Technical Overview

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Performance Characteristics of the Agilent High Sensitivity Genomic DNA ScreenTape Assay for Agilent TapeStation Systems

Introduction

Quality control (QC) of genomic DNA (gDNA) is a critical step in ensuring the success of downstream molecular biology applications such as next-generation sequencing (NGS). The integrity, concentration, and size distribution of input DNA directly influences library preparation efficiency and sequencing outcomes. QC can be performed using Agilent TapeStation systems, which provide rapid and automated electrophoretic separation of nucleic acids through easy-to-use ScreenTape technology. For objective gDNA quality assessment, the Agilent Genomic DNA ScreenTape assay uses a quality metric called the DNA Integrity Number (DIN). The gDNA ScreenTape assay and DIN are well established in NGS QC workflows, enabling users the ability to set quality thresholds for library preparation and sequencing.[1] However, as sequencing technologies advance and workflows become more sensitive, there is an increasing demand for higher sensitivity in gDNA QC.

To address the need for sensitive analysis of low-concentration gDNA samples, Agilent has developed the Agilent High Sensitivity Genomic DNA (HS gDNA) ScreenTape assay.[2] This assay complements the existing gDNA ScreenTape assay by extending the lower limit of detection to 20 pg/µL and enabling quantitative analysis from 0.5 to 10 ng/µL. The standard gDNA assay supports a quantitative range of 10 to 100 ng/µL. Together, these assays enable analysis of a wide range of sample types and concentrations, with a sizing range spanning 200 to over 60,000 base pairs. Table 1 outlines the analytical specifications of both gDNA ScreenTape assays.

Both assays provide an objective assessment of gDNA integrity by assigning the DIN to each sample. The algorithm for this established quality metric has been refined to ensure consistent performance across the entire DIN functional range of both assays, from as low as 0.25 ng/µL with the HS gDNA assay up to 300 ng/µL with the gDNA assay. This technical overview presents the analytical performance of the HS gDNA ScreenTape assay, with a focus on its sensitivity, accuracy, and reproducibility in sizing, quantification, and integrity assessment.

Table 1. Analytical specifications of Agilent Genomic DNA ScreenTape assays for Agilent TapeStation systems.

Analytical Specifications	High Sensitivity Genomic DNA ScreenTape	Genomic DNA ScreenTape
Sizing Range	200 to > 60,000 bp	200 to > 60,000 bp
Sensitivity[1]	20 pg/µL	0.5 ng/µL
Sizing Precision[2]	200 to 15,000 bp: 20% CV	200 to 15,000 bp: 15% CV
Sizing Accuracy[2]	200 to 15,000 bp: ± 20%[3]	200 to 15,000 bp: ± 15%
Quantitative Precision	20% CV	15% CV
Quantitative Accuracy	± 25%	± 20%
Quantitative Range	0.5 to 10 ng/µL	10 to 100 ng/µL
DIN Functional Range[4]	0.25 to 10 ng/µL	5 to 300 ng/µL
Maximum Buffer Concentration in Sample	200 ng/µL glycogen 3% 2-propanol 3% ethanol 0.5 mM NaOAc 2.5 mM NaCl 2.5 mM guanidine thiocyanate or 10 mM Tris 0.1 mM EDTA	1 µg/µL glycogen 10% 2-propanol 10% ethanol 10 mM $MgCl_2$ 10 mM NaOAc 50 mM NaCl

[1] Signal/noise > 3 (single peak)
[2] Determined using ladder as sample
[3] Sizing accuracy applicable for analysis with run ladder
[4] DIN - DNA Integrity Number

Experimental

Genomic DNA samples were analyzed on an Agilent 4150 TapeStation system (part number G2992AA) and Agilent 4200 TapeStation system (part number G2991BA) using Genomic DNA ScreenTape assays. The performance of the HS gDNA ScreenTape (part number 5067-5634), Agilent HS gDNA reagents (part number 5067-5635), gDNA ScreenTape (part number 5067-5365), and Agilent gDNA reagents (part number 5067-5366) were assessed. Data analysis was performed using Agilent TapeStation Analysis software, version 5.2.

Samples tested include:
Agilent OneSeq Reference DNA, Male (part number 5190-8848) and BioChain Control Genomic DNA - Human Male (BioChain Institute, part number D1234999-G01). To obtain samples of varying integrity, the BioChain Control Genomic DNA was mechanically degraded by shaking on a thermomixer at 2,000 rpm at 19 to 20 °C for up to 100 hours. Initial sample concentration was determined using a Qubit 4 Fluorometer with the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, part number Q33231).

Results and discussion

Sensitivity

Genomic DNA serves as the starting material for numerous molecular analysis methods. QC of the material is essential to ensure the success of downstream applications. When sample input is limited, such as for low concentration or precious samples, sensitive detection methods are critical. The HS gDNA ScreenTape assay addresses this need by enabling detection of samples as low as 20 pg/µL, and a lower functional DIN limit of 0.25 ng/µL.

To evaluate the sensitivity of the HS gDNA assay, the OneSeq Reference DNA, Male was analyzed on the 4200 TapeStation system. Figure 1 shows an electropherogram overlay of six replicates of the sample at the 20 pg/µL limit of detection. The sample was clearly visualized, confirming the ability of the assay to detect low concentration gDNA samples.

A dilution series of a reference gDNA sample was prepared and analyzed on the 4200 TapeStation system. The electropherograms exhibited very similar profiles across the concentration range of 0.25 to 10 ng/µL. As shown in the overlay in Figure 2, each sample was composed of a large proportion of high molecular weight material, with a small shoulder and slight smearing to the left of the main peak. The DIN was consistent across all concentrations, with an average score of 8.1, highlighting the suitability of the assay to analyze sample integrity even at low concentrations.

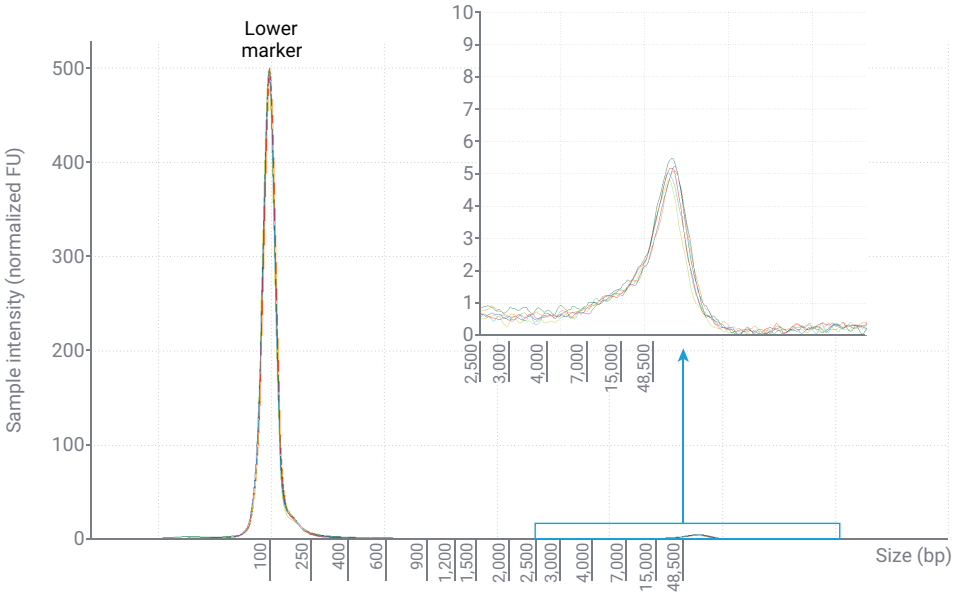

Figure 1. Electropherogram overlay of the Agilent OneSeq Reference gDNA, Male sample analyzed at the specified limit of detection (20 pg/µL) on an Agilent 4200 TapeStation system using the Agilent High Sensitivity Genomic DNA ScreenTape assay. The inset shows a zoom of the resulting peaks (N = 6).

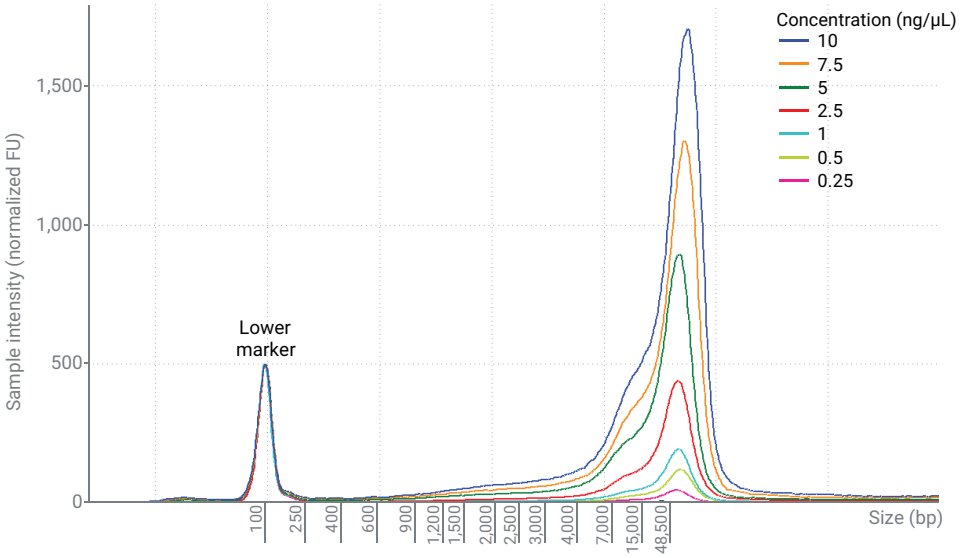

Figure 2. Electropherogram overlay of dilutions of an Agilent OneSeq reference gDNA, Male sample analyzed on an Agilent 4200 TapeStation system using the Agilent High Sensitivity Genomic DNA ScreenTape assay. Samples were assessed at concentrations covering the functional DIN range of the assay from 0.25 to 10 ng/µL, with an average DIN score of 8.1.

Sizing

The HS gDNA ScreenTape assay provides reproducible and accurate sizing across a broad size range from 200 to greater than 60,000 bp, with a precision of 20% CV and accuracy of ± 20% from 200 to 15,000 bp. A run ladder must be analyzed on each ScreenTape device with the samples to provide accurate sizing and molarity calculations. Electropherogram images of a reference gDNA sample and the Agilent HS gDNA ladder demonstrate the sizing range of the assay. Figure 3A shows the lower marker at 100 bp, followed by the HS gDNA ladder, which is composed of several fragments extending from 250 to 48,500 bp. A linear curve allows extrapolation of larger sizes up to 60,000 bp, such as the gDNA sample shown (Figure 3B).

To assess sizing accuracy and precision specifications, the HS gDNA ladder was analyzed as a sample across multiple replicates. The average size of 12 of the ladder fragments (from 250 to 15,000 bp) was calculated. The results demonstrated excellent accuracy with only 2% error or less for each, well below the specification of 20% (Figure 4A). Additionally, the assay displayed robust precision, with 5% CV or less across all fragments, confirming the reproducibility of the assay (Figure 4B).

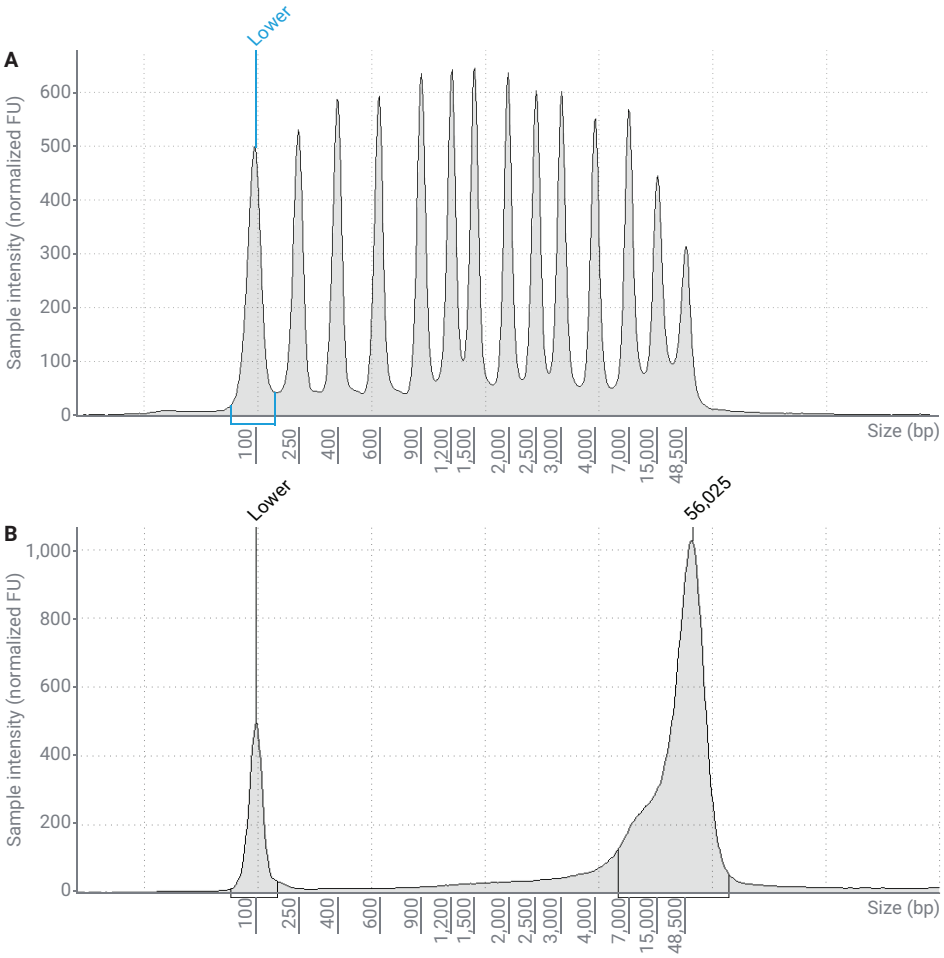

Figure 3. (A) Agilent High Sensitivity Genomic DNA ladder and (B) Agilent OneSeq Reference DNA, Male, analyzed with an Agilent 4150 TapeStation system and the Agilent High Sensitivity Genomic DNA ScreenTape assay.

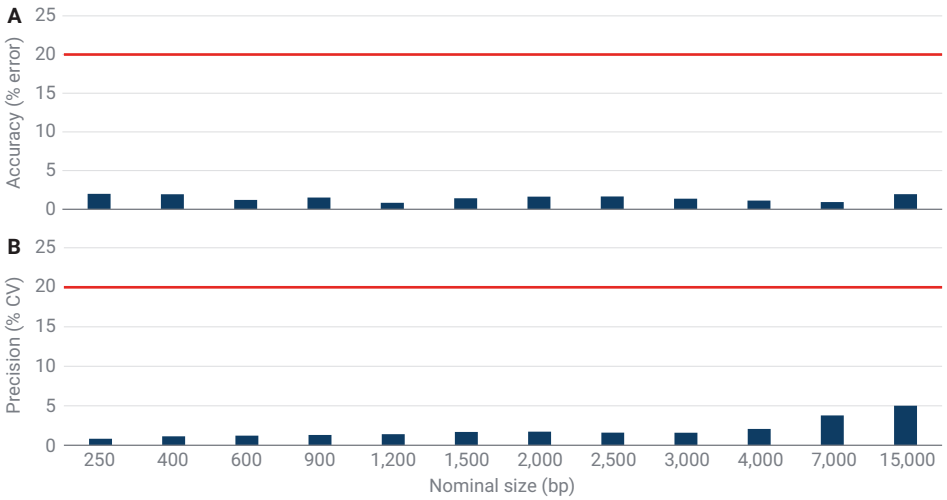

Figure 4. Average sizing (A) accuracy and (B) precision of 12 fragments of an Agilent High Sensitivity Genomic DNA ladder analyzed with an Agilent 4200 TapeStation system and the Agilent High Sensitivity Genomic DNA ScreenTape assay. Red lines indicate the specified sizing accuracy of ± 20% error and sizing precision of 20% CV from 200 to 15,000 bp. N = 18.

Quantification

The specifications of the HS gDNA ScreenTape assay include an average quantitative accuracy of ± 25% and quantitative precision of 20% CV. Dilutions of the reference gDNA sample were assessed on the TapeStation systems within the quantitative range of the assay, from 0.5 to 10 ng/µL. The HS gDNA ScreenTape assay provides highly reliable quantification, as demonstrated by the strong correlation between the concentration measured by the TapeStation compared to Qubit (Figure 5A). Across the dilution range, the TapeStation demonstrated excellent average accuracy with no more than 16% error when compared to the nominal concentration (Figure 5B).

Samples in the low-, mid-, and high-concentration range of the assay were further evaluated on both the 4150 and 4200 TapeStation systems for quantitative precision. As shown in Figure 5C, both systems displayed excellent precision of approximately 6% CV or less, well below the kit specifications of 20% CV. Together these data confirm the highly reliable and reproducible sizing and quantification achieved with the HS gDNA assay and the TapeStation systems.

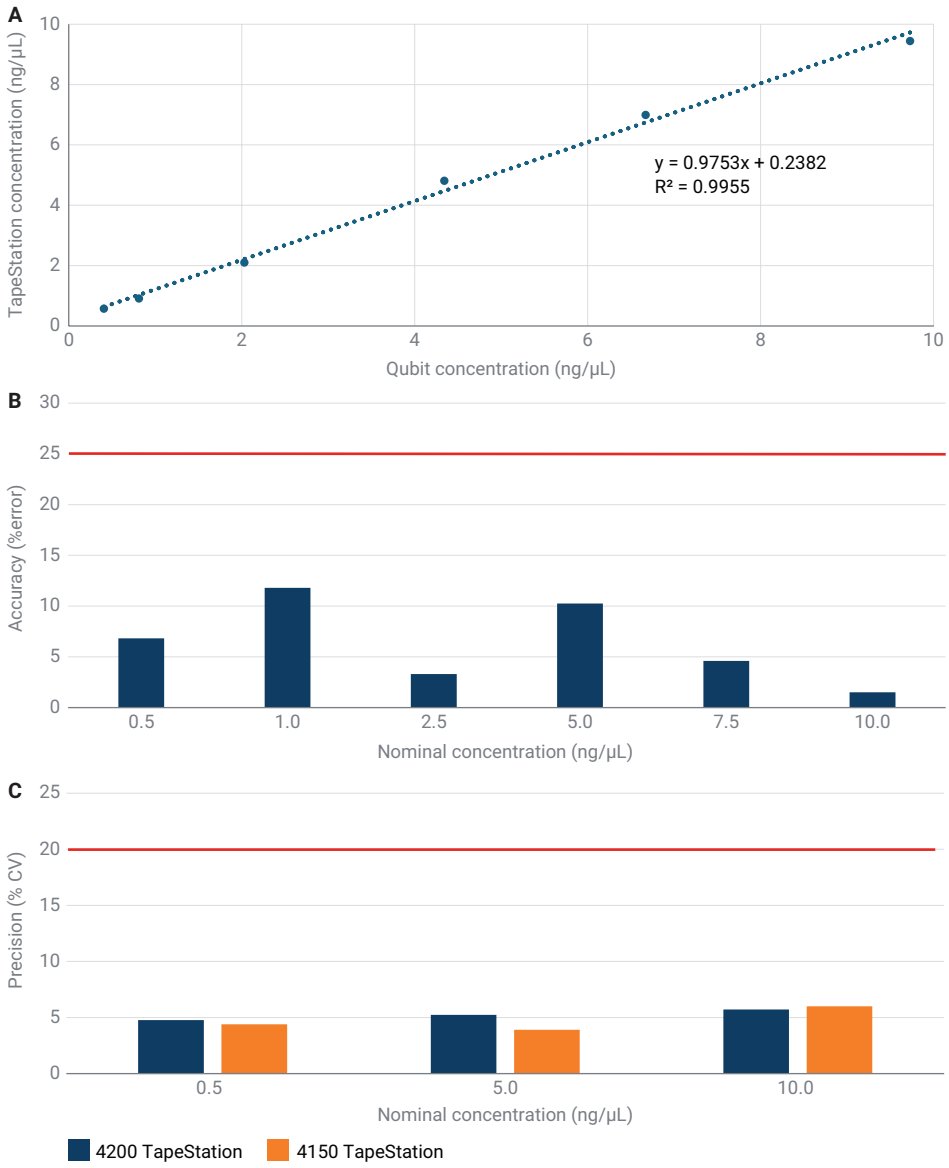

Figure 5. Quantitative analysis of Agilent OneSeq Reference DNA, Male using the Agilent TapeStation systems and the Agilent High Sensitivity Genomic DNA ScreenTape assay. (A) Correlation of sample analyzed on the Agilent 4200 TapeStation system compared to Qubit. (B) Average quantitative accuracy of the Agilent TapeStation systems (red line denotes the specified quantitative accuracy of the High Sensitivity Genomic DNA ScreenTape assay). (C) Assessment of the quantitative precision displayed by the 4150 and 4200 TapeStation systems (red line denotes the specified quantitative precision of the High Sensitivity Genomic DNA ScreenTape assay). 4150 TapeStation: N = 6 per concentration. 4200 TapeStation: N = 18 per concentration. Qubit: N = 2 per concentration (concentration of samples ≤ 1 ng/µL is below the Qubit range and therefore extrapolated).

DIN

Assessment of gDNA integrity is a critical component of input QC, as degraded samples can compromise the efficiency and accuracy of downstream applications or indicate if specific adjustments to the workflow should be made. The TapeStation systems use the DIN, a numerical score ranging from 1 (highly degraded) to 10 (intact), to provide an objective and reproducible measure of gDNA integrity. This metric, previously established for the gDNA ScreenTape assay, has been fully implemented and optimized for the HS gDNA ScreenTape assay.

To evaluate the performance of the DIN in the HS gDNA ScreenTape assay, a degradation series of a reference gDNA sample was analyzed on the 4200 TapeStation system. An overlay of electropherograms and the corresponding digital gel image are shown in Figure 6, illustrating progressing degradation of the sample. The TapeStation analysis software automatically calculates the DIN and displays a customizable, color-coded result flagging below the gel image, allowing for easy and quick visual assessment of sample quality across multiple ScreenTape lanes.

The robustness of the DIN across the concentration range of the assay was demonstrated by assessing a dilution series of representative gDNA samples. As shown in Figure 7, the average DIN score for low-, medium-, and high-quality samples remained consistent across the entire concentration range of the HS gDNA ScreenTape assay.

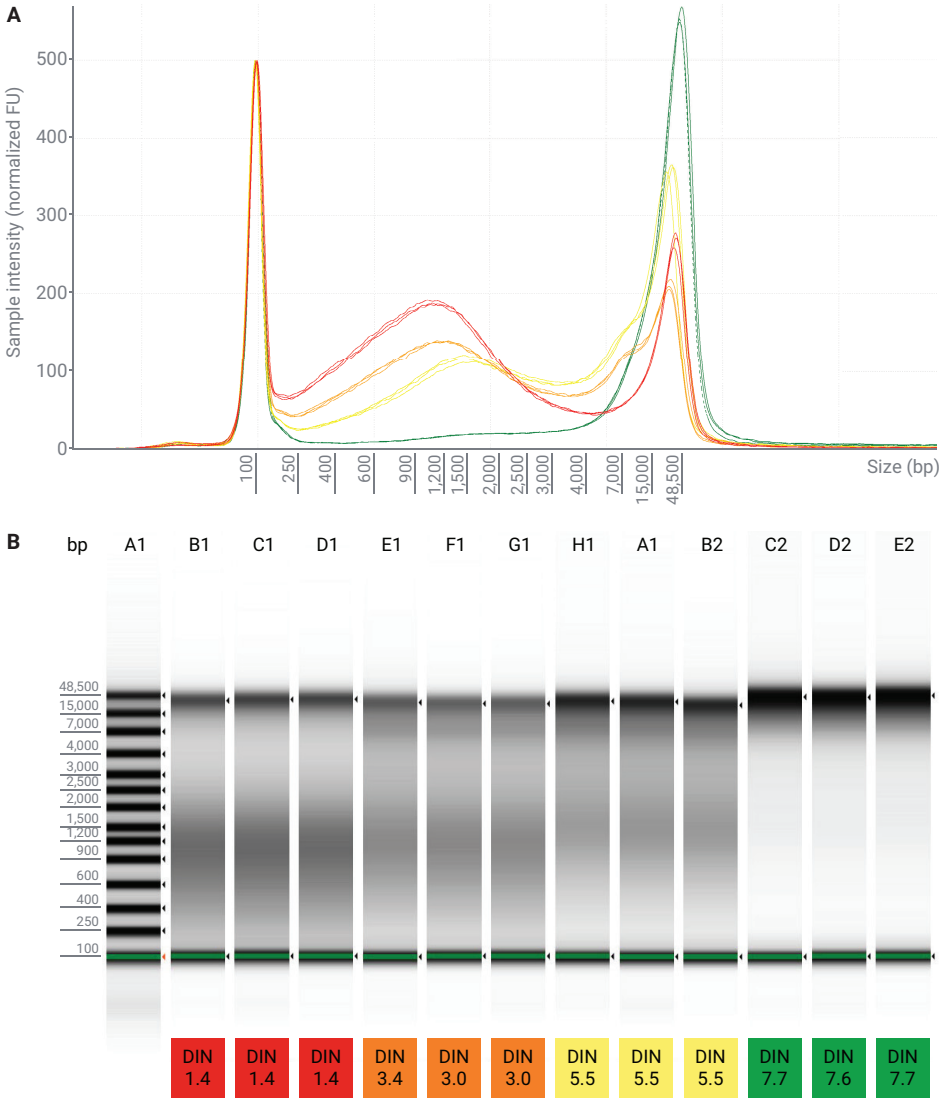

Figure 6. Degradation series of BioChain Control Genomic DNA – Human Male analyzed with an Agilent 4200 TapeStation system and the Agilent High Sensitivity Genomic DNA ScreenTape assay. (A) Electropherogram overlay and (B) digital gel image representative of low (red/orange), medium (yellow), and high (green) DIN scores.

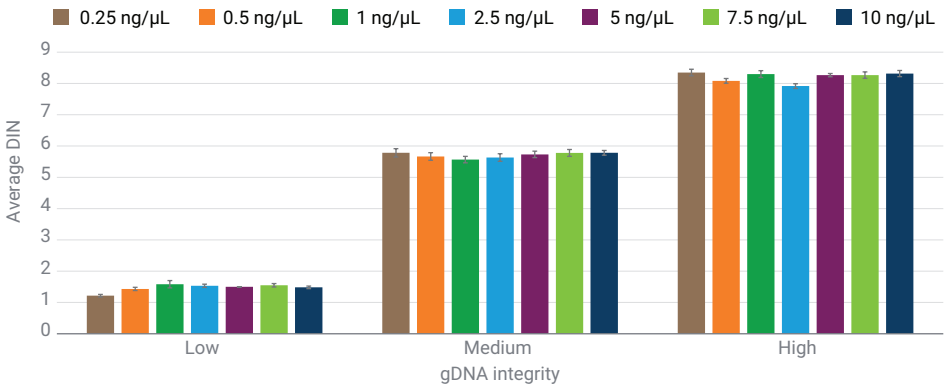

Figure 7. Integrity comparison across the DIN functional range of the Agilent High Sensitivity Genomic DNA ScreenTape assay using a BioChain Control Genomic DNA – Human Male sample at low, medium, and high DIN scores (N ≥ 6).

With the introduction of the DIN to the HS gDNA ScreenTape assay, the algorithm used to determine the score has been improved to ensure stability across the entire concentration range of both assays. The DIN functional range for the HS gDNA ScreenTape assay is 0.25 to 10 ng/µL, while the gDNA ScreenTape assay expands from 5 to 300 ng/µL. Dilutions of the OneSeq Reference DNA, Male that fall within the specified DIN range of both the gDNA ScreenTape and HS gDNA ScreenTape assays, overlapping at 5 to 10 ng/µL, were analyzed on each assay (Figures 8A and 8B). The average DIN for each concentration on the respective assays is shown in Figure 8C, highlighting the consistency of the gDNA ScreenTape assays for integrity analysis.

With the new HS gDNA ScreenTape assay, the DIN can be calculated with an electronic ladder, allowing users to use all 16 lanes of a ScreenTape device for integrity analysis of their samples. For accurate sizing and molarity calculation, a run ladder must be used. Figure 9 provides a comparison of a degradation series analyzed with both an in-run HS gDNA ladder and the electronic ladder. The DIN remains consistent across each sample independent of the ladder used, highlighting the reliability and flexibility of the HS gDNA ScreenTape assay for integrity analysis.

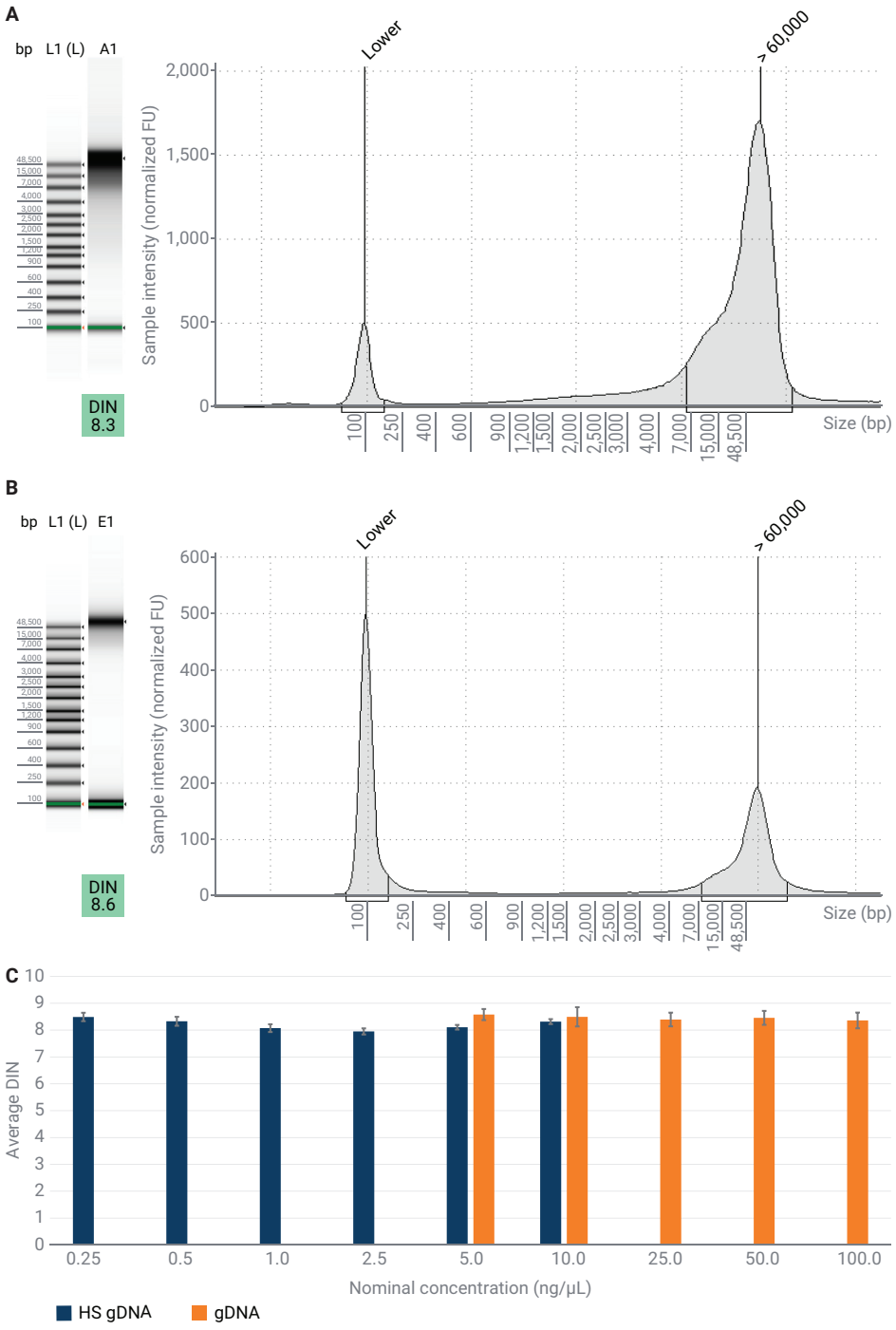

Figure 8. Integrity comparison across the DIN functional range of the Agilent High Sensitivity Genomic DNA ScreenTape assay (0.25 to 10 ng/µL) and Agilent Genomic DNA ScreenTape assay over a range of 5 to 100 ng/µL using a dilution series of a representative Agilent OneSeq Reference DNA, Male sample. Electropherogram of the OneSeq Reference DNA, Male at 10 ng/µL on the (A) High Sensitivity Genomic DNA assay and (B) Agilent Genomic DNA ScreenTape assay. (C) Comparison of DIN across concentration range of both assays (N = 18).

Conclusion

The Agilent High Sensitivity Genomic DNA ScreenTape assay expands the capabilities of the TapeStation platform by enabling reliable analysis of low concentration gDNA samples. This assay demonstrates robust performance across a broad dynamic range, with a lower detection limit of 20 pg/µL and a functional DNA Integrity Number (DIN) range beginning at 0.25 ng/µL. The examples shown in this technical overview highlight the high accuracy and precision of the assay for both sizing and quantification.

Importantly, the integration of an improved DIN algorithm ensures consistent and objective assessment of gDNA integrity across both the HS gDNA ScreenTape and gDNA ScreenTape assays. The reproducibility of DIN values across concentration ranges and degradation series demonstrates the reliability of this metric. Collectively, the HS gDNA ScreenTape and gDNA ScreenTape assays provide a comprehensive and robust solution for gDNA quality assessment, covering an expansive concentration range of 0.25 to 300 ng/µL. This QC step is essential for the success of downstream molecular workflows, including next-generation sequencing.

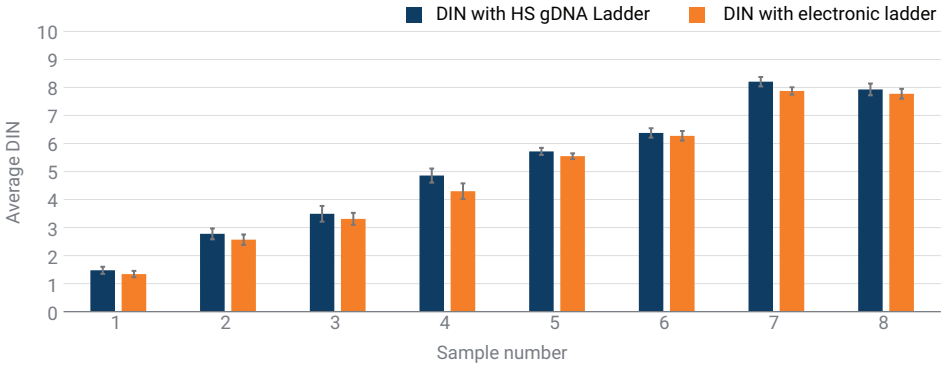

Figure 9. Degradation series of the BioChain Control Genomic DNA – Human Male sample at 5 ng/µL. Average DIN using an Agilent High Sensitivity Genomic DNA ladder in-run, compared to electronic ladder (N = 6).

References

1. Performance Characteristics of the Genomic DNA ScreenTape Assay for the 4150 TapeStation System, *Agilent Technologies technical overview*, publication number 5994-0497EN, **2019**.
2. High Sensitivity Genomic DNA ScreenTape Assay for TapeStation Systems, *Agilent Technologies quick guide*, publication number G2991-90140, **2025**.

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PR7003-83

Printed in the USA, August 28, 2025
5994-8592EN

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