Figure S1  Construction of chromosomal pol3 alleles.

(A) Construction of heterozygous POL3 diploid yeast strains. A DNA fragment containing URA3 (blue), POL3 regulatory sequences (green) and mutant pol3 sequences (white with red lines) was PCR amplified from plasmid templates and integrated (sites of recombination depicted as dashed ‘X’s) at one of two POL3 loci in the diploid strain, AH0401 (Table S1). (B) Genotyping assays. Schematic illustrates two assays used for genotyping pol3 alleles (mutations highlighted in red): the L612M-specific PCR utilizes mismatched primer termini to selectively amplify pol3-L612M; the restriction-fragment-length-polymorphic PCR (RFLP-PCR) assay monitors EcoRV restriction sites lost in pol3-01 and gained in pol3-L612G. Green and blue hashed lines correspond to PCR amplicons. Staggered lines indicate expected fragment sizes for each allele following digestion. (C) Integration efficiency of URA3-pol3 constructs. Agarose gels show results from 10 random transformants of each genotype from the plates pictured in Figure 2A.