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

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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 ¾ 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
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. [Link](http://biorxiv.org/content/early/2016/09/27/077651)
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…

1. Environmental Swab Collection
2. DNA Extraction & Clean-Up
3. MiniPCR Amplification
4. Sample Preparation
5. Loading MiniION Sequencer
6. Successful Sequencing

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

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

**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

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

**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
    - 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?
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