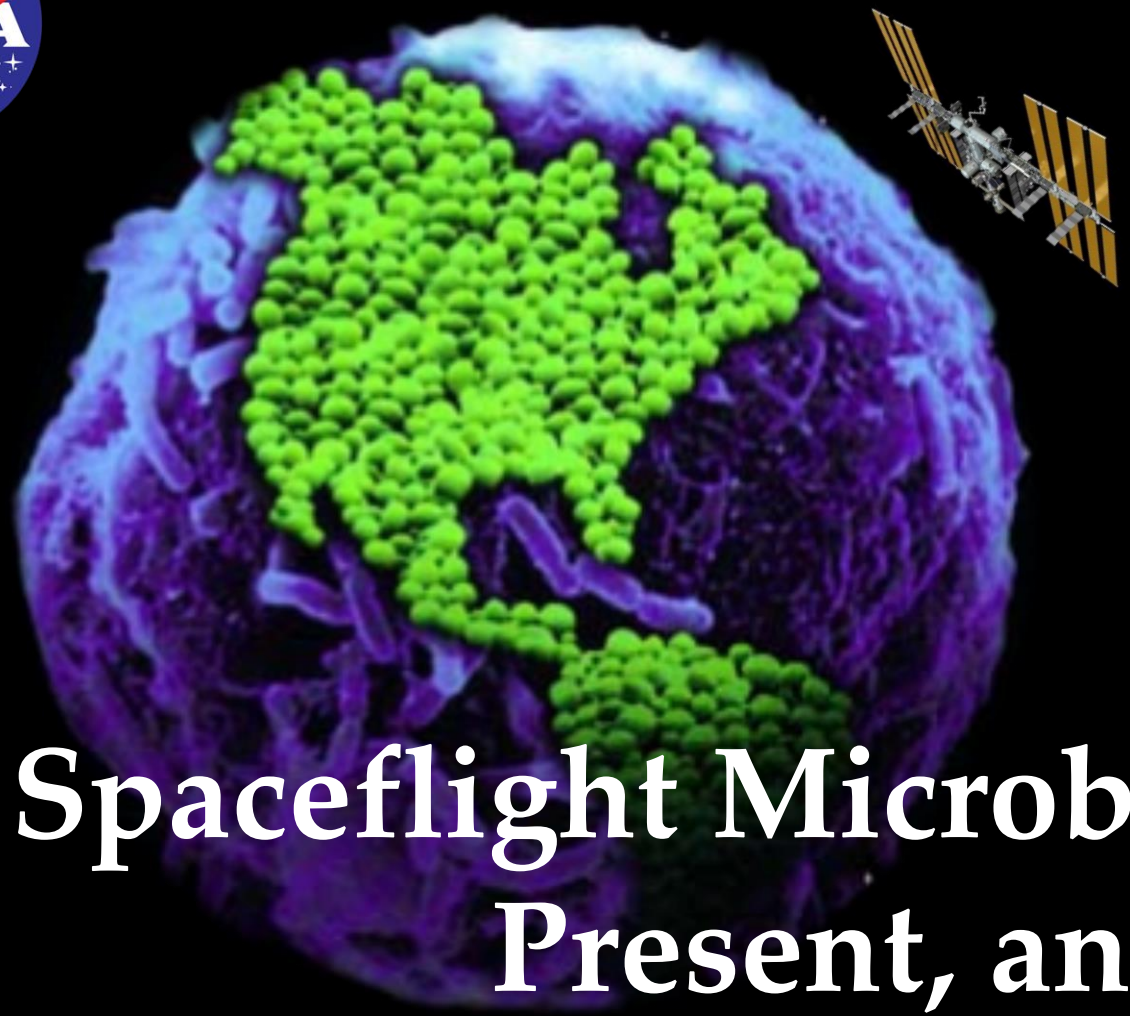
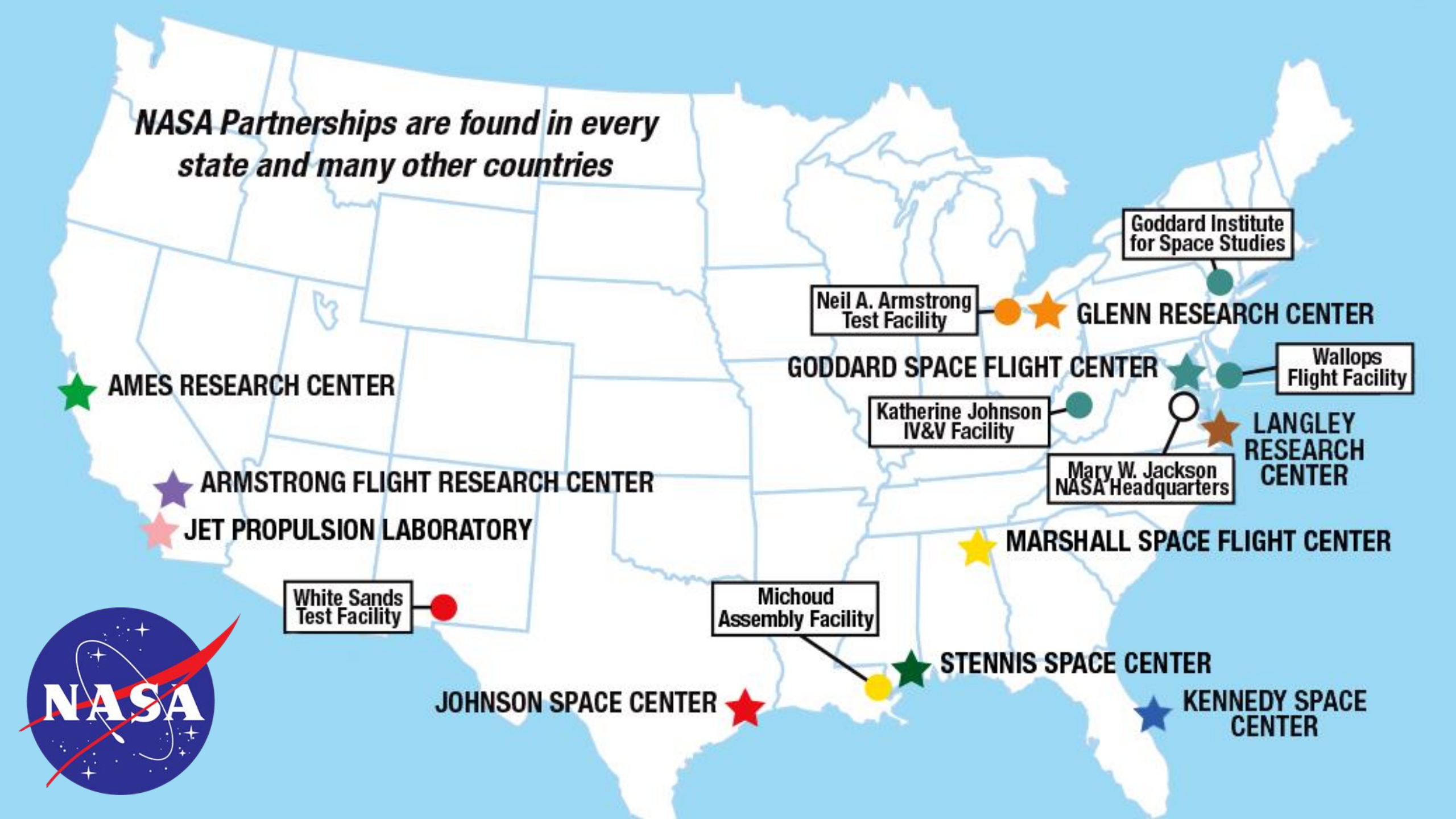


# Spaceflight Microbiology: The Past, Present, and Future

Sarah Wallace, Ph.D.
NASA Johnson Space Center
Microbiology Laboratory


NASA Partnerships are found in every state and many other countries
Goddard Institute for Space Studies
Neil A. Armstrong Test Facility
GLENN RESEARCH CENTER
GODDARD SPACE FLIGHT CENTER
Wallops Flight Facility
AMES RESEARCH CENTER
Katherine Johnson IV&V Facility
LANGLEY RESEARCH CENTER
Mary W. Jackson NASA Headquarters
ARMSTRONG FLIGHT RESEARCH CENTER
JET PROPULSION LABORATORY
MARSHALL SPACE FLIGHT CENTER
White Sands Test Facility
Michoud Assembly Facility
STENNIS SPACE CENTER
JOHNSON SPACE CENTER
KENNEDY SPACE CENTER
NASA




NASA
MISSION CONTROL CENTER

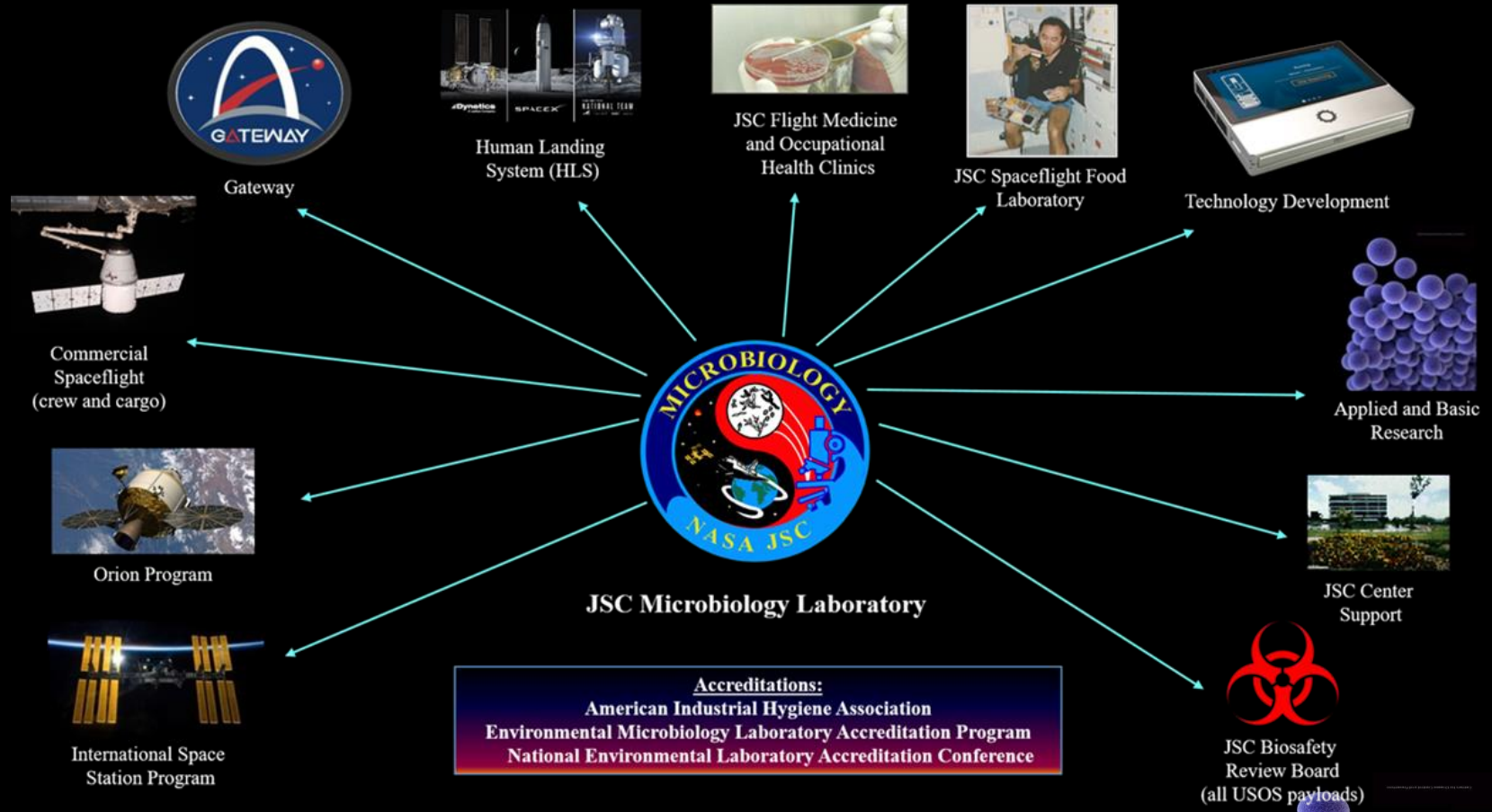

STATES




BOEING
STARLINER
SPACEX

# What is the goal of the NASA Microbiology Laboratory?

- Prevent adverse effects associated with microbes to the crew and vehicle
- Research – understand how the spaceflight environment impacts microbial behavior
- Test new technology to increase our ability to assess microbial risk



# NASA Microbiology

- Hold the requirements that dictate the microbial acceptability limits of the water, air, surfaces, and spaceflight foods
  - How much is there?
  - What is there?
- Responsible for delivering certification of flight readiness status
- Responsible for implementing routine pre-flight and in-flight microbial monitoring practices as a check of contamination controls
- Responsible for remediation when monitoring reveals microbial loads above the acceptability limits or the presence of medically-significant organisms






# When it comes to Micro, we cover it all!

## 1/2 Reference Laboratory, 1/2 Research Laboratory = 1 Amazing Microbiology Laboratory!















# Past / Present







SSK - Surface Sampler Kit





MAS – Microbial Air Sampler





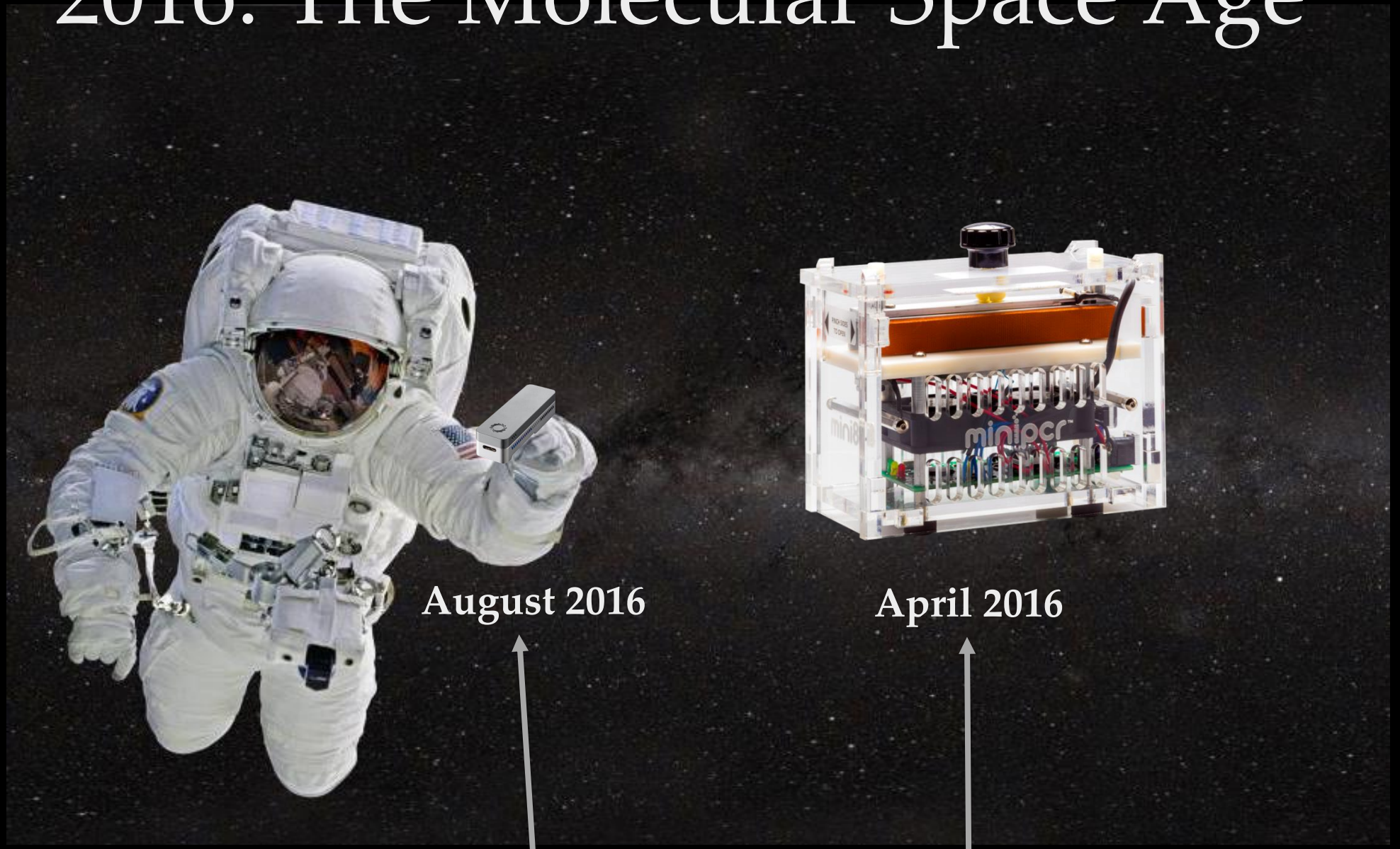




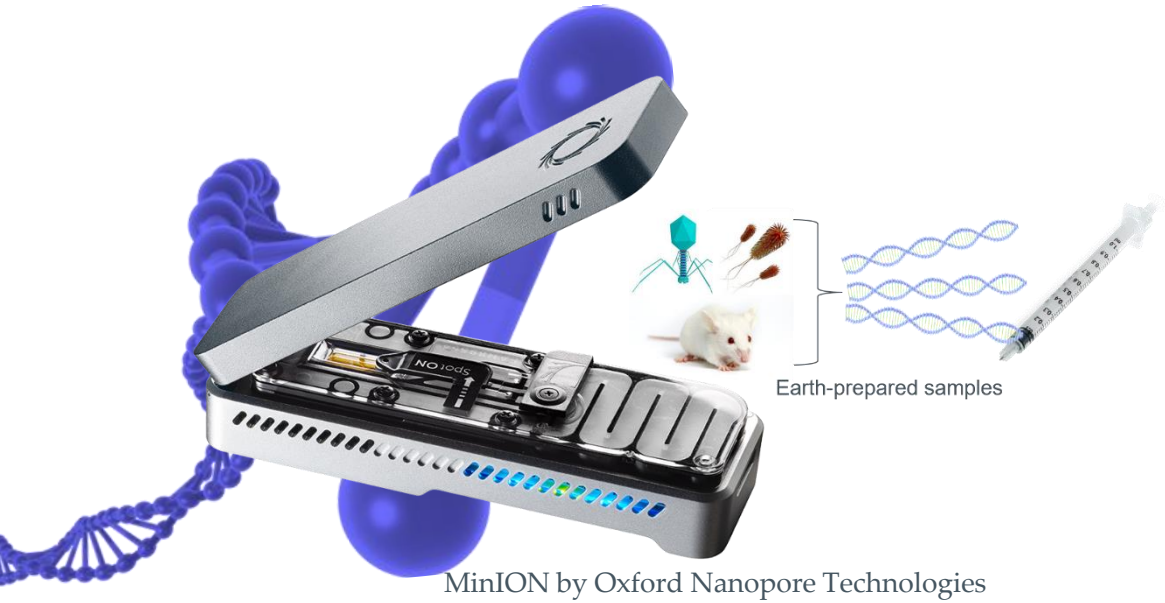
EHS Water Kit – Environmental Health Systems Water Kit

# 2016: The Molecular Space Age

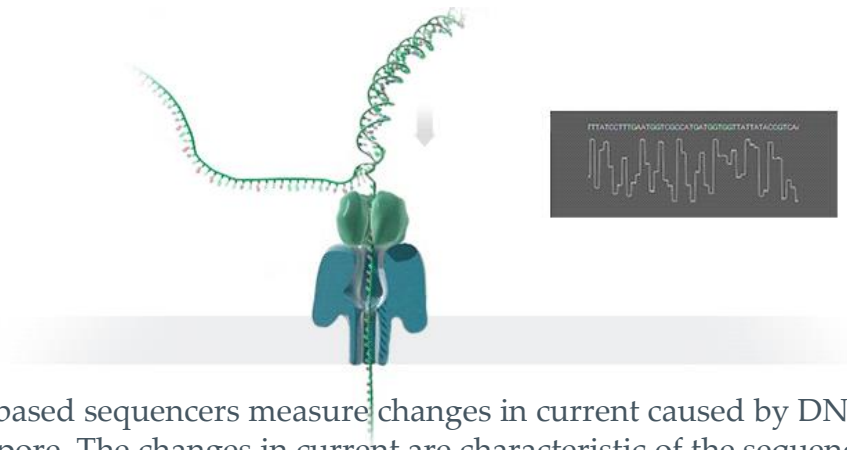
2016



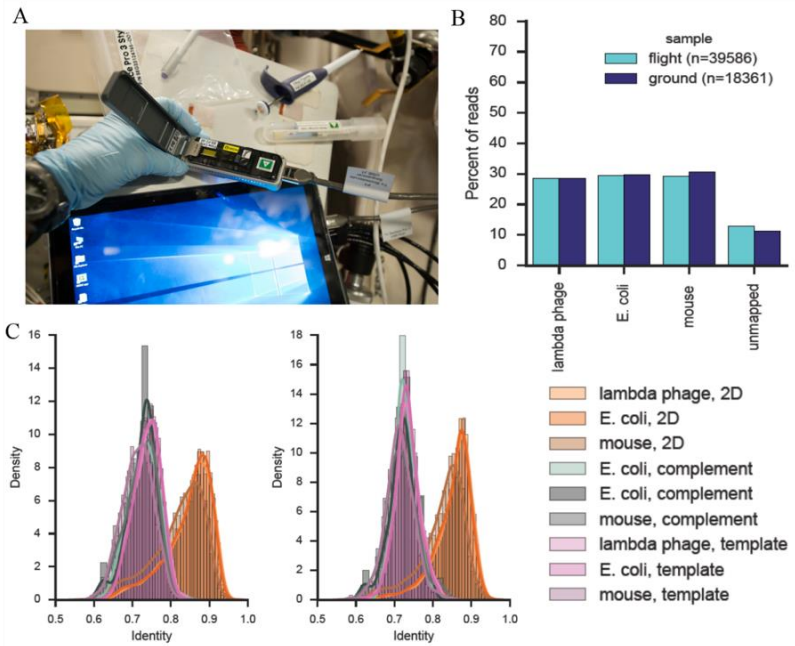
# Biomolecule Sequencer: First DNA Sequencing in Space

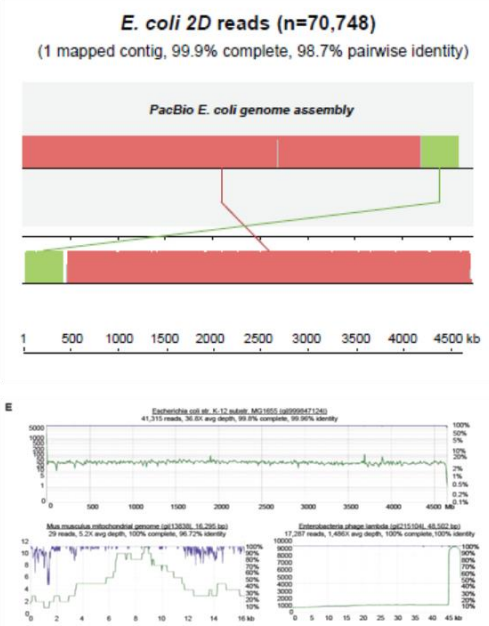


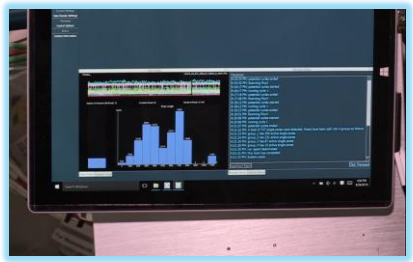

MinION by Oxford Nanopore Technologies



Nanopore-based sequencers measure changes in current caused by DNA strands migrating through the pore. The changes in current are characteristic of the sequence of migrating DNA.



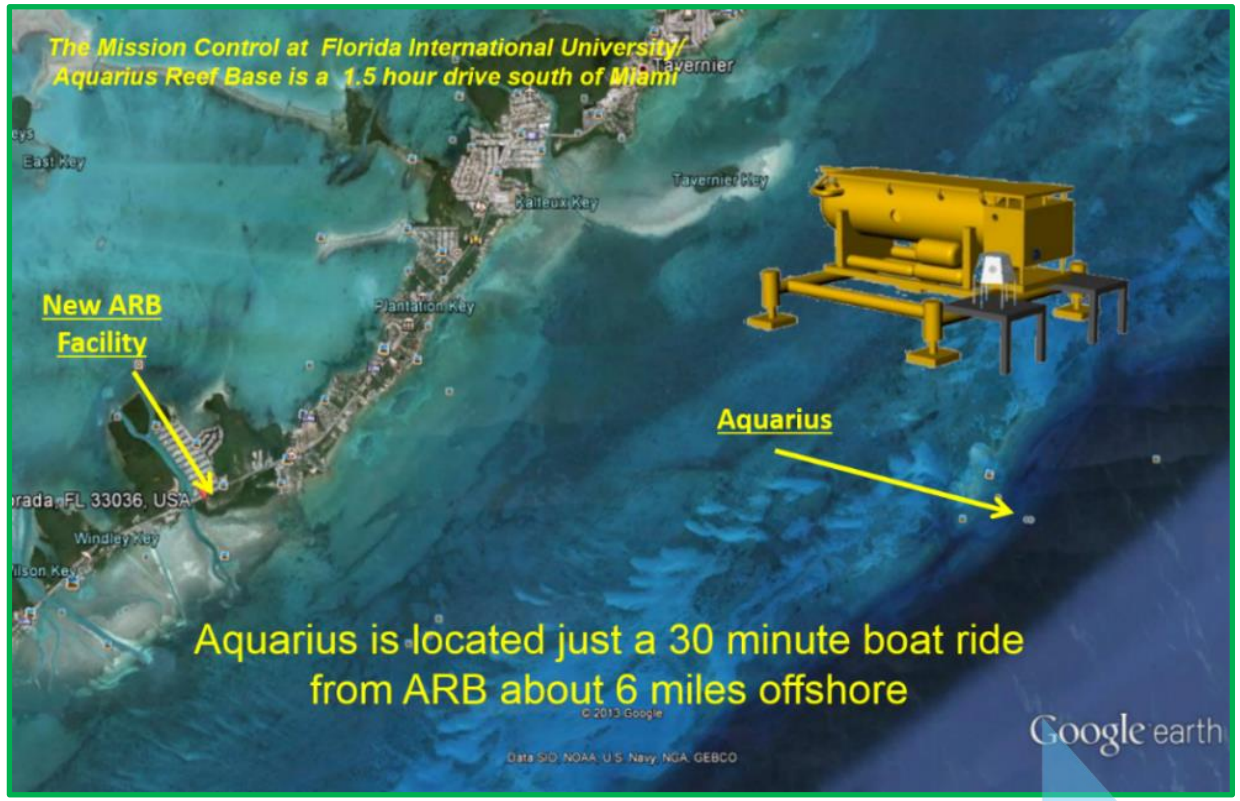


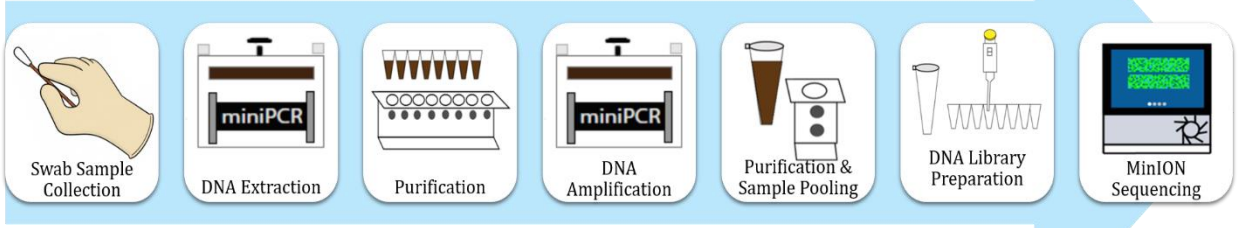



*Castro-Wallace et al.* https://www.nature.com/articles/s41598-017-18364-0

# Swab-to-Sequencer Analog Testing

## NASA Extreme Environments Mission Operations (NEEMO)






# Swab-to-Sequencer Analog Testing: NEEMO 21

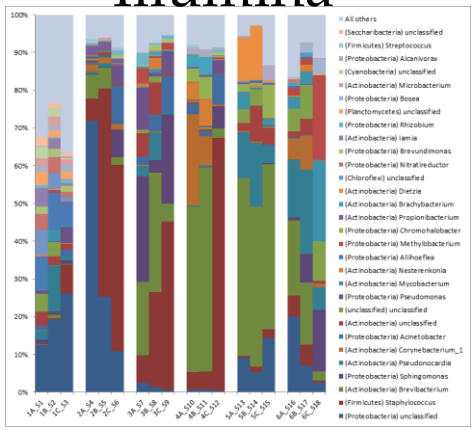



- **Lessons Learned:**
  - Operations / Logistics
  - DNA extraction method needed further optimization
  - Swab type is critical and needed further optimization

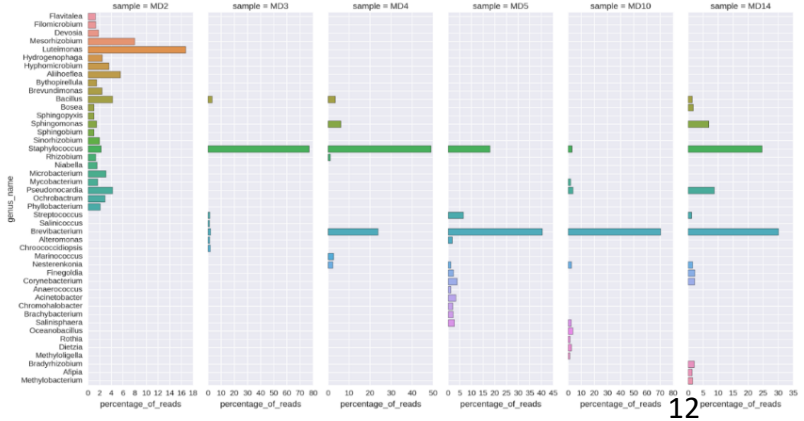


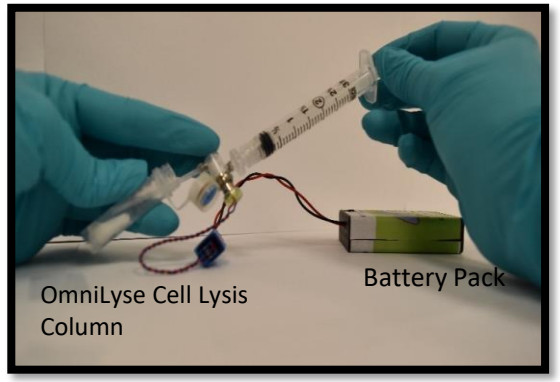


Illumina



MinION





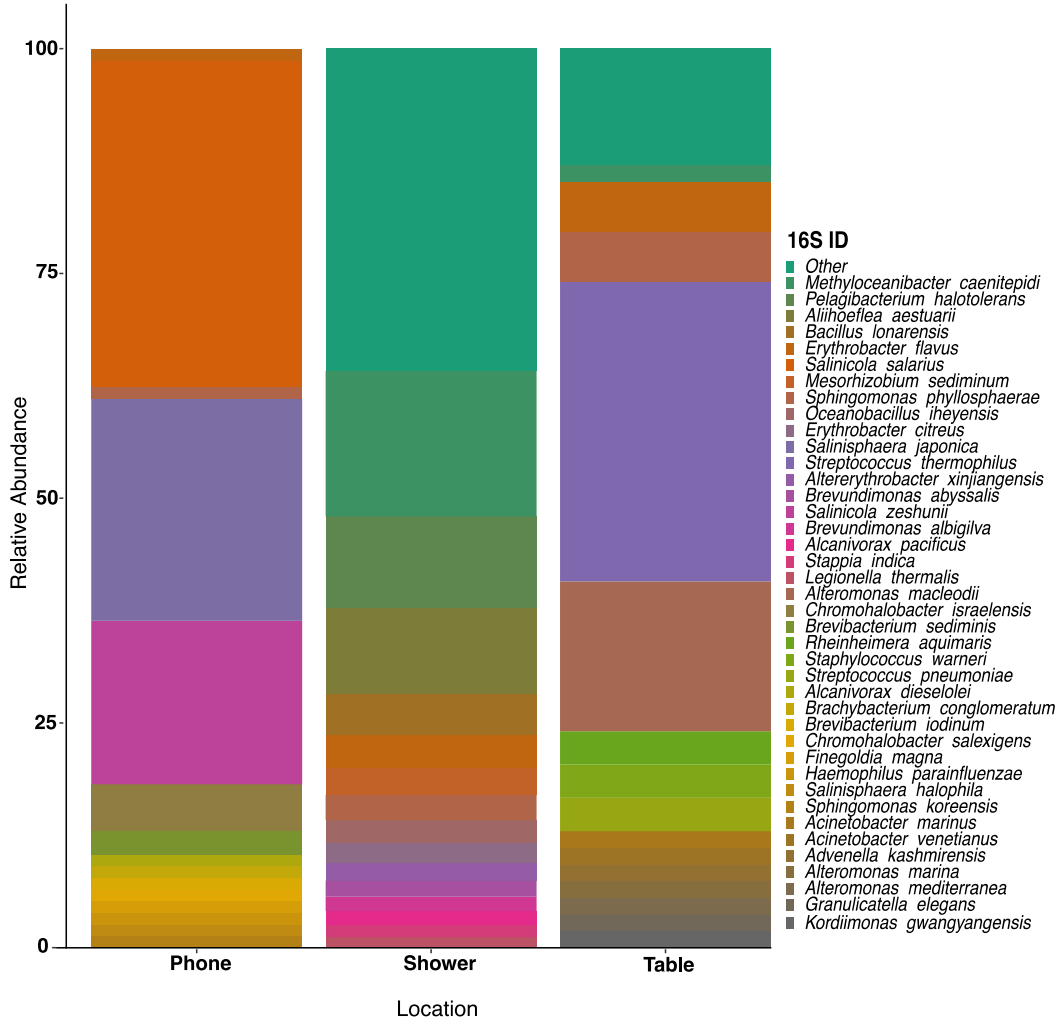


12

# Swab-to-Sequencer Analog Testing: NEEMO 22

- Lessons learned:
  - New, optimized swab works well
  - Streamlined and improved DNA extraction method provides better results
  - Updated crew procedures and kit configurations






*Stahl-Rommel et al.* https://www.mdpi.com/2073-4425/12/1/106/htm

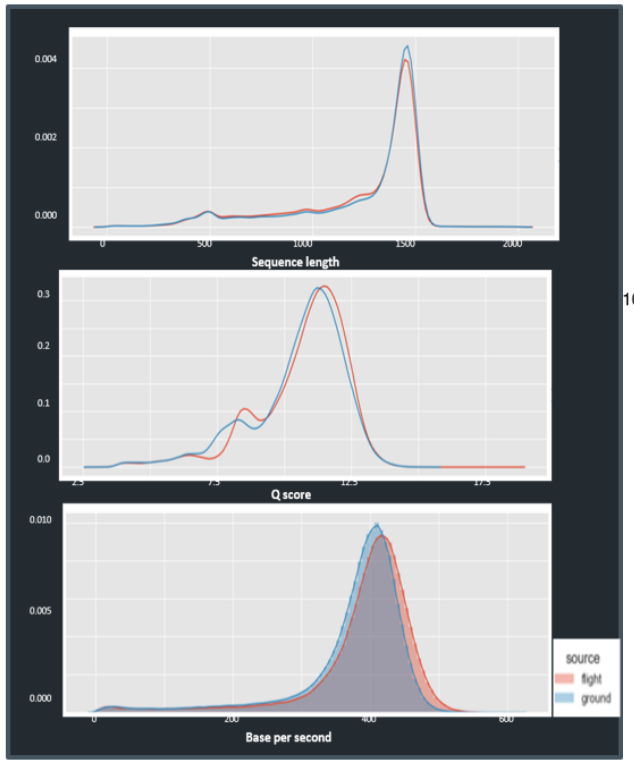
# Genes in Space-3: Culture-to-Sequencer



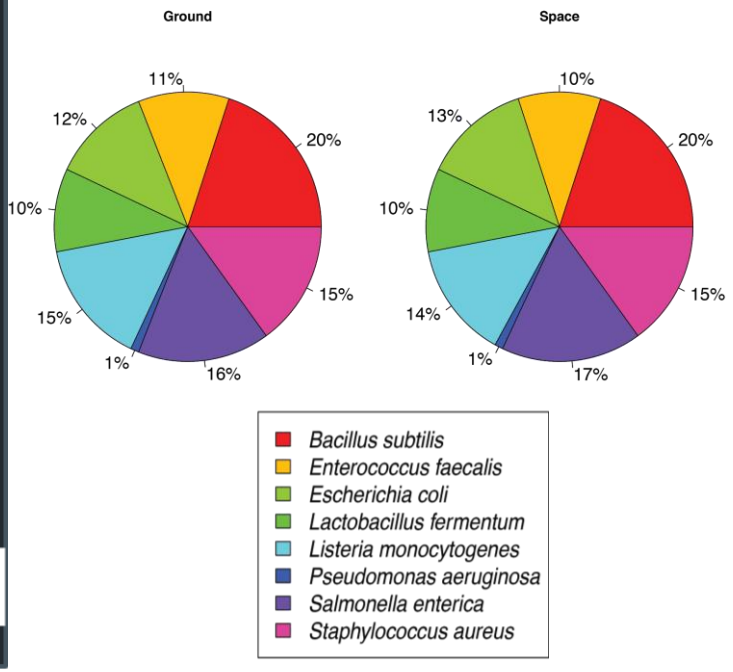


## Transitioning part of the swab-to-sequencer method to the ISS

MinION Metrics



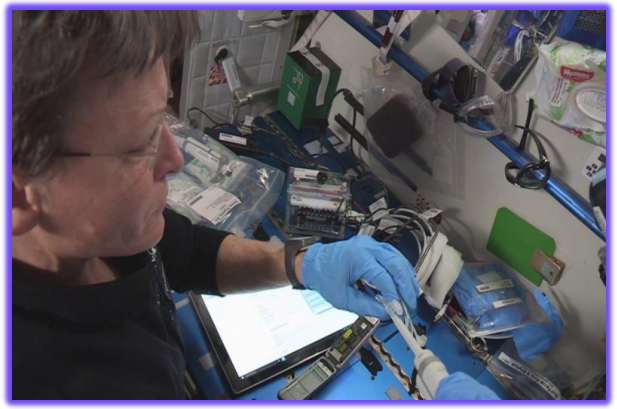

Percentage of reads assigned to ZymoBIOMICS Microbial Community DNA Standard

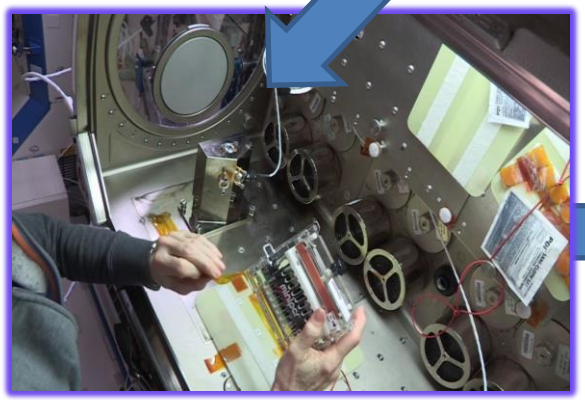



*Burton et al.* https://www.mdpi.com/2073-4425/11/1/76

# Genes in Space-3: Culture-to-Sequencer





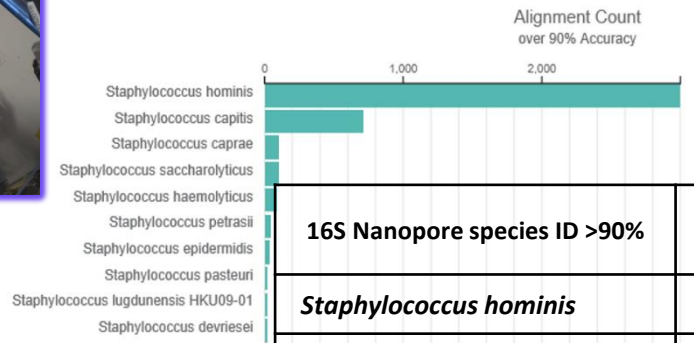




Data Downlinked to Earth



## Ground: Data Analysis




| 16S Nanopore species ID >90% | % Flight reads |
|---|---|
| ***Staphylococcus hominis*** | **71.3%** |
| ***Staphylococcus capitis*** | **17.0%** |

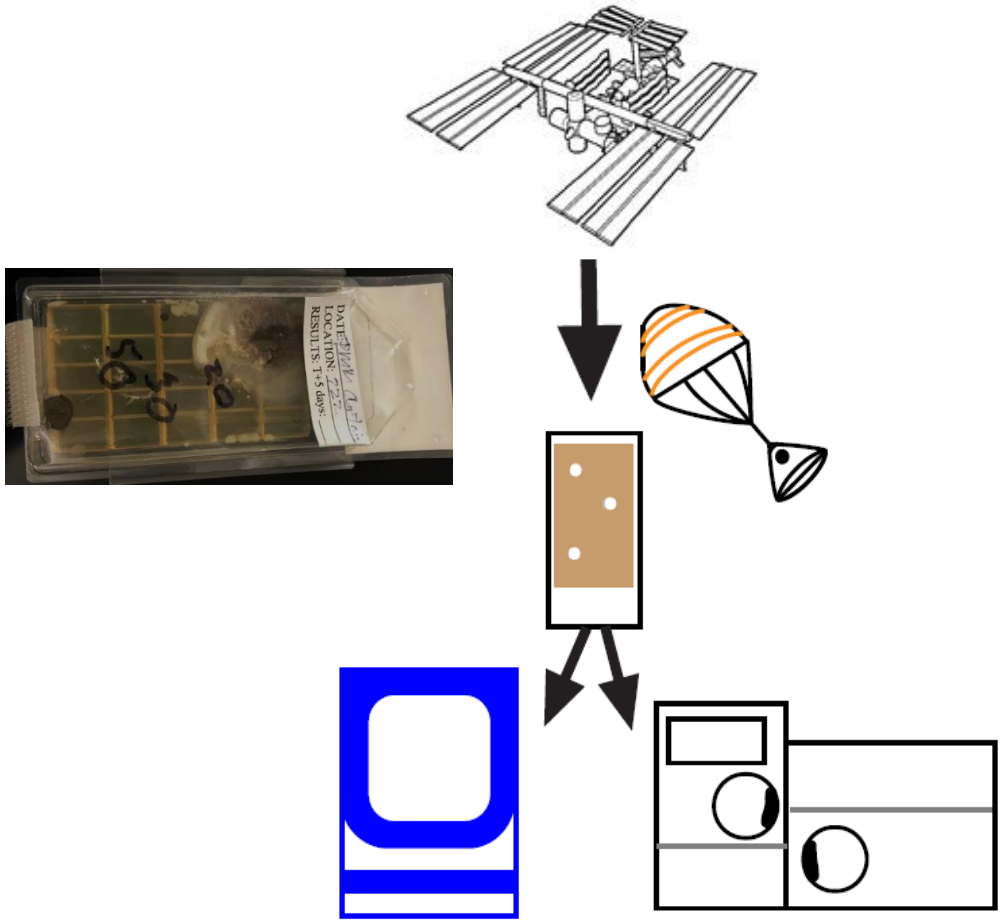
## ISS Identifications

3 colonies selected, 2 identifications

- *Staphylococcus hominis*
- *Staphylococcus capitis*

*Burton et al.* https://www.mdpi.com/2073-4425/11/1/76

# Genes in Space-3: Culture-to-Sequencer




The returned sample is processed via the NASA JSC Microbiology Laboratory's standard methods for microbial identification.

Same 3 colonies analyzed on Earth per standard procedures (biochemical analysis and Sanger sequencing)

| Colony | Detection Method | Sample ID | %ID |
|---|---|---|---|
| 1 | Biochemical | *Staphylococcus hominis hominis* | 97.0 |
| 2 | Biochemical | *Staphylococcus hominis hominis* | 97.0 |
| 3 | Biochemical | *Staphylococcus capitis* | 94.0 |
| 1 | Sanger Sequencing | *Staphylococcus hominis hominis (ATCC=27844)* | 99.9 |
| 2 | Sanger Sequencing | *Staphylococcus hominis hominis (ATCC=27844)* | 100.0 |
| 3 | Sanger Sequencing | *Staphylococcus capitis capitis (ATCC=27840)* | 99.9 |

Earth Identifications

- *Staphylococcus hominis*
- *Staphylococcus hominis*
- *Staphylococcus capitis*

ISS Identifications

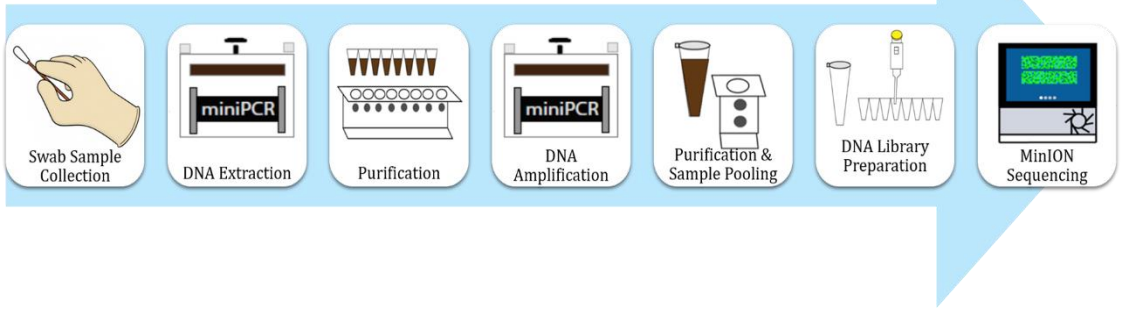
3 colonies selected, 2 identifications

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- *Staphylococcus capitis*

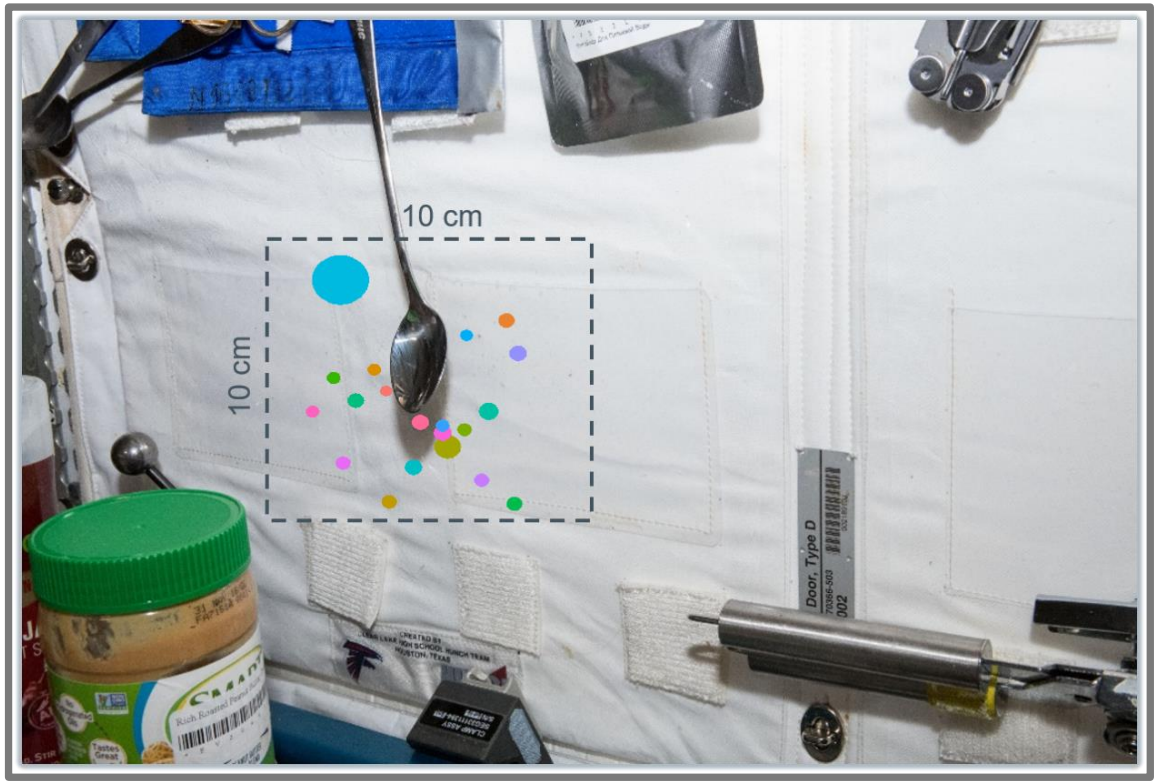
*Burton et al.* https://www.mdpi.com/2073-4425/11/1/76

# BEST: Swab-to-Sequencer



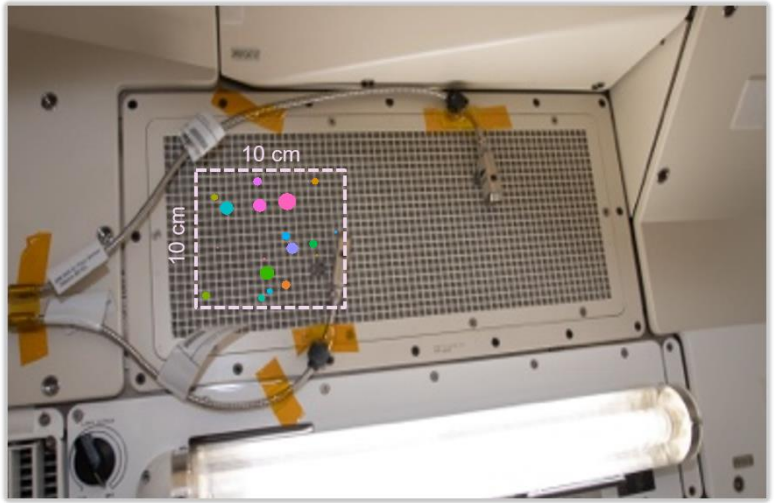

## BEST Payload: Full Swab-to-Sequencer Testing onboard ISS



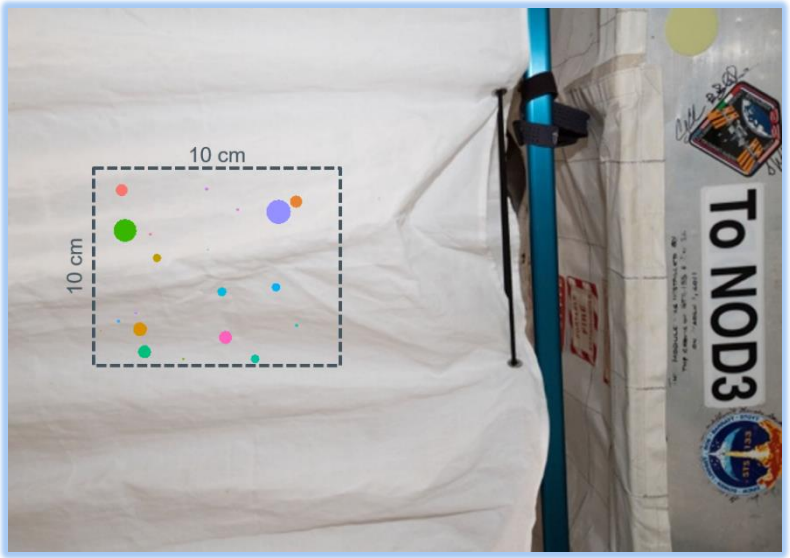

*Stahl-Rommel et al.* https://www.mdpi.com/2073-4425/12/1/106/htm

# BEST: Swab-to-Sequencer

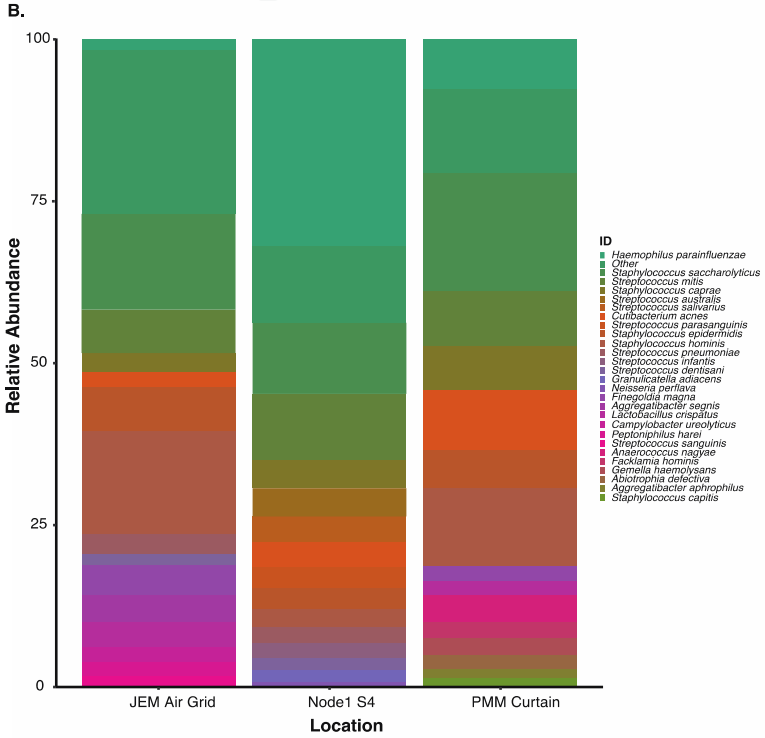







## BEST Payload: Full Swab-to-Sequencer Testing onboard ISS



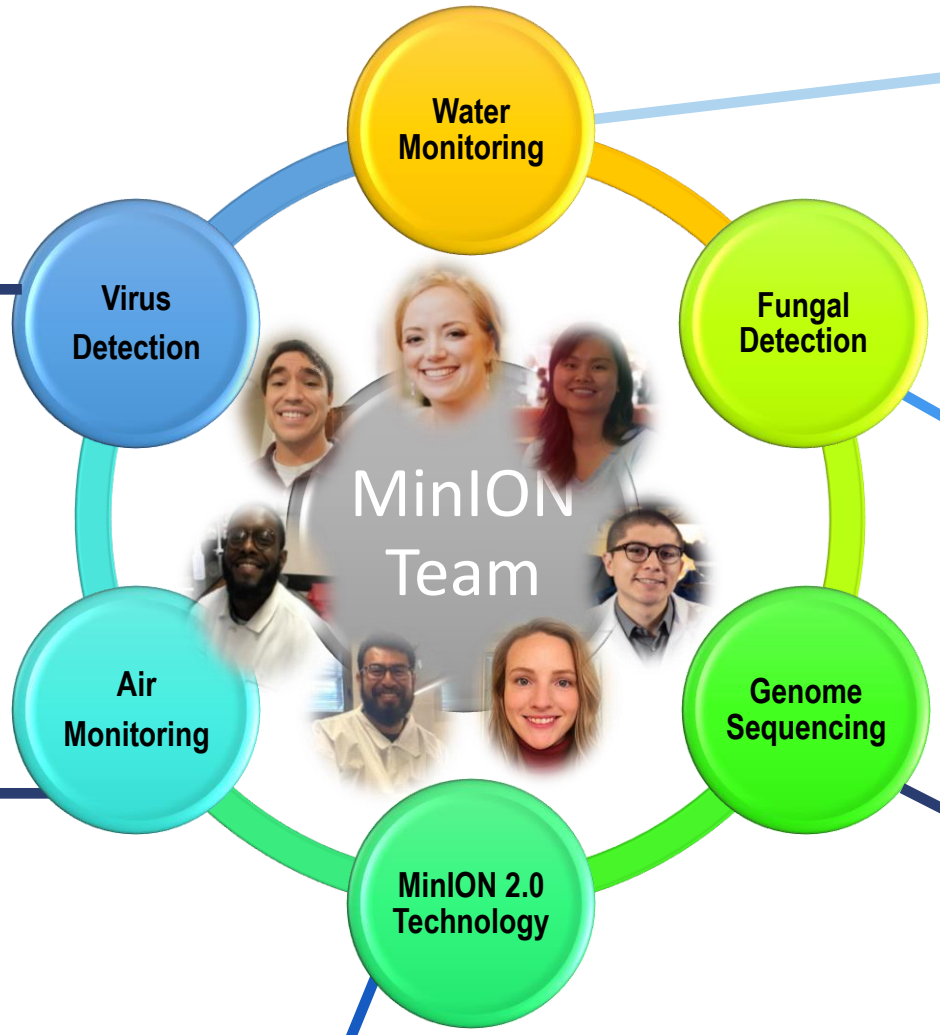

Microbial profile of the top 15 suggested species level identifications present at three distinct locations onboard the International Space Station detected by a culture-independent swab-to-sequencer method. As part of the work, the crew also processed negative swabs. Reads from the negative did not map to 16S (bacteria).

*Stahl-Rommel et al.* https://www.mdpi.com/2073-4425/12/1/106/htm

# Highlight of Current Projects

Expanding swab-to-sequencing protocol to detect microorganisms in water, validation and implementation of filter-to-sequencing protocol

Deploy multiplexed nanopore sequencing method to Palmer Station in Antarctica in Spring 2024 to detect virus from crew saliva as a biomarker of stress and observe countermeasure protocols impact.




Validation of environmental fungal detection using culture-independent nanopore swab-to-sequencing protocol

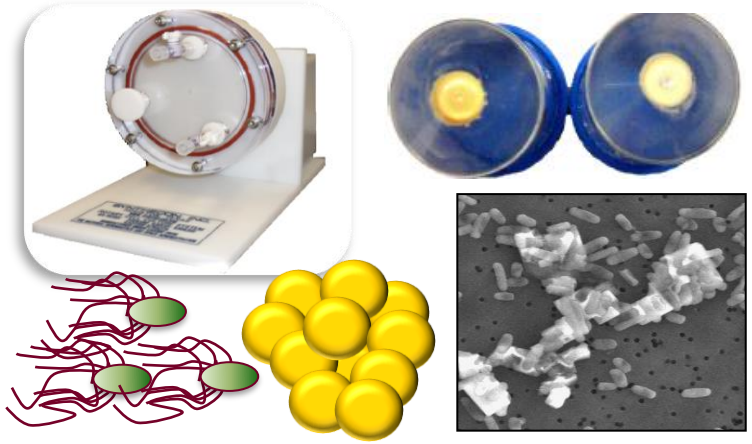
Develop Culture-independent Nanopore Sequencing method for environmental air monitoring.

Develop Hybrid Whole Genome Sequencing capabilities from generating high quality long read DNA, Illumina and Nanopore library preparation, genome assembly and annotation.

Certify MinION Mk1C technology for use on the ISS, expand real-time analysis capabilities

# Areas of Research within the Microbiology Lab

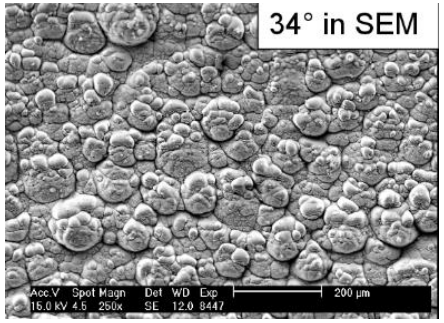
## *Microbial Responses to Spaceflight and Spaceflight -Analog Culture*





- How does the spaceflight and spaceflight-analog culture environment impact bacterial behavior?
- HRP Host Pathogen collaboration with Dr. Cheryl Nickerson (PI) at ASU

## *Engineering Testing: Disinfection and Surface Materials Analysis*




Collaboration with NASA engineering (JSC) and University of Nebraska

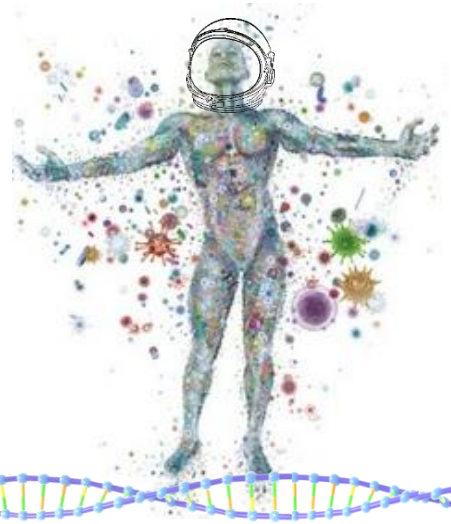
- Leveraging work within academia to develop an antibacterial and antifouling metal surface for the medical industry
- Laser processing of fins for brazing to physically create a hydrophilic surface, thereby bypassing the need for a coating (Next-generation condensing heat exchanger)
- Microgravity Membrane Air Conditioner (MMAC)
- Silver and UV disinfection studies

## *Astromaterials Research and Exploration Sciences Collaborations*



- Technology collaborations with the MinION flight experiments
- Research with the MinION in extreme environments
- Collaborations with the sample return curation group toward microbial monitoring of labs
- Development of standardized processes for both culture-dependent and culture-independent microbial monitoring

## *Astronaut Microbiome: Standard Measures Microbiome & NASA Omics*

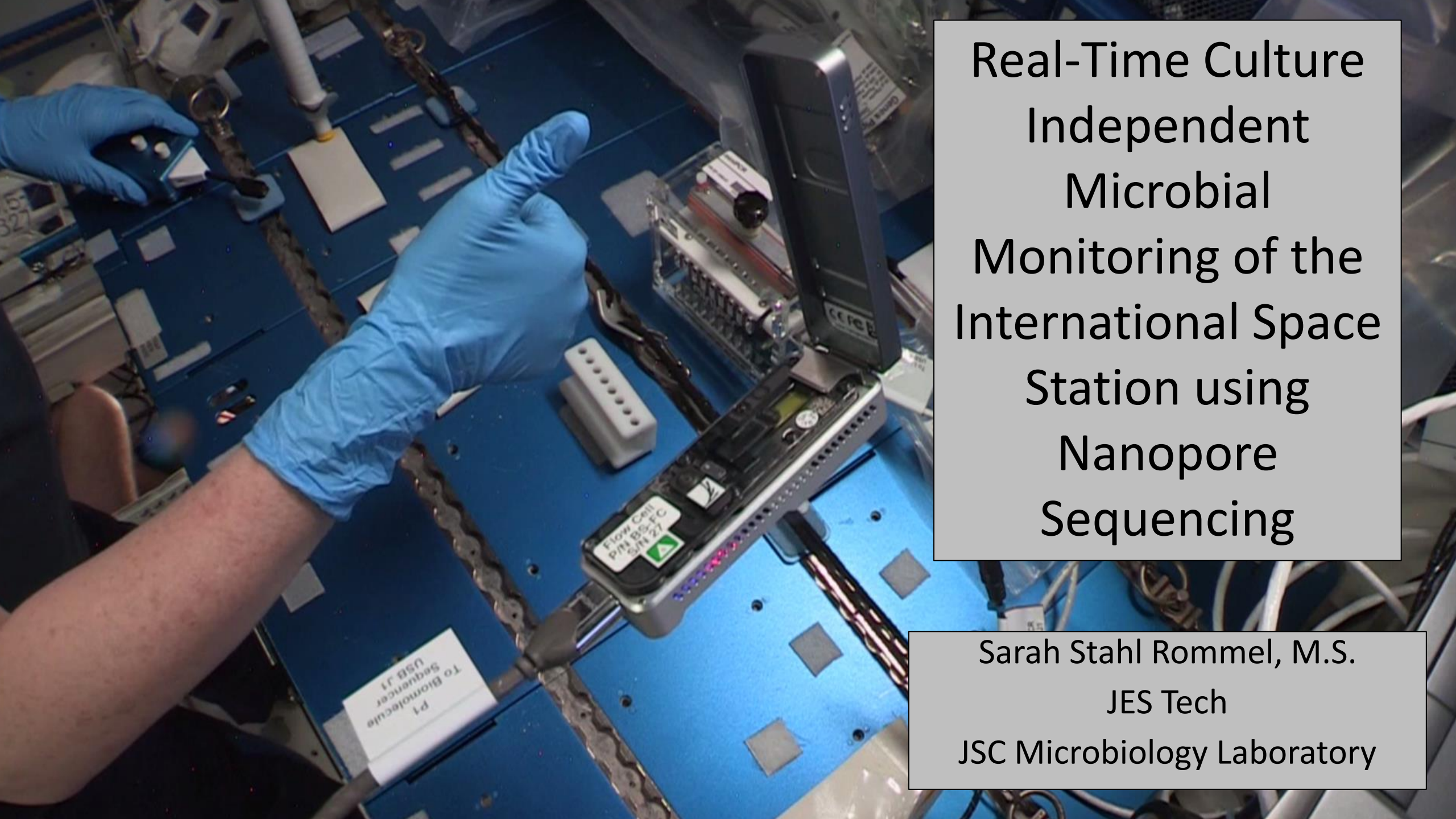


- Investigation designed to determine if the microorganisms associated with the astronaut (skin, nose, GI tract) are altered during spaceflight
- Preflight, in-flight, postflight sample collection
- Full bacterial characterization from all sites for all subjects participating

# NASA JSC Microbiology Laboratory

Mohammad Abu-Awad
Debbie Aldape
Audry Almengor, Ph.D.
Christian Castro
Brandon Dunbar
Todd Elliott
Jane McCourt
Christian Mena
Hang Nguyen, Ph.D.
Lindsey Papineau
**Mark Ott, Ph.D.**
Sarah Stahl Rommel
Patrick Rydzak, Ph.D.
Marie Sharp, Ph.D.
Starla Thornhill, Ph.D.
Melanie Smith
**Sarah Wallace, Ph.D.**






# Real-Time Culture Independent Microbial Monitoring of the International Space Station using Nanopore Sequencing

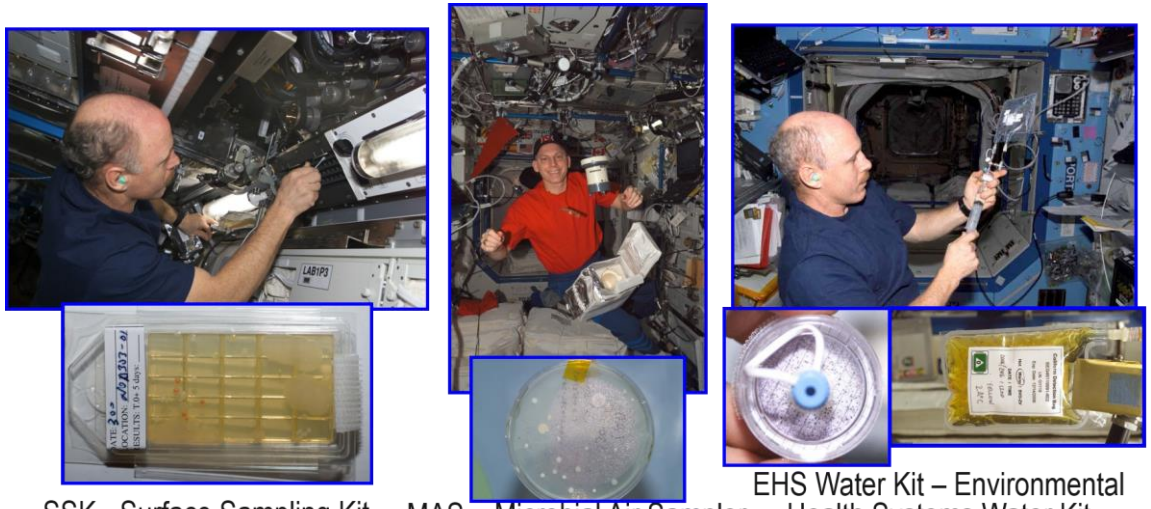
Sarah Stahl Rommel, M.S.
JES Tech
JSC Microbiology Laboratory

# Outline

- Microbial Monitoring past and present
- Timeline to Today
- Swab-to-Sequencer method development: laboratory and analog testing
- Fundamental flight experiments
- BioMole Facility
- Water Monitoring

# Microbial Monitoring on the ISS

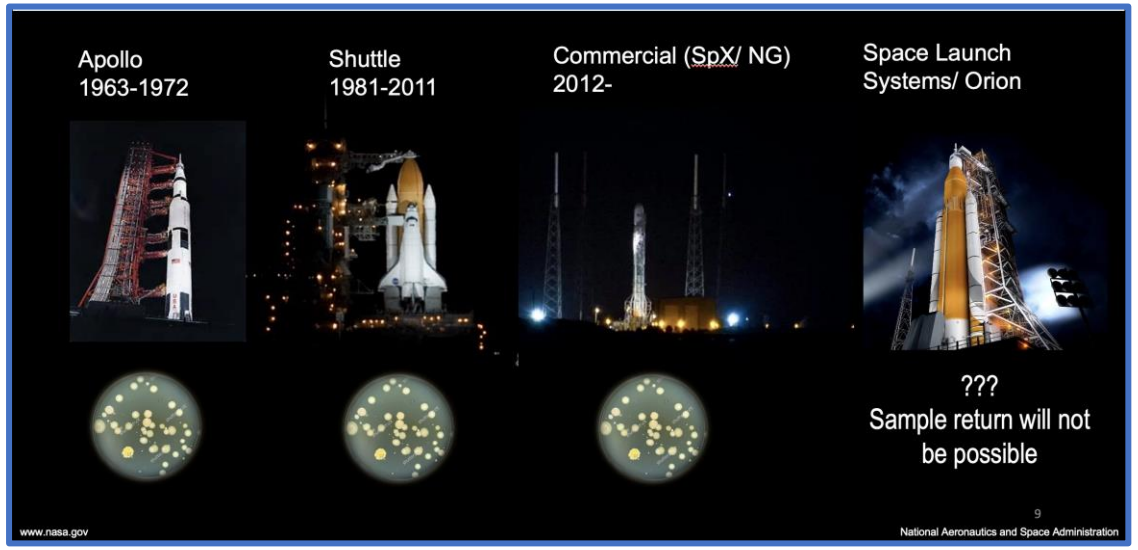
- Current microbial monitoring relies on culture-based methods
  - Requires sample return for ground-based analysis
  - Long turnaround time
  - No ability to rapidly respond to real-time microbial concerns
  - Increased risk of crew exposure to potential pathogens
- What happens to microbial monitoring when culture-based methods and sample return are not possible?
- NASA goal: develop culture-independent, near real-time microbial monitoring method
- BEST Research Payload tested first culture-independent sequencing on ISS in 2018
- The BioMole Facility was launched in 2020 by the ISS Vehicle Office (OB) as a systems demonstration by Crew Health Care Systems (CHeCS) with the goal to replace the current culture-based hardware and required sample return with inflight analysis based on our swab-to-sequencer method



SSK - Surface Sampling Kit

MAS – Microbial Air Sampler

EHS Water Kit – Environmental Health Systems Water Kit



# Timeline to Today



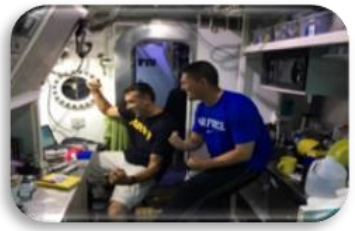
MinION Selected for Flight as Part of JSC 5 x 2015

2/2015



Swab-to-Sequencer Demonstration during NEEMO 21

7/2016



Optimized Swab-to-Sequencer Method Demonstrated during NEEMO 22

6/2017

10/2017 Near Real-Time Data Analysis Pipeline for the MinION Selected as an IRAD by JTWG

11/2018 AES-LSS Funding to Expand Capabilities (Water, Ground Studies to Compare with Culture, New Hardware)

BioMole Launched to ISS

3/2020

2015 | 2016 | 2017 | 2018 | 2019 | 2020

10/2015

Sample Prep Method for the MinION (Swab-to-Sequencer) Selected as an IRAD by JTWG





Biomolecule Sequencer: First DNA Sequencing in Space

8/2016



Genes in Space-3: First Identification of Unknown Microbes Off Earth

8/2017



BEST: Swab-to-Sequencer Demonstrated Onboard the ISS

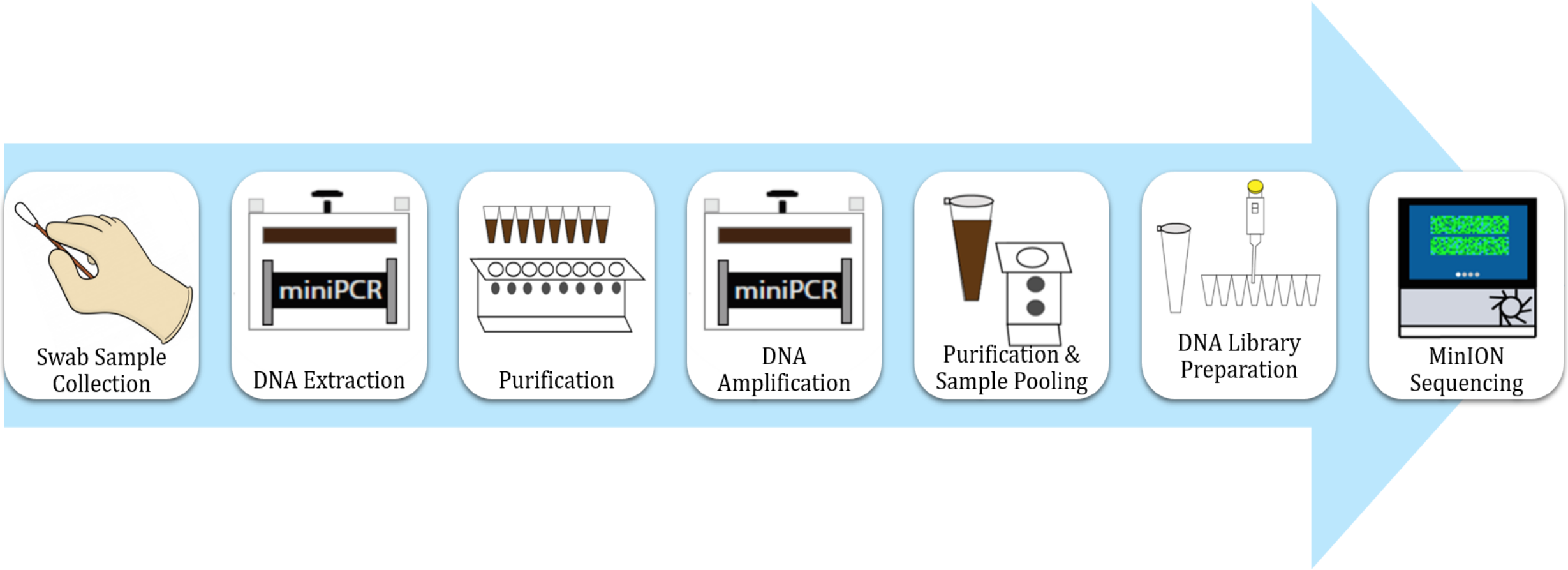
7/2018

AES-LSS Funding to Expand Capabilities (Water, Ground Studies, New Hardware)

10/2019

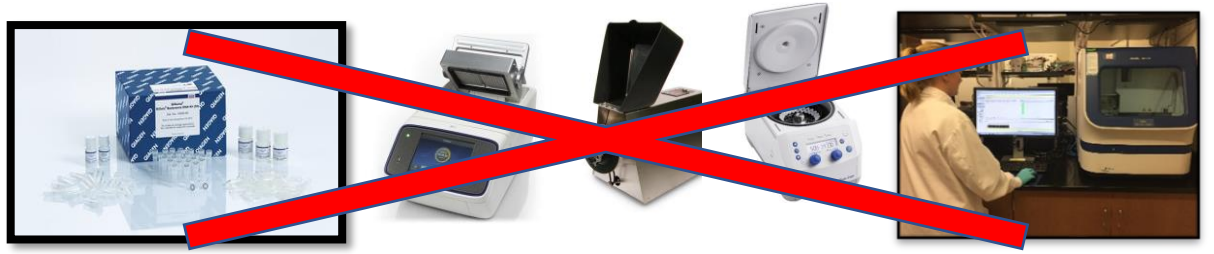
8/2020 BioMole Session 1

# Swab-to-Sequencer Method Development



# Developing Spaceflight-Compatible Sample Preparation

- Adapt laboratory methods for low biomass samples to microgravity
  - We cannot simply fly a commercial kit as used in the lab
- Crew safety, limited crew time and storage conditions
  - Toxicology and flammability
    - Each substance flown receives a toxicology rating, and anything over level 0 requires extra containment
    - No alcohols allowed
  - Compact hardware
  - Temperature constrains and kit configuration
    - Ambient, +4 °C or -90 °C
    - Shelf life
- Extensive low biomass sample testing toward:
    - Swab type
    - Buffers and reagents
    - DNA extraction
    - Purification













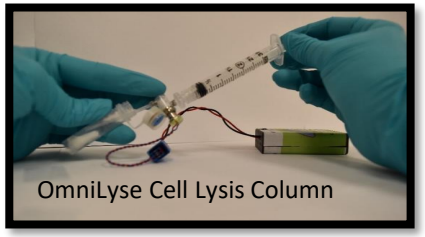








Swabs: Foam, Nylon Flocked, Polyester, Polyester Bicomponent Fiber and Knitted Alpha Polyester



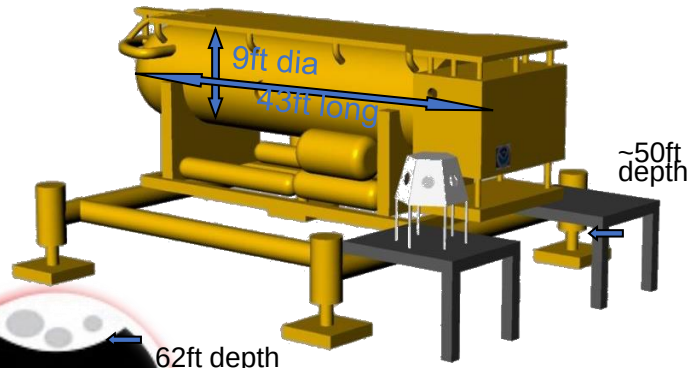






# Analog Testing of Sample Preparation for Sequencing

NASA Extreme Environments Mission Operations (NEEMO)

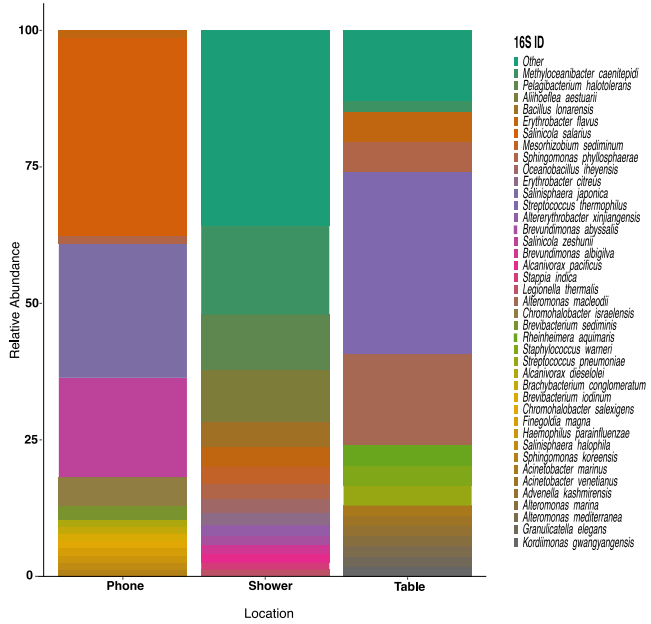





NEEMO 22 (2017)

NEEMO 21 (2016)

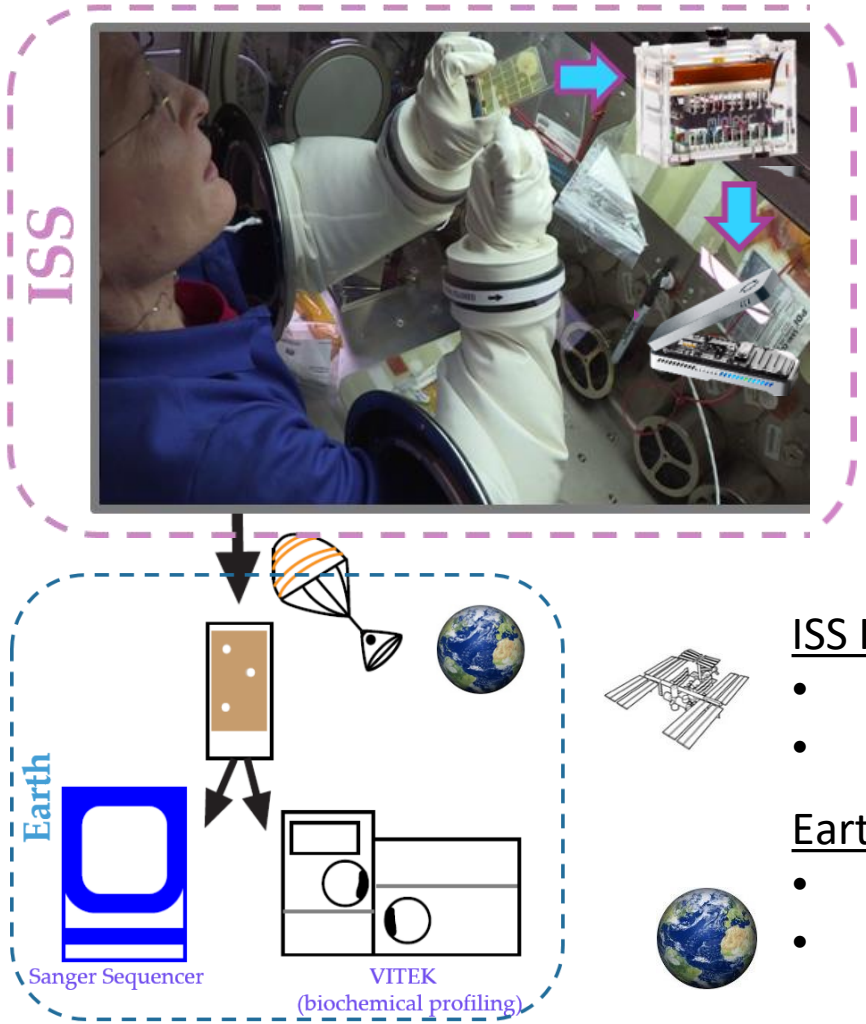



- NEEMO21 Lessons Learned:
  - Operations / Logistics
    - Cold stowage (potting plan)
    - Packaging
  - Crew procedures
  - Swab type and DNA extraction method
  - Crew training and communication
- NEEMO22 Improved Swab-to-Sequencer Protocol:
  - New swab and DNA extraction method
  - Updated crew procedures
  - Experienced cold stowage plan / operations
- NEEMO data used in a subsequent publication

*Stahl-Rommel et al.* Genes | Free Full-Text | Real-Time Culture-Independent Microbial Profiling Onboard the International Space Station Using Nanopore Sequencing | HTML (mdpi.com)

# Transitioning Sample Preparation to Space











ISS Identifications
- *Staphylococcus hominis*
- *Staphylococcus capitis*

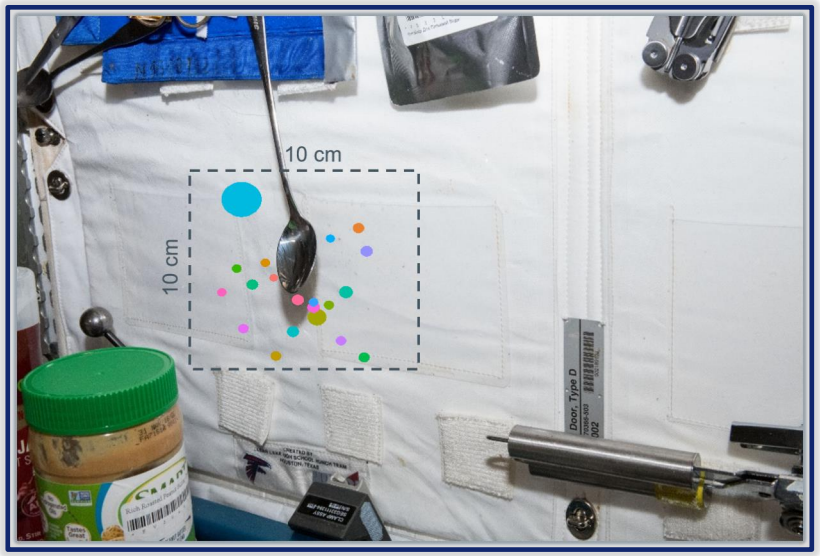
Earth Identifications
- *Staphylococcus hominis*
- *Staphylococcus capitis*

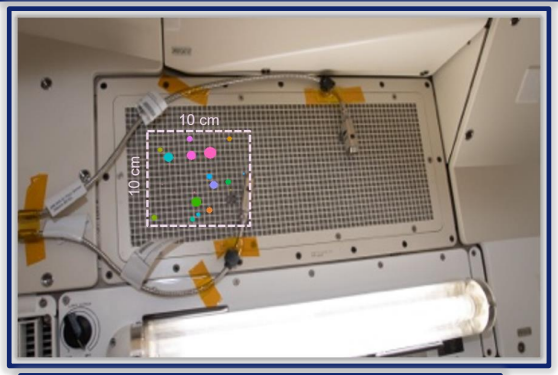
- Genes in Space-3 Payload
  - August 2017 (Exp 52), Astronaut Dr. Peggy Whitson selected 3 colonies from nominal microbial monitoring cultures for identification with the MinION sequencing method
  - First *in-situ* microbial identification on the ISS!
  - First joint use of MinION and MiniPCR
  - Validated pipetting in space
- **Burton *et al.* 2020.** Off Earth Identification of Bacterial Populations Using 16S rDNA Nanopore Sequencing
  - Genes | Free Full-Text | Off Earth Identification of Bacterial Populations Using 16S rDNA Nanopore Sequencing (mdpi.com)

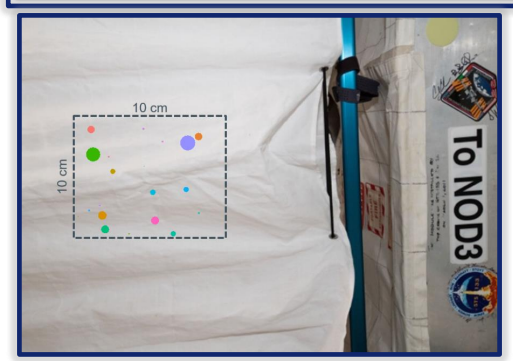
# Culture-Independent Microbial Profiling onboard the ISS



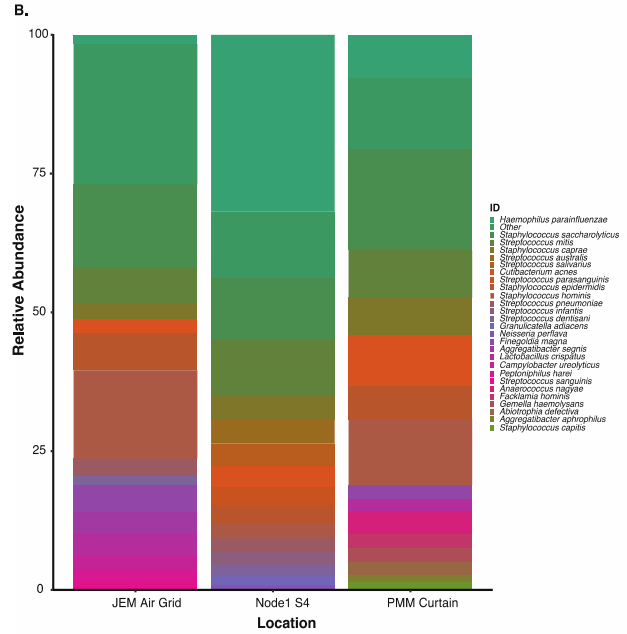





- Biomolecule Extraction and Sequencing Technology (BEST) Payload in 2018
  - 3 successful on orbit swab-to-sequencer runs during Exp 55-57
- Can NASAs microbial risk assessment be performed with this data?
  - Extensive ground studies to validate method

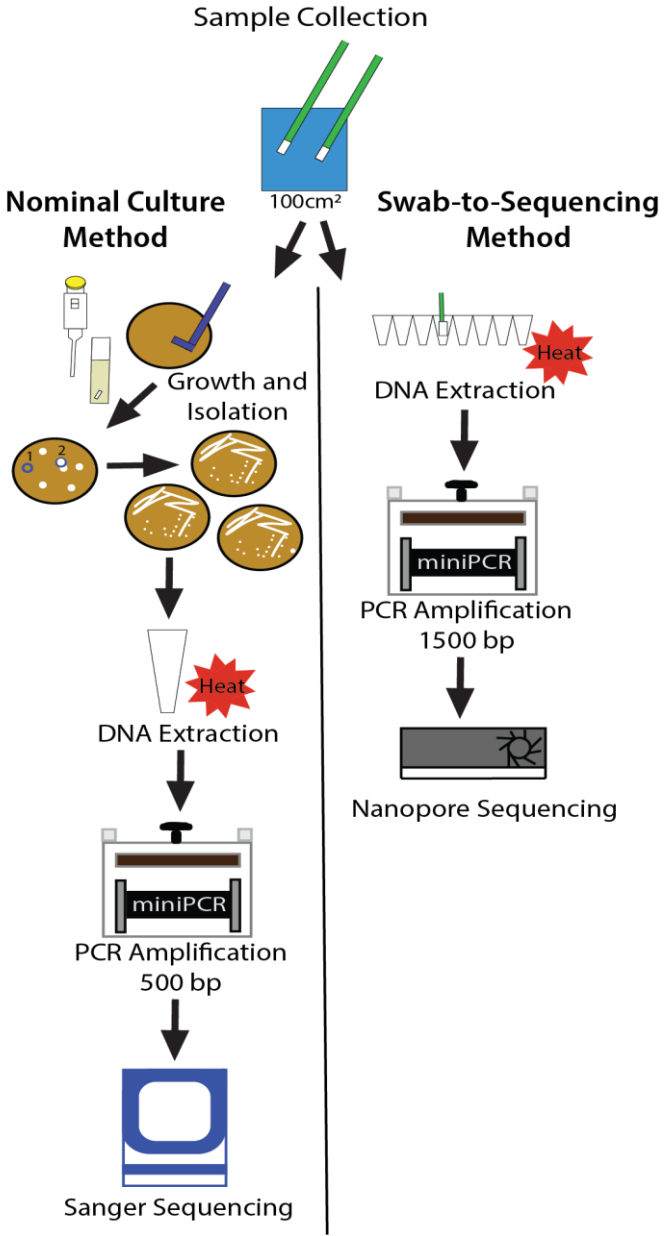


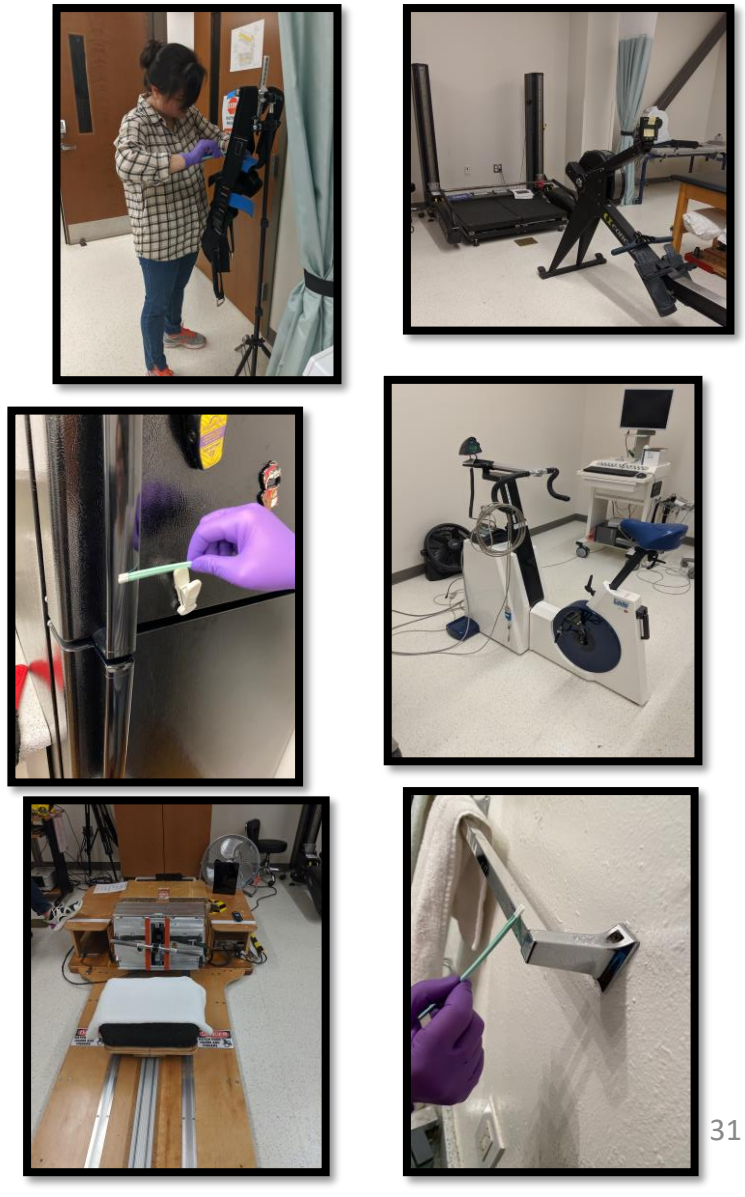

**Ground and flight data:**

*Stahl-Rommel et al.* Genes | Free Full-Text | Real-Time Culture-Independent Microbial Profiling Onboard the International Space Station Using Nanopore Sequencing | HTML (mdpi.com)

# Ground-based Method Validation

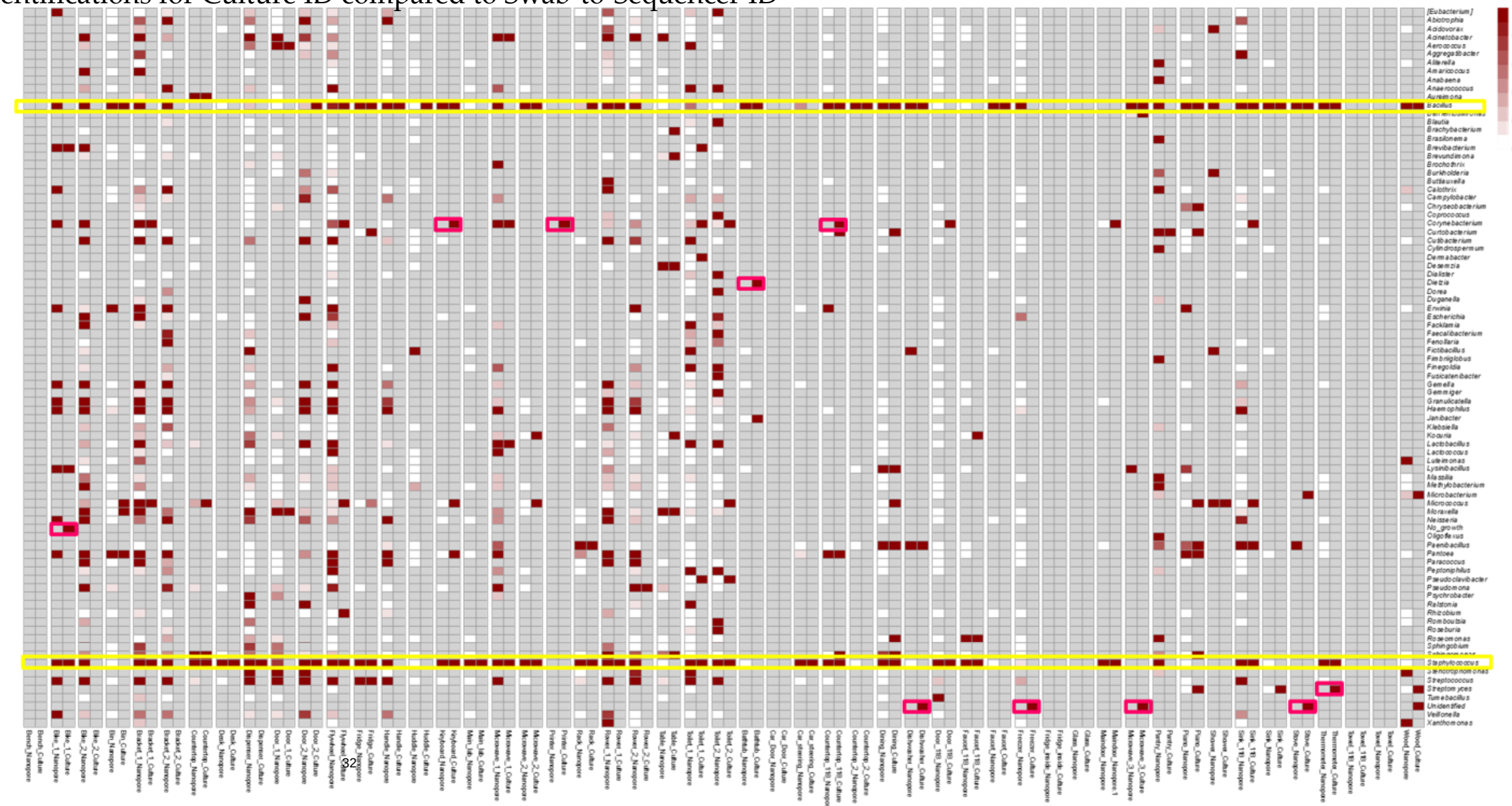
- 2 swabs collected in tandem
  - 1 used for the MinION-based swab-to-sequencer protocol
  - 1 used for culture-based analysis following the lab's standard operating procedures
- 102 total swabs (51 locations)
  - 26 swabs from the Human Health and Performance Laboratory, NASA JSC
  - 25 residential swabs
- 169 culturable bacteria isolates detected
- Different method yield similar results (next slide)



# Ground-based Method Validation

Genus Identifications for Culture ID compared to Swab-to-Sequencer ID




Nanopore = swab-to-sequencer
Culture = standard lab culture and Sanger sequencing

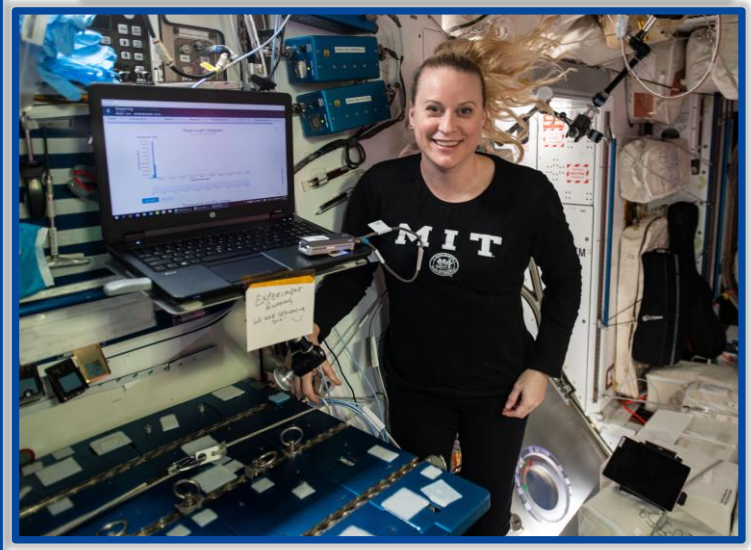
# BioMole: Crew Health Care Systems Demonstration

- **Transition from research payload to operations**
- BioMole Facility goal to perform on orbit side-by-side comparisons of SSK used in nominal microbial monitoring compared to the swab-to-sequencer method
  - 5 operations occurred with 4 crew members from July 2020 to January 2022



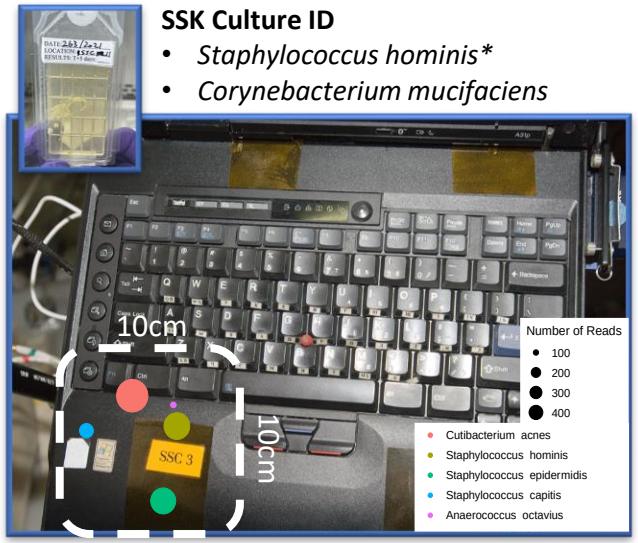






# Results of BioMole Sequencing Operations

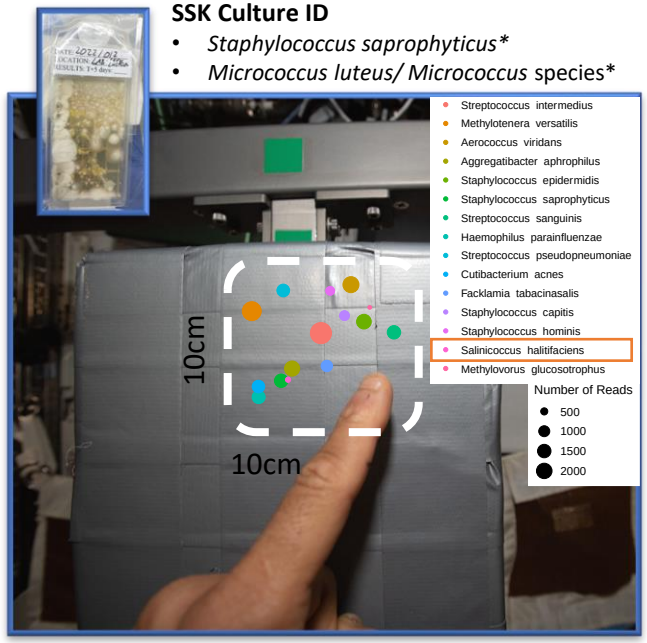
## SSC Keyboard




**SSK Culture ID**

- *Staphylococcus hominis**
- *Corynebacterium mucifaciens*

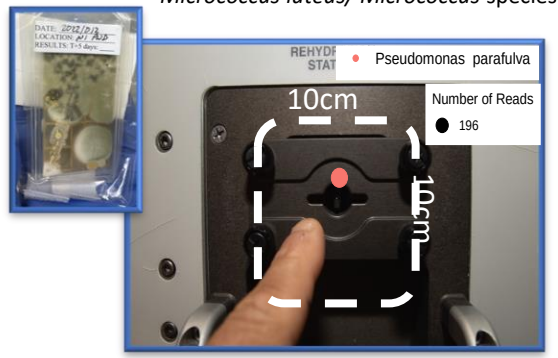
## CEVIS Seat




**SSK Culture ID**

- *Staphylococcus saprophyticus**
- *Micrococcus luteus/ Micrococcus* species*

## PWD Needle




**SSK Culture ID**

- *Staphylococcus saprophyticus*
- *Methylobacterium rhodesianum*
- *Micrococcus luteus/ Micrococcus* species

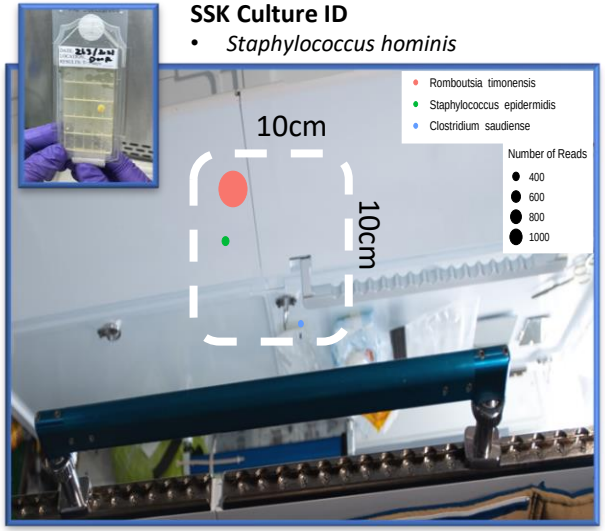
## WHC Handrail




**SSK Culture ID**

- No SSK sample return
- Match historical data

## WHC Door




**SSK Culture ID**

- *Staphylococcus hominis*

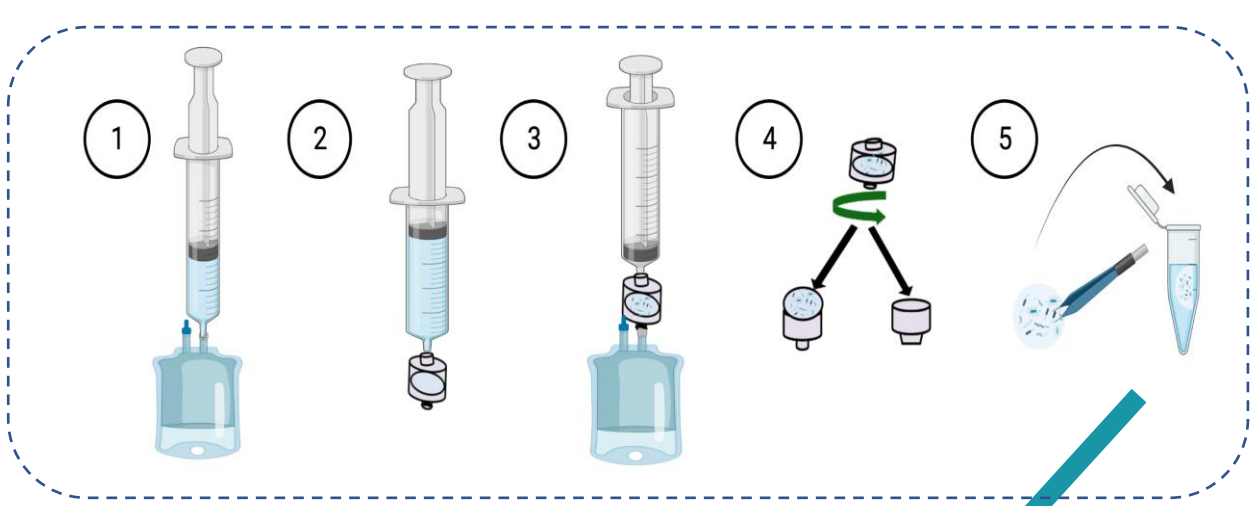
## Overall Conclusions:

- Identifications are associated with:
  - historic culture-based isolates from ISS air and surfaces
  - human and environmental inhabitants
  - Previously-published ISS molecular data (Stahl-Rommel *et al.*, 2021)
- No obligate pathogens
- Negative swabs did not map to bacteria
- *Risk assessment would not have been altered*

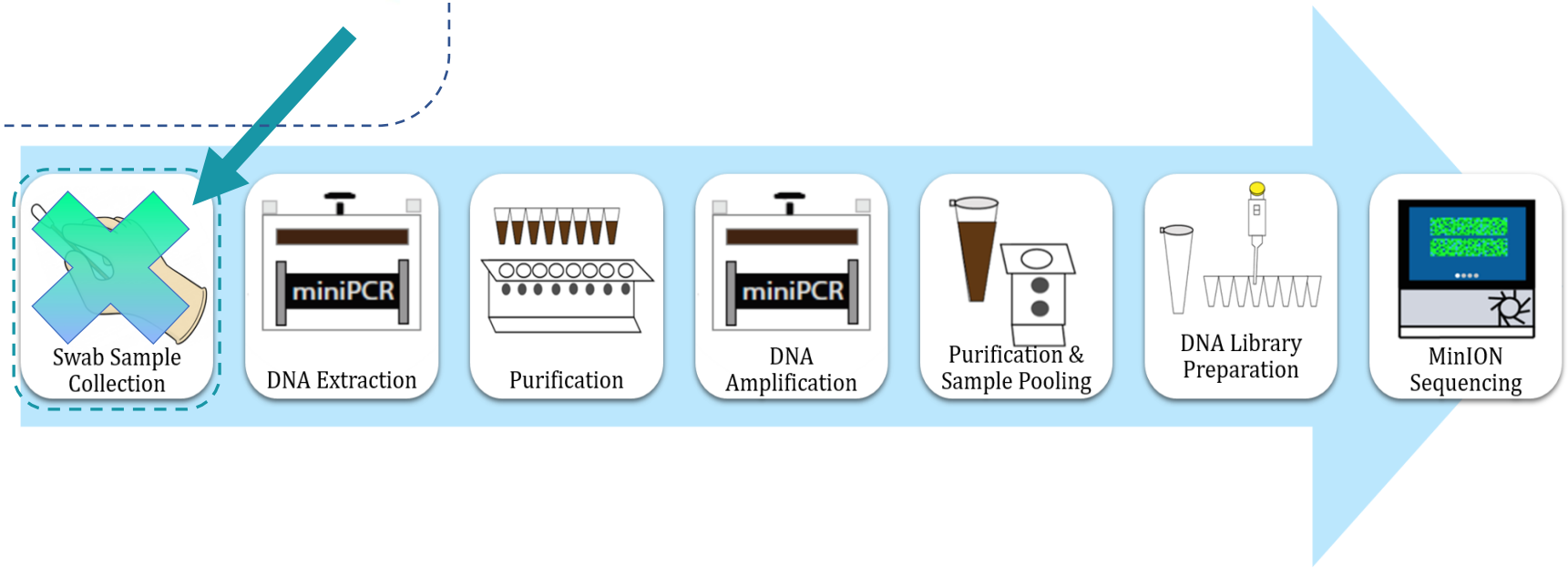

To JEM
To COL
TODAY'S
COFFEE
YESTERDAY'S
COFFEE

# Culture-independent Water Analysis

- Replace surface swabbing with water filtration for a Filter-to-Sequencer method

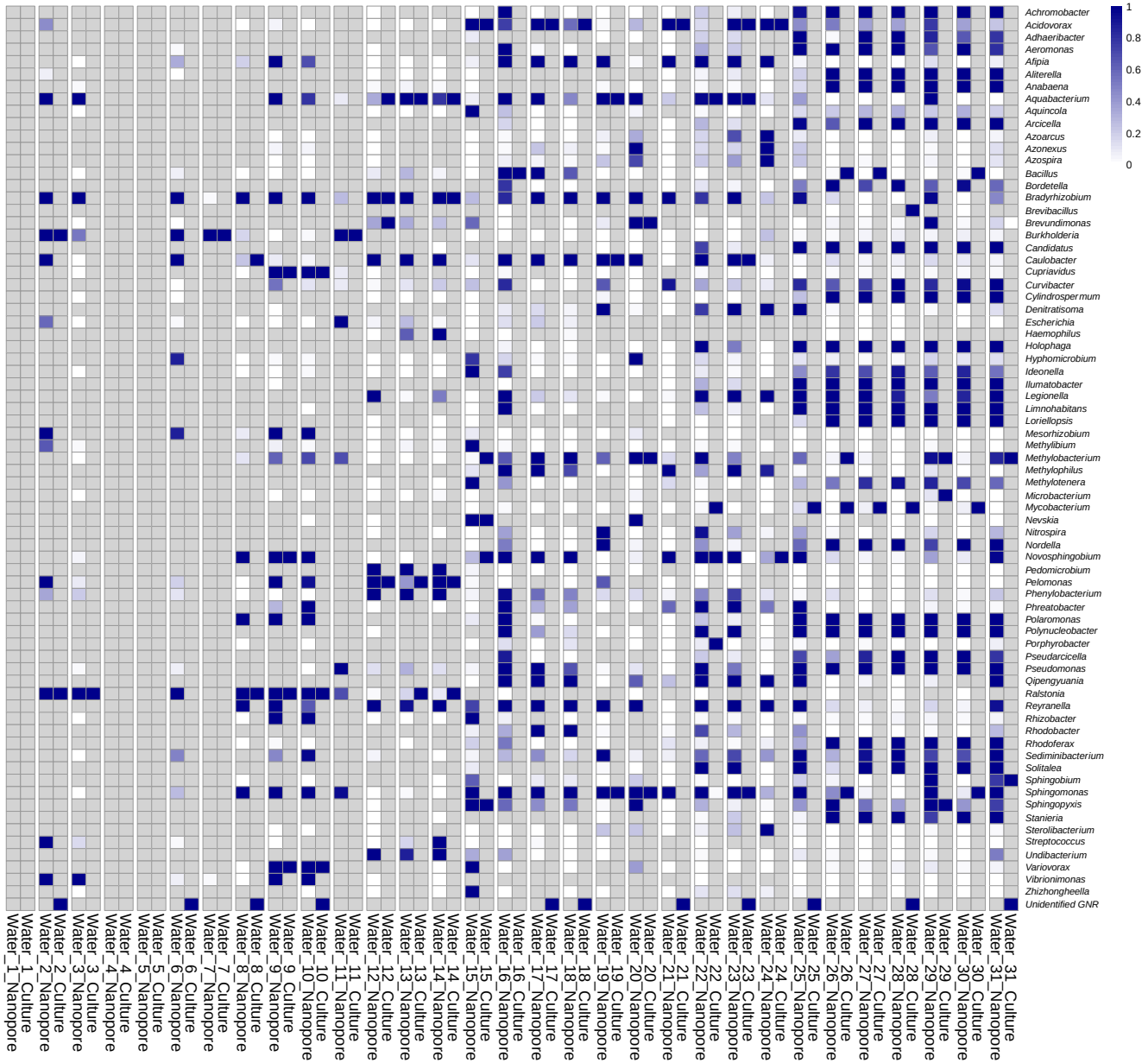



Water Filtration



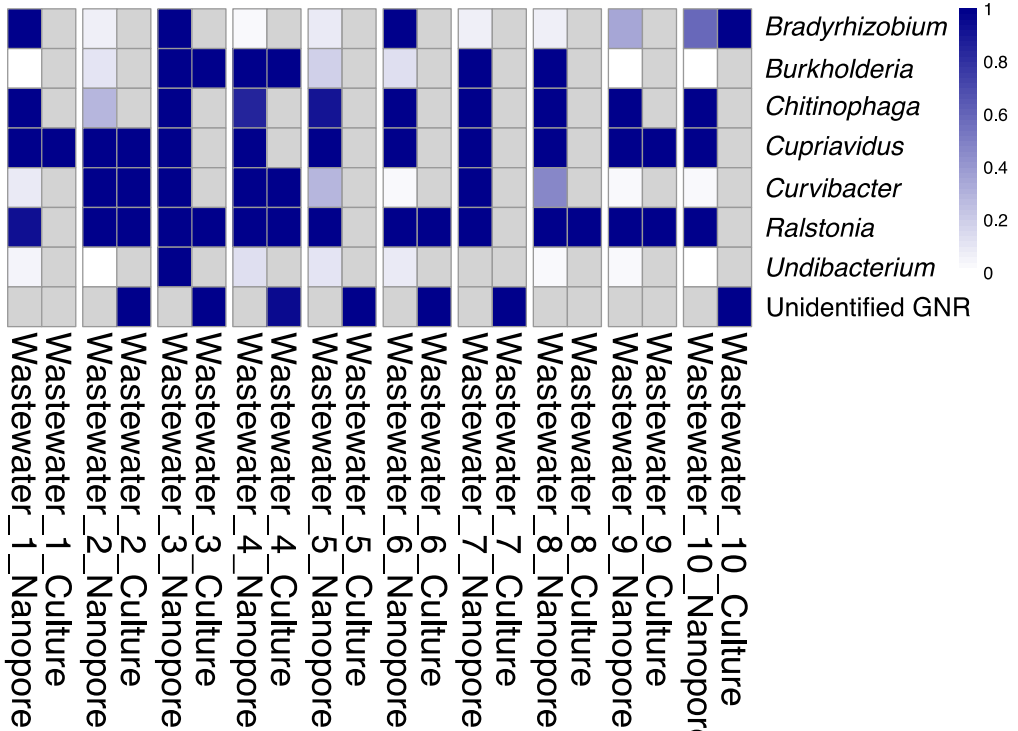
# Culture vs. MinION Water Analysis Comparison

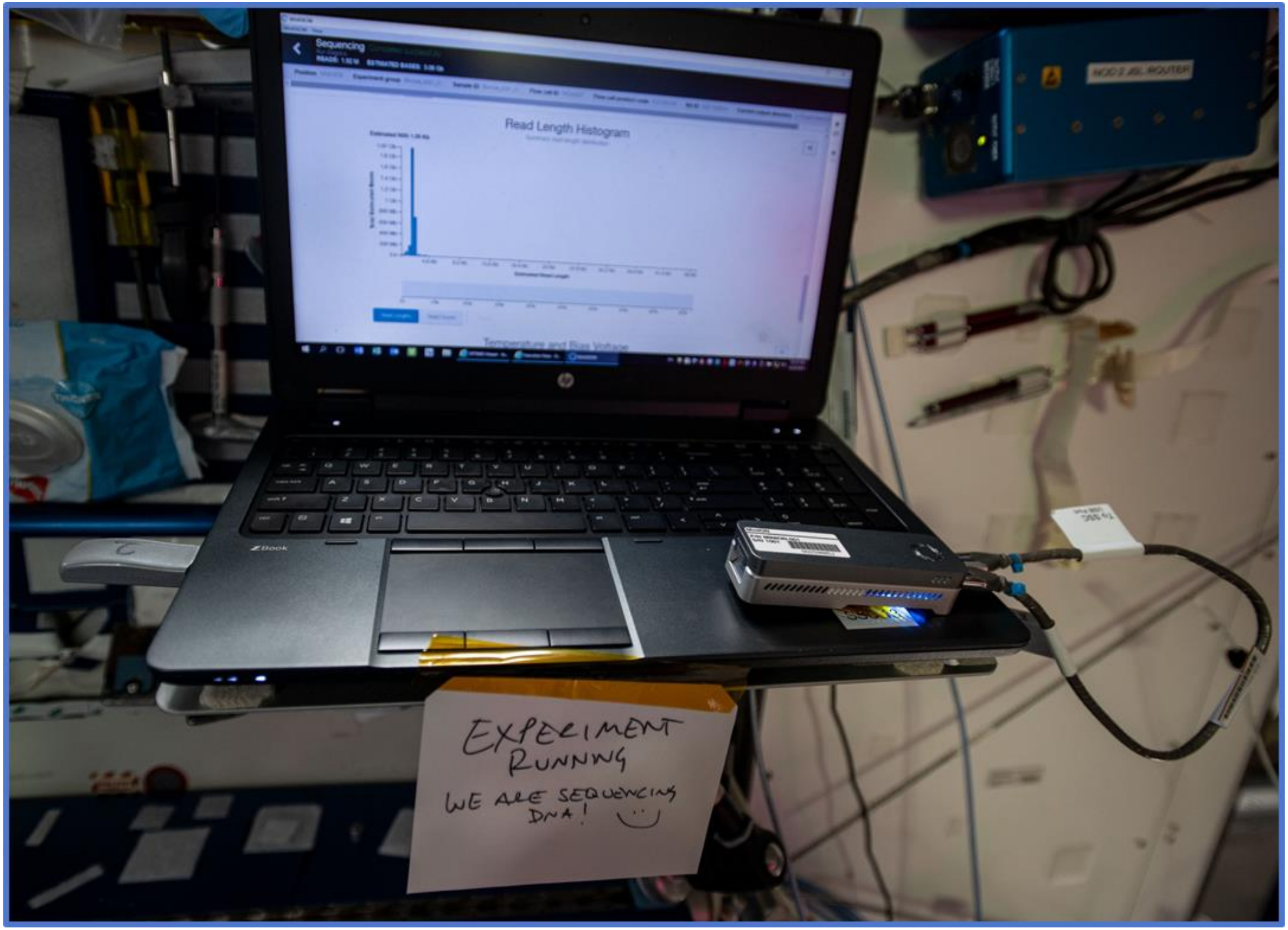
- 31 independent split samples of unknown potable water samples from ISS and ground sources.
  - 3 samples were negative for bacteria in culture and nanopore method.
  - Negative controls were negative, not shown.
- 83 total Culture isolates
  - ISS identifications were mostly *Burkholderia sp., Ralstonia sp. and* unidentified Gram-Negative Rod (UGNR)
  - Ground sample identifications mostly UGNR, *Mycobacterium, Acidovorax, Aquabacterium, Sphingomonas and Methylobacterium*
- 40% culture identifications unable to ID beyond genus level with 14% were UGNR organisms
  - This is common for water samples with the lab's SOPs
- Filter-to-sequencer nanopore IDs matched to culture isolate IDS at 99% to genus and 99% to species when UGNR and unknown species removed
- Missing nanopore ID no impact to risk assessment.
- Results support ability of nanopore to identify wide diversity of microorganisms



# Culture vs. MinION Analysis of Return ISS Wastewater

- 10 samples from ISS return wastewater and condensate.
- Culture identifications were mostly Unidentified Gram-Negative Rod and *Ralstonia sp*.
- Filter-to-sequencer nanopore IDs matched to culture isolate IDs at 100% to genus and 100% to species when UGNR removed
- Nanopore sequencing identified *Chitinophaga sp*. and *Undibacterium sp*. which were not observed in culture data
    - Based on previous laboratory testing, it is likely the Unidentified GNR are *Chitinophaga sp*.




Sequencing
Read Length Histogram
EXPERIMENT RUNNING
WE ARE SEQUENCING DNA!

# Acknowledgements



The Biomolecule Sequencer, Genes in Space-3, and BEST Payload Development Team: Sarah Castro-Wallace, Ph.D., Aaron Burton, Ph.D., and Kristen John, Ph.D.

## NASA JSC Team



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Christian Castro (JES Tech)



Brandon Dunbar (GeoControl)



Christian Mena (JES Tech)



G. Marie Sharp, Ph.D. (KBR)



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Peggy Whitson, Ph.D.



Ricky Arnold



Serena Aunon-Chancellor, MD



Christina Koch



Nick Hague



Chris Cassidy



Aki Hoshide



Raja Chari

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Michael Micorescu, Ph.D.
Fernando Izquierdo
Eoghan Harrington, Ph.D.
Zoe McDougall
Daniel Garalde, Ph.D.
The whole ONT team!






Genes in Space-3 & 6 Collaborators
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New England BioLabs
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BEST / Genes in Space-6 Ops Lead
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Sean Moore



# Culture-Independent Fungal Profiling for the International Space Station using Nanopore Sequencing: Method Development



Hang N. Nguyen[1], Sarah Stahl-Rommel[1], G. Marie Sharp[2], Christian L. Castro[1], Sarah L. Castro-Wallace[3]

[1] JES Tech, Houston, TX, USA
[2] KBR, Houston, TX, USA
[3] Biomedical Research and Environmental Sciences Division, NASA Johnson Space Center, Houston, TX, USA

# Outline

- Spaceflight microbiology and fungal monitoring on International Space Station (ISS)
- Sequencing in space
- Primer selection, optimization, and proposed workflow for validation
- Bioinformatics: optimized pipeline to improve the identifications from the current database
    - Validation with fungal cultures
    - Validation with low biomass swabs
- Fungal DNA the sequencing in Space validation – Gene in Space 6

# Spaceflight Microbiology

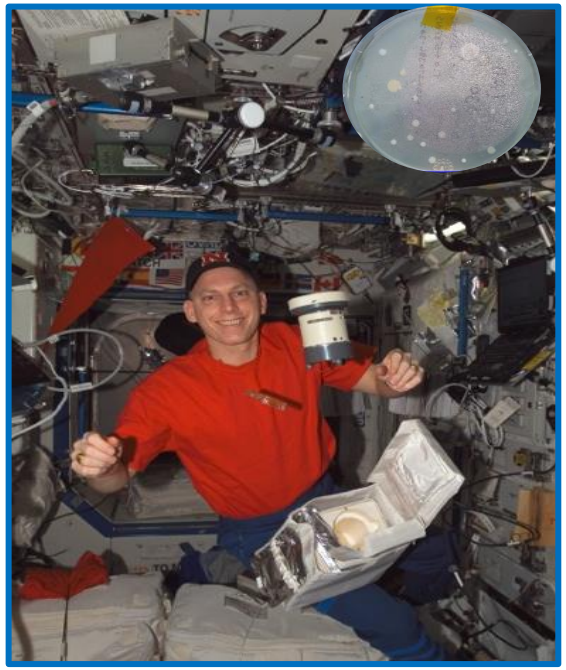
- Pre-flight monitoring of vehicles, hardware, and cargo bags
- Routine Microbial Monitoring onboard ISS

→ Minimizing microbial contamination and immediate remediation to protect crew health

**Surface**




**Air**



**Water**



# Fungal Monitoring

- Fungal growth is observed onboard the ISS
- Culture slides are collected and returned to Earth for analysis

**Cargo Bag**




**FGB Panel**




**Onboard Sampling**



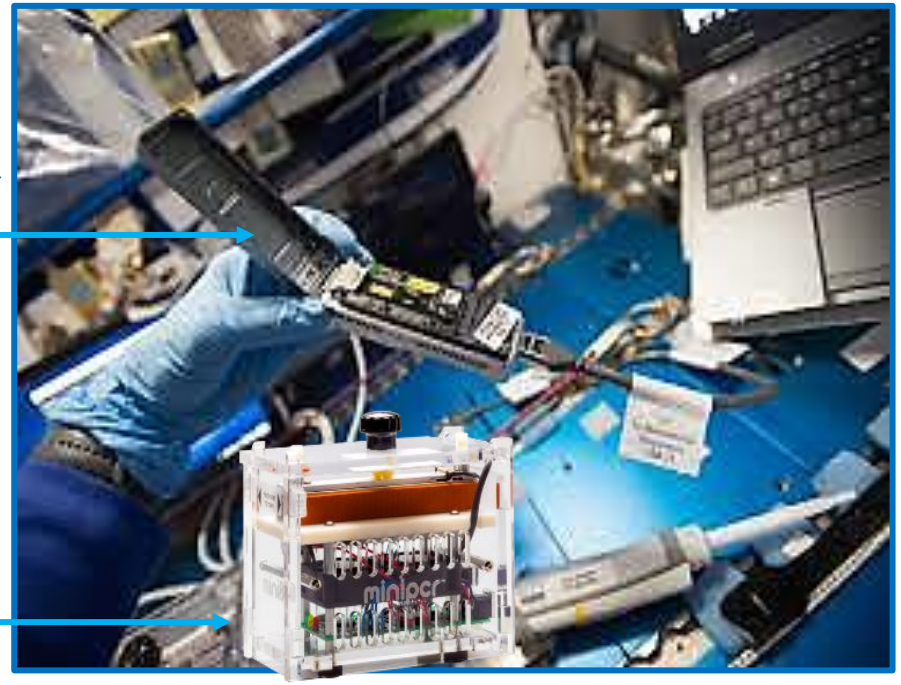
**Returned fungal slides**



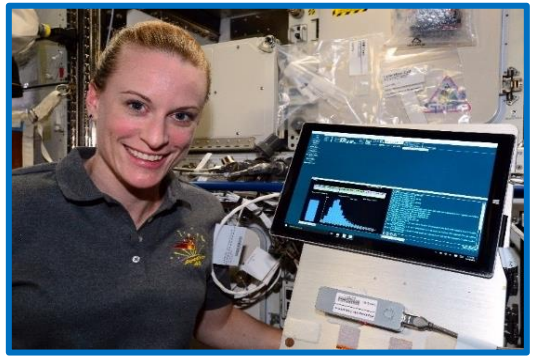


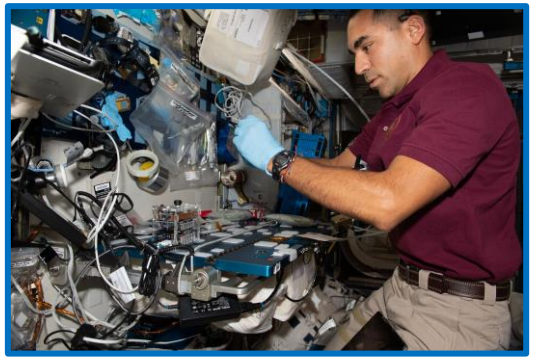

# Sequencing onboard the ISS

- Molecular biology platforms are available and functional onboard the ISS:
- DNA Sequencing in Space is possible:
  - (1) First time DNA was sequenced in space (libraries prepped on Earth)
  - (2) First identification of bacteria collected and cultured onboard the ISS
  - (3) Frist culture-independent (swab-to-sequencer) identification of bacteria onboard the ISS




(1)

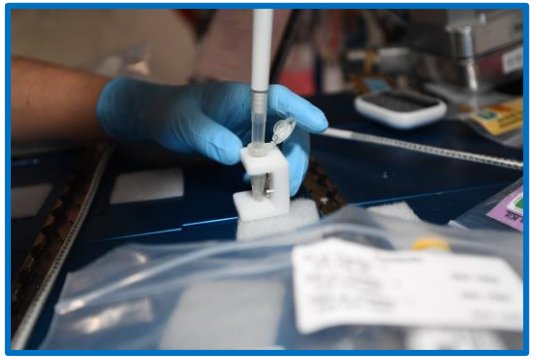




(3)

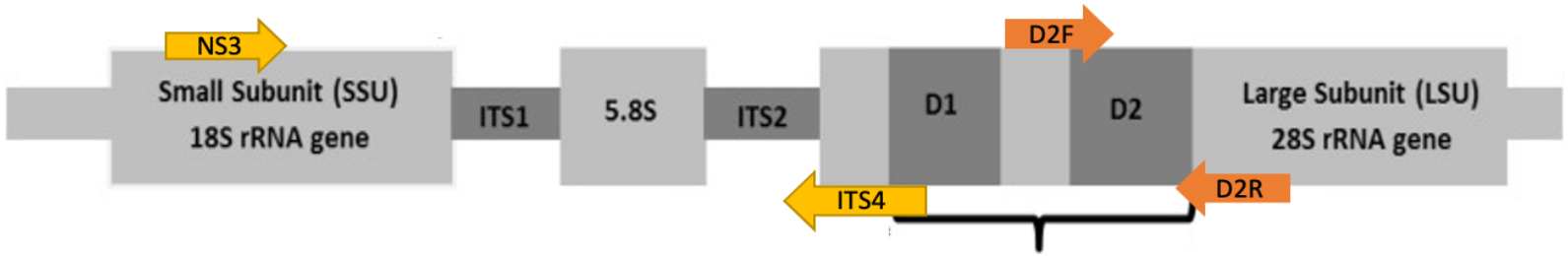
(2)





# Sample Preparation Optimization

- Primer selection
  - 7 pairs of primers
  - Amplicon ~ 1500 bp to 2000 bp
- Nanopore Sequencing Kit
  - SQK-PBK004 – 2 steps PCR was modified to increase the efficiency for low template in the case of low biomass
- PCR Amplification testing with yeast and mold: 18 fungal isolates commonly found on ISS
- Selected primers to extend beyond NS3/ ITS4




D1/D2 hypervariable regions of the LSU rRNA gene

1. *Rhodotorula* sp.*+
2. *Cryptococcus neoformans*+
3. *Cladosporium* sp.*
4. *Acremonium* sp.
5. *Trichosporon mucoides*+
6. *Saccharomyces cerevisae*+
7. *Candida albicans*+
8. *Paecilomyces lilacinus*
9. *Geotrichum candidum*
10. *Pennicilium* sp.*
11. *Aspergillus niger**
12. *Nigrospora* sp.*
13. *Fusarium* sp.*
14. *Pithomyces* sp.*
15. *Exophiala* sp.
16. *Scopulariopsis* sp.*
17. *Aspergillus flavus**
18. *Curvularia* sp.

* ISS isolates
+ Yeast

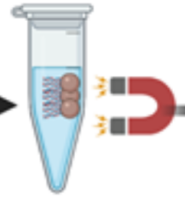
# Proposed validation workflow



## Modified swab-to-Sequencer Method - Spaceflight Compatible ready to use onboard ISS



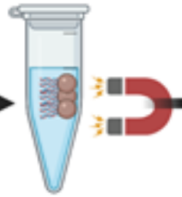
DNA extraction from swab



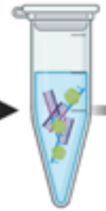
Bead-based purification



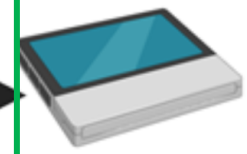
ITS region amplification



Sample pooling



Library preparation



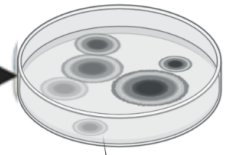


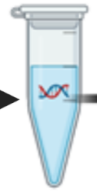
Nanopore Sequencing and analysis



Swab inserted into TSB

Plated onto SABC

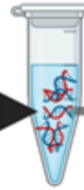






DNA extraction and PCR

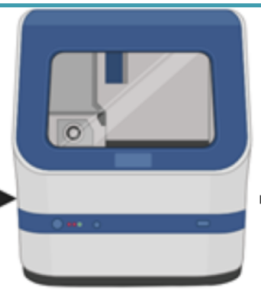
Enzymatic purification







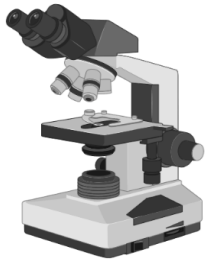
Fluorescent adaption and Purification



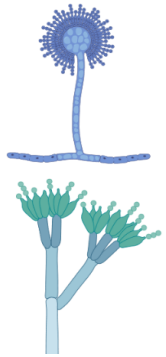
Sanger Sequencing



Fungal culture slide



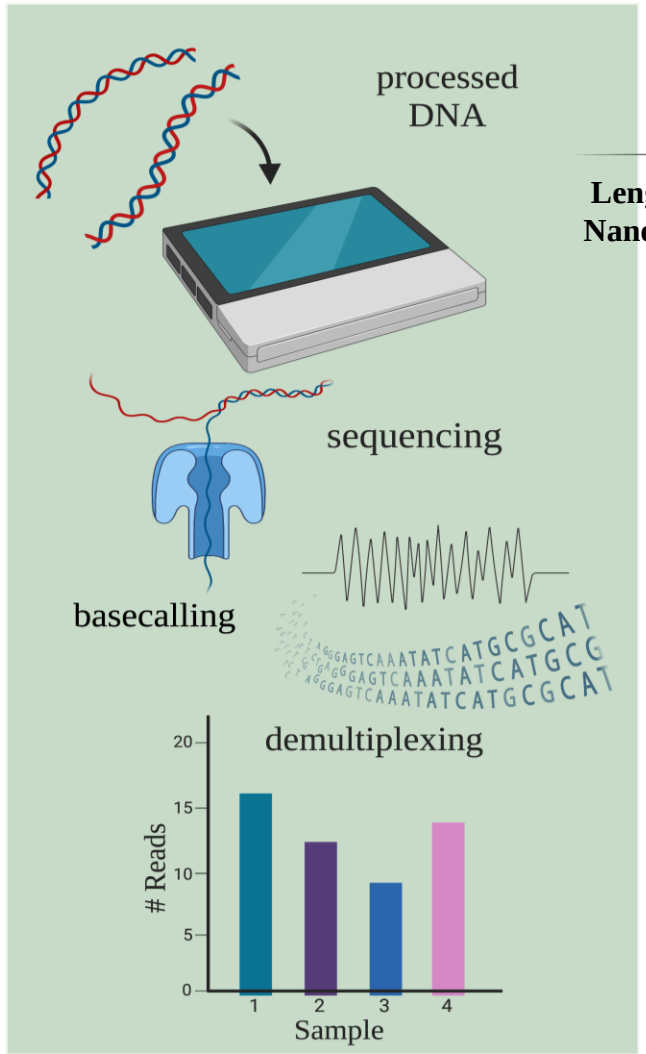
Microscopic morphology



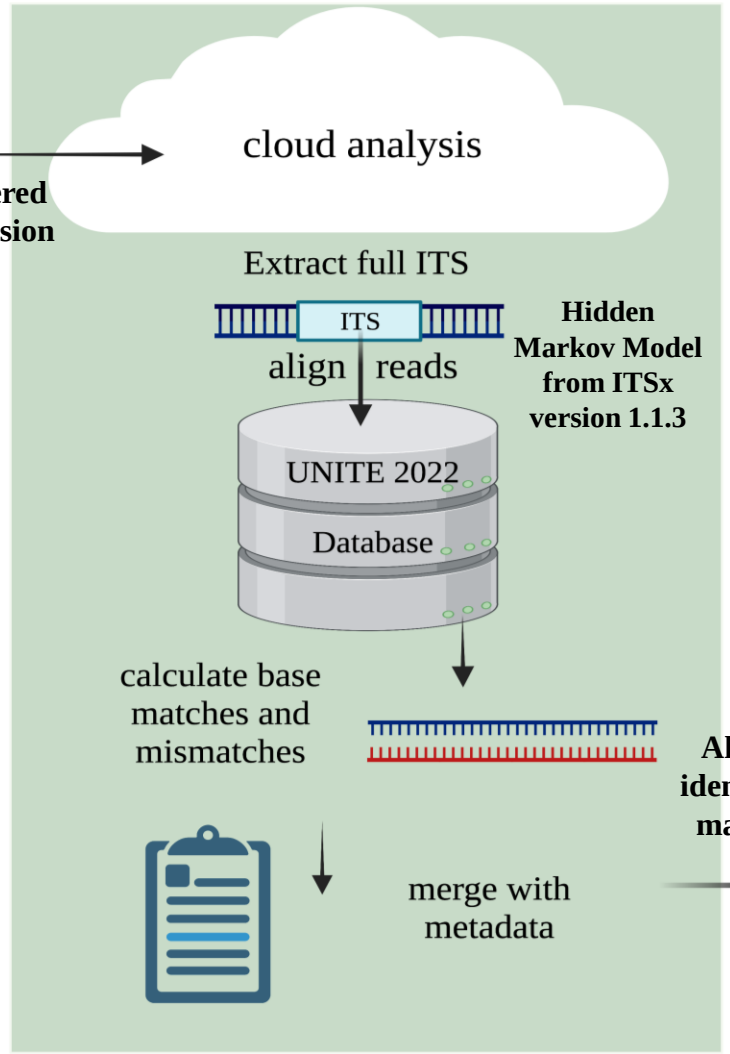
Culture identification confirmed

## NASA JSC Microbiology's Standard Operating Procedure for Fungal Identification

# Fungal Analysis Pipeline Optimization

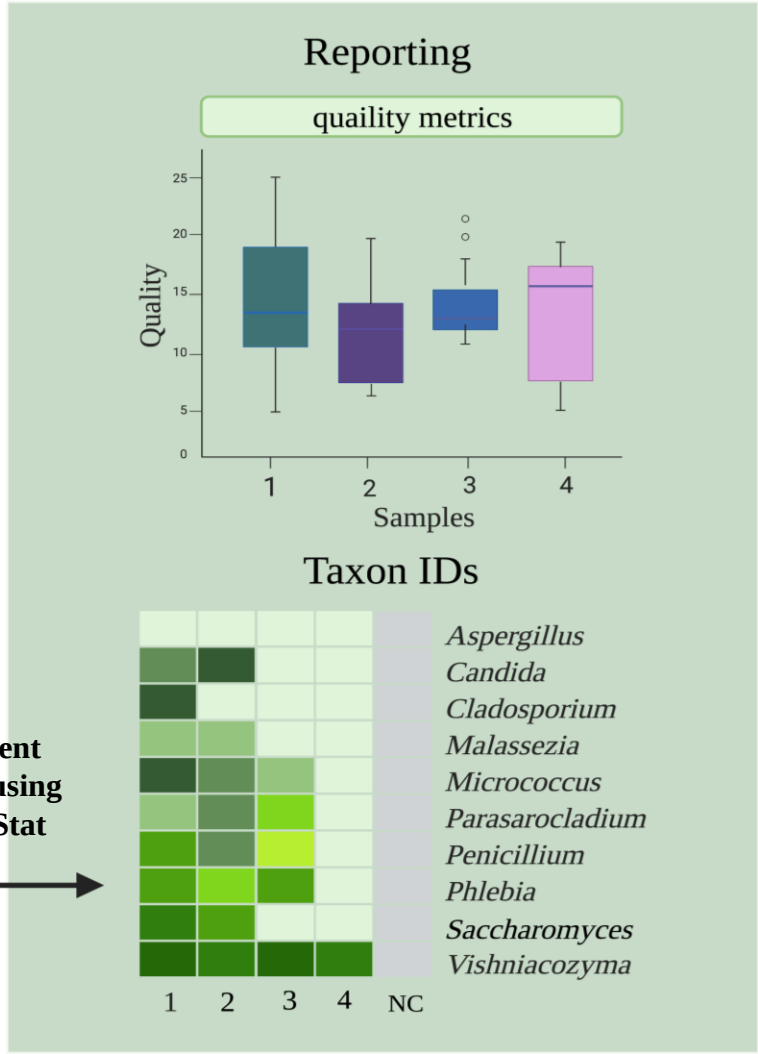



**Length filtered Nanofilt version 2.8.0.**




**Hidden Markov Model from ITSx version 1.1.3**

**Alignment identity using marginStat**




MinKNOW software versions 21.11.6-22.08.6

Mapped using minimap2-2.24 to the UNITE database

> = 90 alignment identity to the UNITE reference sequences minimum nanopore quality score (~phred score) of 10

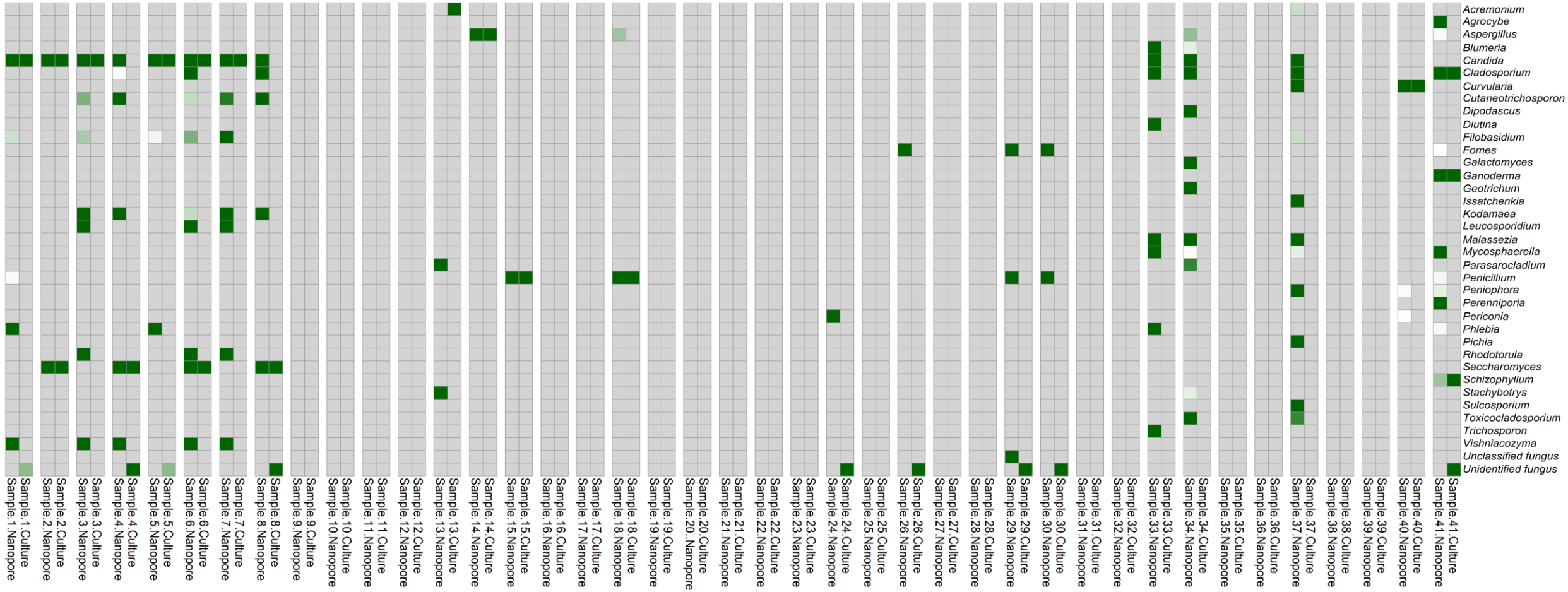
# Fungal Culture Validation

- Identified isolates from the laboratory's archive were used for validation:
  - nanopore followed the previously described workflow from extraction to library prep and sequencing
  - culture method following the JSC microbiology laboratory standard operating procedures

| **Microbiology Lab ID** | **Nanopore ID** |
|---|---|
| *Aspergillus niger* (slide) | *Aspergillus niger* |
| *Aspergillus* species | *Aspergillus clavatus* |
| *Aspergillus* species (slide) | *Aspergillus* sp. |
| *Aureobasidium* species (slide) | *Aureobasidium pullulans* |
| *Cladosporium* species (slide) | *Cladosporium halotolerans* |
| *Curvularia* species (slide) | *Curvularia affinis* |
| *Eurotium* species* | *Aspergillus montevidensis** |
| *Fusarium oxysporum* (slide) | *Fusarium oxysporum* |
| *Fusarium* species (slide) | *Fusarium oxysporum* |
| *Penicillium* species (slide) | *Penicillium commune* |
| *Penicillium* species (slide) | *Penicillium steckii* |
| *Pleurostomaphora richardsiae* | *Pleurostoma richardsiae* |
| *Pseudocercospora* species (slide) | *Pseudocercospora jagerae* |
| *Rhodotorula* species (slide) | *Rhodotorula mucilaginosa* |
| *Tritirachium oryzae* (slide) | *Tritirachium oryzae* |

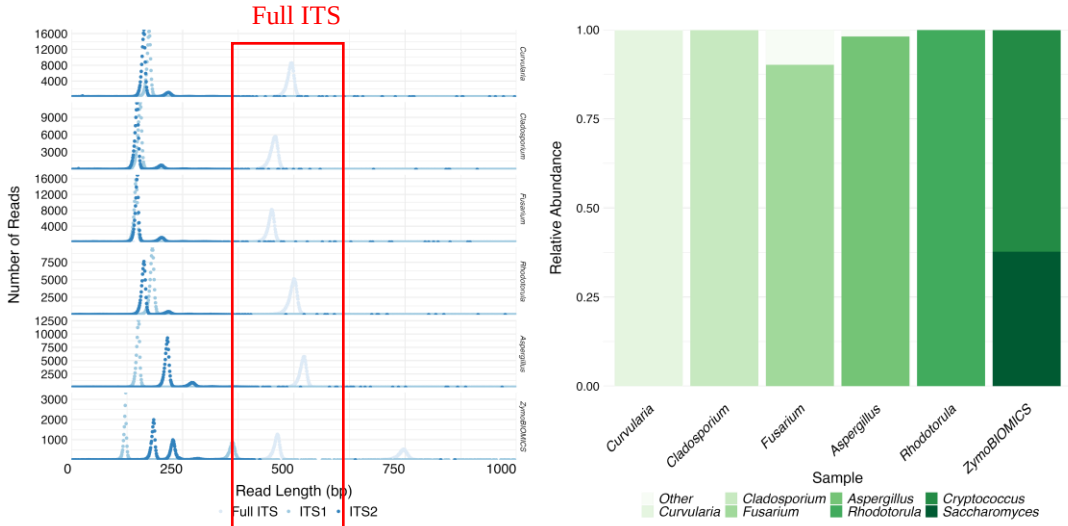
# Environmental Swab Validation

- 41 samples (82 swabs) were collected with 5 negative control swabs
- All negative control swabs were negative for both culture and nanopore
- Fungi was not identified from 19 samples (culture and nanopore)
- Higher diversity noted from nanopore data

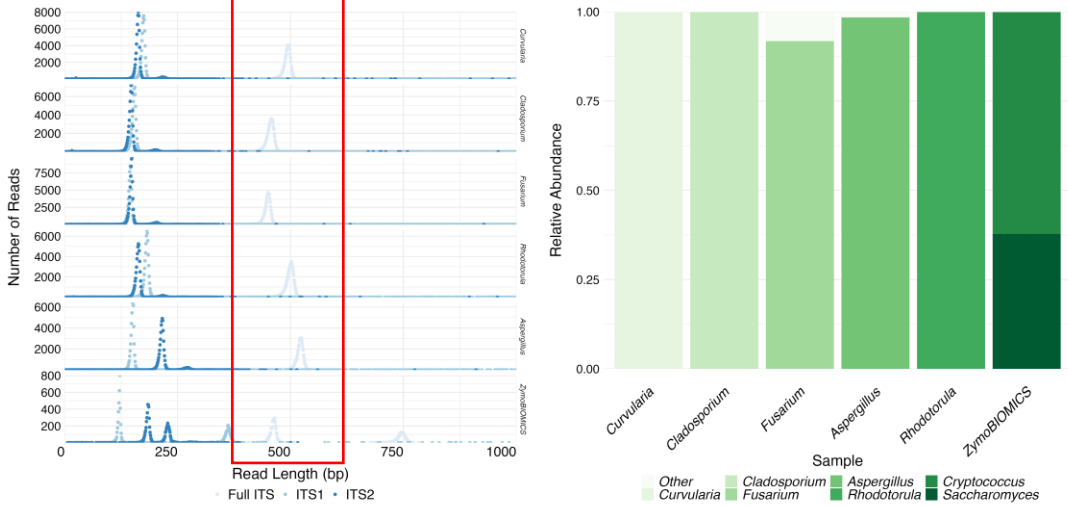


# On Board ISS Fungal Sequencing Validation

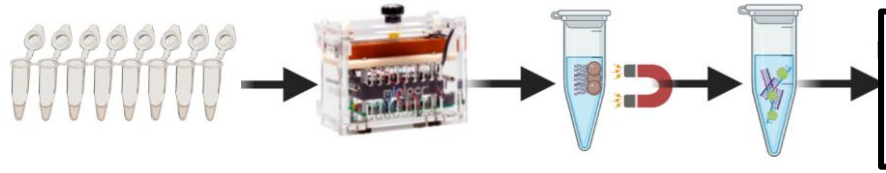
## Ground sequencing




## Flight sequencing




- Five DNA from ISS isolates were prepared
- DNA launched
- Thaw reaction from -80 °C and complete PCR using miniPCR
- Ground and flight samples were prepared and processed using kits prepped at the same time



Sequenced and data downlinked to NASA JSC for analysis



Astronaut Jessica Mier (GIS-6)

| | |
|---|---|
| ***Curvularia* sp.** | Orbital-8 Initial Hardware |
| ***Cladosporium* sp.** | Soyuz 50 Return – Surface Samples |
| ***Fusarium* sp.** | Soyuz 53 Return – Surface Samples |
| ***Aspergillus* sp.** | Soyuz 57 Return – Surface Samples |
| ***Rhodotorula* sp.** | Soyuz 57 Return – Surface Samples |

- The fungal identification identical between ISS and ground controls
- Validated the optimized workflow and data analysis pipeline onboard the ISS
- True unknowns to be performed onboard the ISS in the future

# Conclusions

- Modified method from the already established spaceflight compatible bacterial swab-to-sequencer protocol was developed for fungal identification
- Optimization included data analysis pipeline improvements for fungal identification
- Cultured isolates and environmental swabs were validated with the workflow:
  - Correct identifications were achieved when compared to the laboratory's standard operating procedures
  - Analysis and sequencing were validated with onboard ISS operations

# Acknowledgements

## THE Sequencing in Space Team



The Biomolecule Sequencer, Genes in Space-3, and BEST Payload Development Team: Sarah Stahl-Rommel, Aaron Burton, Ph.D. and Kristen John, Ph.D.

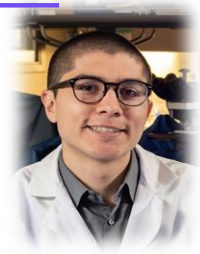
## NASA JSC MinION Team



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Christina Koch



Nick Hague



Chris Cassidy



Aki Hoshide



Raja Chari

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Eoghan Harrington, Ph.D.
Zoe McDougall
Daniel Garalde, Ph.D.
The whole ONT team!




Genes in Space-3 Flight Team
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Melanie Smith



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New England BioLabs
Nicole Nichols, Ph.D.



BEST / Genes in Space-6 Ops Lead
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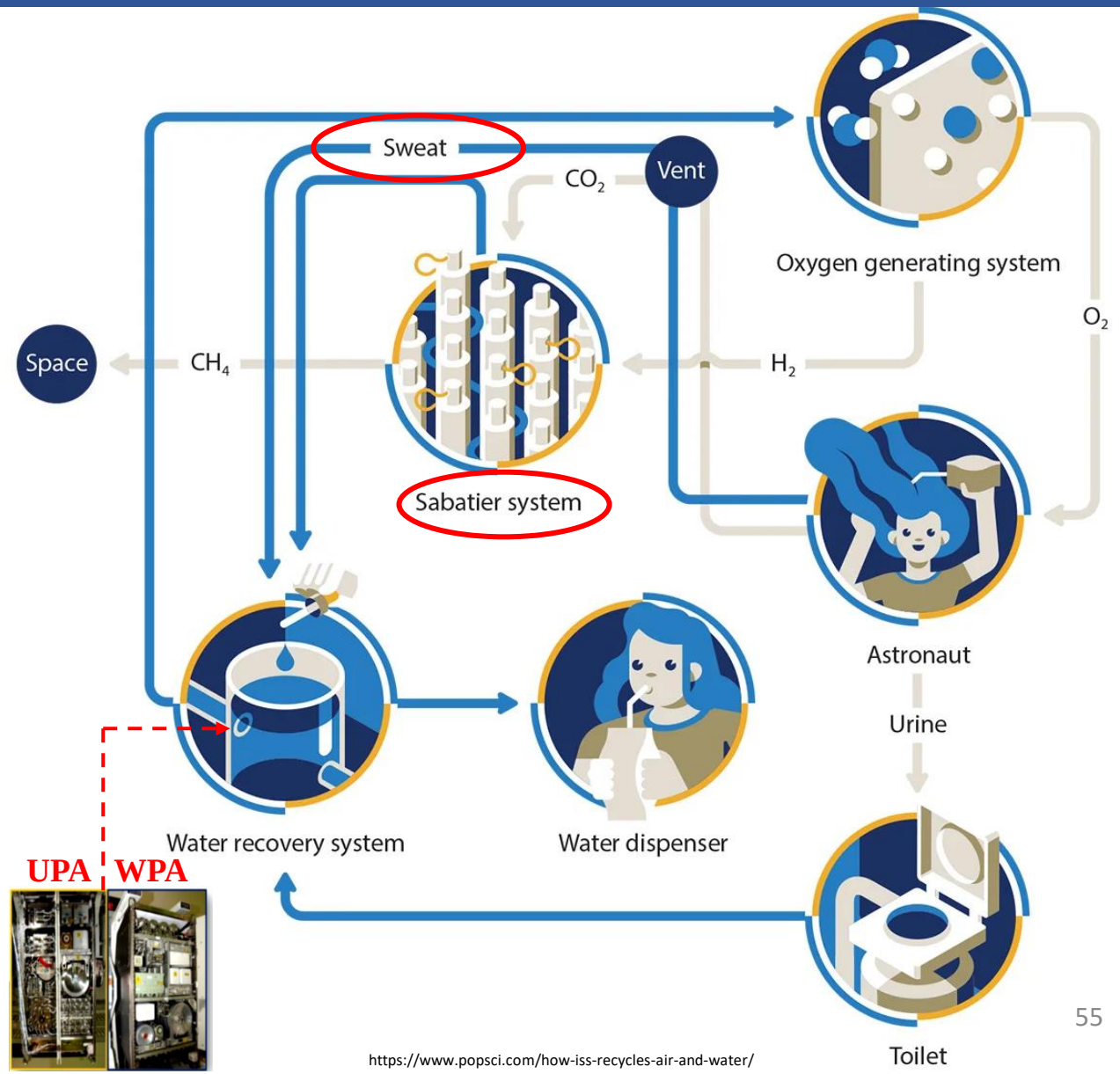
# Biofilm Analysis of Returned Wastewater Samples



**Hang Ngoc Nguyen**, G. Marie Sharp, Sarah Stahl-Rommel, Yo-Ann Velez Justiniano, Christian L. Castro, Mayra Nelman-Gonzalez, Aubrie O'Rourke, Michael D. Lee, Jill Williamson, Chelsea McCool, Brian Crucian, Kenneth W. Clark, Miten Jain, and Sarah L. Castro-Wallace

# Water in Space

- A clean and reliable source of water is critical for human spaceflight
- Launched needed water supply to sustain mission needs, as was the practice during the Space Shuttle Program, was no longer efficient for the ISS crew size and mission duration
- The U.S. Water Recovery System (WRS), consisting of the Urine Processor Assembly (UPA) and the Water Processor Assembly (WPA), launched to the ISS in 2008
- The WRS processes urine distillate, humidity condensate, Sabatier product water, and occasional off-loading of ground supplied water to provide potable water

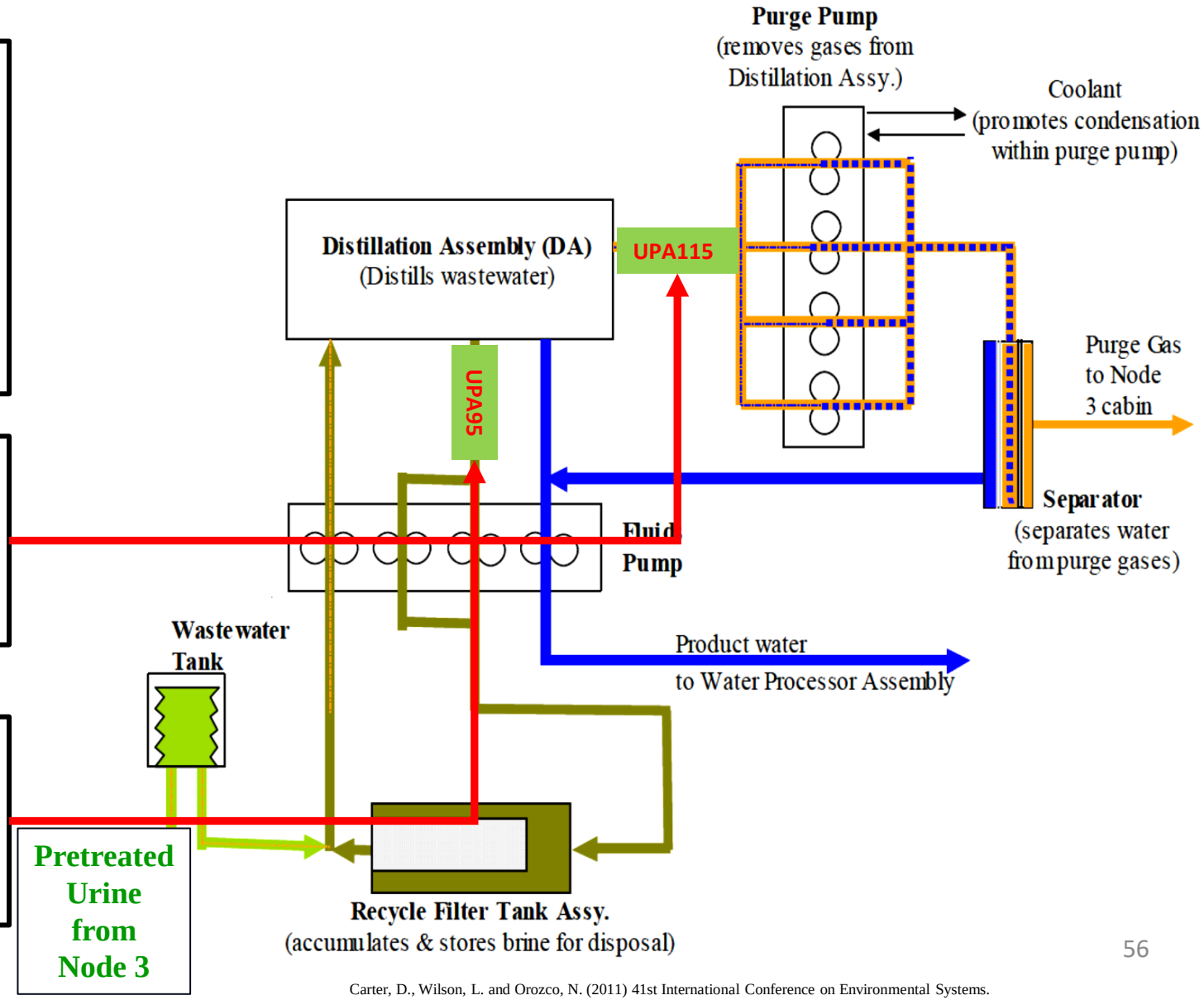



Carter, D.L. (2009) Status of the Regenerative ECLSS Water Recovery System, SAE International.

https://www.popsci.com/how-iss-recycles-air-and-water/

# Urine Processor Assembly (UPA)

- Crew urine has been recycled to potable water since 2008
- Pretreated urine [pretreat = phosphoric acid ($H_3PO_4$) and hexavalent chromium ($Cr^{6+}$)] enters the Distillation Assembly (DA) of the UPA → Two products: **distillate and urine brine**
- Distillation: UPA flex line 115 (UPA115) carries the water vapor to the purge pump where condensation occurs prior to collection for **further processing by the WPA**
- Urine brine is transferred from the DA by UPA flex line 95 (UPA95) into the recycling tank → **further water recovery by processing the brine**






Carter, D., Wilson, L. and Orozco, N. (2011) 41st International Conference on Environmental Systems.

# Sample Collection

**02/2010** Installation UPA95

**05/2015** Installation UPA115

**10/2019** Disconnection

**02/2021** Returned (SpaceX-21)

**04/2021** Processed (MSFC)

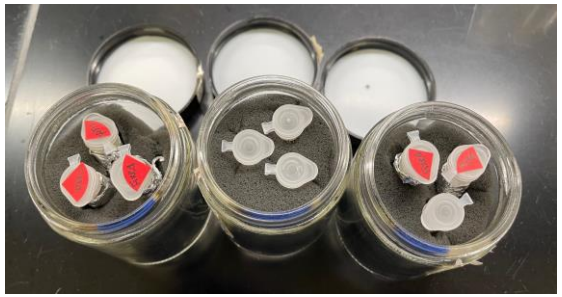






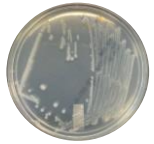
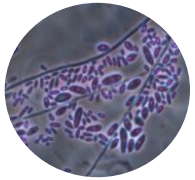
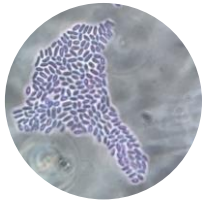


- Samples for:
  - microbial analysis
  - chromium analysis



- Immediately fixed for Environmental Scanning Electron Microscopy (ESEM) imaging

# Identification of Microbial Isolates

| Recycled brine line (UPA95' Flex line 96M 12561-099) | | Purge line (UPA115' Flex line 96M 12561-100) | |
|---|---|---|---|
| **Bacterial Culture** | **Fungal culture** | **Bacterial Culture** | **Fungal culture** |
| 3.1. *Burkholderia* sp._White  | F3.1. *Fusarium* sp.  | 4.1. *Burkholderia fungorum*  | F4.1. *Fusarium* sp.   |
| 3.2. *Burkholderia* sp._Yellow  | | 4.2. *Burkholderia* sp._White  | F4.2. *Lecythophora mutabilis* |
| 3.3. *Burkholderia fungorum*  | | 4.3. *Burkholderia* sp._Yellow  |   |
| | | 4.4. Unidentified Gram-positive species | |
| | | 4.5. *Burkholderia* sp._Wet  | |
| | | 5.2. *Leifsonia lichenia* | |

- Different morphologies
- Higher diversity in UPA115
- *Fusarium* sp. has not previously been isolated from returned PWD or wastewater

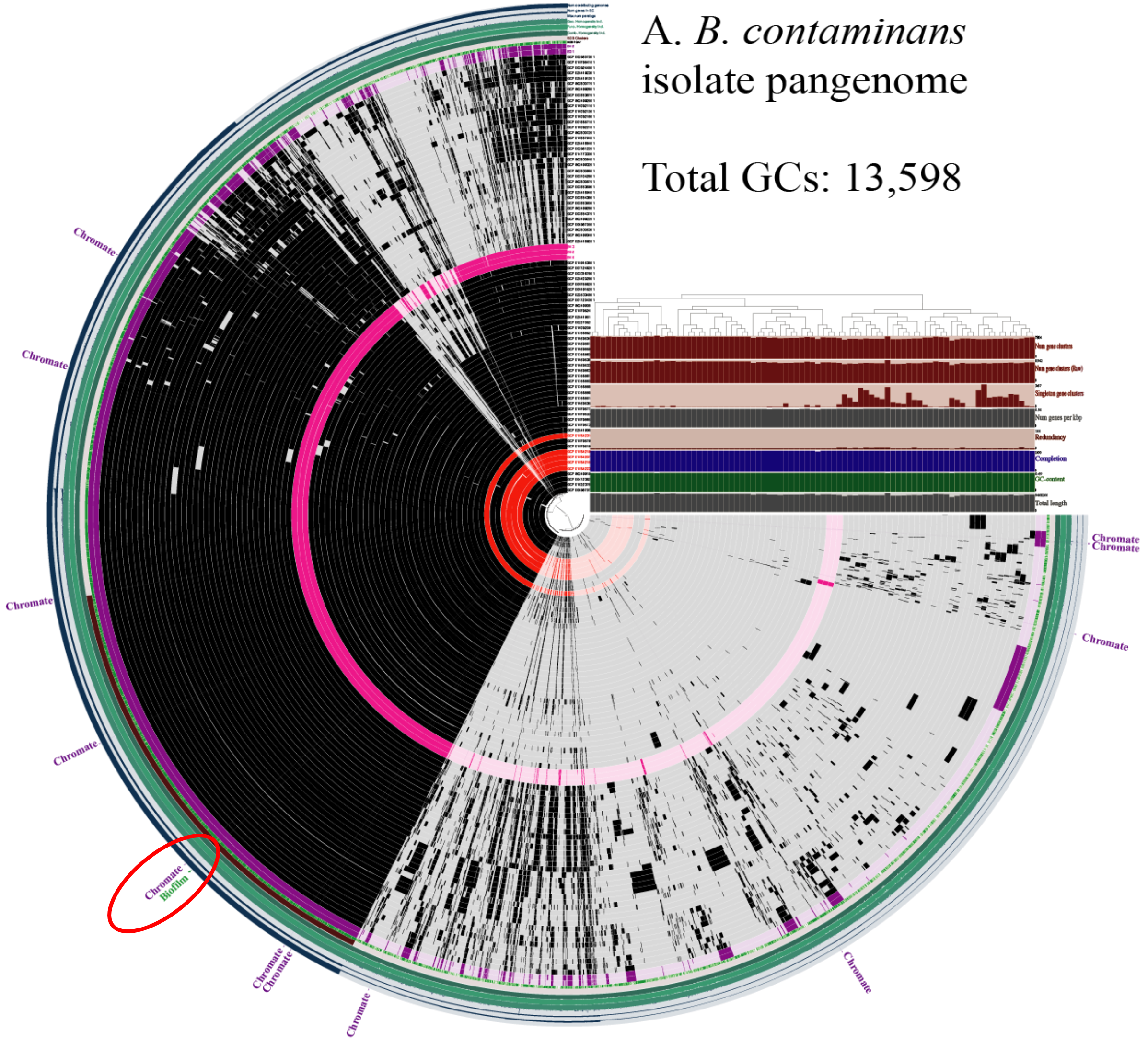
# Hybrid *de novo* whole Genome Analysis

| Sample Source | Sample | Genome size (bp) | Total Genes | Best NCBI Match | ANI |
|---|---|---|---|---|---|
| **NCBI** | *Burkholderia contaminans* SK875 10922 | 8,596,045 | 7,784 | *Burkholderia contaminans* LMG 23361 | 100 |
| **UPA95** | 3.1. *Burkholderia* sp._White | 8,652,817 | 8,026 | ***Burkholderia contaminans* SK875 10922** | **95.6** |
| **UPA95** | 3.2. *Burkholderia* sp._Yellow | 8,726,360 | 7,932 | ***Burkholderia contaminans* SK875 10922** | **99.9** |
| **UPA115** | 4.2. *Burkholderia* sp._White | 8,625,377 | 7,996 | ***Burkholderia contaminans* SK875 10922** | **95.6** |
| **UPA115** | 4.3. *Burkholderia* sp._Yellow | 8,680,200 | 7,876 | ***Burkholderia contaminans* SK875 10922** | **99.9** |
| **UPA115** | 4.5. *Burkholderia* sp._Wet | 8,773,879 | 7,974 | ***Burkholderia contaminans* SK875 10922** | **99.9** |
| **NCBI** | *Burkholderia fungorum* ATCC BAA-463 | 9,058,983 | 8,190 | *Paraburkholderia fungorum* NBRC 102489 | 98.6 |
| **UPA95** | 3.3. *Burkholderia fungorum* | 9,564,261 | 8,799 | ***Paraburkholderia fungorum* NBRC 102489** | **98.6** |
| **UPA115** | 4.1. *Burkholderia fungorum* | 9,745,290 | 8,950 | ***Paraburkholderia fungorum* NBRC 102489** | **98.6** |
| **NCBI** | *Cellulomonas fimi* ATCC 484 | 4,266,344 | 3,851 | *Cellulomonas fimi* ATCC 484 | 100 |
| **UPA115** | 4.4. Unidentified Gram-positive species | 3,862,723 | 3,608 | ***Cellulomonas fimi* ATCC 484** | **80.1** |
| **NCBI** | *Leifsonia* sp. PS1209 | 4,091,164 | 3,916 | *Leifsonia aquatica* ATCC 14665 | 84.0 |
| **UPA115** | 5.2. *Leifsonia lichenia* | 5,084,807 | 4,961 | ***Leifsonia* sp. PS1209** | **81.0** |



* Average Nucleotide Identity (ANI)

# Pangenomic Analysis: *B. contaminans*

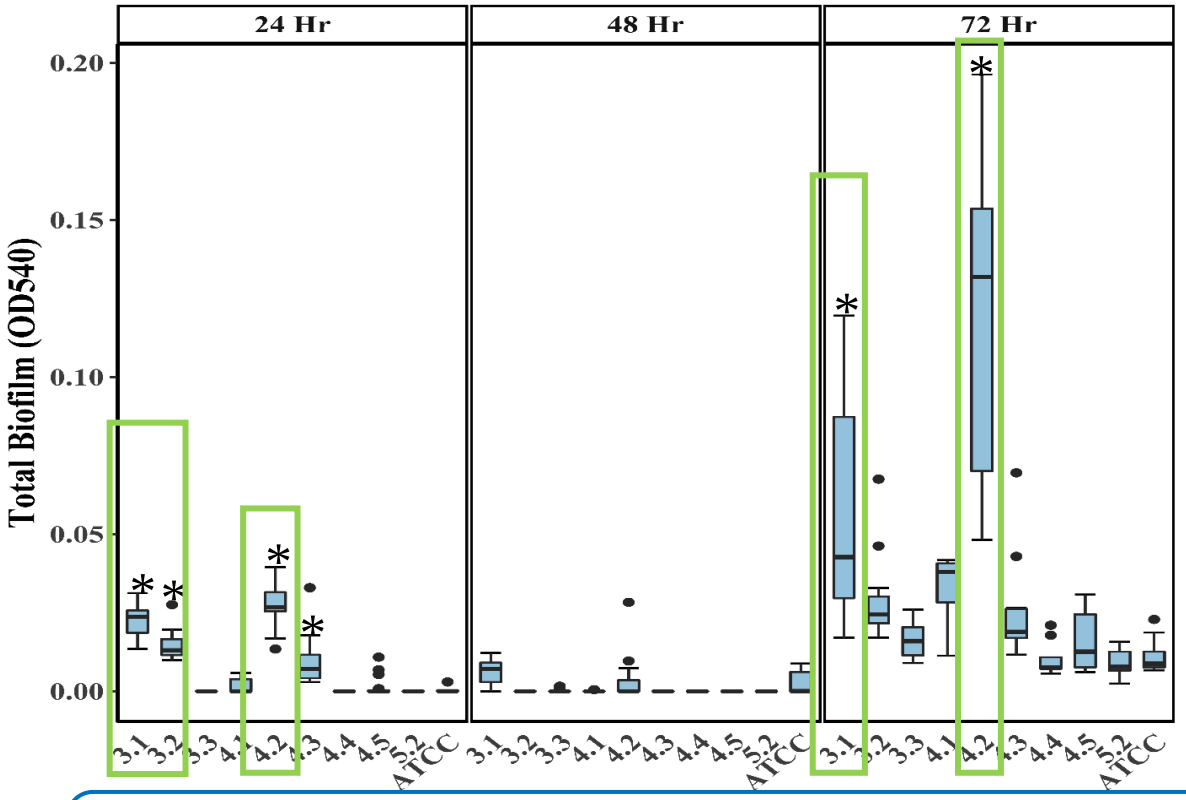



- *B. contaminans*: biofilm, quorum sensing systems, and chromate-resistance gene clusters were observed in all 83 genomes
- *B. contaminans* 3.2, 4.3, and 4.5 (shown in **pink**) are grouped together, while isolates *B. contaminans* 3.1 and 4.2 (shown in **purple**) and other ISS potable water (PWD) isolates are in **red**
- No unique gene clusters were shared between the five *B. contaminans* in this study, but each group did have their own unique gene clusters
    - **Pink** group: cadmium, toxin-antitoxin, flavodoxin, and hemolysin protein gene clusters
    - **Purple** group: high potential activity in cell adhesion, molecule transport, heavy metal resistance, plasmid formation-related, toxin-antitoxin ability gene clusters

# Biofilm Potential

**Biofilm assay:**

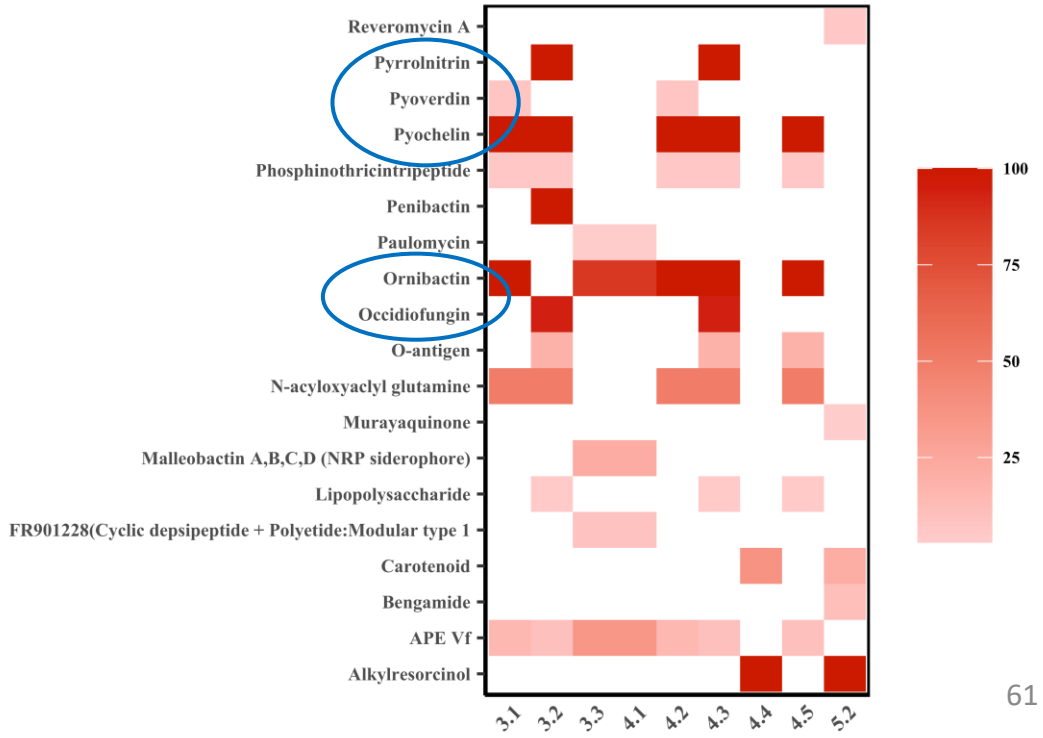
- 24h: significantly-enhanced biofilm *B. contaminans* 3.1, 3.2, and 4.2
- 72h: higher biofilm production from 3.1 and 4.2



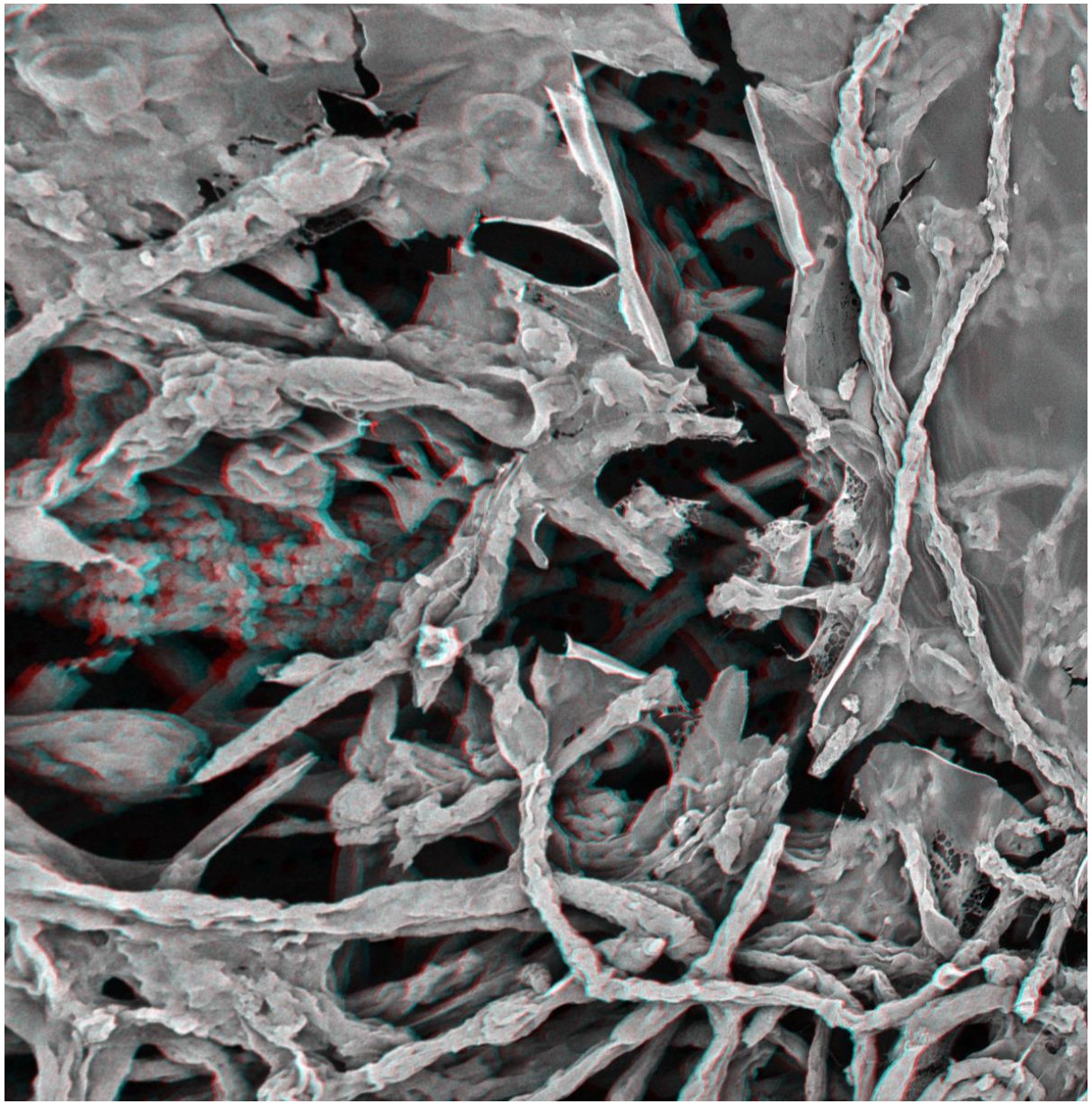

Biofilm potential of the bacterial isolates corresponds to the secondary metabolite gene clusters

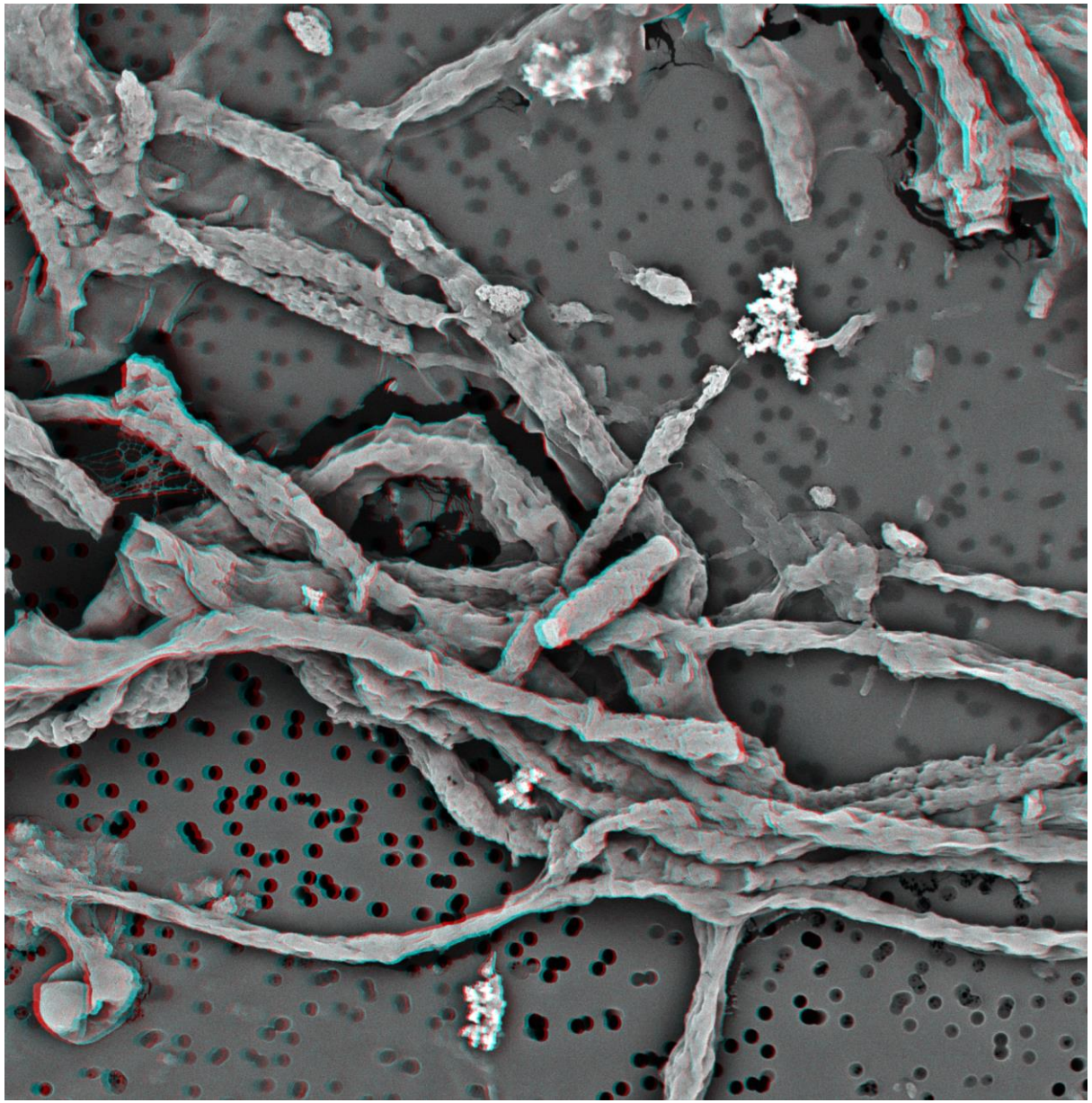
**Secondary metabolites:**

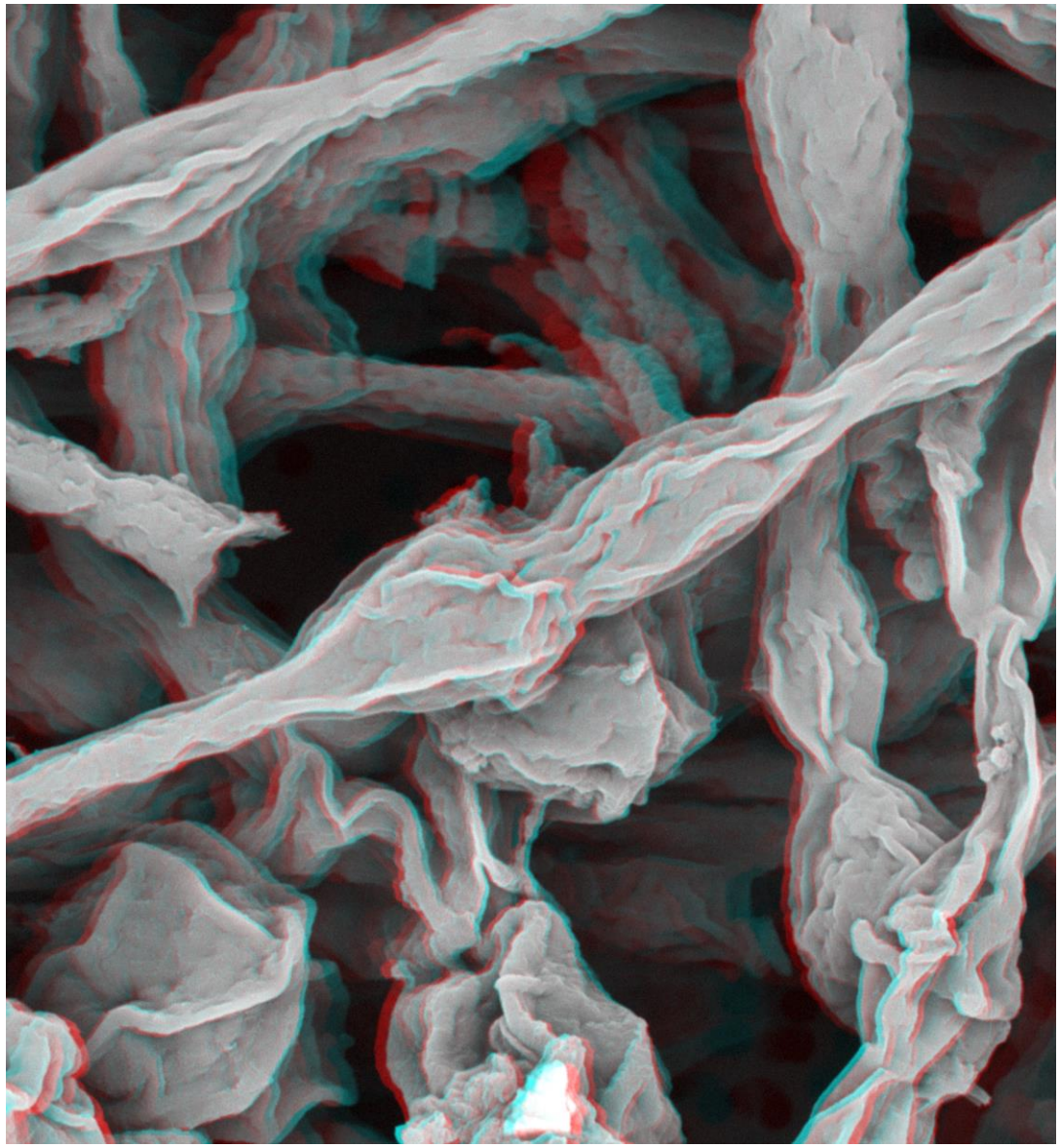
- Pyoverdin production has a strong and significant relationship with biofilm formation
- Iron-binding or antifungal functional groups: occidiofungin, pyrrolnitrin, pyochelin, and ornibactin are regulated by quorum sensing activity

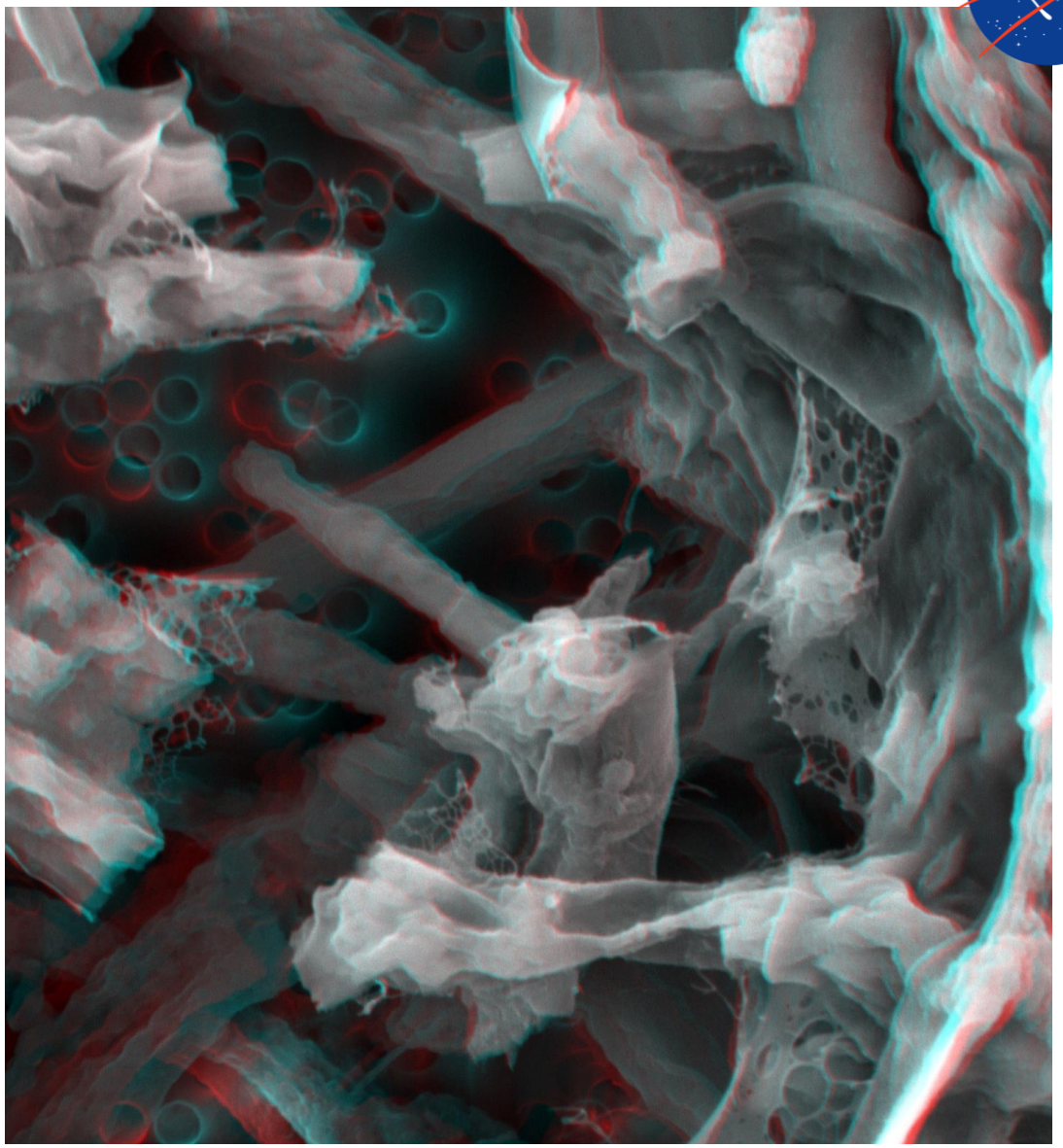


# 3D ESEM Images of the Flex line









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- Co-authors:
  - G. Marie Sharp, Ph.D.
  - Sarah Stahl-Rommel
  - Yo-Ann Velez Justiniano
  - Christian L. Castro
  - Mayra Nelman-Gonzalez
  - Aubrie O'Rourke, Ph.D.
  - Michael D. Lee, Ph.D.
  - Jill Williamson, Ph.D
  - Chelsea McCool
  - Brian Crucian, Ph.D.
  - Kenneth W. Clark
  - Miten Jain, Ph.D.
  - Sarah L. Castro-Wallace, Ph.D, PI

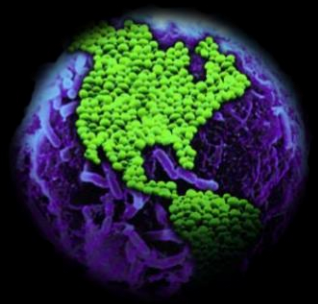
- Ronnie Clayton, JSC Chief Technology Office
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  - Walter Schneider
  - Imelda Stambaugh
  - Caitlin Meyer




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Brandon Dunbar, Hang Nguyen, Christian Mena, Sarah Castro-Wallace, Sarah Stahl-Rommel, G. Marie Sharp, Christian Castro





# Culture-Independent Microbial Air Profiling using a Spaceflight-Compatible Nanopore Sequencing Method

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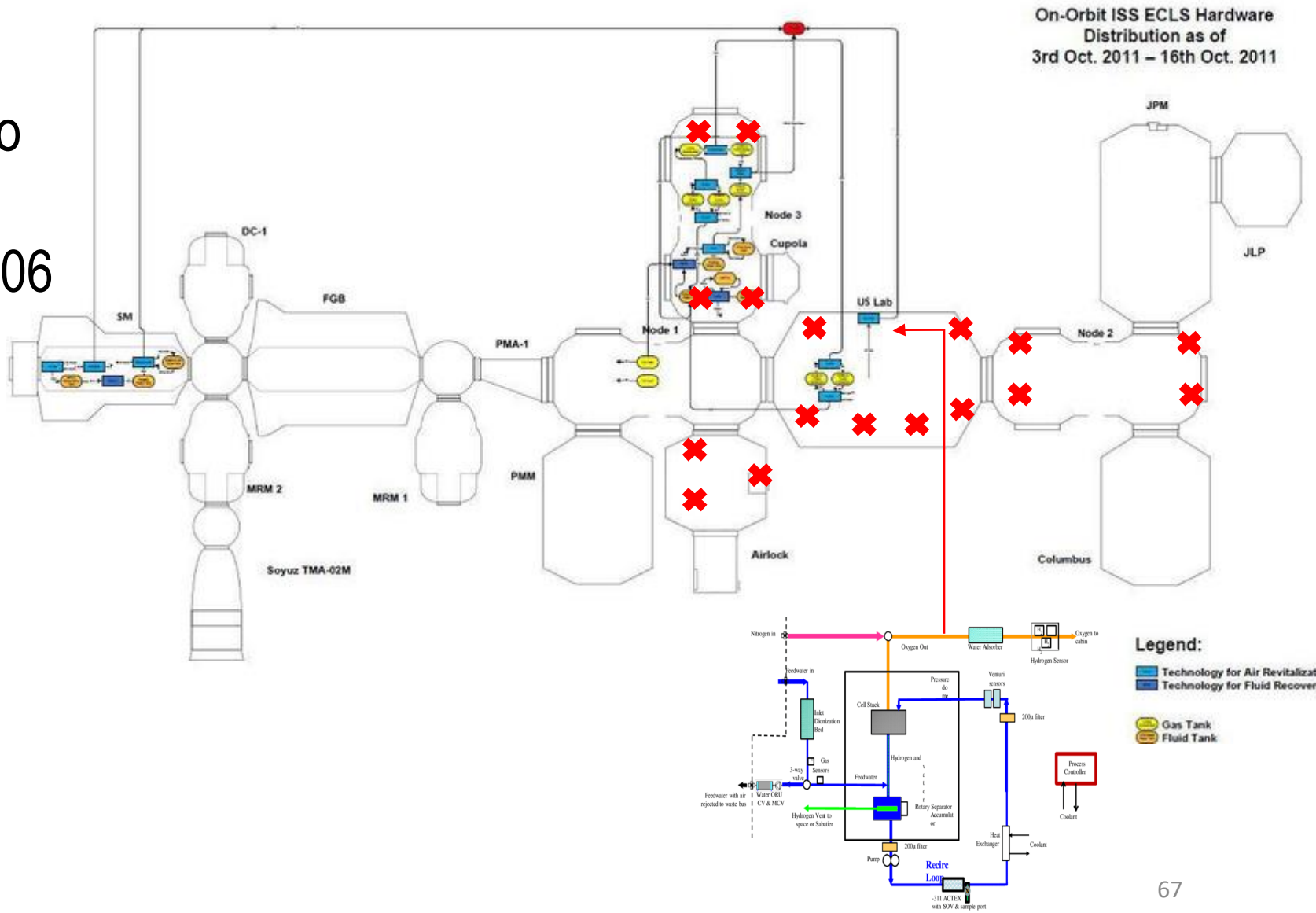
Brandon.Dunbar@nasa.gov

# Outline

- Oxygen Generation Assembly onboard ISS
- Current atmospheric microbial monitoring onboard ISS
- *In-situ* molecular capabilities onboard ISS
- Method development: culture-independent air monitoring
  - Air Samplers
    - Bio-Pump
    - Coriolis Compact
    - SASS 3100
- Results and Conclusion
- Forward work

# The Oxygen Generation Assembly (OGA)

- The OGA supplies oxygen to the ISS cabin
- Delivered on STS-121 in 2006
- Installed in the Destiny module (US Lab)
- Engineering Controls:
  - High volume air transfer
  - 17 HEPA filters are in place throughout the ISS



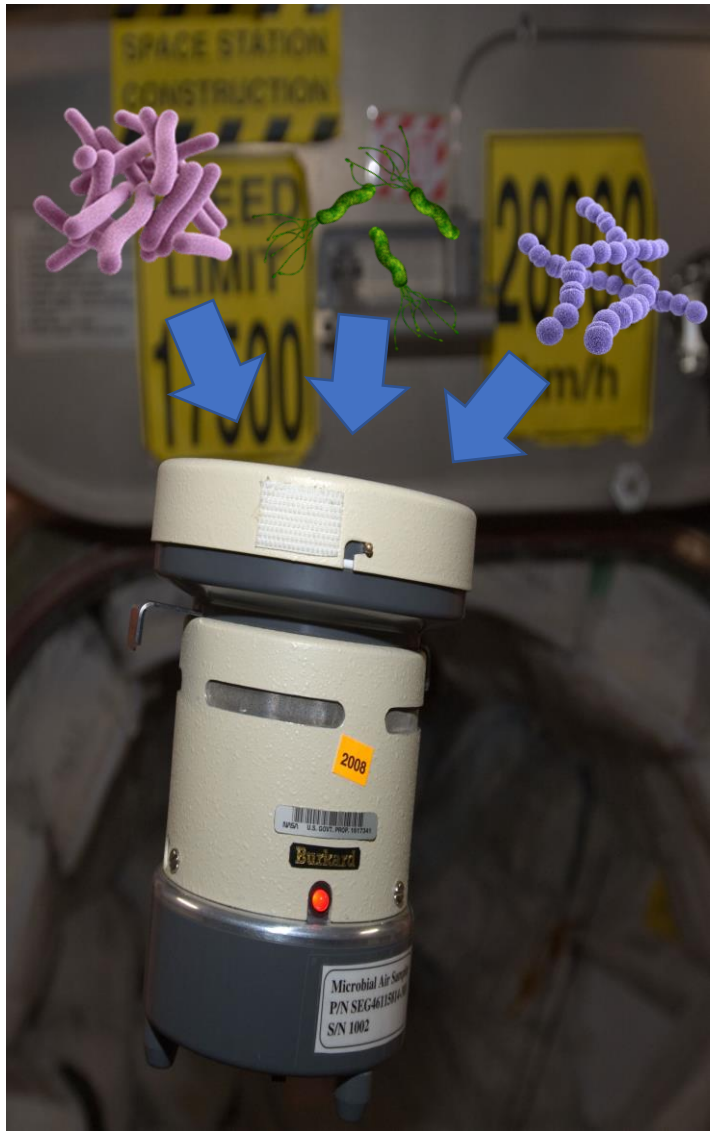
# Microbial Air Sampler (MAS): Current ISS Hardware

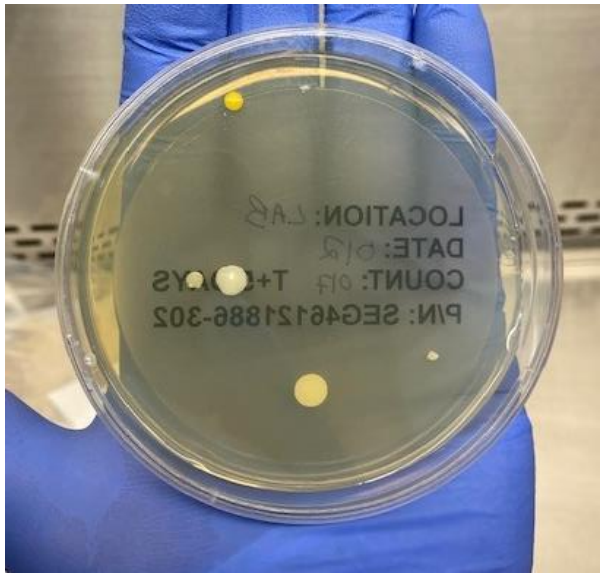
**ISS Medical Operations Requirements Document**

- Monitor every 3 months
- Data used for NASA risk assessment

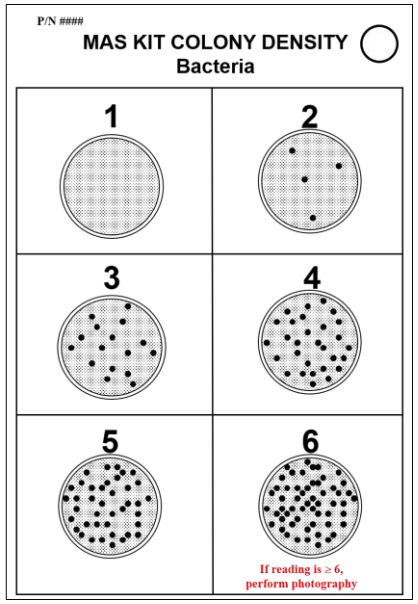
**Sample Collection**

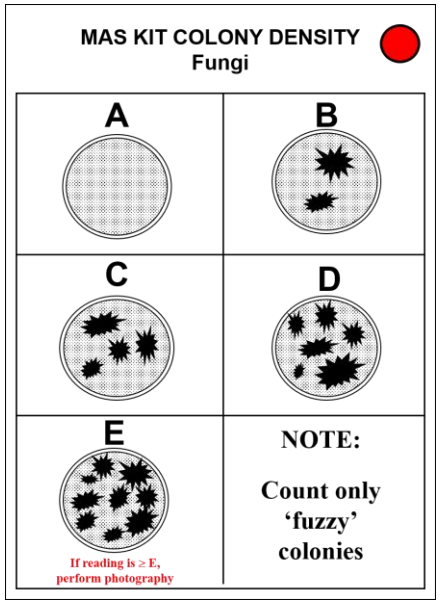
- 60 L of air collected in 3 min on either bacterial or fungal culture media
- Results are observed and reported by astronauts after 5 days at ambient temperature
- Samples are returned to JSC for microbial identifications and final analysis



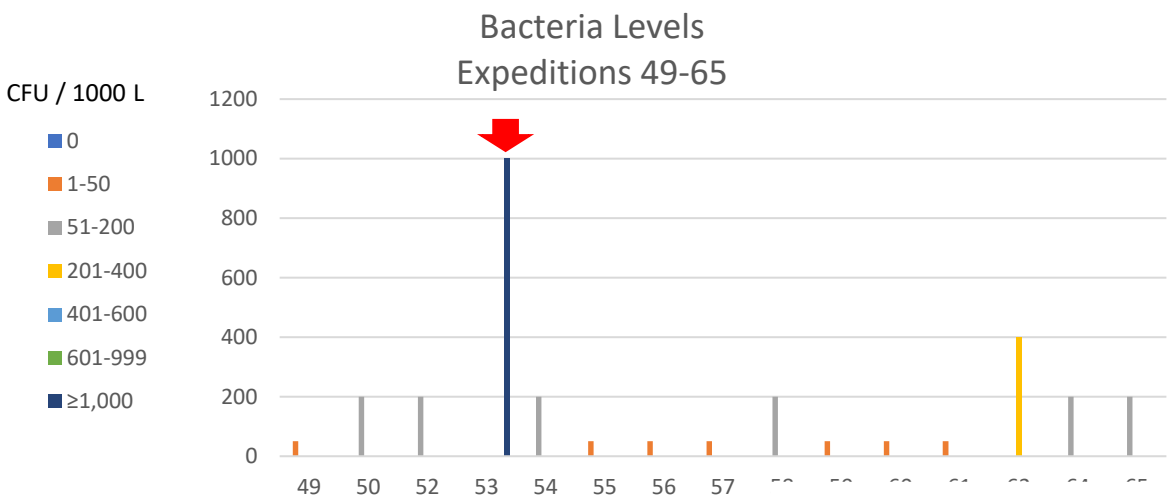



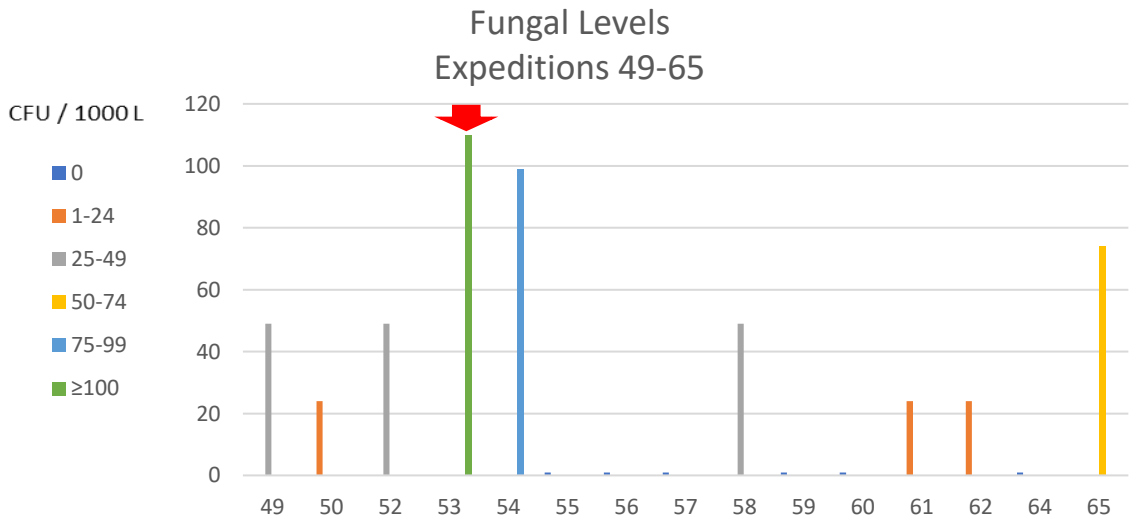


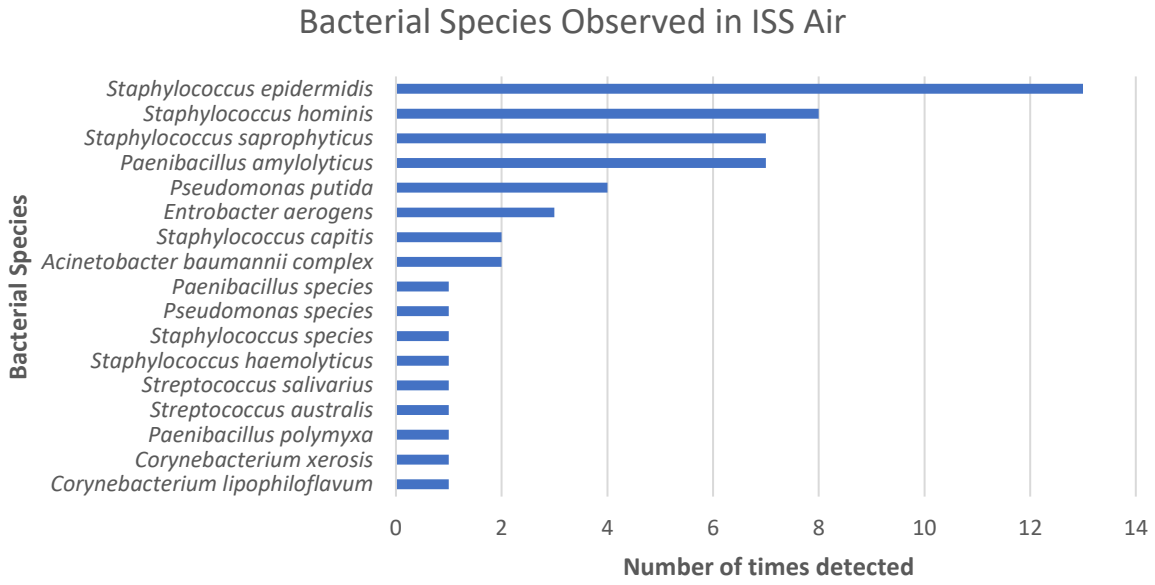


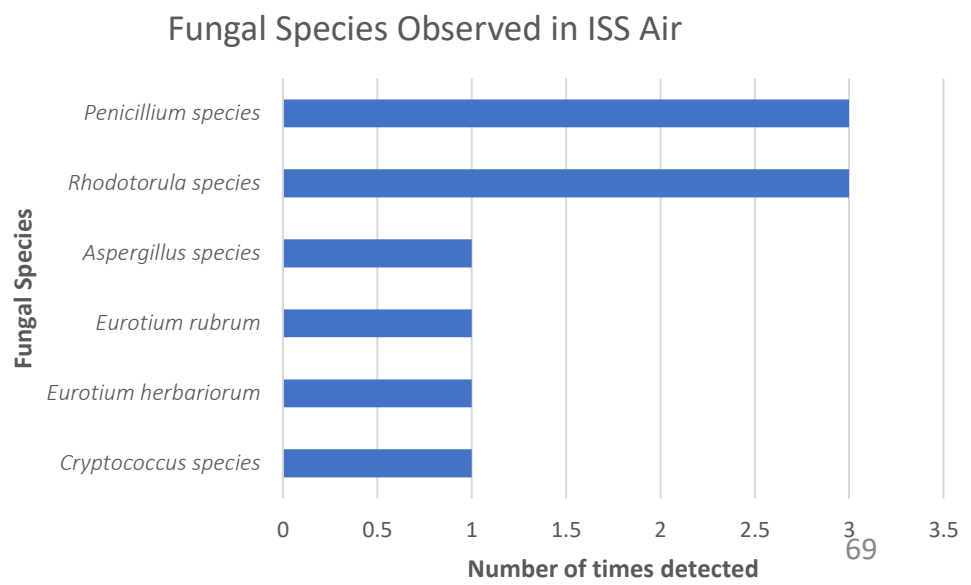



# Microbial Evaluation of ISS Air



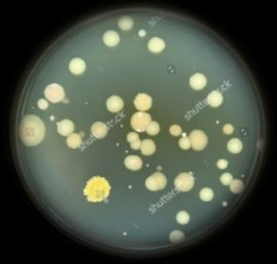



# Microbial Sampling Technology

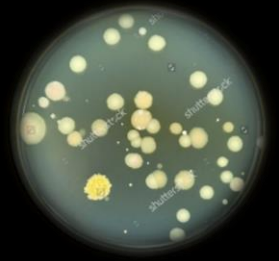
Apollo
1963-1972





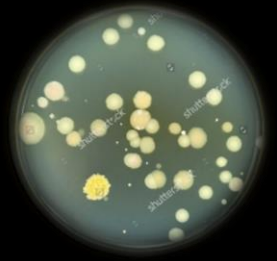
Shuttle
1981-2011





Commercial (SpX / NG / Boeing)
2012-



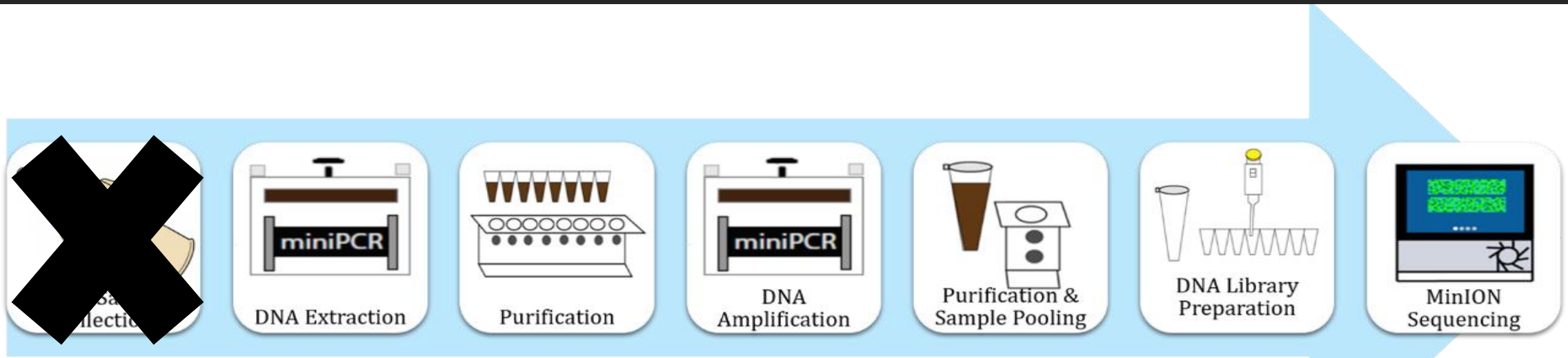


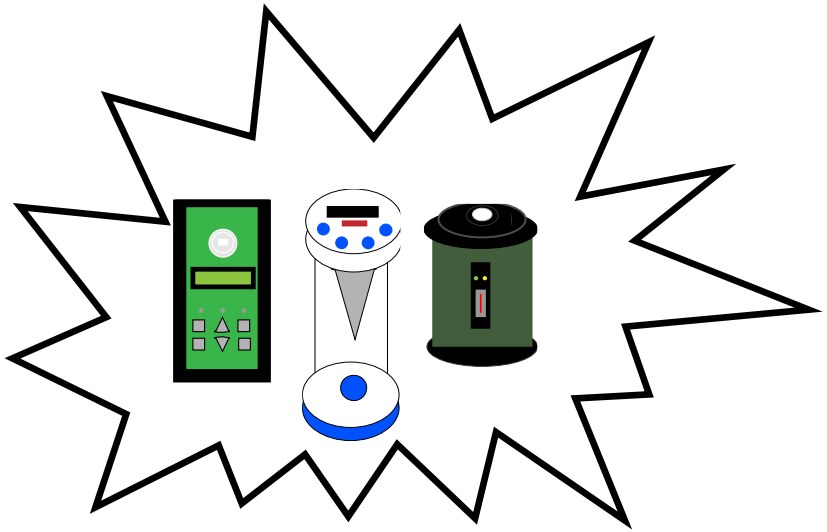
Space Launch System / Orion / Gateway / Lunar



???
Sample return will not be possible

# Building on Swab-to-Sequencer Method



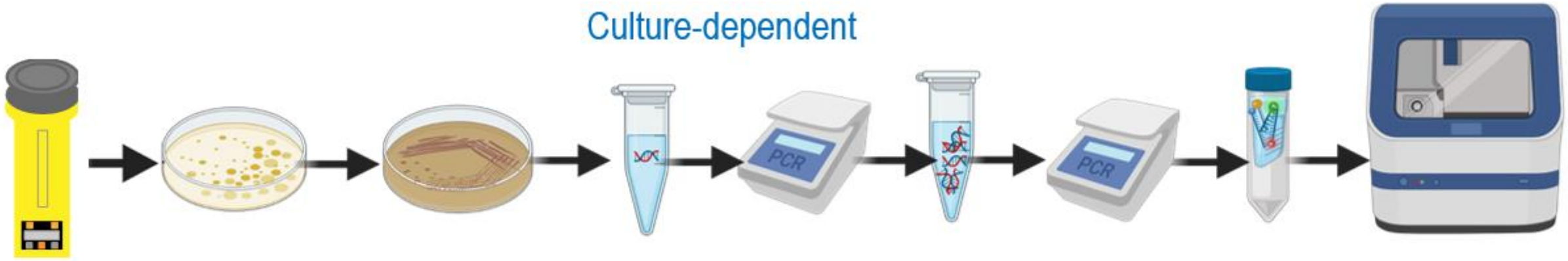



- Based on the validated swab-to-sequencer method
- Portable
- Easy to operate
- Capable of culture-independent sample processing

# Current Standard Method: SAS 180



- Air collection rate – 180 LPM
- Collection method – Impaction onto Growth Media
- Used by the JSC Microbiology for ground-based monitoring
- Functions similarly to the ISS MAS
- Basis for comparison to all samplers in this study

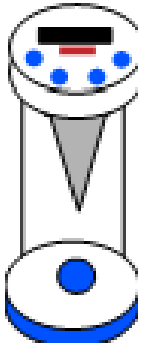



SAS180

NASA JSC Microbiology's Standard Operating Procedure for Bacterial Identification

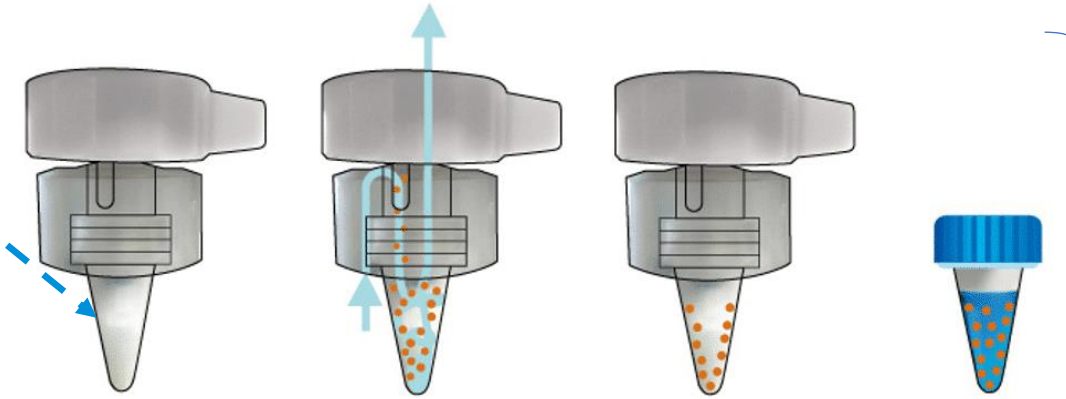
# Air Sampler Selection: Coriolis Compact





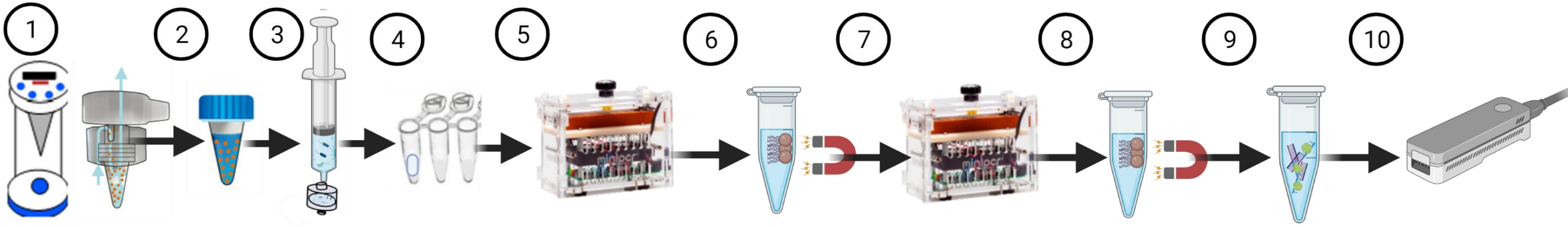
- Weight – 1.4 kg
- Air Collection Rate – 50 LPM
- Collection Method – Polyethylene Cone
- Power Source – Rechargeable Lithium Ion
- Noise Measurement – 68.9 dBA
- Cost - $9,500

Consumable: Polyethylene collection cone



Coriolis force is used centrifuge microbial cells onto the inner wall of the attached cone during sample collection. The cells can then be removed for analysis.

# Coriolis Compact: Concept of Operations



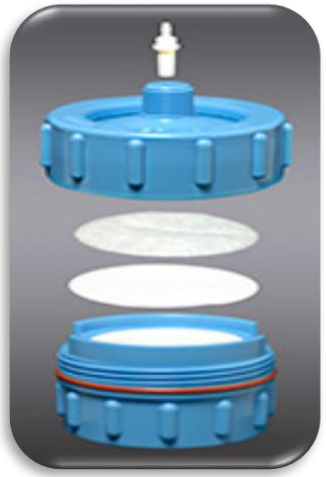

1. Air sample collection (impaction of cells on the cone)
2. Removal of cells (buffer addition, vortexting)
3. Passing buffer through a filter
4. Insert filter into DNA extraction buffer
5. DNA extraction within miniPCR
6. DNA purification
7. DNA amplification within miniPCR
8. Amplified DNA purification
9. Library preparation
10. Sequencing within the MinION

# Air Sampler Selection: Smart Air Sampler System (SASS) 3100



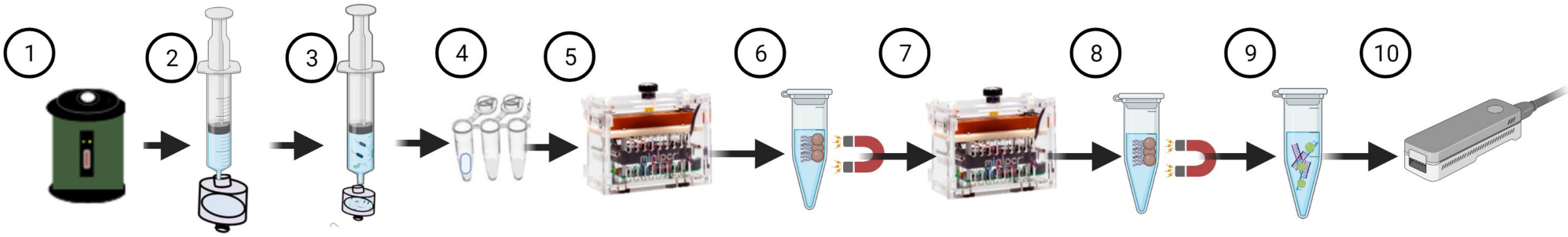
- Weight – 3.3 kg
- Air Collection Rate – 50-300 LPM
- Collection Method – Electret Filter
- Power Source - Rechargeable Lithium Ion
- Noise Measurement – 49.9 dBA
- Cost - $8,250

Consumable: Filter and sterile particle extractor



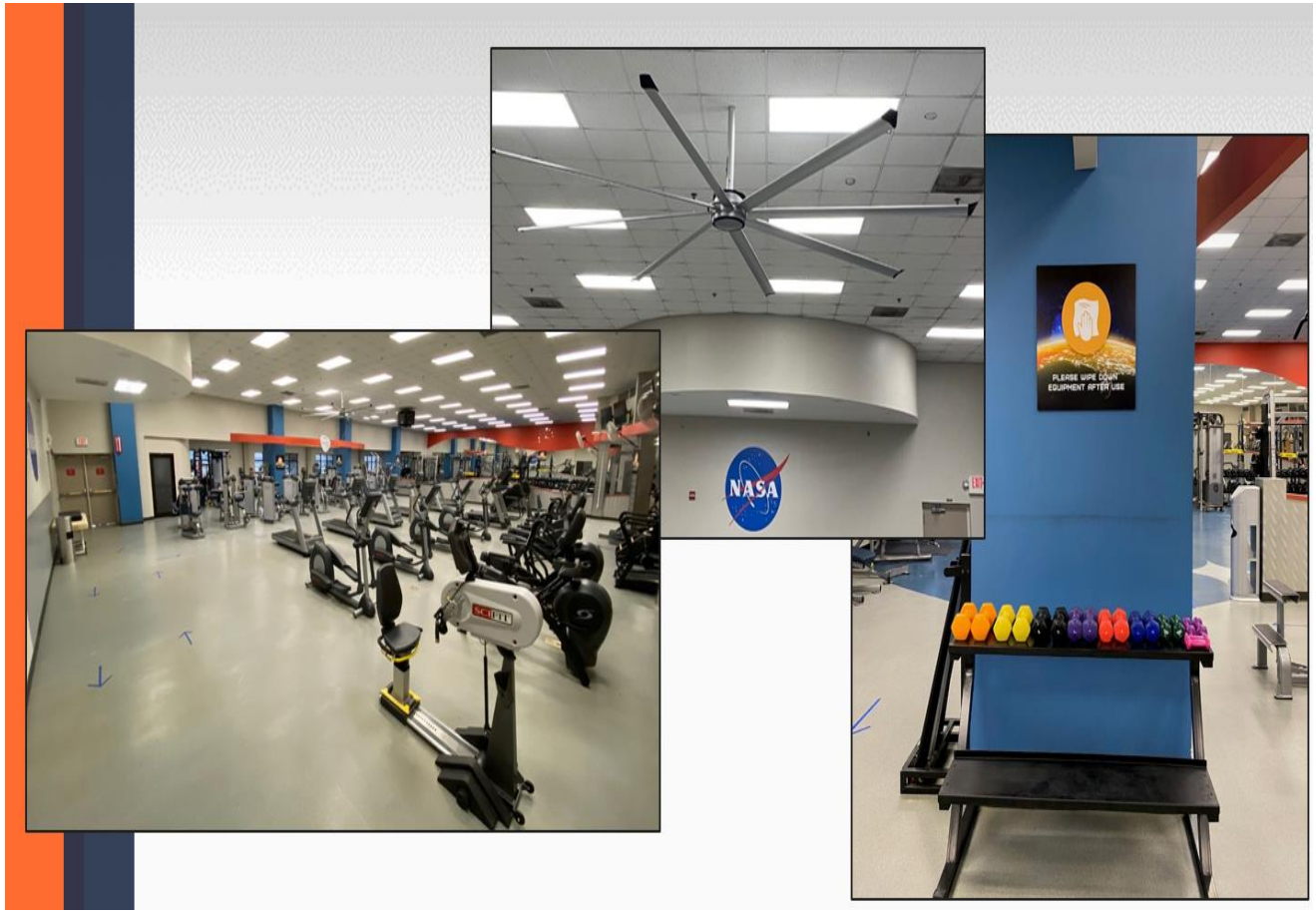
Utilizes a high efficiency centrifugal fan to collect microbial cells onto an electret filter. The cells are removed from the filter by passing particle extraction fluid through it with the particle extractor. The cells are then in liquid for further processing.

# SASS 3100: Concept of Operations




1. Air sample collection (impaction of cells on the filter)
2. Removal of cells (particle extraction fluid passed through the filter)
3. Filtering collected particle extraction fluid to collect cells
4. Insert filter into DNA extraction buffer
5. DNA extraction within miniPCR
6. DNA purification
7. DNA amplification within miniPCR
8. Amplified DNA purification
9. Library preparation
10. Sequencing within the MinION

# Sampling Locations



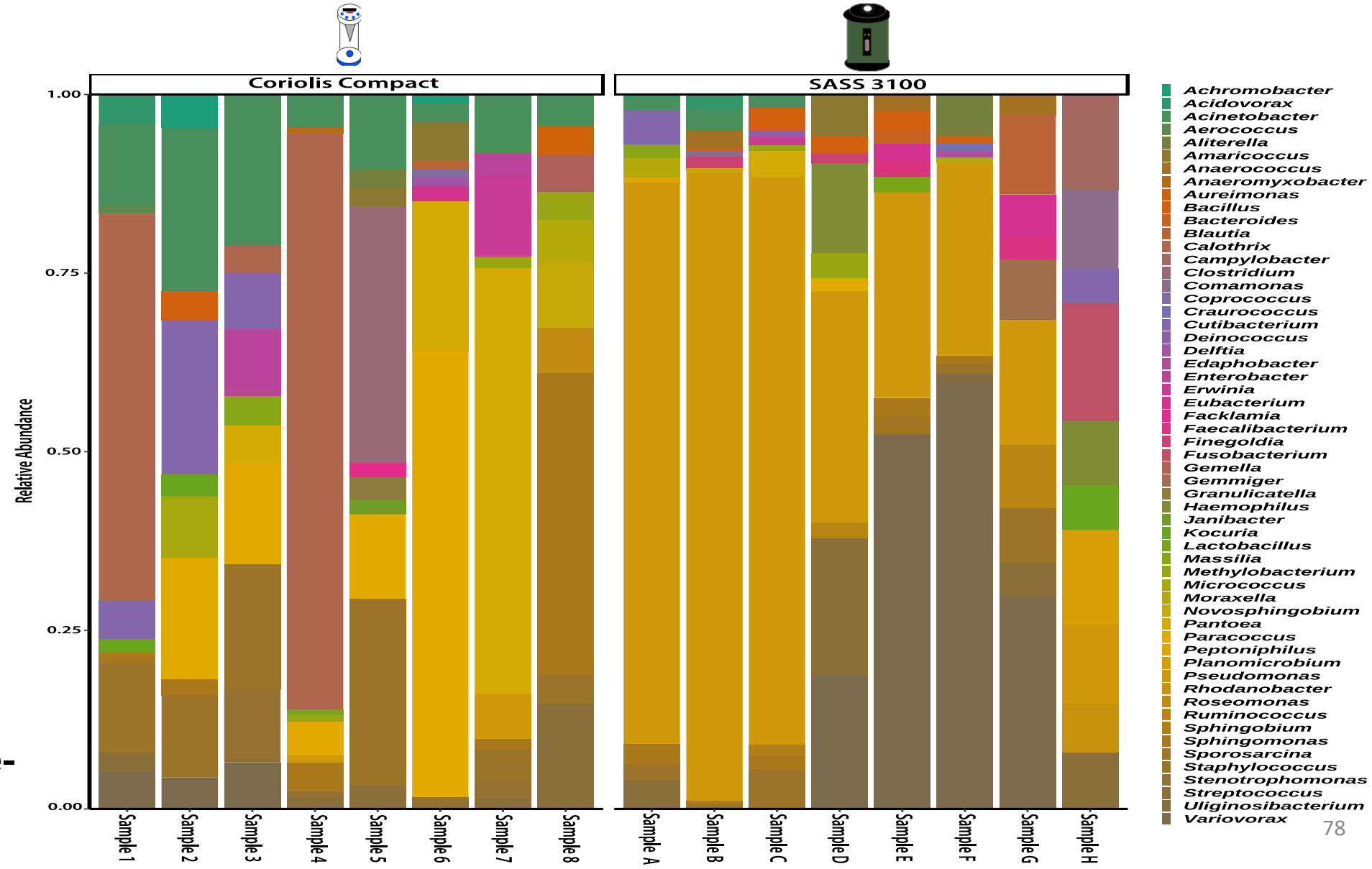

NASA JSC Gilruth Fitness Center

NASA JSC Breakroom B21



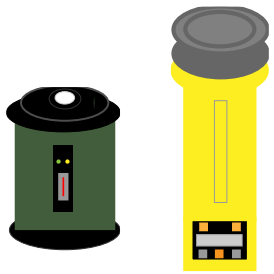
# Sequencing Data from Coriolis and SASS 3100

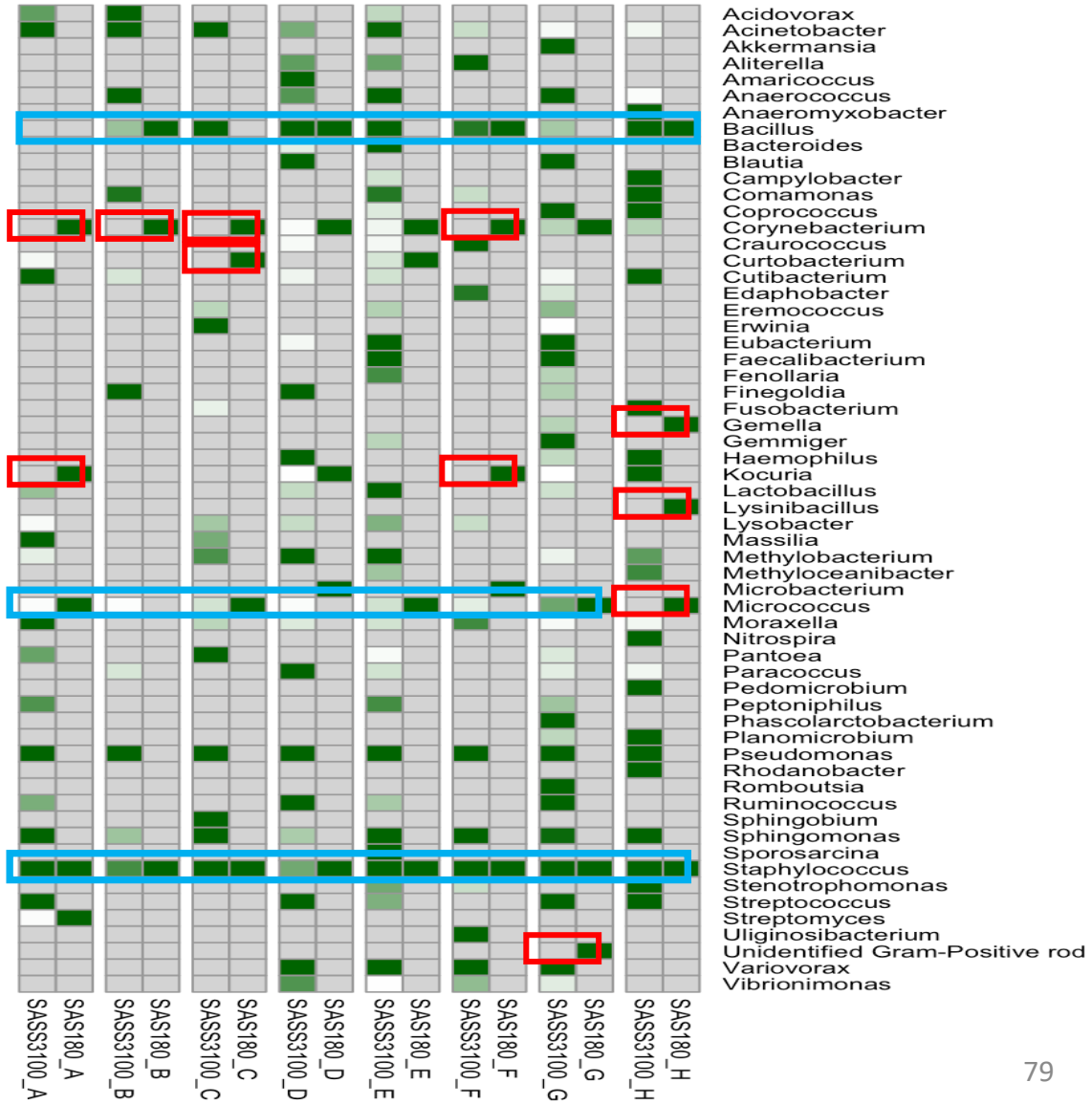
- Data generated from both the Coriolis Compact and SASS 3100 resulted in microbial identifications that are expected in human occupied environment
- *Staphylococcus* spp. and *Streptococcus* spp. were the two most shared organisms between the two culture-independent samplers



# Culture Compared to the Sequencing: SASS 3100

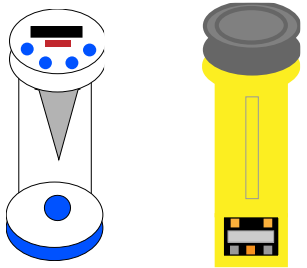
- As expected, more diversity noted with the molecular method as compared to culture
- Frequently matched genera:
  - *Bacillus*
  - *Micrococcus*
  - *Staphylococcus*
- Mismatches (found in culture but not in the molecular method)
  - *Lynsinibacillus*
  - *Corynebacterium* (missed 50% of the time)
  - *Kocuria*
  - *Curteobacterium*
  - *Microbacterium*
  - *Gemella*
  - *Micrococcus*
  - *Macrococcus*

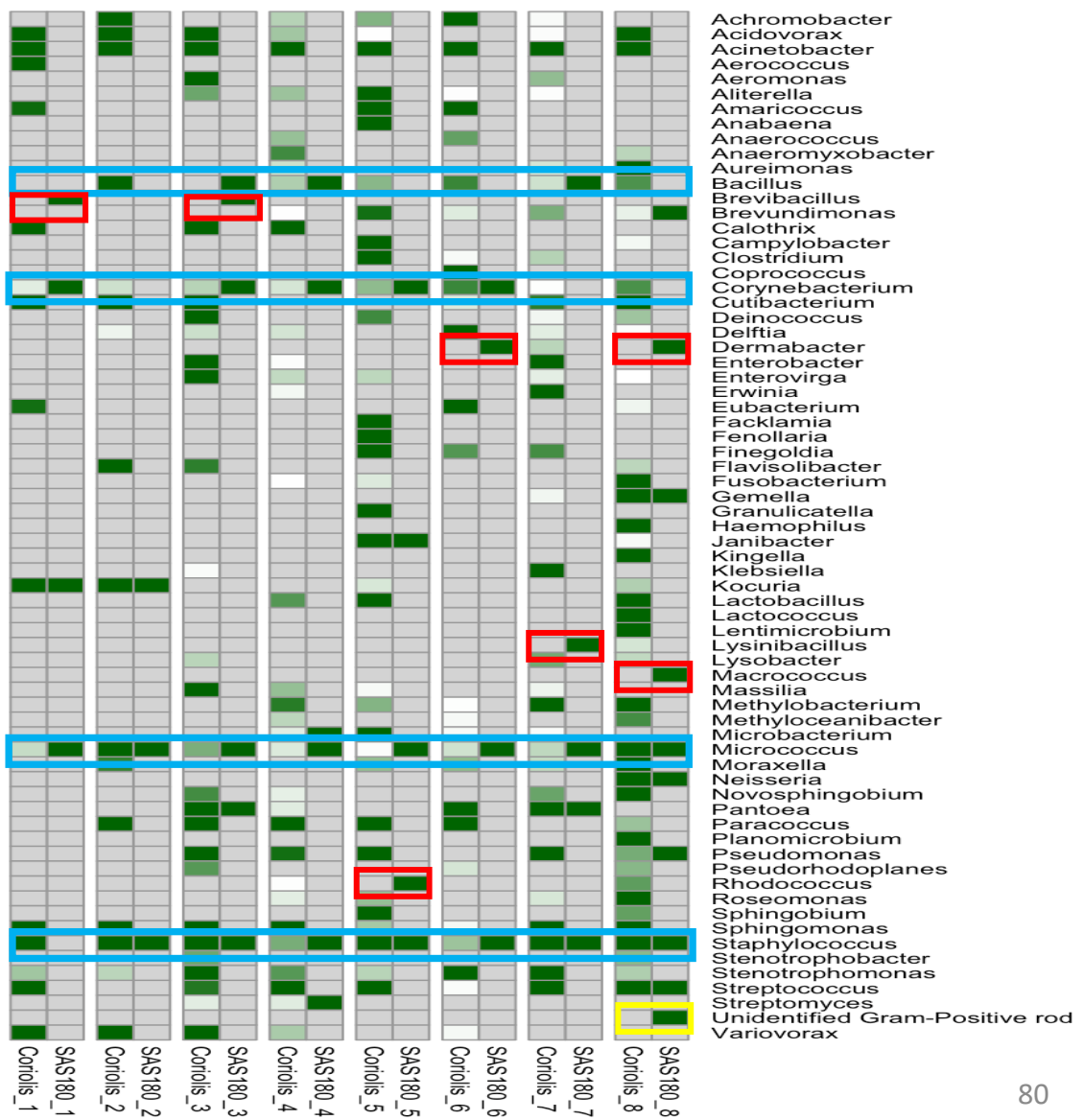




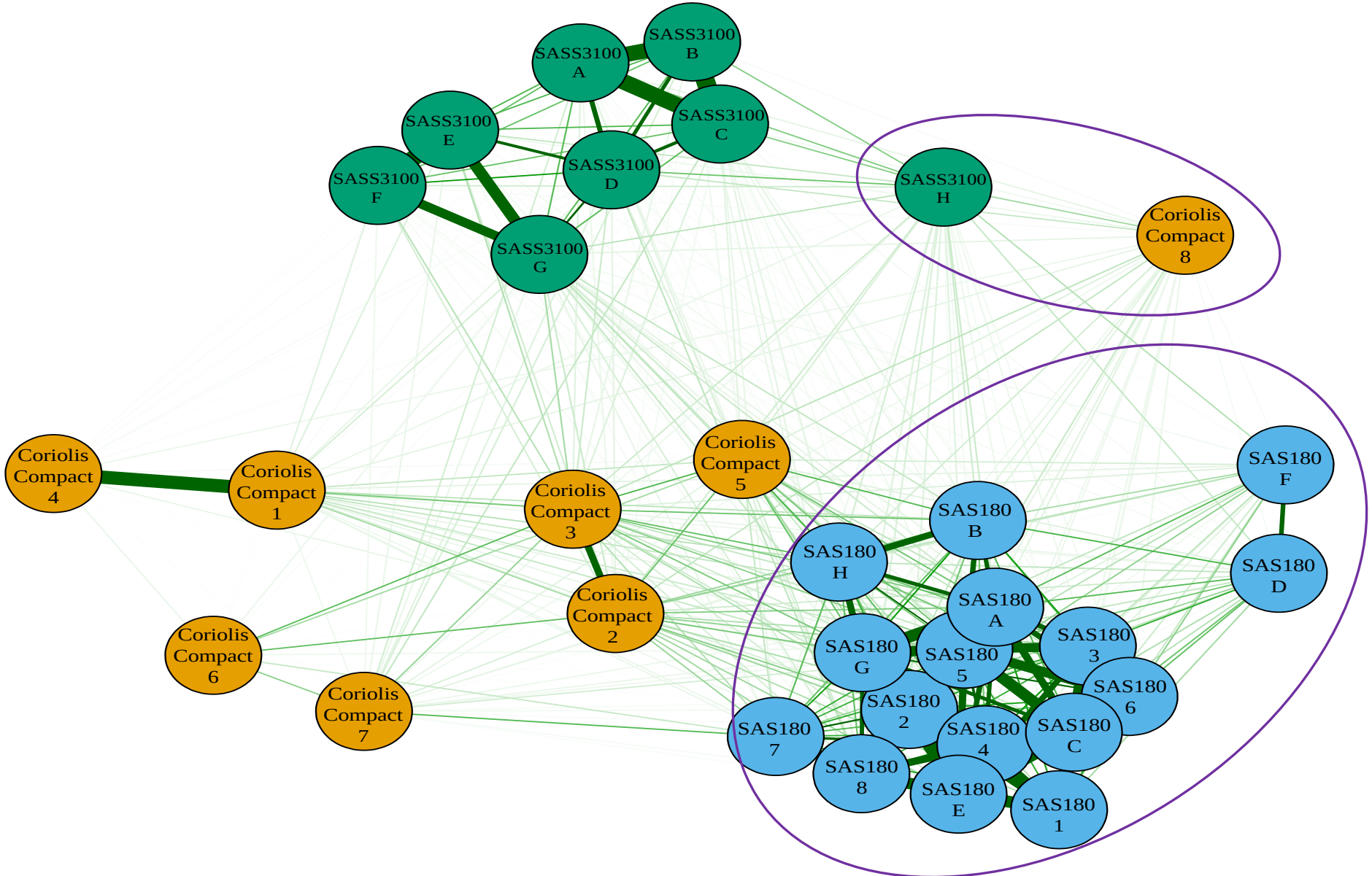
# Culture Compared to the Sequencing: Coriolis Compact

- As expected, more diversity noted with the molecular method as compared to culture
- Frequently matched genera:
  - *Bacillus*
  - *Corynebacterium* (!!)
  - *Micrococcus*
  - *Staphylococcus*
- Mismatches (found in culture but not in the molecular method)
  - *Brevibacillus*
  - *Bacillus*
  - *Macrococcus*
  - *Rhodococcus*
  - *Dermabacter*





# Pearson Correlation: All Samples

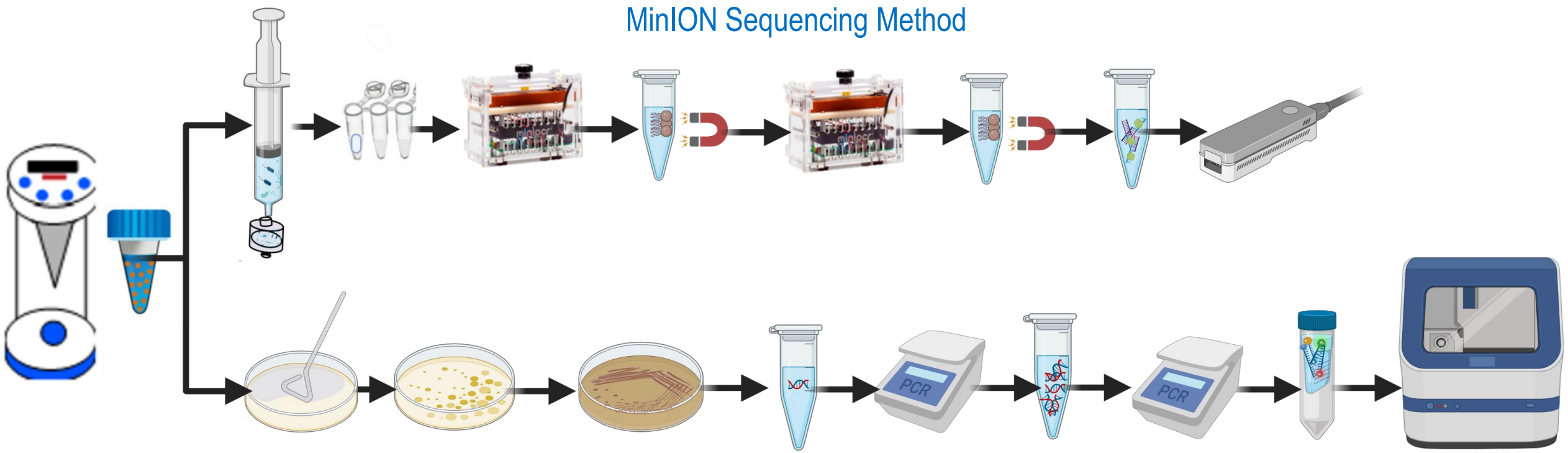


# Conclusion of prior work



- Moving to a culture-independent molecular-based method has many benefits
  - Reduces sample-to-answer time
  - Removes the bias towards the identification of only culturable organisms
  - Reduces crew exposure to potential pathogens
- Three air samplers were investigated for a direct sample-to-sequencer process for atmospheric microbial air profiling
  - Bio-Pump Plus is not compatible with the spaceflight procedure
  - SASS 3100 has a more complicated initial extraction protocol, and the microbial profile is less comparative with culture-dependent data (some bias toward genera may occur)
  - Coriolis Compact has an easy-to-use protocol, and a diverse microbial profile is detected that is highly comparable to culture-based data
- The Coriolis Compact has the most potential for near real-time assessments of the spacecraft atmospheric microbiome

# Forward Work: Split Samples



# Split Samples Results

- 8 samples were split for culture and MinION-based analysis.
- High degree of parallel identifications:
  - 36 matching identifications
  - Most common identifications present in culture were also present in nanopore data: *Staphylococcus, Micrococcus, Bacillus, Microbacterium,* and *Moraxella*
  - 4 total mismatches, but would not impact overall risk assessment
  - 1 culture isolate was unable to be identified (Gram-negative rod)
- Additional sampling in work and to include other environments



# Acknowledgments


## NASA JSC MinION Team / Co-authors



Sarah L. Castro-Wallace, Ph.D.
NASA JSC



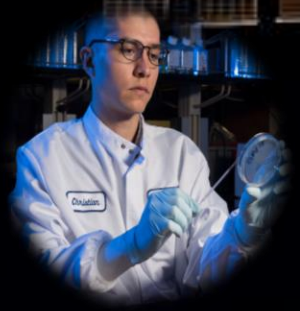
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Christian L. Castro, M.S.
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# Profiling the Astronaut Microbiome

Christian Castro

The 2nd 21st Century Space Microbiology TIM: NASA JSC and the German Aerospace Center (DLR)

November 7thand November 8th, 2022

# Spaceflight Standard Measures







- **Spaceflight Standard Measures (SSM)** is the collection of a set of consistent core measurements taken from U.S. International Space Station crew members to characterize the effects of living and working in space. This collection of data is related to many human spaceflight risks for astronauts before, during, and after long-duration missions. The data is archived and made available for future studies.
- Funded by the Human Research Program



AIMS:

- To ensure consistent capture of an optimized, minimal set of measures from crew members until the end of the International Space Station with the aim of characterizing the adaptive responses to living and working in space.
- To populate a data repository to enable high-level monitoring and meaningful interpretation of countermeasure effectiveness.
- To establish meaningful interpretation of health and performance outcomes and support future research on planetary missions.

# Standard Measures Microbiome



•**Microbiome:** From the bloodstream into the digestive track and the surface of the body, the human body is host to a multitude of microorganisms. These microorganisms ranging from bacteria to fungi play a vital role in human health. This “flora and fauna” create a balanced ecosystem or microbiome. The body may become sick when an imbalance becomes too large and out of control.

•**Research is being conducted to monitor and examine these microcolonies of organisms to better understand the relationship to each other and to the health and well-being of the astronaut.**



# Process Overview

1. Baseline data collection: Pre-flight, In-flight, and Post Flight
   - Saliva
   - Fecal
   - Body swabs

2. Return samples to the Microbiology Laboratory

3. Carry out DNA extraction, sample preparation, and next-generation sequencing

4. Perform data analysis, format data for the Life Science Data Archive (LSDA), and long-term data storage (of raw sequencing data)

# Microbiome

**Spaceflight Standard Measures**

Risk of Adverse Health Effects due to Host-Microorganism Interactions (Microhost)

**Test Sessions:** L-90, FD30, R-30, and R+30

**Body Swabs:**

- Sample forearm, forehead, nostril, and control area using pre-moistened swabs
- Stow in MELFI or home freezer

**Questionnaire:**

- Short environment, health and hygiene survey completed before each collection session

**Saliva Collection:**

- Sample every other day for 7 days (Total: 4 samples)
- Stow in MELFI or home freezer

**Fecal Sampling:**

- Ground collection using Human Stool Sampling Kit (provided)
- In-Flight collection using swab to collect sample from WHC immediately after defecation



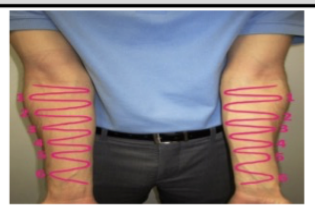




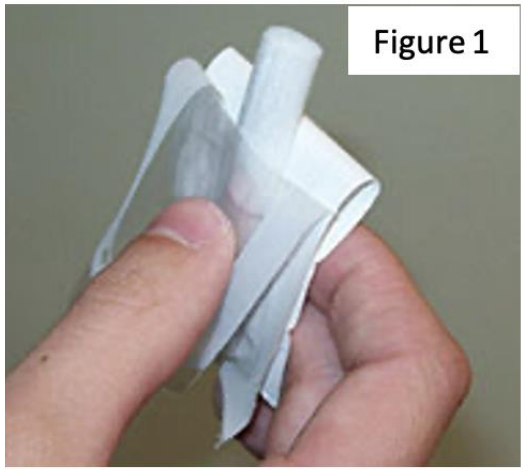

Measures:

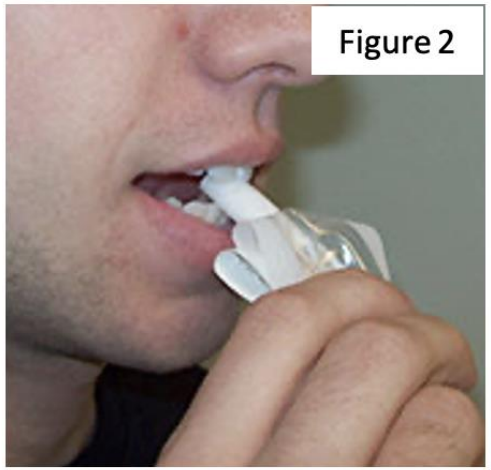
-Types and concentrations of microorganisms on the body

# Protocols

| Body Swab Cue Card | | | |
|---|---|---|---|
| **Sample Location Number** | **Subject Sample Site** | **Description** | **Image** |
| **1** | Either Inner Forearm | Trace at least 5 non-overlapping peaks/valleys on either left or right inner forearm (per crew discretion) using Body Swab designated #1. |  |
| **2** | Forehead | Trace at least 5 non-overlapping peaks/valleys on your forehead using Body Swab designated #2. |  |
| **3** | Either Nostril | Insert Body Swab into either nostril (per crew discretion) and make 6 turns while applying constant pressure using Body Swab designated #3. |  |
| **4** | Control (air) | Remove Body Swab designated #4 from tube, gently wave in the air to a count of five, and return to tube. | N/A |

Body swabs




Saliva

# Sample Collection Overview





Saliva Samples
4 over ~4 days




Body Swabs
3 Locations and a Blank



Fecal Sample

# Sample Processing Overview

1. Extract genomic DNA – Samples are loaded by hand and then transferred to the epMotion
2. QC of extracted DNA – Used to verify amount and size of extracted bacterial genomic DNA.
3. PCR amplification to target bacterial 16S gene
4. QC of amplified 16S DNA – Used to verify quality of amplicons for Illumina Sequencing
5. Normalize and pool library
6. Sequencing



DNA Extraction



PCR Amplification and Purification

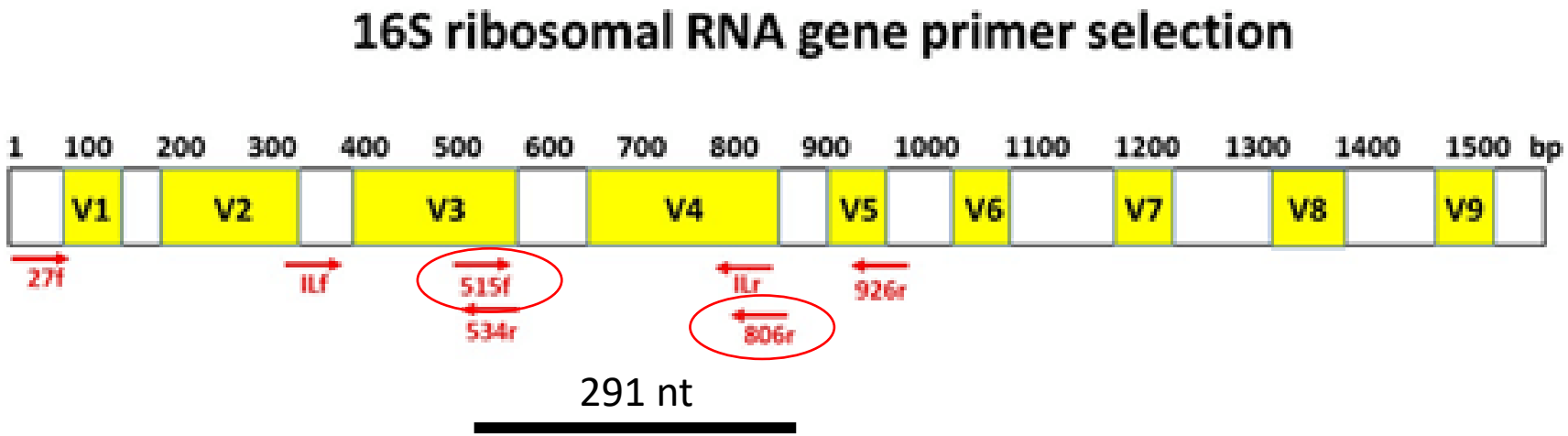


16S Illumina Sequencing

A consistent method that can implemented with little-to-no change across a multi-year study.

# What Data Do We Get?

- 16S Paired End Sequencing data
- Assigned Taxonomy with the SILVA 138 database
- Removed Abundance of less than 1% and Grouped by Genus
- Negative Controls and Positive Control Mock Communities and Genomic DNA at -80C



# Data Analysis

1. Evaluate quality of paired end reads using fastQC, fastp, and multiqc
2. Import data using paired end manifest format QIIME2
3. Trim forward and reverse reads
4. Generate ASVs using DADA2
5. Confirm sequencing depth with a rarefaction curve, determine level for subsampling, and assess diversity statistics





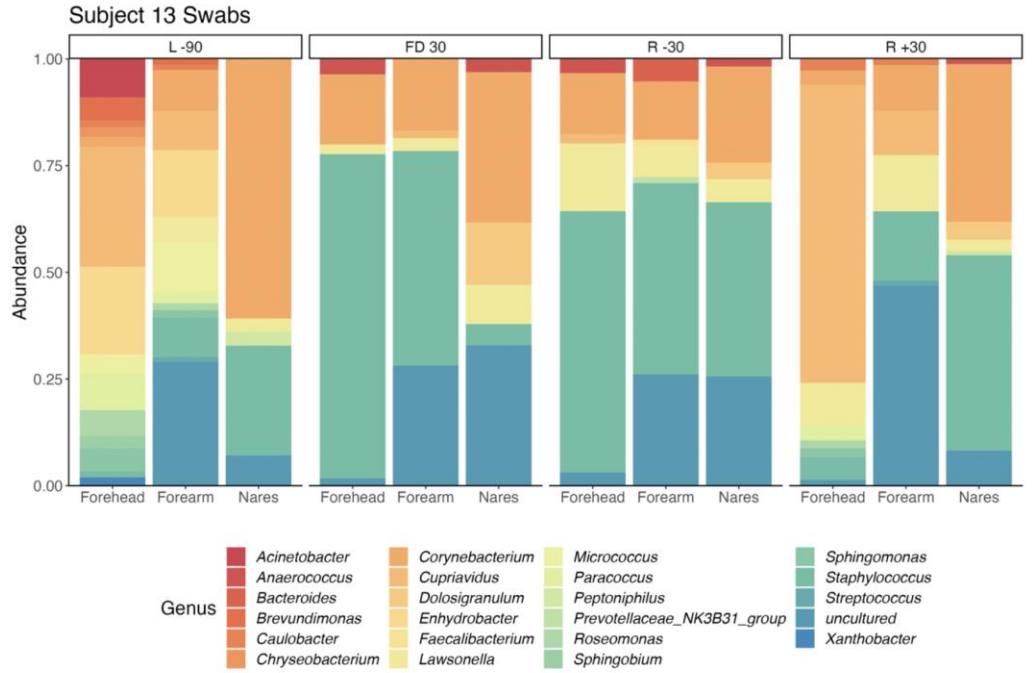


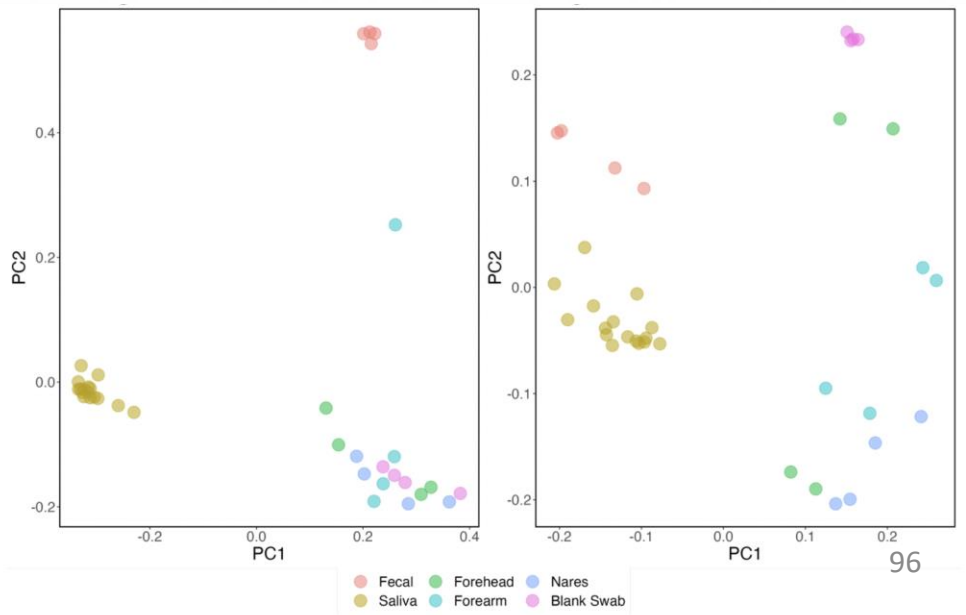




# Microbiology Data

- 18 crew members completed to-date
- Forward analysis:
  - Longitudinal comparison with an in-depth statistical approach
    - Comparing all crew member data against all crew members
    - Comparing in-flight samples against other in-flight samples
  - Differential expression changes of microbes
  - Integration of multiple parameters
  - So much more!



# Next Steps

- Additional Ground Studies - Crew Health and Performance Exploration Analog (CHAPEA)
- Performing the same sample collection and analysis as Standard Measures Microbiome
  - Focus on altered diet



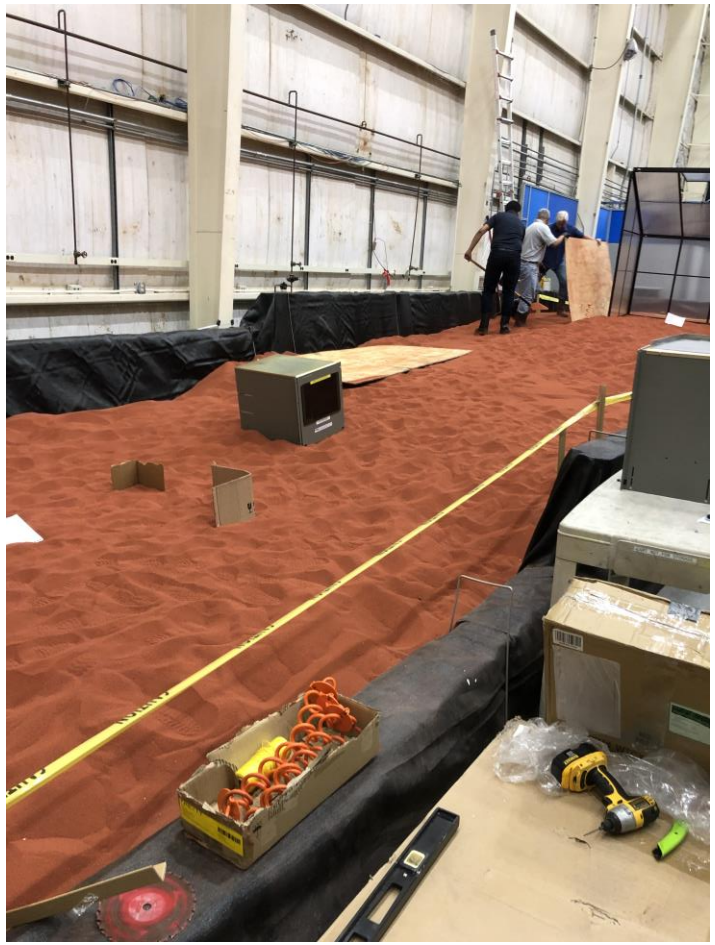
# Background on CHAPEA

- Four crew members living and working in a 1,700-square-foot module for a year long mission.
- 3D printed habitat
- AKA Mars Dune Alpha which includes:
  - Four private crew quarters
  - Dedicated workstations
  - Dedicated medical station
  - Common lounge areas
  - Galley and food growing stations
- 3 x 1-year missions

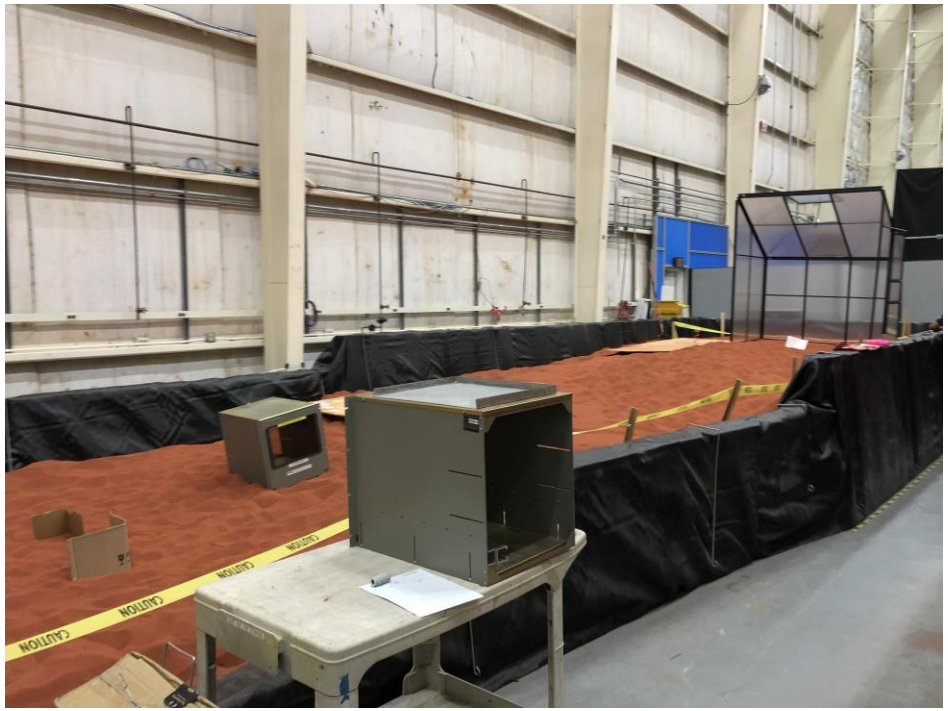
  1) Analog mission 1 – Starting Mid 2023
  2) Analog mission 2 – Starting TBD
  3) Analog mission 3 – Starting TBD


B220
B21
Google


1'
2'
5'
10'
CREW QUARTER 1
CREW QUARTER 2
CREW QUARTER 3
CREW QUARTER 4
AERO G.
AERO G.
STG
CROP AREA
W/D
W/D
W/C
SHOWER
AERO G.
CROP AREA
AERO G.
STG
RECREATION
55" SCREEN
KITCHEN
55" SCREEN
ROWING MACHINE
W/D
FITNESS
STATIC BIKE
W/D
3D PRINTER
SHOWER
W/C
CHANGE RM.
WORK ROOM
STG
roBOT-r STATION
TREATMENT ROOM



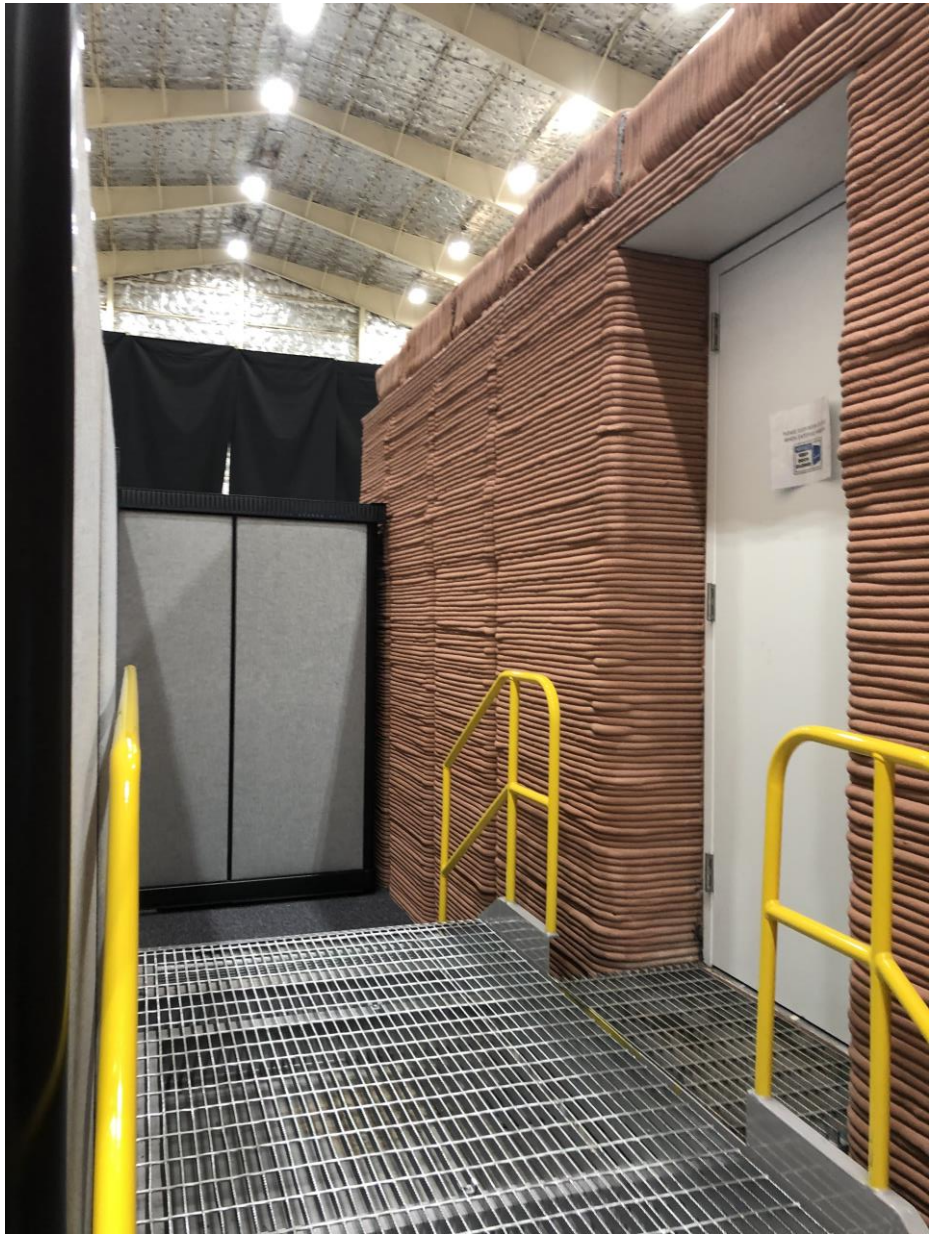
EVA Area




EVA Area



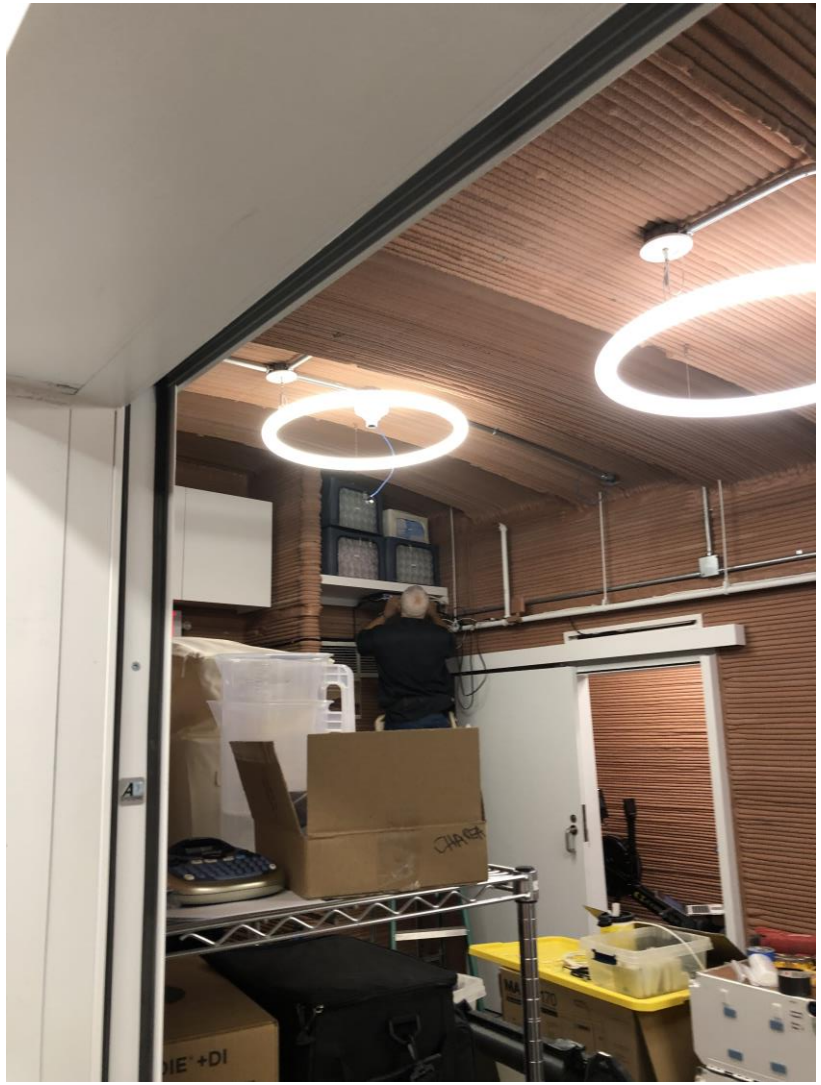
Resupply module

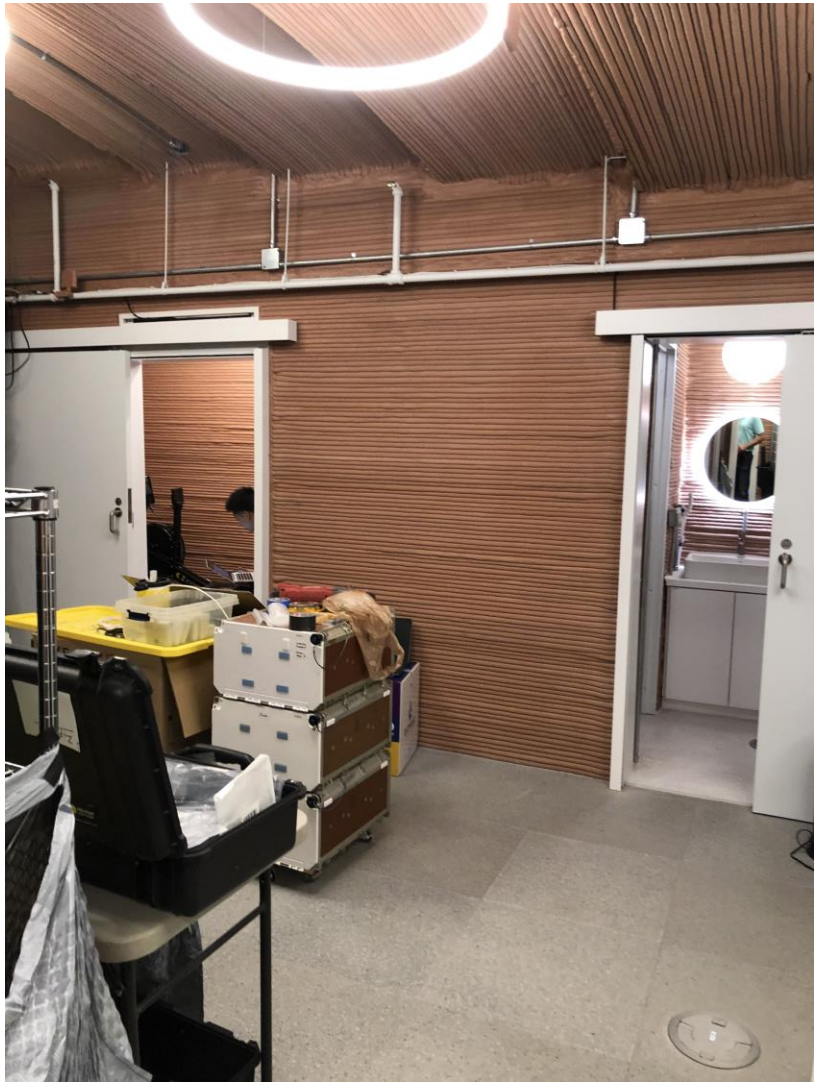


South Side of Habitat

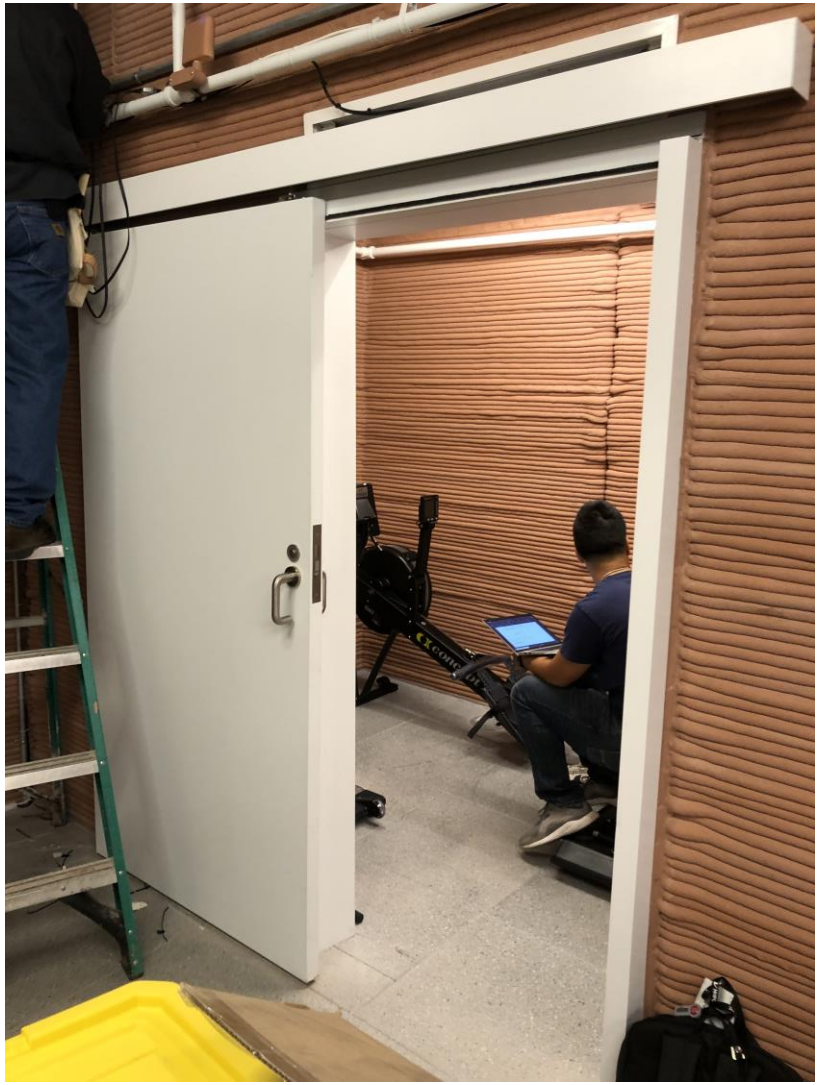


Entrance into Habitat





Work Room



Fitness

Questions?

Thank you

# Development of Real-Time, *in situ* Protocol for the Multiplex Detection of Herpesvirus Reactivation in Astronauts

Patrick Rydzak, Christian Castro, Hang Nguyen, Sarah Rommel, and Sarah Wallace



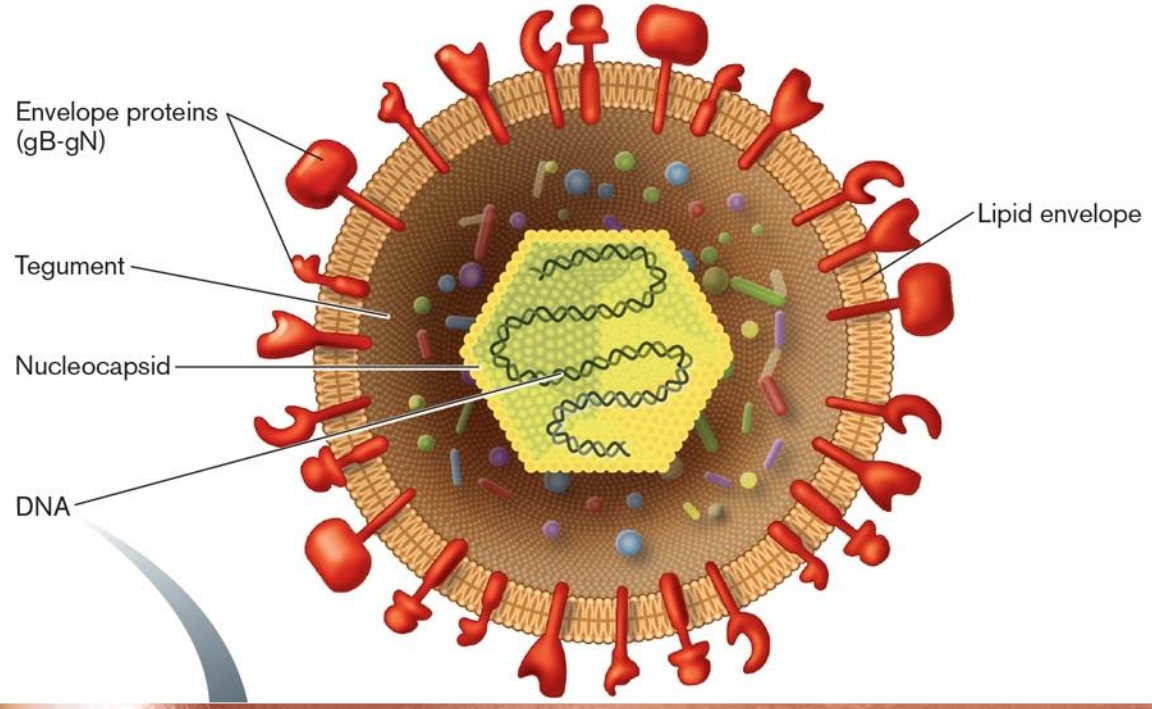
# Stress in Space

- Stressors
  - Anxiety
  - Confinement
  - Isolation
  - Sleep Deprivation
  - Radiation
  - Microgravity
  - Physical
    - altered immune response
    - fluid shift
    - muscle and bone loss
    - GI alterations
    - cardiovascular adaptations
- Increased viral reactivation as a result of spaceflight-associated stress
  - Samples returned to Earth for testing
  - Need for *in situ* monitoring






# Herpes Simplex Virus 1 (HSV-1)

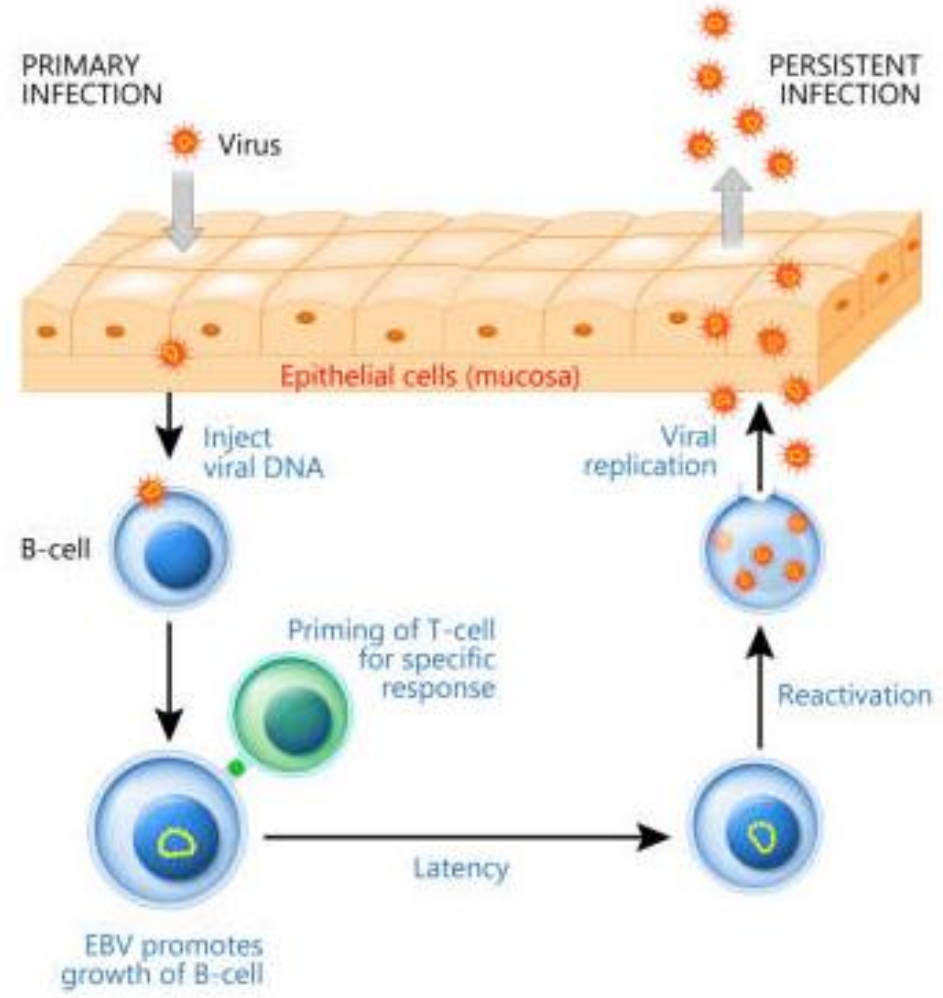



- Primarily infects oral epithelial cells
- Symptoms:
  - oral sores and blisters
- Orally transmitted
  - Mouth to mouth contact
  - Sharing drinkware or cosmetics
- Replicates at infection site
- Travel by retrograde
  - Axon to the dorsal root ganglia
  - Latency is then established
- Latent until reactivation occurs



# Epstein-Barr Virus (EBV)

- Human herpesvirus-4 (HHV4)
- Over 90% infection rate worldwide
- Infects B lymphocyte cells
  - Virus causes differentiation and circulation or latency
- Commonly Transmitted via:
  - Saliva
  - Sharing food and drinks
- Mononucleosis
  - Swelling of the liver, lymph nodes, malaise, fever, sore throat
  - Symptoms may last from two weeks, up to 6 months
- EBV associated cancers (1% of global cancers)

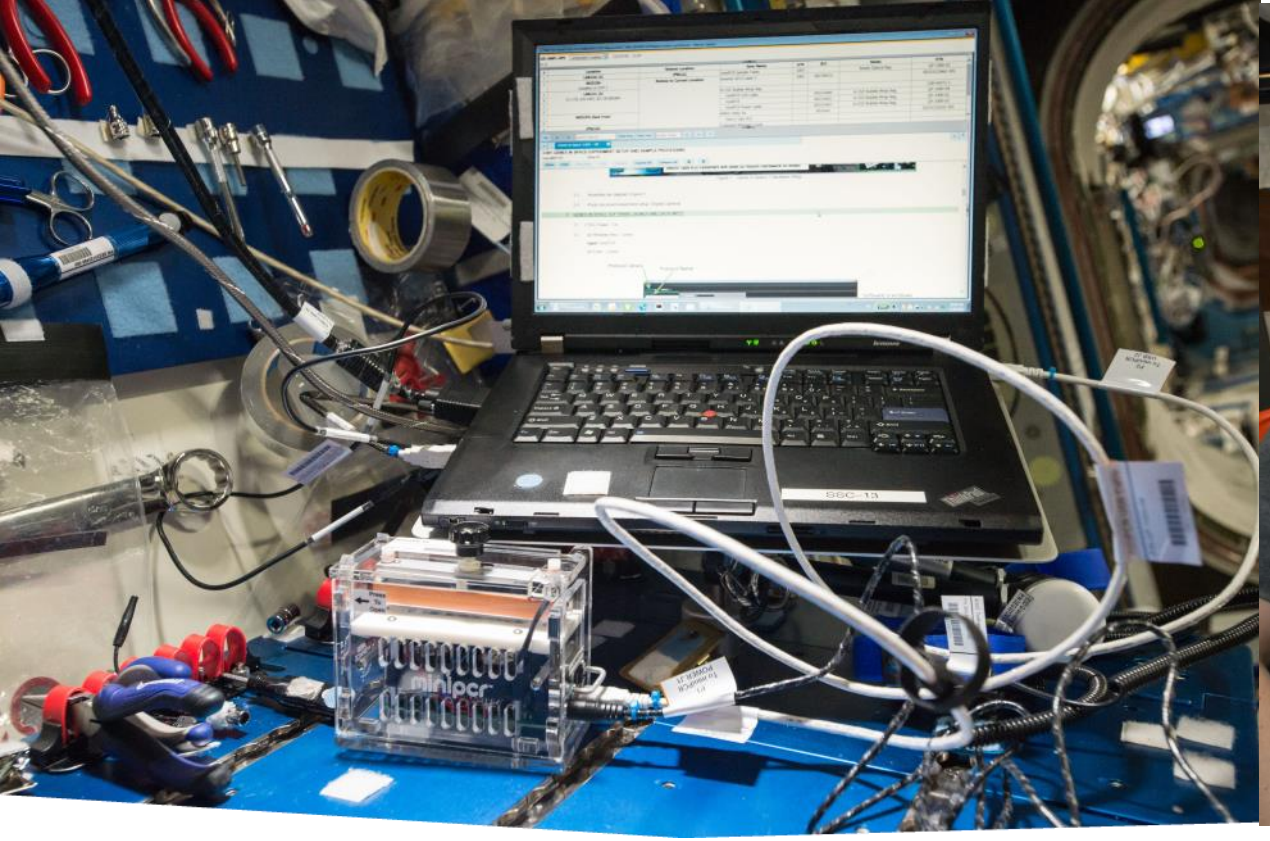


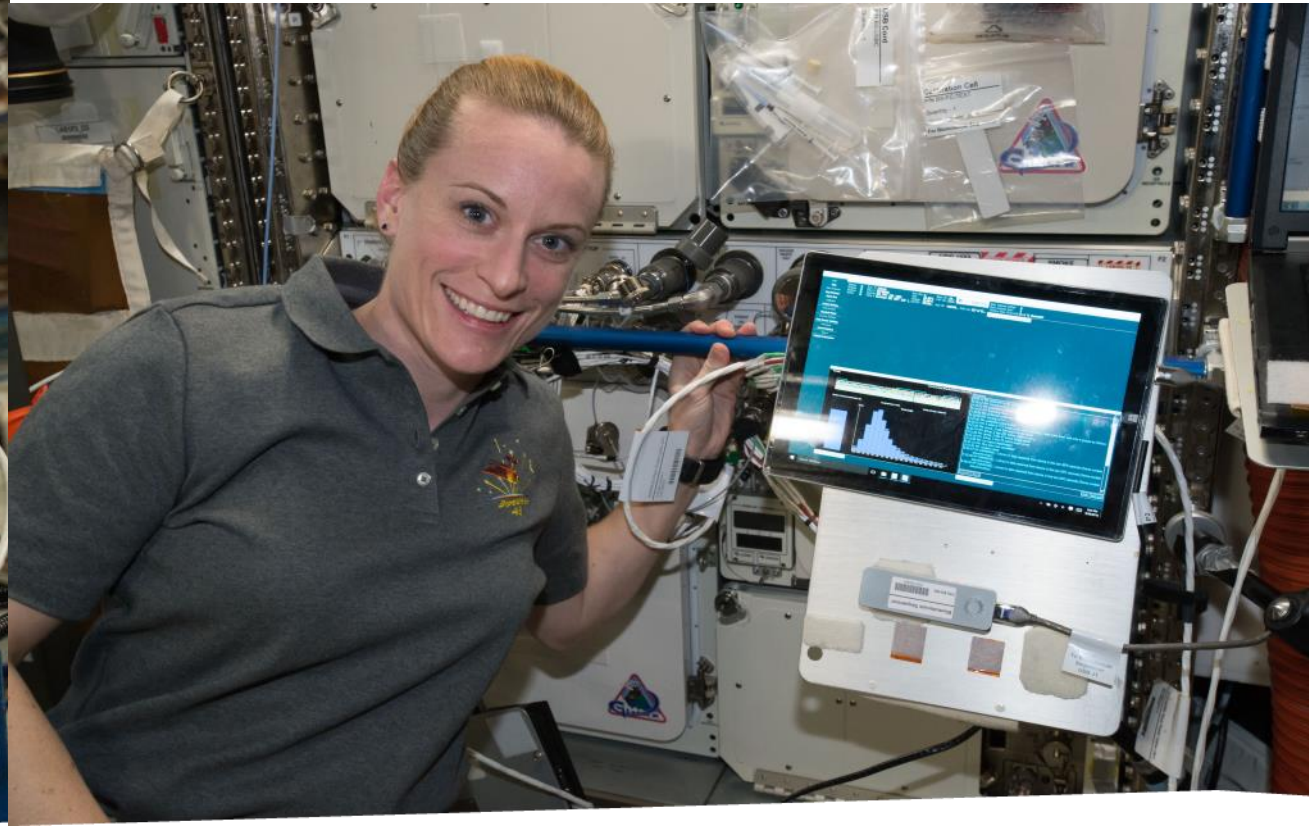
# Varicella-Zoster Virus (VZV)

- Primary infection leads to “chickenpox”
  - Relatively mild symptoms in children
  - Severe symptoms in adults
- Spread through:
  - Contact with skin lesions
  - Airborne respiratory droplets
- Establishes lifelong latency:
  - Cranial nerve
  - Dorsal root ganglia
- Reactivates as shingles
  - Asymmetrical painful rash






miniPCR



# Palmer Station Antarctica

- Located on Anvers Island (64° 46°S, 64° 03°W)
- Nathaniel Palmer, fist American to record sighting Antarctica (1820)
- Bio lab at palmer opened in 1970
- Avg temp -10°C – 2°C
- 4 meters of annual snowfall and 30 in of annual rain
- 20 winter crew, 40+ during the summer
- Similar viral reactivation rates to that on the ISS



# Objectives

1) Develop a multiplex PCR capable of detecting:
   - HSV-1
   - EBV
   - VZV

2) Incorporate an internal control
   - Slatherin (STATH)

3) Combine multiplex with ONT PCR Barcoding Kit

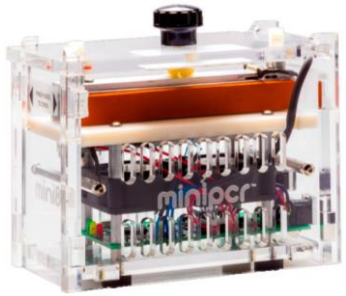
4) Determine assay sensitivity

5) Test assay on saliva samples

# Materials and Methods




Saliva Collection



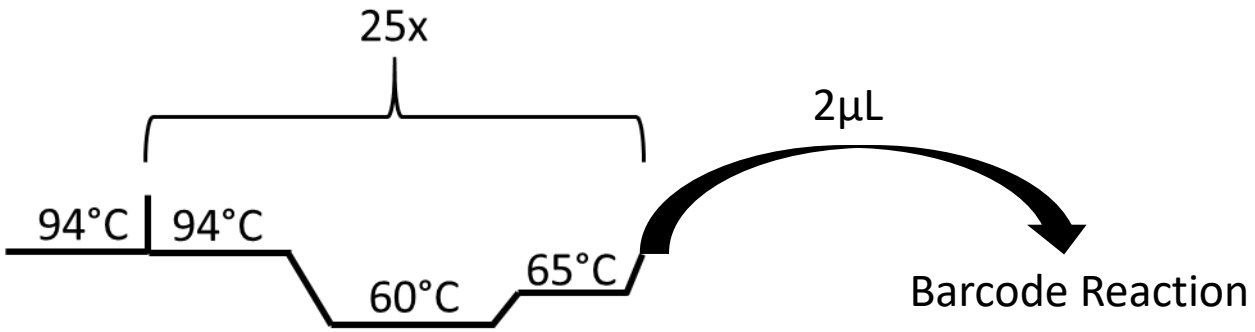


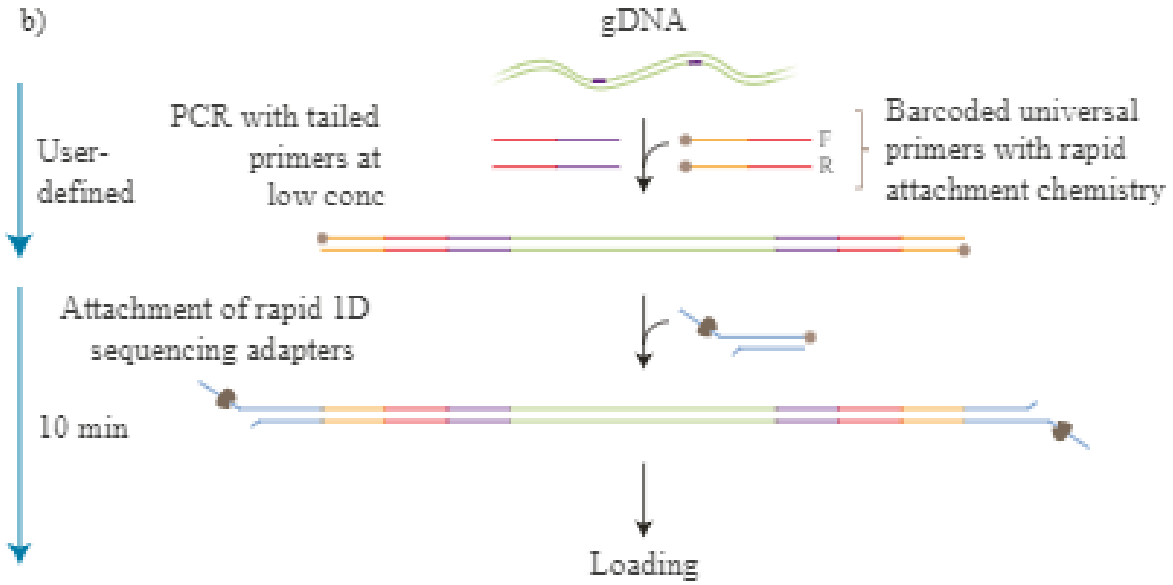


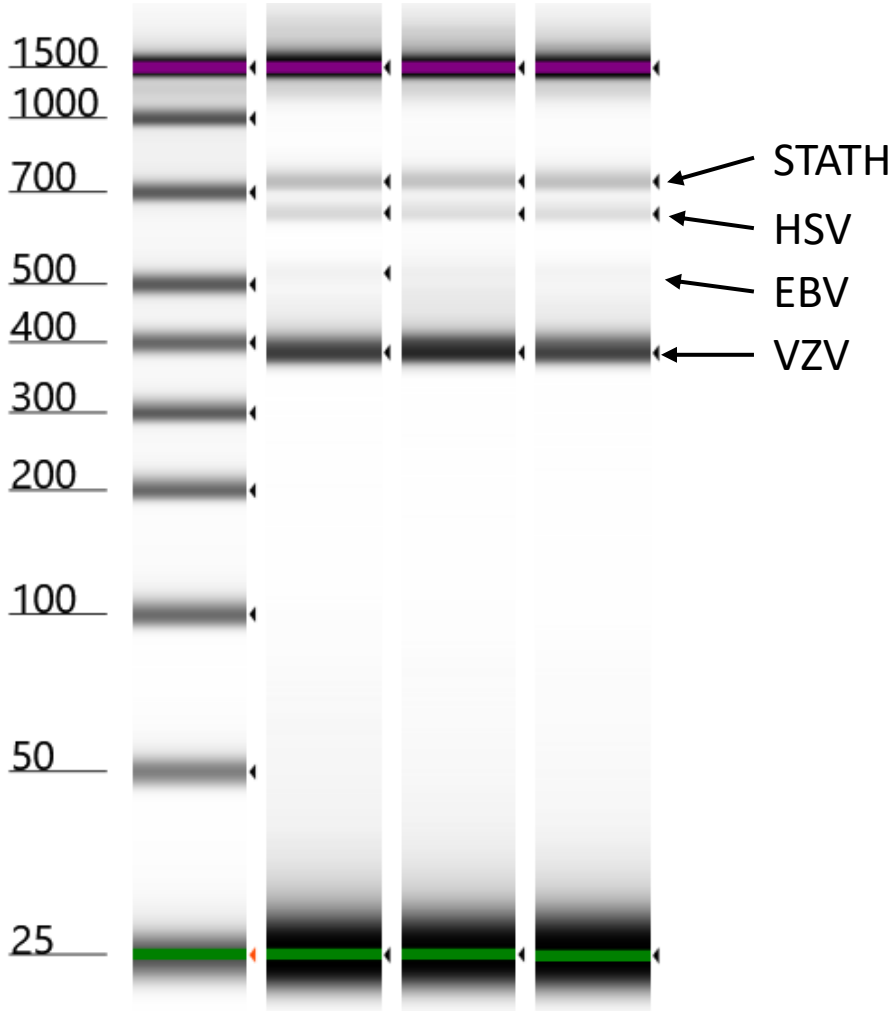




# Multiplex with ONT-“Tailed” Primers



# Objectives

✓1) Develop a multiplex PCR capable of detecting:

- HSV-1
- EBV
- VZV

✓2) Incorporate an internal control

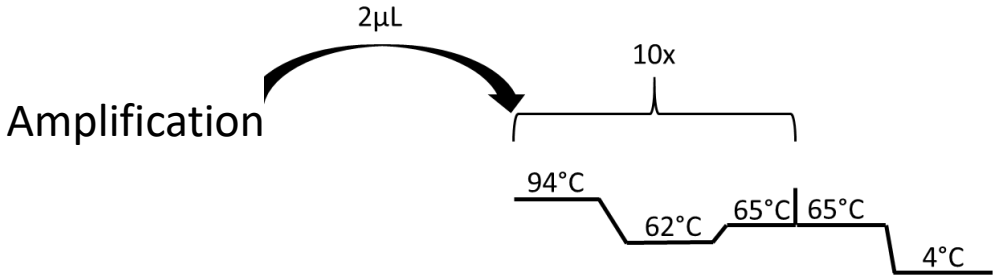
- Slatherin (STATH)

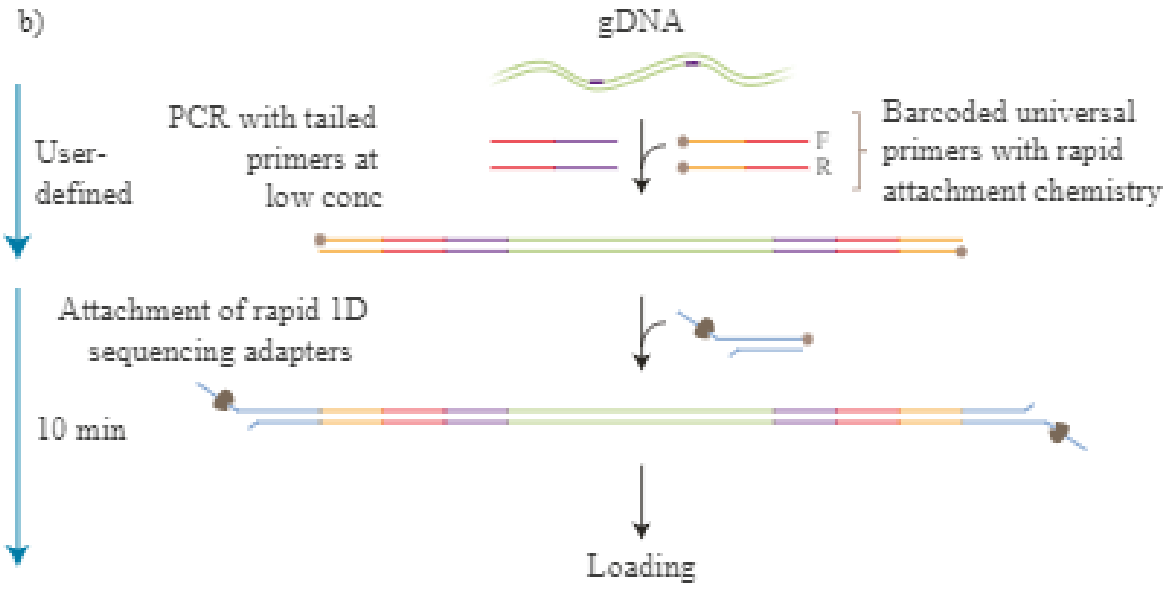
3) Combine multiplex with ONT PCR Barcoding Kit

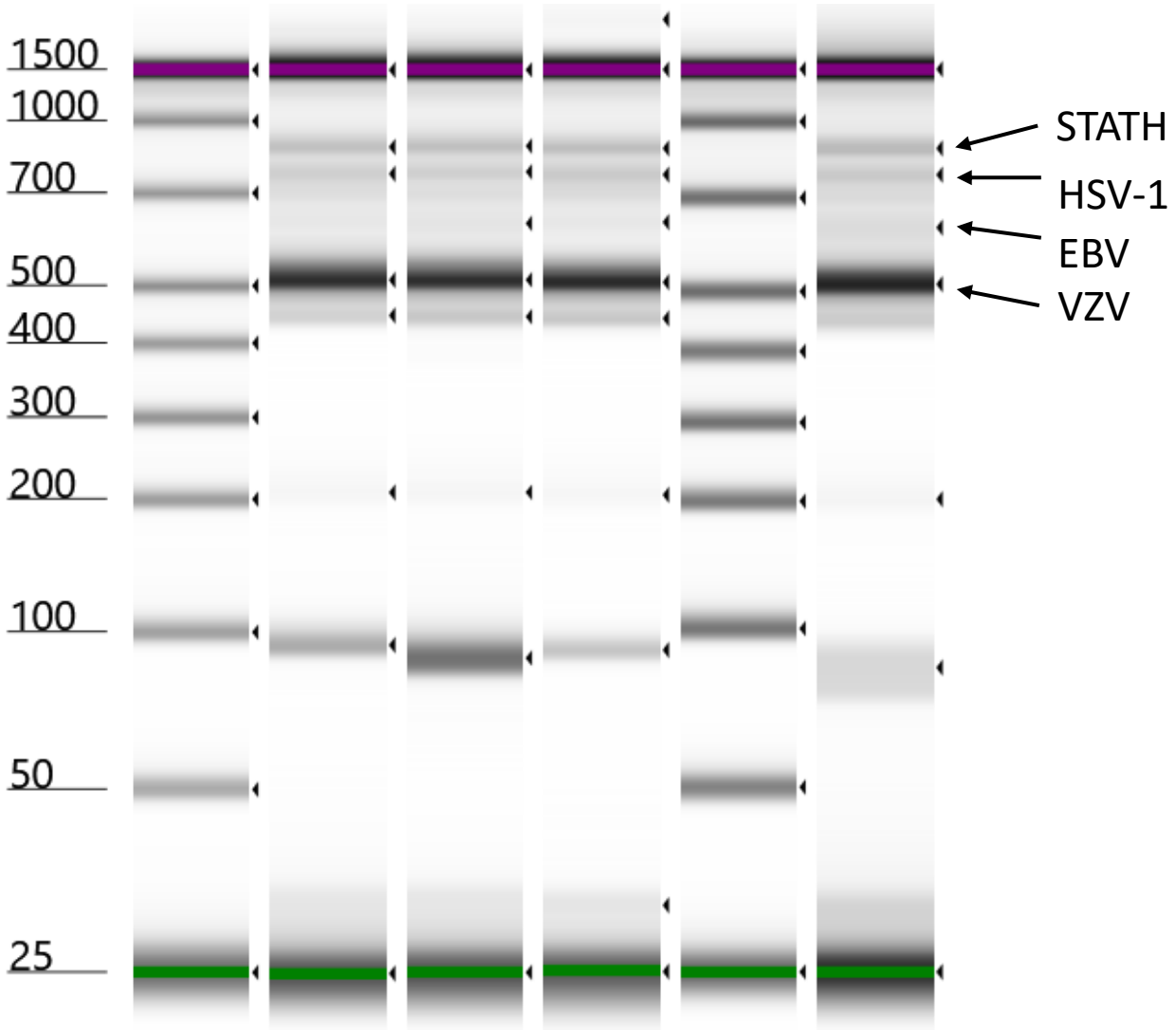
4) Determine assay sensitivity

5) Test assay on saliva samples

# Barcode Reaction



# Objectives

✓1) Develop a multiplex PCR capable of detecting:

- HSV-1
- EBV
- VZV

✓2) Incorporate an internal control

- Slatherin (STATH)

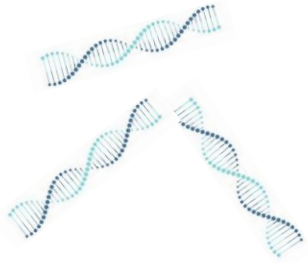
✓3) Combine multiplex with ONT PCR Barcoding Kit

4) Determine assay sensitivity

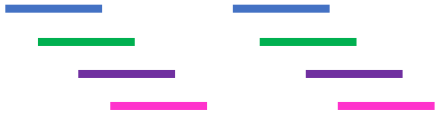
5) Test assay on saliva samples

# Testing sensitivity: Normalizing Template

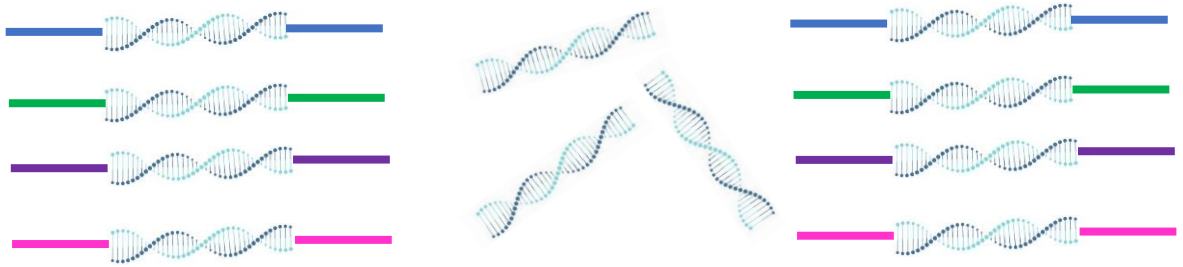
Virus and Human Genomic DNA



Multiplex Primers

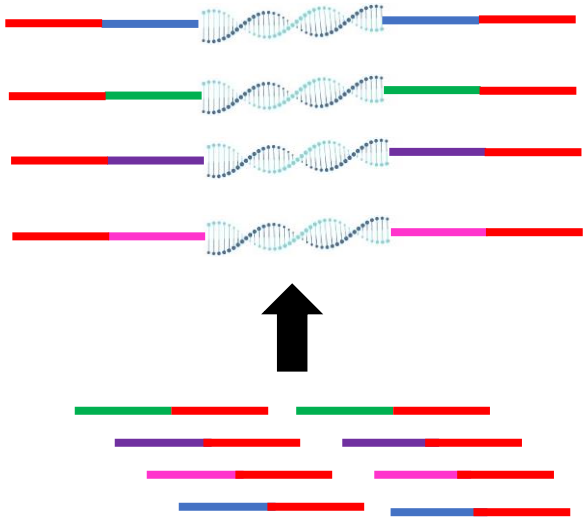


Amplified Product + Genomic DNA

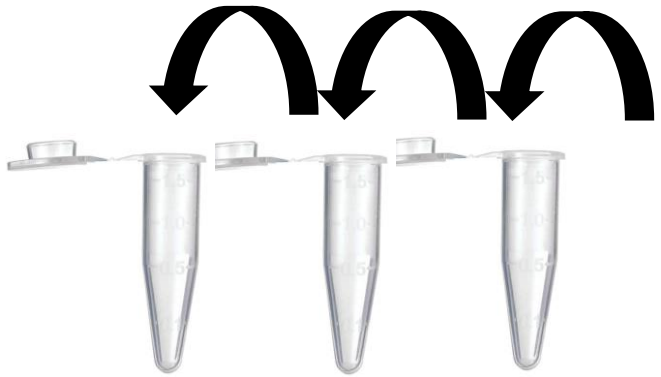


DNA Cleanup

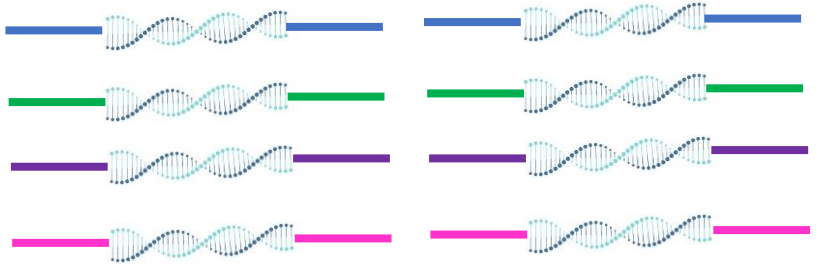
ONT-“Tailed” Amplicon



Serial Dilution

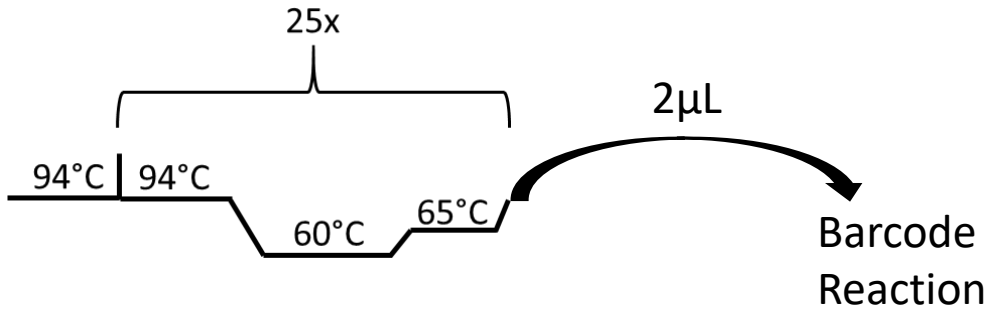


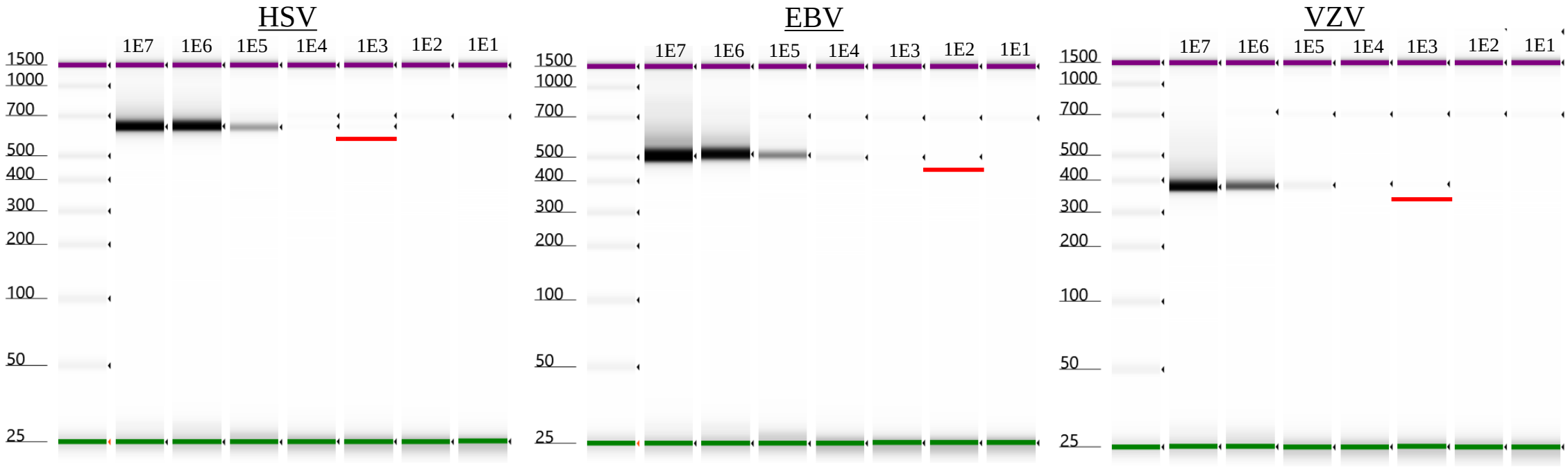
Purified Amplicon (Normalized)



# Testing sensitivity: Reaction 1 of 2

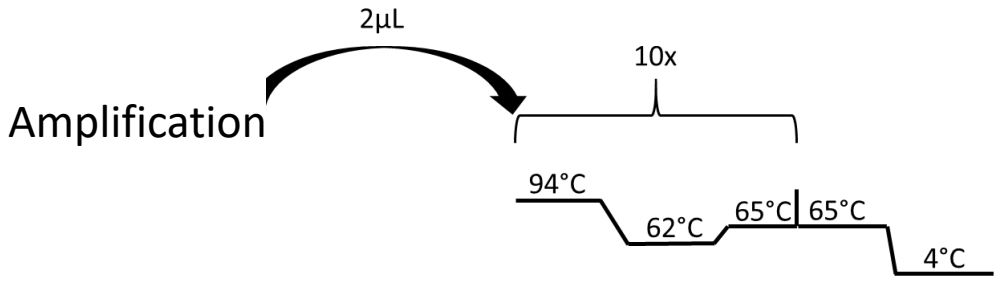
- Isolated, normalized template
  - 1E7 (~200,000 copies/µL) – 1E1 (~0.2 copies/µL)
  - ~16,000 copies of STATH per reaction

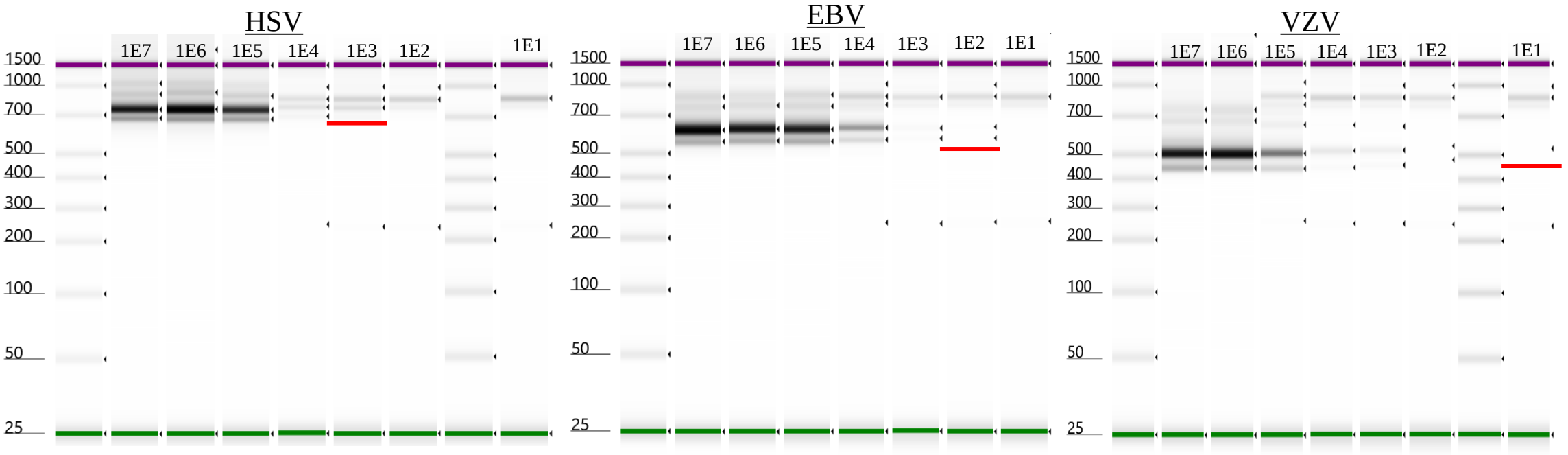


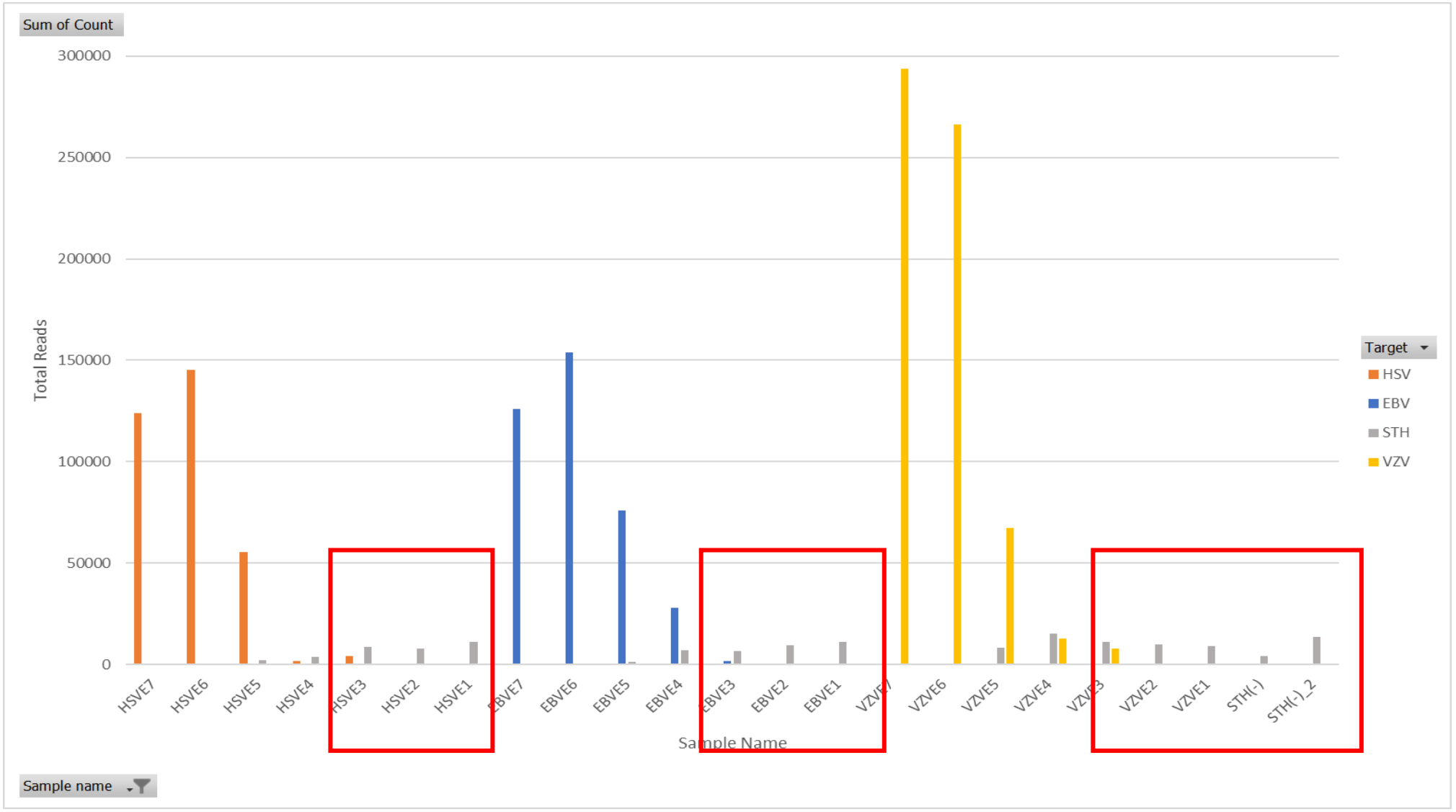
# Testing sensitivity: Reaction 2 of 2

- Transfer 2µL from reaction 1 to reaction 2
  - Amplifies target using adapter-targeted barcodes

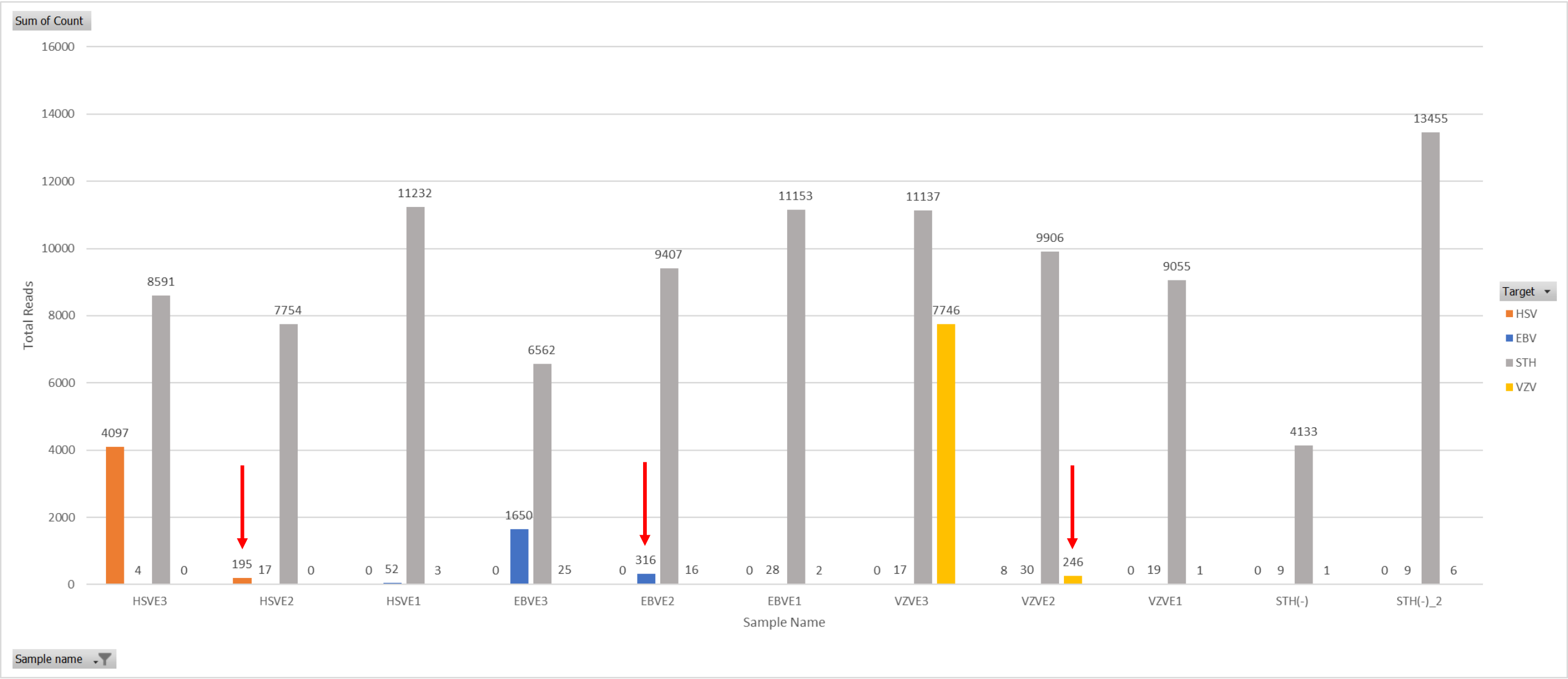


# Testing sensitivity: Sequencing results



# Testing sensitivity: Sequencing results



# Objectives

✓1) Develop a multiplex PCR capable of detecting:

- HSV-1
- EBV
- VZV

✓2) Incorporate an internal control

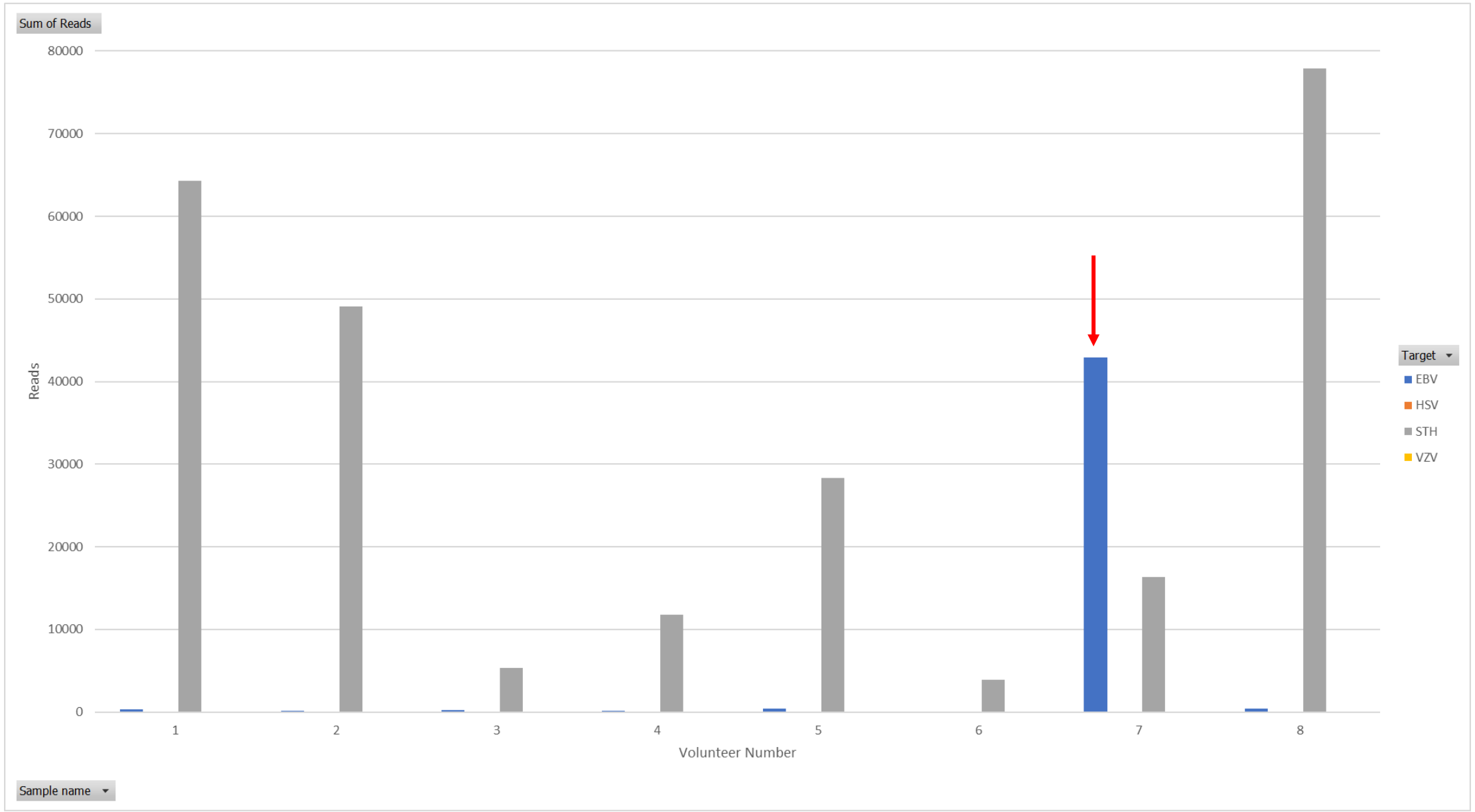
- Slatherin (STATH)

✓3) Combine multiplex with ONT PCR Barcoding Kit

✓4) Determine assay sensitivity

5) Test assay on saliva samples

# Testing Saliva Samples



# Objectives

✓1) Develop a multiplex PCR capable of detecting:

- HSV-1
- EBV
- VZV

✓2) Incorporate an internal control

- Slatherin (STATH)

✓3) Combine multiplex with ONT PCR Barcoding Kit

✓4) Determine assay sensitivity

✓5) Test assay on saliva samples

# Conclusions and Future Work

- Successfully combined multiplex PCR with ONT PCR barcode kit
- Assay will be tested against clinical samples
- Evaluation of assay and crew operability during winter over at Palmer Station
- Potential future application onboard ISS an beyond

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