Technical Overview

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FFPE RNA Quality Assessment with the Agilent Bioanalyzer and Fragment Analyzer Systems

Introduction

Formalin-fixation and paraffin-embedding (FFPE) of tissues have long been a cornerstone in pathology for preserving specimens. This method permits long-term storage of tissue samples while maintaining cellular morphology. However, the fixation process presents challenges for the extraction and analysis of nucleic acids, particularly RNA, a critical component in gene expression studies. Chemical modifications that occur during formalin fixation lead to RNA degradation and fragmentation, posing a considerable hurdle for researchers aiming to retrieve high-quality RNA from FFPE samples. Using a quality control system to check RNA integrity after extraction from FFPE samples helps determine if the sample is of sufficient quality for downstream applications such as sequencing.

The Agilent automated electrophoresis portfolio provides powerful tools for the assessment of RNA quality. Both the Agilent 2100 Bioanalyzer and Agilent Fragment Analyzer systems provide users with quality metrics: RNA integrity number (RIN) for the Bioanalyzer and RNA quality number (RQN) for the Fragment Analyzer.[1] Due to the formalin fixing process damaging RNA, the resulting RNA quality scores will be lower than expected. Despite the degradation and low-quality scores, sequencing may still be possible, which has led to the need for a quality metric that can further assess FFPE RNA. The DV_{200} quality metric helps researchers classify degraded FFPE RNA samples by size, determining which are suitable for next-generation sequencing (NGS) and which are not, saving time and costs. The metric represents the percentage of RNA fragments above 200 nucleotides (nt) and correlates highly with the pre-capture library yield of FFPE samples.[2,3]

This technical overview highlights the capabilities of both the Bioanalyzer and Fragment Analyzer systems in analyzing FFPE RNA samples using the DV_{200} score. The results provide a comparison between the DV_{200} scores from each system.

Experimental

Four FFPE RNA samples from different sources were used for this study (cow liver, cow cerebellum, mouse liver, and pig spleen). Each sample was diluted to a concentration range that fits within the specifications of the RNA kits for both systems (Table 1). The Bioanalyzer system used the Agilent RNA 6000 Nano kit (p/n 5067-1511) and the Agilent RNA 6000 Pico kit (p/n 5067-1513) for assessment. Samples were not heat denatured to prevent further degradation. Data analysis on the Agilent 2100 Bioanalyzer Expert software was completed using the DV_{200} assay configuration file.[2]

The same samples were analyzed on the Fragment Analyzer using the Agilent RNA kit (15nt) (p/n DNF-471) and the Agilent HS RNA kit (15nt) (p/n DNF-472). Data analysis was performed using the Agilent ProSize data analysis software. To use the DV_{200} calculation with the ProSize software, the DV_{200} configuration files for either the RNA kit or the HS RNA kit must be downloaded from the Agilent website.

Table 1. Kits used for analyzing FFPE RNA with the Agilent 2100 Bioanalyzer system and Agilent Fragment Analyzer systems.

	2100 Bioanalyzer System		Fragment Analyzer Systems	
Kit	RNA 6000 Nano kit (p/n 5067-1511)	RNA 6000 Pico kit (p/n 5067-1513)	RNA (15nt) kit (p/n DNF-471)	HS RNA (15nt) kit (p/n DNF-472)
Concentration range	5 – 500 ng/µL	50 – 5,000 pg/µl	5 – 500 ng/µl	50 – 5,000 pg/µl
RNA quality metric	RIN, DV_{200}	RIN, DV_{200}	RQN, DV_{200}	RQN, DV_{200}

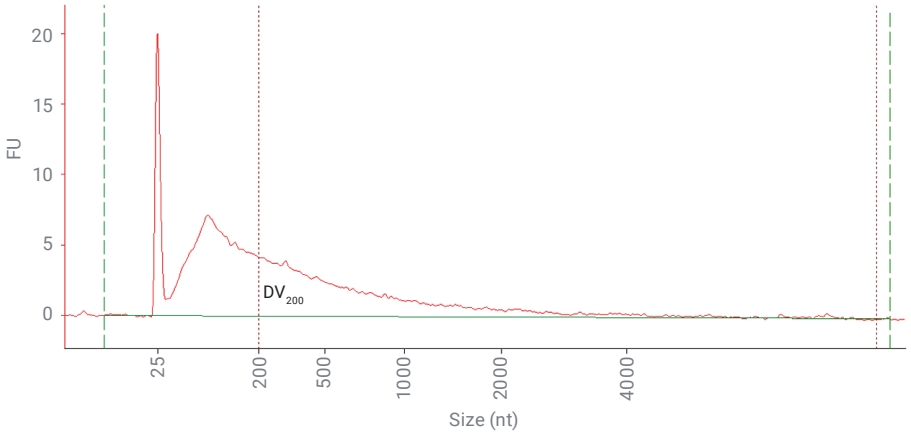
Results

Standard sensitivity kit analysis

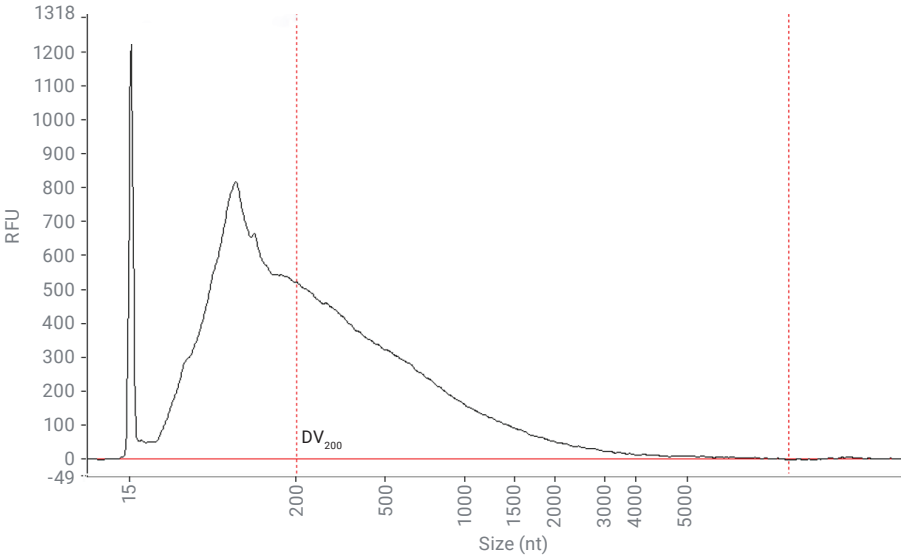
The FFPE RNA samples were analyzed using the standard sensitivity RNA 6000 Nano kit on the Bioanalyzer and the RNA (15nt) kit on the Fragment Analyzer. The resulting electropherograms from each system were compared, with cow liver shown as a representative example in Figures 1A and 1B. The electropherograms from both systems exhibit similar patterns. To the right of the lower marker peak on either system, a peak below 200 nt is visible, indicating a high presence of small RNA fragments. A smear extends from this peak across the rest of the electropherogram, slightly above the baseline. This suggests that the RNA sample also includes fragments of various lengths above 200 nt, spanning the sizing ranges of the kits. The DV_{200} provides a numerical representation of the sample amount above 200 nt, a necessary measurement for sequencing FFPE RNA.

The Bioanalyzer reported an average DV_{200} for the cow liver sample at 46.0%, while the Fragment Analyzer gave a similar average of 45.5%. These values illustrate the comparable performance of the Bioanalyzer and Fragment Analyzer systems in determining the sample quality. Similar comparisons were observed for the other samples measured between systems (Figure 1C). Furthermore, when measuring replicates, each system displayed excellent precision, with all sample types displaying a 5.7 percent coefficient of variation (%CV) or less. The comparable DV_{200} scores provided by each system indicate that they both deliver reliable data that can be used interchangeably.

A) Bioanalyzer



B) Fragment Analyzer



C

	Standard Sensitivity Kit Analysis			
	2100 Bioanalyzer System		Fragment Analyzer Systems	
	Average DV_{200}	Precision (%CV)	Average DV_{200}	Precision (%CV)
Cow liver	45.3	1.3%	45.5	0.4%
Cow cerebellum	44.3	5.7%	45.6	2.0%
Mouse liver	58.0	0.0%	64.1	1.3%
Pig spleen	53.0	1.9%	58.9	0.5%

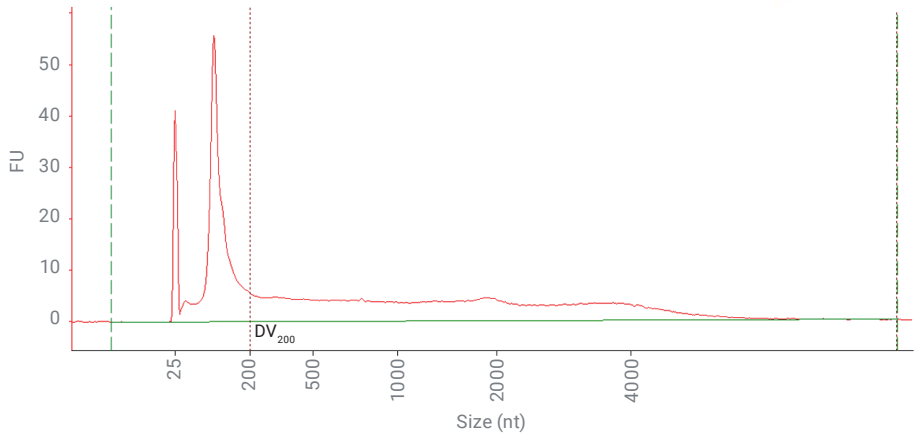
Figure 1. Representative electropherograms of cow liver FFPE RNA analyzed on the (A) Agilent 2100 Bioanalyzer system using the Agilent RNA 6000 Nano kit and (B) Agilent Fragment Analyzer systems using the Agilent RNA (15nt) kit. Red lines in both image represent the DV_{200} range. (C) Average DV_{200} scores and measurement precision for both systems across all sample types. N = 3.

High sensitivity kit analysis

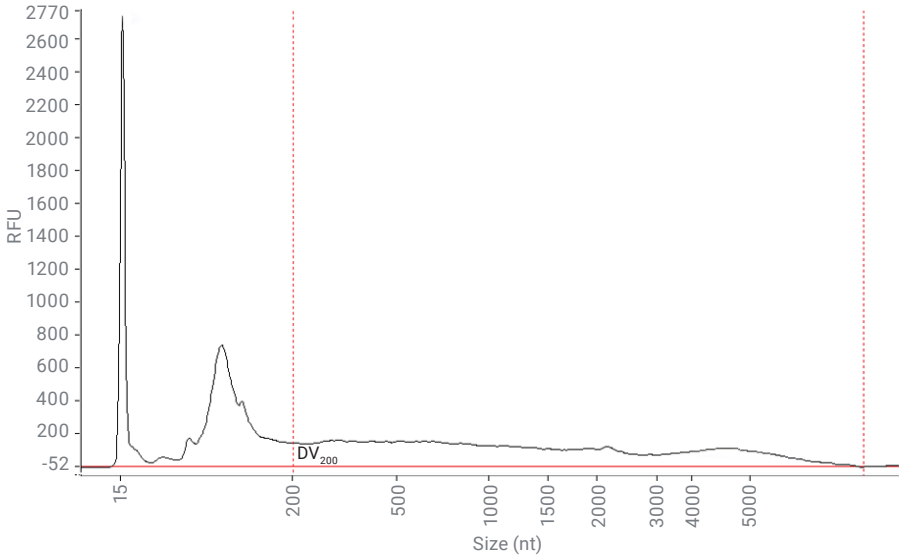
To further demonstrate the comparability of the systems, the FFPE RNA samples were also analyzed using the respective high sensitivity kits: the RNA 6000 Pico kit for the Bioanalyzer and the HS RNA kit (15nt) for the Fragment Analyzer. The resulting electropherograms from each system were compared, with mouse liver shown as a representative example in Figures 2A and 2B. Both electropherograms display a similar overall pattern for the distribution of RNA fragments. Starting from the marker peak, there is an immediate increase in the amount of smaller RNA fragments, which then decrease in abundance as the size of RNA fragments become longer.

The DV_{200} of the mouse liver sample averaged 56.3% on the Bioanalyzer, while the Fragment Analyzer reported a similar average of 57.7%. This shows the similarity in the assessment of DV_{200} metrics by both systems. Similar results were observed for the other sample comparisons between systems. Both systems demonstrated remarkable precision, with no more than 5.7 %CV for each sample type (Figure 2C). These results demonstrate that the high sensitivity kits provide comparable data between systems.

A) Bioanalyzer



B) Fragment Analyzer



C

	High Sensitivity Kit Analysis			
	2100 Bioanalyzer System		Fragment Analyzer Systems	
	Average DV_{200}	Precision (%CV)	Average DV_{200}	Precision (%CV)
Cow liver	46.3	3.3%	38.1	2.8%
Cow cerebellum	47.3	1.2%	35.1	2.5%
Mouse liver	56.3	5.7%	57.7	2.6%
Pig spleen	58.0	0.0%	57.2	1.2%

Figure 2. Representative electropherograms of mouse liver FFPE RNA analyzed on the (A) Agilent 2100 Bioanalyzer system using the Agilent RNA 6000 Pico kit and on the (B) Agilent Fragment Analyzer systems using the Agilent HS RNA (15nt) kit. Red lines in both image represent the DV_{200} range. (C) Average DV_{200} scores and measurement precision for both systems across all sample types. N = 3.

Conclusion

This technical overview compared the analysis of multiple FFPE RNA samples using the Agilent 2100 Bioanalyzer and Agilent Fragment Analyzer systems. The results demonstrate that each system can effectively evaluate the quality of FFPE RNA samples. The similarity in DV_{200} measurements provides users with confidence to measure FFPE RNA on either the Bioanalyzer or the Fragment Analyzer systems and to incorporate the DV_{200} quality metric into their workflows.

References

1. RNA Quality Assessment with the Agilent Automated Electrophoresis Systems. *Agilent Technologies technical overview*, publication number 5994-7327EN, **2024**.
2. Evaluating RNA Quality from FFPE Samples. *Illumina Technical Note*, publication number 470-2014 001, **2016**.
3. Simplified DV_{200} Evaluation with the Agilent 2100 Bioanalyzer System. *Agilent Technologies technical overview*, publication number 5991-8287EN, **2017**.

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