Construction of barcoded *C. albicans* strains to study *in vitro* and *in vivo* competitive fitness

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*Candida albicans* is a species of fungus that typically resides in the oral cavity and gastrointestinal tracts of humans and other warm-blooded animals. Although *C. albicans* normally lives within the human host without causing disease, it can infect a variety of organ systems in hosts with compromised immunity. *C. albicans* is a major fungal pathogen in hospitalized patients around the world and can cause superficial, mucosal, and blood born infections. The same strains of *C. albicans* that inhabit a healthy human host commensally can act as opportunistic pathogens in an immunocompromised host (Nobel and Johnson, 2007). Although *C. albicans* reproduces primarily asexually, high levels of genetic variability are seen among clinical strains of *C. albicans* (Odds et al, 2007).

Previous studies from the Forche lab have shown that passage through a mouse host (*in vivo*) resulted in genetic and phenotypic changes in recovered *C. albicans* strains relative to the initial progenitor strain. The frequency of these changes was higher than what was observed during propagation in the laboratory (Forche et al, 2005 and 2009). Importantly, some genotypic and phenotypic changes were exclusively observed in *C. albicans* strains recovered from mice. This suggests that these changes may be important for adaptation to the host. Within the host, *C. albicans* exists as a population (many cells) rather than just as a single cell. Mutations could potentially confer a growth advantage (fitness gain) to some cells within this population, allowing those cells to outcompete other cells and take over the population. Furthermore, if these mutations increase the survival of the fungus, they could potentially increase virulence as well. Therefore, studying entire populations is crucial to more accurately reflect a true within-host situation. Recent studies in the Forche lab have analyzed many individual *C. albicans* strains from multiple populations recovered from different mouse hosts and have identified many changes in virulence-associated properties such as biofilm formation, secretion of lipases and proteinases, and growth at host temperature (Forche et al. unpublished). To assess whether these changes affect survival, persistence, and/or virulence of *C. albicans*, the Forche lab and its collaborators will be conducting a series of *in vivo* (using mouse hosts) and *in vitro* (using test tubes) studies in which strains with changes in virulence-associated properties are pooled and competed against each other to determine differences in fitness. In order to track the success of each *C. albicans* strain over the course of these competitive tests, each strain will be marked with a unique genomic barcode, which can then be used to quantitatively measure the success of each strain within the competition assay.

Over the summer, I worked on the construction of barcoded strains so that they can be pooled and competed against each other to analyze their fitness differences. In order to create barcoded strains, I used unique reverse primers, containing a different barcode for each strain, and a universal forward primer for all of the strains to amplify a portion of a bacterial plasmid containing the *NAT1* gene, which confers resistance to the antibiotic norceothricin, via polymerase chain reaction (PCR). The resulting *NAT1*-barcode construct was then incorporated into strains via transformation. I was able to identify *C. albicans* strains that successfully incorporated the barcode into the genome, by growth on plates containing norceothricin. I then isolated genomic DNA from the colonies that grew on plates containing norceothricin in order to perform diagnostic PCR and sequencing to confirm that the norceothricin-resistant transformants have the barcode inserted at the correct location in the genome and that no mutations were introduced into the barcode during transformation, respectively. Once constructed, barcoded strains, together with the barcoded progenitor strain, can be pooled and competed using various *in vitro* and *in vivo* growth conditions such different temperature or various antifungal drugs.

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