Yeast 2 Hybrid Analysis of *Candida albicans* Proteins Mbp1, Swi6 and Skn7

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Introduction

Candida albicans is a yeast that can cause opportunistic skin and mucous membrane infections, such as thrush and vaginal yeast infection, as well as systemic infections in immune-compromised patients. It has been well established that morphogenesis, the transition from yeast to filamentous growth forms, is essential for *C. albicans* to cause systemic infections.

Previous work in our lab has shown that the Mbp1 protein is required for morphogenesis under nitrogen-limiting conditions on solid media. In addition, analysis of the amino acid sequence of Mbp1 revealed the presences of ankyrin repeat sequences, which suggests that Mbp1 interacts with other proteins to perform its function.

This study is attempting to elucidate if Mbp1 interacts with itself, Swi6 and/or the Skn7 proteins. To accomplish this, the genes for the *C. albicans* proteins were each transformed into *Saccharomyces cerevisiae* and will be verified via PCR to confirm the presence of the genes. A yeast-2- hybrid analysis will then be performed to test whether Mbp1 interacts with itself, Swi6 and/or Skn7. These results will help to identify proteins that interact with Mpb1 to promote morphogenesis and thus pathogenesis.

Materials and Methods

The pGBKT7 and pGADT7 plasmids were isolated using Qiagen plasmid isolation kit. The *MBP1*, *SKN7* and *SWI6* genes were amplified using primers in Table 1. Homologous ends were added during a secondary PCR reaction using primers given in Table 1.

Plasmid pGBKT7 was digested using *Nde1* and *EcoR1*. The digested pGBKT7 plasmid and genes *MBP1*, *SWI6* or *SKN7* were co-transformed into YPB2a (Trp 1-). Transformants are selected by growing on tryptophan dropout media.

Plasmid pGADT7 was digested using *Sfi1* and *BamH1*. The digested plasmid and gene *MBP1* were co-transformed into LB414α (Leu 2-). Transformants are selected by growing on leucine dropout media.

The transformation was carried out using the TRAFO method (1).

Tranformants were verified by colony PCR using primers in Table 1.

Yeast-2-Hybrid matings were performed by physically crossing the pGADMBP1 transformants with either pGBKMBP1, pGBKSWI6 or pGBKSKN7 transformants to obtain diploids carrying both plasmids. Initial growth from these matings were plated on double dropout media to confirm diploid phenotype.

References

1. Gietz, R.D. and R.A.Woods. (2002) TRANSFORMATION OF YEAST BY THE Liac/SS CARRIER DNA/PEG METHOD. Methods in Enzymology 350:87-96

Figure 1. Yeast-2-Hybrid Procedure

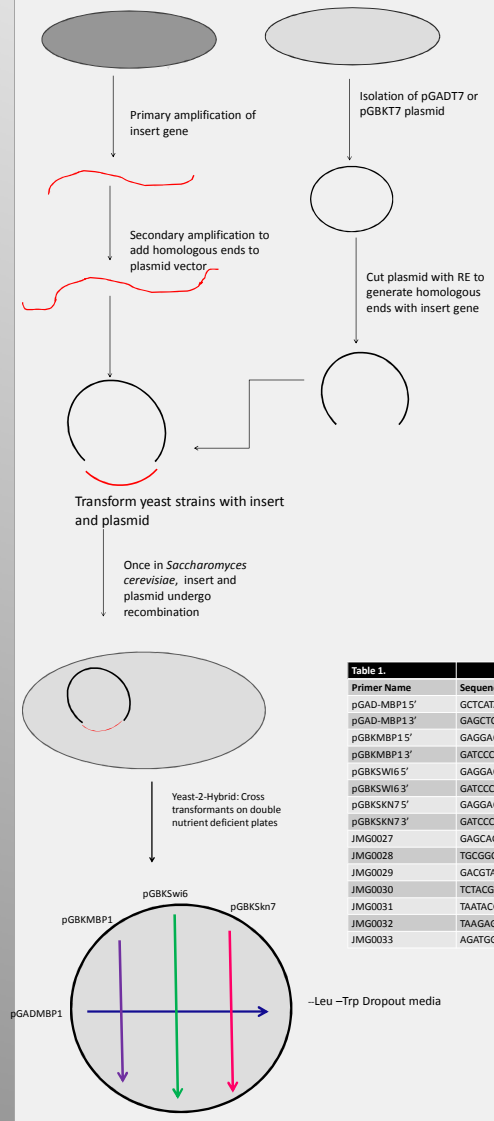

Table 1.

Primer Name	Sequence	Used for
pGAD-MBP1 5′	GCTCATATGGCCATGGAGGCCATGTCAGACTCTCAAATTTAT	Primary amplification of pGADMBP1 gene
pGAD-MBP1 3′	GAGCTCGATGGATCCTTATTTTTTGACCAATGATTCTTC	Primary amplification of pGADMBP1 gene
pGBKMBP1 5′	GAGGACCTGCATATGTCAGACTCTCAAATTTATTCA	Primary amplification of pGBKMBP1 gene
pGBKMBP1 3′	GATCCCCGGGAATTCTTATTTTTTGACCAATGATTCTTC	Primary amplification of pGBKMBP1 gene
pGBKSWI6 5′	GAGGACCTGCATATGGATTCTCCTATACACATTGGT	Primary amplification of pGBKSwi6 gene
pGBKSWI6 3′	GATCCCCGGGAATTCTTACTGTTGTCCTTCCACTGCTTC	Primary amplification of pGBKSwi6 gene
pGBKSKN7 5′	GAGGACCTGCATATGTCTTCATTACAACAACCCATA	Primary amplification of pGBKSkn7 gene
pGBKSKN7 3′	GATCCCCGGGAATTCTTAACTCAATTTCTGTTTCTTTAC	Primary amplification of pGBKSkn7 gene
JMG0027	GAGCAGAAGCTGATCTCAGAGGAGGACCTGCATATG	Secondary amplification of pGBK genes
JMG0028	TGCGGCCGCTGCAGGTCGACGGATCCCCGGGAATCC	Secondary amplification of pGBK genes
JMG0029	GACGTACCAGATTGGCCATGGAGGCC	Secondary amplification of pGAD gene
JMG0030	TCTACGATTCATCGCTCGATGGATCC	Secondary amplification of pGAD gene
JMG0031	TAATACGACTCACTATAGGGC	Colony PCR for pGAD and pGBK
JMG0032	TAAGAGTCACTTTAAAATTTGTAT	Colony PCR for pGBK
JMG0033	AGATGGTGCACGATGCACAG	Colony PCR for pGAD

Results

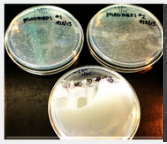

Figure 2. Transformants growing on nutrient deficient plates

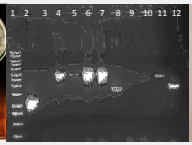

Figure 3. Colony PCR

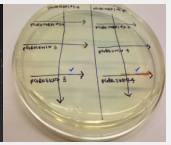

Figure 4. Yeast-2-Hybrid cross growing on double nutrient deficient plates

Discussion

The procedure for creating the strains to be used in the Yeast-2-Hybrid Analysis is diagramed in Figure 1. Transformants were successfully created using this procedure (Figure 2).

Preliminary results from the colony PCR indicate that we have created the strains to be used in Yeast-2-Hybrid Analysis (Figure 3). Lane 1 is a 1Kb ladder. Lanes 2 and 3 are pGADT7 positive and negative controls respectively. Lanes 4-7 are pGADMBP1 transformants. Lanes 7 and 8 are pGBKT7 positive and negative controls respectively while lane 10 is a pGBKSWI6 transformant, lane 11 is a pGBKMBP1 transformant and lane 12 is a pGBKSKN7 transformant.

Preliminary results from mating reactions (Figure 4) have indicated that we have created diploids that can be tested for protein-protein interactions. Confirmation of these results are ongoing. Once we have confirmed our diploid strains, additional analysis will be conducted to detect protein-protein interactions.

Acknowledgements

Special thanks to the UW – Eau Claire Office of Research and Sponsored Programs for their financial support of this project.