RNA Quality Control using the Agilent 2200 TapeStation System – Assessment of the RIN^e Quality Metric.

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Introduction

RNA quality assessment has become a critical step prior to downstream experiments. The integrity of the RNA determines the success rate of experiments that involve microarray workflows, cDNA library construction, and RT-PCR and RT-qPCR. The Agilent 2200 TapeStation system built on the success of the 2100 Bioanalyzer system enables reliable RNA quality assessment 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 consisting of 16 individual lanes. The consumable ScreenTape offers advantage of using unused gel lanes later, for subsequent analyses.

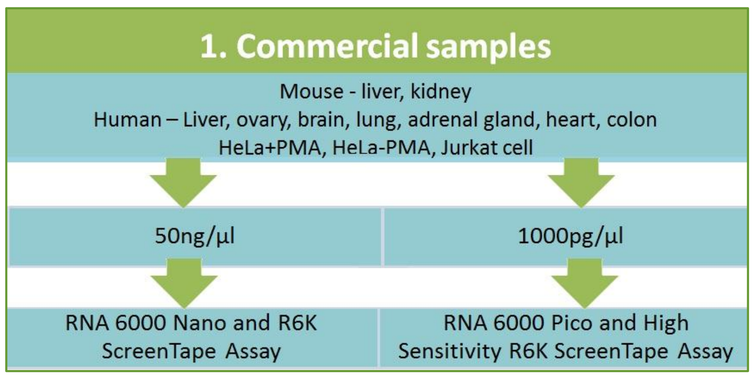
Figure 1: The Agilent 2200 TapeStation System

The Agilent 2200 TapeStation system determines the quality of RNA by the relative ratio of fast zone signal to 18S peak signal and presents the results as the RIN equivalent (RIN^e). RIN^e uses the same scale as RIN to score the RNA quality and presents values between 1 and 10, where 10 is the highest quality RNA and 1 is completely degraded RNA

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.

Materials & Methods

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



All RNA samples were analyzed in replicates of six and each assay was repeated three times on three different days.

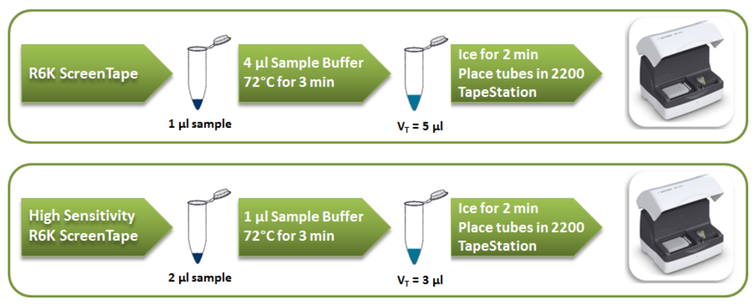


Figure 2: Protocols for sample preparation before analysis on the Agilent R6K ScreenTape and High Sensitivity R6K ScreenTape

Results & Discussion

The commercial, degraded, and extracted sample were prepared and loaded to the Agilent 2200 TapeStation system. The gel image in Figure 3 shows the separation profile of each of the individual samples showing 28S, 18S, small rRNAs and lower marker along with a representative electropherogram. The RNA quality is presented as RIN^e value for each individual sample below the gel image.

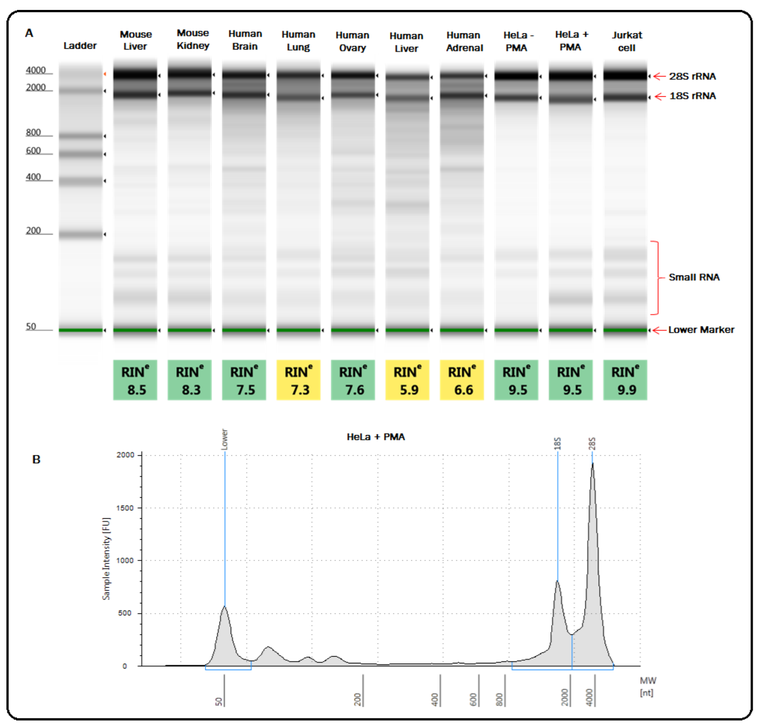


Figure 3: A typical RNA analysis carried out with Agilent 2200 TapeStation system. The gel image in Figure 3A shows the separation profile of each sample along with the RIN^e. Figure 3B shows a representative electropherogram of total RNA from HeLa cells treated with PMA. The electropherogram shows ribosomal RNA peaks of 28S and 18S with annotations, along with small RNA and the lower marker.

The 2200 TapeStation system was assessed for performance in quality scoring of degraded RNA by analyzing heat degraded RNA samples. Jurkat Cell Total RNA which were heat degraded at 90°C was analyzed on R6K ScreenTape and High Sensitivity R6K ScreenTape assays 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.

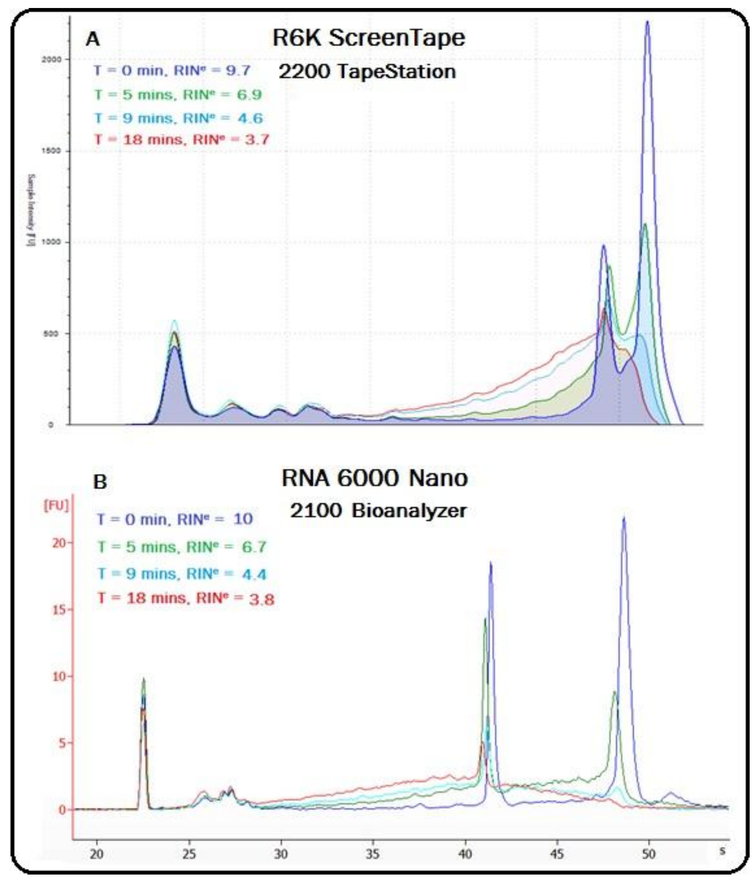


Figure 4: Electropherogram overlays of heat-degraded Jurkat cell RNA samples show increased degradation over time. A) Results from the R6K ScreenTape assay. B) Results from RNA 6000 Nano.

The average RIN value from replicates of all sample type was compared with the average RIN^e value generated by TapeStation system. Figure 5 suggests that both systems show a positive correlation with

- R^2 value of 0.9878 for RNA 6000 Nano and R6K ScreenTape assay
- R^2 value of 0.9474 for RNA 6000 Pico and High Sensitivity R6K ScreenTape assay.

Typically less than 4 percent deviation from RIN was observed for R6K ScreenTape assay and less than 7 percent deviation was observed for High Sensitivity R6K ScreenTape assay.

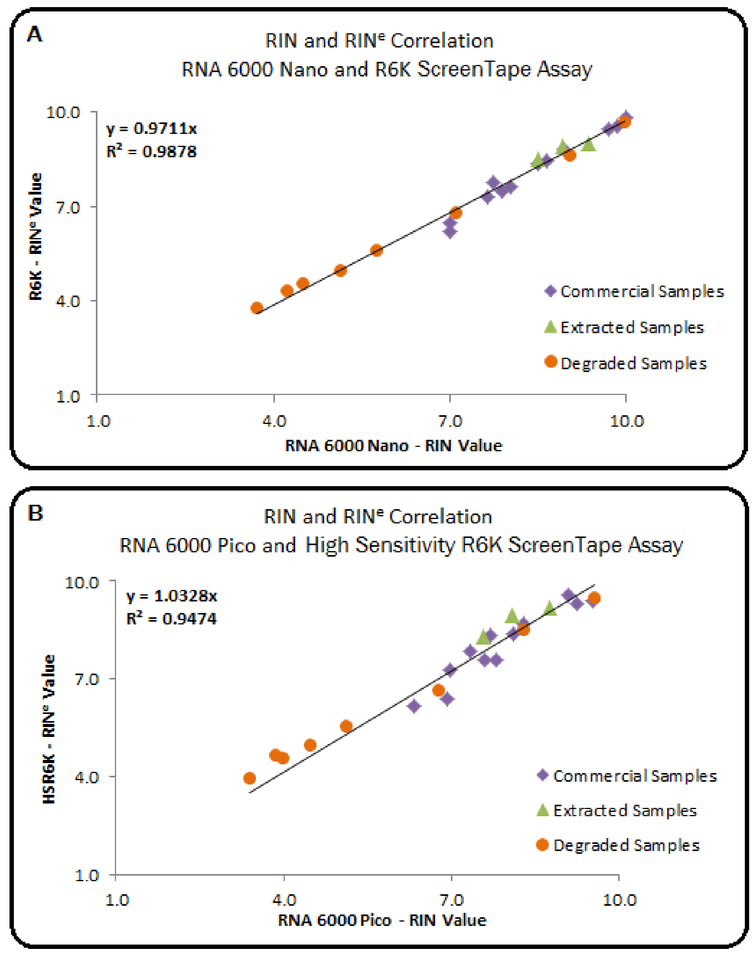


Figure 5: Correlation curves plotted by values obtained from commercial, degraded and extracted RNA samples. A) Plot of RIN and RIN^e values generated by RNA 6000 Nano and R6K ScreenTape assay. B) Plot of RIN and RIN^e values obtained from RNA 6000 Pico and High Sensitivity R6K ScreenTape assay.

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. 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.

Analytical Specification	Mean % CV			
	RNA 6000 Nano (n =30)	R6K ScreenTape (n = 27)	RNA 6000 Pico (n = 36)	High Sensitivity R6K ScreenTape (n = 27)
Intra-assay precision	< 3%	< 2%	< 4%	< 2%
Inter-assay precision	< 3%	< 2.5%	< 6%	< 3%

Table 1: Precision of RIN and RIN^e.

Conclusions

- The performance of the Agilent 2200 TapeStation system is comparable to the industry standard, Agilent 2100 Bioanalyzer for RNA quality assessment.
- The 2200 TapeStation system offers an easy to use system for analyzing RNA samples with minimum manual intervention.
- RIN and RIN^e shows high positive correlation in assessing the RNA samples of different quality
- The 2200 TapeStation system also shows high precision in RNA integrity assessment.
- Scalable throughput, ease of use, constant cost per sample and a fast time to result means that the Agilent 2200 TapeStation system is ideal for RNA quality assessment.