MasterPure™ Yeast DNA Purification Kit
MasterPure™ Yeast RNA Purification Kit

1. What are the MasterPure Yeast Kits, and how do they work?

The MasterPure Yeast DNA Purification Kit and MasterPure Yeast RNA Purification Kit are designed for rapid, high-yield purification of DNA or RNA from yeast and filamentous fungi. These kits provide high yields of pure, intact genomic DNA or total RNA without bead-beating, spin columns, or toxic chemicals. The workflow involves nonenzymatic lysis and a simple desalting process to separate nucleic acids from proteins and other cellular components (Figure 1). The purified DNA is suitable for many molecular biology applications, including PCR, cloning, Southern blotting, genomic library preparation, and fungal identification and typing. The purified RNA is suitable for cDNA synthesis, RT-PCR, gene expression analysis, and other molecular biology applications.

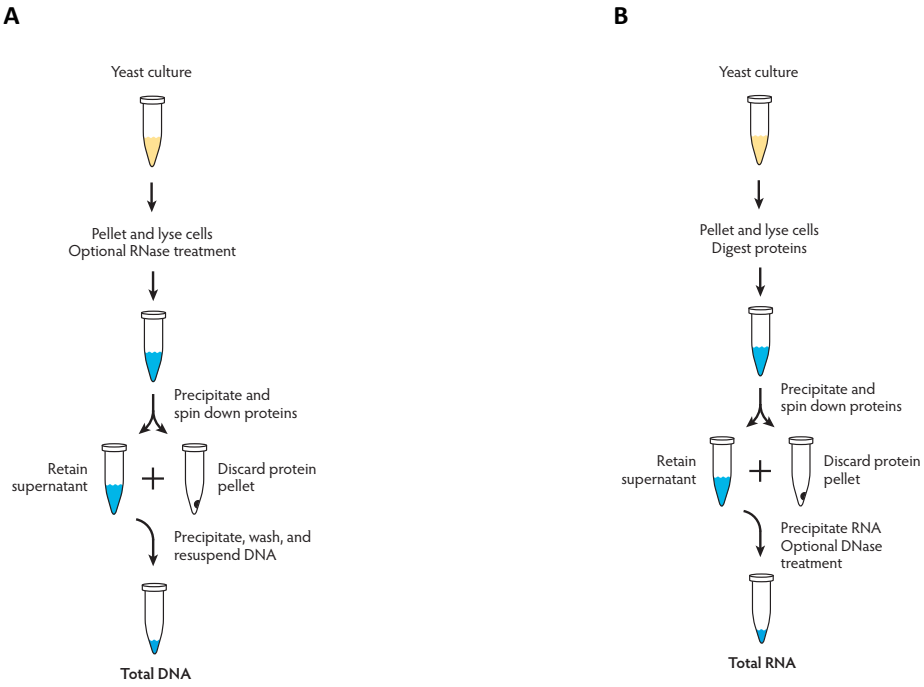

Figure 1. The MasterPure Yeast Kits provide high yields of pure total DNA (A) or RNA (B).

2. What kinds of samples can I use with the MasterPure Yeast Kits?

The kits can be used with a variety of yeast species, including *Candida*, *Saccharomyces*, *Pichia*, and *Schizosaccharomyces.* Additionally, they are also suitable for use with filamentous fungi, such as *Aspergillus* and *Penicillium*.

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3. For what applications can I use the DNA or RNA purified with the MasterPure Yeast Kits?

The purified DNA is suitable for many molecular biology applications, such as cloning, PCR amplification, Southern blotting, genomic library preparation, fungal identification and typing, and next gen sequencing (NGS). Researchers have published many novel applications for the MasterPure Yeast DNA Kit, including: PCR typing of DNA extracted from symbiont *Burkholderia* spp. in the gut of the Southern chinch bug [1]; and single-molecule (PacBio) and Illumina® sequencing of the human skin microbial metagenome [2].

The purified RNA is suitable for a variety of applications, such as cDNA synthesis, RT-PCR (endpoint and real-time), microarray analysis, and RNA-Seq. Researchers have published many novel applications for the MasterPure Yeast RNA Kit, including: cDNA library preparation from single-cell green algae [3]; real-time PCR-based gene expression analysis of yeast strains used in beer fermentation [4]; cDNA synthesis and digital droplet PCR in *S. pombe* [5]; and RNA-Seq analysis of genome-wide splicing efficiency in *S. cerevisiae* [6].

4. How much starting material do I need for the MasterPure Yeast Kits? How much DNA/RNA will I get after purification?

The MasterPure Yeast Kit protocols can be scaled proportionally, depending on the volume of liquid culture harvested. They can also be used with a single colony of yeast or filamentous fungi grown on solid media.

The average total yield of DNA from 1.5 mL of liquid culture is 3-4 µg for *Saccharomyces* spp. and 6-8 µg for *Candida* spp. The maximum theoretical yield of DNA from 1.5 mL of an early stationary-phase culture of haploid yeast (e.g., *Saccharomyces*) is approximately 10 µg (assuming a cell density of 2 x 10^8 cells/mL and a DNA concentration of 0.017 pg/cell).

The average yield of total RNA from 1 mL of mid-log phase cultures is 25 µg for *Candida albicans*, *Saccharomyces cerevisiae*, and *Schizosaccharomyces pombe*.

5. Do I need to perform the optional RNase treatment step in the MasterPure Yeast DNA Purification Kit protocol?

We recommend treating the cell lysate with RNase A (supplied in the kit) if the presence of small amounts of RNA will interfere with downstream applications, such as some PCR applications or sequencing library preparations. However, for applications such as restriction enzyme digestion and cloning, or PCR with primers specific to genomic DNA sequences, RNase treatment is not necessary.

6. Do I need to perform the optional DNase treatment step in the MasterPure Yeast RNA Purification Kit protocol?

The MasterPure Yeast RNA Purification Kit protocol recovers DNA with much lower efficiency than RNA after cell lysis, so removal of contaminating DNA may not be necessary for many applications. For applications such as RT-PCR with gene-specific primers, we recommend following the DNase treatment protocol.

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7. Can I use RNA purified with the MasterPure Yeast RNA Purification Kit directly to prepare an RNA-seq library?

The kit purifies total RNA, of which mRNA constitutes approximately 1-5%. To prepare an RNA-seq library, we recommend treating the purified total RNA with the Ribo-Zero® Gold Yeast rRNA Removal Kits (Illumina) or using an mRNA-specific library prep method.

8. What is the best way to quantify DNA or RNA isolated using the MasterPure Yeast Kits?

Spectrophotometric methods (e.g., A_{260}) to quantify DNA or RNA, although in common use, may overestimate the concentration. The best method to quantify DNA is by fluorometry using a DNA-specific dye, such as Hoechst 332581 (bisbenzimide), or PicoGreen® dye (Thermo Fisher Scientific). These dyes bind specifically to double-stranded DNA and not to nucleotides, single-stranded DNA, or RNA. To quantify RNA, we recommend using a Qubit™ fluorometer (Thermo Fisher Scientific) or a 2100 BioAnalyzer® instrument (Agilent).

References

1. Xu Y et al. 2016. Culturing and characterization of gut symbiont *Burkholderia* spp. from the Southern chinch bug, *Blissus insularis* (Hemiptera: Blissidae). *Appl Environ Microbiol* **82**:3319-3330.
2. Tsai Y-C et al. 2016. Resolving the complexity of human skin metagenomes using single-molecule sequencing. *mBio* 7:e01948-15.
3. Tabrizi ST et al. 2016. GUN4-protoporphyrin IX Is a singlet oxygen generator with consequences for plastid retrograde signaling. *J Biol Chem* **291**:8978-8984.
4. Schneiderbanger H et al. 2016. Gene expression in wheat beer yeast strains and the synthesis of acetate esters. *J Inst Brew* **122**:403-411.
5. Shimada Y et al. 2016. The RNA-induced transcriptional silencing complex targets chromatin exclusively via interacting with nascent transcripts. *Genes Dev* **30**:2571-2580.
6. Prevorovský M et al. 2016. Workflow for genome-wide determination of pre-mRNA splicing efficiency from yeast RNA-seq data. *BioMed Res Intl* **2016**:4783841.

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