JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

RNA Stability and Transportation

3/24/2015

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Shipping RNA samples to the JGI

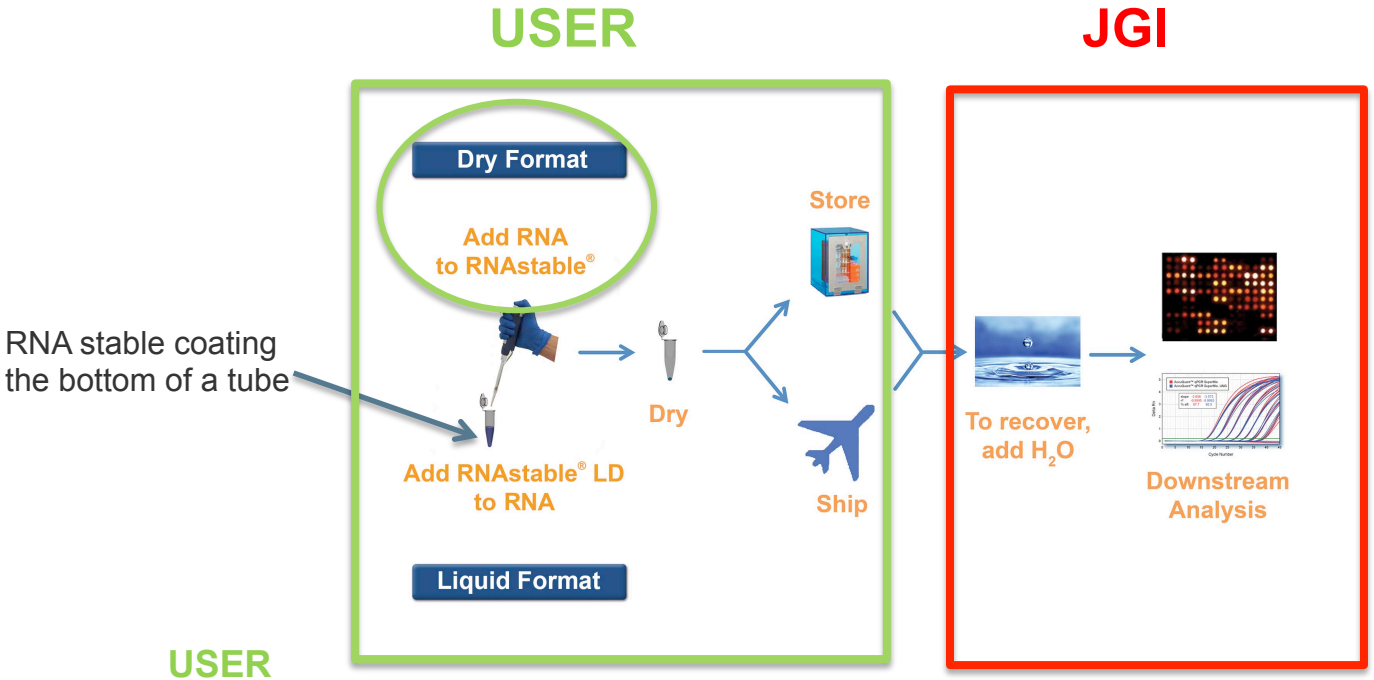
- **Collaborators samples are shipped to the JGI**
  - In liquid format
  - On dry ice

- **Some Countries have stringent shipping requirements**
  - No Dry ice
  - No liquids to be shipped

- **The JGI has tested an alternative shipping format**
  - A commercial product from Biomatrica: RNAstable

What is RNAstable

A synthetic polymer that forms a protective seal around RNA to prevent degradation during storage at room temperature.



USER

1) Add RNA to an RNAstable coated tube.
2) Dry RNA in a speed vacuum with NO HEAT.
3) Store or Ship dried RNA, at room temperature (in heat-sealed foil bag), without dry ice.

JGI

1) Rehydrate with water.
2) The RNA is now ready for downstream analysis without further purification.
3) After resuspension, the RNA is stored at -80C until sample QC.

RNAstable Testing

- Compared RNAstable treated RNA in dry format with the same RNA stored at -80C.
- Did RNAstable affect recovery or quantification?
- Did RNAstable affect quality of the RNA?
- Did RNAstable compounds affect the quantity and quality of the RNA?
- Did RNAstable affect library quality, yield, or sequencing results?

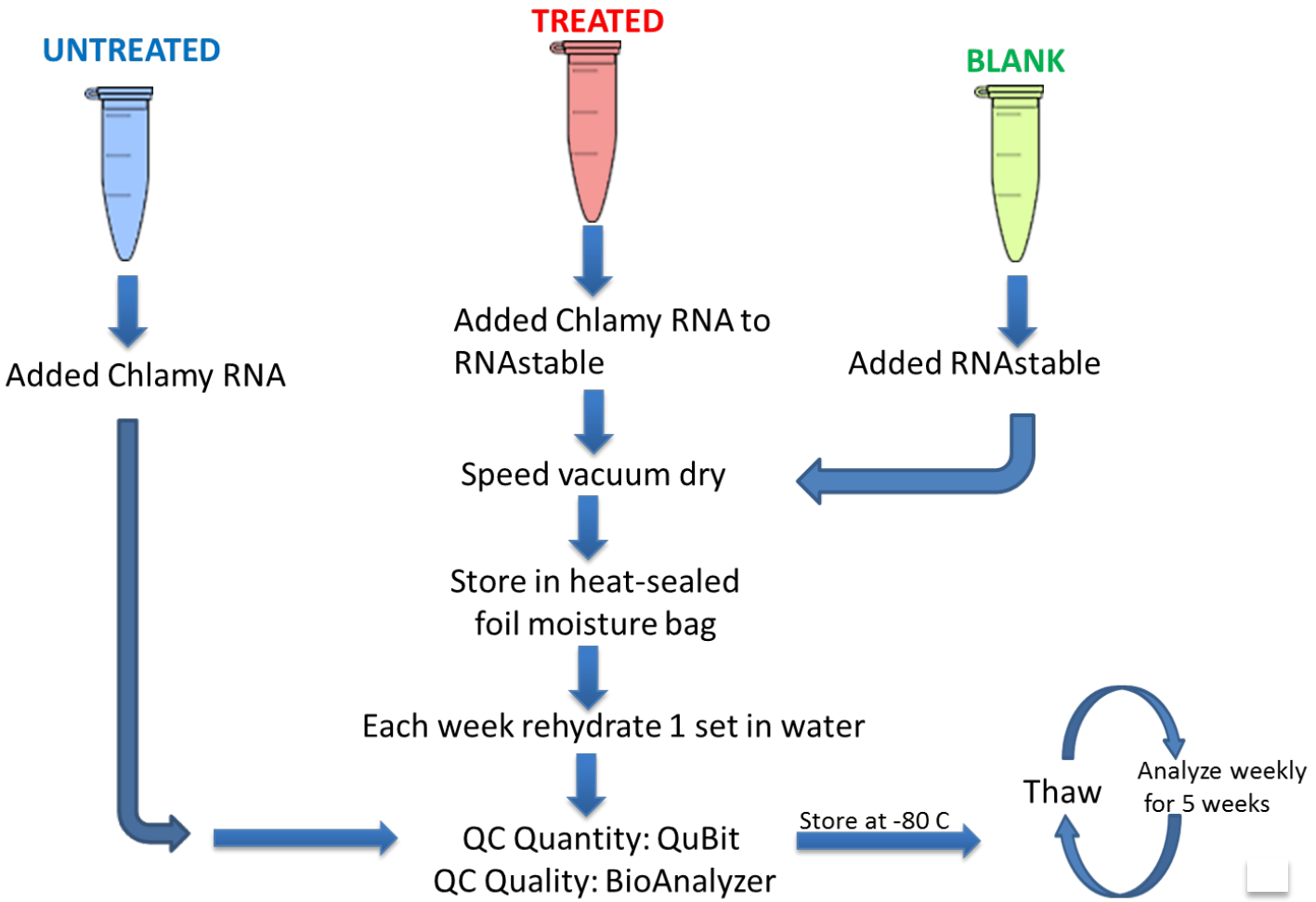
Life cycle of an RNA sample

User Sample Process

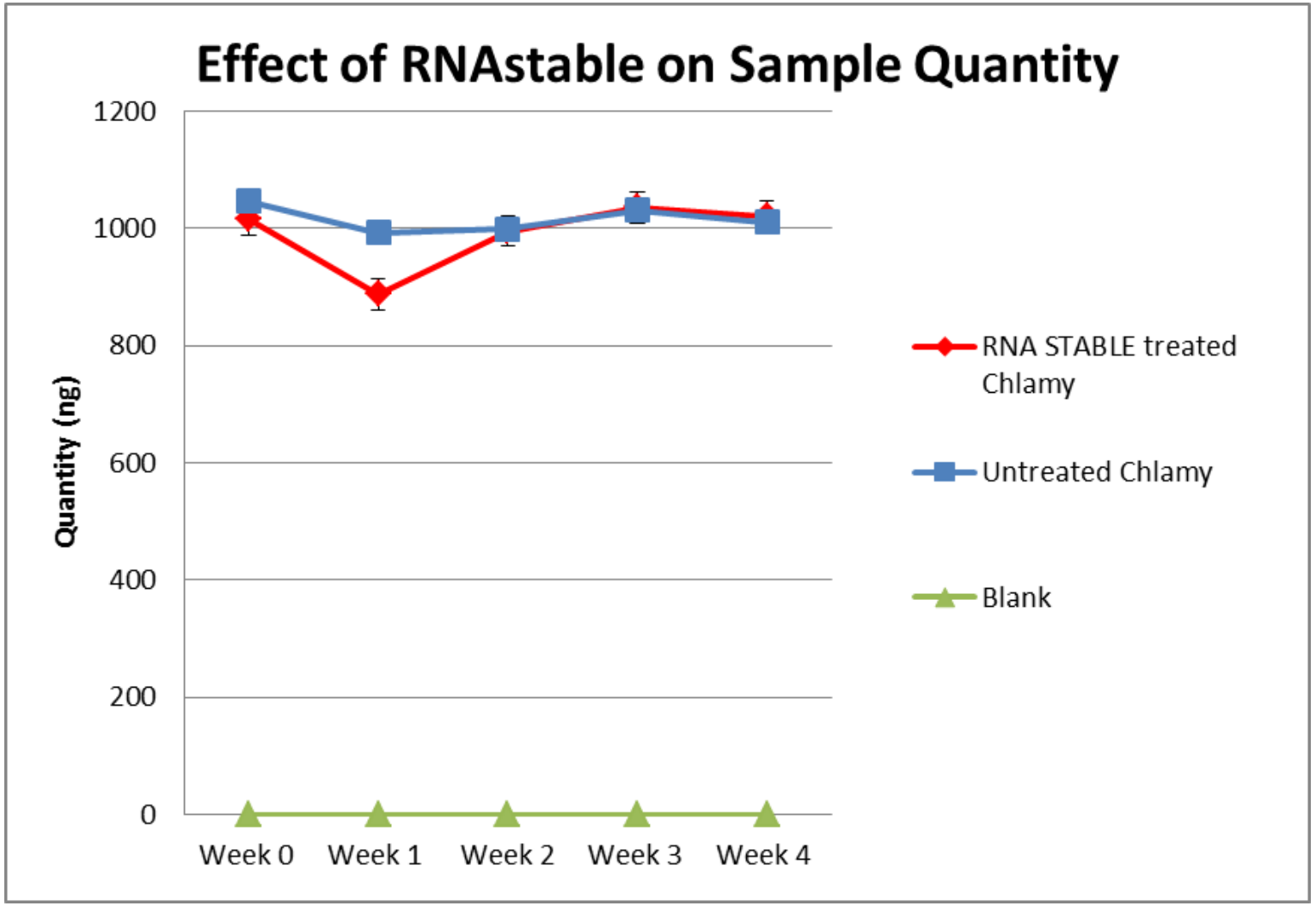
JGI Sample Process

RNAstable and Freeze/Thaw Cycle Experimental Setup

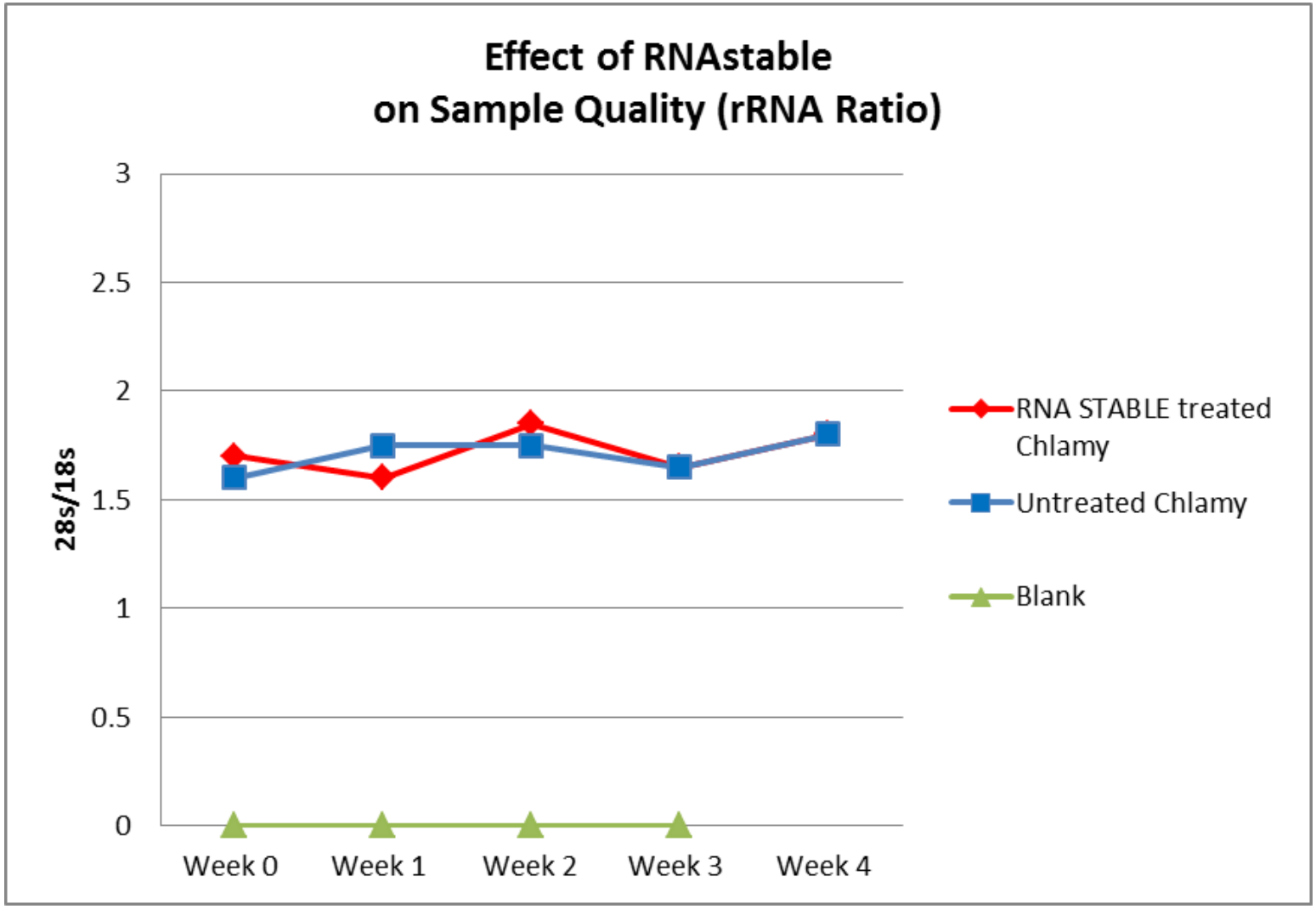
Set up in duplicates for weekly analysis and 4 Freeze/Thaw cycles



Did RNAstable have an effect on RNA Quantity?

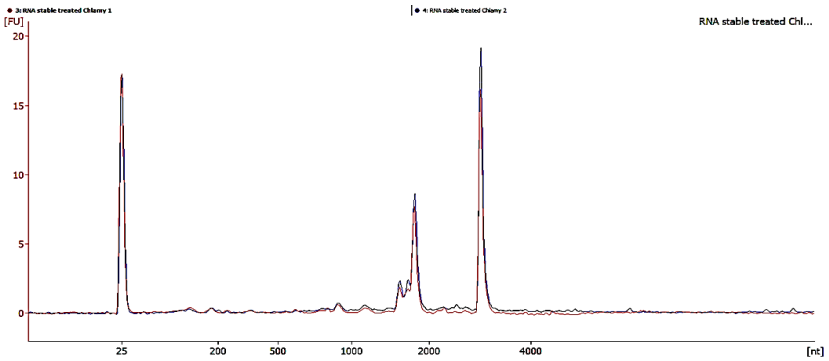


Did RNAstable have an effect on RNA Quality?

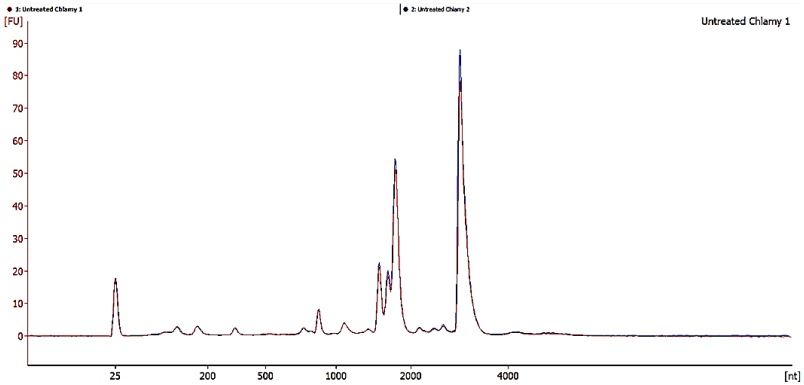


Week 0: Initial Test of RNAstable on Chlamy

TREATED



UNTREATED



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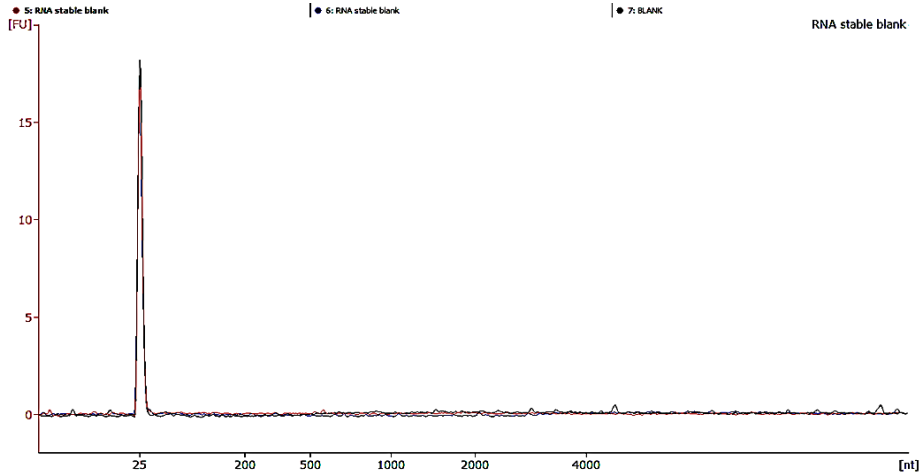
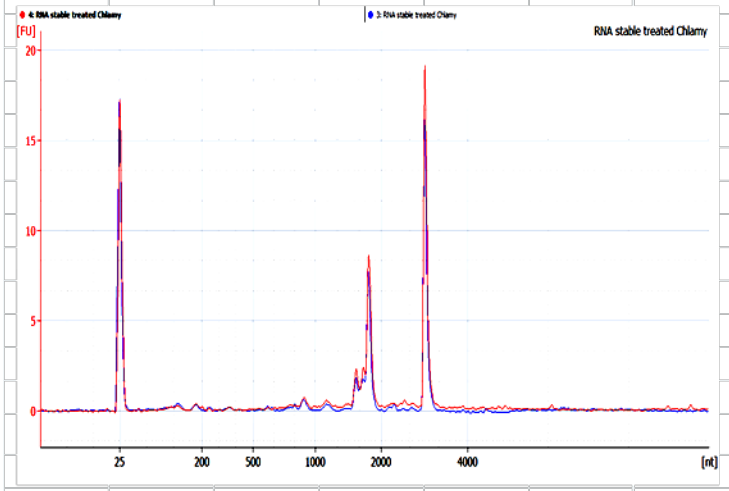


Week 4 compared to Week 0

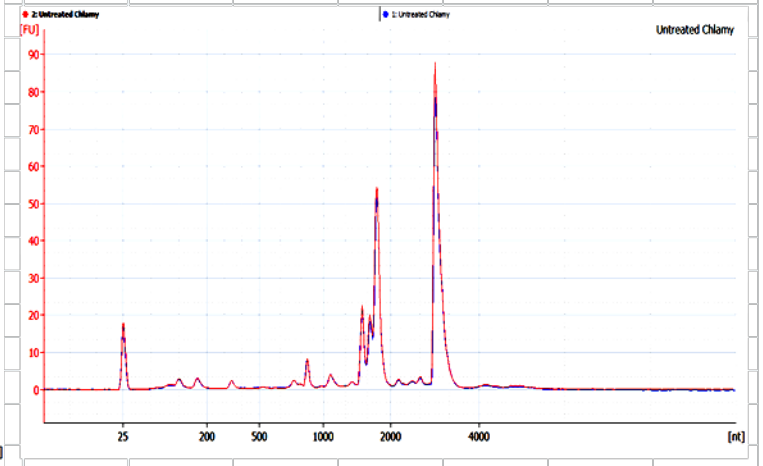
Week 0-TREATED

RNA STABLE treated Chlamy (resusp. in 50uL)



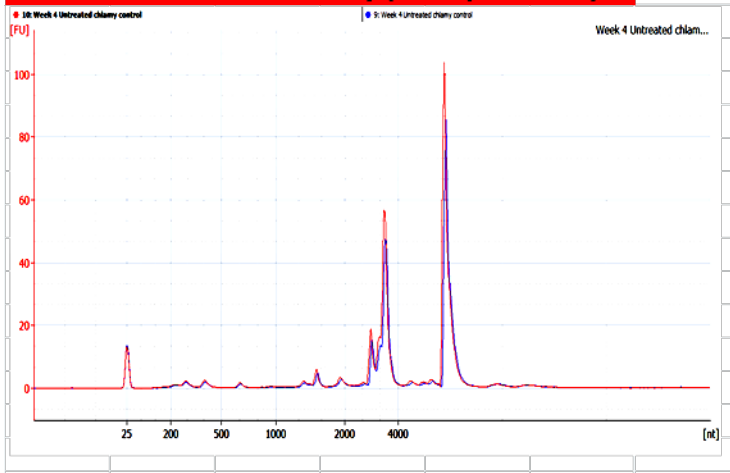
Week 0-UNTREATED

Untreated Chlamy (1.0ug in 10uL)



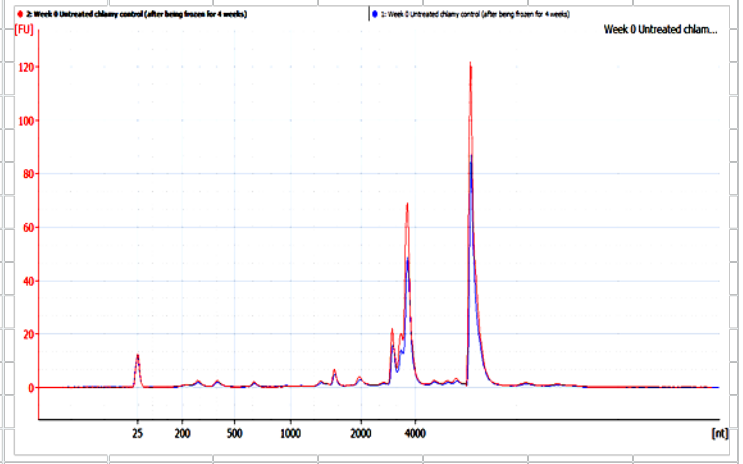
Week 4-TREATED

RNA STABLE treated Chlamy (resusp. in 50uL)



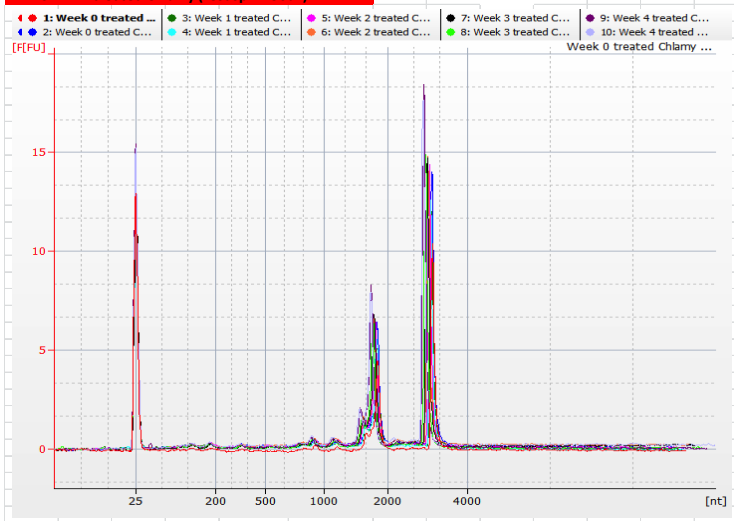
Week 4-UNTREATED

Untreated Chlamy (1.0ug in 10uL)

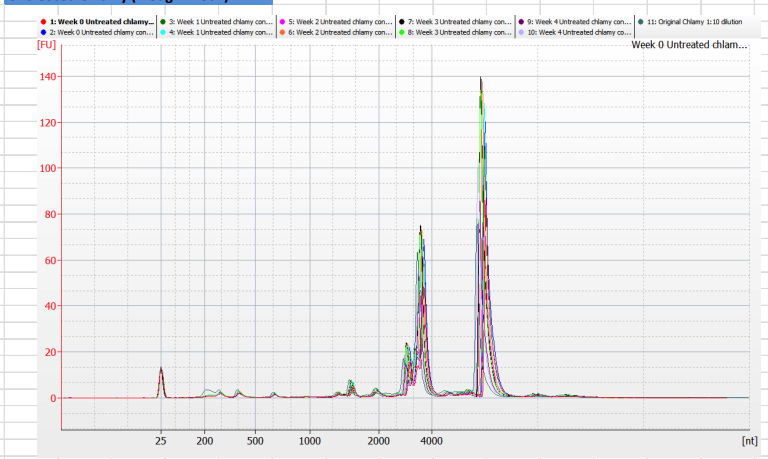


Freeze/Thaw Study and RNAstable on Chlamy

TREATED



UNTREATED



Neg. control: RNA stable

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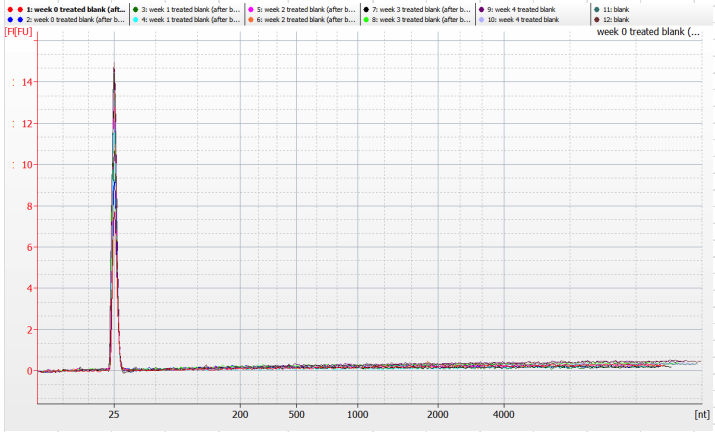
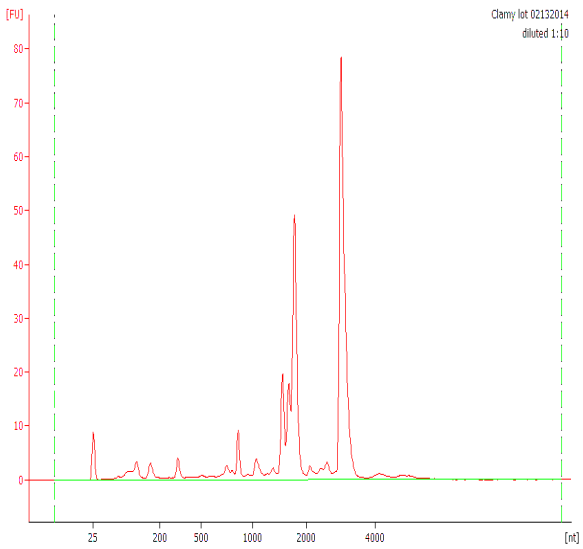
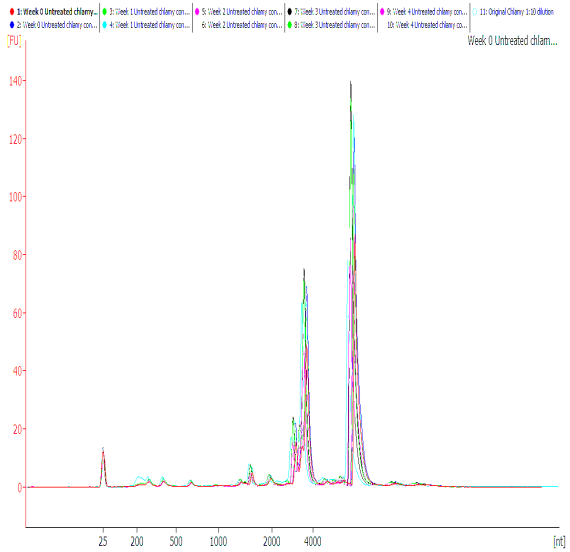


Was there any degradation seen from Freeze Thaw cycles?

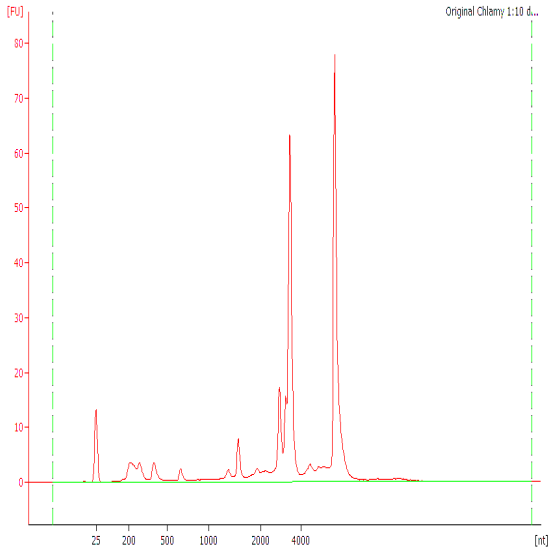
Before time course study



After 4 week time point-with freeze/thaw cycles



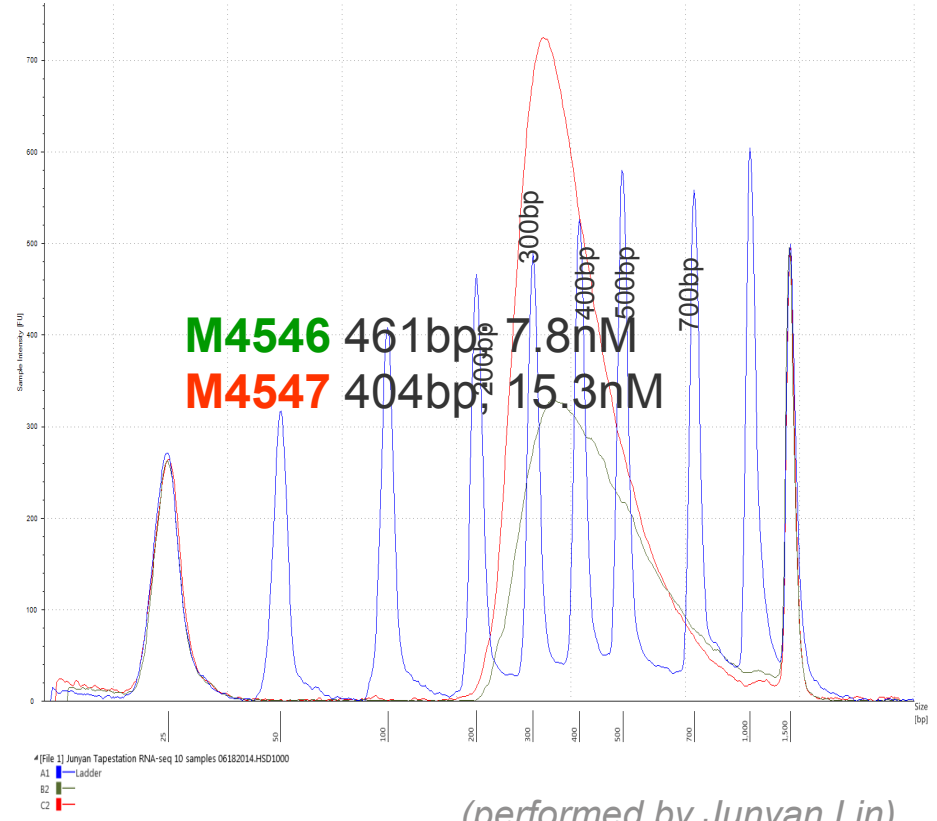
NO freeze/thaw cycles for 4 weeks



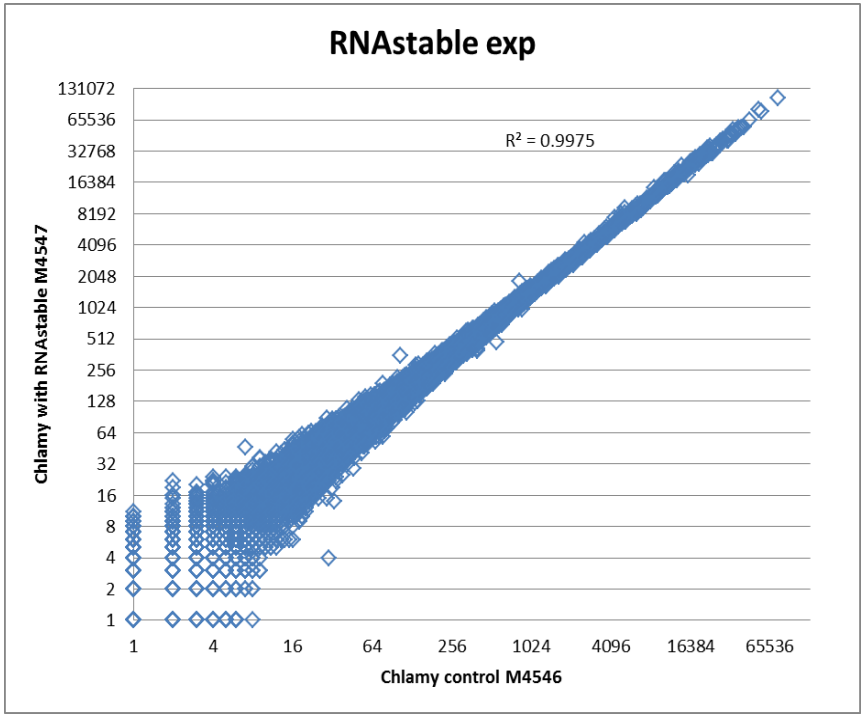
Library Construction and Sequencing QC

Control and test sample were used to generate standard RNA-seq Libraries and sequenced 2x150 on the miseq.

M4546 (control) 8094.1.90087.ATCAC.fastq.gz
M4547 (RNA-stable) 8095.1.90086.CGATG.fastq.gz



(performed by Junyan Lin)



(performed by Anna Lipzen)

Limitations of RNAstable

- **Suitable types of RNA that can be stored in RNAstable include**
  - purified total RNA from cells or tissues
  - poly(A) mRNA.

- **<u>Important</u>: sample purity is critical for RNAstable. Contaminants may affect the quality of RNA while in RNAstable**

- **Starting RNA must be free from any contaminating RNase activity to prevent degradation.**

- **RNA must be completely dry prior to adding RNA stable to prevent degradation.**

- **RNA must not exceed 100ug of total RNA and or 100uL per tube.**

- **Can be used on picogram amounts of RNA.**

- **Cannot repeat more than three times per sample.**

Conclusion

- **QUANTITY**
  - No significant difference seen between RNA treated with RNAstable and untreated.
  - No background was detected from the RNAstable.
  - No significant effect on sample quantity even after 5 freeze/thaw cycles.
- **QUALITY**
  - No difference was seen between RNA treated with RNAstable and untreated.
  - No effect on sample quality even after 5 freeze/thaw cycles.
- **Library Construction**
  - RNAstable had no effect on standard RNAseq library protocol.
- **Sequencing:**
  - RNAstable had no effect on sequencing QC

Stay tuned we are currently testing DNAstable with in-house prepped Chlamy gDNA…

Thank you - Sample Management Group

- **Additional information:**
  - An online hand book is available at:

http://www.biomatrica.com/downloads/FINAL_RNAstable_Handbook.pdf.

- **Supplies can be purchased from:**
  - http://www.biomatrica.com/rnastable.php
  - *Tel*: 1-866-379-6879 (Toll-free in U.S. & Canada)

- **Individual tubes: (1.5 ml screw cap microfuge tubes made of durable polypropylene)**
  - RNA Stable tube kit: 93221-001 (trial tube kit: 93220-001)