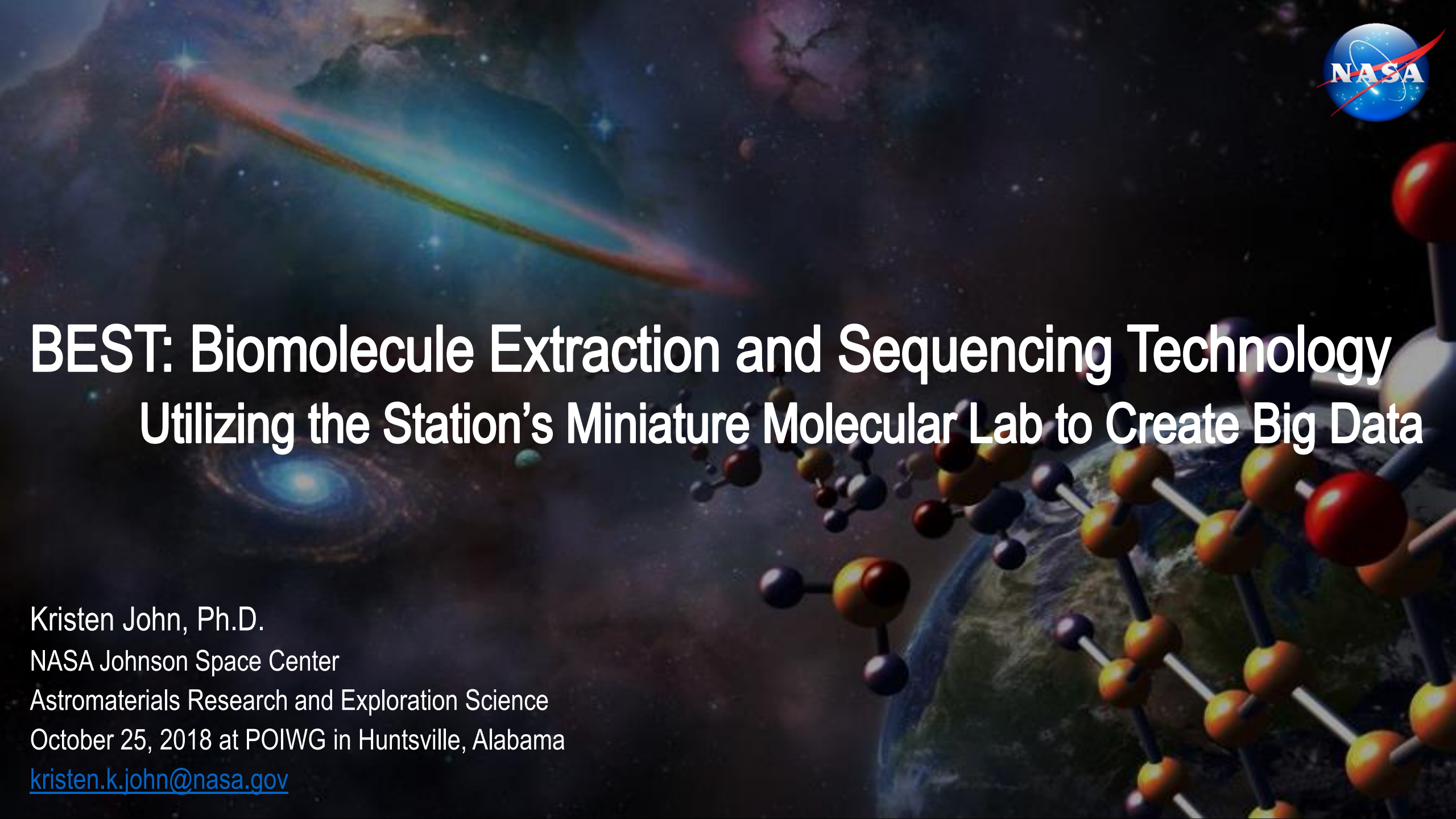


BEST: Biomolecule Extraction and Sequencing Technology

Utilizing the Station's Miniature Molecular Lab to Create Big Data

Kristen John, Ph.D.
NASA Johnson Space Center
Astromaterials Research and Exploration Science
October 25, 2018 at POIWG in Huntsville, Alabama
kristen.k.john@nasa.gov

Outline

- Brief background to how we got here
  - miniPCR
    - Genes in Space
  - Biomolecule Sequencer
- Why molecular biology on station?
- Merging the technology
  - NEEMO
- Genes in Space-3
- Biomolecule Extraction and Sequencing Technology (BEST)
  - Swab-to-sequencer
  - Mutation tracking
  - Direct RNA sequencing

Genes in Space: The Molecular Space Age

Sebastian Kraves, Ph.D.
and Ezequiel (Zeke) Alvarez Saavedra, Ph.D.,
miniPCR, Genes in Space

miniPCR

- Copies targeted pieces of DNA in a process known as the polymerase chain reaction (PCR) (amplifies segment of DNA)
- Serves as a heat block for a range of biochemical enzymatic reactions required in molecular biology-based investigations such as whole genome amplifications

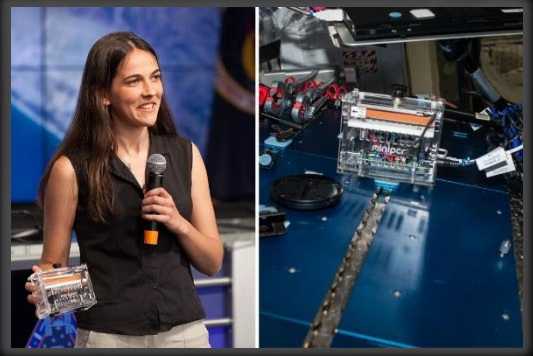
April 19: The first molecular biology assay in space is completed, as DNA is amplified using the miniPCR thermal cycler

2016

GENES IN SPACE

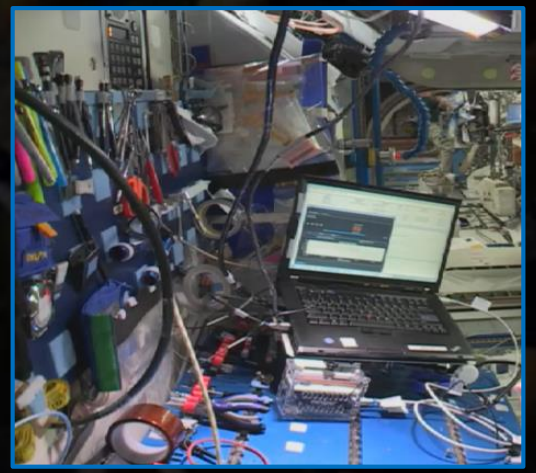
https://www.genesinspace.org/

BOEING minipcr NEW ENGLAND BioLabs Inc. enabling technologies in the life sciences MfA CASIS



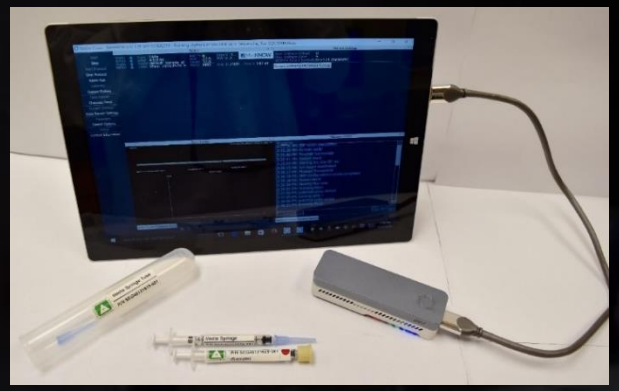
Genes in Space-1 winner Anna-Sophia's investigation was the first molecular biology experiment ever conducted in space, April 19, 2016

Genes in Space-2 winner Julian had his experiment launched to the ISS in March 2017 on OA-7

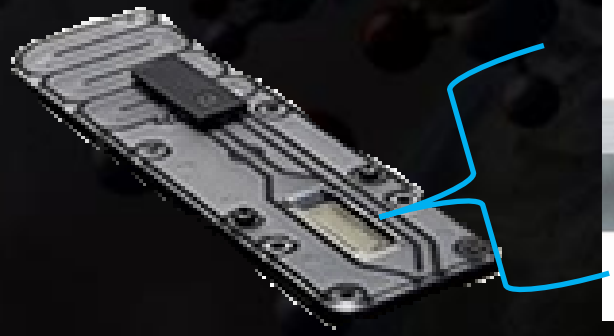


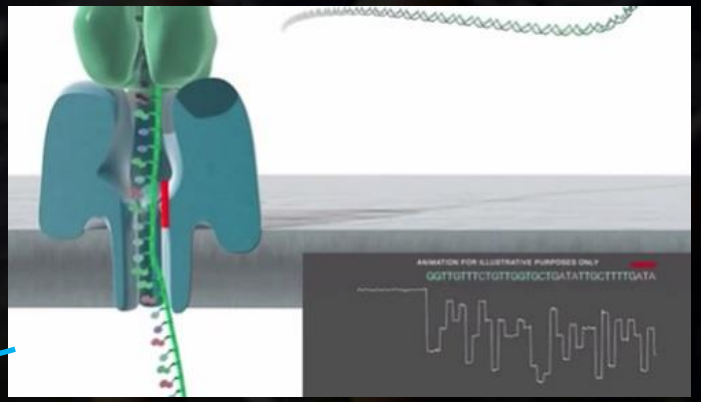
Biomolecule Sequencer

- The first device to assess the capability of DNA sequencing in the microgravity environment of space
- Enabled by the MinION, developed by Oxford Nanopore Technologies
- COTS miniature DNA Sequencer
- 3 ¾ x 1 ¼ x 5/8 inches
- Less than 120 grams (with USB cable)
- Powered via USB connection
- Capable of DNA, RNA, and protein sequencing
- First launched July 18, 2016 (SpaceX-9)



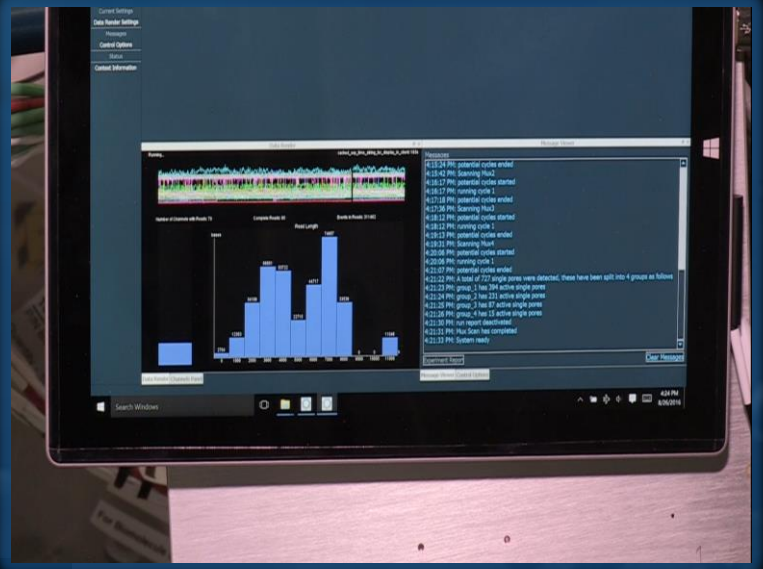
MinION by Oxford Nanopore Technologies

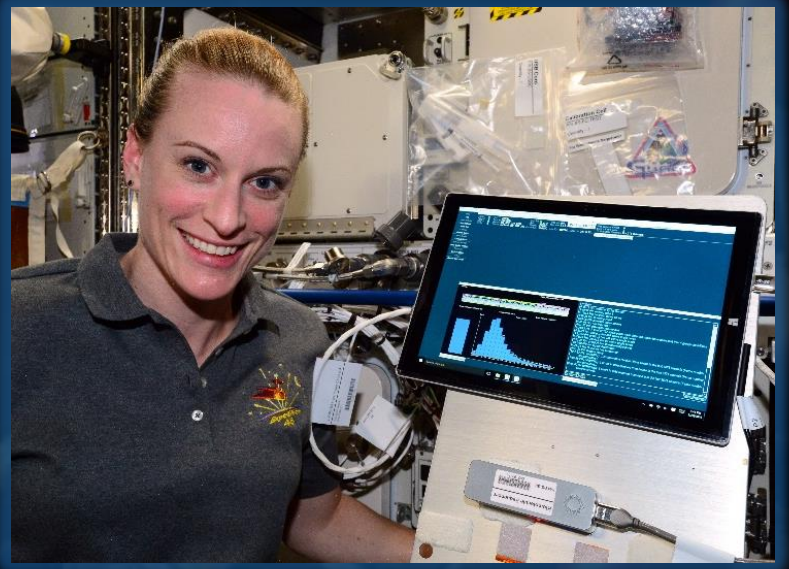


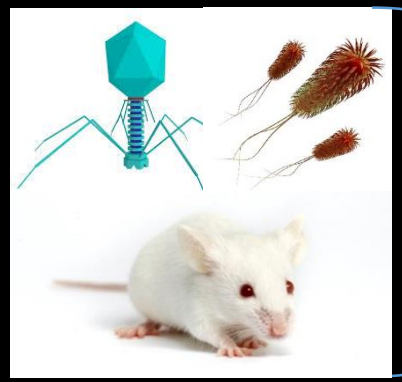


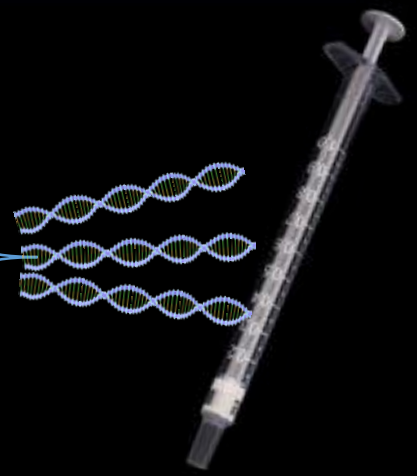
Nanopore-based sequencers measure changes in current caused by DNA strands migrating through the pore. The changes in current are characteristic of the sequence of migrating DNA.

Biomolecule Sequencer









August 26th, 2016

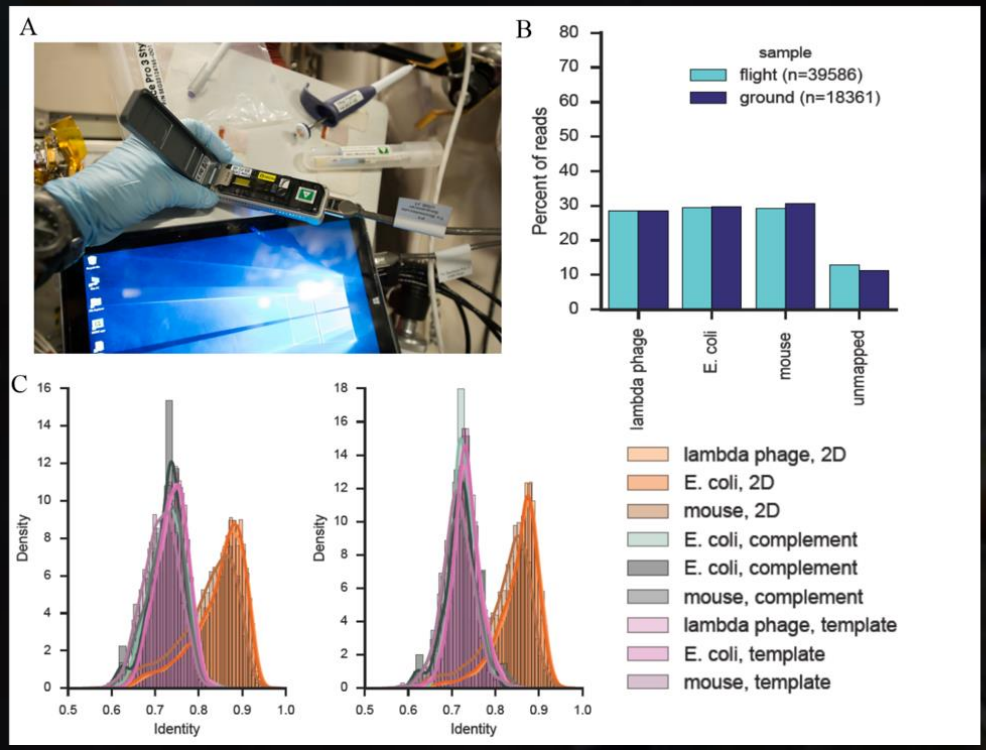
“Welcome to systems biology in space.” – Astronaut Kate Rubins, Ph.D.

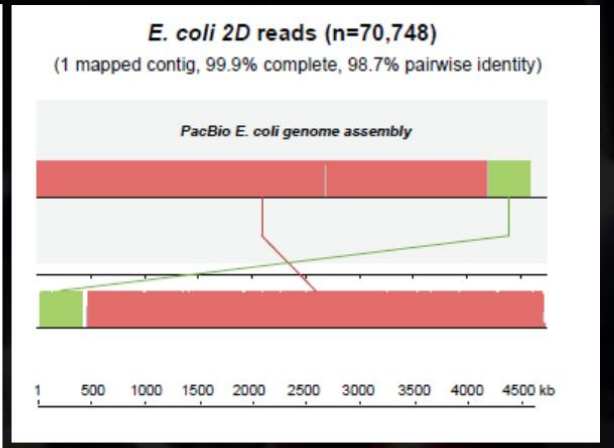
<u>On orbit operations</u>:

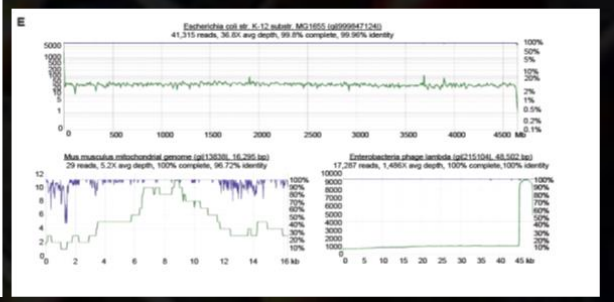
August 26, 2016
September 3, 2016
September 7, 2016
September 13, 2016
October 18, 2016
October 25, 2016
October 26, 2016
November 26, 2016
January 9, 2017

Biomolecule Sequencer

- 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







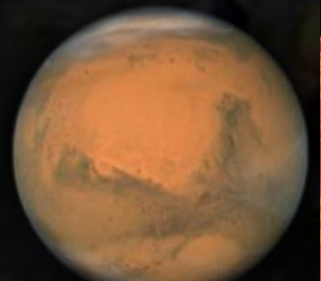
Castro-Wallace et al. https://www.nature.com/articles/s41598-017-18364-0

Why Molecular Biology in Space?

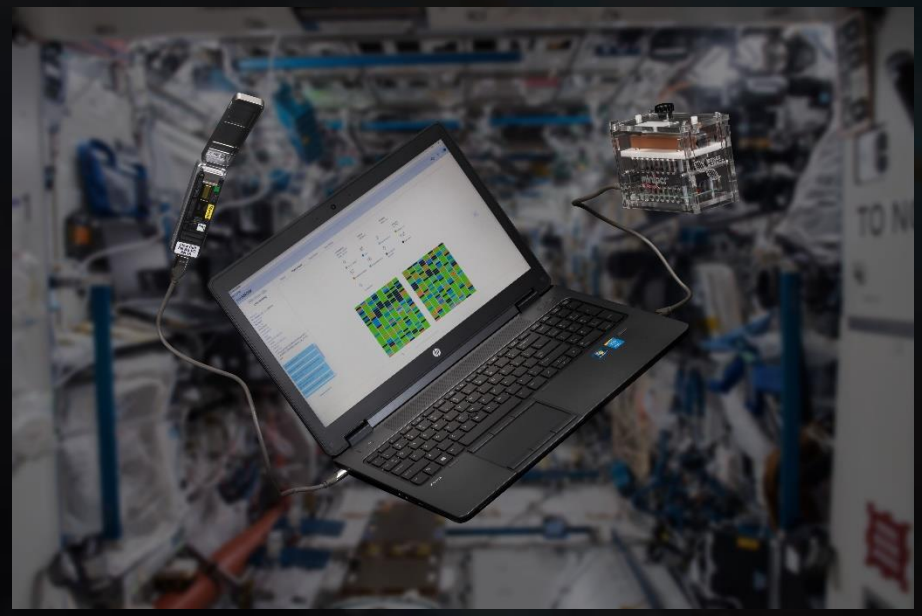
Why do we need molecular biology tools to support the human exploration of space?

- 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

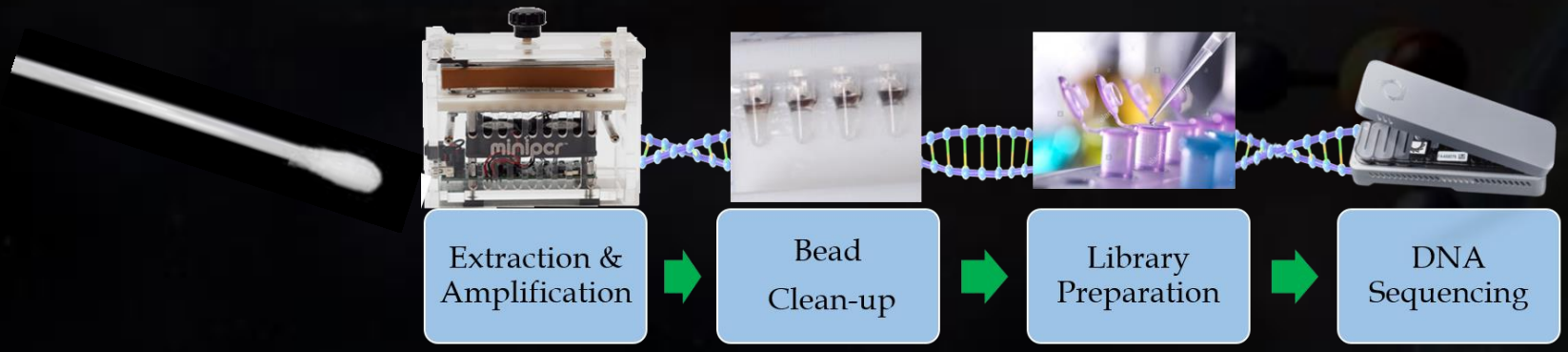
Changes in the genome? Changes in gene expression?



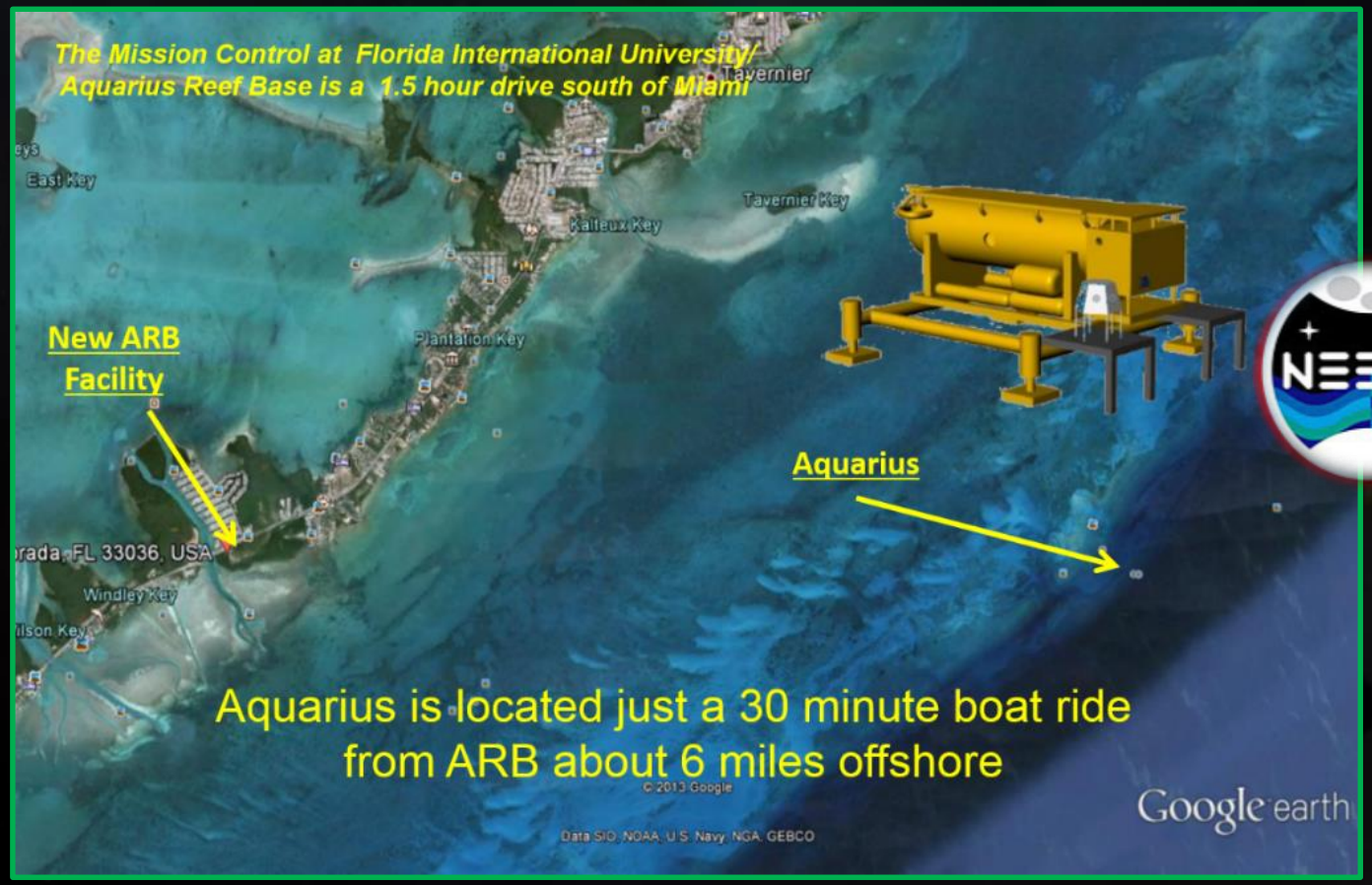
Merging the Technology: Swab-to-Sequencer



- Sample preparation methods are needed to enable use of the technology for research and medical operations.
- Method development began concurrently with the flight certification of the Biomolecule Sequencer.



Merging the Technology: Swab-to-Sequencer

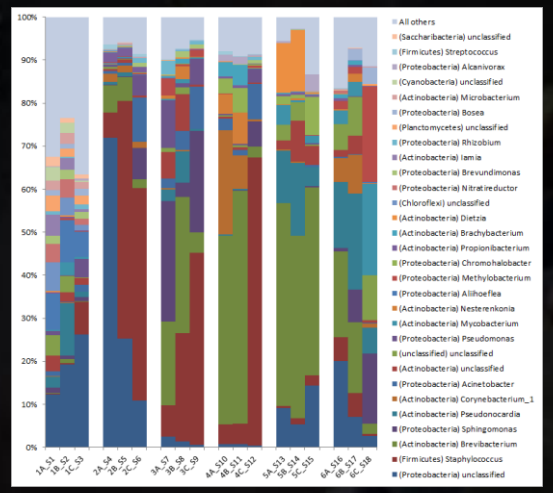


NASA Extreme Environments Mission Operations (NEEMO)

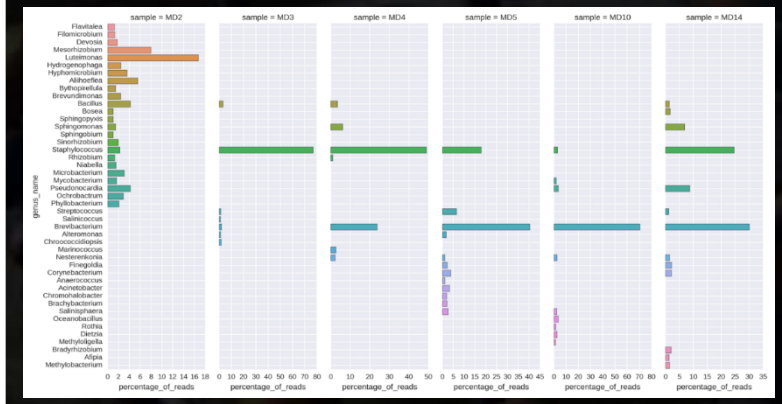
Merging the Technology: Swab-to-Sequencer

Culture-independent microbial profiling: NEEMO 21 & 22

Illumina MiSeq



Oxford Nanopore Technologies MinION



Transitioning Sample Preparation to Space

- Genes in Space-3 built upon the NEEMO 21 demonstration of the joint operations between miniPCR and the MinION, as it transitioned the DNA sample preparation process and sequencing to the spaceflight environment
- Enhanced capabilities available to the Genes in Space student contestants (certified reagents, consumables, and crew procedures)
- Increasing the scientific capacity of the ISS
- 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

The successful implementation of this process resulted in, for the first time, the ability to identify contaminating microbes in-flight.

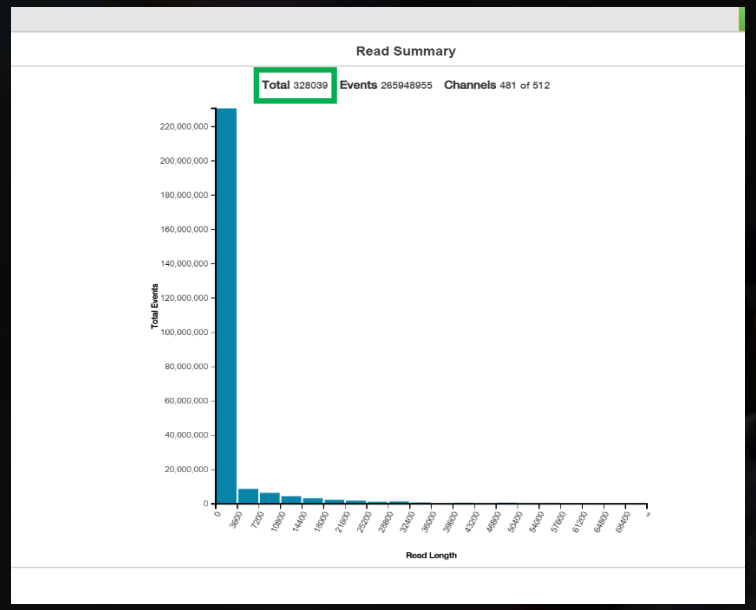
Genes in Space-3

Increasing the scientific capacity of the ISS by facilitating state-of-the-art molecular biology research for both current and next generation ISS researchers.

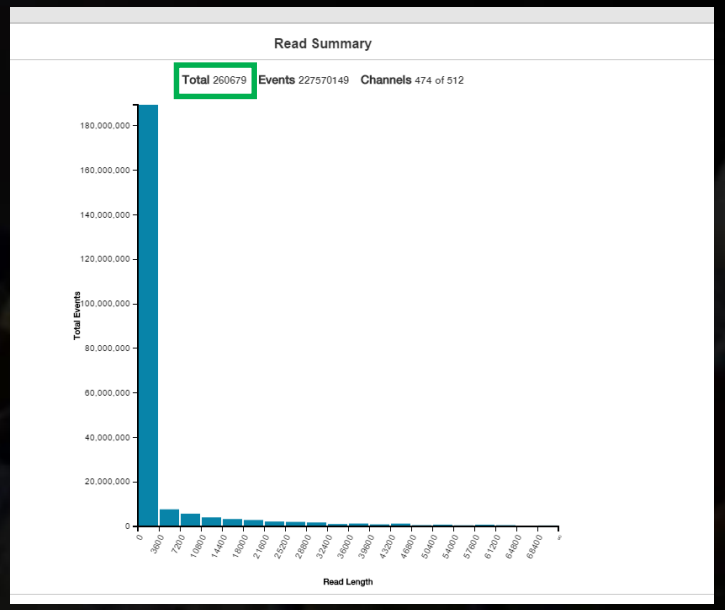
Demonstrating the first identification of ISS-derived unknown microbes in space.

Genes in Space-3

ISS

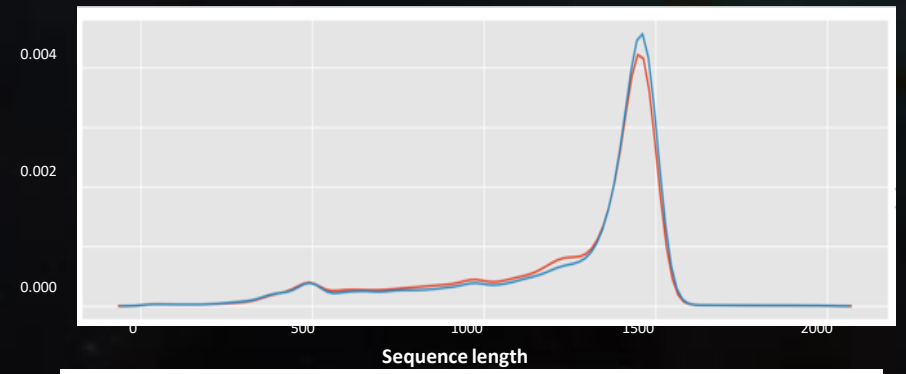


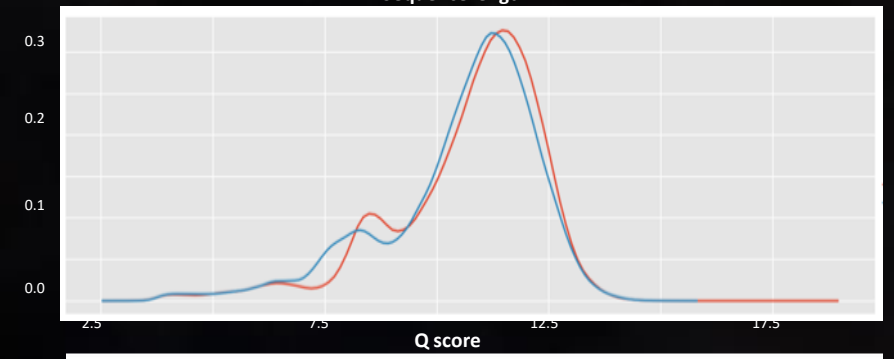
Ground

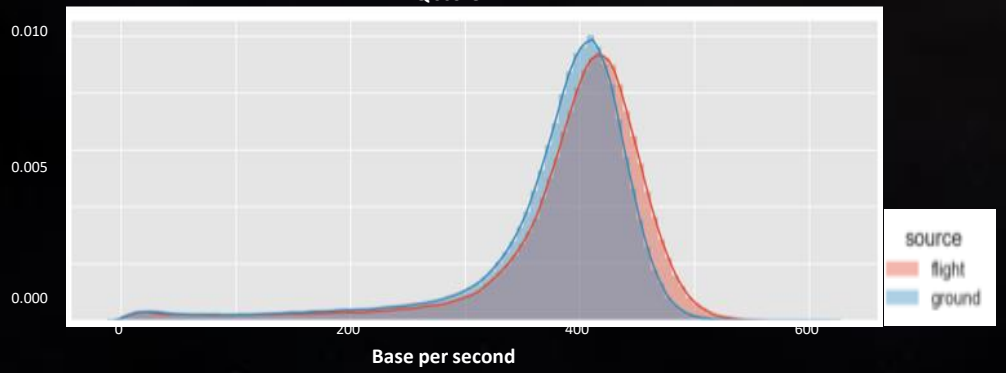


DNA sample to sequencer process successfully implemented – as it would be done on the ground – on the ISS!
Successful: pipetting, DNA amplification, DNA clean up, library preparation, flow cell preparation, and sequencing

Genes in Space-3

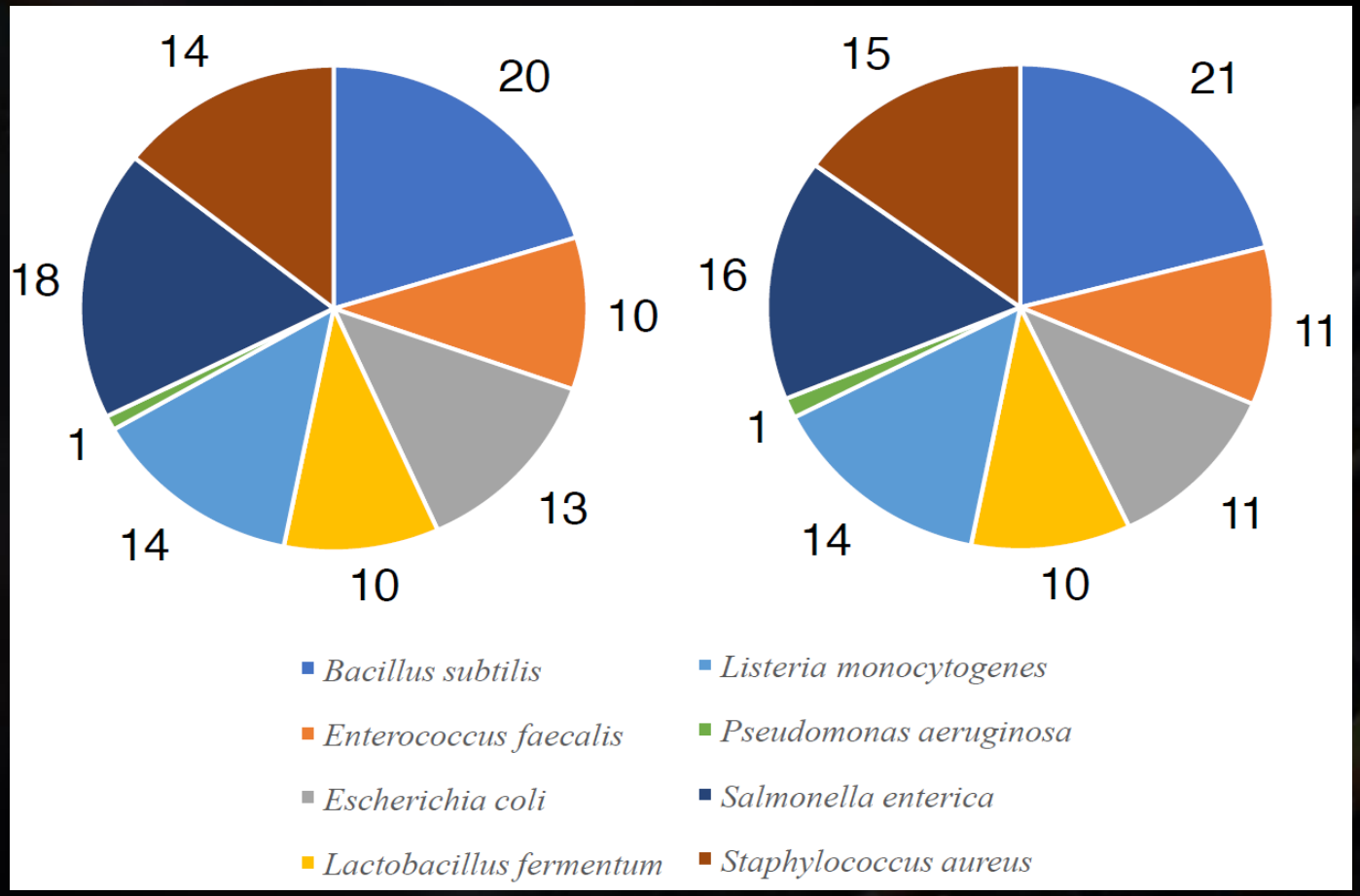




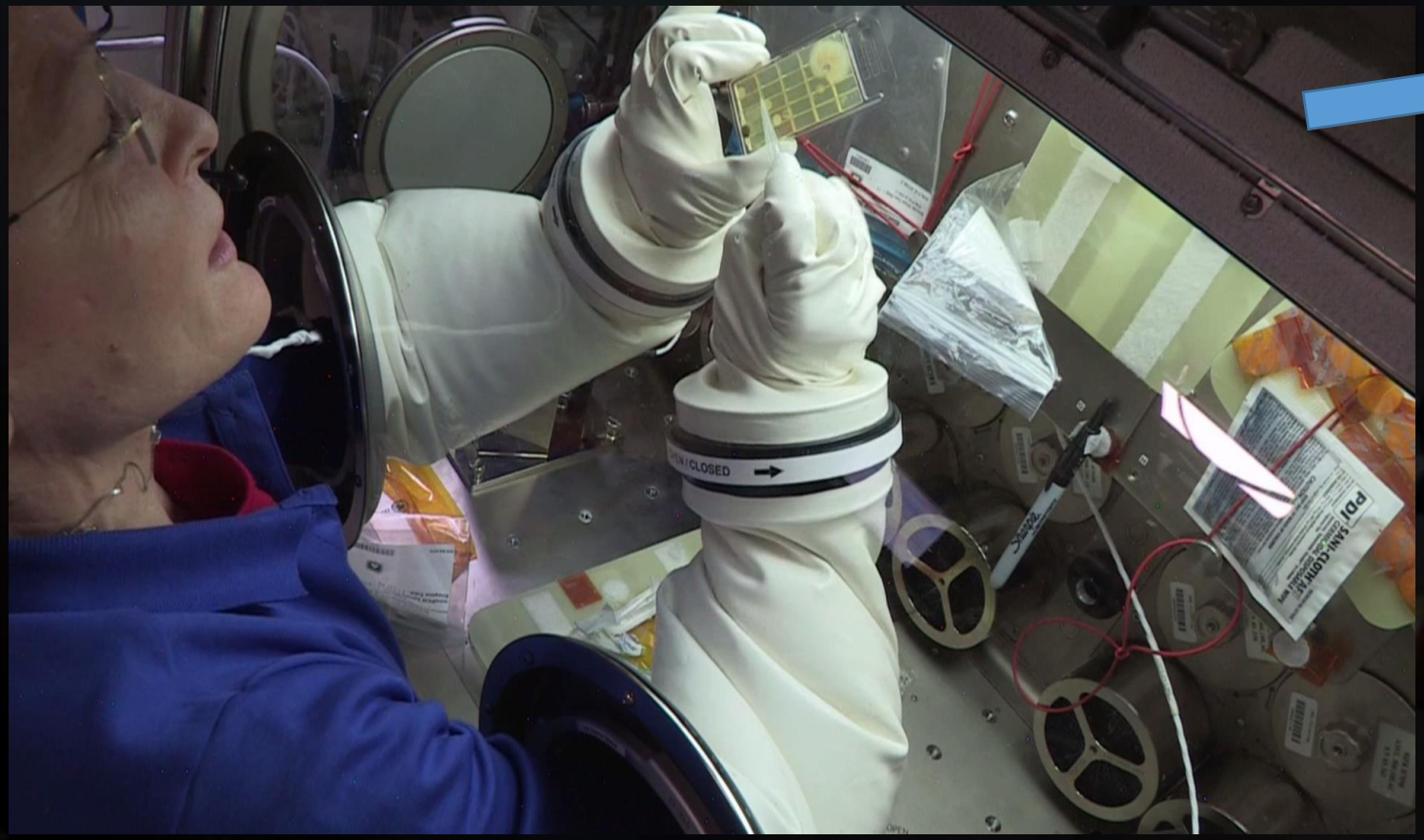


Flight

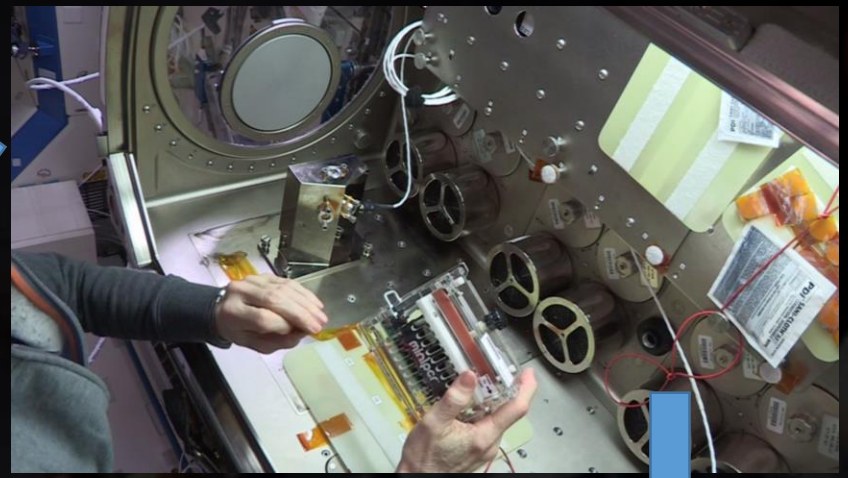
Ground

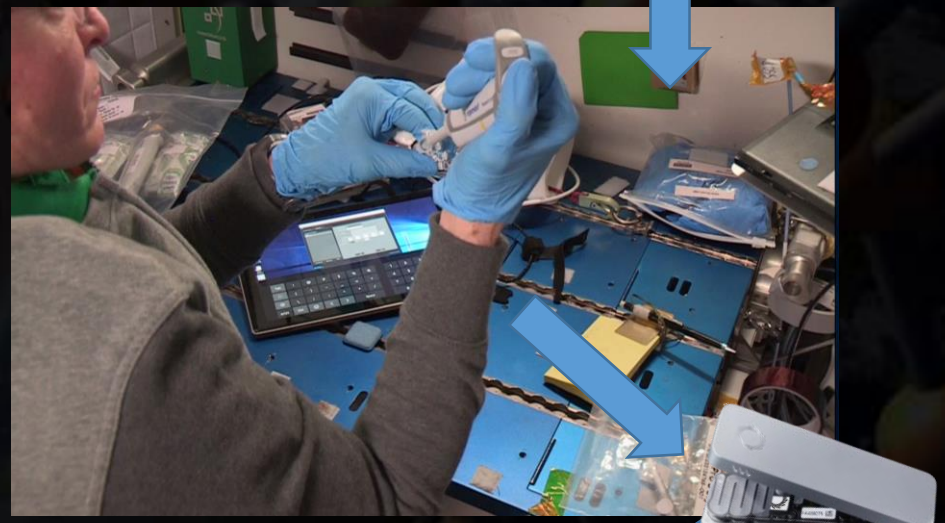


Genes in Space-3



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





Microbial Identifications

Genes in Space-3

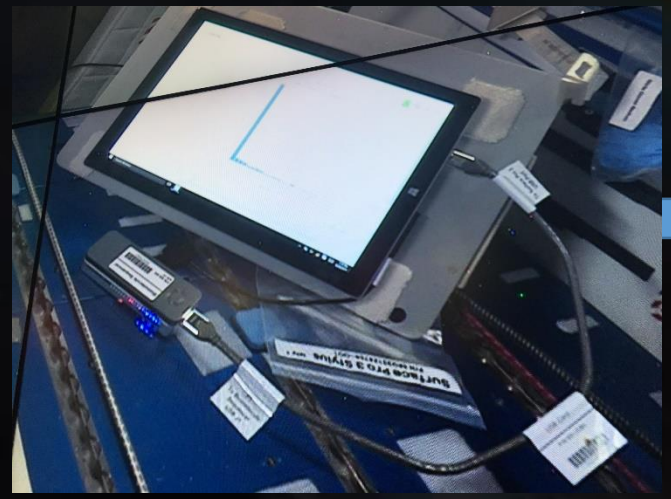
VITEK (biochemical)

ABI 3500 (Sanger sequencing)

NASA JSC Microbiology Laboratory's standard methods for microbial identification

Genes in Space-3

Data obtained in flight downlinked to the ground for analysis:



```
agtcgatcgata
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atcgagggaggt
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attaccccgata
gggtaagatagc
agggacatttac
ggattacggaga
cttcgatacgat
actgtgaccagt
```
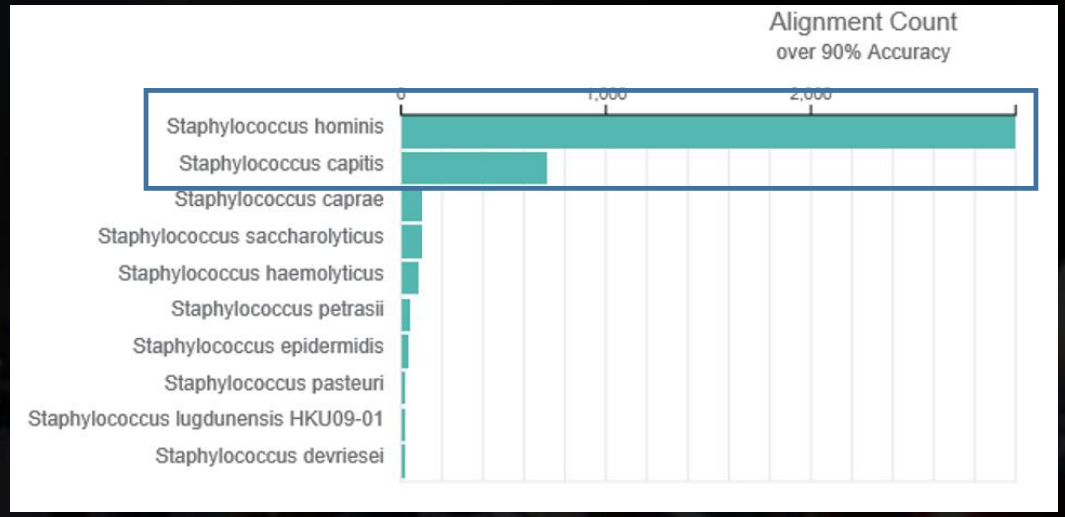



Return sample processed through normal procedures in the Microbiology Lab:

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!

Increasing Molecular Capabilities on Station

- ✓ Biomolecule Sequencer → Sequencing in space is possible!
- ✓ Genes in Space-3 → miniPCR and the Biomolecule Sequencer can be used for an end-to-end, culture-based microbial identification method!

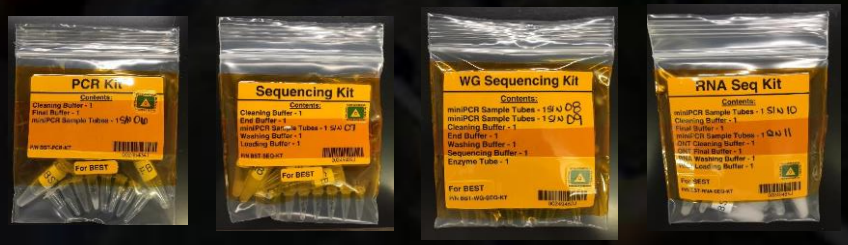
- ➢ What about a rapid means of microbial identification?
- ➢ What about whole genome and epigenome analysis of cells / model organisms?
- ➢ What about transcriptomic analysis (direct RNA sequencing) of cells / model organisms?

The BEST is yet to come...

BEST

Biomolecule Extraction and Sequencing Technology

- Using hardware already on station
  - miniPCR
  - Biomolecule Sequencer
- Consumables launched on OA-9 (and SpX-16)



BEST Experiments

3 separate experiments

1. **Swab-to-sequencer**
   - Perform culture-independent microbiome assessments through sequencing DNA directly from an environmental swab (and return additional swabs)
2. **Cellular evolution**
   - Assess genomic changes as a function of time on the ISS in a cellular population through whole genome sequencing
3. **Direct RNA sequencing**
   - Demonstrate transcriptomic evaluations with the MinION through the sequencing of cDNA and RNA

BEST Experiments Progress to Date

- 3 sessions of the swab-to-sequencer run
- RNA and cDNA sequencing
- RNA sample prep and sequencing

BEST: Swab-to-Sequencer on Station

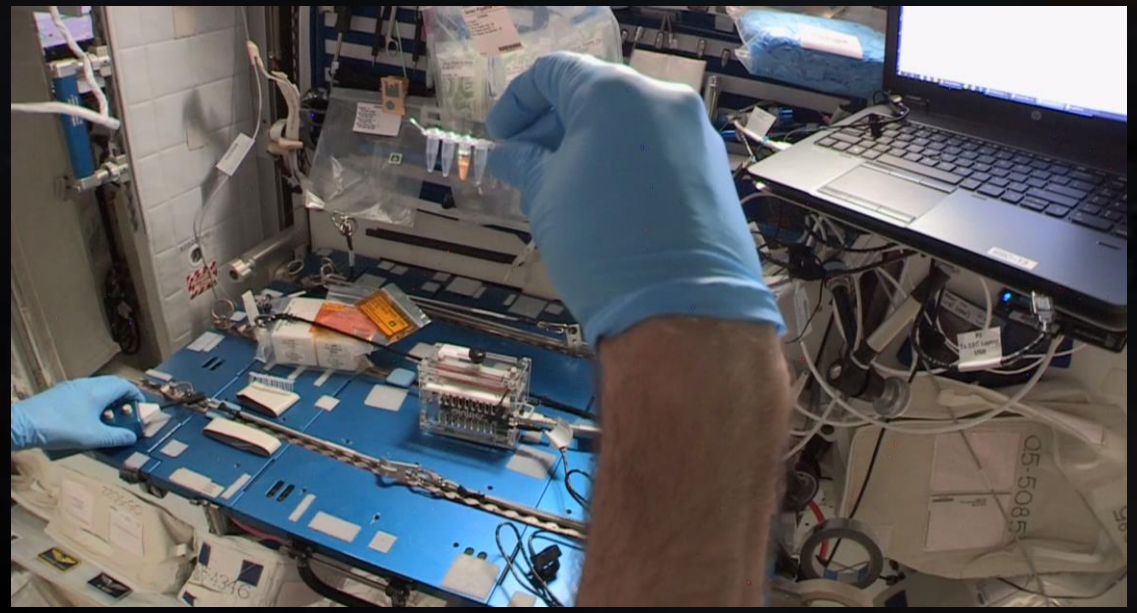
Non-culture based-microbial identification method

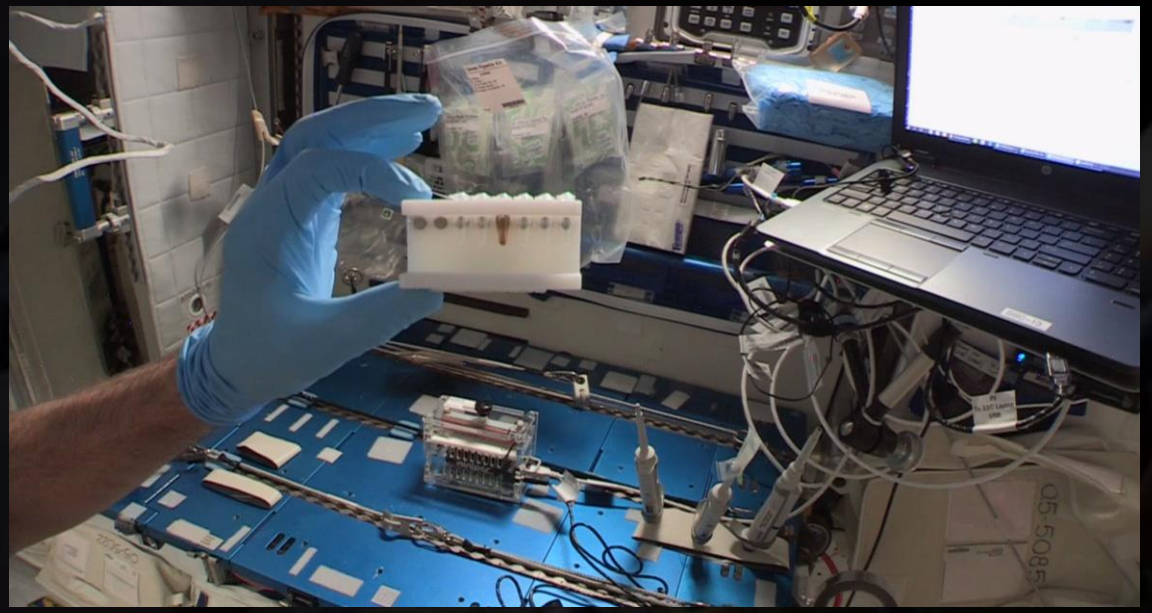
Swab

Extract

July 19, 2018

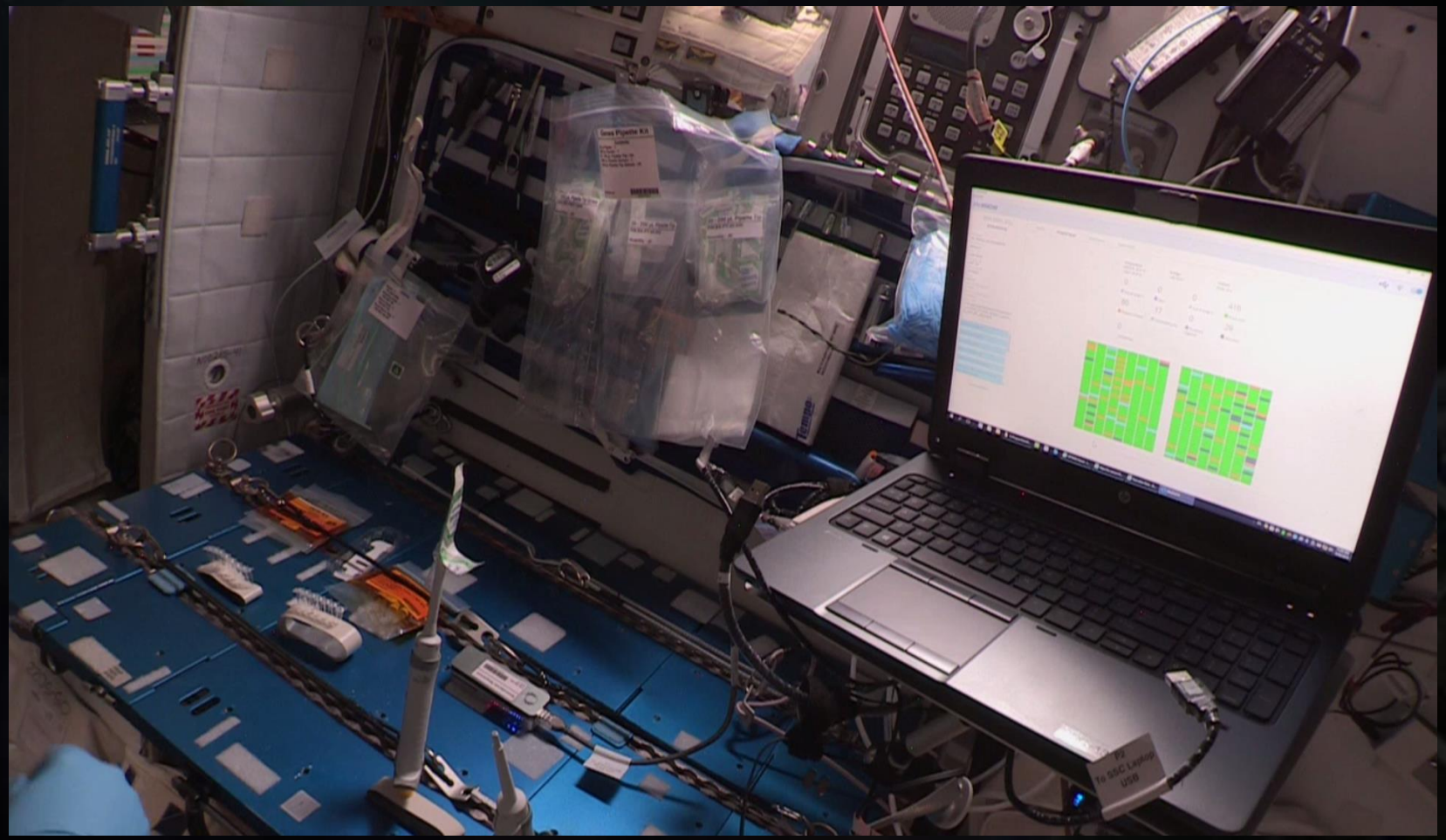
BEST: Swab-to-Sequencer on Station





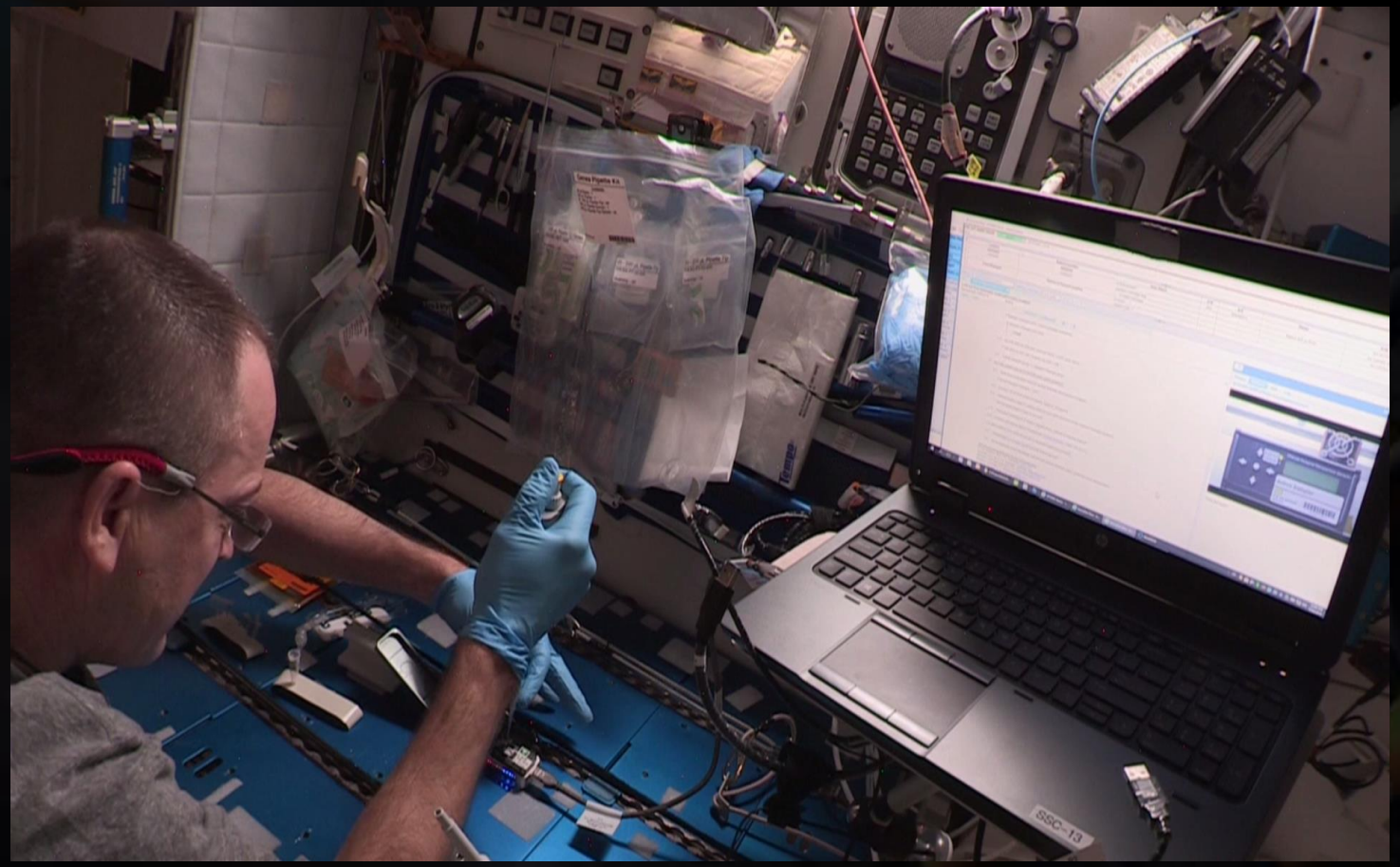
A magnetic stand and magnetic beads were used to purify and concentrate DNA.

BEST: Swab-to-Sequencer on Station



Preparing libraries for sequencing

BEST: Swab-to-Sequencer on Station

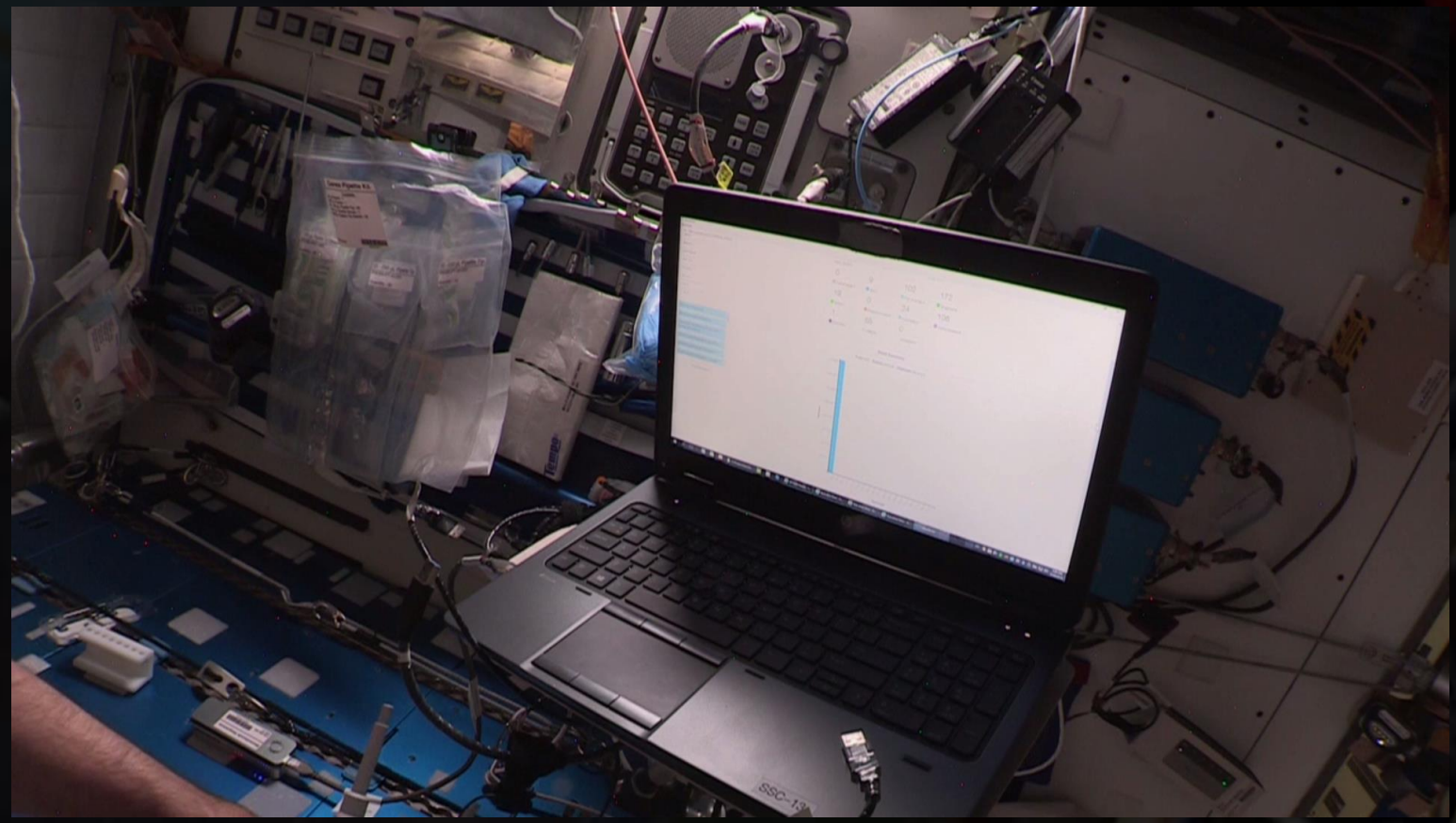


Loading the flow cell of the Biomolecule Sequencer.

July 20, 2018

BEST: Swab-to-Sequencer on Station

- Ricky provided confirmation of success, as ~4,000 reads were sequenced within the first few minutes of the run
- A peak corresponding to the length of the 16S amplicon was immediately apparent
- Data was downlinked days to the ground days later.

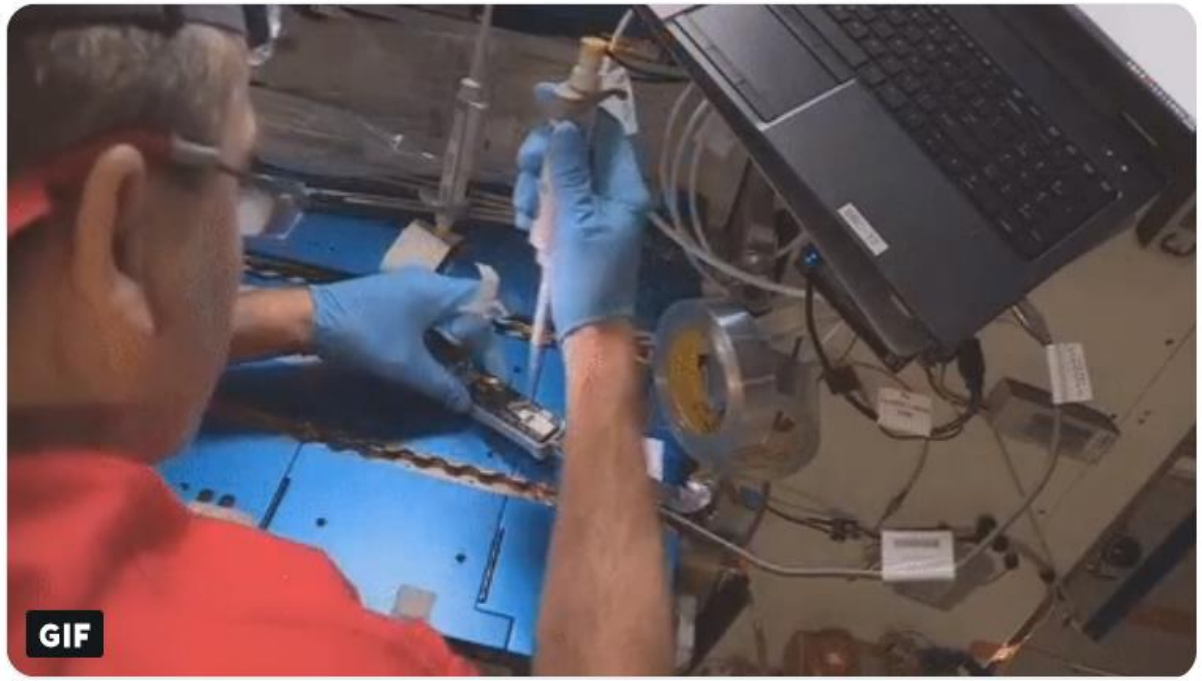


BEST: First Ever RNA Sequencing in Space!

Demonstrating the direct sequencing of RNA (sample prepped on Earth) and demonstrating the preparation of RNA for direct sequencing on station.

ISS Research @ISS_Research · Aug 29

Today, @Astro_Ricky became the first person to sequence RNA in space aboard the @Space_Station! "There is so much to be gained from that real-time snapshot of gene expression." -Dr. Sarah Wallace go.nasa.gov/2tNntKu



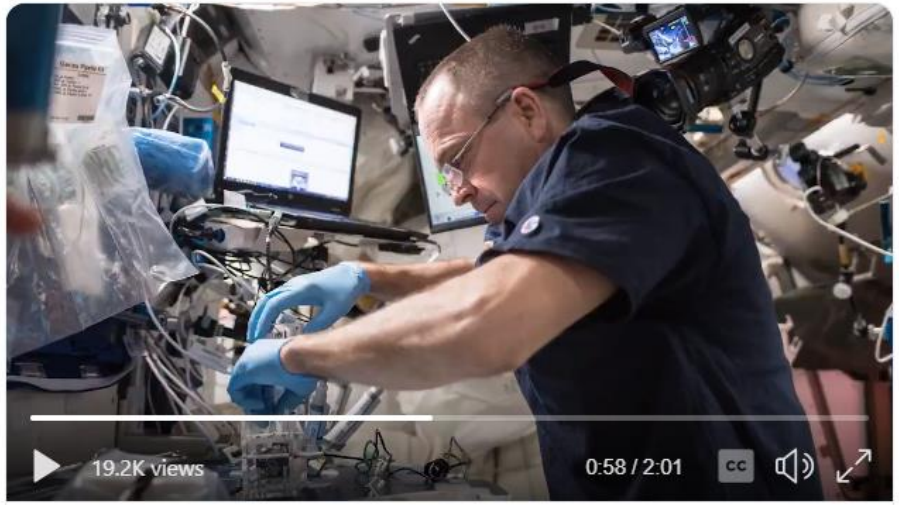
29 666 2.3K

ISS National Lab @ISS_CASIS

Following

An important milestone was achieved on @Space_Station this week -- @Astro_Ricky successfully sequenced RNA in space! Watch latest episode of #SpaceToGround for more details.

Intl. Space Station

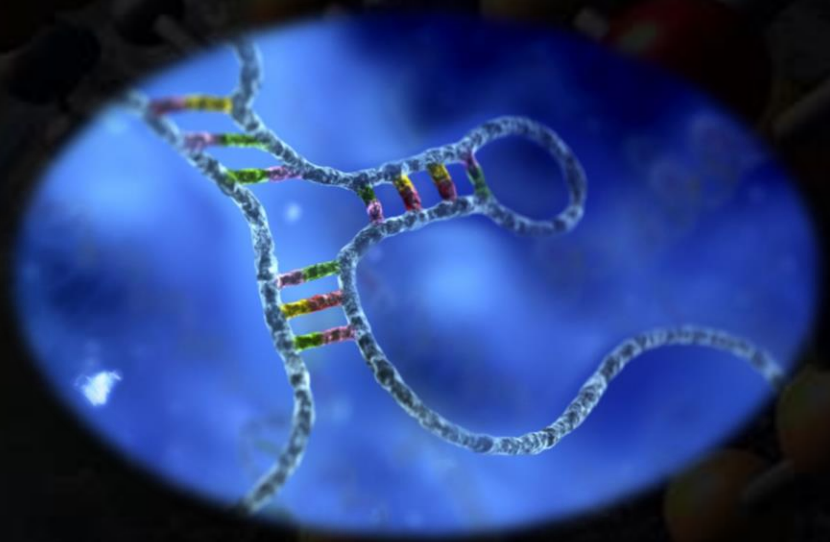


Space to Ground: Potential Game Changer: 08/31/2018

The BEST experiment is a potential game changer studying how life adapts in space . Meanwhile, pressure is holding steady on the station after the crew patched up a small hole on a Soyuz spaceship.

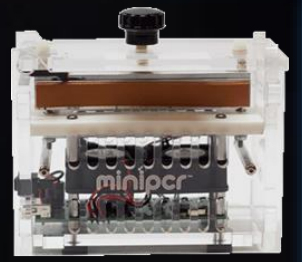
BEST: What's Next?

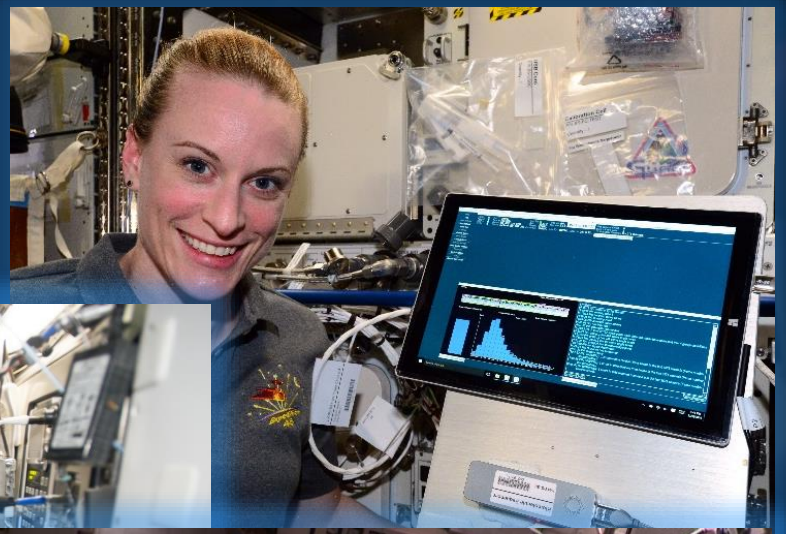
Assessing microbial mutation rates in space through long-term culture and periodic whole genome sequencing on station. Genetic and epigenetic changes will be compared against parallel cultures on the ground.

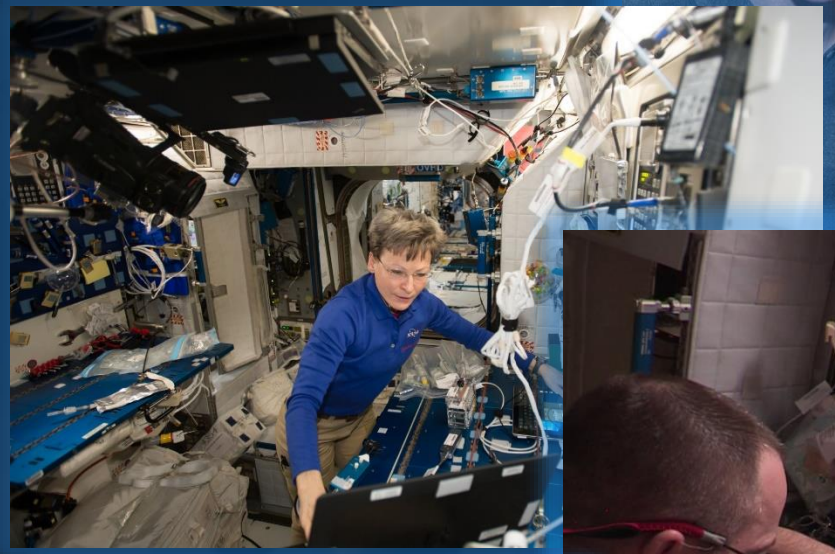


Conclusions

- We have a molecular lab on station!
  - COTS technology has transformed the science that can be accomplished on station
  - Microbes identified in space for the first time!
- Astronauts are amazing molecular biologists
  - Through the optimization of techniques and methods, basic laboratory protocols can be rapidly (and inexpensively) transitioned to ISS
    - Alteration of reagents to Tox 0 solutions allow for open cabin operations
    - Creative use of common laboratory tools alleviates the need for engineering to develop spaceflight-compatible hardware
- If put to the correct use, significant and impactful science can be accomplished during the remaining lifetime of ISS
  - More research to come!







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