Performance of the Agilent RNA ScreenTape and the High Sensitivity RNA ScreenTape Assay for the Agilent 2200 TapeStation System

Technical Overview

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Introduction

The Agilent 2200 TapeStation system provides automated, fast, and reliable DNA, RNA, and protein electrophoresis of up to 96 samples using prepackaged reagents. The RNA ScreenTape and the High Sensitivity RNA ScreenTape assays have been developed to enable robust quantity and quality analysis of total RNA samples from both eukaryotic and prokaryotic sources; all from a sample concentration of as little as 500 pg/µL. The assays additionally benefit from the ability to provide separation of contaminant genomic DNA, allowing more accurate purity assessment of sample material.

This Technical Overview compares the performance of the RNA ScreenTape and High Sensitivity RNA ScreenTape assays against the Agilent 2100 Bioanalyzer as well as the NanoDrop and Qubit systems for RNA quality and quantity determination.

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Experimental

Materials

Mouse genomic DNA was purchased from Promega (Fitchburg, WI, USA). Eukaryotic and prokaryotic total RNA samples from different origins were obtained from Clontech (Mountain View, CA, USA). A Qubit 2.0 Fluorometer and the Qubit RNA Assay kit were purchased from Life Technologies (Carlsbad, CA, USA), and the NanoDrop 2000 system from Thermo Scientific (Wilmington, DE, USA). The 2100 Bioanalyzer system, RNA 6000 Nano kit, 2200 TapeStation system, RNA ScreenTape and reagents, High Sensitivity RNA ScreenTape, and High Sensitivity RNA Reagents were obtained from Agilent Technologies.

RNA degradation

RNA degradation was induced to obtain a full range of RIN and RIN^e scores by incubation of RNA samples at 94 °C for various durations.

RNA analysis

RNA samples were analyzed using RNA ScreenTape assay (5067-5576, 5067-5577, and 5067-5578) or the High Sensitivity RNA ScreenTape assay (5067-5579, 5067-5580, and 5067-5581) on the 2200 TapeStation system (G2964AA and G2965AA). Samples were also analyzed on the RNA Nano (5067-1511) or RNA Pico (5067-1513) with the 2100 Bioanalyzer system (G2943CA), the NanoDrop 2000 (Thermo Scientific), and the Qubit 2.0 Fluorometer (Life Technologies) according to the manufacturer's protocols.

Results and Discussion

The RNA ScreenTape assay is designed to bring the assessment of RNA quality and quantity into alignment with the market-leading 2100 Bioanalyzer application. The RNA ScreenTape assay delivers quantification and separation profiles equivalent to the corresponding 2100 Bioanalyzer assays. To illustrate this, rat kidney total RNA, at a concentration of 500 ng/µL, was analyzed on both systems. Figure 1 shows the obtained electropherograms and demonstrates the comparability of the two systems with good separation of the 18S and 28S peaks. Both the 2100 Bioanalyzer system and the RNA ScreenTape assay on the 2200 TapeStation system gave an assessment of RNA quality and quantity in a single step.

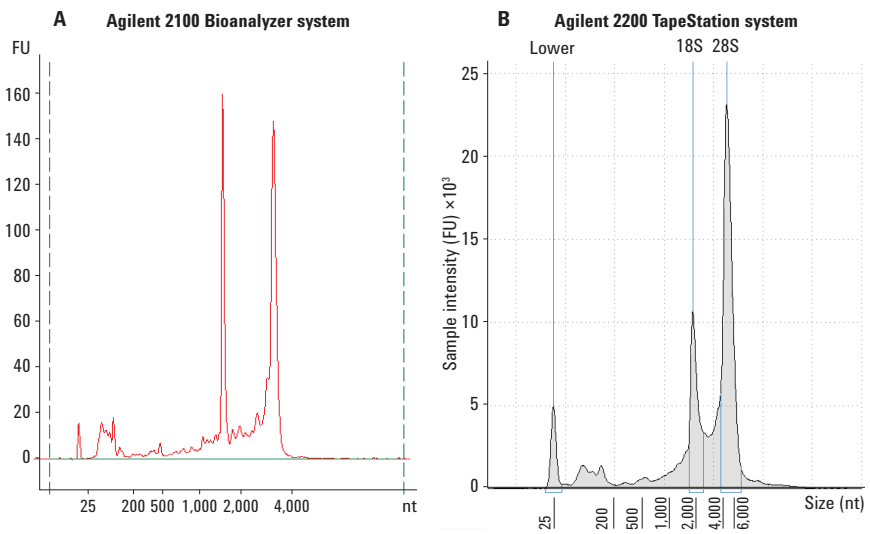

Figure 1. A comparison of the electropherograms obtained for rat kidney RNA (500 ng/µL) on the Agilent 2100 Bioanalyzer instrument and the RNA 6000 Nano assay (A) and the Agilent 2200 TapeStation system and the RNA ScreenTape assay (B).

Comparison of RIN^e and RIN

Agilent pioneered the reliable and robust assessment of total RNA quality with the introduction of the RNA Integrity Number (RIN) for the 2100 Bioanalyzer system. The RIN is widely recognized as a method for objective quality assessment of total RNA samples. Despite the differences in the technologies between the two systems, the 2200 TapeStation system provides the same quality assessment with the RNA Integrity Number equivalent (RIN^e)[1]. To demonstrate that RIN and RIN^e are directly comparable, a large number of RNA samples were analyzed on both the 2100 Bioanalyzer and the 2200 TapeStation systems.

The RNA samples for this study were prepared by five different analysts across three different laboratory sites. Total RNA from three different species, human, mouse, and rat, and from different tissues was degraded to generate a large variation of RNA qualities. The samples (n = 317) were analyzed at different concentrations either as single RNA ScreenTape analyses of 16 samples, or as higher throughput analyses on 96-well plates. In parallel, the same set of samples was also analyzed with the 2100 Bioanalyzer system and the RNA 6000 Nano assay. To verify the comparability of RIN^e with RIN, both values obtained for eukaryotic total RNA were plotted against each other (Figure 2).

The median error calculated versus the 2100 Bioanalyzer system was a 0.4 RIN unit difference, and a standard deviation of 0.28 RIN units. No increased error or bias in the obtained results was observed for a particular tissue or organism (Figure 2). Statistical analysis further showed that the correlated RIN^e data is normally distributed and that greater than 90 % of all observations are within 1 RIN unit.

A similar approach was taken to compare the obtained RIN^e and RIN values from prokaryotic total RNA. For this purpose, total RNA from *E. coli* and *R. solanacearum* was degraded to obtain a range of RNA qualities. Samples (n = 203) were analyzed with the 2100 Bioanalyzer and the 2200 TapeStation systems and the obtained RIN and RIN^e values are plotted against each other (Figure 3).

Similar results were obtained for the prokaryotic RNA with a median error of 0.2 RIN units and a standard deviation over dilution series of 0.16 RIN units. The reduced number of sample types for this analysis (2) accounts for the higher correlation between the systems. Statistical analysis further showed that the spread of the correlated RIN^e data is normally distributed and that greater than 90 % of all observations are within 1 RIN unit.

RNA quantification

To determine the quantification accuracy and reproducibility of the RNA ScreenTape assay, rat kidney total RNA samples were prepared at five different nominal concentrations ranging from 25 to 500 ng/µL. The same set of samples was analyzed with the RNA ScreenTape assay on the 2200 TapeStation system and the NanoDrop 2000 system by four different analysts. The data is shown in Figure 4.

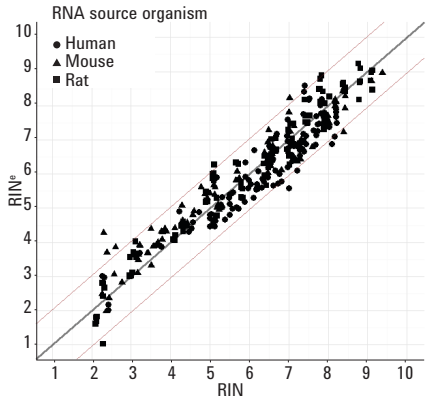

Figure 2. Comparison of RIN and RIN^e values from eukaryotic RNA samples (n = 317) obtained with the Agilent 2100 Bioanalyzer and the Agilent 2200 TapeStation system.

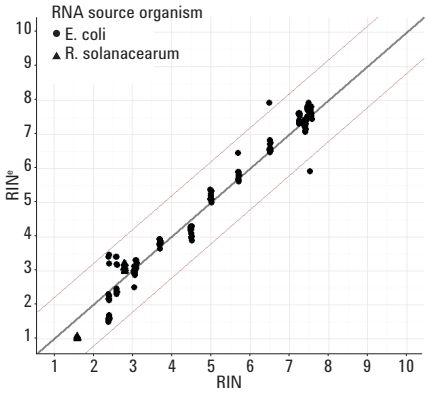

Figure 3. Comparison of RIN and RIN^e values obtained from prokaryotic RNA samples (n = 203) obtained with the Agilent 2100 Bioanalyzer and the Agilent 2200 TapeStation system.

Both quantification methods, the RNA ScreenTape assay and the NanoDrop 2000 system, yielded comparable results for the tested concentrations. The observed average error in accuracy was less than 20 % and the precision had a CV of less than 5 % within a run of one RNA ScreenTape assay.

Similar to above, the quantification accuracy and reproducibility of the High Sensitivity RNA ScreenTape assay was tested. Rat kidney total RNA samples were prepared at five different nominal concentrations ranging from 500 to 10,000 pg/µL. The samples were analyzed with the High Sensitivity RNA ScreenTape assay on the 2200 TapeStation system and this time the Qubit 2.0 Fluorometer was used by two different analysts. The NanoDrop 2000 system could not be used in this comparison as it was not specified to quantify within the range indicated above. The data is shown in Figure 5.

Both quantification methods, the High Sensitivity RNA ScreenTape assay and the Qubit 2.0 Fluorometer, yielded comparable results for the tested samples. The observed average error in accuracy was much less than 30 % and the precision had a CV of less than 10 % within a run of one High Sensitivity RNA ScreenTape assay.

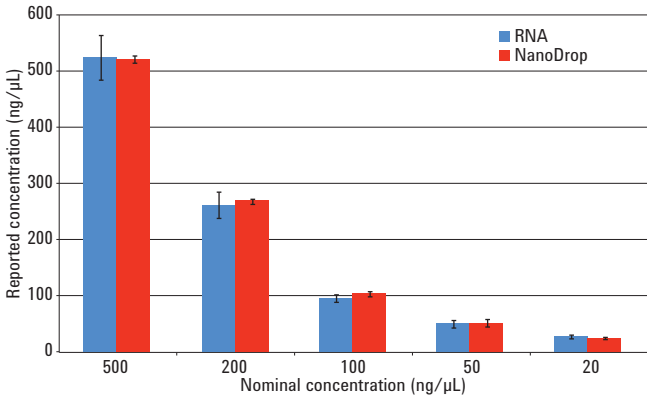

Figure 4. Total RNA quantification with the RNA ScreenTape assay (n = 46), blue bars and the NanoDrop system (n = 60), red bars.

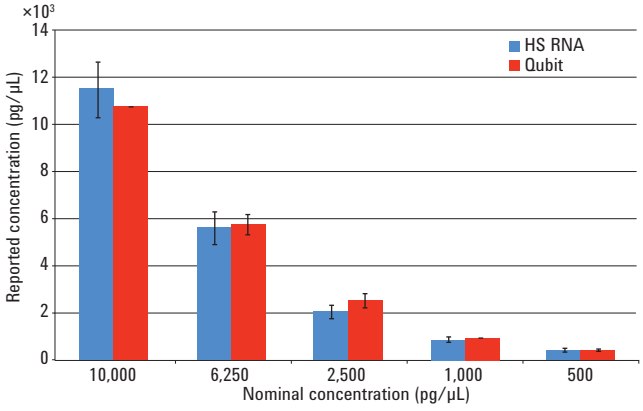

Figure 5. Comparison of concentration values obtained from High Sensitivity RNA ScreenTape assay (n = 48), blue bars and the Qubit 2.0 Fluorometer (n = 6), red bars.

RNA purity

During RNA purification procedures, residual genomic DNA can be present. This can lead to inaccurate RNA quantification or cause issues with downstream applications. The identification of genomic DNA contamination can therefore be useful in deciding if further cleanup of the extracted RNA is required. In contrast to some capillary based systems, the 2200 TapeStation system is capable of resolving intact genomic DNA contaminants from the large ribosomal RNA. Figure 6 shows the analysis of rat kidney total RNA spiked with mouse genomic DNA. The genomic DNA contamination results in an additional peak running above the 28S. Treatment of the RNA sample with DNAse shows that this spiked DNA is removed.

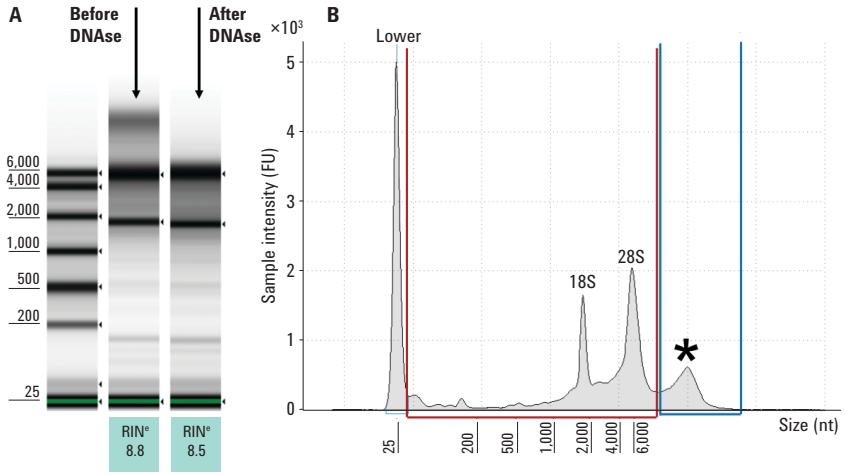

Figure 6. Panel A shows a gel-like image of a total RNA sample with genomic DNA contamination before (lane 1) and after (lane 2) treatment with DNAse. Panel B show the electropherogram of total RNA with genomic DNA contamination (asterisk). The RNA region (red lines) and a seperate genomic DNA region (green lines) were defined within the region analysis mode.

To demonstrate the advantage of the purity analysis with the 2200 TapeStation system, mouse genomic DNA was spiked at varying concentrations from 10 to 90 ng/µL into rat kidney total RNA at a concentration of 200 ng/µL. The samples were analyzed with the NanoDrop 2000 system and the RNA ScreenTape assay (Table 1). The region functionality of the 2200 TapeStation Analysis Software was used to discriminate the RNA region from the genomic DNA region to deliver an accurate RNA concentration that excluded any genomic DNA (Figure 6). The accuracy of the purity determination with the 2200 TapeStation system was calculated against the values determined with the NanoDrop 2000 system. This region analysis of spiked genomic DNA resulted in an approximate 10 % accuracy for the purity determination compared to the nominal values determined from NanoDrop readings.

Table 1. Assessing RNA purity in the presence of genomic DNA with the Agilent 2200 TapeStation and NanoDrop 2000 system.

NanoDrop		Agilent 2200 TapeStation system				
Total conc. (ng/µL)	RNA purity (%)	Total conc. (ng/µL)	RNA region conc. (ng/µL)	gDNA region conc. (ng/µL)	RNA purity (%)	Purity accuracy (%)
290	69.0	276	175	98	63.4	91.9
275	72.7	321	221	95	68.8	94.7
250	80.0	246	177	64	72.0	89.9
225	88.9	237	198	37	83.5	94.0
210	95.2	194	175	16	90.2	94.7

Conclusion

The RNA ScreenTape and High Sensitivity RNA ScreenTape assays demonstrated excellent performance when assessing RNA quality, quantity, and purity.

In contrast to other systems, the Agilent 2200 TapeStation system offers a new level of flexibility for RNA analysis. It not only provides variable throughput from two to 96 samples for eukaryotic and prokaryotic RNA samples, but the 2200 TapeStation Analysis Software also allows these sample types to be analyzed without the need to rerun them. In addition, genomic DNA can be identified, and quantity assessments can be made based only on the RNA portion of the sample by utilizing the region functionality in the Agilent 2200 TapeStation Analysis Software.

Reference

1. "Comparison of RIN and RIN^e algorithms for the Agilent 2100 Bioanalyzer and the Agilent 2200 TapeStation systems", Agilent Technologies Technical Overview, Publication Number 5990-9613EN, **2012**.

www.agilent.com/genomics/tapestation

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