

# Genomic DNA Quantification and Integrity Analysis with the Agilent 2200 TapeStation System in Comparison to Two Other Electrophoresis Systems

## Technical Overview

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### Introduction

The outcome of Next Generation Sequencing (NGS) and other molecular screening assays often rely on the overall quality of the genomic DNA (gDNA) starting material. It is therefore widely recommended to quantify and assess the integrity of the gDNA, especially for costly workflows such as NGS to prevent wasted time and resources.

Traditionally, agarose gel electrophoresis is used for gDNA analysis. This method only offers limited data though, as a second fluorometric or spectrophotometric method is required for gDNA quantification. In addition to being labor intensive and a manual process, it also does not provide a numerical assessment of the gDNA integrity. To address these limitations, automated electrophoresis systems, such as the Agilent 2200 TapeStation system, were developed. The 2200 TapeStation system, in conjunction with the Agilent Genomic DNA ScreenTape assay, provides an excellent automated solution for measuring the quantity and integrity of the gDNA starting material, as well as a numerical assessment of the gDNA integrity, using the DNA Integrity Number (DIN)<sup>1</sup>.

This Technical Overview compares the performance of the quantification analysis and the DNA integrity assessment with the 2200 TapeStation system to two other alternative electrophoresis systems on the market, a chip-based and a capillary-based electrophoresis system.



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## Experimental

### Materials

Commercially available intact human gDNA was purchased from Promega (Madison, WI, USA). A Digital Sonifier was used from Branson Ultrasonics (Danbury, CT, USA). Insulin syringes Omnican 50 (30G × 8 mm) were obtained from B. Braun Melsungen AG (Melsungen, Germany). The Qubit Fluorometer was from Thermo Fischer Scientific Inc. (Waltham, MA, USA). The Agilent 2200 TapeStation system (p/n G2965AA) with the TapeStation Analysis Software (A.01.05 (SR1)), Agilent Genomic DNA ScreenTape consumable (p/n 5067-5365), and Genomic DNA Reagents (p/n 5067-5366) were obtained from Agilent Technologies (Waldbronn, Germany).

### Samples

Commercially available gDNA was degraded using ultrasonication (10 % amplitudes, 2-second treatment, 5-second pause, up to 120-second treatment), or shearing with a fine gauge needle, or a combination of both to generate a set of 15 gDNA samples with a wide range of gDNA degradation. The gDNA concentration was determined using a fluorometer.

### gDNA analysis

The 2200 TapeStation system, in combination with the Genomic DNA ScreenTape assay, including the automatic determination of DIN, was used according to the manufacturer's instructions<sup>2</sup>.

In addition, the same set of samples was analyzed for direct comparison using two competitive systems according to instructions provided by the manufacturers. The concentration of the tested samples was adjusted according to the specified quantification range of the used system.

## Results and Discussion

### System and specification comparison of gDNA analysis

A set of 15 gDNA samples with varying degrees of degradation at a concentration of 60 ng/μL was analyzed using the 2200 TapeStation system and the Genomic DNA ScreenTape assay. The Agilent TapeStation Analysis Software (A.01.05 (SR1)) provides a gel image (Figure 1), an electropherogram for each of the samples, and automatically calculates the gDNA concentration and provides the DIN<sup>1</sup>.

The gel image in Figure 1 clearly shows an expected shift in DNA size and distribution with increasing degradation. Highly intact gDNA samples migrate as a well-defined peak above the largest ladder peak (48,500 bp). With increasing degradation, the main peak is shifted towards smaller sizes. Highly degraded

gDNA migrates as a broad peak with sizes below 2,000 bp. Figure 1 also demonstrates good reproducibility for the sample triplicates.

To allow a direct comparison between the electrophoresis systems, the same set of 15 gDNA samples was analyzed with a chip-based system (Figure 2A) and a capillary-based system (Figure 2B).

Table 1 summarizes the specifications for gDNA analysis using the three different electrophoresis systems as provided by the manufacturers. The 2200 TapeStation system offers a significantly larger sizing range compared to the two other electrophoresis systems. It also provides more flexibility for the number of samples analyzed and shorter analysis times. Only the capillary-based system equipped with a 96-capillary head provides faster analysis for 96 samples.

Table 1. Comparison of the specifications for gDNA analysis with the Agilent 2200 TapeStation system, a chip-based system and a capillary-based system.

| Specifications for gDNA analysis | Agilent 2200 TapeStation system  | Chip-based system   | Capillary-based-system   |
|----------------------------------|--|---|--|
| Quantification                   |  |   |  |
| Quantification range             | 10–100 ng/μL   | 2–50 ng/μL  | 5–200 ng/μL <sup>2</sup>   |
| Accuracy                         | ± 20 % <sup>1</sup>  | ± 30 % <sup>3,4</sup>   | ± 25 % <sup>4</sup>  |
| Precision                        | 15 %CV <sup>1</sup>  | 20 %CV <sup>4</sup>   | 15 %CV <sup>4</sup>  |
| Sizing                           |  |   |  |
| Sizing range                     | 200 to > 60,000 bp   | 50–40,000+ bp   | 50–20,000 bp   |
| Accuracy                         | ± 15 % (200–15,000 bp) <sup>2</sup>  | ± 20 % (up to 10,000 bp) <sup>2</sup>                         | Not provided   |
| Precision                        | 15 %CV (200–15,000 bp) <sup>2</sup>  | 20 %CV (up to 10,000 bp) <sup>2</sup>                         |  |
| Sample volume required           | 1 μL   | 20 μL (undiluted) <sup>5</sup><br>2 μL (diluted) <sup>5</sup> | 2 μL   |
| Samples per run                  | Flexible (1–15 samples + ladder per ScreenTape), Scalable up to 96 samples | 24 or 48 samples (2 workflows)                                | 12-Capillary: 11 + ladder<br>96-Capillary: 95 + ladder   |
| Analysis time                    | 16 samples < 25 minutes<br>96 samples < 150 minutes                        | 24 samples = 90 minutes<br>48 samples = 150 minutes           | <b>12-capillary head</b><br>12 samples = 50 minutes<br>96 samples < 7 hours<br><b>96-capillary head</b><br>96 samples = 50 minutes |

<sup>1</sup> Average results from various gDNA sample types

<sup>2</sup> Based on DNA ladder

<sup>3</sup> Based on PicoGreen and plate reader quantitation of human gDNA

<sup>4</sup> Using human gDNA as standard

<sup>5</sup> 96-well plate

Due to the restricted quantification range of the chip-based electrophoresis system (Table 1), the concentrations of the tested samples had to be selected based on the specified quantification range to enable a fair comparison.

Figure 2A shows the analysis of the gDNA samples as duplicates and blanks to check for capillary contamination between sample injections using the chip-based system. Surprisingly, the lanes with the gDNA samples of highest

integrity (1 and 2), show only a very weak signal, especially in contrast to Figure 1. Evidently, only a small amount of sample was injected, which caused a quantification bias (Figure 3B).

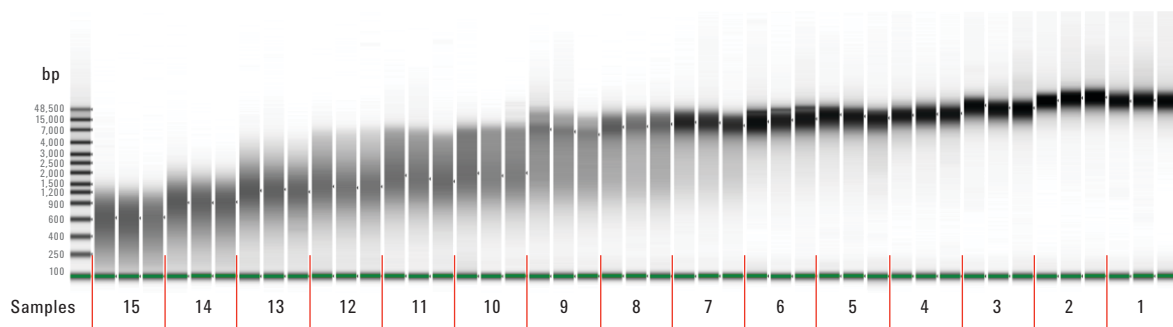


Figure 1. A degradation series of 15 gDNA samples (triplicates at 60 ng/μL) was analyzed using the Agilent 2200 TapeStation system and the Agilent Genomic DNA ScreenTape assay.

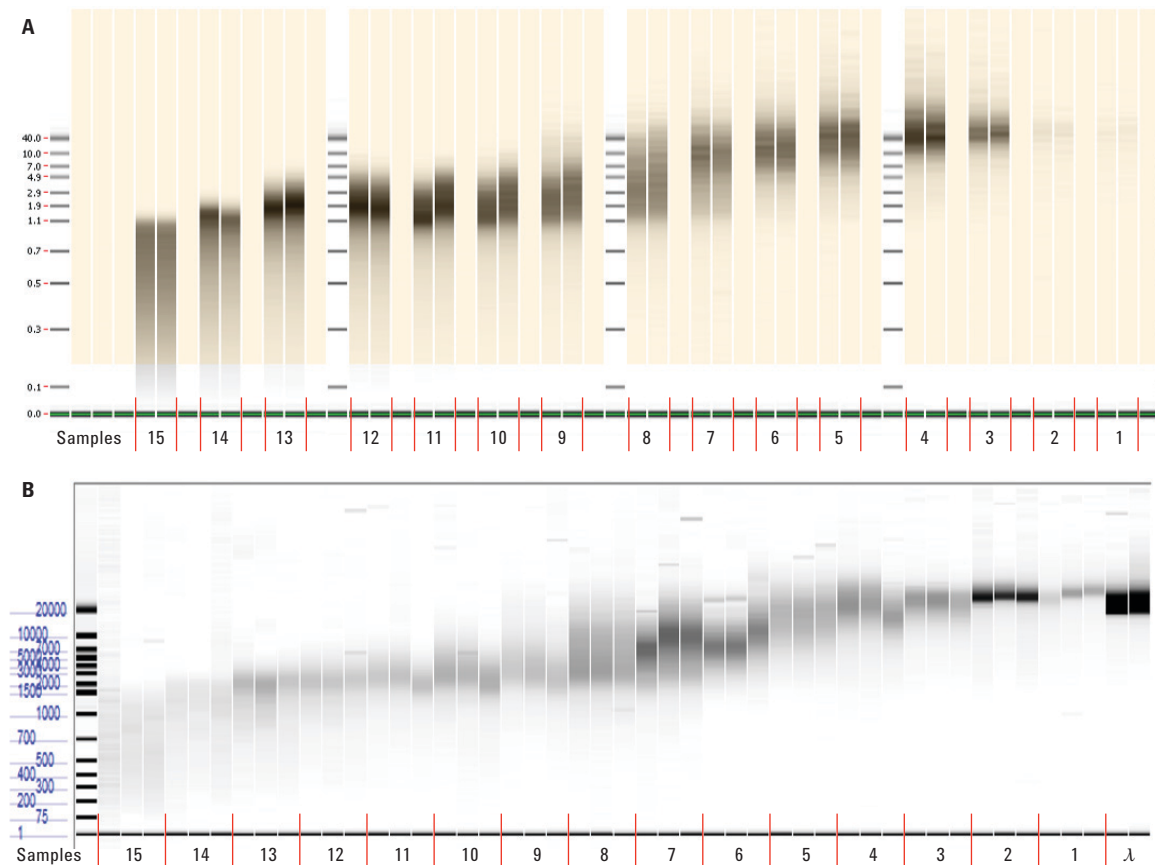


Figure 2. The set of 15 gDNA samples analyzed on the Agilent 2200 TapeStation system was also analyzed on the competitor's systems. The analysis using the chip-based system (panel A) was performed in duplicates (at 30 ng/μL), a blank was run between two samples. Triplicates (at 60 ng/μL) were analyzed using the capillary-based system (panel B). The two last lanes show the analysis of λ DNA.

In contrast to the 2200 TapeStation system (Figure 1), the gel in Figure 2B, obtained with the capillary-based system, shows a relatively high degree of variation between the triplicates. Similar to the observation made for the capillary-based system, the signal for the gDNA sample with the highest DNA integrity (sample 1) is relatively low, indicating an issue with injecting highly intact gDNA. The capillaries are auto-flushed between runs to eliminate cross contamination, which was not observed in blanks between the samples (not shown), but samples and blanks contain several spikes of unknown cause. It is not clear, if and how these spikes affect the gDNA quantification and the DNA integrity analysis.

### Quantification analysis of samples with different degradation stages

Since Figures 2A and 2B indicate a bias in quantification of intact gDNA for the chip-based and the capillary-based system, the quantification results of all systems were compared. Depending on the differences of the specified quantification range of the systems, three to four different concentrations of three gDNA samples with varying DNA integrity were analyzed (Figure 3).

Figure 3 summarizes the concentrations obtained when analyzing three gDNA samples with low DNA integrity (sample 15), intermediate integrity (sample 8), and high integrity (sample 1) at three to four different concentrations (each n = 3).

Figure 3A clearly demonstrates that the DNA quantification with the 2200 TapeStation system is not dependent on DNA integrity, in contrast to the other two systems, which failed to provide a reliable quantification for the sample with the highest integrity (Figures 3B and 3C). Our results indicate that the chip-based system and the capillary system are less suitable to quantify gDNA samples with high integrity.

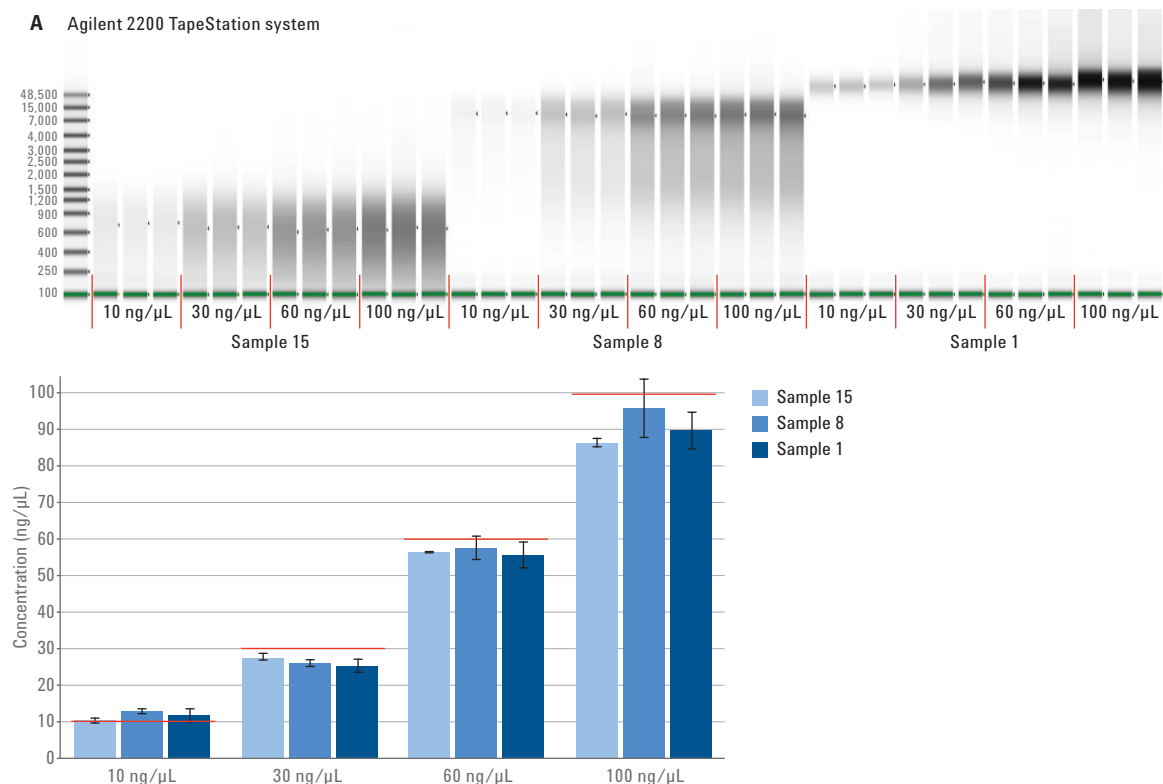


Figure 3A. A subset of three gDNA samples with low DNA integrity (sample 15), intermediate integrity (sample 8) and high integrity (sample 1) was analyzed. To cover the specified quantification ranges for the three electrophoresis systems, the analysis was performed at three to four different concentrations (each n = 3) as indicated in the figure. The nominal concentrations were determined using a fluorometer, and are indicated as red lines in the graphs.

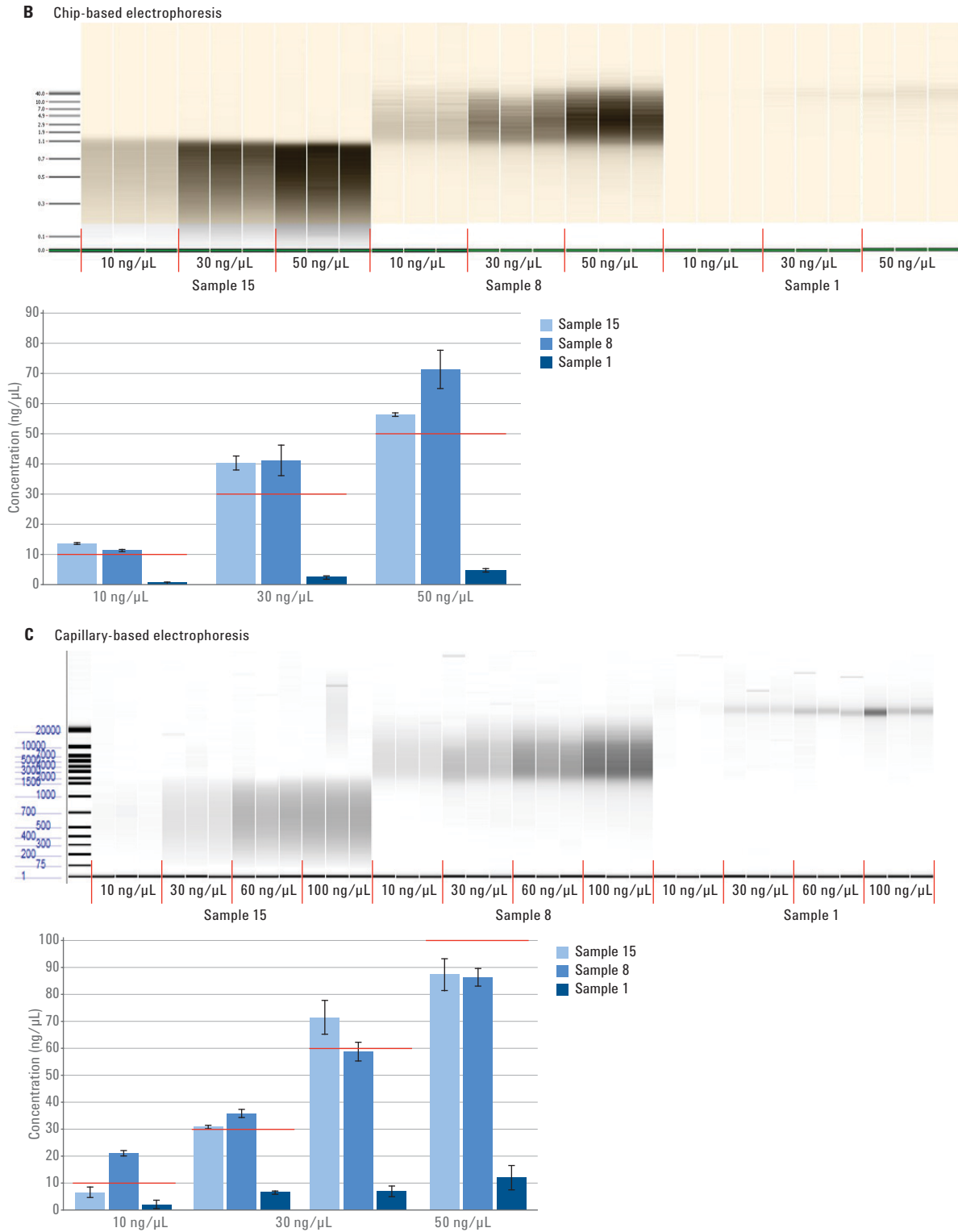


Figure 3. A subset of three gDNA samples with low DNA integrity (sample 15), intermediate integrity (sample 8) and high integrity (sample 1), was analyzed. To cover the specified quantification ranges for the three electrophoresis systems, the analysis was performed at three to four different concentrations (each n = 3) as indicated in the figure. The nominal concentrations were determined using a fluorometer, and are indicated as red lines in the graphs.

### Sample integrity assessment across the entire degradation range

Reliable gDNA integrity analysis is also a critical parameter for experimental success. Therefore, the DIN obtained with the 2200 TapeStation system was directly compared to the numerical DNA integrity assessment with the chip-based system and the capillary-based system to investigate each system's ability to determine qualitative gDNA data.

The 2200 TapeStation system automatically provides DIN, ranging from 1 to 10. A high DIN indicates highly intact gDNA, and a low DIN indicates a strongly degraded gDNA sample (Figure 4). Similarly, the chip-based electrophoresis system provides the gDNA Quality Score (GQS) as a quality metric based on the size distribution of gDNA. The GQS ranges from 0 to 5, with 5 representing the highest gDNA quality. The capillary-based system provides the Genomic Quality Number (GQN) ranging from 0 to 10.

In general, Figure 4 shows that all three systems assign a low DNA integrity value for samples with low gDNA integrity, and a high DNA integrity value is assigned to samples with high gDNA integrity.

The direct comparison of the obtained DIN or GQS values in Figure 4 demonstrates that DIN clearly distinguishes gDNA in the low integrity range (samples 15 to 10), whereas the GQS values do not provide a differentiation. In addition, the GQS value reaches a maximum for sample 3, and the GQS decreases despite increasing gDNA integrity for samples 2 and 1. High molecular weight DNA is probably not fully injected, causing a quantitative and qualitative bias.

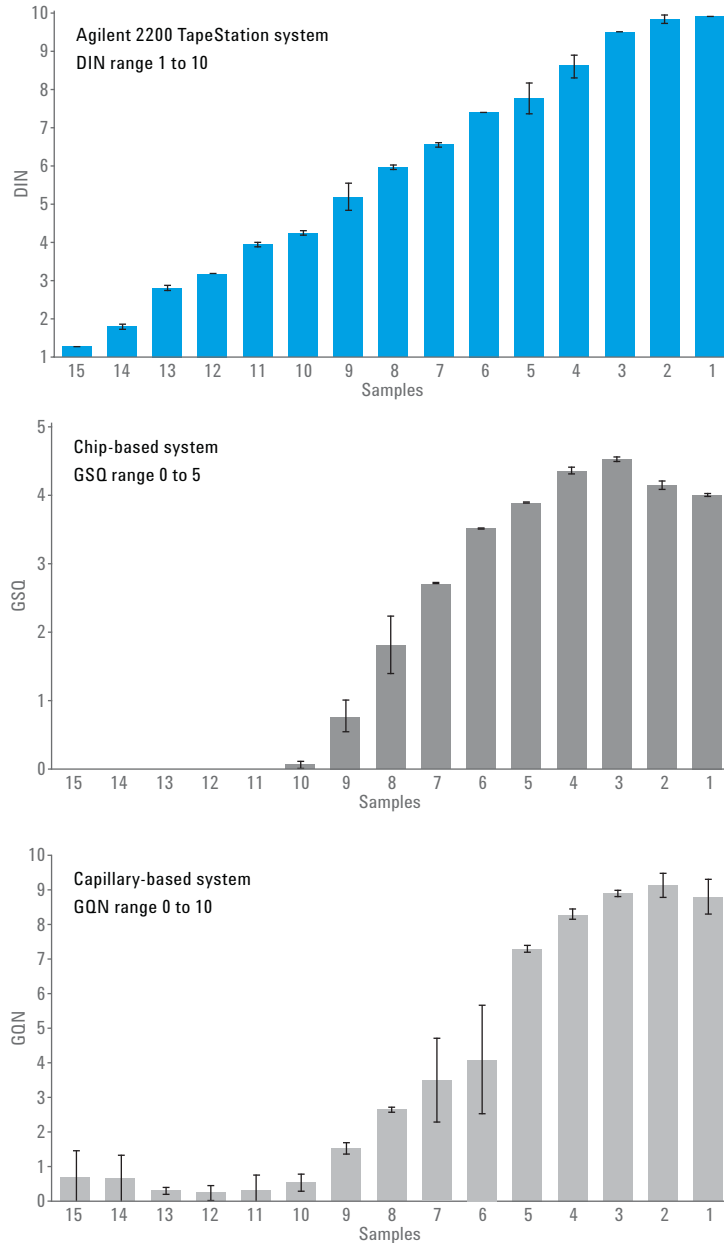


Figure 4. DIN obtained with the Agilent 2200 TapeStation system (n = 4 to 5) was directly compared to the numerical DNA integrity assessment with the chip-based system (n = 2), GQS, and the capillary-based system (n = 3), GQN.

Similarly, the comparison of the DIN or GQN values in Figure 4 shows that only DIN distinguishes gDNA in the low integrity range (samples 15 to 10). The obtained GQN values do not provide a significant differentiation in this integrity range. In addition, the GQN value seems to flatten for the high integrity gDNA samples. GQN is unable to provide sufficient differentiation of sample integrity across the entire degradation range. A relatively large GQN error was observed for several samples, which is in agreement with unspecific spikes observed in the gel images in Figure 2B.

We also verified how blanks or samples with concentrations below the specified concentration range were handled by the three electrophoresis systems. The chip-based system or the 2200 TapeStation system did not assign DNA integrity values to blanks. The 2200 TapeStation system has a specified DIN functional range from 5 to 300 ng/ $\mu$ L. Based on an excellent exception handling, samples outside this exceptionally large DIN functional range are marked with a red alert icon above the gel image, and DIN is not determined.

Conversely, the capillary-based system misleadingly determined GQN in blanks. The average GQN for 43 blanks was  $6.3 \pm 1.7$ . Astonishingly, the average GQN value assigned to blanks was in the upper range and, therefore, usually assigned to a gDNA sample with relatively good integrity. This is critical when analyzing samples with low gDNA concentrations.

## Conclusion

This Technical Overview demonstrates that, in comparison to the chip-based system, and the capillary-based system, only the Agilent 2200 TapeStation system:

- Allows the differentiation of a wide range of samples from highly intact gDNA (DIN 10) to very degraded gDNA (DIN ~1)
- Provides reliable quantification of gDNA samples, even with high integrity
- Uses excellent exception handling for blanks or samples with concentrations outside the functional range (5 to 300 ng/ $\mu$ L), ensuring reliable DIN assessments

Only the superior 2200 TapeStation system provides the required combination of quantitative and qualitative data, ease of use, and shorter analysis times simplifying the critical sample quality control of gDNA starting material without any compromise in performance.

## References

1. DNA Integrity Number (DIN) with the Agilent 2200 TapeStation System & Genomic DNA ScreenTape, *Agilent Technologies*, publication number G5991-5258EN, **2014**.
2. Agilent Genomic DNA ScreenTape System Quick Guide, *Agilent Technologies*, publication number G2964-90040 rev.C, **2014**.

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