

Figure S1, Sampaio *et al.*

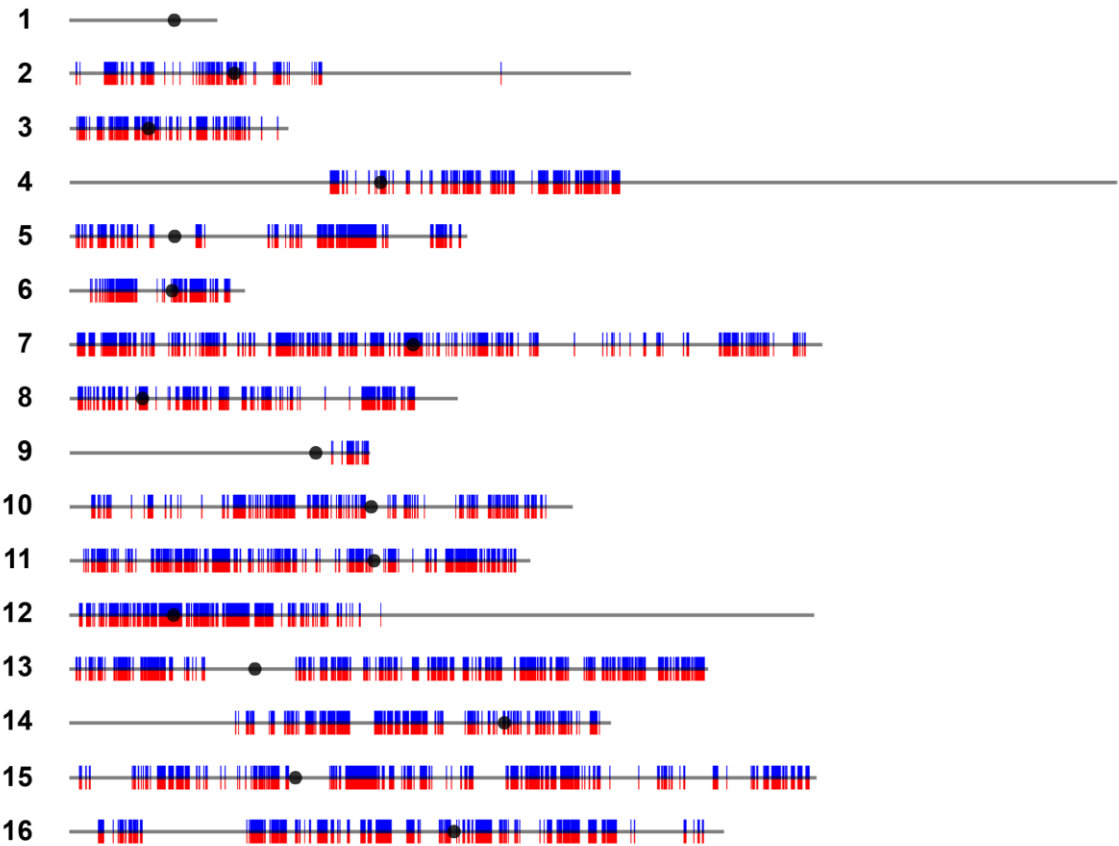
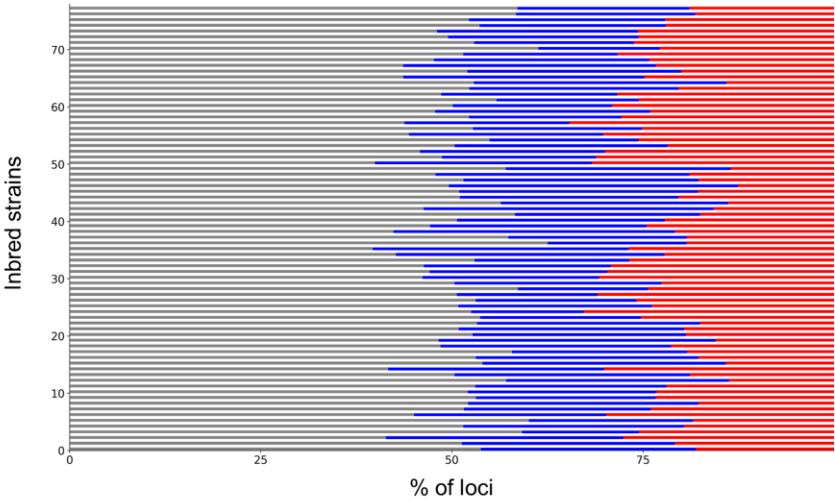


Figure S2, Sampaio *et al.*

A.

| | | MAT α -ACE2 Haploids | | | | | | | | | | | | |
|--------------------------------|------|-----------------------------|------|------|------|------|------|------|------|------|------|------|------|------|
| MAT α -ace2-A7 Haploids | | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 | T11 | T12 | T13 |
| | JAY# | 1520 | 1525 | 1526 | 1531 | 1536 | 1539 | 1545 | 1546 | 1553 | 1557 | 290 | 293 | 328 |
| | T1 | 1521 | 1598 | 1600 | 1602 | 1604 | 1606 | 1608 | 1610 | 1612 | 1614 | 1616 | 1618 | 1620 |
| | T2 | 1522 | | 1622 | 1624 | 1626 | 1628 | 1630 | 1632 | 1634 | 1636 | 1638 | 1640 | 1642 |
| | T3 | 1529 | | | 1644 | 1646 | 1648 | 1650 | 1652 | 1654 | 1656 | 1658 | 1660 | 1662 |
| | T4 | 1533 | | | | 1664 | 1666 | 1668 | 1670 | 1672 | 1674 | 1676 | 1678 | 1680 |
| | T5 | 1535 | | | | | 1682 | 1684 | 1686 | 1688 | 1690 | 1692 | 1694 | 1696 |
| | T6 | 1540 | | | | | | 1698 | 1700 | 1702 | 1704 | 1706 | 1708 | 1710 |
| | T7 | 1543 | | | | | | | 1712 | 1714 | 1716 | 1718 | 1720 | 1722 |
| | T8 | 1549 | | | | | | | | 1724 | 1726 | 1728 | 1730 | 1732 |
| | T9 | 1552 | | | | | | | | | 1734 | 1736 | 1738 | 1740 |
| | T10 | 1554 | | | | | | | | | | 1742 | 1744 | 1746 |
| | T11 | 291 | | | | | | | | | | | 1748 | 1750 |
| | T12 | 295 | | | | | | | | | | | | 1752 |
| | T13 | 326 | | | | | | | | | | | | |

B.



C.

| 78 strains in inbred collection | Genotype Frequency (%) | | |
|------------------------------------|------------------------|-------|-------|
| | M/P | P/P | M/M |
| Average | 51.02 | 26.11 | 22.87 |
| Median | 51.49 | 25.98 | 22.68 |
| Maximum | 62.52 | 37.95 | 34.65 |
| Minimum | 39.64 | 14.72 | 12.57 |

Figure S3, Sampaio *et al.*

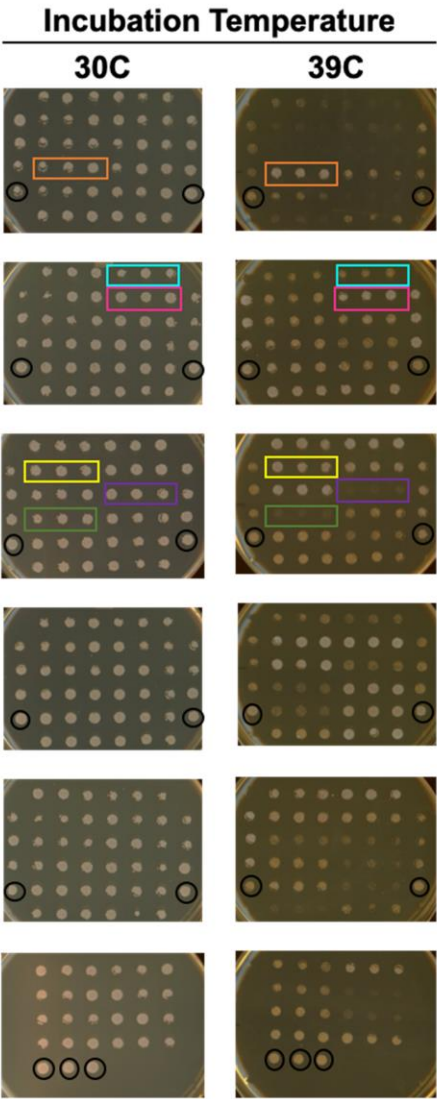


Figure S4, Sampaio *et al.*

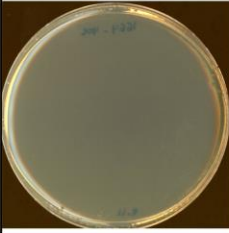
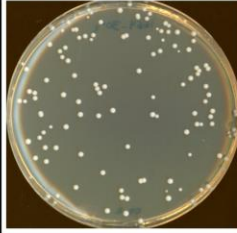
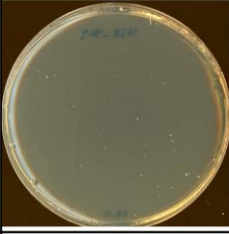
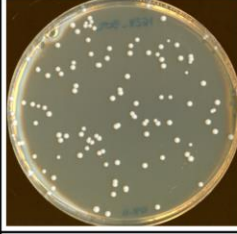
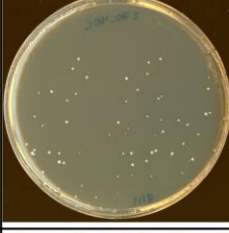
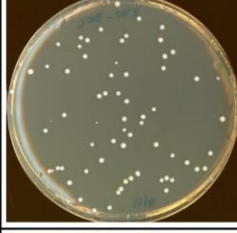
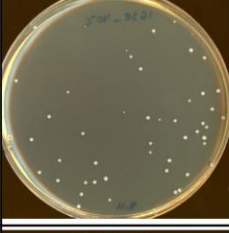
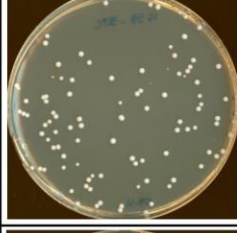

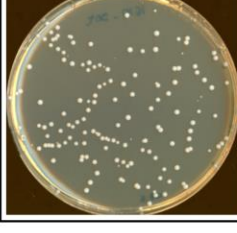
| Phenotype classification | Representative growth at 39C | Representative growth at 30C |
|---|---|--|
| Score 1 (JAY1664) Very small colonies, at the lower limit of unaided visualization. |  |  |
| Score 2 (JAY1628) Small, yet easily visible, colonies. |  |  |
| Score 3 (JAY270) Heterogeneous colony size, ranging from medium to small sizes. |  |  |
| Score 4 (JAY1658) Medium to large colonies, with most having medium size. Narrow variation in colony size. |  |  |
| Score 5 (JAY1610) Large colonies. Homogenous size. |  |  |

Figure S5, Sampaio *et al.*

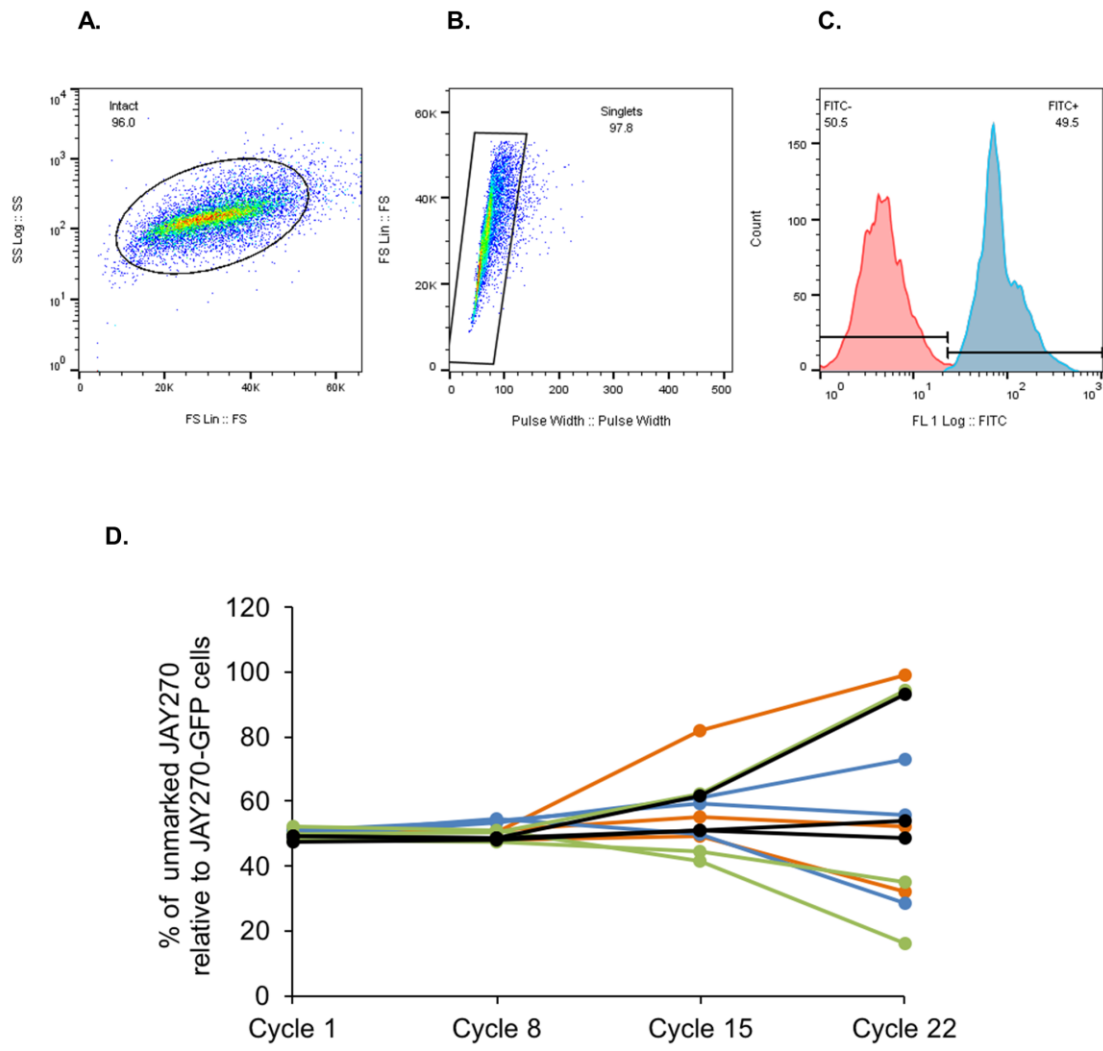


Figure S6, Sampaio *et al.*

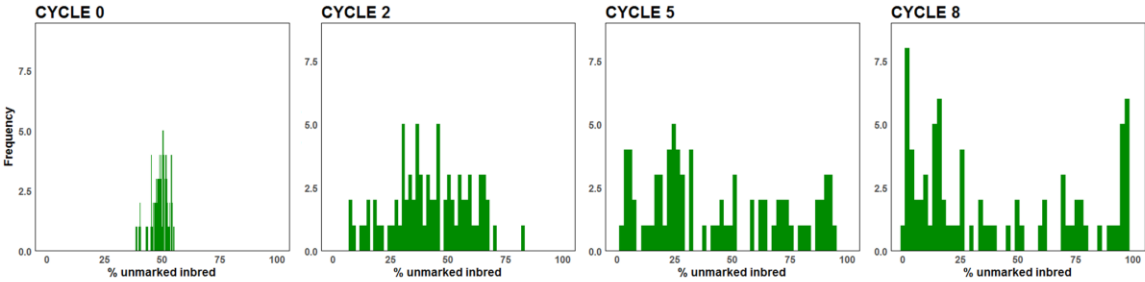


Figure S7, Sampaio *et al.*

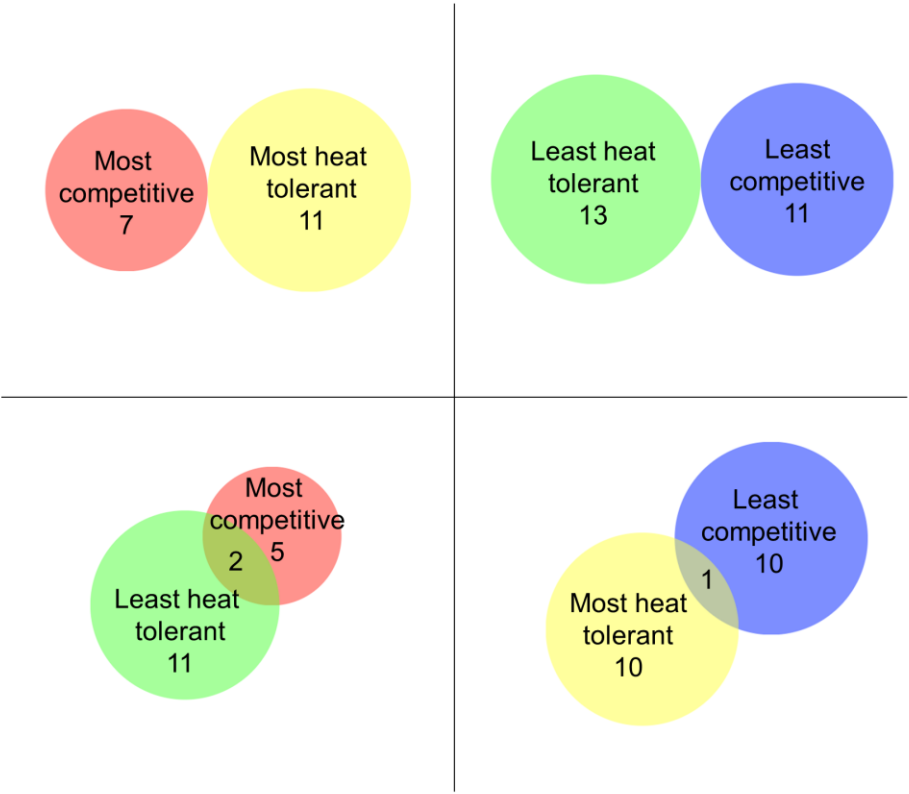


Figure S8, Sampaio *et al.*

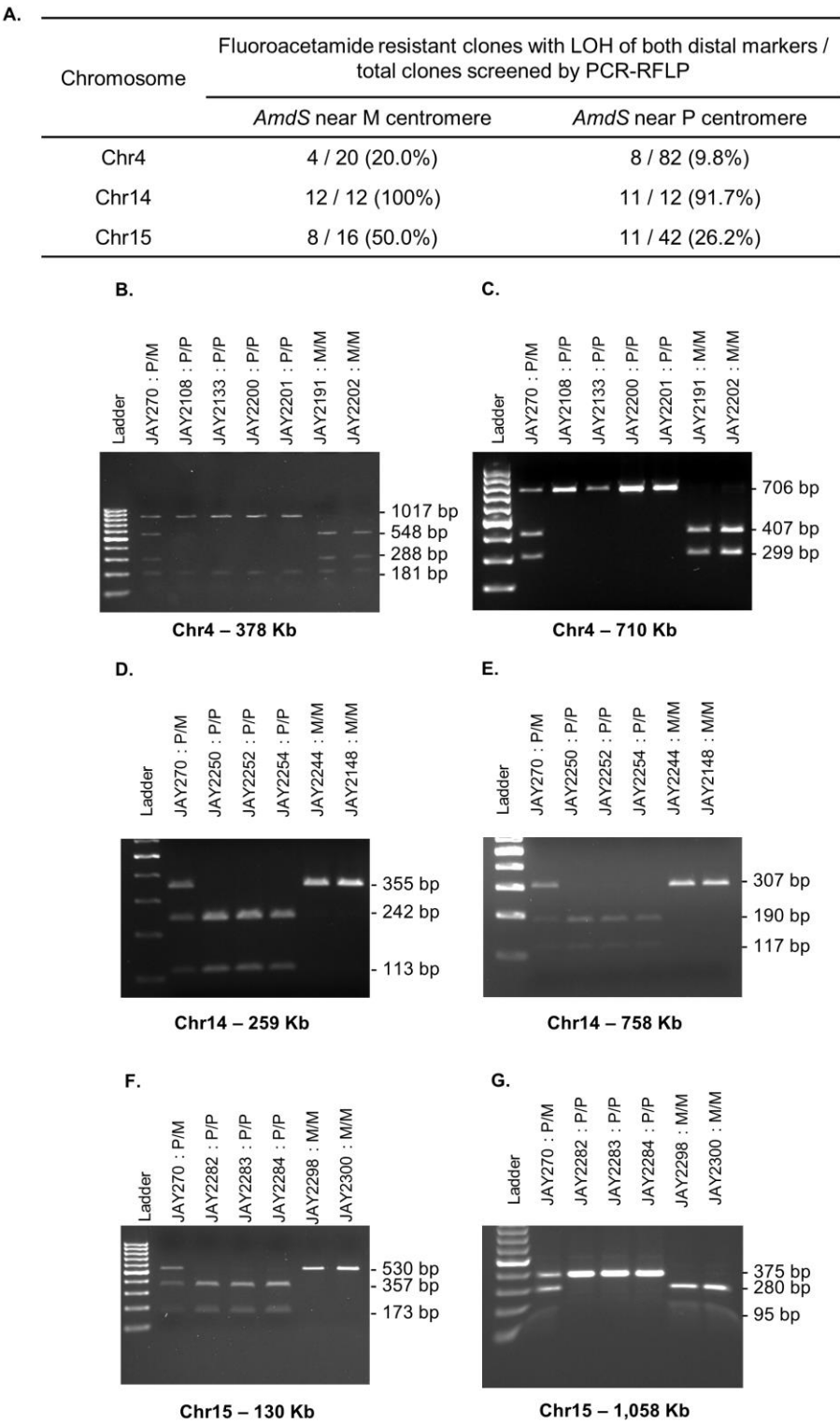


Figure S9, Sampaio *et al.*

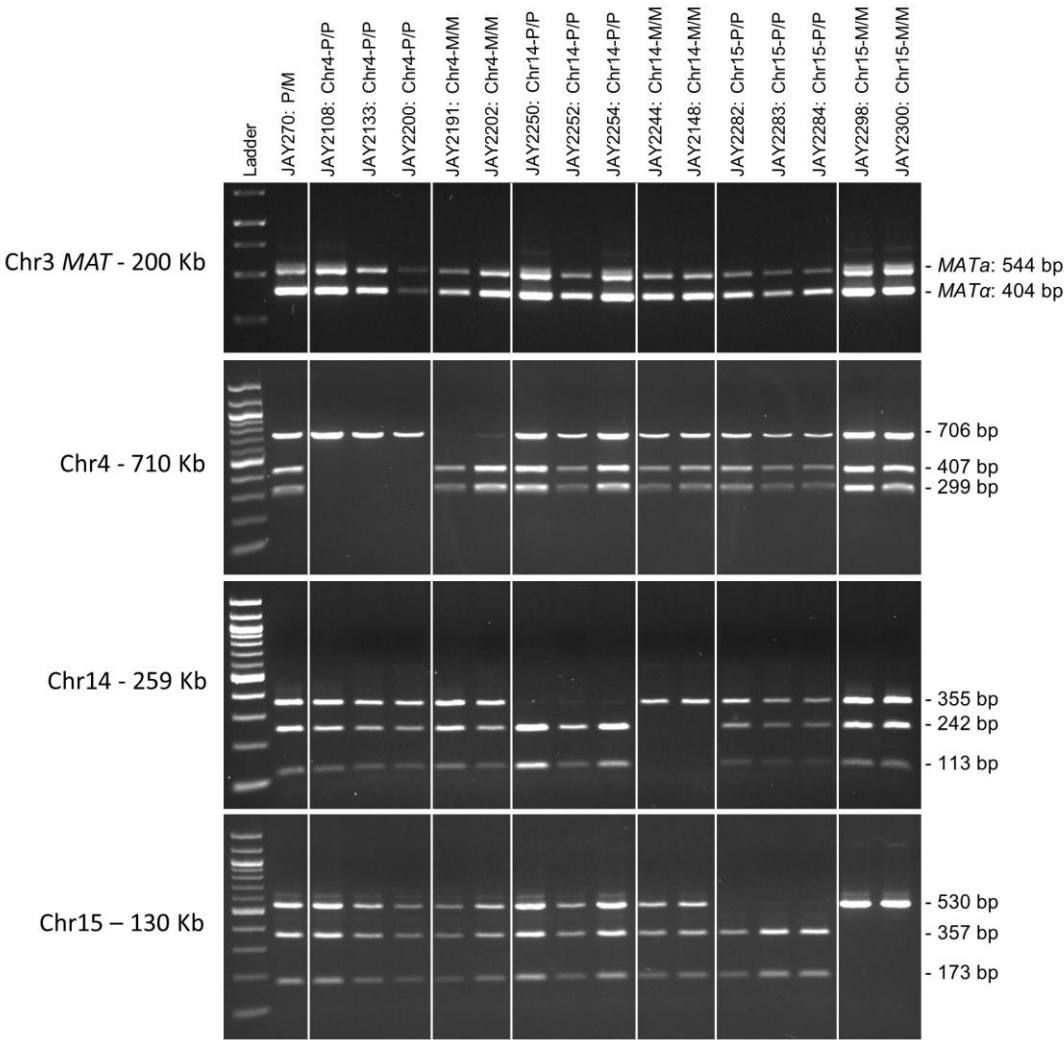
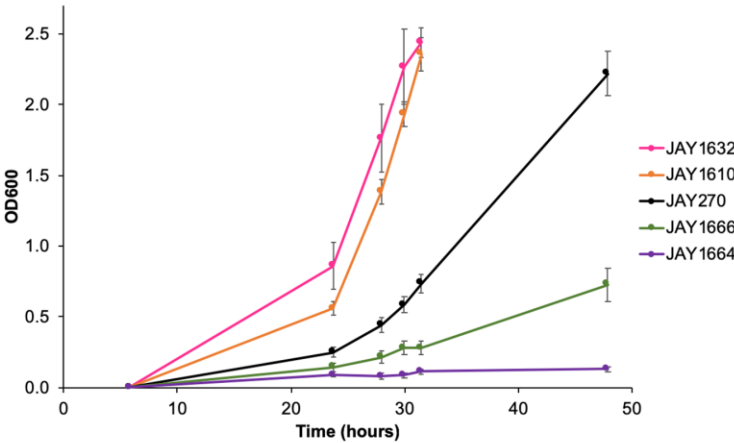


Figure S10, Sampaio *et al.*

A. Colony formation in agar media at 39C heat tolerance scores

| Strain | Description | Heat tolerance phenotype scores (based on colony size) | | | | | |
|---------|-----------------------|--|-----------------------------------|---|---|---|---|
| | | Consensus score | Independent biological replicates | | | | |
| | | | A | B | C | D | E |
| JAY1632 | Heat tolerant inbred | 5 | 4 | 5 | 5 | 5 | 4 |
| JAY1610 | Heat tolerant inbred | 5 | 5 | 4 | 5 | 5 | 4 |
| JAY270 | Parental strain | 3 | 3 | 2 | 3 | — | — |
| JAY1666 | Heat sensitive inbred | 1 | 2 | 2 | 1 | 1 | 1 |
| JAY1664 | Heat sensitive inbred | 1 | 1 | 1 | 1 | 1 | 1 |

B. Liquid media 39C growth curves



Supplemental Figures, Sampaio *et al.*

Figure S1. Draft map of heterozygosity in the JAY270 genome (Rodrigues-Prause *et al.*, 2018), showing the distribution of 12,023 phased heterozygous single nucleotide polymorphisms (HetSNPs; File S1; double colored markers, arbitrarily defined as maternal (red) or paternal (blue) haplotypes). Each horizontal line represents a *S. cerevisiae* chromosome. Black circles indicate the positions of the centromeres. Plots were generated to scale in Python 2.7 using the matplotlib package and a custom script. For size reference, Chr1 is 230 Kb. Note that Chr1 is fully homozygous.

Figure S2. Inter-tetrad matings and genotype distribution in the inbred collection.

A) Schematic representation of the matings between recombinant haploids. Two haploid spores from each of 13 different tetrads (T1 – T13) were selected to generate a collection of diploid inbred strains resulting from inter-tetrad matings. The JAY number (*e.g.*, JAY1520, JAY1598, etc.) corresponding to the strain identifier is displayed for the parent haploids and for the inbreds, and matches the entries in Table S1.

B) The inbreds were expected to contain approximately 50% of the original heterozygosity present in the parental JAY270 genome, and be homozygous for 25% of the paternal alleles and 25% of the maternal alleles. The genotype classes in the inbred collection from panel A and Figure 1B are plotted as cumulative frequencies for all HetSNP markers. Heterozygous M/P (gray); homozygous P/P (blue); homozygous M/M (red). C) Summary of the frequency of genotype classes in the inbred collection.

Figure S3. Variation in tolerance to heat stress in the inbred collection. A plate spotting assay was used for phenotypic screening. The column to the left shows uniform growth among strains incubated for 24 hours at 30C. The column to the right shows variable growth at 39C after four days of incubation. Black circles highlight the control JAY270, which displays an intermediate level of heat tolerance. Rectangles highlight the growth of representative strains shown in Figures S4 and S8 with different levels of heat tolerance, from high to low: JAY1632 (pink), JAY1610 (orange), JAY1658 (yellow), JAY1628 (turquoise), JAY1666 (green), and JAY1664 (purple).

Figure S4. Inbred strains show phenotypic variation in tolerance to heat stress. A scoring system was used to assess the heat stress tolerance phenotype of each inbred strain. The criteria for each score used and the strains chosen as representative examples for each phenotypic category are described next to images of growth on Petri dishes. Incubation at 39C for 4 days and incubations at 30C for 2 days are depicted.

Figure S5. Flow cytometry analysis.

A) False events corresponding to cell debris or other material were eliminated by gating intact cells in a side scatter by forward scatter plot.

B) Potential cell aggregates were subsequently removed from counting by gating single cells in a forward scatter by pulse width plot.

C) Final counts of GFP+ (JAY270 parent strain) and GFP- cells (inbred diploid strains) in each co-culture were determined by gating the FITC+ and FITC- population in a count by FITC signal plot.

D) Co-culture competition assays between four independently-generated GFP-marked JAY270 derivatives and the unmarked JAY270 parent over 22 passage cycles (24 hours per cycle). Each of the four GFP-marked controls (shown in orange, blue, green and black curves) were co-cultured with the original JAY270 parent in triplicates. A neutral and stable ratio of GFP- to GFP-tagged strains was observed in all cases through cycle 8 of competition. Beyond that point the ratios tended to diverge up or down between triplicates, presumably as a result of emergence of beneficial *de novo* mutations either in the GFP- or GFP+ competitors. These results indicated that the GFP marker itself did not confer an advantage or a burden to the cells carrying it, and that any departure from the starting 1:1 ratio detected before cycle 8 could be confidently attributed to the genotypes present in the starting inoculum, and not due to *de novo* mutations arising during the co-culture.

Figure S6. Distributions of the competitive growth phenotypes in the inbred strain collection in cumulative co-culture over time. Histograms showing the change over time of the distributions of phenotypes in the competitive growth assay. By Cycle 8 the cumulative nature of this phenotype can be seen by the higher numbers of strains in the highest and lowest phenotypes.

Figure S7. Venn diagrams displaying pairwise overlap comparisons of inbred strains that displayed extreme phenotypes in heat tolerance and competitive growth (at Cycle 5). Circles represent the groups of inbred strains that were most heat tolerant (yellow) and most competitive (red); and the groups that were least heat tolerant (green) and least competitive (purple). Diagrams were created using BioVenn web application (Hulsen et al., 2008)

Figure S8. Validation of targeted UPD strain constructions.

A) Table showing the frequency of concomitant loss of both (left and right arm) distal PCR-RFLP markers physically linked to the insertion of *AmdS* adjacent to the respective centromere (Figure 5A). A subset of the *AmdS* clones remained heterozygous at one or both distal PCR-RFLP markers, suggesting that they were likely derived from mitotic recombination instead of the desired chromosome loss. All of the chromosome loss clones with bilateral LOH analyzed by tetrad dissection displayed full (4) spore viability, indicating that spontaneous endoduplication leading to UPD occurred very quickly after selection of the monosomic intermediates.

B-G) Agarose gel electrophoresis of PCR-RFLP genotyping of HetSNP markers. Panels B, D and F show genotypes of RFLP markers located at the left arms of Chr4, Chr14 and Chr15, respectively. Panels C, E and G show genotypes of RFLP markers located at the right arms of Chr4, Chr14 and Chr15, respectively. The approximate coordinates of each RFLP marker are indicated below each image. The approximate positions of the relevant centromeres where *AmdS* was inserted are: *CEN4* – 450 Kb; *CEN14* – 629 Kb; *CEN15* – 327 Kb. The

corresponding genotype at each position is indicated for each UPD strain as P/P for homozygous paternal, M/M for homozygous maternal and P/M for heterozygous.

Figure S9. Validation of retention of heterozygosity at non-targeted loci in the UPD strains. PCR-RFLP genotyping of three non-targeted and one targeted HetSNP markers was performed for JAY270 and each of the 15 independently generated UPD strains constructed for this study. Panels show agarose gels used for genotyping of PCR-RFLP markers located at the Chr3 *MAT* locus (not targeted in any of the UPD strains), and at each of the targeted chromosomes. Note that all UPD strains remained heterozygous at the non-targeted chromosomes but were homozygous for the specific allele targeted. Vertical lines were used to facilitate the visualization of the groups of strains with the same targeted UPDs.

Figure S10. Comparison of the two assays used to characterize heat stress tolerance.

A) Representative results from colony size (agar media; 4 days incubation) scoring system described in Figure S4 and used in Figure 3A.

B) Pure culture (liquid media) growth curves and OD600 measurements over a period of 24-48 hours. Error bars shown in SD.

Strains used in the two heat tolerance assays are color coded similarly in A and B and Figure S3.

Table S1. Yeast strains used in this study.

| Strain | Mating type | Relevant genotype | Description | Source |
|----------------------|------------------|--------------------------------------|--|---------------------------------|
| Haploids: | | | | |
| JAY1520 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1521 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1525 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1522 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1526 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1529 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1531 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1533 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1536 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1535 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1539 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1540 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1545 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1543 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1546 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1549 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1553 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1552 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY1557 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | (Rodrigues-Prause et al., 2018) |
| JAY1554 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY290 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | Our strain collection |
| JAY291 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY293 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | Our strain collection |
| JAY295 | <i>MATa</i> | <i>ace2-A7</i> | | |
| JAY328 | <i>MATa</i> | <i>ACE2</i> | Sibling spores from a JAY270 tetrad | Our strain collection |
| JAY326 | <i>MATa</i> | <i>ace2-A7</i> | | |
| Diploids: | | | | |
| JAY270 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Sugarcane bioethanol fermentation strain | Argueso et al., 2009 |
| JAY2208 (JAY270-GFP) | <i>MATa/MATa</i> | <i>ACE2/ace2-A7; CEN5::GFP-KanMX</i> | Hemizygous <i>GFP-KanMX</i> insertion near Chr5 | This study |
| JAY1598 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1525 | This study |
| JAY1600 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1526 | This study |
| JAY1602 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1531 | This study |
| JAY1604 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1536 | This study |
| JAY1606 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1539 | This study |
| JAY1608 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1545 | This study |
| JAY1610 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1546 | This study |
| JAY1612 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1553 | This study |
| JAY1614 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY1557 | This study |
| JAY1616 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY290 | This study |
| JAY1618 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1521 x JAY293 | This study |

Table S1 (continued). Yeast strains used in this study.

[illegible]

Table S1 (continued). Yeast strains used in this study.

| Strain | Mating type | Relevant genotype | Description | Source |
|---------|------------------|-------------------------------------|--|------------|
| JAY1706 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1540 x JAY290 | This study |
| JAY1708 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1540 x JAY293 | This study |
| JAY1710 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1540 x JAY328 | This study |
| JAY1712 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1543 x JAY1546 | This study |
| JAY1714 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1543 x JAY1553 | This study |
| JAY1716 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1543 x JAY1557 | This study |
| JAY1718 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1543 x JAY290 | This study |
| JAY1720 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1543 x JAY293 | This study |
| JAY1722 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1543 x JAY328 | This study |
| JAY1724 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1549 x JAY1553 | This study |
| JAY1726 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1549 x JAY1557 | This study |
| JAY1728 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1549 x JAY290 | This study |
| JAY1730 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1549 x JAY293 | This study |
| JAY1732 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1549 x JAY328 | This study |
| JAY1734 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1552 x JAY1557 | This study |
| JAY1736 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1552 x JAY290 | This study |
| JAY1738 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1552 x JAY293 | This study |
| JAY1740 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1552 x JAY328 | This study |
| JAY1742 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1554 x JAY290 | This study |
| JAY1744 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1554 x JAY293 | This study |
| JAY1746 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY1554 x JAY328 | This study |
| JAY1748 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY291 x JAY293 | This study |
| JAY1750 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY291 x JAY328 | This study |
| JAY1752 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> | Inbred diploid resulting from crossing between JAY295 x JAY328 | This study |
| JAY2108 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr4-UPD P/P | JAY270 derivative containing uniparental disomy for Chr4 (P/P) | This study |
| JAY2133 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr4-UPD P/P | JAY270 derivative containing uniparental disomy for Chr4 (P/P) | This study |
| JAY2191 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr4-UPD M/M | JAY270 derivative containing uniparental disomy for Chr4 (P/P) | This study |
| JAY2200 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr4-UPD P/P | JAY270 derivative containing uniparental disomy for Chr4 (P/P) | This study |
| JAY2201 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr4-UPD M/M | JAY270 derivative containing uniparental disomy for Chr4 (M/M) | This study |
| JAY2202 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr4-UPD M/M | JAY270 derivative containing uniparental disomy for Chr4 (M/M) | This study |
| JAY2250 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr14-UPD P/P | JAY270 derivative containing uniparental disomy for Chr14 (P/P) | This study |
| JAY2252 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr14-UPD P/P | JAY270 derivative containing uniparental disomy for Chr14 (P/P) | This study |
| JAY2254 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr14-UPD P/P | JAY270 derivative containing uniparental disomy for Chr14 (P/P) | This study |
| JAY2244 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr14-UPD M/M | JAY270 derivative containing uniparental disomy for Chr14 (M/M) | This study |
| JAY2248 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr14-UPD M/M | JAY270 derivative containing uniparental disomy for Chr14 (M/M) | This study |
| JAY2282 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr15-UPD P/P | JAY270 derivative containing uniparental disomy for Chr15 (P/P) | This study |
| JAY2283 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr15-UPD P/P | JAY270 derivative containing uniparental disomy for Chr15 (P/P) | This study |
| JAY2284 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr15-UPD P/P | JAY270 derivative containing uniparental disomy for Chr15 (P/P) | This study |
| JAY2298 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr15-UPD M/M | JAY270 derivative containing uniparental disomy for Chr15 (M/M) | This study |
| JAY2300 | <i>MATa/MATa</i> | <i>ACE2/ace2-A7</i> ; Chr15-UPD M/M | JAY270 derivative containing uniparental disomy for Chr15 (M/M) | This study |

Table S2. Oligonucleotides used in this study.

| Name | 5' – 3' sequence ^a | Description |
|---------|--|--|
| JAO14 | AGGAGGGTATTCTGGGCCTCCATG | Inside <i>MX4</i> region. To confirm insertion of <i>MX4</i> cassettes; Rev. |
| JAO15 | ATGCGAAGTTAAGTGCGCAGAAAG | Inside <i>MX4</i> region. To confirm insertion of <i>MX4</i> cassettes; Fwd. |
| JAO1385 | GATTACACCGTACTTCTTTTCAATGCGTAAACAACTAGAGTTGACAAA CTGCTGTGCGATTTCGATAC | To amplify <i>GFP</i> cassette; Fwd. Homology tail to <i>CEN5</i> region. |
| JAO1386 | CACGTCAAGACTGTCAAGGAGGGTATTCTGGGCCTCCATGTC GTCGATGAATTCGAGCTC | To amplify <i>GFP</i> cassette; Rev. Homology tail to <i>KanMX4</i> cassette. |
| JAO1387 | GACATGGAGGCCAGAAATAC | To amplify <i>KanMX4</i> cassette; Fwd. |
| JAO466 | TACGAAGTGCTAGGAGGTATATTATTAATATGATAGTTTTAGTTTATAATA GTGGATCTGATATCATCG | To amplify <i>KanMX4</i> cassette; Rev. Homology tail to <i>CEN5</i> region. |
| JAO464 | CATCGTGTAGTCAAGCAGCA | To confirm integration of <i>GFP-KanMX4</i> cassette; Fwd. <i>CEN5</i> region. |
| JAO467 | CATTTACAGATTCATAGTTC | To confirm integration of <i>GFP-KanMX4</i> cassette; Rev. <i>CEN5</i> region. |
| JAO1563 | ACAGTACTAGCTTTTAACTTGTATCCTAGGTTATCTATGCTGTCTCACCATAGGGAATAT TACCTATTTTCAG CTGCTCGTCCCCGCCG | To amplify <i>AmdS</i> cassette; Fwd. Homology tail to <i>CEN4</i> region. |
| JAO1566 | GTATTTTAAAGTTTTTAAAAAAGTTGATTAAATAGCATGTGAC CTCCAGTATAGCGACCAG | To amplify <i>AmdS</i> cassette; Rev. Homology tail to region immediately upstream to <i>CEN4</i> . |
| JAO1567 | AAAAAGTATTTTAAAGTTTTTAAAAAAGTTGATTAAATAGCAT CTCCAGTATAGCGACCAG | To amplify <i>AmdS</i> cassette; Rev. Integration results in 5bp deletion of <i>CEN4</i> element 1. |
| JAO1568 | GTATTTTAAAGTTTTTAAAAAAGTTGATTAAATAGCATGTGAC AGTTATGGAGTAACAACG | To amplify <i>AmdS</i> cassette excluding its terminator region; Rev. Homology tail to region immediately upstream to <i>CEN4</i> . |
| JAO1569 | CTGCAAAACAGTACTAGCTTTTAACTTG | To confirm integration of <i>AmdS</i> at <i>CEN4</i> region; Fwd. |
| JAO1570 | GGAATATATAGCAGTAGTCAATTTAGCAC | To confirm integration of <i>AmdS</i> at <i>CEN4</i> region; Rev. Used for Sanger sequencing. |
| JAO1574 | TCCAAGGTGGTTGCATCATA | Chr4 710,025 SNP <i>Bgl</i> III RFLP marker Fwd |
| JAO1575 | TGGCTGGAGTTTCGTCTTCT | Chr4 710,025 SNP <i>Bgl</i> III RFLP marker Rev |
| JAO1576 | GTATCTTAATGAACTATGCAATGG | Chr4 378,184 SNP <i>Bam</i> HI RFLP marker Fwd |
| JAO1577 | CCTCATCGGCACATTAAAGCTG | Chr4 378,184 SNP <i>Bam</i> HI RFLP marker Rev |
| JAO1659 | AAAGAAAAAATTACTGCAAAACAGTACTAGCTTTTAACTTGTATCCTAGGTTATCTATG CTGTCTCACCAT CTGCTCGTCCCCGCCG | To amplify <i>AmdS</i> cassette; Fwd. Homology tail to <i>CEN4</i> region |
| JAO1681 | TGGAGGAAAAGCATTGGTATTAAGTACTTTGCATTCTCTTGGAGAAGAACTTGATCAATT GACGGGTATAGC CTGCTCGTCCCCGCCG | To amplify <i>AmdS</i> cassette; Fwd. Homology tail to <i>CEN14</i> region |
| JAO1682 | AAAATGTTTTAAAAATTTTTTAAAAAGCTGCACGTGACTAAC AGTTATGGAGTAACAACG | To amplify <i>AmdS</i> cassette excluding its terminator region; Rev. Homology tail to region immediately upstream to <i>CEN14</i> . |
| JAO1683 | GGACTACTGATGTACTGAAGTTTG | To confirm integration of <i>AmdS</i> at <i>CEN14</i> region; Fwd |
| JAO1684 | CCTGCTGTCTAATCCGTATTCATTC | To confirm integration of <i>AmdS</i> at <i>CEN14</i> region; Rev |
| JAO1685 | CTGAATTGGACGCTTTGGTTC | Inside <i>AmdS</i> gene. To confirm insertion of <i>AmdS</i> cassettes; Fwd. |
| JAO1686 | TCATCACTGTTCTTTTCAGATACTAGTTTCAAAAATTCCTTGACAGAACCATTTTCATGTT AATAATGAAAA CTGCTCGTCCCCGCCG | To amplify <i>AmdS</i> cassette; Fwd. Homology tail to <i>CEN15</i> region |
| JAO1687 | AATGTTTTAAATATTTAATGTATATGACTTCCGAAAAATATAT AGTTATGGAGTAACAACG | To amplify <i>AmdS</i> cassette excluding its terminator region; Rev. Homology tail to region immediately upstream to <i>CEN15</i> . |
| JAO1688 | GGTAGTATATAAGAGAATAAATCTCCC | To confirm integration of <i>AmdS</i> at <i>CEN15</i> region; Fwd |
| JAO1689 | GCTCTCACCACATTTATCACC | To confirm integration of <i>AmdS</i> at <i>CEN15</i> region; Rev |
| JAO1692 | CACAAAATATGTTTCGTACCCATCC | Chr14 259,198 SNP <i>Mfe</i> II RFLP marker Fwd |
| JAO1693 | GTGACGGAGTTTGATATACTTAAC | Chr14 259,198 SNP <i>Mfe</i> II RFLP marker Rev |

Table S2 (continued). Oligonucleotides used in this study.

| Name | 5' – 3' sequence ^a | Description |
|---------|-------------------------------|---|
| JAO1694 | GTTGAAGCAATGAAGGAATCTTCG | Chr14 758,547 SNP <i>MseI</i> RFLP marker Fwd |
| JAO1695 | CCTCAGTCATGCCAAGTGCATCAAC | Chr14 758,547 SNP <i>MseI</i> RFLP marker Rev |
| JAO1696 | CGTCTCGTATTATACTTGTCCTG | Chr15 16,733 SNP <i>SalI</i> RFLP marker Fwd |
| JAO1697 | CATCGAGGAACAACAGCATCTAG | Chr15 16,733 SNP <i>SalI</i> RFLP marker Rev |
| JAO1983 | CAATAATTCTCCTAGTGTGTGC | Chr15 130,295 SNP <i>PstI</i> RFLP marker Fwd |
| JAO1984 | CTGGTGTAAGGCGTACTGAAC | Chr15 130,295 SNP <i>PstI</i> RFLP marker Rev |
| JAO1588 | GAGTTATTGACAGGGAATAC | Chr15 1,058,946 SNP <i>NdeI</i> RFLP marker Fwd |
| JAO1589 | GGAGCTTACTTCACTCATTTT | Chr15 1,058,946 SNP <i>NdeI</i> RFLP marker Rev |
| JAO1063 | AGTCACATCAAGATCGTTTATGG | Chr3, mating type reverse primer; to amplify <i>MATa</i> and/or <i>MATα</i> |
| JAO1064 | GCACGGAATATGGGACTACTTCG | Chr3, <i>MATα</i> forward primer; to amplify <i>MATα</i> |
| JAO1065 | ACTCCACTTCAAGTAAGAGTTTG | Chr3, <i>MATa</i> forward primer; to amplify <i>MATa</i> |

- a. Nucleotides in the 5' end (**bold**) have homology to the respective chromosomal insertion sites. 3' end nucleotides (not in bold) correspond to primer sequences to amplify the desired amplicons.

Table S3. Growth conditions used in phenotypic screening of inbred strain collection through plate spotting assay.

| Base Media | Additional treatment/supplement | Temperature of incubation | Hours of incubation |
|-----------------|---------------------------------|---------------------------|---------------------|
| 2% YPD | - | 30C | 48 |
| 2% YPD | - | 39C | 96 |
| 2% YPGE | - | 30C | 48 |
| 2% YP Galactose | - | 30C | 48 |
| 2% YP Raffinose | - | 30C | 48 |
| 2% YPD | 7 % Ethanol | 30C | 48 |
| 2% YPD | 11 % Ethanol | 30C | 48 |
| 2% YPD | 30 mM furfural | 30C | 48 |
| 2% YPD | 0.75 mM menadione | 30C | 48 |
| 2% YPD | 0.01% MMS | 30C | 48 |
| 2% YPD | 100 mM hydroxyurea | 30C | 96 |
| 2% YPD | 100 J/m ² UV light | 30C | 48 |
| 2% YPD | 150 J/m ² UV light | 30C | 48 |

Table S4. Phenotypic data of inbreds from competition assays (CA) and tolerance to high temperature assays (HT).

| Strain | ^a Mean % inbred cells in the population in CA | | | | ^b Median Score in HT |
|---------|--|--------------|--------------|--------------|---------------------------------|
| | Cycle 0 | Cycle 2 | Cycle 5 | Cycle 8 | |
| JAY1598 | 50.50 ± 0.40 | 33.72 ± 0.41 | 19.15 ± 0.17 | 13.32 ± 0.49 | 3.50 (0.42) |
| JAY1600 | 54.40 ± 0.31 | 36.87 ± 0.12 | 22.27 ± 0.16 | 10.03 ± 0.19 | 1.00 (0.46) |
| JAY1602 | 50.65 ± 0.26 | 18.00 ± 0.18 | 5.72 ± 0.27 | 2.39 ± 0.06 | 3.50 (0.28) |
| JAY1604 | 54.45 ± 0.19 | 38.85 ± 0.30 | 25.68 ± 0.27 | 12.97 ± 0.26 | 1.00 (0.43) |
| JAY1606 | 54.00 ± 0.10 | 59.88 ± 0.40 | 74.95 ± 0.19 | 78.28 ± 0.34 | 1.00 (0.25) |
| JAY1608 | 54.10 ± 0.41 | 43.32 ± 0.15 | 32.38 ± 0.35 | 26.13 ± 0.30 | 3.25 (0.25) |
| JAY1610 | 54.20 ± 0.13 | 39.92 ± 0.40 | 37.22 ± 0.39 | 25.55 ± 0.70 | 5.00 (0.00) |
| JAY1612 | 49.95 ± 0.30 | 36.57 ± 0.28 | 25.97 ± 0.50 | 14.63 ± 0.32 | 4.00 (0.31) |
| JAY1614 | 47.15 ± 0.09 | 11.48 ± 0.14 | 1.27 ± 0.07 | 0.81 ± 0.07 | 3.50 (0.28) |
| JAY1616 | 50.35 ± 0.38 | 57.08 ± 0.54 | 73.27 ± 0.41 | 70.05 ± 0.26 | 3.00 (0.26) |
| JAY1618 | 53.55 ± 0.08 | 57.93 ± 0.32 | 73.47 ± 0.15 | 76.57 ± 0.61 | 1.00 (0.39) |
| JAY1620 | 47.45 ± 0.21 | 22.00 ± 0.06 | 6.85 ± 0.17 | 3.11 ± 0.14 | 4.00 (0.62) |
| JAY1622 | 49.75 ± 0.18 | 52.28 ± 0.15 | 62.38 ± 0.48 | 59.13 ± 0.30 | 0.00 (0.26) |
| JAY1624 | 47.95 ± 0.26 | 38.35 ± 0.43 | 31.53 ± 0.45 | 21.43 ± 0.51 | 3.00 (0.26) |
| JAY1626 | 48.25 ± 0.20 | 53.23 ± 0.74 | 69.08 ± 0.25 | 76.03 ± 0.24 | 1.50 (0.42) |
| JAY1628 | 47.70 ± 0.40 | 67.25 ± 0.46 | 92.93 ± 0.16 | 98.15 ± 0.12 | 2.00 (0.00) |
| JAY1630 | 47.60 ± 0.27 | 50.40 ± 0.64 | 64.62 ± 0.71 | 71.98 ± 0.22 | 3.00 (0.26) |
| JAY1632 | 48.75 ± 0.32 | 48.18 ± 0.79 | 63.85 ± 0.31 | 70.22 ± 0.17 | 5.00 (0.42) |
| JAY1634 | 45.95 ± 0.48 | 46.48 ± 0.91 | 51.25 ± 0.52 | 51.07 ± 0.39 | 3.00 (0.41) |
| JAY1636 | 49.05 ± 0.32 | 33.93 ± 0.99 | 16.63 ± 0.34 | 7.22 ± 0.70 | 3.00 (0.63) |
| JAY1638 | 46.60 ± 1.87 | 58.07 ± 0.44 | 83.22 ± 0.50 | 93.55 ± 0.37 | 2.25 (0.52) |
| JAY1640 | 47.40 ± 0.74 | 63.05 ± 0.98 | 90.82 ± 0.14 | 97.47 ± 0.10 | 1.00 (0.43) |
| JAY1642 | 40.25 ± 1.59 | 28.00 ± 0.82 | 24.68 ± 0.81 | 15.60 ± 0.63 | 3.50 (0.13) |
| JAY1644 | 47.95 ± 0.39 | 45.53 ± 1.02 | 62.88 ± 0.31 | 61.22 ± 0.78 | 4.00 (0.21) |
| JAY1646 | 49.20 ± 0.90 | 55.67 ± 0.72 | 79.45 ± 0.28 | 88.7 ± 0.50 | 3.25 (0.25) |
| JAY1648 | 45.40 ± 0.44 | 65.70 ± 1.03 | 93.83 ± 0.24 | 97.42 ± 0.05 | 4.00 (0.21) |
| JAY1650 | 49.45 ± 0.93 | 46.10 ± 0.72 | 68.23 ± 1.57 | 70.28 ± 1.85 | 4.25 (0.21) |

a. Mean value between 3 replicates plus or minus standard error.

b. Median value between 3 replicates. Standard error of the median indicated in parentheses and calculated by multiplying standard error of mean by 1.253.

Table S4 (continued). Phenotypic data of inbreds from competition assays (CA) and tolerance to high temperature assays (HT).

| Strain | ^a Mean % inbred cells in the population in CA | | | | ^b Median Score in HT |
|---------|--|--------------|--------------|--------------|---------------------------------|
| | Cycle 0 | Cycle 2 | Cycle 5 | Cycle 8 | |
| JAY1652 | 45.75 ± 0.47 | 54.32 ± 0.77 | 81.95 ± 0.74 | 91.70 ± 0.60 | 4.25 (0.91) |
| JAY1654 | 39.90 ± 1.48 | 61.55 ± 0.73 | 88.72 ± 0.42 | 96.30 ± 0.20 | 4.00 (0.13) |
| JAY1656 | 45.40 ± 0.73 | 44.32 ± 0.85 | 49.82 ± 2.32 | 50.20 ± 3.18 | 4.25 (0.39) |
| JAY1658 | 45.35 ± 0.63 | 65.97 ± 0.82 | 90.13 ± 0.33 | 96.32 ± 0.24 | 4.00 (0.00) |
| JAY1660 | 49.60 ± 0.93 | 64.93 ± 0.47 | 87.97 ± 0.53 | 96.30 ± 0.13 | 4.00 (0.26) |
| JAY1662 | 40.60 ± 0.88 | 30.02 ± 0.95 | 20.12 ± 1.01 | 12.95 ± 0.99 | 4.00 (0.26) |
| JAY1664 | 47.05 ± 0.69 | 67.30 ± 1.42 | 92.93 ± 0.49 | 97.93 ± 0.20 | 0.00 (0.00) |
| JAY1666 | 51.83 ± 0.42 | 41.30 ± 0.85 | 28.87 ± 0.30 | 18.93 ± 0.39 | 0.00 (0.00) |
| JAY1668 | 52.20 ± 0.58 | 28.53 ± 0.56 | 5.56 ± 0.46 | 2.29 ± 0.31 | 2.50 (0.75) |
| JAY1670 | 52.02 ± 0.42 | 63.97 ± 0.81 | 71.73 ± 0.68 | 74.48 ± 0.56 | 3.00 (0.10) |
| JAY1672 | 50.53 ± 0.36 | 51.68 ± 0.99 | 45.70 ± 0.93 | 25.42 ± 0.72 | 0.50 (0.42) |
| JAY1674 | 49.07 ± 0.40 | 37.38 ± 0.49 | 18.28 ± 0.53 | 9.05 ± 0.32 | 3.00 (0.13) |
| JAY1676 | 50.70 ± 0.48 | 51.02 ± 1.44 | 45.40 ± 0.58 | 29.93 ± 0.55 | 0.50 (0.42) |
| JAY1678 | 49.55 ± 0.20 | 41.27 ± 1.36 | 50.87 ± 0.58 | 46.52 ± 0.79 | 1.00 (0.39) |
| JAY1680 | 45.35 ± 0.47 | 59.17 ± 0.39 | 28.92 ± 1.55 | 38.80 ± 0.58 | 3.00 (0.21) |
| JAY1682 | 50.23 ± 0.71 | 45.62 ± 1.01 | 47.33 ± 0.62 | 34.42 ± 0.52 | 3.00 (0.42) |
| JAY1684 | 49.00 ± 0.31 | 36.33 ± 0.68 | 14.77 ± 0.68 | 5.20 ± 0.36 | 4.00 (0.26) |
| JAY1686 | 52.93 ± 0.42 | 31.18 ± 0.96 | 28.03 ± 0.29 | 17.82 ± 0.52 | 4.00 (0.26) |
| JAY1688 | 52.82 ± 0.42 | 30.47 ± 1.46 | 24.83 ± 0.45 | 14.92 ± 0.22 | 5.00 (0.00) |
| JAY1690 | 49.32 ± 0.37 | 28.15 ± 0.42 | 8.07 ± 0.38 | 2.89 ± 0.29 | 5.00 (0.13) |
| JAY1692 | 43.13 ± 0.34 | 32.80 ± 1.55 | 24.40 ± 1.12 | 15.27 ± 0.74 | 4.00 (0.00) |
| JAY1694 | 48.32 ± 0.67 | 30.17 ± 1.76 | 27.12 ± 1.13 | 14.50 ± 1.14 | 3.00 (0.00) |
| JAY1696 | 44.93 ± 0.49 | 44.48 ± 0.98 | 31.73 ± 1.56 | 33.77 ± 0.86 | 4.25 (0.25) |
| JAY1698 | 52.42 ± 0.53 | 48.17 ± 1.22 | 31.33 ± 1.40 | 16.68 ± 0.68 | 3.00 (0.00) |
| JAY1700 | 48.68 ± 0.31 | 32.05 ± 1.64 | 26.65 ± 0.45 | 2.93 ± 0.22 | 5.00 (0.00) |
| JAY1702 | 50.48 ± 0.73 | 30.12 ± 1.45 | 17.13 ± 0.34 | 2.96 ± 0.46 | 2.50 (0.51) |

a. Mean value between 3 replicates plus or minus standard error.

b. Median value between 3 replicates. Standard error of the median indicated in parentheses and calculated by multiplying standard error of mean by 1.253.

Table S4 (continued). Phenotypic data of inbreds from competition assays (CA) and tolerance to high temperature assays (HT).

| Strain | ^a Mean % inbred cells in the population in CA | | | | ^b Median Score in HT |
|---------|--|--------------|--------------|--------------|---------------------------------|
| | Cycle 0 | Cycle 2 | Cycle 5 | Cycle 8 | |
| JAY1704 | 49.87 ± 0.45 | 55.72 ± 0.49 | 71.50 ± 0.42 | 84.67 ± 0.30 | 4.50 (0.38) |
| JAY1706 | 50.80 ± 0.96 | 64.72 ± 0.45 | 86.90 ± 0.29 | 96.1 ± 0.41 | 3.50 (0.13) |
| JAY1708 | 53.53 ± 0.48 | 81.92 ± 0.25 | 92.57 ± 0.25 | 97.25 ± 0.21 | 3.00 (0.26) |
| JAY1710 | 46.03 ± 0.37 | 25.37 ± 0.54 | 25.25 ± 0.79 | 24.93 ± 1.63 | 5.00 (0.21) |
| JAY1712 | 51.80 ± 0.32 | 19.22 ± 0.67 | 16.70 ± 0.50 | 16.70 ± 0.72 | 4.00 (0.00) |
| JAY1714 | 52.30 ± 0.34 | 24.70 ± 0.54 | 22.67 ± 0.60 | 23.55 ± 0.37 | 3.00 (0.63) |
| JAY1716 | 51.90 ± 0.22 | 7.05 ± 0.32 | 3.56 ± 0.51 | 4.00 ± 0.56 | 2.50 (0.65) |
| JAY1718 | 51.47 ± 0.27 | 46.05 ± 0.35 | 57.65 ± 0.55 | 75.33 ± 0.87 | 2.00 (0.52) |
| JAY1720 | 49.82 ± 0.28 | 41.17 ± 1.24 | 43.68 ± 1.19 | 35.63 ± 1.01 | 1.50 (0.54) |
| JAY1722 | 46.68 ± 0.39 | 7.64 ± 0.76 | 5.25 ± 0.38 | 4.94 ± 0.53 | 3.00 (0.00) |
| JAY1724 | 43.30 ± 0.75 | 18.58 ± 0.55 | 14.02 ± 0.42 | 8.49 ± 0.40 | 1.50 (0.28) |
| JAY1726 | 43.73 ± 0.59 | 8.52 ± 0.35 | 4.18 ± 0.43 | 5.11 ± 0.45 | 4.00 (0.13) |
| JAY1728 | 39.18 ± 0.94 | 34.82 ± 2.23 | 25.07 ± 1.19 | 16.42 ± 0.36 | 3.00 (0.26) |
| JAY1730 | 40.37 ± 0.27 | 33.60 ± 2.30 | 23.30 ± 0.78 | 17.52 ± 1.46 | 1.00 (0.26) |
| JAY1732 | 38.78 ± 2.23 | 13.20 ± 0.85 | 4.97 ± 0.73 | 1.68 ± 0.17 | 3.00 (0.26) |
| JAY1734 | 51.42 ± 0.35 | 36.17 ± 0.54 | 12.00 ± 0.40 | 3.91 ± 0.49 | 4.00 (0.21) |
| JAY1736 | 50.88 ± 0.33 | 59.33 ± 0.47 | 69.98 ± 0.49 | 79.22 ± 0.42 | 3.00 (0.00) |
| JAY1738 | 51.72 ± 0.35 | 55.88 ± 0.70 | 58.42 ± 1.26 | 61.85 ± 1.41 | 3.00 (0.26) |
| JAY1740 | 47.28 ± 0.35 | 35.82 ± 0.53 | 19.05 ± 0.62 | 10.22 ± 0.56 | 4.00 (0.00) |
| JAY1742 | 53.95 ± 0.25 | 69.72 ± 0.25 | 90.65 ± 0.28 | 97.78 ± 0.10 | 1.50 (0.28) |
| JAY1744 | 55.13 ± 0.27 | 65.48 ± 0.32 | 86.57 ± 0.46 | 95.35 ± 0.05 | 0.50 (0.36) |
| JAY1746 | 48.22 ± 0.24 | 42.45 ± 0.53 | 41.25 ± 0.61 | 38.33 ± 0.80 | 3.00 (0.13) |
| JAY1748 | 51.38 ± 0.38 | 38.30 ± 0.33 | 22.48 ± 0.18 | 12.52 ± 0.36 | 2.50 (0.23) |
| JAY1750 | 48.55 ± 0.59 | 15.60 ± 0.43 | 4.05 ± 0.47 | 1.83 ± 0.33 | 4.00 (0.13) |
| JAY1752 | 48.17 ± 0.81 | 15.15 ± 0.13 | 4.11 ± 0.49 | 2.31 ± 0.50 | 3.00 (0.13) |

a. Mean value between 3 replicates plus or minus standard error.

b. Median value between 3 replicates. Standard error of the median indicated in parentheses and calculated by multiplying standard error of mean by 1.253.

Table S5. List of phased JAY270 HetSNPs interrogated for confirmation of UPD strains.

| Marker SGD coordinates | | PCR primers | Polymorphism detection method | ^a SGD Watson bases | JAY270 Watson phased bases | |
|------------------------|------------|-----------------|-------------------------------|-------------------------------|----------------------------|----------|
| Chromosome | Nucleotide | | | | Chr-P | Chr-M |
| Chr4 | 378,184 | JAO1576/JAO1577 | <i>Bam</i> HI | <u>C</u> | T | <u>C</u> |
| Chr4 | 710,025 | JAO1574/JAO1575 | <i>Bgl</i> II | <u>T</u> | G | <u>T</u> |
| Chr14 | 259,198 | JAO1692/JAO1693 | <i>Mfe</i> I | <u>T</u> | <u>T</u> | A |
| Chr14 | 758,547 | JAO1694/JAO1695 | <i>Mse</i> I | <u>A</u> | G | <u>A</u> |
| Chr15 | 16,733 | JAO1696/JAO1697 | <i>Sal</i> II | <u>C</u> | T | <u>C</u> |
| Chr15 | 130,295 | JAO1983/JAO1984 | <i>Pst</i> I | <u>A</u> | <u>A</u> | C |
| Chr15 | 1,058,946 | JAO1588/JAO1589 | <i>Nde</i> I | T | T | <u>C</u> |

a. Nucleotides that are underlined indicate the sequences that are cut by the respective restriction endonucleases, whereas lack of underlining corresponds to no cut site.

Table S6. Regions showing significant association to the heat tolerance and competitive growth phenotypes and their gene contents.

| Phenotype | Chr | Genome Coordinates | HetSNPs | Max LOD | Total Genes | Genes with HetSNPs | Genes with Nonsynonymous Substitution(s) | List of Genes with Nonsynonymous Substitution(s) |
|--------------------|-----|----------------------------------|---------|---------|-------------|--------------------|--|---|
| Heat Tolerance | 2 | Chr02_207698..214801 (7.1 Kb) | 14 | 4.45 | 2 | 2 | 1 | <i>YBL008W-A</i> |
| | 5 | Chr05_549677..565641 (16.0 Kb) | 40 | 5.47 | 9 | 2 | 2 | <i>ISC10, SLO1</i> |
| | 6 | Chr06_74814..97759 (22.9 Kb) | 91 | 6.23 | 14 | 13 | 8 | <i>HAC1, CAF16, GYP8, STE2, BST1, BUD27, FRS2, GAT1</i> |
| | 7 | Chr07_82753..88718 (6.0 Kb) | 11 | 4.61 | 4 | 2 | 1 | <i>KIP3</i> |
| | 7 | Chr07_673392..730469 (57.1 Kb) | 9 | 7.12 | 30 | 2 | 0 | none |
| | 7 | Chr07_849524..887971 (38.4 Kb) | 14 | 5.56 | 20 | 3 | 0 | none |
| | 12 | Chr12_176348..201613 (25.3 Kb) | 88 | 4.28 | 14 | 10 | 6 | <i>PRS2, YEH2, IRC25, SDO1, UBR2, ADE16</i> |
| | 13 | Chr13_75040..92531 (17.5 Kb) | 49 | 8.67 | 12 | 9 | 4 | <i>TAF13, YML096W, UTP14, RPM2</i> |
| | 13 | Chr13_715996..749967 (34.0 Kb) | 13 | 8.70 | 19 | 5 | 2 | <i>MRE11, BCH1</i> |
| | 13 | Chr13_754723..814438 (59.7 Kb) | 59 | 5.20 | 37 | 19 | 13 | <i>YMR244W, YMR253C, GFD1, PET111, TRM732, TPS3, RSN1, PPA2, PRP24, RRN9, URA10, SCS7, ZDS1</i> |
| | 14 | Chr14_528514..605480 (77.0 Kb) | 66 | 11.06 | 47 | 14 | 9 (1 nonsense) | <i>COG5, SFB2, ALG11, SLM2 (nonsense), YNL034W, KTR5, CRZ1, SAM50, FAP1</i> |
| | 14 | Chr14_691878..695118 (3.2 Kb) | 6 | 5.26 | 3 | 1 | 0 | none |
| | 15 | Chr15_764174..781222 (17.0 Kb) | 13 | 6.01 | 10 | 1 | 1 | <i>YOR228C</i> |
| Competitive Growth | 3 | Chr03_77849..84690 (6.8 Kb) | 44 | 4.34 | 5 | 3 | 2 | <i>KCC4, YCL021W-A</i> |
| | 4 | Chr04_556996..592486 (35.5 Kb) | 99 | 6.00 | 24 | 18 | 11 | <i>DBF4, PST1, MAK21, YDR061W, LCB2, AIM7, RRG1, RRTR2, DOS2, DOA4, IPT1</i> |
| | 8 | Chr08_35149..51314 (16.2 Kb) | 26 | 4.68 | 7 | 2 | 2 | <i>ECM29, WSC4</i> |
| | 10 | Chr10_467589..569699 (102.1 Kb) | 48 | 8.30 | 56 | 17 | 8 | <i>REC107, YJR030C, GEA1, CPR7, RAD26, URB2, TOR1, YAE1</i> |
| | 15 | Chr15_882911..889659 (6.7 Kb) | 3 | 4.13 | 2 | 1 | 0 | none |
| | 15 | Chr15_1024820..1069385 (44.6 Kb) | 138 | 4.31 | 23 | 12 | 10 | <i>YOR365C, GFB1, NDD1, NUD1, YOR376W-A, ATF1, FRE3, YOR381W-A, YOR385W, PHR1</i> |
| | 16 | Chr16_289873..361102 (71.2 Kb) | 134 | 5.93 | 40 | 22 | 15 | <i>SPP1, GIP3, ISU1, COX11, SPO19, TBF1, HHO1, NAN1, KAP120, MRP51, HOS3, CAR1, YPL109C, YPL108W, FMP30</i> |

Table S7. Single locus analysis of variance for heat tolerance and competitive growth phenotypes in the inbred strains.

| Phenotype | Locus | Significance | ^a PVE | ^b Relative PVE | Inheritance model | Parent Contributing high value allele |
|--------------------|---------------|--------------|------------------|---------------------------|-------------------|---------------------------------------|
| Heat Tolerance | Chr02_209988 | <0.0001 | 23.12 | 0.48 | Additive | P |
| | Chr05_564935 | <0.0001 | 27.59 | 0.58 | Dominant | P |
| | Chr06_87106 | <0.0001 | 30.77 | 0.64 | Additive | M |
| | Chr07_86432 | <0.0001 | 23.82 | 0.50 | Additive | M |
| | Chr07_677969 | <0.0001 | 34.34 | 0.72 | Additive | P |
| | Chr07_858098 | <0.0001 | 27.99 | 0.58 | Additive | P |
| | Chr12_188842 | <0.0001 | 22.31 | 0.47 | Dominant | P |
| | Chr13_83905 | <0.0001 | 40.04 | 0.84 | Additive | M |
| | Chr13_743742 | <0.0001 | 40.16 | 0.84 | Additive | P |
| | Chr13_799417 | <0.0001 | 26.45 | 0.55 | Additive | P |
| | Chr14_572098 | <0.0001 | 47.95 | 1.00 | Additive | M |
| | Chr14_691878 | <0.0001 | 26.68 | 0.56 | Dominant | M |
| | Chr15_764348 | <0.0001 | 29.86 | 0.62 | Additive | M |
| Competitive Growth | Chr03_82057 | <0.0001 | 22.6 | 0.58 | Additive | P |
| | Chr04_560433 | <0.0001 | 29.82 | 0.77 | Additive | M |
| | Chr08_49196 | <0.0001 | 24.13 | 0.62 | Additive | P |
| | Chr10_488369 | <0.0001 | 38.74 | 1.00 | Additive | P |
| | Chr15_888521 | <0.0001 | 21.64 | 0.56 | Additive | P |
| | Chr15_1055015 | <0.0001 | 22.47 | 0.58 | Dominant | M |
| | Chr16_340133 | <0.0001 | 29.52 | 0.76 | Additive | M |

- a. The PVE values obtained through this single-QTL model are likely over-estimates of the values.
b. PVE values normalized to highest value PVE for each phenotype.

Table S8. Multi-locus QTL models that best explain variation seen in heat tolerance and competitive growth inbred strains.

| Phenotype | Locus | Significance | Total Percent Variance Explained |
|--------------------|---------------|--------------|----------------------------------|
| Heat Tolerance | Chr06_87106 | <0.0001 | 75.88 |
| | Chr13_743742 | <0.0001 | |
| | Chr14_572098 | <0.0001 | |
| Competitive Growth | Chr04_560433 | 0.0015 | 57.27 |
| | Chr10_488369 | <0.0001 | |
| | Chr15_1055015 | 0.0047 | |