



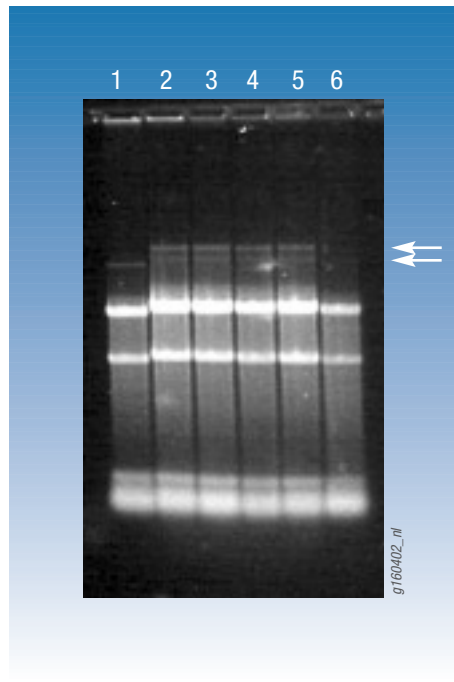
# A Novel, Rapid Method to Release Intact Yeast RNA Using the MasterPure™ Yeast RNA Purification Kit

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## Introduction

Yeast RNA has traditionally been extracted using hot acid phenol.<sup>1-4</sup> Recently, other methods have been introduced that require either physical shear force or enzymatic lysis to break the very resilient yeast cell walls. These methods require special steps to “break open” the cells and release the contents, after which the RNA is purified away from the other cell components. However, the MasterPure™ Yeast RNA Purification Kit uses a revolutionary RNA isolation method that does not require special steps to lyse the yeast cells and that leaves the yeast cells essentially intact, based on observations using a light microscope. The MasterPure Kit protocol releases RNA that is largely free of DNA and protein, without using organic solvents or caustic reagents.

Here, we demonstrate use of the MasterPure Yeast RNA Purification Kit to isolate total RNA from three different yeast species - *Candida albicans*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe* - all grown under standard conditions to mid-log phase. Approximately 25 µg of total RNA is obtained from 1-ml cultures of each of these yeast species - more than enough for synthesis of cDNA. Major component rRNAs are intact, as shown by denaturing gel electrophoresis. The RNA obtained from *Saccharomyces* is shown to be suitable for a variety of other applications, including synthesis of cDNA and dye-labeling for microarray hybridizations (Marilee Morgan, personal communication). Isolated rRNAs, and small nuclear



**Figure 1.** A SYBR® Gold-stained denaturing gel shows the size spectrum of RNA purified using the MasterPure™ Yeast RNA Purification Kit. Each lane contains 1 µg of *S. cerevisiae* (lanes 1 and 6) or *S. pombe* (lanes 2-5) total RNA. Arrows indicate the sizes expected for precursor rRNAs, 35S and 32S, which are approximately 7 kb.

RNAs (snRNAs) are undegraded, based on Northern blot analysis. RT-PCR experiments further demonstrate the quality of this mRNA.

The MasterPure Yeast RNA Purification Kit compares favorably to two other commercially available yeast RNA purification kits

with respect to yield, quality of the isolated mRNA, ease of use, and cost.

**Materials and Methods** can be found online at: [www.epicentre.com/f11-2mmmp.asp](http://www.epicentre.com/f11-2mmmp.asp)

## Results

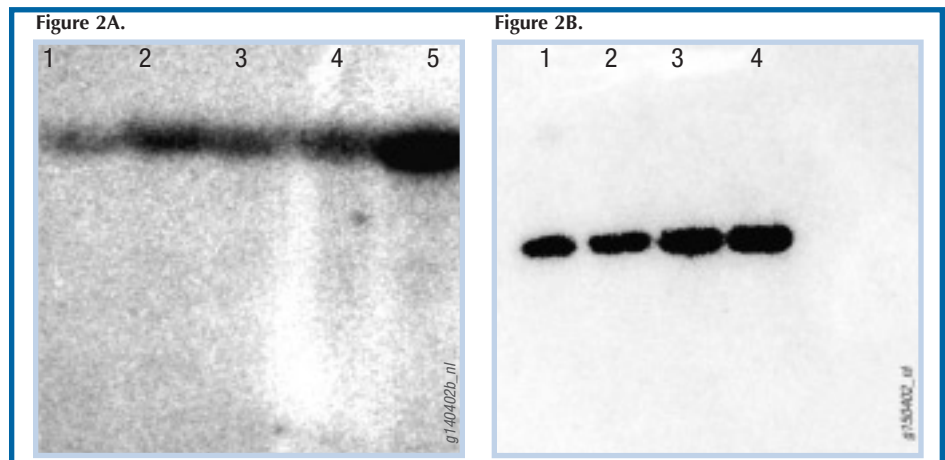
### Electrophoresis and Northern blots show intact RNA

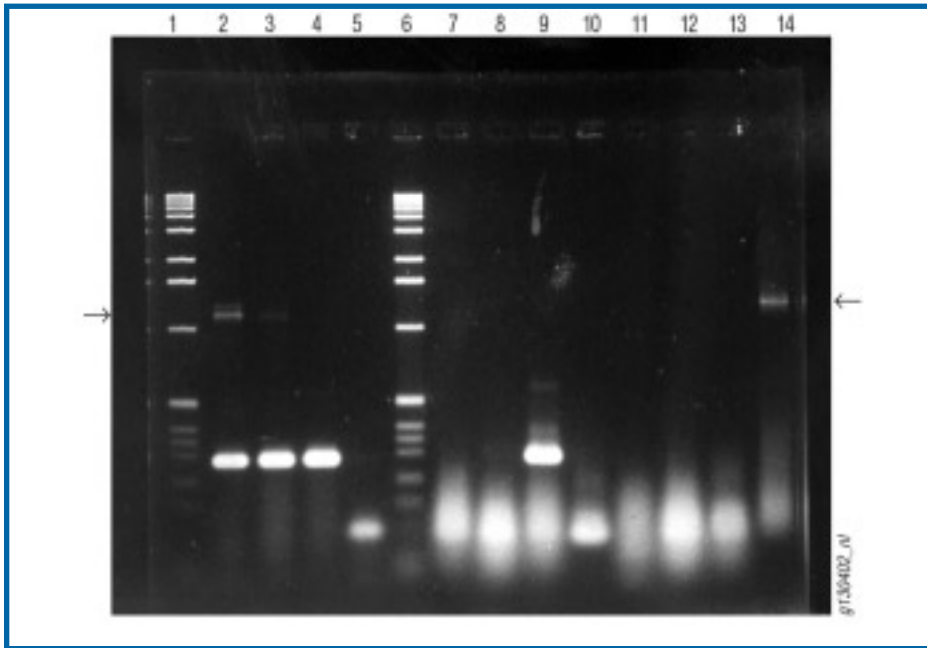
A denaturing agarose gel, stained with SYBR® Gold, shows the size spectrum of RNA isolated from *S. cerevisiae* and *S. pombe* (Figure 1). Arrows indicate the sizes expected for the precursors of rRNAs, 35S and 32S, which are approximately 7 kb.

We demonstrate the integrity of RNA from MasterPure preparations by two different methods, Northern blotting and RT-PCR. For Northern blots, 5 to 10 µg of total RNA, purified using the MasterPure Yeast RNA Purification Kit, was electrophoresed in denaturing gels, blotted to nylon membranes, and sequentially hybridized with probes for the 25S (3391 nucleotides) rRNA of *S. cerevisiae* and the 26S (3497 nucleotides) rRNA of *S. pombe*. The data indicate that the large 25S and 26S rRNAs, from both the budding and fission yeast species, are intact (Figure 2A). Based on the substantial amounts of rRNA present, we would expect to detect any significant degradation of the RNA by Northern blots.

To assess the possible degradation of another yeast RNA, we chose the U2 snRNA. The unusually long *S. cerevisiae*

**Figure 2.** Northern blots indicate the integrity of MasterPure™ Yeast RNA. RNA from *S. cerevisiae* or *S. pombe* (5 to 10 µg) were separated on a 1% agarose/formaldehyde gel and transferred to a nylon membrane. Probes for (A) 25S and 26S rRNA, or (B) U2 snRNA were hybridized to the blots. **2A.** *S. pombe* RNA (lanes 1-4), *S. cerevisiae* RNA (lane 5). **2B.** *S. cerevisiae* AB1380 (lanes 1 and 2), *S. cerevisiae* InvSc2 (lanes 3 and 4).





**Figure 3. Results of RT-PCR, using RNA purified by the MasterPure™ Yeast RNA Purification Kit or two other yeast purification kits, show differences in the quality of purified RNA.** RT-PCR of *DBP2* mRNA was performed using a two step (lanes 2-5) or one step (lanes 7-14) RT-PCR protocol. MMLV-RT was omitted from samples in lanes 11-14. Supplier A RNA is used in lanes 2, 7 and 11; Supplier Q RNA is used in lanes 3, 8, and 12; MasterPure RNA was used in lanes 4, 9, and 13. Control reactions were done with RNA-free yeast DNA in lanes 5, 10, and 14 to amplify only genomic *DBP2* sequences.

U2 snRNA (1175 nucleotides) resides principally in the nucleus, but shuttles between the nucleus and cytosol during its maturation and processing.<sup>5</sup> The Northern blot in Figure 2B shows that U2 snRNAs from 2 strains of *S. cerevisiae* appear full-length after MasterPure isolation.

Another advantage of the MasterPure Yeast RNA Purification Kit is the integrity of low molecular weight RNA, such as 5.8S RNA, 5S RNA, and tRNA, that it yields. Electrophoresis in a 2% agarose gel showed very discreet low molecular weight species in MasterPure yeast RNA (data not shown).

**RT-PCR amplification of mRNA**

RT-PCR is a useful technique to evaluate the quality of mRNA in a total RNA sample. However, if DNA is present, gene-specific primers can produce a false-positive

amplicon. To verify that the amplicon was a product of the cDNA rather than genomic DNA, we chose a gene containing an intron and designed primers to amplify the region containing the intron in genomic DNA. The *S. cerevisiae DBP2* gene contains a 1001 bp intron and codes for an RNA helicase featuring a DEAD-box.<sup>6</sup> Because the RT-PCR product from the mRNA is 1 kb shorter than the PCR product amplified from genomic DNA, it can easily be distinguished from the DNA amplification product by electrophoresis.

Using the MasterPure™ High Fidelity RT-PCR Kit, we compared RT-PCR results of *S. cerevisiae* total RNA that was purified by the MasterPure Yeast RNA Purification Kit or by kits from Suppliers Q and A. Yeast RNA from all of the kits tested amplified by the two-step RT-PCR

(Figure 3, lanes 2-4.). However, the gel also shows DNA amplification of the target gene with RNA prepared with Supplier Q and A kits (see arrow, lanes 2 and 3).

Using one-step RT-PCR conditions, *DBP2* RNA isolated by Suppliers Q and A kits gave no detectable product (Figure 3, lanes 7 and 8), while the MasterPure RNA gave a significant product band (Figure 3, lane 9). To verify that this problem was not attributable in some way to the *DBP2* gene, we also tried to amplify *S. cerevisiae ERD2* mRNA by one-step RT-PCR. Again, The MasterPure RNA gave a strong product band and the RNA from the other two kits gave no detectable product (data not shown).

In one-step RT-PCR negative control reactions, without reverse transcriptase, none of the yeast RNA, purified by any of the kits, gave a product (Figure 3, lanes 11, 12 and 13). Control yeast DNA (RNA-free) produced only the genomic-template amplicon (see arrow, lane 14).

**MasterPure versus other yeast RNA isolation kits**

Spin-column methods of RNA purification rely on the lysis of yeast cells by enzymatic treatment and/or mechanical shearing. RNA yields from these methods are variable, and the RNA requires further clean-up for some applications. In Table 1, we compare the MasterPure Yeast RNA Purification Kit to the two other commercially available kits for *S. cerevisiae* RNA purification. The MasterPure Yeast RNA Purification Kit easily, quickly and safely

**Table 1. Comparison of the MasterPure™ Yeast RNA Purification Kit protocol with other yeast RNA kit protocols.**

	MasterPure	Supplier Q	Supplier A
<b>Method</b>	Aqueous Extraction	Spheroplasting	CHCl <sub>3</sub> /Phenol/Beads
<b>Number of Steps</b>	10	15	19
<b>Time*</b>	40 min	73 min	33 min
<b>Extra Item to Buy</b>	None	Zymolyase	Vortex Adapter

\* Without DNase I digestion

isolated intact total RNA at a lower cost per purification than the other kits.

**Discussion**

The MasterPure Yeast RNA Purification protocol requires no additional enzymes or special equipment purchases. The simple procedure, with fewer steps and safer reagents, means that the MasterPure method is useful for high throughput applications. Because this method does not use spin columns, accurately calculating the starting number of cells is not necessary and scale-up does not require a different kit.

The RNA from *S. cerevisiae* and *S. pombe* was analyzed using Northern blotting. Several RNA types appeared to be intact, up to approximately 7 kb in length. Stained gels suggest that the MasterPure Yeast RNA Purification Kit extracts nuclear precursors of rRNAs. The U2 snRNA of *S. cerevisiae*, which is much longer than most snRNAs in yeast, is recovered as a full-length molecule with MasterPure. U2 is especially susceptible to degradation

during isolation (Stephanie Ruby, personal communication).

The MasterPure RNA was readily converted to cDNA by both one-step and two-step RT-PCR protocols and only produced the smaller, intronless product. RNA isolated with kits from Suppliers Q and A produced the smaller product in the two-step, but not the one-step, RT-PCR protocol, and also produced some of the larger, DNA template product.

Finally, avoiding hot phenol extraction conditions is another principal advantage of the MasterPure Yeast RNA Purification Kit. The dangers of using hot organic solvents are obvious, and this RNA isolation procedure effectively purifies RNA without those conditions.

**Acknowledgments**

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**References**

1. Ausubel, F. et al., eds. (2003) *Current Protocols in Mol. Biol.*, Fall 2003 CD-ROM, chapter 4.9, John Wiley and Sons, New York.
2. Schmitt, M.E. et al. (1990) *Nucleic Acids Res.* **18**, 3091.
3. Manna F. et al. (1996) *Trends Genet.* **12**, 337.
4. Bacci, A. et al. (2002) *J. Immunol.* **168**, 2904.
5. Elela, A.E. and Ares, M. (1998) *EMBO J.* **17**, 3738.
6. Bond, A.T. et al. (2001) *Mol. Cell. Biol.* **21**, 7366.

[www.epicentre.com/masterpure\\_yrna.asp](http://www.epicentre.com/masterpure_yrna.asp)

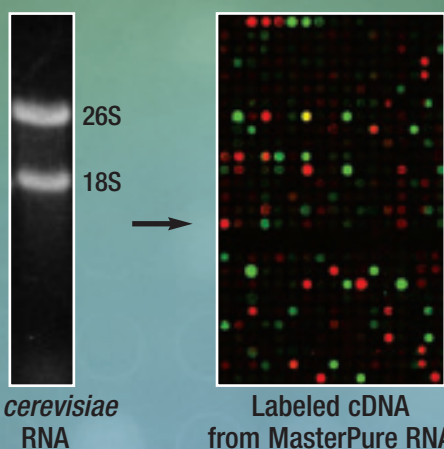
**MasterPure™ Yeast RNA Purification Kit**

MPY03010	10 Reactions
MPY03100	100 Reactions

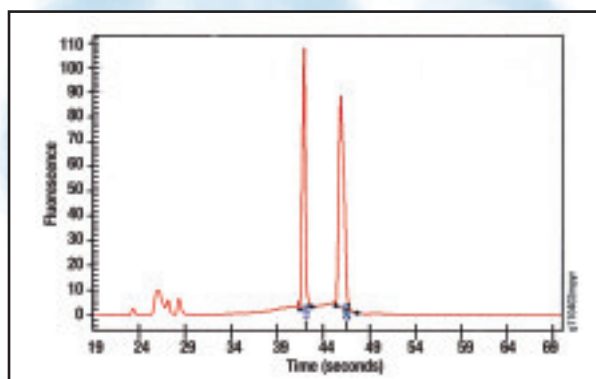
**Contents:**

- Extraction Reagent for RNA
- MPC Protein Precipitation Reagent
- TE Buffer (in 100 rxn kit only)
- Proteinase K (50 µg/µl)
- RNase-Free DNase I (1 Unit/µl)
- 10X DNase Buffer
- 2X T & C Lysis Solution

**Microarray Application Data for MasterPure™ Yeast RNA Purification Kit**



**Figure 1.** RNA purified with the MasterPure™ Yeast RNA Purification Kit was reverse-transcribed, labeled, and hybridized to an *S. cerevisiae* DNA microarray. The cDNA from cells under oxidative stress was labeled with Cy5, and cDNA from control cells was labeled with Cy3.



**Figure 2.** Agilent Technologies 2100 Bioanalyzer® electrophoretogram of the *S. cerevisiae* RNA shown in Figure 1. RNA was purified by the MasterPure™ Yeast RNA Purification Kit and stored at -20°C for 2 months before analysis. Observe the high purity, intact RNA.