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Authors: Luraschi A, Cissé OH, Pagni M, Hauser PM

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Identification and Functional Ascertainment of the *Pneumocystis jirovecii* Potential Drug Targets Gsc1 and Kre6 Involved in Glucan Synthesis

Amanda Luraschi [a], Ousmane H. Cissé [a,b,1], Marco Pagni [b] and Philippe M. Hauser [a]

a Institute of Microbiology, Lausanne University Hospital and University of Lausanne, 1011 Lausanne, Switzerland

b Vital-IT Group, SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland

1 Present address: O. H. Cissé, Critical Care Medicine Department, NIH Clinical Center, National Institutes of Health, Bethesda, Maryland 20892, USA

Correspondance

Philippe M. Hauser, Institute of Microbiology, Lausanne University Hospital and University of Lausanne, 1011 Lausanne, Switzerland.

Tel: +41213144084; Fax: +41213144060; e-mail: Philippe.Hauser@chuv.ch

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ABSTRACT

The most efficient drug against the human pathogenic fungus *Pneumocystis jirovecii* is cotrimoxazole targeting the folate biosynthesis. However, resistance towards it is emerging and adverse effects occur in some patients. Studies in rodent models suggested that echinocandins could be useful to treat *Pneumocystis* pneumonia. Echinocandins inhibit the catalytic subunit Gsc1 of the enzymatic complex ensuring the synthesis of 1,3-β glucan, an essential constituent of cell walls of most fungi. Besides, inhibitors of the enzyme Kre6 involved in the synthesis of 1,6-β glucan, another essential component of fungal walls, were recently described. We identified and functionally characterized these two potential drug targets in the human pathogen *P. jirovecii* by rescue of the null allele of the orthologous gene in *Saccharomyces cerevisiae.* The *P. jirovecii* proteins Gsc1 and Kre6 identified using those of the relative *Pneumocystis carinii* as the query sequence showed high sequence identity to the putative fungal orthologs (53 to 97% in conserved functional domains). The expression of their encoding genes on plasmid rescued the increased sensitivity to respectively caspofungin or calcofluor white of the corresponding *S. cerevisiae* null allele. The uniqueness and likely essentiality of these proteins suggest that they are potential good drug targets.

Keywords

Echinocandins; human pathogen; pathogenic fungus; *Saccharomyces cerevisiae* heterologous complementation; cell wall.

INTRODUCTION

Pneumocystis organisms are fungal parasites that colonize the lungs of mammals (Skalski et al. 2015; Thomas and Limper 2004). Each *Pneumocystis* species displays strict host specificity for a single mammalian species. The species infecting humans is *Pneumocystis jirovecii*. In immuno-compromised patients, *P. jirovecii* can turn into an opportunistic pathogen causing *Pneumocystis jirovecii* pneumonia (PCP), a severe disease which can be fatal. Standard antifungals targeting the fungal cell membrane integrity or synthesis are not effective against *P. jirovecii*, possibly because of the presence of cholesterol instead of ergosterol in its membrane (Kaneshiro et al. 1994; Russian and Kovacs 1998). The most efficient drug currently used is cotrimoxazole, a combination of trimethoprim and sulfamethoxazole. These two molecules are inhibitors of enzymes involved in the biosynthesis of folic acid, a metabolite required for the biosynthesis of essential cellular compounds. Cotrimoxazole is active against both cellular forms present during *Pneumocystis* infection: the trophic form which is the predominant one and apparently devoid of wall, and the ascus form which would be issued from a sexual cycle and has a thick wall (Aliouat et al. 2009; Itatani and Marshall 1988; Thomas and Limper 2007). The use of cotrimoxazole for prophylaxis and treatment of PCP since the late 1980s (Kovacs et al. 2001) correlated with the detection of specific mutations within the active site of the dihydropteroate synthase, the target of sulfamethoxazole (Lane et al. 1997; Ma et al. 1999; Nahimana et al. 2003). Because of the emerging resistance to cotrimoxazole and because this drug can cause side effects such as intolerance and toxicity, it is of the utmost importance to find new treatments and to identify new drug targets to fight *P. jirovecii*.

One promising class of antifungals is echinocandins. Caspofungin (CAS), anidulafungin, and micafungin are cyclic hexapeptides with fatty acyl side chains which are non-competitive inhibitors of the catalytic subunit of the 1,3-β glucan synthase enzymatic complex (Schmatz et al. 1990). β-glucan molecules are homopolymers of β-1,3 linked D-glucose with β-1,6 linked D-glucose side chains in minority. β-glucans are important components of the fungal cell wall, together with mannoproteins and chitins (Bowman and Free 2006). All these components are usually involved in the host immune recognition in typical fungal pathogens (Amarsaikhan and Templeton 2015; Cambi et al. 2008). In contrast, *Pneumocystis* organisms would be unable to synthesize chitin, and harbour only β-glucans and mannoproteins, which, however, lack hyper-mannose glycosylation (Ma et al. 2016). The 1,3-β glucan synthase enzymatic complex is an ideal drug target to fight pathogenic fungi because it (i) is essential for these microorganisms, (ii) has no human counterparts, and (iii) is easily reachable by drugs because of their localization at the fungal surface, anchored in the cellular membrane. Four studies reported initially the efficacy of echinocandins and related compounds against *Pneumocystis carinii,* the species infecting rats (Powles et al. 1998; Schmatz et al. 1990; Schmatz et al. 1995; Sun and Zhaohui 2014). Consistent with their lack of or poor glucan content, trophic forms appeared to be much less sensitive to CAS than asci (Cushion et al. 2010). Combining caspofungin with cotrimoxazole provided an addictive effect, improving the clearance of infection by *Pneumocystis murina*, the species infecting mice (Lobo et al. 2013). However, the response to echinocandins of the human pathogen *P. jirovecii* could be different than those of the models *P. carinii* and *P. murina*. Indeed, the genomic coding sequences of the two latter species present a mean divergence of ca. 20% at nucleotide level from those of *P. jirovecii* (Stringer 1996). The available clinical reports concerning the issue are contradictory so that the efficacy of caspofungin against *P. jirovecii* remains unclear. Indeed, the combination of caspofungin with cotrimoxazole was reported to have cleared several PCP episodes, two in HIV-positive patients (Ceballos et al. 2011; Lee et al. 2016), seven in transplant recipients (Utili et al. 2007; Tu et al. 2013), and one in a pediatric case (Beltz et al. 2006). Caspofungin alone was reported to have been successful in a patient undergoing bone marrow transplantation (Annaloro et al. 2006), in a Wegener's granulomatosis patient (Hof and Schnülle 2008), as well as in eight out of ten HIV-infected patients (Armstrong-James et al. 2011). However, failure of treatment using caspofungin alone was described in four HIV-negative (Kim et al. 2013) and two cancer patients (Kamboj et al. 2006).

In *S. cerevisiae,* the *GSC1*, *GSC2*, and *GSC3* genes (also called *FKS1* to 3) encode different catalytic subunits of the 1,3-β glucan synthase enzymatic complex. The *GSC1* gene is active during the vegetative growth whereas *GSC2* and *GSC3* are expressed during sporulation (Bowman and Free 2006; Mazur et al. 1995). The presence of a unique *gsc1* gene encoding the 1,3-β glucan synthase catalytic subunit in the genome of *P. carinii* and the inhibition of the Gsc1 protein by the pneumocandin L-733,560, a compound structurally very close to echinocandins, were demonstrated (Kottom and Limper, 2000). These observations also suggest that echinocandins might become useful for prevention and treatment of PCP in the future.

1,6-β glucans are less abundant than 1,3-β glucans in fungal cell walls, and act as a flexible glue between the other components of the wall (Kollár et al. 1997). At least ten genes are involved in the biosynthesis of 1,6-glucan in *S. cerevisiae* (Shahinian and Bussey, 2000). These genes have also no homologs in human genome. Inhibitors of the 1,6-β glucan synthesis were recently discovered (Kitamura et al. 2009; Kitamura 2010). These inhibitors target the membrane protein *kre6* and show an important *in vitro* activity against *Candida* species and *S. cerevisiae* (Kitamura 2010). *S. cerevisiae* harbours the Kre6 enzyme as well as a homolog called SknI sharing 66% sequence identity with Kre6. Unlike the *KRE6* gene, the deletion of the *SKN1* gene does not show any effect on growth or 1,6-β glucan levels within the wall, suggesting that the Skn1 enzyme is less important than the Kre6 enzyme, at least in the laboratory conditions (Roemer et al. 1993). The presence of a unique *kre6* gene and the sensitivity of the Kre6 enzyme to the 1,6-β glucan inhibitors were recently demonstrated in *P. carinii* (Kottom et al. 2015). These observations suggest that 1,6-β glucan inhibitors might become useful new drugs against *P. jirovecii.*

The identification and characterization of the *P. jirovecii gsc1* and *kre6* genes has not been carried out yet. Despite that the *in vitro* culture method for this pathogen published by Schlidgen *et al.* (2014) is not widely established yet, this task is now possible because of the recent release of the genome sequence of *P. jirovecii* by two groups (Cissé et al. 2012; Ma et al. 2016). In the present study, we report the identification of these two genes within the *P. jirovecii* genome sequence, as well as the assessment of their function by the complementation of the respective deleted orthologous gene of *S. cerevisiae.*

MATERIALS AND METHODS

Strains and growth conditions

Y05251 is an *S. cerevisiae* haploid strain with a deletion of the 1,3-β glucan synthase catalytic subunit gene (*GSC1*, also called *FKS1)* that encodes the Gsc1 protein (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YLR342w::kanMX4*). This strain is herein named the *gsc1* deletant. It was obtained from Euroscarf (European *Saccharomyces cerevisiae* Archive for Functional Analysis [http://web.uni-frankfurt.de/fb15/mikro/euroscarf]). Strains of *S. cerevisiae* with deletion of *GSC1* exhibit a slow growth rate and impaired growth in the presence of low doses of caspofungin (CAS) (Markovich et al. 2004). The parental strain of the *gsc1* deletant, strain BY4741 from Euroscarf (*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), was used as the control in the complementation tests. This strain is herein named the wild type strain.

Y05574 is an *S. cerevisiae* haploid strain with a deletion of the *KRE6* gene that encodes the Kre6 protein which is one of the genes involved in the 1,6-β glucan biosynthesis (*Mat***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 YPR159w::kanMX4*). This strain is herein named the *kre6* deletant. It was also obtained from Euroscarf. Strains of *S. cerevisiae* with deletion of *KRE6* exhibit defects in cell wall integrity which induces an impaired growth in the presence of Calcofluor White M2R (CFW) (Roemer and Bussey 1991). Strain BY4741 is also the parental strain of the *kre6* deletant and was used as the control in the complementation tests.

Strains were grown on complete yeast extract-peptone-dextrose (YEPD) medium (1% [wt/vol] Difco yeast extract, 2% Difco peptone, 2% glucose).

Source of *P. jirovecii* gene sequence

The *P. carinii* Gsc1 protein (primary accession number UniProt ID Q9HEZ4) was used as the query sequence in BLASTp searches against the two available *P. jirovecii* proteomes at http://blast.ncbi.nlm.nih.gov/Blast.cgi. A single putative ortholog was detected in each proteome, i.e., no homolog with a lower identity was present. The two *P. jirovecii* gene sequences encoding the Gsc1 protein identified were then retrieved from the European Nucleotide Archive (http://www.ebi.ac.uk/ena) (Brooksbank et al. 2014). The *gsc1* gene corresponds to the PNEJI1_001061 locus in the *P. jirovecii* genome assembly version ASM33397v2 (Cissé et al. 2012), and to the T551_02309 locus in the *P. jirovecii* genome assembly version Pneu_jiro_RU7_V2 (Ma et al. 2016).

The *P. jirovecii* gene sequences encoding the Kre6 protein were similarly retrieved using the *P. carinii* Kre6 protein (UniProt ID Q6UEI2) as the query sequence. A single putative ortholog was detected in each proteome. The *kre6* gene corresponds to the PNEJI1_003487 locus in the ASM33397v2 assembly, and to the T551_02808 locus in Pneu_jiro_RU7_V2 assembly.

Multiple sequence alignments of *P. jirovecii*, *P. carinii*, *S. cerevisiae*, and *S. pombe* proteins were generated using T-Coffee (Notredame et al. 2000). Conserved domains were found using the NCBI's search tool (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Sequences identity % of whole proteins and conserved domains has been calculated with Align Sequence Protein BLAST tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Cloning of the *P. jirovecii gsc1* and *kre6* genes

Since the *P. jirovecii gsc1* and *kre6* genes include several introns (three and seven, respectively), the corresponding cDNAs were synthesized by GeneCust Europe (Ellange, Luxembourg). The sequences of the alleles present in the ASM33397v2 assembly were used (primary accession number UniProt ID L0PD34 and L0P8X6, respectively). Their sizes without introns are respectively 5,760 and 2,040 bps.Both cDNAs were synthesized with flanking XbaI and SalI restriction sites for subsequent cloning . Both genes were digested with XbaI and SalI restriction enzymes and cloned using T4 ligase (New England Biolabs, Basel, Switzerland) into the p416GPD yeast expression vector (Mumberg et al. 1995) previously digested with the same restriction enzymes. The recombinant plasmids were introduced into *E. coli* DH5α-competent cells and minipreparations of plasmid DNA was performed according to Birnboim and Doly (Birnboim and Doly 1979).

Cloning of the *S. cerevisiae GSC1* and *KRE6* genes

In order to perform complementation controls, the *S. cerevisiae GSC1* and *KRE6* genes were amplified by PCR from DNA from the wild type strain BY4741 extracted as described previously (Lo Presti et al. 2007). The genes are respectively 5,631 and 2,163 pbs. PCR primers and conditions are given in Tables S1 and S2, respectively. Primers were synthesized by Microsynth (Balgach, Switzerland). For oriented cloning, the primerswere designed to create unique restriction sites at ends of the PCR products. The latter were extracted using the QIAquick gel extraction kit (Qiagen, Basel, Switzerland), digested by BamHI and PstI restriction enzymes and cloned into the p415GPD expression vector (*GSC1*), or digested by BamHI and SalI and cloned into p416GPD (*KRE6*).

Transformation of *S. cerevisiae* deletants

The recombinant plasmids p416GPD.*Pjgsc1*, p416GPD.*Pjkre6*, p415GPD.*ScGSC1*, and p416GPD.*ScKRE6* were introduced into their corresponding *S. cerevisiae* deletant by transformation for uracil (p416GPD) or leucine (p415GPD) prototrophy using the one-step method (Chen et al. 1992). In order to be used as controls in the complementation tests involving selection of plasmids, the deletants and the wild type strain BY4741 were transformed with the empty p416GPD or p415GPD. Transformants were selected on solid yeast nitrogen base (YNB) medium (0.67% [wt/vol] yeast nitrogen base, 2% glucose, 2% Gibco agar) supplemented with complete supplement mixture (CSM) lacking uracil or leucine

(MP Biomedicals Luzern, Switzerland). Transformants clones were randomly chosen and purified by growth on the same selective medium.

Complementation tests

Functional complementation of the *S. cerevisiae gsc1* deletant was assessed by growth on YNB selective medium lacking uracil or leucine and supplemented with or without 75 ng/ml CAS (Fluka, Chemie AG, Buchs Switzerland). Transformant clones were grown overnight in the selective medium in order to avoid the loss of plasmid. Cells were then diluted at an absorbance at 540 nm of 0.5 in NaCl 0.9% (wt/vol)(ca. 3.5 x 10^6 cells), four serial 10-fold dilutions in NaCl 0.9% were prepared, and three µl of each dilution were spotted onto the medium. Spots were observed and photographed after 5 days at 30 °C.

Functional complementation of the *S. cerevisiae kre6* deletant on plasmid was assessed using exactly the same procedure as for *gsc1*, except that the selective medium was supplemented with 35 µg/ml CFW (Sigma Life Science, Buchs, Switzerland). CFW inhibits the growth of the deletant by binding to the residues of chitin remaining exposed due to the improper 1,6-β glucan cell wall deposition (Roemer and Bussey 1991). Since CFW is photosensitive, Petri dishes were wrapped in aluminium paper just after their preparation and kept away from light as much as possible.

PCR primers and conditions used to assess the presence or absence of complementing genes are given in Tables S1 and S2, respectively. Positive controls consisted in the amplification of the genomic genes from DNA extracted using QIAamp DNA blood kit (Qiagen, Basel, Switzerland) from a BAL fluid of a patient with PCP.

RESULTS

Identification and cloning of the *P. jirovecii gsc1* and *kre6* genes

A single Gsc1 protein was identified within each of the two *P. jirovecii* proteomes available by a homology search using the Gsc1 protein of *P. carinii* as the query sequence. The two *gsc1* gene sequences were identical, except (i) three synonymous single-nucleotide polymorphisms (SNP), and (ii) a second exon longer in the Ma *et al.* assembly than in the Cissé *et al.* assembly (Fig. S1A). The supplementary sequence of the exon presented five bp deleted relatively to Cissé *et al.* which eliminate a STOP codon in the frame. This resulted in a segment of 27 amino acids which is absent in the *P. jirovecii* Gsc1 protein predicted by Cissé *et al*., but present and exactly identical in that of *P. carinii* (Fig. S2A). This segment is not part of the conserved glucan synthase domains. The allele of the Cissé *et al.* assembly was investigated in the present study (only this one was described at the beginning of the present study). The translation product of the ORF bears the highest degree of identity with the Gsc1 protein of *P. carinii* (90%), and a weaker degree of identity with that of *S. cerevisiae* (61%) and with the *S. pombe* Bgs4 protein which is orthologous to Gsc1 (60%). The identity was higher in the two conserved functional domains, supporting that the four proteins were orthologs (Table 1). Since *S. cerevisiae* does not process *Pneumocystis* introns, a synthetic *P. jirovecii gsc1* gene without introns was cloned into the expression vector p416GPD.

A single *P. jirovecii* Kre6 protein was identified in each proteome using the Kre6 protein of *P. carinii* as the query sequence. The two *kre6* gene sequences were identical, except (i) four synonymous SNPs, and (ii) an ORF shorter in Ma *et al.* than in Cissé *et al.* which ended within the last intron predicted in the latter assembly (Fig. S1B). This resulted in a predicted protein lacking the last segment of 23 amino acids present in that of Cissé *et al.* (Fig. S2B).”The allele of the Cissé *et al.* assembly was investigated in the present study. The gene encompasses seven introns. The translation product of the ORF bears the highest degree of identity with the Kre6 protein of *P. carinii* (70%), while it shares a weaker degree of identity with those of *S. cerevisiae* (48%) and with the *S. pombe* Yepb protein which is orthologous to Kre6 (51%) (a second homolog Yeob presenting a high identity with *S. cerevisiae* Skn1 was also present in *S. pombe*). The identity was higher in the functional domain (Table 1; Fig. S2B), supporting that the four proteins were orthologs. A synthetic *P. jirovecii kre6* gene without introns was cloned into p416GPD.

Functional complementation of the *S. cerevisiae gsc1* deletant with the *P. jirovecii gsc1* gene

The recombinant plasmids p416GPD.*Pjgsc1* and p415GPD.*ScGSC1* were introduced into the *S. cerevisiae gsc1* deletant, whereas the empty p416GPD and p415GPD vectors were introduced into both the deletant and the wild type strain (*S. cerevisiae GSC1* could not be cloned into p416GPD because of restriction sites issues). The deletant shows a paradoxical phenotype: an increased susceptibility to CAS in absence of the target of this antifungal. This results from the involvement of this gene in the cell wall integrity pathway (Markovich et al.

2004; Reinoso-Martín et al. 2003). On medium containing CAS, a complete restoration occurred in the presence of p415GPD.*ScGSC1*, but not in the presence of the empty vector (Fig. 1A). On the other hand, a partial restoration of the wild type growth was observed with p416GPD.*Pjgsc1, i.e.,* the number of colonies growing at each concentration was reduced. Because of the instability of the plasmids requiring constant selection of their auxotrophy marker as well as of the partial complementation obtained upon expression of the *P. jirovecii gsc1* gene, it was crucial to check by PCR the presence or absence of the complementing gene in the different strains (Fig. 2A). The *P. jirovecii gsc1* gene or the *S. cerevisiae GSC1* gene was present in the functionally complemented strains but not in the deletant harbouring the empty vector. Amplifying the polylinker without insert further identified the empty vector. As a positive control, *P. jirovecii gsc1* gene with introns was amplified from a BAL fluid of a patient with PCP. These observations demonstrated that expression of the *P. jirovecii* gene rescued the function of the deleted *S. cerevisiae GSC1* gene encoding the Gsc1 protein.

Functional complementation of the *S. cerevisiae kre6* deletant with the *P. jirovecii kre6* gene

The assay and PCR controls for complementation of the *kre6* deletant were performed exactly as described above for the *gsc1* deletant, except that the recombinant plasmids p416GPD.*Pjkre6* and p416GPD.*ScGSC1* and CFW in the medium were used. A partial and a complete restoration of the wild type growth occurred in the presence of respectively p416GPD.*Pjkre6* and p416GPD.*ScKRE6*, but not in the presence of the empty vector (Fig. 1B). The reduced size of the colonies revealed that the growth rate of the deletant carrying the recombinant plasmid p416GPD.*Pjkre6* was lower than that of the wild type strain, in presence or not of CFW. PCRs showed that the *P. jirovecii kre6* gene or the *S. cerevisiae KRE6* gene was present in the functionally complemented strains but not in the deletant harbouring the empty vector (Fig. 2B). These observations demonstrated that expression of the *P. jirovecii* gene rescued the function of the deleted *KRE6* gene encoding the Kre6 protein.

DISCUSSION

In the present study, we identified the *P. jirovecii gsc1* gene and proved its function by rescue of the null allele of the orthologous gene in *S. cerevisiae*. We also identified another potential drug target among the enzymes involved in cell wall biosynthesis, the Kre6 protein implicated in the 1,6-β glucan biosynthesis, and also characterized its function by complementation. Both these heterologous complementations were partial, whereas those with the homologous *S. cerevisiae* gene were complete. This could be explained by the fact that we used a vector with a strong promoter for the complementation, and that the high expression of the gene was toxic (Romanos et al. 1992). However, we repeated the experiments using a weaker promoter and the results were similar (data not shown). Such partial complementation is not surprising since it often occurred in our previous complementation studies. Indeed, the *S. cerevisiae dhfs* and *brl1* deletants complemented with the *P. jirovecii* and *P. carinii* genes presented a reduced growth rate (Lo Presti et al. 2007; Luraschi et al. 2015). Partial complementation may result from a poor fitness of the enzyme in the heterologous cellular environment. For example, the reduced growth rate of the complemented *kre6* deletant could be due to a persistent stress in the cell wall, leading to a perturbation of the cell wall integrity. Impaired synthesis due to a different codon usage could also play a role. We reported partial complementation also in homologous complementation of *brl1* (Lo Presti et al. 2009). This suggests the possible importance of a different gene dosage due to the plasmidic rather than chromosomic location, or of the use of a non natural promoter which provides a constitutive rather than temporally regulated expression, and/or an inadequate level of expression.

The Gsc1 subunit catalyzes the polymerization of uridine diphosphate-glucose into the 1,3-β glucan core, an insoluble molecule required for the cell wall assembly (Shematek et al 1980). The *GSC1* and *GSC2* genes are a redundant essential pair, i.e., the double deletion mutant is not viable, while the single mutants is viable (Inoue et al. 1995; Mazur et al. 1995). Thus, the growth of the *gsc1* deletant in absence of CAS in our experiments was probably supported by the expression of *GSC2*. In *P. carinii,* only one *gsc1* gene was found using Southern blot analyses (Kottom and Limper 2000). The *gsc1* gene is also unique in *P. jirovecii* according to our BLAST searches in the two proteomes presently available. Consequently, this gene is likely to be essential in *P. jirovecii* and the protein it encodes a good potential drug target. This is true even if this gene is active mostly in the asci because it is thought that production of asci constitutes an obligate stage of the life cycle (Cushion and Stringer 2010; Hauser 2014), and that these forms might be the particles responsible for the transmission of the fungus between hosts via the airborne route (Cushion et al. 2010; Martinez et al. 2013).

The Kre6 enzyme is anchored to plasma, Golgi, and reticulum endoplasmic membranes. It is involved in the synthesis of 1,6-β glucan which is also an essential component of the fungal cell wall. The function of this enzyme remains unclear. It is thought to be a transglucosidase contributing to the construction of a protein-bound glucan-structure which acts as an acceptor site for the 1,6- β- glucan addition at cell surface (Montijn et al. 1999). In *S. cerevisiae,* this Golgi membrane glycoprotein has a homolog, Skn1, which is structurally related and has a similar function but which plays a less important role. The double deletant of *KRE6* and *SKN1* genes shows a noticeable alteration of the cell wall structure not observed in the single mutants, suggesting the essentiality of the function (Roemer et al 1994). Only one *kre6* gene was found in *P. carinii* using Southern blot analyses (Kottom et al. 2015) and our BLAST searches suggest that this gene is also unique in *P. jirovecii*. Thus, this gene is possibly essential and the protein it encodes a good candidate drug target against *P. jirovecii*, even if it is expressed mostly or exclusively in asci. The pyridobenzimidazole derivatives inhibiting Kre6 enzymes recently described (Kitamura et al. 2009), may become a useful new treatment against *P. jirovecii* as well as against other pathogenic fungi, possibly in combination with cotrimoxazole.

The *gsc1* and *kre6* genes and their encoded proteins predicted in the two available *P. jirovecii* genomes were not identical. In both cases, the few synonymous SNPs observed can be attributed to natural polymorphisms between these isolates originating from Switzerland and USA. As far as the Gsc1 proteins are concerned, the 27 amino acids segment missing within one of them may also be a natural polymorphism because (i) the shortened enzyme is functional as demonstrated in the present work, (ii) the segment is not part of the conserved glucan synthase domains, and (iii) the absence is associated to five bp inserted in a region spanning only 24 bp, a configuration which is unlikely for sequencing errors. As far as the Kre6 proteins are concerned, the 23 amino acids shorter end of one of them may result from a prediction error because (i) the sequence of the gene is identical in both assemblies, and (ii) the missing segment is almost fully conserved in the *P. carinii* enzyme. These observations suggest that further assessment of the two available *P. jirovecii* genomes and proteomes is warranted.

The sensitivities of Gsc1 and Kre6 enzymes toward respectively echinocandins and the specific inhibitors of Kre6 were demonstrated in *P. carinii* (Kottom et al. 2015; Schmatz et al. 1990). Although active sites of enzymes are generally conserved, one cannot exclude that these sensitivities vary between *P. carinii* and *P. jirovecii* because these species are relatively distant. Clinical reports are contradictory but insights can be obtained by studying the *P. jirovecii* enzymes. We plan to use the complemented strains constructed here in order to determine these sensitivities.

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TRANSPARENCY DECLARATION

None to declare.

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FIGURE LEGENDS

Fig. 1. Complementation of the *S. cerevisiae gsc1* (**A**) and *kre6* (**B**) deletants by expression of the *P. jirovecii gsc1* or *kre6* gene on plasmid. Control complementation by expression of the *S. cerevisiae GSC1* or *KRE6* gene is also shown. Log dilutions of a suspension of cells at 3.5×10^6/ml were spotted on minimal selective medium without (left) or with (right) CAS or CFW, and incubated for 5 days at 30 °C. The most concentrated suspension is on the left. YNB + CSM – ura was used for p416GPD selection, YNB + CSM – leu for p415GPD selection.

Fig. 2. PCR assessment of the presence or absence of the *P. jirovecii gsc1* and *kre6* and *S. cerevisiae GSC1* and *KRE6* genes in the constructed strains. **A.** The presence of the *P. jirovecii gsc1* gene (PCR product with introns of 6,017 bp) was confirmed in the DNA from the BAL fluid of a patient with PCP and in the three transformant clones of *S. cerevisiae gsc1* deletant bearing p416GPD.*Pjgsc1* (PCR product without introns of 5,782 pb), whereas the gene was absent in the *gsc1* deletant bearing the empty vector. The presence of the *S. cerevisiae GSC1* gene was confirmed in the DNA of the clone bearing p415GPD.*ScGSC1*, whereas the gene was absent in the clonebearing the empty vector. To confirm the presence of the empty vector in the clones bearing p416GPD or p415GPD, the polylinker without insert (1,106 pb) was amplified. **B.** The presence of the *P. jirovecii kre6* gene (PCR product with introns of 2,341 bp) was confirmed in the DNA from the BAL fluid of a patient with PCP and in the three transformant clones of *S. cerevisiae kre6* deletant bearing p416GPD.*Pjkre6* (PCR product without introns of 2,040 pb), whereas the gene was absent in the kre6 deletant bearing the empty vector. The presence of the *S. cerevisiae KRE6* gene was confirmed in the DNA of the clone bearing p416GPD.*ScKRE6*. The presence of the empty vector was confirmed by amplification of the polylinker without insert. All negative controls did not generate products.

SUPPORTING INFORMATION

Table S1. Oligonucleotide primers used for PCR amplification

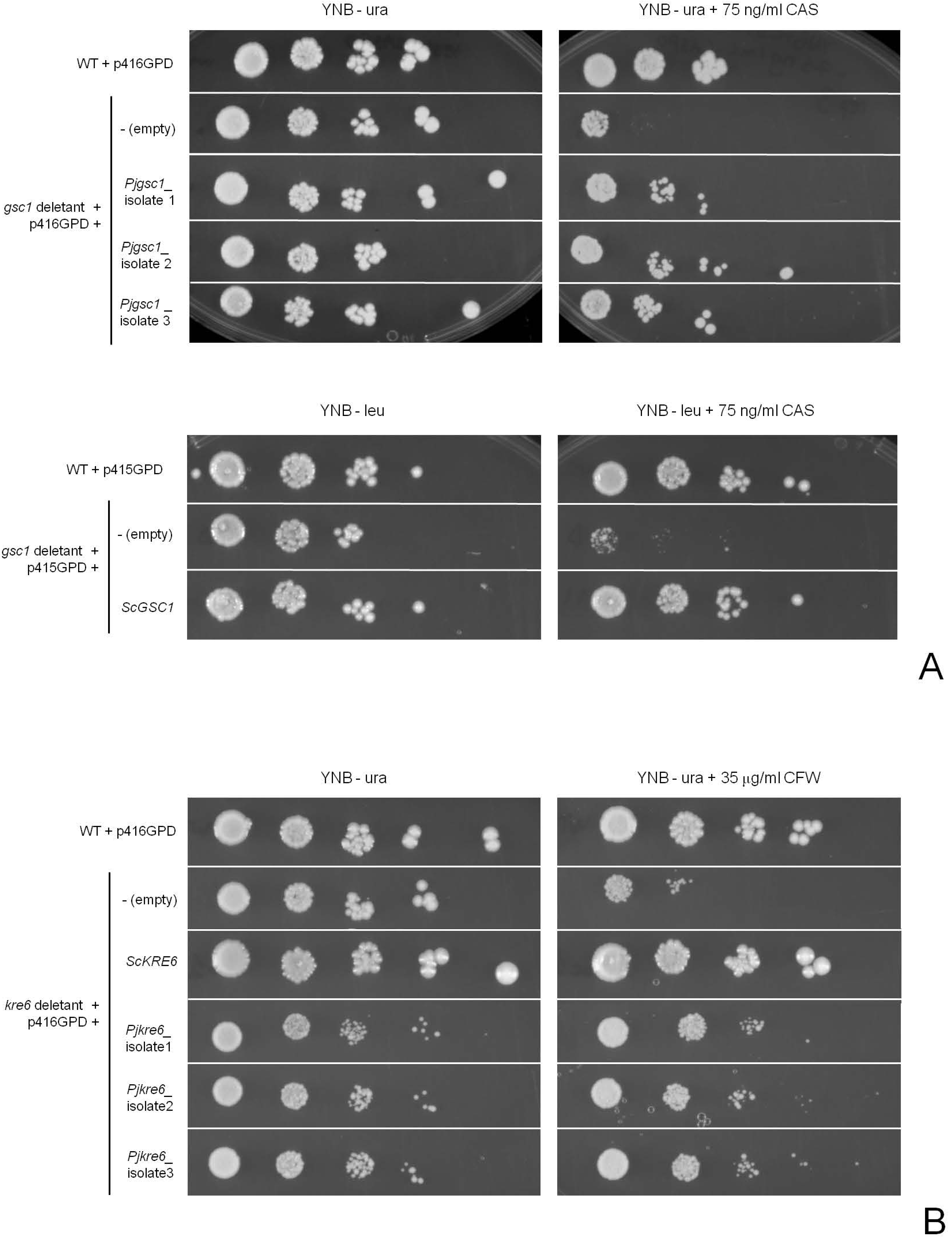
Table S2. PCR conditions

Fig. S1. Multiple sequence alignment of *gsc1* (A) and *kre6* (B) gene sequences of the two *P. jirovecii* genome assemblies (Cissé et al. 2012, indicated as Cisse; Ma et al. 2016, indicated as Ma). T-Coffee was used (Notredame et al. 2000). Residues shown in bold are synonymous SNPs. Dashes indicate gaps. **A.** Alignment of *gsc1* ORF and genomic sequences. The second exon of the allele of Ma *et al*. is 81 bp longer than that of Cissé *et al*., and presents five bp deleted relatively to the genomic sequence of Cissé *et al.* in the region from position 2581 to 2604. **B.** Alignment of *kre6* ORF and genomic sequences. The last exon of the allele of Ma *et al.* ends within the last intron of the allele of Cissé et al., resulting in a ORF 69 bp shorter.

Fig. S2. Multiple-sequence alignment of Gsc1 (A) and Kre6 (B) proteins. T-Coffee was used (Notredame et al. 2000). Identical, strongly and weakly conserved residues are indicated by asterisks, double points, and single points, respectively. Dashes indicate gaps. **A.** Alignment of the Gsc1 proteins of *P. jirovecii* of Cissé *et al.* (primary accession number UniProt ID L0PD34), *P. carinii* (UniProt ID Q9HEZ4), *S. cerevisiae* (UniProt ID P38631), and the *S. pombe* (Bgs4; UniProt ID O74475). The 1,3-β glucan synthase domains 1 and 2 as well as transmembrane helix domains 1 to 16 are shown respectively above and under the alignment. **B.** Alignment of the Kre6 proteins of *P. jirovecii* (UniProt ID L0P8X6), *P. carinii* (UniProt ID Q6UEI2), *S. cerevisiae* (UniProt ID P32486), and *S. pombe* (UniProt ID O13941). The transmembrane signal-anchor for type II membrane protein domain is shown. The two basic residues shown in bold are implicated in the cytoplasmic localization (Roemer and Bussey 1991). The Kre6 glucanase domain related to the glycoside hydrolase family 16 (GH16) and a coiled coil are also shown.

Table1. Sequence identity (%) of the *P. jirovecii* Gsc1 and Kre6 proteins to the putative orthologs of *P. carinii, S. cerevisiae*, and *S. pombe*

	Gsc1			Kre6	
	Whole protein	1,3-β glucan synthase domain 1	1,3-β glucan synthase domain 2	Whole protein	GH16 fungal kre6 glucanase domain
P. carinii	90	94	97	70	83
S. cerevisiae	61	70	71	48	53
S. pombe	60	65	73	51	59


YNB - ura
YNB - ura + 75 ng/ml CAS
WT + p416GPD
gsc1 deletant + p416GPD +
- (empty)
Pjgsc1_ isolate 1
Pjgsc1_ isolate 2
Pjgsc1 _ isolate 3
YNB - leu
YNB - leu + 75 ng/ml CAS
WT + p415GPD
gsc1 deletant + p415GPD +
- (empty)
ScGSC1
A
YNB - ura
YNB - ura + 35 μg/ml CFW
WT + p416GPD
kre6 deletant + p416GPD +
- (empty)
ScKRE6
Pjkre6_ isolate1
Pjkre6_ isolate2
Pjkre6_ isolate3
B

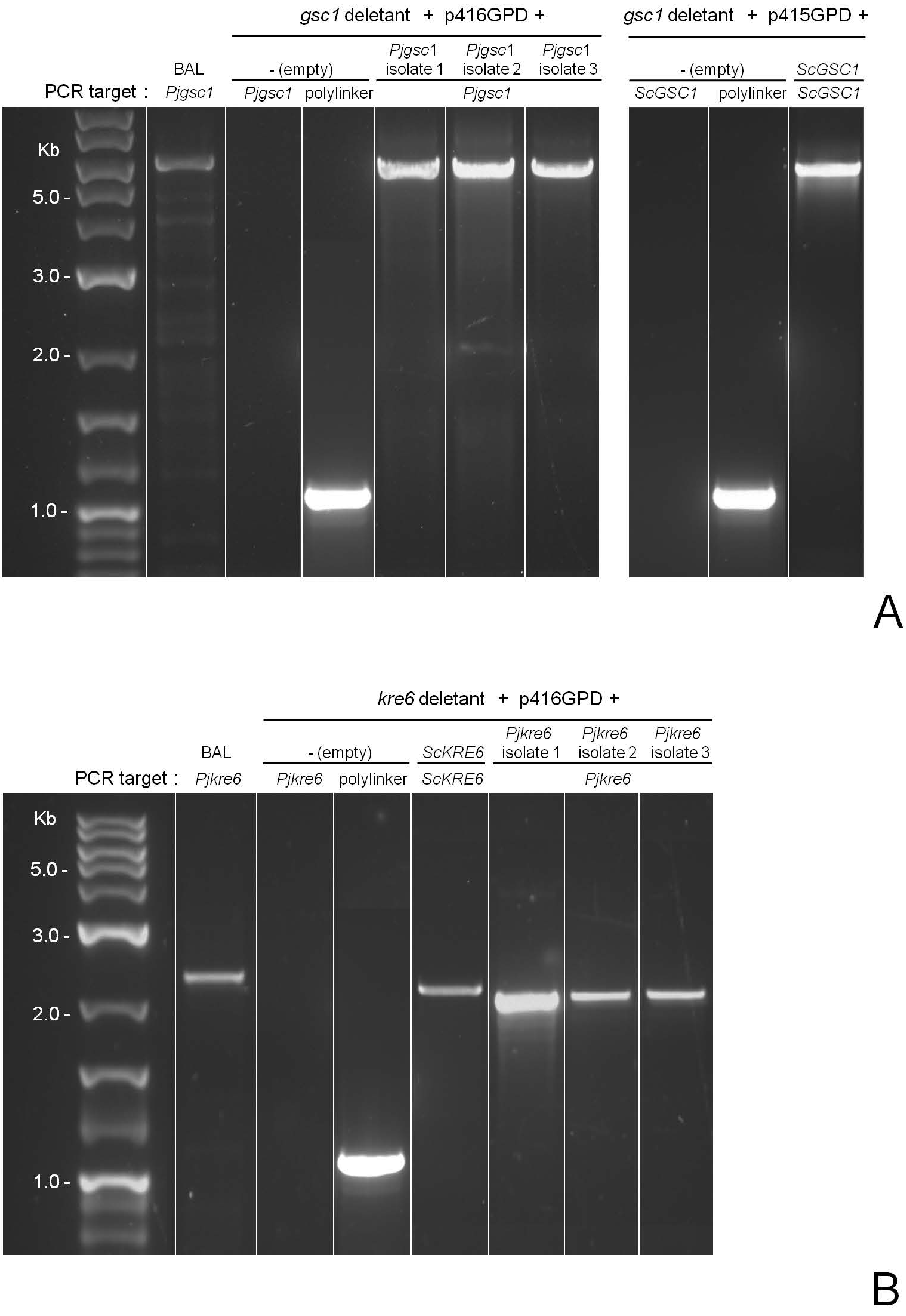

gsc1 deletant + p416GPD +
gsc1 deletant + p415GPD +
BAL
- (empty)
Pjgsc1 isolate 1
Pjgsc1 isolate 2
Pjgsc1 isolate 3
- (empty)
ScGSC1
PCR target :
Pjgsc1
Pjgsc1
polylinker
Pjgsc1
ScGSC1
polylinker
ScGSC1
Kb
5.0 -
3.0 -
2.0 -
1.0 -
A
kre6 deletant + p416GPD +
BAL
- (empty)
ScKRE6
Pjkre6 isolate 1
Pjkre6 isolate 2
Pjkre6 isolate 3
PCR target :
Pjkre6
Pjkre6
polylinker
ScKRE6
Pjkre6
Kb
5.0 -
3.0 -
2.0 -
1.0 -
B

Table S1. Oligonucleotide primers used for PCR amplification

Target[a]	Primer	5' - 3' nucleotide sequence	Fragment amplified size with / without introns (bp)	Position in ORF
Pjgsc1 ORF	Pjgsc1start	CCGCGGTCGACATGTCACAACGACAGC[b]	6017 / 5782	First 16 nt
	Pjgsc1end	CCCCCCCCCGGGCTATGTAAACATCTTATATG[b]		Last 20 nt
Pjkre6 ORF	Pjkre6start	ATGAAAAAAAGTCATTGGTC	2341 / 2040	First 20 nt
	Pjkre6end	TTAACATTCGTTCATAAGTC		Last 20 nt
ScGSC1 ORF	ScGSC1start	GCGGGATCCATGACCACTGATCAACAACC[d]	5652 / NA[c]	First 20 nt
	ScGSC1end	CCCCCCCTGCAGTTATTTTATAGTTGACCAGG[d]		Last 20 nt
ScKRE6 ORF	ScKRE6start	GCGGGATCCATGCCTTTGAGAAAATCTAAC[d]	2184 / NA	First 21 nt
	ScKRE6end	CCCCCCGTCGACAGAGGATAACTTGAACTTCG[d]		Last 20 nt
Plasmids' polylinker	M13 forward	GTAAAACGACGGCCAGT	1106 / NA	NA
	M13 reverse	CAGGAAACAGCTATGAC		

[a] *Pj, P. jirovecii; Sc, S. cerevisiae*.

[b] These primers include the underlined restriction sites and five or six upstream bases allowing restriction because there were intended for an oriented cloning which we did not use in the present work.

[c] NA, not applicable.

[d] These primers include the underlined restriction sites and three or six upstream bases allowing restriction for oriented cloning.

Table S2. PCR conditions

				35 cycles							
		Initial denaturation		Denaturation		Annealing		Elongation		Final extension	
Target[1]	MgCl$_2$ (mM)	Time (s)	Temp (°C)	Time (s)	Temp (°C)	Time (s)	Temp (°C)	Time (s)	Temp (°C)	Time (s)	Temp (°C)
Pjgsc1[2]	1.75	180	94	25	94	15	44	360	68	360	72
Pjkre6	7.5	180	94	30	94	30	52	150	72	600	72
ScGSC1	3.0	180	94	30	94	30	53	360	72	600	72
ScKRE6	3.0	180	94	30	94	30	52	135	72	600	72
Plasmids' polylinker	3.0	180	94	30	94	30	55	70	72	600	72

[1] *Pj, P. jirovecii; Sc, S. cerevisiae*.

[2] The Kapa Long Rage HotStart polymerase (Kapa Biosystems, Baden, Switzerland) was used for amplification of *Pjgsc1*, whereas all the other PCRs were performed using the High Fidelity Expand polymerase (Roche, Basel, Switzerland).

A

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Pjgsc1_Cisse_ORF          1 ATGTCACAACGACAGCATTATTACGATGATTCTTATCCAAGTCAAACAGATCCATATTATGCTGAC
Pjgsc1_Cisse_genomic      1 ATGTCACAACGACAGCATTATTACGATGATTCTTATCCAAGTCAAACAGATCCATATTATGCTGAC
Pjgsc1_Ma_ORF             1 ATGTCACAACGACAGCATTATTACGATGATTCTTATCCAAGTCAAACAGATCCATATTATGCTGAC
Pjgsc1_Ma_genomic         1 ATGTCACAACGACAGCATTATTACGATGATTCTTATCCAAGTCAAACAGATCCATATTATGCTGAC


Pjgsc1_Cisse_ORF         67 AATGGCTATAATAATGCAGATTTTCACGGGTCTTCATATGCACCCGAAGGGTATGATCATCAGGGA
Pjgsc1_Cisse_genomic     67 AATGGCTATAATAATGCAGATTTTCACGGGTCTTCATATGCACCCGAAGGGTATGATCATCAGGGA
Pjgsc1_Ma_ORF            67 AATGGCTATAATAATGCAGATTTTCACGGGTCTTCATATGCACCCGAAGGGTATGATCATCAGGGA
Pjgsc1_Ma_genomic        67 AATGGCTATAATAATGCAGATTTTCACGGGTCTTCATATGCACCCGAAGGGTATGATCATCAGGGA


Pjgsc1_Cisse_ORF        133 GCTTATCATCCTATGGAATACGGACAAGAGTATTATGATGAAGGATATGATAATGGGCAGGTACCA
Pjgsc1_Cisse_genomic    133 GCTTATCATCCTATGGAATACGGACAAGAGTATTATGATGAAGGATATGATAATGGGCAGGTACCA
Pjgsc1_Ma_ORF           133 GCTTATCATCCTATGGAATACGGACAAGAGTATTATGATGAAGGATATGATAATGGGCAGGTACCA
Pjgsc1_Ma_genomic       133 GCTTATCATCCTATGGAATACGGACAAGAGTATTATGATGAAGGATATGATAATGGGCAGGTACCA


Pjgsc1_Cisse_ORF        199 TATGATGCACGTGCTTTTGATATGTATTCACCATCGGATGATGCATATTATCGTCAAGAAAATGCC
Pjgsc1_Cisse_genomic    199 TATGATGCACGTGCTTTTGATATGTATTCACCATCGGATGATGCATATTATCGTCAAGAAAATGCC
Pjgsc1_Ma_ORF           199 TATGATGCACGTGCTTTTGATATGTATTCACCATCGGATGATGCATATTATCGTCAAGAAAATGCC
Pjgsc1_Ma_genomic       199 TATGATGCACGTGCTTTTGATATGTATTCACCATCGGATGATGCATATTATCGTCAAGAAAATGCC


Pjgsc1_Cisse_ORF        265 TATTATGATTATCCTGCAGATGCATATGCAACGGATGTGTATG-----------------------
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Pjgsc1_Cisse_genomic    3136 ACCAGAATATACCTTAAGGACACGTATTTGGGCATCATTGCGTTCTCAAACTTTATACAGAACTGT
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Pjgsc1_Ma_genomic       3131 ACCAGAATATACCTTAAGGACACGTATTTGGGCATCATTGCGTTCTCAAACTTTATACAGAACTGT


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Pjgsc1_Ma_ORF           3090 TTCTGGATTTATGAACTATTCACGAGCTATTAAGCTTCTTTATCGTGTTGAAAATCCTGATGTTGT
Pjgsc1_Ma_genomic       3197 TTCTGGATTTATGAACTATTCACGAGCTATTAAGCTTCTTTATCGTGTTGAAAATCCTGATGTTGT
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Pjgsc1_Ma_ORF           3156 TCAAATGTTTGGTGGAAATACAGATAAGTTAGAACATGAGTTAGAAAGAATGGCCCGTCGGAAATT
Pjgsc1_Ma_genomic       3263 TCAAATGTTTGGTGGAAATACAGATAAGTTAGAACATGAGTTAGAAAGAATGGCCCGTCGGAAATT


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Pjgsc1_Cisse_genomic    3334 CAAATTTGTTATATCAATGCAACGATTTTTTAAATTCAATAAAGAGGAGCAAGAGAATACGGAATT
Pjgsc1_Ma_ORF           3222 CAAATTTGTTATATCAATGCAACGATTTTTTAAATTCAATAAAGAGGAGCAAGAGAATACGGAATT
Pjgsc1_Ma_genomic       3329 CAAATTTGTTATATCAATGCAACGATTTTTTAAATTCAATAAAGAGGAGCAAGAGAATACGGAATT


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Pjgsc1_Cisse_genomic    3400 TCTTCTTCGGGCCTATCCAGATCTTCAAATTGCATATTTGGATGAAGAGCCGCCTTCACATGAAGG
Pjgsc1_Ma_ORF           3288 TCTTCTTCGGGCCTATCCAGATCTTCAAATTGCATATTTGGATGAAGAGCCGCCTTCACATGAAGG
Pjgsc1_Ma_genomic       3395 TCTTCTTCGGGCCTATCCAGATCTTCAAATTGCATATTTGGATGAAGAGCCGCCTTCACATGAAGG


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Pjkre6_Cisse_ORF          1 ATGAAAAAAAGTCATTGGTCTAATGACACTCCGGTGGATTATTCTCCACAAAATAGTGGAAATAAT
Pjkre6_Cisse_genomic      1 ATGAAAAAAAGTCATTGGTCTAATGACACTCCGGTGGATTATTCTCCACAAAATAGTGGAAATAAT
Pjkre6_Ma_ORF             1 ATGAAAAAAAGTCATTGGTCTAATGACACTCCGGTGGATTATTCTCCACAAAATAGTGGAAATAAT
Pjkre6_Ma_genomic         1 ATGAAAAAAAGTCATTGGTCTAATGACACTCCGGTGGATTATTCTCCACAAAATAGTGGAAATAAT


Pjkre6_Cisse_ORF         67 TGGAATGTTCGAAGTTCAGGAACAGTACATAGACCATTACCACCAACACCCTGTCATTTTGAAACT
Pjkre6_Cisse_genomic     67 TGGAATGTTCGAAGTTCAGGAACAGTACATAGACCATTACCACCAACACCCTGTCATTTTGAAACT
Pjkre6_Ma_ORF            67 TGGAATGTTCGAAGTTCAGGAACAGTACATAGACCATTACCACCAACACCCTGTCATTTTGAAACT
Pjkre6_Ma_genomic        67 TGGAATGTTCGAAGTTCAGGAACAGTACATAGACCATTACCACCAACACCCTGTCATTTTGAAACT


Pjkre6_Cisse_ORF        133 CAATATCAAGAGTCATATGGAGATGCTGTATGGAATAGTCAGCAGTCTCCATATAATCAAAGTTAT
Pjkre6_Cisse_genomic    133 CAATATCAAGAGTCATATGGAGATGCTGTATGGAATAGTCAGCAGTCTCCATATAATCAAAGTTAT
Pjkre6_Ma_ORF           133 CAATATCAAGAGTCATATGGAGATGCTGTATGGAATAGTCAGCAGTCTCCATATAATCAAAGTTAT
Pjkre6_Ma_genomic       133 CAATATCAAGAGTCATATGGAGATGCTGTATGGAATAGTCAGCAGTCTCCATATAATCAAAGTTAT


Pjkre6_Cisse_ORF        199 TATTTATTTCAAGATGAAAATGTATCATTTCCAAGAAGAAGAATTGTATCTCATGGAGATTATTCA
Pjkre6_Cisse_genomic    199 TATTTATTTCAAGATGAAAATGTATCATTTCCAAGAAGAAGAATTGTATCTCATGGAGATTATTCA
Pjkre6_Ma_ORF           199 TATTTATTTCAAGATGAAAATGTATCATTTCCAAGAAGAAGAATTGTATCTCATGGAGATTATTCG
Pjkre6_Ma_genomic       199 TATTTATTTCAAGATGAAAATGTATCATTTCCAAGAAGAAGAATTGTATCTCATGGAGATTATTCG


Pjkre6_Cisse_ORF        265 GAAGATGAACCAGCATATCAAACAAAACAAGAAAATGTGGAGTATTATGATAATTCTTTTAGCTCT
Pjkre6_Cisse_genomic    265 GAAGATGAACCAGCATATCAAACAAAACAAGAAAATGTGGAGTATTATGATAATTCTTTTAGCTCT
Pjkre6_Ma_ORF           265 GAAGATGAACCAGCATATCAAACAAAACAAGAAAATGTGGAGTATTATGATAATTCTTTTAGCTCT
Pjkre6_Ma_genomic       265 GAAGATGAACCAGCATATCAAACAAAACAAGAAAATGTGGAGTATTATGATAATTCTTTTAGCTCT


Pjkre6_Cisse_ORF        331 CAGTCTCCTCGAAATGTATATACAGATGGGTATAAAGCTTATAACGATATACAGGATTATGCATCT
Pjkre6_Cisse_genomic    331 CAGTCTCCTCGAAATGTATATACAGATGGGTATAAAGCTTATAACGATATACAGGATTATGCATCT
Pjkre6_Ma_ORF           331 CAGTCTCCTCGAAATGTATATACAGATGGGTATAAAGCTTATAACGATATACAGGATTATGCATCT
Pjkre6_Ma_genomic       331 CAGTCTCCTCGAAATGTATATACAGATGGGTATAAAGCTTATAACGATATACAGGATTATGCATCT


Pjkre6_Cisse_ORF        397 CTTGATTACAAAAGGAAATCTTATATGAATTATCCTGAAGAACCAGATAATAATTATTGGCATGAA
Pjkre6_Cisse_genomic    397 CTTGATTACAAAAGGAAATCTTATATGAATTATCCTGAAGAACCAGATAATAATTATTGGCATGAA
Pjkre6_Ma_ORF           397 CTTGATTACAAAAGGAAATCTTATATGAATTATCCTGAAGAACCAGATAATAATTATTGGCATGAA
Pjkre6_Ma_genomic       397 CTTGATTACAAAAGGAAATCTTATATGAATTATCCTGAAGAACCAGATAATAATTATTGGCATGAA


Pjkre6_Cisse_ORF        463 CCGCAAGAAAGTGTGTATACAGAAGAATATATAGAACCCGAATCAAGAAAAACAAATAAAGGATCT
Pjkre6_Cisse_genomic    463 CCGCAAGAAAGTGTGTATACAGAAGAATATATAGAACCCGAATCAAGAAAAACAAATAAAGGATCT
Pjkre6_Ma_ORF           463 CCGCAAGAAAGTGTATATACAGAAGAATATATAGAACCCGAATCAAGAAAAACAAATAAAGGATCT
Pjkre6_Ma_genomic       463 CCGCAAGAAAGTGTATATACAGAAGAATATATAGAACCCGAATCAAGAAAAACAAATAAAGGATCT


Pjkre6_Cisse_ORF        529 TTTAATACATACAAAAATACCGCTAAATCTGATGTTGGAAATAACCTTGATACATTATGGGATCCT
Pjkre6_Cisse_genomic    529 TTTAATACATACAAAAATACCGCTAAATCTGATGTTGGAAATAACCTTGATACATTATGGGATCCT
Pjkre6_Ma_ORF           529 TTTAATACATACAAAAATACCGCTAAATCTGATGTTGGAAATAACCTTGATACATTATGGGATCCT
Pjkre6_Ma_genomic       529 TTTAATACATACAAAAATACCGCTAAATCTGATGTTGGAAATAACCTTGATACATTATGGGATCCT


Pjkre6_Cisse_ORF        595 ACTGTAACAGAACCCGATGATTATCTTCACAACCCAACCTTTAAGGATAGAAAAAAAGATTATTAT
Pjkre6_Cisse_genomic    595 ACTGTAACAGAACCCGATGATTATCTTCACAACCCAACCTTTAAGGATAGAAAAAAAGATTATTAT
Pjkre6_Ma_ORF           595 ACTGTAACAGAACCCGATGATTATCTTCACAACCCAACCTTTAAGGATAGAAAAAAAGATTATTAT
Pjkre6_Ma_genomic       595 ACTGTAACAGAACCCGATGATTATCTTCACAACCCAACCTTTAAGGATAGAAAAAAAGATTATTAT


Pjkre6_Cisse_ORF        661 TTTTTTACAAAACGTGGTATATTTAATATAGGATCTCTTGTGTTTTTAATACTTGGTGTAATGTTT
Pjkre6_Cisse_genomic    661 TTTTTTACAAAACGTGGTATATTTAATATAGGATCTCTTGTGTTTTTAATACTTGGTGTAATGTTT
Pjkre6_Ma_ORF           661 TTTTTTACAAAACGTGGTATATTTAATATAGGATCTCTTGTGTTTTTAATACTTGGTGTAATGTTT
Pjkre6_Ma_genomic       661 TTTTTTACAAAACGTGGTATATTTAATATAGGATCTCTTGTGTTTTTAATACTTGGTGTAATGTTT


Pjkre6_Cisse_ORF        727 GTTTTTATTGGTTACCCTATTATGTTATATATTCGCAGGGCATATGATGATGCTCATAGTTGTCCT
Pjkre6_Cisse_genomic    727 GTTTTTATTGGTTACCCTATTATGTTATATATTCGCAGGGCATATGATGATGCTCATAGTTGTCCT
Pjkre6_Ma_ORF           727 GTTTTTATTGGTTACCCTATTATGTTATATATTCGCAGGGCATATGATGATGCTCATAGTTGTCCT
Pjkre6_Ma_genomic       727 GTTTTTATTGGTTACCCTATTATGTTATATATTCGCAGGGCATATGATGATGCTCATAGTTGTCCT
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Pjkre6_Cisse_ORF         793 AATTGTATACGAACACTGCCTATAGATTTATTGGATGCAACAAGAAGCCTTATTGATCCGGATACA
Pjkre6_Cisse_genomic     793 AATTGTATACGAACACTGCCTATAGATTTATTGGATGCAACAAGAAGCCTTATTGATCCGGATACA
Pjkre6_Ma_ORF            793 AATTGTATACGAACACTGCCTATAGATTTATTGGATGCAACAAGAAGCCTTATTGATCCGGATACA
Pjkre6_Ma_genomic        793 AATTGTATACGAACACTGCCTATAGATTTATTGGATGCAACAAGAAGCCTTATTGATCCGGATACA


Pjkre6_Cisse_ORF         859 CCGTTAGAATTTTATGAACGGAAGAGTAAAGATGGAAAAATTTATAAAATTGTATTTTCAGATGAA
Pjkre6_Cisse_genomic     859 CCGTTAGAATTTTATGAACGGAAGAGTAAAGATGGAAAAATTTATAAAATTGTATTTTCAGATGAA
Pjkre6_Ma_ORF            859 CCGTTAGAATTTTATGAACGGAAGAGTAAAGATGGAAAAATTTATAAAATTGTATTTTCAGATGAA
Pjkre6_Ma_genomic        859 CCGTTAGAATTTTATGAACGGAAGAGTAAAGATGGAAAAATTTATAAAATTGTATTTTCAGATGAA


Pjkre6_Cisse_ORF         925 TTTAATAAAAATGGAAGAACATTCTATCCAGGTGATGATCAGTTTTG-------------------
Pjkre6_Cisse_genomic     925 TTTAATAAAAATGGAAGAACATTCTATCCAGGTGATGATCAGTTTTGGTATATATTAAATATGTCT
Pjkre6_Ma_ORF            925 TTTAATAAAAATGGAAGAACATTCTATCCAGGTGATGATCAGTTTTG-------------------
Pjkre6_Ma_genomic        925 TTTAATAAAAATGGAAGAACATTCTATCCAGGTGATGATCAGTTTTGGTATATATTAAATATGTCT


Pjkre6_Cisse_ORF         972 ------------------------GGAAGCAGTCGATTTACATTACTGGTCAACTATGAGTATAGA
Pjkre6_Cisse_genomic     991 TTGAATTTTAATAAATTTGAATAGGGAAGCAGTCGATTTACATTACTGGTCAACTATGAGTATAGA
Pjkre6_Ma_ORF            972 ------------------------GGAAGCAGTCGATTTACATTACTGGTCAACTATGAGTATAGA
Pjkre6_Ma_genomic        991 TTGAATTTTAATAAATTTGAATAGGGAAGCAGTCGATTTACATTACTGGTCAACTATGAGTATAGA


Pjkre6_Cisse_ORF        1014 ATGGTATGATCCTGATGCTATAACTACAAATGGAGGTTTTTTGGAGATACGACTTGATGCTTTTCG
Pjkre6_Cisse_genomic    1057 ATGGTATGATCCTGATGCTATAACTACAAATGGAGGTTTTTTGGAGATACGACTTGATGCTTTTCG
Pjkre6_Ma_ORF           1014 ATGGTATGATCCTGATGCTATAACTACAAATGGAGGTTTTTTGGAGATACGACTTGATGCTTTTCG
Pjkre6_Ma_genomic       1057 ATGGTATGATCCTGATGCTATAACTACAAATGGAGGTTTTTTGGAGATACGACTTGATGCTTTTCG


Pjkre6_Cisse_ORF        1080 AAATCATGATCTTAATTATAGATCAG---------------------------------------G
Pjkre6_Cisse_genomic    1123 AAATCATGATCTTAATTATAGATCAGGTATTTTTTGCAATTAAATTTTAATTTTGACATGTATAGG
Pjkre6_Ma_ORF           1080 AAATCATGATCTTAATTATAGATCAG---------------------------------------G
Pjkre6_Ma_genomic       1123 AAATCATGATCTTAATTATAGATCAGGTATTTTTTGCAATTAAATTTTAATTTTGACATGTATAGG


Pjkre6_Cisse_ORF        1107 TATGCTTCAAAGTTGGAATAAACTATGTTTTAAAGGAGGTATTATTGAAGCATCTATTTCACTTCC
Pjkre6_Cisse_genomic    1189 TATGCTTCAAAGTTGGAATAAACTATGTTTTAAAGGAGGTATTATTGAAGCATCTATTTCACTTCC
Pjkre6_Ma_ORF           1107 TATGCTTCAAAGTTGGAATAAACTATGTTTTAAAGGAGGTATTATTGAAGCATCTATTTCACTTCC
Pjkre6_Ma_genomic       1189 TATGCTTCAAAGTTGGAATAAACTATGTTTTAAAGGAGGTATTATTGAAGCATCTATTTCACTTCC


Pjkre6_Cisse_ORF        1173 AGGTAGAGGAGACATTTCTGGATT------------------------------------------
Pjkre6_Cisse_genomic    1255 AGGTAGAGGAGACATTTCTGGATTGTAGGATAATATATGTATATATATATTATATTATTTATTACT
Pjkre6_Ma_ORF           1173 AGGTAGAGGAGACATTTCTGGATT------------------------------------------
Pjkre6_Ma_genomic       1255 AGGTAGAGGAGACATTTCTGGATTGTAGGATAATATATGTATATATATATTATATTATTTATTACT


Pjkre6_Cisse_ORF        1197 -----TTGGCCAGCATTTTGGGCAATGGGAAATCTTGGTCGTCCTGGCTTTGGTGCTTCTACTGAT
Pjkre6_Cisse_genomic    1321 ATTAGTTGGCCAGCATTTTGGGCAATGGGAAATCTTGGTCGTCCTGGCTTTGGTGCTTCTACTGAT
Pjkre6_Ma_ORF           1197 -----TTGGCCAGCATTTTGGGCAATGGGAAATCTTGGTCGTCCTGGCTTTGGTGCTTCTACTGAT
Pjkre6_Ma_genomic       1321 ATTAGTTGGCCAGCATTTTGGGCAATGGGAAATCTTGGTCGTCCTGGCTTTGGTGCTTCTACTGAT


Pjkre6_Cisse_ORF        1258 GGAGTATGGCCATATAGTTATGATACATGTGAT---------------------------------
Pjkre6_Cisse_genomic    1387 GGAGTATGGCCATATAGTTATGATACATGTGATGTAAGATTATATATTTGTTTTTTAAAATCTTAT
Pjkre6_Ma_ORF           1258 GGAGTATGGCCATATAGTTATGATACATGTGAT---------------------------------
Pjkre6_Ma_genomic       1387 GGAGTATGGCCATATAGTTATGATACATGTGATGTAAGATTATATATTTGTTTTTTAAAATCTTAT


Pjkre6_Cisse_ORF        1292 -------GTTGGAATTACACCTAATCAATCAGATTCTAACGGCATATCATCTCTTCCTGGAATGAG
Pjkre6_Cisse_genomic    1453 AATTTAGGTTGGAATTACACCTAATCAATCAGATTCTAACGGCATATCATCTCTTCCTGGAATGAG
Pjkre6_Ma_ORF           1292 -------GTTGGAATTACACCTAATCAATCAGATTCTAACGGCATATCATCTCTTCCTGGAATGAG
Pjkre6_Ma_genomic       1453 AATTTAGGTTGGAATTACACCTAATCAATCAGATTCTAACGGCATATCATCTCTTCCTGGAATGAG


Pjkre6_Cisse_ORF        1350 ATTTCCTAACTGTGTATGTCCTAATTCAGATCATCCAAGCCCAGGAAAAGGACGAGGTGCACCAGA
Pjkre6_Cisse_genomic    1519 ATTTCCTAACTGTGTATGTCCTAATTCAGATCATCCAAGCCCAGGAAAAGGACGAGGTGCACCAGA
Pjkre6_Ma_ORF           1350 ATTTCCTAACTGTGTATGTCCTAATTCAGATCATCCAAGCCCAGGAAAAGGACGAGGTGCACCAGA
Pjkre6_Ma_genomic       1519 ATTTCCTAACTGTGTATGTCCTAATTCAGATCATCCAAGCCCAGGAAAAGGACGAGGTGCACCAGA


Pjkre6_Cisse_ORF        1416 AATAGATATAATTGAAGCATCTGTTGATCTTAGTTTTCGTCTTGGAGAAGCATCACAATCAGTACA
Pjkre6_Cisse_genomic    1585 AATAGATATAATTGAAGCATCTGTTGATCTTAGTTTTCGTCTTGGAGAAGCATCACAATCAGTACA
Pjkre6_Ma_ORF           1416 AATAGATATAATTGAAGCATCTGTTGATCTTAGTTTTCGTCTTGGAGAAGCATCACAATCAGTACA
Pjkre6_Ma_genomic       1585 AATAGATATAATTGAAGCATCTGTTGATCTTAGTTTTCGTCTTGGAGAAGCATCACAATCAGTACA
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Pjkre6_Cisse_ORF        1482 GTTTGCACCTTTTGACGACCTTTATACACCAAATTATGAACATATGAAAATATATAATAAAGAAAA
Pjkre6_Cisse_genomic    1651 GTTTGCACCTTTTGACGACCTTTATACACCAAATTATGAACATATGAAAATATATAATAAAGAAAA
Pjkre6_Ma_ORF           1482 GTTTGCACCTTTTGACGACCTTTATACACCAAATTATGAACATATGAAAATATATAATAAAGAAAA
Pjkre6_Ma_genomic       1651 GTTTGCACCTTTTGACGACCTTTATACACCAAATTATGAACATATGAAAATATATAATAAAGAAAA

Pjkre6_Cisse_ORF        1548 AACTCATATAAATAATTATCGTGGCAATTCTTTTCAACAAA-------------------------
Pjkre6_Cisse_genomic    1717 AACTCATATAAATAATTATCGTGGCAATTCTTTTCAACAAAGTAAAAATATAATATTTCTATTTTT
Pjkre6_Ma_ORF           1548 AACTCATATAAATAATTATCGTGGCAATTCTTTTCAACAAA-------------------------
Pjkre6_Ma_genomic       1717 AACTCATATAAATAATTATCGTGGCAATTCTTTTCAACAAAGTAAAAATATAATATTTCTATTTTT

Pjkre6_Cisse_ORF        1589 ------------------CATTTTCATGTATCACGTACTTAAATAATGAATGGTATGATGGACGTA
Pjkre6_Cisse_genomic    1783 AATTTACTTATTATTTAGCATTTTCATGTATCACGTACTTAAATAATGAATGGTATGATGGACGTA
Pjkre6_Ma_ORF           1589 ------------------CATTTTCATGTATCACGTACTTAAATAATGAATGGTATGATGGACGTA
Pjkre6_Ma_genomic       1783 AATTTACTTATTATTTAGCATTTTCATGTATCACGTACTTAAATAATGAATGGTATGATGGACGTA

Pjkre6_Cisse_ORF        1637 AATTTCAAACATATTCTCTTGAGTATGAACCTGGTAAAAATGGTTTTATTCAATGGTATATTGGTG
Pjkre6_Cisse_genomic    1849 AATTTCAAACATATTCTCTTGAGTATGAACCTGGTAAAAATGGTTTTATTCAATGGTATATTGGTG
Pjkre6_Ma_ORF           1637 AATTTCAAACATATTCTCTTGAGTATGAACCTGGTAAAAATGGTTTTATTCAATGGTATATTGGTG
Pjkre6_Ma_genomic       1849 AATTTCAAACATATTCTCTTGAGTATGAACCTGGTAAAAATGGTTTTATTCAATGGTATATTGGTG

Pjkre6_Cisse_ORF        1703 ATAATCCAACGTGGATGATGAAAGCTGAATCAGTAGGACCAAATGGGAAAATTGGACAAAGGCTCA
Pjkre6_Cisse_genomic    1915 ATAATCCAACGTGGATGATGAAAGCTGAATCAGTAGGACCAAATGGGAAAATTGGACAAAGGCTCA
Pjkre6_Ma_ORF           1703 ATAATCCAACGTGGATGATGAAAGCTGAATCAGTAGGACCAAATGGGAAAATTGGACAAAGGCTCA
Pjkre6_Ma_genomic       1915 ATAATCCAACGTGGATGATGAAAGCTGAATCAGTAGGACCAAATGGGAAAATTGGACAAAGGCTCA

Pjkre6_Cisse_ORF        1769 TATCTGAAGAGCCCATG--------------------------------------------GCATT
Pjkre6_Cisse_genomic    1981 TATCTGAAGAGCCCATGGTGAGATTAACTTATATCTTTGTTTTTTTTTTGACCTGATACAGGCATT
Pjkre6_Ma_ORF           1769 TATCTGAAGAACCCATG--------------------------------------------GCATT
Pjkre6_Ma_genomic       1981 TATCTGAAGAACCCATGGTGAGATTAACTTATATCTTTGTTTTTTTTTTGACCTGATACAGGCATT

Pjkre6_Cisse_ORF        1791 CGTAATTAACCTTGCAATGTCAGAGTCTTTTGCTAAAATAGAATGGGGAAGATTACAATTTCCTGC
Pjkre6_Cisse_genomic    2047 CGTAATTAACCTTGCAATGTCAGAGTCTTTTGCTAAAATAGAATGGGGAAGATTACAATTTCCTGC
Pjkre6_Ma_ORF           1791 CGTAATTAACCTTGCGATGTCAGAGTCTTTTGCTAAAATAGAATGGGGAAGATTACAATTTCCTGC
Pjkre6_Ma_genomic       2047 CGTAATTAACCTTGCGATGTCAGAGTCTTTTGCTAAAATAGAATGGGGAAGATTACAATTTCCTGC

Pjkre6_Cisse_ORF        1857 TATTATGCGAGTAGATTGGGTCCGCATTTACCAAGAAACGCCTATGATTACATGTGATCCTCCTGG
Pjkre6_Cisse_genomic    2113 TATTATGCGAGTAGATTGGGTCCGCATTTACCAAGAAACGCCTATGATTACATGTGATCCTCCTGG
Pjkre6_Ma_ORF           1857 TATTATGCGAGTAGATTGGGTCCGCATTTACCAAGAAACGCCTATGATTACATGTGATCCTCCTGG
Pjkre6_Ma_genomic       2113 TATTATGCGAGTAGATTGGGTCCGCATTTACCAAGAAACGCCTATGATTACATGTGATCCTCCTGG

Pjkre6_Cisse_ORF        1923 ATATCCAACTACAAAGTATATTAAAGA---------------------------------------
Pjkre6_Cisse_genomic    2179 ATATCCAACTACAAAGTATATTAAAGAGTATGTTGTTTTTATTATATAATTTGCAAATTTAACAGA
Pjkre6_Ma_ORF           1923 ATATCCAACTACAAAGTATATTAAAGAGTATGTTGTTTTTATTATATAA-----------------
Pjkre6_Ma_genomic       2179 ATATCCAACTACAAAGTATATTAAAGAGTATGTTGTTTTTATTATATAA-----------------

Pjkre6_Cisse_ORF        1954 ------ACATCCTATAGCTTATTATAACAATAATATTACTACTTGGGAGAATACCGGCTATCAATG
Pjkre6_Cisse_genomic    2245 TATCAGACATCCTATAGCTTATTATAACAATAATATTACTACTTGGGAGAATACCGGCTATCAATG
Pjkre6_Ma_ORF           1965 ------------------------------------------------------------------
Pjkre6_Ma_genomic       2221 ------------------------------------------------------------------

Pjkre6_Cisse_ORF        2010 GCCTAAAAATCGACTTATGAACGAATGTTAA
Pjkre6_Cisse_genomic    2311 GCCTAAAAATCGACTTATGAACGAATGTTAA
Pjkre6_Ma_ORF           1965 -------------------------------
Pjkre6_Ma_genomic       2221 -------------------------------
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Fig. S1.

A

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P.jirovecii        1 MSQRQHYYDDSYPSQTDPYYADNGYNNADFHGSSYAPEGYDHQGAYHPMEYGQEYYDEGYDNGQVPYDARAF
P.carinii          1 MSQQQHYYDDSYGGQNGGYYGEHSYDNTGFNNGSYGSGVYE-QGGYYGPEYGQEYYDE-YDGGGAMYNGQGH
S.cerevisiae       1 MNTDQQ-----------PYQGQTDYTQ----------------------GPGNGQSQE-----------QDY
S.pombe            1 MSGNN-----------ENVSGITGHDAVSDDQYAYDSEVYD-DQNAYQRQPADAYSNE-EFLDQADYD----
                     *.  :               .  .:                          .:   :*

P.jirovecii       73 DMYSPSDDAYYRQENAYYDYPAD--AYATDVYDPYGMPIADQHPLQYFQDHGNY-MYNRKGK-HRGSSEGSE
P.carinii         71 EMYNSGEEGYYRQEEGYYDYPQD--GYVG---DTYGIKKDILRGNGYFQGQDEYYTYDRKGK-RRGSSEASE
S.cerevisiae      29 DQY--GQPLYPSQADGYYD-PNVAAGTEA---DMYGQQPPN---ESYDQDYTNG-EYYGQPP-NMA-AQDGE
S.pombe           56 -------SMYGEGYNG-YDYPTG----VT---ESYGDEYTP--VDTASSGINQY-S-TEKGKFTRPSDEYES
                              *    :. ** *           : **            ..  :      :        :  .

P.jirovecii      141 AFSDFTMRSDMARAAEFDAYGRFDEQYRSYAPSTESLNQMA-SR-RGYYP-DSSQISYTGNRSSGASTPVYG
P.carinii        137 TFSDFTMRSDMVRAAEYDSYGRFDERYRSYEPSTESLNQMA-SRQRGYRP-D-SQISYTGNRSSGASTPIYG
S.cerevisiae      89 NFSDFSSYGPP-GTPGYDSYG------------------------GQYT-A-SQMSYGEPNSSGTSTPIYG
S.pombe          109 EYSDYNAQPSD--ANNF-YNLRGDGRYNAYDPSSDSLANVYNSVPYGSSPYDFSNSSFVGN--SGSGTPLDG
                      :**:.       :  :                             *  .   *: *:     **:.**: *

P.jirovecii      210 MEYNQAAMMTSARSREPYPAWTAENQIPISKEEIEDIFIDLTNKFGFQRDSMRNMYDHMMVLLDSRASRMTP
P.carinii        206 MYYNQAAMMTSARSREPYPTWTAENQIPISKEEIEDIFIDLTNKFGFQRDSMRNMYDHMMVLLDSRASRMTP
S.cerevisiae     133 NYDPNAIAM--ALPNEPYPAWTADSQSPVSIEQIEDIFIDLTNRLGFQRDSMRNMFDHFMVLLDSRSSRMSP
S.pombe          176 DSGSFYADSANLTNREPYPAWTPENELPLTKEEIEDIFIDLTNKLGFQRDSMRNMYDFFMCLLDSRASRMTP
                                   .****:**.:.: *:: *:**********::**********:*.:* *****:***:*

P.jirovecii      282 NQALLSLHADYIGGDNANYRNWYFAAQLDLDDAVGFSNMDFEKN-KKTNHSQKF------------------
P.carinii        278 NQALLSLHADYIGGDNANYRNWYFAAQFDLDDAVGFSNMDLDKN-RKSNYSQKS------------------
S.cerevisiae     203 DQALLSLHADYIGGDTANYKKWYFAAQLDMDDEIGFRNMSLGKLSRKARKAKKK------------------
S.pombe          248 DQALLTLHADYIGSDIANYKKWYFASQMDREDAVGLANVGIYGG-KVTSIKEKGKFFSRNKKAPKVVKPPRK
                     :****:*******.* ***::****:*:* :* :*: *:.:    : :   :*

                                                                        ---------------------
P.jirovecii      335 ------SKS---Q-KNTTAKDILQALESDNPLESAIYRWKTKCSQMSQYDRARELALYLLCWGEANQVRFTP
P.carinii        331 ------SKKF--Q-KNSASKSILQALDGDNSLESAIYRWKTRCTQMSQYDRARELALYLLCWGEANQVRFTP
S.cerevisiae     257 ------NKKAMEEANPEDTEETLNKIEGDNSLEAADFRWKAKMNQLSPLERVRHIALYLLCWGEANQVRFTA
S.pombe          319 SRFKRKKKK---E-QPEEAEDEYIDVNTDDSLESAEYRWRSHMRSMTQFERAQQIALWLLLWGEANNVRFMP
                           .*.   : :   ::.    :: *:.**:* :**::   .::  :*.:.:**:** *****:*** .

                     ----------------------1,3-β glucan synthase domain 1--------------------
P.jirovecii      397 ECLCFIFKCANDYLNSPQCQAMVEPVPEGSYLNDIITPLYIYMRDQGYEIINGKYVRRERDHNKIIGYDDIN
P.carinii        394 ECLCFIFKCANDYLNSPQCQAMVEPAPEGSYLNDVITPLYAYMRDQGYEIINGRYVRRERDHNKIIGYDDIN
S.cerevisiae     323 ECLCFIYKCALDYLDSPLCQQRQEPMPEGDFLNRVITPIYHFIRNQVYEIVDGRFVKRERDHNKIVGYDDLN
S.pombe          387 EVIAFLFKCAYDYIISPEAQNVTEPVPEGYYLDNIVSPLYQYMHDQQFEIINGKYVRRERPHDQLIGYDDIN
                     * :.*::*** **: ** .*   ** *** :*: :::*:* ::::* :**::*::*:*** *::::****:*

                     ---------------
P.jirovecii      469 QLFWYSEGIERIVLSDKTRIIDLPPEQRYLRLKDVVWKKVFFKTYRETRSWFHLFTNFNRIWIIHITVYWFY
P.carinii        466 QLFWYPEGIQRIVLSDKTRMVDLPLDQRYPRFKDVVWKKAFFKTYRETRSWFHLFTNFNRIWIIHITVYWFY
S.cerevisiae     395 QLFWYPEGIAKIVLEDGTKLIELPLEERYLRLGDVVWDDVFFKTYKETRTWLHLVTNFNRIWVMHISIFWMY
S.pombe          459 QLFWHAEGIARLIFEDGTRLIDIPASERFHRLPEVQWNRAFYKTYYESRSWFHLITNFNRIWVIHFGMFWYF
                     ****:.*** ::::.* *:::::* .:*: *: :* *. .*:*** *:*:*:**.*******::*: ::* :
                                                                       --------transmembrane-

P.jirovecii      541 TAANSPTVYTHNYQQSLDNQPPFAYRMSAVSFGGGVASLLMIIATLAEWAYVPRKWAGAQHLTRRLLFLILF
P.carinii        538 TAANSPTVYTHNYQQSLDNQPPFAYRMSAVGFGGGVASLLMIVATLAEWAYVPRKWPGAQHLTRRLLFLILF
S.cerevisiae     467 FAYNSPTFYTHNYQQLVDNQPLAAYKWASCALGGTVASLIQIVATLCEWSFVPRKWAGAQHLSRRFWFLCII
S.pombe          531 TAFNSPTLYTKPFHQRDGPKPTGASQWAAVACTSVVSCIIMAAASLCEYLFVPRRFPGSKPIWKRLCIIVLI
                      * ****.**: ::*  . :*  * : :: .  . *:.::   *:*.*: :***::.*:: : :*: :: ::
                     ----------            ------transmembrane------                  -trans-

P.jirovecii      613 LIINVAPGVYVIKFAPWKPKVSVVTTLISIIHFLIAMFTFLFFAIMPLGGLFGNYLYKKTRRYVASQTFTAN
P.carinii        610 FIINVAPGVYVIKFAPWKPNVSIVTTLISIMHFLIAIFTFLFFAIMPLGGLFGNYLYKKTRRYVASQTFTAN
S.cerevisiae     539 FGINLGPIIFVFAYDKDT-VYSTAAHVVAAVMFFVAVATIIFFSIMPLGGLFTSYMKKSTRRYVASQTFTAA
S.pombe          603 AIINLIPIVYIFGFSSKHQQRSGRRIAVGVVAFLMSIATYVYFSLVPLQSTFGKLSVKDSRKYLANKYFTSN
                       **: * :::: :       *     :. : *:::: * ::*:::** . * .   *.:*:*:*.: **:
                     -membrane------       -----------transmembrane-----------
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P.jirovecii      685 FAKLKGNDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIGHCGIRYLGSILCPYQAKITLGIMY
P.carinii        682 FAKLKGNDLWLSYGLWIAVFACKFAESYFFLSLSLRDPIRYLNTMTIGHCGIRYLGSSLCPYQAKITLGIMY
S.cerevisiae     610 FAPLHGLDRWMSYLVWVTVFAAKYSESYYFLVLSLRDPIRILSTTAMRCTGEYWWGAVLCKVQPKIVLGLVI
S.pombe          675 FAPLKFDNQALSVIIWVCVFTCKFAESYFFLTLSIRDPIIVLSTMRPYLCSIYWAGSRLCFVQPRIILGIMY
                     ** *:  :  :*  :*: **:.*::***:** **:****  *.*      .  : *: **  *.:* **::
                              ------transmembrane------                                    --

P.jirovecii      757 ITDLVLFFLDTYLWYIIWNTICSVARSFYLGVSIWTPWRNIFSRMPKRIYSKILATNDMEIKYKPKVLISQ-
P.carinii        754 ITDLVLFFLDTYLWYIIWNTICSVARSFYLGVSIWTPWRNIFSRMPKRIYSKILATNDMEIKYKPKVLISQV
S.cerevisiae     682 ATDFILFFLDTYLWYIIVNTIFSVGKSFYLGISILTPWRNIFTRLPKRIYSKILATTDMEIKYKPKVLISQV
S.pombe          747 FTDLILFFLDTYLWYIIFNTIFSVLRSFVLGISILTPWRNIFSRMPQRIYGKILATNDMEIKYKPKILISQI
                      **::************ *** ** :** **:** *******:*:*:***.*****.*********:****
                     -transmembrane----

                                                                       ------------------
P.jirovecii      828 --------------------------VPSEQEGKRTLRAPTFFISQEDHSFKTEFFPSHSEAERRISFFAQS
P.carinii        826 WNAVVISMYREHLLAIDHVQKLLYHQVPSEQEGKRTLRAPTFFISQEDHSFKTEFFPSHSEAERRISFFAQS
S.cerevisiae     754 WNAIIISMYREHLLAIDHVQKLLYHQVPSEIEGKRTLRAPTFFVSQDDNNFETEFFPRDSEAERRISFFAQS
S.pombe          819 WNAIVISMYREHLLSIDHVQRLLYHQVPAE-EGRRTLRTPTFFVSQDDNIVHTTFFPANSEAERRLSFFAQS
                                               **:* **:****:****:**:*: ..* *** .******:******

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P.jirovecii      874 LSTPIPEPLPVDNMPTFTVLVPHYGEKILYSLREIIREDDQLSRVTLLEYLKQLHPVEWDCFVKDTKILAEE
P.carinii        898 LSTPIPEPLPVDNMPTFTVLVPHYGEKILYSLREIIREDDQLSRVTLLEYLKQLHPVEWDCFVKDTKILAEE
S.cerevisiae     826 LSTPIPEPLPVDNMPTFTVLTPHYAERILLSLREIIREDDQFSRVTLLEYLKQLHPVEWECFVKDTKILAEE
S.pombe          890 LATPIPEPVPVDNMPTFTVLIPHYAEKILLSLREIIREEDQLSRVTLLEYLKQLHPVEWDCFVKDTKILVEE
                     *:******:*********** ***.*:** ********:**:*****************:*********.**

                     ------------------------------------------------------------------------
P.jirovecii      946 TSLYNGGSSFDKDEKDTVKSKIDDLPFYCVGFKSAAPEYTLRTRIWASLRSQTLYRTVSGFMNYSRAIKLLY
P.carinii        970 TSLYNGGVPFDKDEKDTVKSKIDDLPFYCVGFKSSAPEYTLRTRIWASLRSQTLYRTVSGFMNYSRAIKLLY
S.cerevisiae     898 TAAYEGNEN-EAEKEDALKSQIDDLPFYCIGFKSAAPEYTLRTRIWASLRSQTLYRTISGFMNYSRAIKLLY
S.pombe          962 NAPYENDSV--SEKEGTYKSKVDDLPFYCIGFKSAMPEYTLRTRIWASLRSQTLYRTISGFMNYSRAIKLLY
                     .: *:..     :::.: **::*******:****: *********************:**************

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P.jirovecii     1018 RVENPDVVQMFGGNTDKLEHELERMARRKFKFVISMQRFFKFNKEEQENTEFLLRAYPDLQIAYLDEEPPSH
P.carinii       1042 RVENPDVVQMFGGNTDKLEHELERMARRKFKFDISMQRFFKFSKEELENTEFLLRAYPDLQIAYLDEEPPMN
S.cerevisiae     969 RVENPEIVQMFGGNAEGLERELEKMARRKFKFLVSMQRLAKFKPHELENAEFLLRAYPDLQIAYLDEEPPLT
S.pombe         1032 RVENPEIVQMFGGNTDRLERELDRMARRKFKLVVSMQRYAKFTKEEYENAEFLLRAYPDLQIAYLDEDPPEE
                     *****::*******:: **:**::*******: :****  **. .* **:*****************:**

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P.jirovecii     1090 EGDEPKIYSSLIDGYSEIMEDGRRRPKFRIQLSGNPILGDGKSDNQNHAIIFYRGEYIQLIDANQDNYLEEC
P.carinii       1114 EGDEPKIYSSLIDGYSEIMENGKRRPKFRIQLSGNPILGDGKSDNQNHAIIFYRGEYIQLIDANQDNYLEEC
S.cerevisiae    1041 EGEEPRIYSALIDGHCEILDNGRRRPKFRVQLSGNPILGDGKSDNQNHALIFYRGEYIQLIDANQDNYLEEC
S.pombe         1104 EGAEPQLFAALIDGHSEIMENERRRPKYRIRLSGNPILGDGKSDNQNMSLPFYRGEYIQLIDANQDNYLEEC
                     ** **:::::****:.**::: :****:*::**************** :: *********************

                     ---------------------1,3-β glucan synthase domain 2---------------------
P.jirovecii     1162 LKIRSVLAEFEEMSPLEEFPYNPNEN---SKVNNPVAILGAREYIFSENIGVLGDVAAGKEQTFGTLFARTL
P.carinii       1186 LKIRSVLAEFEEMTPTEESPYNPNEI---SSATNPVAILGAREYIFSENIGVLGDVAAGKEQTFGTLFARTL
S.cerevisiae    1113 LKIRSVLAEFEELNVEQVNPYAPGLRYEEQTTNHPVAIVGAREYIFSENSGVLGDVAAGKEQTFGTLFARTL
S.pombe         1176 LKIRSVLAEFEEMETDNVNPYSESAR---ERNKHPVAILGAREYIFSENIGILGDVAAGKEQTFGTLFSRTL
                     ************:   :  **  .     .  .:****:********** *:****************:***

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P.jirovecii     1231 AQIGGKLHYGHPDFLNGPFMTTRGGVSKAQKGLHLNEDIYAGMTALLRGGRIKHCEYYQCGKGRDLGFGSIL
P.carinii       1255 AQIGGKLHYGHPDFLNGPFMTTRGGVSKAQKGLHLNEDIYAGMTALLRGGRIKHCEYYQCGKGRDLGFGSIL
S.cerevisiae    1185 SQIGGKLHYGHPDFINATFMTTRGGVSKAQKGLHLNEDIYAGMNAMLRGGRIKHCEYYQCGKGRDLGFGTIL
S.pombe         1245 AQIGGKLHYGHPDFLNGIFMTTRGGVSKAQKGLHVNEDIYAGMNAMLRGGRIKHCEYFQCGKGRDLGFGSIL
                     :*************:*. ****************:********.*:***********:***********:**

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P.jirovecii     1303 NFTTKVGTGMGEQMLSREYYYLGTQLPLDRFLSFYYAHPGFHINNLFIILSVQLLMIVMINLGSMYNILLIC
P.carinii       1327 NFTTKVGTGMGEQMLSREYYYLGTQLPLDRFLSFYYAHPGFHINNLFIILSVQLLMIVMINLGSMYNILLIC
S.cerevisiae    1257 NFTTKIGAGMGEQMLSREYYYLGTQLPVDRFLTFYYAHPGFHLNNLFIQLSLQMFMLTLVNLSSLAHESIMC
S.pombe         1317 NFNTKVGTGMGEQMLSREYYYLGTQLQLDRFLSFYFAHPGFHLNNMFIMLSVQLFMVVLINLGAIYHVVTVC
                     **.**:*:****************** :****:**:******:**:** **:*::*:.::**.:: :   :*
                                                                  ------transmembrane-----
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                     ------------------------------------------------------------------------
P.jirovecii     1375 KPRRGQPIT--DPFLPVGCYSLAPVLDWIKRSIISIFIVFFIAFIPLVVQELTERGVWRASTRLAKHFGSLS
P.carinii       1399 RPRRGQPIT--DPYLPVGCYSIAPVLDWIKRSIISIFIVFFIAFIPLVVQELTERGVWRASTRLAKHFGSLS
S.cerevisiae    1329 IYDRNKPKT--DVLVPIGCYNFQPAVDWVRRYTLSIFIVFWIAFVPIVVQELIERGLWKATQRFFCHLLSLS
S.pombe         1389 YYNGNQKLSYDTSIVPRGCYQLGPVLSWLKRCVISIFIVFWISFIPLTVHELIERGVWRATKRFFKQIGSFS
                         .:  :     :* ***.: *.:.*::*  :******:*:*:*:.*:** ***:*:*: *:  :: *:*
                                                    ----transmembrane-----

                     ------------------------------------------------------------------------
P.jirovecii     1445 PLFEVFVSQIYANSLLQNLAFGGARYIGTGRGFATTRIPFSILFSRFAGASIYLGSRTLIMLLFATVTMWIP
P.carinii       1469 PLFEVFVSQIYANSLLQNLAFGGARYIGTGRGFATTRIPFSILFSRFAGASIYLGSRTLIMLLFATVTMWIP
S.cerevisiae    1399 PMFEVFAGQIYSSALLSDLAIGGARYISTGRGFATSRIPFSILYSRFAGSAIYMGARSMLMLLFGTVAHWQA
S.pombe         1461 PLFEVFTCQVYSQAITSDLAYGGARYIGTGRGFATARLPFSILYSRFAVPSIYIGARFLMMLLFGTMTVWVA
                     *:****. *:*:.:: .:** ******.*******:*:*****:**** .:**:*:* ::****.*:: * .
                                                                   ----transmembrane----   --

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P.jirovecii     1517 HLVYFWVSVLALCICPFIFNPHQFSWTDFFVDYREFIRWLSRGNSRSHANSWIGYCRLSRTRITGFKRKALG
P.carinii       1541 HLVYFWVSVLALCISPFIFNPHQFSWTDFFVDYREFIRWLSRGNSRSHANSWIGYCRLSRTRITGFKRKALG
S.cerevisiae    1471 PLLWFWASLSSLIFAPFVFNPHQFAWEDFFLDYRDYIRWLSRGNNQYHRNSWIGYVRMSRARITGFKRKLVG
S.pombe         1533 HLIYWWVSIMALCVAPFLFNPHQFDWNDFFVDYREFIRWLSRGNSRSHANSWIGYCRLTRTRITGYKRRVLG
                      *:::*.*: :* ..**:****** * ***:***::********.: * ****** *::*:****:**: :*
                     --transmembrane-----

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P.jirovecii     1589 QPSEKLSGDIPRAGFNNVFFSEVIGPLILVILSLVPFCFMNSRPGFEPFGK--------SNPARNGSNPLIR
P.carinii       1613 QPSEKLSGDIPRAGFSNVFFSEVIGPMILVLLSLVPYCFINSRPGFEPFGK--------SNPAKNGSNPLIR
S.cerevisiae    1543 DESEKAAGDASRAHRTNLIMAEIIPCAIYAAGCFIAFTFINAQTGVKTTD-------------DDRVNSVLR
S.pombe         1605 QPSDKISMDTPRAKFTNVFFSDVLIPALLAAGAIIPYFFINSQPGNPMFITDPNNPSPYVHDTKTGTNPILR
                     : *:* : * .**  .*:::::::   : .  .::.: *:*::.*                      *.::*
                                       -----transmembrane------                           ---

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P.jirovecii     1653 IAIVSFAPICVNALVAFVFFGMACCMGPILTICCKKFGAVLATISHAIAVIVLVAFFEVLWFLEGWSFSKTI
P.carinii       1677 IAIVSFAPICVNAMVAFVFFGMACCMGPILTICCKKFAAVLATISHAIAVIILVTFFEVLWFLEGWSFSKTI
S.cerevisiae    1602 IIICTLAPIAVNLGVLFFCMGMSCCSGPLFGMCCKKTGSVMAGIAHGVAVIVHIAFFIVMWVLESFNFVRML
S.pombe         1677 LVIISLIPIAAGFGMSGFFGGMACCLGPAFGLCCKKFPSIFAAIAHTIQIFIFIAIFEVCWFLDGWSLPKTV
                     : * :: **...  :  .  **:** ** : :****  :::* *:* : ::: :::* * *.*:.:.: : :
                     --transmembrane----------           -------transmembrane-------        -

P.jirovecii     1725 LGLVTMISLQRAFLKMLTIMILTREFKHDGSNLAWWTGRWYSNNLGVHAMSQPAREFVCKVIELSLFAADFC
P.carinii       1749 LGLVTMISLQRAFLKILTIMILTREFKHDGSNLAWWTGRWYSNNLGVYAMSQPAREFVCKVIELSLFAADFC
S.cerevisiae    1674 IGVVTCIQCQRLIFHCMTALMLTREFKNDHANTAFWTGKWYGKGMGYMAWTQPSRELTAKVIELSEFAADFV
S.pombe         1749 LAFCAVTAIHRFIFKILTLLCLSREVKQDSANISWWSGKWYGKGYGYHAFTLPAREFVCKAIELNLFATDFF
                     :.. :    :* ::: :* : *:**.*:* :* ::*:*:**.:. *  * : *:**:..*.***. **:**
                     -transmembrane--------                                         ---trans-

P.jirovecii     1797 LGHLLLFILTPILAIPYIDRWHSMLLFWLRPSRQIRPPIFSLKQNKLRKRIVRRYATLFFGLFLLFLMIILV
P.carinii       1821 LGHLLLFILTPILAIPYIDRWHSMLLFWLRPSRQIRPPIFSLKQNKLRKRIVRRYATLFFGLFLLFLMIILV
S.cerevisiae    1746 LGHVILICQLPLIIIPKIDKFHSIMLFWLKPSRQIRPPIYSLKQTRLRKRMVKKYCSLYFLVLAIFAGCIIG
S.pombe         1821 LGHLLLFFMLPVICIPYIDRWHSVLLFWLRPSRQIRPPIFSTKQNRLRKRIVRRYSALYFSILVIFLILIIV
                     ***::*:   *:: ** **::**::****:*********:* **.:****:*::*.:*:* :: :*   *:
                     -membrane----------                                   ----transmembrane-

P.jirovecii     1869 PAVGHSKFPKSLNNIPALKNLGLIQPSNDPRGATG---RTTRPANSNGTYKM--F-----------T
P.carinii       1893 PALGHSKFPKSLNNIAFLKNLGLIQPSNDPRGATG---RTTRPGNSNGTYKL--FI----------Y
S.cerevisiae    1818 PAVASAKIHKHIGDSLDGVVHNLFQPINTTNNDTGSQMSTYQSHYYTHTPSLKTWSTIK--------
S.pombe         1893 PLAAGAEIRQGLTASEAVAKG-AV-GWNQTNSSIGSGIIQPRDTNYTANYSF--WYDRYHFEFNTTY
                     *  . ::: : :           .   * ...  *      :    . . .:  :
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B

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P.jirovecii        1 MK-----KSHWSNDTPV-------------DYSPQNSGNNWNV--RSS-GTVHR--PLPPTPCHFETQYQE-
P.carinii          1 MT-----RNHWFNEGFP-------------NYHPPNYEENWNV--QTP--RVHR--PLPPIPCHLETQYQE-
S.cerevisiae       1 MPLRNLTETHNF-----------------SS-------TNLDT--DGT-GDDHDGAPLSSSPS-FGQQNDNS
S.pombe            1 ME-----KGHSDLPRQPERVAQNPFLTFDQDSFPSSYGSSLNVSEQTSGSSSTS--PLPQISCLLRKDD---
                     *      . *                    .        . :.    .        **.  .. :  :

P.jirovecii       49 --------------------SYGDAVWNSQQ--SPYNQSYYLFQDENVSFPRRRIVSHGDYSEDEPAYQTKQ
P.carinii         48 --------------------QCENIVLNQQQQQHLYDKNYRRFRNEDVSFPRRRIVSHGDYSEDEVVHQKQE
S.cerevisiae      45 TNDNAGLTNPFMGSDEESNAR----------------------------------DGESLSSSVHYQPQG
S.pombe           63 ------------------------------------------------------------------------
cons              73

P.jirovecii       99 ENV-EYYD-NSFSSQSPRNVYTDGYKAYNDIQDYASLDYKRKSYMNYPEEPDNNYWHEPQESVYT-------
P.carinii        100 ESA-NHCDYNSFVPHTSRNMSGDIYDDYSDI-DYAILDHKRKSYMNYTQELNNDYYEAE-----R-------
S.cerevisiae      81 SDSSLLHD-NSRLDLSQ-NKGVSDYKGYY----------SRNNSRAVSTANDNSFLQPPHRAIASSPSLNSN
S.pombe           63 -----------------------------------------------------------------VPLANKE

P.jirovecii      162 ------------EEYIEPESRKTNKGSFNTYKNTAKSDV------------------G--------NNLDTL
P.carinii        158 ------------NEYKESKFEKADKISLNAQNGEIKRDI------------------I--------SNLDIM
S.cerevisiae     141 LSKNDILSPPEFDRYPLVGSRVTSMTQLNHHGRSPTSSPGNESSASFSSNPFLGEQDFSPFGGYPASSF--P
S.pombe           70 LSRS-LIHVQELSRYPPF------YNQDHQHLGVPRSRVGSDV-WKMREKSFLSPSQF--------SSIDLS
                                 ..*           . :                                     ..:

                                                           --------transmembrane--------
P.jirovecii      196 WDPTVTEPDDYLHNPTFKDRK--------KDYYFFTKRGIFNIGSLVFLILGVMFVFIGYPIMLYIRRAYDD
P.carinii        192 WNPNIVEPDDYLHNPATKERK--------KDYHIFTKRGILNMGSLVILTFGIISFFMGYPILLYTKKIYED
S.cerevisiae     211 LMIDEKEEDDYLHNPDPEEEARLDRRRFIDDFKYMDKRSASGLAGVLLLFLAAIFIFIVLPALTFTGAIDHE
S.pombe          126 WVYRSKEEDDDFHDPKSSVVSL-MG---EEDYLGWSR--FCDLFFLFVLSLGIGLLFIVFPALTFTGNITPS
                           * ** :*:*  .           .*:    :    .:  :..* :.   .*:  * : :      .

                                                                     ------------------------
P.jirovecii      260 AHSCPN-----CIRTLPIDLLDATRSLIDPDTPLEFYERKSKDGKIYKIVFSDEFNKNGRTFYPGDDQFWEA
P.carinii        256 IHRCPD-----CTKTLPIDLLNATRGLIDPDTPEEFYELKNKDGKIYKLVFSDEFNKNGRTFYPGDDQFWEA
S.cerevisiae     283 SNT-EE--VTYLTQYQYPQLSAIRTSLVDPDTPDTAKTREAMDGSKWELVFSDEFNAEGRTFYDGDDPYWTA
S.pombe          192 KEKFDAIMANQITDHLFAHMRVPRTNLIDKDTPSTAYHRTGYNGRKYNLVFSDEFNKEGRSFYSGNDQFWEA
                      .                .:     .*:* ***         :*  :::******* :**:** *:* :* *

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P.jirovecii      327 VDLHYWSTMSIEWYDPDAITTNGGFLEIRLDAFRNHDLNYRSGMLQSWNKLCFKGGIIEASISLPGRGDISG
P.carinii        323 VDLHYWSTLSLEWYDPDAITTNDGFLEIRLDSFRNHDLNYRSGMLQSWNKLCLKGGIIEASISLPGRGDTSG
S.cerevisiae     352 PDVHYDATKDLEWYSPDASTTVNGTLQLRMDAFKNHGLYYRSGMLQSWNKVCFTQGALEISANLPNYGRVSG
S.pombe          264 VNIHYAATNDLDWYDPDAITTVNGTLAIQLDAFWNRDLNFRSGMLQSWNKLCLKGGIIEVSASLAGSGEHAG
                      ::** :* .::**.*** ** .* * :::*:* *:.* :**********:*:. * :* * .*.. *  :*

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P.jirovecii      399 FWPAFWAMGNLGRPGFGASTDGVWPYSYDTCDVGITPNQSDSNGISSLPGMRFPNCVCPNSDHPSPGKGRGA
P.carinii        395 LWPAFWMMGNLGRPGFGASTEGTWPYSYDNCDIGITPNQSDHTGLSFLPGMKLPGCTCPNSDHPSPGKGRGA
S.cerevisiae     424 LWPGLWTMGNLGRPGYLASTQGVWPYSYESCDAGITPNQSSPDGISYLPGQKLSICTCDGEDHPNQGVGRGA
S.pombe          336 LWPGIWTLGNLARPGYMATTDGVWPYAYSQCDVGITPNQSSYDGISYLPGQKLPNCVCLNEDHPSPGVGRGA
                     :**.:* :***.***: *:*:*.***:*. ** *******.  *:* *** ::. *.* ..***. * ****

                     ------ GH16 fungal kre6 glucanase---------------------------------------
P.jirovecii      471 PEIDIIEASV-----DLSFRLGEASQSVQFAPFDDLYTPNYEHMKIYNKEKTHINNYRGNSFQQTFSCITYL
P.carinii        467 PEIDIIEASV-----DLSLHIGEASQSVQFAPFDIQHKPNYDHMIIYNAEKTHINPYHGNVFQQTFSCITYL
S.cerevisiae     496 PEIDVLEGET-----DTKIGVGIASQSLQIAPFDIWYMPDYDFIEVYNFTTTTMNTYAGGPFQQAVSAVSTL
S.pombe          408 PEIDILEGSTEKLHPDDELDIGVVSQSGQFAPFDFFWLPNYDYLAVYNDSITHMNSYVGGPFQQALSGITTL
                     ****::*...     * .: :* .*** *:****    *:*:.: :**   * :* * *. ***:.* :: *

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P.jirovecii      538 NNEWYD----GRKFQTYSLEYEPG--KNGFIQWYIGDNPTWMMKAESVGPNGKIGQRLISEEPMAFVINLAM
P.carinii        534 NNEWYN----GYKFQTYGLEYRPG--RKGYIEWFIGDQTTWKMKSESVGPNGNIGQRLISEEPMAIIINLAL
S.cerevisiae     563 NVTWYEFGEYGGYFQKYAIEYLND-DDNGYIRWFVGDTPTYTIHAKALHPDGNIGWRRISKEPMSIILNLGI
S.pombe          480 NNTWYG----GNAFQIYGFDYKPGEGTNGYVSWFVGPNYTWSMLGSAVGQNGNVGPRQISEEPMSIIFNLGI
                     *  **     *  ** *.::*  .   :*:: *::*   *: : ..::  :*::* * **:***::::**.:
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P.jirovecii      604 SESFAKIEWGRLQFPAIMRVDWVRIYQET----PMITCDPPGYPTTKYIKEHPIAYYNNNITTWENTGYQWP
P.carinii        600 SETFSKIEWEKLQFPAIMRIDWVRIYQEE----SLITCDPPGYPTTNYIKEHPIAYYNNNVTTWNNTGYEWP
S.cerevisiae     634 SNNWAYIDWQYIFFPVVMSIDYVRIYQPSN--AISVTCDPSDYPTYDYIQSHLNAFQNANLTTWEDAGYTFP
S.pombe          548 SNNWAYYYFRDLSFPAVMYIDYIRIYQDPDDTNSHIGCDPPGYPTTKYIEEHPLAYKNPNATTWEMAGYTWP
                     *:.::   :  : **.:* :*::****        : ***..*** .**:.*  *: * * ***: :** :*

                     coiled coil
P.jirovecii      672 KNRLMNEC---------
P.carinii        668 KNRLMNKC---------
S.cerevisiae     704 KNILTGKCTSSKFKLSS
S.pombe          620 KNSLMHKCNT-------
                     ** *  :*
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Fig. S2.