The Agilent RNA Isolation Kit in Combination with New Agilent DNase Accessory Kit Dramatically Reduces Genomic DNA Contamination of RNA Preparations

Application

Gene Expression

Author

Ilgar Abbaszade, PhD
Agilent Technologies, Inc.
2850 Centerville Road
Wilmington, DE 19808-1610
USA

Abstract

Real time PCR was used to quantify genomic DNA contamination in total RNA samples isolated with the Agilent Total RNA Isolation Kit, both alone and in combination with the new Agilent DNase Accessory Kit. Similarly, genomic DNA in total RNA samples isolated with a leading silica column-based kit, both alone and in combination with the DNase accessory kit from the same supplier, was also quantified. RNA was isolated from mouse thymus, spleen, and pancreas tissues for this study. Comparison of the data obtained from the two techniques clearly demonstrated the advantages of the Agilent method for the reduction of genomic DNA contamination in total RNA samples from these tissues.

Introduction

RNA quality is the single most important determinant of the success of gene expression analysis methods such as microarray hybridization, real time PCR, cDNA synthesis, Northern Blotting, and RNase Protection assay. However, RNA integrity alone, as a measure of RNA quality, cannot insure accurate data if RNA samples are contaminated with significant amounts of genomic DNA (gDNA). For example, due to intrinsic DNA polymerase activity reverse transcriptase could label gDNA during the cDNA labeling reaction used for microarray hybridization. In this case, the label in the form of a nucleotide triphosphate is incorporated into partially degraded gDNA. As a result, false positive signals will be produced due to hybridization of the labeled gDNA to the array. In this application, as well as in many other applications such as real time PCR analysis, the gDNA could contribute to the positive expression signal and lead to data that does not reflect actual expression levels in the RNA sample. Thus, in order to increase fidelity and reproducibility of gene expression data, scientists prefer to work with RNA samples with the lowest possible gDNA contamination.

The Agilent Total RNA Isolation Kit is a rapid and convenient method for the isolation of high purity intact cellular RNA with very low levels of gDNA contamination. This method results in up to 1000-fold lower levels of gDNA contamination compared to a silica column-based method from a leading supplier [1]. A new Agilent DNase Accessory Kit was developed for use with the Agilent Total RNA Isolation Kit and allows additional reduction of the gDNA level in total RNA samples. This additional elimination of gDNA is important for applications that are extremely sensitive to the gDNA contamination.

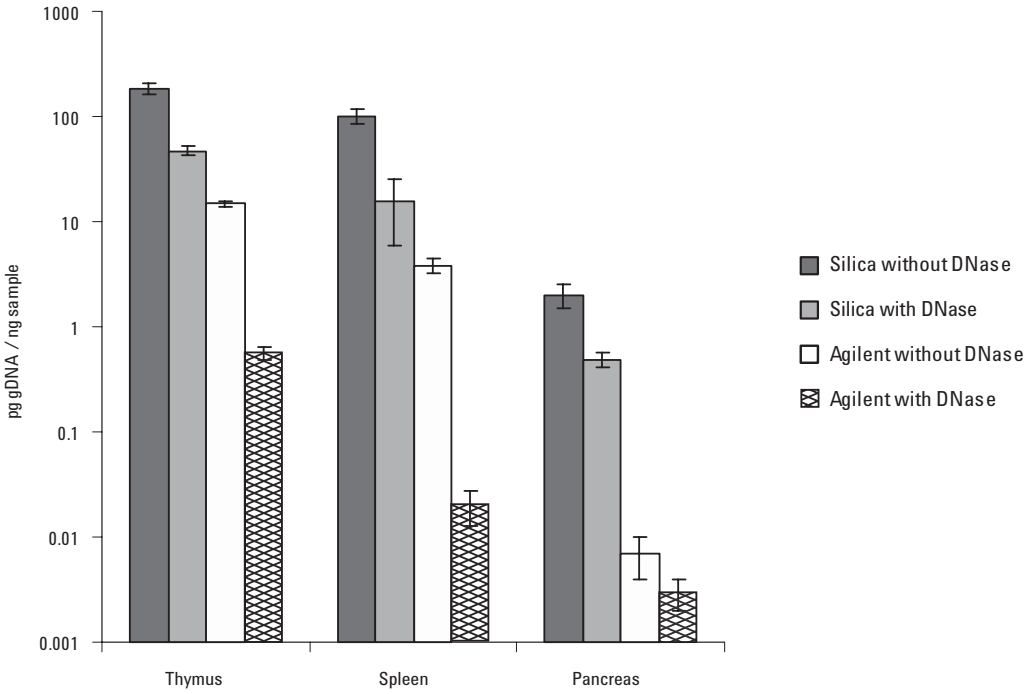
This application note demonstrates that the Agilent Total RNA Isolation Kit in combination with the Agilent DNase Accessory Kit provides scientists with a robust and convenient method for the isolation of total RNA with minimum levels of gDNA contamination.

Materials and Methods

Total RNA was isolated from mouse thymus, spleen, and pancreas tissues using either the Agilent Total RNA Isolation Mini Kit (product number 5185-6000), or a commercially available silica-based kit from a leading supplier. RNA isolation was accomplished according to the manufacturers instructions. On-column DNase treatment with the silica column-based method was performed using a protocol and reagents from the supplier. The Agilent DNase Accessory Kit (product number 5188-2735) was used with the Agilent kit. For thymus and spleen, 15 mg of frozen tissue per column and for pancreas 10 mg frozen tissue per column were used for total RNA isolation. The gDNA contamination was measured using a quantitative real-time PCR assay. This assay was performed on an ABI PRISM® 7000 Sequence Detection System using primers and probes specific to the mouse GAPDH gene. These primers were designed to amplify a 79 bp fragment within an exon, thus enabling the detection of gDNA. Total RNA samples were added directly into the PCR reactions. The sequences were (from 5' to 3'): forward primer - GCAAATTCAACGGCACAGT; reverse primer - CGCTCCTGGAAGATGGTGA; dual labeled (FAM and BHQ) probe - TGATGACAAGCTTCCCATTCTCGGC. No reverse transcription step was performed to insure that detected signals are coming from the gDNA contamination only. For all tissue types the data was obtained from at least three different tissue samples and each measurement was done in triplicate. All values are expressed as mean values ± SD, n = 3.

Results

Since DNA and RNA absorb light equally well at 260 nm, the level of gDNA contamination in RNA samples cannot be estimated by spectrophotometric analysis. The most accurate quantitative method for determining the level of gDNA contamination is quantitative real-time PCR analysis. The TaqMan® assay was used to quantitate the presence of gDNA contamination in our RNA samples. Total RNA was isolated from mouse thymus, spleen, and pancreas tissues using both the Agilent Total RNA Isolation Mini Kit and a commercially available silica-based kit from a leading supplier. RNA was also isolated from the same tissues using these two methods in combination with their respective on-column DNase protocols. Primers specific to the mouse GAPDH gene were designed to amplify signal from gDNA. Levels of gDNA contamination were measured using a real-time PCR assay on an ABI PRISM 7000 Sequence Detection thermocycler. The data for RNA samples isolated using both methods with DNase digestion and without DNase digestion (as indicated) is shown in the chart and the table in Figure 1.



	Silica without DNase	Silica with DNase	Agilent without DNase	Agilent with DNase
Thymus	184.17 ± 19.28	47.31 ± 4.92	14.73 ± 1.10	0.56 ± 0.084
Spleen	100.15 ± 15.46	15.40 ± 9.60	3.82 ± 0.64	0.02 ± 0.007
Pancreas	2.01 ± 0.49	0.49 ± 0.07	0.007 ± 0.003	0.003 ± 0.001

Figure 1. Genomic DNA content of total RNA samples isolated from different mouse tissues (shown in pg of gDNA per ng of total RNA).

As is evident from the presented data, the levels of gDNA contamination in thymus and spleen total RNA samples, isolated using the silica column-based RNA isolation kit are significant (10%–20%). On-column DNase digestion reduces gDNA contamination levels in samples isolated with silica-based columns to match, at best, the contamination levels of RNA isolated with the Agilent Total RNA Isolation Mini Kit without DNase-digestion. For pancreas RNA, the level of gDNA contamination in RNA samples isolated using the Agilent Total RNA Isolation Mini Kit was nearly two orders of magnitude lower than in RNA samples isolated using the silica-based kit with associated on-column DNase digestion. The lowest level of gDNA contamination in all tissues was achieved when total RNA was isolated using the Agilent kit in combination with the Agilent on-column DNase digestion protocol.

Conclusion

- The Agilent Total RNA Isolation Mini Kit combined with Agilent DNase Accessory Kit provides a robust, rapid, and convenient method for the purification of total RNA for those applications requiring the lowest possible level of gDNA contamination.
- As the presented data demonstrates, gDNA contamination levels in the total RNA samples isolated with this method were 0.06% for thymus, 0.002% for spleen, and as low as 0.0003% for pancreas.

Reference

1. “Efficient Method for Isolation of High Quality Concentrated Cellular RNA with Extremely Low Levels of Genomic DNA Contamination” Agilent Technologies publication 5989-0322EN www.agilent.com/chem

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