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# **Preparation of RNA for the 2009-2010 NARG Study**

**Scott Tighe**

**University of Vermont  
Vermont Genetics Network  
Microarray Core Lab**

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## RNA Considerations:

- Prepare (3) RNA's at different RIN values to test the effect of degradation on  $C_q$  value
- RNA included would be Good, Fair, and Poor quality
- RIN values were determined by the processing
- RNA must be in “dried down” format that could be easily shipped and resuspended by participating labs
- Same RNA and lot number as the previous NARG studies.
  - Ambion FirstChoice™ Human Brain Reference [HBR]
  - (RNA Lot 105055201a)

# Methods to Prepare NARG RNA:

## I. Degradation Methods

- Used RNase A titrated to 0.3U/ul
- Added a total of 1.8U/50ug of HBR RNA
- Incubated at 37C for 1-4 hour
- Withdrew aliquot added RNase Inhibitor and ran Bioanalyzer
- Stopped reaction with RNase inhibitor –Fermentas Ribolock
- Took many many trial runs!!!
  - Easy to over-degrade
  - Difficult to get sample to exactly RIN 4-5



# Methods to Prepare NARG RNA:

## II. Dry Down Methods

- Used to Two Products

- RNAstable from BioMatrica

- RNA storage material designed for speedvac applications
    - Designed to reliably store and stabilize total RNA
    - Tested using total RNA from DAOY cells prior to HBR

- Tested by leaving at room temp for several days

- Sent to several NARG members to check on the Bioanalyzer

- RNase Inhibitor (RiboLock)

- Before speedvac- Added RNase inhibitor to final RNA sample prior to combining with RNAstable
    - Speedvac for 30 minutes at 500 mT
    - Stored at -80C

- Took many many trial runs!!!

# Testing RNAsable

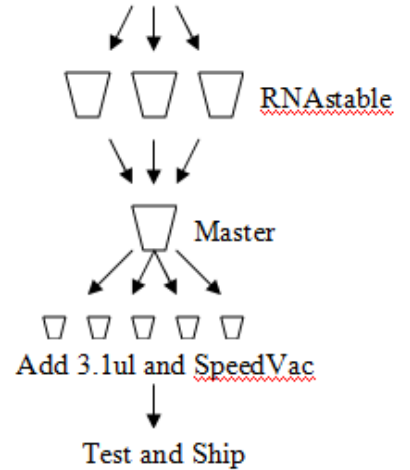
Combine RNA, RNAsable, and RI

SpeedVac 30 minutes

Store at RT 48 Hr

Ship Standard Mail

65 ul RNA (65 ug)  
10 ul RNase Inhibitor (400U)  
160 ul DEPC water

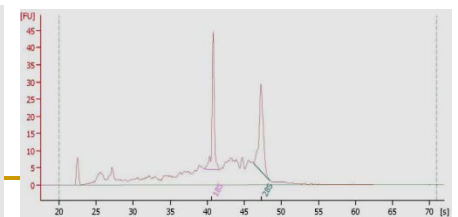
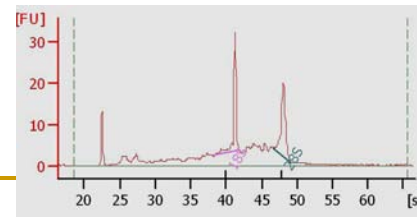
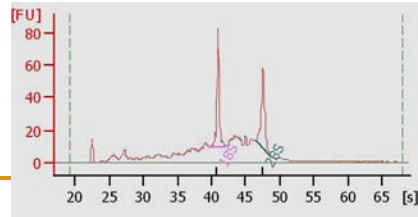
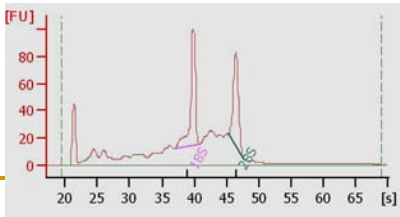
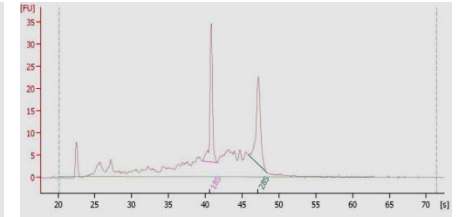
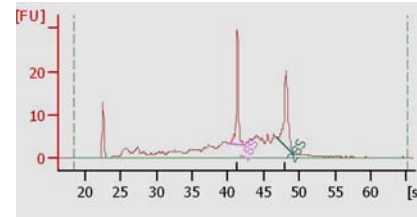
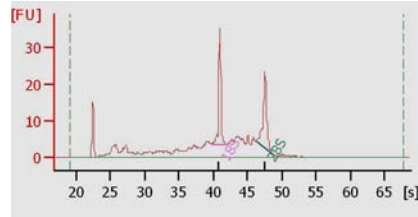
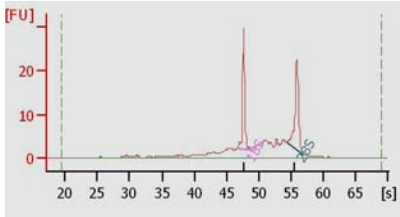


ST 7.8, 7.8

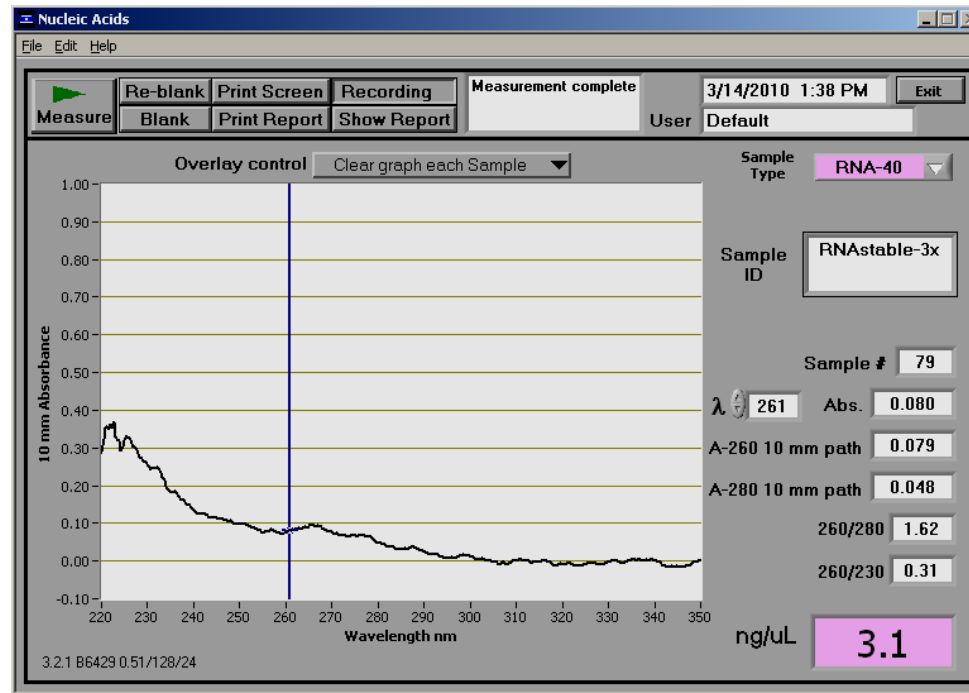
KK-6.8, 7.0

SC-7.0, 7.5

BT-6.9, 6.9



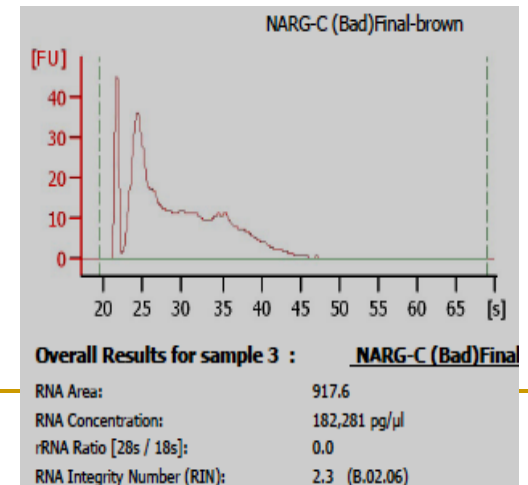
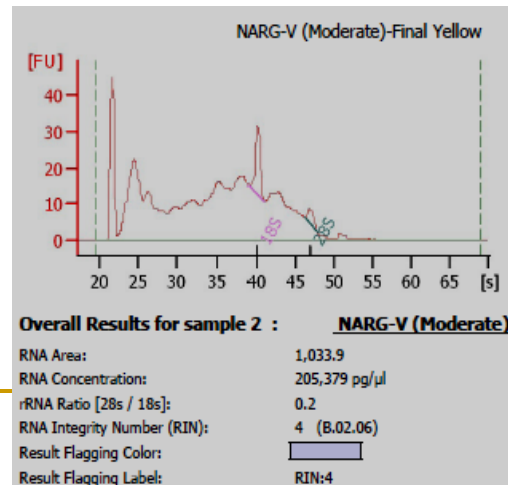
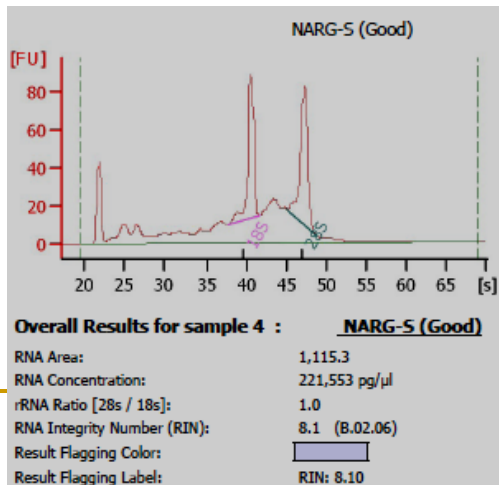
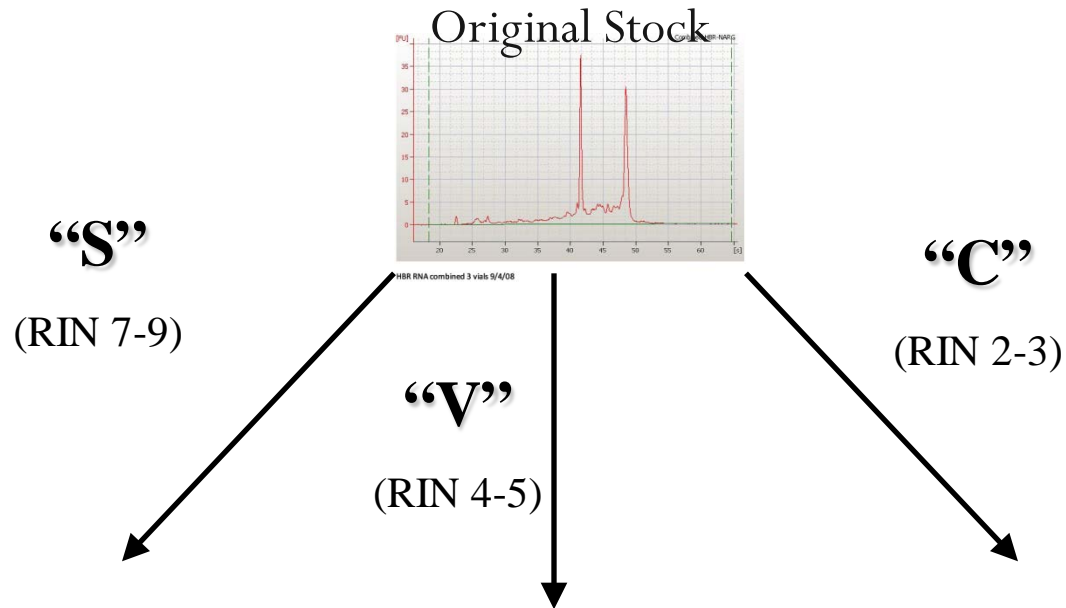
# RNAstable did not contribute to the nanodrop reading



3x concentration of RNAstable

# Range of RIN values from Control Samples

(Taken from same tubes that were sent)



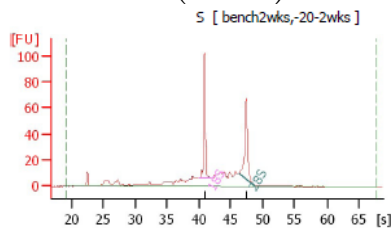
# NanoChip vs PicoChip

Labs may see a difference based on which chip they ran

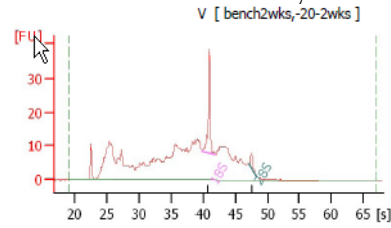
Running the same EXACT RNA does not give the same RIN

These samples were left out (dried) for 2 weeks at RT and rehydrated and stored at -20 for 2 weeks

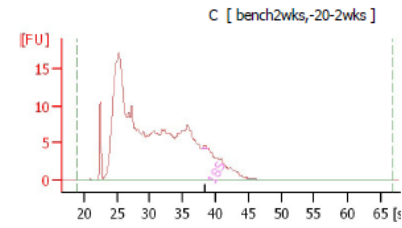
NanoChip



**Overall Results for sample 1 :** S  
 RNA Area: 431.0  
 RNA Concentration: 5,792 ng/μl  
 rRNA Ratio [28s / 18s]: 1.0  
 RNA Integrity Number (RIN): 7.9 (A.01.01)



**Overall Results for sample 2 :** V  
 RNA Area: 516.6  
 RNA Concentration: 6,944 ng/μl  
 rRNA Ratio [28s / 18s]: 0.2  
 RNA Integrity Number (RIN): 4.3 (A.01.01)



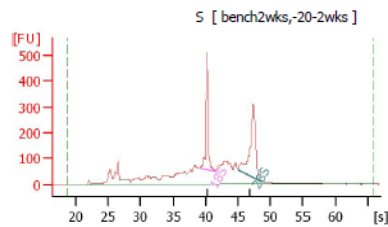
**Overall Results for sample 3 :** C  
 RNA Area: 405.0  
 RNA Concentration: 5,444 ng/μl  
 rRNA Ratio [28s / 18s]: 0.0  
 RNA Integrity Number (RIN): 2.2 (A.01.01)

S

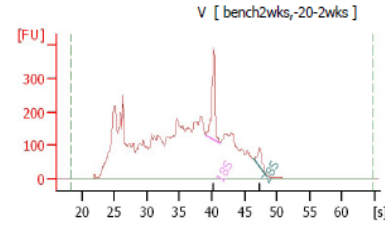
V

C

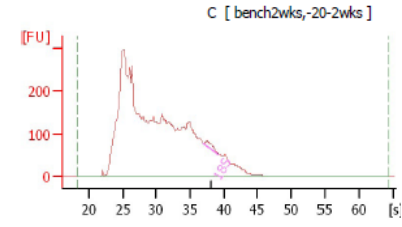
PicoChip



**Overall Results for sample 1 :** S  
 RNA Area: 3,833.3  
 RNA Concentration: 11,489 pg/μl  
 rRNA Ratio [28s / 18s]: 1.2  
 RNA Integrity Number (RIN): 7.2 (A.01.01)



**Overall Results for sample 2 :** V  
 RNA Area: 9,082.9  
 RNA Concentration: 27,222 pg/μl  
 rRNA Ratio [28s / 18s]: 0.3  
 RNA Integrity Number (RIN): 3.6 (A.01.01)



**Overall Results for sample 3 :** C  
 RNA Area: 7,629.3  
 RNA Concentration: 22,866 pg/μl  
 rRNA Ratio [28s / 18s]: 0.0  
 RNA Integrity Number (RIN): 2.1 (A.01.01)

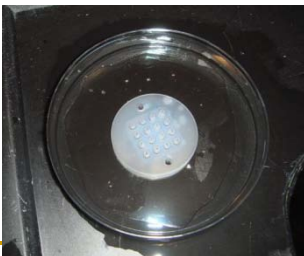
Chips were run within 5 minutes of each other

# Methods to Prepare the Bioanalyzer

- Some differences in how to prepare the bioanalyzer exists
- Some labs removing the probe assembly completely and disinfect

Complete clean off of assembly, DI, RNaseZap, 3x DEPC water rinse, filtered high pressure Nitrogen

- Some labs use the drip and rinse chip method



Please See our Poster of the  
Study  
RG-1



The NARG is actively seeking new members. Please talk with us at the meeting if your are interested in joining the group.

Or Email : Sridar Chittur

[schittur@albany.edu](mailto:schittur@albany.edu)