Sequencing DNA, RNA and related molecules as a tool to advance space exploration

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As humans seek to return to the Moon, and eventually to Mars and beyond, new challenges must be overcome to keep astronauts safe and healthy. This includes protecting crew members from harmful organisms in their environment, treating infections that may arise, monitoring nutrition and understanding how the human body adapts to spaceflight during missions that could last multiple years. Since the International Space Station (ISS) was first occupied in 2000, crew health has been monitored with thorough check-ups before and after flight, and the collection of many samples during flight that are brought back to Earth for analysis. During longer missions to more distant solar system locales, where returning samples to Earth is no longer practical, being able to analyse samples aboard the spacecraft could be very important.

The ISS offers a platform to develop and test new technologies and procedures under actual spaceflight conditions. For molecular biology, 2016 was a banner year on the ISS to show that several key techniques for monitoring crew health could be performed in-flight. This included the polymerase chain reaction (PCR), to multiply copies of DNA, and real-time PCR, which turns PCR into a quantitative technique for measuring DNA and RNA abundances in a cell. The year 2016 also saw the first extraction of DNA and RNA from intact cells on the ISS, and the first DNA sequencing experiments were performed there.

Although each of these techniques are routinely conducted in laboratories on Earth, doing them aboard the ISS is anything but routine, due to the fact the ISS is orbiting some 250 miles above the Earth’s surface, travelling at 18,000 miles per hour. Furthermore, since the ISS is in perpetual free fall, it causes a continuously weightless environment. Removing the force of gravity has a significant effect on all sorts of things, from how fluids like water behave, to how fire burns. It also has a profound effect on living things. People aboard the ISS are no longer tethered to the ground, but instead can float or glide through the air. Words like up, down, floor or ceiling are no longer helpful points of reference because the force of gravity on the ISS is not strong enough to tell you which way is down. The lack of gravity poses clear physical challenges for crew members doing experiments aboard the ISS. Equipment has to be secured to a surface to ensure it does not drift away or move during use, and crew members must find ways to hold themselves in place while performing the experiments so that they do not drift away.

The difficulties associated with reduced gravity extend to the small-scale liquid manipulations that are required for processes like PCR and sequencing. Air bubbles can be significantly more difficult to remove on the ISS than on the ground because they are not being driven to the top of the solution by gravity, but rather can stay suspended in the middle of a test tube or stuck to the side or bottom. These air bubbles can be detrimental for several reasons. First, they can prevent solutions from being properly mixed. Second, optical measurements like fluorescence can be strongly impacted by the presence of air bubbles because they scatter light. Third, air bubbles can disrupt the stability of things like proteins and membranes that are commonly found in biological samples.

The DNA sequencer used aboard the ISS operates in a fundamentally different way than most sequencers used...
on Earth. The more common sequencing platforms use variants of a process called sequencing by synthesis, where the sample being sequenced contains DNA molecules that serve as templates. Those template DNA molecules are copied with nucleotides, the building blocks of DNA which, in contemporary systems, have been modified to include a fluorescent tag. By using different colours for A, G, C and T, the nucleotide that has just been added can be readily identified. Conducting this process in a stepwise fashion allows the DNA sequence to be determined.

In contrast, the MinION™ sequencer (Oxford Nanopore Technologies) used on the ISS determines the nucleotide sequence electrochemically. It contains nanopores, which are proteins that can be embedded in biological or synthetic membranes. These pores are just wide enough in diameter to allow a single strand of DNA to pass through. When there is no DNA in the nanopore, an open current is measured, but when DNA passes through the pore, the current is reduced. This reduction in current is diagnostic of the sequence of DNA occupying the pore, and, with the aid of some sophisticated computing, the resulting DNA sequence can be determined. From the perspective of doing experiments on the ISS, or on a future deep-space mission, the MinION sequencer is very attractive because it is small (about as big as a Snickers bar), does not require much power, and can be run with a laptop computer.

There were two primary concerns regarding operating the MinION on the ISS: whether the nanopores and the membrane assemblies in the consumable flow cells used for sequencing could survive transit to the ISS; and whether crew members aboard the ISS could perform the necessary liquid transferring procedures (like pipetting) to eliminate most or all of the air bubbles in the samples that would be sequenced. During launch and the initial stages of flight to the ISS, the nanopores and membranes experience a significant amount of vibration and shock forces that can disrupt the nanopore and membrane assemblies essential for sequencing. Once in orbit, crew members have to perform several steps involving pipetting liquids between different solutions to prepare the DNA for sequencing. With each step, there is a risk that air bubbles can be introduced, which ultimately have to be removed prior to loading the sample into the flow cell. Excitingly, experiments by four different astronauts since 2016 have demonstrated that MiniION-based sequencing can readily be performed aboard the ISS, making this technology available for research now, and opening the possibility that sequencing could be part of the long-term strategy for human exploration of the Moon and Mars.

The Biomolecule Sequencer (BSeq) project in 2016 was the first test of sequencing on the ISS. During BSeq, astronaut Dr Kate Rubins loaded a sample that had been prepared for sequencing on the ground, and was flown up to the ISS. The sample contained fragments of DNA from the entire genomes of a virus (lambda bacteriophage), a bacteria (Escherichia coli) and a mouse. In all, variations of this experiment were performed nine times, and demonstrated that MinION sequencing worked just as well.
on the ISS as it did on the ground. A second astronaut, Dr Peggy Whitson, performed two of these nine experiments, in preparation for the next set of sequencing experiments on the ISS. This follow-up project began in 2017 and was called Genes in Space 3; the goal was to determine whether astronauts could perform all of the necessary steps to get DNA ready for sequencing on the ISS. Preparing DNA for sequencing requires several chemical reactions to take place, and the astronaut must proficiently handle aqueous solutions in relatively small 1 to 100 microlitre volumes. This is routine on Earth, with the use of micropipettes, but how well these tools would function in microgravity on the ISS had not yet been determined. In particular, there was concern about whether solutions in the pipette tips would ‘creep’ further up the tip without gravity. Although some minor creeping does occur, it does not significantly affect how well astronauts can micropipette. Whitson was able to successfully prepare DNA for sequencing. Initially, she used DNA that had been shipped to the ISS from the Earth. Building on this result, Whitson then sequenced DNA from intact cells that had been isolated from the ISS environment and were grown on a Petri dish, enabling the identification of those bacteria. By targeting the 16S ribosome gene, found in all bacteria, their genus and even species can be identified. As humans travel deeper into space, this is an important capability because it allows us to determine whether microorganisms that are co-habitating with crew members can cause illness, and, if so, identify the best methods to treat any infections that arise and eliminate any harmful organisms in the environment.

One of the limitations of the Genes in Space 3 experiment that identified bacteria aboard the ISS was that the cells had to be cultured (grown on the Petri dish) before they could be analysed. It has been estimated that approximately 99% of bacteria on Earth cannot be grown in this way, meaning that they would not be found using culture-based methods. The Biomolecule Extraction and Sequencing Technology (BEST) project in 2018 sought to overcome this limitation by sequencing the DNA of microorganisms that were collected on a swab that had been wiped over a surface. Astronauts Richard Arnold II and Dr Serena Auñón-Chancellor both performed this swab-to-sequencer process aboard the ISS, enabling better characterization of the microbial ecology (microbiome) of the ISS in a matter of days, rather than the weeks to months it normally takes to return samples to Earth. Since microbes are found everywhere humans go, including
 sequencing approaches that analyse the molecules directly, DNA, RNA and proteins, would be a clear benefit. Hence is an attractive starting point. Having the capability to then, searching for molecules like DNA, RNA and proteins. In terms of searching for extraterrestrial life, in these harsh and disparate environments use DNA, RNA oxygen, and by using sunlight or chemical energy stored in the freezing point to above the boiling point of water, at been found that can live: at temperatures ranging below that life could exist beyond Earth. Organisms have now actually find it. However, our expanding knowledge of know what form alien life would take, if it exists, until we answer the question of whether life exists elsewhere in the solar system, galaxy and universe. Obviously, we cannot

Studies to date have shown that astronauts experience significant physiological changes while they are on the ISS. These changes include: the loss of bone and muscle mass, down-regulation of immune system function, shifting of fluids from the lower body to the upper body and changes in vision. Understanding the basis for these changes at the molecular level, and potentially identifying ways to counteract the changes, is important to keep crew members safe, healthy and able to function well on the surface of the Moon or Mars. RNA sequencing is now a spaceflight-proven technique that can be used to track crew member health at the molecular level.

A driving goal for space exploration is to definitively answer the question of whether life exists elsewhere in the solar system, galaxy and universe. Obviously, we cannot know what form alien life would take, if it exists, until we actually find it. However, our expanding knowledge of terrestrial extreme environments bolsters the possibility that life could exist beyond Earth. Organisms have now been found that can live: at temperatures ranging below the freezing point to above the boiling point of water, at pHs from −1 − 13, in the presence or absence of molecular oxygen, and by using sunlight or chemical energy stored in rocks as an energy source. Strikingly, all known forms of life in these harsh and disparate environments use DNA, RNA and proteins. In terms of searching for extraterrestrial life, then, searching for molecules like DNA, RNA and proteins is an attractive starting point. Having the capability to sequence molecules closely related to, but not exactly like, DNA, RNA and proteins, would be a clear benefit. Hence sequencing approaches that analyse the molecules directly, like nanopore sequencing, would seem to be the leading candidate of currently available technologies to look for DNA- or RNA-like molecules that have different letters (besides A, G, C and T) or sugars (threose or glucose instead of ribose). A number of research groups are working on variations of this approach.

Sequencing has already been demonstrated to be useful for environmental microbial monitoring on the ISS, and could provide a way to track crew health and response to spaceflight on long-duration missions. Because both DNA and RNA are used by all life as we know it, under an incredibly wide range of environmental conditions, they are reasonable candidates to guide the search for life elsewhere. In this respect, sequencing would be a dual-purpose technology, used to monitor astronauts’ health as well as to track down alien life. ■

Further reading

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- Biomolecule Extraction and Sequencing Technology official payload page: https://www.nasa.gov/mission_pages/station/research/experiments/2736.html

Aaron Burton is a planetary scientist within the Astromaterials Research and Exploration Science (ARES) Division at the Johnson Space Center. His research seeks to understand how prebiotic chemical reactions could have led to the origins of life. Such research includes the analysis of organic material in astromaterials such as meteorites. He also studies how informational polymers serve as biomarkers in the search for life beyond Earth. He is a member of the SHERLOC team, who will utilize a spectrometer to characterize organics and minerals on Martian surfaces during the Mars 2020 mission and also is also involved in work directed towards the maintenance of crew health during spaceflight.

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