Technical Overview

DV_{200} Evaluation with RNA ScreenTape Assays

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Abstract

The success of library preparation for RNA sequencing workflows depends highly on the quality of RNA starting material. The RNA integrity number equivalent (RIN^e) obtained with Agilent RNA ScreenTape assays is a reliable and reproducible metric to evaluate sample integrity for standard RNA sequencing. Library preparation of RNA samples originating from formalin-fixed paraffin-embedded (FFPE) tissue is challenging, due to RNA degradation and restricted sample volume, thus tailored protocols are recommended for FFPE RNA library preparation. The fragment size distribution of FFPE RNA samples has a major influence on the library yield, and can be represented as the percentage of RNA fragments above 200 nucleotides by the DV_{200} quality metric[1]. The Agilent RNA ScreenTape and Agilent High Sensitivity RNA ScreenTape assays are convenient for highly reproducible DV_{200} evaluation of degraded RNA samples extracted from FFPE tissue. The Agilent TapeStation Analysis software enables simple assessment and processing of DV_{200} data by region analysis, which can be automated for repeated DV_{200} analysis.

Technical Details

The Agilent 4200 TapeStation system with Agilent TapeStation Analysis software version A.02.02 was used. RNA ScreenTape (p/n 5067-5576) with reagents (p/n 5067-5577 and 5067-5578) and Agilent High Sensitivity RNA ScreenTape (p/n 5067-5579) with reagents (p/n 5067-5580 and 5067-5581) were obtained from Agilent Technologies. Unless stated, the manufacturer's protocols and guidelines were followed. All samples were analyzed with a ladder run on each ScreenTape. RNA samples extracted from FFPE tissue were kindly provided by the tissue bank of the National Center for Tumor Diseases (NCT, Heidelberg, Germany) in accordance with the regulations of the tissue bank, and the approval of the ethics committee of Heidelberg University.

Data Analysis

The DV_{200} represents the percentage of RNA fragments larger than 200 nucleotides with respect to all RNA fragments. TapeStation Analysis software can be used to calculate the DV_{200} by defining a region for fragments larger than 200 nt. The software provides several ways for region setup, suitable for low to high sample throughput. Consult the Agilent Information Center (AIC) for further information and how-to videos about region analysis.

Region Setup for Individual Samples

To define a DV_{200} region for individual samples, click Region, then right click in the electropherogram to add a region. The region borders can be individually adjusted by shifting to the left or to the right.

Region Setup for Data Files

To define a DV_{200} region as default for all samples in an individual data file, click Region, then click Region Settings, and enter the lower and upper limits of the region in the dialog box (Figure 1). To identify the region in the electropherogram, type DV_{200} as region comment. To apply the region to all samples in the active file, select This File.

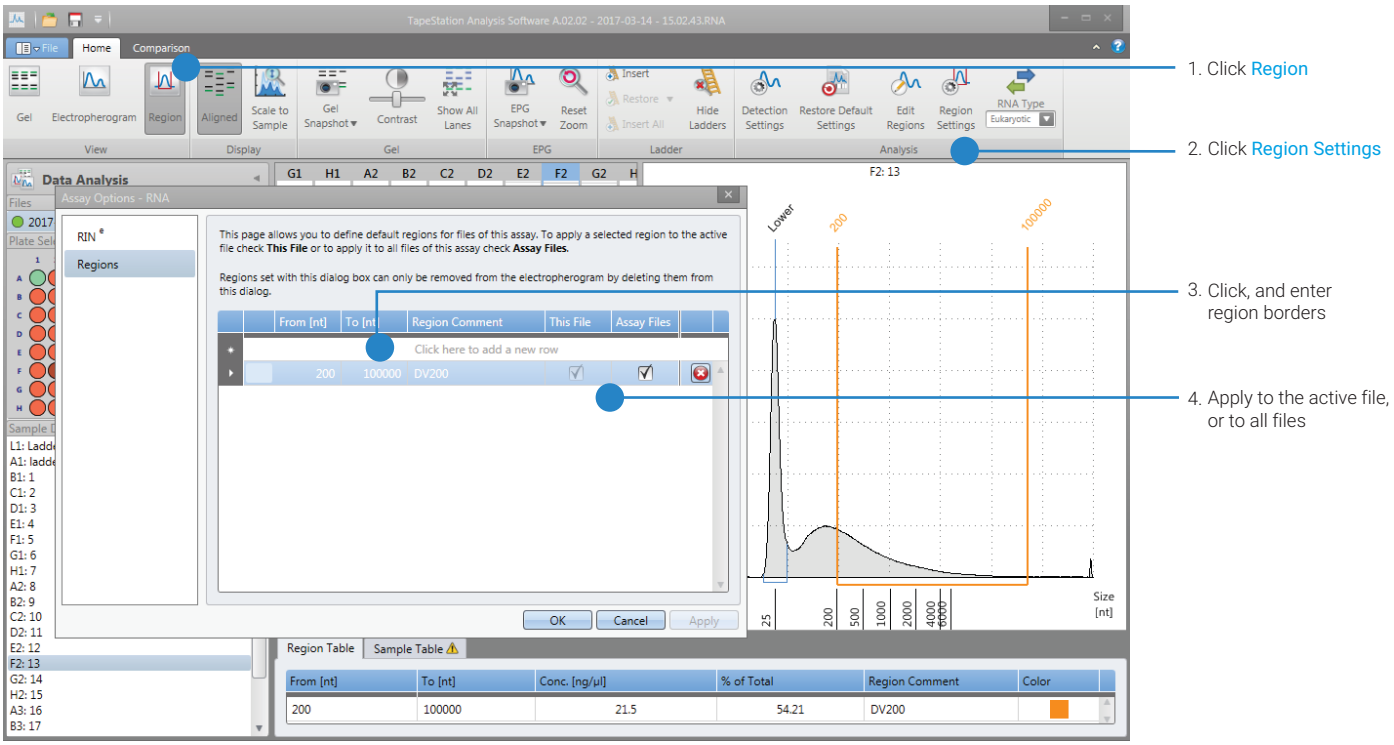

Figure 1. Setting up default regions to calculate DV_{200} with Agilent TapeStation Analysis software.

Automated Region Setup

To define a DV_{200} region as default for reoccurring FFPE RNA sample analysis, click Region, then click Region Settings, and enter the lower and upper limits of the region in the dialog box (Figure 1). To identify the region in the electropherogram, type DV_{200} as region comment. To apply the DV_{200} region to all files of the same assay as the active file, select Assay Files.

When DV_{200} regions are defined, the value is provided in the region table in the % of Total column for each sample (Figure 2). All region data can be exported and reported when selecting the region table. Note that the concentration shown in the region table of the TapeStation Analysis software, as well as in exported files and reports, refers to the concentration of the defined region; the total sample concentration is displayed in the sample table.

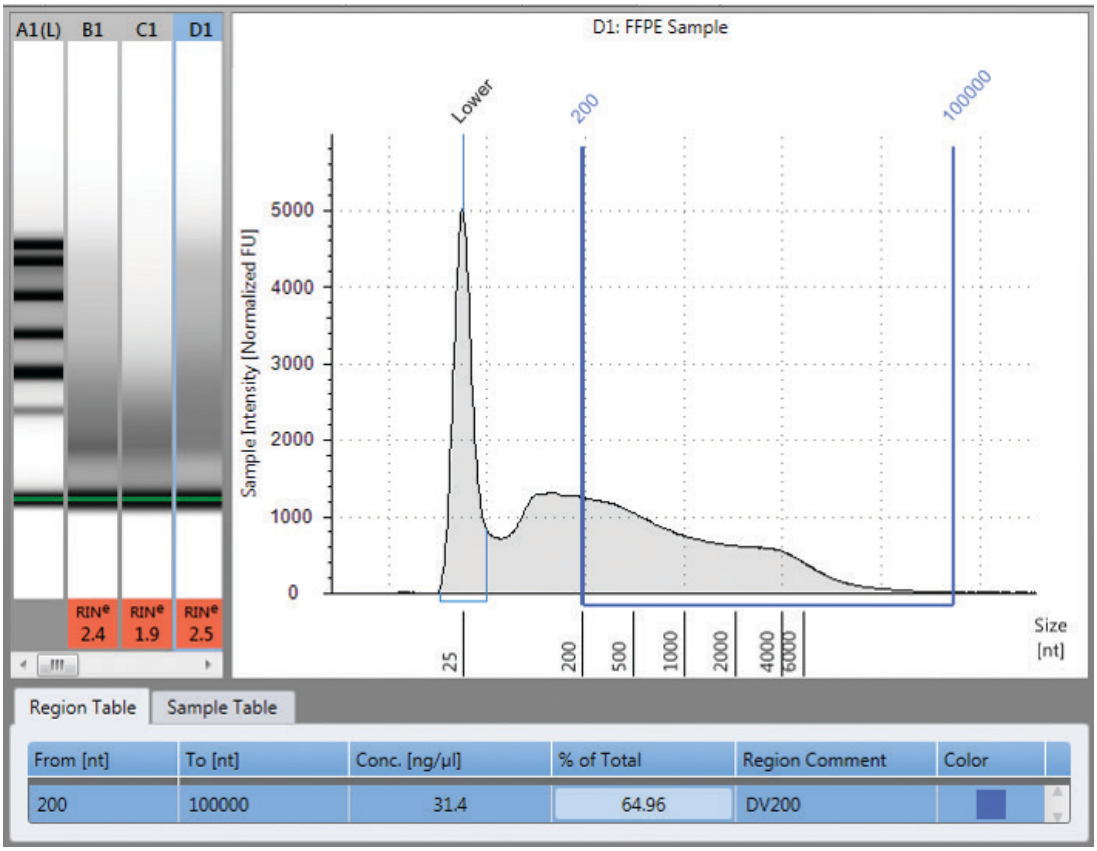

Figure 2. FFPE RNA sample analysis showing DV_{200} as percentage of total in the region table.

Results and Discussion

Reproducibility

A set of three FFPE RNA samples with five individual replicates was used to verify the reproducibility of the DV_{200} with the RNA and the High Sensitivity RNA ScreenTape assay. Both assays showed high reproducibility with a CV of less than 5 % (Figure 3).

Concentration Dependence

A dilution series for three individual FFPE RNA samples was analyzed with the RNA and High Sensitivity RNA ScreenTape assays to determine the consistency of the DV_{200} analysis (Table 1). Both the RNA ScreenTape and High Sensitivity RNA ScreenTape assay yielded comparably high DV_{200} precision of less than 5 % CV.

The dilution series showed that DV_{200} evaluation is most precise when measuring above the lower limit of the specified assay concentration range, as shown in Figure 4.

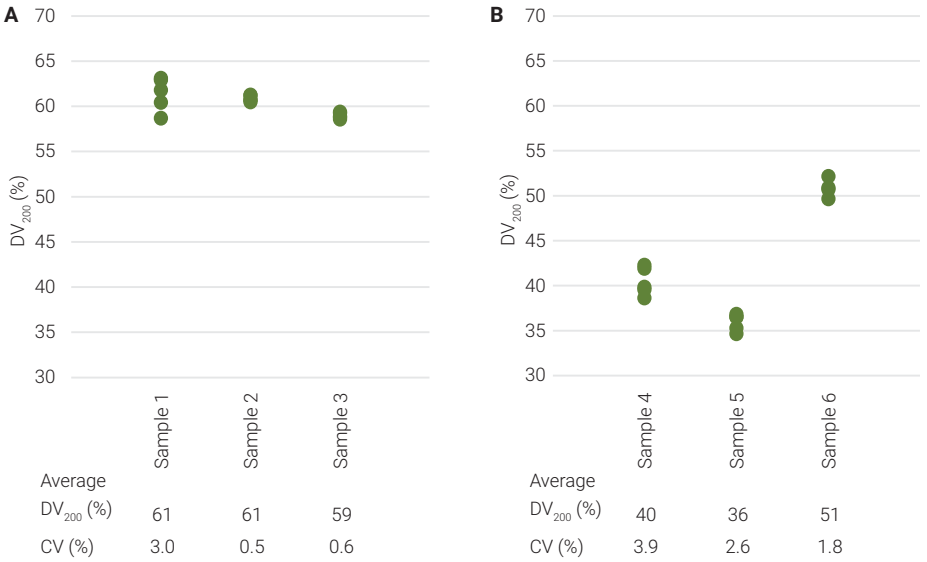

Figure 3. Reproducibility of DV_{200} analysis for three individual samples with (A) the Agilent RNA ScreenTape assay and (B) the Agilent High Sensitivity RNA ScreenTape assay.

Table 1. Consistency of DV_{200} analysis with the Agilent RNA and the Agilent High Sensitivity RNA ScreenTape assay measured in dilution series for three samples per assay.

	Agilent RNA ScreenTape assay			Agilent High Sensitivity RNA ScreenTape assay		
	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12
Average DV_{200} in %	46	52	42	57	66	64
Standard deviation	1.1	1.6	1.7	1.6	0.6	0.9
% CV	2.4	3.1	4.1	2.8	1.0	1.4

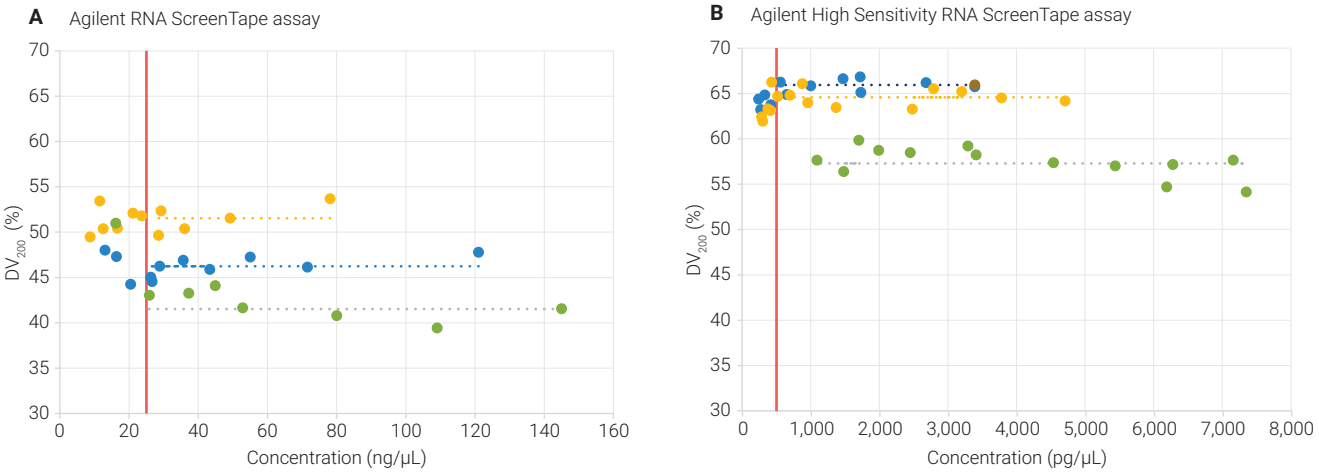

Figure 4. DV_{200} evaluation of a dilution series of three individual FFPE samples. The red line indicates the lower limit of the assay's concentration range. Horizontal lines display the average DV_{200} of each individual sample. Only data within the respective assay concentration range were used for the evaluation.

Severely degraded FFPE samples may migrate closely to the lower marker leading to insufficient separation of the lower marker, especially at sample concentrations close to the upper limit of the specified quantitative range. In this case, diluted samples yielded more accurate results for DV_{200} (see Figure 5).

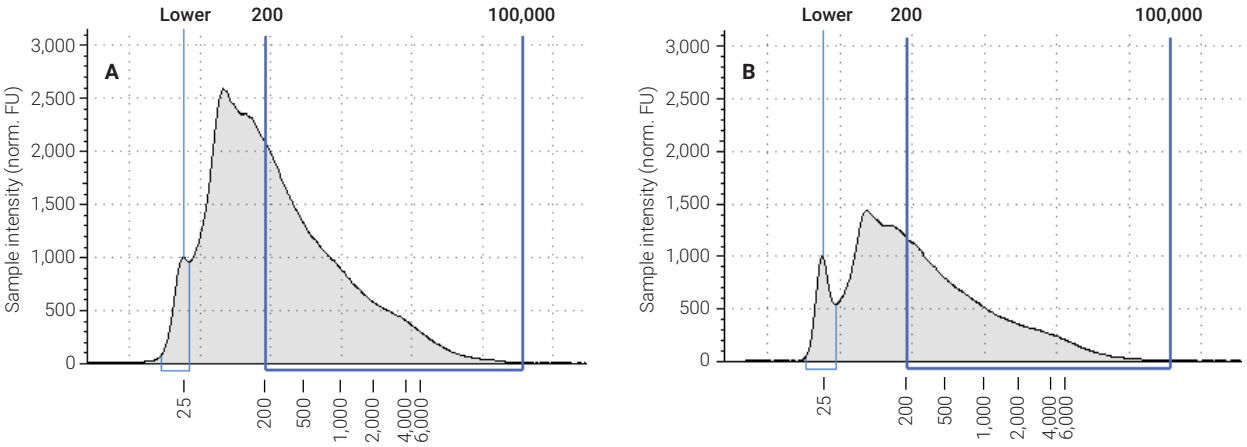

Figure 5. Highly degraded FFPE RNA sample merging with the lower marker (A) and with improved marker separation after 1:2 dilution (B).

Assay Comparison

Twenty FFPE RNA samples from various human tissues were analyzed with the RNA ScreenTape assay, and after 1:20 dilution with the High Sensitivity RNA ScreenTape assay. The DV_{200} obtained with the RNA assay ranged from 40 to 74 %, and the corresponding samples analyzed with the High Sensitivity RNA assay resulted in 101 ± 5 % of these values. To verify the comparability of DV_{200} measured with the RNA and High Sensitivity RNA ScreenTape assays, both values were plotted against each other, as shown in Figure 6.

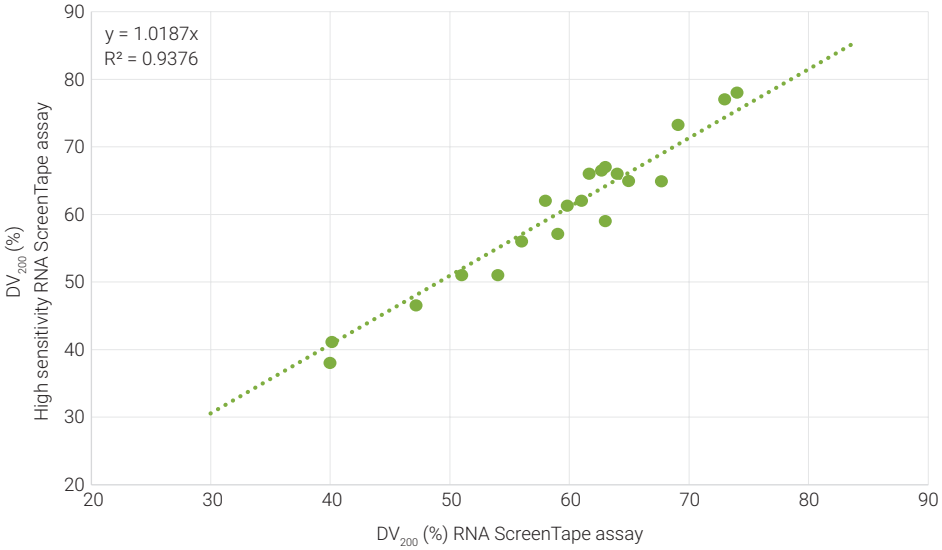

Figure 6. Comparison of DV_{200} data from FFPE RNA samples obtained with the Agilent RNA ScreenTape and the Agilent High Sensitivity RNA ScreenTape assays with a linear regression line forced through the origin.

Conclusion

The Agilent RNA and Agilent High Sensitivity RNA ScreenTape assays together with the Agilent 4200 TapeStation enable fast and easy quality analysis of FFPE RNA samples. The Agilent TapeStation Analysis software displays the DV_{200} after region setup as a percentage of total. The RINe algorithm remains unaffected by the DV_{200} calculation. The DV_{200} evaluation with the RNA and High Sensitivity RNA ScreenTape assays is highly reproducible, and both assays yield highly comparable results. For best performance, it is recommended to run a ladder together with the samples. Within the specified assay ranges, the DV_{200} is consistent in dilution series. Highly degraded samples with insufficient marker separation should be diluted for most accurate results.

Reference

1. Evaluating RNA Quality from FFPE Samples. *Illumina Technical Note*, publication number 470-2014-001. https://www.illumina.com/documents/products/technotes/technote- truseq-rna-access.pdf.

www.agilent.com/chem

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