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Original article

The new modern era of yeast genomics: community sequencing and the resulting annotation of multiple *Saccharomyces cerevisiae* strains at the *Saccharomyces* Genome Database

Stacia R. Engel* and J. Michael Cherry

Department of Genetics, Stanford University, Stanford, CA 94305-5120, USA

***Corresponding author:** Tel: +650 725 8956; Email: stacia@stanford.edu

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The first completed eukaryotic genome sequence was that of the yeast *Saccharomyces cerevisiae*, and the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/) is the original model organism database. SGD remains the authoritative community resource for the *S. cerevisiae* reference genome sequence and its annotation, and continues to provide comprehensive biological information correlated with *S. cerevisiae* genes and their products. A diverse set of yeast strains have been sequenced to explore commercial and laboratory applications, and a brief history of those strains is provided. The publication of these new genomes has motivated the creation of new tools, and SGD will annotate and provide comparative analyses of these sequences, correlating changes with variations in strain phenotypes and protein function. We are entering a new era at SGD, as we incorporate these new sequences and make them accessible to the scientific community, all in an effort to continue in our mission of educating researchers and facilitating discovery.

Database URL: http://www.yeastgenome.org/

Brief history of yeast genomics

A diverse set of *Saccharomyces cerevisiae* genomes have been sequenced, encompassing a variety of commercial and laboratory strains, as well as wild isolates, many of which have been made available from the *Saccharomyces* Genome Database (SGD). Here we present a description of the isolation and uses of these budding yeast strains, their current incorporation into SGD and our plans for future developments in their annotation and analysis.

The first completed eukaryotic genome sequence was that of the yeast *S. cerevisiae* strain S288C, completed through the effort of a worldwide sequencing consortium (1). S288C has a complex genealogy, but is derived primarily (~88% of its genome) from strain EM93, which was isolated from a rotting fig in Central California in 1938 (2). The remaining 12% of the S288C genome comes from five different progenitors: two natural isolates (EM126 isolated in 1939 also from a rotting fig in Central California, and NRRL YB-210 isolated from rotting bananas from Costa Rica in 1942) and three commercial baking strains (Yeast Foam, FLD and LK). S288C is a widely used laboratory strain, designed by Mortimer for biochemical studies, and specifically selected to be non-flocculent with a minimal set of nutritional requirements (2). In the years since the publication of the S288C genome, dozens of yeast genome sequences have been published, laying the groundwork for giant leaps in our understanding of chromosomal evolution and the great plasticity of the eukaryotic genome.

The first new genomes arrived a decade after the consortium completed S288C. In 2005 came RM11-1a, a haploid derivative of Bb32(3), a wild isolate collected from a California vineyard. Published in 2007, YJM789 is the haploid form of an opportunistic pathogen derived from a yeast isolated from the lung of an immunocompromised patient in 1989 (3, 4). YJM789 is useful for infection studies and quantitative genetics owing to its divergent phenotype, which includes flocculence, heat tolerance and deadly virulence (4). With the publication of these second and third *S. cerevisiae* genomes, comparative yeast genomics was born. Researchers began investigating the functional significance of genetic variation on a genomic scale. Wei *et al*. (4) demonstrated almost 60 000 single nucleotide polymorphisms (SNPs) and ~6000 insertion/deletions (indels) between YJM789 and S288C, with heterogeneity in polymorphism density along chromosomes, and also within specific genes. An especially dramatic example of sequence changes contributing to an altered lifestyle, in this case, pathogenicity, is *PDR5*, which encodes an ABC transporter involved in the pleiotropic drug response. Wei *et al*. (4) also published the first chromosome-by-chromosome sequence comparison for yeast, identifying a large inversion in chromosome XIV, spanning a region just >30 kb long and flanked by transposable elements and tRNAs. Similarly, an inversion between RM11-1a and S288C exists on chromosome III, also bounded by long terminal repeats and tRNA genes. These two new genomes also allowed the first comparisons of genome-wide evolutionary rates, with Gu *et al*. (5) reporting increased rates of protein evolution in S288C compared with YJM789, and Ronald *et al*. (6) reporting even faster evolution in RM11-1a relative to the other two strains in pairwise comparisons.

In 2008, three more genomes were published, doubling the number of sequenced yeast genomes from three to six. This is notable because the community had waited 9 years for the completion of the second yeast genome, and another 2 years for the third. Then in the span of just 12 months, the next three new genomes appeared. M22 was collected in an Italian vineyard, whereas YPS163 came from the soil beneath an oak tree in a natural woodland area in southern Pennsylvania in 1999 (7, 8). Not surprisingly, YPS163 is freeze tolerant, a phenotype associated with its increased expression of aquaporin *AQY2* (9). AWRI1631 is Australian wine yeast, a robust fermenter and haploid derivative of industrial wine strain N96 (10). Researchers began comparing three different genomes at a time, with similar findings emerging. Doniger *et al*. (7) reported in excess of 88 000 polymorphisms between the combined genome alignments of M22, YPS163 and S288C. Of these polymorphisms, many of which are strain specific with a decidedly non-random genomic distribution, 93% are SNPs and the remainder are indels. Doniger *et al*. (7) also confirmed a reciprocal translocation between chromosomes VIII and XVI in vineyard isolate M22 relative to S288C. The specific reciprocal translocation identified is one that is common in wine strains, and produces increased sulfite resistance (11), an intriguing result considering that vineyards are routinely dusted with elemental sulfur as a fungicide. Borneman *et al*. (10) saw a mosaic pattern of differences when comparing AWRI1631 with both YJM789 and S288C, such that while substantial conservation exists throughout much of the genome, many regions exhibit high degrees of interstrain variation. Furthermore, Borneman *et al*. (10) also reported a reciprocal translocation, this time in YJM789 as compared with S288C and AWRI1631, between chromosomes VI and X, as well as a large inversion in chromosome XIV in YJM789.

By the end of 2009, sequences of entire yeast genomes were being published one after another. JAY291 is a non-flocculent haploid derivative of Brazilian bioethanol strain PE-2; it produces high levels of ethanol and cell mass, and is tolerant to heat and oxidative stress (12). Argueso *et al*. (12) determined that JAY291 is highly divergent to S288C, RM11-1a and YJM789, and contains well-characterized alleles at several genes of known relation to thermotolerance and fermentation performance. EC1118, a diploid commercial yeast, is probably the most widely used wine-making strain worldwide based on volume produced. In the Northern hemisphere, it is also known as Premier Cuvee or Prise de Mousse; it is a reliably aggressive fermenter, and makes clean but somewhat uninteresting wines. Novo *et al*. (13) found EC1118 more diverged from S288C and YJM789 than from RM11-1a and AWRI1631, and also reported three unique regions from 17 to 65 kb in size in the EC1118 genome on chromosomes VI, XIV and XV, encompassing 34 genes related to key fermentation characteristics, such as metabolism and transport of sugar or nitrogen. They also identified >100 genes present in S288C that are missing from EC1118. The release of the Sigma1278b genome was notable, as it was the second widely used laboratory strain to be sequenced, but also because of the concurrent production of a systematic deletion collection in this strain background. Dowell *et al*. (14) reported 75 genes in Sigma1278b that are absent from S288C, as well as sets of ‘conditional essentials’, which are genes required for viability in one background but not the other, demonstrating decisively that phenotypes are influenced by background-specific modifiers.

The following year, five new *S. cerevisiae* genomes became available (15). Foster’s O and Foster’s B are commercial ale yeasts. VIN13 is a cold-tolerant South African wine strain, a strong fermenter that is good for making aromatic white wines. AWRI796 is another South African wine strain, but ferments more successfully at warmer temperatures and is more suited to the production of reds. CLIB215 was isolated in 1994 from a bakery in Taranaki in the North Island of New Zealand. Borneman *et al*. (15) identified different large chromosomal copy number variations (CNV) in the various industrial strains. Some genomes appear to have whole-chromosome amplifications: chromosome I in AWRI796, chromosome III in Foster's O and chromosomes II, V and XV in Foster's B. Several partial chromosomal CNV amplifications hundreds of kilobases long were also identified, as were some reductions in copy number. Borneman *et al*. (15) also reported dozens of novel open reading frames (ORFs) in each strain, some of which are shared between strains, for a total of 218 in this non-degenerate set of ORFs that are not present in the S288C reference genome (Table 1).

In 2011, the most prolific year, the number of available genomes doubled from 14 to 29. CBS7960 was isolated from a cane sugar ethanol factory in Sao Paulo, Brazil. PW5 came from fermented sap of a Raphia palm tree in Nigeria in 2002. CLIB324 is a Vietnamese baker's strain collected in 1996 from Ho Chi Minh City. CLIB382 came from beer brewed in Ireland sometime before 1952. EC9-8 is a haploid cadmium-resistant derivative of a yeast isolated from the valley bottom of Evolution Canyon at Lower Nahal Oren, Israel (18). T7 was isolated from oak tree exudate in Missouri's Babler State Park. T73 is from a Mourvedre (aka Monastrell) red wine made in Alicante, Spain, in 1987. T73 has low nitrogen requirements, high alcohol tolerance and low volatile acidity production, making it ideal for fermenting robust structured reds grown in hot climates. UC5 came from Sene sake in Kurashi, Japan, sometime before 1974. VL3 was isolated in Bordeaux, France, and is most suited to the production of premium aromatic white wines with high thiol content (citrus and tropical fruit characters). Borneman *et al*. (15) reported a whole-chromosome amplification of chromosome VIII in VL3, as well as >50 ORFs in VL3 that are missing from S288C (Table 1). Kyokai No. 7 (K7) is the most extensively used sake yeast, and was first isolated from a sake brewery in Nagano Prefecture, Japan, in 1946 (17). Akao *et al*. (17) reported two large inversions in K7 on chromosomes V and XIV, both flanked by transposable elements and inverted repeats, two CNV reductions on chromosomes I and VII and a similar mosaic-like pattern and non-random distribution of variation compared with S288C as seen by other researchers in other strains. They also identified 48 ORFs in K7 that are absent in S288C, and 49 ORFs in S288C that are missing from K7 (Table 1). Also in 2011 came the genome of QA23, a cold-tolerant Portuguese wine strain from the Vinho Verde region. QA23 has low nutrient and oxygen requirements, and exhibits high β-glucosidase activity, a combination that makes beautiful Sauvignon blancs. Y10 was isolated from a coconut in the Philippines, sometime before 1973. YJM269 came from red Blauer Portugieser grapes in Austria in 1954. FL100 is the third laboratory strain to be sequenced, and very soon thereafter followed W303. Ralser *et al*. (19) reported that the W303-derivative K6001, a key model organism for research into aging, shares >85% of its genome with S288C, differing at >8000 nucleotide positions, causing changes to the sequences of 799 proteins. These differences are distributed non-randomly throughout the genome, with chromosome XVI being almost identical between the two strains, and chromosome XI the most divergent. Ralser *et al*. (19) also noted that some of the non-S288C regions in W303 are also present in Sigma1278b, which exhibits six times the rate of sequence divergence to S288C as seen in W303, and which is identical to S288C at less than half its genome.

Table 1. Various *S. cerevisiae* genomes contain ORFs that are not present in the S288C reference genome

Strain	ORFs not in S288C	Reference
AWRI796	74	15
CEN.PK113-7D	83	16
EC1118	77	15
FostersB	36	15
FostersO	48	15
JAY291	16	12
Kyokai No.7	48	17
QA23	110	15
RM11-1a	38	15
Sigma1278b	75	14
VIN13	45	15
VL3	54	15
YJM789	34	15

In 2012, genome sequences for an additional four strains became available, such that by now, dozens of genomes have been published, from yeasts with all different kinds of jobs and lifestyles (Figure 1; Table 2). BY4741 and BY4742 are the S288C-deriviative strains used for the systematic deletion collection, and variation between these strains and S288C is miniscule (T. Yamaguchi and F. Roth, personal communication). ZTW1 was isolated from corn mash used for industrial bioethanol production in China in 2007. CEN.PK113-7D is a laboratory strain derived from parental strains ENY.WA-1A and MC996A, and is popular for use in systems biology studies. Nijkamp *et al*. (16) found six duplicated regions in CEN.PK113-7D relative to S288C, two on chromosome II, and one each on chromosomes III, VII, VIII and XV, including an enrichment of maltose

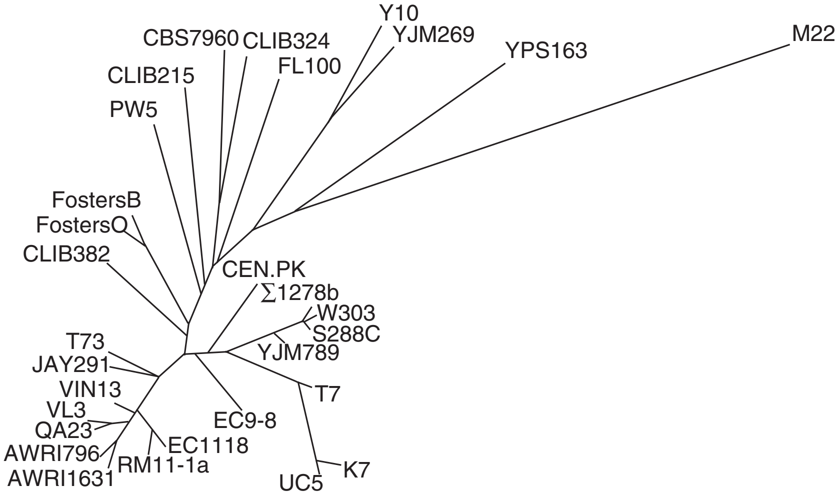


Figure 1. Phylogram depicting relationships among *S. cerevisiae* strains based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering of whole-genomic distances as calculated by Nijkamp *et al*. (16). Redrawn from Nijkamp *et al*. (16).

metabolism genes. Also present in CEN.PK113-7D, which Nijkamp and coworkers found to be a biotin prototroph, are genes required for biotin biosynthesis. They also identified >20 000 SNPs between the two strains, two-thirds of which are within ORFs. Almost 5000 of these result in altered sequences of >1400 proteins. Nijkamp *et al*. (16) also reported >2800 small indels averaging 3 bp each, and more than 400 of these were found in coding regions. An additional 83 genes were identified that are absent from S288C, including the ENA6 sodium pump that is also found in YJM269, and others that are present in both YJM269 and PW5. Nijkamp *et al*. (16) also presented a phylogenetic analysis of whole-genomic distances of the strains mentioned above (Figure 1; Table 2).

Next-generation sequencing methods have, by this time, become so mainstream that whole genomes are now being analyzed *en masse* to answer specific questions. Wenger *et al*. (20) used high-throughput sequencing in conjunction with bulk segregant analysis to investigate the distribution of the ability to ferment xylose among 600+ strains of *S. cerevisiae*, and found that this ‘xylose-positive’ phenotype, which was present in ~5% of the tested strains, clustered within wine yeasts. They further determined the presence of a novel xylitol dehydrogenase gene *XDH1* in the Simi White strain, in the same sub-telomeric 65-kb insert on chromosome XV that Novo *et al*. (13) had previously identified in wine industry workhorse EC1118. Note that while Simi White and EC1118 share the same large insertion, the xylose utilization locus itself in EC1118 is pseudogenic (13). Libkind *et al*. (21) combined comparative genomics with population ecology of >200 natural isolates to resolve questions of taxonomy and systematics, ultimately identifying *S. cerevisiae* and the novel cryotolerant species *Saccharomyces eubayanus* as progenitors of *Saccharomyces pastorianus*, shedding light on the evolution and domestication of lager yeasts. At the same time, Nguyen *et al*. (22) were also using comparative genomics to study the hybridization history of lager *Saccharomyces*, finding mosaic genomes and patterns of introgression between *Saccharomyces bayanus*, *Saccharomyces uvarum* and *S. cerevisiae*. The same novel species named *S. eubayanus* by Libkind and coworkers (21) was identified by Ngyuyen *et al*. (22) and called *Saccharomyces lagerae*. Borneman *et al*. (23) studied the wine yeast VIN7, which is widely used in cool-temperature fermentations to produce premium Sauvignon blancs and Semillons, and confirmed that its genome is a complex allotriploid of *S. cerevisiae* and cryotolerant *Saccharomyces kudriavzevii*. VIN7 most likely arose through a mating between a diploid *S. cerevisiae* and a haploid *S. kudriavzevii*, and exhibits evidence of translocation and recombination events occurring between alleles of both progenitors. Erny *et al*. (24) performed genomic analyses of Alsatian industrial wine yeast Eg8, which tolerates cool temperatures and elevated alcohol concentrations, and is ideal for fermentation of Semillons and Muscats. Erny *et el*. (24) found that Eg8 is also a chimeric allotriploid hybrid between a diploid *S. cerevisiae* and haploid *S. kudriavzevii*, and further identified the same translocation between chromosomes VIII and XVI that Doniger *et al*. (8) reported in vineyard isolate M22 that leads to increased sulfite resistance, and which is common in wine yeast strains. Peris *et al*. (25) investigated genomic compositions of various *S. cerevisiae* × *S. kudriavzevii* natural hybrids isolated from wine and beer fermentations, including VIN7. They found different chromosome complements and rearrangements in the different yeasts, although all shared a common set of *S. kudriavzevii* genes and lacked a common set of *S. cerevisiae* genes. The rich

Table 2. In the years since the publication of the S288C genome, dozens of yeast genome sequences have been published

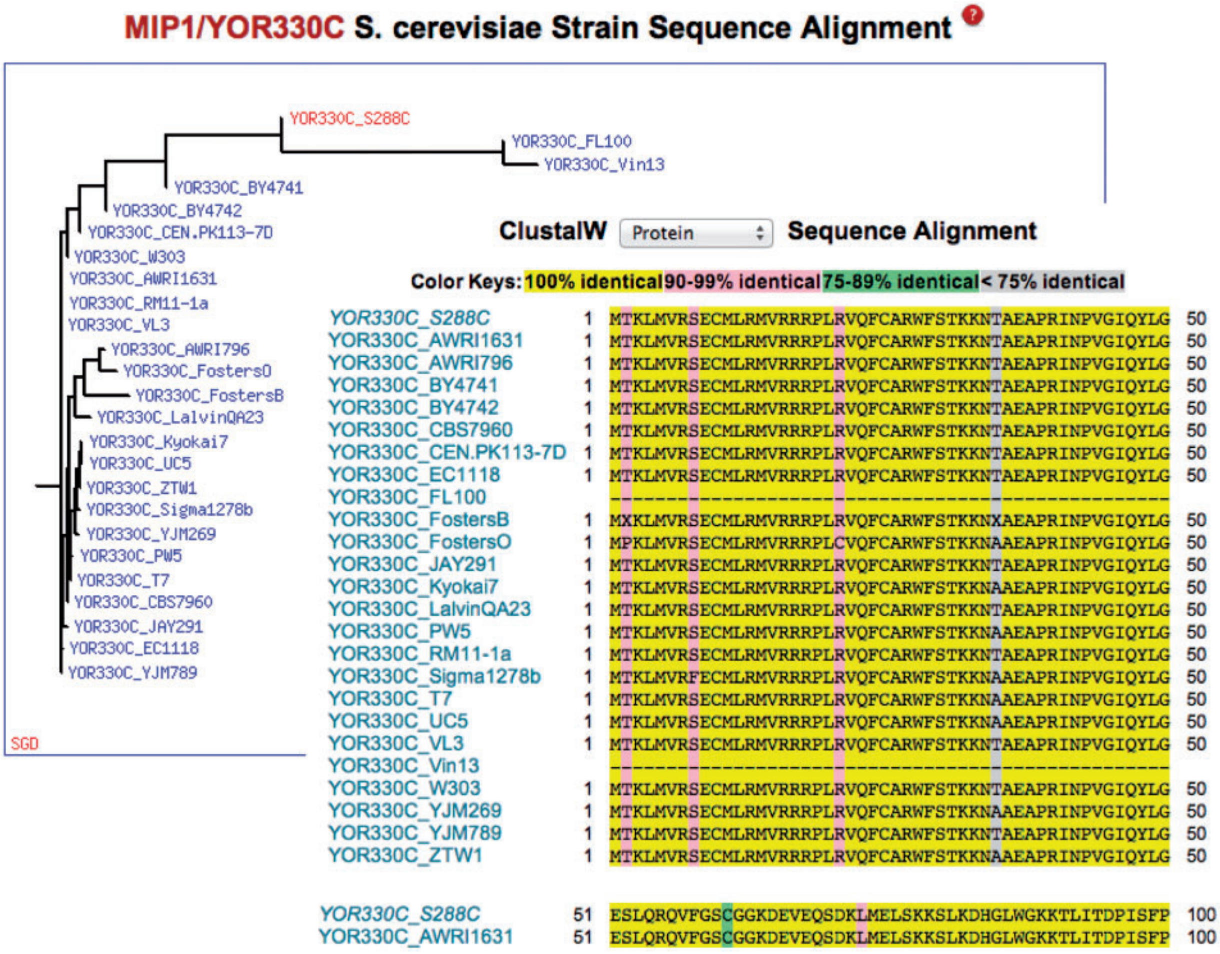
Strain	Year	Provenance	NCBI BioProject	Contig N50[a]	Scaffold N50[a]
S288C	1996	Laboratory strain	PRJNA128	N/A[b]	N/A
RM11-1a	2005	Haploid derivative of California vineyard isolate	PRJNA13674	263 288	795 018
YJM789	2007	Haploid derivative of opportunistic human pathogen	PRJNA13304	429 709	N/A
M22	2008	Italian vineyard isolate	PRJNA28815	2207	N/A
YPS163	2008	Pennsylvania woodland isolate	PRJNA28813	2901	N/A
AWRI1631	2008	Haploid derivative of South African commercial wine strain N96	PRJNA30553	7704	N/A
JAY291	2009	Haploid derivative of Brazilian industrial bioethanol strain PE-2	PRJNA32809	64 336	N/A
EC1118	2009	Commercial wine strain	PRJEA37863	776 014	N/A
Sigma1278b	2009	Laboratory strain	PRJNA39317	365 700	N/A
Foster's O	2010	Commercial ale strain	PRJNA48567	195 316	N/A
Foster's B	2010	Commercial ale strain	PRJNA48569	204 208	626 897
VIN13	2010	South African white wine strain	PRJNA48563	308 189	700 638
AWRI796	2010	South African red wine strain	PRJNA48559	403 341	565 854
CLIB215	2010	New Zealand bakery isolate	PRJNA60143	16 813	47 217
CBS7960	2011	Brazilian bioethanol factory isolate	PRJNA60391	18 761	65 099
CLIB324	2011	Vietnamese bakery isolate	PRJNA60415	4260	24 472
CLIB382	2011	Irish beer isolate	PRJNA60145	840	2711
EC9-8	2011	Haploid derivative of Israeli canyon isolate	PRJNA73985	15 539	541 605
FL100	2011	Laboratory strain	PRJNA60147	4244	26 506
Kyokai No.7	2011	Japanese sake yeast	PRJNA45827	120 978	902 266
QA23	2011	Portuguese Vinho Verde white wine strain	PRJNA48561	182 942	182 942
PW5	2011	Nigerian Raphia palm wine isolate	PRJNA60181	14 234	393 105
T7	2011	Missouri oak tree exudate isolate	PRJNA60387	147 205	476 142
T73	2011	Spanish red wine strain	PRJNA60195	2945	36 287
UC5	2011	Japanese sake yeast	PRJNA60197	17 142	356 094
VL3	2011	French white wine strain	PRJNA48565	293 399	656 188
W303	2011	Laboratory strain	PRJNA167645	149 943	367 966
Y10	2011	Philippine coconut isolate	PRJNA60201	2730	22 204
YJM269	2011	Austrian Blauer Portugieser wine grapes	PRJNA60389	23 452	58 353
BY4741	2012	S288C-derivative laboratory strain	N/A	N/A	N/A
BY4742	2012	S288C-derivative laboratory strain	N/A	N/A	N/A
CEN.PK 113-7D	2012	Laboratory strain	PRJNA52955	48 196	918 791
ZTW1	2012	Chinese corn mash bioethanol isolate	PRJNA174065	556 921	N/A

[a] Contig and scaffold N50 lengths are common genome statistics that indicate the minimum length in the set of individual contiguous sequences (contigs or scaffolds), which contain half of all bases in the assembly.
[b] N/A = not available.

content of genomic sequences available to serve as a background against which to compare has changed the way we study genomes.

New directions

While next-generation sequencing has been taking the yeast genetics community by storm, SGD has been preparing for this shift into the new modern era of yeast genomics. In February 2011, SGD put into place an updated reference sequence of increased quality based on these modern sequencing technologies, and henceforth, we anticipate very few sequence updates for S288C (Engel *et al.*, in preparation). We will continue to provide the definitive reference genome sequence for *S. cerevisiae* as well as variant sequences from these other sequenced strains, and are moving increasingly toward the representation of sequence variation and allelic differences. We have already



MIP1/YOR330C S. cerevisiae Strain Sequence Alignment

ClustalW Protein Sequence Alignment

Color Keys: 100% identical 90-99% identical 75-89% identical < 75% identical

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YOR330C_S288C        1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_AWRI1631     1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_AWRI796      1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_BY4741       1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
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YOR330C_CEN.PK113-7D 1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_EC1118       1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_FL100           --------------------------------------------------
YOR330C_FostersB     1  MXKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNXAEAPRINPVGIQYLG  50
YOR330C_FostersO     1  MPKLMVRSECMLRMVRRRPLCVQFCARWFSTKKNAAEAPRINPVGIQYLG  50
YOR330C_JAY291       1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_Kyokai7      1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNAAEAPRINPVGIQYLG  50
YOR330C_LalvinQA23   1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_PW5          1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNAAEAPRINPVGIQYLG  50
YOR330C_RM11-1a      1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_Sigma1278b   1  MTKLMVRFECMLRMVRRRPLRVQFCARWFSTKKNAAEAPRINPVGIQYLG  50
YOR330C_T7           1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNAAEAPRINPVGIQYLG  50
YOR330C_UC5          1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNAAEAPRINPVGIQYLG  50
YOR330C_VL3          1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_Vin13           --------------------------------------------------
YOR330C_W303         1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_YJM269       1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNAAEAPRINPVGIQYLG  50
YOR330C_YJM789       1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNTAEAPRINPVGIQYLG  50
YOR330C_ZTW1         1  MTKLMVRSECMLRMVRRRPLRVQFCARWFSTKKNAAEAPRINPVGIQYLG  50

YOR330C_S288C       51  ESLQRQVFGSCGGKDEVEQSDKLMELSKKSLKDHGLWGKKTLITDPISFP 100
YOR330C_AWRI1631    51  ESLQRQVFGSCGGKDEVEQSDKLMELSKKSLKDHGLWGKKTLITDPISFP 100
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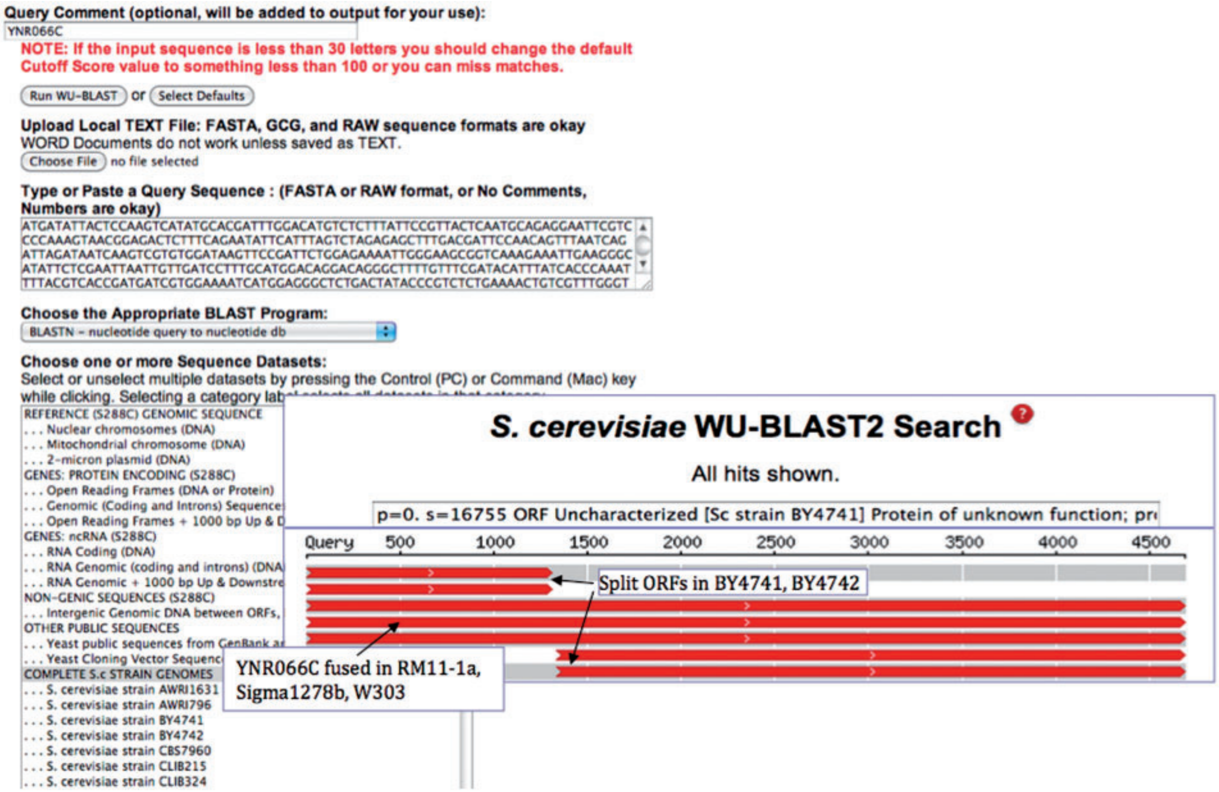
Figure 2. Precomputed ClustalW alignments of both amino acid and coding DNA sequences and ORF-specific dendrograms are available for each ORF at http://www.yeastgenome.org/cgibin/FUNGI/alignment.pl.

incorporated the new *S. cerevisiae* genomes mentioned above into SGD and will continue to expand the amount of information available to researchers for the growing number of laboratory and industrial strains and wild isolates. New tools are being developed that will provide access to this compendium of allelic and variation information and allow a newly determined sequence to be compared with the reference strain, as well as with the sequences of several widely used and commonly studied *S. cerevisiae* strains. Current tools in place include precomputed protein and coding DNA alignments (ClustalW) for each ORF, as well as ORF-specific dendrograms, which depict the degree of similarity of that ORF sequence among the set of strains in which it was identified (Figure 2). From each Locus Summary page, the protein and DNA sequences are accessible via a pair of pull-down menus in the Sequence Information section, while the alignments can be reached via links in the Analyze Sequence section, or through the 'Strains and species' item in the Sequence menu at the top of most SGD web pages. Protein or DNA sequences can also be downloaded in batch from the alignment pages, or one-by-one from each Locus Summary. We also have the genomes of the various strains incorporated into the Basic Local Alignment Search Tool (BLAST) datasets, available for searching against genomic and coding DNA, as well as protein sequences. BLAST can be useful for finding evidence of fission/fusion events in which ORFs, such as YNR066C, are split in some strains but not others (Figure 3). The BLAST tool is accessible via the Sequence menu at the top of most SGD pages. All the strain DNA and protein sequences are available for download so that researchers can perform their own analyses (http://downloads.yeastgenome.org). Furthermore, we continue to associate information regarding sequence variation with functional effects and phenotypic variations. SGD has been curating phenotypes in the different strain backgrounds for several years, as genes shared across strains and species can produce different phenotypes, revealing genetic variation and possibly uncovering new models of disease (26).



Query Comment (optional, will be added to output for your use):

YNR066C

NOTE: If the input sequence is less than 30 letters you should change the default Cutoff Score value to something less than 100 or you can miss matches.

Upload Local TEXT File: FASTA, GCG, and RAW sequence formats are okay
WORD Documents do not work unless saved as TEXT.

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Type or Paste a Query Sequence : (FASTA or RAW format, or No Comments, Numbers are okay)

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Choose the Appropriate BLAST Program:

BLASTN – nucleotide query to nucleotide db

Choose one or more Sequence Datasets:
Select or unselect multiple datasets by pressing the Control (PC) or Command (Mac) key while clicking. Selecting a category lab

REFERENCE (S288C) GENOMIC SEQUENCE
. . . Nuclear chromosomes (DNA)
. . . Mitochondrial chromosome (DNA)
. . . 2-micron plasmid (DNA)
GENES: PROTEIN ENCODING (S288C)
. . . Open Reading Frames (DNA or Protein)
. . . Genomic (Coding and Introns) Sequence
. . . Open Reading Frames + 1000 bp Up & D
GENES: ncRNA (S288C)
. . . RNA Coding (DNA)
. . . RNA Genomic (coding and introns) (DNA
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COMPLETE S.c STRAIN GENOMES
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S. cerevisiae WU-BLAST2 Search

All hits shown.

p=0. s=16755 ORF Uncharacterized [Sc strain BY4741] Protein of unknown function; pr

Query
500
1000
1500
2000
2500
3000
3500
4000
4500
Split ORFs in BY4741, BY4742
YNR066C fused in RM11-1a, Sigma1278b, W303

Figure 3. Genomes of the various *S. cerevisiae* strains have been incorporated into the BLAST datasets at SGD, available for searching against genomic and coding DNA, as well as protein sequences at http://www.yeastgenome.org/cgi-bin/blast-sgd.pl.

New technologies and approaches are pushing *S. cerevisiae* annotation past the limits of a system based exclusively on a single reference sequence. Next-generation sequencing methods will determine the genomic sequences of hundreds, if not thousands, of different *S. cerevisiae* industrial strains, laboratory strains and natural isolates in the coming years. Comparative genomics can provide a clearer picture of the full constituent parts of a species' genome and provide for the identification of sequence features such as binding motifs, regulatory regions and non-coding RNAs. As described above, these new genomes vary not only at specific nucleotides, but also in the complement of genes they carry and the architecture of their chromosomes, as genomic elements can be shuffled, amplified, lost or gained as populations adapt to different environments. To provide a more comprehensive view of the genetic repertoire of yeast, SGD is compiling the virtual *S. cerevisiae* genome, or pan-genome, that will comprise all genes found within the various sequenced *S. cerevisiae* strains.

A pan-genome more accurately describes the genetic content of a species, and can be much larger than any single constituent genome. Each gene can be binned into one of three categories. Core genes are those present in every genome, and include conserved essential genes for proteins such as actin, or polymerases, histones and ribosomal constituents required for some of the most basic cellular processes such as replication and translation. Frequent genes are those found in some genomes but not others; they are commonly involved in adaptation to specific environments or applications, such as metabolism of specific sugars or fermentation of specific carbon sources. In bacterial genomics, this intermediate class goes by various names: 'character', 'dispensable', 'peripheral', 'variable' or 'flexible' genes (27–30). They tend to evolve more quickly than the conserved essential genes, but more slowly than the individual genomes themselves. The *S. cerevisiae* pan-genome contains hundreds of frequent genes that are found in some strains but not others. Examples include the MAL (maltose fermentation) family of multigene loci, each of which encodes a maltose permease, a maltase and a *trans*-acting MAL activator (31). As mentioned earlier, Nijkamp *et al.* (16) found the genome of strain CEN.PK113-7D to be enriched in the MAL genes. Rare genes are those that are present in only a small number of genomes, possibly even unique to a single strain, and often are of unknown function. Rare genes tend to be rapidly evolving and especially mutable, exhibiting high rates of gene birth and death. In bacterial genomics, these genes are sometimes called 'accessory' genes (27). A recently reported rare gene in *S. cerevisiae* is the novel *XDH1* xylose utilization gene mentioned earlier (20). Other examples include *PRM8* and *PRM9*, both of which encode non-essential pheromone-regulated transmembrane proteins of the DUP240 family (32). These three sets together—core, frequent and rare—make up the pan-genome that we want to describe, and will in the future provide a valuable resource for the annotation of newly determined budding yeast genomes and for the functional analysis and comparison of observed variation within *S. cerevisiae.*

The availability of an ever-increasing number of sequenced genomes presents a growing list of clear and present challenges that all genome databases will have to address: How will any particular approach scale up to handling hundreds of genomes? What is the best way to organize and display SNPs, larger polymorphisms and genome rearrangements? How should chromosomal coordinates and mapping information be dealt with in the context of a pan-genome? At SGD, we are expanding our scope to provide annotation and comparative analyses of all major budding yeast strains, and are moving toward providing multiple reference genomes. We are not abandoning a standard sequence, but instead determining how far one can get from a reference while still maintaining utility. It is helpful to be able to 'shift the reference', selecting the genome that is most appropriate and informative for a specific area of study. SGD has actively sought and obtained genome sequences for a set of strains with a substantial history of use and experimental results that will serve as reference genomes. These strains include W303, Sigma1278b, SK1, SEY6210, CEN.PK, JK9-3d and FL100, and are the genomes for which we have the most curated phenotype data, and for which we aim to curate specific functional information. High-quality genome sequences combined with detailed phenotypes and functional annotations will allow dissection of the genomic bases of phenotypic variation.

The meticulous investigation of the complexes and interactions of individual gene products in the yeast cell allows great things to be done in yeast genomics. There is no other model organism that provides such a fertile environment for understanding the basic mechanisms of biology. There is a continued need for this 'small science' investigating the biochemistry and cell biology of eukaryotic cells (33). SGD bridges the experimentally defined knowledge provided from investigations on the small scale over to its application during the annotation of genomic results. The power of yeast genomics is resting squarely on the shoulders of yeast genetics and biochemistry. SGD has a long history of service to yeast researchers and to the broader genetics community as a whole. As we all move through this new era of comparative yeast genomics, SGD maintains its high level of dedication to quality and remains the primary annotation resource for new strains of *S. cerevisiae*. We continue in our mission of educating students, enabling bench researchers and facilitating scientific discovery.

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