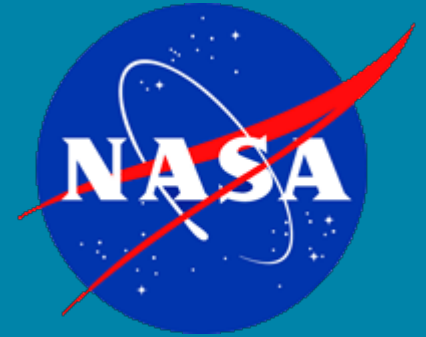


BEYOND NANOPORE SEQUENCING IN SPACE: IDENTIFYING THE UNKNOWN



Sarah Stahl
JES TECH



FIRST DNA SEQUENCING IN SPACE

Biomolecule Sequencer Payload

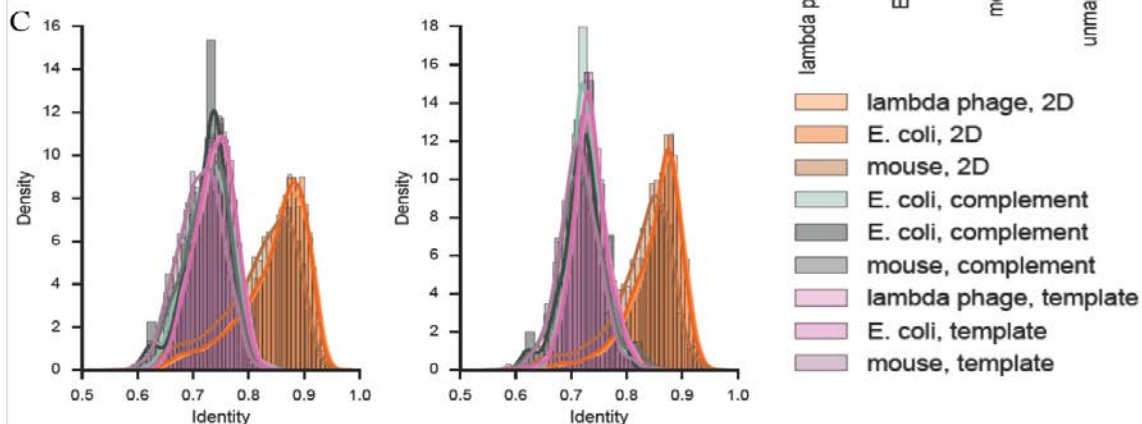
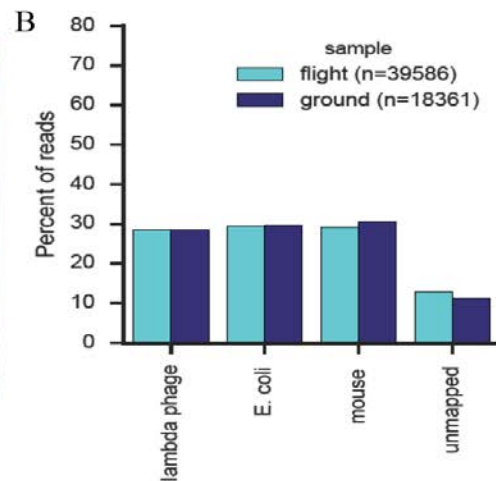
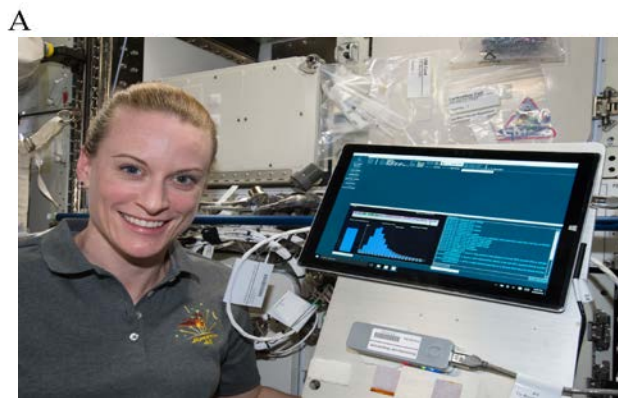


- Completed by Astronaut Kate Rubins, Ph.D.
- Ground-prepared mixture of mouse (BALB/C), lambda phage and *E.coli* DNA
- Total of 9 runs on orbit over 6 months
- Comparable read accuracy and identification
 - ~280,000 reads on orbit
 - ~130,000 reads on ground

Kit

Flow cell

SQK-MAP-006 R7.3





SAMPLE PREPARATION IN SPACE

Genes In Space-3 Payload

- **Goals:**
- Demonstrate sample preparation for sequencing using miniPCR device.
- How does microgravity impact pipetting?
- **Experiments:**
- Benchmark on orbit sequencing preparation with Zymo microbial community standard.
- Sequence and identify and unknown ISS-derived microbes on orbit for the first time.

Kit

Flow cell

SQK-RAS-201

R9.4



Astronaut Peggy Whitson, Ph.D.



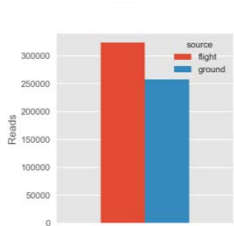
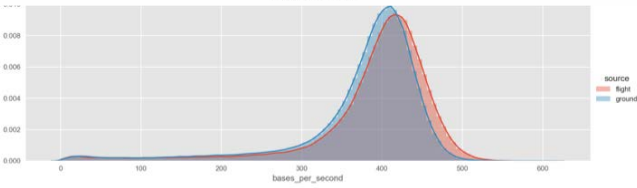
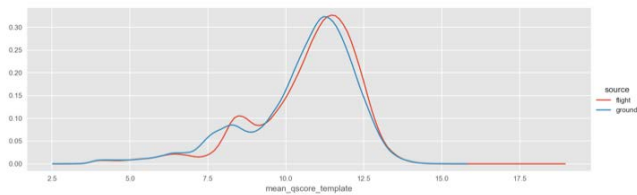
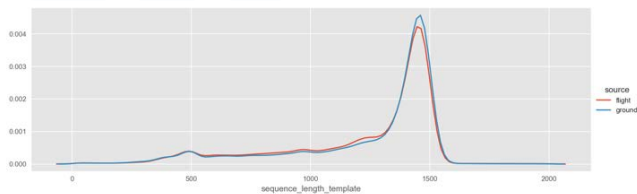
ON ORBIT LIBRARY PREPARATION SEQUENCING

Zymo microbial community standard DNA

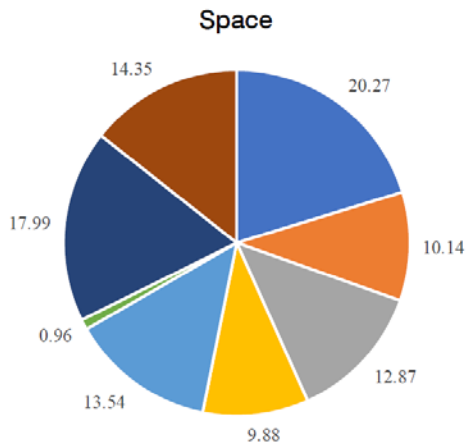


Sequencing was comparable between flight and ground

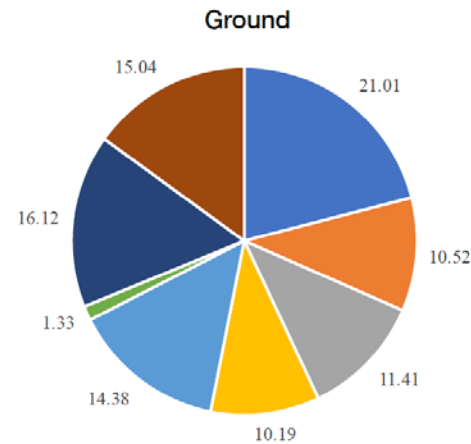
- Similar read length, q score, % species identification of Zymo community



Available Pore Count	
Flight	Ground
485	484
408	375
249	202
78	46
1220	1107

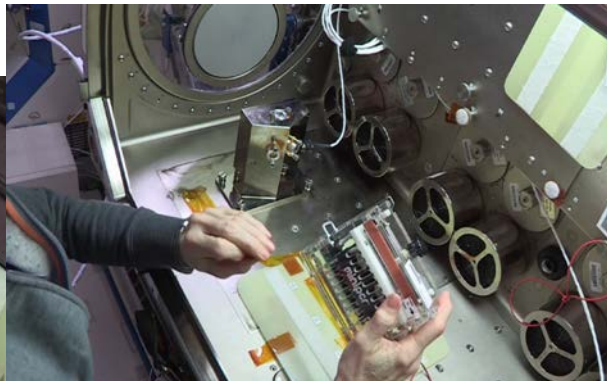


- Bacillus_subtilis_16S
- Enterococcus_faecalis_16S
- Escherichia_coli_16S
- Lactobacillus_fermentum_16S



- Listeria_monocytogenes_16S
- Pseudomonas_aeruginosa_16S
- Salmonella_enterica_16S
- Staphylococcus_aureus_16S

FIRST IDENTIFICATION OF UNKNOWN SPACE-DERIVED MICROORGANISMS



Kit

Flow cell

SQK-RAS-201

R9.4

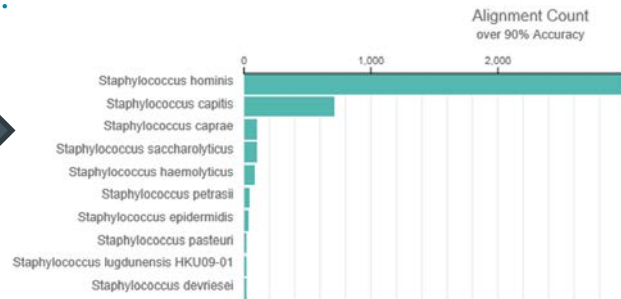


COMPARISON OF FLIGHT AND GROUND DATA ANALYSIS

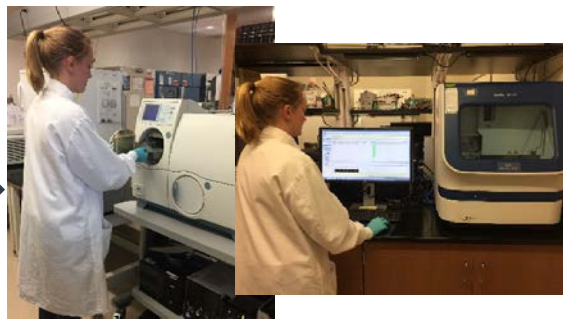
Data obtained in flight downlinked to the ground for analysis:



```
agtcgatcgatagctag
agcatcgatcgagggag
gtaccgattagggtatt
acccgatagggttaaga
tagcaggacatttacg
gattacggagacttcga
tacgatactgtgaccag
```



Return sample processed through operational procedures in the Microbiology Lab:

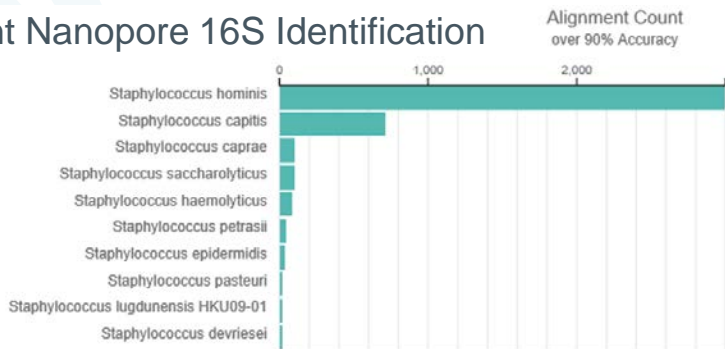


VITEK (biochemical)
ABI 3500 (Sanger sequencing)

Success!
The sequence data
from Station
matches the
identifications
obtained on the
ground!

FIRST IDENTIFICATION OF UNKNOWN SPACE-DERIVED MICROORGANISMS

Flight Nanopore 16S Identification



16S Nanopore species ID <90%	% Flight reads
<i>Staphylococcus hominis</i>	71.3%
<i>Staphylococcus capitis</i>	17.0%

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

ACKNOWLEDGEMENTS

Name

Division/location

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Kristen John PhD.**

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Tunner, PhD, David Stoddart, PhD,
Michael Micorescu, PhD, and Eoghan
Harrington**

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Saavedra, PhD and Scott Copeland**

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**Miten Jain, Benedict Paten, PhD and
Mark Akeson, PhD**

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Research Center, Weill Cornell, University of
California San Francisco

Flight Operations Team

Boeing, Geo Control, JES Tech

NASA Microbiology Laboratory

KBR Wyle, JES Tech, Geo Control



THANK YOU

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