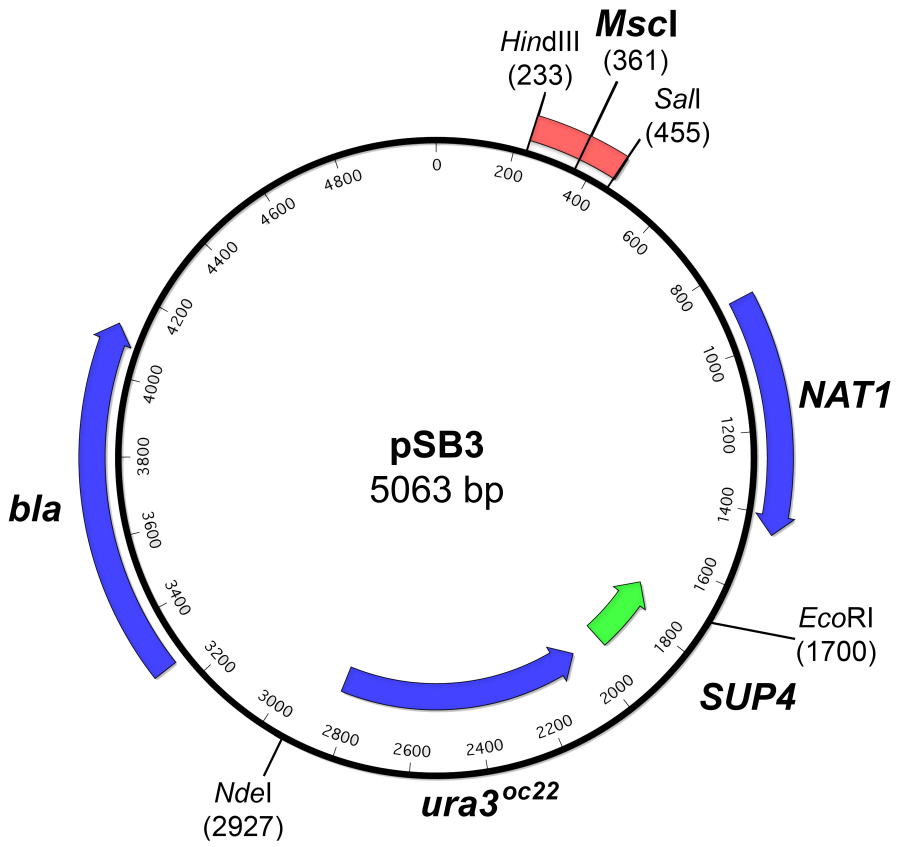
Figure S1

A



B

*Hin*dIII (495)

AAGCTTTGCAGAGGCTAGCAGAATTACCCTCCACGTTGATTGTCTGCGAGGCAAGAATGATCATCA

623 209

CCGTAGTGAGAGTGCGTTCAAGGCTCTTGCGGTTGCCATAAGAGAAGCCACCTCGCCCAATGG|CC

*Msc*I

AAGCATTCCGGCTGGTCGCTAATCGTTGAGTGCATTGGTGACTTACACATAGACGACCATCACACC

ACTGAAGACTGCGGGATTGCTCTCGGTCgac

304 *Sal*I

Figure S1. Construction of a plasmid for targeting single-copy integration of *SUP4* and a *ura3 ochre* allele to the *his3* locus. (A) Plasmid map showing the position of the restriction sites that define the different DNA sequences used in construction of the plasmid, together with the unique *Msc*I site used to target the construct in single copy to the *HIS3* locus. *bla*, ampicillin resistance; *NAT1*, ClonNat resistance (bracketed by *TEF* promoter and terminator sequences that are not shown); $ura3^{oc22}$, *ura3* allele in which codon 22 is substituted by an *ochre* termination codon; *SUP4*, $tRNA^{Tyr}$(UUA) ochre suppressor tRNA. (B) Sequence of the *Hin*dIII-*Sal*I fragment used for *HIS3* targetting. Upper-case letters correspond to the *HIS3* coding sequence positions indicated (numbered from the first base of the *HIS3* open reading frame), lower case letters represent additional bases required to generate the *Sal*I site. Linearization using *Msc*I (|, cut site) generates a fragment flanked by *HIS3* residues 209-304 and 495-623 that integrates in single copy at the *HIS3* locus.