Table S1 Primers

Name	Oligonucleotide Sequence (5' to 3')
M13pr	AATTAACCCTCACTAAAGGGAACAAAAGCTGG
Sacpf1	ACTGGAGCTCATTTTATGATGGAATGAATGGG
*MTL***a**1-F [a]	TTGAAGCGTGAGAGGCTAGGAG
*MTL***a**1-R [a]	GTTTGGGTTCCTTCTTTCTCATTC
MTLα1-F [a]	TTCGAGTACATTCTGGTCGCG
*MTL*α1-R [a]	TGTAAACATCCTCAATTGTACCCGA
ENOpf	GGGATCAAGATTTGTTTACAG
tetRWH11pr	CCATGGTGAGACGCGACAGA
TRpf	CTCGAGCCCGGGTGGACTTCTTCG
TRpr	CTGCAGGTCGACTTTTCTGA G
IMH3pfatg	ATGGTGTTTGAAACTTCAAAAG
IMH3pr	CTCGAGTCTAGAACTCAGTATATCTT CA
TRpIMHp	ATCTCAGAAAAGTCGACCTGCAGATGGTGT TTGAAACTTC AAAAG
TS1pf	GCGGCCGCCCCGGGTCCTTTCTTACTAAAATATAGA
TS1pr	GCGGCCGCGATCTTACACACACAATCAG
TS2pf	AAGCTTAGAAGGAAAGAAGGAAAGAA
TS2pr	AAGCTTCCCGGGCCTTATCATCATGATCACCA
pNZ11pf	CGAATTGGAGCTCATTTTATG
CaNATpr	GGACATGGCATAGACATATAC
pACTFpf	TGCCTCTTACCAACTATTTCA
TS1pr-p	GCGGCCGCGATCTTACACA
TS1Fpf	CACTACTACTACCTACTACTAC
TRsepr	GGTGTGGTCAATAAGAGCGA
TS2Fpr	TACCTATGCACTACTACTACTC
CaACTpr	CTAAAACATACCACCGTCCA
pENOpr	ATCGTTAGTCAACTTTTGCAAC
SSR1-2 [b]	CTAGTTCAGCCAAGGCTTCTTC

Continued on next page

Table S1, continued

Name	Oligonucleotide Sequence (5' to 3')
SSR1-3 [b]	AGAAGAAGCCTTGGCTGAAC
MCEA1outpf2 [c]	TGGGTCATGTGGTATTGGAG
MCEA1outpr2 [c]	ATGGCCGGATGTTTCCAGAA
MC0repf [c]	AACCATCATGACGATCACCA
MC0repr [c]	GATAAATCTCATCTGCAGGC
tetRpf	ATGTCTAGAT TAGATAAAAG TAAA
tetRpr2	AGTCGTCATCAGTACCGGC
TS1se	CTCTCTCCTT TTGTTACGAG
CaNATpf	CTACTACTTTGGATGATACTG

[a] designed by M. Legrand et al. (Legrand *et al.* 2004)

[b] designed by Z. Zhou (Zhou 2010).

[c] designed by N. Zhang et al. (Zhang *et al.* 2009)

Table S2 Parental strains and fusants detected in rat co-colonization model

Mating	Inoculum		Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Fusants Detected [a,b]			
OD8916α x														t = 28
W43**a**			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	d
	white	Rat1	2.7E+03	3.0E+02	8.4E+03	0.0E+00	1.4E+04	0.0E+00	4.3E+03	0.0E+00	70	10	0	0
		Rat2	2.2E+03	2.5E+02	1.1E+04	0.0E+00	1.1E+04	5.8E+02	1.7E+03	0.0E+00	80	20	0	0
		Rat3	2.9E+01	1.5E+00	1.5E+04	7.7E+02	1.8E+04	0.0E+00	4.5E+03	0.0E+00	0	0	0	0
														t = 28
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	d
	white	Rat1	3.7E+02	3.0E+02	6.6E+02	4.4E+02	3.2E+03	3.2E+03	2.1E+02	4.1E+03	10	10	0	0
		Rat2	3.8E+02	4.6E+02	2.4E+02	2.0E+02	1.8E+03	2.8E+03	5.6E+02	5.1E+03	0	0	0	0
		Rat3	3.0E+02	2.0E+02	6.7E+02	1.0E+03	3.3E+03	4.9E+03	3.4E+02	6.5E+03	30	0	0	0
														t = 28
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	d
	opaque	Rat1	3.7E+02	3.0E+02	6.6E+02	4.4E+02	1.2E+03	2.2E+03	1.2E+02	2.3E+03	129	104	30	0
		Rat2	3.8E+02	4.6E+02	2.4E+02	2.0E+02	5.8E+02	8.7E+02	6.3E+02	5.6E+03	82	0	0	0
		Rat3	3.0E+02	2.0E+02	6.7E+02	1.0E+03	1.2E+03	1.8E+03	1.3E+02	2.5E+03	110	50	0	0

Continued on next page

Table S2, continued

Mating	Inoculum		Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Fusants Detected [a,b]			
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	white	Rat1	1.3E+03	8.7E+02	3.7E+03	0.0E+00	1.1E+03	0.0E+00	5.7E+02	0.0E+00	30	30	0	0
		Rat2	2.4E+02	2.0E+02	2.8E+03	3.1E+02	1.0E+03	0.0E+00	3.6E+02	0.0E+00	10	50	10	0
		Rat3	1.1E+03	2.8E+02	4.0E+03	0.0E+00	9.9E+02	0.0E+00	5.0E+02	0.0E+00	30	0	0	0
W17α x W43**a**			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	white	Rat1	3.4E+02	2.8E+02	1.5E+03	0.0E+00	5.0E+03	2.7E+02	4.1E+03	2.2E+02	0	0	0	0
		Rat2	0.0E+00	0.0E+00	1.0E+01	0.0E+00	1.3E+03	6.8E+01	4.5E+03	2.4E+02	0	0	0	0
		Rat3	5.8E+02	3.8E+02	5.1E+02	2.7E+01	2.9E+03	0.0E+00	7.8E+03	0.0E+00	40	0	0	0
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	opaque	Rat1	3.4E+02	2.8E+02	1.5E+03	0.0E+00	1.1E+03	5.6E+01	9.5E+02	5.0E+01	227	0	0	0
		Rat2	0.0E+00	0.0E+00	2.0E+02	0.0E+00	8.4E+02	4.4E+01	7.8E+02	4.1E+01	0	0	0	0
		Rat3	6.5E+02	4.3E+02	5.1E+02	2.7E+01	4.0E+03	0.0E+00	1.0E+03	0.0E+00	70	0	0	0

Continued on next page

Table S2, continued

Mating	Inoculum		Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Fusants Detected [a,b]			
YSU63α x														t = 28
W43**a**			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	d
	white	Rat1	5.0E+03	1.3E+03	2.9E+03	3.2E+02	1.3E+03	6.9E+01	3.8E+03	0.0E+00	0	0	0	0
		Rat2	8.1E+03	9.0E+02	1.8E+03	0.0E+00	4.0E+03	0.0E+00	3.4E+03	0.0E+00	0	0	0	0
		Rat3	1.2E+03	6.5E+01	1.4E+03	1.6E+02	2.7E+03	0.0E+00	3.9E+03	0.0E+00	0	0	0	0
														t = 28
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	d
	white	Rat1	9.1E+02	1.6E+02	6.5E+01	0.0E+00	2.0E+03	1.0E+02	2.3E+03	0.0E+00	0	0	0	0
		Rat2	4.3E+02	1.1E+02	3.2E+01	7.0E+00	7.6E+03	4.0E+02	2.6E+02	0.0E+00	0	0	0	0
		Rat3	1.4E+03	3.5E+02	3.9E+02	0.0E+00	2.7E+03	0.0E+00	2.8E+02	0.0E+00	0	0	0	0
														t = 28
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	d
	opaque	Rat1	3.9E+03	3.2E+03	3.6E+03	2.4E+03	1.3E+03	2.4E+03	2.4E+03	4.5E+04	0	0	0	0
		Rat2	3.2E+03	4.0E+03	4.0E+03	3.3E+03	3.6E+03	5.4E+03	3.2E+04	2.9E+05	0	0	0	0
		Rat3	4.7E+03	3.1E+03	4.3E+02	6.4E+02	3.0E+03	4.4E+03	1.1E+04	2.1E+05	0	0	0	0

Continued on next page

Table S2, continued

Mating	Inoculum		Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Fusants Detected [a,b]			
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	opaque [c]	Rat1	6.1E+02	1.1E+03	7.3E+02	1.1E+03	1.1E+03	4.5E+02	1.2E+03	1.3E+02	0	0	0	0
		Rat2	7.5E+02	1.4E+03	8.8E+02	8.8E+02	8.2E+02	2.1E+02	1.4E+03	7.6E+01	0	0	0	0
		Rat3	4.8E+02	1.1E+03	7.0E+02	8.5E+02	8.4E+02	2.1E+02	9.7E+02	1.1E+02	0	0	0	0
HUN97α x Au90**a**			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	white	Rat1	0.0E+00	7.3E+03	0.0E+00	2.1E+04	1.8E+02	3.5E+03	3.6E+02	6.9E+03	0	0	0	0
		Rat2	1.9E+03	1.1E+04	0.0E+00	4.8E+02	3.2E+02	6.1E+03	3.7E+01	7.0E+02	0	0	0	0
		Rat3	1.1E+03	9.8E+03	4.4E+02	8.4E+03	0.0E+00	5.8E+02	0.0E+00	5.8E+02	0	0	0	0
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	opaque	Rat1	3.7E+02	4.5E+02	0.0E+00	2.2E+03	8.4E+01	1.6E+03	4.6E+01	8.8E+02	0	0	0	0
		Rat2	5.9E+02	5.9E+02	0.0E+00	3.2E+03	9.8E+01	1.9E+03	1.9E+01	3.5E+02	0	0	0	0
		Rat3	5.0E+02	7.5E+02	1.6E+02	3.1E+03	0.0E+00	2.2E+03	0.0E+00	4.1E+03	0	0	0	0

Continued on next page

Table S2, continued

Mating	Inoculum		Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Fusants Detected [a,b]			
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	opaque [d]	Rat1	6.4E+02	1.9E+03	5.5E+02	1.3E+03	7.0E+02	8.5E+02	9.0E+02	3.9E+02	0	0	0	0
		Rat2	2.2E+02	6.0E+02	7.3E+02	1.1E+03	3.9E+02	5.8E+02	1.1E+03	3.7E+02	0	0	0	0
		Rat3	3.7E+02	1.0E+03	4.6E+02	1.0E+03	4.2E+02	6.0E+02	8.7E+02	9.6E+01	0	0	0	0
FJ11α x Au90**a**			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	white	Rat1	1.3E+03	1.5E+02	1.7E+03	0.0E+00	2.6E+03	0.0E+00	2.6E+03	0.0E+00	0	0	0	0
		Rat2	3.1E+03	3.4E+02	3.3E+04	0.0E+00	2.3E+03	1.2E+02	2.4E+03	0.0E+00	0	0	0	0
		Rat3	2.8E+02	1.5E+01	1.9E+03	1.0E+02	3.1E+03	0.0E+00	2.8E+03	0.0E+00	0	0	0	0
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	opaque	Rat1	2.3E+03	4.0E+02	4.9E+03	0.0E+00	3.3E+03	1.7E+02	9.9E+02	0.0E+00	0	0	0	0
		Rat2	2.5E+03	6.3E+02	2.0E+03	2.2E+02	2.1E+03	1.1E+02	5.1E+03	0.0E+00	0	0	0	0
		Rat3	3.0E+03	7.4E+02	2.7E+03	0.0E+00	1.1E+03	0.0E+00	7.6E+02	0.0E+00	0	0	0	0

Continued on next page

Table S2, continued

Mating	Inoculum		Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Parent 1	Parent 2	Fusants Detected [a,b]			
FJ11α x W43**a**			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	white	Rat1	4.0E+02	4.4E+01	4.4E+02	0.0E+00	9.9E+03	0.0E+00	1.0E+04	0.0E+00	0	0	0	0
		Rat2	2.1E+02	2.3E+01	2.3E+02	0.0E+00	8.8E+03	4.6E+02	1.1E+03	0.0E+00	0	0	0	0
		Rat3	8.3E+01	4.4E+00	8.3E+01	4.4E+00	4.8E+03	0.0E+00	9.5E+03	0.0E+00	0	0	0	0
			t = 7 d		t = 14 d		t = 21 d		t = 28 d		t = 7 d	t = 14 d	t = 21 d	t = 28 d
	opaque	Rat1	1.3E+03	3.3E+02	4.7E+03	0.0E+00	2.2E+03	2.5E+02	1.7E+03	0.0E+00	0	0	0	0
		Rat2	1.5E+03	3.6E+02	3.2E+03	3.5E+02	1.5E+03	8.0E+01	3.1E+03	0.0E+00	0	0	0	0
		Rat3	3.3E+03	8.3E+02	5.4E+03	0.0E+00	3.2E+03	0.0E+00	2.7E+03	0.0E+00	0	0	0	0

[a] No fusants were detected when any of the inocula were plated out at t = 0 d

[b] Identification of fusants was not significantly affected by marker loss. When groups of three rats were inoculated with either an OD8916α x W43**a** or a W17α x W43**a** fusant, and cells were recovered from the rats over a period of four weeks and checked for the presence of *MTL***a**, *MTL*□, *NAT*^{r} or *MPA*^{r} markers by PCR, only one out of 469 cells tested had lost one marker (*MPA*^{r})

[c] ratio of YSU63α:W43**a** in initial inoculum was 1:2

[d] ratio of HUN97α:Au90a in initial inoculum was 2:1

Table S3 Effect on growth rate of marking parental *MTL*-homozygotes with resistance cassettes

strain	Growth rate of strain with resistance cassette relative to that of the unmarked ancestral strain[a,] (actual rate in doublings /h) YPD	Harsh conditions [b]
Au35**a**	0.988 (1.019)	1.006 (0.551)
Au7**a**	1.001 (1.065)	n/a
Au90**a**	0.969 (0.932)	0.939 (0.601)
RIHO11**a**	0.819 (0.963)	n/a
W43**a**	0.949 (0.904)	1.001 (0.606)
HUN97α	0.999 (1.098)	0.971 (0.587)
OD8916α	0.997 (1.014)	0.994 (0.562)
W17α	0.612 (0.685)	1[c] (0.000)
Ysu63α	1.000 (1.063)	0.964 (0.583)

[a] Calculated by dividing the average growth rates after ≥ 100 generations of serial propagation of strains with resistance cassettes by those of their unmarked *MTL*- homozygous ancestors.

[b] Rates were only determined for those strains that produced progeny that were tested under harsh conditions

[c] NeitherW17α, nor its marked version W17α –pNZ11 grew under the harsh conditions.

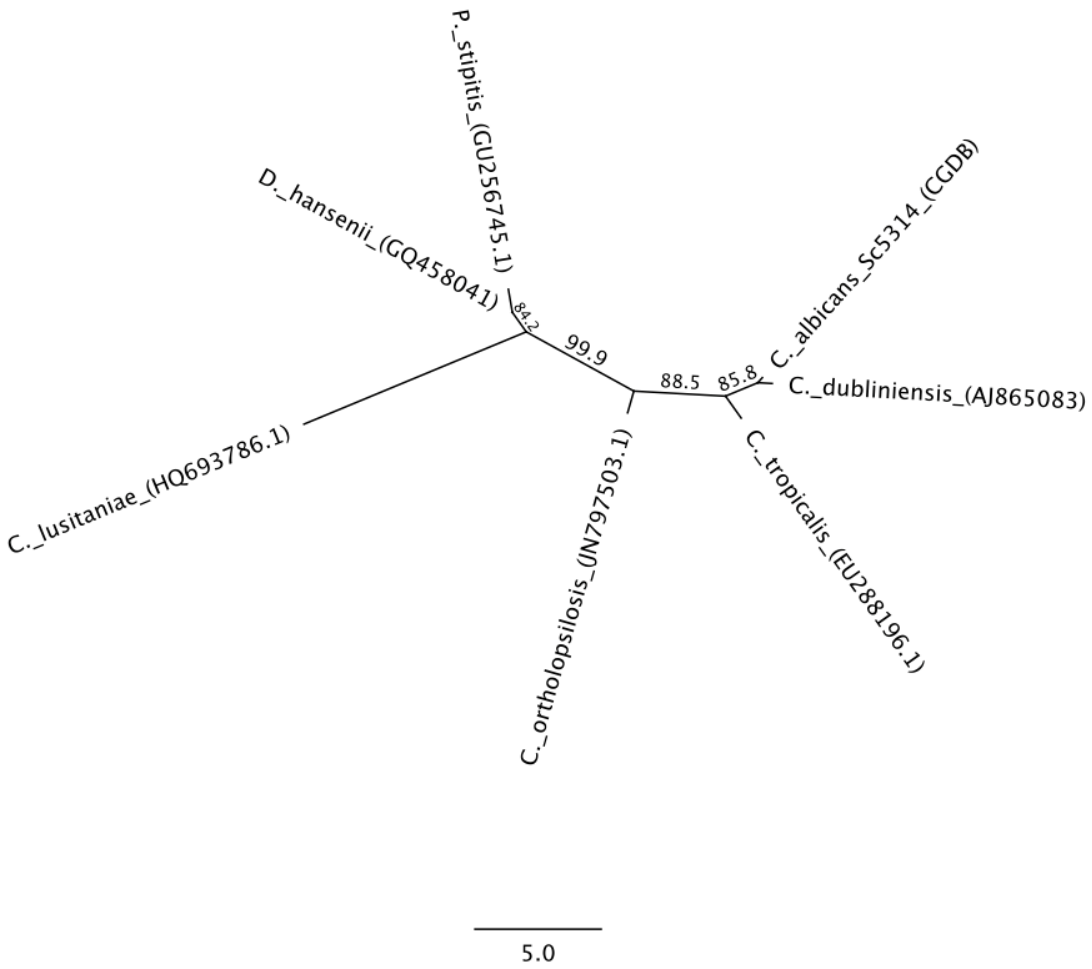

Figure S1 Neighbor-joining consensus tree showing ITS1-based phylogenetic relationships between the species used in d_n/d_s analysis. Percentages of consensus support values of nodes below 100% are shown. The bar indicates 5 substitutions.

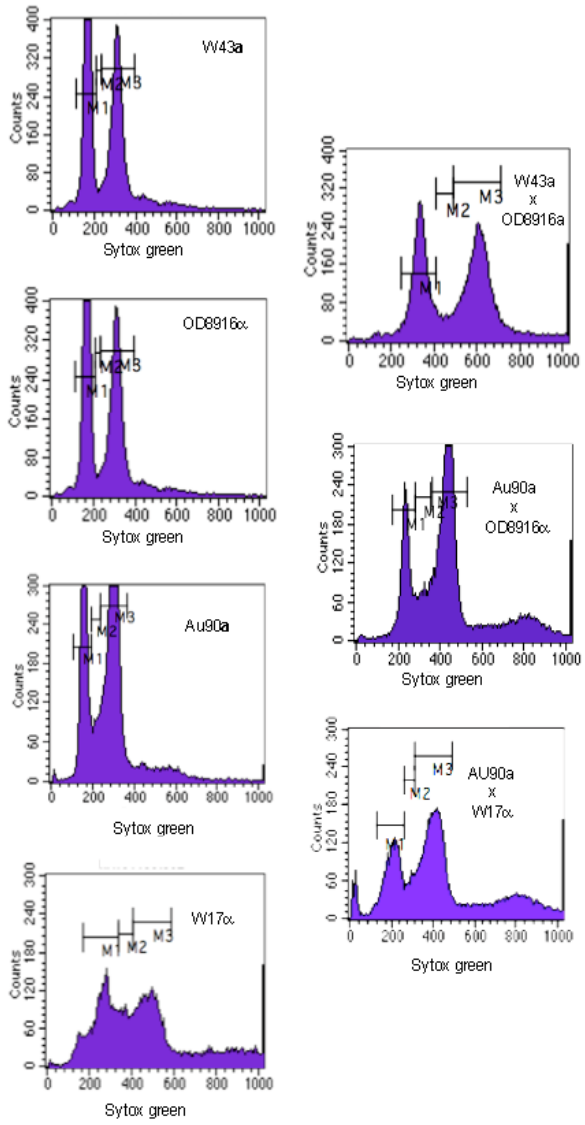

Figure S2 Examples of flow cytometry analyses by SYTOX staining of fusants and parents. Four parents, W43a, OD8916α, AU90a, W17α, and their fusants W43a x OD8916α, AU90a x OD8916α and AU90a x W17α are shown. M1 and M3 are peaks in the DNA content distribution corresponding to exponentially growing cells in their G1 and G2 phases, respectively. M2 corresponds to S phase cells. The peak regions were selected visually based on the shape of the histogram.

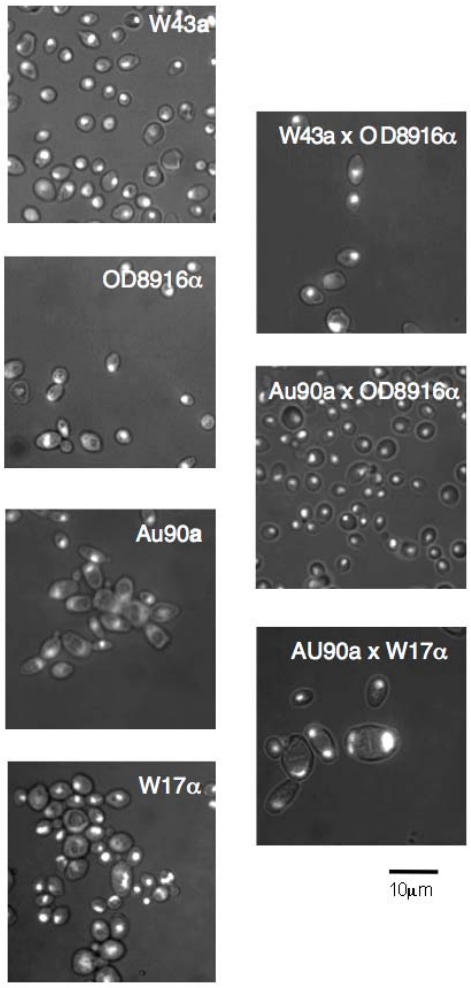

Figure S3 Examples of nuclear staining (DAPI) of parents and fusants. Four parents, W43a, OD8916α, AU90a, W17α, and their fusants W43a x OD8916α, AU90a x OD8916α and AU90a x W17α are shown. The photographic images were generated by combining fluorescent images and bright field images.

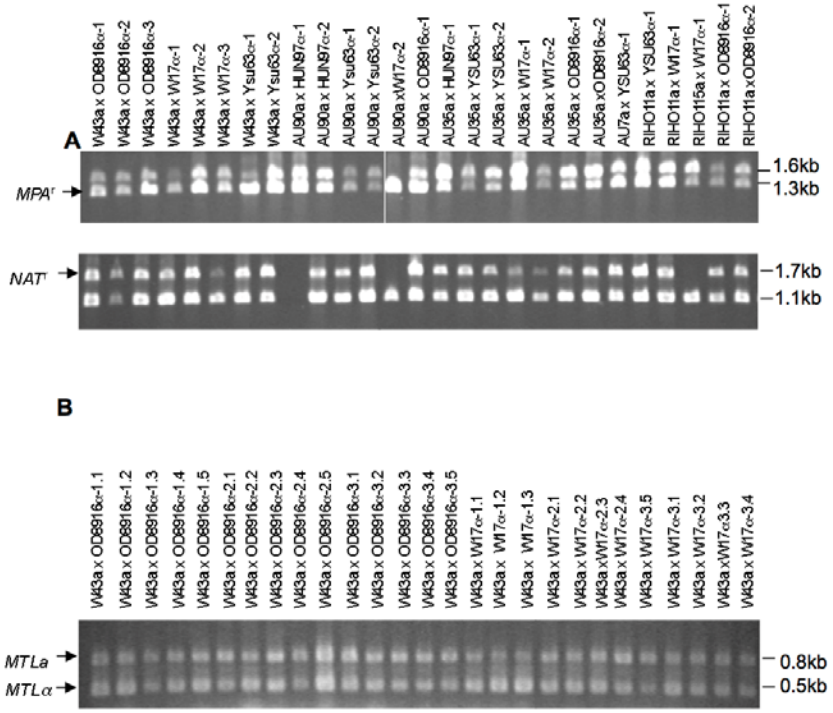

Figure S4 Retention of resistance cassettes and *MTL* alleles in serially propagated fusants. (A) Representative \fusants of each successful mating were serially transferred in YPD medium for 100 generations and the presence of the MPA and NAT resistance markers was investigated in a single colony of each fusant. Three-primer PCRs were used (primers TS2Fpr/pENOpr/TS1se for assays of the *MPA*^{r} cassette and primers pACTFpf/CaACTpr/CaNATpr for assays of the *NAT*^{r} cassettes), to distinguish between the insertion site with a cassette (1.3 kb and 1.7 kb products for the *MPA*^{r} and *NAT*^{r} cassette respectively; marked with arrows), and the insertion site without cassettes (1.6 kb and 1.1 kb, respectively). In 27/27 fusants tested the *MPA*^{r} cassettes were retained and in 24/26 fusants tested the *NAT*^{r} cassettes were still present. (B) In 27/27 fusants tested after serial propagation for 100 generations, both mating type loci (0.8 and 0.5 kb as indicated on the right and marked with arrows on the left), were also still present. The offspring from six fusants of two matings were streaked and 3 to 5 single colonies were tested from each fusant. The names above the figure show the parents, followed by the number of fusants, followed by the number of the colony tested.

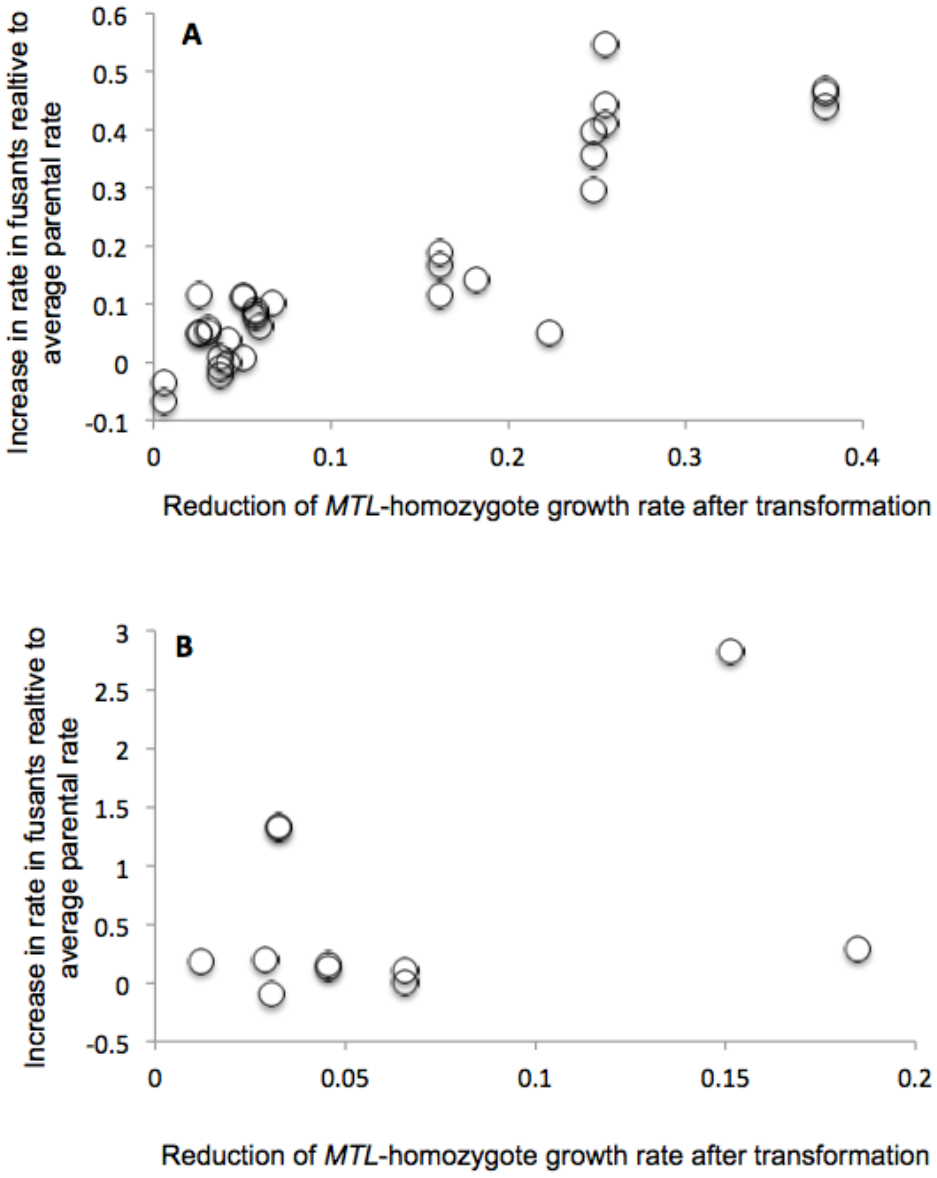

Figure S5 Increase in growth rates of fusants relative to the average rate of their parents (calculated as fusant rate / average parent rate -1) plotted against the reduction of growth rates of *MTL*-homozygotes after transformation with resistance cassettes (the average of the reductions for both parents each calculated as 1-rate of transformed homozygote / rate of original homozygote). Calculations are based on rates determined after ≥ 100 generations of serial propagation. (A) YPD medium at 37ºC; (B) harsh conditions (excluding transformed and untransformed W17α and the fusants it parented, since only the fusants could grow in harsh conditions).

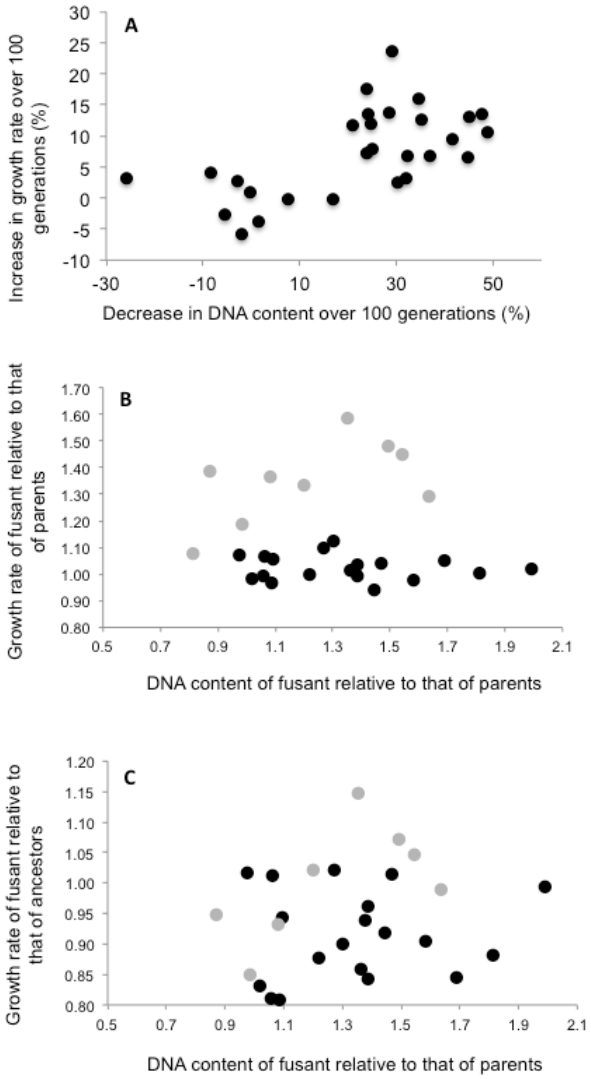

Figure S6 Relationship between reduction in DNA content and increase in growth rate. (A) Fusant growth rate increase over 100 generations of serial propagation versus fusant DNA content decrease over 100 generations of serial propagation, in YPD for 28 representative fusants from all 15 successful matings (names of fusants used are shown in Figure S4). DNA content changes were estimated from flow cytometry. Apparent increases (negative values on x-axis) are most likely attributable to inaccuracy of this method and mainly associated with W17-derived fusants where flow cytometry peaks are ill-defined (Figure S2). (B) Relationship between growth rate and DNA content of fusants, both relative to those of parental averages, after 100 generations of propagation in YPD. Grey symbols represent values for W17-derived fusants. Growth rates of parents prior to propagation in YPD were used in these calculations. (C) Relationship between growth rate, relative to those of *MTL*-heterozygous ancestors, and DNA content of fusants, relative to those of their parents, after 100 generations of propagation in YPD. Growth rates of *MTL*-heterozygous ancestors after propagation in YPD were used in these calculations. Grey symbols in (B) and (C) represent values for W17-derived fusants.

File S1

SUPPORTING METHODS

Estimating the frequency of mutations which abolish mating

The frequency at which mutations can arise which prevent mating (*m*) is the product of several parameters. The first is the mutation frequency per bp (3.3×10^{-10} for point mutations, determined in *S. cerevisiae* (Lynch *et al.* 2008)). The second is the number of genes required for parasexual recombination. The likely number of genes required for mating is between ~25 (genes whose disruption is listed by the *Candida* Genome Database (CGD) as affecting mating) and ~80 (the former plus genes described in CGD as having a role or predicted role in mating) (Arnaud *et al.* 2015). The third and fourth are the average ORF and estimated promoter sizes of these genes which are 1439bp (Braun *et al.* 2005) and ~500 bp, respectively (Kristiansson *et al.* 2009). Lastly the frequency of mutation needs to be multiplied with the probability that they will affect function, which is 0.107 (based on a probabilities of 0.12 for coding regions and 0.07 in promoters, respectively (Doniger *et al.* 2008)).

Based on this, we arrive at an estimate of the frequency with which mutations arise which interfere with *C. albicans*' ability to mate which lies between 1.7×10^{-6} and 5.5×10^{-6} per division.

The accuracy of this estimate is influenced by two factors affecting the rate at which mutations arise that abolish mating ability, which we have not taken into consideration, because suitable estimates are lacking. Fortunately these have opposing effects of comparable size and are likely to largely cancel each other out: Firstly we used in the above calculations probabilities of mutations being deleterious (Doniger *et al.* 2008). To be deleterious, a mutation needs to only reduce functionality, and not necessarily completely destroy it. Thus the probability of a mutation destroying function is less, by an unknown degree, than the figure we used. Secondly ~ 1% of protein-coding *C. albicans* DNA is repetitive (Schmid *et al.* 2012)with a mutation rate of $\sim 10^{-6}$ (Lynch *et al.* 2008), which would increase the overall probability of a mutation in mating-related genes ~30 fold. However repeat regions mutate by insertion and deletion of repeat units and the extent of impact of insertion or deletion of repeat units is uncertain.

PCR amplifications

Most polymerase chain reactions (PCRs), were performed in a final volume of 20 µl containing 1 U *Taq* DNA polymerase (Qiagen Pty Ltd, Clifton Hill Vic, Australia), 4 µl of Q-buffer and 1x PCR buffer supplied by the manufacturer (Qiagen), 10 pmol of each primer, 200 µM of each dNTP (Roche Diagnostics, Auckland, New

Zealand), and 10–100 ng DNA. For amplification of products used in construction of resistance cassettes KOD polymerase (Novagen) was used instead of Taq polymerase. The cycling conditions varied according to primer sets and the size of the products (Ausubel *et al.* 2014) and included an initial incubation for 2 min at 94°C, followed by 30 cycles of 45 s at 94°C, 45 s at 50–60°C, and 30 s to 3 min at 72°C. All PCR protocols included a final 5 min extension step at 72°C. For colony PCR, a portion of a *C. albicans* colony was picked with a 10 µl pipette tip and mixed with 20 µl PCR reaction mixture; the initial step in the cycling program was altered to 5 min at 96°C. Reactions were carried out in an Eppendorf Mastercycler thermocycler (Eppendorf, Hamburg, Germany).

Selection of *MTL*-homozygous derivatives

MTL-homozygous derivatives of clinical isolates were obtained through chr 5 loss by sorbose selection (Rustchenko *et al.* 1994). Approximately 10^6 *C. albicans* cells of each strain were spread on sorbose medium agar plates (2% w/v sorbose, 0.67% w/v yeast nitrogen base w/o amino acids [sterilized by filtration], 1.5% agar) (Rustchenko *et al.* 1994). The plates were incubated at 37°C for one to two weeks. Colonies were checked by multiplex colony PCR for the presence of *MTL***a** and *MTL*α alleles. Primer combinations used were *MTL***a**1-F/*MTL***a**1-R and *MTL*α-1F/*MTL*α1-R (Table S1) (Legrand *et al.* 2004). Colonies lacking one type of *MTL* allele were sub-cultured twice on sorbose plates, tested by PCR once again and then stored in YPD containing 30% glycerol v/v at -80°C.

To obtain spontaneously arising *MTL*-homozygotes, approximately 4-6 x 10^3 *C. albicans* cells were plated at low density (50 to 150 colonies per 85-mm plate) on YPD + phloxine B (5 µg/ml) agar and the plates were incubated at 25°C for 2 weeks, as previously described (Lockhart *et al.* 2002). Red sectors were checked by multiplex colony PCR for the presence of *MTL***a** and *MTL*α alleles. Potential *MTL*-homozygotes were streaked onto YPD plates to obtain single colonies and the colonies were checked again for presence of *MTL* alleles.

Transformation of *MTL*-homozygotes

The most frequently occurring *MTL*-homozygous derivative of a clinical isolate was transformed with a resistance cassette, except for strain W43, where the slightly less frequent derivative was used to balance the *MTL* allele distribution among the derivatives used for mating. The *IMH3*^{r} cassette was removed from plasmid pNZ4 by *Nae*I digestion, gel purified, and used to transform *MTL***a** derivatives, using either the lithium acetate heat shock method (Beckerman *et al.* 2001) or electroporation (De Backer *et al.* 1999). Putative transformants were selected on minimal plates (0.67% w/v yeast nitrogen base without amino acids, 2% w/v glucose, 1.5% w/v agar)

containing MPA (5 μg/ml) and re-streaked on minimal medium with MPA (10 μg/ml) to verify resistance. The presence and correct insertion of the *IMH3*^{r} cassette were tested by colony PCR (Zhang *et al.* 2010) with primer combinations TS1Fpf/TRsepr and TS2Fpr/pENOpr, and with primers TS1Fpf and TS1pr-p to detect tandem integration. Southern hybridization (Ausubel *et al.* 2014) of *EcoR*I digests of transformants' genomic DNA using a 1134 bp fragment amplified from pNZ4 plasmid with primers tetRpf/tetRpr2 as a probe confirmed that insertion had occurred only at the intended locus.

*MTL*α derivatives were transformed with uncut plasmid pNZ11 as described above, except that 100 μl portions of transformation mixture were spread on YPD plates and incubated at 37°C for 7 h, at which time a thin lawn of cells had formed. Putative transformants were selected by replica plating onto YPD plates containing NAT at 200 μg/ml. The presence of a correctly inserted *NAT*^{r} cassette was verified by PCR with primers M13pr/CaACTpr and pACTFpf/CaNATpr. A PCR reaction using primers M13pr and pNZ11pf was used to detect tandem integration. Southern hybridization (Ausubel *et al.* 2014) of *Sal*I digests of transformants' genomic DNA using a 564 bp DNA fragment amplified from pNZ11 using primers CaNATpf/CaNATpr as a probe confirmed that insertion had occurred only at the intended target area.

Serial transfers and growth rate determinations

For serial transfer experiments, cultures were grown in 2ml medium to saturation (for 24 h). Then 10μl were transferred to 2 ml for further propagation.

For growth rate determinations, a pre-culture was made by inoculating 2ml of YPD medium in a 19 mm x 20 cm test tube with one inoculating loop of cells and then placing it in an incubator and shaking it at 150 rpm. After 16 h, 80 μl of culture was used to inoculate 40 ml of medium in a 200 ml Erlenmeyer flask, which was incubated under the same conditions. Growth rates reported are averages of at least two, and usually three, independent experiments (with 8 absorbance measurements each) conducted at different times with different batches of medium. As far as possible, rates that were to be compared with each other were determined in the same experiments. Experiments under harsh conditions were carried out in a similar manner, except that growth rates were determined in 200μl cultures in 96-well plates using a plate reader.

***In vivo* mating in a rat co-colonization model**

All animal husbandry and manipulation was carried out in government-approved facilities and procedures were approved by the University of Otago Animal Ethics Committee (approval 60/2007). Groups of three male Sprague-Dawley rats (~ 200 g in weight) were housed together and fed standard rat chow and water *ad libitium*. Immunosuppressants (doxycycline and dexamethasone) were added to the drinking water of rats one week after admission to the animal facility. Two weeks after admission to the facility, rats were orally inoculated with equal numbers of white- or opaque-phase *C. albicans* cells of the two parental strains that had been grown on YPD agar containing phloxine B (5 µg/ml) at 35°C for 24 h. Cells of each strain were washed and suspended to a concentration of 3.0 x 10^8 cells/ml in sterile water (confirmed by cell counting with a hemocytometer) and an equal volume of each strain suspension was mixed together to form the inoculum. Rats were inoculated by placing 100 µl of the cell suspension on their tongues. The rats were inoculated three times at 48 h intervals. The rats had their mouths swabbed weekly after initial inoculation to sample yeast present. The tongue of each rat was rubbed with a separate sterile swab moistened in sterile saline. Each swab was then vortex mixed in 1 ml sterile saline and portions (5, 50, or 100 µl) of appropriate dilutions of the oral swab samples were plated on YPD agar containing chloramphenicol and on double selection medium (DSM) consisting of 0.67 % yeast nitrogen base with amino acids, 2 % glucose, 1.5 % bacteriological agar, mycophenolic acid (MPA) 5 µg/ml, nourseothricin (NAT) 100 µg/ml. Large colonies on DSM agar were picked and streaked to single colonies on YPD and individual colonies were tested by PCR to detect *MPA*^{r}, *NAT*^{r}, *MTL***a** and *MTL*a markers. Colonies were considered as fusants if both *MTL***a** and *MTL*a markers could be amplified. The number of colonies on YPD + chloramphenicol agar plates was used to calculate the total number of *C. albicans* cells/swab. Individual colonies from these plates were picked at random and checked by PCR amplification of *MTL***a** or *MTL*a markers to determine the numbers of each parent and present in the swab sample. To determine the rate of marker loss in fusants, groups of three rats were inoculated with either an OD8916α x W43**a** or a W17α x W43**a** fusant. The mouths of rats were swabbed weekly and swabs plated on YPD + chloramphenicol agar plates. At each sampling time up to 20 colonies from each rat were selected at random and were tested by PCR to detect *MPA*^{r}, *NAT*^{r}, *MTL***a** and *MTL*a markers.

File S2

SUPPORTING DATA

The inability of FJ11α to mate is most likely caused by a truncated Mtlα1 protein

We were unable to obtain fusants from any matings that involved strain FJ11α, be it with *MTL*-homozygous derivatives of other clinical isolates or with the universal tester strain 3710 (Magee *et al.* 2002), an MPA-resistant auxotrophic *MTL***a** derivative of laboratory strain SC5314 with which fusants can be selected on minimal plates containing MPA. All other nine strains could mate with 3710 or its *MTL*α equivalent 3685 (Magee *et al.* 2002) (data not shown). When we found that the *MTL***a** derivative of FJ11 could mate with 3685, we suspected that a defective Mtlα protein, essential for mating (Tsong *et al.* 2003), might prevent FJ11α from mating. We sequenced the FJ11α *MTL*α locus (Genbank accession number JN099704) and found that it contained a nonsense mutation that led to the truncation of the Mtlα1 protein.

Retention of resistance cassettes in serially propagated fusants

To assess if resistance cassettes carry a fitness cost we assessed their retention in fusants serially propagated for 100 generations in YPD medium containing neither MPA nor NAT. A fitness cost should be apparent as a loss of the cassettes more frequent than expected on the basis of ploidy reduction, as monitored by flow cytometry analysis. For example if a fusant's ploidy had been reduced from 4n to 2n, we would expect on average only one cassette to be lost if they incurred no fitness cost.

We tested how often both resistance markers were still retained in a single randomly-chosen colony from each of 27 fusant cultures (at least one per successful mating) analyzed by flow cytometry after 100 generations. The *MPA*^{r} cassette was present in all of these and the *NAT*^{r} cassette was present in 92%. Figure S4A). The average DNA content reduction of these fusants was 24% (median 21%).

The presence of both resistance markers was also checked in 3-5 individual colonies from each of six fusants (three W43a x OD8916α and three W43a x W17α), that had been transferred for 100 generations on YPD medium, by PCR (primers TS2Fpr/pENOpr/TS1se for assays of the *MPA*^{r} cassette and primers pACTFpf/CaACTpr/CaNATpr for assays of the *NAT*^{r} cassettes). Of 27 colonies analyzed, only one, a W43a x OD8916α fusant, had lost one marker - a NAT resistance cassette (i.e. the frequency of loss was 0% for the MPA resistance cassette and 3.7 % for the NAT resistance cassette; Figure S4B). In contrast, the average DNA content reduction of the six fusants was 36% (median 31%).

Both experiments imply that the resistance cassettes had no negative impact on the fitness of fusants.

REFERENCES

Arnaud, M., D. Inglis, M. Skrzypek, J. Binkley, P. Shah *et al.*, 2015 Candida Genome Database (http://www.candidagenome.org/), accessed 1/2015.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. A. Smith *et al.*, 2014 *Current protocols in molecular biology*. John Wiley and Sons, Inc., New York.

Beckerman, J., H. Chibana, J. Turner and P. T. Magee, 2001 Single-copy *IMH3* allele is sufficient to confer resistance to mycophenolic acid in *Candida albicans* and to mediate transformation of clinical *Candida* species. Infect. Immun. 69**:** 108-114.

Braun, B. R., M. van het Hoog, C. d'Enfert, M. Martchenko, J. Dungan *et al.*, 2005 A human-curated annotation of the *Candida albicans* genome. PLoS Genet. 1**:** 36-57.

De Backer, M. D., D. Maes, S. Vandoninck, M. Logghe, R. Contreras *et al.*, 1999 Transformation of *Candida albicans* by electroporation. Yeast 15**:** 1609-1618.

Doniger, S. W., H. S. Kim, D. Swain, D. Corcuera, M. Williams *et al.*, 2008 A Catalog of Neutral and Deleterious Polymorphism in Yeast. PLoS Genet 4**:** e1000183.

Kristiansson, E., M. Thorsen, M. J. TamV°s and O. Nerman, 2009 Evolutionary forces Act on promoter length: Identification of enriched cis-regulatory elements. Mol. Biol. Evol. 26**:** 1299-1307.

Legrand, M., P. Lephart, A. Forche, F. M. Mueller, T. Walsh *et al.*, 2004 Homozygosity at the *MTL* locus in clinical strains of *Candida albicans*: karyotypic rearrangements and tetraploid formation. Mol. Microbiol. 52**:** 1451-1462.

Lockhart, S. R., C. Pujol, K. J. Daniels, M. G. Miller, A. D. Johnson *et al.*, 2002 In *Candida albicans*, white-opaque switchers are homozygous for mating type. Genetics 162**:** 737-745.

Lynch, M., W. Sung, K. Morris, N. Coffey, C. R. Landry *et al.*, 2008 A genome-wide view of the spectrum of spontaneous mutations in yeast. Proc. Natl. Acad. Sci. U. S. A. 105**:** 9272-9277.

Magee, B. B., M. Legrand, A. M. Alarco, M. Raymond and P. T. Magee, 2002 Many of the genes required for mating in *Saccharomyces cerevisiae* are also required for mating in *Candida albicans*. Mol. Microbiol. 46**:** 1345-1351.

Rustchenko, E. P., D. H. Howard and F. Sherman, 1994 Chromosomal alterations of *Candida albicans* are associated with the gain and loss of assimilating functions. J. Bacteriol. 176**:** 3231-3241.

Schmid, J., M. Wilkins, N. Zhang, R. Bradshaw, M. Cox *et al.*, 2012 Why do so many fungal open reading frames contain repeats? Mycoses 55**:** 67-68.

Tsong, A. E., M. G. Miller, R. M. Raisner and A. D. Johnson, 2003 Evolution of a combinatorial transcriptional circuit: a case study in yeasts. Cell 115**:** 389-399.

Zhang, N., R. D. Cannon, B. Holland, M. Patchett and J. Schmid, 2010 Impact of genetic background on allele selection in a highly mutable *Candida albicans* gene*, PNG2*. PLoS ONE 5**:** e9614.

Zhang, N., J. E. Upritchard, B. R. Holland, L. E. Fenton, M. M. Ferguson *et al.*, 2009 Distribution of mutations distinguishing the most prevalent disease-causing *Candida albicans* genotype from other genotypes. Infect., Genet. Evol. 9**:** 493-500.

Zhou, Z., 2010 Evidence that *SSR1* can act as a hypermutable contingency gene in *Candida albicans*, pp. in *IMBS*. MSc thesis, Massey University, Palmerston North.