Technical Overview

Genomics

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Comparison of the Agilent 2100 Bioanalyzer and the Agilent Fragment Analyzer Systems for Analysis of Plant, Insect, and Bacterial RNA

Introduction

RNA analysis is an important part of life science research. Although much of life science research is focused on human samples, a variety of other research focuses on plants, insects, and bacteria. Regardless of the focus of the life science research, having high-quality RNA samples is necessary for successful downstream applications.

Knowing the quality of an RNA sample can be crucial for downstream applications such as next-generation sequencing (NGS), RNA sequencing, qPCR, microarrays, and more. Factors such as temperature and enzymatic digestion from RNases in the environment can have deleterious effects on the end results of an RNA workflow. The quality of an RNA sample can provide insight into how the sample may perform throughout a workflow, and the quality of data it may yield when analyzed downstream. Agilent offers a portfolio of automated electrophoresis systems used for sample quality control that are fit for many applications and throughput needs.

Evaluating RNA integrity prior to downstream analysis saves time, effort, and resources by ensuring that only samples of sufficient quality are used. To aid in objectively determining the integrity of a sample, quality metrics can be assigned, helping to grade the quality of the RNA independently of user bias. The Agilent 2100 Bioanalyzer and 5200 Fragment Analyzer systems each provide reliable, convenient analysis of samples. Each system also provides a quality metric for RNA samples called the RNA integrity number (RIN), and the RNA quality number (RQN) respectively. The RIN and RQN assign a score from 1 to 10 for a given RNA sample. A score of 1 indicates severely degraded RNA, while a score of 10 indicates highly intact RNA.

The Bioanalyzer and Fragment Analyzer systems have different kits available depending on the concentration of the sample. For RNA at low concentrations, the Bioanalyzer system offers high sensitivity analysis in the form of the Agilent RNA 6000 Pico kit, which has a qualitative analysis input range of 50 to 5,000 pg/µL. Alternatively, the Fragment Analyzer utilizes the Agilent HS RNA kit (15 nt), which has an input range for quantification of 50 to 5,000 pg/µL. If an RNA sample has an abundant concentration, the Bioanalyzer system offers the standard sensitivity Agilent RNA 6000 Nano kit, and the Fragment Analyzer offers the standard sensitivity Agilent RNA kit (15 nt). Both kits have an input range for quantification of 25 to 500 ng/µL (Table 1).

Previously, the RIN and RQN quality-metric scores were shown to be comparable for eukaryotic total RNA at various stages of degradation.[1] In this technical overview, different total RNA samples were evaluated to compare the quality metrics between the Bioanalyzer and Fragment Analyzer systems. Plant, insect, and bacterial total RNA samples were analyzed using both the high and standard sensitivity kits for each system to provide a detailed comparison of the RIN and RQN. In the Agilent 2100 Expert software for the Bioanalyzer, there are assays for each kit and sample type that are used. Using the correct assay for each kit and sample is needed to ensure accurate RNA quality scoring, which is based on the whole electropherogram and not just the ribosomal subunit peaks. When using Agilent ProSize data analysis software for the Fragment Analyzer data, the ability to change the mode of analysis for total RNA is available in the advanced settings. When the mode is changed in ProSize, the appropriate ribosomal subunits for the sample type will be analyzed, and an accurate RNA quality score can be displayed.

By leveraging sample quality information, determinations can be made as to whether time and resources would yield usable results for samples of varying quality. In this technical overview we demonstrate the robust and concordant results observed by the Bioanalyzer and Fragment Analyzer systems.

Experimental

Three different, commercially available total RNA samples were used in this experiment. The total RNA sample types were corn (Zyagen, part number PLR-1002), *E. coli* (Zyagen, part number ECR-310), and *Drosophila* (Zyagen, part number DPR-290). Each sample was diluted to a concentration range to fit either the standard or high sensitivity RNA analysis kits (Table 1) for the Agilent 2100 Bioanalyzer and 5200 Fragment Analyzer systems. Each sample was analyzed in triplicate, on the Bioanalyzer system using the RNA 6000 Nano kit and the RNA 6000 Pico kit. Depending on the kit type (RNA Nano kit or RNA Pico kit) the corresponding assay was selected in the 2100 Expert software. When running corn total RNA, the plant RNA assays were selected. For *Drosophila* the eukaryote assay was chosen. For *E. coli* the prokaryote assay was utilized.

The same samples were also run in triplicate, on a 5200 Fragment Analyzer using the RNA kit (15 nt) and the HS RNA kit (15 nt). When using ProSize data analysis software for corn total RNA, the plant mode was selected, which calculates the ratio of the 25S and 18S ribosomal RNA (rRNA) peaks. *Drosophila* was analyzed using eukaryote mode, which calculates the ratio of the 28S and 18S rRNA peaks. For *E. coli* the prokaryote mode was used, which calculates the ratio of the 23S and 16S rRNA peaks. The RIN and RQN for each sample type were compared among systems for each of the kits tested.

Table 1. Quantitative and qualitative ranges of the total RNA analysis kits for the Agilent 2100 Bioanalyzer system and Agilent Fragment Analyzer systems.

	2100 Bioanalyzer System		Fragment Analyzer Systems	
	RNA 6000 Pico kit (p/n 5067-1513)	**RNA 6000 Nano kit (p/n 5067-1511)**	**HS RNA kit (15 nt) (p/n DNF-472)**	**RNA kit (15 nt) (p/n DNF-471)**
Quantitative Range	-	25–500 ng/µL	50–5,000 pg/µL	25–500 ng/µL
Qualitative Range	50–5,000 pg/µL	5–500 ng/µL	50–5,000 pg/µL	5–500 ng/µL
Quantification Precision (CV%)	20%	10%	20%	10%
Kit Sensitivity	High	Standard	High	Standard

Results and discussion

Total RNA refers to all the RNA molecules that are found within a cell. Analysis of total RNA using the whole electropherogram allows for assessment of the integrity of RNA in a sample. The 2100 Bioanalyzer and 5200 Fragment Analyzer can both be used to analyze total RNA via automated electrophoresis. Often the resulting electropherogram for total RNA will display a combination of peaks and smears. The peaks are representative of the rRNA in the sample, while the smear between the lower marker and these peaks represents the 5S rRNA region. The electropherograms shown in Figures 1 to 3 for each sample type are examples of the results obtained from the high sensitivity kit runs and are also representative of the standard sensitivity kit results.

Corn Total RNA Analysis

RNA from corn was analyzed on both the 2100 Bioanalyzer system and the 5200 Fragment Analyzer. As shown in the electropherograms from both systems, the plant RNA is visualized as four distinct peaks (Figure 1). The two largest peaks shown are the 18S and 25S peaks from the cellular rRNA. The two smaller peaks are the 16S and 23S peaks, which are from chloroplasts in the cells found in plant leaves. Additionally, the Bioanalyzer system and Fragment Analyzer show smears for the 5S region of the electropherograms (Figure 1A and B).

Corn total RNA was run on the Bioanalyzer system using the RNA Nano kit and output an average RIN score of 8.6 and an average quantification of 63.67 ng/µL. The same sample was run on the Fragment Analyzer using the RNA kit and output an average RQN score of 7.8 and an average quantification of 72.40 ng/µL. The RIN and RQN were 0.8 points apart between instruments. The difference in quantification was approximately 13.72% between the Bioanalyzer system and Fragment Analyzer (Table 2).

Corn total RNA was also run on the Bioanalyzer system using the RNA Pico kit and output an average RIN score of 8.2, with an average quantification of 2.80 ng/µL. The same sample was run on the Fragment Analyzer using the HS RNA kit and output an average RQN score of 7.3 with an average quantification of 2.33 ng/µL. The RIN and RQN differed by 0.9 points. The quantification difference between instruments was 16.94% (Table 2).

The similarity between the RIN and RQN scores for the corn total RNA shown in this technical overview is indicative of the ability of the Bioanalyzer and Fragment Analyzer to deliver comparable RNA quality metrics. Both systems also reported similar concentrations for each sample, further highlighting the equivalence of the two systems.

Table 2. Comparison of quality metrics and concentration of corn total RNA from the Agilent 2100 Bioanalyzer and 5200 Fragment Analyzer systems, n = 3.

		Bioanalyzer System (RIN)	Fragment Analyzer (RQN)	Bioanalyzer System (ng/µL)	Fragment Analyzer (ng/µL)
Standard Sensitivity Kit	Average	8.63	7.83	63.67	72.40
	%CV	1.55%	0.74%	2.40%	7.74%
High Sensitivity Kit	Average	8.20	7.33	2.80	2.33
	%CV	3.23%	2.84%	4.35%	2.28%

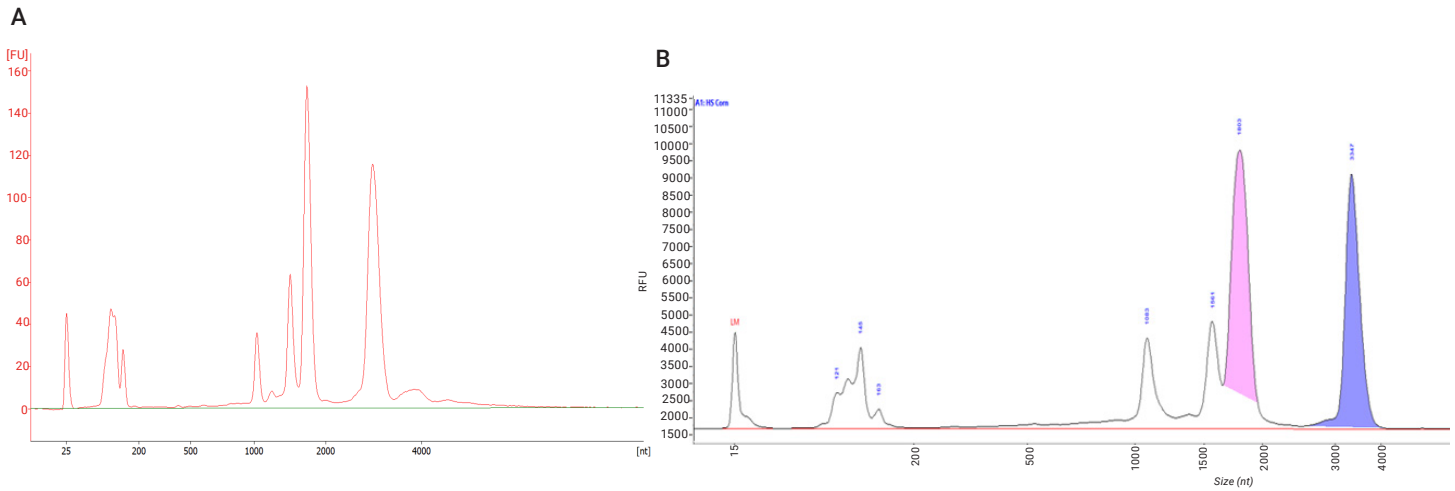

Figure 1. Electropherogram from corn analyzed on the (A) Agilent 2100 Bioanalyzer system and (B) Agilent 5200 Fragment Analyzer system.

Drosophila Total RNA

Drosophila total RNA analyzed on the 2100 Bioanalyzer system and 5200 Fragment Analyzer displayed two peaks, representing the 18S and 28S rRNA. In Figure 2 (A and B), the peaks are visualized close together, and both the Bioanalyzer system and Fragment Analyzer show the peaks to be analogous of each other. Also, the 5S region for the Bioanalyzer system is comparable to the 5S region observed in the electropherogram for the Fragment Analyzer.

An average RIN score for *Drosophila* total RNA of 6.8 was obtained using the RNA Nano kit on the Bioanalyzer system. Similarly, the Fragment Analyzer gave an average RQN score of 6.6 using the RNA kit. The RIN and RQN for the two instruments were 0.2 points apart. The average quantification reported by the Bioanalyzer system was 36 ng/µL. The Fragment Analyzer reported the average quantification of 28.12 ng/µL (Table 3). There was a difference in quantification of 21.88% between the systems.

For the RNA Pico kit and HS RNA kit, the Bioanalyzer system provided an average RIN score of 6.4 and the Fragment Analyzer output an average RQN score of 7.1. The RIN and RQN had a score that differed by 0.7 points. The quantification of the *Drosophila* total RNA using the RNA Pico kit on the Bioanalyzer system was reported to be an average of 2.13 ng/µL. On the Fragment Analyzer, the same sample was reported to be an average of 2.12 ng/µL (Table 3) from using the HS RNA kit. The quantification difference between each instrument was 0.89%.

In this technical overview, the RIN and RQN obtained for the *Drosophila* total RNA showed comparable results with all assays tested. The results demonstrate the ability of the Bioanalyzer system and Fragment Analyzer to objectively determine the integrity of eukaryotic total RNA samples.

Table 3. Comparison of quality metrics and concentration of *Drosophila* total RNA from the Agilent 2100 Bioanalyzer and 5200 Fragment Analyzer systems, n = 3.

		Bioanalyzer System (RIN)	Fragment Analyzer (RQN)	Bioanalyzer System (ng/µL)	Fragment Analyzer (ng/µL)
Standard Sensitivity Kit	Average	6.83	6.60	36.00	28.12
	%CV	0.84%	0.00%	7.35%	2.84%
High Sensitivity Kit	Average	6.43	7.10	2.13	2.12
	%CV	0.90%	1.41%	6.20%	4.88%

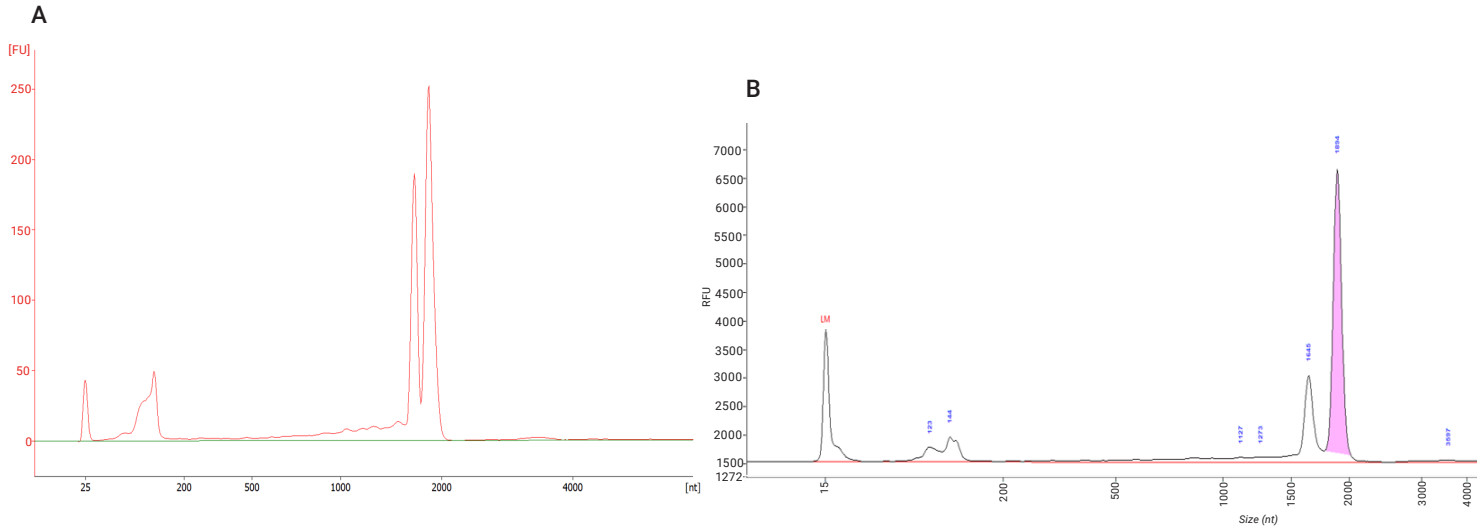

Figure 2. Electropherograms for the *Drosophila* high sensitivity, run on the (A) Agilent 2100 Bioanalyzer and (B) Agilent 5200 Fragment Analyzer systems.

E. coli Total RNA

E. coli total RNA, as shown in the electropherograms from the 2100 Bioanalyzer system and 5200 Fragment Analyzer, displayed two prominent peaks (Figure 3). The peaks are representative of the 16S and the 23S rRNA fragments. The large, split peak seen at the beginning of the electropherogram is in the 5S region and is made up of small RNA fragments. As seen in Figure 3, the Bioanalyzer system and Fragment Analyzer have comparable electropherograms.

Using *E. coli* total RNA, the RNA Nano kit showed an average RIN score of 9.3 on the Bioanalyzer system, with an average quantification of 52 ng/µL. On the Fragment Analyzer, the RNA kit displayed an average RQN score of 9.7 and an average quantification of 51 ng/µL (Table 4). The RIN and RQN were 0.4 points apart. Both instruments quantified within 1.42% of each other.

The *E. coli* RNA analyzed using the RNA Pico kit output an average RIN score of 8.4 and an average quantification of 2.93 ng/µL on the Bioanalyzer system. On the Fragment Analyzer, an average RQN score of 8.9 with an average quantification of 2.38 ng/µL was observed using the HS RNA kit (Table 4). The RIN and RQN differed by 0.4 points. The two instruments had quantification values that differed by 18.74%.

The RIN and RQN results shown in this technical overview demonstrate that prokaryotic total RNA quality metrics are comparable between the Fragment Analyzer and Bioanalyzer systems. Further, the Bioanalyzer system and the Fragment Analyzer quantification of prokaryotic total RNA samples yield comparable results to each other.

Table 4. Comparison of quality metrics and concentration of *E. coli* total RNA from the Agilent Bioanalyzer and Fragment Analyzer systems, n = 3.

		Bioanalyzer System (RIN)	Fragment Analyzer (RQN)	Bioanalyzer System (ng/µL)	Fragment Analyzer (ng/µL)
Standard Sensitivity Kit	Average	9.30	9.67	52.00	51.26
	%CV	1.52%	1.19%	1.92%	2.73%
High Sensitivity Kit	Average	8.43	8.85	2.93	2.38
	%CV	2.98%	0.80%	1.99%	1.82%

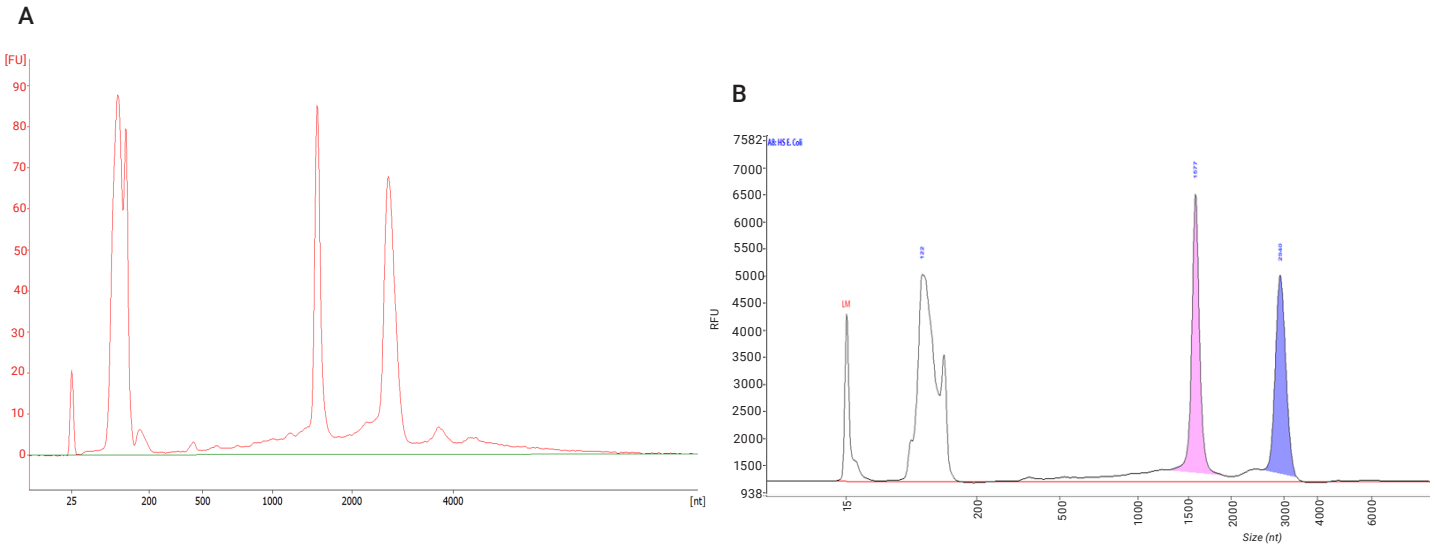

Figure 3.b. Electropherograms for the *E. coli* high sensitivity, run on the (A) Agilent 2100 Bioanalyzer system and (B) Agilent 5200 Fragment Analyzer system.

Conclusion

Knowing the quality of your RNA sample is important for many downstream RNA applications. In this technical overview, three total RNA sample types were analyzed on both the Agilent 2100 Bioanalyzer system and the Agilent 5200 Fragment Analyzer, and the results were compared. The quality scores, quantification, and electropherogram results from each kit analyzed on the Bioanalyzer and Fragment Analyzer showed similar results between sample types across multiple total RNA samples.

References

1. Comparison of RIN and RQN for the Agilent 2100 Bioanalyzer and the Fragment Analyzer Systems. Agilent Technologies *technical overview,* publication number 5994-1860EN, **2020**.

www.agilent.com/genomics/automated-electrophoresis

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