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.