Evaluation of Plant RNA Integrity Number (RIN) generated using an Agilent BioAnalyzer 2100

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INTRODUCTION

The RNA Integrity Number (RIN) is a measure of the integrity and level of degradation of an RNA sample which is obtained via the 2100 Agilent BioAnalyzer (Agilent Technologies, Inc.). Since the integrity of RNA molecules reflects the snapshot of gene expression at the moment of RNA extraction, the RIN value is used to determine if the RNA is acceptable for sensitive downstream applications such as sequencing and microarray. The RIN scale ranges from 1 (degraded) to 10 (intact) and in general RIN > 9 is required in most core facilities. However with plant RNA, the Agilent BioAnalyer often generates low RIN values (< 8) or sometimes absolutely no RIN values. Here we provide a few examples of RIN measurements with different plant species to understand the cause of the problem. In addition, we also tried to indicate how possible variation among the RNA purification methods may affect the RIN value, to provide a general example of the plant RIN value for plant RNA researchers for core facility applications.

MATERIALS AND METHODS

RNA Purification

To compare total RNA purification, three different plant RNA purification kits were tested; Norgen's Plant/Fungi Total RNA Purification Kit (Cat# 25800), Competitor's Plant Kit and Tri-Reagent Kit. Fifty mg of different plant species (peach, grape, tomato, tobacco and vanilla fruit) were used for total RNA purification according to the manufacturer's protocol.

RNA Integrity Measurement using the Agilent BioAnalyzer 2100

To assess the RIN value, purified RNA was prepared according to the Agilent RNA 6000 Nano Kit quick start guide and run on the Agilent BioAnalyzer 2100. The data was analyzed by the 2100 software version B.02.08.SI648 (SR2).

RNA Gel Electrophoresis

The purified plant or fungal RNA was typically run on 1X MOPS, 1.5% formaldehyde-agarose gels for visual inspection. Generally, 5 µL of each 50 µL elution was run on the gel. The purified plant RNAs (Norgen's and the competitor's) were also resolved on a 1X MOPs, 1.0% formaldehyde-agarose gel for visual comparison.

RESULTS AND DISCUSSION

Unlike animal and human cells, plant cells have chloroplast ribosomes in addition to the cytosolic and mitochondrial ribosomes. Therefore, when the RNA was separated on an RNA gel, typically multiple RNA bands appeared. However, a few exceptions can be also found with certain types of tissue and species. As an example, vanilla bean generates less RNA bands and the bioanalyzer could read the RIN value clearly as a high integral RNA (Figure 1).
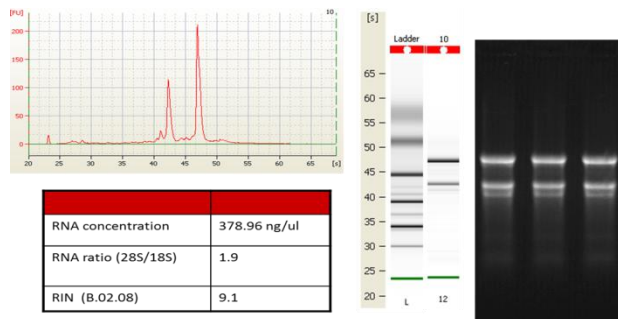

RNA concentration	378.96 ng/ul
RNA ratio (28S/18S)	1.9
RIN (B.02.08)	9.1

Figure 1: Example of a high RIN value sample from vanilla bean. It gives 2 clear ribosomal bands of 25S and 18S resulting in a RIN value of 9.1.

A plant leaf tissue is commonly used for RNA purification and typically generates multiple RNA bands as the result of different ribosomes including large and small subunits. RNA from four different plant species was analyzed for the RIN value. The highest RIN was 6.2 from tobacco, and grape did not show any RIN value (Figure 2) despite the fact that no sign of degradation was found from the RNA gel (Figure 3).
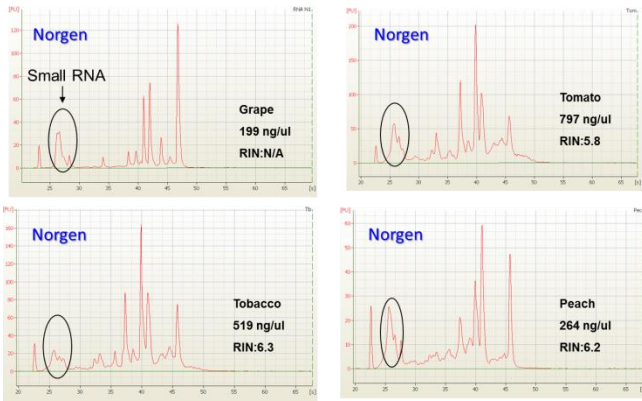
Figure 2. Example of low RIN values from different plant samples showing multiple ribosomal RNA bands. These samples give low RIN values despite no signs of RNA degradation on the RNA MOPS gel.
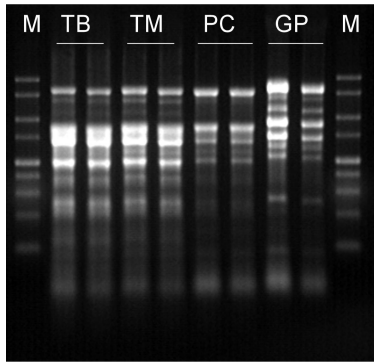

Figure 3. Total RNA profile shows intact RNA isolated using Norgen's kit from tobacco (TB), tomato (TM), peach (PC) and grape (GP) on a 1% MOPS RNA gel.

Three different RNA purification methods were also compared to address any influence of the RNA purification method on the RNA RIN value. Column based methods from Norgen (Figure 4 Panel A) and Competitor (Figure 4 Panel B) did not generate a RIN value, however the purified RNA is intact as evidenced by the RNA gel Figure 4 Panel D). While Tri-reagent (phenol/chloroform) did give a RIN value (=5.2), it can be suggested that the impure background might contribute to the RIN value. The RNA profile shown in Figure 4D agree with this assumption, indicating that RNA purified using Tri-reagent has a smeared background.
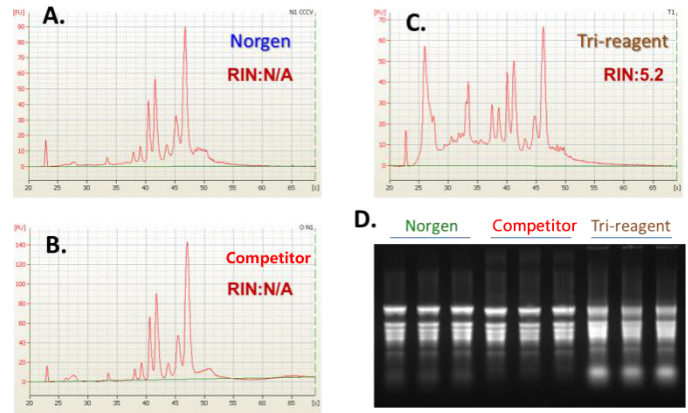

Figure 4. Comparison of the electropherogram generated from RNA isolated using Norgen's kit (Panel A), Competitor's kit (Panel B) and Tri-reagent (Panel C), and the total RNA integrity of the RNA purified with the 3 methods on a 1% MOPS RNA gel (Panel D).

DISCUSSION AND CONCLUSION

With successful RNA purification, human and animal RNA RIN values are typically 9 or higher. As we demonstrated here, many plant species have a variable rRNA size (5S, 8S, 16S. 18S, 23S and 25S) generating a complexity in the RIN reading. Despite the recent update of the Agilent 2100 Expert software (B.02.08 version), we are still experiencing a low RIN (< 6 to 7) or no RIN value at all (grape RNA from leaves). Accumulated data (different plant species and RNA purification methods) indicated that the problem seems to lie in the BioAnalyzer algorithm for the plant RNA having multiple RNA bands rather the RNA quality.

Therefore a more comprehensive approach is needed when the RNA sample is sent to a core facility:

1) Check the sample quality
2) Choose the right RNA purification method
3) Consult with a specialist from the core facility.

SUMMARY

1) Plant RNA has multiple ribosomes from cytosol, mitochondria and chloroplasts, generating multiple RNA bands.
2) Due to the multiple RNA bands, even intact tobacco, tomato, grape and peach RNA can't be analyzed properly by the Agilent Bioanalyzer, resulting in low RIN.
3) The RNA purification method might affect the RIN value. For example, purification using Tri-reagent (phenol/chloroform) resulted in a better RIN value than column-based methods; however this is likely due to the high background. This was demonstrated when comparing the RNA on a gel, as it was clear that the

RIN did not accurately reflect the RNA integrity.

4) RIN values have to be validated by the visualization of RNA band integrity on the RNA gel.