High sensitivity quality control of RNA samples using the RNA 6000 Pico LabChip kit

Application

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Abstract

This Application Note describes the use of the RNA 6000 Pico LabChip® kit for the quality control of small amounts of RNA using the Agilent 2100 bioanalyzer. The main advantages of the RNA 6000 Pico LabChip kit are its high sensitivity combined with its time saving experimental procedure. Here, we show experimental data that demonstrate the sensitivity of the RNA 6000 Pico LabChip kit by the analysis of different amounts of highly dilute RNA samples. The results show the suitability of the RNA 6000 Pico LabChip kit for quality control of total RNA and messenger RNA (mRNA) in a range from 200 pg/µl to 5000 pg/µl and 500 pg/µl to 5000 pg/µl, respectively. The reproducibility of the assay is also demonstrated. In order to provide an insight into the advantages of the RNA 6000 Pico LabChip kit in combination with specific RNA isolation techniques, we present data from the RNA quality control (QC) of microdissected human kidney tissues. The RNA 6000 Pico LabChip kit used with the Agilent 2100 bioanalyzer identified differences in RNA quality referring to different isolation and purification methods. In summary, we provide evidence that the RNA 6000 Pico LabChip kit is useful for the QC of very low amounts of RNA samples. The data clearly indicate a 25-fold increase of assay sensitivity in comparison with the well established RNA 6000 Nano LabChip kit.

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Introduction

During the last decade, the analysis of the transcriptome received a lot of attention, especially the comparison of the transcription products of a large number of expressed genes via microarray technology. By determining changes in the expression profile of several transcriptomes, scientists try to correlate differences in the gene expression patterns with distinctive features of a specific cellular phenotype. At best, results should be conclusive enough to identify similar transcriptional changes that might be responsible for the occurrence of different phenotypical features.

Over the last five years, there has been an increase in the development of new methods, suitable for measuring low changes in gene expression rates. The complexity of these newly developed methods has created a need for a high sensitivity tool for RNA quality control with respect to degradation and contamination. Unidentified poor RNA quality at the beginning of an expression profiling workflow will result in an impairment of data quality, wasting time and money.

The RNA 6000 Nano LabChip kit in combination with the Agilent 2100 bioanalyzer, was the first commercially available lab-on-a-chip based analysis tool for the quantification and quality control of RNA. This RNA assay offers a fast and reliable method for RNA analysis combined with superior data management. Meanwhile, the 2100 bioanalyzer and the RNA 6000 Nano LabChip kit are used in a large number of laboratories. Increasingly sophisticated experimental approaches allow the analysis of expression profiles of different distinct tissues and cell types. Various types of microdissection have proven to be very useful for the isolation of specific tissues that are free of unspecific cell contaminations. One disadvantage of these techniques is that the yield of extractable RNA from microdissected tissues is often very low. Therefore, it is essential to provide a fast and sensitive method that allows quality control of the extracted RNA, combined with low sample consumption. The RNA 6000 Pico LabChip kit was developed to fulfill these analytical requirements. Using this kit, detection of total RNA can be achieved between 200 pg/µl and 5000 pg/µl.

In this Application Note, the suitability of the RNA 6000 Pico LabChip kit for high sensitivity RNA quality control is demonstrated.

Material and methods

RNA 6000 Pico assay protocol

All chips were prepared according to the instructions provided with the RNA 6000 Pico LabChip kit. In short, 550 µl of the RNA 6000 Pico gel matrix were placed on a spin filter, centrifuged at 1500 g and divided in 65 µl aliquots. After the addition of 1 µl of the RNA 6000 Pico dye concentrate, the gel-dye mix was vortexed and centrifuged at 13000 g for 10 minutes prior to analysis. The gel-dye mix was injected into the channel system of the RNA 6000 Pico chip by using the chip priming station followed by the addition of conditioning solution. After the addition of 5 µl marker to the ladder and sample wells, 1 µl of each RNA sample was added in the 11 designated sample wells, followed by the addition of 1 µl of diluted RNA 6000 ladder (Ambion) in the ladder well. The RNA samples and ladder were prepared by heating the samples at 70° C for two minutes.

RNA samples

For this study, human kidney tissue (unaffected part of a tumor nephrectomy) stored in RNAlater™ (Ambion) was used. Renal medullar and tubulo-interstitial tissue was microdissected manually under a stereomicroscope to samples of approximately 0.1 mm^3. The tubulo-interstitial compartment and the medullar tissue were lysed and RNA isolation was performed by using a Qiagen column based protocol according to manufacturer's instructions. In certain cases, RNA samples were additionally treated with DNaseI to exclude DNA contaminations. To avoid salt contamination, which could cause a decrease in sensitivity, the RNA samples were washed two times with 70 % ethanol and eluted with 30 µl RNase-free water. For all other experiments, mouse heart total RNA was purchased from Ambion and diluted with RNase-free water to various concentrations.

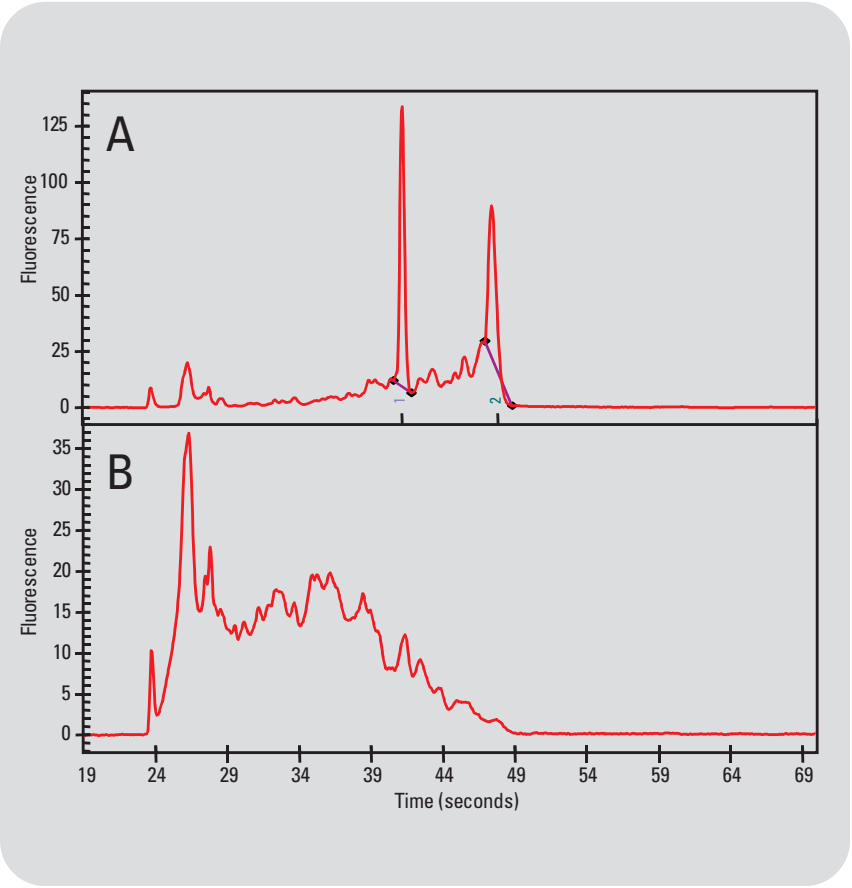

Figure 1
Quality control of total RNA samples using the RNA 6000 Pico LabChip kit. 5 ng of different quality RNA samples were analyzed on the Agilent 2100 bioanalyzer. A) Typical electropherogram of high quality RNA including the clearly visible 18S/28S rRNA peaks. B) Partially degraded sample as indicated by a shift in the electropherogram to shorter fragment sizes.

Results and discussion

Sensitivity of the RNA 6000 Pico LabChip kit

To test the sensitivity of the RNA 6000 Pico LabChip kit, several different experiments were performed. The limit of detection for RNA quality control was determined by analyzing different RNA dilutions on the Agilent 2100 bioanalyzer. RNA quality control

(QC) as well as an estimate of concentration was possible in the 200 pg/µl to 5000 pg/µl concentration range. Total mouse heart RNA was diluted to a concentration of 5 ng/µl and 1 µl aliquots were analyzed using RNA 6000 Pico LabChip kit. The RNA electropherogram of two different samples clearly demonstrates differences in the RNA quality (figure1). Whereas the RNA sample of good quality shows clear 18S and 28S rRNA peaks (figure 1, A), the electropherogram of the partially degraded RNA shows absence of the 18S/28S rRNA peaks combined with a shift of the bands to shorter migration times (figure 1, B). The analysis of further RNA dilutions clearly shows that RNA QC can be performed down to a concentration of 200 pg/µl by using the RNA 6000 Pico LabChip kit (figure 2). The electropherogram identifies distinct features of good quality RNA such as sharp 18S/28S rRNA bands combined with a comparably flat baseline. The signal to noise ratio at 200 pg/µl was 10. The linear dynamic range of the RNA 6000 Pico LabChip kit was determined by analyzing RNA samples ranging between 200 – 5000 pg/µl. Within this range, the regression coefficient was 0.9998 (figure 3) indicating linearity in this concentration range. The reproducibility of quantitation was tested by the analysis of identical samples (data not shown). Using diluted samples, a good degree of reproducibility can be achieved.

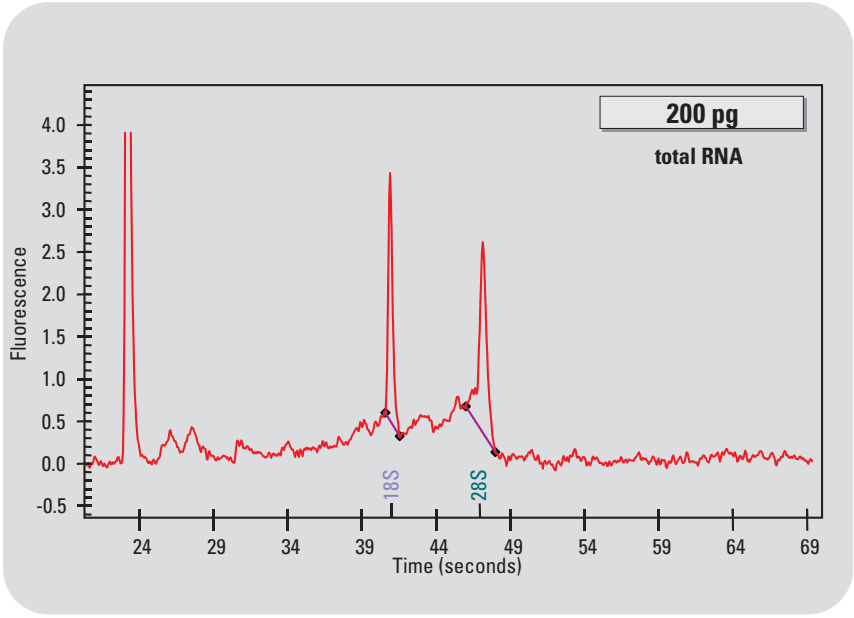

Figure 2
Quality control of low concentration total RNA (diluted total Mouse Heart RNA; Ambion). The electropherogram shows high RNA quality.

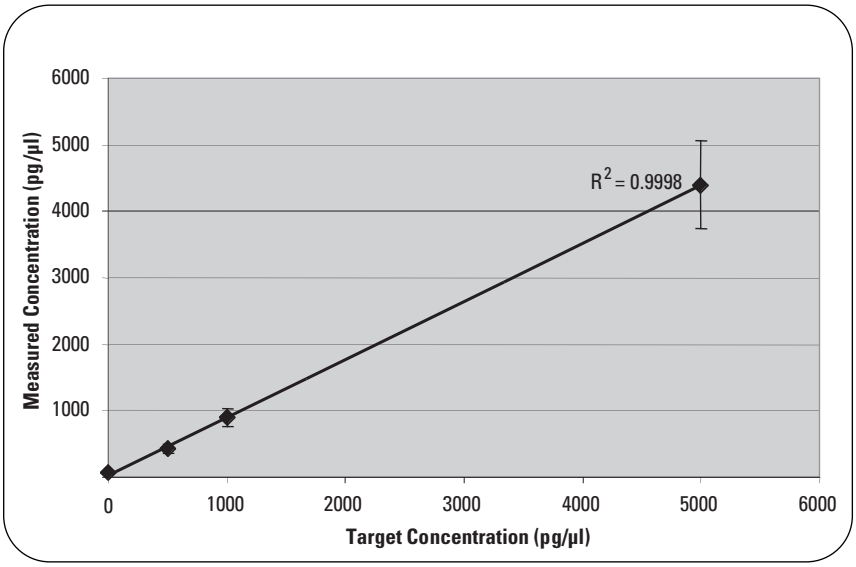

Figure 3
The concentration values of 4 different dilutions from 200 to 5000 picograms are plotted. Under these ideal conditions, the Pico assay gave a linear regression curve with high degree of linearity (regression coefficient: 0.999).

However, any sample contaminants such as salt could potentially reduce the assay sensitivity resulting in interference with the RNA quantitation results. Therefore, it is recommended that the manufacturers instructions are strictly followed and the use of salt buffers for the elution of purified RNA avoided. In general, due to the high sensitivity of the RNA 6000 Pico assay, it does not allow RNA quantitation with the same degree of accuracy and precisions as the RNA 6000 Nano assay and the concentrations given in the software should be regarded as concentration estimations.

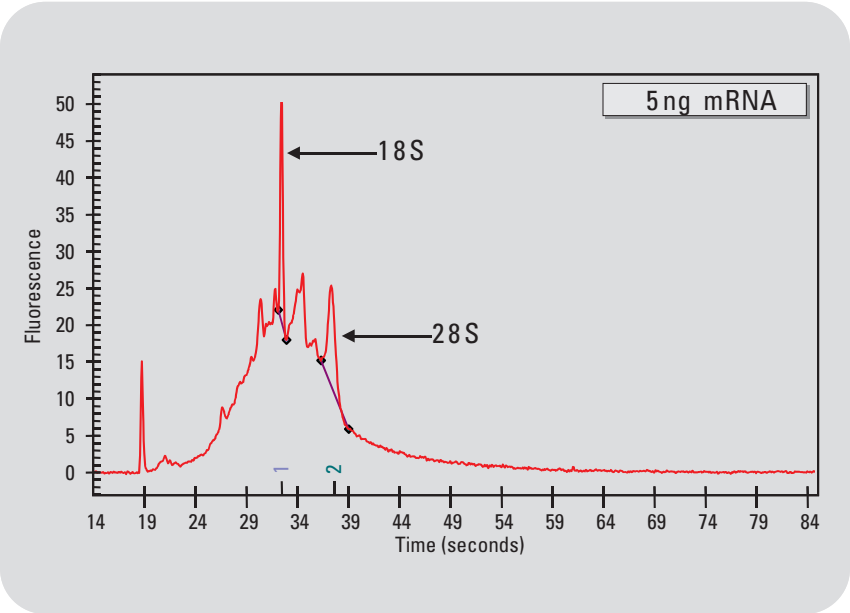

Figure 4
Quality control of mRNA. By the use of the RNA 6000 Pico LabChip kit, rRNA contamination was detected in the electropherogram of a 5 ng mRNA sample (indicated by arrows).

Analysis of mRNA with the RNA 6000 Pico kit

For some array experiments, it is advantageous to work with mRNA instead of total RNA. Since mRNA must be isolated from total RNA by additional purification steps, mRNA yield is typically low and the samples valuable. The use of the RNA 6000 Pico LabChip kit for mRNA quality control was evaluated by the analysis of 5 ng/µl mRNA on the Agilent 2100 bioanalyzer. The electropherogram shown in figure 4 depicts the high sensitivity of the assay. The broad size distribution is typical for an intact mRNA sample, whereas the rRNA peaks above the mRNA distribution pattern indicate a small amount of ribosomal RNA contamination (figure 4). Depending on the amount of ribosomal contamination an additional purification step can be performed. RNA degradation would result in a shift in the electropherogram towards shorter fragment sizes. Using the RNA 6000 Pico kit, mRNA samples can be investigated in a concentration range that is inaccessible by any other method.

Quality control of total RNA from microdissected cells

Microdissection is a powerful technique for the isolation of homogeneous tissues, specific cell types, and/or single cells derived from heterogeneous environments such as organs or tumors. The number of isolated cells is often in the range of several 100 to several 1000 cells depending on the isolation method. Due to the fact that resulting RNA concentration is typically extremely low and, therefore, strictly limited for downstream experiments, quality control of RNA derived from microdissected tissues is in most cases impossible. To test the use

of the RNA 6000 Pico kit with microdissected samples, RNA isolated following microdissection was analyzed. Cartridge isolated RNA was either treated with DNaseI or used untreated and 1 µl of the purified sample was used for analysis. Using the RNA 6000 Pico kit and the Agilent 2100 bioanalyzer, differences in the quality of the very low concentrated RNA (figure 5) can be displayed. Whereas the electropherogram of the untreated RNA results in a trace that shows a broad "hump" of genomic DNA in the electrophoretic trace (figure 5, A), RNA prepared with an additional DNaseI digestion shows a typical high quality electro-pherogram with only a small degree of degradation visible (figure 5, B). These results indicate that the RNA 6000 Pico kit can be used as a quality control tool for RNA extracted during micro-dissection experiments. It should be noted that in the context of this study, it was not intended to test the compatibility of the RNA 6000 Pico kit with all microdissection related cell staining and RNA isolation protocols. Subsequent studies will have to be performed to show any influence of sample matrix components on the performance of the RNA 6000 Pico assay.

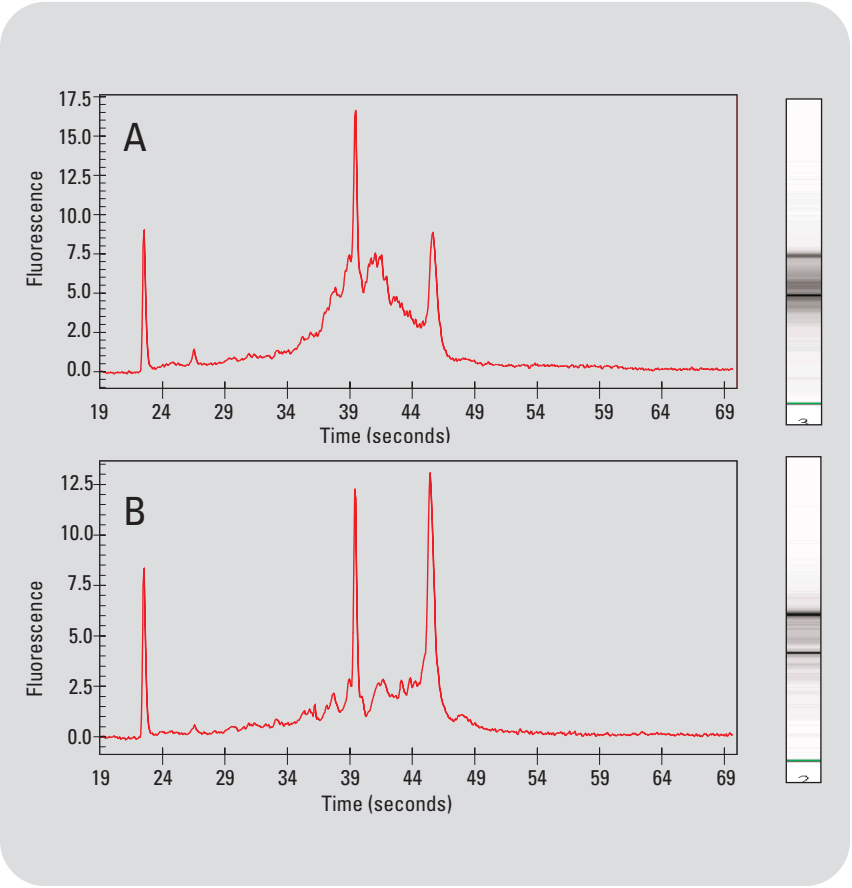

Figure 5
Identification of DNA contaminations in total RNA samples derived from micro-dissected tissues. (A) 1200 pg/µl of renal medulla RNA samples before DNAseI treatment and (B) 650 pg/µl of renal medulla RNA samples after DNase I treatment were analyzed. The DNA contamination related differences in RNA quality before and after DNAseI digest are clearly observable in the electro-pherogram.

Conclusion

The Agilent 2100 bioanalyzer in combination with LabChip kits is a powerful platform for the fast quantification and quality control of biomolecules such as RNA, DNA and proteins. In addition, the versatility of the equipment allows the flow-cytometric analysis of cell fluorescence parameters. The RNA 6000 Pico LabChip kit is an important extension of the existing LabChip kit series. The unparalled sensitivity of this RNA assay allows fast quality control of very low concentrated RNA samples. Here, we presented data indicating that quality control of total and mRNA could be performed in the range of 200 pg/µl to 5000 pg/µl and 500 pg/µl to 5000 pg/µl, respectively. The assays performance was tested on commercially available RNA standards as well as on RNA samples extracted from kidney tissue. The RNA 6000 Pico LabChip kit is the first commercially available tool that provides the economical analysis and quality control of mRNA preparations as well as total RNA samples derived from low cell numbers and/or micro-dissected tissues.

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