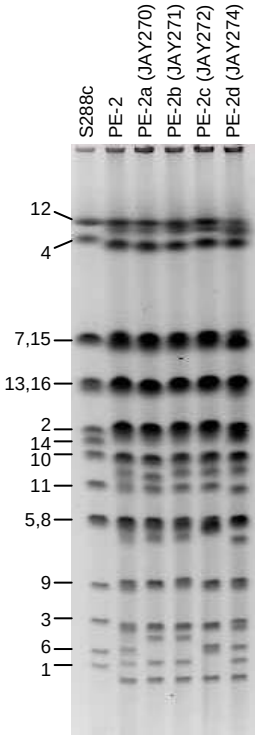
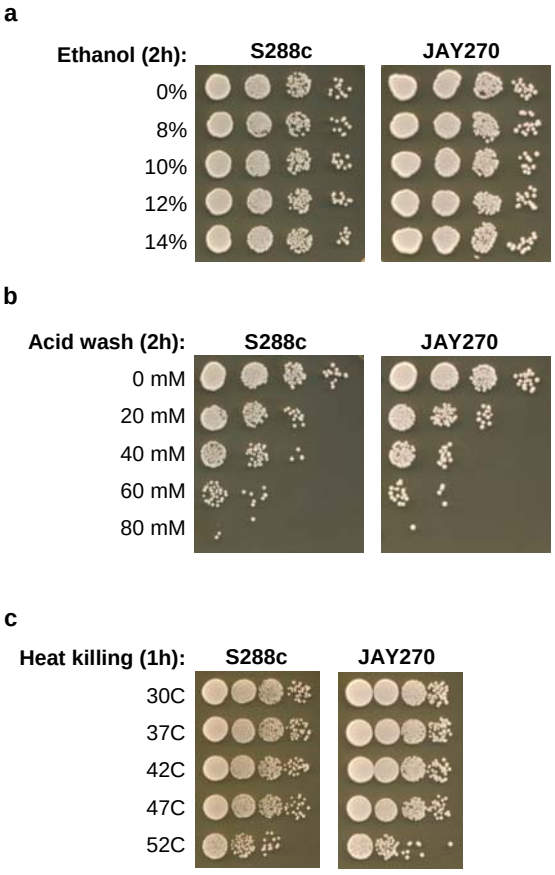


Supplemental Figure 1. Molecular karyotype analysis of PE-2 and its derived pure strains.

a. PFGE analysis of the original PE-2 mixed cultured and of four independent clonal single colony isolates derived from PE-2. Note the diffuse chromosomal banding pattern in PE-2 which was caused by the presence of subpopulations of cells with slightly divergent karyotypes. In contrast, the bands observed in the purified isolates (JAY270 to JAY274) are discrete and sharper as expected for clonal cell lineages, each carrying specific chromosomal variants.

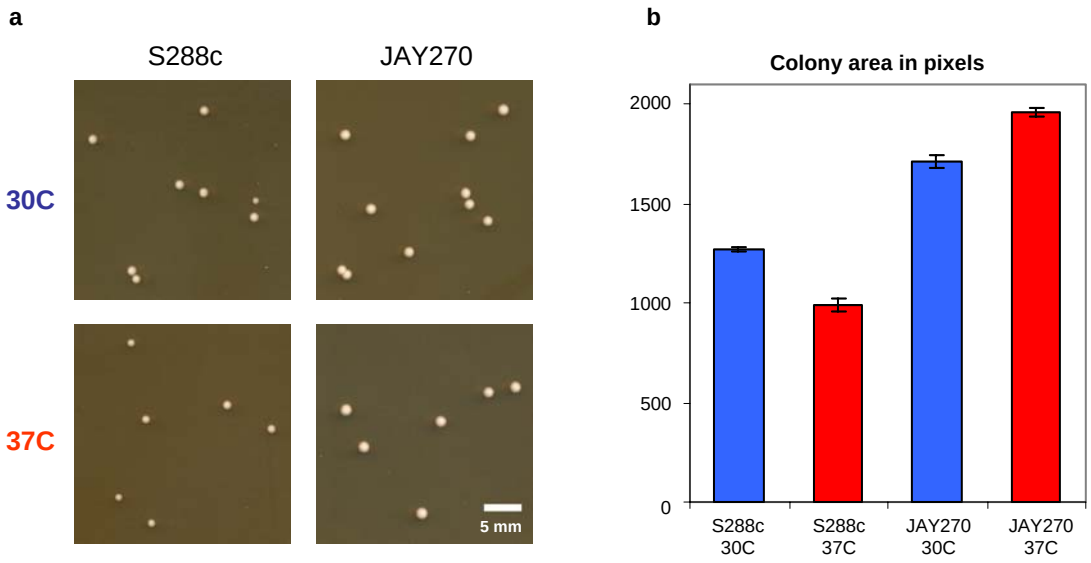


Supplemental Figure 2. Effect of incubation on various stressors on cell viability.

10^{-1} dilutions of saturated overnight cultures of an S288c-derived diploid (JAY309) and JAY270 were incubated in the presence of various concentrations of ethanol (**a**), sulfuric acid (**b**), or at different temperatures (**c**). After the indicated time periods, these aliquots were serially diluted in sterile water in 10^{-1} fold increments, spotted to rich medium and allowed to grow at 30C for 48 hours to assess cell viability.

No significant differences in viability were observed between the two strains for ethanol tolerance up to 14% concentration (**a**). Under standard sugarcane extract fermentation the final ethanol concentration rarely exceeds 12%.

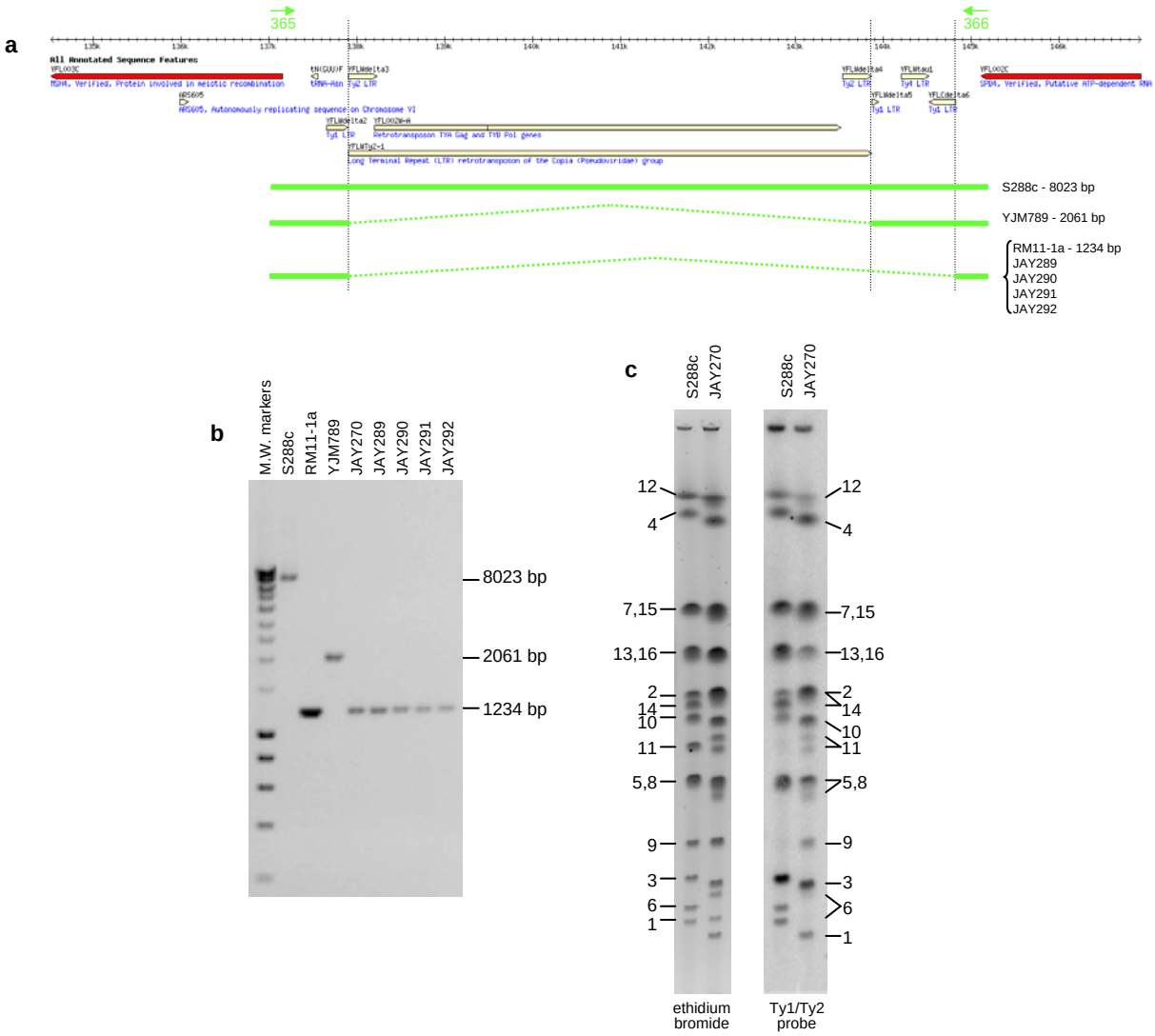
Also, no significant differences were observed in tolerance to short-term acid wash (**b**), or to short-term heat killing (**c**) at high temperatures beyond those compatible with cell growth (42C, 47C and 52C).



Supplemental Figure 3. Effect of incubation temperature on colony size.

a. Cropped sample Petri dish scans showing difference in colony size between S288c and JAY270 grown in rich medium (YEPD) for 48 hours at 30C and 37C. Scale bar is 5x1 mm.

b. Quantification of colony sizes. Cells were plated to a density of about 20 colonies per plate. After 48 hours of incubation, Petri dishes were scanned at 800 dpi and images were analyzed in Adobe Photoshop CS2 to measure the colonies. The average colony area in pixels with standard error are shown. In the images above, 1 mm^2 equals 986 pixels. In the specific experiment shown, the diameter of 13 well-spaced colonies was determined. Colonies were then sorted by size and two smallest and the two largest were ignored for the average area calculation to minimize the influence of possible *petite* colonies in the plate. The same trend was observed in another two independent experimental repetitions.

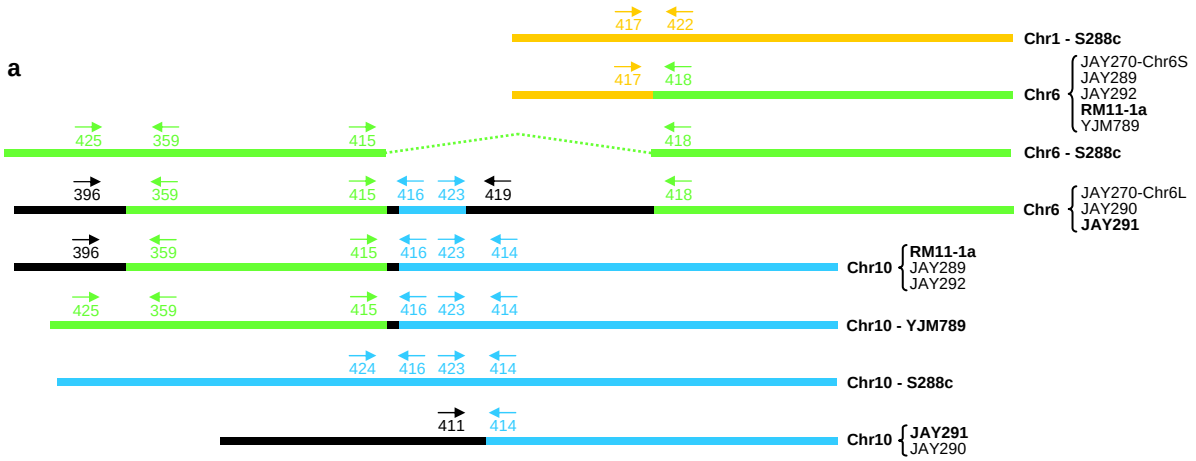


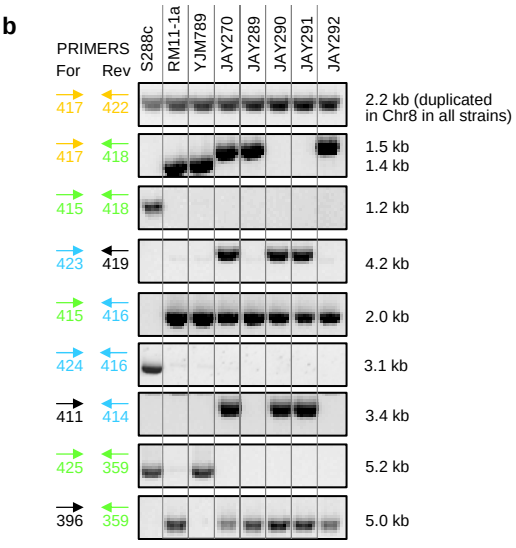
Supplemental Figure 4. PCR mapping of the *YFLWTy2-1* region and Ty distribution in JAY270.

a. Screen capture of GBrowser software showing the *YFLWTy2-1* region in Chr6 of S288c, including the *MSH4* and *SPB4* flanking genes. The forward PCR primer JAO365 is positioned at the 5' end of *MSH4*, and the reverse primer JAO366 is positioned at the 3' end of *SPB4*. The schematics below the map show the expected PCR products from this region in various *S. cerevisiae* strains. S288c contains multiple Ty element insertions upstream of the tN(GUU)f tRNA gene, including the full length *YFLWTy2-1* element. YJM789 has the same LTRs as S288c, but lacks the Ty2 element. Finally, RM11-1a and the JAY270 ascospores contain only the YFLWdelta2 LTR, which likely marks the founding Ty insertion at this site.

b. Ethidium bromide stained agarose gel showing the JAO365 / JAO366 PCR products from (a).

c. PFGE and Southern analysis of Ty1 and Ty2 retrotransposons in JAY270. S288c chromosomes are indicated to the left and JAY270 chromosomes to the right. The probe used hybridizes to both Ty1 and Ty2 elements.





c

Primer	Nucleotide sequence (5'→3')
JAO359	TCGCTGTCTCTTCAATGTCGACAG
JAO365	AAACTAGATAGATTAGATTCACTC
JAO366	GTTTCCAGCAAAGCTATCCAAGGC
JAO396	AGTTTATATTGTAGTGGCATGACTC
JAO411	CTTACTGATAGCGATGCCGCAGAC
JAO414	ACAGCTGTCAAAGGCACTAGCAGG
JAO415	TGTCAGTGTGCGCTTATCACG
JAO416	CAGTTCATTGATCTTGATAATGAC
JAO417	CATTGCTGCGATAGAATGGAG
JAO418	TGCAATTGATGCCTACCGGAG
JAO419	CCCACGACGTGCTTACAACGCACT
JAO422	AACTCAAGGACGAGGAGTATTCGG
JAO423	GTCATTATCAAGATCAATGAACTG
JAO424	AGAGTTCAGTTGTTGCGGACCGAG
JAO425	ACATCACCAAGATTAAGGCGGAG

Supplemental Figure 5. PCR mapping of chromosomal rearrangements at the left arm of Chr6 and Chr10.

a. Map of PCR primer locations in the region examined. This map matches the genome rearrangement breakpoints in Fig. 6b, but it is not drawn to scale. The chromosomal regions and primers are color coded according to their correspondence to S288c's Chr1 (orange), Chr6 (green) and Chr10 (blue), while regions in black correspond to sequences not found in the S288c genome. Arrows indicate primer forward or reverse orientations.

b. Cropped ethidium bromide stained agarose gels with PCR reactions using as template genomic DNA from the indicated strains. The primer pair used in each reaction is shown to the left, and the size of the expected target product is indicated to the right. Only the region of each gel containing the target PCR product is shown. The expected target band was observed in all cases. Occasional unspecific PCR products were also present in a few of the reactions, but were cropped out to facilitate visualization of the results.

c. PCR primers used in mapping of chromosomal rearrangements at the left arm of Chr6 and Chr10, and of the *YFLWTy2-1* insertion (Supplemental Fig. 4).