Development Status of the WetLab-2 Project: New Tools for On-orbit Real-time Gene Expression.

Jimmy Jung, Macarena Parra, Eduardo Almeida, Travis Boone, Tori Chinn, Antonio Ricco, Kenneth Souza, Liz Hyde, Rukhsana Yousuf, C. Scott Richey

Tuesday, Nov 5, 2013
ASGSR Conference

Rationale and Goals for WetLab-2

National Aeronautics and Space Administration

WetLab-2

Rationale

- Retirement of NASA Space Shuttle Program has negatively impacted the downmass of space flown samples.
- To date, analysis has only been conducted on returned samples, leaving the question of the effects of re-entry forces unanswered.
- Optimizes the utilization of the ISS for molecular biology and related technology applications and addresses the 2010 NRC Decadal Survey which strongly encourages the application of molecular biology technologies to ISS.

Goals

- 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 real-time PCR (qRT-PCR) data.
- Wetlab-2 will enable expanded genomic research on ISS by developing tools that support *in situ* sample collection, processing, and analysis.
  - -Capable of researching multiple sample types: bacterial, plant, insects, and mammalian samples.
  - -System can be either an integrated system or made up of modular components.

National Aeronautics and Space Administration

Engagement of Scientific Community WL-2 SWG

WetLab-2

Dr. Cynthia Collins
Assistant Professor
Department of Chemical and Biological Engineering
Rensselaer Polytechnic Institute, Troy, NY
Experiments Flown: **STS-132 (Micro-2), STS-135 (Micro-2A**)

Dr. Mike Delp
Professor and Chair
Department of Applied Physiology and Kinesiology
University of Florida, Gainesville, FL
Experiments Flown: **STS-107, STS-131, STS-133, STS-135**

Dr. John Z. Kiss
Professor, Department of Biology
Dean of Graduate School
University of Mississippi, University, MS
Experiments Flown: **STS-81 (Preplastid), STS-84 (Plastid) STS-121 (TROPI-1), STS-130 (TROPI-2), STS-131 (BRIC-16)**

Dr. Greg Nelson
Professor, Radiation Medicine
Professor, Basic Sciences
School of Medicine
Loma Linda University, Loma Linda, CA
Experiments Flown: **STS-42 (Radiat), STS-76 (Elegans), STS-108 (CBTM-1), STS-118 (CBTM-2), STS-135 (CBTM-3)**

Dr. Cheryl Nickerson
Professor
The Biodesign Institute, Infectious Diseases and Vaccinology
Arizona State University, Tempe, AZ
Experiments Flown: **STS-115 (Microbe), STS-123 (MDRV), STS-131 (STLImmune),STS-135)**

Dr. David Niesel
Professor & Chair
Microbiology & Immunology
University of Texas Medical Branch, Galveston, TX
Experiments Flown: **STS-118 (SPEGIS), STS-123 (MDRV), PharmaSat**

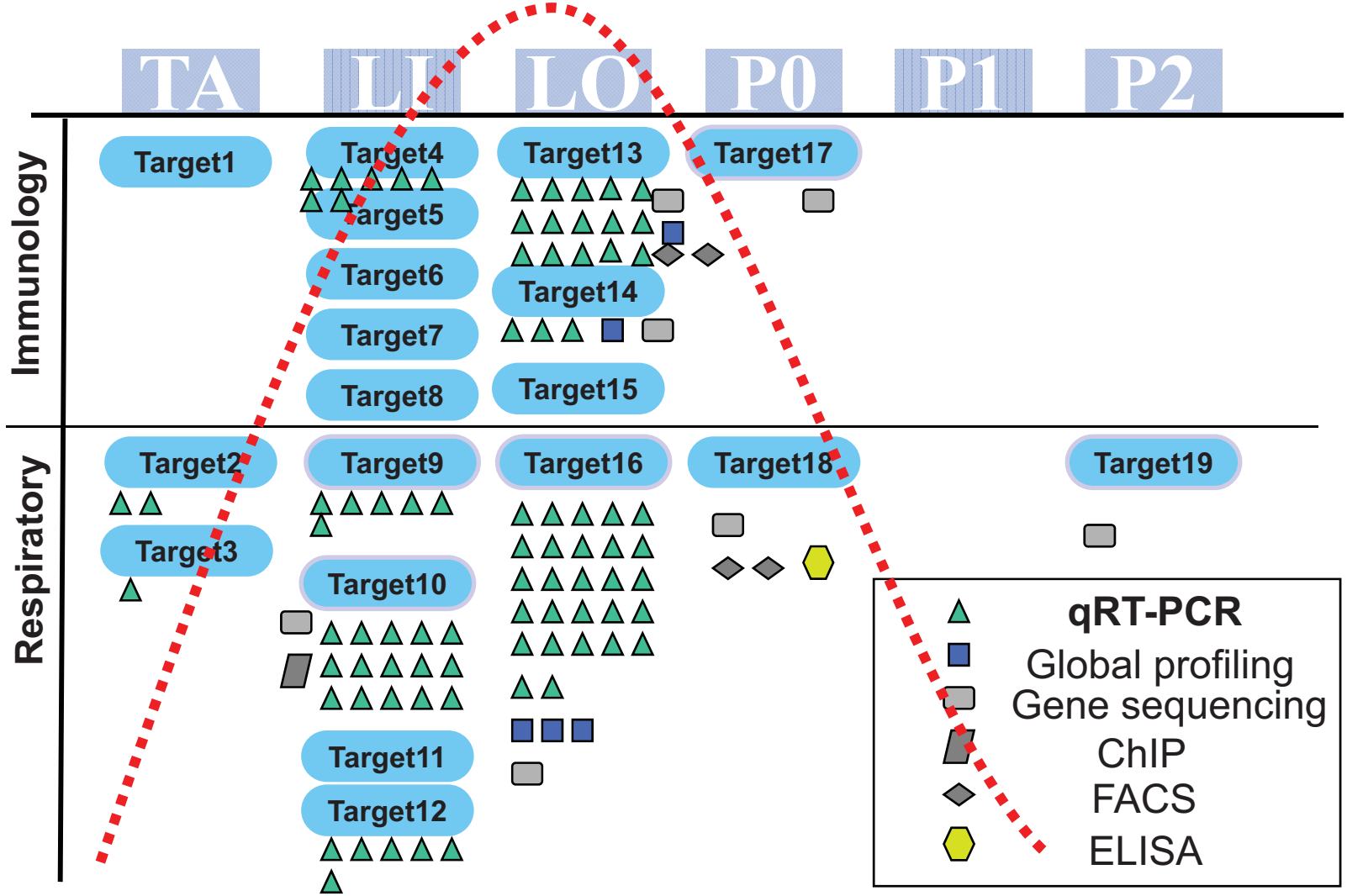
Dr. Michael Roberts
CASIS Representative
Experiments Flown: **STS-135 (SyNRGE)**

At least 24 Total Flight Experiments

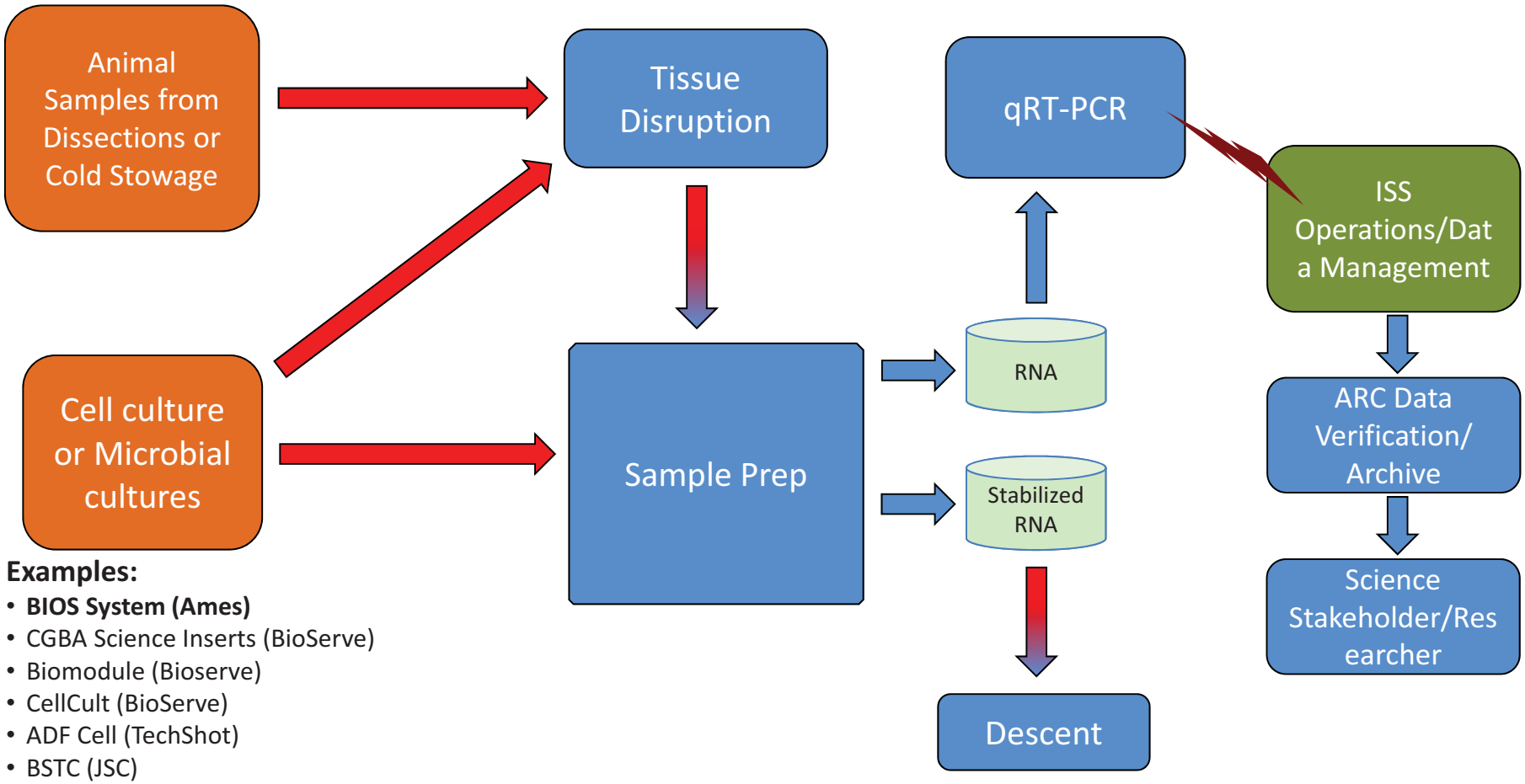
National Aeronautics and Space Administration

qRT-PCR Needed for Validation and Discovery

WetLab-2



Concept of Operations

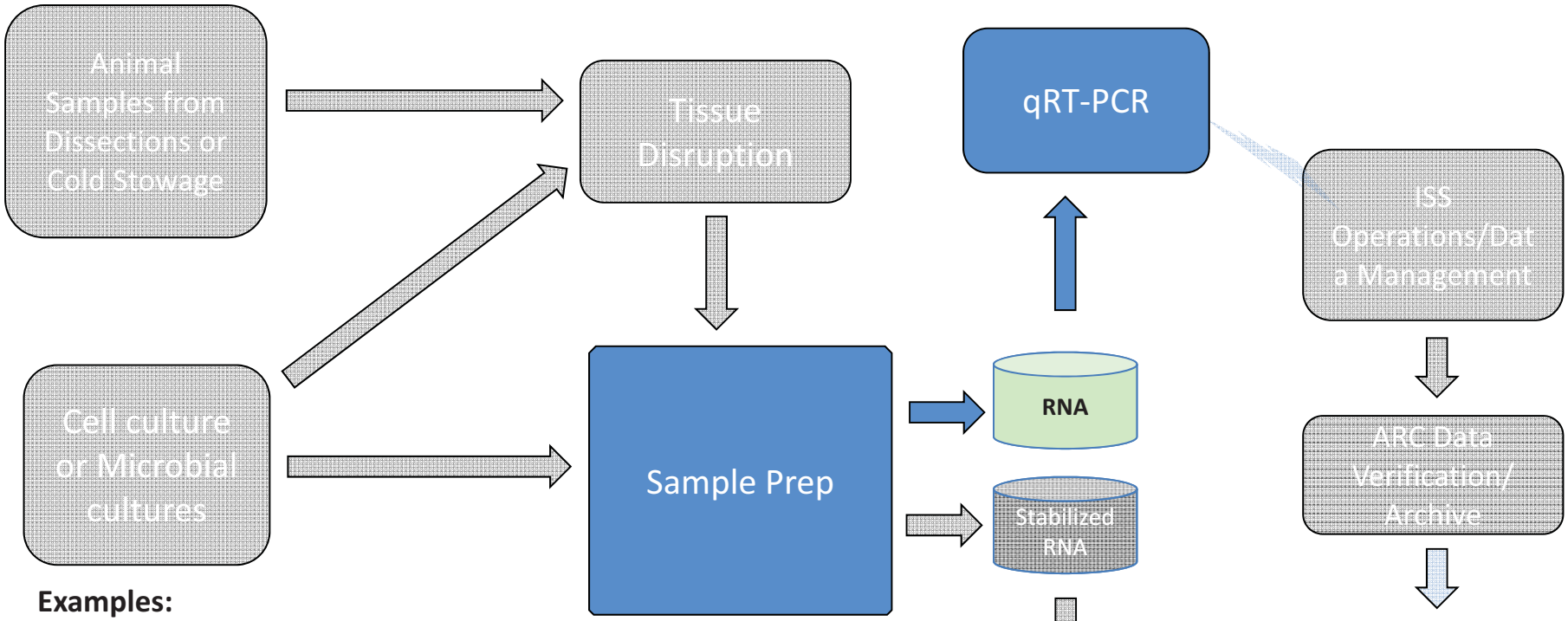


Examples:

- **BIOS System (Ames)**
- CGBA Science Inserts (BioServe)
- Biomodule (Bioserve)
- CellCult (BioServe)
- ADF Cell (TechShot)
- BSTC (JSC)
- RWPS (JSC)
- **Fruit Fly Lab (Ames)**
- **Rodent Habitat (Ames)**
- **Seedling Growth (Ames)**

Concept of Operations

Sample Prep / qRT-PCR Specifics



Examples:

- **BIOS System (Ames)**
- CGBA Science Inserts (BioServe)
- Biomodule (Bioserve)
- CellCult (BioServe)
- ADF Cell (TechShot)
- BSTC (JSC)
- RWPS (JSC)
- **Fruit Fly Lab (Ames)**
- **Rodent Habitat (Ames)**
- **Seedling Growth (Ames)**

National Aeronautics and Space Administration

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

National Aeronautics and Space Administration

Instrument Selection

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

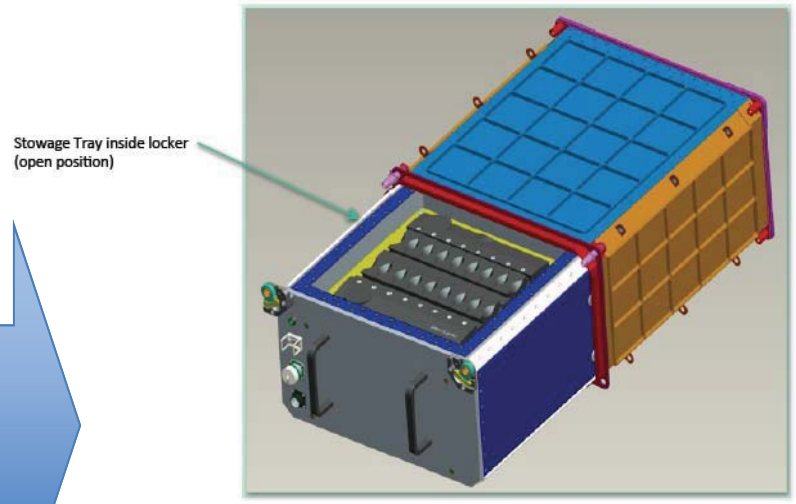
Selection of a standard vs. automated instrument = we need to design a sample prep system

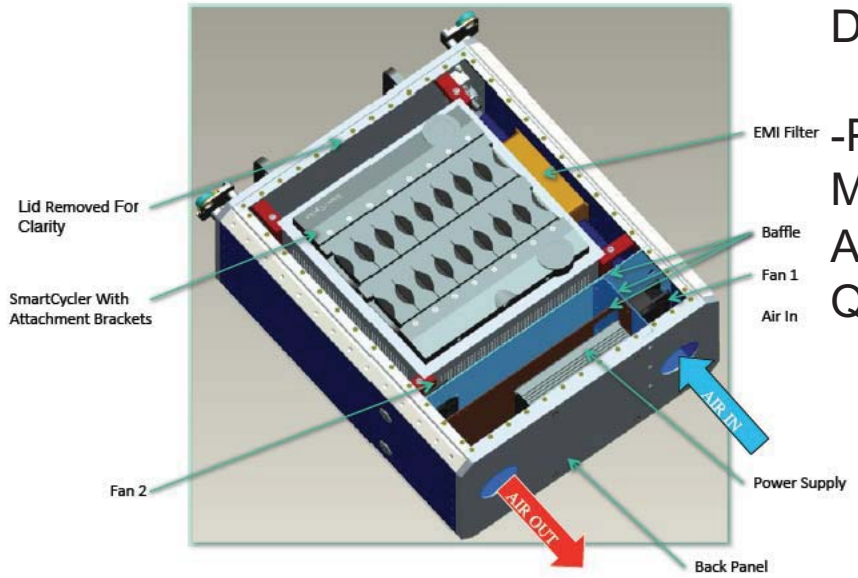
Gene Expression Enabling Tool: COTS Modified for ISS

National Aeronautics and Space Administration

WetLab-2

Benchtop Model qRT-PCR Cycler (Cepheid SmartCycler)





ISS Model Running on Express Rack Laptop

-Completed Engineering Studies for Spaceflight Unit

-Completed Spaceflight Unit Engineering Drawings

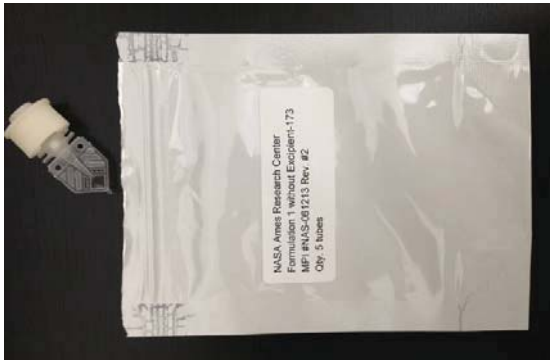
-Pending Manufacture And Flight Qualification

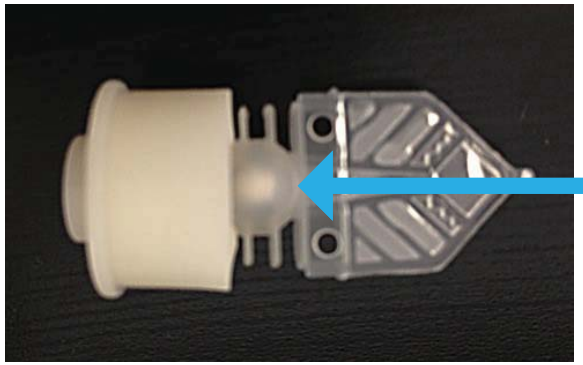
The qRT-PCR Enzyme and Reaction Tube: COTS Modified for ISS

National Aeronautics and Space Administration

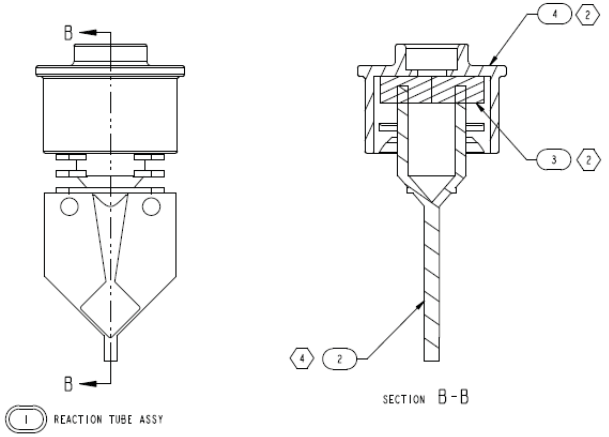
WetLab-2

- Foil Pack with gene assay(s) can be kept at room temperature for 1 year
- Requires only addition of sample through containment septum cap
- Small-scale pilot assay production cost was less than $50 per tube/assay





Lyophilized qRT-PCR Chemistry assay bead



National Aeronautics and Space Administration

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 qRT-PCR

Sample Prep Strategy

Simplified RNA purification

- Column based purification with minimal number of steps and complexity
- Can be used for intronless genes (more sample types)
- Can return excess pure RNA to PI for ground studies

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 qRT-PCR

Expanded approach: design homogenization system that extracts RNA from plants, Drosophila, and rodent tissue

- Limit Toxicity of chemicals
- If possible, avoid alcohols

ClaremontBio System for RNA Purification

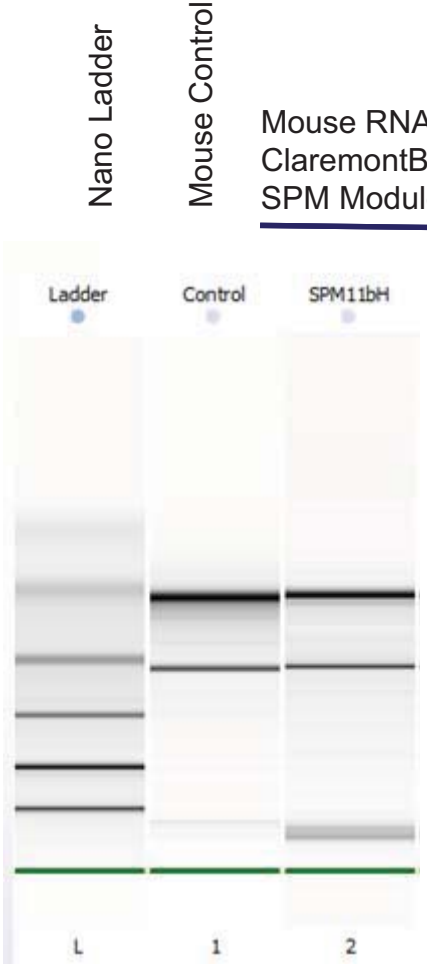
- Commercially available
- Simplified RNA purification procedure
- Small components
- OmniLyse cartridge capable of lysing yeast cells
- RNA was of sufficient quality for qRT-PCR

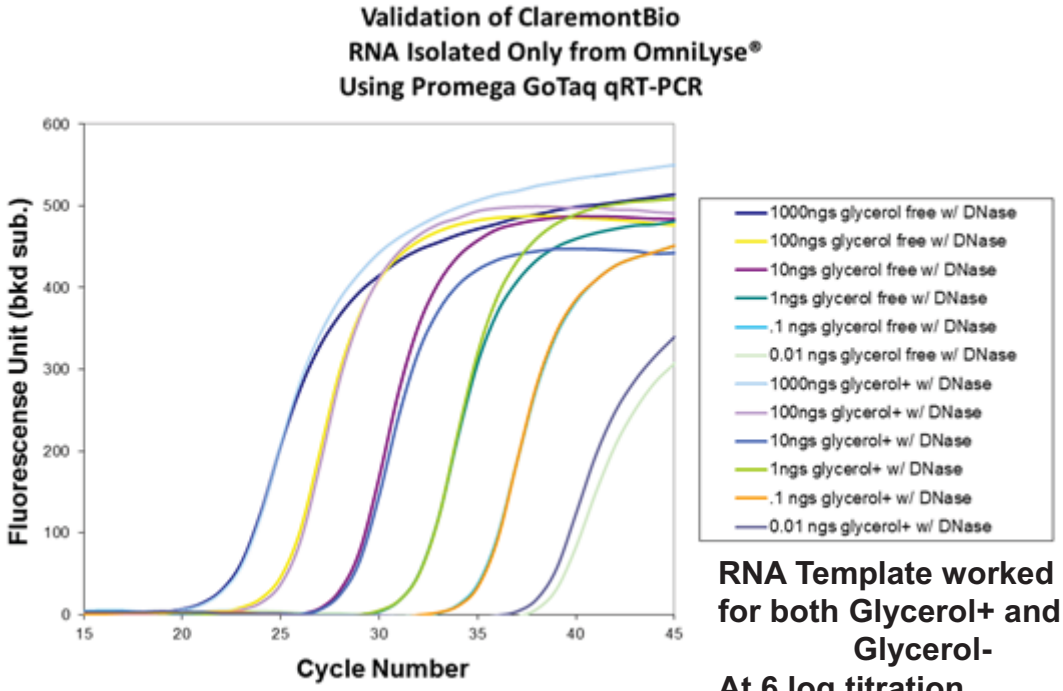
- System tested successfully with yeast, *E. coli,* and mammalian cells
- Company is willing to work with NASA to optimize the protocol for our needs
- Preliminary testing consistent with microgravity compatibility

ClaremontBio Procedure
RNA Isolation

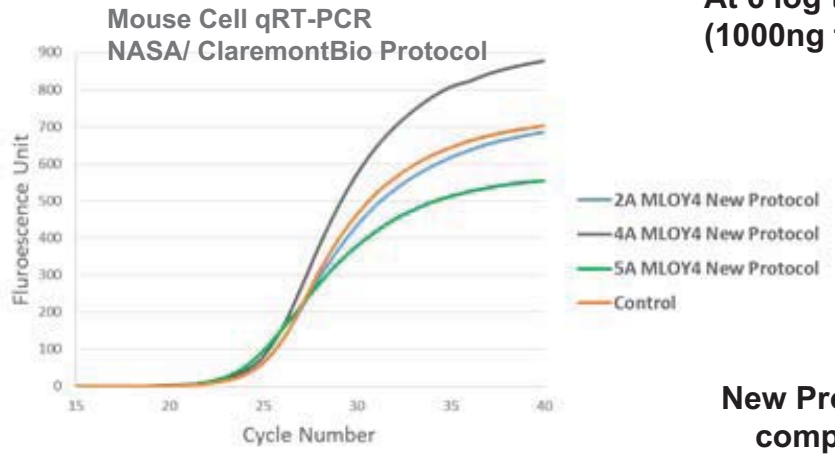
National Aeronautics and Space Administration

WetLab-2





RNA Template worked for both Glycerol+ and Glycerol-
At 6 log titration (1000ng to 10pg)



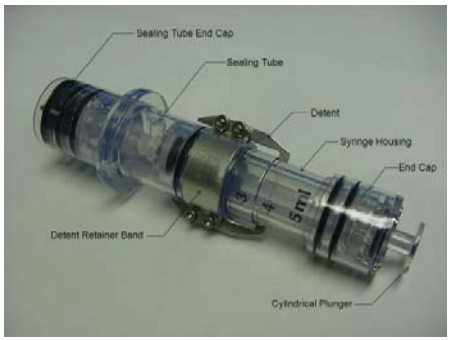
New Protocol Ct comparable to Qiagen Control

WL-2 Integration of Sample to Data Process

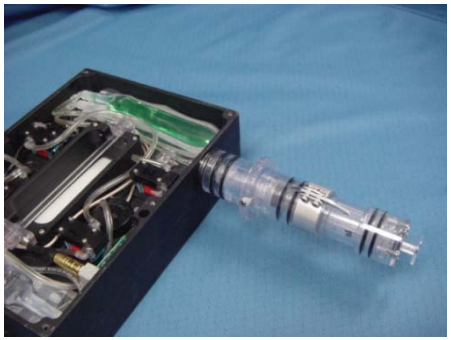
National Aeronautics and Space Administration

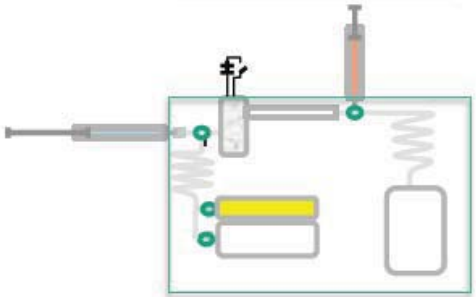
WetLab-2

Sample Hardware → Sample Prep/Fluidic Hardware → Rack Hardware

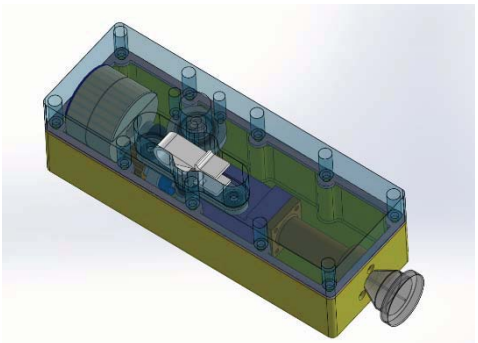


Techshot ACT2

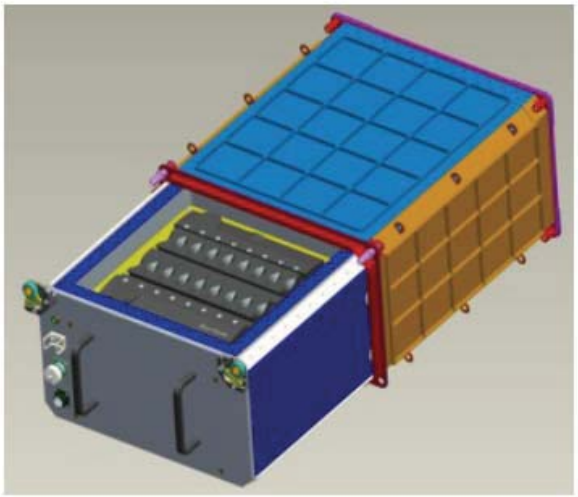




Sample Prep Module



Reaction Assembly Module



Support H/W*

General Purpose hardware for entire operations

Summary and Future Work

- Developed and tested a system capable of measuring gene expression on ISS.
- Components are modified COTS or have been designed and prototypes have been successfully tested.
- Custom assays can be produced economically (less than $50) for various sample type genes and have a shelf-life of one year at room temperature.
- Work with PIs and SWGs on their specific sample type and genes of interest.

National Aeronautics and Space Administration

Acknowledgements

Wetlab-2

Eduardo Almeida

Adrianna Aguilar

Travis Boone

Bob Dahlgren

Matt Everingham

Mike Henschke

Ed Houston

Al Howard

Elizabeth Hyde

Tom Luzod

Tony Ricco

Scott Richey

Wetlab-2(cont'd)

Kenneth Souza

Marla Smithwick

Omar Talavera

Eddie Uribe

Roy Vogler

Diana Wu

Rukhsana Yousuf

Ames Collaborators

Sharmila Bhattacharya- Fruit Fly Lab

Sungshin Choi- Rodent Habitat

Natalya Dvorochkin-BIOS

John Freeman- Seedling Growth

Kara Martin- Rodent Habitat

Thank You!

WetLab-2

National Aeronautics and Space Administration

BACK-UP SLIDES

WetLab-2

National Aeronautics and Space Administration

Experiment Design Examples for Number of Gene Assays

WetLab-2

Biological Test Replicates	Module 1	Module 2	Module 3	Module 4	Module 5	Module 6	Module 7	Module 8	Module 9	Module 10	Module 11	Module 12	Module 13	Module 14	Module 15	Module 16	Total Genes Assays
1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	48
3	3			3			3			3			3				15
4	3				3				3				3				12
8	3								3								6
16	3																3

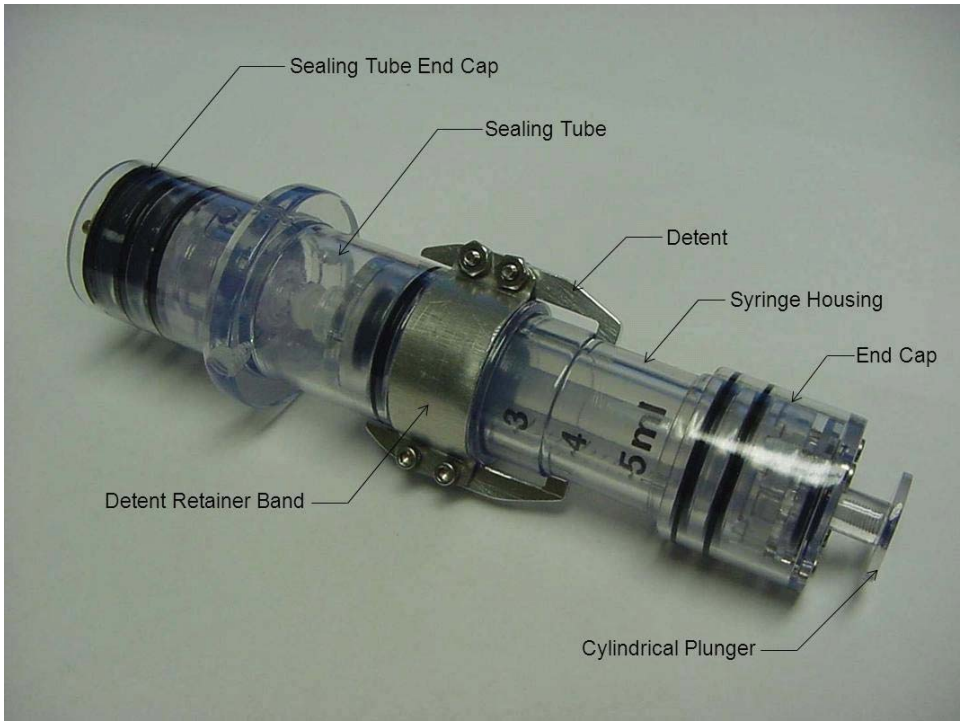
Updates and Accomplishments

- **Completed SRR Dec 2012**
- Addressed 17/19 RIDS
- **Completed First SWG 4/2013**

  SWG in line with Science Developments, test more sample types

  Look to simply operations, RNA Isolation for gene expression.
- **Completed PDR 5/2013**
- **Entered CDR 10/2013**
- **Stress testing reagents, systems, and modules, Finalized qRT-PCR Formulations, RNA Isolation Chemistry, Sample Prep Modules (SPM), Reaction Assembly Modules (RAM).**
- **WL-2 has been put on hold pending NASA review and direction.**

TechShot Syringe for Sample Input

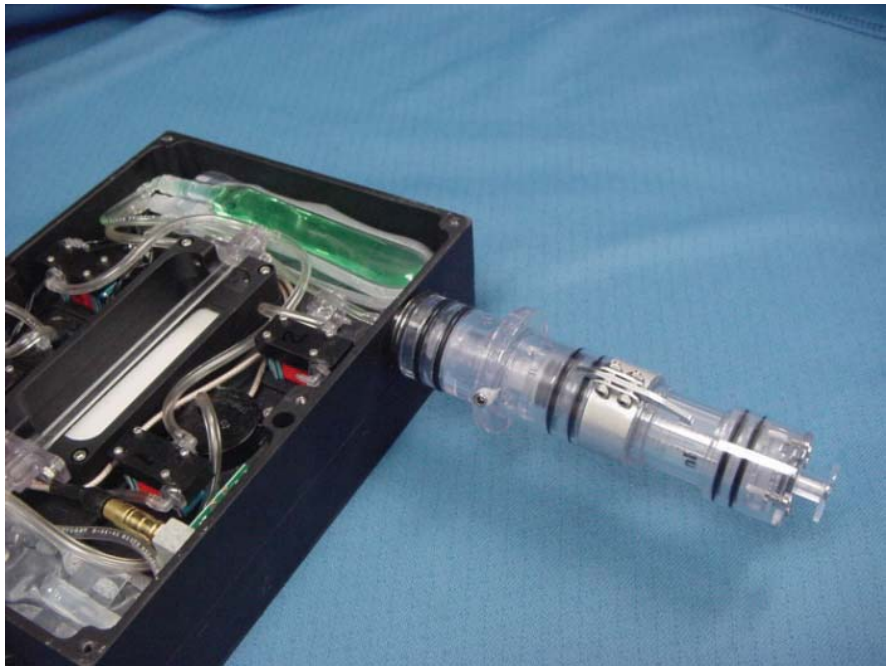


The ACT2 is a disposable device that transfers samples in a safe and contained manner from unique experiment-specific spaceflight hardware to on-orbit analytical tools for real-time analysis.

Sample Prep Module SPM-11

-Modules has the ability to extract RNA template from multiple Sample types (cell based).

-Receives input homogenized tissue samples after using the Homogenization device.

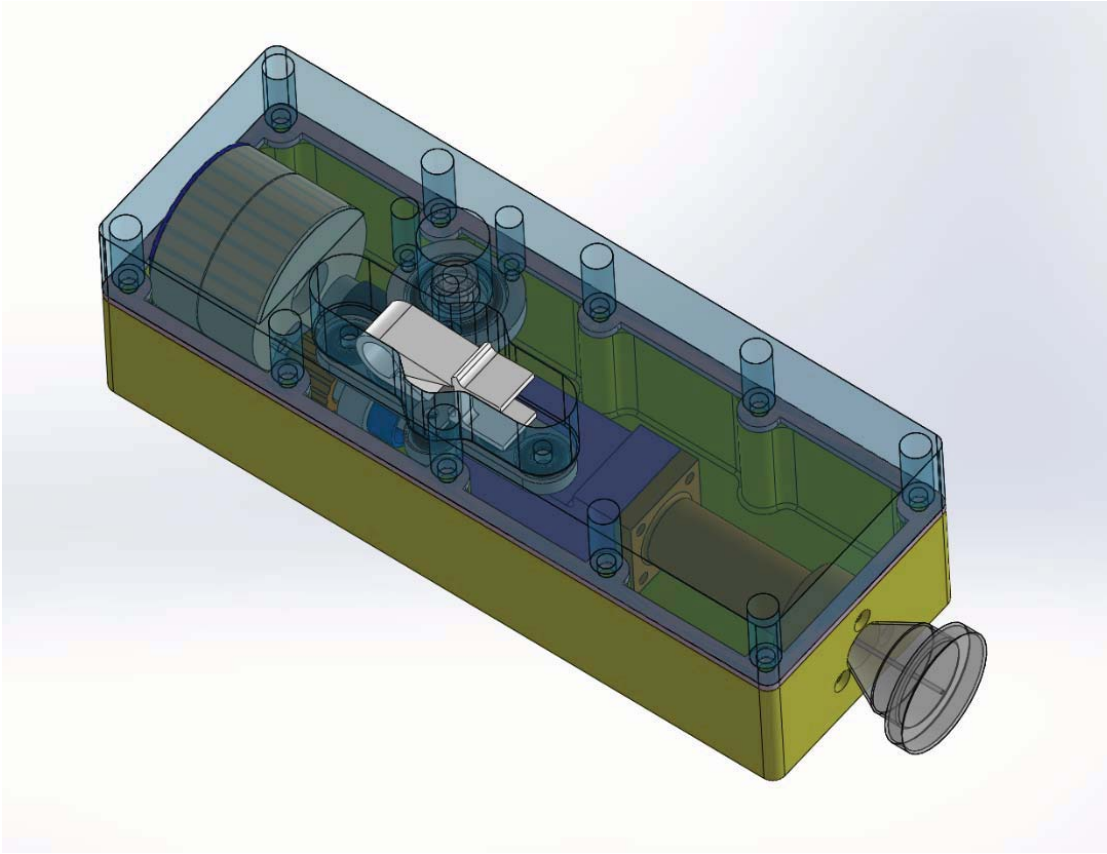


Conceptualized SPM with TechShot Syringe

National Aeronautics and Space Administration

Reaction Assembly Module

WetLab-2



Computer Model of the Reaction Assembly Module