Comparison of two available platforms for the determination of RNA quality

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Introduction

The integrity of RNA is a very critical aspect regarding downstream RNA based analysis. Low-quality RNA can compromise the results of such experiments. To save time, costs and material, platforms for the determination of RNA quality play an important role. Currently two automated systems are available for the analysis of RNA quality and quantity, the Experion (Bio-Rad Laboratories), and the 2100 Bioanalyzer (Agilent Technologies). Both systems are based on an automated and miniaturized electrophoresis system, realized by Lab-on-chip technology. They determine RNA quality either by using the ribosomal 28S /18S ratio, or a numerical system which represents the integrity of RNA. Agilent Technologies offers the RIN algorithm (RNA Integrity Number) on the 2100 Bioanalyzer and Bio-Rad developed a new Experion software version that offers an algorithm for calculating the RNA Quality Index (RQI). The RIN and the RQI are based on a numbering system from 1 to 10 (1 being the most degraded RNA profile, 10 being the most intact RNA).
The aim of these experiments was to compare the 2100 Bioanalyzer and the Experion regarding sensitivity, reproducibility and the influence of individual tissue extractions and different runs on RNA quality determination.

Figure 1

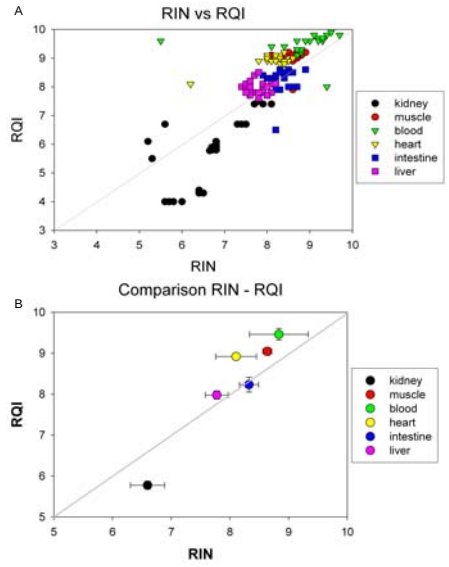

Figure 1: Comparison of the corresponding RIN and RQI values
In figure 1A RIN values were plotted against the associated RQI values for all RNA quality measurements. Figure 1B shows the mean values for each tissue ±SD. The grey lines (y = x) represents the actually expected situation, that the RQI is equivalent to the RIN.

Material and Methods

RNA from six bovine tissues (kidney, muscle, blood, heart, intestine, liver) was extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany). To show the influence of different extractions on RNA quality and quantity measurements RNA from each tissue was extracted six times. Thus 6 x 6 (n = 36) samples were available for later experiments.

Project 1:
The aim of the first project was to show the influence of different extractions on RNA quality determination by the two platforms and to compare the results of the Experion and the 2100 Bioanalyzer with regard to the influence of different chip runs on the results. Therefore the six samples of each tissue were measured two times on one day and two times on another day to verify the influence of different chip runs and of freezing and thawing on RNA quality results. Every chip composition was made and measured simultaneously in the Experion and the 2100 Bioanalyzer.

Project 2:
The purpose of the second project was to analyze and compare the sensitivity of the two platforms in various RNA quality ranges. Therefore total cellular RNA of each tissue was degraded artificially by UV irridation and a dilution series of degraded and intact RNA of the same RNA pool with 6 (Degradation substudy 1) or 11 (Degradation substudy 2) dilution steps respectively was prepared in order to get RNA samples with different degradation levels, but with the identical transcriptome and mRNA distribution.

Figure 3

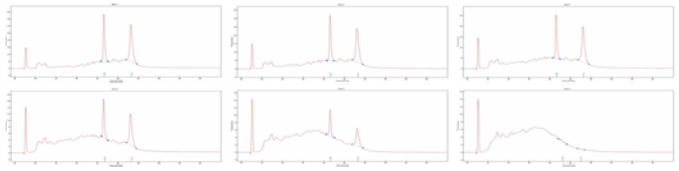

Figure 3: examples of Experion electropherograms of six degradation steps (heart)
Electropherograms of 6 degradation steps obtained by the Experion showing 18 S rRNA and 28 S rRNA peaks decreasing with declining RNA Integrity from top left to bottom right.

Results and Discussion

Project 1:
To find the level of influence of different extractions and different chip runs on RNA quality determination, a “Two Way ANOVA” statistical analysis was employed.
The influence of different extractions on RNA quality determination was significant ($p<0.05$) for kidney ($p<0.001$) in both platforms and for liver ($p<0.001$) in the Experion. Kidney samples show the worst quality results (RIN: 5.2 - 8.1, RQI: 4 – 7.5) followed by liver samples (RIN: 7.4 – 8.2, RQI: 7.6 - 8.4). This indicates that the influence of repeated extraction is dependent on RNA quality.
The influence of different chip runs on RNA quality determination was significant for blood ($p = 0.037$), heart ($p = 0.008$), intestine ($p = 0.008$) and liver ($p<0.001$) in the Bioanalyzer and for non of the tissues in the Experion. This indicates, that the quality determination of the Experion is more reproducible than that of the Bioanalyzer. This may be a result of the more automated chip preparation system of the Experion.
Figure 1A shows all data points obtained from the single quality measurements. It can be observed that all data points scatter around the grey ideal line (ideal line: RIN = RQI). The worse RNA quality results are obtained by kidney samples. Regarding the other tissues it can be observed that the data points are located closer and that most data points are located above the ideal line, which indicates that RQI values are estimated higher that the corresponding RIN. Figure 1B shows, that the standard deviations related to the RIN are higher than those related to the RQI, indicating again, that RQI results are more reproducible.

Project 2:
The purpose of the second project was to analyze and compare the sensitivity of the two platforms in various RNA quality ranges. Therefore, two dilution series with 6 or 11 dilution steps respectively were established to get RNA in different degradation steps from degraded to intact RNA.
Figure 2 shows the results of both degradation experiments. Each spot represents one quality measurement. Actually all spots should show a constant and linear disposition along the grey line. Both platforms clearly show a lack of values in the area between 2 and 6. While the RQI lacks values between 3 and 6, the RIN only lacks values between 2.5 and 4. This indicates sensitivity problems in both platforms in the lower and medial areas of the quality determination. Mainly the Experion overestimates or underestimates RNA with medial quality. Regarding the electropherograms of this dilution series (figure 3), a stepwise and linear decrease of the RNA quality is seen. This shows that the reason for the lack of values is the algorithm, and not the measurement itself.
Also when determination in this medial area of RNA quality is not of great importance for daily laboratory work, where good quality samples are needed, improvements of these under- and over estimations will be very important.
In conclusion, data obtained by the Experion show better results regarding reproducibility and absolute sensitivity, whereas the Bioanalyzer 2100 shows a a higher linearity in the lower RNA quality areas (RIN/RQI 3 to 5). Overall it was shown that both algorithms are very comparable and beneficial for the determination of RNA quality for downstream applications, like qRT-PCR or hybridization arrays.

Figure 2

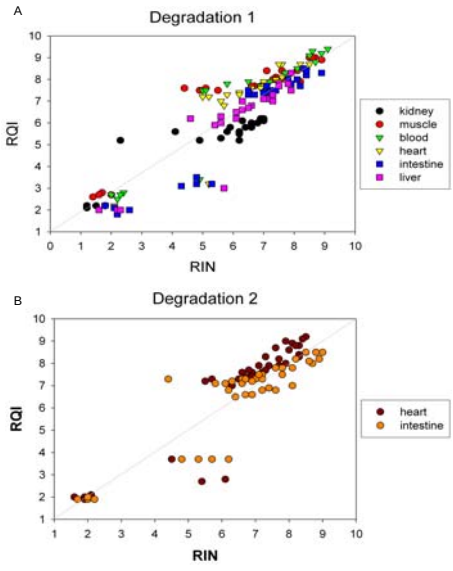

Figure 2: Comparison of the corresponding RIN and RQI values of degradation 1 and 2
In figure 2A RIN values were plotted against the associated RQI values for all RNA quality measurements of six degradation steps for six tissues. In figure 2B RIN values were plotted against the associated RQI values for all RNA quality measurements of eleven degradation steps for heart and intestine.