

# Evaluating the Agilent 4200 TapeStation System for High-Throughput Sequencing Quality Control

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## Abstract

This application note demonstrates the data compatibility for the Agilent DNA and RNA ScreenTape assays using the Agilent 4200 TapeStation system and the Agilent 2200 TapeStation system. Data from both systems are highly reproducible and user-independent. In comparison to the 2200 TapeStation system, the 4200 TapeStation system further reduces hands-on-time, allowing the analysis of 96 samples without manual intervention, an important requirement for high throughput users such as the Genomics and Proteomics Core Facility of the German Cancer Research Center (DKFZ).

## Introduction

The German Cancer Research Center (DKFZ) is the largest biomedical research institution in Germany. The High Throughput Sequencing Unit of the DKFZ Genomics and Proteomics Core Facility provides sequencing services to national and international cancer genome projects for multiple applications such as whole genome or targeted resequencing. To ensure the experimental success of sequencing experiments, quality control (QC) of DNA or RNA samples at various stages of the next-generation sequencing (NGS) workflow is mandatory. The facility is working in a high throughput environment running 10,000 to 16,000 sequencing reactions per year from various biological sources. The sequencing core facility subjects the received DNA or RNA samples to an incoming QC, prepares the libraries including additional QC steps, and performs the sequencing, followed by the final data QC.

The quantification of DNA or RNA samples at the sequencing core facility is currently performed using the Qubit assay based on fluorescence detection with a microplate reader capable of analyzing 96 samples at once. Data are then automatically transferred into a data management system for analysis.

The integrity of the DNA and RNA samples is verified with an Agilent 2200 TapeStation system. The 2200 TapeStation system provides DNA and RNA analysis in a 96-well plate format, which can be combined with a liquid handling system for sample preparation. Another advantage of the 2200 TapeStation system is that each sample is analyzed in a discrete gel lane, so that contamination of a single sample does not affect subsequent samples. However, the tips and the Agilent ScreenTape device need to be exchanged after 16 samples, requiring manual intervention every 15 to 20 minutes.

The Agilent 4200 TapeStation system is designed to reduce hands-on time, analyzing 96 samples without manual intervention, providing a major benefit for laboratories focused on high sample throughput.

This application note evaluates the performance of the 4200 TapeStation system in comparison with the existing 2200 TapeStation system using the four major assays in QC during the library preparation workflow:

- Agilent Genomic DNA assay
- Agilent D1000 assay
- Agilent High Sensitivity D1000 assay
- Agilent RNA ScreenTape assay

In addition, reproducibility between repeated runs and different users was investigated.

## Experimental

### Materials

Two lymphoblastoid cell line derived genomic DNA (gDNA) samples NA12891 (gDNA sample A) and NA12878 (gDNA sample B) were obtained from the Coriell Institute (Camden, NJ, USA). DNA libraries (DNA samples A to D) were prepared from human gDNA. Ambion Human Liver Total RNA was purchased from ThermoFisher Scientific, Inc. (Waltham, MA, USA). The Agilent 2200 TapeStation system (G2965AA), Agilent 4200 TapeStation system (G2991AA), Agilent Genomic DNA ScreenTape (5067-5365), Agilent Genomic DNA reagents (5067-5366), Agilent D1000 ScreenTape (5067-5582), Agilent D1000 reagents (5067-5583), Agilent High Sensitivity D1000 ScreenTape (5067-5584), Agilent High Sensitivity D1000 reagents (5067-5585), Agilent RNA ScreenTape (5067-5576), Agilent RNA ScreenTape sample buffer (5067-5577), Agilent RNA ScreenTape ladder (5067-5578) High Sensitivity

RNA ScreenTape (5067-5579), High Sensitivity RNA sample buffer (5067-5580), and High Sensitivity RNA ScreenTape ladder (5067-5581) were obtained from Agilent Technologies (Waldbronn, Germany).

### Samples

Four DNA libraries were prepared using the Illumina TruSeq Nano protocol with 100 ng input material and the shearing protocol for 350 bp. The gDNA, DNA libraries, and total RNA samples were diluted as indicated.

### DNA and RNA analysis

Analysis of gDNA, DNA libraries, and RNA was performed according to the appropriate ScreenTape Quick Guide1-10 for the 2200 and 4200 TapeStation systems.

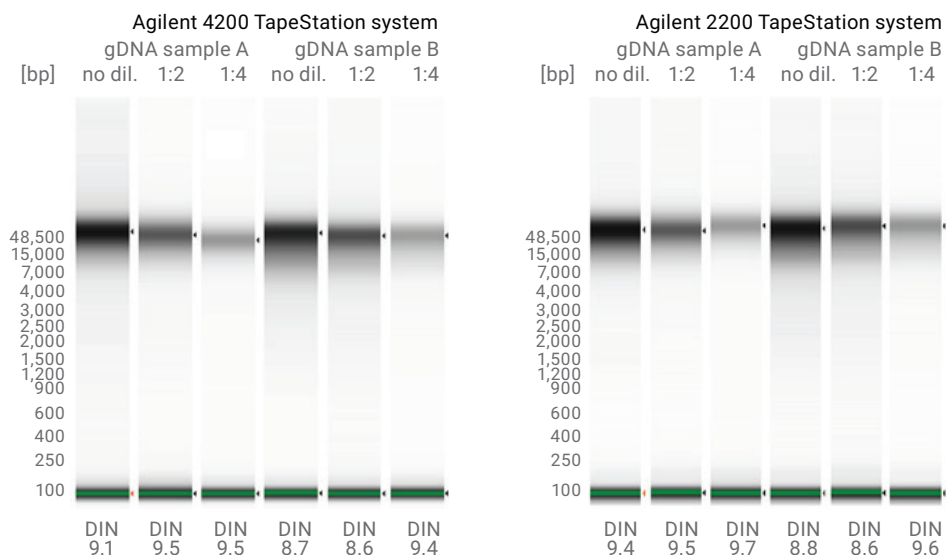
## Results and Discussion

### Genomic DNA ScreenTape Assay

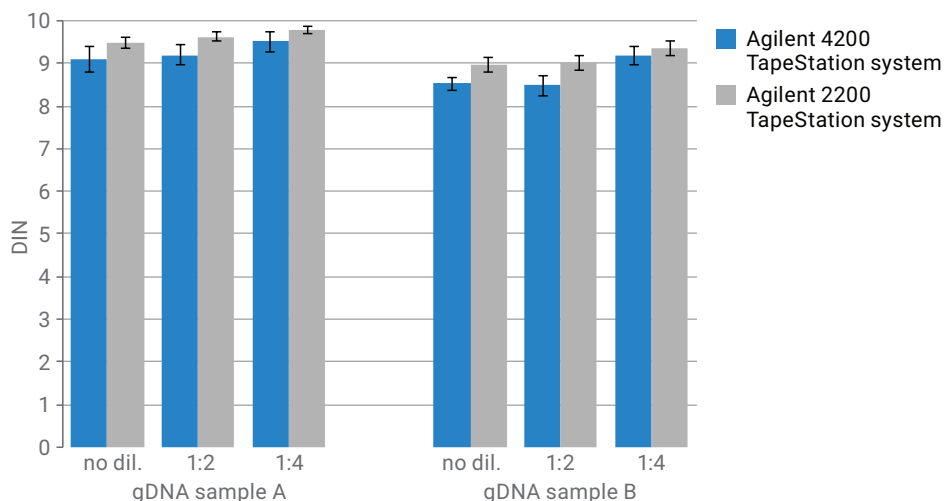
To evaluate the difference between the 2200 TapeStation system and the 4200 TapeStation system, two gDNA samples were analyzed at three different concentrations using the Genomic DNA ScreenTape assay. The DNA Integrity Number (DIN) is automatically determined and directly displayed below the individual lane of the gel image (Figure 1). A DIN is calculated on a scale from 1 to 10. A high DIN indicates highly intact gDNA, whereas a low DIN corresponds to a strongly degraded gDNA sample<sup>11</sup>. Both gDNA samples were highly intact, as indicated by the determined DIN.

Figure 2 demonstrates that the DNA integrity analysis with the 4200 TapeStation system is highly comparable to that of the 2200 TapeStation system. The precision for the DNA integrity analysis for both TapeStation systems is 4% for all tested samples and concentrations.

In addition to the calculation of the DIN as a metric for the integrity of gDNA, the Genomic DNA ScreenTape assay also determines the gDNA concentration. To demonstrate that both TapeStation systems provide comparable quantification information, the gDNA concentration determined with the 4200 TapeStation system was plotted against the concentration measured with the 2200 TapeStation system (Figure 3).



**Figure 1.** Two gDNA samples in different concentrations were analyzed with the Agilent Genomic DNA ScreenTape assay using the Agilent 4200 TapeStation and Agilent 2200 TapeStation systems. The automatically determined DIN is shown under the gel images.

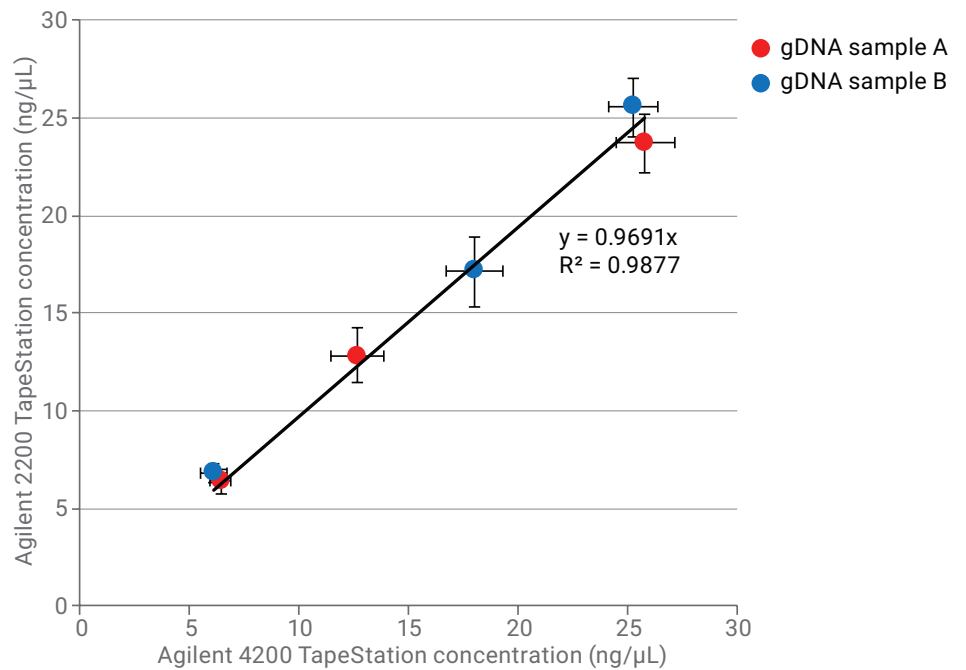


**Figure 2.** Two gDNA samples in different concentrations were analyzed with the Agilent Genomic DNA ScreenTape assay using the Agilent 4200 TapeStation and Agilent 2200 TapeStation systems (n = 15 to 16). The error bars indicate the standard deviation.

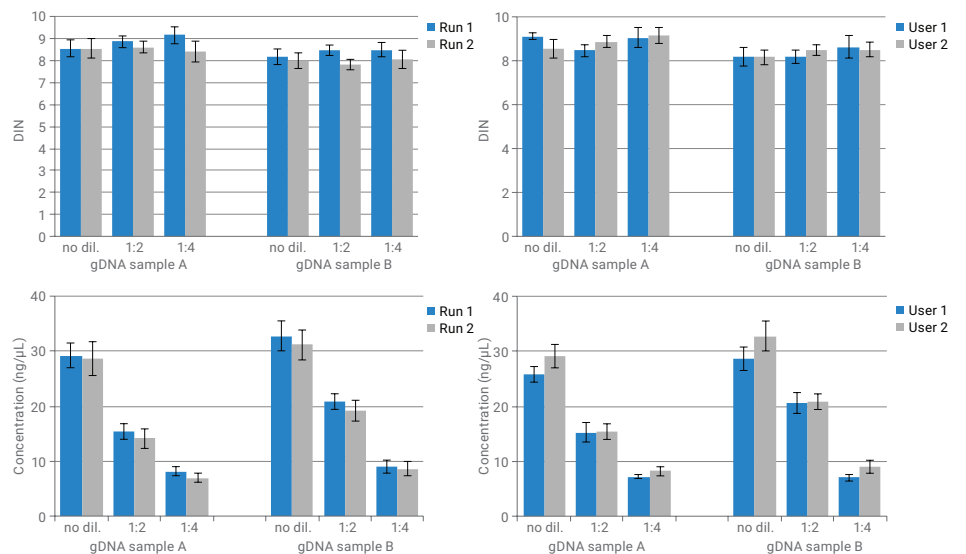
Figure 3 shows a good correlation of the quantification between both gDNA samples for all tested concentrations with both TapeStation systems. The gDNA quantification precision with the 4200 TapeStation system was below 10% for all tested samples and concentrations, which is well within the specification of the assay<sup>2</sup>.

The same set of samples was used to verify the reproducibility of the 4200 TapeStation system and the Genomic ScreenTape assay with special focus on run-to-run or user-to-user variation. To verify the run-to-run variation, the same sample plate was analyzed twice. To determine the user-to-user variation, the same sample set was prepared and analyzed by different users.

Figure 4 demonstrates that gDNA analysis with the 4200 TapeStation system and the Genomic DNA ScreenTape assay is user-independent and highly reproducible between repeated runs. The %CV for the DNA integrity analysis was below 6% and 15% for the gDNA quantification. This is in agreement with the specified quantitative precision of the Genomic DNA ScreenTape assay<sup>2</sup>.



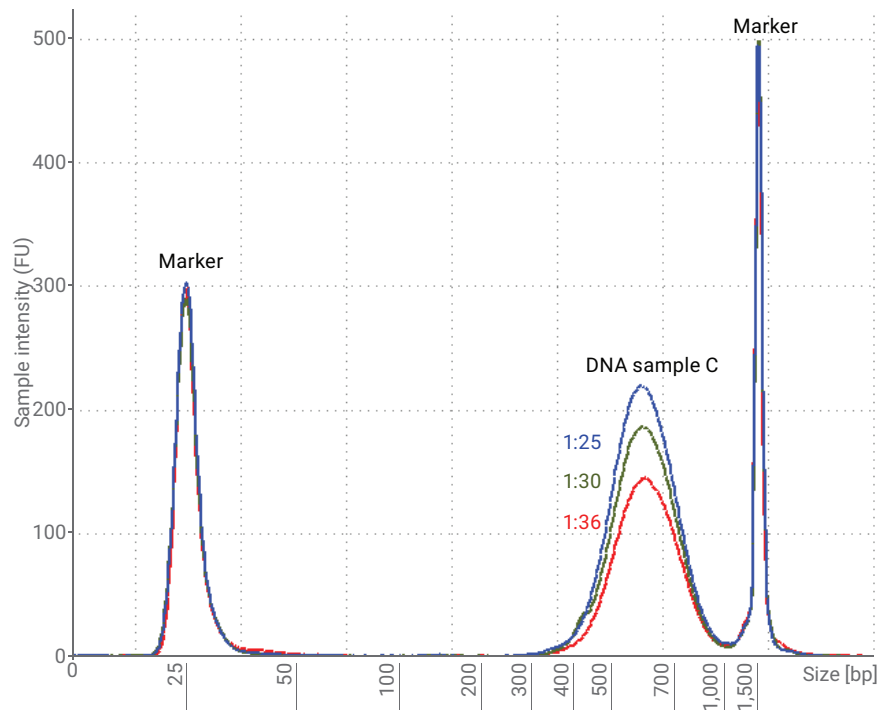
**Figure 3.** Two gDNA samples in different concentrations were quantified with the Agilent Genomic DNA ScreenTape assay using the Agilent 4200 TapeStation and Agilent 2200 TapeStation systems (n = 15).



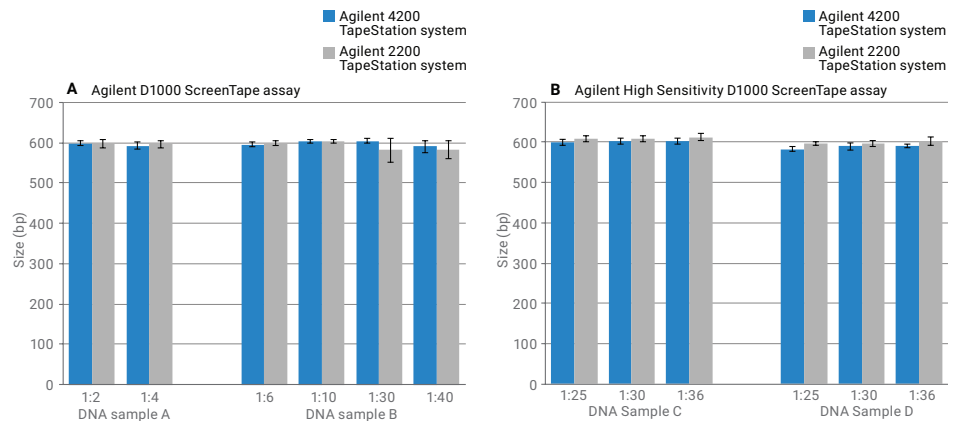
**Figure 4.** Analysis of two gDNA samples in different concentrations ranging from 5 to 30 ng/μL with the Agilent Genomic DNA ScreenTape assay using the Agilent 4200 TapeStation system (n = 16) with focus on run-to-run and user-to-user variation.

## D1000 ScreenTape Assays

In addition to the Genomic DNA ScreenTape assay, the D1000 and High Sensitivity ScreenTape assays are used for quality control, providing DNA sizing and quantification. Figure 5 shows an electropherogram overlay of a DNA sample in different concentrations analyzed with the High Sensitivity D1000 ScreenTape assay and the 4200 TapeStation system. The electropherogram shows the typical size distribution for DNA libraries. To compare the 4200 TapeStation system to the 2200 TapeStation system, four DNA samples were analyzed in different concentrations with the D1000 and the High Sensitivity D1000 ScreenTape assays. Figure 6 demonstrates that the sizing analysis of both TapeStation systems is reproducible and equivalent, independent of the sample concentration. The sizing precision for all samples analyzed with the 4200 TapeStation system was below 2%, which is well within the specified sizing precision of 5% CV for both D1000 ScreenTape assays<sup>5,6</sup>.



**Figure 5.** Electropherogram overlay showing the analysis of DNA sample C in different concentrations ranging from 550 to 1,000 pg/ $\mu$ L with the Agilent High Sensitivity D1000 ScreenTape assay and the Agilent 4200 TapeStation system.



**Figure 6.** DNA sizing of four DNA samples in different concentrations with the Agilent D1000 ScreenTape assay (A) and the Agilent High Sensitivity D1000 ScreenTape assay (B) and both Agilent TapeStation systems (n = 14 to 17). DNA sample A: 6 to 12 ng/ $\mu$ L, DNA sample B: 0.5 to 5 ng/ $\mu$ L, DNA sample C: 550 to 1,000 pg/ $\mu$ L, and DNA sample D: 600 to 1,000 ng/ $\mu$ L.

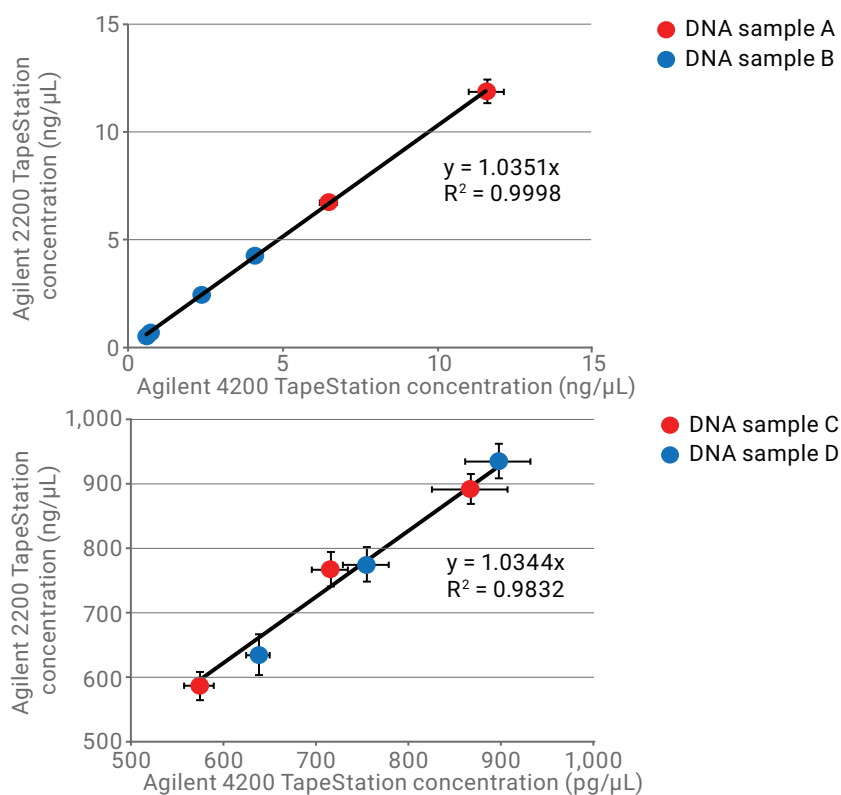
To demonstrate the compatibility of both TapeStation systems for DNA quantification, the DNA concentration determined with the 4200 TapeStation system was plotted against the concentration measured with the 2200 TapeStation system for the D1000 and High Sensitivity D1000 ScreenTape assays (Figure 7).

Figure 7 shows good correlation of the quantification results for both D1000 ScreenTape assays between both TapeStation systems. The DNA quantification precision obtained with the 4200 TapeStation system for the D1000 ScreenTape assay was below 5%, and for the High Sensitivity D1000 ScreenTape assay below 9% for all tested samples and concentrations.

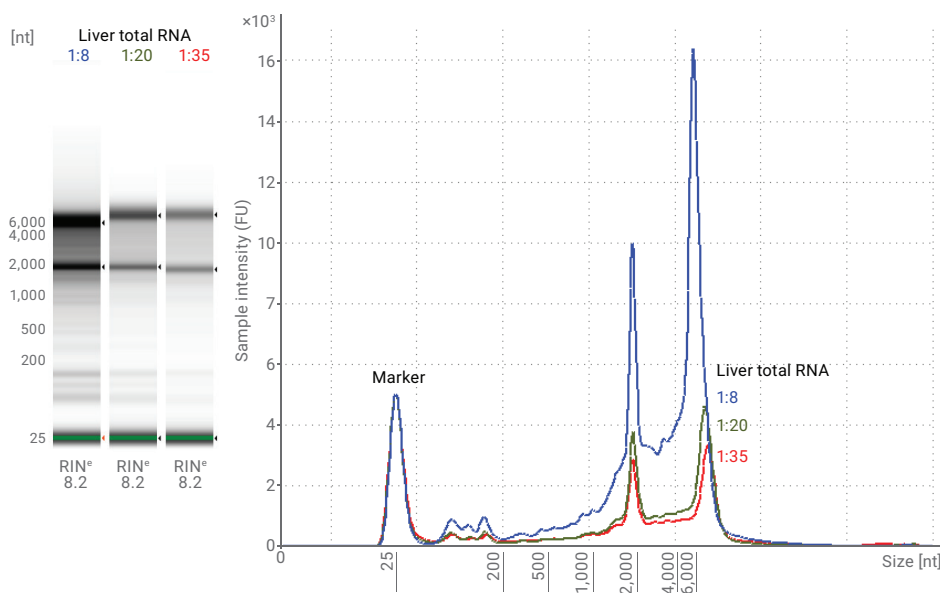
### RNA ScreenTape Assays

In addition to DNA, RNA samples are used for high throughput sequencing, for example in RNAseq workflows. As RNA is highly sensitive to degradation, quality control is of special importance, and usually includes RNA integrity analysis and quantification.

Figure 8 shows the obtained gel image and an electropherogram overlay, when analyzing different dilutions of a total RNA sample with the 4200 TapeStation system.



**Figure 7.** Correlation of the DNA quantification of four DNA samples in different concentrations with the Agilent D1000 ScreenTape assay and the Agilent High Sensitivity D1000 ScreenTape assay using both Agilent TapeStation systems (n = 14).



**Figure 8.** Analysis of liver total RNA in different concentrations (50 to 150 ng/μL) with the Agilent RNA ScreenTape assay using the Agilent 4200 TapeStation system. The gel like image and the electropherogram overlay of the serial dilution is shown.

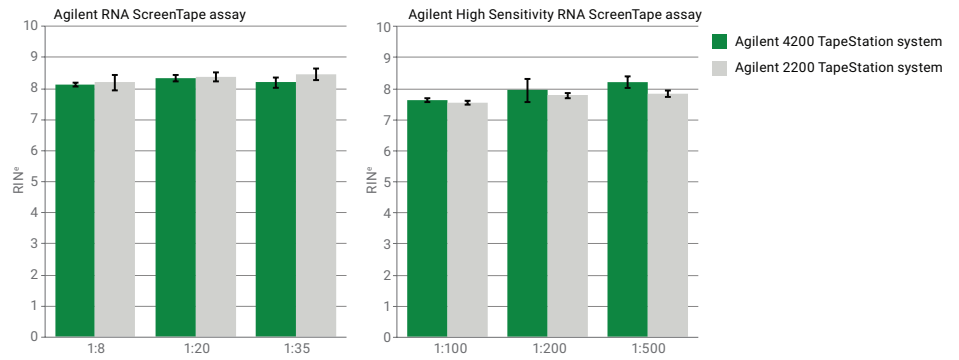
The RNA Integrity Number (RINe), indicating RNA integrity, is automatically determined and directly displayed below the according lane of the gel image (Figure 8). The RINe is calculated on a scale from 1 to 10. A high RINe indicates highly intact total RNA, whereas a low RINe corresponds to a strongly degraded sample<sup>12</sup>. Figure 8 demonstrates the typical gel pattern and electropherogram obtained for a total RNA sample analyzed on the 4200 TapeStation system using the RNA ScreenTape assay.

Figure 9 illustrates that RNA integrity analysis with the 4200 TapeStation system is highly comparable to the 2200 TapeStation system. The RINe is highly reproducible and independent of the analyzed concentration for both TapeStation systems. The precision for the total RNA integrity analysis with the 4200 TapeStation system was below 3% for the RNA ScreenTape assay, and below 6% for the High Sensitivity RNA ScreenTape assay.

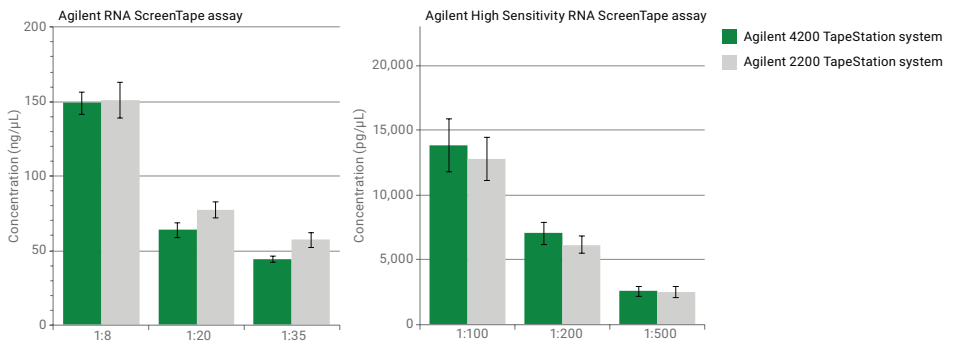
In addition to the total RNA integrity analysis, RNA quantification with both TapeStation systems was also compared (Figure 10).

Figure 10 demonstrates that the total RNA quantification with both TapeStation systems is comparable for the RNA and the High Sensitivity RNA ScreenTape assays.

The total RNA quantification precision with the 4200 TapeStation system was below 15% for the RNA and High Sensitivity RNA ScreenTape assays.



**Figure 9.** RNA integrity analysis of liver total RNA in different concentrations with the Agilent RNA ScreenTape assay and the Agilent High Sensitivity RNA ScreenTape assay using the Agilent 4200 TapeStation system and the Agilent 2200 TapeStation system (n = 15).



**Figure 10.** Quantification of liver total RNA in different concentrations with the Agilent RNA ScreenTape assay and the Agilent High Sensitivity RNA ScreenTape assay using the Agilent 4200 TapeStation system and the Agilent 2200 TapeStation system (n = 15).

## Results and Discussion

This application note demonstrates that the data obtained with the Agilent Genomic DNA assay, Agilent D1000 assay, Agilent High Sensitivity D1000 assay, or Agilent RNA ScreenTape assays using the Agilent 4200 TapeStation system or the Agilent 2200 TapeStation system is directly comparable, highly reproducible, and user-independent. In comparison to the 2200 TapeStation system, the 4200 TapeStation system further reduces hands-on-time, allowing the analysis of 96 samples without manual intervention. This is a primary requirement in a high throughput environment.

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[www.agilent.com/genomics/tapestation](http://www.agilent.com/genomics/tapestation)

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