BIOMOLECULE SEQUENCER: NANOPORE SEQUENCING TECHNOLOGY FOR IN-SITU ENVIRONMENTAL MONITORING AND ASTROBIOLOGY.

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Introduction: Future missions to planetary bodies such as Mars require the next generation of tools and instrumentation. As we venture further, it will become increasingly challenging to transfer materials back to Earth for analysis, creating a need for in-situ diagnostic capabilities. Human missions will present challenges associated with crew health and safety, as well as contamination. One tool in particular, the Biomolecule Sequencer, has applications for in-situ environmental monitoring, medical diagnostics, and even life detection experiments for astrobiology investigations.

Nucleotide Sequencing: Nucleotide sequencing (the process of determining the order of nucleotides within a molecule such as DNA) can be used to: (1) mitigate microbial risks to crew by allowing identification of microbes in water, in air, and on surfaces; (2) identify optimal treatment strategies for infections that arise in crew members; and (3) track how crew members, microbes, and mission-relevant organisms respond to conditions on Mars through transcriptome- and genome-level changes. On the surface of other bodies, sequencing can be used as a tool for identifying Earth-derived contamination in samples. Additionally, if Mars contains indigenous life that is based on nucleic acids or closely related molecules, sequencing could serve as a tool to characterize those molecules. For these reasons, it is necessary to develop spaceflight-compatible nucleic acid sequencing tools.

Nanopore Sequencing: Until recently, sequencing technology has not been space-compatible due to size, power, and operational constraints. However, the development of nanopore-based sequencing has produced devices that are much smaller, consume less power, and use detection methods more conducive to the challenges of spaceflight. Nanopore sequencers measure changes in current caused by DNA passing through nanopores, as shown in Figure 1.

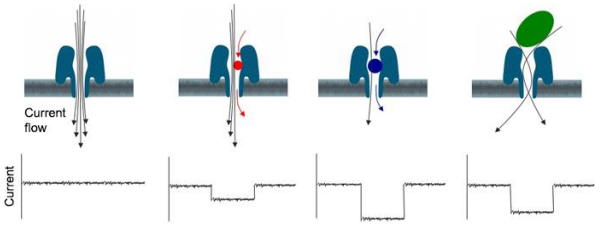

Figure 1. Nanopore-based sequencers measure changes in current caused by molecules (ex. DNA strands) passing through the pore. The changes in current are diagnostic of the sequence of the molecule passing through the pore. This method of measurement means that the sequencing capability is not limited to DNA and RNA with canonical nucleobases (A, G, C, and T/U).

Biomolecule Sequencer Project: In July of 2016, the Biomolecule Sequencer project launched to the International Space Station (ISS), creating history by becoming the first experiment to sequence DNA in space. The Biomolecule Sequencer project is the first step towards evaluating nanopore sequencing as a tool for future exploration [1]. This project involves testing the MinION™ device (see Figure 2), a commercially available product developed by Oxford Nanopore Technologies, to determine how the device performs after flight to the ISS and under continuous microgravity conditions. The MinION™ device is lightweight (< 150 g), small (9.5 x 3.2 x 1.6 cm), and powered by a USB connection. On the ISS, it is powered by a Microsoft Surface Pro3™.

Figure 2: The MinION™ device is a nanopore sequencer that weighs less than 150 g, with a volume of less than 100 cm^3.

Three different types of DNA will be sequenced: lambda bacteriophage, *Escherichia coli* and mouse genomic DNA. The ground-prepared samples are stored frozen until experiment initiation. Identical ground controls will be run in parallel with the flight experiment. Immediately prior to sequencing, the crew member will thaw a frozen sample, inject the thawed sample into a new flow cell, and initiate sequencing. After 6 hours, sequencing will be terminated and the data downlinked to Earth for analysis. The concept of operations, from sample preparation to termination of sequencing, was successfully tested aboard a parabolic flight [2]. The first effort of the Biomolecule Project is a technology demonstration. The next step involves demonstrating end-to-end in-flight sequencing, from sample collection to data analysis. The latest results from the first set of experiments will be presented.

Sequencing as a tool for astrobiology: For the future of astrobiology, nucleic acid sequencing will serve as a critical tool. Sequencing can place extremophiles in evolutionary context [e.g., 3-5], provide insights into the origin and evolution of the ribosome itself [e.g., 6, 7], and give key information regarding organismal metabolism [e.g., 8]. Sequence data have been successfully obtained from samples including: DNA from bones that were hundreds of thousands of years old [9,10]; insects trapped in amber over 100 million years old [11]; and archaea entrained in halite crystals over 400 million years old [12]. The preservation of DNA combined with the amount of possible information obtained from genomic sequences, provide strong support for the inclusion of nucleic acid sequencing as part of a life detection mission.

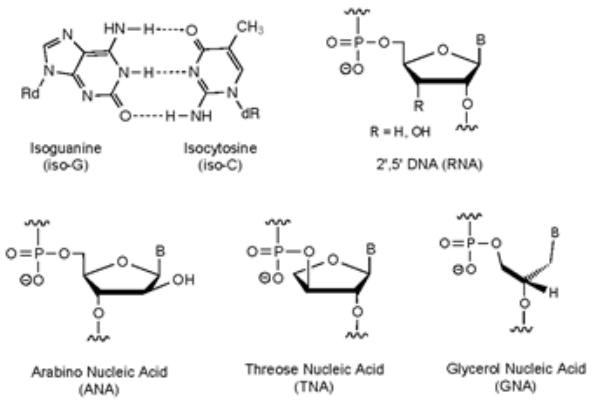

Figure 3. Examples of nucleic acid-like molecules that could support life. Nanopore-based sequencers have the potential to detect these kinds of molecules, whereas sequencers that require synthesis or incorporation of chromophores would not.

Sequencing beyond DNA and RNA: Traditional sequencing methods for life detection can only sequence DNA or RNA. It is possible that life existing elsewhere uses informational molecules other than DNA or RNA. Traditional sequencers would be not able to obtain data, even from closely related nucleic acid analogs such as arabinose or threose nucleic acids shown in Figure 3. However, nanopore-based sequencers measure changes in current based on the polymer passing through them, so they can also read RNA, proteins, and other polymers [13]. Therefore, nanopore-based sequencing is more versatile as a life detection tool than other sequencers that require nucleic acid synthesis or other detection methods.

Figure 4: The Biomolecule Sequencer launched to the ISS on July 18th, 2016 aboard the SpaceX Falcon 9 Dragon capsule. [Photo credit: Ken Kremer, kenkremer.com]

References:

[1] "Sequencing DNA in the palm of your hand"; http://www.nasa.gov/mission_pages/station/research/news/biomolecule_sequencer; September 29, 2015 [2] McIntyre et al., Nanopore Sequencing in Microgravity, (in press) *npj Micrograv.* [3] Ciccarelli F. D. et al. (2006) *Science* 311, 1283-1287. [4] Puigbò P. et al. (2009) *J. Biol.* 8, 59 – 59. [5] Wolf Y. I. et al. (2002) *Trends Gen.* 18, 472-479. [6] Fox G. E. (2010) *Cold Spr. Harb. Persp. Biol.* 2, a003483 [7] Petrov A. S. (2014) *Proc. Natl. Acad. Sci. USA* 111, 10251-10256. [8] Oren A. (2008) *Sal. Syst.* 4, 2-2. [9] Dabney J. et al. (2013) *Proc. Natl. Acad. Sci USA* 110, 15758-15763. [10] Orlando L. et al. (2013) *Nature* 499, 74-78. [11] Cano R. J. et al. (1993) *Nature* 363, 536-538. [12] Park J. S. et al. (2009) *Geobiology* 7, 515-523. [13] Oukhaled A. et al. (2012) *ACS Chem. Biol.* 7, 1935-1949.