Beyond Nanopore Sequencing in Space: Identifying the Unknown

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Background

Astronaut Kate Rubins sequenced DNA on the International Space Station (ISS) for the first time in August 2016 (Figure 1A). A 2D sequencing library containing an equal mixture of lambda bacteriophage, *Escherichia coli*, and *Mus musculus* was prepared on the ground with a SQK_MAP006 kit and sent to the ISS frozen and loaded into R7.3 flow cells. After a total of 9 on-orbit sequencing runs over 6 months, it was determined that there was no decrease in sequencing performance on-orbit compared to ground controls (1). A total of ~280,000 and ~130,000 reads generated on-orbit and on the ground, respectively, identified 90% of reads that were attributed to 30% lambda bacteriophage, 30% *Escherichia coli*, and 30% *M. musculus* (Figure 1B). Extensive bioinformatics analysis determined comparable 2D and 1D read accuracies between flight and ground runs (Figure 1C), and data collected from the ISS were able to construct directed assemblies of *E.coli* and lambda genomes at 100% and *M. musculus* mitochondrial genome at 96.7%. These findings validate sequencing as a viable option for potential on-orbit applications such as environmental microbial monitoring and disease diagnosis.

Current microbial monitoring of the ISS applies culture-based techniques that provide colony forming unit (CFU) data for air, water, and surface samples. The identity of the cultured microorganisms in unknown until sample return and ground-based analysis, a process that can take up to 60 days. For sequencing to benefit ISS applications, spaceflight-compatible sample preparation techniques are required. Subsequent to the testing of the MinION on-orbit, a sample-to-sequence method was developed using miniPCR™ and basic pipetting, which was only recently proven to be effective in microgravity. The work presented here details the in-flight sample preparation process and the first application of DNA sequencing on the ISS to identify unknown ISS-derived microorganisms.

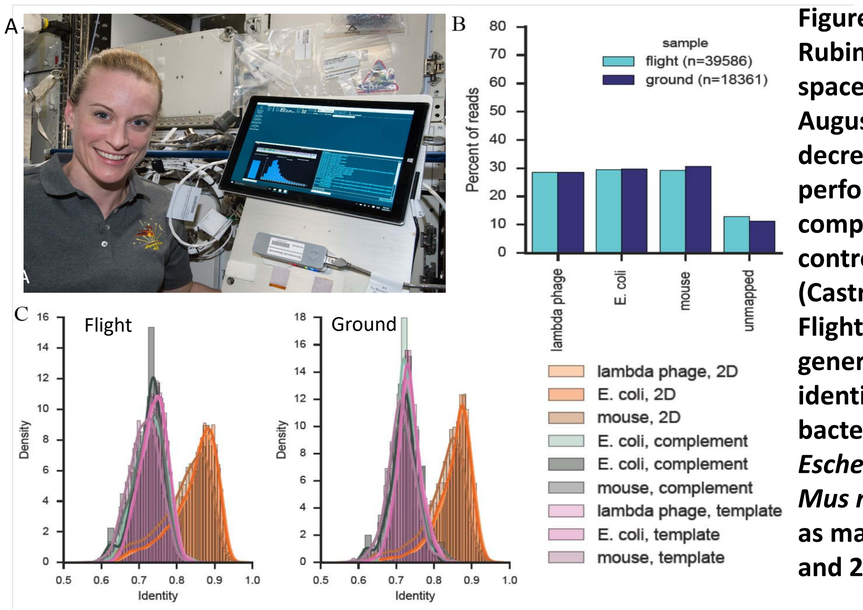


Figure 1. Astronaut Kate Rubins sequenced DNA in space for the first time on August 26, 2016 (A). No decrease in sequencing performance on-orbit compared to ground controls was observed (Castro-Wallace 2017). Flight- and ground-generated reads both identified 30% lambda bacteriophage, 30% *Escherichia coli*, and 30% *Mus musculus* (B) as well as maintained similar 1D and 2D read accuracies (C).

Methods

Astronaut Peggy Whitson prepared sequencing libraries on-orbit using standard laboratory methods and pipettes following our optimized spaceflight-compatible method. A total of 10 ng of microbial community standard DNA (Zymo Research, Irvine, CA) or a scraping of cells from a microbial colony were prepared for sequencing in triplicate using miniPCR™ (miniPCR, Cambridge, MA) and modified procedures from SQK-RAS201 16S Rapid Sequencing Kit (Oxford Nanopore Technologies, Oxford, UK). Pipetting was achieved with Eppendorf Research Plus P20 and P200 pipettes with individually-wrapped epT.I.P.S (Eppendorf, Hauppauge, NY). Triplicate samples were pooled, and 200 µL of the prepared library was loaded into a R9.4 flow cell in the MinION (ONT) and sequenced using MinKNOW version 1.1.21 for 48 hours. Both miniPCR ™ and the MinION were operated by a Surface Pro 3. Sequencing data were returned to ground, basecalled with albacore and analyzed using 16S Sequencing Metrichor workflow as well as undergoing additional analysis by ONT and UCSC.

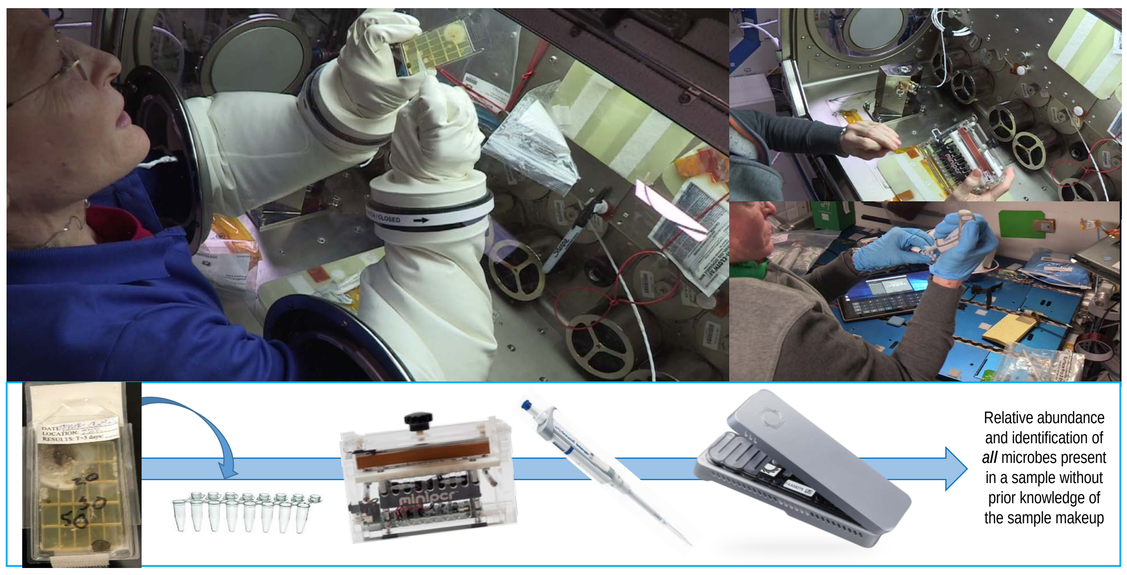
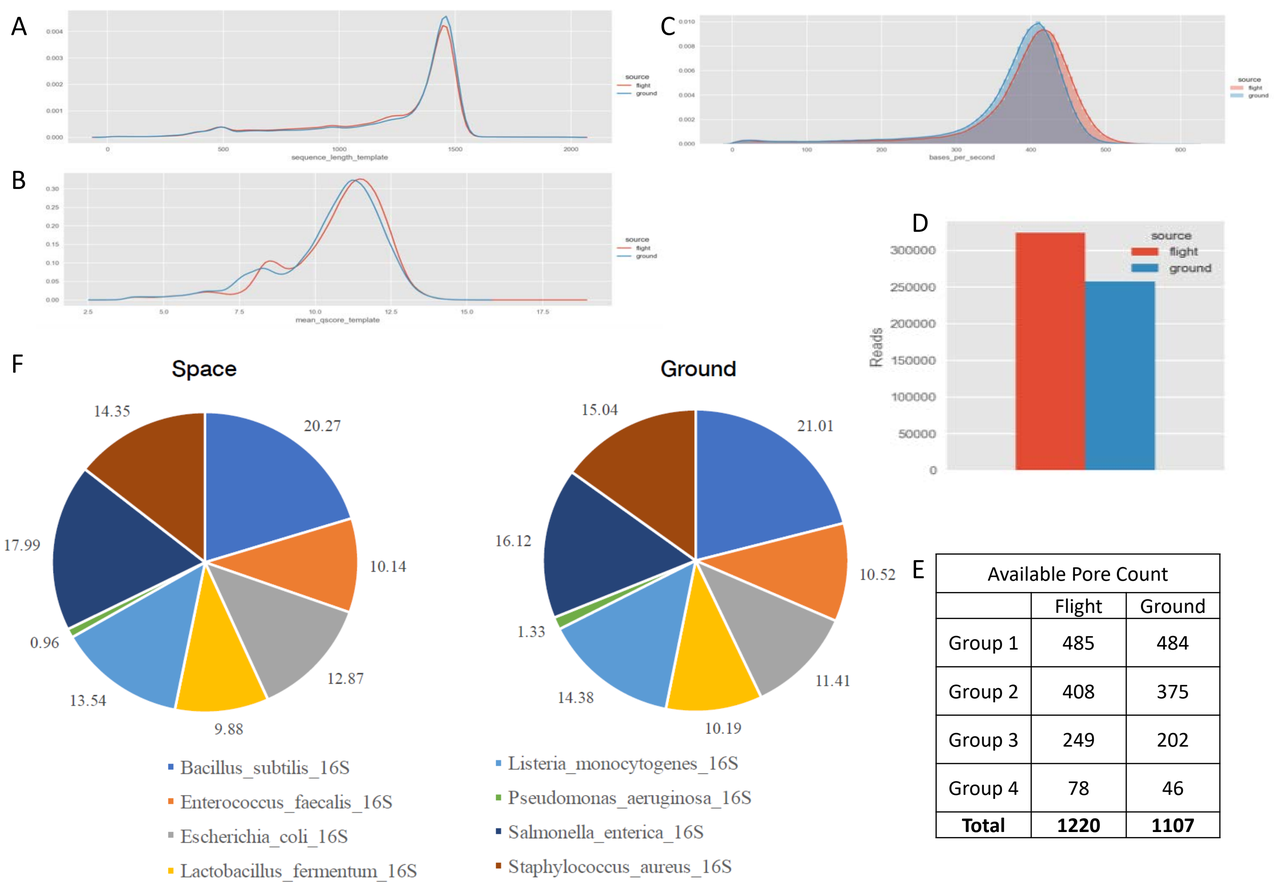


Figure 2. Astronaut Peggy Whitson selecting a microbial colony from the Environmental Health Systems surface sample kit slide for colony PCR with miniPCR ™ and preparing samples for sequencing with the MinION using Eppendorf Research Plus pipettes and the SQK-RAS201 Rapid 16S sequencing kit on the International Space Station on August 21, 2017.

Environmental microbial samples were collected from the ISS on August 10, 2017, using the Environmental Health Systems (EHS) surface sample kit's tryptic soy agar slide. Astronaut Peggy Whitson selected a single colony, collected cells by scraping part of the colony with a pipette tip, and transferred the cells to tubes containing the PCR master mix. This process was repeated three times (Figure 2). EHS microbial samples were returned to Earth on Soyuz 50 on September 5, 2017, and analyzed in the NASA Johnson Space Center's Microbiology laboratory by standard operational methods of biochemical analysis using VITEK (BioMerieux, Durham, NC) and Sanger sequencing with an ABI 3500 genetic analyzer (ThermoFisher Scientific, Waltham, MA).

Results

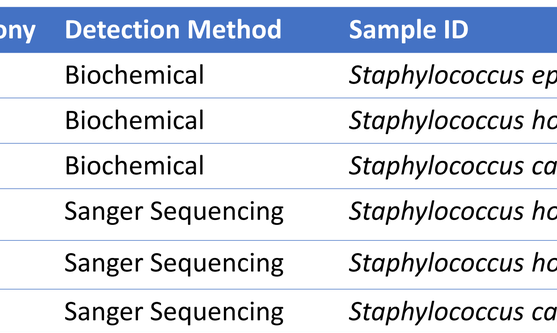
The first on-orbit sample preparation for DNA sequencing confirmed that standard laboratory methods and pipetting can be used in the microgravity environment of space without risk to sequencing performance (Figure 3).



Available Pore Count		
	Flight	Ground
Group 1	485	484
Group 2	408	375
Group 3	249	202
Group 4	78	46
Total	**1220**	**1107**

Figure 3. Sequencing run metrics from the first on-orbit sample preparation and sequencing of Zymo microbial community standard DNA using modified SQK-RAS201 Rapid 16S sequencing kit, miniPCR ™ and the MinION compared to ground control. Read lengths ~1450 bp (A) and q score ~11 (B) were similar between flight and ground control runs. The speed of in-flight sequencing was slightly faster than the ground control run (C) and flight operations generated more total reads (D). This increased flight performance may be related to increased available pore count in flight compared to ground R9.4 flow cells (E). Species level % identification of community were similar between flight and ground (F).

For the first time, microorganisms collected from, and cultured on, the ISS were identified at species level on-orbit through 16S sequencing. Analysis of the returned samples using biochemical methods and Sanger sequencing revealed almost identical identifications to those obtained on the ISS (Figure 4 and Table 1).



16S species level ID >90% accuracy	% reads
S. hominis	71.3%
S. capitis	17.0%

Figure 4. Species level taxonomic identification of unknown ISS-derived microorganisms collected and cultured as part of the Environmental Health Systems routine microbial monitoring efforts sequenced on the ISS using SQK-RAS201 16S Rapid Sequencing Kit, miniPCR ™ and the MinION.

Table 1. Ground-based analysis of the returned sample. The 3 bacterial colonies selected by the crew were identified using biochemical and Sanger sequencing techniques per normal laboratory procedures.

Colony	Detection Method	Sample ID	%ID
1	Biochemical	*Staphylococcus epidermidis*	96.0
2	Biochemical	*Staphylococcus hominis hominis*	97.0
3	Biochemical	*Staphylococcus capitis*	94.0
1	Sanger Sequencing	*Staphylococcus hominis hominis (ATCC=27844)*	99.9
2	Sanger Sequencing	*Staphylococcus hominis hominis (ATCC=27844)*	100.0
3	Sanger Sequencing	*Staphylococcus capitis capitis (ATCC=27840)*	99.9

Conclusion

One year and 2 days following the demonstration of successful nanopore sequencing in space, an end-to-end sample-to-sequence process resulted in the first in-flight identifications of ISS-derived microorganisms. To date, all sequencing experiments in space have produced data that parallel that of ground controls. The ability to prepare and sequence DNA in the spaceflight environment will revolutionize space-based research and in-flight medical operations.

Acknowledgements

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Reference: S.L. Castro-Wallace *et al.*, Nanopore DNA sequencing and genome assembly on the International Space Station, *bioRxiv* (2017) doi: 10.1101/077651