

Table S4. Growth results for colonies from controls lacking at least one PCR product.

| Parental Strain | PCR fragment(s) | PCR | | YPD | YPD | Total Colonies |
|---------------------|-------------------------------|--------|--------|-------|------|----------------|
| | | SD-Ura | SD-His | +G418 | +Hyg | |
| 2002-B ¹ | <i>CDC11</i> | + | - | + | - | 7 |
| | | + | - | + | + | 9 |
| 2002-C | <i>SHS1</i> | + | - | + | - | 2 |
| | | + | - | + | + | 7 |
| 2002-D | - | + | - | + | - | 0 |
| | | + | - | + | + | 3 |
| 2003-B ³ | <i>CDC11</i> & <i>SHS1</i> | + | - | + | - | 0 |
| | | + | - | + | + | 6 |
| 2003-C | <i>CDC11</i> & <i>HIS3</i> | + | - | + | - | 2 |
| | | + | - | + | + | 7 |
| 2003-D | <i>SHS1</i> & <i>HIS3</i> | + | - | + | - | 0 |
| | | + | - | + | + | 6 |
| 2003-E | - | + | - | + | - | 0 |
| | | + | - | + | + | 6 |

Clonal isolates were pooled from multiple experimental trials, in which either 500 bps of flanking homology (Fig. 2) or 30 bps of flanking homology (Fig. S3) were used, and replica-plated onto various growth conditions (SD-Ura, SD-His, YPD+G418, and YPD+Hygromycin). A “+” score indicates growth/resistance whereas “-” designates no growth/sensitivity. For the initial genotypes indicated [strain and PCR fragment(s)], two growth patterns were observed and the number of colonies displaying each pattern were totaled (*far right column*). Yeast were also tested on SD-Leu medium for the presence or absence of the high-copy pRS425::sgRNA[u1] vector. After only two rounds of selection on SD-Ura (no selective pressure for the *LEU2* marker), 40/55 strains had lost the sgRNA-expressing plasmid. Representative isolates were taken from each category for further analysis by diagnostic PCR.

¹B, C, D or E designation is from the experiment in Fig. 2, in which no PCR fragments, one PCR fragment, or two PCR fragments were introduced by transformation, as indicated.

³For simplicity, only combinations of two PCR products (omitting a third) were tested for strain GFY-2003, rather than all possible combinations.