

DOES THE INTERNATIONAL SPACE STATION LEAK DNA? PRELIMINARY RESULTS FROM THE ISS EXTERNAL MICROORGANISMS PAYLOAD

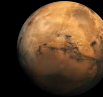
National Aeronautics and
Space Administration



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Mary Sue Bell , Jamie Miccuilla Patrick S.G. Chain, Richard E. Davis, Andrew J. Hatch, Paul Li Martin Tschirschwitz, and Sarah Wallace

05/21/2026

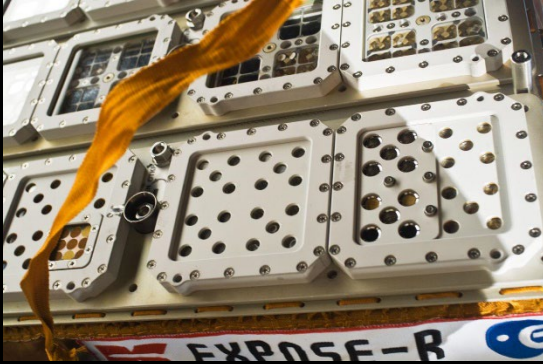


LEAKING VEHICLES AND SUITS COULD IMPEDE OUR SEARCH FOR SIGNS OF LIFE ON OTHER PLANETS



BACKGROUND

Some organisms can survive exposure to space!



Cyano-bacteria, lichen and fungi survived up to 500+ days outside ISS

images-
assets.nasa.gov/image/iss018e03922
7/iss018e039227~orig.jpg

Tardigrades survived extended ISS exposure... *and then reproduced*



We also know that all crewed, pressurized volumes will leak or vent

ISSUE

But we *don't* know what's actually leaking/venting from our current systems, how long those organisms could survive, or how far they may travel under destination conditions



Does proximity to a warm spacecraft matter?



How close can crew get without compromising science?



How far could our hitchhikers spread

The answers will drive element design (i.e. closed vs. open ECLS), where we place elements, and who/how we collect science samples

KNOWLEDGE GAP 2H. WHAT MICROBIAL CONTAMINANTS WOULD VENT FROM AN EXTRAVEHICULAR ACTIVITY (EVA) SUIT OR OTHER VEHICLES?



- Do we need to filter all of our vented products?
- How close can an astronaut get to a sample without contaminating it?
 - Should we use robots to collect and contain sensitive samples?



Apollo 12 Mission image - Astronaut Bean deploys ALSEP Central Station

Kminek G. et al. 2018. *Report of the COSPAR Workshop on Refining Planetary Protection Requirements for Human Missions*, Houston Texas: Lunar Planetary Institute. 28 p.

KNOWLEDGE GAP 2B. WHAT LEVEL OF NON-VIABLE MICROBIAL CONTAMINATION ESCAPE IS ACCEPTABLE?



<https://images.nasa.gov/details/art002e004357>

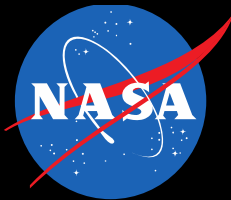
<https://www.nasa.gov/image-article/ammonia-pictured-venting-outside-of-international-space-station/>



- Do we need to filter all of our vented products?
- How close can an astronaut get to a sample without contaminating it?
 - Should we use robots to collect and contain sensitive samples?
- What if our spacecraft create an artificial habitable zone?

Kminek G. et al. 2018. *Report of the COSPAR Workshop on Refining Planetary Protection Requirements for Human Missions*, Houston Texas: Lunar Planetary Institute. 28 p.

COSMONAUTS COLLECT CULTURABLE BACTERIA AND FUNGI FROM THE EXTERIOR OF ISS



BioNanoScience

<https://doi.org/10.1007/s12668-019-00712-1>

Check for updates

Microbiological Investigation of the Space Dust Collected from the External Surfaces of the International Space Station

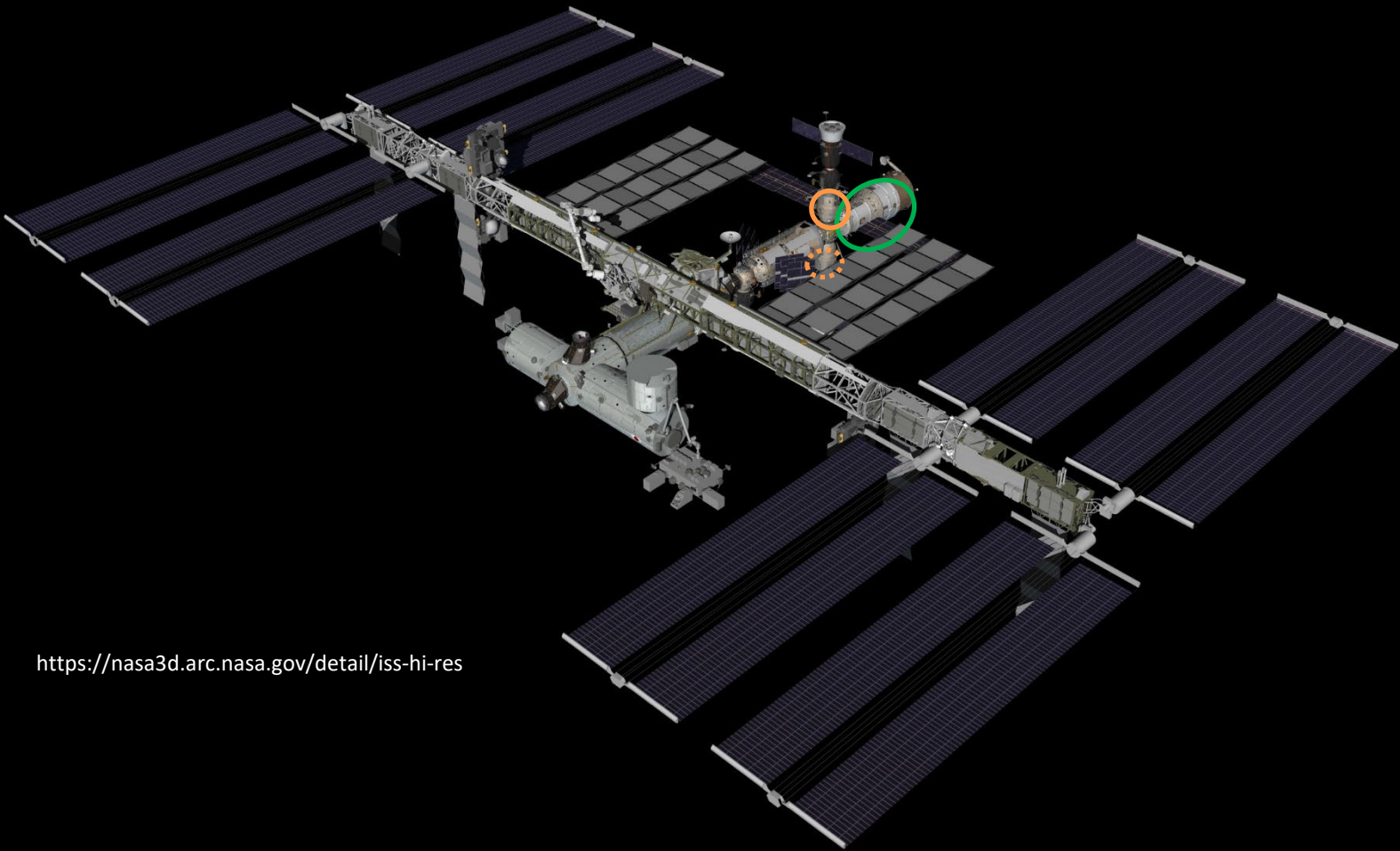
Elena A. Deshevyaya¹ · Elena V. Shubralova² · Svetlana V. Fialkina^{1,3} · Aleksandr A. Guridov¹ · Natalia D. Novikova¹ · Oleg S. Tsygankov⁴ · Pavel S. Lianko² · Oleg I. Orlov¹ · Sergey P. Morzunov² · Albert A. Rizvanov^{5,6} · Irina V. Nikolaeva⁷

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Table 4 Microorganisms isolated on the ISS external surfaces in SE TEST

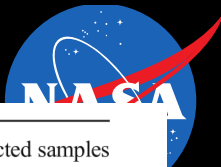
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EVA-42 26 February 2016	The exit porthole of the module “PIRS,” window, and hatch frame	Black and gray spots, bright grains

EVA, extravehicular activities
VL2, exit porthole 2
SM, SERVICE MODULE
STR, thermal control system



<https://nasa3d.arc.nasa.gov/detail/iss-hi-res>

RESULTS FROM THE RUSSIAN SEGMENT



BioNanoScience

<https://doi.org/10.1007/s12668-019-00712-1>

Microbiological Investigation of the Space Dust Collected from the External Surfaces of the International Space Station

Elena A. Deshevaya¹ • Elena V. Shubralova² • Svetlana V. Fialkina^{1,3} • Aleksandr A. Guridov¹ • Natalia D. Novikova¹ • Oleg S. Tsygankov⁴ • Pavel S. Lianko² • Oleg I. Orlov¹ • Sergey P. Morzunov⁵ • Albert A. Rizvanov^{5,6} • Irina V. Nikolaeva⁷



Table 2 The dust sampling chronology

EVA	Date	Cosmonauts who collected samples
EVA-25 (two samples)	15.11.2010	F. Yurchikhin
EVA-35 (two samples)	22.08.2013	A. Misurkin
EVA-38 (two samples)	19.06.2014	O. Artemiev
EVA-39 (two samples)	18.08.2014	O. Artemiev
EVA-40 (two samples)	20.10.2014	A. Samokutiaev
EVA-41 (five samples)	10.08.2015	M. Kornienko
EVA-42 (three samples)	26.02.2016	O. Volkov, Yu. Malenchenko

Table 4 Microorganisms isolated on the ISS external surfaces in SE TEST

EVA/date	Location sampled	Swab appearance	Microorganisms
EVA-25 15 November 2010	Valves of trace contamination removal unit	Black spots	<i>Bacillus licheniformis</i>
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module “SEARCH”	Black spots	<i>Bacillus sphaericus</i> ; <i>B. subtilis</i>
EVA-35 22 August 2013	Porthole of the exit hatch VL2 of the module “SEARCH”	Gray spots	<i>B. subtilis</i>
EVA-38 19 June 2014	SM, window 2	Black spots	<i>Bacillus pumilus</i>
EVA-41 10 August 2015	SM, between tubes of radiator (STR)	Black spots	<i>Bacillus pumilus</i> ; <i>Aureobasidium</i> sp.
EVA-42 26 February 2016	The exit porthole of the module “PIRS” surface	Gray spots	<i>Bacillus sphaericus</i>
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EVA, extravehicular activities

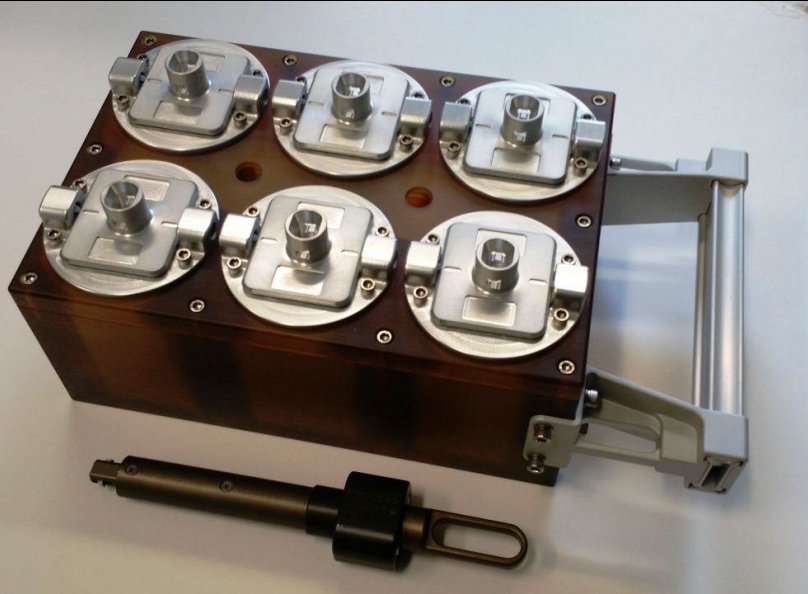
VL2, exit porthole 2

SM, SERVICE MODULE

STR, thermal control system

- All identified organisms also found inside ISS
- Only able to culture organisms from stained samples
- Not able to cultivate microorganisms from beneath thermal blankets

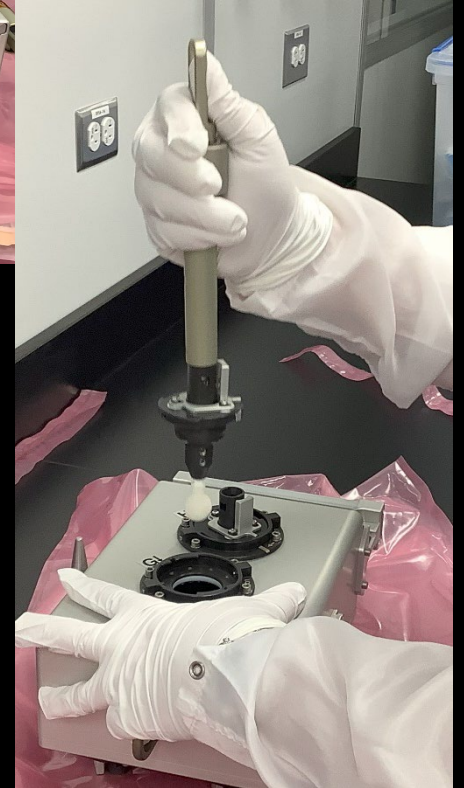
SPACE WALK SAMPLING CADDY



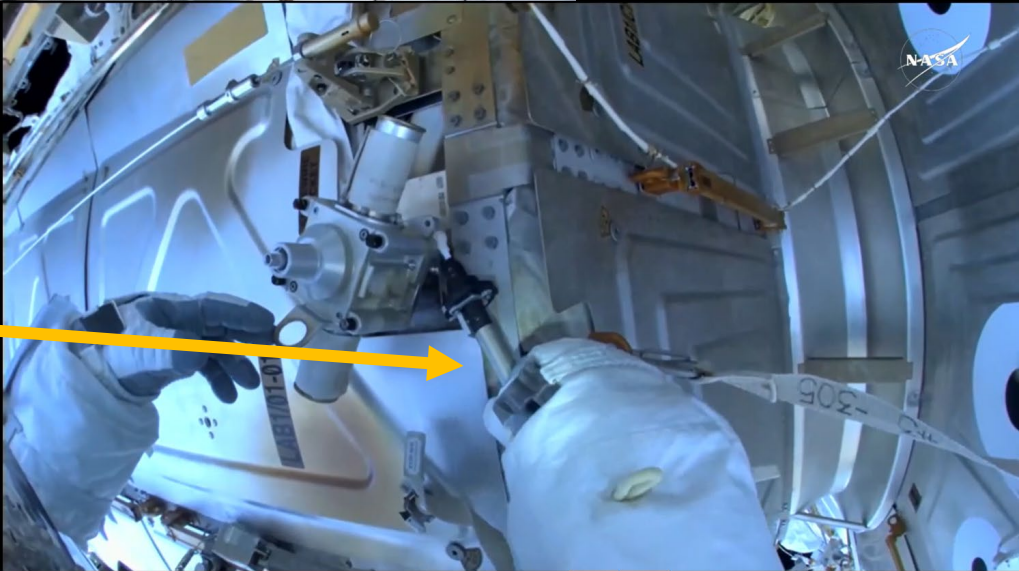
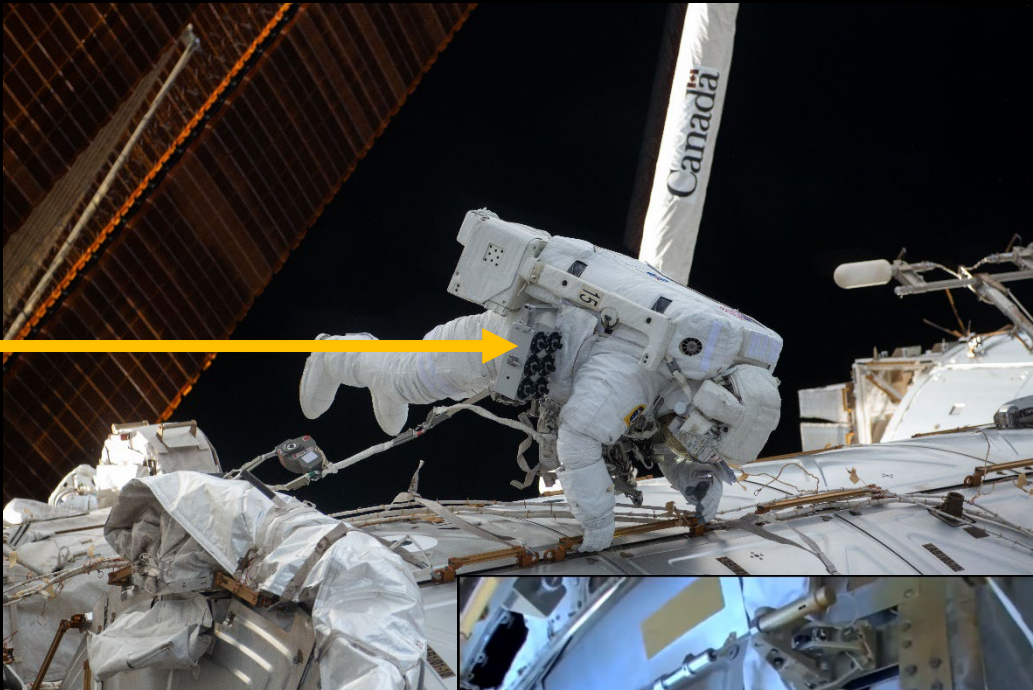
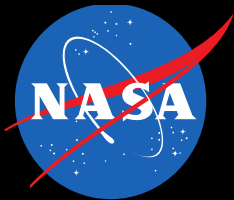
5 years



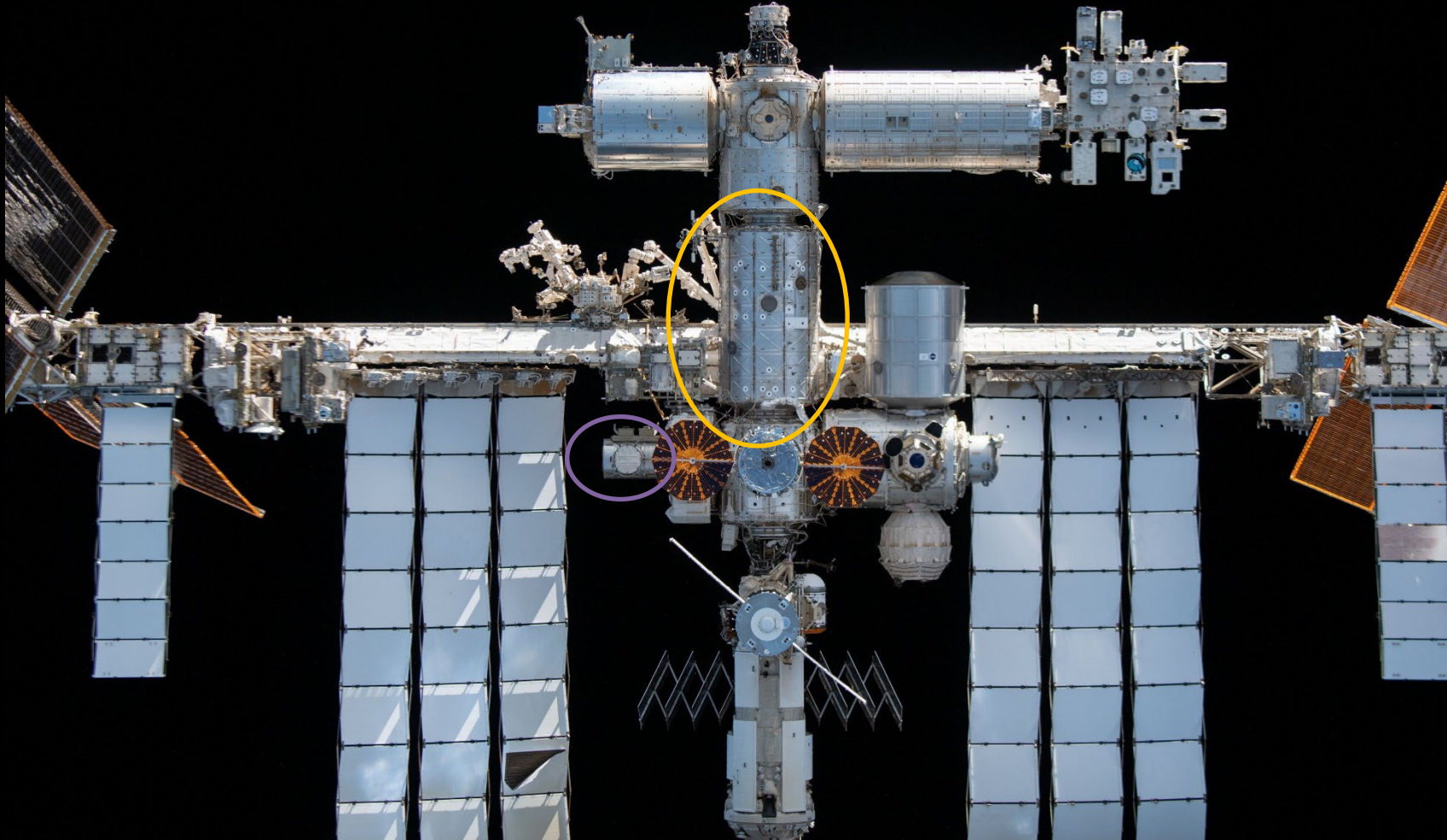
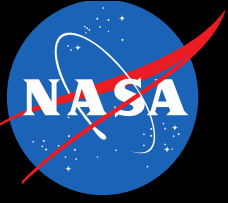
Delivered for launch in August 2023
EVA conducted Jan. 2025



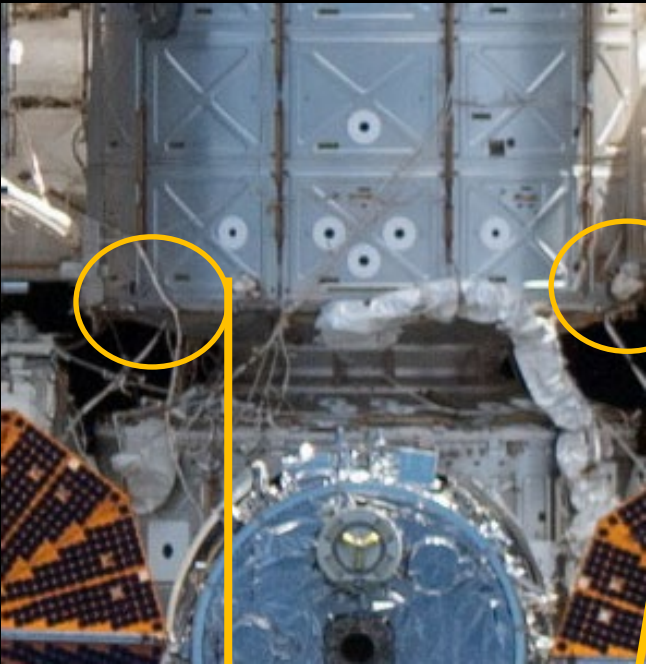
SPACE WALK SAMPLING CADDY HAS 8 SWABS



SAMPLE LOCATIONS NEAR THE AIRLOCK AND ON THE LAB MODULE



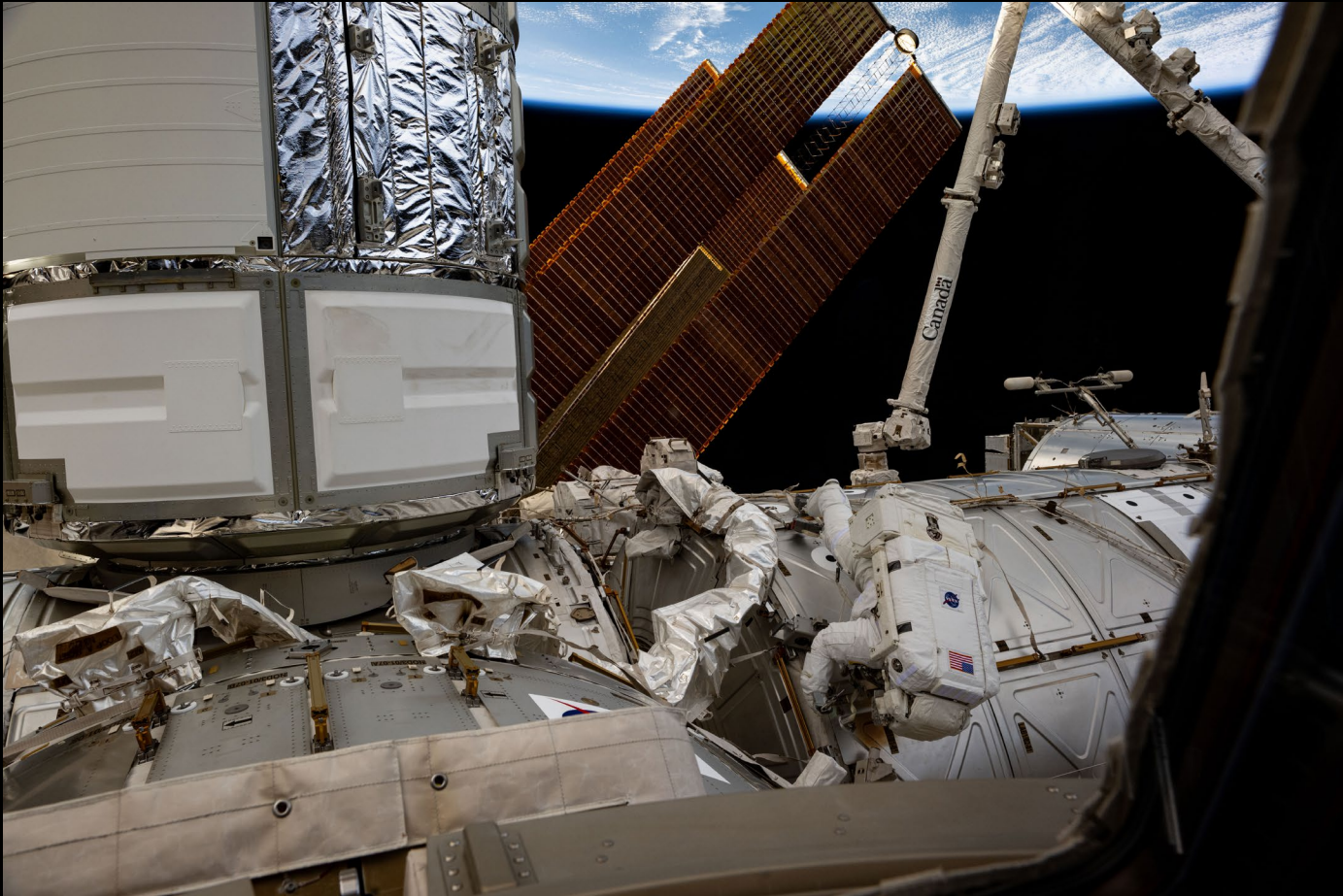
SAMPLE LOCATIONS ON THE LAB MODULE



3) Carbon Dioxide Vent

1) "Atmosphere Sample": Open a swab at this location but do not touch it to any surface

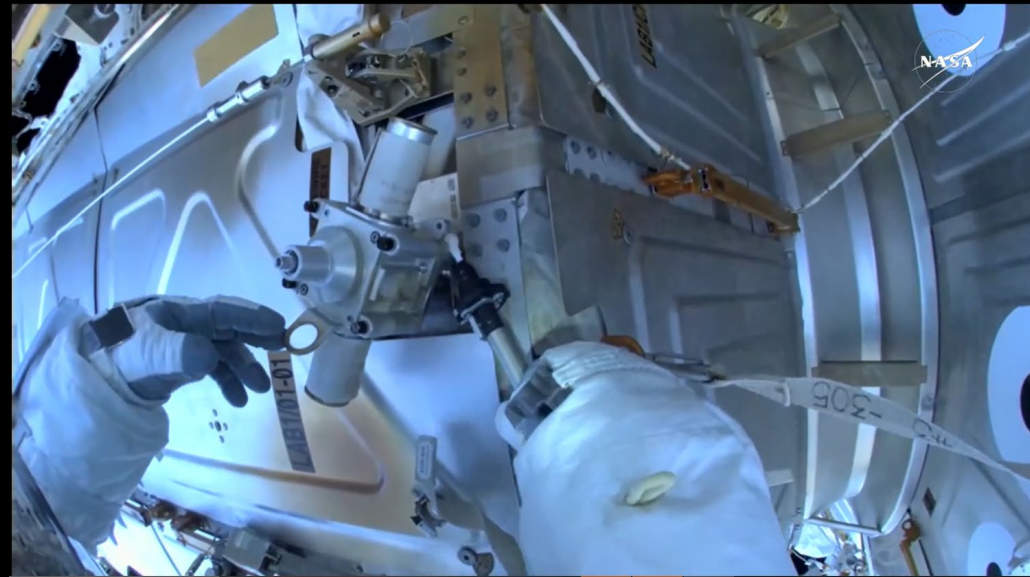
2) Vacuum Exhaust Vent



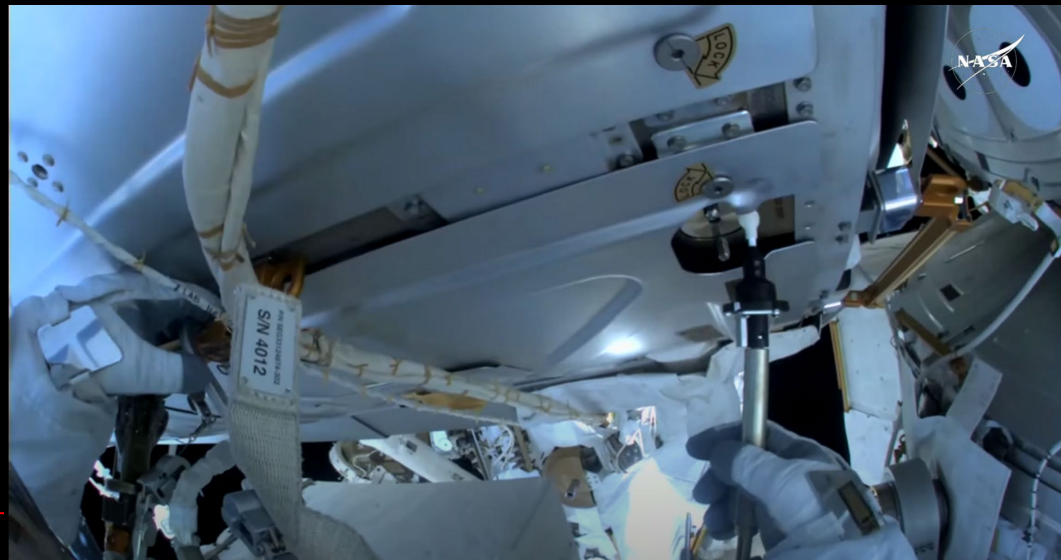
SAMPLE LOCATIONS ON THE LAB



1) "Atmosphere Sample": Open a swab at this location but do not touch it to any surface

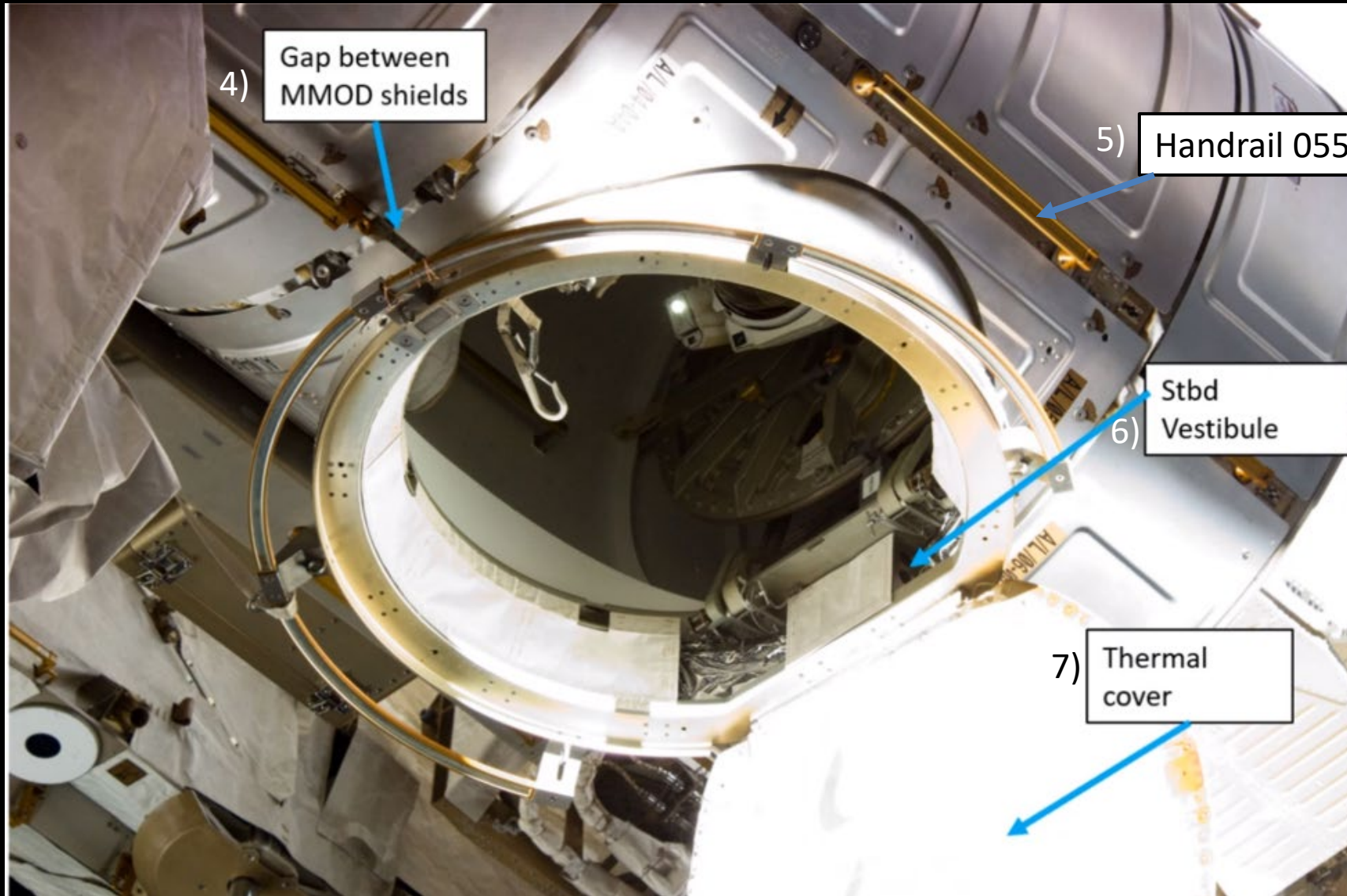


2) Vacuum Exhaust Vent



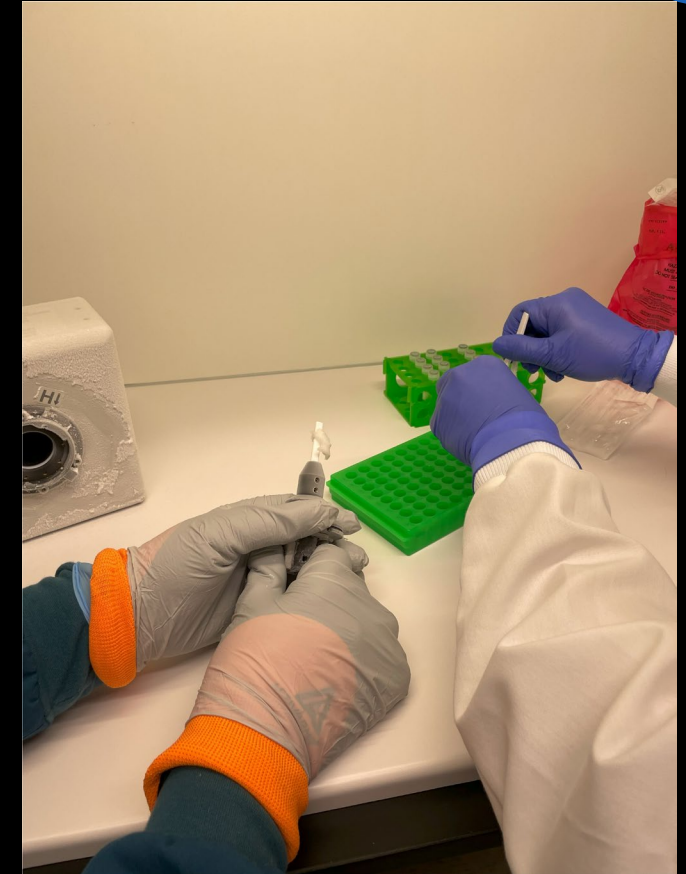
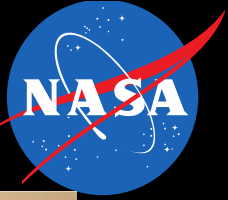
3) Carbon Dioxide Vent

SAMPLE LOCATIONS AROUND THE AIRLOCK



8) Negative Control: Swab never opened in space

DNA EXTRACTION ON THE GROUND

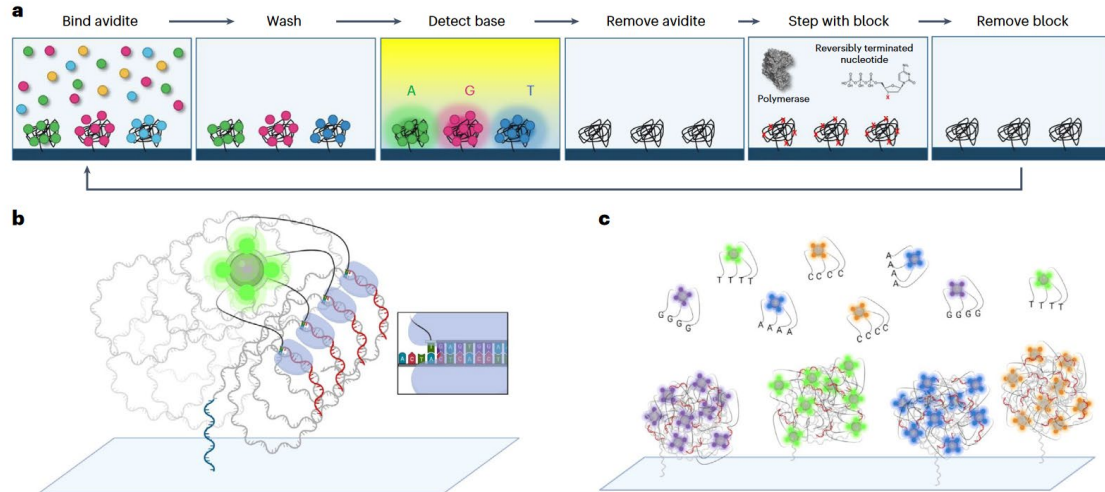


Bead beating to release cells from the swab head

Qiagen UCP DNA Extraction Kit

Spike in controls (zymo) added to several blanks

METAGENOMIC SEQUENCING WITH LOS ALAMOS NATIONAL LAB (PATRICK CHAIN)



- Element Biosciences Aviti Sequencer
- Data shared and processed using EDGE Bioinformatics
- Sequence Based / Taxonomic Analysis
 - BLASTnt, GOTTCHA2, Kraken2, PanGIA
- Metagenome Assembly
 - CONCOCT, MaxBin2, MetaBAT2

Element Biosciences Aviti Sequencing Platform
uses rolling circle amplification to increase signal to noise ratio

Arslan S. et al. 2024. Sequencing by avidity enables high accuracy with low reagent consumption. *Nature Biotechnology* 42:132–138.

DETECTED SIMILAR ORGANISMS AS THOSE CULTURED FROM RUSSIAN SAMPLES

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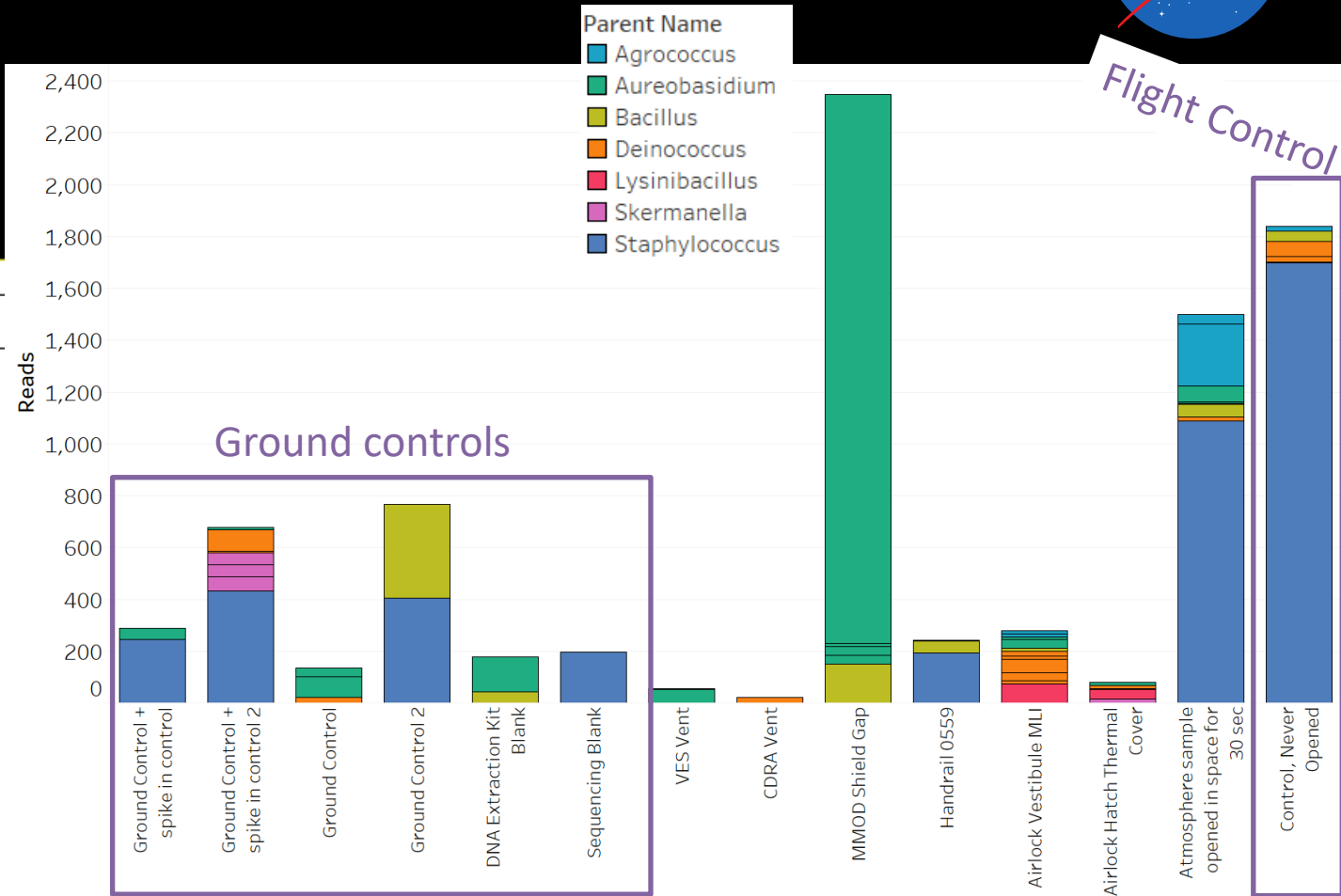
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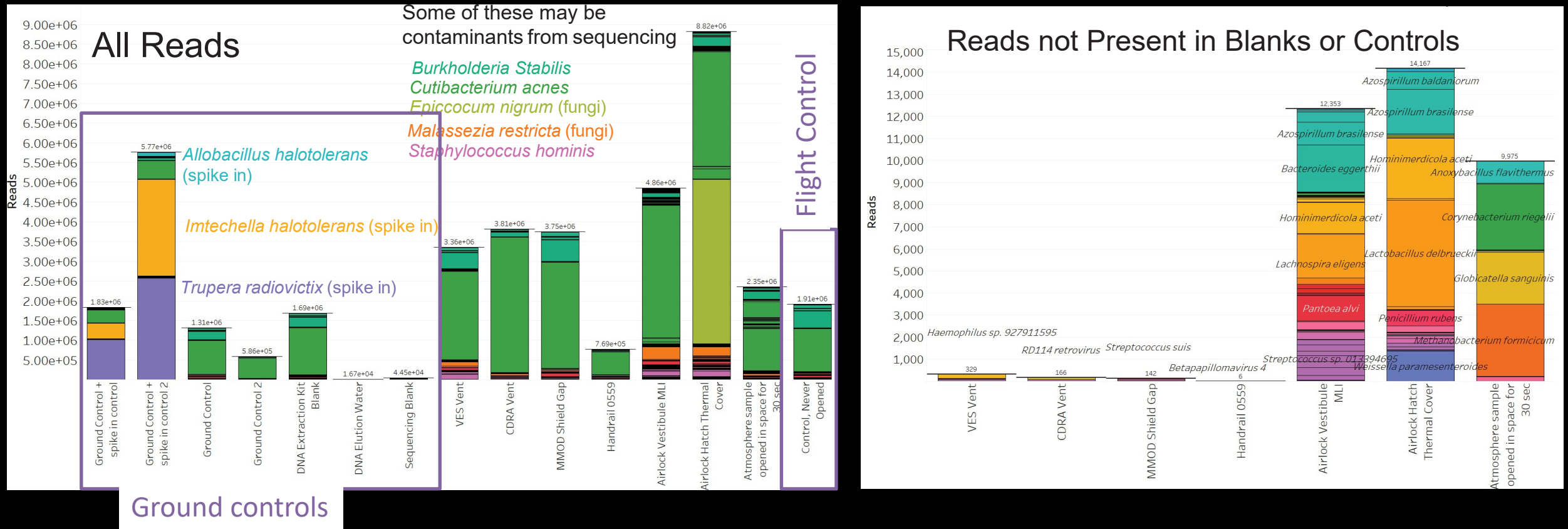
VL2, exit porthole 2

SM, SERVICE MODULE

STR, thermal control system



DOES THE ATMOSPHERE AROUND THE SUIT HAVE ITS OWN MICROBIOME?

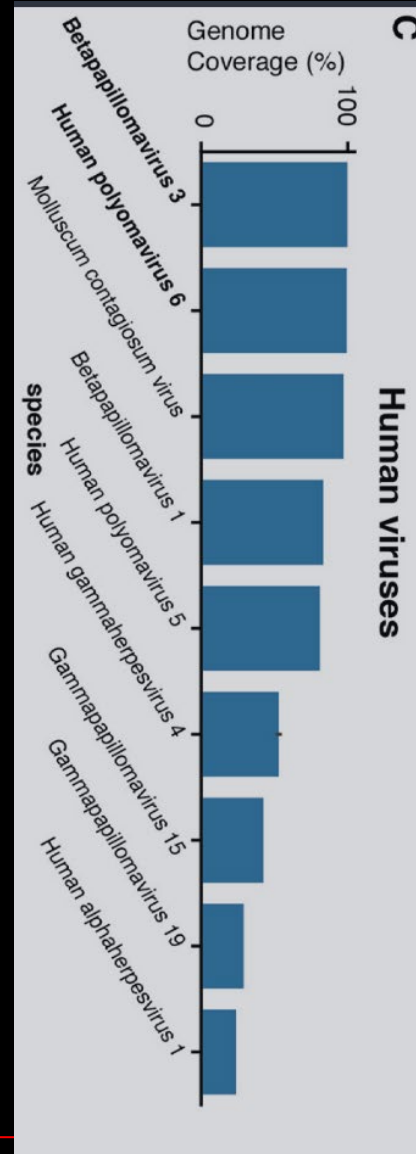
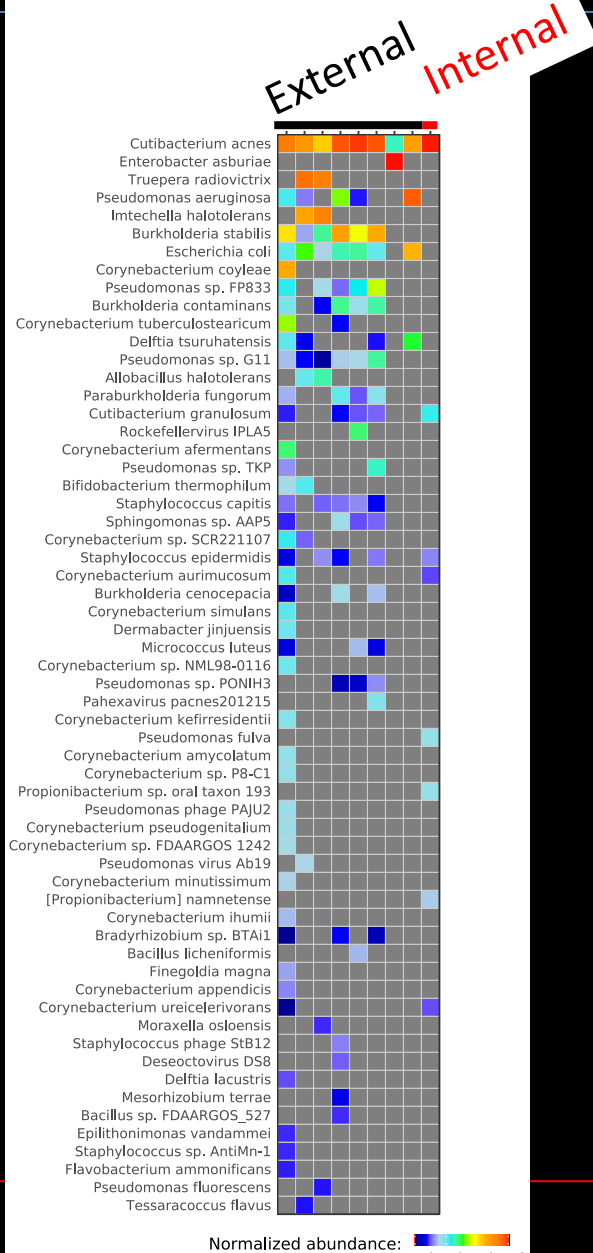


Observe similar clustering using binning methods

NEXT STEPS: COMPARE TO DATA FROM INSIDE ISS

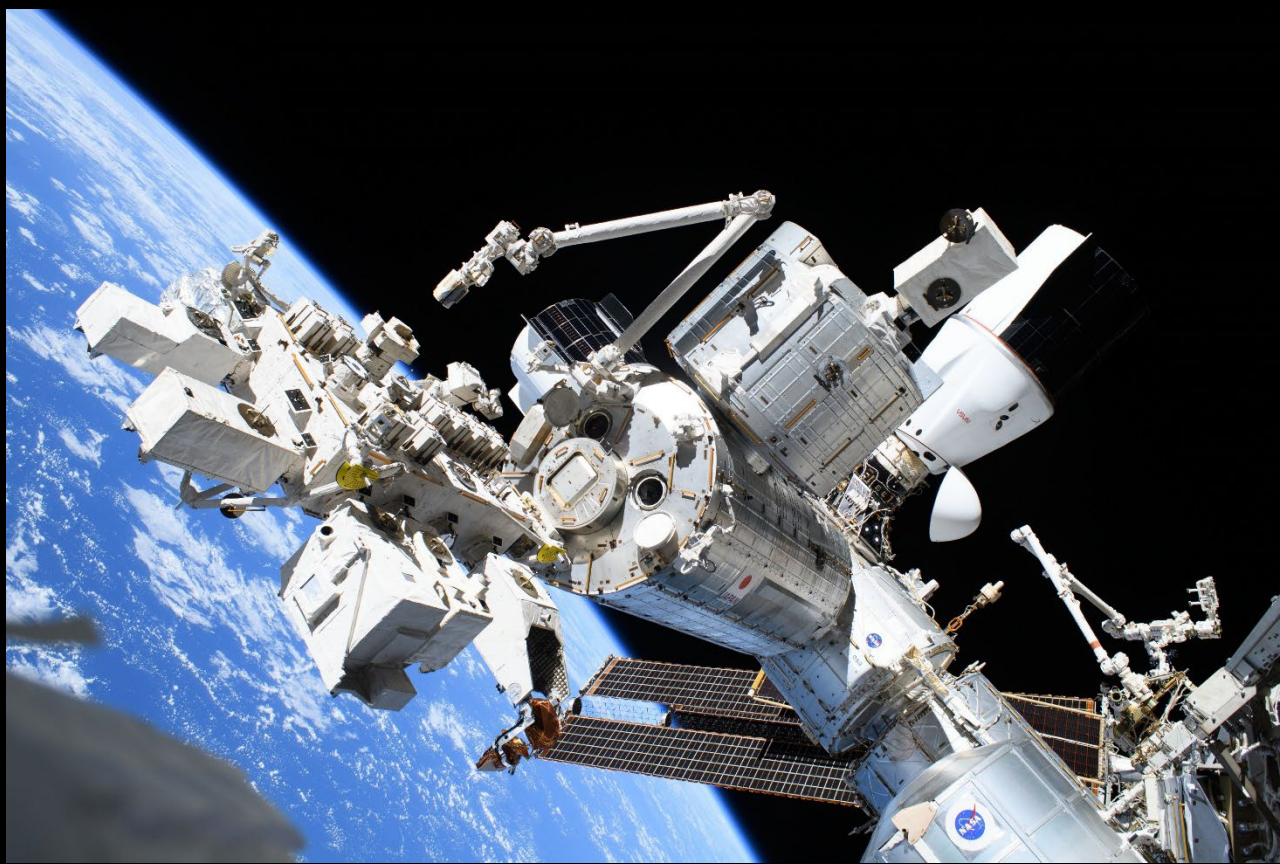


GOTTCHA2-SPEDB-B Merged plot (species)



- Reprocess in EDGE
- Data to Compare
 - Salido R. A. et al. 2025. The International Space Station has a unique and extreme microbial and chemical environment driven by use patterns. *Cell* 0.
 - Singh N. K., Wood J. M., Patane J., Moura L. M. S., Lombardino J., Setubal J. C., and Venkateswaran K. 2023. Characterization of metagenome-assembled genomes from the International Space Station. *Microbiome* 11:125.
 - Danko D. et al. 2021. Characterization of Spacesuit Associated Microbial Communities and Their Implications for NASA Missions. *Frontiers in Microbiology* 12:1900.
- Do External metagenomes look different from internal metagenomes from ground test data?

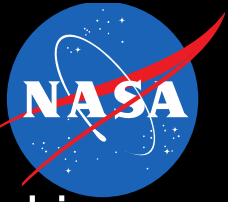
WE ARE PLANNING A SECOND EVA



- Originally Scheduled for 01/08/2026
 - On the timeline for 3/18/2026, but not accomplished
- Planned Sampling Locations
 - Replicate “atmosphere” sample and extend exposure time (30 sec. vs. 2 min.)
 - Replicate airlock samples
 - Sample Spacesuit Glove
- Sample Wishlist
 - Science Airlock on JEM
 - Sample the Human Landing System on the Moon

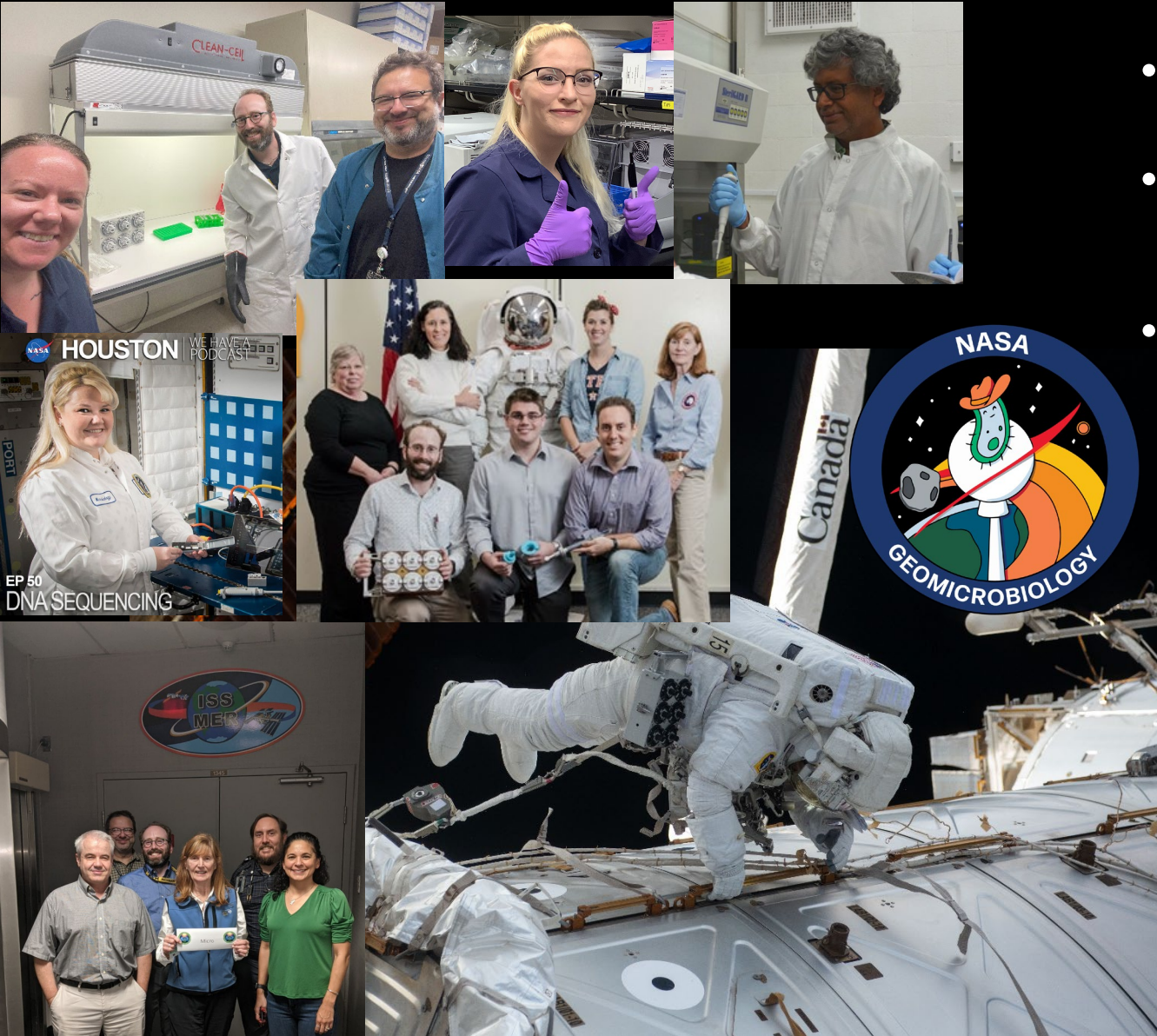
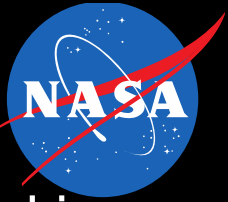
NASA: <https://flic.kr/p/2r2h3WA>

CONCLUSIONS AND FUTURE WORK



- Vents on ISS do not appear to be leaking much if any detectable DNA
- Crewed airlock and the suit itself do appear to be sources of DNA from inside station
- Metagenomic sequencing has improved in recent years and appears to be a viable option for even ultra-low biomass samples.
- Work needed to be able to extract and analyze these samples in space.
- Data processing pipelines need work if we want to use this for routine monitoring.
- How do we use shotgun metagenomics for monitoring?
 - Standardized protocols, methods and bioinformatic pipelines
 - DNA free reagents and consumables

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- Standardized protocols, methods and bioinformatic pipelines
- DNA free reagents and consumables



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Funding Number Type WBS	Funding Number 048290.02.01.01.05	⊖