

## Total RNA Quality Evaluation by RNA Integrity Index (RII) Using MultiNA II

Yuji Sogabe

### User Benefits

- ◆ Possible to automate the electrophoresis operation
- ◆ Automatically calculates RNA Integrity Index (RII) needed to assess the quality of Total RNA
- ◆ Quick and easy measurement of multiple samples

### Introduction

Total RNA extracted from biological samples is the basic material for various experiments including sequencing analysis (next generation, Sanger), and the yield and quality of total RNA are affected by the original sample collection and pretreatment conditions. The quality of the total RNA obtained by extraction will greatly determine the success or failure of subsequent experiments.

Traditionally, total RNA quality has been evaluated by ultraviolet (UV) spectrophotometry and electrophoresis (denaturing agarose gel electrophoresis). With the advent of electrophoresis instruments, the RIN (RNA Integrity Number) obtained by Agilent Technologies' Bioanalyzer in particular has long been used as the de facto standard for total RNA quality assessment. Based on this result, a decision is made whether to use the obtained total RNA for subsequent experiments or to reprepare it.

Shimadzu's microchip electrophoresis system MultiNA II MCE-301 (Fig. 1) can calculate RII (RNA Integrity Index), an index for evaluating the quality of total RNA from electrophoresis results.

Here we introduce the correlation between RII, a total RNA quality index calculated from the results of electrophoresis of total RNA from various tissues by MultiNA II, and RINe (RNA Integrity Number equivalent) obtained by 4150 TapeStation System (Agilent Technologies) will be introduced.

### Sample Preparation and Analysis Conditions

#### Total RNA sample preparation

Seven types of Total RNA samples were used: Rat liver total RNA (Clontech), Rat kidney total RNA (Clontech), Mouse liver total RNA (Clontech), Mouse brain total RNA (Clontech), Human heart total RNA (Thermo Fisher Scientific), Human lung total RNA (Clontech), and Human kidney total RNA (Clontech).

Total RNA samples were prepared to a concentration of 50 ng/μL and then heat treated at 90 °C for 10, 30, 50, 70, 90, and 120 min. A total of seven samples were prepared for analysis, including those without heat treatment (Fig.2).

#### Analysis of Total RNA

Analysis of RNA by MultiNA II is performed in premix mode. Samples for analysis were mixed 1:1 with the marker provided in the RNA kit. For the ladder, RNA 6000 Ladder (Thermo Fisher Scientific) was diluted 6-fold with THE RNA Storage Solution (Thermo Fisher Scientific) and mixed 1:1 with the marker provided in the kit as for the analytical samples. The analytical samples and ladder mixed with markers were heat-treated at 72 °C for 3 min and placed on ice. After treatment, they were placed in the instrument together with the separation buffer for analysis (Fig.2).



Fig. 1 Microchip electrophoresis system MultiNA™ II MCE-301

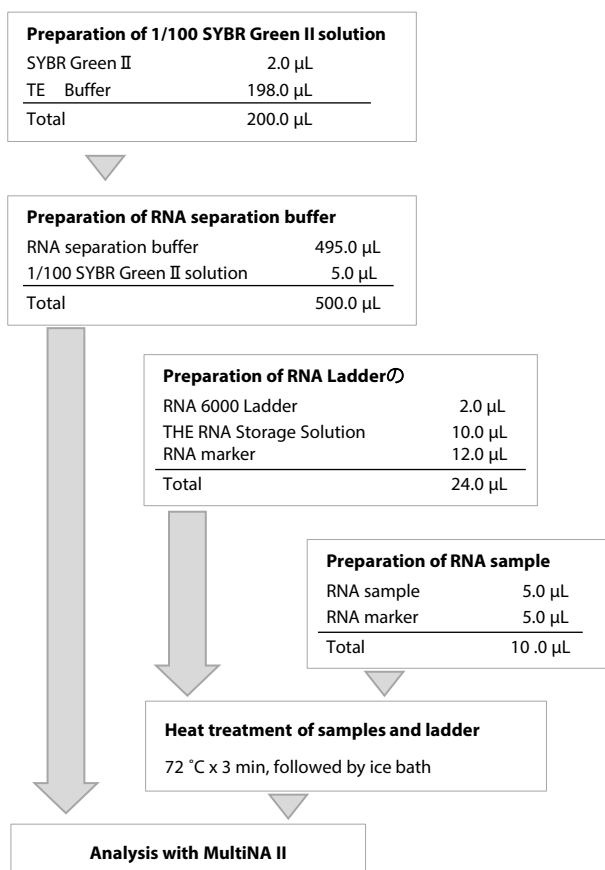
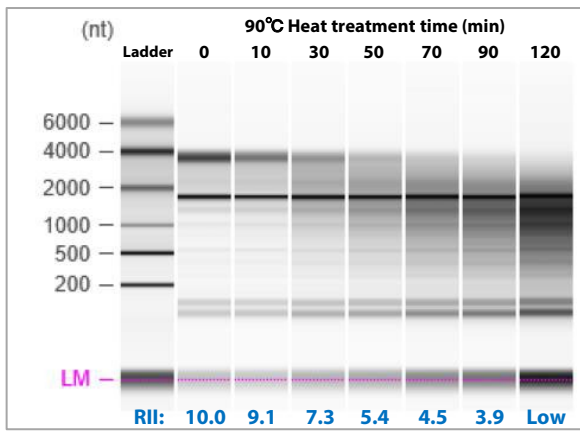
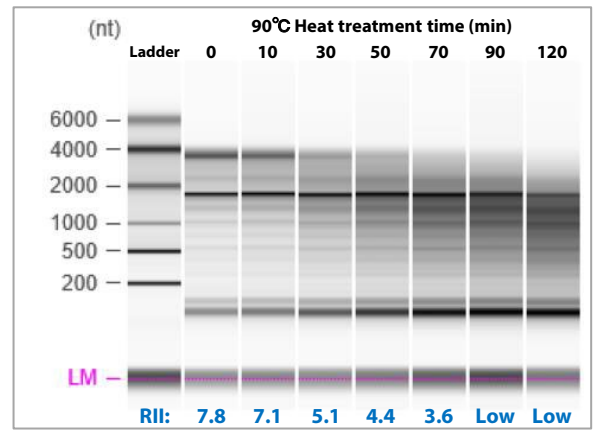


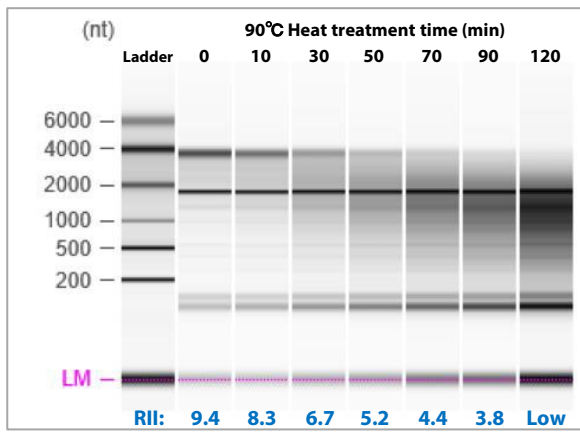
Fig. 2 Analysis procedure of RNA by MultiNA II (6 analyzed samples)



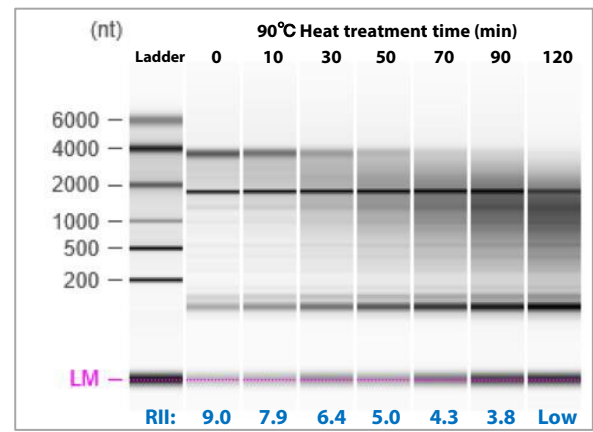
Rat Liver Total RNA



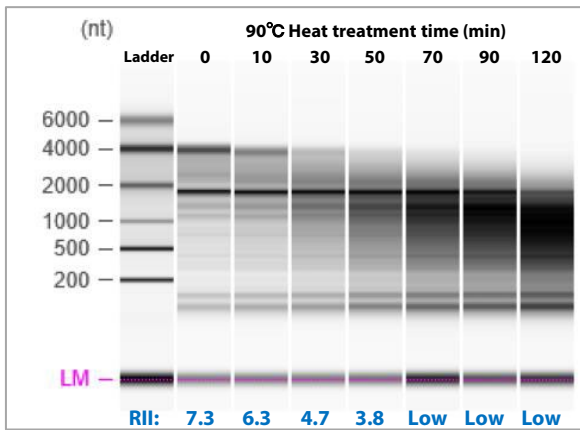
Rat Kidney Total RNA



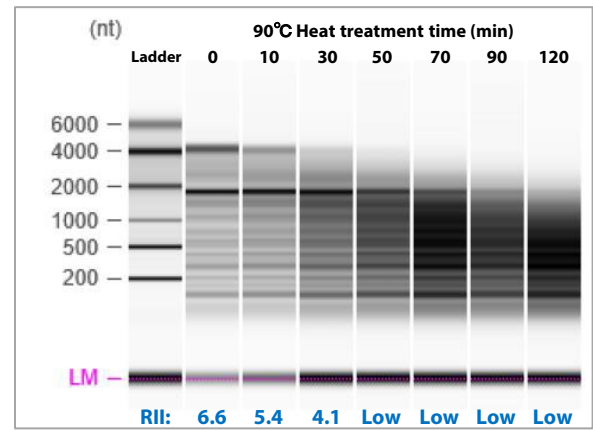
Mouse Liver Total RNA



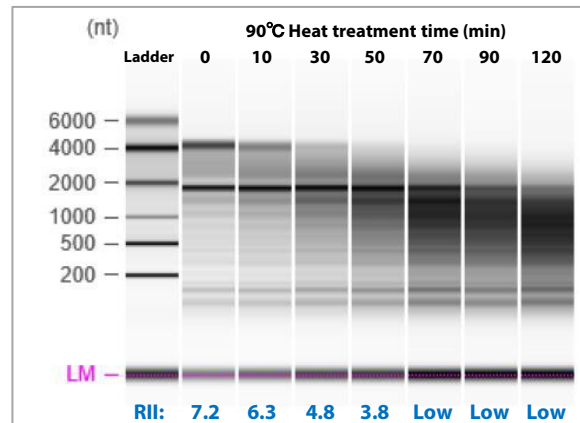
Mouse Brain Total RNA



Human Heart Total RNA



Human Kidney Total RNA



Human Lung Total RNA

Fig. 3 Electrophoresis results of various Total RNA by MultiNA II (gel image)

\* RII less than 3.5 is indicated as Low.

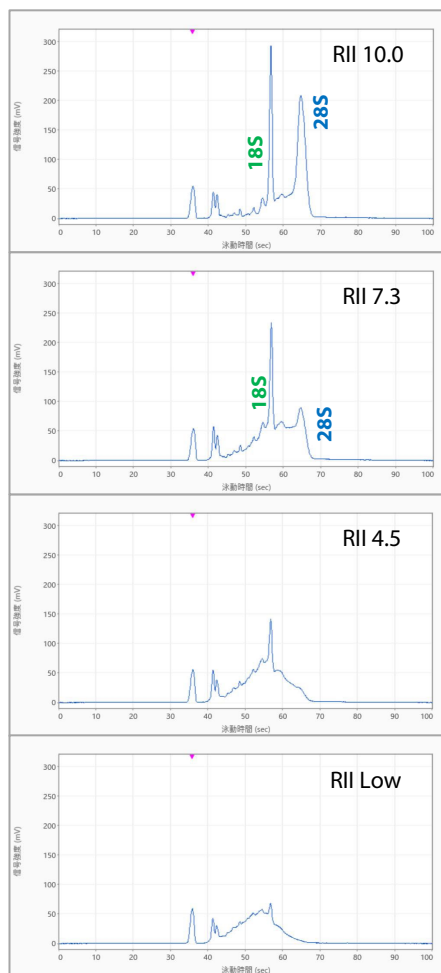


Fig. 4 Electropherograms of RII differences on Rat liver total RNA (RII 10.0 to RII Low)  
\* RII less than 3.5 is indicated as Low.

## Results

### Analysis results with MultiNA II

Fig. 3 shows the results (gel images) of electrophoresis of seven different total RNAs in MultiNA II after seven steps of heat treatment for each. The calculated RII is indicated below the gel image of each sample.

At a heat treatment time of 0 min for each sample, differences in RII were observed, indicating that there were differences in the degree of degradation from the untreated stage. As the heat treatment time increased, the percentage of smear bands below about 2000 nt increased. On the other hand, the percentage of RII decreased as the heat treatment time increased.

We then compared the electropherogram results for different RIIs (Fig. 4).

At RII 10.0, the 18S and 28S rRNA (ribosomal RNA) peaks were clearly observed. At RII 7.3, the 28S rRNA peak was lower. At RII 4.5, 28S rRNA is almost no longer observed and the 18S rRNA peak is lower. In RII Low, 28S rRNA was completely disappeared and 18S rRNA was only slightly observed.

### Comparison of RII and RINe

A comparison of RII calculated from the results of samples analyzed by MultiNA II and RINe calculated by the 4150 TapeStation System is shown in Fig. 5. A good correlation was obtained with a coefficient of determination of  $R^2 = 0.97$ .

## Conclusions

Here seven types of total RNA were each subjected to seven steps of heat treatment and electrophoresed with MultiNA II.

From the electrophoresis results, we compared RII, a quality indicator, to RINe, which is calculated by the 4150 TapeStation System.

As the results, regardless of the degree of RNA degradation, a good correlation was obtained between RII and RINe.

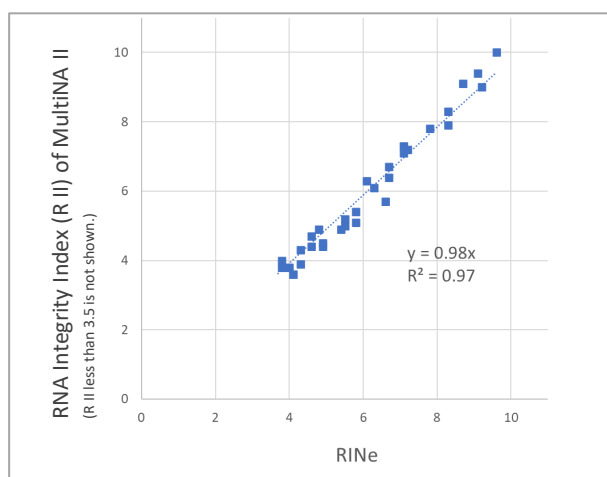


Fig. 5 Comparison of RII and RINe

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