) | Ploidy | GC Content (%) | 16/18S Copy Number | 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 | 1.921 | 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[2] | 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 |

**Notes:**

[1] Several strains within the standard were replaced with similar strains in version 1.10. This update will not affect the species composition of the standard. For analyses that require reference genomes of the standard, please update the reference genomes accordingly, which can be found in Page 2 of this manual.

[2] 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 shotgun sequencing data.

**Notes:**

[1] The table was prepared in April of 2016

## Protocol

1. Thaw the standard on ice. After thawing, vortex and spin down quickly.

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 preparation processes[1]**

| Lib. Prep | 16S Library | Illumina Nextera® XT | Illumina TruSeq® Nano | TruSeq® PCR-free | Kapa HyperPlus |
|---|---|---|---|---|---|
| DNA input (ng) | 10 | 1 | >200 | 2000 | 1-2000 |

## Ordering Information[1]

| Product Description | Catalog No. | Size |
|---|---|---|
| **ZymoBIOMICS™ Microbial Community Standard I (Even, DNA Mix, 200 ng)** | D6305 | 200 ng / 20 µl |
| **ZymoBIOMICS™ Microbial Community Standard I (Even, DNA Mix, 2000 ng)** | D6306 | 2000 ng/20 µl |

## Related Products

| Product Description | Catalog No. | Size |
|---|---|---|
| **ZymoBIOMICS™ Microbial Community Standard I (Even, Cellular Mix)** | D6300 | 10 preps |
| **ZymoBIOMICS™ DNA Miniprep** | D4300 | 50 preps |
| **ZymoBIOMICS™ Microbial Community Standard II (Staggered, Celluar Mix)** | D6310 | 10 preps |
| **ZymoBIOMICS™ Microbial Community Standard II (Staggered, DNA Mix)** | D6311 | 200 ng / 20 µl |

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

**Notes:**

[1] 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

## Appendix A

**Table 3. Additional Strain Information**

| Species | NRRL Accession NO. | Strain Name[1] |
|---|---|---|
| *Bacillus subtilus* | B-354 | *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 ATCC 6633=NRRL B-209=NRS-231=PCI 219 |
| *Cryptococcus neoformans* | Y-2534 | *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 | *Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 ATCC 7080 |
| *Escherichia coli* | B-1109 | Castellani and Chalmers 1919, 01485cm |
| *Lactobacillus fermentum* | B-1840 | *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 | *Listeria monocytogenes* (Murray et al. 1926) Pirie 1940 2847=ATCC 19117 |
| *Psuedomonas aeruginosa* | B-3509 | *Pseudomonas aeruginosa* (Schroeter 1872) Migula 1900 ATCC 15442=NCIB 10421=Pdd-10 |
| *Saccharomyces cerevisiae* | Y-567 | *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 | *Salmonella enterica* subspecies *enterica*, Castellani and Chalmers 1919, TA1536 |
| *Staphylococcus aureus* | B-41012 | *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/).

## Appendix B Reference Sequences

We replaced five strains in the ZymoBiomics standards (D6300, D6305 and D6306) with similar strains beginning on December XXXX from Lot #190633. Simultaneously, the product names of the standards were modified slightly to reflect this update (as shown in the table below). We apologize for any inconvenience that this update might cause.
Key Points:

- No further organism changes will occur.
- The updated standard includes 7 complete genomes and 3 draft genomes.
- Species-level composition of the standards is unchanged.
- For analyses that require the reference genomes or sequences of the strains, please use of the new reference sequences.

**Products Containing New Strains**

| Cat. # | Lot # | Product Name[1] | Reference Genome and 16S/18S sequences |
|---|---|---|---|
| D6300 | ZRC190633 | ZymoBIOMICS™ Microbial Community Standard I (Even, Cellular Mix) | https://s3.amazonaws.com/zymo-files/BioPool/ZymoBIOMICS_STD_v2.refseq.ZR170924.zip |
| D6305, D6306 | ZRC190811 ZRC190812 | ZymoBIOMICS™ Microbial Community Standard I (Even, DNA Mix) | |

**Products Containing Old Strains**

| Cat. # | Lot # | Product Name[1] | Reference Genome and 16S/18S sequences |
|---|---|---|---|
| D6300 | ZRC183430 ZRC187326 | ZymoBIOMICS™ Microbial Community Standard | https://s3.amazonaws.com/zymo-files/BioPool/ZymoBIOMICS.STD.genomes.ZR160406.zip |
| D6305, [ST1]D6306 | | ZymoBIOMICS™ Microbial Community DNA Standard | |

[1] You can differentiate by product name.