

Introduction: Cryptococcosis is a rare and potentially fatal systemic fungal infection caused by the facultative intracellular pathogen, *Cryptococcus neoformans* [2]. The main portal of entry for this pathogen is through inhalation into the lungs. Here, *C. neoformans* causes severe pneumonia in immunocompromised individuals, including acquired immunodeficiency syndrome (AIDS) patients, and if the infection is not adequately controlled it will disseminate to the central nervous system, leading to death [7]. In the lungs of immunocompetent individuals alveolar macrophages (AMs), a type of innate phagocyte, act as a first line of defense against the pathogen [3].

Previous research has shown that the polarization state of macrophages greatly affects the anticryptococcal activity of these cells. Exposure to interferon-gamma (IFN γ) stimulates these cells to transition from the naïve M0 state to the M1 polarization state, also referred to as ‘classical activation’ [4]. M1 macrophages exhibit heightened proinflammatory and antimicrobial activity, which is important for the successful clearance of *C. neoformans* [4].

However, *C. neoformans* infection is known to compromise M1 polarization [3], although the mechanisms by which this is achieved are not fully understood. Improving our understanding of this process will assist with the development of new, much needed therapies to treat cryptococcosis, but this will require the use of appropriate AM cell culture models. While it is possible to isolate murine AMs by bronchoalveolar lavage, the cells can only be maintained in culture for short periods and rapidly lose their AM properties [1]. Other models used by the field, including bone marrow-derived macrophages and the macrophage-like cell lines, such as J774, do not accurately model the AM phenotype. For these reasons a new AM model would be of great use to the field. Within the past two years, the Olive lab (MSU) has developed an alternative approach to produce AM-like cells by differentiating macrophage precursor cells obtained from murine fetal livers in the presence of GM-CSF and TGF- β [1,5]. These fetal liver-

derived alveolar-like macrophages (FLAMs) maintain AM markers and properties in culture for over 40 days [1] and have been shown to ingest opsonized *C. neoformans* (Unpublished data; Nelson lab, MTSU). In this sense, they have the potential to serve as an appropriate model for the AM: *C. neoformans* interaction in the lung.

Purpose: The goal of this research is to understand how intracellular *C. neoformans* infection affects host AM polarization state and gene expression.

Objective 1: Determine how *C. neoformans* infection impacts the macrophage M1

transcriptome: To achieve this, we will leverage transcriptome datasets (generated by Derek Wiggins; Nelson lab) comparing gene expression in FLAMs and J774-like macrophages exposed to the M1 polarizing agent, IFN γ , with and without *C. neoformans* infection. Lists of differentially expressed genes (DEGs) produced by comparing these datasets will be subjected to gene ontology (GO) analysis using the online bioinformatics platform, Database for Annotation, Visualization and Integrated Discovery [8+9]. This will identify biological processes and associated gene sets impacted by *C. neoformans* infection in both cell types.

Objective 2: Identify specific genes affected by *C. neoformans* infection of macrophages.

Here, we will validate a subset of representative DEGs associated with the biological processes identified in objective 1 using qRT-PCR. Successfully validated genes could serve as markers of *C. neoformans* infection and may provide insights into how the pathogen affects gene expression in host macrophages.

Collaboration with faculty mentor: Dr. Nelson will provide training in the use of DAVID for GO analysis and will help me make sense of the resulting data. Derek Wiggins (MOBI; Nelson lab) will provide training in qRT-PCR. I will participate in weekly research meetings with Dr. Nelson and Derek to go over my progress, present my findings, and plan new experiments.

References

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Timeline

<u>Week</u>	<u>Objective 1</u>	<u>Objective 2</u>
Week of February 12th	Bioinformatics training	
Week of February 19th	Bioinformatics training	
Week of February 26th	Analyze FLAM and J774 transcriptome data sets	
Week of March 4th	Spring break	
Week of March 11th		Tissue and yeast culture training
Week of March 18th		Tissue and yeast culture training
Week of March 25th		Harvest FLAM and J774 RNA and make cDNA libraries for qRT-PCR – (Repeat 1)
Week of April 1 st		Repeat 2
Week of April 15 th		Repeat 3
Week of April 22 nd		Perform qRT-PCR using the cDNA libraries produced over the previous 3 weeks
Week of April 29 th		Analyze data and write up results

Budget

1. The online tools used for the GO analysis described in objective 1 are free and no additional resources are required for this.
2. SYBR Green FastMix: This product is necessary for the qRT-PCR experiments described in objective 2. We are ordering the necessary amount to complete each repeat and the project. This is supplied by VWR with no additional shipping costs. Cost = \$251.34 (Fig. 1)
3. Sterile Polystyrene disposable serological pipets: This product is required for the tissue culture work associated with objective 2. This product is supplied by Fisher Scientific with no additional shipping costs. Cost = \$25.98 (Fig. 2)
4. All other materials required for objective 2 are already available within the Nelson lab. We do not anticipate additional costs.

The screenshot shows the Avantor VWR website interface. At the top, there is a search bar and a user greeting: "Hello, David.E.Nelson... My Account". The navigation menu includes "Products", "Applications & Protocols", "Featured Solutions", and "Services". Below the navigation, the breadcrumb trail reads: "Home > Nucleic Acid Reagents > qPCR/RT-qPCR Enzymes and Kits > PerfeCTa® SYBR® Green FastMix® Reaction Mixes, Quantabio". The main heading is "PerfeCTa® SYBR® Green FastMix® Reaction Mixes, Quantabio" with the supplier listed as "Quantabio".

The product description states: "This unique combination of proprietary buffer, stabilizers, and AccuFast™ Taq DNA polymerase delivers maximum PCR efficiency, sensitivity, and specificity, along with a robust fluorescent signal using fast, or conventional, cycling protocols with SYBR® Green qPCR". Key features listed include:

- 2x concentrated reagents minimize pipetting steps and improve accuracy
- Ultrapure, hot start AccuStart enzyme technology and anti-foaming technology
- FastMix formulation supports both fast and standard thermal cycling conditions

Additional text mentions: "AccuFast™ Taq DNA polymerase is a key component of this reaction mix. This hot-start Taq... More Product Information". A prominent blue "Order Now" button is visible.

Under "SPECIFICATIONS", the application is listed as "qPCR dye". Below this, there are tabs for "ORDER", "DOCUMENTATION", and "Q and A". An "Add to Cart" button is also present.

A table titled "Green FastMix®" provides detailed product information:

Description	Cycler compatibility	No. of reactions	Supplier No.	VWR Catalog Number	Unit	Availability	Your Price	Quantity
PerfeCTa® SYBR® Green FastMix®	Agilent AriaMx, BioRad CFX, QIAGEN Rotor-Gene Q, Quantabio Q, Roche LightCycler 480	250	95072-250	101414-276	Each	In Stock at VWR	\$251.34	0

Fig. 1: SYBR Green


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Description Specifications Product Certifications

Fig. 2: Serological Pipets