Researcher : Taneja nee Mahajan, Vibha (2003) Guide :Ganesan, K.(Dr.)

Development of a Transposon Tagging System for Candida Albican and its Use to Identify Drug Targets.

Candida albicans is one of the major causes of the increased incidence of fungal infections. An inhabitant of normal flora of gut, mouth and vagina, it flourishes because of several factors, including lowered immune system, stress, antibiotic overuse, oral contraceptives and steroid therapy. In India, a number of cases have been reported in the last two decades (Chakrabarti *et al.,* 1992). Patients with acute leukemia (Rajendran *et al.*, 1992), bronchopulmonary disorders (Phukan *et al.*, 2000), renal transplantation (Altiparmak *et al.*, 2002), HIV (human immunodeficiency virus) infection (Ghate *et al.*, 2000) and sick (Gupta *et al.*, 1996) and pre-mature neonates (Narang *et al.*, 1998) have been detected to be positive for Candida infections. *C. albicans* has been the major causative agent of these infections. The toxicities associated with the currently available antifungal drugs and the emergence of drug-resistant strains indicated the need for tools to identify novel potential drug targets. Hence, this study was aimed at developing tools that can be employed to identify essential and pathogenic determinants of *C. albicans*. The various chapters in this book portray the failures and successes during our journey towards this goal.

Chapter one describes the genetically refractory nature of *C. albicans* that has led to the delay in understanding the biology and genetics of this clinically important fungus. This chapter further briefly provides the information about the significant advancement that [illegible] been achieved in the last two decades to explore the molecular nature of *C. albicans*. [illegible] gene disruption strategies currently being employed for this diploid organism have [illegible] highlighted. The importance of transposons as an insertional mutagenesis tool has

Acc. No.: TH-142

been pointed out with emphasis on the need of transposon mutagenesis system for *C. albicans*.

Chapter two gives the various reagents and methodologies used during the course of this study. The oligos, plasmids and the strains used in this study have been tabulated for easy reference.

Chapter three starts with the description of an attempt to carry out *en masse* mutagenesis in *C. albicans* using a mini transposon that contains *LEU2* of *S. cerevisiae* as selection marker. The failure with this transposon did not deter our enthusiasm and instead encouraged us to construct a transposon that can be successfully used to create insertional mutants of *C. albicans*. This chapter describes the construction of Tn3-based transposon with *URA3* of *C. albicans* as marker for selection in yeast. The utility of the new transposon mTn[*CaURA3*] was demonstrated by using the *LEU2* of *C. albicans* as the target gene for transposon mutagenesis.

In chapter four, the mini-transposon, mTn[*CaURA3*], has been shown to be useful to identify targets of known drugs. This chapter also describes the haploinsufficiency screen to identify genes critical for the survival of *C. albicans*. A number of genes involved in diverse cellular processes were identified that can be further evaluated for their potential as drug targets. Hence, it was successfully established that transposon mutagenesis of *C. albicans* can be used to identify haploinsufficient phenotypes. However, the main limitation of this approach is that many essential genes might be missed in the screen if

they do not exhibit haploinsufficiency and might require disruption of both the alleles of the gene to exhibit a discernible mutant phenotype.

In order to overcome the limitation of the haploinsufficiency screen and to make the transposon mutagenesis more effective, we decided to engineer transposons to carry an 'outward-facing' promoter for an antisense effect. Instead of further modifying Tn3 transposon, we settled with the modification of Tn5 transposon because an *in vitro* system for Tn5 has been well defined. Furthermore, Tn5 has been reported to exhibit less sequence bias and hence might result in more random insertions. In Chapter five, the construction of two Tn5-based transposons that carried either *SAP2* or *SAP5* promoters is described. The two 'promoter-out' transposons were shown to carry out random insertions in a cloned gene. In addition, this chapter also describes the construction of two 'promoter-out' cassettes. The utility of the cassettes is yet to be demonstrated in the lab.

Finally, chapter six describes a new approach for targeted gene replacements in *C. albicans*. This approach which we refer to as 'directional ligation' is a simple and easy to use approach for synthesizing marker cassettes flanked with long homologous regions for gene disruption in *C. albicans* and has edge over other methods used for gene disruption. We could demonstrate its use by successfully disrupting several genes of *C. albicans*.