From Mark Hickman

Repaired HAP1 allele in S288C strain

The sequence of the repaired HAP1 allele is identical to that of S288C (SGD version) if the Ty sequence is removed. Sequencing was performed from 224 bp upstream of start codon to 554 bp downstream of stop codon.

There are two sequences below:

1. S288C allele from SGD
2. Repaired HAP1 allele

PCR to distinguish wild-type and S288C HAP1 alleles

There are two separate PCR reactions for the two alleles. The antisense primer is the same for the two reactions. However, the two sense primers are not compatible so the reactions must be carried out separately. These primers work well for colony PCR.

(1) S288C allele
Sense CTTTATCAACAATGGAATCCC (in Ty) (primer FO560)
Antisense ATCAGCTTACGGAATGTTAC (460 bp downstream of HAP1 stop codon) (primer MH39 or FO2641)
583 bp product

(2) wild-type allele
Sense GGGAGCTTTACCATCTTTAGATAGG (510 bp upstream of HAP1 stop codon) (primer FO1498)
Antisense ATCAGCTTACGGAATGTTAC (460 bp downstream of HAP1 stop codon) (primer MH39 or FO2641)
970 bp product

hap1 mutant genomic sequence (S288C, from SGD)

ATG and TAG of coding sequence are in bold (these are according to SGD annotation of the sequence).
The actual 3' end of the HAP1 coding sequence is in red text. (The sequence between the bolded TAG and the red text is the Ty insertion.)

GCTTCTGTCACTTAGTAAGCAAATGTACGAAACCCAGAGCATTGTATGTACACCAGCAGTCGTTTTTTG
TAGCTCTTTTCCATGGATGGACGATGATGGCAAGACGCGATCGTGGCGGAGCCTCACTAAGAAAAACA
ATTGTGGAGAATCTATACACACAGAAATCGTACTTCAAGAAATATTGATTAGCGAGCCTTTAAGCACA
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HAP1 genomic sequence (in repaired S288C HAP1 strain)

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