

ATCC Corporate Workshop Association of Molecular Pathology (AMP) Annual Meeting

November 15, 2017



About ATCC

- Founded in 1925, ATCC is a non-profit organization with HQ and core operations in Manassas, VA, and an R&D and services center in Gaithersburg, MD
- World's premiere biological materials resource and standards development organization
 - 5,000 cell lines
 - 80,000 microbes
 - Genomic & synthetic nucleic acids
 - Media/Reagents
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Talented team of 485 employees; over one third with advanced degrees



An innovative global partner for authentic biomaterials, standards, and services



ATCC Microbiology Portfolio



Authenticated biomaterials for diverse applications

The most comprehensive fully authenticated microbial collection:

- >18,000 bacterial strains
- 3,000 human and animal viruses
- Over 50,000 yeast, fungi, and protozoa
- Nearly 1,000 nucleic acids

Featured product categories:

- Respiratory health
- Enteric diseases
- Vector-borne pathogens (Zika, Dengue, Chikungunya)
- Sexually transmitted infections

Research and Development programs:

- Native and synthetic nucleic acids
- Metagenomic standards (human microbiome)
- Highly characterized MDR clinical isolates

Brand recognition:

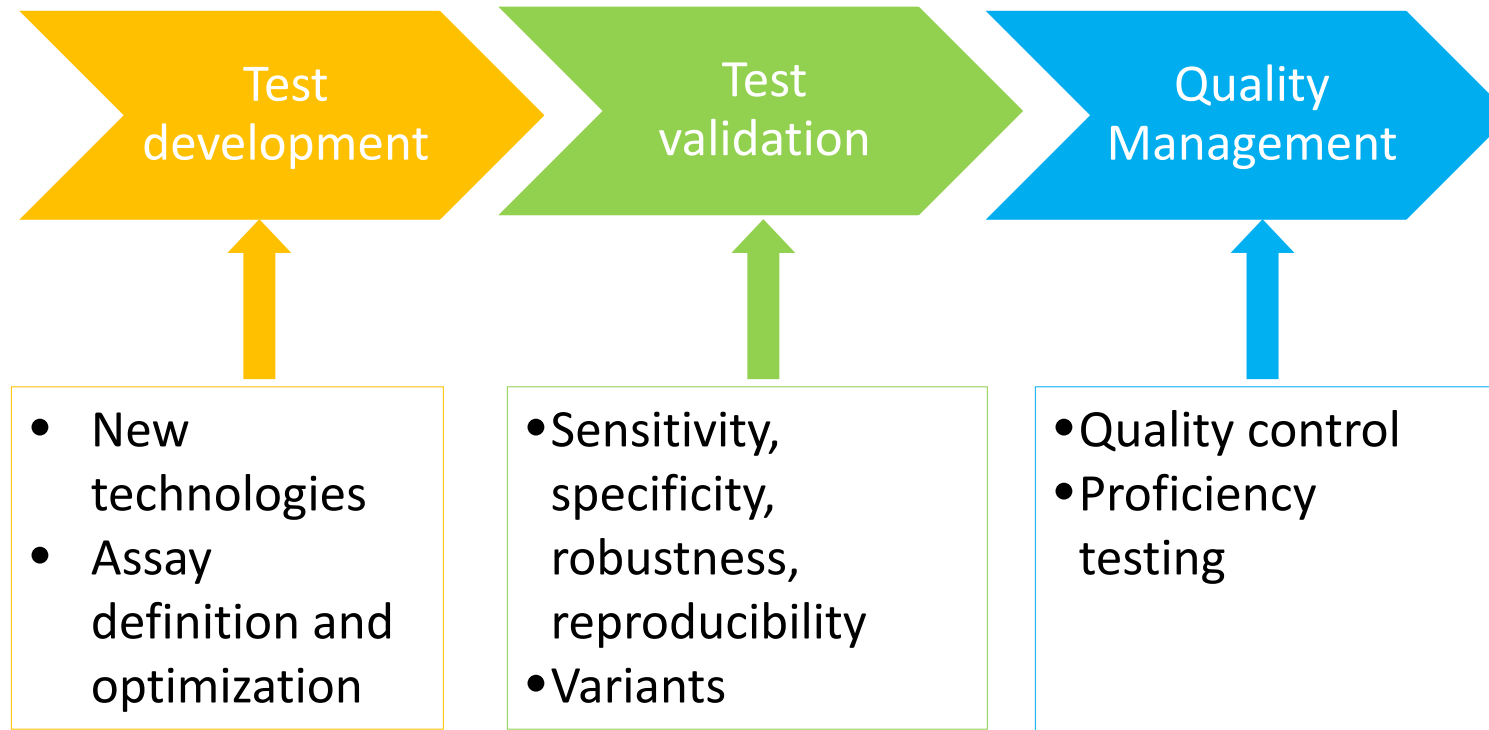
- Organizations and regulatory agencies specify ATCC cultures (ISO, FDA, CLSI, USP, USDA, ASTM, AOAC, and more)
- Over 475 microbial cultures recommended as quality control reference strains

Community of Stakeholders in Assay Development



Importance of Standards/Reference Materials for Assay Development

Key steps in assay development

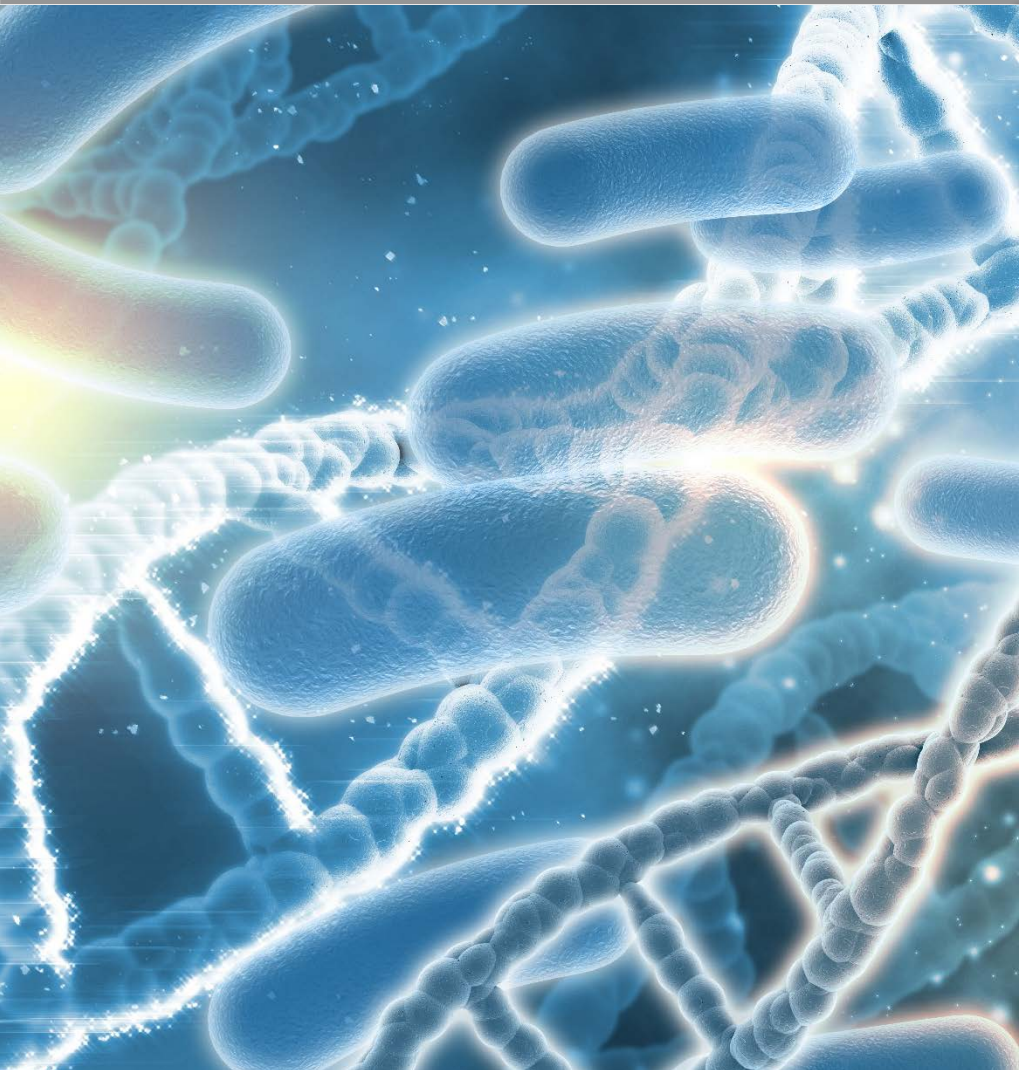


Microbiome and Molecular Diagnostics Standards for Next-Generation Sequencing- based Assays

Dev Mittar, Ph.D., *Lead Scientist, ATCC*



Outline



Microbiome Research

- Importance of microbiome research and the need for standards
- Development of ATCC® Microbiome Standards
- Using ATCC® Microbiome Standards to evaluate bias
- Data analysis on the One Codex platform

Molecular Diagnostics

- ATCC® Molecular Standards
 - Quantitative nucleic acids
 - Genomic DNA/RNA
 - Synthetic DNA/RNA
- Development and quantitation of standards
- Case studies using quantitated standards

Using Reliable Biomaterials as Controls

What is a reference material?

A material or substance, one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of a measuring system, the assessment of a measurement procedure, or for assigning values to materials (ISO 15195:2003)

Properties to consider

- Fully authenticated
- Characterized
- Stable molecular profiles
- Avoid contamination or misidentification
- Reproducible results

Types of materials

Reference Material	Benefit	Disadvantage
Specimens such as stool, blood, <i>etc.</i>	Representative	Not a sustainable source
Whole cells and genomic DNA	Mimics complexity of the whole genome	Genetic stability; rare mutations are difficult to obtain
Synthetic nucleic acids	Easy to design and synthesize	Do not resemble the complexity of the whole genome

Standards for Microbiome Research

Microbiome Research

A microbiome is defined as the totality of microorganisms and their collective genetic material present in or on the human body or in another environment.

The human microbiome is one of the major areas of research in microbiology, with widespread applications in the area of human health, personalized medicine, forensic analyses, and environmental studies, *etc.*

PubMed

- ~36,949 total papers
- ~80% in the last 5 years

Start-up companies

- 24 new companies in 2016



Microbiome Research: Challenges & Need for Standardization

Optimizing methods and dodging pitfalls in microbiome research

Dorothy Kim[†], Casey E. Hofstaedter[†], Chunyu Zhao, Lisa Mattei, Ceylan Tanes, Erik Clarridge, Scott Sherrill-Mix, Christel Chehoud, Judith Kelsen, Máire Conrad, Ronald G. Collman, Ronald Frederic D. Bushman and Kyle Bittinger ✉

[†] Contributed equally

Microbiome 2017 5:52 | <https://doi.org/10.1186/s40168-017-0267-5> | © The Author(s). 2017

Focus on Metagenomics

[Christopher E. Mason](#)¹ and [Scott Tighe](#)²

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Assessing the Accuracy of Quantitative Molecular Microbial Profiling

Denise M. O'Sullivan,^{1,*} Thomas Laver,^{2,†} Sasithon Temisak,^{1,†} Nicholas Redshaw,¹ Kathryn A. Harris,³ Carole A. Foy,¹ David J. Studholme,² and Jim F. Huggett¹

Weizhong Li, External Editor

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International Standards for Genomes, Transcriptomes, and Metagenomes

[Christopher E. Mason](#),^{1,2,3,*} [Ebrahim Afshinnekoo](#),^{1,2,4} [Scott Tighe](#),⁵ [Shixiu Wu](#),⁶ and [Shawn Levy](#)⁷

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Evaluating Bias of Illumina-Based Bacterial 16S rRNA Gene Profiles

Katherine Kennedy^a, Michael W. Hall^a, Michael D. J. Lynch^a, Gabriel Moreno-Hagelsieb^b and Josh D. Neufeld^a

K. E. Wommack, Editor

The truth about metagenomics: quantifying and counteracting bias in 16S rRNA studies

J Paul Brooks ✉, David J Edwards, Michael D Harwich Jr, Maria C Rivera, Jennifer M Fettweis, Myrna G Nihar U Sheth, Bernice Huang, Philippe Girerd, Vaginal Microbiome Consortium (additional members) Kimberly K Jefferson and Gregory A Buck

BMC Microbiology 2015 15:66 | DOI: 10.1186/s12866-015-0351-6 | © Brooks et al.; licensee BioMed Central

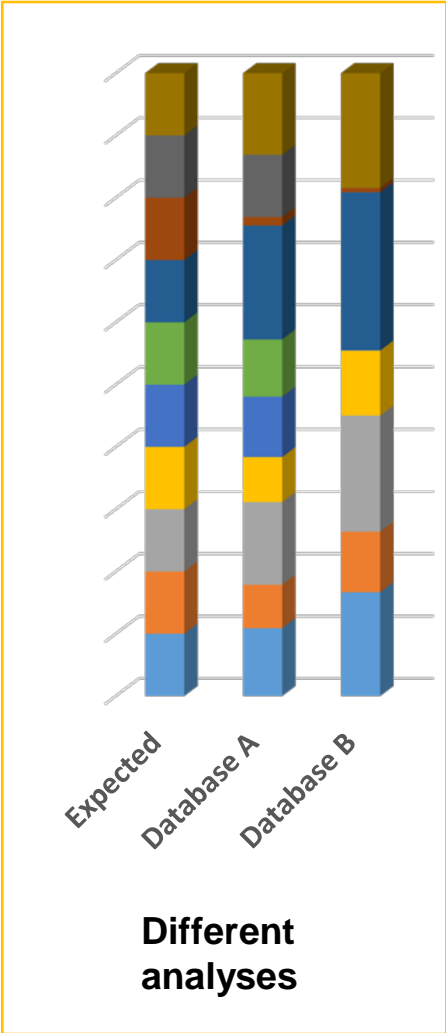
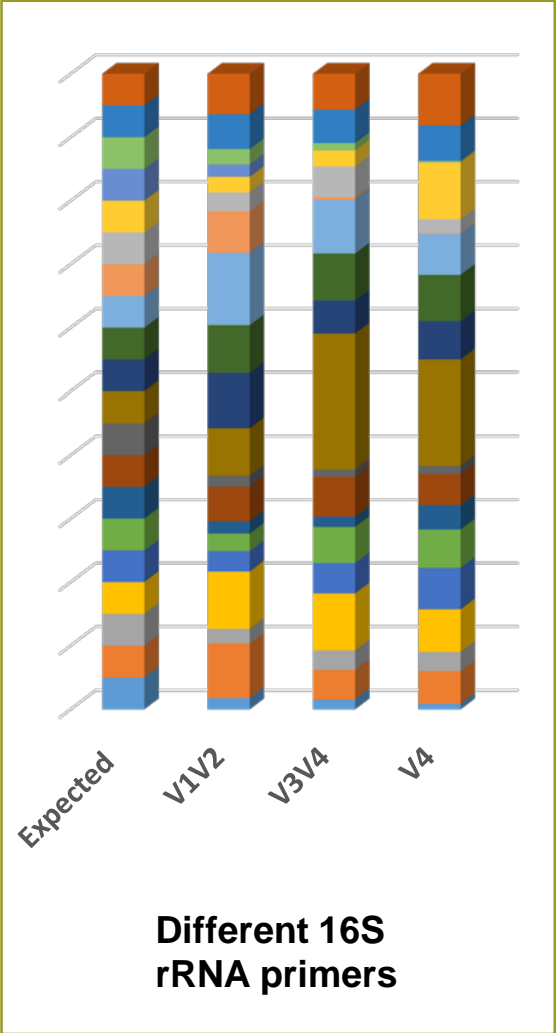
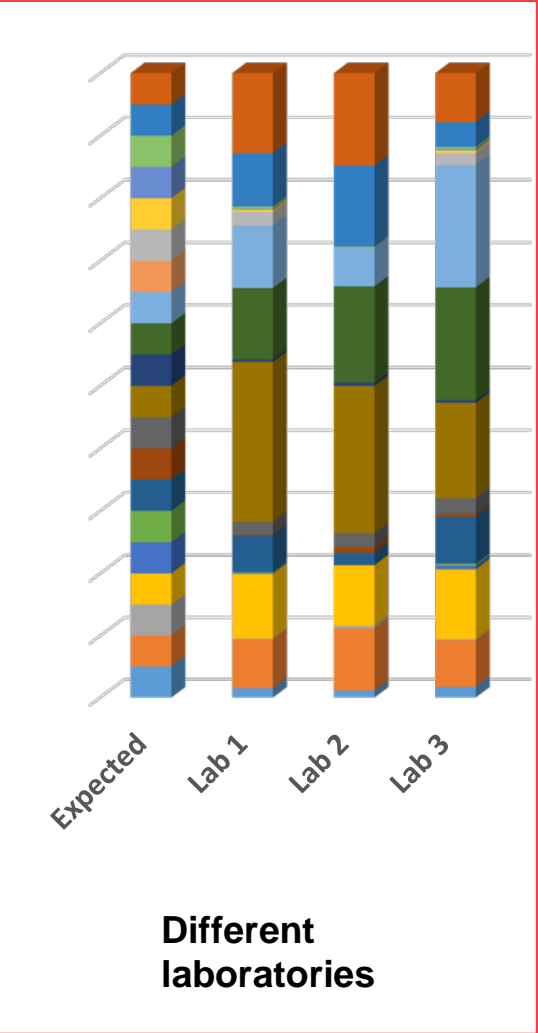
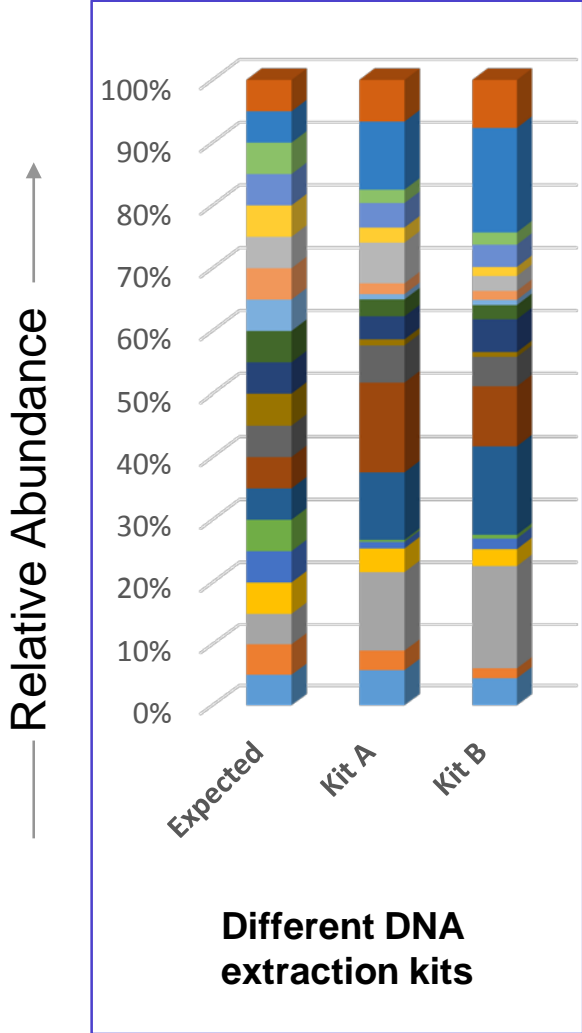
Received: 17 September 2014 | Accepted: 16 January 2015 | Published: 21 March 2015

Reagent and laboratory contamination can critically impact sequence-based microbiome analyses

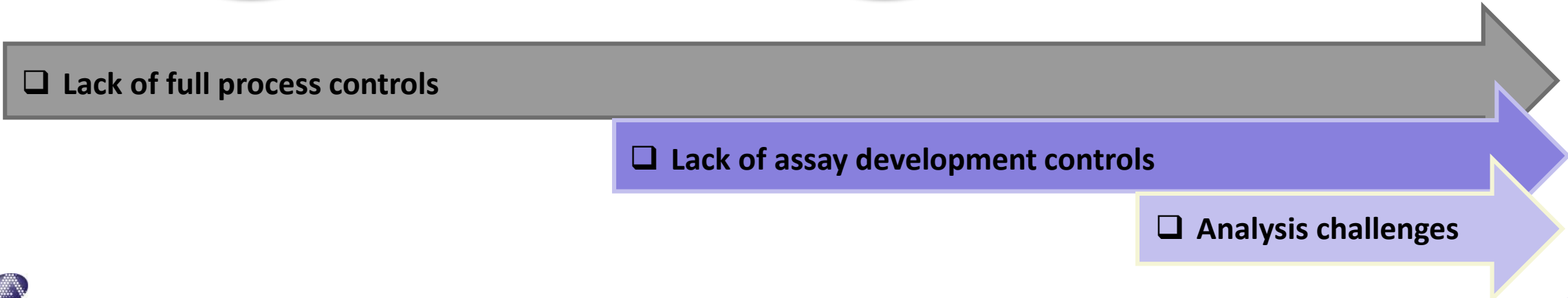
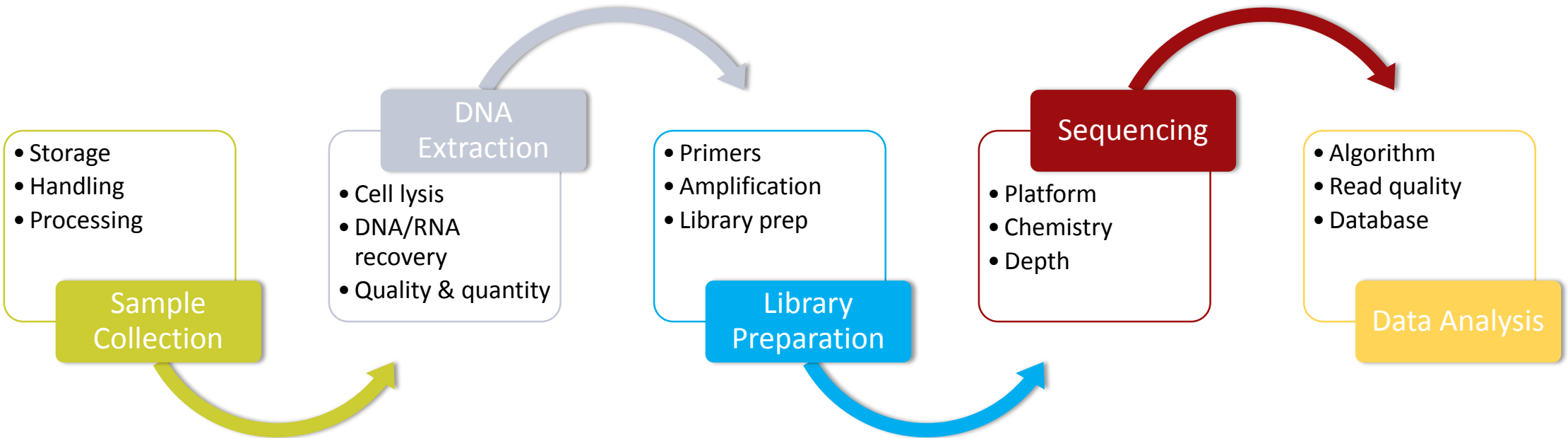
Susannah J Salter,[✉] Michael J Cox, Elena M Turek, Szymon T Calus, William O Cookson, Miriam F Moffatt, Paul Turner, Julian Parkhill, Nicholas J Loman, and Alan W Walker[✉]

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The problem: Different Data From the Same Sample



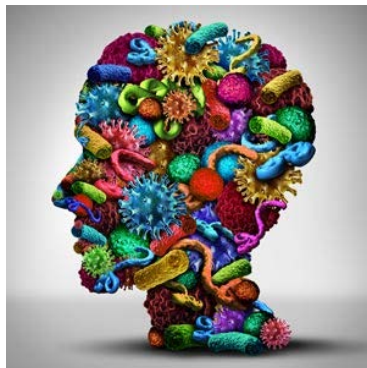
Microbiome Workflow, Biases, and Standardization



ATCC Microbiome Standards: Mixed Mock Communities

Whole Cell Standards

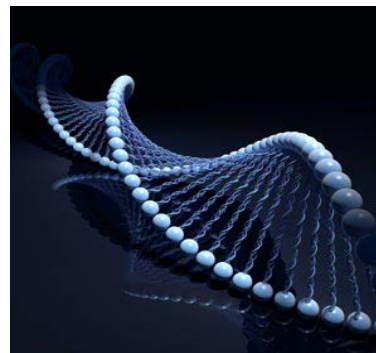
Lyophilized whole cells that are quantified and mixed in equal proportions



Full process controls

Genomic DNA Standards

Genomic DNA isolated from individual strains; preparations are quantified and mixed in appropriate proportions



Assay development & optimization tools

Bioinformatics Solutions

Access to One Codex to analyze quality control data from microbiome standards



End-to-end solution

Selection Attributes for Strains

ATCC® No.	Name	Gram Stain	% GC	Genome Size (Mb)	Special Features	Microbiome	16S rRNA Copies	GenBank ID
10987™	<i>Bacillus cereus</i>	+	35.2	5.42	Endospores former	Soil	12	NC_003909.8
15703™	<i>Bifidobacterium adolescentis</i>	+	59.2	2.09	Anaerobe	Gut	5	NC_008618.1
35702™	<i>Clostridium beijerinckii</i>	+	30	6.49	Spores former	Gut/soil	14	NC_009617.1
BAA-816™	<i>Deinococcus radiodurans</i>	-	66.7	3.29	Thick cell wall	Gut/environment	7	NC_001263.1
47077™	<i>Enterococcus faecalis</i>	+	37.5	3.36	Biofilm producer	Gut	4	NC_017316.1
700926™	<i>Escherichia coli</i>	-	50.8	4.64	Facultative anaerobe	Gut	7	NC_000913.3
33323™	<i>Lactobacillus gasseri</i>	+	35.3	1.89	Nuclease producer	Vaginal/gut	6	NC_008530.1
17029™	<i>Rhodobacter sphaeroides</i>	-	68.8	4.60	Metabolically diverse	Aquatic	3	NZ_AKVVW01000001.1
12228™	<i>Staphylococcus epidermidis</i>	+	31.9	2.56	Thick cell wall	Skin/mucosa	5	NC_004461.1
700610™	<i>Streptococcus mutans</i>	+	36.8	2.03	Facultative anaerobe	Oral	5	NC_004350.2
17978™	<i>Acinetobacter baumannii</i>	-	39	4.34	Filaments, capsule	Environment	6	NZ_CP009257.1
17982™	<i>Actinomyces odontolyticus</i>	+	65.5	2.39	Type 1 fimbriae	Oral	2	NZ_DS264586.1
8482™	<i>Bacteroides vulgatus</i>	-	42.2	5.16	Anaerobe	Gut	7	NC_009614.1
700392™	<i>Helicobacter pylori</i>	-	38.9	1.67	Helix shaped	Stomach/gut	2	NC_000915.1
BAA-335™	<i>Neisseria meningitidis</i>	-	51.5	2.27	Diplococcus	Respiratory tract	4	NC_003112.2
33277™	<i>Porphyromonas gingivalis</i>	-	48.4	2.35	Anaerobe, collagenase	Oral	4	NC_010729.1
11828™	<i>Propionibacterium acnes</i>	+	60	2.56	Aerotolerant anaerobe	Skin	4	NC_006085.1
9027™	<i>Pseudomonas aeruginosa</i>	-	66.6	6.26	Facultative anaerobe	Skin	4	NC_009656.1
BAA-1556™	<i>Staphylococcus aureus</i>	+	32.8	2.82	Thick cell wall	Skin/respiratory	6	NC_007795.1
BAA-611™	<i>Streptococcus agalactiae</i>	+	35.6	2.16	Serogroup B	Vaginal/environment	7	NC_004116.1

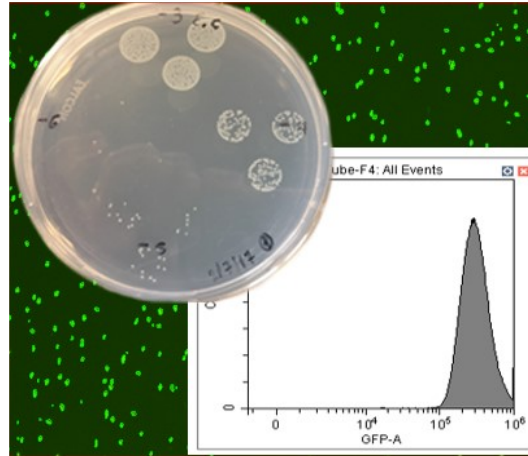
Development of Whole Cell Standards

ATCC Cultures



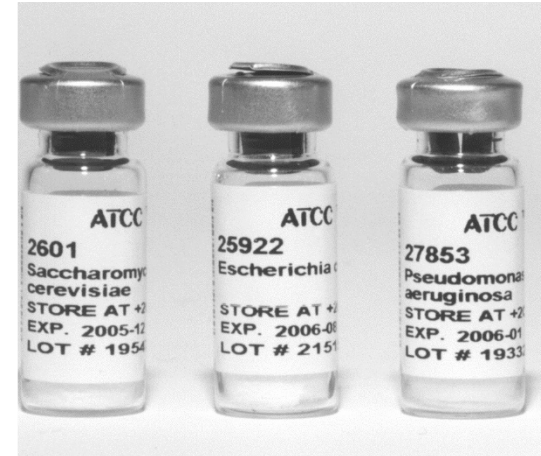
- Authenticated and fully characterized
- Genome sequenced
- Published in multiple databases

Growth & Quantification



- CFU
- Image cytometry
- Flow cytometry

Mix & Lyophilize



- Store at 4°C
- Ship at room temperature
- Mixed in even proportions based on number of cells

Whole Cell Standards



Species (10)	% Abundance
<i>Bacillus cereus</i>	10%
<i>Bifidobacterium adolescentis</i>	10%
<i>Clostridium beijerinckii</i>	10%
<i>Deinococcus radiodurans</i>	10%
<i>Enterococcus faecalis</i>	10%
<i>Escherichia coli</i>	10%
<i>Lactobacillus gasseri</i>	10%
<i>Rhodobacter sphaeroides</i>	10%
<i>Staphylococcus epidermidis</i>	10%
<i>Streptococcus mutans</i>	10%



Species (20)	% Abundance
<i>Acinetobacter baumannii</i>	5%
<i>Actinomyces odontolyticus</i>	5%
<i>Bacillus cereus</i>	5%
<i>Bacteroides vulgatus</i>	5%
<i>Bifidobacterium adolescentis</i>	5%
<i>Clostridium beijerinckii</i>	5%
<i>Deinococcus radiodurans</i>	5%
<i>Enterococcus faecalis</i>	5%
<i>Escherichia coli</i>	5%
<i>Helicobacter pylori</i>	5%
<i>Lactobacillus gasseri</i>	5%
<i>Neisseria meningitidis</i>	5%
<i>Porphyromonas gingivalis</i>	5%
<i>Propionibacterium acnes</i>	5%
<i>Pseudomonas aeruginosa</i>	5%
<i>Rhodobacter sphaeroides</i>	5%
<i>Staphylococcus aureus</i>	5%
<i>Staphylococcus epidermidis</i>	5%
<i>Streptococcus agalactiae</i>	5%
<i>Streptococcus mutans</i>	5%

Even amounts | 2×10^6 cells/organism | Lyophilized cells

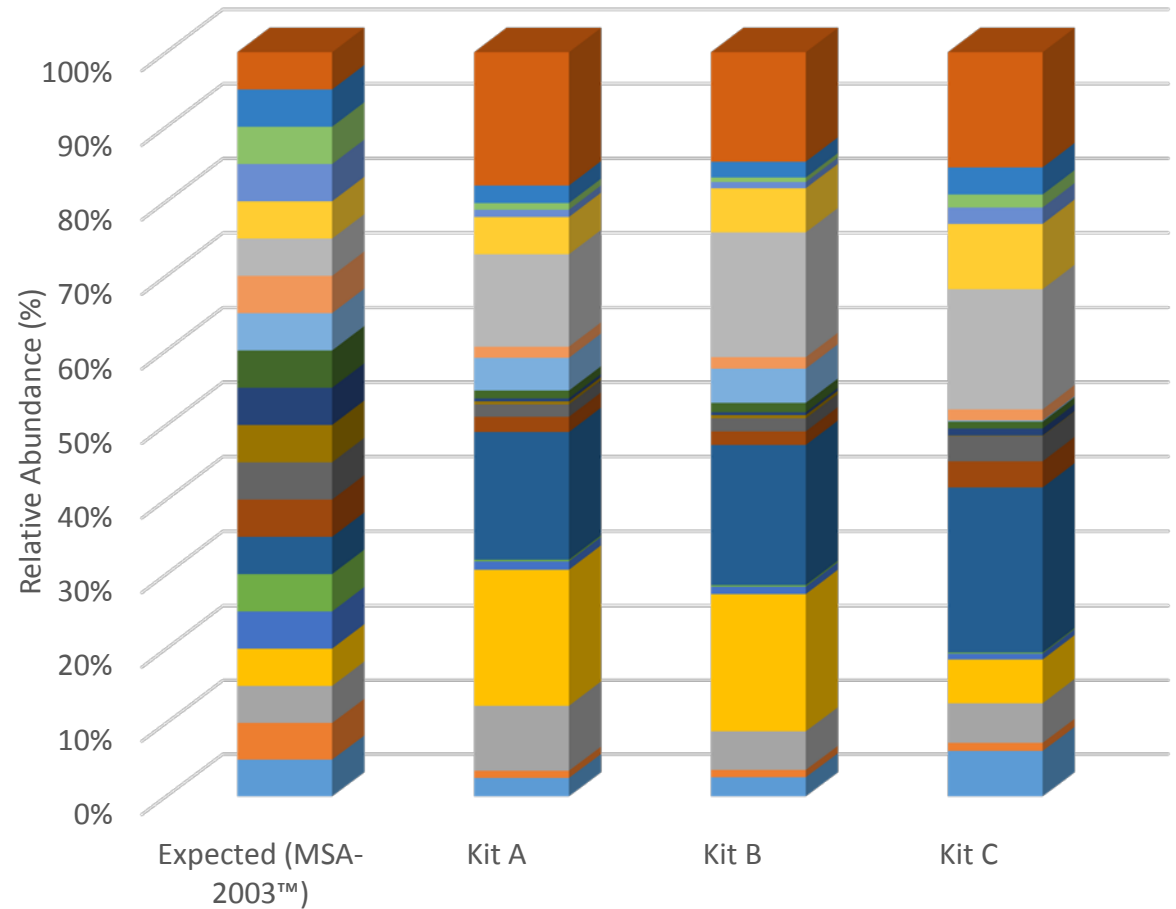
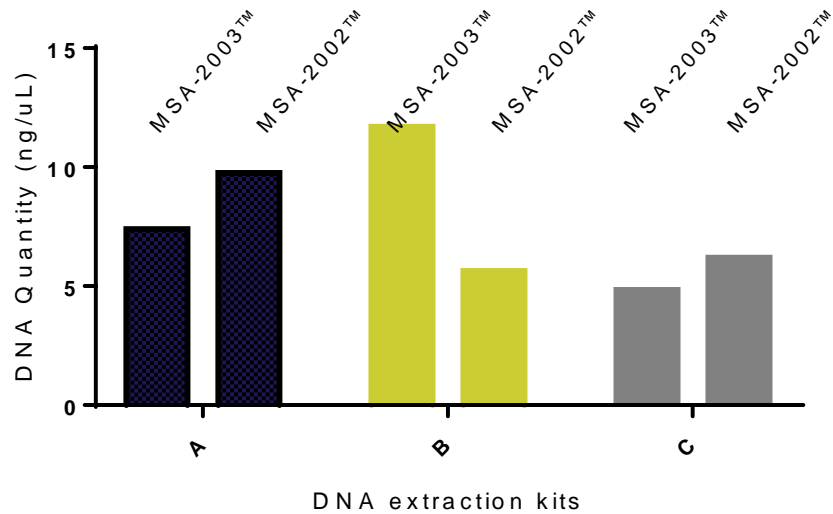
Assay Optimization: DNA Extraction kits | Daily run controls for full-process monitoring

Evaluation of DNA Extraction Methods Using Whole Cell Standards

Reconstitute lyophilized product in 1 mL PBS

Aliquot into 5 vials (200 μ L) and pellet cells (10,000xg for 10 minutes)

Perform DNA extraction or store the pellet (-20°C)



Evaluation of DNA Extraction Methods Using Whole Cell Standards

ATCC® MSA-2002™ Percent of number of reads (Relative abundance) – shotgun metagenomic analysis

Species	Expected	Kit A	Kit B	Kit C
<i>Acinetobacter baumannii</i>	5.00%	2.50%	2.60%	6.19%
<i>Actinomyces odontolyticus</i>	5.00%	1.00%	1.01%	1.11%
<i>Bacillus cereus</i>	5.00%	8.85%	5.26%	5.38%
<i>Bacteroides vulgatus</i>	5.00%	18.25%	18.45%	5.87%
<i>Bifidobacterium adolescentis</i>	5.00%	1.12%	0.99%	0.79%
<i>Clostridium beijerinckii</i>	5.00%	0.22%	0.21%	0.16%
<i>Deinococcus radiodurans</i>	5.00%	17.13%	18.80%	22.13%
<i>Enterococcus faecalis</i>	5.00%	2.04%	1.82%	3.52%
<i>Escherichia coli</i>	5.00%	1.67%	1.75%	3.37%
<i>Helicobacter pylori</i>	5.00%	0.39%	0.43%	0.07%
<i>Lactobacillus gasseri</i>	5.00%	0.42%	0.38%	0.95%
<i>Neisseria meningitidis</i>	5.00%	1.05%	1.26%	0.92%
<i>Porphyromonas gingivalis</i>	5.00%	4.39%	4.60%	0.17%
<i>Propionibacterium acnes</i>	5.00%	1.50%	1.54%	1.47%
<i>Pseudomonas aeruginosa</i>	5.00%	12.39%	16.73%	16.12%
<i>Rhodobacter sphaeroides</i>	5.00%	5.00%	5.92%	8.76%
<i>Staphylococcus aureus</i>	5.00%	0.98%	0.83%	2.21%
<i>Staphylococcus epidermidis</i>	5.00%	0.89%	0.60%	1.73%
<i>Streptococcus agalactiae</i>	5.00%	2.35%	2.12%	3.64%
<i>Streptococcus mutans</i>	5.00%	17.88%	14.69%	15.45%



Development of Genomic DNA Standards

ATCC Cultures



- Authenticated and fully characterized
- Genome sequenced
- Published in multiple databases

Extraction & Quality Control



- Fluorescent dye-based quantification
- Digital PCR
- WGS on individual genomes

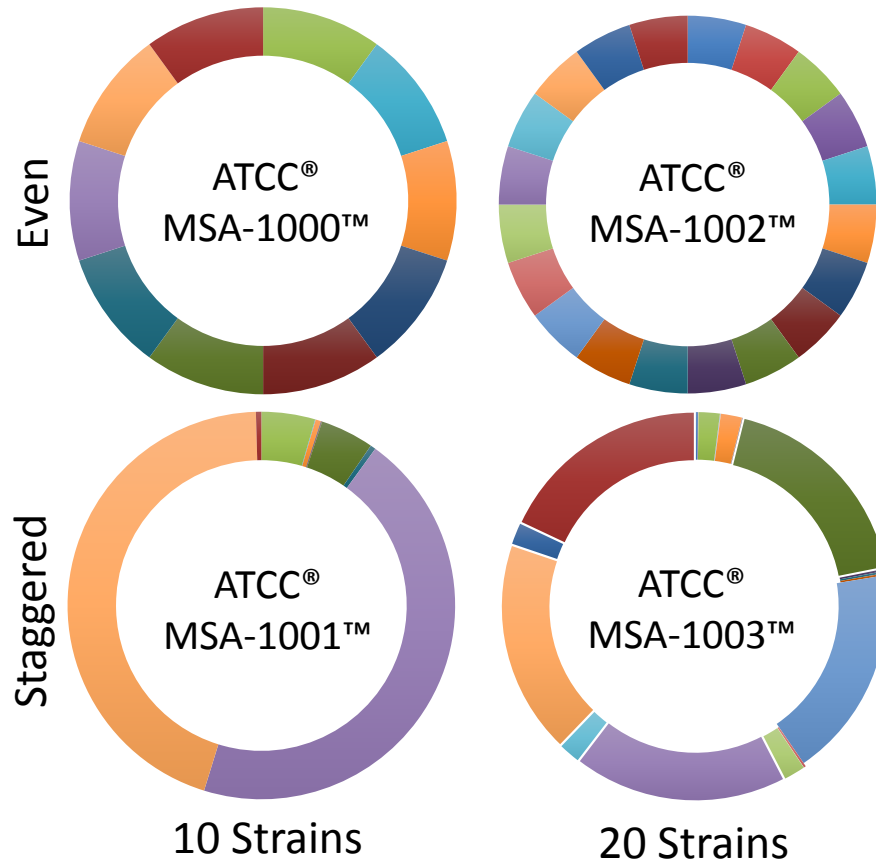
Mixed in Even and Staggered Proportions



- Store at -20°C
- Mixed in even or staggered proportions based on copy number

Development of Genomic DNA Standards

Species (10)	% Abundance	
	MSA-1000™	MSA-1001™
<i>Bacillus cereus</i>	10%	4.48%
<i>Bifidobacterium adolescentis</i>	10%	0.04%
<i>Clostridium beijerinckii</i>	10%	0.45%
<i>Deinococcus radiodurans</i>	10%	0.04%
<i>Enterococcus faecalis</i>	10%	0.04%
<i>Escherichia coli</i>	10%	4.48%
<i>Lactobacillus gasseri</i>	10%	0.45%
<i>Rhodobacter sphaeroides</i>	10%	44.78%
<i>Staphylococcus epidermidis</i>	10%	44.78%
<i>Streptococcus mutans</i>	10%	0.45%



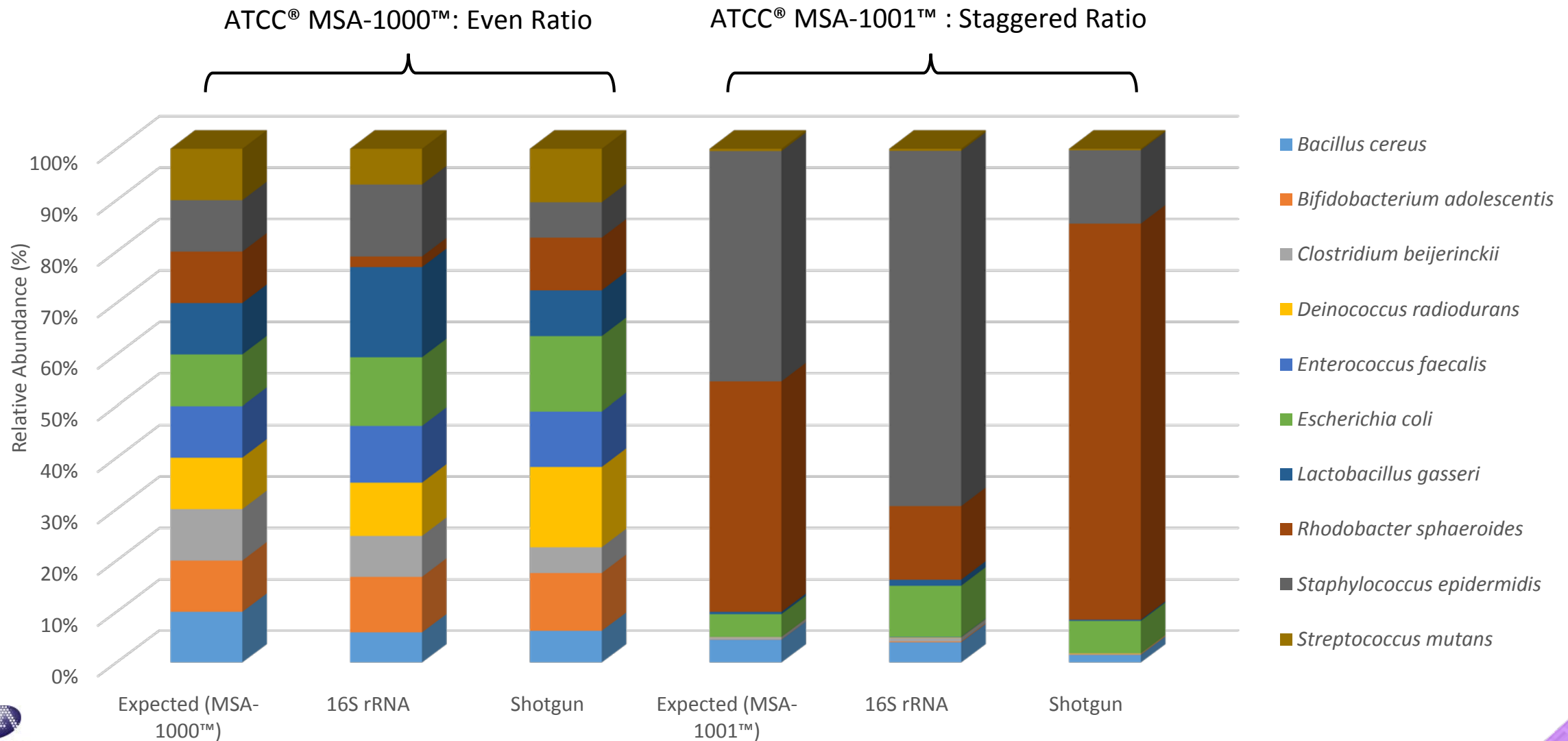
Species (20)	% Abundance	
	MSA-1002™	MSA-1003™
<i>Acinetobacter baumannii</i>	5%	0.18%
<i>Actinomyces odontolyticus</i>	5%	0.02%
<i>Bacillus cereus</i>	5%	1.80%
<i>Bacteroides vulgatus</i>	5%	0.02%
<i>Bifidobacterium adolescentis</i>	5%	0.02%
<i>Clostridium beijerinckii</i>	5%	1.80%
<i>Deinococcus radiodurans</i>	5%	0.02%
<i>Enterococcus faecalis</i>	5%	0.02%
<i>Escherichia coli</i>	5%	18.0%
<i>Helicobacter pylori</i>	5%	0.18%
<i>Lactobacillus gasseri</i>	5%	0.18%
<i>Neisseria meningitidis</i>	5%	0.18%
<i>Porphyromonas gingivalis</i>	5%	18.0%
<i>Propionibacterium acnes</i>	5%	0.18%
<i>Pseudomonas aeruginosa</i>	5%	1.80%
<i>Rhodobacter sphaeroides</i>	5%	18.0%
<i>Staphylococcus aureus</i>	5%	1.80%
<i>Staphylococcus epidermidis</i>	5%	18.0%
<i>Streptococcus agalactiae</i>	5%	1.80%
<i>Streptococcus mutans</i>	5%	18.0%

Even amounts: 2×10^6 genome copies/organism

Staggered amounts: 2×10^4 – 2×10^7 genome copies/organism

Assay development, optimization, reproducibility, verification, and validation

Comparing 16S rRNA and Shotgun Analyses Using Genomic DNA Standards

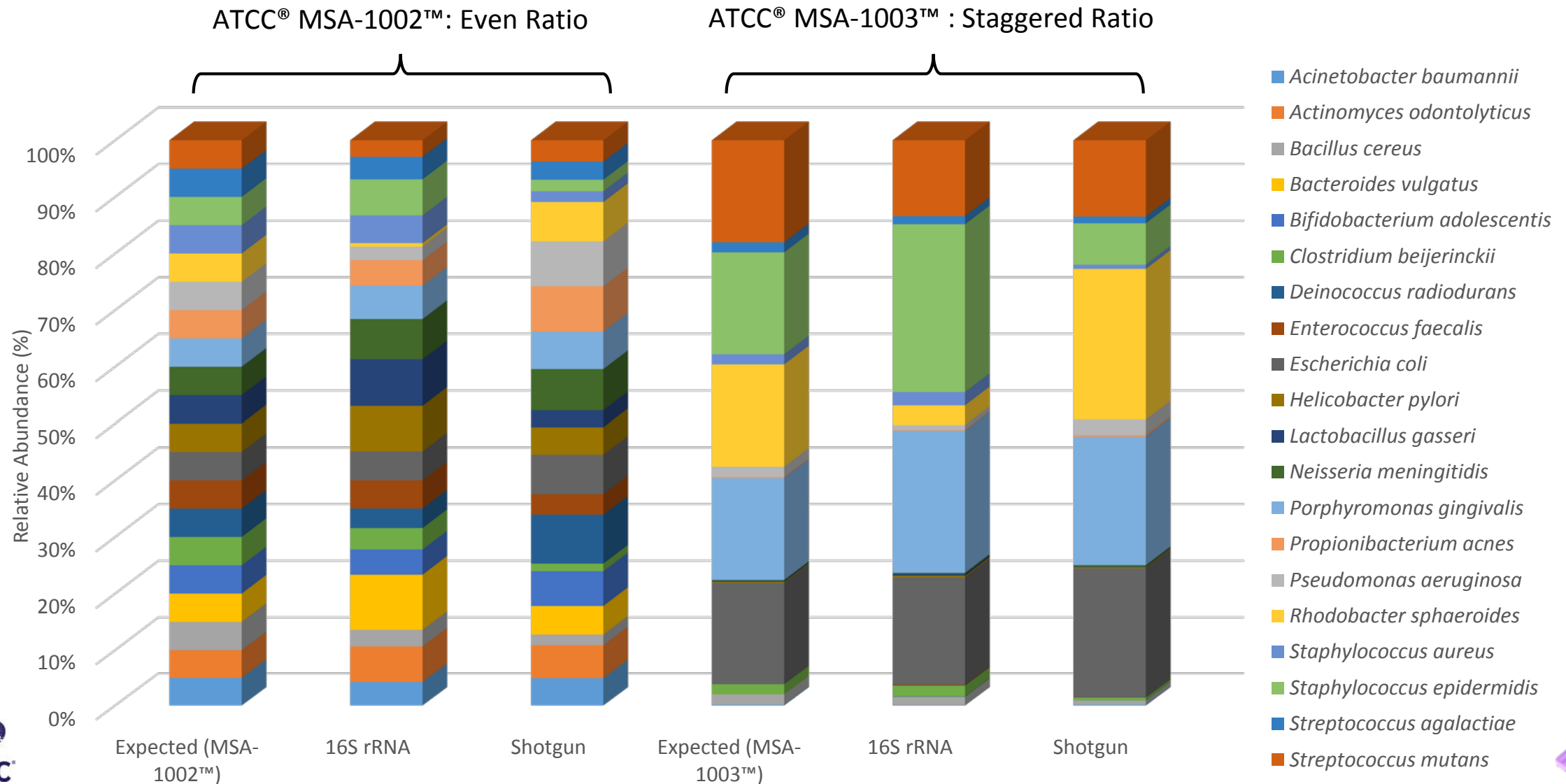


Comparing 16S rRNA and Shotgun Analyses Using Genomic DNA Standards

Percent of number of reads (Relative abundance)

Species	ATCC® MSA-1000™			ATCC® MSA-1001™		
	Expected	16S rRNA	Shotgun	Expected	16S rRNA	Shotgun
<i>Bacillus cereus</i>	10.00%	5.94%	6.24%	4.48%	3.98%	1.49%
<i>Bifidobacterium adolescentis</i>	10.00%	10.90%	11.34%	0.04%	0.15%	0.08%
<i>Clostridium beijerinckii</i>	10.00%	7.94%	5.00%	0.45%	0.69%	0.11%
<i>Deinococcus radiodurans</i>	10.00%	10.36%	15.62%	0.04%	0.08%	0.10%
<i>Enterococcus faecalis</i>	10.00%	11.04%	10.76%	0.04%	0.13%	0.04%
<i>Escherichia coli</i>	10.00%	13.26%	14.60%	4.48%	10.06%	6.37%
<i>Lactobacillus gasseri</i>	10.00%	17.54%	8.90%	0.45%	1.17%	0.24%
<i>Rhodobacter sphaeroides</i>	10.00%	2.07%	10.25%	44.78%	14.32%	77.01%
<i>Staphylococcus epidermidis</i>	10.00%	13.99%	6.88%	44.78%	69.01%	14.27%
<i>Streptococcus mutans</i>	10.00%	6.95%	10.40%	0.45%	0.41%	0.28%

Comparing 16S rRNA and Shotgun Analyses Using Genomic DNA Standards

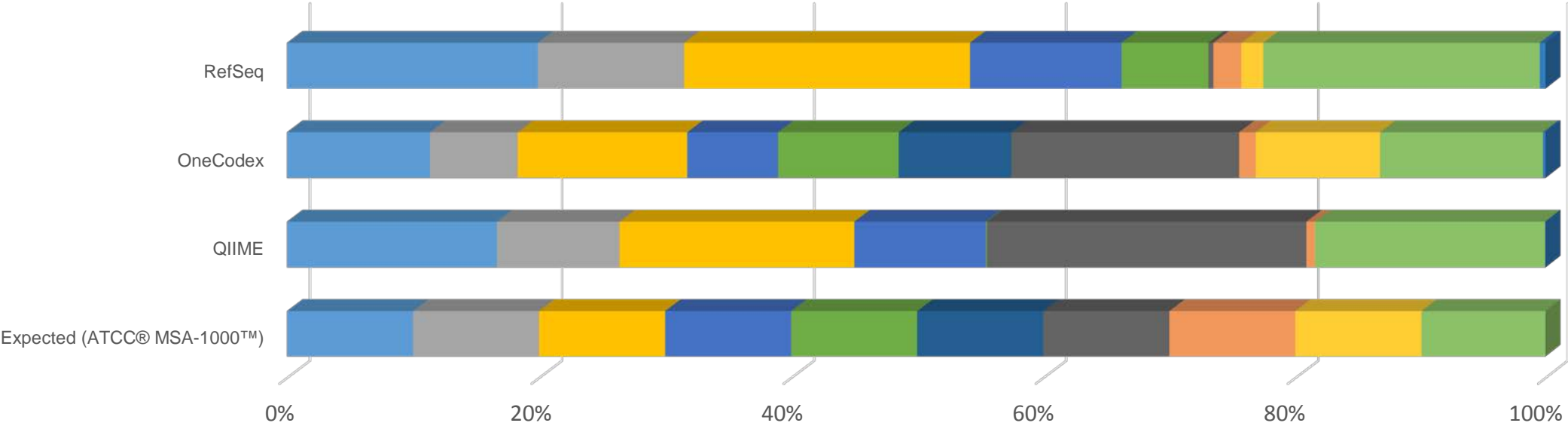


Comparing 16S rRNA and Shotgun Analyses Using Genomic DNA Standards

Species	ATCC® MSA-1002™			ATCC® MSA-1003™		
	Expected	16S rRNA	Shotgun	Expected	16S rRNA	Shotgun
<i>Acinetobacter baumannii</i>	5%	4.27%	4.94%	0.18%	0.13%	0.18%
<i>Actinomyces odontolyticus</i>	5%	6.40%	5.92%	0.02%	0.05%	0.03%
<i>Bacillus cereus</i>	5%	2.92%	1.90%	1.80%	1.30%	0.65%
<i>Bacteroides vulgatus</i>	5%	9.74%	5.04%	0.02%	0.05%	0.02%
<i>Bifidobacterium adolescentis</i>	5%	4.46%	6.15%	0.02%	0.12%	0.03%
<i>Clostridium beijerinckii</i>	5%	3.77%	1.34%	1.80%	1.95%	0.50%
<i>Deinococcus radiodurans</i>	5%	3.45%	8.64%	0.02%	0.07%	0.03%
<i>Enterococcus faecalis</i>	5%	4.99%	3.63%	0.02%	0.14%	0.02%
<i>Escherichia coli</i>	5%	5.09%	6.96%	18.00%	19.06%	23.02%
<i>Helicobacter pylori</i>	5%	7.99%	4.84%	0.18%	0.28%	0.17%
<i>Lactobacillus gasseri</i>	5%	8.22%	2.97%	0.18%	0.34%	0.11%
<i>Neisseria meningitides</i>	5%	7.12%	7.27%	0.18%	0.20%	0.25%
<i>Porphyromonas gingivalis</i>	5%	5.88%	6.61%	18.00%	25.08%	22.68%
<i>Propionibacterium acnes</i>	5%	4.51%	8.00%	0.18%	0.16%	0.29%
<i>Pseudomonas aeruginosa</i>	5%	2.38%	7.94%	1.80%	0.83%	2.69%
<i>Rhodobacter sphaeroides</i>	5%	0.63%	6.95%	18.00%	3.44%	26.58%
<i>Staphylococcus aureus</i>	5%	4.89%	1.91%	1.80%	2.37%	0.76%
<i>Staphylococcus epidermidis</i>	5%	6.37%	2.02%	18.00%	29.62%	7.30%
<i>Streptococcus agalactiae</i>	5%	3.97%	3.21%	1.80%	1.44%	1.19%
<i>Streptococcus mutans</i>	5%	2.93%	3.76%	18.00%	13.39%	13.48%

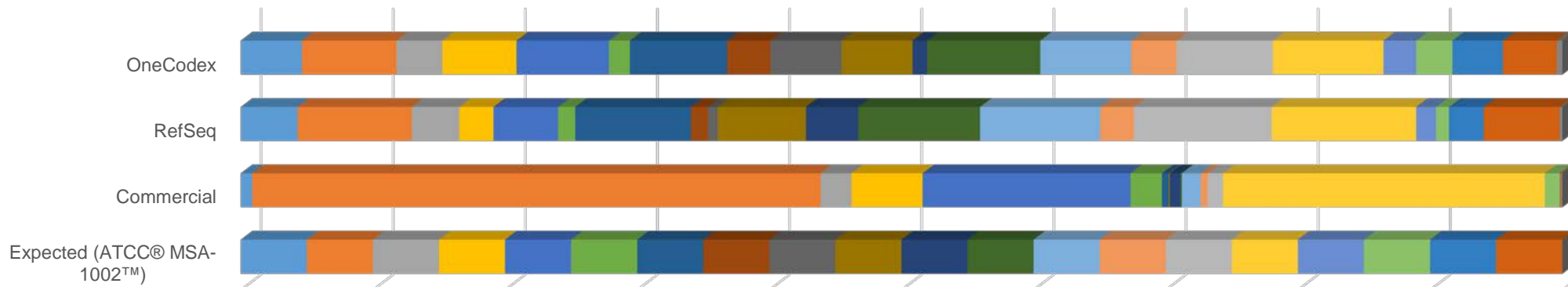


Evaluation of 16S rRNA Databases using the Genomic DNA Standard



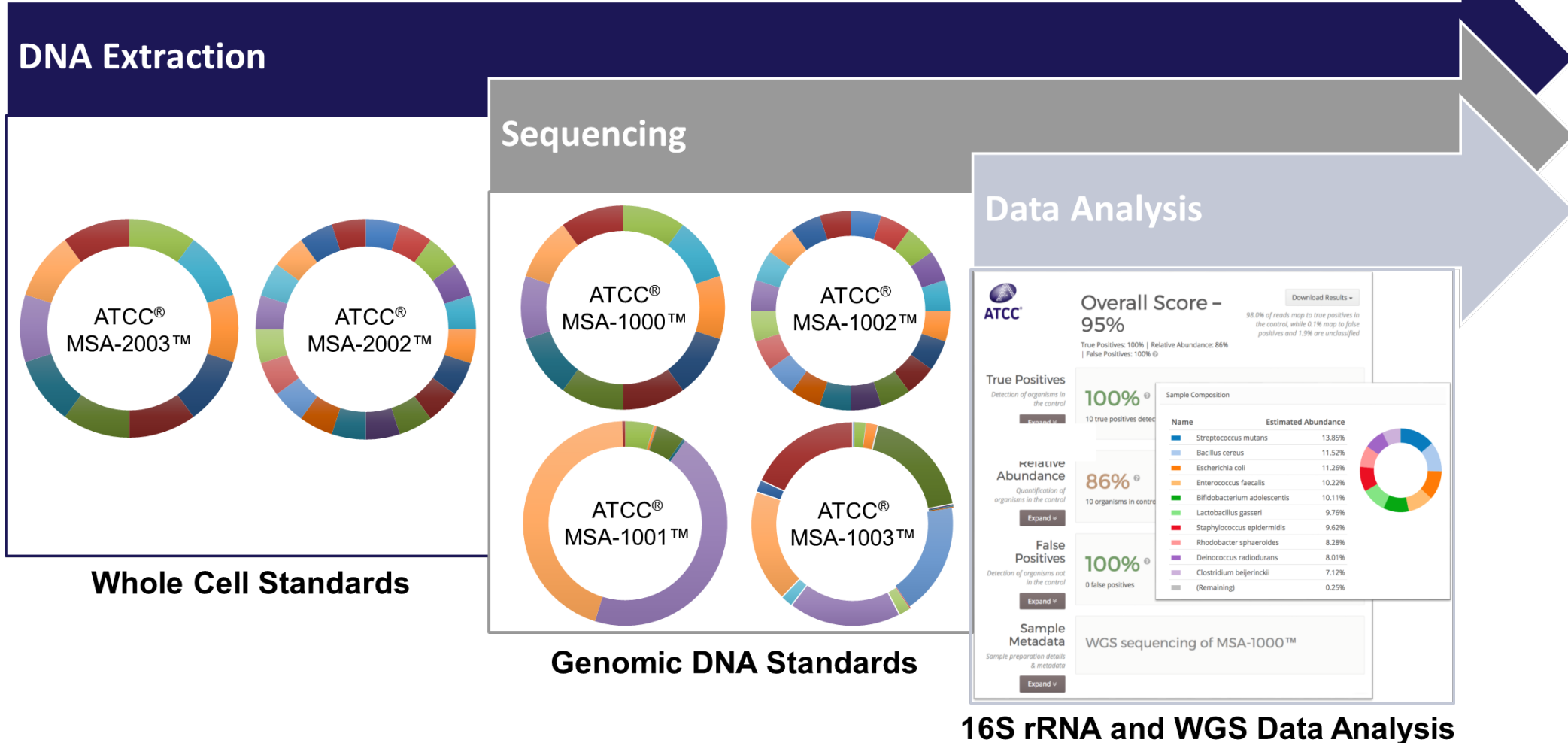
	Expected (ATCC® MSA-1000™)	QIIME	OneCodex	RefSeq
■ <i>Bacillus cereus</i>	10.00%	16.66%	11.35%	19.90%
■ <i>Bifidobacterium adolescentis</i>	10.00%	9.73%	6.96%	11.61%
■ <i>Clostridium beijerinckii</i>	10.00%	18.63%	13.45%	22.68%
■ <i>Deinococcus radiodurans</i>	10.00%	10.44%	7.20%	12.02%
■ <i>Enterococcus faecalis</i>	10.00%	0.08%	9.57%	6.89%
■ <i>Escherichia coli</i>	10.00%	0.00%	8.94%	0.00%
■ <i>Lactobacillus gasseri</i>	10.00%	25.34%	18.07%	0.40%
■ <i>Rhodobacter sphaeroides</i>	10.00%	0.67%	1.33%	2.22%
■ <i>Staphylococcus epidermidis</i>	10.00%	0.02%	9.85%	1.74%
■ <i>Streptococcus mutans</i>	10.00%	18.42%	13.08%	22.08%
■ Remaining		0.02%	0.20%	0.46%

Evaluation of Different Bioinformatics Platforms and Databases for Shotgun Analysis



	Expected (ATCC® MSA-1002™)	Commercial	RefSeq	OneCodex
■ <i>Acinetobacter baumannii</i>	5.00%	0.87%	4.30%	4.63%
■ <i>Actinomyces odontolyticus</i>	5.00%	43.01%	8.64%	7.15%
■ <i>Bacillus cereus</i>	5.00%	2.34%	3.60%	3.48%
■ <i>Bacteroides vulgatus</i>	5.00%	5.38%	2.58%	5.61%
■ <i>Bifidobacterium adolescentis</i>	5.00%	15.73%	4.89%	7.00%
■ <i>Clostridium beijerinckii</i>	5.00%	2.37%	1.29%	1.58%
■ <i>Deinococcus radiodurans</i>	5.00%	0.52%	8.76%	7.36%
■ <i>Enterococcus faecalis</i>	5.00%	0.01%	1.30%	3.29%
■ <i>Escherichia coli</i>	5.00%	0.00%	0.72%	5.37%
■ <i>Helicobacter pylori</i>	5.00%	0.08%	6.68%	5.37%
■ <i>Lactobacillus gasseri</i>	5.00%	0.79%	3.95%	1.10%
■ <i>Neisseria meningitidis</i>	5.00%	0.13%	9.23%	8.56%
■ <i>Porphyromonas gingivalis</i>	5.00%	1.42%	9.10%	6.88%
■ <i>Propionibacterium acnes</i>	5.00%	0.53%	2.54%	3.43%
■ <i>Pseudomonas aeruginosa</i>	5.00%	1.16%	10.44%	7.29%
■ <i>Rhodobacter sphaeroides</i>	5.00%	24.34%	10.93%	8.36%
■ <i>Staphylococcus aureus</i>	5.00%	0.01%	1.51%	2.48%
■ <i>Staphylococcus epidermidis</i>	5.00%	1.12%	0.97%	2.74%
■ <i>Streptococcus agalactiae</i>	5.00%	0.00%	2.63%	3.82%
■ <i>Streptococcus mutans</i>	5.00%	0.08%	5.74%	4.07%
■ Remaining	0.00%	0.12%	0.20%	0.42%

ATCC® Microbiome Standards: Controlled Reference Materials that Mimic Complex Microbiome Specimens



Standards for Molecular Diagnostics

ATCC® Molecular Standards: Genomic and Synthetic Nucleic Acids

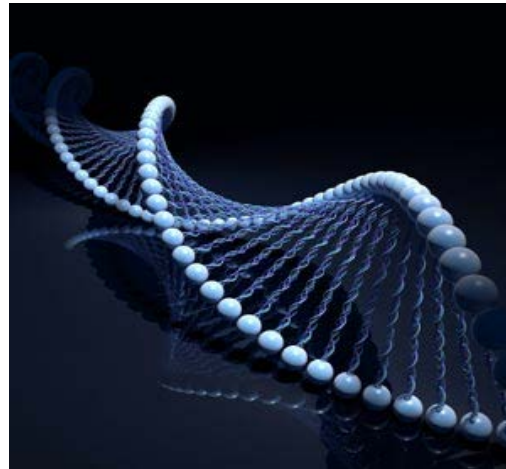
Nucleic Acids

Whole genome preparations
extracted from ATCC Genuine
Cultures®



Quantitative Genomic Molecular Standards

Whole genome preparations
quantified using digital PCR



Quantitative Synthetic Molecular Standards

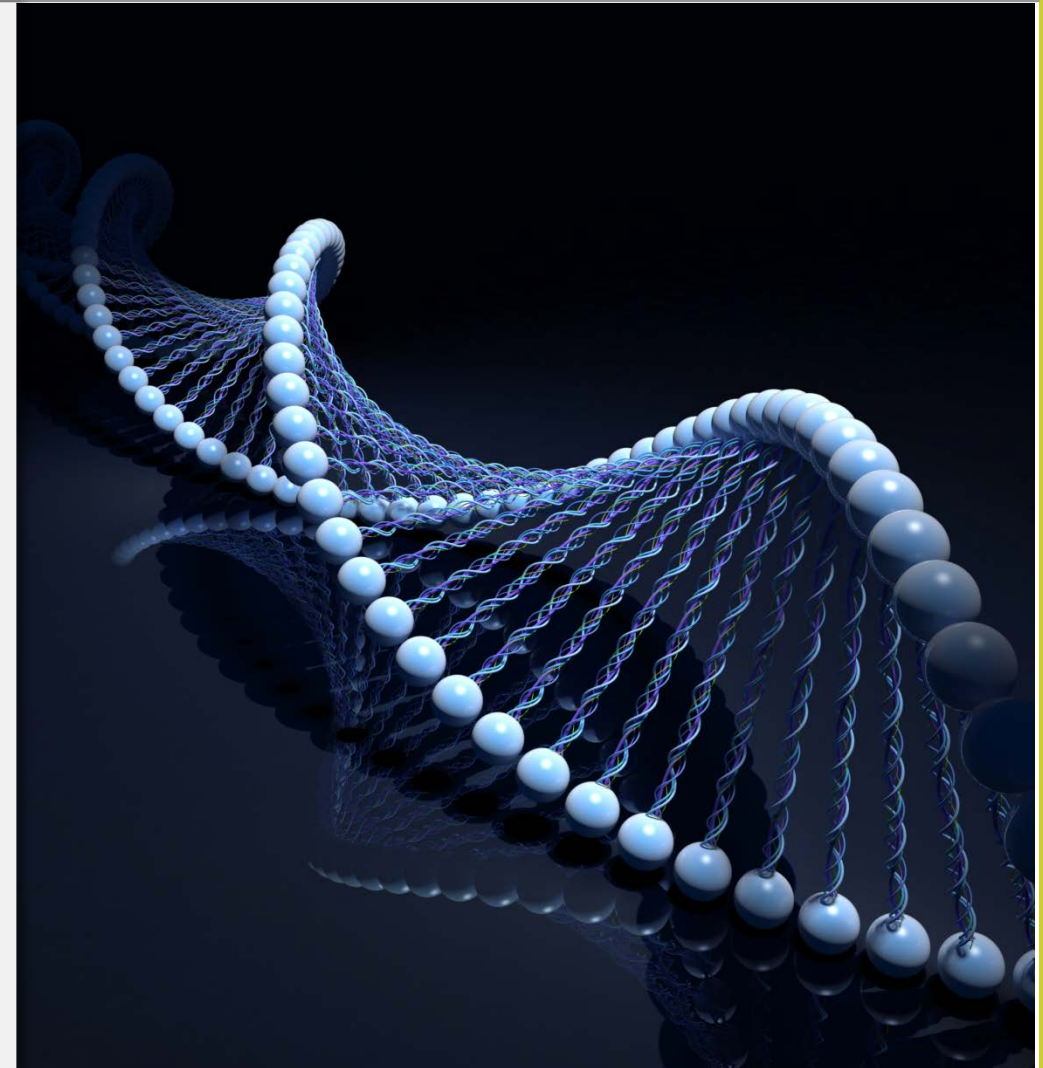
Synthetic DNA or RNA
preparations quantified using
digital PCR



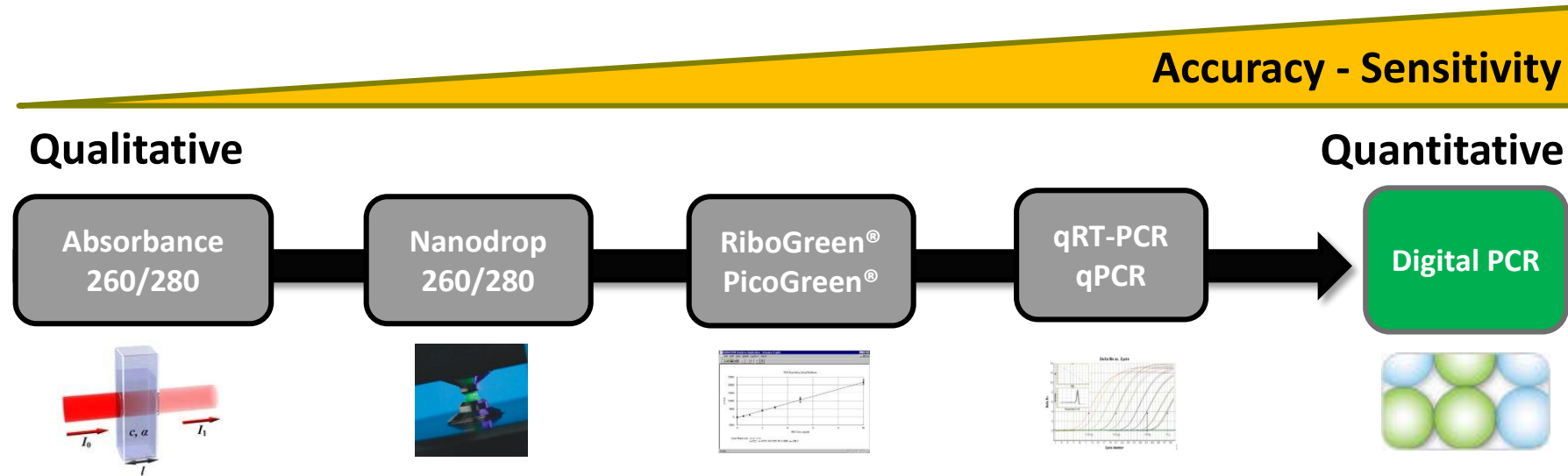
ATCC Genomic Nucleic Acids

Whole genome preparations extracted from ATCC Genuine Cultures®

- More than 800 catalog items
- Agarose gel electrophoresis to ensure integrity
- Spectrophotometry to evaluate purity
- PicoGreen® or RiboGreen® to calculate concentration
- PCR to confirm functional activity
- Sequence analysis of conserved genomic regions to confirm species identity
- Custom preparations available
- DNA from bacteria, fungi, protists, and viral strains
- RNA from viral strains



Quantitative Molecular Standards



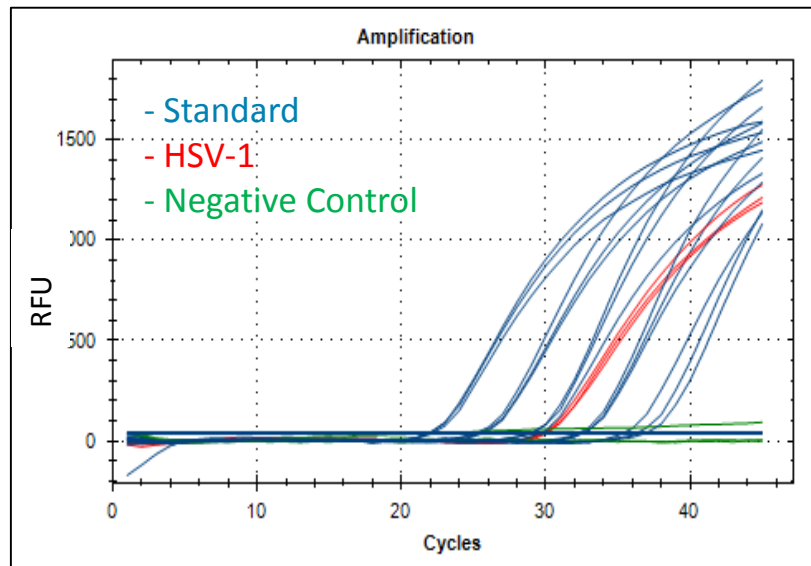
Absolute quantification using digital PCR:

- Allows for absolute quantification of nucleic acids
- High precision and accuracy
- Target-specific quantification
- Copy number of individual genes
- No need to generate cloned standards for standard curve

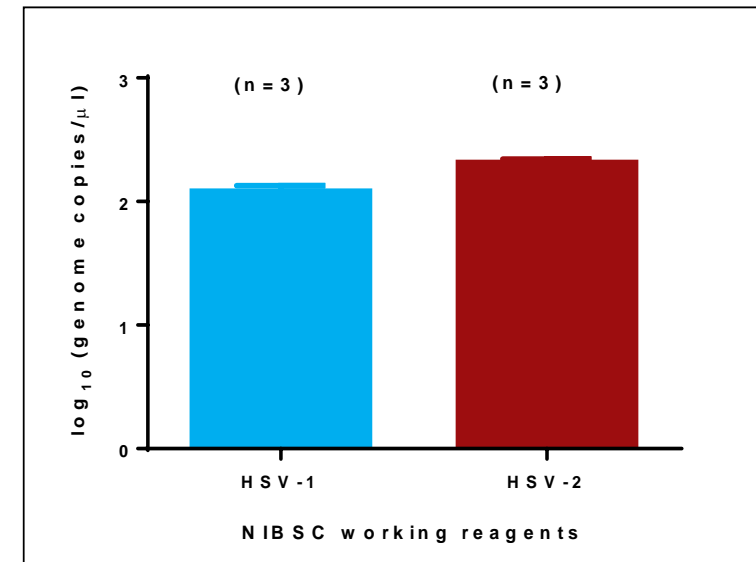
Quantification of NIBSC Working Reagents for HSV-1 and HSV-2 Using ATCC Quantitative Molecular Standards

ATCC® No.	Description
VR-539D™	Genomic DNA from Human herpesvirus 1 (HSV-1)
VR-540D™	Genomic DNA from Human herpesvirus 2 (HSV-2)
VR-539DQ™	Quantitative Genomic DNA from Human herpesvirus 1 (HSV-1)
VR-540DQ™	Quantitative Genomic DNA from Human herpesvirus 2 (HSV-2)

qPCR amplification plot using VR-539DQ™



Quantitation of the working reagent



Quantitative molecular standards can be used in qPCR assays for relative quantification of unknown preparations

ATCC Synthetic Molecular Standards

Can be used as positive controls for:

- Difficult-to-culture or unculturable strains
- Strains requiring BSL-3 containment
- Strains on the commerce control list

Advantages of synthetic nucleic acids:

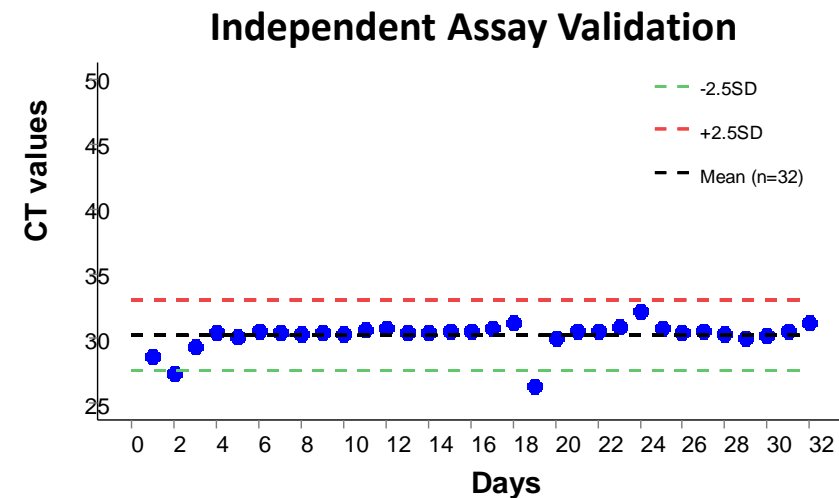
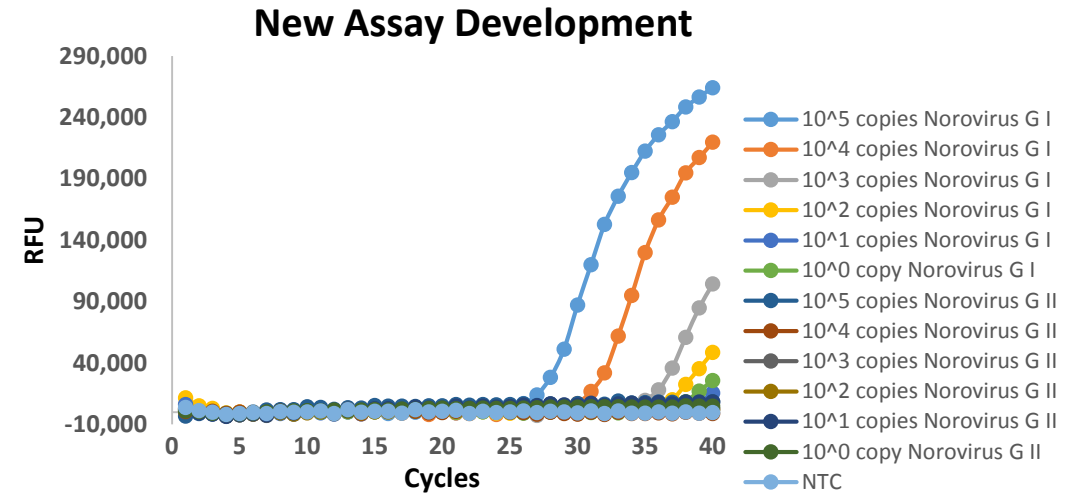
- Eliminate the need to culture microorganisms
- Can be used in a BSL-1 facility
- No shipping restrictions
- Manufacturing under ISO 13485:2003
- Quantified using Droplet Digital™ PCR
- Useful for monitoring assay-to-assay- or lot-to-lot variation



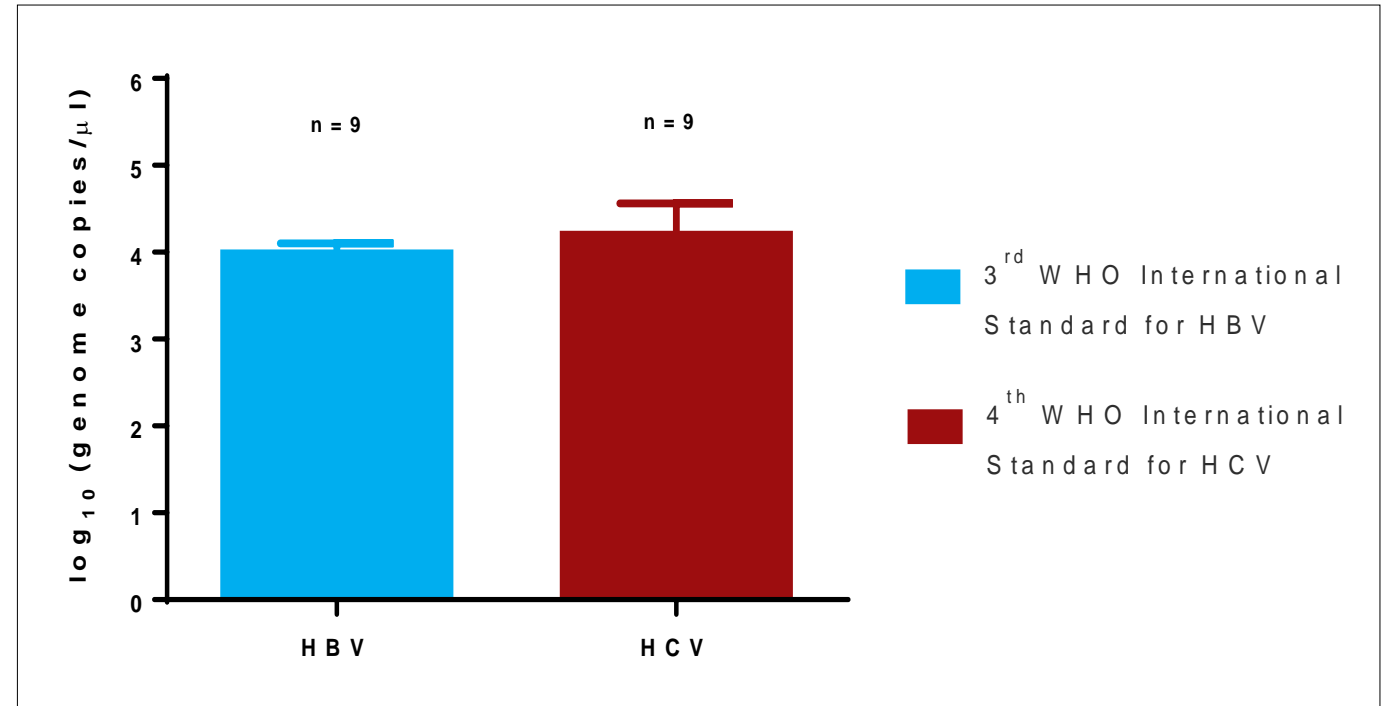
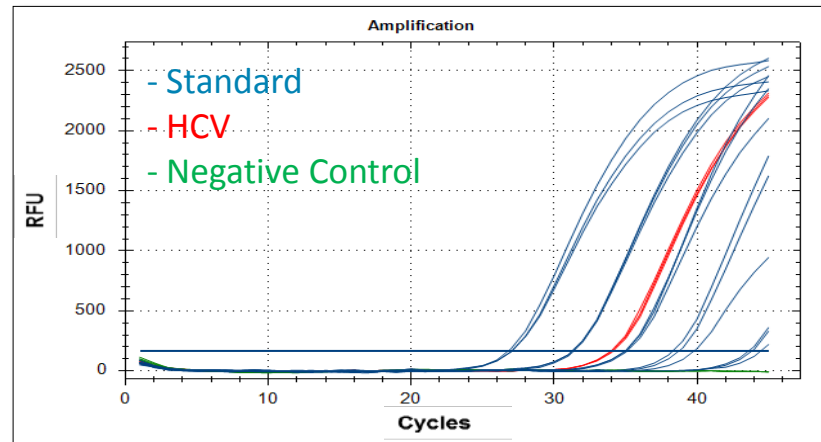
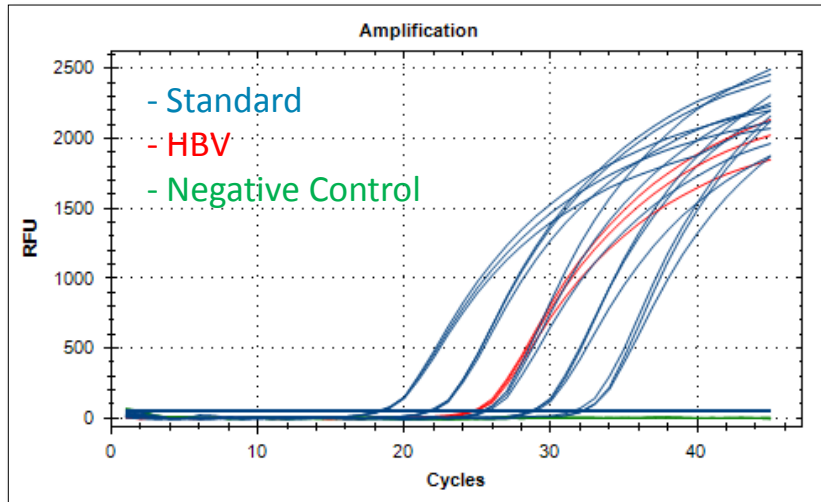
ATCC Synthetic Norovirus RNA

ATCC® No.	Description
VR-3234SD™	Synthetic Norovirus GI RNA (NoV GI)
VR-3235SD™	Synthetic Norovirus GII RNA (NoV GII)

Salient features	Applications
<ul style="list-style-type: none"> Fully authenticated & characterized Generated under ISO 13485:2003 Quantitative format Compatible with several lab-developed and commercially available assays BSL-1 ready-to-use control Stabilized RNA 	<ul style="list-style-type: none"> Generation of a standard curve for qRT-PCR Positive control for RT-PCR assays Independent standard for validation and verification studies Monitor assay-to-assay and lot-to-lot variation New assay development Limit of detection studies

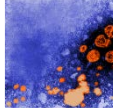


Quantification of WHO International Standards for HBV and HCV Using ATCC Synthetic Molecular Standards



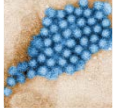
Quantitative synthetic molecular standards can be used in qPCR assays for relative quantification of unknown preparations

ATCC® Quantitative Molecular Standards



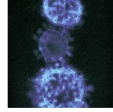
Blood-borne disease

- BK Virus
- Hepatitis B virus
- Hepatitis C virus
- Epstein-Barr virus
- Human immunodeficiency virus 1
- Human T-cell leukemia virus
- Human cytomegalovirus
- Varicella-zoster virus
- *Neisseria meningitidis*



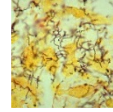
Gastro-Intestinal disease

- Astrovirus
- *Cyclospora cayetanensis*
- Hepatitis A virus
- Hepatitis E virus
- Norovirus GI
- Norovirus GII
- Sapovirus
- *Mycobacterium avium* subsp. *paratuberculosis*
- *Clostridium difficile*
- *Salmonella enterica* subsp. *enterica* serovar Typhimurium
- *Cryptosporidium parvum*
- Human Enterovirus 71
- Rotavirus A
- *Entamoeba histolytica*
- *Enterococcus faecalis*
- *Escherichia coli*
- *Helicobacter pylori*
- *Streptococcus agalactiae*



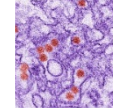
Respiratory disease

- Human bocavirus
- Middle East respiratory syndrome coronavirus
- Human metapneumovirus
- *Bordetella pertussis*
- *Mycobacterium africanum*
- *Mycobacterium bovis*
- *Mycobacterium talmoniae*
- *Mycobacterium microti*
- *Mycobacterium pinnipedii*
- *Mycobacterium tuberculosis*
- *Streptococcus pneumoniae*



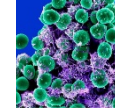
Sexually transmitted infections

- Hepatitis B virus
- Human immunodeficiency virus 1
- Human papillomavirus 16
- Human papillomavirus 18
- Human papillomavirus 31
- Human T-cell leukemia virus 2
- *Treponema pallidum*
- *Chlamydia trachomatis* serovar I
- *Chlamydia trachomatis* serovar II
- *Chlamydia trachomatis* serovar III
- Human herpesvirus 1
- Human herpesvirus 2
- *Neisseria gonorrhoeae*



Vector-borne disease

- Chikungunya virus
- Dengue virus types 1-4
- Eastern equine encephalitis virus
- *Plasmodium malariae*
- St. Louis encephalitis virus
- West Nile virus
- Yellow fever virus
- Zika virus
- *Borrelia burgdorferi*
- *Plasmodium falciparum*
- Yellow fever virus



Epidermal disease

- *Staphylococcus aureus* subsp. *aureus*
- *Staphylococcus epidermidis*
- *Streptococcus pyogenes*

LGC Metagenomics Control Material (MCM) for pathogen detection ATCC® MSA-4000™

Developed in collaboration with LGC

ATCC® MSA-4000™

LGC METAGENOMIC CONTROL MATERIALS



ATCC has partnered with the LGC Group, the UK's designated National Measurement Institute for chemical and bioanalytical measurements and an international leader in the laboratory services, measurement standards, reference materials, genomics and proficiency testing marketplaces, to develop metagenomic control materials for clinically relevant pathogen detection. Each product comprises genomic DNA prepared from ATCC Genuine Cultures® and quantitated using Droplet Digital™ PCR.

[Metagenomic Control Material for Pathogen Detection](#)



Assessing the accuracy of quantitative molecular microbial profiling

National
Measurement
System



Download the paper '[Assessing the accuracy of quantitative molecular microbial profiling](#)' from the Multidisciplinary Digital Publishing Institute website.

Authors: D. M. O'Sullivan, T. Laver, S. Temisak, N. Redshaw, K. A. Harris, C. A. Foy, D. J. Studholme, J. F. Huggett

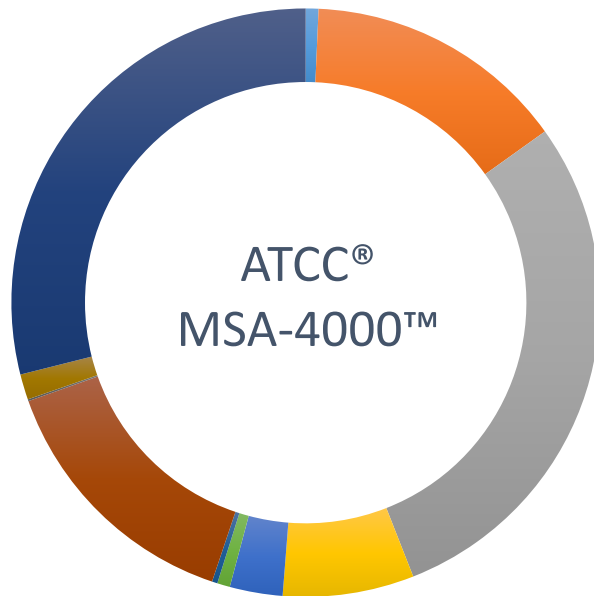
Reference: Int. J. Mol. Sci. 2014, 15(11), 21476-21491

The application of high-throughput sequencing in profiling microbial communities is providing an unprecedented ability to investigate microbiomes. Such studies typically apply one of two methods: amplicon sequencing using PCR to target a conserved orthologous sequence (typically the 16S ribosomal RNA gene) or whole (meta)genome sequencing (WGS). This paper provides a comparison of the inherent precision and bias of the different sequencing approaches.

Selection Attributes for Strains

ATCC® No.	Species	Abundance	Gram Status	% GC	Genome Size (Mb)	GenBank ID	16S copies
17978™	<i>Acinetobacter baumannii</i>	0.10%	-	39.0	4.00	CP000521	5
700802™	<i>Enterococcus faecalis</i>	0.70%	+	37.3	3.34	AE016830	4
700928™	<i>Escherichia coli</i>	1.40%	-	50.6	5.23	AE014075	7
700721™	<i>Klebsiella pneumoniae</i>	14.40%	-	57.1	5.32	CP000647	8
700532™	<i>Neisseria meningitides</i>	28.90%	-	51.7	2.19	AM421808	4
47085™	<i>Pseudomonas aeruginosa</i>	0.30%	-	66.6	6.26	AE004091	4
BAA-1556™	<i>Staphylococcus aureus</i> (MRSA)	0.70%	+	32.8	2.87	CP000255	5
BAA-1718™	<i>Staphylococcus aureus</i> (MSSA)	14.40%	+	32.8	2.87	AASB02000000	5
BAA-611™	<i>Streptococcus agalactiae</i>	2.90%	+	35.6	2.16	AE009948	7
700669™	<i>Streptococcus pneumoniae</i>	28.90%	+	39.6	2.22	FM211187	4
700924™	<i>Streptococcus pyogenes</i>	7.2%	+	38.5	1.85	AE004092	6

Composition of Metagenomic Control Material

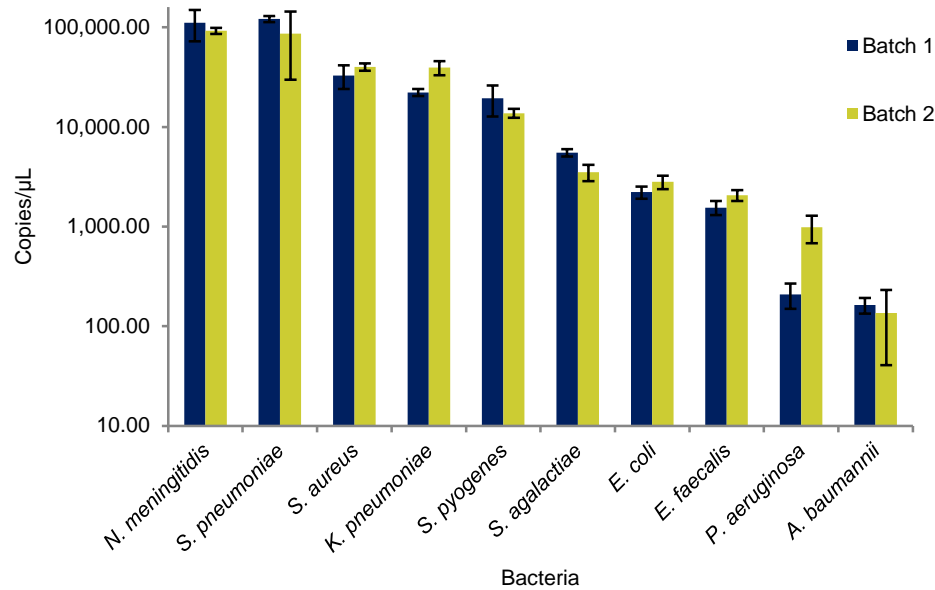


Species (11)	% Abundance
<i>Acinetobacter baumannii</i>	0.10%
<i>Enterococcus faecalis</i>	0.70%
<i>Escherichia coli</i>	1.40%
<i>Klebsiella pneumoniae</i>	14.40%
<i>Neisseria meningitides</i>	28.90%
<i>Pseudomonas aeruginosa</i>	0.30%
<i>Staphylococcus aureus</i> (MRSA)	0.70%
<i>Staphylococcus aureus</i> (MSSA)	14.40%
<i>Streptococcus agalactiae</i>	2.90%
<i>Streptococcus pneumoniae</i>	28.90%
<i>Streptococcus pyogenes</i>	7.2%

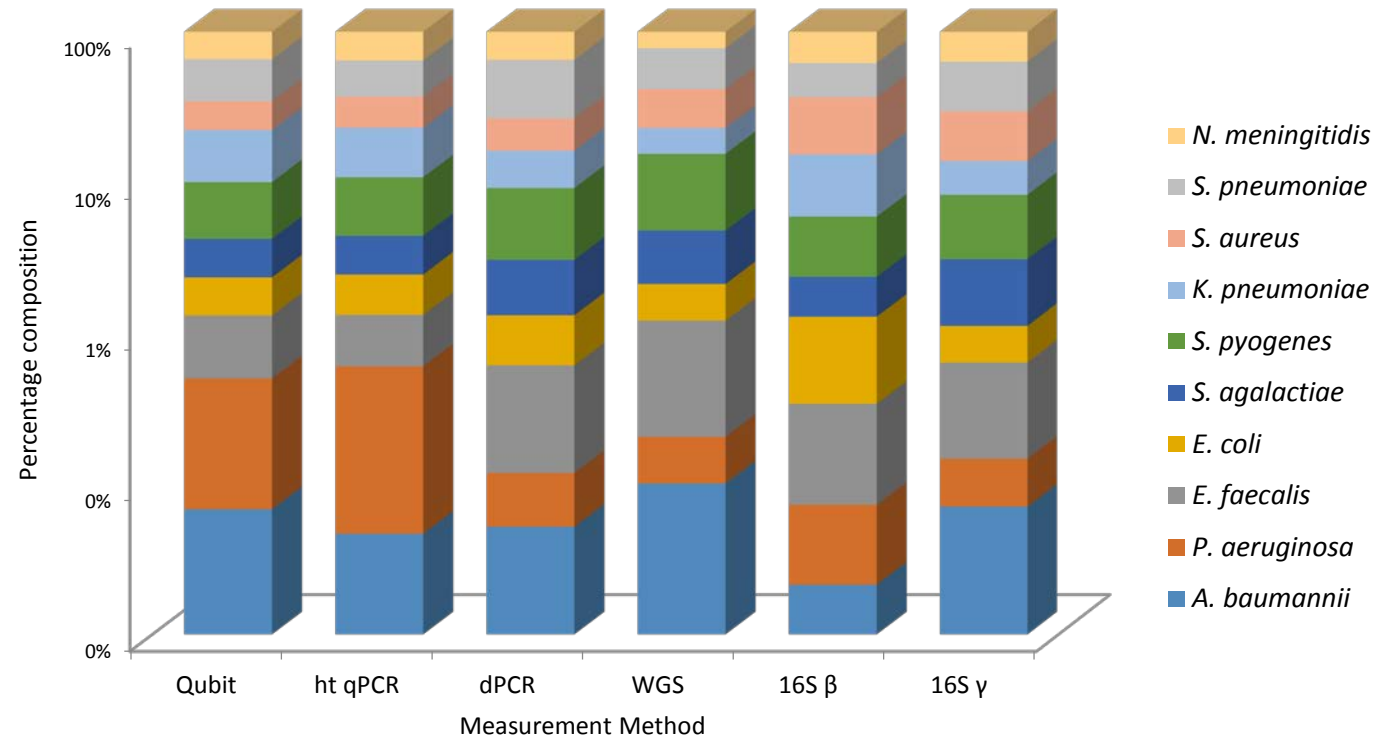
Staggered amounts: 2.5×10^3 – 2×10^5 genome copies/organism

Assay development, optimization, reproducibility, verification, and validation

Development of Standard

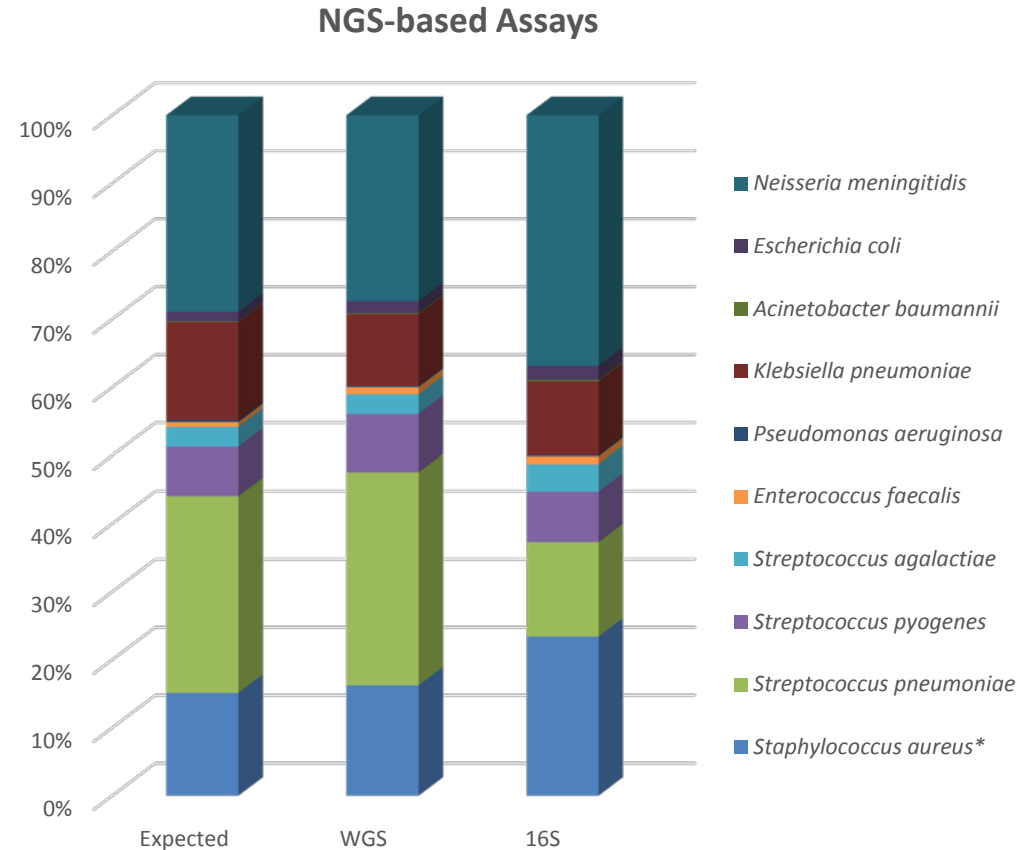
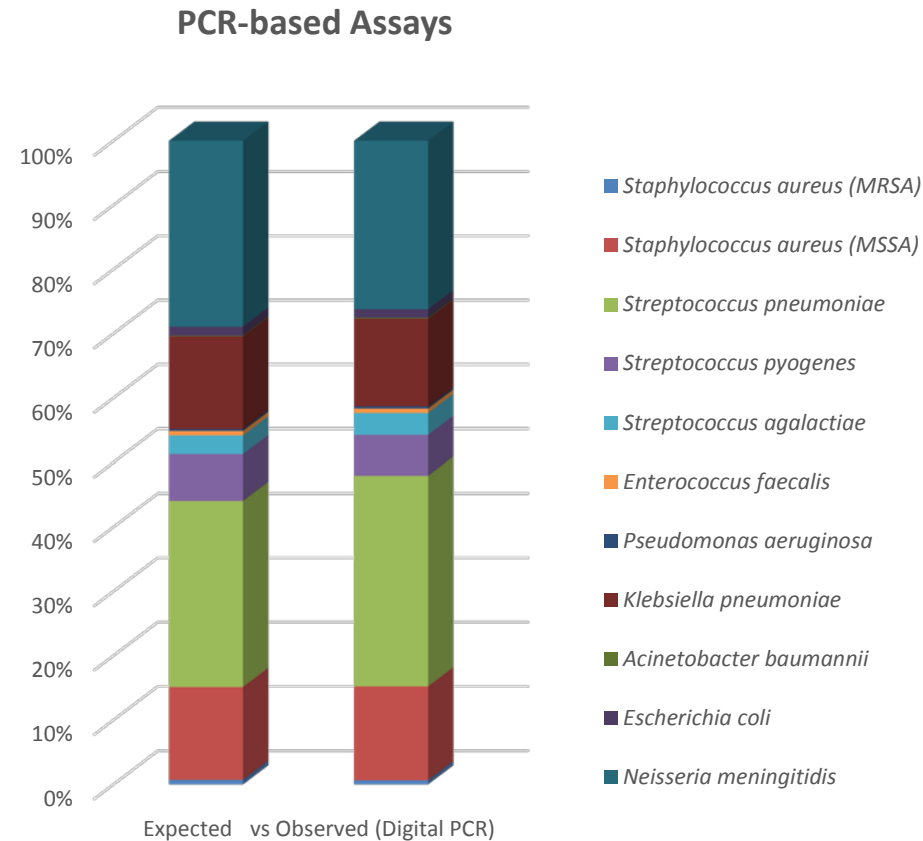


Batch-to-batch production:
Evaluation using ddPCR™



Assessment of the metagenomic control
material using different methods

Assessment of Metagenomic Control Material



ATCC Microbiome Standards Portfolio (7 Products)

Collaboration	Preparation	ATCC® Catalog No.	Number of organisms	Composition	Complexity	Importance
ATCC Microbiome Standards	Genomic DNA	MSA-1000™	10	Even	Medium	Mock microbial communities comprising diverse strains selected on the basis of diversity, genome size, GC content, Gram stain, and other special features
		MSA-1001™	10	Staggered	Medium	
		MSA-1002™	20	Even	High	
		MSA-1003™	20	Staggered	High	
	Whole cells	MSA-2003™	10	Even	Medium	
		MSA-2002™	20	Even	High	
Metagenomic Control Material for Pathogen detection	Genomic DNA	MSA-4000™	11	Staggered	Medium	Metagenomic control material encompassing pathogenic bacterial species commonly observed in clinical infections



Validate & optimize assays




Daily run controls



Bundled analysis

ATCC® Microbiome Standards





Overall Score – 97%

True Positives: 100% | Relative Abundance: 91% | False Positives: 99%

95.8% of reads map to true positives in the control, while 0.2% map to false positives and 4.1% are unclassified

[Download Results](#)

True Positives
Detection of organisms in the control

100%

10 true positives detected (of 10 total)

[Expand](#)

Relative Abundance
Quantification of organisms in the control

91%

10 organisms in control

[Expand](#)

False Positives
of organisms not in the control

99%

1 false positives


[Expand](#)

Sample Metadata
Preparation details & metadata

WGS sequencing of MSA-1001™

[Expand](#)

Name	Estimated Abundance
Micrococcus luteus	17.07%
Pseudomonas aeruginosa	13.92%
Rhodobacter capsulatus	10.19%
Escherichia coli	9.87%
Bacillus amyloliquefaciens	9.87%
Pseudomonas protegens	8.75%
Pseudomonas putida	8.58%
Frankia sp. Ccl3	7.32%
Burkholderia cenocepacia	5.16%
Bacillus cereus	4.49%
(Remaining)	4.79%



Even Distribution

20 Bacteria

ATCC® MSA-1002™
ATCC® MSA-2002™

Staggered Distribution

ATCC® MSA-1003™

10 Bacteria

ATCC® MSA-1000™
ATCC® MSA-2003™

ATCC® MSA-1001™

Disclaimers

The microbiome proof-of-concept data presented in this presentation was generated by whole genome sequencing or amplicon sequencing of the ATCC® Microbiome Standards using the Illumina® Platform. These proof-of-concept datasets are available as examples on the One Codex website along with additional metadata. The information supplied for ATCC® Microbiome Standards on the One Codex website constitutes neither a recommendation nor endorsement of specified methods or materials. ATCC and One Codex do not guarantee identical results to these proof-of-concept datasets when performing similar analyses.

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Thank You



ONE CODEX

A Platform for Microbiome Research & Application

Kashef Qaadri
VP Marketing, One Codex

Agenda

- 1 Platform Overview
- 2 Product Details
- 3 Demo

Agenda

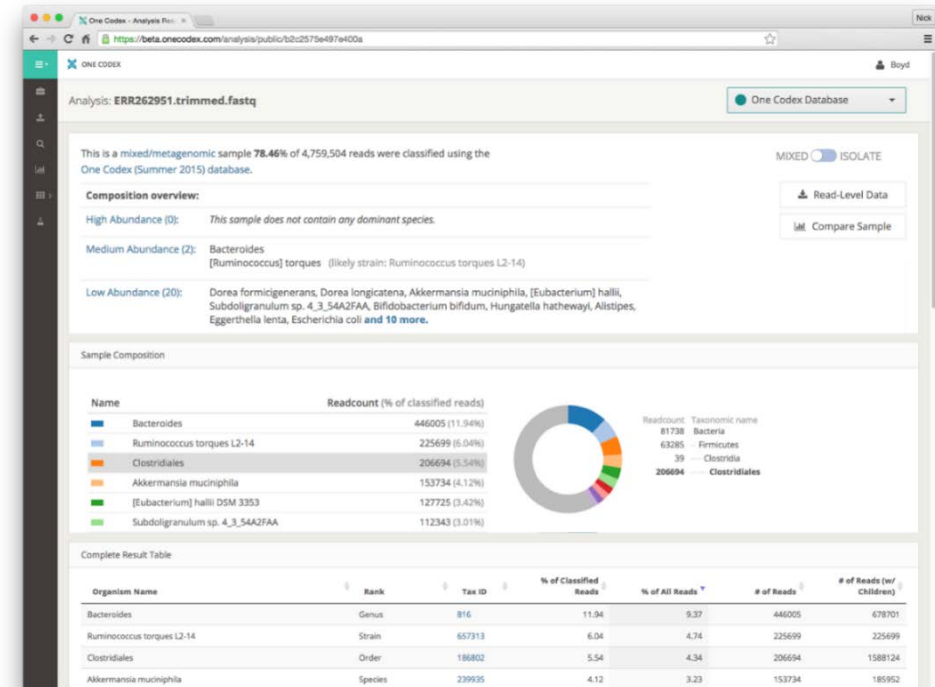
1 Platform Overview

2 Product Details

3 Demo

One Codex – Background

- Leading bioinformatics platform for microbial genomics
- Best-in-class taxonomic & functional analysis of metagenomic (WGS), 16S rRNA, *etc.*
- “Sequence to answer” data platform
- Software engineering with microbiology expertise



A Sample Microbiome Study

Sample Collection*



Sequencing*



Primary Analysis



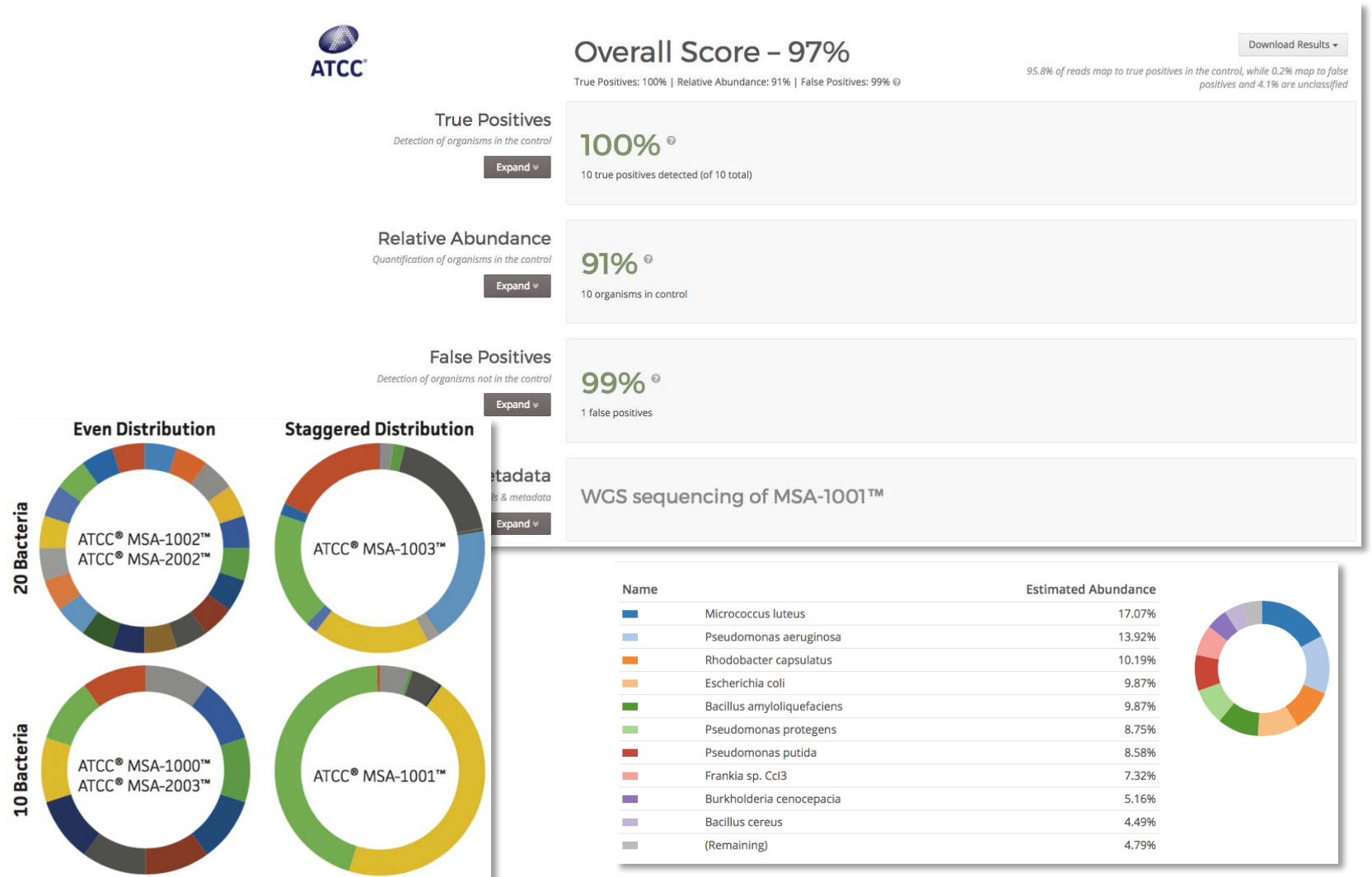
Insight & Discovery



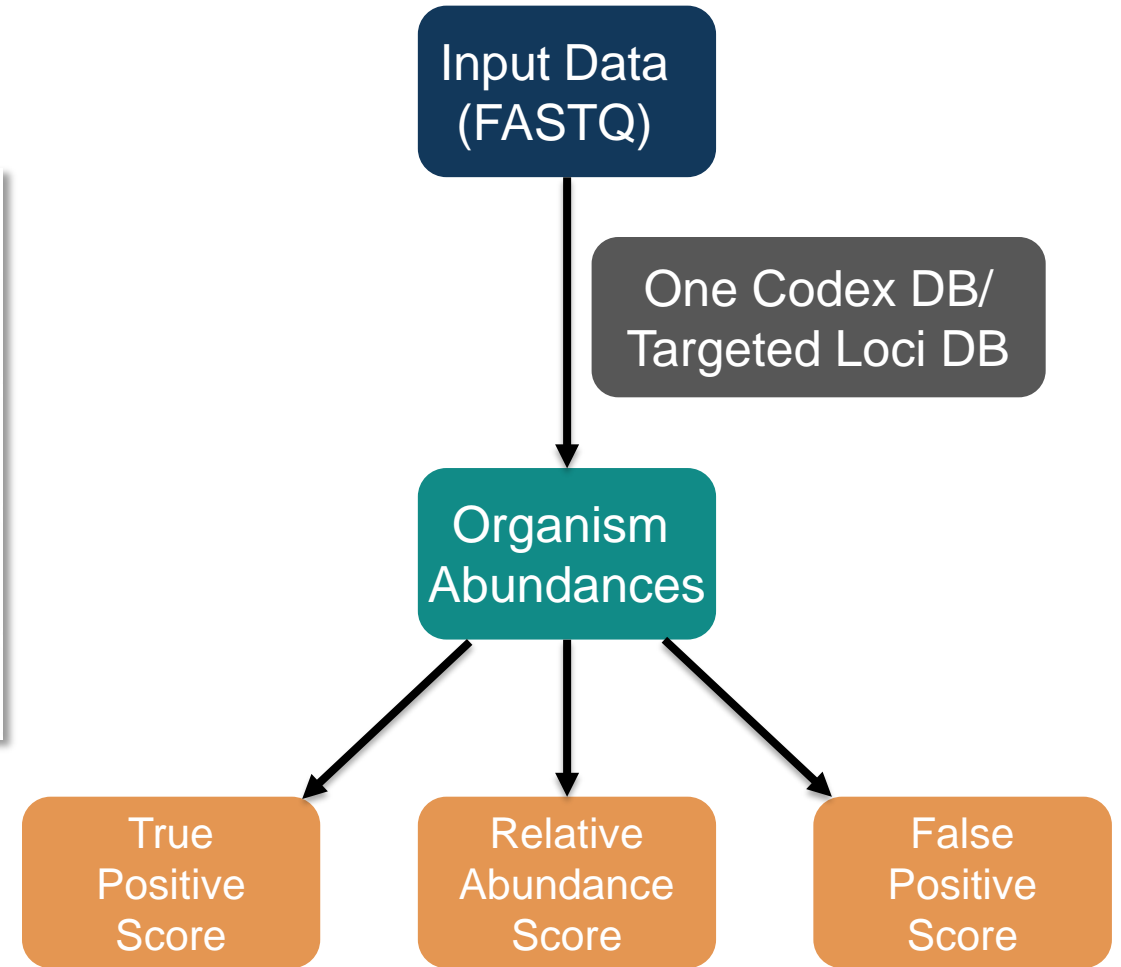
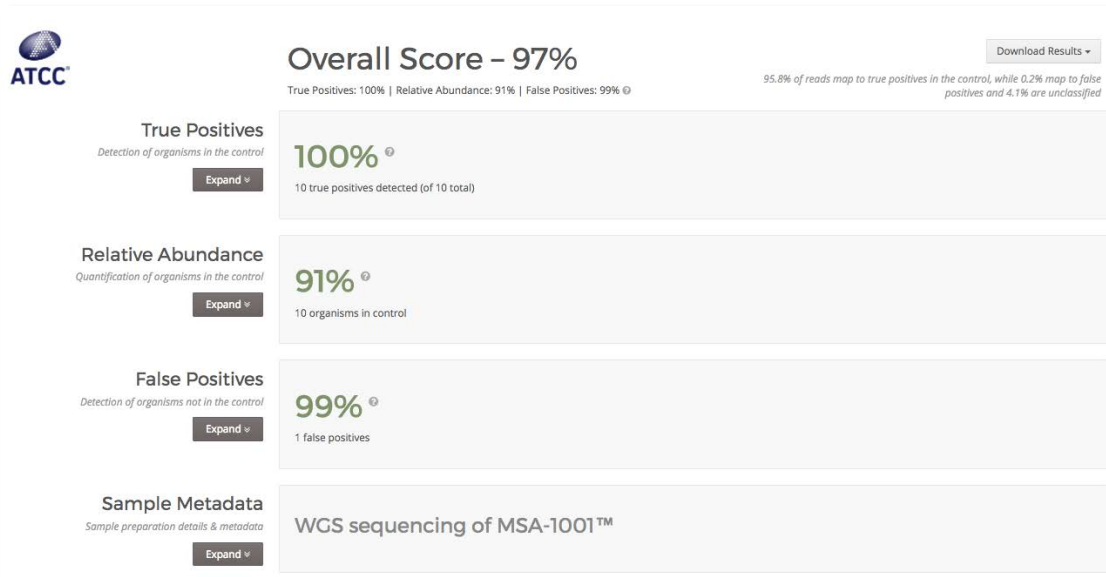
Agenda

- 1 Platform Overview
- 2 Product Details
- 3 Demo

ATCC[®] Microbiome Standards



Bioinformatics Workflow



Agenda

- 1 Platform Overview
- 2 Product Details
- 3 Demo


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Microbiome Reference Standards

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ATCC Microbiome Reference Standards are mock microbial communities for use as controls — optimizing your metagenomics workflows and microbiome research.

[Learn more »](#)

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Product Type


Whole Cell **Genomic DNA**

Sequencing

Shotgun **16S**

ATCC MSA-1000™


10 Strain Even Mix Genomic Material



A mixture of nucleic acids isolated from ten (10) ATCC Genuine Cultures® and prepared with even relative abundance ratios. [Order now »](#)

ATCC MSA-1001™


10 Strain Staggered Mix Genomic Material



A mixture of nucleic acids isolated from ten (10) ATCC Genuine Cultures® and prepared with staggered relative abundance ratios. [Order now »](#)

ATCC MSA-1002™


20 Strain Even Mix Genomic Material



A mixture of nucleic acids isolated from twenty (20) ATCC Genuine Cultures® and prepared with even relative abundance ratios. [Order now »](#)

ATCC MSA-1003™


20 Strain Staggered Mix Genomic Material



A mixture of nucleic acids isolated from twenty (20) ATCC Genuine Cultures® and prepared with staggered relative abundance ratios. [Order now »](#)

ATCC MSA-4000™

Metagenomic Control Material for Pathogen Detection




A mixture of nucleic acids from 10 species (11 strains) isolated from ATCC Genuine Cultures® for clinically relevant pathogen detection. [Order now »](#)

Select an existing sample...

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ATCC Microbiome Reference Standards are mock microbial communities for use as controls — optimizing your metagenomics workflows and microbiome research. [Learn more »](#)

Choose your product type

Product Type

Sequencing

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A mixture of nucleic acids isolated from ten (10) ATCC Genuine Cultures® and prepared with staggered relative abundance ratios. [Order now »](#)

ATCC MSA-1002™

20 Strain Even Mix Genomic Material

A mixture of nucleic acids isolated from twenty (20) ATCC Genuine Cultures® and prepared with even relative abundance ratios. [Order now »](#)

ATCC MSA-1003™

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Product Type


Whole Cell **Genomic DNA**

Sequencing

Shotgun 16S

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ATCC MSA-1001™


10 Strain Staggered Mix Genomic Material



A mixture of nucleic acids isolated from ten (10) ATCC Genuine Cultures® and prepared with staggered relative abundance ratios. [Order now >](#)

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ATCC Microbiome Reference Standards are mock microbial communities for use as controls — optimizing your metagenomics workflows and microbiome research. [Learn more](#)

Choose your ATCC product

Product Type: Whole Cell **Genomic DNA**

Sequencing: Shotgun 16S

ATCC MSA-1000™


10 Strain Even Mix Genomic Material



A mixture of nucleic acids isolated from ten (10) ATCC Genuine Cultures® and prepared with even relative abundance ratios. [Order now »](#)

ATCC MSA-1001™


10 Strain Staggered Mix Genomic Material



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ATCC MSA-1002™


20 Strain Even Mix Genomic Material



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Metagenomic Control Material for Pathogen Detection



A mixture of nucleic acids from 10 species (11 strains) isolated from ATCC Genuine Cultures® for clinically relevant pathogen detection. [Order now »](#)

Select an existing sample...

Find samples...

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Select an existing sample...

Upload in progress...

... or upload a FASTQ file

MSA-1000.16S.example.fastq
File size: 9.3 MB

UPLOADING...



CANCEL UPLOAD

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- Samples
- Upload / Import
- Search
- Compare Analyses
- Cluster View
- Notebooks BETA
- Run Analyses
- ATCC Standards NEW
- Help & Documentation



Microbiome Reference Standards

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- Whole Cell
- Genomic DNA**

Sequencing

- Shotgun
- 16S**

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Select an existing sample...

Find samples...

MSA-1000.16S.example.fastq.gz

... or upload a FASTQ file

Continue with the selected sample, or deselect it to upload a new FASTQ file

[Continue & Add Metadata](#)
Or enter detailed metadata



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1 → What 16S amplicon region was used?*

- A V1-V2
- B V1-V3
- C V3-V4
- D V4
- E V6
- F Other

2 → What sequencing instrument was used?*

Type or select an option

- Illumina MiSeq
- Illumina MiniSeq
- 3 → Illumina NextSeq 500/550
- Illumina HiSeq 2000
- Illumina HiSeq 2500
- Illumina HiSeq 3000/4000
- 4 → Illumina HiSeq X Ten
- Ion S5
- Ion S5 XL
- 5 → Ion PGM

0% completed



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4 → what was the facility or laboratory?

One Codex Sequencing Partner

5 → What is the institutional affiliation? *

Academic

Commercial

Public Health

Core Facility

Other

Submit press ENTER

60% completed





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4 → what was the facility or laboratory?

One Codex Sequencing Partner

5 → What is the institutional affiliation? *

Academic
 Commercial ✓
 Public Health

Core Facility
 Other

Submit press ENTER

100% completed





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Thank you for providing your sample information!

Click here for results

press ENTER



Control — MSA-1000.16S.example.fastq.gz

ATCC Microbiome Standard (16S)



Overall Score – 89%

True Positives: 100% | Relative Abundance: 66% | False Positives: 100%

89.4% of reads map to true positives in the control, while 0.4% map to false positives and 10.2% are unclassified

Download Results

True Positives

Detection of organisms in the control

100%

10 true positives detected (of 10 total)

Expand

Relative Abundance

Quantification of organisms in the control

66%

10 organisms in control

Expand

False Positives

Detection of organisms not in the control

100%

0 false positives

Expand

Sample Metadata

Sample preparation details & metadata

16S sequencing of MSA-1000™

Expand

Please Note: This report, and the information in it, is intended for conduct of research only and is not designed, nor approved, to be used for patient care or diagnostic purposes. Job Version ID: c6a75a42cd704b93.



Control — MSA-1000.16S.example.fastq.gz

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Download Results

True Positives

Detection of organisms in the control

Collapse

100%

10 true positives detected (of 10 total)

Filter organisms...

Organism		# of Reads	% of True Positives	% Expected
<i>Bacillus cereus</i>	Present	12049	5.95	10.00
<i>Bifidobacterium adolescentis</i>	Present	9103	10.79	10.00
<i>Clostridium beijerinckii</i>	Present	18560	7.85	10.00
<i>Deinococcus radiodurans</i>	Present	12217	10.34	10.00
<i>Enterococcus faecalis</i>	Present	7357	10.90	10.00
<i>Escherichia coli</i>	Present	15892	13.45	10.00
<i>Lactobacillus gasseri</i>	Present	17793	17.57	10.00

Relative Abundance

Quantification of organisms in the control

Expand

66%

10 organisms in control

False Positives

Detection of organisms not in the control

Expand

100%

0 false positives



Collapse ^

10 true positives detected (of 10 total) Filter organisms...

Organism		# of Reads	% of True Positives	% Expected
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<i>Bifidobacterium adolescentis</i>	Present	9103	10.79	10.00
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<i>Escherichia coli</i>	Present	15892	13.45	10.00
<i>Lactobacillus gasseri</i>	Present	17793	17.57	10.00

Relative Abundance

Quantification of organisms in the control

Collapse ^

66% [?]
10 organisms in control Filter organisms...

Organism		% of True Positives	Detected / Expected
<i>Bacillus cereus</i>	Moderate	5.95	0.59
<i>Bifidobacterium adolescentis</i>	Good	10.79	1.08
<i>Clostridium beijerinckii</i>	Moderate	7.85	0.79
<i>Deinococcus radiodurans</i>	Good	10.34	1.03
<i>Enterococcus faecalis</i>	Good	10.90	1.09
<i>Escherichia coli</i>	Moderate	13.45	1.35
<i>Lactobacillus gasseri</i>	Poor	17.57	1.76

False Positives

Detection of organisms not in the control

Expand v

100% [?]
0 false positives



Organism		% of True Positives	Detected / Expected
<i>Bacillus cereus</i>	Moderate	5.95	0.59
<i>Bifidobacterium adolescentis</i>	Good	10.79	1.08
<i>Clostridium beijerinckii</i>	Moderate	7.85	0.79
<i>Deinococcus radiodurans</i>	Good	10.34	1.03
<i>Enterococcus faecalis</i>	Good	10.90	1.09
<i>Escherichia coli</i>	Moderate	13.45	1.35
<i>Lactobacillus gasseri</i>	Poor	17.57	1.76

False Positives

Detection of organisms not in the control

Collapse ↕

100% [?]

0 false positives

Filter organisms...

Organism		# of Reads	% of Reads
<i>Corynebacterium</i>	Trace	76	0.062
<i>Vibrio</i>	Trace	60	0.049
<i>Streptomyces</i>	Trace	56	0.046
<i>Porphyromonas</i>	Trace	46	0.038
<i>Methylobacterium</i>	Trace	26	0.021
<i>Paenibacillus</i>	Trace	24	0.020
<i>Thiothrix</i>	Trace	24	0.020

Sample Metadata

Sample preparation details & metadata

Expand ↕

16S sequencing of MSA-1000™



Control — MSA-1000.16S.example.fastq.gz

ATCC Microbiome Standard (16S)



Overall Score – 89%

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Download Results

True Positives

Detection of organisms in the control

100%

10 true positives detected (of 10 total)

Expand

Relative Abundance

Quantification of organisms in the control

66%

10 organisms in control

Expand

False Positives

Detection of organisms not in the control

100%

0 false positives

Expand

Sample Metadata

Sample preparation details & metadata

16S sequencing of MSA-1000™

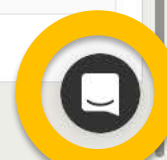
16S Amplicon: V4
Facility Type: Commercial
Technician: Lab Tech OCX

Facility: One Codex Sequencing Partner
Instrument: Illumina MiSeq

Collapse

Edit Detailed Metadata

Edit Metadata



Conclusion



Fast Results



Accurate
Reporting



Easy to Use



Validate &
Optimize Protocols

Kashef Qaadri
VP, Marketing

kashef@onecodex.com
510-552-2876