



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^r* and *NAT^r* 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.