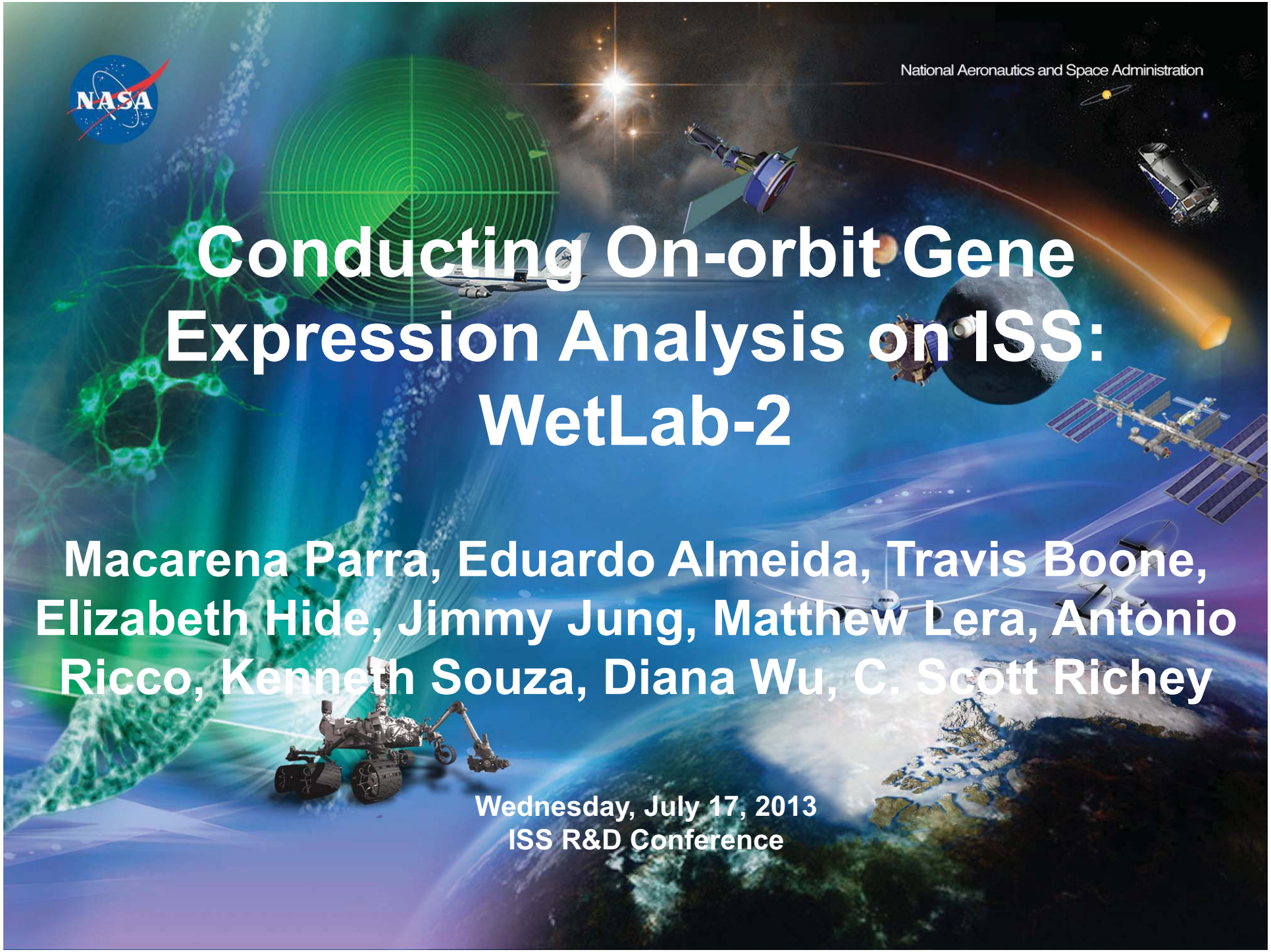

Conducting On-orbit Gene Expression Analysis on ISS: WetLab-2

Macarena Parra, Eduardo Almeida, Travis Boone, Elizabeth Hide, Jimmy Jung, Matthew Lera, Antonio Ricco, Kenneth Souza, Diana Wu, C. Scott Richey

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ISS R&D Conference

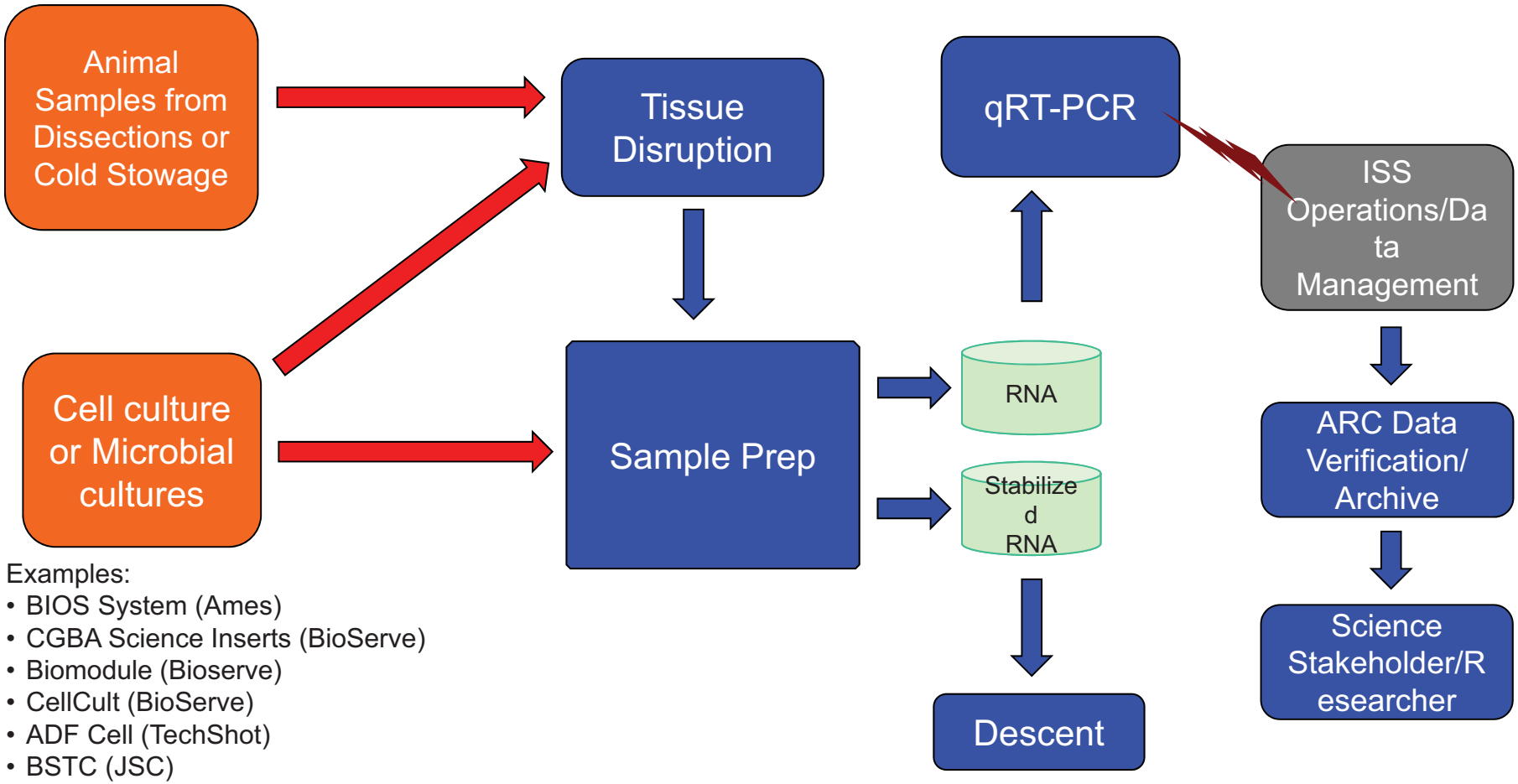
National Aeronautics and Space Administration

Goals of WetLab-2 Project

WetLab-2

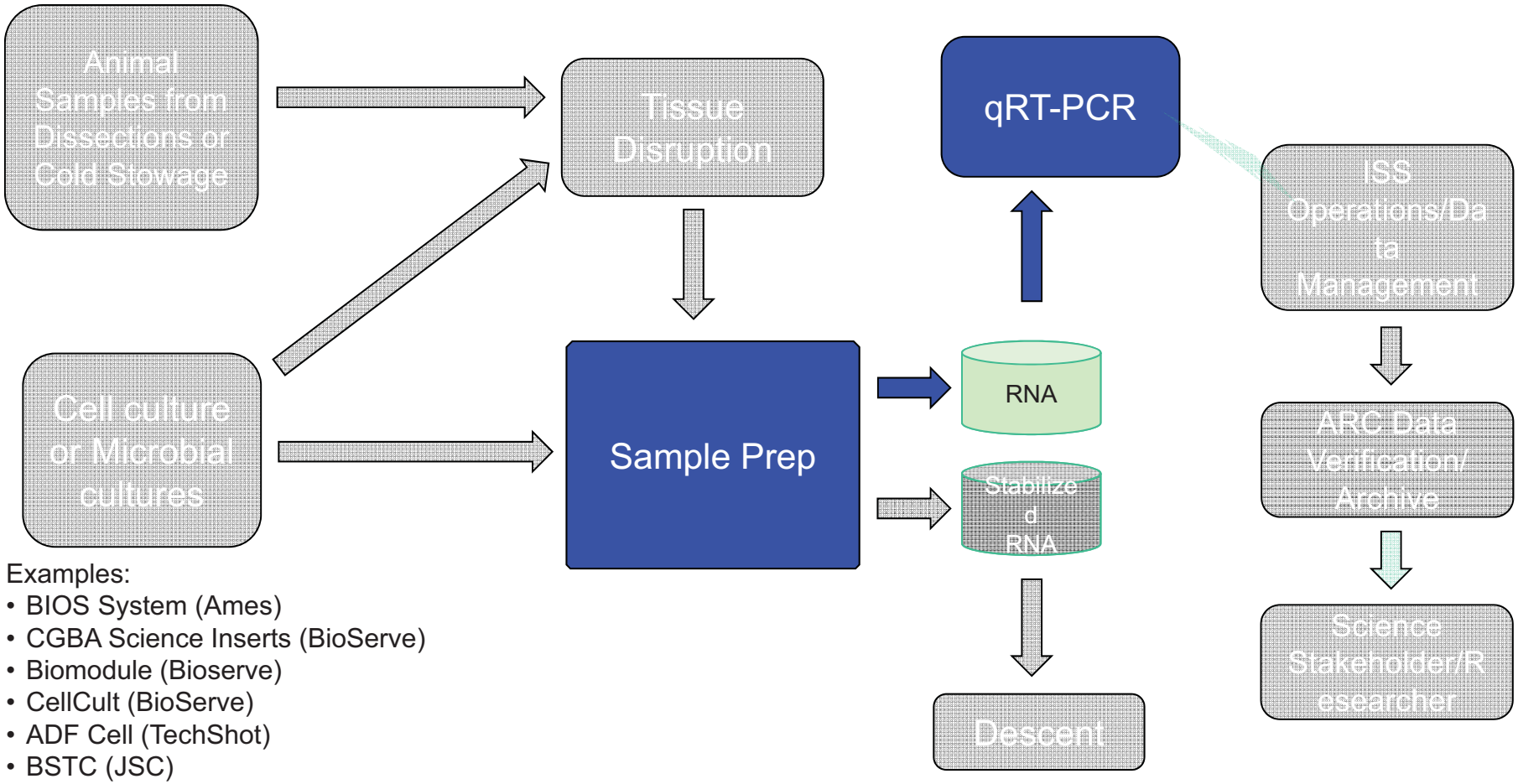
- Provide a system that has the capability to process and analyze samples from various sources and can provide, as its end point, gene expression information to PIs in the form of on-orbit quantitative real-time PCR data.
- System can be either an integrated system or made up of separate modular components.

Concept of Operations



Examples:
- BIOS System (Ames)
- CGBA Science Inserts (BioServe)
- Biomodule (Bioserve)
- CellCult (BioServe)
- ADF Cell (TechShot)
- BSTC (JSC)
- RWPS (JSC)
- ESA or JAXA Experiment Container

Concept of Operations



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Instrument Constraints

WetLab-2

Constraints

- Works in microgravity or can be easily modified to do so
- Low power consumption
- Low heat output
- Low noise levels during operation
- Must have flexibility over primer and probe selection

Preferences

- LED light source
- Few moving parts
- Can be modified to fit in single locker

Instrument Selection

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WetLab-2

Cepheid SmartCycler

Modular Design

- **Total of sixteen modules**
- **Modules can be independently programmed and run**
- **If one module fails, other modules still function**
- **Configurable within ISS rack volume constraints**

Few moving parts (small fan in each module)

Rapid run time

Full flexibility over primer and probe selection and use

Multiplex capability: four fluorescence channels per module

Selection of a standard vs. automated instrument = we need to design a sample prep system

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Sample Prep Constraints

WetLab-2

Crew Time

- Whenever possible minimize the necessary crew manipulations
- Keep crew operations as simple as possible

Cold Stowage

- If possible, reagents should be long lived
- If possible, reagents will not require storage at refrigerated or frozen temperatures

Hazardous chemicals

- Limit Toxicity of chemicals
- If possible, avoid alcohols

Containment

- Plan to provide two levels of containment during sample preparation
- Plan to provide one level of containment during qPCR

Sample Prep Development

Initial approach: design system that extracts RNA from 2-3 common sample types (mouse, yeast, *E. coli*)

RNA must be of high enough purity/quality for qPCR

Pursuing two methods

Simplified RNA purification

Column based purification with minimal number of steps and complexity

Advantages: cleaner, works with intronless genes (more sample types), can return excess pure RNA to PI for ground studies

Disadvantages: more steps, less simple

One Step Lysis

Reagent added to cells to lyse then crude lysate is directly used in qPCR reactions

Advantages: very simple procedure, few steps

Disadvantages: many impurities in mix, may not work with some organisms and intronless genes

ClaremontBio System

Commercially available

Simplified column based RNA purification procedure

Small components

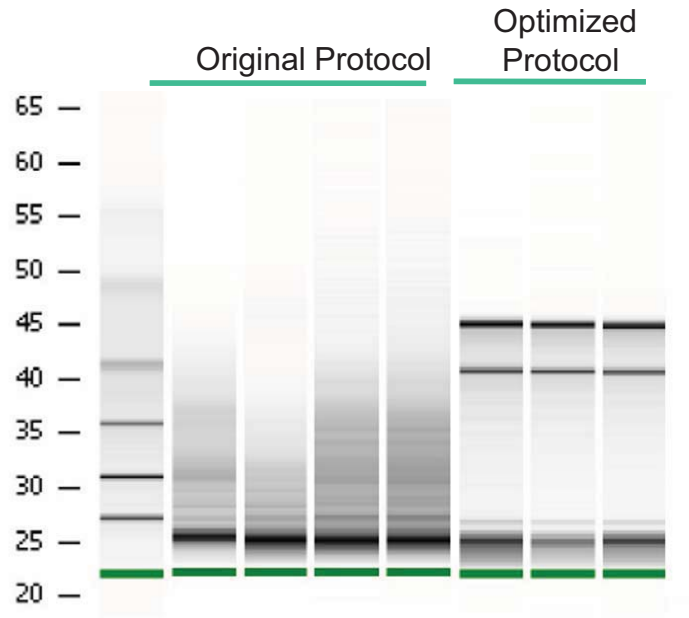
No spin columns, syringe based

OmniLyse cartridge capable of lysing yeast cells and *E. coli*

RNA was of sufficient quality for RT-qPCR

System tested successfully with yeast and *E. coli*

Company worked with us to optimize the protocol for our needs



ClaremontBio System

Final Optimized Procedure

Turn on OmniLyse unit (not needed for mammalian cells)

Lyse sample and bind RNA to column

Performed by passing culture back and forth through OmniLyser and column

Lysis buffer contains Proteinase K

Wash column

Wash buffer contains Proteinase K

Wash column again

Wash buffer does not contain Proteinase K

Purge column with air

Elute RNA

Sample Processing Module

Prototype of closed system with syringes, valves and tubing was developed and tested

Procedure

All liquids (except sample) are preloaded in module

Sample is introduced into module using Transfer Tool

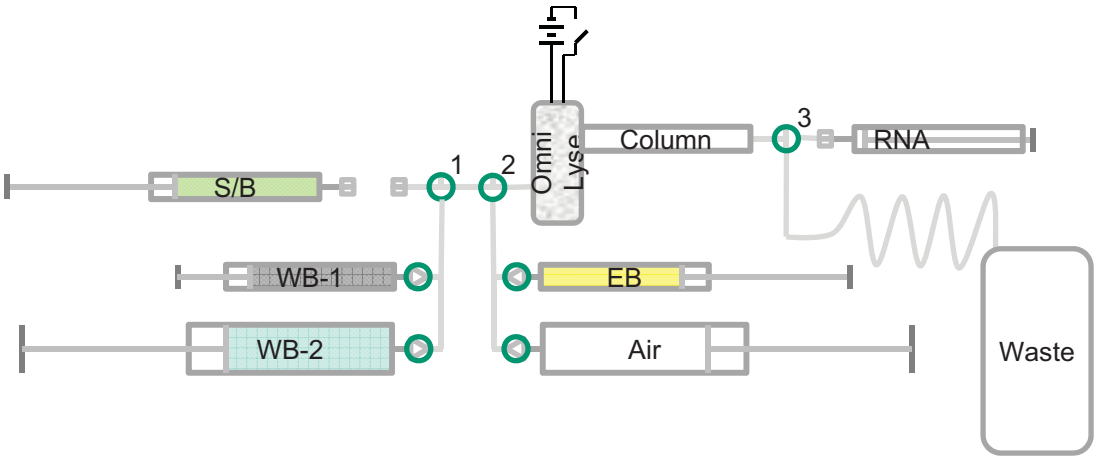
Activate OmniLyse unit and push sample back and forth

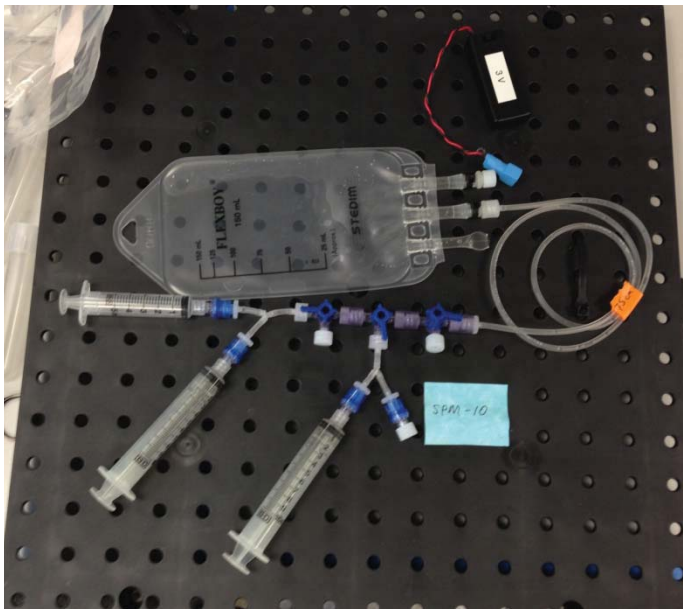
As it is lysed sample moves through the column and the RNA binds

Column is washed twice with wash buffers

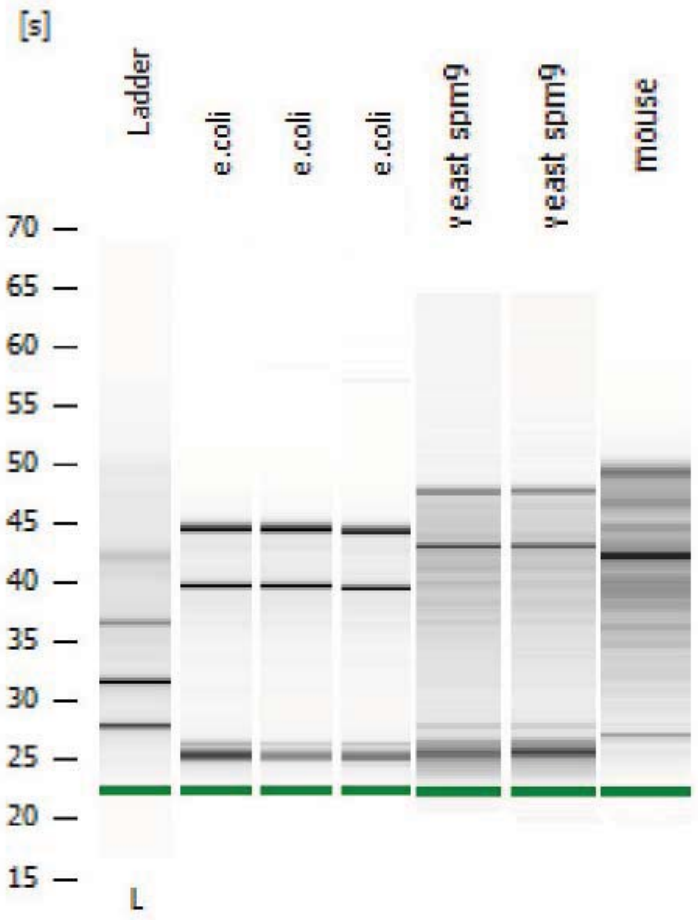
Column is purged with air

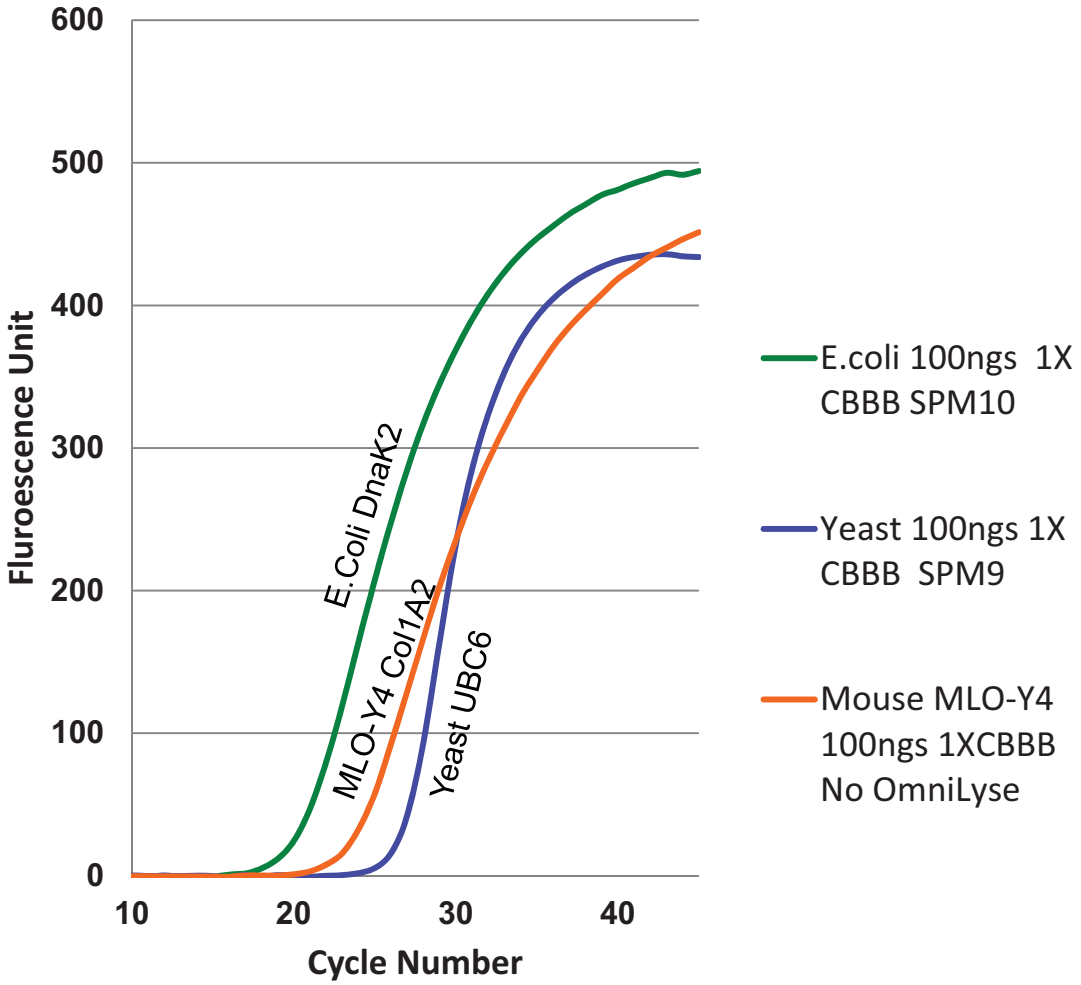
RNA is eluted with elution buffer





SPM Purified RNA





RNA purified using prototype SPM shows compatibility with qRT-PCR.

RNA Sample to qPCR

Eluted RNA in syringe

↓

Mix with qPCR reagents and primers and probes

qPCR reagents (enzymes, buffer, etc.) will be lyophilized

Location of reagents is not finalized – mix after elution or in Reaction Tube

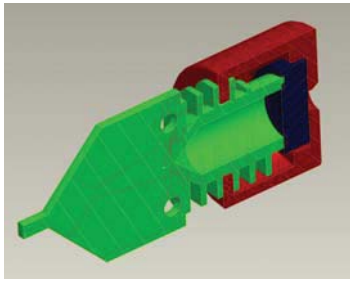
Primers and Probes must be located in the Reaction Tubes

↓

Remove air bubbles

↓

Load Tubes



Reaction Assembly Module

Procedure:

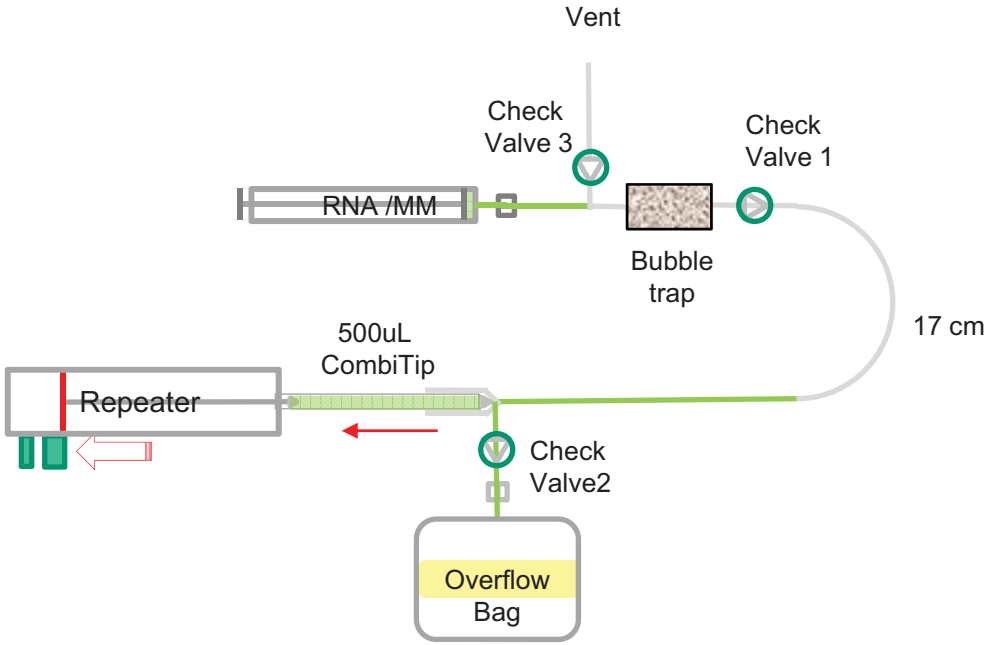
Syringe containing eluted RNA from the SPM is connected to the RAM

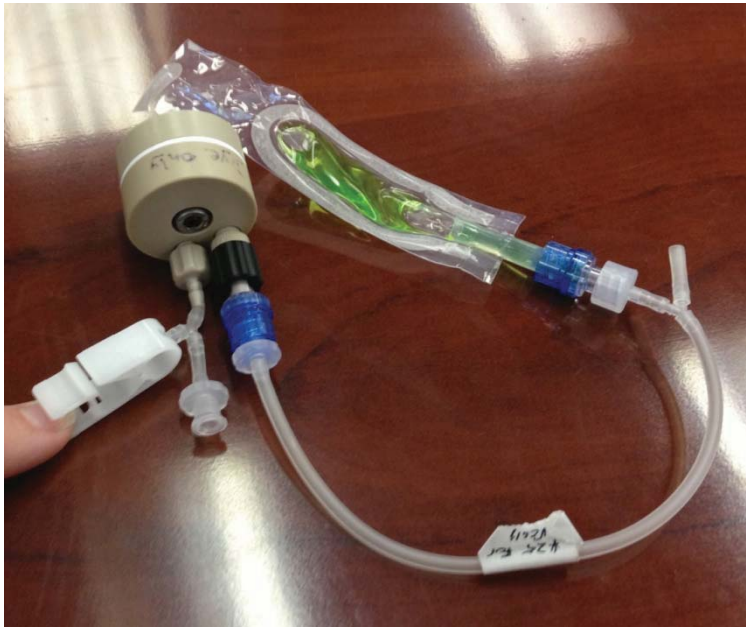
RNA may be mixed with lyophilized Reagent Mix (MM)

Mixture is pushed through line designed to remove air bubbles

Mixture is loaded into Repeater pipetter tip from the Wet Lab Kit

Repeater pipettor is removed and used to load the Reaction Tubes





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Tube Loading

WetLab-2

Procedure:

Tubes have been modified to have a permanent cap with a pierced septa

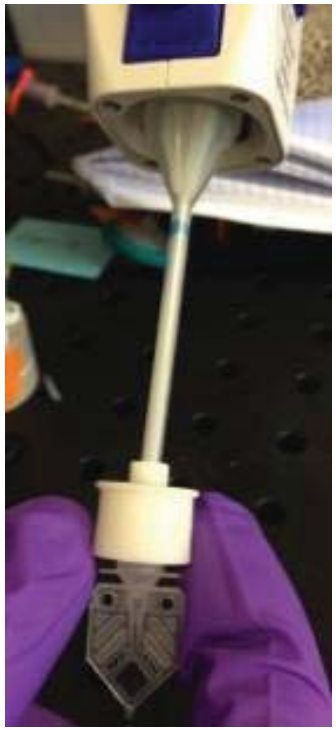
Reaction Tubes will contain dried DNA primers and probes

Repeater pipettor containing RNA from RAM is used to manually load Reaction Tubes

Reaction Tubes are loaded through septum

Liquid must be located in the detection window

Will use centrifugation



RAM and tube load procedure are compatible with qPCR.

EXPRESS Rack Modifications

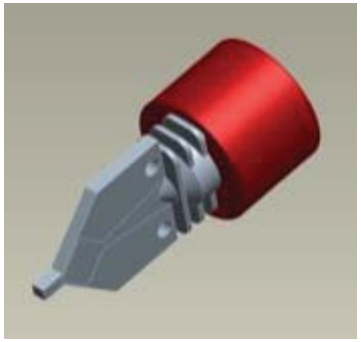
SmartCycler has been modified to fit in one EXPRESS Rack Locker

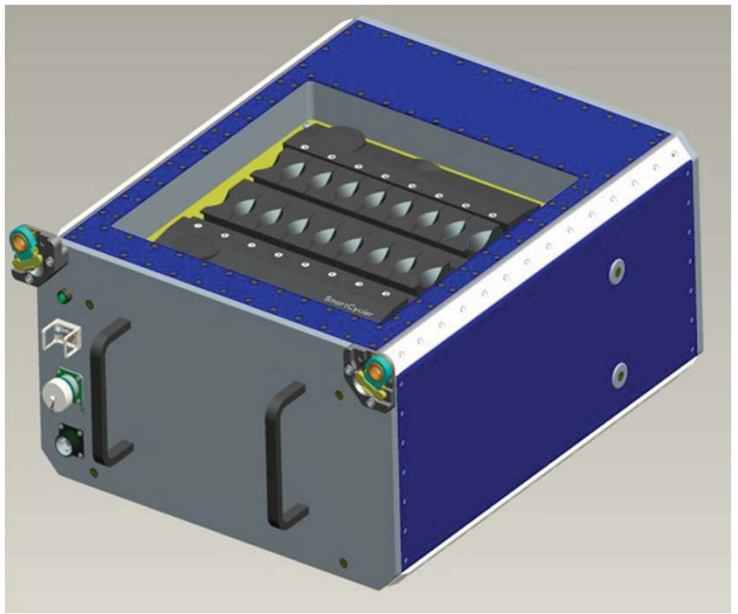
Drawer design – Unit will be pulled out to load tubes

Connected to dedicated laptop during entire run

When run is complete, data will be down-linked to the ground

Fits 16 reaction tubes





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Future Work

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Select reagent lyophilization provider

- Define location of qPCR reagents

Long term testing

- Stability of lyophilized qPCR reagents
- Stability of SPM reagents

Further testing on sample processing

- Test with more sample types (SWG)
- Test using low abundance RNA

Integrated testing

- Continue testing with prototype and final RAM and SPM versions
- End-to-end runs of experiments using hardware

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Verification Flight Plan

WetLab-2

Goal of Verification 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 Verification Flight

Install hardware and software **(Session 1)**

Does real-time PCR data produced on-orbit match data on earth? **(Session 2)**

- **No convection or other issues**

Does the Sample Processing Module function correctly on-orbit? **(Session 3)**

- **All fluidic manipulations function properly**
- **Prove out system with first sample type**

Does the Homogenizer unit function correctly on-orbit? **(Session 4)**

- **All fluidic manipulations function properly**
- **Prove out system with second sample type**

Flight results from each session will be compared to those obtained from the ground controls

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

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BACK-UP SLIDES

WetLab-2

SmartCycler Characterization Fluorophores

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WetLab-2

Fluorophores	Channel	h Exc/Em	Calibrated	Tested	Crosstalk
FAM	1	495/519	Yes	Yes	No
Cy3	2	550/570	Yes	Yes	No
TET	2	521/536	Yes	No	N/A
Alex 532	2	532/554	Yes	No	N/A
VIC	2	538/554	No	Yes	Yes
Texas Red	3	589/610	Yes	No	N/A
Cy5	4	649/670	Yes	No	No
Alexa 647	4	650/665	Yes	No	N/A

Master Mix Selection

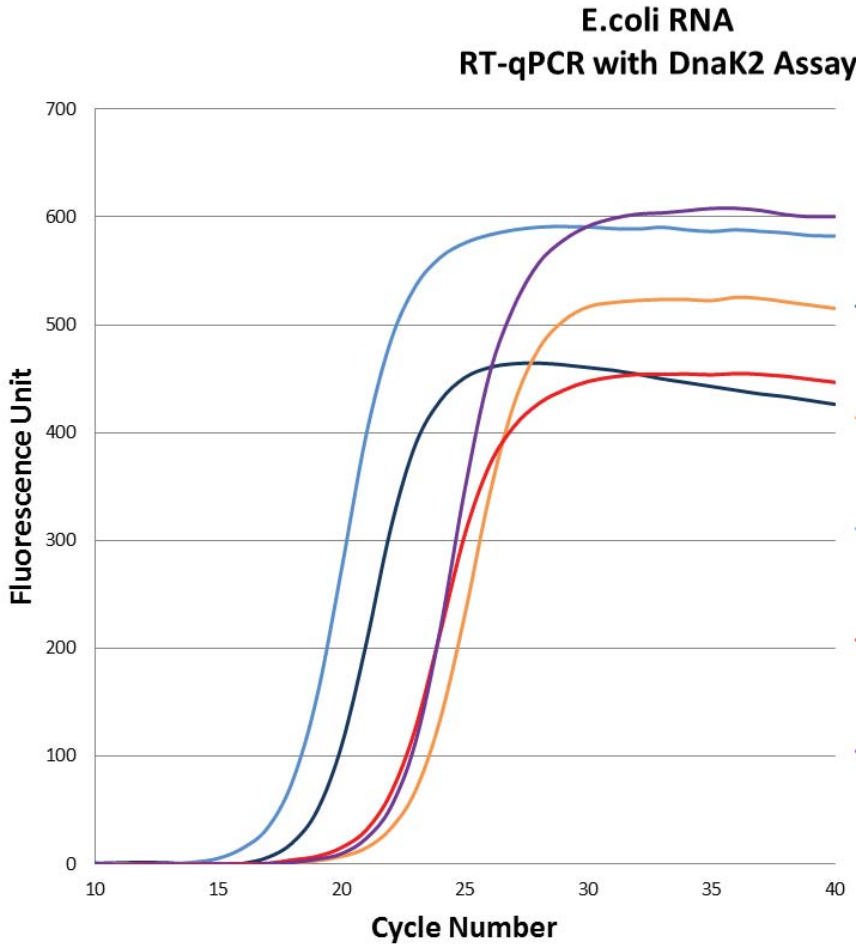
Promega GoTaq® Probe 1-Step RT-qPCR chosen

- Master Mix that includes all reagents needed for Reverse Transcription and qPCR
- Five-Six logs of dynamic range (mouse)
- Four-Six logs of dynamic range (yeast and *E. coli*)
- Company willing to make glycerol free reagents for lyophilization
- Test batch of glycerol-free reagents showed same activity/characteristics
- Promega has working relationship with our Lead lyophilization vendor

Shrimp DNase chosen as an additive

- dsDNA-specific DNase capable of removing residual genomic DNA
- Compatible with Promega Probe 1-Step RT-qPCR system
- Effectively removed residual genomic DNA as effectively as on column DNase 1 digestion

Master Mix Additives
Shrimp DNase



Normalized 100ngs RNA Template	Ct
ClaremontBio (CB)	18.36
CB + Shrimp DNase	21.84
CB + Qiagen clean (Q)	16.82
CB+Q + Shrimp DNase	20.91
CB+Q + DNase 1 digestion	21.22

Shrimp DNase is as effective as on column DNase 1 treatment in removing carryover genomic DNA

SmartCycler Characterization Volume Effect

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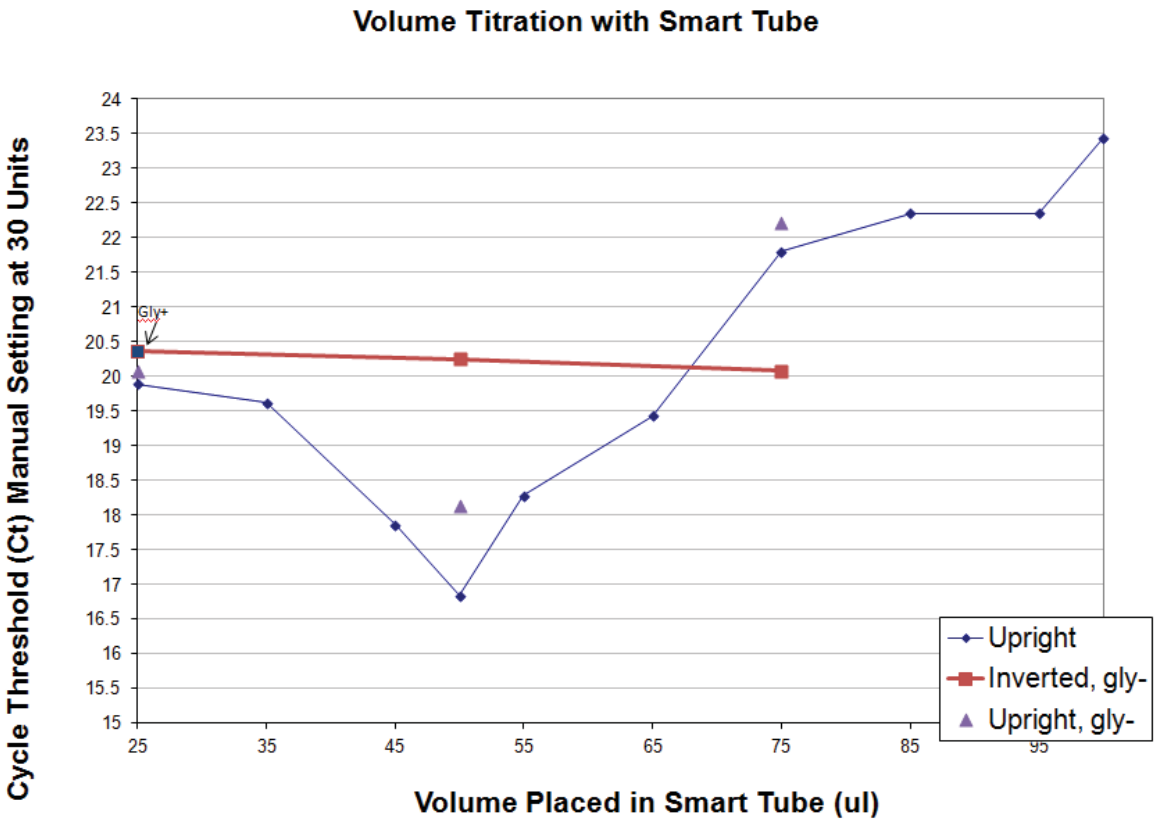
WetLab-2

The upright volume test indicated a Ct dependence on volume. Ct values decreased as volumes increased from 20 to 50 uL. As volumes increased further from 50 uL to 100 uL, Ct values increased (Blue ◆ diamond line).

However, the same volume dependence was not seen in the inverted configuration. (Red ■ square line)

Ct values from extended volume testing with Promega RT-PCR kit

Volume (uL)	Ct	Volume (uL)	Ct
25	19.89	65	19.43
35	19.62	75	21.8
45	17.86	85	22.35
50	16.84	95	22.35
55	18.28	100	23.44



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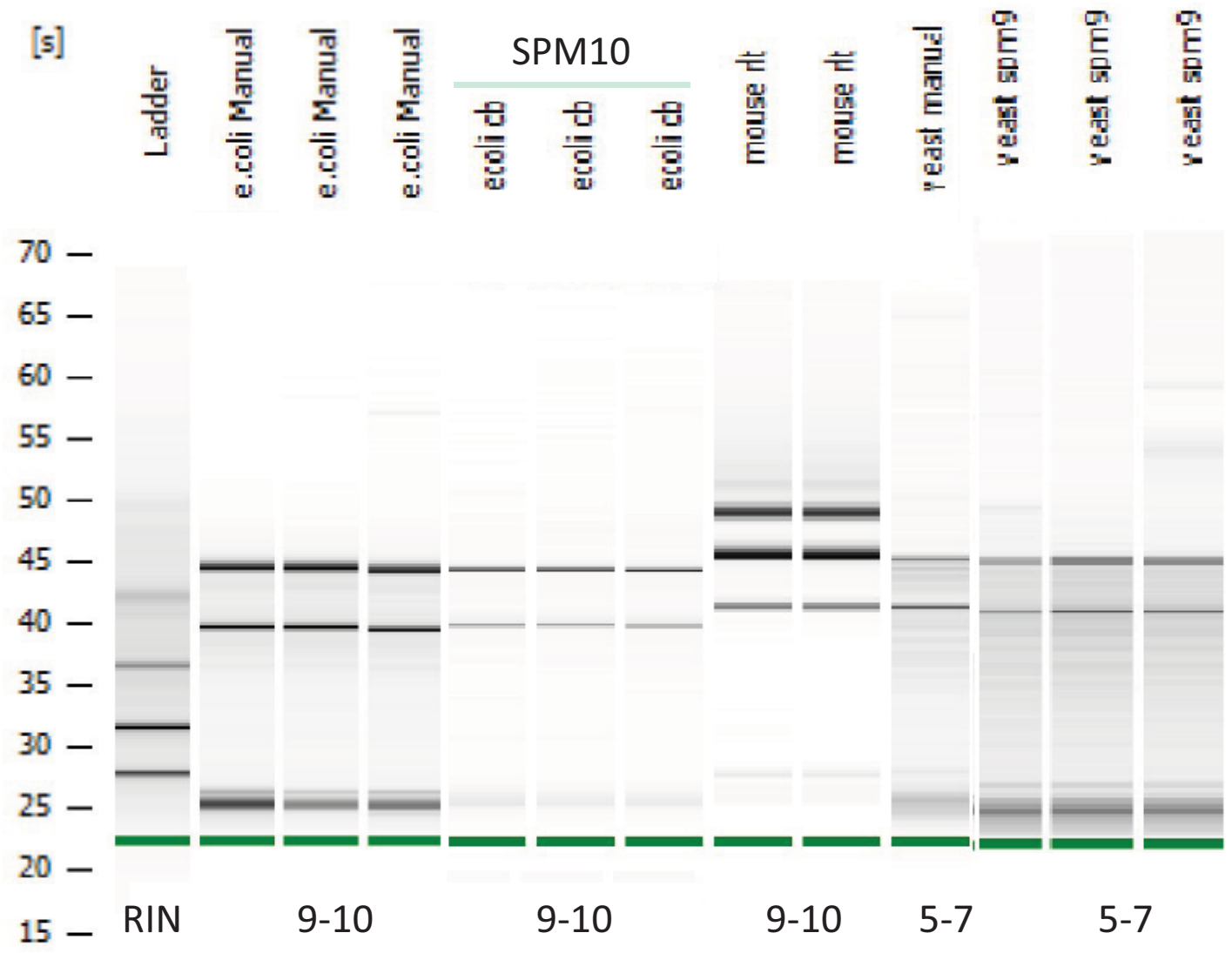

SPM-9/10 Testing on Three Model Organisms

Sample Process	Organism	Elution Volume (ul)	Conc. ng/uL	Total Yield (ugs)	RIN	Ct (100ngs Template)
Manual	Yeast	220	36	7.2	5-7	26.6
SPM	Yeast	750	~6	4.5	5-7	27
Manual	E. *coli*	250	88.6	22.2	9-10	21
SPM	E. *coli*	300	110.2	33	9-10	20.2
Manual	MLO-Y4	220	50	11	5-7	25.3
SPM	MLO-Y4	1,100	15	16.5	1-2.5*	26.65

* = Needs improvement