National Aeronautics and Space Administration

Nanopore Sequencing in Space: One Small Step for a MinION, One Giant Leap for Spaceflight Research

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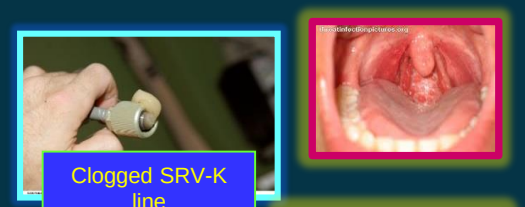
To Sequence Where No One Has Sequenced Before

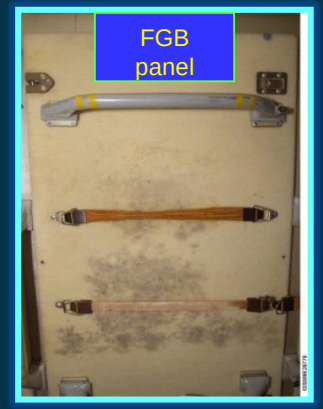
OUTLINE

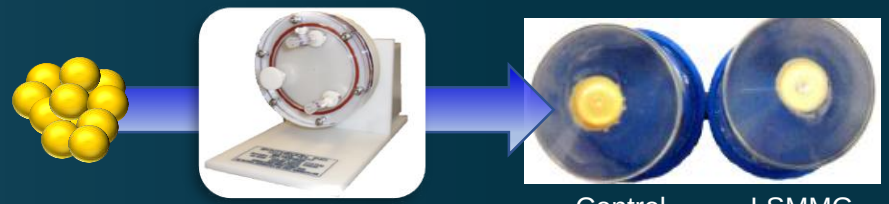
- Why sequence in space?
- The Molecular Space Age
- Biomolecule Sequencer
- Sample Prep
  - NEEMO
- Genes in Space-3
- BEST

Why Molecular Biology in Space?

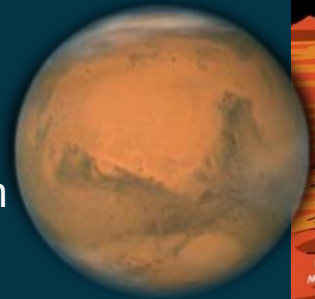
- Operational environmental monitoring
  - Identification of contaminating microbes
  - Infectious disease diagnosis
  - Reduce down mass (sample return for environmental monitoring, crew health, etc.)
- Research
  - Human
  - Animal
  - Microbes/Cell lines
  - Plants
- Med Ops
  - Response to countermeasures
  - Radiation
  - Real-time analysis can influence medical intervention
- Support astrobiology science investigations
  - Technology superiorly suited to *in situ* nucleic acid-based life detection
  - Functional testing for integration into robotics for extra-planetary exploration mission







Changes in the genome?
Changes in gene expression?



Microbial Monitoring on the ISS

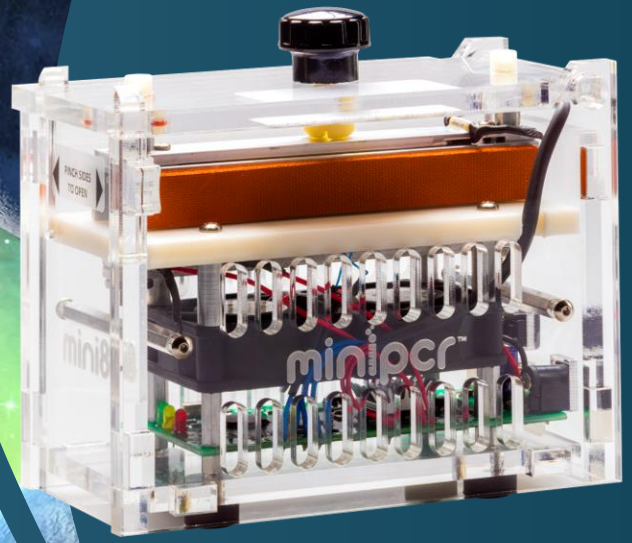
SSK - Surface Sampling Kit

MAS – Microbial Air Sampler

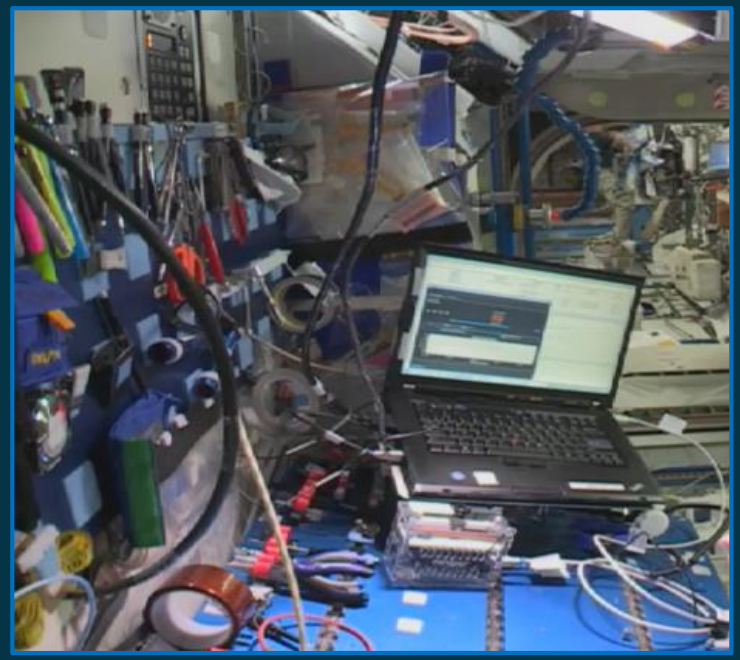
EHS Water Kit – Environmental Health Systems Water Kit

2016: The Molecular Space Age

2016



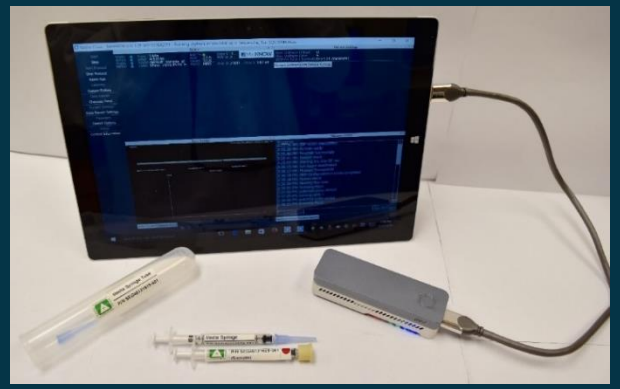
April 19: The first molecular biology assay in space is completed, as DNA is amplified using the miniPCR thermal cycler

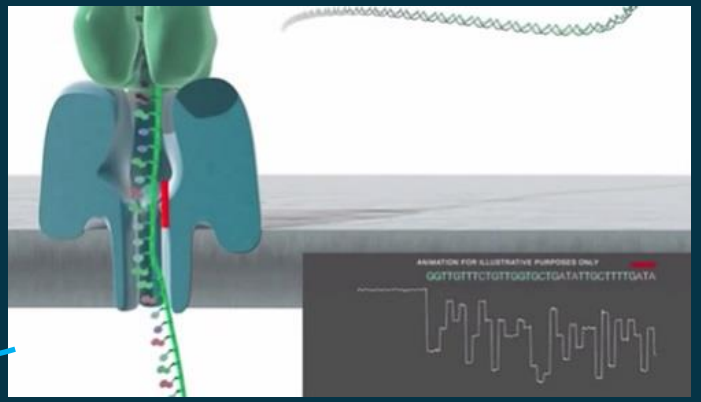


2016

Biomolecule Sequencer

- The first device to assess the capability of DNA and RNA sequencing in the microgravity environment of space
- Capable of DNA, RNA, and protein sequencing
- First launched July 18, 2016 (SpaceX-9)

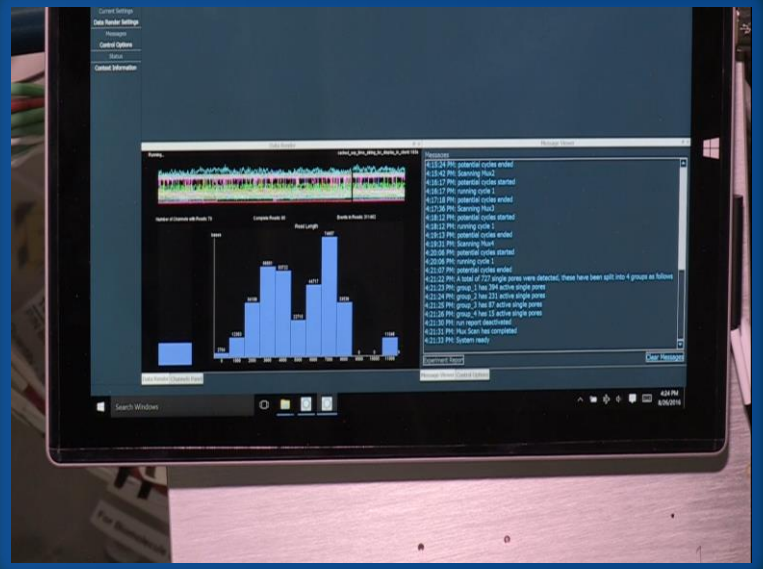


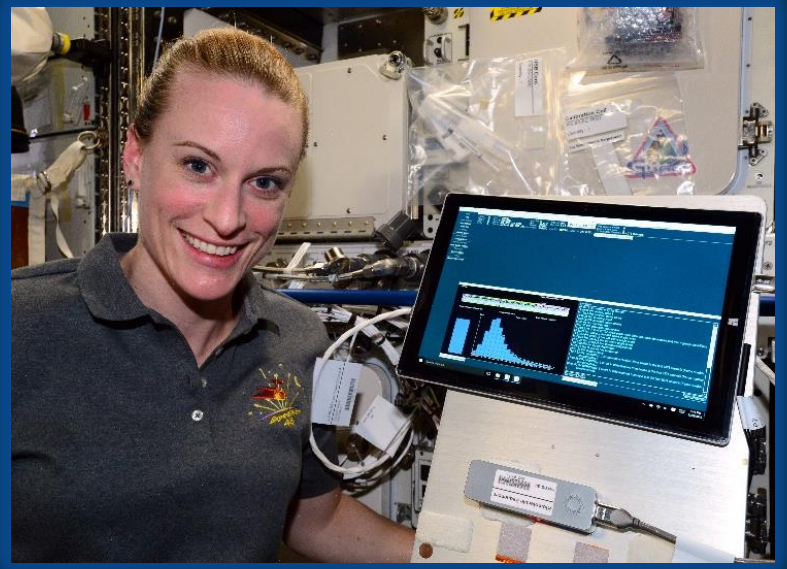


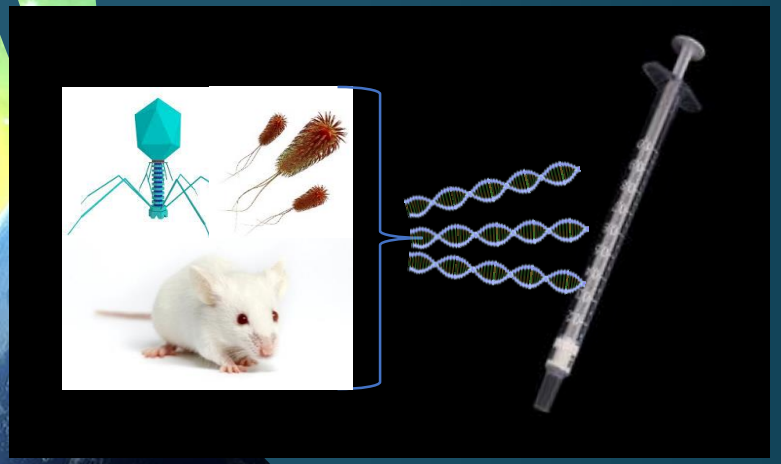
MinION by Oxford Nanopore Technologies

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.

Biomolecule Sequencer





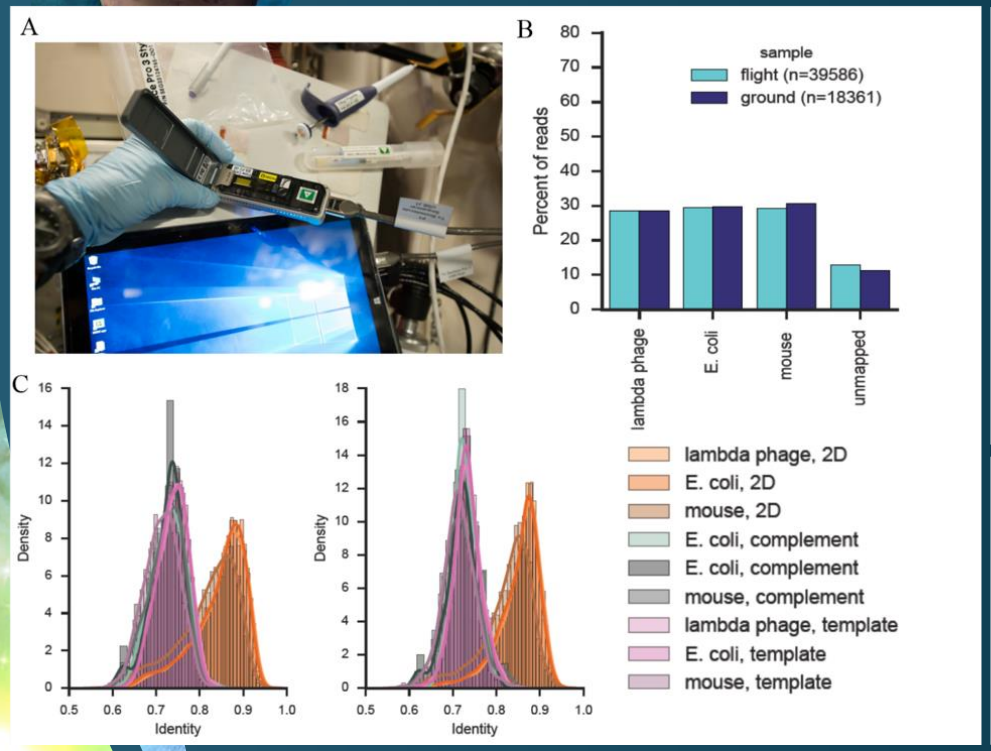


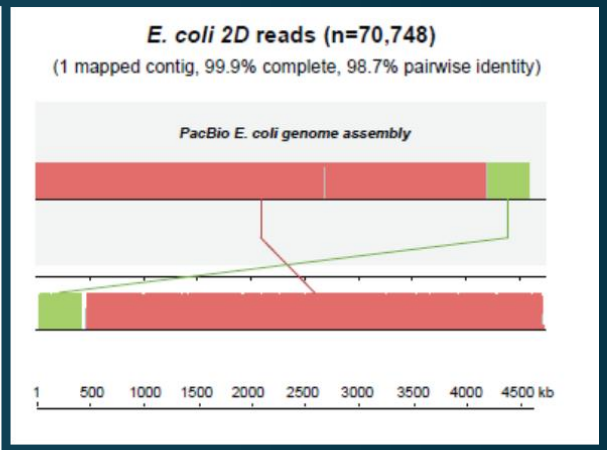
August 26th, 2016

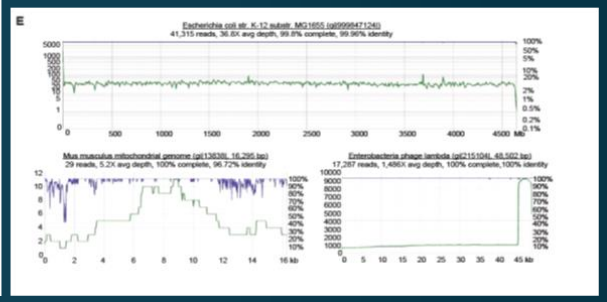
“Welcome to systems biology in space.”
Astronaut Kate Rubins, Ph.D.

On orbit operations:
August 26, 2016
September 3, 2016
September 7, 2016
September 13, 2016
October 18, 2016
October 25, 2016
October 26, 2016
November 26, 2016
January 9, 2017

Biomolecule Sequencer







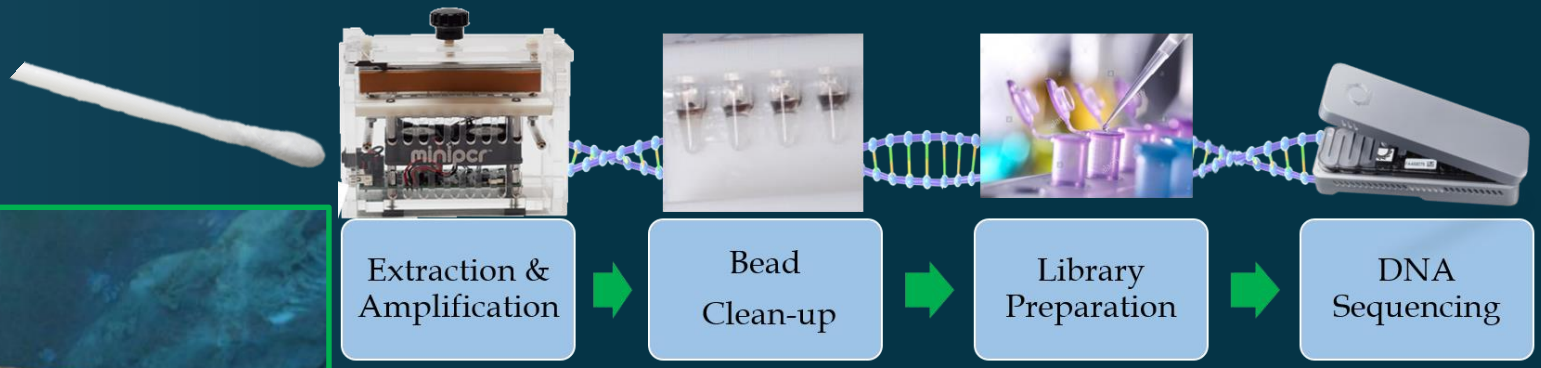
Castro-Wallace et al. https://www.nature.com/articles/s41598-017-18364-0

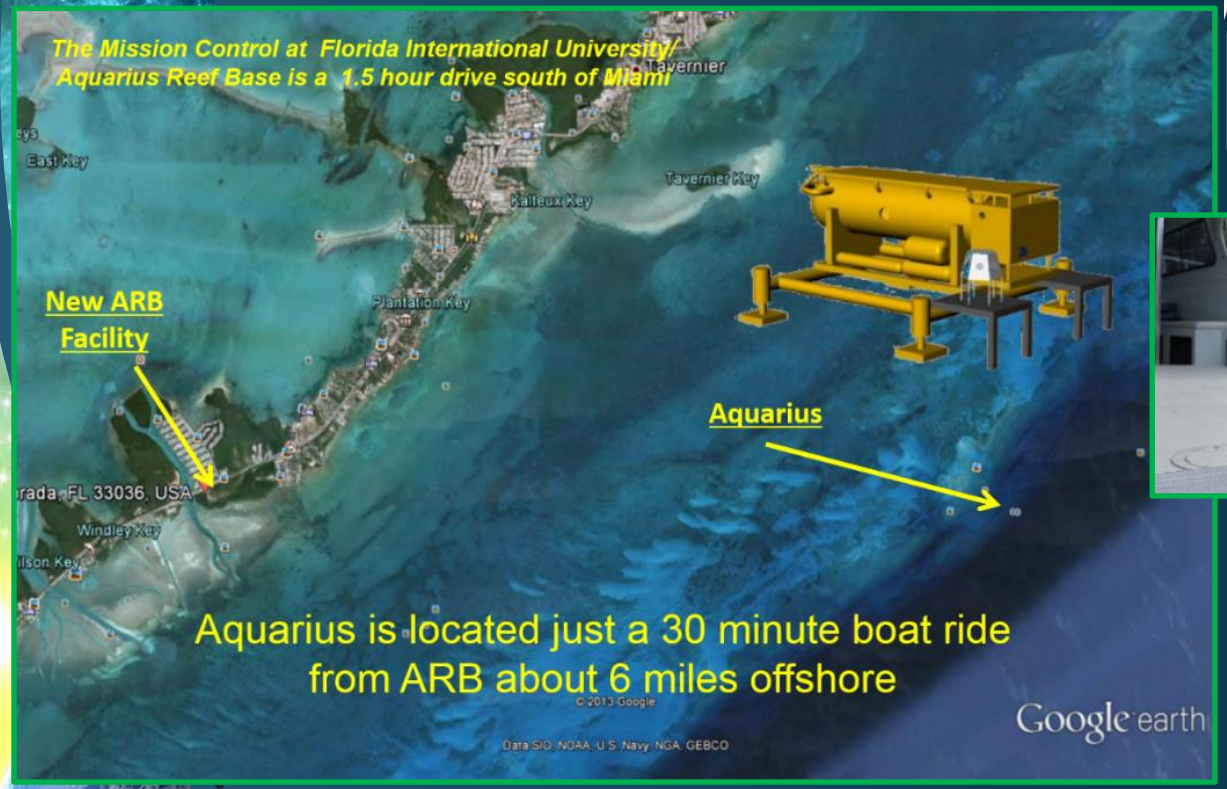
Kit	Flow cell
SQK-MAP-006	R7.3

~280,000 reads on orbit
~130,000 reads on ground

- No decrease in sequencing performance
- Over 284,000 reads were generated on the ISS
- Directed genome assemblies of:
  - Bacteriophage lambda
  - *E. coli*
  - Mouse mitochondrial genome
- *de novo* genome assemblies of:
  - Bacteriophage lambda
  - *E. coli*
- Demonstrated flow cell reuse and shelf-life stability to at least 6 months in space

The Need for Sample Prep



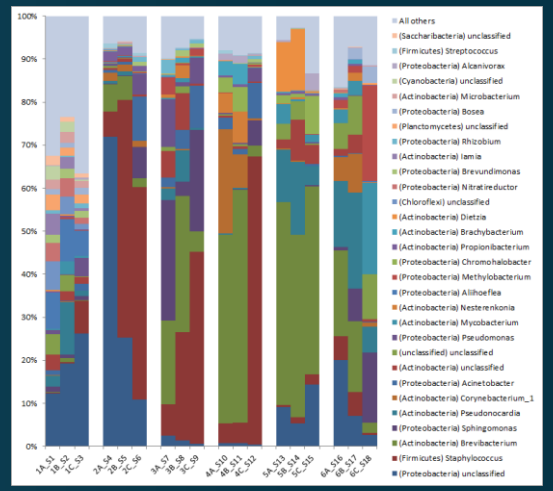


NASA Extreme Environments Mission Operations (NEEMO)

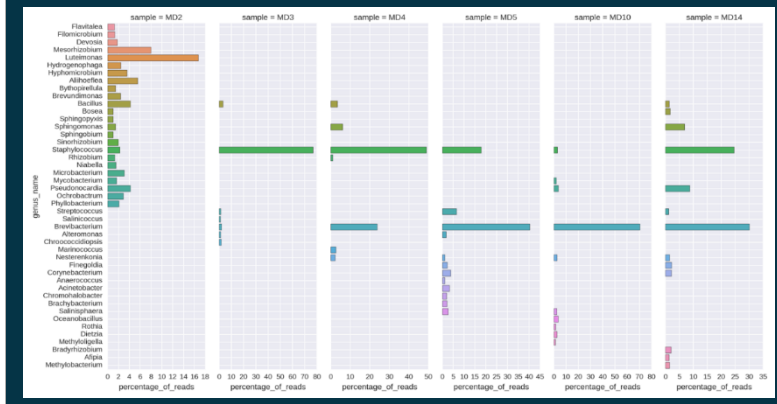
The Need for Sample Prep

Culture-independent microbial profiling: NEEMO 21 & 22

Illumina MiSeq



Oxford Nanopore Technologies MinION



Genes in Space-3

(NASA/Boeing Collaboration)

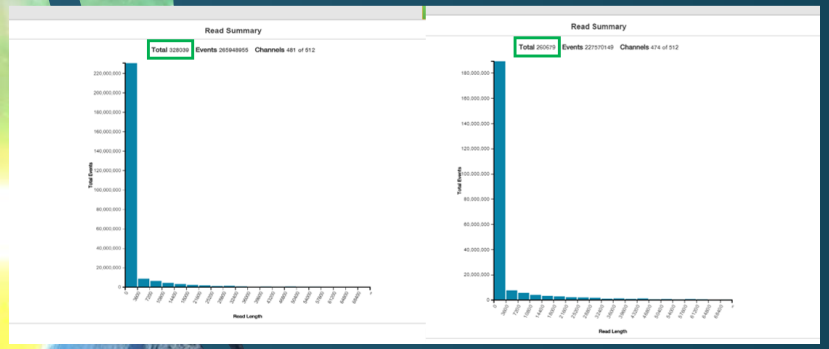
- A series of controlled experiments testing key steps of the DNA preparation process
- Culminated in the sequencing of unknown environmental samples from the ISS
- Genes in Space-3 launched: April 18, 2017 on OA-7

Genes in Space-3

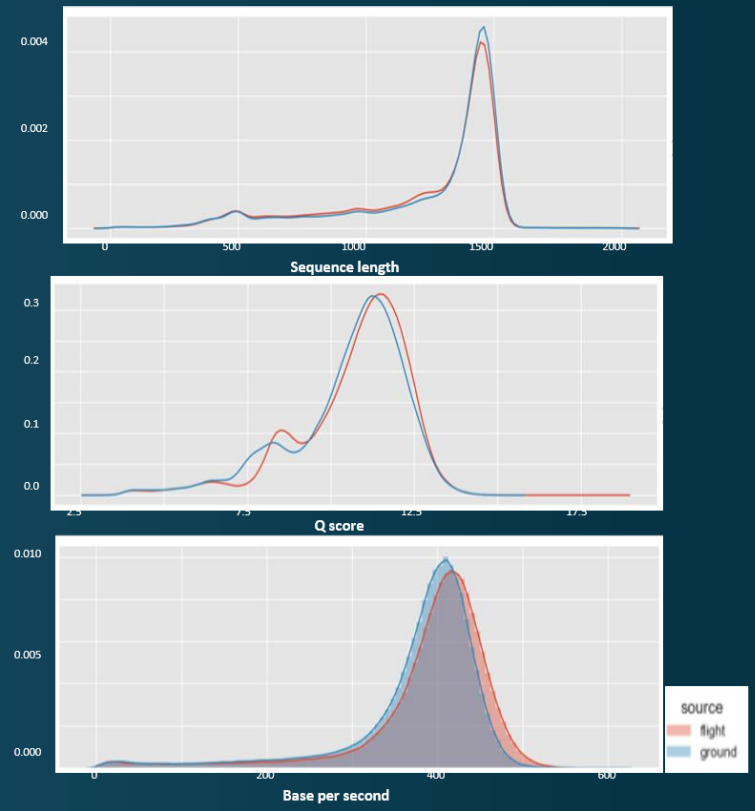
(NASA/Boeing Collaboration)

ISS

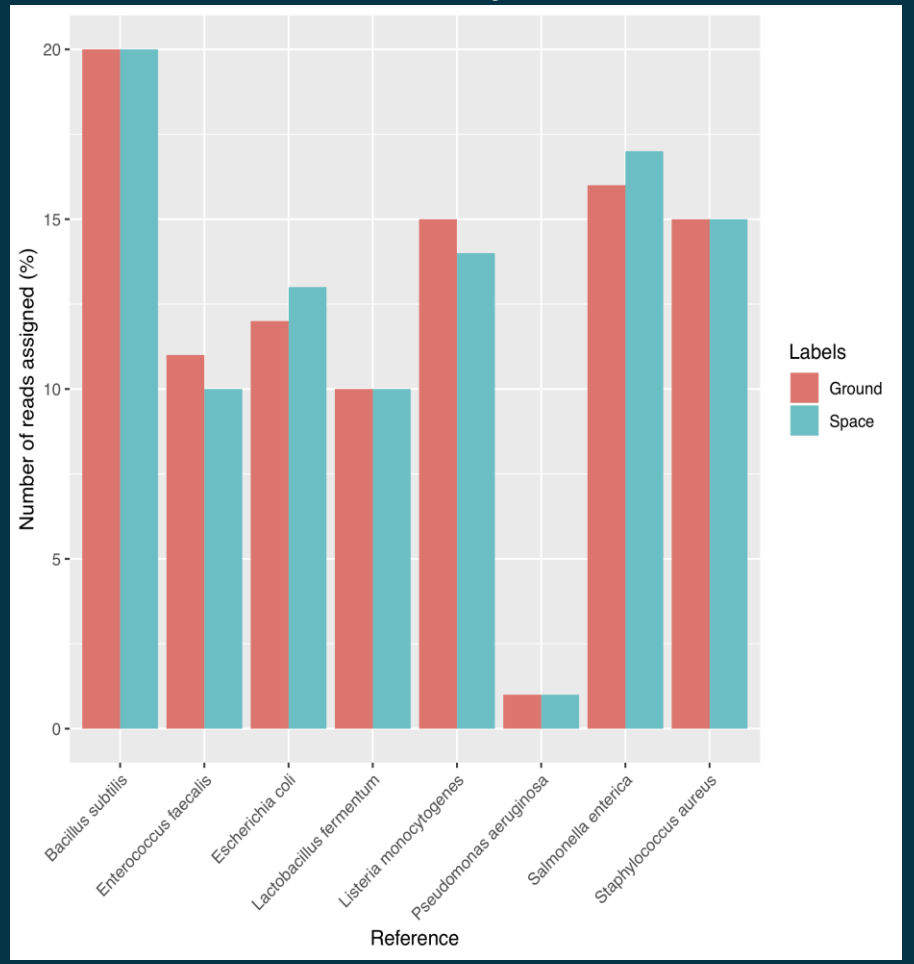
Ground



MinION Metrics



Percentage of reads assigned to ZymoBIOMICS Microbial Community DNA Standard



Kit	Flow cell
SQK-RAS-201	R9.4

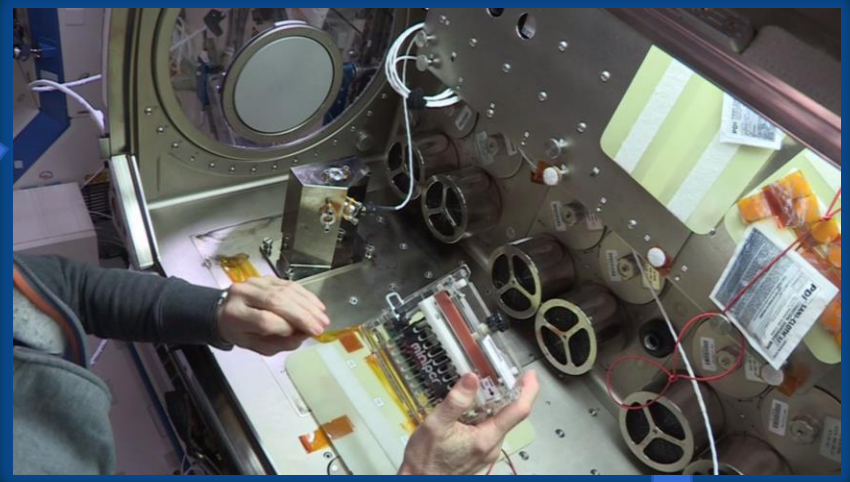
~328,000 reads on orbit

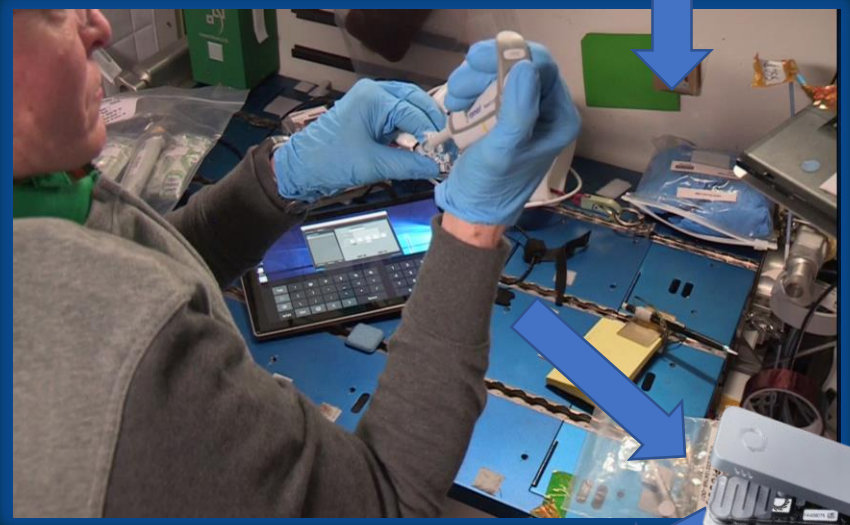
~260,000 reads on ground

Genes in Space-3

(NASA/Boeing Collaboration)

Astronaut Peggy Whitson collecting microbial cells from an Environmental Health Systems (EHS) Surface Sampling Kit (SSK) Slide





Microbial Identifications

Genes in Space-3

(NASA/Boeing Collaboration)

VITEK (biochemical)

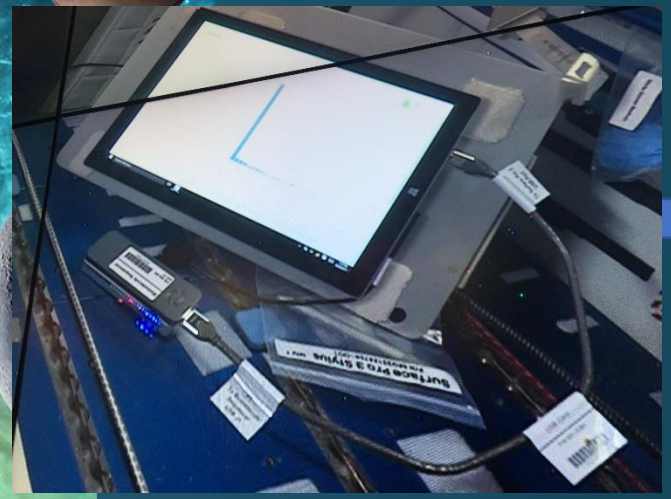
ABI 3500 (Sanger sequencing)

NASA JSC Microbiology Laboratory's standard methods for microbial identification

Genes in Space-3

(NASA/Boeing Collaboration)

Data obtained in flight downlinked to the ground for analysis:



```
agtcgatcgata
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atcgagggaggt
accgattagggt
attaccccgata
gggtaagatagc
agggacatttac
ggattacggaga
cttcgatacgat
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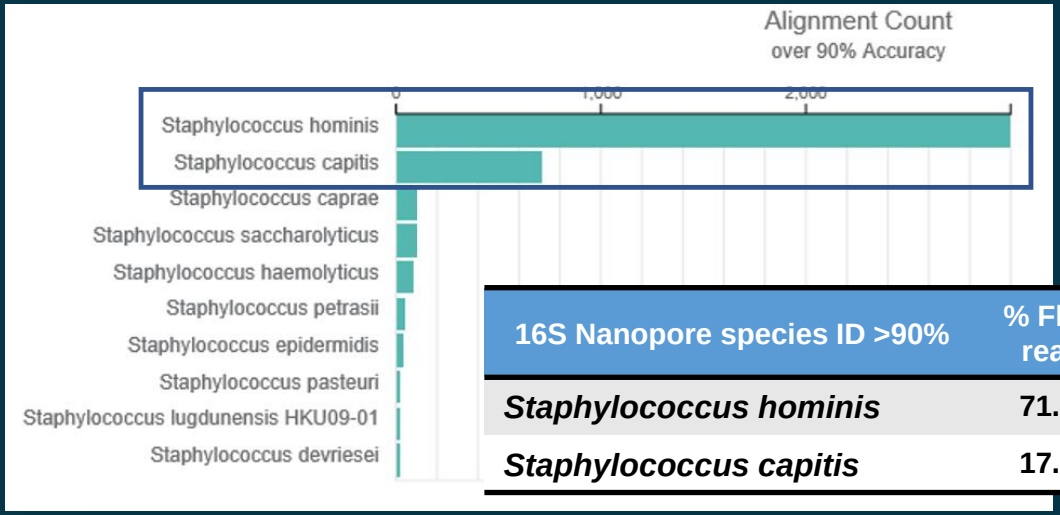


16S Nanopore species ID >90%	% Flight reads
Staphylococcus hominis	**71.3%**
Staphylococcus capitis	**17.0%**

Return sample processed through normal procedures in the Microbiology Lab:

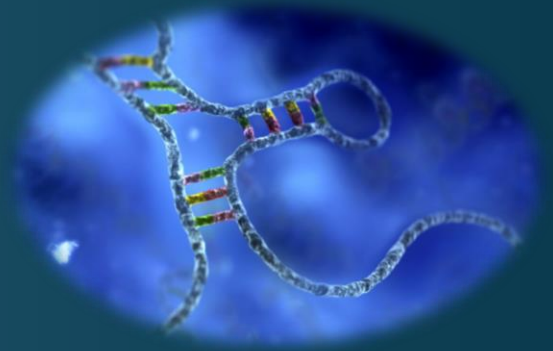
Colony	Detection Method	Sample ID	%ID
1	Biochemical	*Staphylococcus hominis hominis*	97.0
2	Biochemical	*Staphylococcus hominis hominis*	97.0
3	Biochemical	*Staphylococcus capitis*	94.0
1	Sanger Sequencing	*Staphylococcus hominis hominis (ATCC=27844)*	99.9
2	Sanger Sequencing	*Staphylococcus hominis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

Success! The sequence data from Station matched the identifications obtained on the ground. History made!

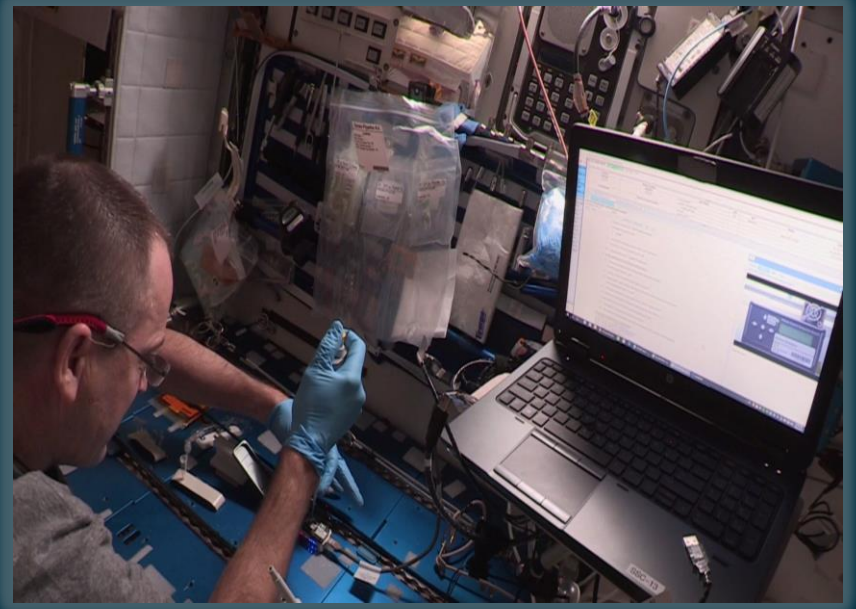
BEST

(Biomolecule Extraction and Sequencing Technology)

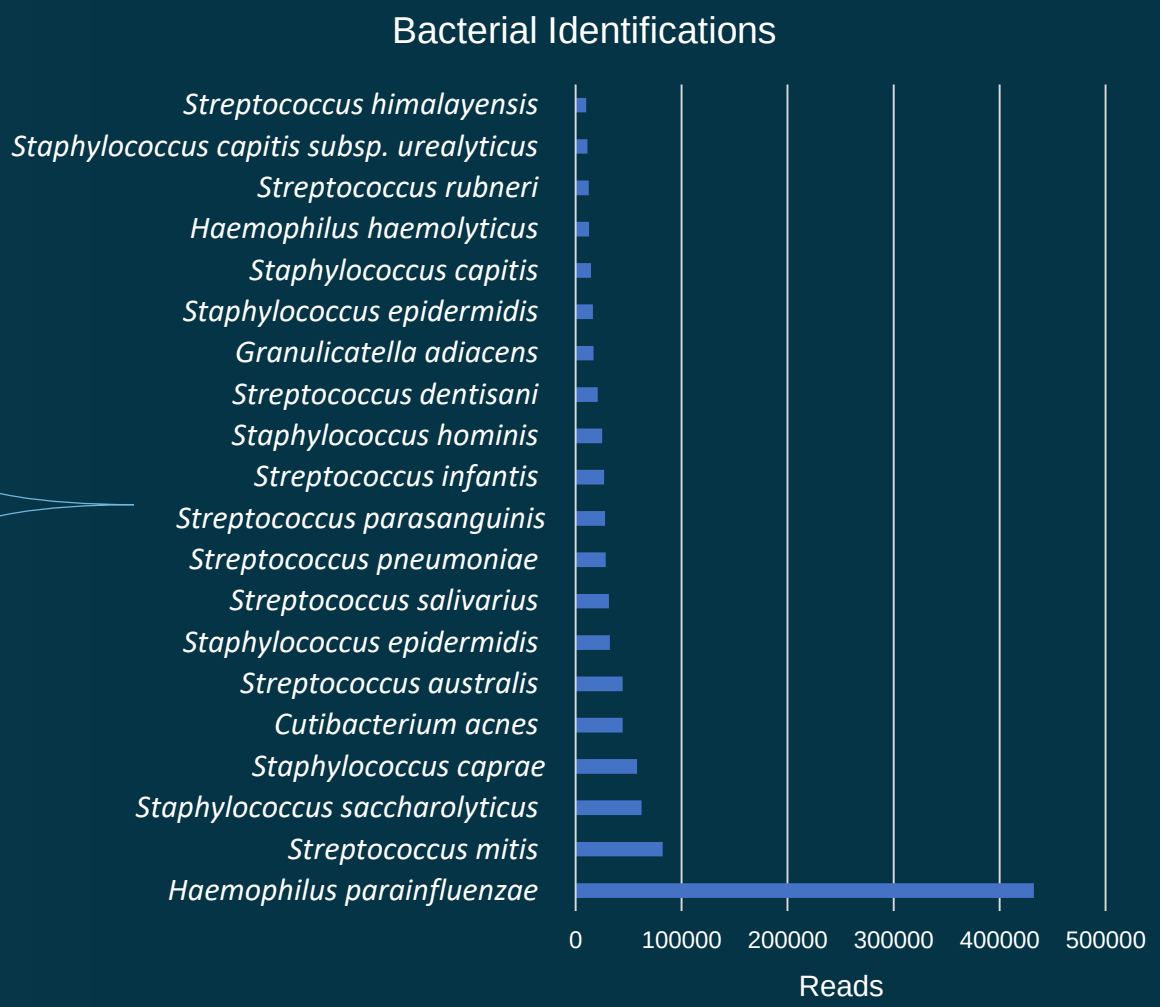
- Consumables launched on OA-9
- 3 separate experiments
  - Swab-to-sequencer
  - Cellular evolution
  - Direct RNA sequencing



BEST
Swab-to-Sequencer



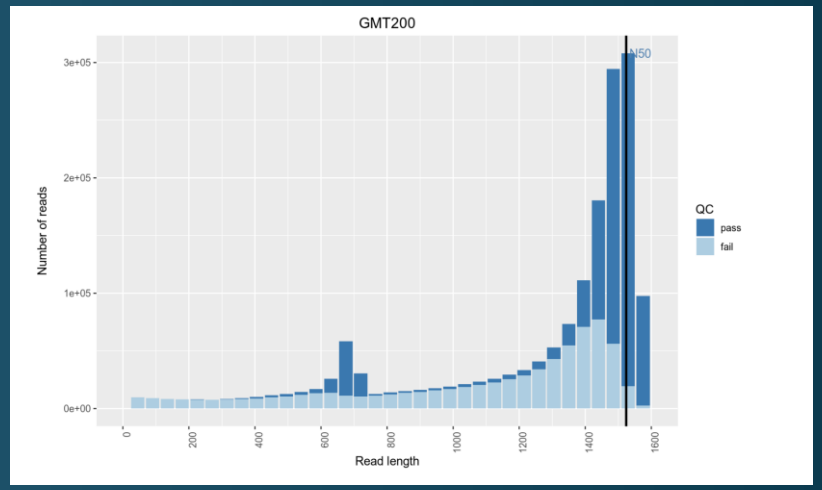
BEST
Swab-to-Sequencer



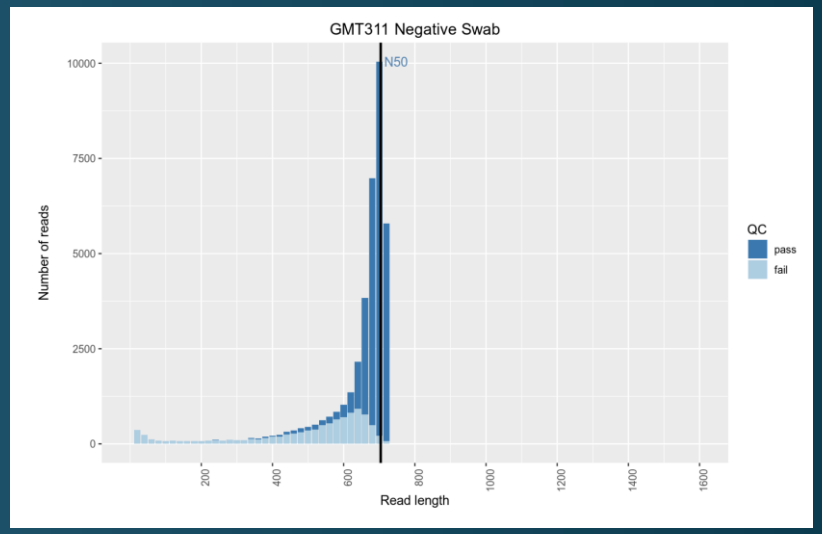
Kit	Flow cell
SQK-RAB-204	R9.4

~1.6 million reads on orbit

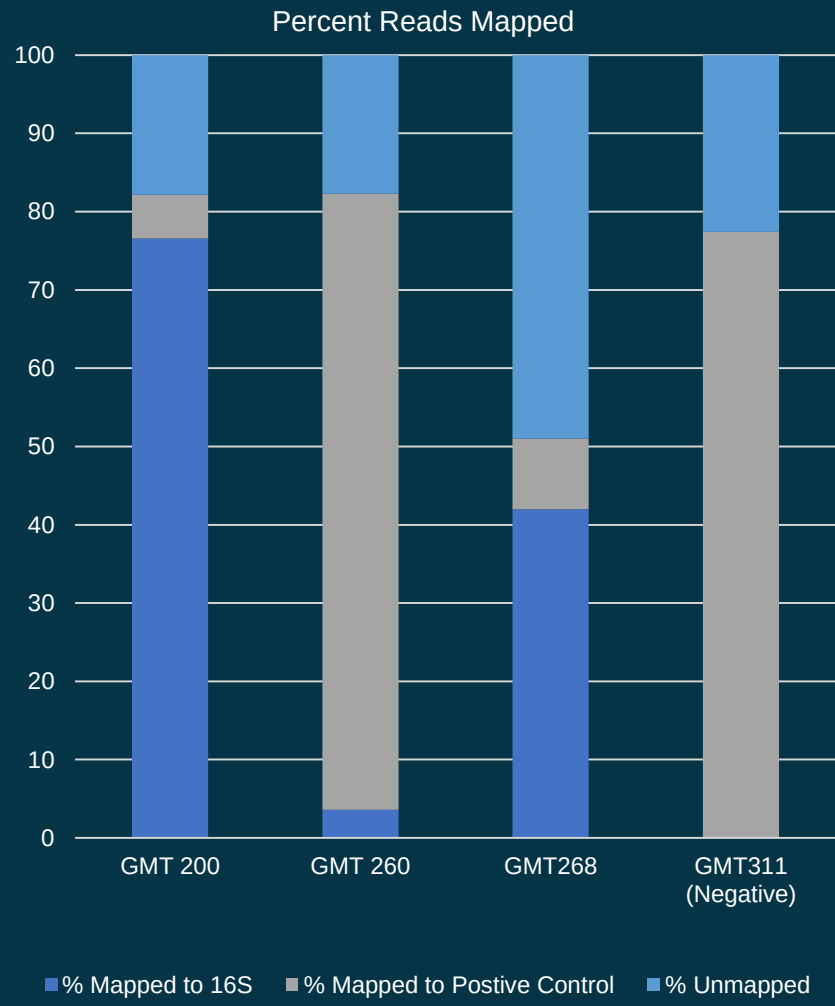
BEST
Swab-to-Sequencer



GMT200



GMT311



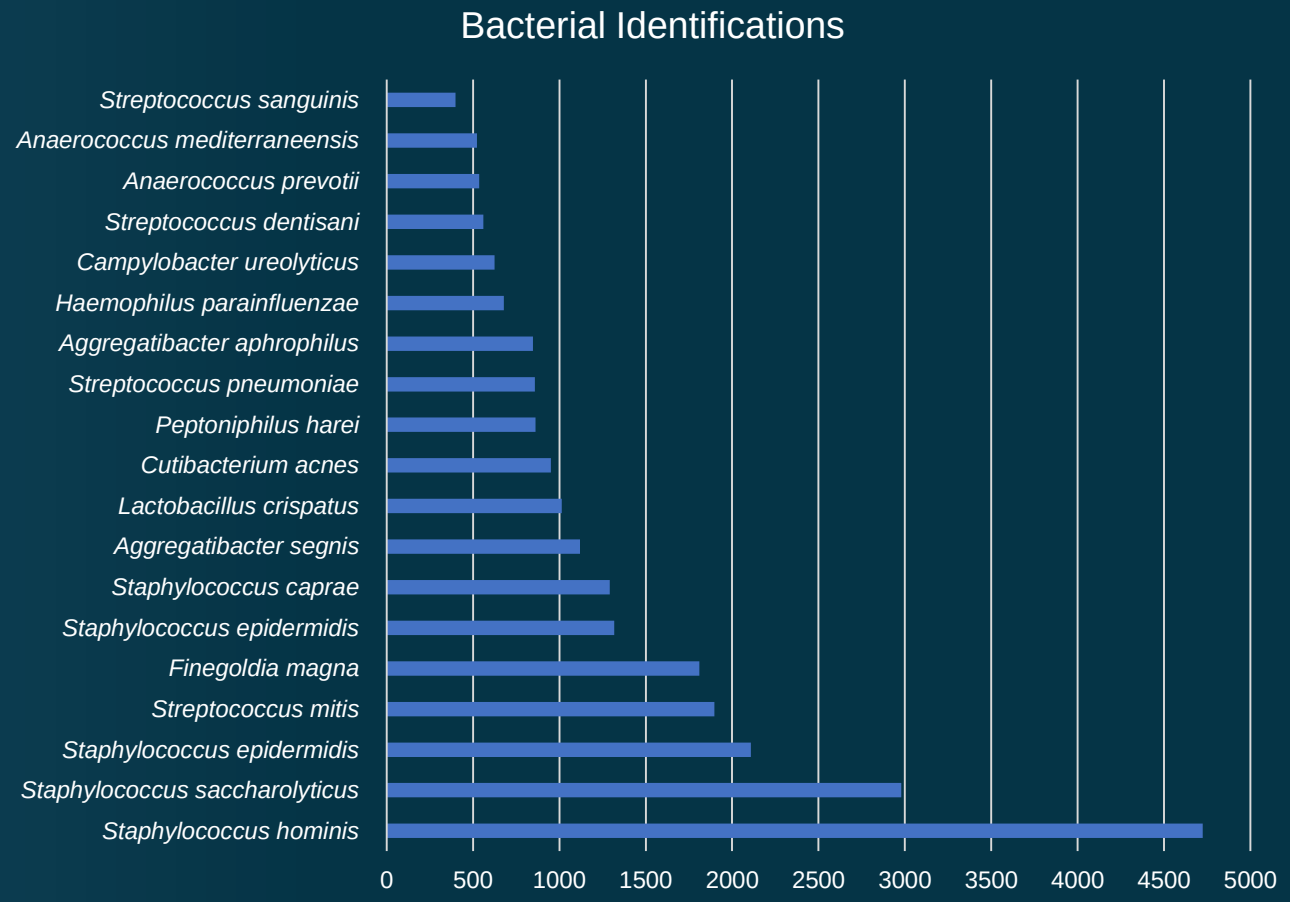
Positive control sample versus 16S distribution

BEST
Swab-to-Sequencer

*Columbus Module: Air Return Grid

Bacterial Identifications
Staphylococcus hominis
Staphylococcus epidermidis
Staphylococcus saccharolyticus
Corynebacterium species

Culture-based identifications detailed by the NASA Microbiology Laboratory's final report



*Sampling of this location occurred on different dates

Swab-to-Sequencer identifications obtained by the crew on ISS

What's Next?

- ~200 ISS swabs to be processed
- ~200 more ISS swabs to be collected (same locations 6 – 8 months later)
- How does this compare to our historical culture-based data?
  - Risk assessment
- What does this data tell us about the microbiome of ISS?
- Culture vs Swab-to-Sequencer ground study
- Bacterial + Fungal analysis
- Microbial water analysis
  - Wastewater
  - Humidity condensate
  - Product water
- RNA!
- Microbial long-term evolution in response to the spaceflight environment

Kate Rubins, Ph.D.

Peggy Whitson, Ph.D.

NEEMO 21 Team
Reid Wiseman, NASA
Megan McArthur, NASA
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Matthias Maurer, ESA
Noel Du Toit
Dawn Kernagis

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Pedro Duque, ESA
Dom D'Agnostion
Mark Hulsbeck
Sean Moore

<u>Genes in Space-3 Collaborators</u>
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<u>New England BioLabs</u>
Nicole Nichols, Ph.D.

<u>Oxford Nanopore Technologies</u>
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<u>Genes in Space-3 Flight Team</u>
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Landon Sommer
Melanie Smith

<u>BEST PIM</u>
Melissa Boyer

<u>BEST Ops Lead</u>
Linda Gibson

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Science Team

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Genes in Space-3 & BEST Co-Investigators

Mark Akeson, Ph.D., Miten Jain, Ph.D., and Benedict Paten, Ph.D.
University of California Santa Cruz

Thank you!


Reid Wiseman
@astro_reid
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This look? It's when an @USNavy pilot sees confirmation from the @nanopore sequencer of successful #DNA extraction
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Kjell Lindgren
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Replying to @astro_reid
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@astro_ricky
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Awesome week of science on @Space_Station last week. My personal highlight was isolating, amplifying, then decoding bacterial DNA. Lots of potential uses in space & remote communities on Earth.
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Intl. Space Station, ISS Research, NASA and NASA Astronauts
23
246
1.2K