Results of the Multi-Mission Metagenomics Technology Development Workshop for Modernizing Planetary Protection Policy

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Rush University, Chicago, IL

Workshop Chair

Stefan Green

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Workshop Co-Chair

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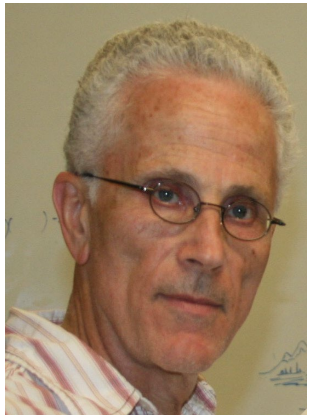


Tamas Torok

BERKELEY LAB

Committee on Planetary Protection 2023 Fall Meeting

October 20, 2023

NATIONAL ACADEMIES *Sciences Engineering Medicine*

Disclaimer

- **NASA Office of Planetary Protection funded JPL to run a workshop addressing the need for molecular approaches to spacecraft bioburden assessment**

Workshop Participants

Independent science review

- **Stefan Green**, Rush University (Chair)
- **Scott Tighe**, UVM (co-Chair)
- **Tamas Torok**, LBNL (co-Chair)

- **Panelists:**
  - **Scott Jackson**, NIST (presenter)
  - **Sunny Jiang**, UC Irvine (presenter)
  - **Emiley Eloe-Fadrosh**, JGI
  - **Jonathan Allen**, LLNL
  - **Shawn Levy**, Hudson Alpha
  - **Stuart Levine**, MIT
  - **Lynn Schriml**, U. Maryland
  - **Kelley Thomas**, UNH

NASA Presenters

- **Nick Benardini**, PPO, NASA
- **Julie Robinson**, HEOMD, NASA
- **Sarah Wallace**, JSC
- **Aaron Regberg**, JSC
- **Jason Wood,** former JPL
- **Lisa Guan**, JPL
- **Nitin Singh**, JPL
- **Kasthuri Venkateswaran**, JPL

Publication

- Green SJ, Torok T, Allen JE, Eloe-Fadrosh E, Jackson S, Jiang SC, Levine S, Levy S, Schriml LM, Thomas WK, Wood JM, and Tighe S. 2023. Metagenomic Methods for Meeting NASA's Planetary Protection Policy Requirements on Future Missions – a Workshop Report. *Astrobiology* 23(8):897-907.

Motivation for New Methods for Bioburden Assessment

- **COSPAR has formulated an international standard Planetary Protection Policy to protect against interplanetary biological and organic contamination**
- **NASA missions with potential biological contamination currently use the NASA Spore Assay (NSA)**
- **Space Studies Board (SSB) recommendations:**
    - **1992/2006**: ***Adapt molecular assays for bioburden and diversity measurement***
    - **2019: *Require collection of phylogenetic data to assess diversity of microbes* in assembly, test, and launch operations (ATLO) environments and on Mars spacecraft**
- **In-house 'genetic inventory' DNA molecular assays have been conducted for certain NASA missions on a research basis.**
- **State-of-the-art techniques are widely used by federal agencies, academia, and industry, but need to be validated for spacecraft-associated surfaces**

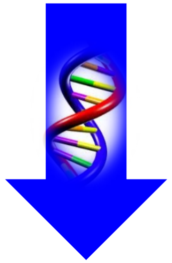
Current Approach for Bioburden Assessment

- NASA Spore Assay (NSA)
  - Labor-intensive and time-consuming (3-day incubation)
  - Targets only **aerobic, bacterial endospore-forming taxa**
    - Such organisms represent fewer than 0.1% of microbiota present on spacecraft surfaces
    - No correlation between NSA counts and total microbiota
    - Does not capture all PP-relevant taxa
  - No taxonomic or functional characterization of isolates

Workshop Objectives and Goals

- Recommend a molecular verification process to supplement the NSA bioburden assessment
  - Rapid quantification of biological contaminants
  - Taxonomic and functional characterization of contaminants from molecular data
  - Understanding the function and risk profile of contaminants
- Evaluate molecular methods for assessing spacecraft bioburden
- Identify knowledge gaps and needed innovations

Current NASA Standard Process



- New Technology
  - Transforms process
  - Faster enumeration and higher resolution

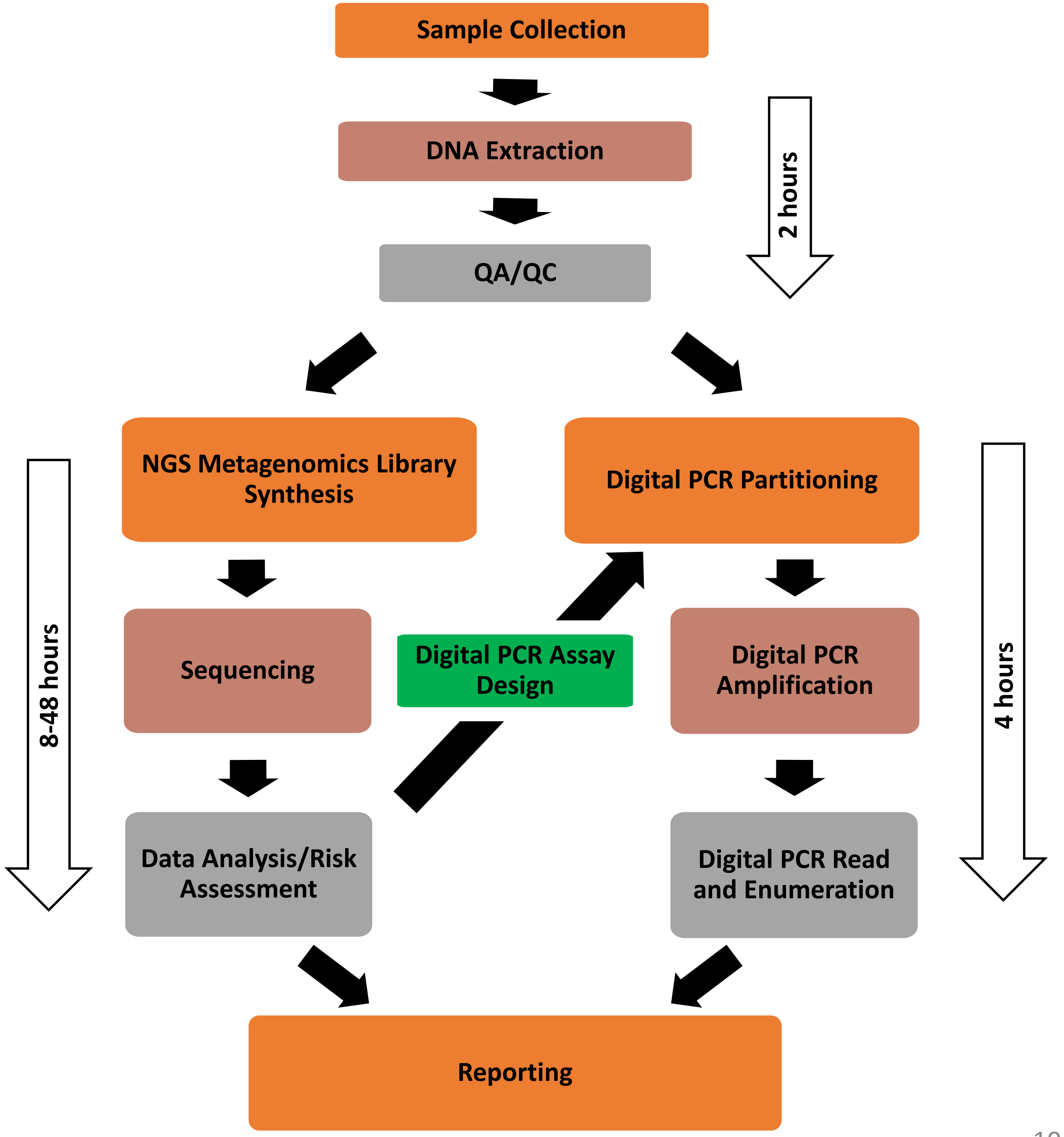
Expected Key Benefits

1. Lower Risk - risk-based decision making for organism types and capabilities.
2. Higher Resolution - >99% fold increase in diversity over NSA.
3. Faster - Aim is to reduce TAT from 72 h to ~6 h.
4. Cost / Schedule Savings - reduction in TAT and tailored microbial reduction.

Workshop Findings

- Objectives are threefold:
  - Rapid results
  - Quantitative results
  - High information content results
- Currently, no single method can achieve all three objectives for low biomass
- Workshop panel recommended a hybrid model of digital PCR and shotgun metagenome sequencing to achieve all objectives
- Bioinformatics serves as an anchor linking metagenomics and digital PCR
- Culturomics is essential for developing appropriate reference databases

Proposed Hybrid Molecular Approach



Technology Gaps

- Nucleic acid-free sampling devices and materials
- Nucleic acid-free reagents and consumables
- Mitigation strategies effective for low biomass samples
- Ultra-low nucleic acid detection (*e.g.*, 10 pg challenge)
- One-click bioinformatics solutions for metagenomic sequence data
- Hybrid risk models combining metagenomic and digital PCR data
- Live/dead evaluation methods for low biomass samples
- Ultra-low bioburden whole cell reference standards

Standardizing Molecular Assessment of Spacecraft Biological Contamination

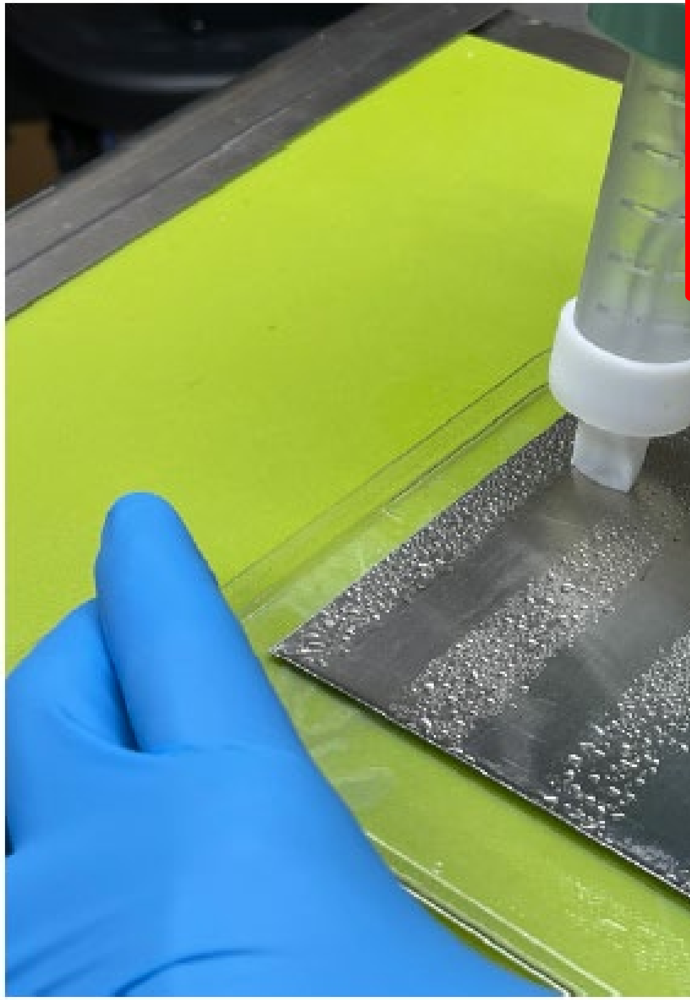
- **Sample Collection**
  - Sample device standardization for large and small surface areas
  - Sample collection from low and high biomass surfaces
- **Sample Processing and Sequencing**
  - Nucleic acid extraction standard process
  - Viable organism enumeration standard process
  - Optimization of sequencing reactions – pre-processing, interim processing, and final benchmarks
- **Bioinformatic Analysis**
  - Broadest spectrum of organisms identified with appropriate analytics
  - Bioinformatic pipeline standardization
  - Populate and standardize database with PP-relevant organisms
- **Establishing Relevant Organism Assessments**
  - Enhanced understanding of microbes in extreme environments and tolerance to sterilization modalities
  - Develop databases of mission-specific microbial traits and biochemical capabilities
  - Rapid assessment of PP-relevant organisms
  - Leverage molecular data to culture PP-relevant organisms
  - Establishment of thresholds and limits of detection for rapid and classical assessments

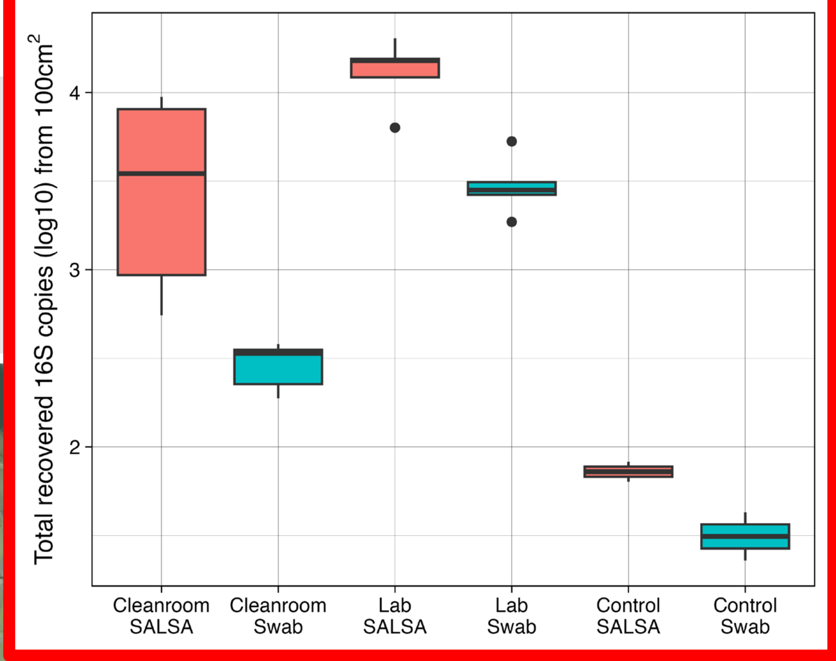
Recommendations and Next Steps

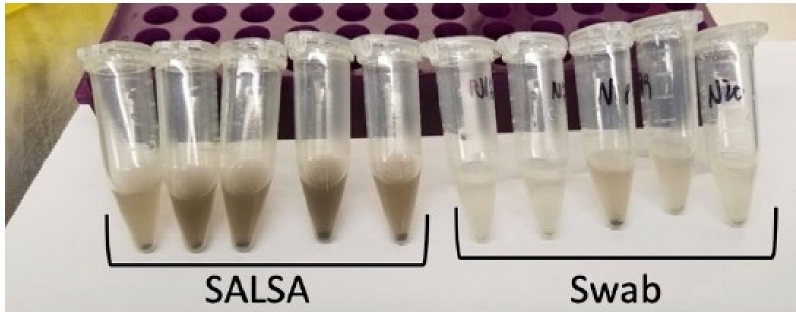
- Optimization of semi-quantitative metagenomics for ultra-low biomass microbial characterization
- Validation of targeted digital PCR for quantitative assessment of spacecraft surfaces
- Encourage NASA to
  - continue regular workshops to evaluate technology development
  - leverage technology roadmaps to support development in the following areas:
    - reagent and instrumentation development
    - cultivation and bioinformatics research
- COSPAR to host international workshops to coordinate technology development between agencies

Developments

- Squeegee-Aspirator for Large Sampling Area (SALSA) surface sampling device (Simpson et al. 2023 – JBT 34(3): https://jbt.pubpub.org/pub/3rzb85v3/release/2)
- Challenge: Need to validate “metagenomics” methods for ~pg DNA samples and no funding available

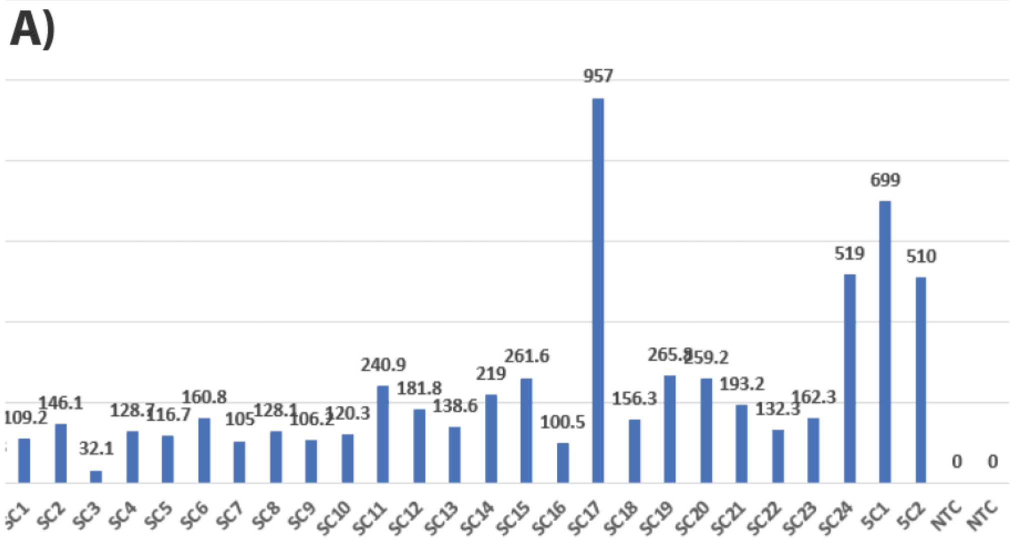


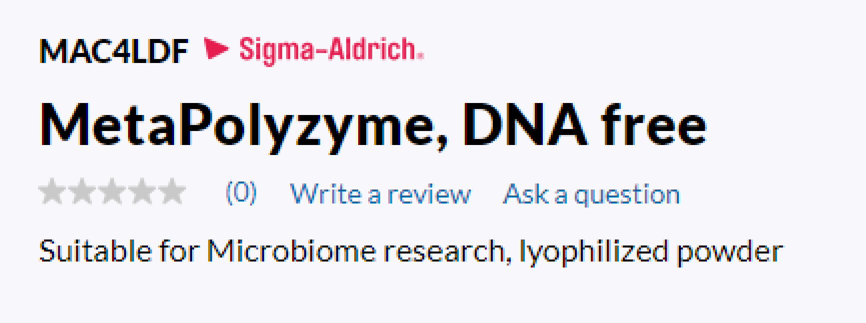




Developments

- Qiagen Ultra-clean reagents
  - [UCP HiFidelity PCR Kit]
- BioSkryb ResolveDNA Microbiome whole genome amplification
  - [ResolveDNA Microbiome (Alpha) - BioSkryb Genomics]
- DNA-free reagents from Sigma
  - DNA-free PBS, Tris, polymerases, MetaPolyzyme





MAC4LDF Sigma-Aldrich

MetaPolyzyme, DNA free

★★★★★ (0) Write a review Ask a question

Suitable for Microbiome research, lyophilized powder

Questions

- Contact
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  - Scott Tighe, Scott.Tighe@uvm.edu
  - Tamas Torok, ttorok@lbl.gov