SUCCESSFUL VALIDATION OF RNA PURIFICATION AND QUANTITATIVE REAL-TIME PCR ANALYSIS OF GENE EXPRESSION ON THE INTERNATIONAL SPACE STATION

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Abstract

The NASA Ames WetLab-2 system was developed to offer new on-orbit gene expression analysis capabilities to ISS researchers and can be used to conduct on-orbit RNA isolation and quantitative real time PCR (RT-qPCR) analysis of gene expression from a wide range of biological samples ranging from microbes to mammalian tissues.

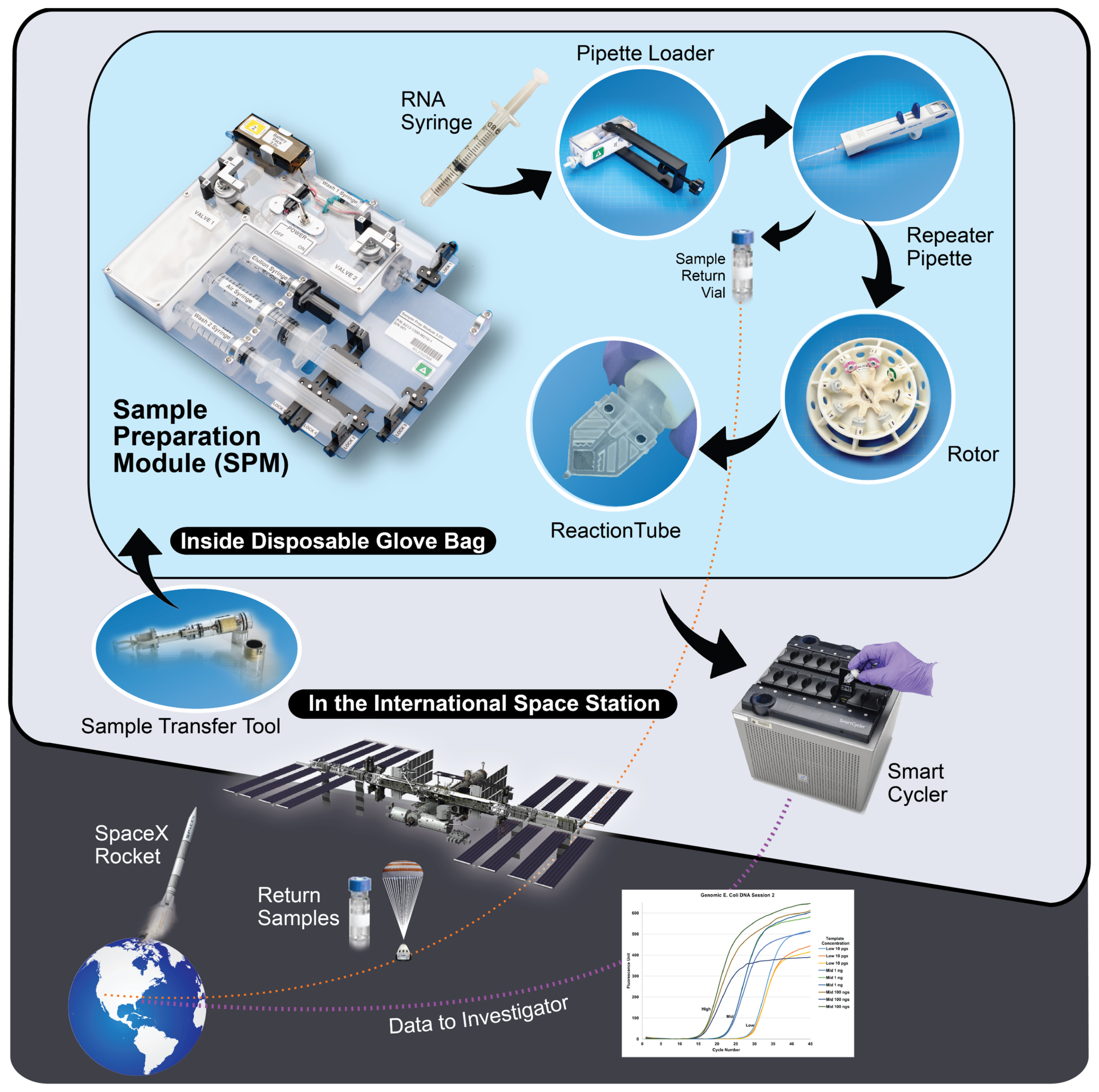
On orbit validation iincluded three quantitative PCR (qPCR) runs using an *E. coli* genomic DNA template pre-loaded at three different concentrations. The flight Ct values for the DNA standards showed no statistically significant differences relative to ground controls although there was increased noise in Ct curves, likely due to microgravity-related bubble retention in the optical windows.

RNA was successfully purified from both *E. coli* and mouse liver samples and successfully generated singleplex, duplex and triplex data although with higher standard deviations than ground controls, also likely due to bubbles. Using volunteer science activities, a potential bubble reduction strategy was tested and resulted in smooth amplification curves and tighter Cts between replicates.

The WetLab-2 validation experiment demonstrates a novel molecular biology workbench on ISS which allows scientists to purify and stabilize RNA, and to conduct RT-qPCR analyses on-orbit with rapid results. This novel ability is an important step towards utilizing ISS as a National Laboratory facility with the capability to conduct and adjust science experiments in real time without sample return, and opens new possibilities for rapid medical diagnostics and biological environmental monitoring on ISS.

Methods

- On-orbit qPCR was verified using four replicates each of assays with pre-loaded *E. coli* genomic DNA template of 0.01, 1.0, or 100 ng/ assay
- RNA Isolation and analyses was performed on both $1x10^8$ E.coli cells and a 30mg mouse liver biopsy and tested in single, duplex, and triplex formats
- dnaK, rpoA, and srlR gene expression was quantified for E.coli and Gapdh, Rpl19, and Fn1 for mouse tissue. All qPCR results were compared to ground controls.



Results

On-orbit qPCR validation

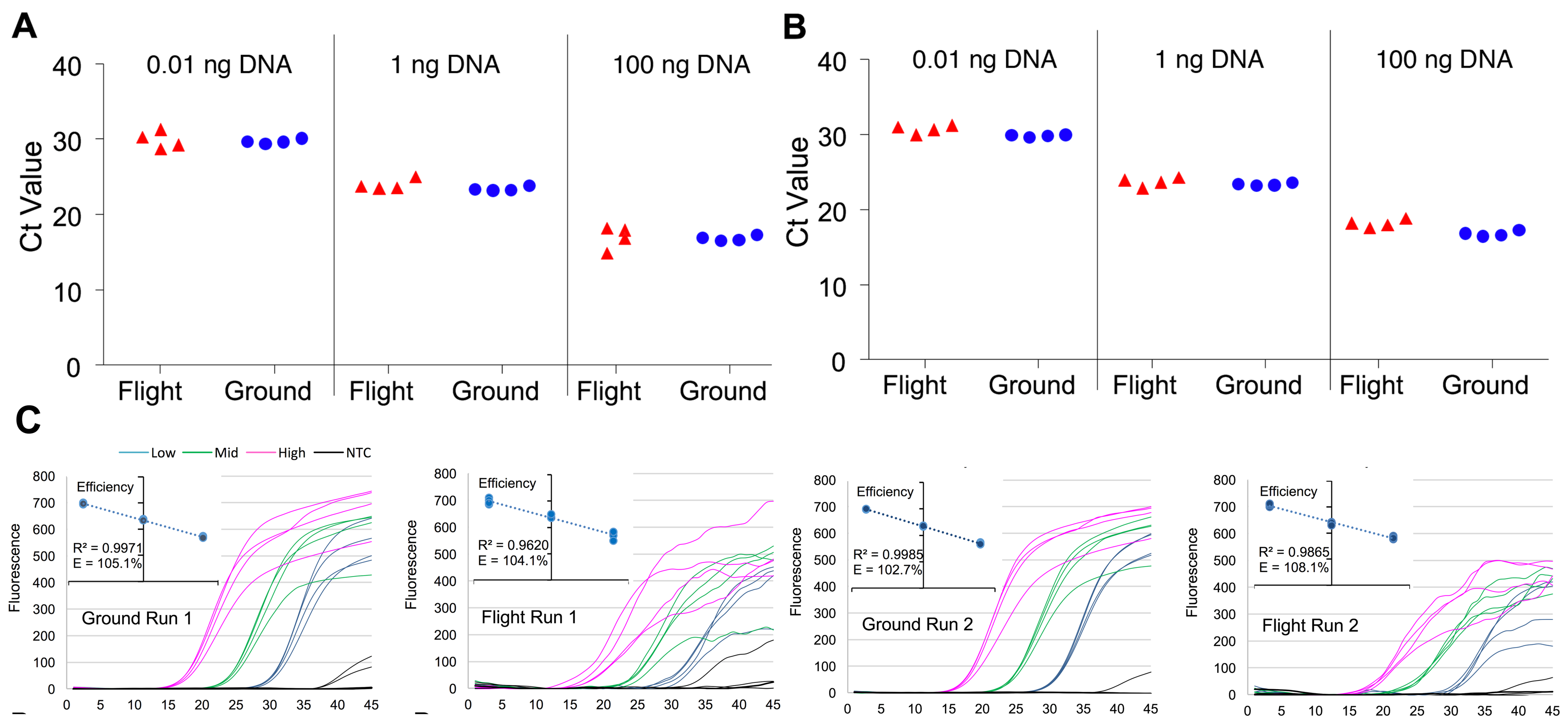


Figure 1. Flight and ground control Ct values and amplification plots. Ct values for low (0.01 ng/test), mid (1 ng/test), and high (100 ng/test) assays during A) the first and B) second experimental runs are shown using scatter plots with jitter. C) Corresponding amplification plots for flight and ground data for both experimental runs.

On-orbit RNA isolation and qPCR

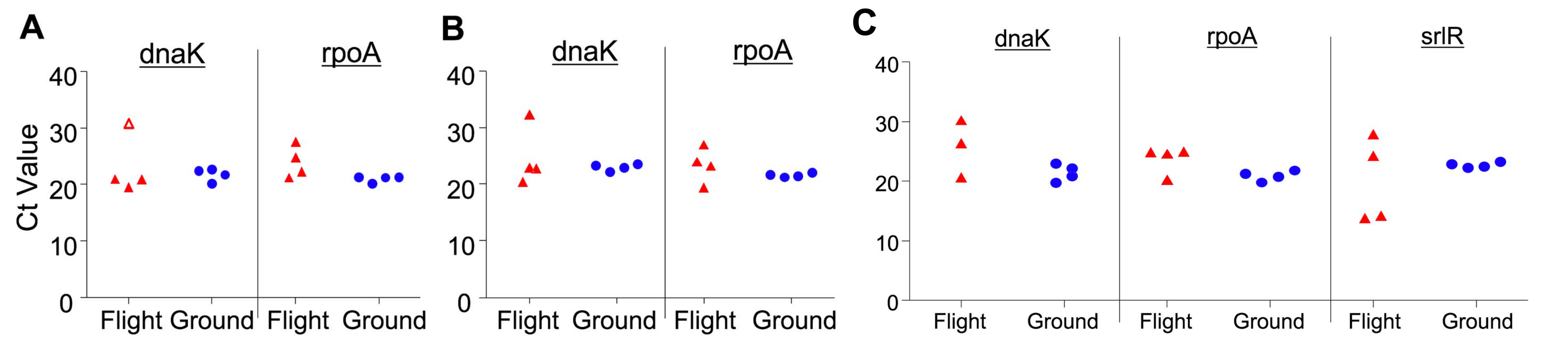


Figure 2. Scatter plots of flight and ground *E. coli* data. Ct values from flight and ground singleplex (A), duplex (B) and triplex (C) reactions are shown. Open marker indicates statistical outliers.

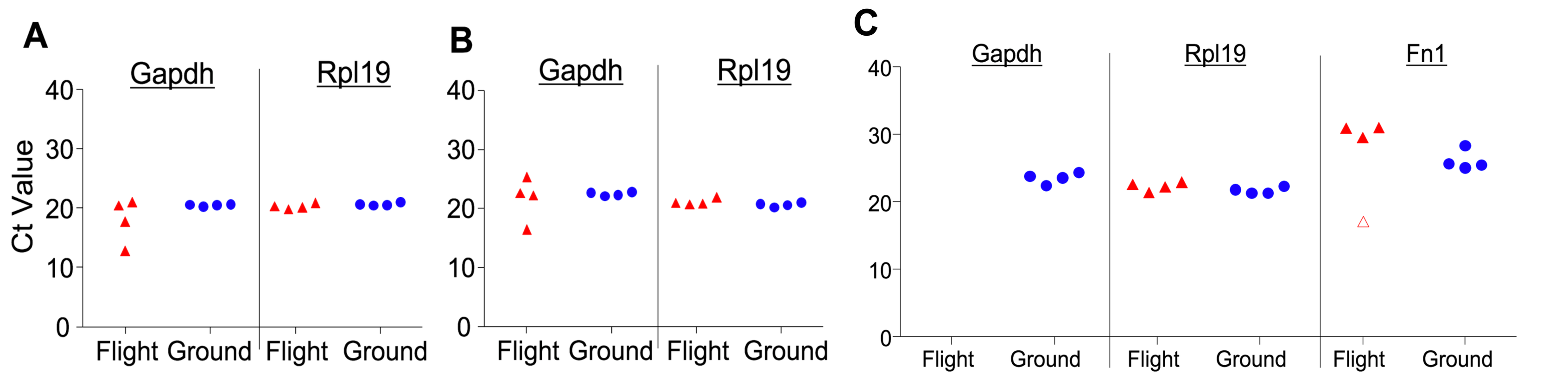


Figure 3. Scatter plots of the flight and ground mouse liver data. Ct values from flight and ground singleplex (A), duplex (B) and triplex (C) reactions are shown. One outlier from the flight triplex Fn1 plot is clearly indicated by the filled marker and in this sample, no Gapdh FAM signal was seen.

Effervescence Mitigation

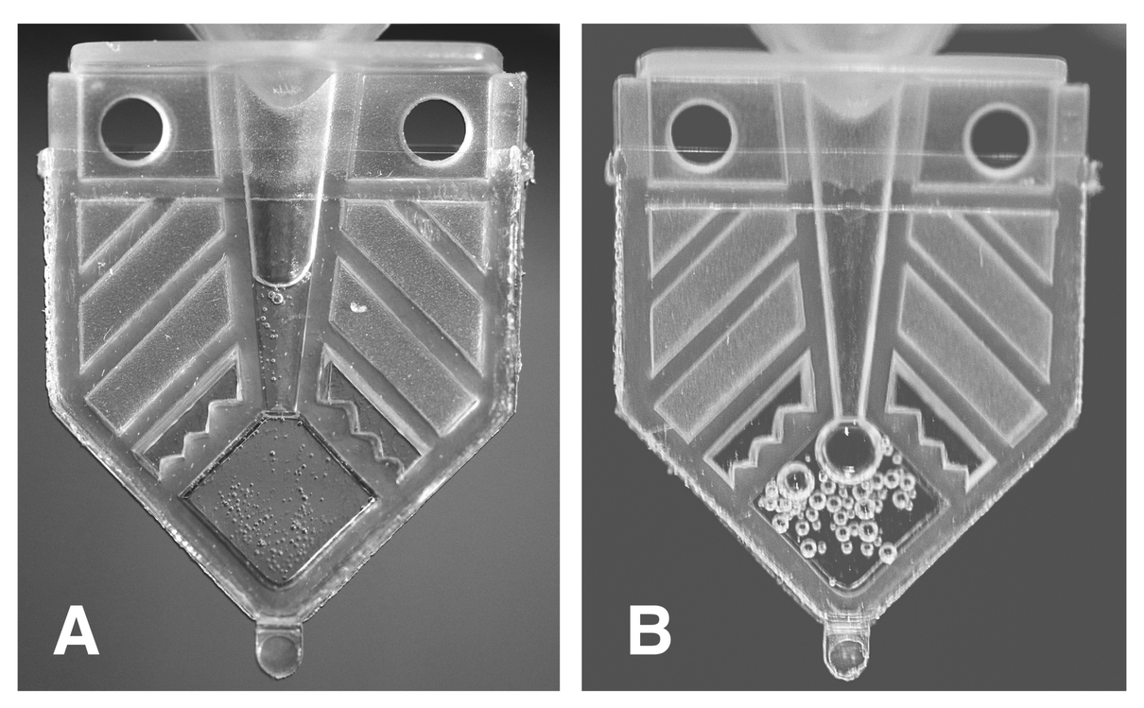


Figure 4. Photos of prepared tubes before and after on-orbit qPCR. Representative photos of on-orbit prepared reactions (A) pre- and (B) post- amplification. Effervescence present in panel B was suspected to cause signal noise seen in previous flight data.

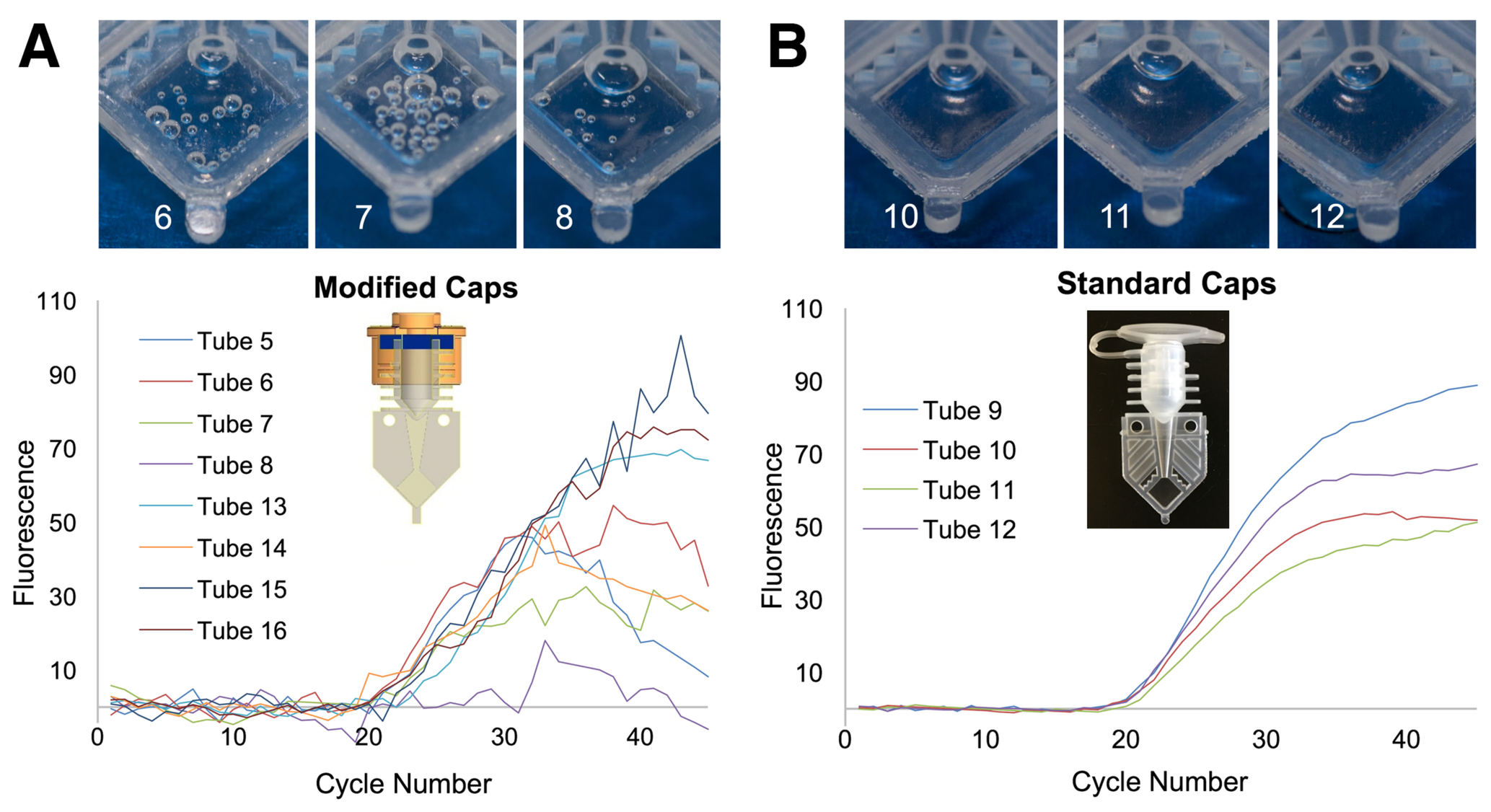


Figure 5. Amplification curves from in-flight reactions comparing different tube caps. A comparison of on-orbit *E.coli* dnaK amplification when using either (A) NASA developed caps or (B) standard commercial SmartTube Caps. Numbered photos were taken post- amplification and represent individual tubes. Inset images show tube cap configurations.

Conclusions

- qPCR in a microgravity environment is not only possible, but can generate data that is statistically indistinguishable from ground data
- In-flight qPCR analyses generates noisier amplification curves, believed to be caused by the presence of effervescence
- The WetLab2 system for on-orbit RNA isolation and gene expression analyses is able to isolate RNA from cell suspensions (*E.coli)* and soft tissues (mouse liver) for the purpose of single or multiplex gene expression analyses
- WetLab2 Isolated samples can be run in single, duplex, or, less reliably, triplex qPCR reactions
- The system facilitates dynamic experimentation on-orbit and allows for flexibility in experimental design, as proven by our effervescence mitigation work

Future Development

- We are currently adapting the system to be compatible with other sample types (Arabidopsis and primary cell cultures)
- The WetLab2 system will be utilized in an upcoming flight experiment
- Feasibility testing of the WetLab2 system with genetic sequencing
- Additional hardware is being developed to allow for further sample processing (incubation at >55ºC for protein denaturation)

Acknowledgements