Evidence for S. cerevisiae Fermentation in Ancient Wine

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Abstract. Saccharomyces cerevisiae is the principal yeast used in modern fermentation processes, including winemaking, breadmaking, and brewing. From residue present inside one of the earliest known wine jars from Egypt, we have extracted, amplified and sequenc**ed** ribosomal DNA from S. cerevisiae. These results indicate that this organism was probably responsible for wine fermentation by at least 3150 B.C. This inference has major implications for the evolution of bread and beer yeasts, since it suggests that S. cerevisiae yeast, which occurs naturally on the surface bloom of grapes, was also used as an inoculum to ferment cereal products.

Key words: Ancient DNA ù cereal products ù fermentation ù Ctztal Alps Iceman ù S. cerevisiae ù winemaking

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Introduction

Hundreds, if not thousands, of years of experimentation in vine cultivation and winemaking technology are needed to explain the level of sophisticated and large-scale wine production that is displayed in mountainous and upland Neolithic settlements of the ancient Near East. The earliest molecular archaeological evidence for large-scale wine production is from the site of Hajji Firuz Tepe in the northern Zagros Mountains, dated to ~5400 B.C. (McGovern et al. 1986). Even earlier chemical evidence, in association with what appear to be remains of domesticated grapes (Vitis vinifera vinifera), has been obtained from the early 6th millennium B.C. in the Neolithic village of Shulaveris-Gora in the Transcaucasus region of

modern Georgia (Ramishvili 1983; McGovern unpublished).
Winemaking enabled humans to produce a beverage with a high content of ethyl
alcohol, whose mind-altering, analgesic, disinfectant, and preservative properties made it the most widespread drug and medicine of antiquity. Winemaking is consequently of great importance in the biocultural evolution of the human species and its societies. Saccharomyces cerevisiae is the principal agent in wine fermentation, converting the simple sugars of grapes to carbon dioxide and alcohol. Once the juices of the fruit have been exuded, an ideal breeding ground with the right mix of water and nutrients is created for S. cerevisiae to begin

and sustain fermentation to produce an alcoholic beverage. By comparing the DNA sequences of modern S. cerevisiae with that of ancient wine yeasts, one stage in the human selection process of the microorganism can be reconstructed in a process likely extending back 8000 or more years.
Materials and Methods
The original experimental material consisted of residue inside one of the earliest wine jars from ancient Egypt, dated to 3000 B.C., which were recovered from tomb U-j of King Scorpion I (Dreyer 1993; McGovern et al. 1997; Hartung 2001). From the residue inside jar 10/115 [catalog
number 156 in Hartung **delete parentheses:**(2001)], three independent DNA extractions were carried out, each
with a 500-mg sample. The DNA extractions employed a procedure designed for soil microorganisms (DNA Spin Kit, Bio101, LaJolla, CA). Strict laboratory protocols were followed throughout the DNA extractions and amplifications to prevent contamination from modern organisms.
All of the polymerase chain reaction (PCR) amplifications were carried out in a new, isolated laboratory at the University of Florence, using equipment not previously exposed to yeast or yeast DNA. The laboratory bench was cleaned of contamination by DNA or RNA using DNAûZap (Ambion, Austin TX). New, thoroughly cleaned Gilson pipettes (Gilson, Middleton, MI) were employed, and air-filtered tips were used for each PCR to prevent contamination from the aerosol. Samples were handled with clean latex gloves, and masks and laboratory gowns were worn at all times. Containers enclosing the samples were opened away from any possible source of yeast contamination, and the contents transferred to sterile Falcon tubes (Becton, Dickinson, Franklin Lakes, NJ). All the materials used for the extraction, including the mortars for crushing the samples, were thoroughly cleaned and sterilized.
The amplified DNA was subjected to gel electrophoresis using equipment not
previously used for other yeast DNA samples. Freshly prepared gel buffers were used, and the gel cast apparatus was previously treated with DNAûZap to eliminate contamination from modern DNA or RNA. DNA blank extractions, serving as negative controls, were carried out in parallel during the extraction and the PCR amplification. A positive control was also carried out by adding 50 ng of modern S. cerevisiae genomic DNA to an aliquot of the

negative control prior to electrophoresis. The amplifications were performed using Hi-Fidelity DNA polymerase (Hoffmann-La Roche, Basel, Switzerland). The amplification reactions conditions were as described in Guillam#n et al. (1998). The DNA amplifications were carried out twice at different times and in different laboratories and resulted in products that were indistinguishable from one sample to the next. After extraction of bands of the

amplified DNA from the gel, DNA sequencing was carried out directly on the PCR products without prior cloning, employing the same primers as used for amplification and internal primers as needed. The putative amplification product from ancient S. cerevisiae was sequenced from samples from two different extractions.
The DNA was amplified with primers ITS1 (5'-GTTTCCGTAGGTGAACCTGC-3'), which corresponds to nucleotide coordinates 455966û455947 in the S. cerevisiae genomic sequence of chromosome 12 (genome-www.stanford.edu/cgi-bin/SGD/chromosomes) and ITS4 (3'-CGTATAGTTATTCGCCTCCT-5'), which corresponds to coordinates 455142û455123.
These primers are located in the ribosomal DNA (rDNA) sequences and can amplify 394 bp of the ITS1 (internal transcribed spacer 1), the 158-bp sequence encoding 5.8S RNA (RDN58-1), and 288 bp of the ITS2 (Guillam#n et al. 1998). The yeast genome contains 100û200 copies of this sequence, embedded in a tandemly repeated unit of 9.1 kb that encodes the 18S, 5.8S, and 25S rRNAs.
To confirm the putative Saccharomyces origin of the 840-bp product amplified by primers ITS1ûITS4, amplifications were also carried out with primers ITS5 (5'-GGTGAGAGATTTCTGTGC-3'), corresponding to coordinates 455776û455759 and ITS6 (3'-GGGTCTCCATTGTTTGTGTT-5'), corresponding to coordinates 455663û455644. These primers
are nested inside the region amplified by ITS1 and ITS4. The IS5 and IS6 oligonucleotides were also used as primers for sequencing.

Results

The yellowish residue that we used for the DNA amplifications is present inside the earliest wine jars from ancient Egypt, as established by molecular archaeological analyses (McGovern et al. 1997, 2001; McGovern 2001). The date of the jarsù3150 B.C.ùis based on radiocarbon determinations (Dreyer 1993). The jar we studied, together with hundreds more, had been
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imported from the southern hill country of Palestine and the Jordan Valley, according to Instrumental Neutron Activation Analysis of the pottery fabrics (McGovern 2001), and deposited in tomb U-j of Scorpion I, one of the first kings of Egypt, at the site of Abydos along the middle Nile River. If all the jars were full when imported, as seems likely, they originally contained approximately 4500 liters of wine, equivalent in volume to 20 modern French Bordeaux barrels. The alcohol and other volatile substances had long ago disappeared, but the large quantity of solid material that had collected on the bottoms and sides of the jars was presumed to include dead cells and cellular debris from yeast.
Lees from the Scorpion I jar was subjected to DNA extractions and amplifications under conditions designed to minimize the risk of contamination with DNA from modern organisms, in a laboratory and using equipment previously unexposed to modern S. cerevisiae. The extracted DNA molecules exhibited a wide distribution of sizes * 150 bp (Fig. 1A). The blank negative control, extracted using the same procedure, yielded no detectable DNA. The quantity of DNA recovered from the wine lees is an order of magnitude greater than that obtained from other ancient soft tissue samples (PEEbo 1989). Part of this high yield we attribute to the large number of dead yeast cells present in the lees. Yeast cells and debris typically make up about half of wine lees. Six liters of wine, approximately the volume of a Scorpion I jar, would contain as much as 12 mg of yeast DNA. A second reason for the high yield we attribute to the conditions of preservation. Because the tomb of Scorpion I was 3

meters deep in desert sand, and was covered over with a roof and more sand in an extremely dry climate, the conditions for long-term organic preservation were excellent.
The extracted DNA was used as a template for PCR amplification using primers ITS1 and ITS4, which amplify a region of ~840 bp across the genomic region between the 18S and 28S ribosomal RNA genes that includes the ITS1 (internal transcribed spacer 1), the 5.8S ribosomal DNA, and the ITS2 (internal transcribed spacer 2). The ITS1 and ITS2 spacer regions differ among species of yeast, and have recently been used to assess the phylogenetic relationship of strains belonging to the genus Saccharomyces and to assign strains to three closely related species of the genus: S. cerevisiae, S. bayanus (= S. uvarum) and S. paradoxus (= S. douglasii) (Montrocher et al. 1998).

Using the primer pair ITS1ûITS4, we obtained products of 540 bp, 580 bp and 840 bp (Fig. 1B). These bands were purified from the gels and sequenced. A BLAST search (Altschul et al. 1990) of Genbank using the 540-bp sequence revealed a close match with that of an ITS1û5.8S rRNAûITS2 sequence from fungal clone T2709 (Rollo et al. 1995) recovered from the clothing of The Iceman "Ctzi" discovered near the Hauslabjoch formation in the Ctztal Alps in 1991, and dated to 3350û3300 B.C. The alignment between T2709 (Genbank accession number X88771) and our Abydos 540-bp product (Ab540) is shown in Fig. 2. Across 496 bp there is 90%
identity between the sequences. The sequence of the 580-bp band was less interesting, but was similar to those of ITS1û5.8S rRNAûITS2 sequences from the fungi Ampelomyces humuli and Phoma glomerata (data not shown).
The observation that the 840-bp PCR product was faint (Fig. 1B) could be due to competition of the mold DNA sequences for the primers or to the rarity of long molecules of DNA in the ancient samples that are sufficiently undamaged by interstrand crosslinks, depurination or other lesions to still support PCR amplification. The 840-bp fragment corresponds in size to the region encompassing S. cerevisiae chromosome 12 coordinates 455966û455123 comprising 394 bp of ITS1, 158 bp of 5.8S rDNA and 288 bp of ITS2. To confirm
the Saccharomyces origin of the 840-bp product, we designed new primers ITS5 and ITS6, internal to the 840-bp S. cerevisiae ITS1û5.8S rDNAûITS2 sequence, which would specifically amplify sequences belonging to the genus Saccharomyces (Montrocher et al. 1998).
Amplifications of the extracted DNA with the primer pair ITS1ûITS6 produced a band of ~320 bp, amplification with ITS5ûITS4 produced a band of ~650 bp, and amplification with ITS5û ITS6 produced a band of ~130 bp. These products correspond to overlapping regions within the 840-bp product and are of the predicted size. The bands were also more intense than the 840-bp band, suggesting that most of the molecules in the extracted DNA able to support amplification are smaller than 800 bp.
The bands of 840 bp, 320 bp, 130 bp and 650 bp were extracted from the gels and used to obtain a consensus sequence across the 840-bp region. A BLAST search of Genbank revealed that the 840-bp sequence most closely matches the sequence from modern S. cerevisiae (Fig. 3). This sequence has no homology with sequences from molds, and it shows no significant alignment longer than 100 bp with sequences of yeasts and other fungi outside of the genus

Saccharomyces. The 748-bp region shown in Fig. 3 corresponds to S. cerevisiae chromosome 12 coordinates 455928-455181. The sequences denoted Sc, Sp and Sb are from modern S.

cerevisiae, S. paradoxus and S. bayanus, respectively, and the nucleotides in boldface correspond to the 5.8S rDNA. The sequence denoted AW (ancient wine) is that from the 5000-year-old wine jar. Compared with the S. bayanus sequence, AW shows 24 nucleotide mismatches including 5 insertions/deletions of 1û2 bp. Compared with the S. paradoxus sequence, AW shows 10 nucleotide mismatches including 5 insertions/deletions of 1 bp. On the other hand, there are only 4 nucleotide mismatches with the sequence from S. cerevisiae, and the alignment requires no insertions/deletions. The sequences in Fig. 3 also include 5 phylogenetically informative sites in which the sequences of S. paradoxus and S. bayanus are identical but differ from that of S. cerevisiae, and these include 2 single-nucleotide insertions/deletions. At all five sites the ancient wine sequence AW matches that of S. cerevisiae.

The similarity between the AW sequence and modern S. cerevisiae sequences is illustrated in the unrooted maximum parsimony dendrogram in Fig. 4. In addition to AW, the sequences analyzed were S. cerevisiae strains CBS1171, HA6, CBS459, CBS400 and CBS382;
S. paradoxus strains CBS406 and CBS432; and S. bayanus strains CBS1513, CBS424, CBS380, IFO1127 and CBS1538. (See the legend for Genbank accession numbers.) The sequence comparisons were generated by ClustalX (Thompson et al. 1997) alignments of the ribosomal DNA region encompassing the region of ITS1û5.8SûITS2 shown in Fig. 3. The number of bootstrap samples among 1000 replicates that support each node in the dendrogram is indicated. The clustering of the yeast strains of the three species is in agreement with that of Montrocher et al. (1998) and Oda et al. (1997). The results in Fig. 4 are also consistent with a dendrogram generated by neighbor joining (Saitou and Nei 1987).

The strong bootstrap support for the clusters in Fig. 4 implies that, based on the ITS1û 5.8SûITS2 sequence, the sequence AW should be regarded as coming from an ancient strain affiliated with modern S. cerevisiae. Hence we implicate S. cerevisiae in the production of the wine buried with Scorpion I.

Ancient DNA: Preservation and possible contamination

One of the major problems in acquiring information from archeological samples is to prevent contamination from modern samples. As stressed in the Materials and Methods, all of the experiments were performed with the necessary precautions to prevent any modern contamination, in accordance with the suggestions recently summarized in Hofreiter et al. (2001). We also carried out three independent extractions and two independent sets of PCR amplifications with the appropriate negative and positive controls. Based on the reproducibility and consistency of the results, we believe that contamination with modern yeast can be ruled out.

But if modern laboratory contamination can be ruled out, how likely is other contamination, either during the post-burial period of 5000 years or when the archaeologists collected the samples, admittedly without sterile gloves and masks? Post-burial contamination seems unlikely in view of the large quantity of DNA extracted from the ancient Abydos samples. One would not expect contamination by stray microorganisms to yield an amount of DNA which, when extracted, could readily be visualized in a gel (Fig. 1A). A single Scorpion I jar would contain as much as 12 mg of yeast DNA, of which more than 7% consists of the ribosomal DNA repeats. We also attribute the large amount and relatively good quality of the extracted DNA to the exceptional environment in which the samples were

preserved. The residues were inside closed pottery containers in an essentially zero-humidity, desert environment. The recovery of whole dried figs and raisins from some of the jars indicates how extraordinary the preservation conditions were inside the tomb. The presence of these fruits also indicates that there was relatively little activity by microorganisms once the air and water present at the time of burial had been consumed.
Contamination by the archaeologists is also very unlikely, because S. cerevisiae is not airborne, and is rare in nature, particularly in desert environments. Even grape skins rarely harbor S. cerevisiae cells unless broken to release the juice (Mortimer and Polsinelli 1999). Moreover, since wine yeasts do not live on human skin, the probability that the archaeologists transferred modern S. cerevisiae to the ancient samples is very low.
The ancient ITS1û5.8SûITS2 sequence shown in Fig. 3 (AW) is very close to that of the modern S. cerevisiae strain S288c used for sequencing, and it clusters along with the sequences

from other modern S. cerevisiae (Fig. 4). The close similarity with modern strains seems reasonable because, over 5000 years, one would not expect a large accumulation of changes in the 100û200 tandemly arrayed copies of the 9.1-kb repeating unit that encodes the 18S, 5.8S, and 25S rRNAs. Militating against rapid sequence evolution is the fact that in nature S. cerevisiae is typically a diploid organism, and it has a highly efficient DNA repair system in which a high rate of gene conversion and homologous recombination is likely to retard sequence evolution. In this context it should also be noted that there is a very close match between the mitochondrial DNA (mt DNA) sequences of 191 contemporary horses with those of 8 horses 12,000û18,000 years old as well as with those of 8 horses 1000û2000 years old (Vila et
al. 2001).
As an additional indication that the DNA extracted from the Abydos jars derived from ancient organisms present at or introduced shortly after the time of burial, we note the striking similarity between the 540-bp fungal sequence in the Abydos samples with that recovered from the remains of the clothing of the 5000-year-old Iceman "Ctzi" discovered in the Ctztal Alps in 1991. This sequence has no significant similarities longer than 300 bp with sequences from modern molds. It seems likely that the fungal organism from which the 540-bp sequence derives, as well as the organism yielding the 580-bp sequence affiliated with the fungi Ampelomyces humuli and Phoma glomerata, were present in the wine at the time of burial. At the beginning of natural fermentation, S. cerevisiae is usually in the minority. Later in the process, the fermentation of sugars by S. cerevisiae produces alcohol that, when the amount exceeds about 5% by volume, kills most of the other yeasts and molds. Under prolonged storage, however, the surviving molds can proliferate and produce repulsive odors and distasteful flavors.

Discussion and conclusions

The finding that the lees inside a ~5000-year-old wine jar contain DNA from an organism that can confidently be assigned to Saccharomyces cerevisiae has important implications for the evolution of wine, bread and beer yeasts, as well as for the emergence of long-term human settlement and civilization based on fermented grain and fruit staples as early as 8500 B.C. (McGovern et al. 1995; McGovern 2003). These results provide the oldest evidence of an

association of a microorganism of the S. cerevisiae species with human activities. Although the chemical and archaeological evidence were compatible with the interpretation that the

Scorpion I jar contained a fermented beverageùnamely, grape wineùuntil the present study was carried out, it could not be established definitively what organism was responsible for the fermentation.

The Neolithic period, from about 8500 to 4000 B.C., is the first time in human prehistory when the necessary preconditions came together for the momentous innovation of viniculture, encompassing a knowledge of viticulture, winemaking, and fermentation along with methods for storage. Numerous year-round villages had been established by this time in the Near East, especially in upland regions bordering the ôFertile Crescentöùthe foothills of the Zagros Mountains bordering the Tigris and Euphrates Rivers on the east, Transcaucasia to the north, and the upland plateaus descending from the Taurus Mountains in eastern Turkey. Using a variety of food processing techniquesùin particular fermentation, but also soaking, heating, spicingùNeolithic peoples produced the first wine, bread, beer as well as an array of meat and grain entr1 es that we continue to enjoy today.

In trial and error fashion, it must have dawned upon the ancient beverage-maker that by adding grapes to any mixture of other less-sweet ingredientsùberries, barley malt, wheatù the prospects of producing a fermented beverage were improved. It is even possible that the frothy yeast that bubbled on the surface of a mixed beverage of grapes and other materials was skimmed off, and used in later fermentations. Gradually, in this seemingly non-hygienic, experimental setting, a single yeast species came to predominate and was shared by the whole range of fermented beverages: S. cerevisiae.

S. cerevisiae is also the bread and beer yeast. Because neither the organism nor its spores are airborne, a practical understanding of the use of S. cerevisiae in fermenting grapes, as well as dates, figs or honey, probably preceded its use for grain-based foods and beverages. Ancient literary evidence implies that grapes generally provided the yeast for making barley beer (Civil 1964). It is thus likely that wine yeasts, which occur naturally in damaged grapes (Mortimer and Polsinelli 1999), were used to ferment other cereal products and, over centuries of human selection, this yeast eventually evolved into specific strains that became the fermenting agents of choice for human food and drink.

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Figure Captions
Fig. 1. Analysis of ancient DNA from Scorpion I wine jar residues. A. Three aliquots of finely ground 500-mg samples were extracted and run on a gel. The DNA recovered is shown in lanes 1û3. A blank negative control, extracted using the same procedure, was run in lane 4. Lane 5 is empty. The molecular weight marker (Gene Ruler 100-bp DNA ladder, M-Medical Genenco, Florence, Italy) is shown in the M lanes. B. The extracted DNA was replicated by PCR with primers ITS1 and ITS4 (lane 2): lane 1 (5 microliters of negative control + 50 ng S. cerevisiae genomic DNA), lane 2 (5 microliters of amplified ancient DNA sample), lane 3 (5

microliters of negative control). A molecular weight marker with bands in 100 bp intervals
from 100û800 bp (Invitrogen, Carlsbad, CA) is shown in the M lanes.
Fig. 2. BLAST alignment of the 540 bp fungal sequence from the Scorpion I wine jar from ancient Abydos (Ab540) compared with fungal clone T2709 (Genbank accession number X88771) from The IcemanÆs clothing. The sequence comprises 151 bp of ITS1, 157 bp of 5.8S rDNA and 187 bp of ITS2.
Fig. 3. ClustalX alignment of the ribosomal DNA region encompassing ITS1û5.8SûITS2 of the ancient wine sequence (AW) and the corresponding sequences of S. cerevisiae strain S288c (Sc), S. paradoxus strain CBS432 (Sp), and S. bayanus strain CBS380 (Sb). The region shown corresponds to S. cerevisiae chromosome 12 coordinates 455928-455181.
Fig. 4. Unrooted dendrogram based on maximum parsimony, with bootstrap scores based on 1000 replications indicated. In addition to the ancient wine sequence AW, the other sequences are S. cerevisiae strains CBS1171 (Genbank accession number D89886), HA6 (U90327), CBS459
(Z95938), CBS400 (Z95939), and CBS382 (Z95936); S. paradoxus strains CBS406 (D89890) and
CBS432 (Z95933); and S. bayanus strains CBS1513 (Z95949), CBS424 (Z95947), CBS380 (Z95945),
IFO1127 (D89887) and CBS1538 (D89888).

F igure 1
a b
M 1 2 3 M M 1 2 3 4 5 M
bp
800
700
600
500
400
300
bp
1000
800
500
600
400
300
200
100

T2709
AAATGAAGCCGGGAAACCGGTCCTTCTAACCCTTGATTATCTTAATTTGTTGCTTTGG
TG
Ab540 DDDDDDDDDDDDDDDDDDDDDDDDDDDDCDDDDDDDDDDDDADDCDCDDDDDDDDDDDDCD
T2709
GGCCGCGCAAGCACCGGCTTCGGCTGGATTGTGCCCGCCAGAGGACCACAACTCTGT

ATT
Ab540 DDDDDDDADDDDGDGDDDDCACDDDCDDGADDDDDDDDDDDDDDDDDDDDDDDDDDDDAD
T2709
AAATGTCGTCTGAGTACTATAAAATAGTTAAAACTTTCAACAACGGATCTCTTGGTT
CTG
Ab540 DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDTDDDDDDD
T2709
GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT
GAAT
Ab540 DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDTDDDDDDD
T2709
CATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTC
GAG
Ab540 DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
T2709
CGTCATAATGACCAAATCACCCCCGTGGTGGACTTGGAGTTGG-CTGTACTAGCCTCT
CT
Ab540 DDDDDDTDDADDDDDCDD-DTDGDAADDDDDDDDDDDGDNDDDCDDDDDD--DDDDDCDD
T2709
TAAAATCAGTGGCGGTGCTCTTAAAGCTCTACGCGTAGTA-ATTTTCTCGCTACAGGG
TC
Ab540 DDNDDDDDDDDDDDDDDDD-NDCDDDDDDDDDDDATDDDDDDDTCDDDDDDDDDDCDDCD
T2709
TTGATTGTACTTGCCAACAACCCCAACTTATCATAAGGTTGACCTCGGATCAGGTAG
GGA
Ab540 DADGDGDDDGDDDDD-DGDDDDDDDDDDD-DTTCDCDDDDDDNDDDADDDDDADDDNDDD
T2709 TACCCGCTGAACTTAA
Ab540 DDDDDDDDDDDDDDDD
Cavalieri et al. Fig. 2
Sc
ATTTAATAATTTTGAAAATGGATTTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTACT
GG
AW DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDTDDDDDDDDDDDDDDDDDDDDDDDD
Sp DDDDDDDDDDDDDDDDDDDDDDDDDDDD-DDDDDDDDDGDDDDDDDDDDDDDDDDDDDDDD
Sb DDDDDDDDDDDDDDDDTDTDDDDDDDDDD-DDDDDDDDDDDDDDGDDDDDDDDDDDDDDDD
Sc
GCAAGAAGACAAGAGATGGAGAGTCCAGCCGGGCCTGCGCTTAAGTGCGCGGTCTT
GCTA
AW DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
Sp DDDDDDDDDDDDDDDDDDDDDDDDDDDDTDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDT
Sb DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
Sc
GGCTTGTAAGTTTCTTTCTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTTTTGTTA
TA
AW DDDDDDDDDDDDDDDDDDDDDDADCDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
Sp DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDADDDDDDDD

Sb DDDDDDCDDDDDDDDDDDDDDDDDDDDDDDDDADDDDDDC--DDDDCDDTDDDDDDDDDD
Sc
GGACAATTAAAACCGTTTCAATACAACACACTGTGGAGTTTTCATATCTTTGCAACT
TTT
AW DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
Sp DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
Sb DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDTDDDCTDDDDDDDDDDDD
Sc
TCTTTGGGCATTCGAGCAATCGGGGCCCAGAGGTAACAAACACAAACAATTTTATTT
ATT
AW DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDCDDDD
Sp DDDDDDDDDDDDDDDDDDDDDDADDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
Sb DDDDDDDDT-DDDDDDDDDDDDDAD-DDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDDD
Sc
CATTAAATTTTTGTCAAAAACAAGAATTTTCGTAACTGGAAATTTTAAAA-TA-TTAA
AA
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Cavalieri et al. Fig. 3

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CBS432
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S. paradoxus
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Cavalieri et al. Fig. 4

CBS380
399
279
370
999
S. bayanus
S. cerevisiae
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421 441
582
CBS382
CBS1538
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