Chemogenomic profiling of the fungal pathogen *Candida albicans*

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Abstract

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Nosocomial blood stream infections and candidemia caused by the opportunistic fungal pathogen, *Candida albicans*, represent a serious medical problem. Hence, there is a great need to better understand the signalling and/or regulatory pathways that confers sensitivity and/or resistance to a given class of antifungal agent for this fungal pathogen. The main aim of the present study was to use a modified version of the gene replacement and conditional expression (GRACE) library to study the interactions between anti-fungal drugs (fluconazole, posaconazole and amphotericin B) and a set of non-conditional mutants in non-essential *C. albicans* genes. Further, the utility of using the modified version (GRACE v1.0) of GRACE library in comparison to the parent GRACE library was assessed. Overall, my findings showed that the chemogenomic profiles within drug classes were highly similar. The small amount of overlap between classes suggests that different drug classes interact with discrete networks of *C. albicans* genes. Importantly, my findings also demonstrate that screening of compounds, specifically azoles, in GRACE parent library may result in false positive findings possibly due to interaction with tetracycline. Future work involving further characterization of non-essential *C. albicans* genes is warranted to better understand the biology this model organism.

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List of Abbreviations

ARG	arginine
ALS	Agglutinin-like sequences
AMB	Amphotericin B
BSI	Nosocomial Bloodstream Infections
Ca	*Candida albicans*
CDC	Centre for Disease Control
CRISPR	Clustered regularly interspaced short palindromic repeat
Cg	*Candida glabrata*
CYP	Cytochrome P
DTT	DL-Dithiothreitol
DMSO	Dimethyl sulfoxide
dNTP	Nucleoside triphosphate
FLU	Fluconazole
FDA	food and drug administration
GRACE	Gene Replacement And Conditional Expression
HIS	histidine
Hwp1	hyphal wall protein

MTL	Mating-type-like
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
PEG	Polyethylene glycol
PCR	Polymerase Chain Reaction
POSA	Posaconazole
SC	Synthetic Complete
Sc	*Saccharomyces cerevisiae*
spp	Species
TE	Tris- EDTA
URA3	Uracil auxotrophic marker
YPD	Yeast Peptone Dextrose
WT	Wild Type
WHO	World Health Organization

CHAPTER ONE

LITERATURE REVIEW

1. Introduction: Fungal Pathogens

Virulence involves a complex interplay between a host and a microbe, a phenomenon that is particularly evident in fungal pathogenesis [1]. The impact of fungal pathogens on the plant and animal species, and associated ecosystem disturbances is well recognized [2, 3]. In contrast, the effect of fungal infections on human health is often overlooked with little or no mycological surveillance conducted by public health agencies such as World Health Organization (WHO) and Centre for Disease Control and Prevention (CDC; United States of America) [3]. In fact, billions of people world-wide are infected by pathogenic fungi every year, with over million deaths reported annually [3].

The human pathogenic fungi are broadly classified into two groups: commensals or opportunistic pathogens, and thermally dimorphic fungi or primary pathogens [1, 4]. The commensals such as *Candida spp*., dermatophytes, *Malassezia spp*., are normal constituents of the human micro-flora that can cause disease when the host defense settings are altered [1]. On the other hand, the primary pathogens, including *Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Penicillium marneffei*, and *Sporothrix schenckii etc.,* reside in specific environmental niches and affect individuals who have either been exposed to the spores or small yeast cells or who are immunologically naïve to fungal pathogen [1, 4].

Over the past two decades, there has been a dramatic increase in the incidence and prevalence of nosocomial (originating in a hospital) fungal infections [5]. Infections caused by the fungal pathogens range from catheter-related localized fungemia to widespread haematogenous dissemination, and differ in their nature, distribution and causative agents [6]. Among the most deadly fungal pathogens, *Candida* species (spp.) remains the most important and common cause of invasive fungal diseases in humans [5], and is discussed in detail in the following sections.

1.1 Candida Species

Candida species are by far the most studied group of opportunistic fungal pathogens and remain the most common group encountered in intensive care units, or during organ or bone marrow transplantation [8]. To date, more than 100 species of *Candida* have been described [6]. Most of the clinically common *Candida* spp. are asexual yeasts of phylum ascomycetes that are genetically diploid (haploid constitution of eight chromosomes) and capable of transitioning between a unicellular yeast form and a filamentous growth form [7]. An exception is the haploid *C. glabrata* which grows only in yeast form [7].

Candida spp. are responsible for causing a large spectrum of diseases, including candidemia with or without endopthalmitis, disseminated haematogeneous infections and chronic hepatosplenic candidiasis [8]. Importantly, *Candida* spp. are the third most common cause of nosocomial bloodstream infections (BSI) in global adult [9] as well as pediatric populations [10]. However, the true prevalence of *Candida*-related BSIs is often markedly underrepresented as the studies predominantly rely only on positivity in blood cultures [5]. This is in addition to the fact that the deep-seated pathogens in organs such as bones, muscles, joints or eyes often remain undetected [11]. Worldwide, ~75% of women suffer from *Candida* infection at least once in their lifetime [12]. It has been estimated that the healthcare costs associated with hematogenously disseminated candidiasis is approximately $2 - 4 billion per year in United States alone [13].

Until the early 1990s, predominant *Candida* infections were caused by *C. albicans,* which was found to adhere avidly to human tissues *in vitro* [14, 15] and to mammalian tissues in preclinical rodent models *in vivo* [16, 17]. Since then, there has been a steady increase in the frequency of non-*albicans Candida* species causing candidiasis [3, 11, 18]. Among the 160 species in the genus *Candida*, recent findings suggest that ~95 – 97% of *Candida*-associated

BSIs in the clinical setting are caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* [11], with the remaining 3% – 5% cases caused by 12 – 14 species, including *C. lusitaniae, C. guilliermondii,* and *C. rugose* [6]. Studies have also shown differences in the incidence rates of various *Candida* spp.-related BSI in various countries such as Spain [19], Iceland [20], Europe [21-25], United States [10, 13] and Australia [26] (reviewed in detail in [12, 18]). The epidemiological differences in the prevalence of these species in the clinical setting could be attributed to the differences in demographic characteristics, healthcare practices and co-morbidities of patients [8]. In addition, considerable differences in the virulence of *Candida* spp. and other factors such as sensitivity to anti-fungal drugs, degree of adherence and propensity to colonize etc. may also play a significant role [11]. This epidemiological trend also has profound consequences in the selection of anti-fungal therapies [18].

Overall, recent studies show that *C. albicans* remain the most common species causing invasive candidiasis worldwide and is detected in more than 50% of clinical isolates in patients with hematogenous candidiasis, thereby making it a dominating fungal pathogen of the genus *Candida* [12, 27-31].

1.1.1 *Candida albicans*

Historically, *C. albicans* is known since 400 BC when renowned Greek physicians, Hippocrates and Galen, identified oral aphthous lesions and named it as “thrush” [32]. Later in the 19th century, Langenbeck discovered a fungi in the gastrointestinal tract of patient [33], the etiology of which was subsequently demonstrated by Berg [34]. In 1843, the pathogen was named as '*Oidium albicans*' by Robin [35] which was later followed by more than 100 synonyms. It was only in 1923 the pathogen was named as *Candida albicans* by Berkhout, a

denomination currently accepted as the name of this species [36]. The first case of *C. albicans* invasive candidiasis was documented in 1861 [37].

Candida albicans is an opportunistic fungal pathogen that exists as a harmless commensal in the oral cavity, skin, gastrointestinal and genitourinary tracts of warm-blooded animals including humans [11]. However, in patients with high risk factors, *Candida* spp. that colonize in the gut invade other organs, either through translocation or through anatomical leakage, and subsequently cause secondary metastatic infections [11]. The incidence of *C. albicans* induced systemic candidiasis in the United States is approximately 20 cases per 100,000 people (or about 60,000 cases per year) with the incidence reportedly higher in patients who have been hospitalized for longer durations [38]. In spite of the advances in the diagnosis and treatment of candidiasis over the past two decades, the infection still causes high mortality rates [11, 39].

a. Virulence and pathogenicity

Pathogenicity is the ability of a fungi to adapt to the host environment and defenses, and cause harm to the host niches [40]. The pathogenicity of *Candida* species is attributed to various virulence factors, including phenotypic switching, the ability to enter and/or evade host defences, the expression of adhesins and invasins, and biofilm formation. These have been reviewed in detail in [7, 40-42] and are discussed in brief in the following sections.

b. Morphogenesis

The morphological diversity of *C. albicans* is a striking feature that promotes its survival, growth and dissemination in the host [40, 43]. The various morphological forms range from unicellular white or opaque budding yeast to true hyphae, with various other forms in the transition collectively termed as pseudohyphae [44, 45]. The yeast cells are characterized by

polar growth followed by isometric expansion of buds. Polarized growth is defined by a crescent-shaped polarisome at the tip of the growing bud [45]. In contrast, buds produced by pseudohyphal cells remain attached to the parent even after septum formation resulting in filaments with constrictions at the junction of the septa [44, 45]. True hyphae form when an unbudded yeast cell extends a germ tube with parallel side-walls, and is defined by both a polarisome and a Spitzenkorper [12, 14, 45]. Occasionally, thick-walled cells called Chlamydospores are formed in response to stress at the ends of pseudohyphae and hyphae [9, 43].

The ability of *C. albicans* to switch between yeast, hyphal and pseudohyphal forms are often considered to be necessary for virulence [44, 46]. It is believed that the filamentous forms, (hyphae and pseudohyphae), are uniquely invasive as they express apical hydrolytic enzymes [47] and that the yeast form is more suited for dissemination via the blood stream [46]. But, it is also important to recognize that most dimorphic fungi exhibit yeast growth in diseased tissues and exist as filamentous fungi in the external environment [44, 46].

Pioneering studies of morphological switching have documented various environmental conditions that could trigger the yeast to hyphal formation [48]. These include addition of serum, *N*-acetyl-glucosamine, temperature of 37°C, neutral pH, 5% carbon-dioxide and high phosphate concentrations [48]. The role of various signaling pathways and the cell cycle in the regulation of morphogenesis have also been elucidated and are discussed in detail elsewhere [44, 45, 48, 49].

c. Adhesins and invasins

Adherence of cells to the host tissues and the subsequent invasion is a complex phenomenon utilizing various adhesins and invasins expressed on morphogenetically changing cell surfaces [50]. The key part of these initial contact events is the adherence of fungal cells to host epithelial cells, whose outcome governs all subsequent interactions between fungus and host [25]. Although various adhesins are known, the best characterised, most studied families of adhesins are agglutinin-like sequences (*ALS*) and the hyphal wall protein (Hwp1) [7, 38]. Following adherence and recognition of the pathogen by host cells, hyphae are engulfed via a clathrin-mediated mechanism. Importantly, the ability to induce this endocytic uptake is restricted only to hyphae and not yeast cells [41]. The latter predominantly follow an active penetration process involving the Secreted Aspartic Protease (SAP) family. See [33, 41] for detailed review on adhesins and invasins.

d. Mating in *C. albicans*

Originally classified as an asexual organism, a novel parasexual reproduction process has also been elucidated for *C. albicans* [50, 51]. The *C. albicans* genome contains open reading frames that are similar to the *S. cerevisiae* mating-type genes, viz. *MAT***a***1*, *MAT*α*1* and *MAT*α*2*, that are organized into two non-homologous mating-type-like (MTL) loci, *MTL*a and *MTL*α, on chromosome 5 [51]. However, unlike *S. cerevisiae***,** **a** and α cells in *C. albicans* must transform to the relatively unstable opaque form to be mating competent [50, 51]. The *C. albicans* mating locus also contains three additional pairs of genes: *PAP*, *PIK*, and *OBP* [50]. The sexual and parasexual reproduction in *C. albicans* has been reviewed in detail in [50, 51].

1.2 ANTI-FUNGAL DRUGS

Since the early 1950s, there have been significant developments in the discovery of anti-fungal drugs. However, the efficacy of all of the exiting anti-fungal drugs are often limited by

inherent toxicity, emergence of resistance, inability to counteract biofilm formation and/or restrictions in drug formulation [38, 52]. Antifungal drug development is complicated by the fact that fungal cells are eukaryotic, like the human host, thereby making it much more difficult to identify selective pathogen-specific targets for drug discovery programs. In addition to this is the difference in sensitivity of various *Candida* species to the commonly used anti-fungal drugs.

Hence, there is a great need to better understand the signalling and/or regulatory pathways that facilitate the growth as well as the virulence of this fungal pathogen. The current armamentarium for treatment of *Candida*-BSIs are primarily limited to three classes of antifungal drugs; polyenes, azoles and echinocandins [11, 38, 52].

1.2.1 Polyenes

The polyenes, such as amphotericin B and nystatin, were the first class of antifungals to reach the clinic [53]. Although amphotericin B was once considered to be the 'gold standard' anti-fungal therapy, infusion-related adverse effects as well as dose-limiting nephrotoxicity limited its use [55]. These molecules bind to the fungal cell membrane sterol, ergosterol, to create pores and allow leakage of intracellular ions as well as inhibit membrane transporters [56]. At low concentrations of amphotericin B, the channels are permeable only to monovalent cations [55]. In contrast, at higher concentrations, the non-aqueous channels interact with ergosterol or cholesterol in the cell membrane to form aqueous pores that are permeable to both chloride and potassium, resulting in intracellular acidification and subsequent membrane damage [55]. Amphotericin B is generally recommended for invasive infections such as fungemia, osteomyelitis and chronic disseminated candidiasis, particularly for patients with neutropenia or critical illness where the organism has not been identified to the species level [52].

1.2.2 Azoles

The discovery of azoles marked a significant milestone in the antifungal drug discovery programs [53]. In comparison to its predecessor (polyenes), azoles are superior in terms of safety, efficacy, and oral bioavailability [57]. The azoles are broadly classified as imidazoles, which contain two nitrogens, and triazoles, which have three nitrogens on the azole ring [55]. Imidazoles are generally topical formulations, with an exception of ketoconazole administered systemically, for the treatment of mucosal and superficial fungal infections [55]. The structural modifications of ketoconazole led to discovery of four triazole molecules that are currently preferred antifungal drugs in the clinical setting: fluconazole, itraconazole, voriconazole, and posaconazole [57]. The mechanism of action of azoles are similar to that of polyenes in that they exert their pharmacological actions within the fungal cell membrane [53]. However, azoles inhibit cytochrome P (CYP) 450-dependent 14-α-demethylase (CYP51) resulting in inhibition of ergosterol formation leading to fungal cell membrane disruption and inhibition of growth [55]. This mechanism of action of azoles also results in cross-inhibition of some CYP-dependent enzymes in humans, which may contribute to toxicity profile seen in humans [52].

1.2.3 Echinocandins

Echinocandins are a recent class of antifungal drugs that are large lipopeptide molecules containing an amphiphilic cyclic hexapeptide with an N-linked acyl lipid side chain [57]. The first echinocandin to be approved by FDA was Caspofungin acetate that is synthesized from a fermentation byproduct of the filamentous fungus Glarea lozoyensis [53, 57]. Subsequently, Micafungin and Anidulafungin also were approved by FDA for various types of candidemia. Their mechanism of action involves inhibition of synthesis of β-(1,3)-D-glucan, an integral component of the fungal cell wall, thereby leading to weakened cell wall integrity and cell lysis [53, 57].

1.3 Genetic screens in *C. albicans*

Functional fungal genome-wide studies facilitate identification of genes essential for the life of a fungal pathogen, which in turn define targets for antifungal drug discovery programs. For many decades *S. cerevisiae* has been an invaluable surrogate model to study the gene function in *C. albicans* [58]. However, unlike *S. cerevisiae*, *C. albicans* is a diploid polymorphic fungus without a full sexual life cycle [59]. These apparent differences have questioned the use of *S. cerevisiae* to study *C. albicans* biology [59].

Completion of *C. albicans* genome sequencing project in 2004 marked a significant milestone in the functional analysis of the *C. albicans* genome. However, the use of molecular tool kits such as forward genetics has been challenging in this model organism as it lacks meiosis [59]. Despite these limitations, researchers have used various efficient gene disruption techniques to create *C. albicans* mutant libraries for studying *C. albicans* biology [59]. Figure 1 provides a brief comparison of the three commonly used mutant libraries of *C. albicans* [60-62]. Although, all these libraries are potentially a useful resource for both basic and applied studies, each of them have their own limitations [59] (See [63, 64] for reviews).

1.4 Rationale

As already noted, the nosocomial blood stream infections and candidemia caused by the opportunistic fungal pathogen, *C. albicans*, is a significant medical problem. Despite the high incidence and mortality rates in humans, relatively few efficacious antifungal drugs currently exist. Furthermore, the emergence of drug-resistant fungi and toxicity limit the clinical usefulness of these drugs. As a result, there is an urgent need for a new generation of antifungal drugs with superior safety and efficacy against a broad fungal spectrum.

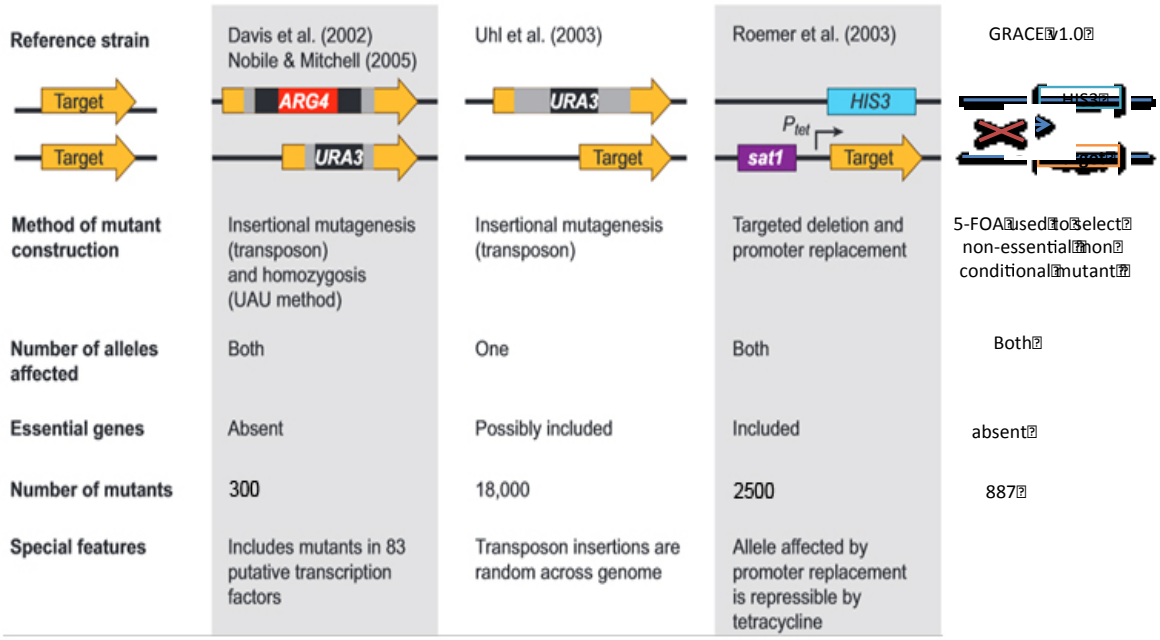


Figure 1. A comparison of four largest mutant collections of *C. albicans*

The completion human genome-sequencing project in 2001 marked the culmination of unprecedented advances in the science of genomics [65]. However, out of the vast majority of genes discovered, only a handful of them have been characterised [65]. Although the functional phenotype of each gene can be studied using classical genetics approaches, it is time consuming [65]. This situation has given rise to a new interdisciplinary field in science called "chemogenomics" - discussed in detail elsewhere [58, 65]. The use of chemogenomic profiling offers promise for characterizing the global cellular response to an arbitrary compound, for predicting the mode of action of a compound, and for inferring the function of genes [58]. The availability of the *C. albicans* deletion libraries such as gene replacement and conditional expression (GRACE) has enabled researchers to investigate the functional relationships between genes and chemical compounds in a systematic and unbiased fashion.

However, the requirement of tetracycline treatment to shut off gene expression is a significant limitation as the observed interactions may be consequence of the interaction of compound screened with tetracycline [61]. Hence, to avoid these potential false-positive findings, previous work in our laboratory involved construction of a derivative library (GRACE v1.0) from the GRACE collection to create a collection of non-conditional non-essential inactivated genes. Identification of *C. albicans* genes whose inactivation leads to either sensitivity or resistance of commercial antifungal drugs using GRACE v1.0 library could facilitate identification of drug targets for new anti-fungal agents.

1.5 Objective and Aims

1. To use the GRACE v1.0 library to study the interactions between specific anti-fungal drugs (fluconazole, posaconazole and amphotericin B) and a set of non-conditional mutants in non-essential *C. albicans* genes.

2. To characterise the utility of our modified version of GRACE library (GRACE v1.0) and compare it with the parent GRACE library.

CHAPTER TWO

MATERIALS AND METHODS

2. Materials and Methods

2.1 Strains, Oligonucleotides and Plasmids

The plasmids, strains, and oligonucleotides used are summarized in Table 1.

Table 1. List of oligonucleotides, strains and plasmids

	Oligonucleotides	
Experiment	**Oligo**	**Sequence 5'-3'**
SN76 *NPY1 C. albicans* strain construction *(See Section 2.6)*	NPY1-S1	CCACCAAAAAAAAAATATTTTTCTCTTTCTATTTTA ACATAGCTGCAATTTTTATTTCAACTTGTTCTTTTC TTTCTTTCTTTCTTTTGCTCTTCTTTGTGaagcttcgtacgct gcaggtc*
	NPY1-exF	AAAACTATTGACTTAAACTC
	NPY1-S2	CTAAATTTTTTTTTTTTGCAGCAAAAAGTTGAAAAA AGAAAAAGAACTCTTAAAACAACTCTAATGTATTT AATAATAATCTATCTCCTGTTCTACtctgatatcatcgatgaat tcgag*
	NPY1-exR	ATTTTAACAAACACTGATTT
	X2-CaHIS1	CAACGAAATGGCCTCCCCTACCACAG
	X3-CaHIS1	GGACGAA TTGAAGAAAGCTGGTGCAACCG
	X2-CaARG4	AAT GGA TCA GTG GCA CCG GTG
	X3-CaARG4	GAGGAGTACGACCTCAAGCGC
SN148 *PAA11* strain construction *(See section 2.7)*	LR182F	atttgGTGGCCCCTGAATTGTGTGCg*
	LR182R	aaaacGCACACAATTCAGGGGCCACc*
	LR183F	AGAAAGATGTTCCCGTGAAAAGTTCAACTACCGCT TGACGGTGGCCCCTtAATaGTGTGC*
	LR183R	AGCATTGTATTTGTAGTCGTACTCTCTGACAAACA AGCttGCACACtATTaAGGGGCCAC*
	LR184F	GTGTTGGAGAATAGACAGCG
	LR184R	GCGGGTACATTCATTGTTGG

Strains

Strain	**Genotype**	**Source**
SN148	*arg4Δ/arg4Δ*, *leu2Δ/leu2Δ*, *his1Δ/his1Δ*, *ura3Δ/ura 3Δ*	[62]

SN76	*arg4Δ/arg4Δ*, *his1Δ/his1Δ*, *ura3Δ/ura3Δ*	[62]
CaSS1	his3::hisG/his3::hisG leu2::tetR-GAL4AD-URA3/LEU2	[61, 66]
GRACE library	Collection of regulated gene shut-off constructions	[61]
GRACE1.0 library	Collection of null, non-regulated non-essential genes	Made by one of my lab member (Yuan)
Plasmids		
S.No	**Plasmid**	**Source**
1	pFA Arg4	[67]
2	pFA His1	[67]

**In the oligonucleotide sequence, capital letters represent those that are homologous to the NPY1 sequence in SN76 and the small letters represent sequences homologous to the plasmid. *Lowercase letters in LR182 primers represents the nucleotides necessary to attach the gRNA sequence into the plasmid by complementarity. Lowercase letters in LR183 primers indicates the changes in nucleotides compared with wild type sequence. These changes introduce stop codons; an enzyme restriction site and also they disrupt the PAM sequence.*

2.2 Drugs

Fluconazole (FLU) and posaconazole (POSA) were purchased from Sigma-Aldrich, and Amphotericin B (AMB) was purchased from MP. For these studies FLU and POSA were dissolved in 99% ethanol and 99% methanol, respectively, and AMB was dissolved in 90 % dimethyl sulfoxide (DMSO).

2.3 Growth Media

Strains were grown in liquid mixture of 1% yeast extract, 2% peptone, 2% glucose and 50mg/L uridine (YPD plus uridine; nutrient supplement) at 30°C, and the synthetic plates were supplemented with 100 mg/L of arginine, or histidine to allow optimal growth of *his1*- or *arg4*- auxotrophs.

For screening purposes, 200µl liquid YPD with specific concentrations of each compound (discussed in later sections) was used. Liquid YPD was used for overnight cell cultures as well. Solid YPD plus uridine mixture was used for cell culture studies. For transformation experiments, solid synthetic complete –his or -arg was used.

2.4 Compound screening in GRACE v1.0 library

The GRACE (Gene Replacement and Conditional Expression) library represents approximately 2500 strains that include regulated expression constructs for both essential as well as non-essential genes. Much of the work in the literature has been focused on the role of essential genes. Hence, to characterize the role of non-essential *C. albicans* genes, a modified version of GRACE library (GRACE v1.0) was designed previously in our laboratory. Additionally, unlike the conditional mutants, these non-conditional mutants do not require the addition of tetracycline. This in turn prevents any potential drug-drug interactions that may occur otherwise.

2.4.1 Experiment One

A total of 887 non-essential mutants of *C. albicans* from GRACE (gene replacement and conditional expression) v1.0 (Appendix; Table A1) library collection were organized in 96-well microtiter plates. Briefly, approximately 2 µl of the each strain of cells from saturated cultures were transferred to fresh 200 µl of YPD with uridine using a replicator-pinning tool. Then, the same tool was used to transfer approximately 2 µl samples of cells from the intermediate dilution in YPD to 200 µl fresh YPD without drug to function as controls. Similarly, 2 µl samples of cells were transferred from the intermediate dilution of YPD to 200µl fresh YPD media with known concentrations of each drug (Figure 2). The concentration of FLU and POSA used in this study were 10 and 0.1 µg/ml, respectively, and that of AMB was 1 µg/ml. These initial drug concentrations were selected to allow saturated

growth of the control strains after 3 days, while drug free controls showed saturated growth in less than 2 days. Each well had an approximately 10^{-4} dilution of the saturated culture to provide the starting density. The plates were then incubated at 30°C for five days (Figure 3). If a strain showed saturated growth before 3 days in the presence of the drug it was classified as resistant. If a strain failed to grow or showed saturation of growth after 5 days, it was called as sensitive. Each experiment was repeated at least 3 times. The growth was quantified by Tecan Sunrise machine. A semi-quantitative scoring scale represented the day that saturation growth was observed. A score of 1 and 2 represented resistance, and 5 and 6 represented sensitivity.

2.4.2 Experiment Two

Strains that showed consistent sensitivity in all 3 replicates for both FLU and POSA were then tested again using different concentrations of the drug; FLU (10 μg/ml, 7 μg/ml and 3 μg/ml) and POSA (0.1 μg/ml, 0.07 μg/ml and 0.03 μ g/ml). Similarly, the strains that were consistently resistant to both drugs were once again tested at different concentrations of FLU (10 μg/ml , 15 μg/ml and 20 μ g/ml) and POSA (0.1 μg/ml, 0.15 μg/ml and 0.20 μ g/ml). Subsequently, 5 of the most sensitive and resistant strains were chosen from the highly sensitive and resistant strains and their growth curves were measured in presence of FLU (3 μg/ml) and POSA (0.03 μg/ml) and these strains were designated super-sensitive or super-resistant. Likewise, strains that showed consistent sensitivity to AMB were tested again using different concentrations of the drug, viz. 1 μg/ml, 0.7 μ g/ml and 0.3 μg/ml, and 5 of the most sensitive strains were randomly chosen from the most sensitive strains and their growth curves were measured in presence of 0.3 μg/ml of AMB.

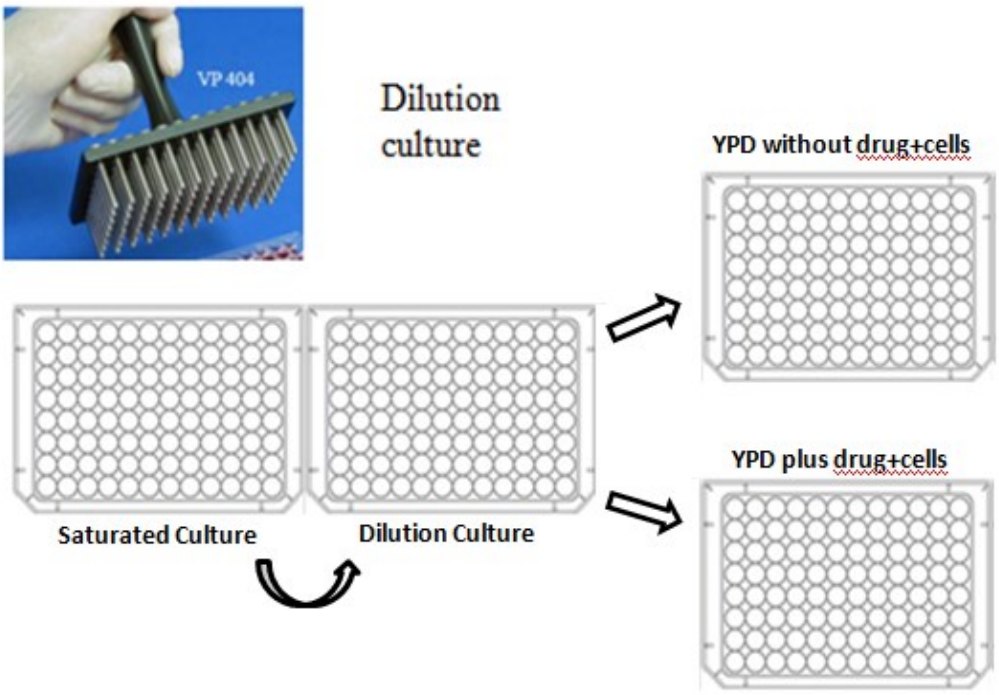


Figure 2. The experiment of screening of the GRACE v1.0 in 96 well plates with replicator-pinning tool.

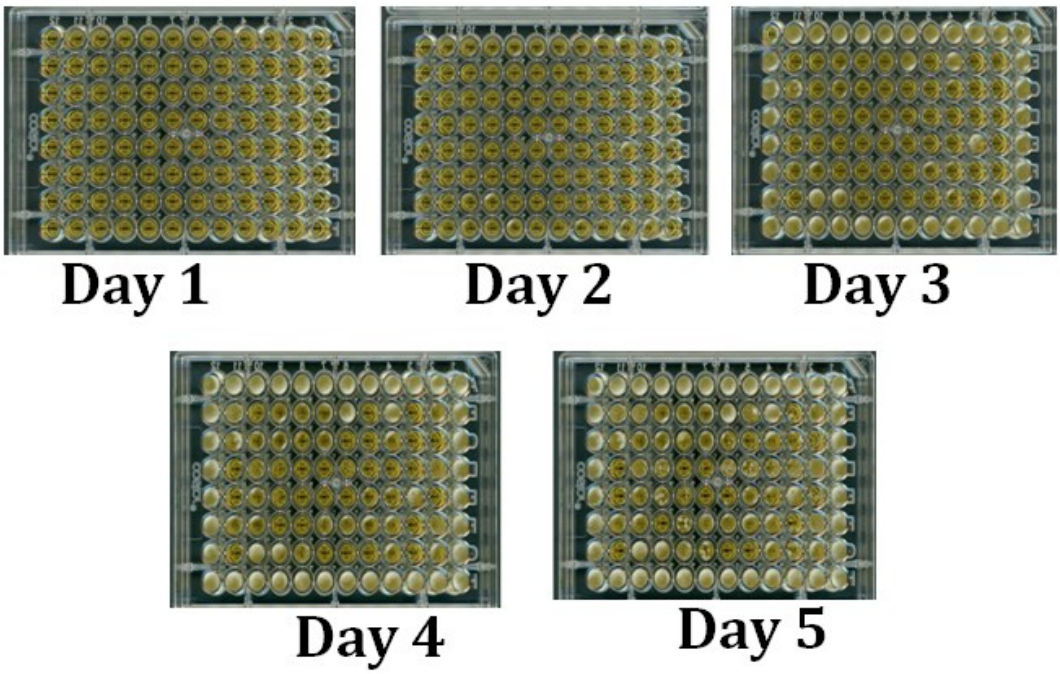


Figure 3. Screening of GRACE v1.0 in 96 well plates for five days to select sensitive and resistance mutants.

2.5 Comparison of sensitive and resistant strains in GRACE vs GRACE v1.0 library

On completion of Experiment Two, the top 5 sensitive and resistant strains were chosen for comparison. Because of the appearance of unrelated loss of heterozygosity events in a subset of the GRACE v1.0 library, candidates of the sensitive and resistant classes for each drug were tested with the original GRACE strain under tetracycline inactivation. The protocol used for this study as well as the doses for FLU, POSA and AMB were similar to that of Experiment One (Section 2.4.1).

2.6 Construction and validation of SN76 NPY1 strain

2.6.1 Plasmid extraction

Escherichia coli strain DH5alpha was used for plasmid amplification. Lysogeny Broth (LB) media was prepared and supplemented with 100 µg/mL Ampicillin (LB plus Amp) after autoclaving and cooling the agar to 55°C. The strains of *E. coli* were then streaked onto media and incubated overnight at 37°C. The following day, single colonies of *E. coli* were cultured in 5ml of LB plus Amp overnight at 37C, and were stored in 4°C until further use. The following day, plasmid DNA was extracted using QIAprep Spin Miniprep Kit (Qiagen) as per manufacturer's protocol. However, in the final step of extraction, the plasmid DNA was eluted in sterile distilled water instead of EB buffer provided along with the kit.

2.6.2 PCR amplification of deletion cassette

A 500-µL reaction mixture, with appropriate 120bp foreword primer-S1 (100 bp are homologous of the *NPY1 (NAD(+) diphosphatase)* sequence in SN76 and S1 is homologous of the plasmid as well for 120bp reverse primer-S2 (mentioned in Table 1). The size of pFA-ARG4 plasmid is 2017bp. The mixture for PCR was made in a 1.5 mL sterile tube, and is summarised in Table 2. I expected the size of the band 2257bp. pFA means backbone of the

plasmid and it is similar in all other plasmid like pFA-*HIS1* this allowed the "open" construction of pFA-modules for *Candida albicans* in which single components can easily be exchanged for new ones upon availability [67].

The thermal cycling parameters for PCR are as follows:

- Denaturation at 98°C for 2 min
- 25 Cycles of 98°C for 30 sec and annealing/extension at 55°C for 30 sec.
- Final extension at 72°C for 10 min*

*If the sample was to be left overnight in the machine, a final step at 4°C for unlimited time was added to the program to prevent degradation of the samples.

In order to confirm the amplification of the desired template, the final PCR products were run on a 1% agarose gel to confirm the size of the final product.

Table 2. PCR reaction mix for amplification of deletion cassette

REAGENT	For 50 µL reaction	For 500 µL reaction
Q5 Buffer (New England Bio labs)	10	100
Sterile distilled water	31.5	315
10 mM dNTPs	2	20
10 µL Forward primer (10nM)	2	20
10 µL Reverse primer (10nM)	2	20
Plasmid (diluted 1:20; 100 µg/ml)	2	20
Q5 enzyme (New England Bio labs)	0.5	5

2.6.3 Precipitation of DNA

On confirmation of the amplification of the desired template, 495 µl of each PCR product was mixed with 1/10 volume of 3 M sodium acetate (pH 5.3). To precipitate the DNA with

ethanol, the sample was then mixed with 2X volume of 99% ethanol and gently shaken for 5 - 10 min. The samples were then precipitated overnight at -80°C. The following day, the samples were centrifuged at 4°C for 30 min (14000 rpm). The supernatant was discarded and the DNA pellet was washed in 70% ethanol and re-centrifuged. The washed pellet was then air dried and re-suspended in 100 µl of sterile distilled water. The DNA was quantified using a nanospec instrument.

2.6.4 Strain Transformation

The SN76 *C. albicans* strain was grown in a Falcon tube with 5mL of YPD at 30°C overnight with shaking. The following day, 200 µl of the overnight culture was centrifuged (30 sec @ 9000 rpm) and the pellet was washed using 200 µL of 1 M lithium acetate in Tris- EDTA (TE) to permeablize the cell wall of *Candida albicans* to permit DNA transformation. After re-centrifugation, the pellet was re-suspended in 100 µL of freshly prepared ice cold one-step buffer (1 mL of buffer contains 200 µL of 1 M lithium acetate in TE and 800 µL of 55 % w/v PEG (polyethylene glycol), 15 mg DTT (DL-Dithiothreitol), 25 µL single-stranded salmon sperm DNA). Next, 10 µl of precipitated DNA (@ 2, 6 and 10 µg) collected from *Section 2.6.3* was added and the transformation mixture was incubated at 30°C for 1h to overnight on a shaker. The mixture was then incubated at 44 °C for 15 – 30 min and cells were plated onto solid synthetic complete media (SC) plus uridine plates without arginine and incubated for at least 3 days at 30°C. Single colonies of the transformed strain were then isolated and streaked onto separate plates with the same media (SC plus uridine without arginine) and incubated at 30°C. A colony PCR with a reaction mixture (summarized in Table 3) and primer (X2-CaARG4which is 20 bp of ARG4 plasmid was used as reverse primer and *NPY1*-exF, which is 20 bp of *NPY1* was used as a forward primer, or X3-CaARG4 and *NPY1*-exR; summarized in Table 1) was performed to confirm the knockout of one of the alleles of the *NPY1* gene (the *NPY1* gene has two alleles) as shown in figure 4 B [67]. The thermal cycling parameters

used were: 94°C for 3min, 25 cycles of 94°C for 30 sec, 51°C for 1 mins, 72°C for 3 mins, and 72°C for 10min. After confirmation of the first knockout allele with *ARG4* cassette, strains heterozygous at *NPY1* were taken to transfer the second allele with the *His1* cassette. I PCR amplified the *His1* cassette (his1 cassette size is 1429 bp) with flanking sequences and transform *C. albicans* followed by selecting His+ transformants and confirmed deletion of the second allele of *NPY1* by doing colony PCR as shown in figure 4(C). Then I confirmed that the his+ insertion has not replaced the arg+ insertion allele, and also there is no sequence of *NPY1* left in the genome by colony PCR using ether primers: X2- CaHIS1 and *NPY1*-exF or X3- CaHIS1 and *NPY1*-exR; summarized in Table 1).

Table 3. Reaction mixture for transformed strain PCR

REAGENT	For 50 µL reaction
10 x Taq Buffer	5
Sterile distilled water	38
50 mM $MgCl_2$	1.5
10 mM dNTPs	1
10 µL Forward primer	1
10 µL Reverse primer	1
Strain (diluted 1:20)	2
Taq enzyme (5U/µl)	0.5

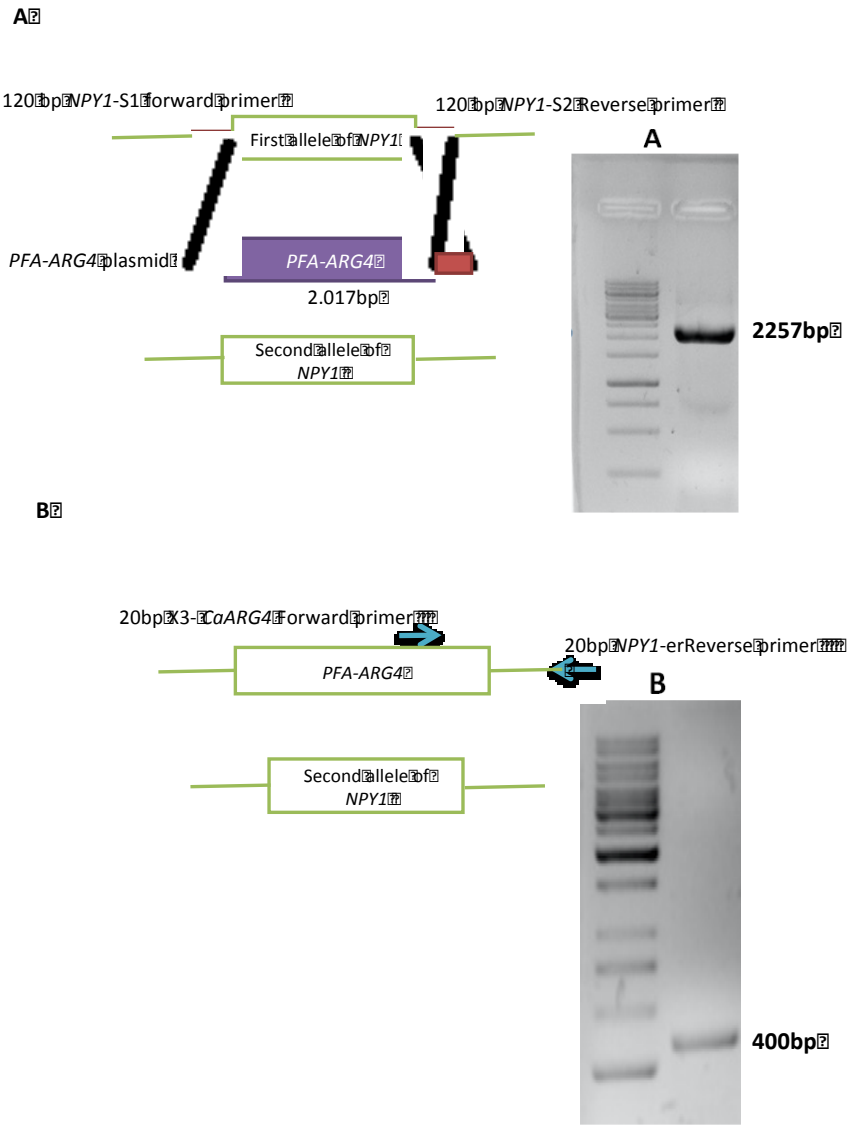


Figure 4: *NPY1* Transformation.

(A) Amplification of pFA-ARG4 deletion cassette for disruption of the first allele of *NPY1* with 120bp foreword primer-S1and 120bp reverse primer-S2 and 2.017bp of *ARG4* sequence from the pFA-ARG4 plasmid. This generates an expected band size of 2257bp.

(B) To confirm the first knockout, a colony PCR with a reaction mixture primers (X2-CaARG4 which is 20 bp internal to the *ARG4* gene was used as reverse primer and 20 bp of the flanking sequence of *NPY1* was used as foreword primer. The expected size of the band is 400bp.

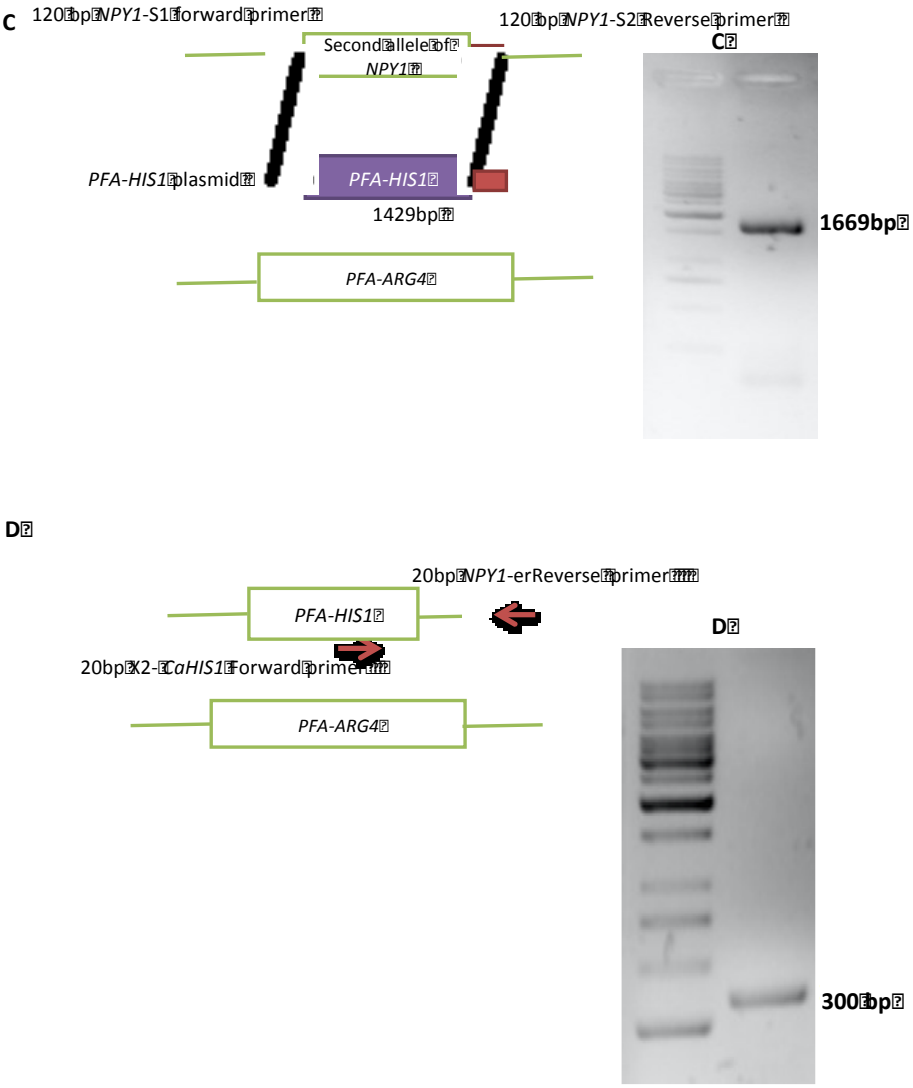


Figure 4: *NPY1* Transformation.

(C) Amplification of pFA-HIS1 cassette for disruption of the second allele of *NPY1* .120bp foreword primer-S1, 120bp reverse primer-S2, and 1429 bp of HIS1 from the pFA-HIS1 plasmid. This generates an expected band of 1669 bp.

(D) To confirm the second knockout, a colony PCR with a reaction mixture primers (20 bp X2-CaHIS1 of *HIS1* gene used as foreword primer and 20 bp of the flanking sequence of *NPY1* was used as reverse primer. The size of the band is 300bp.

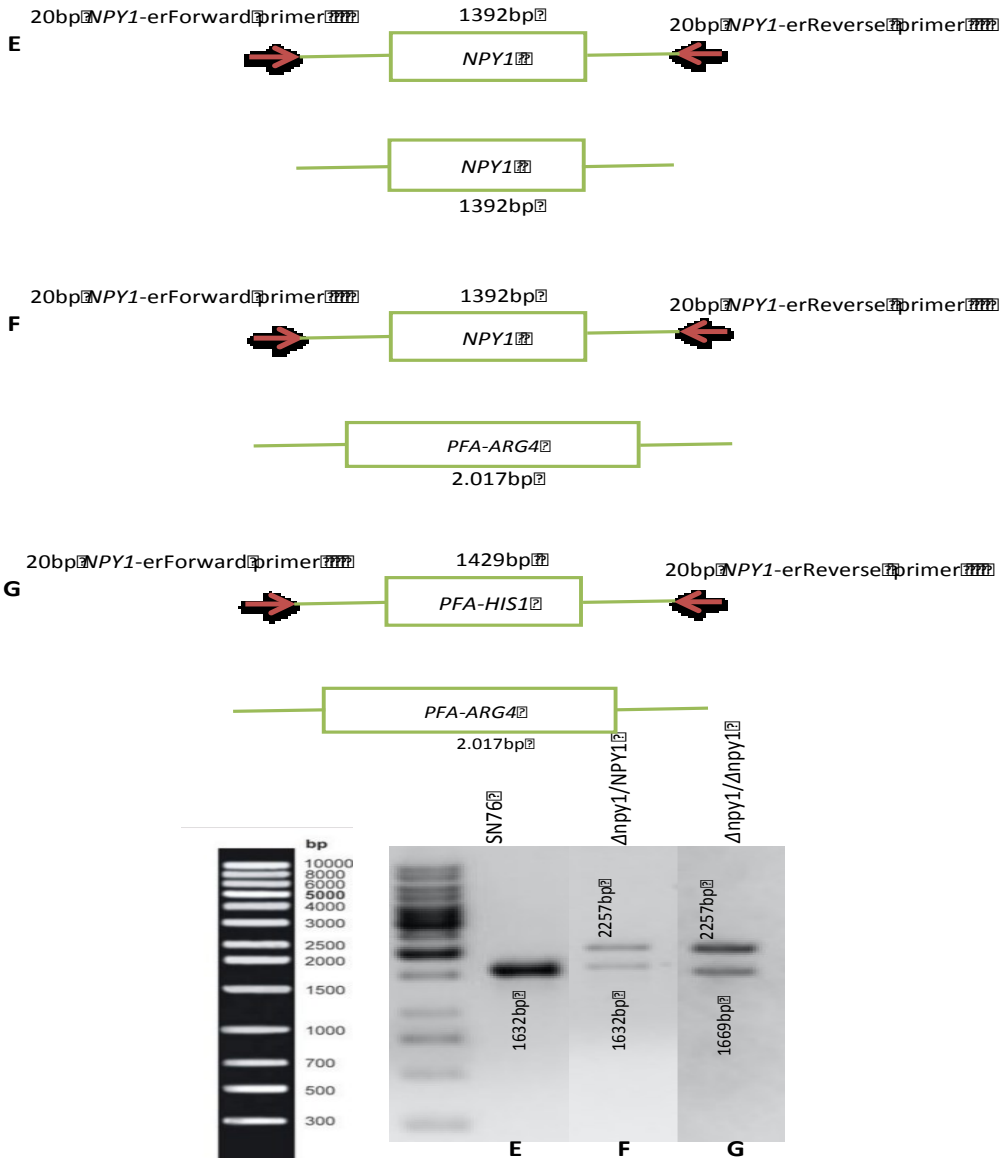


Figure 4: *NPY1* Transformation.

(E) Conformation *NPY1* Transformation with *SN76* using 20 bp primers upstream and downstream in the flanking sequence of *NPY1*. Expecting one band of 1632 bp.

(F) Conformation *NPY1* Transformation generating *heterozygous NPY1* using 20 bp up and down of the flanking sequence of *NPY1*. Expecting two bands,(1632 bp (*SN76*) and 2257bp (*ARG4*)).

(G) Conformation *NPY1* Transformation generating *homozygous npy1* using 20 bp up and down of the flanking sequence of *NPY1*. Expecting two bands (2257bp (*ARG4*) and 1669bp (*HIS1*)).

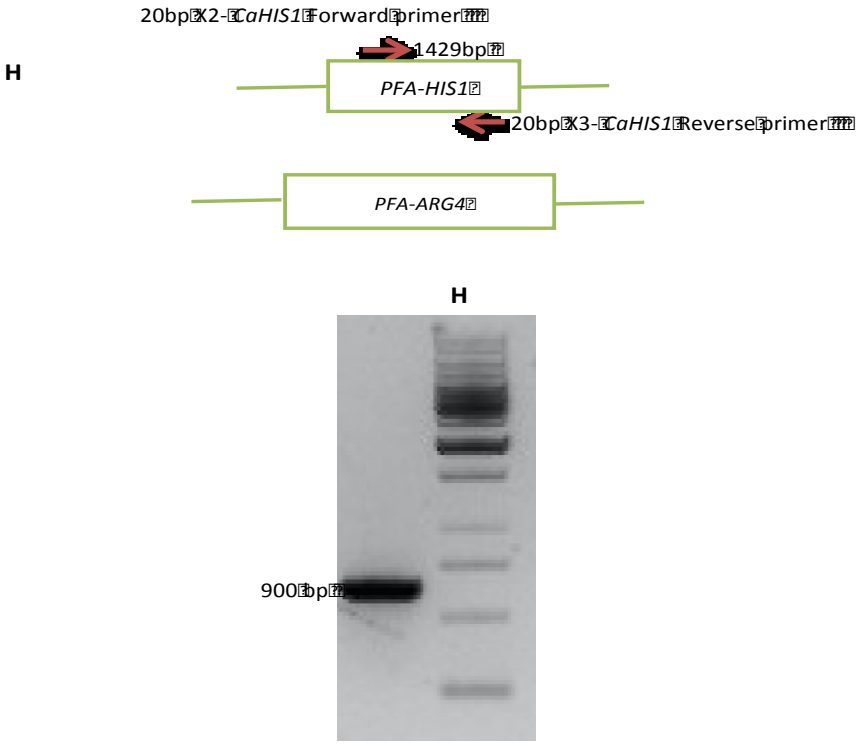


Figure 4: *NPY1* Transformation.

(H) Two 20 bp primers within *HIS1* in homozygous *npy1*. The expected size of the band is 900 bp.

2.7 Construction of SN148 PAA11 strain

The SN148 *PAA11* (Potential polyamine N-acetyl transferase) strain was constructed using CRISPR, as previously documented [68]. Briefly, phosphorylated and annealed *PAA11* guide RNA primers (LR182F and LR182R; Table 1) were ligated to CIP-treated BsmBI-digested pV1093 vector. Mutagenic double stranded oligonucleotide (LR183F and 183R) was used as a repair DNA. This oligonucleotide is complementary to *PAA11* and contains a mutation on PAM sequence, two premature stop codons (UAA, UAG) and a *Hind*III restriction site. Standard LiAc transformation was done on the SN148 strain. Transformants obtained on YPD Nat plates were screened by PCR using the primers LR184F and LR184R followed by *Hind*III digestion. Correct clones were verified by sequencing.

2.8 Bioinformatics analyses

To analyse different genes within a class, I used a Venn diagram program (http://www.bioinformatics.lu/venn.php)**.** The ortholog comparisons among fungal species were done using a Venn diagram program available for comparing 4 lists of data (http://bioinfogp.cnb.csic.es/tools/venny/) [69].

CHAPTER THREE

RESULTS

3. Results

3.1 Compound screening in GRACE v1.0 library

3.1.1 Experiment One: Azoles

Among 887 non-essential mutants of *C. albicans* from the GRACE v1.0 library, 119 strains were resistant and 81 strains were sensitive to FLU (10 µg/ml). Testing of POSA (0.1 µg/ml) showed 101 resistant and 145 sensitive strains. Both compounds gave clear sensitivity and resistance profiles, and the overlap between the two different azole compounds was considerable (Figure 5A and B). The list of strains that were resistant and sensitive to FLU, POSA and both compounds are tabulated in Table A2, A3 and A4, respectively.

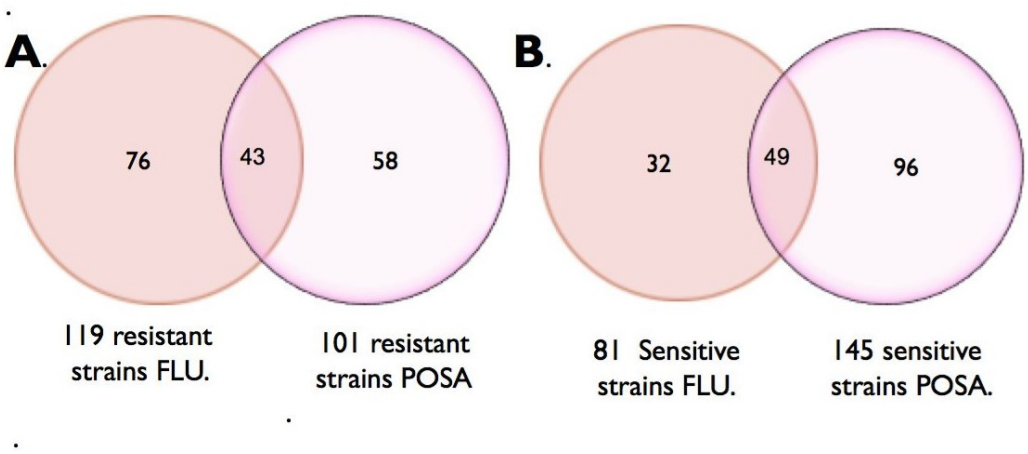


Figure 5. Comparisons of genes showing sensitivity or resistance to two azoles, FLU and POSA. A) 43 strains showed a common resistance to both FLU and POSA. B) 49 strains showed a common sensitivity to both FLU and POSA.

3.1.2 Experiment One: AMB

In contrast, among 887 non-essential mutants of *C. albicans* from the GRACE v1.0 library, 268 strains were sensitive and zero strains (Table A5) were resistant to AMB (1 µg/ml).

3.1.3 Experiment Two: Azoles

A set of 37 strains out of the 49 consistently sensitive strains was then tested at diminishing concentrations of both FLU (10 μg/ml, 7 μg/ml and 3 μg/ml) and POSA (0.1 μg/ml , 0.07 μg/ml and 0.03 μg/ml). Likewise, 37 resistant strains out of the 43 commonly resistant strains were tested with increasing concentrations of FLU (10 μg/ml, 15 μg/ml and 20 μg/ml) and POSA (0.1 μg/ml , 0.15 μg/ml and 0.2 μg/ml). Table 4 summarizes the five of the most sensitive and resistant strains to both FLU and POSA. The growth curves measured by Tecan machine for the 5 super resistant, and 5 super sensitive strains to azoles are shown in Figure 6A and B. The comparative growth curves of the super sensitive and resistant strains and *cass1* WT strain in the presence of FLU and POSA over 5 day period are shown in Figure 7, 8, 9 and 10.

Table 4. Strains showing super-sensitivity or super-resistance to both FLU and POSA.

Super Sensitive Strains		**Super Resistant Strains**	
Orf name	**Gene name**	**Orf name**	**Gene name**
orf19.2557	*SEC65*	orf19.767	*ERG3*
orf19.3482	*NPY1*	orf19.6199	*HCS1*
orf19.4631	*ERG251*	orf19.260	*CaORF6_1455*
orf19.7269	*PAA11*	orf19.1773	*RAP1*
orf19.4448	*SOG2*	orf19.459	*ADP1*

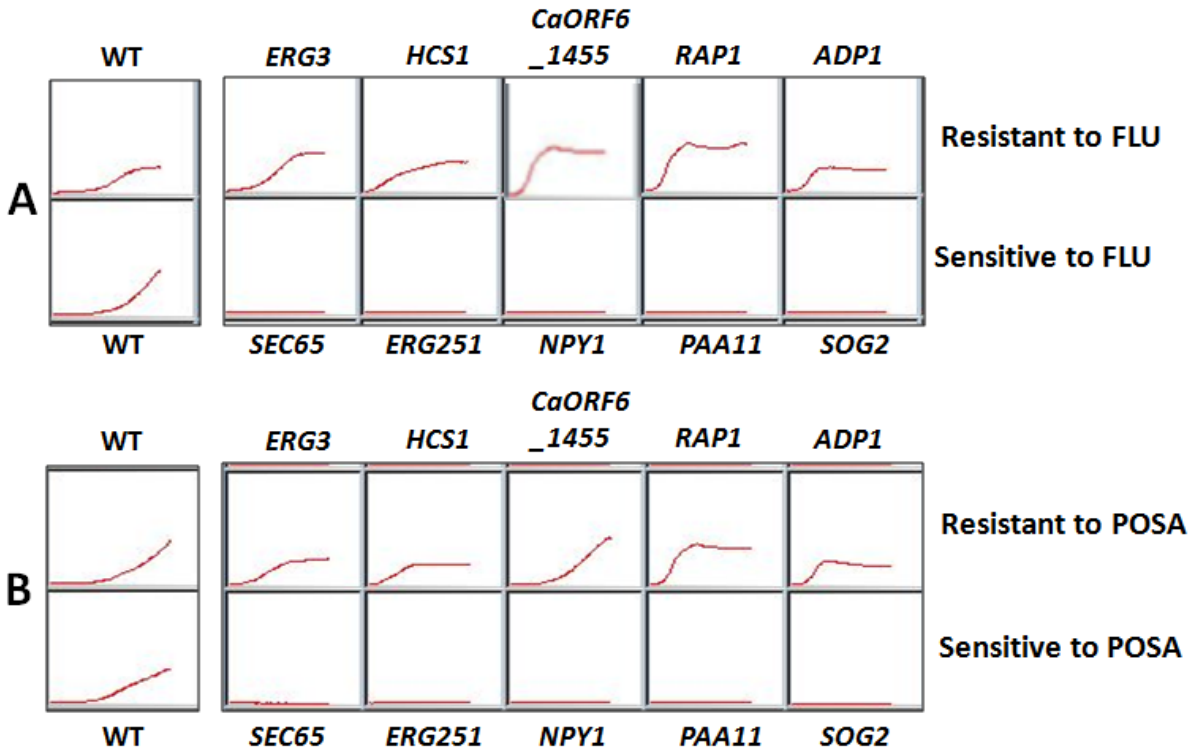


Figure 6. The growth curves of super resistant and super sensitive strains in presence of (A) FLU (10 µg/ml) and (B) POSA (0.1 µg/ml).

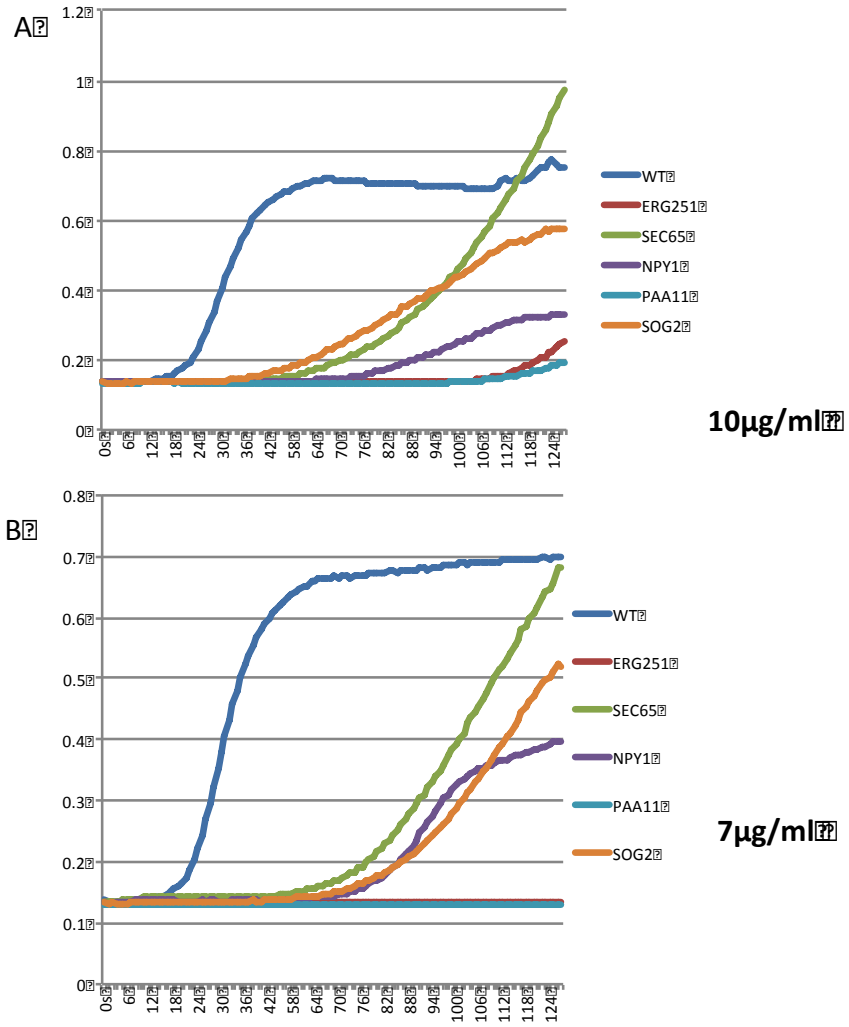


Figure 7. Growth curves of the sensitive *C. albicans* strains and cass1 WT strain in presence of FLU (A: 10µg/ml and B: 7µg/ml) over 5 days period.

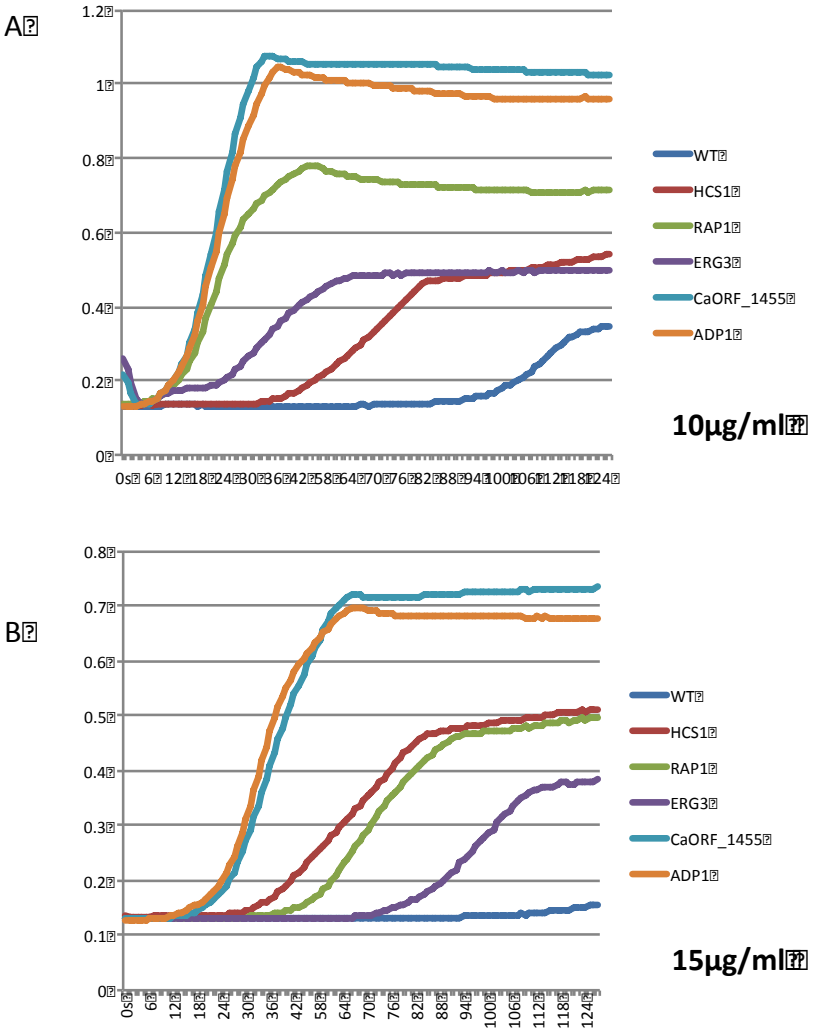


Figure 8. Growth curves of the resistant *C. albicans* strains and cass1 WT strain in presence of FLU (A: 10µg/ml and B: 15µg/ml) over 5 days period.

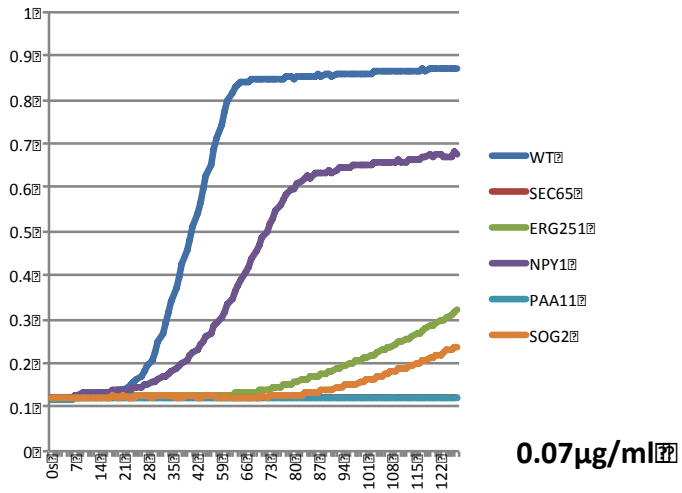


Figure 9. Growth curves of the super sensitive *C. albicans* strains and cass1 WT strain in presence of POSA (0.07µg/ml) over 5 day period.

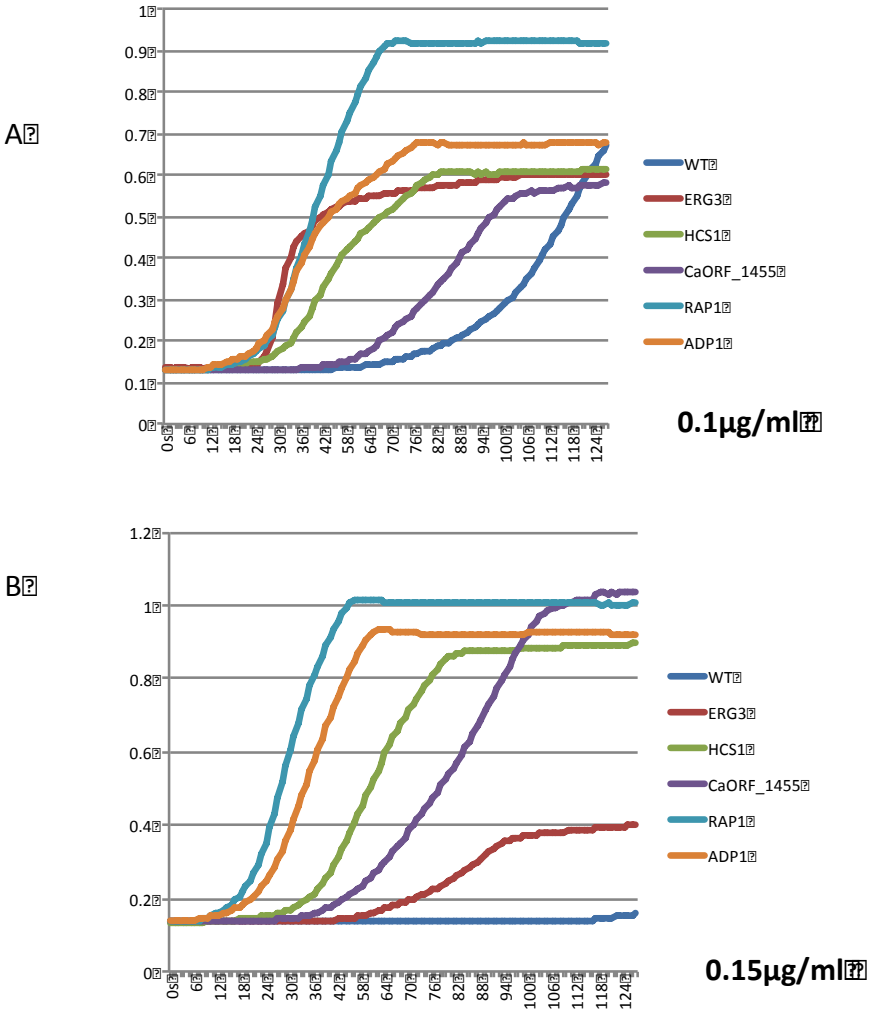


Figure 10. Growth curves of the super resistant *C. albicans* strains and cass1 WT strain in presence of POSA (A: 0.1µg/ml and B: 0.15µg/ml) over 5 day period

3.1.4 Experiment Two: AMB

Figure 11 summarizes the growth curves of 5 of the super sensitive strains in the absence and presence of AMB at 1µg/ml. Table 5 summarizes the five of the most sensitive strains to AMB. Growth curves of the super sensitive strains and *cass1* WT strain in presence of AMB over 5 days (0.7µg/ml and 1µg/ml) or 8 days (1µg/ml) are shown in Figure 12.

Table 5. Strains showing super-sensitivity to AMB.

Super Sensitive Strains	
Orf name	**Gene name**
orf19.5013	*PCM1*
orf19.2216	*PDS5*
orf19.7655	*RPO21*
orf19.5667	*MNR2*
orf19.147	*YAK1*

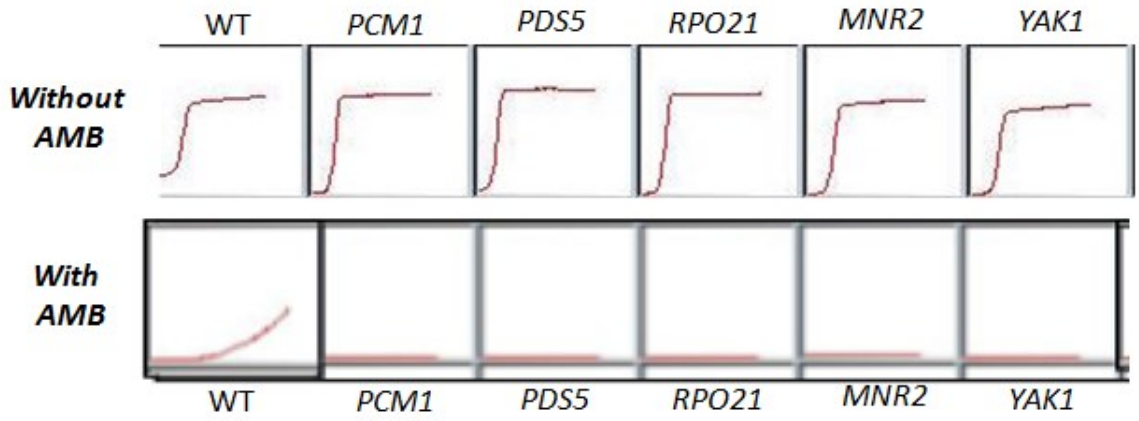
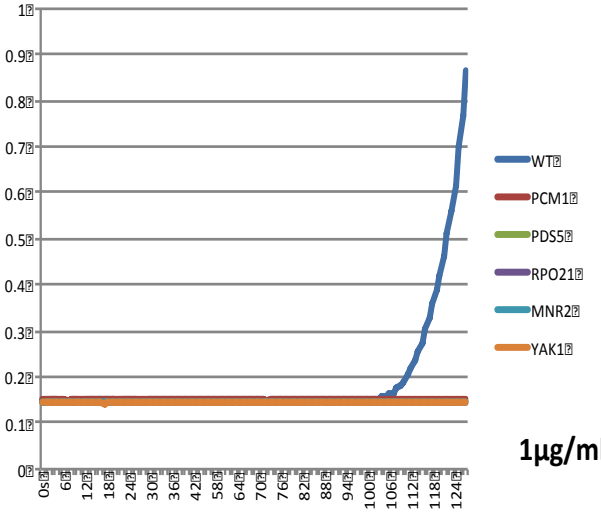


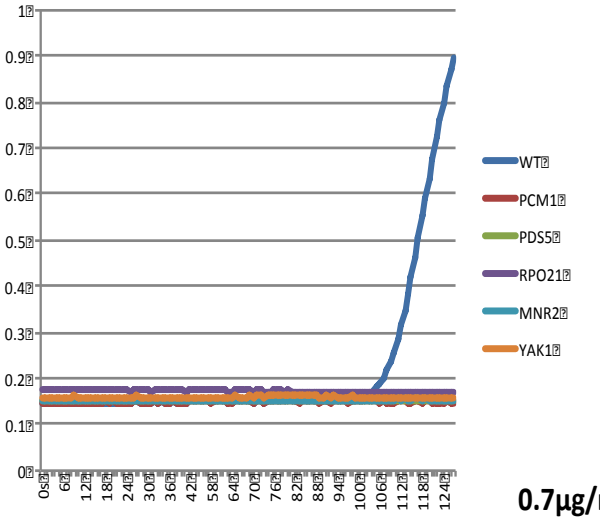
Figure 11. Growth curves of super sensitive strains in the absence and presence of 1µg/ml AMB.

A



1μg/ml

B



0.7μg/ml

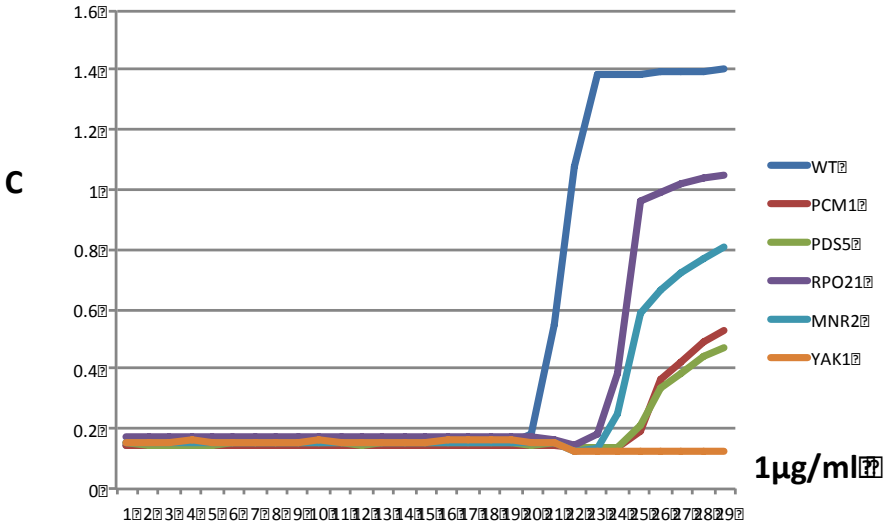


Figure 12. Growth curves of the super sensitive strains and cass1 WT strain in presence of AMB (A) 1µg/ml, WT is red color and (B) 0.7µg/ml over 5 days, WT is red color, and (C) 1µg /ml over 8 days.

3.2 Comparison: GRACE v1.0 vs GRACE library

3.2.1 Azoles

Figure 13 shows the growth profile of the five of the super sensitive and resistant strains in GRACE and GRACE v1.0 library, in the absence of drug. The results for the 5 strains that were resistant to azoles (both FLU and POSA) when tested in GRACE v1.0 library were also resistant when tested in the GRACE library where strains were inactivated by tetracycline. In contrast, 3 out of the 5 strains (null mutants for *NPY1, PAA11*, and *SOG2)* that are sensitive to FLU and POSA in GRACE v1.0 library were shown to not be sensitive when tested in the GRACE library (Figure 14).

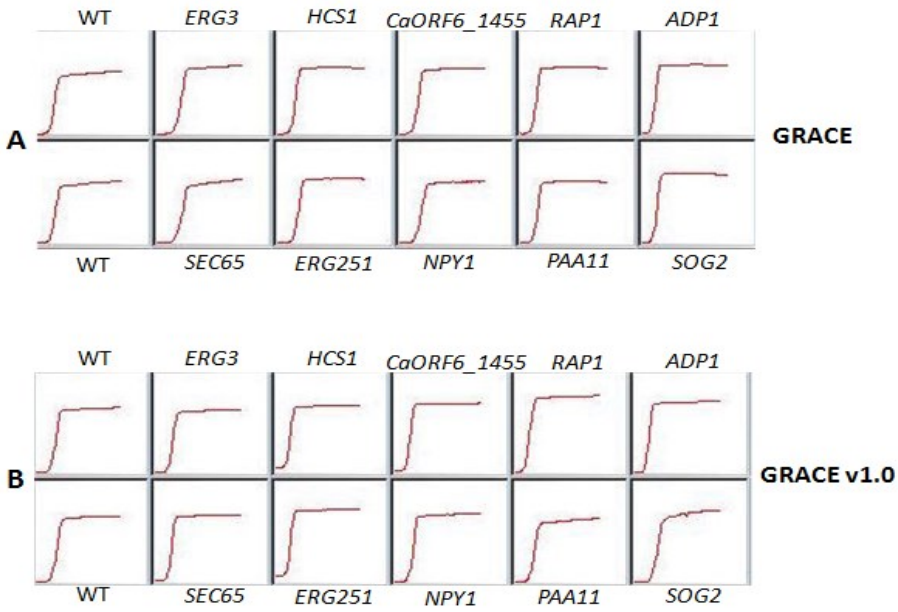


Figure 13. Growth curves of five super sensitive and resistant strains in (A) GRACE and (B) GRACE v1.0 library in the absence of drug.

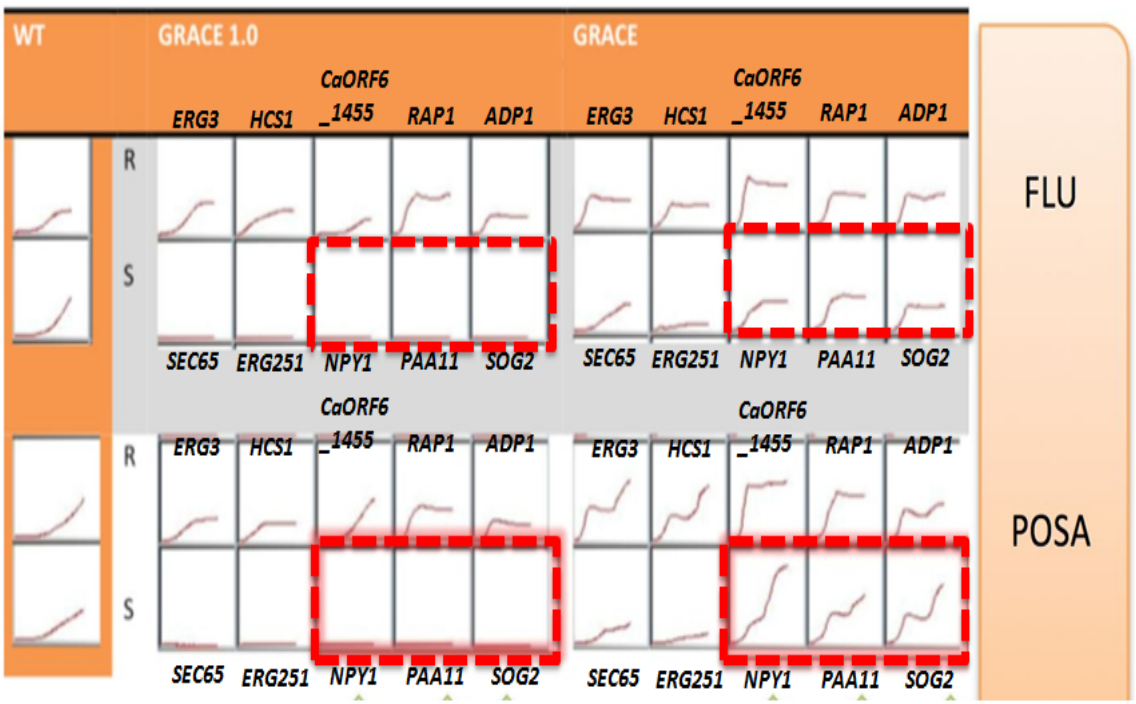


Figure 14. Comparison of five strains that are super sensitive and super resistant to FLU and POSA in GRACE v1.0 library and GRACE library inactivated by tetracycline. Dotted red box indicates the differences in sensitivity/resistance profiles between GRACE v1.0 and GRACE library for NPY1, PAA11 and SOG2 mutants.

3.2.2 AMB

Consistent with the results of GRACE v1.0 library, the 5 strains that were super sensitive to AMB were also sensitive when tested in GRACE library (Figure 15).

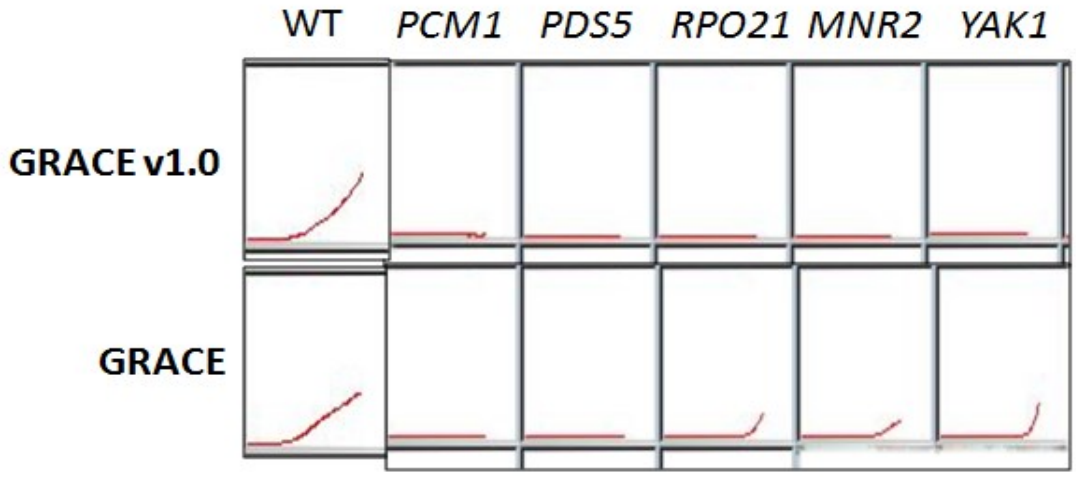


Figure 15. Comparison of five strains that are super sensitive to AMB in GRACE v1.0 library and GRACE library inactivated by tetracycline.

3.3 Screening of Azoles in SN76 NPY1 mutant and SN148 PAA11 mutant strains

3.3.1 NPY1 mutant SN76 strain

The knockout of *NPY1* gene in both the alleles of SN76 *C. albicans* strain was confirmed using colony PCR for the Arg marker (Figure 4A) and the His marker (Figure 4B). The testing of *NPY1* mutant SN76 strain with 10 µg/ml FLU (Figure 16A) and 0.1 µg/ml POSA (Figure 16B) over 5 day period showed sensitivity profile for both azoles *c.f.* SN76 WT. These findings are consistent with that of the results obtained in GRACE v1.0 library.

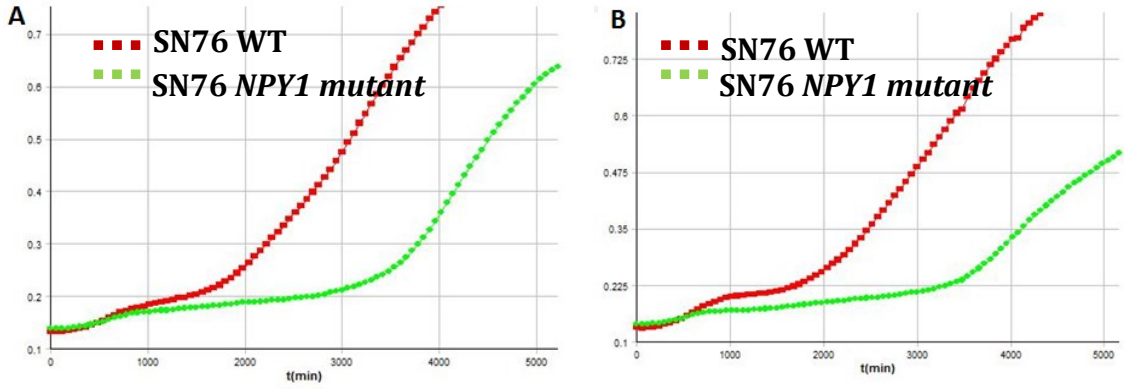


Figure 16. Growth curves showing sensitive profiles for SN76 NPY1 mutant strain in the presence of (A) 10 µg/ml FLU and (B) 0.1 µg/ml POSA c.f. WT SN76 strain.

3.3.2 PAA11 mutant SN148 strain

The *PAA11* mutant SN148 strain generated using CRISPR and the respective WT SN148 strain were tested in the presence of 10 µg/ml FLU (Figure 17A) and 0.1 µg/ml POSA (Figure 17B) over a 5 day period. My findings showed that the *PAA11* mutant SN148 strain was sensitive to both FLU and POSA *c.f.* SN148 WT strain, as seen in GRACE v1.0 library,

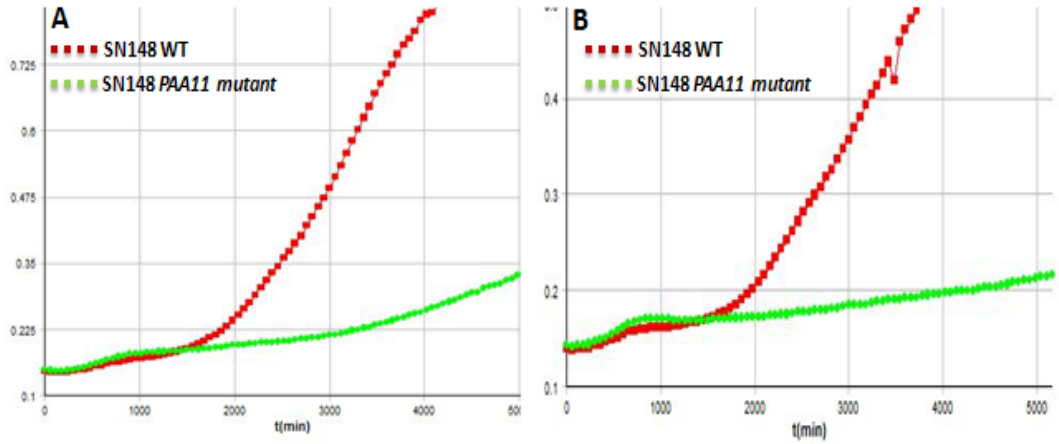


Figure 17. Growth curves showing sensitive profiles for SN148 PAA11 mutant strain in the presence of (A) 10 µg/ml FLU and (B) 0.1 µg/ml POSA c.f. WT SN148 strain.

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

4.1 Discussion

In the present study, I screened an in-house derived collection of approximately 900 non-essential, transactivator-defective disruption *Candida albicans* strains (GRACE v1.0 library) against classic anti-fungal drugs to identify genes that confer either enhanced sensitivity or increased resistance. Specifically, I examined the effects of two azoles, fluconazole (FLU) and posoconazole (POSA), as well as the polyene, amphotericin B (AMB). Overall, the chemogenomic profiles suggest that different drug classes interact with discrete networks of *C. albicans* genes.

To date, *Candida* spp., most notably *C. albicans*, has been known to cause a substantial fraction of human fungal disease [3]. These fungal pathogens are the third most common cause of nosocomial infections in the global adult, [77] as well as pediatric, populations [78]. The major classes of antifungal drugs include polyenes, azoles, and echinocandins [29,43,48]. However, poor efficacy and/or dose-limiting side effects limit the existing antifungal agents. Futhermore, there is an increase in the growth of populations of drug resistant strains [3]. Hence, there is a great need to better understand the signalling and/or regulatory pathways that confers sensitivity and/or resistance to a given class of antifungal agent in this fungal pathogen.

The completion of *C. albicans* genome sequencing project in 2004 gave rise to various molecular tool kits, including various *C. albicans* mutant libraries [27,57]. These libraries have served as invaluable tools to investigate the genes that confer resistance or sensitivity to antifungal drugs and to study the pathobiology of this model organism [27,57]. Among these libraries, the gene replacement and conditional expression (GRACE) set has been widely used to study various *C. albicans* functions. However, a significant limitation of this approach includes the use of tetracycline, which may potentially interact with the compound

used or the process investigated in the study [56]. Hence, a modified version of this library involving a collection of non-conditional non-essential inactivated genes was recently constructed and validated in our laboratory, and was used for the experiments herein.

In the present study, first I screened the 887 non-essential mutant *C. albicans* strains from GRACE v1.0 library in the presence of commercially available azole antifungal drugs fluconazole (FLU) and posaconazole (POSA). Among the 887 mutant strains, 49 and 43 strains were found to be sensitive and resistant, respectively, to both FLU and POSA (see Table A4). Next, 37 strains that were both sensitive (or resistant) to FLU and POSA were randomly chosen and were screened again using various concentrations of the azole drugs. The top 5 strains that were sensitive (*SEC65*, *ERG251*, *NPY1*, *PAA11* and *SOG2* mutants) and resistant (*ERG3*, *HCS1*, *CaORF6_1455*, *RAP1*, *ADP1* mutants) to both FLU and POSA were chosen for subsequent studies. A brief description of the role of these genes has been summarized in Table 6.

My findings for *SOG2* and *ERG3* are consistent with that of reported previously [60,64]. However, the functions of the other genes remain uncharacterized in *C. albicans*. Additionally, my findings showing that *ERG3* mutant strain was resistant to azole, but sensitive to AMB, further implies that different classes of drugs targeting the same biosynthetic pathway also may have different chemogenomic profile. Although, the function of *RAP1* has been characterized in *C. glabrata* [16] and *S. cerevisiae* [59], comparison of genetic disruptions conferring drug sensitivity in other fungi should be carefully done. In order to compare the genomic profile for azole sensitivity among different fungi, datasets of azole sensitive *S. cerevisae* and *C. glabrata* strains from previously published large-scale chemogenomic profiling studies were collected [45,62,65]. Since there were two large-scale

studies involving *S. cerevisae* [45,65], first I determined the common set of azole sensitive genes in *S. cerevisae* using the Venn diagram program (http://www.bioinformatics.lu/venn.php). My analysis revealed that out of 330 and 403 azole-sensitive genes from Dr. Boone's lab [45] and Dr. Tyers lab [65], respectively, only 91 genes overlapped (Appendix A8). The dataset generated was further compared with that of the 14 azole sensitive *C. glabrata* strains from Dr. Kuchler lab [62] (Appendix A10) and the 49 azole sensitive *C. albicans* strains from my study (Appendix A9) using the Venn diagram program. Interestingly, the comparison of the genomic profile for azole sensitivity among different fungi showed no common genes (Figure 18). Hence, this suggests that the chemogenomic profile of a given antifungal azole drug can differ significantly among organisms even when the apparent target of the drug is the same [62,65]. Furthermore, as shown in Figure 18, the number of strains that are sensitive to azoles in *S. cerevisiae* is much higher than the other fungal organisms suggesting that azoles affects numerous signalling pathways in *S. cerevisiae*. In support of this notion, recent work by Epp and colleagues involving screening of *S. cerevisiae* to identify synergistic drug interactions that render FLU fungicidal and validation of the same in *C. albicans* showed that out of 22 predicted genes in *S.cerevisiae* only one gene mediated FLU tolerance in *C. albicans* [19].

Of specific interest, null mutants of two genes involved in the ergosterol biosynthetic pathway, *ERG3* and *ERG251/ERG25*, seem to result in resistant and sensitive profiles, respectively, to azoles. There is still some confusion as to how to address why deletion of *ERG3* causes resistance to azoles and *ERG251/ERG25* causes sensitivity. *ERG3* encodes the Δ5, 6 desaturase (also known as Erg3p), an enzyme that acts late in the ergosterol biosynthesis pathway [2]. Erg3p is responsible for converting tolerated 14-methyl intermediates, which accumulate because of azole inhibition of the 14C-lanosterol

demethylase, into the toxic sterol 14-methylergosta-8,24(28)-dien-3,6-diol [2]. Therefore, *ERG3* inactivation should and does confer azole resistance. Or in other words, *ERG3* destroys a beneficial compound and replaces it with a toxic variant if active, in the presence of azoles, so the absence of Erg3p prevents the beneficial compound from being destroyed and keeps the cells living and thereby makes them resistant to azoles. The explanation of *ERG251/25* is more complicated. In *S. cerevisiae*, *ERG25* deletion confers resistance to cells in presence of azoles [2]. In Candida biofilms, *ERG25* expression is upregulated by the presence of azoles in general and fluconazole in particular [40]. The difference in biofilms in comparison to the yeast is that growth happens in anaerobic conditions in the former. This could be one reason why the expression of *ERG251/25* increases in this case. However others like Boreca-Melkusova and colleagues [10] have shown that while expression of *ERG25* increases in *Candida dubliniensis* biofilms on fluconazole exposure, the same is not observed in *Candida albicans*. The reason they give for this is strain specificity. The detailed mechanism underpinning these observations remains for future investigation.

Next, I screened 887 non-essential mutant *C. albicans* strains from GRACE v1.0 library in the presence of AMB. My findings showed that 268 strains were sensitive to AMB (Table A5) and that no strains from the GRACE v1.0 library were resistant. My finding of no AMB resistant strains is intriguing and previous studies have also failed to show good candidates for AMB resistance. It has been speculated that AMB-induced accumulation of reactive oxygen species could possibly explain the low rate of resistance to AMB [34]. However, the molecular mechanisms underlying this phenomenon are unclear. Similar to comparison of genome profile of azole sensitivity, the genome profile for AMB sensitivity was compared using dataset generated from my study in *C. albicans* and from previously published papers in *S. cerevisiae and C. glabrata* (Figure 19). My analysis showed that out of 273 AMB

sensitive genes in *S. cerevisiae* (Appendix A7) [45], 268 genes in *C. albicans* (Appendix A6) and 13 genes in *C. glabrata* (Appendix A10) [62], only one common gene (*SAC7;* Putative GTPase activating protein for Rho1) was involved, further implying the same as observed in azole sensitivity among different fungal pathogens.

Subsequently, the top 5 of the sensitive strains (*PCM1*, *PDS5*, *RPO21*, *MNR2* and *YAK1* mutants) were chosen and were screened again using various concentrations of AMB. A brief description of the role of these genes has been summarized in Table 6. To the best of my knowledge, this is the first study that has demonstrated the sensitivity profile for the aforementioned mutant strains to AMB.

Following the selection of the top 5 super-sensitive and super-resistant strains to FLU, POSA and/or AMB using GRACE v1.0 library, I then screened the same mutant strains from tetracycline-inactivated parent GRACE library. This was undertaken to ensure that there were no differences in the sensitivity or resistant profiles for a given drug when screened using GRACE v1.0 and the parent GRACE library. My findings showed that the sensitivity/resistant profiles for the 5 strains from GRACE library that were super-resistant to azoles (FLU and POSA) and super sensitive to AMB were similar to that observed in GRACE v1.0 library. However, 3 out of the 5 strains (null mutants for *NPY1, PAA11*, and *SOG2)* that were super sensitive to FLU and POSA in GRACE v1.0 library were found to be resistant when tested in the parent GRACE library.

Table 6. Description of genes those are sensitive and/or resistant to FLU, POSA and AMB (adapted from CGD [26]).

Gene	Description
ERG3	C-5 sterol desaturase; introduces C-5(6) double bond into episterol; some clinical isolates show increased azole resistance and defects in hyphal growth and virulence
HCS1	Ortholog(s) have ATP-dependent 5'-3' DNA helicase activity and DNA helicase A complex, alpha DNA polymerase:primase complex, cytosol localization
RAP1	Transcription factor; binds telomeres and regulatory sequences in DNA; involved in telomere maintenance; represses hyphal growth under yeast-favoring conditions
ADP1	Putative PDR-subfamily ABC transporter; similar to WHITE subfamily proteins; gene used for strain identification by multilocus sequence typing
SEC65	Component of the protein-targeting Signal Recognition Particle (SRP)
ERG251	C-4 sterol methyl oxidase; role in ergosterol biosynthesis; Hap43-induced; ketoconazole-induced; amphotericin B, caspofungin repressed; possibly essential gene, disruptants not obtained by UAU1 method
NPY1	Ortholog(s) have NAD+ diphosphatase activity, role in NADH metabolic process and cytosol, nucleus, peroxisome localization
PAA11	Putative polyamine acetyltransferase; acetylates polyamines (e.g. putrescine, spermidine, spermine) and aralkylamines (e.g. tryptamine, phenylethylamine)
SOG2	Leucine-rich-repeat domain protein of RAM cell wall integrity signaling network; role in cell separation, azole sensitivity; required for hyphal growth; lacks orthologs in higher eukaryotes
PCM1	Phosphoacetylglucosamine mutase (N-acetylglucosamine-phosphate mutase); enzyme of UDP-N-acetylglucosamine (UDP-GlcNAc) biosynthesis
PDS5	Putative protein with a predicted role in establishment and maintenance of sister chromatid condensation and cohesion; cell-cycle regulated periodic mRNA expression
RPO21	RNA polymerase II; ortholog of S. cerevisiae Rpo21, transposon mutation affects filamentous growth; flow model biofilm repressed
MNR2	Putative ion transporter; fungal-specific (no human or murine homolog)
YAK1	Predicted serine-threonine protein kinase; involved in hyphal growth regulation and biofilm formation; flow model biofilm induced; induced in core caspofungin response

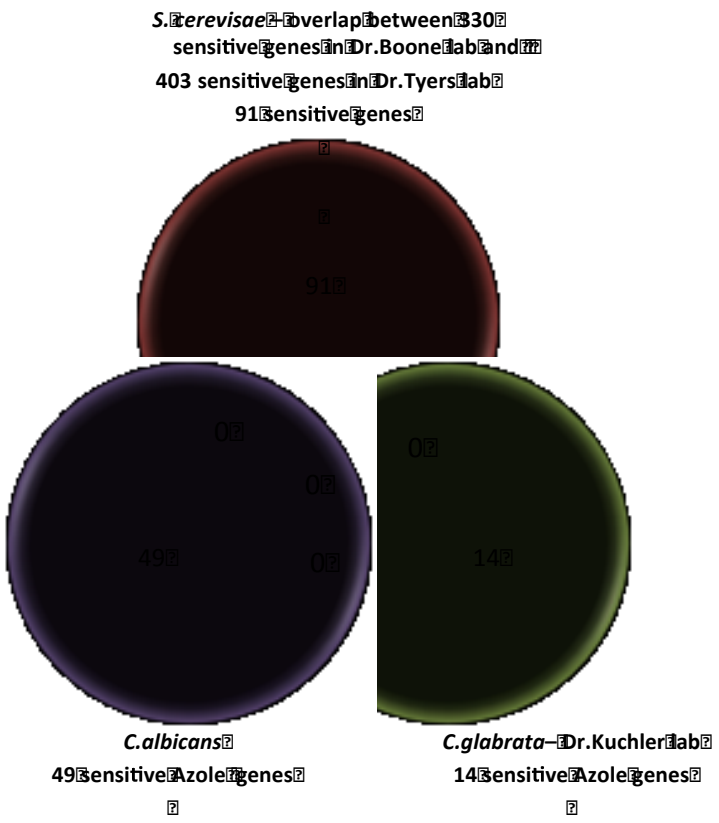


Figure 18. Comparison of genomic profile for azole sensitivity among different fungi [45,62,65]

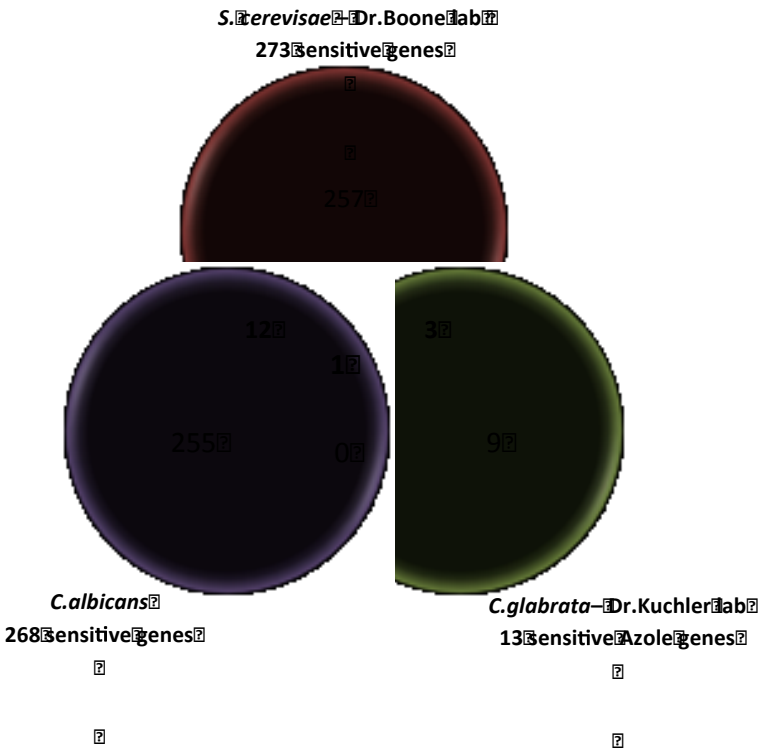


Figure 19. Comparison of genomic profile for AMB sensitivity among different fungi.

Previous work by others using *C. albicans* strain knockout experiments have demonstrated the sensitive profile for *SOG2* mutants in the presence of azoles similar to that observed in GRACE v1.0 library [64]. Hence, in order to understand the apparent differences in the profile of supersensitive *NPY1* and *PAA11* strains in GRACE and GRACE v1.0, I then constructed null mutants for *NPY1* and *PAA11* (CRISPR allele was obtained from a colleague) using the *SN76* and *SN148 C. albicans* strains. My findings showed that the *NPY1* and *PAA11* mutant strains were indeed sensitive to FLU and POSA, validating my findings in GRACE v1.0 library. This in turn corroborates the utility of GRACE v1.0 library and supports my hypothesis that screening of compounds in GRACE parent library may result in false positive findings possibly due to interaction with tetracycline.

In conclusion, screening an in-house derived collection of approximately 900 non-essential, transactivator-defective disruption *Candida albicans* strains against classic anti-fungal drugs demonstrated genes that confer either enhanced sensitivity and resistance to azoles and AMB. An important limitation of the current study is that only 5 of the most super sensitive and super resistant strains in each anti-fungal drug class were investigated in detail. Future studies to better understand other genes that confer sensitivity and resistance to azoles and AMB are warranted. Furthermore, the present study also has characterized the utility of using GRACE v1.0 library over the parent tetracycline inactivated GRACE library.

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APPENDIX

APPENDIX A1: GRACE v1.0 *C. albicans* library

Strain	*Gene Mutant*
orf19.5507	*ENP1*
orf19.665	*EMG1*
orf19.7291	*GCD14*
orf19.6026	*ERG2*
orf19.767	*ERG3*
orf19.6199	*HCS1*
orf19.6716	*ABD1*
orf19.5013	*PCM1*
orf19.1387	*CFD1*
orf19.6860	*PIS1*
orf19.2835	*AOS1*
orf19.1295	*VAS1*
orf19.4903	*GPI12*
orf19.7136	*SPT6*
orf19.122	*CDC20*
orf19.1304	*RRP4*
orf19.7101	*TEL2*
orf19.3366	*SHR3*
orf19.6025	*YGL047W*
orf19.2216	*PDS5*
orf19.7413	*MMS21*
orf19.4005	*YDR196C*
orf19.5379	*ERG4*
orf19.6422	*SSY5*
orf19.1631	*ERG6*
orf19.2116	*NAT2*
orf19.7363	*KRE6*
orf19.6818	*YLR419W*
orf19.6356	*PRP6*
orf19.1192	*DNA2*
orf19.4336	*RPS5*
orf19.7655	*RPO21*
orf19.3996	*GPI10*
orf19.4015	*GPA1*
orf19.6294	*MYO1*
orf19.586	*ERV46*
orf19.5825	*NCB2*
orf19.4258	*FIP1*
orf19.5148	*CYR1*
orf19.3859	*YBR159W*
orf19.6640	*TPS1*
orf19.4697	*MDN1*
orf19.2761	*GPI11*

Strain	*Gene Mutant*
orf19.4635	*NIP1*
orf19.7035	*RFC2*
orf19.2313	*PEX30*
orf19.3447	*PBN1*
orf19.4230	*PRE4*
orf19.3058	*COQ6*
orf19.6075	*CDC36*
orf19.1232	*VRG4*
orf19.5895	*YEL023C*
orf19.6707	*YNL217W*
orf19.6168	*USO1*
orf19.7184	*VPS55*
orf19.4941	*TYE7*
orf19.4414	*orf19.4414*
orf19.7567	*YDR124W*
orf19.5067	*LRP1*
orf19.2671	*NDI1*
orf19.7073	*YCL002C*
orf19.2653	*orf19.2653*
orf19.1125	*orf19.1125*
orf19.645	*HXT5*
orf19.703	*CPD1*
orf19.3536	*GNT1*
orf19.2659	*YER067W*
orf19.4730	*YNL050C*
orf19.4528	*YLR065C*
orf19.1479	*PIB2*
orf19.4629	*BUL2*
orf19.5812	*YOR051C*
orf19.4023	*MRP2*
orf19.7017	*YOX1*
orf19.6055	*BUL1*
orf19.811	*YLR283W*
orf19.4294	*CYC2*
orf19.2706	*CRH1*
orf19.2075	*DFG5*
orf19.158	*APD1*
orf19.3548	*YBL095W*
orf19.642	*SAP155*
orf19.4798	*EFR3*
orf19.5634	*FRE5*
orf19.2426	*YGL101W*
orf19.3442	*OYE3*

Strain	*Gene Mutant*
orf19.6685	*ISY1*
orf19.3612	*PST2*
orf19.1310	*YDR314C*
orf19.4309	*GRE2*
orf19.1264	*FRE4*
orf19.3505	*SLM2*
orf19.246	*YIL108W*
orf19.5826	*UGA4*
orf19.5087	*BUD6*
orf19.4231	*YEL007W*
orf19.5655	*orf19.5655*
orf19.3071	*MIH1*
orf19.1910	*orf19.1910*
orf19.7444	*YOR352W*
orf19.260	*CaORF6_1455*
orf19.2842	*GZF3*
orf19.551	*CaORF6_1932*
orf19.5499	*orf6.4658*
orf19.5595	*SHE3*
orf19.667	*CaORF6_6560*
orf19.1587	*YBR241C*
orf19.7069	*YBR261C*
orf19.3608	*MSH3*
orf19.1069	*RPN4*
orf19.4761	*HST1*
orf19.1564	*YDL072C*
orf19.5323	*MDH1*
orf19.5171	*PMT1*
orf19.5627	*HEK2*
orf19.829	*SCH9*
orf19.5808	*YBR271W*
orf19.3792	*PAT1*
orf19.6460	*PEX1*
orf19.2281	*CaORF6_2249*
orf19.2462	*orf6.4973*
orf19.5294	*PDB1*
orf19.4927	*BNI1*
orf19.2501	*YGL139W*
orf19.6323	*HPA3*
orf19.5917	*STP2*
orf19.639	*RAI1*
orf19.1862	*YHR087W*
orf19.3533	*EMI1*

Strain	*Gene Mutant*
orf19.652	*YEN1*
orf19.696	*STE2*
orf19.4816	*YMR209C*
orf19.4887	*ECM21*
orf19.203	*STB3*
orf19.6717	*FSH1*
orf19.4176	*MRPS17*
orf19.5441	*ULP2*
orf19.4357	*YMR115W*
orf19.1533	*YKL077W*
orf19.4844	*AIR2*
orf19.5278	*YJR111C*
orf19.3022	*RSM24*
orf19.2199	*PHO86*
orf19.2363	*YOR286W*
orf19.3659	*YER139C*
orf19.3263	*ADY2*
orf19.4722	*RTG1*
orf19.4958	*ECM25*
orf19.5270	*YJR111C*
orf19.7385	*LEE1*
orf19.3624	*MSU1*
orf19.3574	*MDJ2*
orf19.3937	*SDF1*
orf19.4011	*YGR263C*
orf19.6681	*YGR263C*
orf19.5601	*orf19.5601*
orf19.612	*YML050W*
orf19.1773	*RAP1*
orf19.2060	*SOD1*
orf19.944	*orf19.944*
orf19.3282	*orf19.3282*
orf19.3362	*NDI1*
orf19.5016	*YAL044W-A*
orf19.1632	*DCW1*
orf19.1681	*orf19.1681*
orf19.2303	*PRP38*
orf19.2973	*VTS1*
orf19.2064	*orf19.2064*
orf19.2343	*STP22*
orf19.752	*orf19.752*
orf19.3170	*BUD27*
orf19.4577	*GPB1*

Strain	*Gene Mutant*
orf19.5250	*orf19.5250*
orf19.3627	*YER182W*
orf19.2418	*orf19.2418*
orf19.1058	*RPN13*
orf19.927	*YML036W*
orf19.4922	*YIP5*
orf19.4701	*ELP6*
orf19.2564	*YMR269W*
orf19.6035	*orf19.6035*
orf19.2961	*MIG2*
orf19.4639	*orf19.4639*
orf19.713	*YMR074C*
orf19.5213	*PCF11*
orf19.273	*YNL156C*
orf19.3012	*ARO80*
orf19.7503	*CDA2*
orf19.2308	*PFK26*
orf19.6869	*AST1*
orf19.2660	*EAF3*
orf19.577	*YDL057W*
orf19.2400	*YNL224C*
orf19.4062	*NSR1*
orf19.1224	*ADY2*
orf19.1844	*FRE4*
orf19.6143	*orf6.5671*
orf19.2839	*orf6.4500*
orf19.1568	*VAM6*
orf19.4370	*orf6.4535*
orf19.2881	*MNN4*
orf19.3226	*NPC2*
orf19.1040	*MAD2*
orf19.4758	*orf6.4597*
orf19.2551	*MET6*
orf19.445	*YNR040W*
orf19.804	*YPR011C*
orf19.1149	*ETR1*
orf19.5645	*MET17*
orf19.2580	*HST2*
orf19.578	*MSB3*
orf19.5610	*ARG3*
orf19.1362	*SMM1*
orf19.1404	*DUS1*
orf19.3983	*YGR266W*

Strain	*Gene Mutant*
orf19.3131	*OYE3*
orf19.4035	*GAS5*
orf19.7016	*PPN1*
orf19.5702	*YOR093C*
orf19.2107	*MUQ1*
orf19.5628	*DIC1*
orf19.1720	*YFR038W*
orf19.3330	*RHO4*
orf19.3325	*GLG2*
orf19.2133	*orf6.5367*
orf19.3854	*SAT4*
orf19.6730	*YCR016W*
orf19.4526	*HSP30*
orf19.5994	*RHB1*
orf19.908	*FEN1*
orf19.3407	*RAD18*
orf19.459	*ADP1*
orf19.7330	*PET18*
orf19.1671	*UTR2*
orf19.6578	*PHO84*
orf19.6740	*PRP19*
orf19.308	*SNG1*
orf19.4160	*QRI7*
orf19.4001	*MSS2*
orf19.5038	*TRM3*
orf19.6498	*IWR1*
orf19.3606	*SNA4*
orf19.7188	*RPP1B*
orf19.5061	*ADE5,7*
orf19.6901	*DCS1*
orf19.7520	*POT1*
orf19.6502	*YDL114W*
orf19.4416	*VPS13*
orf19.4889	*HOL1*
orf19.5234	*BSC1*
orf19.1747	*KIP2*
orf19.873	*CaORF6_7304*
orf19.4461	*CaORF6_2028*
orf19.314	*STB6*
orf19.5337	*UBC13*
orf19.5079	*PDR5*
orf19.1606	*SWI1*
orf19.5727	*CaORF6_4906*

Strain	*Gene Mutant*
orf19.2172	*ARA1*
orf19.4534	*UBX7*
orf19.3613	*PAF1*
orf19.3064	*MRPL27*
orf19.1204	*APM3*
orf19.7074	*SGF29*
orf19.3292	*YCL033C*
orf19.3736	*KAR4*
orf19.548	*CDC10*
orf19.7522	*BNA3*
orf19.2194	*APM4*
orf19.1795	*PUF3*
orf19.5640	*PEX5*
orf19.1578	*RRP5*
orf19.6908	*FOL3*
orf19.5260	*RPN2*
orf19.487	*SPT14*
orf19.1701	*RKI1*
orf19.5436	*UTP8*
orf19.7511	*NUP192*
orf19.2672	*NCP1*
orf19.2751	*MCH4*
orf19.2781	*RCK2*
orf19.2665	*MSN5*
orf19.1114	*YML030W*
orf19.6736	*YOR205C*
orf19.1339	*PRC1*
orf19.4466	*ERF2*
orf19.2474	*PRC1*
orf19.2538	*PTC2*
orf19.6538	*VMA11*
orf19.1161	*SPO14*
orf19.799	*STE4*
orf19.1734	*INO80*
orf19.4778	*LYS14*
orf19.2094	*KAP122*
orf19.7460	*YOL125W*
orf19.6082	*GSF2*
orf19.7096	*YLR001C*
orf19.2040	*TAH18*
orf19.5174	*TAF13*
orf19.3785	*CAJ1*
orf19.1634	*orf19.1634*

Strain	*Gene Mutant*
orf19.4195	*orf19.4195*
orf19.719	*HRD1*
orf19.5078	*orf19.5078*
orf19.6508	*LST7*
orf19.4242	*STE20*
orf19.1490	*MUC1*
orf19.5447	*ITR1*
orf19.814	*SSY1*
orf19.7433	*NUP116*
orf19.4013	*YHR045W*
orf19.5680	*orf19.5680*
orf19.4373	*FMN1*
orf19.202	*CDC47*
orf19.6234	*IPI3*
orf19.677	*CHO1*
orf19.379	*IPI1*
orf19.3898	*TLG1*
orf19.5722	*YDR026C*
orf19.7449	*DML1*
orf19.6369	*RIO2*
orf19.6903	*RPC37*
orf19.2557	*SEC65*
orf19.6917	*YNL310C*
orf19.6512	*EXO70*
orf19.6923	*TAF11*
orf19.124	*CIC1*
orf19.7624	*BFR2*
orf19.1941	*NUF2*
orf19.4353	*ULP1*
orf19.3531	*YOR060C*
orf19.24	*RSB1*
orf19.4026	*HIS1*
orf19.6995	*ADY2*
orf19.2137	*orf6.5371*
orf19.942	*KRE6*
orf19.2065	*DAL2*
orf19.1805	*PEX14*
orf19.4524	*YIL130W*
orf19.2666	*MSN5*
orf19.1793	*HSV2*
orf19.4898	*YER004W*
orf19.2810	*GAP1*
orf19.1523	*orf6.1172*

Strain	*Gene Mutant*
orf19.153	*YKL174C*
orf19.1442	*PLB2*
orf19.3247	*YJL207C*
orf19.318	*CAF17*
orf19.3051	*YJR116W*
orf19.2458	*SIP5*
orf19.3023	*NGG1*
orf19.4003	*TIP20*
orf19.5498	*SOK2*
orf19.1991	*PTM1*
orf19.805	*YGR021W*
orf19.4520	*YDR248C*
orf19.4850	*YPL260W*
orf19.2217	*orf6.4262*
orf19.6348	*SWI1*
orf19.3302	*GAC1*
orf19.511	*NRK1*
orf19.7033	*PPS1*
orf19.4923	*YFL040W*
orf19.1585	*ZRT2*
orf19.473	*TPO4*
orf19.2445	*DIP5*
orf19.2350	*YOR378W*
orf19.5815	*SCT1*
orf19.3083	*CDC1*
orf19.2003	*HNM1*
orf19.3895	*CTS1*
orf19.5676	*MAG2*
orf19.2175	*YNR074C*
orf19.3991	*ROG1*
orf19.7310	*MSC1*
orf19.2575	*YLR063W*
orf19.3441	*YNR002C*
orf19.541	*orf6.6753*
orf19.7403	*YML020W*
orf19.5292	*AXL2*
orf19.7518	*TEA1*
orf19.5839	*PDR16*
orf19.7370	*YOL092W*
orf19.5970	*HPR5*
orf19.1445	*RTT107*
orf19.2045	*HSD1*
orf19.6444	*RMD11*

Strain	*Gene Mutant*
orf19.5837	*YMR289W*
orf19.6882	*OSM1*
orf19.6899	*YMR315W*
orf19.644	*HXT5*
orf19.2983	*CDC73*
orf19.4396	*YKR016W*
orf19.7437	*YJL218W*
orf19.4617	*MAK3*
orf19.778	*PIL1*
orf19.2055	*NPL6*
orf19.5426	*YKR089C*
orf19.3066	*DSE4*
orf19.4833	*MLS1*
orf19.2072	*HNM1*
orf19.1980	*GIT1*
orf19.1979	*GIT1*
orf19.4781	*GRE2*
orf19.988	*YGR149W*
orf19.3149	*PIL1*
orf19.3352	*YOR246C*
orf19.2113	*PEX30*
orf19.3926	*RNY1*
orf19.5663	*YMR034C*
orf19.3764	*GSG1*
orf19.3592	*JEM1*
orf19.1185	*DMA2*
orf19.2848	*ATG13*
orf19.3212	*MID1*
orf19.7176	*NPT1*
orf19.7243	*DCD1*
orf19.151	*YKL174C*
orf19.1800	*YPR157W*
orf19.2573	*FRS1*
orf19.329	*MSL5*
orf19.4146	*SMD3*
orf19.1833	*CBF5*
orf19.717	*HSP60*
orf19.5493	*GSP1*
orf19.7454	*TAF6*
orf19.4631	*ERG25*
orf19.2917	*NUG1*
orf19.2183	*YER036C*
orf19.6375	*RPS20*

Strain	*Gene Mutant*
orf19.2991	*HOL1*
orf19.3546	*PEX2*
orf19.4655	*OPT2*
orf19.6551	*GOS1*
orf19.2242	*PRB1*
orf19.5023	*DAL5*
orf19.4985	*GUP1*
orf19.943	*FET3*
orf19.7551	*ALO1*
orf19.2943	*DIP5*
orf19.2555	*URA5*
orf19.2444	*CHS7*
orf19.2602	*OPT1*
orf19.309	*DAL5*
orf19.4063	*UGA4*
orf19.2170	*PHM7*
orf19.1311	*SPO75*
orf19.5037	*orf6.3314*
orf19.2488	*FAL1*
orf19.4191	*RLP24*
orf19.6039	*SED5*
orf19.7657	*POP3*
orf19.5074	*UBA2*
orf19.4154	*SPB4*
orf19.5528	*MOB1*
orf19.5076	*PFY1*
orf19.1814	*STT4*
orf19.6131	*TSC10*
orf19.4863	*PDC2*
orf19.3256	*SLN1*
orf19.4448	*SOG2*
orf19.7037	*YAE1*
orf19.302	*BET5*
orf19.7597	*PMU1*
orf19.5131	*GID7*
orf19.4106	*YJR141W*
orf19.7382	*TEF4*
orf19.7561	*DEF1*
orf19.5926	*ORT1*
orf19.6169	*ADY2*
orf19.6858	*EDC3*
orf19.7269	*YDR071C*
orf19.4195.1	*FCY1*

Strain	*Gene Mutant*
orf19.4274	*PUT1*
orf19.2095	*YLL029W*
orf19.3047	*SIP3*
orf19.2143	*YPL183C*
orf19.6322	*SPS19*
orf19.28	*TPC1*
orf19.2028	*MXR1*
orf19.2098	*ARO8*
orf19.7231	*FTR1*
orf19.2909	*ERG26*
orf19.583	*BNA2*
orf19.3567	*BIO3*
orf19.1180	*YER152C*
orf19.4899	*ROT2*
orf19.4128	*YOR059C*
orf19.2626	*RGD2*
orf19.1891	*PEP4*
orf19.1881	*YOR175C*
orf19.1477	*YGL010W*
orf19.1468	*CDC55*
orf19.6904	*GCN3*
orf19.3298	*CCH1*
orf19.3307	*FMO1*
orf19.350	*PRE9*
orf19.897	*VPS20*
orf19.6933	*RRD2*
orf19.4845	*TVP18*
orf19.6981	*YKR051W*
orf19.6990	*CPR5*
orf19.3035	*CHD1*
orf19.3049	*SPS1*
orf19.7029	*YDL238C*
orf19.5025	*MET3*
orf19.7156	*FAA2*
orf19.5720	*MCH4*
orf19.1719	*SGA1*
orf19.6195	*RNH70*
orf19.2484	*YDR415C*
orf19.2008	*YPR118W*
orf19.5658	*MNN10*
orf19.744	*GDB1*
orf19.5377	*HOS2*
orf19.3542	*LEM3*

Strain	*Gene Mutant*
orf19.299	*ECM14*
orf19.4940	*HIP1*
orf19.607	*RAD26*
orf19.2092	*STR3*
orf19.2073	*YDR338C*
orf19.3822	*SCS7*
orf19.3841	*ATG1*
orf19.6558	*SEC23*
orf19.6070	*ENA2*
orf19.6533	*MSK1*
orf19.5280	*MUP1*
orf19.5263	*SER3*
orf19.5253	*YAK1*
orf19.4297	*CKB2*
orf19.606	*YLR352W*
orf19.1324	*RAD2*
orf19.2251	*AAH1*
orf19.2031	*VPS24*
orf19.2046	*POT1*
orf19.4945	*MSH6*
orf19.6063	*UBP6*
orf19.6237	*CDC42*
orf19.6306	*ALD4*
orf19.6011	*SIN3*
orf19.2425	*YBR241C*
orf19.6287	*AAT2*
orf19.7390	*REV3*
orf19.5986	*THI4*
orf19.3482	*NPY1*
orf19.5862	*CAR1*
orf19.3710	*YHB1*
orf19.6152	*orf6.5662*
orf19.7213	*YDR291W*
orf19.5641	*CAR2*
orf19.354	*YER078C*
orf19.4446	*MEP3*
orf19.6928	*YPS1*
orf19.4610	*CPS1*
orf19.7218	*PRY2*
orf19.7336	*AZR1*
orf19.5656	*orf6.6896*
orf19.5661	*PTC7*
orf19.5673	*OPT2*

Strain	*Gene Mutant*
orf19.5160	*SAP190*
orf19.2436	*SKY1*
orf19.4186	*PCT1*
orf19.763	*CBC2*
orf19.3089	*YLR168C*
orf19.742	*ALD4*
orf19.4233	*THR4*
orf19.6230	*RAI1*
orf19.6328	*ACN9*
orf19.1333	*SNG1*
orf19.6522	*YIL166C*
orf19.5495	*NAB6*
orf19.5517	*ADH7*
orf19.4506	*LYS21*
orf19.1235	*HOM3*
orf19.7298	*CHS1*
orf19.4633	*YMR226C*
orf19.2693	*URE2*
orf19.6766	*NOP13*
orf19.2703	*YMR171C*
orf19.4475	*KTR4*
orf19.2007	*VPS54*
orf19.3770	*ARG8*
orf19.313	*FUR4*
orf19.2771	*BEM3*
orf19.3208	*DAL5*
orf19.3649	*FES1*
orf19.7327	*PHO88*
orf19.5869	*BSD2*
orf19.4548	*MAK32*
orf19.3558	*ERP3*
orf19.7367	*UBP1*
orf19.403	*PCL2*
orf19.52	*MMT1*
orf19.3139	*YML131W*
orf19.1236	*GVP36*
orf19.212	*VPS28*
orf19.4183	*MUC1*
orf19.5903	*RAX1*
orf6.2225	*YJL064W*
orf19.6877	*YHR217C*
orf19.1285	*YPR091C*
orf19.4240	*PER1*

Strain	*Gene Mutant*
orf19.1639	*JLP1*
orf19.6727	*RIT1*
orf19.349	*NCA2*
orf19.1130	*orf6.780*
orf19.5925	*YDL237W*
orf19.4211	*FET3*
orf19.4760	*YIL110W*
orf19.216	*orf6.5157*
orf19.5849	*RDS2*
orf19.2423	*YBR239C*
orf19.991	*DJP1*
orf19.1493	*RAD7*
orf19.4546	*HOL1*
orf19.4287	*XYL2*
orf19.5592	*CaORF6_1762*
orf19.6449	*orf6.7805*
orf19.3929	*YJR100C*
orf19.6398	*JLP1*
orf19.2151	*SEY1*
orf19.7158	*YIL166C*
orf19.857	*orf6.1227*
orf19.5780	*YIL067C*
orf19.5859	*DAL5*
orf19.4688	*WSC2*
orf19.1663	*KRE2*
orf19.105	*MET22*
orf19.4171	*orf6.6065*
orf19.1583	*HOL1*
orf19.4142	*AVT2*
orf19.6185	*orf6.5406*
orf19.592	*YNL092W*
orf19.7247	*RIM101*
orf19.183	*HIS3*
orf19.3151	*YGL157W*
orf19.6772	*ECM29*
orf19.1946	*YMR099C*
orf19.5971	*YHR151C*
orf19.7257	*MLH3*
orf19.5894	*YEL023C*
orf19.4743	*AFG1*
orf19.7219	*FTR1*
orf19.835	*IES1*
orf19.1345	*orf6.2643*

Strain	*Gene Mutant*
orf19.2798	*YDR332W*
orf19.4376	*orf6.4541*
orf19.7629	*YOL138C*
orf19.6096	*TRP1*
orf19.851	*MNN4*
orf19.4560	*BFR1*
orf19.1698	*APP1*
orf19.3705	*YCR079W*
orf19.4779	*YKR105C*
orf19.2945	*PUT4*
orf19.5103	*PMU1*
orf19.5307	*JEN1*
orf19.3458	*VPS68*
orf19.5071	*NRP1*
orf19.4783	*orf6.1419*
orf19.23	*RSB1*
orf19.1160	*SVP26*
orf19.5662	*PEP7*
orf19.2942	*DIP5*
orf19.6020	*ATG3*
orf19.1159	*MET2*
orf19.5496	*AVT1*
orf19.2198	*YAL053W*
orf19.2411	*SYN8*
orf19.1449	*YNL335W*
orf19.234	*PHA2*
orf19.983	*YOR322C*
orf19.6167	*AYR1*
orf19.1363	*YOR338W*
orf19.1381	*LSB5*
orf19.6297	*DEG1*
orf19.1832	*TPN1*
orf19.5789	*ADE8*
orf19.304	*YOR378W*
orf19.5437	*RHR2*
orf19.3852	*CaORF6_2313*
orf19.6464	*CaORF6_2212*
orf19.2882	*orf6.2894*
orf19.4229	*DDP1*
orf19.6592	*PHO84*
orf19.841	*COY1*
orf19.4288	*STB4*
orf19.3586	*YBR005W*

Strain	*Gene Mutant*
orf19.1853	*HHT2*
orf19.754	*YBR025C*
orf19.4519	*SUV3*
orf19.6358	*MMS2*
orf19.478	*MON1*
orf19.4855	*BUD31*
orf19.1394	*YCR090C*
orf19.3915	*YFR044C*
orf19.1940	*CAT5*
orf19.3980	*SLH1*
orf19.2984	*MST1*
orf19.1659	*ALG8*
orf19.7324	*THI13*
orf19.1078	*AGX1*
orf19.656	*DPP1*
orf19.4339	*VPS4*
orf19.4334	*MUC1*
orf19.2160	*TPO3*
orf19.4131	*YCL045C*
orf19.5667	*MNR2*
orf19.2886	*FUS3*
orf19.1836	*APN2*
orf19.517	*HAP3*
orf19.3369	*MOH1*
orf19.5530	*NAB3*
orf19.7611	*TRX1*
orf19.2114	*orf6.4747*
orf19.3290	*CaORF6_1780*
orf19.7594	*GPR1*
orf19.5784	*orf6.4856*
orf19.4822	*orf6.7389*
orf19.10	*DIT2*
orf19.211	*orf6.5162*
orf19.5921	*ANT1*
orf19.7002	*orf6.7748*
orf19.4325	*MUC1*
orf19.4905	*MAD1*
orf19.2772	*HOS3*
orf19.4006	*PAN5*
orf19.5553	*YKL069W*
orf19.2762	*AHP1*
orf19.1797	*SWI1*
orf19.5924	*BNR1*

Strain	*Gene Mutant*
orf19.1911	*YJL171C*
orf19.3230	*BOI2*
orf19.2792	*IST2*
orf19.5295	*orf6.3939*
orf19.7479	*NTH1*
orf19.2467	*orf6.4968*
orf19.4804	*orf6.5210*
orf19.3901	*orf6.5147*
orf19.1070	*YMR010W*
orf19.604	*orf6.4827*
orf19.2553	*PMR1*
orf19.2248	*ARE2*
orf19.6059	*TTR1*
orf19.2434	*NPL4*
orf19.4950	*AKR1*
orf19.4593	*RGA2*
orf19.4755	*KEX2*
orf19.732	*SPS19*
orf19.5352	*orf6.8341*
orf19.6794	*orf6.8837*
orf19.895	*HOG1*
orf19.7281	*YIL042C*
orf19.7513	*DIT2*
orf19.3100	*USO1*
orf19.6327	*orf6.5520*
orf19.7512	*DIT2*
orf19.1042	*POR1*
orf19.6225	*PCL6*
orf19.4088	*GLO2*
orf19.2747	*RGT1*
orf19.1154	*EGD1*
orf19.1648	*RAD50*
orf19.5043	*USO1*
orf19.5728	*orf6.4907*
orf19.3874	*orf6.5845*
orf19.4658	*NAB3*
orf19.4831	*orf6.7380*
orf19.5090	*TAD3*
orf19.1989	*DCW1*
orf19.4764	*PAN2*
orf19.287	*orf6.4816*
orf19.3152	*CaORF6_1742*
orf19.2285	*CaORF6_2253*

Strain	*Gene Mutant*
orf19.1857	*SPT10*
orf19.4984	*CTS2*
orf19.3329	*LCB3*
orf19.147	*YAK1*
orf19.7355	*SSN8*
orf19.4112	*YJR142W*
orf19.4981	*ECM27*
orf19.4906	*MUC1*
orf19.2990	*EXG1*
orf19.4316	*YHL021C*
orf19.2282	*CaORF6_2250*
orf19.4147	*GLR1*
orf19.5411	*UBC12*
orf19.7238	*NPL3*
orf19.2514	*orf6.3919*
orf19.4372	*YDL206W*
orf19.2838	*orf6.4499*
orf19.4773	*orf6.3984*
orf19.728	*TSC11*
orf19.4921	*NOP1*
orf19.3720	*BCY1*
orf19.7061	*YFR007W*
orf19.916	*YNL305C*
orf19.3846	*LYS4*
orf19.6898	*orf6.7162*
orf19.6912	*CKI1*
orf19.2059	*orf6.7496*
orf19.7337	*YBL113C*
orf19.7071	*PHO84*
orf19.5393	*orf6.8629*
orf19.723	*YPL230W*
orf19.5827	*BUB2*
orf19.3042	*CaORF6_7779*
orf19.330.1	*RUB1*
orf19.6562	*RNH201*
orf19.5730	*YDR539W*
orf19.7440	*STE6*
orf19.889	*THI20*
orf19.2618	*MET2*
orf19.7115	*SAC7*
orf19.3815	*UBP7*
orf19.7585	*INO1*
orf19.5729	*CHA4*

Strain	*Gene Mutant*
orf19.1887	*YLL012W*
orf19.4324	*CaORF6_3262*
orf19.2926	*PSO2*
orf19.7668	*YGR287C*
orf19.4772	*SHO1*
orf19.3804	*YMR134W*
orf19.1419	*SEC15*
orf19.500	*GCD10*
orf19.4232	*YGR046W*
orf19.2790	*SWD2*
orf19.1066	*YPL067C*
orf19.6012	*YOR296W*
orf19.300	*DLD2*
orf19.1062	*orf6.4927*
orf19.782	*YBR204C*
orf19.3912	*GLN3*
orf19.4668	*SCW10*
orf19.5605	*YJL084C*
orf19.5611	*GRE2*
orf19.3432	*YCR023C*
orf19.3679	*YNL200C*
orf19.4175	*TOK1*
orf19.6653	*YHR168W*
orf19.26	*PNG1*
orf19.6606	*orf6.6218*
orf19.6271	*YPR045C*
orf19.4943	*PSA1*
orf19.3010	*LIP2*
orf19.2312	*FRE4*
orf19.6344	*RBK1*
orf19.2988	*NFS1*
orf19.4381	*VTC2*
orf19.809	*NOP12*
orf19.3122	*ARR3*
orf19.3106	*MET16*
orf19.5838	*SER2*
orf19.1421	*DAL3*
orf19.2952	*EXG1*
orf19.3227	*FTH1*
orf19.669	*PRM1*
orf19.6992	*QDR1*
orf19.6596	*YJL068C*
orf19.2517	*HOL1*

Strain	*Gene Mutant*
orf19.5058	*SMI1*
orf19.7514	*PCK1*
orf19.3267	*SWF1*
orf19.2077	*ARG81*
orf19.5164	*ECM39*
orf19.1427	*YLR004C*
orf19.4545	*SWI4*
orf19.6847	*YPL030W*
orf19.7427	*YML018C*
orf19.6602	*YLR201C*
orf19.5832	*HPT1*
orf19.1124	*orf6.1199*
orf19.3686	*ATP12*
orf19.7490	*BSC6*
orf19.7319	*MAL13*
orf19.7322	*YPL225W*
orf19.5029	*YDR061W*
orf19.3232	*TNA1*
orf19.7565	*GNP1*
orf19.7619	*YHR194W*
orf19.7666	*SEO1*
orf19.5919	*YOR129C*
orf19.3995	*RIM13*
orf19.3308	*STB5*
orf19.7369	*YML005W*
orf19.3219	*SIA1*
orf19.387	*STO1*
orf19.3445	*HOC1*
orf19.388	*CAF16*
orf19.690	*PLB3*
orf19.2970	*LYS2*
orf19.4197	*YHM2*
orf19.3222	*YGR125W*
orf19.3412	*ATG15*
orf19.3065	*orf6.7802*
orf19.1538	*TLG2*
orf19.4579	*ERV29*
orf19.843	*DCR2*
orf19.5102	*PLB3*
orf19.7320	*orf6.8401*
orf19.7306	*YPR127W*
orf19.4624	*HRT2*
orf19.2541	*YBL055C*

Strain	***Gene Mutant***
orf19.864	*NMD2*
orf19.240	*YKR065C*
orf19.5534	*TVP38*
orf19.4119	*ATG2*
orf19.1112	*YMR237W*
orf19.5532	*orf6.1547*
orf19.2186	*SEH1*
orf19.3964	*BRE2*
orf19.1723	*YJL055W*
orf19.698	*YIL090W*

APPENDIX A2: Strains resistant and sensitive to FLU

Strains resistant to FLU	Strains sensitive to FLU
orf19.6199	orf19.665
orf19.2671	orf19.6716
orf19.4629	orf19.7136
orf19.5595	orf19.2216
orf19.3792	orf19.6294
orf19.3533	orf19.5825
orf19.2363	orf19.273
orf19.3263	orf19.3325
orf19.3574	orf19.4461
orf19.1773	orf19.2538
orf19.908	orf19.2557
orf19.459	orf19.4524
orf19.4160	orf19.2666
orf19.4001	orf19.3247
orf19.6502	orf19.5676
orf19.1805	orf19.644
orf19.5986	orf19.4146
orf19.3710	orf19.4631
orf19.5869	orf19.6039
orf19.4142	orf19.5076
orf19.5894	orf19.4448
orf19.2160	orf19.7269
orf19.1836	orf19.4274
orf19.3369	orf19.5720
orf19.6327	orf19.299
orf19.3329	orf19.3482
orf19.7115	orf19.6152
orf19.500	orf19.5641
orf19.3227	orf19.4610
orf19.6602	orf19.4186
orf19.7619	orf19.3649
orf19.3995	orf19.403
orf19.2970	orf19.52
orf19.3412	orf19.3139
orf19.767	orf19.4240
orf19.5379	orf19.2423
orf19.1631	orf19.991
orf19.7017	orf19.4688
orf19.2706	orf19.1663
orf19.260	orf19.7257
orf19.551	orf19.5667

Strains resistant to FLU	Strains sensitive to FLU
orf19.829	orf19.732
orf19.652	orf19.1042
orf19.696	orf19.4088
orf19.3659	orf19.7337
orf19.3624	orf19.5393
orf19.4722	orf19.723
orf19.612	orf19.5827
orf19.3282	orf19.6012
orf19.2973	orf19.5919
orf19.5250	orf19.4624
orf19.2660	orf19.1112
orf19.2551	orf19.3243
orf19.1720	orf19.2762
orf19.3330	orf19.6025
orf19.6578	orf19.2842
orf19.7520	orf19.1069
orf19.6908	orf19.5702
orf19.4863	orf19.5337
orf19.6904	orf19.2665
orf19.607	orf19.6538
orf19.763	orf19.318
orf19.1235	orf19.3051
orf19.349	orf19.2458
orf19.4287	orf19.3302
orf19.6185	orf19.1585
orf19.7247	orf19.151
orf19.6772	orf19.1833
orf19.5971	orf19.717
orf19.1345	orf19.5493
orf19.2798	orf19.7561
orf19.851	orf19.3298
orf19.5662	orf19.7298
orf19.2411	orf19.4633
orf19.2882	orf19.1236
orf19.1659	orf19.6727
orf19.4131	orf19.6449
orf19.2886	orf19.1066
orf19.7594	orf19.6653
orf19.4905	orf19.7319
orf19.1070	orf19.7565
orf19.5352	

Strains resistant to FLU	Strains sensitive to FLU
orf19.6794	
orf19.895	
orf19.7281	
orf19.7513	
orf19.6225	
orf19.3874	
orf19.4831	
orf19.4984	
orf19.7355	
orf19.4316	
orf19.3720	
orf19.7061	
orf19.3846	
orf19.6898	
orf19.6912	
orf19.2059	
orf19.5730	
orf19.6562	
orf19.889	
orf19.6596	
orf19.2517	
orf19.5058	
orf19.7514	
orf19.3267	
orf19.5164	
orf19.1427	
orf19.3686	
orf19.7666	
orf19.387	
orf19.690	
orf19.1538	
orf19.2541	
orf19.5532	
orf19.5294	
orf19.4887	
orf19.5447	
orf19.656	

APPENDIX A3: Strains resistant and sensitive to POSA

Strains sensitive to POSA	Strains resistant to POSA
orf19.1295	orf19.5507
orf19.3996	orf19.665
orf19.6640	orf19.767
orf19.5087	orf19.6199
orf19.2842	orf19.1387
orf19.5270	orf19.4903
orf19.944	orf19.1304
orf19.1632	orf19.6025
orf19.2881	orf19.7413
orf19.5702	orf19.6356
orf19.4526	orf19.1192
orf19.5994	orf19.5148
orf19.4461	orf19.7035
orf19.1204	orf19.2313
orf19.3292	orf19.3447
orf19.2781	orf19.4230
orf19.2538	orf19.6075
orf19.6538	orf19.1232
orf19.2094	orf19.5895
orf19.379	orf19.6707
orf19.6369	orf19.7567
orf19.2557	orf19.2671
orf19.2137	orf19.7073
orf19.942	orf19.703
orf19.4524	orf19.1479
orf19.2666	orf19.4629
orf19.3247	orf19.2706
orf19.318	orf19.4798
orf19.2458	orf19.6685
orf19.4850	orf19.260
orf19.3302	orf19.5595
orf19.3083	orf19.5294
orf19.6444	orf19.4176
orf19.644	orf19.2199
orf19.2055	orf19.2363
orf19.3592	orf19.3263
orf19.151	orf19.4722
orf19.4631	orf19.3574

Strains sensitive to POSA	Strains resistant to POSA
orf19.943	orf19.1773
orf19.2555	orf19.3282
orf19.4063	orf19.1224
orf19.1311	orf19.2839
orf19.4191	orf19.4370
orf19.6039	orf19.1720
orf19.5076	orf19.908
orf19.3256	orf19.459
orf19.4448	orf19.4160
orf19.7269	orf19.4001
orf19.4274	orf19.5038
orf19.2098	orf19.873
orf19.4128	orf19.1795
orf19.1891	orf19.1578
orf19.1468	orf19.6908
orf19.3298	orf19.1634
orf19.4845	orf19.6348
orf19.5720	orf19.5292
orf19.2484	orf19.7518
orf19.299	orf19.5839
orf19.4940	orf19.7370
orf19.2073	orf19.1980
orf19.3841	orf19.4781
orf19.6558	orf19.7551
orf19.3482	orf19.1814
orf19.5862	orf19.4863
orf19.6152	orf19.5926
orf19.7213	orf19.6904
orf19.5641	orf19.6933
orf19.4446	orf19.3049
orf19.4610	orf19.7029
orf19.5160	orf19.5377
orf19.1333	orf19.607
orf19.5517	orf19.5986
orf19.4633	orf19.3710
orf19.2703	orf19.742
orf19.313	orf19.1235
orf19.3649	orf19.5869
orf19.3558	orf19.7367
orf19.403	orf19.4171
orf19.52	orf19.6185

Strains sensitive to POSA	Strains resistant to POSA
orf19.6877	orf19.5662
orf19.4240	orf19.2882
orf19.3139	orf19.5894
orf19.2423	orf19.754
orf19.991	orf19.6358
orf19.1493	orf19.1078
orf19.3929	orf19.2160
orf19.2151	orf19.1836
orf19.5780	orf19.4822
orf19.5859	orf19.6225
orf19.7257	orf19.4831
orf19.7219	orf19.4984
orf19.4376	orf19.3329
orf19.3458	orf19.4316
orf19.23	orf19.2282
orf19.1159	orf19.4232
orf19.5496	orf19.5164
orf19.2198	orf19.2970
orf19.1832	orf19.1538
orf19.656	orf19.7306
orf19.5667	orf19.2541
orf19.5530	orf19.5532
orf19.5921	
orf19.4325	
orf19.2762	
orf19.5924	
orf19.1911	
orf19.2792	
orf19.7479	
orf19.2467	
orf19.3901	
orf19.604	
orf19.732	
orf19.3100	
orf19.1042	
orf19.4088	
orf19.5043	
orf19.5728	
orf19.3874	
orf19.4658	
orf19.287	

Strains sensitive to POSA	
orf19.7585	
orf19.1887	
orf19.5827	
orf19.1066	
orf19.6012	
orf19.1062	
orf19.6606	
orf19.4943	
orf19.3106	
orf19.1421	
orf19.669	
orf19.6992	
orf19.2077	
orf19.6847	
orf19.7319	
orf19.7322	
orf19.5029	
orf19.7565	
orf19.7666	
orf19.388	
orf19.4624	
orf19.4119	
orf19.1112	
orf19.698	
orf19.3243	

APPENDIX A4: Strains resistant and sensitive to both FLU and POSA

Strains sensitive to both FLU and POSA	Strains resistant to both FLU and POSA
orf19.1042	orf19.1235
orf19.1066	orf19.1538
orf19.1112	orf19.1720
orf19.151	orf19.1773
orf19.2423	orf19.1836
orf19.2458	orf19.2160
orf19.2538	orf19.2363
orf19.2557	orf19.2541
orf19.2666	orf19.260
orf19.2762	orf19.2671
orf19.2842	orf19.2706
orf19.299	orf19.2882
orf19.3139	orf19.2970
orf19.318	orf19.3263
orf19.3243	orf19.3282
orf19.3247	orf19.3329
orf19.3298	orf19.3574
orf19.3302	orf19.3710
orf19.3482	orf19.4001
orf19.3649	orf19.4160
orf19.403	orf19.4316
orf19.4088	orf19.459
orf19.4240	orf19.4629
orf19.4274	orf19.4722
orf19.4448	orf19.4831
orf19.4461	orf19.4863
orf19.4524	orf19.4984
orf19.4610	orf19.5164
orf19.4624	orf19.5294
orf19.4631	orf19.5532
orf19.4633	orf19.5595
orf19.5076	orf19.5662
orf19.52	orf19.5869
orf19.5641	orf19.5894
orf19.5667	orf19.5986
orf19.5702	orf19.607
orf19.5720	orf19.6185
orf19.5827	orf19.6199

orf19.6012	orf19.6225
Strains sensitive to both FLU and POSA	**Strains resistant to both FLU and POSA**
orf19.6152	orf19.6908
orf19.6039	orf19.6904
orf19.644	orf19.767
orf19.6538	orf19.908
orf19.7257	
orf19.7269	
orf19.7319	
orf19.732	
orf19.7565	
orf19.991	

APPENDIX A5: Strains sensitive to AMB in *C. albicans*

Strains sensitive to AMB				
orf19.665	orf19.5720	orf19.52	orf19.6020	orf19.895
orf19.5013	orf19.1719	orf19.1236	orf19.1159	orf19.3100
orf19.1387	orf19.6195	orf19.212	orf19.5496	orf19.7512
orf19.6860	orf19.2484	orf19.6877	orf19.2411	orf19.1042
orf19.2835	orf19.2008	orf19.4240	orf19.1449	orf19.5043
orf19.1295	orf19.5658	orf19.6727	orf19.234	orf19.5728
orf19.7136	orf19.744	orf19.1130	orf19.6167	orf19.4658
orf19.3366	orf19.3542	orf19.5849	orf19.1832	orf19.4831
orf19.6025	orf19.299	orf19.2423	orf19.5789	orf19.5090
orf19.2216	orf19.2092	orf19.991	orf19.6464	orf19.1989
orf19.7413	orf19.2073	orf19.4546	orf19.2882	orf19.4764
orf19.4005	orf19.3822	orf19.4287	orf19.4229	orf19.287
orf19.5379	orf19.3841	orf19.5592	orf19.6592	orf19.3152
orf19.6422	orf19.6558	orf19.3929	orf19.841	orf19.1857
orf19.7363	orf19.6533	orf19.6398	orf19.3586	orf19.147
orf19.4336	orf19.5263	orf19.2151	orf19.4519	orf19.7355
orf19.7655	orf19.2251	orf19.7158	orf19.478	orf19.4112
orf19.4015	orf19.2031	orf19.857	orf19.1394	orf19.4981
orf19.6294	orf19.6237	orf19.5780	orf19.3980	orf19.4906
orf19.4258	orf19.6011	orf19.5859	orf19.1659	orf19.2990
orf19.5148	orf19.2425	orf19.4688	orf19.7324	orf19.4316
orf19.6640	orf19.6287	orf19.1663	orf19.1078	orf19.5411
orf19.4697	orf19.7390	orf19.105	orf19.4339	orf19.2514
orf19.2761	orf19.3482	orf19.183	orf19.5667	orf19.4372
orf19.4635	orf19.6152	orf19.1946	orf19.517	orf19.728
orf19.3058	orf19.4446	orf19.835	orf19.5530	orf19.916
orf19.6075	orf19.6928	orf19.2798	orf19.2114	orf19.7337
orf19.1232	orf19.7218	orf19.4376	orf19.5784	orf19.5393
orf19.5895	orf19.5673	orf19.7629	orf19.4325	orf19.723
orf19.6707	orf19.4186	orf19.4560	orf19.2772	orf19.5827
orf19.6168	orf19.5495	orf19.1698	orf19.2792	orf19.7440
orf19.2666	orf19.1235	orf19.3705	orf19.5295	orf19.2618
orf19.4128	orf19.6766	orf19.2945	orf19.2467	orf19.7115
orf19.1891	orf19.2703	orf19.5103	orf19.4804	orf19.5729
orf19.3298	orf19.4475	orf19.3458	orf19.604	orf19.1887
orf19.350	orf19.2007	orf19.5071	orf19.2553	orf19.2926
orf19.897	orf19.313	orf19.4783	orf19.2248	orf19.7668
orf19.4845	orf19.3649	orf19.23	orf19.6059	orf19.4772
orf19.6981	orf19.5869	orf19.1160	orf19.4950	orf19.3804
orf19.3035	orf19.3558	orf19.5662	orf19.4593	orf19.1419
orf19.7156	orf19.403	orf19.2942	orf19.732	orf19.500

Strains sensitive to AMB				
orf19.2790	orf19.3432	orf19.2312	orf19.2952	orf19.7322
orf19.1066	orf19.3679	orf19.6344	orf19.669	orf19.5029
orf19.300	orf19.4175	orf19.2988	orf19.6992	orf19.3232
orf19.1062	orf19.6653	orf19.4381	orf19.3267	orf19.7565
orf19.782	orf19.26	orf19.3122	orf19.2077	orf19.7619
orf19.3912	orf19.6606	orf19.3106	orf19.6847	orf19.7666
orf19.4668	orf19.6271	orf19.5838	orf19.1124	orf19.5919
orf19.5611	orf19.4943	orf19.1421	orf19.7490	orf19.3995
orf19.3308	orf19.4197	orf19.4579	orf19.4624	orf19.1723
orf19.3219	orf19.3222	orf19.843	orf19.240	orf19.698
orf19.387	orf19.3412	orf19.5102	orf19.5534	orf19.3243
orf19.3445	orf19.3065	orf19.7320	orf19.4119	
orf19.2970	orf19.1538	orf19.7306	orf19.2186	

APPENDIX A6: Systemic Name of sensitive Strains to AMB in *C. albicans*

Strains sensitive to AMB	YKR088C	YER164W	YGL100W
YMR077C	YDR126W	YKL209C	YPR173C
YLR106C	YGR208W	YDR264C	YLR306W
YIL067C	YJL141C	YNL323W	YOR067C
YMR177W	YPL067C	YDL167C	YER052C
YLR027C	YOR137C	YPL065W	YEL058W
YNL277W	YMR137C	YGL184C	YIL124W
YHR005C	YMR027W	YOR165W	YCR090C
YBL021C	YNL141W	YEL019C	YCR036W
YKL041W	YGL047W	YPR201W	YDR261C
YNL305C	YGR255C	YDL244W	YGR271W
YJR123W	YGR276C	YGL180W	YGR094W
YBR005W	YBR290W	YJL156C	YPL030W
YPR113W	YOL138C	YDR483W	YBR239C
YDL140C	YLR120C	YIR004W	YPL096W
YIL003W	YPL265W	YFL013C	YMR241W
YDR335W	YGR116W	YER040W	YNL055C
YPL029W	YGL094C	YPR184W	YPR127W
YPL167C	YDR408C	YHR168W	YBR126C
YMR309C	YPR088C	YOR129C	YGL225W
YDR061W	YHR178W	YLL012W	YJR093C
YMR099C	YDL018C	YER081W	YDR415C
YOL004W	YER167W	YMR125W	YFL030W
YGL124C	YMR154C	YGR135W	YBR086C
YGR217W	YBR101C	YCR079W	YKR051W
YPL230W	YPL133C	YGR287C	YBR115C
YIR028W	YMR071C	YPR180W	YHR181W
YGR284C	YNL175C	YLR300W	YNL073W
YNL094W	YEL023C	YOR163W	YOR198C
YMR283C	YNL180C	YKR013W	YDR338C
YJL005W	YMR134W	YOL018C	YNL316C
YNL242W	YPL190C	YDR513W	YNL062C
YPL116W	YLR113W	YMR055C	YHR132C
YJR142W	YLR186W	YGL186C	YER118C
YGR125W	YJL055W	YPR118W	YDR302W
YMR076C	YNL217W	YNL200C	YPL225W
YIL099W	YKL046C	YIL090W	YOL129W
YOL151W	YPL154C	YCR068W	YDR332W
YBR199W	YIR032C	YJR100C	YKR065C
YBR204C	YGL067W	YDL178W	YKL179C
YJR075W	YIL041W	YPR167C	YDL127W
YDR379W	YMR171C	YDR027C	YLR316C
YDR245W	YJL093C	YNR019W	YOR202W
YNL279W	YKL018W	YNR007C	YHR035W
YNL025C			

YDL212W
YNL335W
YGL233W
YOL137W
YGL012W
YCR044C
YGR202C
YFL004W
YAL014C
YPR045C
YDR323C
YKL064W
YDR196C
YPR159W
YDL165W
YER093C
YJR106W
YHR023W
YDR389W
YBR241C
YLR361C
YMR272C

APPENDIX A7:

Strains sensitive to AMB

in *Saccharomyces cerevisiae*

Systemic Name	YLR039C	YDR392W	YER092W
YDR276C	YPL057C	YIL056W	YOR067C
YDR326C	YGR197C	YPR051W	YIL111W
YNL257C	YDR310C	YAL012W	YIL092W
YJR118C	YLR200W	YGR252W	YHR168W
YHR155W	YNL015W	YJR059W	YPR124W
YMR102C	YGL020C	YBR041W	YEL062W
YDR207C	YDR418W	YBR168W	YBL042C
YLR372W	YKL081W	YAL056W	YHL006C
YJL056C	YNR029C	YNL190W	YLR090W
YLR182W	YDR297W	YNR031C	YIL161W
YLR315W	YGL035C	YHL043W	YCR045C
YPL105C	YGR122W	YDL232W	YBR021W
YNL041C	YJL139C	YAL013W	YDR293C
YOR008C	YJL183W	YDR530C	YMR185W
YMR109W	YDR484W	YJL128C	YDR071C
YDR497C	YGR143W	YNR075W	YOL103W
YBL086C	YMR060C	YBL067C	YNL197C
YBR126C	YPR095C	YJR152W	YNL099C
YPR097W	YJR139C	YMR216C	YCR019W
YGR285C	YPL056C	YHR100C	YIL116W
YGL253W	YLR110C	YFL001W	YGL066W
YKR028W	YER110C	YMR038C	YNR001C
YGR229C	YOR371C	YHL028W	YDR028C
YIR033W	YBR200W	YNL051W	YOR109W
YBL072C	YPR040W	YIL073C	YPR139C
YNL307C	YMR264W	YML074C	YLR006C
YKL037W	YJL198W	YHL047C	YHL040C
YER048C	YDR389W	YIL077C	YKL216W
YGL005C	YNL323W	YGL167C	YCR017C
YLR418C	YGL062W	YPL262W	YBR295W
YDR072C	YLR113W	YIL042C	YPL214C
YLR262C	YGL219C	YIR016W	YGL252C
YOR035C	YML123C	YNL259C	YMR283C
YLR454W	YDL002C	YDR448W	YNL154C
YDR320C	YIL124W	YDL021W	YGR037C
YML048W	YKR029C	YCR003W	YJL181W
YDL100C	YLR242C	YNL064C	YDR146C
YJR043C	YIL160C	YAL010C	YLR439W
YBR103W	YOR304W	YLR420W	YDL101C
YGL033W	YGL027C	YOR360C	YPL203W
YML041C	YJL180C	YCR036W	YNL010W
YJL062W	YCR068W	YNL271C	YDR202C
YIL034C	YOL089C	YGL133W	YCL050C
YGL194C	YBL006C	YMR081C	YDL020C

YBR283C	YGR155W	YLR436C	YNL047C
YNL229C	YCL056C		
YMR037C	YHL011C		
YPR133W-A	YMR303C		
YHL029C	YMR294W		
YER027C	YPL042C		
YBL103C	YIL055C		
YHR025W	YMR315W		
YMR021C	YLR375W		
YLL038C	YNL194C		
YJR054W	YHR017W		
YML014W	YCR026C		
YGR056W	YOL067C		
YBR023C	YLR205C		
YNR052C	YHL019C		
YHL023C	YHR001W-A		
YOL087C	YFR036W		
YKR014C	YDR528W		
YBR036C	YDR173C		
YMR307W	YAR029W		
YLR055C	YLR373C		
YHL016C	YDR346C		
YNL076W	YDR371W		
YML109W	YKL174C		
YMR022W	YML112W		
YPL055C	YDR176W		
YCL037C	YOL076W		
YMR304W	YKL054C		
YPL144W	YJL134W		
YHL031C	YGR276C		
YOL088C	YDR524C		
YBL104C	YDL117W		
YKL023W	YOR381W		
YCL045C	YGL236C		
YLR046C	YPL174C		
YIL112W	YNL055C		
YPL234C	YGL231C		
YCR077C	YOR073W		
YML013W	YBR284W		
YDL176W	YIL057C		
YMR164C	YJR073C		
YMR031C	YDR067C		
YFL053W	YDR153C		
YDR245W			
YLR278C			
YDR439W			

YMR306W	
YKR096W	
YLR085C	

APPENDIX A8:

Overlap sensitive strains to Azole

in *Saccharomyces cerevisiae* in two different labs.

YAL059W	YKL166C	YPL195W
YBL089W	YKR030W	YPL273W
YBR114W	YKR074W	YPR133W-A
YBR126C	YLR015W	YPR179C
YBR166C	YLR055C	
YCR028C	YLR065C	
YCR088W	YLR082C	
YDL020C	YLR083C	
YDL224C	YLR119W	
YDL226C	YLR176C	
YDR073W	YLR239C	
YDR202C	YLR330W	
YDR213W	YLR408C	
YDR392W	YLR451W	
YDR415C	YML041C	
YDR461W	YML075C	
YDR486C	YMR053C	
YEL007W	YMR186W	
YER002W	YMR238W	
YER020W	YNL021W	
YER056C-A	YNL064C	
YER177W	YNL111C	
YFL014W	YNL122C	
YFL018C	YNL230C	
YGL025C	YNL231C	
YGL151W	YNL294C	
YGL253W	YNL299W	
YGR122W	YNL323W	
YGR252W	YOR067C	
YGR286C	YOR068C	
YHL012W	YOR069W	
YHL022C	YOR089C	
YHL024W	YOR106W	
YHR045W	YOR196C	
YHR087W	YOR221C	
YHR158C	YPL041C	
YIL103W	YPL055C	
YIL165C	YPL061W	
YJL046W	YPL090C	
YJL053W	YPL138C	
YJL206C	YPL145C	
YJL208C	YPL170W	
YJL216C	YPL179W	

APPENDIX A9:

Systemic Name of sensitive Strains to Azole in *C. albicans*

YNL055C	YOR296W
YPL067C	YLR026C
YMR237W	YDL194W
YMR237W	YPL234C
YKL174C	YPL164C
YMR140W	YDR071C
YER089C	YGR288W
YML105C	YNL202W
YDR335W	YCL025C
YLR109W	YIR004W
YJL110C	
YHR132C	
YML131W	
YJR122W	
YPR088C	
YJL207C	
YGR217W	
YOR178C	
YGL067W	
YBR101C	
YDL127W	
YDR272W	
YCR044C	
YLR142W	
YOR353C	
YIL130W	
YJL172W	
YMR027W	
YGR060W	
YMR226C	
YOR122C	
YMR177W	
YLR438W	
YKL064W	
YOR093C	
YOL119C	
YMR055C	

APPENDIX A10:

Strains sensitive to Azole and AMB

in *Candida glabrata*

Sensitive Azole Standard Name	Sensitive Azole Systemic Name
PDR1	YGL013C
PDR5	YOR153W
KTR2	YKR061W
CWH41	YGL027C
HAP1	YLR256W
MYO2	YOR326W
CKA2	YOR061W
YPK1	YKL126W
SSD1	YDR293C
SLG1	YOR008C
SLT2	YHR030C
CNB1	YKL190W
CNA1	YLR433C

Sensitive AMB Standard Name	Sensitive AMB Systemic Name
VPS15	YBR097W
KTR2	YKR061W
CKA2	YOR061W
YPK1	YKL126W
SLA1	YBL007C
SNF6	YHL025W
DEP1	YAL013W
CWH41	YGL027C
SSD1	YDR293C
SNF7	YLR025W
ROX1	YPR065W
SAC7	YDR389W
KRE1	YNL322C

APPENDIX A11:

Overlap of sensitive strains between *C. albicans* , *S. cerevisiae and C. glabrata*

to AMB

Overlap between *C. albicans* and *S. cerevisiae*	**Overlap between *S. cerevisiae and C. glabrata***	**Overlap between *C. albicans and C. glabrata***	**Overlap between *C. albicans* , *S. cerevisiae and C. glabrata***
YBR126C	YAL013W	Non	YDR389W
YCR036W	YDR293C		
YCR068W	YGL027C		
YDR245W			
YGR276C			
YHR168W			
YIL124W			
YLR113W			
YMR283C			
YNL055C			
YNL323W			
YOR067C			