

# INSTRUCTION MANUAL

## ZymoBIOMICS™ HMW DNA Standard

Catalog No. D6322

### Highlights

- **Long-Read Sequencing Ready:** Ideal to benchmark 3<sup>rd</sup> generation sequencing and metagenomic measurements (e.g. Oxford Nanopore & PacBio).
- **High Molecular Weight:** DNA standard is >50 kb in size.
- **Accurate Composition:** Composition is cross-validated with multiple types of measurements.

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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	D6322	Storage Temp.
ZymoBIOMICS™ HMW DNA Standard	5000 ng/50 µl	-20°C

Note: The DNA standard is shipped at ambient temperature which does not affect product quality. Recommended long term storage at -20°C upon receiving.

## Product Specifications

- **Source:** seven bacteria (3 gram-negative and 4 gram-positive) and 1 yeast.
- **Reference genomes and 16S&18S rRNA genes:**  
<https://s3.amazonaws.com/zymo-files/BioPool/D6322.refseq.zip>.
- **Storage solution:** 10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0.
- **DNA concentration:** 100 ng/µl.
- **Impurity level:** < 0.01% foreign microbial DNA.
- **Average relative-abundance deviation:** <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: <http://www.zymoresearch.com/microbiomics/microbial-standards/zymbiomics-microbial-community-standards>.

**Table 1: Microbial Composition**

Species	Theoretical Composition (%)				
	Genomic DNA	16S Only <sup>1</sup>	16S & 18S <sup>1</sup>	Genome Copy <sup>2</sup>	Cell Number <sup>3</sup>
<i>Pseudomonas aeruginosa</i>	14	5.1	4.6	7.8	7.9
<i>Escherichia coli</i>	14	12.4	11.2	10.9	11.0
<i>Salmonella enterica</i>	14	12.7	11.4	11.2	11.2
<i>Enterococcus faecalis</i>	14	12.1	10.9	18.8	18.8
<i>Staphylococcus aureus</i>	14	19	17.1	19.6	19.6
<i>Listeria monocytogenes</i>	14	17.3	15.6	17.8	17.9
<i>Bacillus subtilis</i>	14	21.4	19.2	13.2	13.2
<i>Saccharomyces cerevisiae</i>	2	NA	10	0.63	0.32

<sup>1</sup> 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.

<sup>2</sup> 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.

<sup>3</sup> 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.

### Notes:

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.

™ Trademarks of Zymo Research Corporation. TapeStation® is a registered trademark of Agilent Technologies, Inc. MinION™ is a trademark of Oxford Nanopore Technologies, Ltd.

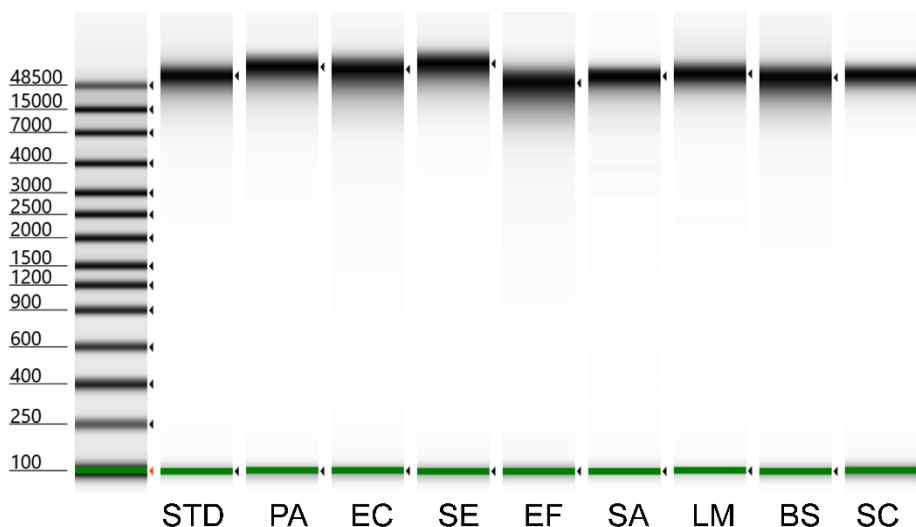
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## Product Description

**ZymoBIOMICS™ HMW DNA Standard** is a mixture of high molecular weight genomic DNA isolated from pure cultures of seven bacterial and one fungal strains (see Figure 1).<sup>1</sup> It has an accurately defined composition, negligible impurities (<0.01%) and contains genomes of a wide range of GC content (15%-85%). This can be used to assess and benchmark the performance of long read/3<sup>rd</sup> generation sequencing for microbiomics and metagenomics analysis and can also be used as a routine quality control (see Figure 2).

Details regarding the eight microbial strains (including species name, genome size, ploidy, average GC content, 16S/18S copy number, and phylogeny) can be found on page 4. The 16S/18S rRNA sequences (fasta format) and genomes (fasta format) of these strains are available at: <https://s3.amazonaws.com/zymo-files/BioPool/D6322.refseq.zip>. Feel free to contact us if we can help analyze the sequencing data generated from this standard.

**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.

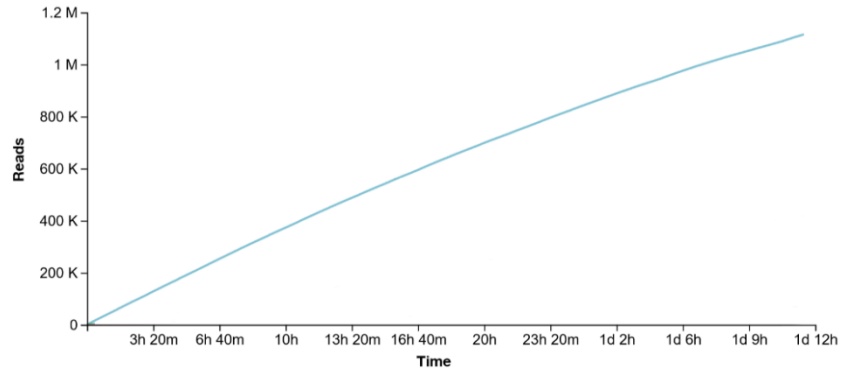


**Figure 1. Genomic DNA from each species is >50 kb size.** Sizing assessed by Agilent 2200 TapeStation® (Genomic DNA ScreenTape). The ZymoBIOMICS™ HMW DNA Standard (STD) is composed of the following organisms: (PA) *Pseudomonas aeruginosa*, (EC) *Escherichia coli*, (SE) *Salmonella enterica*, (EF) *Enterococcus faecalis*, (SA) *Staphylococcus aureus*, (LM) *Listeria monocytogenes*, (BS) *Bacillus subtilis*, (SC) *Saccharomyces cerevisiae*.

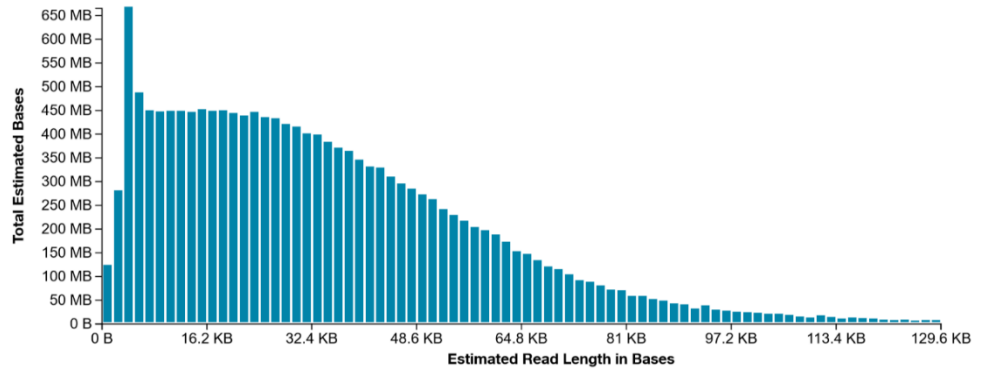
### Notes:

<sup>1</sup> Genomic DNA from each culture was extracted and quantified before mixing so this DNA standard was independent and not a direct derivative of the ZymoBIOMICS™ Microbial Community Standard.

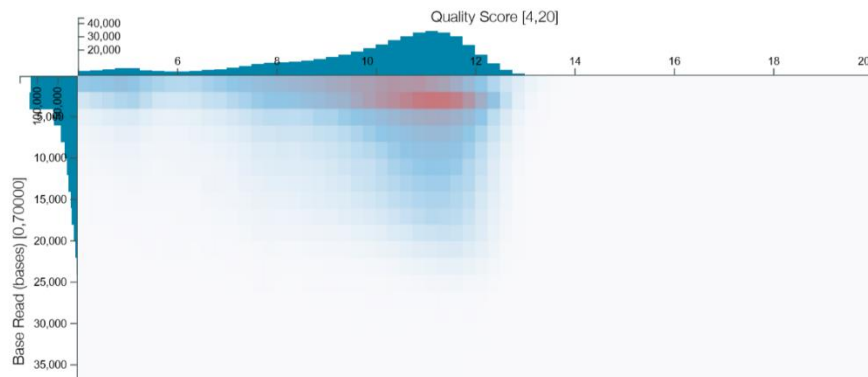
### A) Cumulative Throughput



### B) Read Length Histogram



### C) Basecalling Quality



**Figure 2. High Quality Long Read Sequencing.** Long read sequencing was performed on Oxford Nanopore MinION™ using the Ligation Sequencing kit (LSK109) for library preparation. A) Cumulative throughput yield was 15.71 Gb (run for 36 hours). B) Read length histogram shows an approximate average of 24 kb with >125 kb recorded. C) Basecalling quality of number of reads shows overall good q-score of 11.

## Strain Information

Species	NRRL Accession NO.	Genome Size (Mb)	Ploidy	GC Content (%)	16/18S Copy Number	Gram Stain
<i>Pseudomonas aeruginosa</i>	B-3509	6.792	1	66.2	4	-
<i>Escherichia coli</i>	B-1109	4.875	1	46.7	7	-
<i>Salmonella enterica</i>	B-4212	4.760	1	52.2	7	-
<i>Enterococcus faecalis</i>	B-537	2.845	1	37.5	4	+
<i>Staphylococcus aureus</i>	B-41012	2.730	1	32.9	6	+
<i>Listeria monocytogenes</i>	B-33116	2.992	1	38.0	6	+
<i>Bacillus subtilis</i>	B-354	4.045	1	43.9	10	+
<i>Saccharomyces cerevisiae</i>	Y-567	12.1	2	38.3	109 <sup>1</sup>	Yeast

### Notes:

<sup>1</sup> 18S rRNA gene copy numbers in a haploid genome of the strain of *Saccharomyces cerevisiae* were estimated based on read depth information from mapping shotgun sequencing data.

Species	NCBI Phylogeny Database
<i>Pseudomonas aeruginosa</i>	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas; Pseudomonas aeruginosa group
<i>Escherichia coli</i>	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia
<i>Salmonella enterica</i>	Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Salmonella
<i>Enterococcus faecalis</i>	Bacteria; Firmicutes; Bacilli; Lactobacillales; Enterococcaceae; Enterococcus
<i>Staphylococcus aureus</i>	Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus
<i>Listeria monocytogenes</i>	Bacteria; Firmicutes; Bacilli; Bacillales; Listeriaceae; Listeria
<i>Bacillus subtilis</i>	Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus; Bacillus subtilis group
<i>Saccharomyces cerevisiae</i>	Eukaryota; Opisthokonta; Fungi; Dikarya; Ascomycota; saccharomyceta; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycetaceae; Saccharomyces

## Protocol

1. Thaw the standard on ice. After thawing, flick and spin down quickly.
2. The amount of DNA used depends on the library preparation process being evaluated.

**Appendix: Additional Strain Information**

<b>Species</b>	<b>NRRL Accession NO.</b>	<b>Strain Name<sup>1</sup></b>
<i>Bacillus subtilis</i>	B-354	<i>Bacillus subtilis</i> (Ehrenberg 1835) Cohn 1872 ATCC 6633=NRRL B-209=NRS-231=PCI 219
<i>Enterococcus faecalis</i>	B-537	<i>Enterococcus faecalis</i> (Andrewes and Horder 1906) Schleifer and Kilpper-Bälz 1984 ATCC 7080
<i>Escherichia coli</i>	B-1109	Castellani and Chalmers 1919, 01485cm
<i>Listeria monocytogenes</i>	B-33116	<i>Listeria monocytogenes</i> (Murray et al. 1926) Pirie 1940 2847=ATCC 19117
<i>Pseudomonas aeruginosa</i>	B-3509	<i>Pseudomonas aeruginosa</i> (Schroeter 1872) Migula 1900 ATCC 15442=NCIB 10421=Pdd-10
<i>Saccharomyces cerevisiae</i>	Y-567	<i>Saccharomyces cerevisiae</i> 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
<i>Salmonella enterica</i>	B-4212	<i>Salmonella enterica</i> subspecies <i>enterica</i> , Castellani and Chalmers 1919, TA1536
<i>Staphylococcus aureus</i>	B-41012	<i>Staphylococcus aureus</i> Rosenbach 1884

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

**Ordering Information**

Product Description	Size	Catalog No.
ZymoBIOMICS™ HMW DNA Standard	5000 ng	D6322

**Related Products**

Related Products	Size	Catalog No.
ZymoBIOMICS™ DNA Miniprep Kit	50 preps	D4300
ZymoBIOMICS™ Microbial Community Standard	10 preps	D6300
ZymoBIOMICS™ Microbial Community DNA Standard (200ng)	200 ng	D6305
ZymoBIOMICS™ Microbial Community DNA Standard (2000ng)	2000 ng	D6306
ZymoBIOMICS™ Microbial Community Standard II (Log Distribution)	10 preps	D6310
ZymoBIOMICS™ Microbial Community DNA Standard II (Log Distribution)	220 ng	D6311
ZymoBIOMICS™ Spike-in Control I (High Microbial Load)	25 preps	D6320
ZymoBIOMICS™ Spike-in Control I (High Microbial Load)	250 preps	D6320-10
ZymoBIOMICS™ Spike-in Control II (Low Microbial Load)	25 preps	D6321
ZymoBIOMICS™ Spike-in Control II (Low Microbial Load)	250 preps	D6321-10

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*The Beauty of Science is to Make Things Simple*

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