Technical Overview

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Comparison of RNA Quality Analysis with the Qubit RNA IQ Assay and Agilent Automated Electrophoresis Systems

Introduction

The quality of an RNA sample can be crucial to many downstream applications. For example, achieving reliable RNA sequencing results relies upon initiating each analysis run with highly intact RNA samples. RNA integrity can be determined using electrophoresis methods that separate the sample based on size, allowing for the distinction of the ribosomal peaks, small RNA, and any degradation products. The Agilent automated electrophoresis portfolio, including the Agilent 2100 Bioanalyzer system and the Agilent Fragment Analyzer systems, are used for RNA quality control (QC), providing information about both the quantity and the quality of a given sample. The systems both generate an objective, reliable quality metric score for each sample analyzed[1]. These scores are known as the RNA integrity number (RIN) for the Bioanalyzer and the RNA quality number (RQN) for the Fragment Analyzers. Each score is based on a scale of 1 to 10, with 1 identifying significantly degraded samples and 10 identifying high-quality, intact RNA specimens. Many workflows for downstream applications state that only the highest quality scores are sufficient for use. For example, many laboratories performing RNA sequencing will only sequence samples with a quality score of 7 or above to ensure robust results[2,3].

The RNA Integrity and Quality (IQ) Assay kit from Thermo Fisher Scientific also offers an RNA quality score. This kit is used with the Qubit 4.0 fluorometer and utilizes two dyes to selectively bind to either large and intact RNA or degraded RNA. The RNA IQ score is therefore representative of the ratio of small and large RNAs present in the sample. In contrast to the Agilent automated electrophoresis systems, the Qubit RNA IQ kit is not quantitative, and thus does not provide concentration data for the sample. Here, the RIN and RQN scores from the Agilent automated electrophoresis instruments are compared to the Qubit RNA IQ score across a series of RNA reference samples from multiple species.

Methods

Preparation of intact and degraded RNA

Commercially available human heart (Thermo Fisher Scientific, p/n AM7966), mouse CD1 Embryo-E17 (Zyagen, p/n MR-104-17), and corn (Zyagen, p/n PLR-1002) total RNA samples were obtained and diluted to approximately 200 ng/µL to fit the input concentration range of the Bioanalyzer, Fragment Analyzer, and Qubit analysis kits. Aliquots of each sample were stored on ice to maintain integrity, or intentionally degraded at 90 °C to obtain samples that were mildly or severely degraded.

RNA degradation series sample preparation

Commercially available human heart (Thermo Fisher Scientific, p/n AM7966) total RNA samples were obtained and diluted to approximately 200 ng/µL to fit the input concentration range of the Bioanalyzer, Fragment Analyzer, and Qubit analysis kits. Aliquots of each sample were stored on ice to maintain integrity, or intentionally degraded at 75 °C at intervals between 5 and 100 minutes.

Analysis of RNA quality

Each sample was analyzed in triplicate using both the Bioanalyzer with the RNA 6000 Nano kit (p/n 5067-1511) and the Fragment Analyzer with the RNA kit (15 nt) (p/n DNF-471). For comparison, the same samples were analyzed using the Qubit 4.0 with the RNA Integrity and Quality (IQ) Assay kit (Thermo Fisher Scientific, p/n Q33221).

Results and Discussion

RNA quality score comparison

RNA quality can be essential to successful downstream applications. The Bioanalyzer and Fragment Analyzer are well established systems for determining RNA integrity, with quality metric scores (RIN and RQN, respectively) that can help identify the quality of a sample and give an indication of how well the sample may perform in downstream workflows. The RIN and RQN consider the area of the ribosomal peaks and the presence of any small RNAs or degradation products. Additionally, the Bioanalyzer and Fragment Analyzer kits allow for sizing and quantification of RNA in the same assay. The Qubit instrument with the RNA IQ assay kit generates an RNA quality score based on the percentage of large RNAs in a sample and is not quantitative. The specifications of the kits used in this study are shown in Table 1.

Table 1. Specifications of the Agilent 2100 Bioanalyzer system, Agilent Fragment Analyzer system, and Qubit 4.0 kits used in this study for qualification of RNA.

System	Agilent Bioanalyzer system	Agilent Fragment Analyzer system	Qubit 4.0
Kit	**RNA 6000 Nano kit**	**RNA kit (15 nt)**	**RNA Integrity and Quality (IQ) Assay kit**
Quality metric	RIN	RQN	RNA IQ
Qualitative range	5 to 500 ng/µL	25 to 500 ng/µL	25 to 1,500 ng/µL
Quantitative range	25 to 500 ng/µL	25 to 500 ng/µL	NA
Quantitative precision	10% CV	10% CV	NA
Quantitative accuracy	±20%	±20%	NA
Volume required	1 µL	2 µL	1 to 20 µL

Human RNA

Commercially available human heart RNA was heated at 90 °C for 5 and 12 minutes to achieve mild and severe degradation. The intact, mildly degraded, and severely degraded RNA was analyzed on the Bioanalyzer, Fragment Analyzer, and Qubit. Electropherogram overlays of the three samples on the Bioanalyzer are shown in Figure 1A and on the Fragment Analyzer in Figure 1B.

Both the Bioanalyzer and Fragment Analyzer display similar profiles for each sample. The intact RNA is presented as two large ribosomal peaks, the 18S and 28S rRNA, with very little smearing in the fast and inter-regions, indicating high-quality RNA (Figure 1A-B). The smaller sized peaks in the 5S region indicate the presence of small RNAs in the sample. Slight degradation of the sample resulted in shorter peak heights and increased smearing in the fast and inter-regions (Figure 1A-B). Severe degradation of the sample is shown by the absence of the 28S peak and increased smearing (Figure 1A-B).

The average quality scores from each instrument are shown in Figure 1C. The Bioanalyzer resulted in an average RIN of 9.1 for the intact sample, which decreased to 6.0 for the mildly degraded sample, and 4.2 for the severely degraded sample. The Fragment Analyzer displayed average RQNs of 9.0, 6.2, and 4.2, respectively. The average Qubit RNA IQ score was 8.5 for the intact sample, which remained consistent despite degradation, with an average score of 8.6 for both mild and severely degraded RNA.

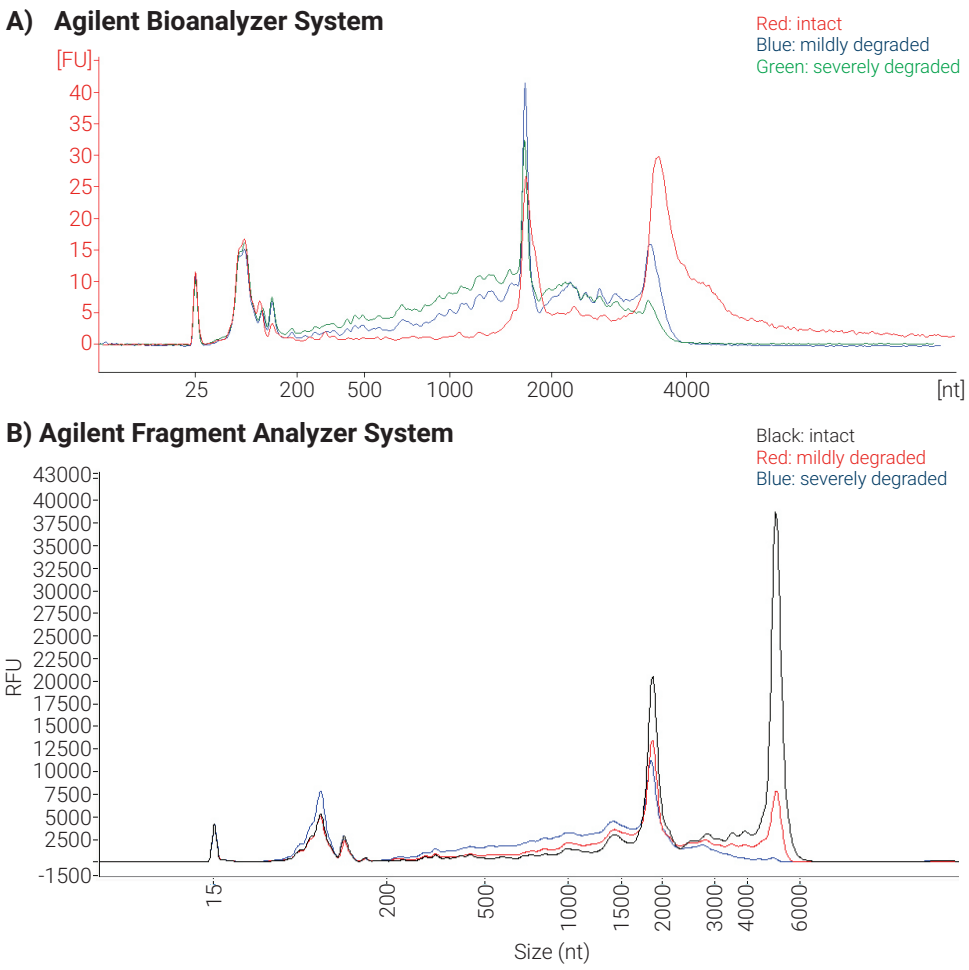


Figure 1. Human heart RNA was analyzed on A) the Agilent 2100 Bioanalyzer system and B) the Agilent 5200 Fragment Analyzer system. Representative electropherograms of intact, mildly degraded, and severely degraded sample are overlaid for comparison. C) The average RNA quality scores reported by the Bioanalyzer, Fragment Analyzer, and Qubit (n=3).

Mouse RNA

To intentionally obtain mildly and severely degraded mouse RNA, the sample was heated at 90 °C for 20 and 40 minutes. The samples display similar profiles to the human RNA on the Bioanalyzer and Fragment Analyzer. The intact RNA appears as two sharp fragments, characteristic of the 18S and 28S RNA peaks, with the fast and inter-regions returning to the baseline. As the sample is degraded, the peak heights decrease and the area under the fast and inter-region lines increases, representative of degradation.

Figure 2A shows representative samples analyzed on the Bioanalyzer, with the intact and degraded samples overlaid for comparison. Similarly, Figure 2B shows an electropherogram overlay from the Fragment Analyzer.

Additionally, the RNA quality numbers are similar between the Bioanalyzer and Fragment Analyzer. Analysis of the intact, mildly degraded, and severely degraded samples on the Bioanalyzer resulted in average RIN scores of 9.2, 6.2, and 4.2, respectively. On the Fragment Analyzer, the RQN scores decreased from 10.0 to 7.1 and 4.2 as the sample was degraded. In contrast, the Qubit RNA IQ score remained at an average of 8.9, indicating highly intact RNA despite the lengthy heat degradation of the sample.

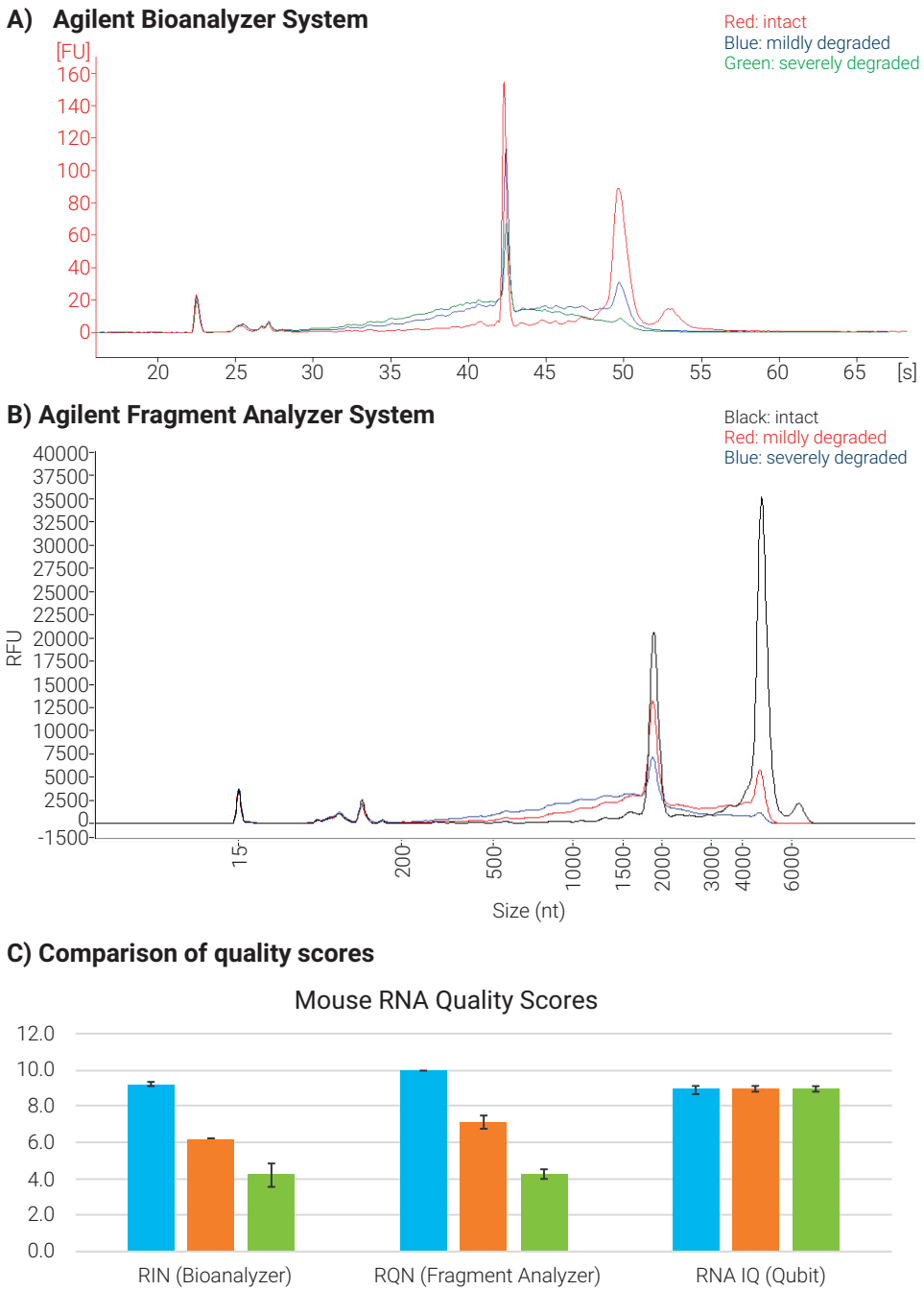


Figure 2. Mouse RNA was analyzed on A) the Agilent 2100 Bioanalyzer system and B) the Agilent 5200 Fragment Analyzer system. Representative electropherograms of intact, mildly degraded, and severely degraded sample are overlaid for comparison. C) The average RNA quality scores reported by the Bioanalyzer, Fragment Analyzer, and Qubit (n=3).

Corn RNA

Intact, mildly degraded, and severely degraded corn RNA was analyzed on the Bioanalyzer, Fragment Analyzer, and Qubit. Electropherograms of plant RNA can display several ribosomal peaks due to the presence of cytosolic, chloroplast, and mitochondrial ribosomal subunits.

As shown in Figures 3A and 3B, the electropherograms of corn RNA from the Bioanalyzer and Fragment Analyzer display four peaks. Similar to the human and mouse profiles, the heights of the four peaks decrease and smearing is evident between the peaks as the sample is heat degraded. Analysis with the Bioanalyzer displays an average RIN for the intact RNA of 9.0, which decreases to 7.1 and 5.4 with mild and severe degradation. The Fragment Analyzer reports an average RQN of 8.4, 6.7, and 3.4, respectively. The average Qubit RNA IQ score for both the intact and mildly degraded RNA was 8.8, with the severely degraded sample at 9.0 (Figure 3C).

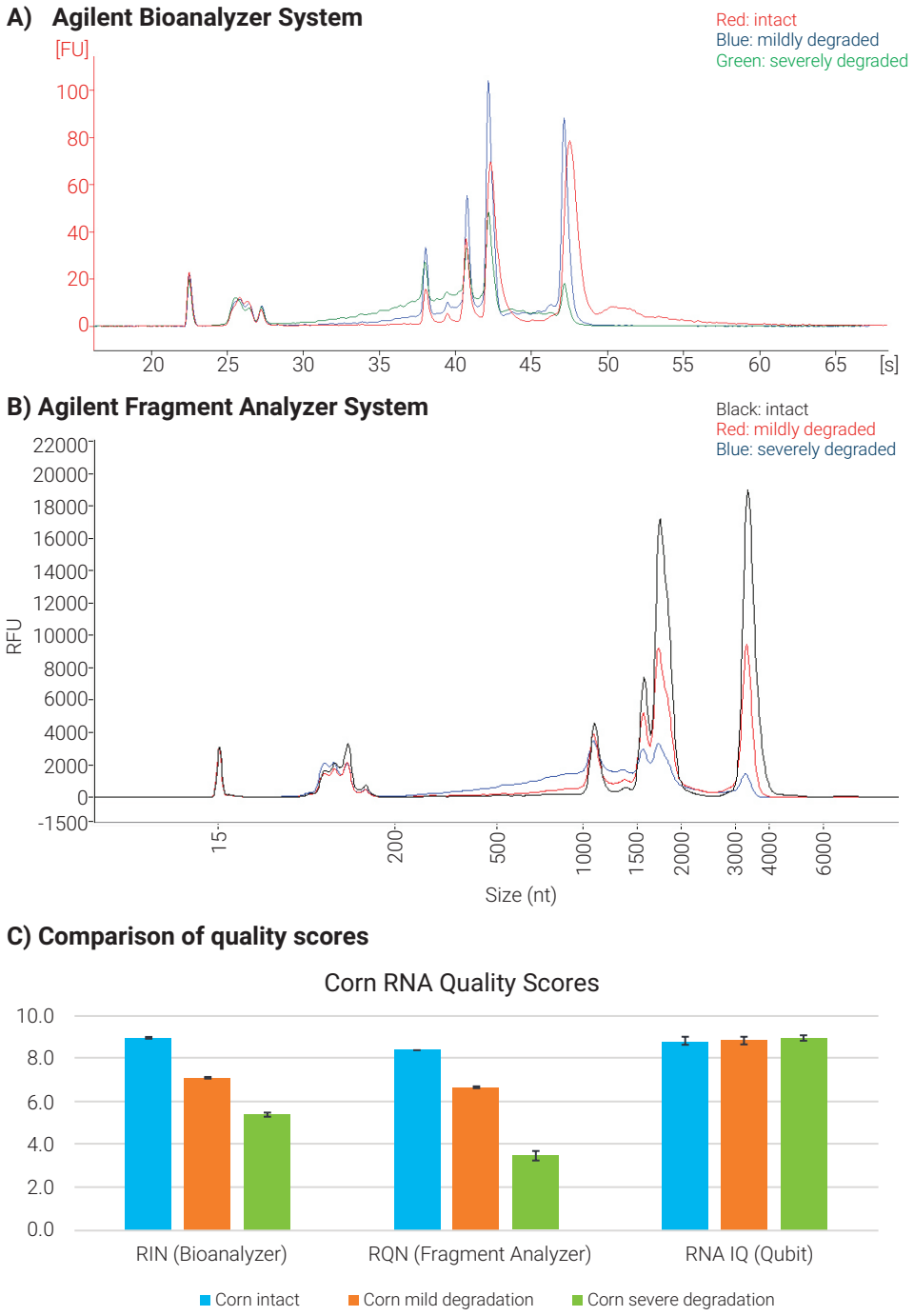


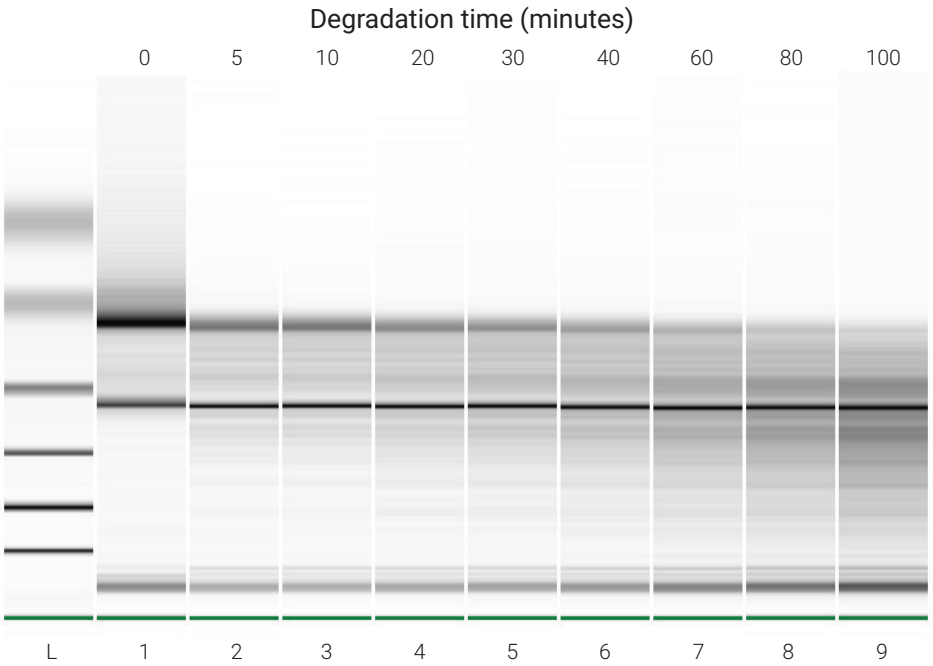
Figure 3. Corn RNA was analyzed on A) the Agilent 2100 Bioanalyzer system and B) the Agilent 5200 Fragment Analyzer system. Representative electropherograms of intact, mildly degraded, and severely degraded sample are overlaid for comparison. C) The average RNA quality scores reported by the Bioanalyzer, Fragment Analyzer, and Qubit (n=3).

Degradation time series

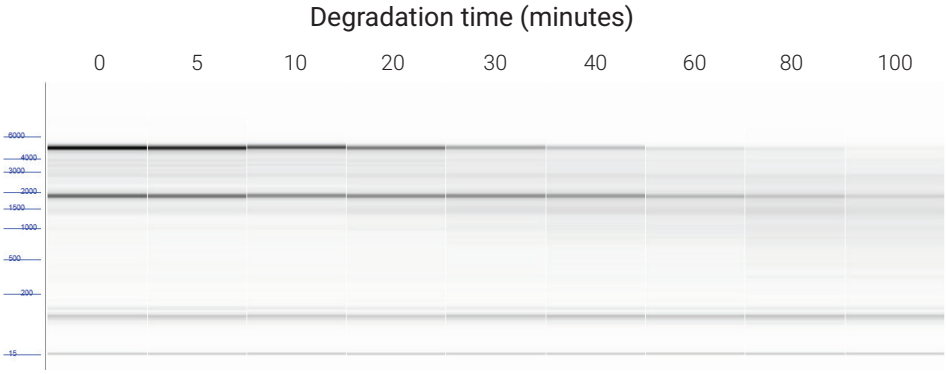
To further evaluate the quality scores obtained from the Bioanalyzer, Fragment Analyzer, and Qubit, commercially available human heart total RNA was degraded at 75 °C for time intervals ranging from 5 to 100 minutes, and the samples were analyzed in triplicate on each system. To illustrate the integrity of the samples over time, digital gel images from the Bioanalyzer (Figure 4A) and Fragment Analyzer (Figure 4B) are shown.

The Bioanalyzer and Fragment Analyzer both display similar results, with the two ribosomal peaks present in the intact sample. The larger ribosomal peak slowly diminishes as the sample is heated for longer times. The increased presence of smaller fragments and a more prevalent smear indicates degradation of the sample over time. The Bioanalyzer RIN and Fragment Analyzer RQN quality scores decrease consistently with the degradation time; the RIN decreases from 9.2 to 3.8, and the RQN from 8.47 to 4.30 (Figure 4C). Alternately, the Qubit RNA IQ score remains between 5.9 and 7.9, with no correlation between the score and time (Figure 4C).

A) Agilent Bioanalyzer System RIN



B) Agilent Fragment Analyzer System RQN



C) Comparison of quality scores

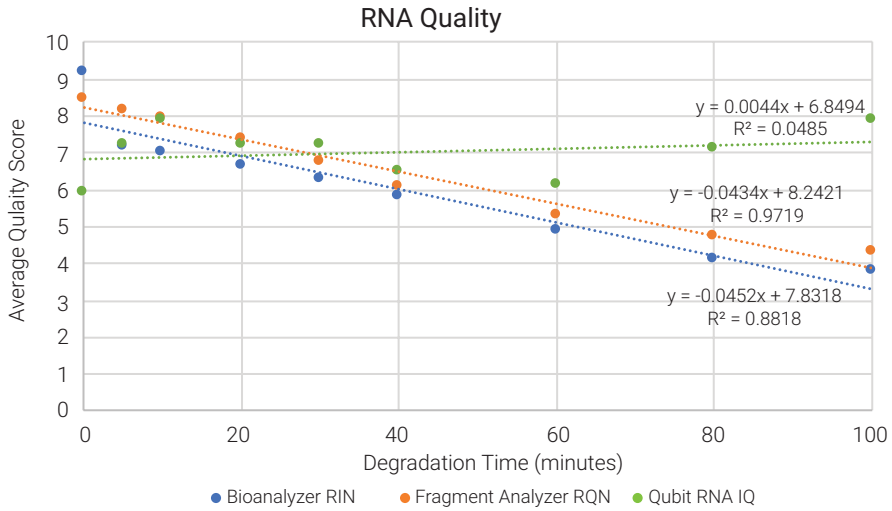


Figure 4. Human heart RNA was analyzed on A) the Agilent 2100 Bioanalyzer system and B) the Agilent 5200 Fragment Analyzer system. Digital gel images of the degradation series are shown, with the most intact samples on the left and the most degraded on the right. C) The average RNA quality scores reported by the Bioanalyzer, Fragment Analyzer, and Qubit for each time point throughout the degradation series (n=3).

Summary

High-quality RNA can be essential for successful downstream workflows, such as qPCR and RNA sequencing. The Agilent automated electrophoresis instruments provide reliable and accurate data about sample quality, including a gel and electropherogram image, quality score, and quantification of the sample. The digital gel and electropherogram provide a visual illustration of the quality of a sample, while the RIN or RQN give a user-independent score representative of the integrity of the sample.

This technical overview compared the RNA quality scores generated from the Agilent automated electrophoresis instruments to the score generated by the Qubit RNA IQ assay. As RNA samples are exposed to increasingly harsh conditions, it is anticipated that the quality score of the sample will decrease. Despite intentional degradation of the sample, the Qubit IQ score remained similar across all samples, while the Agilent systems showed an expected decrease in the quality score as the sample was further degraded.

These results indicate that the Agilent automated electrophoresis systems provide a more accurate assessment of the quality of RNA samples to be used in sensitive downstream applications. Additionally, this overview demonstrates that the Agilent 2100 Bioanalyzer and Fragment Analyzer systems provide similar quality scores for RNA samples from different species and of varying degrees of degradation.

References

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