Assessing integrity of plant RNA with the Agilent 2100 Bioanalyzer

Application Note

Genomics

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Abstract

High quality RNA, free of genomic DNA is a critical determinant for the success of many downstream techniques used in functional genomics, such as RT-PCR and microarray-based experiments. The Agilent 2100 Bioanalyzer and its unique RNA Integrity Number (RIN) algorithm have become the industry standard for quality analysis of total RNA because of the capability of providing sample and user-independent assessment of RNA quality from minimal sample amounts.

This Application Note demonstrates the use of a dedicated Plant RNA assay with the Agilent 2100 Bioanalyzer to assess the integrity of plant RNA samples from multiple plant sources and differing degradative states.

Introduction

Plant tissues are comprised of three types of rRNAs – cytosolic, chloroplastic, and mitochondrial – which vary in size from 5S to 25S (Table 1[1]). Green tissue may contain additional rRNAs in contrast to non-green tissues, such as roots. A dedicated method to determine plant RIN values was introduced in the B.02.07 version of the Agilent 2100 Expert software allowing reliable RIN values to be determined from these complex types of plant tissues. This assay was validated with 454 plant samples to improve the detection of appropriate plant RNA peaks for RIN determination. We describe the use of this assay to provide easy, user-independent assessment of plant RNA quality with as little as picogram to nanogram amounts of RNA[2].

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Materials and Methods

RNA samples

Total RNA (Plant Normal Tissue) samples were purchased from BioChain (Hayward, CA, USA) and from the University of Hohenheim (Stuttgart, Germany). Water (cell culture tested) and all other chemicals were obtained from Sigma (St. Louis, MO, USA).

Instrument and kits

Separations were performed on the Agilent 2100 Bioanalyzer, controlled by the Agilent 2100 Expert software, using the Plant RNA Nano assay in accordance with the RNA 6000 Nano kit protocol (Agilent Technologies, Waldbronn, Germany). Data analysis was performed on the 2100 Expert software, version B.02.07

Degradation study

Tobacco tissue homogenates were incubated at room temperature for 0, 5, 10, 20, and 40 minutes followed by centrifugation to remove insoluble compounds. RNA was extracted using suitable solvents and subjected to analysis on the Agilent 2100 Bioanalyzer for degree of degradation. Degradation and extractions were performed by Jens Göpfert at the University of Hohenheim (Stuttgart, Germany).

Results and Discussion

Plant RNA assay

The utility of the Agilent 2100 Bioanalyzer Plant RNA assay was demonstrated with multiple commercially available plant total RNA samples. Figure 1 illustrates typical electropherograms from sunflower root, sunflower leaf, lettuce, and potato RNA samples, with respective RINs. In all samples, the abundant 25S and 18S rRNA peaks are well resolved and automatically identified by the software. Compared to the root samples, the leaf and lettuce extracts exhibit additional fast migrating peaks corresponding to smaller chloroplast ribosomal RNAs showing that total RNA profiles can vary depending on species and tissue types.

RIN values of various plant RNA samples across different chips on the same (intra-) and different (inter-) days show high concordance (Table 2). All RNA samples had a RIN value above 6.4. The coefficients of variation (CV) of the RIN measurements were in the range of 1.54% to 5.46%, illustrating the assay precision of the RNA integrity measurements.

Ribosomal RNA	Types of RNA
Cytosolic ribosomes	
Larger subunit	25S, 8S, 5S
Smaller subunit	18S
Chloroplasts ribosomes	
Larger subunit	23S, 5S
Smaller subunit	16S
Mitochondrial ribosomes	
Larger subunit	24S, 5S
Smaller subunit	18S, 5S

Table 1
Plant ribosomal RNA.

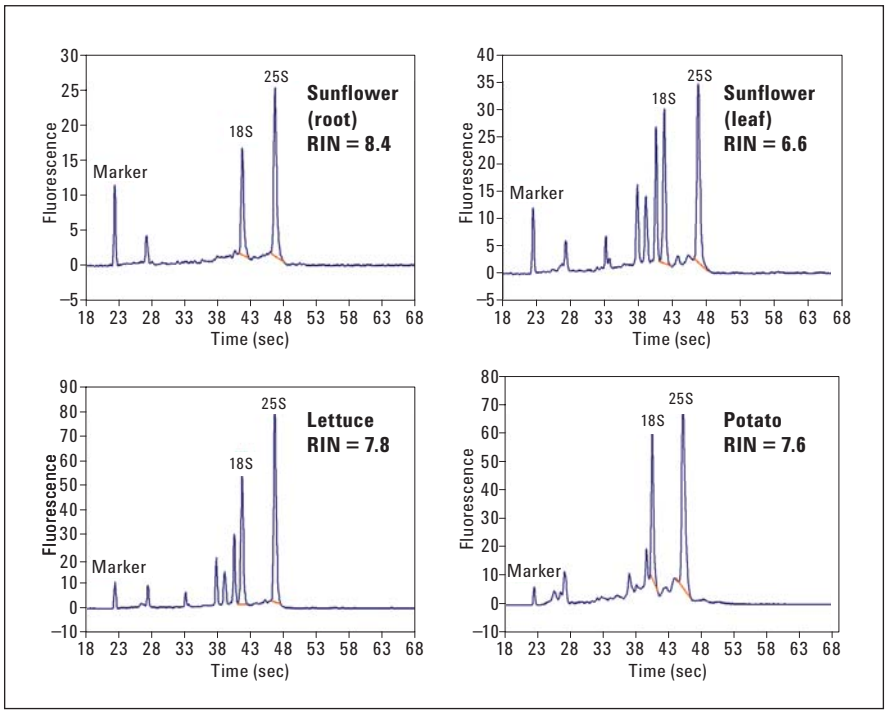

Figure 1
Electrophoretic separation of different plant total RNA using the RNA 6000 Nano Kit with the Plant RNA assay. Representative electropherograms indicate the position of 18S and 25S rRNA peaks. Unlabeled peaks correspond to additional ribosomal RNA. RIN values are shown.

Time-dependent degradation of plant RNAs

RNA is sensitive to degradation with many factors (cellular RNases, temperature or chemical treatment) contributing to this process. To mimic a natural degradation process, tobacco samples were incubated at room temperature over 0 to 40 minutes. The degree of degradation was monitored through analysis on the Agilent 2100 Bioanalyzer and RNA Nano 6000 kit. Figure 2 shows representative tobacco RNA electropherograms at different time points. As expected, the RIN decreases as degradation progresses. The Agilent 2100 Bioanalyzer profiles show minimal change in 25S/18S rRNA ratio (data not shown) or RIN at time points 0 and 5 minutes. With longer incubations, the RNA size distribution shifts toward smaller fragments resulting in decreased RINs (Figures 2 and 3). As expected, the samples showed the highest degree of degradation with a mean RIN value of 3.6 after 40 minutes at room temperature.

Conclusion

The Agilent 2100 Bioanalyzer Plant RNA assay allows rapid, automated analysis of plant RNA samples with excellent precision. The RIN algorithm of the Plant RNA assay provides a user-independent assessment of total plant RNA integrity.

	Inter chip (intra-day, n = 15)			Inter chip (inter-day, n = 30)		
	Mean RIN	SD	%CV	Mean RIN	SD	%CV
Arabidopsis	**7.29**	0.13	1.78	**7.31**	0.15	2.05
Lettuce	**7.61**	0.19	2.49	**7.76**	0.12	1.54
Sunflower (root)	**8.02**	0.29	3.61	**8.41**	0.46	5.46
Sunflower (leaf)	**6.88**	0.13	1.88	**6.87**	0.12	1.74
Tomato	**6.48**	0.12	1.85	**6.51**	0.12	1.84
Tobacco	**6.75**	0.16	2.37	**6.78**	0.15	2.21

Table 2
Precision of plant RNA assay method.

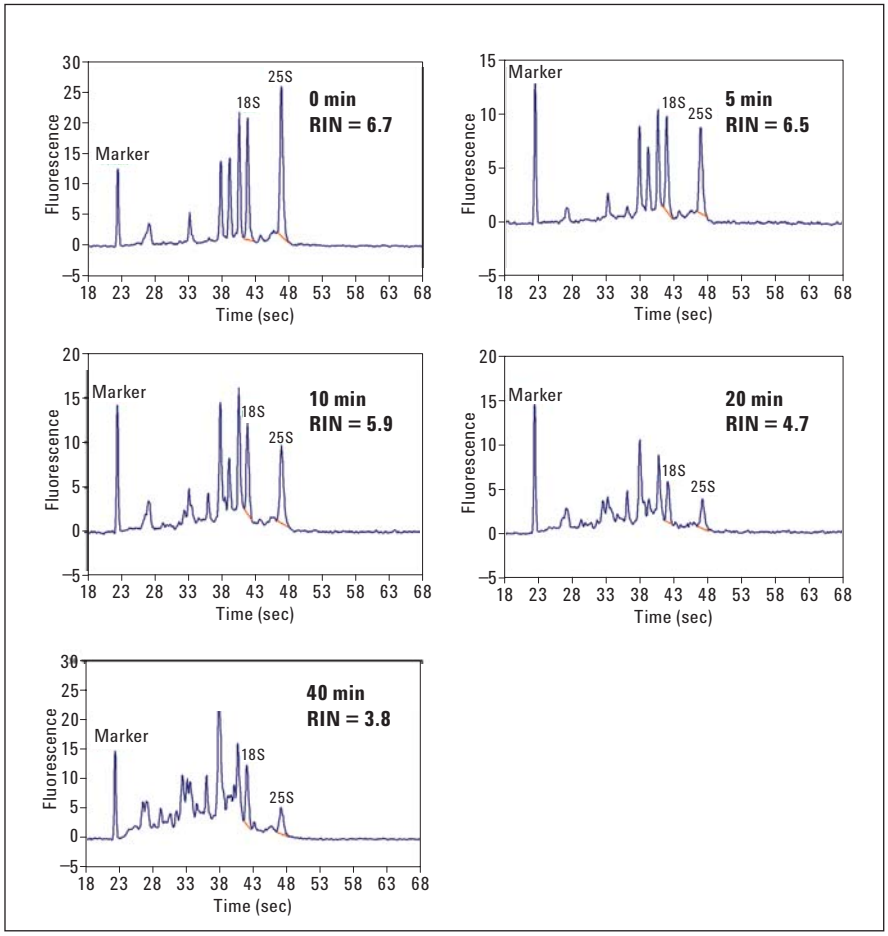

Figure 2
Time course degradation of tobacco RNA. Total RNA samples were incubated at room temperature at selected time points and separated with RNA Nano 6000 kit. Electropherograms indicate the position of 18S and 25S rRNA peaks. RIN values of individual samples are shown.

References

1.
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2.
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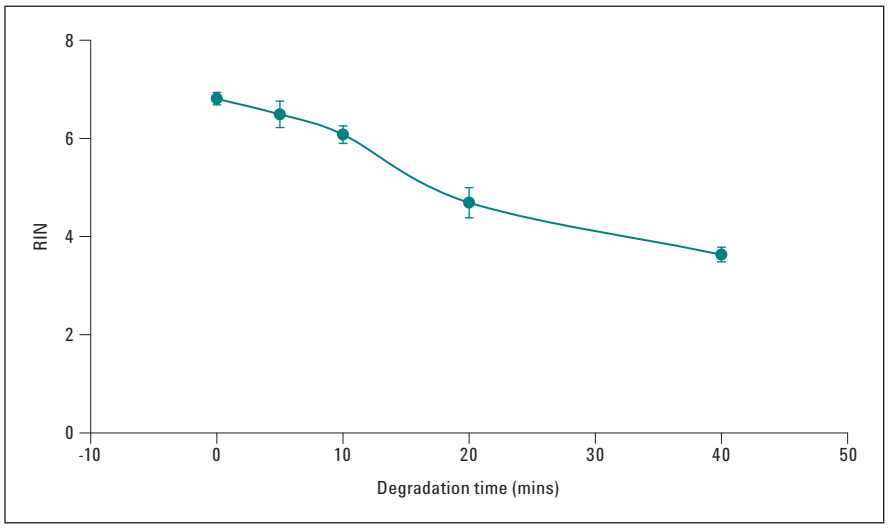

Figure 3
Assessment of plant RNA degradation. Different degradative states of tobacco RNA samples were analyzed with the Plant RNA assay and RNA 6000 Nano kit (average of forty seven measurements, data represent mean ± SD).

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