Viral metagenomics and the use of standards: from biology to clinical applications

Tasha M. Santiago-Rodriguez, Ph.D.
Bioinformatician R&D
Diversigen, Houston, Texas
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 cells and 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, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees
Agenda

• Diversigen overview
• What do we know so far about virome research?
• Biases in virome research
• Application of mock communities in virome research
• Potential applications of standards in the detection of ongoing and future pathogenic human viruses: considerations from SARS-CoV 2
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Diversigen as part of OraSure Technologies

MICROBIOME SERVICES
Metagenomic sequencing pipelines | Germ-free | qPCR | Project consulting | Bioinformatics

2014
YEAR FOUNDED
Operational since January 2015

LOCATION
Houston, Texas - located in the heart of the Texas Medical Center

PEOPLE
Highly talented and experienced staff, including 12 PhD scientists

ORIGIN

Centre for Metagenomics and Microbiome Research (CMMR)
Microbiome Exploration | Microbial ecology, modeling, and dissection
Therapeutic development | Policy and outreach | Education | Translation
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Metagenomic studies focus on bacteria

- Viruses are more difficult to study
- No universal gene
- DNA or RNA
- Databases are biased towards pathogens and known phages
Why are viruses important?

Viruses can be found throughout the body (gut, oral cavity, skin, bladder, blood, respiratory tract)

Viruses, particularly phages, are estimated to outnumber bacteria by a factor of 10:1 in the human gut

Viral profiles of stool can provide insight into diets (i.e., dairy product-associated phage, plant pathogens)

Viral communities vary in the context of health and disease, even in the absence of known pathogens

New studies are shedding light on the ability of the virome to influence immune response

Viral communities change in response to external factors (antibiotic-treatment, intimate contact and diet)

Virus-mediated approaches show promise in treating antimicrobial resistant infections

Vireome may provide answers to microbially-mediated phenomena where bacterial studies have come up short
Viruses are acquired from birth and modulated by diet
Viruses in samples previously thought to be sterile
Viruses in samples previously thought to be sterile

The blood DNA virome in 8,000 humans

Human viruses can predict disease risk

Prospective virome analyses in young children at increased genetic risk for type 1 diabetes

Kendra Vehik*, Kristian F. Lynch†, Matthew C. Wong‡, Xiangjun Tian†, Matthew C. Ross§, Richard A. Gibbs*, Nadim J. Ajami†, Joseph F. Petrosino†, Marian Rewers*, Jorma Toppila†,‡, Anette G. Ziegler†, Alan Jin-Xiong She†, Ake Lernmark*†, Beena Akolkar†, William A. Hagopian§, Desmond A. Schatz†, Jeffrey P. Krischer†, Heikki Hyöty†,§, Richard E. Lloyd† and the TEDDY Study Group

(1) Child was negative for EV-B (reference group)
(2) Child had a single sample positive for EV-B
(3) Child had multiple, independent, non-consecutive EV-B samples
(4) Child had consecutive EV-B with unconfirmed prolonged shedding
(5) Child had multiple samples and prolonged shedding of the same EV-B serotype

Positive for EV-B serotype (not consecutively positive and no prolonged shedding)

<table>
<thead>
<tr>
<th>Virus</th>
<th>N</th>
<th>Case (%)</th>
<th>Control (%)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>CVB1</td>
<td>2</td>
<td>39.7</td>
<td>27.2</td>
<td>0.02</td>
</tr>
<tr>
<td>CVB2</td>
<td>3</td>
<td>50.0</td>
<td>33.3</td>
<td>0.59</td>
</tr>
<tr>
<td>CVB3</td>
<td>6</td>
<td>66.7</td>
<td>41.7</td>
<td>0.04</td>
</tr>
<tr>
<td>CVB4</td>
<td>2</td>
<td>10.0</td>
<td>6.7</td>
<td>0.08</td>
</tr>
<tr>
<td>CVB5</td>
<td>10</td>
<td>39.0</td>
<td>19.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Echo0</td>
<td>6</td>
<td>50.0</td>
<td>33.3</td>
<td>0.25</td>
</tr>
<tr>
<td>Echo11</td>
<td>3</td>
<td>66.7</td>
<td>41.7</td>
<td>0.24</td>
</tr>
<tr>
<td>Echo18</td>
<td>3</td>
<td>100.0</td>
<td>66.7</td>
<td>0.08</td>
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<tr>
<td>Echo25</td>
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<td>39.0</td>
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<td>Echo30</td>
<td>4</td>
<td>50.0</td>
<td>33.3</td>
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<tr>
<td>CVB1</td>
<td>3</td>
<td>66.7</td>
<td>41.7</td>
<td>0.02</td>
</tr>
<tr>
<td>CVB3</td>
<td>1</td>
<td>66.7</td>
<td>41.7</td>
<td>0.14</td>
</tr>
<tr>
<td>CVB5</td>
<td>1</td>
<td>66.7</td>
<td>41.7</td>
<td>0.13</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td>0.07</td>
</tr>
</tbody>
</table>
Viruses can be used to treat diseases

Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease

https://doi.org/10.1038/s41586-019-1742-x
Received: 9 April 2019
Accepted: 2 October 2019
Published online: 13 November 2019


Serum levels of ALT (U/L)

C. crescentus phages
Cytolysin-positive E. faecalis phages

Hepatic triglyceride content (mg per g liver)

C. crescentus phages
Cytolysin-positive E. faecalis phages
Ongoing and future applications of viral metagenomics

- Natural history – what is present, where, and when?
- Identifying novel viral relationships with health and disease
- Identifying viral associations as risk factors for disease
- Identifying potential viral associations in unexplained illnesses
- Veterinary medicine – identifying potential novel, emerging and re-emerging pathogens
- Surveillance for zoonotic diseases
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Biases in virome research

Virome pipeline

Sample collection
- Collection method
- Storage
  - Temperature
  - Buffer
  - Freeze/thaw cycles

Processing
- Filtration/concentration
- Host nucleic acid depletion
- Nucleic acid recovery
- Amplification of nucleic acids
- Library prep

Sequencing
- Platform
- Sequencing depth

Bioinformatics
- In-silico host removal
- Assembly
- Annotation tool
Biases in virome research - Sample collection

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OMNiogene®-GUT accurately captures and stabilizes the human fecal dsDNA virome

Biases in virome research - Sample collection

OMNiogene®-GUT

- Unstabilized
  - Baseline
  - Room temperature after 14 days
  - -80°C after 14 days

- Stabilized OMNiogene.GUT
  - Baseline
  - Room temperature after 60 days
  - 50°C after 3 days
  - Six (6) freeze/thaw cycles

\[ T_5 \text{ baseline (T$_5$)} \]
\[ crAssphage \text{ baseline (T$_5$)} \]

A

- Unstabilized
  - 80°C - 14 days
  - RT - 14 days
  - 6 Freeze/thaw
  - RT - 60 days
  - 50°C - 3 days

B

- Unstabilized
  - 80°C - 14 days
  - RT - 14 days
  - 6 Freeze/thaw
  - 50°C - 3 days
  - RT - 60 days
Biases in virome research - Processing

Virome pipeline

Sample collection
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Bioinformatics
- In-silico host removal
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Sample collection → Processing → Sequencing → Bioinformatics
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>No treatment</td>
</tr>
<tr>
<td>C</td>
<td>Two consecutive centrifugation rounds at 3000×g for 10 min</td>
</tr>
<tr>
<td>0.45</td>
<td>Filtered through 0.45um membranes</td>
</tr>
<tr>
<td>0.22</td>
<td>Filtered through 0.22um membranes</td>
</tr>
<tr>
<td>I</td>
<td>Iodixanol cushion</td>
</tr>
<tr>
<td>C+0.45+I</td>
<td>Centrifugation+Filtration+Iodixanol</td>
</tr>
<tr>
<td>SISPA</td>
<td>Sequence-independent, single-primer amplification</td>
</tr>
<tr>
<td>MDA</td>
<td>Multiple displacement amplification</td>
</tr>
<tr>
<td>C+0.45+I+MDA</td>
<td>Centrifugation+Filtration+Iodixanol+MDA</td>
</tr>
</tbody>
</table>
Biases in virome research - Sequencing

Virome pipeline

Sample collection
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Bioinformatics
- In-silico host removal
- Assembly
- Annotation tool
Biases in virome research - Sequencing

Comparison of Illumina MiSeq and the Ion Torrent PGM and SS platforms for whole-genome sequencing of picornaviruses and calcicoviruses

Rachel L. Marine (Conceptualization) (Investigation) (Writing - original draft) (Writing - review and editing), Laura C. Magaha (Conceptualization) (Investigation) (Writing - original draft) (Writing - review and editing), Christina J. Castro (Formal analysis) (Data curation), Kun Zhao (Formal analysis) (Visualization), Anna M. Montmayeur (Resources) (Writing - review and editing), Alexander Schmidt (Resources) (Writing - review and editing), Marta Diez-Valcarce (Resources) (Writing - review and editing), Terry Fei Fan Ng (Conceptualization) (Resources) (Writing - review and editing), Jan Vinjé (Supervision) (Writing - review and editing), Cara C. Burns (Supervision) (Writing - review and editing), W. Allan Nix (Supervision) (Writing - review and editing), Paul A. Rota (Supervision) (Writing - review and editing) (Funding acquisition), M. Steven Oberste (Supervision) (Writing - review and editing) (Funding acquisition)

PII: S0168-0024(20)30117-8
DOI: https://doi.org/10.1016/j.jviromet.2020.113865
Reference: VIRMET 113865

To appear in: Journal of Virological Methods
Biases in virome research - Sequencing
Biases in virome research - Bioinformatics

Virome pipeline

Sample collection
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- Diversigen overview
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- **Application of mock communities in virome research**
- Potential applications of standards in the detection of ongoing and future pathogenic human viruses: considerations from SARS-CoV 2
Mock communities in microbiome studies

• The realization of including reference materials, such as mock communities, arose very recently

• In articles published in two important microbiome and microbiology journals in 2018, only 30% reported a negative control, and 10% reported the use of a positive control

• Mock communities can be used as positive controls
  • Are still not implemented on a regular basis
  • Available through institutions, laboratories and commercial facilities
  • Most are intended for microbiome analysis
  • Very few have been developed for virome analysis
# Viral mock communities (ATCC)

![Image of Viral mock communities (ATCC)](image_url)

## Table 1: Selection attributes for strains included in the ATCC® Virome Standards.

<table>
<thead>
<tr>
<th>Virus Name</th>
<th>ATCC® No.</th>
<th>Genome Type</th>
<th>Host (ATCC® No.)*</th>
<th>Virion Structure</th>
<th>Reference GenBank ID</th>
<th>Genome Size (Kbp)</th>
<th>Relevance</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human herpesvirus 5</td>
<td>VR-538™</td>
<td>ds DNA</td>
<td>MRC-5 (CCL-171™)</td>
<td>Enveloped</td>
<td>X17403.1</td>
<td>229.4</td>
<td>Ubiquitous infection in adult humans, and significant pathogen within immunocompromised populations¹⁵</td>
<td></td>
</tr>
<tr>
<td>Human mastadenovirus F</td>
<td>VR-931™</td>
<td>ds DNA</td>
<td>HEK-293 (CRL-1573™)</td>
<td>Unenveloped</td>
<td>NC_001454.1</td>
<td>34.2</td>
<td>Human gastrointestinal infection and severe infection in children and immunocompromised patients¹¹</td>
<td></td>
</tr>
<tr>
<td>Influenza B virus B/Florida/4/2006</td>
<td>VR-1804™</td>
<td>ss (-) RNA (8 segments)</td>
<td>SPF embryonated chicken eggs</td>
<td>Enveloped</td>
<td>CY0183651-CY018372.1</td>
<td>14.2</td>
<td>Causes worldwide human epidemics of influenza with high rates of illness and death¹⁲</td>
<td></td>
</tr>
<tr>
<td>Zika virus</td>
<td>VR-1838™</td>
<td>ss (+) RNA</td>
<td>Vero (CCL-81™)</td>
<td>Enveloped</td>
<td>KX830960.1</td>
<td>10.8</td>
<td>Mosquito-borne viral infection that can cause congenital microcephaly in fetuses and infants¹³</td>
<td></td>
</tr>
<tr>
<td>Human respiratory syncytial virus</td>
<td>VR-3540™</td>
<td>ss (-) RNA</td>
<td>HEP-2 (CCL-23™)</td>
<td>Enveloped</td>
<td>KT992094.1</td>
<td>15.2</td>
<td>Causes severe respiratory tract infections in humans¹⁴</td>
<td></td>
</tr>
<tr>
<td>Reovirus 3</td>
<td>VR-824™</td>
<td>ds RNA (10 segments)</td>
<td>LLC-MK2 Derivative (CCL-7.1™)</td>
<td>Capsids</td>
<td>HM159613.1-HM159622.1</td>
<td>23.6</td>
<td>Human respiratory and gastrointestinal infection; oncolytic virus⁵,¹⁶</td>
<td></td>
</tr>
</tbody>
</table>

## Diagram

- **DNA Viruses**
  - **Herpes**
  - **Adeno**
- **RNA Viruses**
  - **Zika**
  - **Influenza B & RSV**
  - **Reo**
  - **Capsids**
  - **Unenveloped**
  - **Enveloped**

1. MRC-5: Human embryonic lung fibroblast cell line.
2. HEK-293: Human embryonic kidney cell line.
3. SPF: Specific pathogen-free.
4. CCL: American Type Culture Collection (ATCC) cell line.

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Beta-testing ATCC viral mock community (nucleic acids)

**Sample collection**
- Nucleic acids (ATCC-MSA 1008)

**Sequencing**
- Viral RNA converted to cDNA
- DNA and cDNA are pooled
- Pooled library is amplified with a barcoded semi-random primer (virome library only)
- Library prep (Nextera DNA Flex)
- Shotgun sequencing (NovaSeq 2X150bp)

**Data Analysis**
- Adapter trimming
- Host read alignment and filtering
- Bioinformatic analysis
Beta-testing ATCC viral mock community (whole virus)

Sample collection
- Whole virus (ATCC-MSA 2008)
- Nucleic acid extraction (MagAttract)

Sequencing
- Viral RNA converted to cDNA
- DNA and cDNA are pooled
- Pooled library is amplified with a barcoded semi-random primer (virome library only)
- Library prep (Nextera DNA Flex)
- Shotgun sequencing (NovaSeq 2X150bp)

Data Analysis
- Adapter trimming
- Host read alignment and filtering
- Bioinformatic analysis
ATCC viral mock communities - MetaPhlAn2

MetaPhlAn 2.0

MetaPhlAn (Metagenomic Phylogenetic Analysis) is a computational tool for profiling the composition of microbial communities from metagenomic shotgun sequencing data. MetaPhlAn relies on unique clade-specific marker genes identified from ~17,000 reference genomes (~73,000 bacterial and archaeal, ~3,500 viral, and ~110 eukaryotic), allowing:

- up to 25,000 reads-per-second (on one CPU) analysis speed (orders of magnitude faster compared to existing methods);
- unambiguous taxonomic assignments as the MetaPhlAn markers are clade-specific;
- accurate estimation of organismal relative abundance (in terms of number of cells rather than fraction of reads);
- species-level resolution for bacteria, archaea, eukaryotes and viruses;
- extensive validation of the profiling accuracy on several synthetic datasets and on thousands of real metagenomes.

<table>
<thead>
<tr>
<th></th>
<th>Nucleic acids (MSA1008)</th>
<th>Whole viruses (MSA2008)</th>
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<tbody>
<tr>
<td></td>
<td>Nextera Only</td>
<td>Semi-random primers</td>
</tr>
<tr>
<td>False positives</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>False negatives</td>
<td>1</td>
<td>2</td>
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</tbody>
</table>

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ATCC viral mock communities - Kraken2

Improved metagenomic analysis with Kraken 2

Derrick E. Wood, Jennifer Lu & Ben Langmead

Genome Biology 20, Article number: 257 (2019) | Cite this article

6610 Accesses | 12 Citations | 75 Altmetric | Metrics

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<td></td>
<td>Nextera Only</td>
<td>Semi-random primers</td>
</tr>
<tr>
<td>False positives</td>
<td>356</td>
<td>258</td>
</tr>
<tr>
<td>False negatives</td>
<td>1</td>
<td>1</td>
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</tbody>
</table>
ATCC viral mock communities - VirMap

Maximal viral information recovery from sequence data using VirMAP

Nadim J Ajami, Matthew C. Wong, Matthew C. Ross, Richard E. Lloyd & Joseph F. Petrosino

*Nature Communications* 9, Article number: 3205 (2018) | Cite this article

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<td>Semi-random primers</td>
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<tr>
<td>False positives</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>False negatives</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
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• Potential applications of standards in the detection of ongoing and future pathogenic human viruses: considerations from SARS-CoV 2
Viral metagenomics as a surveillance tool of SARS-CoV 2

Capabilities in SARS-CoV 2 detection reside in building a custom database with the virus sequence, as well as in the annotation tool used.

To confirm the ability to detect SARS-COV-2, we conducted multiple tests:
- Profiling known positive samples (NCBI SRA)*
- Profiling suspected negative samples (NCBI SRA)

The availability of sequence libraries of varying depth allowed us to evaluate the effects of sequencing depth on virus detection and genome recovery.

https://www.cdc.gov/media/subtopic/images.htm

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3486 Accesses | 8 Citations | 29 Altmetric | Metrics
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### Profiling SARS-CoV2 positive and negative samples

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>SRA RUN IDENTIFIER(S)</th>
<th>EXPECTED SARS-COV-2 STATUS</th>
<th>SARS-COV-2 DETECTED</th>
<th>DNA VIRUSES DETECTED</th>
<th>OTHER RNA VIRUSES DETECTED</th>
</tr>
</thead>
<tbody>
<tr>
<td>WIV02</td>
<td>SRR11092058, SRR11092063</td>
<td>+</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>WIV04</td>
<td>SRR11092057, SRR11092062</td>
<td>+</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>WIV05</td>
<td>SRR11092061</td>
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<td>✓</td>
<td>✓</td>
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<td>COPD18</td>
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<td>−</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>COPD25</td>
<td>SRR5677642</td>
<td>−</td>
<td>−</td>
<td>✓</td>
<td>−</td>
</tr>
</tbody>
</table>
Sequencing depth affects genome coverage of SARS-CoV-2

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- Profiling suspected negative samples (NCBI SRA)

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Sequencing depth: 1.04 Gb
% of SARS-COV-2 genome recovered: 83%
% Hominid reads in library (NCBI): 56%
Average depth of coverage: 3.35x

Sequencing depth: 8.90 Gb
% of SARS-COV-2 genome recovered: 99.89%
% Hominid reads in library (NCBI): 60%
Average depth of coverage: 11.79x

Sequencing depth: 18.4 Gb
% of SARS-COV-2 genome recovered: 100%
% Hominid reads in library (NCBI): 67%
Average depth of coverage: 105x

The availability of sequence libraries of varying depth allowed us to evaluate the effects of sequencing depth on virus detection and genome recovery.
Credible solutions for critical public health emergencies

The outbreak of severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in December 2019 has put the health and safety of the global community at risk. The virus has already spread beyond the borders of China, with numerous countries reporting confirmed cases of coronavirus disease (COVID-19). With the virus continuing to spread and the number of confirmed cases and deaths rising, the World Health Organization has declared the outbreak a public health emergency of international concern.

As in previous public health emergencies such as Zika, SARS, MERS, and the H1N1 2009 pandemic, ATCC stands ready to partner with the dedicated scientists working toward preventing and containing this devastating outbreak. Only through the combined efforts of the global scientific community can we discover the tools and treatments needed to keep humankind healthy and safe. View our resources below to discover how we can support your work toward the development of novel diagnostics and effective therapeutics.
Application of viral standards moving forward

- Expansion of viral databases and surveillance activities
- Continued improvements related to collection, stabilization, extraction
- New reagents and approaches
- Layering virome information onto metagenomic studies to provide new biological and clinical insights
- Viral standards for viral metagenomics and clinical applications will continue to evolve
QUESTIONS?
ATCC Microbiome Research Solutions

Human Microbiome Research
- Virome Standards
- Site-specific Standards
- Mycobiome Standards
- Pathogen Detection Standards

Assay Standardization
- Mock Microbial Communities
- Spike-in Standards

Environmental Microbiome Research
- ABRF-MGRG Standards

www.atcc.org/microbiome