

Brian Wickes, University of Texas Health Science Center at San Antonio, a MasterPure™ Yeast DNA Purification Customer, Outlines His Fungal Research

My laboratory is interested in the biology and molecular biology of medically important fungi.

Cryptococcus neoformans

The fungus that we spend the most time studying is *Cryptococcus neoformans*, a basidiomycetous yeast that is closely related to mushrooms and a number of plant pathogens. *C. neoformans* is ubiquitous in the environment and is found globally. Infections typically occur via a pulmonary route after spores or yeast cells are inhaled. Once the lung is colonized, the fungus can remain there or disseminate to other parts of the body. A wide variety of mammals are susceptible to *C. neoformans* infections. In humans, the most frequent manifestation of infection is meningitis. Typically, immunosuppressed individuals, such as AIDS patients, are at the highest risk for infection, although the fungus can infect healthy individuals. These patients are at greatest risk for cryptococcosis, with 5-10% infected at the peak of the AIDS epidemic. In undeveloped countries, the number can be as high as 30%. If untreated, cryptococcosis is almost 100% fatal.

Our major interest in *C. neoformans* concerns the role that mating plays in virulence. The fungus is heterothallic with a bipolar mating system containing two mating types, *MATa* and *MATalpha*. In nature, *MATalpha* cells significantly outnumber *MATa* cells in population surveys. This bias is also reflected in clinical isolates where *MATa* cells are rarely

isolated from patients. Studies in our laboratory have shown that *MATalpha* cells are more virulent than *MATa* cells. In addition, we, and others, have found that a number of genes that are required for fertility are also required for virulence of the organism. More importantly, some genes that are involved in mating also control certain aspects of fungal morphology. Since it still remains unclear as to what morphology is responsible for infections, researchers will continue to dissect the mating pathway and other pathways that regulate fertility and development.

Candida albicans

A second fungus that we work with in our laboratory is *Candida albicans*. *C. albicans* is an ascomycete, which is closely related to the model yeast *Saccharomyces cerevisiae*. *C. albicans* is the most important and most frequently isolated human fungal pathogen and is capable of infecting virtually every site in the body. Although *C. albicans* is a normal commensal of humans and other mammals, infections can be life threatening. Our specific interests in *C. albicans* concern its ability to form biofilms. *C. albicans* can form biofilms on almost any implantable device. Similar to bacterial biofilms, once a *C. albicans* biofilm is formed, it is virtually impossible to treat with antibiotics, consequently the device must be removed, sometimes at great expense or risk. We are interested in the mechanisms that underlie biofilm formation and are presently using microarrays to search for genes required for biofilm

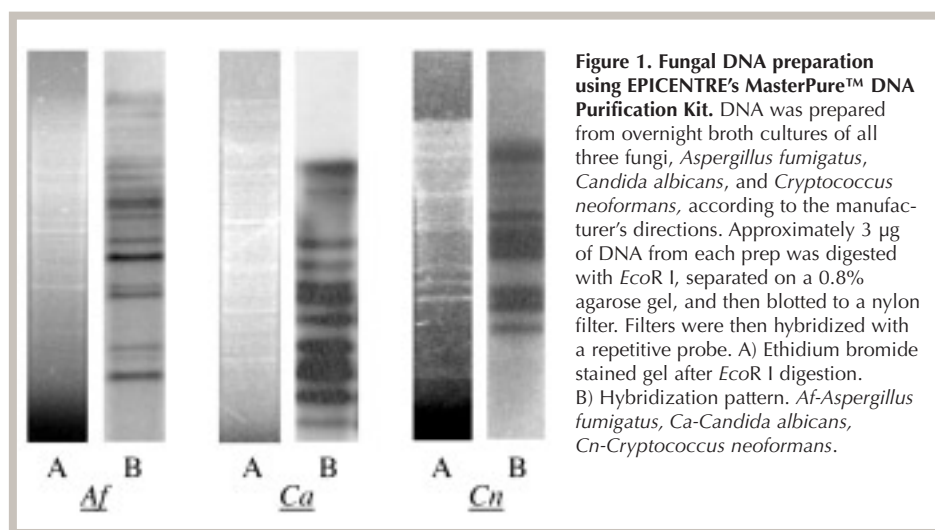
formation. We are also interested in the genetic basis for the increased drug resistance of cells that are growing in the biofilm phenotype. The use of microarrays to study *C. albicans* biofilms has allowed us to identify a number of genes that are only expressed when cells grow as a biofilm. The genes are currently being disrupted in order to study their effect on the ability of the strain to form a biofilm, as well as any change in antifungal susceptibility.

Aspergillus fumigatus

A third fungus that we work with is *Aspergillus fumigatus*. *A. fumigatus* is a ubiquitous fungus that grows exclusively in a mold-like or filamentous morphology. The fungus is classified as an ascomycete and is related to *Aspergillus nidulans* and *Neurospora crassa*, two model filamentous fungi. Although *A. fumigatus* is one of the most common fungi that humans encounter, it poses little problems for healthy people. However, for certain types of immunosuppressed patients, infections can be fatal. In fact, we perform a number of epidemiological studies on *A. fumigatus* because it is a frequent cause of outbreaks in hospitals. The fungus is particularly dangerous for patients undergoing bone marrow transplantations since infections have a very low cure rate and can be uniformly fatal. We perform studies to determine the relatedness of populations of strains recovered from outbreaks using a variety of fingerprinting techniques.

DNA purification

All aspects of our research require the ability to isolate DNA from different fungi. Since we work with a diverse collection of fungi, a universal method that would work with any fungus would be ideal. Presently, the only method that works for most fungi is a method based on the physical breakage of cells using some form of mechanical shearing by beating with glass beads and phenol. This method is convenient for lysing most fungal cells, unfortunately the results are that the DNA itself usually gets sheared. Sheared DNA is usually not a problem for PCR-based experiments, however, when high quality DNA is needed, such as for restriction digests, it is imperative to use gentler procedures that do not result in



significant breakage of large molecules. For fungi, a gentle DNA preparation method involves spheroplasting with enzymes that digest the cell wall, and then lysis. After additional steps to remove proteins and RNA, the DNA is usually high molecular weight and high quality. The drawback of a spheroplasting-based protocol is that the spheroplasting enzymes need to be specific for the fungus, and are not applicable to all fungi. The technique also can be time-consuming and laborious. We have applied EPICENTRE's MasterPure™ Yeast DNA Purification Kit to a number of different

fungus species and have been quite successful in getting high quality DNA from each species. Importantly, while we expected the kit to easily isolate *C. albicans* DNA, we have found that the kit also works well for *C. neoformans* DNA isolation and *A. fumigatus* DNA isolation. In all three cases the DNA is recovered as high molecular weight molecules and is also of sufficient purity to digest easily with standard restriction enzymes. By scaling up using multiple preps, we have been able to recover sufficient quantities to perform multiple digestions. Since the cost per sample is relatively low and eas-

ily comparable to the spheroplasting method, we now use the EPICENTRE kit for all routine DNA isolations.

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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

EPICENTRE Completes Validation of New cGMP Compliant Manufacturing Facility

EPICENTRE announces the validation of its new 2,000 square-foot, cleanroom manufacturing facility. Since 1997, EPICENTRE has been manufacturing raw materials for diagnostic kits and pharmaceuticals in development. With the validation of the new 100 L cGMP compliant fermentor, EPICENTRE's capacity has increased to produce kilogram quantities of microbial enzymes.

"The incorporation of our segregated Class 100,000 and 10,000 clean rooms, with Class 100 work benches and USP PW (United States Pharmacopeia Purified Water) and WFI (Water For Injection) System, makes our new facility one of the best for a non-pharmaceutical biotechnology company" says George Nielander,

Regulatory Affairs Officer. "Based on ISO 9000 and QSR (Quality System Requirement) guidelines, our documented Quality System meets or exceeds the most demanding client's requirements."

"Validation of materials is a major expense in bringing a kit or drug to market" reports Gary Wolfe, Sales Manager. "A key benefit EPICENTRE now offers to its clients is that the researcher can start with EPICENTRE enzymes in the early development stage and be fully confident that these same raw materials will be available, scalable, and compliant up through Phase II trials. There will be no need for a costly revalidation of a new supplier as the product moves out of research and development into production."



Where To Find Us

	<u>Date</u>	<u>Conference</u>	<u>Location</u>
2003	September 28 – October 2	11th Int'l Conf. on Microbial Genomes www.esd.ornl.gov/microbial_genomes/agenda.html	Millennium Hotel - Durham NC
	October 14 – 17	National Institutes of Health Research Festival http://festival03.nih.gov	National Institutes of Health - Bethesda MD
	November 4 – 7	American Society of Human Genetics www.faseb.org/genetics/ashg/menu-annmeet.shtml	Los Angeles Convention Center - Los Angeles CA
	December 13 – 17	American Society of Cell Biology https://www.ascb.org/ascbsec/advregistration/advregsfrm03.cfm	Moscone Convention Centre - San Francisco CA
2004	January 10 – 14	Plant & Animal Genome www.intl-pag.org/pag/	Town & Country Convention Center - San Diego CA
	March 27 – 31	American Society of Cancer Research www.aacr.org/2004am.asp	Orlando Convention Center - Orlando FL
	May 23 – 27	American Society of Microbiologists www.asm.org/meetings/index.asp?bid=697	New Orleans Convention Center - New Orleans LA
	August 8 – 13	Drug Discovery Technology www.drugdisc.com	Hynes Convention Center - Boston MA