Biomolecule Sequencer:
Next-Generation DNA Sequencing Technology for In-flight Environmental Monitoring, Research, and Beyond

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2016: The Molecular Space Age

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 Continues

Biomolecule Sequencer Payload

- First attempt at 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
Johnson Space Center Class 1E Payload

- ~1 year to certify a payload for flight
- Class 1E is a streamlined certification process
- Reduce the time it takes to get scientific payloads to the ISS and increase utilization as a National Laboratory

- Authority to proceed February, 2015
- Hardware delivered on December 18, 2015
- Launched July 18, 2016 (SpaceX CRS-9)
- Technology Demonstration operations occurred on August 26, Sept. 3 and Sept. 7, 2016
Biomolecule Sequencer: The Team

Payload Development
• Aaron Burton, Ph.D. (PI)
  NASA JSC
• Sarah Castro-Wallace, Ph.D.
  NASA JSC
• Kristen John, Ph.D. (Deputy PI and PE)
  NASA JSC
• Sarah Stahl, M.S., (PS)
  NASA JSC

Astronaut
• Kate Rubins, Ph.D.
  NASA JSC

External Science Team
• Charles Chiu, Ph.D. (UCSF)
• Scot Federman
• Sneha Somasekar
• Doug Stryke
• Guixia Yu
• Chris Mason, Ph.D. (WCMC)
• Noah Alexander
• Alexa McIntyre
Biomolecule Sequencer: The Need for In-flight Sequencing

Why do we need a DNA sequencer to support the human exploration of space?

- Operational environmental monitoring
  - Identification of contaminating microbes
  - Infectious disease diagnosis
- Research
  - Human
  - Animal
  - Microbes/Cell lines
  - Plant
- Med Ops
  - Response to countermeasures
  - Radiation
- Functional testing for integration into robotics for Mars exploration missions
Biomolecule Sequencer: The Benefits

- **Benefits to In-flight Sequencing**
  - Sequencing on the ISS can inform real-time decisions (remediation strategies, research, med ops, etc.)
  - Unlike other technologies, sequencing is not limited to the detection of specific targets, but rather will provide data on the entirety of a sample
  - Reduce down mass (sample return for environmental monitoring, crew health, etc.)
  - Real-time analysis can influence medical intervention
  - Support astrobiology science investigations
    - Technology superiorly suited to *in situ* nucleic acid-based life detection
Biomolecule Sequencer: The Hardware

Flow Cell: Contains the nanopore sensing technology that is required to perform the sequencing reaction.
Biomolecule Sequencer: Nanopore Sequencing

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: Comparison to other Sequencers

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Model</th>
<th>Sequencing methodology</th>
<th>Mass-based cost to transport hardware to the ISS</th>
<th>Mass of sequencer hardware (lb)</th>
<th>Volume (cu in)</th>
<th>Power requirements</th>
<th>Special requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illumina</td>
<td>MiSeq</td>
<td>Fluorescence: each nucleotide has a different fluorophore</td>
<td>$1,200,000</td>
<td>120</td>
<td>6,940</td>
<td>100 - 240V AC, 10A, 400 W</td>
<td></td>
</tr>
<tr>
<td>Illumina</td>
<td>MiniSeq</td>
<td>Fluorescence: each nucleotide has a different fluorophore</td>
<td>$990,000</td>
<td>99</td>
<td>12,350</td>
<td>100 - 240V AC, 15A</td>
<td></td>
</tr>
<tr>
<td>PacBio</td>
<td>Sequel</td>
<td>Fluorescence: each nucleotide has a different fluorophore</td>
<td>$7,800,000</td>
<td>780</td>
<td>81,900</td>
<td>208 - 240V AC, 30A</td>
<td>Pressurized N₂ (50 PSI)</td>
</tr>
<tr>
<td>IonTorrent</td>
<td>Ion PGM</td>
<td>Electrochemical: measures voltage changes caused by nucleotide addition</td>
<td>$650,000</td>
<td>65</td>
<td>10,080</td>
<td>100 - 240V AC, 9A, 200 - 300 W</td>
<td>Pressurized N₂ (35 - 45 PSI)</td>
</tr>
<tr>
<td>Oxford Nanopore Technologies</td>
<td>MinION</td>
<td>Electrochemical: measures DNA-mediated changes in current passing through nanopores</td>
<td>$2,000</td>
<td>0.2</td>
<td>5</td>
<td>USB3 (5V, &lt; 1A) 1 W</td>
<td></td>
</tr>
</tbody>
</table>
Biomolecule Sequencer: DNA Samples

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

Experimental Design:
Sequence a ground-prepared sample containing a mixture of genomic DNA from:
- Bacteriophage lambda
- Escherichia coli
- Mouse – BALB/C (female)
1. Launch packaged items

2. Stowage (Ambient, -90°C, +4°C)

3. Remove flow cell and sample syringe from cold stowage and allow to equilibrate to ambient ISS temperature

4. Destow and connect MinION to Surface Pro3

5. Sample injection

6. Dispose of sample syringe

7. Initiate the sequencing experiment

8. Data collection

9. Stow used flow cell for return

10. Data downlink

11. Stow MinION, Surface Pro3, power & USB cords

12. Return of payload

Sequencing stops after 6 hrs

The First DNA Sequencer in Space!
4 batches of libraries were prepared containing the genomes to be sequenced
From the 4 batches, 18 samples were produced: 9 for ISS and 9 identical ground controls
Aliquots of all libraries were sequenced for quality control
Synchronous ground controls were performed
3 sequencing experiments have been conducted to date (Aug. 26, Sept. 3, Sept. 7, 2016); additional runs are planned
Biomolecule Sequencer: Results

The emergence of nanopore-based sequencers greatly expands the reach of sequencing into low-resource field environments, enabling in situ molecular analysis. In this work, we evaluated the performance of the MinION DNA sequencer (Oxford Nanopore Technologies) in-flight on the International Space Station (ISS), and benchmarked its performance off-Earth against the MinION, Illumina MiSeq, and PacBio RS II sequencing platforms in terrestrial laboratories. The samples contained mixtures of genomic DNA extracted from lambda bacteriophage, Escherichia coli (strain K12) and Mus musculus (BALB/c). The in-flight sequencing experiments generated more than 80,000 total reads with mean 2D accuracies of 85 to 90%, mean 1D accuracies of 75 to 80%, and median read lengths of approximately 6,000 bases. We were able to make directed assemblies of the ~4.7 Mb E. coli genome, ~48.5 kb lambda genome, and a representative M. musculus sequence (the ~16.3 kb mitochondrial genome), at 100%, 100%, and 96.7% pairwise identity, and de novo assemblies of the lambda and E. coli genomes solely with yielded 100% and 99.8% genome coverage, respectively, at 100% and 98.5% pairwise identity. Across all surveyed metrics (base quality, throughput, stays/base, skips/base), no observable decrease in MinION performance was observed while sequencing DNA in space. Simulated runs of in-flight nanopore data using an automated bioinformatic pipeline demonstrated the feasibility of real-time sequencing analysis and metagenomic identification of microbes in space. Additionally, cloud and laptop based-assembly illustrated the plausibility of automated, de novo genomic assembly from nanopore data on the ISS. Applications of sequencing for space exploration include infectious disease diagnosis, environmental monitoring, evaluating biological responses to spaceflight, and even potentially the detection of extraterrestrial life on other planetary bodies.

Did it work? Yes!
(A) A mixture of equimolar DNA from mouse, *E. coli* and lambda phage genomes was sequenced in parallel on Earth (“Ground”) and in-flight on the ISS (after being delivered by a SpaceX Dragon capsule). Synchronous nanopore sequencing runs were performed from August 26 to September 13, 2016.

(B) Plot of mean current intensity in picoAmperes (pA; Y-axis) against k-mers (x-axis) in order of increasing mean current based on a model distribution from Oxford Nanopore Technologies (black). Current distributions are tightly clustered with the exception of lower-quality ground #2.

(C) Density plots showing pairwise identity of nanopore reads collected on the ISS (left panel) and on the ground (right panel) relative to lambda (left box), *E. coli* (middle box) and mouse (right box) genomes. Abbreviations: 2D, high-quality two-dimensional reads; template, 1D template read; complement, complement read.

(D) Pie charts of the read distributions corresponding to each ISS run and pooled ISS runs 1 – 4, in comparison to that obtained from a ground Illumina MiSeq run of the same sample mixture.
(A) Flow chart of the SURPIrti bioinformatics pipeline for real-time microbial detection from nanopore data.

(B) Donut charts of read distributions corresponding to all reads (left), viruses, (upper right), and bacteria (upper right) from ISS run 1. These charts were generated dynamically as part of a real-time sequencing analysis simulation using SURPIrt.
(C) Stacked distributions of reads from ISS runs 1 through 4 aligning to mouse, *E. coli*, and lambda. Subgroup 1 shows raw SURPIrt output in the absence of taxonomic classification, while subgroups 2 and 3 show the effects of classification using the GenBank NT database and separate viral or bacterial databases, respectively. The relative proportions of read counts from SURPIrt differ from those obtained by GraphMap alignment to the most closely matched reference genome in NCBI NT (subgroup 4).

(D) Coverage (green) and pairwise identity plots (purple) of raw nanopore reads mapped the *E. coli* (upper panel), the mouse mitochondrial (lower left panel), and lambda genomes (lower left panel). Reads are mapped to the most closely matched reference genome identified by SURPIrt.
Looking Ahead: Need for Sample Preparation

Swab to Sequencer Sample Preparation Process

DNA Extraction → DNA Amplification → Library Preparation → DNA Sequencing
Biomolecule Sequencer: The Future

• Maintain the Biomolecule Sequencer on the ISS as a permanent operational and research facility.
• Continue initiatives to develop the capabilities to perform the sample collection and preparation on orbit, allowing an endless number of potential experiments.
• Nanopore sequencing can go far beyond DNA and can enable methylation, epigenetics, RNA, modified bases, and protein studies.
Biomolecule Sequencer: The Team

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- Chris Mason, Ph.D.
  - Noah Alexander
  - Alexa McIntyre
  - WCMC

Oxford Nanopore Technologies
- James Brayer
- Sissel Juul, Ph.D.
- Daniel Turner, Ph.D.
- Michael Micorescu, Ph.D.

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Biomolecule Sequencer: Questions?

Thank you!

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