Title - Gene deletion in Cryptococcus neoformans using In-Fusion™ Technology

Program of Study – Cell & Molecular Biology

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Abstract:

Cryptococcus neoformans is a fungal pathogen that infects many immunocompromised individuals, especially those suffering from HIV/AIDS. C. neoformans first infects the lungs, and if not treated can travel to the central nervous system, where it causes a deadly meningitis. What makes C. neoformans so deadly is that it is particularly skilled at adapting to the human host in order to avoid being cleared by the immune system. An important aspect of host immune function involves changes in pH; in fungi, pH adaptation occurs via the Rim pathway. The Rim pathway senses the extracellular pH fluctuation when the pathogen enters the neutral/alkaline environment of the human host, and induces production of alkaline proteins in order to survive the pH change. Four specific C. neoformans genes, CNAG_01580, CNAG_02291, CNAG_05866, and CNAG_06473, were recently identified in a screen of Rim pathway-related mutants by collaborators at Duke University. In order to characterize these mutants, gene deletion constructs will be made using In-Fusion™ Technology, which fuses DNA fragments together into a cloning vector using DNA recombination. Once the deletion constructs are made, they will be transformed into wild-type C. neoformans cells using biolistic transformation. The resulting transformed strains will be assessed for alterations in virulence and pH-related phenotypes to characterize the function of each of these genes. Potentially, if these specific genes
are shown to have a significant effect on the disease, then they may serve as targets for antifungal therapy and lead to new treatments and help those afflicted with this and other fungal diseases.