Making Sense Out of Microbiome Data – The Importance of Standards

Briana Benton, BS
Technical Manager, ATCC
Agenda

- Background on American Type Culture Collection
- Challenges in microbiome research
  - Discuss why standards are essential
- Development of microbiome standards
- The ATCC® Microbiome Standards portfolio and upcoming new products
- Applications of standards in microbiome research
  - Extraction method, assay variability, NGS library preparation, and bioinformatics analysis
- Microbiome assay development
- Show the best data
- Recommend any specific assay, kit, protocol, or instrument
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 largest, most diverse biological materials and information resource for microbes – the “gold standard”
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository

- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 18 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees
The microbiome field is rapidly moving toward translational research pertinent to human health and disease, therapeutics, and personalized medicine.
Challenges in Microbiome Research

MICROBIOME WORKFLOW

- Sample Collection
  - Storage
  - Handling
  - Processing

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

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

- Sequencing
  - Platform
  - Chemistry
  - Depth

- Data Analysis
  - Read quality
  - Algorithm
  - Database
Development of Mock Microbial Communities

**Strain selection criteria**
- Aerobic/anaerobic
- Diagnostic relevance
- Assembled genomes
- ITS variability
- Genomic complexity
- Ability to lyse
- Gram stain
- GC content
- Genome size
- Spore formation
- Microbiome site
- 16S rRNA copy number
- 6

**Whole Cell Standards**
- Authenticated ATCC cultures
- Growth and image cytometry cell counting
- Mixed in even proportions.
- Store at 4°C until ready to use

**Genomic DNA Standards**
- Authenticated ATCC nucleic acids
- Fluorescent dye-based quantification
- Mixed in even or staggered proportions based on genome copy number
- Store at -20°C

**Assay development, optimization, verification, and quality control**
## ATCC® Microbiome Standards Portfolio

<table>
<thead>
<tr>
<th>Preparation</th>
<th>ATCC® Catalog No.</th>
<th>Number of Organisms</th>
<th>Composition</th>
<th>Complexity</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic DNA</td>
<td>MSA-1000™</td>
<td>10</td>
<td>Even</td>
<td>Medium</td>
<td>Standards for assay development and optimization</td>
</tr>
<tr>
<td></td>
<td>MSA-1001™</td>
<td>10</td>
<td>Staggered</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSA-1002™</td>
<td>20</td>
<td>Even</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSA-1003™</td>
<td>20</td>
<td>Staggered</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Whole cell</td>
<td>MSA-2003™</td>
<td>10</td>
<td>Even</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSA-2002™</td>
<td>20</td>
<td>Even</td>
<td>High</td>
<td></td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>MSA-4000™</td>
<td>11</td>
<td>Staggered</td>
<td>Medium</td>
<td>NGS-based pathogen detection</td>
</tr>
<tr>
<td>Genomic DNA</td>
<td>MSA-3000™</td>
<td>6</td>
<td>Even</td>
<td>Low</td>
<td>Environmental studies</td>
</tr>
<tr>
<td></td>
<td>MSA-3001™</td>
<td>10</td>
<td>Even</td>
<td>Medium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MSA-3002™</td>
<td>10</td>
<td>Staggered</td>
<td>Medium</td>
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</table>
# Site-specific Microbiome Standards

<table>
<thead>
<tr>
<th>Standard</th>
<th>Preparation</th>
<th>ATCC® Catalog No.</th>
<th>Number of Organisms</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>Whole cell</td>
<td>MSA-2004™</td>
<td>6</td>
<td>• Mock microbial communities representing the oral, skin, gut, and vaginal microbiomes</td>
</tr>
<tr>
<td></td>
<td>Genomic DNA</td>
<td>MSA-1004™</td>
<td></td>
<td>• Comprises normal and atypical flora</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Anaerobic and aerobic microbial strains</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• A combination of Gram-positive and -negative bacterial cultures</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Even composition</td>
</tr>
<tr>
<td>Skin</td>
<td>Whole cell</td>
<td>MSA-2005™</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genomic DNA</td>
<td>MSA-1005™</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gut</td>
<td>Whole cell</td>
<td>MSA-2006™</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genomic DNA</td>
<td>MSA-1006™</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>Whole cell</td>
<td>MSA-2007™</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Genomic DNA</td>
<td>MSA-1007™</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## ATCC Virome Standards

### Composition of Virome Standards

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATCC Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human herpesvirus 5 strain AD169</td>
<td>ATCC VR-538™</td>
</tr>
<tr>
<td>Human mastadenovirus strain F</td>
<td>ATCC VR-931™</td>
</tr>
<tr>
<td>Influenza B virus strain B/Florida/4/2006</td>
<td>ATCC VR-1804™</td>
</tr>
<tr>
<td>Zika virus strain MR 766</td>
<td>ATCC VR-1838™</td>
</tr>
<tr>
<td>Reovirus 3 strain Dearing</td>
<td>ATCC VR-824™</td>
</tr>
<tr>
<td>Human respiratory syncytial virus strain A2</td>
<td>ATCC VR-1540™</td>
</tr>
</tbody>
</table>

### Virome Standards

<table>
<thead>
<tr>
<th>Standard</th>
<th>Preparation</th>
<th>ATCC® Catalog No.</th>
<th>Number of Organisms</th>
<th>Specification (ddPCR™)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virome</td>
<td>Virus Mix</td>
<td>MSA-2008™</td>
<td>6</td>
<td>2 x 10^3 genome copies/µL per virus</td>
<td>Standards for virome assay development, optimization, verification, and validation; evaluating reproducibility; and use as a daily run quality control</td>
</tr>
<tr>
<td></td>
<td>Nucleic Acid Mix</td>
<td>MSA-1008™</td>
<td>6</td>
<td>2 x 10^4 genome copies/µL per virus</td>
<td></td>
</tr>
</tbody>
</table>
Spike-in Standards (3 Strain Tagged Mix)

<table>
<thead>
<tr>
<th>Standard</th>
<th>Preparation</th>
<th>ATCC® Catalog No.</th>
<th>Number of Organisms</th>
<th>Importance</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Strain Tagged Mix</td>
<td>Whole cell</td>
<td>MSA-2014™</td>
<td>3</td>
<td>• Microbiome measurements and data normalization</td>
</tr>
<tr>
<td></td>
<td>Genomic</td>
<td>MSA-1014™</td>
<td></td>
<td>• 16S rRNA and shotgun assay verification, validation, and quality control</td>
</tr>
</tbody>
</table>

Spike-in Composition

<table>
<thead>
<tr>
<th>Species</th>
<th>Gram Stain</th>
<th>Genome size (Mb)</th>
<th>Tag size (bp)</th>
<th>G/C Content (%)</th>
<th>16S Copies</th>
<th>Tag copies</th>
<th>Cells per vial</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> Tag1</td>
<td>Negative</td>
<td>4.59</td>
<td>829</td>
<td>50.8</td>
<td>7</td>
<td>1</td>
<td>2 x 10^7</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> Tag2</td>
<td>Positive</td>
<td>3.25</td>
<td>799</td>
<td>29.0</td>
<td>10</td>
<td>1</td>
<td>2 x 10^7</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> Tag3</td>
<td>Positive</td>
<td>2.70</td>
<td>833</td>
<td>32.8</td>
<td>6</td>
<td>1</td>
<td>2 x 10^7</td>
</tr>
</tbody>
</table>
# Engineering Synthetic 16S Tag Into Bacterial Genome

## Popular regions: V1 to V4

### Bacterial Strains and Synthetic 16S Tag Insertion

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Gram Stain</th>
<th>Genome G/C (%)</th>
<th>Insertion loci</th>
<th>BSL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> Tag1</td>
<td>Negative</td>
<td>50.8</td>
<td>Beta galactosidase</td>
<td>1</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em> Tag2</td>
<td>Positive</td>
<td>29.0</td>
<td>Theta-toxin</td>
<td>2</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> Tag3</td>
<td>Positive</td>
<td>32.8</td>
<td>O-antigen polymerase</td>
<td>2</td>
</tr>
</tbody>
</table>

### Synthetic 16S Tag Locations

- **E. coli Tag1 (7+1)**
- **C. perfringens Tag2 (10+1)**
- **S. aureus Tag3 (6+1)**
Spike-ins of known quantity can provide a baseline number for comparison with the number from unknown quantity, such that the data can be normalized within a sequencing assay and between different assays.
Spike-in: Calculating Absolute Numbers in a Sample

In sample X, if 0.99% Spike-in S = 100, then 19.80% B = 19.80% ÷ 0.99% x 100 = 2,000
In sample Y, if 8.33% Spike-in S = 100, then 20.83% B = 20.8% ÷ 8.33% x 100 = 250

**Total cell#:** In sample X, if 0.99% Spike-in S = 100, then total cell# of the sample X = 99% ÷ 0.99% x 100 = 10,000

Criterium of a spike-in bacterial sequence: Uniqueness
Resources

Technical Data Sheet: 3 Strain Tagged Whole Cell Even Mix
## Coming Soon - Mycobioime (MSA-2010™ and MSA-1010™)

<table>
<thead>
<tr>
<th>Species Name</th>
<th>ATCC® No.</th>
<th>Genome size (Mb)</th>
<th>Relevancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus Af293</td>
<td>MYA-4609™</td>
<td>28.8</td>
<td>Opportunistic, airborne pathogen that is responsible for 90% of fungal infections in immunocompromised patients</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>10231™</td>
<td>17.1</td>
<td>Commensal fungus of the oral cavity that can form biofilms on denture surfaces leading to mucosal infections</td>
</tr>
<tr>
<td>Candida glabrata</td>
<td>2001™</td>
<td>12.3</td>
<td>Commensal fungus of the oral cavity and human gut that can acquire resistance to azole antifungals leading to infection</td>
</tr>
<tr>
<td>Cryptococcus neoformans var. grubii</td>
<td>208821™</td>
<td>18.9</td>
<td>Responsible for cryptococcal meningitis in immunosuppressed patients</td>
</tr>
<tr>
<td>Malassezia globosa CBS 7966</td>
<td>MYA-4612™</td>
<td>9.0</td>
<td>Part of the normal skin flora but can be responsible for skin diseases such as dandruff, dermatitis, and folliculitis</td>
</tr>
<tr>
<td>Saccharomyces cerevisiae</td>
<td>201390™</td>
<td>12.2</td>
<td>Eukaryotic model organism used to study gene expression, signal transduction, the cell cycle, and metabolism</td>
</tr>
<tr>
<td>Trichophyton interdigitale</td>
<td>9533™</td>
<td>21.9</td>
<td>Can infect skin and nails to cause chronic infections such as athlete’s foot and ringworm</td>
</tr>
<tr>
<td>Cutaneotrichosporon dermatis (CBS2043)</td>
<td>204094™</td>
<td>23.3</td>
<td>Emerging opportunistic agent of invasive fungal infections, particularly in severely immunocompromised patients</td>
</tr>
<tr>
<td>Penicillium chrysogenum Thom</td>
<td>10106™</td>
<td>32.5</td>
<td>Spore-former and source of beta-lactam antibiotics such as penicillin</td>
</tr>
<tr>
<td>Fusarium keratoplasticum (F solani)</td>
<td>36031™</td>
<td>48.6</td>
<td>Filamentous opportunistic pathogen that causes fungal keratitis</td>
</tr>
</tbody>
</table>
Microbiome Workflow, Biases, and Standardization

- Sample Collection
  - Storage
  - Handling
  - Processing

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

- Library Preparation
  - Primers
  - Amplification
  - Library prep

- Sequencing
  - Platform
  - Chemistry
  - Depth

- Data Analysis
  - Algorithm
  - Read quality
  - Database

- Ideal use - Whole Cell Microbiomes (full process control)

- Ideal use – Genomic Microbiomes (assay control)

- One Codex Modules
Applications of standards in microbiome research
- DNA extraction
- 16S rRNA amplification and library kits
- NGS platforms
- Bioinformatics and databases
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
Assess Biases in DNA Extraction

Compare different pre-treatments and extraction methods, optimize protocols, and validate different kits.

DNA extraction from individual components of the Oral Whole Cell Mix

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of Cells per Component</th>
<th>Gram Stain</th>
<th>Genome size Mb</th>
<th>%GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces odontolyticus</td>
<td>~2x10^7</td>
<td>+</td>
<td>2.39</td>
<td>65.5</td>
</tr>
<tr>
<td>Fusobacterium nucleatum</td>
<td>-</td>
<td>-</td>
<td>2.17</td>
<td>27.2</td>
</tr>
<tr>
<td>Haemophilus parainfluenzae</td>
<td>-</td>
<td>-</td>
<td>2.12</td>
<td>39.3</td>
</tr>
<tr>
<td>Prevotella melaninogenica</td>
<td>-</td>
<td>-</td>
<td>3.17</td>
<td>35.1</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>+</td>
<td>-</td>
<td>1.83</td>
<td>40.5</td>
</tr>
<tr>
<td>Veillonella parvula</td>
<td>-</td>
<td>-</td>
<td>2.16</td>
<td>38.6</td>
</tr>
</tbody>
</table>
The Gut Whole Cell Microbiome Standard (ATCC® MSA-2006™) can be used as a full process control for shotgun and 16S rRNA assays.
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

The LoopSeq™ 16S rRNA long-read method allows highest sequence accuracy and species-level taxonomy

Loop Genomics

Overall Score – 99%

True Positives: 100%  Relative Abundance: 98%  False Positives: 100% 0

Overall Score – 94%

True Positives: 100%  Relative Abundance: 93%  False Positives: 100% 0

Genomic DNA (ATCC® MSA-1003™)

- Acinetobacter baumannii
- Actinomyces odontolyticus
- Bacillus cereus
- Bacteroides vulgatus
- Bifidobacterium adolescentis
- Clostridium beijerinckii
- Cutibacterium acnes
- Deinococcus radiodurans
- Enterococcus faecalis
- Escherichia coli
- Helicobacter pylori
- Lactobacillus gasseri
- Neisseria meningitidis
- Porphyromonas gingivalis
- Pseudomonas aeruginosa
- Rhodobacter sphaeroides
- Staphylococcus aureus
- Staphylococcus epidermidis
- Streptococcus agalactiae
- Streptococcus mutans

LoopSeq data courtesy of Tony Lialin, Loop Genomics
### MSA-1004™/MSA-2004™ Oral Microbiome

<table>
<thead>
<tr>
<th>Name</th>
<th>Gram Stain</th>
<th>% GC</th>
<th>Genome Size (Mb)</th>
<th>16S rRNA Copies</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinomyces odontolyticus</em></td>
<td>POS</td>
<td>65.5</td>
<td>2.39396</td>
<td>2</td>
</tr>
<tr>
<td><em>Fusobacterium nucleatum</em></td>
<td>NEG</td>
<td>27.2</td>
<td>2.1745</td>
<td>5</td>
</tr>
<tr>
<td><em>Haemophilus parainfluenzae</em></td>
<td>NEG</td>
<td>39.3</td>
<td>2.12476</td>
<td>9</td>
</tr>
<tr>
<td><em>Prevotella melaninogenica</em></td>
<td>NEG</td>
<td>35.1</td>
<td>3.16828</td>
<td>4</td>
</tr>
<tr>
<td><em>Streptococcus mitis</em></td>
<td>POS</td>
<td>40.5</td>
<td>1.83108</td>
<td>4</td>
</tr>
<tr>
<td><em>Veillonella parvula</em></td>
<td>NEG</td>
<td>38.6</td>
<td>2.16347</td>
<td>4</td>
</tr>
</tbody>
</table>
Comparing Library Preparation Kits

Nextera Flex enables uniform coverage of genomes of low GC content

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

**Nextera™ XT Workflow**
- Transposomes
- PCR Amplification
- Normalization
- Sequencing
- Analysis

**Nextera™ Flex Workflow**
- Bead-Linked Tagmentation
- PCR Amplification
- Sequencing
- Analysis

*Data courtesy of Illumina*
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)
Short-read Sequencing Platform: Ion Torrent™

16S rRNA and shotgun data on the Ion GPM Platform (ATCC® MSA-1003™)

Shotgun vs 16S rRNA assay (V1/V2) (ATCC® MSA-1000™)

Data courtesy of Dr. Pat Gillevet and Rohan Patil (Microbiome Analysis Center, GMU)
Shotgun Metagenomic Analysis: Short vs Long Reads

ATCC Microbiome Standards are technology agnostic.
The Gut Microbiome Whole Cell Standard (ATCC® MSA-1006™) was analyzed via shotgun sequencing on the MinION platform.

One hour sequencing coverage was enough to identify all organisms in the mix with sufficient genome coverage.
Long-read Sequencing Platform: PacBio®

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

One Codex Analysis 16S rRNA run 1 16S rRNA run 2 Shotgun run 1 Shotgun run 2

<table>
<thead>
<tr>
<th></th>
<th>True positives</th>
<th>Relative abundance</th>
<th>False positives</th>
<th>Overall score</th>
</tr>
</thead>
<tbody>
<tr>
<td>One Codex</td>
<td>100%</td>
<td>95%</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td>Analysis</td>
<td>100%</td>
<td>95%</td>
<td>100%</td>
<td>98%</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>100%</td>
<td>95%</td>
<td>88%</td>
<td>95%</td>
</tr>
<tr>
<td>run 1</td>
<td>100%</td>
<td>95%</td>
<td>88%</td>
<td>95%</td>
</tr>
<tr>
<td>run 2</td>
<td>100%</td>
<td>97%</td>
<td>84%</td>
<td>95%</td>
</tr>
</tbody>
</table>

Percent of reads Expected 16S Shotgun

Data courtesy of Dr. Joan Wong, PACBIO®
Comparing Bioinformatics and Databases
Data Analysis Using Different Databases

Evaluation of NGS data from microbiome standards in multiple analysis platforms and databases

Nephele vs One Codex
Short-read sequencing data from the Skin Genomic DNA Mix (ATCC® MSA-1005™)

Epi2Me vs One Codex
Long-read sequencing data from the Gut Genomic DNA Mix (ATCC® MSA-1006™)
Mycobiome Standards
Data analysis platform impacts strain identification and taxonomic resolution

### Shotgun Analysis of Genomic DNA standards (ATCC® MSA-1010™)

<table>
<thead>
<tr>
<th></th>
<th>Expected</th>
<th>Databases A1</th>
<th>Databases A2</th>
<th>Databases B1</th>
<th>Databases B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>10%</td>
<td>13.88%</td>
<td>13.99%</td>
<td>8.60</td>
<td>8.16</td>
</tr>
<tr>
<td>Candida</td>
<td>20%</td>
<td>8.17%</td>
<td>8.22%</td>
<td>12.31</td>
<td>12.61</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>10%</td>
<td>10.52%</td>
<td>10.51%</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Cutaneotrichosporon</td>
<td>10%</td>
<td>6.53%</td>
<td>6.61%</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Fusarium</td>
<td>10%</td>
<td>19.00%</td>
<td>18.94%</td>
<td>7.32</td>
<td>7.78</td>
</tr>
<tr>
<td>Malassezia</td>
<td>10%</td>
<td>5.53%</td>
<td>5.55%</td>
<td>10.86</td>
<td>11.74</td>
</tr>
<tr>
<td>Penicillium</td>
<td>10%</td>
<td>9.81%</td>
<td>9.76%</td>
<td>11.17</td>
<td>11.25</td>
</tr>
<tr>
<td>Saccharomyces</td>
<td>10%</td>
<td>6.63%</td>
<td>6.62%</td>
<td>11.48</td>
<td>11.35</td>
</tr>
<tr>
<td>Trichophyton</td>
<td>10%</td>
<td>12.01%</td>
<td>11.98%</td>
<td>0.05</td>
<td>0.29</td>
</tr>
<tr>
<td>Unclasified</td>
<td>0%</td>
<td>7.93%</td>
<td>7.82%</td>
<td>38.21</td>
<td>36.82</td>
</tr>
</tbody>
</table>
WORKFLOW:

1. Drag and drop Fastq files or export via cloud
2. Choose your ATCC product and analysis (16s and shotgun)
3. Download your reports
RESULTS ARE PROVIDED ON A SCORECARD REPORTS:

1. **True positives**: Percentage of organisms detected from the control

2. **False positives**: Detection of organisms not in the control

3. **Relative abundance**: Quantification of organisms in the control
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

New Products
- Genomic DNA and whole cell mock communities representing:
  - Virome
  - Mycobiome

Bundled with data analysis on the One Codex platform

www.atcc.org/Microbiome
Conclusion

✓ The use of standards in all areas of research is absolutely essential.
✓ Microbiome research is challenging and flush with biases
✓ The ATCC® Microbiome Standards portfolio and upcoming new products
✓ Applications of standards in microbiome research
  ✓ Extraction method, assay variability, NGS library preparation, and bioinformatics analysis
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• Tony Lialin, Loop Genomics
Thank you!
Questions?

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Cultivating collaboration to support global health

Upcoming webinars

- STR Profiling for Mouse Cell Lines: Another Tool to Combat Cell Line Misidentification | September 12, 12:00 ET

www.atcc.org/webinars

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