Beyond DNA Sequencing in Space: Current and Future Omics Capabilities of the Biomolecule Sequencer Payload

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April 11, 2017
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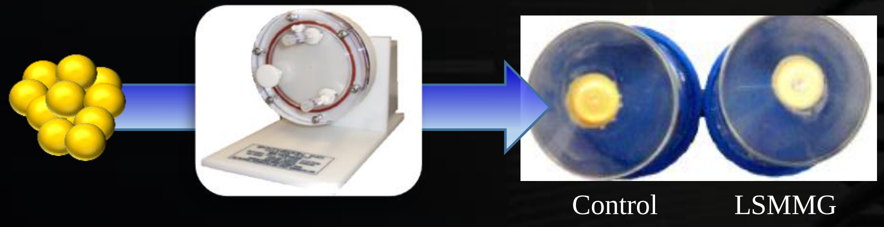
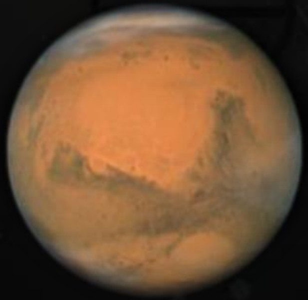
Why a DNA Sequencer in Space?

Why do we need a DNA sequencer 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
  - Plant
- 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 extraplanetary exploration mission



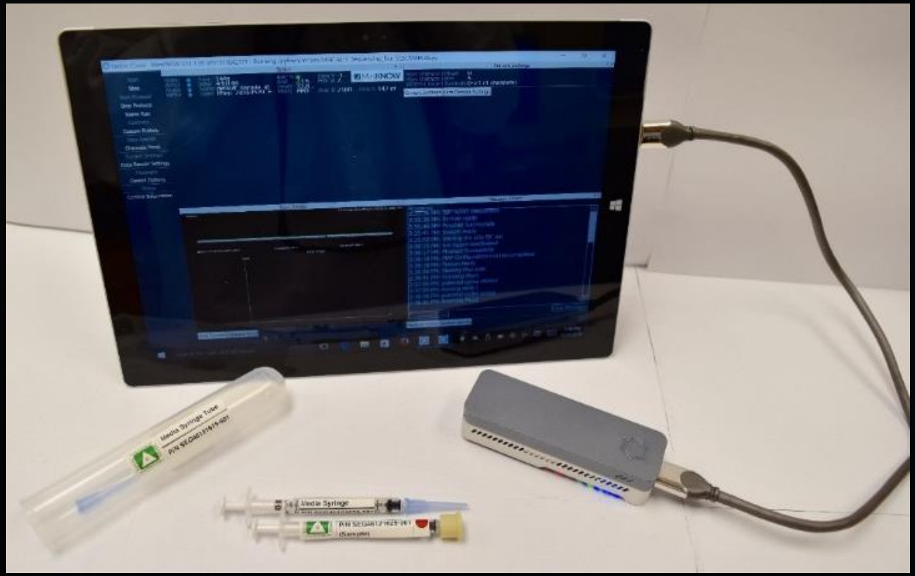


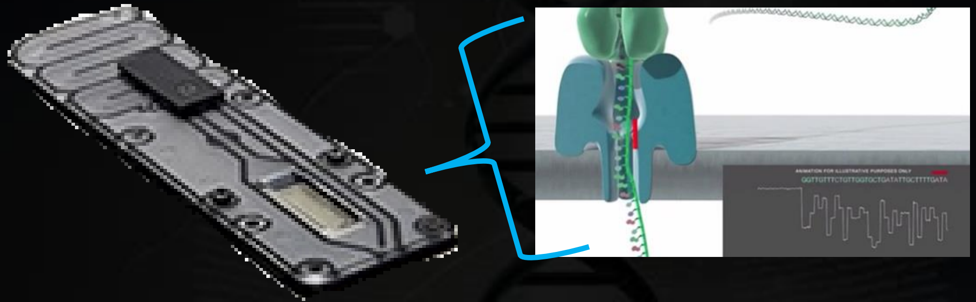
Biomolecule Sequencer Payload

- Would be 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
- Manifested to SpaceX-9
    - Launched July 17th, 2016

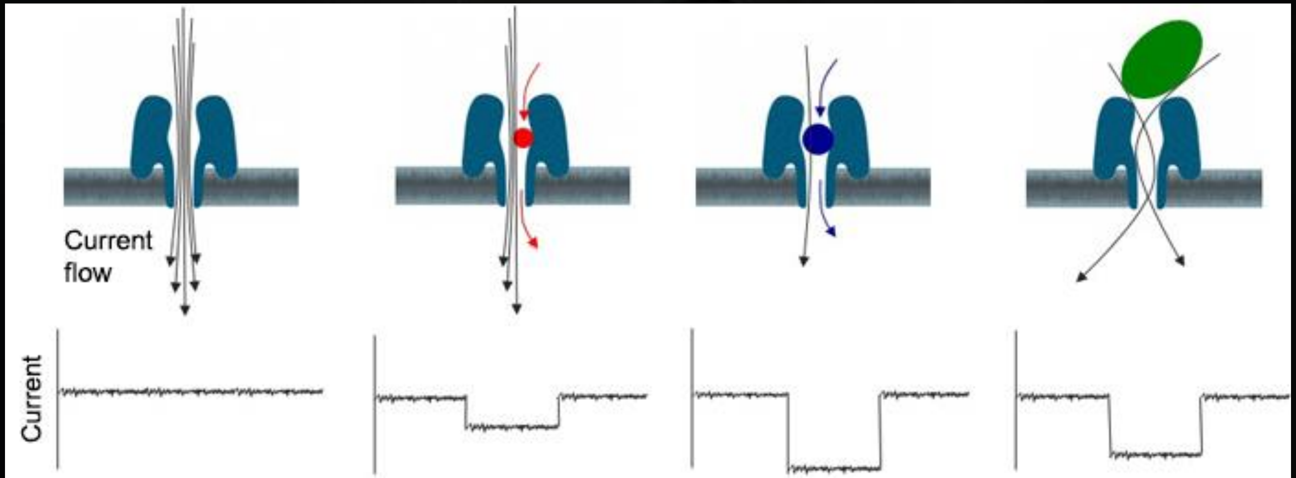
MinION™ by Oxford Nanopore Technologies

Biomolecule Sequencer: the Hardware





Flow Cell: Contains the nanopore sensing technology that is required to perform the sequencing reaction



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.

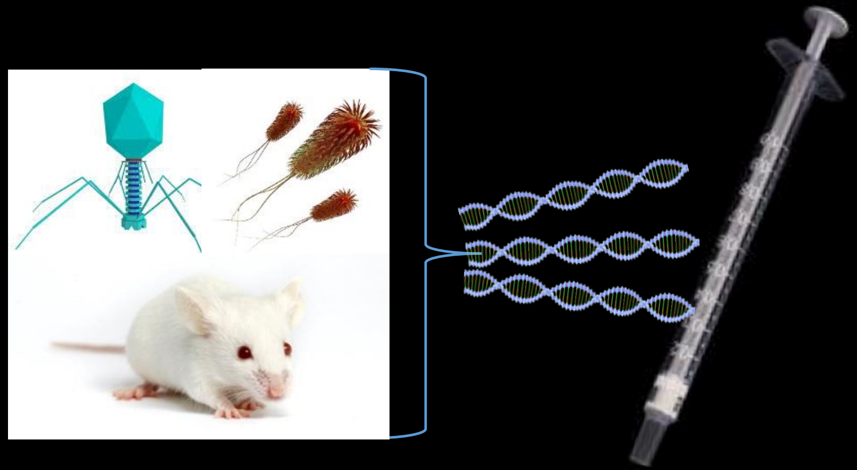
Biomolecule Sequencer: the Experiment

Goals:

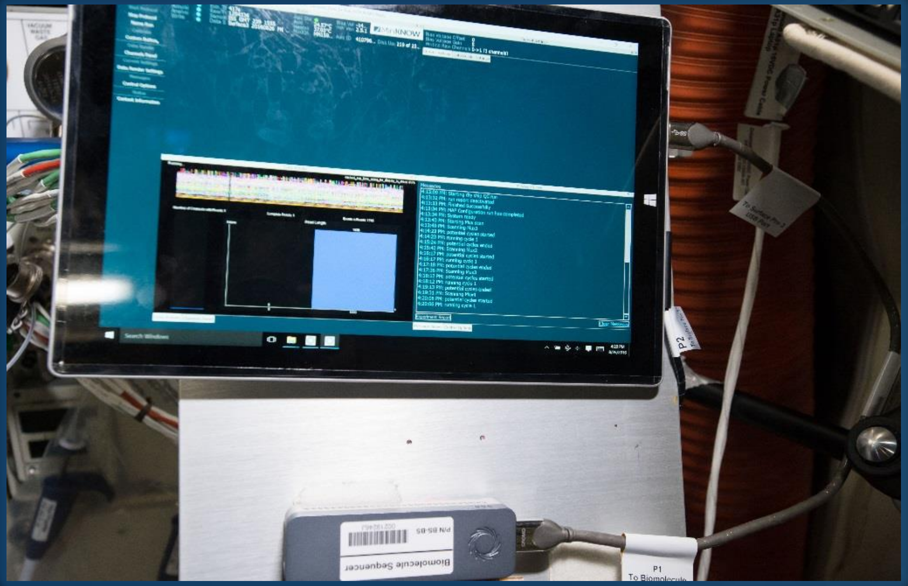
- Test the basic functionality by comparing ISS sequencing results of pre-determined samples to ground results
- Evaluate crew operability and potential for degrees of autonomy

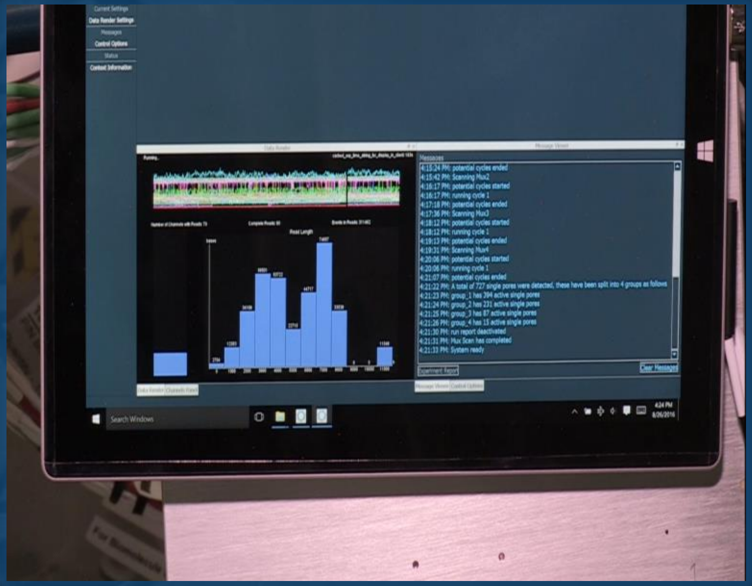
Experiment:

- Sequence a ground-prepared sample containing a mixture of genomic DNA from:
    - Bacteriophage lambda
    - *Escherichia coli*
    - Mouse – BALB/C (female)



Biomolecule Sequencer: the Data

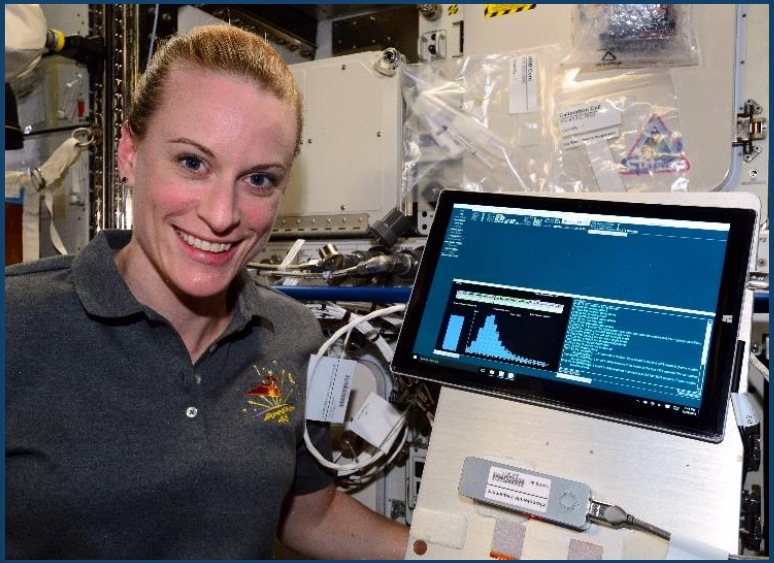




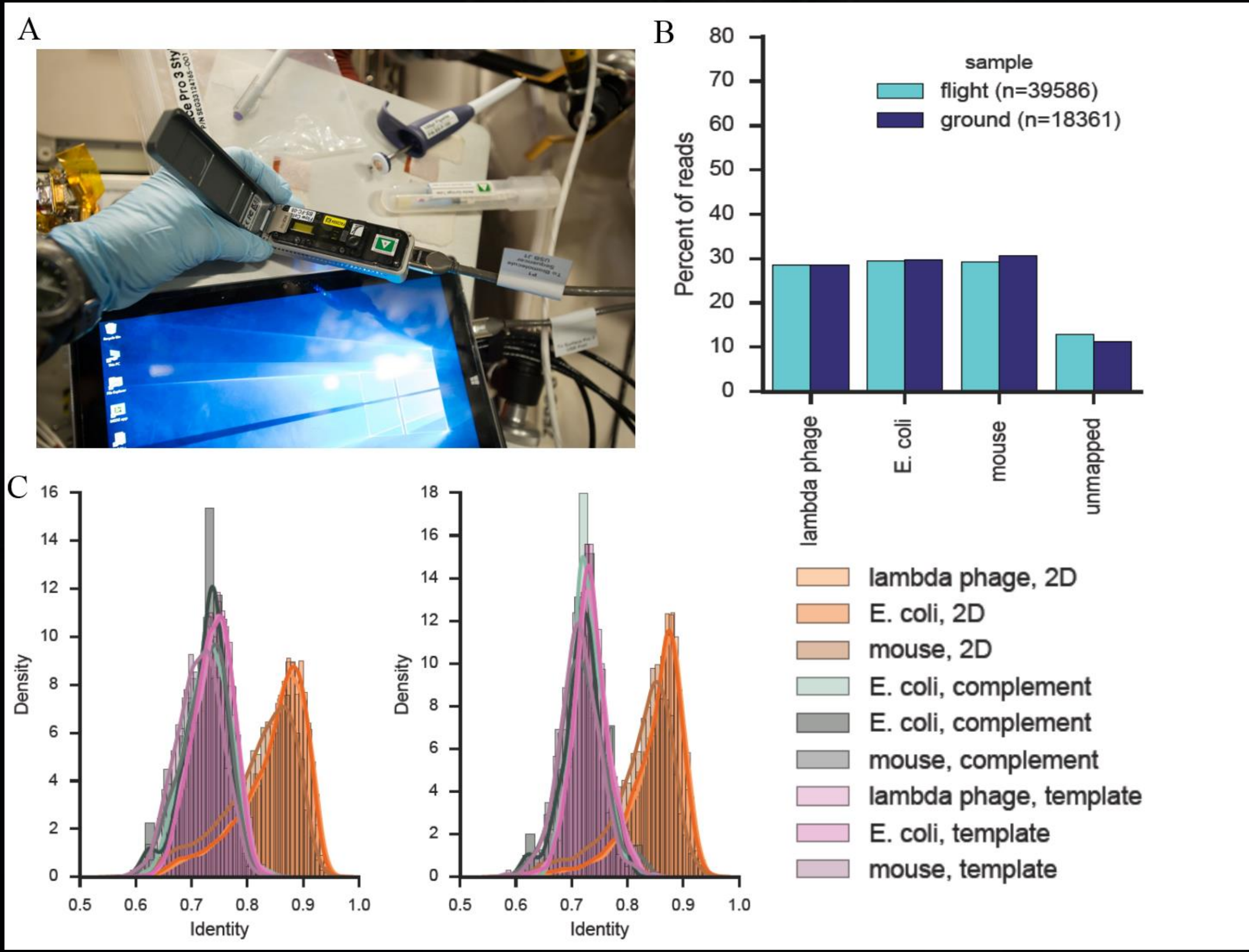
August 26th, 2016

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

Biomolecule Sequencer: the Data



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

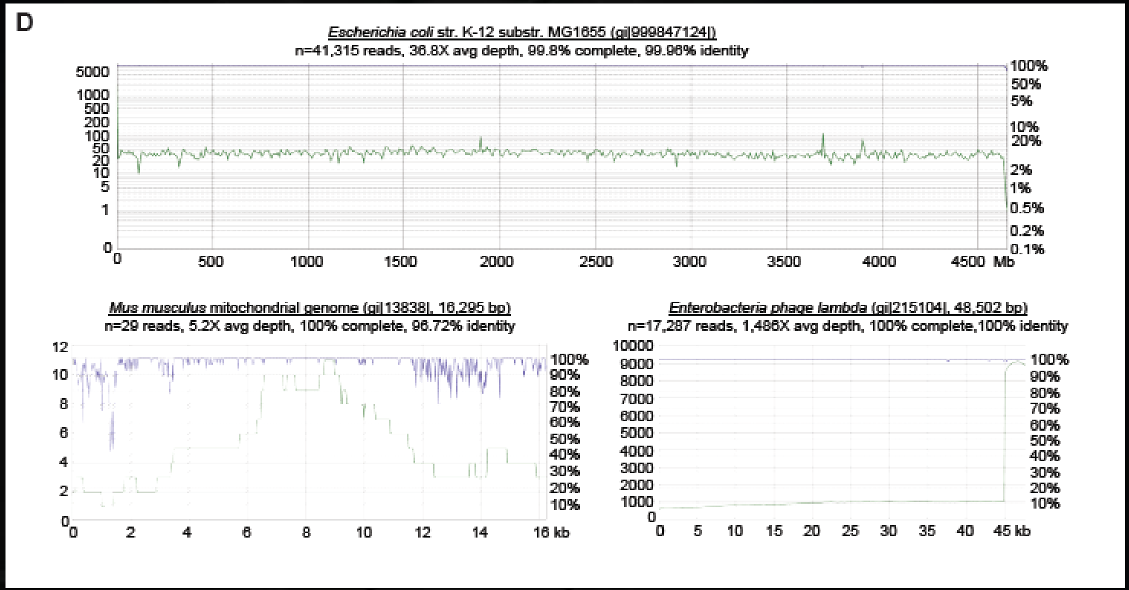


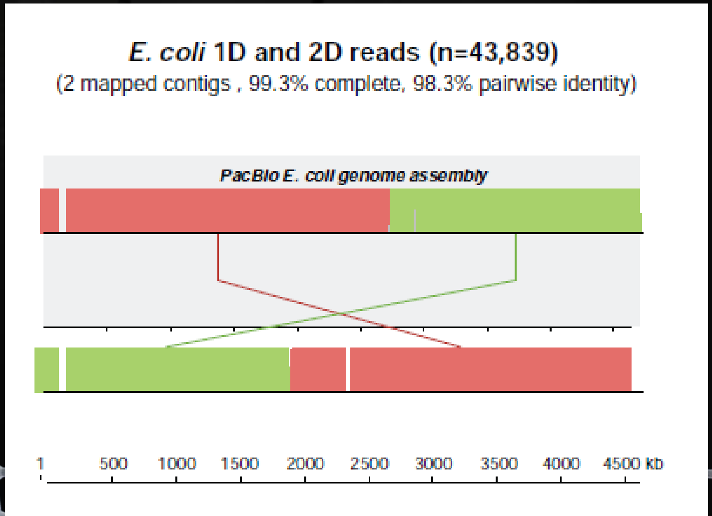
Castro-Wallace et al. http://biorxiv.org/content/early/2016/09/27/077651

Biomolecule Sequencer: Current Omics Capabilities

The “dawn of genomics” in space (Kate Rubins)

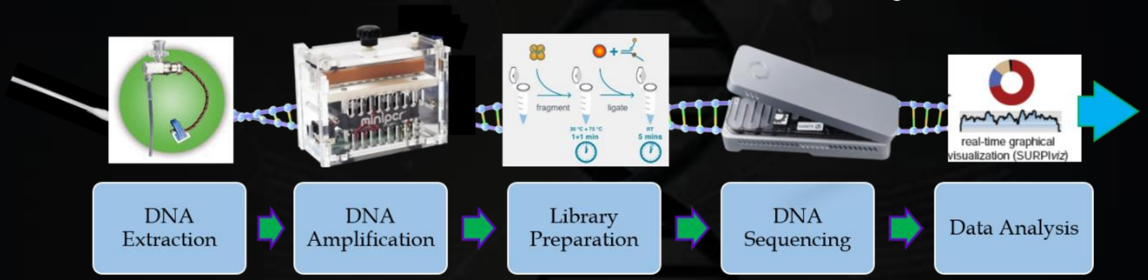
- No decrease in sequencing performance
- Over 284,000 reads were generated on the ISS
- Directed genome assemblies of:
    - Bacteriophage lambda
    - *E. coli*
    - Mouse mitochondrial
- de novo genome assemblies of:
    - Bacteriophage lambda
    - *E. coli*
- Demonstrated flow cell reuse and shelf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



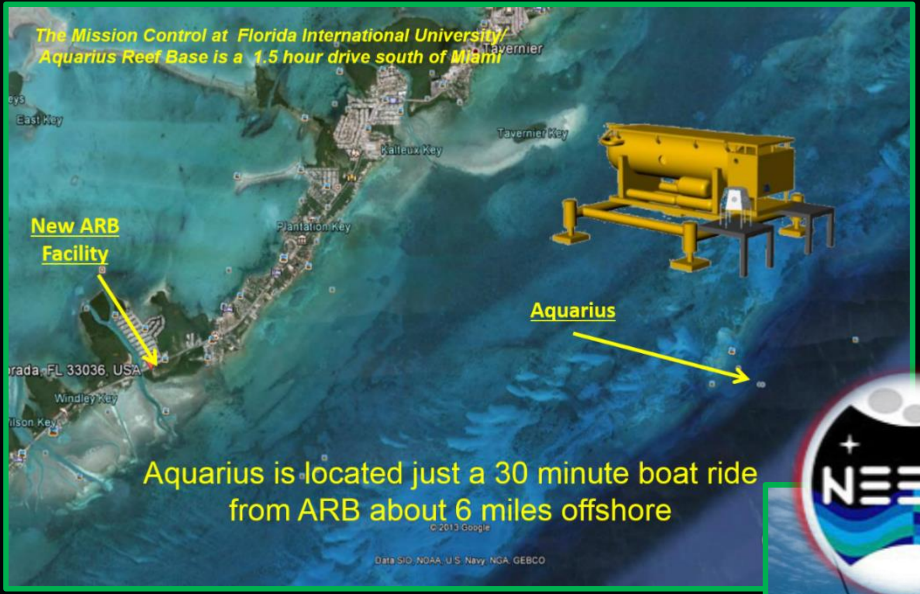


Meanwhile on the Ocean Floor…

Swab-to-Sequencer: July 2016



A full sample-to-sequencer process on the ocean floor and the analog testing of the joint operations of miniPCR and the MinION



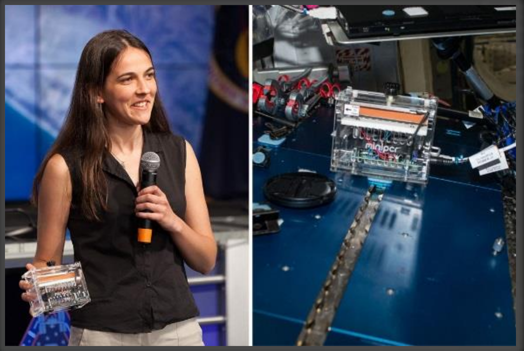
NASA Extreme Environments Mission Operations (NEEMO)

Meanwhile on the Ocean Floor…

The Molecular Space Age

Genes in Space Program

- "We invite students in grades 7 through 12 to design DNA experiments that address challenges in space exploration"
- The winner gets:
  - mentorship from Zeke and Sebastian (Ph.D.s from MIT and Harvard)
  - miniPCR DNA discovery system for their school
  - their experiment conducted on the ISS



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 will have his experiment launched to the ISS in March 2017 on OA-7

Genes in Space moves beyond the US with GiS-4 UAE winner Alia

GENES IN SPACE

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

Scott Copeland, Boeing

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

Just as we are using the ISS as a research platform and test bed for exploration, the Genes in Space Program is using it to inspire and engage the next generation of researchers and explorers.

https://www.genesinspace.org/

Genes in Space-3

- Will build upon the NEEMO 21 demonstration of the joint operations between miniPCR and the MinION, as it will transition 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
- Will culminate in the sequencing of unknown environmental samples from the ISS
- Genes in Space-3 is headed to the ISS on Orbital-7

The successful implementation of this process will result in, for the first time, the ability to identify contaminating microbes in-flight. However, this means so much more than microbiology…

Current Capabilities

We are working to develop a spaceflight-certified catalogue of general laboratory consumables and molecular reagents to be used by ISS researchers.

Reagents

All Tox 0!

- PCR Master Mix (NEB)
- Exonuclease (NEB)
- Fragmentation Mix (ONT)
- Rapid Adaptor Mix (ONT)
- Running Buffer (ONT)

Consumables

- 1.5 ml LoBind Tubes
- 0.2 ml PCR Tube Strips
- Rainin Positive Displacement Pipettes: 10 ml, 100 ml, and 1000 ml and associated tips
- Eppendorf Pipettes: 20 ml, 200 ml, and 1000 ml and associated tips
- MinION Flow Cells

Crew Procedure Techniques

- Pipetting between numerous vessels
- Running a PCR
- Using miniPCR as a heat block
- Running the MinION

All of the operations products have been developed and are available. Reaction conditions for miniPCR and the MinION are easily customizable to any experiment. Different enzymes and reactions can be tested with certified consumables and substituted into procedures.

Future Capabilities: What can the MinION do for your research?

- Full genome assessments of model organisms
  - What is the genomic impact of radiation?
- Complete transcriptomic investigations
  - How is gene expression altered as a result of mission duration?
  - Biomarker tracking through changes in gene expression
    - Bone and muscle
    - Cardiovascular
    - Wound healing
    - CO_2 exposure
- Direct RNA Sequencing
  - How are active transcriptional processes impacted as a result of spaceflight culture?
- Epigenetic Studies
  - How are methylation patterns affected by the spaceflight environment?


Benchmarking of the Oxford Nanopore MinION sequencing for quantitative and qualitative assessment of cDNA populations
Detecting DNA Methylation using the Oxford Nanopore Technologies MinION sequencer
Highly parallel direct RNA sequencing on an array of nanopores
Nanopore sequencing detects structural variants in cancer
Whole genome sequencing and assembly of a Caenorhabditis elegans genome with complex genomic rearrangements using the MinION sequencing device

How would receiving data from your in-flight investigation impact your study?

Acknowledgments

The Biomolecule Sequencer and Genes in Space-3 Payload Development Team: Sarah Stahl, Aaron Burton, Ph.D., and Kristen John, Ph.D.

BSeq Flight Team
Dave Voss
Linda Gibson
Teresa Tan

BSeq Astronauts
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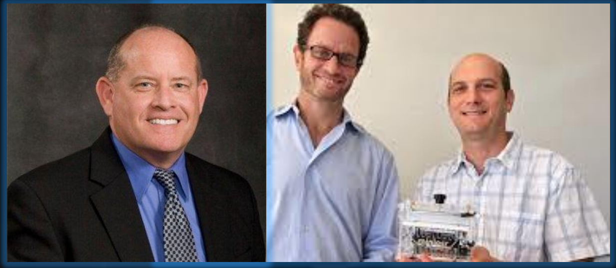
David Smith, Ph.D. – ARC
Kate Rubins, Ph.D. – JSC
Charles Chiu, Ph.D. – UCSF
Scot Federman
Sneha Somasekar
Doug Stryke
Guixia Yu
Chris Mason, Ph.D. – Weill Cornell Medicine
Noah Alexander
Alexa McIntyre

Oxford Nanopore Technologies
James Brayer
Sissel Juul, Ph.D.
Dan Turner, Ph.D.
David Stoddart, Ph.D.
Michael Micorescu, Ph.D.

New England BioLabs
Nicole Nichols, Ph.D.



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