Assessing genomic DNA contamination of total RNA isolated from kidney cells obtained by Laser Capture Microdissection using the Agilent RNA 6000 Pico assay

Application

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Abstract

Total RNA isolated by Laser Capture Microdissection (LCM) from embryonic kidney cells was analyzed for integrity and relative concentration using the Agilent 2100 bioanalyzer and the RNA 6000 Pico assay. An unexpected peak between the 18S and 28S ribosomal RNA peaks was observed and unambiguously proven, by DNase digestion and genomic DNA spike-in experiments, to be caused by genomic DNA contamination. A simple on-column DNase digestion method is suggested to eliminate contaminating genomic DNA from total RNA samples obtained by LCM, fluorescent-activated cell sorting (FACS), or manual dissection.

Introduction

In this era of functional genomics it is feasible to determine gene expression profiles of sub-populations of cells within complex tissue and organ structures. Numerous methods have been developed to isolate such cells, including manual dissection, fluorescent-activated cell sorting (FACS) and laser-capture microdissection (LCM)[1, 2]. LCM involves the minimum amount of manipulation of the sample, thus maximizing the chances of obtaining high quality RNA vital to achieving reliable gene expression profiles using microarray technology.

Cell samples collected by LCM are usually small and yield low amounts of total RNA. Quantification and quality determination can be difficult, time-consuming, and sample-consuming using standard procedures such as linear amplification of the RNA coupled with UV spectrophotometry and/or gel electrophoresis. This report describes the application of the Agilent 2100 bioanalyzer in conjunction with the RNA 6000 Pico assay to assess total RNA quality from LCM isolated cells from mouse kidney cryosections. Genomic DNA (gDNA) contamination of total RNA samples was unambiguously demonstrated

through a series of DNase digestion and gDNA spike-in experiments that were analyzed using the bioanalyzer and RNA 6000 Pico assay.

Materials and methods

Cells from mouse embryonic kidney sections were isolated using the PixCell II laser capture microdissection microscope (Arcturus Engineering, Mountain View, CA). Total RNA was extracted using the RNeasy Mini kit from QIAGEN (cat # 74104, Hilden, Germany). The Agilent 2100 bioanalyzer was subsequently employed, in conjunction with the RNA 6000 Pico assay, to determine the quantity and quality of 1 µL (0.2–5 ng) of total RNA. Genomic DNA (gDNA) used to spike the RNA samples was obtained from mouse tail biopsies using overnight proteinase K digestion at 55 °C followed by a high salt extraction method as previously described[3]. 1 µL of a 1/10 dilution (approximately 60 ng) of the gDNA was added to a 10 µL RNA sample.

Results and discussion

RNA quality control is a critical step in the workflow for assessing gene expression profiles using DNA microarrays. The RNA QC process took approximately 30 minutes with the Agilent 2100 bioanalyzer versus a much longer time that is required to perform linear amplification, UV-spectrophotometry and gel electrophoresis – traditional methods for assessing the quality of small RNA samples.

Electropherograms obtained from the Agilent 2100 bioanalyzer revealed that total RNA extracted from LCM samples contained the expected 18S and 28S ribosomal peaks, evidence of intact RNA. However, an unexpected peak was observed between the 18S and 28S peaks (figure 1). The contaminating peak was thought to be gDNA and a series of simple experiments were devised to validate this hypothesis. Fresh total RNA was isolated from mouse embryonic kidneys using the RNeasy kit and aliquots were spiked with gDNA. The gDNA was then removed from selected spiked samples by DNase digestion followed by DNase removal using the RNeasy kit, to yield total RNA free from gDNA. Three sample types were analyzed using the RNA 6000 Pico LabChip® kit on the Agilent 2100 bioanalyzer (figure 2):

A. Total RNA before gDNA spike-in
B. Total RNA after gDNA spike-in
C. Total RNA after gDNA spike-in, DNAse digestion, and clean-up.

Prior to the addition of gDNA, the total RNA was devoid of peaks between the 18S and 28S rRNAs. Following the addition of gDNA, a large peak was evident between the rRNAs, in the same position as the unexpected signal observed in LCM total RNA (figure 1). The gDNA was removed from aliquots of the spiked samples by DNase digestion followed by column-based clean-up (QIAGEN RNeasy kit). The column-based clean-up reduced the yield of total RNA from 2.19 ng/µl in figure 2A to 0.36 ng/µL in figure 2C.

In order to recover more of the total RNA and to further validate that the contaminating signal in the LCM RNA sample was indeed caused by gDNA, captured cells were lysed and divided into two equal aliquots. One aliquot was processed using the RNeasy Mini RNA extraction kit with the inclusion of the on-column DNase digestion (using RNase-free

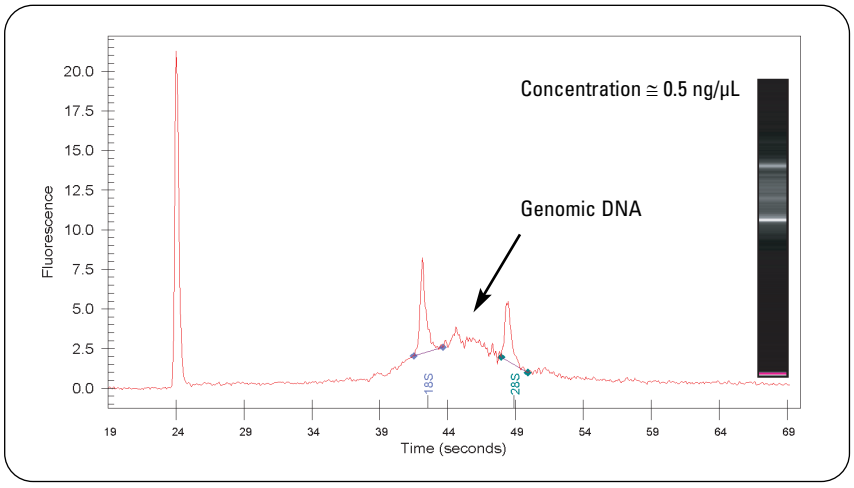

Figure 1
Electropherogram and gel-like image of total RNA isolated from cells captured by LCM of embryonic kidney sections and analyzed on the Agilent 2100 bioanalyzer (RNA 6000 Pico assay). An unexpected signal, potentially gDNA, is evident between the rRNA peaks. RNA was isolated using the QIAGEN RNeasy Mini kit.

DNase cat # 79254), while the second aliquot received no DNase but was otherwise put through the same procedure. This yielded two sample types (figure 3):

A. Column-based total RNA isolation (from LCM) with on-column DNase digestion.
B. Column-based total RNA isolation (from LCM) without on-column DNase digestion.

On-column DNase digestion removed the contaminating peak between the 18S and 28S rRNA peaks, indicating that gDNA was the causative agent. The on-column DNase digestion method also offered an improvement in the yield and integrity of total RNA compared to alternative methods that require heat inactivation of the DNase (data not shown). The presence of gDNA in RNA isolates can result in erroneous concentration determination of total RNA samples. As a final observation, using the bioanalyzer we detected genomic DNA contamination in total RNA extracted from FACS and manually dissected kidney cells (small samples), but not from RNA extracted from whole kidney (large samples).

Conclusions

Limited total RNA obtained from LCM mouse kidney cells can be rapidly assessed for integrity and relative concentration using the Agilent 2100 bioanalyzer and RNA 6000 Pico LabChip kit. This enables the determination of RNA quality and quantity prior to performing RNA linear amplification for downstream microarray

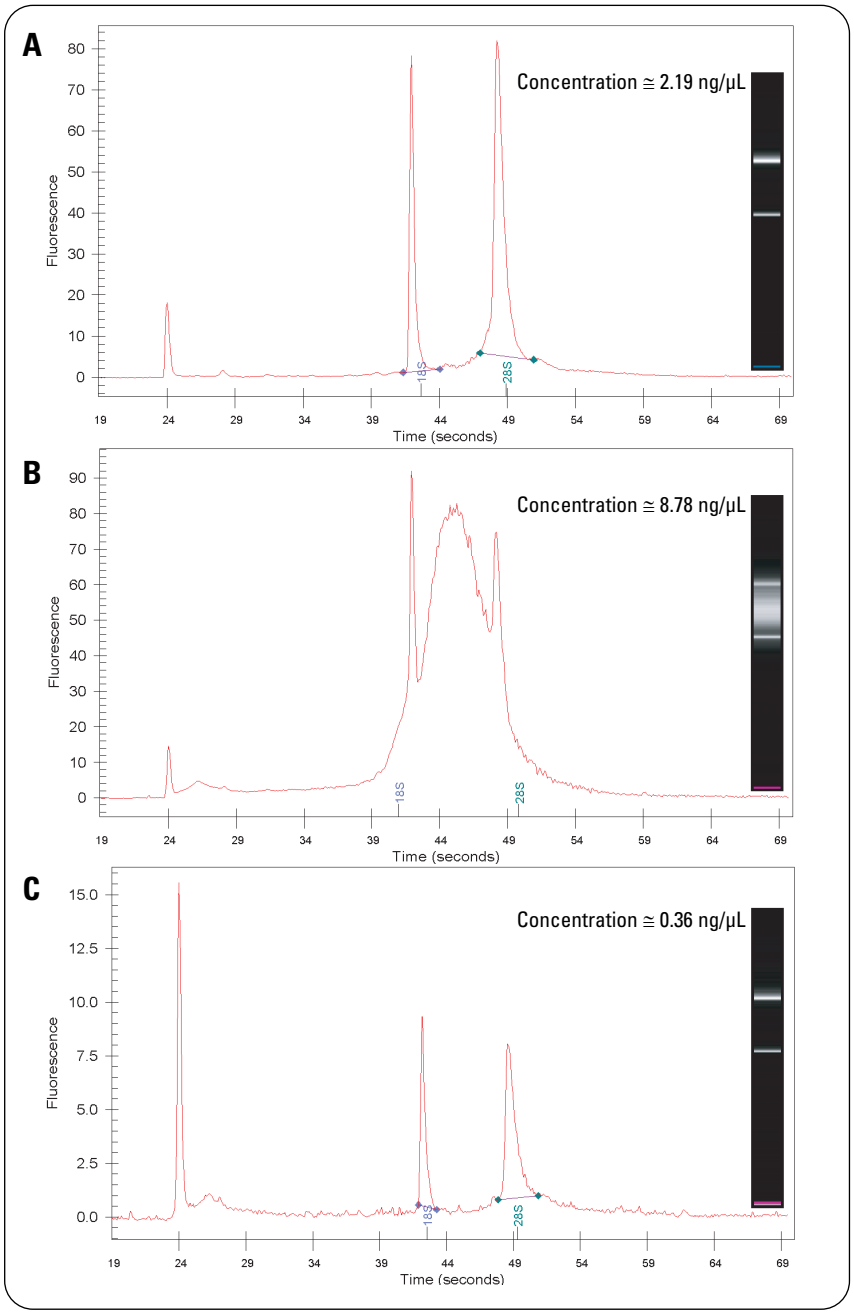

Figure 2
Analysis of gDNA spiking experiment. Total RNA from embryonic mouse kidney was analyzed on the Agilent 2100 bioanalyzer using the RNA 6000 Pico assay. A. 18S and 28S rRNA peaks are present and no degradation is evident in the total RNA. B. Following the spike-in of gDNA (1/10 dilution) an extra peak is evident between the 18S and 28S rRNA peaks. C. Contaminating gDNA is absent and yield is reduced dramatically after the total RNA spiked with gDNA is subjected to DNase digestion and the DNase removed using the RNeasy protocol for RNA cleanup.

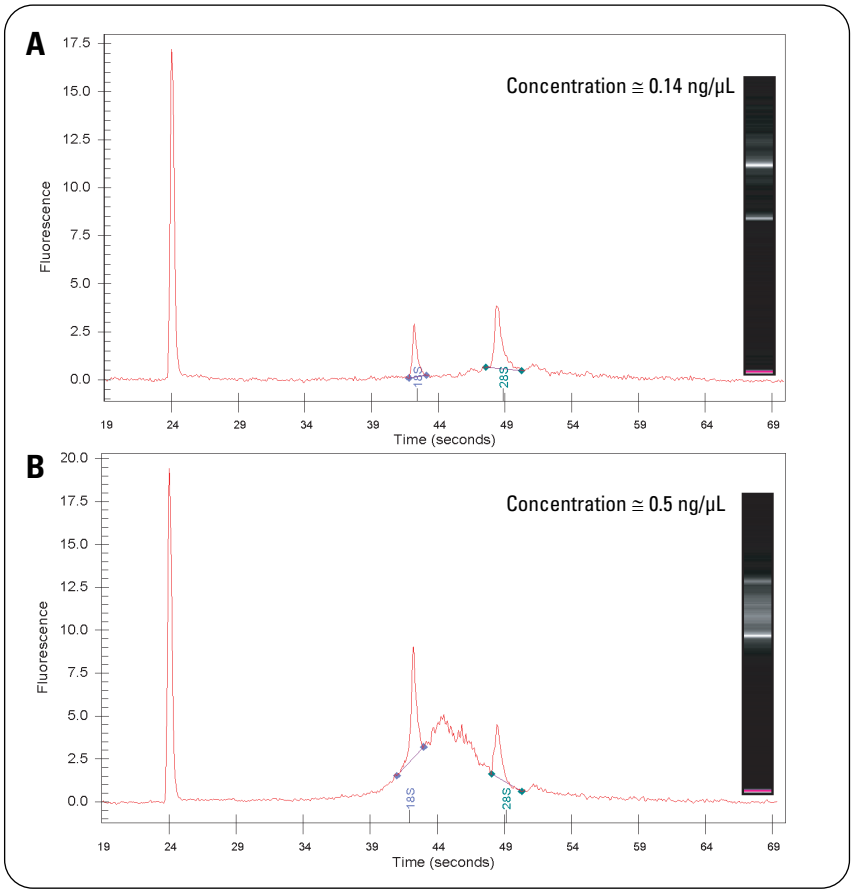

Figure 3
Total RNA isolated from cells captured by LCM from embryonic kidney sections was analyzed on the Agilent 2100 bioanalyzer using the RNA 6000 Pico assay. A. Total RNA isolated using the QIAGEN RNeasy Mini kit with the inclusion of an on-column DNase digestion step with RNase-free DNase. B. Total RNA isolated using the same procedure without the on-column DNase digest step.

experiments. Genomic DNA contamination of total RNA samples was demonstrated by a series of gDNA spike-in and DNase digestion experiments. We conclude that an on-column DNase digestion during RNA purification of small samples with the Qiagen RNeasy Mini RNA extraction kit can eliminate contaminating gDNA when the source tissue is mouse kidney cells obtained by FACS, LCM and manual dissection.

References

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A complete list of all Application Notes for the Agilent 2100 bioanalyzer and more information about the system can be found at:
www.agilent.com/chem/labonachip

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