Isolation of High-Purity Total Cellular RNA from Muscle Tissues Using the Agilent Total RNA Isolation Mini Kit

Application

Genomics

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Abstract

A method was recently developed a for the isolation of high-purity RNA from cardiac and skeletal muscle tissue using the Agilent Total RNA Isolation Mini Kit. High-purity RNA is characterized by the absence of cellular contaminants such as proteins, carbohydrates, and genomic DNA. Removal of these cellular components by the Agilent novel prefiltration column results in a purer RNA product that is suitable for sensitive downstream applications including real time PCR. Efficient isolation of RNA from muscle tissue is dependent upon complete homogenization of the sample followed by Proteinase K digestion to remove the high levels of protein and connective tissue present. The muscle tissue RNA isolated with the Agilent method contained significantly less genomic DNA compared to RNA isolated with a competitor's silica-based kit. Furthermore, analysis of the RNA using the Agilent 2100 Bioanalyzer resulted in a higher RNA Integrity Number (RIN), between 8.5 and 9, when using the Agilent kit.

Introduction

The Agilent Total RNA Isolation Mini Kit (p/n 5185-6000) is a phenol-free, spin-column method for isolation of total cellular RNA (Figure 1). The method employs a unique prefiltration column that removes cellular contaminants including genomic DNA (gDNA). RNA prepared with this kit contains significantly lower levels of gDNA contamination without DNase treatment.

The standard protocol used for most mammalian cell lines and tissues was modified to obtain improved results when isolating RNA from muscle and other fibrous tissue. A Proteinase K digestion step was included to remove the abundant contractile proteins and connective tissues present in muscle fibers prior to capturing the RNA on the mini kit isolation column. After washing, RNA is eluted from the column with quantitative recovery in as little as 10 µL of nuclease-free water.

Analysis with the Agilent 2100 Bioanalyzer demonstrates that the RNA isolated using this method is very pure. Quantitative PCR assays indicate that the RNA contains significantly less gDNA contamination than RNA prepared with a commercially available silica-based kit.

Materials and Methods

Rat heart and skeletal muscle samples were obtained from Pel-Freez (Rogers, AR). Proteinase K was obtained from Sigma (St. Louis, MO). RNA*later* was obtained from Ambion (Houston, TX). All RNA isolations were from ≅ 10 mg of tissue. RNA was isolated with the Agilent Total RNA Isolation Mini Kit following the detailed protocol described in Appendix A, or by a commercially available silica-based method. RNA was quantified by measuring A_{260nm} on the ND-1000 spectrophotometer (Nanodrop Technologies, Rockland, DE) and quality assessed with the RNA 6000 Nano Assay using Agilent Technologies 2100 Bioanalyzer. Genomic DNA levels were measured using a real time quantitative PCR assay with serially diluted rat gDNA used to generate the standard curve and a primer/probe set specific for rat GAPDH. Primer and probe sequences were selected using ABI Primer Express software (Applied Biosystems, Foster City, CA), and synthesized by Biosearch Technologies (Novato, CA). Quantity of gDNA was measured using the ABI Prism 7000 Sequence Detector and sequence detection software.

Results

The quality of the RNA isolated from heart and skeletal muscle was assessed on the Agilent 2100 Bioanalyzer using the Eukaryotic RNA Assay with the RNA 6000 Nano LabChip® Kit. The results are shown in Figure 2 (heart) and Figure 3 (skeletal muscle).

As seen in Figure 2, heart RNA isolated with the Agilent kit is intact as indicated by the sharp 18s and 28s ribosomal RNA peaks with the appropriate migration pattern. The Bioanalyzer profile also shows the Agilent isolated RNA to have a relatively flat baseline, especially between the ribosomal peaks. Furthermore, both the gel-like image and the electropherogram display some smaller RNA species not captured by the silica method. The RNA isolated with the silica-based method, while containing intact RNA as evidenced by the appropriate ribosomal bands, contains high levels of gDNA not found in the samples isolated with the Agilent method.

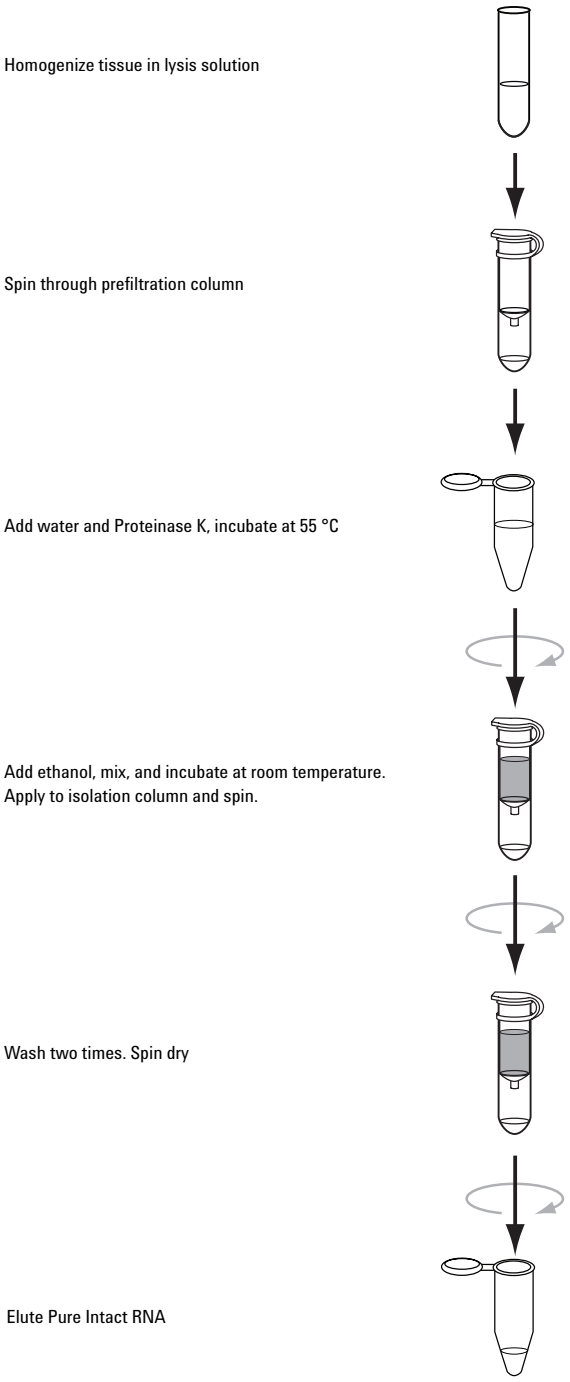

Figure 1. Agilent Total RNA Isolation Method for muscle tissue.

Agilent 2100 Bioanalyzer comparison of heart muscle RNA isolated using the Agilent Total RNA Isolation Mini Kit and a competitor's kit

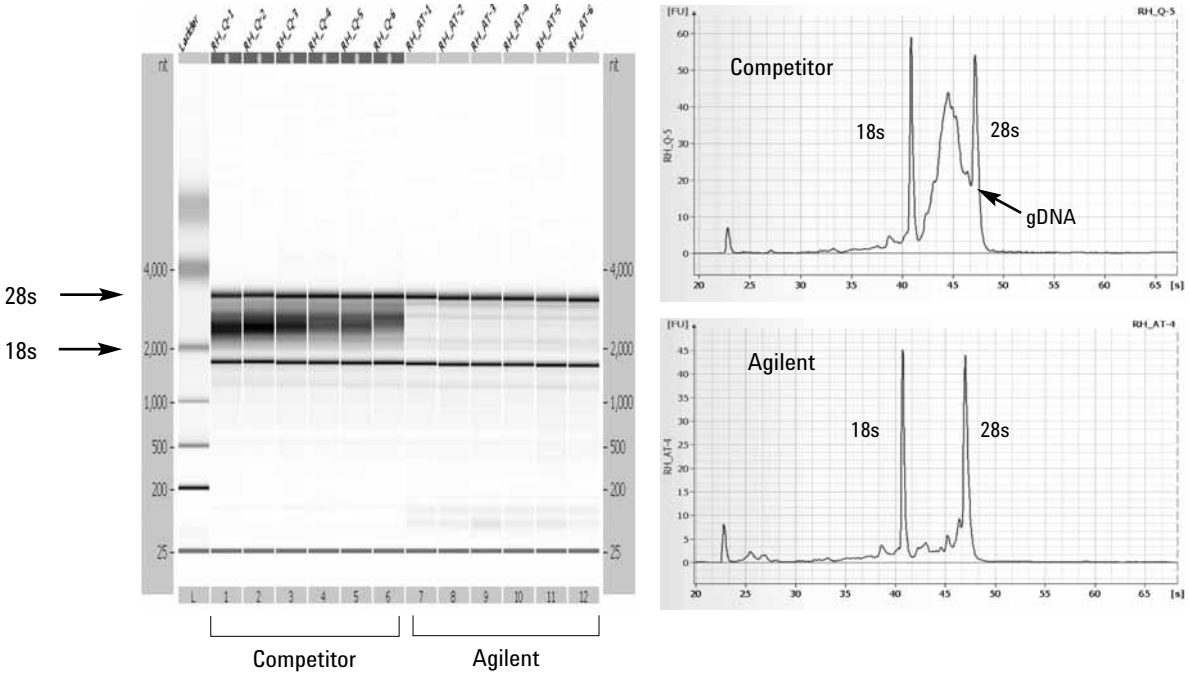

Figure 2. Gel-like images and electropherograms of total RNA isolated from rat heart tissue using a silica-based kit or the Agilent method.

As seen in Figure 3, rat skeletal muscle RNA isolated using either the Agilent kit or a competitor's silica-based kit is intact as demonstrated by the appropriate 18s and 28s ribosomal RNA peaks. The Agilent RNA appears cleaner in the gel-like image and in the intrapeak region of the electropherogram. As with the RNA isolated from rat heart, the Agilent method appears to capture some of the smaller skeletal muscle RNA species that the silica-based kit does not.

Agilent 2100 Bioanalyzer comparison of skeletal muscle RNA isolated using the Agilent Total RNA Isolation Mini Kit and a competitor's kit

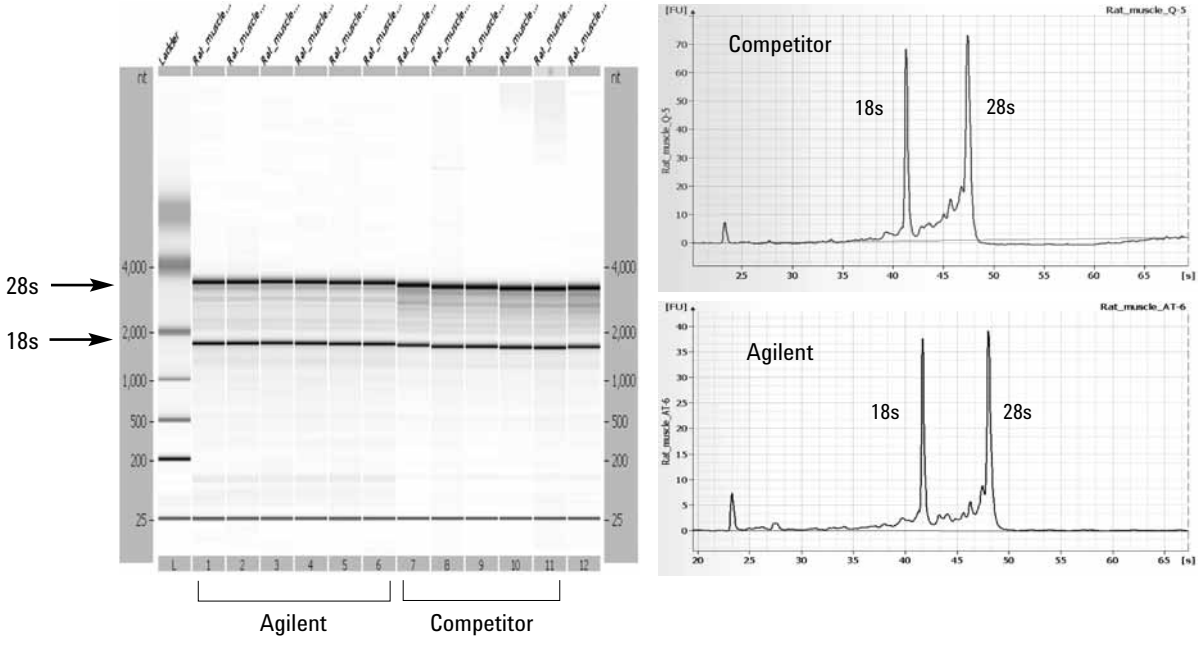

Figure 3. Gel-like images and electropherograms of total RNA isolated from rat skeletal muscle using the Agilent method or a silica-based kit.

RNA was quantified by measuring A_{260}. All A_{260}/A_{280} ratios were 1.9 or greater. Genomic DNA contamination was measured by quantitative PCR using a TaqMan real time PCR assay. Rat gDNA was used to generate the standard curve and rat GAPDH specific primers and FAM probe were used for amplification and detection. Typical RNA yields and gDNA content for heart muscle are shown in Figure 4. Yields were approximately the same for the two methods. However, RNA isolated with the silica-based method contained up to 250-fold more gDNA.

Conclusions

The Agilent Total RNA Isolation Mini Kit provides a rapid and efficient, phenol-free, spin-column based method for the preparation of concentrated, high-purity, intact total cellular RNA.

- RNA yield was essentially equivalent to a silica-based method.
- Levels of gDNA contamination were extremely low compared to the silica-based method.
- RNA analyzed on the Agilent 2100 Bioanalyzer appeared to be of higher quality when using the Agilent method.

Ordering Information

- Agilent Total RNA Isolation Mini Kit (50), part number 5185-6000
- Agilent Mini Prefiltration Columns and Collection Tubes (50), part number 5188-2736

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem

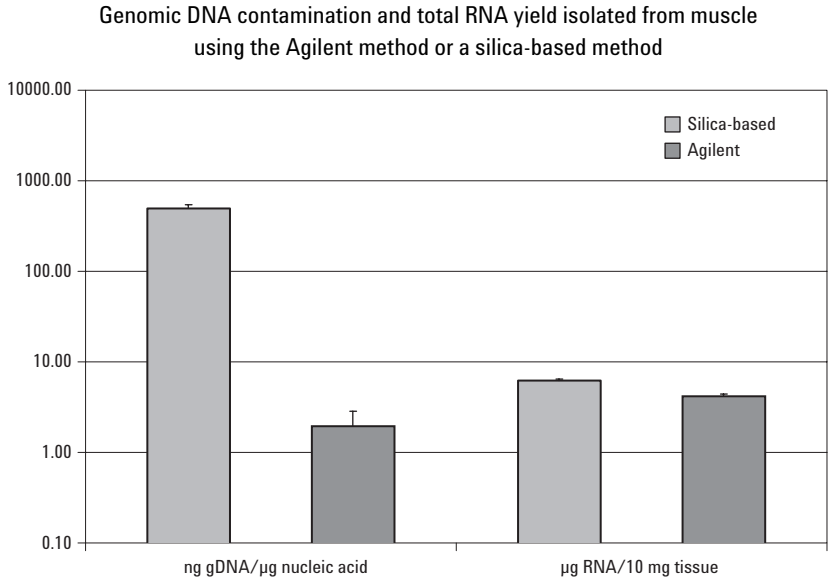

Figure 4. Genomic DNA contamination and RNA yield from muscle tissue isolated with the Agilent Total RNA Isolation Mini Kit or a silica-based kit. Values represent the average of six replicates.

A

RNA Isolation from Cardiac and Skeletal Muscle Tissue

This protocol is for use with the Agilent Total RNA Isolation Mini Kit (p/n 5188-6000).

Additional Materials Required

You will need the following materials not included with the kit:

- Microcentrifuge
- 100% Ethanol
- 14.4M β-Mercaptoethanol
- Proteinase K (for example, Sigma P-5568)

For additional information please refer to the Agilent Total RNA Isolation Mini Kit Manual (p/n 5188-2710)

Protocol

Before you begin, set a water bath or heat block to 55 °C

1 Collect the tissue sample, then process it immediately or flash freeze it in liquid nitrogen. Store flash-frozen tissue at –70 °C. Alternatively, tissues may be preserved in RNA*later* (Cat number 7020, Ambion, Austin, TX) according to manufacturer's directions.

2 Weigh the sample (samples stored in RNA*later* may be thawed), then place tissue in a tube containing prepared Lysis Solution (to which ß-ME was added). Use 20 µL of Lysis Solution per milligram of tissue to be homogenized. Tube must be large enough to accommodate probe of homogenizer. The homogenizer probe should reach the bottom of tube for best homogenization.

Samples smaller than 5 mg must be homogenized in a minimum of 100-µL Lysis solution.

3 Immediately and vigorously homogenize using a conventional rotor-stator homogenizer with a stainless steel probe for **1 minute** at 15,000 rpm. This is 50% of speed for an Omni International TH homogenizer (OMNI International, Warrenton, VA). To reduce foaming, move the probe from side to side rather than up and down. Larger volumes (more than 10 mL) may require slightly longer homogenization times. If you will not process the homogenate immediately, **store** it at –70 °C. To process frozen homogenate, **thaw** it at 37 °C for **15–20 minutes** and vortex vigorously.

4 Centrifuge up to 200 µL of homogenate to a mini-prefiltration column (natural) for **3 minutes** at full speed (for a typical microcentrifuge, approx 16,000 × g). This step ensures complete homogenization of the tissue and removes cellular contaminants. Mini-prefiltration columns **cannot** be reused. If processing more than 200 µL, use a new prefiltration column.

5 Discard column and **add** 390 µL of nuclease-free water to the filtrate. **Mix** well and add 10 µL of 20 mg/mL Proteinase K. **Mix** and **incubate 15 minutes** at 55 °C.

6 Add 600 µL of 100% ethanol, **mix** well and **incubate 5 minutes** at room temperature.

7 Add the ethanol/lysis mixture (up to 600 µL) onto the mini-isolation column (blue) and **centrifuge** for **30 seconds** at full speed. **Discard** flow-through and **replace** the RNA-loaded column in the 2-mL collection tube. **Add** the remaining ethanol/lysis mixture onto the mini-isolation column, then **centrifuge**, and **discard** flow-through as described above. Centrifugation times may be increased to 1 minute to ensure complete flow-through of viscous samples.

8 Add 500 µL of prepared Wash Solution (to which ethanol was added) to the mini-isolation column, then **centrifuge** for **30 seconds** at full speed. **Discard** the flow-through, then **replace** the mini-isolation column in the same collection tube. **Repeat** step 8 one more time for a total of two washes with Wash Solution.

9 Centrifuge the mini-isolation column for **2 minutes** at full speed to completely remove trace amounts of Wash Solution.

10 To elute the RNA, transfer the mini-isolation column into a new 1.5-mL RNase-free final collection tube. **Add** 10–50 µL of nuclease-free water to the top center of membrane (without touching membrane). **Incubate 1 minute**, then **centrifuge** for **1 minute** at full speed.

The quantity and quality of the isolated RNA may be assessed by UV/Vis spectrophotometry and Agilent 2100 Bioanalyzer.

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