

ThermoFisher
S C I E N T I F I C

Fast and simple fluorescent RNA quality assessment

Chris Vonnegut

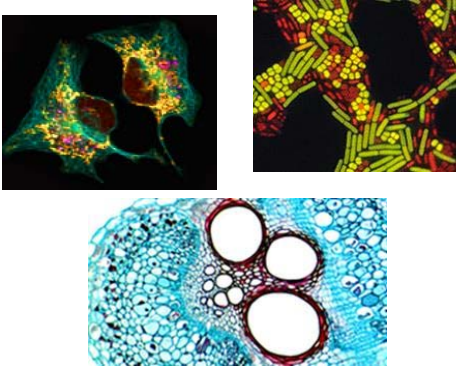
Presented at ASHG 2017
Laboratory CoLab Session
18 October, 2017

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RNA Quality and Quantity

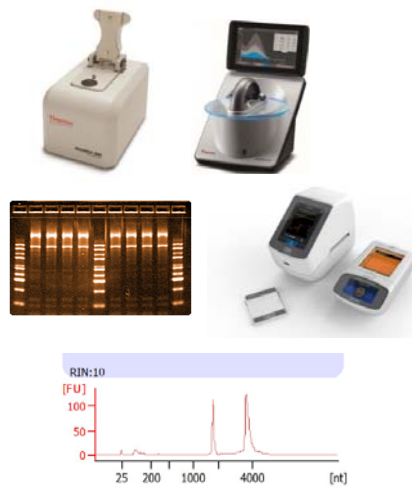
RNA Sample Isolation

Cells
Tissue
Blood / biological samples
Bacteria
Plants



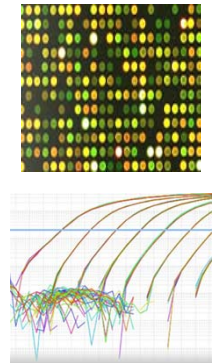
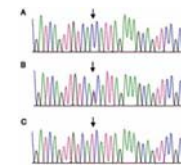
RNA Quantity & Quality

UV-Vis spectroscopy
Electrophoresis



RNA Sample Analysis

qPCR/qRT-PCR
RT-PCR
RNA-Seq
Microarrays
Sequencing
NGS
Cloning



RNA quantity & quality measurements – Essential steps post-purification

Current Techniques for Measuring RNA integrity and quality

UV-Absorbance



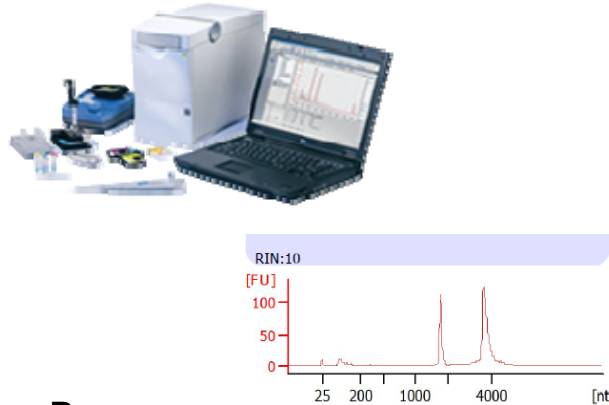
Pros:

- Time to result
- 260/280 (protein contamination)
- 260/230 (chemical contamination)
- No reagents required

Cons:

- Cannot differentiate between intact or degraded RNA
- No size or distribution info.

Capillary electrophoresis



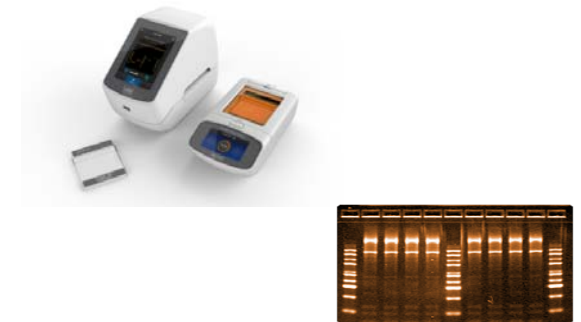
Pros:

- Industry standard
- Size & distribution info.

Cons:

- Time-consuming/tedious workflow
- Time to result
- Price per sample
- Reagent shelf-life
- Sample # flexibility

Gel electrophoresis



Pros:

- Simple
- Size & distribution info.

Cons:

- Time-consuming / tedious workflow
- Time to result
- Sample # flexibility
- ~1 μ g sample required

NEW: Qubit™ RNA Integrity & Quality (IQ) Assay and Qubit™ 4 Fluorometer

- Detect viable from degraded RNA with 2-dye assay:
 - Unique dye for large and/or structured RNA
 - Unique dye for small, degraded RNA
- Simple
 - Add RNA sample to Qubit™ RNA IQ buffer
 - Measure on Qubit™ 4 Fluorometer
- Rapid time to result
 - ~5 minutes sample preparation
 - ~4 seconds per sample



Qubit™ RNA Integrity & Quality (IQ) Assay: RNA Sample Preparation

USE: 1-20 μL of 0.5-1.5 μg RNA Sample

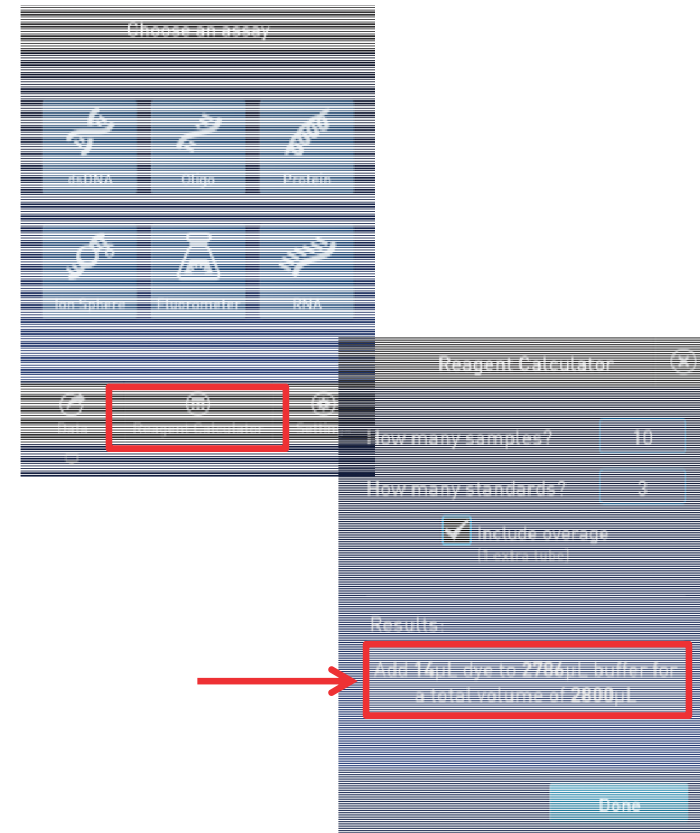
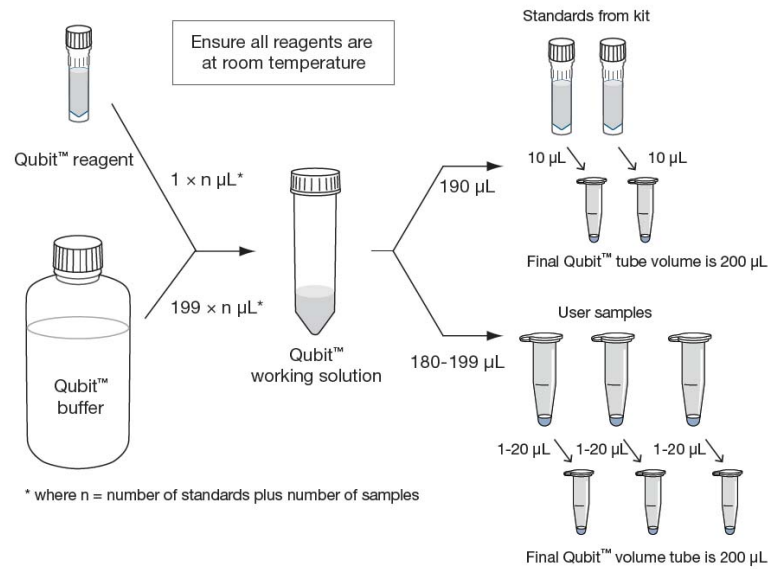
NEW: Qubit™ 4 Reagent Calculator



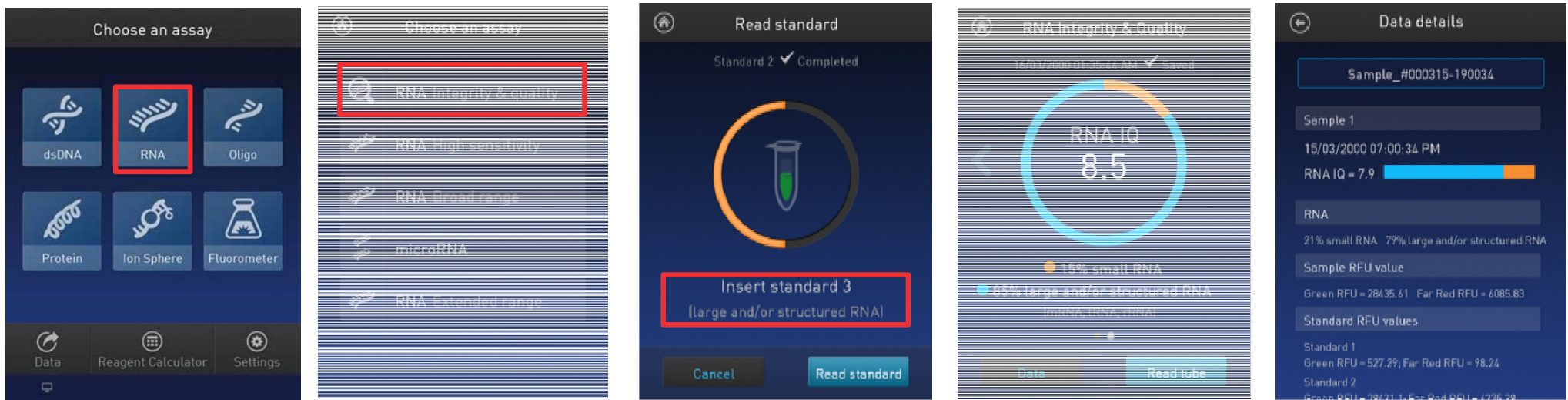
Q33221: 75 Assays



Q33222: 275 Assays

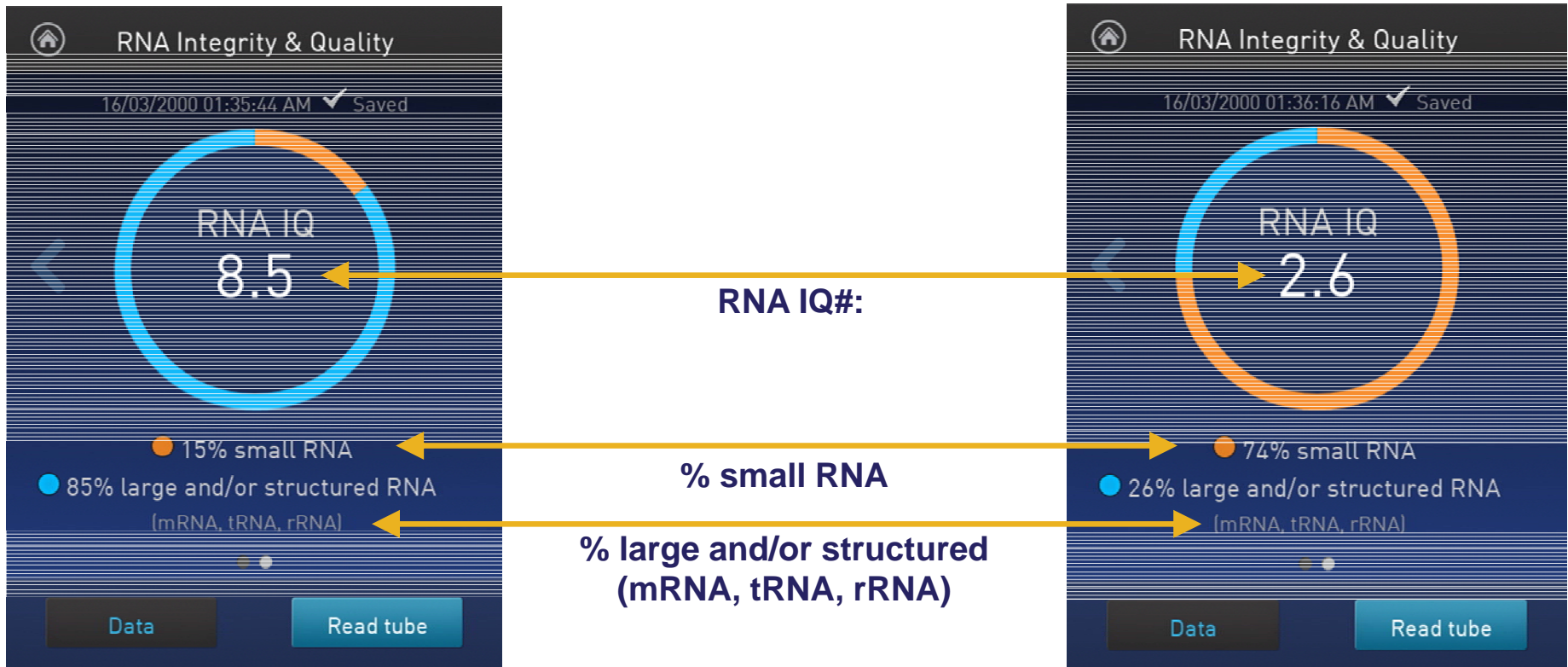


Qubit™ RNA Integrity & Quality (IQ) Assay: Qubit™ 4 Fluorometer Workflow

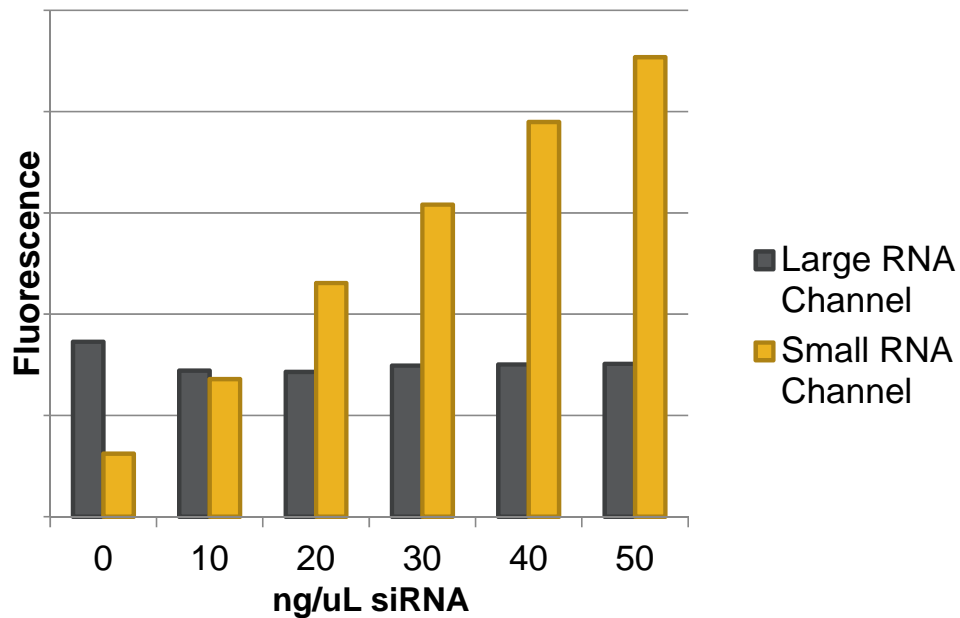


**Qubit™ Fluorometer
Intuitive Workflow**

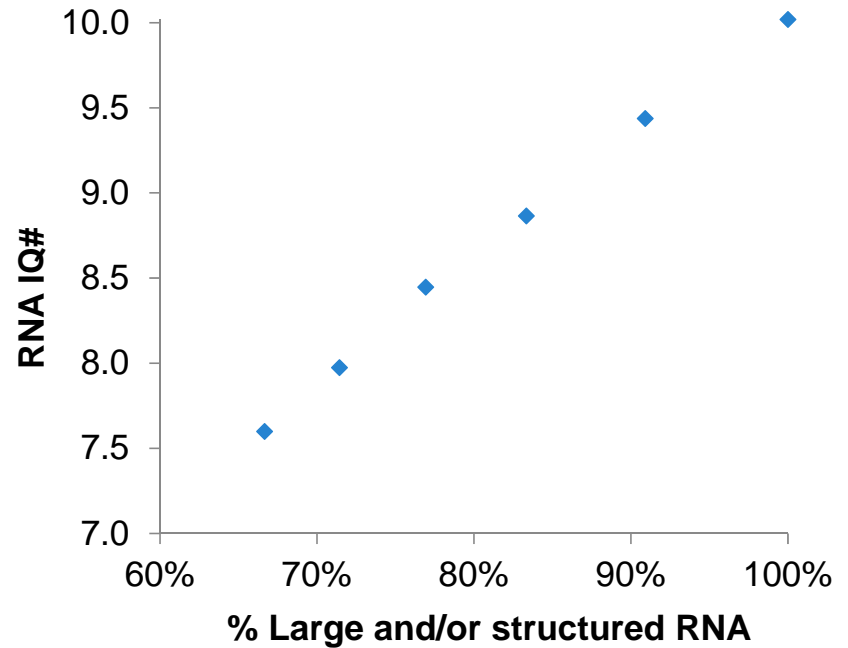
Qubit™ RNA IQ results



Selectivity of the RNA IQ reagents for large and/or structured & small RNA

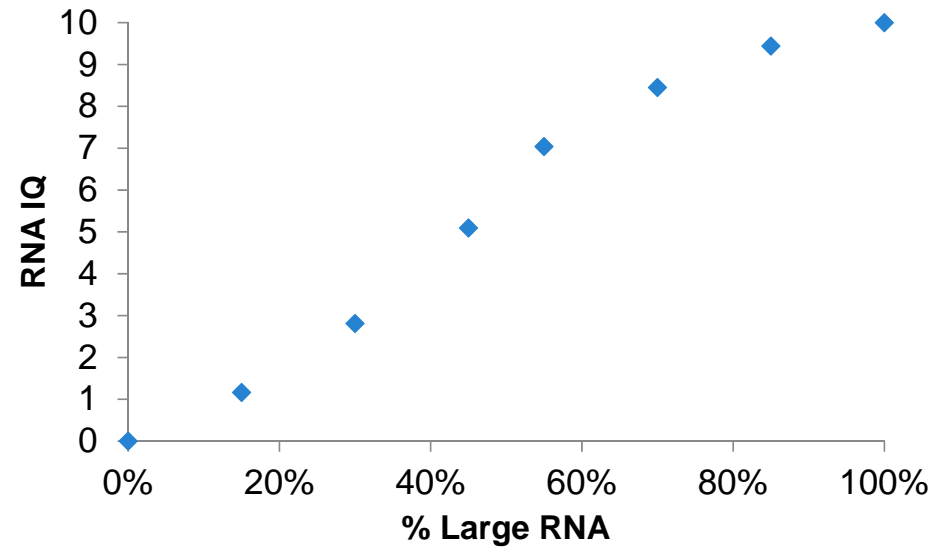
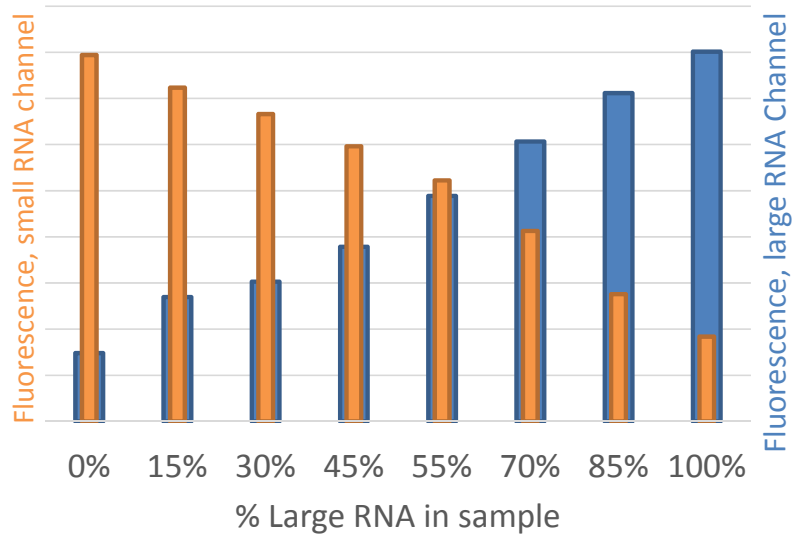


(Large RNA held constant at 100 ng/uL in sample)



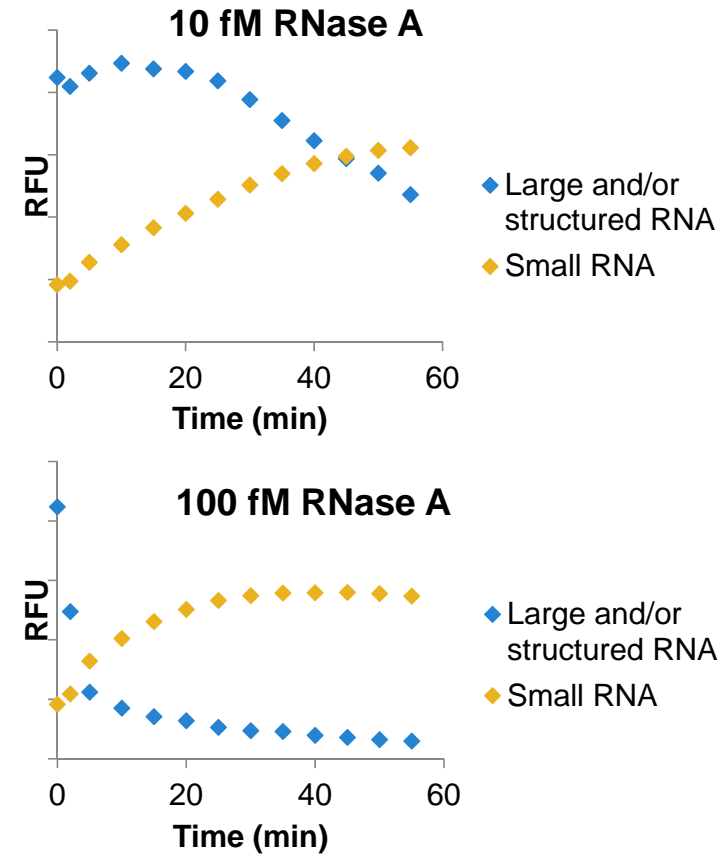
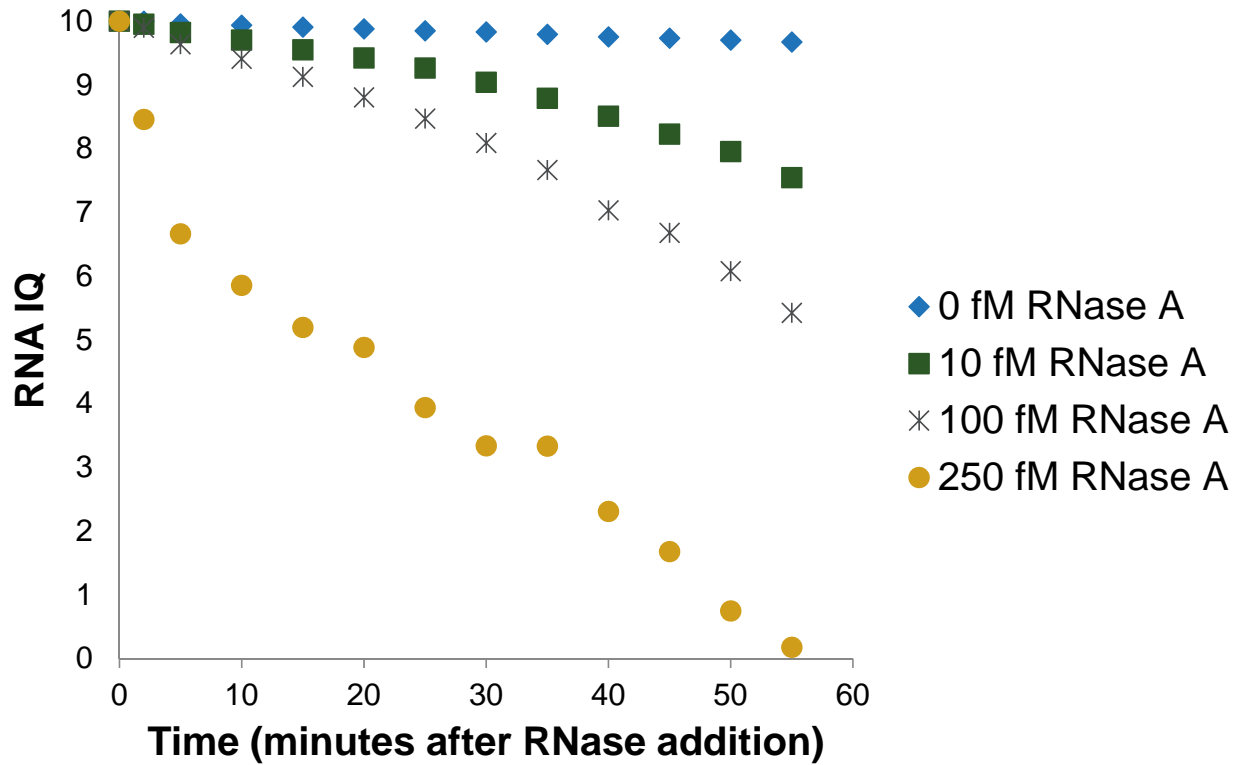
Triplicate samples containing 100 ng/ μ L rRNA (*E. coli*) and varying amounts of siRNA (0 to 50 ng/ μ L) were assayed with the Qubit™ RNA IQ assay on the Qubit™ 4 Fluorometer.

Qubit™ RNA IQ with solutions containing various amounts of large and small RNA



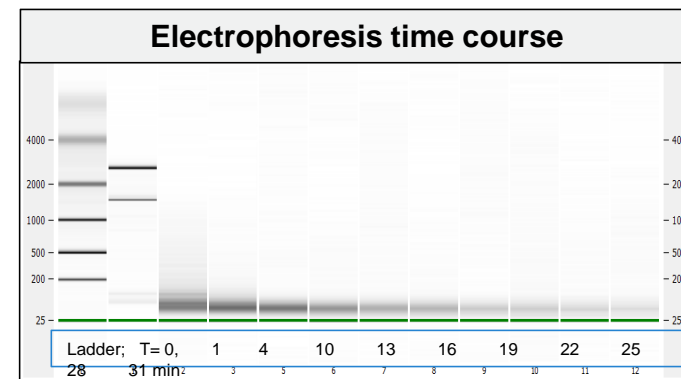
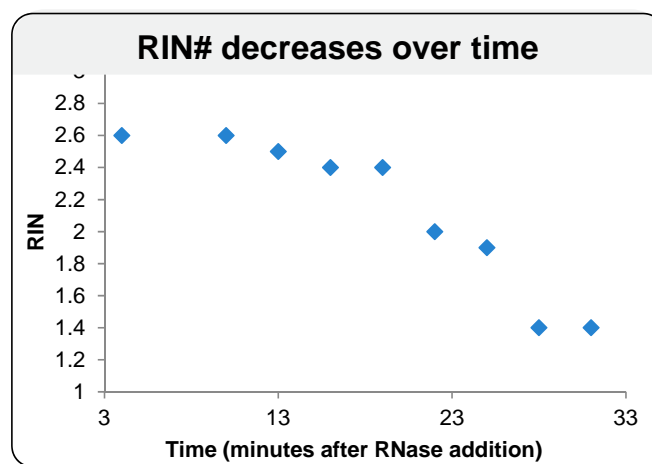
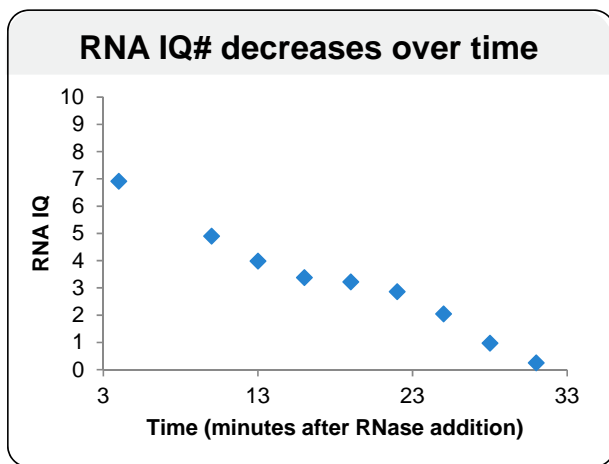
Triplicate samples containing a total of 100 ng/ μ L RNA comprised of small RNA (Silencer™ Select GAPDH siRNA) and large RNA (rRNA, *E.coli*) were assayed with the Qubit™ RNA IQ assay on the Qubit™ 4 Fluorometer.

Real-time RNA degradation monitored with Qubit™ RNA IQ Assay



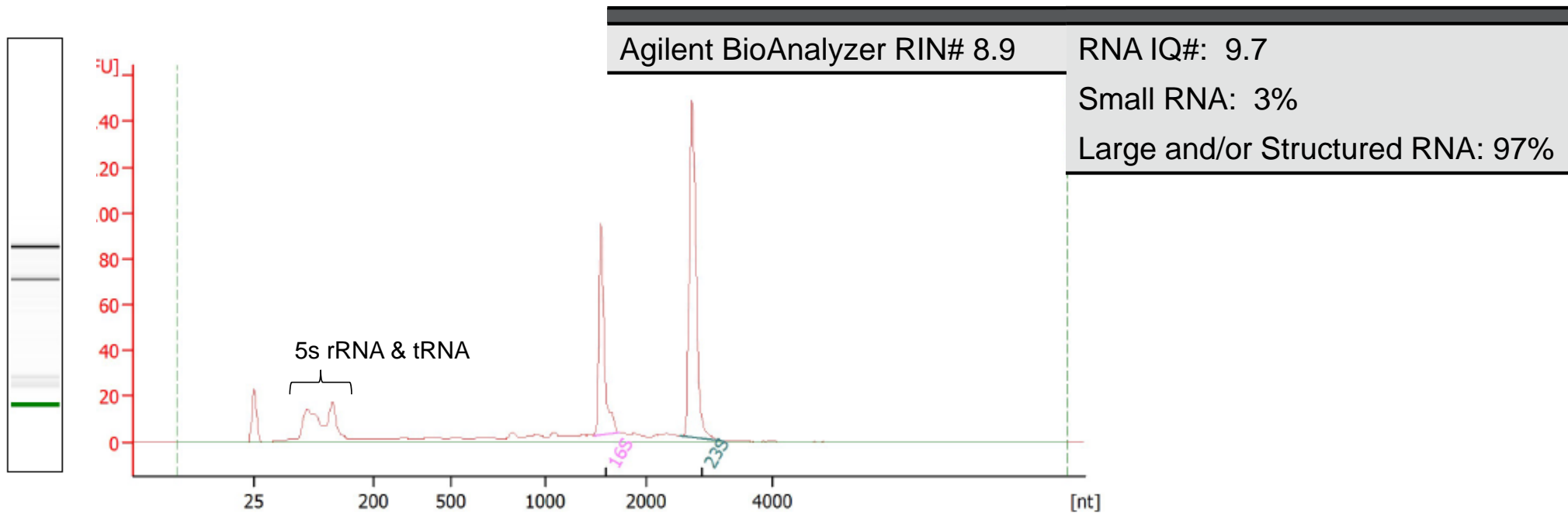
Triplicate samples of 100 ng/μL rRNA solutions were incubated with RNase A in the final assay solution. rRNA degradation by RNase A was measured in real-time using the assay.

RNA assessment by Qubit™ RNA IQ or Agilent BioAnalyzer following RNase treatment



750 fM RNase A was added to aliquots of a 100 ng/ μ L solution of rRNA (*E.coli*), and at various time points treated with RNaseOUT then measured with either the Qubit™ RNA IQ Assay with a Qubit™ 4 Fluorometer or Agilent BioAnalyzer Prokaryote Total RNA Nano chip.

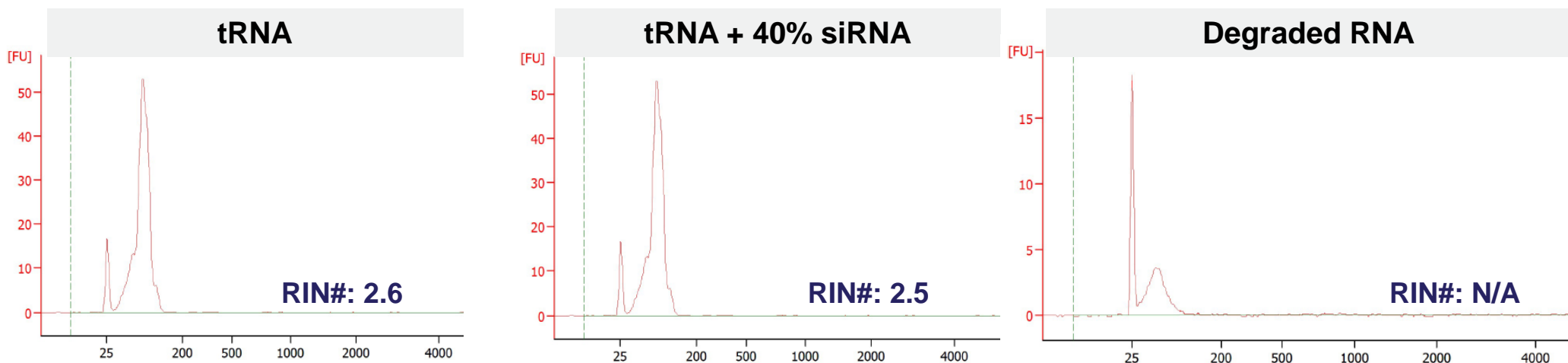
RNA measured by BioAnalyzer & Qubit™ RNA IQ Assay after 1 min. RNase



rRNA was treated with 0.5pg/ μ L RNase A, followed by the addition of RNaseOUT after 1 minute. Samples were measured either on a Prokaryote Total RNA Nano chip or with the Qubit™ RNA IQ Assay.

Quality scores differences, IQ#: 9.8 and RIN#: 8.9 is the result of rRNA, mRNA & structured 5s rRNA and tRNA which bind the large and/or structured RNA dye. Peaks in this area can be misinterpreted as degradation in some electrophoretic analyses.

Small, degraded and/or structured RNA via Agilent™ BioAnalyzer & Qubit™ RNA IQ Assay



RNA IQ#: 7.5

RNA IQ#: 1.0

RNA IQ#: 0.0

Small RNA: 25%

Small RNA: 90%

Small RNA: 100%

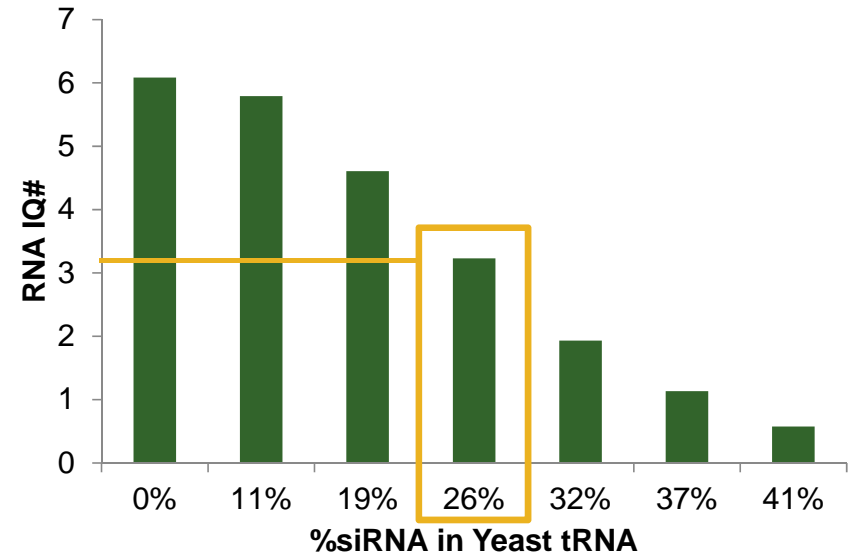
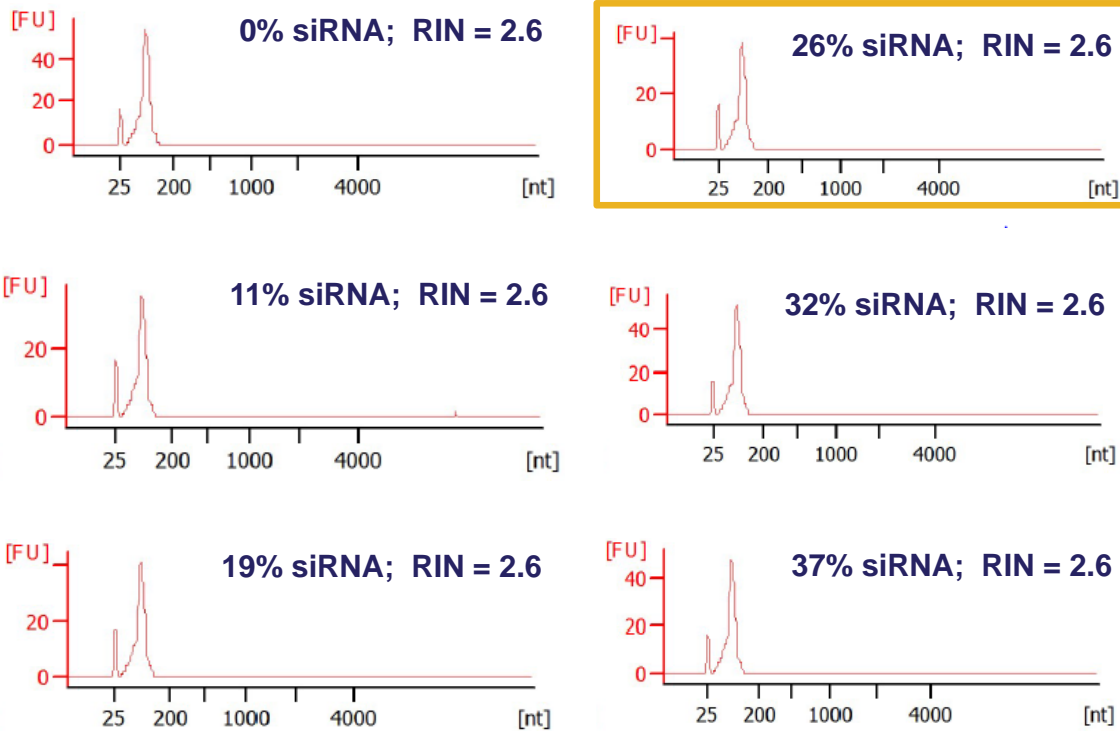
Large and/or Structured RNA: 75%

Large and/or Structured RNA: 10%

Large and/or Structured RNA: 0%

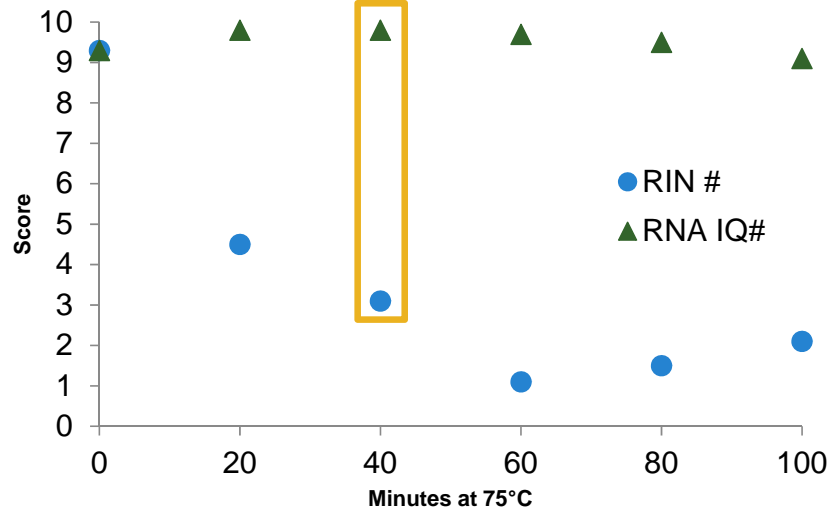
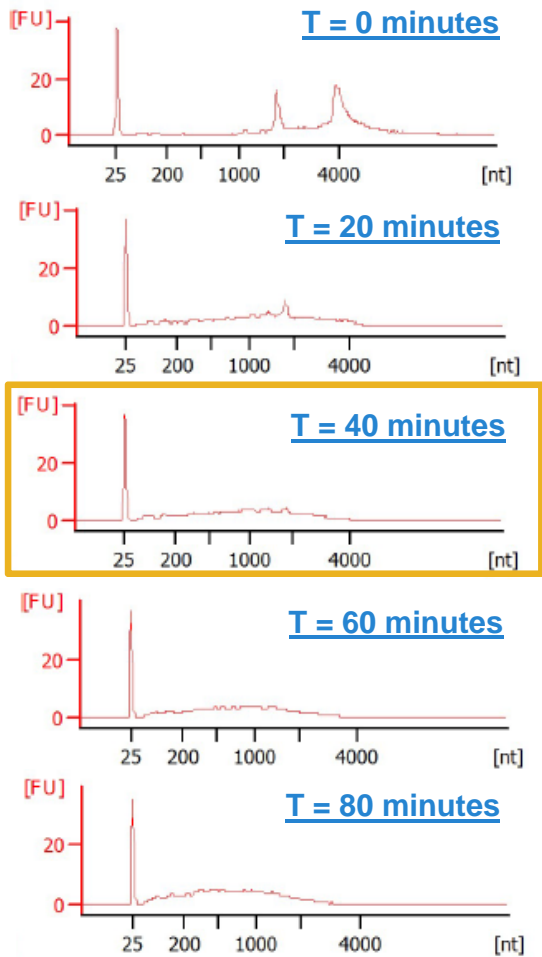
Samples of tRNA, tRNA with 40% siRNA in solution, or RNA exhaustively degraded by RNase were analyzed by Agilent™ Bioanalyzer and the Qubit™ RNA IQ assay on the Qubit™ 4 Fluorometer.

Impact of highly structured RNA on small RNA samples



tRNA was combined with siRNA in varying ratios and assayed, demonstrating the RNA IQ assay's sensitivity to different types of small RNA combined in the same sample.

qRT-PCR following RIN and IQ analysis of heat treated human liver samples

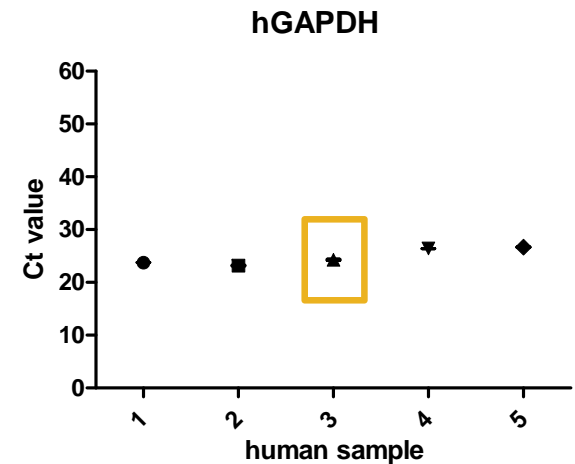
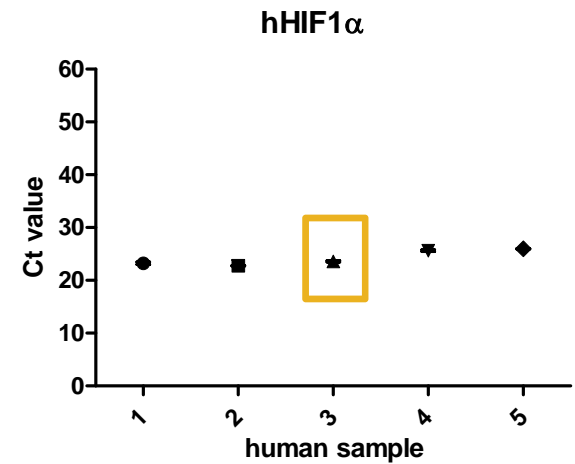


Agilent BioAnalyzer RIN# 3.1

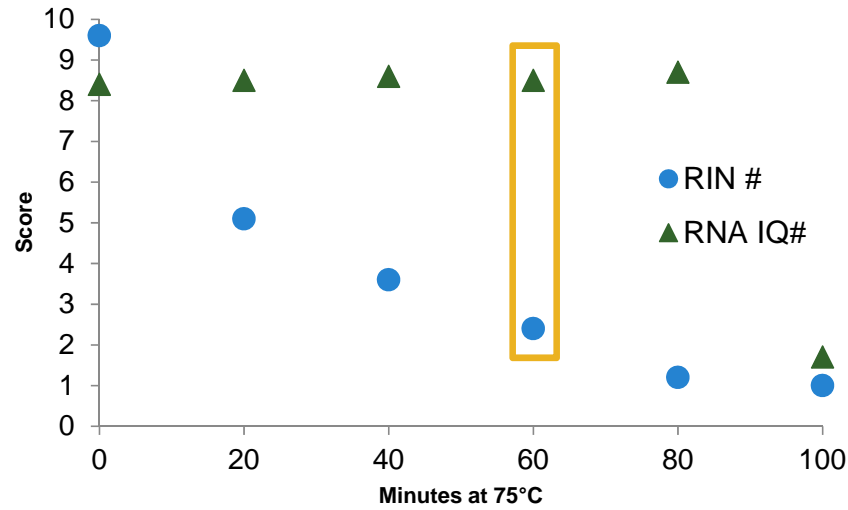
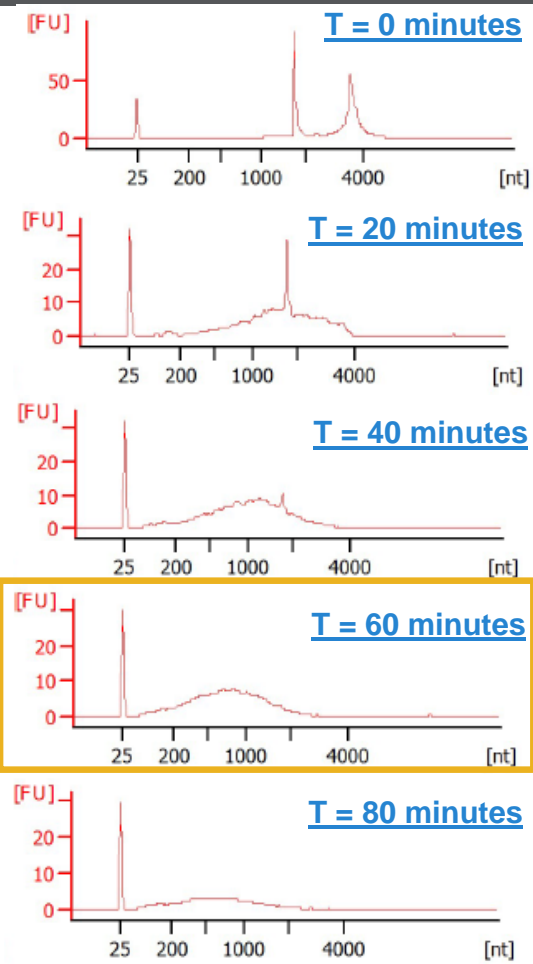
RNA IQ#: 9.8

Small RNA: 2%

Large and/or Structured RNA: 98%



qRT-PCR following RIN and IQ analysis of heat treated mouse liver samples

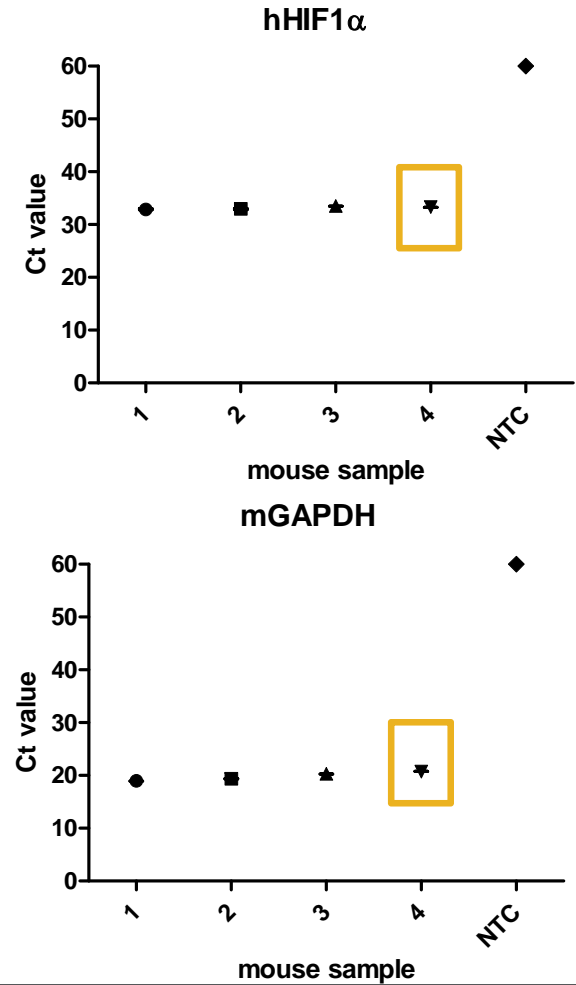


Agilent BioAnalyzer RIN# 2.4

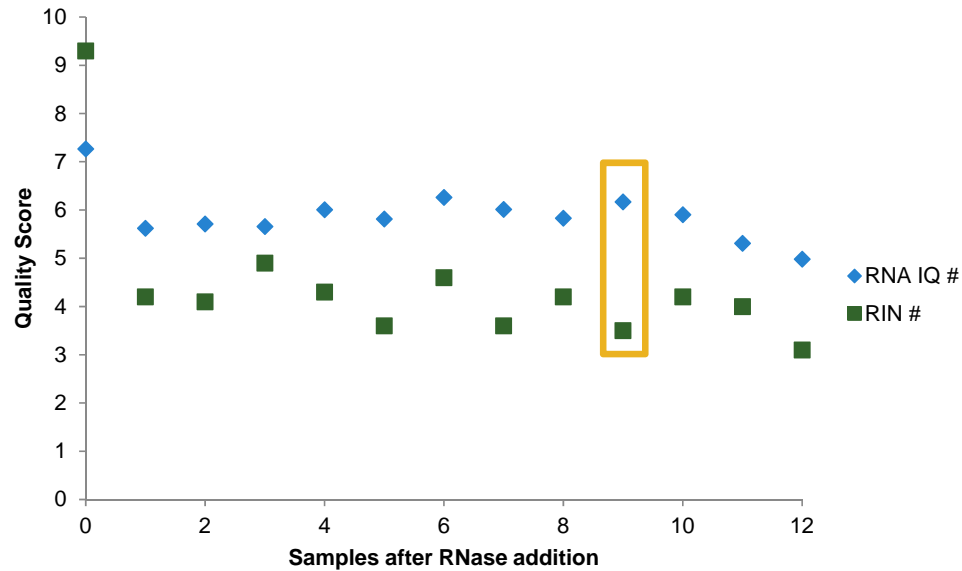
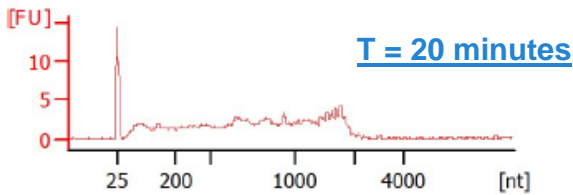
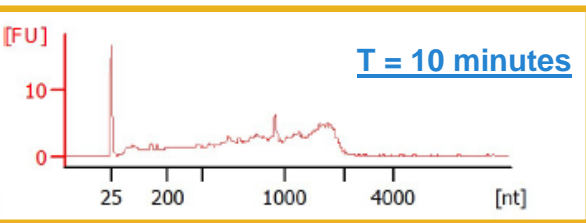
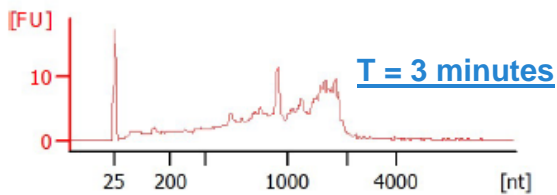
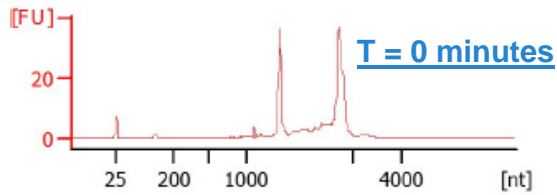
RNA IQ#: 8.5

Small RNA: 15%

Large and/or Structured RNA: 85%



qRT-PCR following RIN and IQ analysis of RNase treated human liver samples

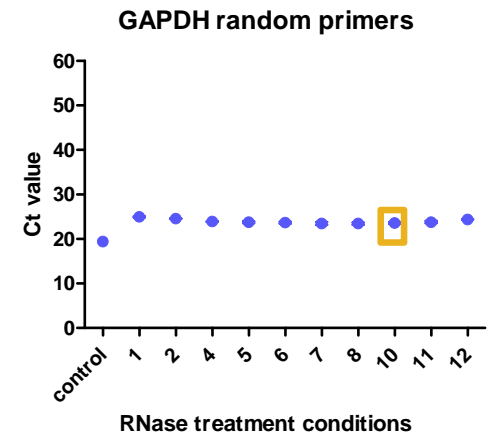
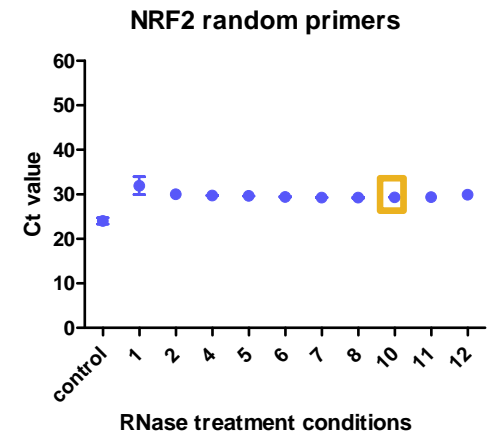


Agilent BioAnalyzer RIN# 3.1

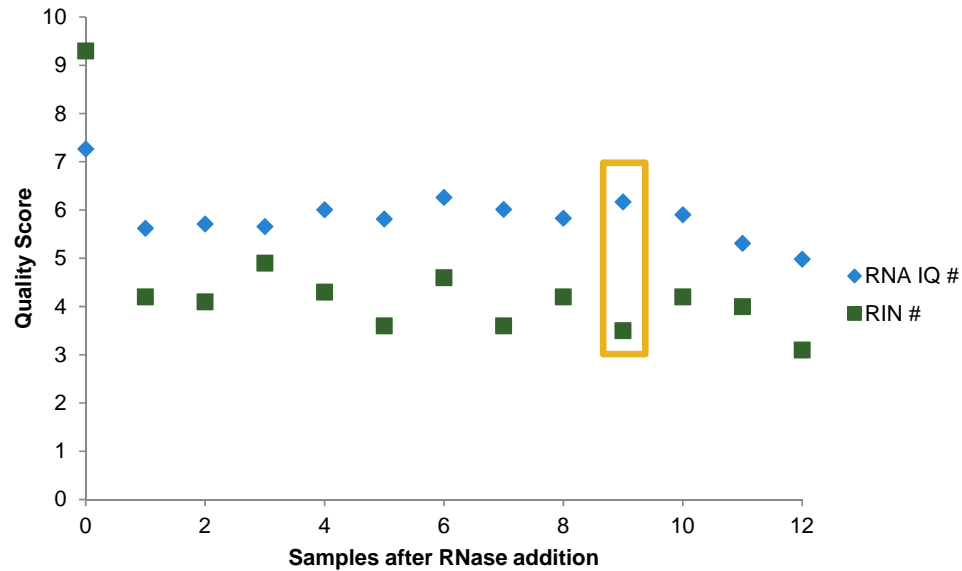
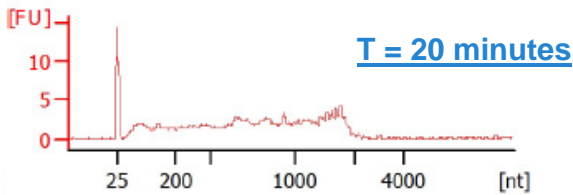
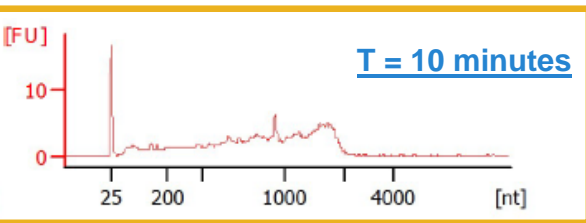
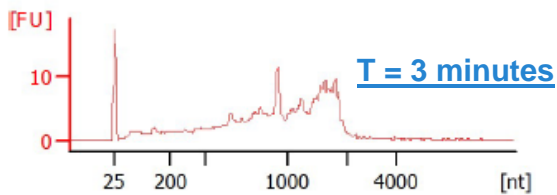
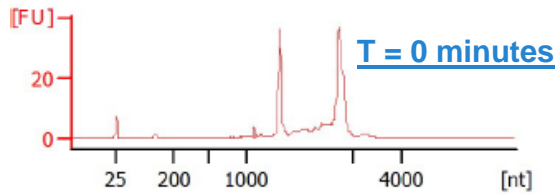
RNA IQ#: 6.2

Small RNA: 38%

Large and/or Structured RNA: 62%



qRT-PCR following RIN and IQ analysis of RNase treated human liver samples

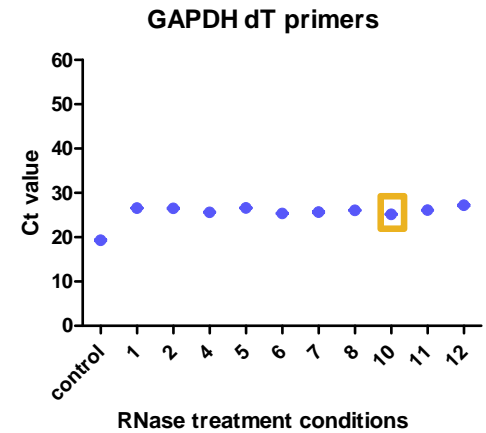
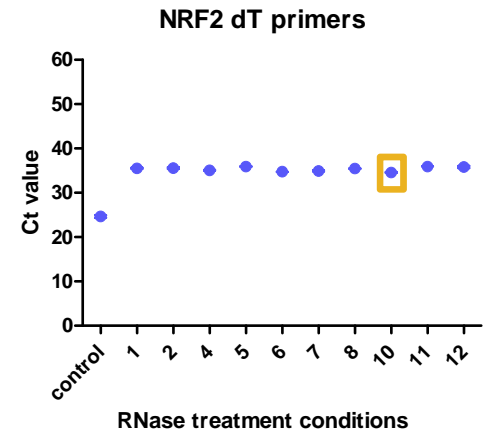


Agilent BioAnalyzer RIN# 3.1

RNA IQ#: 6.2

Small RNA: 38%

Large and/or Structured RNA: 62%



NEW: Qubit™ RNA Integrity & Quality (IQ) Assay and Qubit™ 4 Fluorometer

Qubit™ 4 & RNA IQ Assay

- Detect viable from degraded RNA with multiplexed dye assay:
 - Unique dye for large and/or structured RNA
 - Unique dye for small, degraded RNA
- Simple
 - Add RNA sample to Qubit™ RNA IQ buffer
 - Measure on Qubit™ 4 Fluorometer
- Rapid time to result
 - ~5 minutes sample preparation
 - ~4 seconds per sample



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