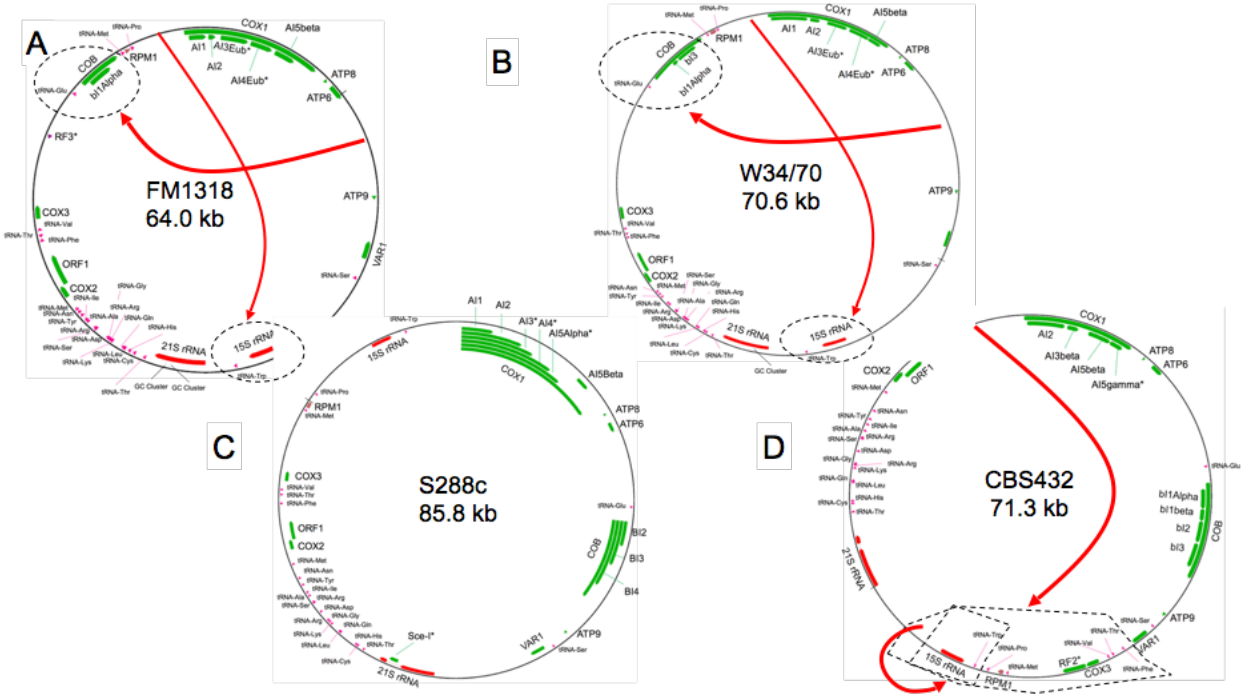
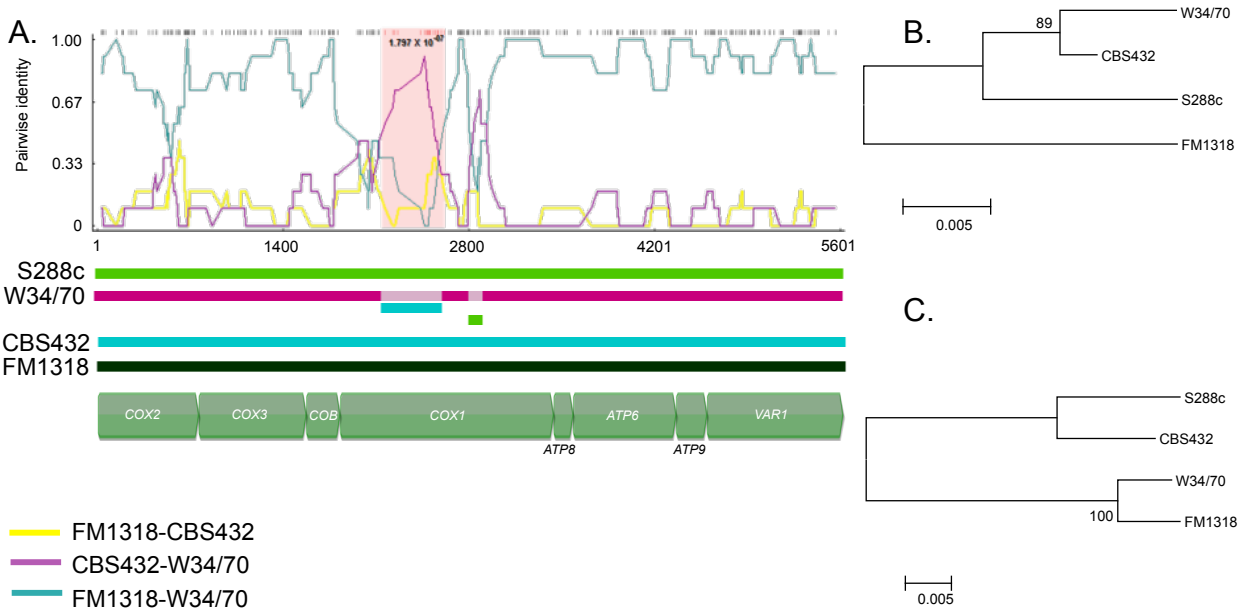
Fig. S1



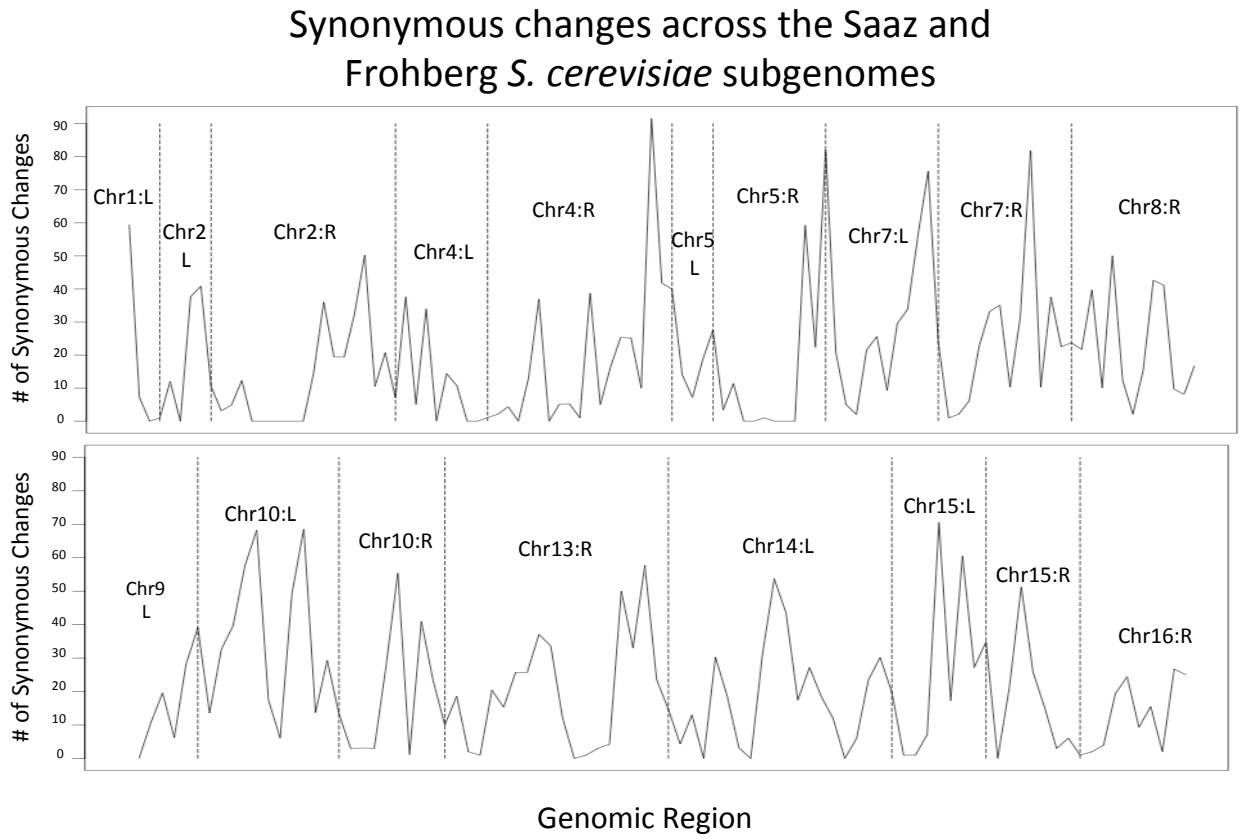
Schematic representation of *S. eubayanus* (FM1318), lager-brewing yeast (Frohberg strain W34/70), *S. cerevisiae* (S288c), and *S. paradoxus* (CBS 432) mitochondrial genome annotations. Mitochondrial genes, rRNAs, tRNAs, and non-coding RNAs are represented in green, red, pink, and brown, respectively. Genes with asterisks are additional elements or gene sequences in each mitochondria compared with *S. cerevisiae* S288c mtDNA. Red arrows represent rearrangements compared to S288c. *S. paradoxus RF2* was annotated in this study.

Fig. S2



Lager-brewing yeast (Frohberg strain W34/70) mtDNA shows an introgression from *S. paradoxus* in the *COX1* gene. A) The output results from RDPv4 shows an introgression in the *COX1* gene in W34/70 involving exon 4. Other potential introgressions, such as in *COB* and the 5' end of *COX1,* were also detected but not well supported. A Neighbor-Joining tree for the introgressed (B) and non-introgressed regions (C) is shown.

Fig. S3

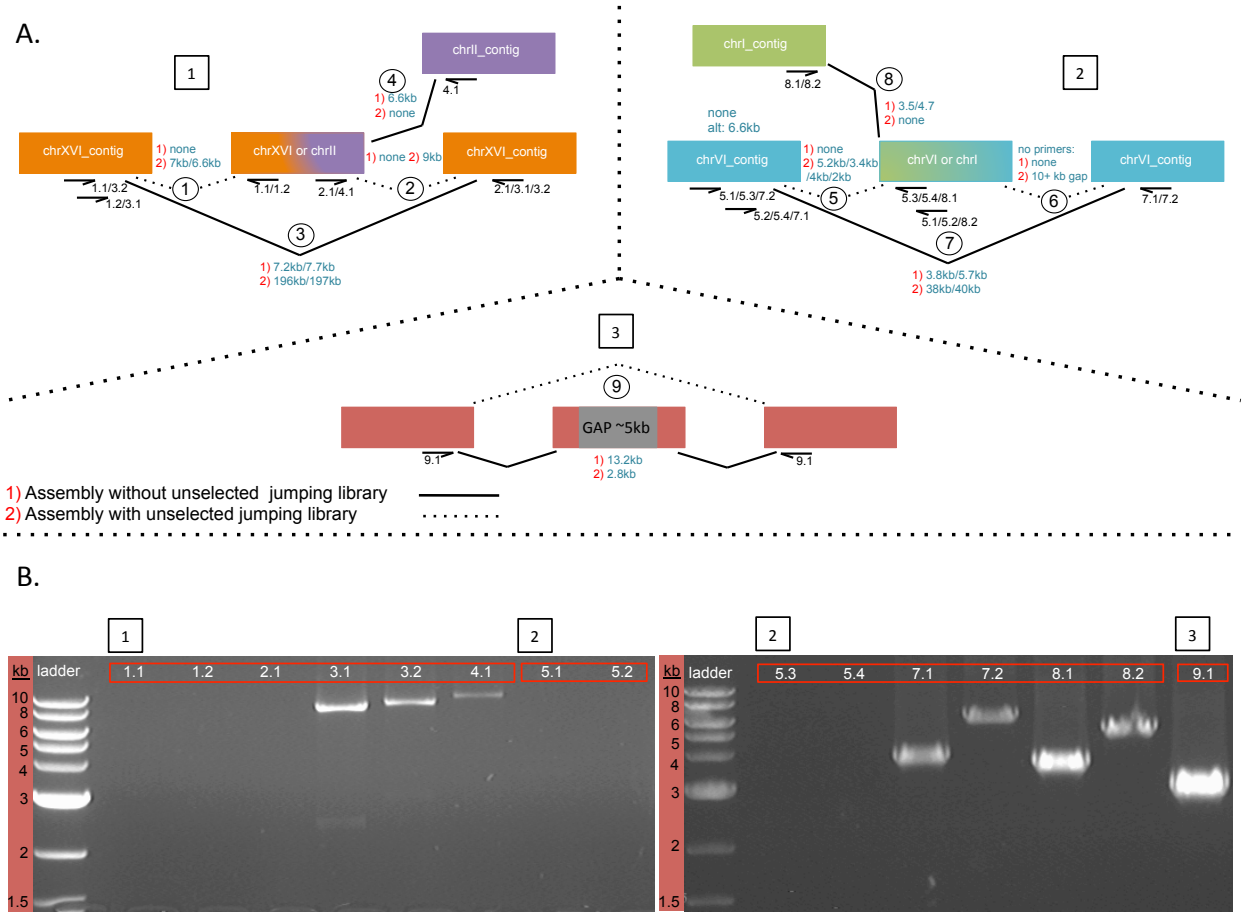


Sliding window (10 gene window) of the number of synonymous changes between the *S. cerevisiae* subgenomes of the Saaz and Frohberg lineages of lager-brewing yeasts. Only chromosome arms with at least 40 conserved genes are shown. Due to differences in genome content between the two lineages, not all genomic regions are represented. With multiple origins of lager yeasts from haploid or low heterozygosity *S. cerevisiae* parents drawn from a meiotically reproducing population, comparisons of the genomes would be expected to reveal a range of diversity values, including some haplotype blocks of low diversity. In contrast, widespread loss of heterozygosity could explain the previous observations of low heterozygosity (Dunn and Sherlock 2008) if a single heterozygous *S. cerevisiae* individual had given rise to the both lineages. Under this model, approximately half of all sites that were heterozygous in the parent would

have had the same alleles fixed in both lineages by chance. As a result, some segments of the genome would have high diversity between the Saaz and Frohberg lineages, and others would have almost no diversity. Examination of d_S along shared portions of the Saaz and Frohberg *S. cerevisiae* genomes failed to demonstrate the expected proportion of segments with low diversity expected under this scenario, indicating that a single diploid heterozygous individual was not the *S. cerevisiae* donor for the lager-brewing yeast lineages.

Dunn B, Sherlock G. 2008. Reconstruction of the genome origins and evolution of the hybrid lager yeast Saccharomyces pastorianus. Genome Res. 18:1610–1623.

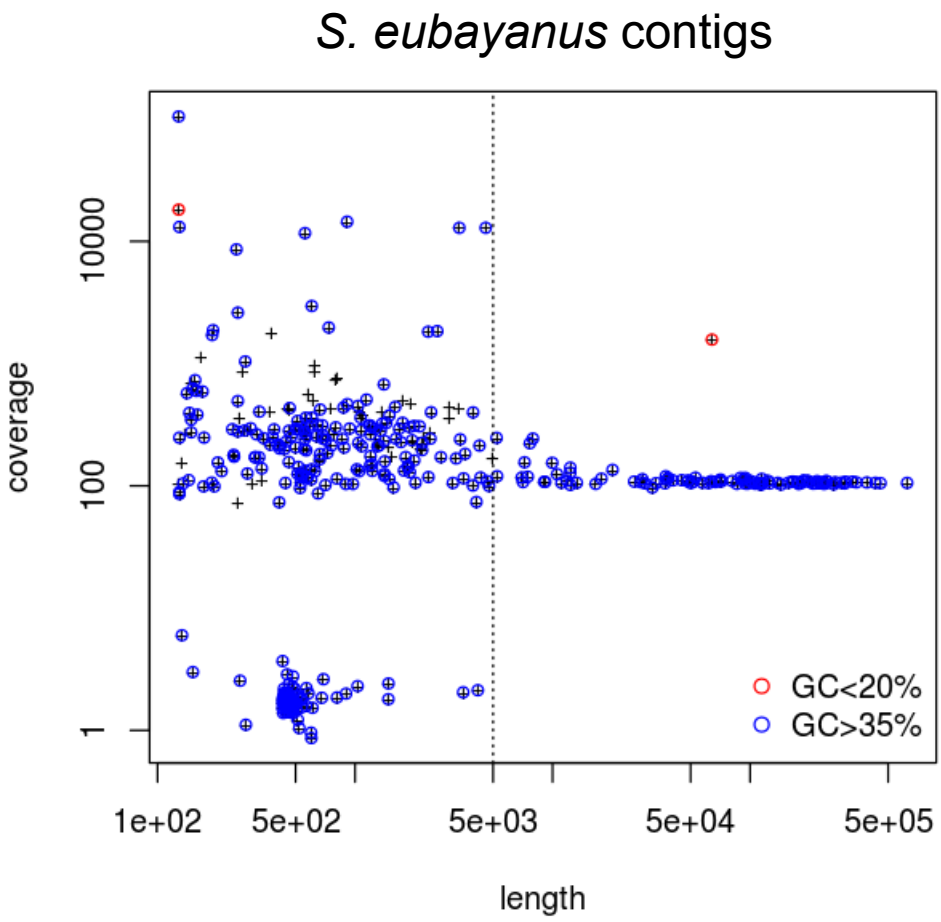
Fig. S4



Verification of alternative *S. eubayanus* genome assemblies by PCR. A) shows the regions, numbered 1-3 (boxed numbers), of the *S. eubayanus* genome with alternative configurations between the assembly made without the unselected jumping library (solid line) and the one made with the unselected jumping library (dashed line). Each alternative connection is numbered (circled numbers), and primer pairs are numbered after the connection they test. Combinations of primers and their directions are indicated by half arrows, and the size of resulting bands that would occur under different assemblies are placed where alternative configurations are possible. B) shows the resulting bands from each pair of primers. Reactions that test the same set of

alternative connections are outlined in red with the number of the region they represent from part A) placed above. For alternative configuration 3 (covered by PCR reaction 9.1), the assembly with the unselected jumping library fully closed the gap.

Fig. S5



GC content, length, and coverage of SPAdes contigs. Distribution of contigs (>200 bp) relative to their read coverage and length. Contigs with GC content > 35% and <20% marked in blue and red, respectively. The mtDNA contig of 64 kb was detected with GC content 17.5% and 14.6-fold greater coverage relative to the remaining contigs >5 kb (vertical dashed line).