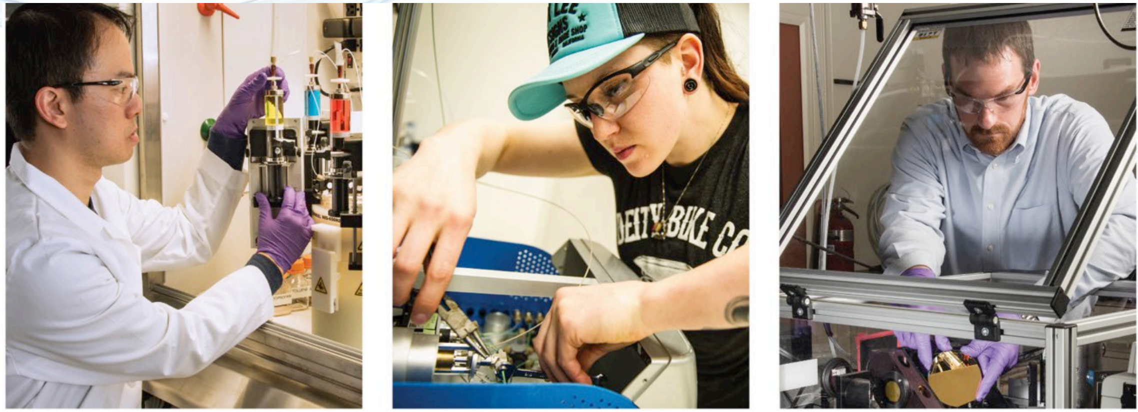
# Measurement Assurance for Metagenomic Innovation



**Stephanie Servetas, PhD**

**Group Leader, Complex Microbial Systems Group**

**Biosystems and Biomaterials Division**

**MML**

MML Microbial Metrology Mission Statement

“To develop advanced measurements to foster the utilization of microbes to support One health, precision medicine, and advanced manufacturing”

NIST
National Institute of Standards and Technology
U.S. Department of Commerce

MATERIAL
MEASUREMENT
LABORATORY

# Disclaimers

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# Measurement Assurance

**BBD Mission:** To promote U.S. biosciences and biotechnology innovation and industrial competitiveness by advancing measurement science, standards, and technology in ways that enhance economic security and improve our quality of life

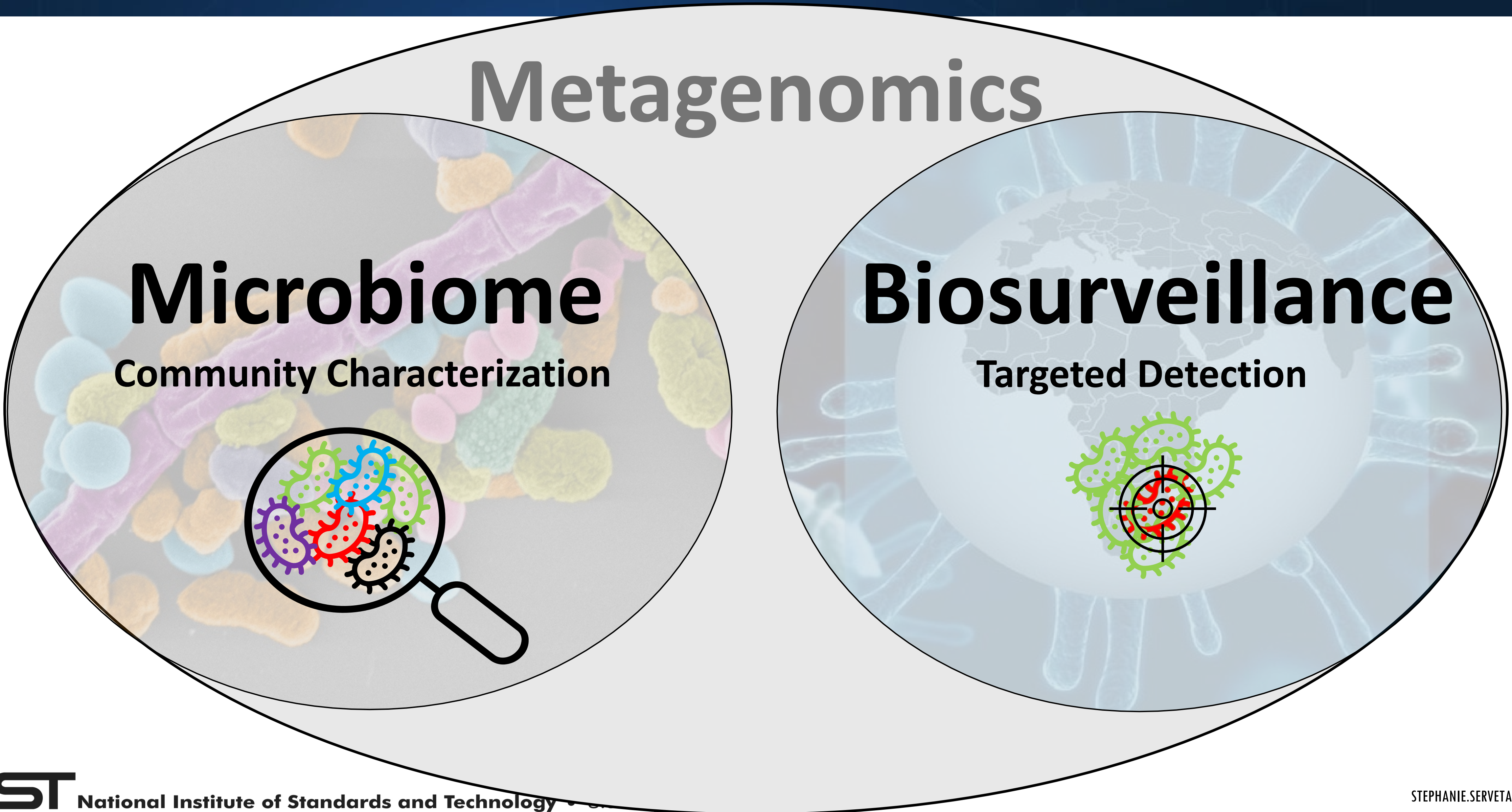
**New methods/technologies require validation**

❯

**Validation requires reference materials, benchmarking studies, validation studies, measurement innovation, etc.**

**Measurement Science & Biometrology**

# NIST Microbial Metrology



Metagenomics

**Microbiome**

Community Characterization

**Biosurveillance**

Targeted Detection

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# Metagenomics Applications Microbial Testing

Metagenomics is increasing being turned to as a technique for microbial detection in numerous applications, specifically  those that require microbial testing prior to release

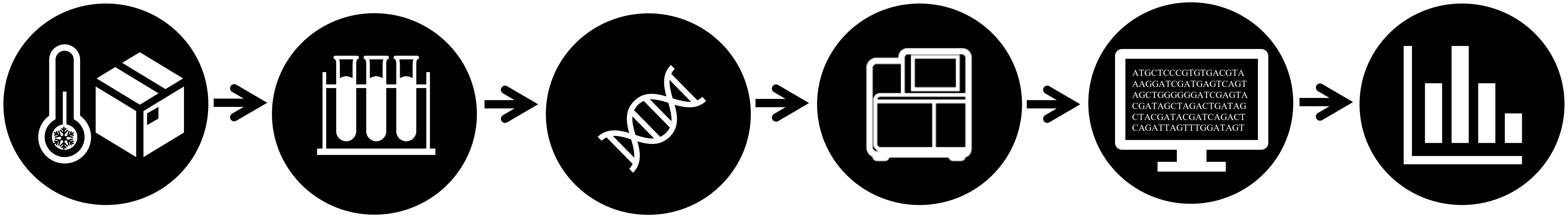
Food safety

Gene and Cell Therapy Manufacturing

Medical/Health care products

Water reuse/reclamation

# Understanding the Metagenomics Workflow



sample collection & storage → DNA/RNA extraction → library preparation → sequencing → read processing → statistical analysis & reporting

- This is a general workflow, at a bare minimum these are the steps that must occur to get from a sample to an actionable results
- Each of the steps, and consideration for additional steps (e.g. a PMA treatment), needs to be considered in context of the sample type, environment, and end goal (e.g. what is type of data do you need to make a decision)
- Standards enable validation of a workflow and confidence in the results – when included correctly

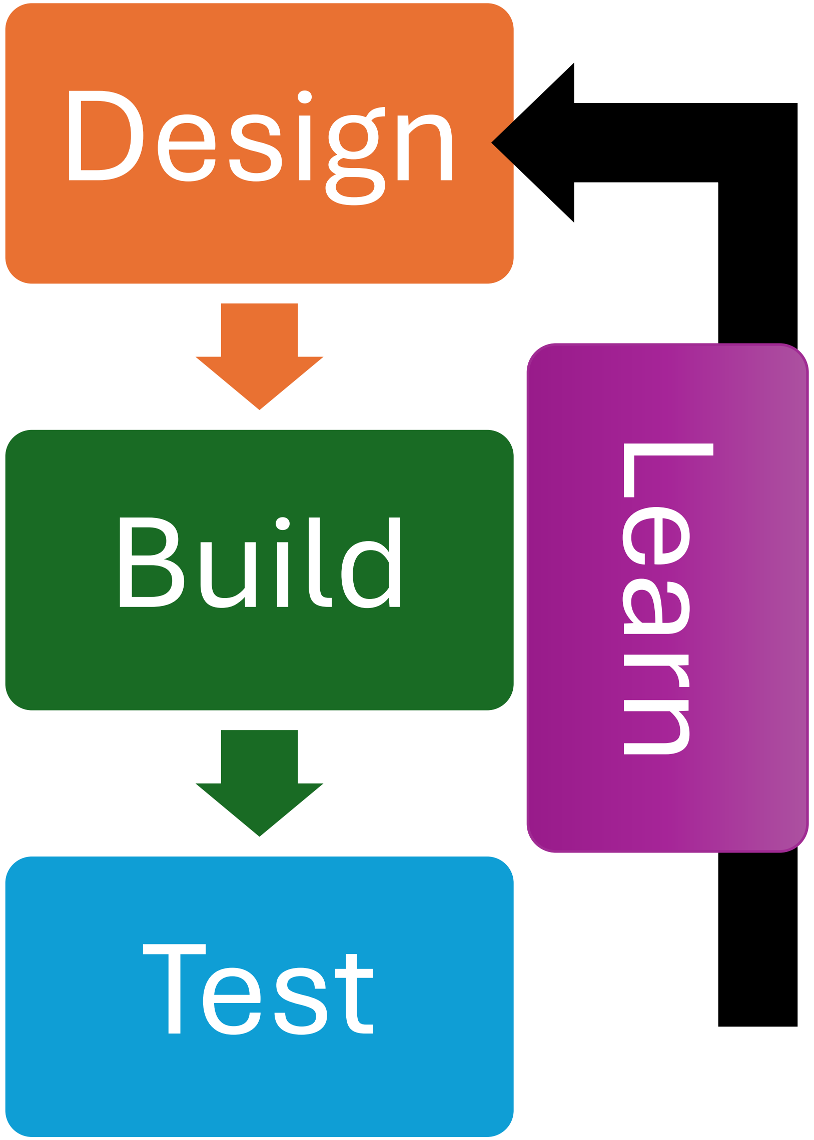
# Considerations for workflow adoption

What question are you asking and what data do you need to answer it?

Outline/Map a workflow

Identify controls for each step of the workflow

Develop a set of minimum guidelines [e.g. GSC MIxS guidelines, STREAMS]



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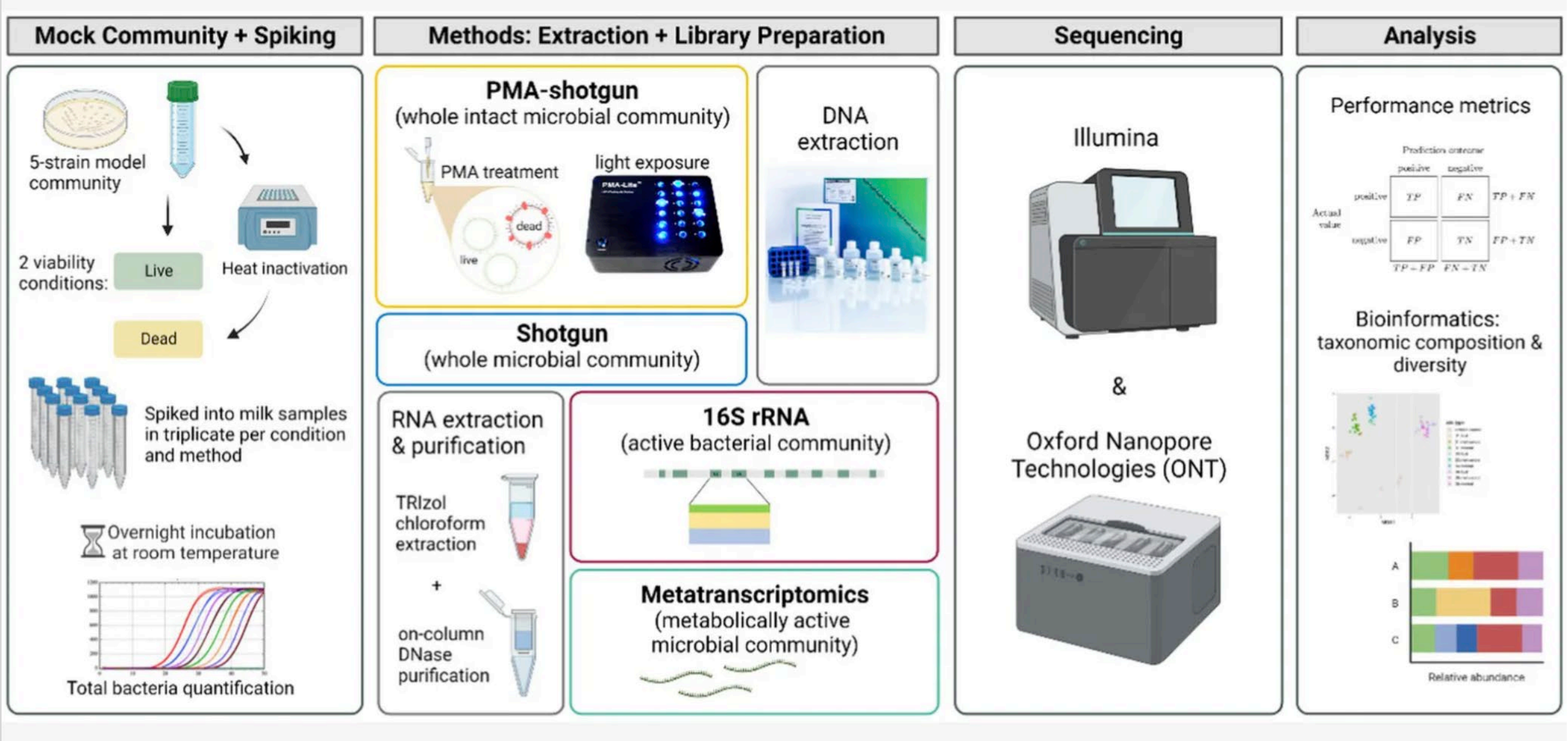
# Workflow Examples from other fields – food science

Development of sequencing-based methodologies to distinguish viable from non-viable cells in a bovine milk matrix: A pilot study

Min Yap [1,2] Orla O'Sullivan [1,3]
Paul D. Cotter [1,3*]

1. Teagasc Food Research Centre, Moorepark, Fe
2. School of Microbiology, University College Co



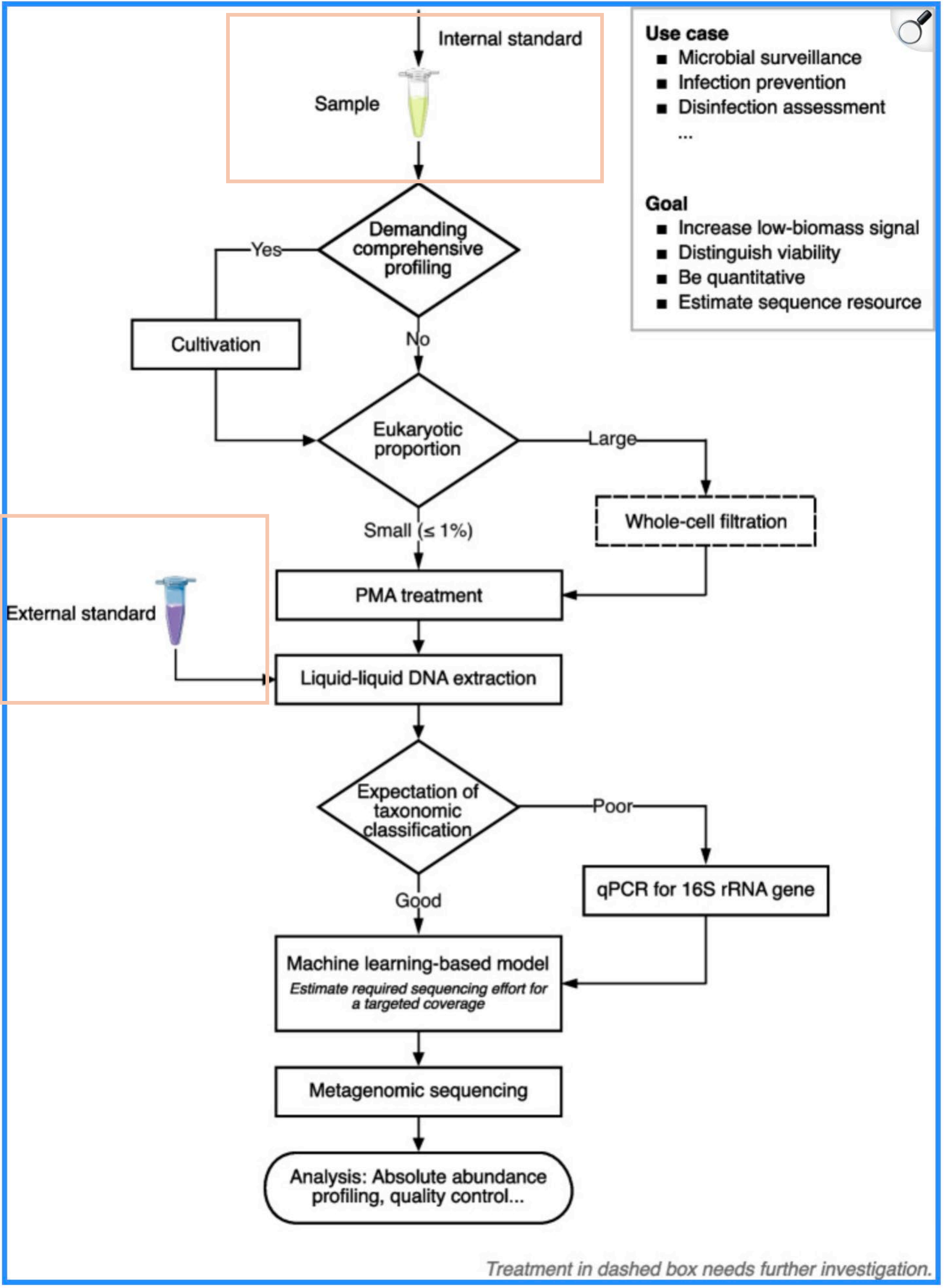
# Workflow Examples from other fields – health care

Microbiome. 2022 Dec 2;10:206. doi: 10.1186/s40168-022-01412-x

**An improved workflow for accurate and robust healthcare environmental surveillance using metagenomics**

Jiaxian Shen [1,✉], Alexander G McFarland [1], Ryan A Blaustein [2], Laura J Rose [3], K Allison Perry-Dow [3], Anahid A Moghadam [1], Mary K Hayden [4], Vincent B Young [5], Erica M Hartmann [1]

"A workflow for metagenomics-based environmental surveillance that is appropriate for **low-biomass** samples, distinguishes **viability**, is quantitative, and estimates sequence resources"



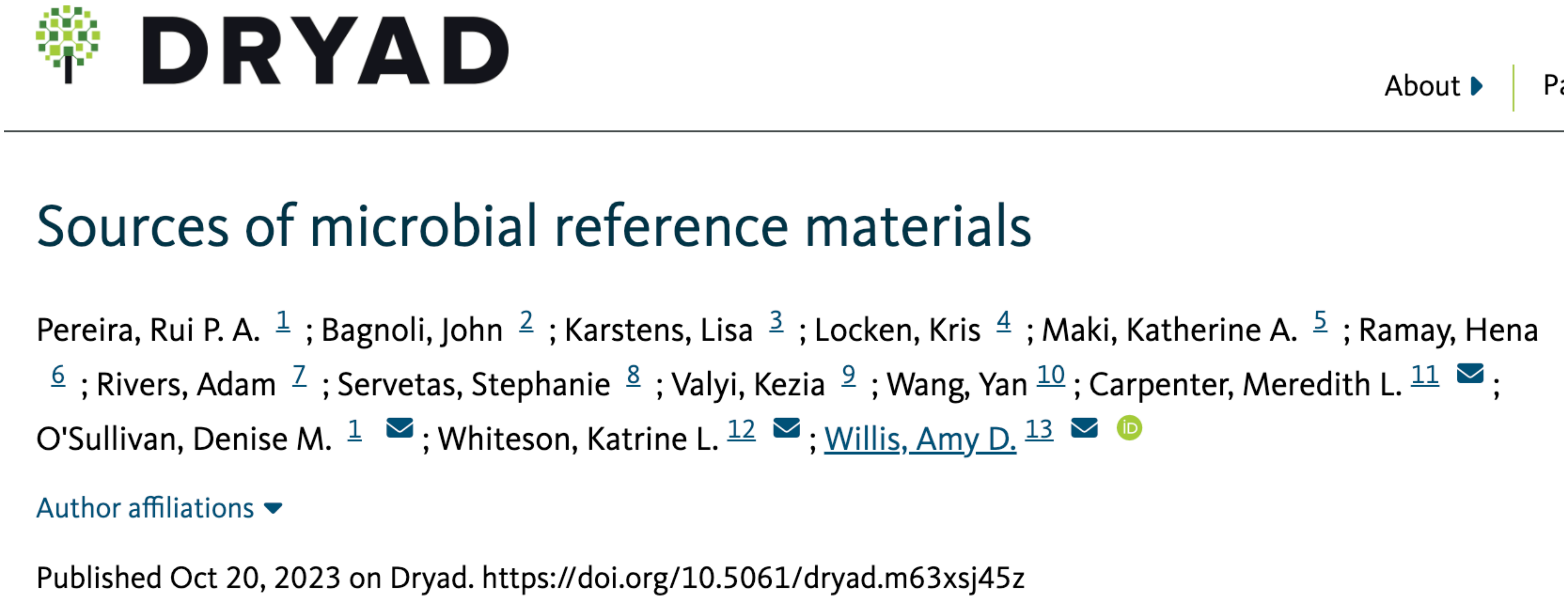
# Types of Controls

| Internal controls | Parallel process controls | Positive controls | Negative controls |
|---|---|---|---|
| - AKA Spike-ins<br>- Whole organisms or nucleic acids<br>- Can be added to a sample at different steps and assessed at the end | | | |

*NOTE: genetically labeled sequences and/or synthetic positive controls (engineered fragments that contain unique intermediary sequences) that can be easily distinguished from a true target based on sequencing should be implemented

# Positive control considerations

**There are many sources for positive controls**



## Sources of microbial reference materials

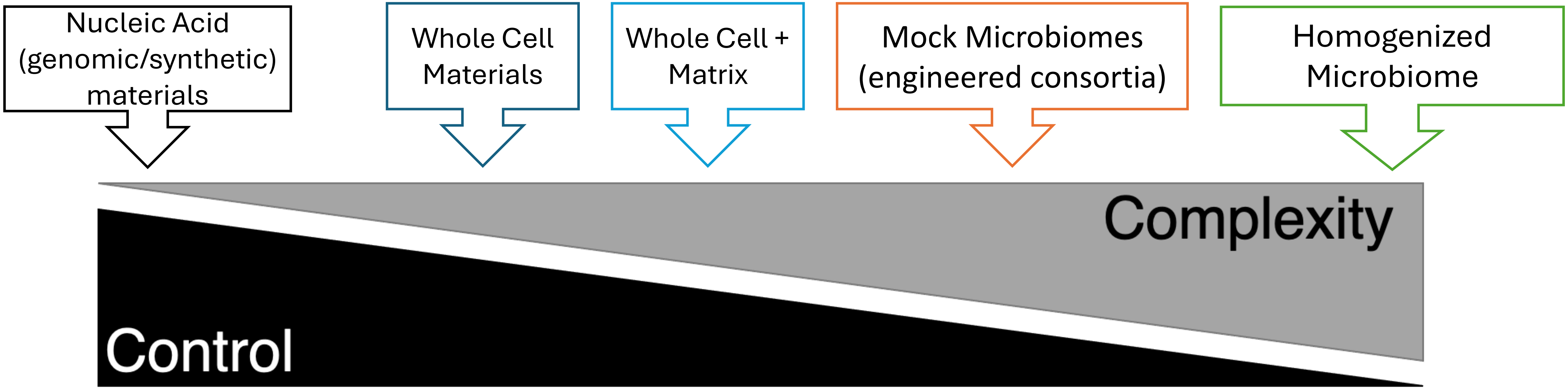
Pereira, Rui P. A. [1] ; Bagnoli, John [2] ; Karstens, Lisa [3] ; Locken, Kris [4] ; Maki, Katherine A. [5] ; Ramay, Hena [6] ; Rivers, Adam [7] ; Servetas, Stephanie [8] ; Valyi, Kezia [9] ; Wang, Yan [10] ; Carpenter, Meredith L. [11] ; O'Sullivan, Denise M. [1] ; Whiteson, Katrine L. [12] ; Willis, Amy D. [13]

Author affiliations

Published Oct 20, 2023 on Dryad. https://doi.org/10.5061/dryad.m63xsj45z

# Positive control considerations

**Materials come in several flavors – depending on the use they will provide different information**



Nucleic Acid (genomic/synthetic) materials

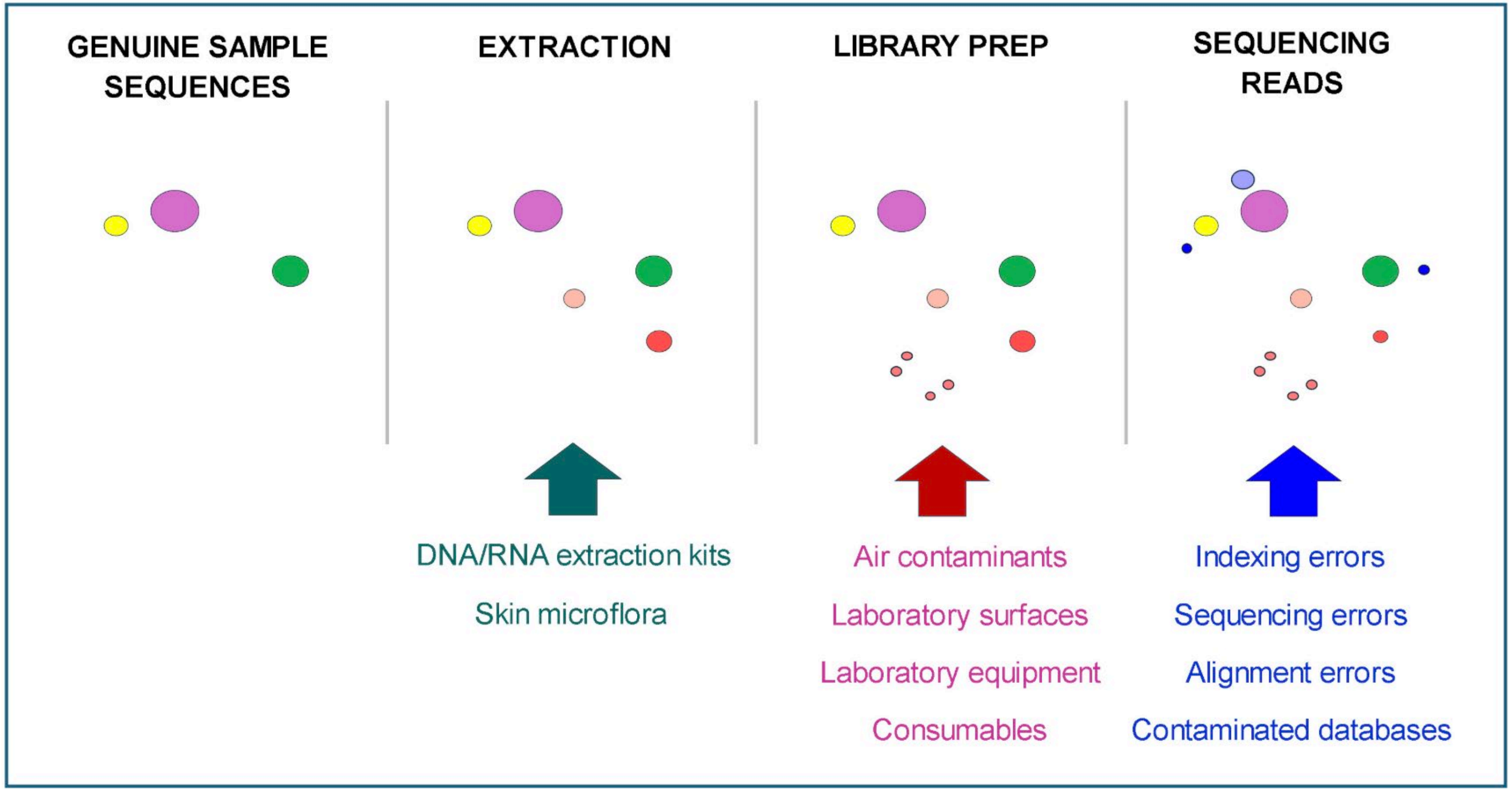
Whole Cell Materials

Whole Cell + Matrix

Mock Microbiomes (engineered consortia)

Homogenized Microbiome

# Negative control considerations



GENUINE SAMPLE SEQUENCES

EXTRACTION

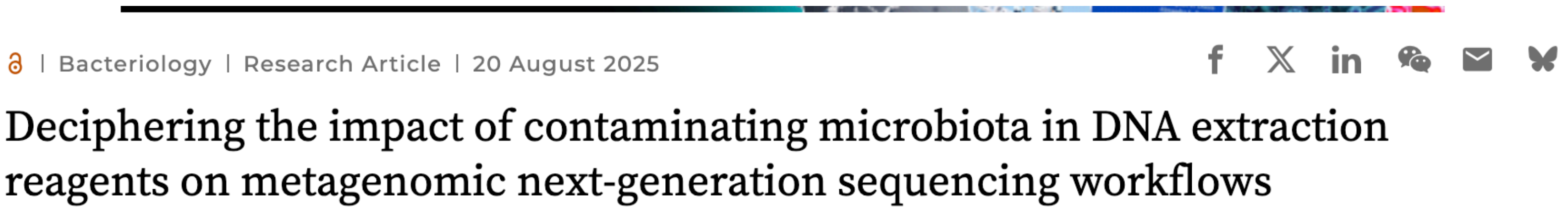
LIBRARY PREP

SEQUENCING READS

DNA/RNA extraction kits
Skin microflora

Air contaminants
Laboratory surfaces
Laboratory equipment
Consumables

Indexing errors
Sequencing errors
Alignment errors
Contaminated databases



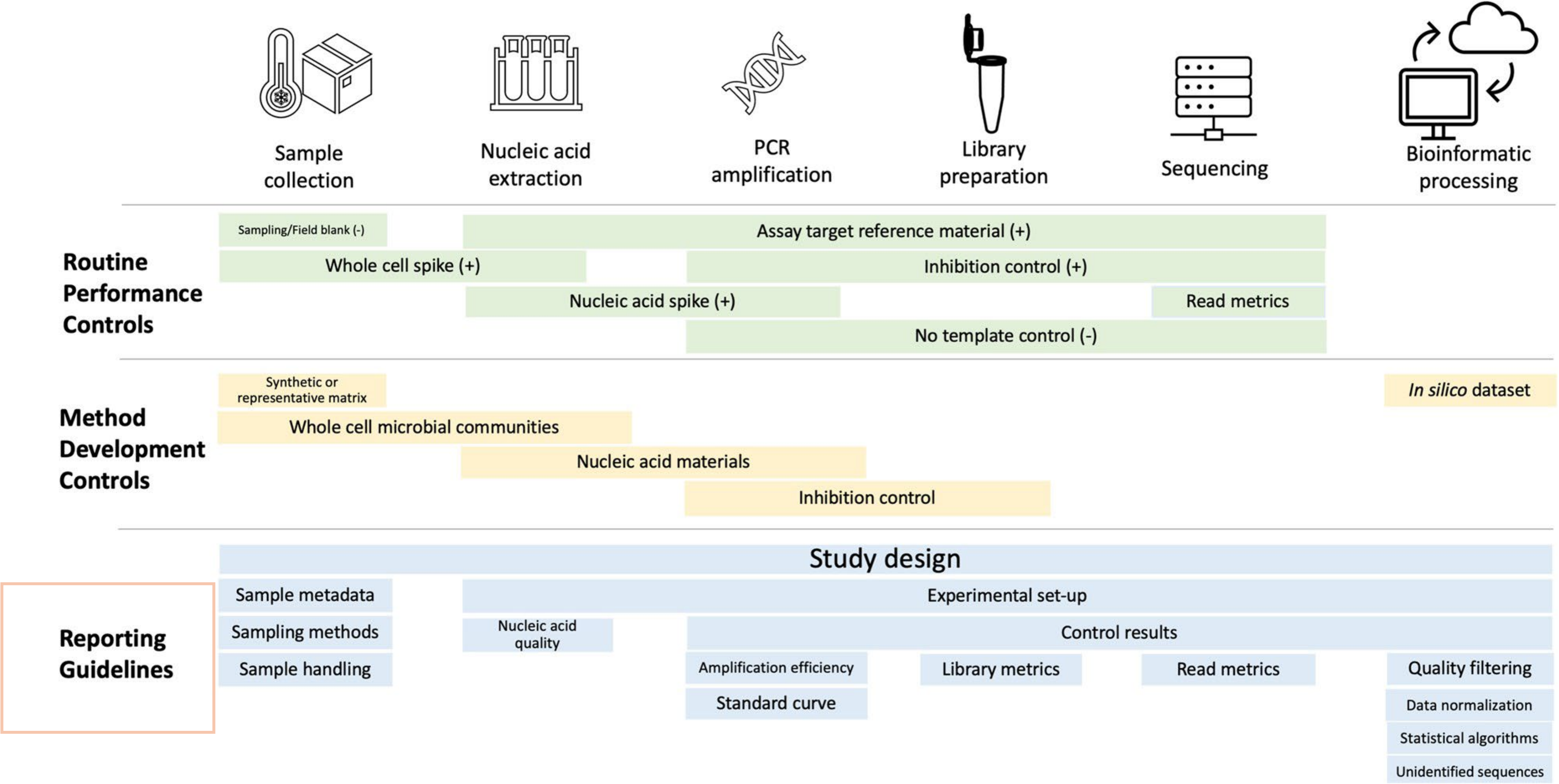
| Bacteriology | Research Article | 20 August 2025

Deciphering the impact of contaminating microbiota in DNA extraction reagents on metagenomic next-generation sequencing workflows

# Negative control considerations

- Each step in a workflow should have a corollary negative control to serve to identify contamination at that stage of the workflow
  - ➢ will help capture kitome
- Should enable the identification of “background noise” or the natural variation present in negative samples
  - ➢ consider adding matched volumes to positive controls
- When chosen and incorporated correctly can enable setting cutoff values for positive confirmation, identify potential contamination and/or false positives in a workflow.

# Example: Controls for Actionable Calls in Biodefense Applications



Sample collection | Nucleic acid extraction | PCR amplification | Library preparation | Sequencing | Bioinformatic processing

**Routine Performance Controls**

- Sampling/Field blank (-)
- Assay target reference material (+)
- Whole cell spike (+)
- Inhibition control (+)
- Nucleic acid spike (+)
- Read metrics
- No template control (-)

**Method Development Controls**

- Synthetic or representative matrix
- *In silico* dataset
- Whole cell microbial communities
- Nucleic acid materials
- Inhibition control

**Reporting Guidelines**

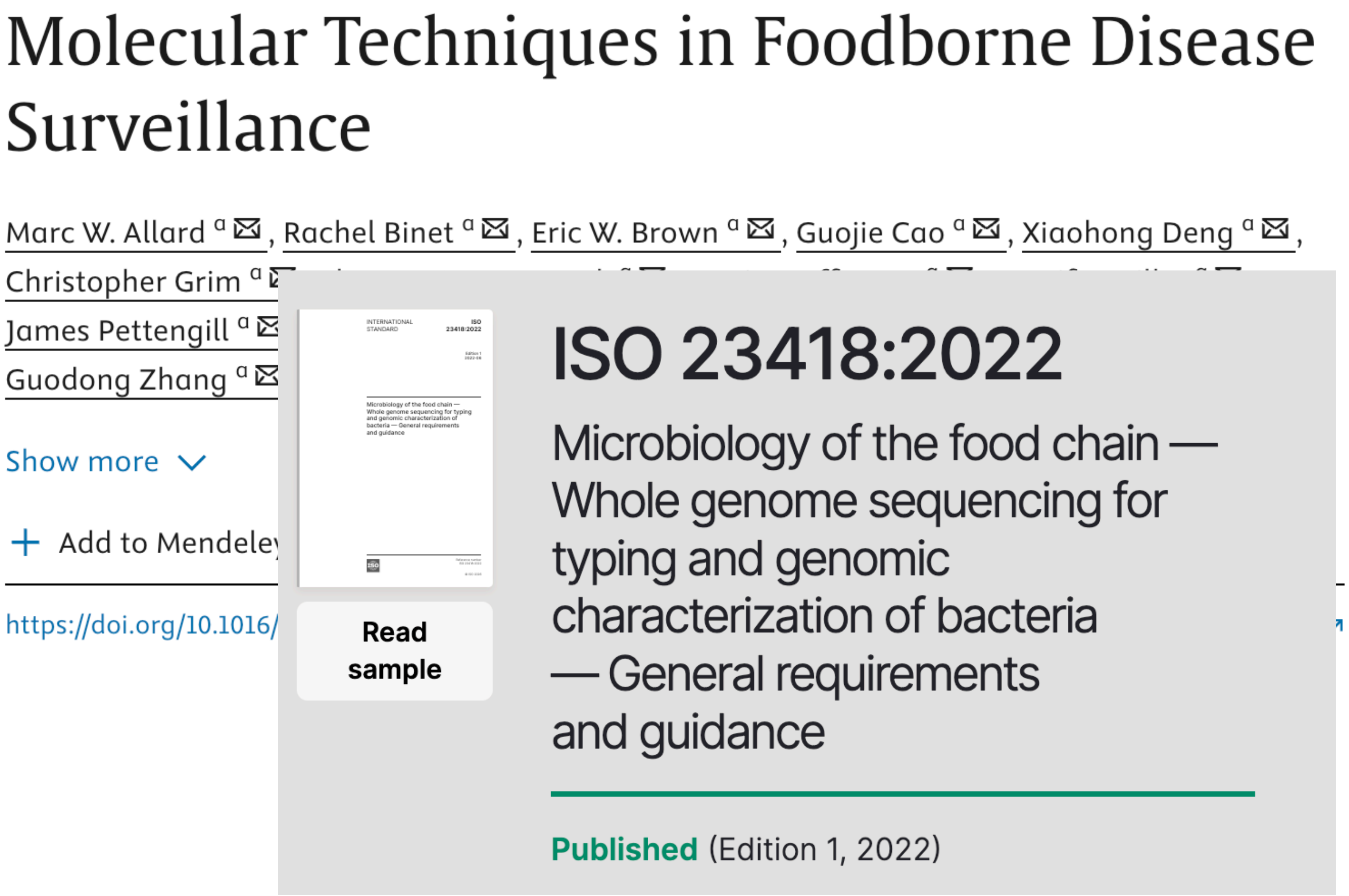
- Study design
- Sample metadata
- Experimental set-up
- Sampling methods
- Nucleic acid quality
- Control results
- Sample handling
- Amplification efficiency
- Library metrics
- Read metrics
- Quality filtering
- Standard curve
- Data normalization
- Statistical algorithms
- Unidentified sequences

**Figure 2.** Summary of controls and reporting guidelines for an AS workflow.

*J AOAC Int*, Volume 106, Issue 5, September-October 2023, Pages 1424–1430, https://doi.org/10.1093/jaoacint/qsad047

# Guidelines for Metagenomics

Food safety



## Molecular Techniques in Foodborne Disease Surveillance

Marc W. Allard [a] ✉, Rachel Binet [a] ✉, Eric W. Brown [a] ✉, Guojie Cao [a] ✉, Xiaohong Deng [a] ✉,
Christopher Grim [a] ✉
James Pettengill [a] ✉
Guodong Zhang [a] ✉

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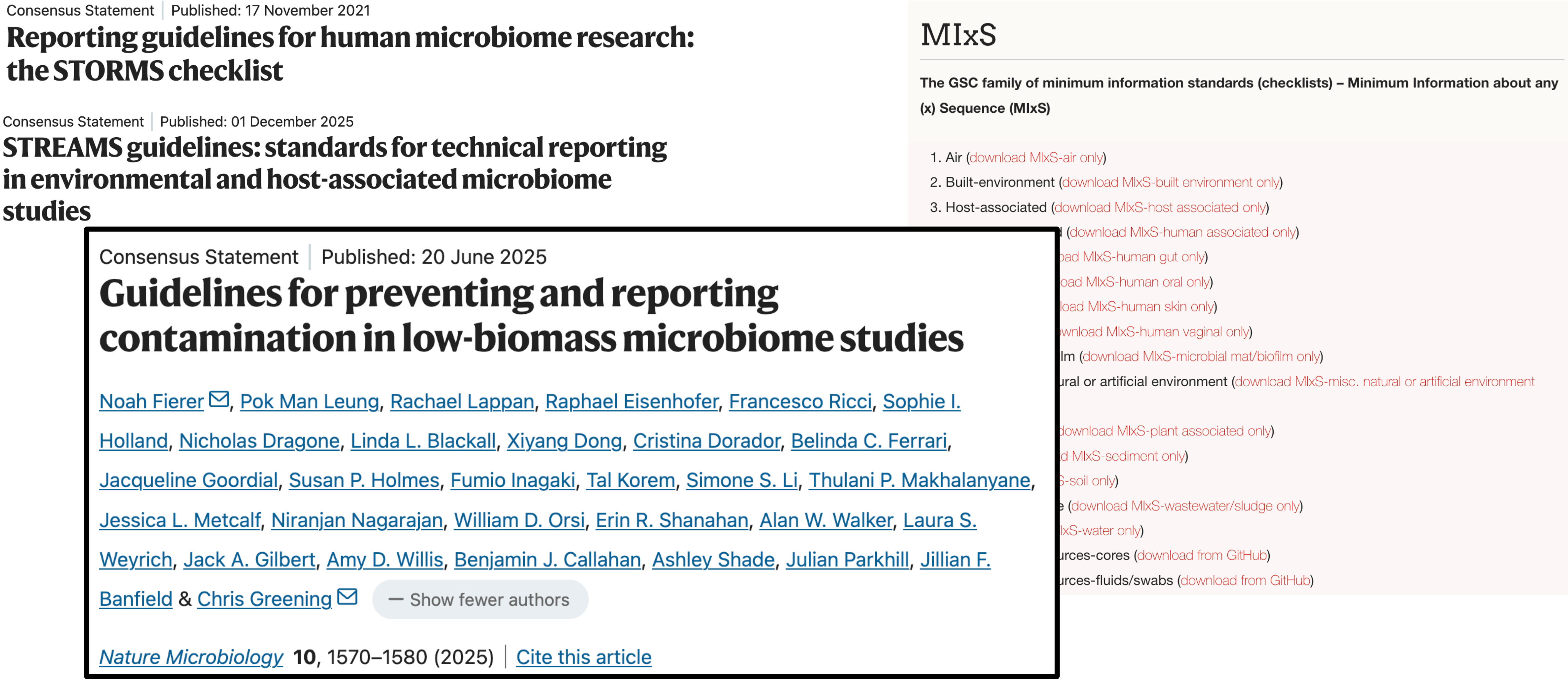
https://doi.org/10.1016/

## ISO 23418:2022

Microbiology of the food chain — Whole genome sequencing for typing and genomic characterization of bacteria — General requirements and guidance

**Published** (Edition 1, 2022)

# Guidelines for Metagenomics



Consensus Statement | Published: 17 November 2021

**Reporting guidelines for human microbiome research: the STORMS checklist**

Consensus Statement | Published: 01 December 2025

**STREAMS guidelines: standards for technical reporting in environmental and host-associated microbiome studies**

Consensus Statement | Published: 20 June 2025

**Guidelines for preventing and reporting contamination in low-biomass microbiome studies**

Noah Fierer, Pok Man Leung, Rachael Lappan, Raphael Eisenhofer, Francesco Ricci, Sophie I. Holland, Nicholas Dragone, Linda L. Blackall, Xiyang Dong, Cristina Dorador, Belinda C. Ferrari, Jacqueline Goordial, Susan P. Holmes, Fumio Inagaki, Tal Korem, Simone S. Li, Thulani P. Makhalanyane, Jessica L. Metcalf, Niranjan Nagarajan, William D. Orsi, Erin R. Shanahan, Alan W. Walker, Laura S. Weyrich, Jack A. Gilbert, Amy D. Willis, Benjamin J. Callahan, Ashley Shade, Julian Parkhill, Jillian F. Banfield & Chris Greening

*Nature Microbiology* **10**, 1570–1580 (2025) | Cite this article

MIxS

**The GSC family of minimum information standards (checklists) – Minimum Information about any (x) Sequence (MIxS)**

1. Air (download MIxS-air only)
2. Built-environment (download MIxS-built environment only)
3. Host-associated (download MIxS-host associated only)

d (download MIxS-human associated only)
oad MIxS-human gut only)
oad MIxS-human oral only)
load MIxS-human skin only)
wnload MIxS-human vaginal only)
lm (download MIxS-microbial mat/biofilm only)
ural or artificial environment (download MIxS-misc. natural or artificial environment
download MIxS-plant associated only)
d MIxS-sediment only)
S-soil only)
e (download MIxS-wastewater/sludge only)
IxS-water only)
urces-cores (download from GitHub)
urces-fluids/swabs (download from GitHub)

# Considerations for workflow adoption

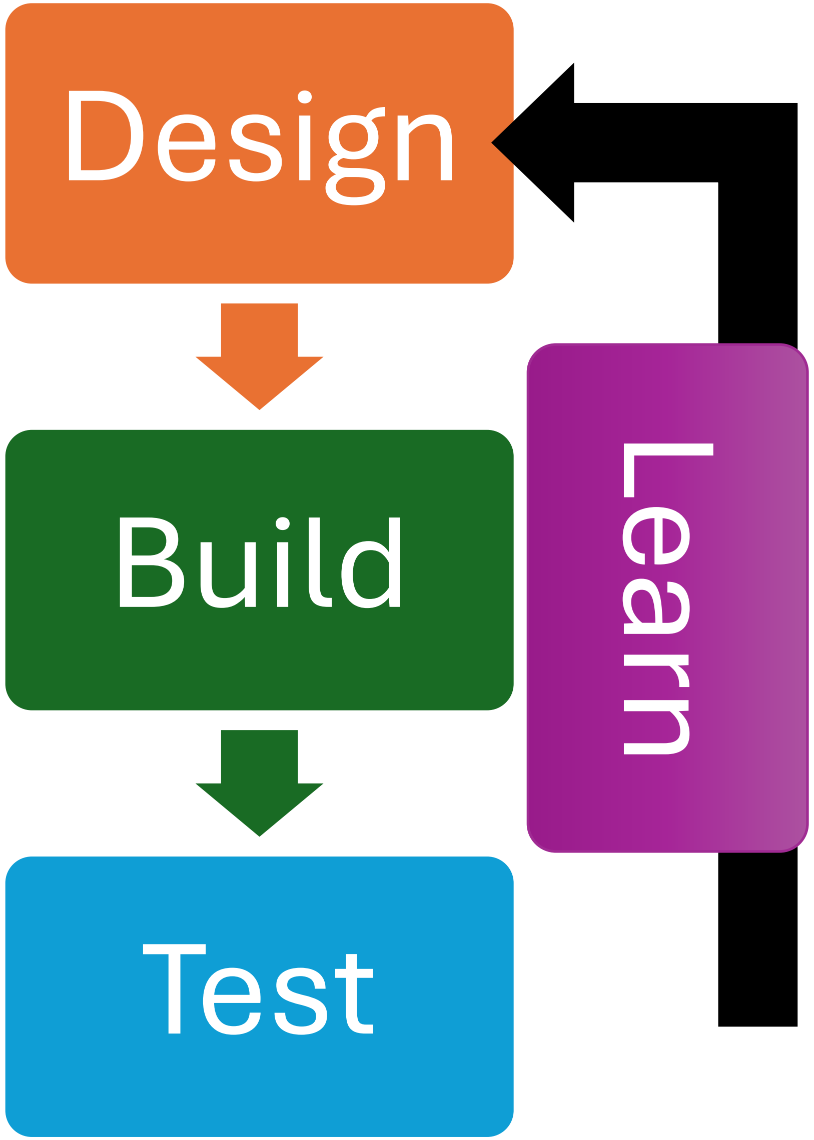
What question are you asking and what data do you need to answer it?

**Design (and draw out) workflow for PP use case**

**Identify controls specific to PP for each step of the workflow**

**Develop or adopt a set of minimum guidelines [e.g. low bio-mass guidelines published in 2025]**

**What is NIST role?**



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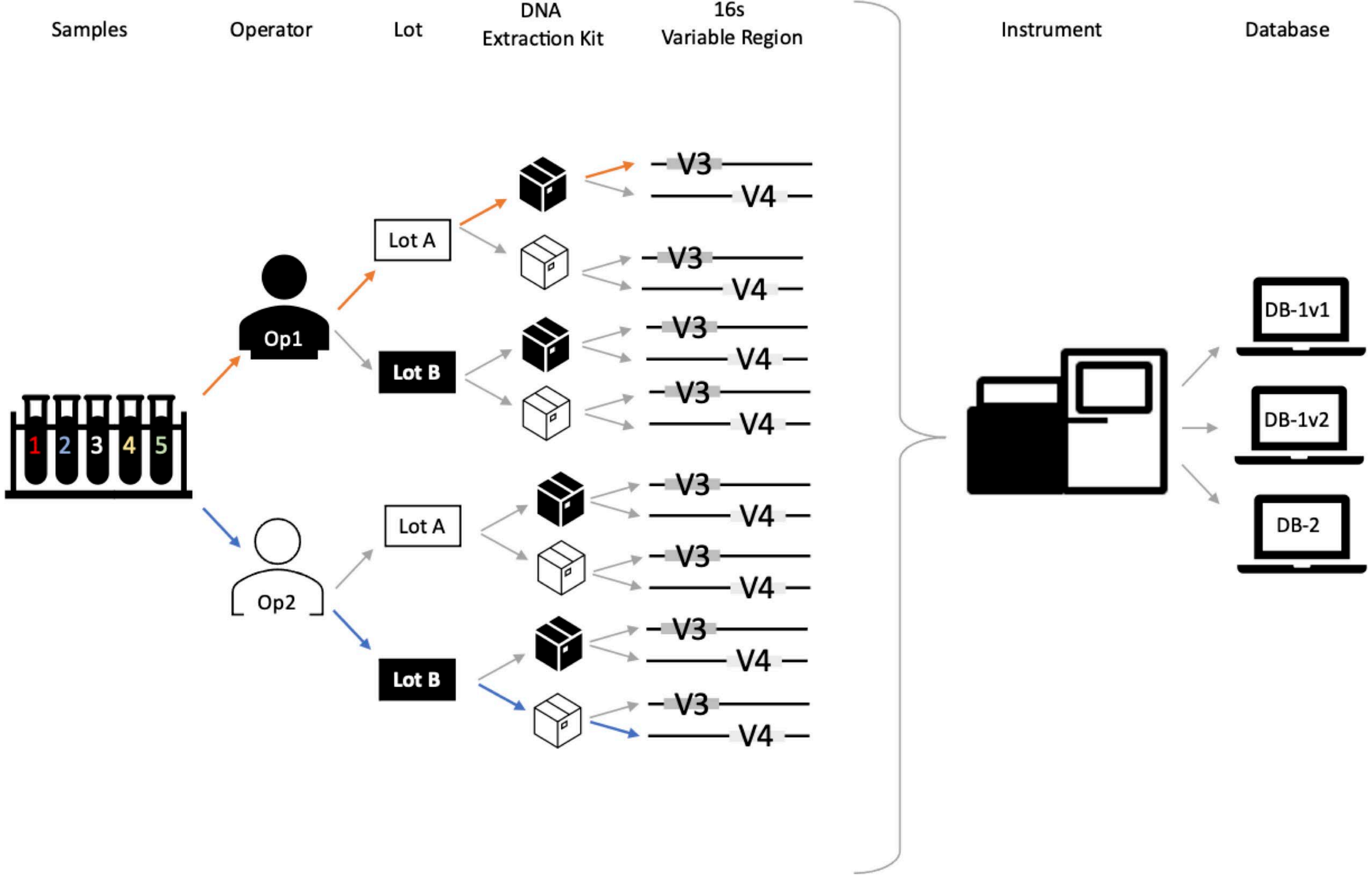
# Experimental Design

Open Peer Review | Human Microbiome | Research Article | 14 January 2025

## A sensitivity analysis of methodological variables associated with microbiome measurements

**Authors:** Samuel P. Forry, Stephanie L. Servetas, Jennifer N. Dootz, Monique E. Hunter, Jason G. Kralj, James J. Filliben, Scott A. Jackson | AUTHORS INFO & AFFILIATIONS

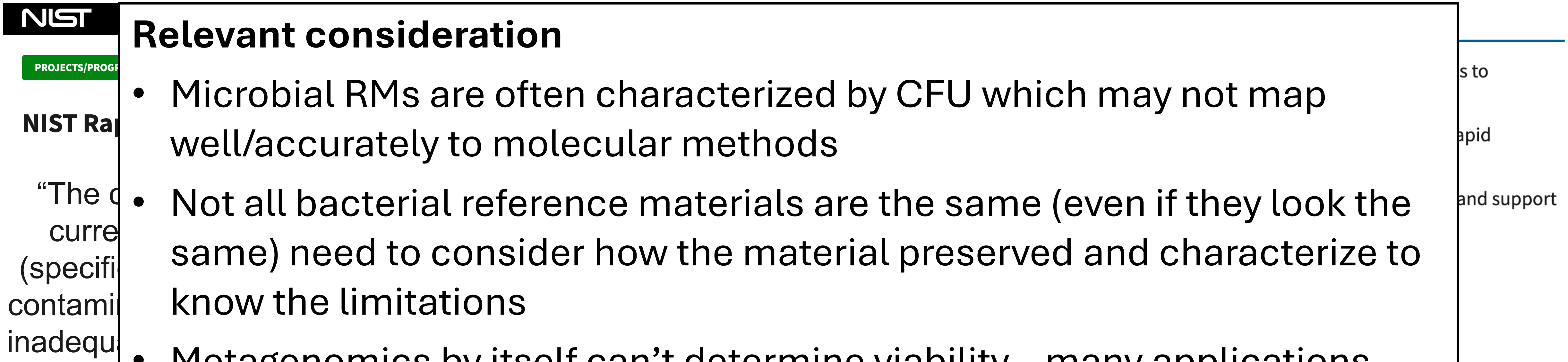


# Reference Materials

A catalog of **Reference Materials**

**Bespoke Materials** to support specific studies (e.g. Interlab studies, Interagency Asks)

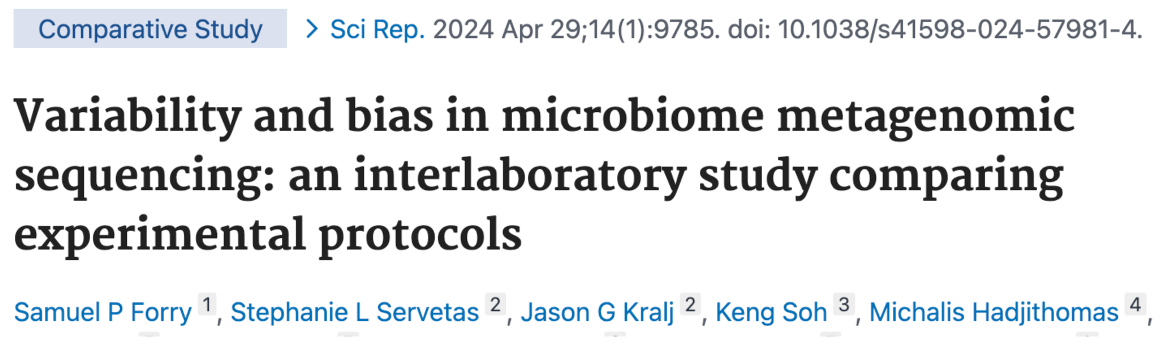
Evaluation of **commercial materials** fitness-for-purpose



**Relevant consideration**

- Microbial RMs are often characterized by CFU which may not map well/accurately to molecular methods
- Not all bacterial reference materials are the same (even if they look the same) need to consider how the material preserved and characterize to know the limitations
- Metagenomics by itself can’t determine viability – many applications mentioned today (RMTM, food safety) are grappling with that issue

# Convening + Standards Development



Comparative Study > Sci Rep. 2024 Apr 29;14(1):9785. doi: 10.1038/s41598-024-57981-4.

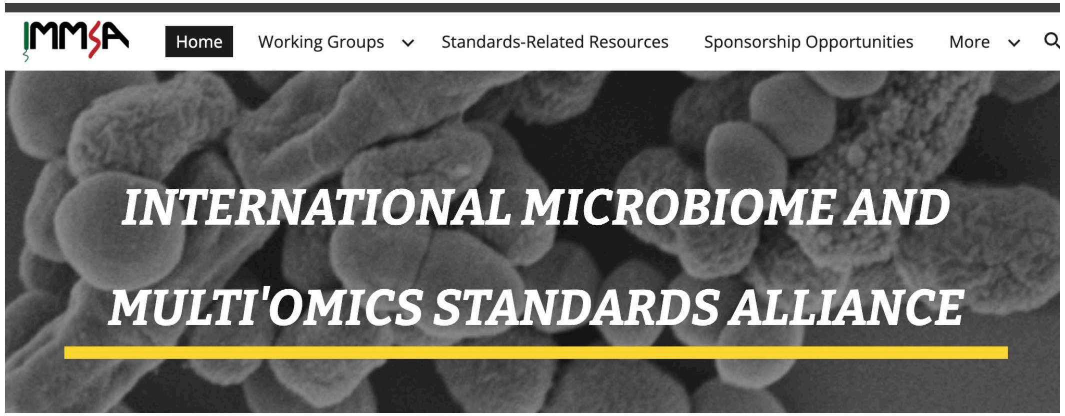
**Variability and bias in microbiome metagenomic sequencing: an interlaboratory study comparing experimental protocols**

Samuel P Forry[1], Stephanie L Servetas[2], Jason G Kralj[2], Keng Soh[3], Michalis Hadjithomas[4],

J AOAC Int. 2023 Apr 17;106(5):1424–1430. doi: 10.1093/jaoacint/qsad047

**Amplicon Sequencing Minimal Information (ASqMI): Quality and Reporting Guidelines for Actionable Calls in Biodefense Applications**

Ishi Keenum[1,✉], Robert Player[2,3], Jason Kralj[4], Stephanie Servetas[5], Michael D Sussman[6], Joseph A Russell[7], Jennifer Stone[8], Sailaja Chandrapati[9], Shanmuga Sozhamannan[10,11]



INTERNATIONAL MICROBIOME AND MULTI'OMICS STANDARDS ALLIANCE

**NIST Rapid Microbial Testing Methods Consortium**

# NIST's Role

- ☐ We work on developing methods and supporting the use of reference materials and other standards in MGS workflows
- ☐ NIST has a portfolio of reference materials to support MGS and experience developing bespoke/small batch materials
- ☐ We love to collaborate!

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# Complex Microbial System Group (CMSG)


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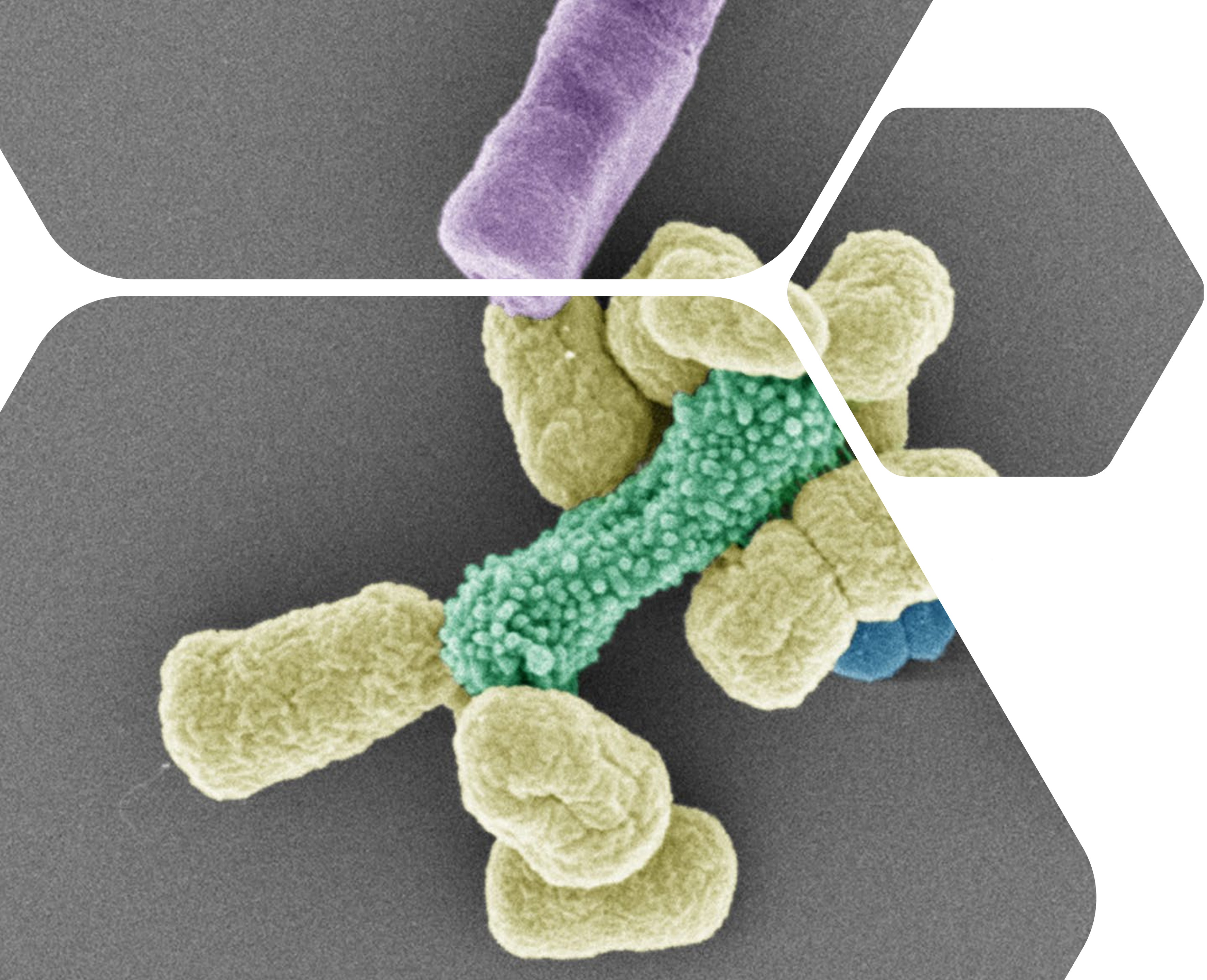

# QUESTIONS

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U.S. Department of Commerce