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

Application

Genomics

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Abstract

A method was recently developed for the isolation of high-purity RNA from yeast cells 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 Agilent's novel prefiltration column results in a purer RNA product that is suitable for sensitive downstream applications. Efficient isolation of RNA from yeast is dependent upon careful preparation of spheroplasts followed by complete homogenization of the sample to remove the high levels of cell wall components and other proteins present. The yield of yeast total RNA isolated with the Agilent method was comparable to other commercial methods and contained significantly less genomic DNA compared to RNA isolated with a competitor's silica-based kit. Analysis using the Agilent 2100 Bioanalyzer demonstrated the RNA was of high quality and integrity.

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 the need for DNase treatment.

The standard protocol used for most mammalian cell lines and tissues was modified to obtain improved results when isolating RNA from yeast cells. Treatment with a lyticase-containing buffer was included to degrade the abundant polysaccharides and proteins present in yeast cell walls prior to lysis and extraction. The RNA is then captured 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 highly purified. Quantitative polymerase chain reaction (PCR) assays indicate that the RNA contains significantly less gDNA contamination than RNA prepared with a commercially-available silica-based kit.

Materials and Methods

Saccharomyces cerevisiae (strain 2601) was obtained from ATCC (Manassas, VA). Liquid cultures were grown at 30 °C in YM medium and harvested at mid to late log-phase (OD_{600nm} ~1.0–1.5). Serial dilutions were plated on YM agar to determine cell counts. All RNA isolations were from $2.5–5 \times 10^7$ cells. 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. Lyticase (Cat number L2524) and Sorbitol (Cat number 240850) were obtained from Sigma-Aldrich (St. Louis, MO). 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 determined using a quantitative PCR assay using serially diluted yeast gDNA as a standard curve and a primer/probe set specific for yeast GAPDH sequences (Biosearch Technologies, Novato, CA). Quantity of gDNA was measured using the ABI Prism 7000 Sequence Detector and software (Applied Biosystems, Foster City, CA).

Results

The quality of the RNA isolated from yeast cells 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.

Yeast 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 RNA isolated by both methods to have a relatively flat baseline, especially between the ribosomal peaks. However, both the gel-like image and the electropherogram of the Agilent isolated RNA display some smaller RNA species not captured by the silica method.

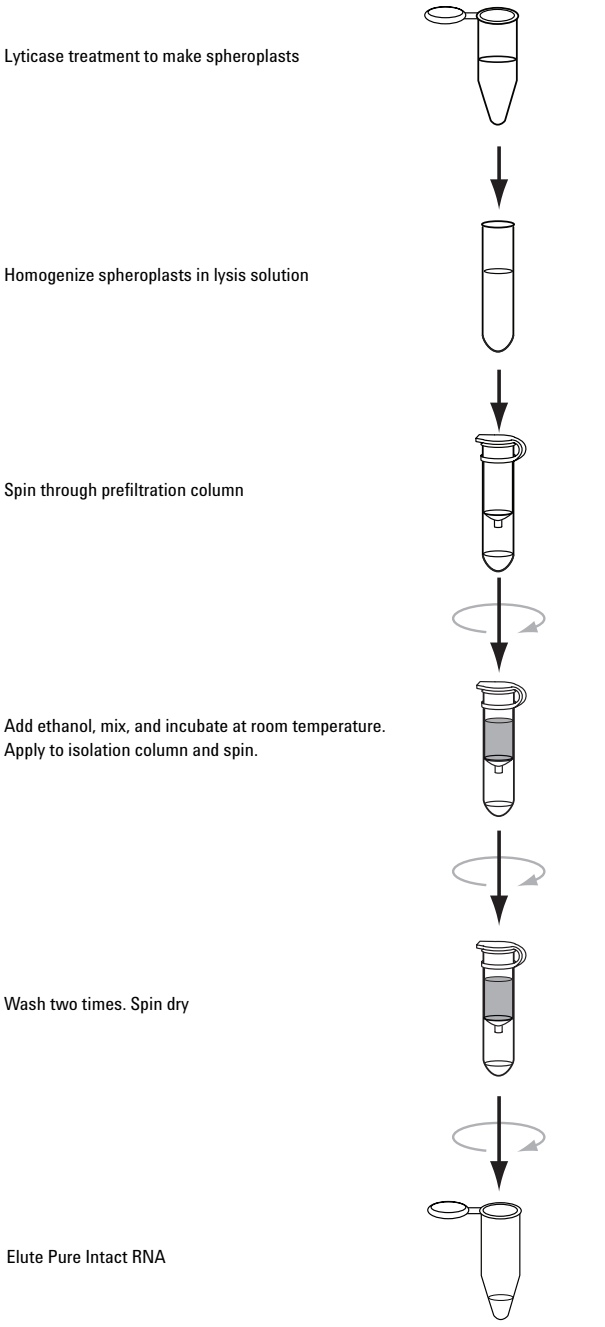

Figure 1. Agilent Total RNA Isolation Method for yeast cells.

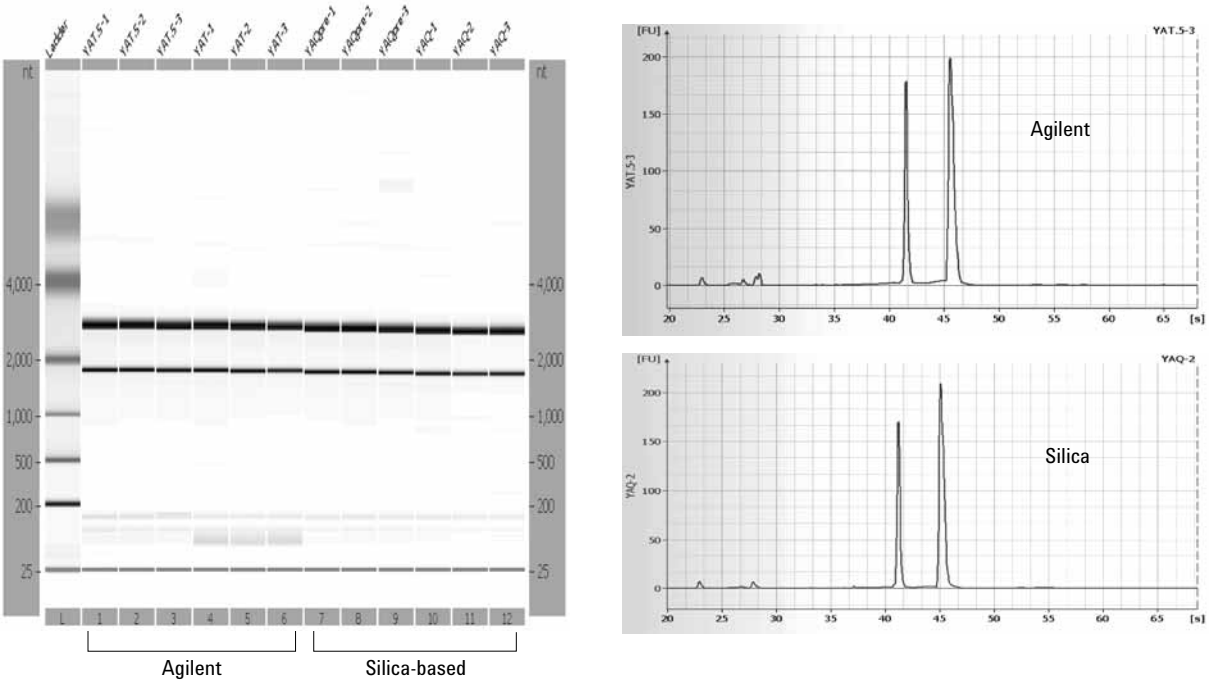

Figure 2. Agilent 2100 Bioanalyzer gel-like image and electropherogram trace of yeast RNA isolated with the Agilent Total RNA Mini Kit or a silica-based method.

RNA was quantified by measuring A_{260nm}. All A_{260nm}/A_{280nm} ratios were 1.9 or greater. Genomic DNA contamination was measured by quantitative polymerase chain reaction (PCR) using a TaqMan realtime PCR assay. Yeast gDNA was used as a standard and yeast GAPDH specific primers and FAM probe were used for detection. Typical RNA yields and gDNA content are shown in Figure 3. Yields were approximately the same for the two methods. However, RNA isolated with the silica-based method contained 5- to 20-fold more gDNA.

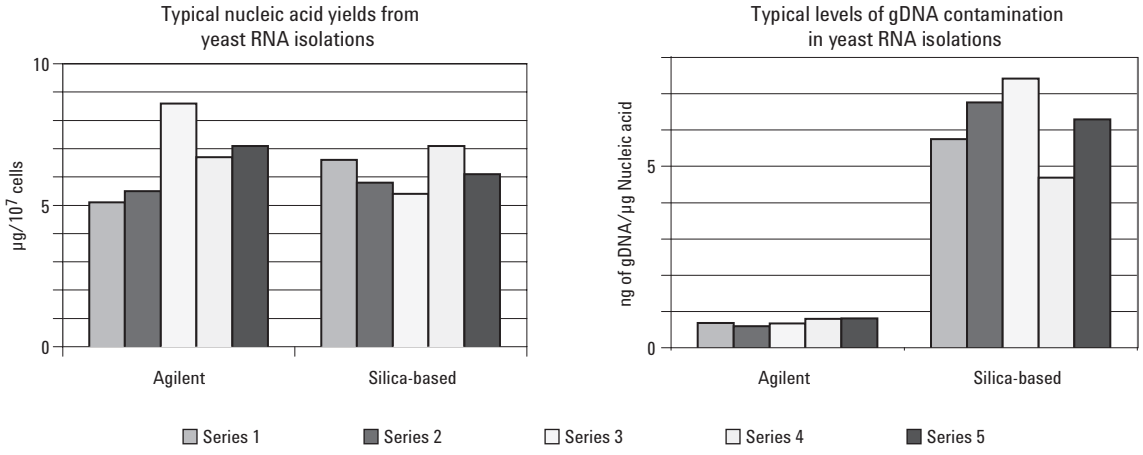

Figure 3. Yields and relative gDNA levels in *S. cerevisiae* RNA isolations.

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 was of high quality and integrity.

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

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RNA Isolation from Yeast Cells

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

Additional Materials Required

Please read entire protocol before beginning.

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

- Microcentrifuge
- 100% Ethanol
- 14.4M β-Mercaptoethanol
- Lyticase (for example, Sigma L-2524)
- D-Sorbitol (for example, Sigma-Aldrich 240850)
- EDTA
- Refrigerated centrifuge
- Shaking incubator at 30 °C

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

Protocol for Isolation of Yeast Total Cellular RNA Using Agilent's Total RNA Isolation Mini Kit

Yeast cultures are typically grown overnight at 30 °C. Growth conditions are dependent on the particular strain used. Avoid isolating RNA from saturated cultures. $OD_{600\ nm}$ readings can approximate culture density (a reading of 1 at 600 nm is approximately 10^7 yeast cells). Serial dilutions of culture should be plated to confirm culture density at a particular absorbance reading.

Before you begin, set a shaking waterbath or incubator at 30 °C and a refrigerated centrifuge at 4 °C.

Prepare 100 mL of Sorbitol/EDTA buffer (1 M Sorbitol, 0.1 M EDTA, pH 7.5) in nuclease-free water and sterile-filter. Each yeast RNA isolation requires 2 mL of buffer.

Lyticase should be dissolved in cold nuclease-free water to a final concentration of 500 units/mL and can be stored at 4 °C up to 1 week.

All steps are to be done at room temperature, unless indicated otherwise. Use RNase Free Technique.

1 Collect the yeast. Centrifuge cultures at 1000 × g for 5 minutes at 4 °C. The growth medium must be thoroughly removed from the cell pellet by draining or aspirating. Additionally, an absorbent material can be used to remove final traces of medium from the centrifuge tube or bottle. Set centrifuge to 25 °C.

2 Resuspend pellet in 2 mL of Sorbitol/EDTA buffer per 5×10^7 cells. Add 2 µL of ß-ME and 500 µL Lyticase per 5×10^7 cells. **Mix** gently by inversion.

3 Incubate for **15 minutes** at 30 °C, shaking at 100 rpm. Handle spheroplasts gently to avoid lysis until step 6.

4 Centrifuge spheroplasts for 5 minutes at 300 × g at room temperature.

5 Carefully remove supernatant by aspirating. An absorbent material can be used to remove final traces of supernatant from tube.

6 Add 350 µL of prepared Lysis Solution per 5×10^7 spheroplasts. Homogenize with a rotor-stator homogenizor with a stainless steel probe for 30 seconds at 15000 rpm. To reduce foaming, move the probe side to side rather than up and down. Alternatively, the lysate may be vortexed vigorously for 1 minute, although this may result in reduced yields.

7 Centrifuge 350 µL of lysate through a mini-prefiltration column (natural) for **3 minutes** at full speed (for a typical microcentrifuge, approximately 16,000 × g). This step ensures complete homogenization and removes cellular contaminants. Mini-prefiltration columns cannot be reused. Use an additional prefiltration column if processing more than 350 µL .

8 Mix an equal volume of 100% ethanol with the clarified lysate. Use a volume equal to the volume of homogenate added to the mini-prefiltration column in step 7. **Mix** until the solution appears homogenous. **Incubate** mixture for **20 minutes** at room temperature.

9 Add the ethanol/lysis mixture to an Isolation Column, **centrifuge 30 seconds** at full speed. Discard the flow-through, and replace the RNA-loaded column in the 2-mL collection tube. Centrifugation time may be increased to 1 minute to ensure complete flow-through of sample.

10 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 10 one more time for a total of two washes with Wash Solution.

11 Centrifuge Isolation Column for **2 minutes** at 16,000 × g to remove final traces of Wash Solution.

12 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.

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