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Comparison of RIN and RQN for the Agilent 2100 Bioanalyzer and the Fragment Analyzer Systems

Introduction

Good quality RNA is crucial to many applications including qPCR, next-generation sequencing (NGS), microarray analysis, and Northern blot analysis. Determining RNA integrity is essential to the success of downstream workflows.

The Agilent 2100 Bioanalyzer system is a microfluidic-based technology dedicated to sample quality control (QC) of nucleic acids. The Bioanalyzer is well established for providing a reliable, automated RNA integrity number, RIN. The RIN provides objective assessment of RNA integrity[1]. The Agilent 5200 Fragment Analyzer system is an automated parallel capillary electrophoresis-based technology that offers reliable QC for nucleic acids. The ProSize data analysis software for the Fragment Analyzer automatically provides an RNA quality number, RQN. The RQN assists in assessing the presence or absence of degradation[2]. Both the RIN and RQN consider the entire electropherogram with scoring from 10 to 1, where 10 indicates the highest possible RNA quality.

This technical overview examines the RIN from the Bioanalyzer and the RQN from the Fragment Analyzer. Comparison of the RIN and RQN is performed with several eukaryotic and prokaryotic RNA samples that have various degrees of degradation.

Analytical specifications

Table 1. Specifications of the Agilent 2100 Bioanalyzer system and 5200 Fragment Analyzer system RNA assays.

	Standard Sensitivity RNA Kits		**High Sensitivity RNA Kits**	
Instrument	Fragment Analyzer	Bioanalyzer	Fragment Analyzer	Bioanalyzer
Kit	RNA kit (15 nt)	RNA 6000 Nano kit	HS RNA kit (15 nt)	RNA 6000 Pico kit
Quantitative range	25 to 500 ng/µL	25 to 500 ng/µL	50 to 5,000 pg/µL	50 to 5,000 pg/µL
Sample volume	2 µL	1 µL	2 µL	1 µL
Quality score	RQN	RIN	RQN	RIN

Experimental

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

Different total RNA samples from human, rat, mouse, and E. coli were obtained from commercial vendors Zyagen and Agilent Technologies. Various animal total RNA samples were also independently analyzed by BGI, China. Samples were diluted with RNase-free water to 400 ng/µL and heat degraded with the Agilent SureCycler 8800 thermal cycler (p/n G880A) for 0 to 20 minutes at 94 °C in order to generate a variety of degraded RNA from completely intact, to mildly and strongly degraded. An aliquot was taken from the stock solution at the appropriate time point and immediately placed on ice. Samples were diluted to 200 ng/µL and analyzed on the 5200 Fragment Analyzer and the 2100 Bioanalyzer systems. The RNA kit (15 nt) (p/n DNF-471) and the Agilent RNA 6000 Nano kit (p/n 5067-1511) were analyzed on the same day. The 200 ng/µL samples were diluted with RNase-free water to 2,000 pg/µL or 4,000 pg/µL and analyzed with the Agilent HS RNA kit (15 nt) (p/n DNF-472) and Agilent RNA 6000 Pico kit (p/n 5067-1513) on the same day (Table 1).

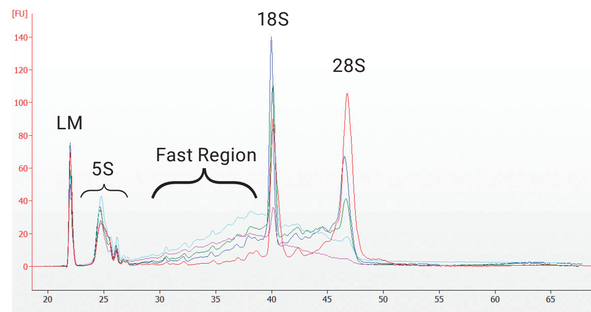
Results and discussion

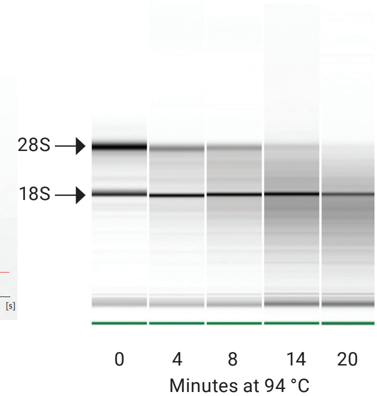
Total RNA quality

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. RNA serves as the input material for workflows including RT-qPCR, microarray analysis, and RNA-Seq. High-quality input RNA is critical for successful outcomes in these workflows, rendering QC an important first step in the process. The Bioanalyzer system uses the RIN and the Fragment Analyzer system uses the RQN to assign a numerical value to the quality of an RNA sample. While different algorithms are used for determining the RIN and RQN, both are based on the entire electrophoretic trace for a given total RNA sample the including 18S to 28S ribosomal peak ratio, separation between these peaks, and the presence or absence of degradation products in the fast region. RIN and RQN have values from 10 to 1, where 10 indicates the highest possible RNA quality and 1 indicates strongly degraded RNA.

A. Bioanalyzer system

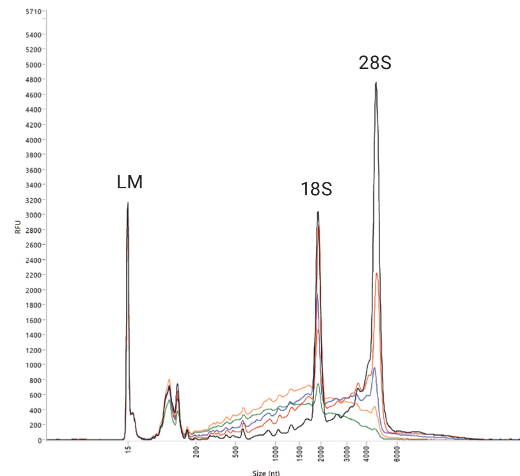
RIN* 9.5 7.4 6.3 4.2 3.5





B. Fragment Analyzer system

RQN* 9.5 7.6 6.3 4.6 3.8



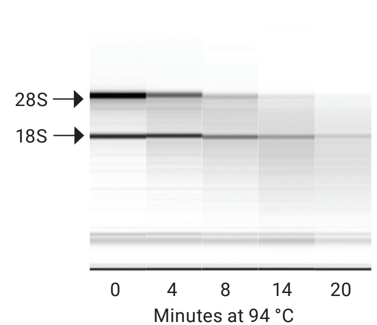
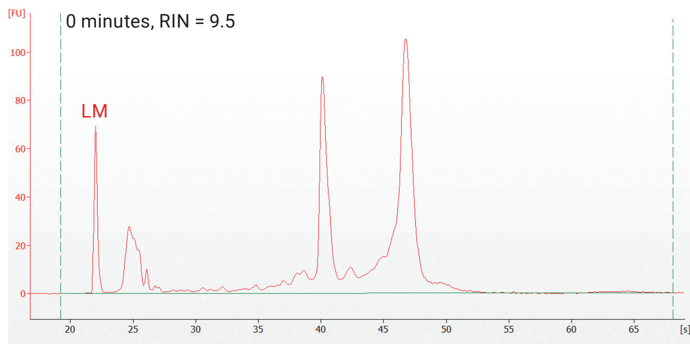
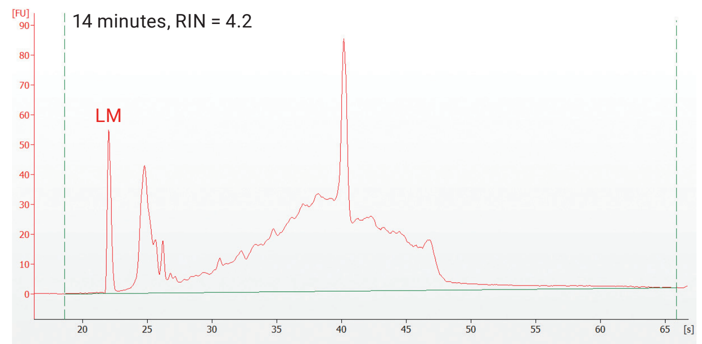


Figure 1. Mouse kidney total RNA heat degraded over time on the (A) Agilent 2100 Bioanalyzer system and (B) the Agilent 5200 Fragment Analyzer system. Typical degradation pattern was seen on both instruments with the 28S and 18S ribosomal peaks disappearing over time. Similar RIN and RQN numbers were assigned by both instruments throughout the RNA degradation series. *n = 4.

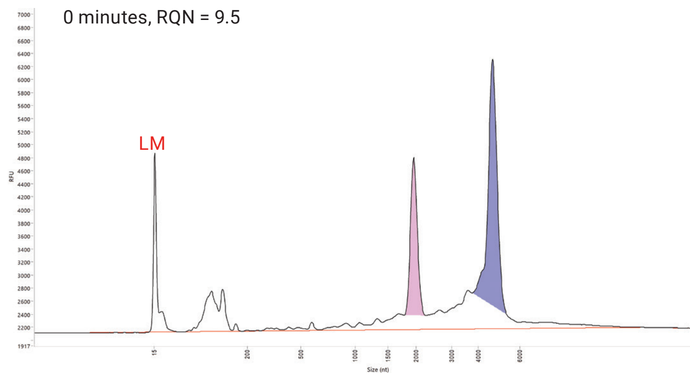
High-quality eukaryotic total RNA samples display two prominent peaks, the 18S and 28S ribosomal fragments. As total RNA degrades, the 28S peak disappears quickly while the 18S peak disappears at a slower pace. As these peaks disappear, the regions between the 18S to 28S peaks and the fast region accumulate degraded material. This typical degradation pattern was observed with mouse kidney total RNA after heat degradation with both the Bioanalyzer and the Fragment Analyzer systems (Figure 1). The digital gel images and electropherogram overlays show the 28S top gel band completely disappearing while the 18S band is reduced over time. Similar RIN and RQN numbers were assigned by both instruments throughout the RNA degradation series (Figure 1 digital gel images). Comparison of the electropherograms at 0- and 14-minute degradation time points demonstrates the similarities in the total RNA degradation patterns presented by the two instruments (Figure 2).

A. Bioanalyzer system





B. Fragment Analyzer system



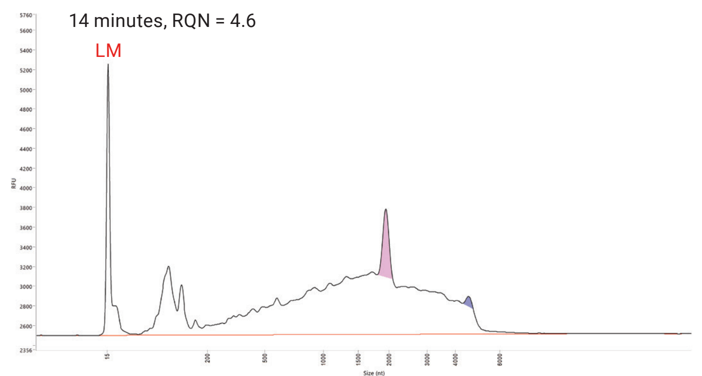


Figure 2. Mouse kidney total RNA heat degraded at 94 °C for 0 and 14 minutes on the (A) Agilent 2100 Bioanalyzer system and (B) the Agilent 5200 Fragment Analyzer system. Similar total RNA degradation patterns are seen on both instruments.

Comparative analysis of RIN and RQN

Total RNA quality was compared between the RIN on the Bioanalyzer system and the RQN on the Fragment Analyzer system. The standard sensitivity (SS) RNA kits included the Fragment Analyzer RNA kit (15 nt) and the Bioanalyzer RNA 6000 Nano kit at 200 ng/µL. The high sensitivity (HS) RNA kits included the Fragment Analyzer HS RNA kit (15 nt) and Bioanalyzer RNA 6000 Pico kit at 2,000 pg/µL. Eukaryotic RNA samples included liver, kidney, spleen, and total RNA tissue from human, mouse, and rat. Data for the standard sensitivity RNA kits also included independently measured animal RNA samples from BGI China. Replicates were averaged, resulting in 30 RIN/RQN pairs ranging from 1.6 to 10. A slope and R^2 value of 1 indicates a perfect linear correlation. Comparison of the standard sensitivity RNA kits provided a slope = 0.96 with an R^2 = 0.96 (Figure 3A). Comparison of the high sensitivity RNA kits provided a slope = 0.99 with an R^2 = 0.97 (Figure 3B). Both RNA kits demonstrated excellent correlation and linear regression between the Bioanalyzer and Fragment Analyzer systems. The average difference between data points was ± 0.4 units for the standard sensitivity and ± 0.3 units for the high sensitivity kits.

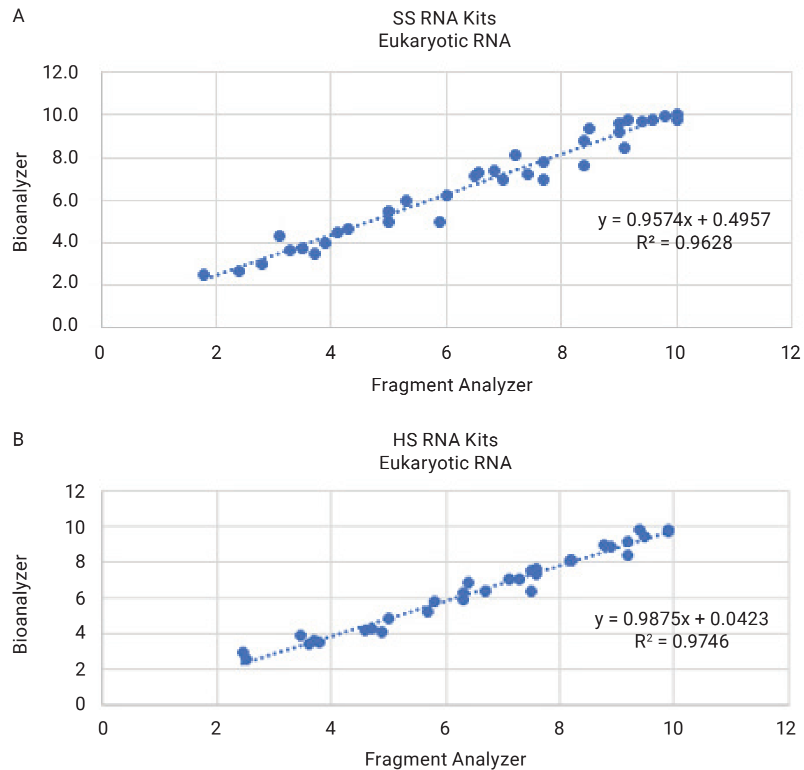


Figure 3. Comparison of the Bioanalyzer RIN and the Fragment Analyzer RQN with RNA eukaryotic samples demonstrated excellent correlation and linear regression between both systems. (A) The standard sensitivity RNA kits included the Fragment Analyzer RNA kit (15 nt) and the Bioanalyzer RNA 6000 Nano kit. (B) The high sensitivity RNA kits included the Fragment Analyzer HS RNA kit (15 nt) and Bioanalyzer RNA 6000 Pico kit.

Prokaryotic RNA *E. coli* samples were also compared throughout the degradation series with both the standard sensitivity (200 ng/µL) and high sensitivity RNA (4,000 pg/µL) kits on the Bioanalyzer and Fragment Analyzer systems (Figure 4). Both sets of kits demonstrated excellent correlation between the two instruments with an average difference of ± 0.3 units for the standard sensitivity RNA kits and ± 0.4 units for the high sensitivity RNA kits throughout the degradation series.

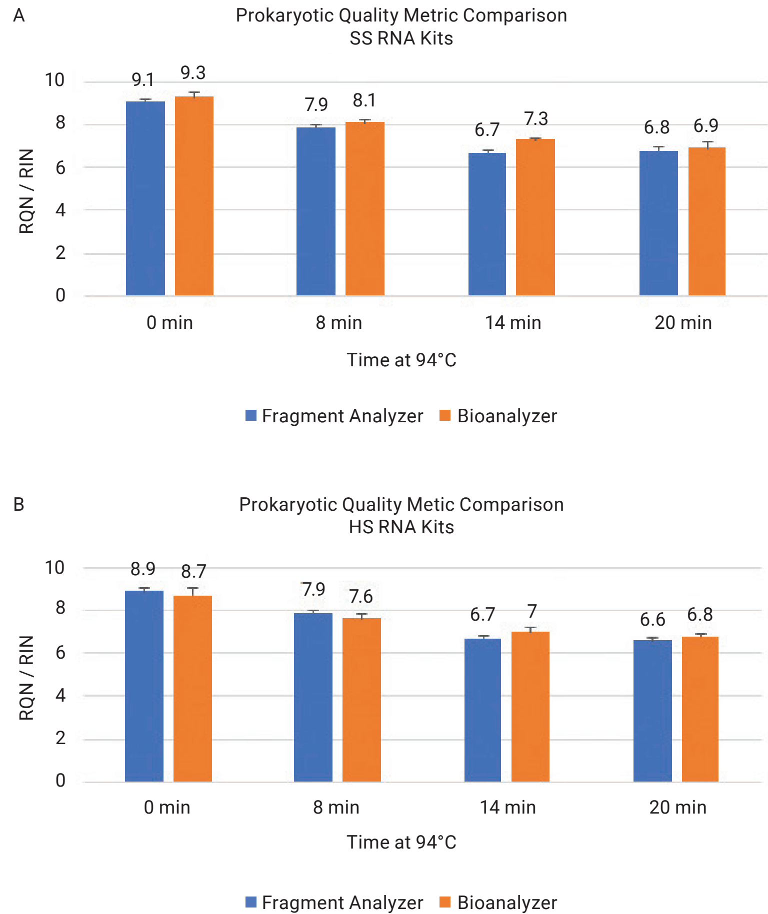


Figure 4. Comparison of the Bioanalyzer RIN and the Fragment Analyzer RQN with RNA prokaryotic *E. coli* samples demonstrated excellent correlation between both systems. (A) The standard sensitivity RNA kits included the Fragment Analyzer RNA kit (15 nt) and the Bioanalyzer RNA 6000 Nano kit. (B) The high sensitivity RNA kits included the Fragment Analyzer HS RNA kit (15 nt) and Bioanalyzer RNA 6000 Pico kit. n = 6.

Conclusion

The 2100 Bioanalyzer and the 5200 Fragment Analyzer systems displayed similar total RNA patterns for high-quality RNA and RNA at different levels of degradation. In addition, the RIN and RQN quality metrics were demonstrated to be equivalent on both the standard sensitivity and high sensitivity kits for the Bioanalyzer and Fragment Analyzer systems with eukaryotic and prokaryotic RNA samples. This confirms the ability to use the RIN and RQN interchangeably for determining the quality and integrity of total RNA samples.

Acknowledgments

We would like to thank BGI China for providing data.

References

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2. Quality Analysis of Eukaryotic Total RNA with the Agilent 5200 Fragment Analyzer System, Application note, *Agilent Technologies,* publication number 5994-0519EN, **2019**.

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www.agilent.com/en/product/automated-electrophoresis/fragment-analyzer

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