METHODS

Strain/plasmid construction. The parental strains for SC5314-derived mutants were based on auxotrophic strains described previously[10]. Gene deletions were constructed using the fusion PCR or *SAT1* flipper methods described previously[27,28]. All strains are listed in Supplementary Table 1. The *bar1* mutant strains CAY320/321 were generated as described previously[10] except an *ARG4* marker was used in place of the *HIS1* marker. To delete *STE2*, oligonucleotides 1/2 were used to amplify the 5′ flank and oligonucleotides 3/4 to amplify the 3′ flank of the gene from SC5314. Oligonucleotides 1/4 were then used to generate a disruption cassette containing either the *LEU2* or *HIS1* selectable marker[27]. The cassette was transformed into RBY1118 and integration checked using oligonucleotides 5 or 6 together with primers internal to the marker, and loss of the ORF was confirmed by PCR using oligonucleotides 7/8, resulting in strains RBY1107 and RBY1108. A similar method was used to delete *STE3* and *MFα* genes. For *STE3*, oligonucleotides 9/10 and 11/12 were used to amplify the 5′ and 3′ flanks, respectively, whereas for *MFα*, oligonucleotides 17/18 and 19/20 were used to amplify the flanking regions. Disruption cassettes were then generated using oligonucleotides 9/12 and 17/20 to amplify the selective marker together with flanking gene sequences. Correct integration of the cassette at *STE3* was confirmed using flanking primers 13/14 and loss of the ORF using 15/16, resulting in strains RBY1113 and RBY1114. For *MFα*, integration was checked using oligonucleotides 21/22 and deletion of the ORF was confirmed using oligonucleotides 23/24 resulting in strains RBY1081 and RBY1082. To generate the plasmid pDS1 for deletion of *BAR1*, oligonucleotides 25/26 were used to generate a 5′ flank that was digested with ApaI and XhoI and ligated into pSFS2a. Subsequently, oligonucleotides 27/28 were used to generate a 3′ flank that was digested with SacI and SacII and ligated into the vector to generate plasmid pDS1. ApaI/SacI-digested pDS1 was then used to transform strains RBY1107, RBY1113 and RBY1081, to generate *bar1* mutant strains DSY760/761, DSY792/793 and DSY762/768, respectively. Integration was checked using oligonucleotides 29/30 paired with oligonucleotides internal to pSFS2a, and loss of the ORF was confirmed using oligonucleotides 31/32. To generate plasmids for integration of GFP under the control of a test promoter, GFP was amplified from plasmid pNIM1[31] using oligonucleotides 33/34, cut with ApaI and SalI and ligated into an ApaI/XhoI-digested pSFS2a, generating pRS2. The promoters of *FUS1* and *FIG1* were then amplified using oligonucleotides 35/36 and 37/38, respectively. The PCR products were digested with KpnI/ApaI and ligated into pRS2 to generate pDS2 and pDS3 respectively. The plasmid pDS2 was linearized with AflII and transformed into RBY1117 and RBY1197 and integration checked using oligonucleotides 39/40 to generate DSY702 and DSY690, respectively. The plasmid pDS3 was linearized with AflII and transformed into RBY1117 and RBY1220, and integration checked using oligonucleotides 40/41 to generate DSY700 and DSY696, respectively. To generate the mating tester strains DSY170/171, RBY1118 was transformed with a fragment containing *SAT1* from the plasmid pNIM1. This fragment targets an intergenic region of chromosome 5 near *MTL* and was created using oligonucleotides 42/43. To generate the strain CAY371, a *WOR1–YFP* fusion construct (white–opaque regulator 1–yellow fluorescent protein) was transformed into CAY320 (ref. 32). To generate fluorescently labelled nuclei, strains RBY1197 and DSY168 were transformed with *HTB–RFP* and *HTB–YFP* constructs as described previously[21,33] to generate strains DSY906 and DSY908, respectively. Select clinical isolates were also obtained from D. MacCallum of the Aberdeen Fungal group and grown on sorbose media to generate homozygous **a** and α derivatives, as described previously[34].

Clinical mating crosses. All clinical isolates were crossed with the SC5314 Δ*bar1* **a** strain, CAY371. Clinical α isolates correspond as follows: 1, AM2003/0165; 2, L1086 derivative (DSY159); 3, YSU751 derivative (DSY161); 4, Hun92 derivative (DSY165); 5, IHEM16614 derivative (DSY183); 6, 19F; 7, 78048; 8, J981315 derivative (DSY167); CAY371 with WO-1 is not shown . Clinical **a** isolates correspond as follows: 9, L1086 derivative (DSY164); 10, P37005; 11, 12C; 12, L26; 13, AM2003/0191 derivative (DSY162); 14, IHEM16614 derivative (DSY168); 15, T101; 16, J981315 derivative (DSY194); 17, RIH09; 18, AM2005/0377.

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