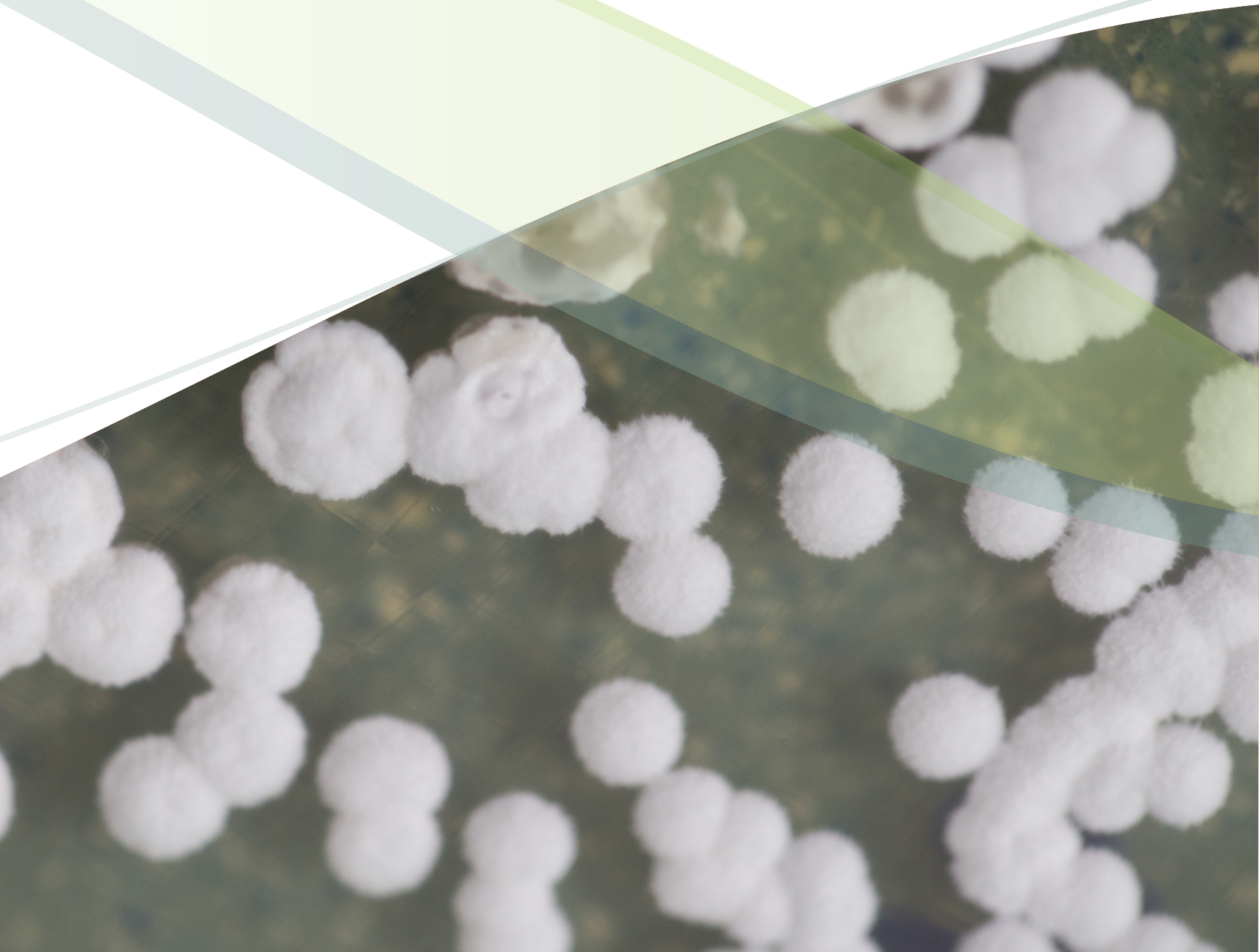
ATCC®

Microbiome Research Solutions



Top 5 reasons to use standards

1. Perform quality control analyses
2. Promote scientific credibility
3. Develop and optimize assays
4. Ensure data reproducibility
5. Eliminate bias

Human Microbiome by the Numbers

10-100 TRILLION
Microorganisms

2-5 LBS
In weight

>1000
Bacterial species

3.3 MILLION
Unique genes in the gut

1.3:1
Ratio of bacteria to human cells

A Growing Field of Research

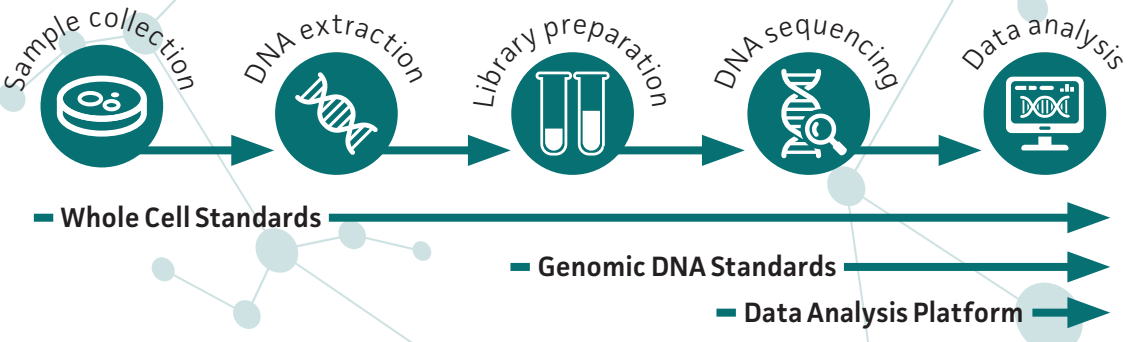
$3.2 BILLION NET WORTH
Over 100 microbiome companies and investors

>48,000 PUBLICATIONS
Over 75% were published in the last 5 years

>1200 CLINICAL TRIALS
Evaluating the microbiome in human health

Start with Credible Standards

NGS standards with known compositions are essential for ensuring assay optimization and data reproducibility throughout each stage of the workflow—from sample collection to data analysis



RAISE THE STANDARDS OF YOUR MICROBIOME RESEARCH

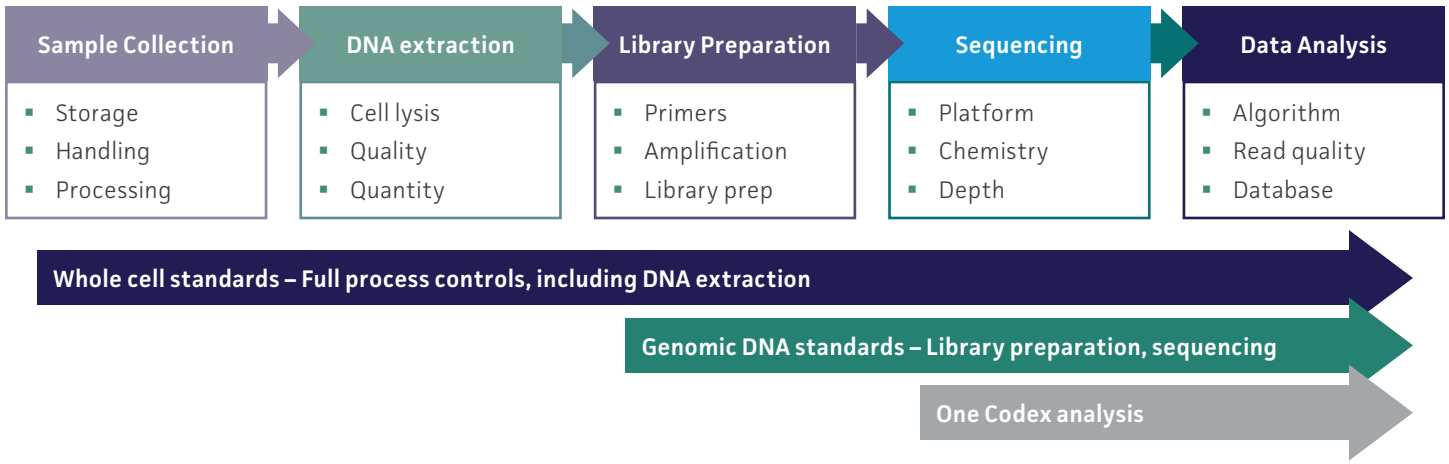
Although there is already a wealth of information on the human microbiome, much is still unknown. As scientists begin to move toward the next wave of methods, such as metatranscriptomics and metabolomics, research will be challenged with managing burgeoning data sets and parsing information into meaningful insights through translational research. Optimization at each step of the analytical process via authenticated standards then becomes essential to the validity and reproducibility of scientific outcomes. It is only with authenticated standards and optimized workflows that scientists will be able to achieve a clear understanding of how the human microbiome changes and develops over time, ultimately relating back to a deeper knowledge of human physiology.

CREDIBLE STANDARDS, INCREDIBLE RESULTS

The complexities associated with microbiome research have made assay standardization challenging. ATCC has the solution: NGS standards. Throughout each stage of your microbiome workflow, NGS Standards enable you to optimize your diverse research applications with confidence and improve the consistency and reproducibility of your data run after run. From sample collection to data analysis, ATCC offers a variety of assay optimization and quality control tools to support your microbiome research, including:

- Mock microbial communities
- Environmental and pathogen mixtures
- Site-specific standards
- Spike-in controls
- Virome and mycobiome standards
- State-of-the-art bioinformatics

Our portfolio contains a variety of reference materials that are completely manufactured from high-quality, authenticated, fully sequenced ATCC cultures that are characterized by polyphasic testing, so you can start your research with credible materials you can trust.



HARMONIZE GLOBAL RESEARCH EFFORTS WITH THE RIGHT CONTROLS

NGS Standards support a broad array of applications ranging from method optimization to data interpretation, and they serve as superior controls for microbial community testing and assay development on any platform. To best support your research, NGS Standards are provided in a variety of formats and levels of complexity:

- Whole cell and nucleic acid preparations
- Even or staggered gDNA abundance
- General and site-specific standards
- Low, medium, or high levels of complexity
- Mixtures comprising 3 to 20 strains per sample
- Parallel and spike-in controls

The robust applicability of these controls, combined with the ATCC commitment to authentication and characterization, make NGS Standards the ideal tool for standardizing data from a wide range of sources and generating consensus among microbiome applications and analyses.

DISCOVER MORE ONLINE AT WWW.ATCC.ORG/MICROBIOME

IMPROVE ASSAY CONSISTENCY

When performing your microbiome research, the tools and protocols you choose can significantly affect your results. By using NGS Standards combined with the One Codex data analysis module in your research, you can compare the performance and overall accuracy of your methods and ensure the validity of your results.

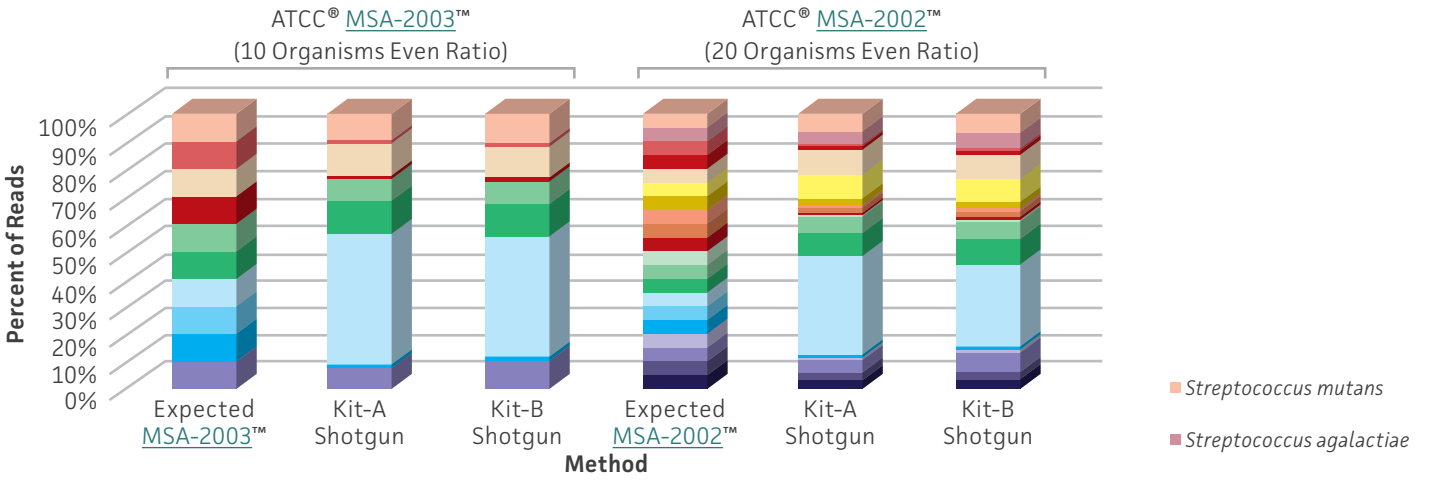


Figure 1: The whole cell mock microbial communities can be used to compare different DNA extraction kits. Here, we show a proof-of-concept study. Total DNA from two aliquots of the whole cell standards was extracted using two commercial extraction kits. Following extraction, the genomic DNA was analyzed using shotgun genomic sequencing analysis on the Illumina® MiSeq® platform, and the resulting data were analyzed using the ATCC bioinformatics tool in One Codex.

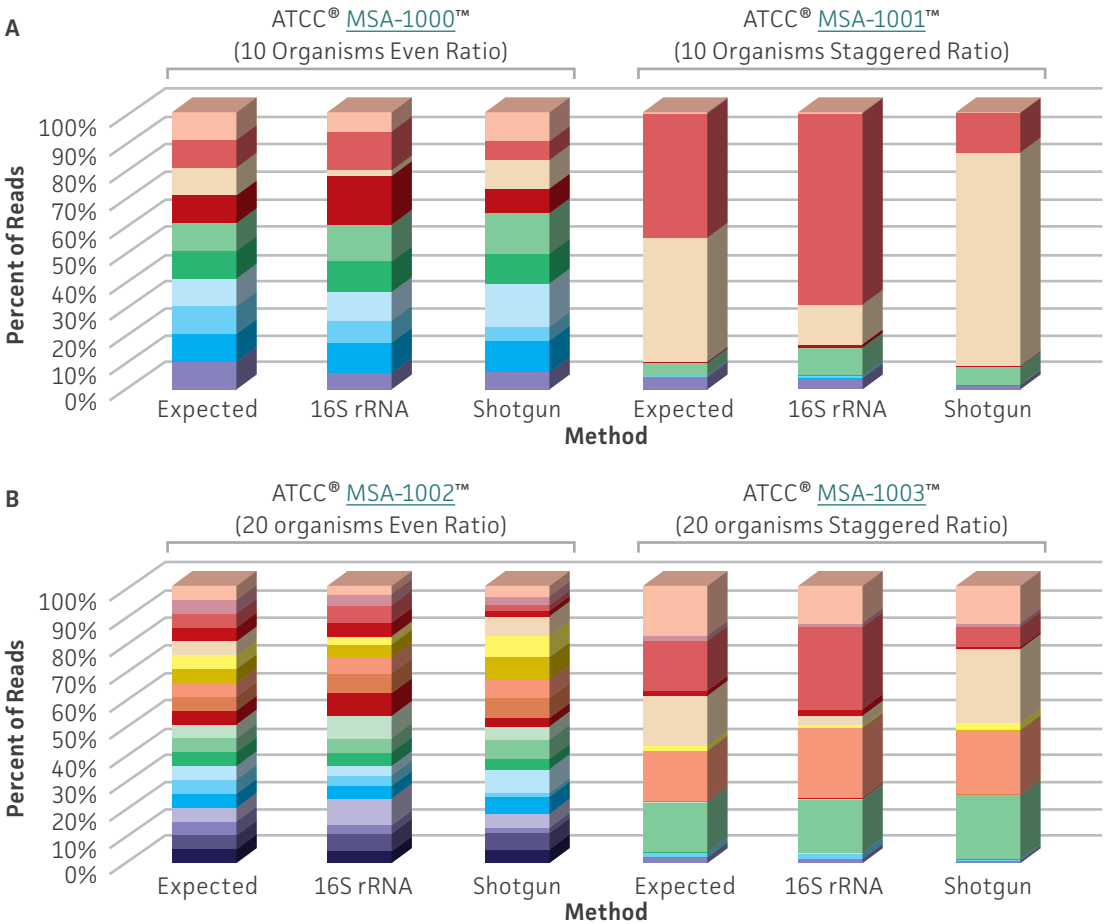


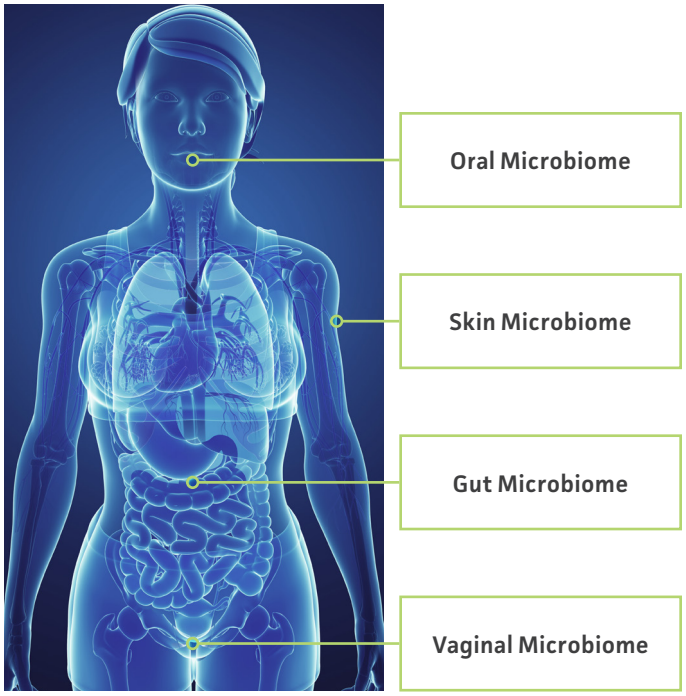
Figure 2: The genomic DNA mock microbial communities can be used to compare 16S rRNA and shotgun metagenomics assays. (A, B) The performance and overall accuracy of the quantitative genomic DNA mock microbial communities were tested using the Illumina® MiSeq® platform, and the resulting data were analyzed using the ATCC bioinformatics tool in One Codex.

SOURCE STANDARDS BASED ON BODY SITE

Although there is already a wealth of knowledge on the human microbiome, much of it still remains an enigma. ATCC is committed to supporting this incredible field of research by creating the cutting-edge site-specific NGS standards needed for studies on the oral, skin, gut, and vaginal microbiomes. With these standards, you can expect:

- Mock microbial communities comprising normal and atypical flora
- Genomic DNA or whole cells prepared from fully sequenced and authenticated ATCC cultures
- Anaerobic and aerobic microbial strains
- A combination of Gram-positive and -negative bacterial cultures

These standard are ideal for use as controls in microbial profiling of mixed populations or for research on the effects of dysbiosis on human health.



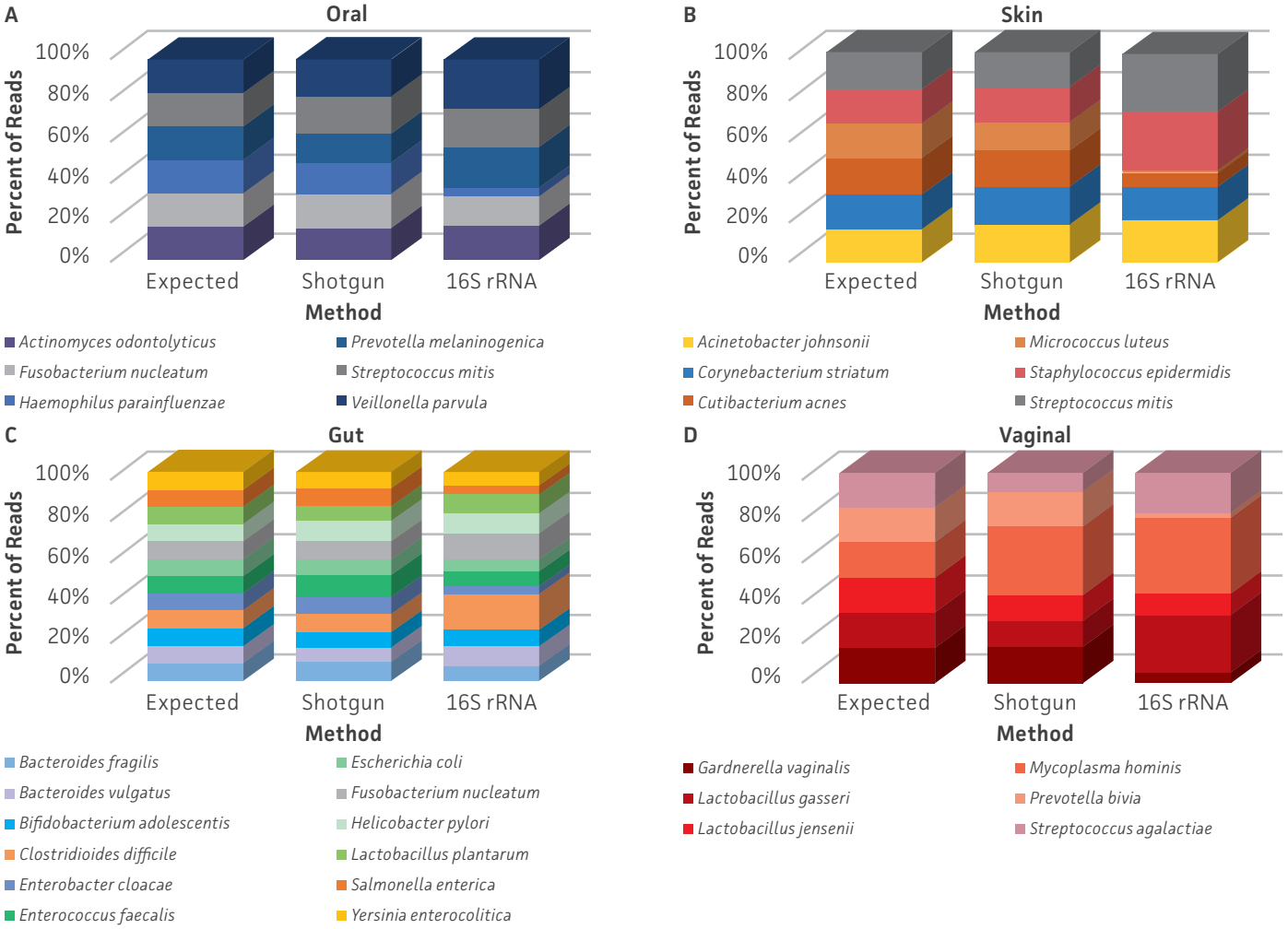


Figure 3: Genomic DNA mock microbial communities analyzed via 16S rRNA and shotgun metagenomics sequencing methods. Genomic DNA Mixes representing the (A) oral genomic mix (ATCC® MSA-1004™), (B) skin genomic mix (ATCC® MSA-1005™), (C) gut genomix mix (ATCC® MSA-1006™), and (D) vaginal genomix mix (ATCC® MSA-1007™) were analyzed via shotgun metagenomics and 16S rRNA sequencing. Data analyses were performed on the One Codex platform.

SOURCE STANDARDS BASED ON SPECIFIC APPLICATIONS

PATHOGEN DETECTION

ATCC has developed a mock microbial community for clinically relevant pathogen detection in partnership with the LGC Group, an international leader in genomics, measurement standards, and reference materials. With the ATCC Metagenomic Control Material for Pathogen Detection (ATCC® MSA-4000™), you can expect:

- Genomic DNA prepared from fully sequenced ATCC cultures
- A combination of strains observed in clinical infections, including antimicrobial-resistant organisms
- Absolute quantification of genomic DNA and assignment of genome copy number using Droplet Digital™ PCR to improve analytical precision

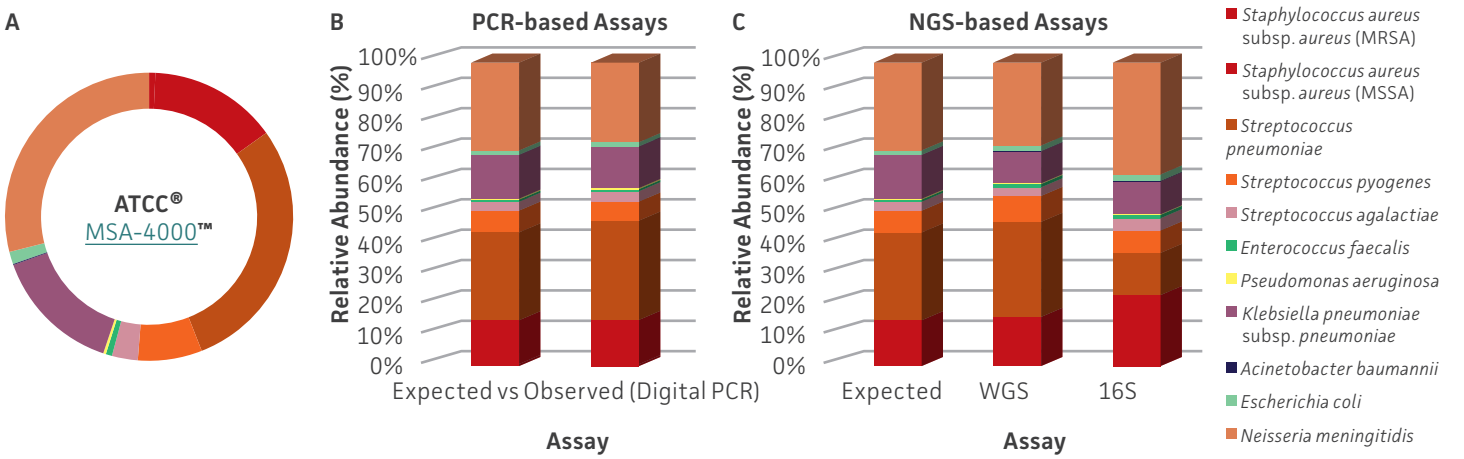


Figure 4: The ATCC Metagenomic Control Material for Pathogen Detection (ATCC® MSA-4000™) can be used to compare various molecular diagnostic methods. Here, we used the (A) control material to evaluate (B) digital PCR and compare (C) 16S rRNA and shotgun metagenomics analysis methods. *The percent relative abundance of *Staphylococcus aureus* includes both MRSA and MSSA.

ENVIRONMENTAL TESTING

The ABRF-MGRG metagenomics reference standards were developed and packaged in partnership with the Association of Biomolecular Resource Facilities Metagenomics Research Group (ABRF-MGRG) as part of the Extreme Microbiome Project (XMP). With the ABRF-MGRG metagenomics reference standards (ATCC® MSA-3000™, MSA-3001™, MSA-3002™), you can expect:

- Genomic DNA prepared from fully sequenced ATCC cultures representing bacterial and archaeal species found in extreme environments
- Mock communities that are human DNA-free, RNA-free, and tested to ensure purity via sequencing
- Access to One Codex, the leading bioinformatics platform for microbial genomics

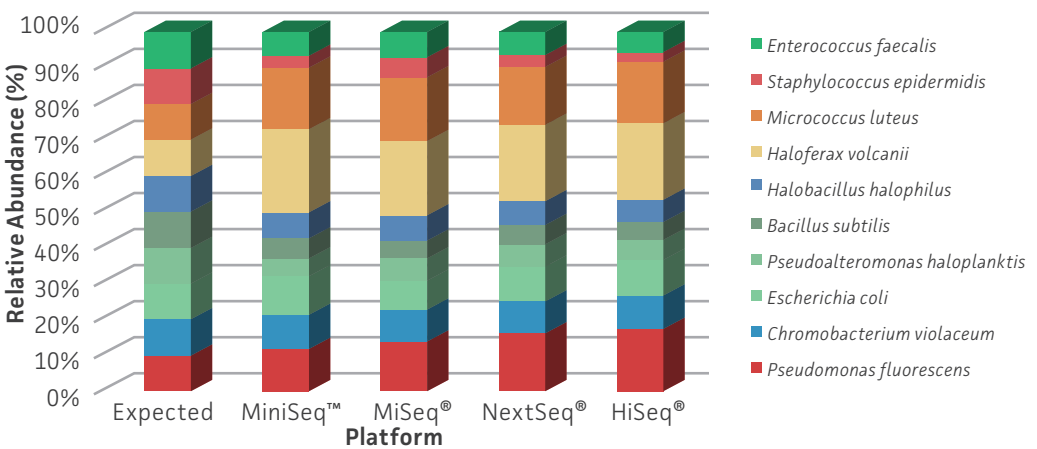


Figure 5: The ABRF-MGRG metagenomics reference standards can be used to compare different sequencing platforms. As a proof-of-concept, ATCC® MSA-3001™ was used a control material to compare the performance of the Illumina® MiniSeq™, MiSeq®, NextSeq®, and HiSeq® platforms. Data were analyzed using the ATCC bioinformatics tool was used as control material. ABRF-MGRG metagenomics reference standards can also be used to evaluate run-to-run reproducibility (data not shown).

NGS STANDARDS ARE PLATFORM AGNOSTIC

While the affordablility of next-generation sequencing technology has significantly enhanced microbiome and metagenomics analyses, many of the common sequencing methods used in microbiome research demonstrate significant challenges. NGS Standards can help account for these biases by enabling the optimization of 16S rRNA and shotgun metagenomics assays performed on any sequencing platform.

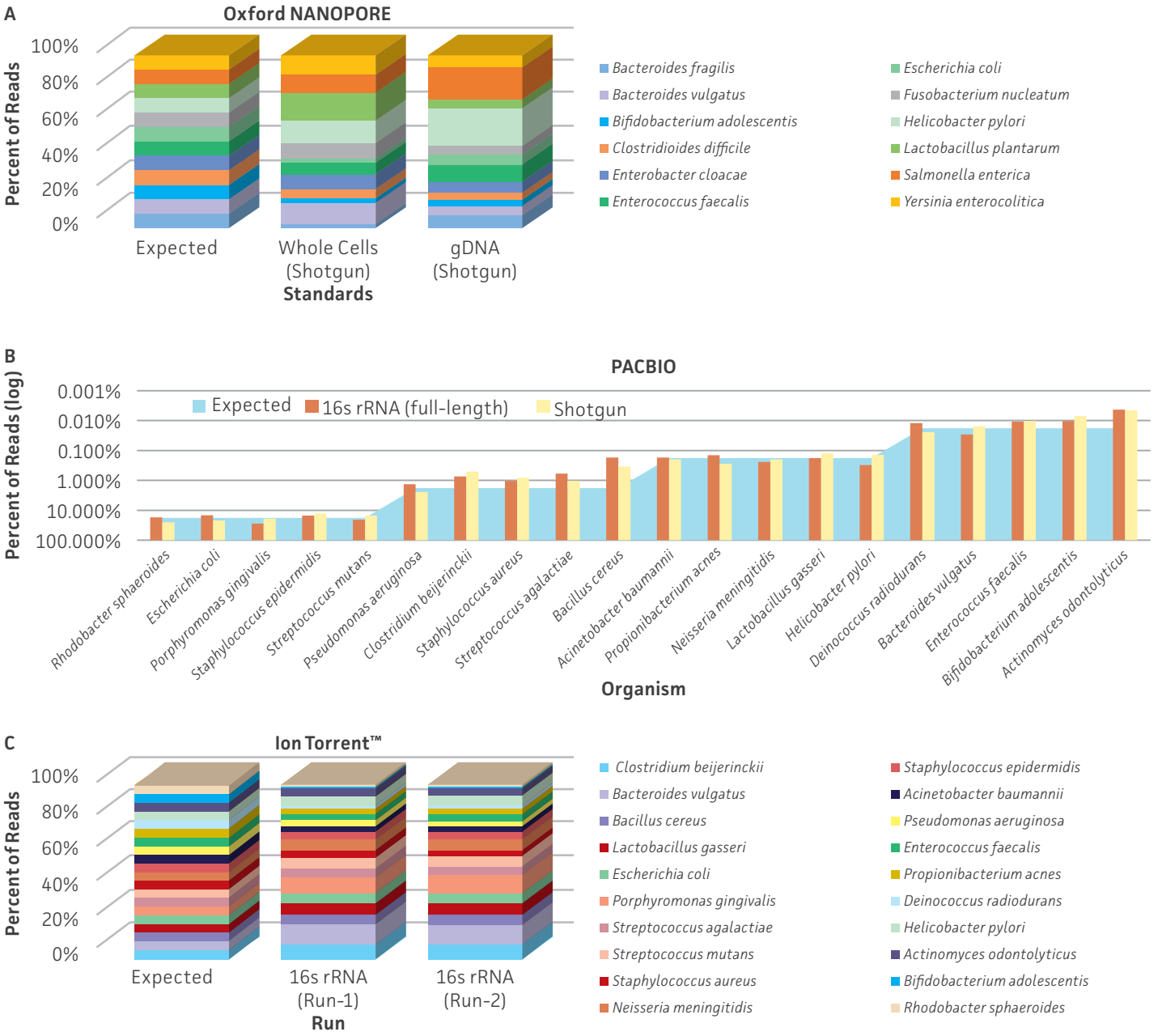


Figure 6: Evaluation of different sequencing platforms with ATCC NGS Standards. (A) The Gut Microbiome Standards (ATCC® MSA-1006™ and MSA-2006™) were analyzed via shotgun sequencing on the Nanopore MinION platform. Results showed that one hour sequencing was enough to identify all organisms in the mix with sufficient genome coverage (read length N50 > 5 kb and average coverage = 5x). (B) Comparison of 16S rRNA (full-length amplicon) and shotgun data on the PacBio Sequel Platform with the 20 organism staggered genomic DNA mix (ATCC® MSA-1003™). Results showed concordance between 16S rRNA (100% false negative) and Shotgun (100% false negative) analysis, which highlight a full-amplicon 16S analysis using a long-read platform to reduce 16S primers biases and improve taxonomic resolution. (C) 16S rRNA (V1V2) metagenomics profiling of 20 organism even genomic DNA mix (ATCC® MSA-1002™) on the Ion Torrent Ion PGM platform. Results showed all organisms were detected in the mix (100% true positive).

EXPLORE THE HUMAN MYCOBIOME

Most of the initial studies on the human microbiome have been primarily focused on bacterial communities. Given that fungi are ubiquitous and live in symbiosis with the human body, researchers are now actively looking into the role of the mycobiome in human health and disease. Recent advancements in sequencing technologies have enabled the community profiling of fungi; however, the complexities associated with metagenomics sequencing analyses highlight the need for reference material. To address this, ATCC have developed genomic and whole cell fungal mock community standards consisting of 10 diverse and clinically relevant fungi.

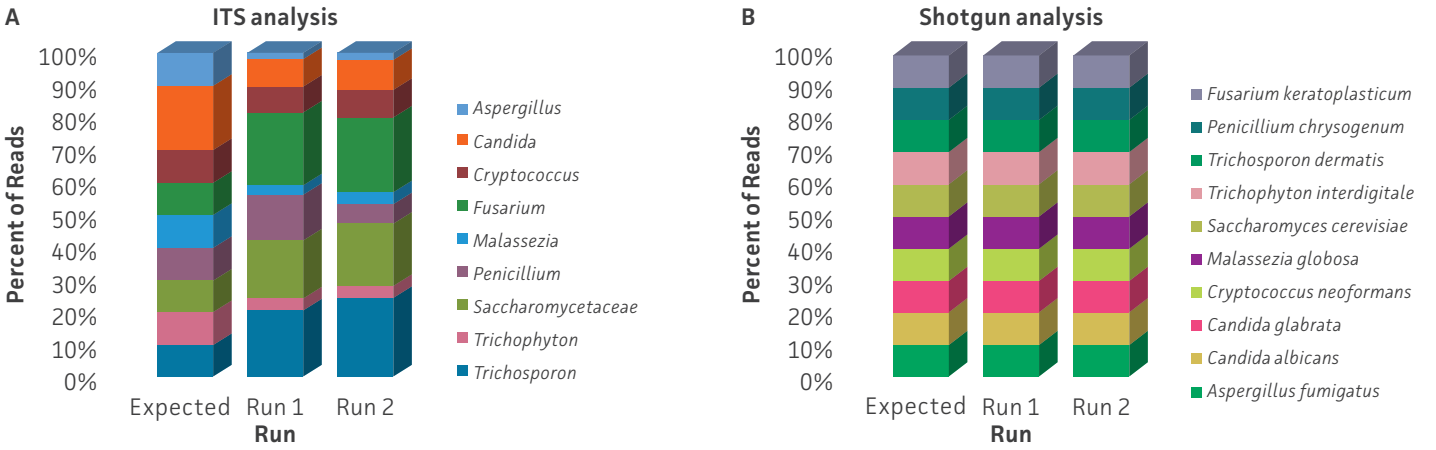


Figure 7: Mycobiome standards can be used with both internal transcribed spacer (ITS) and shotgun metagenomic sequencing assays. The Mycobiome Genomic DNA Mix (ATCC® MSA-1010™) was analyzed by (A) ITS and (B) shotgun metagenomic assays using the Illumina platform, and data were analyzed using OneCodex platform. ITS analysis could profile all the fungi in the mix to genus level where as shotgun metagenomic assay could identify all fungi to the species level. The results also demonstrated run-to-run reproducibility when performing both sequencing assays.

EXPLORE THE HUMAN VIROME

The human virome plays a key role in modulating host responses during homeostasis and disease. By better understanding this unique community, researchers can identify factors that affect infectivity and discover how these organisms impact human health. In support of this burgeoning field of research, ATCC has created mock microbial communities representing genetically diverse and clinically relevant viruses. These standards are ideal tools for assay development or as a daily run control for molecular diagnostics.

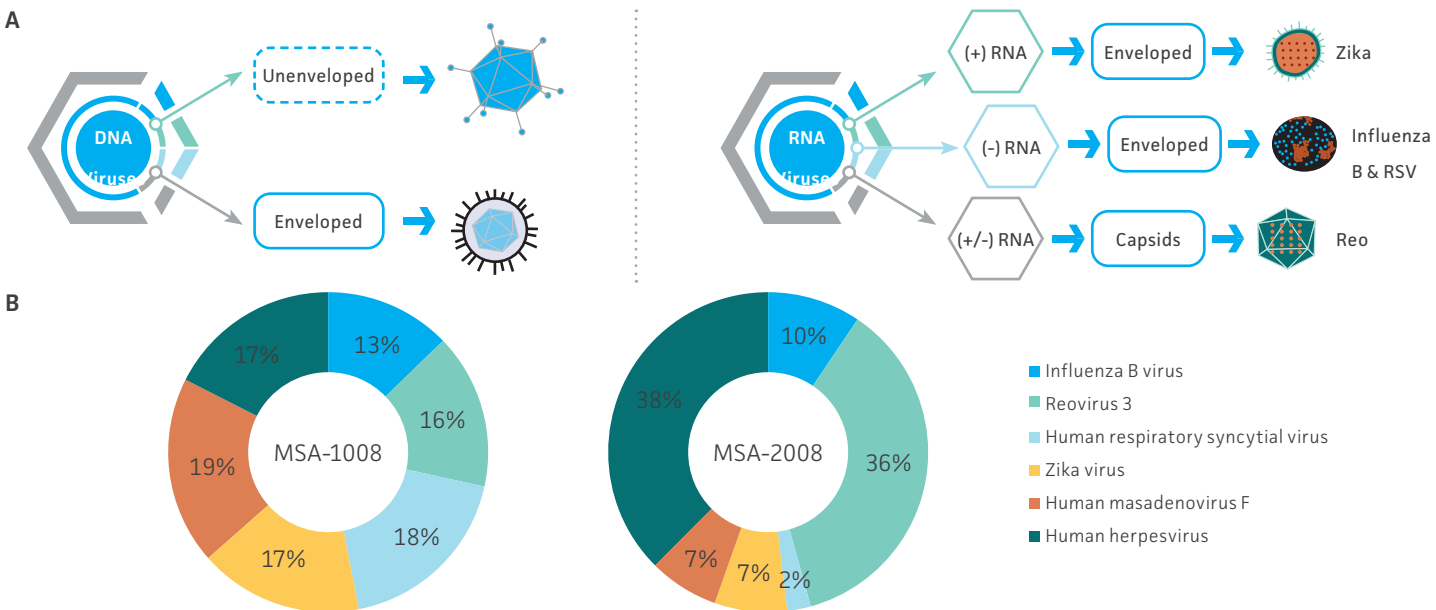


Figure 8: Composition of genomic DNA and whole cell virome standards. (A) ATCC Virome Standards comprise genomic nucleic acids or whole cells prepared from fully sequenced, characterized, and authenticated viral strains that are selected on the basis of genomic size, DNA or RNA genome, envelope/non-envelop, and other special features. (B) The genome copy number for each virus in the virome mixture was determined by using individual digital PCR assays. The variation observed in ATCC® MSA-2008™ could be attributed to extraction efficiency.

OPTIMIZE AND VALIDATE YOUR EXPERIMENTS WITH SPIKE-IN CONTROLS

Evaluating the accuracy and reproducibility of your methods and ensuring standardization across your microbiome research is essential. To address these needs, ATCC has developed two spike-in standards that comprise an even mix of genomic DNA or whole cells of three genetically engineered bacteria strains (*Escherichia coli*, *Clostridium perfringens*, and *Staphylococcus aureus*) that contain a unique synthetic DNA tag that can be detected via 16S rRNA profiling and shotgun metagenomics sequencing assays. These standards offer a valuable way to evaluate the accuracy and reproducibility of microbiome NGS data analysis. Additionally, the whole cell standard can be used as a true run control from sample storage all the way through data analysis. Some additional applications include:

- Evaluate the efficiency of DNA extraction methods
- Compare and validate NGS platforms
- Optimize 16S rRNA primers and shotgun library preparations
- Assess sequence coverage and the level of detection
- Normalize metagenomics 16S and shotgun data

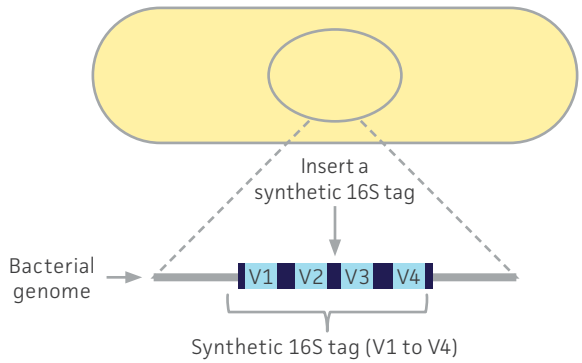


Figure 9: Design of the synthetic tag and production or tagged strains. Each tag consists of 4 artificial variable regions that correspond to V1 through V4 in the 16S rRNA gene. Each variable region is flanked by conserved sequence for PCR amplification. Each tag sequence was integrated into the genome of their cognate strains to create three recombinant strains.

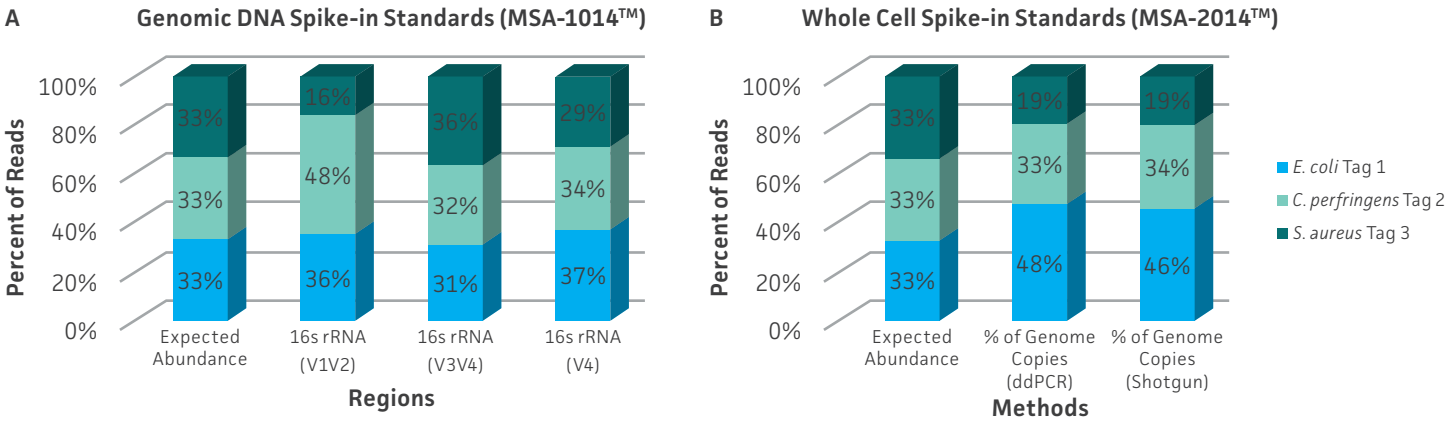


Figure 10: Detection and measurement of three unique synthetic tags from the Spike-in Standards. (A) Percent of recovered reads of individual tags showed that synthetic tags can be identified and quantified by using three commercial primer sets that target the variable regions of the 16S rRNA gene. (B) Percent of genome copies (ddPCR) and percent of reads (shotgun) of individual tags detected in the Whole Cell Spike-in standards.

DATA ANALYSIS SOLUTION

ATCC has collaborated with One Codex to bring microbiome research to a completely new level by combining the power of both physical laboratory standards with the leading bioinformatics platform for microbial genomics and metagenomics.

1. Drag and drop Fastq files or export via cloud
2. Accurate identification of reference organisms via the One Codex Database (whole genome) or the Targeted Loci Database (16S rRNA)
3. Conversion of read counts to relative abundance by using genome size or 16S rRNA copy number

A PORTFOLIO OF PRODUCTS TO MEET YOUR NEEDS

ATCC has been at the heart of microbiological research for over 95 years, delivering consistent controls for reliable data. Whether you are testing out the sensitivity or specificity of a new microbiome assay, performing next-generation sequencing, or developing primers for a set of drug resistance genes in a clinical sample, ATCC has the products you need to ensure the accuracy and reproducibility of your research, including:

- Individual strains relevant to the diseased microbiome
- Antimicrobial-resistant strains
- Environmental isolates
- Nucleic acid standards
- Whole-genome sequencing data on the ATCC Genome Portal

Visit us online to learn more about our products for next-generation sequencing and metagenomics analysis.

EXPLORE ATCC'S MICROBIOME RESEARCH SOLUTIONS AT

WWW.ATCC.ORG/MICROBIOME

Table 1: ATCC® NGS Standards

Collaboration	Preparation	ATCC® No.	Number of organisms	Composition	Complexity	Importance
Mock Microbial Communities	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	
Mock Viral Communities	Genomic DNA/ RNA	MSA-1008™	6	Even	Medium	Mock viral communities comprising diverse strains selected on the basis of genomic size, DNA/RNA genome, envelope/non-envelope, and other special features **- New!**
	Whole Virus	MSA-2008™	6	Even	Medium	
Mock Fungal Communities	Genomic DNA	MSA-1010™	10	Even	Medium	Mock fungal communities comprising diverse strains selected on the basis of genome size, ITS variability, and other special features **- New!**
	Whole Cells	MSA-2010™	10	Even	Medium	
Metagenomic Control Material for Pathogen Detection	Genomic DNA	MSA-4000™	11	Staggered	Medium	Metagenomic control material (MCM) encompassing pathogenic bacterial species commonly observed in clinical infections
ABRF-MGRG Metagenomics Reference Standard	Genomic DNA	MSA-3000™	6	Even	Low	Genomic DNA microbiome standards comprising strains observed in soil, freshwater, seawater, feces, and high salinity ecosystems
		MSA-3001™	10	Even	Medium	
		MSA-3002™	10	Staggered	Medium	
Site-Specific Microbiome Standards	Genomic DNA	MSA-1004™	6	Even	Medium	Oral mock community DNA standard
		MSA-1005™	6	Even	Medium	Skin mock community DNA standard
		MSA-1006™	12	Even	Medium	Gut mock community DNA standard
		MSA-1007™	6	Even	Medium	Vaginal mock community DNA standard
	Whole Cells	MSA-2004™	6	Even	Medium	Oral mock community whole cell standard
		MSA-2005™	6	Even	Medium	Skin mock community whole cell standard
		MSA-2006™	12	Even	Medium	Gut mock community whole cell standard
		MSA-2007™	6	Even	Medium	Vaginal mock community whole cell standard
Microbiome Spike-in Controls	Genomic DNA	MSA-1014™	3	Even	Medium	Spike-in controls for optimizing assay development and metagenomics-based profiling **- New!**
	Whole Cells	MSA-2014™	3	Even	Medium	

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