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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, multiplexed assay for the Invitrogen™ Qubit™ 4 Fluorometer 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 another 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 as the Qubit 4 Fluorometer, allowing multiplexed assays and new user interface features on the instrument, which already has an integral role 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 consisting of: 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).

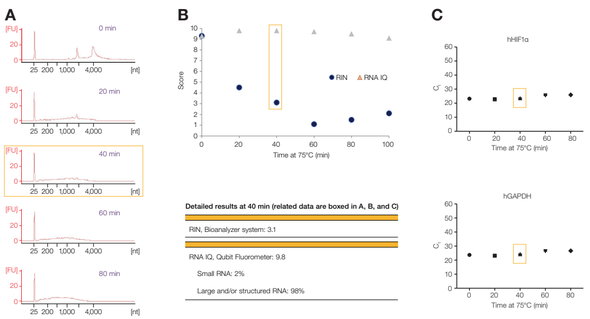

Figure 2. RNA IQ is a better predictor of RT-qPCR performance than RIN. (A) Data from the Bioanalyzer system show rapidly decreasing rRNA peaks over time. **(B)** A comparison of RIN and RNA IQ values is shown, including more detailed results at the 40 min time point. **(C)** In agreement with the RNA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

Measurement of RNA degradation

Triplicate samples of 100 ng/mL 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).

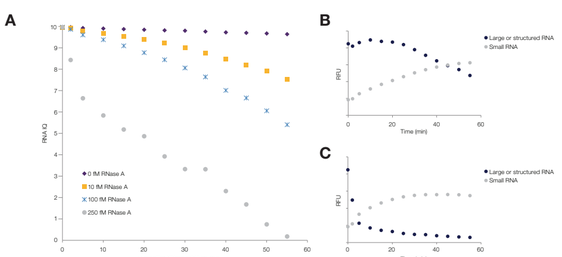

Figure 3. Real-time measurement of rRNA degradation by RNase A, using the RNA IQ assay. Results are plotted for **(A)** various concentrations of RNase A, **(B)** 10 fM RNase A, and **(C)** 100 fM RNase A.

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

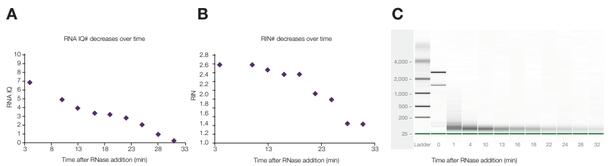

Figure 4. RNA assessment by either RNA IQ or RIN following RNase treatment. Both **(A)** RNA IQ and **(B)** RIN values decrease over time. **(C)** RNA size rapidly decreases, as shown with the Bioanalyzer electropherogram.

Correlation to RNA sequencing (RNA-Seq) results

RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue was subjected to RNA-Seq on the Ion Torrent™ Oncomine™ platform, and the results 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).

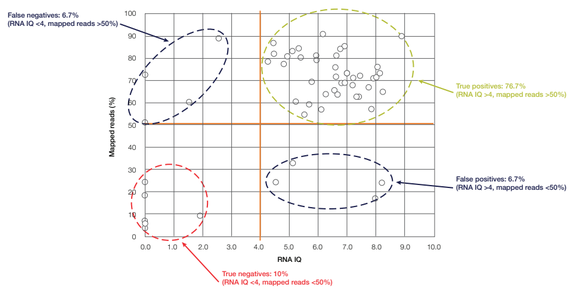

Figure 5. Correlation of RNA IQ values and RNA-Seq mappable reads from FFPE clinical research samples.

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).

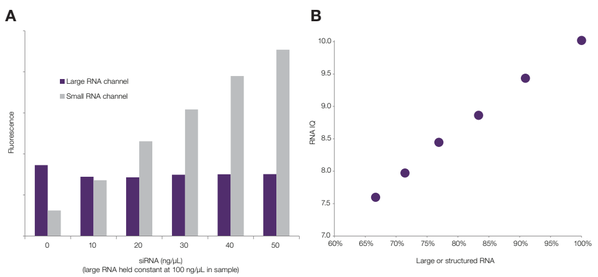

Figure 6. RNA IQ selectivity for large and small RNAs. (A) Fluorescence values obtained by the Qubit 4 Fluorometer are plotted for each type of RNA. **(B)** As expected, RNA IQ value increases with increasing percentage of large RNA.

Conclusion

The Qubit RNA IQ Assay is a fast and easy method to measure RNA quality in under 5 minutes on the Qubit 4 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 lower cost and with an easier, simpler, and faster workflow than with other solutions currently on the market.

- **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 Fluorometer
- **Rapid time-to-results**—about 5 minutes for sample preparation and 4 seconds for sample measurement

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