Title
Core gene set of the species *Saccharomyces cerevisiae*

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Abstract
Examination of the genome sequence of *Saccharomyces cerevisiae* strain S288c and 93 additional diverse strains allows identification of the 5873 genes that make up the core set of genes in this species and gives a better sense of the organization and plasticity of this genome. *S. cerevisiae* strains each contain dozens to hundreds of strain specific genes. In addition to a variable content of retrotransposons, some strains contain a novel transposable element, Ty7. Examination further shows that some annotated putative protein coding genes are likely artifacts. We propose altering approximately 5% of the current annotations in the widely used reference strain S288c. Potential null alleles are common and found in all 94 strains examined, with these potential null alleles typically containing a single stop codon or frameshift. There are also gene remnants, pseudogenes, and variable arrays of genes. Among the core genes there are now only 373 protein coding genes of unknown function, classified as uncharacterized in the Saccharomyces Genome Database. This work suggests that there is a role for carefully edited and annotated genome sequences in understanding the genome organization and content of a species. We propose that gene remnants be added to the repertoire of features found in the *S. cerevisiae* genome, and likely other fungal species.

Introduction

It has now been more than 25 years since the publication and public release of the annotated eukaryotic genome sequence, that of the *Saccharomyces cerevisiae* reference strain S288c. One of the questions answered when that first genome was completed was, "only 5885 protein encoding genes are believed to exist (Goffeau et al. 1996)". Because the genome sequences of dozens of hemiascomycetes and more than 1000 genomes of strains of *S. cerevisiae* have been generated to varying degrees of completeness since then, it is now possible to ask a slightly different question, "what is the set of genes in the species *S. cerevisiae*?". To give a preliminary answer to this question we have re-examined 94 strains, the genome sequence of S288c as well as that of the 93 genomes published in Strope et al (Strope et al. 2015). In addition, this work depends on the tremendous amount of published data available on this species and closely related species. The work presented here would not be feasible without the compilation of this data into a single curated and searchable space, the Saccharomyces Genome Database (SGD) (Cherry et al. 2012). Additional data and analysis used here comes from work on *Ashbya gossypii* (Dietrich et al. 2004, Dietrich et al. 2013) the Yeast Gene Order Browser (YGOB) (Byrne and Wolfe 2005), and genomic and RNA-seq data from NCBI (Sayers et al. 2022).

Much of what is reported here has long been known, but with the addition of whole genome sequences it is possible now to analyze the genome structure in greater depth with greater resolution of the features found in those genomes. For example, in the 1960's it was known that there were at least five sets of genes for maltose utilization (then *MA*, now *MAL*), and that they are found at the ends of linkage groups (Halvorson et al. 1963, Mortimer and Hawthorne 1966). Thus the idea that the distal ends of linkage groups, now telomeres, of *S. cerevisiae* have more extensive variation than exists in the central areas of the linkage groups, now chromosomes, can be traced back nearly 60 years. Among the 94 strains, MAL11 and paralogs can be found at eleven chromosome ends (chromosome IL chromosome IIR chromosome IVL chromosome VIL chromosome VIIR chromosome IXL chromosome XL chromosome XR chromosome XIR chromosome XVL chromosome XVR). In this work we identify the location of the boundary between the less variable central region of each chromosome and the more variable subtelomeric region.

Another characteristic of *S. cerevisiae* strains is the presence of defective alleles. A pivotal event in the development of yeast genetics in the 1940's was the discovery of an *S. cerevisiae* strain lacking homothallism (Winge 1949), making it possible to do crosses and study mating. It is now known that the *D* gene, now *HO*, is a null allele in strain S288c, the result of missense mutations(Meiron et al. 1995). All strains of *S. cerevisiae* that we are examining in this work contain multiple genes where the allele present is a potential null allele, containing a frameshift or a stop codon. Understanding that there are naturally occurring potential null alleles and that there are highly diverged genes often resulting from introgression are keys to establishing a list of the core genes for this species. It is important to note that these potentially null alleles are here considered alleles, not pseudogenes which is a different phenomenon.

One aspect of moving from having a single genome sequence of *S. cerevisiae* to having thousands of genome sequences is the importance of having well defined terms to use in genome annotation. Fortunately for this species rules for chromosome numbering, gene names, and other needed terms have been adopted and are almost universally followed by the community, with a gene name registry formerly maintained by Robert Mortimer and now by SGD (von Borstel 1963, Mortimer and Hawthorne 1966, Mortimer et al. 1989, Cherry et al. 1997, SGD 1998, Engel et al. 2022). Some of the terms used in this work are defined in Table 1, following as closely as possible to the established terminology in the field. We propose the following terms for inclusion in the annotation of these genomes: central region of genome, core genes, and gene remnants, as they provide additional insight into the genome plasticity of *S. cerevisiae* strains. Furthermore, it is important to have a highly accurate reference genome annotation, so the reference genome does not become a source of propagation of errors.

Materials and Methods

Initial genome sequences of the 93 *S. cerevisiae* strains has been previously described (Strope et al. 2015). The sequences when originally submitted to GenBank in 2015 contained thousands of misassembles, gaps, and point errors. Sequences were reassembled with many gaps closed, many errors corrected, and the annotations updated in 2016. To further improve the assemblies by closing gaps and correcting errors, a more exhaustive assembly of the data was undertaken. All 1488 chromosomes contained one or more gaps or errors that have now been corrected. We are currently in the process of updating the sequences and the annotations in GenBank. Initial assemblies of the data using ABySS for the 93 genomes results, all together, in more than 300,000 contigs, with the exact number being difficult to determine as different assemblies using different parameters were used for the mitochondrial genome and rDNA. Alignment of these contigs with the S288c reference genomes identified that the majority of these *de novo* generated contigs are colinear with regions from the reference genome, though more than 12,000 contigs had unusual junctions. Localized assembly identified that while many of these unusual junctions were the result of deletion, inversion, translocation or other phenomena and were correct, about half of these contigs were incorrect junctions of fragments from different regions of the genome. These contigs were then broken. Localized assemblies were then carried out on all fragment ends, resulting in closing nearly all gaps.

Gap closure required multiple rounds of sequence correction and examination of remaining gaps. The most useful tools were the use of BWA (Li and Durbin 2009), samtools and bcftools (Li et al. 2009) to align the raw reads for each genome back on the assembled genome. Pilon (Walker et al. 2014) is then used to identify errors in the sequence based on the sorted BAM files. Pilon is very good at finding errors in the assembly, though the suggested corrections are less accurate, so all corrections were individually checked. Correcting point errors adjacent to gaps often allowed assembly across those gaps. It appears that many of the gaps resulted from failure by both velvet (Zerbino and Birney 2008) and ABySS (Simpson et al. 2009) to extend a contig past a region where multiple point errors had been introduced. Furthermore, from each BWA alignment, reads that failed to align to the assembled genome were extracted and built into a new fastq file. These fastq files were then *de novo* assembled with either velvet or ABySS. Nearly all of these reads are low quality sequence reads that fail to assemble into contigs, though some small contigs are generated. Blast identifies that many of these small contigs are missing from the genome being analyzed, but present in other *S. cerevisiae* genomes, suggesting that they are left out of the assembly. Addition of these fragments back into the genome often results in extension at the site of a gap, or sometimes gap closure. Corrections at a site within the existing sequence also occur.

Depth of coverage analysis was also used in each round of sequence editing. The depth of coverage of the Illumina sequence data used in this project is fairly uniform across the genome, and was previously used to identify aneuploidy, the number of rDNA repeats, and other aspects of the genome (Strope et al. 2015). Sequence regions as small as 200 bases that occur twice in the genome can be distinguished from sequences present only a single time. Regions present only a single time in the genome sequence that, based on depth of coverage analysis, should be present two or more times can thus be identified and the appropriate corrections made. Also, sequences included twice in the assembled genome that by depth of coverage should only have been present a single time were identified and similarly could be corrected.

Illumina read pair pairing information was also examined. From the BWA alignments, scripts were used to extract the pairing information. Singlets, cases where one pair sequence pair aligned in an unexpected way, were skipped. When there were multiple read pairs anomalies in the same region of unique sequence where either the pairs were in the wrong orientation, or the pair length was two large, or they paired to different chromosomes, then these regions are examined. Similarly all locations that were not spanned by any read pairs were examined.

Sites of errors were primarily repetitive sequence regions, regions of unusually low sequence coverage, and regions where there are a high number of low confidence base calls. The transposable elements Ty and the solo long terminal repeat (LTR) sequences from these elements are particularly problematic. Significant effort was made to verify the insertion location for each Ty and LTR element. Less effort was made to get the sequences of the Ty elements themselves correct, and there are still numerous sequence

errors within the sequences of the Ty elements of these genomes Furthermore, while the majority of gaps for each chromosome could be closed, the chromosome ends were problematic due to the high number of repeats, high sequence divergence from the reference genome, and that subtelomeric and telomeric regions are sometimes duplicated, sometimes lost, and sometimes translocated to other chromosomes. As described previously, (Strope et al. 2015), crosses were carried out so the number of translocations for each strain, usually zero, is known and so the assembled genomes should be colinear. This collinearity does not apply to the subtelomeric and telomeric sequences further complicating the editing of the sequences of these regions.

Additional tools used in this analysis include blast (Altschul et al. 1990), fasta (Pearson and Lipman 1988), Smith-Waterman alignment (Smith and Waterman 1981) clustal (Higgins and Sharp 1988), tRNA_scan_SE-1.3.1 (Lowe and Eddy 1997), PlasMapper (Wishart et al. 2023), EMBOSS (Rice et al. 2000), perl (Christiansen et al. 2012), C (Kernighan and Ritchie 1988), LAGAN (Brudno et al. 2003), and MAFFT (Katoh et al. 2002).

The S288c sequence and annotation were taken from SGD (Cherry et al. 2012, Engel et al. 2022) in April, 2023, where gene information was downloaded using the yeastmine tool (Balakrishnan et al. 2012), with additional information from YGOB (Byrne and Wolfe 2005), and from NCBI, including from the Short Read Archive (Sayers et al. 2022), and the genome sequences of other hemiascomycetes, particularly *Saccharomyces paradoxus* and *A. gossypii*. The genomes of *A. gossypii, Zygosaccharomyces rouxii, Kluyveromyces lactis, Kluyveromyces marxianus Torulaspora delbrueckii, Lachancea kluyveri* (formerly *Saccharomyces kluyveri*), *Lachancea waltii* (formerly *Kluyveromyces waltii*), *Lachancea thermotolerans, Zygosaccharomyces bailii,* and *Eremothecium cymbalariae* were used to identify orthologs present before the whole genome duplication (WGD). Genome sequences of additional *Saccharomyces sensu stricto* species *Saccharomyces mikatae, Saccharomyces kudriavzevii, Saccharomyces uvarum, Saccharomyces jurei, Saccharomyces eubayanus, Saccharomyces pastorianus, and Saccharomyces bayanus* were also used extensively.

For identifying genes currently annotated as verified or uncharacterized that are likely to be dubious the following criteria were used. Lack of conservation of the open reading frame in other species. Lack of conservation of the open reading frame in *S. cerevisiae* strains. Overlap with adjacent genes. Lack of RNA-seq data supporting that the gene is transcribed with reads on the plus strand with reads starting in the 5' region. Lack of evidence of a polyadenylation region. Unusual sequence, such as long homopolymer runs. Annotated to contain an intron where there is no evidence of intron splicing. Starting methionine missing in *S. cerevisiae* strains. Location unusually close to an adjacent gene. Lack of published data as to function. Another useful phenomenon is the ratio of non-synonymous to non-synonymous plus synonymous polymorphisms in a gene across many strains. For real genes, this ratio tends to be small. It should be noted that no combination of the above criteria proves that a gene should be considered dubious. It is the opinion of the authors that it is time to raise the bar and that published evidence that a gene is real should be necessary to keep questionable open reading frames from being relegated to dubious.

All sequence and annotation corrections are being submitted to GenBank with the same accession numbers as in Strope et al. Table S19 (Strope et al. 2015).

Results

Central Region of Chromosomes

Examination of the genomes of the 94 strains, S288c and the 93 strains (Strope et al. 2015), confirms a well-known pattern where the gene content of the central region of each chromosome in the genome is highly conserved between strains, but where the subtelomeric regions are significantly more variable in gene location and content (Teunissen and Steensma 1995, Li et al. 2014, Monerawela et al. 2015, McIlwain et al. 2016, Stefanini et al. 2022). To determine the core gene set, the central regions of all 93 strains were carefully edited with all gaps closed and numerous errors resolved. Significant effort was made to close the final gaps in the central regions of chromosomes. At the current time, there are no remaining gaps in what is described in this work as the central regions of the chromosomes, except for gaps in some FLO1, FLO5, FLO9 genes, genes that are at the terminus of the central regions of three chromosome ends. For the 1488 chromosomes of the 93 strains from Strope et al, there are still 2200 gaps in the subtelomeric regions, telomeric regions, and at the edge of the central region in genes FLO1, FLO5, FLO9, and FLO12. Furthermore, 2172 chromosome ends out of 2976 are Ns, not the expected terminal repeat sequence.

To identify the boundary of the central, conserved portion of the genome, the most distal gene on each end of each chromosome where 95% or more of the 94 strains have the same gene at that position and orientation was identified. The choice of 95% was arbitrary. Table 2 shows the most distal gene of the central region of the genomes for each chromosome end.

In most cases an annotated gene marked the boundary of the central region of the genome. An exception is the left end of the central region of chromosome VI that is marked by *YFL051C*, a gene of unknown function. Examination of the 94 strains identifies that *YFL051C* is in S288c and some other strains a fragment of a gene of the *FLO1*, *FLO5*, *FLO9*, and *FLO10* family, while in other strains it is a full length flocculin gene. This gene is intact in more than half of the 93 strains. We propose that *YFL051C* should be called *FLO12*, where the allele in S288c is a truncated allele (Figure S1).

An analysis of the essential genes in *S. cerevisiae*, taken from SGD using the yeastmine tool (Skrzypek and Hirschman 2011), shows that all essential genes are in the central region of the genome, as well as all tRNA genes found in S288c and all other known RNA coding genes. In addition, all 5' UTR and coding region introns in S288c are found in the central region of the chromosomes, other than 14 introns currently annotated in putative subtelomeric genes.

Examination of RNA-seq data for these 14 putative subtelomeric introns using the RNA-Seq dataset SRR488142 (*Saccharomyces cerevisiae* S288c Project jgi.doe.gov) finds RNA spanning the splice sites but no evidence that these introns are spliced. These 14 introns shown in Table S1 are here considered dubious. Excluding tRNA introns, there are currently annotated 285 nuclear introns. Among these eight have a 5' GC splice site, rather than the 5' GT splice site found for the other 277 introns. Six of these are among the dubious subtelomeric introns. A seventh one is in the gene *YJR079W*, which in this work is proposed to be a dubious gene. The eighth case is a real gene *YIL111W* (*COX5B*), and there is evidence in the SRR488142 RNA-seq dataset that this intron is spliced as annotated. The 5' <u>GC</u>ATGT splice site is conserved in 94/94 of the genomes considered here and appears to be the only real case of an intron in S288c with a 5' GC splice site.

The mitochondrial genome of *S. cerevisiae* is circular or circularly permuted and thus does not have variable sequence ends in the sense that chromosomes do. We consider the entire mitochondrial genome to be central. There is significant variation between *S. cerevisiae* mitochondrial genomes, but it is distributed across the genome in these 94 strains, as has been previously described (Vijayraghavan et al. 2019).

Core Genes

The set of core genes in *S. cerevisiae* is here defined as those genes (1) found in the central region of the genome, (2) present at the same position and orientation, relative to flanking genes, in more than 95% of strains, and (3) includes both protein coding genes and genes for which the final product is an RNA molecule. Non-transcribed features such as centromeres and origins of replication are not included. The mitochondrial genome, being circular or circularly permuted, is entirely central and encodes both core and strain specific genes. As previously reported the gene order within the 93 mitochondrial genomes is the same as in S288c, except for a region duplicated in YJM1242 (Vijayraghavan et al. 2019). Thus the conserved mitochondrial genes are core genes by the definition used here. In this current analysis a gene is considered present even if in some strains it is an apparently defective allele, a truncated allele, or an introgressed copy of the gene from a *sensu stricto* species at the conserved location and orientation. While most genes in any strain are core genes, there are also non-core genes including ubiquitous genes of variable genomic location and strain specific genes, those found in less than 95% of strains.

A total of 5873 core genes have been identified, the 5495 verified genes in Table S2, and the 378 uncharacterized genes in Table S3. Of the core genes, 41 are in the mitochondrial genome, and the remaining 5832 are encoded on the sixteen chromosomes. The core set of genes reported here is somewhat larger than the core set of genes reported in Peter et al. (Peter et al. 2018) of 4990 and the 4900 gene core set reported by McCarthy and Fitzparick (McCarthy and Fitzpatrick 2019). This is due to differences in the definition of what is a core gene in these papers and how a core gene is defined here. The subtelomeric regions, as the term is used in this work, refers to the regions outside the central region, but not including the terminal telomeric repeat. These subtelomeric regions contain transporters, genes in synthetic and catabolic pathways, duplicate copies of genes, genes acquired by horizontal gene transfer, and other important genes that contribute to the diversity of this species, yet they are strain and positionally variable and as such are not here considered core genes.

Genes are here identified as being part of the core set even in cases where the sequence is quite diverged, as in the case of *FIT1*, where the S288c allele and the YJM248 allele share only 52.7% identity (Figure S2), or the case of *NTC20* where the S288c allele and the YJM1400 allele share 38% identity (Figure S3). The sequence divergence of *FIT1* and *NTC20* is primarily the result of introgression.

Tandem Gene Duplication

In addition to single genes, tandem arrays of genes in the same orientation are also found in *S. cerevisiae.* For the analysis of core genes, tandemly duplicated genes where the copies are identical or nearly identical are counted only once in this analysis. For example, the 94 strains in this analysis contain from one to more than 10 copies of the *CUP1* locus (Zhao et al. 2014). *YHR053C* (*CUP1-1*) and *YHR055C* (*CUP1-2*) in strain S288c, are counted once *YHR053C*/*YHR055C* (*CUP1*) as a core gene. *ENA1*/*ENA2*/*ENA5*, *HXT6*/*HXT7*, and rDNA genes are similarly counted only once as a core gene for each array. For tandem genes where there are diverged copies such as *HXT4*/*HXT1*/*HXT5* and *PLB1*/*PLB2*, each is counted as a core gene as they are each found as diverged tandem genes in greater than 95% of strains. In three cases, *PHO3*/*PHO5*, *DOG1*/*DOG2*, and *ALD2*/*ALD3*, there are two diverged genes in S288c and many strains, but other strains have only one or the other or a hybrid of these genes. For these three genes, as 100% of strains have at least one gene, but less than 95% have both genes, these genes are here labeled as one core gene and one variable gene. The reason identical tandem duplicated genes and diverged tandem duplicated genes are treated differently is that identical tandem genes can potentially change copy number at higher frequency by unequal crossover.

Another example of diverged tandem genes is the DUP240 family of protein membrane genes. For *S. cerevisiae* there is a single copy of a DUP240 family gene *YCR007C* (*DFP3*), on chromosome III, found in all 94 strains, though in seven strains (YJM320, YJM248, YJM1304, YJM555, YJM689, YJM541,

YJM554) the allele appears to be introgressed from another *sensu stricto* species. In S288c on chromosomes I there is a single DUP240 core gene *YAR023C* (*DFP1*) found in all 94 strains Adjacent to *YAR023C* (*DFP1*) is a tandem array in the opposite orientation of four DUP240 family genes *YAR027W* (*UIP3*), *YAR028W* (*KTD1*), *YAR031W* (*PRM9*), *YAR033W* (*MST28*). The 93 strains have zero to nine members of this array, with four of the strains (YJM270 YJM453 YJM1419 YJM1460) missing the array entirely, and strain YJM1434 containing a single gene in this array. Therefore, this array has one core gene. Thirty strains have the same four genes as found in S288c. In addition to the four genes found in S288c, a total of 23 different genes are found in the arrays of the other 59 strains, some of which may be introgressed from *S. paradoxus*. In some cases there are stop codons in the genes. A significant amount of genome variation can thus be attributed to the chromosome I DUP240 array. There is another DUP240 array on chromosome VII. In S288c this array contains two members, YGL051W (MST27) and YGL053W (PRM8). 67 of the 93 strains are missing this array, with three strains (YJM1208 YJM1386 YJM1419) containing the same two genes as in S288c, and the remaining 23 strains containing a varied combination of 16 different genes other than the S288c genes. Strain YJM1573 had six genes in the chromosome VII array, the most of any of these strains. These genes are not core genes, by the 95% rule. This array further contributes to genome diversity. Additional diversity for this DUP240 family is found in the 1011 *S. cerevisiae* strain sequences (Peter et al. 2018).
A summary of the tandem genes, including some of those not found in S288c is in Table S4. An example of a non-tandem gene duplication is shown in Figure S4 and includes *YJR108W* (*ABM1*), *YIL014C-A*, and *YIL102C* and possibly additional genes including some strain specific genes and subtelomeric genes. These genes do not appear to be duplicate genes arising from the WGD event.

Genes not in the Current Annotation
While some core genes are missing in up to 4 of the 93 strains from Strope et. al., we did not identify any core genes missing from S288c, and only one core gene is present in S288c but not in the current annotation. In Peter et al. it was reported that 3 additional genes are core genes that are not annotated in S288c. These they named *610-snap_masked-2999-BGP_1*, *584-snap_masked-1700-AIE_1*, and *611-snap_masked-3001-BGP_1* (Peter et al. 2018).
Gene 610-snap_masked-2999-BGP_1 is a real protein coding gene that is a syntenic ortholog of *A. gossypii AER178W-A*. This gene is referred to in the YGOB browser in S288c as *YGOB_YBL026W-A* (Byrne and Wolfe 2005). The gene we are calling *YBL026W-A* is a core gene of unknown function in our analysis that was overlooked in the original annotation of *S. cerevisiae.*

In our analysis *584-snap_masked-1700-AIE_1*, located between *YPR148C* and *YPR149W* (*NCE102*), is only an intact reading frame in two of the 94 strains, YJM1078 and YJM248, where it appears to be an introgressed sequence from *S. paradoxus.* Though this sequence is found in two species, it appears that this is a dubious gene. The sequence is conserved, but the open reading frame is not conserved.

Gene Remnants
In addition to genes, disrupted genes, and potentially null alleles, there are cases of genes that have accumulated mutations to the point where all that is left is fragments of the gene, at the location where the gene was once found. The term "gene remnant" has been used previously, initially to refer to gene fragments left over by V-J joining (Selsing et al. 1984).
The Peter et al gene *611-snap_masked-3001-BGP_1* is a complicated case. This putative gene is only intact in about half of the 94 strains we examined. Furthermore, it overlaps with uncharacterized gene *YFL041W-A*. Analysis of *YFL041W-A* shows that is has similarity to the C-terminal end of *YLR070C* (*XYL2*). In *S. paradoxus* strains N44 and CBS432, *YFL041W-A* is a full-length paralog of *YLR070C* (*XYL2*), and they are a WGD pair of genes (Philippsen et al. 1997, Wolfe and Shields 1997). Both dubious hypothetical gene *610-snap_masked-2999-BGP_1* and dubious gene *YFL041W-A* overlap the *S. paradoxus* gene, but in different reading frames.

In addition, in *S. paradoxus* strains UWOPS91, UFRJ50816, and YPS138 (Bergstrom et al. 2014), *YFL041W-A* is a disrupted open reading frame, though much more intact than the *S. cerevisiae* copies. This is a rare example of a duplicate gene pair from the WGD where one of the members was relatively recently lost, since the divergence of the closely related species *S. cerevisiae* and *S. paradoxus*. Thus *YFL041W-A*/*611-snap_masked-3001-BGP_1* are not uncharacterized genes nor pseudogenes but are parts of a gene remnant (Figure S5). There is no intact *S. cerevisiae* copy of the *YFL041W-A* paralog of *YLR070C* in the 94 strains examined here or in the sequences from Peter et al. *YFL041W-A* is thus dubious, and part of the gene remnant we are calling REMYFL041W-A. This gene remnant extends on S288c chromosome IV from 47982 to 48942 on the Watson strand. This remnant includes seven frame shifts and ten in frame stop codons as well as numerous synonymous and non-synonymous polymorphisms. The remnant is somewhat shorter than the *S. paradoxus* gene and the orthologs found in other species, due to multiple deletions in *S. cerevisiae*.

An additional remnant we are calling REMYKL162C-A (REMPIR6) in S288c on the Crick strand of chromosome XI from 145880 to 146662 (Figure S6), overlaps the dubious hypothetical gene *YKL162C-A*. *A. gossypii* has three PIR genes, some *sensu stricto* strains have six, but *S. cerevisiae* has only five with a gene remnant of the ancestral location of the sixth gene, REMYKL162C-A (REMPIR6), a WGD duplicate of *YJL158C* (*CIS3*). REMYKL162C-A contains four frameshifts and four in frame stop codons when compared with *PIR6* of *S. mikatae* strain IFO1815.

Another gene remnant we are calling REMYCR099C (REMVTH4) is paralogous to gene *YBL017C* (*PEP1*). *YBL017C* (*PEP1*) is the one syntenic copy of the gene *AFR018C* in *A. gossypii*. In S288c there are two additional full-length copies, *YIL173W* (*VTH1*) and *YJL222W* (*VTH2*). There is an additional disrupted copy we are calling *YNR066C_YNR065C* (*VTH3*), disrupted by a single stop codon, with an intact open reading frame in 30 of the 93 strains. In this work we are distinguishing between potential null alleles, disrupted protein codon genes with a single disruption by a frameshift or stop codon that could easily revert, and disrupted protein coding genes where multiple disruptions are present. The gene remnant REMYCR099C (REMVTH4) is near the right telomere of chromosome III, on the Crick strand from 300104 to 303030 (Figure S7), spanning proposed dubious genes *YCR099C*, *YCR100C*, and *YCR101C*, and disrupted by numerous frameshifts and stop codons in strain S288c. This region is a gene remnant, even though there is an intact copy in strain YJM1252. The YJM1252 *VTH4* gene appears not to be an *S. cerevisiae* gene, but an introgressed *VTH4* gene from *S. paradoxus* that is 99% identical to that gene in *S. paradoxus* strain CBS432. No intact *S. cerevisiae VTH4* gene was identified. The multiple stop and frameshift mutations in *S. cerevisiae* REMYCR099C (REMVTH4) suggests that introgression would be the primary means of reviving *VTH4*. REMYCR099C (REMVTH4) is also the final conserved element on the right end of Chromosome III, marking the boundary between the central region and subtelomeric region.

YCL001W-A and *YCL001W-B* are also in S288c dubious genes part of a gene remnant, a WGD paralog of *YNL001W* (*DOM34*). In seven of the 93 strains (YJM320, YJM248, YJM1304, YJM555, YJM689, YJM541, YJM554) there is a full length *YCL001W-A* allele that appears to have been acquired by introgression and is 100% identical to the allele found in *S. paradoxus* strain CBS432. This is similar to the case of *VTH4* described above where the gene was recovered by introgression. The allele found in S288c and the other 86 of the 93 strains contains multiple stop codons and a frame shift (Figure S21).

A gene remnant we are calling REMYHR054C arose differently and is a duplicate 3' end fragment of *YHR056C* (*RSC30*) that results from the tandem duplication of *CUP1* and is only found in strains with two or more tandem copies of *CUP1*. It is here proposed that hypothetical gene *YHR054C* is dubious, and a gene remnant not a protein coding gene. As previously published, different *S. cerevisiae* strains contain at least five independent duplications of the *CUP1* locus, so the length of REMYHR054C varies (Zhao et al. 2014).

Because a region is called a gene remnant does not imply that none of the residual pieces are functional genes. It has been known for 50 years that in *S. cerevisiae* you can have a gene with multiple point mutations, and with deletions and still have function or partial function, as has been shown for *HIS4*, a gene encoding three enzymes (Fink and Styles 1974), where gene fragments can still have one of the activities.

Variably located ubiquitous genes and strain specific genes, plasmids, and transposable elements
In these 94 strains, there are also a few variably located ubiquitous genes and strain specific genes. These include variably located ubiquitous genes in the central region of the chromosomes and those located in both the central region and the subtelomeric regions, as described in Table S5. These include sixteen non-core central region genes found in S288c. Strain specific genes can be found both in the central region of the chromosomes, and more frequently in the subtelomeric regions.

A special case is the plasmid 2µ found only in 67 of the 93 strains(Strope et al. 2015). The four genes found on this plasmid are not part of the core set. Similarly, the single and double strand RNA virus genes though found in most strains are variable among strains and not core (Vijayraghavan et al. 2023, Vijayraghavan et al. 2023). Another special case is the transposable elements. While all 94 strains in this analysis contain the ubiquitous Ty element Ty2, a variable number of strains contain Ty1, Ty3, Ty4, and Ty5 (Table S6). As the location of those elements varies significantly, the Ty *GAG* and *POL* genes found in these transposable elements do not meet the definition of core genes used here, even in the case of Ty2. Only these five types of known Ty elements were found among the 94 strains, though a long terminal repeat (LTR) from an element we are calling Ty6 is located near the left telomere of chromosome II of strain YJM451, where Ty6 is a Ty element found in *S. cerevisiae* on contigs AFQ_1-6592, CDM_4-1998, and YDB-17588 from the 1011 genomes project (Peter et al. 2018), and found in many *S. paradoxus* strains including YPS138 and UFRJ50816 (Bergstrom et al. 2014). This element appears to be related to Ty4, and has sometimes been referred to as TSU4 (Bergman 2018).

A Novel *S. cerevisiae* Transposon
A curious case of strain specific genes is a circularly permuted 17kb set of five genes derived by horizontal gene transfer from *Z. bailii* or a closely related species. Complete copies of this 17kb region are found in sixteen of the 93 strains, with partial portions of this 17kb unit found in an additional ten strains. Several strains contain more than one copy. The partial and full copies are at 22 genomic locations, a mixture of central and subtelomeric (Table S7). Among the 1011 strains from Peter et al. a blast search suggests at least 383 strains contain partial or complete copies of this 17kb element. This appears to be a novel transposon that we are calling Ty7 shown in Figure 1. Ty7 encodes 5 protein coding genes, including an ortholog of *OXP1.* In strain YJM1478, the circle is recombined into the *S. cerevisiae OXP1* locus at a site of DNA homology within this protein coding gene, creating two hybrid *OXP1* genes flanking the 17kb insert. Some locations of the Ty7 insertion sites are shown in Table S7 and Figure 1. The remaining insertion sites do not have homology and do not have a duplication or deletion at the insertion site of more than 5 bases. The 17kb circularly permuted sequence does not contain direct or inverted repeat sequences. In one central region case, found in strains YJM271 and YJM193, two complete tandem copies of the 17kb unit were inserted into a site on chromosome XIII. The largest gene in this 17kb sequence, and the one most similar to an *S. cerevisiae* gene is an ortholog of *OXP1*, a 5-oxoprolinase. The other four genes are weakly similar to a flocculin gene, a transporter, and two are weakly similar to transcription factors. In *Zygosaccharomyces* species from which this transposon was acquired this gene cluster is located at a single location near a telomere; there is no evidence that this element is transposable in those species. Curiously, the *S. cerevisiae OXP1* gene is also located near a telomere, though the non-syntenic *A. gossypii* ortholog *ABR114C* is in the central region of the *A. gossypii* genome. *T. delbrueckii* also has a subtelomeric *OXP1* gene (*TDEL_0D00250*) that is not syntenic with the

A. gossypii, S. cerevisiae, or *Zygosaccharomyces* genes, and in *L. kluyveri* the gene is at yet another non-syntenic location. *OXP1* thus seems to be unusual in the lack of conservation of location at which the gene is found . Although Ty1 through Ty6 elements are retrotransposons this was not known when Ty1 was first described (Cameron et al. 1979); Ty7 does not appear to be a retrotransposon. We are calling the encoded genes *TYG71* for the *OXP1* ortholog, and *TYG72*, *TYG73*, *TYG74*, and *TYG75* for the other four genes. No introns or ribosomal frame shifts are found in these genes. A single copy of Ty7 from strain YJM193 has been deposited in GenBank with accession number OR078387. An example of a mutation caused by this transformation is in strain YJM453 where the gene *YOL030W* (*GAS5*) is disrupted by a Ty7 element.

Proposed Dubious Genes

In this analysis, 237 protein-coding genes in the current annotation of *S. cerevisiae* strain S288c are likely annotation artifacts, and thus these are not included in the lists of core genes and non-core genes for *S. cerevisiae*, and we consider them to be dubious. A list of these dubious genes is in Table S8. This list does not include genes previously described as dubious in *S. cerevisiae* (Hirschman et al. 2006, Cherry et al. 2012). For most of these genes proposed as dubious, the open reading frame is quite short, they are not conserved in other species, and in many cases they are not even conserved among strains of *S. cerevisiae.* For all 237 proposed dubious hypothetical orfs, genes there is little to no experimental data supporting that they are actual genes. For the purposes of describing the set of genes in the species *S. cerevisiae*, they are dubious. There have been cases of genes listed as dubious that have later turned out to be genuine, such as *YIL156W-B*, a syntenic ortholog of *A. gossypii AFR298C* once listed in SGD as dubious. With sufficient evidence it is possible that one or more of these dubious genes may turn out to be genuine genes and not annotation errors. Without supporting evidence they should be considered dubious. One current dubious gene, *YBR012C*, appears not to be a dubious gene but instead *YBR012C* and *YBR013C* appear to be a single gene disrupted by a Ty1 element in S288c (Table S12), and thus we propose that *YBR012C* and *YBR013C* should be considered a single gene, disrupted in S288c. Most of these dubious genes date back to the original annotation of the genome of *S. cerevisiae* strain S288c, when it was the first eukaryotic genome sequenced and thus limited data was available for comparative analysis.

One case of a gene here proposed as dubious, *YJR079W*, contains a putative intron with a non-consensus 5' splice site (GCATGT versus the consensus GTATGT). Out of 329 sequence reads at the putative splice site, there were no spliced transcripts found in SRR488142. The intron as well as the gene is dubious.

Another gene proposed here as dubious is *YER014C-A* (*BUD25*). This gene is not conserved in other species as a spliced open reading frame, likely due to the limited conservation of the overlapping, conserved *YER014W* (*HEM14*) gene. *HEM14* has only 44% protein identity between *S. cerevisiae* and *A. gossypii*. The putative *YER014C-A* open reading frame is disrupted in other species. While the open reading frame is present in 90/93 of the Strope et al strains, of 237 RNA-seq sequences spanning the putative *BUD25* splice site from the SRR488142 RNA-seq data set, all are unspliced.

Another case of a proposed dubious gene that contains an intron is more interesting. For proposed dubious gene *YBR090C*, the only conserved portion of this gene is the intron splice sites, branch site, and the intron's location immediately upstream of *YBR089C-A* (*NHP6B*) (Figure S8). The putative coding region sequence is not conserved. This is a real intron in a dubious gene, a 5' UTR intron of *YBR089C-A* (*NHP6B*), not a separate protein-coding gene. Examination of RNA-Seq data SRR488142 shows evidence that this intron is spliced as expected. This 5' UTR intron of *YBR089C-A* (*NHP6B*) is conserved in multiple species, and the gene is a syntenic ortholog of *ADL310W* in *A. gossypii*, which also has this 5' UTR intron.

An interesting case of two proposed dubious genes are *YHR052C-B* and *YHR054C-B* recently published by Cuitong He (He et al. 2018). These putative genes were discovered using proteomics, showing quite

convincingly that these proteins can be detected experimentally. During proteomic analysis the most common approach is to use the set of annotated proteins and identify those present or absent. There are generally multiple spectra that do not correspond to the annotated protein set. The work by He et al shows the value of examining these unexplained fragments. *YHR052C-B* and *YHR054C-B* appear to be real proteins, but here we are proposing that these are dubious genes (Figure S9). The reason for this is as follows.
These proteins are on the same strand and overlap with the identical tandem duplicate genes *YHR053C* (*CUP1-1*) and *YHR055C* (*CUP1-2*) over approximately the entire length of both proteins. *YHR052C-B* is in the +1-reading frame relative to *CUP1*. These are very short proteins, *YHR053C* 62 residues, *YHR052C-B* 58 residues. While the protein sequence of *YHR053C* is identical for all 94 strains, there are four polymorphisms in *YHR052C-B* among the sequences from the 94 strains changing the protein sequence, one is a conservative change, one loss of start codon, and two non-conservative changes. More strikingly, when comparing these open reading frames across the *Saccharomyces sensu stricto* species within *CUP1* there are forty-five positions where the protein sequences are identical, and eight positions with only conservative amino acid substitutions. In contrast for the *YHR052C-B* orthologs, there are only 14 conserved positions, and four positions with only conservative changes. Loss of start codon is found in several species. It thus appears that selection has been acting on *CUP1*, but not on *YHR052C-B*, with most of the polymorphism being in the 3rd position, the wobble position, of residues of *CUP1*, but most of the polymorphism are in the 2nd position of residues of *YHR052C-B*, a very unusual situation for a protein coding gene. We are proposing that *YHR052C-B* and *YHR054C-B* encode proteins, but these are not genes, but are more analogous to the case of uORFs where translation occurs, but they seem to have a role in the regulation of translation of the associated gene and are not independent genetic elements. *YHR052C-B* and *YHR054C-B* thus appear to be features of *YHR053C* (*CUP1-1*) and *YHR055C* (*CUP1-2*).

A similar case is *YKL104W-A* (He et al. 2018). *YKL104W-A* is on the opposite strand of *YKL103C* (*GFA1*), and is conserved in *S. cerevisiae*, and to a much lesser extent in *sensu stricto* species. As shown in Figure S10, when *YKL104W-A* is aligned to the S288c sequence, of the seventeen polymorphisms sixteen are in codon position one and one is in codon position two. None are in the third codon position, the wobble position, where polymorphisms are generally found in protein coding genes. Reading frame one of *YKL104W-A* is on the opposite strand from reading frame three of *YKL103C* (*GFA1*). Those sixteen polymorphisms are in the wobble position of *YKL103C* (*GFA1*). It appears that *YKL103C* is under selective pressure, not *YKL104W-A*. Thus, although *YKL104W-A* appears to encode a real protein, it is not a real protein coding gene.

For gene *YLR379W-A*, this also appears to be dubious based on lack of conservation of the reading frame in other species, as well as limited conservation in *S. cerevisiae* (Figure S11). Multiple strains have two base frameshifts at the sequence AGAGAGAGAGAGA which occurs at fourteen locations in the S288c genome, though only three times in coding regions. The three coding region occurrences are dubious gene *YLR311C*, gene *YLR255C* proposed as dubious, and *YLR379W-A*, also proposed as dubious. In addition, single nucleotide polymorphisms in this gene are a mix of all three codon positions, not the usual pattern where most polymorphism are in the wobble position.

Yet another case of an erroneous intron annotation is *YJR112W-A*. In this case this is a real, core gene, but instead of a non-consensus sequence intron this gene contains a consensus plus one ribosomal frameshift site, CTTAGGC (Belcourt and Farabaugh 1990) (Figure S12). This gene has a conserved plus one ribosomal frameshift site also found at the same position in orthologs from several other species, including *A. gossypii, S. paradoxus,* and *S. bayanus.* Some other related species including *K. lactis* have neither an intron nor ribosomal frameshift in their ortholog. Supporting this being a case of ribosomal frameshifting in *S. cerevisiae* and not an intron is the non-consensus splice and branch sites, and the lack of evidence from RNA-seq data of splicing of the putative intron. For sequence reads from SRR488142

RNA-Seq data set, 0/171 reads matching the 5' side of the putative splice site were spliced, and 0/492 reads matching the 3' side of the putative splice site were spliced.

In *S. cerevisiae* there are only 285 annotated introns, not including the tRNA introns, the ones from *YER014C-A* (*BUD25*), *YJR079W*, *YJR112W-A*, and the dubious subtelomeric introns. All real introns in *S. cerevisiae* S288c are in core genes, and in no case among the 94 strains is an intron present in a core gene in some of these strains but the intron is missing from the gene in other strains. While the nuclear encoded protein and tRNA introns are highly conserved, the intron composition of the mitochondrial genomes is quite variable, as previously described (Vijayraghavan et al. 2019). Variation in the presence of mitochondrial introns, and the encoding of genes within mitochondrial introns is another phenomena that has long been known (Jacquier and Dujon 1985), but is much more clear with the availability of large numbers of mitochondrial genomes to compare.

The *NAG1* gene is on the minus strand and is completely contained within the *YGR031W* (*IMO32*) gene. While *NAG1* was reported to be highly conserved (Ma et al. 2008), it appears that the open reading frame is only conserved in *S. cerevisiae*, *S. uvarum*, and *S. arboricola*. In other *Saccharomyces sensu stricto* species, and other hemiascomycetes the open reading frame is not conserved (Figure S13), though segments of the sequence are conserved, corresponding to conserved regions of *IMO32*.

Of the genes proposed here as dubious, 122 genes were previously proposed as dubious by a different method (Zhang and Wang 2000) and are marked in Table S8. The approach of Zhang and Wang is a version of codon usage, whereas the method used here to identify dubious genes is based on open reading frame conservation, protein sequence conservation, RNA-seq data, lack of known phenotype. These are two independent methods. Over 1000 additional small open reading frames have been identified from the sequence of the 93 strains from Strope et al. that are at least equally likely to be species and strain specific genes as the open reading frames that here have been relegated to dubious. Naming these 1000+ open reading frames as novel uncharacterized genes would serve no purpose. Among those 1000+ open reading frames a small number of these species and strain specific open reading frames may eventually be shown experimentally to be protein coding genes involved in specific processes, carrying out specific functions. It would then be appropriate to welcome them into the pantheon of *S. cerevisiae* genes.

Overlapping protein coding regions

Among these proposed dubious genes, many overlap with other genes. Seven overlap with Ty elements, seventeen overlap with RNA coding genes, mostly tRNA genes, and thirty-four overlap with protein coding genes, shown in Table S9. Among the genes here described as dubious is *YLR154W-C* (*TAR1*) (Coelho et al. 2002). While the published evidence suggests there is transcript and possibly even protein produced, not surprising considering there are over 100 copies of this region in the genome, the lack of conservation of the open reading frame (Figure S14 and Figure 1C of the Coelho et all paper), and the lack of a known function suggests that this open reading frame is unlikely to be a gene. A gene of unknown function overlapping with another gene is not on its own conclusive of an annotation error, but when combined with a general lack of evidence supporting these annotations, it contributes to the conclusion that these genes should be considered dubious. An additional three pairs of real genes currently annotated as overlapping (*YDR512C*, *YDR513W*; *YCR038C*, *YCR039C*) appear to overlap only due to errors in identification of the start codon. Until recently there was another pair of annotated overlapping genes, *YJR012C* and *YJR013W* but this has been resolved by mapping and correction of the start codon of *YJR012C* (Sadhu et al. 2018, Engel et al. 2022). For *YDR512C*, it appears that an internal start codon is the conserved start codon (Figure S15). The case of *YCR038C* is more complicated, and while it appears the current start codon is not correct, it will need to be investigated further.

Hidden among the numerous dubious cases there are real overlapping protein coding regions in *S. cerevisiae*, with seven overlapping gene pairs identified (Table S10). In these cases of well-characterized

overlapping genes, as with the 26 pairs of overlapping genes in *A. gossypii* (Dietrich et al. 2013), they are all pairs of convergent protein coding genes. Two interesting cases are shown in Figure S16 where the overlapping region is conserved among related species. Interestingly, the relative frame of the overlap appears to be more conserved than the actual sequence of the overlap, particularly in the case of *VPS38* and *DCR2*.

Putative disrupted alleles

A phenomenon that has long been known and is quite widespread is that in *S. cerevisiae* there are genes with a stop codon or frameshift or disabling single nucleotide polymorphisms in the coding region. Well known cases of non-functional genes in S288c include *HO* and *HAP1* (Meiron et al. 1995, Gaisne et al. 1999). While the *HO* gene is a null allele in strain S288c due to non-synonymous base substitutions (Meiron et al. 1995), many potential null alleles are the results of frameshifts or stop codons or transposon insertions. Some 444 core genes have a stop codon or frameshift (insertion or deletion) or transposon insertions, in one or more of the 94 strains, shown in Table S11. All 94 strains have some genes with potential null alleles, with the number of genes with potential null alleles per strain ranging from four to forty-four, with a median of 21. S288c is on the low end with only 16 genes with potential null alleles. In 95% percent of these potential null alleles there is only a single stop codon or frameshift. These genes with a single stop codon or frameshift are likely functional in the sense that a single point mutation could revert them. In the definition used here, a gene is still a core gene if it is present at the same location in 95% of the strains, even if in some cases it is a potential null allele. Furthermore, even when there is a frameshift or stop codon in a protein-coding gene, this should be considered a single gene and given a single name. When the genome of S288c was first annotated, some genes were mistakenly given two names due to a frameshift or stop codon because at that time in was not known that those fragments comprised a single gene. This is now misleading. These genes should be given a single name, with the second name relegated to an alias, and these genes identified as a potentially null allele. Exactly how to define these genes with potential null alleles is complicated as some cases involve frameshifts or stop codons very close to the carboxy-terminal end of the protein, and thus may be fully functional, just with an alternative carboxy-terminal end.

An example of a gene with an elevated chance of a disabling mutation is *YLR046C* where a 9bp inverted micro-homology has led to a small inversion and introduction of a stop codon in two strains, YJM1592 and YJM1388, that theoretically can be reversed by a second inversion at the microhomology (Figure S17). An example of a gene with reduced likelihood of contracting a potentially null allele is *HMO1*, where approximately half of the lysine residues are encoded by AAA, and half by AAG. The one run of seven consecutive lysines is encoded by $(AAG)^7$, where a three base frameshift is possible, but a one base frameshift is not as likely as it would be if the seven consecutive lysines were encoded by $(AAA)^7$. Overall, homopolymer runs are underrepresented in S288c protein coding regions in both essential and nonessential genes (Figure S18).

Not all potentially null alleles are stop codons/frameshifts/transposon insertions. An example of a potentially null allele that is a non-synonymous substitution is the glycine to aspartic acid substitution at position 331 in uncharacterized gene *YDR111C* (*ALT2*) in strain S288c. In the WGD paralog *YLR089C* (*ALT1*), and in *ALT2* in all 93 strains there is a glycine at that position. In addition, the ortholog in *A. gossypii*, *Candida albicans, Schizosaccharomyces pombe*, *Cryptococcus neoformans*, *Homo sapiens*, *Arabidopsis thaliana, and even Escherichia coli* all have the glycine as well (Figure S19). It has been shown that the S288c *ALT2* is a non-functional paralog of *ALT1*, an alanine transaminase (Penalosa-Ruiz et al. 2012). Multiple sequence alignment suggests that residue 331 is the only highly conserved position between plants, animals, fungi, and bacteria that is mutated in S288c *ALT2*. The glycine to aspartic acid change at position 331 results in a potentially null allele, though experimental validation is needed to identify if this is the actual change that makes this a null allele.

Pseudogenes
Alleles with a stop codon or a frameshift where the gene is at the same location in other strains where the gene is intact are not pseudogenes. At times these have been mistakenly referred to as pseudogenes but they are instead alleles. For example the conserved gene *SDC25*, a paralog of *CDC25* is a disrupted allele, or a potentially null allele, or a gene remnant, but it is not a pseudogene as reported (Sanchez et al. 2019) as it is at the same location where *CDC25*/*SDC25* is in the pre-SGD strains. There are also pseudogenes in *S. cerevisiae*, but this is a different phenomenon. For example, strain YJM1208 contains a 376 base pair fragment of 25S rDNA gene located distal to *YPL275W* (*FDH2*). This is not a functional rDNA array, but is a small piece integrated at a location where rDNA is not usually found; a 25S pseudogene. In some strains small pieces of mitochondrial DNA are inserted into the nuclear genome, such as the mitochondrial DNA fragment found in strain S288c on chromosome X at bases 6998-7227 and the same piece on chromosome IX at 7015-7244. This same fragment is also found near telomeres in strains YJM1381, YJM1083, YJM326, and YJM1460. Another pseudogene derived from mitochondrial DNA is 95 base pair fragment from the middle of the *COX3* gene found near the left end of chromosome VII in several strains including YJM689, YJM683, YJM682. An example of a chromosomal protein coding gene derived pseudogene is the 830 bases derived from the 5' end of *YDL015C* (*TSC3*) on chromosome IV that is inserted between *YGR177C* (*ATF2*) and *YGR178C* (*PBP1*) on chromosome VII in strains YJM1381 and YJM1199. Another example of a chromosomal protein coding gene derived pseudogene is a copy of *YMR096W* inserted into the chromosome XIII right end subtelomeric region containing frame shifts and a stop codon. These are pseudogenes, following the usage of the term in higher eukaryotes. The initial identification of eukaryotic pseudogenes came from identification of globin genes. In a review by Proudfoot it is reported that "Studies from a variety of mammalian species reveal that in each case more gene sequences are present in the genome than necessary to encode the known globin polypeptides… the term 'pseudogene' – defined as a region of DNA that displays significant homology to a functional gene but has mutations that prevent its expression – has been coined for these aberrant genes" (Proudfoot 1980). To be a pseudogene a DNA segment is at a location distinct from the normal locus of the gene. In *S. cerevisiae* it is important to distinguish between a gene that in some strains a potential null allele, and a pseudogene.

Start Codon Errors
While identifying the set of core genes in *S. cerevisiae*, it was clear that there are some errors in the current annotation, in addition to those listed above. There are 22 genes where it appears that the wrong start codon is annotated (Figure S15) as well as genes currently annotated as two genes (Table S12). This is based on sequence conservation among the 94 strains as well as sequence conservation with other species. These misannotated genes, as with the dubious genes, provide a somewhat inaccurate picture of the set of genes in *S. cerevisiae*. There are also genes where it's unclear as to which start codon is the real start codon, or whether more than start codon is used. It has long been known that there are real protein coding genes in *S. cerevisiae* where more than one start codon is used such as *HTS1* (Natsoulis et al. 1986).

tRNA Genes
The set of tRNA genes is highly conserved among these strains. S288c has 299 tRNA genes with 41 anticodons among the nuclear encoded tRNAs; the other 93 genes have very similar numbers. All tRNA genes are in the central region of the chromosomes, except for several non-core copies. This high level of conservation of the tRNA genes was not obvious in the original assembly of the 93 genomes, as Ty elements often insert adjacent to tRNA genes, so numerous assembly errors were associated with tRNA genes. The tRNA gene content is summarized in Table S13. Two serine tRNA's, *YNCD0024C tX(XXX)D* and *YNCL0039W tX(XXX)L*, are currently misannotated in S288c due to a sequence error in each gene that likely arose from a problem with the ABI dye-primer sequencing originally used to sequence the genome that resulted in G/C compression errors (Figure S20).

KHS1* and *KHR1

An interesting example of how examining multiple genome sequences can resolve uncertainties in the annotation of the S288c genome is the case of *YSC0044* (*KHS1*) and *YSC0002* (*KHR1*) (Goto et al. 1990, Goto et al. 1991). In the current S288c annotation in SGD, these two genes are annotated as not in the reference strain. For *KHR1*, this gene is found in 66 of the 93 strains, and always at the same position. It is located on the complementary strand, between *YIL029C* and *tK(CUU)I*. It is a non-core gene in the central region of chromosome IX. The sequence is somewhat variable, so the predicted protein in some strains is more similar to that in P22313.1 (Goto et al. 1990) and sometimes more similar to that in KZV10588.1 (McIlwain et al. 2016). *KHS1* is a more complicated issue. The gene is a core gene *YER187W* currently annotated as an uncharacterized gene, found in S288c and in 92/93 strains from Strope et. al. However the gene in S288c and many other strains contains a stop codon and is apparently non-functional. In addition, it appears that the original sequence of *KHS1* P39690.1 contains multiple sequence errors, mostly insertions, as well as an inverted DNA fragment connected at a HindIII site. While nearly all strains from the 1011 genome data set contain *KHS1*, in no case does the sequence closely match P39690.1, supporting the notion that the original sequence contained multiple errors, and that *YER187W* is *KHS1*as previously reported by Frank and Wolfe(Frank and Wolfe 2009).

An examination of the core genes in *S. cerevisiae* shows that out of 5873, 5371 have orthologs in *A. gossypii*, and an additional 328 genes have orthologs in *K. lactis*, and other pre-duplication species. More than 98% of core genes in *S. cerevisiae* are of vertical inheritance as they were in the ancestral hemiascomycete pre-WGD genome. For the 174 genes in S288c that do not appear to have a pre-WGD ortholog, a few appear to have been acquired by horizontal gene transfer since the WGD (Hall et al. 2005, Hall and Dietrich 2007, Tapia et al. 2023), though most are of unclear origin.

Genes of unknown function

Following the above description of the set of core genes in *S. cerevisiae*, the following question arises: how many core *S. cerevisiae* genes are of unknown function, those listed as "uncharacterized" in SGD? The current number is 378, of which 291 have orthologs in *A. gossypii* or other pre-WGD species suggesting that most of these 378 genes of unknown function are real genes. This number is decreasing as researchers identify function of previously uncharacterized genes. Some recent examples being the identification of the function of *YNR062C* and *YNR063W* as involved in resistance to pulcherrimin (Krause et al. 2018), *YBR063C* being identified as a nuclear membrane tethering protein (Eisenberg-Bord et al. 2021), *YDL180W* being identified as having a role in TORC1 signaling and localization (Wallace et al. 2022), *YGL036W* required for mRNA m6A methylation (Park et al. 2023), and YIL156W-B being identified as a mitochondrial fission factor (Fukuda et al. 2023). Nearly 94% of the core genes of *S. cerevisiae* are of known function. Among the genes of unknown function, 5 appear to have potential null alleles in the *S. cerevisiae* reference strain S288c. The community of *S. cerevisiae* researchers is thus not that far from the point of being able to say that "we know something about the function of every core gene in *S. cerevisiae*". *S. cerevisiae* continues to be the standard for model organisms; In the future the standard for model organisms will be to know with some accuracy the set of core genes in the organism, and something about the function of every core gene. *S. cerevisiae* will likely be the first eukaryote to meet this threshold.

The total number of genes in the haploid genome of an *S. cerevisiae* strain is thus approximately 6000 genes, 5850 to 5855 core genes, 5 to 50 central, non-core genes including the 2µ and RNA virus encoded genes, Ty element *GAG* and *POL* genes, and 50-300 subtelomeric genes. These numbers are quite different than those reported in Peter et al (Peter et al. 2018), where they report the genome in this species being composed of some 4990 core genes, and a total of 7222 genes. Their numbers, while quite accurate, give the sense that a haploid yeast strain selected from a wild isolate will likely contain the 4990 core genes, and several hundred, perhaps a thousand or more variable genes. Our analysis suggests genomes in this species have a more conserved gene set, and that a strain will likely contain approximately 5885 core genes, plus 50-300 variable genes. This more constrained view of the gene set suggests that among strains

much of the phenotypic variation is due to variation within the core set of genes with a contribution from the fewer variable genes, as opposed to a smaller set of core genes and a large set of variable genes, as seen in some bacteria such as *Escherichia coli* (Hayashi et al. 2001).

Discussion

When the genome of *S. cerevisiae* was first sequenced in the 1990's sequencing a eukaryotic genome was an expensive and labor-intensive process. Thus for *S. cerevisiae* as well as for *Caenorhabditis elegans*, *Drosophila melanogaster*, human, and other species there was the concept of a single reference genome sequence. That is, an *S. cerevisiae* gene came to mean the gene from the reference strain. With the technological advances in the nearly three decades since the first eukaryotic genome sequence, it is now possible to ask the question about a specific gene in a species, or about the set of genes for a species. This paper is in part an investigation into how the genome of *S. cerevisiae* is the same as and differs from the genome of *S. cerevisiae* strain S288c.

The set of genes for a species, the extent of potentially null alleles, the extent of introgression from sibling species, the distinction between core genes and strain specific genes, pseudogenes, and other aspects can best be identified from many genomic sequences and additional "omics" data and published data on specific genes.

While there is still a long way to go in understanding the genome structure and organization of *S. cerevisiae*, comparing multiple genomes of a single species can identify many aspects of the genome not clear from analysis of a single strain, such as transposon diversity, the extent of strain specific genes, the set of core genes, and many other features of the genomes of this species. It should be noted that the term "core genes" as used in this paper refers to the core genes in this species. This is not the same as the core genes of the *Saccharomyces sensu stricto*, or the core genes of the hemiascomycetes. These are somewhat more complicated concepts.

It is clear from this analysis that 94 strains are far too few to get an accurate view of the genome of *S. cerevisiae.* Many features were found in only a single strain, suggesting that there are many additional features that will be identified by analysis of a larger number of strains. Some initial analysis suggests there are potential null alleles, pseudogenes, introgressed genes, and additional strain specific genes in the 1011 *S. cerevisiae* genomes (Peter et al. 2018) that are not present in the 94 strains discussed here.

This work focusses on the core genes, not the strain specific genes. The reason for this is that these genome sequences are still not complete in the subtelomeric regions where many strain specific genes are found, so a list of the strain specific genes for the 93 genomes is not yet possible. Improvements in sequencing technology since the data used here was generated, particularly recent advances in long sequence reads, means that complete, highly accurate genome sequences can now be more easily generated.

Another feature of this analysis is that the 94 strains examined here are haploids, or in the case of the 93 strains it is more accurate to say they are switched and mated homozygous diploids by the action of *HO*. Wild strains are typically diploid and often have extensive heterozygosity. In this analysis potentially null alleles are found in over 400 protein coding genes; it is unclear if these potential null alleles are typically found in the population in a heterozygous or homozygous state. Phased diploid sequencing would potentially answer this question, and other questions about the composition of wild strains of *S. cerevisiae.*

In this paper we propose that the *S. cerevisiae* S288c genome contains 5495 core genes of known function, more than 98% of which have orthologs in pre-WGD species. An additional 378 core genes of unknown function, labeled as uncharacterized in SGD, are present for a total of 5873 core genes, a number amazingly close to the 5885 genes reported by André Goffeau (Goffeau et al. 1996). Eleven of the core genes are potentially null alleles in S288c, disrupted by a frameshift, a stop codon, or a transposon. There are also 22 non-core genes in the central regions of the genome, plus additional non-core genes in the subtelomeric regions. In the current annotation of 681 dubious genes, we conclude 680

are dubious, but *YBR012C* is not dubious but is a segment of a disrupted gene, the other half currently annotated as *YBR013C*. Here it is proposed that another 237 currently annotated genes should be relegated to dubious as they appear unlikely to be real genes but are instead annotation errors. In the absence of solid experimental evidence identifying a function for these open reading frames, their annotation as genes is misleading. Furthermore, there are five gene remnants in S288c, fragments of former genes that have not completely disappeared. With more careful analysis, more gene remnants may be found. Two Serine-tRNA genes appear to have sequence errors.

This work uncovers that there are seven real cases of convergent protein coding genes that overlap, and exactly one intron with a GC 5' splice site, the remaining introns having the typical eukaryotic GT splice site, and that some *S. cerevisiae* strains carry a novel transposon. More interestingly, reanalysis of the *S. cerevisiae* S288c genome suggests that, for example, the gene sets of *Candida albicans* and *S. cerevisiae* are more similar than has been previously thought due to the disrupted genes, the gene remnants, and the 237 proposed dubious genes, and other issues with the annotation.

No new data was presented in this paper. The data presented here has been publicly available in the fastq files deposited in in the NCBI short read archive for the initial publication of the 93 genomes (Strope et al. 2015), along with other publicly available data. There is now a massive amount of fungal data available, but the challenge is it is a labor-intensive process to extract information from data. That is what we have attempted to do here.

Data Availability
The 93 *S. cerevisiae* strains are available through the Fungal Genetics Stock Center. Additional information on the strains is available (Strope et al. 2015).
The Illumina sequence data and annotated chromosomes are available through GenBank at the accession numbers in table S19 of Strope et al (Strope et al. 2015).

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Conflicts of interest
The authors declare no conflict of interest.

Table 1 Nomenclature used in this paper.

Chromosome Names	These follow the chromosome numbering as described in the genetic maps of Robert Mortimer (Mortimer and Hawthorne 1966, Mortimer and Schild 1980, Mortimer and Schild 1985, Mortimer et al. 1989, Mortimer et al. 1992, Cherry et al. 1997), with the addition that in cases where there are translocations, the name of the chromosome is based on the origin of the centromere. In the 94 strains examined here, there has been no gain or loss of centromeres.
Gene Names	Gene names follow the establish rules for *S. cerevisiae* as described originally described and coordinated by Robert Mortimer, and more recently by SGD (SGD 1998). There are several difficulties with this naming convention that will need to be addressed in the future. These include names of genes not in the reference strain, names of tandemly duplicated genes, names of Ty/LTR elements, names of introgressed genes, and the names of subtelomeric genes that are located on different chromosomes in different strains.
Gene	While sometimes gene is used to mean protein coding gene, in general and in this work gene refers to DNA elements encoding RNA products, such as tRNA molecules as well as genes encoding proteins. Genomic structural features such as centromeres are not included here as genes.
Allele	This term is commonly used in reference both to naturally occurring alleles, and those engineered. In this work alleles refers to gene variants found at the location and in the same orientation. Alleles include those with, relative to other strains, synonymous and non-synonymous changes, as well as introgressed orthologs at the usual location for the gene and changes resulting in a premature stop codon, Ty insertion, and insertions and deletions, including those that are out of frame. While synthetic gene deletions are usually referred to as null alleles, naturally occurring alleles are sometimes mistakenly referred to as pseudogenes, and in this work we try to carefully distinguish between alleles and pseudogenes.
Potentially null allele; disrupted gene	An allele containing one or more stop codons or frameshift mutations or non-synonymous changes likely to result in the gene being non-functional. These are sometimes referred to as disrupted genes. These should be considered potentially null alleles as a single stop codon or frameshift can revert, and frameshift and stop codon suppression occur in *S. cerevisiae.*
Truncated allele	An allele where a portion of the gene is missing due to deletion or Ty insertion.
Pseudogene	A non-functional copy of DNA at a location where that DNA is not usually found. This can be a fragment of mitochondrial DNA inserted in the nuclear genome, a partial tRNA gene, a piece of rDNA, or a piece of a protein coding gene or intergenic region. In *S. cerevisiae* many of these pseudogenes are found in the subtelomeric regions.
Gene Remnant	Not a gene, but a region of chromosomal DNA with similarity to an ancestral gene that was at that location in the ancestor of *S. cerevisiae*, but is no longer there, though recognizable fragments of the gene persist, and can be recognized by similarity with genes in related species, or in some cases the paralog from the whole genome duplication. These gene fragments cannot easily be resurrected to a gene by a small number of mutations, or by gene conversion as the gene is generally not present in this species, and insufficient homology exists with other genes. In general, the most likely chance of resurrection is introgression. In some cases these gene remnants are the result of loss of genes that were duplicates from the WGD. In other cases they result from the process of gene duplication, where duplication of one gene can carry along a fragment of an adjoining gene.
Whole Genome Duplication (WGD) Duplicate Gene	While in most cases one gene was lost from pairs of genes resulting from the whole genome duplication (Philippsen et al. 1997, Wolfe and Shields 1997), some 600 pairs of duplicate genes remain from that event in the ancestry of *S. cerevisiae.*
Tandem Duplicate Gene	Cases of tandem gene duplication exist. In some cases, the gene copies are identical or nearly identical, and in other cases the copies are diverged. Some tandem duplicate genes are ubiquitous, while others are found in a subset of strains. Nomenclature for variable tandem arrays of genes is problematic.

Dispersed Duplicate Gene	Duplicate genes, not adjacent, often located in the subtelomeric regions of chromosomes. These are generally intact genes, as opposed to pseudogenes that are gene fragments. In some cases it is unclear if a DNA segment encodes a pseudogene or a dispersed duplicate gene.
Telomeric Sequence	The sequence found at telomeres of all *S. cerevisiae* chromosomes that is encoded by the TLC1 RNA (Walmsley et al. 1984).
Subtelomeric Region	The region from the telomeric sequence to but not including the most distal gene in the central region of the chromosome. Includes a mixture of genes and repeat sequences. This definition of the subtelomeric region is based on genes. The alternative definition sometimes used is based on the extent of gene silencing, a well-studied phenomena in *S. cerevisiae.*
Central Region of Chromosome	The region of the chromosome extending out from the centromere in both directions until and including the most distal gene that is conserved at the same location and orientation in 95% of the strains being examined. There are several criteria that could be used to determine the central region of the genome, and the 95% threshold used in these definitions is arbitrary, so some future thought needs to go into identifying criteria that reflect the organism.
Core Gene	A gene in the central region of the chromosomes, or in the Mitochondrial genome, where an allele is at the same position and orientation in 95% of the strains examined.
Variably Located Ubiquitous Gene	A gene found in more than 95% of strains, and in less than 95% of strains at a single central region location and at variable locations either in the central region of the genome, the subtelomeric portion of the genome, or both.
Strain Specific Genes	Genes found in less than 95% of strains in either the central region of the genome or the subtelomeric portion of the genome.
Verified Gene	A term pioneered by SGD to identify protein coding genes where something is known about the function of the gene. In this work we use the SGD assignment of which genes are verified to support that something is known about the function. In the future it may be desirable to further define what is meant by a gene of known function.
Uncharacterized Gene	A term pioneered by SGD to identify protein coding genes where evidence suggests they are real, but where the function is unknown. Also referred to as genes of unknown function.
Dubious Gene	A term pioneered by SGD to identify regions previously annotated as a protein coding gene where there is little to no evidence that this putative gene is real. This strictly speaking does not mean that a region is not a gene, only that there is insufficient data. If evidence is forthcoming, then it is possible for a dubious gene to be resurrected to the status of verified or uncharacterized gene.

Table 2 Final conserved gene for each chromosome arm.

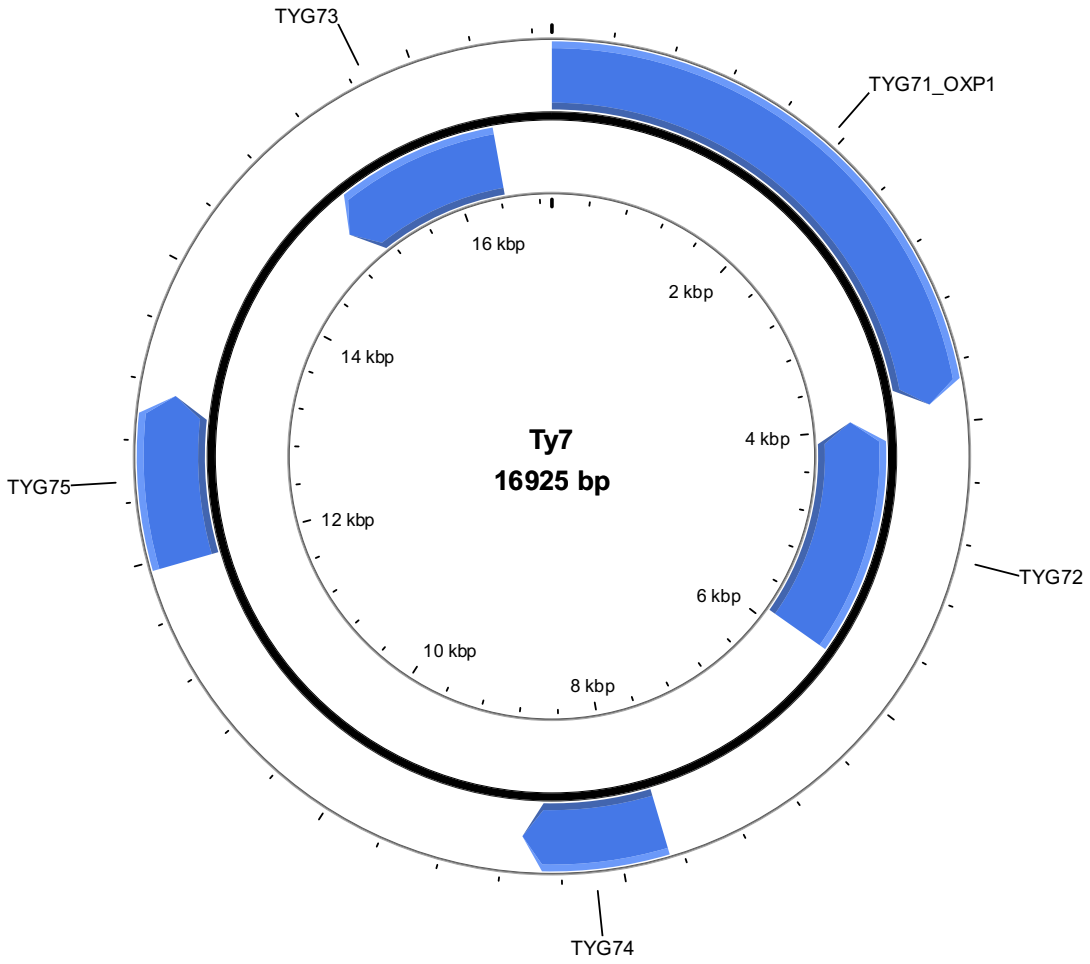
Chromosome	Final conserved gene	Common name	Number of strains out of 93 containing this final central region gene.	first variable gene	Common name	Number of strains out of 93 containing this first variable gene
I L	YAL063C	FLO9	92; not in YJM996, GDH3 at end	YAL064W		17
I R	YAR050W	FLO1	92; not in YJM428	YAR066W		87
II L	YNCB0001W	tL(UAA)B1	93	YBL108C-A	PAU9	33
II R	YBR297W	MAL33	93	YBR298C	MAL31	56
III L	YCL073C	GEX1	93	Ty element, Telomere		
III R	REMYCR099C	YCR099C YCR100C YCR101C	92; not in YJM1078, YDR541C at end	YCR102C		87 missing from YJM1078 YJM1252 YJM1381 YJM1399 YJM1444 YJM248
IV L	YDL243C	AAD4	92; YJM1355 - YDL243C internal; large fragment of chromosome XI ending in YKL222C at left end	YDL244W	THI3	81
IV R	YDR541C		93	YDR542W	PAU10	36
V L	YEL070W	DSF1	93	YEL071W	DLD3	83 sometimes located on other chromosomes
V R	YER187W	KHS1	92 not in YJM326 YER186C present	YER188W		78
VI L	YFL051C*	FLO12	91 not in YJM1389 YJM1592 both have YFL050C	YFL052W	ZNF1	36
VI R	YFR055W	IRC7	93	YFR057W		61
VII L	YGL258W	VEL1	93	YGL258W-A		80 some moved to right telomere chrXV, some both positions, some neither
VII R	YGR287C	IMA1	93	YGR288W	MAL13	52
VIII L	YHL044W [YPL274W]	DFP4	93 (11 YPL274W due to chrVIII/chrXVI translocation)	YHL046C	PAU13	51
VIII R	YHR211W	FLO5	93	YHR213W-A		66
IX L	YIL169C [YIR039C]	CSS1	91; YJM1341 ends YIL162W YIL169C end of chrI YJM195 very large inversion	YIL170W	HXT12	56
IX R	YIR039C [YIL169C]	YPS6	92; YJM195 very large inversion	YIR041W	PAU15	34 YJM195 PAU15 at opposite telomere
X L	YJL213W		93	YJL214W	HXT8	88
X R	YJR152W	DAL5	93	YJR153W	PGU1	83
XI L	YKL222C		93	YKL224C	PAU16	10
XI R	YKR101W	SIR1	93	YKR102W	FLO10	87
XII L	YLL062C	MHT1	93	YLL063C	AYT1	80
XII R	YLR460C		92; not in YJM689; ends at YOR380W	YLR461W	PAU4	14
XIII L	YML131W		93	YML132W	COS3	60
XIII R	YMR321C		92 not in YJM270; ends at YMR319C	YMR322C	SNO4	44
XIV L	YNL332W	THI12	93	YNL333W	SNZ2	74
XIV R	YNR071C		89 YJM1078 YJM248 end YNR069C YJM1387 YJM1478 end YNR065C	YNR072W	HXT17	88
XV L	YOL154W	ZPS1	93	YOL155C	HPF1	84
XV R	YOR381W	FRE3	92; YJM470 ends YOR380W	YOR381W-A		87
XVI L	YPL274W [YHL044W]	SAM3	93 11 on chrVIII due to translocation	YPL275W	FDH2	85
XVI R	YPR201W	ARR3	89; YJM627 ends YPR194C; YJM1400, YJM1447, YJM1479 end YPR198W	telomere		

* YFL051 is a gene fragment in S288c, though an intact member of the FLO1 FLO5 FLO9 FLO10 family of genes in most strains

Figure 1 Ty7
A) Map of Ty7. Start codon of *TYG71* was arbitrarily selected as nucleotide one of this map. The five protein coding genes found in this transposon are shown.

B) Insertion sites of Ty7. S288c is used for comparison as it contains no Ty7 elements. "Ty7" is the consensus Ty7 sequence. A_ and B_ are the two ends of the inserted Ty7. Note that Ty7 elements are circularly permuted and different sites in the element are found at the insertion junctions.

A)



B)

```
Ty7               GAATCTGCTTACATCTGTAGAGGTGCCACCCATGTCGAATCCTATGAGGGGAATCCGGTTATTATCATTGTAACAAGTCCTAGAGTAGCCTACAACACCTCCAGCC
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM1549_chrII   TATTGTCATATTATACTAAAGTTGAAATGGTGGAGTCGACCCTAAGAATTTATTTCGGTTATTATCATTGTAACAAGTCCTAGAGTAGCCTACAACACCTCCAGCC
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrII       TATTGTCATATTATACTAAAGTTGAAATGGTGGAGTCGACCCTAAGAATTTATTTCTTCCAGACA-GAGGAGGTGTGTAGTCACTTTGGTTCCACTAATAGACAAC
                                                                                                        ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
B_YJM1549_chrII   GAATCTGCTTACATCTGTAGAGGTGCCACCCATGTCGAATCCTATGAGGGGAATTCTTCCAGACAAGAGGAGGTGTGTAGTCACTTTGGTTCCACTAATAGACAAC
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               GAATCTGCTTACATCTGTAGAGGTGCCACCCATGTCGAATCCTATGAGGGGAATCCGGTTATTATCATTGTAACAAGTCCTAGAGTAGCCTACAACACCTCCAGCC

Ty7               GCAATGTTATTTCTCAGCCGTTAATTTTGCTCAATATTTGGTTCTCGCTCTATTTTGTGTTACCTTCCTCGCGTTTAACAAGCTTCGTTTCTGCAGCTTTAAGAAA
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM1415_chrIV   TCCTCTATTTATATGACCTCACCAGCAGCGATCTCAGGCACCGTACCATCAATTTTGTGTTACCTTCCTCGCGTTTAACAAGCTTCGTTTCTGCAGCTTTAAGAAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrIV       TCCTCTATTTATATGACCTCACCAGCAGCGATCTCAGGCACCGTACCATCAATTTTCTAAGTGGTGTATATATATGTTCGAAGGTATTGTTGTAATATTTTCATAG
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B_YJM1415_chrIV   GCAATGTTATTTCTCAGCCGTTAATTTTGCTCAATATTTGGTTCTCGCTCTATTTTCTAAGTGGTGTATATATATGTTCGAAGGTATTGTTGTAATATTTTCATAG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               GCAATGTTATTTCTCAGCCGTTAATTTTGCTCAATATTTGGTTCTCGCTCTATTTTGTGTTACCTTCCTCGCGTTTAACAAGCTTCGTTTCTGCAGCTTTAAGAAA

Ty7               TCCGATGCGCAACTAGCAGTACCGCCACTCGATCCTGGTATCGACCTGGATCTTG-CACGAAAGTTTGATCTCTCAAGAATATTGCATGACATCGGGGATAACTCA
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM1574_chrXIV  CAAGAAAAACCCCTGAGCAGACCAAAAGATTGAACATCAAATTATCATTACAGAAACACGAAAGTTTGATCTCTCAAGAATATTGCATGACATCGGGGATAACTCA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrXIV      CAAGAAAAACCCCTGAGCAGACCAAAAGATTGAACATCAAATTATCATTACAGAAAATCTTGAGACATTTTGGTATATACTTATATTTTAGTGTATGTTTATATAT
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B_YJM1574_chrXIV  TCCGATGCGCAACTAGCAGTACCGCCACTCGATCCTGGTATCGACCTGGATCTTCAATCTTGAGACATTTTGGTATATACTTATATTTTAGTGTATGTTTATATAT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               TCCGATGCGCAACTAGCAGTACCGCCACTCGATCCTGGTATCGACCTGGATCTTG-CACGAAAGTTTGATCTCTCAAGAATATTGCATGACATCGGGGATAACTCA

Ty7               GGAAGAAAATTTCATCATTCTGTAGGTGCGCTTAGCATATATTTTGGAAACCGTAATCGAGCTGAATGTTGGTACAGCTACTTTCTCATGCACTACTTTCAACTTA
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM189_chrXII   CGATTGAAGGCAGGAGCTCAACAAACACGAAACAAAACAACGAAGAAAGAAAAGAATCGAGCTGAATGTTGGTACAGCTACTTTCTCATGCACTACTTTCAACTTA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrXII      CGATTGAAGGCAGGAGCTCAACAAACACGAAACAAAACAACGAAGAAAGAAAAGAAATATATCAGAGAACAGAGACCGCCTCGTTTAGGGCCGCACGTATACCGCG
                                                                                                                  ||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
B_YJM189_chrXII   GGAAGAAAATTTCATCATTCTGTAGGTGCGCTTAGCATATATTTTGGAAACCGTAAATATATCAGAGGACAGAGACCGCCTCGTTTAGGGCCGCACGTATACCGCG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               GGAAGAAAATTTCATCATTCTGTAGGTGCGCTTAGCATATATTTTGGAAACCGTAATCGAGCTGAATGTTGGTACAGCTACTTTCTCATGCACTACTTTCAACTTA
```

Special case - recombination of Ty7 *TYG71* (*OXP1*) into *YKL215C* (*OXP1*) resulting in two hybrid genes.

```
Ty7               TCTAACAACCTTCTAATGCCTTCCAAAGGTGCATCTGGGTAGTTCTTGGGATCCACTGACAGCAACTTGATGAATACATCATCCTCAATTTTACCAGTCCCGGGGT
                  || || |  | ||| |||||||| |  || |||||||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM1478_chrXI   TCCAAGAGACGTCTTATGCCTTCAAGGGGAGCATCTGGATAGTTTTTGGGATCCACTGACAGCAACTTGATGAATACATCATCCTCAATTTTACCAGTCCCGGGGT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrXI       TCCAAGAGACGTCTTATGCCTTCAAGGGGAGCATCTGGATAGTTTTTGGGATCCACCGACAAGAGCTTGATGACAGTATCATGTTCTTGTTTACCTGTACCAATAT
                  || || |  | ||| |||||||| |  || |||||||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B_YJM1478_chrXI   TCTAACAACCTTCTAATGCCTTCCAAAGGTGCATCTGGGTAGTTCTTGGGATCCACCGACAAGAGCTTGATGACAGTATCATGTTCTTGTTTACCTGTACCAATAT
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               TCTAACAACCTTCTAATGCCTTCCAAAGGTGCATCTGGGTAGTTCTTGGGATCCACTGACAGCAACTTGATGAATACATCATCCTCAATTTTACCAGTCCCGGGGT

Ty7               AAAAGCCGAATACGTATGGTCCAAGATAGATGTTCCAGAAGCTTTTCAACACATTTCTGAGTATTGACTGCCCCTGTTTCTGAGGGTATACTCTTACCTTGCTAGT
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM271_chrXIII  CACGAGAAATTTGAGCAAAGAGCACGTTGCGAATACGTTTTATTCATTCATCGTTTCTGAGTATTGACTGCCCCTGTTTCTGAGGGTATACTCTTACCTTGCTAGT
                  ||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrXIII     CACGAGAAATTTGAGCAAAGAGCACGTTGCGAATACGTTTTATCCATTCATCGTTTTTAATTTATTACCTTTTCTTTCGATGCTGTTTATTGTCACTCATGAGTGC
                                                                                                                  |||||||||||||||||||| ||||||||||||||||||||||||||||||||||
B_YJM271_chrXIII  AAAAGCCGAATACGTATGGTCCAAGATAGATGTTCCAGAAGCTTTTCAACACATTTTAATTTATTACCTTTTATTTCGATGCTGTTTATTGTCACTCATGAGTGC
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               AAAAGCCGAATACGTATGGTCCAAGATAGATGTTCCAGAAGCTTTTCAACACATTTCTGAGTATTGACTGCCCCTGTTTCTGAGGGTATACTCTTACCTTGCTAGT

Ty7               CTGCTTGTAATTTCAATAAGCAGCTCACTGAGATTATAGAATTAATTGGTTCCACAAATGTGCATATTCCCCAGGAACACCCTTTTCCGAACCCATCTGTAGGCAA
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM453_chrXV    TACGAATATTCCAATGAAACTAACAATTACGGGCTCGTTCAAATTGATGGTGACAAAATGTGCATATTCCCCAGGAACACCCTTTTCCGAACCCATCTGTAGGCAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrXV       TACGAATATTCCAATGAAACTAACAATTACGGGCTCGTTCAAATTGATGGTGACAAGGTCACTAAATTGACAGATTTTGAAAACTTGAAAAATGAATACAGCAAAG
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B_YJM453_chrXV    CTGCTTGTAATTTCAATAAGCAGCTCACTGAGATTATAGAATTAATTGGTTCCACAGGTCACTAAATTGACAGATTTTGAAAACTTGAAAAATGAATACAGCAAAG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               CTGCTTGTAATTTCAATAAGCAGCTCACTGAGATTATAGAATTAATTGGTTCCACAAATGTGCATATTCCCCAGGAACACCCTTTTCCGAACCCATCTGTAGGCAA

Ty7               CTATCTGAAGTTTGTGTTAAGATGTATAGAGTGATTTATTATCGTATTATCTTTTACTCTTGCAGCGTTTGTGTTAAGCTTATGTATAGAGTGATTTATTATCTTA
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM555_chrXI    TTAAAGCCGCAAAAGATGAAGGGTTTTCAAAATTTTCATCTCTTGCAGTATAAGGACTCTTGCAGCGTTTGTGTTAAGCTTATGTATAGAGTGATTTATTATCTTA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_chrXI       TTAAAGCCGCAAAAGATGAAGGGTTTTCAAAATTTTCATCTCTTGCAGTATAAGGATGCAGTGAATCTATCTACAGACAGTTGATAAAAACGTCTACCTTGCCTAG
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B_YJM555_chrXI    CTATCTGAAGTTTGTGTTAAGATGTATAGAGTGATTTATTATCGTATTATCTTTTACGCAGTGAATCTATCTACAGACAGTTGATAAAAACGTCTACCTTGCCTAG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               CTATCTGAAGTTTGTGTTAAGATGTATAGAGTGATTTATTATCGTATTATCTTTTACTCTTGCAGCGTTTGTGTTAAGCTTATGTATAGAGTGATTTATTATCTTA
```

Special case - insertion into Ty1 element. This was either an insertion into a Ty1 element with a deletion at the position marked by / at the deletion site, or the insertion resulted in a deletion in the Ty1 sequence, or the deletion in Ty1 induced translocation of Ty7 possibly through a DNA repair mechanism.

```
Ty7               TTAGAATAGGGCGTGCGGCGTCAATTAGATGTATGCTGCCCCTTTCATTCCTCTGTTCATTCGGGCAGTGGCCACAGTTTTCGTAAAATGAATAGGGGAGATCATG
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
A_YJM451_chrXVI   TGATAATATAACTATTAGTTGATAGACGATAGTGGATTTTTATTCCAACACTCTGTTCATTCGGGCAGTGGCCACAGTTTTCGTAAAATGAATAGGGGAGATCATG
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
S288c_Ty1         TGATAATATAACTATTAGTTGATAGACGATAGTGGATTTTTATTCCAACATA/CATGAGAAATGGGGGAATGTTGAGATAATTGTTGGGATTCCATTGTTGATAAA
                                                                                                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
B_YJM451_chrXVI   TTAGAATAGGGCGTGCGGCGTCAATTAGATGTATGCTGCCCCTTTCATTCCTCTGTGAGAAATGGGGGAATGTTGAGATAATTGTTGGGATTCCATTGTTGATAAA
                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Ty7               TTAGAATAGGGCGTGCGGCGTCAATTAGATGTATGCTGCCCCTTTCATTCCTCTGTTCATTCGGGCAGTGGCCACAGTTTTCGTAAAATGAATAGGGGAGATCATG
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References

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman (1990). "Basic local alignment search tool." J Mol Biol **215**(3): 403-410.

Balakrishnan, R., J. Park, K. Karra, B. C. Hitz, G. Binkley, E. L. Hong, J. Sullivan, G. Micklem and J. M. Cherry (2012). "YeastMine--an integrated data warehouse for *Saccharomyces cerevisiae* data as a multipurpose tool-kit." Database (Oxford) **2012**: bar062.

Belcourt, M. F. and P. J. Farabaugh (1990). "Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site." Cell **62**(2): 339-352.

Bergman, C. M. (2018). "Horizontal transfer and proliferation of *Tsu4* in *Saccharomyces paradoxus*." Mob DNA **9**: 18.

Bergstrom, A., J. T. Simpson, F. Salinas, B. Barre, L. Parts, A. Zia, A. N. Nguyen Ba, A. M. Moses, E. J. Louis, V. Mustonen, J. Warringer, R. Durbin and G. Liti (2014). "A high-definition view of functional genetic variation from natural yeast genomes." Mol Biol Evol **31**(4): 872-888.

Brudno, M., C. B. Do, G. M. Cooper, M. F. Kim, E. Davydov, N. C. S. Program, E. D. Green, A. Sidow and S. Batzoglou (2003). "LAGAN and Multi-LAGAN: efficient tools for large-scale multiple alignment of genomic DNA." Genome Res **13**(4): 721-731.

Byrne, K. P. and K. H. Wolfe (2005). "The Yeast Gene Order Browser: combining curated homology and syntenic context reveals gene fate in polyploid species." Genome Res **15**(10): 1456-1461.

Cameron, J. R., E. Y. Loh and R. W. Davis (1979). "Evidence for transposition of dispersed repetitive DNA families in yeast." Cell **16**(4): 739-751.

Cherry, J. M., C. Ball, S. Weng, G. Juvik, R. Schmidt, C. Adler, B. Dunn, S. Dwight, L. Riles, R. K. Mortimer and D. Botstein (1997). "Genetic and physical maps of *Saccharomyces cerevisiae*." Nature **387**(6632 Suppl): 67-73.

Cherry, J. M., E. L. Hong, C. Amundsen, R. Balakrishnan, G. Binkley, E. T. Chan, K. R. Christie, M. C. Costanzo, S. S. Dwight, S. R. Engel, D. G. Fisk, J. E. Hirschman, B. C. Hitz, K. Karra, C. J. Krieger, S. R. Miyasato, R. S. Nash, J. Park, M. S. Skrzypek, M. Simison, S. Weng and E. D. Wong (2012). "*Saccharomyces* Genome Database: the genomics resource of budding yeast." Nucleic Acids Res **40**(Database issue): D700-705.

Christiansen, T., B. D. Foy, L. Wall and L. Wall (2012). Programming Perl. Beijing ; Sebastopol, O'Reilly.

Coelho, P. S., A. C. Bryan, A. Kumar, G. S. Shadel and M. Snyder (2002). "A novel mitochondrial protein, Tar1p, is encoded on the antisense strand of the nuclear 25S rDNA." Genes Dev **16**(21): 2755-2760.

Dietrich, F. S., S. Voegeli, S. Brachat, A. Lerch, K. Gates, S. Steiner, C. Mohr, R. Pohlmann, P. Luedi, S. Choi, R. A. Wing, A. Flavier, T. D. Gaffney and P. Philippsen (2004). "The *Ashbya gossypii* genome as a tool for mapping the ancient *Saccharomyces cerevisiae* genome." Science **304**(5668): 304-307.

Dietrich, F. S., S. Voegeli, S. Kuo and P. Philippsen (2013). "Genomes of *Ashbya* fungi isolated from insects reveal four mating-type loci, numerous translocations, lack of transposons, and distinct gene duplications." G3 (Bethesda) **3**(8): 1225-1239.

Eisenberg-Bord, M., N. Zung, J. Collado, L. Drwesh, E. J. Fenech, A. Fadel, N. Dezorella, Y. S. Bykov, D. Rapaport, R. Fernandez-Busnadiego and M. Schuldiner (2021). "Cnm1 mediates nucleus-mitochondria contact site formation in response to phospholipid levels." J Cell Biol **220**(11).

Engel, S. R., E. D. Wong, R. S. Nash, S. Aleksander, M. Alexander, E. Douglass, K. Karra, S. R. Miyasato, M. Simison, M. S. Skrzypek, S. Weng and J. M. Cherry (2022). "New data and collaborations at the Saccharomyces Genome Database: updated reference genome, alleles, and the Alliance of Genome Resources." Genetics **220**(4).

Fink, G. R. and C. A. Styles (1974). "Gene conversion of deletions in the his4 region of yeast." Genetics **77**(2): 231-244.

Frank, A. C. and K. H. Wolfe (2009). "Evolutionary capture of viral and plasmid DNA by yeast nuclear chromosomes." Eukaryot Cell **8**(10): 1521-1531.

Fukuda, T., K. Furukawa, T. Maruyama, S. I. Yamashita, D. Noshiro, C. Song, Y. Ogasawara, K. Okuyama, J. M. Alam, M. Hayatsu, T. Saigusa, K. Inoue, K. Ikeda, A. Takai, L. Chen, V. Lahiri, Y. Okada, S. Shibata, K. Murata, D. J. Klionsky, N. N. Noda and T. Kanki (2023). "The mitochondrial intermembrane space protein mitofissin drives mitochondrial fission required for mitophagy." Mol Cell.
Gaisne, M., A. M. Becam, J. Verdiere and C. J. Herbert (1999). "A 'natural' mutation in *Saccharomyces cerevisiae* strains derived from S288c affects the complex regulatory gene *HAP1* (*CYP1*)." Curr Genet **36**(4): 195-200.
Goffeau, A., B. G. Barrell, H. Bussey, R. W. Davis, B. Dujon, H. Feldmann, F. Galibert, J. D. Hoheisel, C. Jacq, M. Johnston, E. J. Louis, H. W. Mewes, Y. Murakami, P. Philippsen, H. Tettelin and S. G. Oliver (1996). "Life with 6000 genes." Science **274**(5287): 546, 563-547.
Goto, K., H. Fukuda, K. Kichise, K. Kitano and S. Hara (1991). "Cloning and nucleotide sequence of the *KHS* killer gene of *Saccharomyces cerevisiae*." Agric Biol Chem **55**(8): 1953-1958.
Goto, K., Y. Iwatuki, K. Kitano, T. Obata and S. Hara (1990). "Cloning and nucleotide sequence of the *KHR* killer gene of *Saccharomyces cerevisiae*." Agric Biol Chem **54**(4): 979-984.
Hall, C., S. Brachat and F. S. Dietrich (2005). "Contribution of horizontal gene transfer to the evolution of *Saccharomyces cerevisiae*." Eukaryot Cell **4**(6): 1102-1115.
Hall, C. and F. S. Dietrich (2007). "The reacquisition of biotin prototrophy in *Saccharomyces cerevisiae* involved horizontal gene transfer, gene duplication and gene clustering." Genetics **177**(4): 2293-2307.
Halvorson, H. O., S. Winderman and J. Gorman (1963). "Comparison of the alpha-glucosidases of *Saccharomyces* produced in response to five non-allelic maltose genes." Biochim Biophys Acta **67**: 42-53.
Hayashi, T., K. Makino, M. Ohnishi, K. Kurokawa, K. Ishii, K. Yokoyama, C. G. Han, E. Ohtsubo, K. Nakayama, T. Murata, M. Tanaka, T. Tobe, T. Iida, H. Takami, T. Honda, C. Sasakawa, N. Ogasawara, T. Yasunaga, S. Kuhara, T. Shiba, M. Hattori and H. Shinagawa (2001). "Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12." DNA Res **8**(1): 11-22.
He, C., C. Jia, Y. Zhang and P. Xu (2018). "Enrichment-Based Proteogenomics Identifies Microproteins, Missing Proteins, and Novel smORFs in Saccharomyces cerevisiae." J Proteome Res **17**(7): 2335-2344.
Higgins, D. G. and P. M. Sharp (1988). "CLUSTAL: a package for performing multiple sequence alignment on a microcomputer." Gene **73**(1): 237-244.
Hirschman, J. E., R. Balakrishnan, K. R. Christie, M. C. Costanzo, S. S. Dwight, S. R. Engel, D. G. Fisk, E. L. Hong, M. S. Livstone, R. Nash, J. Park, R. Oughtred, M. Skrzypek, B. Starr, C. L. Theesfeld, J. Williams, R. Andrada, G. Binkley, Q. Dong, C. Lane, S. Miyasato, A. Sethuraman, M. Schroeder, M. K. Thanawala, S. Weng, K. Dolinski, D. Botstein and J. M. Cherry (2006). "Genome Snapshot: a new resource at the *Saccharomyces* Genome Database (SGD) presenting an overview of the *Saccharomyces cerevisiae* genome." Nucleic Acids Res **34**(Database issue): D442-445.
Jacquier, A. and B. Dujon (1985). "An intron-encoded protein is active in a gene conversion process that spreads an intron into a mitochondrial gene." Cell **41**(2): 383-394.
Katoh, K., K. Misawa, K. Kuma and T. Miyata (2002). "MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform." Nucleic Acids Res **30**(14): 3059-3066.
Kernighan, B. W. and D. M. Ritchie (1988). The C programming language. Englewood Cliffs, N.J., Prentice Hall.
Krause, D. J., J. Kominek, D. A. Opulente, X. X. Shen, X. Zhou, Q. K. Langdon, J. DeVirgilio, A. B. Hulfachor, C. P. Kurtzman, A. Rokas and C. T. Hittinger (2018). "Functional and evolutionary characterization of a secondary metabolite gene cluster in budding yeasts." Proc Natl Acad Sci U S A **115**(43): 11030-11035.
Li, H. and R. Durbin (2009). "Fast and accurate short read alignment with Burrows-Wheeler transform." Bioinformatics **25**(14): 1754-1760.
Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin and S. Genome Project Data Processing (2009). "The Sequence Alignment/Map format and SAMtools." Bioinformatics **25**(16): 2078-2079.

Li, Y., W. Zhang, D. Zheng, Z. Zhou, W. Yu, L. Zhang, L. Feng, X. Liang, W. Guan, J. Zhou, J. Chen and Z. Lin (2014). "Genomic evolution of Saccharomyces cerevisiae under Chinese rice wine fermentation." Genome Biol Evol **6**(9): 2516-2526.
Lowe, T. M. and S. R. Eddy (1997). "tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence." Nucleic Acids Res **25**(5): 955-964.
Ma, J., C. J. Dobry, D. J. Krysan and A. Kumar (2008). "Unconventional genomic architecture in the budding yeast *Saccharomyces cerevisiae* masks the nested antisense gene *NAG1*." Eukaryot Cell **7**(8): 1289-1298.
McCarthy, C. G. P. and D. A. Fitzpatrick (2019). "Pan-genome analyses of model fungal species." Microb Genom **5**(2).
McIlwain, S. J., D. Peris, M. Sardi, O. V. Moskvin, F. Zhan, K. S. Myers, N. M. Riley, A. Buzzell, L. S. Parreiras, I. M. Ong, R. Landick, J. J. Coon, A. P. Gasch, T. K. Sato and C. T. Hittinger (2016). "Genome Sequence and Analysis of a Stress-Tolerant, Wild-Derived Strain of *Saccharomyces cerevisiae* Used in Biofuels Research." G3 (Bethesda) **6**(6): 1757-1766.
Meiron, H., E. Nahon and D. Raveh (1995). "Identification of the heterothallic mutation in HO-endonuclease of S. cerevisiae using HO/ho chimeric genes." Curr Genet **28**(4): 367-373.
Monerawela, C., T. C. James, K. H. Wolfe and U. Bond (2015). "Loss of lager specific genes and subtelomeric regions define two different Saccharomyces cerevisiae lineages for Saccharomyces pastorianus Group I and II strains." FEMS Yeast Res **15**(2).
Mortimer, R. K., C. R. Contopoulou and J. S. King (1992). "Genetic and physical maps of *Saccharomyces cerevisiae*, Edition 11." Yeast **8**(10): 817-902.
Mortimer, R. K. and D. C. Hawthorne (1966). "Genetic mapping in *Saccharomyces*." Genetics **53**(1): 165-173.
Mortimer, R. K. and D. Schild (1980). "Genetic map of *Saccharomyces cerevisiae*." Microbiol Rev **44**(4): 519-571.
Mortimer, R. K. and D. Schild (1985). "Genetic map of *Saccharomyces cerevisiae*, edition 9." Microbiol Rev **49**(3): 181-213.
Mortimer, R. K., D. Schild, C. R. Contopoulou and J. A. Kans (1989). "Genetic map of *Saccharomyces cerevisiae*, edition 10." Yeast **5**(5): 321-403.
Natsoulis, G., F. Hilger and G. R. Fink (1986). "The HTS1 gene encodes both the cytoplasmic and mitochondrial histidine tRNA synthetases of S. cerevisiae." Cell **46**(2): 235-243.
Park, Z. M., E. Belnap, M. Remillard and M. D. Rose (2023). "Vir1p, the yeast homolog of virilizer, is required for mRNA m6A methylation and meiosis." Genetics **224**(1).
Pearson, W. R. and D. J. Lipman (1988). "Improved tools for biological sequence comparison." Proc Natl Acad Sci U S A **85**(8): 2444-2448.
Penalosa-Ruiz, G., C. Aranda, L. Ongay-Larios, M. Colon, H. Quezada and A. Gonzalez (2012). "Paralogous ALT1 and ALT2 retention and diversification have generated catalytically active and inactive aminotransferases in Saccharomyces cerevisiae." PLoS One **7**(9): e45702.
Peter, J., M. De Chiara, A. Friedrich, J. X. Yue, D. Pflieger, A. Bergstrom, A. Sigwalt, B. Barre, K. Freel, A. Llored, C. Cruaud, K. Labadie, J. M. Aury, B. Istace, K. Lebrigand, P. Barbry, S. Engelen, A. Lemainque, P. Wincker, G. Liti and J. Schacherer (2018). "Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates." Nature **556**(7701): 339-344.
Philippsen, P., K. Kleine, R. Pohlmann, A. Dusterhoft, K. Hamberg, J. H. Hegemann, B. Obermaier, L. A. Urrestarazu, R. Aert, K. Albermann, R. Altmann, B. Andre, V. Baladron, J. P. Ballesta, A. M. Becam, J. Beinhauer, J. Boskovic, M. J. Buitrago, F. Bussereau, F. Coster, M. Crouzet, M. D'Angelo, F. Dal Pero, A. De Antoni, F. Del Rey, F. Doignon, H. Domdey, E. Dubois, T. Fiedler, U. Fleig, M. Floeth, C. Fritz, C. Gaillardin, J. M. Garcia-Cantalejo, N. N. Glansdorff, A. Goffeau, U. Gueldener, C. Herbert, K. Heumann, D. Heuss-Neitzel, H. Hilbert, K. Hinni, I. Iraqui Houssaini, M. Jacquet, A. Jimenez, J. L. Jonniaux, L. Karpfinger, G. Lanfranchi, A. Lepingle, H. Levesque, R. Lyck, M. Maftahi, L. Mallet, K. C. Maurer, F. Messenguy, H. W. Mewes, D. Mosti, F. Nasr, J. M. Nicaud, R. K. Niedenthal, D. Pandolfo, A. Pierard, E. Piravandi, R. J. Planta, T. M. Pohl, B. Purnelle, C. Rebischung, M. Remacha, J. L. Revuelta,

M. Rinke, J. E. Saiz, F. Sartorello, B. Scherens, M. Sen-Gupta, A. Soler-Mira, J. H. Urbanus, G. Valle, L. Van Dyck, P. Verhasselt, F. Vierendeels, S. Vissers, M. Voet, G. Volckaert, A. Wach, R. Wambutt, H. Wedler, A. Zollner and J. Hani (1997). "The nucleotide sequence of *Saccharomyces cerevisiae* chromosome XIV and its evolutionary implications." Nature **387**(6632 Suppl): 93-98.
Proudfoot, N. (1980). "Pseudogenes." Nature **286**(5776): 840-841.
Rice, P., I. Longden and A. Bleasby (2000). "EMBOSS: the European Molecular Biology Open Software Suite." Trends Genet **16**(6): 276-277.
Sadhu, M. J., J. S. Bloom, L. Day, J. J. Siegel, S. Kosuri and L. Kruglyak (2018). "Highly parallel genome variant engineering with CRISPR-Cas9." Nat Genet **50**(4): 510-514.
Sanchez, M. R., C. Payen, F. Cheong, B. T. Hovde, S. Bissonnette, A. P. Arkin, J. M. Skerker, R. B. Brem, A. A. Caudy and M. J. Dunham (2019). "Transposon insertional mutagenesis in Saccharomyces uvarum reveals trans-acting effects influencing species-dependent essential genes." Genome Res **29**(3): 396-406.
Sayers, E. W., E. E. Bolton, J. R. Brister, K. Canese, J. Chan, D. C. Comeau, R. Connor, K. Funk, C. Kelly, S. Kim, T. Madej, A. Marchler-Bauer, C. Lanczycki, S. Lathrop, Z. Lu, F. Thibaud-Nissen, T. Murphy, L. Phan, Y. Skripchenko, T. Tse, J. Wang, R. Williams, B. W. Trawick, K. D. Pruitt and S. T. Sherry (2022). "Database resources of the national center for biotechnology information." Nucleic Acids Res **50**(D1): D20-D26.
Selsing, E., J. Voss and U. Storb (1984). "Immunoglobulin gene 'remnant' DNA--implications for antibody gene recombination." Nucleic Acids Res **12**(10): 4229-4246.
SGD (1998). "*Saccharomyces cerevisiae*." Trends Genet **14**(11 Supplement): S.10-S.11.
Simpson, J. T., K. Wong, S. D. Jackman, J. E. Schein, S. J. Jones and I. Birol (2009). "ABySS: a parallel assembler for short read sequence data." Genome Res **19**(6): 1117-1123.
Skrzypek, M. S. and J. Hirschman (2011). "Using the Saccharomyces Genome Database (SGD) for analysis of genomic information." Curr Protoc Bioinformatics **Chapter 1**: 1 20 21-21 20 23.
Smith, T. F. and M. S. Waterman (1981). "Identification of common molecular subsequences." J Mol Biol **147**(1): 195-197.
Stefanini, I., M. Di Paola, G. Liti, A. Marranci, F. Sebastiani, E. Casalone and D. Cavalieri (2022). "Resistance to Arsenite and Arsenate in Saccharomyces cerevisiae Arises through the Subtelomeric Expansion of a Cluster of Yeast Genes." Int J Environ Res Public Health **19**(13).
Strope, P. K., S. G. Kozmin, D. A. Skelly, P. M. Magwene, F. S. Dietrich and J. H. McCusker (2015). "2mu plasmid in Saccharomyces species and in Saccharomyces cerevisiae." FEMS Yeast Res **15**(8).
Strope, P. K., D. A. Skelly, S. G. Kozmin, G. Mahadevan, E. A. Stone, P. M. Magwene, F. S. Dietrich and J. H. McCusker (2015). "The 100-genomes strains, an *S. cerevisiae* resource that illuminates its natural phenotypic and genotypic variation and emergence as an opportunistic pathogen." Genome Res **25**(5): 762-774.
Tapia, S. M., L. G. Macias, R. Perez-Torrado, N. Daroqui, P. Manzanares, A. Querol and E. Barrio (2023). "A novel aminotransferase gene and its regulator acquired in *Saccharomyces* by a horizontal gene transfer event." BMC Biol **21**(1): 102.
Teunissen, A. W. and H. Y. Steensma (1995). "Review: the dominant flocculation genes of Saccharomyces cerevisiae constitute a new subtelomeric gene family." Yeast **11**(11): 1001-1013.
Vijayraghavan, S., S. G. Kozmin, P. K. Strope, D. A. Skelly, Z. Lin, J. Kennell, P. M. Magwene, F. S. Dietrich and J. H. McCusker (2019). "Mitochondrial Genome Variation Affects Multiple Respiration and Nonrespiration Phenotypes in Saccharomyces cerevisiae." Genetics **211**(2): 773-786.
Vijayraghavan, S., S. G. Kozmin, P. K. Strope, D. A. Skelly, P. M. Magwene, F. S. Dietrich and J. H. McCusker (2023). "RNA viruses, M satellites, chromosomal killer genes, and killer/non-killer phenotypes in the 100-genomes S. cerevisiae strains." G3 (Bethesda).
Vijayraghavan, S., S. G. Kozmin, W. Xi and J. H. McCusker (2023). "A novel narnavirus is widespread in Saccharomyces cerevisiae and impacts multiple host phenotypes." G3 (Bethesda) **13**(2).

von Borstel, R. C. (1963). Carbondale Yeast Genetics Conference. Carbondale Yeast Genetics Conference, Laboratoire de Genetique Physiologique, Centre National de la Recherche Scientifique Gifsur-Yvette
Walker, B. J., T. Abeel, T. Shea, M. Priest, A. Abouelliel, S. Sakthikumar, C. A. Cuomo, Q. Zeng, J. Wortman, S. K. Young and A. M. Earl (2014). "Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement." PLoS One **9**(11): e112963.
Wallace, R. L., E. Lu, X. Luo and A. P. Capaldi (2022). "Ait1 regulates TORC1 signaling and localization in budding yeast." Elife **11**.
Walmsley, R. W., C. S. Chan, B. K. Tye and T. D. Petes (1984). "Unusual DNA sequences associated with the ends of yeast chromosomes." Nature **310**(5973): 157-160.
Winge, O. a. R., C. (1949). "A gene for diploidization in yeast." Carlsberg Reasearch Laboratory Communications **24**: 341-346.
Wishart, D. S., L. Ren, J. Leong-Sit, S. Saha, J. R. Grant, P. Stothard, U. Singh, A. Kropielnicki, E. Oler, H. Peters and V. Gautam (2023). "PlasMapper 3.0-a web server for generating, editing, annotating and visualizing publication quality plasmid maps." Nucleic Acids Res.
Wolfe, K. H. and D. C. Shields (1997). "Molecular evidence for an ancient duplication of the entire yeast genome." Nature **387**(6634): 708-713.
Zerbino, D. R. and E. Birney (2008). "Velvet: algorithms for de novo short read assembly using de Bruijn graphs." Genome Res **18**(5): 821-829.
Zhang, C. T. and J. Wang (2000). "Recognition of protein coding genes in the yeast genome at better than 95% accuracy based on the Z curve." Nucleic Acids Res **28**(14): 2804-2814.
Zhao, Y., P. K. Strope, S. G. Kozmin, J. H. McCusker, F. S. Dietrich, R. J. Kokoska and T. D. Petes (2014). "Structures of naturally evolved *CUP1* tandem arrays in yeast indicate that these arrays are generated by unequal nonhomologous recombination." G3 (Bethesda) **4**(11): 2259-2269.