Assessing RNA integrity in FFPE samples with the DV_{200} quality metric for automated electrophoresis systems

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Introduction

Formalin-fixation and paraffin-embedding (FFPE) is essential in pathology for preserving tissue specimens long-term while maintaining cellular structure and allowing for repeated analyses. However, FFPE complicates RNA extraction and analysis due to RNA degradation during fixation, posing challenges for high-quality requiring downstream analyses like next-generation sequencing (NGS). Implementing a quality control system to evaluate RNA integrity post-FFPE extraction is crucial for determining the viability of a sample. Quality control systems, like the Agilent Bioanalyzer and TapeStation, assess RNA integrity post-FFPE extraction. The DV_{200} metric helps classify degraded RNA, ensuring only high-quality samples are used for next-generation sequencing (NGS), thus enhancing the reliability of gene expression studies.

DV_{200} analysis with Agilent TapeStation systems

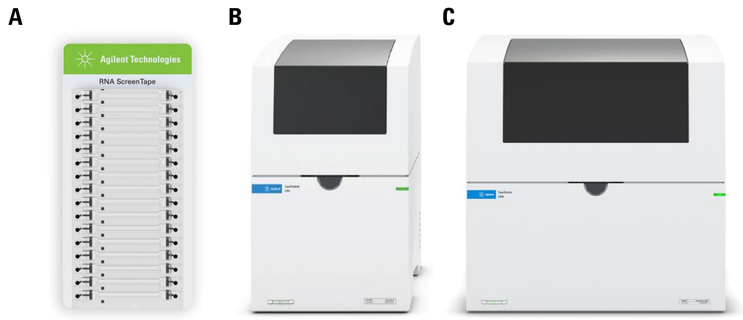

Figure 1. Components of the Agilent TapeStation system. A) RNA ScreenTape device with 16 individual gel lanes. B) 4150 TapeStation instrument for automated electrophoresis of 1-16 samples per run. C) 4200 TapeStation instrument for automated high-throughput electrophoresis of up to 96 samples per run.

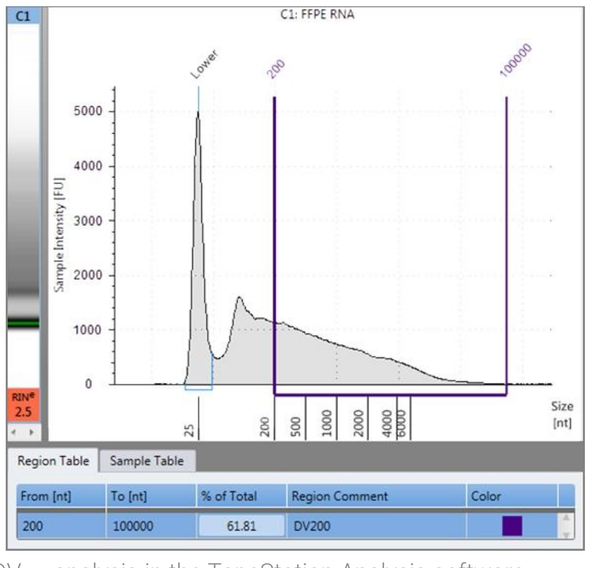

Figure 2. DV_{200} analysis in the TapeStation Analysis software

The DV_{200} represents the percentage of RNA fragments larger than 200 nucleotides relative to the total RNA fragments in a sample. The TapeStation Analysis software can be used to calculate the DV_{200} by defining a region for fragments larger than 200 nt. The value is provided in the region table in the % of total column for each sample.

Methods

Four FFPE RNA samples (cow liver, cow cerebellum, mouse liver, and pig spleen) were analyzed in triplicates on the 4200 TapeStation using the RNA ScreenTape, sample buffer, and ladder and the High Sensitivity RNA ScreenTape, sample buffer, and ladder and the Agilent 2100 Bioanalyzer systems using the RNA 6000 Nano kit and the RNA 6000 Pico kit. Data analysis was performed using the DV_{200} assay configuration file on the Bioanalyzer and region mode (200-10,000 nucleotides) on the TapeStation to obtain the DV_{200} metric.

Conclusions

- The DV_{200} quality metric provides researchers the knowledge to modify their workflows to enable optimal use of FFPE RNA samples in NGS workflows.
- Both the Agilent TapeStation and Agilent 2100 Bioanalyzer systems can similarly assess the quality of FFPE RNA samples.
- Consistent measurement can be obtained regardless of the used assay, ensuring reliable sample QC across the whole concentration range.

Results

RNA Analysis

The obtained FFPE RNA samples were analyzed with both the Bioanalyzer RNA 6000 Nano kit and the TapeStation RNA ScreenTape assay.

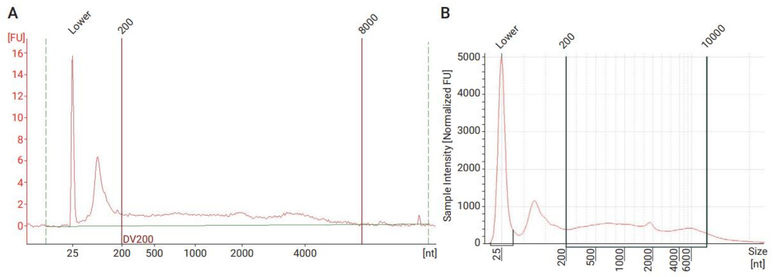

Figure 3. Electropherograms of the mouse liver FFPE RNA sample analyzed with the RNA 6000 Nano kit on the 2100 Bioanalyzer system (A) and the RNA ScreenTape assay on the 4200 TapeStation system (B).

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. The Bioanalyzer reported an average DV_{200} for the mouse liver at 58.0%, while the TapeStation gave a similar average of 62.3%.

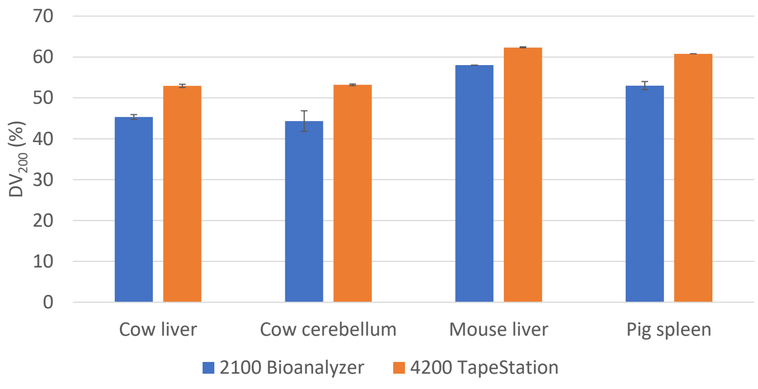

Figure 4. DV_{200} values for the FFPE RNA samples analyzed with the RNA 6000 Nano kit on the 2100 Bioanalyzer system and the RNA ScreenTape assay on the 4200 TapeStation system, demonstrating the comparable performance of the Bioanalyzer and TapeStation in determining the sample quality.

High Sensitivity RNA Analysis

The FFPE RNA samples were further analyzed using both the Bioanalyzer RNA 6000 Pico kit and TapeStation High Sensitivity RNA ScreenTape assay.

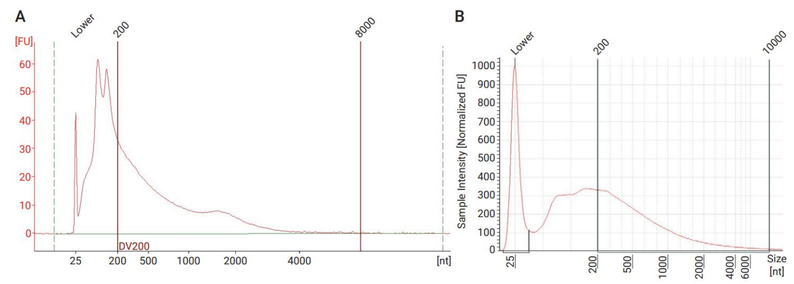

Figure 5. Electropherograms of the cow liver FFPE RNA sample analyzed with the RNA 6000 Pico kit on the 2100 Bioanalyzer system (A) and the High Sensitivity RNA ScreenTape assay on the 4200 TapeStation system (B).

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 sample was measured at 46.3% by the Bioanalyzer, while the measurement on the TapeStation was slightly higher at 50.0%.

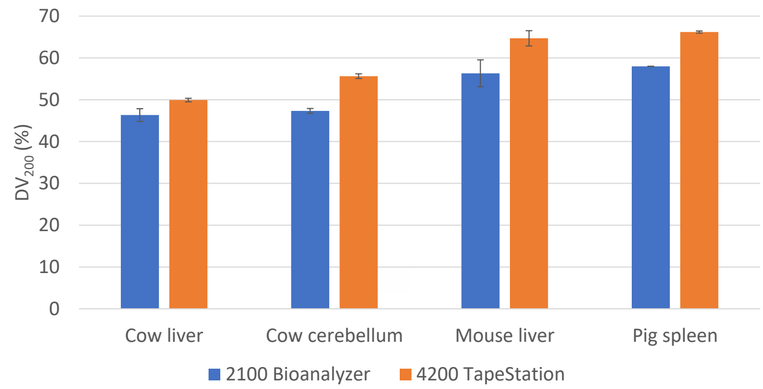

Figure 6. DV_{200} values for the FFPE samples, analyzed with the RNA 6000 Pico kit on the 2100 Bioanalyzer system and the High Sensitivity RNA ScreenTape assay on the 4200 TapeStation system, demonstrating the comparable performance of the Bioanalyzer and TapeStation in determining the sample quality.

Comparison of RNA and High Sensitivity RNA ScreenTape assay

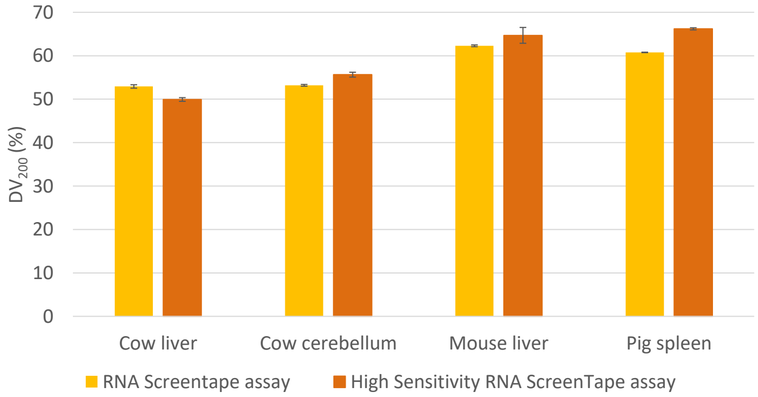

Figure 7. DV_{200} values compared between the RNA ScreenTape and High Sensitivity RNA ScreenTape assays for TapeStation systems.

The two different assays for the TapeStation systems (RNA ScreenTape and High Sensitivity RNA ScreenTape assay) were compared for consistency (Figure 7). For this comparison, all samples were assessed in appropriate concentrations for the respective assays. Both assays demonstrated consistent performance across different concentration levels for all samples tested. This data emphasizes the reliability of the TapeStation system for DV_{200} analysis regardless of the assay used.