Application Note

Genomics

Agilent

Trusted Answers

Plant RNA Degradation Detection Using the Agilent 5200 Fragment Analyzer System

Authors

Chava Pocernich,
Denise Warzak, and
Kit-Sum Wong
Agilent Technologies, Inc.

Abstract

Assessment of RNA quality is a crucial component in the success of genomic techniques such as RT-PCR, microarray analysis, and next-generation sequencing (NGS). The Agilent 5200 Fragment Analyzer system and Agilent RNA kits enable easy analysis of RNA quality. The Agilent ProSize data analysis software provides an electropherogram and a digital gel image for visual inspection, automatically reports the ribosomal ratio, and assigns an RNA quality number (RQN) for total RNA to every sample. ProSize software includes a dedicated plant mode for evaluating complex plant RNA. In this Application Note, we demonstrate the ease of assessing plant RNA integrity with excellent RQN precision on the 5200 Fragment Analyzer system.

Introduction

RNA integrity is a constant concern because of how easily RNA degrades due to temperature, enzymatic digestion, and improper handling. High-quality RNA is crucial for successful outcomes in RT-PCR, microarray analysis, and next-generation sequencing (NGS). Conventionally, only the ribosomal ratio (25S/18S) was used to assess the quality of RNA, but Agilent ProSize data analysis software uses the RNA quality number (RQN) as a quality metric indicator. ProSize software considers the entire electropherogram and has a dedicated plant mode that can be selected under the Advanced Settings tab for evaluating complex plant RNA. Calculation of the RQN includes the 5S region and the fast region where the small and chloroplastic RNA separate, as well as the 25S/18S region for assessing the RNA quality. The RQN is based on a scale from 1 to 10, where 1 represents completely degraded total RNA and 10 represents fully intact total RNA. The RQN and 25S/18S ribosomal ratio are automatically reported, providing for easy evaluation of total plant RNA quality.

Experimental

The experiments in this study were done using an Agilent 5200 Fragment Analyzer system and can be replicated with comparable results on Agilent 5300 and 5400 Fragment Analyzer systems.

The Agilent 5200 Fragment Analyzer system equipped with the Agilent FA 12-capillary array short, 33 cm (short array) (p/n A2300-1250-3355) was used to analyze various plant total RNA samples with the Agilent HS RNA kit (15 nt) (p/n DNF-472) (Figures 1 and 4). Total RNA was extracted from several

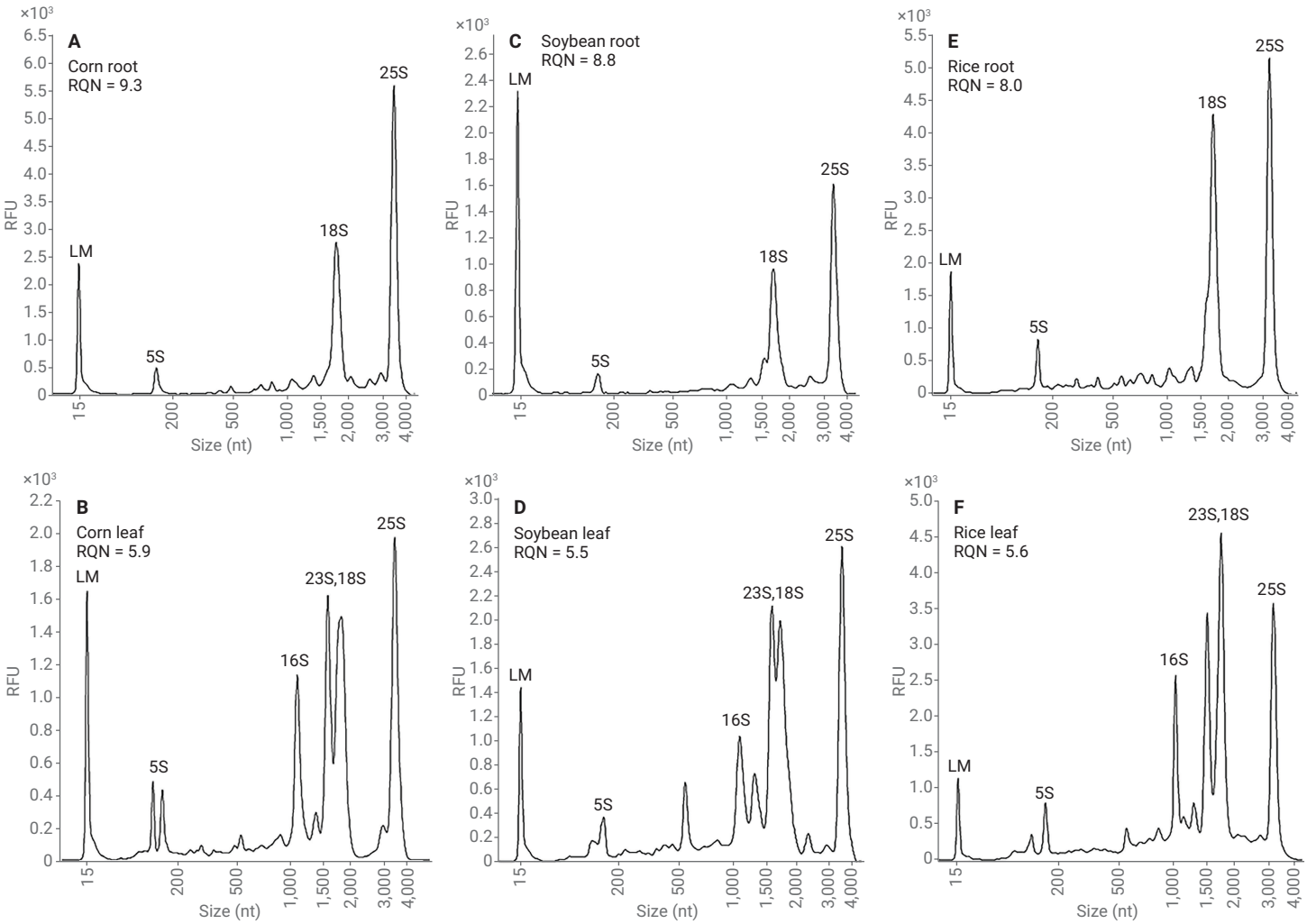

Figure 1. Separation of various plant leaf and root total RNA on the Agilent 5200 Fragment Analyzer system with the Agilent HS RNA kit (15 nt). LM = lower marker.

plant tissues with the RNeasy plant mini kit (Qiagen, #74903). *Arabidopsis thaliana* leaf total RNA was degraded at 90 °C for 0 to 20 minutes. Rice leaf and root total RNA (Figure 2) were analyzed by 2 % agarose gel electrophoresis with the RNA ladder (p/n DNF-382-U020) and the 5200 Fragment Analyzer system with the Agilent RNA kit (15 nt) (p/n DNF-471).

Results and discussion

Plant RNA separation profiles

Plant tissues have three types of ribosomal RNAs (rRNA); chloroplast, cytosolic, and mitochondrial. Chloroplastic RNA is unique to the green tissue, such as the leaf or stem of a plant, changing the total RNA fragment profile when compared to root total RNA. Plant RNA samples from the roots and leaves of corn, soybean, and rice were separated on the 5200 Fragment Analyzer system (Figure 1). In all samples, the 25S and 18S cytosolic rRNA fragments were easily identified and the RQN, ribosomal ratio, concentration, and percent concentration for each peak were automatically reported by the ProSize software. Compared to the root RNA, the leaf RNA samples all exhibited additional fragments corresponding to the 23S and 16S chloroplastic rRNA fragments. Total RNA separation profiles vary greatly depending on the origin, leaf or root and the species of plant, and because of this the RQN for total RNA from each plant may vary. As seen in Figure 1, root total RNA consistently has a higher RQN than leaf total RNA from the same plant.

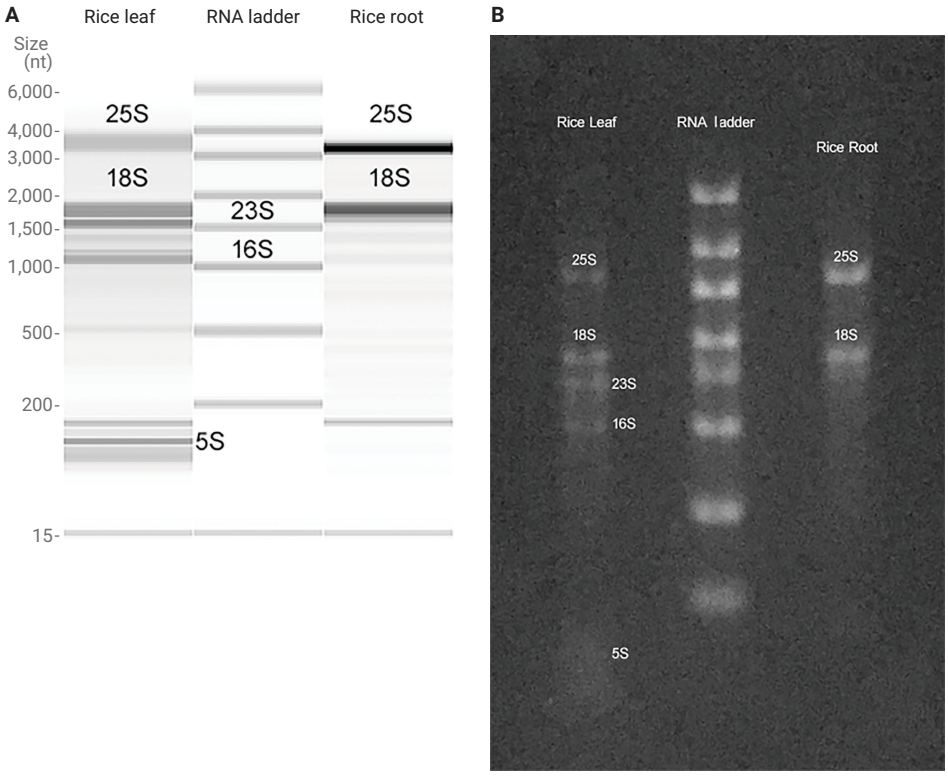

Figure 2. Rice leaf and root total RNA; (A) separated on the Agilent 5200 Fragment Analyzer system with the Agilent RNA kit (15 nt) and displayed as a digital gel image by the Agilent ProSize data analysis software, and (B) separated by agarose gel electrophoresis.

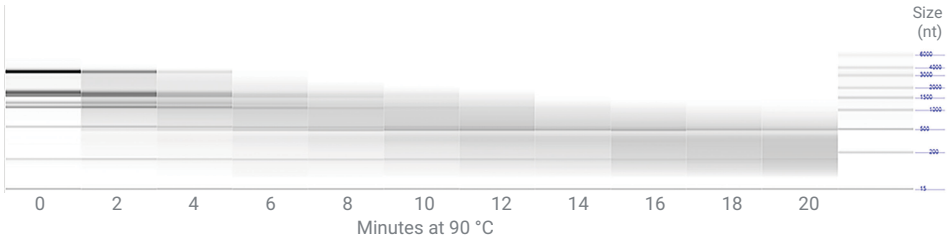

Figure 3. Digital gel image from the Agilent 5200 Fragment Analyzer system depicting the degradation of the *Arabidopsis* leaf total RNA at 90 °C for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 minutes. The 25S peak (top band) decreases in concentration and disappears with increased degradation.

Rice leaf and root total RNA were separated by agarose gel electrophoresis and with the 5200 Fragment Analyzer system with the RNA kit (15 nt) to compare total RNA fragment separation profiles (Figure 2). The 25S, 18S, 23S, 16S, and 5S rRNA fragments were separated with the same ladder on both the 5200 Fragment Analyzer system and agarose gel. The 5200 Fragment Analyzer system achieved better resolution of total RNA fragments (Figure 2, 1E, and 1F), especially in the 5S and fast region compared to the agarose gel. The ProSize software automatically provided user-independent RNA quantification and quality assessment, while agarose gel electrophoresis only conveys an estimation with further software analysis.

Degradation of plant RNA

Arabidopsis leaf total RNA was extracted and incubated at 90 °C for 0 to 20 minutes. Samples were taken every two minutes and run on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt). The ProSize software provided a digital gel image of the RNA degradation over time (Figure 3). The rRNA 25S fragment (top band) decreased in concentration and disappeared with increased heat degradation over time resulting in an increase of small RNA fragments in the fast region of the electropherogram. Separation profiles on the 5200 Fragment Analyzer system offered easy visualization of the degree of total RNA degradation with a strong correlation to the RQN (Figure 4). Increased RNA degradation over time resulted in a corresponding decrease in RQN assignment (Figure 5). The degree of total RNA degradation can easily be determined by the assigned RQN and visually assessing the electropherogram.

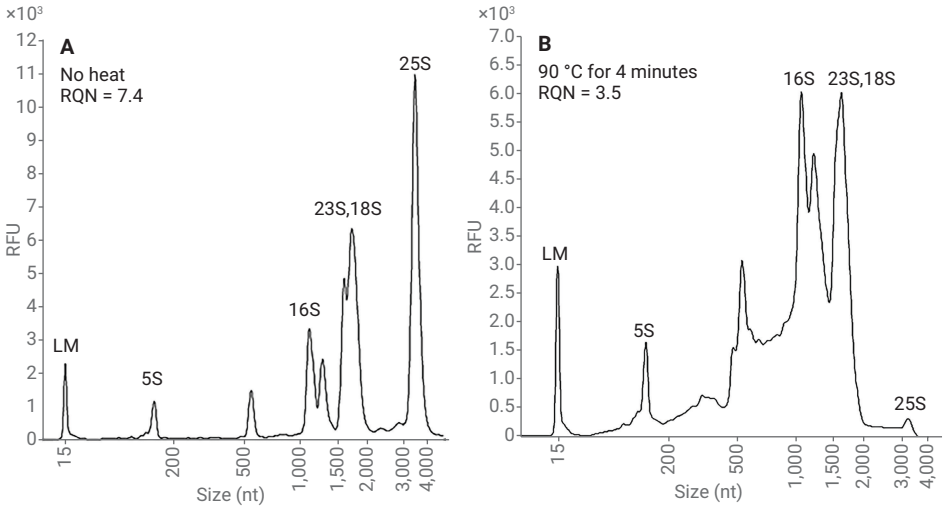

Figure 4. Separation profile of *Arabidopsis* leaf total RNA on the Agilent 5200 Fragment Analyzer system with the Agilent HS RNA kit (15 nt). (A) No heat, RQN = 7.4. (B) 4 minutes at 90 °C, RQN = 3.5. LM = lower marker.

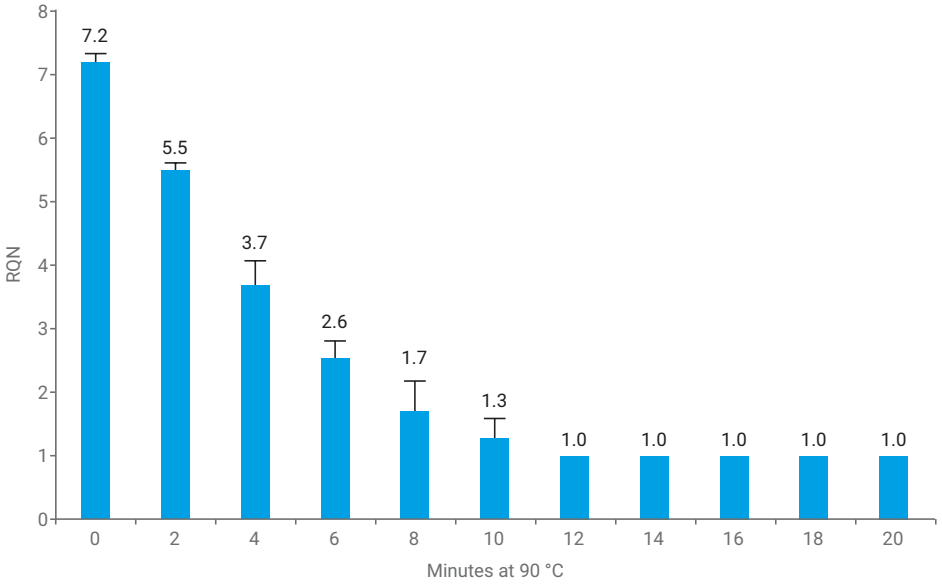

Figure 5. *Arabidopsis* leaf total RNA degradation at 90 °C for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 minutes. The RNA quality number (RQN) was analyzed on the Agilent 5200 Fragment Analyzer system with the Agilent HS RNA kit (15 nt).

Reproducibility of RQN

RQN reproducibility was determined between several 5200 Fragment Analyzer systems over a range of RNA concentrations (Table 1). The ProSize software reported a consistent RQN value for the Arabidopsis leaf RNA dilution series varying from 5.9 to 0.38 ng/µL. All RNA samples had an RQN number above 7.1, with the coefficient of variation (%CV) ranging from 2.2 to 3.3 % between instruments.

gDNA Contamination

On occasion, RNA extractions can become contaminated with genomic DNA (gDNA). Corn leaf RNA was extracted and then separated on the 5200 Fragment Analyzer system with the HS RNA kit (15 nt) (Figure 6). A high molecular weight gDNA smear was observed after the 25S RNA peak. gDNA has a high enough molecular weight as to not interfere with RNA separation on the 5200 Fragment Analyzer system.

Table 1. RQN remains consistent between Agilent 5200 Fragment Analyzer systems and the HS RNA kit (15 nt) with varied concentrations of *Arabidopsis* leaf total RNA.

RQN inter-instrument precision			
ng/µL	Average	Std dev	%CV
5.92	7.4	0.25	3.3
3.02	7.1	0.18	2.6
1.51	7.4	0.18	2.5
0.75	7.4	0.22	3.0
0.38	7.2	0.16	2.2

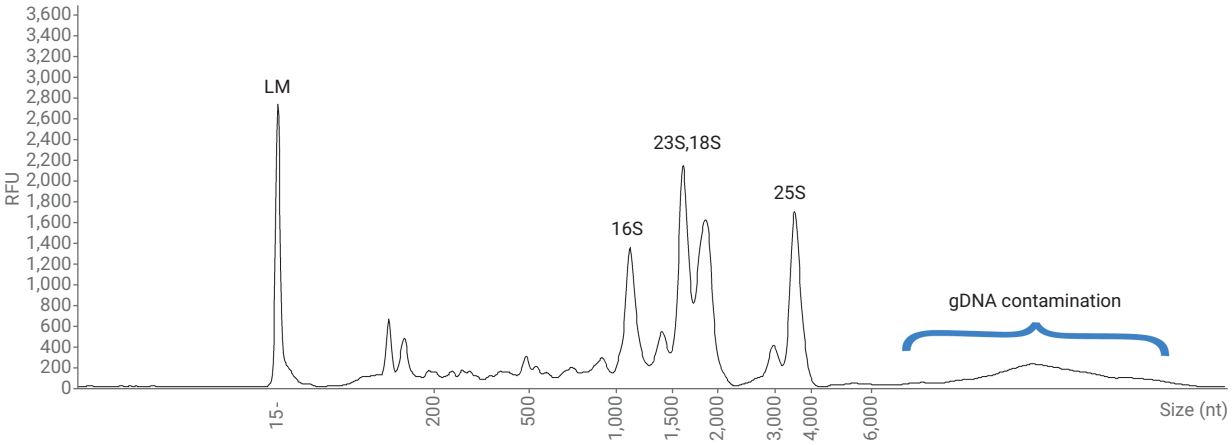

Figure 6. Corn leaf RNA was separated on the Agilent 5200 Fragment Analyzer systems with the Agilent HS RNA kit (15 nt). A gDNA smear separated out after the RNA. LM = lower marker.

Conclusions

The 5200 Fragment Analyzer system facilitated easy parallel capillary electrophoresis analysis of plant RNA quality with excellent precision. The RNA quality number (RQN) provided by the ProSize software reflected the degree of total RNA degradation and remained consistent between instruments and over a range of total RNA concentrations. The 5200 Fragment Analyzer system can be used in place of agarose gel electrophoresis for better resolution of plant total RNA, especially in the 5S and fast regions.

www.agilent.com

Printed in the USA, February 13, 2019
5994-0518EN

Agilent
Trusted Answers