

# Comparison of Small RNA Analysis using the Agilent Bioanalyzer and Agilent Fragment Analyzer Systems

## Introduction

Small RNAs are considered to be RNA molecules that are approximately 200 nucleotides or less, and are often noncoding. A variety of small RNA types have been identified, including microRNA (miRNA), piwi-interacting RNA (piRNA), and small-interfering RNA (siRNA). These small RNAs are known to be involved in gene silencing, and potentially other mechanisms of gene regulation<sup>1</sup>. Research into the biological roles of these small molecules is novel and ongoing. Analysis of these samples can help with optimization of small RNA isolation and purification protocols, and ensure successful downstream applications such as small RNA next-generation sequencing (NGS) (RNA-Seq), miRNA microarrays, and qRT-PCR<sup>2</sup>.

The systems of the automated electrophoresis portfolio from Agilent have been well-established for the analysis of total RNA, and uniquely offer quality analysis of small RNAs with either the Agilent 2100 Bioanalyzer system<sup>3,4</sup>, or the Agilent Fragment Analyzer systems<sup>5</sup>. Both instruments use kits that focus on small RNA and miRNA quality control, concentrating on a narrow range of 200 nt and below, and allowing for high-separation resolution of small RNAs. Separations from different small RNA samples on each of the automated electrophoresis systems have been detailed in previous Agilent publications<sup>3,4</sup>. In this paper, samples are compared across both the Bioanalyzer and the Fragment Analyzer Small RNA kits to demonstrate the equivalency of the systems to analyze small RNAs.

## Methods

### Total RNA integrity

Total RNA samples were obtained from Thermo Fisher Scientific (Human Heart Total RNA, p/n AM7966) (Sample A) and analyzed on the 2100 Bioanalyzer and 5200 Fragment Analyzer systems using the respective total RNA analysis kits for each system. Samples processed with the Agilent RNA Nano 6000 kit (p/n 5067-1511) and the Eukaryotic Total RNA Nano Series II assay were analyzed with the Bioanalyzer. Samples prepared with the Agilent RNA kit (15 nt) (p/n DNF-471) were analyzed with the Fragment Analyzer. Integrity of the total RNA was assessed using the RIN and RQN, which are objective quality scores determined by each system. Visual inspection of the electropherograms from each system indicated the presence of small RNA within the commercial sample.

### Small RNA isolation

The small RNAs present within the total RNA were isolated using two commercially available kits, the mirVana miRNA isolation kit (Invitrogen, p/n AM1561) (Sample B) and the Quick-RNA Miniprep kit (Zymo, p/n R1054) (Sample C), with modifications for extraction of small RNAs, as noted in the respective manuals.

### Small RNA analysis isolation

Following isolation of the small RNA from the total RNA, both the total and purified samples were prepared using the small RNA kits and were analyzed on the Bioanalyzer and Fragment Analyzer systems for comparison. The Agilent Bioanalyzer Small RNA kit (p/n 5067-1548) and the Agilent Fragment Analyzer Small RNA kit (p/n DNF-470) were used. The analytical specifications of the small RNA kits are compared in Table 1. The concentration of the total and small RNA samples was determined by Qubit. The samples were diluted to fit the quantitative range of the Bioanalyzer and Fragment Analyzer kits and were analyzed in triplicate with each kit.

**Table 1.** Analytical specifications of the Small RNA kits for the Agilent Bioanalyzer and Agilent Fragment Analyzer systems.

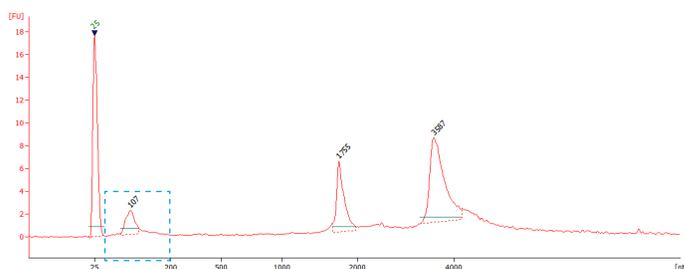
Analytical Specification	Bioanalyzer	Fragment Analyzer
	Small RNA kit (p/n 5067-1548)	Small RNA kit (p/n DNF-470)
Sizing Range	6 – 150 nt	15 – 200 nt
Quantitative Range	50 – 2,000 pg/μl	50 – 2,000 pg/μl
Quantitative Precision	25 %CV	25 %CV

## Results and discussion

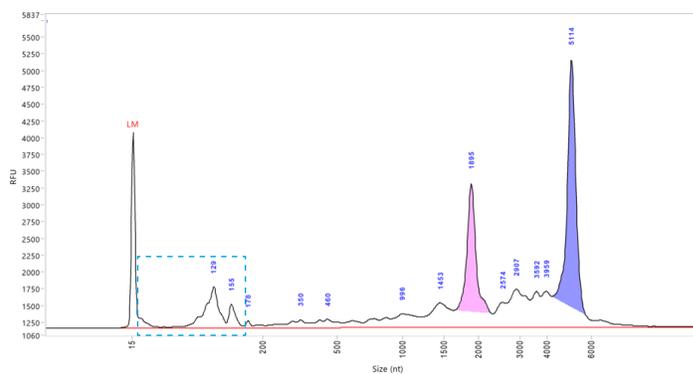
### Total RNA analysis

Total RNA (Sample A) was analyzed to ensure the integrity of the sample prior to small RNA isolation. Analysis was performed on both the Bioanalyzer with the RNA Nano kit and the Fragment Analyzer with the RNA kit (15 nt). Both systems display the results in digital gel and electropherogram formats, and report the integrity of a sample through a user-independent quality metric. The Bioanalyzer uses the well-established RNA integrity number (RIN), while the Fragment Analyzer gives the RNA quality number (RQN). These quality metric scores have been demonstrated to be equivalent and can be used interchangeably for determining the quality and integrity of total RNA samples<sup>5</sup>. The electropherograms from Sample A, the total RNA, are shown in Figure 1. Analysis of the total RNA showed an average RIN of 9.3 and an RQN of 8.5 (n=3), indicating highly intact RNA that is of good quality for any potential downstream application. The peaks below 200 nt indicate the presence of small RNAs in the total RNA (dashed boxes).

#### A) Agilent Bioanalyzer system



#### B) Agilent Fragment Analyzer system



**Figure 1.** Sample A, a commercially available total RNA, was analyzed on **A)** the Agilent Bioanalyzer system with the Agilent RNA 6000 Nano kit and **B)** the Agilent Fragment Analyzer system with the RNA kit (15 nt). The two larger peaks on each electropherogram correspond to the 18S and 28S ribosomal peaks of eukaryotic RNA (pink and purple peaks, respectively, on the Fragment Analyzer). The smaller peaks below 200 nt (dashed boxes) show the presence of some small RNAs. An average RIN of 9.3 and RQN of 8.5 indicate high-quality RNA.

## Small RNA analysis

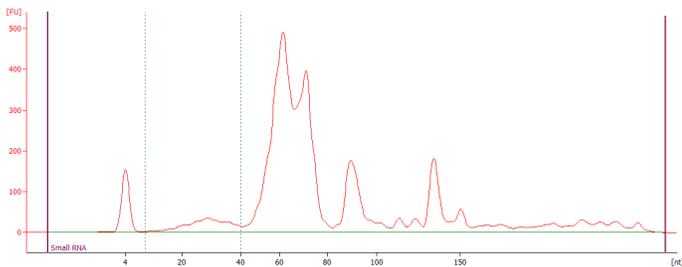
For better visualization of the small RNA area, specialized small RNA analysis kits are available for both systems. Each kit uses different gel chemistries, sizing ladders, dye, and electrophoresis conditions that account for small differences between the electropherogram profiles. As shown in Figure 2, Sample A was analyzed with the Small RNA kits on the Bioanalyzer (Figure 2A) and the Fragment Analyzer (Figure 2B) to examine the small RNA region more closely. Automated smear analysis regions are utilized with each system for the analysis of the small and microRNA regions. For the Bioanalyzer, the small RNA region is set arbitrarily to cover the entire range of the electropherogram, while the Fragment Analyzer defines this region as 10 to 200 nt. Both systems define the microRNA region as the area between 10 and 40 nt.

To further demonstrate the equivalency of the Bioanalyzer and Fragment Analyzer, the small RNAs were isolated from the total RNA using commercially available purification kits (Sample B and C) and analyzed with both the total and small

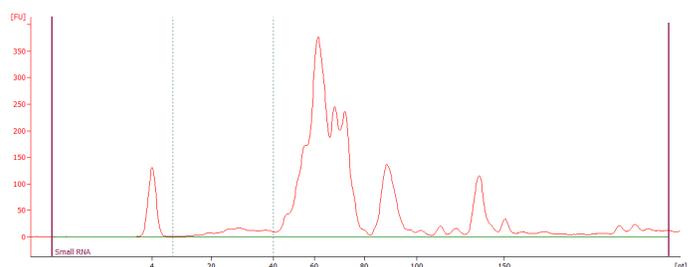
RNA analysis kits. When analyzed on the total RNA kits, the patterns of the small RNA samples B and C (data not shown) are similar to the small RNA portion of Sample A highlighted in Figure 1, demonstrating the successful isolation of the small RNA from the total RNA.

The electropherograms of the small RNA analysis kits for the Bioanalyzer and Fragment Analyzer show that the small RNA portion within the total RNA Sample A (Figure 2A-B) and the isolated small RNA Samples B (Figure 2C-D) and C (data not shown) display similar, although slightly different patterns. For example, samples A and B have very similar electropherogram patterns on the Bioanalyzer (Figure 2 A, C). Examination of these same samples on the Fragment Analyzer show slightly different patterns than with the Bioanalyzer, although the samples look similar to one another (Figure 2 B, D). Together these data demonstrate the differences between the small RNA kits for each automated electrophoresis instrument, and highlight that the small RNA portion visualized in the total RNA is a good representation of the small RNA present in the sample.

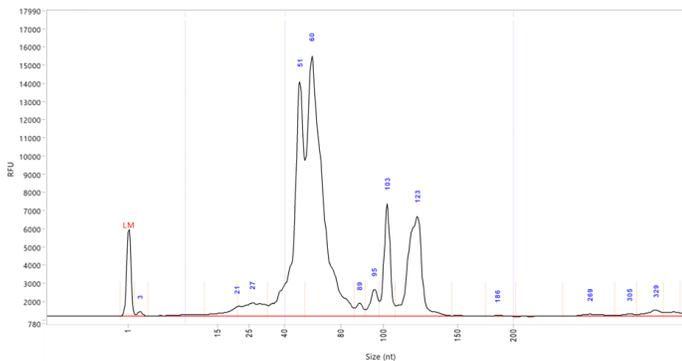
**A) Sample A, Agilent Bioanalyzer system**



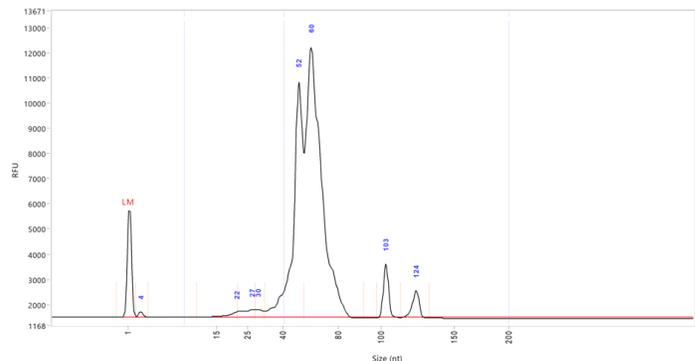
**C) Sample B, Agilent Bioanalyzer system**



**B) Sample A, Agilent Fragment Analyzer system**



**D) Sample B, Agilent Fragment Analyzer system**



**Figure 2.** Sample A, a commercially available total RNA, was further analyzed on **A)** the Agilent Bioanalyzer Small RNA kit and **B)** the Agilent Fragment Analyzer Small RNA kit to allow for higher separation resolution of the small RNA portion of the sample below 200 nt. The region from 10 to 40 nt (dashed lines) indicate the miRNA region. Sample B, the small RNA isolated from the total RNA, was analyzed on **C)** the Agilent Bioanalyzer Small RNA kit and **D)** the Agilent Fragment Analyzer Small RNA kit for comparison between the two systems.

## Small RNA quality

When analyzed on the total RNA kits, Sample B displayed an average RIN of 2.6 and RQN of 2.1, and Sample C showed a RIN of 2.6 and an RQN of 3.0 (data not shown). While a low-quality score could indicate degradation of the RNA sample, in this case it is indicative of the absence of the larger ribosomal peaks, demonstrating successful isolation of the small RNAs. Examination of the samples on the small RNA kits allows for better separation and qualification of only the small RNA region, and verifies the success of the small RNA purification kits.

## Small RNA quantification

The automated smear analysis for the small RNA kits provides quantification information of both the microRNA and the small RNA regions. For both the Bioanalyzer and Fragment Analyzer, the kit specifications state a 25 %CV for quantification precision. Both samples displayed a precision below 10 %CV, well within both kit specifications (n=3) (Table 2). These smear analysis regions also allow for the calculation of the percent microRNA within a sample, which can give researchers an indication of if a sample contains a sufficient amount of microRNA for sensitive downstream applications.

**Table 2.** Analytical specifications of the Small RNA kits for the Agilent Bioanalyzer and Agilent Fragment Analyzer systems.

	Agilent Bioanalyzer system Small RNA kit (p/n 5067-1548)			Agilent Fragment Analyzer system Small RNA kit (p/n DNF-470)		
	small RNA (ng/μl)	microRNA (ng/μl)	%microRNA	small RNA (ng/μl)	microRNA (ng/μl)	%microRNA
<b>Average</b>						
<b>Sample A</b>	18.9	1.9	10.3	14.3	1.2	8.2
<b>Sample B</b>	14.4	1.1	7.7	9.7	0.6	6.8
<b>%CV</b>						
<b>Sample A</b>	5.91	4.69	5.59	4.65	9.90	2.55
<b>Sample B</b>	3.26	3.45	7.53	2.59	9.12	6.41

## Summary

As research into small RNAs continues to become prevalent in molecular biology laboratories, knowing the quality of these samples can help ensure the success of applications and be used to optimize isolation and purification protocols for small RNA. The Agilent automated electrophoresis portfolio offers two instruments which can be used in the analysis, the Agilent Bioanalyzer and Agilent Fragment Analyzer systems. Each instrument is compatible with a unique small RNA analysis kit. Both kits focus on the small RNA region of 200 nt and below, allowing for high resolution separations, and quantitation of both the small RNA and the smaller microRNA region. In this application note, total RNA and isolated small RNA samples were analyzed on both the Bioanalyzer and Fragment Analyzer with the respective Small RNA kits to allow for comparison between the instruments. The kits utilize different gels and separation methods, accounting for slight differences in the electropherogram images. Despite these differences, the quantification precision for both the small RNA and miRNA regions was excellent, and the calculated percent microRNA was similar between the two kits. Either kit can be used for precise quality control of small RNA samples, allowing researchers to seamlessly move between instruments and have confidence in their sample analyses.

## References

1. Pritchard, C. C.; Cheng, H. H.; Tewari, M. MicroRNA Profiling: Approaches and Considerations. *Nat. Rev. Genet.* **2012**, *13* (5), 358–369. <https://doi.org/10.1038/nrg3198>.
2. Becker, C.; Hammerle-Fickinger, A.; Riedmaier, I.; Pfaffl, M. W. mRNA and MicroRNA Quality Control for RT-QPCR Analysis. *Methods* **2010**, *50* (4), 237–243. <https://doi.org/10.1016/j.ymeth.2010.01.010>.
3. RNA Quality Control in miRNA Expression Analysis. *Agilent Technologies application note*, publication number 5990-5557EN, **2016**.
4. Analysis of miRNA content in total RNA preparations using the Agilent 2100 Bioanalyzer. *Agilent Technologies application note*, publication number 5989-7870EN, **2008**.
5. Comparison of RIN and RQN for the Agilent 2100 Bioanalyzer and the Fragment Analyzer Systems. *Agilent Technologies application note*, publication number 5994-1860EN, **2020**.

[www.agilent.com/genomics/sample-qc](http://www.agilent.com/genomics/sample-qc)

PR7000-8633

This information is subject to change without notice.

© Agilent Technologies, Inc. 2022  
Published in the USA, May 03, 2022  
5994-4860EN