RNA quality control using the Agilent 2200 TapeStation system – Assessment of the RIN^e quality metric

Application Note

Nucleic Acid Analysis

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Abstract

This Application Note describes the performance comparison and benchmark of the Agilent 2200 TapeStation system with the Agilent 2100 Bioanalyzer system, the industry standard for RNA quality control. The 2200 TapeStation system performs automated analysis of up to 96 samples with approximate analysis time of 1 minute per sample. This Application Note demonstrates the high correlation between RIN^e generated by the 2200 TapeStation system with the RIN quality assessment of the 2100 Bioanalyzer system. The high reproducibility of RIN^e achieved with the 2200 TapeStation system in RNA quality assessment is also shown. The 2200 TapeStation system is, therefore, a reliable system for RNA quality control.

Agilent Technologies

Introduction

The structural integrity of RNA is of absolute importance for assuring success for procedures like microarray, cDNA library constructions, RT-PCR and RT-qPCR[1]. An increased focus on microarray technologies and qPCR to study gene expression demands a reliable and robust method to analyze RNA integrity[2]. The Agilent 2100 Bioanalyzer system and its RNA Integrity Number (RIN) is the industry standard for the assessment of RNA quality. The recently introduced Agilent 2200 TapeStation system builds on the success of the 2100 Bioanalyzer system with faster analysis times, more flexibility of throughput at a constant cost per sample, and 96-well plate capabilities.

Analyses on the 2200 TapeStation system are performed using the ready-to-use consumable ScreenTape. The Agilent R6K ScreenTape and the Agilent High Sensitivity R6K ScreenTape consists of 16 individual lanes. As unused lanes of the ScreenTape consumable can be stored for subsequent analysis, variable throughputs from 2 to 96 samples are delivered at a constant cost per sample. The 2200 TapeStation instrument automatically loads the prepared samples from either of the 8-way tube strips or a 96 well plate onto the R6K ScreenTape. Electrophoresis and imaging are also automated within the instrument. Results are presented as gel image and electropherogram for each individual sample in the 2200 TapeStation software and can be viewed in as little as 1 minute per sample. For RNA quality analysis on R6K ScreenTape and High Sensitivity R6K ScreenTape a RIN equivalent (RIN^e) is indicated. RIN^e is presented as a value between 1 and 10, where 10 represent the highest quality RNA sample. Here, we present a comparative study between the RIN^e quality score obtained from R6K ScreenTape and High Sensitivity R6K ScreenTape compared to the RIN quality metric obtained from the 2100 Bioanalyzer system.

Equipment

Instrumentation and reagent kits

RNA 6000 Nano and RNA 6000 Pico kits, 2100 Bioanalyzer system, 2200 TapeStation system, R6K ScreenTape and High Sensitivity R6K ScreenTape kits were obtained from Agilent Technologies (Waldbronn, Germany) and used in accordance to manufacturer instructions[3,4,5]. The Total RNA Isolation Mini Kit from Agilent Technologies (Santa Clara, CA, USA) was used to extract RNA from the HEK 293 and HEP G2 cell line following the manufacturer guidelines[6].

Samples

A total of 23 RNA samples were used for the benchmark study and were grouped into three categories:

Commercial samples

Commercially available total RNA extracts from different mammalian tissues were obtained from Agilent Technologies (Santa Clara, USA). The standards were supplied at a concentration of 1 µg/µL and include RNA extracted from human lung, brain, ovary, adrenal gland, heart, colon, fetal liver, and mouse liver and kidney. RNA extracted from human Jurkat cell line and HeLa cell line treated and untreated with phorbol myristyl acetate (PMA) was also obtained from Agilent Technologies. A 1-µL amount of sample was used for RNA 6000 Nano and R6K kit and 2 µL was used for RNA 6000 Pico and High Sensitivity R6K kits. Each of these were run in replicates of four at a concentration of 50 ng/µL for RNA 6000 Nano and R6K kits and then diluted to 1,000 pg/µL for RNA 6000 Pico and High Sensitivity R6K kit.

Degraded samples

Human Jurkat RNA sample at 50 ng/µL was serially heat degraded at 90 °C. The RNA was sampled at time points t = 0, 2, 5, 7, 8, 9, 15, and 18 minutes and incubated on ice to arrest the degradation and immediately processed for analysis. The samples were analyzed in replicates of six for RNA 6000 Nano and R6K kits and then diluted 1:50 for RNA 6000 Pico and High Sensitivity R6K kit.

Extracted samples

Two cell lines, HEK 293 from ATCC (ATCC, USA) and HEP G2 (Connexious, Bangalore, India) were cultured using MEM medium containing 10% FBS and 1% Pen/Strep and incubated at 37 °C in a 5% CO_2 atmosphere. The cells were harvested at 80% confluent and frozen at -80 °C until further use. The RNA was extracted from the cell population of 1.76 × 106 cells/mL for HEK 293 and 2.2 × 106 cells/mL for HEP G2 culture. RNA sample extracted from human gastrointestinal tract cancer tissue was obtained from IOB -Institute of Bioinformatics (Bangalore, India). Total RNA from these cell lines and tissues were analyzed on both 2100 Bioanalzyer and 2200 TapeStation systems in replicates of six.

All analyses were repeated three times on three different days

Results and discussion

RNA analysis

The commercial, degraded, and extracted sample sets were analyzed on the 2200 TapeStation system. The samples were prepared and loaded to the 2200 TapeStation system. Figure 1A illustrates a result obtained by running different RNA samples on the R6K ScreenTape. The gel image shows the separation profile of each of the individual samples showing 28S, 18S, small rRNAs and lower marker. The RNA quality is presented as RIN^e value for each individual sample below the gel image. Figure 1B shows a representative electropherogram of total RNA from HeLa cells treated with PMA. The 28S and 18S peaks are annotated for easy interpretation.

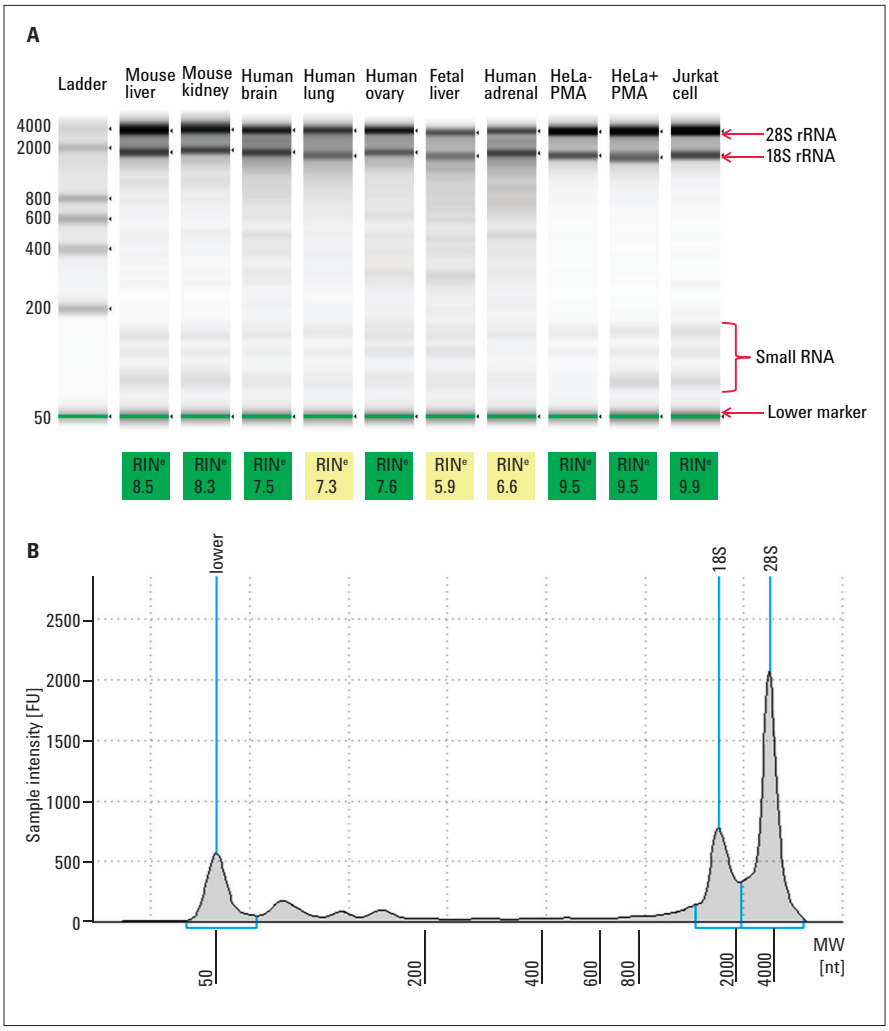

Figure 1
RNA analysis carried out using the Agilent 2200 TapeStation system. A) Gel image showing different RNA samples. B) Representative electropherogram of total RNA from HeLA cell treated with PMA, the 18/28S peaks are annotated.

Heat degraded RNA analysis

The 2200 TapeStation system was assessed for performance in quality scoring of degraded RNA by analyzing heat degraded samples. Degraded Jurkat total RNA samples were analyzed on R6K ScreenTape and High Sensitivity R6K ScreenTape and compared with the 2100 Bioanalyzer system. Both analyses on the 2200 TapeStation and 2100 Bioanalyzer systems showed stepwise degradation of 28S peak and increase in degradation products over the time course.

Figure 2 shows selected electropherogram overlay of heat degraded RNA samples analyzed on R6K ScreenTape and RNA 6000 Nano kit.

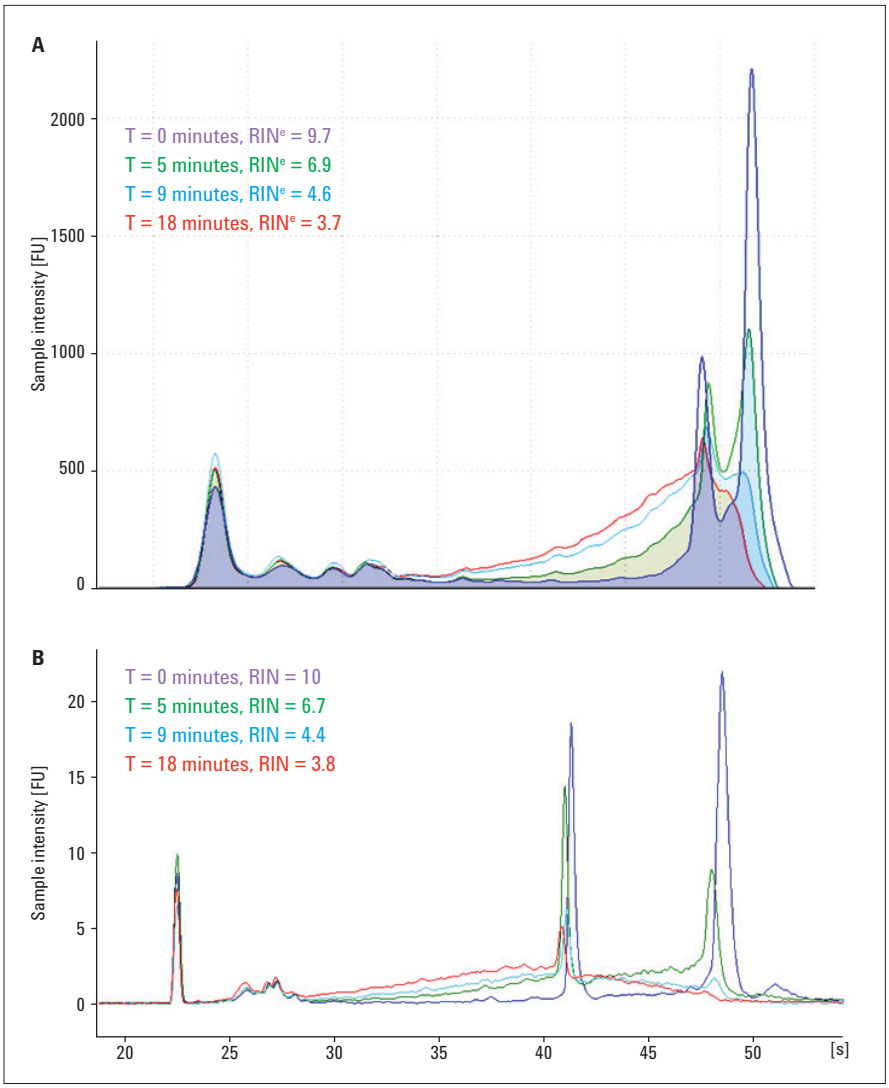

Figure 2
Electropherogram overlay of heat degraded Jurkat cell RNA samples. A) Results from R6K ScreenTape (Agilent 2200 TapeStation). B) Results from RNA 6000 Nano kit (Agilent 2100 Bioanalyzer).

RIN and RIN^e correlation

RNA samples including commercial samples, degraded and extracted samples were analyzed on the 2100 Bioanalyzer and the 2200 TapeStation systems. The average RIN value of the replicates were taken for each sample and compared against the average RIN^e value generated by TapeStation (Figure 3). This analysis suggests that both systems show a positive correlation with R^2 value of 0.9878 for RNA 6000 Nano kits and R6K ScreenTape and R^2 value of 0.9474 for RNA 6000 Pico kits and High Sensitivity R6K ScreenTape. Typically <4% deviation from RIN was observed for R6K ScreenTape and <7% deviation was observed for High Sensitivity R6K ScreenTape.

One way ANOVA analysis shows that there is no significant difference between RNA 6000 Nano kits and R6K ScreenTape [F (5, 132) = 0.104, p = 0.991] and also between RNA 6000 Pico kits and High Sensitivity R6K ScreenTape [F (5, 132) = 0.295, p = 0.914].

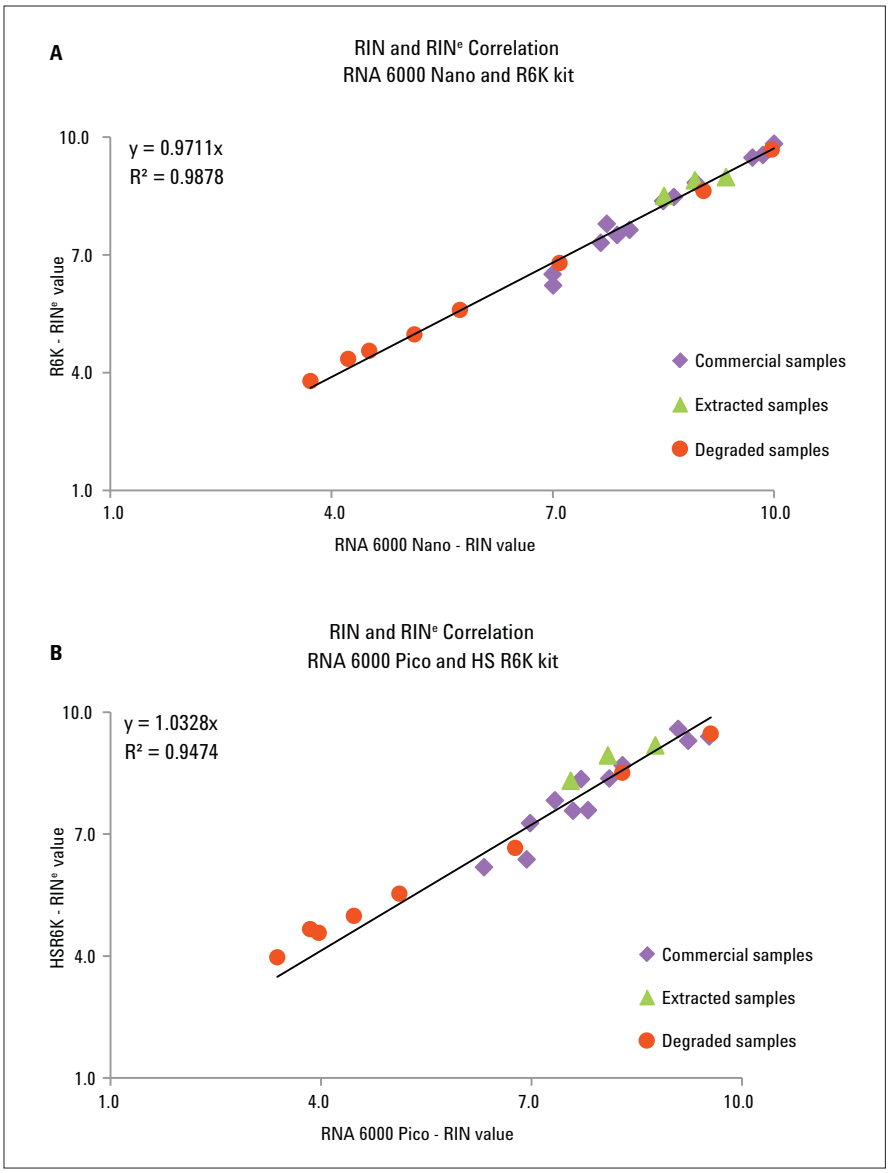

Figure 3
Correlation curves plotted by values obtained from commercial, extracted and degraded RNA samples
A) Plot of RIN and RIN^e values generated by RNA 6000 Nano and R6K kits. B) Plot of RIN and RIN^e values obtained from RNA 6000 Pico and an High Sensitivity R6K kit.

Reproducibility

The reproducibility of the 2200 TapeStation system was assessed against the 2100 Bioanalyzer system by collating results from 30 RNA 6000 Nano chips, 27 R6K ScreenTape similarly from 36 RNA 6000 Pico chips and 27 High Sensitivity R6K ScreenTape. The reproducibility CV over the wide range of samples and degradation levels for RIN and RIN^e values were calculated. Intra-assay and inter-assay precisions were calculated by taking averages from samples ran in single chip or ScreenTape and across multiple chips or ScreenTape respectively.

The Table 1 shows obtained %CV for both systems. 2200 TapeStation system shows a higher precision compared with 2100 Bioanalyzer system due to the automated sample handling and loading that reduces manual pipetting errors.

	Mean % CV			
Analytical specification	**RNA 6000 Nano**	**R6K ScreenTape**	**RNA 6000 Pico**	**High Sensitivity R6K ScreenTape**
Intra-assay precision	< 3%	< 2%	< 4%	< 2%
Inter-assay precision	< 3%	< 2.5%	< 6%	< 3%

Table 1
Precision of RIN and RIN^e.

Conclusion

The Agilent 2200 TapeStation system offers an easy to use system for analyzing RNA samples with minimum manual intervention. R6K and High Sensitivity R6K ScreenTape demonstrated accurate and reliable RNA quality control comparable to the Agilent 2100 Bioanalyzer system. The 2200 TapeStation system also shows high precision in sample reproducibility.

The 2200 TapeStation system, therefore, offers comparable performance to the industry standard 2100 Bioanalyzer system for quality assessment of RNA. Combined with the added advantages of high throughput compatibility, ease of use, minimal manual intervention, and constant cost per sample regardless of throughput, the 2200 TapeStation system is ideal for the assessment of RNA quality.

References

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