Technical Overview

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RNA Quality Assessment with the Agilent Automated Electrophoresis Systems

A comparison of the RIN, RQN, and RIN^{e}

Introduction

Assessing total RNA quality is vital in molecular biology and genomics research, as the integrity of RNA samples influences downstream analyses. Electrophoresis methods are commonly used to determine RNA quality. These techniques separate samples based on size, enabling the identification of ribosomal RNA peaks, small RNA, and any potential degradation products. The Agilent automated electrophoresis systems, including the Agilent 2100 Bioanalyzer instrument, Agilent Fragment Analyzer systems, and Agilent TapeStation systems, streamline total RNA quality analysis. Each system evaluates total RNA quality and assigns an individual RNA quality score on a scale from 1 to 10, with 1 indicating very degraded RNA and 10 implying highly intact RNA. This simplifies the process of total RNA quality assessment for each sample.

Microfluidic electrophoresis employed by the Bioanalyzer uses miniaturized channels and wells on a chip to enable precise analysis of small volumes of biological samples. This allows for efficient electrophoresis-based separation and characterization of nucleic acids. Following the separation, the system assigns an RNA Integrity Number (RIN) to each sample.

Using capillary electrophoresis, the Fragment Analyzer systems separate nucleic acids based on their size. This technology uses narrow capillary tubes to enable precise and rapid characterization of fragment sizes and concentrations in small sample volumes for diverse research applications. Additionally, the Fragment Analyzer accommodates up to three 96-well plates for automated sample quality control (QC). The Fragment Analyzer provides an RNA quality assessment in the form of the RNA Quality Number (RQN).

The TapeStation systems use specialized ScreenTape devices containing 16 lanes filled with gel. The automated sample transfer built into the system in conjunction with the ScreenTape devices enables the precise loading and analysis of small sample volumes and accurate assessment of nucleic acids. Additionally, the systems offer full sample scalability from 1 to 96 samples, which results in a streamlined and flexible solution for quality control in genomics research. When using the TapeStation for analysis of RNA, the system assigns an RNA integrity number equivalent (RIN^e) to each sample.

Each of the Agilent automated electrophoresis instruments provide digital gel images and electropherograms for each sample being assessed. An electropherogram for total RNA displays distinct areas that can influence a sample's quality metrics, as depicted in Figure 1: the ribosomal peaks, the fast region, and the inter region. The ribosomal peaks correspond to the ribosomal RNA found within cells. Eukaryotes have 18S and 28S rRNA peaks, whereas prokaryotes display 16S and 23S rRNA peaks. Plant samples may have different profiles depending upon the species and tissue. Regardless of the cell type, each of the rRNA peaks within a sample are representative of the major components of total RNA, and their integrated areas can be used to calculate a ribosomal ratio, which can be used as an indicator of RNA integrity. The fast region is the area left of the ribosomal peak, where small RNA molecules such as the 5S and 5.8S peaks migrate. Large or sharp peaks in this region indicate contamination or degradation of the RNA. The inter region is located between the two ribosomal peaks and is usually without other major peaks. However, when the larger rRNA peak degrades, it becomes smaller fragments which can show up in the inter region. This indicates that the sample has degraded, and therefore the quality of the total sample is affected[1,2]. Collectively, the distinct regions observed in the electropherogram play a crucial role in QC of RNA. These regions provide valuable information about sample integrity, contamination, and degradation, allowing researchers to assess the overall quality of the RNA sample.

In this technical overview, multiple sample types were analyzed across the three different Agilent automated electrophoresis instruments. Despite using different technologies, the RNA quality scores reported by each instrument were comparable across all platforms, providing researchers with confidence in the interchangeability of the systems between laboratories.

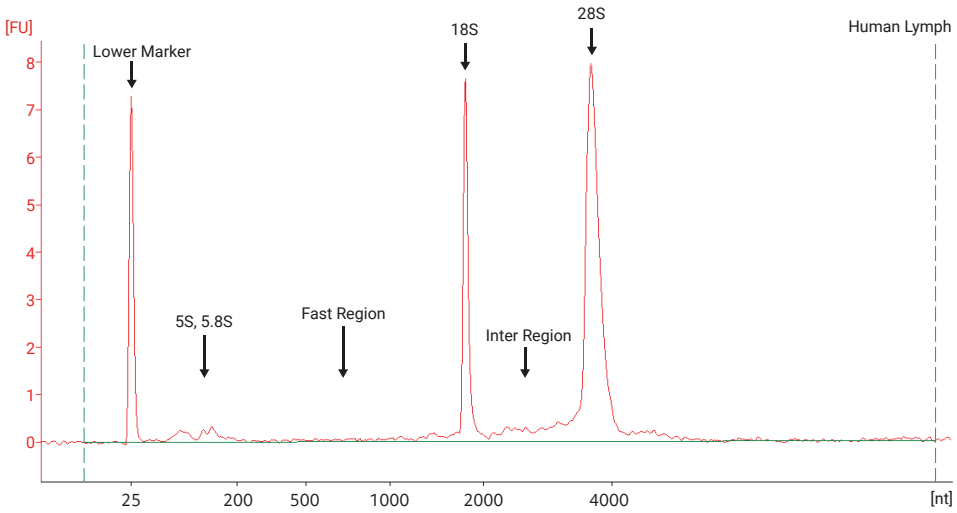

Figure 1. Example electropherogram from the Agilent 2100 Bioanalyzer system that highlights important features including ribisomal peaks (18S and 28S) and regions indicative of RNA quality.

Methods

Four commercially available total RNA samples are used as examples in this technical note: human heart (Thermo, p/n AM7966), mouse (Zyagen, p/n MR-104-17), corn (Zyagen, p/n PLR-1002), and *E. coli* (Zyagen, p/n ECR-310). Each sample was diluted to a concentration of approximately 200 ng/µl to fit the RNA assays for each of the Agilent automated electrophoresis systems (Table 1). An aliquot of each sample was then heat-degraded using a thermal cycler. The intact and degraded samples were analyzed in triplicate on the Bioanalyzer, Fragment Analyzer, and TapeStation systems. The RIN, RQN, and RIN^e for each sample type were compared.

For the Bioanalyzer system, the Agilent RNA 6000 Nano kit (p/n 5067-1511) was used. The eukaryote RNA assay was used when running human heart and mouse samples. The plant RNA assay was chosen when running corn total RNA, and the prokaryote assay was employed for *E. coli*.

Samples analyzed on the 5200 Fragment Analyzer used the Agilent RNA kit (15 nt) (p/n DNF-471). The Agilent ProSize data analysis software used the eukaryote setting when analyzing human heart and mouse samples, plant mode for analysis of corn, and prokaryote mode for *E. coli*.

The Agilent 4200 TapeStation analyzed samples using the Agilent RNA ScreenTape (p/n 5067-5576), Agilent RNA ScreenTape sample buffer (p/n 5067-5577), and Agilent RNA Screen-Tape ladder (p/n 5067-5578). The Agilent TapeStation analysis software was configured for human heart, mouse, and corn using the Eukaryotic RNA type setting, while *E. coli* was analyzed using the Prokaryotic RNA type setting.

Table 1. A comparison of the different RNA assays used in this technical overview for each of the Agilent automated electrophoresis systems.

	Reagent Kits	Sizing Range	Concentration Range	Quality Metric
Bioanalyzer system	RNA 6000 Nano kit (p/n 5067–1511)	--	25 to 500 ng/µL	RIN, DV_{200}
Fragment Analyzer systems	RNA kit (15 nt) (p/n DNF-471)	200 to 6,000 nt	25 to 500 ng/µL	RQN, DV_{200}
TapeStation systems	RNA ScreenTape (p/n 5067–5576) and Reagents (5067-5577, 5067-55578)	--	25 to 500 ng/µL	RIN^e, DV_{200}

Results

Human heart

Human heart total RNA was analyzed on all three systems (2100 Bioanalyzer, 5200 Fragment Analyzer, and 4200 TapeStation). The electropherograms from each system exhibit similarities in their general patterns and peak distributions (Figure 2). Particularly, each instrument displayed two prominent peaks representing the 18S and 28S ribosomal RNA fragments. When an RNA sample degrades, the larger 28S-sized fragment breaks apart more quickly[3].

This is evident by the 18S to 28S rRNA peak ratio changing after degradation, with the 28S peak significantly decreasing in area under the curve compared to the 18S peak which displayed a smaller reduction in area. Additionally, the amount of smaller RNA fragments increased, as shown by the elevated line in the fast region on each system's electropherogram. All the electropherograms display changes in the fast region, an increase in fragments in the inter region, and alterations in ribosomal ratios between the intact and degraded samples. Despite slight variations in peak heights and shapes, all three electropherograms effectively illustrate the distribution of RNA fragments within the samples. Notably, their RNA quality scores remained similar across the different platforms. For example, with the intact human heart samples, the Bioanalyzer, Fragment Analyzer, and TapeStation systems yielded average scores of 7.8, 7.5, and 6.9, respectively. The precision of each system, represented by the coefficient of variation (%CV), was 1% or less for all systems when analyzing the intact sample. When the degraded samples were run, the average scores decreased to 6.7, 6.5, and 5.8, respectively with %CVs of 2% or less (Table 2).

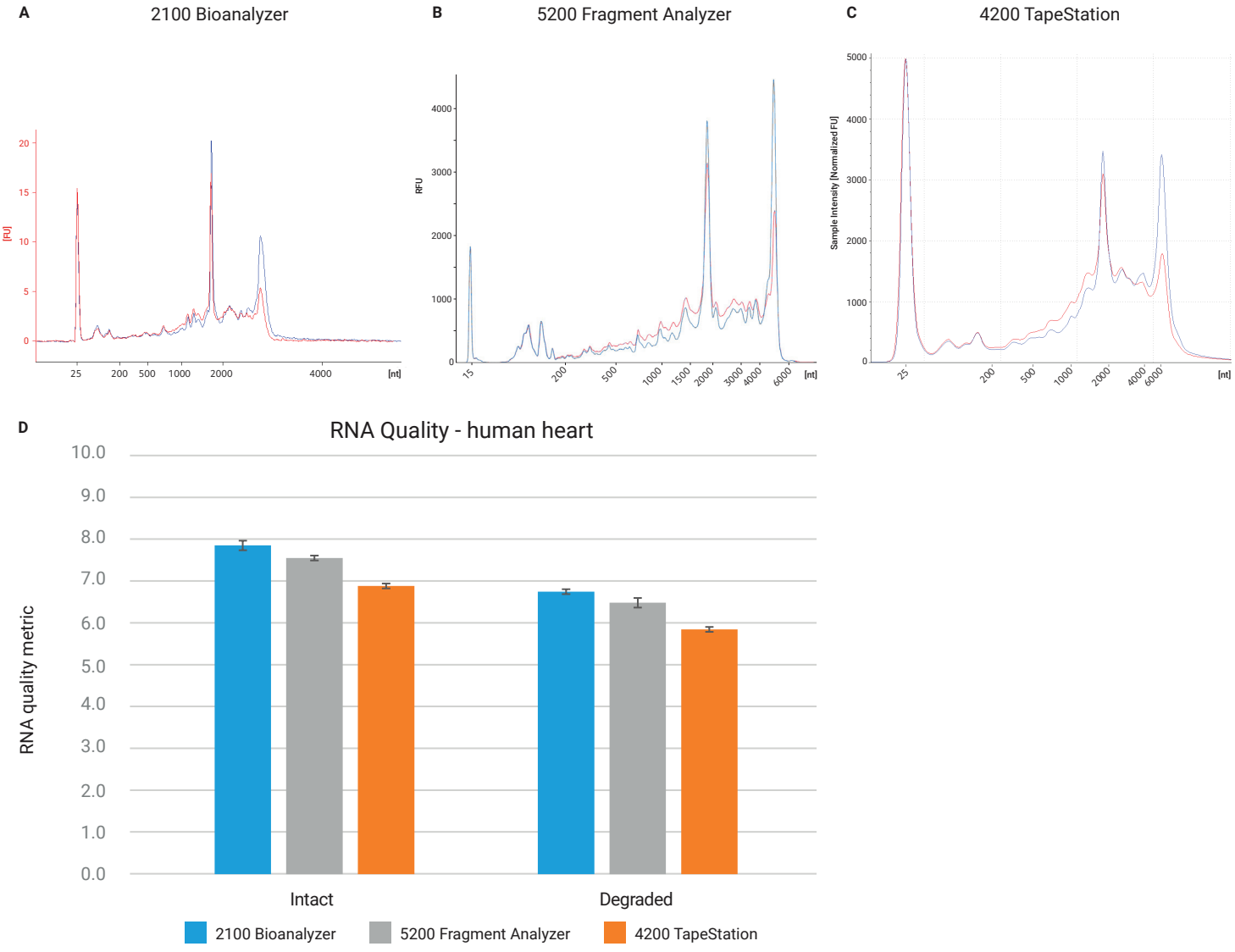

Figure 2. Electropherograms of intact (blue) and degraded (red) human heart total RNA run on the **A)** Agilent 2100 Bioanalyzer system using the Agilent RNA 6000 Nano kit, **B)** Agilent 5200 Fragment Analyzer using the Agilent RNA kit (15 nt), and on the **C)** Agilent 4200 TapeStation using the Agilent RNA ScreenTape assay. **D)** Resulting RNA quality metrics provided by each system for intact and degraded samples. Error bars represent standard deviation. N = 3.

Mouse

Mouse total RNA assessed on all three systems showed electropherograms with similarities in their general patterns and peak distributions (Figure 3). Each system showed the eukaryotic peaks for the 18S and 28S ribosomal RNA fragments. Few additional smaller peaks and smears throughout the electropherograms are visible in the intact samples, indicating good quality. Analysis of the degraded samples showed a decrease in the 28S rRNA peak in all instruments. There is also a significant increase in the amount of smaller RNA fragments, indicated by the smear-like curve present in the fast region of the degraded sample for all systems. Although there are slight differences in the appearance of the electropherograms between systems, such as variations in peak heights and shapes, the RNA quality scores remained highly similar to one another. The intact mouse total RNA, assessed on the Bioanalyzer, Fragment Analyzer, and TapeStation systems resulted in average scores of 9.0, 9.7, and 9.3, respectively. The precision of each system represented with %CV values were of 2% or less. The degraded samples also had highly similar quality scores with averages of 6.1, 6.2, and 6.1 with %CV values of 3 %CV or less (Table 2).

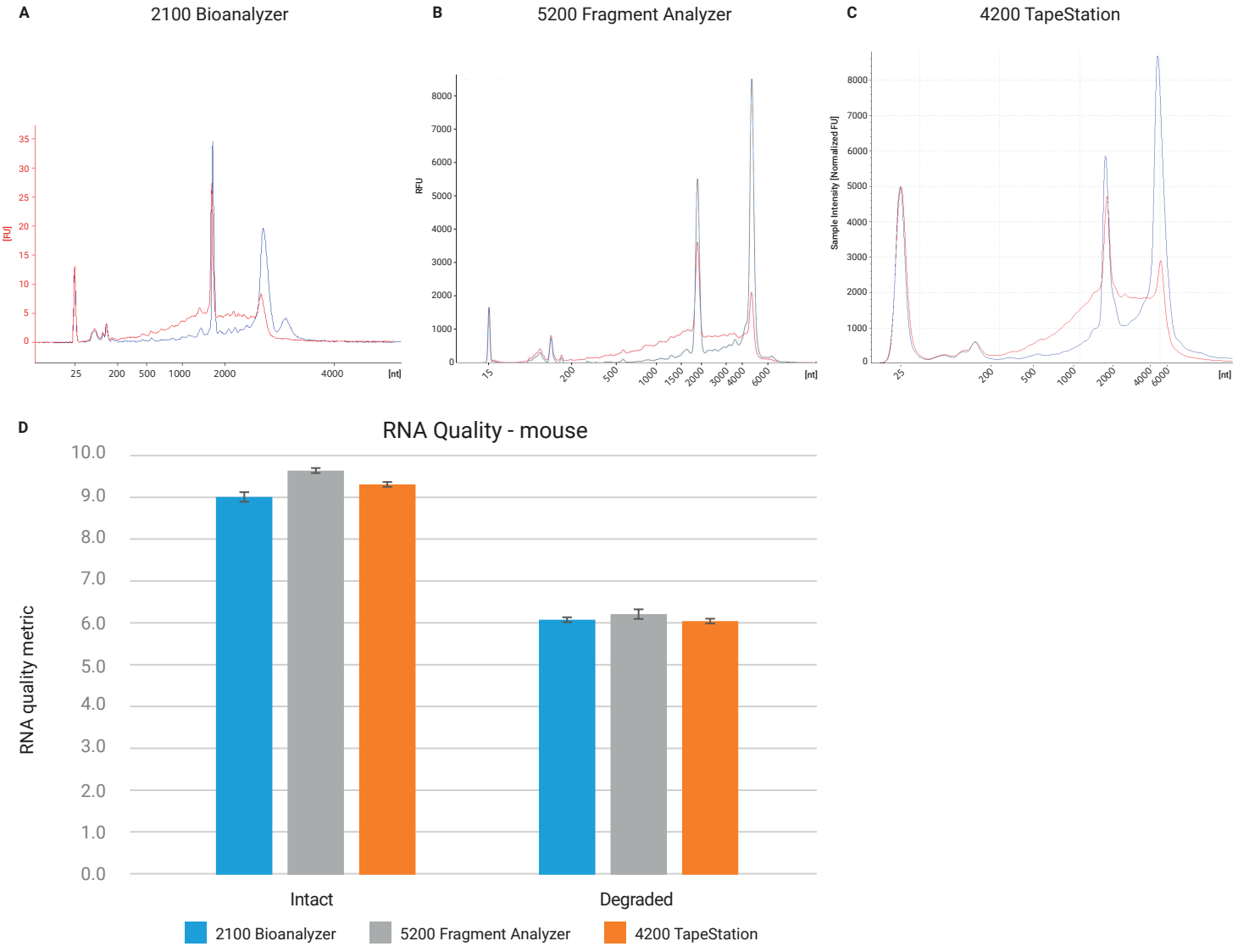

Figure 3. Electropherograms of intact (blue) and degraded (red) mouse total RNA run on the **A)** Agilent 2100 Bioanalyzer system using the Agilent 6000 RNA Nano kit, **B)** Agilent 5200 Fragment Analyzer using the Agilent RNA kit (15 nt), and on the **C)** Agilent 4200 TapeStation using the Agilent RNA ScreenTape Assay. **D)** Resulting RNA quality metrics provided by each instrument for the intact and degraded samples. Error bars represent standard deviation. N = 3.

Corn

Corn total RNA was evaluated using each system, and their electropherograms are shown in Figure 4. Unlike eukaryotic RNA, plant RNAs can have several ribosomal RNA peaks due to the presence of chloroplast rRNA. The two fast-migrating peaks correspond to smaller chloroplast ribosomal RNAs, while the two larger peaks that migrate slower are from the original cell, and are identified as the 18S and 25S rRNA peaks. The electropherograms from each system depict the overall changes between the intact and degraded samples. This change is characterized by an increase in smaller RNA fragments, as evidenced by the heightened line away from the baseline for the degraded sample, located in the fast region. Additionally, each system shows a change in the ratio between the 18S and 25S rRNA peak heights.

For the intact sample, the Bioanalyzer, Fragment Analyzer, and TapeStation systems showed comparable average RNA quality scores of 7.9, 7.4, and 8.2, respectively, with %CVs of 1% or less, representing the high precision of each system. After degradation of the sample, the quality scores decreased similarly across the systems, with average scores of 6.3, 5.2, and 5.9, respectively, with precision of 3 %CV or less (Table 2).

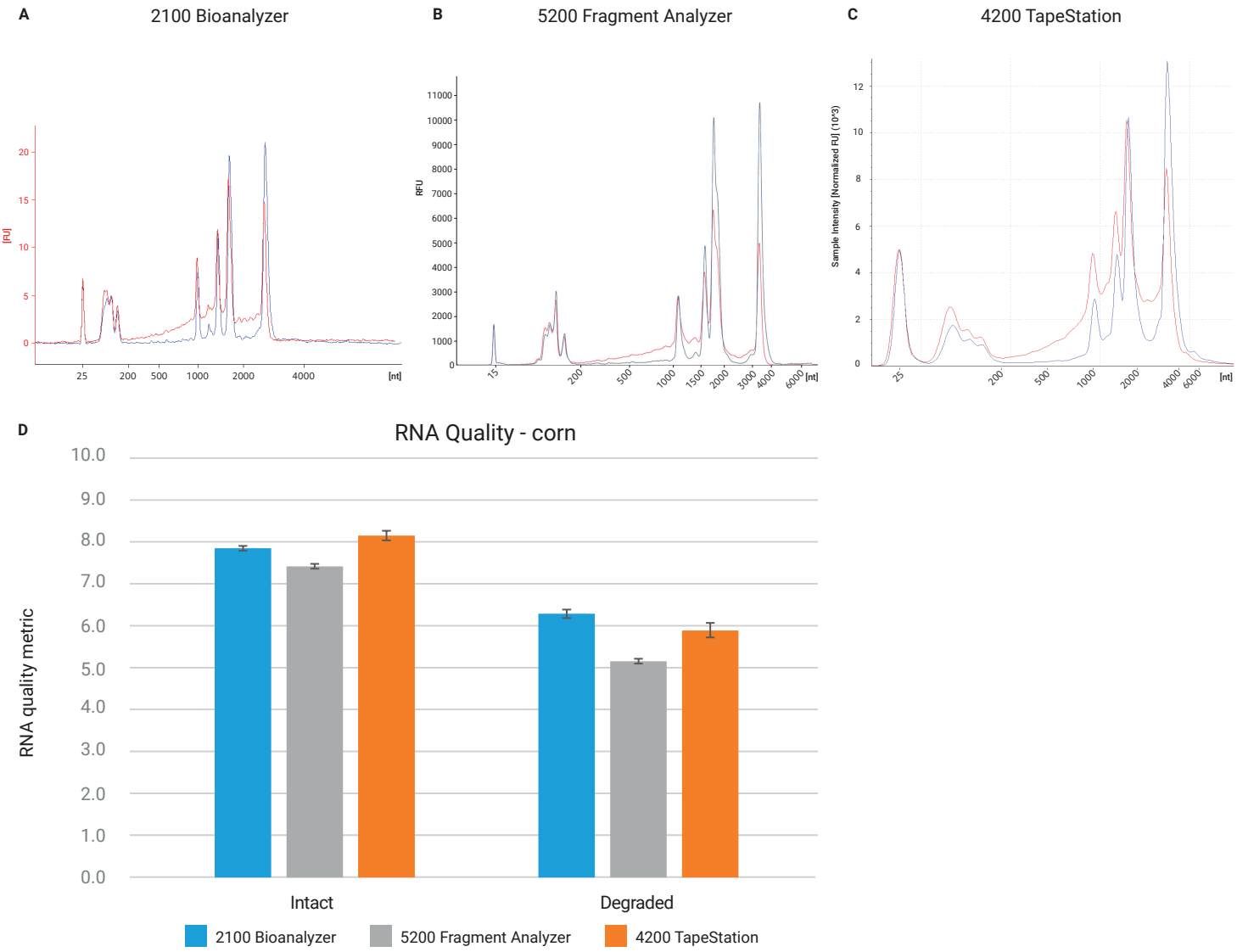

Figure 4. Electropherograms of intact (blue) and degraded (red) corn total RNA run on the **A)** Agilent 2100 Bioanalyzer system using the Agilent 6000 RNA Nano assay, **B)** Agilent 5200 Fragment Analyzer using the Agilent RNA kit (15 nt), and on the **C)** Agilent 4200 TapeStation using the Agilent RNA ScreenTape assay. **D)** Resulting RNA quality metrics provided by each instrument for intact and degraded samples. Error bars represent standard deviation. N = 3.

E. coli

The total RNA of *E. coli* assessed on all three systems displayed similar patterns for both the intact and degraded versions of the sample. Specifically, the electropherograms showed two dominant peaks representing the 16S and 23S rRNA (Figure 5). The degraded samples on each system showed an increase in smaller RNA fragments in the fast region, indicating lower quality. This is visualized by the elevated line in each system that is present in the fast region of the degraded sample but not the intact sample. Additionally, each system exhibits a change in the ratio between the 16S and 23S rRNA peaks. While some slight differences are apparent, the RNA quality scores were still comparable. Using the Bioanalyzer, Fragment Analyzer, and TapeStation systems, the intact sample had average RNA quality scores of 8.4, 9.3, and 8.5, respectively. The calculated level of precision was found to be 2 %CV or less. After degradation of the sample, the quality scores decreased on each system, scoring on average 5.4, 5.7, and 5.7 respectively with 3% CV or less (Table 2).

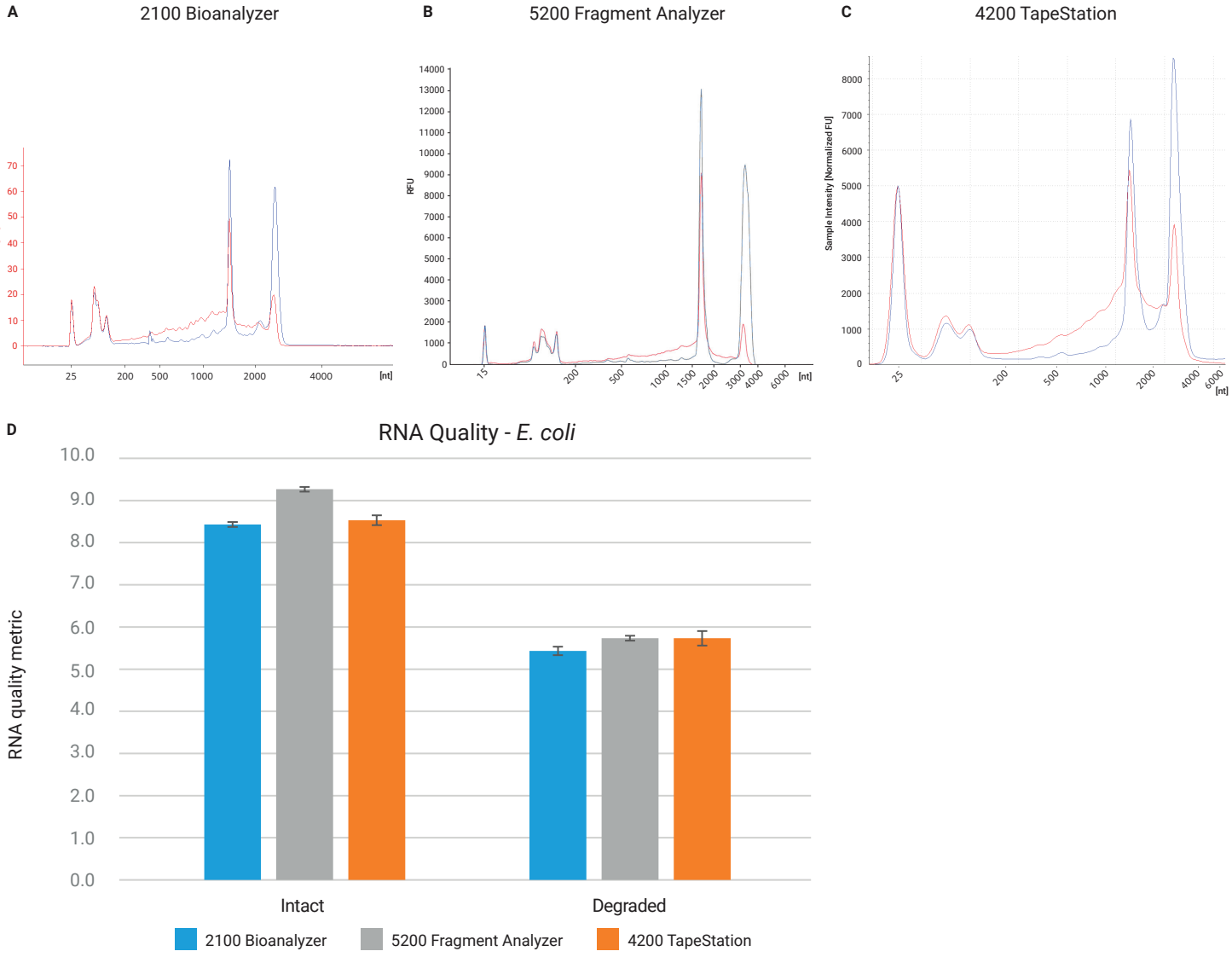

Figure 5. Electropherograms of intact (blue) and degraded (red) *E. coli* total RNA run on the **A)** Agilent 2100 Bioanalyzer system using the Agilent RNA 6000 Nano assay, **B)** Agilent 5200 Fragment Analyzer system using the Agilent RNA kit (15 nt), and on the **C)** Agilent 4200 TapeStation system using the Agilent RNA ScreenTape assay. **D)** Resulting RNA quality metrics provided by each instrument for intact and degraded samples. Error bars represent standard deviation. N = 3.

RNA quality score comparison

Despite the different technologies employed by each instrument, the RNA quality metrics remained consistent between systems within each tested sample type. As demonstrated in Table 2, only slight differences in the average RNA quality metrics were observed between systems, regardless of the sample. These differences can be attributed to the distinct technologies. Furthermore, the %CVs for each system underscore their high precision in assessing RNA quality. The high-sensitivity versions of the RNA assays used in this paper provided similar results (data not shown).

Conclusion

This technical overview demonstrates the comparability of the Agilent automated electrophoresis instruments in assessing intact and degraded total RNA samples from various sources, including human heart, mouse, corn, and *E. coli*. Although each instrument employs different technologies, the RNA quality scores provided between systems remained similar. This provides researchers with confidence in the interchangeability of these systems across laboratories.

Table 2. Average RNA quality scores and precision (%CV) observed for the Agilent 2100 Bioanalyzer system using the Agilent RNA 6000 Nano assay, Agilent 5200 Fragment Analyzer system using the Agilent RNA kit (15 nt), and the Agilent 4200 TapeStation system using the Agilent RNA ScreenTape assay. N=3.

Sample Name	Sample Integrity	2100 Bioanalyzer		5200 Fragment Analyzer		4200 TapeStation	
		Average RNA Integrity Number (RIN)	%CV	Average RNA Quality Number (RQN)	%CV	Average RNA Integrity Number equivalent (RIN^e)	%CV
Human Heart	Intact	7.8	1.5%	7.5	0.8%	6.9	0.8%
Human Heart	Degraded	6.7	0.9%	6.5	1.8%	5.8	1.0%
Mouse	Intact	9.0	0.6%	9.7	1.6%	9.3	0.6%
Mouse	Degraded	6.1	1.6%	6.2	0.9%	6.1	2.5%
Corn	Intact	7.9	0.7%	7.4	0.8%	8.2	1.4%
Corn	Degraded	6.3	1.6%	5.2	1.1%	5.9	2.9%
E. coli	Intact	8.4	0.7%	9.3	2.5%	8.5	0.7%
E. coli	Degraded	5.4	1.1%	5.7	2.7%	5.7	1.0%

References

1. Comparison of RIN and RQN for the Agilent 2100 Bioanalyzer and the Fragment Analyzer Systems. *Agilent Technologies technical overview*, publication number 5994-1860, **2020**.
2. RNA Integrity Number (RIN) – Standardization of RNA Quality Control. *Agilent Technologies application note*, publication number 5989-1165, **2016**.
3. Quality Analysis of Eukaryotic Total RNA with the Agilent 5200 Fragment Analyzer System. *Agilent Technologies application note*, publication number 5994-0519, **2019**.

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