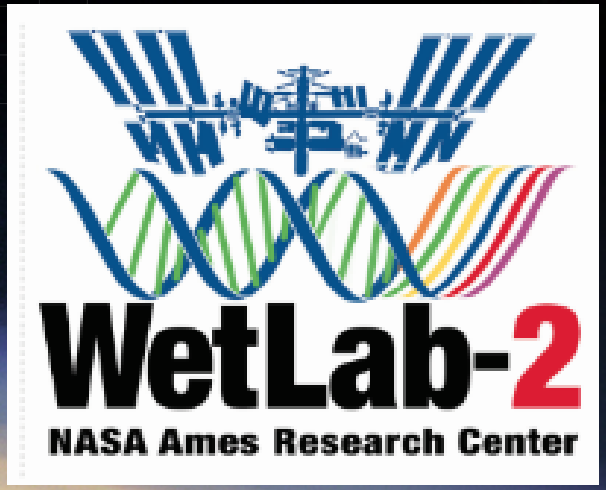
Successful Validation of Sample Processing and qRT-PCR Capabilities on the International Space Station



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Ames Research Center
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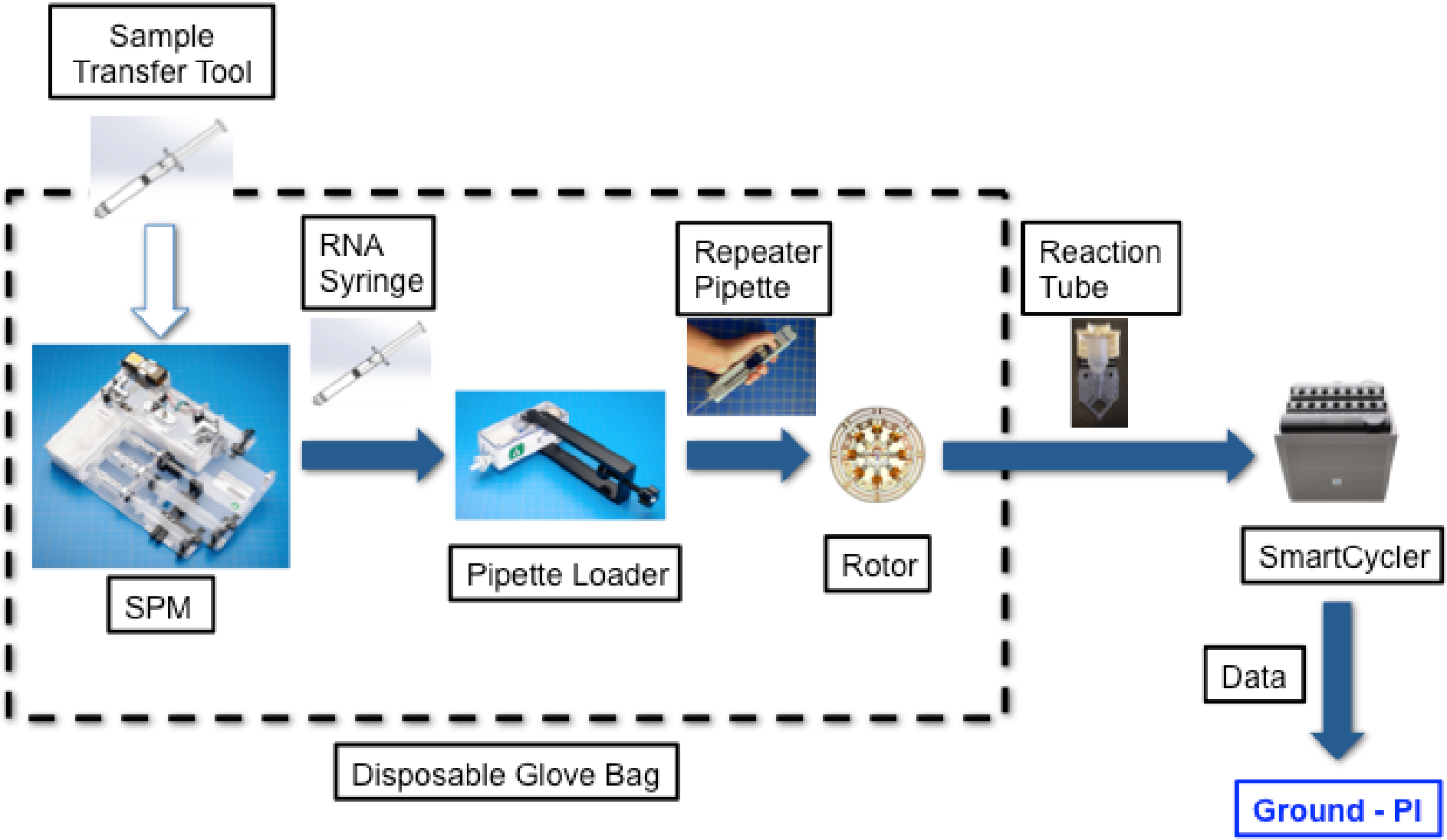
WetLab-2 Objectives

- Provide on-orbit RNA extraction and qRT-PCR analysis capabilities on ISS
- Facility will support multiple sample types
  - Bacteria, cells, tissue
  - Intent of expanding to plant, blood, etc.
- Also capable of supporting analysis of air, surface, water, and crew health.

The analyzer will remain on ISS, while experiment-specific (primers and probes, RNA isolation chemistry) disposable hardware will launch with the experiments.

WetLab-2 Operations Overview



Validation Flight – SpaceX-8

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

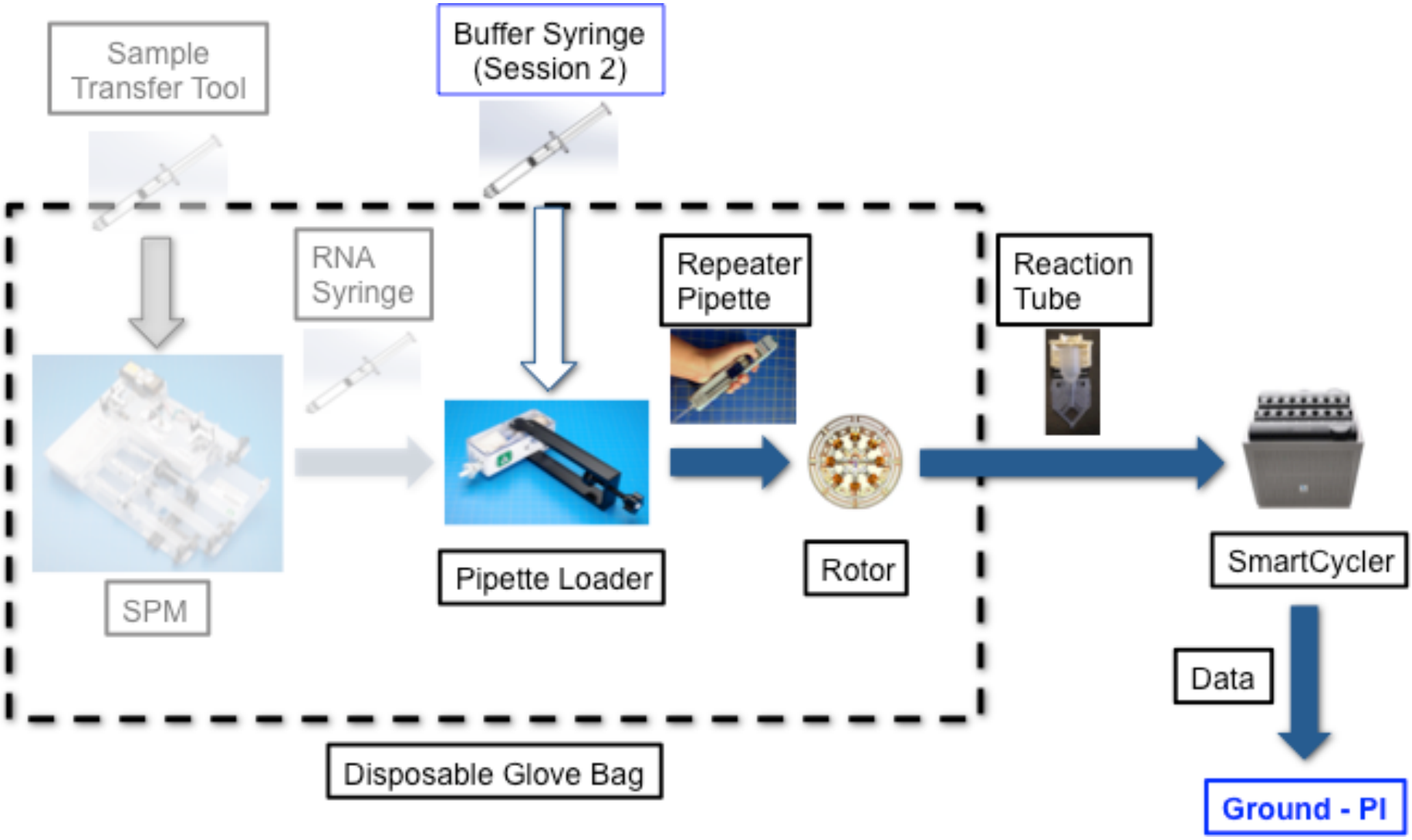
Objectives of Validation Flight:

- Install software and set-up hardware (Session 1) – **April 15**
- Does on-orbit qPCR data match data on earth? (Session 2) – **April 19, 22, and 26**
  - No effect from microgravity related issues (*i.e.* convection)
  - Validate SmartCycler, Pipette Loader, tube loading and rotor functions
- Does the Sample Prep Module function correctly on-orbit? (Session 3) – **April 29**
  - 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) – **May 2**
  - 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

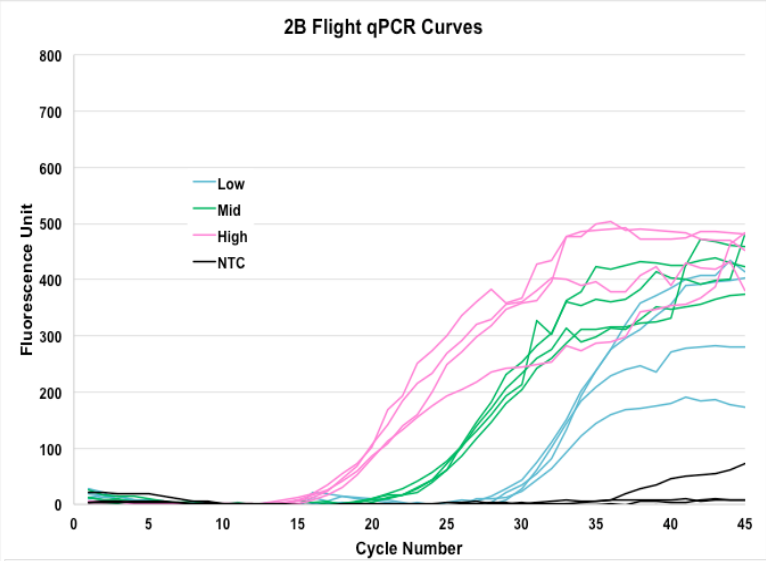
Ground controls will be run with a 2-24 hour delay from the flight samples

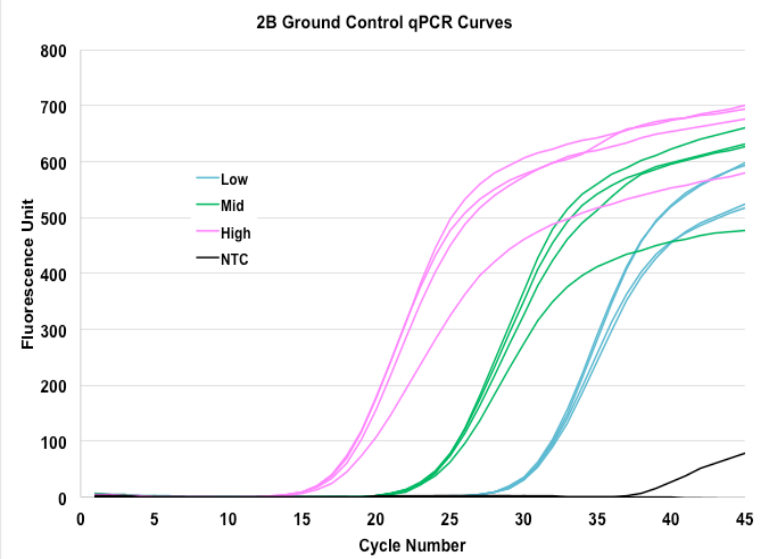
Operations Overview for QC Runs



Summary of qPCR QC Runs

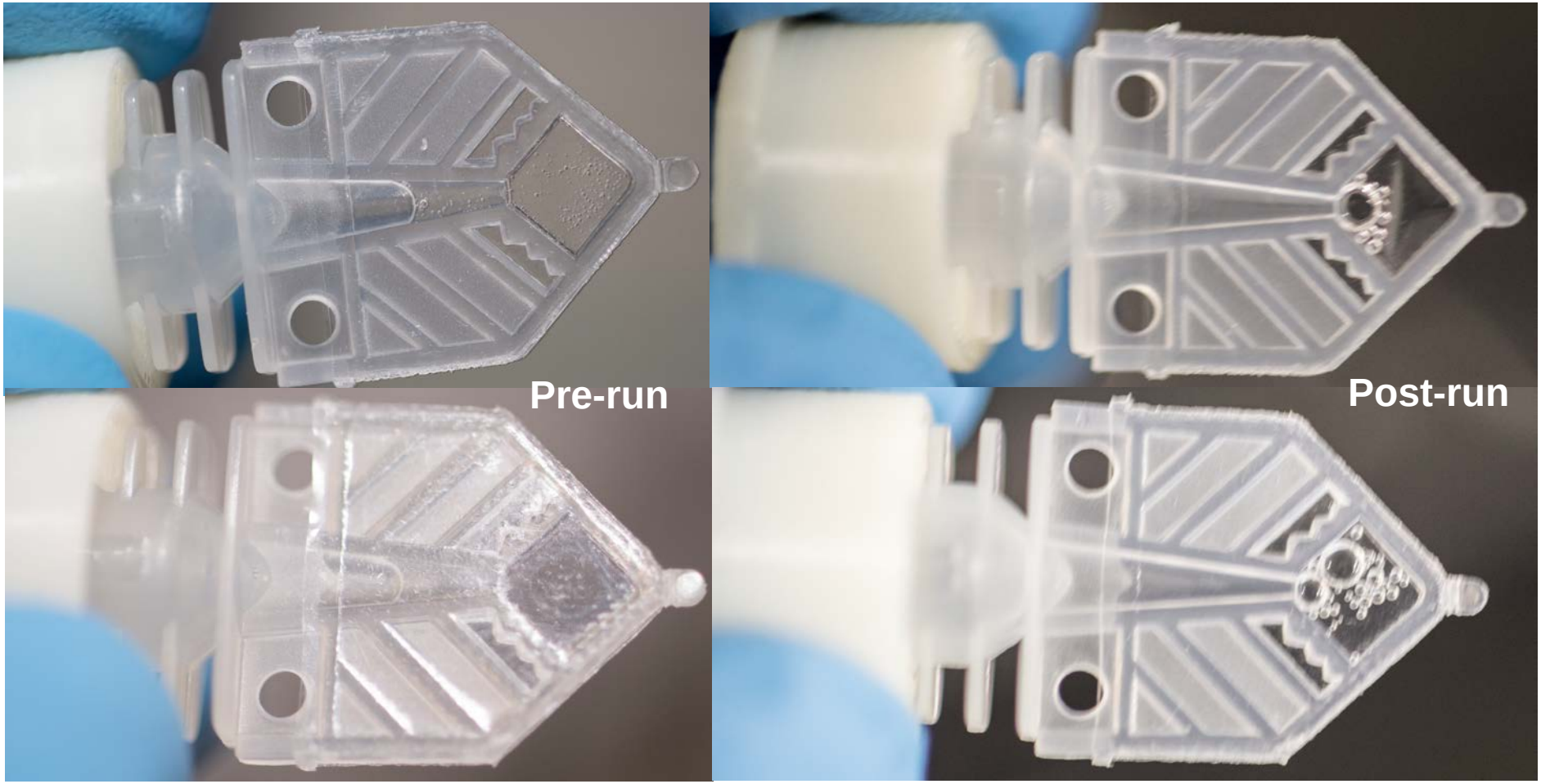
- First two genomic DNA runs – April 19 and 22
  - Nominal operations
  - Good data from all tubes
  - Successful amplification
  - Data comparable to ground runs
  - Raw curves – qPCR works in microgravity
  - Flight data is noisy – to air bubbles?
  - Requested photos of tubes post-run
- Third genomic DNA run – April 26
  - Hardware anomaly in pipette loader
  - Many tubes were not hydrated (14/16)





Tube Photos

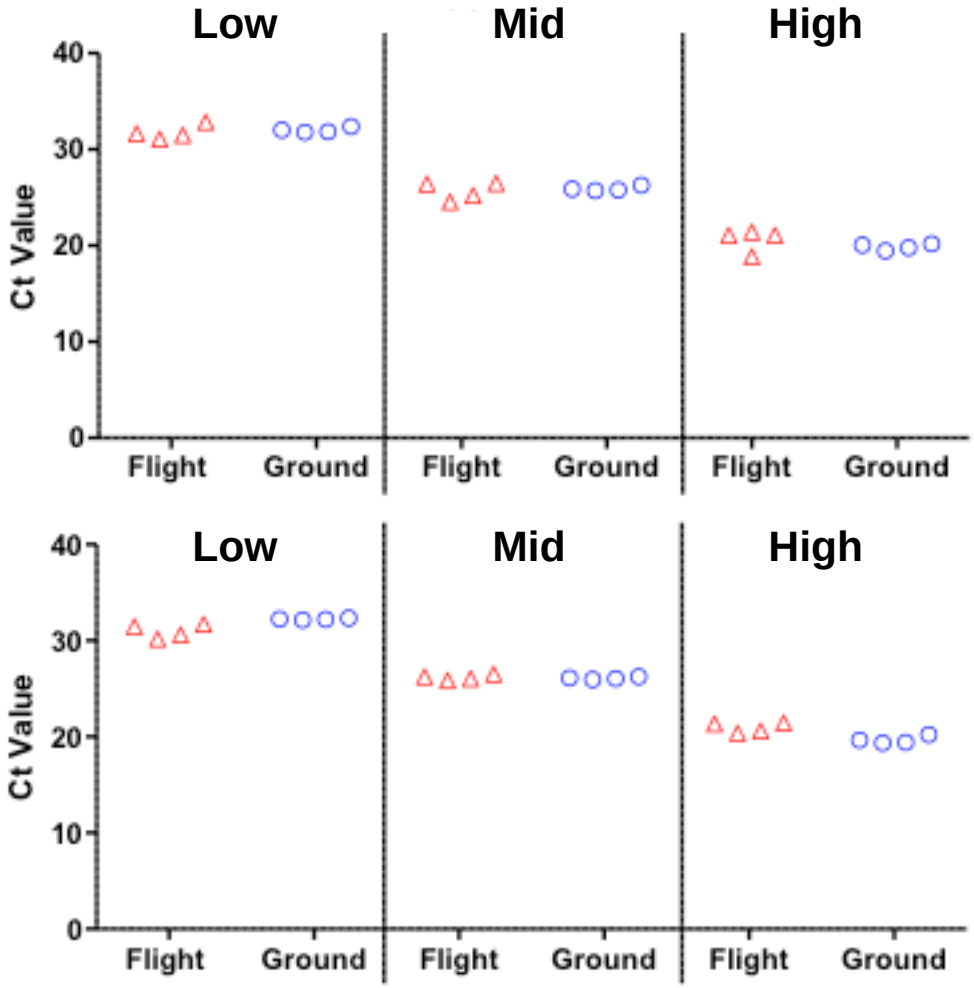
- Pre and post-run photos were taken during last session:



- Applied boxcar averaging correction to both flight and ground data to reduce noise

Cycle Threshold (Ct) Comparison

- Good correlation of Ct's between flight and ground; flight data has higher variance



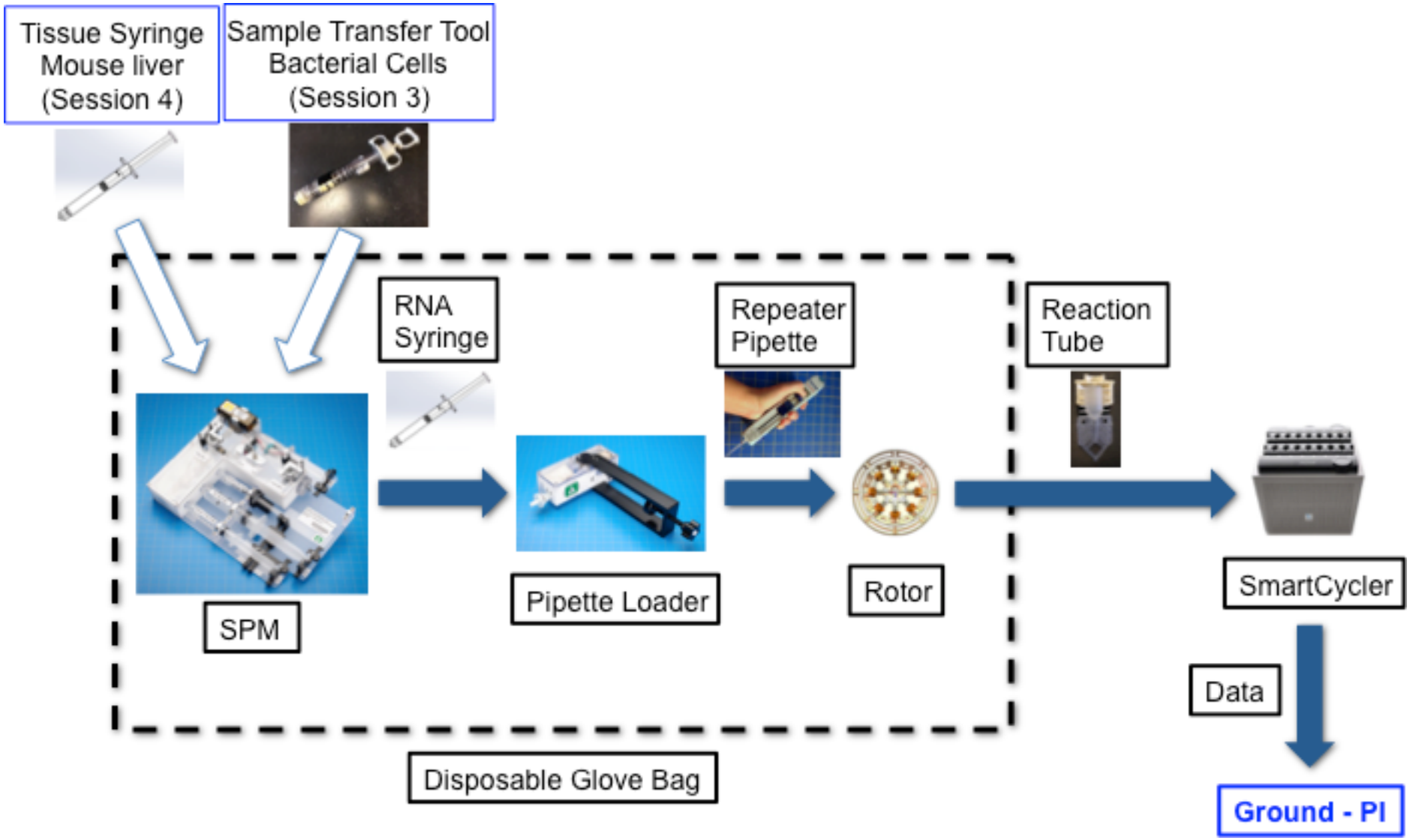
First Run	Efficiency	R squared
Flight	104%	0.962
Ground	105%	0.997

Second Run	Efficiency	R squared
Flight	108%	0.987
Ground	103%	0.999

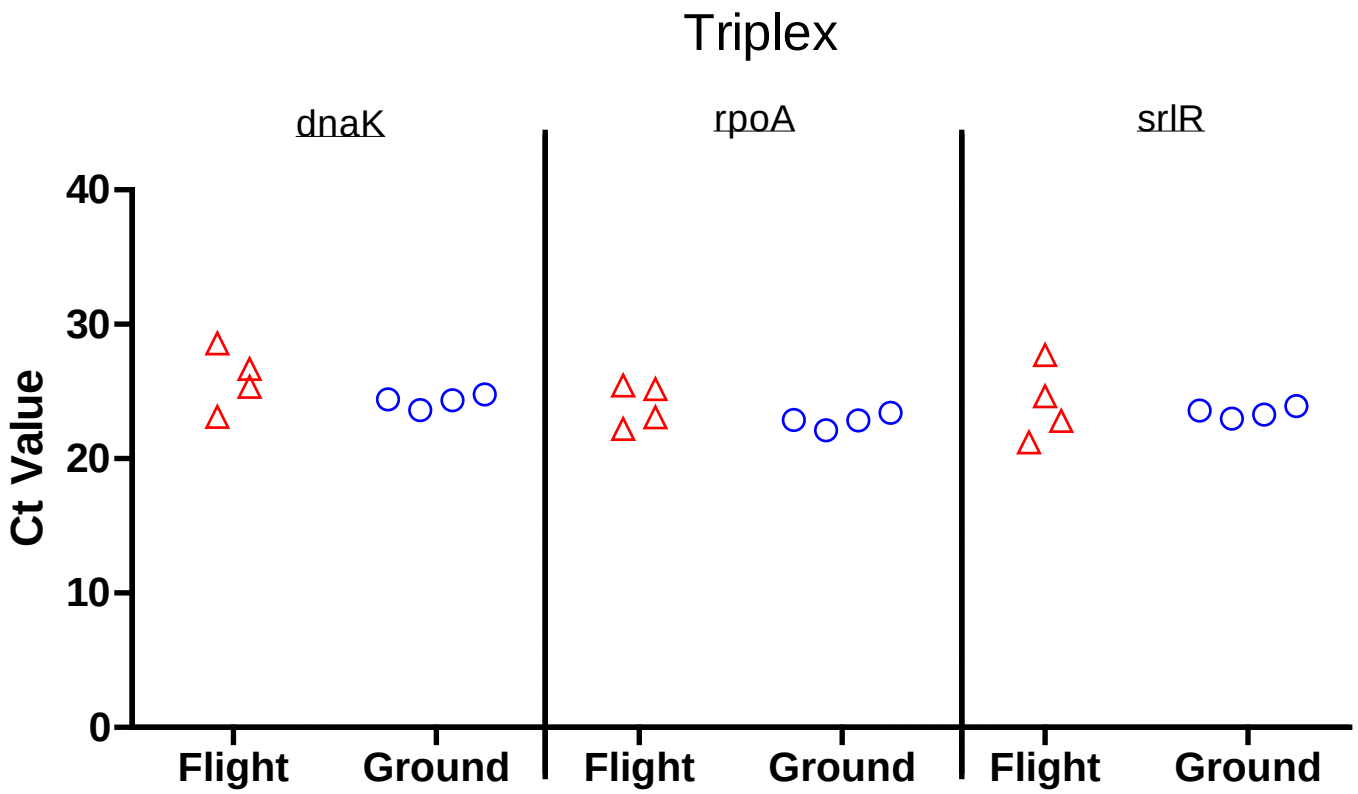
Conclusions from QC Runs

- Data shows that qPCR works in microgravity
- Flight data has noticeable inflections in Ct curves
  - Attributed to air bubbles that form during the thermal cycling
- Slightly larger standard deviation in flight Ct values
- Good PCR efficiencies in flight: 104% and 108%

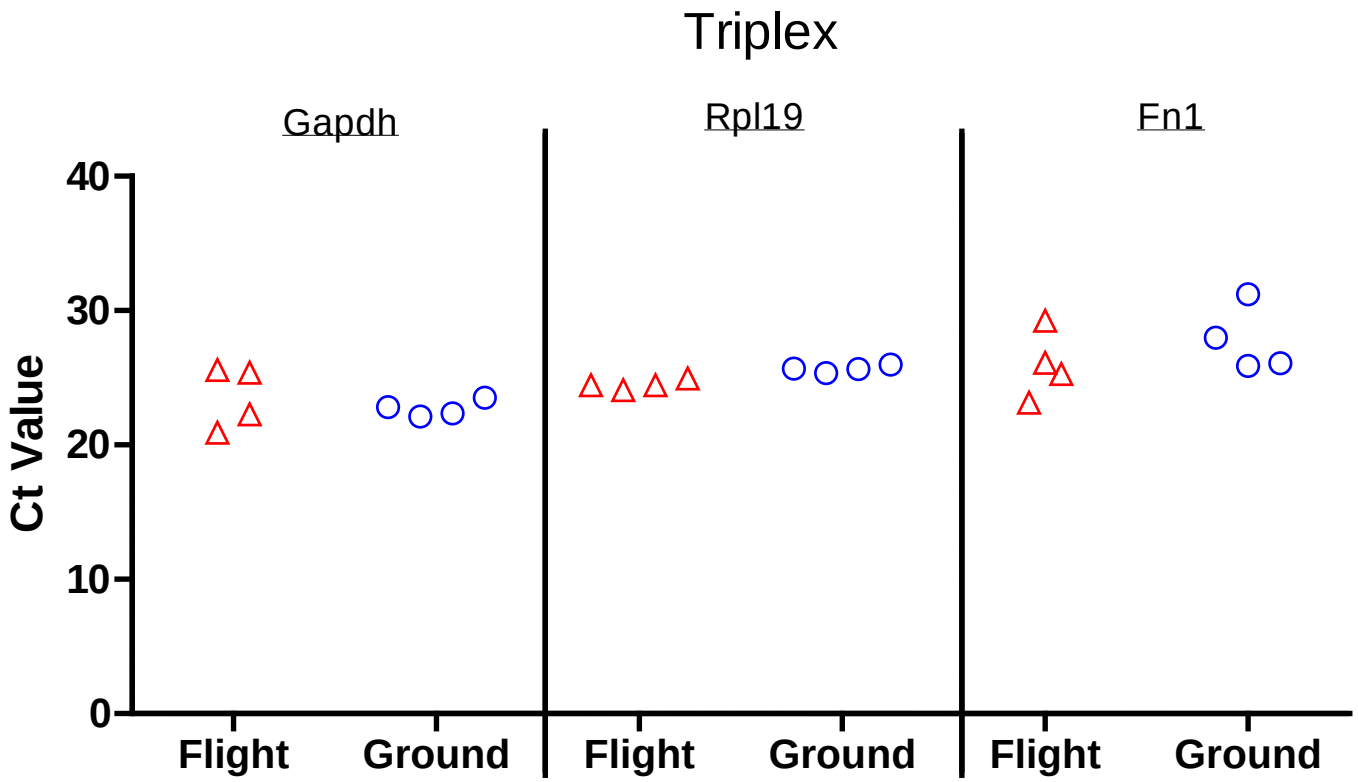
Operations Overview for *E. coli* and Mouse Runs



E. coli qRT-PCR Triplex Results



Mouse liver qRT-PCR Triplex Results



Conclusions from RNA Purification Runs

- Data shows that RT-qPCR analysis was successful in microgravity
  - RNA was successfully purified from both bacterial and mouse liver samples
- Successful Singleplex, Duplex and Triplex reactions from both bacterial and mouse samples
- Flight data has more variability than ground due to air bubbles

Follow up:

- Kate Rubins performed volunteer science activities over past month
  - First two tests were aimed at using COTS SmartTubes to increase assay longevity
  - Data suggested a mitigation strategy to reduce air bubble formation
  - Strategy was tested successfully on 10-19

WetLab-2 Advantages

- Researchers can receive results less than 24 hours after experiment run
- On-orbit analysis is especially useful in cases where fixation or freezing of samples is problematic
- On-orbit data can be used to guide the experiment in real-time
  - On-orbit time-course results can be used to guide experiment actions
  - Allow researcher to change details (timeline, etc.) of future runs without need for sample return, ground analysis and re-flight
  - Provide indicators of best time to fix or otherwise conclude experiment
- System can be used to provide verification of results from ground analysis
- System can be used to produce purified RNA or DNA for analysis on the ground

Conclusion

- WetLab-2 provides the following capabilities:
  - Establishes a qRT-PCR analytical instrument on the ISS for research purposes
  - Microgravity Sample Preparation of minimal complexity (RNA, DNA isolation) that can be completed on orbit
- Allow researchers to begin to utilize the ISS as a fully working laboratory
- Provide on-orbit analysis of air, surface, water, and clinical samples to monitor environmental contaminants and crew health.
  - Will indicate if harmful bacteria are present in water supply, surfaces, etc.
  - 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
- On-orbit analysis has the potential to reduce the need for downmass

WetLab-2 Team

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BACK UP SLIDES