Technical Overview

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FFPE RNA Quality Assessment with the Agilent Bioanalyzer and TapeStation 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, allowing researchers to continue to go back to a sample numerous times without having to commit to another sample collection. However, the fixation process causes 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 when using the RNA for sequencing. By employing a quality control system that can assess the RNA integrity after an FFPE extraction, the integrity of the sample can be determined.

The Agilent automated electrophoresis portfolio has emerged as a powerful tool for the assessment of RNA quality. Specifically, the Agilent 2100 Bioanalyzer and Agilent TapeStation systems both assess RNA and provide users with an equivalent quality metric. The quality metrics are the RNA Integrity Number (RIN) for the Bioanalyzer and the RNA Integrity Number equivalent (RIN^e) for the TapeStation. Both the RIN and the RIN^e are metrics allowing the user to easily assess the quality of a sample before RNA sequencing. However, RNA from FFPE tissue samples are inherently of low quality, based on the RIN and RIN^e, but are often still able to be sequenced. This characteristic has led to the development of an RNA sequencing quality metric that can further assess these degraded RNA samples. To address this unique QC challenge, the DV_{200} was developed to provide researchers with a tool to reliably classify degraded RNA by size, and to effectively parse samples suitable for next-generation sequencing (NGS) from unsuitable samples, conserving time and costs[1,2]. The DV_{200} quality metric represents the percentage of RNA fragments above 200 nucleotides (nt) and shows a high correlation to the precapture library yield of FFPE samples[3]. Furthermore, the DV_{200} score provides researchers the knowledge to modify their workflows to still enable the use of FFPE samples in downstream applications.

This technical overview highlights the capabilities of both the Bioanalyzer and TapeStation 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 200 ng/µl and 3 ng/µl concentrations to fit within the specifications of the RNA kits for assessment on the Agilent 2100 Bioanalyzer system (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. Each sample was prepared in triplicate according to the Bioanalyzer quick guide, omitting the optional heat denaturing step. Data analysis on the Agilent 2100 Bioanalyzer Expert software was completed using the DV_{200} assay configuration file[2].

The same samples assessed on the Bioanalyzer were analyzed on the 4200 TapeStation using the Agilent RNA ScreenTape (p/n 5067-5576), Agilent RNA ScreenTape sample buffer (p/n 5067-5577), and Agilent RNA ScreenTape ladder (p/n 5067-5578), along with the Agilent High Sensitivity RNA ScreenTape (p/n 5067-5579), Agilent High Sensitivity RNA ScreenTape sample buffer (p/n 5067-5580), and Agilent High Sensitivity RNA ScreenTape ladder (p/n 5067-5581). The samples were run in triplicate with the assay- specific ladder as outlined in the TapeStation quick guide. Analysis using the TapeStation as previously described[1] requires the region mode to be selected, a region of 200 to 10,000 nucleotides to be set, and then using the "% of Total" column under the Region Table tab to obtain the DV_{200} metric.

Table 1. Concentration ranges of each assay used in this technical overview and the provided RNA quality metrics.

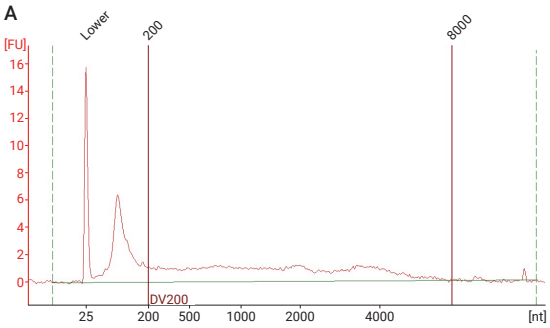
	2100 Bioanalyzer		4200 TapeStation	
Kit/Assay	RNA 6000 Nano	RNA 6000 Pico	RNA ScreenTape	High Sensitivity RNA ScreenTape
Qualitative Range	5 to 500 ng/µL	50 to 5,000 pg/µL	25 to 500 ng/µL	1,000 to 25,000 pg/µL
RNA Quality Metric	RIN, DV_{200}	RIN, DV_{200}	RIN^e, DV_{200}	RIN^e, DV_{200}

Results

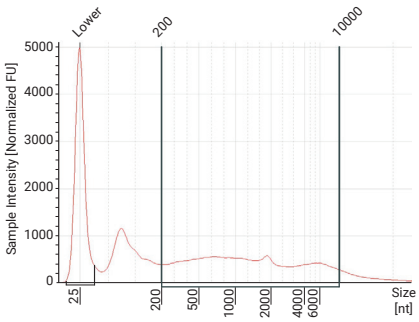
The FFPE RNA samples were analyzed with both the RNA 6000 Nano kit and TapeStation RNA ScreenTape assay. The resulting electropherograms from each system were compared, with mouse liver shown as a representative example in Figures 1A and 1B. The electropherograms from both systems exhibit comparable patterns, suggesting a level of similarity in the analysis of the same FFPE RNA sample between systems. To the right of the lower marker peak on either system is a prominent peak below 200 nt, representative of abundant small RNA fragments. A smear extends from this peak through the rest of the electropherogram, slightly above the baseline, suggesting that the RNA sample also contains fragments of various lengths above 200 nt and spanning throughout the sizing ranges of the kits. Thus, it is useful to use the DV_{200} score to get a numerical representation of the amount of sample above 200 nt.

The Bioanalyzer reported an average DV_{200} for the mouse liver at 58.0%, while the TapeStation gave a similar average of 62.3%. These values illustrate the comparable performance of the Bioanalyzer and TapeStation in determining the sample quality. Similar measurements were observed for the other samples, demonstrating excellent comparability between the systems (Figure 1C). Further, when measuring replicates, each system displayed excellent precision with calculated %CVs across all sample types being 5.7% or less (Figure 1D). Collectively, this data indicates that each system can measure the quality of FFPE RNA samples comparably, which gives confidence in the interchangeability between the DV_{200} measurements of each system when using these assays.

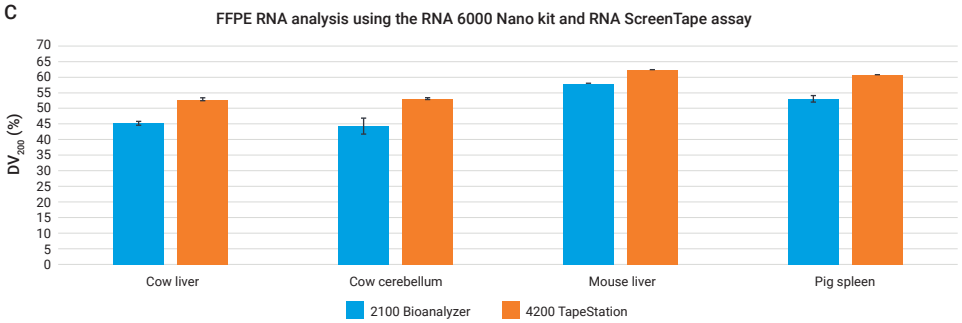
A



B



C



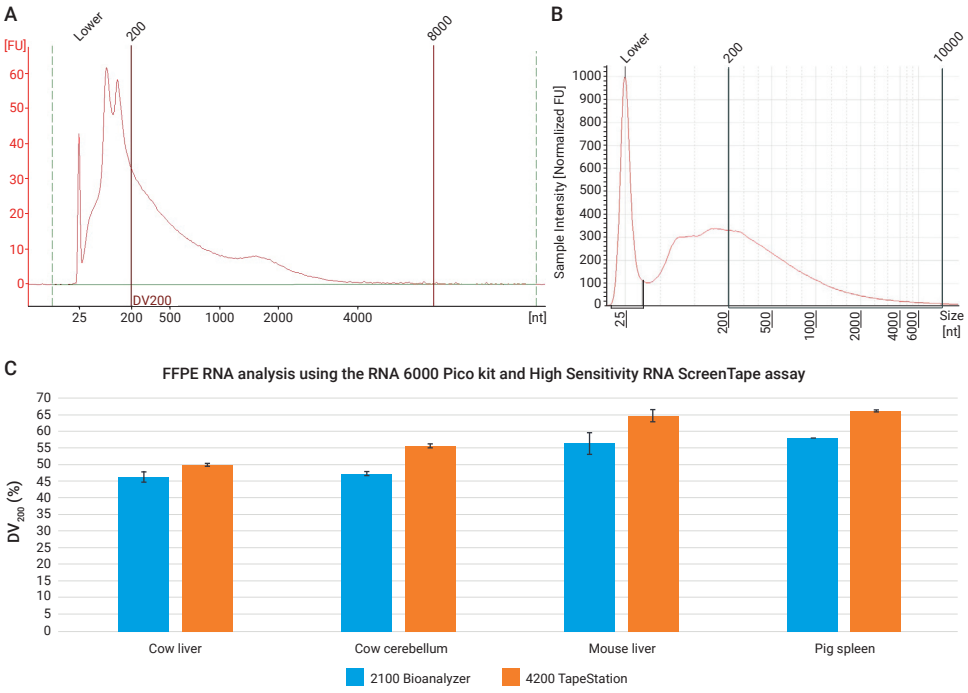
D

	%CV	
	2100 Bioanalyzer	4200 TapeStation
Cow liver	1.3%	0.8%
Cow cerebellum	5.7%	0.4%
Mouse liver	0.0%	0.3%
Pig spleen	1.9%	0.1%

Figure 1. Representative electropherograms of mouse liver FFPE RNA analyzed on both the A) Agilent 2100 Bioanalyzer system using the Agilent RNA 6000 Nano kit and the B) Agilent 4200 TapeStation system using the Agilent RNA ScreenTape assay. C) The average DV_{200} percentage for all samples is shown and D) shows the calculated %CV values. Error bars represent standard deviation. N = 3.

The FFPE RNA samples were analyzed using both the Agilent Bioanalyzer RNA 6000 Pico kit and Agilent TapeStation High Sensitivity RNA ScreenTape assay. The resulting electropherograms from each system were compared, with cow liver shown as a representative example in Figures 2A and 2B. Both electropherograms display a similar overall pattern for the distribution of RNA fragments. There is an immediate increase in the amount of smaller RNA fragments that then decreases in abundance as the size of RNA fragments become longer.

The DV_{200} of the cow liver was measured at 46.3% by the Bioanalyzer, while the measurement on the TapeStation was slightly higher at 50.0%. This indicates a similar assessment of DV_{200} metrics by both systems. Some samples showed a marginally greater difference, with the most pronounced being from 56.3 to 64.7%. Both systems demonstrated remarkable precision, with the calculated %CVs for all sample types being no more than 5.7%.



D

	%CV	
	2100 Bioanalyzer	4200 TapeStation
Cow liver	3.3%	0.8%
Cow cerebellum	1.2%	1.0%
Mouse liver	5.7%	2.8%
Pig spleen	0.0%	0.4%

Figure 2. Representative electropherograms of cow liver FFPE RNA run on both the A) Agilent 2100 Bioanalyzer and B) Agilent 4200 TapeStation systems using their respective high sensitivity kits. C) The average DV_{200} percentage for all samples is shwon and D) shows the calculated %CV values.

Comparison within systems

The two different kits (RNA 6000 Nano and RNA 6000 Pico) for the Bioanalyzer, along with the two assays for the TapeStation (RNA ScreenTape and High Sensitivity RNA ScreenTape) were compared for consistency within their respective systems. For this comparison all samples were assessed with cow cerebellum being used as an example.

The Bioanalyzer results showed that the RNA 6000 Nano kit measured a DV_{200} percentage of 44.3%, while the RNA 6000 Pico kit measured 47.3%. On the other hand, the TapeStation yielded a DV_{200} percentage of 53.2% with the RNA ScreenTape assay, and 55.6% with the High Sensitivity RNA ScreenTape assay.

Both instruments demonstrated consistent performance across different concentration levels for all samples tested (Figure 3). This data emphasizes the reliability of each system's results regardless of the assay used.

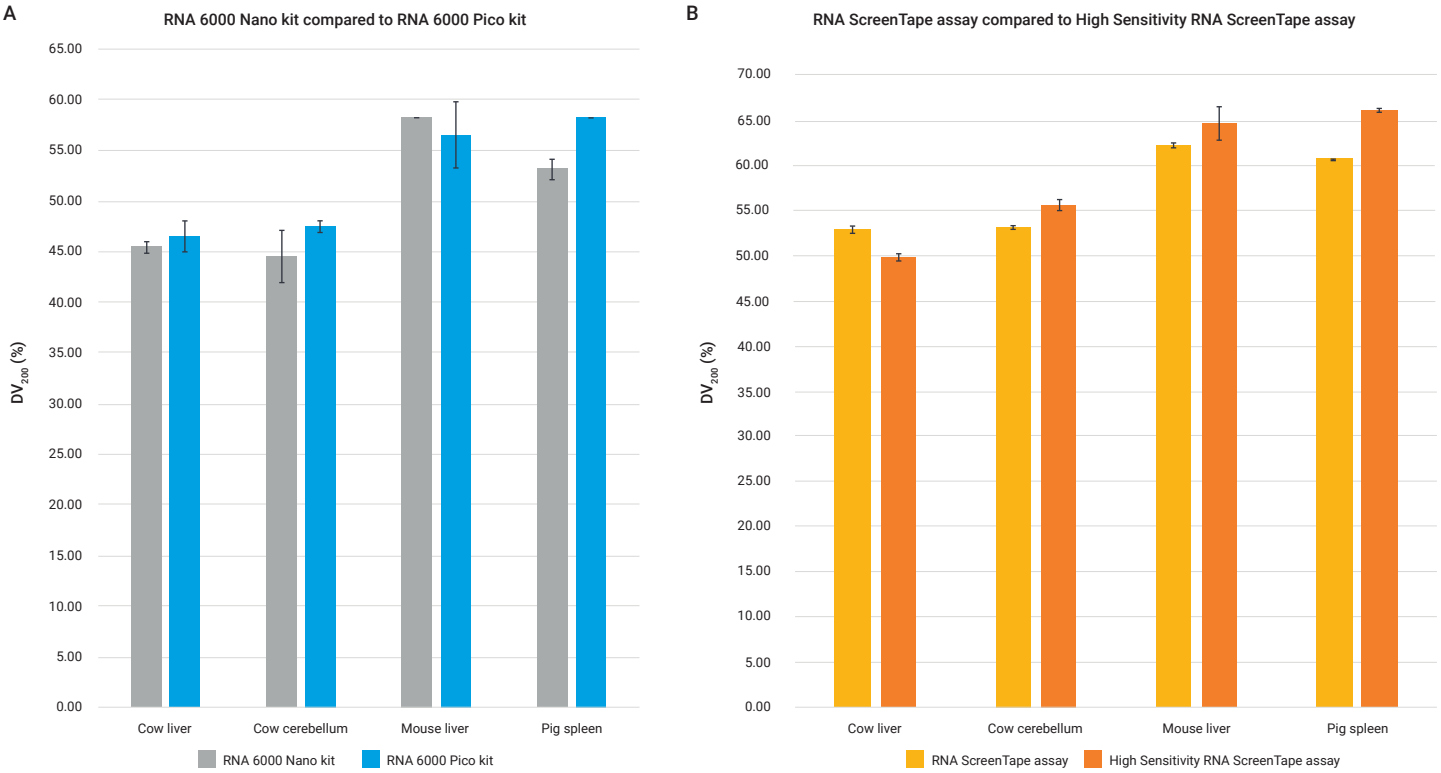


Figure 3. Each plot displays the average DV_{200} percent. The first plot, A) shows a comparison between the Agilent RNA 6000 Nano and Agilent RNA 6000 Pico kits for the Agilent Bioanalyzer system. The second plot, B) shows a comparison between the Agilent RNA ScreenTape and Agilent High Sensitivity RNA ScreenTape assays for the Agilent TapeStation system.

Conclusion

This technical overview compared the analysis of multiple FFPE RNA samples on the Agilent 2100 Bioanalyzer and Agilent TapeStation systems. The results demonstrate that each system is capable of similarly assessing the quality of FFPE RNA samples, as reflected in the results of the DV_{200} measurements. Additionally, the data points out that a consistent measurement can be obtained regardless of the assay or kit being used within each system. This comparison allows users the confidence to measure FFPE RNA on the Bioanalyzer and TapeStation systems and to incorporate the DV_{200} quality metric into their workflows.

References

1) DV_{200} Evaluation with RNA ScreenTape Assays. *Agilent Technologies technical overview*, publication number 5991-8355EN, **2017**.

2) Simplified DV_{200} Evaluation with the Agilent 2100 Bioanalyzer System. *Agilent Technologies technical overview*, publication number 5991-8287EN, **2017**.

3) Evaluating RNA Quality from FFPE Samples. Illumina, Technical Note, publication number 470-2014 001, **2016**.

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