Comparison of RIN and RINe algorithms for the Agilent 2100 Bioanalyzer and the Agilent 2200 TapeStation systems

Technical Overview

Introduction

A typical application for the 96-well plate compatible Agilent 2200 TapeStation system is automated, efficient, and reliable RNA analysis, including RNA characterization and quality assessment with Agilent R6K ScreenTape or Agilent High Sensitivity R6K ScreenTape.

When separated by electrophoresis, eukaryotic total RNA samples typically display two major peaks representing the 18S and 28S ribosomal RNA (rRNA). Depending on the purification method, additional smaller peaks can also been seen, which correspond to small rRNAs as well as tRNAs. As total RNA degrades, the 18S and 28S rRNA peaks slowly disappear and the degradation products emerge in the region between the 18S and small RNAs, termed as the fast zone.

The RNA integrity number (RIN) functionality of the Agilent 2100 Expert software for the Agilent 2100 Bioanalyzer system uses a proprietary neural network trained algorithm to make an assessment of the entire electrophoretic trace for a given total RNA sample, including ribosomal peak ratios, separation, and the presence or absence of degradation products. RIN values from 1 to 10 can be obtained, where 10 indicates the highest possible RNA quality.

Due to fundamental differences in the nature of the raw data, a dedicated software algorithm has been developed for the 2200 TapeStation system and R6K ScreenTape to determine the RNA integrity number equivalent (RINe).

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The RIN^e algorithm is based on a mathematical model that calculates an objective quantitative measurement of RNA degradation[1]. This mathematical model has been refined and optimized to analyze total RNA profiles obtained from R6K ScreenTape. In contrast to RIN, RIN^e represents the relative ratio of the signal in the fast zone to the 18S peak signal. The values obtained are presented in the same format as RIN from 1 to 10, whereby highest quality RNA is assigned a RIN^e value of 10. Calculated automatically by the 2200 TapeStation software, RIN^e provides an objective and reliable numerical assessment of the integrity of eukaryotic total RNA degradation.

This Technical Overview describes the validation of RIN^e obtained with the 2200 TapeStation system on R6K ScreenTape against the gold standard, the RIN obtained with the 2100 Bioanalyzer system.

Experimental

Material

Different total RNA samples from different species and sources, including various human, rat and mouse tissues (Table 1) were obtained from typical vendors. R6K ScreenTape, R6K Reagents, a 2200 TapeStation instrument, Agilent RNA 6000 Nano kit, a 2100 Bioanalyzer instrument, and the Agilent Total RNA Isolation Mini kit were from Agilent Technologies (Waldbronn, Germany).

Tissue	Human	Rat	Mouse
Adipose	X		
Bladder	X		
Brain	X	X	X
Cervix	X		
Colon	X		
Embryo		X	X
Esophagus	X		
Heart	X	X	X
Intestines	X		
Kidney	X	X	X
Liver	X	X	X
Lung	X	X	X
Ovary	X	X	X
Placenta	X		
Prostate	X		
Skeletal muscle	X		
Spleen	X	X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid	X		
Trachea	X		

Table 1
Total RNA samples from various human, rat and mouse tissues used for the evaluation.

RNA preparation

Total RNA from HEK 293, Hep G2, and Jurkat cells was purified using the Total RNA Isolation Mini kit following the recommended protocol.

RNA degradation

Total RNA samples were heat degraded by incubation in a PCR block for various times (0 to 120 minutes) to generate a large set of various total RNA samples with different RNA integrity. All RNA samples were analyzed using the 2100 Bioanalyzer and the 2200 TapeStation systems as described below.

RNA 6000 Nano kit with the 2100 Bioanalyzer system

There were 387 total RNA samples analyzed with the 2100 Bioanalyzer system and the RNA 6000 Nano kit according to manufacturer's instructions.

R6K ScreenTape analysis procedure with the 2200 TapeStation system

There were 482 total RNA samples analyzed with the 2200 TapeStation system and the R6K ScreenTape according to manufacturer's instructions.

Results and discussion

RNA integrity measurement

The RNA integrity of total RNA samples was assessed using the 2100 Bioanalyzer and the 2200 TapeStation systems. Figure 1 shows gel-like images obtained for total RNA from Jurkat cells with RIN values ranging from 3 to 10.

High quality eukaryotic total RNA samples display various bands representing small RNAs, 18S and 28S rRNA. As total RNA degrades, the 18S and 28S peaks slowly disappear while peaks from degraded material emerge in the fast zone, the region between the 18S and small RNAs. This typical pattern was observed with the 2100 Bioanalyzer and the 2200 TapeStation systems (Figure 1).
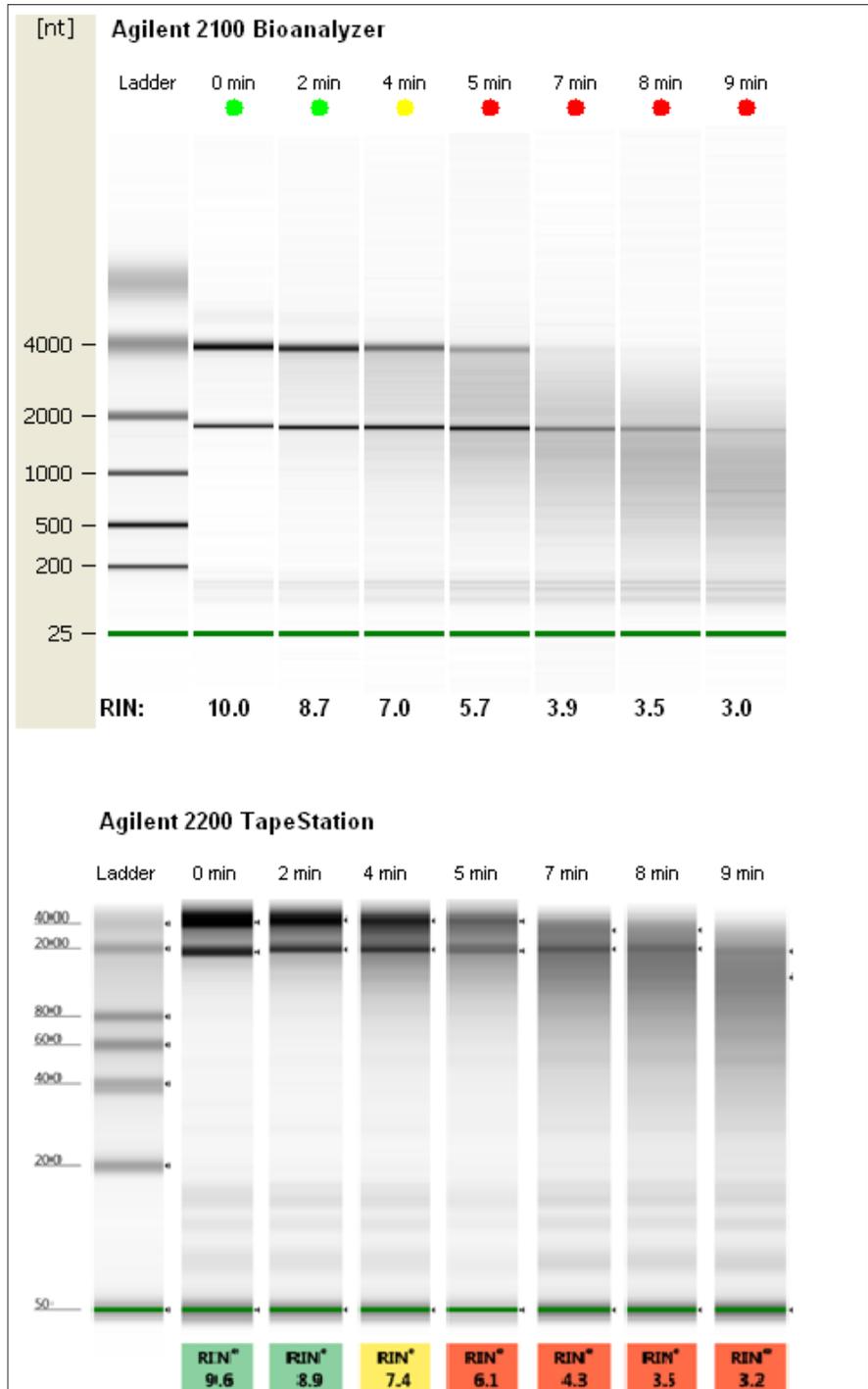

Figure 1
Heat degradation of total RNA from Jurkat cells heat degraded at 90 °C for various times as indicated (0 to 9 minutes). The gel-like image obtained with the Agilent 2100 Bioanalyzer and with the Agilent 2200 TapeStation systems is shown. The automatically determined RIN and RIN[e] values are shown below the gel-like image.

Comparative analysis of RIN and RIN^e

To compare RIN from the 2100 Bioanalyzer and RIN^e from the 2200 TapeStation system, approximately 400 total RNA samples were analyzed with both the 2100 Bioanalyzer and the 2200 TapeStation system including up to 15 replicates. The obtained results for replicate RNA samples were averaged resulting in a dataset of 105 RIN^e/RIN pairs. This dataset covered over 40 different total RNA types with RIN values ranging from 1.2 to 10.0. The plotted data is shown in Figure 2. A linear regression line forced through 0 provides a good fit with $R^2>0.9$.

The median error for this dataset is 0.3 RIN units, and is evenly distributed across all sample qualities (not shown). This observed error is comparable to the median error of 0.3 RIN obtained from all samples on the 2100 Bioanalyzer.

Conclusion

This Technical Overview demonstrates that RIN^e values obtained from the Agilent 2200 TapeStation system and the Agilent R6K ScreenTape are equivalent to RIN values obtained from the Agilent 2100 Bioanalyzer. The 2200 TapeStation system and R6K ScreenTape therefore provide a faster and more automated alternative to determine total RNA integrity, with results comparable to the 2100 Bioanalyzer system.
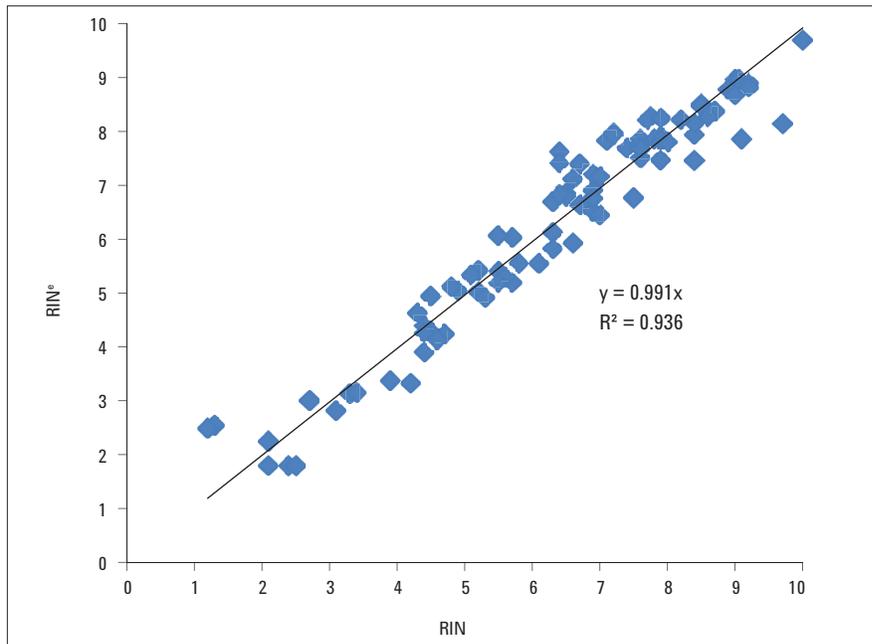
Figure 2
Comparison of RIN and RIN^e. The RIN measured with the 2100 Bioanalyzer was plotted against the calculated RIN^e, a linear regression line forced through origin is shown.

Reference

1.
H. Auer, S. Lyianarachchi, D. Newsom, M. I. Klisovic, G. Marcucci, and K. Kornacker, "Chipping away at the chip bias: RNA degradation in microarray analysis," Nature Genetics, 35 (4), 292-3, **2003**.

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