National Aeronautics and Space Administration

NASA's Multicenter Planetary Protection Metagenomics Study

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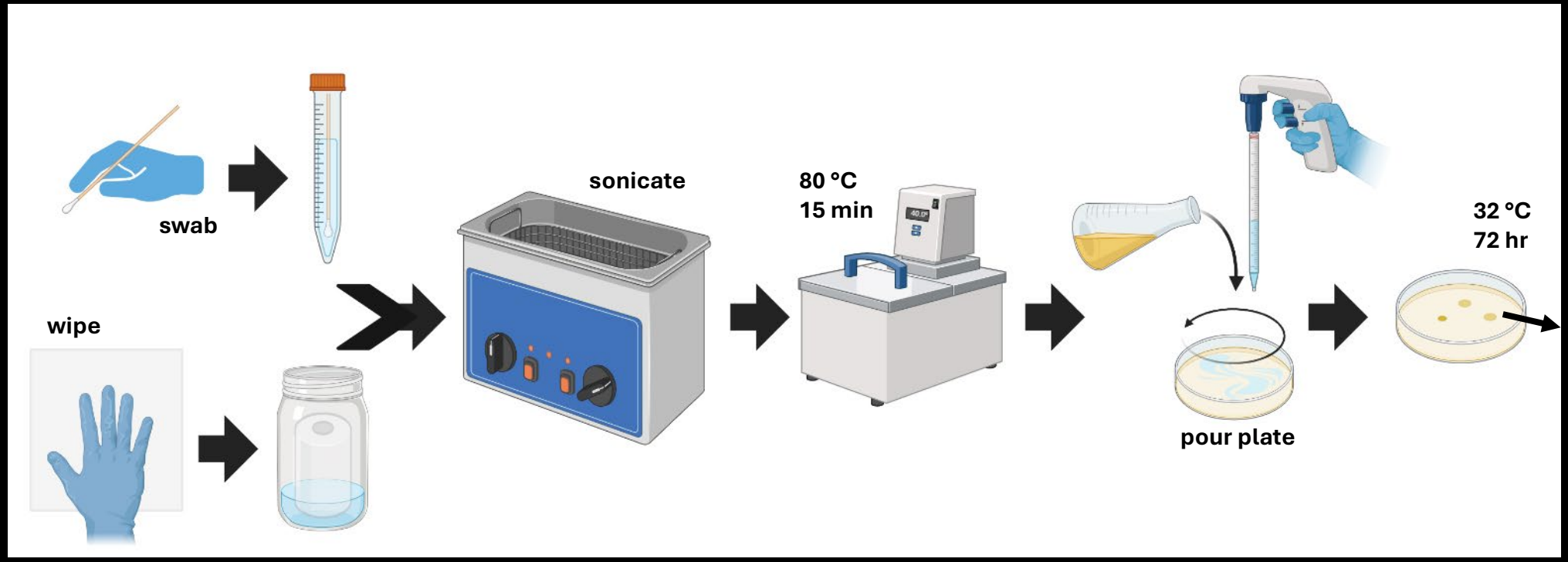
NASA Standard Spore Assay (NSA)

- Fast, Reproducible, Easy, Quantifiable



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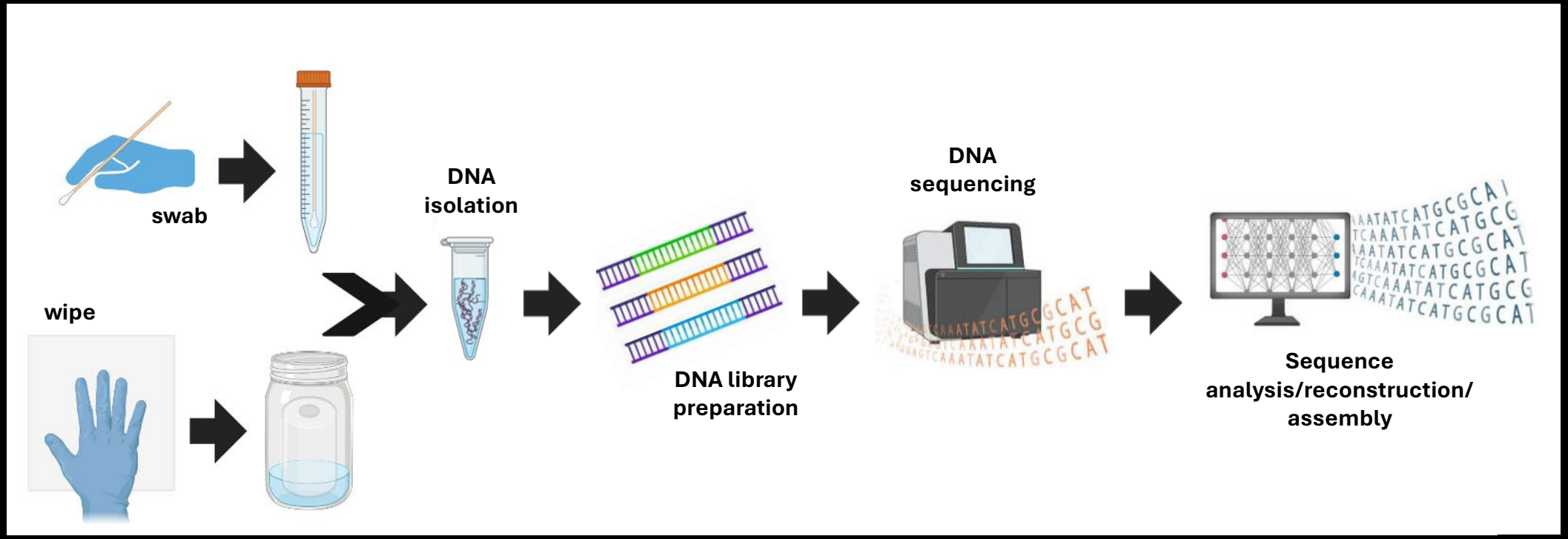
Modernization of PP Methods

- **Metagenomics** is the study of all genetic material (DNA) from all organisms in a particular environment.



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Details and Objectives

- Timeframe: September 2024 – ongoing
- What / Vision
  - To collect cleanroom environmental samples from multiple NASA Centers to compare metagenomic analysis with NASA Standard Spore Assay (NSA)

- Objectives
  - Evaluate cleanrooms across NASA Centers using metagenomics and the NSA
  - Strengthen NASA's PP SME community and build collaboration
  - Publication of findings
  - Eventual: collaborate with the international community on an international standard

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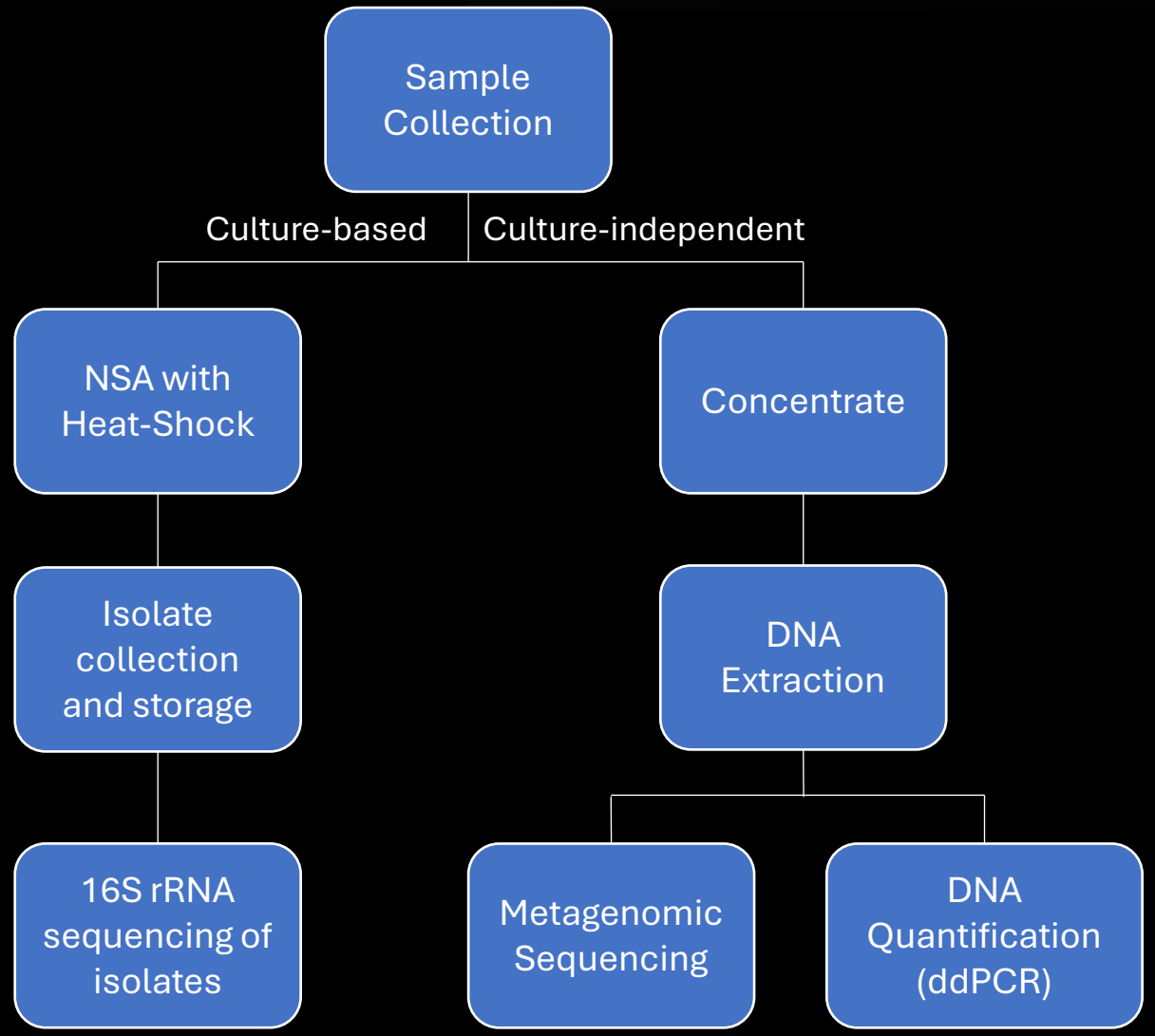
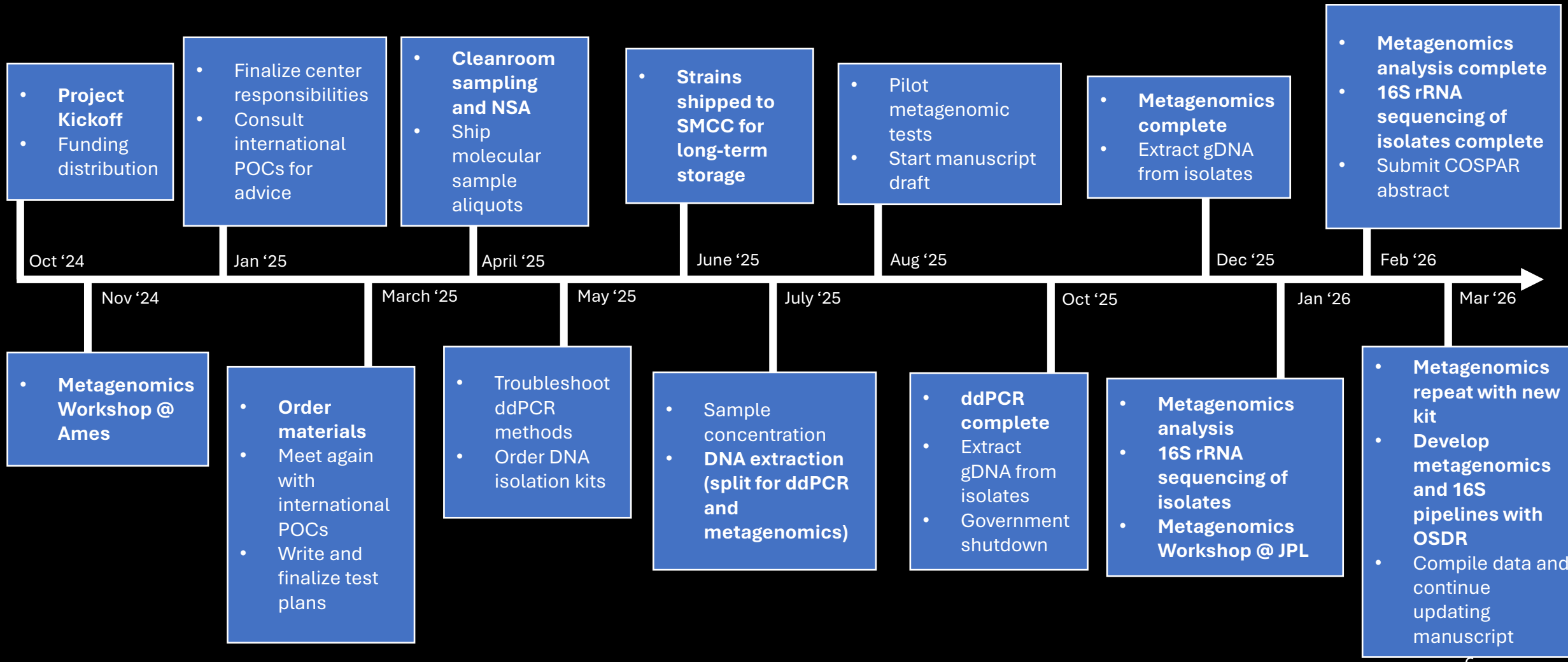
Key Tasks

- Develop/edit test plan (including shipment procedures)
- Cleanroom wipe sampling
- Sample processing
- NSA
- Isolate archiving
- Isolate ID (amplicon sequencing)
- Sample concentration
- DNA extraction
- ddPCR
- Metagenomics
- Bioinformatics



Workflow Timeline

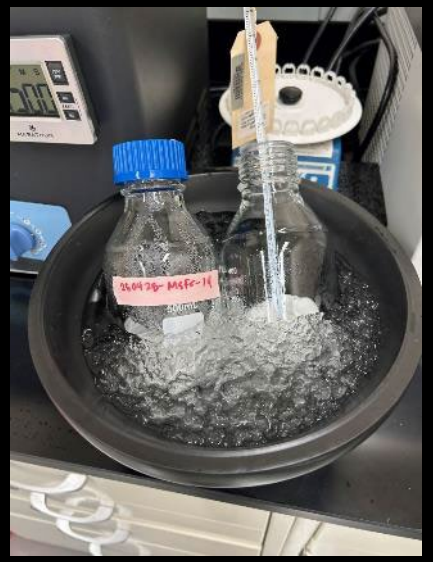


Test Plan Development and Cleanroom Sampling

ISO 8 Cleanroom Sampling

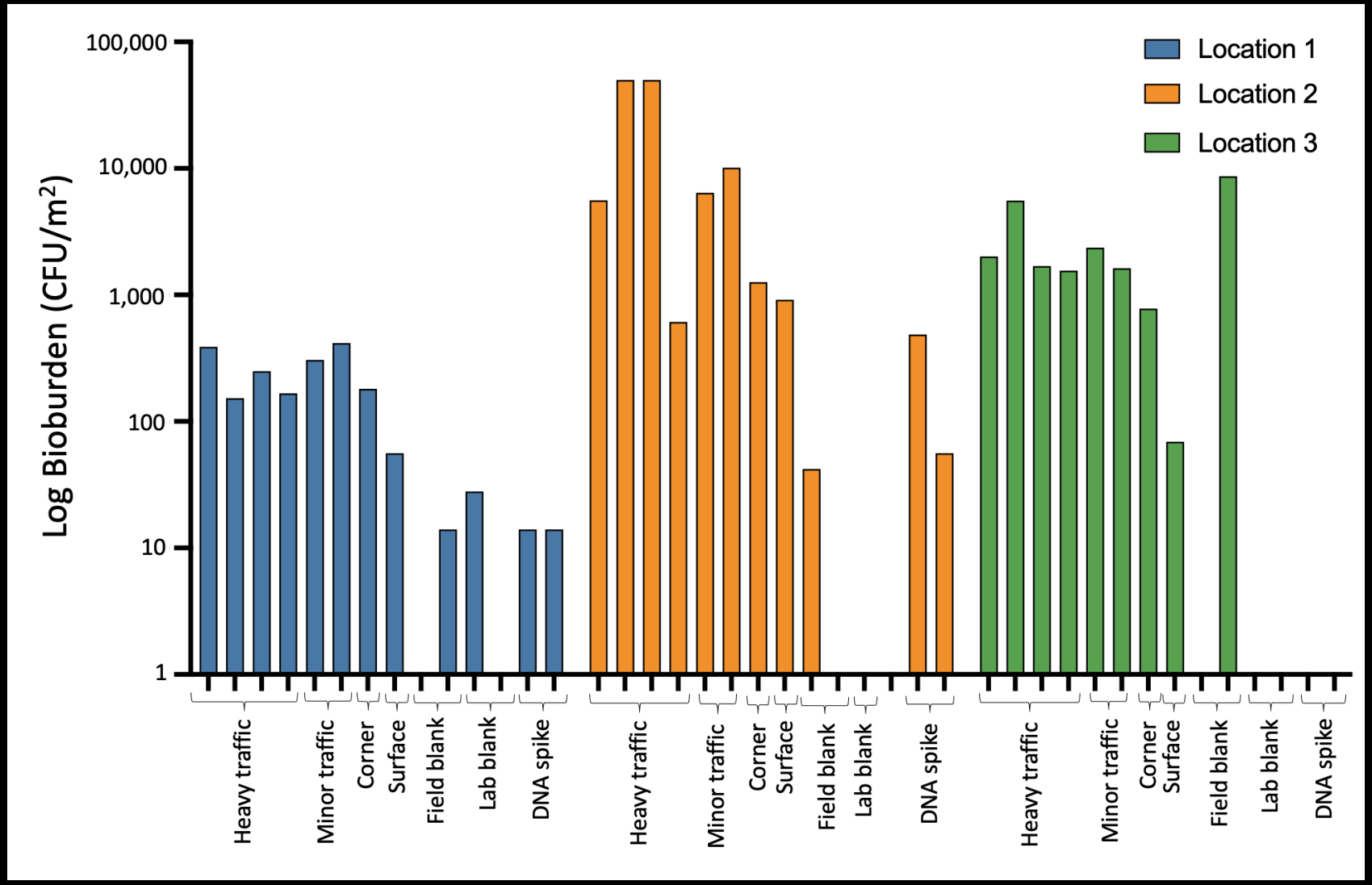
- Developed test plan and procedure
- 3 centers selected a cleanroom for sampling:
  - 4 heavy traffic walkways
  - 2 minor trafficked areas
  - 1 corner
  - 1 surface or table
  - 2 field blank
  - 2 DNA Spike controls (Zymo D6321 Mock Community)

Sample Processing and NSA



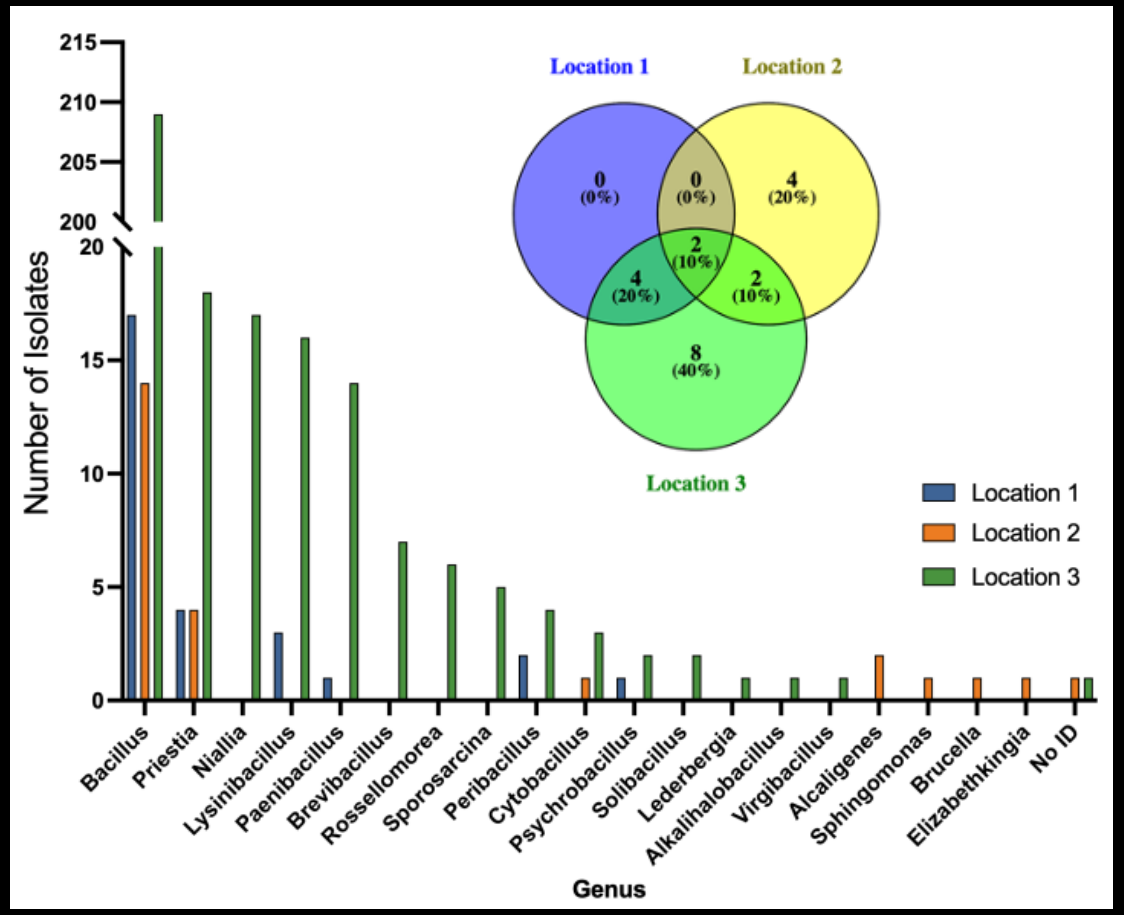
- Samples were processed at two different centers
- Prior to heat shock, 100 ml of each sample was removed for molecular analysis and frozen at -80 °C
- Remaining 100 ml was processed via NSA
- After 72 hours of incubation, representative isolates were streaked

Sample Processing and NSA



Isolate Archiving and Identification

- Pure cultures were collected and frozen at -80 °C
- Over 360 isolates were shipped to ARC's Space Microbial Culture Collection (SMCC)
- Sequenced 16S rRNA gene to identify
  - Working with OSDR to develop standardized pipeline for taxonomy calling



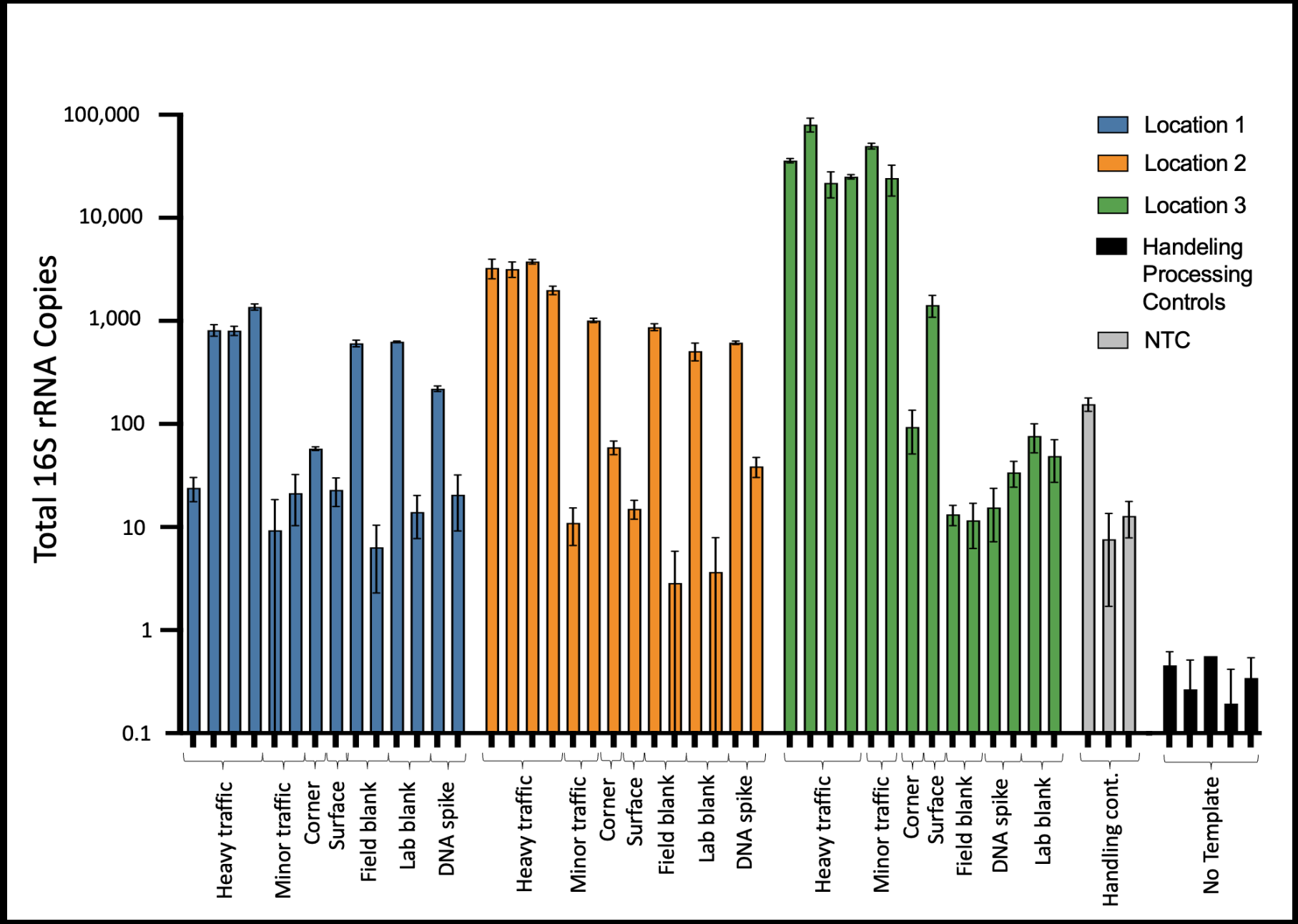
Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html

Sample Concentration and DNA Extraction

- 100 ml aliquots saved from samples were shipped blind on dry ice
- Samples were concentrated using an InnovaPrep FluidPrep Concentrating Pipette System
- DNA was extracted from concentrated samples using the ZymoBIOMICS DNA Microprep Kit
- All DNA was shipped for ddPCR or metagenomics

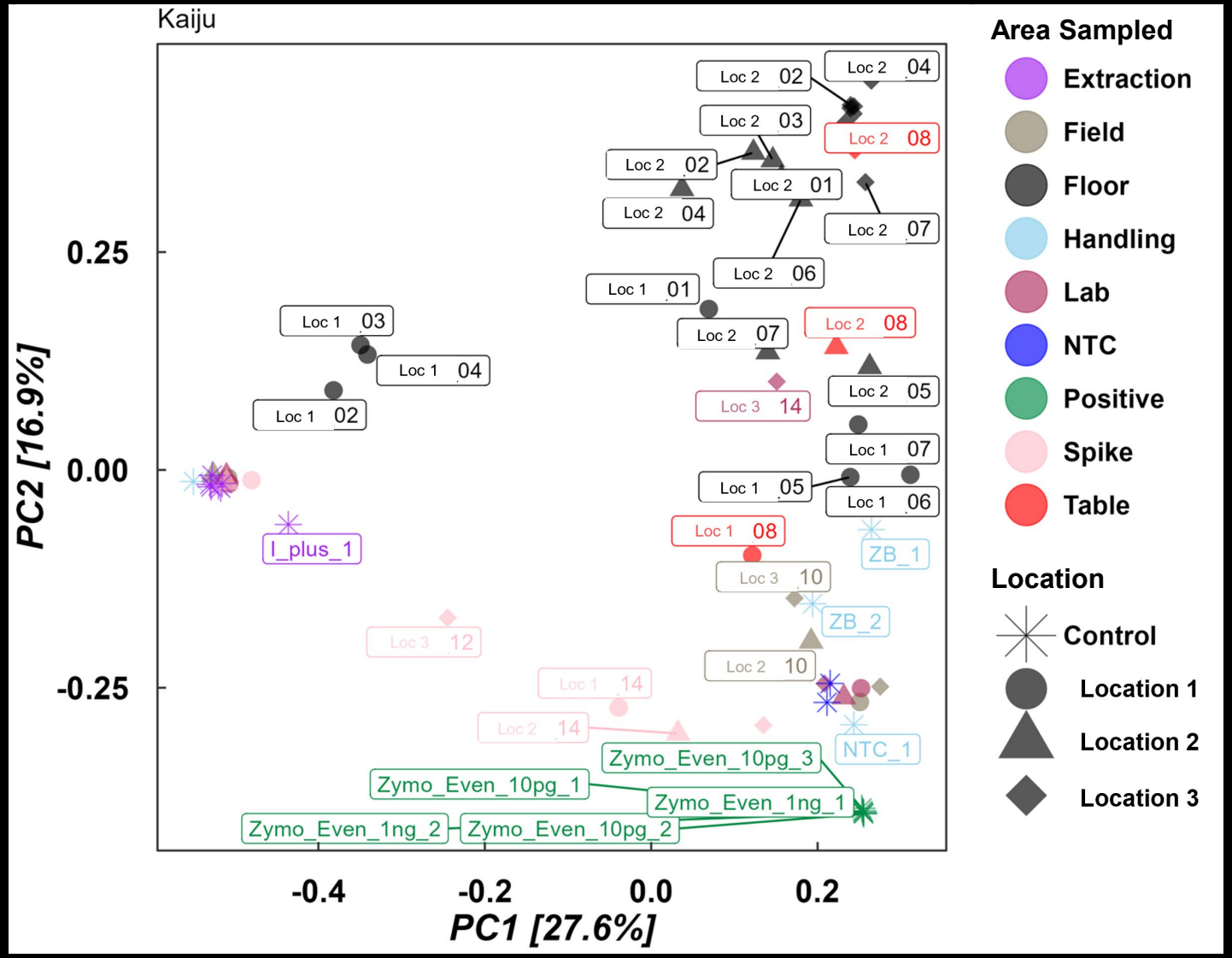
ddPCR

- ddPCR of 16S rRNA performed on BioRad QX200 with Manual Droplet Generator



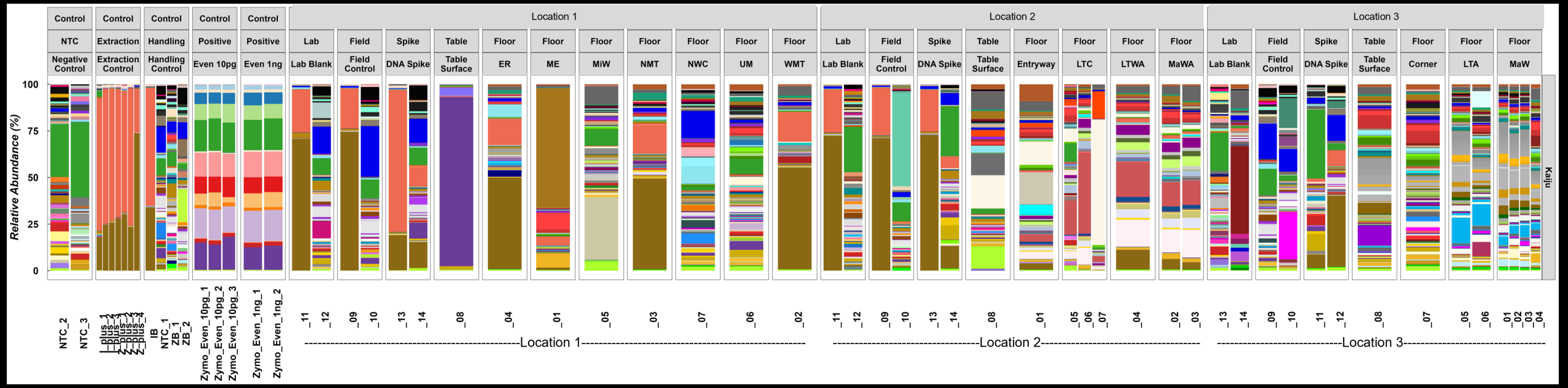
Metagenomics and Bioinformatics

- TapeStation & Qubit showed that the DNA concentration was below detection for all samples
- Libraries were constructed using the Illumina DNA Prep Kit and pooled.
- Sequencing was performed on an Illumina NovaSeq 6000 using a SP 500 cycle flow cell
- Area sampled and location may explain some clustering



Metagenomics and Bioinformatics

- **ME** - Main entrance floor
- **WMT** - Floor west of main traffic flow
- **NMT** - Floor north of main traffic flow
- **ER** - Floor on east side of room
- **MiW** - Minor walkway floor, between hardware cages
- **UM** - Upper mezzanine floor
- **NWC** - Floor of northwest corner
- **Entryway** - Entryway floor
- **MaWA** - Major work area floor
- **LTWA** - Less trafficked work area floor
- **LTC** - Less trafficked corner floor
- **MaW** - Major walkway floor
- **LTA** - Less trafficked area floor
- **Corner** - Corner floor



- Cleanroom samples were dominated by extremophiles, human associated microbes, and plants
- Microbial composition was different depending on area sampled and location (appears that ISO 7 has less diversity than the ISO 8 cleanrooms with Location 3 having the most)
- The dominant species were different for each location

Metagenomics and Bioinformatics

- Still interpreting the data, but our use of blanks, and controls is allowing us to identify steps in the process where contamination is occurring
- Variable read depth and quality for samples using Illumina library prep. kit.
- Trying a Takara DNA Prep Kit currently
- Data from both kit runs will be available publicly in OSDR
- Pipelines for metagenomic data analysis in preparation

Takeaways and Next Steps

- Sample processing for molecular analysis matters
  - Variability between centers
  - Assessing differences in Takara vs. Illumina library prep kits
- Working with OSDR to develop standardized pipeline for metagenomics analysis and 16S taxonomy calling to increase efficiency and decrease data processing timeline
- Manuscript in preparation to submit soon
- Submitted abstract to present this work at COSPAR (1 – 9 August)
- Open to additional follow-up studies and collaborations
  - Strengthen statistical considerations and use of data for decision making

We have a great team!
Limited resources, backorders, layoffs/personnel changes, new administration, 4 parental leave considerations, Government shutdown, Software installation delays, Shipment constraints, etc.

- **NASA MSFC**
  - Samantha Marcella
  - Carly Parker
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- **NASA GSFC**
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  - Dr. Philip Hochendoner
- **NASA JSC**
  - Dr. Aaron Regberg
  - Dr. Jamie Micciulla
  - Dr. Richard Davis

+ MANY OTHERS

- **NASA ARC**
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  - Samrawit Gebre
  - Dr. Jonathan Galazka
  - Dr. Jessica Lee
  - Valery Stanylov
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- **JPL**
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