Application Note

Genomics

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Assessing FFPE Genomic DNA Integrity at Low Concentrations

Using the Agilent High Sensitivity Genomic DNA ScreenTape assay on the Agilent TapeStation system

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Abstract

The Integrated Biobank of Luxembourg (IBBL), like many other biobanks, stores formalin-fixed, paraffin-embedded (FFPE) tissue for research. Nucleic acids extracted from FFPE tissue are compromised in yield and integrity compared to those from cryopreserved tissue due to the formalin fixation process. Researchers need a simple and standardized way to check the integrity of the nucleic acids extracted from these stored samples before moving onto downstream analyses. The Agilent High Sensitivity Genomic DNA (gDNA) ScreenTape assay and the Agilent Genomic DNA ScreenTape assay used with the Agilent TapeStation systems provide the DNA integrity number (DIN), an objective measure of DNA fragmentation.

In this application note, the IBBL assessed a large set of extracted FFPE DNA samples for integrity. Previously, integrity assessment was only possible using the Agilent gDNA ScreenTape assay but was limited if the sample concentration was too low. The Agilent High Sensitivity gDNA ScreenTape assay was developed to expand the range of DNA integrity analyses for low input concentrations. The results from the samples tested demonstrate that the High Sensitivity gDNA ScreenTape assay provides reliable DIN measurements at low concentrations, helping biobanks and researchers assess the integrity of low yield FFPE-derived DNA and make better-informed decisions about downstream analyses.

Introduction

Biobanks often store large numbers of tissue samples preserved using various methods, with formalin-fixed, paraffin-embedding (FFPE) being one of the most common. This technique is a standard method that preserves tissue architecture excellently and enables long-term archiving without the need for cold storage. While FFPE is the standard for preserving tissue structural integrity, the fixation process fragments genomic DNA (gDNA) compromising its its amenability to downstream molecular biological analyses. Additional degradation can occur during long-term storage, further impacting its quality. Nevertheless, the development of FFPE-friendly platforms has enabled DNA from FFPE tissue to be applied to most analytical methods. However, before beginning downstream workflows such as next-generation sequencing, gene expression analysis, or targeted DNA assays, it is important to establish that the DNA is still sufficiently intact for the intended application.

To support this need, the Agilent TapeStation system provides fast, easy, reliable, and scalable automated electrophoresis for sizing, quantification, and integrity assessment. Both the Agilent Genomic DNA ScreenTape assay and the Agilent High Sensitivity Genomic DNA ScreenTape assay are run on the TapeStation system, automatically generating a DNA integrity number (DIN). The DIN is a numerical score ranging from 1, signifying highly degraded DNA, to 10, signifying highly intact DNA, providing researchers with an objective method of comparing DNA quality across different storage conditions and tissue types. DIN has become a widely used metric in biobanking and molecular biology workflows for rapid assessment of sample suitability for downstream applications.

The DIN functional range of the gDNA ScreenTape assay is 5 to 300 ng/µL. However, extraction from FFPE tissues regularly yields low concentrations of DNA that fall below this range. To address this, Agilent developed the High Sensitivity gDNA ScreenTape assay, which expands the TapeStation assay portfolio to include reliable DIN assessment for low concentration DNA, with a DIN functional range of 0.25 to 10 ng/µL (Table 1).[1] This capability is valuable for biobanks, where limited quantity tissue specimens (for example, tissue biopsies) cannot be subjected to the re-extraction of DNA and must be evaluated as they are. Knowing this information is essential for downstream planning, as molecular analyses can often be tailored to the quality and quantity of available DNA.

In this application note, the Integrated Biobank of Luxembourg (IBBL) used the High Sensitivity gDNA ScreenTape assay and the gDNA ScreenTape assay with the 4200 TapeStation system to assess DNA extracted from various FFPE tissue samples. DIN values from each assay were compared to assess the degree of consistency across a range of tissue types, extraction methods, and DNA concentrations. The performance of the electronic ladder for the High Sensitivity gDNA assay was also examined relative to the run ladder to show the impact of ladder choice on DIN reporting. This evaluation was performed because some users may choose to use the electronic ladder as a means of lowering costs. Together, these results illustrate how DIN analysis using the High Sensitivity gDNA ScreenTape assay can help biobanks and researchers evaluate the condition of stored FFPE DNA and make informed decisions about sample usability when DNA quantity is limited.

Table 1. Agilent High Sensitivity Genomic DNA ScreenTape assay and Agilent Genomic DNA ScreenTape assay selected specifications.

Analytical Specifications	High Sensitivity Genomic DNA ScreenTape	Genomic DNA ScreenTape
Sizing range	200 to > 60,000 bp	200 to > 60,000 bp
Sensitivity	20 pg/µL	0.5 ng/µL
DIN functional range	0.25 to 10 ng/µL	5 to 300 ng/µL

Experimental

Analyses were performed on the Agilent 4200 TapeStation system (p/n G2991AA) using the Agilent High Sensitivity Genomic DNA ScreenTape (p/n 5067-5634) and High Sensitivity Genomic DNA reagents (p/n 5067-5635), along with the Agilent Genomic DNA ScreenTape (p/n 5067-5365) and Genomic DNA reagents (p/n 5067-5366). All FFPE tissue samples were extracted with the QIAamp DNA FFPE kit (Qiagen p/n 56404), the Chemagic FFPE DNA kit (Revvity p/n CMG-1099), or the Exscale FFPE DNA Purification kit (Exscale Biospecimen Solutions p/n ES-K110FP). Fresh-frozen tissue samples were extracted using the AllPrep DNA/RNA/Protein Mini kit (Qiagen p/n 80004). Extracted DNA samples were diluted to the specified working concentration ranges of each Agilent ScreenTape assay using 1x TE buffer. FFPE DNA from multiple human tissues (human ovary, human breast, human neck, human fallopian tubes, human uterus, human colon, human stomach, human small bowel, human jejunum, and pooled DNA from various other organs) was evaluated. The gDNA extracted from fresh-frozen tissues (human myometrium and rat lung) was also assessed for DIN correlation between the Genomic DNA and High Sensitivity Genomic DNA ScreenTape assays across diverse sample types, integrity levels, and DNA concentrations. DIN values generated with the High Sensitivity Genomic DNA ScreenTape assay were also assessed using either a run ladder loaded on the ScreenTape or the electronic ladder available in the TapeStation software.

Results and discussion

DIN assessment across sample types and integrities

An expansive dataset of 76 FFPE DNA samples from various tissue sources, different extraction methods, and a wide range of sample integrities was used to provide a broad evaluation of quality analysis between the High Sensitivity gDNA ScreenTape assay and the gDNA ScreenTape assay. The samples ranged in integrity, with DIN values of approximately 2 to 7. DIN values from the two assays were compared by plotting them on a scatter plot shown in Figure 1A (green dots), which demonstrated a strong linear relationship with an R^2 of 0.95. This indicates that the High Sensitivity gDNA ScreenTape assay produced DIN values consistent with those from the gDNA ScreenTape assay, even when DNA input was limited. Representative electropherograms of high-, medium-, and low-integrity samples (fresh-frozen and FFPE) analyzed using the High Sensitivity gDNA ScreenTape assay are shown in Figure 1B–D, illustrating DNA quality across the integrity range. Intact DNA is characterized by a dominant high molecular weight peak, whereas increasing degradation results in a shift toward smaller DNA fragments on the left side of the electropherogram and a corresponding loss of the high molecular weight peak.

To further demonstrate that DIN assessment is applicable across different sample types, a smaller set of five non-FFPE samples were evaluated. This set of samples was stored as fresh-frozen and had DIN values that ranged from 6.0 to 9.3. The two assays produced highly comparable DIN values with differences of less than 0.4 between the assays. When added to the scatter plot showing the DIN values of the 76 FFPE samples on both assays, the combined R^2 of both FFPE and fresh-frozen samples was 0.96, signifying a high degree of linearity between assay DIN values (Figure 1A, red dots). Together, these results demonstrate that DIN values are consistent between assays and reflect DNA integrity across a wide range of sample types and input conditions.

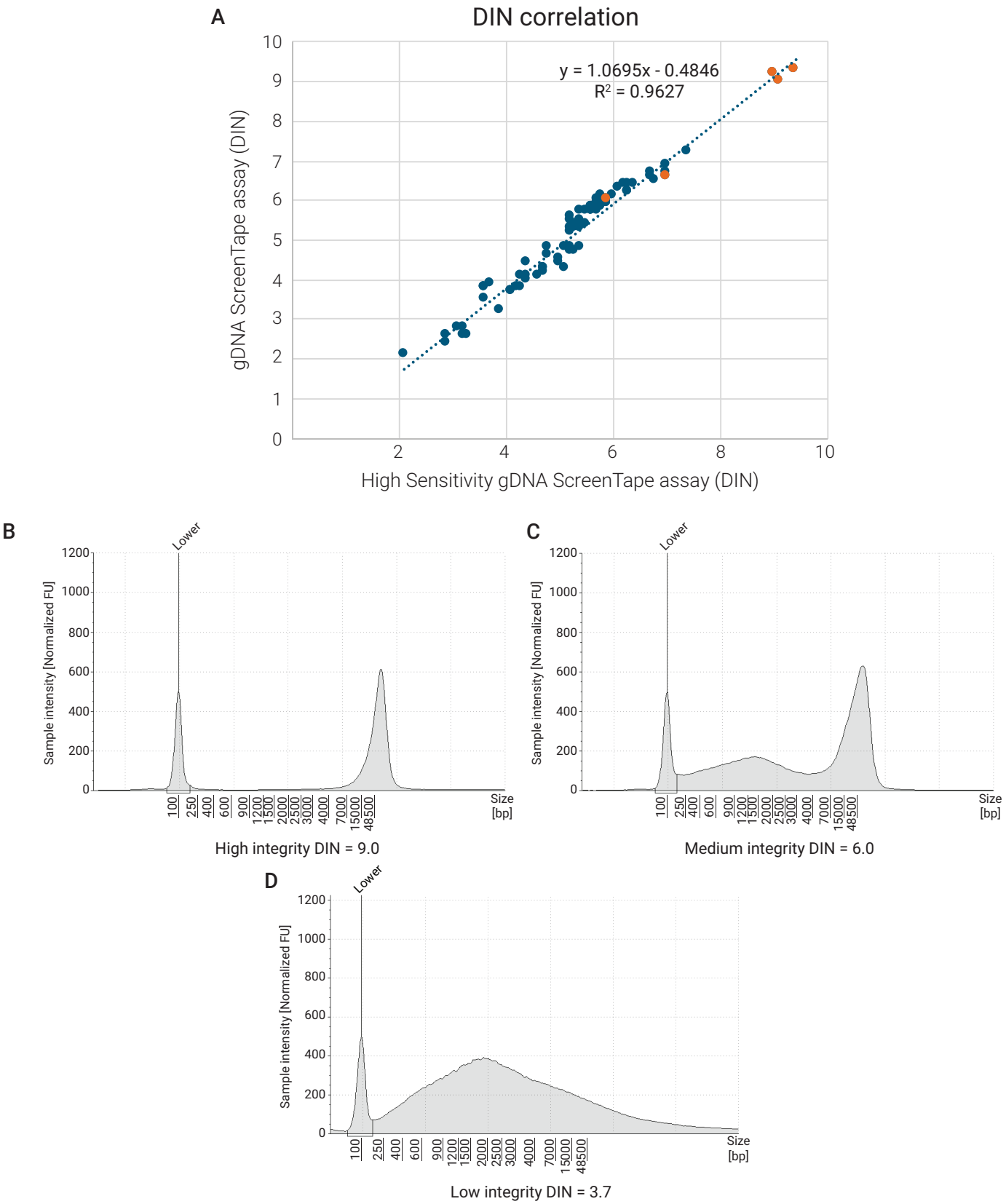

Figure 1. Comparison of DNA integrity number (DIN) values generated using the Agilent High Sensitivity Genomic DNA ScreenTape assay and the Agilent Genomic DNA ScreenTape assay on the Agilent 4200 TapeStation system. A) Scatter plot showing DIN correlation for 76 FFPE DNA samples (blue dots) and five fresh-frozen tissue DNA samples (orange dots), analyzed by IBBL, with linearity shown for all samples combined. Representative electropherograms from samples with B) high (DIN = 9.0), C) medium (DIN = 6.0), and D) low (DIN = 3.7) integrity across sample sets.

Evaluation of electronic ladder usage for DIN analysis

The High Sensitivity gDNA ScreenTape assay has the option of supporting DIN analysis using an electronic ladder, replacing the need for the run ladder. To demonstrate the feasibility of this for the High Sensitivity gDNA ScreenTape assay, datasets from the 76 FFPE samples and five fresh-frozen samples were analyzed using both the electronic ladder and the in-run ladder provided with the assay. The DIN values for each type of ladder were plotted on a scatter plot shown in Figure 2. The results show that the DIN values generated using each ladder type were highly similar, producing a line of best fit with an R^2 of 0.99 (Figure 2). The electronic ladder simplifies the assay setup, increases lab efficiency by freeing additional ScreenTape lanes, and provides greater flexibility for routine quality control (QC) workflows, all while providing consistent and reliable DIN interpretation.

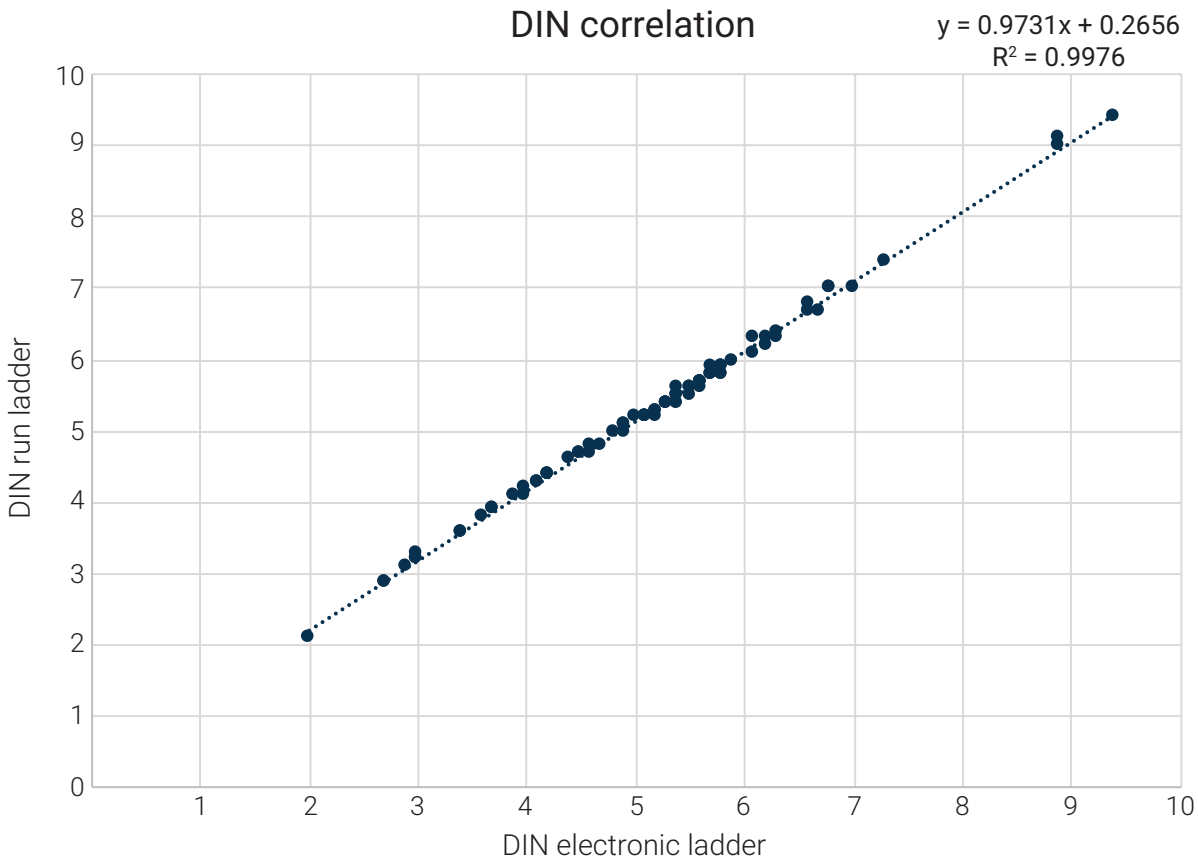

Figure 2. Correlation of DIN values generated using the Agilent High Sensitivity Genomic DNA ScreenTape assay on the Agilent 4200 TapeStation system with the electronic ladder in the Agilent TapeStation analysis software and the run ladder for the 76 sample FFPE and additional five fresh-frozen sample datasets analyzed by the IBBL.

Conclusion

As demonstrated in this application note, the IBBL has adopted the use of either the gDNA ScreenTape assay or the High Sensitivity gDNA ScreenTape assay for routine FFPE extraction and QC workflows due to the reliable integrity assessment across a broad range of sample types and input concentrations. Additionally, the DIN values generated using the electronic ladder were consistent with those obtained using the run ladder, enabling simplified assay setup, improved laboratory efficiency, and increased flexibility for routine QC workflows without impacting DIN interpretation. Together, these capabilities support dependable integrity based decision making during routine FFPE QC, particularly when DNA input is limited.

References

1. Performance Characteristics of the Agilent High Sensitivity Genomic DNA ScreenTape Assay for Agilent TapeStation Systems, *Agilent Technologies technical overview*, publication number 5994-8592EN, **2025**.

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