

Quick and Efficient Purification of Single- or Double-Stranded RNA Transcripts Using the MasterPure™ RNA Purification Kit

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Introduction

In vitro transcription reactions are performed to produce RNA transcripts of various sizes and types including both single- and double-stranded RNA. For traditional applications, a simple precipitation step is sufficient purification of the RNA, but for many new techniques it is important to remove the DNA template, RNA polymerase, and buffer components, before proceeding. The MasterPure™ RNA Purification Kit is a quick and effective solution to transcript purification. Designed for the efficient recovery of even the smallest amounts of RNA, this salt-based extraction method can be used to purify RNA transcripts of any type or length.

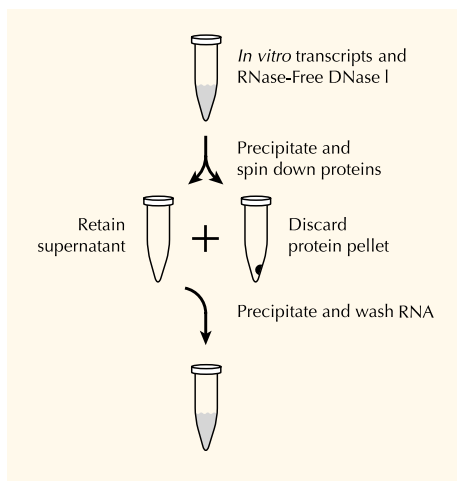


Figure 1. Quick and efficient recovery of RNA transcripts with the MasterPure™ RNA Purification Kit. Strategy for transcript purification.

The MasterPure RNA Purification protocol uses RNase-Free DNase I to completely and safely remove the transcription template. Then, the RNA polymerase and DNase I are inactivated and removed with the MasterPure Protein Precipitation Reagent, and the RNA is precipitated, removing remaining transcription reaction components (Figure 1). Here we demonstrate the purification of single-stranded and double-stranded RNA transcripts and even transcripts less than 100 bases in length using the MasterPure RNA Purification Kit.

Methods

High yield transcription reactions

Transcripts of various sizes were produced, in yields of up to 150 µg of RNA,

using the AmpliScribe™ T7 or T3 High Yield Transcription Kits as per protocol. Single-stranded sense RNA transcripts were annealed to anti-sense RNA transcripts at 37°C, in AmpliScribe Reaction Buffer, to produce double-stranded RNA (dsRNA) transcripts.

RNA purification

The MasterPure RNA Purification protocol is depicted in Figure 1. To remove the DNA template, the 20-µl transcription reactions were treated with 1 Unit of RNase-Free DNase I for 15 minutes at 37°C, in AmpliScribe Reaction Buffer. Then, 280 µl of Tissue & Cell Lysis Solution were added, followed by 150 µl of MPC Protein Precipitation Solution. The reactions were vortexed and then spun for 10 minutes at full speed in a microcentrifuge. The supernatant was transferred to a new tube and the RNA was precipitated with 500 µl of isopropanol. The RNA was spun down for 10 minutes at full speed, and the pellets were washed twice with 70% ethanol. The RNA was resuspended in 100 µl of TE Buffer.

RNA transcript probe

To demonstrate the functionality of the RNA transcript, a 1.4-kb RNA probe was generated using the DuraScribe™ T7 Transcription Kit, which generates RNase A resistant transcripts. Unlike standard T7 RNA Polymerase, DuraScribe T7 RNA Polymerase efficiently incorporates 2'-deoxy-NTPs and 2'-modified-NTPs such as 2'-Fluoro-dNTPs into full length transcripts. The RNA transcript was labeled with biotin, by partial substitution of 2'-Fluoro-UTP (3.5 mM) with biotin-dUTP (to 0.42 mM) in the reaction. The transcript was purified using the MasterPure RNA Purification Kit by the protocol described above and was then used to detect various amounts of DNA template blotted to a membrane.

Results

Identical amounts of 1.4-kb RNA transcripts before and after clean-up with the MasterPure RNA Purification Kit were analyzed by gel electrophoresis. Figure 2 demonstrates the efficient removal of the *in vitro* transcription template and the recovery of the single-stranded RNA transcript with MasterPure RNA Purification. The RNA recovery, as estimated by gel electrophoresis, approached a complete

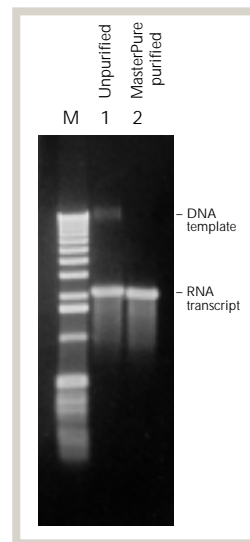


Figure 2. Complete removal of the transcription template with the MasterPure™ RNA Purification Kit. The 1.4-kb transcript, produced from a linear 3-kb DNA template, was purified using the MasterPure Purification Kit protocol. Lane M, kb ladder; Lane 1, unpurified transcript; Lane 2, purified RNA transcript.

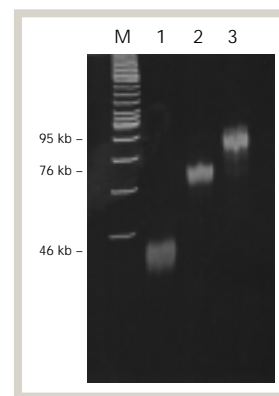


Figure 3. Efficient recovery and purification of even small RNA transcripts. Lane M, 100-bp ladder; Lane 1, 46-base transcript; Lane 2, 76-base transcript; Lane 3, 95-base purified RNA transcript.

recovery. The MasterPure RNA Purification Kit efficiently purifies even RNA transcripts of less than 100 bases in length. Three AmpliScribe transcripts of 46, 76 and 95 bases in length were purified and recovered using this procedure (Figure 3). The purified transcripts all had 260/280 absorbance ratios of 1.9 to 2.0.

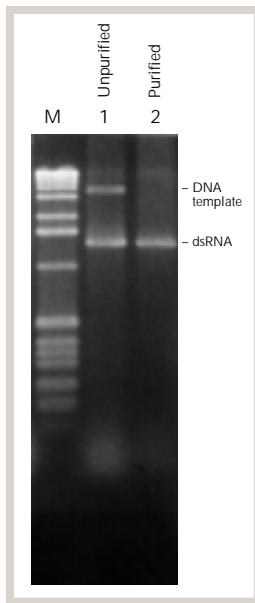


Figure 4. Double-stranded RNA is purified effectively with the MasterPure™ RNA Purification Kit. Lane M, kb ladder; Lane 1, dsRNA transcript before purification; Lane 2, dsRNA transcript purified with the MasterPure Purification Kit.

A double-stranded RNA transcript, produced by annealing two single-stranded AmpliScribe control transcripts together, was treated with RNase-Free DNase I and purified using the same protocol described above. The purified double-stranded RNA shows no remaining DNA template and resulted in only a slight loss of RNA during purification (Figure 4). Alternatively, to improve the RNA recovery, single-stranded RNA transcripts could be purified and subsequently annealed after MasterPure RNA Purification. The MasterPure purified biotin-labeled DuraScribe™ transcript detected various amounts of DNA template blotted to a membrane when used as a probe (Figure 5).

Conclusion

This simple and quick, solution-based clean-up method was designed to maximize the recovery of RNA from even the smallest amount of starting material. The easily scaleable, MasterPure RNA Purification Kit works effectively to remove proteins, including transcription

enzymes and nucleases, as well as DNA templates, and transcription buffer components from single-stranded and double-stranded RNA transcripts.

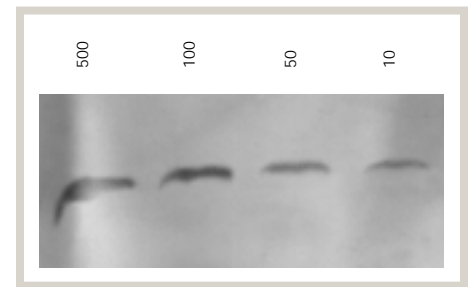


Figure 5. Detection of decreasing amounts of DNA template using a MasterPure™ purified biotin-labeled DuraScribe™ transcript as probe. Lane 1, 500 pg; Lane 2, 100 pg; Lane 3, 50 pg; Lane 4: 10 pg of DNA template.

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MasterPure™ RNA Purification Kit

MCR85102 100 Purifications

Obtain the Highest DNA Yields from Yeast with the MasterPure™ Yeast DNA Purification Kit

The MasterPure™ Yeast DNA Purification Kit is a simple and nonenzymatic approach to yeast genomic DNA purification. High quality yeast DNA can be obtained in less than 40 minutes without columns, resins or organic extractions. Recover DNA from a wide variety of yeast species including *Candida*, *Saccharomyces*, *Pichia*, and *Schizosaccharomyces*, and filamentous fungi such as *Aspergillus* and

Penicillium. The MasterPure Kit produces high-quality DNA that can be used directly for many applications including PCR amplification, restriction endonuclease digestion, Southern blotting, and genomic library preparation.

Higher yield than the competition

Using a simple, short protocol, DNA yields obtained with the MasterPure Kit were consistently above those of two competing kits (Figure 1). For example, the kit from supplier F averaged 0.25 µg of DNA from 1.5 ml of *S. cerevisiae*, whereas the MasterPure Kit produced an average of 2.94 µg, almost twelve times as much. The supplier Q kit produced 1.8 µg of *S. cerevisiae* DNA from the same culture volume.

High molecular weight DNA

The MasterPure Kit yields high molecular weight yeast DNA. As determined by pulsed field gel electrophoresis, the size of the *S. cerevisiae* DNA isolated with the MasterPure Yeast DNA Purification Kit is approximately 40-50 kb, while DNA of the same yeast species purified with the kit from supplier Q was mostly degraded to fragments smaller than 40 kb (Figure 2). A similar size discrepancy was observed for *C. albicans* DNA (data not shown).

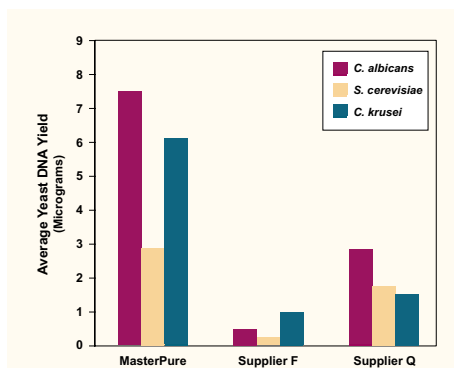


Figure 1. The MasterPure™ Yeast DNA Purification Kit gives higher yields of DNA than other kits. DNA yields were from 1.5 ml cultures and quantitated by fluorometry with Hoescht 33258 dye. The data represent the average of duplicate extractions from either one (*C. krusei*) or two (*S. cerevisiae* and *C. albicans*) experiments.

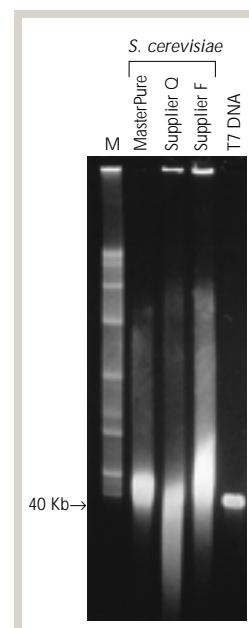


Figure 2. Yeast DNA purified using the MasterPure™ Kit has a higher molecular weight than DNA purified using other kits. 500 ng of purified yeast DNA was analyzed by pulsed field gel electrophoresis on a 1% agarose gel. Lane M, lambda DNA ladder.

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MasterPure™ Yeast DNA Purification Kit

MPY80010 10 Purifications
MPY80200 200 Purifications

Contents:

Yeast Cell Lysis Solution, MPC Protein Precipitation Reagent, TE Buffer, and RNase A.