

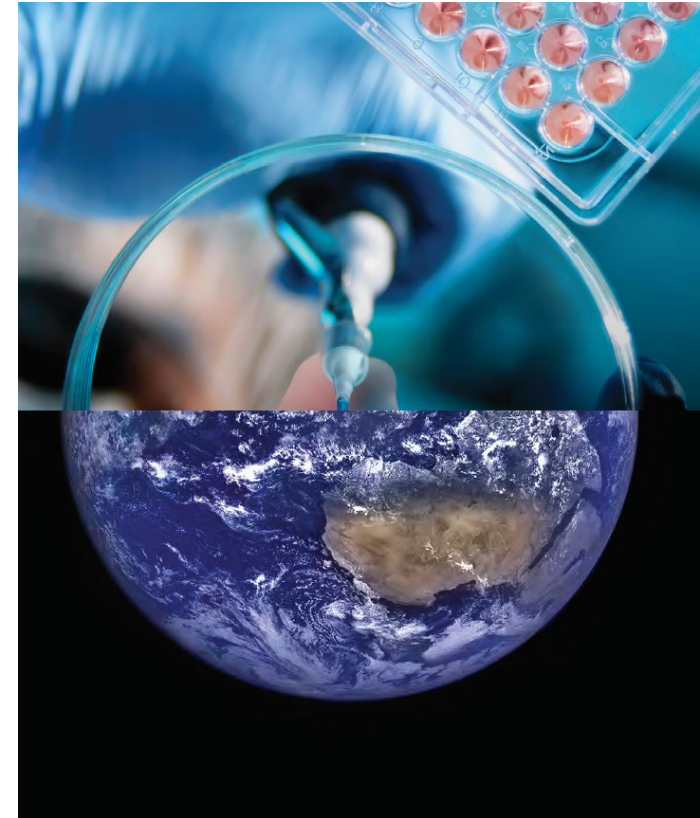
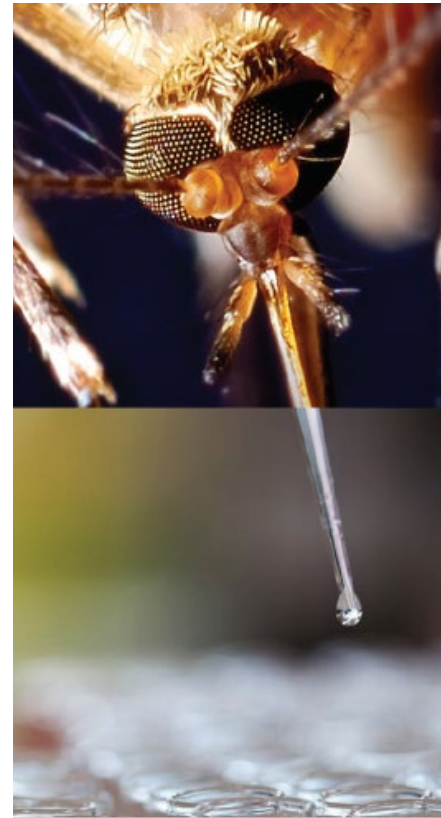
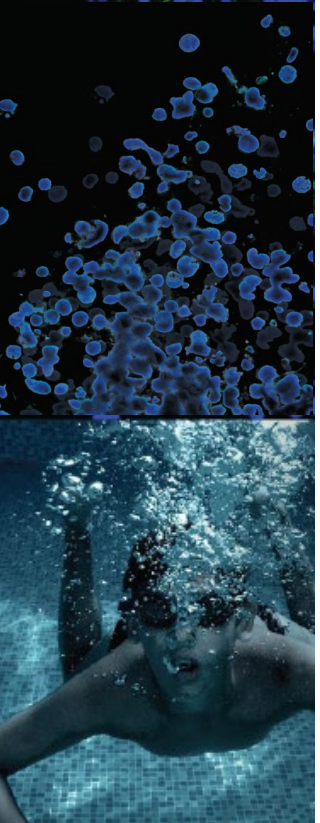


ATCC® NGS Standards and Their Applications

How Standards Help Establish Reliable Workflows for Microbiome Analysis

Leka Papazisi, DVM, PhD
Principal Scientist, ATCC

Credible Leads to InCredible™



About ATCC®

- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
 - 5,000 cell lines
 - 80,000 microorganisms
 - Genomic & synthetic nucleic acids
 - Media/reagents
- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 600+ employees, over one-third with advanced degrees



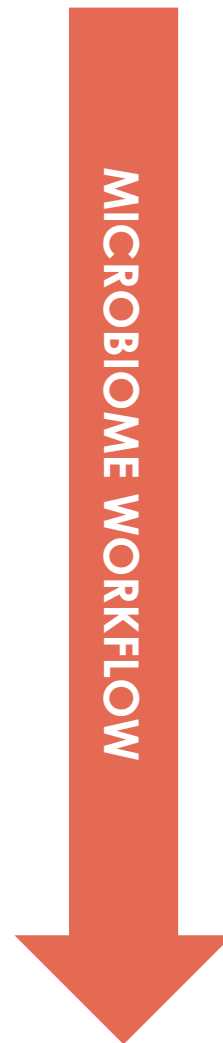
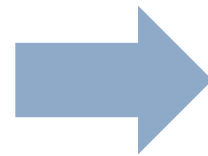
Microbiome Research and Applications

The microbiome field is rapidly moving toward translational research pertinent to human health and disease, therapeutics, and personalized medicine

Challenges in Microbiome Research and Applications

sequencing viability coverage
extraction technology
amplification
depth bioinformatics

bias



Sample Collection

- Storage
- Handling
- Processing

DNA Extraction

- Cell lysis
- DNA/RNA recovery
- Quality & quantity

16S

Shotgun

- Amplicon vs non-amplicon
- Choice of primers
- Library preparation

Library Preparation

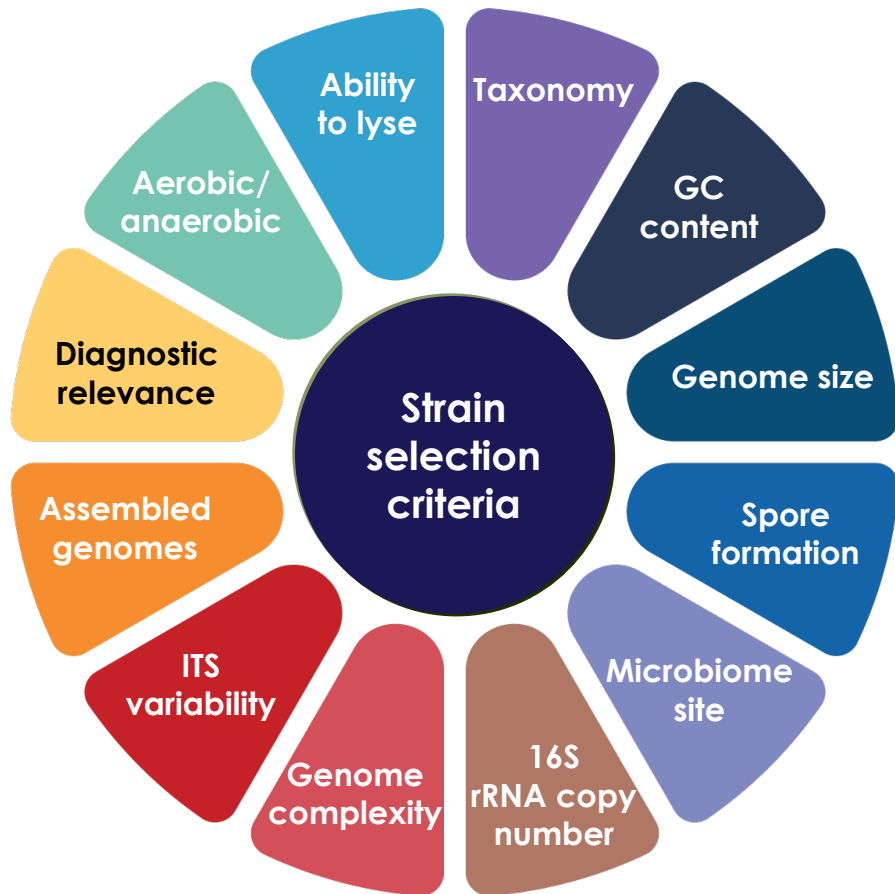
Sequencing

- Platform
- Chemistry
- Depth

Data Analysis

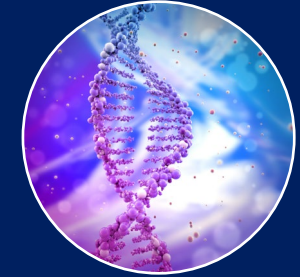
- Read quality
- Algorithm
- Database

Development of ATCC NGS Standards



Whole Cell Standards

- Authenticated ATCC cultures
- Growth and image cytometry cell counting
- Mixed in even proportion-based cell numbers cells
- Storage at 4°C



Genomic DNA Standards

- Authenticated ATCC nucleic acids
- Fluorescent dye-based quantification
- Mixed in even proportions-based genome copy number
- Storage at -20°C

Assay development, optimization, verification, and quality control

ATCC® Genome Portal

A cloud-based platform that enables users to easily browse authenticated and traceable reference genomes and metadata.



Download whole-genome sequences and annotations of ATCC materials



Search for nucleotide sequences or genes within genomes



View genome assembly metadata and quality metrics

genomes.atcc.org

Yarmosh DA, et al. Comparative Analysis and Data Provenance for 1,113 Bacterial Genome Assemblies. Msphere 7(3): e00077-22, 2022

3,238 Authenticated Microbial Reference Genomes

2,778 bacteria
250 viruses
206 fungi
4 protists

New genomes released every month!

REST-API for bioinformatics applications available

Free for non-commercial research use (RUO) purposes. Commercial use licenses available. Registration required.

ATCC® NGS Standards Portfolio



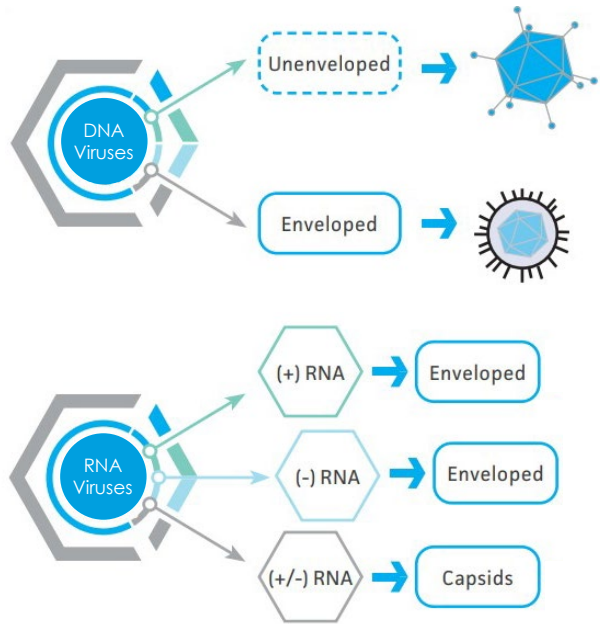
| Preparation | ATCC® Catalog No. | Number of Organisms | Composition | Complexity | Utility |
|-------------|-------------------|---------------------|-------------|------------|--|
| Genomic DNA | MSA-1000™ | 10 | Even | Medium | Standards for assay development and optimization |
| | MSA-1001™ | 10 | Staggered | Medium | |
| | MSA-1002™ | 20 | Even | High | |
| | MSA-1003™ | 20 | Staggered | High | |
| Whole cell | MSA-2003™ | 10 | Even | Medium | |
| | MSA-2002™ | 20 | Even | High | |
| Genomic DNA | MSA-4000™ | 11 | Staggered | Medium | NGS-based pathogen detection |
| Genomic DNA | MSA-3000™ | 6 | Even | Low | Environmental studies |
| | MSA-3001™ | 10 | Even | Medium | |
| | MSA-3002™ | 10 | Staggered | Medium | |

ATCC® Site-specific NGS Standards



| Standard | Preparation | ATCC® Catalog No. | Number of Organisms | Importance |
|----------|-------------|-------------------|---------------------|---|
| Oral | Whole cell | MSA-2004™ | 6 | <ul style="list-style-type: none"> Mock microbial communities representing the oral, skin, gut, and vaginal microbiomes Comprises normal and atypical flora Anaerobic and aerobic microbial strains A combination of Gram-positive and Gram-negative bacterial cultures Even composition |
| | Genomic DNA | MSA-1004™ | | |
| Skin | Whole cell | MSA-2005™ | 6 | |
| | Genomic DNA | MSA-1005™ | | |
| Gut | Whole cell | MSA-2006™ | 12 | |
| | Genomic DNA | MSA-1006™ | | |
| Vaginal | Whole cell | MSA-2007™ | 6 | |
| | Genomic DNA | MSA-1007™ | | |

ATCC® Virome Standards



Composition of Virome Standards

Human herpesvirus 5 strain AD169 (ATCC® VR-538™)

Human mastadenovirus strain F (ATCC® VR-931™)

Influenza B virus strain B/Florida/4/2006 (ATCC® VR-1804™)

Zika virus strain MR 766 (ATCC® VR-1838™)

Reovirus 3 strain Dearing (ATCC® VR-824™)

Human respiratory syncytial virus strain A2 (ATCC® VR-1540™)

| Standard | Preparation | ATCC® Catalog No. | Number of Organisms | Specification (ddPCR™) | Applications |
|----------|------------------|-------------------|---------------------|--|--|
| Virome | Virus Mix | MSA-2008™ | 6 | 2×10^3 genome copies/ μ L per virus | Standards for virome assay development, optimization, verification, and validation; evaluating reproducibility; and use as a daily run quality control |
| | Nucleic Acid Mix | MSA-1008™ | 6 | 2×10^4 genome copies/ μ L per virus | |

ATCC® Spike-in and Mycobiome Standards

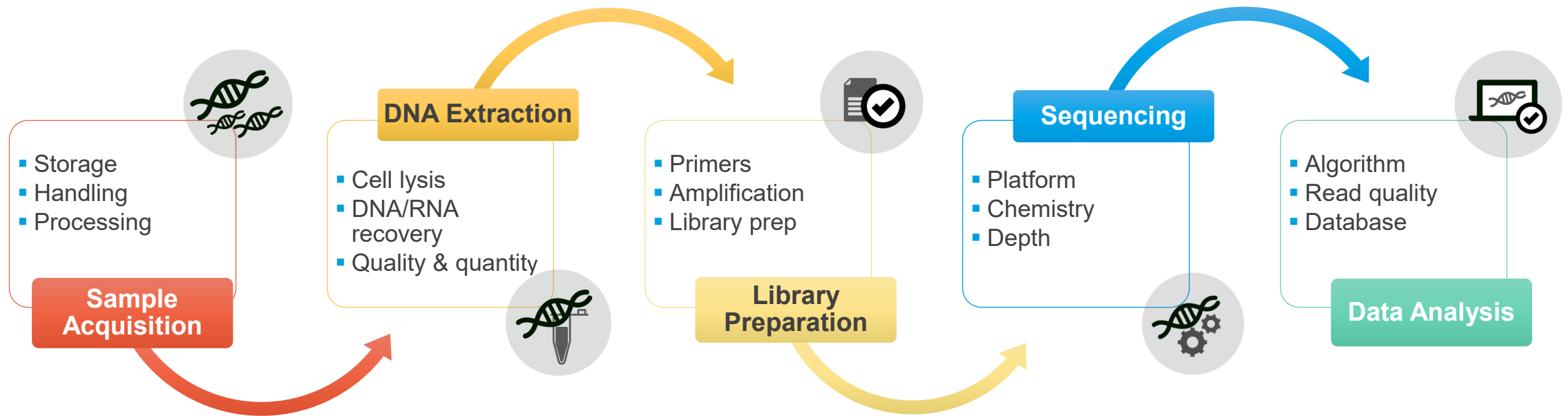


| Standard | Preparation | ATCC® Catalog No. | Number of Organisms | Application |
|----------|-------------|-------------------|---------------------|--|
| Spike-in | Whole cell | MSA-2014™ | 3 | <ul style="list-style-type: none"> Microbiome measurements and data normalization 16S rRNA and shotgun assay verification, validation, and quality control |
| | Genomic | MSA-1014™ | | |



| Standard | Preparation | ATCC® Catalog No. | Number of Organisms | Application |
|-----------|-------------|-------------------|---------------------|---|
| Mycobiome | Whole cell | MSA-2010™ | 10 | <ul style="list-style-type: none"> Fungal mock community standards for assay development, optimization, verification, and validation; evaluating reproducibility; and use as a daily run quality control |
| | Genomic | MSA-1010™ | | |

Microbiome Workflow, Biases, and Standardization



Whole cell standards – Full process controls, including DNA Extraction

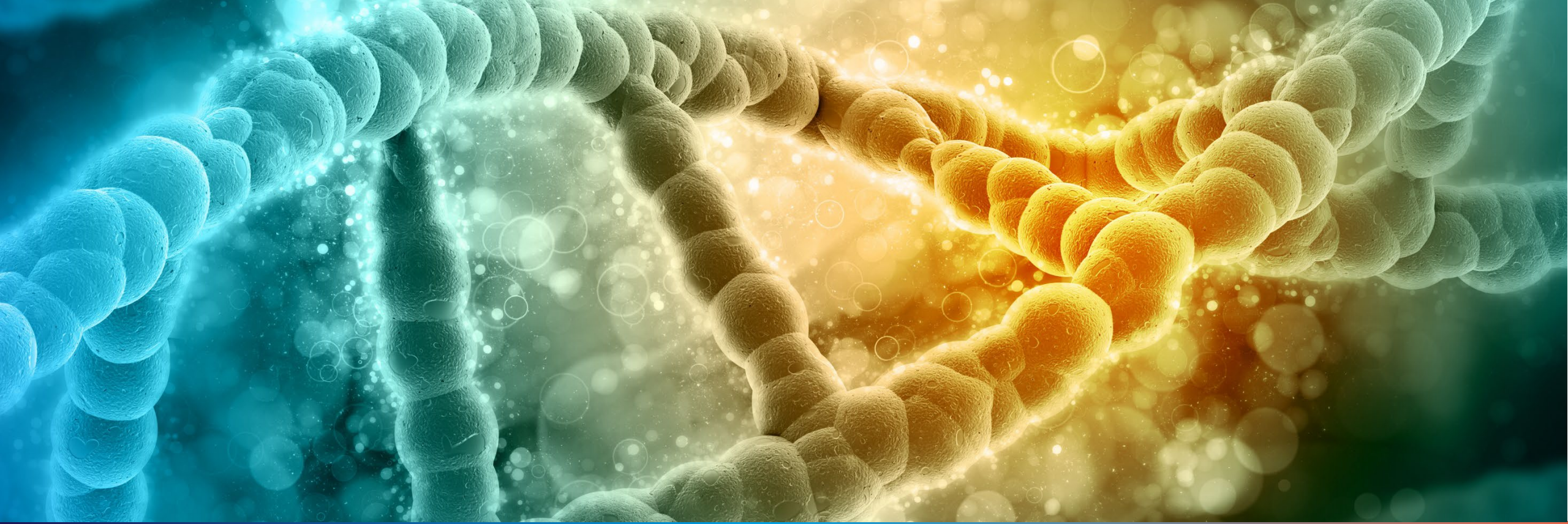
Genomic DNA standards – Library preparation, sequencing

One Codex analysis



Utility and Application of NGS Standards

- Evaluating DNA extraction methods and kits
- Evaluating 16S rRNA and WGS library kits
- Evaluating NGS platforms

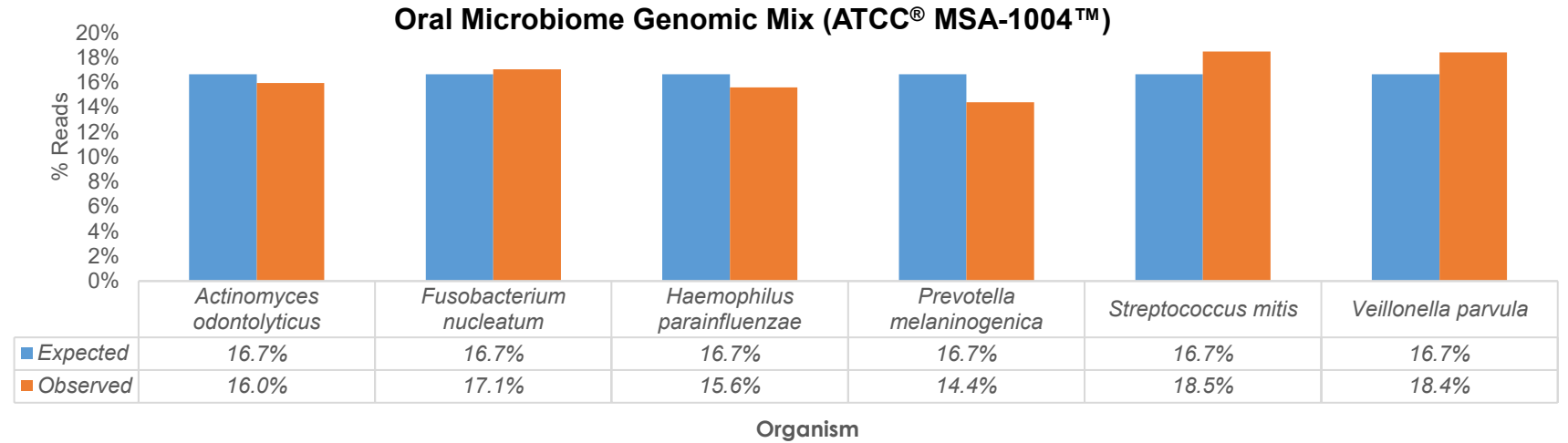


Evaluating DNA Extraction Methods and Kits

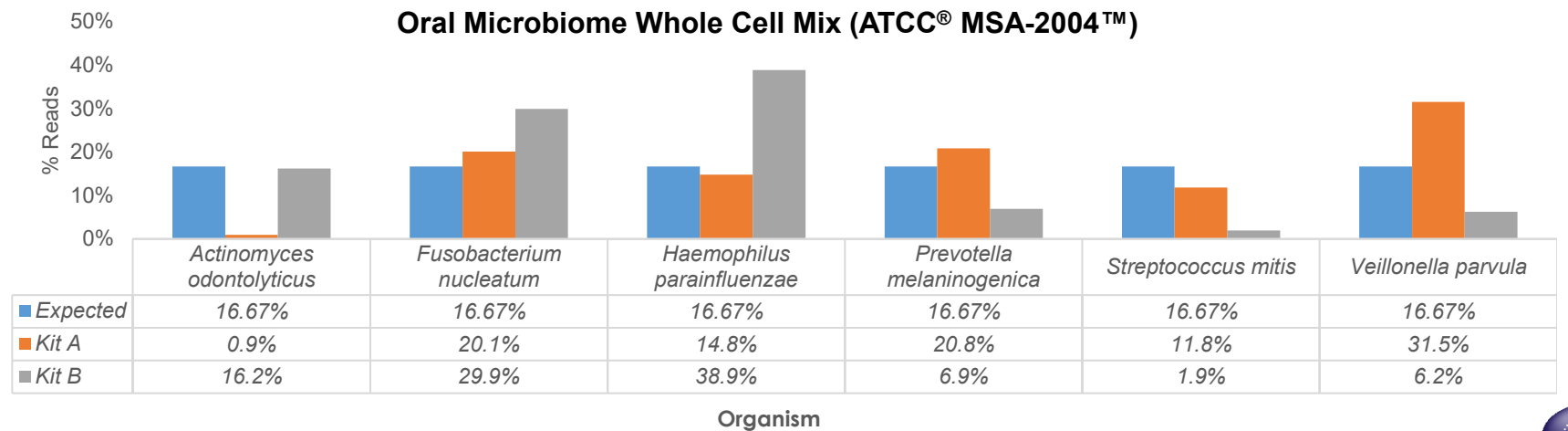
Genomic Versus Whole Cell Standards

DNA extraction methods are not perfect

Shotgun metagenomic analysis of the Oral Microbiome Genomic Mix



DNA extraction from the Oral Microbiome Whole Cell Mix with two different kits followed by shotgun metagenomic analysis



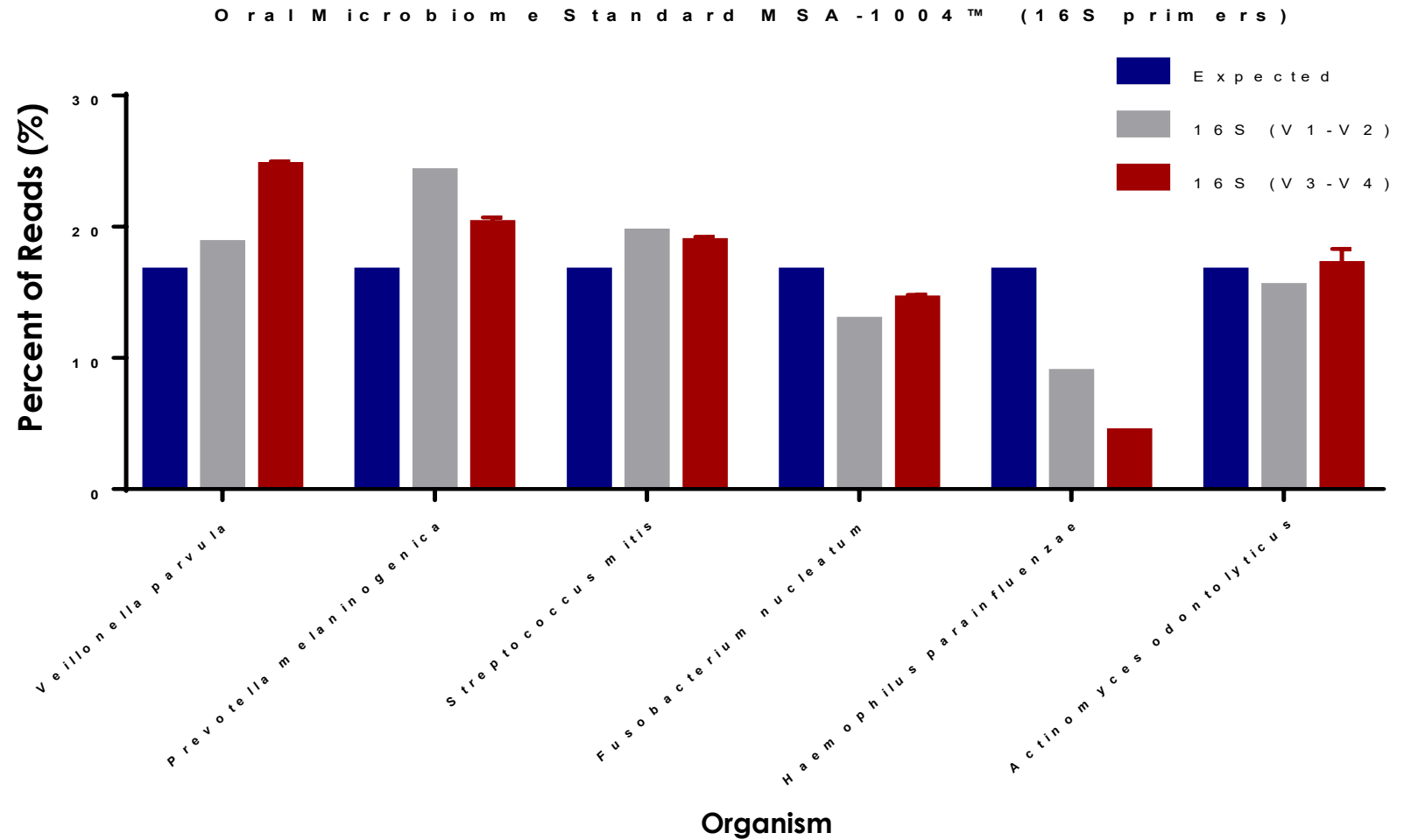


Evaluating 16S rRNA and WGS Library Kits

16S Amplicon-based Analysis: Primer Selection

Compare different primer sets, optimize amplification steps, and validate 16S analysis methods

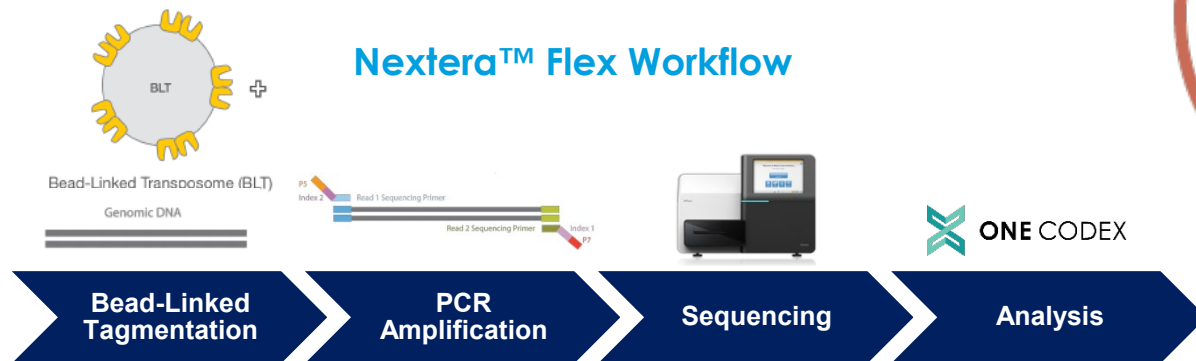
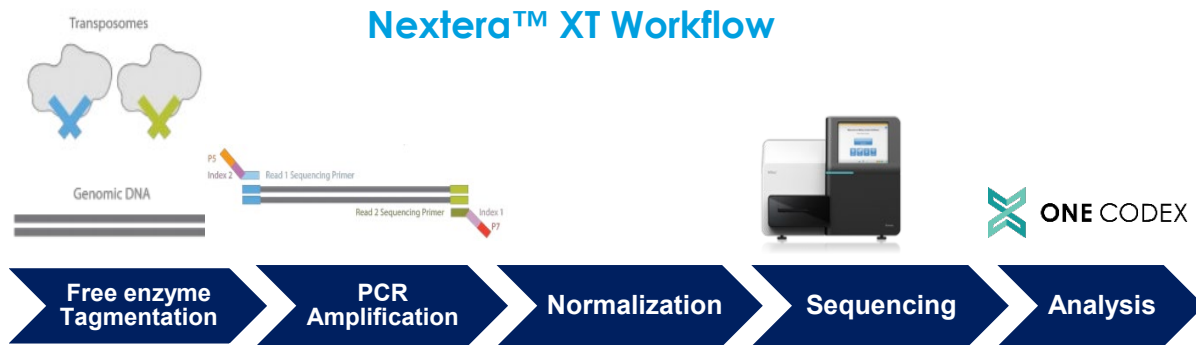
16S rRNA analysis of the Oral Genomic DNA Standard via two primer sets



Comparing Library Preparation Kits

Nextera Flex enables uniform coverage of genomes of low GC content

Oral Microbiome Genomic DNA (ATCC® MSA-1004™)



Sample Composition

| Name | Estimated Abundance |
|----------------------------|---------------------|
| Actinomyces odontolyticus | 46.07% |
| Prevotella melaninogenica | 16.09% |
| Streptococcus mitis | 16.04% |
| Veillonella parvula | 12.65% |
| Haemophilus parainfluenzae | 7.80% |
| Fusobacterium nucleatum | 1.34% |



Sample Composition

| Name | Estimated Abundance |
|----------------------------|---------------------|
| Streptococcus mitis | 18.83% |
| Veillonella parvula | 18.42% |
| Fusobacterium nucleatum | 17.13% |
| Haemophilus parainfluenzae | 15.68% |
| Actinomyces odontolyticus | 15.54% |
| Prevotella melaninogenica | 14.40% |



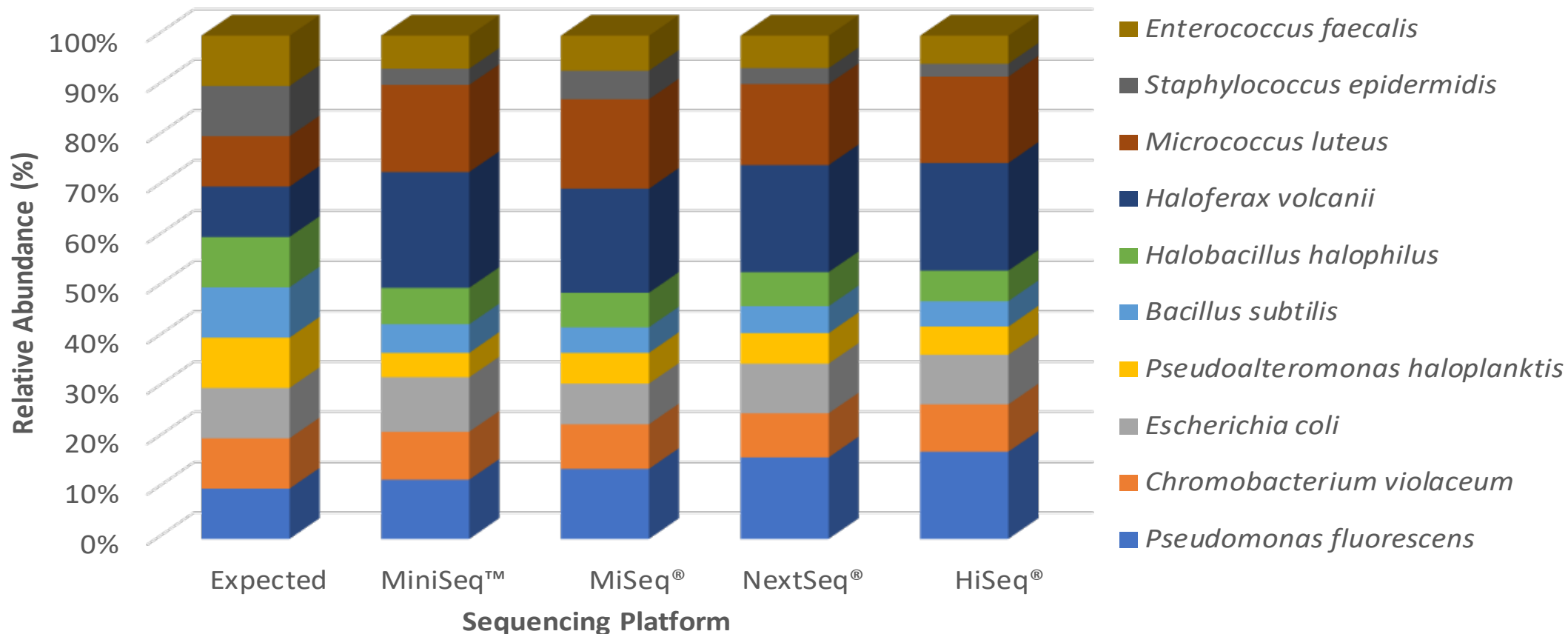


Evaluating NGS Platforms

Short-read Sequencing Platform: Illumina®

Assay reproducibility through different Illumina sequencing platforms

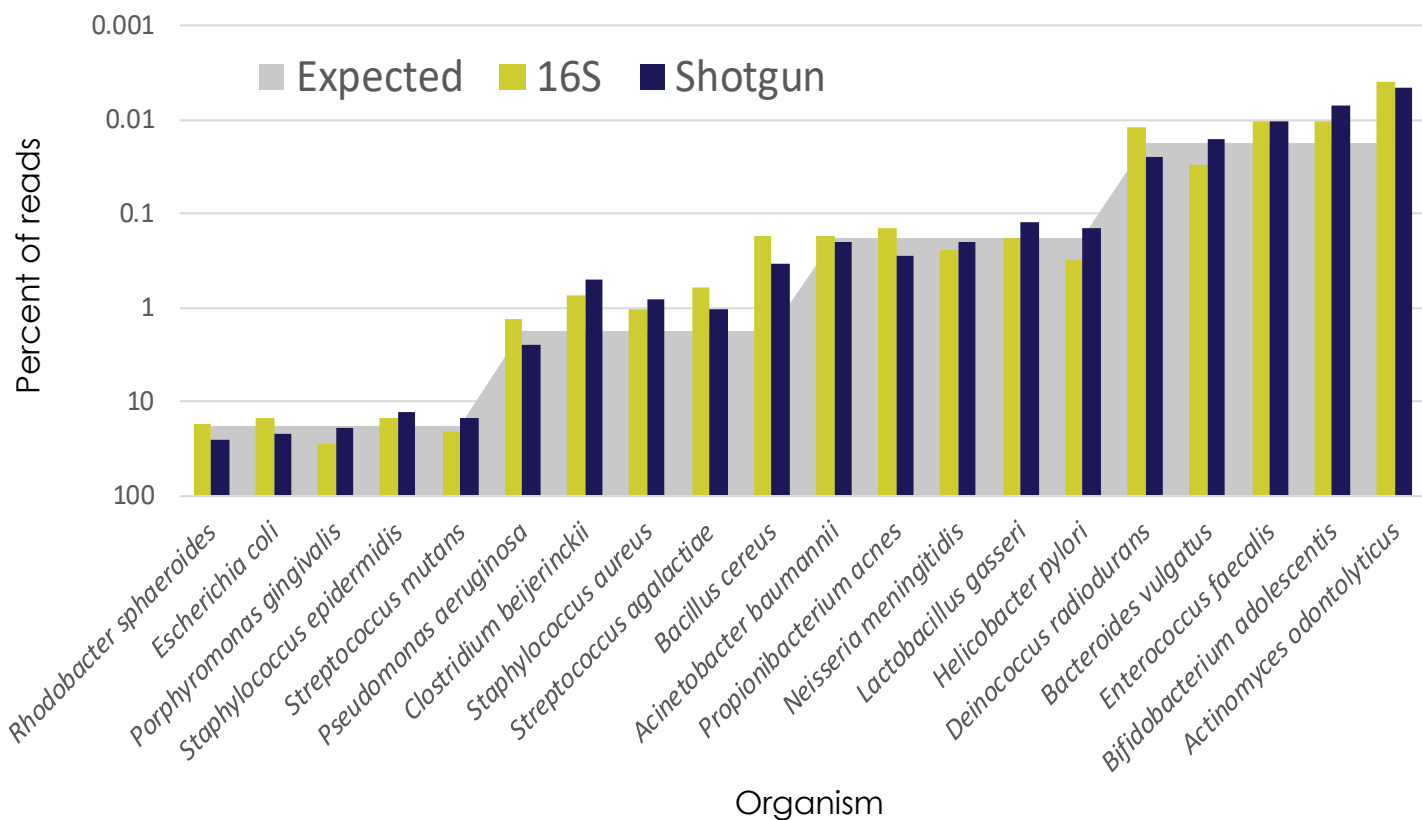
Shotgun Metagenomic Data (ATCC® MSA-3001™)



Data courtesy of Dr. Stefan Green, UIC (ABRF-MGRG)

Long-read Sequencing Platform: PACBIO®

16S rRNA (full-length) and shotgun data on the PacBio Sequel Platform using ATCC® MSA-1003™



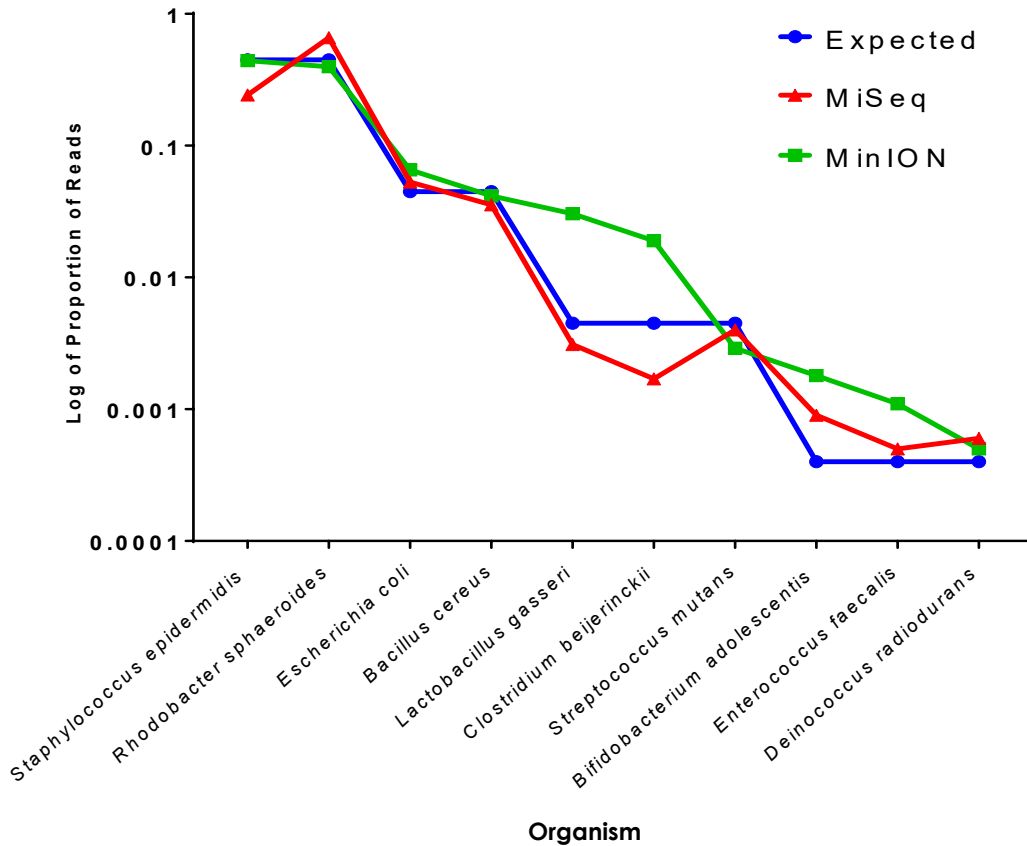
ATCC quality control score (One Codex)

| One Codex Analysis | 16S rRNA run 1 | 16S rRNA run 2 | Shotgun run 1 | Shotgun run 2 |
|--------------------|----------------|----------------|---------------|---------------|
| True positives | 100% | 100% | 100% | 100% |
| Relative abundance | 95% | 95% | 97% | 97% |
| False positives | 0% | 0% | 12% | 16% |
| Overall score | 98% | 98% | 95% | 95% |

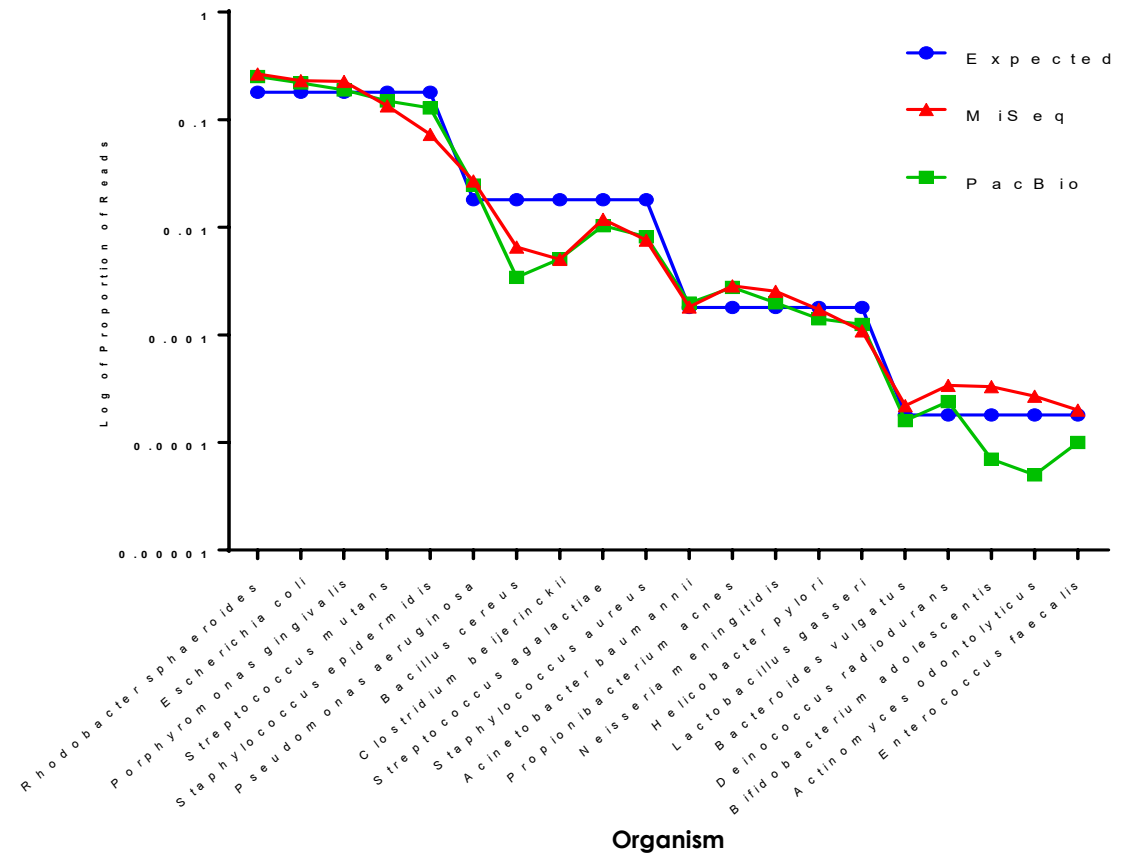
Shotgun Metagenomic Analysis: Short vs Long Reads

ATCC® NGS Standards are technology agnostic

MSA-1001™ (Staggered 10 Strains)



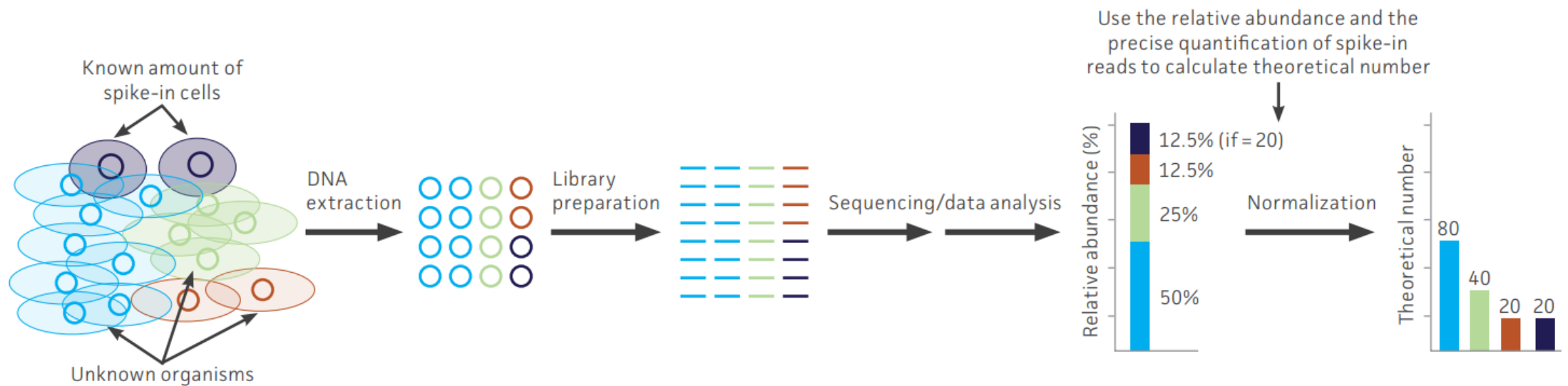
MSA-1003™ (Staggered 20 Strains)





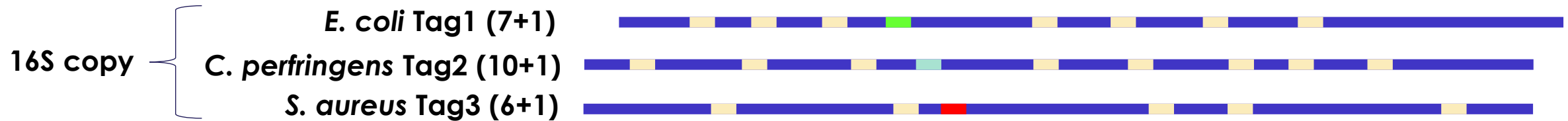
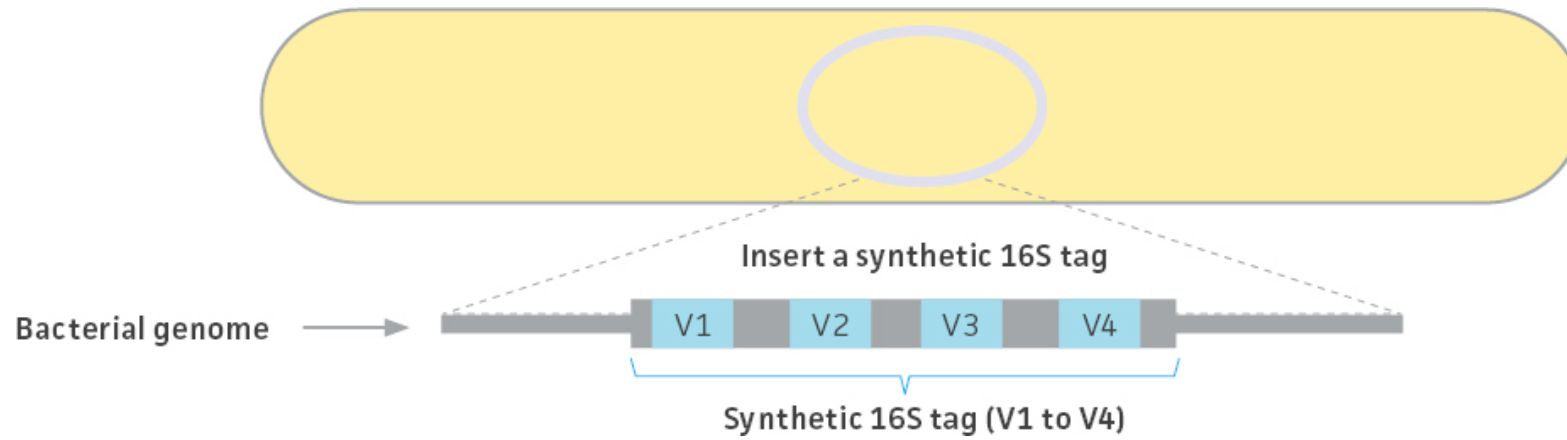
Spike-in Internal Controls: Synthetic 16S Tagged Strains

The Output of a Metagenomic Data is a Relative Abundance



Relative abundance does not reflect the quantity of the microbial community and the inter-sample differences among taxa.

Engineering Synthetic 16S Tag Into Bacterial Genome



Spike-in Standards (3 Strain Tagged Mix)



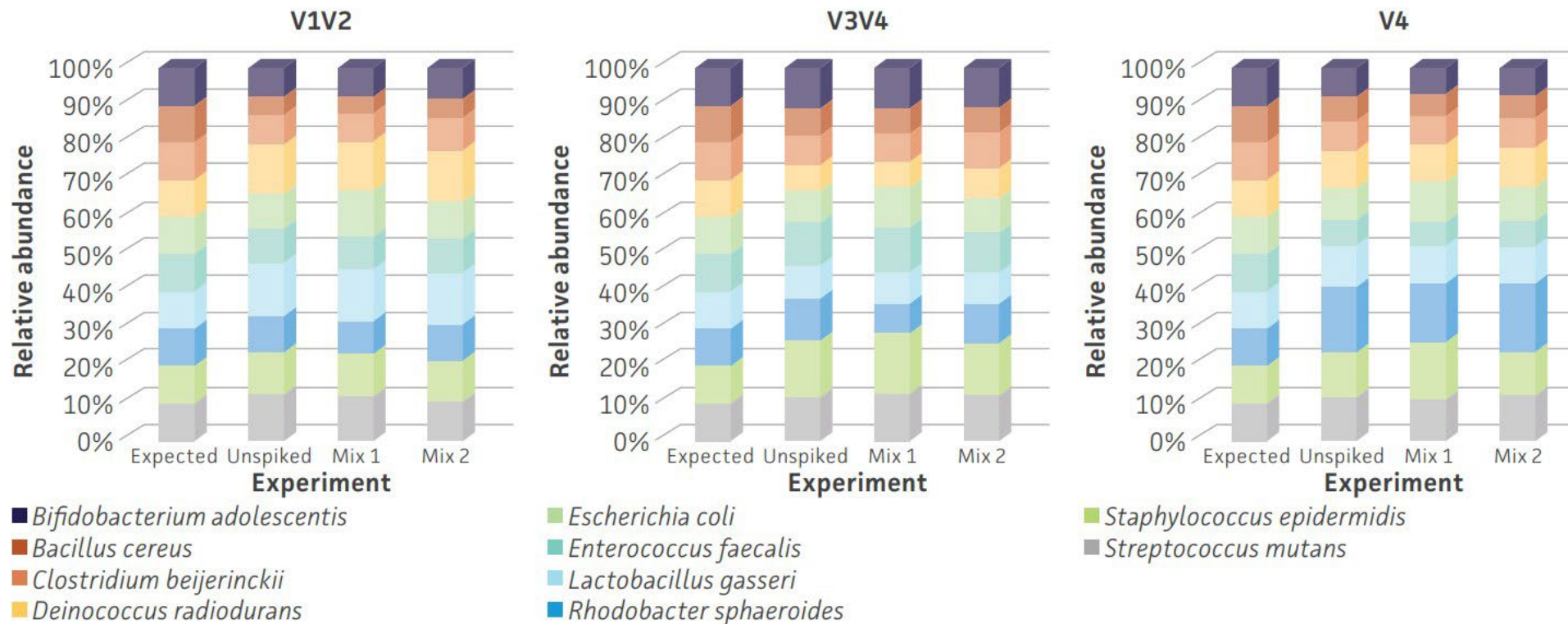
| ATCC® Catalog No. | Preparation | Specification | Importance |
|-------------------|-------------|---|--|
| MSA-1014™ | Genomic DNA | 6×10^7 genomes copies/vial ± 1 log | <ul style="list-style-type: none"> Microbiome measurements and data normalization 16S rRNA and shotgun assay verification, validation, and quality control |
| MSA-2014™ | Whole cells | 6×10^7 cells/vial ± 1 log | |

Spike-in Composition

| Species | Gram Stain | Genome size (Mb) | Tag size (bp) | G/C Content (%) | 16S Copies | Tag copies | Cells per vial |
|--|------------|------------------|---------------|-----------------|------------|------------|-----------------|
| <i>Escherichia coli</i> Tag1 | Negative | 4.59 | 829 | 50.8 | 7 | 1 | 2×10^7 |
| <i>Clostridium perfringens</i> Tag2 | Positive | 3.25 | 799 | 29.0 | 10 | 1 | 2×10^7 |
| <i>Staphylococcus aureus</i> Tag3 | Positive | 2.70 | 833 | 32.8 | 6 | 1 | 2×10^7 |

Relative Abundance of a Mock Community with Spike-in

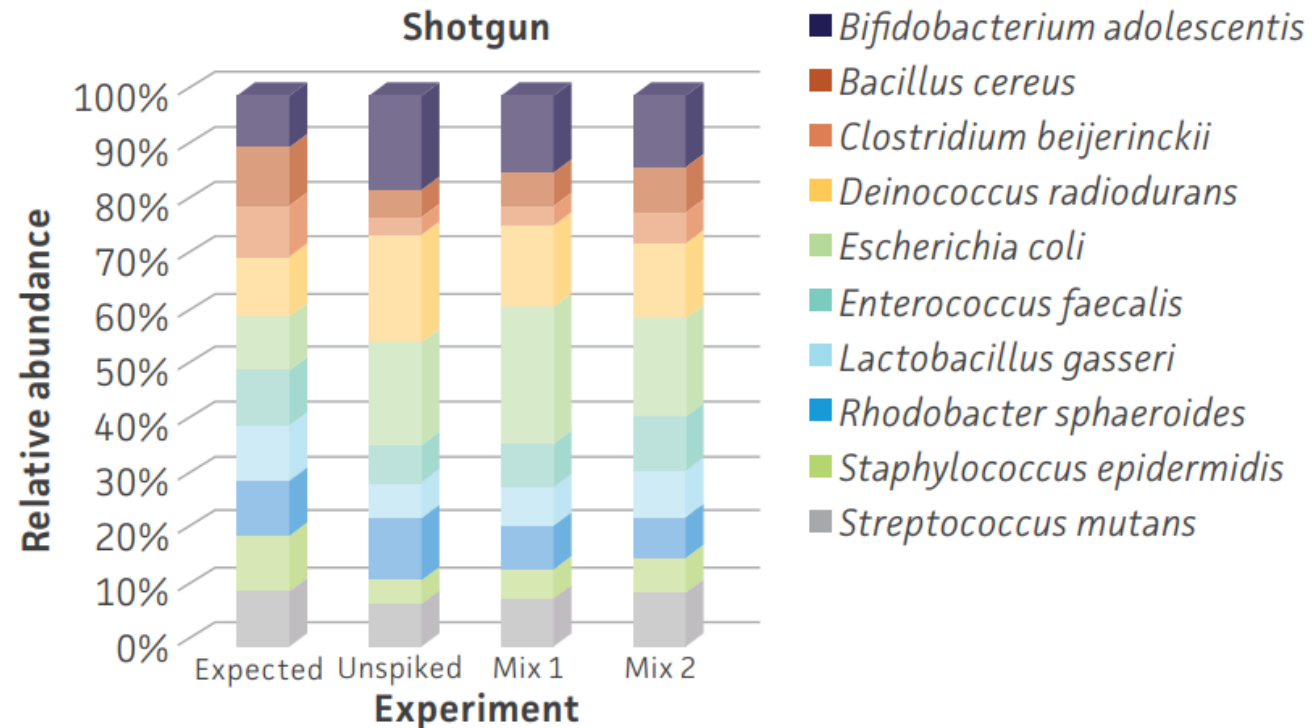
The spike-in doesn't have obvious impact on 16S relative abundance



ATCC[®] MSA-1014[™] was mixed with MSA-1000[™] at ~ 1:10 and ~1:100

Relative Abundance of a Mock Community with Spike-in

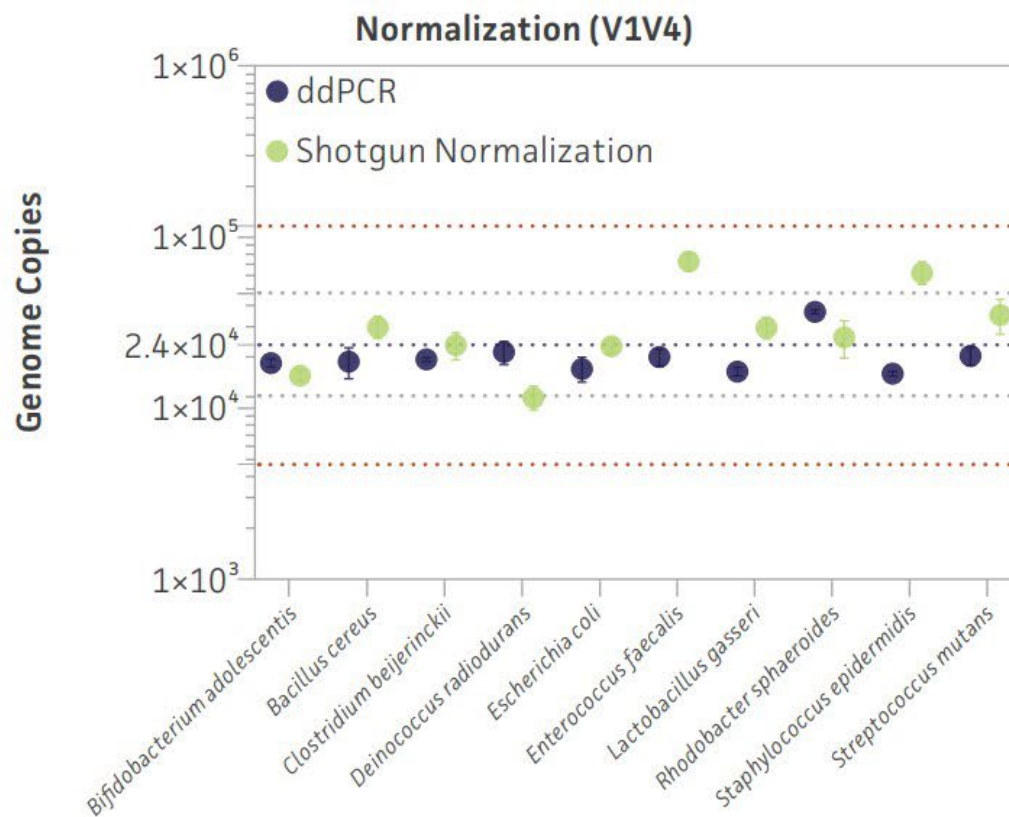
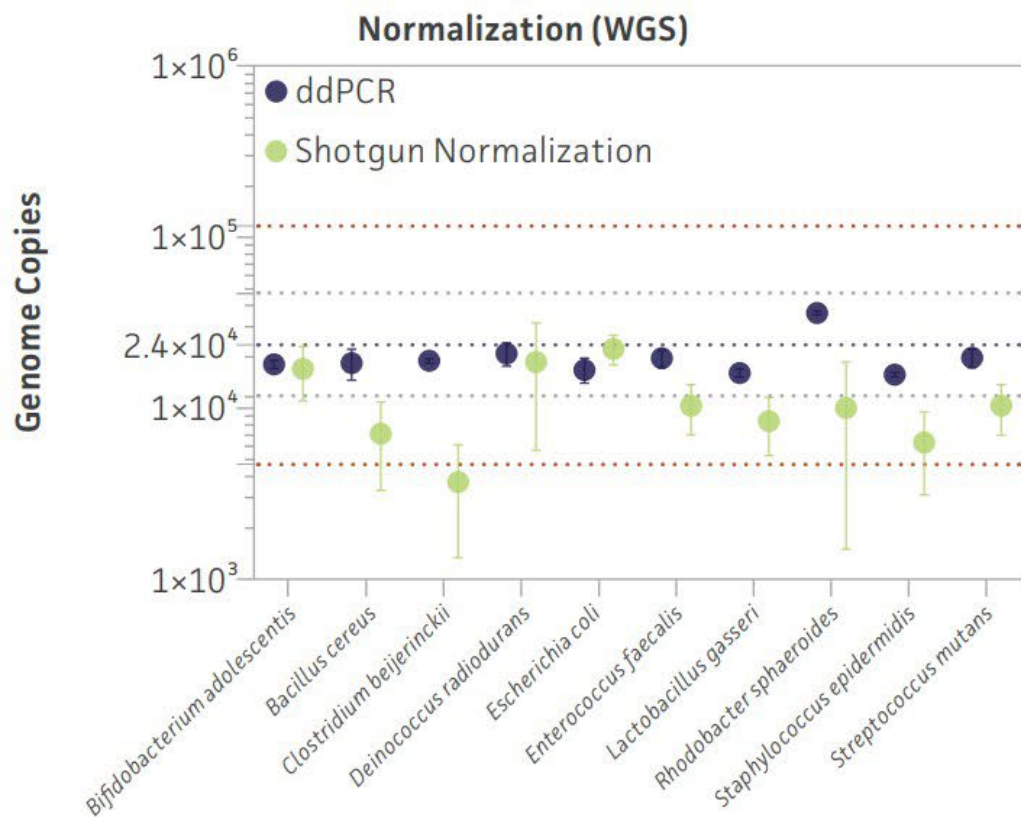
The spike-in doesn't have an obvious impact on whole genome shotgun analysis



ATCC[®] MSA-1014[™] was mixed with MSA-1000[™] at ~ 1:10 and ~1:100

Comparison of Absolute Quantitation by ddPCR and Normalized WGS

Three tagged genomic DNA mixed with 10 even genomic DNA (ATCC[®] MSA-1000[™])



ATCC Data Analysis Solution



Mock Microbial Communities

- Genomic DNA and whole cell standards
- Even and staggered mixtures comprising 10 or 20 strains
- Environmental and pathogen mixtures



Site-specific Standards

- Genomic DNA and whole cell standards
- Even mixtures of 6-12 strains
- Bacterial strains prevalent in the oral, skin, gut, and vaginal microbiome



Spike-In Standards

- Recombinant strains with a unique DNA tag stably integrated into the chromosome
- Recombinant standards include the Gram-negative and Gram-positive bacteria



Virome and Mycobiome Standards

- Genomic DNA and whole cell standards comprising diverse and clinically relevant strains
- Even mixtures of 6-10 strains

Bundled with data analysis on the One Codex platform



1. UPLOAD YOUR
DATA



2. SELECT YOUR NGS
STANDARD



3. ENTER YOUR
METADATA



4. REVIEW YOUR
RESULTS

ATCC NGS Standards for Microbiome Research

www.atcc.org/Microbiome

Microbiome Research

Raising the standards for
microbiome research

Optimize your research with the right controls

The complexities involved in 16S rRNA community profiling and shotgun metagenomics methods pose significant challenges for microbiome research. Significant biases can be introduced at each stage of the microbiome workflow, affecting data interpretation and reproducibility.

[NGS Standards](#) provide a solution to this problem. From sample collection to data analysis, NGS Standards enable you to optimize your diverse research applications with confidence and improve the consistency and reproducibility of your data, run after run.

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





Questions?

Credible Leads to Incredible™

