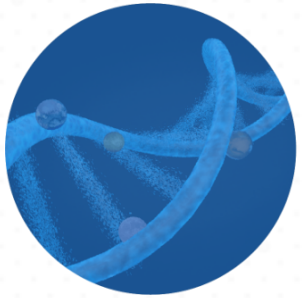

PLANETARY PROTECTION

METAGENOMICS IN SPACEFLIGHT:

ESTABLISHING AN IMPLEMENTATION ROADMAP

NASA AMES | NOVEMBER 19-22

Low Biomass Metagenomics and Implications for Planetary Protection

Christopher E. Carr

Assistant Professor, School of Aerospace Engineering & School of Earth and Atmospheric Sciences, Georgia Institute of Technology

Co-Director, Astrobiology Program, Georgia Institute of Technology

cecarr@gatech.edu

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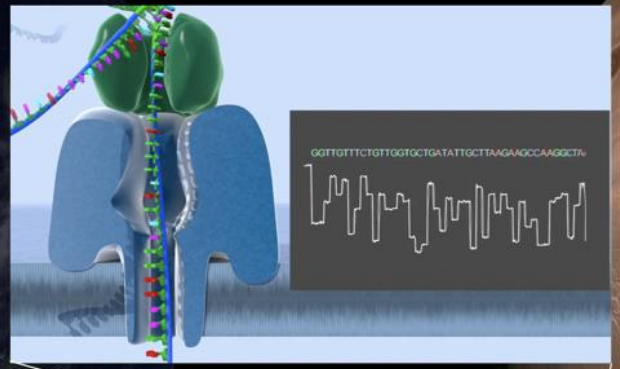
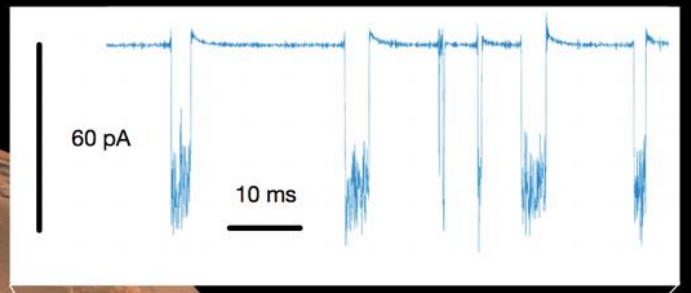
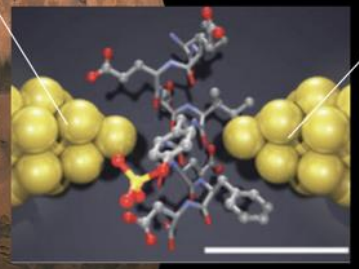
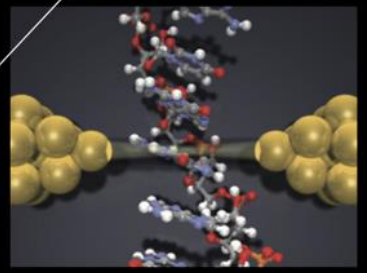
Sensitive, specific, and agnostic methods for life detection

Search for Extra-Terrestrial Genomes (SETG)









Electronic Life-detection Instrument [for Enceladus / Europa (ELIE)]

Solid-state Agnostic Life-detection Instrument (SALI)

Atacama Low Biomass Metagenomics

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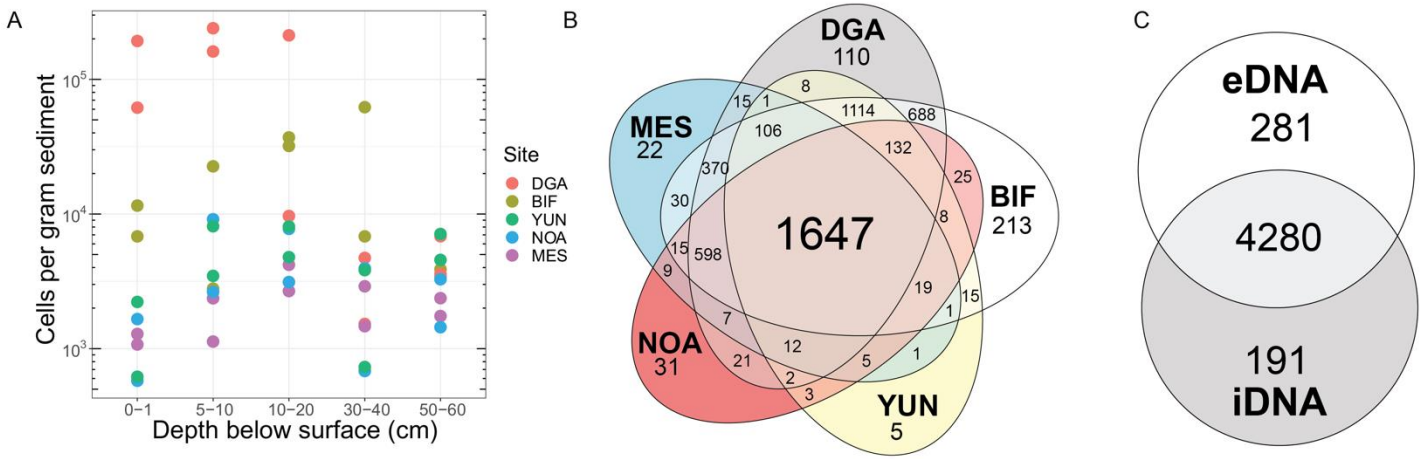
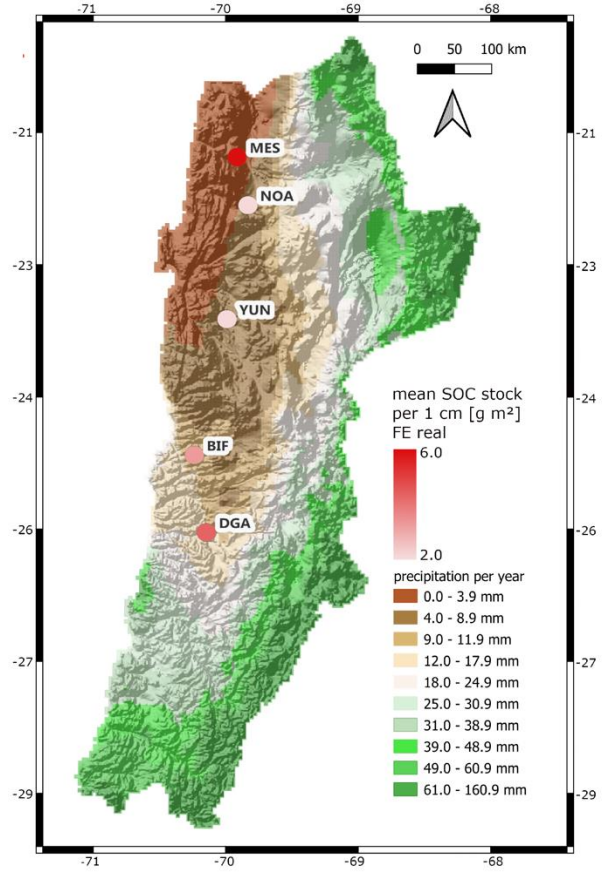
Ultra-low biomass metagenomics in the Atacama hyperarid core

- How do microbes adapt to dry conditions?
- Prior work (e.g., Schulze-Makuch et al. *PNAS* 2018) counted 16S genes but inadequate DNA for metagenomics
- Carried out expedition in 2022 via South-North transect in the hyper-arid core, followed by metagenomic sequencing





Rachel A. Moore, Diana Boy, Jens Boy, Marcus A. Horn,
Georg Guggenberger, Armando Azua-Bustos, Christopher E. Carr (*in review*)
Preprint: ResearchSquare https://doi.org/10.21203/rs.3.rs-5241557/v1

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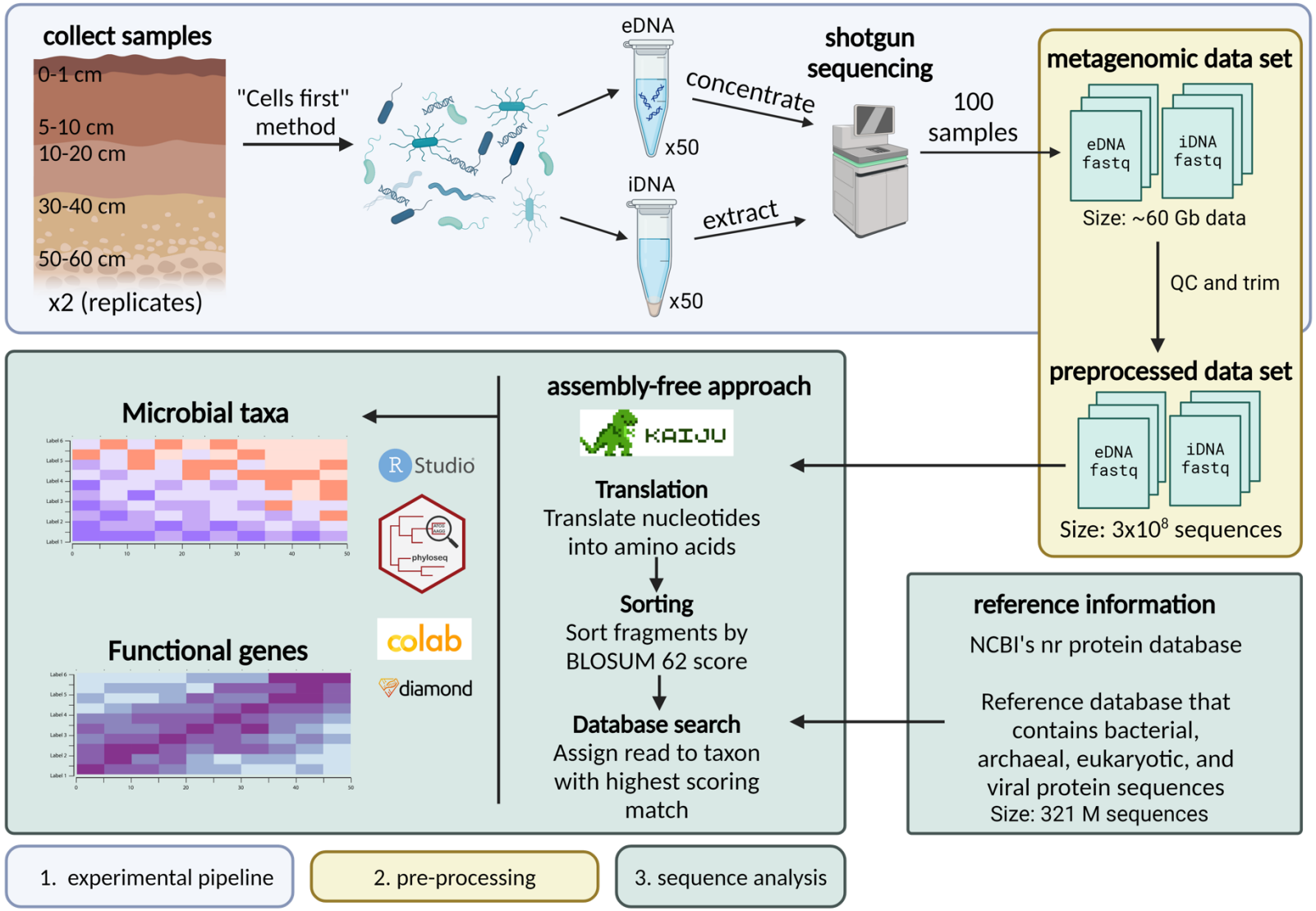
Methods

- **How?**
- Big sample size, up to 100 g
- Elaborate extraction process
- Quantification of sub-ng extracted DNA (off-label Qubit usage via calibration c/o Dr. Christina Davis)
- NEB Next Ultra II library prep + Illumina (sub-ng libraries)



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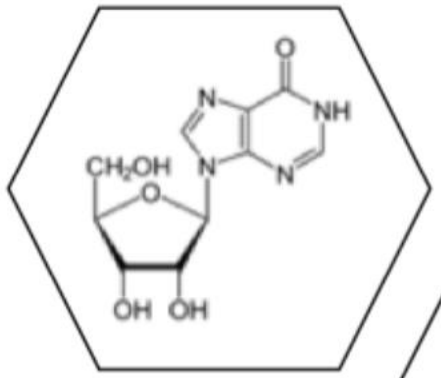
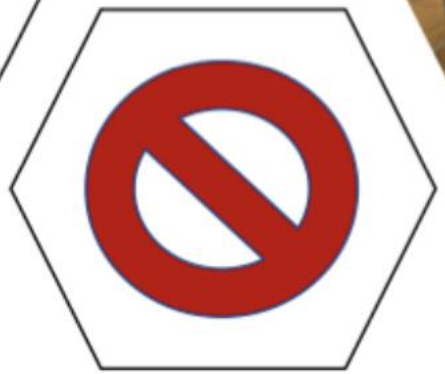
Low-input nanopore sequencing

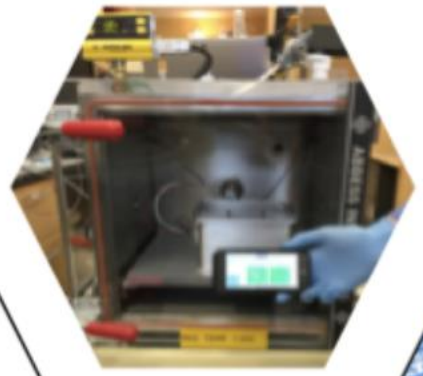
SETG Selected Highlights



Non-standard base detection: Inosine detection

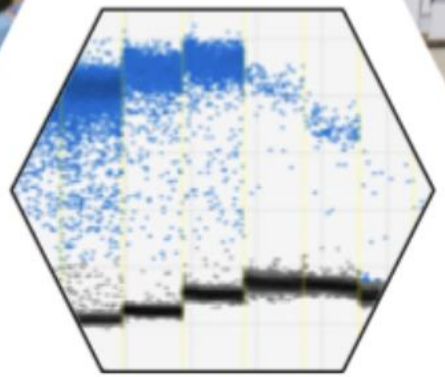
Sequencing nothing: Low false positive rate





Thermal Vac: Environmental control

Low Input Sequencing: *B. subtilis* 2 pg input DNA (~ 1 ppb)



Parabolic Flight: Robust to vibration, g-level

GEARS: Genomic Enumeration of Antibiotic Resistance in Space (on ISS)

- Tested limits of MinION nanopore sequencing.
- Automated sample-in to sequence out on the bench (TRL4)
- Can we move this technology closer to in-space use?

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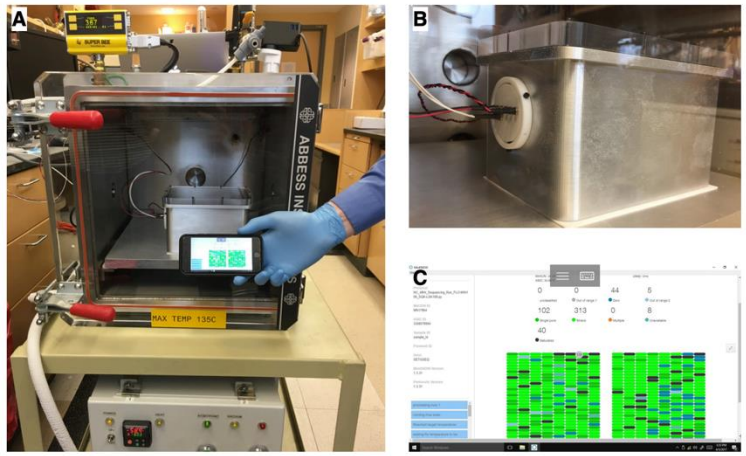
Low-input nanopore sequencing under Mars-like conditions

- Library: 1100 ng Lambda, 0.2 ng (200 pg) *B. subtilis* W23 (equivalent to ~5 x 10^4 spores)
- “Mars”: -60˚C, 400-500 Pa
- 2.4 hrs at “Mars” then to 48 hrs in the lab.



	On "Mars"	On "Desk"	Total	
Sequencing Data				
Sequencing Time	2.38	37.93	40.31	hours
Total Reads	59,649	718,433	778,082	
Total Bases	450,391,859	6,022,122,138	6,472,513,997	bases
Mean Length	7550	8382	-	bases
Min Length	6	5	5	bases
Max Length	131,307	158,250	158,250	bases
Mapping (BWA)				
Lambda Reads	59,461	717,249	776,710	bases
Lambda Bases	450,247,113	6,020,985,496	6,471,232,609	bases
B. subtilis (W23) Reads	188	1,184	1,372	bases
B. subtilis (W23) Bases	144,746	1,126,642	1,271,388	bases
Ratio by Reads	316	606	566	Lambda / B. subtilis
Ratio by Bases	3111	5344	5090	

Ratio by bases similar to DNA ratio of 5500:1

Carr et al. *IEEE Aerospace (2019)* Nucleic Acid Sequencing Under Mars-like Conditions

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Nanopore sequencing with 2 pg DNA (circa 2018)

- Mars regolith simulants extraction from ~10^4 spores in 50 mg regolith
- Defined threshold performance as achieving 5% extraction yield
- Sequencing: 1000 ng lambda (8 kb) + 2 pg *B. subtilus* DNA to ~5 x 10^2 spores
- Used digital droplet PCR for spore counting
- MinION Mk1B, R9.4 flowcells, SQK-LSK108 kit, Albacore 1.1 basecalling

TABLE 1. LOW-INPUT CARRIER SEQUENCING METRICS

	Number of reads		*Min length*		*Max length*		*Median length*		*Total bases*	
All reads (Lambda + *B. subtilis* + Contamination + HQNRs)	1,303,007	reads	16	bases	501,249	bases	6,493	bases	8,698,026,598	bases
Target reads (*B. subtilis* + Contamination + HQNRs)	29	reads	267	bases	1,559	bases	595	bases	19,981	bases
B. subtilis reads	5	reads	848	bases	1,559	bases	967	bases	5,270	bases
Contamination reads HQNRs	6	reads	267	bases	865	bases	453	bases	2,933	bases

actual ratio 8698026598/5270 = 1.65M:1
theoretical ratio: 1000 ng / 2 pg = 0.5M:1

Mojarro et al. *Astrobiology (2019)*

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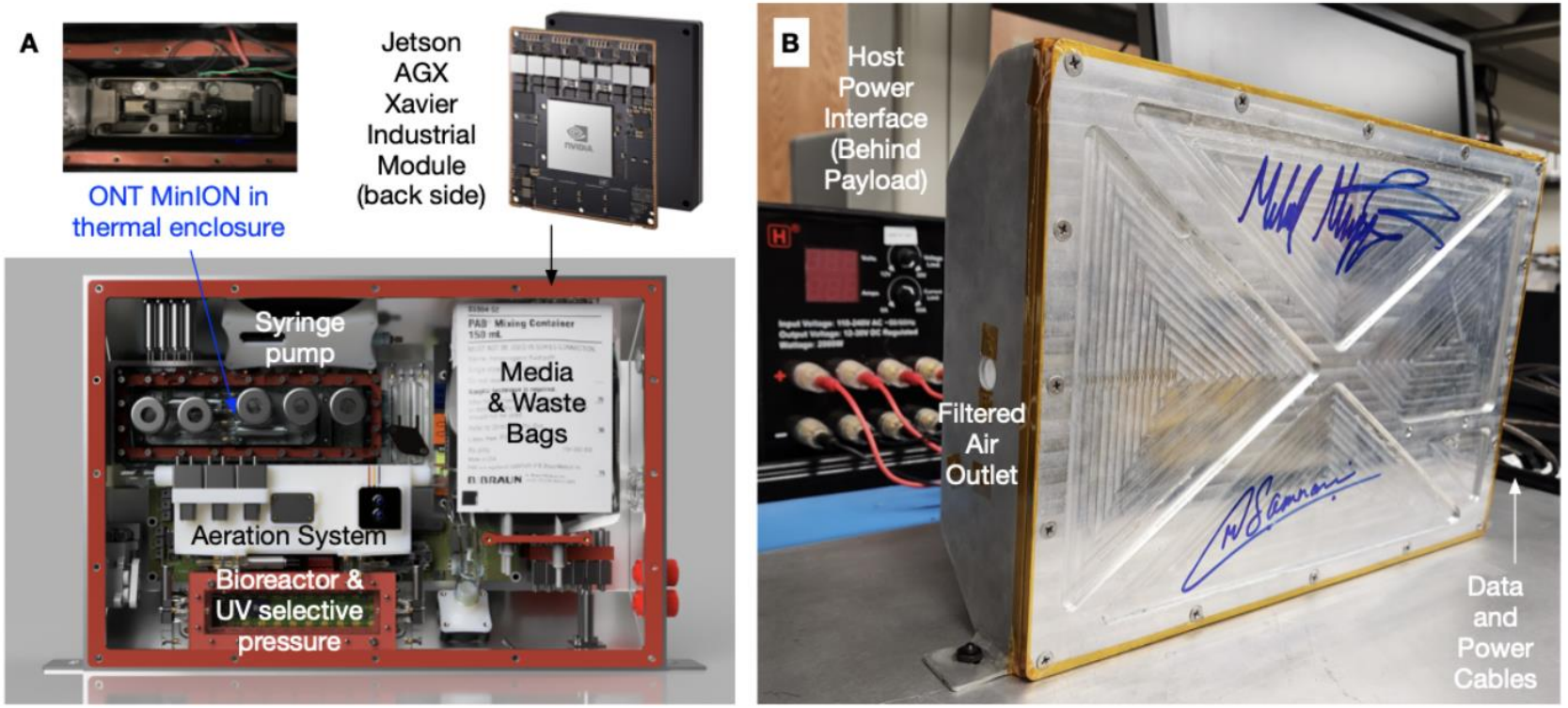
Biological Exploration 2 (BioX2) Payload



- Bioreactor
- UV selective pressure (LEDs)
- Isolated lysis module
- Isolated MinION with loading fluidics
- GPU computer for basecalling

- Ground-based validation of automated sequencing, zero-mass payloads
- Fall 2022 launch; MinION USB cable sheared on launch (inadequate stress relief)
- On-orbit basecalling and genome assembly (pre-loaded reads)

Carr et al. (in prep)

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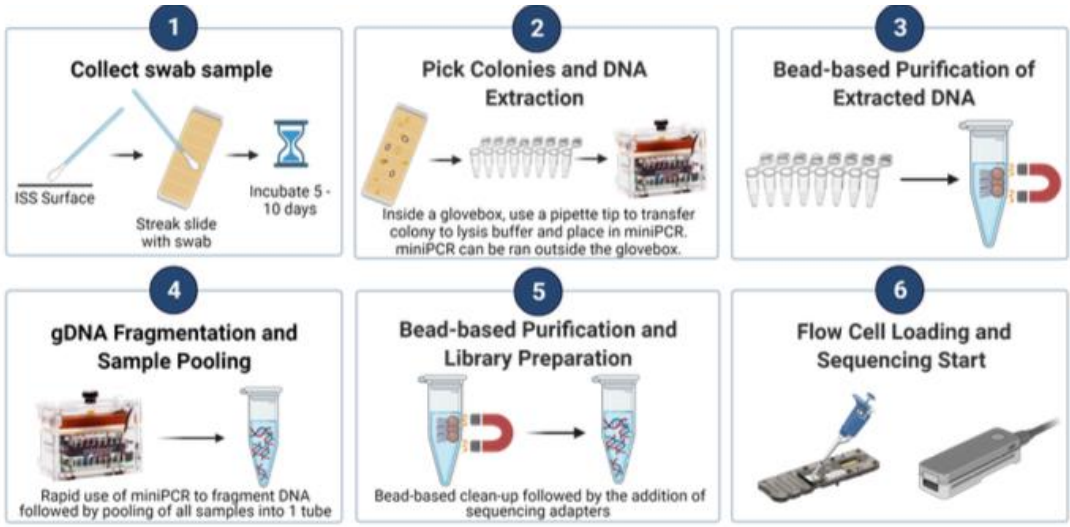
Genomic Enumeration of Antibiotic Resistance in Space (GEARS)

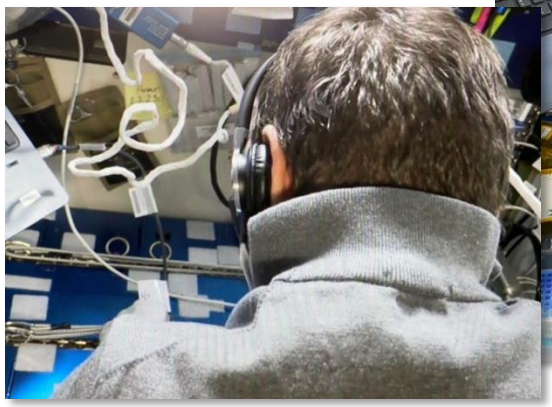
- The first of up to 4 GEARS missions launched (Mar 21) and returned on SpX-30 (Apr 30).
- GEARS is quantifying the abundance of antibiotic-resistant bacterial strains on ISS surfaces.
- GEARS leverages on-orbit genomic sequencing and complementary ground analyses.
- Subsequent GEARS missions will enable longitudinal analyses of antibiotic resistance (Next iterations on SpX-31 and SpX-32).



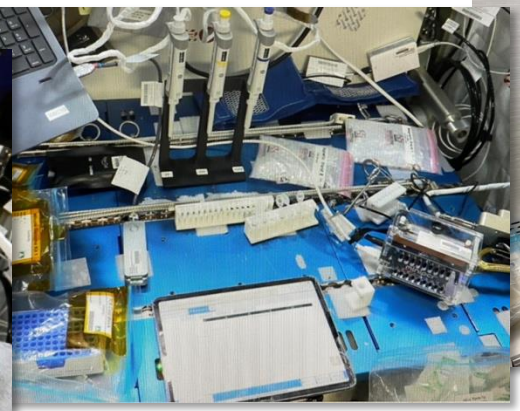
Co-PI: Sarah Wallace, Ph.D.
NASA JSC

PhD student; Jordan McKaig
Georgia Tech

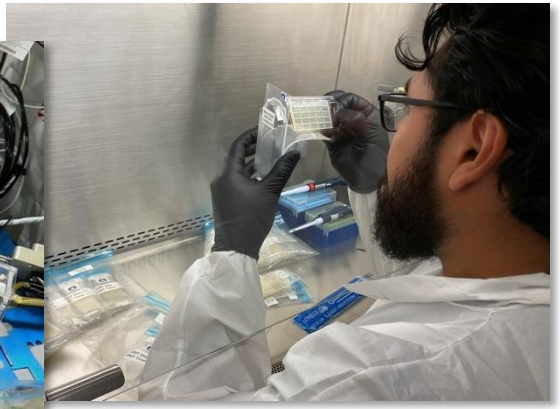
Highlighted team members



Astronaut Dr. Michael Barrett preparing sequencing libraries



GEARS samples being sequenced on-orbit



GEARS samples being inspected by Christian Mena after return

The future

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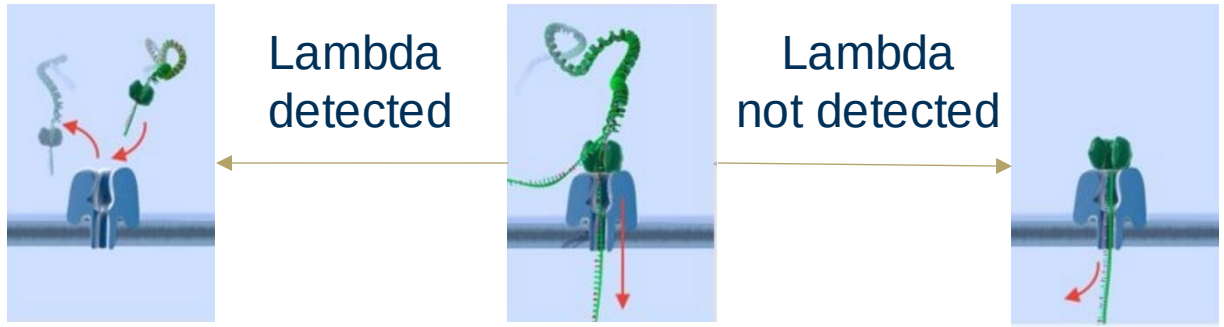
Future studies needed

- IDT xGen™ ssDNA & Low-Input DNA Library Preparation
  - Down to 10 pg input, works with both ssDNA and dsDNA, multiplex up to 1536 samples
- Make library for Illumina, optionally sequence on MinION (short fragment mode)
- ONT TraxION – hands-off library prep & optimize for low-input (minimize external contamination)
- ONT MinION adaptive sampling with depletion mode (lambda background)
  - Lambda is most of the reads; if read maps to lambda the molecule is rejected
  - Could result in low pore occupancy; requires testing to validate efficacy

MinION adaptive sampling with depletion mode



Images: Oxford Nanopore Technologies

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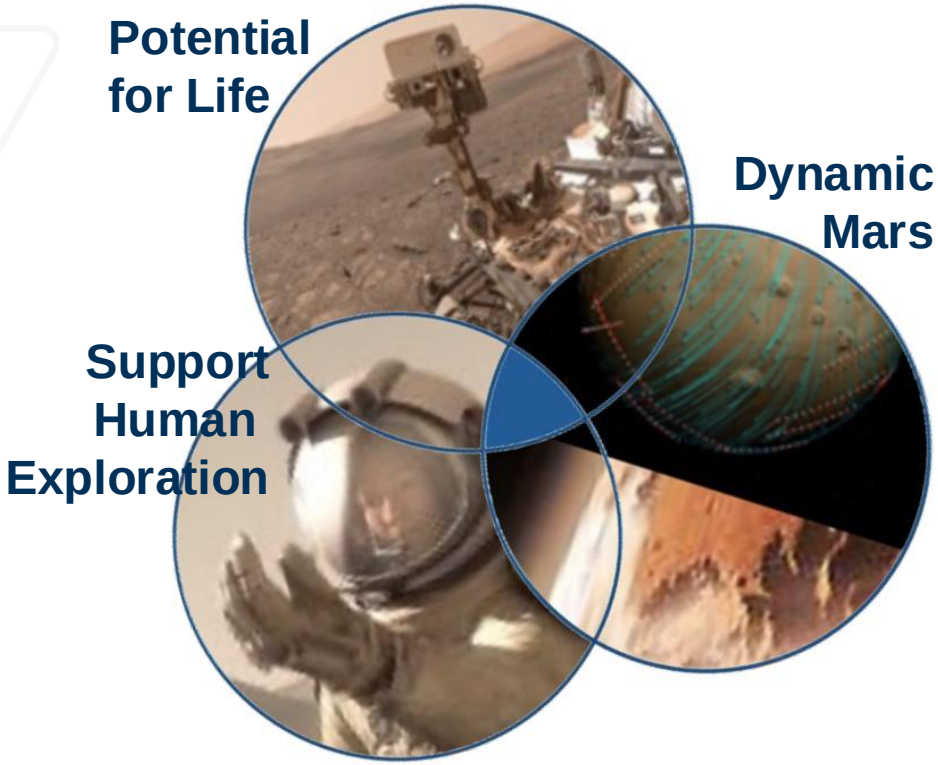
Preparing for multiple life detection missions

- Near-earth payloads can advance cis-lunar exploration and help us prepare for astrobiology and planetary science missions – **and make PP assessments**

Low-cost & life detection missions at Mars



Use the moon as a testbed for Planetary Protection
Example: Lee et al., “SOTERIA: Searching for Organisms Through Equipment Recovery at Impact Areas” (2020); https://ntrs.nasa.gov/citations/20205007157.

Image credit: NASA/Ianson, Exploring Mars Together (URL); NASA/APL/Enceladus Orbilander; Europa Lander SDT Report.

Incorporating growth into risk assessment

- Prior risk assessment (e.g., McCoy et al. 2021, Europa Clipper) considers detailed mission events leading to sterilization and transfer to habitable environment
- Current standard (e.g., NASA-STD-8719.27) based on occurrence of inoculation of viable microbe into habitable environment and/or total bioburden (see Table 4-3)
- Should we also consider **growth potential in habitable environment**?
- If yes, then **metagenomics** may be able to be used to **infer growth temperature on a population basis**
- Could we change "contamination avoidance" to "consequence avoidance"?
- Proposal: Prediction and Verification of Growth Temperature Range for Spacecraft Cleanroom Associated Microbes
- Challenge: Predict growth temperature from genome – especially poor for psychrophiles

Mission Description	Probability Assessment		Value Less Than	Duration or Timing
Category III or IV missions conducting trajectories and maneuvers in the vicinity of Mars (e.g., fly-by, gravity assist, or orbital) or launches from the surface of Mars	Contamination Avoidance of Mars	Inadvertent Impact of Mars	1.0×10^{-2} Probability	20 Years after Launch[a]
			AND	
			5.0×10^{-2} Probability	20 to 50 Years after Launch[a]
		OR		
		Total Bioburden Level	5.0×10^{5} Spores	At Launch
				OR
				At Impact at the Martian Surface
Category III or IV missions conducting a fly-by or gravity assist of Europa, Enceladus, or other sensitive icy worlds to be determined	Contamination Avoidance (occurrence for a biological inoculation event into a potentially habitable aqueous environment)		1.0×10^{-4} Probability	1,000 Years[a]

a. Current period of biological exploration as defined by COSPAR approved by the COSPAR Bureau on 17 June 2020.

Contacting Europa, Enceladus, or Mars → **Surface fails to Sterilize** → **Transfer to Habitable Environment** → **Proliferation in Habitable Environment**

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Final thoughts

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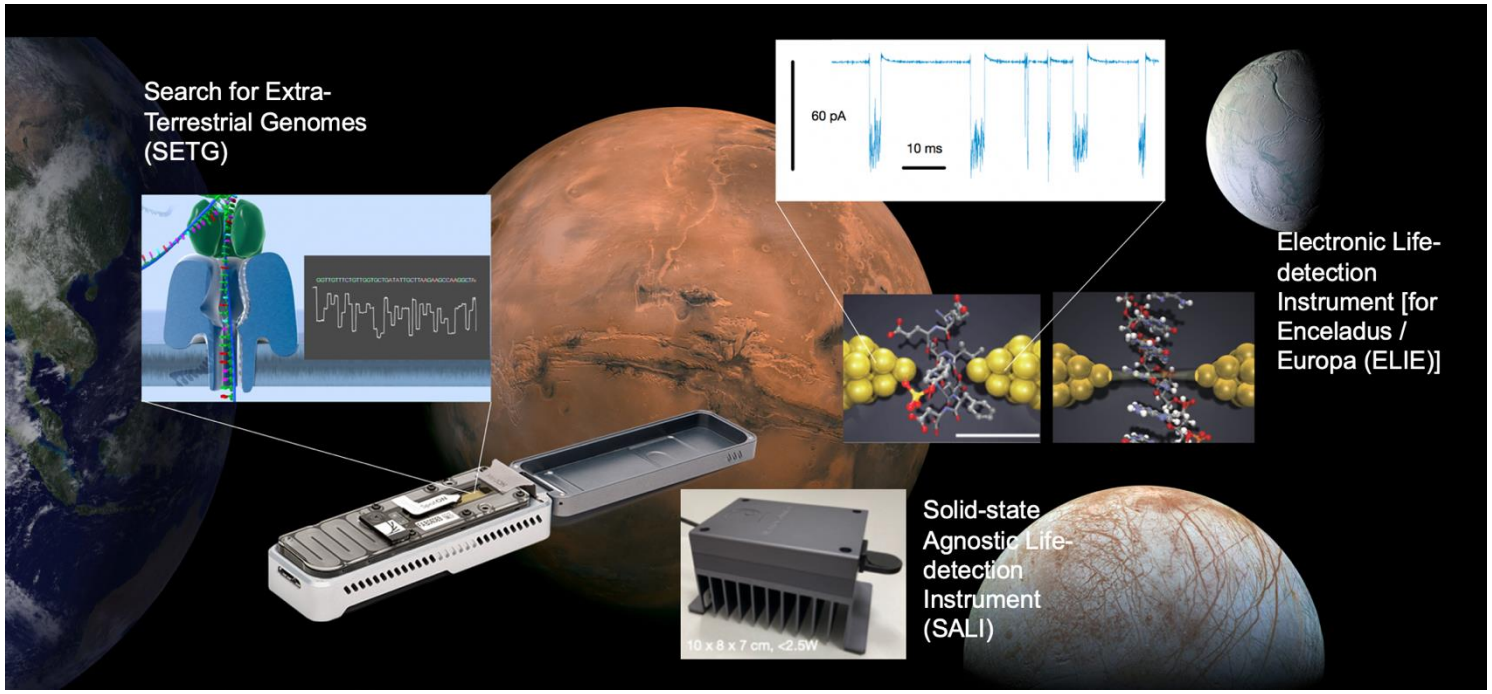
Future planetary protection policies

- Metagenomics can be used to support science-driven probabilistic risk assessment (PRAs)
  - Relatively “standard” Illumina methods can achieve sub-ng sequencing
  - Continued advances in nanopore sequencing are likely to make it competitive for planetary protection monitoring via metagenomics
  - Future missions require working out credits carefully (e.g., Orbilander, Mars)
- Prediction of growth temperature range from genome may support limiting statistical consequence of contamination via inferred growth potential
- Challenge: What measurements would allow planetary protection policies for Mars (or elsewhere) to evolve beyond current rules?

Thank you

- Dr. Christina Davis and conference organizers
- PXL lab members
- We gratefully acknowledge support from
  - NASA MATISSE, PICASSO, Astrobiology/Exobiology, PSTAR, and Space Biology programs.
  - NSF Arctic program
  - Schmidt Futures



NASA NSF SCHMIDT FUTURES PXL

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