Technical Overview

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Sample Measurement and Analysis Best Practices for Agilent TapeStation Systems

Introduction

Quality control (QC) is essential in most workflows for optimal sample results. The Agilent TapeStation systems are specifically designed for the QC of nucleic acids, offering scalable throughput and rapid results. These instruments are ideal for applications such as next-generation sequencing (NGS), where they can assess the condition of incoming samples and monitor quality throughout the library preparation process. The systems provide dependable performance in quantification and sizing.

Operating the system is straightforward: select the appropriate Agilent ScreenTape assay, prepare the samples as instructed, and set up and analyze the run in the TapeStation software. The 4150 TapeStation system is an economical, low-throughput automated electrophoresis platform that can handle up to 16 samples per run. In contrast, the 4200 TapeStation system is a high-throughput platform capable of processing up to 96 samples per run. Each system completes separation, imaging, analysis, and result presentation in approximately one to two minutes per sample.

For optimal results, it is advised to strictly follow the instructions for reagent and sample preparation, as well as instrument maintenance, during assay preparation. This technical overview outlines best practices for achieving reliable quantification and sizing results using the assays available for the 4150 and 4200 TapeStation systems.

Calibrated tools and validated equipment

Using calibrated pipettes of the appropriate volume size is crucial for optimal sample and reagent setup. Accurate pipetting technique is crucial to maintain precision in the volumes used for each assay, allowing for correct concentration calculations.

For best results, use Agilent-supplied consumables, such as loading tips (p/n 5067-5598), tube strips (p/n 401428), tube strip caps (p/n 401425), and 96-well sample plates (p/n 5042-8502) with 96-well plate foil seals (p/n 5067-5154). The TapeStation systems are calibrated to the dimensions of these validated consumables. Using other consumables may lead to blockage errors or insufficient volume being drawn by the instrument pump.

Sample preparation guidelines and assay selection

To obtain optimal results, follow the quick guides and use the exact volumes for both the sample and sample buffer, as depicted in Figure 1. Deviations from the specified volumes can negatively impact the results generated by the system.

The systems are compatible with DNA and RNA analysis assays. When selecting an assay, consider the sample type, expected size, and sample concentration. Table 1 outlines the available assays and their recommended applications. Each assay covers a specific concentration range, so users must choose the appropriate assay for their expected sample concentration. For example, if an RNA sample has an expected concentration of 50 ng/µL, the Agilent RNA ScreenTape assay should be used. Conversely, if the RNA sample has an expected concentration of 1 ng/µL, the Agilent High Sensitivity RNA ScreenTape assay should be used. Using concentrations outside the specified quantitative ranges for each assay will result in suboptimal analysis results.

Table 1. Agilent DNA and RNA ScreenTape assays compatible with the Agilent 4150 and 4200 TapeStation systems, along with their corresponding applications.

ScreenTape Assay Type	ScreenTape Assay Name	Quantitative Range	Applications
DNA	Genomic DNA	10 to 100 ng/µL (DIN functional range: 5 to 300 ng/µL)	Analysis of genomic DNA quality and quantity, incl. sizing for samples ranging from 200 to over 60,000 bp
	D1000	0.1 to 50 ng/µL	Sizing and quantification of DNA fragments and smears from 35 to 1,000 bp
	D5000	0.1 to 50 ng/µL	Sizing and quantification of DNA fragments and smears from 100 to 5,000 bp
	High Sensitivity D1000	10 to 1,000 pg/µL	High sensitivity analysis (sizing and quantification) of DNA fragments and smears from 35 to 1,000 bp
	High Sensitivity D5000	10 to 1,000 pg/µL	High sensitivity analysis (sizing and quantification) of DNA fragments and smears from 100 to 5,000 bp
	Cell-free DNA	100 to 4,000 pg/µL (%cfDNA functional range: 100 to 5,000 pg/µL)	Analysis of cell-free DNA quality and quantity, incl. sizing from 50 to 800 bp
RNA	RNA	25 to 500 ng/µL (identical for RIN^e functional range)	Analysis of total RNA quality and quantity
	High Sensitivity RNA	500 to 10,000 pg/µL (RIN^e functional range: 1,000 to 25,000 pg/µL)	High sensitivity analysis of total RNA quality and quantity

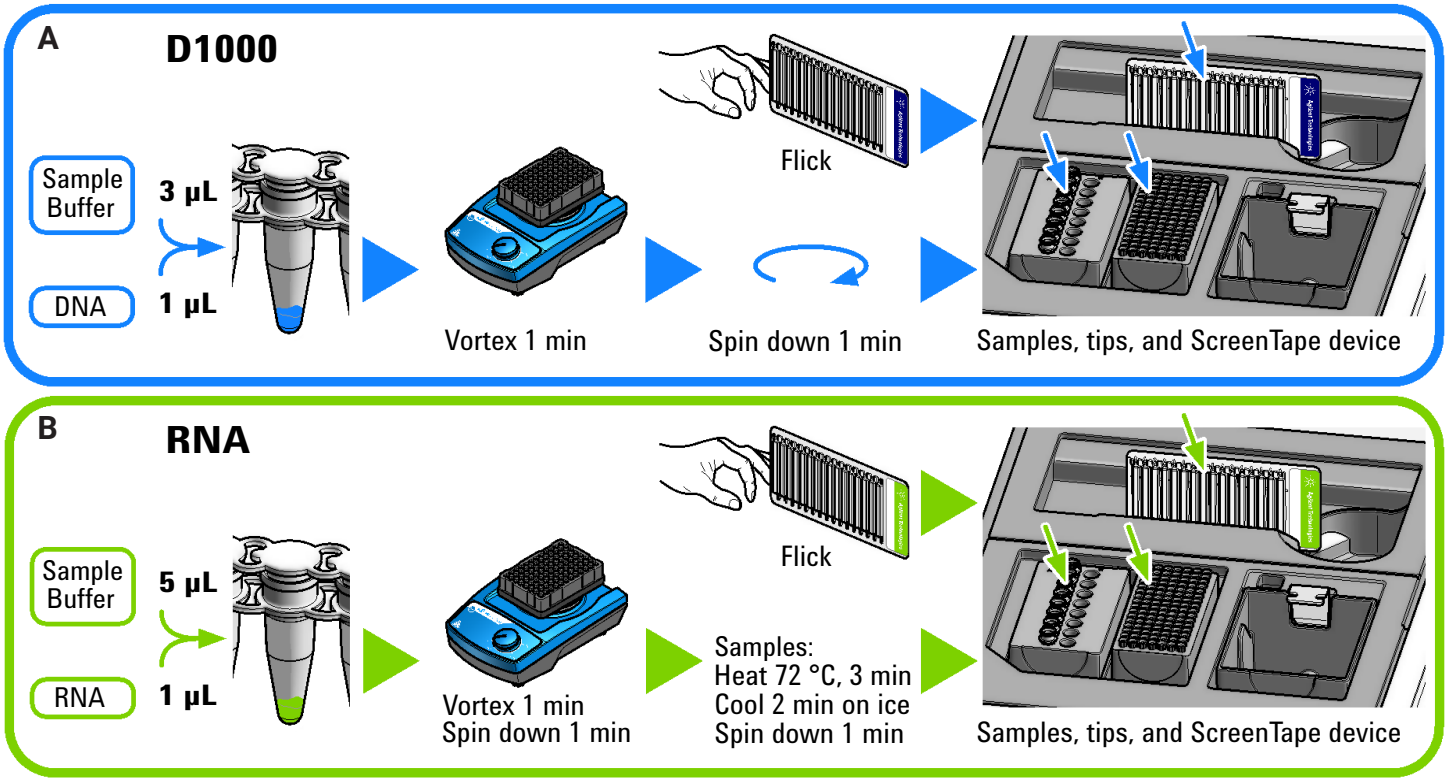

Figure 1. Example workflows for Agilent DNA and RNA ScreenTape assays on the Agilent 4150 TapeStation system, specifically the (A) Agilent D1000 ScreenTape assay and the (B) Agilent RNA ScreenTape assay.

Optimizing sample mixing

For mixing purposes, it is recommended to use a vortex mixer specifically designed for 8-well PCR tube strips or a 96-well plate. An IKA MS3 vortex mixer, bundled with the TapeStation systems, includes a 96-well plate adaptor suitable for both 8-well PCR tube strips and 96-well plates. While optional, this vortex mixer is recommended for use with all ScreenTape assays. If using a different vortex mixer, ensure thorough mixing by vortexing for one minute at maximum speed. Moreover, when using a 96-well plate, employ the 96-well plate foil seals (p/n 5067-5154) to prevent sample escape during vortexing. After vortexing, spin down the samples using a centrifuge to ensure all the sample settles at the bottom of the tube before analysis on the TapeStation system to avoid injection errors.

To emphasize the importance of proper mixing, samples were combined with buffer using various methods and analyzed on the TapeStation with the Agilent D1000 ScreenTape assay. Insufficient mixing resulted in discrepancies in the reported sample concentration, as indicated by the optical intensity of the gel image and the peak heights in the electropherograms (Figure 2). This outcome demonstrates the need to follow the recommended mixing procedure for accurate quantification results.

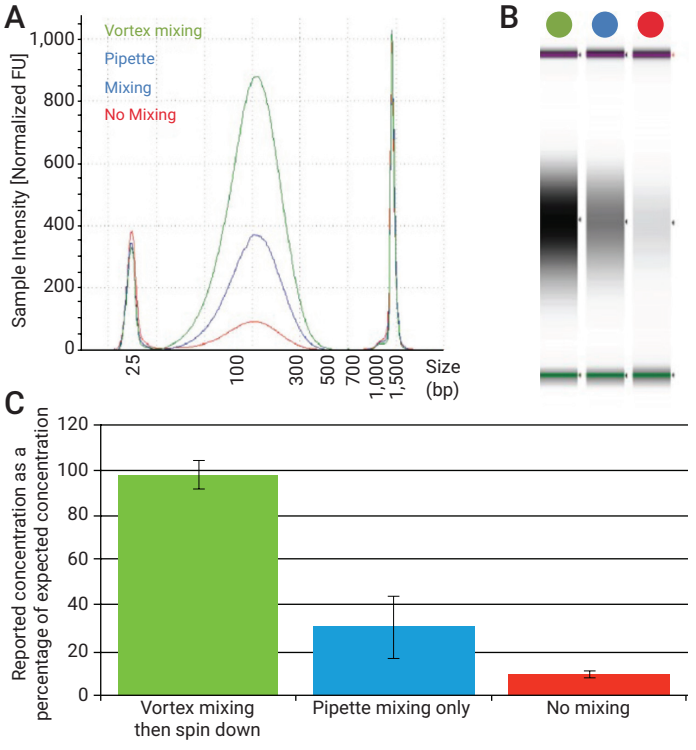

Figure 2. (A) Representative electropherogram and (B) gel image of the Agilent D1000 ScreenTape assay using different mixing methods. The green trace from the electropherogram and the green dot from the gel image show the results of the recommended protocol of vortex mixing using the IKA vortexer and adaptor at 2,000 rpm for 1 minute, followed by brief centrifugation. The blue trace shows results for pipette mixing only, and the red trace shows the effect of no mixing. Images were taken from the Agilent TapeStation software. (C) Reported concentrations for the D1000 ScreenTape mixing tests, where concentrations are expressed as a percentage of the theoretical for the three methods.

Consumables preparation

ScreenTape devices must be stored properly at temperatures between 2 to 8 °C. Frozen ScreenTape devices must be discarded, as their performance will be significantly altered after freezing (Figure 3A). When using the ScreenTape, be aware of potential bubbles in the buffer chamber. Bubbles at the gel interface can compromise the run's performance, resulting in smudged or diagonal bands (Figure 3B), which may affect band identification in the TapeStation software.

To mitigate this, gently flick the ScreenTape device before inserting it into the TapeStation instrument. This action helps move any bubbles to the top of the chamber, ensuring they do not interfere with sample loading (see Figure 3C).

Before using TapeStation reagents (ladder and buffer), ensure they are equilibrated to room temperature for 30 minutes, vortex mixed, and spun down. ScreenTape devices can be used directly from the fridge with no equilibration time.

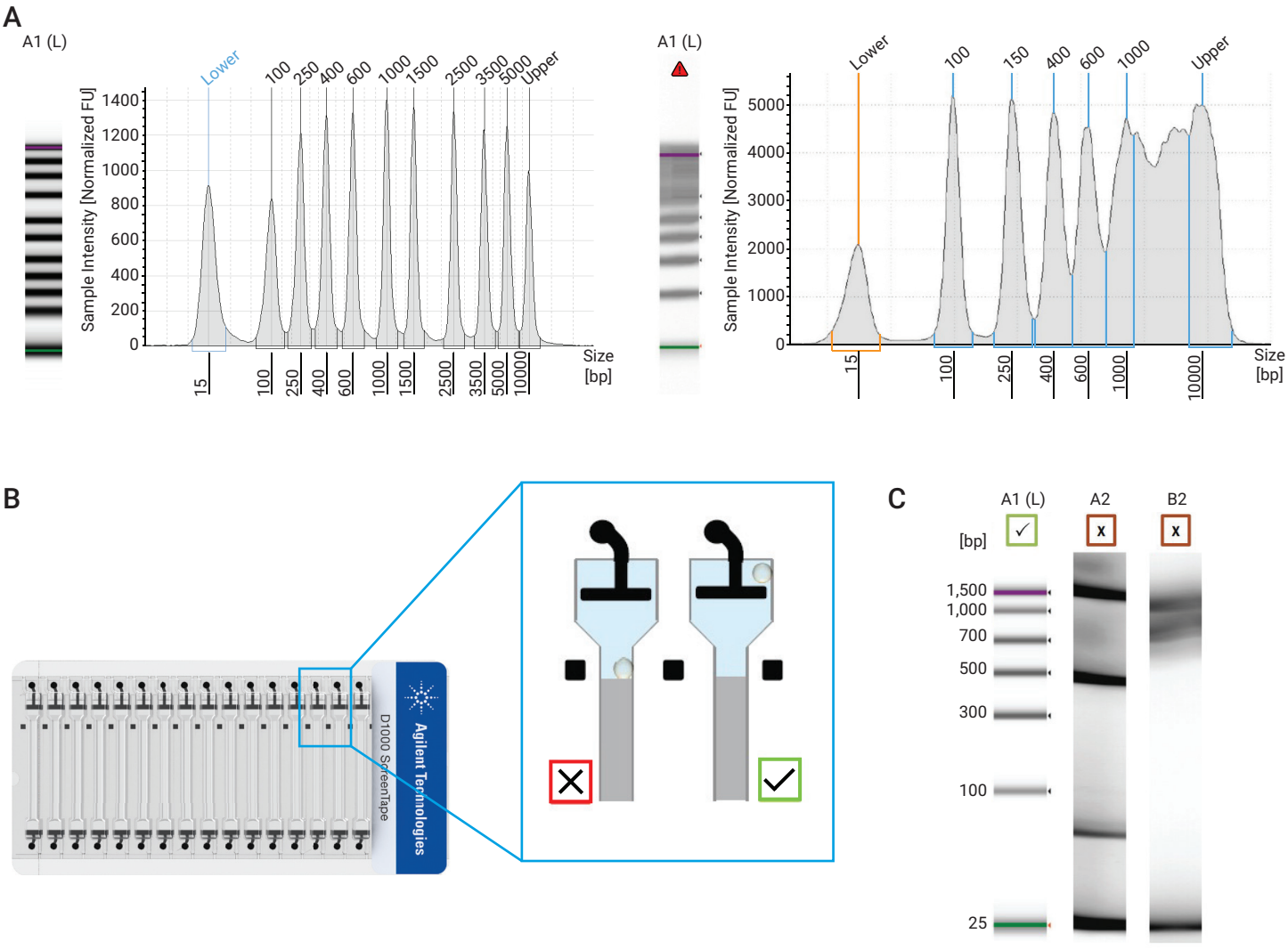

Figure 3. (A) A properly stored Agilent ScreenTape device (left) and the effect of using Agilent ScreenTape devices that have been frozen (right). (B) Flicking the ScreenTape device causes any bubbles trapped at the top of the gel lane to migrate to the top of the device, no longer hindering sample loading. (C) A side-by-side comparison of ScreenTape gel images: one that has been flicked, causing any present bubbles to move out of the way of sample injection, resulting in a clear ladder in lane A1. In contrast, a ScreenTape that was not flicked shows the result of a bubble blocking the loading of the sample, as seen in lanes A2 and A3.

Salt concentration

Samples that are extracted or stored may be suspended in buffers containing salt, which adds ionic components to the sample. If the salt concentration is too high, it can affect electrophoresis. ScreenTape assays are designed to compensate for these potential negative effects; however, the sample matrix must not exceed a maximum salt concentration. Specifically for the RNA assays NaCl should not be in excess of 50 mM for the RNA assay and not above 0 mM for the HS RNA assay.

As shown in Figure 4, a sample was run multiple times with increasing salt concentrations. The results indicate that increased salt slows the migration of the sample, causing improper alignment with the ladder and leading to inaccurate sizing assessment. Therefore, it is imperative to check the buffer compatibility for every ScreenTape assay to ensure ideal assay performance.

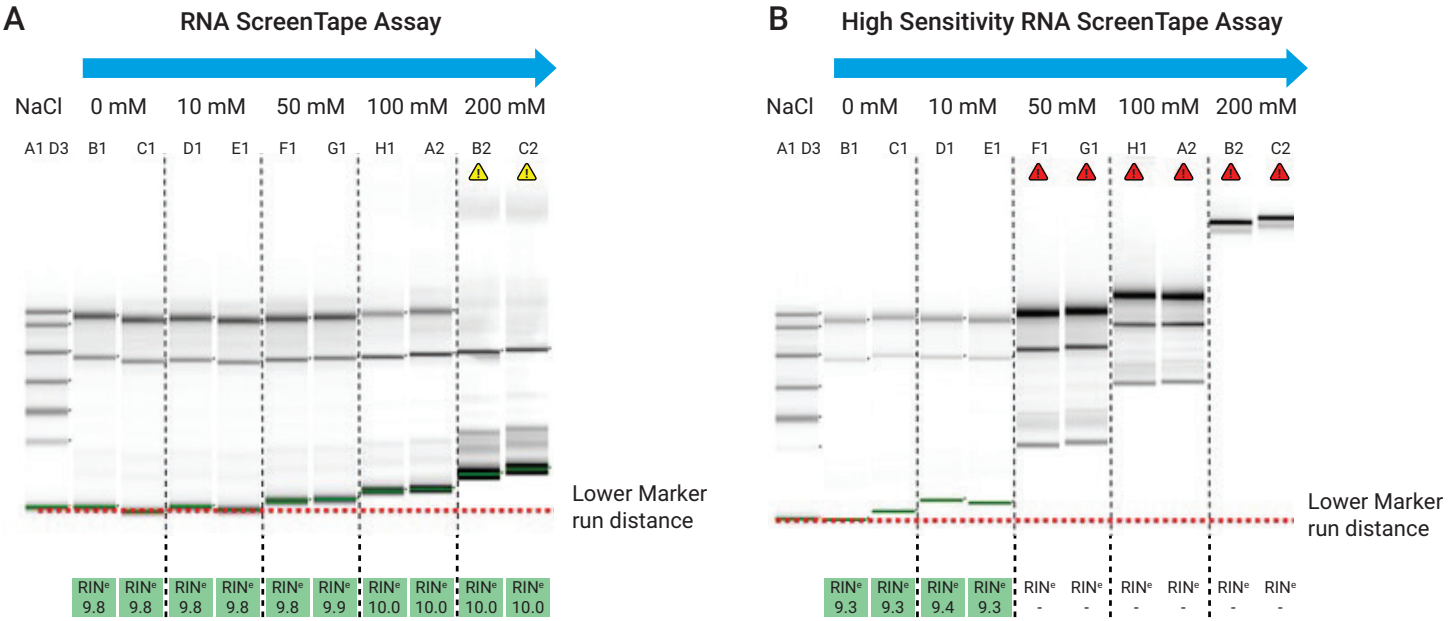

Figure 4. Representative examples of how increasing salt concentrations in the sample matrix affect the Agilent (A) RNA and (B) High Sensitivity RNA ScreenTape assays, shown in the unaligned gel view of the Agilent TapeStation software.

Assay specific best practices

Before pipetting the ladder into the TapeStation-approved tube strips or 96-well plate, handle the ladder vial with care. For example, when handling the genomic DNA ladder, avoid shaking the vial and mix only by vortexing. Excessive shaking can potentially degrade the largest ladder fragment.

RNA assays

Heat denaturation is an essential step in the sample preparation workflow for RNA ScreenTape assays. For optimal results, it is recommended to use an accurately calibrated heat block or a thermal cycler that fits 200 µL vials for the heat denaturing process. Accurate temperature control is necessary to ensure the integrity and quality of the RNA samples.

Before initiating the heating step, properly close the tube strips with the tube strip caps (p/n 401425) or seal the 96-well sample plates with the supplied foil seal (p/n 5067-5154). This step is critical to prevent evaporation during the heating process, which can lead to sample loss and concentration changes. Ensuring a tight seal will maintain the sample volume, leading to more reliable and reproducible results. Additionally, following these steps helps to minimize the risk of contamination and degradation of the RNA, which is particularly sensitive to environmental conditions.

Best practices for TapeStation data analysis

In the TapeStation software, accurate assignment of the upper and lower markers is necessary. These markers act as internal references, enabling the software to determine the molecular weight and size of each sample peak. Incorrect marker identification can lead to inaccuracies when reporting sizing values and concentrations. Therefore, precise marker assignment is key for reliable results.

Discrepancies such as misalignment of sample peaks, unexpected fragment sizes, or bands running further or shorter than parallel runs can occur. Figure 5 illustrates an instance of incorrect lower marker identification alongside a corrected file where the lower marker is properly identified. Both gel images depict lanes representing the same sample at varying concentrations. The left image in Figure 5 demonstrates the incorrect lower marker identification, resulting in sample misalignment and incorrect fragment size reporting. By manually assigning the correct lower marker (right-clicking on the peak and selecting "assign marker"), as shown in the right image in Figure 5, accurate sizing information is obtained.

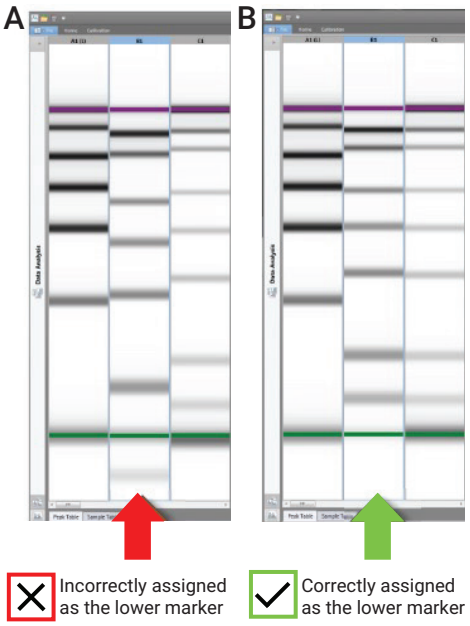

Figure 5. Screenshots of the Agilent TapeStation software displaying (A) incorrect lower marker identification in the middle lane of the gel image, and (B) corrected lower marker in the middle lane. The lower marker is highlighted with green.

Electropherogram vs. region view analysis

Within the TapeStation software, users can choose between the electropherogram view and the region view of the sample. Select the appropriate view based on the type of sample and desired analysis.

The electropherogram view is designed for use with discrete peaks, reporting the size at the highest point of the peak. In contrast, the region view is tailored for samples that appear as a smear, such as NGS libraries. This view calculates data over the entire smear or region and reports the average size of the region, providing an idea of the distribution of sizes within the sample (Figure 6).

By adding or modifying regions in the region settings, users can address the specific analysis needs of different sample types. This also allows for the individual analysis of several regions in the same sample.

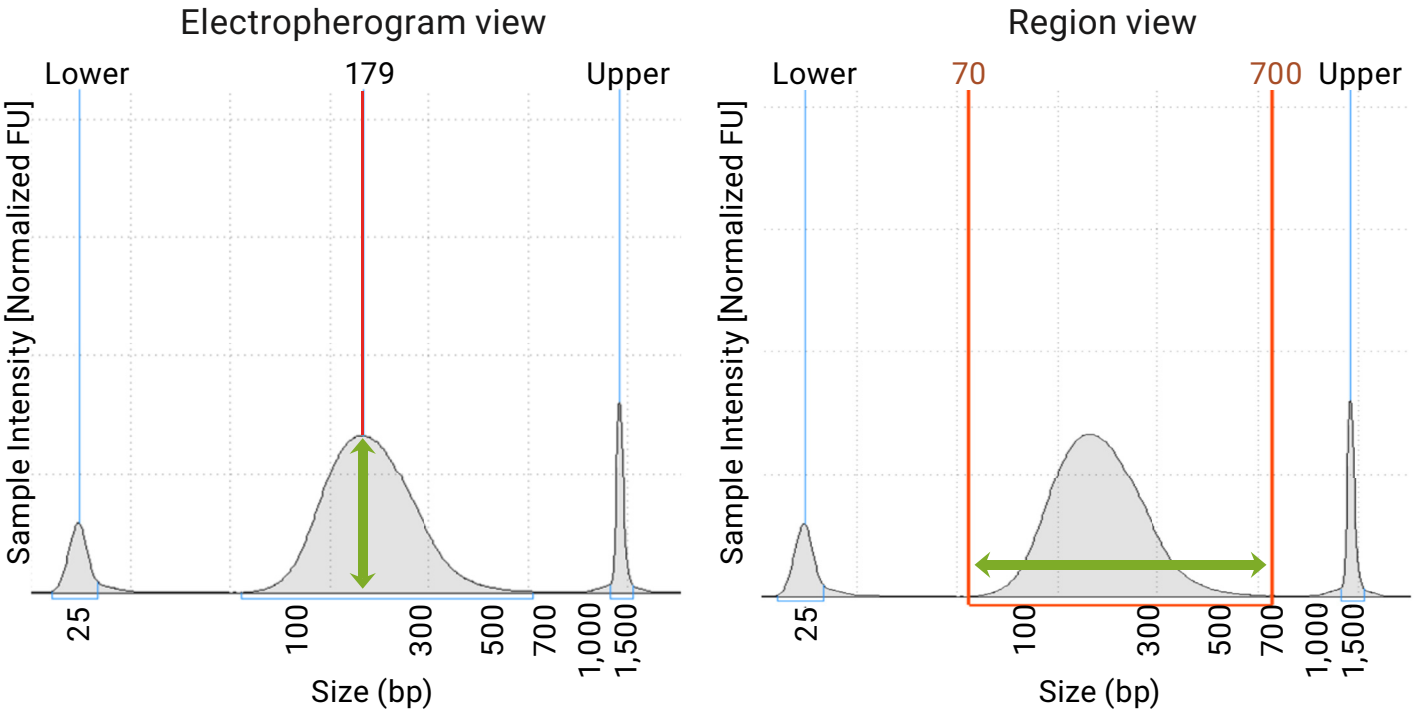

Figure 6. Sizing data displayed in electropherogram and region views within the Agilent TapeStation software.

Electropherogram appearance

The TapeStation software offers flexibility in customizing the electropherogram's appearance for better visualization. For instance, the electropherogram can be made to resemble the electropherogram appearance of the Agilent Bioanalyzer system. To do this, select the "File" tab in the TapeStation software and then choose "Settings." The electropherogram color can be set to either black with filled peak areas or red with empty peak areas. In addition, there is an option to modify the background to display either equidistant grid lines or ladder-based grid lines (Figure 7).

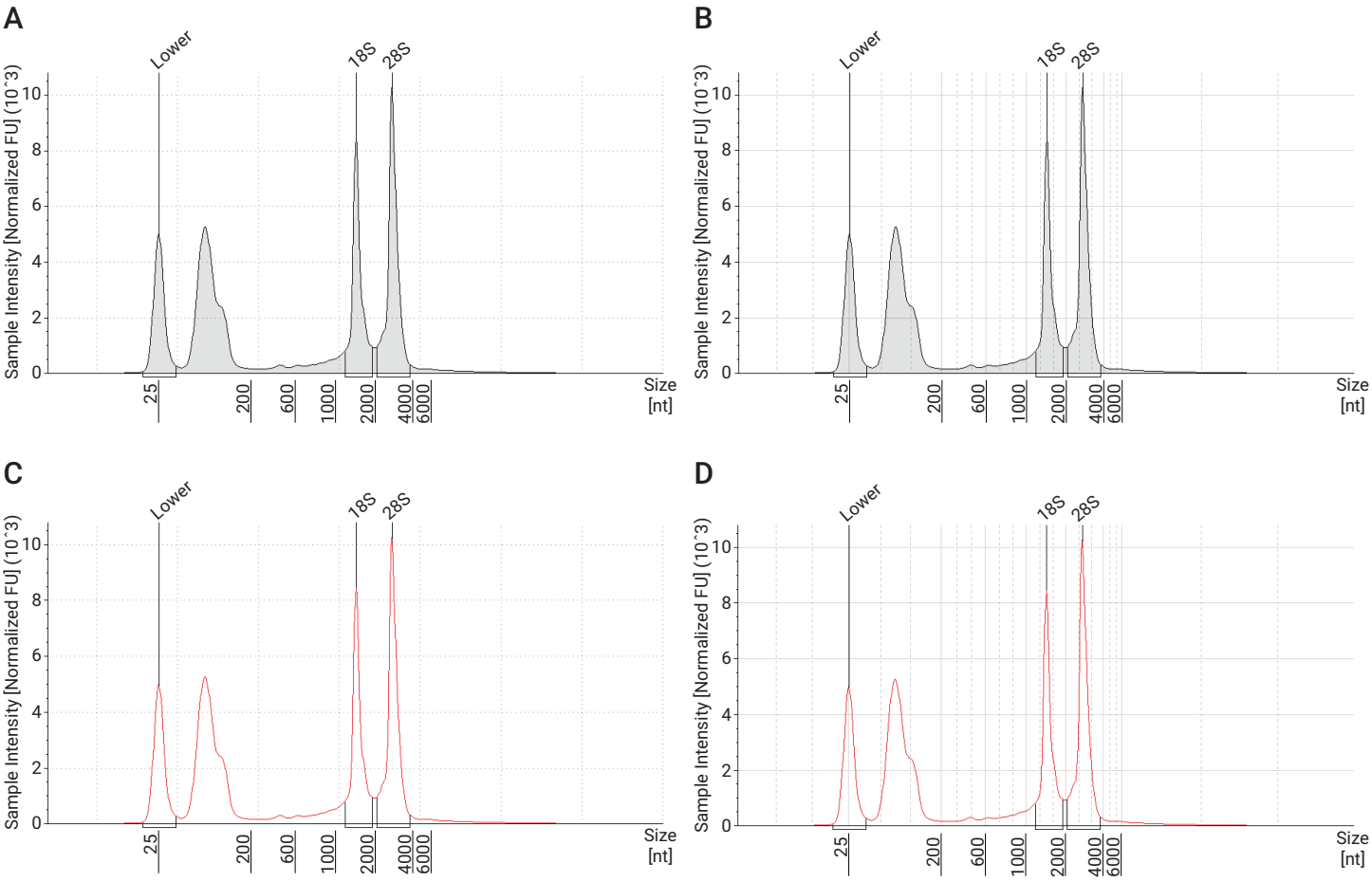

Figure 7. Agilent TapeStation system electropherograms showing different visual appearances: (A) Black, filled peak area with equidistant grid lines, (B) Black, filled peak area with ladder-based grid lines, (C) Red, empty peak area with equidistant grid lines, and (D) Red, empty peak area with ladder-based grid lines.

How to obtain further support

For detailed technical information, see the TapeStation User Manual[1,2] and other supporting documents.

For additional support, contact our sales/support team by phone or email (https://www.agilent.com/en/contact-us/page). Join the Agilent Community (https://community.agilent.com) to access self-help articles, connect with customers, and share insights.

Conclusion

The Agilent TapeStation systems offer scalable throughput and automation, making the instruments an excellent choice for QC of biological samples in NGS, microarray, and quantitative PCR workflows. By adhering to the recommended best practices outlined in this technical overview, users can achieve reliable and accurate quantification and sizing with ease.

References

1. Agilent 4150 TapeStation System, *Agilent System Manual*, Document No: SD-UF0000165, **2022**
2. Agilent 4200 TapeStation System, *Agilent System Manual*, Document No: SD-UF0000087, **2022**

www.agilent.com/genomics/tapestation

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