Development of *Streptococcus thermophilus* *lacZ* as a reporter gene for *Candida albicans*

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The study of gene regulation in many organisms has been facilitated by the development of reporter genes. The authors report the use of *lacZ* from *Streptococcus thermophilus*, a gene encoding a β -galactosidase, as a reporter for the fungal pathogen *Candida albicans*. As test cases, *Strep. thermophilus lacZ* was placed under control of three different *C. albicans* promoters: *MAL2* (maltase), inducible by maltose; *HWP1* (hyphal cell wall protein), induced by conditions that promote filamentous growth; and *ACT1* (actin). These constructs were each integrated into the *C. albicans* genome and β -galactosidase activity was readily detected from these strains, but only under the appropriate growth conditions. β -Galactosidase activity could be detected by several methods: quantitative liquid assays using permeabilized cells, colorimetric assays of colonies replicated to paper filters, and *in situ* coloration of colonies growing on medium containing the indicator X-Gal. These results show the usefulness of *Strep. thermophilus lacZ* as a monitor of gene regulation in this medically important yeast.

Keywords: β -galactosidase, yeast, fungal pathogen, gene regulation

INTRODUCTION

Candida albicans is a commensal yeast of warm-blooded animals and has been recognized as an opportunistic pathogen for many years. *C. albicans* can assume a variety of morphologies, including budding yeast (blastospores) as well as pseudohyphal and hyphal forms; all of these are found at sites of infection (Odds, 1988). A number of growth conditions can be utilized *in vitro* to induce the transition between morphological forms of *C. albicans*. Signals which influence the yeast to hyphal transition include temperature, pH and the nutrient composition of the growth media (Odds, 1988), and several genes with roles in the morphological transition have been identified (Liu *et al.*, 1994; Braun & Johnson, 1997; Csank *et al.*, 1997, 1998; Lo *et al.*, 1997; Newport & Agabian, 1997; Alex *et al.*, 1998; Gale *et al.*, 1998; Timpel *et al.*, 1998; Stoldt *et al.*, 1997; Leberer *et al.*, 1996, 1997; Calera *et al.*, 2000; Brown & Gow, 1999).

Studies of transcriptional regulation in *C. albicans* have

been limited by several properties of the organism; e.g. it is diploid with a little-understood sexual cycle, making conventional genetic analysis impossible (Odds, 1988). Additionally, expression of foreign genes, including reporter genes, in *C. albicans* has been complicated by the alternative codon usage of the organism. The codon CTG is read as a serine in *C. albicans* but as a leucine in other organisms (Ohama *et al.*, 1993). *Escherichia coli lacZ*, which has been successfully adapted as a reporter gene in many organisms, contains 51 CTG codons (Gilbert & Maxam, 1973); therefore successful expression of a functional enzyme seems unlikely without extensive alteration. Other common reporter genes, such as *uidA* (β -glucuronidase) also contain several CTG codons (Blanco *et al.*, 1985). We were particularly interested in developing a β -galactosidase as a reporter for *C. albicans* because of the extensive variety of substrates available for this enzyme. In addition, *C. albicans* is not known to have an endogenous β -galactosidase and cannot utilize lactose as a carbon source (Kwon-Chung, 1992).

Here we report the use of *lacZ* from *Streptococcus thermophilus* as a versatile reporter gene for *C. albicans*. We show that β -galactosidase activity can be readily detected by a variety of methods when *lacZ* is expressed from several *C. albicans* promoters.

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METHODS

Strains and plasmids. pRH116 was obtained from Larry McKay (Dept of Food Science and Nutrition, University of Minnesota, St Paul) and contains *Strep. thermophilus lacZ* in a pBR322-based vector (Schroeder *et al.*, 1991). The vectors utilized for LacZ expression were constructed as follows. pAU13 contains the *URA3* gene from *C. albicans* inserted into *XbaI/Ecl136II*-digested pBluescript KS+ (Stratagene) as an *XbaI-ScaI* fragment from pDBV52 (Brown *et al.*, 1996). pAU15, the MAL2 expression vector, was created by amplifying a 550 bp region upstream of the MAL2 coding sequence with the primers MAL2-5' (GAAGGTACCAGATTGATATTTTGTCTAGTACC) and MAL2p3' (TCTCACTCGAGATGTAGTTGATTATTAGTTAAAC) using *C. albicans* CAI4 genomic DNA. A 250 bp downstream region of MAL2 was amplified from *C. albicans* CAI4 genomic DNA using the primers UTR5' (CCTGGGATCCTAAATATACACTAGATGCTATC) and MAL2-3' (CTGTCTAGAAGACATACGCTTTGCAGGTGGTGT). The MAL2 upstream and downstream fragments were cloned into pAU13 to create pAU15, which is a MAL2 expression vector containing a *XhoI-SalI-HindIII-SmaI-BamHI* polylinker following the MAL2 promoter. The HWP1 expression vector was created in a similar fashion, by amplifying an upstream region of HWP1 with the primers HWP1-P5' (GTCCTGGTACCAAAAACAAGGAATTCGAAATTC) and HWP1-P3' (CTCCTTCTCGAGATTGACGAACTAAAAGCGAGTG). The downstream region of HWP1 was amplified with the primers HWP1-U5' (GAAGAGGATCCTTCTAATAACTGATACTAAGTTTT) and HWP1-U3' (CTCTTTCTAGAATTGTAAAAAAGGGGTATTTATT). The resulting fragments were cloned into pAU13, resulting in pAU64, which contains a *HindIII-EcoRV-PstI-SmaI* polylinker following the HWP1 promoter. The ACT1 expression vector was created by amplifying a 1.1 kb region upstream of ACT1 from *C. albicans* CAI4 genomic DNA with the primers ACT1-P5' (CTCTCGGTACCAGAGCTATTAAGATCACCAGCCT) and ACT1-P3' (CTCTTCTCGAGTTTGAATGATTATATTTTTTAATATTA). The ACT1 downstream region was amplified with the primers ACT1-U5' (GAAGAGGATCCGAGTGAAATTCGGAAATCTGGAA) and ACT1-U3' (CTCTTTCTAGAATTTTATGATGGAATGAATGGATG). These fragments were cloned into pAU13 to create pAU34, which contains an *XhoI-ClaI-HindIII-EcoRV-PstI-SmaI-BamHI* polylinker following the ACT1 promoter region.

The *Strep. thermophilus lacZ* gene was amplified from pRH116 using the primers LacZ5' (GTGTCCCGGGTCCATGAACATGACTGAAAAATTCAA) and LacZ3' (TCCACGAGGATCCCTAATTTAGTGGTTCAATCATGAG) and cloned into the various expression constructs to create the following plasmids: MAL2-*lacZ* (pAU22), HWP1-*lacZ* (pAU95) and ACT1-*lacZ* (pAU36). Alteration of the CTG codon to TTA in *lacZ* was done by PCR-based mutagenesis using the primer pairs LacZ-1631 (ATGGTGTCTGAGTTTGA) and LacZm3' (CAGTTGCTTCTCTTAAGACACAAGCAACTTCGTAAATTTG), and LacZm5' (GTTGCTTGTGTCTTAAGAGAAGCAACTGAATGGGCTCC) and LacZ3'. The resulting PCR fragments were mixed and served as a template for amplification with the primers LacZ-1631 and LacZ3'; this was cloned into pAU22 to give pAU106. Presence of the mutation was screened for by creation of an *AflII* site and confirmed by sequencing. For expression of *Strep. thermophilus lacZ* in *Saccharomyces cerevisiae*, *lacZ* was amplified with Pfu DNA polymerase (Stratagene) using linearized pAU22 as a template and the

primers LacZ5' and MAL2-3'Bln (CCTGCCTAGGAGACATACGCTTTGCAGGTGGTGT). The resulting product was digested with *XmaI* and *BlnI* and cloned into *XmaI/XbaI*-cut pDK20 (a gift from Doug Kellogg, Sinsheimer Laboratories, Dept of Biology, University of California, Santa Cruz).

C. albicans methods. *C. albicans* strain CAI4 (*ura3::imm434/ura3::imm434*) served as the parent strain for all manipulations (Fonzi & Irwin, 1993). Linearized DNA was transformed into CAI4 by the modified lithium acetate method (Hill *et al.*, 1991; Gietz *et al.*, 1995), and transformants were selected on medium lacking uridine (Guthrie & Fink, 1991). Standard recipes were utilized for all media, and carbon sources such as maltose, glucose, galactose or lactose were added at a final concentration of 2% (v/v) (Guthrie & Fink, 1991). For expression of *lacZ* in *Sacch. cerevisiae*, strain W303 (*ade2-1 trp1-1 cau1-100 leu2-3,112 his3-11 ura3 psi+*) was utilized (Guthrie & Fink, 1991). Plasmids were introduced into W303 by lithium acetate transformation (Hill *et al.*, 1991; Gietz *et al.*, 1995).

β -Galactosidase assays. *C. albicans* β -galactosidase assays were performed as described by Ausubel *et al.* (1992) for *Sacch. cerevisiae* with minor modifications. For liquid assays, 1 ml cells was resuspended in an equal volume of Z buffer (Ausubel *et al.*, 1992) and placed on ice. The OD₆₀₀ was determined for each sample. Then 10–100 μ l of cells was added to Z buffer to a final volume of 1 ml, and the cells were permeabilized with 15 μ l 0.1% SDS and 30 μ l chloroform. There was no appreciable change in activity when alternative methods such as toluene or glass beads were used for permeabilizing cells. Cells were equilibrated at 37 °C for 5 min, then 0.2 ml ONPG (4 mg ml⁻¹) was added and the cells were mixed and incubated at 37 °C. Reactions were stopped by addition of 0.5 ml 1 M Na₂CO₃, spun for 5 min at 10000 g and the A₄₂₀ and A₅₅₀ were read. Units of activity were determined by the standard equation given by Ausubel *et al.* (1992).

Filter assays were performed as described by Ausubel *et al.* (1992). Patched colonies of *C. albicans* were replica-plated onto solid medium with an overlay of a circular Whatman filter and grown overnight. Filters were frozen in liquid nitrogen and incubated in 3 ml Z buffer with 20 μ l 3% X-Gal and incubated at 37 °C.

Visual screens for *C. albicans* were carried out by patching colonies onto X-Gal plates (Ausubel *et al.*, 1992). The standard formulation for X-Gal plates was utilized for most applications. A slightly modified recipe proved more sensitive for detection of β -galactosidase, although strains grew more slowly on this medium (X-Gal Modified Medium, XMM). XMM contained 1.7 g Yeast Nitrogen Base (without amino acids or ammonium sulfate), 20 g glucose, 5 g ammonium sulfate and 20 g agar in 930 ml H₂O. After autoclaving, 70 ml 1 M potassium phosphate pH 7.0 and 2 ml of a 20 mg ml⁻¹ X-Gal solution were added.

RESULTS AND DISCUSSION

Assays of β -galactosidases in *C. albicans*

We reasoned that β -galactosidases from organisms with high percentages of adenosine/thymine genomic content might be biased against CTG codons; a search against various databases revealed two β -galactosidases that contained relatively few CTG codons. *LAC4* from

Fig. 1. β -Galactosidase genes tested for activity in *C. albicans*. *K. lactis* LAC4 (Poch *et al.*, 1992) and *Strep. thermophilus* lacZ (Schroeder *et al.*, 1991) were placed under control of the maltose-inducible *C. albicans* MAL2 promoter (Brown *et al.*, 1996) and integrated into the *C. albicans* genome. β -Galactosidase was scored by visual assays on medium containing X-Gal and maltose and by liquid assays on cells grown in medium with maltose (Ausubel *et al.*, 1992). For the LAC4 and lacZ genes, mutant derivatives were also constructed in which the CTG codons were changed to TTA. CTG is decoded as serine in *C. albicans* and as leucine in other organisms (Ohama *et al.*, 1993). The approximate locations of the CTG codons in LAC4 and lacZ are shown.

the yeast *Kluyveromyces lactis* contains only two CTG codons, while lacZ from the thermophilic bacterium *Strep. thermophilus* has only one CTG codon (Schroeder *et al.*, 1991; Poch *et al.*, 1992). The single leucine encoded by CTG in the *Strep. thermophilus* lacZ is at the extreme C-terminus of the LacZ protein in a non-conserved region of β -galactosidases, while one of the leucines encoded by a CTG codon in the *K. lactis* LAC4 gene is in a position conserved as a hydrophobic residue in β -galactosidases (Jacobson *et al.*, 1994) (Fig. 1). Both CTG codons in LAC4 were changed to TTA in order to specify leucines at these positions. Either wild-type LAC4 or the mutant derivatives were integrated into the *C. albicans* genome and expressed from the MAL2 (maltase) promoter. MAL2 is induced by maltose and repressed by glucose (Brown *et al.*, 1996). No activity was detected in strains with the MAL2-LAC4 wild-type and MAL2-LAC4 mutant derivatives under inducing conditions using both liquid assays and qualitative plate assays (Fig. 1). Although LAC4 has previously been described as a possible reporter gene in *C. albicans* (Leuker *et al.*, 1992), its usefulness in our hands appeared limited. When LAC4 was expressed from the ACT1 promoter, β -galactosidase activity could only be detected in a limited number of transformants; these presumably represented strains with multiple integration events (Leuker *et al.*, 1992).

When *Strep. thermophilus* lacZ (unaltered in its CTG codon) or lacZ with the single CTG codon altered to TTA was expressed from the MAL2 promoter in *C. albicans*, β -galactosidase activity could be readily detected by several means (Fig. 1). Colonies of *C. albicans* expressing MAL2-lacZ turned blue on solid medium containing both maltose and the chromogenic

substrate X-Gal after a few days' growth. No such activity was detected when colonies were grown on the same medium containing glucose instead of maltose. Maltose-dependent activity was also detected by liquid assays (see below and Table 1).

Expression of lacZ from a variety of promoters

Since lacZ expression could be detected from the MAL2 promoter, we examined its use for monitoring promoter activity from two other *C. albicans* genes, ACT1 and HWP1 (Fig. 2). The promoter for the ACT1 (actin) gene has previously been used for expression of foreign genes in *C. albicans* (Leuker *et al.*, 1992). ACT1 promoter expression is constant under many conditions but has been shown to be regulated by starvation and growth phase in *C. albicans* (Delbruck & Ernst, 1993). HWP1 expression is minimal when *C. albicans* is growing in the budding yeast form and highly induced during conditions that promote filamentous growth (Staab *et al.*, 1996). Quantitative liquid assays were performed on *C. albicans* strains expressing MAL2-lacZ, ACT1-lacZ or HWP1-lacZ from constructs that had been integrated in the genome (Table 1). Strains were grown at 30 °C in YEP-glucose or YEP-maltose (budding yeast morphology) or at 37 °C in YEP-glucose with 10 % fetal calf serum (hyphal/pseudohyphal morphology).

In the MAL2-lacZ strains, β -galactosidase activity was significantly induced (> 20-fold) by growth in maltose compared to growth in glucose; activity was detected at only background levels in glucose (Table 1), where background levels are defined as the very low levels of β -galactosidase activity observed in the absence of the *Strep. thermophilus* lacZ reporter gene. Expression of

Fig. 2. Qualitative assays of β -galactosidase activity in *C. albicans*. The expression constructs indicated on the left were integrated into the *C. albicans* genome, and individual transformants were plated on X-Gal modified medium (see Methods) and photographed after 5 d growth at 30 °C or 37 °C.

Table 1. Expression of *lacZ* from various promoters in *C. albicans*

Cultures were grown in YEP + glucose (Glc), YEP + maltose (Mal) or YEP + glucose + 10 % fetal calf serum. Cells were permeabilized and β -galactosidase assays were performed in Z buffer using ONPG as a substrate (Ausubel *et al.*, 1992). Values represent the mean of three independent transformants; units are expressed according to the standard protocol for *Sacch. cerevisiae* (Ausubel *et al.*, 1992). *MAL2-lacZ** refers to the *Strep. thermophilus lacZ* gene in which the single CTG codon was changed to TTA.

Construct	β -Galactosidase activity		
	+ Mal	+ Glc	+ Glc + serum
MAL2	< 0.1	< 0.1	< 0.1
MAL2-LacZ	1.7 $\pm$ 0.1	< 0.1	< 0.1
MAL2-LacZ*	1.6 $\pm$ 0.1	< 0.1	
ACT1	< 0.1	< 0.1	< 0.1
ACT1-LacZ	7.0 $\pm$ 0.5	9.0 $\pm$ 0.5	10 $\pm$ 0.5
HWP1	< 0.1	< 0.1	< 0.1
HWP1-LacZ	1.1 $\pm$ 0.1	< 0.1	24 $\pm$ 4
SC5314 (WT)	< 0.1	< 0.1	< 0.1

MAL2-lacZ was not induced significantly under conditions that promoted filamentous growth (Table 1). We noticed that approximately 10 % of strains transformed with our *lacZ* constructs expressed significantly higher levels of β -galactosidase than did the majority; these strains were assumed to represent multiple integrants and were not studied further. Examples of this are shown in Fig. 2. Additionally, approximately 5 % of strains did not express detectable levels of β -galactosidase; these presumably resulted from integration into the genome at sites other than the intended locus or from rearrangements that occurred subsequent to transformation.

Expression of the *ACT1-lacZ* construct was constant during all growth conditions, varying less than 1.5-fold during all growth conditions (Table 1). The *ACT1* gene is known to be downregulated by starvation and during growth-phase changes; these conditions were not tested for the *ACT1-lacZ* fusion (Delbruck & Ernst, 1993; Swoboda *et al.*, 1994). In contrast, the *HWP1-lacZ* construct was strongly induced by growth under filamentous conditions, including YEP-glucose + 10 % fetal calf serum at 37 °C. Compared to growth in YEP-glucose, the *HWP1-lacZ* construct was induced approxi-

mately 250-fold by growth in YEP-glucose + 10% fetal calf serum. This is consistent with measurements of RNA levels of *HWP1* in cells grown in YEP-glucose or YEP-glucose + 10% fetal calf serum at 37 °C (data not shown). *HWP1-lacZ* was also induced to a lesser degree by substitution of maltose for glucose in the growth medium (see Table 1 and Fig. 2), and utilization of maltose as a carbon source has been reported to be an inducer of filamentous growth in *C. albicans* (Odds, 1988).

In order to optimize enzymic assays for the *Strep. thermophilus* β -galactosidase, we performed assays at various temperatures and pH values. The temperature optimum of *Strep. thermophilus* LacZ was found to lie between 45 and 50 °C; enzymic activity fell sharply at temperatures higher than 50 °C. Activity of the enzyme was 2.2-fold higher at 45 °C than at 30 °C. The pH optimum of the enzyme was between 6.8 and 7.2.

Expression of *lacZ* does not allow growth on lactose as a sole carbon source

Since expression of *ACT1-lacZ* was independent of carbon source, this allowed a test of whether *C. albicans* expressing β -galactosidase could utilize lactose as a sole carbon source. We grew *C. albicans* SC5314 (wild-type) and *C. albicans* CA361 (*ACT1::lacZ*) on minimal medium with glucose (SG), minimal medium with lactose (SL) and minimal medium without any carbon source (S). No growth of SC5314 or CA361 was observed on SL or S medium, while both strains grew on SG medium (data not shown). A similar phenotype has been noticed for *Sacch. cerevisiae* strains expressing β -galactosidase, and expression of a lactose permease was necessary before lactose could be utilized as a carbon source (Sreekrishna & Dickson, 1985).

Qualitative assays of β -galactosidase activity

Growth of cells on medium containing X-Gal provides a useful method of screening large numbers of cells for the expression of β -galactosidase activity under particular sets of conditions. When cells were plated on the appropriate medium containing X-Gal, activity was easily detectable from *C. albicans* strains expressing *MAL2-lacZ* (CAU221), *HWP1-lacZ* (CAU951) and *ACT1-lacZ* (CAU361) (Fig. 2). After 2 d, colonies expressing the *HWP1-lacZ* fusions turned blue on X-Gal + 10% fetal calf serum, but remained white on X-Gal + glucose or X-Gal + maltose (not shown). However, after 5 d, strains with the *HWP1-lacZ* fusion began to turn blue on all media (Fig. 2). This result was expected since *HWP1* expression is highly induced under a variety of conditions that promote filamentous growth, including starvation, a condition that arises in colonies upon prolonged growth (see below). Colonies of the *ACT1-lacZ* fusion strain turned visibly blue on all media as soon as 2 d, and developed further after 5 d (Fig. 3). Colonies of the *MAL2-lacZ* fusion strain turned blue on X-Gal + maltose medium in 4–5 d, but

	Budding yeast	Hypthal/pseudohyphal
Edge of colony	97 %	3 %
Centre, wrinkled part of colony	53 %	47 %

Fig. 3. Expression of the *HWP1-lacZ* construct in particular regions of a streak of *C. albicans*. A *C. albicans* strain with the integrated *HWP1-lacZ* construct was grown on X-Gal + maltose at 30 °C for 3 d. β -Galactosidase activity is noticeable in regions of the streak that appear wrinkled and in the centre of the streak; these regions contain a higher percentage of filamentous cells than the edge of the streak, which contains *C. albicans* primarily in the budding yeast form. Cell morphology was quantified by resuspending sections of colonies in liquid culture and scoring 300 cells for morphology.

remained white on X-Gal + glucose medium (Fig. 2). The longer developing time of the *MAL2-lacZ* fusion strains compared with the other strains is consistent with the lower level of expression as detected by the liquid assays (Table 1).

In addition to its usefulness in distinguishing expressing from non-expressing colonies, growth on X-Gal medium can also be used to discern changes in gene expression that occur within a colony. Fig. 3 depicts a streak of a *C. albicans* strain containing the *HWP1-lacZ* fusion that had been grown on X-Gal + maltose for 3 d. Most of the streak is white, consistent with the lack of expression of *HWP1* by budding cells. However, in the centre of the streak, particularly in areas of wrinkled appearance, groups of cells have begun to express *lacZ*. Microscopic analysis revealed that many of the cells taken from the centre of the streak were filamentous while almost all those taken from around the edge were in the budding form. It seems likely that conditions in the centre of the streak, perhaps depletion of nutrients, had induced filamentous growth, which is easily monitored by expression of the *HWP1-lacZ* fusion. No such patterns of expression were seen in colonies of *C. albicans* expressing the *ACT1-lacZ* fusion.

Although growth on X-Gal medium is a useful monitor of *lacZ* expression, it can require several days for the colour to develop. For more rapid detection of β -galactosidase activity, colonies grown on solid medium can be transferred to paper filters, permeabilized by freezing in liquid nitrogen and incubated with X-Gal (Ausubel *et al.*, 1992). Results with filter assays matched results of the liquid β -galactosidase assays and plate assays (not shown). Positive reactions were obtained after only a few hours' incubation at 37 °C for *C.*

albicans strains with integrated MAL2-*lacZ*, HWP1-*lacZ* or ACT1-*lacZ* fusions.

Strep. thermophilus lacZ* in *Sacch. cerevisiae

Since it is often useful to compare regulatory pathways in *C. albicans* and *Sacch. cerevisiae* (Gimeno *et al.*, 1992), we tested whether *Strep. thermophilus lacZ* also functioned in *Sacch. cerevisiae*. *Sacch. cerevisiae* strains that contain the *Strep. thermophilus lacZ* fused to the GAL1,10 promoter and integrated in the genome readily exhibit regulated β -galactosidase activity as determined by filter assays (Guthrie & Fink, 1991). When grown on YEP-galactose, 88% of the transformants produced β -galactosidase activity within 1 h, while no activity was observed when transformants were grown on YEP-glucose. No activity was detected from *Sacch. cerevisiae* strains containing the GAL1,10 vector alone. Thus, *Strep. thermophilus lacZ* can be shuttled between *C. albicans* and *Sacch. cerevisiae* and used as a reporter gene in both yeasts.

The results presented in this paper show that *lacZ* from *Strep. thermophilus* is a useful reporter gene for the human pathogen *C. albicans*. Other reporter genes have been developed for *C. albicans*, including the sea pansy luciferase (Srikantha *et al.*, 1996), the *K. lactis* β -galactosidase LAC4 (Leuker *et al.*, 1992), the URA3 gene from *C. albicans* (Myers *et al.*, 1995) and the yeast-enhanced green fluorescent protein (yEGFP; Cormack *et al.*, 1997). These reporter genes have been used effectively for studies of transcriptional regulation in *C. albicans* (Wirsching *et al.*, 2000; Srikantha *et al.*, 1996, 1997; Stoldt *et al.*, 1997; Leuker *et al.*, 1997). *Strep. thermophilus lacZ* combines the advantages of sensitivity, simplicity of qualitative assays, and ease of colorimetric visual screens in growing colonies of *C. albicans*.

E. coli lacZ has proved to be a highly versatile reporter gene in *Sacch. cerevisiae* and has been used to study many aspects of signal-transduction pathways, gene regulation and other cellular processes (Guarente & Ptashne, 1981; Rose *et al.*, 1981; Guarente, 1983; Burns *et al.*, 1994). We believe that the *Strep. thermophilus lacZ* reporter gene can be used for many of these same purposes in *C. albicans*.

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