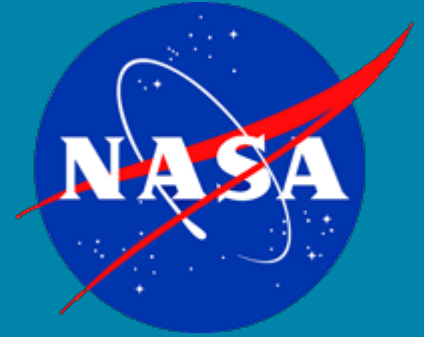


BEYOND NANOPORE SEQUENCING IN SPACE: IDENTIFYING THE UNKNOWN

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

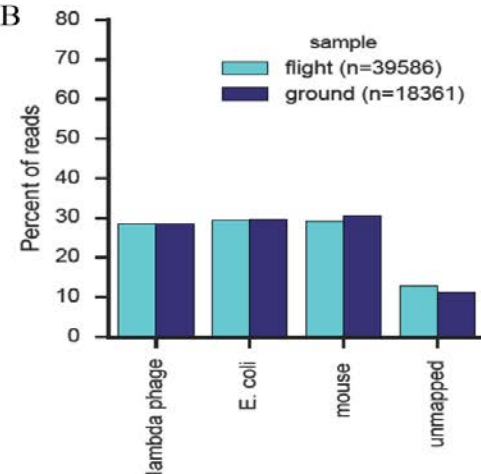
Flow cell

SQK-MAP-006 R7.3

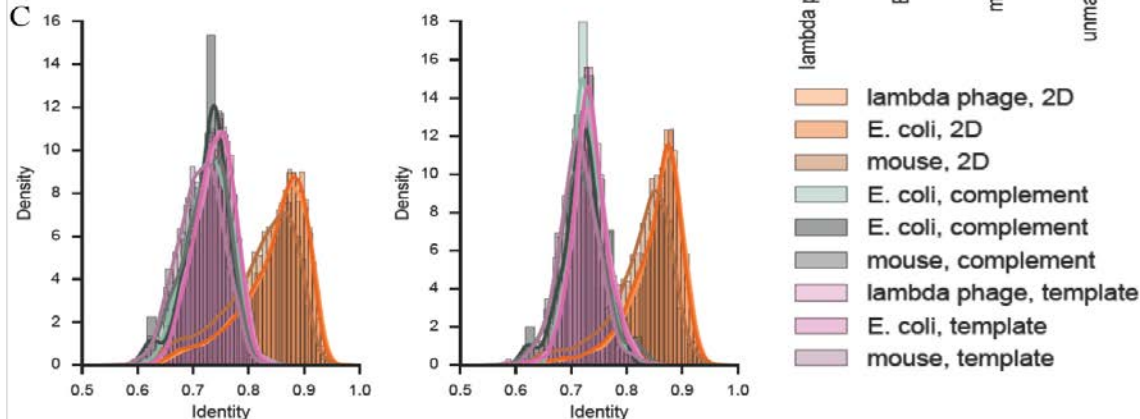
A



B



C



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.

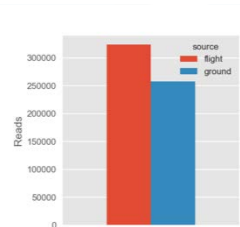
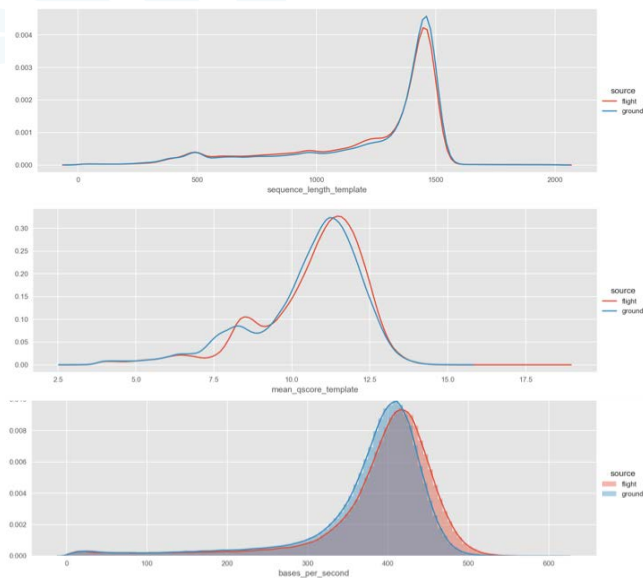
ON ORBIT LIBRARY PREPARATION SEQUENCING

Zymo microbial community standard DNA

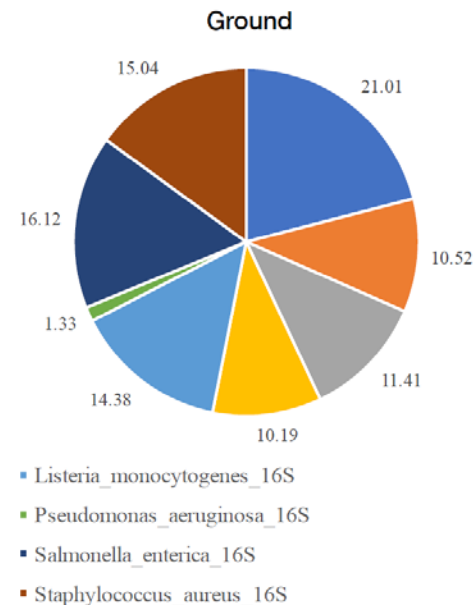
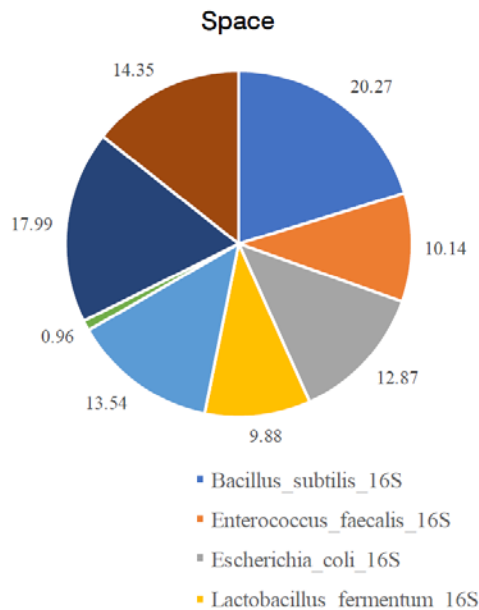


Sequencing was comparable between flight and ground

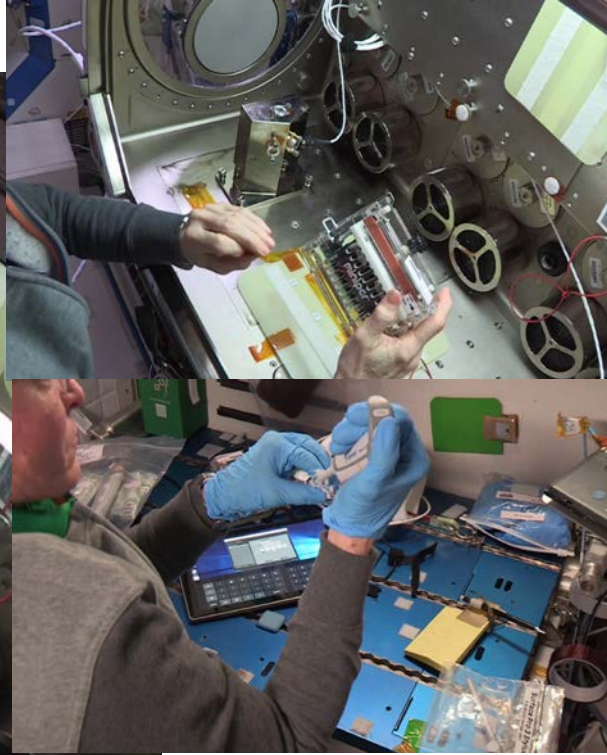
- Similar read length, q score, % species identification of Zymo community



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



FIRST IDENTIFICATION OF UNKNOWN SPACE-DERIVED MICROORGANISMS



Kit

Flow cell

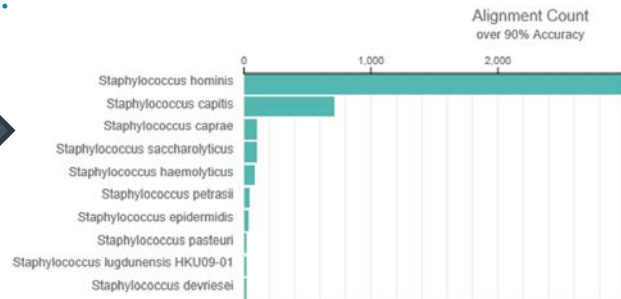
COMPARISON OF FLIGHT AND GROUND DATA ANALYSIS



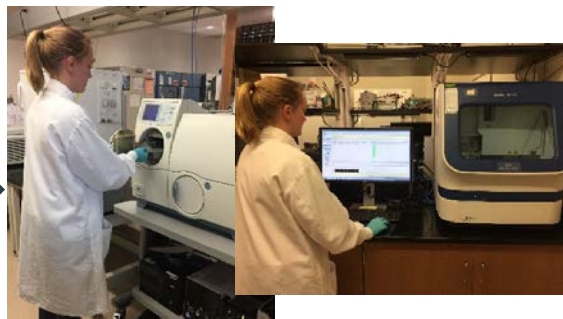
Data obtained in flight downlinked to the ground for analysis:



agtcgatcgatagctag
agcatcgatcgaggag
gtaccgattagggtatt
acccgatagggttaaga
tagcaggacattttacg
gattacggagacttcga
tacgatactgtgaccag



Return sample processed through operational procedures in the Microbiology Lab:

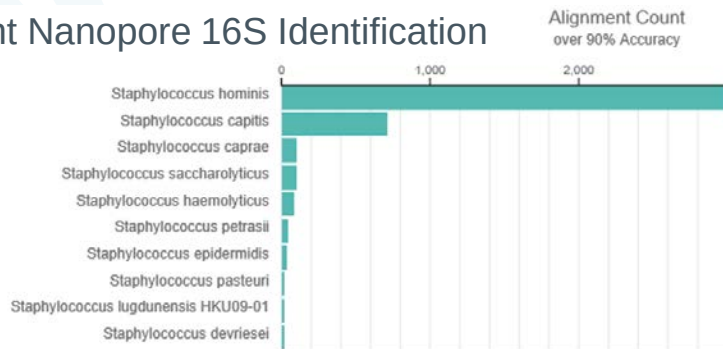


VITEK (biochemical)
ABI 3500 (Sanger sequencing)

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**

Staphylococcus hominis

71.3%

Staphylococcus capitis

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

Sarah Wallace, PhD, Aaron Burton, PhD, Kristen John PhD.

NASA Johnson Space Center

Astronauts Kate Rubin, PhD and Peggy Whitson, PhD

NASA Johnson Space Center

James Brayer, Sissel Juul, PhD, Dan Tunner, PhD, David Stoddart, PhD, Michael Micorescu, PhD, and Eoghan Harrington

Oxford Nanopore Technologies

Sebastian Kraves, PhD, Zeke Alvarez Saavedra, PhD and Scott Copeland

miniPCR/Genes in Space-3 Collaborators

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

University of California Santa Cruz

Biomolecule Sequencer Team

NASA Goddard Space Flight Center, Ames 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

The content contained in this presentation should not be reproduced without permission of the speaker.

© Copyright 2017 Oxford Nanopore Technologies. The MinION, GridION, PromethION and VolTRAX are for research use only.