Table S1. Strains and *ADE2* allele descriptions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Strain number** | ***ADE2* allele** | **Mating type** | **descriptiona** | **Nucleotide sequenceb** | **positionc** |
| Wsc24 | *+* | **a** | wild-type | N.A. | N.A. |
| Wsc55 | 1004 | **a** | *ura3Kl-kanMX4* | N.A. | 95d |
| Wsc59 | 1005 |  | *ura3Kl-kanMX4* | N.A. | 96-1120e |
| Wsc64 | 1006 | **a** | *M26* | A**T**GACG**TC**A**T** | 82-91 |
| Wsc66 | 1007 | **a** | 5'-stop | **T**AA | 103-105 |
| Wsc70 | 1008 | **a** | 3x-*M26* | (ATGACGTCAT)3 | 82-111 |
| Wsc72 | 1003 |  | 3'-stop | **T**AA | 1111-1113 |
| Wsc92 | 1014 | **a** | *CCAAT* | **CC**GACC**AA**T**C**ATA**T** | 82-94 |
| Wsc94 | 1015 | **a** | *Oligo-C* | AGA**AC**C**CCGC**A | 82-91 |
| Wsc97 | 1016 | **a** | *4095* | **GGTCTG**G**AC**C | 81-89 |
| Wsc103 | 1017 | **a** | *4156* | T**C**G**G**C**C**G**A** | 83-89 |
| Wsc110 | 1021 | **a** | *M26* (no 5'-stop) | ATG**ACGTC**AT | -(98-89) |
| Wsc115 | 1025 | **a** | *M26* | ATG**ACGTC**AT | -(98-89) |
| Wsc116 | 1026 | **a** | *M26* control | ATG**GTACC**AT | -(98-89) |
| Wsc117 | 1027 | **a** | *4156* | **AA**T**TCG**G**CCG**A**G**A**C** | -(102-89) |
| Wsc118 | 1028 | **a** | *CCAAT* | **G**T**CCTCCAATC**AA**T**T | -(103-89) |
| Wsc120 | 1030 | **a** | 3x-*M26* control | (ACTAGCTAGT)3 | 82-111 |
| Wsc121 | 1031 | **a** | *4095* | **GGTCTGG**A**CC** | -(98-89) |
| Wsc125 | 1032 | **a** | *oligo-C* | **G**A**ACCCCGC**A**C** | -(99-89) |
| Wsc126 | 1003 |  | 3'-stop *sko1* | **T**AA | 1111-1113 |
| Wsc127 | 1008 | **a** | 3x-*M26* *sko1* | (ATGACGTCAT)3 | 82-111 |
| Wsc128 | 1030 | **a** | 3x-*M26* control *sko1* | (ACTAGCTAGT)3 | 82-111 |
| Wsc129 | 1025 | **a** | *M26 sko1* | ATG**ACGTC**AT | -(98-89) |
| Wsc130 | 1026 | **a** | *M26* control *sko1* | ATG**GTACC**AT | -(98-89) |
| Wsc134 | 1034 | **a** | *4156* control | **AAGT**A**G**G**CG**T**C**A**CC** | -(102-89) |
| Wsc135 | 1035 | **a** | *oligo-C* control | **G**A**CCACC**A**GCC** | -(99-89) |
| Wsc136 | 1036 | **a** | *oligo-C* control | G**CC**AC**CAGC** | 83-90 |
| Wsc143 | 1003 |  | 3'-stop *cst6* | **T**AA | 1111-1113 |
| Wsc144 | 1025 | **a** | *M26 cst6* | ATG**ACGTC**AT | -(98-89) |
| Wsc145 | 1026 | **a** | *M26* control *cst6* | ATG**GTACC**AT | -(98-89) |
| Wsc156 | 1038 | **a** | *4095* control | **GTCGGTCGC** | -(97-89) |
| Wsc157 | 1008 | **a** | 3x-*M26* *cst6* | (ATGACGTCAT)3 | 82-111 |
| Wsc166 | 1047 | **a** | Bas1/Reb1 BSKOf  (no stop) | **AGT**…**AAC**…**AGT** | -(195-193…169-167…152-150) |
| Wsc167 | 1048 | **a** | *CCAAT* control | **TTAC**TC**TCA**T**GC**A**C**A**C** | -(104-89) |
| Wsc168 | 1049 | **a** | Bas1/Reb1 BSKOf | **AGT**…**AAC**…**AGT** | -(195-193…169-167…152-150) |
| Wsc169 | 1050 | **a** | *M26* control | **T**C**T**G**CT** | 85-90 |
| Wsc172 | 1003 |  | 3'-stop *cst6* *sko1* | **T**AA | 1111-1113 |
| Wsc173 | 1025 | **a** | *M26 cst6 sko1* | ATG**ACGTC**AT | -(98-89) |
| Wsc174 | 1030 | **a** | 3x-*M26* control *cst6* | (ACTAGCTAGT)3 | 82-111 |
| Wsc179 | 1008 | **a** | 3x-*M26* *cst6* *sko1* | (ATGACGTCAT)3 | 82-111 |
| Wsc183 | 1003 |  | 3'-stop *aca1* | **T**AA | 1111-1113 |
| Wsc184 | 1008 | **a** | 3x-*M26* *aca1* | (ATGACGTCAT)3 | 82-111 |
| Wsc185 | 1030 | **a** | 3x-*M26* control *aca1* | (ACTAGCTAGT)3 | 82-111 |
| Wsc186 | 1025 | **a** | *M26 aca1* | ATG**ACGTC**AT | -(98-89) |
| Wsc187 | 1026 | **a** | *M26* control *aca1* | ATG**GTACC**AT | -(98-89) |
| Wsc197 | 1030 | **a** | 3x-*M26* control *sko1 cst6* | (ACTAGCTAGT)3 | 82-111 |
| Wsc200 | 1026 | **a** | *M26* control *sko1 cst6* | ATG**GTACC**AT | -(98-89) |
| Wsc202 | 1021 | **a** | *M26* (no 5'-stop) *sko1* | ATG**ACGTC**AT | -(98-89) |

aName of hotspot motifs and control alleles in *ADE2* gene and transcription factor (TF) gene knockouts, if present. TF gene knockouts are insertion-deletions that substitute *kanMX4* for the coding sequence. In addition to the listed mutations, all Mat **a** strains are also *trp1-289 ura3-52 his7* and all Mat  strains are also *leu2-3,112 ura3-52 his7 can1 cyh2*. In addition, all *ade2* alleles except *ade2-1003, -1004, -1005, -1008, and -1030* contain the same stop mutation as the *ade2-1007* allele unless otherwise indicated (no stop). *ade2-1008* and *-1030* contain different stop mutations incorporated in their sequence substitutions.

bThe relevant sequence of *ADE2* is shown. Nucleotide substitutions are shown in boldface type. Nucleotide insertions are underlined. The *ade2-1008* and *ade2-1030* alleles contain multiple substitutions, which are not indicated, and no insertions. N.A. Not applicable.

cThe position of the sequence shown in the proceeding column, with the first nucleotide of the coding sequence considered as +1 and the preceding nucleotide as -1. Insertions are not counted.

dAn insertion of the *Gal1-I-SceI kanMX4 KlURA3* cassette after nucleotide 95 of *ADE2*. This construct allows for a galactose-inducible double-strand break within the cassette to facilitate the introduction of *ADE2* sequence substitutions by homologous recombination ([32](#_ENREF_32)).

e*ADE2 *96-1120 with insertion of the *Gal1-I-SceI kanMX4 KlURA3* cassette.

fBinding Site KnockOuts.