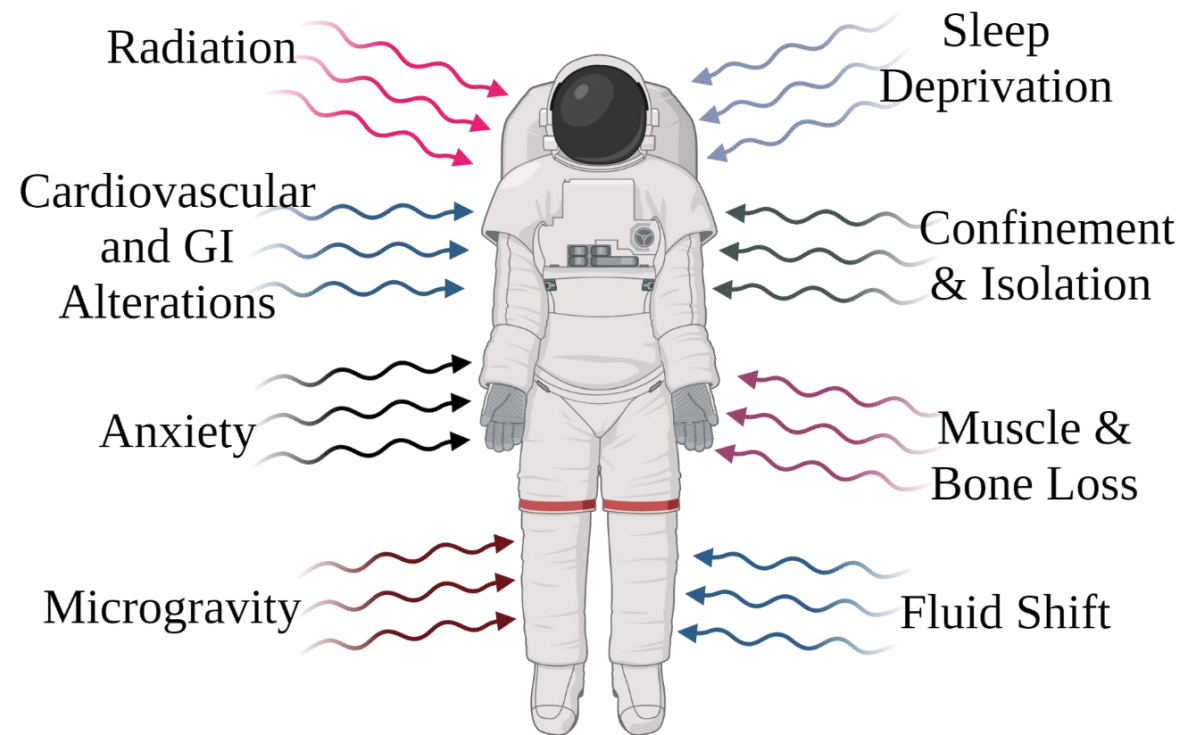




METHOD DEVELOPMENT FOR MULTIPLEX, *IN-SITU*, AND REAL-TIME DETECTION OF HERPESVIRUS REACTIVATION IN SPACEFLIGHT CREWS USING NANOPORE SEQUENCING

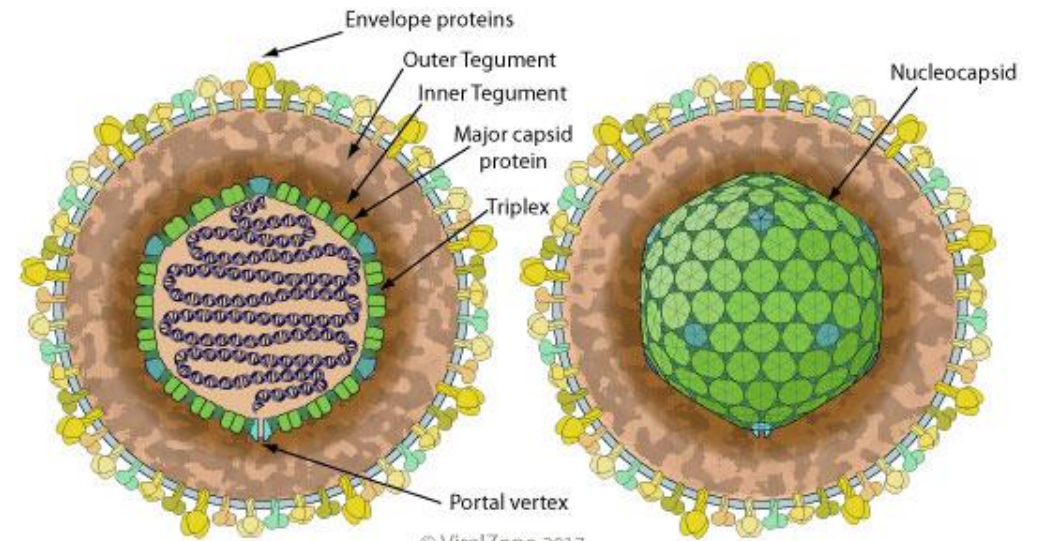
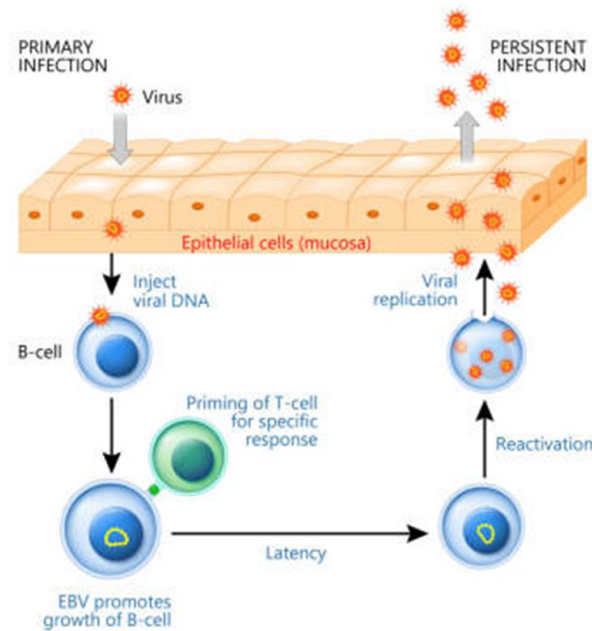
Patrick M. Rydzak, Christian L. Castro, Hang N. Nguyen, Audry C. Almengor, Sarah Stahl-Rommel, Satish Mehta, Douglass M. Diak, Miten Jain, Brian E. Crucian, and Sarah L. Castro-Wallace

Stress in Spaceflight



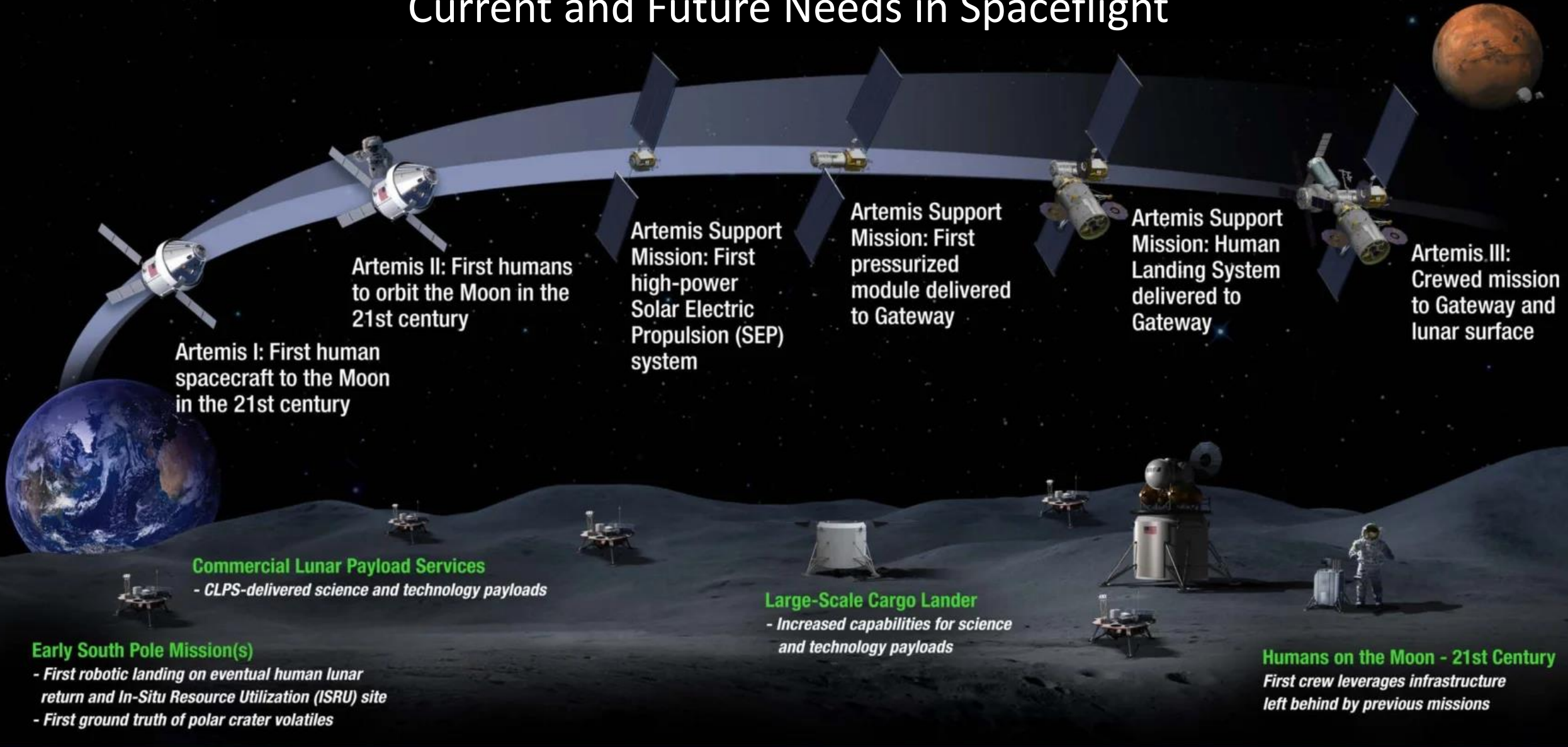
Herpesviruses Reactivation / Shedding as an Indirect Measure of Stress

- Target Viruses
 - Herpes Simplex Virus-1 (HSV-1)
 - Epstein Barr Virus (EBV)
 - Varicella-Zoster Virus (VZV)
- Why these viruses?
 - DNA Viruses
 - Latency -> Reactivation
 - Crew health consequences
 - Detectable in Saliva
 - Actionable data for countermeasures

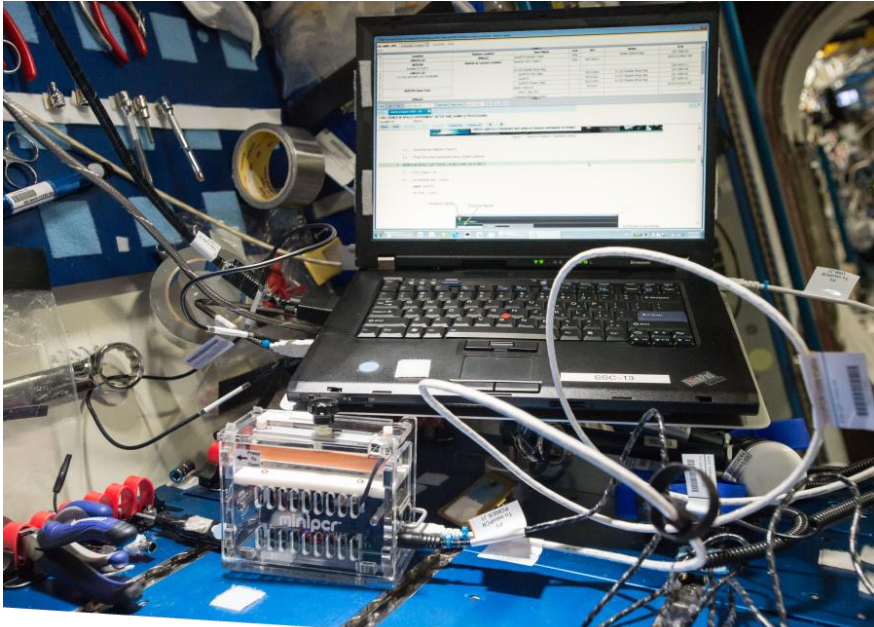
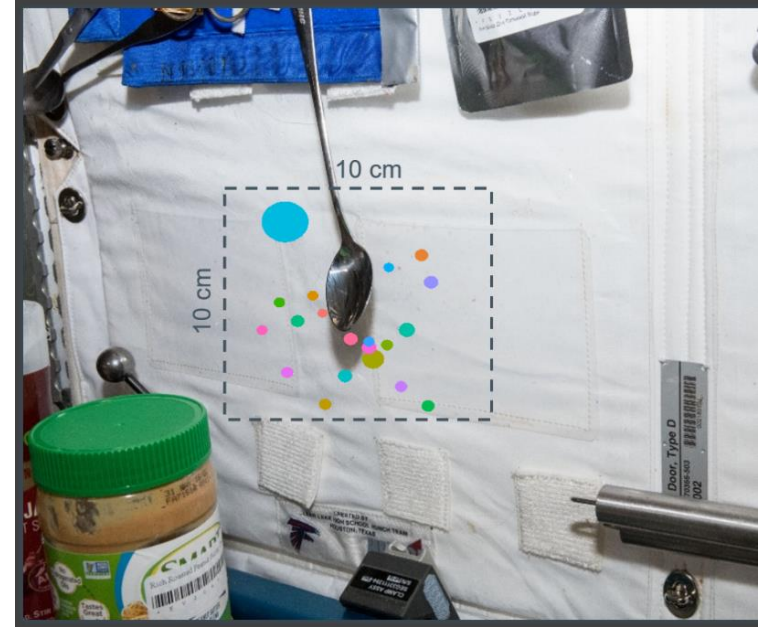
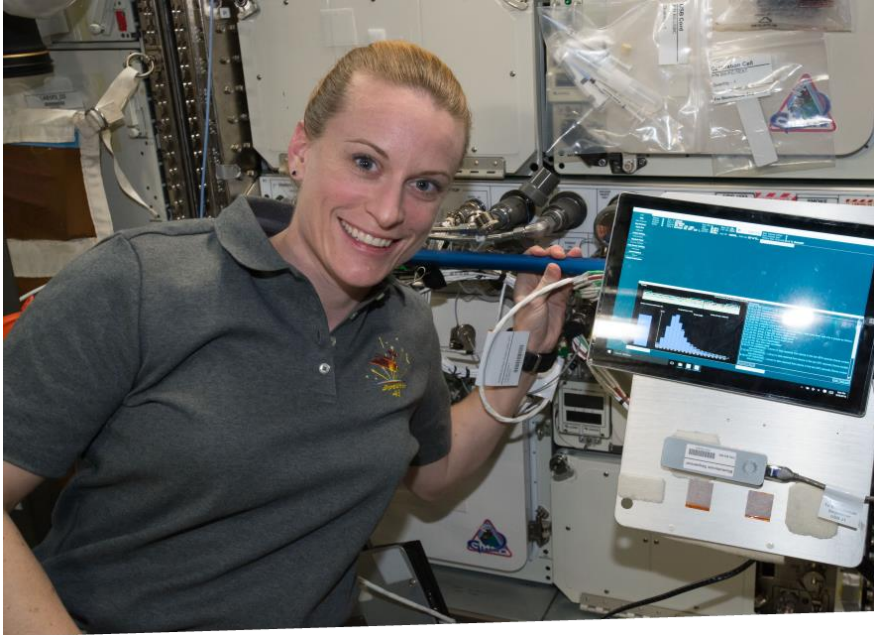


T=16

Current and Future Needs in Spaceflight



LUNAR SOUTH POLE TARGET SITE



DNA Sequencing in Space

Biomolecule Sequencer
Genes in Space-3
BEST
BioMole

Objectives

Develop

- Develop a multiplex PCR capable of detecting
 - HSV-1
 - EBV
 - VZV
 - Internal control
 - Statherin (STATH)

Combine

- Combine multiplex PCR with ONT PCR Barcoding Kit

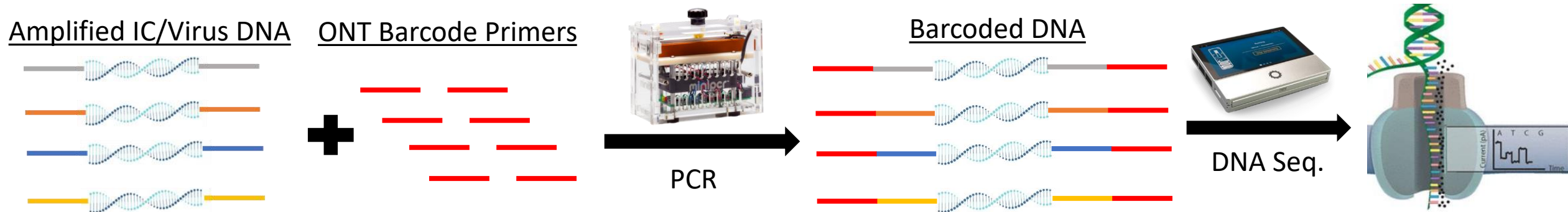
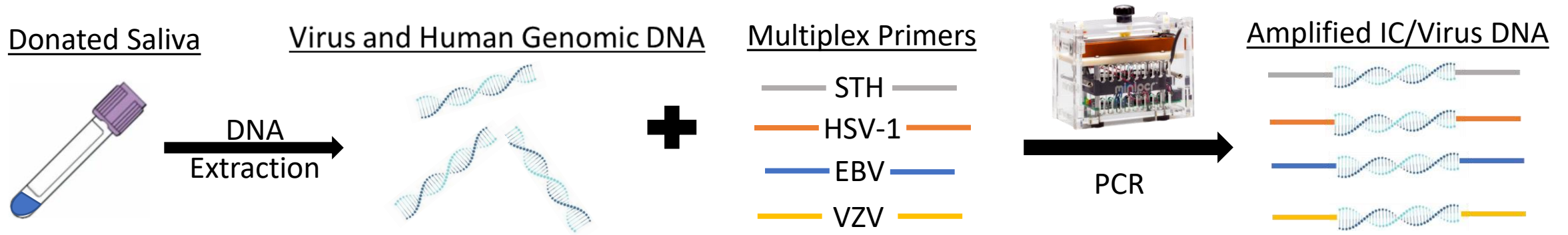
Test

- Test multiplex on positive controls
- Test multiplex on suspected Herpesvirus positive clinical samples

Generate

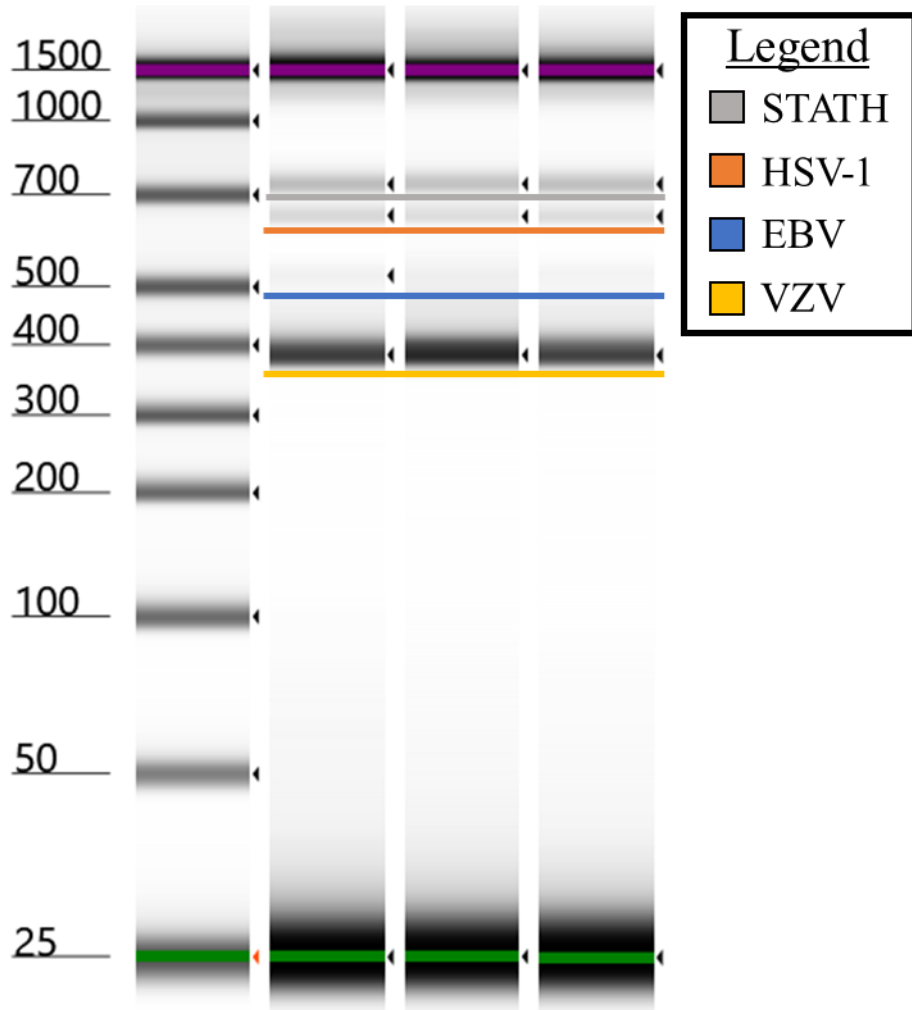
- Generate protocol and kits for use in terrestrial flight analog
 - Palmer Station, Antarctica

Methods

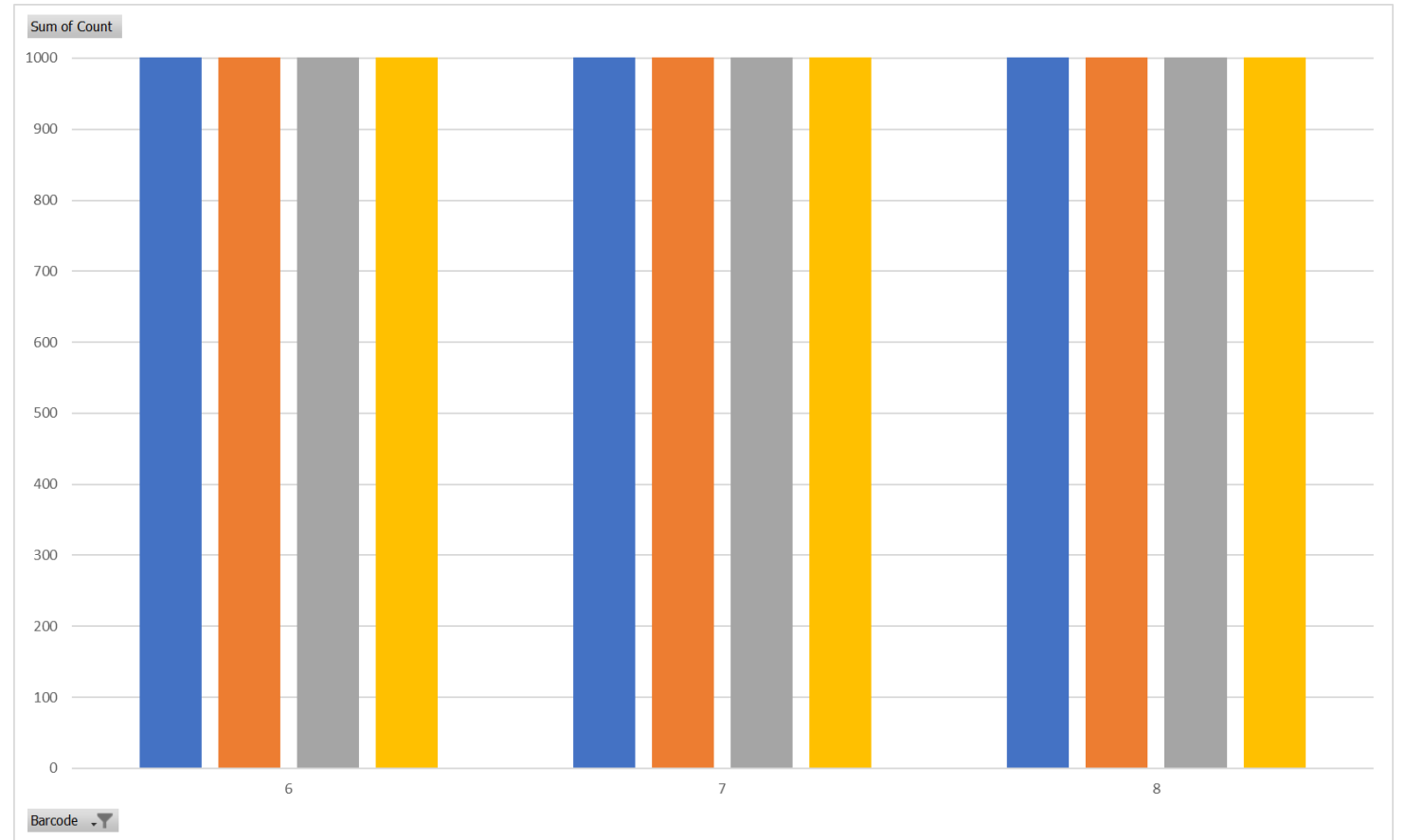


Results: Positive Control Multiplex




Gel Electrophoresis



Sequencing Data

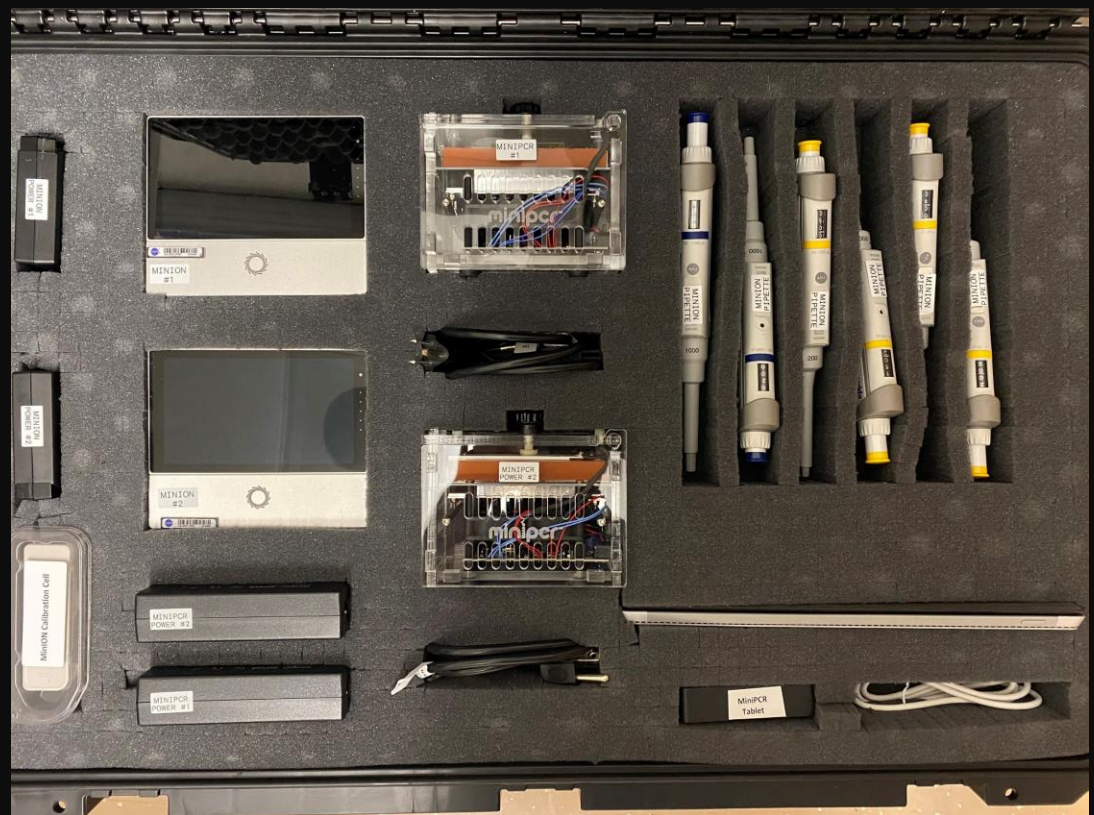
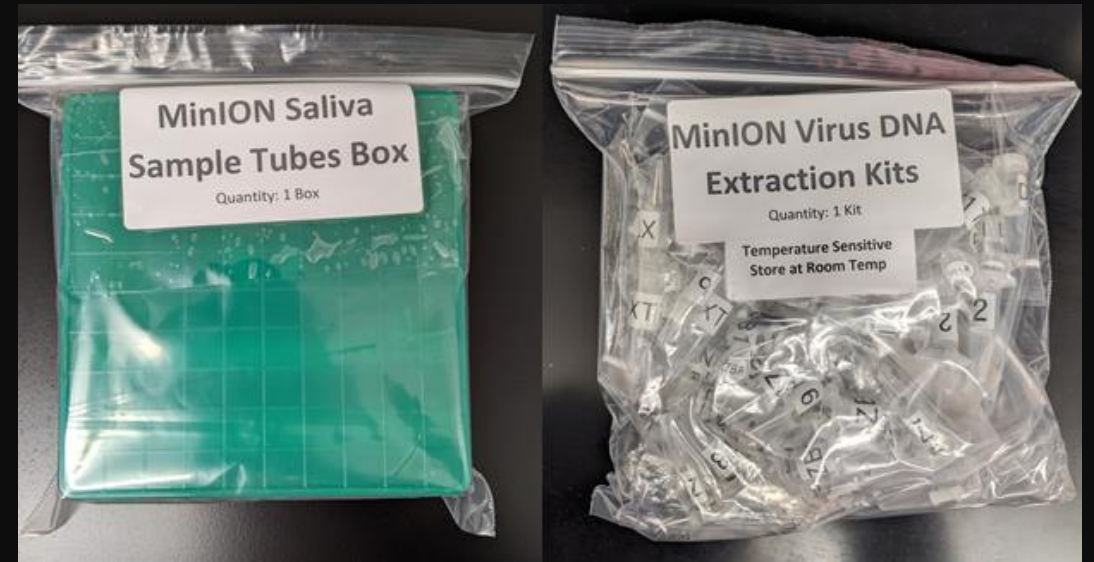


Results: Clinical Saliva Samples

Virus											
		1	2	3	4	5	6	7	8	9	10
 EBV	Seq.	+	-	+	+	+	+	+	+	-	-
	qPCR	-	+	+	+	+	+	+	+	-	-
 HSV-1	Seq.	+	-	-	-	-	-	+	-	-	-
	qPCR	+	-	-	-	-	-	+	-	-	-
 VZV	Seq.	-	-	+	-	+	-	-	-	+	+
	qPCR	-	-	+	-	+	-	-	-	+	+

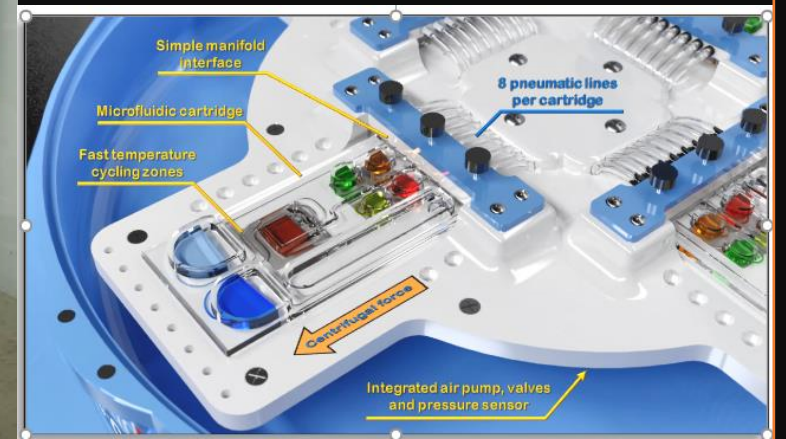
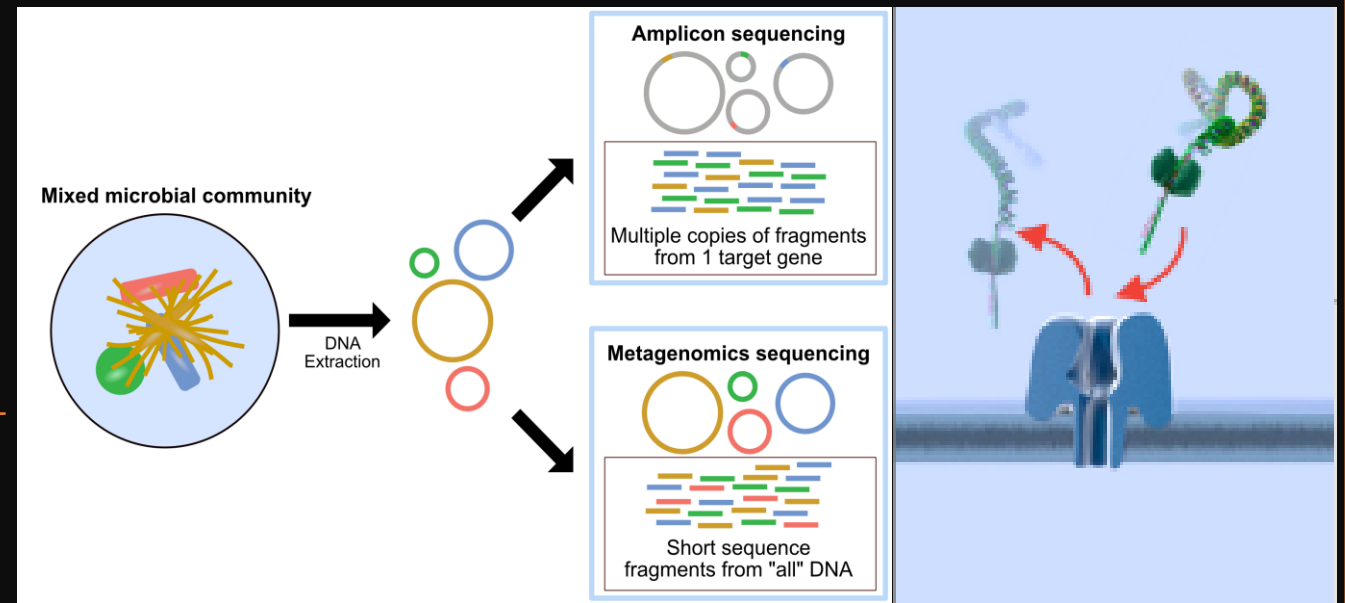
Palmer Station, Antarctica

- Terrestrial spaceflight analog
- In Situ monitoring of viral reactivation in overwinter crew
- Prepared protocol and kits for four sampling sessions
- Materials shipped 2022 for Antarctica winter, 2023



Conclusions and Future Work

- Continue to improve current method
 - Sensitivity
 - Accuracy
 - Ease of use/speed
 - Automation
 - Spaceflight compatibility



Images courtesy of Dr. Teodor Veres, National Research Council of Canada

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