

# DNA sequencing at picogram level for extremely low biomass detection

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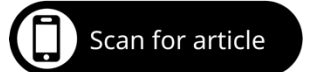
Supervisors:

Prof. Maria-Paz Zorzano (CAB, Spain)

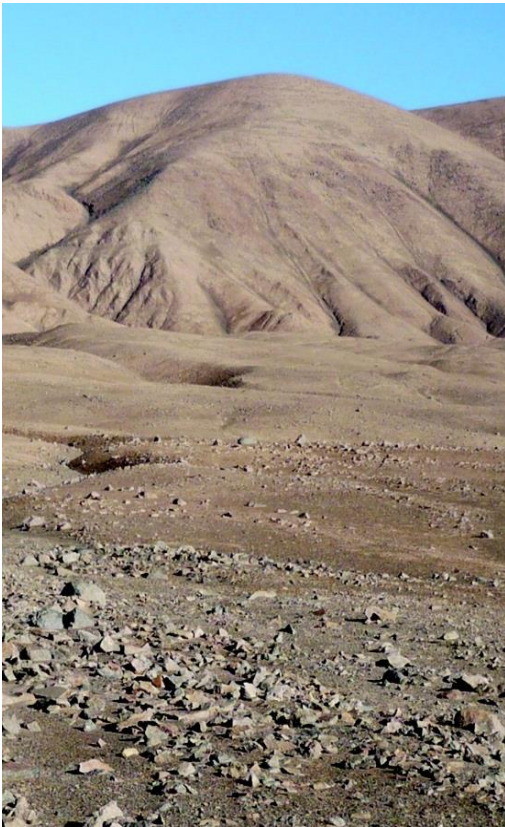
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## Low biomass environments



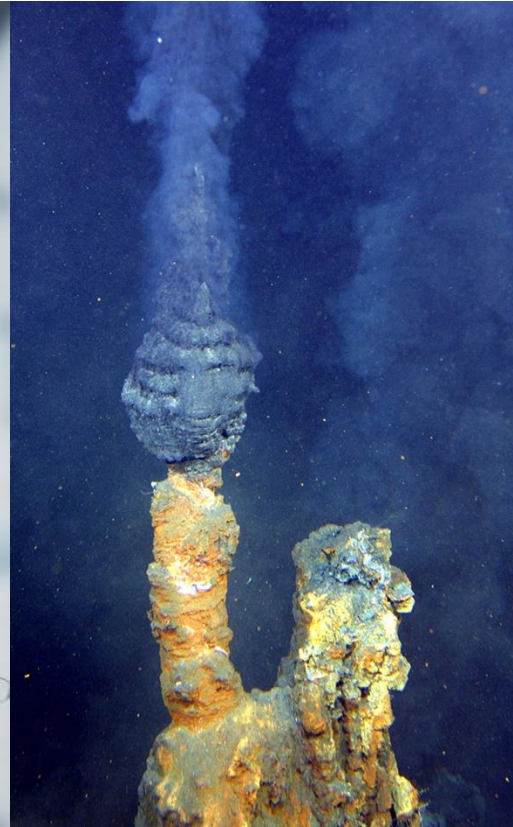
**Atacama desert**  
( $\sim 10^6$  cells/g)

(Azua-Bustos, A *et al.*, 2012)



**Bioaerosols**  
( $\sim 10^5$  cells/L)

(Gong, J *et al.*, 2020)



**Hydrothermal vents**  
( $\sim 10^4$  cells/ml)

(Yanagawa, K *et al.*, 2017)



**Planetary Protection  
(Cleanroom)**  
( $\sim 300$  spores/m<sup>2</sup>)



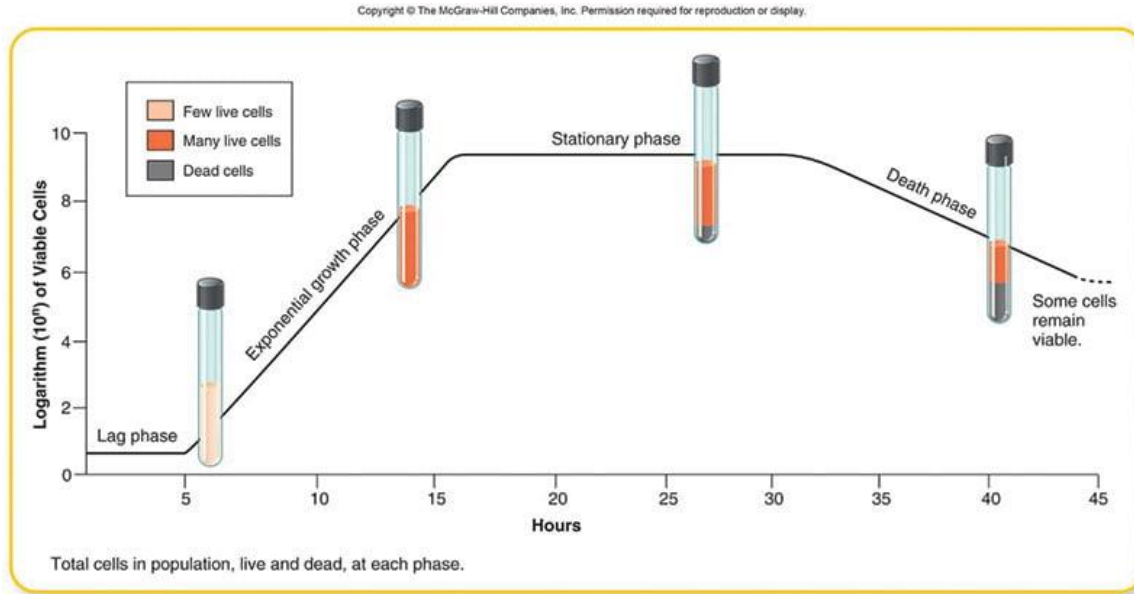
**DNA/nucleobases on  
Mars?**

(Azua-Bustos, A *et al.*, 2012. Life at the dry edge: microorganisms of the Atacama Desert. *FEBS letters*)

(Yanagawa, K *et al.*, 2017. Defining boundaries for the distribution of microbial communities beneath the sediment-buried, hydrothermally active seafloor, *ISME*)



## Traditional growth monitoring methods



Colony forming units (CFU)

Optical density (O.D)

Less than 2% of environmental bacteria are culturable in lab (Wade. W *et al.*, 2002)

Need accurate molecular methods that can detect low-biomasses and distinct microbes

## Low detection with Nanopore so far

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### The Limits, Capabilities, and Potential for Life Detection with MinION Sequencing in a Paleochannel Mars Analog

Catherine Maggiori,<sup>1</sup> Jessica Stromberg,<sup>2</sup> Yolanda Blanco,<sup>3</sup> Jacqueline Goordial,<sup>1,4</sup>  
Edward Cloutis,<sup>5</sup> Miriam García-Villadangos,<sup>3</sup> Victor Parro,<sup>3</sup> and Lyle Whyte<sup>1</sup>

frontiers  
in Microbiology

ORIGINAL RESEARCH  
published: 20 December 2017  
doi: 10.3389/fmicb.2017.02594



### *In Situ* Field Sequencing and Life Detection in Remote (79°26'N) Canadian High Arctic Permafrost Ice Wedge Microbial Communities

J. Goordial<sup>1,2\*</sup>, Ianina Altshuler<sup>1</sup>, Katherine Hindson<sup>1</sup>, Kelly Chan-Yam<sup>1</sup>,  
Evangelos Marcollef<sup>1</sup> and Lyle G. Whyte<sup>1\*</sup>

All of them had an amplification  
step (PCR) included

GCAT  
TACG  
GCAT  
*genes*



Article

### Real-Time Culture-Independent Microbial Profiling Onboard the International Space Station Using Nanopore Sequencing

Sarah Stahl-Rommel<sup>1</sup>, Miten Jain<sup>2</sup> , Hang N. Nguyen<sup>1</sup> , Richard R. Arnold<sup>3</sup>, Serena M. Aunon-Chancellor<sup>3</sup>,  
Gretta Marie Sharp<sup>4</sup>, Christian L. Castro<sup>1</sup>, Kristen K. John<sup>5</sup>, Sissel Juul<sup>6</sup> , Daniel J. Turner<sup>7</sup>, David Stoddart<sup>7</sup>,  
Benedict Paten<sup>2</sup>, Mark Akeson<sup>2</sup> , Aaron S. Burton<sup>8</sup> and Sarah L. Castro-Wallace<sup>9,\*</sup>

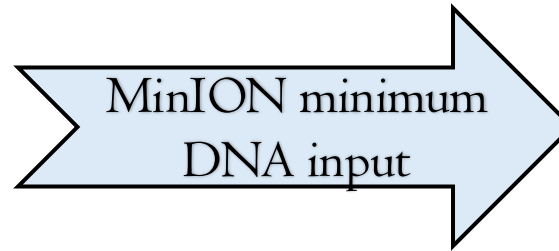
To define new limits of concentration for DNA sequencing **without the need of any amplification** that could be applicable in diverse research areas

A clean room of ISO class 5 was built by our team at UoA for low detectability experiments

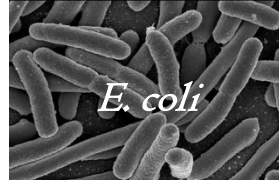




# Test I: Single taxa lowest detection

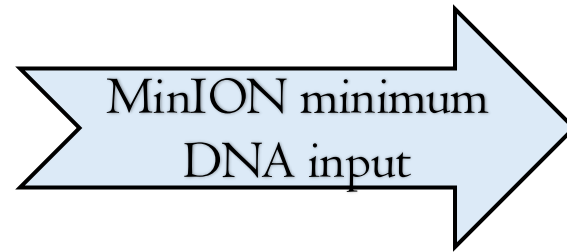


400 ng – without amplification  
1 ng – with amplification

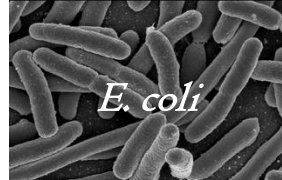


Sample type	Qscore passed reads			Taxonomic classification	Total reads (pass and fail)		
	R1	R2	R3		R1	R2	R3
10 pg of <i>E. coli</i> DNA	4	3	1	<i>Escherichia coli</i>	224	73	313
	2	1	0	<i>Homo sapiens</i>			
10 pg of YSC-2 yeast DNA	4	4	2	<i>Saccharomyces cerevisiae</i>	337	37	190
	1	1	0	<i>Homo sapiens</i>			
Nuclease-free water (control)	2	5	3	<i>Homo sapiens</i>	180	72	269

# Testing the limits of MinION

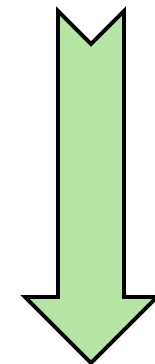


400 ng – without amplification  
1 ng – with amplification



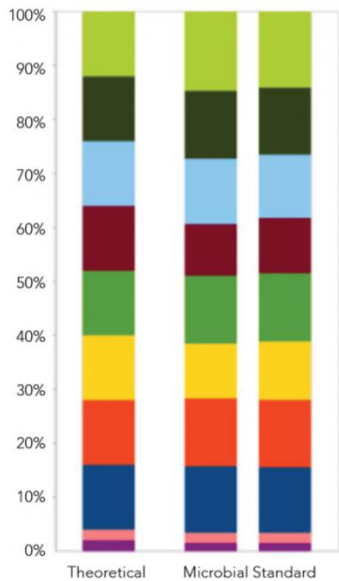
Sample type	Qscore passed reads		Taxonomic classification	Total reads (pass and fail)	
	R1	R2		R1	R2
10 pg of <i>E. coli</i> + 2 pg of yeast	2	120	<i>Escherichia coli</i>	411	1180
	2	17	<i>Saccharomyces cerevisiae</i>		
	1	7	<i>Homo sapiens</i>		
10 pg of yeast + 2 pg of <i>E. coli</i>	243	263	<i>Saccharomyces cerevisiae</i>	2110	4700
	88	78	<i>Escherichia coli</i>		
	19	53	<i>Homo sapiens</i>		

2 pg without amplification



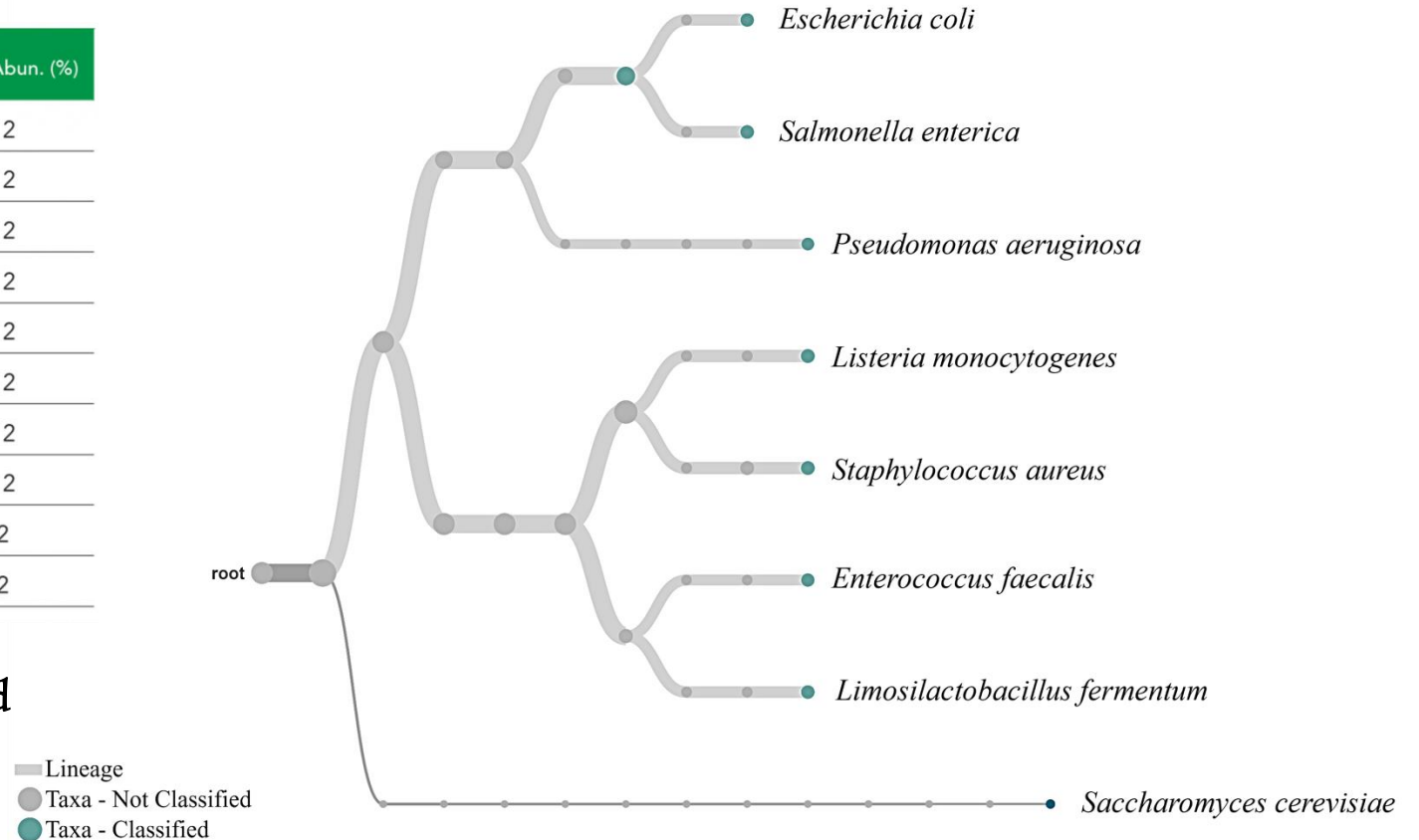
Set a record for lowest  
detection limit

# Test 3: Multiple taxa lowest detection



Species	Avg. GC (%)	gDNA Abun. (%)
<i>Pseudomonas aeruginosa</i>	66.2	12
<i>Escherichia coli</i>	56.8	12
<i>Salmonella enterica</i>	52.2	12
<i>Lactobacillus fermentum</i>	52.8	12
<i>Enterococcus faecalis</i>	37.5	12
<i>Staphylococcus aureus</i>	32.7	12
<i>Listeria monocytogenes</i>	38.0	12
<i>Bacillus subtilis</i>	43.8	12
<i>Saccharomyces cerevisiae</i>	38.4	2
<i>Cryptococcus neoformans</i>	48.2	2

ZymoBiomix, Microbial Community standard

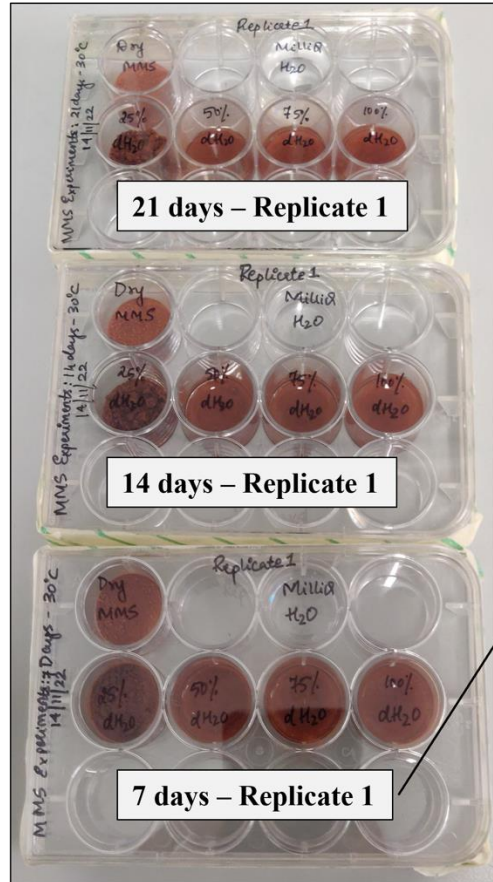


EPI2ME, WIMP analysis

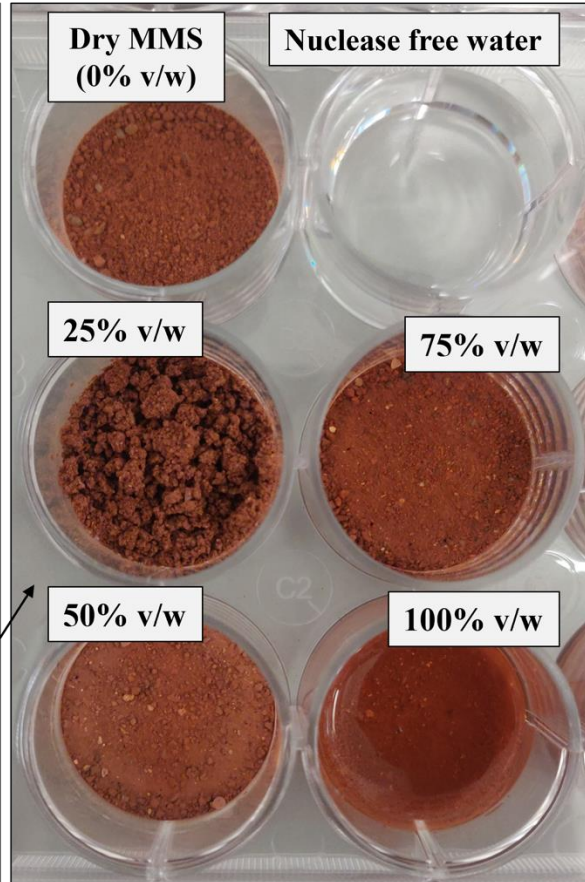
Minimum of 100 pg to detect atleast 8 microbes  
from the community standard



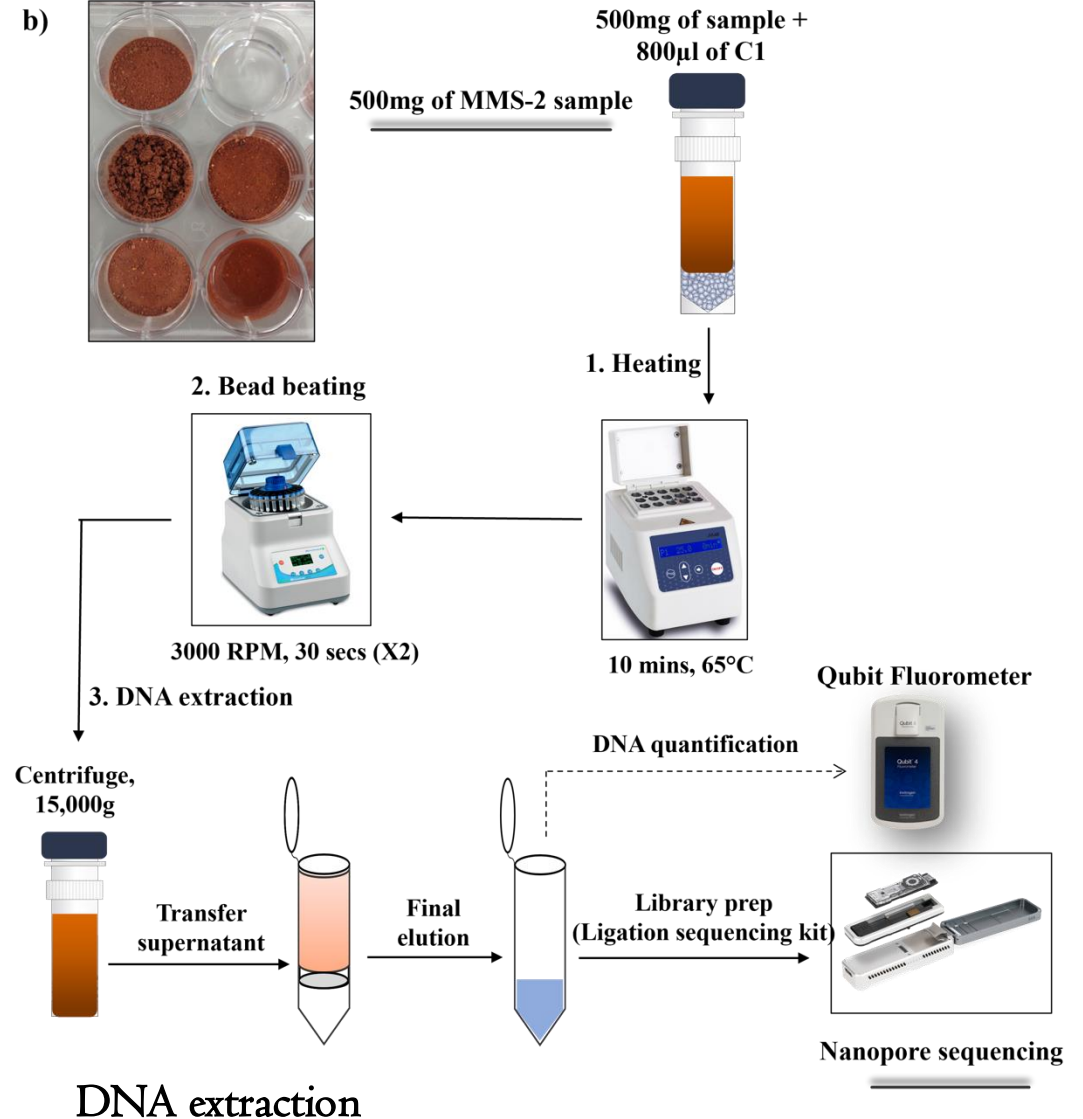
a) 3 weeks replicate set-up

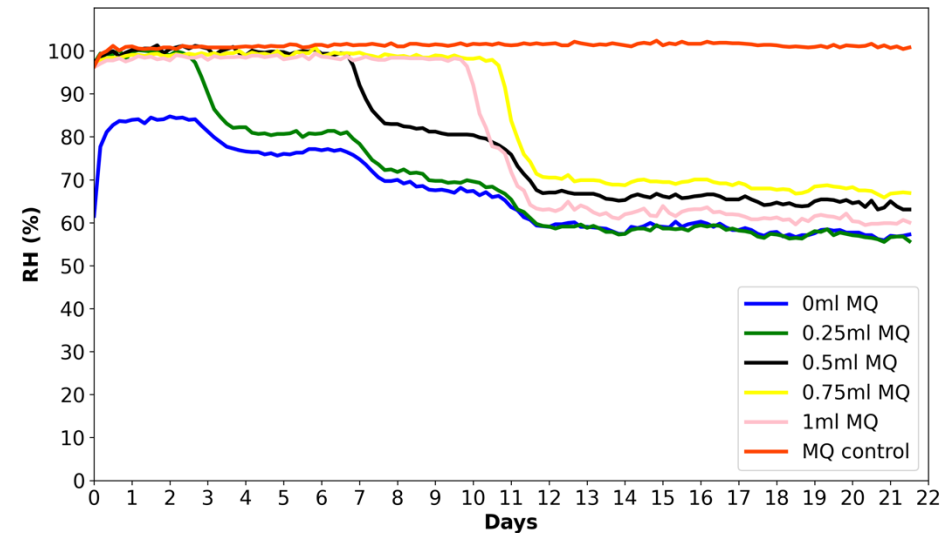
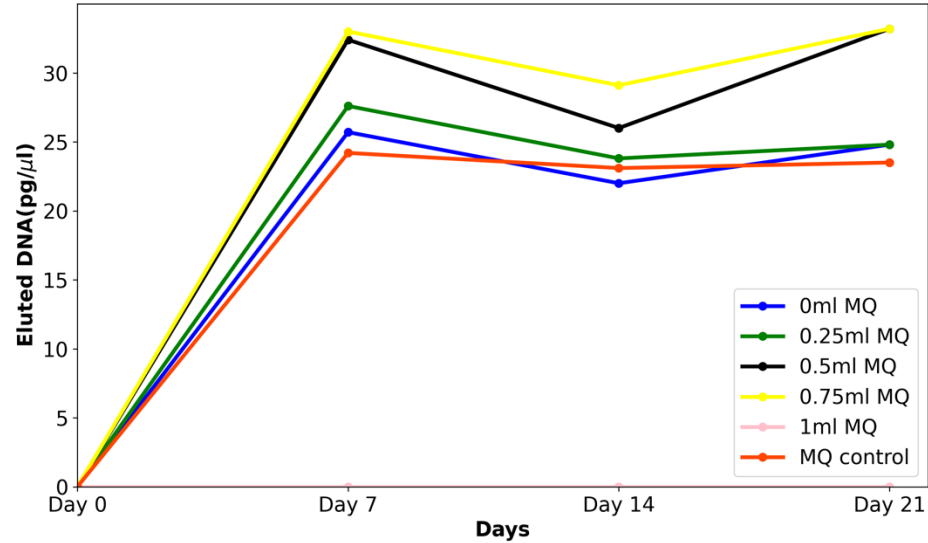


b) 7 days post incubation

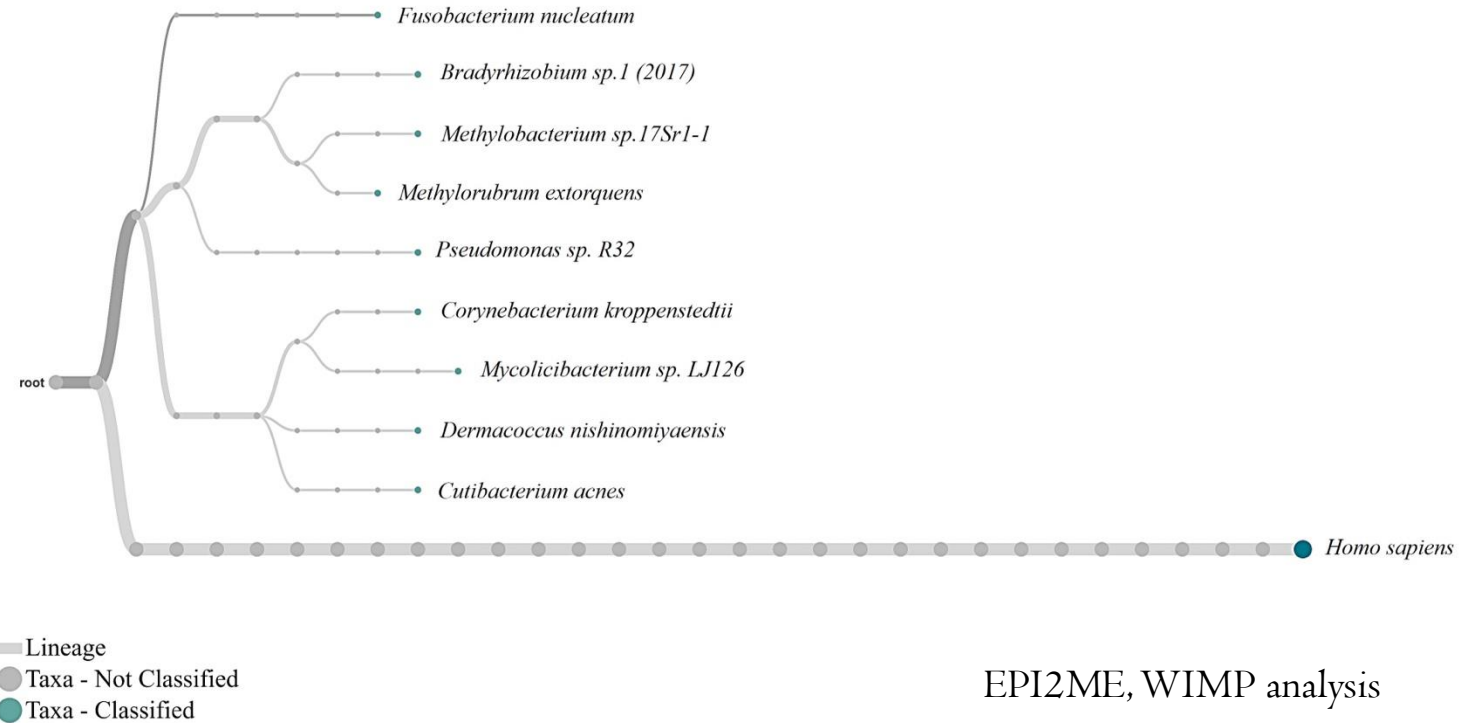


Non-sterile Mojave Martian Simulant (MMS-2) soil



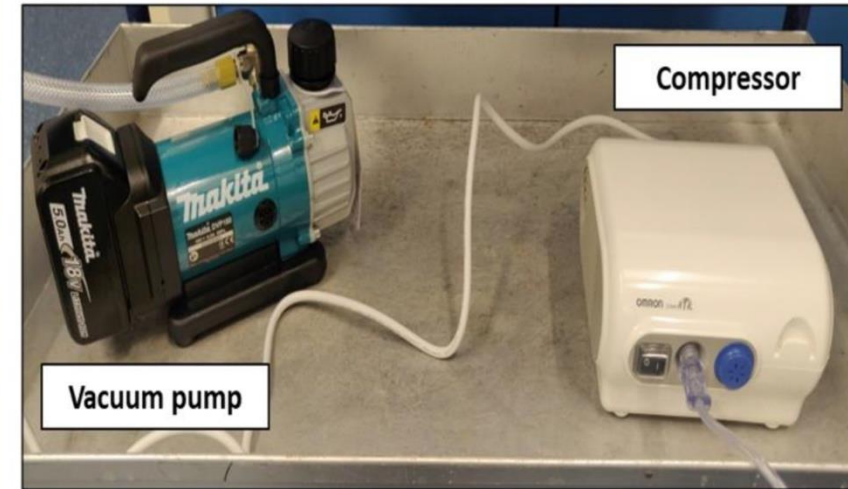
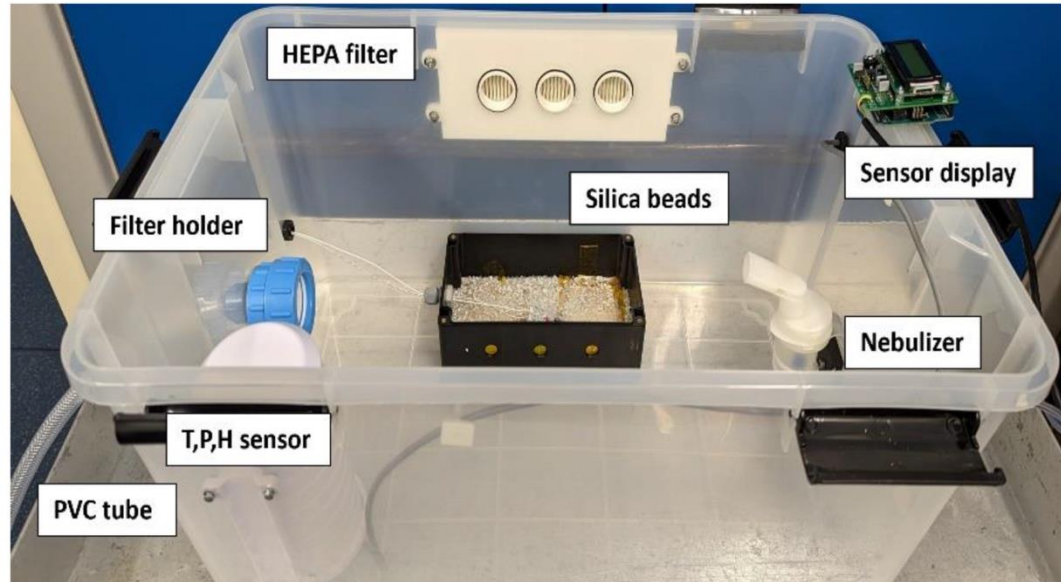


600 pg

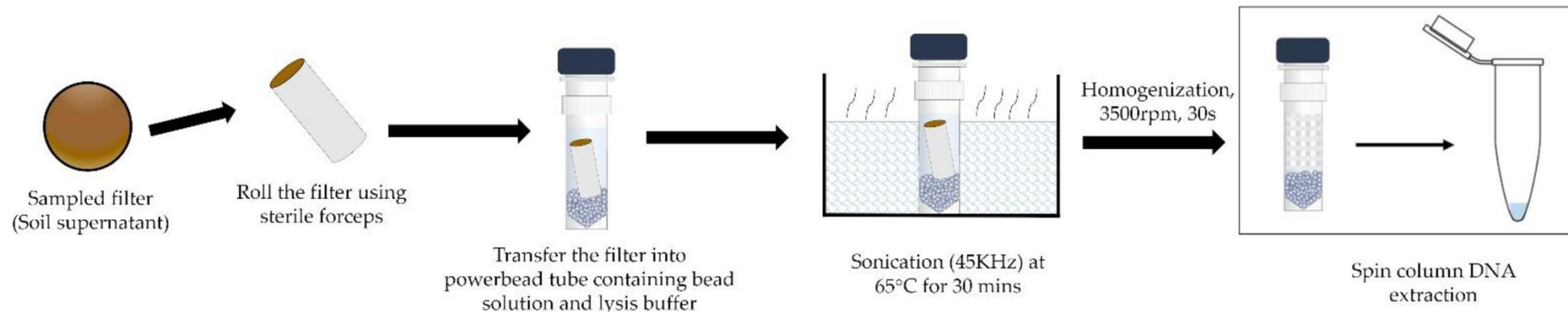


EPI2ME, WIMP analysis





Bioaerosol sampler developer: Thasshwin Mathanlal

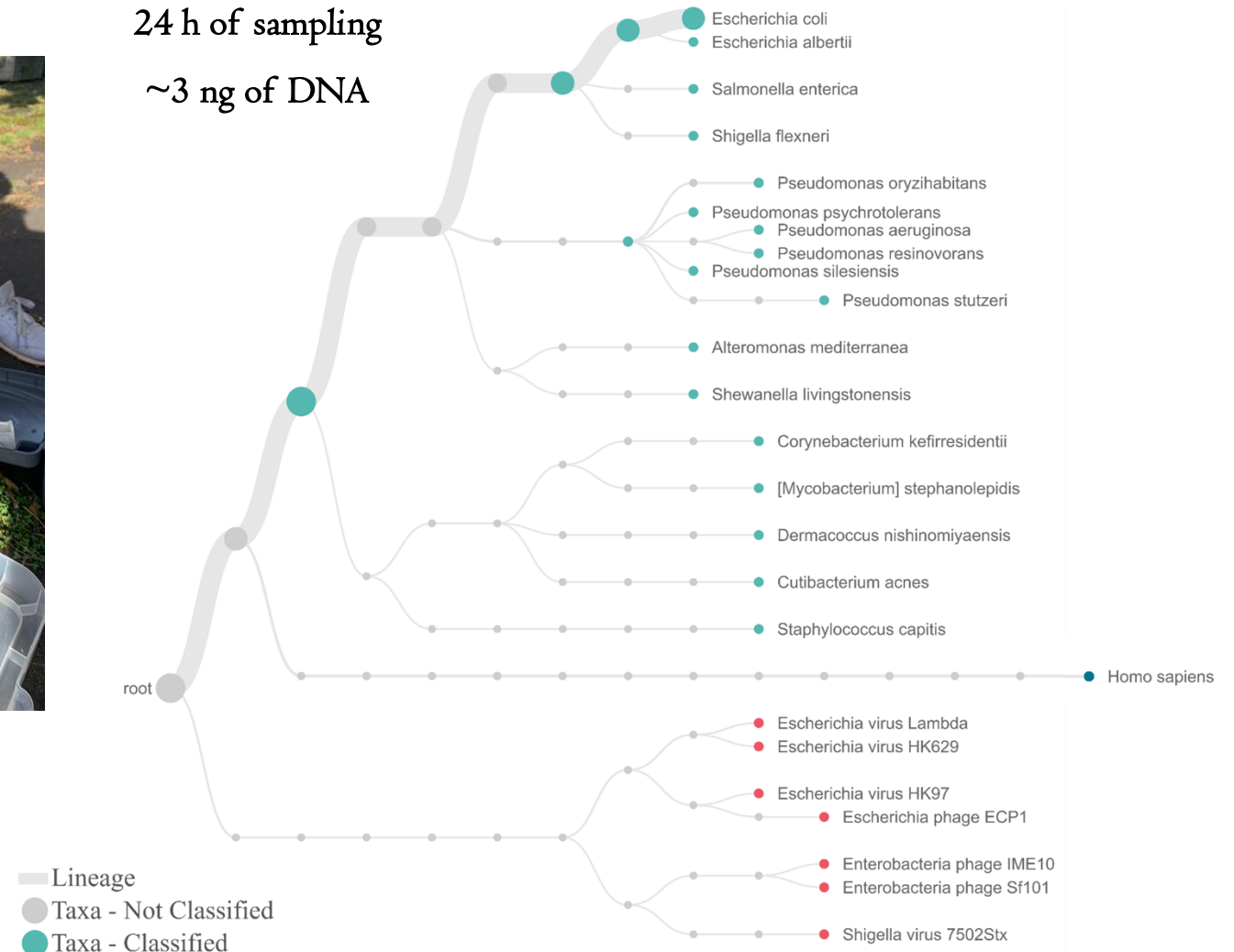






Bioaerosol sampler developer: Thasshwin Mathanlal

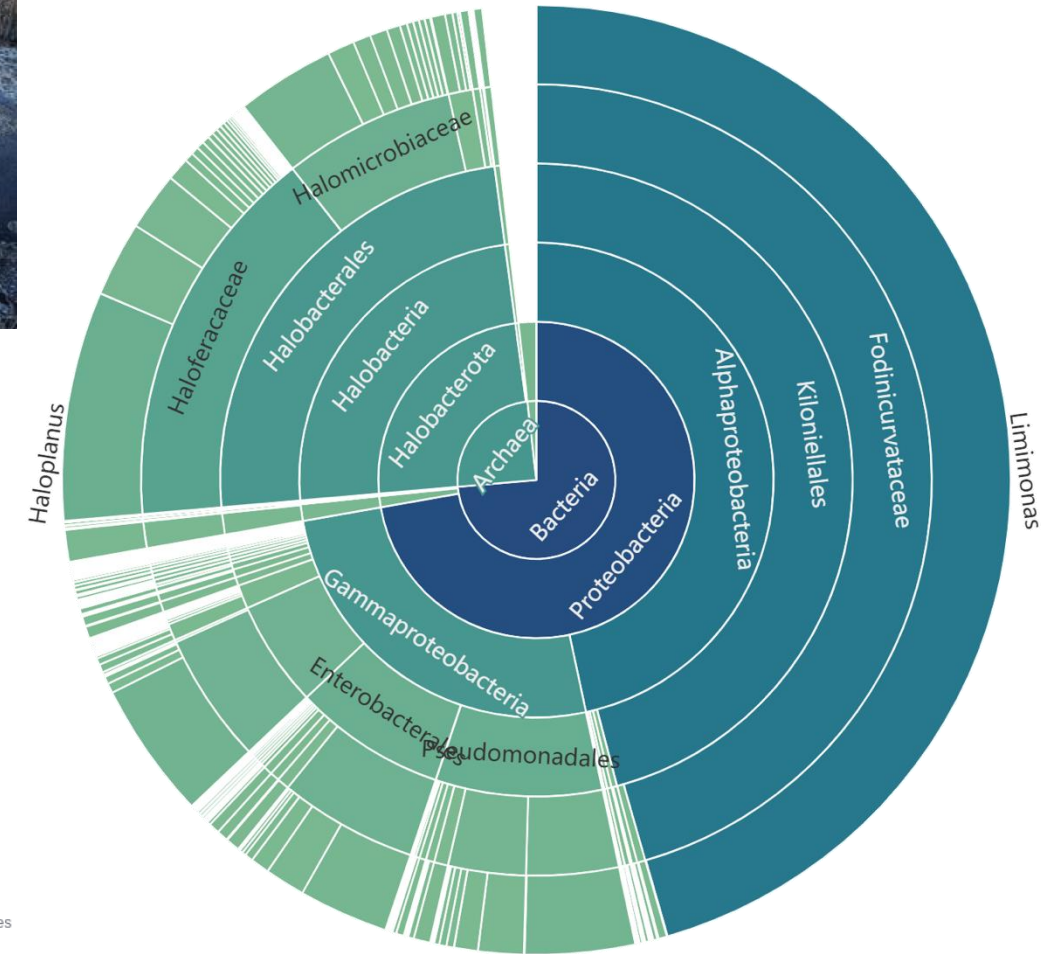
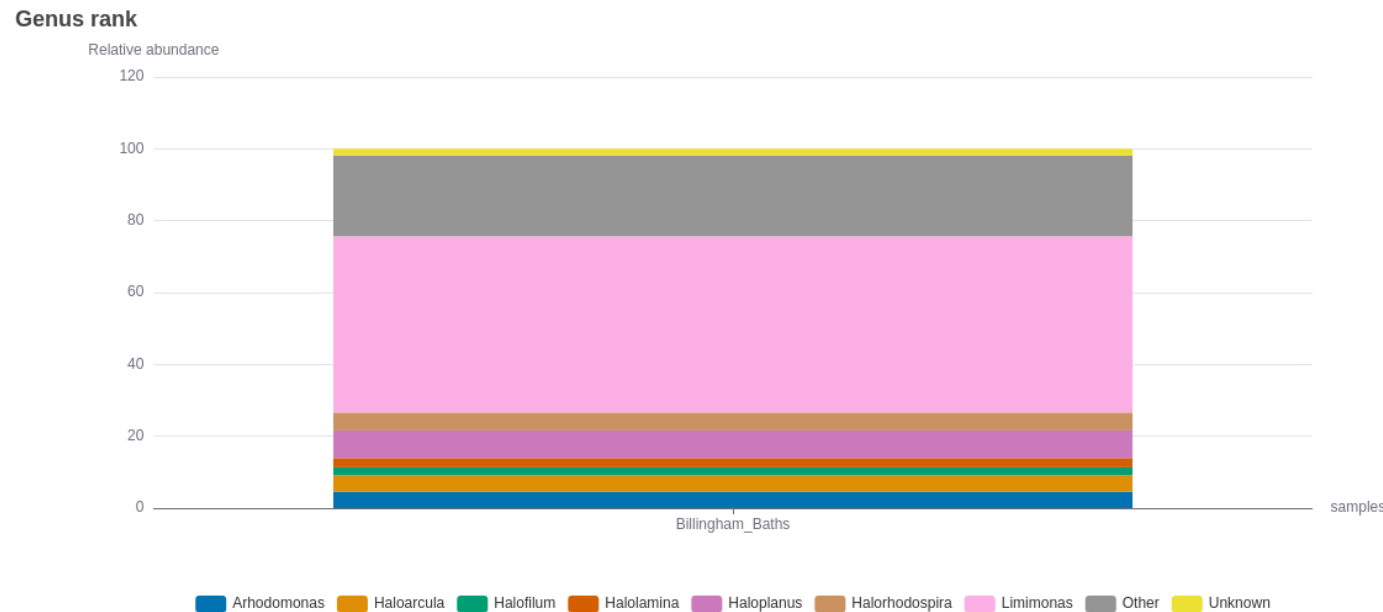
24 h of sampling  
~3 ng of DNA



Brine samples  
(Boulby Mine, UK)



Nazarious, M *et al*, 2023

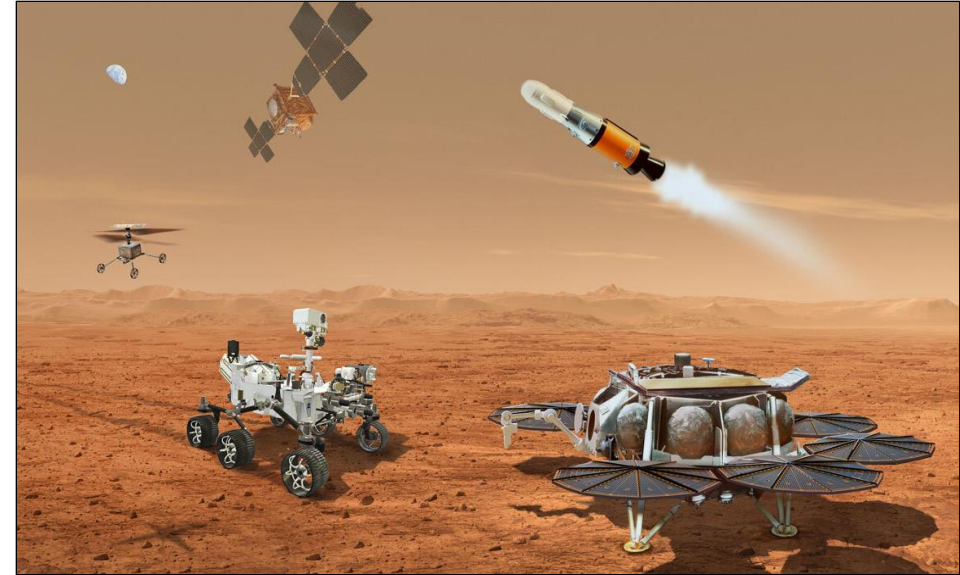
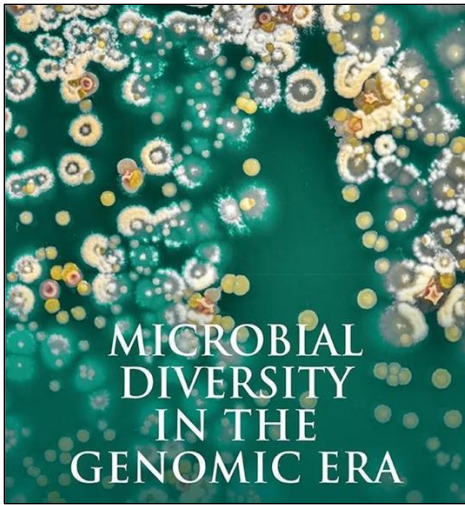


EPI2ME Labs, NCBI

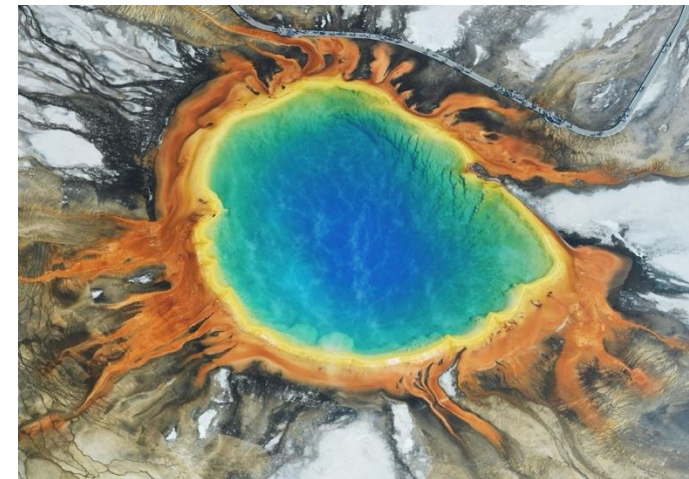
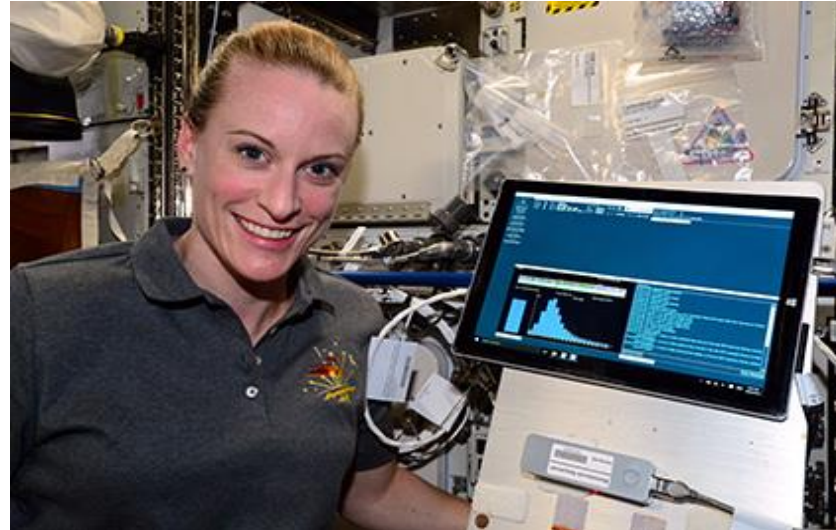
(Nazarious, M *et al*, In preparation)



# Metagenomics application - planetary protection



(MSR, NASA)

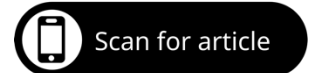


(Photo credits: NASA)



## Potential of MinION nanopore technology:

- Can detect DNA strands from a monoculture with an input of 10 pg and mixed culture with 2 pg
- Characterise with an ISO5 cleanroom environment, the possible ambient Earth contamination/kitome microbiome
- Can negate false positives due to mis-amplification of DNA primer sets.
- Identify microorganisms with extremely low biomass
- Applicable for monitoring clean room environments, planetary protection protocols, future Mars exploration studies and missions.



# Acknowledgements

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Dr. Thasshwin Mathanlal

Juan Antonio Ramirez Luque

Department of Biological Sciences, Queen's University

Dr. Deepak Kumaresan



QUADRAT DTP (UKRI)



I DID IT !!!

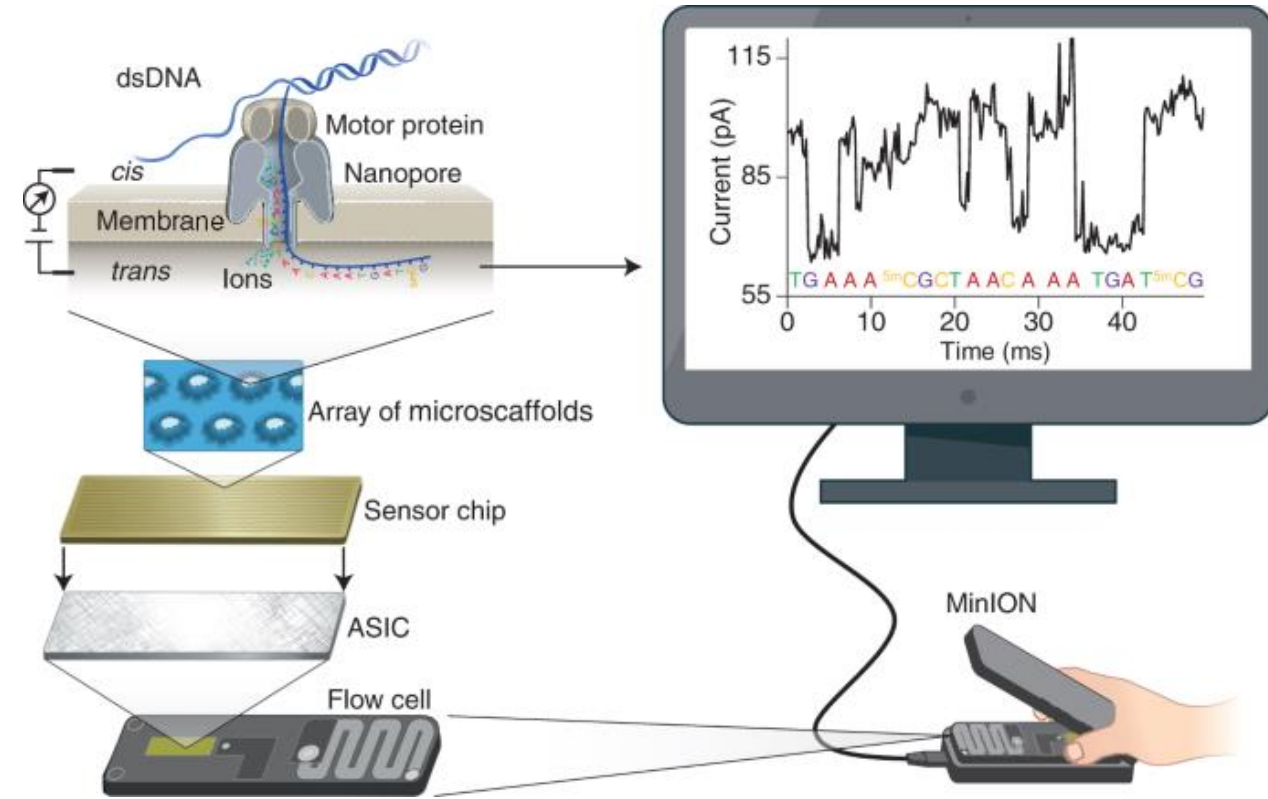
NO, I DID  
IT !!!



Any  
**Question**



# Why Nanopore technology?



(Wang, Y *et al.*, 2021)



Portable