

# DNA Isolation from a Filamentous Fungus Using the MasterPure™ Yeast DNA Purification Kit

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## Introduction

Methods for isolation of fungal DNA generally can be grouped into enzymatic, chemical, and physical. Large yields of high molecular weight DNA are most frequently recovered using cell wall-degrading enzymes to spheroplast cells, followed by gentle lysis and additional purification steps. Filamentous fungi offer much more diversity in their cell walls, as well as different morphologies (i.e., conidia vs. hyphae), all of which can make choosing and optimizing a method for a spheroplasting enzyme laborious. Consequently DNA preps for filamentous fungi typically employ bead beating or grinding in liquid nitrogen. Unfortunately, these methods can lead to sheared DNA (bead beating) or can be hazardous and laborious (grinding pathogenic fungi in an open mortar and pestle, or prepping multiple isolates). In an effort to circumvent these issues, we used EPICENTRE's MasterPure™ Yeast DNA Purification Kit to recover DNA from *Aspergillus fumigatus*, a filamentous fungus that is an opportunistic pathogen of humans and animals.<sup>1</sup>

## Methods and Results

Multiple isolates of *A. fumigatus*, from a variety of backgrounds (laboratory strains, clinical isolates, environmental isolates), were tested under variable conditions, that included preparing DNA from hyphae or conidia, using broth or agar cultures, and growing isolates for varying lengths of time prior to preparing DNA.<sup>2</sup> The standard preparation consisted of 200 mg of hyphae harvested from an overnight broth culture, although 72-hour conidia harvested from a single plate also worked well. The protocol accompanying the kit was followed with some modifications. The amount of Yeast Cell Lysis

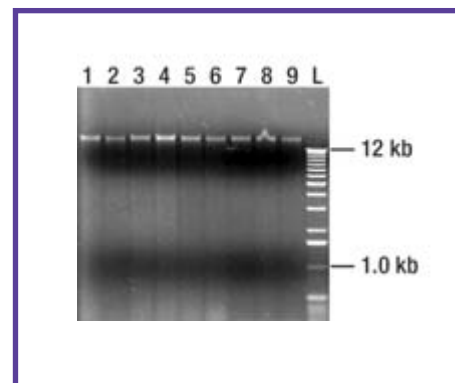
Solution was increased to 450 µl and successive steps were scaled accordingly. Isolates were heated at 65°C for 1 hour, and in some cases, a phenol-chloroform extraction was included, however, grinding was not needed under any circumstance. The yield from these conditions was typically 500-700 µg, by OD<sub>260</sub> (Figure 1) and the DNA was easily digested with restriction enzymes (Figure 2).

## Summary

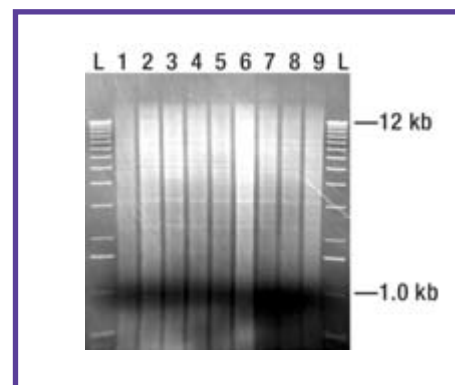
The MasterPure Yeast DNA Purification Kit has proven to be extremely robust and versatile in our laboratory. We have found that it works equally well on both yeast and filamentous fungal species. In addition to the ease of use, the amount of DNA recovered in a single preparation was enough to perform numerous Southern blots. We also found the DNA is of high molecular weight and suitable as a template for PCR reactions. In addition to working for *A. fumigatus*, we have found that the kit is suitable for other members of the aspergilli. Periodically we have also used the kit for DNA recovery from non-aspergilli and observed comparable results. The MasterPure Kit has proven particularly valuable for working with biohazardous fungi and for preparing DNA from multiple species in microtiter tube formats.

## References

- Marr K.A. et al. (2002) *Infect. Dis. Clin. N. Am.* **16**, 875.
- Jin, J. et al. (2004) *J. Clin. Microbiol.* **42** 4293.



**Figure 1. Quality of genomic DNA prepared from nine different strains of *Aspergillus fumigatus*.** Lane 1, AF293 (used for genomic sequence); Lane 2, ATCC 64746; Lane 3, ATCC 14110; Lane 4, Clinical isolate; Lane 5, Environmental isolate; Lanes 6-9, Clinical isolates.



**Figure 2. Eco RI digestion of genomic *Aspergillus fumigatus* DNA from Figure 1.**

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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  
RNase A