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

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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

Sample Transfer Tool -> RNA Syringe -> SPM -> Pipette Loader -> Rotor

Reaction Tube -> SmartCycler

Data -> Ground - PI

Disposable Glove Bag
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

- Sample Transfer Tool
- Buffer Syringe (Session 2)
- RNA Syringe
- Repeater Pipette
- Reaction Tube
- Pipette Loader
- Rotor
- Disposable Glove Bag
- SmartCycler
- Data
- Ground - PI
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)
• Pre and post-run photos were taken during last session:

![Tube Photos](image)

• 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

<table>
<thead>
<tr>
<th>First Run</th>
<th>Efficiency</th>
<th>R squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight</td>
<td>104%</td>
<td>0.962</td>
</tr>
<tr>
<td>Ground</td>
<td>105%</td>
<td>0.997</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Second Run</th>
<th>Efficiency</th>
<th>R squared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flight</td>
<td>108%</td>
<td>0.987</td>
</tr>
<tr>
<td>Ground</td>
<td>103%</td>
<td>0.999</td>
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</table>
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

- Tissue Syringe: Mouse liver (Session 4)
- Sample Transfer Tool: Bacterial Cells (Session 3)

Diagram:
- RNA Syringe
- Repeater Pipette
- Reaction Tube
- Pipette Loader
- Rotor
- SPM
- Disposable Glove Bag
- SmartCycler
- Data

**Ground - PI**
E. coli qRT-PCR Triplex Results

<table>
<thead>
<tr>
<th>Triplex</th>
<th>Flight</th>
<th>Ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>dnaK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rpoA</td>
<td></td>
<td></td>
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<tr>
<td>srlR</td>
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Ct Value

- Flight
- Ground
Mouse liver qRT-PCR Triplex Results

<table>
<thead>
<tr>
<th></th>
<th>Flight</th>
<th>Ground</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gapdh</td>
<td>△△△△△</td>
<td>○○○○○</td>
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<tr>
<td>Rpl19</td>
<td>△△△△△</td>
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<tr>
<td>Fn1</td>
<td>△△△△△</td>
<td>○○○○○</td>
</tr>
</tbody>
</table>
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
• 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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