Novel End-to-End Molecular Biology Approach for Direct Nanopore 1D cDNA Sequencing of Reverse Transcribed mRNAs Purified from Cell Cultures by the NASA ISS WetLab2 SPM.

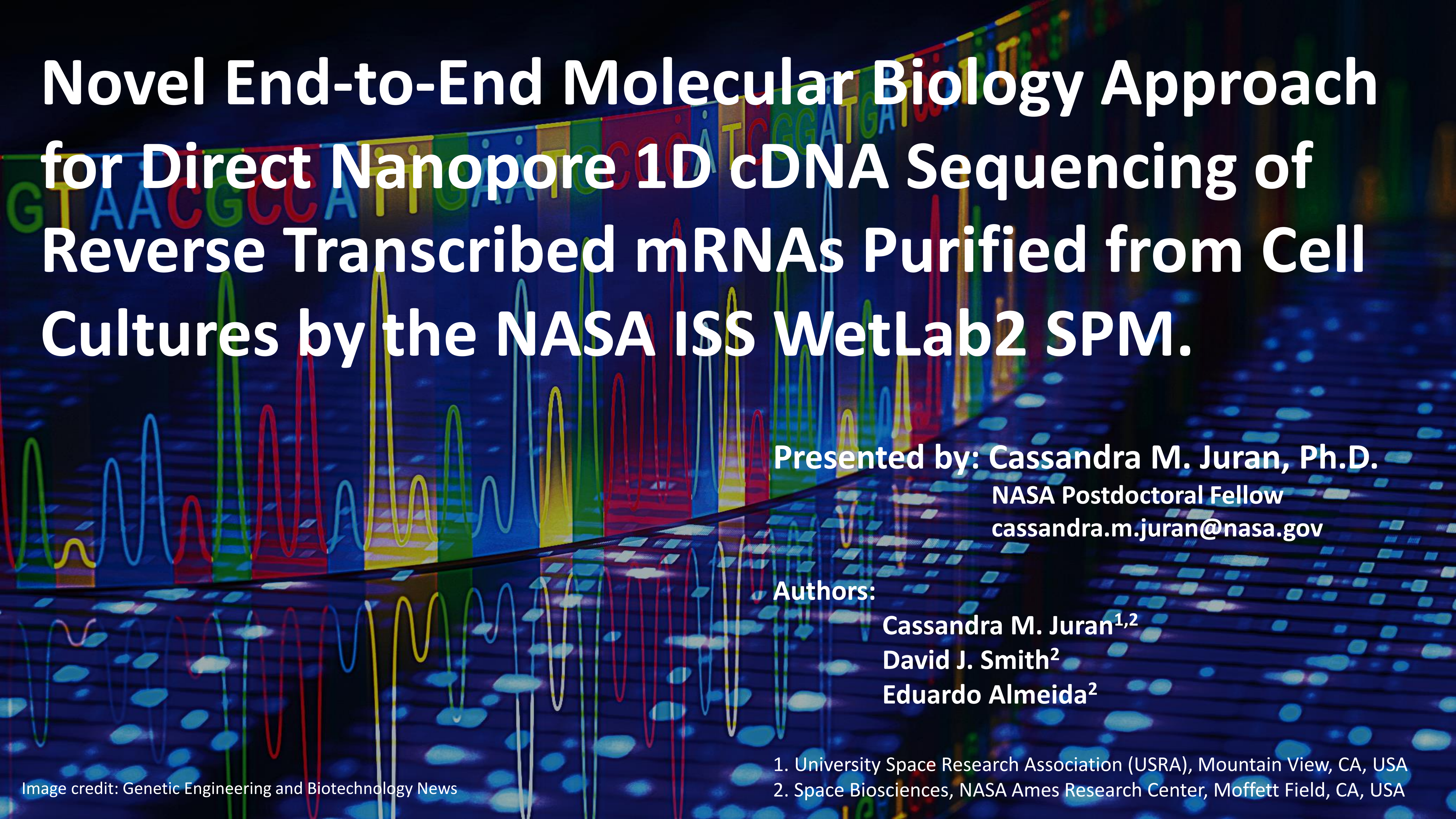

Image credit: Genetic Engineering and Biotechnology News

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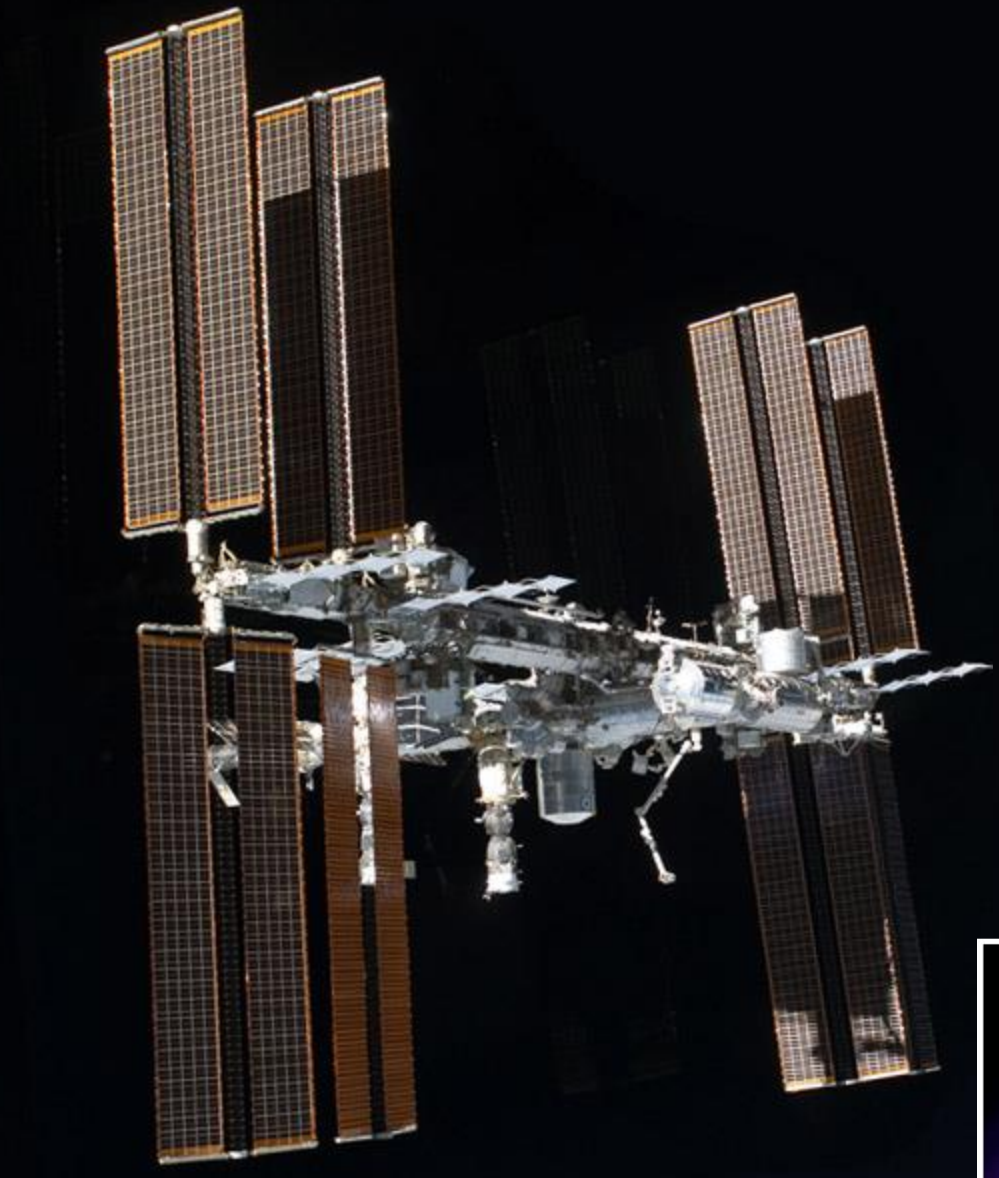


ISS

U.S. NATIONAL LABORATORY

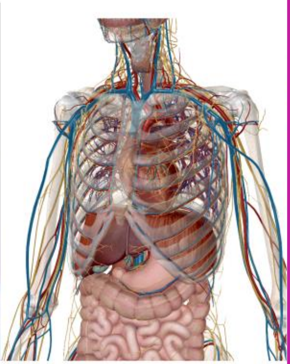
NASA

CASIS™

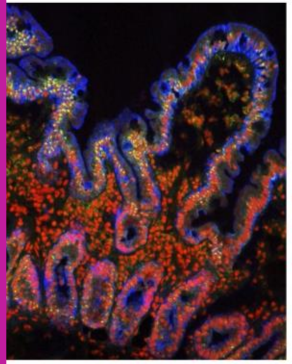
POWERED THROUGH PARTNERSHIP

Organism

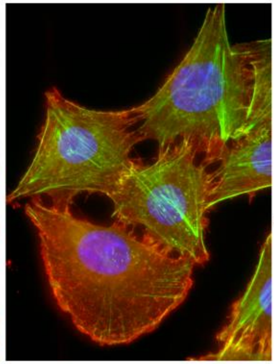
Systems



Tissues



Cells



Molecules

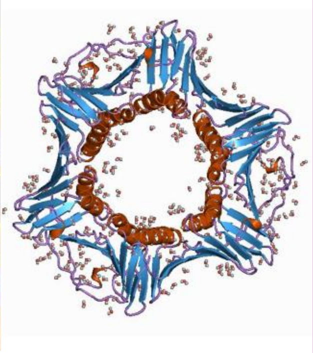


Image credit: CASIS website

What has been done on-board the ISS National Lab

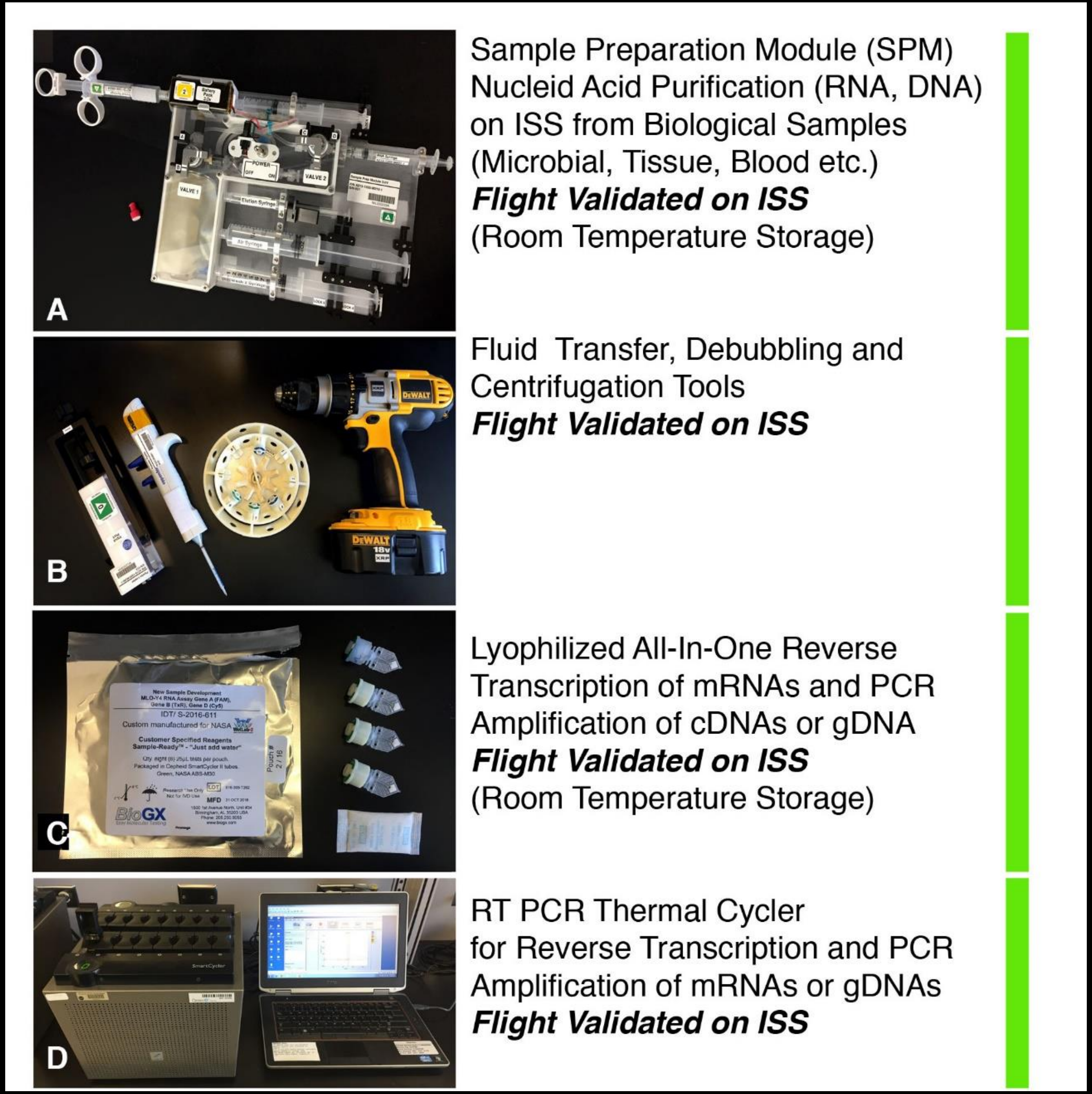
WetLab-2
NASA Ames Research Center

BURTON | WALLACE | JOHN | STAHL | BOTKIN | RUBINS | OXFORD NANOPORE
NASA BIOMOLECULE SEQUENCER PROJECT

- g-DNA/total-RNA isolation from eukaryote, prokaryote and viral samples (WL2 – SpaceX-8) and PCR analysis tech demo.
- Real time sequencing with the MinION (Bio-Seq) from pre-prepared library.

Image credit: First DNA Sequencing in Space a Game Changer on NASA.gov

WetLab-2 Platform Components



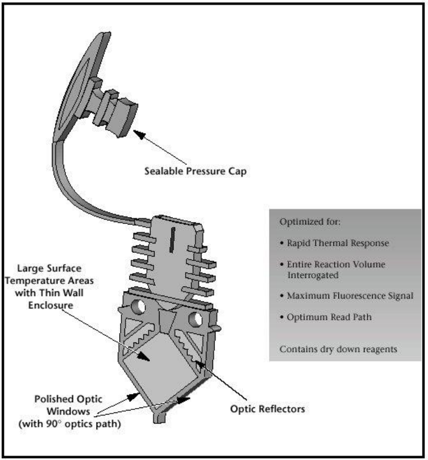
Sample Preparation Module (SPM)
Nucleid Acid Purification (RNA, DNA)
on ISS from Biological Samples
(Microbial, Tissue, Blood etc.)
Flight Validated on ISS
(Room Temperature Storage)

Fluid Transfer, Debubbling and
Centrifugation Tools
Flight Validated on ISS

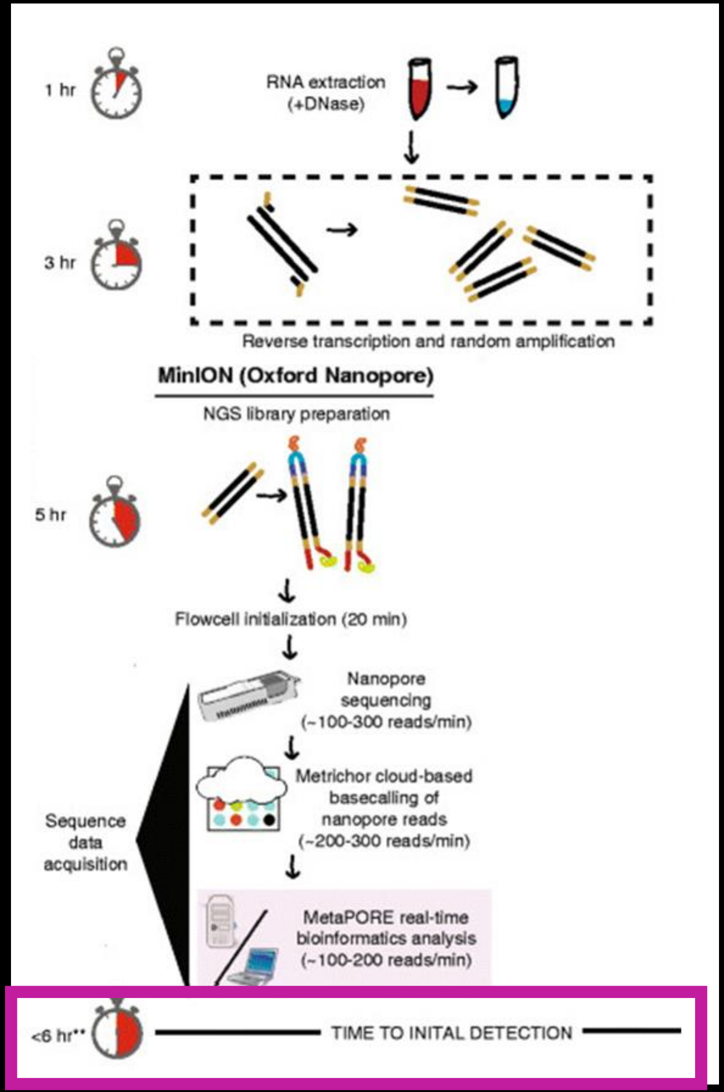
Lyophilized All-In-One Reverse
Transcription of mRNAs and PCR
Amplification of cDNAs or gDNA
Flight Validated on ISS
(Room Temperature Storage)

RT PCR Thermal Cycler
for Reverse Transcription and PCR
Amplification of mRNAs or gDNAs
Flight Validated on ISS

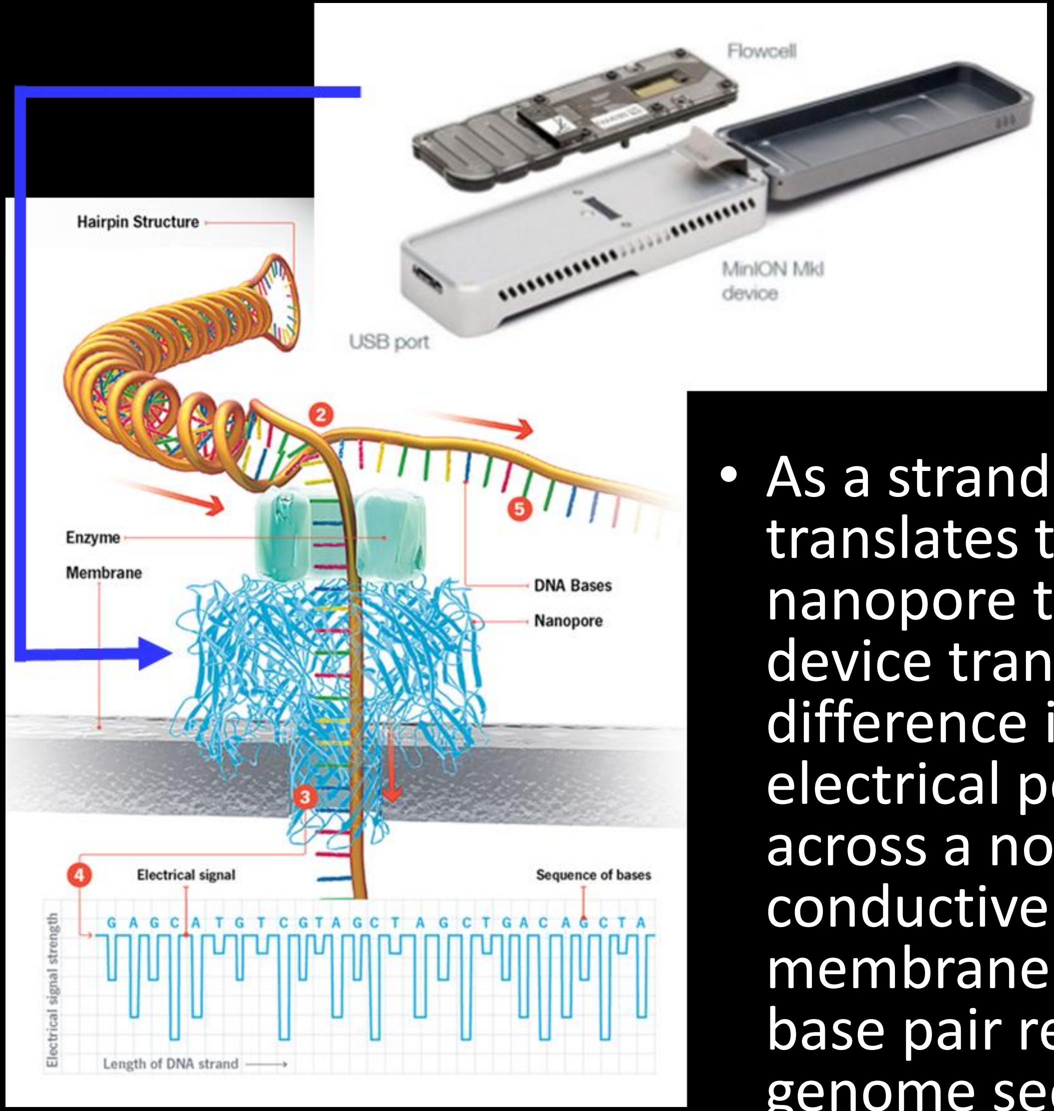
- Sample Preparation Module (SPM)
  - Nucleic acid purification (RNA, DNA) on ISS from biological samples.
  - (Microbrial, Tissue, Cells, Blood, etc.)
- Cyphid Thermal Cycler (Smartcycler)
  - Controlled thermal cycling
  - 16 sample capacity
  - 16 independently controlled ports.



MinION Biomolecule Sequencer



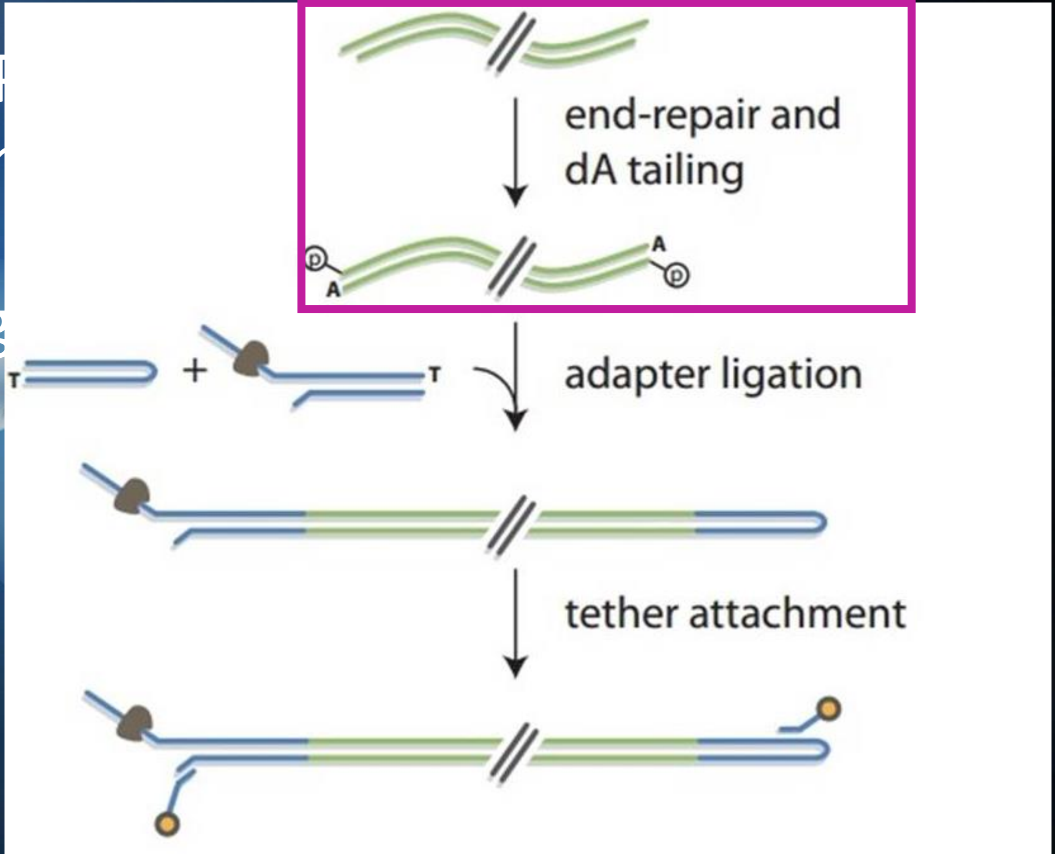
Greninger, Alexander L., et al. "Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis." Genome Medicine 7 (2015).



- As a strand of DNA translates the nanopore the MinION device translates the difference in electrical potentials across a non-conductive membrane into single base pair resolution genome sequences.

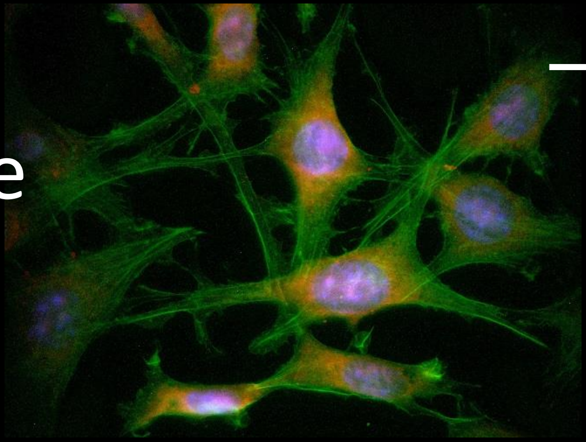
End-to-End Methodology

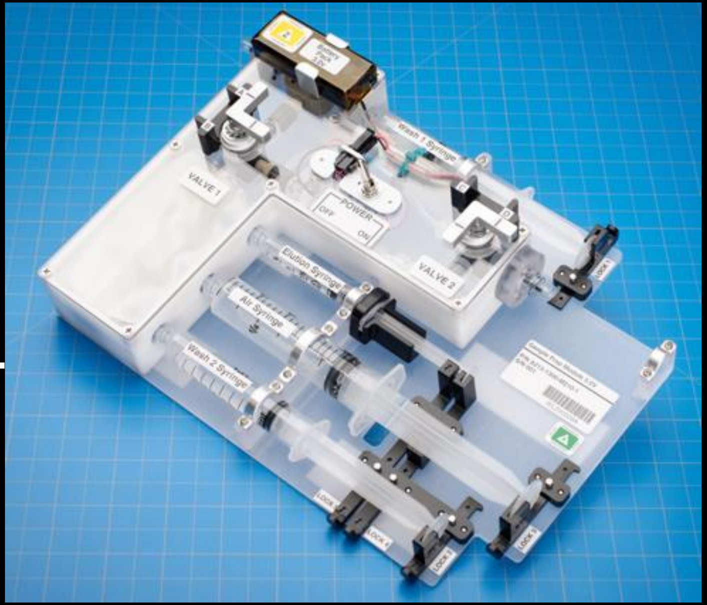
- Utilize the Sample Preparation Module (S
given sample of tissue, cells, or microbiom
- Purify the mRNA from the total RNA using
isolate sample with poly-A tailing.
- Synthesize cDNA and amplify using the W
- Generate pre-sequencing library
  - General 3 step system
- Purify sample and load into the MinION for sequencing and live basecalling



Proof of Concept Experiment: End-to-End Library Isolation and Purification

- Sample Preparation Module (SPM) Total RNA Isolation from MLOY4 mouse osteocyte-like cell colonies.





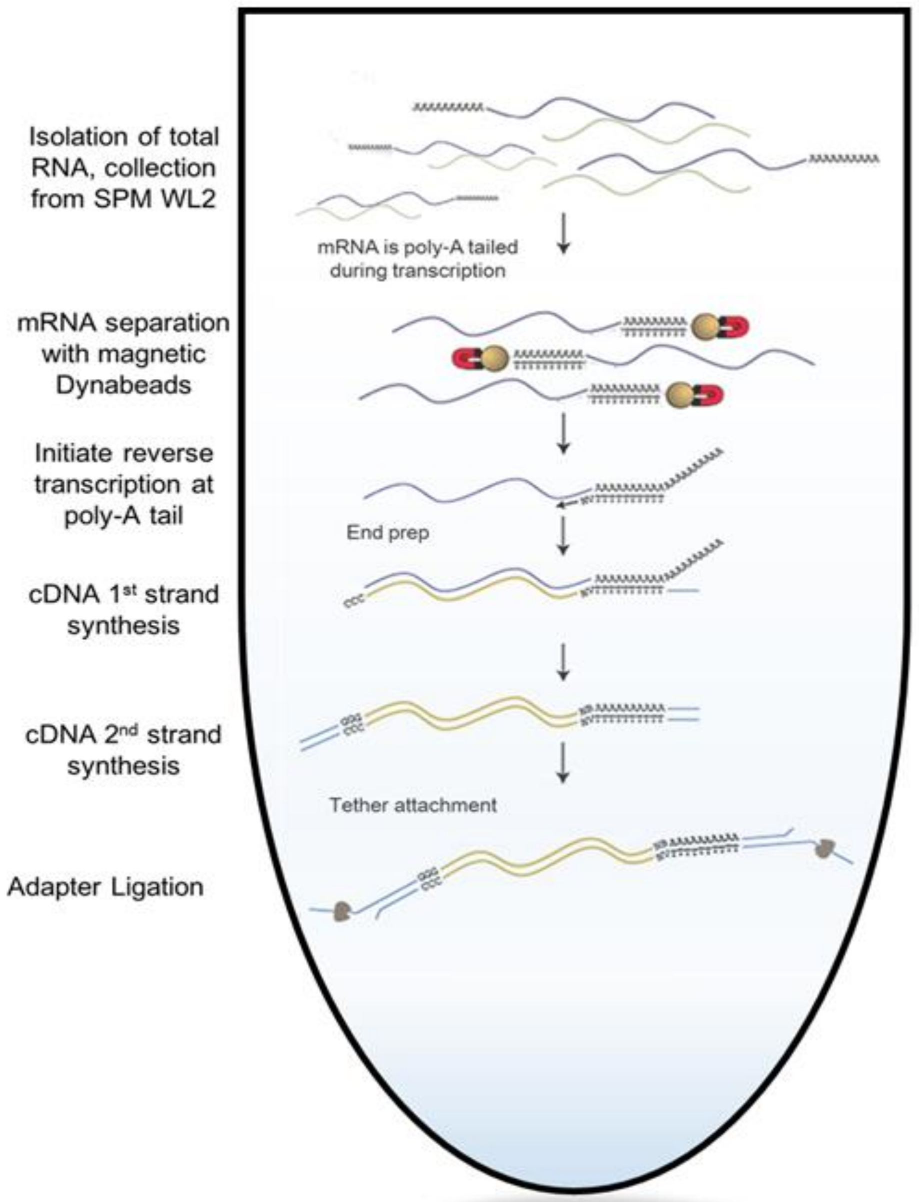
- Transfer tube

- Magnetic Bead Clean-Up mRNA preferential binding

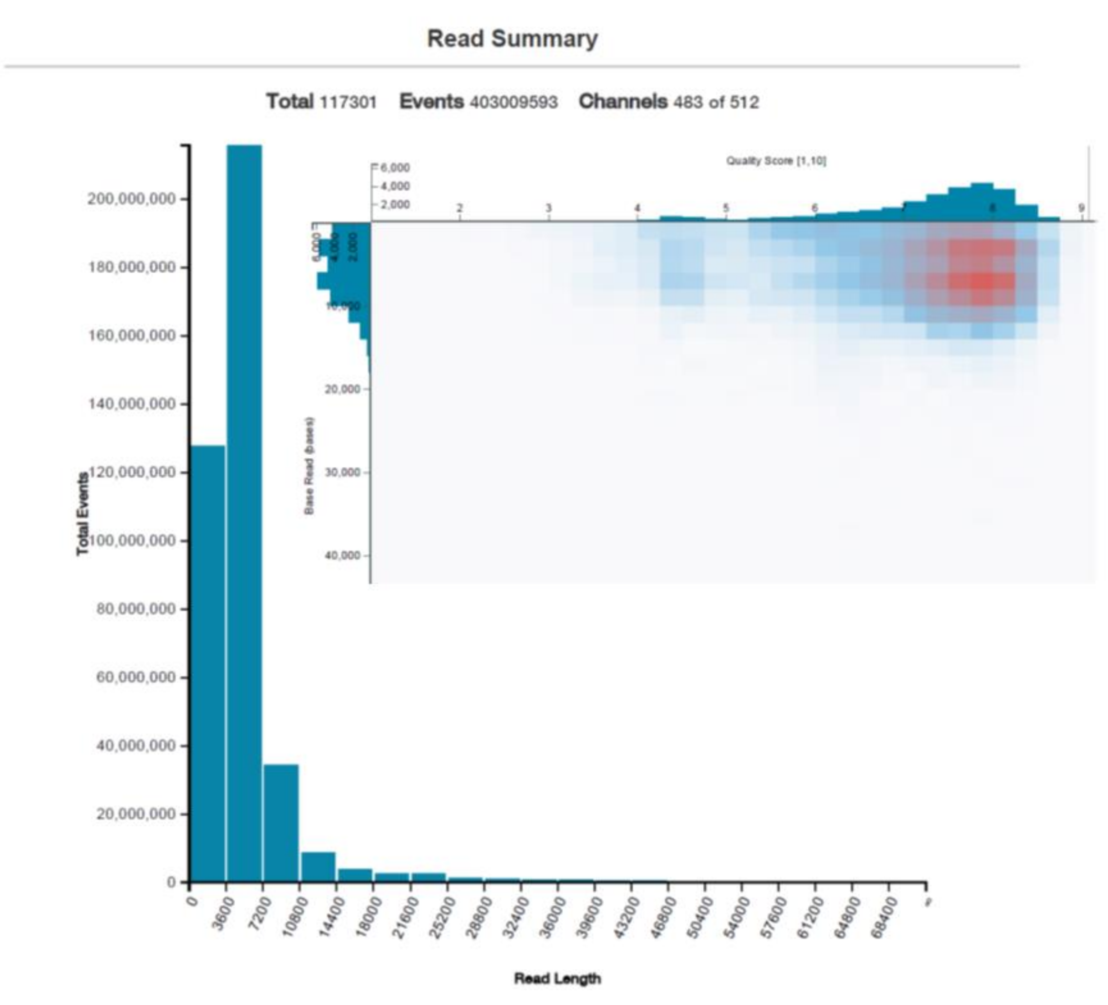
POC: End-to-End pre-sequencing library prep

- Our protocol takes advantage of mRNA’s native poly(A) tail to eliminate end-prep for adapter ligation.
- After magnetic bead cleanup

1. Synthesize 1st and 2nd strand cDNA using SuperScript II and WL2 SmartCycler for thermal cycling
2. Attach Tether and Adapter via ligation to the polyA and polyT endcaps respectively.



POC: End-to-End Sequencing Run MinION 6 hour with Local Basecalling Enabled



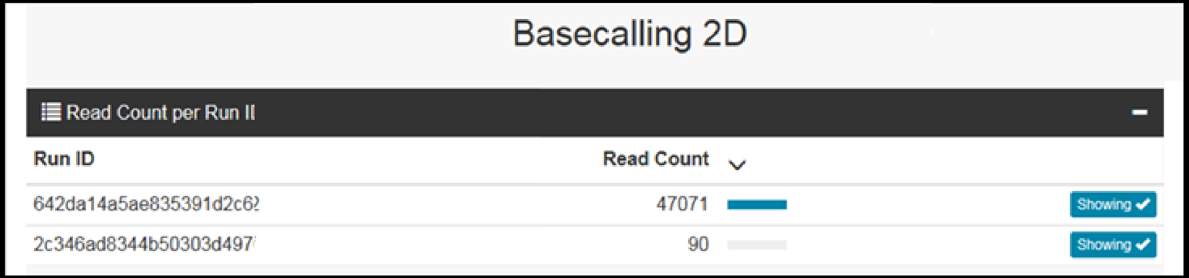
Read Summary

Total 117301 **Events** 403009593 **Channels** 483 of 512

- The MinION device translates the difference in electrical potentials across a non-conductive membrane into single base pair resolution genome sequences.

- MinKNOWN, the MinION software, identifies sequence lengths and basecalling reads in real time.

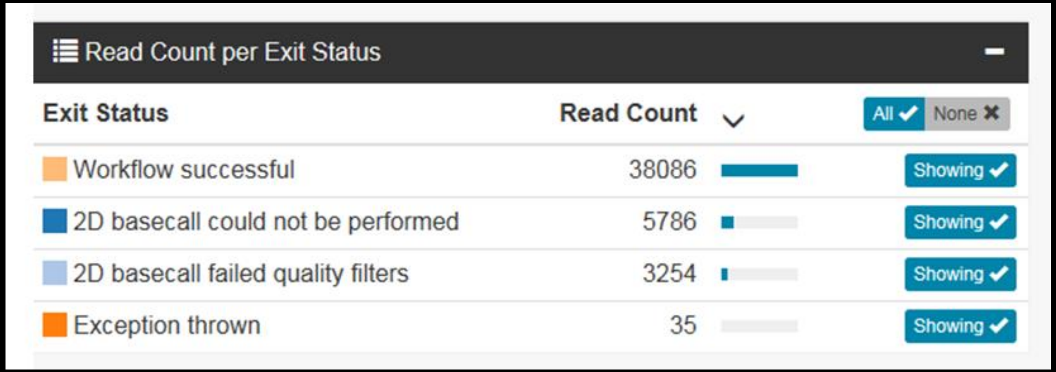
POC: Metrichor Analysis of Sequencing run Success



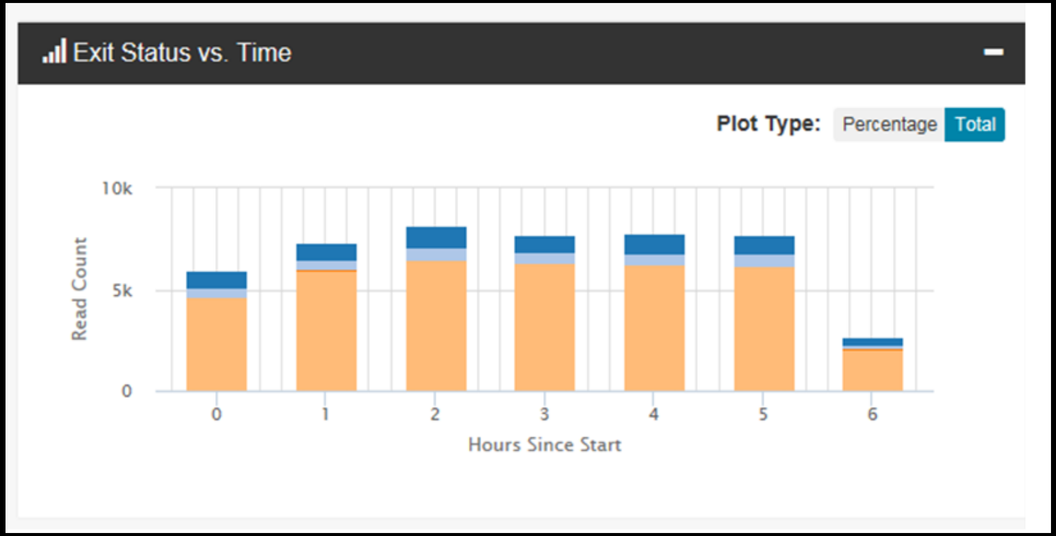
Basecalling 2D

Run ID	Read Count		
642da14a5ae835391d2c6	47071		Showing ✓
2c346ad8344b50303d497	90		Showing ✓

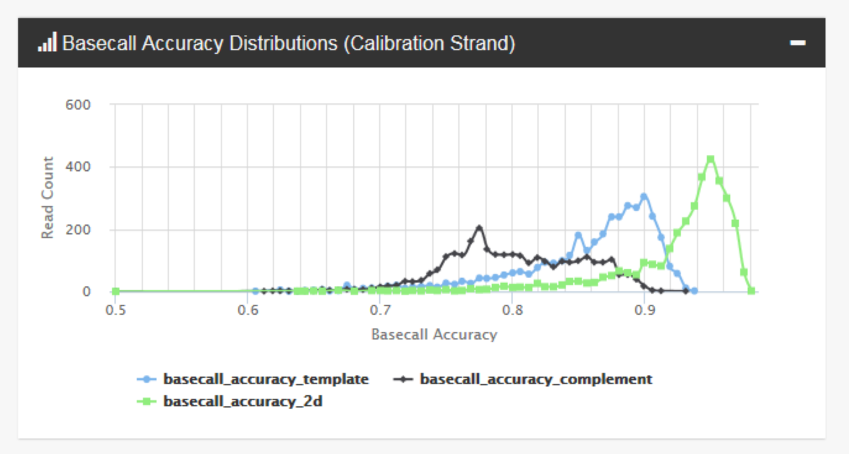
- After basecalling data was uploaded into Metrichor (a cloud based analysis program) and evaluated for
  1. Successful basecall
  2. Exit Status – associated with successful basecalling



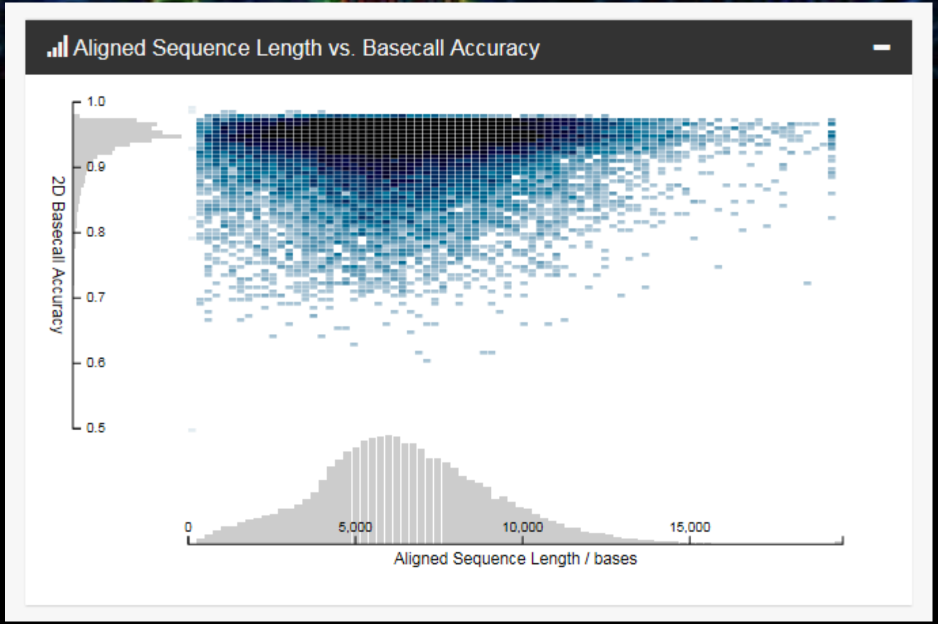
Exit Status	Read Count		
🟧 Workflow successful	38086		Showing ✓
🟦 2D basecall could not be performed	5786		Showing ✓
🟦 2D basecall failed quality filters	3254		Showing ✓
🟧 Exception thrown	35		Showing ✓

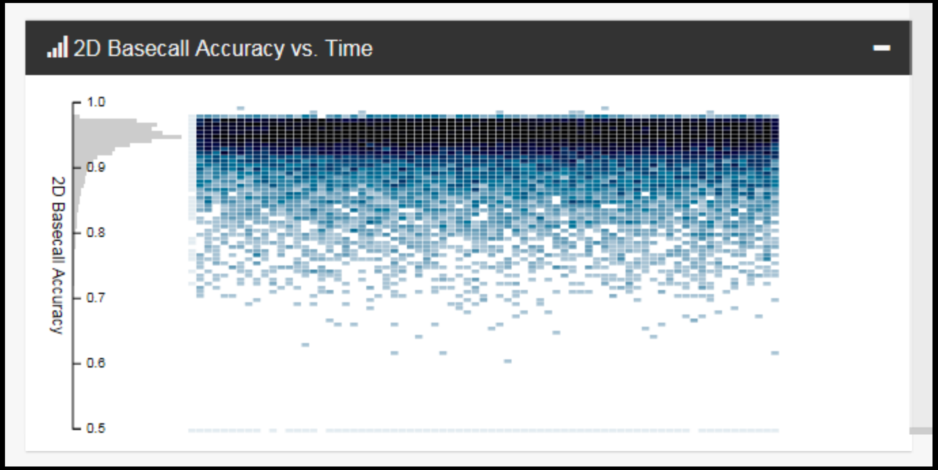


POC: Metrichor Analysis of Sequencing run Success



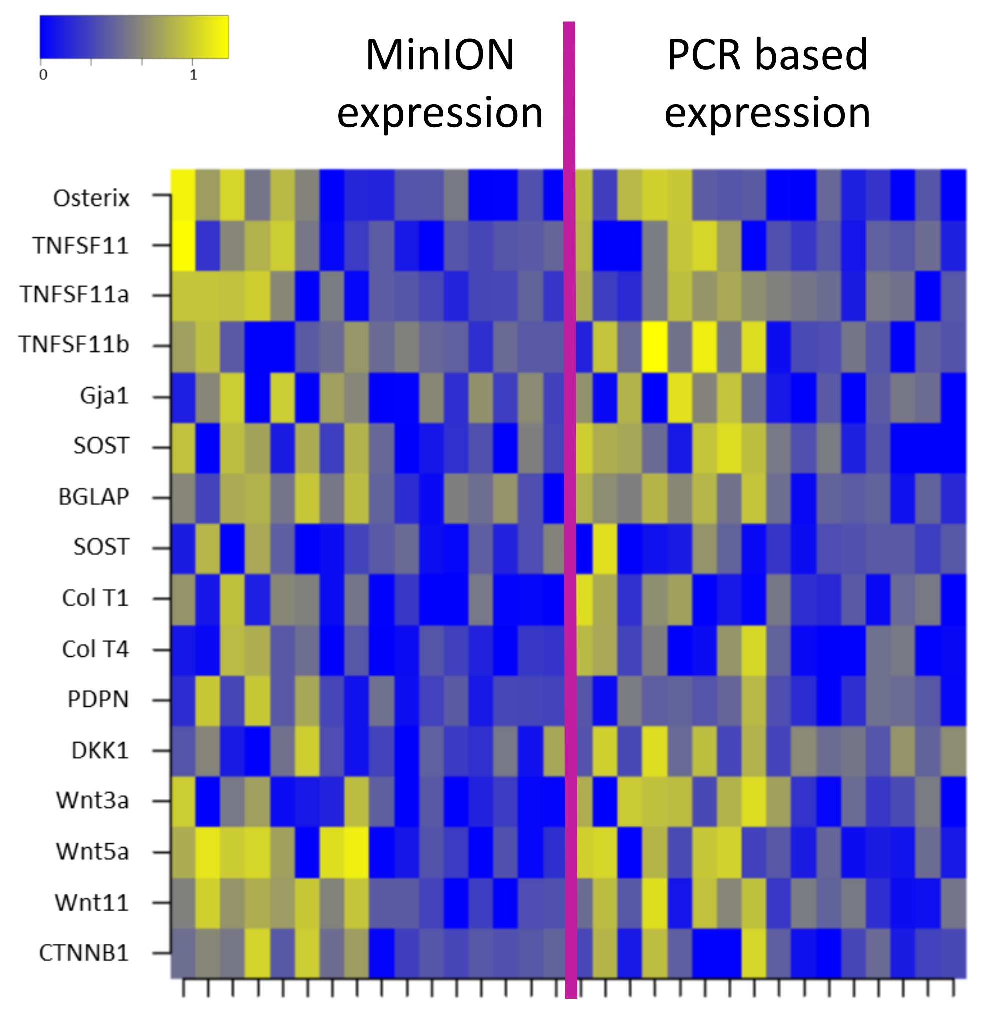
- Data is compared to a Calibration Strand for accuracy and alignment.
- 2D accuracy is above 95% for all Aligned reads.
- Average sequence length is 6,400 bases.
- Long read with high accuracy





POC: End-to-End Expression of Key Gene Sets

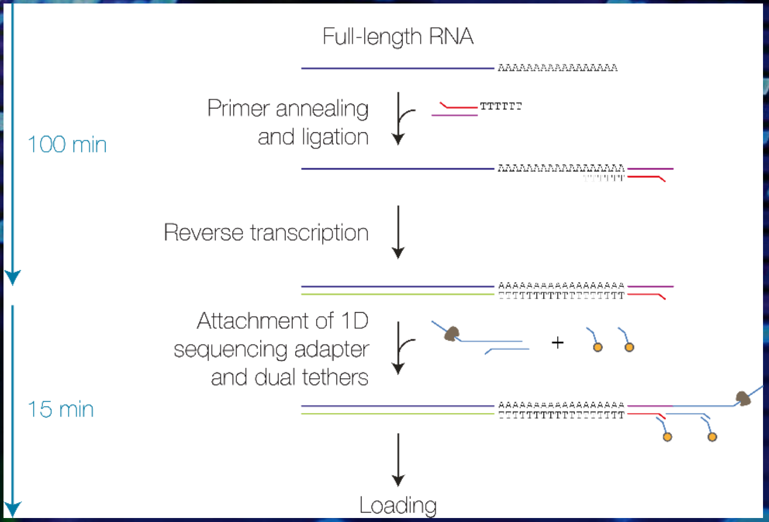
- Last using a Command Line based open source analytic tool called **poRe** visualization of expression information was established for 16 mouse genes (expression compared to GAPDH control).



Proof-of-Concept Conclusions

- We have established proof of concept for novel end-to-end molecular biology approach to sample sequencing with technology already on-board the ISS.
- Our method will enable more fundamental space bioscience investigations. Eliminate the effects of sample recovery and delay in processing.
- **Real example of continuous science in space!**

Caviat: Oxford Nanopore has greatly improved the chemistry supporting library preparation and there are now kits available which accomplish similar outcomes to those presented herein.



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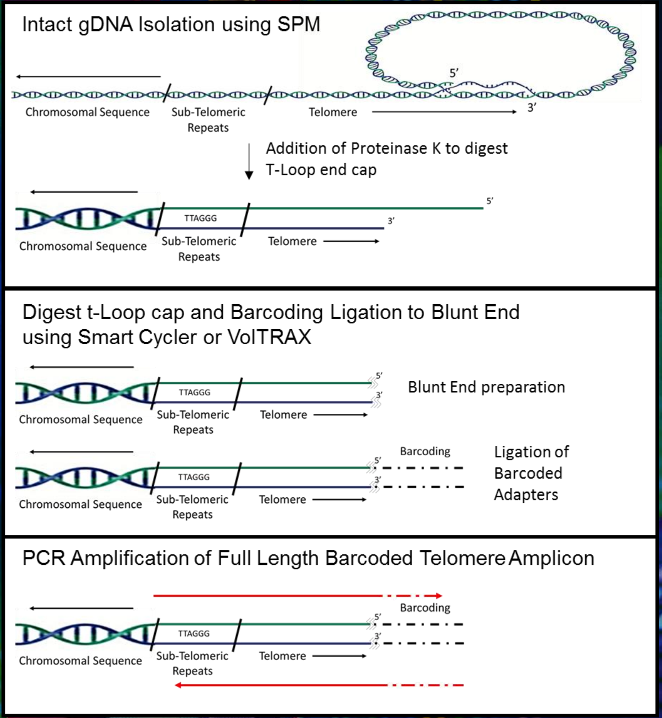
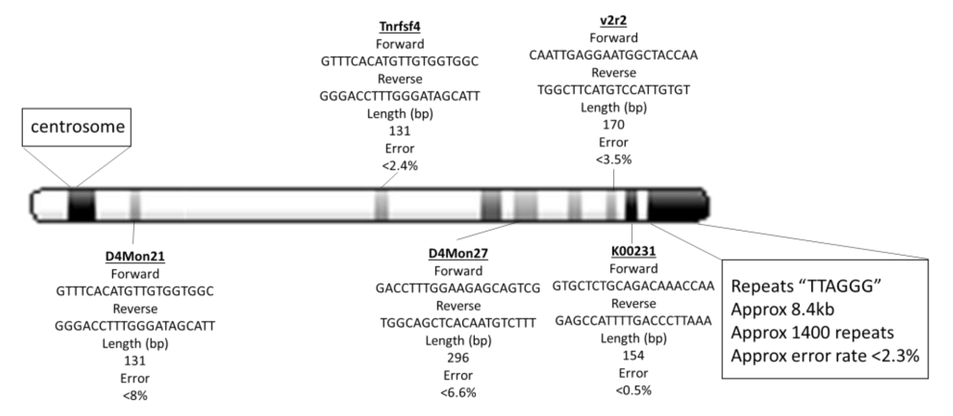
Next steps: **Automation and practical application**

VolTRAX automated library preparation device. Uses magnetic ferrofluids to mix and thermal cycle reagents and prepare the sequencing library



Quantification of individual chromosome telomere length at single base-pair resolution and development of a health metric to monitor astronaut general health in real time.



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