

Standards for Optimizing Microbiomics Workflows



Standardizing Microbiomics



Does your DNA extraction method reflect reality or is it biased? Evaluate your method.

Use ZymoBIOMICSTM Microbial Community Standard to compare different DNA extraction protocols. DNA samples were profiled by 16S rRNA gene targeted sequencing. HMP Protocol stands for the fecal DNA extraction protocol used by the Human Microbiome Project.

ZymoB OM CS

Assess GC-Bias in Shotgun Metagenomics



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.

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Track PCR Chimera in 16S rRNA Gene Sequencing



PCR chimera increases with increasing PCR cycle number in the library preparation process of 16S rRNA gene targeted sequencing. 20 ng ZymoBIOMICS™ Microbial Community DNA Standard was used as a template. The PCR reaction was performed with ZymoTaq™ master mix and with primers that target v34 region of 16S rRNA gene. Chimera rate in percentage was determined with Uchime and using the 16S rRNA gene of the 8 bacterial strains in the standard as reference.



Accurate Characterization

Species	GC %	Gram Stain	gDNA Abun. (%)
Pseudomonas aeruginosa	66.2	-	12
Escherichia coli	56.8	-	12
Salmonella enterica	52.2	-	12
Lactobacillus fermentum	52.8	+	12
Enterococcus faecalis	37.5	+	12
Staphylococcus aureus	32.7	+	12
Listeria monocytogenes	38.0	+	12
Bacillus subtilis	43.8	+	12
Saccharomyces cerevisiae	38.4	Yeast	2
Cryptococcus neoformans	48.2	Yeast	2

•	Well defined and characterized standards		
	composed of 5 Gram-Positive and 3 Gram-		
	Negative bacteria plus 2 yeast species with		
	wide GC range (15%-85%).		

- Quality control for Microbiomics workflows (16s rRNA seq. and Shotgun seq.)
- Impurities are less than 0.01%

gDNA by Shotgun Sequencing Bacillus subtilis Listeria Monocytoge Staphylococcus aureus 70% ococcus faecalis Lactobacillus fermentu 60% 50% Escherichia coli 40% ccharomyces cerevis 30% occus neoforma 20% 10%

Accurate composition

16S Counts by 16S Sequencing



Negligible impurity (<0.01%)

Species	mOTU counts	mOTU Abun. (%)
Bacillus subtilis	9048	11.86
Enterococcus faecalis	11322	14.84
Escherichia coli	6994	9.17
Lactobacillus fermentum	17081	22.39
Listeria monocytogenes	11454	15.01
Pseudomonas aeruginosa	4484	5.88
Salmonella enterica	7939	10.41
Staphylococcus aureus	7960	10.43
Propionibacterium acnes (contaminant)	1	0.0013

Microbial composition was profiled with shotgun metagenomic sequencing (178 million reads). Taxonomy identification was performed with mOTU (http://www.bork.embl.de/software/mOTU/)

For reliable evaluation of shotgun seq. and 16S rRNA gene seq.

Join The Beta Test

Receive a free sample of the ZymoBIOMICS™ Microbial Community Standards and the ZymoBIOMICS™z DNA Mini Kit. Sign up and submit your evaluation at: http://www.zymoresearch.com/beta-test/zymobiomics-standards

For your professional feedback, you will receive a \$5 Amazon gift code and be entered to win an iPad Air 2!



