Qubit RNA IQ Assay: a fast and easy fluorometric RNA quality assessment

Abstract

The quality of RNA samples is paramount to any downstream application involving this nucleic acid. The ability to quickly and easily measure RNA quality is enabled by chip-based electrophoresis approaches. However, these methods are time-consuming, expensive, and prone to errors in handling. To overcome these challenges, our expertise in nucleic acid dyes was leveraged to generate a solution-based, multiplex assay for the Invitrogen™ Qubit™ 4 and Qubit™ Flex Fluorometers that enables fast and easy measurement of RNA quality.

Introduction

Utilizing two dyes with two separate emission channels, one that selectively binds to degraded RNA and one that selectively binds to large and intact RNA, we have developed a ratiometric fluorescence-based method to quickly assess the integrity of RNA within a sample. To enable this assay, the Qubit platform was updated, allowing multiplex assays and new user interface features on the instruments, which already have integral roles in nucleic acid workflows. As a result, we offer an RNA assessment assay that enables the measurement of RNA quality in as little as 5 minutes.

Results

Assay overview

The RNA integrity and quality (IQ) assay utilizes three standards: a blank; a small, degraded RNA; and a large, intact RNA. Samples are interrogated using the multiplexed dye mixture, and the two emission signals are combined using a proprietary algorithm to yield a quality score representative of the ratio of small and large RNAs in the sample. The touchscreen interface of the Qubit 4 Fluorometer makes it easy to select, run, and interpret the RNA IQ assay (Figure 1).

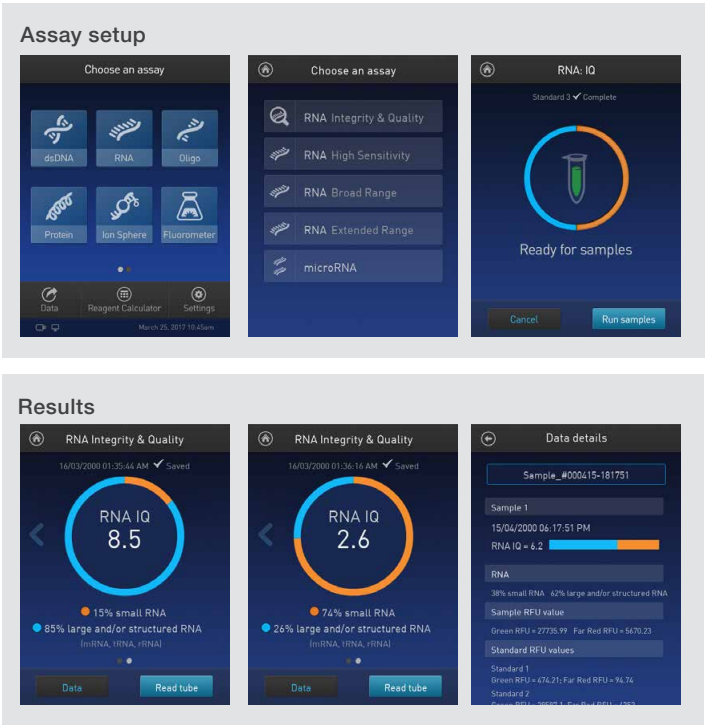

Figure 1. RNA IQ user interface on the Qubit 4 Fluorometer.

Comparison of RNA IQ to RNA integrity number (RIN)

Analysis using the Agilent™ Bioanalyzer™ system, Qubit RNA IQ Assay, and RT-qPCR was performed on total RNA (isolated from human liver) that was heat-treated at 75°C for various amounts of time. RT-qPCR analysis was performed using Invitrogen™ RETROScript™ reverse transcriptase and Applied Biosystems™ TaqMan™ hHIF1α and hGAPDH assays. Of note is the rapidly decreasing RIN, while C_t and RNA IQ values remain largely consistent across the series (Figure 2).

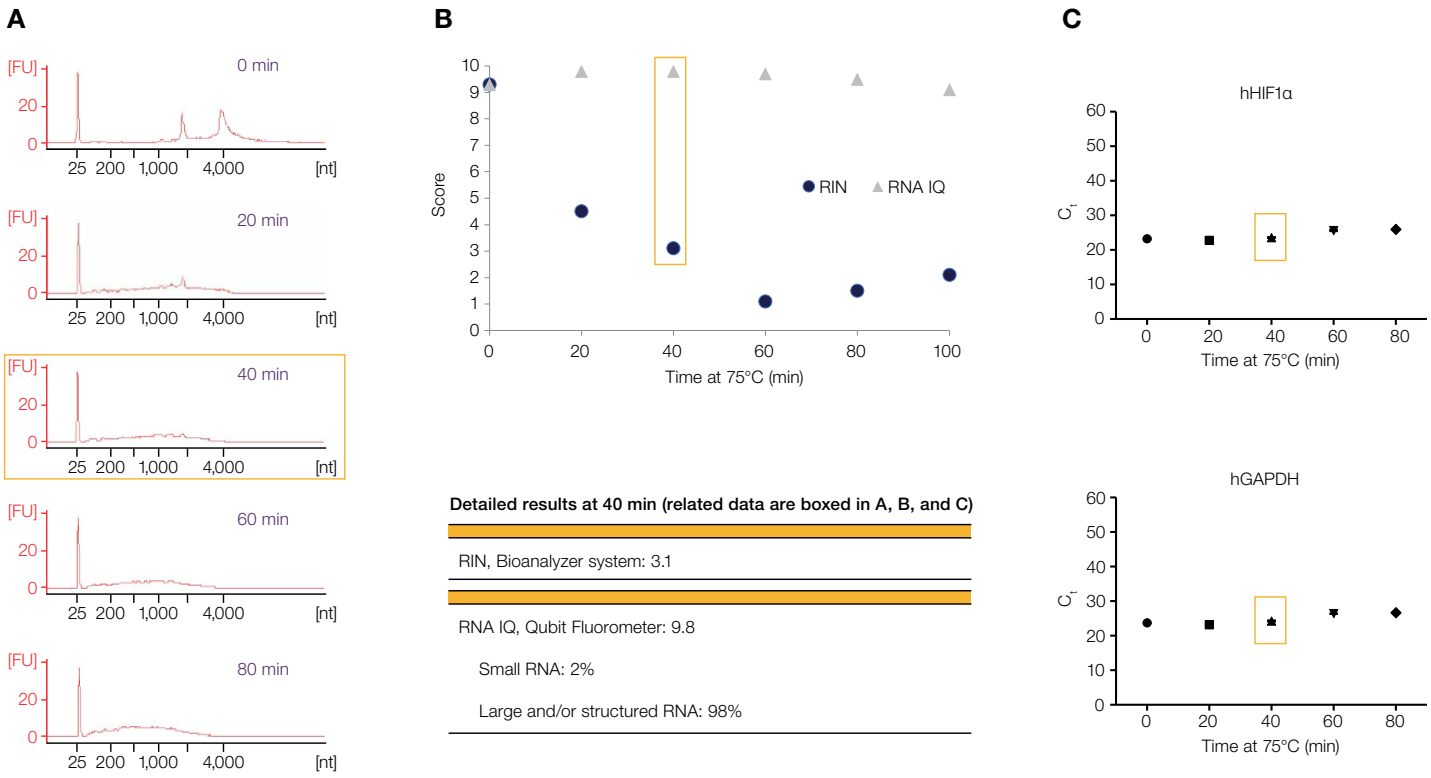

Figure 2. RNA IQ is a better predictor of RT-qPCR performance than RIN. (A) Data from the Bioanalyzer system show rapidly decreasing rRNA peaks over time. **(B)** A comparison of RIN and RNA IQ values is shown, including more detailed results at the 40 min time point. **(C)** In agreement with the RNA IQ assay, RT-qPCR results are largely consistent over time.

Qubit 4 Fluorometer

The Qubit 4 Fluorometer is designed to quickly and specifically quantitate DNA or RNA.

Key features:

- Qubit assay dyes bind selectively to DNA or RNA, making it more sensitive than UV absorbance
- Uses as little as 1 µL of sample, even for very dilute samples
- Fast, reliable detection of degraded RNA with the Qubit RNA IQ Assay
- New integrated reagent calculator to quickly generate working solution calculations

Measurement of RNA degradation

Triplicate samples of 100 ng/µL rRNA solutions were incubated with RNase A in the final assay solution containing multiplexed dyes and assay buffer. rRNA degradation by RNase A was measured in real time using the RNA IQ assay via the two fluorescence channels (Figure 3).

To compare RNA IQ and RIN measurements, various amounts of RNase A were added to aliquots of a 100 ng/µL solution of rRNA and at various time points treated with Invitrogen™ RNaseOUT™ Recombinant Ribonuclease Inhibitor to stop the reaction. Results were measured using either the Qubit RNA IQ Assay or Agilent™ RNA 6000 Nano Kit (Figure 4).

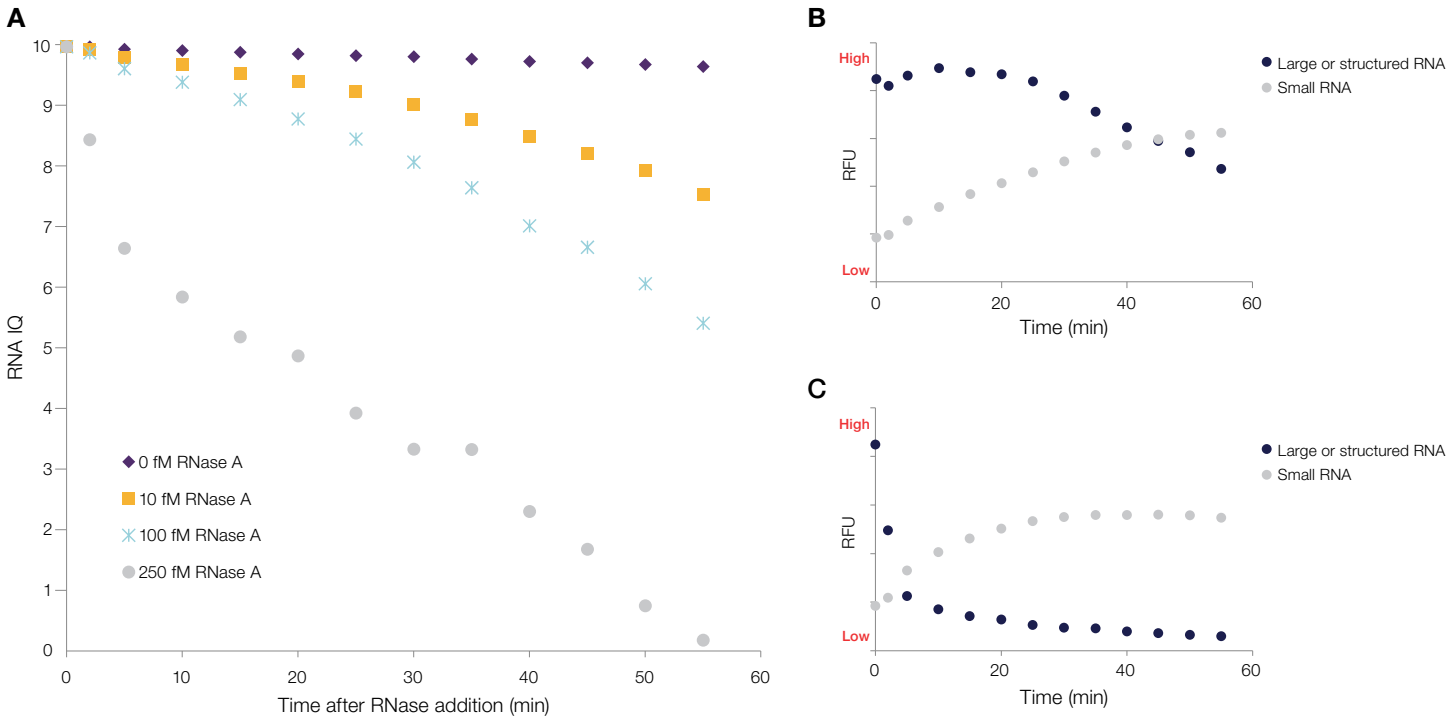

Figure 3. Real-time measurement of rRNA degradation by RNase A, using the RNA IQ assay. Results for **(A)** various concentrations of RNase A, **(B)** 10 fM RNase A, and **(C)** 100 fM RNase A.

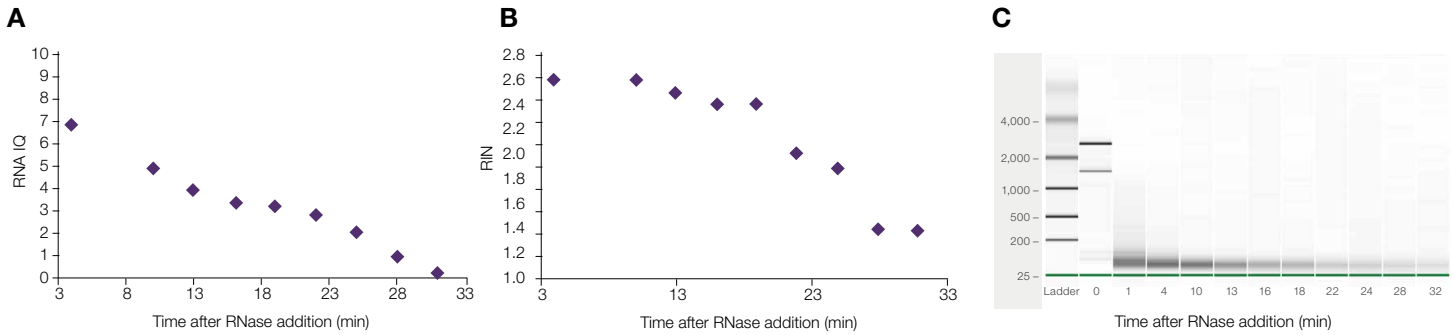

Figure 4. RNA assessment by either RNA IQ or RIN following RNase treatment. Both **(A)** RNA IQ and **(B)** RIN values decrease over time. **(C)** RNA size rapidly decreases, as shown with the electropherogram from the Bioanalyzer instrument.

Correlation to RNA sequencing (RNA-Seq) results

RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue was used for RNA-Seq on the Ion Torrent™ Oncomine™ platform, and the results were compared to RNA IQ results. Sufficiently mapped reads (>50% mappable reads) were found to correlate to RNA IQ >4. With this guideline, only 4 out of 60 samples resulted in a false-negative result, a 6.7% failure rate (Figure 5).

Demonstration of dye selectivity

Triplicate samples containing *E. coli* rRNA (100 ng/µL) and varying amounts of siRNA (0 to 50 ng/µL) were assayed with the Qubit RNA IQ Assay on the Qubit 4 Fluorometer. The results show the selectivity of the two dyes in binding to different RNAs (Figure 6).

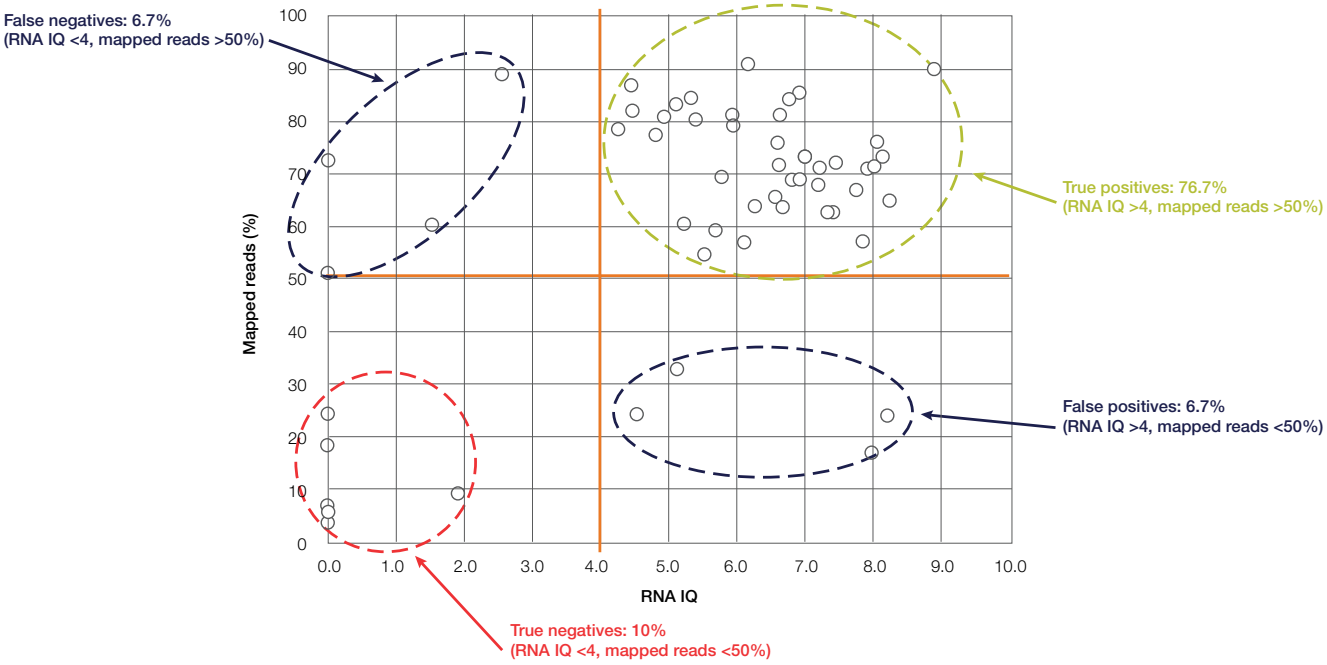

Figure 5. Correlation of RNA IQ values and RNA-Seq mappable reads from FFPE clinical research samples.

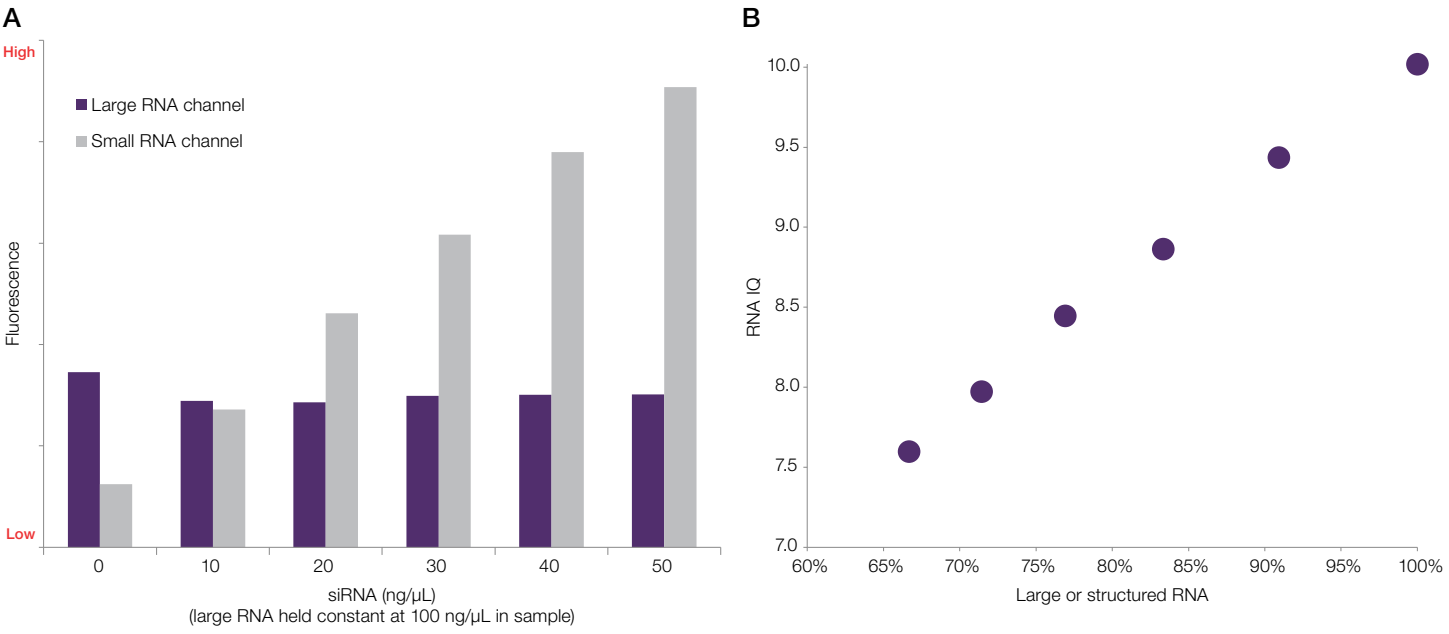

Figure 6. RNA IQ selectivity for large and small RNAs. (A) Fluorescence values obtained by the Qubit 4 Fluorometer for each type of RNA. **(B)** As expected, RNA IQ value increases with increasing percentage of large RNA.

Conclusion

The Qubit RNA IQ Assay is a fast and easy method to measure RNA quality in under 5 minutes on the Qubit 4 or Qubit Flex Fluorometer. We have shown correlation to performance in RNA-Seq and RT-qPCR applications, and the ability to assess RNA degradation via enzymatic and thermodynamic processes. This assay allows assessment of RNA quality at a low cost and with an easy, simple, and fast workflow.

- **Easy assessment of RNA integrity**—two unique dyes, one for large RNA and one for small, degraded RNA
- **Simple protocol**—add RNA sample to Qubit RNA IQ Buffer and measure on the Qubit 4 or Qubit Flex Fluorometer
- **Rapid time-to-results**—about 5 minutes for sample preparation and 4 seconds for sample measurement

Ordering information

Product	Quantification range	Quantity	Cat. No.
RNA integrity and quality kit			
Qubit RNA IQ Assay Kit*	NA	75 assays	Q33221
		275 assays	Q33222
RNA quantification assays			
Qubit RNA HS Assay Kit	4–200 ng	100 assays	Q32852
		500 assays	Q32855
Qubit RNA BR Assay Kit	10–1,200 ng	100 assays	Q10210
		500 assays	Q10211
Qubit RNA XR Assay Kit	100 ng–20 µg	100 assays	Q33223
		500 assays	Q33224
Qubit microRNA Assay Kit	0.5–150 ng	100 assays	Q32880
		500 assays	Q32881
Instruments and accessories			
Qubit 4 Fluorometer with WiFi		1	Q33238
Qubit 4 RNA IQ Starter Kit with WiFi		1 kit	Q33241
Qubit 4 Quantitation Starter Kit with WiFi		1 kit	Q33239
Qubit 4 NGS Starter Kit with WiFi		1 kit	Q33240
Qubit Assay Tubes		500 tubes	Q32856
Qubit Flex Fluorometer		1	Q33327
Qubit Flex Quantitation Starter Kit		1 kit	Q45894
Qubit Flex NGS Starter Kit		1 kit	Q45893
Qubit Flex Assay Tube Strips		125 tube strips	Q33252

* The Qubit RNA IQ Assay for the detection of degraded RNA can only be run on the Qubit 4 and Qubit Flex Fluorometers and cannot be performed on the original Qubit, Qubit 2.0, or Qubit 3.0 Fluorometers.

Learn more at **thermofisher.com/qubit**

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