

Expanded RIN^e Range for the Agilent TapeStation Systems

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Abstract

Assessing RNA quality is a critical step in many molecular biology workflows. Agilent has developed objective metrics to support RNA integrity assessment. The Agilent 2100 Bioanalyzer system established the RNA integrity number (RIN), while the Agilent TapeStation systems report the RNA integrity number equivalent (RIN^e) to support RNA quality control (QC). While the RIN and RIN^e scores are comparable,¹ RIN^e scoring with the Agilent High Sensitivity RNA ScreenTape assay has been validated for a functional range of 1,000 to 25,000 pg/μL, with the RIN^e currently being displayed down to 400 pg/μL. An update to the TapeStation software (version 5.3) now allows a RIN^e score to be reported down to 100 pg/μL. This expanded range enables reliable RNA integrity assessment across a broader range of sample concentrations and RNA types without altering the RIN^e algorithm, providing users confidence in their sample quality and improving comparability between the Bioanalyzer and TapeStation systems.

Introduction

RNA integrity strongly influences performance in downstream workflows such as RT-qPCR, RNA sequencing, and gene expression profiling. Since RNA can degrade during sample collection, storage, and extraction, RNA quality control (QC) is recommended before proceeding to time- and cost-intensive analyses. To support objective RNA integrity assessment, Agilent offers automated electrophoresis systems and associated RNA quality metrics, including the Agilent Bioanalyzer and Agilent TapeStation platforms.¹

The Bioanalyzer system established broad adoption of the RNA integrity number (RIN), a numerical integrity score from 1 to 10 based on the electrophoretic profile, where higher values indicate more intact RNA. The TapeStation systems report the RNA integrity number equivalent (RIN^e), which uses a similar scale to provide comparable RNA integrity scoring with a streamlined workflow for routine RNA QC.²

The Agilent High Sensitivity RNA ScreenTape assay provides quantitative analysis across an established range of 500 to 10,000 pg/μL, with sensitivity down to 100 pg/μL.^{3,4} However, the supported RIN^e functional range has previously been 1,000 to 25,000 pg/μL, with the RIN^e being displayed down to 400 pg/μL,^{3,4} limiting quality assessment at low concentrations. The updated Agilent TapeStation software can now apply a RIN^e score to samples below the functional range, as low as 100 pg/μL, bringing low-concentration measurements into closer alignment with Bioanalyzer RIN scores and helping bridge the previous gap between the two systems. This application note evaluates eukaryotic and prokaryotic RNA samples spanning different integrity levels across the expanded range of the TapeStation High Sensitivity RNA ScreenTape assay.

Methods

Commercially available Human Universal and *E. coli* RNA samples were degraded to generate medium- and low-integrity material. Intact and degraded samples were diluted to 20 ng/μL in 1X TE. Five sample dilutions, ranging from approximately 100 to 5,000 pg/μL, were prepared for analysis on the Agilent 4200 TapeStation system (p/n G2991BA) using the Agilent High Sensitivity RNA ScreenTape assay: ScreenTape (p/n 5067-5579), ladder (p/n 5067-5581), and sample buffer (p/n 5067-5580). Each dilution was analyzed across all integrity levels in multiple replicates. Data analysis was performed using Agilent TapeStation software version 5.3.

For comparison, the Human Universal RNA samples were also analyzed in triplicate on the Agilent Bioanalyzer system using the Agilent RNA 6000 Pico kit (p/n 5067-1513).

Results

To demonstrate the capabilities of the updated TapeStation software, Human Universal total RNA samples were analyzed across concentrations ranging from 100 to 5,000 pg/μL. Digital gel images and electropherogram overlays of high-integrity Human Universal RNA are shown in Figure 1. At the highest concentration, the sample displayed two distinct ribosomal peaks and yielded a RIN^e score of 7.9, consistent with high-quality RNA. Below 1,000 pg/μL, the 28S ribosomal peak migrates more slowly with dilution, complicating RIN^e assessment. With the software update, RIN^e values can now be displayed for concentrations down to 100 pg/μL; however, samples outside the specified RIN^e functional range^{3,4} receive a warning flag to indicate that the samples should be reviewed carefully for suitability in downstream workflows.

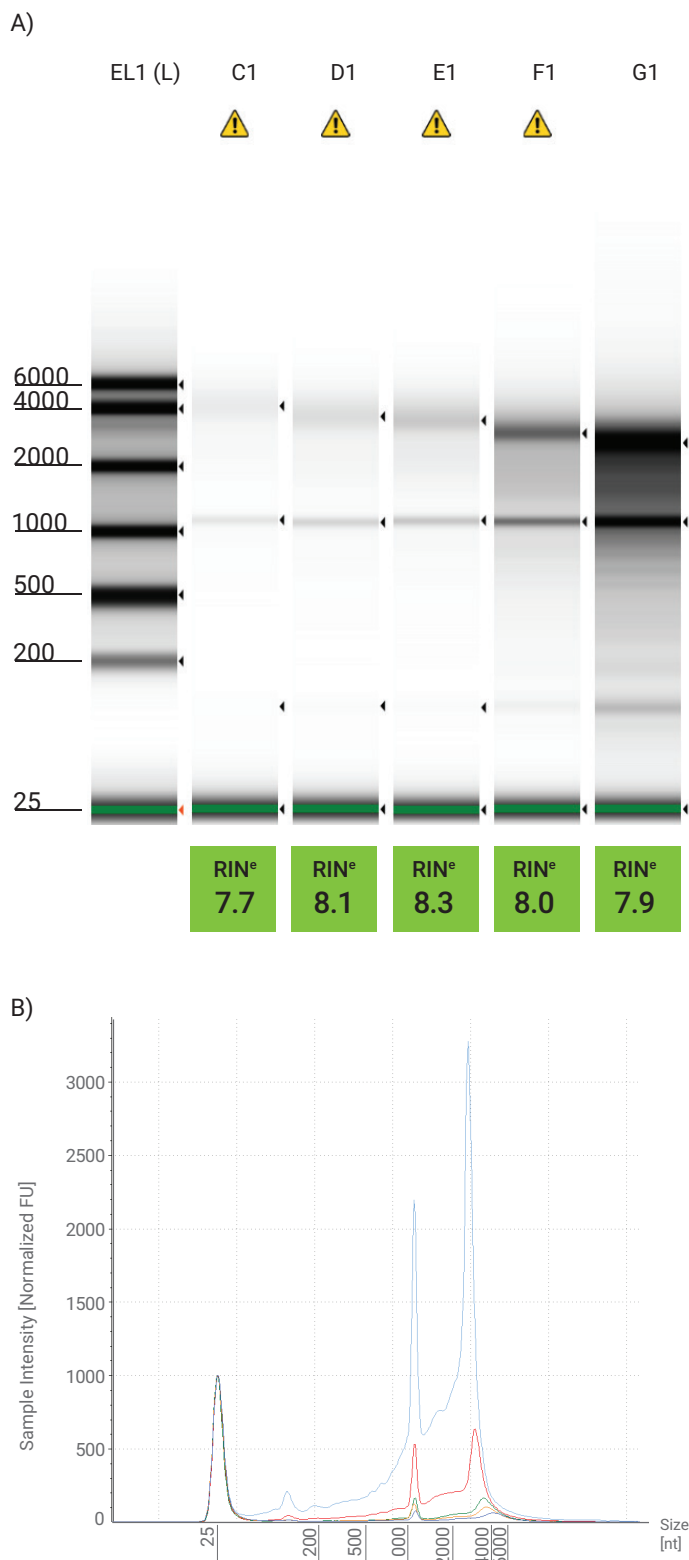
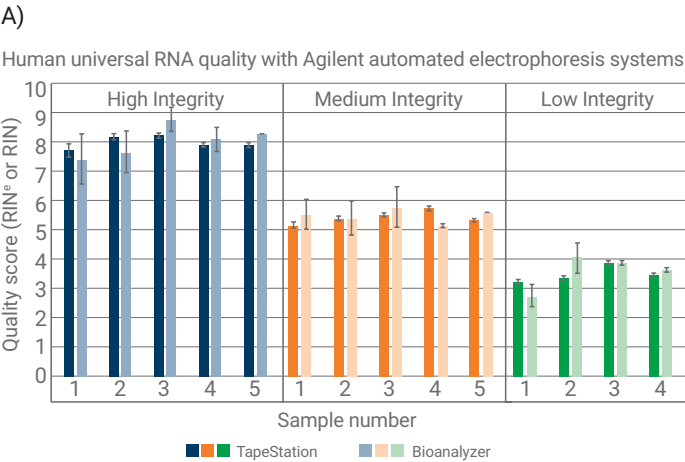


Figure 1. (A) Digital gel image and (B) electropherogram overlay of Human Universal RNA analyzed on the Agilent TapeStation system with the Agilent High Sensitivity RNA ScreenTape assay. Sample concentrations are shown in ascending order from approximately 100 to 5,000 pg/μL. RIN^e scores remain consistent across the dilution series. The warning symbol indicates that the concentration is outside the specified RIN^e functional range.

Human Universal RNA samples spanning high, medium, and low integrity were prepared by heat degradation and analyzed across a dilution series from 100 to 5,000 pg/ μ L on both the TapeStation and Bioanalyzer systems. As expected, decreasing RNA integrity was associated with loss of well-defined ribosomal peaks and lower integrity scores. Across the dilution series, RIN^e and RIN values remained consistent within each integrity level, demonstrating reliable scoring independent of sample concentration. Comparison of the two systems showed strong agreement in quality scores across all concentrations (Figure 2). For example, across all concentrations the intact RNA samples had overall average quality scores of 8.1 for RIN^e and 8.1 for RIN. Together, these results demonstrate robust and comparable RNA integrity assessment across the expanded RIN^e range, down to 100 pg/ μ L.



B)

Integrity Level	Sample #	Average Concentration (pg/ μ L)	Average TapeStation RIN ^e	Average Bioanalyzer RIN
High	1	146	7.8	7.4
	2	219	8.2	7.7
	3	275	8.3	8.8
	4	843	8.0	8.1
	5	3,715	7.9	8.3
Medium	1	139	5.2	5.5
	2	212	5.4	5.4
	3	285	5.6	5.8
	4	694	5.8	5.2
	5	4,480	5.3	5.6
Low	1	147	3.2	2.7
	2	216	3.4	4.1
	3	543	3.9	3.9
	4	3,175	3.5	3.7

Figure 2. Human Universal RNA of varying quality and concentrations ranging from 100 to 5,000 pg/ μ L were analyzed on the Agilent TapeStation system with the Agilent High Sensitivity RNA ScreenTape assay and the Agilent Bioanalyzer system with the Agilent RNA 6000 Pico kit to demonstrate the equivalency of RIN^e and RIN scores. Concentrations are the average measurements recorded by the TapeStation. Error bars represent standard deviation. N = 4.

In a second sample set, *E. coli* RNA spanning different integrity levels was prepared by heat degradation and analyzed on the TapeStation across the same dilution series. As with the Human Universal RNA samples, decreasing integrity was reflected by progressively lower RIN^e scores, while values remained consistent within each integrity level across concentrations from 100 to 5,000 pg/ μ L (Figure 3). These data further support the ability of the updated TapeStation software to provide reliable RNA integrity assessment down to 100 pg/ μ L across different RNA sample types.

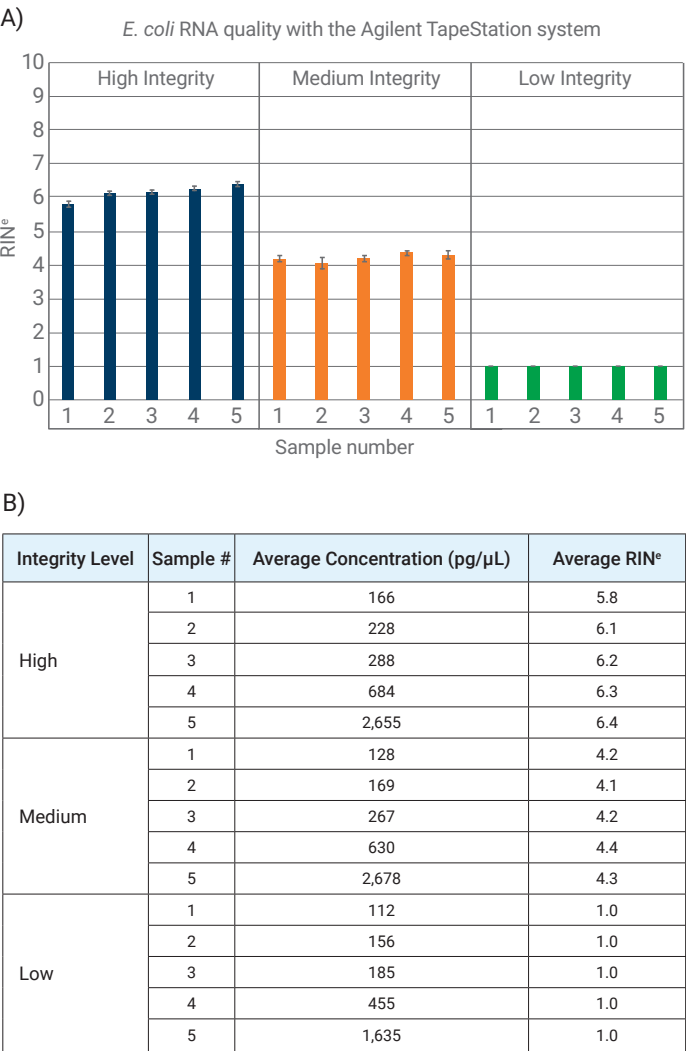


Figure 3. *E. coli* RNA of varying quality and concentrations ranging from 100 to 5,000 pg/ μ L were analyzed on the Agilent TapeStation system with the Agilent High Sensitivity RNA ScreenTape assay. Concentrations are the average values recorded by the TapeStation. Blue bars indicate high-quality samples, orange bars indicate medium-quality samples, and green bars indicate low-quality samples. Error bars represent standard deviation. N = 4.

Conclusion

This application note demonstrates that a TapeStation software update enables the reporting of RIN^e scores for the High Sensitivity RNA ScreenTape assay down to 100 pg/μL, below the validated functional RIN^e range of 1,000 to 25,000 pg/μL. This expanded range enables RNA integrity assessment for low-input samples without changes to the existing workflow. Across the extended reporting range, RIN^e values remained consistent within each integrity level, at low concentrations, and showed strong agreement with Bioanalyzer RIN values for Human Universal RNA. Results from both Human Universal and *E. coli* RNA further support reliable integrity assessment down to 100 pg/μL across different RNA sample types. By extending integrity scoring to lower concentrations, the updated Agilent TapeStation software version 5.3 provides greater flexibility for sample types where RNA is limited, including low-yield extractions, precious samples, and dilute preparations.

References

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