

Nanopore sequencing and the search for life in the solar system

Aaron Burton

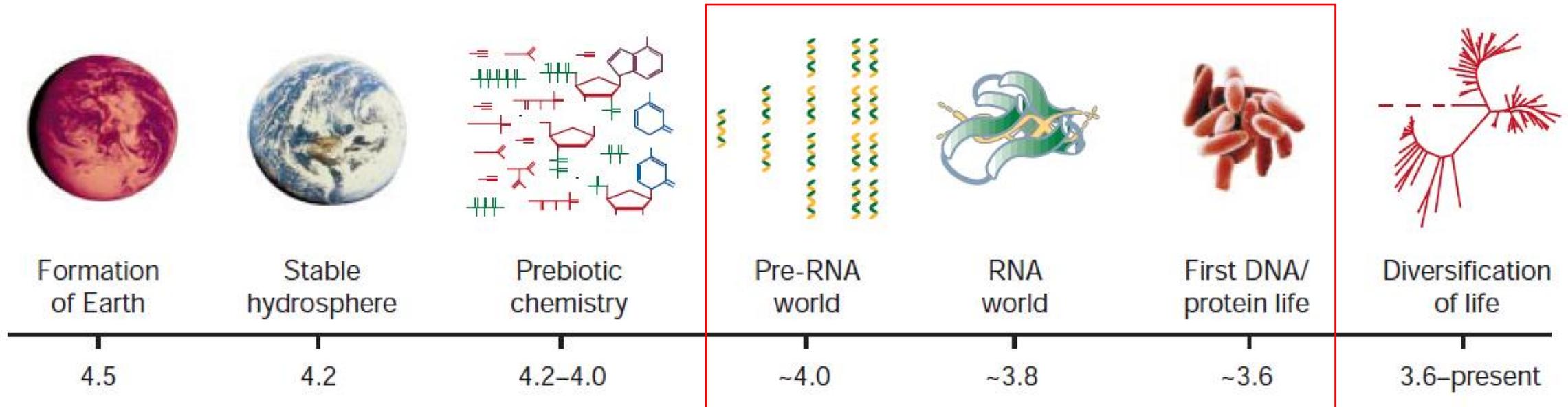
NASA Johnson Space Center

November 1, 2017

Overview

- Nanopore sequencing as a life detection tool
- Nanopore sequencing for human exploration
- Experiments on the international space station
- Next steps

Timeline for the origins of life on Earth

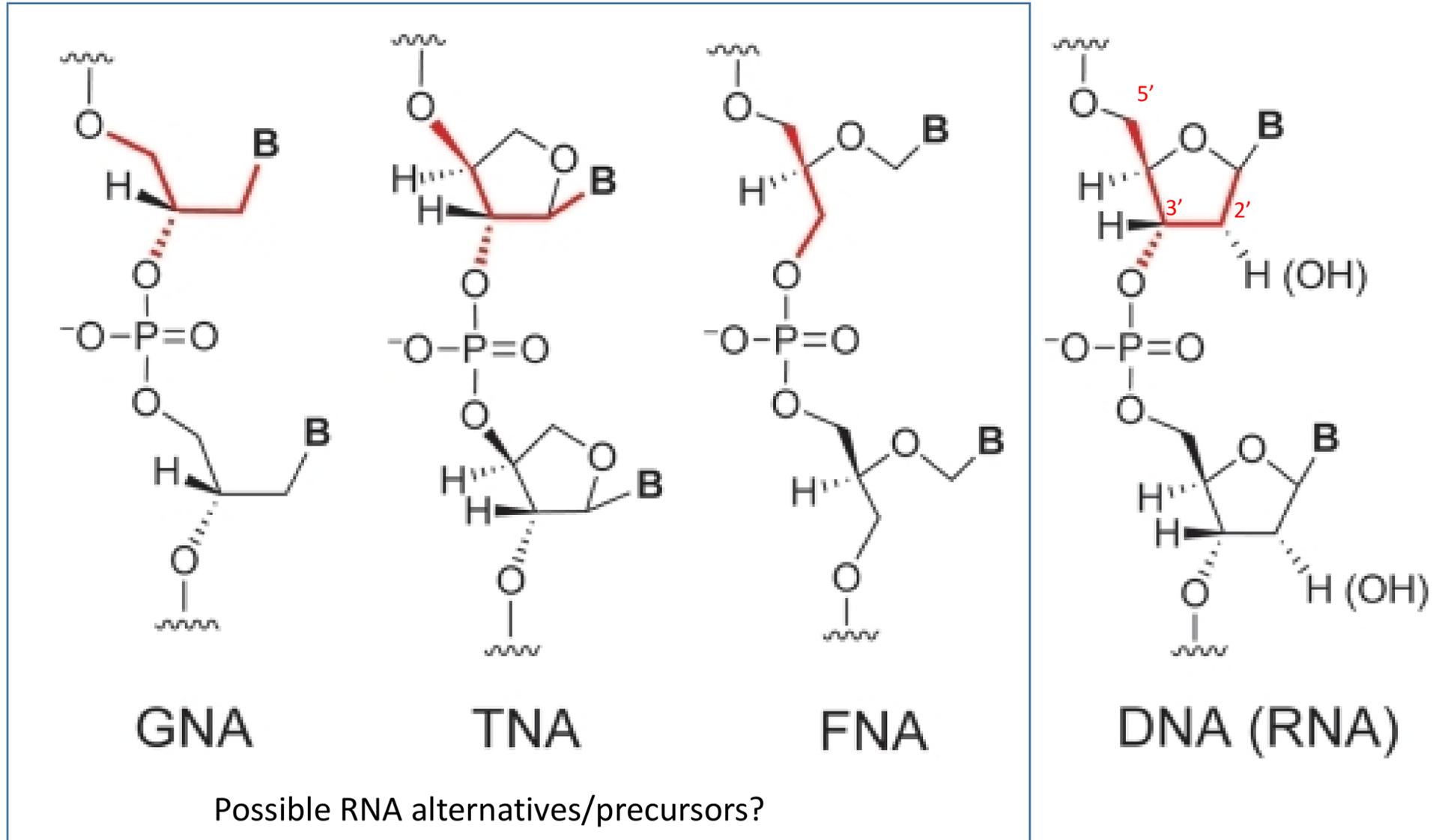


Joyce GF 2002. *Nature* 418, 214-221.

The RNA World Hypothesis (Gilbert 1986)

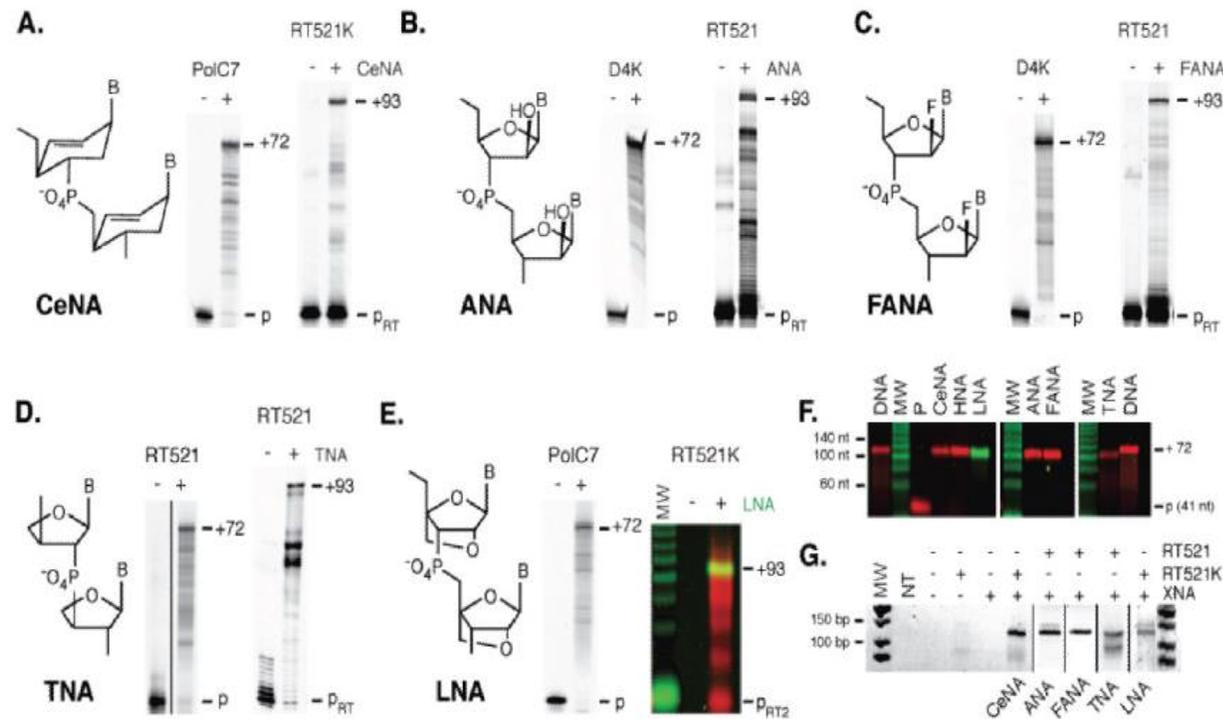
- RNA carries information in contemporary biology
 - Example: mRNA contains codes for proteins
- RNA performs catalytic functions in contemporary biology
 - Example: cis- and trans-splicing RNAs (Cech, Altman), catalytic center of the ribosome is RNA (Steitz)
- So RNA can do the job of both DNA and proteins, but not as well
- Conclusion: RNA preceded both DNA and proteins
 - DNA emerged as more stable informational macromolecule
 - proteins emerged as better catalysts
 - RNA maintained lesser informational and catalytic roles

But where did the RNA come from?



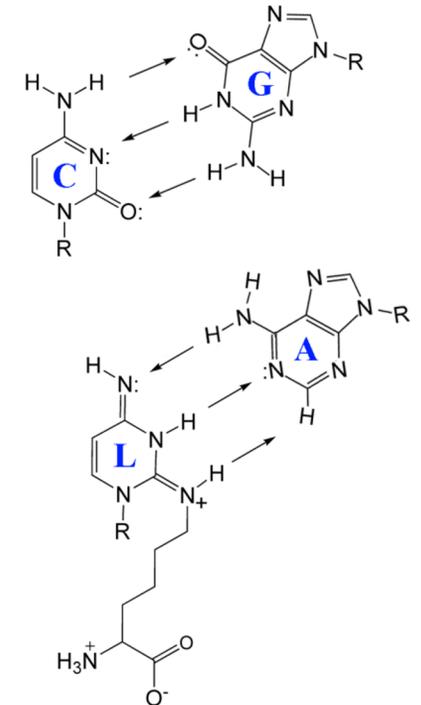
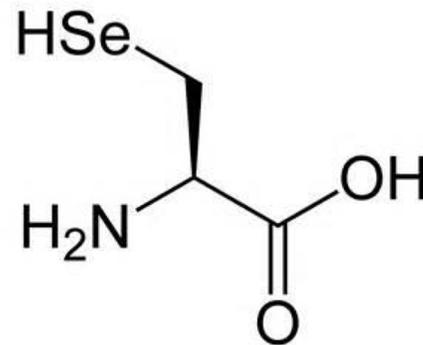
DNA and RNA are not the only game in town...

- Chaput + Holliger labs (2012) showed that threose nucleic acid (TNA), hexose nucleic acid (HNA) and other XNAs could carry information and be evolved to bind a ligand



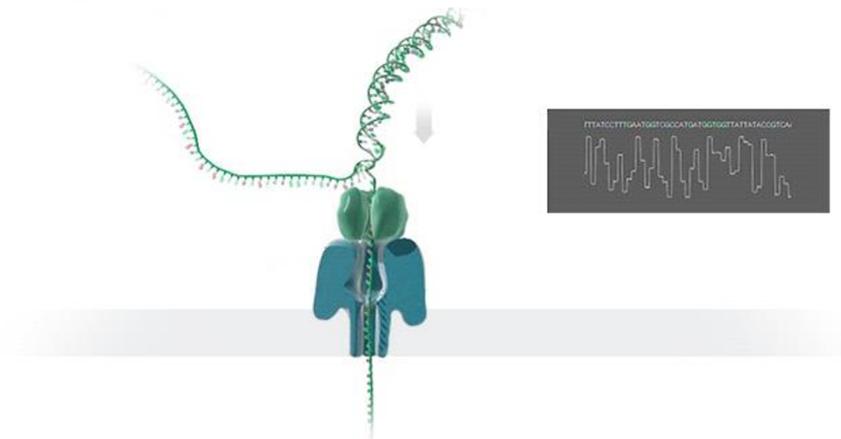
...even on Earth

- Methylation of DNA
- 2,6-diaminopurine in DNA of bacteriophage
- 112 base modifications used in RNA
- Proteins use 20 (or 22, or 25...) amino acids
- **Need capability to look beyond just standard DNA/RNA/proteins in the search for life**



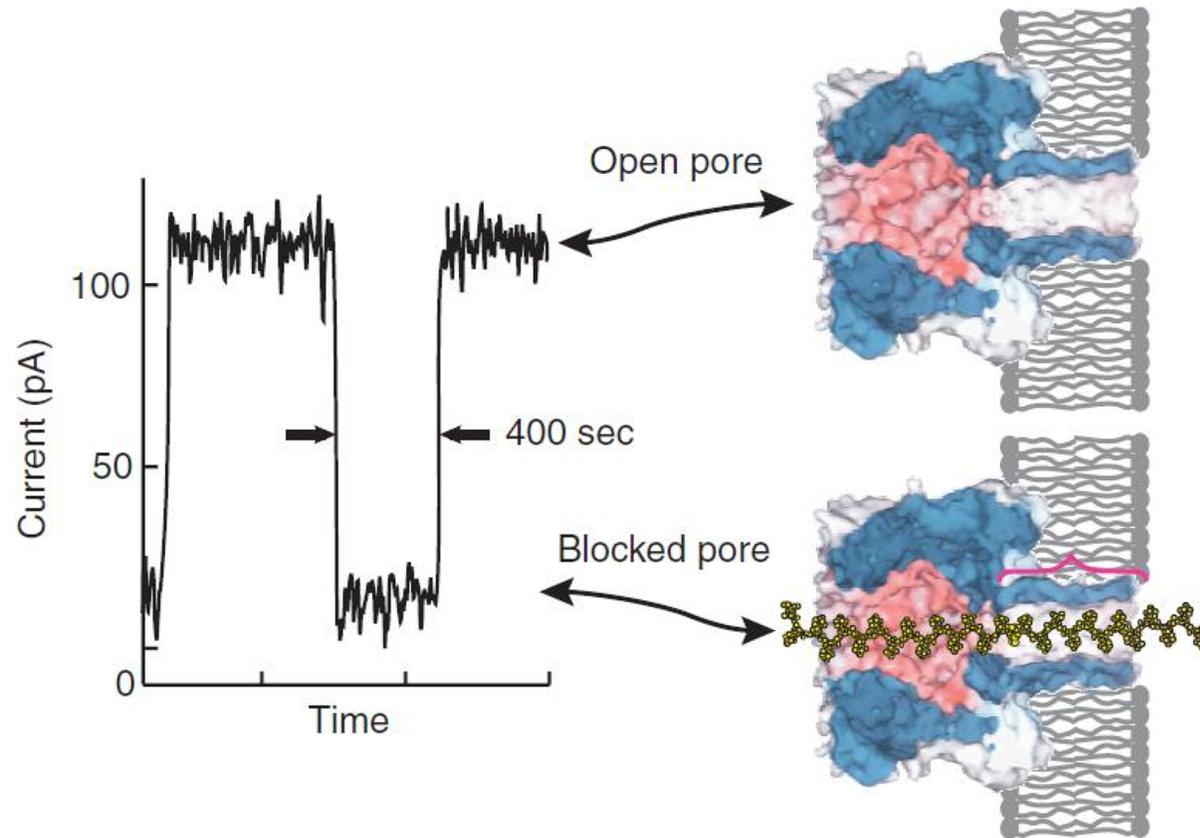
Why are nanopores suited for *in situ* Astrobiology?

- Can detect broad range of charged molecules
 - Many biomolecules are charged (nucleic acids, proteins, lipids, small molecules)
- Nanopore (electrochemical) sensors are inherently small
 - Instruments can be small without sacrificing performance
- Sample preparation is faster and simpler than other commercially available sequencing platforms



Versatility of Nanopore sensing

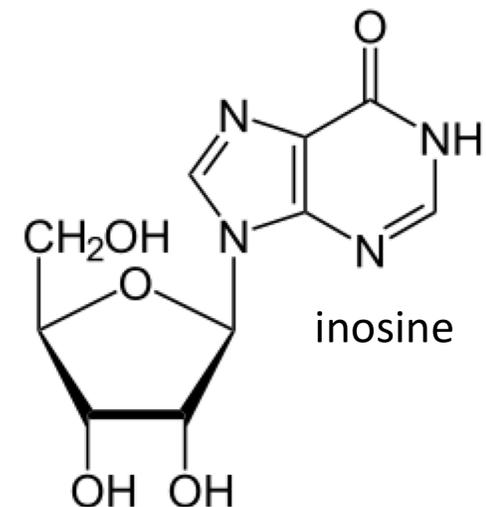
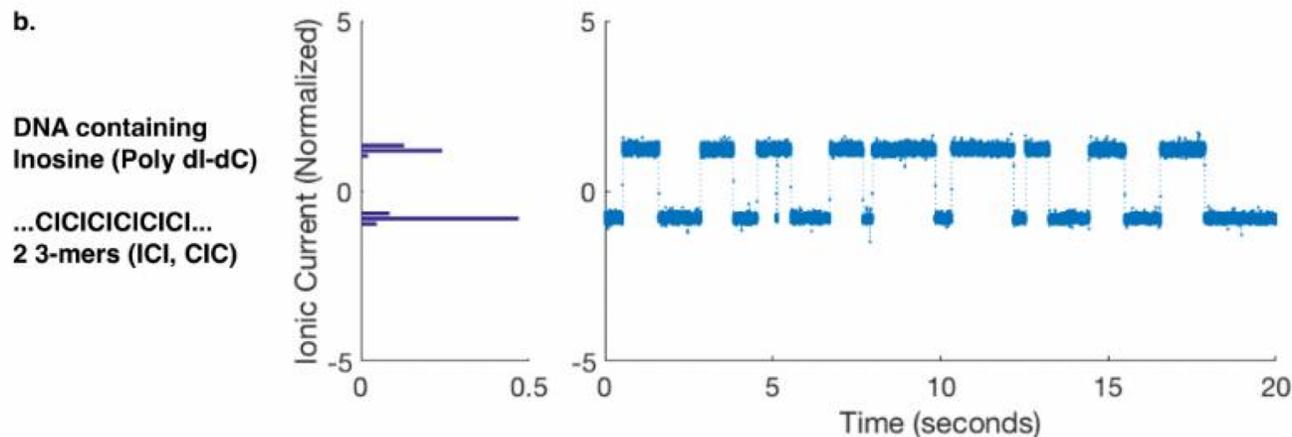
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- Possible to analyze any polymer that obstructs the pore
- Currently being applied to DNA and direct RNA sequencing, and protein analysis
- Need to modulate rate through pore and change in current depending on template

Nanopore analysis is not limited to canonical AGCT DNA

- The MinION has also been used for:
 - Direct RNA sequencing (Garalde et al. bioRxiv 2016)
 - Sequencing inosine-containing oligos (Carr lab)
 - Epigenetic sequencing with the detection of methylated bases (eg., Rand et al. 2017; Simpson et al. 2017)
 - Nanopores have been used to characterize proteins (e.g., Oukhaled et al. 2007)



Next steps for nanopore sequencing in the search for life

- Evaluate stability for long-duration missions
 - Mars is within reach with current technology
 - Europa or other ocean world would require ruggedization
- Expand analytical approaches to ensure a broad range of DNA/RNA/protein-like molecules could be analyzed
 - Funded efforts in COLDTech, other projects in development



Nanopore sequencing on the International Space Station

Slides courtesy of Sarah Wallace, Ph.D.
NASA Johnson Space Center

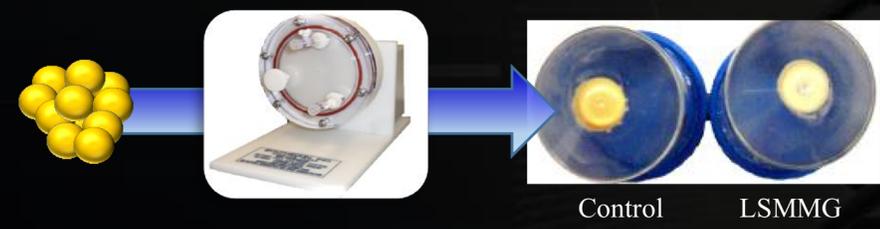


Why a DNA Sequencer in Space?



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 extra-planetary exploration mission





2016: The Molecular Space Age

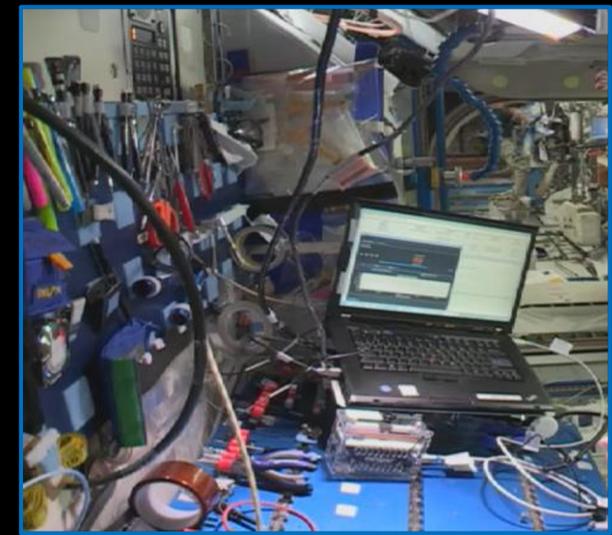


2
0
1
6

2
0
1
6



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



April 29: RNA isolation, reverse transcription, and DNA amplification data obtained on the ISS using the Wetlab-2 qPCR platform





2016: The Molecular Space Age



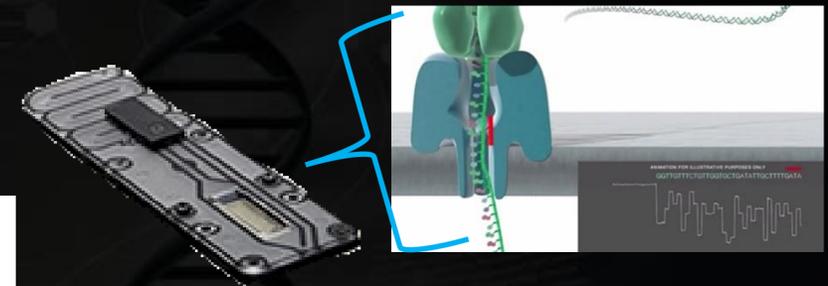
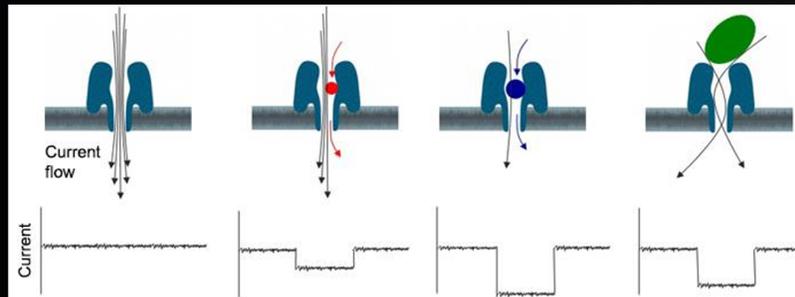
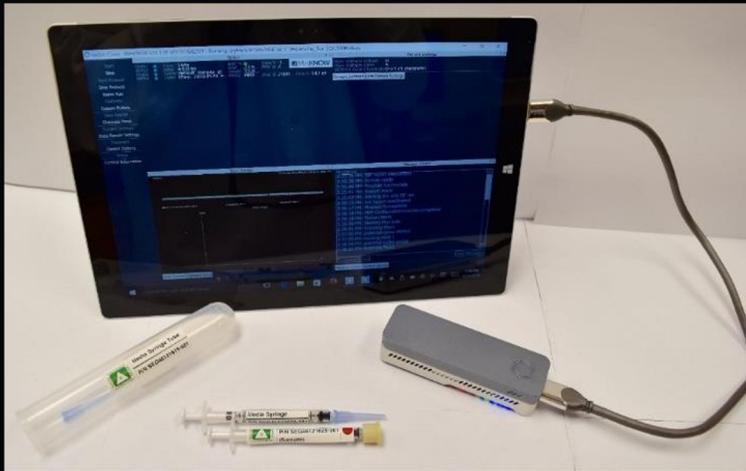
MinION by Oxford Nanopore Technologies

Biomolecule Sequencer Payload

- 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 ³/₄ x 1 ¹/₄ x 5/8 inches
- Less than 120 grams (with USB cable)
- Powered via USB connection
- Capable of DNA, RNA, and protein sequencing
- Launched July 18, 2016 (SpaceX-9)



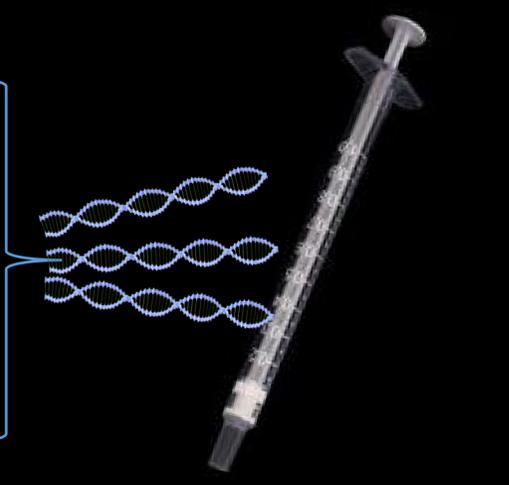
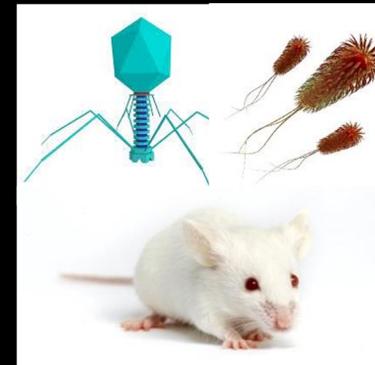
Biomolecule Sequencer: the Hardware



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

Experiment:

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





Biomolecule Sequencer: the Experiment



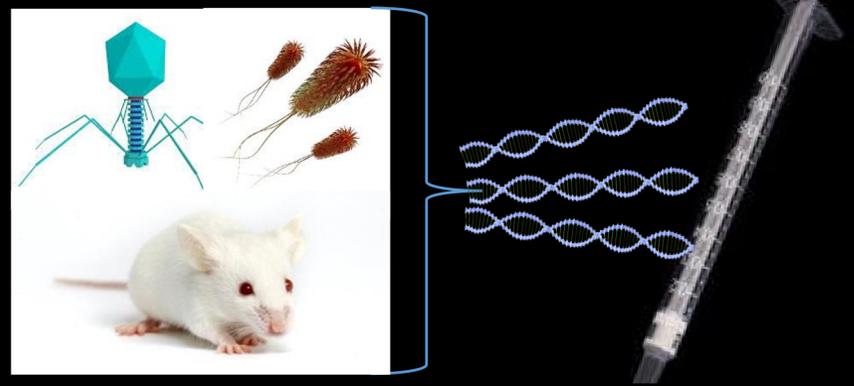
Goals:

- 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
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Biomolecule Sequencer: the Data

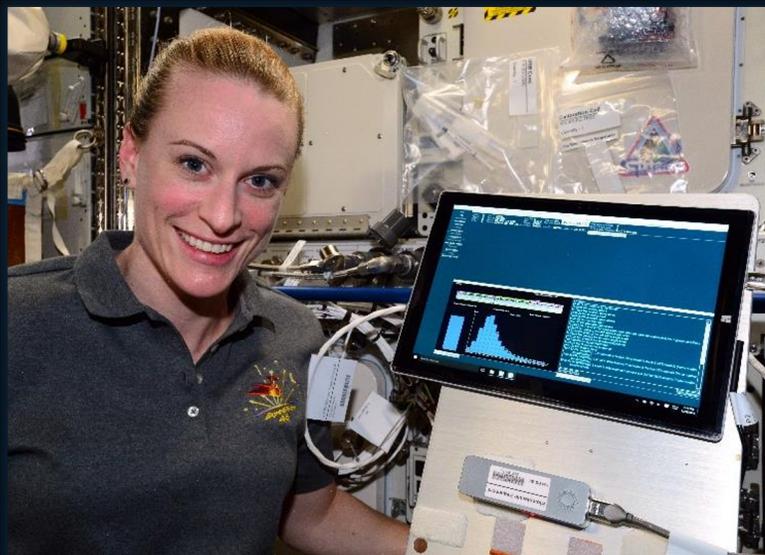


August 26th, 2016

“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

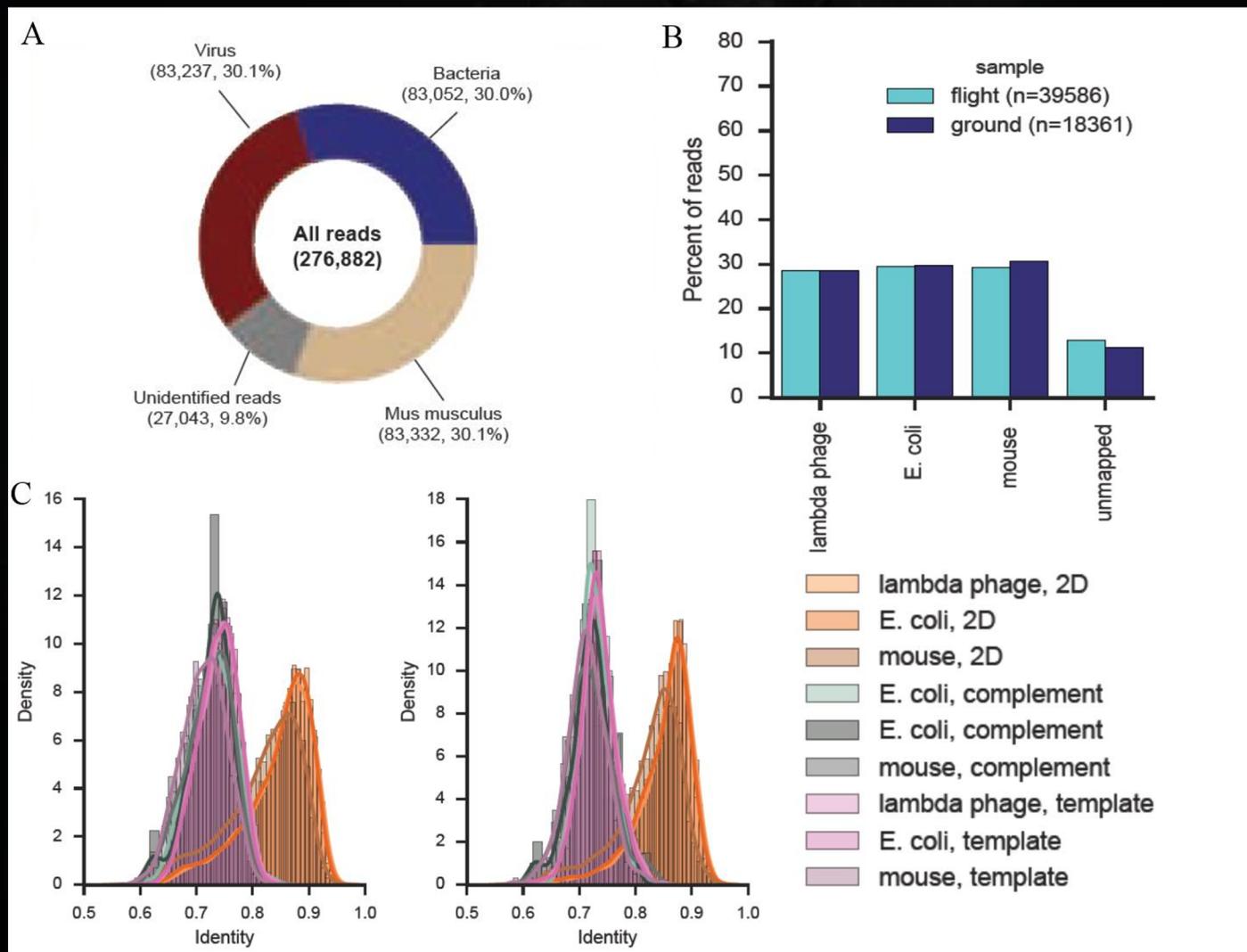
October 18, 2016

October 25, 2016

October 26, 2016

November 26, 2016

January 9, 2017



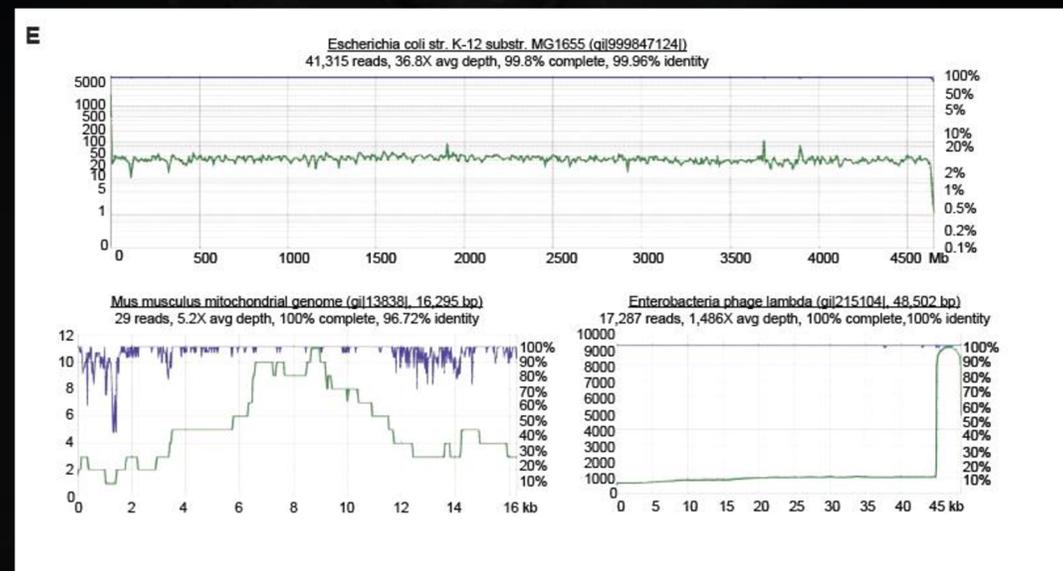


Biomolecule Sequencer: the Data

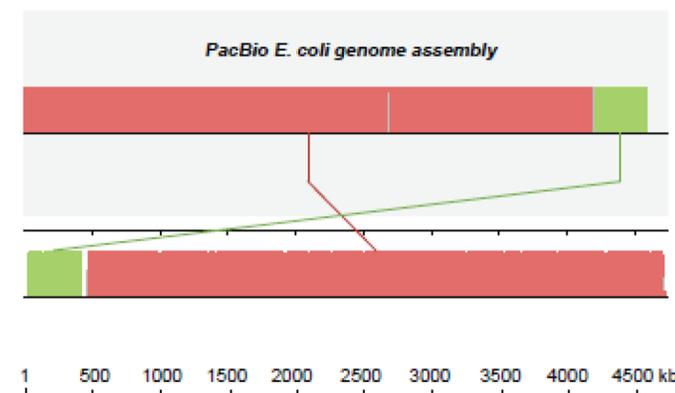


The “dawn of genomics” in space (Kate Rubins)

- No decrease in sequencing performance
- Over 284,000 reads were generated on the ISS
- Directed genome assemblies of:
 - Bacteriophage lambda
 - *E. coli*
 - Mouse mitochondrial 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



***E. coli* 2D reads (n=70,748)**
(1 mapped contig, 99.9% complete, 98.7% pairwise identity)

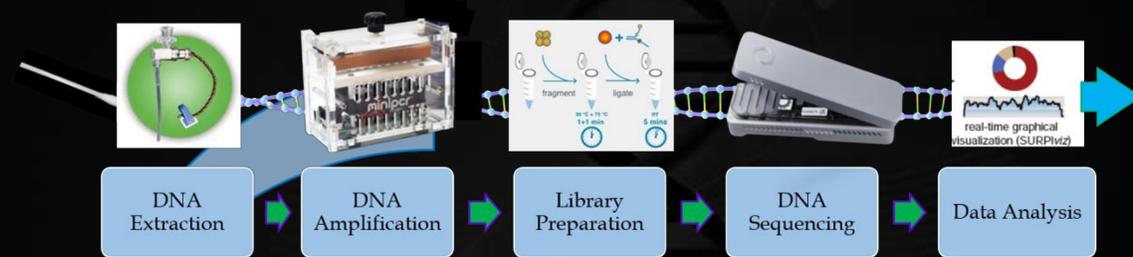




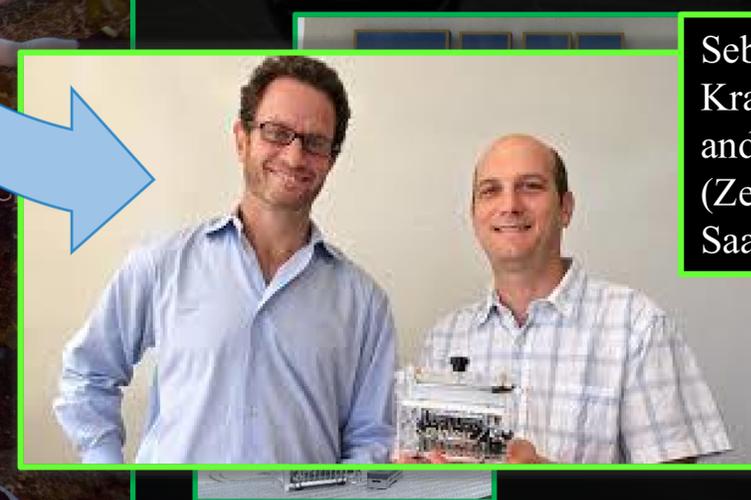
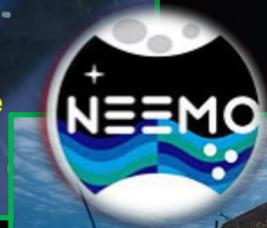
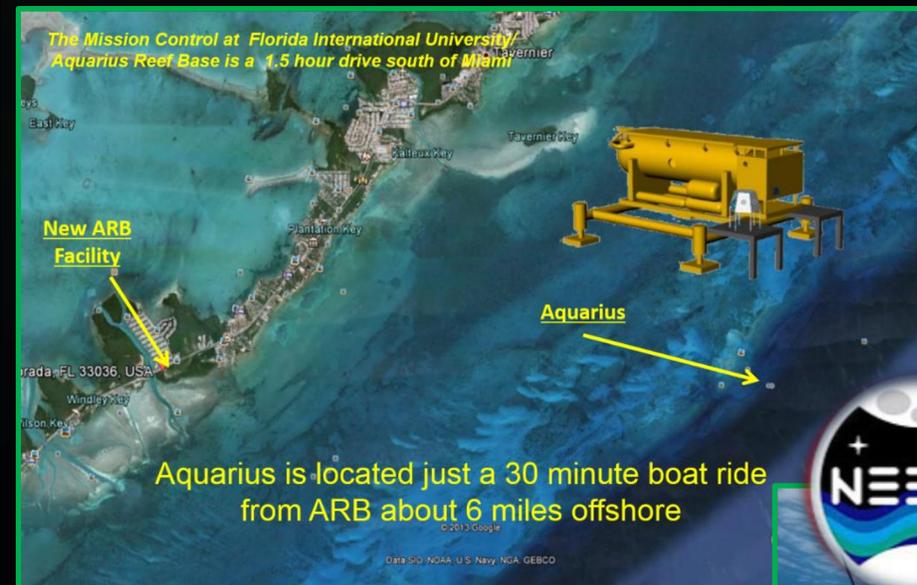
Meanwhile on the Ocean Floor...



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



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



NASA Extreme Environments Mission Operations (NEEMO)



Meanwhile on the Ocean Floor...



1. Environmental Swab Collection



2. DNA Extraction & Clean-Up



3. MiniPCR Amplification



4. Sample Preparation



5. Loading MinION Sequencer



6. Successful Sequencing

 **Reid Wiseman**  @astro_reid 

This look? It's when an [@USNavy](#) pilot sees confirmation from the [@nanopore](#) sequencer of successful [#DNA](#) extraction



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12:45 PM - 25 Jul 2016

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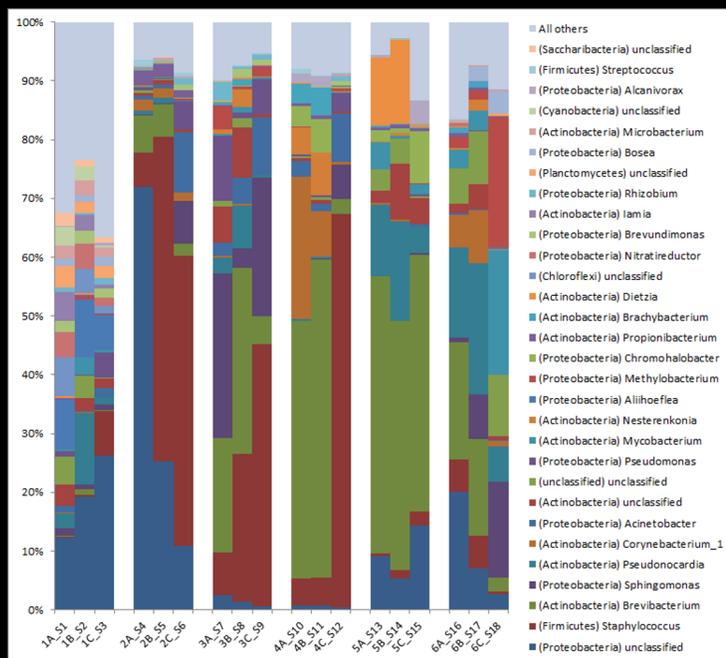


A Molecular Biology Lab on the Ocean Floor

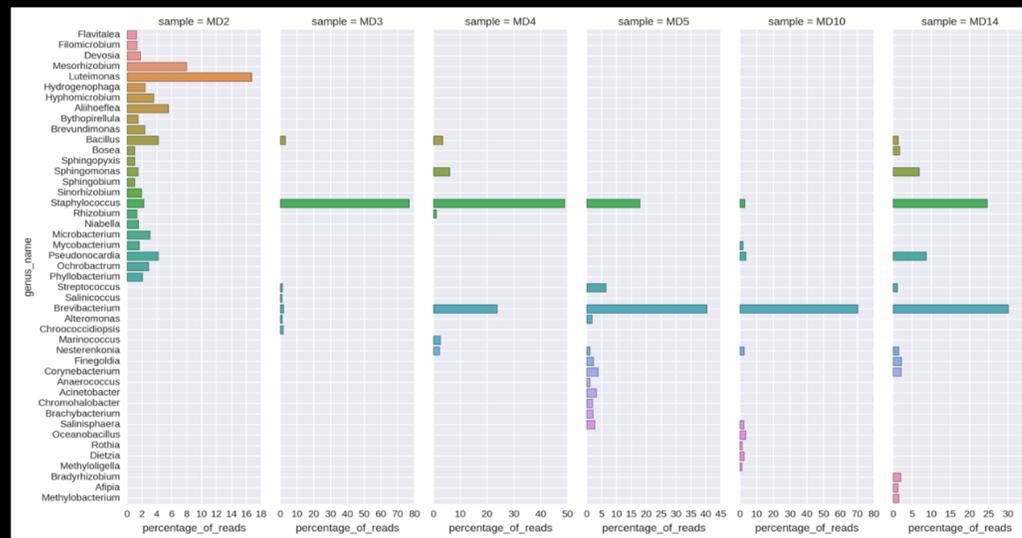


Culture-independent analysis: NEEMO 21

Illumina MiSeq



Oxford Nanopore Technologies MinION



Analog tested, Analog approved.





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 launched: April 18, 2017 on OA-7



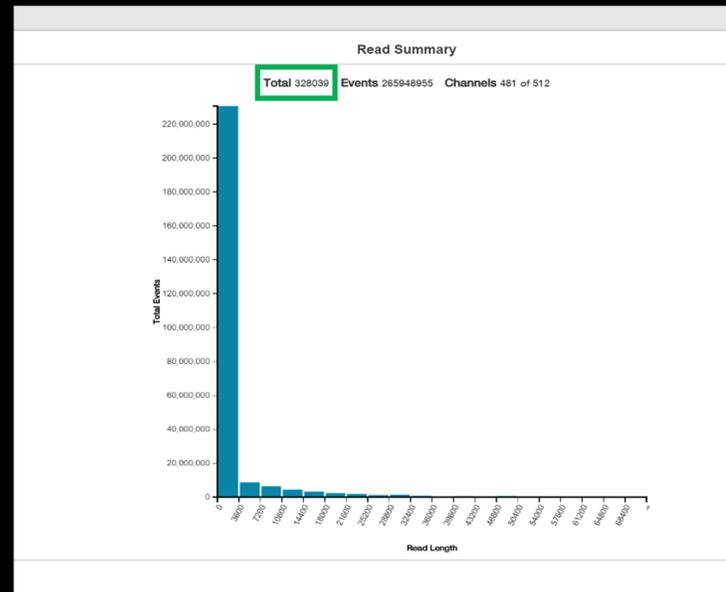
The successful implementation of this process will result in, for the first time, the ability to identify contaminating microbes in-flight. However, this means so much more than microbiology...



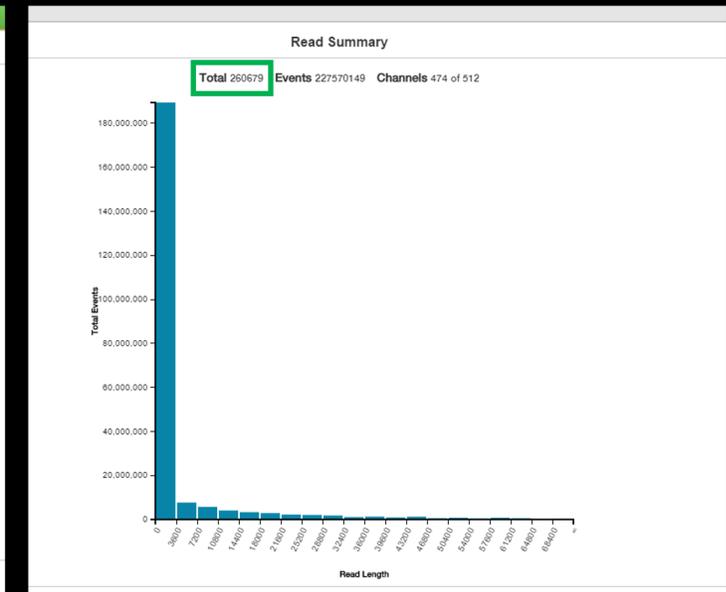
Genes in Space-3



ISS



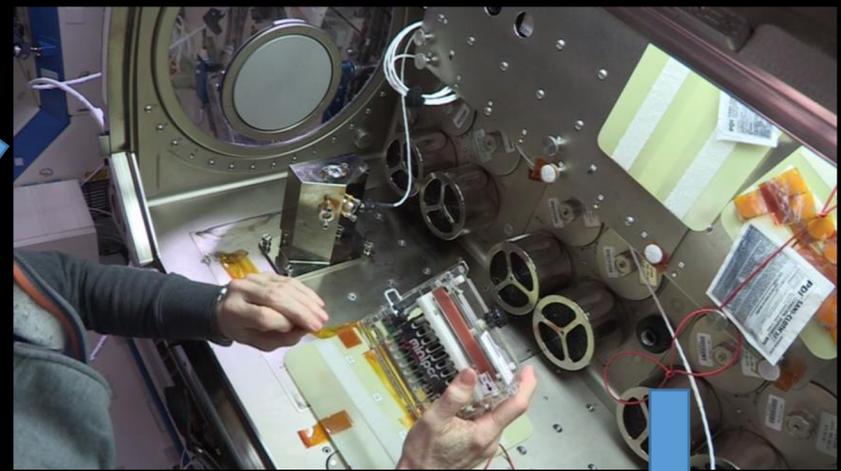
Ground



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: Med Ops Sample



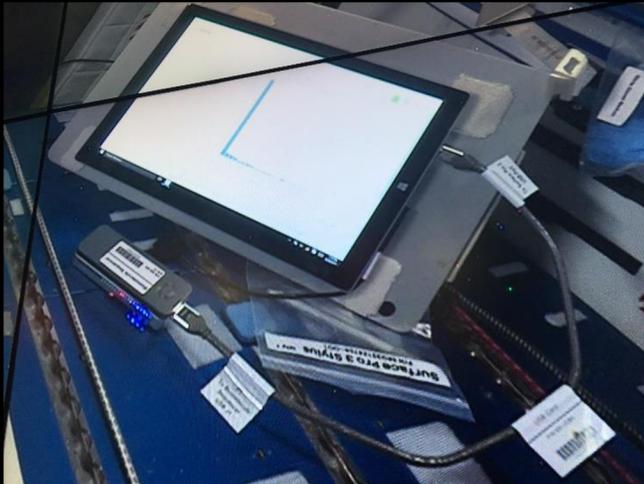
Astronaut Peggy Whitson collecting microbial cells from an Environmental Health Systems (EHS) Surface Sample Kit (SSK) Slide.



Genes in Space-3: Med Ops Sample



Data obtained in flight downlinked to the ground for analysis:



```
agtcgatcgatagctag
agcatcgatcgagggag
gtaccgattagggtatt
acccgatagggttaaga
tagcagggacatttacg
gattacggagacttcga
tacgatactgtgaccag
```

Microbial identifications
(not authorized to share yet)

Return sample processed through normal procedures in the Microbiology Lab:



Peggy selected 3 colonies
for analysis (2, 3, and 5):
not authorized to share yet

Success! The sequence
data from Station
matches the
identifications obtained
on the ground!



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

- 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 Tox 0!

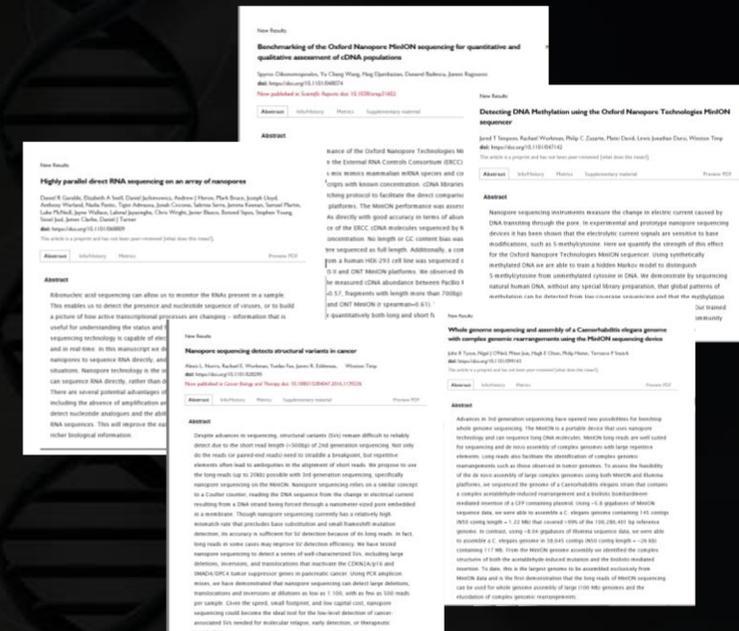
All of the operations products have been developed and are available. Reaction conditions for miniPCR and the MinION are easily customizable to any experiment. Different enzymes and reactions can be tested with certified consumables and substituted into procedures.



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



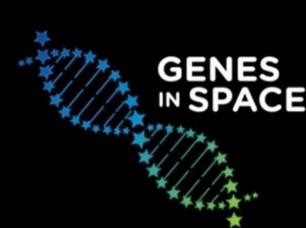
- Full genome assessments of model organisms
 - What is the genomic impact of radiation?
- Complete transcriptomic investigations
 - How is gene expression altered as a result of mission duration?
- Biomarker tracking through changes in gene expression
 - Bone and muscle
 - Cardiovascular
 - Wound healing
 - CO₂ 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?



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 Science Team

Jason Dworkin, Ph.D. – GSFC
Mark Lupisella, Ph.D. – GSFC
David Smith, Ph.D. – ARC
Kate Rubins, Ph.D. – JSC
Chris Mason, Ph.D. – Weill

BSeq Flight Team

Dave Voss
Linda Gibson
Teresa Tan

Astronauts

Kate Rubins, Ph.D.
Peggy Whitson, Ph.D.



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.



NEEMO 21 Team

Marc Reagan
Reid Wiseman, NASA
Megan McArthur, NASA
Marc O Griofa
Matthias Maurer, ESA
Noel Du Toit
Dawn Kernagis



Genes in Space-3 Collaborators Scott Copeland, Sebastian Kraves, Ph.D., and Ezequiel (Zeke) Alvarez Saavedra, Ph.D.



Genes in Space-3 Flight Team

Teresa Tan
Melissa Boyer
Landon Sommer
Melanie Smith



NEEMO 22 Team

Marc Reagan
Bill Todd
Kjell Lindgren, NASA
Trevor Graff
Pedro Duque, ESA
Dom D'Agnostion
Mark Hulsbeck
Sean Moore





Thank you!



Reid Wiseman @astro_reid Following

This look? It's when an @USNavy pilot sees confirmation from the @nanopore sequencer of successful #DNA extraction



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"First DNA sequencing in space." #AstroKate #genomics go.nasa.gov/2bV2UnD



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11:49 AM - 29 Aug 2016

472 676

Kjell Lindgren @astro_kjell

Replying to @astro_reid

This look? More DNA sequenced during #NEEMO22! Go Science!



7:29 pm · 24 Jun. 17

Jack Fischer @Astro2fish Follow

Just an average morning in our awesome Space-Lab...Here's our resident Space-Ninja @AstroPeggy sequencing DNA. Info at go.nasa.gov/2tqwtUD



12:23 PM - 5 Jul 2017