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' cassette and primers pACTFpf/CaACTpr/CaNATpr for assays of the NAT' cassettes), to distinguish between the insertion site with a cassette (1.3 kb and 1.7 kb products for the MPA' and NAT' 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' cassettes were retained and in 24/26 fusants tested the NAT' 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.