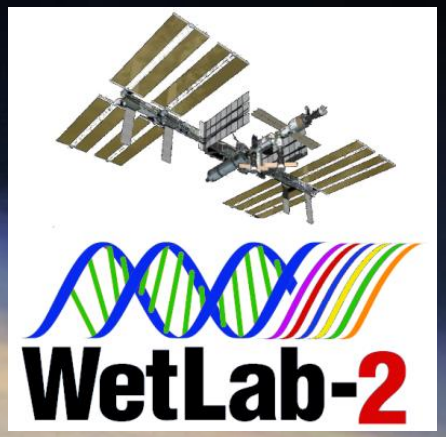
WetLab-2: Providing Quantitative PCR Capabilities on ISS



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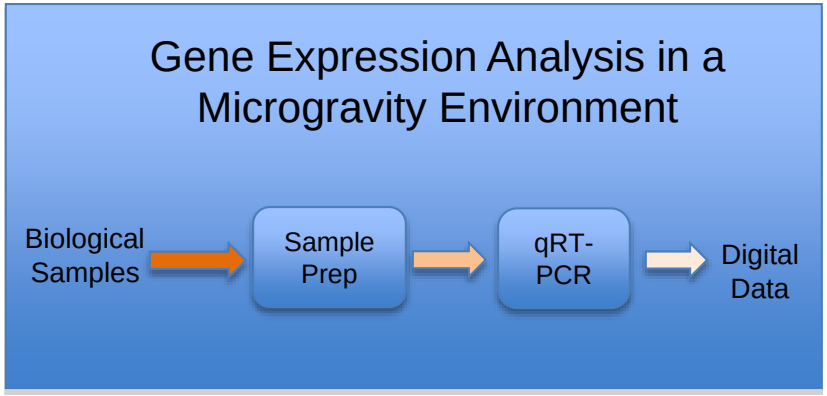
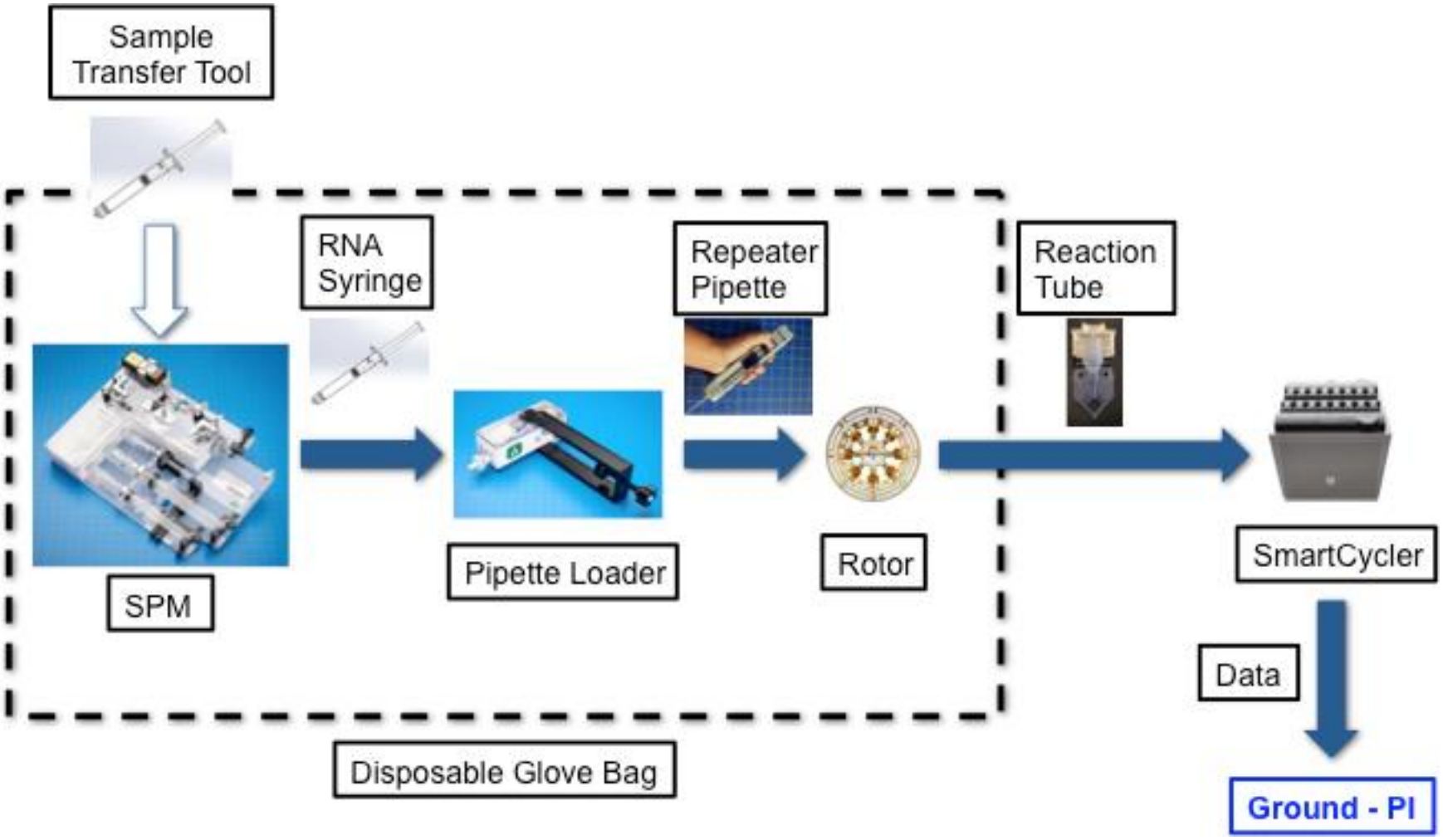
WetLab-2 Objectives

- To place on the ISS a research platform to facilitate space biology gene expression research.
  - Capability to process samples and perform qRT-PCR
- Facility will support multiple sample types (bacteria, cells, tissue)
- The analyzer will remain on ISS, while the experiment-specific disposable hardware will launch with the experiments.
- Also capable of supporting analysis of air, surface, water, and crew health.
- Validation Flight: SpaceX-7



WetLab-2 Operations Overview



Sample Transfer Tool

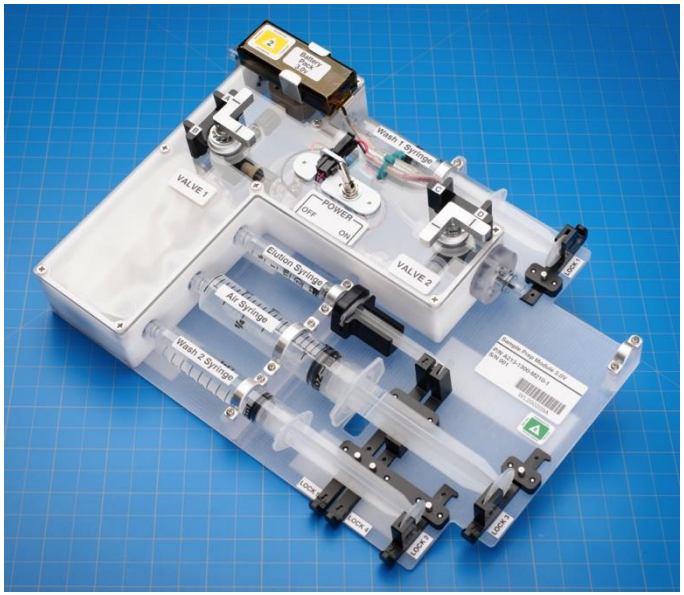
- WL2 uses a Luer-lok connection to accept samples
- Techshot Analytical Containment Transfer Tool (ACT2)
  - Uses luer-lok
  - Provides two levels of containment
  - Maintained throughout sample transfer process
- Standard syringe can be used if two containment levels are not needed

For Validation Flight:

- Will use the 5ml configuration of the ACT2
- Sample (*E. coli*) will be frozen at -80C after loading in unit
- Crew will thaw the sample then use it as input to the SPM
- Will use a standard luer-lok syringe to introduce the tissue

Sample Preparation Module (SPM)

- Function of SPM
  - Breaks open cells and binds RNA to column
  - Washes RNA
  - Elutes RNA into removable RNA syringe



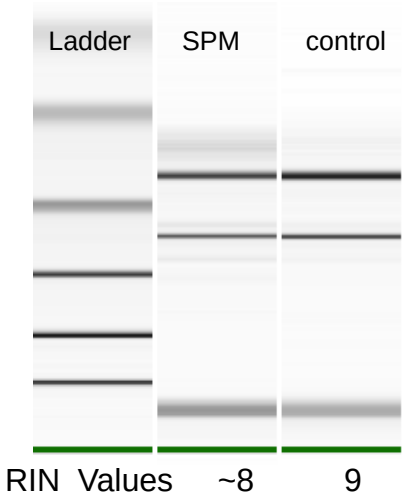
- Two versions: mammalian, bacterial
- Closed system
- Fluids are pre-loaded in syringes
- No alcohols or organic solvents
- Disposable one time use
- Designed to be run in Disposable Glove Bag (DGB)
- Crew manipulations consist of:
  - Attaching and removing syringes
  - Turning OmniLyser on and off
  - Pushing syringe plungers
  - Switching valves

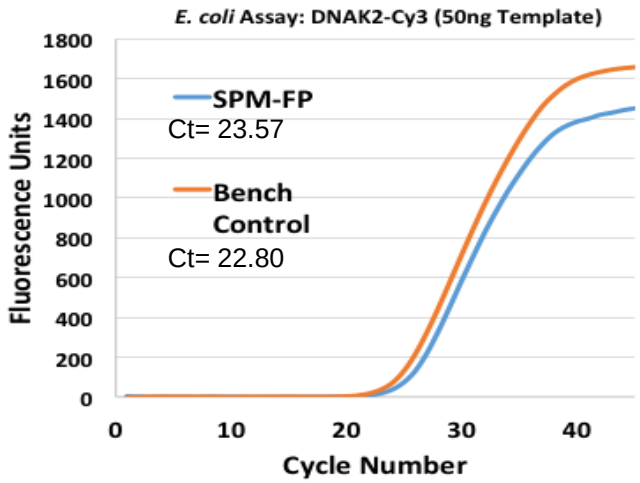
System has been successfully tested on the ground with bacterial and mammalian cells

Sample Preparation Module (SPM)

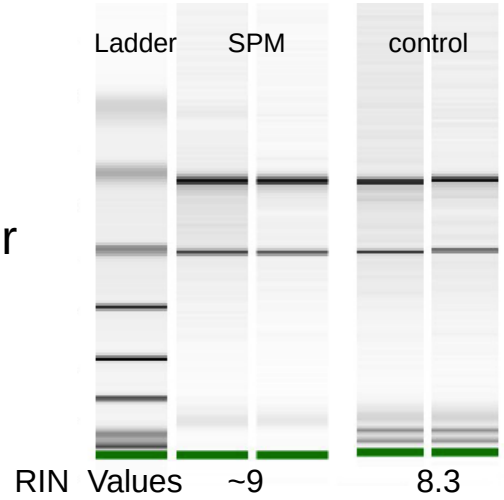
SPM runs give comparable results to those from the already proven ClaremontBio bench procedure

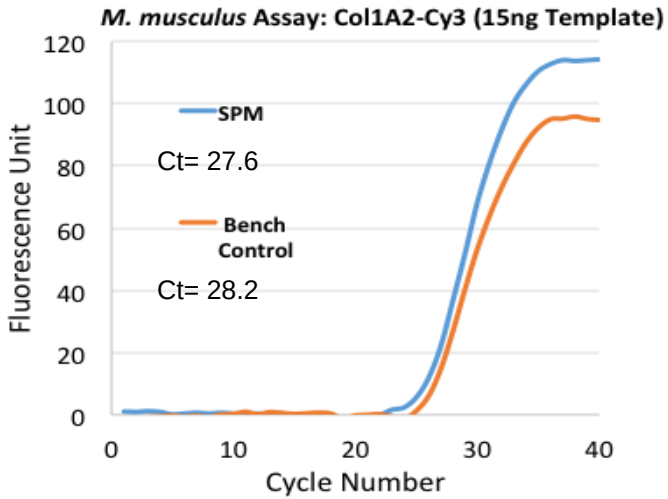
E. coli





Mouse liver

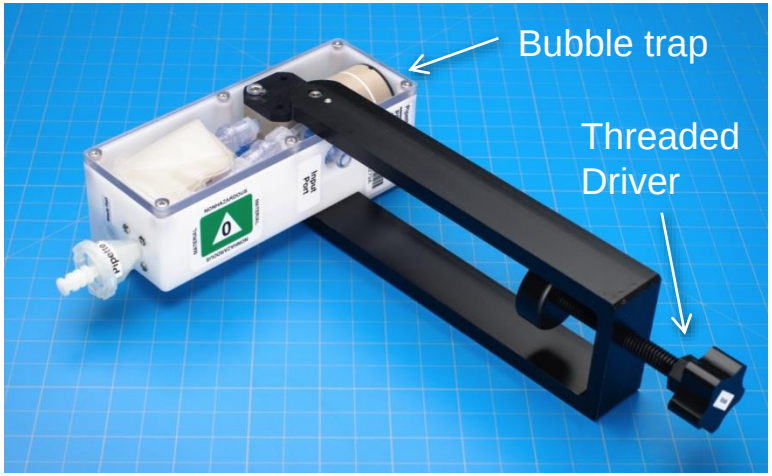




Pipette Loader

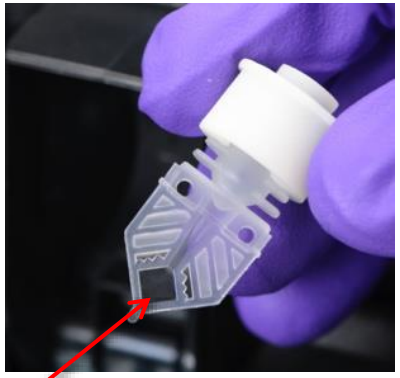
- Function of RAM (Pipette Loader)
  - Removes air bubbles from the RNA sample
  - Loads pipette tip

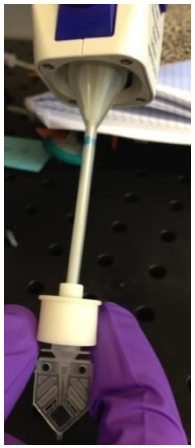
- Closed system
- Designed to be run in the Disposable Glove Bag (DGB)
- No fluids pre-loaded (long shelf life)
- Sample must be driven slowly through the bubble trap to be effective
- Loads the sample into the Repeater Pipette Tip for downstream tube loading

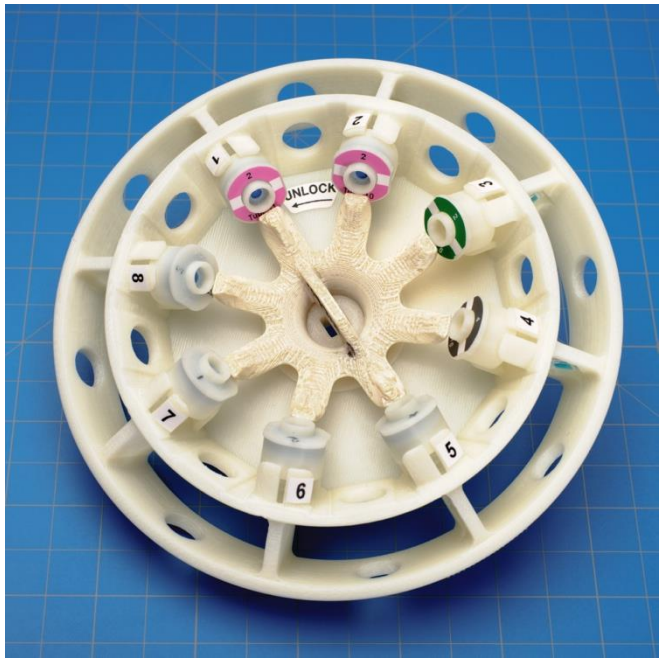


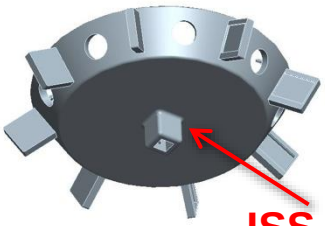
Reaction Tube Loading and Rotor

- Reaction Tube Loading
  - Pipette is used to load 25ul into each Reaction Tube
  - Loading occurs through septum of modified cap
  - Prototypes tested on Parabolic Flight
- Reaction Tube Rotor
  - Holds 8 Reaction Tubes
  - Rotor is attached to drill on ISS
  - Spin to get RNA into the sample window
  - Prototypes used on Parabolic Flight – design changes made based on experience



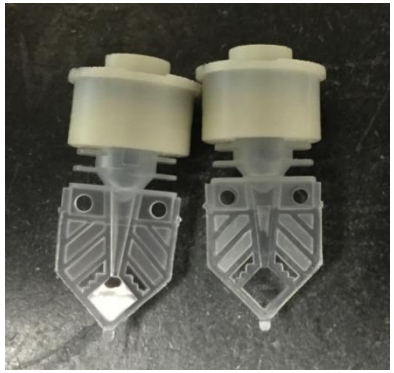




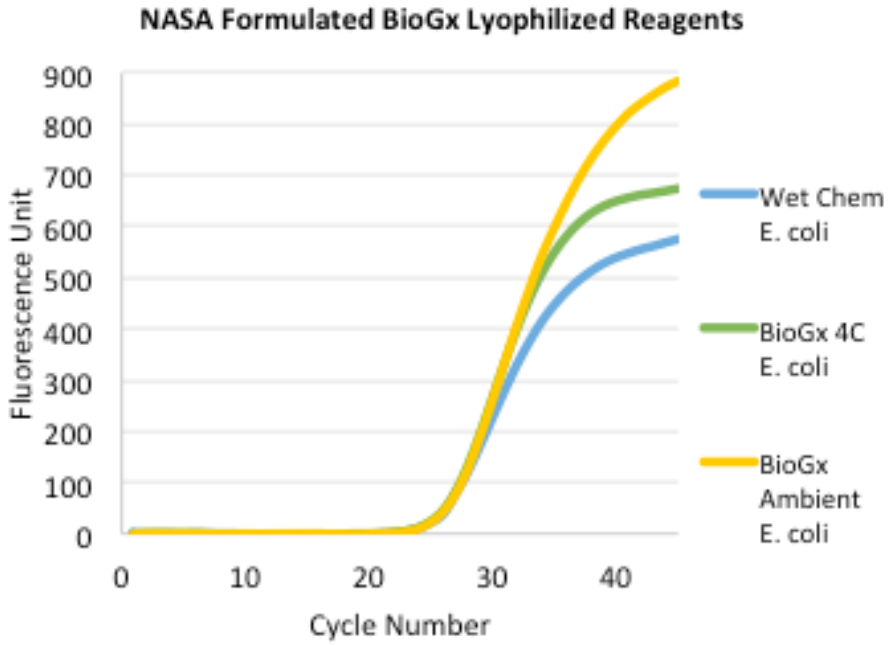


Pre-filled Reaction Tubes

- Pre-filled Reaction Tubes
  - Tubes are a COTS SmartTube with a custom septa cap for loading in microgravity
  - Tubes contain lyophilized primers, probes, enzymes and Master Mix
  - Tubes will be foil packed to protect from moisture and light
  - Lyophilized Reagents give comparable data to commercial wet chemistry reagents

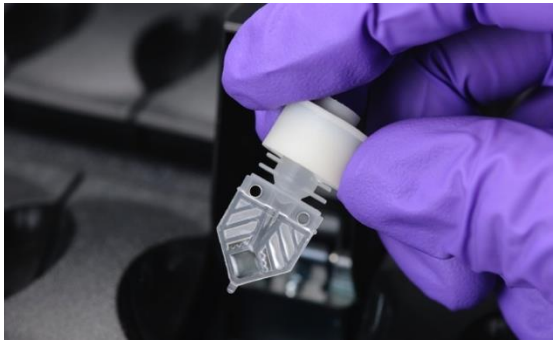


Lyophilized qPCR Assay

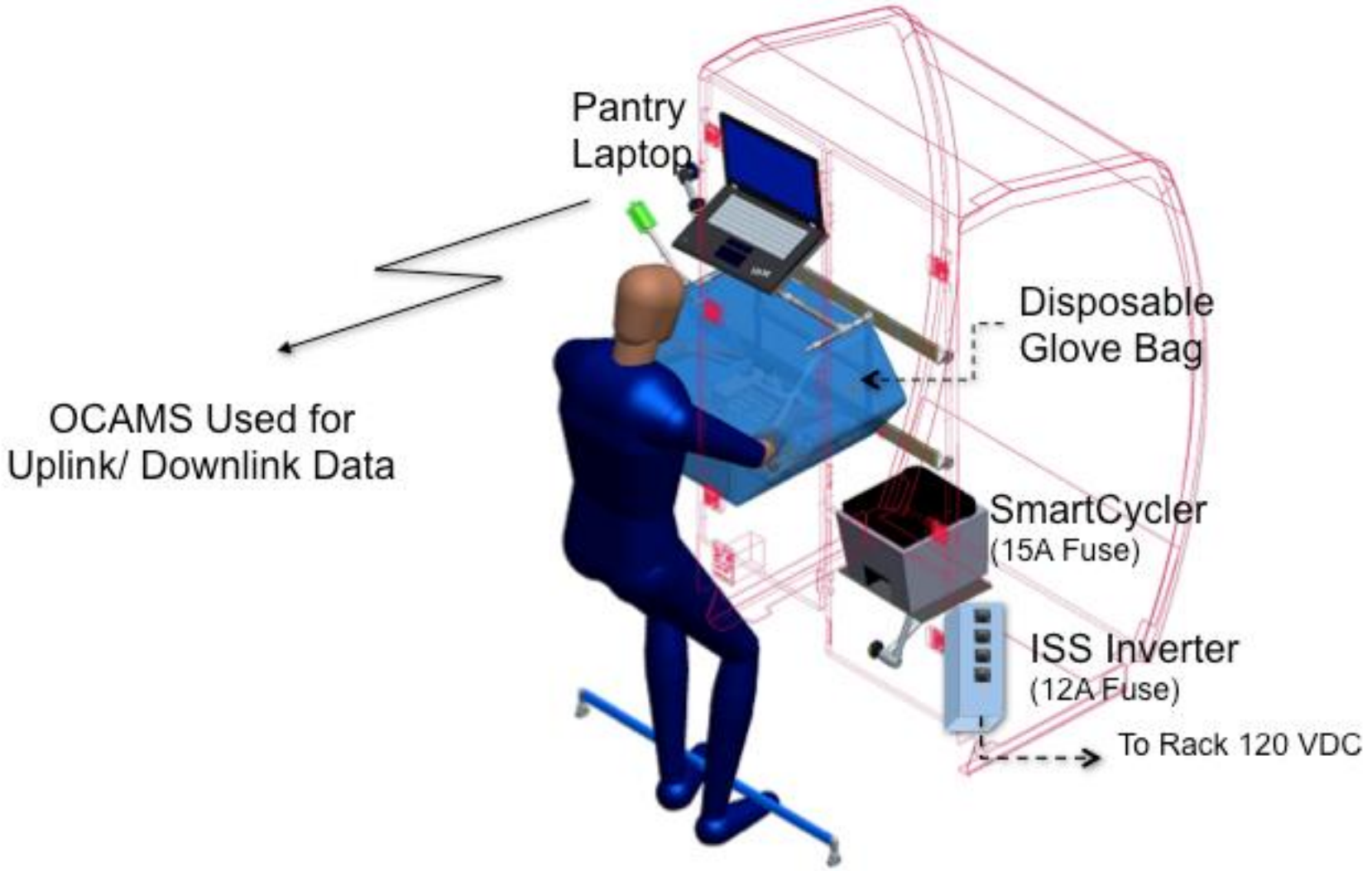


SmartCycler Hardware

- SmartCycler
  - qRT-PCR system
  - Instrument is designed for field work
  - Will fly as a COTS item
  - Will be used in the aisle
  - Mounted on a Bogun Arm on the rack
  - Provides 16 wells and multiplexing capability
  - Thermal programs can be uploaded from the ground
  - Data can be downlinked to ground after run



On-Orbit Configuration



Validation Flight – SpaceX-7

Goal of Validation Flight: On-orbit test and check-out of the WetLab-2 system in a systematic way to ensure it will return valid data to future researchers

Objectives of Validation Flight:

- Install software and set-up hardware (Session 1)
- Does real-time PCR data generated on-orbit match data on earth? (Session 2)
  - No convection or other microgravity related issues
  - Validate SmartCycler, RAM, tube loading and rotor functions
- Does the Sample Processing Module function correctly on-orbit? (Session 3)
  - All fluidic manipulations function properly
  - Prove out system with first sample type (*E. coli*)
  - Test system using on-orbit isolated RNA as input to SmartCycler
- Does system function correctly on-orbit with tissues? (Session 4)
  - All fluidic manipulations function properly
  - Prove out system with second sample type: mouse tissue

Flight results from each session will be compared to results from ground controls

Ground controls will be run with a 2-24 hour delay from the flight samples

End to End Test Data

Session 3: *E. coli* qRT-PCR

Site ID	Protocol	Sample ID	Sample Type	FAM Ct	Cy3 Ct	Cy5 Ct	Avg A	SD	Avg B	SD	Avg C	SD
A1	Session 3	Gene A	E. coli RNA	19.87	0	0						
A2	Session 3	*singleplex*	E. coli RNA	20.06	0	0	20.08	0.25				
A3	Session 3		E. coli RNA	20.44	44.44	0						
A4	Session 3		E. coli RNA	19.94	35.29	0						
A5	Session 3	Gene B	E. coli RNA	0	20.44	0						
A6	Session 3	*singleplex*	E. coli RNA	0	20.13	0			20.29	0.41		
A7	Session 3		E. coli RNA	0	20.78	0						
A8	Session 3		E. coli RNA	0	19.82	0						
A9	Session 3	Genes A&B	E. coli RNA	20.03	20.35	0						
A10	Session 3	*duplex*	E. coli RNA	19.32	20.58	0	19.75	0.42	20.40	0.15		
A11	Session 3		E. coli RNA	19.47	20.22	0						
A12	Session 3		E. coli RNA	20.19	20.43	0						
A13	Session 3	Genes A, B, & C	E. coli RNA	20.24	20.75	22.55						
A14	Session 3	*triplex*	E. coli RNA	20.09	21.71	23.29	19.96	0.89	20.97	0.69	22.98	1.02
A15	Session 3		E. coli RNA	20.81	21.3	24.24						
A16	Session 3		E. coli RNA	18.71	20.12	21.85						

Successful singleplex, duplex and triplex results

This data is typical for post-optimization runs

End to End Test Data

Session 4: mouse liver qRT-PCR

Site ID	Protocol	Sample ID	Sample Type	FAM Ct	Cy3 Ct	Cy5 Ct	Avg A	SD	Avg B	SD	Avg C	SD
A1	Session 4	Gene A	mouse liver RNA	22.68	0	0						
A2	Session 4	*singleplex*	mouse liver RNA	21.83	0	0	22.29	0.35				
A3	Session 4		mouse liver RNA	22.3	0	0						
A4	Session 4		mouse liver RNA	22.36	0	0						
A5	Session 4	Gene B	mouse liver RNA	0	22.15	0						
A6	Session 4	*singleplex*	mouse liver RNA	0	21.07	0			21.62	0.44		
A7	Session 4		mouse liver RNA	0	21.66	0						
A8	Session 4		mouse liver RNA	0	21.61	0						
A9	Session 4	Genes A&B	mouse liver RNA	23.47	21.61	0						
A10	Session 4	*duplex*	mouse liver RNA	23.79	21.48	0	23.86	0.72	21.55	0.15		
A11	Session 4		mouse liver RNA	24.89	21.72	0						
A12	Session 4		mouse liver RNA	23.27	21.37	0						
A13	Session 4	Genes A, B, & C	mouse liver RNA	26.51	23.32	25.83						
A14	Session 4	*triplex*	mouse liver RNA	25.64	23.2	25.71	26.58	0.85	23.38	0.38	26.41	1.23
A15	Session 4		mouse liver RNA	27.7	23.06	25.84						
A16	Session 4		mouse liver RNA	26.48	23.93	28.26						

Successful singleplex, duplex and triplex results

This data is typical for post-optimization runs

Conclusion

- WetLab-2 will facilitate space biology gene expression research
  - Establishes a qRT-PCR analytical instrument on the ISS.
  - Provides a Standard Transfer System for sampling among Wetlab-2 and other bioprocessing & analytical systems (Techshot ACT2)
  - Sample preparation of minimal complexity, can be completed by crew in <2 hours
- Reduce need for downmass of samples due to on-orbit analysis
- Allow researchers to begin to utilize the ISS as a fully working laboratory
  - Results will be available to researchers within hours of run completion allowing for the potential for interactivity with experiments driven by the analysis of results
- Provide on-orbit analysis of air, surface, water, and clinical samples to monitor environmental contaminants and crew health.
  - Results would be available in as little as 90 min compared to current testing that takes 3-6 months due to the need for sample return
- Looking for users of the system after completion of validation flight

WetLab-2 Team

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RIM: Melissa Wallace (Brienne Shkedi)
RPM: Jessica Curry

Note: Wetlab-2 = Wet Lab RNA SmartCycler

Backup Slides

End to End Test Data

Session 2: q-PCR using QC DNA

Site ID	Protocol	Sample ID	Sample Type	FAM Ct	Avg	SD
A1	Session 2	Low Template	E.coli DNA	30.25	29.72	0.38
A2	Session 2		E.coli DNA	29.71		
A3	Session 2		E.coli DNA	29.35		
A4	Session 2		E.coli DNA	29.57		
A5	Session 2	Mid Template	E.coli DNA	22.6	22.55	0.15
A6	Session 2		E.coli DNA	22.59		
A7	Session 2		E.coli DNA	22.67		
A8	Session 2		E.coli DNA	22.33		
A9	Session 2	High Template	E.coli DNA	16.04	16.30	0.45
A10	Session 2		E.coli DNA	16.21		
A11	Session 2		E.coli DNA	16.95		
A12	Session 2		E.coli DNA	15.99		
A13	Session 2	No Template	E.coli DNA	0	0	0
A14	Session 2		E.coli DNA	0		
A15	Session 2		E.coli DNA	0		
A16	Session 2		E.coli DNA	0		

No carryover from tube to tube

This data is typical

Post-Validation/Post-Flight

- Fluidics components are disposable
- SmartCycler to remain on board ISS
- SLPS Principle Investigators who propose to use the SmartCycler with launch fluidics components with science hardware
  - Current estimates indicate SmartCycler use 2-4 investigations per year
  - Reagents loaded in SPM and SmartTubes to be experiment-specific