Analysis of miRNA content in total RNA preparations using the Agilent 2100 bioanalyzer

Application Note

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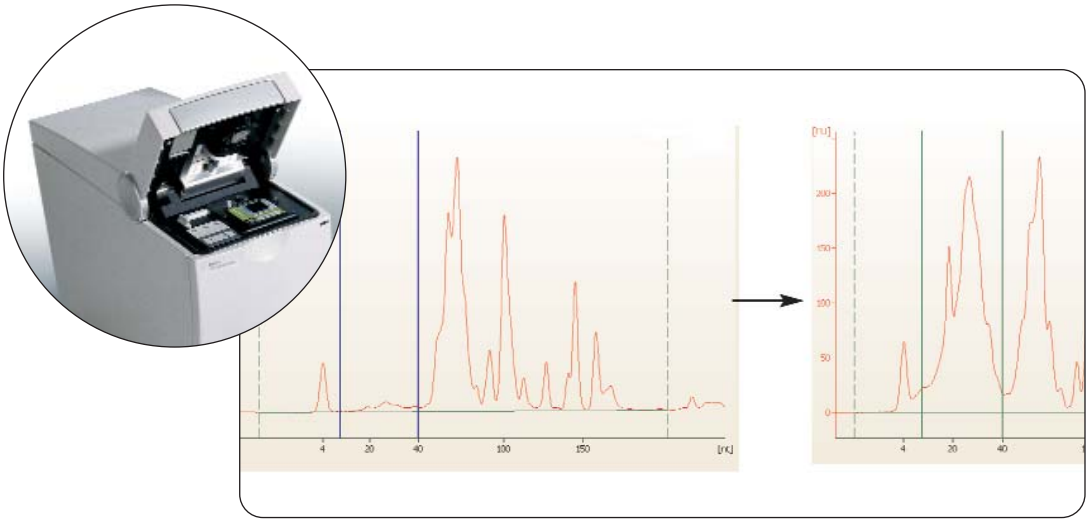

Agilent Equipment

- Agilent 2100 bioanalyzer
- Agilent RNA Nano kit
- Agilent Small RNA kit

Application Area

- Gene expression

Abstract

Advances in miRNA research have led to the increased demand for techniques appropriate for the analysis of this small RNA subclass. This Application Note describes the use of the Small RNA assay with the Agilent 2100 bioanalyzer to quickly and efficiently analyze and monitor miRNA content in total RNA samples.

Agilent Technologies

Introduction

Over the past years the scientific community has seen an explosion in the number of research studies related to small RNA molecules, and in particular the microRNAs (miRNA). Due to the high interest in investigating miRNA expression patterns by microarray or qPCR, the demand for precise and accurate measurements of miRNA samples has increased significantly. At the same time, the Agilent 2100 bioanalyzer with the RNA Nano and Pico assays and associated RNA integrity number (RIN) became the industry standard for the assessment of total RNA integrity. The Small RNA assay, an extension to the established portfolio for RNA quality control, provides the first analytical solution for the identification and characterization of small RNA molecules. The assay is fast, highly sensitive and offers visualization and quantification of small RNA[1]. These features allow researchers to identify and monitor the ratio of miRNA fractions in total RNA extracts and to optimize small RNA isolation and purification protocols. In this Application Note we present the results obtained for miRNA analysis using two new Small RNA applications:

- measurement of miRNA content in total RNA samples, and
- monitoring miRNA isolation from total RNA samples.

Materials and methods

RNA sources

FirstChoice Total RNA (Ambion Inc.) from a variety of human, mouse and rat tissues were used.

Small RNA isolation and size fractionation

Small RNA was extracted from total RNA samples by utilizing a column based isolation method (*mir*Vana™ miRNA Isolation Kit, Ambion Inc.). Enriched small RNA samples were size fractionated, purified and concentrated by using a gel-based fractionation system (flashPAGE™ fractionator, Ambion Inc.) according to manufacturer's protocol.

RNA analysis

All RNA samples were analyzed on the Agilent 2100 bioanalyzer. The RNA 6000 Nano kit was used for quantification and integrity assessment of total RNA samples and the Small RNA kit was used for separation and quantification of miRNA.

Software

All RNA 6000 Nano and Small RNA analyses were carried out using the Agilent 2100 expert software (Rev. B.02.05).

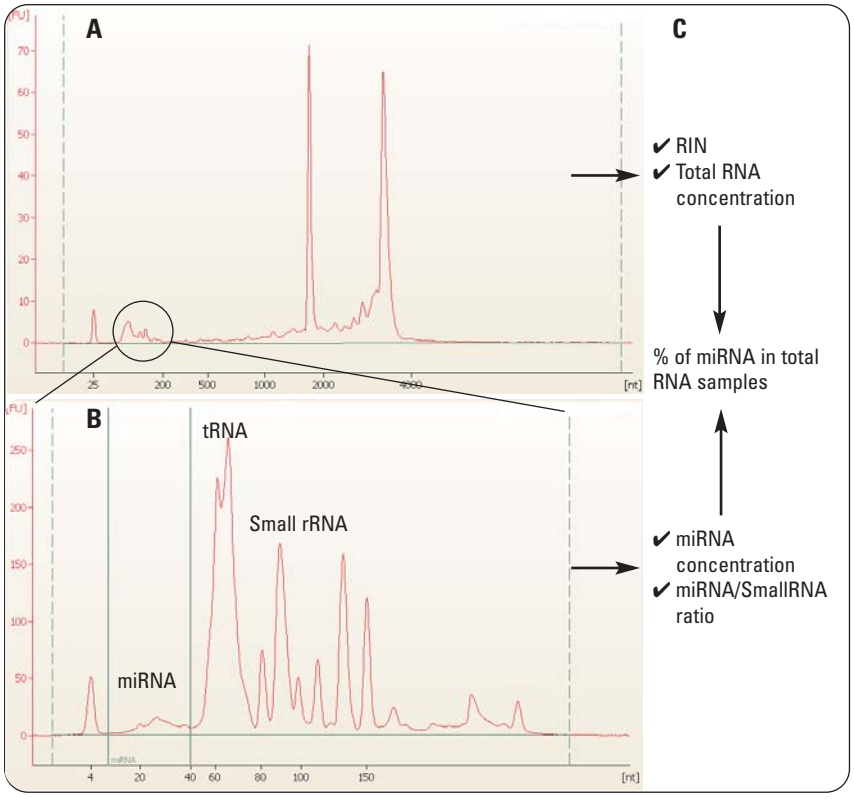

Figure 1
Total and small RNA analysis. A) The electropeherogram shows the total RNA pattern analyzed with the RNA 6000 Nano assay. B) The electropherogram shows the small RNA pattern analyzed with the Small RNA assay. C) miRNA analysis workflow. All RNA quantities were used to estimate the % of miRNA in total RNA samples.

Results and discussion

Analysis of miRNA content in total RNA from 40 different tissues samples

In order to determine the miRNA content in total RNA samples, commercially available total RNA samples containing small RNAs (including miRNAs, siRNA, and snRNA) were analyzed on the Agilent 2100 bioanalyzer. Altogether, 40 different samples extracted from a variety of rat, mouse and human tissues were selected to conduct this study.

The concentration and quality of all total RNA samples were analyzed using the RNA 6000 Nano assay (figure 1 A). Integrity of the RNA samples was assessed with the RNA Integrity Number (RIN) tool, a dedicated algorithm available in the Agilent 2100 Expert software[2]. The RIN of the samples varied between 6.6 and 10, showing clear differences in the quality of total RNA derived from different tissues samples (figure 2). The human RNA samples were found to have lower RIN values, whereas the RIN scores of mouse and rat RNA provided higher values.

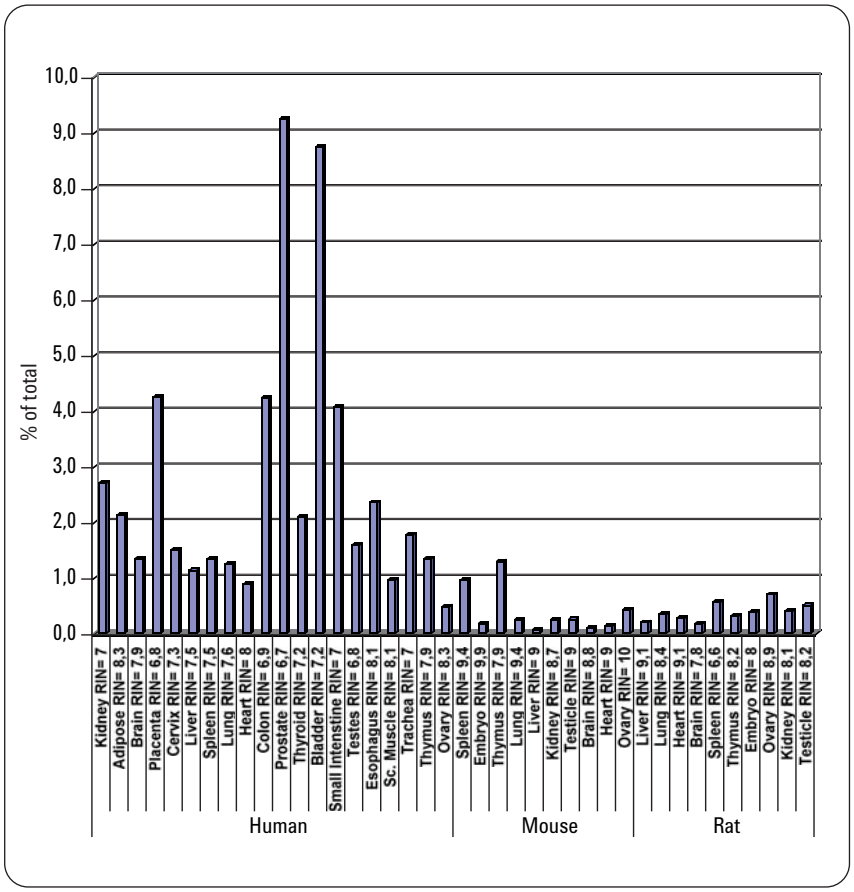

Figure 2
miRNA content in total RNA of 40 different tissues. In the bar chart, the miRNA ratio is displayed as % of total RNA. The corresponding RIN values of all tissues are listed.

All total RNA samples were then analyzed with the Small RNA assay to measure the miRNA concentration. The relative amount of miRNA in all the tissues samples were manually calculated as a ratio of the concentration of miRNA in total RNA. The workflow of this experiment is shown in figure 1 C. The miRNA ratio varies significantly among different tissues, and species types as shown in figure 2. Whereas the rat and mouse miRNA ratio range between 0.1 and 1.3 % of total RNA, the miRNA ratios of the human samples are highly elevated (0.5 - 9.2 % of total). These differences may be due to the quality of the total RNA samples and the way the samples were prepared.

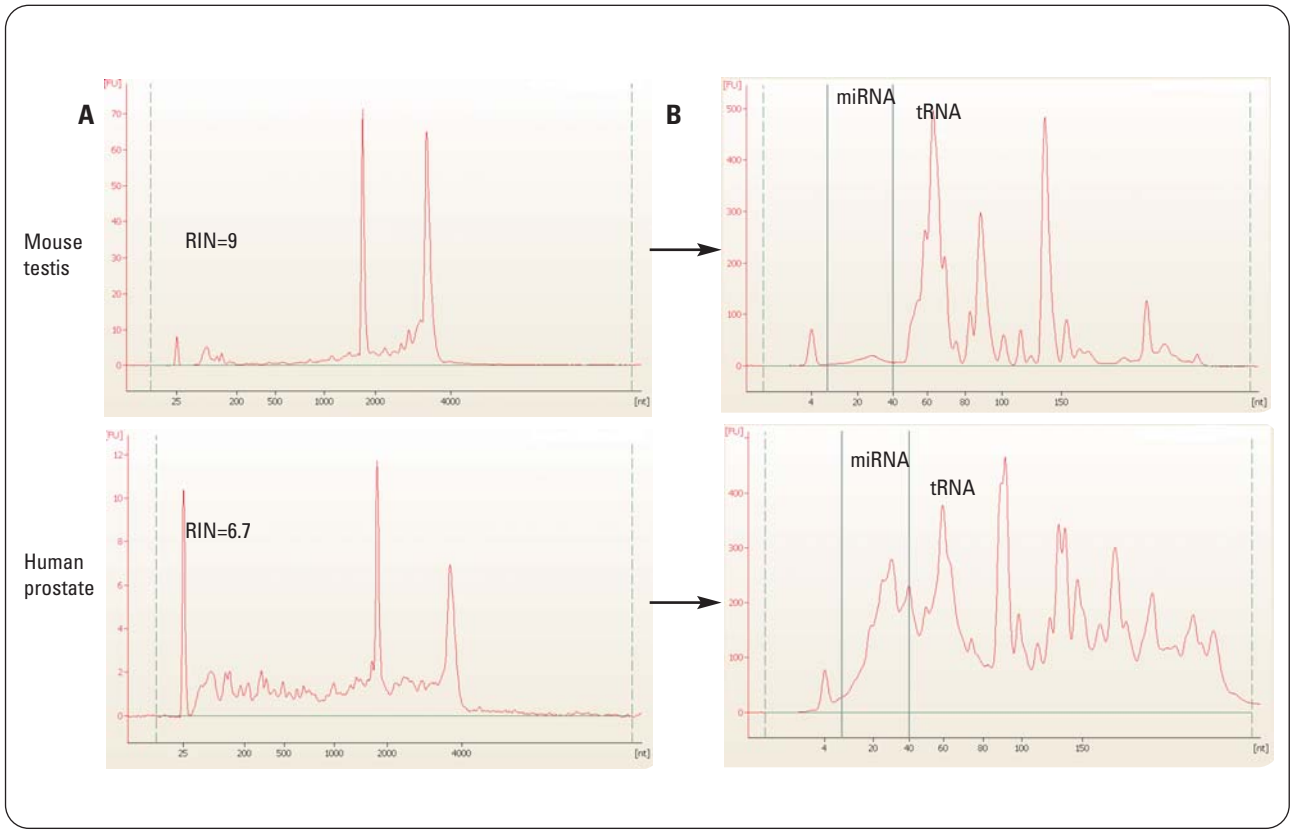

Figure 3
Effect of sample quality on the miRNA profiles. A) RNA Nano 6000 Assay: The electropherograms shows highly intact mouse testis (RIN = 9) and slightly degraded human prostate (RIN = 6.7) total RNA samples. B) Small RNA Assay: Corresponding small RNA electropherograms are shown.

Figure 3 shows a comparison example between two different total RNA samples with differing RIN values. The electropherogram of the mouse testis total RNA (RIN 9) shows all features of a highly intact RNA sample. The human prostate total RNA (RIN 6.7) provides evidence of sample degradation. The results are also reflected in the small RNA electropherograms (figure 3B). The miRNA region of the mouse RNA sample is clearly separated from larger RNA fractions as tRNA or small rRNA. In contrast, the baseline of the human miRNA profile is elevated and fractions are not well resolved indicating an overlap of small RNA with RNA degradation products.

The differences in the quality of human and rat/mouse total RNA can be reasonably explained by the modified extraction method of human total RNA as mentioned by the supplier.

Monitoring miRNA isolation with the Small RNA assay

An important step in many miRNA experiments is to obtain high yields of miRNA from biological samples. Several kits are available on the market to extract and/or enrich miRNA from total RNA samples. Here we show how the Small RNA assay can be used to monitor miRNA content obtained by different miRNA isolation methods.

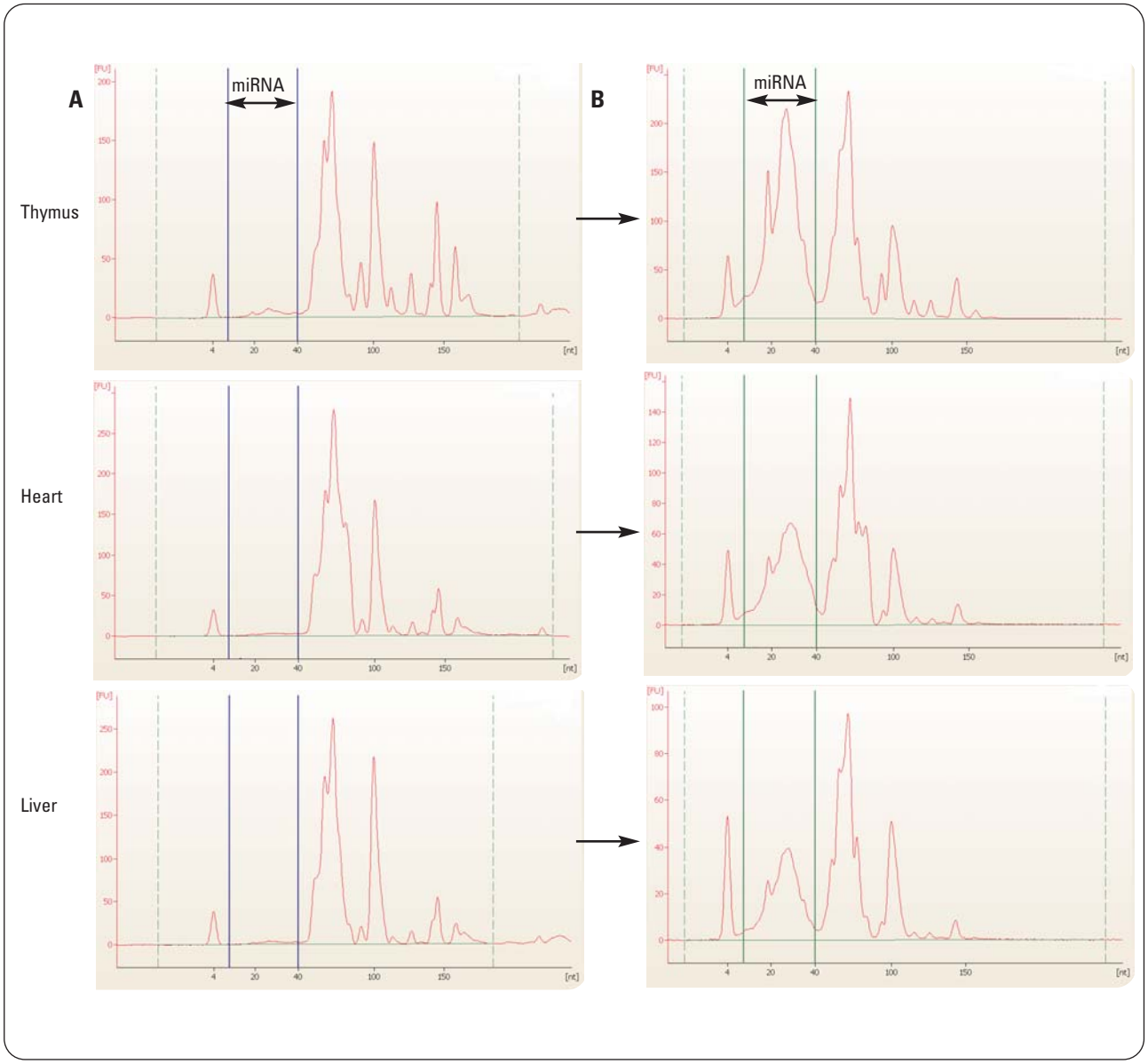

Figure 4
Electropherograms of small RNA samples isolated from 3 different mouse tissues (thymus, heart and liver).
The limits of the miRNA region spanning from 10 and 40 nt are represented by blue and green lines, respectively.
A) Electropherograms of small RNA samples isolated by utilizing a column based method.
B) Electropherograms of small RNA samples enriched by utilizing a gel based method

Firstly, miRNA was extracted from mouse total RNA samples from three different tissues (thymus, heart, and liver) by using a column based method. In addition, the obtained miRNA fractions were further processed by using a gel based enrichment step. All miRNA extractions were analyzed with the Small RNA assay. The enrichment of miRNA could be easily monitored by comparing the calculated miRNA ratio of the different sample extractions or by visual inspection of the electropherograms (figure 4). The samples extracted with the column based method showed low miRNA concentrations (1 to 5 % of the small RNA content).

In contrast, the implementation of an additional gel-based purification step enriched the miRNA/small RNA ratio from 10 to 30 fold (figure 5). This data shows the ability of the Agilent 2100 bioanalyzer to evaluate the miRNA quantity and quality during the miRNA extraction workflow.

Conclusion

The results show that the Agilent 2100 bioanalyzer in conjunction with the Small RNA assay are valuable tools for the visualization and quantitation of miRNA derived from different tissues. The assay provides a rapid method for evaluating the miRNA content of RNA samples, making it the ideal tool for both assessing miRNA percentage of total RNA samples and for monitoring enrichment and purification in miRNA extraction protocols.

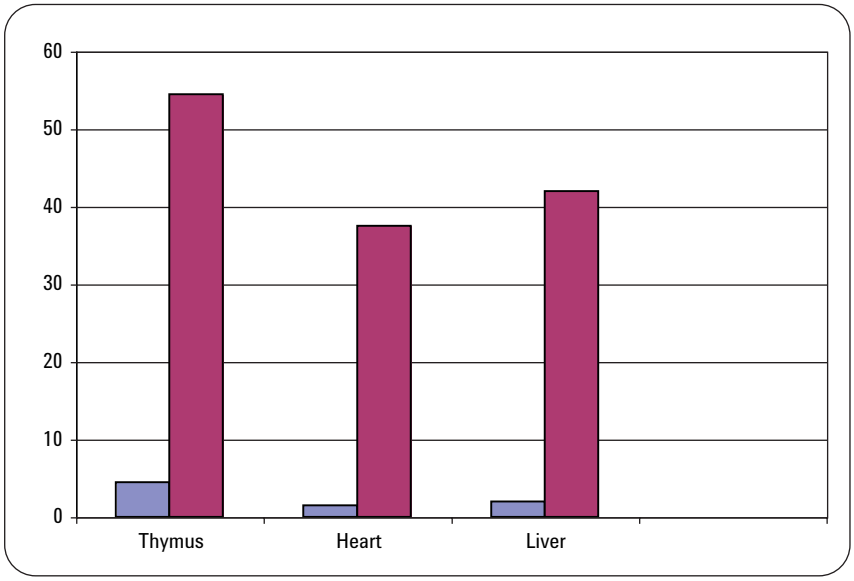

Figure 5
Results of miRNA quantification. On the y-axis, the miRNA ratio is displayed as % of small RNA. Blue bars: percentage of miRNA after column based purification; Purple bars: percentage of miRNA after gel based purification.

References

1.
Performance of the Agilent Small RNA assay. *Publication Number 5989-7002EN*

2.
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