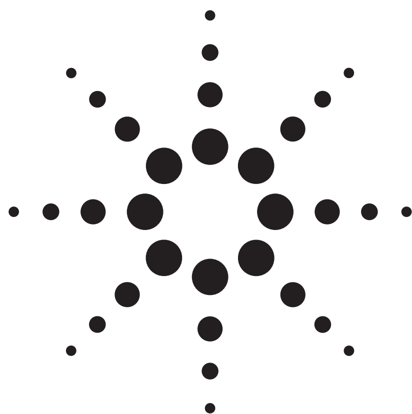
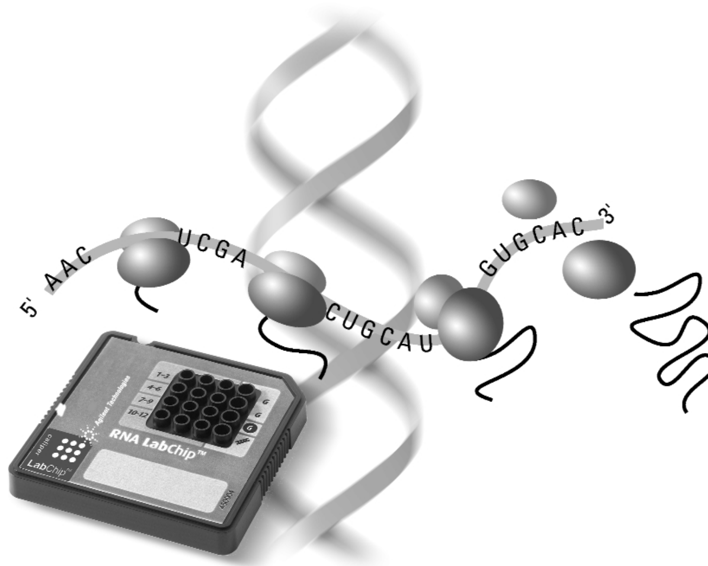


Analysis of messenger RNA using the Agilent 2100 Bioanalyzer and the RNA 6000 LabChip® kit

Application Note

Meike Kuschel



Abstract

The RNA 6000 LabChip® kit was used with the Agilent Technologies 2100 bioanalyzer to determine the integrity and measure the concentration of mRNA samples from various species and tissues. For each sample the data analysis software automatically reported the mRNA concentration in ng/µl. The resolution of the mRNA separations on the chip was better than that achieved using native agarose gels across a quantitation range of 25 to 250 ng/µl.

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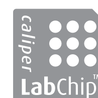


Introduction

Isolation of pure and intact mRNA is essential for many applications. Depending on the purification process, the quality and integrity of mRNA preparations can vary significantly. Information regarding possible ribosomal RNA contamination and the distribution of mRNA fragment lengths within a particular preparation can be very useful, especially for expression profiling applications.

Agarose gel electrophoresis followed by ethidium bromide staining can be used to check the purity and quality of an mRNA preparation. However, for accurate analysis, this traditional technology typically requires significant amounts of sample and an additional method for quantitation. RNA concentration is typically determined by measuring the absorbance at 260 nm in a spectrophotometer. The ratio between the absorbance at 260 and 280 nm gives a rough estimate of RNA purity with respect to contaminants that absorb at a different wavelength, such as proteins, but provides no information regarding possible ribosomal RNA contamination nor potential degradation by nucleases during the purification process.

With the increased interest in expression profiling applications, there has been a steadily growing demand for faster, more automated analysis tools that consume minimal sample amounts. Lab-on-a-Chip technology[1-5] is particularly suited for the rapid analysis of nucleic acids because it integrates multiple experimental steps. With the introduction of the Agilent 2100 bioanalyzer utilizing the LabChip technology from Caliper Technologies Corp., sample handling, separation, and analysis has been integrated in a more automated manner. Chip-based analysis offers several benefits over existing technology including reduced sample consumption, minimized manual intervention, increased analysis speed and data precision, and minimized exposure to hazardous materials.

The RNA 6000 LabChip kit can be used with the Agilent 2100 bioanalyzer to measure both the concentration and the integrity/ purity of mRNA samples. The integration of these two measurements provides a simple and quick assay that consumes minimal sample amounts.

Materials and methods

Agilent 2100 bioanalyzer instrument and software

All chip-based separations were performed on the Agilent 2100 bioanalyzer, which was controlled by dedicated software running on a PC. The Agilent 2100 biosizing software includes data collection, presentation and interpretation functions. Data can be displayed as a gel-like image and/or as electropherogram(s). The RNA quantitation data is automatically displayed on the individual electropherograms. An additional data evaluation tool is available for data comparison. The chip reader contains high voltage power supplies, each of which is connected to a platinum electrode, which allows the instrument to perform multiple and precisely controlled separations. For the RNA applications, the instrument uses fluorescence detection, monitoring the fluorescence between 670 nm and 700 nm.

Chip Preparation

All chips were prepared according to the chip preparation protocol provided with the RNA 6000 LabChip kit. The kit includes 25 chips, syringe, spin filters and the following reagents: sample buffer, gel matrix, and dye concentrate. In addition, the custom-made RNA 6000 ladder for use with the instrument/assay was purchased from Ambion, Inc. The gel-dye mix was prepared by mixing 400 µl of the gel matrix with 4 µl of the dye concentrate and filtering through a spin filter. The chip was filled with the gel-dye mixture and 5 µl of the sample buffer was then added to each sample well. 12 samples (1 µl each) in the concentration range from 25 to 250 ng/ µl were loaded into the sample wells of the chip. Finally, 1 µl of the RNA ladder was loaded into the assigned ladder well. The chip was then vortexed and run on the Agilent 2100 bioanalyzer.

Reagents

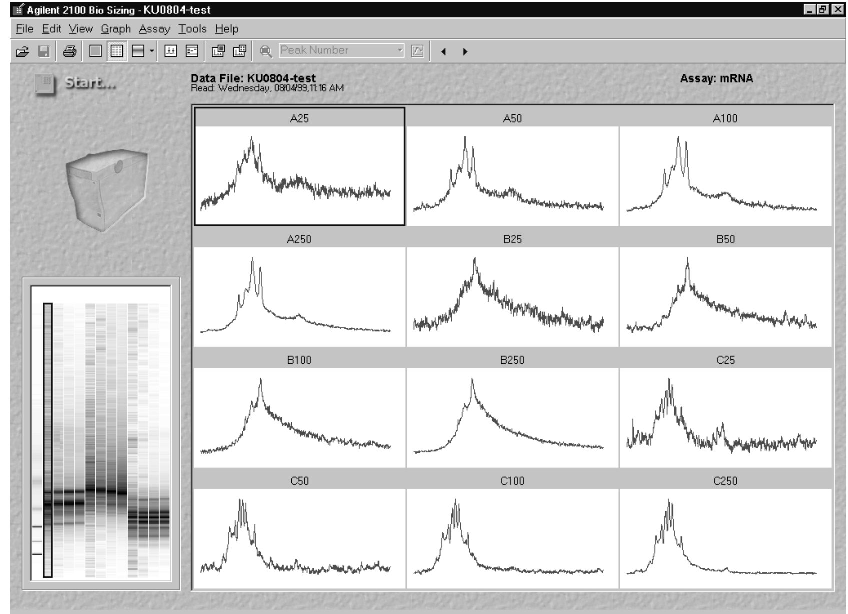
Poly (A)+ RNA (highly enriched mRNA) samples were purchased from Ambion Inc., USA and Clontech Laboratories, Inc., USA and diluted in DEPC-treated water (Research Genetics Inc., USA). RNA ladders were from Ambion Inc.,USA and Sigma Chemical Co., USA. The RNA 6000 LabChip kit was obtained from Agilent Technologies GmbH, Germany. The custom-made RNA 6000 ladder for use with the RNA 6000 LabChip kit was purchased from Ambion, Inc., USA.

Results and discussion

The RNA 6000 LabChip kit was used with the Agilent 2100 bioanalyzer to analyze various mRNA samples. Twelve samples can be sequentially separated on a chip through a single separation channel. The chip run is completed within approximately 25 minutes and the data for each sample can be accessed in real-time after each separation. Sample concentration is displayed on each electropherogram. Significant advantages over manual gel analysis are the data analysis and comparison functions, which are embedded in the software. No additional time consuming steps, such as scanning or densitometric analysis are required.

Quantitation of mRNA samples

The analysis of mRNA samples from chicken liver, bovine brain, and S. cerevisiae (yeast) are shown in figure 1. mRNA samples tested ranged in concentration from approximately 25 to 250 ng/µl. The expected effect of migration time variation due to the different sample origin was only minimal when mRNA samples from different species, tissues, and concentrations were separated on the same chip. The separation pattern was reproducible within a chip and between chips.



mRNA samples	Concentration (ng/µl)	Chip concentration (ng/µl)
chicken liver	250	264
bovine brain	50	51
S. cerevisiae	25	21

Figure 1
Agilent 2100 biosizing software. Data are displayed as electropherograms (samples 1-12) as well as a gel-like image. mRNA samples (25 to 250 ng/µl) from chicken liver (A), bovine brain (B) and S. cerevisiae (C) were separated through one single separation channel. The table shows the quantitation data for representative samples from one chip.

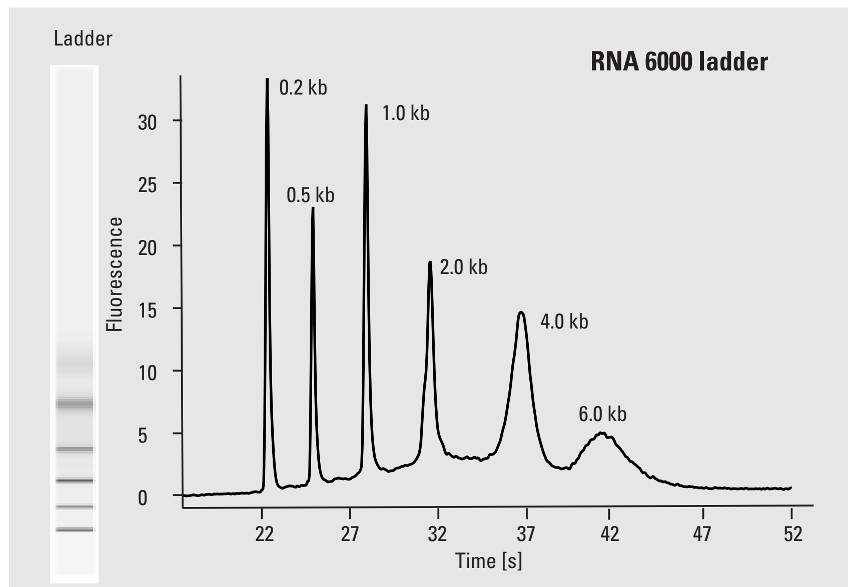


Figure 2
Electrophoretic separation of the RNA 6000 ladder using the RNA 6000 LabChip kit.

RNA 6000 ladder standard

The RNA 6000 ladder is run on every chip from a specified well and is used as a reference for data analysis. The RNA 6000 ladder contains six RNA fragments ranging in size from 0.2 to 6 kb at a total concentration of 150 ng/µl (figure 2). The software automatically compares the unknown samples (1-12) to the ladder to determine the concentration of the samples. The ladder also serves as a built-in quality control measure of system performance under standard conditions.

Ribosomal contamination in mRNA samples

Figure 3 shows analyses of 200 ng of five different mouse and rat mRNA preparations from different tissues. The electropherograms of the mouse embryo and kidney mRNA samples are shown alongside the gel-like image of all five samples. The electropherograms for the two samples highlight the characteristic peaks indicating the presence of contaminating ribosomal RNA. The mouse embryo mRNA sample has a significant amount of contaminating ribosomal RNA. In contrast, the electropherogram for the kidney mRNA indicates that the sample is enriched in mRNA and only contains a minor amount of contaminating ribosomal RNA.

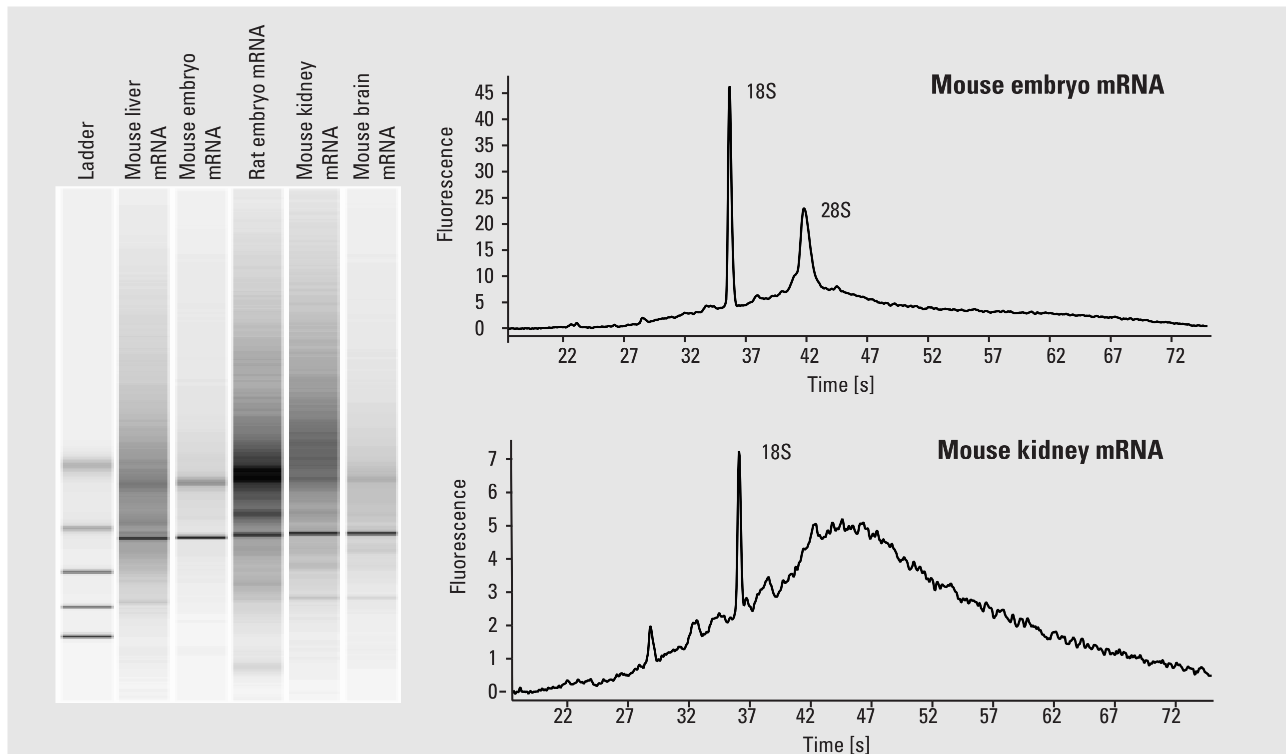


Figure 3
Five different mouse and rat mRNA preparations were analyzed with the Agilent 2100 bioanalyzer. The electropherograms of the mouse embryo and kidney mRNA samples are shown alongside the gel-like image of all five samples in addition to the RNA 6000 ladder.

Chip versus gel analysis of mRNA samples

mRNA preparations from different species and tissues (Drosophila, rabbit lung and brain, and mouse kidney and brain) were analyzed using the RNA 6000 LabChip kit (figure 4-A) and compared to the analysis of the same samples on a 1.2% agarose gel stained with ethidium bromide (figure 4-B). The mRNA separation obtained under native conditions employing the Agilent 2100 bioanalyzer gave comparable results with better resolution compared to the native agarose gel. An additional advantage of the chip-based mRNA analysis is the low sample volume required. 15 µl (1.25 µg) mRNA per lane was loaded on the agarose gel in comparison to 1 µl (250 ng) of RNA sample, which was separated on the chip. Because of the low sensitivity of ethidium bromide, a relatively high concentration of purified RNA has to be loaded onto agarose gels. In contrast, the combination of the intercalating dye used in the assay and the fluorescent detection used by the Agilent 2100 bioanalyzer enables detection of small concentration differences between samples that may not be detected using traditional gel electrophoresis. Additionally, the system provides a more precise quantitation of the mRNA compared to the rough estimate obtained from gel electrophoresis.

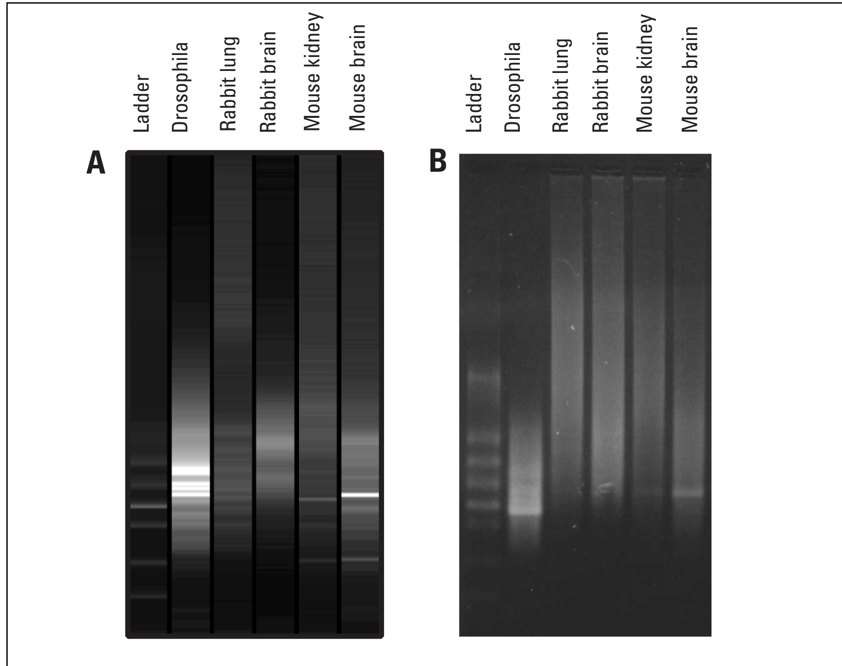


Figure 4
Different mRNA preparations from Drosophila, rabbit lung and brain, and mouse kidney and brain were analyzed using the Agilent 2100 bioanalyzer (A) and a conventional native 1.2 % agarose gel electrophoresis followed by ethidium bromide staining (B).

Conclusion

The LabChip-based mRNA analysis integrates the quantitation of RNA samples with a quality assessment in one quick and simple assay. The use of Lab-on-a-Chip technology allows the rapid analysis of multiple samples through a single separation channel with excellent reproducibility of the system. Data precision is comparable or superior to agarose gel electrophoresis, whereas analysis time is greatly reduced. Automation of separation and data analysis makes the system versatile and results in less consumption of sample compared to conventional methods. In addition to the mRNA analysis with the RNA 6000 LabChip kit, the instrument platform can also be used for other nucleic acid analyses. Initial kits are available for sizing of restriction fragment digests (DNA 12000 LabChip kit) and for sizing and quantitation of PCR fragments (DNA 7500 LabChip kit).

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For more information, visit our website at http://www.agilent.com/chem/labonachip

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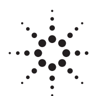
Printed 04/2000
Publication Number 5968-7495E



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