June 22, 2019
SATURDAY - MBP-7

Evaluation of ATCC® Site-Specific Microbiome Standards on Long-Read Sequencing Platforms

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Background

A predominant limitation in microbiome research is the lack of appropriate and relevant standards to control the technical biases introduced throughout the metagenomics workflow. To address this, ATCC has developed a set of genomic DNA and whole cell mock microbial communities from fully sequenced and characterized ATCC strains that represent species found in the oral, skin, gut, or vaginal microbiome. Here, we demonstrate the utility of these standards as reference materials for 16S and shotgun analyses performed on long-read sequencing platforms. This proof-of-concept analysis demonstrate that ATCC® Microbiome Standards are platform agnostic and can be used for the development and optimization of assays performed on both short-read and long-read sequencing platforms.

Selection and Relevance of Strains in Site-specific Microbiome Standards

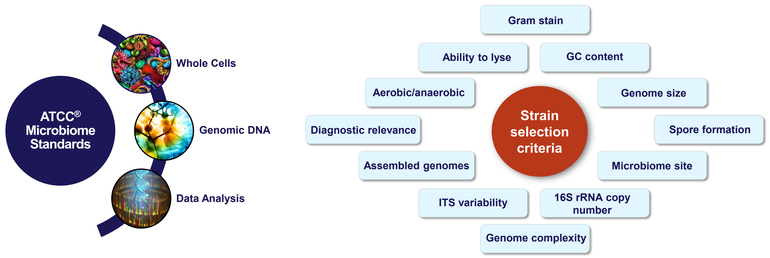
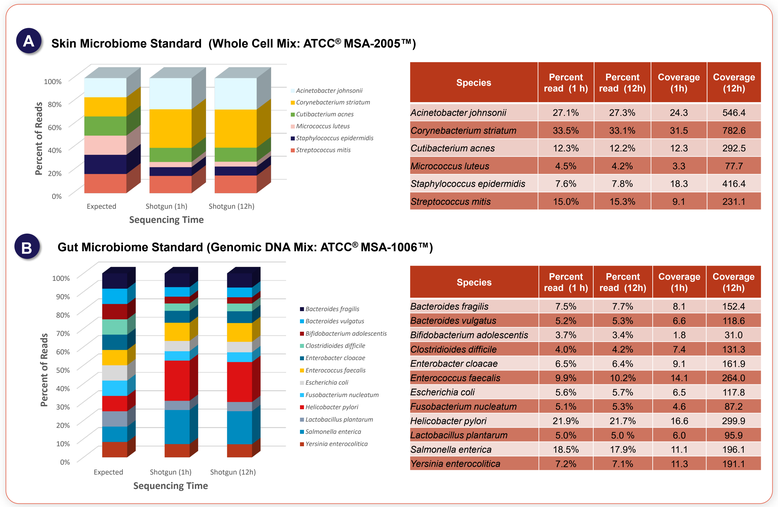


Table 1. The strains selected for the site-specific microbiome standards were chosen on the basis of their relevance in the normal and atypical flora of the oral, skin, gut, and vaginal microbiomes

Species	Gram Stain	Genome Size (Mb)	% GC	Relevance
Oral Microbiome Standard (Genomic Mix: ATCC® MSA-1004™ and Whole Cell Mix: ATCC® MSA-2004™)				
Actinomyces odontolyticus	Positive	2.39	65.5	Anaerobic bacterium associated with dental plaques
Fusobacterium nucleatum	Negative	2.17	27.2	Periodontal pathogen
Haemophilus parainfluenzae	Negative	2.12	39.3	Common oral commensal
Prevotella melaninogenica	Negative	3.17	35.1	Associated with dental caries
Streptococcus mitis	Positive	1.83	40.5	Associated with dental caries
Veillonella parvula	Negative	2.16	38.6	Prevalent on all oral surfaces
Skin Microbiome Standard (Genomic Mix: ATCC® MSA-1005™ and Whole Cell Mix: ATCC® MSA-2005™)				
Acinetobacter johnsonii	Negative	3.47	41.9	Frequently encountered in the skin microbiota
Corynebacterium striatum	Positive	2.72	59.7	Nosocomial opportunistic pathogen
Cutibacterium acnes	Positive	2.49	60.1	Causative agent of acne
Micrococcus luteus	Positive	2.50	73.0	High GC content
Staphylococcus epidermidis	Positive	2.50	32.0	Common cause of nosocomial infections
Streptococcus mitis	Positive	1.83	40.5	Commensal but occasionally pathogenic
Gut Microbiome Standard (Genomic Mix: ATCC® MSA-1006™ and Whole Cell Mix: ATCC® MSA-2006™)				
Bacteroides fragilis	Negative	5.21	43.3	Symbiotic but occasionally opportunistic in the peritoneal cavity
Bacteroides vulgatus	Negative	5.16	42.2	Most common fecal isolate from humans
Bifidobacterium adolescentis	Positive	2.09	59.2	Found in breast-fed newborns
Clostridioides difficile	Positive	4.11	28.6	May colonize the gut following antibiotic therapy
Enterobacter cloacae	Negative	5.31	55.1	Opportunistic pathogen following antibiotic exposure
Enterococcus faecalis	Positive	3.36	37.4	Opportunistic pathogen, produces cytolysin toxin
Escherichia coli	Negative	4.64	50.6	Typically found in the lower intestine of humans
Fusobacterium nucleatum	Negative	2.17	27.0	Belongs to normal microflora of oral and gastrointestinal tracts
Helicobacter pylori	Negative	1.67	38.9	Associated with peptic ulcers and chronic gastritis
Lactobacillus plantarum	Positive	3.31	44.5	Commonly found in probiotics to regulate intestinal microflora
Salmonella enterica	Negative	4.59	52.1	Not considered to be normal microflora
Yersinia enterocolitica	Negative	4.55	47.0	Foodborne and waterborne pathogen that causes gastroenteritis
Vaginal Microbiome Standard (Genomic Mix: ATCC® MSA-1007™ and Whole Cell Mix: ATCC® MSA-2007™)				
Gardnerella vaginalis	Negative	1.67	41.9	Sexually transmitted pathogen associated with bacteremia and UTIs
Lactobacillus gasseri	Positive	1.89	34.9	Found in the oral, intestinal, and vaginal microflora of humans
Lactobacillus jensenii	Positive	1.67	34.4	Found in the normal flora of the human female urogenital tract
Mycoplasma hominis	Negative	0.67	27.0	Causative agent of urogenital infections
Prevotella bivia	Negative	2.52	39.9	Associated with endometritis and septic arthritis
Streptococcus agalactiae	Positive	2.16	35.4	Associated with septicemia, meningitis, and pneumonia in newborns

Identification of microbiome components over time



Species	Percent read (1 h)	Percent read (12h)	Coverage (1h)	Coverage (12h)
Acinetobacter johnsonii	27.1%	27.3%	24.3	546.4
Corynebacterium striatum	33.5%	33.1%	31.5	782.6
Cutibacterium acnes	12.3%	12.2%	12.3	292.5
Micrococcus luteus	4.5%	4.2%	3.3	77.7
Staphylococcus epidermidis	7.6%	7.8%	18.3	416.4
Streptococcus mitis	15.0%	15.3%	9.1	231.1

Species	Percent read (1 h)	Percent read (12h)	Coverage (1h)	Coverage (12h)
Bacteroides fragilis	7.5%	7.7%	8.1	152.4
Bacteroides vulgatus	5.2%	5.3%	6.6	118.6
Bifidobacterium adolescentis	3.7%	3.4%	1.8	31.0
Clostridioides difficile	4.0%	4.2%	7.4	131.3
Enterobacter cloacae	6.5%	6.4%	9.1	161.9
Enterococcus faecalis	9.9%	10.2%	14.1	264.0
Escherichia coli	5.6%	5.7%	6.5	117.8
Fusobacterium nucleatum	5.1%	5.3%	4.6	87.2
Helicobacter pylori	21.9%	21.7%	16.6	299.9
Lactobacillus plantarum	5.0%	5.0 %	6.0	95.9
Salmonella enterica	18.5%	17.9%	11.1	196.1
Yersinia enterocolitica	7.2%	7.1%	11.3	191.1

Figure 1. Time-course data obtained during one and twelve hours of sequencing on a long-read sequencing platform. Analysis of percent of reads and genome coverage of individual organisms from the (A) whole cell skin microbiome standard and (B) genomic DNA gut microbiome standard. The shotgun metagenomics sequencing results obtained during the first 60 minutes of sequencing were enough to detect all of the organisms in the mock communities at the species level. After 12 hours of sequencing, depth increased considerably and no significant differences in percent of reads were observed. Data analyses were performed on the One Codex platform.

Long-reads analysis of whole cell and genomic DNA Standards

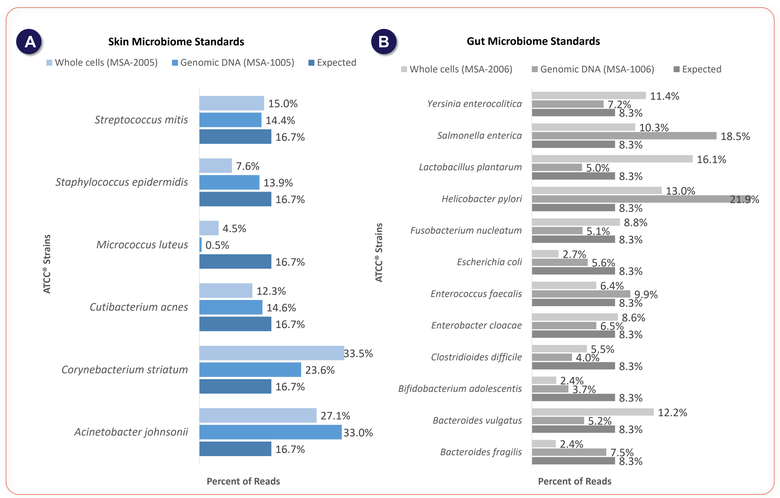


Figure 2. Comparison of genomic DNA and whole cell microbiome standards via long-reads sequencing platform. Evaluation of (A) skin and (B) gut microbiome standards via long-read shotgun metagenomics sequencing. Data analyses were performed on the One Codex platform.

Database analysis and comparison

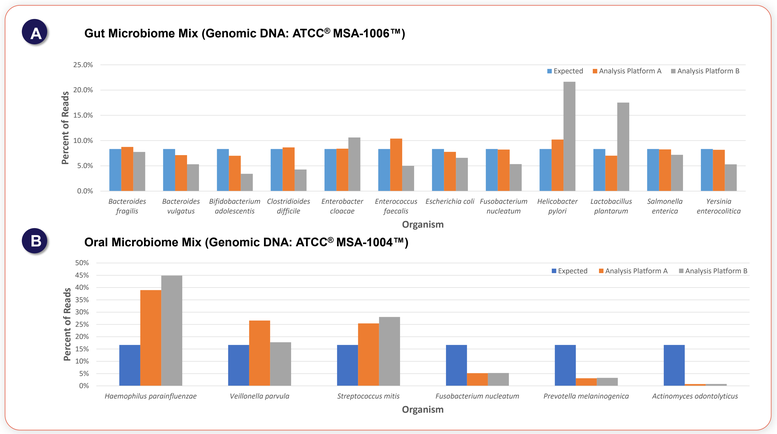


Figure 3. ATCC Site-specific microbiome standards can be used to compare bioinformatics platforms. The same datasets generated from sequencing the (A) gut genomic DNA mix and the (B) oral genomic DNA mix were analyzed via two different commercially available bioinformatics databases. Our data demonstrates that both analysis platforms were able to identify all organism at the species level.

Short-read versus long-read sequencing platforms

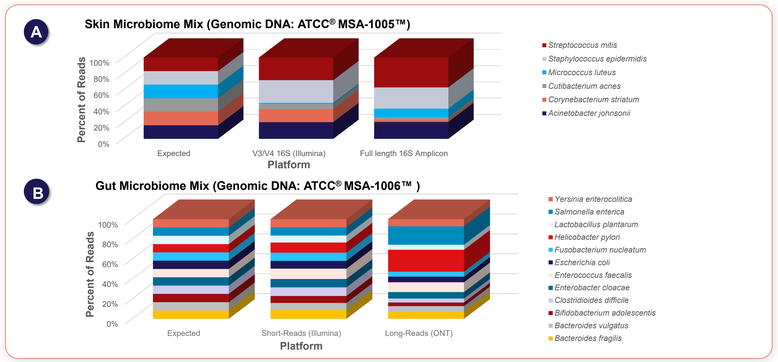


Figure 3. Evaluation of short- and long-read NGS sequencing platforms by using ATCC Site-specific Microbiome Standards. (A) Analysis of the skin microbiome whole cell mix; the V3/V4 region of the16S rRNA was evaluated on the Illumina® MiSeq® platform and the full-length 16S amplicon was evaluated on the Oxford Nanopore (ONT) MinION® platform. (B) Shotgun metagenomics analysis of the gut microbiome genomic standard by using the short-read (2 x 250 bp) Illumina platform and long-read (average ~5 kb) ONT platform. Data analyses were performed in the One Codex platform.

Summary

This proof-of-concept study demonstrates the application of site-specific microbiome standards as controls for evaluating and optimizing full-length 16S rRNA and shotgun metagenomic sequencing assay performance on long-read sequencing platforms. Additionally, our results show that both genomic DNA and whole cell ATCC® Microbiome Standards can be used to compare various 16S rRNA primers sets, metagenomics assays, and sequencing platforms.

Acknowledgements: We would like to thank Nick Greenfield, One Codex, for data analysis; Nezar Al-hebshi, Temple University, for assistance with oral microbiome standard design; and Jonathan Hetzel, Illumina, for scientific input and sequencing expertise.

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