Technical Overview

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Performance Characteristics of the RNA and the High Sensitivity RNA ScreenTape Assays for the 4150 TapeStation System

Introduction

The Agilent 4150 TapeStation system is an automated electrophoresis solution for fast and reliable nucleic acid sample quality control. The system offers individual analysis of sample numbers from 1 to 16 samples together with ready to use consumables. The entire Agilent RNA and DNA ScreenTape portfolio is applicable on the 4150 TapeStation system. Furthermore, software and workflow compatibility ensure seamless transition between the 4150 and the Agilent 4200 TapeStation systems. The Agilent RNA ScreenTape assays enable separation, integrity, and quantity analysis of total RNA samples from both eukaryotic and prokaryotic origin. The RNA integrity number equivalent (RIN^e) provides objective assessment of RNA degradation. The 4150 TapeStation system together with the RNA ScreenTape assays is perfectly suited for RNA quality control at low throughput need.

This Technical Overview focuses on the performance characteristics of the RNA ScreenTape assay and the Agilent High Sensitivity RNA ScreenTape assay (HS RNA ScreenTape assay) on the 4150 TapeStation system regarding RNA integrity analysis, sensitivity, and quantification. The performance of the 4150 TapeStation system was compared to the 4200 TapeStation system using the same assays to demonstrate full compatibility. Furthermore, the Agilent 2100 Bioanalyzer system, together with the corresponding RNA assays as widely accepted standards for RNA quality control, were applied for a benchmarking of all three systems.

Analytical specifications

Table 1 summarizes the analytical specifications of the RNA and the HS RNA ScreenTape assays for both the 4150 and the 4200 TapeStation systems as well as the specifications of the Agilent RNA 6000 Nano and the RNA 6000 Pico assays for the 2100 Bioanalyzer system.[1]

Experimental

Material

The 4150 TapeStation system (part number G2992AA) and the 4200 TapeStation system (part number G2991AA) with the RNA ScreenTape (part number 5067-5576), Agilent RNA ScreenTape sample buffer (part number 5067-5577), Agilent RNA ScreenTape ladder (part number 5067-5578), HS RNA ScreenTape (part number 5067-5579), Agilent HS RNA ScreenTape sample buffer (part number 5067-5580), and Agilent HS RNA ScreenTape ladder (part number 5067-5581), the 2100 Bioanalyzer system (part number G2939BA) using the RNA 6000 Nano kit (part number 5067-1511) and RNA 6000 Pico kit (part number 5067-1513) as well as the Total RNA, Kidney, Sprague-Dawley Rat, Male (part number 737007) were obtained from Agilent Technologies (Waldbronn, Germany). *E. coli* Total RNA was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA).

Sample preparation

The intact rat kidney total RNA was diluted with TE buffer that the resulting concentrations covered the entire specified quantitative range of the RNA assays (Table 1). For RNA integrity analyses, both rat kidney and *E. coli* total RNA were incubated at 94 °C for different times to generate RNA samples of different degradation level.

RNA analysis

Total RNA samples were analyzed using the RNA and HS RNA ScreenTape assays on the 4150 and 4200 TapeStation systems or the RNA 6000 Nano and RNA 6000 Pico assays together with the 2100 Bioanalyzer system, according to manufacturer instructions.[2,3,4,5] The rat kidney total RNA samples were analyzed in replicates of 12 to 22 on three different 4150 and 4200 TapeStation instruments each and in replicates of six on two different 2100 Bioanalyzer instruments. The *E. coli* total RNA samples were analyzed in replicates of six on each instrument type. For data analysis, Agilent TapeStation software revisions 3.1 (4150 TapeStation system), A.02.02 (SR1) (4200 TapeStation system), and Agilent 2100 Expert software revision B.02.10 (2100 Bioanalyzer system) were applied.

Table 1. Analytical specifications of the Agilent RNA and HS RNA ScreenTape assays (Agilent 4150 and 4200 TapeStation systems) and the Agilent RNA 6000 Nano and RNA 6000 Pico assays (Agilent 2100 Bioanalyzer system).

	Agilent 4150 and 4200 TapeStation Systems		Agilent 2100 Bioanalyzer System	
Analytical Specifications	Agilent RNA ScreenTape Assay	Agilent High Sensitivity RNA ScreenTape Assay	Agilent RNA 6000 Nano Assay (total RNA)	Agilent RNA 6000 Pico Assay (total RNA)
Quality Score	RIN^e	RIN^e	RIN	RIN
RIN^e Functional Range	25 to 500 ng/µL	1,000 to 25,000 pg/µL	–	–
Sensitivity[1]	5 ng/µL	100 pg/µL	5 ng/µL in water	50 pg/µL in water
Quantitative Range	25 to 500 ng/µL	500 to 10,000 pg/µL	25 to 500 ng/µL	–
Quantitative Accuracy	±20%	±30%	±20%[2]	±30%[2]
Quantitative Precision	10% CV	15% CV	10% CV[3]	20% CV[3]
Maximum Sample Buffer Strength	200 mM Tris, 20 mM EDTA, or 50 mM NaCl	10 mM Tris, 1 mM EDTA	100 mM Tris, 0.1 mM EDTA, or 125 mM NaCl, or 15 mM $MgCl_2$	50 mM Tris, 0.1 mM EDTA, or 50 mM NaCl, or 15 mM $MgCl_2$

[1] S/N >3 for a single peak
[2] Determined analyzing the RNA ladder as sample
[3] Within a chip

Results and discussion

Total RNA integrity analysis

RNA serves as input material within various gene expression analysis techniques like RNASeq, microarray, or RT-qPCR workflows. Since RNA is very sensitive to degradation and the integrity of RNA critically affects the success of the downstream application, quality control of RNA input material is crucial for ensuring high-quality results.

For the TapeStation systems and the RNA ScreenTape assays, a software algorithm was established to reliably determine RNA integrity by the RNA integrity number equivalent (RIN^e).

The objective quality metric RIN^e was demonstrated to be equivalent to RIN delivered by the 2100 Bioanalyzer system, which is a widely accepted standard for RNA quality assessment.[6,7] In the same manner as for RIN, RIN^e values are presented as numerical values ranging from 10 (highly intact) to 1 (strongly degraded). RIN^e can be assessed within the large concentration range from 1 to 500 ng/µL (Table 1).

Figure 1 shows the electrophoretic separation of four rat kidney total RNA samples at different degradation stages in triplicate analyzed with the 4150 TapeStation system and the RNA ScreenTape assay. The TapeStation software displays the results as a gel image along with the automatically assessed RIN^e, as an electropherogram, and in the sample table.

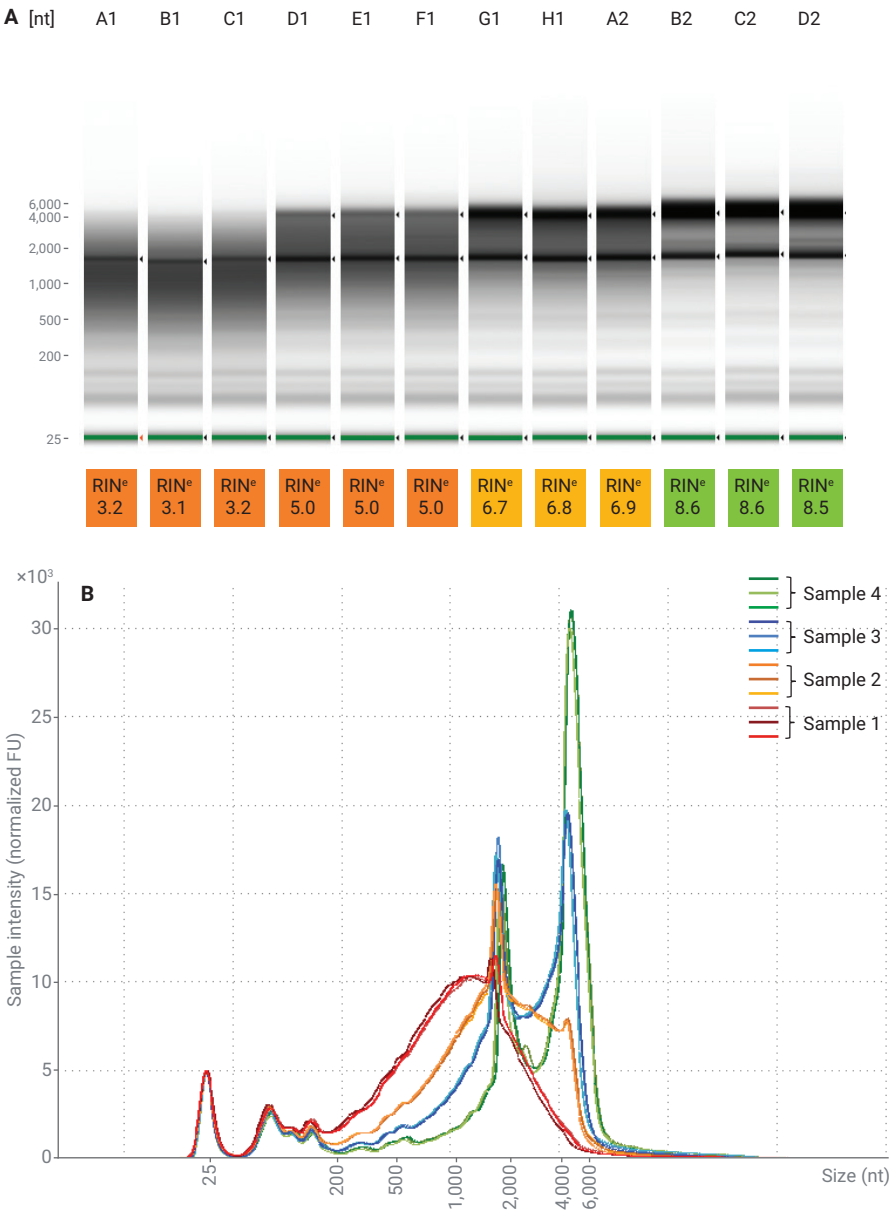

Figure 1. Four rat kidney total RNA samples (300 ng/µL) at different degradation stages were analyzed on the Agilent 4150 TapeStation system together with the Agilent RNA ScreenTape assay to determine RNA integrity by the RNA integrity number equivalent (RIN^e). A) Gel image with corresponding RIN^e displayed below each lane including color coded result flagging. B) Electropherogram overlay of all 12 RNA samples.

For evaluation of RNA integrity assessment on the 4150 TapeStation system and a comparison to the 4200 TapeStation and 2100 Bioanalyzer systems the differently degraded rat kidney total RNA samples were analyzed on the two TapeStation systems using the RNA and the HS RNA ScreenTape assays (n = 18) as well as on the 2100 Bioanalyzer system with the corresponding RNA 6000 Nano and RNA 6000 Pico assays (n = 6). In addition, a prokaryotic RNA degradation series from *E. coli* total RNA was prepared and analyzed on all three systems (n = 6).

Figure 2 summarizes the RIN^e results obtained with the 4150 TapeStation system correlated with the corresponding RIN values of the 2100 Bioanalyzer system as well as the RIN^e values of the 4200 TapeStation system. Since eukaryotic and prokaryotic RNA integrity assessment occurred in full

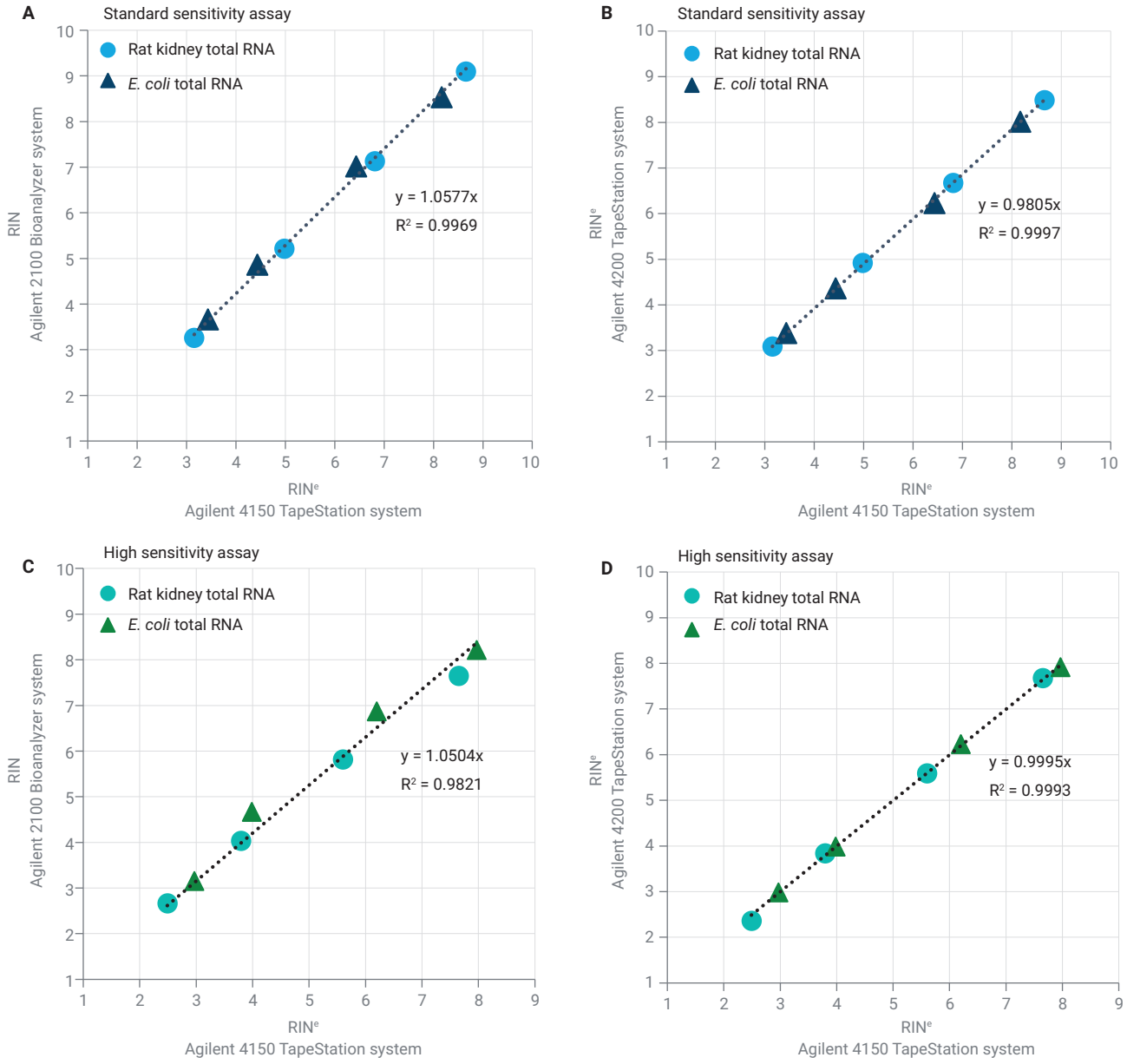

Figure 2. Correlation of RNA integrity assessment of rat kidney total RNA (eukaryotic) and *E. coli* total RNA (prokaryotic) samples at different degradation stages with the Agilent 4150 TapeStation, 4200 TapeStation, and the Agilent 2100 Bioanalyzer systems. The RIN^e results from the 4150 TapeStation system (X-axis) are plotted against the RIN values obtained with the 2100 Bioanalyzer system (Y-axis) using the respective standard sensitivity (A) and high sensitivity assays (C). RIN^e-RIN^e correlation of the 4150 TapeStation (X-axis) and the 4200 TapeStation system (Y-axis) using the Agilent RNA ScreenTape (B) and the Agilent HS RNA ScreenTape assays (D).

accordance without any difference on all three systems, the results were pooled. The RNA integrity analysis performed with the two TapeStation systems can be considered equivalent, demonstrated by R^2 values above 99.9% for both assays. Furthermore, the RIN-RIN^e comparison showed excellent correlation between the 4150 TapeStation and the 2100 Bioanalyzer systems.

In addition, the RIN and RIN^e determination were highly reproducible. For the standard sensitivity assay, standard deviation of all samples was below 4% for both TapeStation systems and below 6% for the 2100 Bioanalyzer system (Figure 3). The reproducibility for the high sensitivity assays on all systems showed standard deviations below 0.4 (data not shown).

Taken together, the RIN^e values obtained with the 4150 TapeStation system are confirmed to be equivalent to RIN of the 2100 Bioanalyzer system, which is in accordance with the previously demonstrated performance of RIN^e assessed with the 2200 and the 4200 TapeStation systems.[6,7]

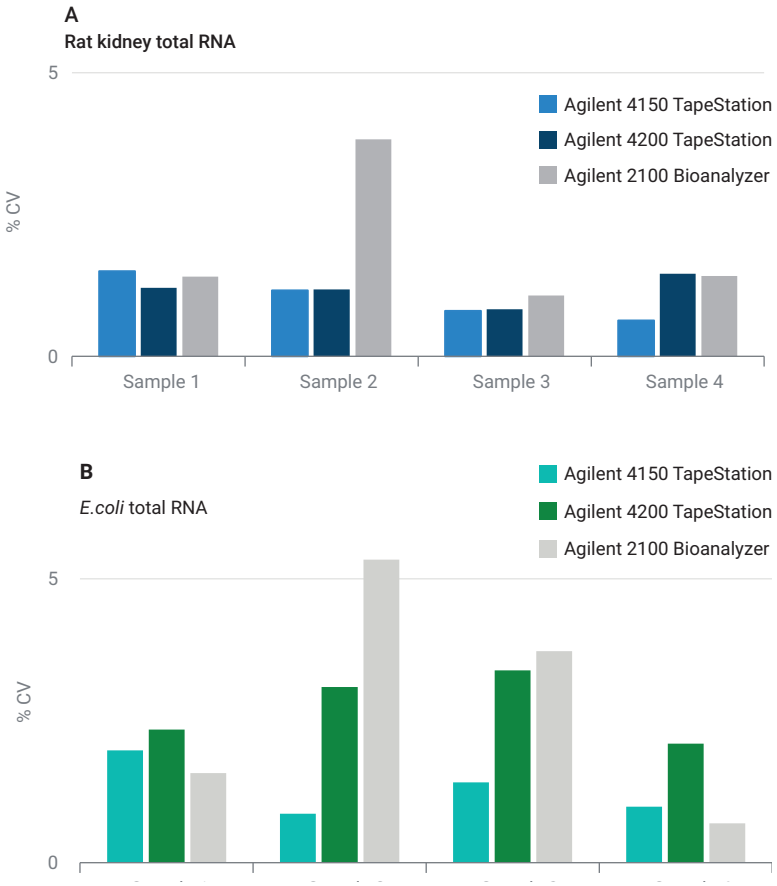

Figure 3. Reproducibility of RNA quality assessment on the Agilent 4150 TapeStation, 4200 TapeStation, and the Agilent 2100 Bioanalyzer systems (standard sensitivity assays). A) RIN^e/RIN reproducibility of four rat kidney total RNA samples analyzed with the 4150 and the 4200 TapeStation systems (n = 18) and the 2100 Bioanalyzer system (n = 6). B) Precision of the RIN^e/RIN values of four *E. coli* total RNA samples (n = 6).

Sensitivity

An intact rat kidney total RNA sample at 100 pg/µL was analyzed with the 4150 TapeStation system using the HS RNA ScreenTape assay for sensitivity evaluation.

Figure 4 shows the electropherogram overlay of multiple analyses of the sample at the specified limit of detection of 100 pg/µL focusing on the enlarged peaks in the insert section. Since the rat kidney total RNA is clearly detected, exhibiting a signal-to-noise ratio (S/N) greater than 3, the sensitivity of 100 pg/µL for the assay is verified on the 4150 TapeStation system.

Quantification

The RNA ScreenTape assays enable quantification of total RNA. The quantification range of the RNA ScreenTape assay accounts from 25 to 500 ng/µL, which is equal to that of the RNA 6000 Nano assay of the 2100 Bioanalyzer system. The HS RNA ScreenTape assay quantification ranges from 500 to 10,000 pg/µL, whereas the corresponding RNA 6000 Pico assay of the 2100 Bioanalyzer system provides only a qualitative range from 50 to 5,000 pg/µL. Therefore, quantification analyses on the 2100 Bioanalyzer system were restricted to the RNA 6000 Nano assay.

To demonstrate the quantification specifications of both assays on the 4150 TapeStation system, serial dilutions of intact rat kidney total RNA samples covering the entire concentration range were analyzed. For a direct comparison, the same dilution series were also quantified on the 4200 TapeStation and the 2100 Bioanalyzer system.

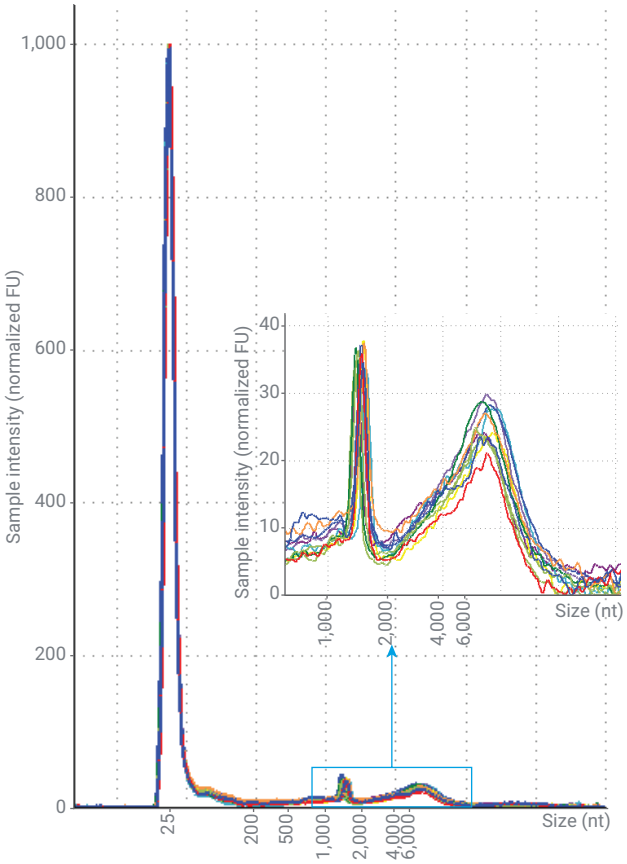

Figure 4. Electropherogram overlay of a rat kidney total RNA sample at the specified limit of detection (100 pg/µL) on the Agilent 4150 TapeStation system using the Agilent HS RNA ScreenTape assay. The enlarged image shows a close-up of the resulting peaks (n = 12).

The quantitative results of the 4150 TapeStation system were plotted against the concentrations obtained with the 4200 TapeStation system for both assays and the standard sensitivity assay on the 2100 Bioanalyzer system (Figure 5). The correlation results exhibited excellent R^2 values of 99.6% and higher and accuracy was below 20% deviation between all three systems.

The average quantitative precision, which was evaluated with 12 replicates per sample for the TapeStation systems or six replicates per sample for the 2100 Bioanalyzer system is displayed in Figure 6. The relative standard deviation (% CV) was below 6% for the standard sensitivity and below 10% for the High Sensitivity RNA ScreenTape assay on the 4150 TapeStation system. Precision was comparable on the 4200 TapeStation and the 2100 Bioanalyzer system and the specifications were met for all assays.

The specified quantitative accuracy and precision of the RNA ScreenTape assays, already successfully demonstrated on the 4200 TapeStation system,[7] were confirmed on the 4150 TapeStation system.

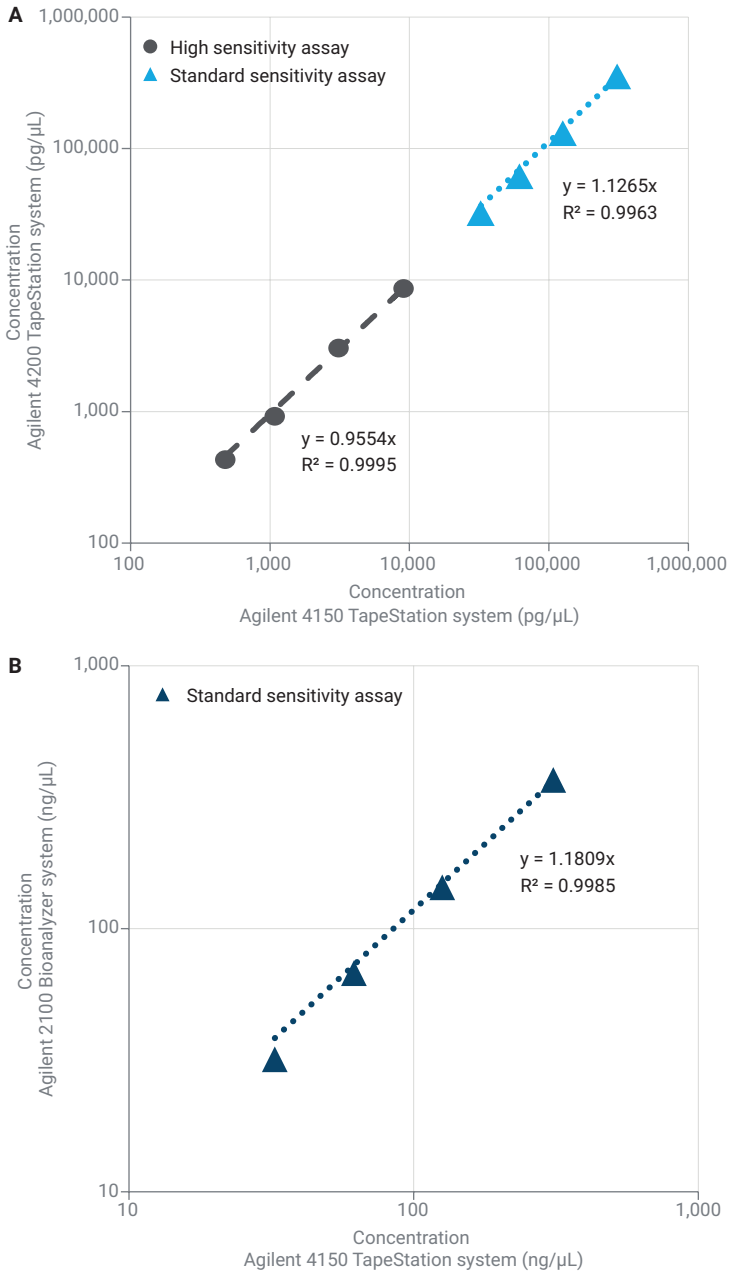

Figure 5. Quantification of a rat kidney total RNA sample in dilution series from 0.5 to 9 ng/µL and 35 to 360 ng/µL. The RNA samples were analyzed with the Agilent HS RNA ScreenTape assay (Agilent TapeStation systems) as well as the standard sensitivity assays RNA ScreenTape assay (TapeStation systems) and Agilent RNA 6000 Nano assay (Agilent 2100 Bioanalyzer system). A) Quantification results from the Agilent 4150 TapeStation system (X-axis) compared to the Agilent 4200 TapeStation system (Y-axis). B) Comparison of the quantification on the 4150 TapeStation system (X-axis) and the Agilent 2100 Bioanalyzer system (Y-axis).

Conclusion

This Technical Overview demonstrates efficient and reliable RNA analysis performed with the Agilent RNA ScreenTape assays on the Agilent 4150 TapeStation system in terms of sensitivity down to 100 pg/µL as well as quality and quantity information of RNA samples. Moreover, the RIN[e] quality metric enabling standardized assessment of RNA integrity for samples from both eukaryotic and prokaryotic sources is confirmed to be equivalent to the well-established RIN of the Agilent 2100 Bioanalyzer system. The performance of the 4150 TapeStation system was in very good agreement with the 4200 TapeStation system, ensuring full compatibility of the two systems, as well as being highly comparable to the 2100 Bioanalyzer system. Altogether, excellent performance of the RNA and the HS RNA ScreenTape assays was confirmed on the 4150 TapeStation system, achieving consistent results compared to the two established systems.

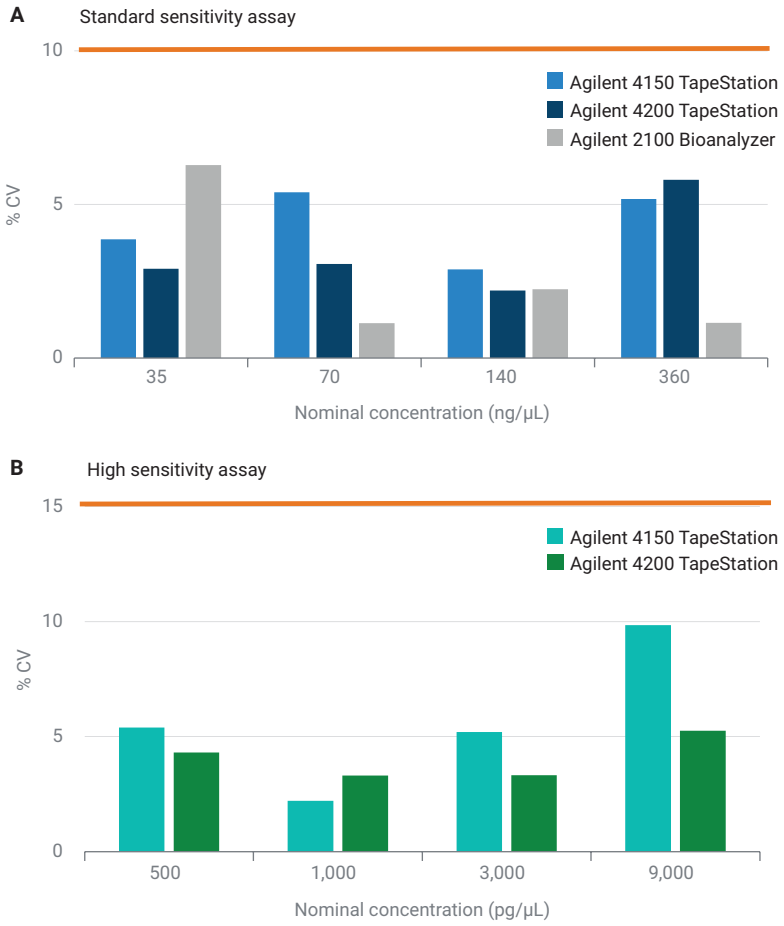

Figure 6. Quantification precision of rat kidney total RNA samples in four concentrations analyzed with the Agilent 4150 TapeStation, 4200 TapeStation (n = 12), and Agilent 2100 Bioanalyzer systems (n = 6). The orange line indicates the corresponding specified quantitative precision. A) Quantification precision of the Agilent RNA ScreenTape assay and the Agilent RNA 6000 Nano assay. B) Quantification precision of the Agilent HS RNA ScreenTape assay.

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