VALIDATION OF NANOPORE SEQUENCING ABOARD THE INTERNATIONAL SPACE STATION AND POTENTIAL APPLICATIONS FOR ASTROBIOLOGY.

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Introduction: Life on Earth universally relies on nucleic acids for information storage and transfer, and orchestrating biochemical pathways. Nucleic acid sequencing offers a powerful tool to characterize organisms, including evolutionary and metabolic signatures, and, accordingly, sequencing is a fundamental step in in the study of newly discovered species on Earth. Sequencing would also be powerful in the search for life beyond Earth, assuming those life forms also contain sequenceable nucleic acids, such as DNA, RNA or related molecules. There are also several arguments that can be made for the plausibility of life based on DNA or DNA-like molecules beyond Earth. First, Martian meteorites provide us with direct evidence of transfer of material between planets, so it is at least possible that Earth meteorites could have also reached Mars. Second, DNA is well-suited to support and enable a diverse Tree of Life across a wide range of environmental conditions, including temperatures from -15°C to +113°C [1,2] exploiting a vast array of energy sources. Given the versatility of DNA and the plausibility of transfer of Earth material to Mars, DNA is a logical target molecule in the search for extraterrestrial life.

Despite the ubiquity of DNA and RNA on Earth, some exceptions to the universal A,G,C,T/U alphabet are made. For examples: the S-2L cyanophage incorporates 2,6-diaminopurine into its DNA in place of adenine [3]; DNA can be significantly modified by methylation for epigenetic control of gene expression; and ribosomal and transfer RNAs are extensively modified during post-transcriptional processing. Furthermore, it has been argued that during the origins of life on Earth, simpler nucleotides, such as threose nucleic acid or glycerol nucleic acid (xenonucleic acids or XNAs) could have preceeded RNA. Intriguingly, many of these XNAs have been observed to be capable of forming double-stranded helices and even of adopting functional tertiary conformations [4,5]. In light of these considerations, the most robust sequencing platform for searching for extraterrestrial life would be able to analyze not only DNA and RNA, but also a range of nucleic acids including XNAs.

The MinION™ (Oxford Nanopore Technologies) is a nanopore-based sequencer that has been used to analyze DNA, RNA [6] and a DNA polymer containing inosine in place of guanine [7]. The Biomolecule Sequencer project [8] tested the performance and stability of the commercially-available nanopore DNA sequencing platform aboard the International Space Station (ISS), the results of which will be discussed.

Methods: Nine ground-prepared sequencing libraries, containing mixtures of mouse, lambda bacteriophage and *E. coli* genomic DNA, and nine MinION flow cells (R7 chemistry) were flown to the ISS at -90°C and +4°C respectively. Over the course of 6 months, samples were periodically removed from the freezer aboard the ISS, thawed, and loaded on a new MinION flow cell for sequencing. Synchronous control samples were run in parallel on Earth. Data were downloaded from the ISS and analyzed using commercially-available and custom software.

Results: In total, more than 284,000 reads were generated in space, and more than 130,000 were generated in the corresponding ground controls. Accuracies on the R7 flow cells were ~85-90% for 2D reads and 75-80% for 1D reads, and read lengths were typically 5,000–7,000 bases. The data were of sufficient quality to permit de novo assemblies of the entire lamda bacteriophage and *E. coli* genomes. No drop in performance beyond flow cell to flow cell variation was observed even after 6 months in space. Based on the successful outcome of the Biomolecule Sequencer Project 1.0, the team is preparing for a second flight to demonstrate in-flight sample preparation of genomic DNA from organisms of astrobiological interest, amplification and sequencing of DNA from microbial community standards, and actual environmental samples obtained from the ISS. The team is also working to make the sequencer an ISS research facility for general use.

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