

# Direct Genomic DNA Sequencing for Rapid Fungal Identification

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## Introduction

There are several methods available for taxonomic classification of clinically occurring fungi. Chief among these are methods based on biochemical tests<sup>1</sup> and those based on DNA sequence identity. Among the DNA sequencing methods are Pyrosequencing™\*,<sup>2</sup> PCR-based technologies<sup>3</sup>, and Random Amplified Polymorphic DNA (RAPD).<sup>4</sup> We have found that direct sequencing of genomic DNA purified with EPICENTRE Biotechnologies' MasterPure™ Yeast DNA Purification Kit provides nine-fold longer read lengths than pyrosequencing with only a 1 hour increase in time. Genomic DNA (gDNA) sequencing can be performed on standard capillary-based instruments commonly found in molecular biology laboratories. In contrast, the PCR-based methods require several more hours of time to provide equivalent sequence read lengths, and pyrosequencing requires highly specialized equipment. Fungal identification by direct genomic DNA sequencing centers on the highly repetitive ribosomal

gene (rDNA) tandem repeats (FIG 1), which are repeated up to 200 times in fungal genomes.<sup>5,6</sup> We have discovered that multiple copies of this region provide enough template for direct sequencing.

## Methods

Genomic DNA was extracted from yeast, filamentous fungi, and mushrooms using EPICENTRE's MasterPure Yeast DNA Purification Kit according to the kit protocol. Optimal sequencing was obtained with 1/4X BigDye® sequencing reactions (ABI, Foster City, CA) and 100 ng of gDNA templates. Primer sequences were: LROR (5'-ACC CGC TGA ACT TAA GC), NL-1 (5'-GCA TAT CAA TAA GCG GAG GAA AAG), NL-4 (5'-GGT CCG TGT TTC AAG ACG G), and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC). Reactions were ethanol precipitated and sequenced on an ABI PRISM® 310 Genetic Analyzer.

## Results and Conclusions

The MasterPure™ Yeast DNA Purification Kit provides impressive yields of high molecular weight DNA (>25 kb) from

yeasts and filamentous fungi,<sup>7</sup> which can be used in direct sequencing. As shown in Table 1, direct sequencing with the ITS4 primer enabled positive identification of each of the ten clinical fungal isolates and one environmental isolate. Thus, direct sequencing of DNA purified from fungal cultures using the MasterPure Kit offers a rapid, convenient, and relatively inexpensive method for identifying medically significant fungi. The additional time and expense of PCR are also avoided. The multiple copies (50-200) of fungal rDNA genes<sup>6</sup> provide a sufficient homogeneous template for direct sequencing without amplification. Sequencing with commonly available instrumentation yields read lengths nine-fold longer than those obtained from typical pyrosequencing. As a result, the accuracy of BLAST sequence alignments is improved.

## References

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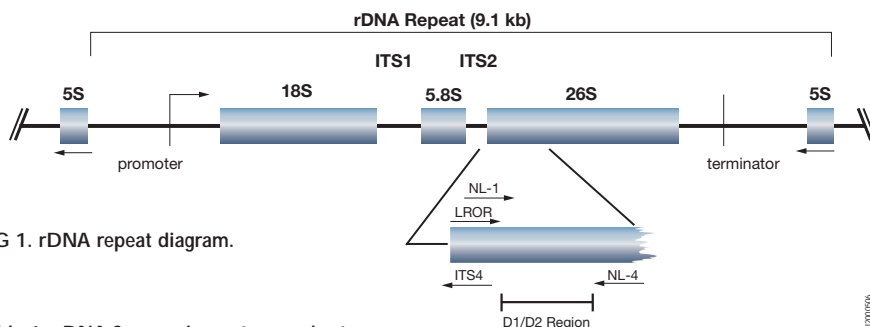


FIG 1. rDNA repeat diagram.

Table 1. gDNA Sequencing outcome chart.

Sample Identity	[gDNA] ng/μl	Primers							
		LROR	e value	NL-1	e value	NL-4	e value	ITS4	e value
<i>Cryptococcus neoformans</i>	39	yes	4e-91*	yes	0.0	yes	0.0	yes	0.0
<i>Yarrowia (Candida) lipolytica</i>	46	no		yes	0.0	yes	4e-9	yes	2e-156
<i>Candida tropicalis</i>	43	no		no		yes	2e-32	yes	2e-29
<i>Candida parapsilosis</i>	34	yes	7e-110	no		no		yes	0.0
<i>Cryptococcus laurentii</i>	116	yes	0.0	no		no		yes	8e-100
<i>Clavispora (Candida) lusitanae</i>	37	no		no		yes	2e-29	yes	3e-173
<i>Cryptococcus neoformans</i>	25	yes	3e-119	yes	0.0	yes	3e-96	yes	0.0
<i>Aspergillus fumigatus</i>	48	no		yes	2e-63	yes	8e-14	yes	6e-61
<i>Candida albicans</i>	25	nd		nd		nd		yes	0.0
<i>Candida glabrata</i>	20	nd		nd		nd		yes	0.0
<i>Coprinus comatus</i>	50	nd		nd		nd		yes	0.0

nd = not determined

\*Expect Value

"... E value describes the random background noise that exists for matches between sequences. For example, an E value of 1 assigned to a hit can be interpreted as meaning that in a database of the current size one might expect to see 1 match with a similar score simply by chance. This means that the lower the E-value, or the closer it is to "0" the more "significant" the match is." See

[http://www.ncbi.nlm.nih.gov/blast/blast\\_FAQs.shtml](http://www.ncbi.nlm.nih.gov/blast/blast_FAQs.shtml)

[www.EpiBio.com/masterpure\\_yeast.asp](http://www.EpiBio.com/masterpure_yeast.asp)

**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.

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