Application Note

Genomics

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Reliable DNA Integrity Assessment Across Low-Concentration and FFPE-Derived Samples

Using the Agilent High Sensitivity Genomic DNA ScreenTape assay for quality control of genomic DNA

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Abstract

Accurate quality control (QC) of low-concentration genomic DNA (gDNA) is increasingly important in modern molecular biology workflows, particularly in applications such as next-generation sequencing (NGS). Samples derived from limited material or challenging sources such as formalin-fixed paraffin-embedded (FFPE) tissue often present unique analytical challenges due to degradation and chemical modification. The Agilent High Sensitivity Genomic DNA (HS gDNA) ScreenTape assay offers a solution for reliable assessment of gDNA quality for these low concentrations and challenging samples. The assay enables sensitive and consistent analysis across a broad range of sample types, delivering DNA integrity number (DIN) values that support informed decisions in downstream workflows. With minimal sample input and automated data analysis, the TapeStation systems with the HS gDNA assay offer a streamlined and reproducible approach to gDNA QC, even for degraded or limited-abundance samples.

Introduction

QC of gDNA is essential for the success of downstream molecular biology applications such as NGS. The integrity, concentration, and size distribution of input gDNA directly influence library preparation efficiency and sequencing outcomes[1]. This is particularly important when working with low-concentration DNA samples, which are increasingly common in modern workflows, including those derived from limited material such as blood spots, saliva, buccal swabs, microdissected tissue, or challenging sources such as for FFPE tissues.

To address the growing need for sensitive analysis of low-concentration DNA, Agilent developed the HS gDNA ScreenTape assay[2]. This assay utilizes the DIN, a numerical score from 1 (highly degraded) to 10 (highly intact), to objectively assess gDNA quality. The assay has a lower limit of detection of 20 pg/µL and provides the DIN quality metric analysis from 0.25 to 10 ng/µL.

The HS gDNA ScreenTape assay is particularly capable of assessing low-input samples, including those extracted from FFPE tissues. While FFPE preservation is widely used in clinical research, it often results in fragmented or chemically modified DNA. The FFPE nucleic acid extraction workflow includes deparaffinization, washing, and elution (Figure 1). The deparaffinization step is very important to DNA integrity. Too long or too short of deparaffinization may lead to nucleic acid degradation. In addition, other variables can result in low extraction efficiency and impact DNA integrity. The TapeStation systems, using the DIN metric, enable objective evaluation of FFPE DNA quality after extraction. Even at low concentrations, the HS gDNA assay provides a DIN, enabling researchers to confidently assess sample quality and determine suitability for NGS and other downstream applications.

This application note evaluates the performance of the HS gDNA ScreenTape assay in analyzing low-concentration DNA samples, including FFPE-derived DNA. Examples include assessment across different sources, sample concentrations, and extraction methods. The results demonstrate the consistency and reliability of using the DIN for QC, confirming its usefulness in both routine and challenging sample contexts.

Experimental

DNA was extracted from different tissue types using various kits and diluted with 1 × TE buffer to concentrations of 10, 5, and 0.5 ng/µL if needed. Specifically, DNA was extracted from whole blood using the DNeasy Blood and Tissue Kit from Qiagen (p/n 69504), dried blood spots using QiAamp DNA micro Kit (p/n 56304), FFPE heart tissue using the QIAamp DNA FFPE Tissue Kit for DNA Extraction (p/n 56404), buccal swabs using the ReliaPrep gDNA Tissue Miniprep System from Promega (p/n A2051), FFPE heart tissue using MagMAX FFPE DNA/RNA Ultra Kit (p/n A31881), and saliva using the MagMAX Saliva gDNA Isolation Kit (p/n A39059).

To assess DIN using different extraction kits, FFPE DNA from two sample types were extracted. FFPE DNA from heart tissue was extracted using the QIAamp DNA FFPE Tissue kit (p/n 56404), QIAamp DNA FFPE Advanced kit (p/n 56604), and MagMAX FFPE kit (p/n A31881). FFPE DNA from kidney tissue was extracted using the GeneJET FFPE kit (p/n K0881) and Quick-DNA/RNA FFPE kit (p/n R1009). All samples were assessed on the Agilent 4200 TapeStation system (p/n G2991BA) using the Agilent High Sensitivity Genomic DNA ScreenTape (p/n 5067-5634) and High Sensitivity Genomic DNA reagents (p/n 5067-5635).

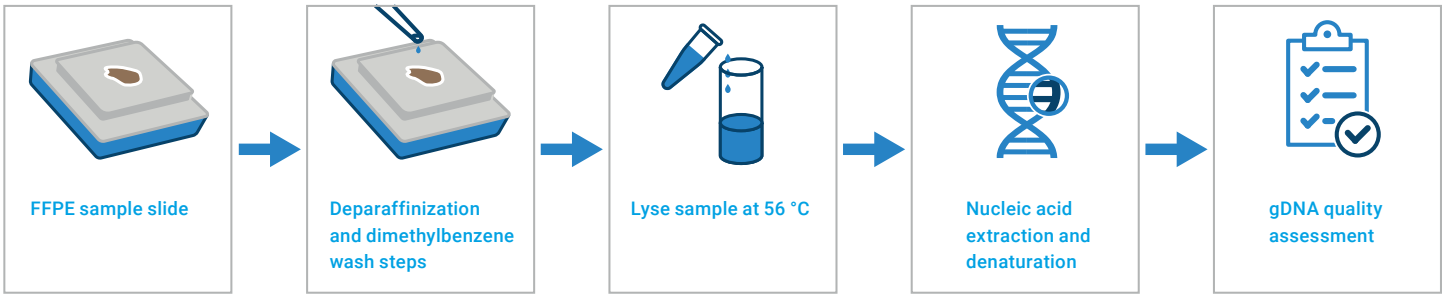

Figure 1. Representative FFPE genomic DNA extraction kit workflow.

Results and discussion

Quality control of low-concentration samples

Extraction of gDNA from dried blood spots and FFPE heart tissue resulted in low yields. Each was evaluated using the HS gDNA ScreenTape assay. The DNA from the dried blood spot sample had a concentration of 0.7 ng/µL, which is near the lower end of the assay concentration range. Despite the low concentration, the electropherogram (Figure 2A) revealed a clearly visible peak with a shoulder preceding it to the left, indicating partial degradation of the sample. The sample was assigned an average DIN of 6.8, reflecting moderately intact DNA. In contrast, the 1.1 ng/µL FFPE heart tissue sample exhibited a broad smear across the electropherogram, demonstrating a wide distribution of fragment sizes. This pattern indicates degraded DNA, and the TapeStation system assigned it an average DIN of 2.6, which is low and expected for a sample with this broad distribution.

These results demonstrate that the HS gDNA ScreenTape assay on the TapeStation system enables assessment of DNA quality in low-concentration samples. The assay provides clear visualization of fragment patterns and generates reliable DIN values that complement the electropherograms, even near the lower detection limit. This makes it especially useful for evaluating sample integrity in workflows involving limited or degraded DNA inputs.

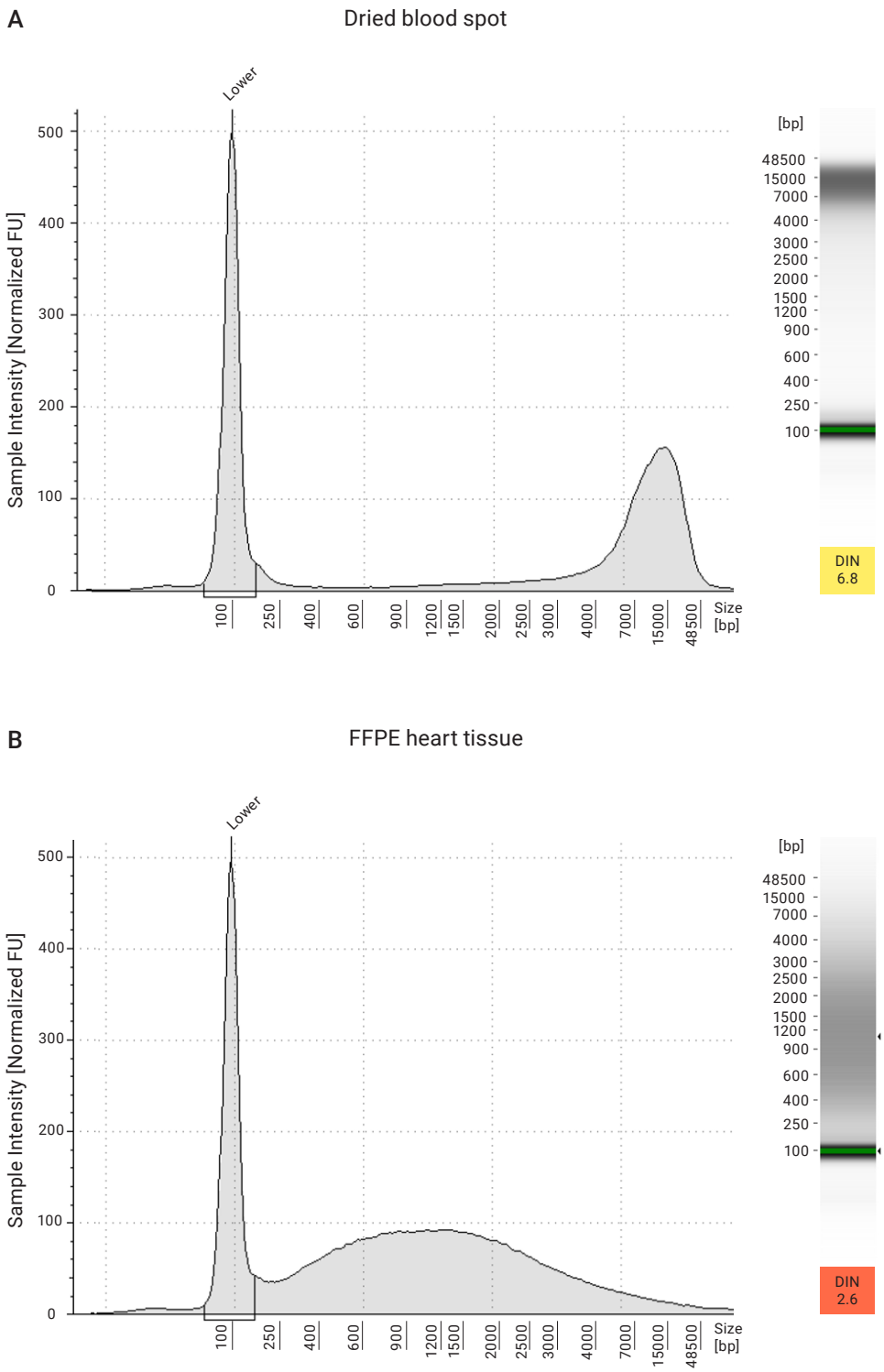

Figure 2. Representative electropherograms showing A) dried blood spot DNA with an average DIN of 6.8 and B) FFPE heart DNA with an average DIN of 2.6, analyzed on the Agilent 4200 TapeStation system with the Agilent High Sensitivity Genomic DNA ScreenTape assay. N=6

Reproducibility of DIN across the concentration range

To evaluate the performance of the HS gDNA ScreenTape assay across its full concentration range, DNA from whole blood, buccal swabs, and FFPE heart tissue were extracted. Each extracted sample was diluted into replicate samples of 10, 5, and 0.5 ng/µL and then analyzed. The varying tissue types and concentrations allowed for assessment of the assay robustness across different levels of DNA integrity and input amounts.

Figure 3A shows electropherograms of whole blood gDNA analysis with the HS gDNA assay. Across all concentrations, the sample profile remained consistent. A prominent gDNA peak and minor shouldering between the lower marker and the primary fragment are shown, indicating a small amount of degradation. DIN scores were stable across concentrations, ranging from an average of 7.4 to 7.8 (Figure 3D).

Buccal swab gDNA (Figure 3B) displayed broad peaks, with a large signal near the lower marker and multiple smaller peaks leading up to the primary fragment peak. These visual results indicated some amount of degradation in the sample. Despite the lower integrity, the electropherogram profile remained consistent across concentrations, and DIN scores ranged from an average of 5.5 to 6.2 (Figure 3D).

Heart tissue FFPE gDNA (Figure 3C) showed a broad, sloping peak with smearing on both sides, indicating a mix of different fragment sizes due to degradation or other impurities. The overall profile remained stable across concentrations, and DIN scores ranged from an average of 3.8 to 4.1 (Figure 3D).

These results demonstrate that the HS gDNA ScreenTape assay delivers reliable DNA quality assessment across samples with varying degrees of degradation and across the assay's full concentration range (Figure 3D). Within each sample type, the electropherogram profiles showed the same pattern, and DIN values showed minimal variation, confirming the assay's robustness across concentrations.

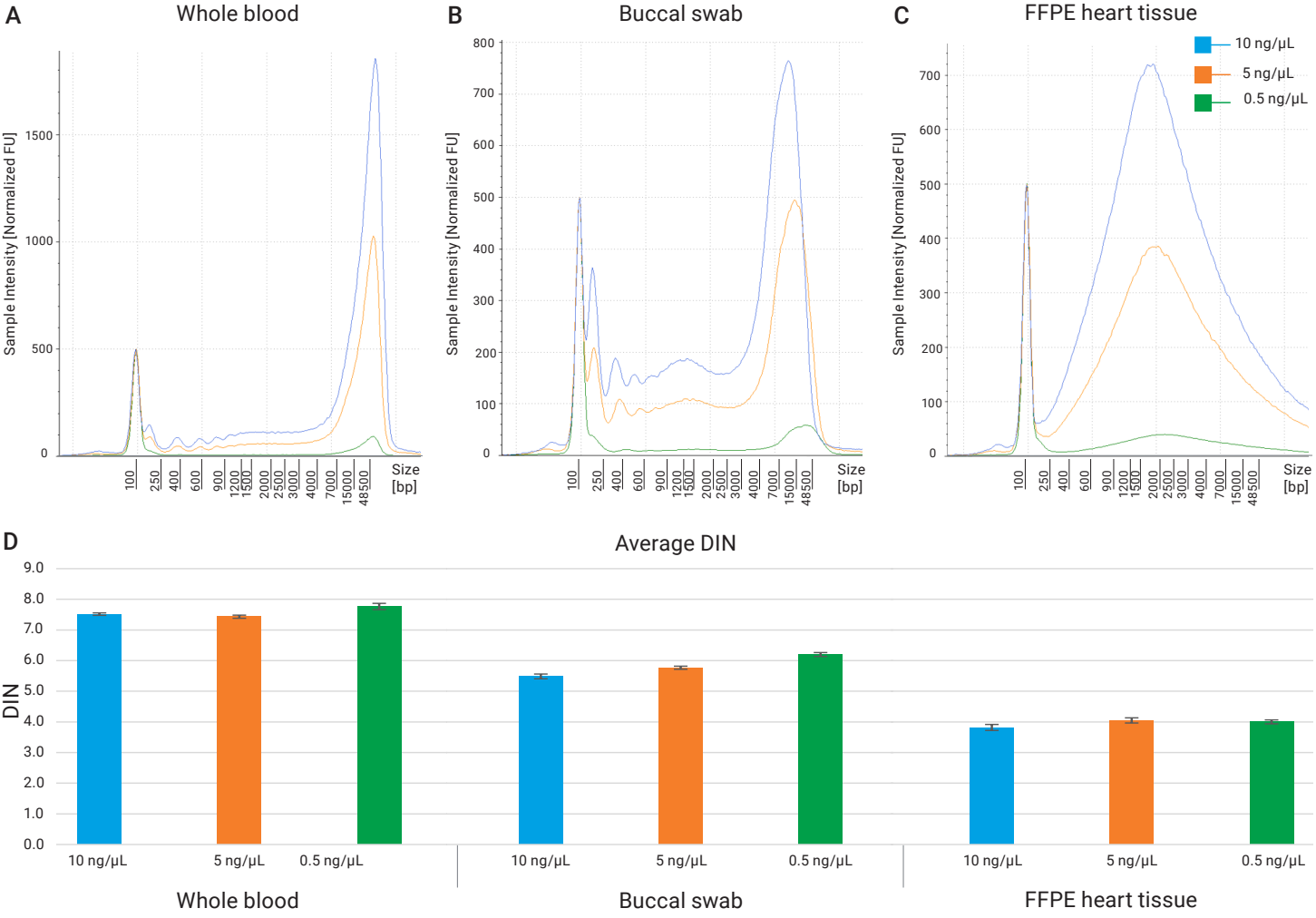

Figure 3. Representative electropherograms of gDNA samples analyzed on the Agilent 4200 TapeStation system using the Agilent High Sensitivity Genomic DNA assay from highest to lowest DIN quality value. The samples assessed were A) whole blood, B) buccal swab, and C) FFPE heart tissue. D) The average DIN quality metric for each sample type and concentration is shown by sample type. N=6

Comparative evaluation of FFPE DNA extraction kits

To further assess the performance of the HS gDNA ScreenTape assay, gDNA was extracted from human heart and kidney FFPE samples using different commercial extraction kits. These kits vary in their chemistries, including the efficiency of paraffin removal, protein digestion, DNA purification steps, and elution buffer composition, all of which can influence the integrity of the extracted DNA. The resulting DNA quality was evaluated using the TapeStation system with the HS gDNA ScreenTape assay.

Electropherogram overlays of FFPE DNA from heart tissue from each extraction kit are shown in Figure 4. Regardless of the kit used, the samples showed an overall smear pattern indicative of a low-quality sample. Extraction kit 1 had the highest DIN value of 3.8. Extraction kits 2 and 3 displayed more smearing with smaller fragments, indicating degradation and lower quality samples. This visual analysis of the quality is confirmed by the DIN values of 2.9 and 2.5, respectively.

Figure 5 shows the electropherogram and DIN results of analyzing FFPE DNA from kidney tissue extracted with kits 4 and 5. The electropherogram of the gDNA from the fourth extraction kit shows a shift towards smaller fragments. Extraction kit 5 showed a higher portion of the sample towards larger fragments comparatively, signifying more intact sample. The differences in the electropherograms correspond with the varying degrees of degradation and are verified by the DIN values of 3.9 and 5.3 respectively.

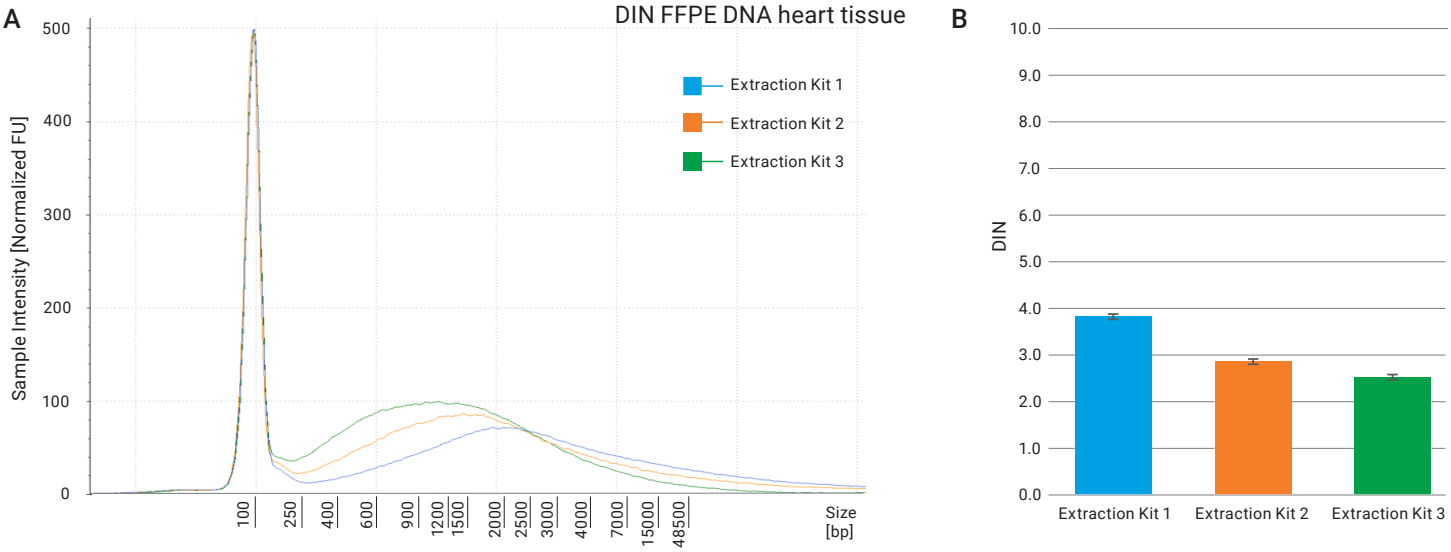

Figure 4. FFPE heart tissue extracted DNA was analyzed using the Agilent 4200 TapeStation system with the Agilent High Sensitivity Genomic DNA ScreenTape assay. The A) electropherogram shows the DNA fragment distribution and the B) DNA integrity number (DIN) quality analysis quantifies the level of degradation, offering a standardized measure of DNA quality. N=3

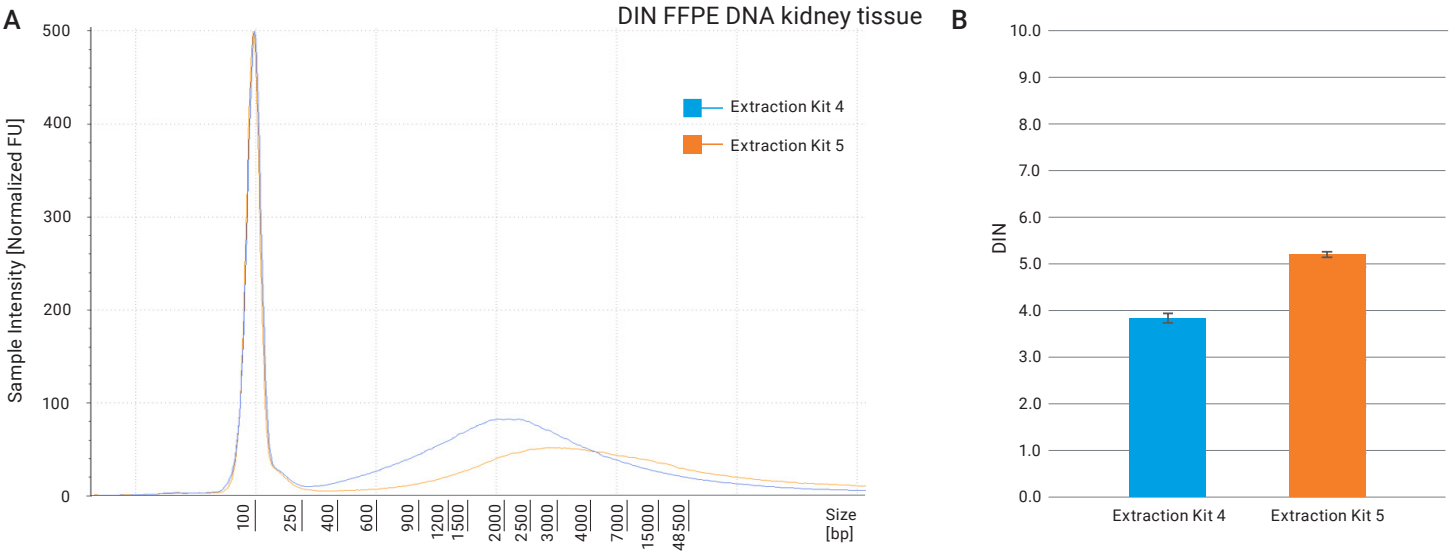

Figure 5. FFPE kidney tissue extracted DNA was analyzed using the Agilent 4200 TapeStation system with the Agilent High Sensitivity Genomic DNA ScreenTape assay. The A) electropherogram shows the DNA fragment distribution and the B) DNA integrity number (DIN) quality analysis quantifies the level of degradation, offering a standardized measure of DNA quality. N=3

Conclusion

This application note demonstrates the effectiveness of the Agilent TapeStation system using the Agilent High Sensitivity Genomic DNA ScreenTape assay for assessing DNA quality in various low-concentration samples. The assay consistently delivered reliable DIN values across a range of sample types and concentrations and shows differences in extraction methods. These results underscore the value of the TapeStation system as a dependable solution for low-concentration DNA quality assessment. Its ability to provide consistent DIN values with minimal sample input allows researchers to make informed decisions in workflows such as NGS, where input quality is critical for success.

References

1. Correlating NGS Success with Sample Input Quality: a Large Scale Study. *Agilent Technologies technical overview*, publication number 5994-7119EN, **2024**.
2. Performance Characteristics of the Agilent High Sensitivity Genomic DNA ScreenTape Assay for Agilent TapeStation Systems. *Agilent Technologies technical overview*, publication number 5994-8529EN, **2025.**

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