

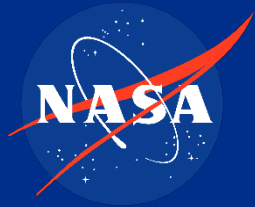
# One-Step Gene Extraction Tool



AAGGTCGATACGCTAGC

*Rapid RNA purification and Genetic Analysis on ISS*

Gergana G. Nestorova, Ph.D.  
Louisiana Tech University



# Conventional RNA purification



**Specimen lysis**



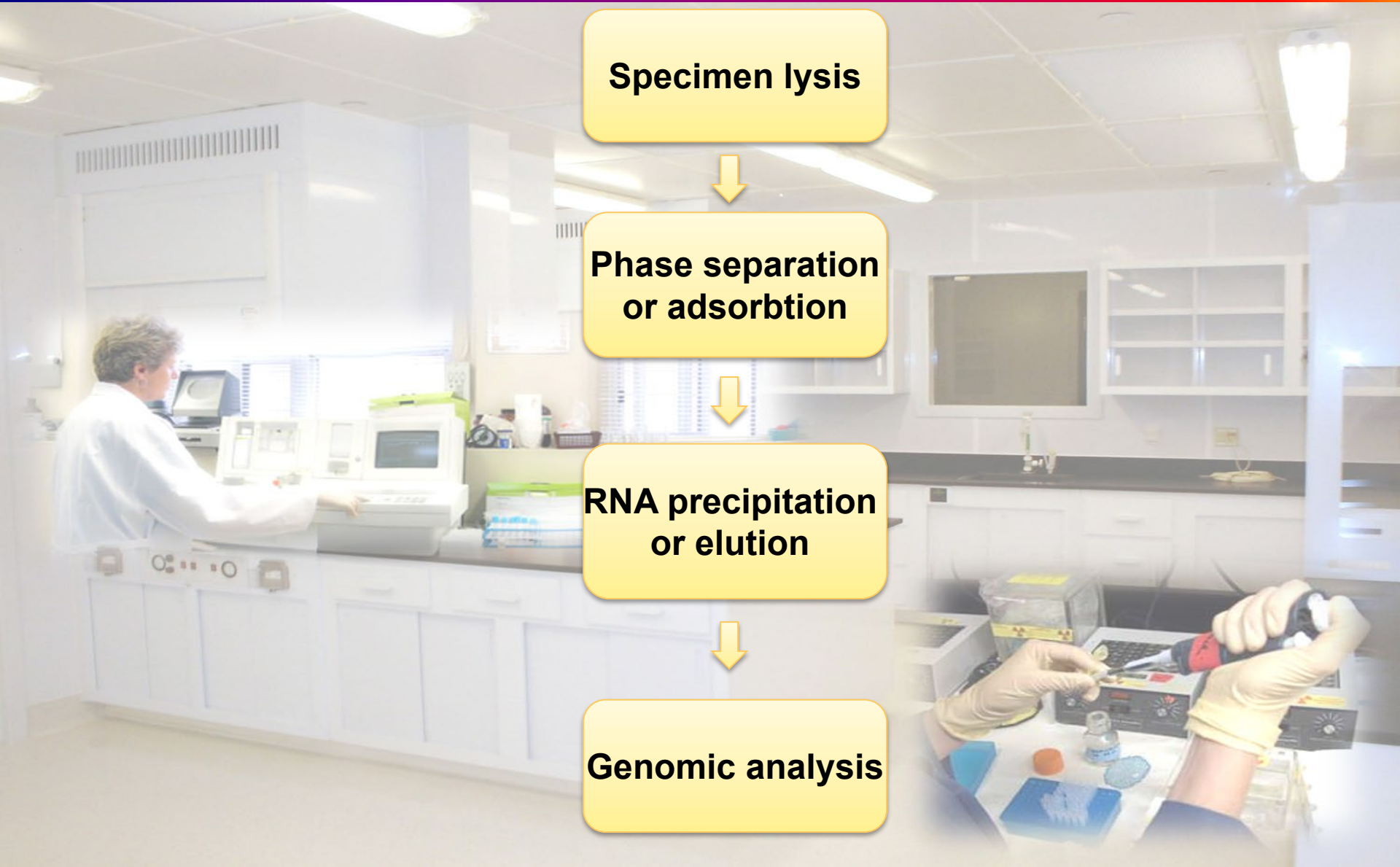
**Phase separation  
or adsorption**

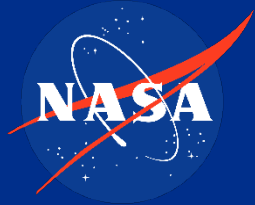


**RNA precipitation  
or elution**

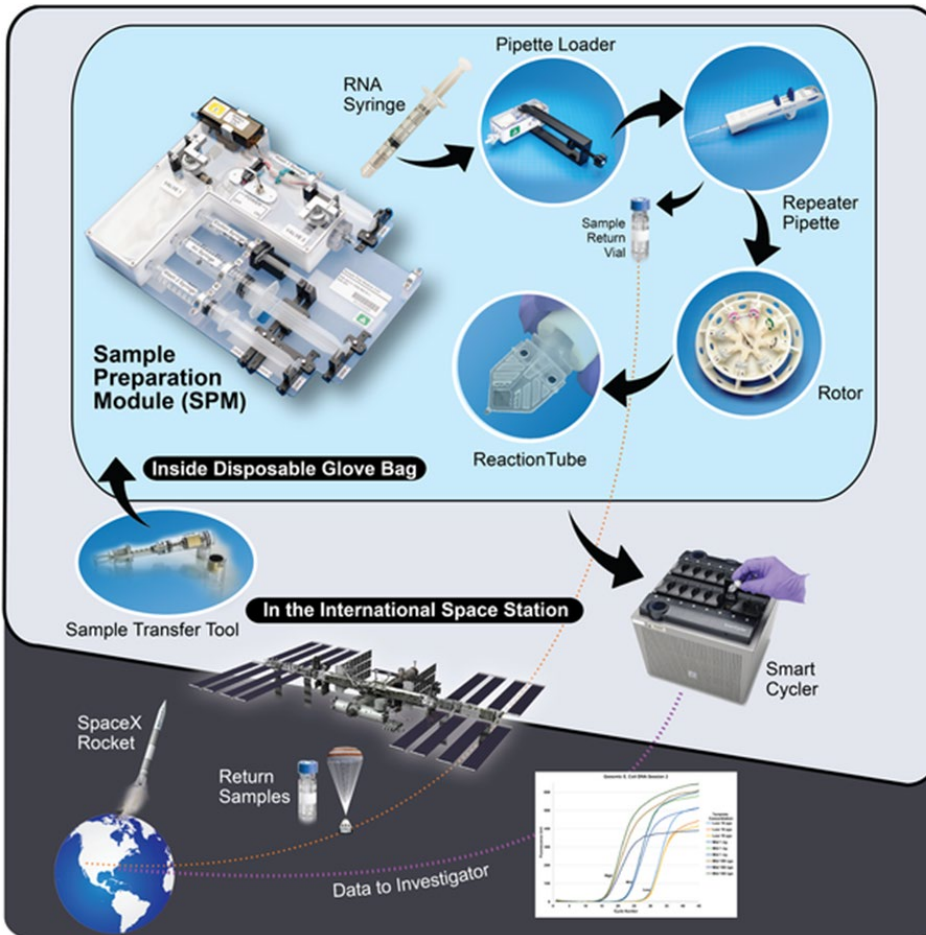


**Genomic analysis**

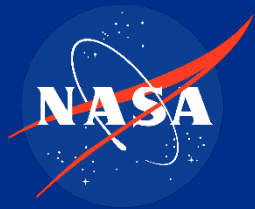




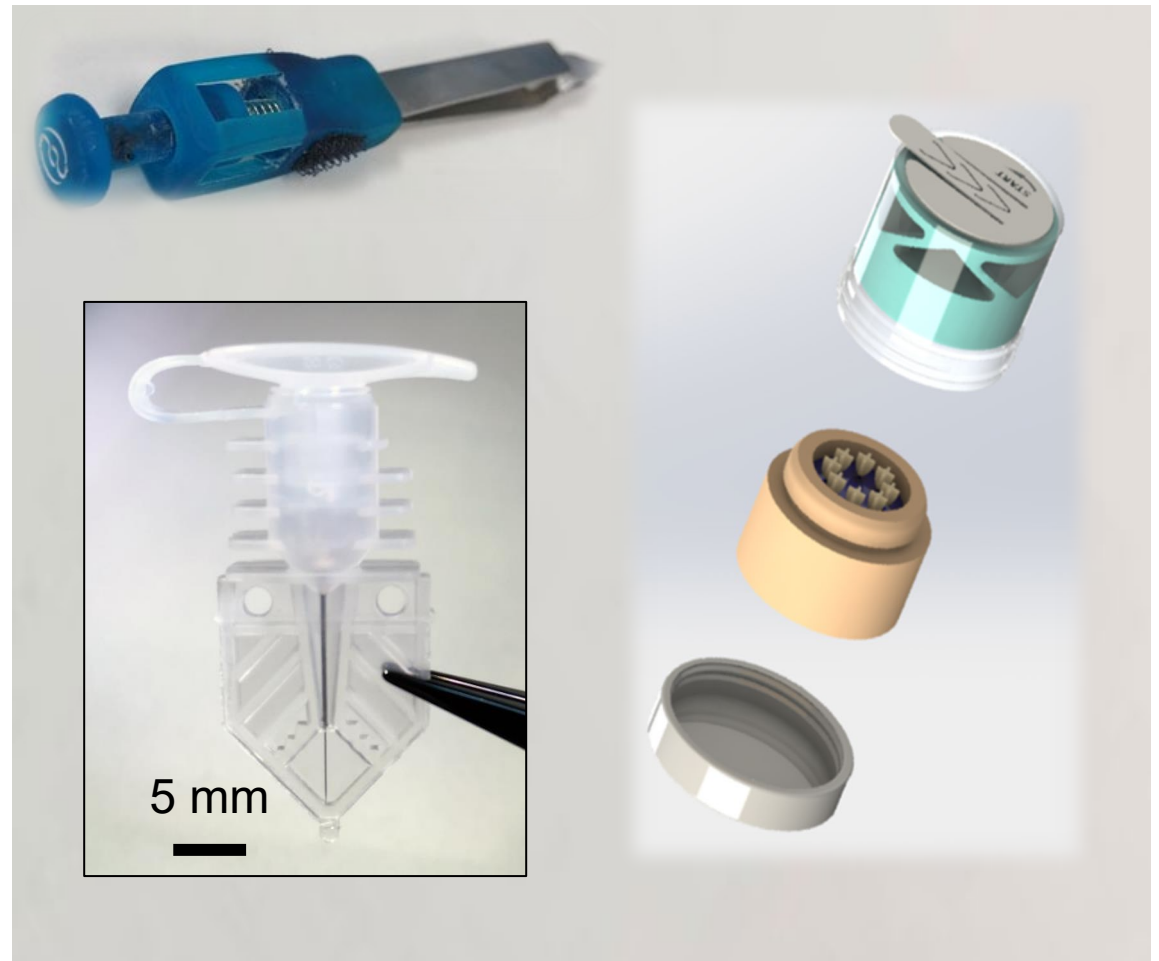
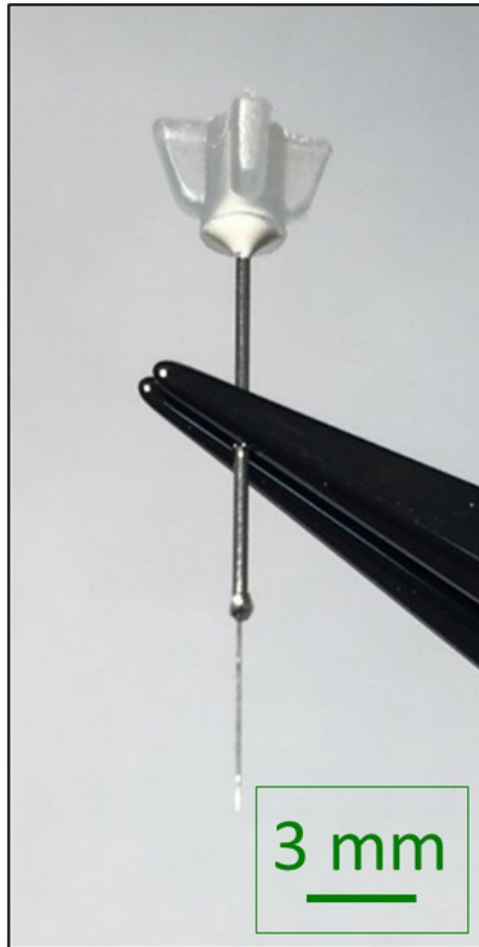
# WetLab-2 System

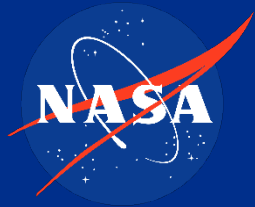


Parra, Macarena, et al.; *PloS one* 12, no. 9 (2017).

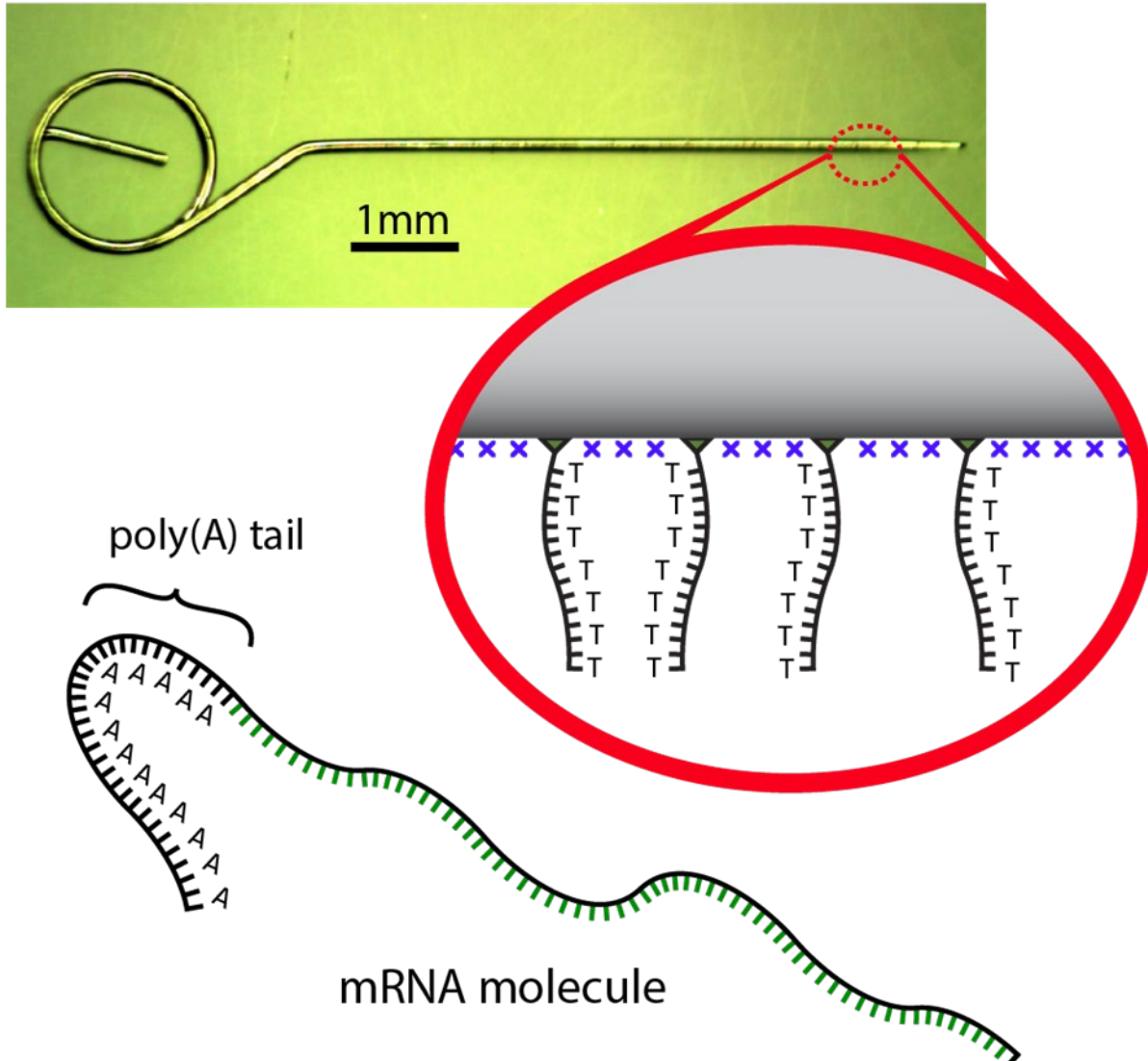


# One-step Gene Sampler

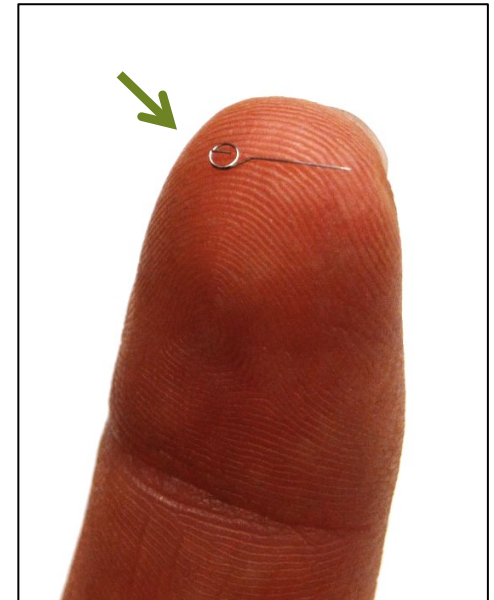


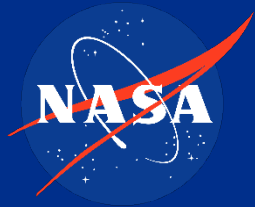


# Dry mRNA purification

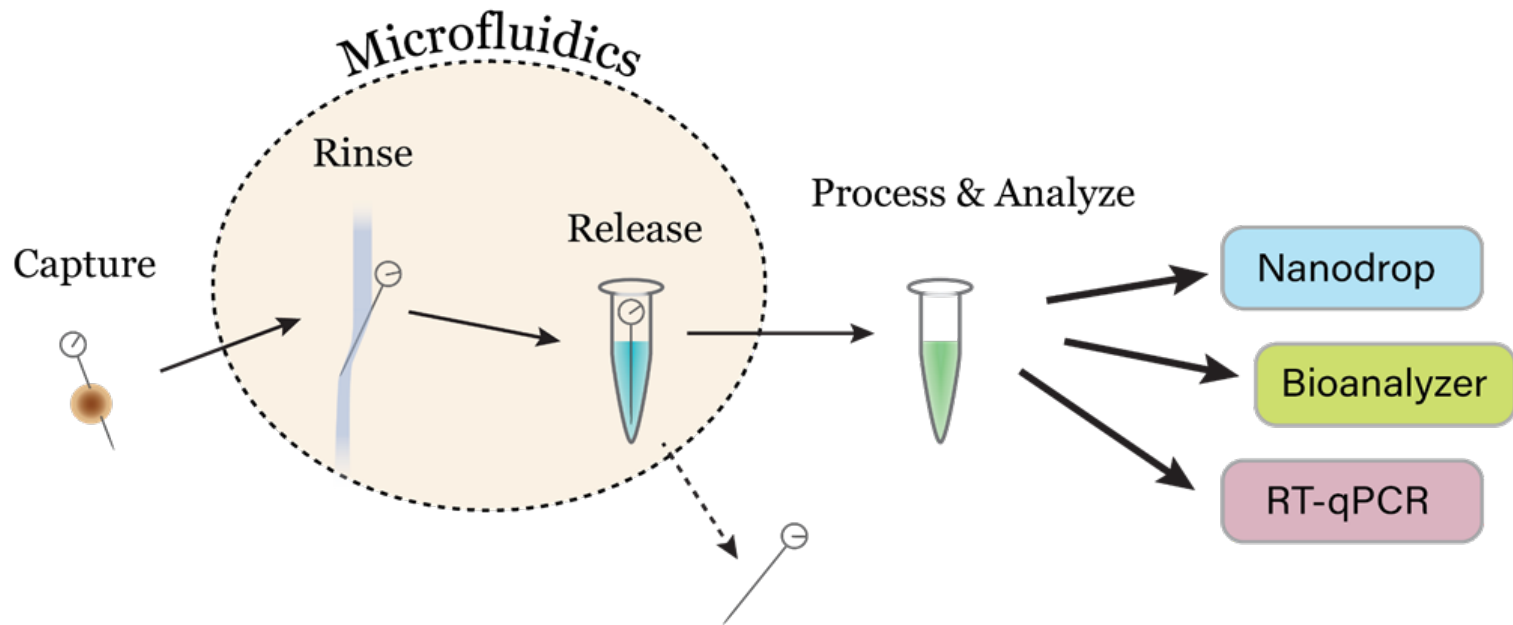
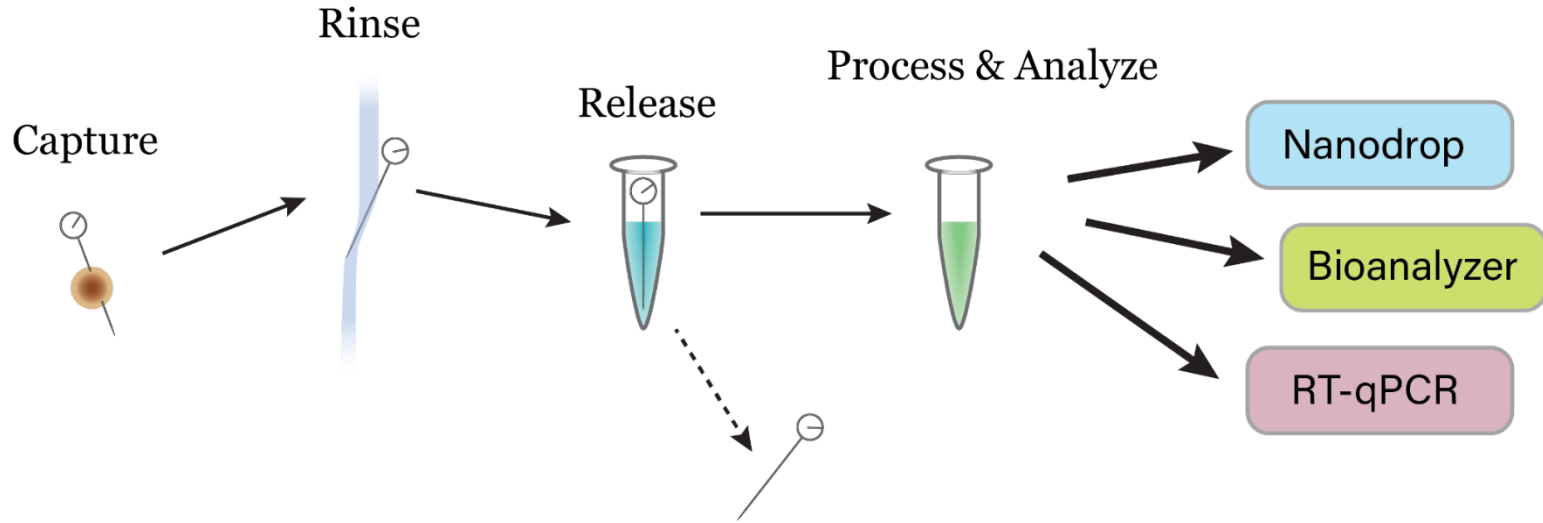


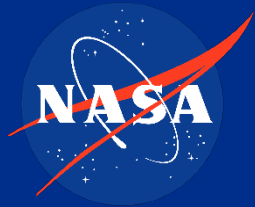
Yield  
~2 ng/mm



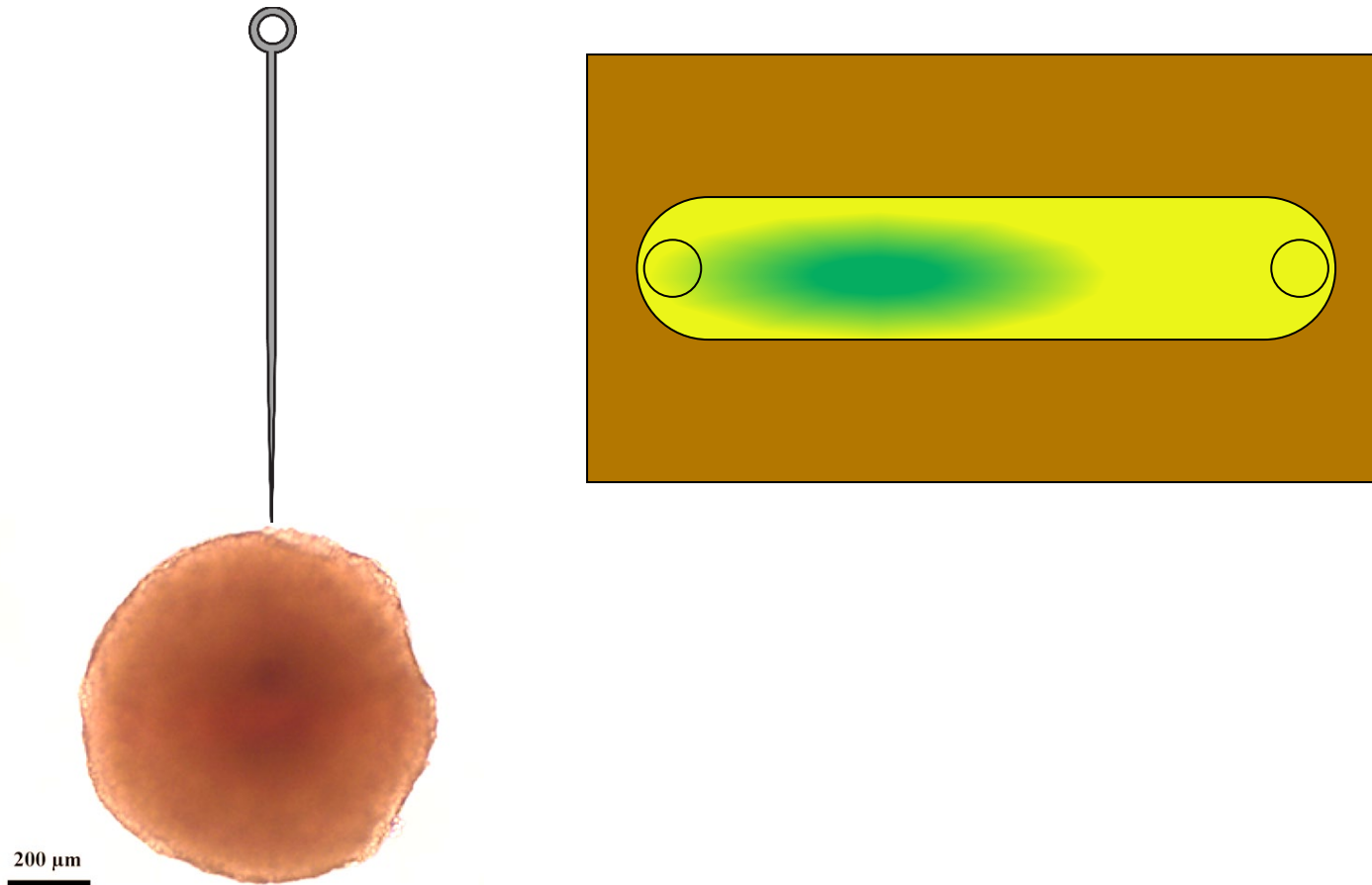


# Microfluidic Integration

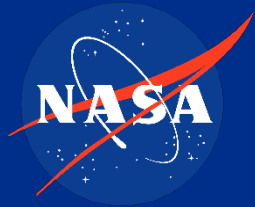




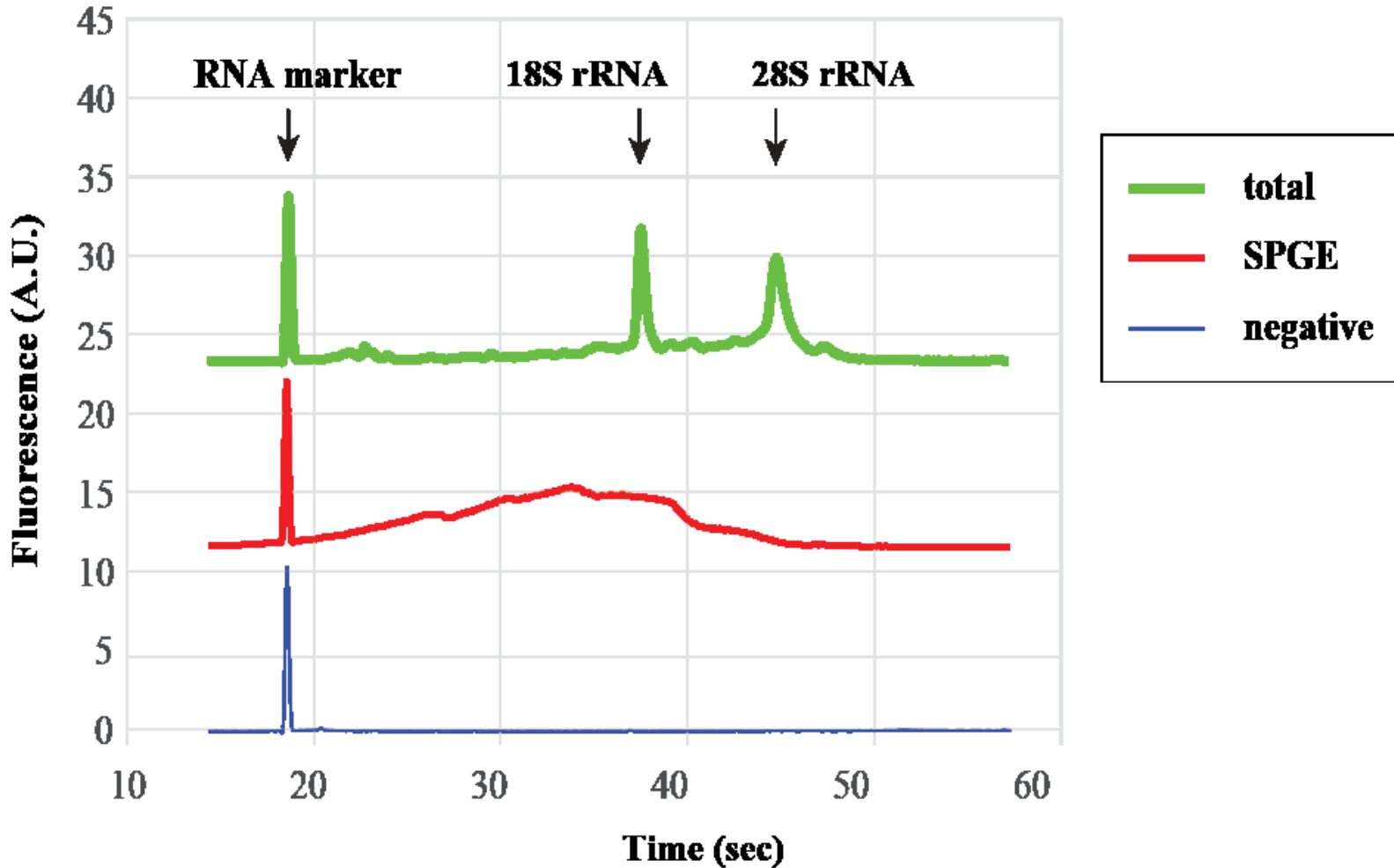
# RNA purification and on chip RT from 3D cell line



Nestorova, Gergana G., et al.; *Lab on a Chip* 17, no. 6 (2017): 1128-1136.

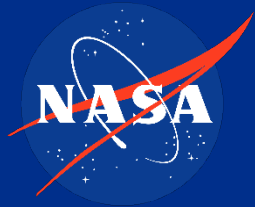


# Specificity Studies

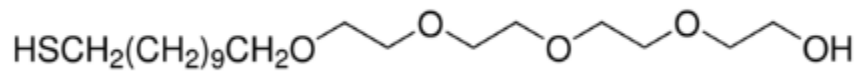
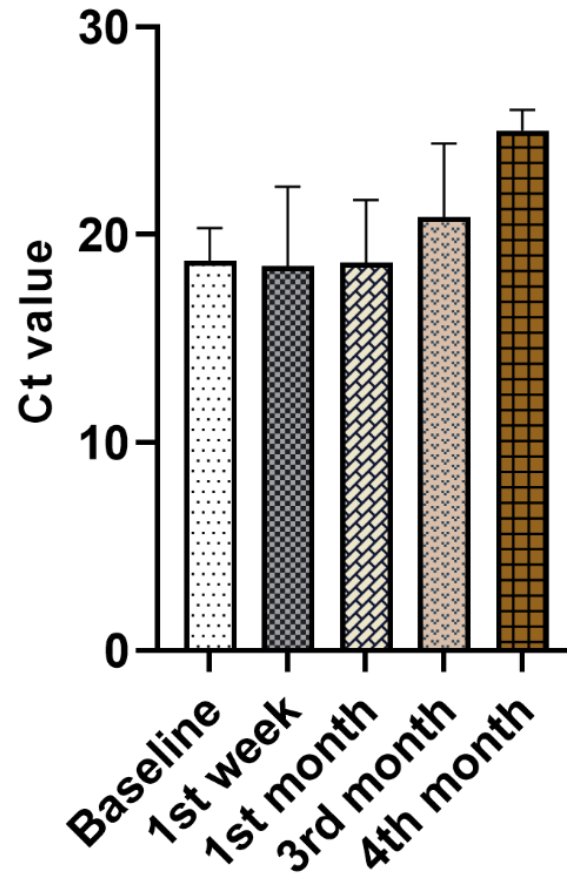


Nestorova, Gergana G., et al.; *Lab on a Chip* 17, no. 6 (2017): 1128-1136.

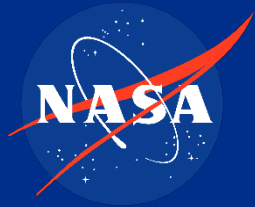




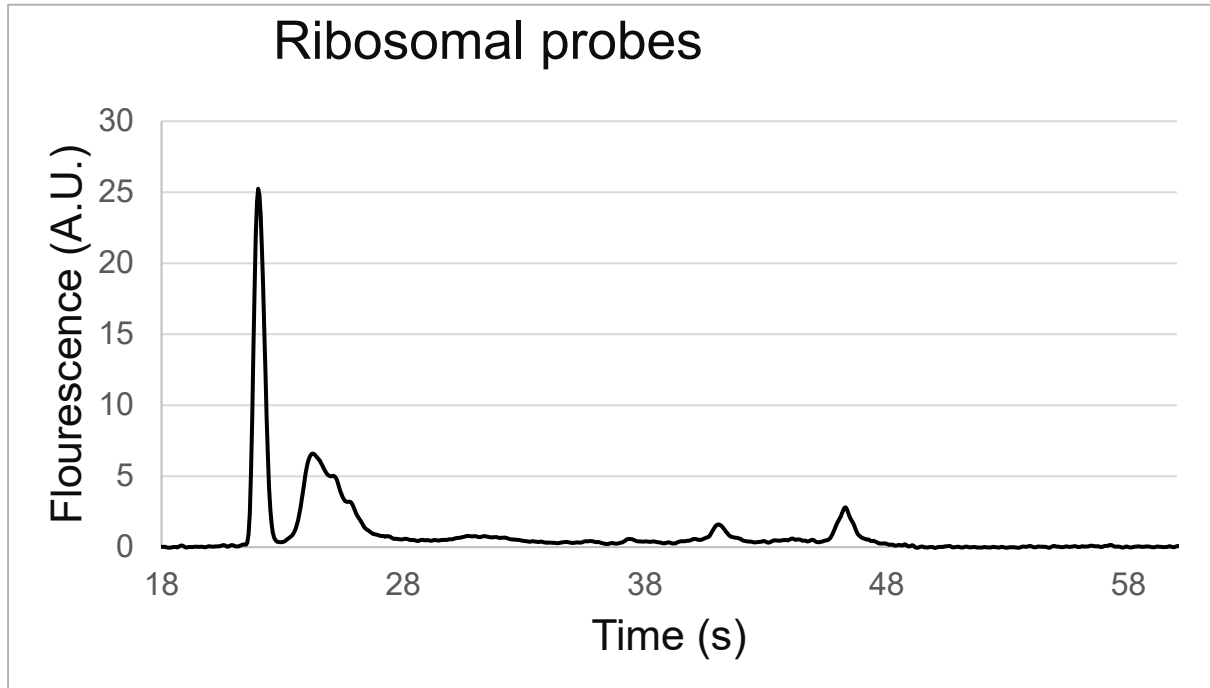
# Long term stability: Au-Thiol



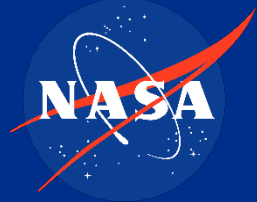
(11-Mercaptoundecyl)tetra(ethylene glycol)



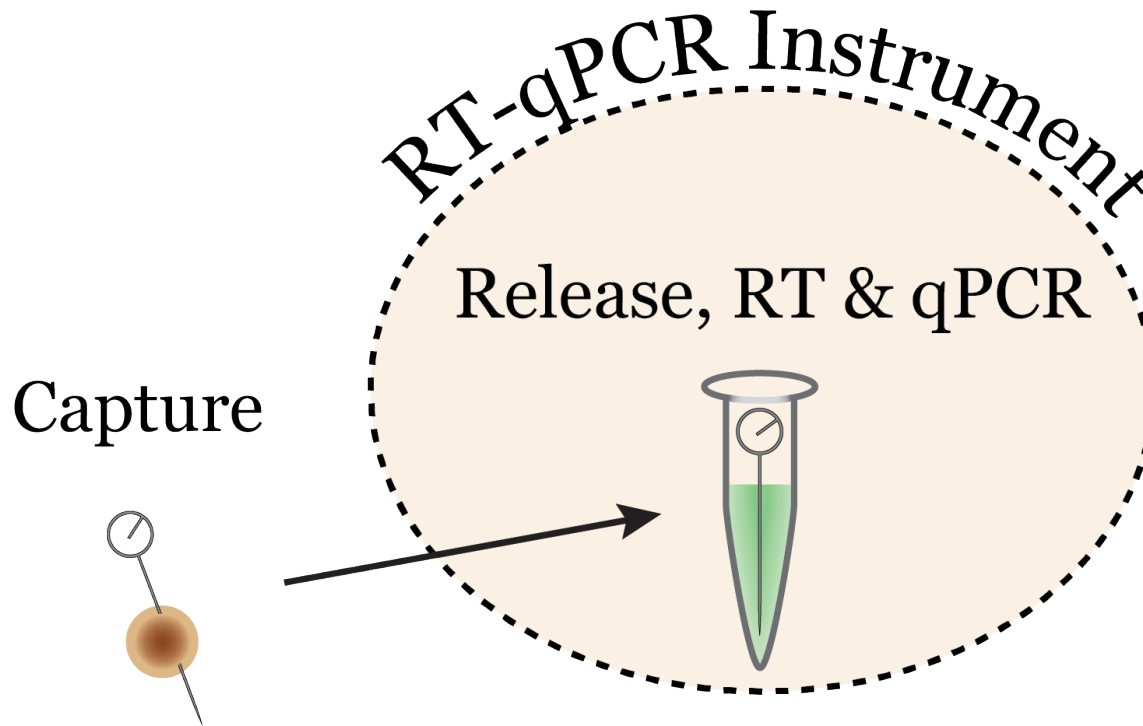
# rRNA Specificity

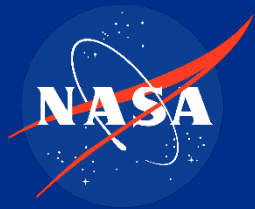


- Custom RNA Depletion Design Tool
- Eukaryotic 18S rRNA

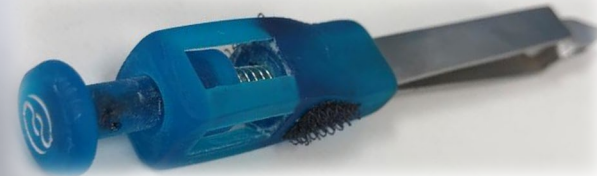
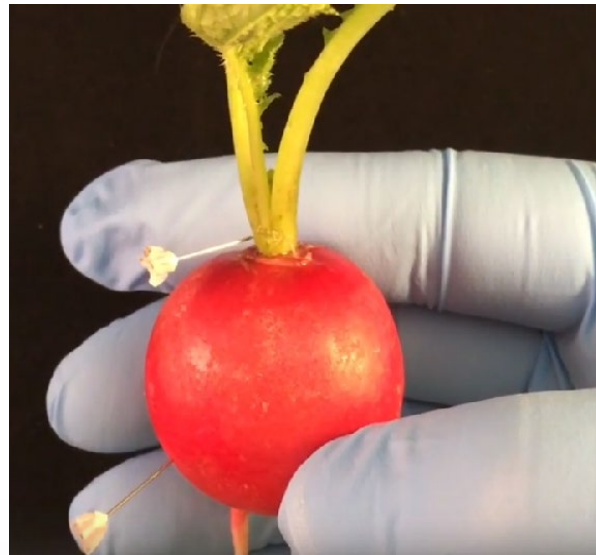
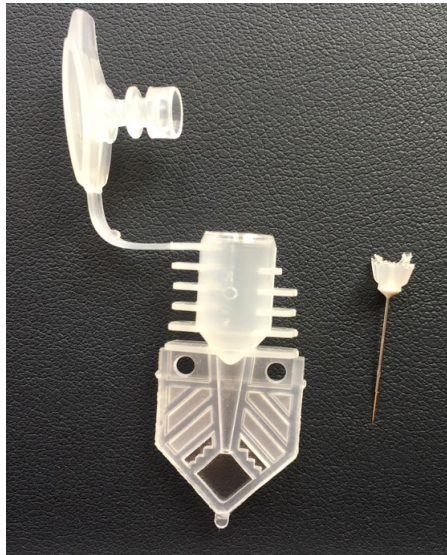


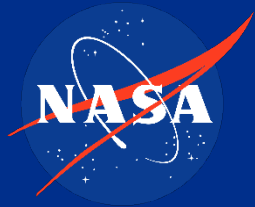
# One Step Gene Sampler Workflow



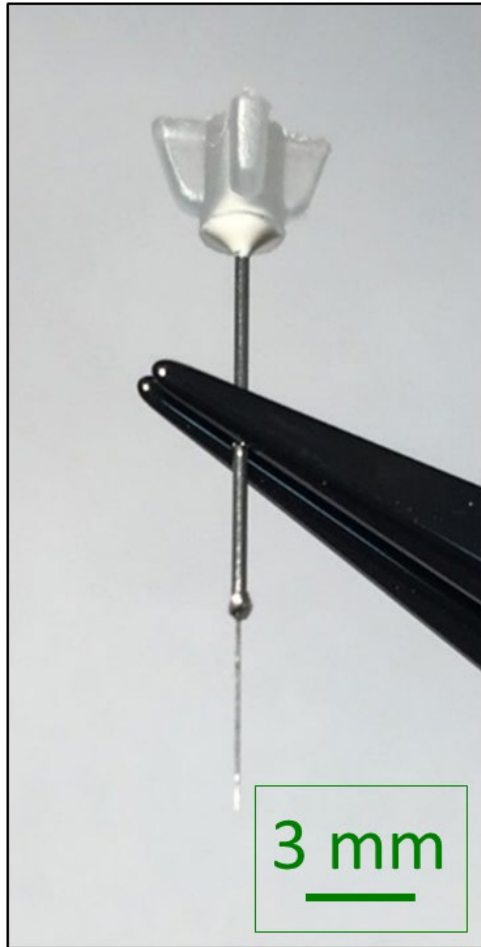


# Gene Sampler Components

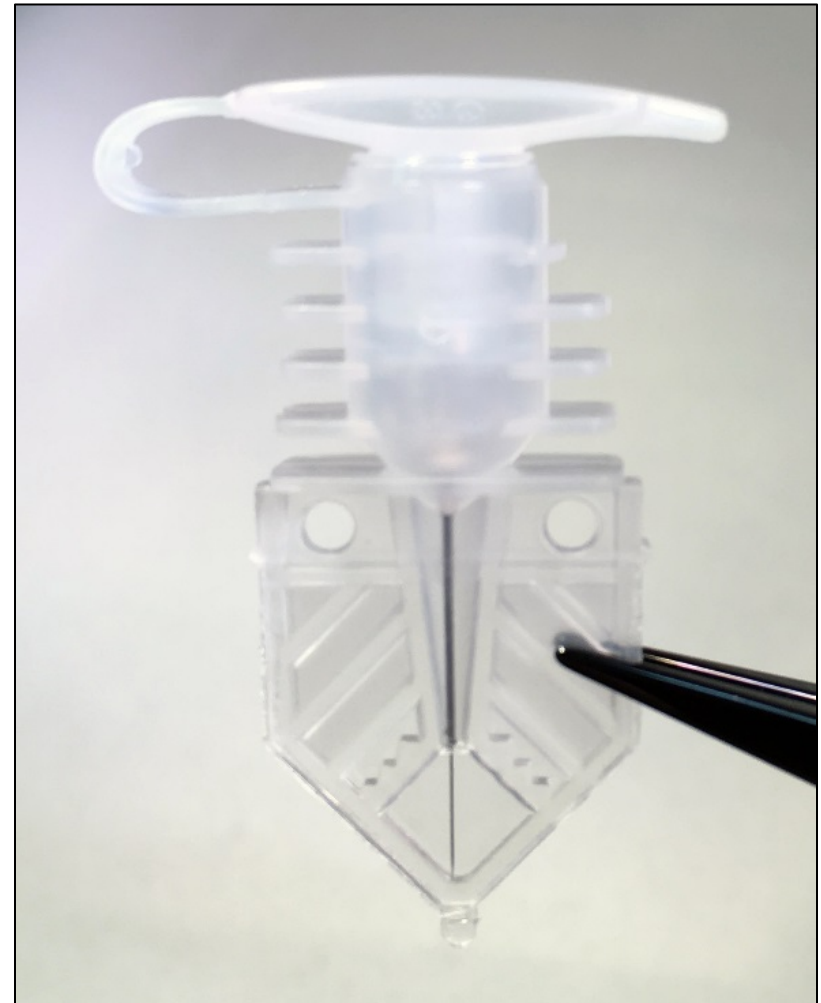


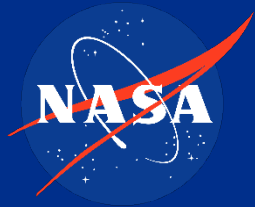


# RNA Capture Pin

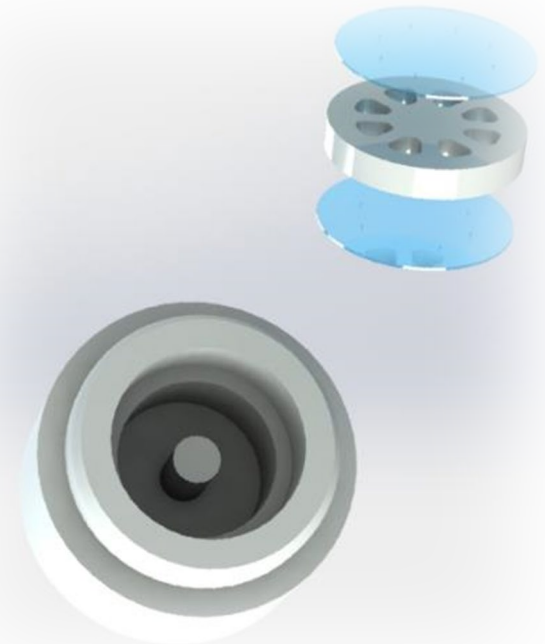
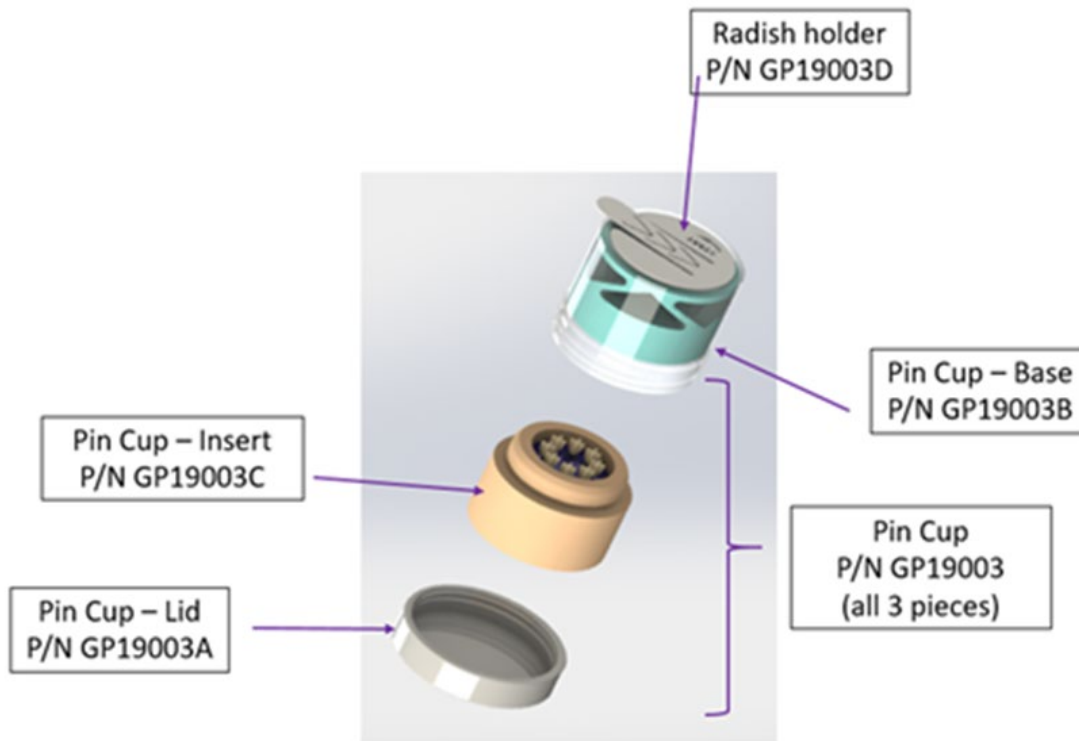


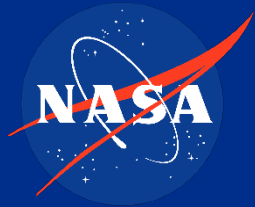
RNA Capture  
Pin (RCP) P/N  
GP19001





# Pin Cup Design

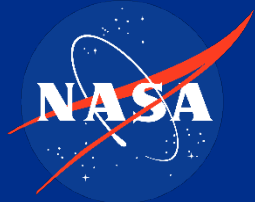




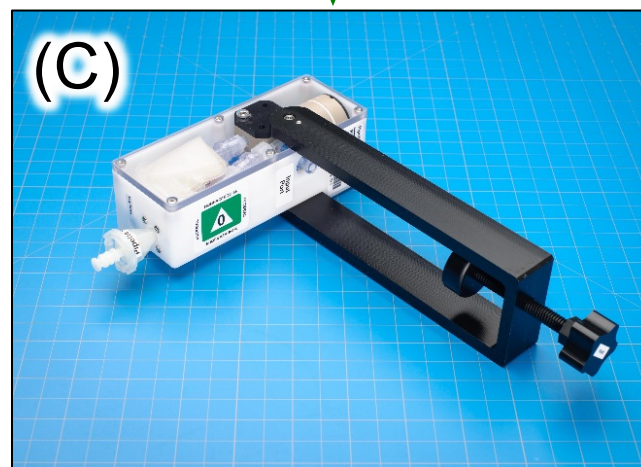
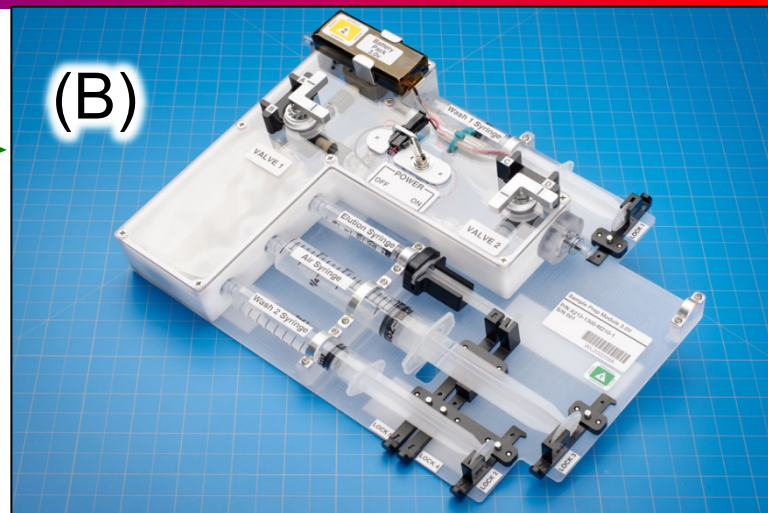
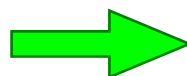
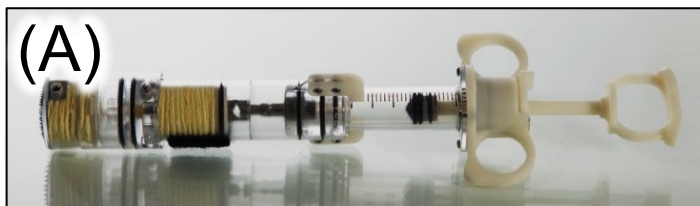
# Pin Tool



The Pin Tool is a pair modified COTS reverse action tweezers used to grip RCPs during capture and insertion into SmartCycler Reaction Tubes.

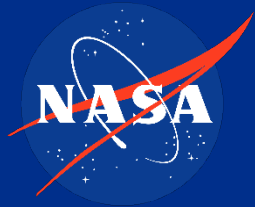


# WetLab-2 workflow

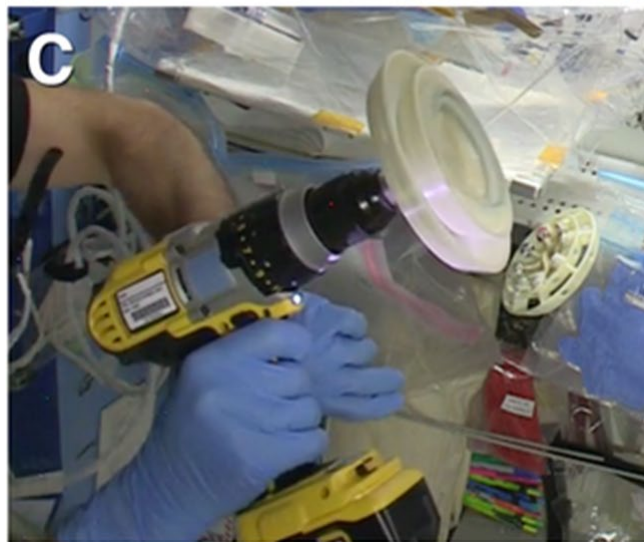
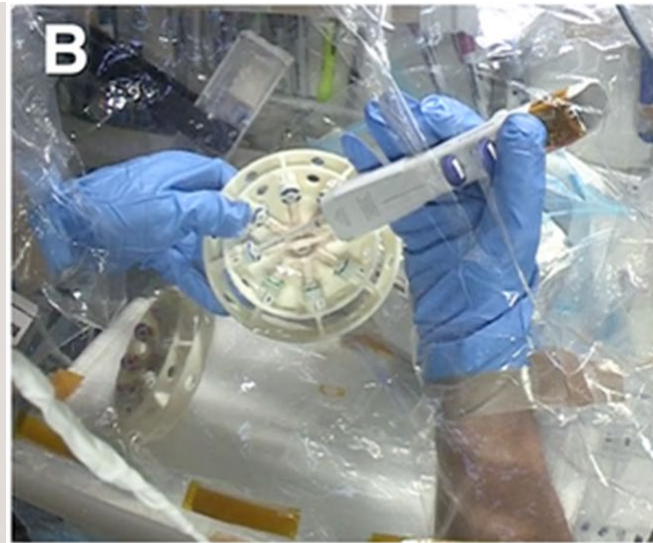
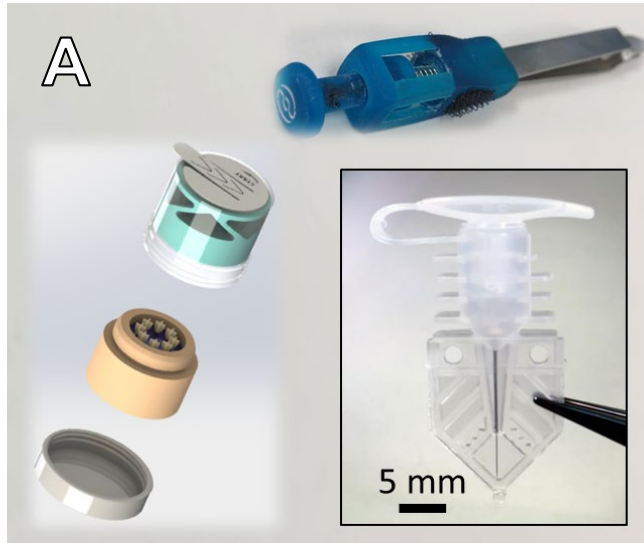


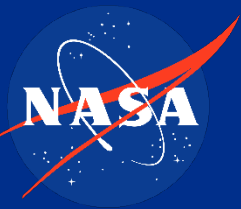
Yield  
~5,000 ng





# Gene Sampler Integration





# Hardware and operations process flow

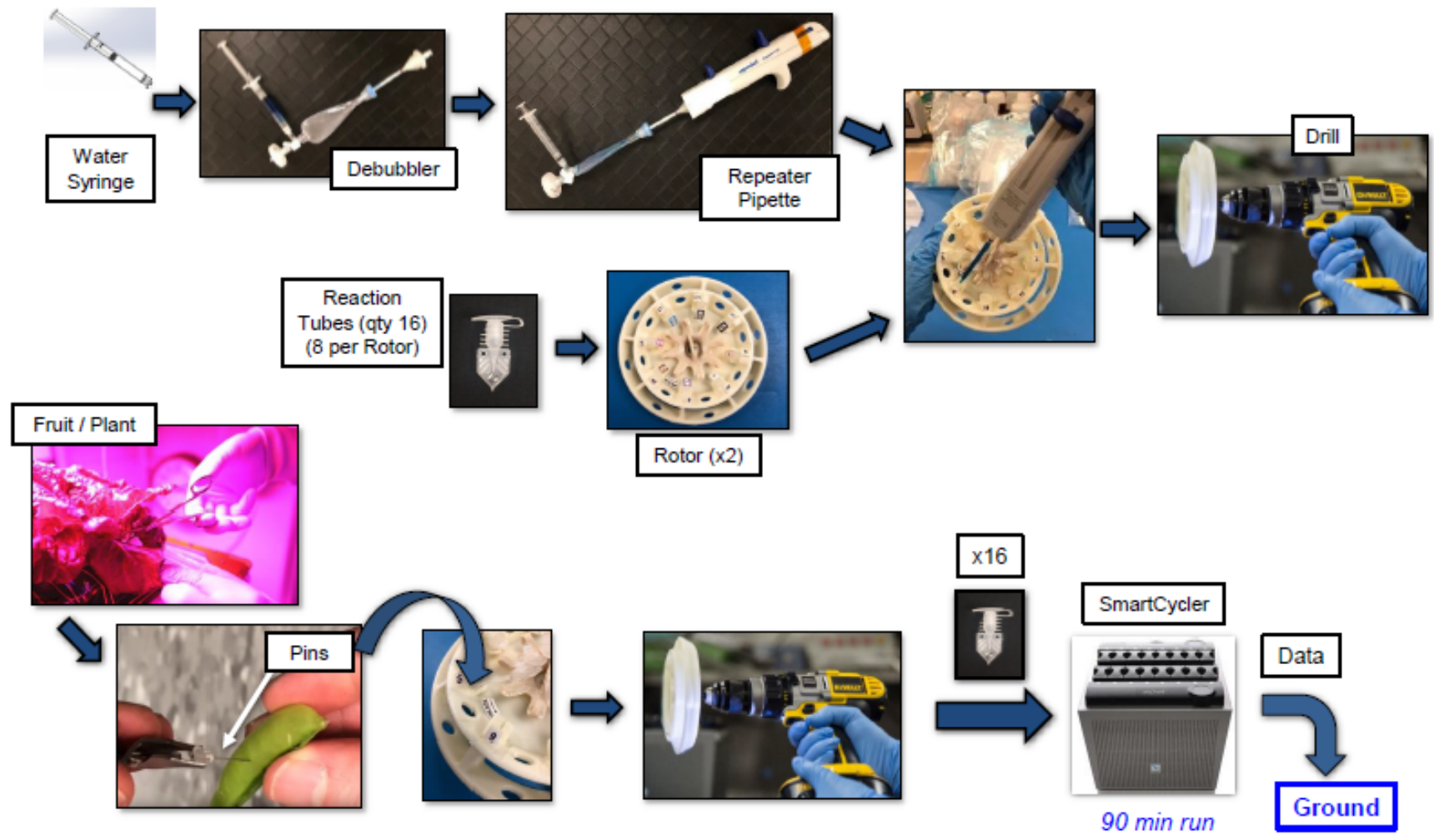
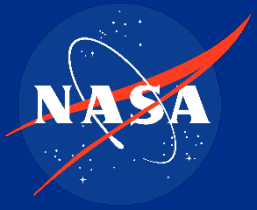


Image credit: Lisa Anderson and Travis Boone



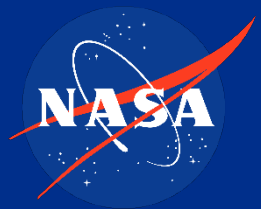
# Gene Sampler Validation on ISS



- Mission ops run GMT 047 (set up and experiment run) and gmt 048 (clean up)/ 16 Feb 21 and 17 Feb 21

Astronaut Dr. Kathleen Rubins performing RNA extraction and gene expression analysis on radish harvested from APH-02

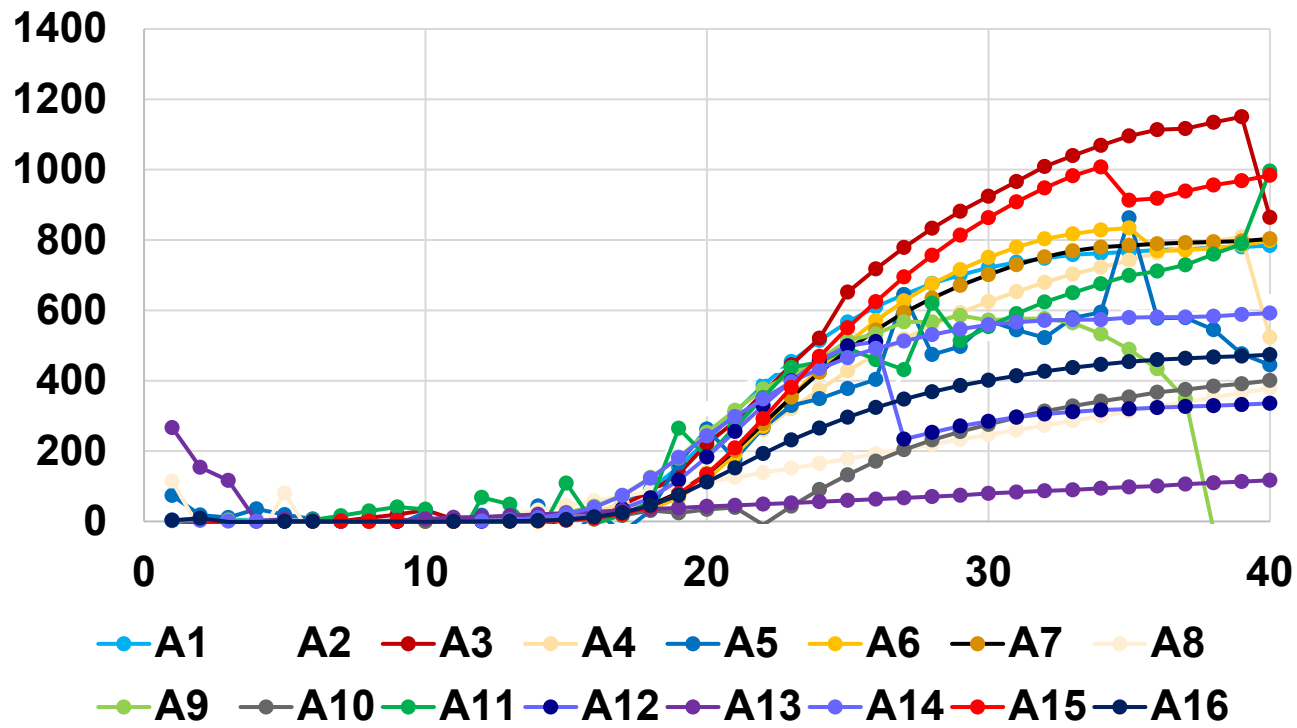




# Gene Sampler OPS Data

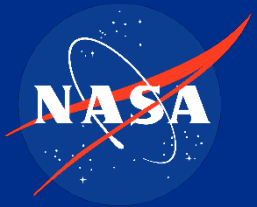


## One-Step Gene Sampler Amplification Plots



Ct by site ID

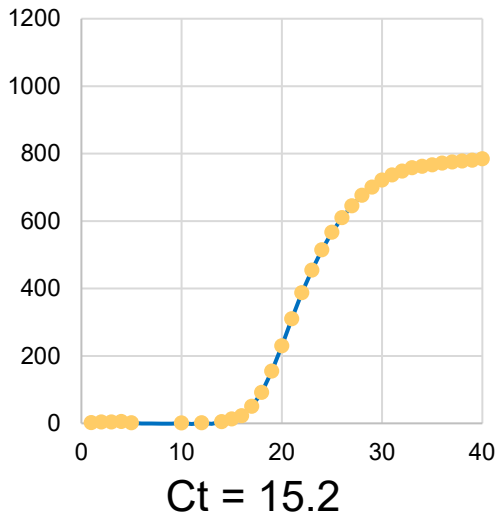
Site ID	Ct
A1	15.2
A2	12.7
A3	15.7
A4	14.9
A5	13.8
A6	16.8
A7	16.2
A8	12.6
A9	13.9
A10	16.8
A11	14.4
A12	15.6
A13	12.6
A14	14.4
A15	16.5
A16	16.2



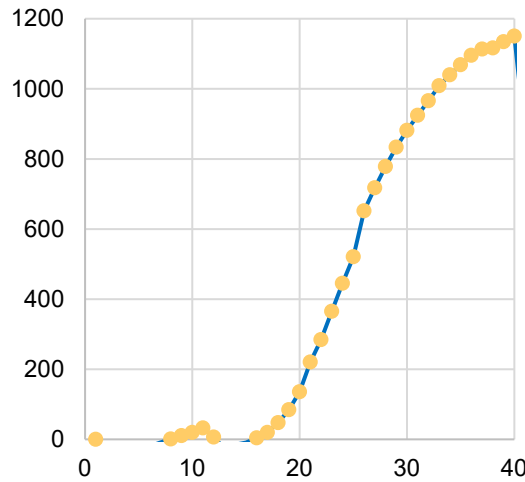
# Individual Amplification Plots



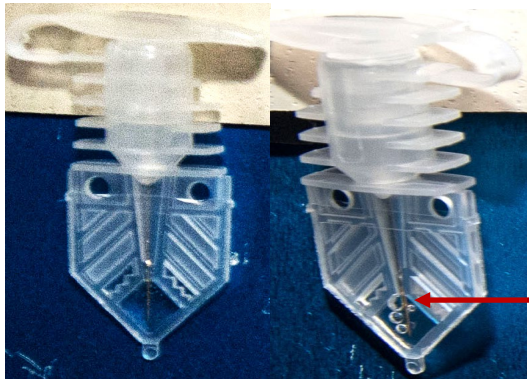
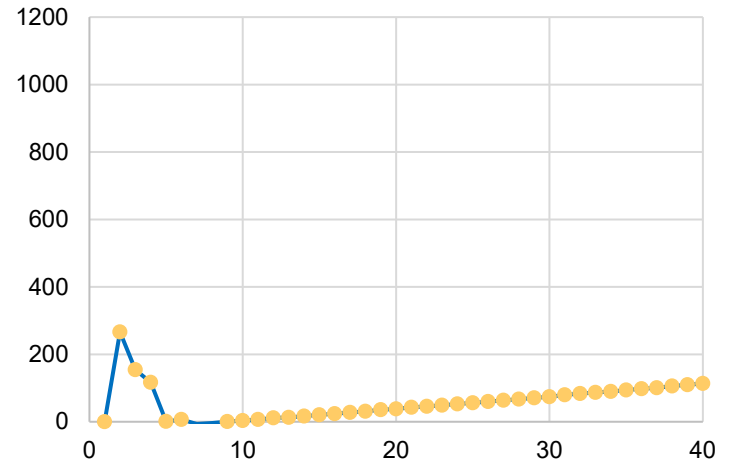
Amplification plot - A1



Amplification plot - A3

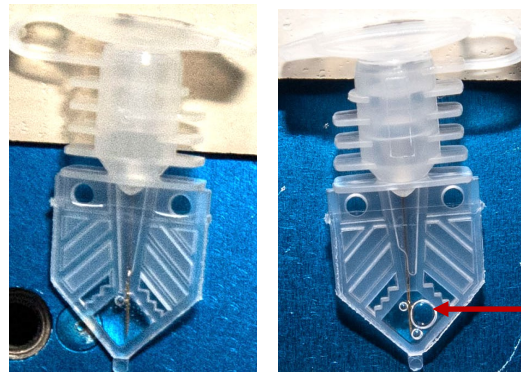


Amplification plot - A13



before

after

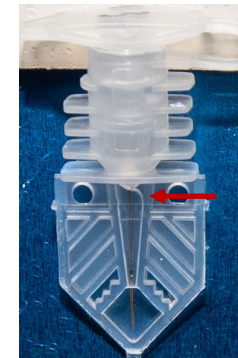


before

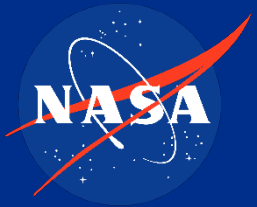
after



before



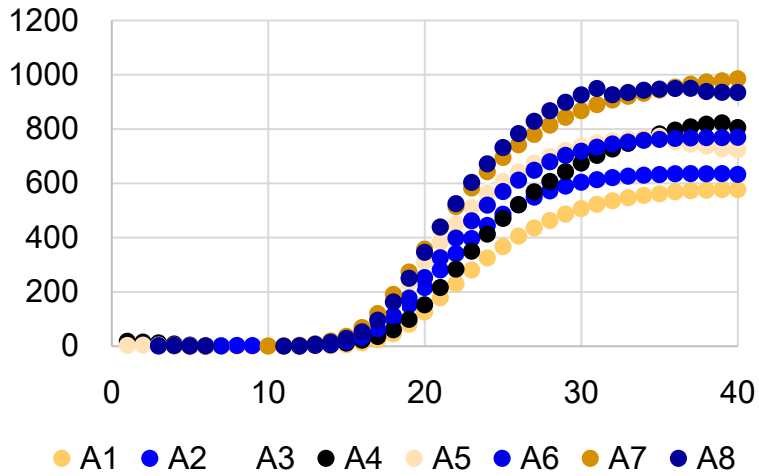
after



# Gene Sampler Ground Control



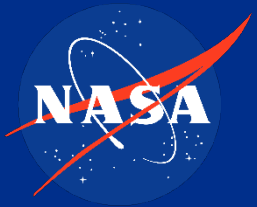
Gene Sampler amplification plot



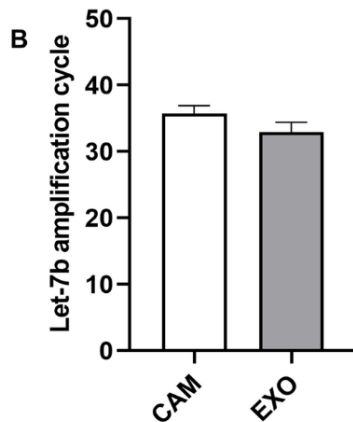
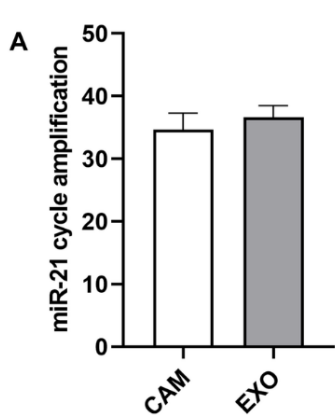
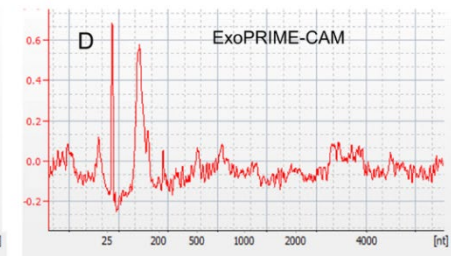
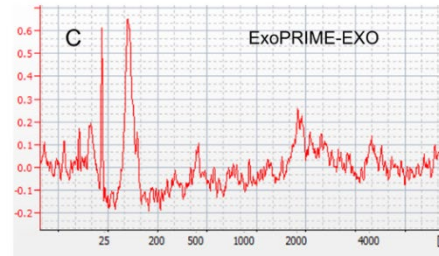
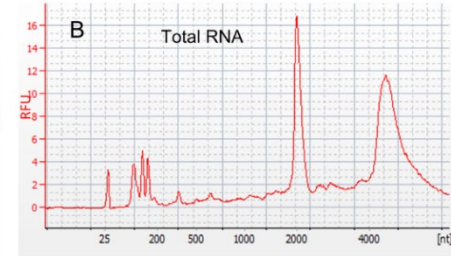
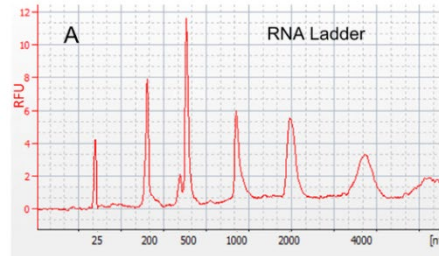
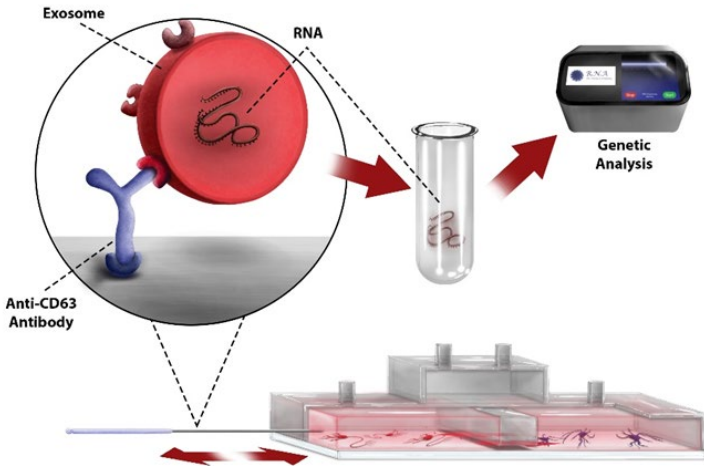
before



after

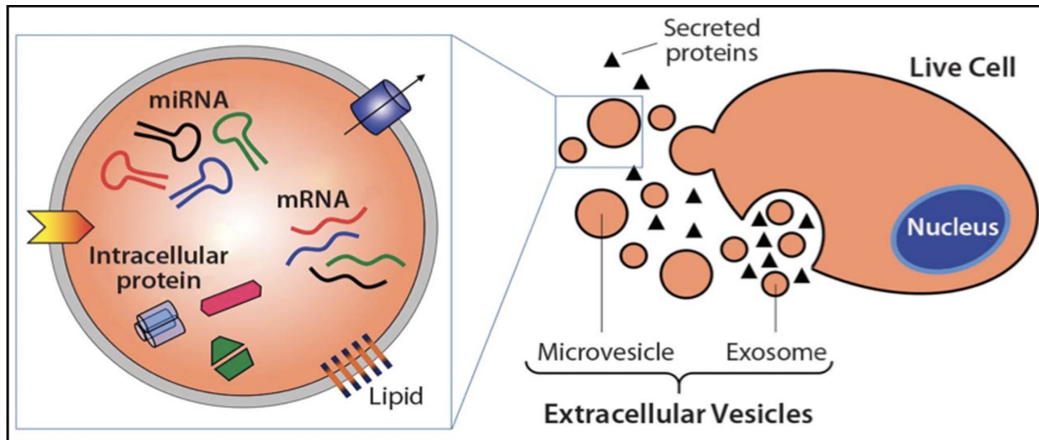


# ExoPRIME Technology



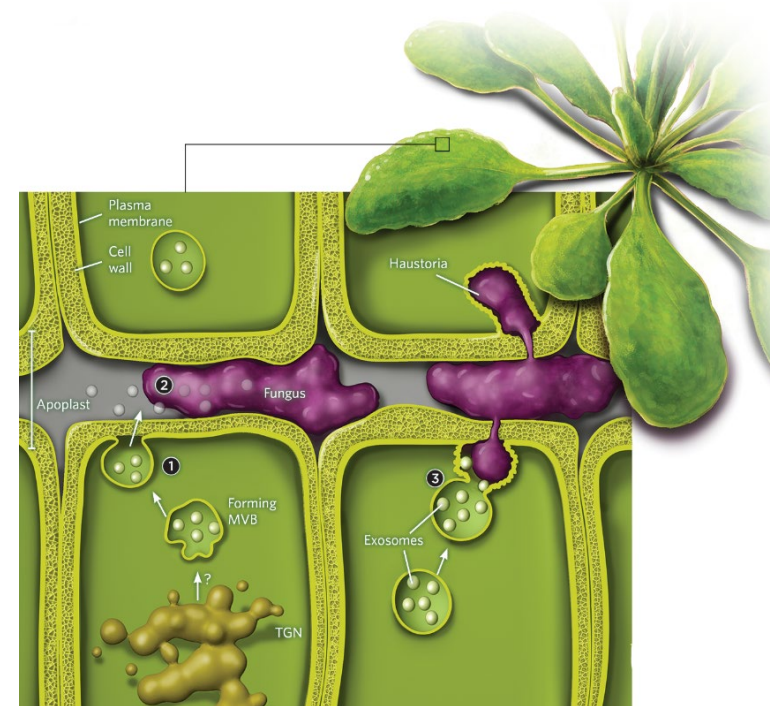
Nwokwu, Chukwumaobim D., et al. "ExoPRIME: Solid-phase immunisolation and OMICS analysis of surface-marker-specific exosomal subpopulations." *Talanta* (2021): 122870.

# Why exosomes?

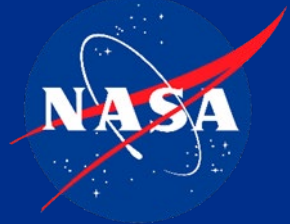


Smith, J. et al. (2015). *BioProcess International*

- Biomarkers of radiation exposure
- Mediate cell immunity
- Cell-to-cell communication
- Plant immunity







# Gene Sampler Team



- Dr. Niel D. Crews- Payload Developer and Senior Engineer  
Nou Systems, Inc., Huntsville, AL

## WetLab-2 Team

- Rudolph A. Aquilina-Wet Lab-2 Project Manager  
NASA Ames Research Center, Moffett Field, CA
- Lisa Anderson- Operations Lead, developed procedures and Flight Operations lead  
ASRC Federal Space and Defense, Inc., NASA Ames Research Center, Moffett Field, CA
- Annmarie Schramm- Project Scientist  
KBR, NASA Ames Research Center, Moffett Field, CA
- Travis Boone- Micro-Fluidics Consultant- Crew Interface  
MEI, NASA Ames Research Center, Moffett Field, CA
- Matt Chin- Lead Systems Engineer- and Verification lead for WetLab-2  
NASA Ames Research Center, Moffett Field, CA
- Tori Chinn- Lead Mechanical Engineer  
NASA Ames Research Center, Moffett Field, CA 94035
- Leonard Hee- System Safety and Mission Assurance  
Bastion Technologies Inc, NASA Ames Research Center, Moffett Field, CA 94035
- Dov Jelen- Systems Engineer-  
MEI, NASA Ames Research Center, Moffett Field, CA 94035
- Jared Shimada- Mechanical Engineer- Completed all kit manufacturing and procedures for SmartCycler operations  
MEI, NASA Ames Research Center, Moffett Field, CA 94035

Renee Bell and Brett Early-Payload Integration Manager  
Dr. Jennifer Scott Williams-Science Integration Manager  
NASA Johnson Space Center

Dr. Kathleen Rubins  
NASA Jonson Space Center

Michael Vickery and Bill Marion  
BioGX, Birmingham, AL

APH-02 and Dr. Karl Hasenstein  
Kennedy Space Center and ULL

Thomas Holland and Deriesha Gaines  
Louisiana Tech University



Any  
**Questions?**