

Quality Analysis of Eukaryotic Total RNA with the Agilent 5200 Fragment Analyzer System

Authors

Chava Pocernich,
Denise Warzak, and
Kit-Sum Wong
Agilent Technologies, Inc.

Abstract

The Agilent 5200 Fragment Analyzer system and associated RNA kits allow for easy and reliable analysis of RNA quality and concentration. Agilent ProSize data analysis software provides an electropherogram and a digital gel image for visual inspection of RNA. In addition, ProSize automatically reports the concentration, ribosomal ratio, and assigns an RNA quality number (RQN). The 5200 Fragment Analyzer system delivers consistent and reliable RNA concentration measurements and RQN values between instruments and throughout the RNA dilution series.

Introduction

Total RNA quality is a constant concern because of how easily RNA degrades due to temperature, enzymatic digestion from the abundance of RNase in the environment, and improper handling. High-quality total RNA is critical for successful outcomes in RT-PCR, microarray analysis, and next-generation sequencing (NGS). Previously, only the ribosomal ratio (28S/18S) was used to assess the quality of RNA, but the Agilent 5200 Fragment Analyzer system and Agilent ProSize data analysis software use an RNA quality number (RQN) as a quality metric indicator. ProSize considers the entire electropherogram. This includes the 5S and fast region where the small RNA separates, as well as the small and large ribosomal peaks, as well as their ratios. The degradation in front of the small ribosomal peak when calculating the RQN is also analyzed. The 5200 Fragment Analyzer system offers high separation resolution to easily distinguish between small RNA fragments and degradation in the small RNA region. This feature enables ProSize to assign an RQN that justly represents the sample quality. The RQN is based on a scale from 1 to 10, where 1 represents completely degraded total RNA, and 10 represents intact total RNA. A large RQN value indicates higher-quality RNA with minimal degradation in the sample. The concentration, RQN, and ribosomal ratio are automatically reported in ProSize, allowing for easy evaluation of total RNA quality.

ProSize provides easy and flexible data analysis with the option of advanced settings for unique circumstances. The data analysis mode is automatically selected based on the kit utilized. However, the advanced settings tab allows users to select between five different RNA analysis options. Selecting the specific type of RNA allows the unique software parameters to be applied to each separation for data processing. There are five different settings for RNA analysis including:

- Eukaryotic - Calculates the ratio of the 28S and 18S ribosomal RNA peaks.
- Prokaryotic - Calculates the ratio of the 23S and 16S ribosomal RNA peaks.
- Plant - Calculates the ratio of the 25S and 18S ribosomal RNA peaks.
- mRNA - Analyzes messenger RNA (mRNA) smears from ribosomal depleted total RNA or isolated polyadenylated mRNA with the HS RNA kit (15 nt). Calculates the % rRNA contamination from total RNA.
- Small RNA - Analyzes small and microRNA (miRNA) samples. Calculates % miRNA, small RNA (pg/ μ L), and size in nucleotide (nt).

Experimental

The experiments in this study were done using a 5200 Fragment Analyzer system and can be replicated with comparable results on Agilent 5300 and 5400 Fragment Analyzer systems.

Agilent universal mouse reference total RNA (p/n 740100) was separated on the 5200 Fragment Analyzer system with the Agilent HS RNA kit (15 nt) (p/n DNF-472). Human brain total RNA (BioChain, #R1234035-P) and human liver total RNA (Ambion, #AM7960) were separated on the 5200 Fragment Analyzer system with the Agilent RNA kit (15 nt) (p/n DNF-471). Qubit 2.0 with the RNA HS assay kit (ThermoFisher Scientific, #Q32852) was utilized.

Results and discussion

Dilution series

Universal mouse reference total RNA was separated on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt) throughout the concentration range of the kit (Figure 1A). Excellent separation resolution was seen between the 18S and 28S ribosomal peaks and in the small RNA region, with several small RNA peaks discernible from the baseline. As shown in the overlay (Figure 1), the sizing of the 18S and 28S ribosomal peaks remained constant and was unchanged by the concentration range. The RQN also remained consistent across the dilution series, averaging 9.2 ± 0.1 (Figure 1B). The concentration of each sample (around 5, 2.5, 1.25, and 0.5 ng/ μ L) was analyzed and compared between the Qubit and 5200 Fragment Analyzer systems (Figure 1C). Both the Qubit and the 5200 Fragment Analyzer systems reported similar concentrations throughout the dilution series with a percent error of 8 % or less. Concentration precision on the 5200 Fragment Analyzer system was tight, with a coefficient of variation (CV) less than 4 %.

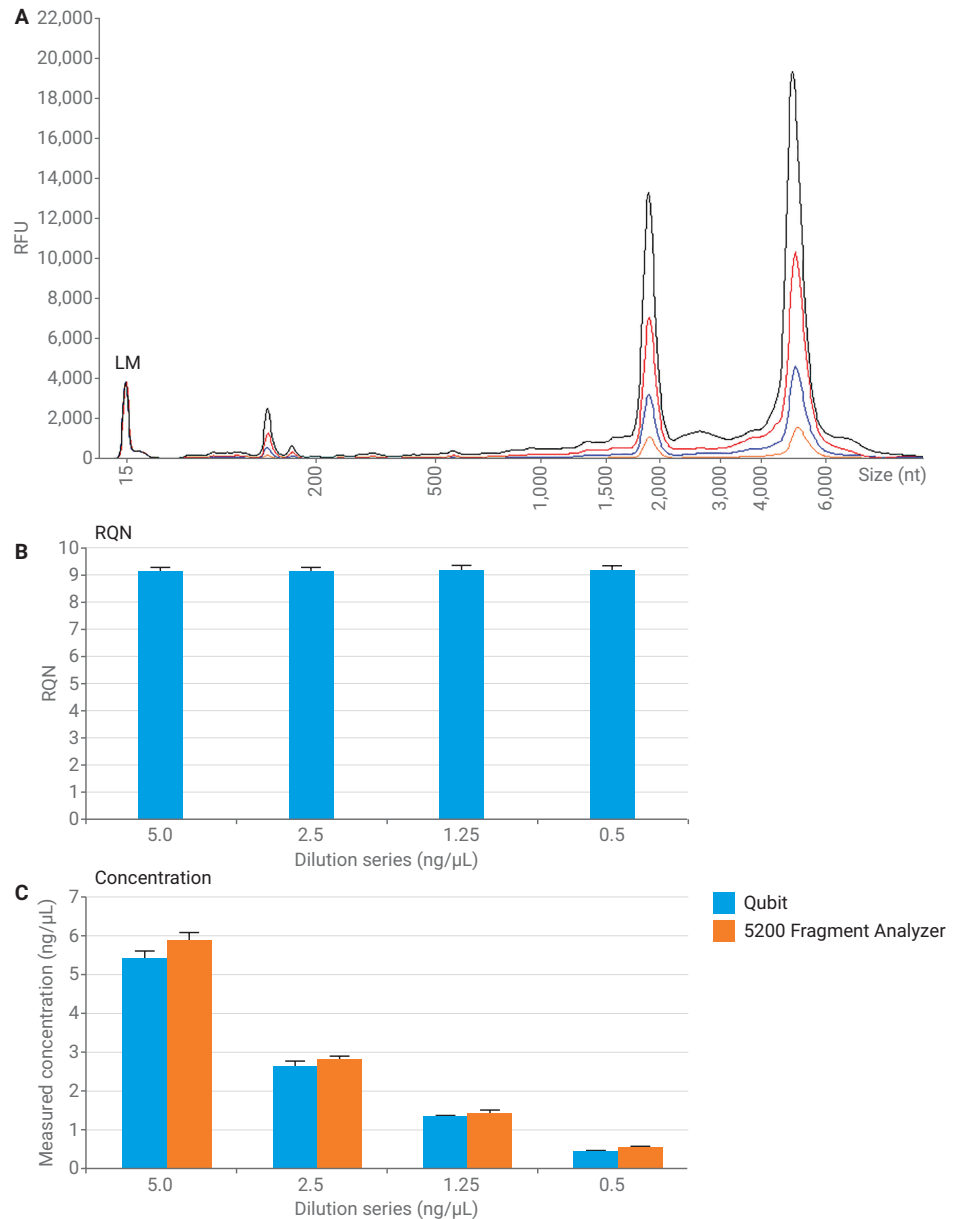


Figure 1. Universal mouse reference total RNA was separated on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt). (A) Electropherogram overlay of the dilution series. (B) RQN remained consistent throughout the dilution series. (C) Concentration measured on the Qubit (blue) and on the 5200 Fragment Analyzer system (orange) was similar across the entire dilution series (8 % error). n=16; LM = lower marker.

Reliability between instruments

A dilution series of partially degraded universal human reference total RNA was analyzed with the HS RNA kit (15 nt) on two different 5200 Fragment Analyzer systems to demonstrate reliability and consistency of quantification and RQN quality metrics (Figure 2). The RQN remained steady, ranging from 7.1 to 7.3 between the two instruments and throughout the dilution series. Quantification between the two instruments and throughout the dilution series remained similar with the precision well below the kit specifications.

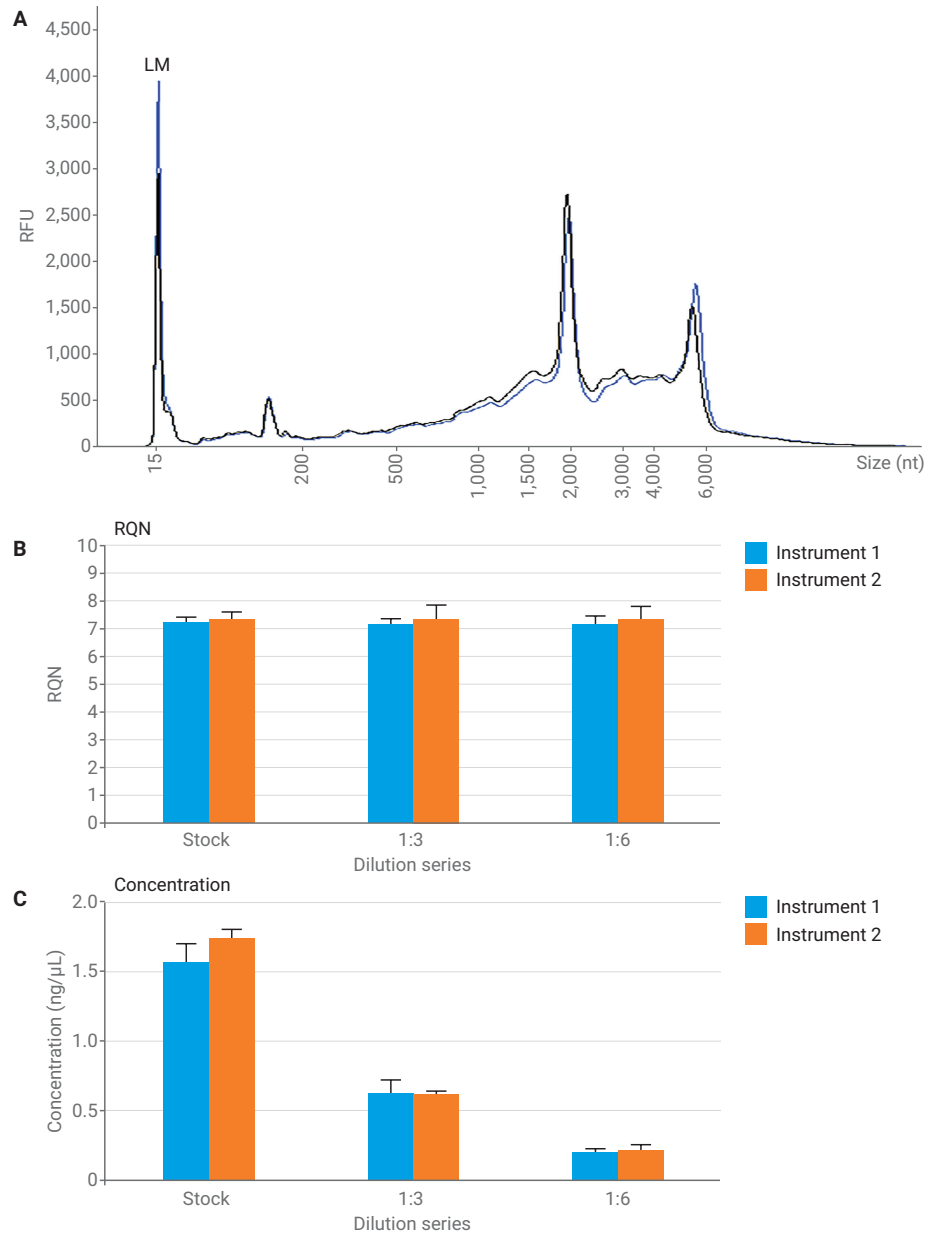


Figure 2. Universal human reference total RNA was separated on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt). (A) Overlay of instrument 1 (blue) and instrument 2 (black) at 1:3 dilution. (B) RQN remains consistent between the instruments throughout the dilution series. $n=8$; LM = lower marker.

RQN over time

Degradation by RNase is an ever-present concern when handling RNA. RNase is found everywhere and does not require cofactors for activation. When preparing RNA for analysis on the 5200 Fragment Analyzer system, the RNA sample is mixed with the marker containing 50 % formamide, which is known to protect RNA from RNase degradation¹. To evaluate the quality of RNA throughout a full plate analysis, a freshly prepared 96-well plate of universal mouse reference RNA was separated on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt). The RQN was compared between the first run (row 1) and the last run, eight hours later (row 8). The RQN only decreased by 0.4 from the first run to the last run over the entire dilution series, indicating little to no RNA degradation occurred overnight (Figure 3).

Degradation series

Universal mouse reference total RNA was incubated at 70 °C for 0 to 20 minutes. Samples were taken every two minutes and analyzed on the 5200 Fragment Analyzer system with the HS RNA kit. The electropherogram overlay and digital gel image revealed the heat degradation of RNA over time (Figure 4A and B). The 28S ribosomal fragment decreased in concentration and completely disappeared with increased heat degradation over time, resulting in an increase of low molecular weight RNA fragments in the fast region of the electropherogram. Separation profiles from the 5200 Fragment Analyzer system offered easy visualization of the degree of total RNA degradation. This degradation strongly correlated with the decrease in RQN (Figure 4C). The degree of total RNA degradation can easily be determined by the RQN and visually assessed with the electropherogram and digital gel image.

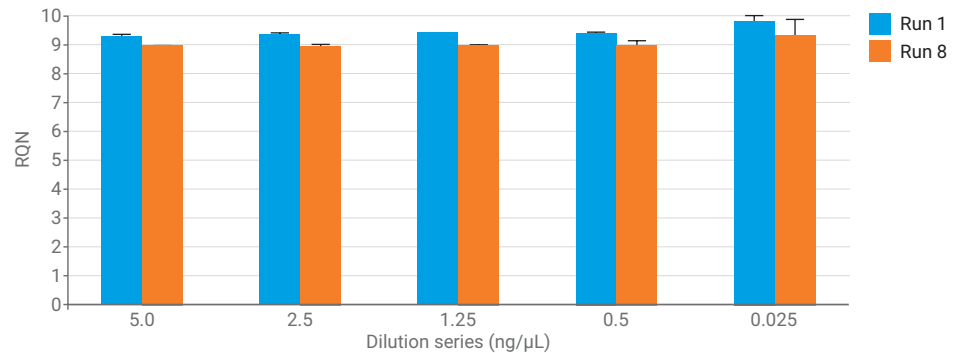


Figure 3. Universal mouse reference total RNA was separated on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt). RQN decreased slightly (0.4 RQN) after eight hours (orange) throughout the dilution series, indicating that RNA prepped for overnight analysis is stable. n = 2.

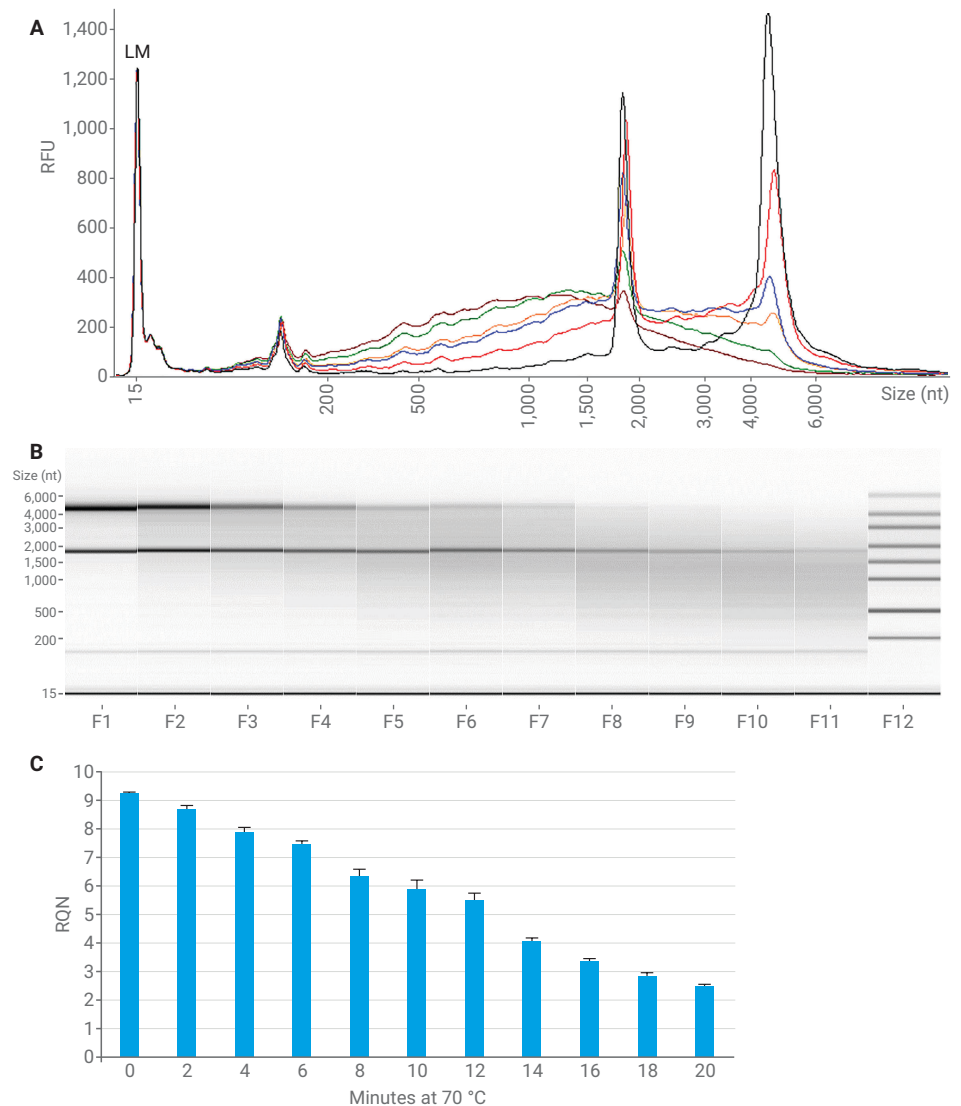


Figure 4. Universal mouse reference total RNA was separated on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt). (A) Electropherogram overlay of the degradation series. (B) Digital gel image of degradation series. (C) RQN decreases throughout the degradation series. n=3; LM = lower marker.

RNA examples

RNA profiles can vary from species to species and between tissue types². Human liver and brain total RNA were analyzed on the 5200 Fragment Analyzer system with the RNA kit (15 nt) at two different concentrations (Figure 5A and B). The excellent separation resolution of the 5200 Fragment Analyzer system allows the user to compare the fast region containing the small RNA fragments and the region between the ribosomal peaks between the different tissue types. Regardless of the tissue type, the RQN remained the same between the two concentrations (Figure 5C).

Conclusion

The 5200 Fragment Analyzer system offers excellent separation resolution for all regions of eukaryotic RNA, including the small RNA fast region. The degree of degradation was easily visualized with the electropherogram and digital gel image. The RQN provided by ProSize reflected the degree of total RNA degradation and remained consistent between instruments, across the RNA dilution series, and during overnight analysis. Concentration of total RNA remained consistent between 5200 Fragment Analyzer systems and between the 5200 Fragment Analyzer system and Qubit 2.0. The 5200 Fragment Analyzer system offers reliable and consistent analysis of eukaryotic RNA.

References

1. Chomczynski, P. Solubilization in Formamide Protects RNA from Degradation, *Nucleic Acids Res.* **1992**, *20*(14), 3791-92.
2. Pocernich, C.; Warzak, D.; Wong, K-S. Plant RNA degradation detection with the Agilent 5200 Fragment Analyzer system. *Agilent Technologies Application Note*, publication number 5994-0518EN, **2017**.

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Printed in the USA, February 14, 2019
5994-0519EN

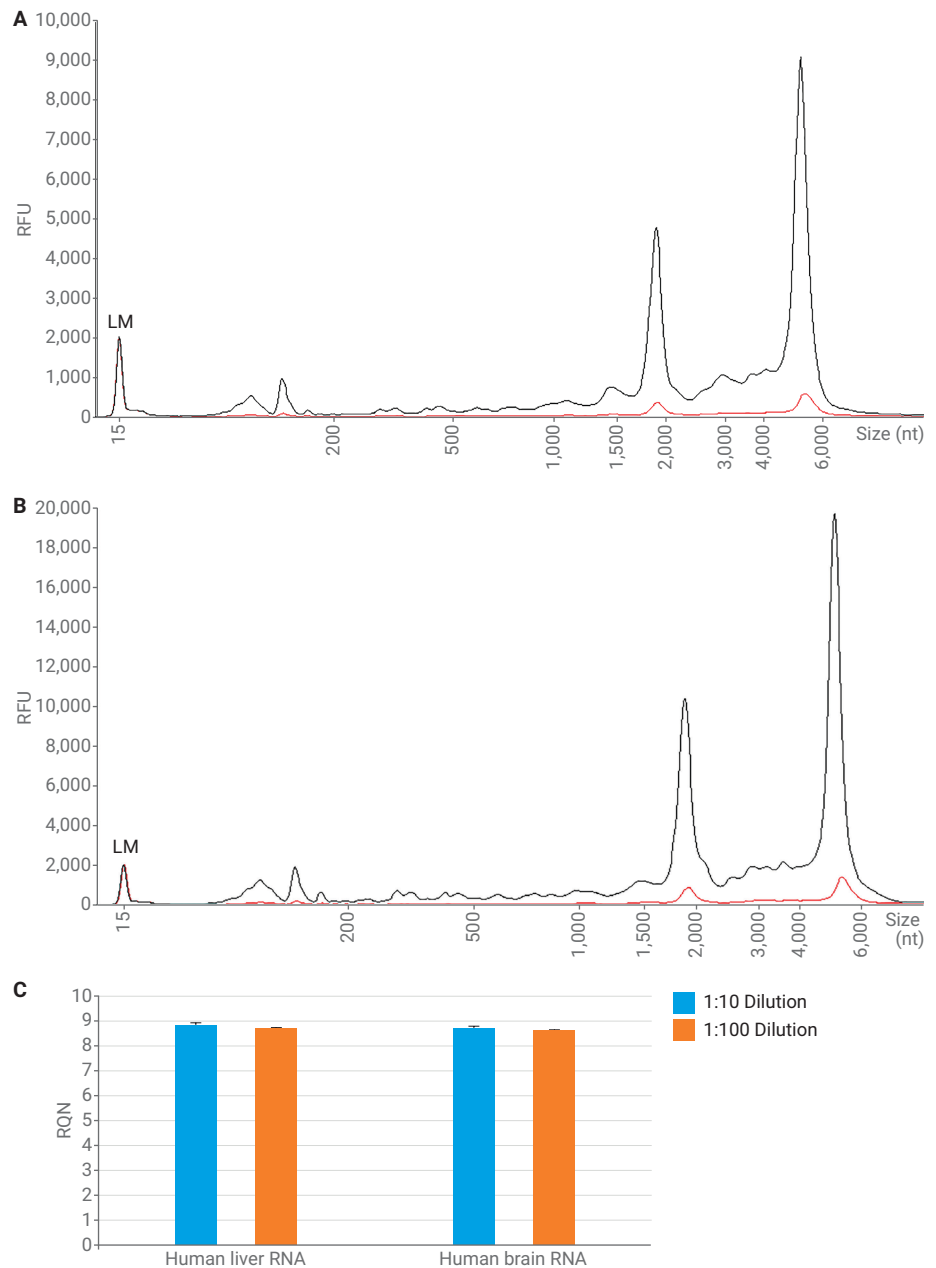


Figure 5. Total RNA samples separated on the 5200 Fragment Analyzer system with the RNA Kit (15 nt); 1:10 dilution (black), 1:100 dilution (orange). (A) Human liver total RNA. (B) Human brain total RNA. (C) RQN remained the same for human liver and brain samples at both concentrations. n=9; LM = lower marker.