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Study of the volatile phenol metabolism and role of SO_2 as stress agent in *Brettanomyces/Dekkera bruxellensis* under wine conditions

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Non aspettare di finire l'università,

di innamorarti,

di trovare lavoro,

di sposarti,

di avere figli,

di vederli sistemati,

di perdere quei dieci chili,

che arrivi il venerdì sera o la domenica mattina,

la primavera,

l'estate,

l'autunno o l'inverno.

Non c'è momento migliore di questo per essere felice.

La felicità è un percorso, non una destinazione.

Lavora come se non avessi bisogno di denaro,

ama come se non ti avessero mai ferito e balla, come se non ti vedesse nessuno.

Ricordati che la pelle avvizzisce,

i capelli diventano bianchi e i giorni diventano anni.

Ma l'importante non cambia: la tua forza e la tua convinzione non hanno età.

Il tuo spirito è il piumino che tira via qualsiasi ragnatela.

Dietro ogni traguardo c'è una nuova partenza. Dietro ogni risultato c'è un'altra sfida.

Finché sei vivo, sentiti vivo.

Vai avanti, anche quando tutti si aspettano che lasci perdere.

Madre Teresa di Calcutta

CONTENT

1. INTRODUCTION

1.1 Wine spoilage by *Brettanomyces/Dekkera* species - The Brett character

Yeasts and bacteria, beside being the main actors of the transformation of grape must into "Bacchus nectar", may also become protagonists of wine spoilage. Although technological advances in oenology and the implementation of Good Manufacturing Practices in winemaking have led to a significant reduction in the risk of wine alteration by bacteria, spoilage linked to yeast contamination is still a potential threat. Most common alterations involve the formation of films or sediments, turbidity, gas production and sensory injuries such as the release of volatile phenols. These undesirable metabolites (off-flavours) can appear, especially during the aging of red wines and mainly if they are stored in barrels, due to contaminating yeasts, such as *Brettanomyces/Dekkera bruxellensis* species (Silva et al., 2004). Precursors of these compounds are represented by hydroxycinnamic acid in the free form, that is, *p*-cumaric acid, ferulic acid and caffeic acid. These molecules are found in grape in the esterified form with tartaric acid and their free form is released by cinnamoyl-esterase activity or grape juice heating (Gerbaux et al., 2002; Smit et al., 2003). These weak acids are converted in less harmful compunds through an enzymatic conversion, thus allowing a detoxification of the environment (Baranowski et al., 1980; Edlin et al., 1998; Stead, 1995; Suárez et al., 2007). This conversion follows two-step: a decarboxylation occurs on hydroxycinnamic acids, determining the production of 4-vinyl phenol, 4-vinyl guaiacol, and 4-vinyl catechol (Edlin et al., 1998), that are subsequentially reduced into 4-ethyl phenol, 4-ethyl guaiacol and 4-ethyl catechol, respectively (Dias et al., 2003b). Depending on their concentration levels in wines, volatile phenols can be considered to improve the aroma complexity or to be detrimental. The negative impact of these metabolites on wine has been largely revised in literature (Aguilar-Uscanga et al., 2003; Caruso et al., 2002; Chatonnet et al., 1992, 1995, 1997; Dias et al., 2003a; Fugelsang et al., 1993, Fugelsang, 1997; Joseph and Bisson, 2004; Loureiro and Malfeito-Ferreira, 2003; Mansfield et al., 2002; Romano, 2007; Snowdon et al., 2006). Descriptors such as "horse sweat", "leather", "medicinal", "barnyard", "bacon" are used to define the sensory modification resulting in wine defect and termed "Brett Charatcter".

As far the enzymatic activity, in particular the first reaction, a cinnamate decarboxylase (CD), was identified in *B. bruxellensis* LAMAP2480 by Godoy et al., (2014) as phenylacrylic acid decarboxylase (PAD1p) encoded by the corresponding *DbPAD* gene. The enzyme catalysing the reduction of the vinyl phelnols to the ethyl derivatives, is a vinyl phenols reductase (VPR), that is considered the key enzyme of the transformation, because contrary to the former is found in a smaller number of species

(Chatonnet et al., 1995, 1997; Dias et al., 2003a; Edlin et al., 1995; Saez et al., 2011). Although Godoy el al. (2008) and Tchobanov et al. (2008) isolated a potential VPR protein from *D. bruxellensis*, the full lengths sequence of the enzyme has been only recently released (Granato et al., 2014). While the protein sequence showed a high similarity of the amino acid sequence of the superoxide dismutase SOD1p of *S. cerevisiae*, a deeper bioinformatics investigation reveals that the enzyme is a dehydrogenase/reductase protein hosting a Rossmann fold domain (Lesk, 1995) and a structurally conserved C-terminal region (Oppermann et al., 2003; Jçrnvall et al., 1995), regions that are both required to bind NAD(P)H. Moreover, researchers studied some wine components (i.e. ethanol, sugars, volatile phenols) and some chemical factors (i.e. pH and sulphur dioxide) in relation with the production of off-flavours by *B./D. bruxellensis*, demonstrating their influence on the formation of this metabolites. In addition, the effect of interactions due to the presence of *p*-coumaric acid, ferulic acid and ethanol on CD activity and the expression of its putative gene has been studied (Ganga *et al.,* 2011). Results outlined that although oenological concentrations of *p*-coumaric and ferulic acids alone did not produced any significant effect on the enzyme activity, this was influenced by interactions between ethanol and cinnamic acid or temperature. Recently, Chandra et al., (2014) analysed the effect of glucose, ethanol and SO_2 on the growth and volatile phenol production by *B. bruxellensis* ISA 2211. A negative linear and quadratic effect triggered by SO_2 occurred on growth and 4-ethyl phenol production; in particular, a SO_2 concentrations higher than 20 mg/L, at pH 3.50, induced immediate loss of cell culturability even under growth permissive levels of ethanol.

1.2 Evolutionary aspects and genetic features of *Brettanomyces/Dekkera bruxellensis* species

In the last decades, the interest of oenologists about the contribution of yeasts, given their important role on wine quality, to the winemaking process has been steadily increasing. In addition to *Saccharomyces cerevisiae*, the most important species for alcoholic fermentation, non-*Saccharomyces* yeasts, such as *Candida zemplinina*, *Hanseniaspora uvarum* and *Torulaspora delbrueckii*, are involved in the first phase of alcoholic fermentation and play a decisive role on taste and aroma of wine (Herraiz et al., 1990). Contrarywise, other species might negatively affect wine flavour. Among yeasts responsible for wine spoilage, *B./D. bruxellensis* is the species in which the scientific community has been more interested. This fact is documented by an increase in international publications in the last two decades, and the beginning of genome sequencing in 2007 (Woolfit et al.), followed in 2012 by

obtaining the full sequense (Piskur et al.; Curtin et al., a). In *B./D. bruxellensis* the karyotype is strongly variable, in fact, the number of the chromosomes ranges from 4 to at least 9, as well as the size shows notable intraspecific differences (0,75-6,5 Mbp).

When single-gene molecular probes were used in Southern analysis, the corresponding genes were mapped to at least two chromosome bands, excluding a simple aploid organization of the genome. In addition, in most cases, when different loci of a single strain were sequenced, different haplotypes were obtained belonging to two subtypes. Phylogenetic reconstruction based on the haplotypes revealed that the sequences of several isolates belonging to a subtype were more similar to each other than the sequences belonging to the other subtype of the same isolate. The size of the genome was defined in 13.4 Mb as far *B./D. bruxellensis* CBS2499 strain (Piskur et al., 2012), while 12.7 Mb is the value obtained by Curtin et al. (2012a) working on *D. bruxellensis* AWRI1499.

In order to obtain an accurate view of the evolutionary pattern of *B./D. bruxellensis*, analysis were conducted using two different approaches. Both of the study strategies have led to a single surprising result that places *B./D. bruxellensis* in close relationship with *Pichia* (*Komagataella*) *pastoris*, (as it is shown in the phylogenetic tree, in Fig. 1, Piskur et al., 2012) and far distant from *S. cerevisiae*, contrary to what was previously believed, despite the clearly different observable characteristics.

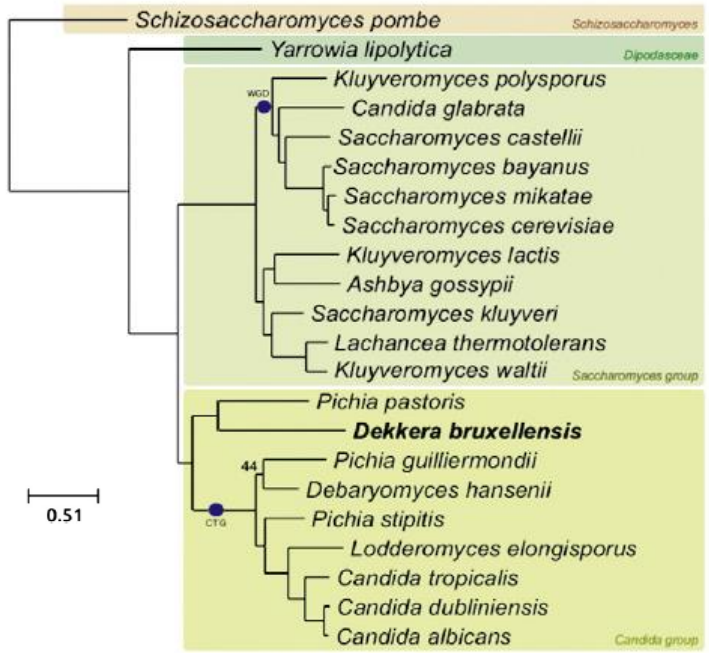

Fig. 1 Phylogenetic relationships within the *Schizosaccharomyces/Dipodasceae/Candida/Saccharomyces* group. Position of *D. bruxellensis* is in bold. (Piskur et al., 2012)

This result has been partially confirmed and partially denied by Curtin and colleagues (2012a) who have highlighted the evolutionary distance between *B./D. bruxellensis* and *S. cerevisiae* as well, but finding the maximum likelihood of *B./D. bruxellensis* with *Pichia angusta*, that has been defined as the closest relative of the yeasts of which genomic sequencing is available so far. Yeasts belonging to *Komagataella* genus are known as aerobic and low ethanol producers, contrary to what is observed in *B./D. bruxellensis* and *S. cerevisiae*.

From the analysis of *B./D. bruxellensis* genome sequence, a relatively high frequency of single nucleotide polymorphisms (SNPs) was observed. The haplotype analysis of about 1.2% of ORF suggested that the genome of *D. bruxellensis* AWRI1499 consists of a diploid and moderately heterozygous genome combined with a divergent and haploid genome. An average of 27 single-nucleotide (SNPs) polymorphisms per 1000 nucleotides - a total of 342,900 sites across the genome, were found. However, this density is not uniform, with some regions exhibiting significantly lower heterozygous rates. Within the ORFs, fewer SNPs were observed than in the whole genome, with an average of 1.9%. Most SNPs in the coding regions are synonymous, however, 0.66% of all nucleotides are expected to produce non-synonym substitutions within *B./D. bruxellensis* ORF. In many regions there were more than two distinct sequences between the reading frames.

1.3 *Brettanomyces/Dekkera bruxellensis* species metabolism and physiology: Custer effect and VBNC state

B./D. bruxellensis produces considerable amounts of ethanol under aerobic conditions and can also growth anaerobically, like *S. cerevisiae*. Thus, it can be classified as a Crabtree-positive and facultative anaerobic yeast. When glucose has been exausted *B./D. bruxellensis* is capable of catabolise ethanol efficiently, as well as acetic acid. This "make-accumulate-consume" life strategy, sustained by the Crabtree effect, and common with *S. cerevisiae*, helps yeasts to out-compete other microorganisms in natural habitats (Rozpędowska et al., 2011). A characteristic metabolic feature of *B./D. bruxellensis* is the inhibition of the glucose fermentation to ethanol under strictly anaerobic conditions and its stimulation in the presence of oxygen, defined as "Custers effect" (Custers, 1940; Henrici, 1941; Skinner, 1947; Barnet et al., 2005). High amount of acetic acid is produced during aerobic growth, while no appreciable amounts are formed under anaerobic fermentation. Normally, in yeasts acetate is formed via acetaldehyde oxidation troughout acetaldehyde dehydrogenase activity, a NAD+ or NADP+ dependent enzyme whose gene is constitutively expressed. This last characteristic induces the subsequent Custers effect, determined by the continued drainage of NAD+ through the irreversible conversion of acetaldehyde to acetic acid bringing glycolysis to a stop due to the lack of NAD+. Custers effect is caused by the inability of cells in restoring the redox balance via production of reduced metabolites, specifically glycerol, possibly attributed to the lack of glycerol 3-phosphate phosphatase activity (Wijsman et al., 1984). It has been observed that the majority of strains belonging to *Brettanomyces* spp. utilize both glucose and ethanol in producing acetic acid,

nonetheless not all strains could use both as carbon sources, with high variability seen in the levels of acetic acid produced by different strains (Freer, 2002).

In the oenological field, the isolation and the count of *B./D. bruxellensis* are performed using media for selective/differential growth. However, these protocols have limited efficacy due to the low rate of growth of these yeasts and their low cell density compared to other microbial species present in the must/wine. In addition, stress conditions that may occur during fermentation of wine may induce cells to enter a "viable but not culturable" state (VBNC) (Millet and Lonvaud-Funel, 2000; du Toit et al., 2005; Agnolucci et al., 2010; Serpaggi et al., 2012) thus preventing cell detection by cultural-method analysis. VBNC state is characterized by the inability of cells to divide, even though they are still alive and capable of maintaining metabolic activity. Moreover, in 2012 Serpaggi and collaborators demonstrated the resuscitation fenomenon, thus the ability of cells in VBNC state to exit this condition. Stress, and thus cells entering the VBNC state, was induced with addition of SO_2. Cell size resulted decreased, as well a reduction in the glycolytic flux was observed. Cells recoverd culturability after stressor removal by pH modification in the growth medium. As well Agnolucci et al., assessed (2010) the maintained production of vinyl phenols both in media containing VBNC or not viable *B. bruxellensis* cells, Serpaggi et al., (2012) also confirmed a peristance in spoilage compounds production in VBNC cells. Agnolucci et al. (2014) observed, in wine, a strain dependent character with regards to lack of viability and culturability once cells were exposed to SO_2. Moreover, comparing results to what found in synthetic wine medium, a higher amount of molecular SO_2 was needed to induce the not culturable state, and the wine spoilage activity resulted affected by medium composition.

Considering that, the VPR gene represents a suitable target to approach the early detection of *B./D. bruxellensis* in wine contamination control. Till now few studies are focused on the development of Real-Time protocols for the detection of this species (Tofalo et al. 2012; Willenburg & Divol, 2012).

2. AIMS

Despite other approaches proposed to prevent and/or control microbial contamination in food matrixes, the use of sulphur dioxide is still the most common method worldwide because of its ease use. Unfortunately, SO_2 is not always effective on all the spoilage microorganisms (Barata et al., 2008; Agnolucci et al., 2010; Curtin et al., 2012b; Vigentini et al., 2013). Due to a multitude of adverse reactions to sulphite exposure in humans (Vally et al., 2012), limits about the maximum permitted amount of SO_2 in foods have been set (Reg. EU 1129/2011).

Among the various detrimental effects caused by the presence of *B./D. bruxellensis* yeast in wine, the production of volatile phenols is probably the most relevant.

A deep understanding on the production of these compounds and the mechanisms implemented by the yeast to counteract the SO_2 stress will be helpful for improving the sulphur dioxyde management in a context of a more sustainable winemaking.

Specifically, aims of this study are to:

I. Demonstrate the VPR activity and investigate the volatile phenol production with regards to the genetic features at strain level;
II. Identify a proper/suitable HKG for the subsequent evaluation of the expression of the two genes mainly involved in the VPs production, as a response of variation of SO_2, pH and ethanol parameters and the related VP production;
III. Investigate the genetic mechanisms involved in SO_2 stress resistance, under oenological conditions.

3. RESULTS

3.1 Cloning the putative gene of vinyl phenol reductase of *Dekkera bruxellensis* in *Saccharomyces cerevisiae*

3.1.1 Recombinant strains of *S. cerevisiae* express *D. bruxellensis* VPR

In order to verify the activity of the putative VPR of *D. bruxellensis*, *S. cerevisiae* W303 was genetically engineered with expression vectors YEp112TVPR and YEp112TVPRT. The difference between the two vectors is that the latter allowed an easy protein purification due to a poly-histidine amino acid motif at C-terminus end. In both cases, the VPR gene was amplified by PCR from the genome of *D. bruxellensis* CBS4481, the strain that Granato and collaborators (2014) have showed to exhibit the highest vinyl phenol reductase activity toward 4-vinyl guaiacol, and ligated in the plasmid backbone (YEp112T).

Thus, to determine the suitability of *S. cerevisiae* as a host, the function of VPR was preliminary addressed in recombinant clones expressing the native nt sequence and transformed with the vector YEp112TVPR (Fig. 2).

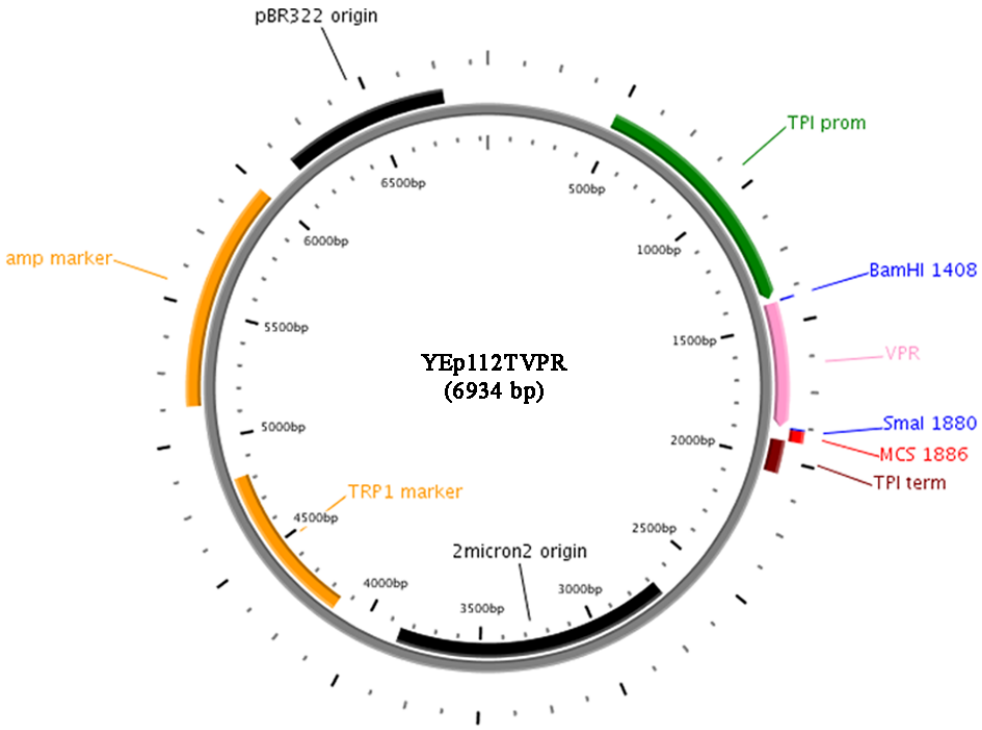

Fig. 2 Expression vector YEp112TVPR designed using PlasMapper Version 2.0.

After cell transformation with plasmid YEp112TVPR, 20 yeast colonies were isolated on selective medium and checked for the presence of the VPR gene using primer pairs TPIpromF/VPRR by PCR amplification. Among the clones producing a band at about 465 bps, corresponding to VPR gene, one clone (#17) was selected for further analysis. PCR product from clone #17 was submitted to the

sequencing in order to check for any synthesis mistake and to confirm the presence of the consensus binding pattern for NAD(P)H and the cofactor binding motif (Granato et al. 2014). Results, obtained by *in silico* translation of the nt sequences, ruled out the occurrence of both domains, supporting the thesis that the VPR enzyme belongs to the short chain dehydrogenases/reductases (SDRs) family. Finally, the presence of the VPR transcript was investigated in the recombinant strain #17 using the couple of primers vprRTF/vprRTR that specifically target the gene of *D. bruxellensis* (Fig. 3). As expected, a single band at 125 bps was generated for strain #17 and *D. bruxellensis* CBS4481. Genomic DNAs of *S. cerevisiae* and *P. pastoris*, this latter the closest relative of *D. bruxellensis* (Curtin et al., 2012a; Piskur et al., 2012), enclosed as negative controls were not amplified (Fig. 3).

Once the specific protein activity was verified in crude cellular extracts, new *S. cerevisiae* transformants were obtained using the vector YEp112TVPRT, which allowed for easy protein purification. PCR amplification of the VPR gene from 20 yeast colonies isolated on selective medium confirmed the presence of the expected construct (one band of 465 bps). One clone, named #62, was maintained for further experiments. Finally, the analysis of the transcript showed the expression of the heterologous gene (Fig. 3).

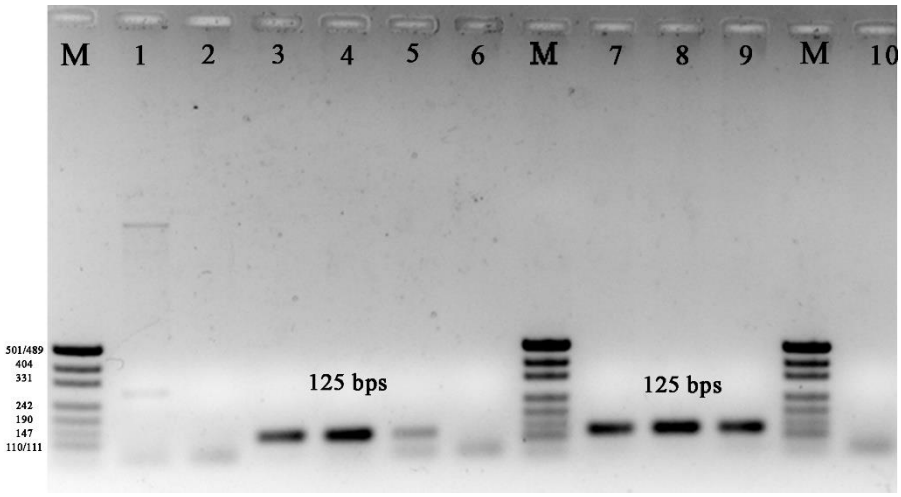

Fig. 3 Specificity of primer pair vprRTF/vprRTR to target the VPR gene of *D. bruxellensis* in different genomic DNAs. From the left: lane 1, *P. pastoris* GS115; lane 2, *S. cerevisiae* W303; lane 3, *D. bruxellensis* CBS4481; lane 4: transformed strain *S. cerevisiae* W303 clone #17; lane 5, transformed strain *S. cerevisiae* W303 clone #62; lane 6, negative control. Analysis of the transcripts of VPR gene in: lane 7, cDNA of *D. bruxellensis* CBS4481; lane 8, cDNA of the transformed strain *S. cerevisiae* W303 clone #17; lane 9, cDNA of the transformed strain *S. cerevisiae* W303 clone #62, lane 10, cDNA of *S. cerevisiae* W303. M: molecular marker, pUC19 DNA/MspI (HpaII) (Fermentas, Vilnius, Lithuania).

3.1.2 *S. cerevisiae* recombinants can trigger the reduction of the 4-vinyl guaiacol

With the aim of testing the capability of transformed cells to release volatile phenols in the supernatant of a cultural medium, cells were grown in presence of p-cumaric acid or ferulic acid or 4-vynil guaiacol or 4-vynil phenol as precursors of 4-ethyl phenol or 4-ethyl guaiacol. Cell cultures with not transformed strain of *S. cerevisiae* W303 were carried out as control. Volatile phenols failed to be detected by HPLC analysis both in transformed and, as expected, not transformed strains (data not shown). The same result was obtained from bioconversions with cells in stationary phase of growth. Thus, this study measures the VPR activity in cellular extracts. The specific activity of VPR was assessed in crude cellular extracts of the recombinant clone #17 of *S. cerevisiae,* of *D. bruxellensis* CBS4481 and of the sequenced strain *D. bruxellensis* CBS2499. No activity was detected from *S. cerevisiae* cultures grown in Verduyn medium (Verduyn et al., 1992), which contains only traces of inorganic salts; consequently, assuming that VPR enzyme belongs to the class of the Zn/Cu SODs, a higher Zn and/or Cu concentration could be necessary to obtain the functional heterologous enzyme.

A biologically active protein able to trigger the reduction of the 4-vinyl guaiacol substrate was detected by UV assays when the recombinant cells were cultivated using a modified Verduyn medium (see Material &Methods paragraph). The calculated specific activity of the protein produced by the clone #17 (9 ± 0.6 mU/mg) was found to be lower in the recombinant strain in comparison to the one obtained in the same growth conditions for the native strain *D. bruxellensis* CBS4481 (57 ± 12 mU/mg). A closer value was obtained from *D. bruxellensis* CBS2499 that reached 4.7 ± 1.9 mU/mg. The VPR protein was then purified from *S. cerevisiae* W303 clone #62, the one expressing the gene for VPR fused with an his-tag for protein isolation. According to literature (Granato et al., 2014), the purification allowed the separation of a single band in denaturing condition with a molecular mass of about 20 kDa (Fig.

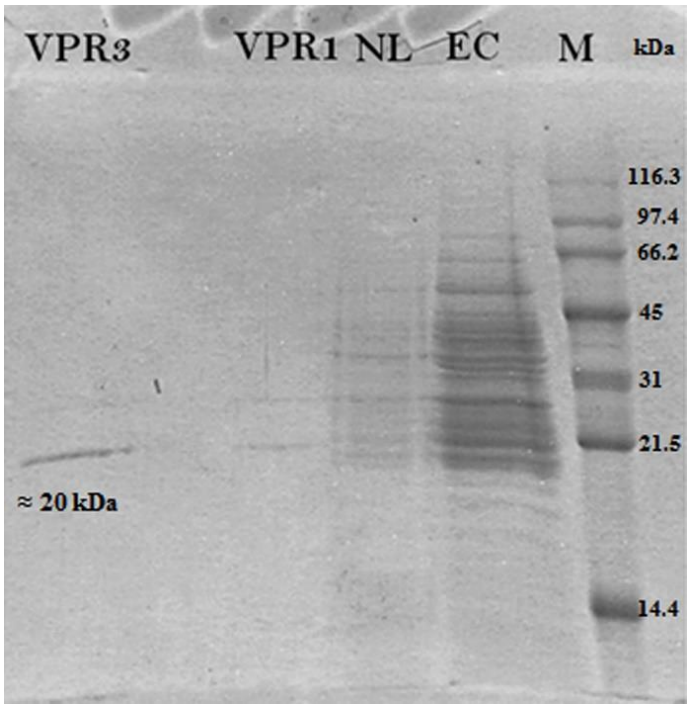

Fig. 4 SDS-PAGE gel image showing the fractions obtained from the cell extract of the recombinant strain #62 of *S. cerevisiae* carrying the his-tagged VPR protein. M: Broad Range Molecular Markers (Biorad); CE: crude extract of *S. cerevisiae* W303 clone #62; NL: not bound fraction; VPR1: first fraction of VPR; VPR3; third fraction of VPR (showing the highest concentration of protein).

4). The eluted fractions containing the highest protein concentration (0.040 ± 0.017 mg/mL) were used to test the activity of VPR (as an example, lane VPR3 in Fig. 4). No absorbance decrease was observed in the unbound fractions (Fig. 4, lane NL) (data not shown). Since no variation in absorbance was detected in absence of the substrate, results confirmed that the purified protein specifically catalyses the reaction from 4-vinyl guaiacol to 4-ethyl guaiacol (Fig. 5). The biotransformation was confirmed by following HPLC analysis, although low overall conversion (7 %) was observed. VPR-active fractions showed a specific activity of 0.97 ± 0.01 U/mg at pH 7.5. Moreover, an optimization of the UV assay using a phosphate buffer at pH 6.0 rather than 7.5 almost doubled the value of the specific activity of VPR that increased at 1.83 ± 0.03 U/mg of protein. As expected, the crude extracts of *S. cerevisiae* W303 clone #62 confirmed the order of magnitude of the specific activity of the VPR obtained in *S. cerevisiae* W303 clone #17 (4 ± 1.1 mU/mg).

Finally, according to Granato et al. (2014), the purified protein was also tested for SOD activity (Fig. 5), showing a specific activity of 3.41 U/mg in the fraction VPR3 (Fig. 4 and 5).

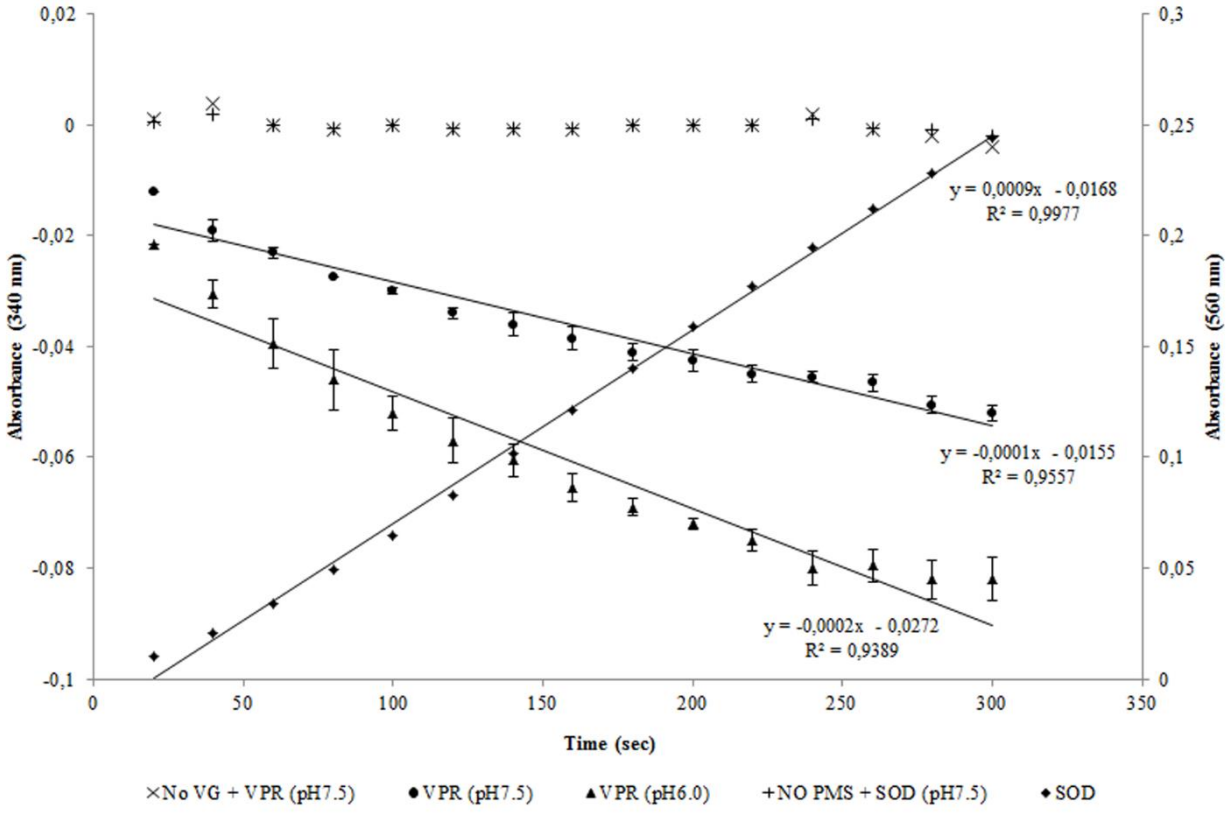

Fig. 5 VPR and SOD activities of the purified protein extracted from *S. cerevisiae* W303 clone #62. VPR pH 6.0, activity measured in phosphate buffer at pH 6.0; VPR pH 7.5, activity measured in phosphate buffer at pH 7.5; No VG + VPR (pH 7.5), absorbance variation in absence of 4-vinyl guaiacol (VG); No PMS + SOD (pH 7.5), absorbance variation in absence of phenazine methosulfate (PMS); SOD, absorbance variation in presence of PMS at pH 7.5.

3.1.3 The polymorphism of VPR gene in *D. bruxellensis* does not justify a different production of volatile phenols

A comparative genetic analysis of the partial VPR sequences from 17 different *D. bruxellensis* strains of oenological origin was carried out. The aim of this evaluation was to investigate if production of volatile phenol is correlated with a polymorphic state of the genetic *locus* of VPR, and in particular in the sites of the Rossmann fold-like region potentially involved in the reductase activity of the enzyme. As already observed for *D. bruxellensis* CBS4481 strain, the analysis revealed that a conserved consensus binding pattern for NAD(P)H and a cofactor binding motif (Oppermann et al. 2003; Jçrnvall et al. 1995; Lesk 1995) is present in all strains of the collection. Eight potential SNPs, on 354 scored nucleotides, were recognized; these nucleotide modifications allow the subdivision of the yeast collection in 10 genotypes. Furthermore, all polymorphic positions, though with different frequencies (from 0.72 to 78.6%) showed allelic heterozygosity. No triple o multiple peak was detected at each single nucleotide position of the pherograms; this data suggests that, in the investigated strains, the gene is in a diploid state. As far the heterozygosity state of the single strains, it ranged from 0 to a maximum of 1.7%. The majority of these SNPs were synonymous; two SNP positions (95 and 329) led to a new codon, producing non-synonymous amino acid substitutions across *D. bruxellensis* VPR gene (Table 1). In detail, the SNP at position 95 determined a switch from asparagine to serine, still belonging to the group of neutral-polar amino acids and, at position 329, the SNP led from alanine to valine, as alanine a neutral-nonpolar amino acid. Most significant protein changing was detected in three strains (*D. bruxellensis* CBS1941, CBS2547 and CBS5206).

Table 1 Comparative genetic analysis of the partial sequence of VPR gene and relative volatile phenol productions by different *D. bruxellensis* wine strains. SNP positions are calculated from the starting codon ATG. n.d., not detectable.

Strain (CBS#)	Origin	SNP position								Allelic heterozygosity (% at strain level)	Genotype	Production (µg/cell) x10^{-6}		
		95	120	126	156	228	297	329	351			4-ethyl phenol (A)	4-ethyl guaiacol (B)	Volatile phenols (A+B)
73	France/Must	A/G	G	T	T/C	C/T	C	C	C	**0.85**	**1**	2.66	1.86	4.52
74	Belgium/Lambic beer	A	A/G	T/C	C	C	C	C	C	**0.56**	**2**	n.d.	n.d.	-
1941	France/Sour wine	A/G	A/G	T/C	C	T/C	C	T/C	C	**1.41**	**3**	2.36	2.31	4.67
1942	France/Sour wine	A/G	G	C	C	T/C	C	C	C	**0.56**	**4**	1.62	1.45	3.07
1943	France/Sour wine	A/G	A/G	C	C	T/C	C	C	C	**0.85**	**5**	2.45	1.88	4.33
2499	France/Wine	A	A/G	C	T/C	T/C	T/C	C	C	**1.13**	**6**	2.10	1.95	4.05
2547	France/Sour wine	A/G	A/G	T/C	T/C	T/C	C	T/C	C	**1.69**	**7**	1.26	0.88	2.14
2796	Germany/Sparkling wine	A/G	G	C	T/C	T/C	T/C	C	C	**1.13**	**8**	1.95	1.57	3.52
4459	South Africa/White dry wine	A	A/G	C	T/C	T/C	T/C	C	C	**1.13**	**6**	2.23	1.89	4.12
4481	South Africa/Sparkling wine	A	A/G	C	T/C	T/C	T/C	C	C	**1.13**	**6**	2.62	2.06	4.68
4482	South Africa/Sherry wine	A/G	G	C	T/C	T/C	T/C	C	C	**1.13**	**8**	1.97	1.47	3.44
4601	South Africa/Wine	A	G	C	C	C	C	C	C	**0**	**9**	3.76	2.62	6.38
4602	South Africa/Wine	A	A/G	C	T/C	T/C	T/C	C	C	**1.13**	**6**	1.66	1.04	2.7
5206	South Africa/Must	A/G	A/G	T/C	C	C	C	T/C	T/C	**1.41**	**10**	1.78	2.37	4.15
	Allelic heterozygosity (% at SNP position level)	**57.1**	**64.3**	**28.6**	**57.1**	**78.6**	**42.9**	**21.4**	**0.72**					

3.2 A Response Surface Methodology approach to investigate the effect of sulphur dioxide, pH and ethanol on *DbCD* and *DbVPR* gene expression and on the volatile phenol production in *Brettanomyces/Dekkera bruxellensis* CBS2499

The aim of the study was to investigate the expression of *DbCD* and *DbVPR* genes and the production of VPs in a range of oenological conditions. To do that, we defined the experimental conditions at the realistic concentrations of some factors found in wines along with the requirement to have conditions compatible with cell growth. Different runs (Table 2) were performed to obtain gene expression values workable through a Response Surface Methodology approach under the tested conditions: SO_2 levels ranged from 0 mg/L to 0.25 mg/L, pH varied between 3.5 and 4.5 units and ethanol concentrations between 5% (v/v) and 12.5% (v/v).

3.2.1 Identification of *DbPDC* and *DbALD* genes in *D. bruxellensis*

*Db*PDC gene was identified in the scaffold 1 at 1700 bps (e_gw1.1.1485.1) of *D. bruxellensis* CBS2499 genome; in particular, the nucleotide sequence showed about 55% identity with the *S. cerevisiae* genes encoding for *PDC1*, *PDC5* and *PDC6* (55.1%, 55.8%, 55.5%, respectively). Due to the similar level of identity found among the three isoforms, *PDC1* sequence was chosen for a further investigation in the genome of *B. bruxellensis* AWRI1499. The nucleotide sequence with accession number "EIF49850.1" was identified as a possible homologous of *S. cerevisiae PDC* gene with an identity of 55% (identity of 96.9% with e_gw1.1.1485.1). In *K. phaffii* genome, the gene codifying for *KpPDC* showed two potential isoforms differently located in *K. phaffii* CBS7435 (chromosomes 3 and 4). Only the sequence on the chromosome 3 identified the homologous gene (identity of 100%) on the genome of the strain *K. phaffii* GS115, with accession number XM_002492352.1. Thus, this gene was aligned against *D. bruxellensis* CBS2499 and the sequence in the scaffold 1 (e_gw1.1.1485.1) was confirmed as the potential homologous gene of *KpPDC* (55.5%identity). In conclusion, the open reading frames represented by the accessions e_gw1.1.1485.1 and EIF49850.1 of *D. bruxellensis* CBS2499 and *B. bruxellensis* AWRI1499, respectively, were identified as the homologous genes of *ScPDC1* and *KpPDC*. As regards *DbALD*, among the three genes (*ScALD3, ScALD2* and *ScALD6*) encoding for the sequence of *ScALD6* of *S. cerevisiae* S288c genome led to the identification of a possible homologous gene in *D. bruxellensis* CBS2499 genome in the scaffold 4 at 1523 bps

Table 2 Runs of Box-Behnken experimental design, normalized relative expression values of *DbCD* and *DbVPR* genes, expressed as fold change, and quantification of vinyl phenol, vinyl guaiacol, ethyl phenol and ethyl guaiacol, expressed as ratios between μmoles of product (volatile phenols) on μmoles of relative consumed precursor (coumaric and ferulic acids) for the different trials.

Run	mol. SO_2 (mg/L)	pH	Ethanol (v/v)	*DbCD*	*DbVPR*	*p*-cumaric acid	Ferulic acid	Vinyl phenol	Vinyl guaiacol	Ethyl phenol	Ethyl guaiacol	Vinyl phenol	Vinyl guaiacol	Ethyl phenol	Ethyl guaiacol
						Conc. (mg/L)						Yield (μM product/μM consumed acid)			
1	0	3.5	8.75	0.49	1.22	2.35	1.64	1.09	0.036	2.45	2.29	0.25	0.01	0.55	0.58
2	0.25	3.5	8.75	0.45	1.33	2.39	1.92	0.71	0.029	4.15	3.46	0.16	0.01	0.94	0.93
3	0	4.5	8.75	0.52	1.47	3.81	1.81	1.13	0	2.67	1.92	0.35	0.00	0.80	0.51
4	0.25	4.5	8.75	0.66	1.80	4.11	2.82	0.35	0	2.54	1.57	0.12	0.00	0.83	0.53
5	0	4	5	0.30	1.47	0.86	0.42	0.40	0	5.73	4.29	0.07	0.00	1.02	0.87
6	0.25	4	5	0.14	0.80	3.86	2.66	0.10	0	2.53	1.96	0.03	0.00	0.77	0.63
7	0	4	12.5	0.24	0.67	3.43	2.43	1.87	0.075	2.16	2.00	0.53	0.02	0.60	0.60
8	0.25	4	12.5	0.38	0.91	3.45	2.78	2.24	0.324	2.52	2.28	0.64	0.11	0.70	0.75
9	0.125	3.5	5	0.33	0.99	0.39	0.37	0.14	0	6.45	5.18	0.02	0.00	1.08	1.04
10	0.125	4.5	5	0.39	1.11	1.26	0.66	0.26	0	5.65	3.90	0.05	0.00	1.07	0.82
11	0.125	3.5	12.5	0.40	0.94	2.34	2.33	1.75	0.092	2.41	2.70	0.40	0.03	0.54	0.80
12	0.125	4.5	12.5	0.65	0.92	4.08	2.07	1.55	0.021	2.06	1.37	0.51	0.01	0.66	0.38
13	0.125	4	8.75	0.21	0.85	3.16	2.13	0.59	0	3.56	2.62	0.16	0.00	0.93	0.74
14	0.125	4	8.75	0.15	0.89	2.29	1.61	0.51	0.024	2.60	2.00	0.11	0.01	0.58	0.50
15	0.125	4	8.75	0.18	0.72	3.17	2.06	0.76	0	3.37	2.61	0.20	0.00	0.88	0.72

(e_gw1.4.403.1) with an identity of 55.6%. *ScALD6* sequence was also aligned against the genome of *B. bruxellensis* AWRI1499 and the resulting amino acid sequence with the accession number "EIF46557.1" showed an identity of 56% (99.4% identity with e_gw1.4.403.1). In *K. phaffii* genome, the gene encoding for *KpALD* was identified on different chromosomes; the nucleotide sequence in the scaffold 20 at 1496 bps (e_gw1.20.29.1) of the chromosome 3 of the strain CBS7435 showed the highest identity (67.8%) with both e_gw1.4.403.1 and EIF46557.1 open reading frames of *D. bruxellensis* CBS2499 and *B. bruxellensis* AWRI1499 genome, respectively. Thus, these last genes were used for primer design being considered the homologous genes of *ScALD6* and *KpALD*.

3.2.2 Primer validation in standard PCRs and optimization of qPCR experiments

The primer pairs designed on *DbALD*, *DbPDC* and *DbCD* were evaluated for their ability to produce a specific fragment through a standard PCR and further sequencing of the amplified products. A unique amplification product of 140 bps for all the three genes investigated was obtained (data not shown). This value corresponds to the expected product length on the base of the size (Table 3) of *in vitro* primers design. No aspecific products were detected and no amplification was observed with *S. cerevisiae* S288C and *K. phaffii* GS115 used as negative controls. Primer specificity was confirmed by sequencing with a 100% identity with the target sequences.

Table 3 Primer pairs used for qPCRs.

Oligo name	Sequence (5' -> 3')	Tm (°C)	Reference
*DbALD*_F	CTATCAAGGTCGGAAACCCA	57.3	This study
*DbALD*_R	TCTCTCACCACCAGTAAGGA	57.3	This study
*DbACT*_F	TTATTGATAACGGTTCTGGTATGT	55.9	Nardi et al., (2010)
*DbACT*_R	ACCCATACCGACCATGATAC	57.3	Nardi et al., (2010)
*DbEF*_F	CTCCAGTTGTTGACTGCCA	56.7	Nardi et al., (2010)
*DbEF*_R	CATCTTAACCATAGCAGCATCAC	58.9	Nardi et al., (2010)
*DbPDC*_F	GTGGTTTGCTTTCCGACTAC	57.3	This study
*DbPDC*_R	AAACAGCGGACTTGACCTTAC	57.9	This study
*DbTUB*_F	GTATCTGCTACCAGAAACCAACC	60.6	Rozpędowska et al., (2011)
*DbTUB*_R	CCCTCACTAACATACCAGTGGAC	62.4	Rozpędowska et al., (2011)
*DbCD*_F	CACAGACTCGAACGGAAAAC	57.3	Godoy et al., (2014)
*DbCD*_R	CCAGGGCGTACACATTGATA	57.3	Godoy et al., (2014)
*DbVPR*_F	CTAAGGGCACTATCAAGGACA	57.9	Romano et al., 2017
*DbVPR*_R	CTGCAAAGAACCAGCATCA	54.5	Romano et al., 2017

All primers designed for the amplification of the potential HKGs (*DbALD, DbPDC, DbEF, DbTUB, DbACT*) and the target genes (*DbCD, DbVPR*) were validated to assess whether the qPCR reactions were really optimized. Five dilutions of cDNA samples obtained from cell culture of *D. bruxellensis* CBS2499 grown in SWM at LS condition [0 mg/L. SO_2, pH 4.5, 5% (v/v) ethanol] were tested to evaluate the ones containing from 10^3 to 10^6 copies of template that were able to give amplification curves between 30 and 20 C_T values, respectively. The obtained C_Ts values were relatively low and similar; the lowest one (about 13) was given by *DbEF* gene, while the highest (about 20) was obtained for *DbCD* gene, thus revealing similar expression levels among the amplified genes. Then, a standard curve was created to assess primer efficiency of both the target genes and potential HKGs, as well as to be used as "standard" within the normalization plate used for HKG identification by qPCR. The R^2 values obtained for all primer pairs ranged from 0.980 to 0.999.

3.2.3 Analysis of the gene expression stability of potential HKGs

Five genes were evaluated for this purpose (Table 3): two genes encoding for metabolic enzymes, pyruvate decarboxylase (PDC) and acetaldehyde dehydrogenase (ALD), were chosen based on their important role on fermentative metabolism and on NAD(P)H supply. The three others, encoding for elongation factor (EF), tubulin (TUB) and actin (ACT), have been already used as housekeeping genes in other studies (Nardi et al., 2010; Rozpędowska et al., 2011; Moktaduzzaman et al., 2015).

DbALD, DbPDC, DbEF, DbTUB, DbACT were analyzed by a qPCR multiplex assay to identify the reference gene with a constant expression level across the experimental conditions under study. Expression stability of potential HKG genes were assessed at the two extreme growth conditions of the used experimental design, LS [0 mg/L. SO_2, pH 4.5, 5% (v/v) ethanol] and HS [0.25 mg/L. SO_2, pH 3.5, 12.5% (v/v) ethanol]. The cultures showed a negligible lag phase reaching a similar final biomass (1.4-1.7 OD_{600nm}) in 8 days. The absolute quantification approach was employed to obtain the qPCR results from the assayed normalization plate. Thus, a direct comparison between C_Ts of each sample and C_Ts of the standards (corresponding to the transcript copy number of each serial dilution of the HKG candidates) was accomplished. Overall, genes presented C_Ts spanning from 11 to 20, with *DbEF* and *DbPDC* having the lower values (Table 4). C_T data were submitted to GeNorm (Vandesompele et al., 2002) and Normfinder analysis. Because of the elimination process, GeNorm algorithm cannot identify an optimum reference gene and ended up by suggesting a pair of genes having the best same M-value of 0.186, *DbACT* and *DbTUB* (Table 4). For a single gene discrimination, Normfinder

was employed along with GeNorm algorithm. Since samples came from two different treatment groups, Normfinder algorithm separated the variation into an intragroup and an intergroup contribution. The analysis was then repeated without considering the groups and this allowed to estimate a robust standard deviation; the lowest SD (0.0929) was assigned to *DbTUB* (Table 4). A minimal value of the accumulated standard deviation was a great indicator of the optimal number of reference genes to be used for normalization. The highest expression stability revealed by *DbTUB*, attributed by both the lowest M-value and the standard deviation, identifying this gene as the HKG for this study.

Table 4 Candidate genes for their potential as HKGs.

Gene	C_T values								M-Value	Acc.SD
	LSA	LSA	LSB	LSB	HSA	HSA	HSB	HSB		
DbALD	19.51	19.77	20.10	20.12	19.05	18.62	18.6	18.5	0.373	0.2398
DbPDC	14.2	13.98	14.04	13.94	14.46	14.65	15.06	15.13	0.564	0.1523
DbEF	11.77	11.65	12.22	12.19	12.66	12.64	13.42	13.19	0.741	0.2762
DbTUB	16.71	16.83	16.86	16.49	16.89	17.04	17.28	17.34	0.186	0.0929
DbACT	17.58	17.23	17.27	17.53	18.17	18.13	18.01	17.75	0.186	0.1443

The second row indicates the two tested conditions: LS, low stringent growth condition and HS, high stringent growth condition, performed in two independent replicates (A and B). From each replicate three mRNAs were extracted and analysed in qPCR assays. M-value is calculated by the GeNorm analysis while Normfinder algorithm and GenEx software calculate the accumulated Standard Deviation (Acc. SD) that is the expected SD if multiple reference genes are used for normalization.

3.2.4 Effect of SO_2, pH and ethanol on *DbCD* and *DbVPR* gene expression

Real-time quantitative PCR assays were carried out to test all conditions of the experimental design in order to study the role of SO_2, pH and ethanol on *DbCD* and *DbVPR* genes expression. All the assays produced amplification curves in the range of the best sensitivity of the qPCR (20-30 C_T values) and a high reproducibility within a single test and among tests was obtained; indeed, an overlapping of the amplification curves of the replicates of both each run and the calibrator was observed. This was particularly evident in the case of *DbTUB* amplification that showed a constant gene expression (C_T value of 23) among the 15 conditions evaluated, confirming once again its reliable role as HKG.

Although the experimental design has to be considered functional to only apply the RSM approach and data cannot be individually interpreted as not obtained from biological replicates (except for runs 13, 14 and 15), it was possible to observe that *DbCD* gene was downregulated in all the tested conditions with fold-change values ranging between 0.14 and 0.66 (Table 2). The application of the Box-Behnken results to the RSM approach allowed to analyze how the *DbCD* gene expression was

influenced by SO_2, pH and ethanol by predicting further expression values inside the environment of the tested variables. Indeed, as regards the *DbCD* gene expression, a high R-squared values indicated a good fit of the model to the experimental data explaining the 98.3% (R-squared) of the *DbCD* gene variability (Table 5). Main and interaction effects (linear and quadratic) of the factors on the gene expressions are reported in Table 5 and shown in the standardized Pareto chart (Figure 6). While pH and ethanol factors produced a significant effect (*P*-value < 0.01) on the *DbCD* gene expression, SO_2 did not affect it. On the contrary, linear interactions between SO_2 and pH and SO_2 and ethanol revealed a substantial influence (*P*-value < 0.05) (Table 6 and Figure 6) thus concurring to define the response represented as three-dimensional surface (Figure 7a and 7b).

Table 5 Regression equations which fitted to the data of the Box-Behnken experimental design

Variable (*y*)	Regression model equation	R^2 (%)
***DbCD* gene**	*y* = 17.797 - 5.704*A - 8.5935*B – 0.0976963*C + 5.47467*AA + 0.756*AB + 0.159467*AC + 1.05217*BB + 0.0249333*BC – 0.000245926*CC	98.3
***DbVPR* gene**	*y* = 21.2275 – 12.6293*A – 10.3565*B + 0.186785*C + 19.4453*AA + 0.88*AB + 0.4864*AC + 1.32733*BB – 0.0189333*BC – 0.011603*CC	87.3
Vinyl phenol yield	*y* = -0.10787 + 0.203333*A + 0.173333*B - 0.10463*C + 4.34667*AA - 0.56*AB + 0.08*AC - 0.0183333*BB + 0.0106667*BC + 0.00660741*CC	94.5
Vinyl guaiacol yield	*y* = -0.619907 - 0.523333*A + 0.346667*B - 0.0109259*C + 0.773333*AA + 0.0*AB + 0.048*AC - 0.0416667*BB - 0.00266667*BC + 0.00121481*CC	81.2
Ethyl phenol yield	*y* = 2.62032 + 5.05*A - 0.6825*B - 0.161407*C - 2.61333*AA - 1.44*AB + 0.186667*AC + 0.0966667*BB + 0.0173333*BC + 0.00118519*CC	71.8
Ethyl guaiacol yield	*y* = 2.08079 + 4.24667*A - 0.385833*B - 0.060037*C - 2.02667*AA - 1.32*AB + 0.208*AC + 0.0633333*BB - 0.0266667*BC + 0.00645926*CC	78.2

Factors are mol SO_2 (A), pH (B) and Ethanol (C). The second-order equations show main (A, B and C), linear (AB, AC and BC) and quadratic effects (AA, BB and CC). Coefficients are the regression coefficients for the considered variable. R-Squared statistic indicates that the model as fitted explains a certain % of the variability in the considered variable.

The shape of the surface obtained for SO_2 and pH interaction (Figure 7a) on the response reflected the predominant inhibition by pH, since the expression of the gene decrease rapidly up to pH 4. In particular, the change in *DbCD* expression occurring from the lowest to the highest level of pH (Figure 8a) was the same for both 0.125 mg/L and 0.250 mg/L levels of SO_2; the parallel trend of lines indicated that the effect of the pH on the response is probably not dependent from these SO_2 values. Even when pH was in the range 3.5-4 and SO_2 at 0 mg/L, the observed lines were almost parallel with respect to the other lines (with an overlapping between 0 mg/L and 0.250 mg/L of SO_2). On the

contrary, when pH was set between 4 and 4.5 a moderate interaction of this factor with SO_2 occurred (lines are not parallel) (Figure 8a).

Table 6 Statistical analysis (values are expressed as *P*) of main effect of three variables and their interaction for *DbCD* and *DbVPR* expression levels and volatile phenol productions. Bold values are those considered statistically significant (*P* <0.005).

Factor	*DbCD* gene	*DbVPR* gene	Vinyl phenol	Vinyl guaiacol	Ethyl phenol	Ethyl guaiacol
			Yield (µM product/µM consumed acid)			
Mol SO_2 (A)	0.456	0.989	0.3090	0.1805	0.5915	0.5164
pH (B)	**0.003**	0.190	0.4067	0.5201	0.6185	**0.0395**
Ethanol (C)	**0.004**	0.146	**0.0003**	**0.0323**	**0.0283**	0.0934
AA	**0.006**	**0.029**	0.1556	0.3078	0.6576	0.6859
BB	**0.000**	**0.021**	0.9146	0.3727	0.7917	0.8387
CC	0.8635	0.164	0.0710	0.1694	0.8552	0.2733
AB	**0.050**	0.593	0.4111	1.0000	0.3294	0.2973
AC	**0.010**	0.064	0.3810	0.0791	0.3417	0.2277
BC	0.052	0.727	0.6303	0.6456	0.7126	0.5124

On the other hand, the interaction between SO_2 and ethanol produced a response that changed faster as function of ethanol (Figure 7b). In detail, considering ethanol from 5% (v/v) to 12.5% (v/v) and SO_2 at the concentration of 0 mg/L, 0.125 mg/L or 0.250 mg/L, the observed lines were not parallel indicating that an interaction between ethanol and SO_2 exists (Figure 8b). If ethanol at 5% (v/v) interacted with 0.25 mg/L SO_2, the *DbCD* expression was lower than the one revealed by the condition at 0 mg/L SO_2. This is probably due to the effect of ethanol along with SO_2 in determining more stress to the cell. Moreover, the expression at 0 mg/L SO_2 and 8.75% (v/v) ethanol was slightly lower than the one revealed at 0 mg/L SO_2 and 5% (v/v) ethanol.

The comparison between the two interaction plots (Figure 8a and 8b) allowed identifying the SO_2-ethanol as the stronger interaction to define the expression of *DbCD*, as also showed by the *p*-value of this linear interaction (AC, Table 6 and Figure 6).

Finally, based on the response surfaces for *DbCD* gene expression and the model equation it is also possible to predict further responses in addition to those obtained in this study; according to this

prediction approach, the combination of the factor levels that maximizes the *DbCD* expression (0.834 fold-change) is at 0.25 mg/L, 4.5 and 12.5% (v/v), respectively for SO_2, pH and ethanol.

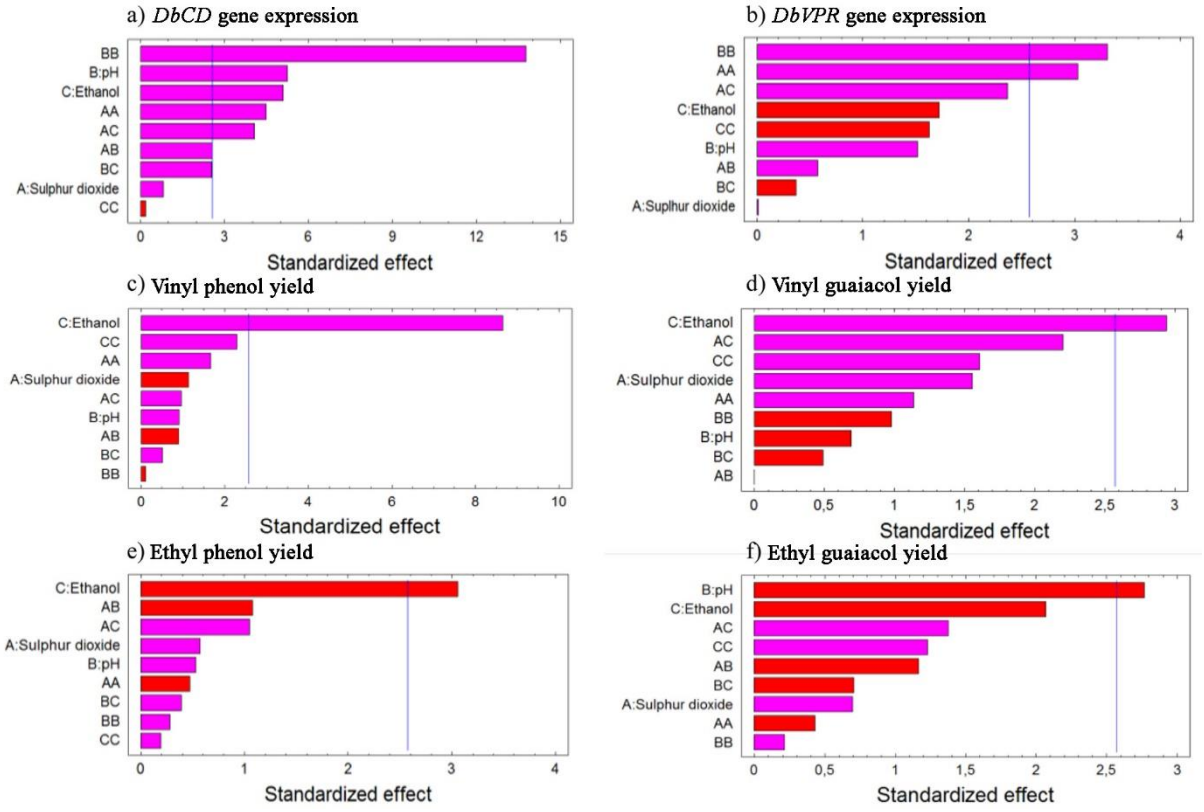

Figure 6 Standardized Pareto charts for each analysed variable [a) *DbCD* and b) *DbVPR* gene expression, c) vinyl phenol, d) vinyl guaiacol, e) ethyl phenol and f) ethyl guaiacol yields]. The color of the bars shows whether an effect is positive (pink) or negative (red). A line is drawn on the chart beyond which an effect is statistically significant at the specified significance level of 5%.

As far the *DbVPR* gene expression, it showed a different trend in regulation in comparison to *DbCD* gene. Even if data of the experimental design cannot be singularly interpreted, *DbVPR* seemed to be upregulated in runs 1, 2, 3, 4, 5, and 10 with fold-change value ranging between 1.11 and 1.80, whereas in the other cases it was slight downregulated, being values lower than 1. Interestingly, following the results in Table 1, although *DbCD* and *DbVPR* genes were expressed at their maximum level under the same growth condition corresponding to SO_2 0.25 mg/L, pH 4.5, ethanol 8.75% (v/v) (run 4). The statistical processing of expression data provided a regression equation of the proposed model with a goodness of fit of 87.3% (R-squared) (Table 5). In this case only a positive quadratic effect of SO_2 and pH resulted statistically significant on the *DbVPR* gene expression being all the other factors, main and interactions, characterized by *p*-values higher than 0.05 (Table 6). In agreement with the RSM approach, *DbVPR* expression was maximizes (1.80 fold-change) at 0.25 mg/L SO_2, pH 4.5 and 12.5% (v/v) ethanol, as observed for the *DbCD*.

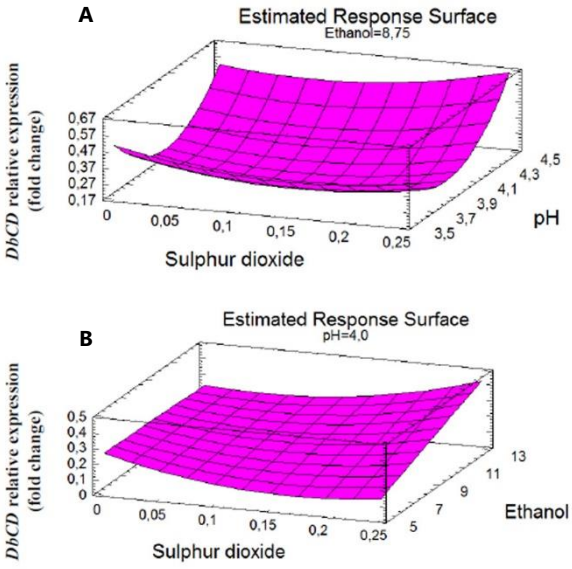

Figure 7 Response surface fitted to experimental data points corresponding to: **(A)** *Db*CD expression as function of SO_2 and pH interaction (AB); **(B)** *Db*CD expression as function of SO_2 and ethanol interaction (AC).

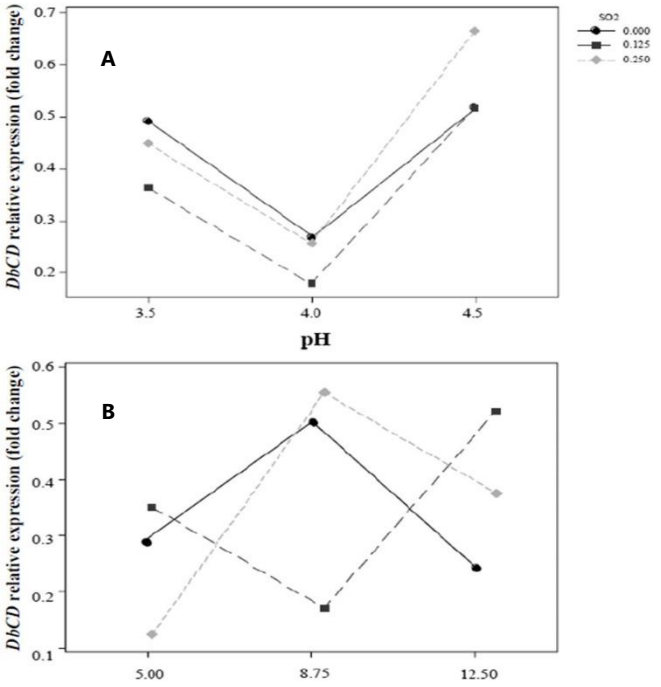

Figure 8 Interaction plots for the expression of *DbCD* gene. **(A)** SO_2 and pH (AB); **(B)** SO_2 and ethanol concentration (AC). Lines represent the predicted responses at further experimental combinations among the analyzed factors. Continuous line (•), 0 mg/L mol. SO_2; long-dashed line (■), 0.125 mg/L mol. SO_2; short-dashed line (♦),0.25 mg/L mol. SO_2.

3.2.4 Effect of SO_2, pH and ethanol on VP production

The release of VPs was determined in the experimental conditions adopted in the Box-Behnken experimental design. Although 10 mg/L of each hydroxycinnamic acid were added to the SWM, the initial concentrations of p-coumaric acid and ferulic acid were estimated at 8.40±0.07 mg/L and 6.71±0.25 mg/L, respectively. As expected, these compounds proportionally decreased as the VPs increased (data not shown). The highest concentration of VPs was reached under a condition that is more permissive the yeast growth [SO_2 0.125 mg/L, pH 3.5 and ethanol 5% (v/v)] in comparison to the expression of *DbCD* and *DbVPR* genes [SO_2 0.25 mg/L, pH 4.5 and ethanol 12.5% (v/v)]. Indeed, VPs are released at a final concentration of 6.45 and 5.18 mg/L of ethyl phenol and ethyl guaiacol, respectively, in run 9 whereas *DbCD* and *DbVPR* genes were approximatively half of the expression values detected in run 4.

In general, some considerations arose from the calculated yields of VPs (Table 2). First, the lowest conversion of acids in the corresponding vinyl compounds was detected for the vinyl guaiacol that was mostly produced at trace level in all the analyzed runs (Table 2). We could speculate that this behavior could be linked to a higher activity of *Db*CDp toward the cumaric acid rather than the ferulic

acid. On the contrary, ethyl phenol and ethyl guaiacol yields were found relatively balanced each other suggesting a similar capability of the *DbVPR* enzyme to transform its two substrates, the vinyl derivates. However, for this observation studies are required to analyze the activity of *Db*CDp and *Db*VPRp in the metabolic pathway of VPs under enological conditions.

Data processing by the RSM approach released four second-order equations with R-Squared values indicating that the model as fitted explained 94.5%, 81.2%, 71.8% and 78.2% of the variability in vinyl phenol, vinyl guaiacol, ethyl phenol and ethyl guaiacol molar ratios, respectively (calculated against the corresponding substrates of hydroxycinnamic acids). Main and interaction effects (linear and quadratic) of the factors on the VP production are reported in Table 6 and shown in the standardized Pareto charts (Figure 2). Considering the influence of individual factors, ethanol and pH produced a significant effect (*P*-value < 0.05) on the production of such aromatic compounds whereas SO_2 did not result involved in. In particular, ethanol influenced the release of vinyl phenol, ethyl phenol and vinyl guaiacol while pH was important in determining the variability of ethyl guaiacol. No linear interaction between factors resulted statistically significant for the synthesis of VPs.

3.3 SO_2 stress resistance in *Brettanomyces/Dekkera bruxellensis*: investigation at transcriptome level

3.3.1 Growth behavior

Optical density (OD) was measured daily until 1±0.1 OD was reached, and the stress factor added. Optical density evolution and plating results are shown in Fig. 9. As it is possible to observe in the figure, growth profile of AWRI1499_1 recovered later than the other fermentations of the same set of experiments. However, taking into account that all replicates were carried out simultaneously, it was considered a reliable repetition. As it is possible to see, almost immediately after the addition of SO_2, growth got stuck. A slight difference between the two strains here investigated was observed immediately after the stress was applied: OD in the AWRI1499 start to decrease faster than in the other strain, where at T5h the optical density was still slightly increasing. As far the all count results, both yeasts had a drop in the culturability after cells were exposed to the stress. At T5h, culturability decreased of about 2 orders of magnitude, and in later time points the culturability felt from 10^7 up to $10^4/10^2$, depending on the replicate. Cell biomass slowly decreased over 5 days/one week, except for one replicate of AWRI1499 strain where the lag-phase took 4-5 days more. After this "latency" period, OD started to increase again likewise the culturability. In the "late period" after the SO_2 addition, the evaluation of glucose and fructose quantity revealed a possible difference in sugars consumption by the two strains. Before the all recovered, for AWRI1499 strain 0.5 g/L of glucose were still available in the media, and 2 g/L of fructose were remaining still. On the contrary, for CBS2499 strain both sugars were consumed, with glucose being almost completely exhausted and fructose concentration resulting in 1 g/L.

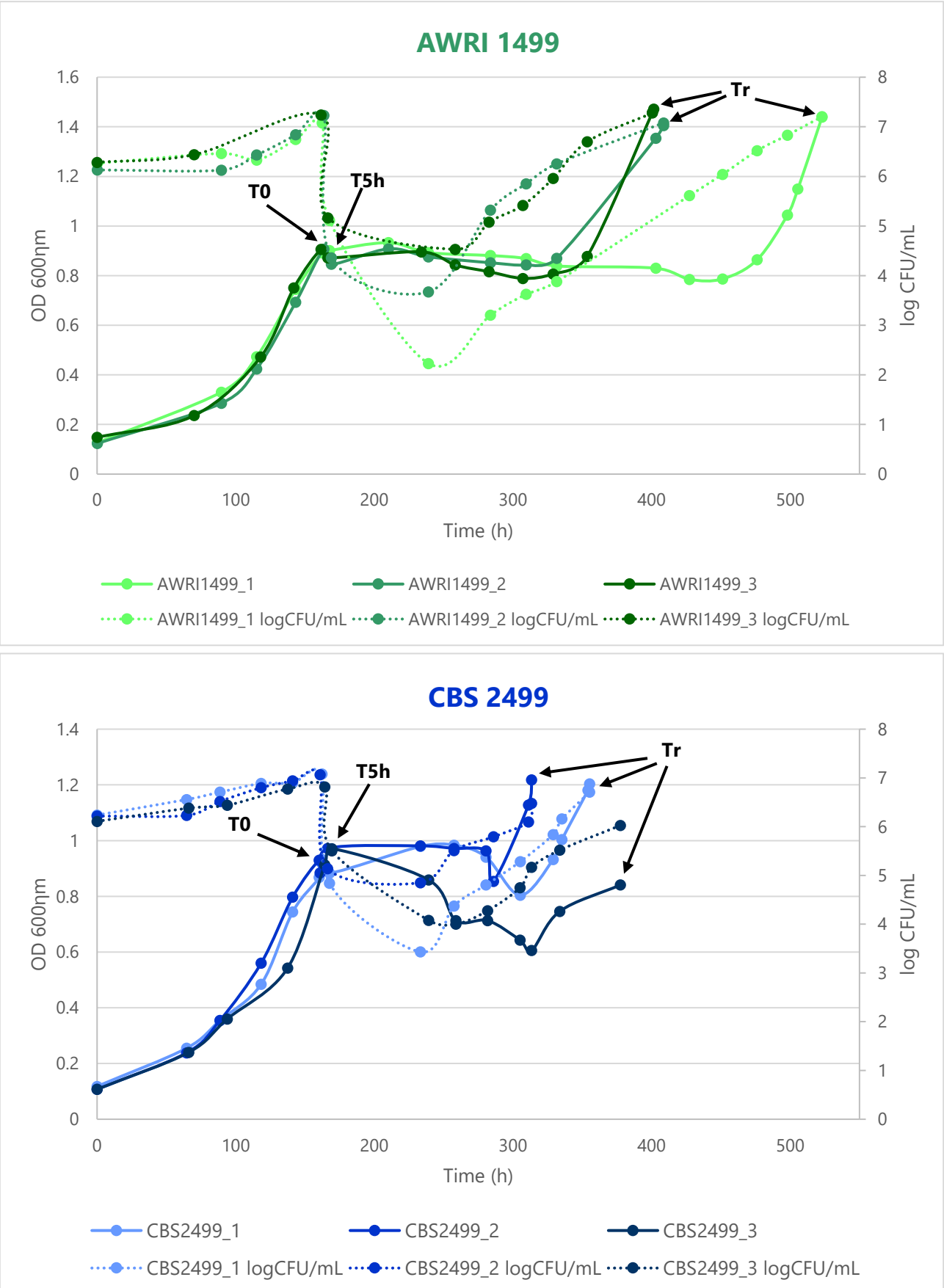

Fig. 9 Graphical representation of growth curves of AWRI1499 and CBS2499 *Brettanomyces bruxellensis* strains in SWM medium (10% of Ethanol, pH 3.5) under O_2 limitation. Sampling time indicated: T0 (sample before SO_2 addition), T5h (sample 5h after pulse with sulphur dioxide), Tr (samples after cell recovered).

3.3.2 Transcriptomic variation in response to SO_2 stress

In order to evaluate the genetic mechanisms activated by *B. bruxellensis* to counteract the stress caused by the addiction of sulphur dioxide to the wine, a RNA-seq approach was applied. Three sample types, untreated cells (T0), cells collected 5 hours after the SO_2 pulse (T5h) and once cells recovered (Tr) were analysed. All sequencing results submitted to the quality analysis resulted, as it is possible to see in the example reported in Fig. 10, of good quality, being the mean quality score (blue line in the figure) higher than 30.

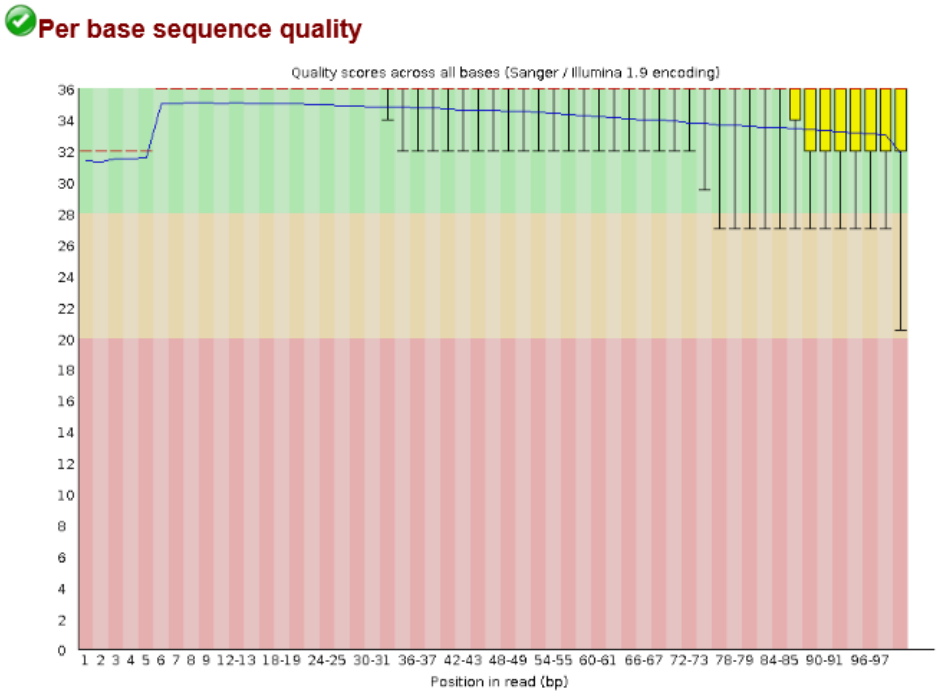

Fig. 10 Graphical representation of "per base sequence quality".

For each position a BoxWhisker type plot is drawn. The elements of the plot are as follows:

- The red line is the median value;
- The yellow box represents the inter-quartile range (25-75%);
- The upper and lower whiskers represent the 10% and 90% points;
- The blue line represents the mean quality.

The y-axis on the graph shows the quality scores. The higher the score the better the base call. The background of the graph divides the y axis into three quality calls-different area: very good quality calls (green), calls of reasonable quality (orange), and calls of poor quality (red). The quality of calls on most platforms will degrade as the run progresses, so it is common to see base calls falling into the orange area towards the end of a read.

A PCA analysis has been conducted on TPM value and dispersion of the samples is reported in Fig. 11.

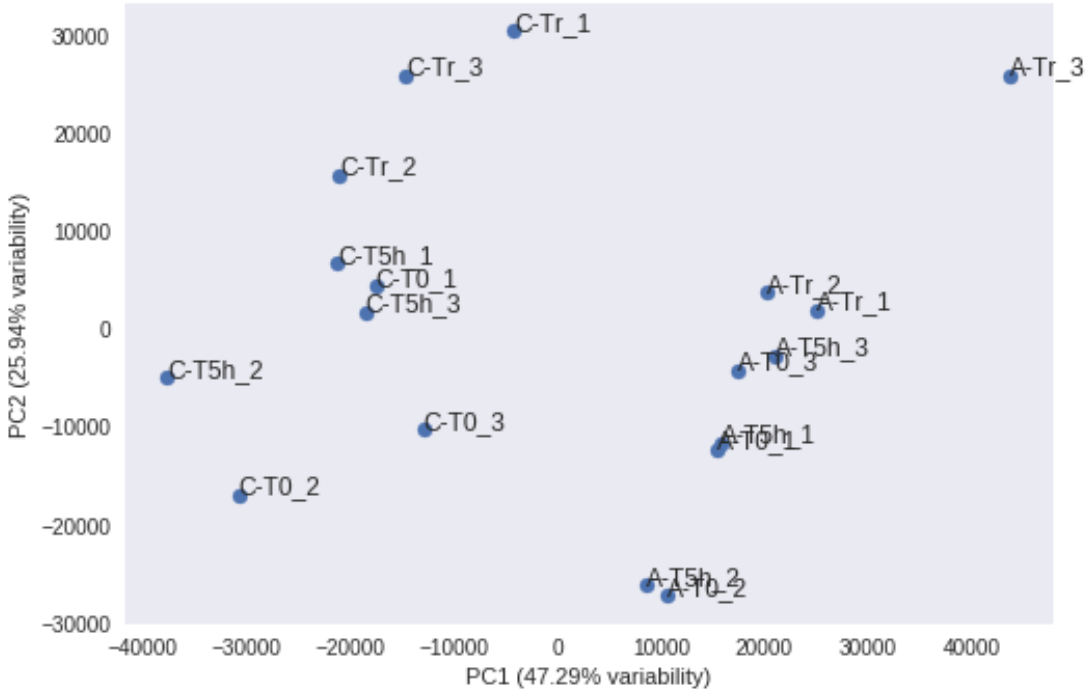

Fig. 11 Pincipal Component Analysis on TPM value. In the code of the samples is reported the strain (A= AWRI1499, C=CBS2499), time point (T0=untreated cells, collected immediately before the SO_2 addition, T5h=sample collected 5h after the pulse, Tr=cells collected at the recovery phase). "_1/2/3" represent the sample replicates.

The analysis covered almost the 70% of the variability, with more than 47% explained by component 1 and about 26% by component 2, for a total explanation around 73%. Strains were completely differentiated on the base of component 1.

Global transcriptional changes, later mentioned as a "short" or a "long" response, have been evaluated comparing transcriptomes from cells collected at T5h and Tr time points with untreated (T0) cells. As far the AWRI1499 strain (A) 4855 genes were expressed at T0, 4854 at T5h and 4851 at Tr, while for CBS2499 strain (C), the genes found expressed were 4835, 4834 and 4836, respectively. The number of genes showing a significant change, statistically based, in the expression (Differentially Expressed Genes, DEG), is shown in Table 7. In bracket is reported the number of those genes with an absolute log_2fold-change (log_2FC) greater than 1. For the long response comparison is also reported the number of genes having a correspondant homolog in the reference yeast genome, *S. cerevisiae*. The outcome showed only few genes significantly changing the expression in the short time response, and mainly down-regulated, while in the long response a higher number of genes resulted significantly up- or down- regulated.

Table 7 Number of genes with log_2fold-change value significant. In bracket is reported the number of DEG having an absolute log_2fold-change greater than 1.

	T5h-vs-T0 (short response)		Tr-vs-T0 (long response)			
			AWRI1499-identifiers		*S. cerevisiae* homolog	
	UP	DOWN	UP	DOWN	UP	DOWN
AWRI1499	19 (n.d.)	149 (3)	571 (170)	573 (138)	448(126)	425(96)
CBS2499	2 (n.d.)	7 (1)	536 (107)	549 (85)	387(81)	388(52)

3.3.2.1 Significant Up- and Down-regulated genes and relative category terms enriched within AWRI1499 and CBS2499 strains

In strain AWRI1499, nine of the Significantly Up-Regulated Genes (SURGs) in the short response were still up-regulated in the long response, but none with a log_2FC above 1. Regarding the Significant Down-Regulated Genes (SDRGs), out of the 149 found (Table 7), 45 were still down-regulated in the long response. As far the CBS2499 strain, only one (AWRI1499_1038) of the two SURGs was still significant in the long response SURGs, while 3 out of 7 SDRGs maintained the significant down-regulation.

The list of all the AWRI genes resulted significantly differentially expressed (up or down regulated) with the correspondent log_2fold-change value and the annotation to *S. cerevisiae* genome, for both strains and time comparison is reported in Appendix 2. In Table 8 are reported the genes with a log_2fold-change greater than |1|.

Table 8 Statistically differentially expressed genes with log_2fold-change≥|1|, separate for strain and time response. Up-regulated genes were colored with yellow, down-regulated genes with blue. When the regulation was greater (absolute fold-change values being above 4, log_2FC≥|2|) dark yellow and dark blue were used for highly up-regulated and highly down-regulated genes, respectively.

Strain and comparison time	**log_2(FC)**	**AWRI identifiers**	**Protein name**	***S. cerevisiae* homologous gene (Standard name)**
A-T5h	-1.07	AWRI1499_3326	protein kinase and ribonuclease	*IRE1*
A-T5h	-1.10	AWRI1499_1252	pcl1p	*PCL1*
A-T5h	-1.11	AWRI1499_2222	hypothetical protein AWRI1499_2222	
A-Tr	7.05	AWRI1499_1841	ynr018w-like protein	*RCF2*
A-Tr	6.13	AWRI1499_1936	alpha-glucoside permease	*MPH2*
A-Tr	5.57	AWRI1499_0080	membrane transporter	*SSU1*
A-Tr	4.43	AWRI1499_3505	sorbitol dehydrogenase	*SOR1*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	3.94	AWRI1499_4725	high-affinity glucose transporter	*HXT13*
A-Tr	3.92	AWRI1499_3717	anaerobic ribonucleoside-triphosphate reductase	
A-Tr	3.89	AWRI1499_4728	gal10 bifunctional protein	*GAL10*
A-Tr	3.74	AWRI1499_4351	isomaltase	*IMA1*
A-Tr	3.36	AWRI1499_0109	hxk2p	*HXK1*
A-Tr	3.26	AWRI1499_0089	family integral membrane protein	
A-Tr	3.24	AWRI1499_2435	forkhead box protein l2	
A-Tr	3.22	AWRI1499_2713	glycerol dehydrogenase	*BDH1*
A-Tr	3.20	AWRI1499_4733	beta-glucosidase	
A-Tr	3.20	AWRI1499_3271	neutral trehalase	*NTH1*
A-Tr	3.14	AWRI1499_3429	hypothetical protein AWRI1499_3429	
A-Tr	3.11	AWRI1499_4884	maltase	*IMA1*
A-Tr	3.11	AWRI1499_4727	galactokinase	*GAL1*
A-Tr	3.02	AWRI1499_4109	acetyl- hydrolase	*ACH1*
A-Tr	2.99	AWRI1499_3210	phosphoglucomutase	*PGM2*
A-Tr	2.93	AWRI1499_2202	glycogen synthase	*GSY1*
A-Tr	2.93	AWRI1499_0332	succinyl- ligase alpha- mitochondrial precursor	*LSC1*
A-Tr	2.90	AWRI1499_4832	putative carbohydrate kinase	*YDR109C*
A-Tr	2.90	AWRI1499_3771	succinyl-CoA ligase beta-chain, mitochondrial precursor	*LSC2*
A-Tr	2.87	AWRI1499_1045	gata-type sexual development transcription factor	*GAT2*
A-Tr	2.87	AWRI1499_1608	respiratory growth induced protein 1	*RGI2*
A-Tr	2.82	AWRI1499_4172	hexaprenyldihydroxybenzoate methyltransferase	
A-Tr	2.80	AWRI1499_4194	oleate-induced peroxisomal protein	
A-Tr	2.76	AWRI1499_2432	inositol 1-phosphate synthase	*INO1*
A-Tr	2.64	AWRI1499_1615	peroxisomal hydratase-dehydrogenase-epimerase	*FOX2*
A-Tr	2.57	AWRI1499_4297	atpase-stabilizing factor 15 kda protein	*TMA10*
A-Tr	2.53	AWRI1499_0430	alcohol dehydrogenase	*ADH3*
A-Tr	2.52	AWRI1499_0599	6-phosphofructo-2-kinase, putative	*YLR345W*
A-Tr	2.52	AWRI1499_4492	dihydrolipoamide dehydrogenase -binding protein	*PDX1*
A-Tr	2.49	AWRI1499_2013	dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex	*LAT1*
A-Tr	2.47	AWRI1499_2863	nad-dependent epimerase dehydratase	*YLL056C*
A-Tr	2.43	AWRI1499_1614	peroxisomal hydratase-dehydrogenase-epimerase	*FOX2*
A-Tr	2.40	AWRI1499_2608	pdb1p	*PDB1*
A-Tr	2.34	AWRI1499_3343	high-affinity copper transporter of the plasma membrane	*CTR1*
A-Tr	2.31	AWRI1499_0623	s-formylglutathione hydrolase	*YJL068C*
A-Tr	2.27	AWRI1499_1678	hypothetical protein AWRI1499_1678	
A-Tr	2.10	AWRI1499_2466	gluconolactonase ly involved in growth regulation	*YBR053C*
A-Tr	2.10	AWRI1499_2002	mitochondrial carrier protein leu5	*LEU5*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	2.10	AWRI1499_3162	e1 alpha subunit of the pyruvate dehydrogenase complex	*PDA1*
A-Tr	2.08	AWRI1499_0238	oxidoreductase domain protein	*YMR315W*
A-Tr	2.04	AWRI1499_3824	medium chain alcohol dehydrogenase	*ADH7*
A-Tr	2.04	AWRI1499_1840	hypothetical protein AWRI1499_1840	
A-Tr	2.03	AWRI1499_2675	glucosidase ii catalytic subunit required for normal cell wall synthesis	*ROT2*
A-Tr	2.03	AWRI1499_1545	transcriptional activator	*VHR1*
A-Tr	2.02	AWRI1499_2876	nrma-like protein	
A-Tr	1.98	AWRI1499_4726	galactose-1-phosphate uridylyltransferase	*GAL7*
A-Tr	1.98	AWRI1499_3675	hexaprenyldihydroxybenzoate methyltransferase	
A-Tr	1.98	AWRI1499_1318	lactose permease	*HXT13*
A-Tr	1.95	AWRI1499_0088	yol022c-like protein	*TSR4*
A-Tr	1.94	AWRI1499_1937	sucrose-6-phosphate hydrolase	*SUC2*
A-Tr	1.93	AWRI1499_1209	sco1p	*SCO1*
A-Tr	1.92	AWRI1499_2674	alpha-glucosidase catalytic	*ROT2*
A-Tr	1.91	AWRI1499_2717	glycerol dehydrogenase	*BDH1*
A-Tr	1.91	AWRI1499_4231	phosphatidyl synthase	*YKR070W*
A-Tr	1.90	AWRI1499_3802	adh6p	*ADH6*
A-Tr	1.90	AWRI1499_4413	rna binding protein	*RTC3*
A-Tr	1.88	AWRI1499_4046	iron copper transporter	*ATX1*
A-Tr	1.84	AWRI1499_4456	putative ldg family protein 7	
A-Tr	1.82	AWRI1499_0804	lipoate-protein ligase a family member	
A-Tr	1.79	AWRI1499_1315	beta-mannosidase precursor	
A-Tr	1.78	AWRI1499_3058	hypothetical protein AWRI1499_3058	
A-Tr	1.77	AWRI1499_1126	hypothetical protein AWRI1499_1126	
A-Tr	1.77	AWRI1499_3222	hypothetical protein AWRI1499_3222	
A-Tr	1.76	AWRI1499_1316	beta-mannosidase precursor	
A-Tr	1.75	AWRI1499_0110	hypothetical protein AWRI1499_0110	
A-Tr	1.74	AWRI1499_0515	protein phosphatase regulatory subunit	*PIG2*
A-Tr	1.73	AWRI1499_0040	mannosyl-oligosaccharide glucosidase	*CWH41*
A-Tr	1.71	AWRI1499_4880	beta-galactosidase	
A-Tr	1.69	AWRI1499_2771	succinate-semialdehyde dehydrogenase	*UGA2*
A-Tr	1.69	AWRI1499_4196	polyadenylate-binding protein	*PAB1*
A-Tr	1.67	AWRI1499_3896	1,4-alpha-glucan branching enzyme	*GLC3*
A-Tr	1.67	AWRI1499_2897	glutathione reductase	*GLR1*
A-Tr	1.66	AWRI1499_4419	dicarboxylic amino acid permease	*DIP5*
A-Tr	1.66	AWRI1499_3238	permease of basic amino acids in the vacuolar membrane	*VBA4*
A-Tr	1.65	AWRI1499_4451	ygl080w-like protein	*FMP37*
A-Tr	1.65	AWRI1499_1406	hypothetical protein AWRI1499_1406	

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	1.63	AWRI1499_3716	tip1p	*TIP1*
A-Tr	1.62	AWRI1499_0997	ubiquitin-protein ligase e3	*ETP1*
A-Tr	1.61	AWRI1499_4965	ion transporter	*HOL1*
A-Tr	1.61	AWRI1499_2758	putative spindle-pole body protein	*SDS24*
A-Tr	1.61	AWRI1499_3239	putative mfs-mdr transporter	*VBA1*
A-Tr	1.60	AWRI1499_4516	duf833 domain-containing protein	*YGR127W*
A-Tr	1.60	AWRI1499_0692	subunit of a heterodimeric peroxisomal atp-binding cassette transporter complex (pxa1p-pxa2p)	*PXA1*
A-Tr	1.60	AWRI1499_2006	nad(+) salvage pathway protein	*PNC1*
A-Tr	1.56	AWRI1499_3043	duf1237 domain protein	
A-Tr	1.56	AWRI1499_1726	lip2p	*LIP2*
A-Tr	1.53	AWRI1499_2089	putative translation initiation factor subunit	*CLU1*
A-Tr	1.52	AWRI1499_2862	glycerol kinase	*GUT1*
A-Tr	1.51	AWRI1499_0803	hypothetical protein AWRI1499_0803	
A-Tr	1.50	AWRI1499_4350	ymr315w-like protein	*YMR315W*
A-Tr	1.49	AWRI1499_0998	ring finger protein	*ETP1*
A-Tr	1.48	AWRI1499_3431	ubiquitin-conjugating enzyme e2g 2	*UBC7*
A-Tr	1.48	AWRI1499_1003	mls1	*MLS1*
A-Tr	1.46	AWRI1499_2040	2-enoyl thioester reductase	*ETR1*
A-Tr	1.46	AWRI1499_2548	zinc cluster transcriptional activator	*CAT8*
A-Tr	1.42	AWRI1499_4966	mannosyl phosphorylinositol ceramide synthase sur1	*SUR1*
A-Tr	1.42	AWRI1499_3511	acyl-coenzyme a oxidase 4	*POX1*
A-Tr	1.41	AWRI1499_3221	hypothetical protein AWRI1499_3221	
A-Tr	1.40	AWRI1499_2234	protein mida mitochondrial	
A-Tr	1.38	AWRI1499_2547	zinc cluster transcriptional activator	*CAT8*
A-Tr	1.37	AWRI1499_0441	yhr131c-like protein	*YNL144C*
A-Tr	1.37	AWRI1499_4081	dihydroxyacetone kinase	*DAK2*
A-Tr	1.37	AWRI1499_4242	mfs efflux	*BSC6*
A-Tr	1.34	AWRI1499_4368	phosphoenolpyruvate carboxykinase	*PCK1*
A-Tr	1.34	AWRI1499_3059	ada regulatory protein o6-methylguanine-dna methyltransferase	
A-Tr	1.33	AWRI1499_0320	putative mitochondrial protein fmp35	*COA1*
A-Tr	1.33	AWRI1499_2321	short chain dehydrogenase	
A-Tr	1.32	AWRI1499_4430	hypothetical protein AWRI1499_4430	*FIG2*
A-Tr	1.31	AWRI1499_2476	mitochondrial peculiar membrane protein 1	*MPM1*
A-Tr	1.30	AWRI1499_3136	low affinity glucose transporter	*HXT6*
A-Tr	1.29	AWRI1499_3014	adh3p	*ADH3*
A-Tr	1.29	AWRI1499_2467	nascent polypeptide-associated complex subunit beta	
A-Tr	1.28	AWRI1499_0987	hypothetical protein AWRI1499_0987	

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	1.26	AWRI1499_0098	ymr027w-like protein	*YMR027W*
A-Tr	1.25	AWRI1499_0715	hypothetical protein AWRI1499_0715	
A-Tr	1.25	AWRI1499_4255	ybr269c-like protein	*SDH8*
A-Tr	1.24	AWRI1499_4369	phosphoenolpyruvate carboxykinase	*PCK1*
A-Tr	1.24	AWRI1499_2733	carbohydrate kinase, putative	*YKL151C*
A-Tr	1.21	AWRI1499_3558	succinate dehydrogenase flavoprotein subunit	*SDH1*
A-Tr	1.21	AWRI1499_1474	yjr096w-like protein	*YJR096W*
A-Tr	1.21	AWRI1499_3584	protoplast secreted protein 2 precursor	*PST2*
A-Tr	1.21	AWRI1499_0121	c6 transcription factor	*STB5*
A-Tr	1.20	AWRI1499_3825	adh6p, partial	*ADH6*
A-Tr	1.20	AWRI1499_3682	mitochondrial tryptophanyl-trna synthetase	*MSW1*
A-Tr	1.19	AWRI1499_1368	abhydrolase domain-containing protein	*IMO32*
A-Tr	1.19	AWRI1499_1715	glycogen phosphorylase	*GPH1*
A-Tr	1.19	AWRI1499_0483	trna(1-methyladenosine) methyltransferase subunit	
A-Tr	1.18	AWRI1499_0511	homocysteine s-methyltransferase	*MHT1*
A-Tr	1.18	AWRI1499_0463	hypothetical protein AWRI1499_0463	
A-Tr	1.18	AWRI1499_2554	malate nad-dependent	*MDH1*
A-Tr	1.18	AWRI1499_2105	hypothetical protein AWRI1499_2105	
A-Tr	1.18	AWRI1499_4241	mfs efflux	*BSC6*
A-Tr	1.18	AWRI1499_3615	sol3p	*SOL3*
A-Tr	1.17	AWRI1499_3758	hypothetical protein AWRI1499_3758	
A-Tr	1.17	AWRI1499_0604	hypothetical protein AWRI1499_0604	*REG1*
A-Tr	1.16	AWRI1499_0104	protoporphyrinogen oxidase	*HEM14*
A-Tr	1.16	AWRI1499_3169	hypothetical protein AWRI1499_3169	*HXT7*
A-Tr	1.16	AWRI1499_4820	hypothetical protein AWRI1499_4820	
A-Tr	1.15	AWRI1499_3820	hypothetical protein AWRI1499_3820	
A-Tr	1.15	AWRI1499_2294	pyruvate dehydrogenase kinase	*PKP1*
A-Tr	1.15	AWRI1499_1904	emi2p	*GLK1*
A-Tr	1.14	AWRI1499_4414	protein mitochondrial precursor	*MGM1*
A-Tr	1.12	AWRI1499_0934	hypothetical protein AWRI1499_0934	
A-Tr	1.12	AWRI1499_4250	isocitrate lyase	*ICL1*
A-Tr	1.12	AWRI1499_1296	hypothetical protein AWRI1499_1296	
A-Tr	1.11	AWRI1499_4934	d-arabinitol 2-dehydrogenase	*SPS19*
A-Tr	1.11	AWRI1499_3209	aspartate aminotransferase	*AAT2*
A-Tr	1.11	AWRI1499_3590	protein ure2	*URE2*
A-Tr	1.10	AWRI1499_0018	hypothetical protein AWRI1499_0018	
A-Tr	1.10	AWRI1499_1171	mitochondrial distribution and morphology protein 31	*MDM31*
A-Tr	1.09	AWRI1499_0479	glycerol-3-phosphate dehydrogenase	*GPD1*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	1.09	AWRI1499_4659	lipoic acid mitochondrial precursor	*LIP5*
A-Tr	1.08	AWRI1499_2233	protein mida mitochondrial	
A-Tr	1.07	AWRI1499_4522	hypothetical protein AWRI1499_4522	
A-Tr	1.07	AWRI1499_3936	putative proteasome-interacting protein	*NAS6*
A-Tr	1.06	AWRI1499_3589	ybr096w-like protein	*YBR096W*
A-Tr	1.06	AWRI1499_2995	ygr110w-like protein	*CLD1*
A-Tr	1.05	AWRI1499_2515	hypothetical protein AWRI1499_2515	*TIP1*
A-Tr	1.04	AWRI1499_1366	glycogen synthase kinase	*RIM11*
A-Tr	1.03	AWRI1499_3135	hexose transporter	*GAL2*
A-Tr	1.03	AWRI1499_0598	hypothetical protein AWRI1499_0598	
A-Tr	1.02	AWRI1499_3786	nadh-cytochrome b-5 reductase	*CBR1*
A-Tr	1.01	AWRI1499_4861	ydl025c-like protein	*RTK1*
A-Tr	1.01	AWRI1499_4106	fad dependent oxidoreductase superfamily	
A-Tr	1.00	AWRI1499_3478	zn2+-dependent endopeptidase	*CYM1*
A-Tr	1.00	AWRI1499_1227	protein serine threonine kinase expressed at the end of meiosis	
A-Tr	-1.00	AWRI1499_4360	allantoate permease	*THI73*
A-Tr	-1.00	AWRI1499_1356	ribosome biogenesis protein bms1	*BMS1*
A-Tr	-1.02	AWRI1499_1873	protein bfr2	*BFR2*
A-Tr	-1.02	AWRI1499_1099	phosphatidylserine decarboxylase	*PSD1*
A-Tr	-1.02	AWRI1499_1159	putative u3 snornp protein utp6p	*UTP6*
A-Tr	-1.02	AWRI1499_2034	msg5p	*MSG5*
A-Tr	-1.03	AWRI1499_1269	u3 small nucleolar rna-associated protein	*UTP15*
A-Tr	-1.03	AWRI1499_2727	putative dead box atp-dependent rna helicase	*DBP10*
A-Tr	-1.03	AWRI1499_3372	putative u3 snornp protein utp20p	*UTP20*
A-Tr	-1.03	AWRI1499_4776	5-aminolevulinate mitochondrial precursor	*HEM1*
A-Tr	-1.03	AWRI1499_1483	constituent of 66s pre-ribosomal required for large ribosomal subunit biogenesis	*MAK21*
A-Tr	-1.04	AWRI1499_2051	purine-cytosine permease	*FCY2*
A-Tr	-1.04	AWRI1499_0464	hypothetical protein AWRI1499_0464	
A-Tr	-1.04	AWRI1499_2952	hypothetical protein AWRI1499_2952	
A-Tr	-1.04	AWRI1499_1796	periodic tryptophan protein 2	*PWP2*
A-Tr	-1.04	AWRI1499_2305	enp2p	*ENP2*
A-Tr	-1.04	AWRI1499_4418	acid phosphatase phoa	
A-Tr	-1.05	AWRI1499_4095	fungal-specific transcription factor	
A-Tr	-1.05	AWRI1499_4754	amino acid permease	*UGA4*
A-Tr	-1.05	AWRI1499_3115	putative trna dihydrouridine synthase	*DUS3*
A-Tr	-1.05	AWRI1499_1198	uracil phosphoribosyltransferase	*FUR1*
A-Tr	-1.06	AWRI1499_3814	putative nadh-dependent flavin oxidoreductase	*OYE2*
A-Tr	-1.06	AWRI1499_3079	protein mak16	*MAK16*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	-1.06	AWRI1499_0041	protein involved in the transcription of 35s rrna genes by rna polymerase i	*RRN6*
A-Tr	-1.06	AWRI1499_2837	fact complex subunit ssrp1	
A-Tr	-1.07	AWRI1499_1954	deoxyhypusine synthase	*DYS1*
A-Tr	-1.08	AWRI1499_0712	hypothetical protein AWRI1499_0712	
A-Tr	-1.08	AWRI1499_4252	mrna export factor elf1	*NEW1*
A-Tr	-1.08	AWRI1499_1695	dynein-related aaa-type	*MDN1*
A-Tr	-1.08	AWRI1499_4626	general amino acid permease	*GAP1*
A-Tr	-1.09	AWRI1499_4126	ydl144c-like protein	*YDL144C*
A-Tr	-1.10	AWRI1499_4879	hypothetical protein AWRI1499_4879	
A-Tr	-1.10	AWRI1499_1006	putative rrna methyltransferase	*RRP8*
A-Tr	-1.11	AWRI1499_4361	allantoate permease	*THI73*
A-Tr	-1.11	AWRI1499_1638	nucleolar component of the small subunit processome containing the u3 snorna	*UTP10*
A-Tr	-1.12	AWRI1499_1000	part of small ribosomal subunit processosome (contains u3 snorna)	*RRP5*
A-Tr	-1.12	AWRI1499_2369	x-pro dipeptidyl-peptidase family protein	
A-Tr	-1.13	AWRI1499_4359	hypothetical protein AWRI1499_4359	
A-Tr	-1.14	AWRI1499_4507	ymr259c-like protein	*TRM732*
A-Tr	-1.14	AWRI1499_4708	maltose permease	*MAL31*
A-Tr	-1.14	AWRI1499_4796	outer membrane autotransporter	
A-Tr	-1.14	AWRI1499_3074	ydl063c-like protein	*SYO1*
A-Tr	-1.16	AWRI1499_0532	putative zinc finger protein	
A-Tr	-1.16	AWRI1499_3373	putative u3 snornp protein utp20p	*UTP20*
A-Tr	-1.16	AWRI1499_3038	inosine-5 -monophosphate dehydrogenase imd2	*IMD4*
A-Tr	-1.17	AWRI1499_2317	hypothetical protein AWRI1499_2317	
A-Tr	-1.17	AWRI1499_3534	conserved fungal protein	
A-Tr	-1.17	AWRI1499_3913	electron transfer flavoprotein-ubiquinone mitochondrial precursor	*CIR2*
A-Tr	-1.17	AWRI1499_0870	delta 1-pyrroline-5-carboxylate reductase	*PRO3*
A-Tr	-1.18	AWRI1499_0750	u3 small nucleolar rna-associated u3 snornp	*NAN1*
A-Tr	-1.18	AWRI1499_1863	putative pyridoxine transport protein	*TPN1*
A-Tr	-1.19	AWRI1499_2912	essential nucleolar protein that is a component of the ssu (small subunit) processome	*UTP23*
A-Tr	-1.20	AWRI1499_1896	hypothetical protein AWRI1499_1896	
A-Tr	-1.20	AWRI1499_3720	hypothetical protein AWRI1499_3720	*FLO1*
A-Tr	-1.21	AWRI1499_4438	gtp-binding rho subfamily	*JIP5*
A-Tr	-1.21	AWRI1499_2388	cysteine transporter	*YCT1*
A-Tr	-1.21	AWRI1499_3399	gamma-aminobutyric acid transporter	*UGA4*
A-Tr	-1.22	AWRI1499_4136	hypothetical protein AWRI1499_4136	
A-Tr	-1.23	AWRI1499_0976	nucleolar protein	*NOP4*
A-Tr	-1.23	AWRI1499_3917	activator of forkhead-like transcription factor	*IFH1*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	-1.24	AWRI1499_2936	hypothetical protein AWRI1499_2936	
A-Tr	-1.26	AWRI1499_3527	purine nucleoside permease	
A-Tr	-1.26	AWRI1499_4323	5-oxoprolinase	*OXP1*
A-Tr	-1.27	AWRI1499_1519	putative dioxygenase	
A-Tr	-1.28	AWRI1499_2827	hypothetical protein AWRI1499_2827, partial	
A-Tr	-1.28	AWRI1499_1082	serine threonine protein kinase	
A-Tr	-1.28	AWRI1499_2004	plasma membrane atp-binding cassette transporter	*YOR1*
A-Tr	-1.29	AWRI1499_3916	activator of forkhead-like transcription factor	
A-Tr	-1.30	AWRI1499_2138	ybr238c-like protein	*YBR238C*
A-Tr	-1.31	AWRI1499_4317	flavin-containing localized to the cytoplasmic face of the er membrane	*FMO1*
A-Tr	-1.31	AWRI1499_4461	class iii	*ARG8*
A-Tr	-1.32	AWRI1499_1563	indoleamine -dioxygenase subfamily	*BNA2*
A-Tr	-1.33	AWRI1499_0294	adenosine deaminase	
A-Tr	-1.36	AWRI1499_4897	tkp3 protein	*YGR109W-B*
A-Tr	-1.36	AWRI1499_3879	atp sulfurylase	*MET3*
A-Tr	-1.38	AWRI1499_4425	hypothetical protein AWRI1499_4425	
A-Tr	-1.39	AWRI1499_0462	homoserine o-acetyltransferase	*MET2*
A-Tr	-1.40	AWRI1499_2082	gtp cyclohydrolase ii	
A-Tr	-1.41	AWRI1499_3899	carrier protein mitochondrial precursor	*YMC1*
A-Tr	-1.41	AWRI1499_1987	peroxisomal copper amine oxidase	
A-Tr	-1.42	AWRI1499_0859	phosphatidylinositol transfer protein	*PDR16*
A-Tr	-1.42	AWRI1499_4138	lactate transporter	*JEN1*
A-Tr	-1.43	AWRI1499_0704	faf1 (forty s assembly factor)	*FAF1*
A-Tr	-1.43	AWRI1499_4051	glycine mitochondrial precursor	*GCV2*
A-Tr	-1.46	AWRI1499_3193	elongation factor 3	*HEF3*
A-Tr	-1.47	AWRI1499_1734	hypothetical protein AWRI1499_1734	
A-Tr	-1.47	AWRI1499_4313	hypothetical protein AWRI1499_4313	
A-Tr	-1.48	AWRI1499_2748	essential nucleolar dead-box rna helicase	*MAK5*
A-Tr	-1.50	AWRI1499_0534	mfs transporter of unknown specificity	*AMF1*
A-Tr	-1.51	AWRI1499_4061	hypothetical protein AWRI1499_4061	
A-Tr	-1.55	AWRI1499_3844	glucose transporter of the major facilitator sugar	*STL1*
A-Tr	-1.57	AWRI1499_3400	intersectin 1 (sh3 domain protein)	*LSB1*
A-Tr	-1.57	AWRI1499_1919	thiamine metabolism	*NRT1*
A-Tr	-1.58	AWRI1499_0411	mitochondrial malate dehydrogenase	*MAE1*
A-Tr	-1.58	AWRI1499_0766	nuclear localization sequence binding protein	*NSR1*
A-Tr	-1.60	AWRI1499_4956	capsule synthesis	
A-Tr	-1.65	AWRI1499_0342	putative protein kinase	*SWE1*
A-Tr	-1.65	AWRI1499_2371	inorganic phosphate transporter	

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	-1.65	AWRI1499_3518	aldehyde dehydrogenase	*ALD4*
A-Tr	-1.66	AWRI1499_1819	ammonium transporter mep2	*MEP2*
A-Tr	-1.67	AWRI1499_1933	c6 transcription	*YLR278C*
A-Tr	-1.69	AWRI1499_3438	urea active transporter	*DUR3*
A-Tr	-1.73	AWRI1499_4963	amidase family protein	*HER2*
A-Tr	-1.76	AWRI1499_4474	mitochondrial cytochrome	*CYB2*
A-Tr	-1.78	AWRI1499_2113	plasma membrane iron permease	*FTR1*
A-Tr	-1.80	AWRI1499_1337	d-lactate dehydrogenase	*DLD1*
A-Tr	-1.84	AWRI1499_0077	monocarboxylate transporter	*MCH2*
A-Tr	-1.89	AWRI1499_3462	sugar transporter family protein	*JEN1*
A-Tr	-1.92	AWRI1499_3753	trna methyltransferase	*TRM2*
A-Tr	-1.95	AWRI1499_4962	amidase family protein	
A-Tr	-1.95	AWRI1499_4964	hypothetical protein AWRI1499_4964	
A-Tr	-1.96	AWRI1499_2368	peptidase s15	
A-Tr	-1.97	AWRI1499_1468	hypothetical protein AWRI1499_1468	
A-Tr	-1.98	AWRI1499_1930	n-acetylglucosamine kinase	*HXK2*
A-Tr	-1.98	AWRI1499_4615	hypothetical protein AWRI1499_4615	
A-Tr	-2.01	AWRI1499_1957	mitochondrial peroxiredoxin prx1	*PRX1*
A-Tr	-2.01	AWRI1499_1432	d-aspartate oxidase	
A-Tr	-2.09	AWRI1499_1922	c6 transcription	*YLR278C*
A-Tr	-2.18	AWRI1499_3564	c6 transcription	
A-Tr	-2.24	AWRI1499_1929	duf895 domain membrane protein	
A-Tr	-2.24	AWRI1499_2083	gtp cyclohydrolase	
A-Tr	-2.25	AWRI1499_1808	allantoin permease	*DAL4*
A-Tr	-2.29	AWRI1499_4953	major facilitator superfamily transporter	*SEO1*
A-Tr	-2.31	AWRI1499_0196	iron transport multicopper oxidase	*FET3*
A-Tr	-2.33	AWRI1499_3463	putative lactate pyruvate transporter	*JEN1*
A-Tr	-2.33	AWRI1499_1923	putative allantoate permease	*DAL5*
A-Tr	-2.39	AWRI1499_3760	sulfate permease 1	*SUL1*
A-Tr	-2.43	AWRI1499_1336	taurine catabolism dioxygenase	*JLP1*
A-Tr	-2.45	AWRI1499_4954	d-lactate dehydrogenase	*DLD1*
A-Tr	-2.50	AWRI1499_0959	mfs allantoate	*THI73*
A-Tr	-2.77	AWRI1499_1353	inorganic phosphate transporter	*PHO84*
A-Tr	-2.99	AWRI1499_0257	fun19p	*FUN19*
A-Tr	-3.03	AWRI1499_0988	hypothetical protein AWRI1499_0988	
A-Tr	-3.04	AWRI1499_4319	major facilitator superfamily transporter	*FEN2*
A-Tr	-3.14	AWRI1499_1484	aro10p	*ARO10*
A-Tr	-3.29	AWRI1499_1931	hypothetical protein AWRI1499_1931	
A-Tr	-3.50	AWRI1499_1308	hypothetical protein AWRI1499_1308	

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
A-Tr	-4.23	AWRI1499_1461	general amino acid permease	*GAP1*
C-T5h	-1.27	AWRI1499_3932	hypothetical protein AWRI1499_3932	
C-Tr	4.81	AWRI1499_4109	acetyl- hydrolase	*ACH1*
C-Tr	4.65	AWRI1499_0332	succinyl- ligase alpha- mitochondrial precursor	*LSC1*
C-Tr	4.47	AWRI1499_2863	nad-dependent epimerase dehydratase	*YLL056C*
C-Tr	4.35	AWRI1499_1936	alpha-glucoside permease	*MPH2*
C-Tr	4.07	AWRI1499_1841	ynr018w-like protein	*RCF2*
C-Tr	3.98	AWRI1499_4725	high-affinity glucose transporter	*HXT13*
C-Tr	3.75	AWRI1499_1545	transcriptional activator	*VHR1*
C-Tr	3.67	AWRI1499_4728	gal10 bifunctional protein	*GAL10*
C-Tr	3.58	AWRI1499_2995	ygr110w-like protein	*CLD1*
C-Tr	3.47	AWRI1499_3771	succinyl-CoA ligase beta-chain, mitochondrial precursor	*LSC2*
C-Tr	3.37	AWRI1499_4492	dihydrolipoamide dehydrogenase -binding protein	*PDX1*
C-Tr	3.29	AWRI1499_2861	hypothetical protein AWRI1499_2861	
C-Tr	3.26	AWRI1499_4726	galactose-1-phosphate uridylyltransferase	*GAL7*
C-Tr	3.25	AWRI1499_0746	carbonic anhydrase	*NCE103*
C-Tr	3.22	AWRI1499_4193	nadp-specific glutamate dehydrogenase	*GDH1*
C-Tr	3.11	AWRI1499_4734	hexose transporter	*HXT2*
C-Tr	2.93	AWRI1499_2608	pdb1p	*PDB1*
C-Tr	2.72	AWRI1499_4727	galactokinase	*GAL1*
C-Tr	2.60	AWRI1499_4194	oleate-induced peroxisomal protein	
C-Tr	2.44	AWRI1499_2013	dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex	*LAT1*
C-Tr	2.39	AWRI1499_2713	glycerol dehydrogenase	*BDH1*
C-Tr	2.37	AWRI1499_4451	ygl080w-like protein	*FMP37*
C-Tr	2.32	AWRI1499_2435	forkhead box protein l2	
C-Tr	2.24	AWRI1499_4419	dicarboxylic amino acid permease	*DIP5*
C-Tr	2.18	AWRI1499_3720	hypothetical protein AWRI1499_3720	*FLO1*
C-Tr	2.13	AWRI1499_3162	e1 alpha subunit of the pyruvate dehydrogenase complex	*PDA1*
C-Tr	2.09	AWRI1499_2592	delta-1-pyrroline-5-carboxylate dehydrogenase	*PUT2*
C-Tr	2.06	AWRI1499_4322	5-oxoprolinase	*OXP1*
C-Tr	2.05	AWRI1499_0080	membrane transporter	*SSU1*
C-Tr	2.03	AWRI1499_1321	glutamyl-trna amidotransferase subunit a	*HER2*
C-Tr	2.00	AWRI1499_2054	hsp12p	*HSP12*
C-Tr	2.00	AWRI1499_1757	aldehyde dehydrogenase	*ALD5*
C-Tr	1.98	AWRI1499_2002	mitochondrial carrier protein leu5	*LEU5*
C-Tr	1.94	AWRI1499_1937	sucrose-6-phosphate hydrolase	*SUC2*
C-Tr	1.91	AWRI1499_4733	beta-glucosidase	
C-Tr	1.91	AWRI1499_4822	phenylpyruvate decarboxylase	*ARO10*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
C-Tr	1.90	AWRI1499_3505	sorbitol dehydrogenase	*SOR1*
C-Tr	1.70	AWRI1499_4323	5-oxoprolinase	*OXP1*
C-Tr	1.68	AWRI1499_4162	yhl008c-like protein	*YHL008C*
C-Tr	1.68	AWRI1499_2715	hypothetical protein AWRI1499_2715	
C-Tr	1.63	AWRI1499_3668	d-lactate dehydrogenase	*DLD1*
C-Tr	1.57	AWRI1499_0599	6-phosphofructo-2-kinase, putative	*YLR345W*
C-Tr	1.56	AWRI1499_4297	atpase-stabilizing factor 15 kda protein	*TMA10*
C-Tr	1.54	AWRI1499_4163	yhl008c-like protein	*YHL008C*
C-Tr	1.50	AWRI1499_0803	hypothetical protein AWRI1499_0803	
C-Tr	1.49	AWRI1499_4932	molybdenum cofactor sulfurase	
C-Tr	1.46	AWRI1499_4878	hypothetical protein AWRI1499_4878	
C-Tr	1.46	AWRI1499_3836	ribose-5-phosphate ketol-isomerase	*RKI1*
C-Tr	1.44	AWRI1499_3719	nad-dependent formate dehydrogenase	*FDH1*
C-Tr	1.40	AWRI1499_1452	hypothetical protein AWRI1499_1452	
C-Tr	1.38	AWRI1499_3822	medium chain alcohol dehydrogenase	*ADH7*
C-Tr	1.36	AWRI1499_2921	4-aminobutyrate aminotransferase	*UGA1*
C-Tr	1.36	AWRI1499_2040	2-enoyl thioester reductase	*ETR1*
C-Tr	1.34	AWRI1499_1194	cysteine synthase	*YGR012W*
C-Tr	1.32	AWRI1499_1614	peroxisomal hydratase-dehydrogenase-epimerase	*FOX2*
C-Tr	1.32	AWRI1499_4474	mitochondrial cytochrome	*CYB2*
C-Tr	1.32	AWRI1499_3429	hypothetical protein AWRI1499_3429	
C-Tr	1.32	AWRI1499_1227	protein serine threonine kinase expressed at the end of meiosis	
C-Tr	1.32	AWRI1499_0213	nadph-dependent medium chain alcohol dehydrogenase with broad substrate specificity	*ADH6*
C-Tr	1.30	AWRI1499_3271	neutral trehalase	*NTH1*
C-Tr	1.30	AWRI1499_1233	phosphatidylinositol-specific phospholipase c	
C-Tr	1.30	AWRI1499_3365	hypothetical protein AWRI1499_3365	
C-Tr	1.30	AWRI1499_4832	putative carbohydrate kinase	*YDR109C*
C-Tr	1.29	AWRI1499_3058	hypothetical protein AWRI1499_3058	
C-Tr	1.27	AWRI1499_1038	hypothetical protein AWRI1499_1038	
C-Tr	1.26	AWRI1499_1615	peroxisomal hydratase-dehydrogenase-epimerase	*FOX2*
C-Tr	1.25	AWRI1499_4559	cell wall glucanase	*UTR2*
C-Tr	1.24	AWRI1499_1325	nmra-like protein	
C-Tr	1.24	AWRI1499_3766	putative d-isomer specific 2-hydroxyacid dehydrogenase	*GOR1*
C-Tr	1.23	AWRI1499_1315	beta-mannosidase precursor	
C-Tr	1.23	AWRI1499_4618	p-type atpase sodium involved in na+ and li+ efflux to allow salt tolerance	*ENA1*
C-Tr	1.23	AWRI1499_4196	polyadenylate-binding protein	*PAB1*
C-Tr	1.22	AWRI1499_3376	histone h2b	*HTB1*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
C-Tr	1.21	AWRI1499_3347	ady2p	*ADY2*
C-Tr	1.20	AWRI1499_1115	sugar transporter family protein	*JEN1*
C-Tr	1.20	AWRI1499_0804	lipoate-protein ligase a family member	
C-Tr	1.20	AWRI1499_0109	hxk2p	*HXK1*
C-Tr	1.19	AWRI1499_3001	rna binding protein	
C-Tr	1.18	AWRI1499_1933	c6 transcription	*YLR278C*
C-Tr	1.18	AWRI1499_3584	protoplast secreted protein 2 precursor	*PST2*
C-Tr	1.16	AWRI1499_4934	d-arabinitol 2-dehydrogenase	*SPS19*
C-Tr	1.16	AWRI1499_2878	threonine aldolase	*GLY1*
C-Tr	1.16	AWRI1499_3825	adh6p, partial	*ADH6*
C-Tr	1.14	AWRI1499_0089	family integral membrane protein	
C-Tr	1.13	AWRI1499_4879	hypothetical protein AWRI1499_4879	
C-Tr	1.13	AWRI1499_2549	ydl124w-like protein	*YDL124W*
C-Tr	1.12	AWRI1499_0306	cortical actin cytoskeletal component	*CRN1*
C-Tr	1.10	AWRI1499_1807	beta alanine synthase	
C-Tr	1.10	AWRI1499_2529	bop3p	*BOP3*
C-Tr	1.10	AWRI1499_3518	aldehyde dehydrogenase	*ALD4*
C-Tr	1.09	AWRI1499_3111	iah1p	*IAH1*
C-Tr	1.07	AWRI1499_2949	sulfite reductase alpha subunit	*MET10*
C-Tr	1.07	AWRI1499_2157	histone h4	*HHF2*
C-Tr	1.07	AWRI1499_2090	transcription factor	
C-Tr	1.06	AWRI1499_4413	rna binding protein	*RTC3*
C-Tr	1.06	AWRI1499_1368	abhydrolase domain-containing protein	*IMO32*
C-Tr	1.06	AWRI1499_4520	hypothetical protein AWRI1499_4520	
C-Tr	1.05	AWRI1499_1316	beta-mannosidase precursor	
C-Tr	1.05	AWRI1499_3765	putative d-isomer specific 2-hydroxyacid dehydrogenase	*GOR1*
C-Tr	1.05	AWRI1499_4849	hmo1p	*HMO1*
C-Tr	1.04	AWRI1499_2548	zinc cluster transcriptional activator	*CAT8*
C-Tr	1.04	AWRI1499_4752	prd1p	*PRD1*
C-Tr	1.03	AWRI1499_0561	type ii myosin heavy required for wild-type cytokinesis and cell separation	*MYO1*
C-Tr	1.03	AWRI1499_3031	zinc-finger inhibitor of ho transcription	*ASH1*
C-Tr	1.02	AWRI1499_1649	translational activator gcn1	*GCN1*
C-Tr	1.01	AWRI1499_0593	hypothetical protein AWRI1499_0593	
C-Tr	1.00	AWRI1499_4490	noc3p	*NOC3*
C-Tr	-1.01	AWRI1499_1000	part of small ribosomal subunit processosome (contains u3 snorna)	*RRP5*
C-Tr	-1.01	AWRI1499_0970	purine permease	
C-Tr	-1.01	AWRI1499_2063	subunit of the rave complex (skp1p) which promotes assembly of the v-atpase holoenzym	*RAV1*

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
C-Tr	-1.02	AWRI1499_3332	zinc finger protein	*ZAP1*
C-Tr	-1.02	AWRI1499_0411	mitochondrial malate dehydrogenase	*MAE1*
C-Tr	-1.02	AWRI1499_2081	hypothetical protein AWRI1499_2081	
C-Tr	-1.02	AWRI1499_4252	mrna export factor elf1	*NEW1*
C-Tr	-1.02	AWRI1499_2266	hypothetical protein AWRI1499_2266	
C-Tr	-1.03	AWRI1499_4743	mitochondrial processing peptidase	*YOL098C*
C-Tr	-1.03	AWRI1499_4390	hypothetical protein AWRI1499_4390	
C-Tr	-1.03	AWRI1499_3235	acetyl-coenzyme a synthetase 1	*ACS1*
C-Tr	-1.03	AWRI1499_2410	hypothetical protein AWRI1499_2410	
C-Tr	-1.03	AWRI1499_3753	trna methyltransferase	*TRM2*
C-Tr	-1.03	AWRI1499_0174	izh2p	*IZH2*
C-Tr	-1.04	AWRI1499_2304	enp2p	*ENP2*
C-Tr	-1.05	AWRI1499_2704	hypothetical protein AWRI1499_2704	
C-Tr	-1.05	AWRI1499_3373	putative u3 snornp protein utp20p	*UTP20*
C-Tr	-1.05	AWRI1499_0564	hypothetical protein AWRI1499_0564	
C-Tr	-1.06	AWRI1499_2726	hypothetical protein AWRI1499_2726	
C-Tr	-1.06	AWRI1499_3126	dna repair helicase rad25	*SSL2*
C-Tr	-1.06	AWRI1499_2849	protein kinase	*KNS1*
C-Tr	-1.07	AWRI1499_4926	poly polymerase	*PAP1*
C-Tr	-1.07	AWRI1499_0061	nuclear architecture related protein	*NAR1*
C-Tr	-1.07	AWRI1499_2685	hypothetical protein AWRI1499_2685	
C-Tr	-1.08	AWRI1499_1673	putative ubiquitin conjugating factor	
C-Tr	-1.09	AWRI1499_4061	hypothetical protein AWRI1499_4061	
C-Tr	-1.10	AWRI1499_1012	glutamate synthase	*GLT1*
C-Tr	-1.10	AWRI1499_0494	hypothetical protein AWRI1499_0494	
C-Tr	-1.10	AWRI1499_2292	putative membrane-bound transcriptional activator	*MGA2*
C-Tr	-1.11	AWRI1499_1133	nuclear protein required for actin cytoskeleton	*SDA1*
C-Tr	-1.12	AWRI1499_2748	essential nucleolar dead-box rna helicase	*MAK5*
C-Tr	-1.12	AWRI1499_4100	phosphoribosylformylglycinamidine cyclo-ligase	*ADE5,7*
C-Tr	-1.12	AWRI1499_4051	glycine mitochondrial precursor	*GCV2*
C-Tr	-1.13	AWRI1499_3844	glucose transporter of the major facilitator sugar	*STL1*
C-Tr	-1.13	AWRI1499_1734	hypothetical protein AWRI1499_1734	
C-Tr	-1.15	AWRI1499_0258	putative fe 2-oxoglutarate-dependent dioxygenase	*TPA1*
C-Tr	-1.15	AWRI1499_1463	single-stranded dna cleaves single-stranded dna during nucleotide excision repair	*RAD2*
C-Tr	-1.15	AWRI1499_2807	hypothetical protein AWRI1499_2807	
C-Tr	-1.16	AWRI1499_0426	pyridoxine biosynthesis protein	*SNZ1*
C-Tr	-1.17	AWRI1499_2706	dna repair protein rad16	*RAD16*
C-Tr	-1.17	AWRI1499_0211	hypothetical protein AWRI1499_0211	

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
C-Tr	-1.17	AWRI1499_4897	tkp3 protein	*YGR109W-B*
C-Tr	-1.19	AWRI1499_0404	ubiquitin carrier protein	*HMS1*
C-Tr	-1.20	AWRI1499_0349	rna-binding protein nob1p involved in 26s proteasome assembly	*NOB1*
C-Tr	-1.21	AWRI1499_4770	isocitrate mitochondrial precursor	*IDP1*
C-Tr	-1.22	AWRI1499_0729	carnitine acetyltransferase	*YAT2*
C-Tr	-1.22	AWRI1499_1863	putative pyridoxine transport protein	*TPN1*
C-Tr	-1.22	AWRI1499_1686	putative protein kinase	*IME2*
C-Tr	-1.23	AWRI1499_2582	hypothetical protein AWRI1499_2582	
C-Tr	-1.23	AWRI1499_2004	plasma membrane atp-binding cassette transporter	*YOR1*
C-Tr	-1.24	AWRI1499_4095	fungal-specific transcription factor	
C-Tr	-1.26	AWRI1499_1563	indoleamine -dioxygenase subfamily	*BNA2*
C-Tr	-1.26	AWRI1499_2143	hypothetical protein AWRI1499_2143	
C-Tr	-1.30	AWRI1499_4962	amidase family protein	
C-Tr	-1.30	AWRI1499_0007	protein that recognizes and binds damaged dna in an atp-dependent manner (with rad16p)	*RAD7*
C-Tr	-1.31	AWRI1499_3686	hypothetical protein AWRI1499_3686	
C-Tr	-1.31	AWRI1499_2703	hypothetical protein AWRI1499_2703	
C-Tr	-1.32	AWRI1499_0093	atp-dependent rna helicase ded1	*DBP1*
C-Tr	-1.32	AWRI1499_2936	hypothetical protein AWRI1499_2936	
C-Tr	-1.33	AWRI1499_1424	biotin synthase	*BIO2*
C-Tr	-1.34	AWRI1499_4754	amino acid permease	*UGA4*
C-Tr	-1.37	AWRI1499_1717	hypothetical protein AWRI1499_1717	
C-Tr	-1.39	AWRI1499_0832	kynurenine 3-mono oxygenase	*BNA4*
C-Tr	-1.39	AWRI1499_4543	putative c1-tetrahydrofolate synthase	*ADE3*
C-Tr	-1.42	AWRI1499_3916	activator of forkhead-like transcription factor	
C-Tr	-1.44	AWRI1499_3913	electron transfer flavoprotein-ubiquinone mitochondrial precursor	*CIR2*
C-Tr	-1.44	AWRI1499_0176	putative autophagy-related protein kinase atg1	*ATG1*
C-Tr	-1.46	AWRI1499_3932	hypothetical protein AWRI1499_3932	
C-Tr	-1.49	AWRI1499_4359	hypothetical protein AWRI1499_4359	
C-Tr	-1.51	AWRI1499_3191	general amino acid permease	*GAP1*
C-Tr	-1.56	AWRI1499_0959	mfs allantoate	*THI73*
C-Tr	-1.57	AWRI1499_3980	s-adenosylmethionine decarboxylase	*SPE2*
C-Tr	-1.60	AWRI1499_1707	hypothetical protein AWRI1499_1707	
C-Tr	-1.62	AWRI1499_2075	fatty acid synthase alpha subunit, partial	*FAS2*
C-Tr	-1.63	AWRI1499_0077	monocarboxylate transporter	*MCH2*
C-Tr	-1.68	AWRI1499_2636	putative oligopeptide transporter, partial	*OPT2*
C-Tr	-1.73	AWRI1499_2638	acyl-coenzyme a:6-aminopenicillanic acid acyl-transferase	
C-Tr	-1.73	AWRI1499_2106	hypothetical protein AWRI1499_2106	

Strain and comparison time	log_2(FC)	AWRI identifiers	Protein name	*S. cerevisiae* homologous gene (Standard name)
C-Tr	-1.99	AWRI1499_4952	c2h2 transcription factor, partial	*CRZ1*
C-Tr	-2.05	AWRI1499_1353	inorganic phosphate transporter	*PHO84*
C-Tr	-2.19	AWRI1499_2139	hypothetical protein AWRI1499_2139	
C-Tr	-2.40	AWRI1499_2142	hypothetical protein AWRI1499_2142	
C-Tr	-2.42	AWRI1499_2141	hypothetical protein AWRI1499_2141	
C-Tr	-2.57	AWRI1499_2140	hypothetical protein AWRI1499_2140	
C-Tr	-2.80	AWRI1499_2637	putative oligopeptide transporter	*OPT2*

Gene Ontology analysis was conducted on genes listed in Table 8. Since the analysis was done against *S. cerevisiae* genome annotation and not all the genes in AWRI1499 genome are annotated with the correspondent function in *S. cerevisiae*, the number of genes processed was lower than the one resulted significant (Table 7, "*S. cerevisiae* homolog" column). No enrichment analysis has been done for the short-time response, neither the up or down regulation, since the number of genes exceeding the fixed threshold (absolute log_2fold-change value of 1) was null or too little. In both strains no genes had log_2fold-change value grater then |1| regarding the up-regulation, and just few exceed the threshold in the down-regulation. No genes could be analysed for the short response-upregulation, nonetheless were obtained genes with a statistical significant change in the expression (Appendix 2). With regard to the long-time response, 126 and 96 genes were submitted to the enrichment analysis for the AWRI strain up and down-regulation, respectively, resulting in 113 and 87 unique identifiers, due to 13 (*ADH3, ADH6, BDH1, BSC6, CAT8, ETP1, FOX2, HXT13, IMA1, PCK1, ROT2, TIP1, YMR315W*) and 7 (*DLD1, GAP1, JEN1, THI73, UGA4, UTP20, YLR278C*) genes duplicated in the correspondent list of *S. cerevisiae* homologus protein. As far for the CBS strain the genes submitted to the Gene Ontology analysis were 81 for the up-regulation and 52 for the down-regulation, resulting in 76 and 51 unique identifiers, respectively, because of 5 (*ADH6, FOX2, GOR1, OXP1, YHL008C*) and 1 (*OPT2*) identifiers duplicated in the lists.

Biological process

The enrichment analysis of the SURGs in AWRI1499 resulted in 50 terms with a corrected *p*-value lower than 0.01 out of 496 total terms found. Similarly, for the CBS2499 strain the outcome consisted of 28 terms significantly enriched out of 383 terms found. Considering the down-regulated genes, the terms significantly enriched in the AWRI1499 and CBS2499 strains, were 31 out of 423 and 7 out 380 processes found, respectively.

As shown in Fig. 12, a treemap obtained using REVIGO visualization, the main processes arising from SURGs in AWRI1499 strain mainly belong to the metabolism of the monocarboxylic acids. Other groups were the carbohydrate metabolism, and the coenzyme and the cellular carbohydrate metabolism, followed by other processes such as those concerning to glucose import and sulphur compound, with a lower *p*-value.

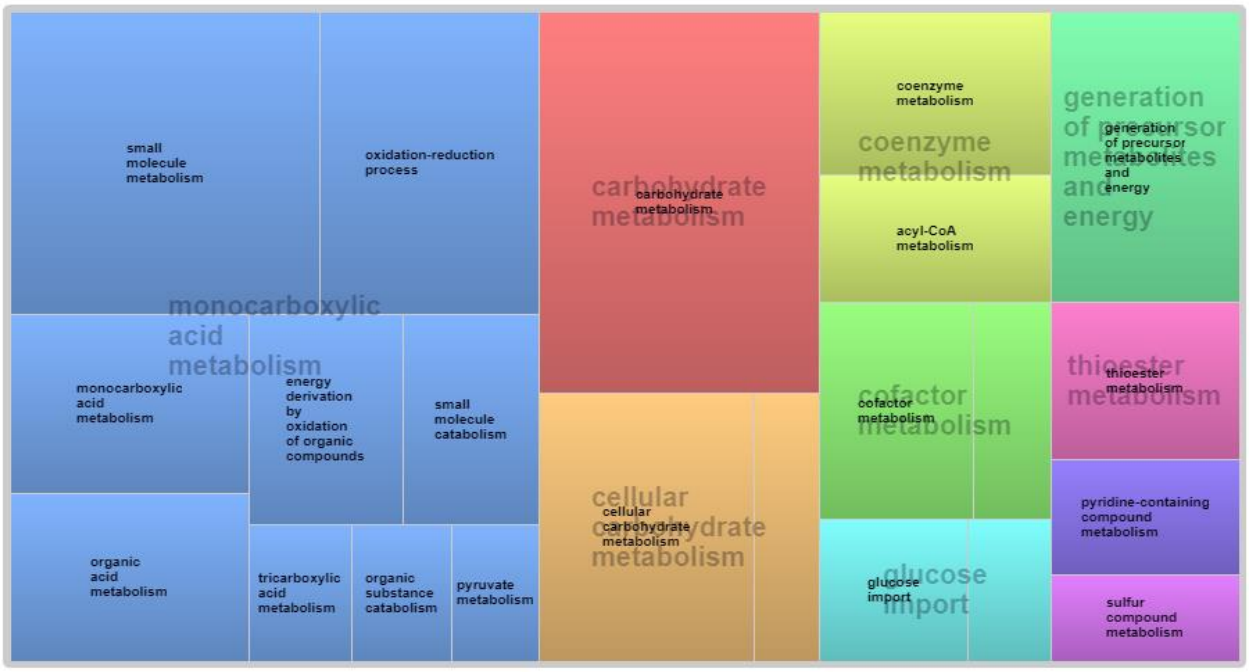

Fig. 12 Treemap view of the main terms from REVIGO. AWRI1499 Biological Processes enriched by SURGs. Each rectangle is a cluster representative. The representativr are joined into "supercluster" of loosely related terms, visualised with different colors. Size of the rectangle reflectsthe *p*-value of the GO term in the underlying database.

With regards to processes enriched by SDRGs (Fig. 13), in the AWRI1499 outcome emerged the anion transmembrane transport, followed by the ribosomal small subunit biogenesis and the RNA 5'end-processing.

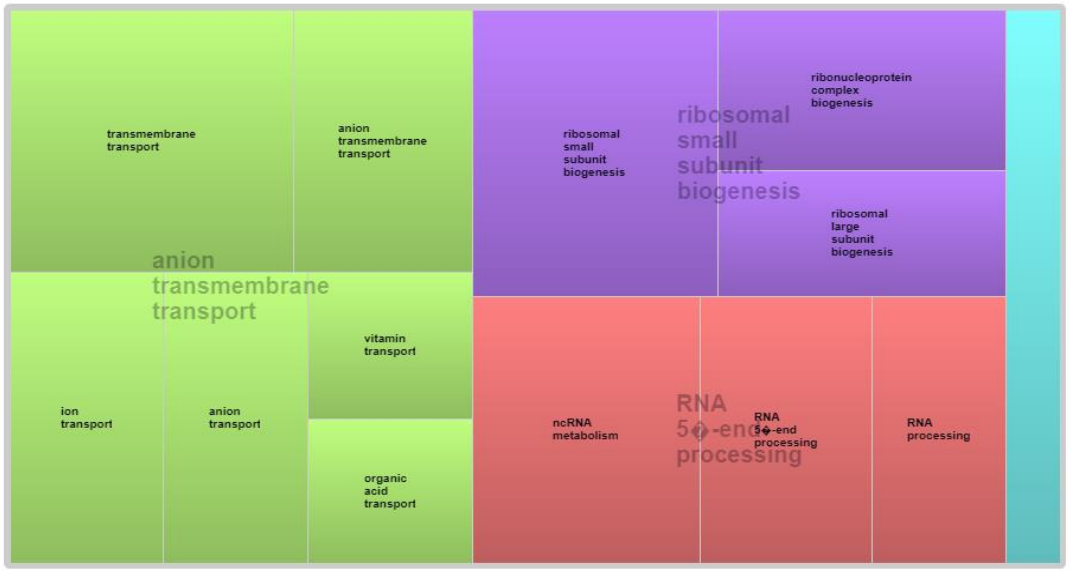

Fig. 13 Treemap from REVIGO showing the main clusters (each rectangle represents a cluster), eventually group in supercluster, visualised with different colors, of AWRI1499 Biological Processes enriched by SDRGs. The size of the rectangles returns the *p*-value.

The monocarboxylic acid metabolism was the main supercluster for the SURGs in CBS2499 as well, with single underlying clusters. The second bigger group was represented by the acyl-CoA metabolism, then the carbohydrate, sulphur compound and organic hydroxy compound metabolisms were also found (Fig. 14). As far processes enriched by SDRGs, results reported only the supercluster of monocarboxylic acid metabolism for the CBS2499 strain (Data not shown); it was found also among the processes enriched by SURGs, but different genes were involved.

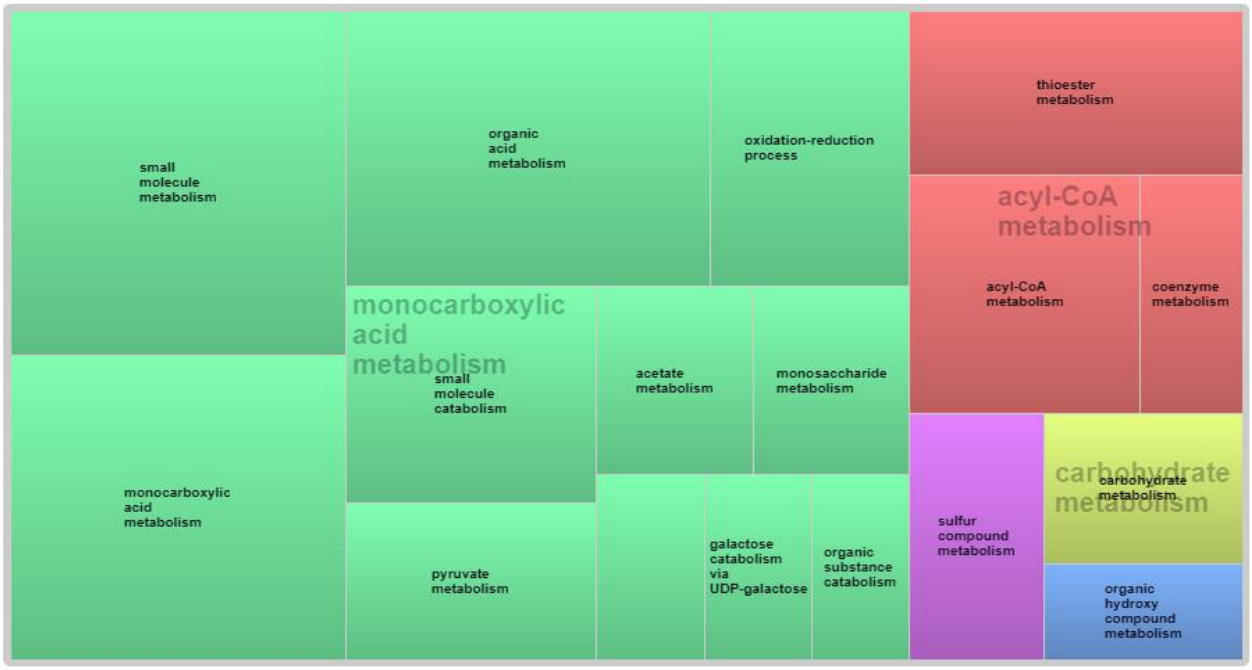

Fig. 14 Treemap view of the main terms from REVIGO. CBS2499 Biological Processes enriched by SURGs. Each rectangle is a cluster representative. The representativr are joined into "supercluster" of loosely related terms, visualised with different colors. Size of the rectangle reflectsthe *p*-value of the GO term in the underlying database.

Cellular Components

SURGs in AWRI1499 strain enriched significantly 8 cellular components out of 93, while 4 were the significant ones originating from up-regulated genes in CBS2499 strain of 104 found. As well, SDRGs defined 14 out of 94 and 2 out of 84 significantly enriched terms in AWRI1499 and CBS2499 strains, respectively.

Of the terms resulting in the Australian strain from the GO enrichment analysis based on SURGs was possible to identify superclusters of peroxisome and mitochondrial pyruvate dehydrogenase complex, other than the cytoplasm. With regards to genes down-regulated, these were belonging and significantly enriching mainly the preribosome, the nucleolus and the integral component of plasma membrane (Fig. 15).

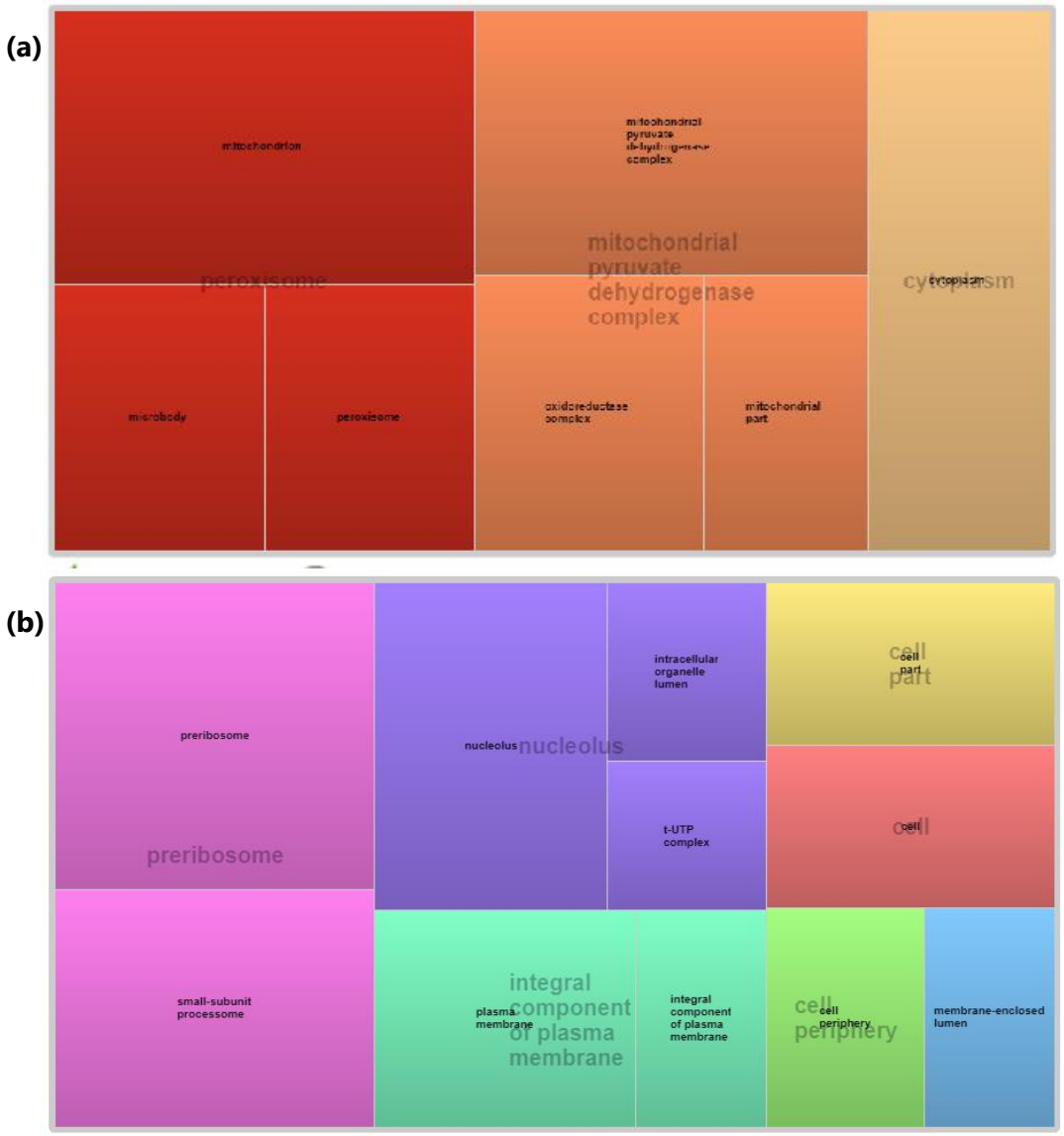

Fig. 15 Treemap view of the main terms from REVIGO. AWRI1499 Cellular Component enriched by SURGs (a) and SDRGs (b). Each rectangle is a cluster representative. The representativr are joined into "supercluster" of loosely related terms, visualised with different colors. Size of the rectangle reflectsthe *p*-value of the GO term in the underlying database.

The enrichment analysis of SURGs and SDRGs derived from CBS2499 strain, resulted in the main component of the mitochondrial pyruvate dehydrogenase complex and the nucleotide-excision repair complex, respectively (Data not shown).

Molecular Functions

Likewise, the analysis looking for the significantly enriched molecular functions displayed 6 terms, out of 154 identified, with corrected *p*-value below 0.01 as regard of genes up-regulated in the AWRI1499 strain, and 21 out of 147 considering SDRGs. Functions observed up-regulated consisted mainly in the oxidoreductase activity, other than the catalytic activity and the carbohydrate binding,

while among the functions enriched by down-regulated genes, were noticed the transporter activity, together with snoRNA and cofactor binding. (Fig. 16).

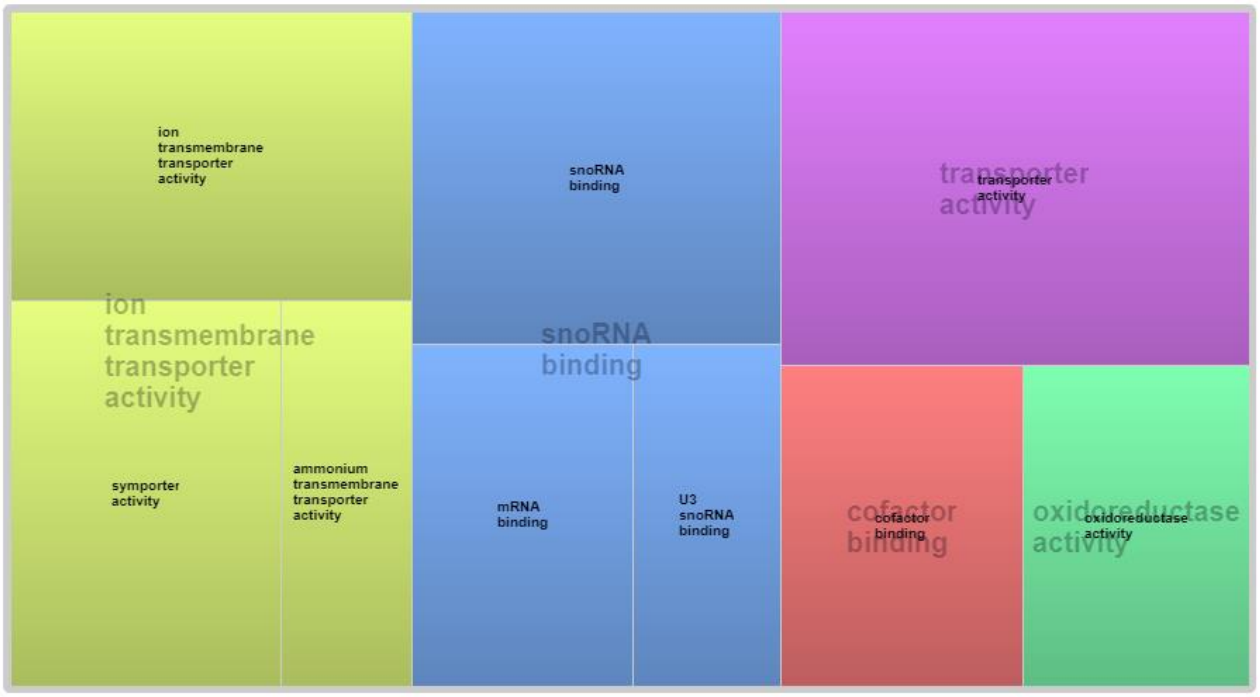

Fig. 16 Treemap view of the main terms from REVIGO. AWRI1499 Molecular Function enriched by SDRGs. Each rectangle is a cluster representative. The representativr are joined into "supercluster" of loosely related terms, visualised with different colors. Size of the rectangle reflectsthe *p*-value of the GO term in the underlying database.

As far CBS2499 strain, 9 out of 132 were the terms highlighted as significant for the enrichment analysis based on SURGs while 8 terms of 119 resulted significant on the base of SDRGs. The oxidoreductase activity resulted enriched (by SURGs), together wih catalytic and cofactor binding, while other genes as well referring to some oxidoreductase activity enriched that function along with the ion binding.

For both strains a detailed list of all the GO terms resulted significant in the different categories is reported in Appendix 3.

3.3.2.2 *B./D. bruxellensis* coral response against SO_2 exposure

In order to define the general response associated to SO_2 stress in *B./D. bruxellensis* species, a comparison between each time-point-response per strains was carried out on homologous genes revealed in *S. cerevisiae* (Table 8). The analysis was referred to the genes with FC I1I in the long response since in the short response no SURGs showed a FC> I1I and none of the few SDRGs with FC> I1I (Table 7) were found in common between the two analysed strains. *B./D. bruxellensis* AWRI1499 and CBS2499 strains had in common 66 genes significantly changing the expression (Fig. 17): 38 and 19 were those showing the same regulation in both strains, being up- and down-

regulated, respectively. The remaining 9 genes switched the regulation between the strains being down-regulated in AWRI1499 strain and down-regulated in the CBS2499 strain.

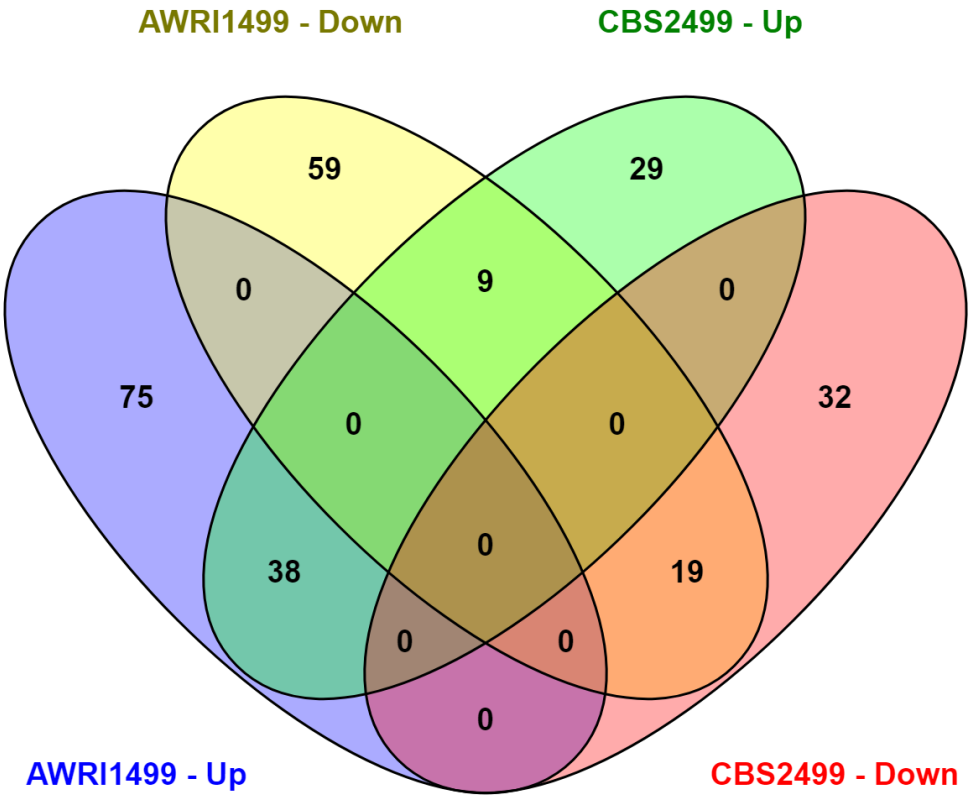

Fig. 17 Venn diagram representing distribution of genes with log_2fold-change greater than |1|, and statistically significant, in the long response, up- and down-regulated, for both strains.

Common homologous genes are listed in Table 9.

Table 9 Standard names in *S. cerevisiae* of AWRI1499 gene identifiers shared by the two strains.

Up-regulated genes:	*ACH1, ADH6, ADH7, BDH1, CAT8, CLD1, DIP5, ETR1, FMP37, FOX2, GAL1, GAL10, GAL7, HXK1, HXT13, IMO32, LAT1, LEU5, LSC1, LSC2, MPH2, NTH1, PAB1, PDA1, PDB1, PDX1, PST2, RCF2, RTC3, SOR1, SPS19, SSU1, SUC2, TMA10, VHR1, YDR109C, YLL056C, YLR345W.*
Down-regulated genes:	*BNA2, CIR2, ENP2, GAP1, GCV2, MAE1, MAK5, MCH2, NEW1, PHO84, RRP5, STL1, THI73, TPN1, TRM2, UGA4, UTP20, YGR109W-B, YOR1.*
AWRI Down/CBS Up:	*ALD4, ARO10, CYB2, DLD1, FLO1, HER2, JEN1, OXP1, YLR278C*

Thus, the set of shared genes were submitted to the GO analysis with the aim of identifying the process, component and function enriched terms. Up-regulated genes enriched processes referred

to the main supercluster of monocarboxylic acid, acyl-CoA and carbohydrate metabolisms. Out of a total of 224 terms found, 31 were significant (Corrected *p*-value cut-off: 0.01) and they were submitted to REVIGO. In detail, as shown in Fig. 18, the monocarboxylic acid metabolism grouped smaller terms such as the pyruvate metabolism and the oxidation-reduction process. Moreover, the cofactor and sulphur compound metabolism were also represented.

Fig. 18 Treemap from REVIGO showing the main clusters (each rectangle represents a cluster), eventually group in supercluster, visualised with different colors, of AWRI1499 and CBS2499 strains biological processes enriched by shared up-regulated genes. The size of the rectangles reflects the *p*-values of the GO term in the underlying GOA database.

The only process resulted enriched, analysing down-regulated genes, is the "transmembrane transport". However, by submitting to the GO the SDRGs of each strain, separately (Fig. 13, Appendix 3) it was not observed in CBS2499. This was probably due to the fact that 8 genes are in common and related to the transmembrane transport out of a total number of down-regulated genes shared of 19 (equivalent to 42%). In the AWRI1499 strain 24 genes are involved in the enrichment of the transmembrane transport, out of a total of 96 genes considered for the analysis (25%). Considering the CBS2499, the 8 genes in common with AWRI1499 would be the only ones (out of 52, 15%) to enrich the transmembrane transport process, which is therefore not significant.

With regards to the Cellular Component category, enriched by the up-regulated genes in common between the strains studied, 4 terms were significant, mainly represented by the mitochondrial pyruvate dehydrogenase complex (the most significant one), out of 63 found.

No terms based on the down-regulated genes resulted significant with regard to the Cellular Component category.

The GO analysis of Functions shared by the two strains resulted in 9 significant terms out of 90 found enriched by SURGs. As illustrate in Fig. 19, those were attributable to the pyruvate dehydrogenase, carbohydrate kinase, catalytic and succinate-CoA ligase activities.

Fig. 19 Treemap from REVIGO showing the main clusters (each rectangle represents a cluster), eventually group in supercluster, visualised with different colors, of AWRI and CBS strains molecular functions enriched by shared up-regulated genes. The size of the rectangles reflects the *p*-values of the GO term in the underlying GOA database.

Regarding the functions enriched by down-regulated genes, the outcome of the GO analysis was of 6 significant terms (corrected *p*-value cutoff 0.01) out of 58 found, linked to the transporter activity (Data not shown).

4. DISCUSSION

4.1 Cloning the putative gene of vinyl phenol reductase of *Dekkera bruxellensis* in *Saccharomyces cerevisiae*

In order to improve our knowledge about vinil phenol production, the putative VPR of *D. bruxellensis* has been cloned into a non-conventional host and characterised trough a quantitative analysis.

A(poly)histidine tag has been added to allow an easy purification, nonetheless has been reported to produce sometime unforeseen changes in protein structure, that can negatively affect its function (Arnau et al., 2006; Halliwell et al., 2001).

The presence of the VPR transcript was investigated in the recombinant strain #17 using the couple of primers vprRTF/vprRTR that specifically target the gene of *D. bruxellensis* (Fig. 4). A single band was generated, as expected at 125 bps for strain #17 and *D. bruxellensis* CBS4481. Genomic DNAs of *S. cerevisiae* and *P. pastoris*, this latter the closest relative of *D. bruxellensis* (Curtin et al., 2012a; Piskur et al., 2012), enclosed as negative controls were not amplified (Fig. 4).

Once the specific protein activity was verified in crude cellular extracts, new *S. cerevisiae* transformants were obtained using the vector YEp112TVPRT, which allowed for easy protein purification. PCR amplification of the VPR gene from 20 yeast colonies isolated on selective medium confirmed the presence of the expected construct (one band of 465 bps). One clone, named #62, was maintained for further experiments. Finally, the analysis of the transcript showed the expression of the heterologous gene (Fig. 4).

Faling the measure volatile phenols both in transformed and, as expected, not transformed strains, VPR activity has been measured in cellular extracts.

No activity was detected from *S. cerevisiae* cultures grown in Verduyn medium (Verduyn et al., 1992), which contains only traces of inorganic salts; consequently, assuming that VPR enzyme belongs to the class of the Zn/Cu SODs, a higher Zn and/or Cu concentration could be necessary to obtain the functional heterologous enzyme. A biologically active protein able to trigger the reduction of the 4-vinyl guaiacol substrate was detected by UV assays when the recombinant cells were cultivated using a modified Verduyn medium. The calculated specific activity of the protein produced by the clone #17 (9 ± 0.6 mU/mg) was found to be lower in the recombinant strain in comparison to the one obtained in the same growth conditions for the native strain *D. bruxellensis* CBS4481 (57 ± 12 mU/mg). A closer value was obtained from *D. bruxellensis* CBS2499 that reached 4.7 ± 1.9 mU/mg.

The VPR protein was then purified from *S. cerevisiae* W303 clone #62, the one expressing the gene for VPR fused with an his-tag for protein isolation. According to literature (Granato et al., 2014), the purification allowed the separation of a single band in denaturing condition with a molecular mass

of about 20 kDa (Fig. 4). The eluted fractions containing the highest protein concentration (0.040 ± 0.017 mg/mL) were used to test the activity of VPR (as an example, lane VPR3 in Fig. 4). No absorbance decrease was observed in the unbound fractions (Fig. 5, lane NL) (data not shown). Since no variation in absorbance was detected in absence of the substrate, results confirmed that the purified protein specifically catalyses the reaction from 4-vinyl guaiacol to 4-ethyl guaiacol (Fig. 6). The biotransformation was confirmed by following HPLC analysis, although low overall conversion (7 %) was observed; this may be due to the low stability of the enzyme as isolated protein (Harris et al.; 2009). VPR-active fractions showed a specific activity of 0.97 ± 0.01 U/mg at pH 7.5. Moreover, an optimization of the UV assay using a phosphate buffer at pH 6.0 rather than 7.5 almost doubled the value of the specific activity of VPR that increased at 1.83 ± 0.03 U/mg of protein.

Actually, *D. bruxellensis* species appears to tolerate and proliferate under extreme conditions (Galafassi et al., 2013; Galafassi et al 2015). In particular, a recent work of Moktaduzzaman and collaborators (2016) provides new insights into the *Brettanomyces* physiology, emphasizing the ability of this species to grow in acid environments and to modulate the metabolism of acetic acid in a strain-dependent way. As expected, the crude extracts of *S. cerevisiae* W303 clone #62 confirmed the order of magnitude of the specific activity of the VPR obtained in *S. cerevisiae* W303 clone #17 (4 ± 1.1 mU/mg).

Finally, according to Granato et al. (2014), the purified protein was also tested for SOD activity (Fig. 5), showing a specific activity of 3.41 U/mg in the fraction VPR3 (Fig. 4 and 5).

A comparative genetic analysis of the partial VPR sequences from 17 different *D. bruxellensis* strains of oenological origin was carried out. The aim of this evaluation was to investigate if production of volatile phenols is correlated with a polymorphic state of the genetic locus of VPR, and in particular in the sites of the Rossmann fold-like region potentially involved in the reductase activity of the enzyme. As already observed for *D. bruxellensis* CBS4481 strain, the analysis revealed that a conserved consensus binding pattern for NAD(P)H and a cofactor binding motif (Oppermann et al. 2003; Jçrnvall et al. 1995; Lesk 1995) is present in all strains of the collection. The level of gene polymorphism (2.3%) found was slightly higher in comparison to what recently reported for the genome of *D. bruxellensis* AWRI1499 (1.9%) (Curtin et al., 2012a). Furthermore, all polymorphic positions, though with different frequencies (from 0.72 to 78.6%) showed allelic heterozygosity. No triple o multiple peak was detected at each single nucleotide position of the pherograms; this data suggests that, in the investigated strains, the gene is in a diploid state. As far the heterozygosity state of the single strains, it ranged from 0 to a maximum of 1.7%. The majority of these SNPs were

synonymous; two SNP positions (95 and 329) led to a new codon, producing non-synonymous amino acid substitutions across *D. bruxellensis* VPR gene (Table 1). In detail, the SNP at position 95 determined a switch from asparagine to serine, still belonging to the group of neutral-polar amino acids and, at position 329, the SNP led from alanine to valine, as alanine a neutral-nonpolar amino acid. Most significant protein changing was detected in three strains (*D. bruxellensis* CBS1941, CBS2547 and CBS5206); however, the observed genetic polymorphism of VPR does not justify the strain-dependent production of volatile phenols extensively discussed in literature (Table 1) (Conterno et al., 2006; Vigentini et al., 2008; Agnolucci et al., 2009). The highest production of volatile phenols was detected in strain *D. bruxellensis* CBS4601 ($6.4x10^{-7}$ µg/cell, 9.0 mg/L) that, due to the absence of heterozygosity in the VPR gene, could indicate the presence of the gene in a single copy or in an allelic homologous state. This outcome could suggest that the genetic sequence of the VPR in CBS4601 represents the most efficient version of the gene, in the collection under study, in terms of off-flavour production. However, except for the polymorphic positions 95 and 329, other 6 strains have two potentially functional alleles since they show an analogous amino acidic sequence to *D. bruxellensis* CBS4601 (CBS74, CBS2499, CBS4459, CBS4481, CBS4602). The fact that all these strains present a lower production of volatile phenols (ranging between $4.7x10^{-6}$ and 0 µg/cell) demonstrates that other factors could affect the final production, such as strain-dependent transcriptional/post-translational mechanisms. However, we cannot rule out that an intraspecific codon usage bias could also affect the translation of strain-specific allelic variants.

4.2 A Response Surface Methodology approach to investigate the effect of sulphur dioxide, pH and ethanol on *DbCD* and *DbVPR* gene expression and on the volatile phenol production in *Brettanomyces/Dekkera bruxellensis* CBS2499

Wine spoilage by *B./D. bruxellensis* has increased in frequency because of the use of less-severe processing conditions, the great variety of diverse vinification techniques and the tendency to reduce the use of preservatives, such as sulfur dioxide. In particular, the sustainable perspective that to limit SO_2 in bottled wines can reduce undesirable allergenic effects on humans drives the latter action.

The capability of *B./D. bruxellensis* to survive and to grow in wine can be partially ascribed to its high resistance to SO_2; one of the main research question that can be addressed regarding the prevention of this spoilage yeast species is: "how the SO_2 addition can be managed in order to counteract the yeast occurrence during winemaking and in the final product?". Unfortunately, since the active form of SO_2 against microbial proliferation depends on pH, ethanol concentration and temperature (Ribéreau-Gayon et al., 2006; Usseglio-Tomasset and Bosia, 1984), the answer has to take into consideration that wine is an extremely heterogeneous environment.

Although some wine factors/constituents are reported to play a key role on the off-flavor synthesis by *B./D. bruxellensis*, most of the works carried out to date have independently studied the factors without considering their interactions (Dias et al., 2003b; Godoy et al., 2008; Sturm et al., 2014). With the RSM approach used in this study, the simultaneous effects produced by SO_2, pH and ethanol on *DbCD* and *DbVPR* gene expression and volatile phenols production have been investigated. Two specific aims are issued in this investigation: i) the identification of a suitable HKG to assess the relative expression of *DbCD* and *DbVPR* genes; and, ii) the setup of an experimental design in order to predict factors and/or possible factor interactions affecting the pathway of VP production.

Regarding the first goal, since real-time qPCR represents the protocol for highly sensitive and reproducible gene expression analysis, accurate and reliable expression results cannot exclude the normalization of real-time qPCR data against a "confident" reference gene in the condition under study. In this work, five genes were evaluated for this purpose and the GeNorm and Normfinder algorithm were used to assay the RNA transcription level of each candidate gene. Despite to the large literature reporting real-time qPCR expression data of several *B./D. bruxellensis* genes, only one manuscript has searched for adequate HKGs to be involved in the data normalization of gene expression assays under oenological conditions (Nardi et al., 2010). In particular, Nardi and co-authors choose actin (*ACT1*) and translational elongation factor EF-1α (*TEF1*) genes as housekeeping

references. The finding that tubulin (*DbTUB*) was the best reference gene in the present study proves the need of include, as a specific objective of the work, preliminary transcriptional assays to validate the "housekeeping" status of a candidate reference gene under particular experimental conditions.

As concern the second goal, different considerations can be done on the analysis of possible factors that influence the expression of *DbCD* and *DbVPR* genes and the production of VPs.

In general, the main outcome of this study reveals that the highest variability of the response, as a function of the studied factors, was obtained with the expression of *DbCD* that resulted repressed in all the conditions tested by the experimental design in comparison with the condition used as "calibrator". Indeed, being the first enzyme of the metabolic pathway of VPs, the *DbCD* gene is probably more influenced by change of the environmental/oenological conditions in comparison to the *DbVPR* gene.

The expression of *DbCD* is strongly affected by pH and the linear interactions between pH and SO_2, SO_2 and ethanol. Regarding the effect exerted by pH on *DbCD* expression, is important to consider that pH plays an important role on the enzyme substrates, determining the dissociation/undissociation of hydroxycinnamic acids. At wine pH both p-coumaric and ferulic acids are mainly under undissociated form (pKa = 4.5), that, due to their lipophilic properties, easily cross the periplasmic membrane and decrease cytoplasmic pH by dissociation into cytosol (Agnolucci et al., 2010). This means that in our study, *DbCD* expression would be expected to increase in the entire range of pH 3.5-4.5, and not only from pH 4 to 4.5, in order to convert acids into the corresponding vinyls. Interestingly, the maximal downregulation can be observed under conditions of pH 4. A hypothesis of this behavior of *DbCD* expression could be related to different mechanisms of the hydroxycinnamic acids uptake in *D. bruxellensis* CBS2499, by passive as well as by active transport, which would deserve more detailed analysis. However, we cannot also exclude the possibility of a strong downregulation resulting from the presence of higher level of SO_2 at low pH.

Although it has been suggested that the entry of the hydroxycinnamic acids into cells is facilitated by the localization of ethanol close to the dehydrated membrane (Sousa et al., 1996), a high ethanol concentration can generate a cessation of the *Db*CD enzyme activity reducing the conversion of the hydroxycinnamic acids into vinyl phenols (Benito et al., 2009). Moreover, ethanol can also determine a post-transcriptional regulation of the cinnamate decarboxylase affecting the protein activity (Clausen et al., 1994; Cavin et al., 1998). Thus, the same effect that ethanol produces on the membrane permeability is possibly the same exerted on enzyme's conformation since this last depends mainly on the hydrophobic interactions among the amino acid residues of the protein

(post-transcriptional regulation). We could speculate that the relative lower level of downregulation of *DbCD* gene observed in cells growing in presence of higher concentration of ethanol [0.25 mg/L, 4.5 and 12.5% (v/v)] could allow the cells compensating, by a transcriptional regulation of *DbCD* gene, a decreased enzyme activity.

Neither a main nor an interaction effect seem to influence *DbVPR* gene expression in the growth conditions under our study. However, the quadratic effect of pH and SO_2 show a significant role in its expression. Indeed, under oenological conditions, SO_2 causes undoubtedly oxidative stress, and we cannot forget that VPR enzyme has been identified in *D. bruxellensis* CBS4481 as a Zn/Cu superoxide dismutase (SOD1) (Granato et al., 2014).

The present study shows that the observed production of VPs, in the tested conditions, depends mainly on ethanol, as single factor, although pH is important in modulating the ethyl guaiacol yield. Moreover, a higher gene expression (run 4, Table 1) did not lead to a higher release of VPs (run 9, Table 1). This finding suggests that the transformation yield could be affected by factors other than *DbCD* and *DbVPR* regulation.

Ethanol plays a positive linear effect in the transformation of hydroxycinnamic acids to vinyl derivates. This result can support the finding that a lower dowregulation of the *DbCD* gene occurs at a high ethanol concentration when cells have to counteract a possible lost in enzyme conformation. Contrarily to what has been observed by Chandra and co-authors (2014), the SO_2 factor seems to have no effect on the effective production of ethyl phenols, and in general on the off-flavour yields. Nevertheless, different wines and winemaking procedures can affect the content of this chemical and, usually, a higher level is reached during aging, due to a mismanaging use of SO_2 by oenologists. Further experiments are so required to investigate the pathway of VPs by *B./D. bruxellensis* in real wines or under more severe conditions. Finally, due to a diverse capability to counteract the SO_2 stress, different *B./D. bruxellensis* strains could behave differently (Curtin et al., 2012b; Vigentini et al., 2013); however, this work suggests that the uncontrolled use of sulfur dioxide, besides not representing a sustainable choice, may not be an adequate strategy to protect wine from spoilage.

4.3 SO_2 stress resistance in *B./D. bruxellensis*: investigation at transcriptome level

Brettanomyces/Dekkera bruxellensis is a "well-known" spoilage yeast determining quality and, subsequently, economic damages once it contaminates wines (Chatonnet et al., 1992; Suárez et al., 2007). From the first description of the species (Gilliland,1961) the knowledge about the performance of growth and the spoilage feature of *B./D. bruxellensis* in wine environment has been continuously expanded; however, several research questions regarding its activity as contaminant remain to be clarified.

The wine sensory profile (Chatonnet et al., 1992) can be seriously affected by *B./D. bruxellensis* contamination; indeed, it does not need to growth to be dangerous (Agnolucci et al., 2010). Moreover, the ability to resist to the SO_2 addition in wine represents a further issue to counteract the spoilage. As previous studies have suggested (Curtin et al., 2012b; Vigentini et al., 2013), the response to SO_2 is a strain-dependent trait in *B./D. bruxellensis* and differences in both culturability and viability can arise depending on the investigated yeast haplotype (Agnolucci et al., 2014).

In order to improve understanding about the genetic mechanisms acting in response to SO_2 stress and to evaluate a possible species-response-activation, two different strains of *B./D. bruxellensis* have been investigated using a transcriptomic approach.

The environmental conditions in which this species is more likely to be found have been simulated using a wine-model medium. The yeast growth trend appears similar in the two investigated strains; in general, both yeasts showed a growth perturbation in the period after sulphur treatment and before the cellular recovery when more severe biomass oscillations were registered. This result supports the previous observation reported by Vigentini and collaborators (2008) which shown that pre-cultured *B./D. bruxellensis* cells in ethanol added medium are able to adapt themselves to the harsh medium conditions (low pH, low sugars availability, high ethanol concentration, anaerobiosis) easier that yeasts not exposed to an adaptation phase. With regards to sugar usage a difference between the two strains here studied has been observed; in particular, *B./D. bruxellensis* AWRI1499 consumed glucose and fructose sequentially contrary to *B./D. bruxellensis* CBS2499 that exhausted them simultaneously. As already described in other non-conventional yeasts these data could suggest that, although glucose and fructose are metabolised through the same route, different fluxes through the same metabolic pathways can occur (Merico et al., 2003). A combination of different effects exerted by the two sugars on transcriptional regulation of the involved genes, and/or different

kinetic properties of sugar carriers and enzymes involved in the transport and in the metabolism, could give rise to the observed sugar consumption (Barbin et al., 2008).

Recently, Capozzi and co-authors (2016) used a transcriptomic approach to investigate the molecular mechanisms involved in the Viable but non Culturable (VBNC) state in *B./D. bruxellensis*, condition in which *B./D. bruxellensis* appears to enter when environmental conditions became not suitable for its growth (Millet and Lonvaud-Funel, 2000; du Toit et al., 2005). The work analysed the transcriptional changing of cells entering and exiting this particular condition, inducing the stress by way of a SO_2 treatment; in particular, the stress factor could be removed by increasing the pH in order to eliminate SO_2 fraction responsible for antimicrobial activity. The discussion here presented is focused on Capozzi et al. (2016) being the only available reference using a transcriptomic approach on *B./D. bruxellensis* cells exposed to SO_2, unless otherwise specified. The exposure of cells to suboptimal growth conditions or any environment that negatively affect parameters such as cell viability or fitness can be considered stresses. Nonetheless different kind of stress, defined as mild, chronic or acute stresses, exist. Cell responses depend on the organism, the environment in which the stress is arising from and the physiological state of cells. Responses are usually defined by two components: a generic response, common to various types of stresses, and a specific adaptive response, characteristic of particular stresses. Both stress-specific and general responses are the reflection of mechanisms acting over a series of timescales. Post-translational effects provide immediate responses, while regulation of gene expression is essential for the slower, long-term adaptation and recovery phases (de Nadal et al., 2011).

The analysis of results arising from the study of a short and a long response against SO_2 exposure in *B./D. bruxellensis* highlights that, in both strains, the number of statistically differentially expressed genes is considerably smaller in the short response in comparison to the long response. Indeed, only a low number of genes results differentially expressed in the short time response, and few (3 in the AWRI1499 strain and 1 in the CBS2499, all being down-regulated) are those changing expression more than twofold. On the other hand, in the long response, genes that were found significantly differentially expressed were around a thousand, but approximatively the 10% had a $\log_2$ fold-change greater than |1|. This finding is in general consistent with the previous investigation of Capozzi and co-authors (2016) and it confirms that a primary response evolving in a more stable long response is missing in *B./D. bruxellensis* under SO_2 stress. Possibly due to the different SO_2 stress strategy applied to cells in Capozzi et al. (2016), a huge difference in the number of genes differentially

expressed was found between the time of cell recovery and untreated cells in respect to the number found in this study.

Viable but not culturable (VBNC) state has been described occurring in cells exposed to SO_2 (Agnolucci et al, 2010; Serpaggi et al., 2012; Agnolucci et al., 2014). Unfortunately, the use of fluorescent dyes used to detect cells affected by this *status* did not lead to consistent results (data not shown). Thus, because of the lack of reliable data about the viability of the *B./D. bruxellensis* cells, it cannot be said whether cells entered a viable but not culturable (VBNC) state after the SO_2 pulse and recovered, or if a small "resistant sub-population" of cells could overcoming the stress and slowly became dominant. Nonetheless, Serpaggi and collaborators (2012) defined the VBNC state being characterised by a reduced glycolytic flux coupled to changes in redox homeostatis/protein turnover-related processes. Considering that at the short response cells do not undergo to any change in gene expression in comparison to untreated cells, we could speculate that the SO_2 treatment led to death some "sensitive cells" and that, the remaining "resistant cells" are able to adapt themselves to new environmental conditions throughout the transcriptional modification recorded in the long response. However, more investigations are required in order to clarify this hypothesis.

Regarding the transcriptional events arising at the growth cell recovery, *B./D. bruxellensis* results adapted to the encountered environmental conditions; in fact, the gene expression detected in the long response might be defined as the occurrence of an "Environmental Stress Response" (ESR) where genes are similarly regulated in response to different stresses and are observed, among others, to refer to carbohydrate metabolism, transport and detoxification (de Nadal et al., 2011).

In general, cells modify their metabolism in the long response being different metabolic processes enriched by up- or down- regulated genes. The main changes regard the up-regulation of genes related to processes involving monocarboxylic acid, carbohydrate, acyl-CoA and sulphur compound, and functions such as the pyruvate dehydrogenase, carbohydrate kinase, succinate-CoA ligase.

As far the carbohydrate metabolism and transport, we observed that some genes of the sugars metabolism are up-regulated. In particular, *HXT13, GAL1/7/10, HXK1, YLR345W, YDR109C* of the sugar transport increase their expression. The combination of these genes with an increase in the dehydrogenase activity (*ADH6, BDH1, PDB1, SOR1*) and genes related to the pyruvate decarboxylase complex/carrier (*PDX1* and *LAT1*, *FMP37*), as well as genes involved in fatty acid oxidation (*FOX2, SPS19*), might let to make the assumption that the yeast is active in fermentative growth. Indeed, *HXT13* gene (AWRI1499_4725, AWRI identifiers code corresponding) is shared in both strains with a fold-change above 15 and it has been described as a putative transmembrane polyol transporter

that can uptake mannitol and sorbitol and supports growth, being induced by non-fermentable carbon sources and in low glucose concentrations (Greatrix and van Vuuren, 2006). Moreover, other than in response to sugar limitation, Tiukova et al., (2013) also found it expressed in conditions of oxygen limitation, being both conditions found also here. *SOR1*, highly up-regulated in this study, is reported to be induced in sorbitol or xylose containing media (Sarthy et al., 1994; Toivari et al., 2004) and with *BDH1*, a NAD-dependent (R,R)-butanediol dehydrogenase (González et al., 2000), are two zinc-containing enzymes part of the medium-chain dehydrogenase/reductase (MDR) family (Riveros-Rosas et al., 2003). Many MDR members are basic metabolic enzymes acting on alcohols or aldehydes, and thus these enzymes may have roles in detoxifying alcohols and related compounds, protecting against environmental stresses such as osmotic shock, reduced or elevated temperatures, or oxidative stress (Nordling et al., 2002).

Regarding the detoxification processes of the ESR in relation to SO_2 stress, the main gene involved in sulphite removal from cells in *S. cerevisiae* is *SSU1,* which encod a plasma membrane sulphite pump that can determine the sensitivity/resistance to the toxic action of sulphur compounds at strain level (Avram and Bakalinsky, 1997; Divol et al., 2006; Nardi et al., 2010; Nadai et al., 2016). Ssu1p is part of the major facilitator superfamily involved in efflux of toxic compounds, specifically mediating efflux of the free form of sulphite (Park and Bakalinsky, 2000). AWRI1499_0080, homologous of *SSU1*, shows a very high expression in the long term response, resulting increased more than 4 and 47 times in CBS2499 and AWRI1499, respectively. For a while, sulphite was not observed an inducer of *SSU1* activity in *S. cerevisiae* and it has been reported to be regulated by anaerobiosis (Yuasa et al., 2005). Contrarily, Nardi and collaborators, in 2010, gave evidence that sulphite is the main regulator of *SSU1* expression. The present study confirms that SSU1 exerts a strong detoxification of *B./D. bruxellensis* cells exposed to sulphur, pumping SO_2 out of the cell, as also observed by other researchers (Capozzi et al., 2016; Godoy et al., 2016; Nadai et al., 2016). However, the reason of such a regulation might be due to both factors, sulphur addition first, combined with the oxygen limitation.

In conclusion, this study confirms the great capability of *B./D. bruxellensis* yeasts to growth under highly restrictive conditions such as those found in wine, acidic pH, low sugar as well as oxygen availability and high ethanol concentration. Moreover, the ability of this yeast to counteract the stress induced by the addition of sulphur dioxide to the medium/wine, determines that a deep attention in the management of the winemaking process in the winery, is needed starting from maintaining a high level of cleaning of the vinification system (tanks and barriques as first considering) and

equipment as well (pumps, pipes etc.), in order to eliminate or at least minimize the potential for contamination of the product.

5. CONCLUSIONS

Volatile phenol metabolic pathway characterises the *B. bruxellensis* species. The production of these off-flavours is often considered detrimental to wine and their release is linked to the ability of *B. bruxellensis* to counteract the antimicrobial effect of sulphur dioxide and thus colonise winemaking equipment of the wineries. Thus, the deep investigation of the VP pathway in relation to the pressure of SO_2 and the molecular mechanisms governing the SO_2 resistance is required.

Whit this study has been demonstrated that the Rossmann fold-like region of VPR is conserved in *D. bruxellensis* wine yeasts, although nucleotide polymorphism of the gene is present and, apparently, it is no directly linked with the production of volatile phenols. This comment arises from the evidence that no correlation exists between genotype membership and off-flavours production. Moreover, VPR is a SOD; this evidence appears particularly interesting in terms of adaptive evolution of the species to wine environment. One of the most important physiological characteristics of *B. bruxellensis* allowing its survival and proliferation in wine is undoubtedly the resistance to sulphur dioxide (Curtin et al. 2012b; Vigentini et al., 2013), usually added in vinification and aging, which is known to generate oxidative stress (Niknahad and O'Brien, 2008; Kong et al., 1999; Ranguelova et al., 2010). On the other hand, this species has to manage a redox imbalance in strictly anaerobic conditions, also called "Custer effect" (Wijsman et al., 1984). Thus, in wine, SOD activity might serve to counteract the oxidative stress and VPR activity could help in cope the NAD^+/$NADP^+$ requirement to maintain active the metabolism; actually, Tchobanov et al. (2008) described the VPR as a NADH/NADPH-dependent enzyme. In addition, it has to be considered that although wine is a low nutrient environment, as far sugars and amino acids, it is still rich in reducible compounds such as hydroxycinnamic acids, the precursors of volatile phenols, and salts like zinc and copper, ions requested for SODs (SOD1) enzyme, at the end of the alcoholic fermentation. In this context, expressing a single protein suitable to exert a dual biological activity, dehydrogenases (SOD)/reductases (VPR), could represent a metabolic benefit for *B. bruxellensis*, thus limiting the biosynthetic burden.

With regards to the VP production it has been observed that, in the conditions here tested, it depends mainly on ethanol, as single factor, although pH is important in modulating the ethyl guaiacol yield. Moreover, a higher gene expression did not lead to a higher release of VPs, thus suggesting that the transformation yield could be affected by factors other than *DbCD* and *DbVPR* regulation. Ethanol plays a positive linear effect in the transformation of hydroxycinnamic acids to vinyl derivates, while, contrarily to what has been observed by Chandra and co-authors (2014), the SO_2 factor seems to have no effect on the effective production of ethyl phenols, and in general on the off-flavour yields.

Nevertheless, different wines and winemaking procedures can affect the content of this chemical and, usually, a higher level is reached during aging, due to a mismanaging use of SO_2 by oenologists. Further experiments are so required to investigate the pathway of VPs by *B./D. bruxellensis* in real wines or under more severe conditions. Finally, due to a diverse capability to counteract the SO_2 stress, different *B./D. bruxellensis* strains could behave differently (Curtin et al., 2012b; Vigentini et al., 2013). Regarding the sulphur stress, the main mechanism of resistance involves the *SSU1* gene, confirming what already seen in *S. cerevisiae*, (Nadai et al., 2016; Nardi et al., 2010; Divol et al., 2012). The heterogeneity within strains *B./D. bruxellensis* is characterized of, it is probably shown here also. Nonetheless only two strains have been investigated, the up-regulation involving *SSU1*, besides common is extremely enhanced in AWRI1499, where in the long-term response the gene changes its expression more than 47-fold. Further analysis in the way to understand whether or not this is the only mechanism, or at least the most important one for intracellular sulphur detoxification, will be useful to evaluate the possibility of an engineering of the species to make it is not able to resist those harsh conditions, thus avoiding quality and economic losses due to its presence. These results suggest that, unfortunately, the solution to "Brett-problems" is still quite far. In winemaking, the use of sulphur dioxide is widespread throughout the world. *B./D. bruxellensis* is, in general, a sulphur-resistant yeast and, whether alive or not, it is still able to affect wines in a detrimental way. Volatile phenol production does not seem to be affected by SO_2. Besides not representing a sustainable choice and neither a "safe additive", human health considering, the immoderate and uncontrolled use of sulphur dioxide may not be a suitable strategy to protect wine from spoilage.

6. MATERIALS AND METHODS

6.1 Cloning the putative gene of vinyl phenol reductase of *Dekkera bruxellensis* in *Saccharomyces cerevisiae*

6.1.1 Identification of the nucleotide sequence of putative VPR gene

The amino acid sequence of VPR enzyme of *D. bruxellensis* CBS4481 (Granato et al., 2014) was used to obtain the corresponding nucleotide (nt) sequence in the available genomes of *D. bruxellensis* CBS2499 and AWRI1499 strains. The putative VPR gene was identified through the BLASTP algorithm by comparison with the translated whole genome sequences listed in databases (www.ncbi.nlm.nih.gov).

6.1.2 Construction of VPR gene-expression vectors for *S. cerevisiae*

Commercial plasmid YEplac112 (ATCC® 87590™, Manassas, VA, USA) and pYX012 (R&D System, Wiesbaden, Germany) were used to build the expression vectors for cloning VPR in *E. coli* and in *S. cerevisiae* W303. YEplac112 is a multicopy plasmid (2µm origin) and it contains the TRP1 gene of *S. cerevisiae* as selective marker. pYX012 is an integrative vector and contains an expression cassette for heterologous protein production in *S. cerevisiae* constituted by the strong promoter of the triose phosphate isomerase gene of *S. cerevisiae* (*TPI1*), a multiple cloning site (MCS) and a polyA tail as terminator (Fig. 1). *Hind*III and *Eco*RI restriction enzymes (Thermo Scientific, Hudson, NH, USA) were used to create the appropriate ends in YEplac112 for inserting the expression cassette (*TPI1* promoter-MCS- polyA) extracted by PCR amplification from pYX012 with primers TPIpromF (5'-AAGCTTGGGAATAAGGGCGACACG-3') and TPItermR (5'-GAATTCCGCCATTCGCCATTCAGG-3') carrying the sequences of *Hind*III and *Eco*RI at the 5'end, respectively. The reaction mix contained 10 ng of pYX012 DNA, 0.1 µM of each primer, 200 µM dNTPs, 1X reaction buffer $MgCl_2$ free, 2.5 mM $MgCl_2$ and 1 U Taq polymerase (5 Prime, Hilden, Germany). The amplification was performed in a Mastercycler ep gradient S (Eppendorf, Hamburg, Germany). After a denaturation step for 5 min at 95°C, 35 cycles of amplification (consisting of 1 min at 95 °C, 1.5 min at 68 °C and 1 min at 72 ◦C) were carried out and were followed by a cycle at 72°C for 10 min. Amplification fragment was resolved in 1% (w/v) agarose gel (Agarose electrophoresis grade, Invitrogen, Carlsbad, CA, USA) at 100 V for 1 h using 1 × TAE (40 mM Tris pH 8.6, 20 mM acetic acid, 1 mM EDTA) as running buffer and detected by Ethidium Bromide staining (0.5 µg/mL). The band was visualised under UV exposition (GelDOC, Bio-Rad) and the PCR product was submitted for sequencing to verify the correct nucleotide sequence (Eurofins genomics, Ebersberg, Germany). The restricted vector

(YEplac112 cut *Hind*III/*Eco*RI) and the amplified fragment containing the *TPI1* promoter were ligated in a reaction mix containing 1x ligase buffer in presence of 1-2 U of T4 DNA ligase (Hoffmann La Roche, Basel, Switzerland). The reaction was incubated overnight at 16°C. A 1:4 molar ratio of vector to insert was maintained to obtain the expression vector YEp112T, according to manufacturer's instructions. Then, YEp112T was linearized to verify the expected final length in base pairs (6,494 bps).

The sequence encoding for the VPR protein (465 nt) was obtained by PCR amplification from *D. bruxellensis* CBS4481 genome (Fig. 20). Protocol for DNA extraction was the one described by Vigentini et al. (2012). Primers were designed to allow the insertion of VPR gene in *Bam*HI and *Sma*I restriction sites of YEp112T. Forward primer carries the recognition site for *Bam*HI at 5'end (VPRF: 5'-GTCGGATCCATGGTTAAAGCAGTTGCAG-3') (Fig. 20). As far the reverse primer, two oligonucletides with the sequence of *Sma*I at 5'end were built: VPRR (5'-ATTCCCGGGTTATGCAGACAAGCCAATG-3') (Fig. 20) and VPRRTAG (5'-ATTCCCGGGTTAATGATGATGATGATGATGTGCAGACAAGCCAA TG-3'). The latter allows the protein purification because of the presence of a polyhistidine-tag, an amino acid motif that consists of at least six histidine residues, at the C-terminus of the protein. The PCR amplifications were performed in a mixture containing 80-100 ng of genomic DNA from *D. bruxellensis* CBS4481, 50 pmol of each oligonucleotide primer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 2 U Taq DNA polymerase (5 Prime, Hilden, Germany) in a final volume of 50 µL. The temperature profile used the following cycling parameters: 5 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 63 °C, 1 min at 72 °C, and final extension 10 min at 72 °C. The amplified fragments were sequenced and nucleotide sequences checked to rule out the occurrence of any mutation during synthesis (Eurofins genomics). Amplicons were then *Bam*HI/*Sma*I double digested and cloned into the same sites of the restricted YEp112T expression vector. Two new vectors named YEp112TVPR (6934 bps PlasMapper Version 2.0) (Fig. 1) and YEp112TVPRT (6952 bps) were generated.

6.1.3 Yeasts and bacteria strains, media and protocols for microbial transformation

One Shot® TOP10 Chemically Competent *Escherichia coli* (Cat. No. C4040-10, Invitrogen Life Technologies, Carlsbad, CA, USA) cells were used as host for VPR gene cloning. Cells were routinely grown in LB broth (10 g/L NaCl, 10 g/L peptone, 5 g/L yeast extract, 1 g/L glucose) at 37°C. For

Fig. 20 Nucleotide sequence of VPR gene (5'- 3' strand) and position of primers used for the cloning (VPRF and VPRR) and the transcript analysis (vprRTF and vprRTR) of the gene in *S. cerevisiae* W303. Restriction sites for *BamH*I (5' end) and *Sma*I (3' end) are bold typed.

genetic manipulations, *E. coli* competent cells were transformed with $CaCl_2$/RbCl protocol as described by Kushner (1978). Transformed clones were check on LB medium supplemented with 2% (w/v) agar and 100 µg/mL ampicillin. Plasmid extraction was performed by QIAprep Miniprep Kit according to manufacturing's instructions (Qiagen, Hilden, Germany). *S. cerevisiae* W303 strain (MATα ade2-1 his3-11,15 trp1-1 ura3-1 leu2-3,112 can1-100) was genetically modified for expression the VPR gene by lithium acetate method (Hill et al., 1991). Two transformations were done using YEp112TVPR and YEp112TVPRT expression vectors. Transformants were selected in YNB basic medium (Difco Laboratories, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) without amino acids added with 20 g/L glucose, 5 g/L ammonium sulphate, 100 µg/L adenine, 50 µg/L histidine, leucine and uracil at 30°C; tryptophan was used as selective marker. To confirm successful transformation of the yeast strains, the VPR gene was amplified by PCR using the primer TPIpromF, designed on the TPI promoter of the expression cassette, and VPRR or VPRRTAG. The PCR conditions for the amplification: initial denaturation 5 min at 95°C; 35 cycles of denaturation 1 min at 95°C; 1 min annealing at 63°C; 1 min extension at 72°C and final extension 10 min at 72°C. After check on 1% (w/v) agarose gel and UV visualization of the corresponding expected bands (approx. 1648 bps),

the nt sequences were obtained by sequencing the negative DNA strand (Eurofins genomics). All strains were maintained in useful media added with 20% (v/v) glycerol at -80°C.

6.1.4 RNA extraction, cDNA synthesis and VPR expression

D. bruxellensis CBS4481, *P. pastoris* GS115, *S. cerevisiae* W303 and the transformed strains of *S. cerevisiae* #17 and #62 were grown in a chemically defined medium described by Verduyn (1992) modified adding a three times higher amount of traces and vitamins, at 25°C up to approximatively 3 OD_{600nm}. To avoid RNA degradation, biomass was quickly recovered by centrifugation at 15000 g for 1 min at 4°C and immediately frozen with liquid nitrogen. RNAs were extracted with the RNeasy Plus MINI Kit (Cat. No.74134, Qiagen, Hilden, Germany) with few modifications. Briefly, pellets were re-suspended in 500 µL RB buffer and added with 5 µL of 14 mM β-mercaptoethanol. An isovolume of sterile acid-washed glass beads (425-600 µm, Sigma Aldrich, Saint Louis, MO, USA) was added to cell suspensions and samples were treated for 3 cycles in a TissueLyser LT (Qiagen) alternating 2 min ON and 1 min on ice at maximum shaking speed. Supernatants were centrifuged 3 min at 16000 *g*, transferred in a new vial and immediately added with 500 µL 70% (v/v) ethanol. A treatment with DNAse I (Cat. No. 79254, Qiagen) was applied; the extracted RNA samples were treated with 10 µL DNaseI (0.2 KunitzU), to digest any contaminating genomic DNA following the manufacturers' instructions (RNase-Free DNase Set, Quiagen). RNA concentrations were quantified using the NanoDrop®ND-1000 Spectrophotometer (Wilmington, DE, USA) and integrity of RNA was controlled by agarose gel electrophoresis in denaturing conditions using 1% (v/v) formaldehyde. First strand cDNA was synthesized from 1 µg of RNA using QuantiTect Reverse Transcription Kit (cat. num. 205311, Qiagen) according to the supplied protocol and using the Oligo(dT)15 primers (Qiagen). cDNA was stored at -20˚C for further analysis. cDNA samples were amplified by PCR using the primer pair vprRTF (CTAAGGGCACTATCACGGACA) (Fig. 20) and vprRTR (CTGCAAAGAACCAGCATCA) (Fig. 20). PCR reactions were performed in a 20 µL reaction mixture consisting of 1× *Taq* Buffer with 1.5 mM $MgCl_2$ (5 Prime), 1 mM $MgCl_2$, 10 mM dNTPs, 0.5 µM of each primer, 1 µL of cDNA (10 times diluted cDNA synthesized from 1 µg of RNA) and 1 U *Taq* polymerase (5 Prime). All amplifications were carried out in a Mastercycler ep realplex (Eppendorf, Hamburg, Germany). PCR conditions were: initial denaturation at 95°C, 5 min; 35 cycles of denaturation at 95°C, 30 s; annealing at 54°C, 45 s; extension at 72°C, 1 min and final extension at 72°C, 5 min.

6.1.5 Preparation of cell extract, protein analysis and enzymatic activities of VPR and SOD

The determination of VPR enzymatic activity was performed using transformed cells of *S. cerevisiae* grew in the medium used for RNA extraction and described above. *S. cerevisiae* W303 was analysed as control. A volume of culture in stationary phase of growth (at approximatively 5 OD_{600nm}) corresponding to a total cell amount of 250 OD_{600nm} was centrifuged for 15 min at 4000 rpm (Hettich Zentrifugen, 380r) at 4°C. The pellet was washed twice with distilled water and resuspended in 500 mL of 50 mM potassium phosphate buffer pH 7.4 or pH 6.0, 10 mM β-mercaptoethanol. When *S. cerevisiae* strains producing the HIS-tagged VPR protein was used, three aliquots of a total cell amount of 250 OD_{600nm} in the same growth condition as above were collected, and resuspended in 1.5 mL of 50 mM TRIS-HCl, 100 mM NaCl and 6 mM imidazole pH 8. Cells were mechanically disrupted as for the RNA extraction protocol mentioned above but treating the cell suspension for 8 cycles. Then, the supernatant was collected and centrifuged at 13000 rpm (Hettich Zentrifugen, micro 200) at 4 °C for 30 minutes. In the case of extracts containing the HIS-tagged VPR protein, supernatants were 0.22 µm filtered. Protein purification was carried out on HIS-Select Nickel Affinity Gel #P6611 column (Sigma-Aldrich, St. Louis, USA). After ethanol removing, 50 mM TRIS-HCl, 100mM NaCl 6 mM imidazole pH 8 was added to the column allowing the gel packing and equilibrating. Cell extracts were slowly injected and a first fraction, representing the total protein pool of extract except the VPR, was collected eluting with 50 mM TRIS-HCl, 100mM NaCl 6 mM imidazole pH 8. Then, VPR fraction was collected eluting 50 mM TRIS-HCl, 100mM NaCl 250 mM imidazole pH 8. In total, six fractions were pooled. Imidazole was finally removed by ultrafiltration using Vivaspin centrifugal concentrators (Cut-Off 10,000 kDa, Sigma-Aldrich, St. Louis, USA) at 5,000 g, 5 °C, 30 minutes. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on 12% (33:1 acrylamide to bis-acrylamide) cross-linked polyacrylamide gels using a Miniprotein apparatus (Bio-Rad Laboratories). The gels were stained with 0.1% Coomassie blue R-250 in methanol–acetic acid–water, 40:10:50 (v/v) followed by destaining with methanol–acetic acid–water (40:10:50, v/v). Broad Range Molecular Markers (Biorad) were used for calibration.

Protein content of cell extracts and VPR purified protein was determined with Bio-Rad Protein Assay kit (500-002, Bio-Rad, Munich, Germany), using bovine serum albumin as standard.

VPR and SOD activities were assayed in duplicate as described by Granato et al., 2014. Specific activities were calculated considering that a unit (U) of enzyme activity is defined as 1 mmol of substrate transformed per minute using an extinction coefficient for NADH of 6.22 mmol/L/cm. Data

are reported as U/mg of total proteins when crude cell extracts were analysed or as U/mg of total protein if the purified VPR protein was used in the reaction mixture. As far biotransformations, they started in the same reaction mix used for the VPR activity assay at 28°C for 24h. Supernatants were filtered 0.22 µm and injected for HCPL analysis (see paragraph *HPLC analysis of volatile phenols*). Retention times of 4-vinyl-guaiacol and 4-ethyl-guaiacol were 26.8 min and 27.3 min, respectively.

6.1.6 HPLC analysis of volatile phenols

The HPLC analysis of volatile phenols was performed with a commercially available HPLC system composed by a Hitachi LaChrom L-7100 quaternary pump (Merck-Hitachi, USA) equipped with autosampler Hitachi LaChrom L-7400 (Merck Hitachi, USA) and UV-VIS single wavelength detector Hitachi L-4000 (Merck Hitachi, USA) set at 260 ŋm. The chromatographic separation of products was performed with a XTerra RP18 3.9*150mm 5µm 125 Å (Waters, USA) column at room temperature. The following gradient was used: 0-'water + 0.05% TFA/ MeOH + 0.05% TFA (90:10 v/v); 0-15'switch to water + 0.05% TFA/ MeOH + 0.05% TFA (50:50 v/v); 15'-25' switch to water + 0.05% TFA/ MeOH + 0.05% TFA (0:100 v/v); 25'-28' water + 0.05% TFA/ MeOH + 0.05% TFA (0:100 v/v); 28'-28.5' switch to water + 0.05% TFA/ MeOH + 0.05% TFA (90:10 v/v); 28.5'-36' water + 0.05% TFA/ MeOH + 0.05% TFA (90:10 v/v). The flow rate was set to 1 mL/min and EZ Chrome Elite software by Agilent was used for data management. Calibrations curves of each volatile phenols were carried out in a range of concentration of compound between 1 and 0.01 g/L. All the analyses were performed in triplicate.

6.1.7 Volatile phenol production in *D. bruxellensis* wine strains

The production of volatile phenols was evaluated in *Brettanomyces* Medium (BM) (Vigentini et al., 2013), a cultural broth simulating the wine composition, using the yeast collection provided in Table 1. The medium was prepared at two different ethanol concentrations, (v/v) 5% and 10%. In particular, the former was used to allow yeast adaptation to ethanol; then, yeast growth was carried out in BM supplemented with (v/v) 10% ethanol and 100 mg/L p-cumaric and ferulic acids, respectively. Briefly, as far the inoculum fresh cultures were obtained in YPD medium. At approximatively 3-4 OD_{600nm}, cells were transferred to sterile vials containing BM added with 5% ethanol and maintaining a 1% (v/v) percentage of inoculum, at 22°C. At approximatively 1 OD_{600nm}, cells were inoculated in BM added with 10% ethanol and volatile phenol precursors in 24-well plates at 1% (v/v) as inoculum. Plates were sealed with parafilm and incubated at 22 °C. Experiments were performed in duplicate. After reaching an OD_{600nm} at about 1-3, 1.5mL of cell cultures were collected and centrifuged at 13000 rpm (Hettich Zentrifugen, mikro 200) for 5 min. Supernatant were stored at -20°C until the HPLC

analysis. The production of volatile phenols per cell has been calculated as ratio between the total volumetric production of ethyl phenols and the total cell count (Table 1 and S1).

6.1.8 Sequencing of VPR gene from *D. bruxellensis* collection and SNPs identification

Single nucleotide polymorphisms (SNPs) and the heterozygosity degree of VPR gene in *D. bruxellensis* strains were investigated. Genomic DNAs were extracted as reported by Vigentini et al. (2012) and PCR reactions were carried out using primer VPRF and primers VPRR or VPRRTAG, depending on the vector used to generate the recombinant strains, and setting the conditions described in the paragraph "*Construction of VPR gene-expression vectors for* S. cerevisiae". A partial sequence of the VPR gene of 354 bps (on 465 bps of the full-length gene) was obtained by sequencing both DNA strands (Eurofins genomics). For sequence analysis, nucleotide alignments were performed using ClustalX 2.1 software (UCD Conway Institute, Dublin, Ireland). SNP frequency was calculated as the ratio between the number of SNPs and the total number of analysed nucleotides. In particular, frequency of heterozygosity was calculated as the ratio between the number of heterozygous SNPs and the total number of SNPs. The partial sequence of VPR of each strain was deposited in GenBank (www.ncbi.nlm.nih.gov/Genbank/) with accession numbers from KX611321to KX611336 (sequences will be released soon).

6.2 A Response Surface Methodology approach to investigate the effect of sulphur dioxide, pH and ethanol on *DbCD* and *DbVPR* gene expression and on the volatile phenol production in *Brettanomyces/Dekkera bruxellensis* CBS2499

6.2.1 Yeast strain and maintenance

Dekkera bruxellensis CBS2499 was used in this study. Its whole genome sequence is available at http://genome.jgi.doe.gov/Dekbr2/Dekbr2.home.html (Piskur *et al.,* 2012). Cells were stored in YPD medium (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, 5.5 pH) supplemented with 20% (v/v) glycerol at -80°C. Cell revitalization was performed inoculating the glycerol stock at 1% (v/v) in YPD broth. Cultures were placed into an incubator (Heidolph, Schwabach, Germany) at 30°C for 3 days.

6.2.2 Growth media and culture conditions

Experiments were run to collect yeast biomass for RNA extraction, retrotranscription and the analysis of gene expression by real-time quantitative PCR (qPCR). All fermentations were carried out in Simil-Wine Medium (SWM) (2.50 g/L glucose, 2.50 g/L fructose, 5 g/Lglycerol, 5 g/L tartaric acid, 0.50 g/L malic acid, 0.20 g/L citric acid, 4 g/L L-lactic acid, 1.70 g/L yeast nitrogen base w/o AA and ammonium sulfate (Difco, Sparks, USA), 0.005 g/L oleic acid, 0.50 mL tween 80, 0.015 g/L ergosterol, 0.020 g/L uracil, 0.010 g/L *p*-coumaric, 0.010 g/L ferulic acid, 1.50 g/L ammonium sulfate). Variants of SWM were prepared at different molecular SO_2 (below: SO_2) and ethanol concentration and pH value, adjusted with NaOH, depending on the conditions set by the chosen RSM (Table 1). Media was sterilized with 0.20 µm cellulose-nitrate filters. Cultural media were stored at 22°C prior the cell inoculation. SO_2 was added immediately before the inoculum from a 4 g/L sodium metabisulphite in mQ water. The theoretical content of molecular SO_2 was calculated according to Duckitt, 2012, Ribéreau-Gayon *et al.,* (2006) and Usseglio-Tomasset and Bosia (1984). Cellular growth was monitored by OD at 600nm. Fresh cells in YPD broth were centrifuged at 3500 rpm for 15 min (Hettich, ROTINA 380R, Tuttlingen, Germany); then, cells were washed in 0.9% (w/v) NaCl and inoculated at 0.1 OD_{600nm} in flask in SWM adjusted at 5% (v/v) ethanol, pH 4.5 and maintaining an air/medium ratio of at least 40% in order to ensure aerobic condition. Cellular pre-cultures were grown at 25°C for 3 days, in aerobic condition. An aliquot of the fresh cultures was analysed by plate count to calculate the exact number of viable cells transferred into each variant of the SWM for the RSM (Table 1). The inoculum was carried out at 0.25 OD_{600nm} in SWM modified as required by the RSM scheme (Table 1). The inoculated media were divided into 10 mL aliquots in sterile and

hermetically closed tubes with no headspace volume, and cultivated at 22°C in static condition. Each aliquot sample was used once for analyses. Cellular growth was monitored daily by total plate count and OD_{600nm} measurement. At 1.00 ± 0.2 OD_{600nm} cells two aliquots were pelleted by centrifugation (11000 rpm, 1 min, 4°C) (Hettich, ROTINA 380R, Tuttlingen, Germany), collecting a total cell amount of 20 OD_{600nm}, immediately frozen with liquid nitrogen and stored at -80°C until use. For the RSM scheme, the cultures were arranged according to the chosen experimental design.

6.2.3 Extraction of total RNA and cDNA synthesis

The extraction of total RNA from pellets was carried out using Presto Mini RNA Yeast Kit (Geneaid, New Taipei City, Taiwan) with few modifications. Briefly, cell lysis through mechanic disruption was performed in 500 µL Buffer RB, 5 µL β-mercaptoethanol and an iso-volume of glass beads (425-600 µm, 154 Sigma Aldrich, Saint Louis, MO, USA). Three breaking cycles with TissueLyzer (Qiagen, Hilden, Germany) for 2 min at the maximum oscillation frequency, interchanged with 1 minute on ice, were applied. The supernatant was centrifuged at 16000 *g* for 3 min (Hettich, Tuttlingen, Germany). The genomic DNA residue was degraded using 100 µL of 2KU/mL DNase (Sigma-Aldrich, Missouri, United States) for 15 min at room temperature. Following steps were carried out according to the manufacturing's instructions. RNA concentration was determined by measuring the absorbance at 260 nm (BioTek, Winooski, Vermont, United States). The integrity of RNA sample (0.3 µg RNA, 2 µL RNA loading Buffer 5X, H_2O DEPC up to 10 µL) was assessed, after 5 min treatment at 65°C, by electrophoresis on 1.2% agarose gel [90 mL DEPC water, 10 mL 10X formaldehyde gel buffer (200 mM MOPS, 50 mM sodium acetate, 10 mM EDTA) adjusted at 7 pH with NaOH prepared in DEPC water 37% (v/v) formaldehyde added. The electrophoretic run was carried out at 100 V for 1 hour and bands were UV visualized (Bio-Rad, Berkeley, California). RNAs were stored at -80°C until cDNA synthesis. The RNA retrotrascription was obtained with the QuantiTect Reverse Trascription Kit (Qiagen, Hilden, Germany). cDNAs were stored at -20°C until used for the qPCR assays.

6.2.4 Primer design

Five genes, pyruvate decarboxylase (*DbPDC*), aldehyde dehydrogenase (*DbALD*), actin (*DbACT*), eukariotic translational elongation factor (*DbEF*) and tubulin (*DbTUB*), were analyzed to identify a housekeeping gene suitable in the normalization process of the gene expression of cinnamate decarboxylase (*DbCD*) and vinylphenol reductase (*DbVPR*) (Table 2). Gene sequences of *DbPDC* and *DbALD* were identified using *S. cerevisiae* S288C (Schifferdecker *et al.,* 2014), *Komagataella phaffii* CBS7435 and GS115, *D. bruxellensis* CBS2499 (Piskur *et al.,* 2012) and *B. bruxellensis* AWRI1499 (Curtin

et al., 2012a) genomes. SGD (http://www.yeastgenome.org), NCBI (https://www.ncbi.nlm.nih.gov) and ENA (http://www.ebi.ac.uk/ena) databases were used as sequence sources. All alignments were performed through BLAST and ClustalIX2. Primer pairs were obtained at NCBI website (https://www.ncbi.nlm.nih.gov/tools/primer-blast) and validated for no forming neither self nor cross-dimers (https://www.thermofisher.com) (Table 2). The *DbCD* gene sequence for primer design was deduced by Godoy *et al.,* (2014).

6.2.5 PCR assays

Two sets of gene expression analysis were set up under different oenological conditions: i) to identify a suitable housekeeping (HKG) gene for gene expression normalization; ii) to analyze the relative expression of *DbCD* and *DbVPR*, by using the gene identified in i). As far the primers couples designed in this study for *DbCD*, *DbALD* and *DbPDC* genes, they were also validated by a standard PCR amplification in a 25 µL reaction composed by: 1U Taq, 200 µM dNTPs (Biotech rabbit, Dusseldorf, Germany), 1X Taq Buffer (Genscript, Piscataway, USA), 1 mM $MgCl_2$ (5Prime, Hilden, Germany), 0.1 µM primer forward and 0.1 µM primer reverse (Eurofins genomics, Ebersberg, Germany) and 80-100 ng DNA. The amplification cycle was: 95°C for 6 min, 95°C for 45 sec/54°C for 30 sec/72°C for 1 min (repeated 34 times), and 72°C for 10 min. Results were visualized on a 2% agarose gel prepared in TAE 1X buffer (20 mL TAE 50X, 980 mL demineralized water) and 0.5 µg/mL ethidium bromide. Electrophoresis was set at 80 V for 1.30 hour. PCR products were sequenced by an external provider (Eurofins genomics, Ebersberg, Germany).

As far qPCRs, they were performed in a Realplex Mastercycler Epgradient Thermocycler (Eppendorf, Hamburg, Germany) using a 15 µL reaction mix composed as follow: 2X SYBR green Master-Mix (Biotech rabbit, Dusseldorf, Germany), 200 nM-100 nM-50 nM primer forward and primer reverse (Eurofins genomics, Ebersberg, Germany), 10-fold dilution cDNA. The qPCR amplification cycle was set at 95°C for 30 seconds, 54°C for 30 sec and 65°C for 30 sec; repeated for 40 times. At the end of the reaction (95°C for 15 seconds), a melt-curve was generated by increasing the temperature from 60°C to 95°C, with a step at 0.5°C. All cDNAs were run as technical duplicates in a 96-well plate (Eppendorf, Hamburg, Germany). For each gene, three decimal serial dilutions at least were prepared into DNA LoBind tubes (Eppendorf, Hamburg, Germany) and stored at -20°C. The amplification curves were analyzed with Realplex software (Eppendorf, Hamburg, Germany).

The $2^{-\Delta\Delta Ct}$ method was applied on the basis of Livak and Schmittgen (2001) to calculate the relative expression of *DbCD* and *DbVPR* respect the chosen HKG expression. Results were expressed as fold-changes whereas the expression value of the target gene (normalized against *DbTUB* expression) was expressed as increase or decrease respect to its expression in the calibrator (for equivalent amount of samples) corresponding to the growth condition "LS" [0 mg/L mol. SO_2, pH 4.5 and 5% (v/v) ethanol] described in the paragraph "*Gene expression stability*".

6.2.6 Gene expression stability

The expression of *DbPDC*, *DbALD*, *DbACT*, *DbEF* and *DbTUB* genes was evaluated setting up a qPCR multiplex assay under two different oenological conditions of the SWM called "low-" and "high-" stringent (LS and HS, respectively) growth conditions. In particular, the LS condition was characterized by 0 mg/L mol. SO_2, pH 4.5 and 5% (v/v) ethanol while the HS condition by 0.25 mg/L mol. SO_2, pH 3.5 and 12.5% (v/v) ethanol. Yeast cultures were prepared in duplicate; three RNA extractions and the following cDNA synthesis were performed from each independent culture.

GeNorm analysis (Vandesompele *et al.*, 2002) (Genex software version 4.3.6, MultiD analyses, Goteborg, Sweden) was used to determine the stability of gene expression (termed M value), by analyzing each reference gene against the others in a pairwise variation that serially excludes the least stable gene (highest M-value) from the analysis. At the end, genes are ranked with an accepted cut-off value of 0.50 according to their expression stability. Normfinder algorithm (Genex software version 4.3.6, MultiD analyses, Goteborg, Sweden) separates the variation into an intragroup and an intergroup contribution. The analysis is repeated without considering the groups and this, estimates a robust standard deviation (SD) for each gene. The accumulated standard deviation (Acc. SD) is a reliable indicator of the number of reference genes to be used. All the genes were analyzed in the same assay to reduce any further experimental variability.

6.2.7 Experimental design and Response Surface Methodology

In order to investigate the expression of *DbCD* and *DbVPR* genes and the production of VPs in oenological conditions a Box-Behnken experimental design and Response Surface Methodology were applied. SWM samples were formulated with different level % ethanol (v/v) (5 – 8.75 – 12.5), pH values (3.5 – 4.0 - 4.5) and molecular SO_2 (mg/L) (0 – 0.125 – 0.25) (Table 1). The 15 trials provided

by Box-Behnken experimental design were analyzed using Statgraphics plus 5.1 software. The expression values of investigated genes were normalized with the HKG expression.

The fit of the model was evaluated by the linearity coefficient (R-squared). The regression approach was used to determine the effects produced by SO_2, pH and ethanol variables. The main effects (A, B, C) and both the linear (AB, AC, BC) and quadratic effects (AA, BB, CC) were statistically validated by analysis of variance. To identify the most important factors, a standardized Pareto chard is drawn. In particular, each effect is converted to a t-statistic by dividing it by its standard error (data not shown). These standardized effects are then plotted in decreasing order of absolute magnitude. Statistically relevant effects with a *p*-value less than 0.05 (95% confidence level) were reported in a response surface graph where the three-dimensional surface is described by a second-order polynomial equation.

6.2.8 Determination of VPs

The content of hydroxycinnamic acids, namely p-coumaric and ferulic acids, vinyl phenol, vinyl guaiacol, ethyl phenol and ethyl guaiacol in the cultures of the 15 runs of Box-Behnken experimental design was assessed in the obtained samples by an Acquity HClass UPLC (Waters, Milford, MA, USA) system equipped with a photo diode array detector 2996 (Waters). Chromatographic separations were performed with a Kinetex C18 150 x 3 mm, 2.6 µm particle size, 100 Å pore size (Phenomenex, Torrence, CA, USA). Eluting solvents were (A) trifluoroacetic acid 0.05% (v/v) and (B) methanol. The gradient program was 0.1 min, 20% B; 0.1-2 min, 35% B; 2-14 min, 58.5% B. The separation run was followed by 7 min of column rinsing and conditioning. The flow rate was 0.5 mL/min and the column temperature was 28°C. The samples were filtered with PVDF 0.22 µm filter prior the injection. Calibration curves were obtained for p-coumaric and ferulic acids, vinyl phenol, vinyl guaiacol, ethyl-phenol and ethyl guaiacol concentrations in the range from 0.1 mg/L to 20 mg/L. Quantification was performed according to the external standard method. Data acquisition and processing were carried out by Empower 2 software (Waters) at 320, 280 and 260 nm for hydroxycinnamic acids, ethyl phenols and vinyl phenols, respectively. Yield values of VPs were calculated as the molar ratio between each product (vinyl phenol, vinyl guaiacol, ethyl phenol and ethyl guaiacol) and the corresponding hydroxycinnamic acid potentially used as substrate. Data were analyzed with Statgraphics plus 5.1 using the RSM approach.

6.3 SO_2 stress resistance in *Brettanomyces/Dekkera bruxellensis*: investigation at transcriptome level

6.3.1 Yeasts, media and culturing conditions

Two completely sequenced strains of *B./D. bruxellensis*, AWRI1499 (Curtin et al., 2012a; https://www.ncbi.nlm.nih.gov/genome/11901?genome_assembly_id=40324) and CBS2499 (Piskur et al., 2012; http://genome.jgi-psf.org/Dekbr2), were used in this study. Yeasts were maintained in YPD medium [1% (w/v) Yeast extract, 2% (w/v) Peptone, 2% (w/v) Glucose, pH 5.6] 20% (v/v) glycerol supplemented, at -80°C. Fresh yeast cultures were prepared inoculating glycerol stocks at 1% (v/v) in YPD medium at 25°C for 3 days with shaking. Cells were collected (5000rpm x 5 min - Hettich, MIKRO 200, Tuttlingen, Germany) and washed once with saline isovolume (15 min at room temperature). Fifty mL of Simil-Wine Medium (Glucose 2.5 g/L, Fructose 2.5 g/L, Glycerol 5 g/L, Tartaric acid 5 g/L, Malic acid 0.5 g/L, Citric acid 0.2 g/L, L-lactic acid 4 g/L, YNB w/o AA & Ammonium sulphate (Difco, Sparks, USA) 1.7 g/L, Ammonium sulphate 1.5 g/L, Tween 80 0.5 mL/L, Uracil 20 mg/L, *p*-coumaric acid 10 mg/L, Ferulic acid 10 mg/L, Ergosterol 15 mg/L, Oleic acid 5 mg/L, pH 3.5) with ethanol 5% (v/v), were inoculated in flasks (100mL volume) at 25°C with shaking, at 0.1 Optical Density (Spectrophotometer Jenway, Staffordshire, UK) at 600nm (OD_{600}). At about 5 OD_{600} cells were collected by centrifugation (5000rpm x 5 min - Hettich, MIKRO 200, Tuttlingen, Germany) and inoculated at 0.1 OD_{600} in batches (800 mL) filled with SWM ethanol 10% (v/v).

6.3.2 Batch cultivations

Triplicate batch cultures for both strains were carried out in a Biostat-Q system (B-Braun, Melsungen, Germany) Fig. 21. Anaerobic conditions were obtained with N_2 insufflation before the inoculum and the concentration of dissolved oxygen was maintained below 20% of air saturation all over the duration of the experiment. Temperature was controlled at 22°C and 200 rpm was the stirring speed setting. Cellular growth was monitored daily by OD determination at 600 nm until reaching

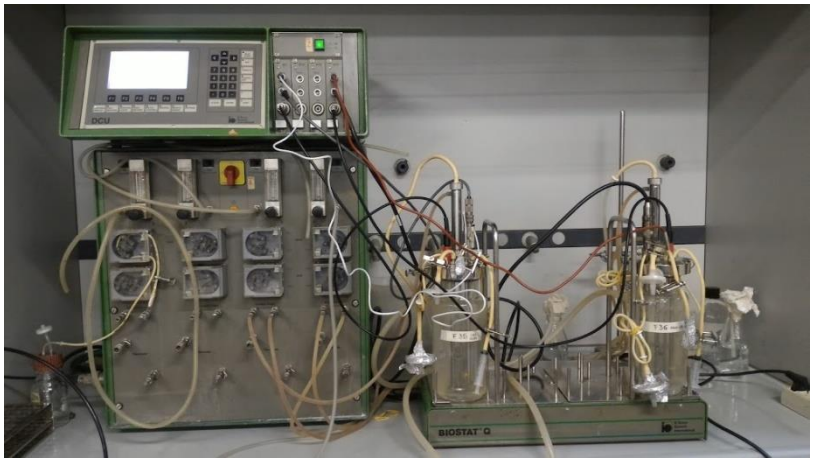

Fig. 21 Batch cultivation system.

1±0.1 OD_{600} then an SO_2 pulse was carried out. $Na_2S_2O_5$ stock solution was prepared according to Valdetara et al., (2017). The optical density (600 nm) was continuously monitored until cells recover their growth. The experiments were concluded when 0.5 ±0.1 g/L of fructose remained in the medium. Immediately before the pulse, 5 hours after it and at growth recovery, a cell amount of twenty OD_{600} per culture have been collected for further transcriptomic analysis.

6.3.3 Microbial and chemical analysis

Cells enumeration and chemical analysis were performed for each sample. In particular, yeast cultures were centrifuged (13000rpm x 3 min - Hettich, MIKRO 200, Tuttlingen, Germany) and supernatants were stored at -20°C for further analysis. Glucose and fructose determinations were obtained using Megazyme D-fructose/D-glucose assay kit according to manufactory instructions. Colony formant unit (CFU/mL) were obtained by plating 100 µL of useful decimal serial dilutions on WL medium, after 5-7 days incubation at 30°C.

6.3.4 Transcriptomic analysis

Before the SO_2 pulse, 5h later and at cell recovery samples were collected for further transcriptomic analysis. A cell amount of twenty OD_{600} per sample was frozen with liquid nitrogen immediately after a centrifugation step (adaptors for 50 mL tubes were previously cool down in order to maintain RNA integrity) at 11000 rpm for 1 minute at 4° C (Hettich, ROTINA 380R, Tuttlingen, Germany). All pellets were stored at -80°C until use. Samples were collected in triplicate. RNA extractions were carried out on the basis of Presto Mini RNA Yeast Kit protocol (Geneaid, New Taipei City, Taiwan) manufactory's instructions with few modifications. Breafly, pellets were resuspended with RB Buffer 500 µL and β-mercaptoethanol 5 µL. An isovolume of glass beads (425-600 µm, 154 Sigma Aldrich, Saint Louis, MO, USA) was added and cells lysis was obtained through mechanic disruption with TissueLyser LT (Qiagen, Hilden, Germany) with 3 cycles (2min ON - 1 min OFF, in ice) at the maximum oscillation frequency. Genomic DNA residue degradation was achieved using 100 µL of 2KU/mL DNase I (Sigma-Aldrich, Missouri, United States) in 15 min at room temperature. Following steps were carried out according to the manufacturing's instructions. RNAs were quantified measuring the absorbance at 260 ηm in a PowerWave XS2 spectrophotometer (BioTek, Winooski, Vermont, United States). The integrity of RNA sample (0.3 µg RNA, 2 µL RNA loading Buffer 5X, H_2O DEPC up to 10 µL) was assessed, after 5 minutes treatment at 65°C, by electrophoresis on 1.2% agarose-FA gel [90 mL DEPC water, 10 mL 10X formaldehyde gel buffer (200 mM MOPS, 50 mM sodium acetate, 10 mM EDTA) adjusted at pH 7 with NaOH prepared in DEPC water 37% (v/v) formaldehyde added]. The

electrophoretic run was carried out at 100 V for 1 hour and then bands were visualized placing gel under UV irradiation (Bio-Rad, Berkeley, California). RNAs were maintained at -80°C until sample were submitted to sequencing. Transcriptome analysis were conducted by an external provider (CNR, Istituto di Biomembrane e Bioenergetica, Bari, Italy). RNAs were purified and then submitted to NGS-sequencing [NextSeq® 500/550 Mid Output Kit, v2 (150 cycles), FC-404-2001Illumina].

6.3.5 RNA-seq data elaboration

First, raw reads obtained from sequencer were submitted to FastQC for quality evaluation, then reads were mapped to a reference genome *Brettanomyces bruxellensis* AWRI1499, (Curtin et al., 2012a) with hisat2 (v2-2.1.0) (Kim et al., 2015) and subsequently a quantification was done using Cufflinks package (v2.2.1) (Trapnell et al., 2013). Results obtained from quantification (Cuffquant) were normalized (Cuffnorm) and tested for differential expression (Cuffdiff), thus obtaining FPKM (Fragments per Kilobase of Million mapped reads) gene expression and $\log_2$fold-change values, respectively. TPM (Transcript Per Million) values were calculated from FPKM values: the formula for TPM calculation has been derived from Pachter (2011) and the R script below has been used to obtain them for the PCA analysis.

```
mat_sum = df_mat.sum(axis=1)

tpm_mat = (df_mat.T / mat_sum).T * (10**6)

where "df_mat" is matrix of FPKM values
```

Genes statistically (FDR-adjusted *p*-value <0.05) differentially expressed more than twofold were used to identify Gene Ontology (GO) categories significantly (Bonferroni corrected *p*-value <0.01) enriched. Gene Ontology enrichment analysis was performed and visualised using http://go.princeton.edu/cgi-bin/GOTermFinder and REVIGO (Supek et al., 2011) e-tools. With the aim of removing redundant GO terms from the list of processes/functions/components below the *p*-value cutoff and summarizing the outcome, results from the enrichment analysis were submitted to REVIGO, applying the default setting [a medium allowed similarity (0.7); the functional similarity measure *simRel* (Schlicker et al., 2006) as semantic similarity score; *S. cerevisiae* GO term as database].

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Appendix 1

Table S1 Volumetric productions of 4-vynil and 4-ethyl phenols expressed in g/L.

Strain (CBS#)	UFC/mL (x 10^7)	*p*-cumaric acid	Ferulic acid	Volumetric production (g/L)				
				4-vinyl phenol	4-vinyl guaiacol	4-ethyl phenol	4-ethyl guaiacol	Total ethyl phenols
Not inoculated	-	0.109 ± 0.002	0.095 ± 0.003	nd	nd	nd	nd	-
73	1.99	nd	nd	nd	nd	0.053 ± 0.006	0.037 ± 0.006	0.090
74	0.11	0.107 ± 0.003	0.095 ± 0.006	<0.005	<0.005	nd	nd	n.d.
1941	1.95	0.015 ± 0.000	0.012 ± 0.000	nd	nd	0.046 ± 0.004	0.045 ± 0.013	0.091
1942	2.41	<0.005	<0.005	nd	nd	0.039 ± 0.001	0.035 ± 0.012	0.074
1943	2.29	<0.005	<0.005	nd	nd	0.056 ± 0.005	0.043 ± 0.004	0.099
2499	2.62	<0.005	<0.005	nd	nd	0.055 ± 0.009	0.051 ± 0.012	0.106
2547	2.85	nd	nd	nd	nd	0.036 ± 0.002	0.025 ± 0.001	0.061
2796	2.61	nd	nd	nd	nd	0.051 ± 0.004	0.041 ± 0.003	0.092
4481	2.38	<0.005	0.012 ± 0.001	nd	nd	0.052 ± 0.009	0.041 ± 0.006	0.098
4459	1.99	<0.005	<0.005	nd	nd	0.053 ± 0.001	0.045 ± 0.003	0.093
4482	2.38	nd	nd	nd	nd	0.047 ± 0.002	0.035 ± 0.003	0.082
4601	1.41	<0.005	<0.005	nd	nd	0.053 ± 0.004	0.037 ± 0.001	0.090
4602	2.59	nd	nd	nd	nd	0.043 ± 0.002	0.027 ± 0.002	0.070
5206	0.84	0.077 ± 0.006	0.069 ± 0.006	<0.005	<0.005	0.015 ± 0.001	0.020 ± 0.002	0.035

Appendix 2

AWRI1499 ~ T5h-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.891005	5.00E-05	3.54E-04	AWRI1499_4045	*CPR3*
0.773886	1.50E-03	6.97E-03	AWRI1499_2114	*FCY1*
0.687059	5.00E-05	3.54E-04	AWRI1499_4656	*HSP26*
0.683857	1.43E-02	4.61E-02	AWRI1499_2715	
0.661391	6.25E-03	2.33E-02	AWRI1499_4854	
0.583571	5.00E-03	1.93E-02	AWRI1499_3023	*IPI3*
0.577116	2.00E-03	8.90E-03	AWRI1499_0727	*HSP26*
0.57147	6.30E-03	2.34E-02	AWRI1499_3742	*AAH1*
0.518253	4.50E-03	1.77E-02	AWRI1499_4307	*NOP7*
0.506659	1.17E-02	3.91E-02	AWRI1499_3741	*RPP2b*
0.491955	1.07E-02	3.63E-02	AWRI1499_0268	*HCH1*
0.486331	8.25E-03	2.93E-02	AWRI1499_1371	*BOS1*
0.486013	8.20E-03	2.92E-02	AWRI1499_3589	*YBR096W*
0.485346	9.85E-03	3.39E-02	AWRI1499_4378	*UTP8*
0.483169	9.00E-03	3.15E-02	AWRI1499_3729	*YPL199C*
0.477412	1.07E-02	3.63E-02	AWRI1499_3806	*ERT1*
0.47685	1.17E-02	3.91E-02	AWRI1499_4780	*TUL1*
0.473359	1.36E-02	4.42E-02	AWRI1499_1798	
0.454684	1.24E-02	4.10E-02	AWRI1499_1617	*SUB1*
-0.440447	1.44E-02	4.62E-02	AWRI1499_4539	*KIN1*
-0.443779	1.56E-02	4.95E-02	AWRI1499_0308	
-0.451025	1.26E-02	4.15E-02	AWRI1499_3777	*KCS1*
-0.45449	1.52E-02	4.85E-02	AWRI1499_0307	*TGL4*
-0.457685	1.35E-02	4.40E-02	AWRI1499_1763	*FAB1*
-0.459079	1.24E-02	4.09E-02	AWRI1499_1402	*DNF3*
-0.459452	1.53E-02	4.86E-02	AWRI1499_0741	*SAC3*
-0.460785	1.17E-02	3.90E-02	AWRI1499_1680	
-0.460816	1.41E-02	4.56E-02	AWRI1499_3324	*MDM1*
-0.462394	1.16E-02	3.87E-02	AWRI1499_1653	*GCN1*

AWRI1499 ~ T5h-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.462906	1.24E-02	4.09E-02	AWRI1499_2223	*YPK9*
-0.463111	1.35E-02	4.38E-02	AWRI1499_0802	*POP4*
-0.46501	1.17E-02	3.91E-02	AWRI1499_4463	
-0.46673	1.10E-02	3.71E-02	AWRI1499_3121	
-0.47005	1.08E-02	3.66E-02	AWRI1499_1987	
-0.470499	1.03E-02	3.51E-02	AWRI1499_4556	*STE20*
-0.47115	1.43E-02	4.61E-02	AWRI1499_1045	*GAT2*
-0.47254	1.22E-02	4.05E-02	AWRI1499_3367	
-0.474348	7.85E-03	2.81E-02	AWRI1499_3969	*DNM1*
-0.480245	9.45E-03	3.28E-02	AWRI1499_0172	*MON1*
-0.48115	8.40E-03	2.97E-02	AWRI1499_2799	*TAF2*
-0.48396	8.75E-03	3.07E-02	AWRI1499_4826	*CIT1*
-0.485801	1.51E-02	4.81E-02	AWRI1499_0561	*MYO1*
-0.487975	6.30E-03	2.34E-02	AWRI1499_3987	
-0.49433	9.40E-03	3.26E-02	AWRI1499_3205	
-0.495389	1.52E-02	4.85E-02	AWRI1499_4071	
-0.496417	1.33E-02	4.35E-02	AWRI1499_2037	*CCR4*
-0.49716	5.95E-03	2.23E-02	AWRI1499_4771	*SLN1*
-0.500161	1.02E-02	3.48E-02	AWRI1499_1611	*USO1*
-0.501047	1.10E-02	3.70E-02	AWRI1499_2548	*CAT8*
-0.501111	1.30E-02	4.27E-02	AWRI1499_2945	
-0.506976	7.35E-03	2.66E-02	AWRI1499_0551	*TRK2*
-0.512954	5.90E-03	2.22E-02	AWRI1499_4868	
-0.515582	4.55E-03	1.79E-02	AWRI1499_1555	*MNN4*
-0.517438	5.25E-03	2.01E-02	AWRI1499_3608	*KHA1*
-0.518905	4.90E-03	1.90E-02	AWRI1499_0406	
-0.519025	4.85E-03	1.88E-02	AWRI1499_2615	*MET32*
-0.520144	5.00E-03	1.93E-02	AWRI1499_3886	*DYN1*
-0.52215	6.05E-03	2.27E-02	AWRI1499_3198	*IQG1*

AWRI1499 ~ T5h-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.524246	5.60E-03	2.12E-02	AWRI1499_2257	
-0.524407	4.30E-03	1.70E-02	AWRI1499_1618	*MNR2*
-0.524495	3.95E-03	1.59E-02	AWRI1499_0343	*OAF1*
-0.526291	8.70E-03	3.06E-02	AWRI1499_0441	*YNL144C*
-0.527849	9.35E-03	3.25E-02	AWRI1499_2604	*TRE2*
-0.529448	7.80E-03	2.80E-02	AWRI1499_1104	*YGR021W*
-0.529772	9.40E-03	3.26E-02	AWRI1499_1629	*CHS3*
-0.531145	3.70E-03	1.50E-02	AWRI1499_0318	*SBE2*
-0.531529	6.25E-03	2.33E-02	AWRI1499_2672	*SVF1*
-0.535598	4.30E-03	1.70E-02	AWRI1499_3861	*PFK27*
-0.536878	4.25E-03	1.69E-02	AWRI1499_1679	
-0.537905	4.95E-03	1.92E-02	AWRI1499_1612	*USO1*
-0.539561	2.95E-03	1.24E-02	AWRI1499_0345	
-0.539599	7.95E-03	2.84E-02	AWRI1499_2063	*RAV1*
-0.540769	8.05E-03	2.87E-02	AWRI1499_1628	*CHS3*
-0.541107	2.20E-03	9.65E-03	AWRI1499_3692	*YML002W*
-0.542098	8.75E-03	3.07E-02	AWRI1499_0888	*PBN1*
-0.543401	2.70E-03	1.15E-02	AWRI1499_0405	
-0.543919	2.90E-03	1.22E-02	AWRI1499_2605	*TRE2*
-0.545286	4.25E-03	1.69E-02	AWRI1499_2637	*OPT2*
-0.546065	2.60E-03	1.11E-02	AWRI1499_0144	*MDL2*
-0.548424	2.60E-03	1.11E-02	AWRI1499_2658	*PSK2*
-0.550126	3.75E-03	1.52E-02	AWRI1499_3885	
-0.555835	2.05E-03	9.08E-03	AWRI1499_4399	*NUP170*
-0.560682	7.30E-03	2.65E-02	AWRI1499_0923	*PDE2*
-0.561526	3.90E-03	1.57E-02	AWRI1499_3163	*BRR2*
-0.562327	6.50E-03	2.41E-02	AWRI1499_1472	*STE4*
-0.5644	4.35E-03	1.72E-02	AWRI1499_4028	*POL2*
-0.569723	2.40E-03	1.04E-02	AWRI1499_1946	*YPR117W*

AWRI1499 ~ T5h-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.569829	8.55E-03	3.02E-02	AWRI1499_2094	*YAK1*
-0.57035	1.44E-02	4.63E-02	AWRI1499_0389	
-0.570906	1.23E-02	4.06E-02	AWRI1499_4557	
-0.571683	2.70E-03	1.15E-02	AWRI1499_0016	
-0.571691	2.10E-03	9.28E-03	AWRI1499_0176	*ATG1*
-0.571979	1.12E-02	3.76E-02	AWRI1499_2193	*PTR3*
-0.581294	7.30E-03	2.65E-02	AWRI1499_2796	*HSL7*
-0.581875	3.20E-03	1.33E-02	AWRI1499_2700	
-0.584263	8.60E-03	3.03E-02	AWRI1499_3412	*SNU114*
-0.584787	1.35E-03	6.38E-03	AWRI1499_0450	*YFL042C*
-0.586187	4.20E-03	1.67E-02	AWRI1499_2252	*EPL1*
-0.588428	1.75E-03	7.94E-03	AWRI1499_0862	
-0.588961	4.00E-03	1.60E-02	AWRI1499_0015	*VAM6*
-0.597603	8.50E-04	4.27E-03	AWRI1499_4199	*PTC6*
-0.598879	7.50E-04	3.83E-03	AWRI1499_0522	*BCK1*
-0.601366	9.00E-04	4.49E-03	AWRI1499_0585	*MET1*
-0.601385	1.20E-03	5.76E-03	AWRI1499_4869	*YPL150W*
-0.610474	1.65E-03	7.56E-03	AWRI1499_4186	*SAM50*
-0.610903	2.25E-03	9.84E-03	AWRI1499_1638	*UTP10*
-0.611595	9.00E-03	3.15E-02	AWRI1499_0041	*RRN6*
-0.615166	1.50E-03	6.97E-03	AWRI1499_1285	*MRH4*
-0.615782	2.30E-03	1.00E-02	AWRI1499_1700	
-0.617528	4.00E-04	2.24E-03	AWRI1499_2237	*CDC15*
-0.623043	1.50E-03	6.97E-03	AWRI1499_2999	
-0.623772	6.50E-04	3.39E-03	AWRI1499_2034	*MSG5*
-0.62587	1.00E-03	4.91E-03	AWRI1499_3351	*CSF1*
-0.628168	1.20E-03	5.76E-03	AWRI1499_3373	*UTP20*

AWRI1499 ~ T5h-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.633937	8.00E-04	4.06E-03	AWRI1499_4952	*CRZ1*
-0.634757	4.50E-04	2.48E-03	AWRI1499_3933	*CCH1*
-0.641231	4.60E-03	1.80E-02	AWRI1499_1734	
-0.641362	2.15E-03	9.47E-03	AWRI1499_2865	
-0.645429	8.50E-04	4.27E-03	AWRI1499_4037	*GIS3*
-0.645662	7.50E-04	3.83E-03	AWRI1499_0133	*MAG2*
-0.657185	2.50E-04	1.48E-03	AWRI1499_3199	*IQG1*
-0.660698	4.00E-04	2.24E-03	AWRI1499_3204	
-0.663768	6.65E-03	2.45E-02	AWRI1499_3883	
-0.676627	5.50E-04	2.94E-03	AWRI1499_4738	*KOG1*
-0.67663	2.00E-04	1.22E-03	AWRI1499_2215	
-0.678536	2.30E-03	1.00E-02	AWRI1499_2251	*EPL1*
-0.687003	5.50E-04	2.94E-03	AWRI1499_2718	
-0.699647	2.20E-03	9.65E-03	AWRI1499_0390	*RAD28*
-0.701283	6.50E-04	3.39E-03	AWRI1499_1695	*MDN1*
-0.704549	1.50E-04	9.48E-04	AWRI1499_3520	
-0.705133	3.80E-03	1.53E-02	AWRI1499_3973	
-0.722859	3.50E-04	1.99E-03	AWRI1499_3964	*MUM2*
-0.724391	1.50E-04	9.48E-04	AWRI1499_4282	
-0.724493	2.90E-03	1.22E-02	AWRI1499_2547	*CAT8*
-0.725235	1.00E-04	6.63E-04	AWRI1499_4175	*YPR097W*
-0.733919	7.00E-03	2.56E-02	AWRI1499_4136	
-0.738554	1.00E-03	4.91E-03	AWRI1499_2195	
-0.748171	1.80E-03	8.14E-03	AWRI1499_2138	*YBR238C*
-0.749516	7.20E-03	2.62E-02	AWRI1499_1590	*BUD14*
-0.751014	1.00E-04	6.63E-04	AWRI1499_1607	*PTP2*
-0.755507	5.00E-05	3.54E-04	AWRI1499_1739	

AWRI1499 ~ T5h-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.764159	1.57E-02	4.98E-02	AWRI1499_3429	
-0.767835	1.50E-04	9.48E-04	AWRI1499_3963	
-0.774344	2.00E-04	1.22E-03	AWRI1499_1818	*VPS34*
-0.777439	1.00E-04	6.63E-04	AWRI1499_2937	
-0.783106	5.00E-05	3.54E-04	AWRI1499_4305	
-0.78676	4.00E-04	2.24E-03	AWRI1499_2214	*LST4*
-0.791103	5.00E-05	3.54E-04	AWRI1499_0867	*RTC1*
-0.794557	1.50E-03	6.97E-03	AWRI1499_4306	
-0.79538	6.65E-03	2.45E-02	AWRI1499_1655	
-0.812411	5.00E-05	3.54E-04	AWRI1499_3418	
-0.814235	5.00E-05	3.54E-04	AWRI1499_0224	*CLB3*
-0.815842	5.00E-05	3.54E-04	AWRI1499_0404	*HMS1*
-0.823651	5.00E-05	3.54E-04	AWRI1499_0805	*ALY2*
-0.835444	1.15E-03	5.55E-03	AWRI1499_0782	
-0.838482	5.00E-05	3.54E-04	AWRI1499_4158	*MDM31*
-0.87122	5.00E-05	3.54E-04	AWRI1499_0333	
-0.873601	1.50E-04	9.48E-04	AWRI1499_3090	*GAL4*
-0.898912	5.00E-05	3.54E-04	AWRI1499_3884	*DYN1*
-0.902178	5.00E-05	3.54E-04	AWRI1499_2469	*AGC1*
-0.920929	5.00E-05	3.54E-04	AWRI1499_1540	
-0.924281	1.06E-02	3.61E-02	AWRI1499_0803	
-0.929805	5.00E-05	3.54E-04	AWRI1499_3755	*YSR3*
-0.943889	5.00E-05	3.54E-04	AWRI1499_3931	
-0.964914	5.00E-05	3.54E-04	AWRI1499_1287	*PLC1*
-1.07307	5.00E-05	3.54E-04	AWRI1499_3326	*IRE1*
-1.10199	5.00E-05	3.54E-04	AWRI1499_1252	*PCL1*
-1.11089	5.00E-05	3.54E-04	AWRI1499_2222	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
7.05137	5.00E-05	3.54E-04	AWRI1499_1841	*RCF2*
6.12709	5.00E-05	3.54E-04	AWRI1499_1936	*MPH2*
5.56535	5.00E-05	3.54E-04	AWRI1499_0080	*SSU1*
4.42582	5.00E-05	3.54E-04	AWRI1499_3505	*SOR1*
3.93668	5.00E-05	3.54E-04	AWRI1499_4725	*HXT13*
3.91687	5.00E-05	3.54E-04	AWRI1499_3717	
3.88639	5.00E-05	3.54E-04	AWRI1499_4728	*GAL10*
3.73598	5.00E-05	3.54E-04	AWRI1499_4351	*IMA1*
3.36327	5.00E-05	3.54E-04	AWRI1499_0109	*HXK1*
3.26361	5.00E-05	3.54E-04	AWRI1499_0089	
3.2369	5.00E-05	3.54E-04	AWRI1499_2435	
3.21597	5.00E-05	3.54E-04	AWRI1499_2713	*BDH1*
3.20285	5.00E-05	3.54E-04	AWRI1499_4733	
3.20093	5.00E-05	3.54E-04	AWRI1499_3271	*NTH1*
3.14129	5.00E-05	3.54E-04	AWRI1499_3429	
3.10826	5.00E-05	3.54E-04	AWRI1499_4884	*IMA1*
3.10809	5.00E-05	3.54E-04	AWRI1499_4727	*GAL1*
3.01971	5.00E-05	3.54E-04	AWRI1499_4109	*ACH1*
2.99262	5.00E-05	3.54E-04	AWRI1499_3210	*PGM2*
2.92652	5.00E-05	3.54E-04	AWRI1499_2202	*GSY1*
2.92531	5.00E-05	3.54E-04	AWRI1499_0332	*LSC1*
2.89695	5.00E-05	3.54E-04	AWRI1499_4832	*YDR109C*
2.89616	5.00E-05	3.54E-04	AWRI1499_3771	*LSC2*
2.8735	5.00E-05	3.54E-04	AWRI1499_1045	*GAT2*
2.86917	5.00E-05	3.54E-04	AWRI1499_1608	*RGI2*
2.81902	5.00E-05	3.54E-04	AWRI1499_4172	
2.80076	5.00E-05	3.54E-04	AWRI1499_4194	
2.76277	5.00E-05	3.54E-04	AWRI1499_2432	*INO1*
2.63891	5.00E-05	3.54E-04	AWRI1499_1615	*FOX2*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
2.57004	5.00E-05	3.54E-04	AWRI1499_4297	*TMA10*
2.53274	5.00E-05	3.54E-04	AWRI1499_0430	*ADH3*
2.52381	5.00E-05	3.54E-04	AWRI1499_0599	*YLR345W*
2.51737	5.00E-05	3.54E-04	AWRI1499_4492	*PDX1*
2.48904	5.00E-05	3.54E-04	AWRI1499_2013	*LAT1*
2.46616	8.00E-04	4.06E-03	AWRI1499_2863	*YLL056C*
2.43195	5.00E-05	3.54E-04	AWRI1499_1614	*FOX2*
2.39964	5.00E-05	3.54E-04	AWRI1499_2608	*PDB1*
2.34071	5.00E-05	3.54E-04	AWRI1499_3343	*CTR1*
2.30745	5.00E-05	3.54E-04	AWRI1499_0623	*YJL068C*
2.26916	5.00E-05	3.54E-04	AWRI1499_1678	
2.10457	5.00E-05	3.54E-04	AWRI1499_2466	*YBR053C*
2.09964	5.00E-05	3.54E-04	AWRI1499_2002	*LEU5*
2.09652	5.00E-05	3.54E-04	AWRI1499_3162	*PDA1*
2.08373	5.00E-05	3.54E-04	AWRI1499_0238	*YMR315W*
2.03828	5.00E-05	3.54E-04	AWRI1499_3824	*ADH7*
2.03699	5.00E-05	3.54E-04	AWRI1499_1840	
2.02619	5.00E-05	3.54E-04	AWRI1499_2675	*ROT2*
2.02506	5.00E-05	3.54E-04	AWRI1499_1545	*VHR1*
2.01528	5.00E-05	3.54E-04	AWRI1499_2876	
1.97941	5.00E-05	3.54E-04	AWRI1499_4726	*GAL7*
1.9792	5.00E-05	3.54E-04	AWRI1499_3675	
1.97873	5.00E-05	3.54E-04	AWRI1499_1318	*HXT13*
1.95469	5.00E-05	3.54E-04	AWRI1499_0088	*TSR4*
1.94399	5.00E-05	3.54E-04	AWRI1499_1937	*SUC2*
1.92615	5.00E-05	3.54E-04	AWRI1499_1209	*SCO1*
1.92257	5.00E-05	3.54E-04	AWRI1499_2674	*ROT2*
1.91168	1.50E-04	9.48E-04	AWRI1499_2717	*BDH1*
1.90734	5.00E-05	3.54E-04	AWRI1499_4231	*YKR070W*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
1.89944	5.00E-05	3.54E-04	AWRI1499_3802	*ADH6*
1.89901	5.00E-05	3.54E-04	AWRI1499_4413	*RTC3*
1.88481	5.00E-05	3.54E-04	AWRI1499_4046	*ATX1*
1.83866	5.00E-05	3.54E-04	AWRI1499_4456	
1.82004	5.00E-05	3.54E-04	AWRI1499_0804	
1.78719	5.00E-05	3.54E-04	AWRI1499_1315	
1.78102	5.00E-05	3.54E-04	AWRI1499_3058	
1.7736	5.00E-05	3.54E-04	AWRI1499_1126	
1.7696	5.00E-05	3.54E-04	AWRI1499_3222	
1.75816	5.00E-05	3.54E-04	AWRI1499_1316	
1.74608	5.00E-05	3.54E-04	AWRI1499_0110	
1.74342	5.00E-05	3.54E-04	AWRI1499_0515	*PIG2*
1.73415	5.00E-05	3.54E-04	AWRI1499_0040	*CWH41*
1.71441	5.00E-05	3.54E-04	AWRI1499_4880	
1.68737	1.00E-04	6.63E-04	AWRI1499_2771	*UGA2*
1.68509	5.00E-05	3.54E-04	AWRI1499_4196	*PAB1*
1.66957	5.00E-05	3.54E-04	AWRI1499_3896	*GLC3*
1.66532	5.00E-05	3.54E-04	AWRI1499_2897	*GLR1*
1.65891	5.00E-05	3.54E-04	AWRI1499_4419	*DIP5*
1.65597	5.00E-05	3.54E-04	AWRI1499_3238	*VBA4*
1.65146	5.00E-05	3.54E-04	AWRI1499_4451	*FMP37*
1.64558	5.00E-05	3.54E-04	AWRI1499_1406	
1.634	5.00E-05	3.54E-04	AWRI1499_3716	*TIP1*
1.61983	5.00E-05	3.54E-04	AWRI1499_0997	*ETP1*
1.6086	5.00E-05	3.54E-04	AWRI1499_4965	*HOL1*
1.60768	5.00E-05	3.54E-04	AWRI1499_2758	*SDS24*
1.60605	1.05E-03	5.12E-03	AWRI1499_3239	*VBA1*
1.6033	5.00E-05	3.54E-04	AWRI1499_4516	*YGR127W*
1.60252	5.00E-05	3.54E-04	AWRI1499_0692	*PXA1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
1.59646	5.00E-05	3.54E-04	AWRI1499_2006	*PNC1*
1.56361	5.00E-05	3.54E-04	AWRI1499_3043	
1.56025	5.00E-05	3.54E-04	AWRI1499_1726	*LIP2*
1.5293	5.00E-05	3.54E-04	AWRI1499_2089	*CLU1*
1.51615	5.00E-05	3.54E-04	AWRI1499_2862	*GUT1*
1.50776	5.00E-05	3.54E-04	AWRI1499_0803	
1.50428	5.00E-05	3.54E-04	AWRI1499_4350	*YMR315W*
1.48888	5.00E-05	3.54E-04	AWRI1499_0998	*ETP1*
1.48208	5.00E-05	3.54E-04	AWRI1499_3431	*UBC7*
1.48033	5.00E-05	3.54E-04	AWRI1499_1003	*MLS1*
1.46179	5.00E-05	3.54E-04	AWRI1499_2040	*ETR1*
1.45961	5.00E-05	3.54E-04	AWRI1499_2548	*CAT8*
1.42467	5.00E-05	3.54E-04	AWRI1499_4966	*SUR1*
1.41518	5.00E-05	3.54E-04	AWRI1499_3511	*POX1*
1.40671	5.00E-05	3.54E-04	AWRI1499_3221	
1.40082	5.00E-05	3.54E-04	AWRI1499_2234	
1.38138	5.00E-05	3.54E-04	AWRI1499_2547	*CAT8*
1.37431	5.00E-05	3.54E-04	AWRI1499_0441	*YNL144C*
1.37212	1.00E-04	6.63E-04	AWRI1499_4081	*DAK2*
1.36619	5.00E-05	3.54E-04	AWRI1499_4242	*BSC6*
1.33743	5.00E-05	3.54E-04	AWRI1499_4368	*PCK1*
1.33558	2.00E-04	1.22E-03	AWRI1499_3059	
1.33022	5.00E-05	3.54E-04	AWRI1499_0320	*COA1*
1.32913	5.00E-05	3.54E-04	AWRI1499_2321	
1.32078	5.00E-05	3.54E-04	AWRI1499_4430	*FIG2*
1.31188	5.00E-05	3.54E-04	AWRI1499_2476	*MPM1*
1.30087	5.00E-05	3.54E-04	AWRI1499_3136	*HXT6*
1.29203	5.00E-05	3.54E-04	AWRI1499_3014	*ADH3*
1.28843	5.00E-05	3.54E-04	AWRI1499_2467	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
1.2842	4.20E-03	1.67E-02	AWRI1499_0987	
1.25754	5.00E-05	3.54E-04	AWRI1499_0098	*YMR027W*
1.25247	5.00E-05	3.54E-04	AWRI1499_0715	
1.2515	5.00E-05	3.54E-04	AWRI1499_4255	*SDH8*
1.23814	5.00E-05	3.54E-04	AWRI1499_4369	*PCK1*
1.23603	5.00E-05	3.54E-04	AWRI1499_2733	*YKL151C*
1.21211	5.00E-05	3.54E-04	AWRI1499_3558	*SDH1*
1.21183	5.00E-05	3.54E-04	AWRI1499_1474	*YJR096W*
1.20677	5.00E-05	3.54E-04	AWRI1499_3584	*PST2*
1.20577	5.00E-05	3.54E-04	AWRI1499_0121	*STB5*
1.20424	6.00E-04	3.17E-03	AWRI1499_3825	*ADH6*
1.19716	5.00E-05	3.54E-04	AWRI1499_3682	*MSW1*
1.19402	5.00E-05	3.54E-04	AWRI1499_1368	*IMO32*
1.19094	5.00E-05	3.54E-04	AWRI1499_1715	*GPH1*
1.18626	5.00E-05	3.54E-04	AWRI1499_0483	
1.18018	5.00E-05	3.54E-04	AWRI1499_0511	*MHT1*
1.17879	5.00E-03	1.93E-02	AWRI1499_0463	
1.17821	5.00E-05	3.54E-04	AWRI1499_2554	*MDH1*
1.17778	5.00E-05	3.54E-04	AWRI1499_2105	
1.17776	5.00E-05	3.54E-04	AWRI1499_4241	*BSC6*
1.17641	5.00E-05	3.54E-04	AWRI1499_3615	*SOL3*
1.17468	5.00E-05	3.54E-04	AWRI1499_3758	
1.16701	5.00E-05	3.54E-04	AWRI1499_0604	*REG1*
1.16471	5.00E-05	3.54E-04	AWRI1499_0104	*HEM14*
1.15992	5.00E-05	3.54E-04	AWRI1499_3169	*HXT7*
1.15619	5.00E-05	3.54E-04	AWRI1499_4820	
1.15442	5.00E-05	3.54E-04	AWRI1499_3820	
1.14791	5.00E-05	3.54E-04	AWRI1499_2294	*PKP1*
1.1458	1.35E-03	6.38E-03	AWRI1499_1904	*GLK1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
1.13638	5.00E-05	3.54E-04	AWRI1499_4414	*MGM1*
1.12358	5.00E-05	3.54E-04	AWRI1499_0934	
1.11957	5.00E-05	3.54E-04	AWRI1499_4250	*ICL1*
1.11844	5.00E-05	3.54E-04	AWRI1499_1296	
1.1093	5.00E-05	3.54E-04	AWRI1499_4934	*SPS19*
1.10882	5.00E-05	3.54E-04	AWRI1499_3209	*AAT2*
1.10858	5.00E-05	3.54E-04	AWRI1499_3590	*URE2*
1.10251	5.00E-05	3.54E-04	AWRI1499_0018	
1.09686	5.00E-05	3.54E-04	AWRI1499_1171	*MDM31*
1.0936	5.00E-05	3.54E-04	AWRI1499_0479	*GPD1*
1.09331	5.00E-05	3.54E-04	AWRI1499_4659	*LIP5*
1.07935	2.00E-04	1.22E-03	AWRI1499_2233	
1.06836	5.00E-05	3.54E-04	AWRI1499_4522	
1.06758	5.00E-05	3.54E-04	AWRI1499_3936	*NAS6*
1.05934	5.00E-05	3.54E-04	AWRI1499_3589	*YBR096W*
1.05817	5.00E-05	3.54E-04	AWRI1499_2995	*CLD1*
1.04695	3.90E-03	1.57E-02	AWRI1499_2515	*TIP1*
1.04216	5.00E-05	3.54E-04	AWRI1499_1366	*RIM11*
1.03079	1.90E-03	8.52E-03	AWRI1499_3135	*GAL2*
1.02784	5.00E-05	3.54E-04	AWRI1499_0598	
1.01802	5.00E-05	3.54E-04	AWRI1499_3786	*CBR1*
1.01381	5.00E-05	3.54E-04	AWRI1499_4861	*RTK1*
1.00805	5.00E-05	3.54E-04	AWRI1499_4106	
1.00331	5.00E-05	3.54E-04	AWRI1499_3478	*CYM1*
1.0013	4.10E-03	1.64E-02	AWRI1499_1227	
0.997291	5.00E-05	3.54E-04	AWRI1499_2136	*IKS1*
0.996128	5.00E-05	3.54E-04	AWRI1499_3513	*PIR3*
0.994784	7.50E-04	3.83E-03	AWRI1499_3062	*MET5*
0.984136	5.00E-05	3.54E-04	AWRI1499_3988	*YMR226C*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.98275	1.50E-04	9.48E-04	AWRI1499_4521	*YIL082W-A*
0.977601	5.00E-05	3.54E-04	AWRI1499_0363	*HSP10*
0.975111	4.50E-04	2.48E-03	AWRI1499_4439	*STL1*
0.974133	5.00E-05	3.54E-04	AWRI1499_4376	*HTB1*
0.971217	5.00E-05	3.54E-04	AWRI1499_3158	
0.9706	5.00E-05	3.54E-04	AWRI1499_3356	*PKP1*
0.966568	1.50E-04	9.48E-04	AWRI1499_0167	*HSP60*
0.965235	5.00E-05	3.54E-04	AWRI1499_2184	*ALD2*
0.962549	5.00E-05	3.54E-04	AWRI1499_1596	*HSP150*
0.960289	5.00E-05	3.54E-04	AWRI1499_2593	
0.959929	6.00E-04	3.17E-03	AWRI1499_1979	
0.958609	5.00E-05	3.54E-04	AWRI1499_3512	*PIR1*
0.954616	5.00E-05	3.54E-04	AWRI1499_0443	
0.946072	5.00E-05	3.54E-04	AWRI1499_2236	
0.946071	3.50E-04	1.99E-03	AWRI1499_1190	*TAL1*
0.940836	5.00E-05	3.54E-04	AWRI1499_3365	
0.940191	5.00E-05	3.54E-04	AWRI1499_0065	*SDH7*
0.939272	5.00E-05	3.54E-04	AWRI1499_3821	*MDV1*
0.935252	5.00E-05	3.54E-04	AWRI1499_0208	*ITR2*
0.934427	6.35E-03	2.36E-02	AWRI1499_3822	*ADH7*
0.93397	5.00E-05	3.54E-04	AWRI1499_0918	*MTH1*
0.931058	5.00E-05	3.54E-04	AWRI1499_2436	*FPR1*
0.930751	5.00E-05	3.54E-04	AWRI1499_0045	*FMT1*
0.930197	1.50E-04	9.48E-04	AWRI1499_1876	*YGR109W-B*
0.923883	5.00E-05	3.54E-04	AWRI1499_4567	
0.919484	1.00E-04	6.63E-04	AWRI1499_0630	
0.914447	5.00E-05	3.54E-04	AWRI1499_2168	*PMI40*
0.91253	5.00E-05	3.54E-04	AWRI1499_3641	
0.910275	3.00E-04	1.74E-03	AWRI1499_4620	*TIP1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.910211	5.00E-05	3.54E-04	AWRI1499_0732	*AQY1*
0.906995	9.00E-04	4.49E-03	AWRI1499_2949	*MET10*
0.901253	5.00E-05	3.54E-04	AWRI1499_0736	*AIM23*
0.898435	3.75E-03	1.52E-02	AWRI1499_1942	
0.896771	5.00E-05	3.54E-04	AWRI1499_4494	*CPR1*
0.89629	5.00E-05	3.54E-04	AWRI1499_3732	*GAP1*
0.888621	5.00E-05	3.54E-04	AWRI1499_0213	*ADH6*
0.885743	5.00E-05	3.54E-04	AWRI1499_1550	*PSA1*
0.885552	5.00E-05	3.54E-04	AWRI1499_3353	
0.884735	5.00E-05	3.54E-04	AWRI1499_2699	*MRPL10*
0.881968	5.00E-05	3.54E-04	AWRI1499_0587	*MRPL1*
0.880489	5.00E-05	3.54E-04	AWRI1499_3622	*LAP3*
0.880166	5.00E-05	3.54E-04	AWRI1499_2622	*SBA1*
0.865344	5.00E-05	3.54E-04	AWRI1499_3757	
0.864993	5.00E-05	3.54E-04	AWRI1499_4163	*YHL008C*
0.864917	5.00E-05	3.54E-04	AWRI1499_4534	*DLD1*
0.851248	2.50E-04	1.48E-03	AWRI1499_2341	
0.844869	2.80E-03	1.19E-02	AWRI1499_4935	
0.844359	5.00E-05	3.54E-04	AWRI1499_4193	*GDH1*
0.842866	1.30E-03	6.17E-03	AWRI1499_2661	
0.838949	2.00E-04	1.22E-03	AWRI1499_3401	
0.836549	5.00E-05	3.54E-04	AWRI1499_4367	*OPT2*
0.827487	5.00E-05	3.54E-04	AWRI1499_4365	*OPT2*
0.825449	1.00E-04	6.63E-04	AWRI1499_2185	*ALD4*
0.82509	2.65E-03	1.13E-02	AWRI1499_0269	*GND2*
0.824837	5.00E-05	3.54E-04	AWRI1499_4366	*OPT2*
0.824544	1.50E-04	9.48E-04	AWRI1499_4298	*ECM4*
0.821644	5.00E-05	3.54E-04	AWRI1499_3624	*YNL200C*
0.820059	5.00E-05	3.54E-04	AWRI1499_3207	*ACP1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.81663	3.00E-04	1.74E-03	AWRI1499_1319	*QDR3*
0.815558	5.00E-04	2.71E-03	AWRI1499_0442	*RPM2*
0.814574	5.00E-05	3.54E-04	AWRI1499_1442	
0.813765	2.00E-04	1.22E-03	AWRI1499_0449	*YGR053C*
0.811497	5.00E-05	3.54E-04	AWRI1499_4559	*UTR2*
0.810669	1.50E-04	9.48E-04	AWRI1499_1276	*MRP7*
0.809938	5.00E-05	3.54E-04	AWRI1499_0693	*ISA1*
0.808774	5.00E-05	3.54E-04	AWRI1499_2823	*YDR248C*
0.808205	5.00E-05	3.54E-04	AWRI1499_1826	
0.804054	5.00E-05	3.54E-04	AWRI1499_1403	*COX15*
0.801519	5.00E-05	3.54E-04	AWRI1499_4947	
0.797267	5.00E-05	3.54E-04	AWRI1499_4642	*MRPL23*
0.796826	5.00E-05	3.54E-04	AWRI1499_3525	*MST1*
0.784592	1.00E-03	4.91E-03	AWRI1499_4045	*CPR3*
0.784543	3.00E-04	1.74E-03	AWRI1499_0632	*RDL1*
0.77563	2.50E-04	1.48E-03	AWRI1499_0236	
0.775502	4.00E-04	2.24E-03	AWRI1499_3756	
0.773837	1.00E-04	6.63E-04	AWRI1499_1625	*MRPS8*
0.773423	5.00E-05	3.54E-04	AWRI1499_2829	
0.770611	5.00E-05	3.54E-04	AWRI1499_4902	
0.77004	5.00E-05	3.54E-04	AWRI1499_2001	
0.769888	5.00E-05	3.54E-04	AWRI1499_2679	*UGO1*
0.769754	5.00E-05	3.54E-04	AWRI1499_2378	*DCW1*
0.769437	1.20E-03	5.76E-03	AWRI1499_3381	*SLX1*
0.768529	6.00E-04	3.17E-03	AWRI1499_0597	
0.768509	1.50E-04	9.48E-04	AWRI1499_4162	*YHL008C*
0.767549	5.00E-05	3.54E-04	AWRI1499_2244	*MSS51*
0.766423	7.50E-04	3.83E-03	AWRI1499_2225	*YCF1*
0.763429	1.00E-04	6.63E-04	AWRI1499_0490	*AGP2*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.760975	5.00E-05	3.54E-04	AWRI1499_1964	*ALK2*
0.759361	4.25E-03	1.69E-02	AWRI1499_2802	
0.75765	5.00E-05	3.54E-04	AWRI1499_2066	*PHB1*
0.756379	1.00E-04	6.63E-04	AWRI1499_2454	*KGD2*
0.756018	2.60E-03	1.11E-02	AWRI1499_0622	*TKL1*
0.754416	5.00E-05	3.54E-04	AWRI1499_4375	*HTA1*
0.753845	3.05E-03	1.27E-02	AWRI1499_1745	*SSC1*
0.752473	4.00E-04	2.24E-03	AWRI1499_0409	
0.752052	5.00E-05	3.54E-04	AWRI1499_4677	*YLR290C*
0.750877	7.00E-04	3.62E-03	AWRI1499_1083	*MRPL39*
0.750009	1.50E-04	9.48E-04	AWRI1499_2780	*PTA1*
0.748816	4.00E-04	2.24E-03	AWRI1499_3107	
0.74806	2.50E-04	1.48E-03	AWRI1499_2891	*YBR219C*
0.746601	1.05E-03	5.12E-03	AWRI1499_1560	*UGA4*
0.746487	2.00E-04	1.22E-03	AWRI1499_4197	*NFU1*
0.743618	1.50E-04	9.48E-04	AWRI1499_1138	*TRX1*
0.733974	4.60E-03	1.80E-02	AWRI1499_4731	*MAL12*
0.733126	1.30E-03	6.17E-03	AWRI1499_1556	*HEM13*
0.732352	1.00E-04	6.63E-04	AWRI1499_1677	*SNF3*
0.732221	4.00E-04	2.24E-03	AWRI1499_4835	*CAR1*
0.732213	1.50E-04	9.48E-04	AWRI1499_1383	*YJR142W*
0.730324	1.50E-04	9.48E-04	AWRI1499_3694	*TAH18*
0.730275	3.00E-04	1.74E-03	AWRI1499_1217	
0.728642	2.50E-04	1.48E-03	AWRI1499_4918	*ALG12*
0.728298	3.00E-04	1.74E-03	AWRI1499_3944	*TOM40*
0.727306	2.00E-04	1.22E-03	AWRI1499_4069	*GSF2*
0.727286	1.03E-02	3.51E-02	AWRI1499_2765	*PGI1*
0.727139	4.20E-03	1.67E-02	AWRI1499_2529	*BOP3*
0.726383	5.00E-05	3.54E-04	AWRI1499_2555	*RIB5*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.725447	5.00E-05	3.54E-04	AWRI1499_2137	*YPT52*
0.722343	5.00E-04	2.71E-03	AWRI1499_4948	*GDE1*
0.721069	3.50E-04	1.99E-03	AWRI1499_4227	*YPS7*
0.720739	3.90E-03	1.57E-02	AWRI1499_4293	*BGL2*
0.717889	2.00E-04	1.22E-03	AWRI1499_0421	
0.716958	3.00E-04	1.74E-03	AWRI1499_4067	*SCW11*
0.715084	1.05E-03	5.12E-03	AWRI1499_4849	*HMO1*
0.714813	5.00E-04	2.71E-03	AWRI1499_3009	
0.71436	1.00E-04	6.63E-04	AWRI1499_1183	*GRX5*
0.713417	1.45E-03	6.77E-03	AWRI1499_3245	
0.712663	5.50E-04	2.94E-03	AWRI1499_4212	*YOR192C-B*
0.711956	4.00E-04	2.24E-03	AWRI1499_2336	*MGE1*
0.709597	5.00E-04	2.71E-03	AWRI1499_3614	
0.709078	5.00E-04	2.71E-03	AWRI1499_1973	*TOM22*
0.703406	1.65E-03	7.56E-03	AWRI1499_2787	*MRP17*
0.701717	1.00E-04	6.63E-04	AWRI1499_4410	*MSC7*
0.695387	2.00E-04	1.22E-03	AWRI1499_1317	*IAH1*
0.694565	4.00E-04	2.24E-03	AWRI1499_1065	
0.694387	3.00E-04	1.74E-03	AWRI1499_4001	
0.691483	6.00E-04	3.17E-03	AWRI1499_4752	*PRD1*
0.69046	1.75E-03	7.94E-03	AWRI1499_1945	*GPA2*
0.688819	2.50E-04	1.48E-03	AWRI1499_3201	*UBP16*
0.688718	1.32E-02	4.31E-02	AWRI1499_3904	*FBP1*
0.687581	2.00E-04	1.22E-03	AWRI1499_2772	*ECM15*
0.687296	3.80E-03	1.53E-02	AWRI1499_0272	*PLB2*
0.686486	6.00E-04	3.17E-03	AWRI1499_0848	*GDA1*
0.686411	5.00E-04	2.71E-03	AWRI1499_2000	*OCH1*
0.68572	5.00E-04	2.71E-03	AWRI1499_4243	*MRPL28*
0.68544	1.01E-02	3.47E-02	AWRI1499_0676	*QDR3*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.684824	4.50E-04	2.48E-03	AWRI1499_0556	*MRPS12*
0.68477	1.50E-04	9.48E-04	AWRI1499_4802	
0.683205	6.95E-03	2.54E-02	AWRI1499_3668	*DLD1*
0.682614	1.50E-03	6.97E-03	AWRI1499_1375	*MSM1*
0.682337	9.50E-04	4.70E-03	AWRI1499_2954	*INO80*
0.682263	2.25E-03	9.84E-03	AWRI1499_4107	
0.678141	3.00E-04	1.74E-03	AWRI1499_3430	
0.677936	6.00E-04	3.17E-03	AWRI1499_0596	*STE23*
0.6769	2.00E-03	8.90E-03	AWRI1499_0577	*GAS1*
0.675601	3.00E-04	1.74E-03	AWRI1499_2539	*RSM7*
0.675469	1.50E-04	9.48E-04	AWRI1499_1244	*NCA2*
0.673807	3.65E-03	1.48E-02	AWRI1499_1320	
0.672973	1.40E-03	6.57E-03	AWRI1499_4700	*PEP4*
0.672676	1.00E-03	4.91E-03	AWRI1499_0273	*PLB1*
0.671616	3.55E-03	1.45E-02	AWRI1499_4211	*YDR261W-B*
0.66913	6.50E-04	3.39E-03	AWRI1499_3695	*MSF1*
0.668035	3.00E-04	1.74E-03	AWRI1499_3806	*ERT1*
0.667885	1.00E-04	6.63E-04	AWRI1499_2099	*MRP51*
0.666567	2.00E-04	1.22E-03	AWRI1499_3042	*SPC2*
0.666492	2.50E-04	1.48E-03	AWRI1499_3497	*YIR007W*
0.666415	3.10E-03	1.29E-02	AWRI1499_1595	*HSP150*
0.66475	7.00E-04	3.62E-03	AWRI1499_2134	
0.664491	5.00E-04	2.71E-03	AWRI1499_1496	*ERP5*
0.663755	3.50E-03	1.43E-02	AWRI1499_1245	*CEM1*
0.662624	7.00E-04	3.62E-03	AWRI1499_3336	*AIM10*
0.66231	9.00E-04	4.49E-03	AWRI1499_1131	*MTG2*
0.662139	9.00E-04	4.49E-03	AWRI1499_2200	*DSE1*
0.66166	4.00E-04	2.24E-03	AWRI1499_2174	*COQ5*
0.661377	8.50E-04	4.27E-03	AWRI1499_2961	*OXA1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.660902	5.00E-04	2.71E-03	AWRI1499_3357	*RSM18*
0.659543	2.50E-03	1.08E-02	AWRI1499_3252	*ITR1*
0.658531	2.20E-03	9.65E-03	AWRI1499_1643	*SIP5*
0.657367	1.00E-03	4.91E-03	AWRI1499_0032	*CHS2*
0.653906	7.50E-04	3.83E-03	AWRI1499_0022	*MRP21*
0.653697	7.00E-04	3.62E-03	AWRI1499_3211	*NCR1*
0.653203	7.00E-03	2.56E-02	AWRI1499_0952	*CBP4*
0.648978	1.45E-03	6.77E-03	AWRI1499_4877	*ATM1*
0.64847	4.00E-04	2.24E-03	AWRI1499_1523	*SIL1*
0.647941	6.00E-04	3.17E-03	AWRI1499_2169	*PMI40*
0.646739	5.00E-04	2.71E-03	AWRI1499_2090	
0.645438	2.65E-03	1.13E-02	AWRI1499_3382	*CNE1*
0.644909	7.00E-04	3.62E-03	AWRI1499_3871	*ADH6*
0.644238	1.45E-03	6.77E-03	AWRI1499_0317	*DPL1*
0.643532	1.50E-03	6.97E-03	AWRI1499_2508	*DAP1*
0.643197	4.50E-04	2.48E-03	AWRI1499_3658	*GRE3*
0.642268	5.00E-04	2.71E-03	AWRI1499_1187	*AIM45*
0.640222	1.36E-02	4.42E-02	AWRI1499_2026	
0.639754	5.50E-04	2.94E-03	AWRI1499_0108	*BUD17*
0.638439	5.00E-04	2.71E-03	AWRI1499_2858	*RSM26*
0.637974	7.50E-04	3.83E-03	AWRI1499_4223	
0.636475	1.05E-03	5.12E-03	AWRI1499_1152	*LYP1*
0.632326	1.10E-03	5.34E-03	AWRI1499_3244	*DUG1*
0.63024	1.45E-03	6.77E-03	AWRI1499_2461	
0.62885	1.45E-03	6.77E-03	AWRI1499_3325	*GPT2*
0.628549	6.50E-04	3.39E-03	AWRI1499_4135	*SLC1*
0.627798	1.25E-03	5.97E-03	AWRI1499_2731	*MRPL8*
0.625907	2.10E-03	9.28E-03	AWRI1499_1825	
0.625277	1.02E-02	3.48E-02	AWRI1499_4854	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.625134	7.50E-04	3.83E-03	AWRI1499_4058	*MRPL24*
0.624686	1.25E-03	5.97E-03	AWRI1499_3225	
0.623327	4.40E-03	1.74E-02	AWRI1499_0637	
0.623092	3.00E-04	1.74E-03	AWRI1499_1077	*TNA1*
0.621548	1.40E-03	6.57E-03	AWRI1499_4482	*CDC8*
0.619822	1.55E-03	7.16E-03	AWRI1499_3017	
0.6137	1.33E-02	4.35E-02	AWRI1499_0595	*CCW14*
0.612167	1.45E-03	6.77E-03	AWRI1499_2433	*VMA3*
0.611443	4.15E-03	1.65E-02	AWRI1499_1552	*FRD1*
0.6106	3.35E-03	1.38E-02	AWRI1499_0929	*SEC21*
0.607918	1.50E-03	6.97E-03	AWRI1499_0793	*DIA4*
0.606814	1.15E-03	5.55E-03	AWRI1499_3390	*TIM17*
0.604298	3.30E-03	1.36E-02	AWRI1499_1059	*MAM33*
0.604146	1.60E-03	7.36E-03	AWRI1499_2533	*YIR035C*
0.603945	1.35E-03	6.38E-03	AWRI1499_2044	*YPC1*
0.602836	2.05E-03	9.08E-03	AWRI1499_1948	*YPR117W*
0.602473	1.65E-03	7.56E-03	AWRI1499_1944	*GPA2*
0.601186	1.50E-03	6.97E-03	AWRI1499_3417	*NTA1*
0.598819	2.05E-03	9.08E-03	AWRI1499_4493	
0.597908	5.15E-03	1.98E-02	AWRI1499_4797	*TCB1*
0.597609	1.50E-03	6.97E-03	AWRI1499_2857	*AIM25*
0.594169	3.65E-03	1.48E-02	AWRI1499_4274	*NIT2*
0.593791	1.90E-03	8.52E-03	AWRI1499_1294	*MRPL6*
0.59368	1.70E-03	7.75E-03	AWRI1499_1558	*PNS1*
0.593568	1.60E-03	7.36E-03	AWRI1499_2207	*UBP7*
0.593047	3.60E-03	1.46E-02	AWRI1499_3696	*MRPL16*
0.592687	5.15E-03	1.98E-02	AWRI1499_3476	*AMS1*
0.592499	1.30E-03	6.17E-03	AWRI1499_0580	
0.590264	1.25E-03	5.97E-03	AWRI1499_3273	*COQ1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.588591	1.50E-03	6.97E-03	AWRI1499_0286	*VBA1*
0.588574	4.20E-03	1.67E-02	AWRI1499_3766	*GOR1*
0.587335	2.55E-03	1.09E-02	AWRI1499_1435	*YMR31*
0.587245	9.00E-04	4.49E-03	AWRI1499_2398	*MRP4*
0.586813	3.45E-03	1.41E-02	AWRI1499_4653	*MEF1*
0.586711	3.30E-03	1.36E-02	AWRI1499_4729	*GAL10*
0.586541	2.70E-03	1.15E-02	AWRI1499_2879	*YEL043W*
0.585925	1.45E-03	6.77E-03	AWRI1499_4346	*VMA16*
0.584704	2.55E-03	1.09E-02	AWRI1499_4041	
0.584117	2.55E-03	1.09E-02	AWRI1499_4834	*ECM14*
0.583603	2.00E-03	8.90E-03	AWRI1499_1946	*YPR117W*
0.581495	1.60E-03	7.36E-03	AWRI1499_1280	*PGS1*
0.581194	3.85E-03	1.55E-02	AWRI1499_3737	
0.581157	3.75E-03	1.52E-02	AWRI1499_1407	*CIR1*
0.580761	1.55E-03	7.16E-03	AWRI1499_4780	*TUL1*
0.579334	1.65E-03	7.56E-03	AWRI1499_2079	*SEC7*
0.578437	1.27E-02	4.17E-02	AWRI1499_2484	*RNY1*
0.578205	1.95E-03	8.72E-03	AWRI1499_4638	*MRP1*
0.577567	2.40E-03	1.04E-02	AWRI1499_3157	*GDE1*
0.576543	1.42E-02	4.58E-02	AWRI1499_0863	
0.572104	1.95E-03	8.72E-03	AWRI1499_2439	*YFH1*
0.570425	3.35E-03	1.38E-02	AWRI1499_4847	*RSM24*
0.565673	1.70E-03	7.75E-03	AWRI1499_0740	*BST1*
0.565383	3.15E-03	1.31E-02	AWRI1499_3937	*PHB2*
0.56309	2.35E-03	1.02E-02	AWRI1499_3499	*YDL206W*
0.560393	2.50E-03	1.08E-02	AWRI1499_0571	*YJL132W*
0.55886	7.75E-03	2.79E-02	AWRI1499_2250	*MSD1*
0.558838	3.15E-03	1.31E-02	AWRI1499_3725	*OMS1*
0.555201	6.80E-03	2.50E-02	AWRI1499_1624	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.554852	2.20E-03	9.65E-03	AWRI1499_3498	*YIR007W*
0.554421	5.25E-03	2.01E-02	AWRI1499_0063	*RNH1*
0.55424	5.50E-03	2.09E-02	AWRI1499_2744	
0.551961	5.05E-03	1.95E-02	AWRI1499_1111	*MRF1*
0.551875	8.45E-03	2.99E-02	AWRI1499_3509	*XKS1*
0.551515	3.85E-03	1.55E-02	AWRI1499_2402	*CYC3*
0.549652	6.30E-03	2.34E-02	AWRI1499_0057	*PRS3*
0.549325	3.40E-03	1.40E-02	AWRI1499_1127	*SRB4*
0.548948	6.40E-03	2.37E-02	AWRI1499_3137	*TUF1*
0.548013	5.80E-03	2.19E-02	AWRI1499_3836	*RKI1*
0.547911	2.85E-03	1.20E-02	AWRI1499_4736	*ARC18*
0.547062	3.30E-03	1.36E-02	AWRI1499_1112	
0.545751	3.65E-03	1.48E-02	AWRI1499_2298	*ESBP6*
0.543381	5.05E-03	1.95E-02	AWRI1499_0582	*CCS1*
0.542894	3.65E-03	1.48E-02	AWRI1499_2088	*TMN2*
0.541048	1.52E-02	4.83E-02	AWRI1499_0568	*CDA2*
0.540117	4.55E-03	1.79E-02	AWRI1499_4894	*MRPL4*
0.539552	3.30E-03	1.36E-02	AWRI1499_4764	*PKH3*
0.538281	9.00E-03	3.15E-02	AWRI1499_0629	*BUB2*
0.538148	1.23E-02	4.08E-02	AWRI1499_3013	
0.537072	2.95E-03	1.24E-02	AWRI1499_2069	*PMU1*
0.536777	3.35E-03	1.38E-02	AWRI1499_2460	*NAM9*
0.53666	3.80E-03	1.53E-02	AWRI1499_1781	*MRPL27*
0.534678	4.15E-03	1.65E-02	AWRI1499_2617	*PBI2*
0.533339	9.60E-03	3.32E-02	AWRI1499_3679	*PEP12*
0.533272	6.85E-03	2.51E-02	AWRI1499_2741	
0.532153	7.85E-03	2.81E-02	AWRI1499_0835	
0.52902	4.90E-03	1.90E-02	AWRI1499_4650	
0.527323	1.07E-02	3.63E-02	AWRI1499_0274	*PLB3*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.526393	1.27E-02	4.17E-02	AWRI1499_3881	*ROY1*
0.525529	1.10E-02	3.70E-02	AWRI1499_0738	
0.525411	4.10E-03	1.64E-02	AWRI1499_4358	*CTS1*
0.524908	5.40E-03	2.06E-02	AWRI1499_0265	*COQ3*
0.524594	1.10E-02	3.70E-02	AWRI1499_1333	*POR1*
0.523606	9.30E-03	3.23E-02	AWRI1499_4409	*MSC7*
0.523569	4.55E-03	1.79E-02	AWRI1499_1524	
0.522975	5.15E-03	1.98E-02	AWRI1499_0639	
0.522812	1.38E-02	4.48E-02	AWRI1499_3338	*PIN4*
0.522174	6.50E-03	2.41E-02	AWRI1499_3078	*MXR1*
0.522101	7.30E-03	2.65E-02	AWRI1499_4134	*SWS2*
0.522036	4.60E-03	1.80E-02	AWRI1499_1151	*YGR026W*
0.521784	5.70E-03	2.16E-02	AWRI1499_3031	*ASH1*
0.521715	8.00E-03	2.86E-02	AWRI1499_3632	*SHY1*
0.521552	5.45E-03	2.08E-02	AWRI1499_0480	*PAM17*
0.520734	1.53E-02	4.86E-02	AWRI1499_4697	
0.520698	1.03E-02	3.52E-02	AWRI1499_2549	*YDL124W*
0.520443	5.25E-03	2.01E-02	AWRI1499_4002	
0.519953	6.30E-03	2.34E-02	AWRI1499_3514	*MRPS35*
0.518628	7.15E-03	2.60E-02	AWRI1499_2167	
0.518568	7.00E-03	2.56E-02	AWRI1499_1169	*YML6*
0.51769	4.60E-03	1.80E-02	AWRI1499_3765	*GOR1*
0.514521	3.95E-03	1.59E-02	AWRI1499_4234	*PTC7*
0.51318	5.50E-03	2.09E-02	AWRI1499_1119	*OCT1*
0.512636	4.60E-03	1.80E-02	AWRI1499_0134	*PMT1*
0.512539	6.85E-03	2.51E-02	AWRI1499_4204	*ECM31*
0.51139	5.85E-03	2.20E-02	AWRI1499_2882	*YLR149C*
0.51044	8.55E-03	3.02E-02	AWRI1499_1993	*YME1*
0.510327	5.45E-03	2.08E-02	AWRI1499_4338	*MSE1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.50952	1.01E-02	3.45E-02	AWRI1499_4422	*ERG7*
0.509396	6.75E-03	2.48E-02	AWRI1499_0510	
0.506482	7.75E-03	2.79E-02	AWRI1499_4213	
0.506078	7.25E-03	2.63E-02	AWRI1499_4844	*SDH4*
0.505793	9.75E-03	3.37E-02	AWRI1499_1913	*ALE1*
0.504257	6.55E-03	2.42E-02	AWRI1499_1243	*URK1*
0.503355	1.22E-02	4.03E-02	AWRI1499_0572	*SGA1*
0.502682	1.20E-02	3.99E-02	AWRI1499_1237	*MDH1*
0.501667	7.95E-03	2.84E-02	AWRI1499_0066	*YCR043C*
0.501101	6.80E-03	2.50E-02	AWRI1499_2716	
0.498196	6.20E-03	2.31E-02	AWRI1499_2941	*DGA1*
0.497849	1.46E-02	4.69E-02	AWRI1499_2060	
0.496741	5.90E-03	2.22E-02	AWRI1499_4130	
0.496593	7.70E-03	2.77E-02	AWRI1499_0771	
0.496224	6.30E-03	2.34E-02	AWRI1499_2927	*CTS2*
0.496127	9.40E-03	3.26E-02	AWRI1499_3465	*QRI7*
0.495905	1.34E-02	4.36E-02	AWRI1499_0809	*YDL157C*
0.495713	1.01E-02	3.47E-02	AWRI1499_1947	*YPR117W*
0.495129	1.28E-02	4.21E-02	AWRI1499_2462	*RCK2*
0.494808	8.45E-03	2.99E-02	AWRI1499_3213	*SKO1*
0.49427	1.32E-02	4.31E-02	AWRI1499_4254	*LAS21*
0.493703	6.35E-03	2.36E-02	AWRI1499_2320	*ACB1*
0.493459	1.49E-02	4.75E-02	AWRI1499_3510	*SPC1*
0.493381	8.20E-03	2.92E-02	AWRI1499_2489	*CHL1*
0.489773	8.10E-03	2.89E-02	AWRI1499_2788	
0.48938	7.50E-03	2.71E-02	AWRI1499_4022	*HNT1*
0.489373	1.24E-02	4.09E-02	AWRI1499_3852	*CTI6*
0.487264	1.04E-02	3.55E-02	AWRI1499_3105	*MRPL40*
0.486347	1.20E-02	3.99E-02	AWRI1499_0062	*ZWF1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.485572	1.19E-02	3.96E-02	AWRI1499_3228	*TAF1*
0.485555	1.34E-02	4.36E-02	AWRI1499_2929	*RSM10*
0.484748	8.80E-03	3.09E-02	AWRI1499_4364	*YMR210W*
0.484435	8.65E-03	3.05E-02	AWRI1499_4257	*ERO1*
0.483786	1.38E-02	4.48E-02	AWRI1499_4185	*YTA12*
0.48327	1.06E-02	3.59E-02	AWRI1499_1259	*CBP3*
0.483159	1.11E-02	3.74E-02	AWRI1499_0372	*EEB1*
0.483084	1.38E-02	4.46E-02	AWRI1499_4798	*TCB1*
0.482405	1.00E-02	3.44E-02	AWRI1499_0368	*MRPS17*
0.481511	7.80E-03	2.80E-02	AWRI1499_4827	*PCM1*
0.480984	1.10E-02	3.70E-02	AWRI1499_0552	*MRPS5*
0.479894	1.18E-02	3.92E-02	AWRI1499_2874	
0.478215	1.32E-02	4.32E-02	AWRI1499_0327	*YOL057W*
0.477674	1.02E-02	3.48E-02	AWRI1499_1829	*MRPL7*
0.47716	1.33E-02	4.33E-02	AWRI1499_3364	
0.475934	9.65E-03	3.34E-02	AWRI1499_3304	*SHH3*
0.474429	1.32E-02	4.32E-02	AWRI1499_1575	*TIM9*
0.472485	1.20E-02	3.98E-02	AWRI1499_0521	*MRPL49*
0.472347	9.10E-03	3.18E-02	AWRI1499_0873	*STE24*
0.471659	8.90E-03	3.12E-02	AWRI1499_4187	*TOM20*
0.470027	9.75E-03	3.37E-02	AWRI1499_1279	*RIB2*
0.469622	1.50E-02	4.79E-02	AWRI1499_0060	*YTP1*
0.469123	1.19E-02	3.95E-02	AWRI1499_3335	*AIM10*
0.467191	1.53E-02	4.87E-02	AWRI1499_1250	*NCE102*
0.467109	1.04E-02	3.55E-02	AWRI1499_3545	*MTO1*
0.466906	1.47E-02	4.71E-02	AWRI1499_3280	*PIG2*
0.465289	1.06E-02	3.61E-02	AWRI1499_3958	*YPR089W*
0.463772	1.07E-02	3.63E-02	AWRI1499_4758	*ALD2*
0.46333	1.41E-02	4.56E-02	AWRI1499_4354	*VNX1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.462919	1.53E-02	4.86E-02	AWRI1499_4065	*RBG2*
0.462158	1.24E-02	4.09E-02	AWRI1499_4570	*KRE5*
0.462074	1.05E-02	3.58E-02	AWRI1499_2921	*UGA1*
0.4619	1.15E-02	3.86E-02	AWRI1499_3355	*LDB17*
0.4617	1.18E-02	3.94E-02	AWRI1499_3458	*DCD1*
0.460197	1.44E-02	4.62E-02	AWRI1499_2012	
0.459883	1.18E-02	3.94E-02	AWRI1499_1530	
0.459269	1.16E-02	3.87E-02	AWRI1499_0451	*YOR296W*
0.458415	1.10E-02	3.71E-02	AWRI1499_3403	*YNK1*
0.455777	1.44E-02	4.63E-02	AWRI1499_0330	
0.453818	1.29E-02	4.23E-02	AWRI1499_0922	*TWF1*
0.45222	1.29E-02	4.23E-02	AWRI1499_4062	*YDR338C*
0.451975	1.56E-02	4.95E-02	AWRI1499_2038	*NTG2*
0.451863	1.30E-02	4.27E-02	AWRI1499_0138	*SNF4*
0.451158	1.35E-02	4.40E-02	AWRI1499_1980	*MDG1*
0.450648	1.35E-02	4.40E-02	AWRI1499_2262	*ELF1*
0.449484	1.46E-02	4.67E-02	AWRI1499_0960	*AMD2*
0.448419	1.48E-02	4.73E-02	AWRI1499_4337	*OPI10*
0.448081	1.40E-02	4.53E-02	AWRI1499_3212	*SKO1*
0.447593	1.43E-02	4.60E-02	AWRI1499_3113	*YET3*
-0.444234	1.29E-02	4.23E-02	AWRI1499_0162	*YLR419W*
-0.447822	1.42E-02	4.57E-02	AWRI1499_1298	*MED11*
-0.448182	1.24E-02	4.10E-02	AWRI1499_3204	
-0.449118	1.48E-02	4.74E-02	AWRI1499_1755	*KRI1*
-0.4494	1.29E-02	4.24E-02	AWRI1499_3469	*UTP7*
-0.453518	1.43E-02	4.61E-02	AWRI1499_2464	*UTP18*
-0.455107	1.23E-02	4.06E-02	AWRI1499_2179	
-0.455871	1.39E-02	4.50E-02	AWRI1499_3565	*PUF6*
-0.458059	1.16E-02	3.87E-02	AWRI1499_1084	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.458338	1.56E-02	4.95E-02	AWRI1499_2385	*FMO1*
-0.458434	1.51E-02	4.82E-02	AWRI1499_1739	
-0.458461	1.57E-02	4.98E-02	AWRI1499_2806	*ALT1*
-0.460432	1.45E-02	4.65E-02	AWRI1499_1661	*INH1*
-0.461287	1.19E-02	3.95E-02	AWRI1499_1064	*AVO2*
-0.464075	1.07E-02	3.62E-02	AWRI1499_1680	
-0.465681	1.10E-02	3.70E-02	AWRI1499_1668	*RDI1*
-0.469651	9.15E-03	3.19E-02	AWRI1499_2148	*PTI1*
-0.470547	9.55E-03	3.31E-02	AWRI1499_4077	*CFD1*
-0.471422	1.02E-02	3.49E-02	AWRI1499_1555	*MNN4*
-0.471599	1.13E-02	3.80E-02	AWRI1499_2612	*GIT1*
-0.472163	1.31E-02	4.29E-02	AWRI1499_0610	
-0.475023	1.07E-02	3.63E-02	AWRI1499_3301	*RPC53*
-0.475834	1.18E-02	3.92E-02	AWRI1499_4675	
-0.476356	9.60E-03	3.32E-02	AWRI1499_0585	*MET1*
-0.477392	1.11E-02	3.74E-02	AWRI1499_2703	
-0.477622	1.34E-02	4.36E-02	AWRI1499_3818	*RPA190*
-0.477661	1.22E-02	4.03E-02	AWRI1499_1427	
-0.4784	1.56E-02	4.95E-02	AWRI1499_0126	*MSH1*
-0.478644	9.60E-03	3.32E-02	AWRI1499_4510	*TSL1*
-0.479177	1.44E-02	4.63E-02	AWRI1499_3809	*RIB1*
-0.480695	1.23E-02	4.08E-02	AWRI1499_3964	*MUM2*
-0.481141	1.06E-02	3.61E-02	AWRI1499_3898	*VPS15*
-0.481831	1.04E-02	3.55E-02	AWRI1499_1652	*GCN1*
-0.482016	9.60E-03	3.32E-02	AWRI1499_4032	*PNO1*
-0.482275	1.16E-02	3.87E-02	AWRI1499_1092	
-0.482437	1.16E-02	3.88E-02	AWRI1499_1019	*AUR1*
-0.482998	1.37E-02	4.45E-02	AWRI1499_1459	*VTC4*
-0.487852	8.00E-03	2.86E-02	AWRI1499_4786	*MPP10*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.489385	8.40E-03	2.97E-02	AWRI1499_1688	*YKR023W*
-0.489957	7.05E-03	2.57E-02	AWRI1499_3333	
-0.490027	1.06E-02	3.61E-02	AWRI1499_3326	*IRE1*
-0.490478	7.50E-03	2.71E-02	AWRI1499_0144	*MDL2*
-0.491088	9.70E-03	3.35E-02	AWRI1499_4440	*MET18*
-0.491332	8.50E-03	3.00E-02	AWRI1499_0224	*CLB3*
-0.491978	6.65E-03	2.45E-02	AWRI1499_2511	*NFI1*
-0.492472	7.45E-03	2.69E-02	AWRI1499_0861	*BNI4*
-0.49258	7.30E-03	2.65E-02	AWRI1499_0006	*RAD7*
-0.493028	7.65E-03	2.76E-02	AWRI1499_0241	*UGA3*
-0.493082	7.80E-03	2.80E-02	AWRI1499_4131	*TMA108*
-0.494238	8.05E-03	2.87E-02	AWRI1499_4850	*UPS2*
-0.496161	9.90E-03	3.41E-02	AWRI1499_4102	*SGS1*
-0.496582	7.00E-03	2.56E-02	AWRI1499_3389	*YAK1*
-0.500972	6.50E-03	2.41E-02	AWRI1499_3931	
-0.501775	9.25E-03	3.22E-02	AWRI1499_4903	*ERB1*
-0.503092	5.95E-03	2.23E-02	AWRI1499_2237	*CDC15*
-0.503792	8.60E-03	3.03E-02	AWRI1499_1256	*UTP9*
-0.504112	8.55E-03	3.02E-02	AWRI1499_3597	*CCC2*
-0.504164	5.95E-03	2.23E-02	AWRI1499_3520	
-0.504199	9.00E-03	3.15E-02	AWRI1499_0768	*NDI1*
-0.504845	7.10E-03	2.59E-02	AWRI1499_1402	*DNF3*
-0.505992	1.30E-02	4.25E-02	AWRI1499_2440	*ARB1*
-0.507172	5.15E-03	1.98E-02	AWRI1499_0206	*MCM7*
-0.507453	7.50E-03	2.71E-02	AWRI1499_2272	*CCA1*
-0.507709	5.55E-03	2.11E-02	AWRI1499_1007	
-0.509197	1.15E-02	3.84E-02	AWRI1499_0090	*SCP160*
-0.509355	6.25E-03	2.33E-02	AWRI1499_1702	
-0.509424	7.55E-03	2.73E-02	AWRI1499_2218	*SMC6*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.510251	5.00E-03	1.93E-02	AWRI1499_0428	*PUT3*
-0.512049	8.85E-03	3.10E-02	AWRI1499_1489	*ORT1*
-0.513057	4.90E-03	1.90E-02	AWRI1499_0142	*POL5*
-0.513228	5.65E-03	2.14E-02	AWRI1499_1073	*REI1*
-0.513805	4.65E-03	1.82E-02	AWRI1499_0494	
-0.516267	5.55E-03	2.11E-02	AWRI1499_1204	*CNS1*
-0.516355	1.42E-02	4.58E-02	AWRI1499_0758	*FIG4*
-0.516482	5.10E-03	1.96E-02	AWRI1499_4623	
-0.517629	9.60E-03	3.32E-02	AWRI1499_3588	*STP3*
-0.518774	5.05E-03	1.95E-02	AWRI1499_4869	*YPL150W*
-0.519626	5.80E-03	2.19E-02	AWRI1499_1917	
-0.52055	9.40E-03	3.26E-02	AWRI1499_1651	*GCN1*
-0.520719	4.95E-03	1.92E-02	AWRI1499_0177	*ATG1*
-0.520885	5.85E-03	2.20E-02	AWRI1499_2458	*NOP2*
-0.521071	5.85E-03	2.20E-02	AWRI1499_4487	*STE12*
-0.521428	4.45E-03	1.75E-02	AWRI1499_4552	*UFD4*
-0.521736	1.43E-02	4.61E-02	AWRI1499_0717	
-0.522191	6.05E-03	2.27E-02	AWRI1499_1627	*MCM2*
-0.523179	5.45E-03	2.08E-02	AWRI1499_2842	*MCH5*
-0.524151	9.80E-03	3.38E-02	AWRI1499_0505	*TIF3*
-0.525338	6.95E-03	2.54E-02	AWRI1499_1604	*KRS1*
-0.526788	6.00E-03	2.25E-02	AWRI1499_2247	*UBA3*
-0.527053	3.85E-03	1.55E-02	AWRI1499_1288	*ENP1*
-0.527386	5.80E-03	2.19E-02	AWRI1499_1601	*RPA34*
-0.528133	6.65E-03	2.45E-02	AWRI1499_2773	*YOR093C*
-0.528197	3.85E-03	1.55E-02	AWRI1499_3093	*ESF2*
-0.529116	7.45E-03	2.69E-02	AWRI1499_3586	*DCC1*
-0.529366	1.41E-02	4.56E-02	AWRI1499_0703	
-0.529375	4.65E-03	1.82E-02	AWRI1499_0244	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.530317	4.30E-03	1.70E-02	AWRI1499_2015	*NOP15*
-0.533016	2.85E-03	1.20E-02	AWRI1499_2217	*SMC6*
-0.533263	3.90E-03	1.57E-02	AWRI1499_4508	*TIF11*
-0.534161	9.85E-03	3.39E-02	AWRI1499_3686	
-0.535765	4.35E-03	1.72E-02	AWRI1499_3302	*MNN2*
-0.536508	3.90E-03	1.57E-02	AWRI1499_4315	
-0.538817	3.60E-03	1.46E-02	AWRI1499_3327	*YMR196W*
-0.540503	1.28E-02	4.21E-02	AWRI1499_2807	
-0.54095	3.60E-03	1.46E-02	AWRI1499_3414	*EBP2*
-0.542943	3.40E-03	1.40E-02	AWRI1499_2704	
-0.543936	1.27E-02	4.19E-02	AWRI1499_1166	*YOX1*
-0.544253	1.09E-02	3.69E-02	AWRI1499_0039	*RPL8b*
-0.545232	1.17E-02	3.91E-02	AWRI1499_4183	
-0.545687	1.06E-02	3.61E-02	AWRI1499_0215	
-0.545703	3.55E-03	1.45E-02	AWRI1499_2719	*MCM6*
-0.548227	3.30E-03	1.36E-02	AWRI1499_1460	*IKI1*
-0.548244	9.45E-03	3.28E-02	AWRI1499_1392	*TFC7*
-0.548739	5.00E-03	1.93E-02	AWRI1499_3561	
-0.549045	1.35E-02	4.38E-02	AWRI1499_4277	*YCR061W*
-0.551519	3.50E-03	1.43E-02	AWRI1499_3249	*ESBP6*
-0.553546	2.65E-03	1.13E-02	AWRI1499_1146	*TBF1*
-0.558979	5.55E-03	2.11E-02	AWRI1499_0840	*TDA4*
-0.559436	3.55E-03	1.45E-02	AWRI1499_0118	*SAP190*
-0.560707	2.20E-03	9.65E-03	AWRI1499_4871	*MET7*
-0.561843	3.30E-03	1.36E-02	AWRI1499_0903	*BUD23*
-0.561993	8.45E-03	2.99E-02	AWRI1499_2278	*SGD1*
-0.562626	2.85E-03	1.20E-02	AWRI1499_3026	*NOP6*
-0.563376	2.95E-03	1.24E-02	AWRI1499_1292	*YIL067C*
-0.563733	1.75E-03	7.94E-03	AWRI1499_1354	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.563945	6.30E-03	2.34E-02	AWRI1499_4020	*FUI1*
-0.564622	2.45E-03	1.06E-02	AWRI1499_3219	*RRP7*
-0.565643	4.40E-03	1.74E-02	AWRI1499_3057	*BAS1*
-0.565796	2.15E-03	9.47E-03	AWRI1499_4141	
-0.568376	2.00E-03	8.90E-03	AWRI1499_2128	*NSA2*
-0.569373	3.90E-03	1.57E-02	AWRI1499_0718	*RPA43*
-0.571072	9.45E-03	3.28E-02	AWRI1499_1161	*RPS17b*
-0.571084	6.70E-03	2.47E-02	AWRI1499_2521	
-0.5722	2.80E-03	1.19E-02	AWRI1499_0795	*RMT2*
-0.572967	3.25E-03	1.34E-02	AWRI1499_3506	*ODC2*
-0.573454	4.65E-03	1.82E-02	AWRI1499_4132	*YDJ1*
-0.575217	2.70E-03	1.15E-02	AWRI1499_4952	*CRZ1*
-0.577365	3.70E-03	1.50E-02	AWRI1499_2139	
-0.579822	8.50E-04	4.27E-03	AWRI1499_4096	
-0.581907	1.75E-03	7.94E-03	AWRI1499_0969	
-0.58221	1.20E-03	5.76E-03	AWRI1499_4676	
-0.583329	1.40E-03	6.57E-03	AWRI1499_3039	
-0.583633	6.00E-03	2.25E-02	AWRI1499_3835	*RPS7b*
-0.584466	1.70E-03	7.75E-03	AWRI1499_1925	
-0.585329	6.35E-03	2.36E-02	AWRI1499_0579	*PSE1*
-0.585932	3.20E-03	1.33E-02	AWRI1499_3549	*CSE1*
-0.58676	1.50E-03	6.97E-03	AWRI1499_0186	*MAK10*
-0.587253	2.75E-03	1.17E-02	AWRI1499_3834	
-0.587877	2.10E-03	9.28E-03	AWRI1499_1872	*LTV1*
-0.588423	1.65E-03	7.56E-03	AWRI1499_3112	*SFP1*
-0.588597	1.15E-03	5.55E-03	AWRI1499_3126	*SSL2*
-0.589082	7.60E-03	2.74E-02	AWRI1499_3730	*RPL7b*
-0.590159	2.00E-03	8.90E-03	AWRI1499_0123	*KRE33*
-0.590483	1.55E-03	7.16E-03	AWRI1499_1134	*SDA1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.590522	1.80E-03	8.14E-03	AWRI1499_3311	*YNL193W*
-0.594252	1.03E-02	3.52E-02	AWRI1499_4103	
-0.59436	5.60E-03	2.12E-02	AWRI1499_0258	*TPA1*
-0.59454	3.85E-03	1.55E-02	AWRI1499_0212	
-0.596284	8.10E-03	2.89E-02	AWRI1499_3742	*AAH1*
-0.596393	1.15E-03	5.55E-03	AWRI1499_0079	*CLF1*
-0.597074	1.15E-03	5.55E-03	AWRI1499_3594	*IOC4*
-0.598	1.35E-02	4.38E-02	AWRI1499_0429	*PAB1*
-0.599602	1.60E-03	7.36E-03	AWRI1499_0282	
-0.600183	7.50E-04	3.83E-03	AWRI1499_3543	
-0.600522	1.70E-03	7.75E-03	AWRI1499_4149	*FUN30*
-0.601183	3.20E-03	1.33E-02	AWRI1499_1833	*LYS4*
-0.6018	2.25E-03	9.84E-03	AWRI1499_1683	*LEU4*
-0.603302	1.90E-03	8.52E-03	AWRI1499_2750	*SUP45*
-0.603635	2.30E-03	1.00E-02	AWRI1499_3676	*REX2*
-0.60463	1.25E-03	5.97E-03	AWRI1499_3027	*CDC42*
-0.608009	2.20E-03	9.65E-03	AWRI1499_2856	*SOD1*
-0.609674	7.60E-03	2.74E-02	AWRI1499_1264	
-0.614553	1.40E-03	6.57E-03	AWRI1499_3861	*PFK27*
-0.615952	7.95E-03	2.84E-02	AWRI1499_4579	*ILV2*
-0.616305	9.50E-04	4.70E-03	AWRI1499_0999	*RRP5*
-0.617026	2.85E-03	1.20E-02	AWRI1499_1815	*CAK1*
-0.618537	1.10E-03	5.34E-03	AWRI1499_4178	*EMW1*
-0.620788	7.50E-04	3.83E-03	AWRI1499_1283	*PUS1*
-0.623717	1.05E-03	5.12E-03	AWRI1499_2334	*MCM3*
-0.627987	2.65E-03	1.13E-02	AWRI1499_4115	*TIF4631*
-0.628587	2.20E-03	9.65E-03	AWRI1499_1797	*PWP2*
-0.628879	7.00E-04	3.62E-03	AWRI1499_3647	
-0.62905	4.95E-03	1.92E-02	AWRI1499_1248	*NOP14*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.629904	1.10E-03	5.34E-03	AWRI1499_2296	*ESBP6*
-0.630086	1.70E-03	7.75E-03	AWRI1499_4743	*YOL098C*
-0.630942	2.40E-03	1.04E-02	AWRI1499_4028	*POL2*
-0.63216	1.45E-03	6.77E-03	AWRI1499_0207	*ITR2*
-0.633872	1.30E-03	6.17E-03	AWRI1499_2290	*MAK11*
-0.63434	2.25E-03	9.84E-03	AWRI1499_4542	*MIS1*
-0.635565	6.50E-04	3.39E-03	AWRI1499_0137	*RRN3*
-0.639205	3.20E-03	1.33E-02	AWRI1499_0949	
-0.641939	1.00E-04	6.63E-04	AWRI1499_4436	
-0.64201	6.00E-04	3.17E-03	AWRI1499_3833	
-0.644025	5.50E-04	2.94E-03	AWRI1499_4208	
-0.644501	3.05E-03	1.27E-02	AWRI1499_1824	*YMC1*
-0.645654	1.09E-02	3.69E-02	AWRI1499_1772	
-0.646307	6.50E-04	3.39E-03	AWRI1499_4866	*RSB1*
-0.646546	6.50E-04	3.39E-03	AWRI1499_0404	*HMS1*
-0.646938	5.00E-04	2.71E-03	AWRI1499_0405	
-0.648104	4.00E-04	2.24E-03	AWRI1499_2140	
-0.649314	4.40E-03	1.74E-02	AWRI1499_1654	
-0.650533	6.00E-04	3.17E-03	AWRI1499_3827	*BRX1*
-0.653168	6.00E-04	3.17E-03	AWRI1499_0862	
-0.653292	2.50E-04	1.48E-03	AWRI1499_0686	*SWC3*
-0.653947	7.20E-03	2.62E-02	AWRI1499_1686	*IME2*
-0.654274	5.50E-04	2.94E-03	AWRI1499_3188	*RRP12*
-0.654465	1.17E-02	3.90E-02	AWRI1499_2042	*SSE1*
-0.654888	1.00E-03	4.91E-03	AWRI1499_0468	*DML1*
-0.655238	3.00E-04	1.74E-03	AWRI1499_3901	*JID1*
-0.655349	4.30E-03	1.70E-02	AWRI1499_3859	*RTT10*
-0.655767	5.85E-03	2.20E-02	AWRI1499_4635	*SER1*
-0.656062	9.40E-03	3.26E-02	AWRI1499_3826	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.656116	1.90E-03	8.52E-03	AWRI1499_0751	*NAN1*
-0.657688	5.50E-04	2.94E-03	AWRI1499_0160	*ZRT2*
-0.658653	5.50E-04	2.94E-03	AWRI1499_0200	*RPS22a*
-0.660406	4.50E-04	2.48E-03	AWRI1499_3028	
-0.66469	1.65E-03	7.56E-03	AWRI1499_2859	*URA7*
-0.664788	2.50E-04	1.48E-03	AWRI1499_0513	*DBP9*
-0.666966	1.05E-03	5.12E-03	AWRI1499_3418	
-0.667216	5.00E-04	2.71E-03	AWRI1499_3050	*RRP1*
-0.670173	2.40E-03	1.04E-02	AWRI1499_1357	*BMS1*
-0.670626	5.50E-04	2.94E-03	AWRI1499_0727	*HSP26*
-0.670853	5.50E-04	2.94E-03	AWRI1499_3371	*UTP20*
-0.671763	9.00E-03	3.15E-02	AWRI1499_1502	
-0.671936	4.00E-04	2.24E-03	AWRI1499_1480	*TEC1*
-0.674523	8.50E-04	4.27E-03	AWRI1499_2394	*RAD57*
-0.676154	2.00E-03	8.90E-03	AWRI1499_2009	*CLG1*
-0.676721	1.00E-03	4.91E-03	AWRI1499_1431	*ERG12*
-0.679124	6.00E-04	3.17E-03	AWRI1499_4094	
-0.679662	3.50E-04	1.99E-03	AWRI1499_1011	*PWP1*
-0.679919	4.50E-04	2.48E-03	AWRI1499_2142	
-0.680768	4.00E-03	1.60E-02	AWRI1499_4550	
-0.684286	2.20E-03	9.65E-03	AWRI1499_4606	
-0.684429	5.00E-04	2.71E-03	AWRI1499_4940	*CYB2*
-0.684682	3.00E-04	1.74E-03	AWRI1499_0037	*PIN4*
-6.86E-01	7.50E-04	3.83E-03	AWRI1499_3702	
-0.686651	1.00E-04	6.63E-04	AWRI1499_3332	*ZAP1*
-0.688059	2.65E-03	1.13E-02	AWRI1499_3348	*ATO2*
-0.689899	2.35E-03	1.02E-02	AWRI1499_2849	*KNS1*
-0.691037	3.75E-03	1.52E-02	AWRI1499_1165	
-0.691895	1.00E-04	6.63E-04	AWRI1499_1790	*HST2*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.692624	4.50E-04	2.48E-03	AWRI1499_3819	*RPA190*
-0.693533	1.00E-03	4.91E-03	AWRI1499_0261	*YDR341C*
-0.693746	1.50E-04	9.48E-04	AWRI1499_4019	*FUR4*
-0.694841	4.50E-04	2.48E-03	AWRI1499_0691	*NOP53*
-0.696026	3.50E-04	1.99E-03	AWRI1499_1639	*UTP10*
-0.696425	1.25E-03	5.97E-03	AWRI1499_2557	*PRP46*
-0.699101	2.00E-04	1.22E-03	AWRI1499_3842	
-0.699868	2.00E-04	1.22E-03	AWRI1499_0061	*NAR1*
-0.701681	7.50E-04	3.83E-03	AWRI1499_0888	*PBN1*
-0.702202	3.50E-04	1.99E-03	AWRI1499_0887	*SPB1*
-0.702955	7.00E-04	3.62E-03	AWRI1499_2728	*DBP10*
-0.704244	3.00E-04	1.74E-03	AWRI1499_1178	*RLP7*
-0.704668	2.00E-04	1.22E-03	AWRI1499_0874	*ARG2*
-0.70476	4.70E-03	1.83E-02	AWRI1499_4100	*ADE5,7*
-0.704898	2.50E-04	1.48E-03	AWRI1499_0014	*UTP21*
-0.707498	6.50E-04	3.39E-03	AWRI1499_1642	*GLN3*
-0.709126	4.50E-04	2.48E-03	AWRI1499_3535	
-0.709171	1.50E-04	9.48E-04	AWRI1499_0352	*DRE2*
-0.709786	1.00E-04	6.63E-04	AWRI1499_1323	
-0.710351	4.80E-03	1.87E-02	AWRI1499_2706	*RAD16*
-0.710763	5.00E-05	3.54E-04	AWRI1499_0146	*DIM1*
-0.711016	2.00E-04	1.22E-03	AWRI1499_2447	
-0.714254	2.50E-04	1.48E-03	AWRI1499_2129	*LCP5*
-0.714731	6.50E-04	3.39E-03	AWRI1499_0007	*RAD7*
-0.714868	1.20E-02	3.99E-02	AWRI1499_2536	*GPA1*
-0.714957	1.35E-03	6.38E-03	AWRI1499_3528	*MRS6*
-0.716754	5.00E-04	2.71E-03	AWRI1499_1926	
-0.718309	2.00E-04	1.22E-03	AWRI1499_1326	*LOS1*
-0.718496	5.00E-05	3.54E-04	AWRI1499_0083	*BEM2*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.719514	1.85E-03	8.32E-03	AWRI1499_3377	
-0.722142	5.00E-05	3.54E-04	AWRI1499_0617	
-0.723369	1.50E-04	9.48E-04	AWRI1499_1485	
-0.723445	1.50E-04	9.48E-04	AWRI1499_2483	*NOC2*
-0.725789	1.00E-04	6.63E-04	AWRI1499_4269	*RAD51*
-0.726771	2.50E-04	1.48E-03	AWRI1499_1281	*ARO3*
-0.728622	2.00E-04	1.22E-03	AWRI1499_3976	
-0.729826	2.00E-04	1.22E-03	AWRI1499_2580	*PUT4*
-0.730729	5.00E-05	3.54E-04	AWRI1499_1492	*UTP14*
-0.731076	1.00E-04	6.63E-04	AWRI1499_3454	*UBP3*
-0.734392	2.80E-03	1.19E-02	AWRI1499_0383	*GCV1*
-0.73535	2.00E-04	1.22E-03	AWRI1499_2569	*RNR1*
-0.735544	1.00E-04	6.63E-04	AWRI1499_4396	*FSF1*
-0.736132	5.00E-05	3.54E-04	AWRI1499_1537	*FCF2*
-0.736166	1.50E-04	9.48E-04	AWRI1499_1327	*LOS1*
-0.736876	1.00E-04	6.63E-04	AWRI1499_1777	*CBF5*
-0.738913	1.50E-04	9.48E-04	AWRI1499_4886	
-0.739997	2.00E-04	1.22E-03	AWRI1499_4216	*DBP2*
-0.741298	5.00E-04	2.71E-03	AWRI1499_4078	*YMR010W*
-0.742115	5.00E-05	3.54E-04	AWRI1499_3600	*RKM3*
-0.742262	2.50E-04	1.48E-03	AWRI1499_0484	*GCD14*
-0.742739	1.80E-03	8.14E-03	AWRI1499_1130	*ADE2*
-0.74338	1.50E-04	9.48E-04	AWRI1499_3938	*KAP123*
-0.744706	2.00E-04	1.22E-03	AWRI1499_2560	
-0.744916	5.00E-05	3.54E-04	AWRI1499_0689	*ABZ1*
-0.745089	1.00E-04	6.63E-04	AWRI1499_0506	*TIF3*
-0.74598	1.50E-04	9.48E-04	AWRI1499_4150	*FUN30*
-0.747616	3.00E-04	1.74E-03	AWRI1499_0259	*TPA1*
-0.749536	1.00E-04	6.63E-04	AWRI1499_4307	*NOP7*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.750028	2.50E-04	1.48E-03	AWRI1499_4530	*FPR3*
-0.750098	5.00E-05	3.54E-04	AWRI1499_2543	*SOK1*
-0.750306	5.00E-04	2.71E-03	AWRI1499_1246	*NOP14*
-0.75169	2.00E-04	1.22E-03	AWRI1499_0009	
-0.752084	5.00E-05	3.54E-04	AWRI1499_0245	*NOP56*
-0.753434	1.15E-03	5.55E-03	AWRI1499_3654	*LTP1*
-0.754165	1.50E-04	9.48E-04	AWRI1499_3577	*ISW2*
-0.756393	2.50E-04	1.48E-03	AWRI1499_0618	
-0.756403	5.00E-05	3.54E-04	AWRI1499_3978	*NOC4*
-0.760877	3.00E-04	1.74E-03	AWRI1499_4485	*NOP9*
-0.761799	3.00E-04	1.74E-03	AWRI1499_0313	*RLP24*
-0.762443	1.00E-04	6.63E-04	AWRI1499_1063	*TNA1*
-0.765149	5.00E-05	3.54E-04	AWRI1499_1928	
-0.766893	5.00E-05	3.54E-04	AWRI1499_0176	*ATG1*
-0.767856	8.50E-04	4.27E-03	AWRI1499_1713	*TIF1*
-0.768465	5.00E-05	3.54E-04	AWRI1499_3507	*ISU1*
-0.769439	2.00E-04	1.22E-03	AWRI1499_2700	
-0.770091	5.00E-05	3.54E-04	AWRI1499_3815	*OYE2*
-0.774151	5.00E-05	3.54E-04	AWRI1499_2451	*TIF6*
-0.775696	1.50E-04	9.48E-04	AWRI1499_1312	*DAL5*
-0.77598	2.00E-04	1.22E-03	AWRI1499_0967	*ECM16*
-0.782457	2.00E-04	1.22E-03	AWRI1499_3467	*URA1*
-0.785398	5.00E-05	3.54E-04	AWRI1499_1653	*GCN1*
-0.785679	5.00E-05	3.54E-04	AWRI1499_1692	*MDN1*
-0.786764	3.00E-03	1.26E-02	AWRI1499_2625	*SSB2*
-0.787562	4.50E-04	2.48E-03	AWRI1499_4724	*TPO1*
-0.789393	1.50E-04	9.48E-04	AWRI1499_0713	
-0.794363	2.55E-03	1.09E-02	AWRI1499_1486	
-0.795145	5.00E-05	3.54E-04	AWRI1499_2610	*PRP43*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.796594	5.00E-05	3.54E-04	AWRI1499_1454	*DBP3*
-0.800414	6.00E-03	2.25E-02	AWRI1499_0948	*VBA2*
-0.801253	5.00E-05	3.54E-04	AWRI1499_3114	*DUS3*
-0.803527	1.50E-04	9.48E-04	AWRI1499_4685	*AXL2*
-0.804064	2.00E-04	1.22E-03	AWRI1499_2274	*TMA19*
-0.804454	5.00E-05	3.54E-04	AWRI1499_3845	
-0.804567	8.55E-03	3.02E-02	AWRI1499_4652	
-0.807258	1.00E-04	6.63E-04	AWRI1499_1352	*PHO84*
-0.807457	5.00E-05	3.54E-04	AWRI1499_2003	*YOR1*
-0.807732	5.00E-05	3.54E-04	AWRI1499_3914	*CIR2*
-0.809004	1.50E-04	9.48E-04	AWRI1499_4270	*YEL1*
-0.809395	2.05E-03	9.08E-03	AWRI1499_0777	*SHM1*
-0.810968	1.00E-04	6.63E-04	AWRI1499_2141	
-0.811744	5.00E-05	3.54E-04	AWRI1499_1404	*NCL1*
-0.813452	2.80E-03	1.19E-02	AWRI1499_3995	
-0.81695	5.00E-05	3.54E-04	AWRI1499_4785	*MPP10*
-0.818361	1.40E-03	6.57E-03	AWRI1499_1846	*FSH1*
-0.818498	5.00E-05	3.54E-04	AWRI1499_3596	*CCC2*
-0.818562	5.00E-05	3.54E-04	AWRI1499_2222	
-0.821724	5.00E-05	3.54E-04	AWRI1499_3566	*PUF6*
-0.824792	2.00E-04	1.22E-03	AWRI1499_1135	*DBP8*
-0.825257	5.00E-05	3.54E-04	AWRI1499_4383	*OPT1*
-0.826527	3.30E-03	1.36E-02	AWRI1499_1877	*LYS21*
-0.830958	1.00E-04	6.63E-04	AWRI1499_1544	
-0.83168	5.00E-05	3.54E-04	AWRI1499_2626	*YNL095C*
-0.834776	8.00E-03	2.86E-02	AWRI1499_3453	
-0.836493	5.50E-04	2.94E-03	AWRI1499_3959	*THI4*
-0.841045	3.00E-04	1.74E-03	AWRI1499_0660	*SAM1*
-0.842221	1.50E-04	9.48E-04	AWRI1499_0980	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.842222	5.00E-05	3.54E-04	AWRI1499_0369	*NOG1*
-0.84434	2.25E-03	9.84E-03	AWRI1499_4390	
-0.845254	1.00E-04	6.63E-04	AWRI1499_3918	
-0.848379	5.00E-05	3.54E-04	AWRI1499_1340	*FRA1*
-0.849349	1.00E-04	6.63E-04	AWRI1499_1718	*HAS1*
-0.850285	5.00E-05	3.54E-04	AWRI1499_3202	*DUR1,2*
-0.851471	1.80E-03	8.14E-03	AWRI1499_0192	*PIB2*
-0.853016	5.00E-05	3.54E-04	AWRI1499_4709	
-0.853495	5.00E-05	3.54E-04	AWRI1499_0189	*YAP1*
-0.854102	5.00E-05	3.54E-04	AWRI1499_4553	*MRT4*
-0.854343	5.00E-05	3.54E-04	AWRI1499_1048	*ROK1*
-0.857068	5.00E-05	3.54E-04	AWRI1499_4424	
-0.85987	5.00E-05	3.54E-04	AWRI1499_2761	*MET16*
-0.860317	5.00E-05	3.54E-04	AWRI1499_4318	
-0.860619	5.00E-05	3.54E-04	AWRI1499_0625	*NOP58*
-0.861861	1.50E-04	9.48E-04	AWRI1499_2624	*RIO2*
-0.864524	5.00E-05	3.54E-04	AWRI1499_2937	
-0.865321	5.00E-05	3.54E-04	AWRI1499_4826	*CIT1*
-0.866487	1.00E-03	4.91E-03	AWRI1499_2888	*ADE13*
-0.866619	5.00E-05	3.54E-04	AWRI1499_3872	
-0.870662	5.00E-05	3.54E-04	AWRI1499_1424	*BIO2*
-0.876938	5.00E-05	3.54E-04	AWRI1499_3127	*SRP40*
-0.881737	5.00E-05	3.54E-04	AWRI1499_4253	*NEW1*
-0.887046	4.50E-04	2.48E-03	AWRI1499_3030	*SPE1*
-0.887295	5.00E-05	3.54E-04	AWRI1499_1364	*LSG1*
-0.887755	5.00E-05	3.54E-04	AWRI1499_4637	*IKI3*
-0.890873	5.00E-05	3.54E-04	AWRI1499_3508	*HOL1*
-0.891275	1.75E-03	7.94E-03	AWRI1499_0896	
-0.89333	5.00E-05	3.54E-04	AWRI1499_0885	*SPB1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.893735	5.00E-05	3.54E-04	AWRI1499_4882	
-0.893907	1.50E-04	9.48E-04	AWRI1499_3713	*PET18*
-0.898183	5.00E-05	3.54E-04	AWRI1499_3203	*DUR1,2*
-0.904363	2.00E-04	1.22E-03	AWRI1499_1429	*GUA1*
-0.909312	5.00E-05	3.54E-04	AWRI1499_1910	*RCL1*
-0.911309	5.00E-05	3.54E-04	AWRI1499_0968	*RAD5*
-0.915787	5.00E-05	3.54E-04	AWRI1499_2177	*NUG1*
-0.915936	5.00E-05	3.54E-04	AWRI1499_0578	*PXR1*
-0.921327	5.00E-05	3.54E-04	AWRI1499_3791	*YCR016W*
-0.923584	5.00E-05	3.54E-04	AWRI1499_1927	
-0.924662	5.00E-05	3.54E-04	AWRI1499_0526	*FLO11*
-0.927332	3.50E-04	1.99E-03	AWRI1499_1061	
-0.932618	5.00E-05	3.54E-04	AWRI1499_0093	*DBP1*
-0.934879	5.00E-05	3.54E-04	AWRI1499_2669	*CTP1*
-0.936394	5.00E-05	3.54E-04	AWRI1499_2304	*ENP2*
-0.937815	5.00E-05	3.54E-04	AWRI1499_2459	*NOP2*
-0.941924	5.00E-05	3.54E-04	AWRI1499_0769	*NDE2*
-0.947391	5.00E-05	3.54E-04	AWRI1499_0067	*YDJ1*
-0.948914	5.00E-05	3.54E-04	AWRI1499_4403	*GAP1*
-0.951103	5.00E-05	3.54E-04	AWRI1499_4090	*SSF1*
-0.952146	5.00E-05	3.54E-04	AWRI1499_2615	*MET32*
-0.956815	5.00E-05	3.54E-04	AWRI1499_1717	
-0.957475	1.00E-04	6.63E-04	AWRI1499_2726	
-0.958565	1.55E-03	7.16E-03	AWRI1499_0293	
-0.962262	5.00E-05	3.54E-04	AWRI1499_2070	*YMC1*
-0.962292	5.00E-05	3.54E-04	AWRI1499_1897	*PHO4*
-0.964011	5.00E-05	3.54E-04	AWRI1499_3069	*TSR1*
-0.965136	5.00E-05	3.54E-04	AWRI1499_1708	*SHB17*
-0.968569	5.00E-05	3.54E-04	AWRI1499_0183	*MIS1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.970337	9.75E-03	3.37E-02	AWRI1499_4874	*FMS1*
-0.970525	2.00E-04	1.22E-03	AWRI1499_0767	
-0.972208	1.70E-03	7.75E-03	AWRI1499_1133	*SDA1*
-0.975251	5.00E-05	3.54E-04	AWRI1499_0467	*RRP14*
-0.976257	5.00E-05	3.54E-04	AWRI1499_2691	*MET14*
-0.976919	5.00E-05	3.54E-04	AWRI1499_0975	*NOP4*
-0.977759	5.00E-05	3.54E-04	AWRI1499_2329	*RSA4*
-0.980691	5.00E-05	3.54E-04	AWRI1499_0886	*SPB1*
-0.981208	5.00E-05	3.54E-04	AWRI1499_1902	*MTD1*
-0.982793	5.00E-05	3.54E-04	AWRI1499_4543	*ADE3*
-0.983252	5.00E-05	3.54E-04	AWRI1499_4210	*OYE2*
-0.983981	5.00E-05	3.54E-04	AWRI1499_1379	
-0.984743	5.00E-05	3.54E-04	AWRI1499_4656	*HSP26*
-0.987764	5.00E-05	3.54E-04	AWRI1499_0436	*TNA1*
-0.989116	5.00E-05	3.54E-04	AWRI1499_3711	
-0.990015	5.00E-05	3.54E-04	AWRI1499_3920	*UTP13*
-0.990599	5.00E-05	3.54E-04	AWRI1499_1682	*LEU4*
-0.993538	9.50E-04	4.70E-03	AWRI1499_1247	*NOP14*
-0.994912	7.00E-04	3.62E-03	AWRI1499_0679	*MET6*
-0.995717	5.00E-05	3.54E-04	AWRI1499_2383	
-1.00047	5.00E-05	3.54E-04	AWRI1499_4360	*THI73*
-1.0013	5.00E-05	3.54E-04	AWRI1499_1356	*BMS1*
-1.0171	5.00E-05	3.54E-04	AWRI1499_1873	*BFR2*
-1.01743	5.00E-05	3.54E-04	AWRI1499_1099	*PSD1*
-1.02189	5.00E-05	3.54E-04	AWRI1499_1159	*UTP6*
-1.02311	5.00E-05	3.54E-04	AWRI1499_2034	*MSG5*
-1.02509	5.00E-05	3.54E-04	AWRI1499_1269	*UTP15*
-1.03097	5.00E-05	3.54E-04	AWRI1499_2727	*DBP10*
-1.03207	5.00E-05	3.54E-04	AWRI1499_3372	*UTP20*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-1.03272	5.00E-05	3.54E-04	AWRI1499_4776	*HEM1*
-1.03425	5.00E-05	3.54E-04	AWRI1499_1483	*MAK21*
-1.03839	1.50E-04	9.48E-04	AWRI1499_2051	*FCY2*
-1.04114	5.00E-05	3.54E-04	AWRI1499_0464	
-1.04178	5.00E-05	3.54E-04	AWRI1499_2952	
-1.04184	5.00E-05	3.54E-04	AWRI1499_1796	*PWP2*
-1.04483	5.00E-05	3.54E-04	AWRI1499_2305	*ENP2*
-1.04495	5.00E-05	3.54E-04	AWRI1499_4418	
-1.05262	5.00E-05	3.54E-04	AWRI1499_4095	
-1.05338	5.00E-05	3.54E-04	AWRI1499_4754	*UGA4*
-1.0534	6.50E-04	3.39E-03	AWRI1499_3115	*DUS3*
-1.05456	5.00E-05	3.54E-04	AWRI1499_1198	*FUR1*
-1.0552	5.00E-05	3.54E-04	AWRI1499_3814	*OYE2*
-1.056	5.00E-05	3.54E-04	AWRI1499_3079	*MAK16*
-1.06305	5.00E-05	3.54E-04	AWRI1499_0041	*RRN6*
-1.06484	5.00E-05	3.54E-04	AWRI1499_2837	
-1.07001	5.00E-05	3.54E-04	AWRI1499_1954	*DYS1*
-1.07683	5.00E-05	3.54E-04	AWRI1499_0712	
-1.07827	5.00E-05	3.54E-04	AWRI1499_4252	*NEW1*
-1.08454	5.00E-05	3.54E-04	AWRI1499_1695	*MDN1*
-1.08486	5.00E-05	3.54E-04	AWRI1499_4626	*GAP1*
-1.0875	2.50E-03	1.08E-02	AWRI1499_4126	*YDL144C*
-1.09565	1.00E-04	6.63E-04	AWRI1499_4879	
-1.10339	5.00E-05	3.54E-04	AWRI1499_1006	*RRP8*
-1.10521	5.00E-05	3.54E-04	AWRI1499_4361	*THI73*
-1.11214	5.00E-05	3.54E-04	AWRI1499_1638	*UTP10*
-1.11782	5.00E-05	3.54E-04	AWRI1499_1000	*RRP5*
-1.12007	5.00E-05	3.54E-04	AWRI1499_2369	
-1.12853	5.00E-05	3.54E-04	AWRI1499_4359	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-1.14259	2.25E-03	9.84E-03	AWRI1499_4507	*TRM732*
-1.14412	1.47E-02	4.71E-02	AWRI1499_4708	*MAL31*
-1.14418	7.00E-04	3.62E-03	AWRI1499_4796	
-1.14498	5.00E-05	3.54E-04	AWRI1499_3074	*SYO1*
-1.15545	5.00E-05	3.54E-04	AWRI1499_0532	
-1.15717	5.00E-05	3.54E-04	AWRI1499_3373	*UTP20*
-1.16211	5.00E-05	3.54E-04	AWRI1499_3038	*IMD4*
-1.16661	5.00E-05	3.54E-04	AWRI1499_2317	
-1.16772	5.00E-05	3.54E-04	AWRI1499_3534	
-1.16971	5.00E-05	3.54E-04	AWRI1499_3913	*CIR2*
-1.17352	5.00E-05	3.54E-04	AWRI1499_0870	*PRO3*
-1.17941	5.00E-05	3.54E-04	AWRI1499_0750	*NAN1*
-1.18209	1.00E-04	6.63E-04	AWRI1499_1863	*TPN1*
-1.18566	5.00E-05	3.54E-04	AWRI1499_2912	*UTP23*
-1.19968	5.00E-05	3.54E-04	AWRI1499_1896	
-1.20066	3.50E-04	1.99E-03	AWRI1499_3720	*FLO1*
-1.20685	5.00E-05	3.54E-04	AWRI1499_4438	*JIP5*
-1.20705	4.25E-03	1.69E-02	AWRI1499_2388	*YCT1*
-1.20787	5.00E-05	3.54E-04	AWRI1499_3399	*UGA4*
-1.21928	2.00E-04	1.22E-03	AWRI1499_4136	
-1.22685	5.00E-05	3.54E-04	AWRI1499_0976	*NOP4*
-1.22729	5.00E-05	3.54E-04	AWRI1499_3917	*IFH1*
-1.24131	5.00E-05	3.54E-04	AWRI1499_2936	
-1.25507	5.00E-05	3.54E-04	AWRI1499_3527	
-1.2587	5.00E-05	3.54E-04	AWRI1499_4323	*OXP1*
-1.26722	5.00E-05	3.54E-04	AWRI1499_1519	
-1.27871	5.00E-05	3.54E-04	AWRI1499_2827	
-1.27969	2.15E-03	9.47E-03	AWRI1499_1082	
-1.28399	5.00E-05	3.54E-04	AWRI1499_2004	*YOR1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-1.28945	5.00E-05	3.54E-04	AWRI1499_3916	
-1.30317	5.00E-05	3.54E-04	AWRI1499_2138	*YBR238C*
-1.31315	5.00E-05	3.54E-04	AWRI1499_4317	*FMO1*
-1.31365	5.00E-05	3.54E-04	AWRI1499_4461	*ARG8*
-1.31587	5.00E-05	3.54E-04	AWRI1499_1563	*BNA2*
-1.3346	5.00E-05	3.54E-04	AWRI1499_0294	
-1.36126	5.00E-05	3.54E-04	AWRI1499_4897	*YGR109W-B*
-1.36206	5.00E-05	3.54E-04	AWRI1499_3879	*MET3*
-1.37646	5.00E-05	3.54E-04	AWRI1499_4425	
-1.38792	5.00E-05	3.54E-04	AWRI1499_0462	*MET2*
-1.39944	1.00E-04	6.63E-04	AWRI1499_2082	
-1.409	5.00E-05	3.54E-04	AWRI1499_3899	*YMC1*
-1.41129	5.00E-05	3.54E-04	AWRI1499_1987	
-1.4161	5.00E-05	3.54E-04	AWRI1499_0859	*PDR16*
-1.41911	5.00E-05	3.54E-04	AWRI1499_4138	*JEN1*
-1.42503	5.00E-05	3.54E-04	AWRI1499_0704	*FAF1*
-1.43389	5.00E-05	3.54E-04	AWRI1499_4051	*GCV2*
-1.45956	5.00E-05	3.54E-04	AWRI1499_3193	*HEF3*
-1.46651	5.00E-05	3.54E-04	AWRI1499_1734	
-1.46693	5.00E-05	3.54E-04	AWRI1499_4313	
-1.47599	5.00E-05	3.54E-04	AWRI1499_2748	*MAK5*
-1.4974	5.00E-05	3.54E-04	AWRI1499_0534	*AMF1*
-1.50921	5.00E-05	3.54E-04	AWRI1499_4061	
-1.55376	5.00E-05	3.54E-04	AWRI1499_3844	*STL1*
-1.56994	5.00E-05	3.54E-04	AWRI1499_3400	*LSB1*
-1.5703	7.35E-03	2.66E-02	AWRI1499_1919	*NRT1*
-1.57537	5.00E-05	3.54E-04	AWRI1499_0411	*MAE1*
-1.58101	5.00E-05	3.54E-04	AWRI1499_0766	*NSR1*
-1.59801	5.00E-05	3.54E-04	AWRI1499_4956	

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-1.64618	5.00E-05	3.54E-04	AWRI1499_0342	*SWE1*
-1.64631	5.00E-05	3.54E-04	AWRI1499_2371	
-1.65033	5.00E-05	3.54E-04	AWRI1499_3518	*ALD4*
-1.6649	5.00E-05	3.54E-04	AWRI1499_1819	*MEP2*
-1.67091	5.00E-04	2.71E-03	AWRI1499_1933	*YLR278C*
-1.68675	5.00E-05	3.54E-04	AWRI1499_3438	*DUR3*
-1.73053	5.00E-05	3.54E-04	AWRI1499_4963	*HER2*
-1.75845	5.00E-05	3.54E-04	AWRI1499_4474	*CYB2*
-1.78093	5.00E-05	3.54E-04	AWRI1499_2113	*FTR1*
-1.80177	5.00E-05	3.54E-04	AWRI1499_1337	*DLD1*
-1.84036	5.00E-05	3.54E-04	AWRI1499_0077	*MCH2*
-1.8858	5.00E-05	3.54E-04	AWRI1499_3462	*JEN1*
-1.92402	5.00E-05	3.54E-04	AWRI1499_3753	*TRM2*
-1.94821	1.70E-03	7.75E-03	AWRI1499_4962	
-1.95394	5.00E-05	3.54E-04	AWRI1499_4964	
-1.95737	5.00E-05	3.54E-04	AWRI1499_2368	
-1.97376	5.00E-05	3.54E-04	AWRI1499_1468	
-1.97798	5.00E-05	3.54E-04	AWRI1499_1930	*HXK2*
-1.97815	5.00E-05	3.54E-04	AWRI1499_4615	
-2.00605	5.00E-05	3.54E-04	AWRI1499_1957	*PRX1*
-2.00772	5.00E-05	3.54E-04	AWRI1499_1432	
-2.08608	5.00E-05	3.54E-04	AWRI1499_1922	*YLR278C*
-2.17747	5.00E-05	3.54E-04	AWRI1499_3564	
-2.23552	5.00E-05	3.54E-04	AWRI1499_1929	
-2.24375	5.00E-05	3.54E-04	AWRI1499_2083	
-2.24767	1.00E-04	6.63E-04	AWRI1499_1808	*DAL4*
-2.29468	5.00E-05	3.54E-04	AWRI1499_4953	*SEO1*
-2.31351	5.00E-05	3.54E-04	AWRI1499_0196	*FET3*
-2.32638	5.00E-05	3.54E-04	AWRI1499_3463	*JEN1*

AWRI1499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-2.32817	5.05E-03	1.95E-02	AWRI1499_1923	*DAL5*
-2.38519	5.00E-05	3.54E-04	AWRI1499_3760	*SUL1*
-2.42991	5.00E-05	3.54E-04	AWRI1499_1336	*JLP1*
-2.45064	5.00E-05	3.54E-04	AWRI1499_4954	*DLD1*
-2.50199	5.00E-05	3.54E-04	AWRI1499_0959	*THI73*
-2.76874	5.00E-05	3.54E-04	AWRI1499_1353	*PHO84*
-2.99437	5.00E-05	3.54E-04	AWRI1499_0257	*FUN19*
-3.03003	5.00E-05	3.54E-04	AWRI1499_0988	
-3.04479	1.10E-03	5.34E-03	AWRI1499_4319	*FEN2*
-3.13796	5.00E-05	3.54E-04	AWRI1499_1484	*ARO10*
-3.29252	5.00E-05	3.54E-04	AWRI1499_1931	
-3.49718	5.00E-05	3.54E-04	AWRI1499_1308	
-4.22658	5.00E-05	3.54E-04	AWRI1499_1461	*GAP1*

CBS2499 ~ T5h-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.661682	1.00E-03	4.91E-03	AWRI1499_1038	
0.650109	7.65E-03	2.76E-02	AWRI1499_4045	*CPR3*
-0.469609	1.16E-02	3.88E-02	AWRI1499_4869	*YPL150W*
-0.510258	7.00E-03	2.56E-02	AWRI1499_2222	
-0.550136	3.30E-03	1.36E-02	AWRI1499_3326	*IRE1*
-0.558696	1.16E-02	3.87E-02	AWRI1499_3885	
-0.686429	6.80E-03	2.50E-02	AWRI1499_1734	
-0.747817	1.70E-03	7.75E-03	AWRI1499_0888	*PBN1*
-1.27345	1.56E-02	4.95E-02	AWRI1499_3932	

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
4.8078	5.00E-05	3.54E-04	AWRI1499_4109	*ACH1*
4.65339	5.00E-05	3.54E-04	AWRI1499_0332	*LSC1*
4.46718	5.00E-05	3.54E-04	AWRI1499_2863	*YLL056C*
4.35229	5.00E-05	3.54E-04	AWRI1499_1936	*MPH2*
4.06796	5.00E-05	3.54E-04	AWRI1499_1841	*RCF2*
3.97709	5.00E-05	3.54E-04	AWRI1499_4725	*HXT13*
3.74889	5.00E-05	3.54E-04	AWRI1499_1545	*VHR1*
3.66858	5.00E-05	3.54E-04	AWRI1499_4728	*GAL10*
3.58068	5.00E-05	3.54E-04	AWRI1499_2995	*CLD1*
3.47168	5.00E-05	3.54E-04	AWRI1499_3771	*LSC2*
3.36636	5.00E-05	3.54E-04	AWRI1499_4492	*PDX1*
3.29109	5.00E-05	3.54E-04	AWRI1499_2861	
3.26127	5.00E-05	3.54E-04	AWRI1499_4726	*GAL7*
3.24603	5.00E-05	3.54E-04	AWRI1499_0746	*NCE103*
3.22198	5.00E-05	3.54E-04	AWRI1499_4193	*GDH1*
3.1105	5.00E-05	3.54E-04	AWRI1499_4734	*HXT2*
2.92561	5.00E-05	3.54E-04	AWRI1499_2608	*PDB1*
2.72112	5.00E-05	3.54E-04	AWRI1499_4727	*GAL1*
2.60395	1.00E-03	4.91E-03	AWRI1499_4194	
2.43964	5.00E-05	3.54E-04	AWRI1499_2013	*LAT1*
2.39409	5.00E-05	3.54E-04	AWRI1499_2713	*BDH1*
2.37264	5.00E-05	3.54E-04	AWRI1499_4451	*FMP37*
2.32393	5.00E-05	3.54E-04	AWRI1499_2435	
2.2431	5.00E-05	3.54E-04	AWRI1499_4419	*DIP5*
2.18479	5.00E-05	3.54E-04	AWRI1499_3720	*FLO1*
2.12744	5.00E-05	3.54E-04	AWRI1499_3162	*PDA1*
2.08585	5.00E-05	3.54E-04	AWRI1499_2592	*PUT2*
2.05772	5.00E-05	3.54E-04	AWRI1499_4322	OXP1
2.04798	5.00E-05	3.54E-04	AWRI1499_0080	SSU1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
2.03188	2.25E-03	9.84E-03	AWRI1499_1321	HER2
1.99973	5.00E-05	3.54E-04	AWRI1499_2054	HSP12
1.99583	5.00E-05	3.54E-04	AWRI1499_1757	ALD5
1.98012	5.00E-05	3.54E-04	AWRI1499_2002	*LEU5*
1.94485	5.00E-05	3.54E-04	AWRI1499_1937	*SUC2*
1.90837	5.00E-05	3.54E-04	AWRI1499_4733	
1.90806	5.00E-05	3.54E-04	AWRI1499_4822	*ARO10*
1.89616	5.00E-05	3.54E-04	AWRI1499_3505	*SOR1*
1.70483	5.00E-05	3.54E-04	AWRI1499_4323	*OXP1*
1.68298	5.00E-05	3.54E-04	AWRI1499_4162	*YHL008C*
1.67838	3.00E-03	1.26E-02	AWRI1499_2715	
1.63166	5.00E-05	3.54E-04	AWRI1499_3668	*DLD1*
1.56991	5.00E-05	3.54E-04	AWRI1499_0599	*YLR345W*
1.56208	5.00E-05	3.54E-04	AWRI1499_4297	*TMA10*
1.54379	5.00E-05	3.54E-04	AWRI1499_4163	*YHL008C*
1.50044	1.00E-04	6.63E-04	AWRI1499_0803	
1.48532	7.50E-04	3.83E-03	AWRI1499_4932	
1.45714	5.00E-05	3.54E-04	AWRI1499_4878	
1.4566	5.00E-05	3.54E-04	AWRI1499_3836	*RKI1*
1.44446	5.00E-05	3.54E-04	AWRI1499_3719	*FDH1*
1.39771	5.00E-05	3.54E-04	AWRI1499_1452	
1.37765	2.55E-03	1.09E-02	AWRI1499_3822	*ADH7*
1.36384	5.00E-05	3.54E-04	AWRI1499_2921	*UGA1*
1.36294	5.00E-05	3.54E-04	AWRI1499_2040	*ETR1*
1.33875	5.00E-05	3.54E-04	AWRI1499_1194	YGR012W
1.3242	5.00E-05	3.54E-04	AWRI1499_1614	FOX2
1.32304	5.00E-05	3.54E-04	AWRI1499_4474	CYB2
1.31718	5.00E-05	3.54E-04	AWRI1499_3429	
1.31593	5.00E-05	3.54E-04	AWRI1499_1227	

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
1.31544	5.00E-05	3.54E-04	AWRI1499_0213	ADH6
1.30336	5.00E-05	3.54E-04	AWRI1499_3271	NTH1
1.30306	5.00E-05	3.54E-04	AWRI1499_1233	
1.29955	5.00E-05	3.54E-04	AWRI1499_3365	
1.2965	5.00E-05	3.54E-04	AWRI1499_4832	YDR109C
1.2866	5.00E-05	3.54E-04	AWRI1499_3058	
1.2741	5.00E-05	3.54E-04	AWRI1499_1038	
1.25646	1.14E-02	3.81E-02	AWRI1499_1615	FOX2
1.25181	5.00E-05	3.54E-04	AWRI1499_4559	UTR2
1.23611	2.50E-04	1.48E-03	AWRI1499_1325	
1.23536	5.00E-05	3.54E-04	AWRI1499_3766	GOR1
1.23164	5.00E-05	3.54E-04	AWRI1499_1315	
1.23106	1.00E-04	6.63E-04	AWRI1499_4618	ENA1
1.23033	5.00E-05	3.54E-04	AWRI1499_4196	PAB1
1.21869	5.00E-05	3.54E-04	AWRI1499_3376	HTB1
1.2055	1.05E-03	5.12E-03	AWRI1499_3347	ADY2
1.19657	5.00E-05	3.54E-04	AWRI1499_1115	JEN1
1.19615	5.00E-05	3.54E-04	AWRI1499_0804	
1.19604	3.80E-03	1.53E-02	AWRI1499_0109	HXK1
1.19176	5.00E-05	3.54E-04	AWRI1499_3001	
1.1759	5.50E-04	2.94E-03	AWRI1499_1933	YLR278C
1.17581	5.00E-05	3.54E-04	AWRI1499_3584	PST2
1.16304	5.00E-05	3.54E-04	AWRI1499_4934	SPS19
1.16082	5.00E-05	3.54E-04	AWRI1499_2878	GLY1
1.1575	5.00E-05	3.54E-04	AWRI1499_3825	ADH6
1.13674	5.00E-05	3.54E-04	AWRI1499_0089	
1.13431	5.00E-05	3.54E-04	AWRI1499_4879	
1.13111	5.00E-05	3.54E-04	AWRI1499_2549	YDL124W
1.1191	3.10E-03	1.29E-02	AWRI1499_0306	CRN1

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
1.1018	1.10E-03	5.34E-03	AWRI1499_1807	
1.09843	5.00E-05	3.54E-04	AWRI1499_2529	BOP3
1.09764	5.00E-05	3.54E-04	AWRI1499_3518	ALD4
1.08932	5.00E-05	3.54E-04	AWRI1499_3111	IAH1
1.07492	1.00E-04	6.63E-04	AWRI1499_2949	MET10
1.06798	5.00E-05	3.54E-04	AWRI1499_2157	HHF2
1.06537	5.00E-05	3.54E-04	AWRI1499_2090	
1.05767	5.00E-05	3.54E-04	AWRI1499_4413	RTC3
1.0571	5.00E-05	3.54E-04	AWRI1499_1368	IMO32
1.05552	2.30E-03	1.00E-02	AWRI1499_4520	
1.04773	5.00E-05	3.54E-04	AWRI1499_1316	
1.0467	5.00E-05	3.54E-04	AWRI1499_3765	GOR1
1.04598	5.00E-05	3.54E-04	AWRI1499_4849	HMO1
1.04169	5.00E-05	3.54E-04	AWRI1499_2548	CAT8
1.03694	5.00E-05	3.54E-04	AWRI1499_4752	PRD1
1.03198	5.00E-05	3.54E-04	AWRI1499_0561	MYO1
1.02651	5.00E-05	3.54E-04	AWRI1499_3031	ASH1
1.02408	5.00E-05	3.54E-04	AWRI1499_1649	GCN1
1.01369	5.00E-05	3.54E-04	AWRI1499_0593	
1.00328	1.50E-04	9.48E-04	AWRI1499_4490	NOC3
0.996266	5.00E-05	3.54E-04	AWRI1499_0997	ETP1
0.993975	5.00E-05	3.54E-04	AWRI1499_4853	
0.992941	5.00E-05	3.54E-04	AWRI1499_3059	
0.989557	5.00E-05	3.54E-04	AWRI1499_2089	CLU1
0.987185	5.00E-05	3.54E-04	AWRI1499_1338	JLP1
0.984392	3.70E-03	1.50E-02	AWRI1499_2697	
0.983983	6.80E-03	2.50E-02	AWRI1499_3119	
0.977159	5.00E-05	3.54E-04	AWRI1499_1030	PUT1
0.971843	5.00E-05	3.54E-04	AWRI1499_3375	HTA1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.969815	5.00E-05	3.54E-04	AWRI1499_3820	
0.966107	5.00E-05	3.54E-04	AWRI1499_3513	PIR3
0.946835	5.00E-05	3.54E-04	AWRI1499_3423	MOT1
0.946557	5.00E-05	3.54E-04	AWRI1499_2501	
0.943385	1.00E-04	6.63E-04	AWRI1499_4376	HTB1
0.93597	5.00E-05	3.54E-04	AWRI1499_2862	GUT1
0.933635	5.00E-05	3.54E-04	AWRI1499_0825	RPS26a
0.933175	5.00E-05	3.54E-04	AWRI1499_2453	SWI5
0.932192	5.00E-05	3.54E-04	AWRI1499_2386	
0.932162	5.00E-05	3.54E-04	AWRI1499_1217	
0.930919	5.00E-05	3.54E-04	AWRI1499_3754	
0.930128	1.00E-04	6.63E-04	AWRI1499_1966	POL1
0.928273	5.00E-05	3.54E-04	AWRI1499_4884	IMA1
0.927308	5.00E-05	3.54E-04	AWRI1499_3615	SOL3
0.923714	1.00E-04	6.63E-04	AWRI1499_2547	CAT8
0.921996	2.00E-04	1.22E-03	AWRI1499_2389	YCT1
0.918055	5.45E-03	2.08E-02	AWRI1499_3062	MET5
0.909874	2.00E-04	1.22E-03	AWRI1499_2384	URE2
0.905779	5.00E-05	3.54E-04	AWRI1499_0637	
0.903269	5.00E-05	3.54E-04	AWRI1499_1045	GAT2
0.903102	5.00E-05	3.54E-04	AWRI1499_2967	
0.900588	5.00E-05	3.54E-04	AWRI1499_0768	NDI1
0.900199	5.00E-05	3.54E-04	AWRI1499_3741	RPP2b
0.897485	8.00E-04	4.06E-03	AWRI1499_3743	
0.891948	5.00E-05	3.54E-04	AWRI1499_0178	
0.890412	5.00E-05	3.54E-04	AWRI1499_3478	CYM1
0.881897	1.15E-03	5.55E-03	AWRI1499_0072	MET17
0.878638	1.00E-04	6.63E-04	AWRI1499_3540	
0.869063	4.60E-03	1.80E-02	AWRI1499_1942	

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.868384	1.00E-04	6.63E-04	AWRI1499_1493	YHR112C
0.867924	3.00E-04	1.74E-03	AWRI1499_2277	RPP0
0.860256	5.00E-05	3.54E-04	AWRI1499_0566	THI20
0.854767	5.00E-05	3.54E-04	AWRI1499_4839	RPL8b
0.852113	5.00E-05	3.54E-04	AWRI1499_0443	
0.849005	1.50E-04	9.48E-04	AWRI1499_1733	
0.846441	5.00E-05	3.54E-04	AWRI1499_0098	YMR027W
0.846431	5.00E-05	3.54E-04	AWRI1499_4375	HTA1
0.836207	5.00E-05	3.54E-04	AWRI1499_1216	RPL38
0.833978	1.50E-04	9.48E-04	AWRI1499_4048	YPR011C
0.828604	1.50E-04	9.48E-04	AWRI1499_2225	YCF1
0.818465	5.00E-05	3.54E-04	AWRI1499_4195	HHO1
0.815119	5.00E-05	3.54E-04	AWRI1499_2925	
0.813919	1.00E-04	6.63E-04	AWRI1499_3801	RPL28
0.81114	5.00E-05	3.54E-04	AWRI1499_4414	MGM1
0.805906	5.00E-05	3.54E-04	AWRI1499_1063	TNA1
0.804615	5.00E-05	3.54E-04	AWRI1499_1334	
0.804012	5.00E-05	3.54E-04	AWRI1499_0583	MIH1
0.800728	5.00E-05	3.54E-04	AWRI1499_2851	
0.799422	4.85E-03	1.88E-02	AWRI1499_3666	
0.799054	1.50E-04	9.48E-04	AWRI1499_1077	TNA1
0.798822	5.00E-05	3.54E-04	AWRI1499_1530	
0.796405	5.00E-05	3.54E-04	AWRI1499_1885	ATP4
0.790865	3.00E-04	1.74E-03	AWRI1499_0217	SNQ2
0.789139	5.00E-05	3.54E-04	AWRI1499_2215	
0.788047	1.50E-04	9.48E-04	AWRI1499_1964	ALK2
0.787415	5.00E-05	3.54E-04	AWRI1499_4473	BUB3
0.787143	6.40E-03	2.37E-02	AWRI1499_4245	
0.784699	1.50E-04	9.48E-04	AWRI1499_1428	RPL43a

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.779508	7.50E-04	3.83E-03	AWRI1499_2466	YBR053C
0.779267	5.00E-05	3.54E-04	AWRI1499_3618	CAB1
0.775835	5.00E-05	3.54E-04	AWRI1499_0040	CWH41
0.77423	5.00E-05	3.54E-04	AWRI1499_0361	TOM1
0.772764	5.00E-05	3.54E-04	AWRI1499_2167	
0.769171	1.00E-04	6.63E-04	AWRI1499_4210	OYE2
0.768932	1.80E-03	8.14E-03	AWRI1499_2156	HHT2
0.767839	2.00E-04	1.22E-03	AWRI1499_4756	JLP1
0.767754	2.10E-03	9.28E-03	AWRI1499_4620	TIP1
0.766715	5.00E-05	3.54E-04	AWRI1499_0878	SEC16
0.76636	5.00E-05	3.54E-04	AWRI1499_2788	
0.762868	1.00E-04	6.63E-04	AWRI1499_0274	PLB3
0.762188	1.00E-04	6.63E-04	AWRI1499_2615	MET32
0.761105	1.50E-03	6.97E-03	AWRI1499_2234	
0.760105	5.00E-05	3.54E-04	AWRI1499_2060	
0.760033	5.00E-05	3.54E-04	AWRI1499_1548	MSH6
0.753723	5.00E-05	3.54E-04	AWRI1499_3785	HOM3
0.753048	3.50E-04	1.99E-03	AWRI1499_0629	BUB2
0.752109	2.00E-04	1.22E-03	AWRI1499_3170	
0.751376	5.00E-05	3.54E-04	AWRI1499_1244	NCA2
0.750802	6.50E-04	3.39E-03	AWRI1499_4410	MSC7
0.749523	5.00E-05	3.54E-04	AWRI1499_3123	
0.748655	5.00E-04	2.71E-03	AWRI1499_4825	
0.746476	5.00E-04	2.71E-03	AWRI1499_0630	
0.745462	5.00E-05	3.54E-04	AWRI1499_4041	
0.741202	2.00E-04	1.22E-03	AWRI1499_4489	
0.739365	2.25E-03	9.84E-03	AWRI1499_1532	
0.737029	6.50E-04	3.39E-03	AWRI1499_4001	
0.736005	2.00E-03	8.90E-03	AWRI1499_1957	PRX1

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.735457	7.50E-04	3.83E-03	AWRI1499_2568	RNR1
0.732859	1.00E-04	6.63E-04	AWRI1499_1880	RPS9a
0.731324	3.50E-04	1.99E-03	AWRI1499_1683	LEU4
0.729864	5.00E-05	3.54E-04	AWRI1499_0740	BST1
0.729221	6.50E-04	3.39E-03	AWRI1499_0516	
0.727824	4.50E-04	2.48E-03	AWRI1499_3512	PIR1
0.727272	2.40E-03	1.04E-02	AWRI1499_2663	PDS5
0.726762	3.00E-04	1.74E-03	AWRI1499_0594	
0.725669	5.00E-05	3.54E-04	AWRI1499_0032	CHS2
0.725263	2.50E-04	1.48E-03	AWRI1499_0200	RPS22a
0.725216	1.00E-04	6.63E-04	AWRI1499_0038	QCR6
0.724753	1.00E-04	6.63E-04	AWRI1499_1128	YDL144C
0.724566	2.00E-04	1.22E-03	AWRI1499_3786	CBR1
0.723124	5.00E-05	3.54E-04	AWRI1499_1911	MVP1
0.721012	6.00E-04	3.17E-03	AWRI1499_3210	PGM2
0.719935	1.00E-04	6.63E-04	AWRI1499_3800	YGL101W
0.71756	1.30E-03	6.17E-03	AWRI1499_1226	SPS1
0.714458	4.85E-03	1.88E-02	AWRI1499_2341	
0.708075	8.75E-03	3.07E-02	AWRI1499_3109	ECM3
0.70644	3.00E-04	1.74E-03	AWRI1499_4354	VNX1
0.704815	2.00E-04	1.22E-03	AWRI1499_4046	ATX1
0.703312	1.15E-03	5.55E-03	AWRI1499_2094	YAK1
0.701245	3.50E-04	1.99E-03	AWRI1499_2385	FMO1
0.6995	4.50E-04	2.48E-03	AWRI1499_4919	QCR8
0.698679	2.50E-04	1.48E-03	AWRI1499_4659	LIP5
0.698329	4.50E-04	2.48E-03	AWRI1499_2570	RPL26a
0.698261	5.65E-03	2.14E-02	AWRI1499_0971	MOD5
0.697155	1.00E-04	6.63E-04	AWRI1499_0355	RPS22a
0.69189	1.00E-04	6.63E-04	AWRI1499_0018	

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.690897	5.00E-04	2.71E-03	AWRI1499_2518	
0.686873	2.50E-04	1.48E-03	AWRI1499_2640	
0.686856	7.50E-04	3.83E-03	AWRI1499_0076	
0.686081	3.00E-04	1.74E-03	AWRI1499_0960	AMD2
0.685387	1.30E-03	6.17E-03	AWRI1499_3483	RPL13a
0.683035	4.45E-03	1.75E-02	AWRI1499_2977	
0.682743	2.75E-03	1.17E-02	AWRI1499_2202	GSY1
0.682095	2.00E-04	1.22E-03	AWRI1499_2910	OSH3
0.681217	1.58E-02	4.99E-02	AWRI1499_4821	ARO10
0.680996	1.00E-04	6.63E-04	AWRI1499_1449	
0.680555	2.95E-03	1.24E-02	AWRI1499_2097	BOR1
0.680495	2.50E-04	1.48E-03	AWRI1499_3343	CTR1
0.680111	1.10E-03	5.34E-03	AWRI1499_0169	BIT61
0.67544	1.44E-02	4.62E-02	AWRI1499_0334	
0.673307	1.42E-02	4.58E-02	AWRI1499_3934	
0.673142	1.35E-03	6.38E-03	AWRI1499_1490	RPP2b
0.671395	6.95E-03	2.54E-02	AWRI1499_2802	
0.670978	1.60E-03	7.36E-03	AWRI1499_2683	DNF1
0.670566	3.00E-04	1.74E-03	AWRI1499_1620	HOF1
0.670476	5.00E-04	2.71E-03	AWRI1499_1726	LIP2
0.669987	4.00E-04	2.24E-03	AWRI1499_0812	TRL1
0.669423	1.35E-03	6.38E-03	AWRI1499_3043	
0.667338	2.50E-04	1.48E-03	AWRI1499_3878	RRT12
0.666553	5.00E-04	2.71E-03	AWRI1499_4455	SMC2
0.665164	6.00E-04	3.17E-03	AWRI1499_2793	MEC1
0.664939	5.50E-04	2.94E-03	AWRI1499_3289	HIS1
0.663673	4.50E-04	2.48E-03	AWRI1499_2581	
0.663011	2.50E-04	1.48E-03	AWRI1499_3988	YMR226C
0.662692	1.35E-03	6.38E-03	AWRI1499_3721	

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.661824	8.50E-03	3.00E-02	AWRI1499_2913	LSM6
0.661426	9.50E-04	4.70E-03	AWRI1499_0483	
0.660644	8.50E-04	4.27E-03	AWRI1499_3758	
0.659557	1.80E-03	8.14E-03	AWRI1499_2723	RPS19b
0.657964	1.70E-03	7.75E-03	AWRI1499_0572	SGA1
0.65739	2.50E-04	1.48E-03	AWRI1499_4471	
0.655092	1.85E-03	8.32E-03	AWRI1499_4546	
0.654949	4.50E-04	2.48E-03	AWRI1499_0387	
0.654291	5.00E-04	2.71E-03	AWRI1499_0475	RPL9b
0.653777	1.40E-03	6.57E-03	AWRI1499_1525	RPL22a
0.651144	2.50E-04	1.48E-03	AWRI1499_2322	RAP1
0.648073	4.00E-04	2.24E-03	AWRI1499_3529	RPS12
0.647045	2.20E-03	9.65E-03	AWRI1499_0496	
0.644857	6.00E-04	3.17E-03	AWRI1499_1826	
0.64467	9.00E-04	4.49E-03	AWRI1499_3852	CTI6
0.64423	8.50E-04	4.27E-03	AWRI1499_1948	YPR117W
0.643735	7.50E-04	3.83E-03	AWRI1499_3912	CDC24
0.643493	8.00E-04	4.06E-03	AWRI1499_3782	SEC24
0.643318	8.50E-04	4.27E-03	AWRI1499_0302	SEN1
0.642787	8.50E-04	4.27E-03	AWRI1499_0906	YCR051W
0.64258	7.00E-04	3.62E-03	AWRI1499_3550	MYO5
0.642518	3.50E-04	1.99E-03	AWRI1499_1394	EPO1
0.642296	5.55E-03	2.11E-02	AWRI1499_1846	FSH1
0.641623	6.00E-04	3.17E-03	AWRI1499_1049	ATP20
0.641389	3.50E-04	1.99E-03	AWRI1499_3355	LDB17
0.640136	1.50E-03	6.97E-03	AWRI1499_2380	BLM10
0.638523	4.75E-03	1.85E-02	AWRI1499_1919	NRT1
0.638431	3.75E-03	1.52E-02	AWRI1499_4023	
0.637695	1.20E-03	5.76E-03	AWRI1499_3701	PRY3

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.636762	5.00E-04	2.71E-03	AWRI1499_4047	
0.635752	1.45E-03	6.77E-03	AWRI1499_3438	DUR3
0.634308	6.50E-04	3.39E-03	AWRI1499_2772	ECM15
0.634008	3.40E-03	1.40E-02	AWRI1499_3472	RPS2
0.632601	1.30E-03	6.17E-03	AWRI1499_2397	LAG1
0.632404	7.50E-04	3.83E-03	AWRI1499_0187	RPL12a
0.632341	3.50E-03	1.43E-02	AWRI1499_4713	PFF1
0.632058	1.85E-03	8.32E-03	AWRI1499_4877	ATM1
0.63191	3.50E-04	1.99E-03	AWRI1499_3974	DBF2
0.631378	1.00E-03	4.91E-03	AWRI1499_2348	MLC2
0.630915	8.00E-04	4.06E-03	AWRI1499_2675	ROT2
0.630124	1.50E-03	6.97E-03	AWRI1499_0502	RPS13
0.629406	1.65E-03	7.56E-03	AWRI1499_4427	COR1
0.62927	4.00E-04	2.24E-03	AWRI1499_3444	CDC39
0.628562	2.85E-03	1.20E-02	AWRI1499_2722	RPL18a
0.628026	2.00E-03	8.90E-03	AWRI1499_1596	HSP150
0.626543	6.50E-04	3.39E-03	AWRI1499_4381	CHS1
0.626208	8.00E-04	4.06E-03	AWRI1499_2291	
0.625991	1.40E-03	6.57E-03	AWRI1499_2865	
0.625852	5.00E-04	2.71E-03	AWRI1499_0053	
0.622913	1.35E-03	6.38E-03	AWRI1499_1630	CHS3
0.621508	1.00E-03	4.91E-03	AWRI1499_4640	RPS27b
0.619297	3.95E-03	1.59E-02	AWRI1499_2780	PTA1
0.618126	1.00E-03	4.91E-03	AWRI1499_4358	CTS1
0.617269	7.00E-04	3.62E-03	AWRI1499_2716	
0.616135	1.55E-03	7.16E-03	AWRI1499_1317	IAH1
0.614752	8.50E-04	4.27E-03	AWRI1499_3590	URE2
0.614344	9.50E-04	4.70E-03	AWRI1499_0613	
0.613761	9.50E-04	4.70E-03	AWRI1499_1989	ATP16

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.613393	1.00E-03	4.91E-03	AWRI1499_0088	TSR4
0.613043	3.35E-03	1.38E-02	AWRI1499_1318	HXT13
0.611601	3.95E-03	1.59E-02	AWRI1499_2087	RPL27b
0.611598	1.20E-03	5.76E-03	AWRI1499_4838	RPL29
0.611514	3.05E-03	1.27E-02	AWRI1499_0697	RFA1
0.610538	1.80E-03	8.14E-03	AWRI1499_1336	JLP1
0.610351	9.50E-04	4.70E-03	AWRI1499_4165	
0.608408	3.90E-03	1.57E-02	AWRI1499_1531	
0.604691	7.10E-03	2.59E-02	AWRI1499_3700	
0.604512	1.10E-03	5.34E-03	AWRI1499_2376	CYT1
0.604264	3.60E-03	1.46E-02	AWRI1499_4493	
0.603724	1.39E-02	4.50E-02	AWRI1499_1025	
0.602878	1.12E-02	3.76E-02	AWRI1499_3756	
0.601917	1.05E-03	5.12E-03	AWRI1499_0408	
0.601863	3.15E-03	1.31E-02	AWRI1499_0389	
0.600422	6.00E-03	2.25E-02	AWRI1499_2354	SMX3
0.599394	1.60E-03	7.36E-03	AWRI1499_1613	
0.59881	1.15E-03	5.55E-03	AWRI1499_3806	ERT1
0.597796	2.05E-03	9.08E-03	AWRI1499_2953	PEX14
0.597379	3.15E-03	1.31E-02	AWRI1499_3517	GRX8
0.597128	6.35E-03	2.36E-02	AWRI1499_2108	TRM3
0.596644	3.60E-03	1.46E-02	AWRI1499_1103	YPR010C-A
0.595676	1.50E-03	6.97E-03	AWRI1499_0199	ACO2
0.595177	2.00E-03	8.90E-03	AWRI1499_3157	GDE1
0.594259	1.03E-02	3.52E-02	AWRI1499_4148	
0.593941	2.00E-03	8.90E-03	AWRI1499_3536	RPS20
0.593666	3.35E-03	1.38E-02	AWRI1499_1215	HIR2
0.593617	2.55E-03	1.09E-02	AWRI1499_4447	RAD1
0.592056	1.70E-03	7.75E-03	AWRI1499_4586	KEL1

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.591352	3.05E-03	1.27E-02	AWRI1499_3606	MSB2
0.590745	2.05E-03	9.08E-03	AWRI1499_1644	
0.586898	6.50E-03	2.41E-02	AWRI1499_0322	TFB1
0.586309	1.40E-03	6.57E-03	AWRI1499_0665	
0.585864	7.90E-03	2.83E-02	AWRI1499_0872	ATP2
0.584858	5.35E-03	2.04E-02	AWRI1499_3889	
0.584538	2.45E-03	1.06E-02	AWRI1499_3641	
0.584427	1.70E-03	7.75E-03	AWRI1499_2200	DSE1
0.583036	8.15E-03	2.90E-02	AWRI1499_3024	AFM1
0.582836	1.65E-03	7.56E-03	AWRI1499_1314	DAL5
0.582814	1.45E-03	6.77E-03	AWRI1499_2170	
0.582242	1.55E-03	7.16E-03	AWRI1499_2124	
0.581181	4.05E-03	1.62E-02	AWRI1499_4797	TCB1
0.580796	4.90E-03	1.90E-02	AWRI1499_3509	XKS1
0.579311	1.40E-03	6.57E-03	AWRI1499_2079	SEC7
0.578831	9.75E-03	3.37E-02	AWRI1499_4611	
0.577045	5.15E-03	1.98E-02	AWRI1499_2973	RPS10b
0.57642	1.75E-03	7.94E-03	AWRI1499_3356	PKP1
0.575818	2.20E-03	9.65E-03	AWRI1499_1209	SCO1
0.574387	1.65E-03	7.56E-03	AWRI1499_2243	
0.573875	2.65E-03	1.13E-02	AWRI1499_4541	
0.572139	6.60E-03	2.44E-02	AWRI1499_0508	RPL32
0.571564	4.90E-03	1.90E-02	AWRI1499_1197	ACT1
0.57149	3.90E-03	1.57E-02	AWRI1499_4263	PIC2
0.571239	1.85E-03	8.32E-03	AWRI1499_0087	DBF4
0.571132	5.30E-03	2.03E-02	AWRI1499_3116	RPL31b
0.568794	2.20E-03	9.65E-03	AWRI1499_0351	ASE1
0.568677	1.75E-03	7.94E-03	AWRI1499_2961	OXA1
0.567308	5.95E-03	2.23E-02	AWRI1499_1150	RPS25a

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.56629	9.30E-03	3.23E-02	AWRI1499_3231	
0.565945	1.02E-02	3.48E-02	AWRI1499_1455	CRH1
0.565755	4.55E-03	1.79E-02	AWRI1499_3787	SKY1
0.564822	1.85E-03	8.32E-03	AWRI1499_2838	ATP17
0.564443	2.20E-03	9.65E-03	AWRI1499_2900	
0.562787	4.55E-03	1.79E-02	AWRI1499_1881	RPL21b
0.562311	3.20E-03	1.33E-02	AWRI1499_4918	ALG12
0.560684	4.75E-03	1.85E-02	AWRI1499_4625	RNA14
0.560252	1.56E-02	4.95E-02	AWRI1499_4714	
0.560059	6.65E-03	2.45E-02	AWRI1499_2911	RPS14a
0.559739	2.40E-03	1.04E-02	AWRI1499_2752	RTS1
0.559647	3.00E-03	1.26E-02	AWRI1499_3139	
0.558308	3.10E-03	1.29E-02	AWRI1499_3146	TRA1
0.557619	3.60E-03	1.46E-02	AWRI1499_3107	
0.557499	1.75E-03	7.94E-03	AWRI1499_4452	
0.556902	6.30E-03	2.34E-02	AWRI1499_3761	
0.556761	6.75E-03	2.48E-02	AWRI1499_3760	SUL1
0.555796	4.85E-03	1.88E-02	AWRI1499_2438	UGA4
0.555332	6.85E-03	2.51E-02	AWRI1499_2891	YBR219C
0.555166	6.60E-03	2.44E-02	AWRI1499_0486	RPL23b
0.554494	3.10E-03	1.29E-02	AWRI1499_3837	ARF3
0.554421	2.95E-03	1.24E-02	AWRI1499_0452	TRI1
0.553684	1.55E-02	4.92E-02	AWRI1499_3424	MOT1
0.553439	4.70E-03	1.83E-02	AWRI1499_0329	
0.55291	5.45E-03	2.08E-02	AWRI1499_0708	RPS23b
0.552213	8.40E-03	2.97E-02	AWRI1499_0110	
0.552163	9.10E-03	3.18E-02	AWRI1499_0568	CDA2
0.55074	4.70E-03	1.83E-02	AWRI1499_3083	CDC5
0.550107	3.75E-03	1.52E-02	AWRI1499_2133	SLA1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.547877	2.75E-03	1.17E-02	AWRI1499_1112	
0.54766	2.70E-03	1.15E-02	AWRI1499_1426	SKT5
0.547371	5.15E-03	1.98E-02	AWRI1499_0511	MHT1
0.547267	2.85E-03	1.20E-02	AWRI1499_4494	CPR1
0.546711	1.03E-02	3.52E-02	AWRI1499_0119	
0.545815	4.85E-03	1.88E-02	AWRI1499_2758	SDS24
0.545764	3.90E-03	1.57E-02	AWRI1499_0769	NDE2
0.544561	2.60E-03	1.11E-02	AWRI1499_3205	
0.543902	2.55E-03	1.09E-02	AWRI1499_2262	ELF1
0.542591	5.35E-03	2.04E-02	AWRI1499_4462	CRC1
0.542088	8.80E-03	3.09E-02	AWRI1499_2596	FHL1
0.541752	9.55E-03	3.31E-02	AWRI1499_0926	
0.539878	4.65E-03	1.82E-02	AWRI1499_0822	
0.538787	3.00E-03	1.26E-02	AWRI1499_4804	TAO3
0.537919	4.35E-03	1.72E-02	AWRI1499_2461	
0.537857	8.70E-03	3.06E-02	AWRI1499_2426	RPS24b
0.537482	4.30E-03	1.70E-02	AWRI1499_1060	RPP1a
0.537141	4.15E-03	1.65E-02	AWRI1499_0543	
0.535785	1.31E-02	4.28E-02	AWRI1499_2344	TED1
0.535471	9.65E-03	3.34E-02	AWRI1499_2184	ALD2
0.534395	4.35E-03	1.72E-02	AWRI1499_2331	RPL33a
0.534111	8.20E-03	2.92E-02	AWRI1499_2207	UBP7
0.53391	2.75E-03	1.17E-02	AWRI1499_4256	
0.533399	3.65E-03	1.48E-02	AWRI1499_4570	KRE5
0.533368	4.40E-03	1.74E-02	AWRI1499_0908	NPL3
0.531688	7.80E-03	2.80E-02	AWRI1499_0875	ELP3
0.531602	3.35E-03	1.38E-02	AWRI1499_1701	BEM1
0.530513	4.05E-03	1.62E-02	AWRI1499_1505	UBP2
0.530221	4.35E-03	1.72E-02	AWRI1499_4059	RPL36a

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.530006	4.10E-03	1.64E-02	AWRI1499_0732	AQY1
0.529162	1.19E-02	3.96E-02	AWRI1499_1998	EFB1
0.527573	5.45E-03	2.08E-02	AWRI1499_0671	YNR021W
0.526473	6.40E-03	2.37E-02	AWRI1499_0934	
0.52498	4.10E-03	1.64E-02	AWRI1499_0263	RIB3
0.523655	1.28E-02	4.20E-02	AWRI1499_0819	SMI1
0.523429	1.28E-02	4.21E-02	AWRI1499_1229	
0.522484	5.05E-03	1.95E-02	AWRI1499_1187	AIM45
0.521297	8.05E-03	2.87E-02	AWRI1499_2332	
0.520295	9.00E-03	3.15E-02	AWRI1499_3733	
0.516465	9.90E-03	3.41E-02	AWRI1499_0547	CAB2
0.515901	1.35E-02	4.38E-02	AWRI1499_2691	MET14
0.514608	1.08E-02	3.65E-02	AWRI1499_0055	RPS8b
0.514574	1.03E-02	3.52E-02	AWRI1499_3578	
0.513447	4.90E-03	1.90E-02	AWRI1499_3166	BRR2
0.512865	4.65E-03	1.82E-02	AWRI1499_2210	GUT2
0.511106	7.00E-03	2.56E-02	AWRI1499_0919	IRA1
0.511106	8.45E-03	2.99E-02	AWRI1499_1640	MEX67
0.510662	1.38E-02	4.48E-02	AWRI1499_0289	SYF1
0.510262	8.10E-03	2.89E-02	AWRI1499_1587	MSH2
0.510118	9.40E-03	3.26E-02	AWRI1499_3815	OYE2
0.509819	5.35E-03	2.04E-02	AWRI1499_4560	QCR7
0.509539	6.35E-03	2.36E-02	AWRI1499_3092	RIB4
0.50923	9.95E-03	3.42E-02	AWRI1499_4739	KOG1
0.506796	4.75E-03	1.85E-02	AWRI1499_3225	
0.506782	1.01E-02	3.45E-02	AWRI1499_3286	RPL34a
0.506604	8.75E-03	3.07E-02	AWRI1499_0706	RPB3
0.504203	9.30E-03	3.23E-02	AWRI1499_2070	YMC1
0.503911	6.80E-03	2.50E-02	AWRI1499_4829	BUD5

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.503207	7.30E-03	2.65E-02	AWRI1499_2898	NMA1
0.502322	1.42E-02	4.58E-02	AWRI1499_0444	RPL42a
0.502052	6.20E-03	2.31E-02	AWRI1499_2168	PMI40
0.501608	8.70E-03	3.06E-02	AWRI1499_0810	CMR1
0.501266	1.02E-02	3.48E-02	AWRI1499_3137	TUF1
0.500626	1.03E-02	3.51E-02	AWRI1499_2744	
0.499593	8.50E-03	3.00E-02	AWRI1499_4512	CDC23
0.499331	6.20E-03	2.31E-02	AWRI1499_4101	
0.498593	6.50E-03	2.41E-02	AWRI1499_0700	PMC1
0.497583	7.05E-03	2.57E-02	AWRI1499_4006	
0.497398	9.05E-03	3.16E-02	AWRI1499_1491	
0.49694	1.32E-02	4.32E-02	AWRI1499_1360	RPL1a
0.496631	1.12E-02	3.77E-02	AWRI1499_2972	HOS2
0.495988	7.30E-03	2.65E-02	AWRI1499_1955	
0.494977	9.15E-03	3.19E-02	AWRI1499_4067	SCW11
0.494474	1.57E-02	4.98E-02	AWRI1499_4678	RPS28b
0.494117	1.45E-02	4.66E-02	AWRI1499_1344	SLH1
0.493427	9.05E-03	3.16E-02	AWRI1499_4791	EMC1
0.4931	7.35E-03	2.66E-02	AWRI1499_0527	KIP2
0.492909	7.95E-03	2.84E-02	AWRI1499_4285	YDL124W
0.490835	1.08E-02	3.66E-02	AWRI1499_3732	GAP1
0.490267	1.10E-02	3.70E-02	AWRI1499_2944	POL3
0.489544	1.28E-02	4.21E-02	AWRI1499_4966	SUR1
0.489211	6.10E-03	2.28E-02	AWRI1499_4645	TRP3
0.488385	7.00E-03	2.56E-02	AWRI1499_0993	GCN2
0.487406	1.50E-02	4.79E-02	AWRI1499_3740	RPS15
0.487314	8.85E-03	3.10E-02	AWRI1499_1058	CLB4
0.487077	7.85E-03	2.81E-02	AWRI1499_4664	SLK19
0.486941	7.15E-03	2.60E-02	AWRI1499_3035	TEM1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.486118	7.50E-03	2.71E-02	AWRI1499_0363	HSP10
0.485729	7.70E-03	2.77E-02	AWRI1499_3248	
0.484811	1.19E-02	3.96E-02	AWRI1499_1043	
0.483975	1.10E-02	3.70E-02	AWRI1499_3612	
0.48318	7.85E-03	2.81E-02	AWRI1499_2885	BPH1
0.479037	1.18E-02	3.94E-02	AWRI1499_3484	RPS16a
0.478207	8.55E-03	3.02E-02	AWRI1499_1474	YJR096W
0.477857	8.30E-03	2.95E-02	AWRI1499_0278	HTZ1
0.477769	1.13E-02	3.78E-02	AWRI1499_4342	GEM1
0.476521	1.08E-02	3.66E-02	AWRI1499_3075	UBC9
0.474882	1.15E-02	3.86E-02	AWRI1499_0057	PRS3
0.474826	9.50E-03	3.29E-02	AWRI1499_4436	
0.470172	1.27E-02	4.19E-02	AWRI1499_1447	HGH1
0.470057	1.02E-02	3.48E-02	AWRI1499_0240	UGA3
0.468262	1.24E-02	4.10E-02	AWRI1499_3350	CSF1
0.467253	1.24E-02	4.09E-02	AWRI1499_3042	SPC2
0.466676	1.19E-02	3.95E-02	AWRI1499_4812	SEC3
0.465424	1.23E-02	4.06E-02	AWRI1499_4742	
0.464499	1.09E-02	3.69E-02	AWRI1499_2158	IDP2
0.463136	1.03E-02	3.52E-02	AWRI1499_0460	
0.462928	1.29E-02	4.24E-02	AWRI1499_3403	YNK1
0.462746	1.53E-02	4.86E-02	AWRI1499_4532	VMA10
0.46097	1.08E-02	3.66E-02	AWRI1499_4223	
0.460058	1.45E-02	4.66E-02	AWRI1499_4467	STI1
0.459117	1.08E-02	3.66E-02	AWRI1499_3216	SLY1
0.458058	1.58E-02	4.99E-02	AWRI1499_1383	YJR142W
0.457543	1.20E-02	3.98E-02	AWRI1499_3025	AFM1
0.455237	1.42E-02	4.58E-02	AWRI1499_4599	GYP1
0.453642	1.30E-02	4.27E-02	AWRI1499_0756	

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
0.453633	1.39E-02	4.49E-02	AWRI1499_0998	ETP1
0.45355	1.40E-02	4.52E-02	AWRI1499_4015	TFC4
0.452963	1.22E-02	4.03E-02	AWRI1499_2436	FPR1
0.452669	1.31E-02	4.28E-02	AWRI1499_3336	AIM10
0.452021	1.37E-02	4.45E-02	AWRI1499_4848	
0.449705	1.41E-02	4.54E-02	AWRI1499_3651	MET8
0.449669	1.51E-02	4.81E-02	AWRI1499_3201	UBP16
0.448207	1.41E-02	4.56E-02	AWRI1499_3479	PTM1
0.448193	1.37E-02	4.44E-02	AWRI1499_1778	
0.445297	1.53E-02	4.86E-02	AWRI1499_0249	KTR4
0.444128	1.31E-02	4.28E-02	AWRI1499_3431	UBC7
0.443547	1.56E-02	4.94E-02	AWRI1499_4806	MXR2
0.439998	1.55E-02	4.91E-02	AWRI1499_4130	
0.438057	1.57E-02	4.98E-02	AWRI1499_1714	RPS21a
-0.438142	1.49E-02	4.75E-02	AWRI1499_3600	RKM3
-0.440107	1.56E-02	4.95E-02	AWRI1499_4510	TSL1
-0.44107	1.55E-02	4.91E-02	AWRI1499_3538	BNA6
-0.442072	1.55E-02	4.91E-02	AWRI1499_1110	CAN1
-0.443829	1.45E-02	4.65E-02	AWRI1499_2188	SMF3
-0.446514	1.30E-02	4.25E-02	AWRI1499_1562	ESA1
-0.44751	1.40E-02	4.53E-02	AWRI1499_3369	
-0.449103	1.49E-02	4.77E-02	AWRI1499_0852	OXP1
-0.449288	1.39E-02	4.49E-02	AWRI1499_0190	SEC23
-0.449362	1.33E-02	4.33E-02	AWRI1499_2690	
-0.451332	1.44E-02	4.63E-02	AWRI1499_0450	YFL042C
-0.453562	1.48E-02	4.73E-02	AWRI1499_1867	RTF1
-0.453801	1.35E-02	4.38E-02	AWRI1499_2041	SKY1
-0.454251	1.22E-02	4.05E-02	AWRI1499_4695	
-0.454816	1.12E-02	3.76E-02	AWRI1499_0509	DRS2

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.457062	1.53E-02	4.87E-02	AWRI1499_3716	TIP1
-0.460986	1.36E-02	4.41E-02	AWRI1499_1702	
-0.461549	1.19E-02	3.96E-02	AWRI1499_4230	SAF1
-0.462142	1.31E-02	4.29E-02	AWRI1499_1011	PWP1
-0.462886	1.24E-02	4.09E-02	AWRI1499_4084	SAC6
-0.464021	1.18E-02	3.94E-02	AWRI1499_2974	YOR292C
-0.464425	1.55E-02	4.92E-02	AWRI1499_3938	KAP123
-0.46536	1.10E-02	3.71E-02	AWRI1499_1261	SNT2
-0.465672	1.09E-02	3.67E-02	AWRI1499_3793	VMS1
-0.466123	1.17E-02	3.91E-02	AWRI1499_3095	CTR9
-0.466426	9.25E-03	3.22E-02	AWRI1499_4131	TMA108
-0.470566	9.90E-03	3.41E-02	AWRI1499_4039	EFR3
-0.476094	7.55E-03	2.73E-02	AWRI1499_3637	
-0.476525	1.34E-02	4.36E-02	AWRI1499_4690	SPT8
-0.47712	1.28E-02	4.20E-02	AWRI1499_3125	SSL2
-0.477895	1.17E-02	3.90E-02	AWRI1499_3791	YCR016W
-0.479614	1.08E-02	3.66E-02	AWRI1499_3326	IRE1
-0.47992	1.08E-02	3.65E-02	AWRI1499_2425	SEC6
-0.479978	1.51E-02	4.82E-02	AWRI1499_2065	
-0.480945	1.24E-02	4.09E-02	AWRI1499_2842	MCH5
-0.481406	7.80E-03	2.80E-02	AWRI1499_1739	
-0.481709	1.50E-02	4.79E-02	AWRI1499_1074	KIN28
-0.481796	1.36E-02	4.41E-02	AWRI1499_0661	PSP1
-0.483805	9.40E-03	3.26E-02	AWRI1499_3436	ORM1
-0.484562	1.05E-02	3.58E-02	AWRI1499_4019	FUR4
-0.485011	8.55E-03	3.02E-02	AWRI1499_3148	FEX1
-0.485031	9.20E-03	3.21E-02	AWRI1499_0862	
-0.485368	8.00E-03	2.86E-02	AWRI1499_3253	
-0.486253	1.57E-02	4.96E-02	AWRI1499_4159	

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.486281	1.34E-02	4.36E-02	AWRI1499_4438	JIP5
-0.487089	8.20E-03	2.92E-02	AWRI1499_4384	CNA1
-0.487827	1.05E-02	3.56E-02	AWRI1499_4872	IZH3
-0.488275	7.85E-03	2.81E-02	AWRI1499_2511	NFI1
-0.489793	9.20E-03	3.21E-02	AWRI1499_3367	
-0.491083	7.50E-03	2.71E-02	AWRI1499_2928	DAS2
-0.491096	7.45E-03	2.69E-02	AWRI1499_2442	TGL1
-0.492723	1.25E-02	4.12E-02	AWRI1499_2512	
-0.492881	1.12E-02	3.76E-02	AWRI1499_0468	DML1
-0.494066	1.44E-02	4.63E-02	AWRI1499_0897	GCD6
-0.494868	7.35E-03	2.66E-02	AWRI1499_0343	OAF1
-0.495403	8.65E-03	3.05E-02	AWRI1499_1853	SEC17
-0.496634	7.05E-03	2.57E-02	AWRI1499_0048	VPS13
-0.497652	6.05E-03	2.27E-02	AWRI1499_1460	IKI1
-0.498067	8.45E-03	2.99E-02	AWRI1499_3925	UBC12
-0.49815	6.95E-03	2.54E-02	AWRI1499_4258	ARG81
-0.500445	9.10E-03	3.18E-02	AWRI1499_1345	SLH1
-0.501111	8.65E-03	3.05E-02	AWRI1499_4082	NOT3
-0.50209	9.35E-03	3.25E-02	AWRI1499_1446	TFG1
-0.502228	7.80E-03	2.80E-02	AWRI1499_3392	
-0.503019	1.31E-02	4.29E-02	AWRI1499_4123	GIN4
-0.50326	9.30E-03	3.23E-02	AWRI1499_2095	
-0.503503	1.06E-02	3.59E-02	AWRI1499_1713	TIF1
-0.50392	7.50E-03	2.71E-02	AWRI1499_3440	BRE5
-0.504054	5.35E-03	2.04E-02	AWRI1499_4753	PPZ1
-0.504557	7.80E-03	2.80E-02	AWRI1499_1301	YKU80
-0.505197	6.30E-03	2.34E-02	AWRI1499_4901	MLH1
-0.505456	5.25E-03	2.01E-02	AWRI1499_4315	
-0.506472	4.70E-03	1.83E-02	AWRI1499_3920	UTP13

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.506591	5.15E-03	1.98E-02	AWRI1499_0312	SEC2
-0.50739	5.40E-03	2.06E-02	AWRI1499_0642	ULS1
-0.508319	1.40E-02	4.52E-02	AWRI1499_4580	VPS53
-0.508907	7.70E-03	2.77E-02	AWRI1499_0800	YHR020W
-0.510154	6.00E-03	2.25E-02	AWRI1499_0016	
-0.510494	1.26E-02	4.15E-02	AWRI1499_0807	
-0.511791	7.35E-03	2.66E-02	AWRI1499_1799	CKI1
-0.515015	9.85E-03	3.39E-02	AWRI1499_2301	
-0.515054	8.70E-03	3.06E-02	AWRI1499_2587	
-0.515631	1.49E-02	4.77E-02	AWRI1499_1246	NOP14
-0.515687	8.30E-03	2.95E-02	AWRI1499_0210	SNX4
-0.518447	1.04E-02	3.55E-02	AWRI1499_2159	
-0.518468	4.85E-03	1.88E-02	AWRI1499_4468	SNF1
-0.519896	1.07E-02	3.62E-02	AWRI1499_0634	
-0.520021	6.40E-03	2.37E-02	AWRI1499_2064	RAV1
-0.520079	5.25E-03	2.01E-02	AWRI1499_4556	STE20
-0.521065	5.65E-03	2.14E-02	AWRI1499_3474	
-0.521415	7.90E-03	2.83E-02	AWRI1499_0984	TMC1
-0.521569	6.25E-03	2.33E-02	AWRI1499_4056	GYP5
-0.522464	5.90E-03	2.22E-02	AWRI1499_1410	
-0.52306	5.65E-03	2.14E-02	AWRI1499_1478	
-0.523506	9.45E-03	3.28E-02	AWRI1499_4132	YDJ1
-0.525979	7.65E-03	2.76E-02	AWRI1499_4784	
-0.528025	5.05E-03	1.95E-02	AWRI1499_1241	PHO91
-0.529418	7.95E-03	2.84E-02	AWRI1499_2832	
-0.529636	5.10E-03	1.96E-02	AWRI1499_0678	SHP1
-0.530263	3.05E-03	1.27E-02	AWRI1499_3680	CIA1
-0.530267	6.95E-03	2.54E-02	AWRI1499_0151	YSH1
-0.530806	5.10E-03	1.96E-02	AWRI1499_1974	SNT1

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.533724	6.05E-03	2.27E-02	AWRI1499_3961	ROG3
-0.534292	3.60E-03	1.46E-02	AWRI1499_1488	YEA6
-0.53598	4.45E-03	1.75E-02	AWRI1499_2311	
-0.537512	4.00E-03	1.60E-02	AWRI1499_1984	BDP1
-0.537594	4.05E-03	1.62E-02	AWRI1499_0296	
-0.538568	6.75E-03	2.48E-02	AWRI1499_0282	
-0.539704	3.25E-03	1.34E-02	AWRI1499_4016	TFC4
-0.540321	3.30E-03	1.36E-02	AWRI1499_4378	UTP8
-0.540481	9.80E-03	3.38E-02	AWRI1499_1173	GLG2
-0.541277	6.00E-03	2.25E-02	AWRI1499_4890	REV3
-0.542069	6.65E-03	2.45E-02	AWRI1499_4360	THI73
-0.542254	3.05E-03	1.27E-02	AWRI1499_4794	MDV1
-0.542318	1.27E-02	4.17E-02	AWRI1499_4225	
-0.545635	3.45E-03	1.41E-02	AWRI1499_4269	RAD51
-0.545991	6.35E-03	2.36E-02	AWRI1499_0967	ECM16
-0.546198	5.15E-03	1.98E-02	AWRI1499_0160	ZRT2
-0.547151	3.20E-03	1.33E-02	AWRI1499_1094	RPO41
-0.548107	2.60E-03	1.11E-02	AWRI1499_3548	CSE1
-0.548188	7.65E-03	2.76E-02	AWRI1499_1326	LOS1
-0.548274	3.20E-03	1.33E-02	AWRI1499_0867	RTC1
-0.548414	1.08E-02	3.66E-02	AWRI1499_4684	
-0.548628	7.95E-03	2.84E-02	AWRI1499_1248	NOP14
-0.550462	3.05E-03	1.27E-02	AWRI1499_4771	SLN1
-0.551351	5.60E-03	2.12E-02	AWRI1499_4744	YOL098C
-0.551852	5.45E-03	2.08E-02	AWRI1499_2305	ENP2
-0.551942	4.25E-03	1.69E-02	AWRI1499_0467	RRP14
-0.552299	2.25E-03	9.84E-03	AWRI1499_2381	BLM10
-0.55271	1.01E-02	3.47E-02	AWRI1499_0798	FMP40
-0.55314	3.65E-03	1.48E-02	AWRI1499_2610	PRP43

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.555044	3.35E-03	1.38E-02	AWRI1499_4249	PRP4
-0.555087	2.80E-03	1.19E-02	AWRI1499_3044	GCG1
-0.555977	2.30E-03	1.00E-02	AWRI1499_4445	RAD1
-0.557463	2.45E-03	1.06E-02	AWRI1499_0578	PXR1
-0.558399	1.80E-03	8.14E-03	AWRI1499_2228	YCF1
-0.560945	8.90E-03	3.12E-02	AWRI1499_4240	AMD1
-0.561038	7.15E-03	2.60E-02	AWRI1499_2278	SGD1
-0.563161	5.80E-03	2.19E-02	AWRI1499_4591	YPL260W
-0.563682	2.05E-03	9.08E-03	AWRI1499_0298	URA4
-0.564106	3.90E-03	1.57E-02	AWRI1499_1269	UTP15
-0.564508	2.30E-03	1.00E-02	AWRI1499_2605	TRE2
-0.564901	4.40E-03	1.74E-02	AWRI1499_1469	ERG13
-0.565094	2.20E-03	9.65E-03	AWRI1499_2179	
-0.56553	3.00E-03	1.26E-02	AWRI1499_2686	FLO8
-0.567579	2.10E-03	9.28E-03	AWRI1499_2658	PSK2
-0.567636	2.80E-03	1.19E-02	AWRI1499_4479	PHO23
-0.570871	3.30E-03	1.36E-02	AWRI1499_3933	CCH1
-0.572949	1.11E-02	3.74E-02	AWRI1499_4267	PRX1
-0.573284	1.85E-03	8.32E-03	AWRI1499_1721	SLM3
-0.573402	1.65E-03	7.56E-03	AWRI1499_1522	VPS27
-0.57343	1.75E-03	7.94E-03	AWRI1499_2129	LCP5
-0.573913	1.75E-03	7.94E-03	AWRI1499_1896	
-0.575047	1.25E-02	4.13E-02	AWRI1499_1500	RIO1
-0.576641	1.60E-03	7.36E-03	AWRI1499_2342	CAJ1
-0.576776	2.00E-03	8.90E-03	AWRI1499_3328	VTI1
-0.577593	1.30E-03	6.17E-03	AWRI1499_0699	RFA1
-0.57816	1.60E-03	7.36E-03	AWRI1499_4871	MET7
-0.578499	1.60E-03	7.36E-03	AWRI1499_0627	STB2
-0.578925	3.15E-03	1.31E-02	AWRI1499_0374	YHB1

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.579508	6.90E-03	2.53E-02	AWRI1499_2859	URA7
-0.579566	2.90E-03	1.22E-02	AWRI1499_1153	LYP1
-0.580411	5.70E-03	2.16E-02	AWRI1499_1327	LOS1
-0.581241	1.15E-03	5.55E-03	AWRI1499_2217	SMC6
-0.583669	1.60E-03	7.36E-03	AWRI1499_3998	
-0.584196	7.20E-03	2.62E-02	AWRI1499_3956	NAS6
-0.58462	5.10E-03	1.96E-02	AWRI1499_3528	MRS6
-0.584703	1.25E-03	5.97E-03	AWRI1499_3561	
-0.584751	8.00E-03	2.86E-02	AWRI1499_4153	DNA2
-0.589401	1.10E-03	5.34E-03	AWRI1499_1688	YKR023W
-0.5898	1.10E-03	5.34E-03	AWRI1499_0664	RGR1
-0.589862	1.05E-03	5.12E-03	AWRI1499_2245	
-0.590855	5.20E-03	2.00E-02	AWRI1499_2474	CLD1
-0.592513	1.30E-03	6.17E-03	AWRI1499_0345	
-0.592528	1.15E-03	5.55E-03	AWRI1499_4063	
-0.593415	1.15E-03	5.55E-03	AWRI1499_2455	SRV2
-0.593584	4.55E-03	1.79E-02	AWRI1499_0579	PSE1
-0.59422	1.41E-02	4.54E-02	AWRI1499_3866	
-0.594342	6.30E-03	2.34E-02	AWRI1499_2424	
-0.59558	1.60E-03	7.36E-03	AWRI1499_0388	NET1
-0.597723	8.20E-03	2.92E-02	AWRI1499_4942	
-0.598144	3.45E-03	1.41E-02	AWRI1499_3038	IMD4
-0.599938	2.25E-03	9.84E-03	AWRI1499_3817	
-0.601161	8.50E-04	4.27E-03	AWRI1499_4626	GAP1
-0.601762	1.35E-03	6.38E-03	AWRI1499_3180	MET13
-0.602129	9.25E-03	3.22E-02	AWRI1499_4233	
-0.602789	9.50E-04	4.70E-03	AWRI1499_1205	DIS3
-0.603783	8.35E-03	2.96E-02	AWRI1499_2394	RAD57
-0.605468	5.70E-03	2.16E-02	AWRI1499_2700	

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.607133	6.10E-03	2.28E-02	AWRI1499_3412	SNU114
-0.607328	3.10E-03	1.29E-02	AWRI1499_2626	YNL095C
-0.60788	7.45E-03	2.69E-02	AWRI1499_0410	PIM1
-0.608786	8.00E-04	4.06E-03	AWRI1499_2464	UTP18
-0.612627	2.20E-03	9.65E-03	AWRI1499_4424	
-0.613328	5.25E-03	2.01E-02	AWRI1499_0050	VPS13
-0.616931	1.65E-03	7.56E-03	AWRI1499_0849	
-0.61758	5.50E-04	2.94E-03	AWRI1499_3156	GDE1
-0.617857	3.30E-03	1.36E-02	AWRI1499_4140	FUN12
-0.618212	1.85E-03	8.32E-03	AWRI1499_4119	TAE1
-0.619218	7.00E-04	3.62E-03	AWRI1499_1952	ERG11
-0.619355	3.50E-04	1.99E-03	AWRI1499_0360	TOM1
-0.619635	1.40E-03	6.57E-03	AWRI1499_0235	SQS1
-0.620411	2.45E-03	1.06E-02	AWRI1499_1431	ERG12
-0.620479	8.50E-04	4.27E-03	AWRI1499_0935	BUD2
-0.620716	4.95E-03	1.92E-02	AWRI1499_3819	RPA190
-0.621253	5.30E-03	2.03E-02	AWRI1499_2992	VPS54
-0.624502	1.00E-03	4.91E-03	AWRI1499_1664	CDC53
-0.624636	1.45E-03	6.77E-03	AWRI1499_0391	
-0.625395	8.00E-04	4.06E-03	AWRI1499_1728	CSC1
-0.625554	4.05E-03	1.62E-02	AWRI1499_1602	ADE4
-0.625709	3.00E-03	1.26E-02	AWRI1499_3834	
-0.625842	1.00E-03	4.91E-03	AWRI1499_2642	GEF1
-0.626528	1.05E-03	5.12E-03	AWRI1499_4843	
-0.626561	1.05E-03	5.12E-03	AWRI1499_3639	YNL247W
-0.627266	1.35E-03	6.38E-03	AWRI1499_3047	RAD4
-0.627417	1.00E-03	4.91E-03	AWRI1499_1292	YIL067C
-0.628461	9.50E-04	4.70E-03	AWRI1499_4623	
-0.628609	9.80E-03	3.38E-02	AWRI1499_0777	SHM1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.628692	1.60E-03	7.36E-03	AWRI1499_3833	
-0.628868	2.60E-03	1.11E-02	AWRI1499_1818	VPS34
-0.629312	6.45E-03	2.39E-02	AWRI1499_4311	RNA1
-0.629531	2.35E-03	1.02E-02	AWRI1499_0401	
-0.629736	9.00E-04	4.49E-03	AWRI1499_0831	LEU1
-0.630532	1.42E-02	4.58E-02	AWRI1499_1705	IDH1
-0.630926	3.50E-03	1.43E-02	AWRI1499_2138	YBR238C
-0.631005	8.00E-04	4.06E-03	AWRI1499_1492	UTP14
-0.631473	6.00E-04	3.17E-03	AWRI1499_1379	
-0.632536	1.15E-03	5.55E-03	AWRI1499_0091	
-0.63364	3.40E-03	1.40E-02	AWRI1499_1534	SRB8
-0.635571	4.85E-03	1.88E-02	AWRI1499_0843	
-0.636126	3.50E-04	1.99E-03	AWRI1499_2798	PIK1
-0.638467	7.50E-04	3.83E-03	AWRI1499_4544	INP53
-0.638709	1.45E-03	6.77E-03	AWRI1499_4433	
-0.63989	6.50E-04	3.39E-03	AWRI1499_4361	THI73
-0.641093	1.25E-03	5.97E-03	AWRI1499_3372	UTP20
-0.641413	5.50E-04	2.94E-03	AWRI1499_3188	RRP12
-0.642675	9.50E-04	4.70E-03	AWRI1499_1652	GCN1
-0.644888	8.60E-03	3.03E-02	AWRI1499_1700	
-0.645262	2.35E-03	1.02E-02	AWRI1499_2276	OPT1
-0.647243	3.00E-04	1.74E-03	AWRI1499_2682	DNF1
-0.648565	5.50E-04	2.94E-03	AWRI1499_4776	HEM1
-0.648682	2.60E-03	1.11E-02	AWRI1499_4221	
-0.648706	3.25E-03	1.34E-02	AWRI1499_4461	ARG8
-0.65328	1.01E-02	3.45E-02	AWRI1499_4648	ERG11
-0.654636	2.45E-03	1.06E-02	AWRI1499_1202	
-0.655382	3.50E-04	1.99E-03	AWRI1499_0423	YKU70
-0.655958	1.18E-02	3.92E-02	AWRI1499_3051	

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.656036	6.50E-04	3.39E-03	AWRI1499_4420	
-0.657654	2.00E-04	1.22E-03	AWRI1499_2574	
-0.658194	3.50E-04	1.99E-03	AWRI1499_1376	MGS1
-0.660239	3.00E-04	1.74E-03	AWRI1499_1621	ECM10
-0.660361	5.50E-04	2.94E-03	AWRI1499_2766	
-0.660914	4.00E-04	2.24E-03	AWRI1499_0433	
-0.660917	4.50E-04	2.48E-03	AWRI1499_3039	
-0.661203	7.50E-04	3.83E-03	AWRI1499_4418	
-0.66191	4.95E-03	1.92E-02	AWRI1499_3527	
-0.66445	1.49E-02	4.77E-02	AWRI1499_2368	
-0.665072	4.50E-04	2.48E-03	AWRI1499_1300	MGR3
-0.665578	1.07E-02	3.62E-02	AWRI1499_3193	HEF3
-0.665648	7.15E-03	2.60E-02	AWRI1499_0464	
-0.666844	8.50E-04	4.27E-03	AWRI1499_4465	RKM4
-0.667546	9.00E-04	4.49E-03	AWRI1499_1073	REI1
-0.66838	1.90E-03	8.52E-03	AWRI1499_4963	HER2
-0.66986	6.00E-04	3.17E-03	AWRI1499_4150	FUN30
-0.673023	7.50E-04	3.83E-03	AWRI1499_2154	
-0.673599	2.50E-04	1.48E-03	AWRI1499_4663	
-0.673643	2.00E-04	1.22E-03	AWRI1499_3649	NAM8
-0.674007	6.00E-04	3.17E-03	AWRI1499_3177	PRP19
-0.674036	1.50E-04	9.48E-04	AWRI1499_3677	
-0.675324	1.50E-04	9.48E-04	AWRI1499_4021	FUR4
-6.76E-01	1.75E-03	7.94E-03	AWRI1499_1374	POL31
-0.67687	2.50E-04	1.48E-03	AWRI1499_1873	BFR2
-0.677341	2.85E-03	1.20E-02	AWRI1499_3020	
-0.681002	1.50E-04	9.48E-04	AWRI1499_2811	
-0.681307	4.50E-04	2.48E-03	AWRI1499_2329	RSA4
-0.684269	5.50E-04	2.94E-03	AWRI1499_4158	MDM31

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.687043	1.50E-04	9.48E-04	AWRI1499_3566	PUF6
-0.688391	7.00E-04	3.62E-03	AWRI1499_4066	
-0.68841	3.30E-03	1.36E-02	AWRI1499_4603	ATG26
-0.689076	1.37E-02	4.45E-02	AWRI1499_4036	GPM1
-0.69057	9.00E-04	4.49E-03	AWRI1499_3067	VAC14
-0.691809	1.00E-04	6.63E-04	AWRI1499_3302	MNN2
-0.69187	4.50E-04	2.48E-03	AWRI1499_1340	FRA1
-0.695096	8.00E-04	4.06E-03	AWRI1499_4155	
-0.695351	4.00E-04	2.24E-03	AWRI1499_0400	
-0.697019	1.50E-04	9.48E-04	AWRI1499_2223	YPK9
-0.697233	1.00E-03	4.91E-03	AWRI1499_0261	YDR341C
-0.697387	4.50E-04	2.48E-03	AWRI1499_3813	NBP35
-0.69752	2.50E-04	1.48E-03	AWRI1499_4485	NOP9
-0.698745	3.00E-04	1.74E-03	AWRI1499_0405	
-0.699236	1.50E-04	9.48E-04	AWRI1499_1849	PSP2
-0.699442	3.00E-04	1.74E-03	AWRI1499_0942	HRQ1
-0.701891	3.50E-04	1.99E-03	AWRI1499_2404	ANR2
-0.702224	1.50E-04	9.48E-04	AWRI1499_2479	SPF1
-0.703294	2.50E-04	1.48E-03	AWRI1499_2459	NOP2
-0.704669	7.55E-03	2.73E-02	AWRI1499_0679	MET6
-0.704712	6.20E-03	2.31E-02	AWRI1499_2888	ADE13
-0.707087	7.00E-04	3.62E-03	AWRI1499_4115	TIF4631
-0.707426	1.70E-03	7.75E-03	AWRI1499_3377	
-0.709929	4.35E-03	1.72E-02	AWRI1499_4606	
-0.712953	1.00E-04	6.63E-04	AWRI1499_3773	DBP7
-0.714557	1.00E-04	6.63E-04	AWRI1499_2325	GYP7
-0.714639	8.25E-03	2.93E-02	AWRI1499_2988	DRS1
-0.715711	3.00E-04	1.74E-03	AWRI1499_3057	BAS1
-0.715735	1.00E-04	6.63E-04	AWRI1499_0357	MHO1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.717523	3.00E-04	1.74E-03	AWRI1499_4766	
-0.717993	1.50E-03	6.97E-03	AWRI1499_1157	ERG1
-0.721893	2.50E-04	1.48E-03	AWRI1499_1604	KRS1
-0.725491	9.50E-04	4.70E-03	AWRI1499_3549	CSE1
-0.726348	1.00E-04	6.63E-04	AWRI1499_1685	LDH1
-0.728578	3.00E-04	1.74E-03	AWRI1499_1653	GCN1
-0.729347	1.00E-04	6.63E-04	AWRI1499_4687	AGP2
-0.731887	2.00E-04	1.22E-03	AWRI1499_3898	VPS15
-0.733506	1.50E-04	9.48E-04	AWRI1499_0189	YAP1
-0.733814	1.05E-03	5.12E-03	AWRI1499_2251	EPL1
-0.734052	5.00E-05	3.54E-04	AWRI1499_2056	NPL4
-0.734142	1.00E-04	6.63E-04	AWRI1499_4237	RLI1
-0.734626	2.50E-04	1.48E-03	AWRI1499_0689	ABZ1
-0.734688	3.00E-04	1.74E-03	AWRI1499_0691	NOP53
-0.734718	6.50E-04	3.39E-03	AWRI1499_1220	
-0.735963	5.50E-04	2.94E-03	AWRI1499_3121	
-0.737746	1.50E-04	9.48E-04	AWRI1499_0615	
-0.738577	2.70E-03	1.15E-02	AWRI1499_1417	
-0.740711	5.00E-05	3.54E-04	AWRI1499_0013	VIP1
-0.741211	1.50E-04	9.48E-04	AWRI1499_2028	
-0.74357	1.00E-04	6.63E-04	AWRI1499_2020	PPN1
-0.743575	2.00E-04	1.22E-03	AWRI1499_0257	FUN19
-0.745095	1.00E-04	6.63E-04	AWRI1499_4785	MPP10
-0.746447	4.35E-03	1.72E-02	AWRI1499_4209	PDR5
-0.747085	2.00E-03	8.90E-03	AWRI1499_1638	UTP10
-0.748516	1.30E-03	6.17E-03	AWRI1499_3318	SCS7
-0.748956	3.25E-03	1.34E-02	AWRI1499_3828	
-0.749655	1.09E-02	3.67E-02	AWRI1499_3471	SER33
-0.74978	2.00E-04	1.22E-03	AWRI1499_1672	UFD2

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.750401	4.15E-03	1.65E-02	AWRI1499_1398	ADE17
-0.750443	3.00E-04	1.74E-03	AWRI1499_2279	MSP1
-0.751348	5.30E-03	2.03E-02	AWRI1499_0778	
-0.751853	5.00E-05	3.54E-04	AWRI1499_0669	SAC7
-0.755772	5.00E-05	3.54E-04	AWRI1499_0049	VPS13
-0.759042	3.00E-04	1.74E-03	AWRI1499_1093	RPO41
-0.76045	3.00E-03	1.26E-02	AWRI1499_3899	YMC1
-0.760712	5.00E-05	3.54E-04	AWRI1499_3963	
-0.760818	1.00E-04	6.63E-04	AWRI1499_0352	DRE2
-0.760913	4.55E-03	1.79E-02	AWRI1499_3983	
-0.762964	2.50E-04	1.48E-03	AWRI1499_4191	TUL1
-0.763712	5.00E-05	3.54E-04	AWRI1499_4035	
-0.76463	2.00E-04	1.22E-03	AWRI1499_1570	
-0.766981	5.00E-05	3.54E-04	AWRI1499_3420	SPE3
-0.768463	9.50E-04	4.70E-03	AWRI1499_2328	ESBP6
-0.769021	1.00E-04	6.63E-04	AWRI1499_2483	NOC2
-0.76961	3.50E-04	1.99E-03	AWRI1499_4886	
-0.769856	4.50E-04	2.48E-03	AWRI1499_2130	MPT5
-0.770617	1.00E-04	6.63E-04	AWRI1499_0968	RAD5
-0.771064	1.00E-04	6.63E-04	AWRI1499_1352	PHO84
-0.771091	1.00E-04	6.63E-04	AWRI1499_0206	MCM7
-0.772617	5.00E-05	3.54E-04	AWRI1499_1432	
-0.773764	8.00E-04	4.06E-03	AWRI1499_0148	FAS1
-0.784651	5.00E-05	3.54E-04	AWRI1499_2218	SMC6
-0.785436	2.00E-04	1.22E-03	AWRI1499_2848	
-0.785901	5.00E-05	3.54E-04	AWRI1499_3874	ATG15
-0.786117	5.50E-04	2.94E-03	AWRI1499_0003	
-0.787221	5.00E-05	3.54E-04	AWRI1499_1356	BMS1
-0.788496	5.00E-05	3.54E-04	AWRI1499_4883	

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.790535	3.00E-04	1.74E-03	AWRI1499_0840	TDA4
-0.795914	2.10E-03	9.28E-03	AWRI1499_2521	
-0.796487	1.15E-03	5.55E-03	AWRI1499_4557	
-0.799186	1.00E-04	6.63E-04	AWRI1499_2296	ESBP6
-0.801476	9.00E-04	4.49E-03	AWRI1499_2994	
-0.802621	5.00E-05	3.54E-04	AWRI1499_3917	IFH1
-0.805537	5.00E-05	3.54E-04	AWRI1499_1099	PSD1
-0.807455	5.00E-05	3.54E-04	AWRI1499_0069	
-0.808273	4.50E-04	2.48E-03	AWRI1499_1429	GUA1
-0.808632	2.90E-03	1.22E-02	AWRI1499_1346	SLH1
-0.810601	5.00E-05	3.54E-04	AWRI1499_0149	NTE1
-0.81124	5.00E-05	3.54E-04	AWRI1499_0750	NAN1
-0.814208	7.00E-04	3.62E-03	AWRI1499_3685	
-0.814958	1.50E-03	6.97E-03	AWRI1499_4730	HXT13
-0.817022	6.50E-04	3.39E-03	AWRI1499_0183	MIS1
-0.817134	5.00E-05	3.54E-04	AWRI1499_1796	PWP2
-0.820078	3.05E-03	1.27E-02	AWRI1499_4828	
-0.821463	5.00E-05	3.54E-04	AWRI1499_3508	HOL1
-0.82271	1.00E-04	6.63E-04	AWRI1499_0506	TIF3
-0.825715	2.50E-04	1.48E-03	AWRI1499_1061	
-0.826846	2.50E-04	1.48E-03	AWRI1499_4250	ICL1
-0.827092	5.00E-05	3.54E-04	AWRI1499_0293	
-0.831424	5.00E-05	3.54E-04	AWRI1499_3093	ESF2
-0.831762	2.00E-04	1.22E-03	AWRI1499_0660	SAM1
-0.833119	1.90E-03	8.52E-03	AWRI1499_1247	NOP14
-0.833863	2.50E-04	1.48E-03	AWRI1499_2052	PRB1
-0.83393	5.00E-05	3.54E-04	AWRI1499_3046	
-0.834924	5.00E-05	3.54E-04	AWRI1499_1526	YFL034W
-0.836316	1.85E-03	8.32E-03	AWRI1499_1590	BUD14

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log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.837719	5.00E-05	3.54E-04	AWRI1499_4956	
-0.83967	1.50E-04	9.48E-04	AWRI1499_2492	WSC4
-0.839927	5.00E-05	3.54E-04	AWRI1499_3915	MCP2
-0.840411	5.00E-05	3.54E-04	AWRI1499_0259	TPA1
-0.843292	5.00E-05	3.54E-04	AWRI1499_3030	SPE1
-0.843306	5.00E-05	3.54E-04	AWRI1499_1092	
-0.843573	5.00E-05	3.54E-04	AWRI1499_3021	POF1
-0.843671	6.00E-04	3.17E-03	AWRI1499_0657	
-0.845389	7.30E-03	2.65E-02	AWRI1499_0354	GAS4
-0.845725	1.00E-04	6.63E-04	AWRI1499_3692	YML002W
-0.845985	5.00E-05	3.54E-04	AWRI1499_2267	
-0.850341	5.00E-05	3.54E-04	AWRI1499_3931	
-0.850472	5.00E-05	3.54E-04	AWRI1499_4309	MRD1
-0.8506	5.00E-05	3.54E-04	AWRI1499_3596	CCC2
-0.850723	5.00E-05	3.54E-04	AWRI1499_0854	UBP15
-0.851902	5.00E-05	3.54E-04	AWRI1499_2719	MCM6
-0.851981	5.00E-05	3.54E-04	AWRI1499_1480	TEC1
-0.852654	1.00E-04	6.63E-04	AWRI1499_0979	BRO1
-0.853094	7.00E-04	3.62E-03	AWRI1499_0904	ARE2
-0.855672	5.00E-05	3.54E-04	AWRI1499_4247	HDA3
-0.856236	5.00E-05	3.54E-04	AWRI1499_1170	FET4
-0.857309	4.50E-04	2.48E-03	AWRI1499_2872	
-0.860179	5.00E-05	3.54E-04	AWRI1499_4552	UFD4
-0.860518	5.00E-05	3.54E-04	AWRI1499_0885	SPB1
-0.861928	5.00E-05	3.54E-04	AWRI1499_4154	BBC1
-0.869468	7.00E-04	3.62E-03	AWRI1499_0234	SQS1
-0.870765	1.80E-03	8.14E-03	AWRI1499_0193	UGA4
-0.870901	5.00E-05	3.54E-04	AWRI1499_1519	
-0.871314	5.00E-05	3.54E-04	AWRI1499_1402	DNF3

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.879562	9.00E-04	4.49E-03	AWRI1499_0794	
-0.88031	5.00E-05	3.54E-04	AWRI1499_2420	
-0.886892	2.45E-03	1.06E-02	AWRI1499_4624	ADE6
-0.890218	2.00E-04	1.22E-03	AWRI1499_1130	ADE2
-0.890856	5.00E-05	3.54E-04	AWRI1499_0976	NOP4
-0.893254	5.00E-05	3.54E-04	AWRI1499_2727	DBP10
-0.898305	5.00E-05	3.54E-04	AWRI1499_1084	
-0.899971	5.00E-05	3.54E-04	AWRI1499_1287	PLC1
-0.900658	5.00E-05	3.54E-04	AWRI1499_0954	KEX1
-0.900903	5.00E-05	3.54E-04	AWRI1499_0558	YGR149W
-0.905083	5.00E-05	3.54E-04	AWRI1499_2364	
-0.909414	5.00E-05	3.54E-04	AWRI1499_3300	SAS10
-0.912895	5.00E-05	3.54E-04	AWRI1499_3842	
-0.917333	5.00E-05	3.54E-04	AWRI1499_1954	DYS1
-0.917791	5.00E-05	3.54E-04	AWRI1499_0192	PIB2
-0.918702	5.00E-05	3.54E-04	AWRI1499_4169	
-0.920448	1.25E-03	5.97E-03	AWRI1499_2083	
-0.921933	5.00E-05	3.54E-04	AWRI1499_3648	NGR1
-0.92756	5.00E-05	3.54E-04	AWRI1499_3060	ERG5
-0.928553	5.30E-03	2.03E-02	AWRI1499_1736	GLN1
-0.928988	2.00E-04	1.22E-03	AWRI1499_4943	
-0.929264	5.00E-05	3.54E-04	AWRI1499_1931	
-0.931325	5.00E-05	3.54E-04	AWRI1499_3007	SAH1
-0.93882	5.00E-05	3.54E-04	AWRI1499_3183	VTC2
-0.944201	3.50E-04	1.99E-03	AWRI1499_4656	HSP26
-0.94873	5.00E-05	3.54E-04	AWRI1499_2837	
-0.957099	5.00E-05	3.54E-04	AWRI1499_1483	MAK21
-0.957701	6.35E-03	2.36E-02	AWRI1499_4507	TRM732
-0.958521	7.25E-03	2.63E-02	AWRI1499_3400	LSB1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-0.960847	5.00E-05	3.54E-04	AWRI1499_4141	
-0.963603	5.00E-05	3.54E-04	AWRI1499_3399	UGA4
-0.968977	1.00E-04	6.63E-04	AWRI1499_2718	
-0.973264	5.00E-05	3.54E-04	AWRI1499_4953	SEO1
-0.976122	5.00E-05	3.54E-04	AWRI1499_0436	TNA1
-0.983303	1.27E-02	4.19E-02	AWRI1499_3097	
-0.985015	5.00E-05	3.54E-04	AWRI1499_4303	NCP1
-0.985793	5.00E-05	3.54E-04	AWRI1499_0196	FET3
-0.98856	5.00E-05	3.54E-04	AWRI1499_3659	PNS1
-0.989793	5.00E-05	3.54E-04	AWRI1499_4439	STL1
-0.990531	5.00E-05	3.54E-04	AWRI1499_0610	
-0.990865	5.00E-05	3.54E-04	AWRI1499_3854	
-0.996522	5.00E-05	3.54E-04	AWRI1499_0383	GCV1
-0.997518	1.50E-04	9.48E-04	AWRI1499_2449	HSP104
-1.00538	5.00E-05	3.54E-04	AWRI1499_1000	RRP5
-1.01008	5.00E-05	3.54E-04	AWRI1499_0970	
-1.0108	5.00E-05	3.54E-04	AWRI1499_2063	RAV1
-1.01503	1.30E-02	4.25E-02	AWRI1499_3332	ZAP1
-1.01572	5.50E-04	2.94E-03	AWRI1499_0411	MAE1
-1.01741	4.00E-04	2.24E-03	AWRI1499_2081	
-1.02393	5.00E-05	3.54E-04	AWRI1499_4252	NEW1
-1.02442	2.00E-04	1.22E-03	AWRI1499_2266	
-1.02584	5.00E-05	3.54E-04	AWRI1499_4743	YOL098C
-1.02835	1.55E-03	7.16E-03	AWRI1499_4390	
-1.02882	5.00E-05	3.54E-04	AWRI1499_3235	ACS1
-1.03192	5.00E-05	3.54E-04	AWRI1499_2410	
-1.03246	5.00E-05	3.54E-04	AWRI1499_3753	TRM2
-1.03251	5.00E-05	3.54E-04	AWRI1499_0174	IZH2
-1.03791	5.00E-05	3.54E-04	AWRI1499_2304	ENP2

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-1.0459	5.00E-05	3.54E-04	AWRI1499_2704	
-1.04691	5.00E-05	3.54E-04	AWRI1499_3373	UTP20
-1.05268	5.00E-05	3.54E-04	AWRI1499_0564	
-1.05898	5.00E-05	3.54E-04	AWRI1499_2726	
-1.05921	5.00E-05	3.54E-04	AWRI1499_3126	SSL2
-1.0604	5.00E-05	3.54E-04	AWRI1499_2849	KNS1
-1.06744	1.20E-03	5.76E-03	AWRI1499_4926	PAP1
-1.07165	5.00E-05	3.54E-04	AWRI1499_0061	NAR1
-1.07171	5.00E-05	3.54E-04	AWRI1499_2685	
-1.08422	5.00E-05	3.54E-04	AWRI1499_1673	
-1.08884	5.00E-05	3.54E-04	AWRI1499_4061	
-1.09972	5.00E-05	3.54E-04	AWRI1499_1012	GLT1
-1.1023	5.00E-05	3.54E-04	AWRI1499_0494	
-1.10372	5.00E-05	3.54E-04	AWRI1499_2292	MGA2
-1.11245	2.35E-03	1.02E-02	AWRI1499_1133	SDA1
-1.11597	5.00E-05	3.54E-04	AWRI1499_2748	MAK5
-1.1173	5.00E-05	3.54E-04	AWRI1499_4100	ADE5,7
-1.1222	8.50E-04	4.27E-03	AWRI1499_4051	GCV2
-1.12824	5.00E-05	3.54E-04	AWRI1499_3844	STL1
-1.13007	5.00E-05	3.54E-04	AWRI1499_1734	
-1.14584	5.00E-05	3.54E-04	AWRI1499_0258	TPA1
-1.14945	5.00E-05	3.54E-04	AWRI1499_1463	RAD2
-1.15329	5.00E-05	3.54E-04	AWRI1499_2807	
-1.16398	5.00E-05	3.54E-04	AWRI1499_0426	SNZ1
-1.16784	5.00E-05	3.54E-04	AWRI1499_2706	RAD16
-1.16886	5.00E-05	3.54E-04	AWRI1499_0211	
-1.17188	5.00E-05	3.54E-04	AWRI1499_4897	YGR109W-B
-1.18646	5.00E-05	3.54E-04	AWRI1499_0404	HMS1
-1.19967	1.00E-04	6.63E-04	AWRI1499_0349	NOB1

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-1.20553	5.00E-05	3.54E-04	AWRI1499_4770	IDP1
-1.22047	5.00E-05	3.54E-04	AWRI1499_0729	YAT2
-1.22155	5.00E-05	3.54E-04	AWRI1499_1863	TPN1
-1.22317	5.00E-05	3.54E-04	AWRI1499_1686	IME2
-1.23246	5.00E-05	3.54E-04	AWRI1499_2582	
-1.23431	5.00E-05	3.54E-04	AWRI1499_2004	YOR1
-1.24009	5.00E-05	3.54E-04	AWRI1499_4095	
-1.26054	5.00E-05	3.54E-04	AWRI1499_1563	BNA2
-1.26115	1.00E-04	6.63E-04	AWRI1499_2143	
-1.29708	5.00E-05	3.54E-04	AWRI1499_4962	
-1.30013	5.00E-05	3.54E-04	AWRI1499_0007	RAD7
-1.30641	5.00E-05	3.54E-04	AWRI1499_3686	
-1.30734	5.00E-05	3.54E-04	AWRI1499_2703	
-1.31871	5.00E-05	3.54E-04	AWRI1499_0093	DBP1
-1.32095	5.00E-05	3.54E-04	AWRI1499_2936	
-1.33341	5.00E-05	3.54E-04	AWRI1499_1424	BIO2
-1.33891	5.00E-05	3.54E-04	AWRI1499_4754	UGA4
-1.36644	5.00E-05	3.54E-04	AWRI1499_1717	
-1.38774	5.00E-05	3.54E-04	AWRI1499_0832	BNA4
-1.39229	5.00E-05	3.54E-04	AWRI1499_4543	ADE3
-1.41612	5.00E-05	3.54E-04	AWRI1499_3916	
-1.438	5.00E-05	3.54E-04	AWRI1499_3913	CIR2
-1.44049	5.00E-05	3.54E-04	AWRI1499_0176	ATG1
-1.45985	8.70E-03	3.06E-02	AWRI1499_3932	
-1.49304	5.00E-05	3.54E-04	AWRI1499_4359	
-1.51214	5.00E-05	3.54E-04	AWRI1499_3191	GAP1
-1.56038	2.00E-03	8.90E-03	AWRI1499_0959	THI73
-1.57495	5.00E-05	3.54E-04	AWRI1499_3980	SPE2
-1.60127	1.47E-02	4.70E-02	AWRI1499_1707	

CBS2499 ~ Tr-vs-T0				
log_2fold-change	***p*-value**	***q*-value**	**AWRI identifier**	***S. cerevisiae* homologous gene (Standard name)**
-1.62495	5.00E-05	3.54E-04	AWRI1499_2075	FAS2
-1.63445	5.00E-05	3.54E-04	AWRI1499_0077	MCH2
-1.67835	5.00E-05	3.54E-04	AWRI1499_2636	OPT2
-1.72885	5.00E-05	3.54E-04	AWRI1499_2638	
-1.73481	5.00E-05	3.54E-04	AWRI1499_2106	
-1.98561	5.00E-05	3.54E-04	AWRI1499_4952	CRZ1
-2.0456	5.00E-05	3.54E-04	AWRI1499_1353	PHO84
-2.1938	5.00E-05	3.54E-04	AWRI1499_2139	
-2.40165	5.00E-05	3.54E-04	AWRI1499_2142	
-2.42165	5.00E-05	3.54E-04	AWRI1499_2141	
-2.57013	5.00E-05	3.54E-04	AWRI1499_2140	
-2.79727	5.00E-05	3.54E-04	AWRI1499_2637	OPT2

Appendix 3

Biological Processes enriched by SURGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
carbohydrate metabolic process	36 of 113 genes, 31.9%	314 of 7166 genes, 4.4%	1.75e-19	0.00	0.00	PGM2, PIG2, GLC3, GAL1, DAK2, GSY1, PDA1, MLS1, GAL7, PCK1, GAL10, IMA1, REG1, CAT8, MDH1, MPH2, YJR096W, ICL1, YBR053C, GPH1, SOL3, NTH1, PKP1, YDR109C, CWH41, GAL2, YLR345W, ROT2, INO1, HXK1, SUC2, GPD1, SOR1, GLK1, PDB1, GUT1
small molecule metabolic process	51 of 113 genes, 45.1%	812 of 7166 genes, 11.3%	3.25e-17	0.00	0.00	PGM2, ADH3, PIG2, MHT1, GAL1, ADH6, SPS19, LAT1, DAK2, LSC1, YMR315W, ETR1, UGA2, ACH1, PDA1, MLS1, GAL7, PCK1, GAL10, YJL068C, LSC2, PDX1, YKL151C, CAT8, POX1, MDH1, ADH7, YJR096W, URE2, ICL1, SOL3, YBR053C, SDH1, BDH1, PKP1, YDR109C, GAL2, YLR345W, INO1, HXK1, PNC1, MSW1, LIP5, GPD1, FOX2, AAT2, GLK1, SOR1, LIP2, PDB1, GUT1
oxidation-reduction process	34 of 113 genes, 30.1%	454 of 7166 genes, 6.3%	2.18e-12	0.00	0.00	PGM2, ADH3, PIG2, GLC3, ADH6, SPS19, DAK2, LSC1, GLR1, YMR315W, HEM14, ETR1, UGA2, GSY1, PDA1, MLS1, REG1, RGI2, LSC2, PST2, CBR1, POX1, MDH1, ADH7, YJR096W, ICL1, URE2, GPH1, SDH1, BDH1, GPD1, FOX2, SOR1, PDB1
cellular carbohydrate metabolic process	23 of 113 genes, 20.4%	215 of 7166 genes, 3.0%	7.86e-11	0.00	0.00	PGM2, CAT8, PIG2, MPH2, GLC3, GAL1, ICL1, GPH1, DAK2, NTH1, YDR109C, CWH41, YLR345W, ROT2, GSY1, MLS1, HXK1, INO1, SUC2, IMA1, GLK1, REG1, GUT1
generation of precursor metabolites and energy	22 of 113 genes, 19.5%	203 of 7166 genes, 2.8%	2.18e-10	0.00	0.00	PGM2, CBR1, ADH3, PIG2, MDH1, GLC3, ICL1, GPH1, SDH1, DAK2, LSC1, ETR1, YLR345W, GSY1, PDA1, MLS1, HXK1, GLK1, REG1, RGI2, LSC2, PDB1
monosaccharide metabolic process	16 of 113 genes, 14.2%	99 of 7166 genes, 1.4%	9.37e-10	0.00	0.00	PGM2, GAL2, CAT8, YLR345W, PIG2, GAL7, HXK1, YJR096W, PCK1, GAL1, GAL10, YBR053C, GLK1, SOR1, PKP1, YDR109C
carbohydrate catabolic process	16 of 113 genes, 14.2%	120 of 7166 genes, 1.7%	1.96e-08	0.00	0.00	PGM2, YJR096W, GAL1, GPH1, DAK2, NTH1, YLR345W, PDA1, GAL7, HXK1, SUC2, GAL10, IMA1, GLK1, PDB1, GUT1
monocarboxylic acid metabolic process	19 of 113 genes, 16.8%	185 of 7166 genes, 2.6%	2.68e-08	0.00	0.00	CAT8, POX1, ICL1, SPS19, LAT1, ETR1, UGA2, YLR345W, ACH1, PDA1, MLS1, HXK1, LIP5, FOX2, AAT2, LIP2, GLK1, PDX1, PDB1
oxoacid metabolic process	28 of 113 genes, 24.8%	450 of 7166 genes, 6.3%	8.47e-08	0.00	0.00	CAT8, ADH3, POX1, MDH1, MHT1, URE2, ICL1, SPS19, YBR053C, LAT1, SDH1, LSC1, ETR1, UGA2, YLR345W, ACH1, PDA1, MLS1, HXK1, MSW1, LIP5, FOX2, AAT2, GLK1, LIP2, PDX1, LSC2, PDB1
organic acid metabolic process	28 of 113 genes, 24.8%	451 of 7166 genes, 6.3%	8.92e-08	0.00	0.00	CAT8, ADH3, POX1, MDH1, MHT1, URE2, ICL1, SPS19, YBR053C, LAT1, SDH1, LSC1, ETR1, UGA2, YLR345W, ACH1, PDA1, MLS1, HXK1, MSW1, LIP5, FOX2, AAT2, GLK1, LIP2, PDX1, LSC2, PDB1
carboxylic acid metabolic process	27 of 113 genes, 23.9%	433 of 7166 genes, 6.0%	1.84e-07	0.00	0.00	CAT8, ADH3, POX1, MDH1, MHT1, ICL1, SPS19, YBR053C, LAT1, SDH1, LSC1, ETR1, UGA2, YLR345W, ACH1, PDA1, MLS1, HXK1, MSW1, LIP5, FOX2, AAT2, GLK1, LIP2, PDX1, LSC2, PDB1
coenzyme metabolic process	18 of 113 genes, 15.9%	185 of 7166 genes, 2.6%	2.23e-07	0.00	0.00	YKL151C, ADH3, SOL3, LAT1, LSC1, YMR315W, YLR345W, ACH1, PDA1, HXK1, PNC1, LIP5, GPD1, LIP2, GLK1, PDX1, LSC2, PDB1
hexose metabolic process	13 of 113 genes, 11.5%	88 of 7166 genes, 1.2%	4.10e-07	0.00	0.00	PGM2, GAL2, CAT8, YLR345W, PIG2, GAL7, HXK1, PCK1, GAL1, GAL10, GLK1, SOR1, PKP1
cofactor metabolic process	19 of 113 genes, 16.8%	232 of 7166 genes, 3.2%	1.32e-06	0.00	0.00	YKL151C, ADH3, SOL3, LAT1, LSC1, YMR315W, HEM14, YLR345W, ACH1, PDA1, HXK1, PNC1, LIP5, GPD1, LIP2, GLK1, PDX1, LSC2, PDB1
energy derivation by oxidation of organic compounds	16 of 113 genes, 14.2%	163 of 7166 genes, 2.3%	2.00e-06	0.00	0.00	PGM2, ADH3, PIG2, GSY1, MDH1, MLS1, GLC3, ICL1, GPH1, REG1, SDH1, DAK2, LSC1, RGI2, LSC2, ETR1
acyl-CoA metabolic process	7 of 113 genes, 6.2%	20 of 7166 genes, 0.3%	6.52e-06	0.00	0.00	LAT1, LSC1, ACH1, PDA1, PDX1, LSC2, PDB1
thioester metabolic process	7 of 113 genes, 6.2%	20 of 7166 genes, 0.3%	6.52e-06	0.00	0.00	LAT1, LSC1, ACH1, PDA1, PDX1, LSC2, PDB1
small molecule catabolic process	14 of 113 genes, 12.4%	133 of 7166 genes, 1.9%	8.25e-06	0.00	0.00	PGM2, ADH3, POX1, GAL7, YJR096W, GAL1, GAL10, FOX2, AAT2, SPS19, DAK2, YJL068C, UGA2, GUT1
acetyl-CoA biosynthetic process from pyruvate	4 of 113 genes, 3.5%	4 of 7166 genes, 0.1%	2.90e-05	0.00	0.00	LAT1, PDA1, PDX1, PDB1
pyridine nucleotide metabolic process	11 of 113 genes, 9.7%	86 of 7166 genes, 1.2%	4.13e-05	0.00	0.00	YKL151C, YLR345W, ADH3, PDA1, PNC1, HXK1, GPD1, SOL3, GLK1, YMR315W, PDB1
pyridine-containing compound metabolic process	11 of 113 genes, 9.7%	98 of 7166 genes, 1.4%	0.00016	0.00	0.00	YKL151C, YLR345W, ADH3, PDA1, PNC1, HXK1, GPD1, SOL3, GLK1, YMR315W, PDB1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
glucose import	7 of 113 genes, 6.2%	31 of 7166 genes, 0.4%	0.00019	0.00	0.00	GAL2, GLK1, HXT6, HXT7, HXK1, HXT13, MPH2
oxidoreduction coenzyme metabolic process	11 of 113 genes, 9.7%	101 of 7166 genes, 1.4%	0.00022	0.00	0.00	YKL151C, YLR345W, ADH3, PDA1, PNC1, HXK1, GPD1, SOL3, GLK1, YMR315W, PDB1
nicotinamide nucleotide metabolic process	10 of 113 genes, 8.8%	85 of 7166 genes, 1.2%	0.00036	0.00	0.00	YKL151C, YLR345W, ADH3, PDA1, HXK1, GPD1, SOL3, GLK1, YMR315W, PDB1
acetyl-CoA biosynthetic process	4 of 113 genes, 3.5%	6 of 7166 genes, 0.1%	0.00042	0.00	0.00	LAT1, PDA1, PDX1, PDB1
hexose transport	7 of 113 genes, 6.2%	35 of 7166 genes, 0.5%	0.00046	0.00	0.00	GAL2, GLK1, HXT6, HXT7, HXK1, HXT13, MPH2
monosaccharide transport	7 of 113 genes, 6.2%	35 of 7166 genes, 0.5%	0.00046	0.00	0.00	GAL2, GLK1, HXT6, HXT7, HXK1, HXT13, MPH2
glucose transport	7 of 113 genes, 6.2%	35 of 7166 genes, 0.5%	0.00046	0.00	0.00	GAL2, GLK1, HXT6, HXT7, HXK1, HXT13, MPH2
galactose metabolic process	5 of 113 genes, 4.4%	13 of 7166 genes, 0.2%	0.00051	0.00	0.00	PGM2, GAL1, GAL2, GAL10, GAL7
acetyl-CoA metabolic process	5 of 113 genes, 4.4%	13 of 7166 genes, 0.2%	0.00051	0.00	0.00	LAT1, ACH1, PDA1, PDX1, PDB1
carbohydrate phosphorylation	5 of 113 genes, 4.4%	14 of 7166 genes, 0.2%	0.00079	0.00	0.00	GAL1, YLR345W, GLK1, YDR109C, HXK1
energy reserve metabolic process	7 of 113 genes, 6.2%	38 of 7166 genes, 0.5%	0.00084	0.00	0.00	GLC3, PGM2, GPH1, PIG2, REG1, RGI2, GSY1
phosphorus metabolic process	31 of 113 genes, 27.4%	824 of 7166 genes, 11.5%	0.00114	0.00	0.00	PGM2, YKL151C, RTK1, ADH3, GAL1, SOL3, LAT1, SDH1, DAK2, LSC1, YMR315W, PKP1, YDR109C, YMR027W, YKR070W, RIM11 , YLR345W, ACH1, PDA1, PNC1, HXK1, INO1, MDM31, CLD1, GPD1, SUR1, GLK1, LSC2, PDX1, GUT1, PDB1
sulfur compound metabolic process	12 of 113 genes, 10.6%	146 of 7166 genes, 2.0%	0.00136	0.00	0.00	ACH1, PDA1, MHT1, URE2, LIP5, LAT1, LIP2, LSC1, LSC2, PDX1, GLR1, PDB1
galactose catabolic process	4 of 113 genes, 3.5%	8 of 7166 genes, 0.1%	0.00193	0.00	0.00	PGM2, GAL1, GAL10, GAL7
thioester biosynthetic process	4 of 113 genes, 3.5%	8 of 7166 genes, 0.1%	0.00193	0.00	0.00	LAT1, PDA1, PDX1, PDB1
acyl-CoA biosynthetic process	4 of 113 genes, 3.5%	8 of 7166 genes, 0.1%	0.00193	0.00	0.00	LAT1, PDA1, PDX1, PDB1
monosaccharide catabolic process	5 of 113 genes, 4.4%	17 of 7166 genes, 0.2%	0.00235	0.00	0.00	YJR096W, PGM2, GAL1, GAL10, GAL7
tricarboxylic acid cycle	6 of 113 genes, 5.3%	30 of 7166 genes, 0.4%	0.00291	0.00	0.00	ICL1, SDH1, LSC1, LSC2, MDH1, MLS1
citrate metabolic process	6 of 113 genes, 5.3%	30 of 7166 genes, 0.4%	0.00291	0.00	0.00	ICL1, SDH1, LSC1, LSC2, MDH1, MLS1
hexose catabolic process	4 of 113 genes, 3.5%	9 of 7166 genes, 0.1%	0.00344	0.00	0.00	PGM2, GAL1, GAL10, GAL7
tricarboxylic acid metabolic process	6 of 113 genes, 5.3%	31 of 7166 genes, 0.4%	0.00356	0.00	0.00	ICL1, SDH1, LSC1, LSC2, MDH1, MLS1
cellular polysaccharide metabolic process	9 of 113 genes, 8.0%	87 of 7166 genes, 1.2%	0.00398	0.00	0.00	CWH41, PGM2, PIG2, ROT2, GSY1, SUC2, GLC3, GPH1, REG1
organic substance catabolic process	28 of 113 genes, 24.8%	744 of 7166 genes, 10.4%	0.00407	0.00	0.00	PGM2, ADH3, POX1, YJR096W, PAB1, GAL1, SPS19, GPH1, DAK2, NTH1, UBC7, UGA2, YLR345W, CYM1, PDA1, GAL7, HXK1, SU C2, GPD1, FOX2, GAL10, AAT2, IMA1, GLK1, REG1, YJL068C, GUT1, PDB1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
cellular glucan metabolic process	8 of 113 genes, 7.1%	67 of 7166 genes, 0.9%	0.00444	0.00	0.00	CWH41, PGM2, PIG2, ROT2, GSY1, GLC3, GPH1, REG1
glucan metabolic process	8 of 113 genes, 7.1%	67 of 7166 genes, 0.9%	0.00444	0.00	0.00	CWH41, PGM2, PIG2, ROT2, GSY1, GLC3, GPH1, REG1
carbohydrate biosynthetic process	10 of 113 genes, 8.8%	118 of 7166 genes, 1.6%	0.00739	0.00	0.00	CWH41, PGM2, CAT8, PIG2, ROT2, GSY1, INO1, PCK1, GLC3, YBR053C
pyruvate metabolic process	7 of 113 genes, 6.2%	52 of 7166 genes, 0.7%	0.00743	0.00	0.00	YLR345W, LAT1, GLK1, PDA1, PDX1, PDB1, HXK1
carbohydrate transport	7 of 113 genes, 6.2%	53 of 7166 genes, 0.7%	0.00845	0.04	0.02	GAL2, GLK1, HXT6, HXT7, HXK1, HXT13, MPH2
glycogen metabolic process	6 of 113 genes, 5.3%	36 of 7166 genes, 0.5%	0.00884	0.04	0.02	GLC3, PGM2, GPH1, PIG2, REG1, GSY1

Biological Processes enriched by SURGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
small molecule metabolic process	38 of 76 genes, 50.0%	812 of 7166 genes, 11.3%	1.98e-14	0.00	0.00	CYB2, ALD5, GOR1, MET10, GAL1, ADH6, SPS19, LAT1, LSC1, ETR1, IAH1, ACH1, PDA1, GAL7, GDH1, PUT2, YGR012W, DLD1, ALD4, GAL10, PDX1, LSC2, HER2, CAT8, YDL124W, ARO10, ADH7, RKI1, FDH1, BDH1, UGA1, YDR109C, GLY1, YLR345W, HXK1, FOX2, SOR1, PDB1
carboxylic acid metabolic process	28 of 76 genes, 36.8%	433 of 7166 genes, 6.0%	4.10e-13	0.00	0.00	CAT8, CYB2, ALD5, GOR1, ARO10, MET10, FDH1, SPS19, LAT1, LSC1, ETR1, UGA1, GLY1, YLR345W, IAH1, ACH1, PDA1, GDH1, HXK1, PUT2, YGR012W, DLD1, ALD4, FOX2, PDX1, LSC2, HER2, PDB1
monocarboxylic acid metabolic process	20 of 76 genes, 26.3%	185 of 7166 genes, 2.6%	7.33e-13	0.00	0.00	CAT8, CYB2, ALD5, GOR1, ARO10, FDH1, SPS19, LAT1, ETR1, UGA1, YLR345W, IAH1, ACH1, PDA1, HXK1, DLD1, ALD4, FOX2, PDX1, PDB1
oxoacid metabolic process	28 of 76 genes, 36.8%	450 of 7166 genes, 6.3%	1.11e-12	0.00	0.00	CAT8, CYB2, ALD5, GOR1, ARO10, MET10, FDH1, SPS19, LAT1, LSC1, ETR1, UGA1, GLY1, YLR345W, IAH1, ACH1, PDA1, GDH1, HXK1, PUT2, YGR012W, DLD1, ALD4, FOX2, PDX1, LSC2, HER2, PDB1
organic acid metabolic process	28 of 76 genes, 36.8%	451 of 7166 genes, 6.3%	1.17e-12	0.00	0.00	CAT8, CYB2, ALD5, GOR1, ARO10, MET10, FDH1, SPS19, LAT1, LSC1, ETR1, UGA1, GLY1, YLR345W, IAH1, ACH1, PDA1, GDH1, HXK1, PUT2, YGR012W, DLD1, ALD4, FOX2, PDX1, LSC2, HER2, PDB1
oxidation-reduction process	22 of 76 genes, 28.9%	454 of 7166 genes, 6.3%	2.98e-07	0.00	0.00	YDL124W, CYB2, ALD5, GOR1, ADH7, MET10, ADH6, FDH1, SPS19, LSC1, BDH1, ETR1, PDA1, GDH1, PUT2, DLD1, ALD4, FOX2, SOR1, LSC2, PST2, PDB1
acyl-CoA metabolic process	7 of 76 genes, 9.2%	20 of 7166 genes, 0.3%	3.02e-07	0.00	0.00	LAT1, LSC1, ACH1, PDA1, PDX1, LSC2, PDB1
thioester metabolic process	7 of 76 genes, 9.2%	20 of 7166 genes, 0.3%	3.02e-07	0.00	0.00	LAT1, LSC1, ACH1, PDA1, PDX1, LSC2, PDB1
small molecule catabolic process	13 of 76 genes, 17.1%	133 of 7166 genes, 1.9%	3.71e-07	0.00	0.00	GLY1, ALD5, GOR1, GAL7, ARO10, PUT2, GAL1, DLD1, FDH1, FOX2, GAL10, SPS19, UGA1
acetyl-CoA biosynthetic process from pyruvate	4 of 76 genes, 5.3%	4 of 7166 genes, 0.1%	4.47e-06	0.00	0.00	LAT1, PDA1, PDX1, PDB1
pyruvate metabolic process	8 of 76 genes, 10.5%	52 of 7166 genes, 0.7%	2.17e-05	0.00	0.00	YLR345W, PDA1, HXK1, ARO10, ALD4, LAT1, PDX1, PDB1
acetyl-CoA metabolic process	5 of 76 genes, 6.6%	13 of 7166 genes, 0.2%	5.41e-05	0.00	0.00	LAT1, ACH1, PDA1, PDX1, PDB1
acetyl-CoA biosynthetic process	4 of 76 genes, 5.3%	6 of 7166 genes, 0.1%	6.60e-05	0.00	0.00	LAT1, PDA1, PDX1, PDB1
sulfur compound metabolic process	11 of 76 genes, 14.5%	146 of 7166 genes, 2.0%	0.00012	0.00	0.00	ACH1, PDA1, ARO10, MET10, YGR012W, LAT1, OXP1, LSC1, LSC2, PDX1, PDB1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
organic acid catabolic process	9 of 76 genes, 11.8%	92 of 7166 genes, 1.3%	0.00017	0.00	0.00	GLY1, FDH1, DLD1, FOX2, SPS19, GOR1, UGA1, ARO10, PUT2
carboxylic acid catabolic process	9 of 76 genes, 11.8%	92 of 7166 genes, 1.3%	0.00017	0.00	0.00	GLY1, FDH1, DLD1, FOX2, SPS19, GOR1, UGA1, ARO10, PUT2
carbohydrate metabolic process	15 of 76 genes, 19.7%	314 of 7166 genes, 4.4%	0.00028	0.00	0.00	CAT8, YLR345W, UTR2, PDA1, GAL7, HXK1, MPH2, GAL1, SUC2, RKI1, GAL10, NTH1, SOR1, YDR109C, PDB1
acetate metabolic process	4 of 76 genes, 5.3%	8 of 7166 genes, 0.1%	0.00030	0.00	0.00	ALD4, IAH1, ACH1, ALD5
thioester biosynthetic process	4 of 76 genes, 5.3%	8 of 7166 genes, 0.1%	0.00030	0.00	0.00	LAT1, PDA1, PDX1, PDB1
acyl-CoA biosynthetic process	4 of 76 genes, 5.3%	8 of 7166 genes, 0.1%	0.00030	0.00	0.00	LAT1, PDA1, PDX1, PDB1
monosaccharide metabolic process	9 of 76 genes, 11.8%	99 of 7166 genes, 1.4%	0.00032	0.00	0.00	CAT8, YLR345W, GAL7, HXK1, GAL1, RKI1, GAL10, SOR1, YDR109C
coenzyme metabolic process	11 of 76 genes, 14.5%	185 of 7166 genes, 2.6%	0.00128	0.00	0.00	YLR345W, ACH1, PDA1, HXK1, RKI1, ALD4, LAT1, LSC1, LSC2, PDX1, PDB1
carbohydrate catabolic process	9 of 76 genes, 11.8%	120 of 7166 genes, 1.7%	0.00162	0.00	0.00	YLR345W, PDA1, GAL7, HXK1, GAL1, SUC2, GAL10, NTH1, PDB1
monocarboxylic acid catabolic process	6 of 76 genes, 7.9%	42 of 7166 genes, 0.6%	0.00172	0.00	0.00	FDH1, DLD1, FOX2, SPS19, GOR1, UGA1
carbohydrate phosphorylation	4 of 76 genes, 5.3%	14 of 7166 genes, 0.2%	0.00413	0.00	0.00	GAL1, YLR345W, HXK1, YDR109C
galactose catabolic process via UDP-galactose	3 of 76 genes, 3.9%	5 of 7166 genes, 0.1%	0.00432	0.00	0.00	GAL1, GAL10, GAL7
organic hydroxy compound metabolic process	9 of 76 genes, 11.8%	139 of 7166 genes, 1.9%	0.00543	0.00	0.00	CYB2, ALD5, ADH7, ARO10, DLD1, RKI1, ADH6, ALD4, BDH1
organic substance catabolic process	21 of 76 genes, 27.6%	744 of 7166 genes, 10.4%	0.00725	0.00	0.00	ALD5, GOR1, ARO10, PAB1, GAL1, FDH1, SPS19, NTH1, UGA1, GLY1, YLR345W, IAH1, PDA1, GAL7, HXK1, PUT2, SUC2, DLD1, GAL10, FOX2, PDB1

Biological Processes enriched by SDRGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
transmembrane transport	24 of 87 genes, 27.6%	471 of 7166 genes, 6.6%	3.52e-07	0.00	0.00	GAP1, YCT1, TPN1, YMC1, THI73, FEN2, SUL1, AMF1, MAL31, FCY2, MCH2, FET3, FTR1, DAL4, MEP2, STL1, DUR3, SEO1, JEN1, DAL5, YOR1, UGA4, NRT1, PHO84
ribosomal small subunit biogenesis	14 of 87 genes, 16.1%	147 of 7166 genes, 2.1%	7.94e-07	0.00	0.00	NEW1, UTP23, NAN1, PWP2, UTP6, UTP20, UTP15, ENP2, UTP10, RRP5, NSR1, FAF1, BFR2, BMS1
ncRNA processing	23 of 87 genes, 26.4%	469 of 7166 genes, 6.5%	1.82e-06	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, TRM732, MAK5, NSR1, BFR2, DUS3, UTP23, UTP6, PWP2, UTP15, ENP2, UTP10, RRP5, TRM2, NOP4, FAF1, MDN1, DBP10, BMS1
maturation of SSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	12 of 87 genes, 13.8%	108 of 7166 genes, 1.5%	2.16e-06	0.00	0.00	UTP23, NAN1, PWP2, UTP6, UTP20, UTP15, ENP2, UTP10, RRP5, FAF1, BFR2, BMS1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
rRNA processing	20 of 87 genes, 23.0%	355 of 7166 genes, 5.0%	2.21e-06	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, MAK5, NSR1, BFR2, UTP6, PWP2, UTP23, UTP15, ENP2, UTP10, RRP5, NOP4, FAF1, MDN1, D BP10, BMS1
rRNA metabolic process	21 of 87 genes, 24.1%	400 of 7166 genes, 5.6%	3.01e-06	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, MAK5, NSR1, BFR2, UTP6, PWP2, UTP23, UTP15, ENP2, RRN6, UTP10, RRP5, NOP4, FAF1, M DN1, DBP10, BMS1
ribosome biogenesis	23 of 87 genes, 26.4%	482 of 7166 genes, 6.7%	3.08e-06	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, MAK5, NSR1, BFR2, NEW1, UTP23, UTP6, PWP2, UTP15, MAK21, ENP2, UTP10, RRP5, SYO1, NOP4, FAF1, MDN1, DBP10, BMS1
ncRNA metabolic process	25 of 87 genes, 28.7%	584 of 7166 genes, 8.1%	5.30e-06	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, TRM732, MAK5, NSR1, BFR2, DUS3, UTP23, UTP6, PWP2, UTP15, ENP2, RRN6, UTP10, RRP5, TRM2, NOP4, FAF1, MDN1, HER2, DBP10, BMS1
maturation of SSU-rRNA	12 of 87 genes, 13.8%	119 of 7166 genes, 1.7%	6.60e-06	0.00	0.00	UTP23, NAN1, PWP2, UTP6, UTP20, UTP15, ENP2, UTP10, RRP5, FAF1, BFR2, BMS1
endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	7 of 87 genes, 8.0%	33 of 7166 genes, 0.5%	4.26e-05	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5, BMS1
endonucleolytic cleavage to generate mature 5'-end of SSU-rRNA from (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	7 of 87 genes, 8.0%	35 of 7166 genes, 0.5%	6.57e-05	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5, BMS1
rRNA 5'-end processing	7 of 87 genes, 8.0%	36 of 7166 genes, 0.5%	8.08e-05	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5, BMS1
ncRNA 5'-end processing	7 of 87 genes, 8.0%	36 of 7166 genes, 0.5%	8.08e-05	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5, BMS1
anion transmembrane transport	10 of 87 genes, 11.5%	96 of 7166 genes, 1.3%	8.36e-05	0.00	0.00	FTR1, GAP1, YCT1, YMC1, JEN1, FEN2, SUL1, UGA4, PHO84, FET3
ribonucleoprotein complex biogenesis	23 of 87 genes, 26.4%	577 of 7166 genes, 8.1%	8.86e-05	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, MAK5, NSR1, BFR2, NEW1, UTP23, UTP6, PWP2, UTP15, MAK21, ENP2, UTP10, RRP5, SYO1, NOP4, FAF1, MDN1, DBP10, BMS1
RNA 5'-end processing	7 of 87 genes, 8.0%	37 of 7166 genes, 0.5%	9.87e-05	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5, BMS1
ion transport	19 of 87 genes, 21.8%	410 of 7166 genes, 5.7%	0.00013	0.00	0.00	GAP1, YCT1, YMC1, FEN2, SUL1, AMF1, MAL31, MCH2, FET3, FTR1, PDR16, MEP2, STL1, DUR3, JEN1, DAL5, YOR1, UGA4, PHO8 4
anion transport	13 of 87 genes, 14.9%	195 of 7166 genes, 2.7%	0.00022	0.00	0.00	FTR1, PDR16, GAP1, YCT1, YMC1, JEN1, FEN2, SUL1, DAL5, UGA4, YOR1, PHO84, FET3
maturation of 5.8S rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	9 of 87 genes, 10.3%	88 of 7166 genes, 1.2%	0.00041	0.00	0.00	UTP23, PWP2, UTP6, UTP20, MAK16, UTP10, RRP5, MAK5, DBP10
ion transmembrane transport	14 of 87 genes, 16.1%	241 of 7166 genes, 3.4%	0.00041	0.00	0.00	FTR1, GAP1, MEP2, DUR3, YCT1, YMC1, JEN1, FEN2, SUL1, AMF1, UGA4, MCH2, PHO84, FET3
maturation of 5.8S rRNA	9 of 87 genes, 10.3%	89 of 7166 genes, 1.2%	0.00045	0.00	0.00	UTP23, PWP2, UTP6, UTP20, MAK16, UTP10, RRP5, MAK5, DBP10
ribosomal large subunit biogenesis	10 of 87 genes, 11.5%	121 of 7166 genes, 1.7%	0.00073	0.00	0.00	RRP8, JIP5, MAK16, MAK21, RRP5, SYO1, MAK5, NOP4, MDN1, DBP10
RNA processing	23 of 87 genes, 26.4%	652 of 7166 genes, 9.1%	0.00077	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, TRM732, MAK5, NSR1, BFR2, DUS3, UTP23, UTP6, PWP2, UTP15, ENP2, UTP10, RRP5, TRM2, NOP4, FAF1, MDN1, DBP10, BMS1
cation transport	13 of 87 genes, 14.9%	240 of 7166 genes, 3.3%	0.00230	0.00	0.00	FTR1, GAP1, MEP2, DUR3, STL1, YCT1, YMC1, JEN1, AMF1, UGA4, MAL31, PHO84, FET3

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
endonucleolytic cleavage involved in rRNA processing	7 of 87 genes, 8.0%	58 of 7166 genes, 0.8%	0.00234	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5, BMS1
endonucleolytic cleavage of tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	7 of 87 genes, 8.0%	58 of 7166 genes, 0.8%	0.00234	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5, BMS1
oxidation-reduction process	18 of 87 genes, 20.7%	454 of 7166 genes, 6.3%	0.00265	0.00	0.00	CYB2, FMO1, BNA2, PRO3, IMD4, JLP1, FET3, OYE2, DUS3, MET3, GCV2, YDL144C, MAE1, PRX1, DLD1, YBR238C, ALD4, CIR2
endonucleolytic cleavage in ITS1 to separate SSU-rRNA from 5.8S rRNA and LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	6 of 87 genes, 6.9%	46 of 7166 genes, 0.6%	0.00722	0.00	0.00	UTP23, PWP2, UTP6, UTP20, UTP10, RRP5
carboxylic acid transport	8 of 87 genes, 9.2%	95 of 7166 genes, 1.3%	0.00741	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4
vitamin transport	4 of 87 genes, 4.6%	14 of 7166 genes, 0.2%	0.00782	0.00	0.00	FEN2, FCY2, NRT1, TPN1
organic acid transport	8 of 87 genes, 9.2%	97 of 7166 genes, 1.4%	0.00864	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4

Biological Processes enriched by SDRGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
oxoacid metabolic process	15 of 51 genes, 29.4%	450 of 7166 genes, 6.3%	0.00010	0.00	0.00	ADE3, ACS1, GCV2, MGA2, IDP1, SPE2, MAE1, BNA4, YAT2, BNA2, GLT1, FAS2, PHO84, IZH2, BIO2
organic acid metabolic process	15 of 51 genes, 29.4%	451 of 7166 genes, 6.3%	0.00011	0.00	0.00	ADE3, ACS1, GCV2, MGA2, IDP1, SPE2, MAE1, BNA4, YAT2, BNA2, GLT1, FAS2, PHO84, IZH2, BIO2
carboxylic acid metabolic process	14 of 51 genes, 27.5%	433 of 7166 genes, 6.0%	0.00043	0.67	0.02	ADE3, ACS1, GCV2, MGA2, IDP1, SPE2, MAE1, BNA4, YAT2, BNA2, GLT1, FAS2, IZH2, BIO2
monocarboxylic acid metabolic process	9 of 51 genes, 17.6%	185 of 7166 genes, 2.6%	0.00190	1.00	0.04	ACS1, MGA2, IDP1, SPE2, MAE1, YAT2, FAS2, IZH2, BIO2
dicarboxylic acid metabolic process	5 of 51 genes, 9.8%	46 of 7166 genes, 0.6%	0.00624	0.80	0.04	ADE3, GLT1, IDP1, MAE1, BNA4
small molecule metabolic process	17 of 51 genes, 33.3%	812 of 7166 genes, 11.3%	0.00947	0.67	0.04	ADE3, ADE5,7, ACS1, MGA2, BNA4, YAT2, BNA2, GLT1, SNZ1, IZH2, BIO2, GCV2, IDP1, MAE1, SPE2, FAS2, PHO84
dicarboxylic acid biosynthetic process	4 of 51 genes, 7.8%	25 of 7166 genes, 0.3%	0.00979	0.57	0.04	ADE3, GLT1, IDP1, BNA4

Cellular Component enriched by SURGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
mitochondrion	45 of 113 genes, 39.8%	1210 of 7166 genes, 16.9%	4.24e-07	0.00	0.00	ADH3, RCF2, MPM1, HOL1, LAT1, YNL144C, LSC1, IMO32, GLR1, HEM14, ETR1, UGA2, YKR070W, ACH1, GSY1, PDA1, MDM31, IMA1, PDX1, LSC2, PST2, COA1, CBR1, SDH8, MDH1, TMA10, SDH1, PKP1, MGM1, CYM1, ROT2, SCO1, HXT6, FMP37, HXK1, SUC2, MSW1, CLD1, LIP5, GPD1, LEU5, LIP2, HXT7, PDB1, GUT1
mitochondrial pyruvate dehydrogenase complex	5 of 113 genes, 4.4%	7 of 7166 genes, 0.1%	1.69e-06	0.00	0.00	LAT1, PDA1, PDX1, PKP1, PDB1
pyruvate dehydrogenase complex	5 of 113 genes, 4.4%	7 of 7166 genes, 0.1%	1.69e-06	0.00	0.00	LAT1, PDA1, PDX1, PKP1, PDB1
cytoplasm	100 of 113 genes, 88.5%	4739 of 7166 genes, 66.1%	3.27e-06	0.00	0.00	PGM2, ADH3, CTR1, GLC3, GAL1, MPM1, HOL1, SPS19, SSU1, YNL144C, LSC1, IMO32, YMR315W, HEM14, VHR1, RTC3, YMR027W, VBA4, ATX1, YKR070W, ACH1, TSR4, MDM31, PCK1, GAL10, IMA1, SDS24, YJL068C, LSC2, PST2, CLU1, YKL151C, TIP1, RTK1, CBR1, SDH8, NAS6, YJR096W, URE2, GPH1, MGM1, YLR345W, CYM1, VBA1, PNC1, INO1, GLK1, LEU5, PIG2, MHT1, RCF2, LAT1, GLR1, ETR1, YBR096W, UGA2, RIM11, GSY1, PDA1, GAL7, MLS1, HXT13, SUR1, REG1, RGI2, PDX1, COA1, CAT8, BSC6, DIP5, POX1, MDH1, PAB1, ICL1, TMA10, SOL3, SDH1, NTH1, BDH1, UBC7, PKP1, CWH41, ROT2, HXT6, SCO1, ETP1, FMP37, HXK1, MSW1, SUC2, CLD1, LIP5, GPD1, FOX2, AAT2, PXA1, LIP2, HXT7, PDB1, GUT1
oxidoreductase complex	7 of 113 genes, 6.2%	42 of 7166 genes, 0.6%	0.00032	0.00	0.00	GPD1, LAT1, SDH1, PDA1, PDX1, PKP1, PDB1
peroxisome	9 of 113 genes, 8.0%	87 of 7166 genes, 1.2%	0.00074	0.00	0.00	MGM1, POX1, MLS1, PNC1, GPD1, FOX2, PXA1, SPS19, AAT2
microbody	9 of 113 genes, 8.0%	87 of 7166 genes, 1.2%	0.00074	0.00	0.00	MGM1, POX1, MLS1, PNC1, GPD1, FOX2, PXA1, SPS19, AAT2
mitochondrial part	24 of 113 genes, 21.2%	629 of 7166 genes, 8.8%	0.00315	0.00	0.00	COA1, CBR1, ADH3, SDH8, MDH1, RCF2, MPM1, LAT1, SDH1, LSC1, PKP1, HEM14, ETR1, MGM1, CYM1, SCO1, PDA1, FMP37, MDM31, MSW1, CLD1, LEU5, PDX1, PDB1

Cellular Component enriched by SURGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
mitochondrial pyruvate dehydrogenase complex	4 of 76 genes, 5.3%	7 of 7166 genes, 0.1%	4.15e-05	0.00	0.00	LAT1, PDA1, PDX1, PDB1
pyruvate dehydrogenase complex	4 of 76 genes, 5.3%	7 of 7166 genes, 0.1%	4.15e-05	0.00	0.00	LAT1, PDA1, PDX1, PDB1
mitochondrion	30 of 76 genes, 39.5%	1210 of 7166 genes, 16.9%	0.00022	0.00	0.00	CYB2, ALD5, GCN1, GOR1, RCF2, TMA10, LAT1, LSC1, IMO32, ETR1, ADY2, ACH1, PDA1, FMP37, HXK1, JEN1, PUT2, SUC2, YGR012W, DLD1, PRD1, ALD4, CLD1, NCE103, LEU5, PDX1, LSC2, HER2, PST2, PDB1
mitochondrial part	19 of 76 genes, 25.0%	629 of 7166 genes, 8.8%	0.00213	0.00	0.00	CYB2, ALD5, RCF2, LAT1, LSC1, ETR1, PDA1, FMP37, PUT2, DLD1, YGR012W, PRD1, ALD4, CLD1, NCE103, LEU5, PDX1, HER2, PDB1

Cellular Component enriched by SDRGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
preribosome	18 of 87 genes, 20.7%	186 of 7166 genes, 2.6%	4.75e-10	0.00	0.00	RRP8, NAN1, UTP20, MAK16, BFR2, NEW1, UTP23, UTP6, PWP2, UTP15, MAK21, ENP2, UTP10, RRP5, NOP4, MDN1, DBP10, BMS1
nucleolus	22 of 87 genes, 25.3%	386 of 7166 genes, 5.4%	5.70e-08	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, MAK5, NSR1, IFH1, BFR2, UTP6, PWP2, UTP23, UTP15, MAK21, ENP2, RRN6, UTP10, RRP5, NOP4, FAF1, DBP10, BMS1
small-subunit processome	10 of 87 genes, 11.5%	54 of 7166 genes, 0.8%	5.96e-08	0.00	0.00	UTP23, NAN1, PWP2, UTP6, UTP20, UTP15, ENP2, UTP10, RRP5, BFR2
90S preribosome	12 of 87 genes, 13.8%	92 of 7166 genes, 1.3%	7.31e-08	0.00	0.00	RRP8, NAN1, PWP2, UTP6, UTP20, UTP15, ENP2, MAK21, UTP10, RRP5, BFR2, BMS1
plasma membrane	23 of 87 genes, 26.4%	532 of 7166 genes, 7.4%	4.42e-06	0.00	0.00	GAP1, YCT1, TPN1, THI73, FEN2, SUL1, AMF1, FCY2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, JEN1, LSB1, DAL5, YOR1, UGA4, FLO1, NRT1, PHO84
cell part	86 of 87 genes, 98.9%	5844 of 7166 genes, 81.6%	3.44e-05	0.00	0.00	NAN1, CYB2, YCT1, MCH2, DYS1, OYE2, FTR1, MET3, YDL144C, MAK21, MAE1, UTP10, RRP5, SYO1, ALD4, DBP10, BMS1, GAP1, RRP8, MAK16, YMC1, PSD1, ARO10, SUL1, FMO1, BNA2, IFH1, ARG8, NEW1, DAL4, JEN1, YOR1, UGA4, FLO1, CIR2, YGR109W-B, TPN1, TRM732, AMF1, MAK5, HEF3, IMD4, JLP1, FET3, DUS3, MEP2, UTP15, STL1, FUN19, SEO1, LSB1, PRX1, DLD1, NOP4, YLR278C, YBR238C, OXP1, MET2, SWE1, MDN1, HER2, NRT1, UTP20, JIP5, THI73, FEN2, HXK2, MAL31, NSR1, FCY2, MSG5, PRO3, BFR2, PDR16, UTP6, PWP2, UTP23, GCV2, DUR3, ENP2, RRN6, DAL5, HEM1, FAF1, FUR1, PHO84
cell	86 of 87 genes, 98.9%	5846 of 7166 genes, 81.6%	3.54e-05	0.00	0.00	NAN1, CYB2, YCT1, MCH2, DYS1, OYE2, FTR1, MET3, YDL144C, MAK21, MAE1, UTP10, RRP5, SYO1, ALD4, DBP10, BMS1, GAP1, RRP8, MAK16, YMC1, PSD1, ARO10, SUL1, FMO1, BNA2, IFH1, ARG8, NEW1, DAL4, JEN1, YOR1, UGA4, FLO1, CIR2, YGR109W-B, TPN1, TRM732, AMF1, MAK5, HEF3, IMD4, JLP1, FET3, DUS3, MEP2, UTP15, STL1, FUN19, SEO1, LSB1, PRX1, DLD1, NOP4, YLR278C, YBR238C, OXP1, MET2, SWE1, MDN1, HER2, NRT1, UTP20, JIP5, THI73, FEN2, HXK2, MAL31, NSR1, FCY2, MSG5, PRO3, BFR2, PDR16, UTP6, PWP2, UTP23, GCV2, DUR3, ENP2, RRN6, DAL5, HEM1, FAF1, FUR1, PHO84
cell periphery	25 of 87 genes, 28.7%	803 of 7166 genes, 11.2%	0.00053	0.00	0.00	GAP1, YCT1, TPN1, THI73, FEN2, SUL1, AMF1, MAL31, FCY2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, SEO1, JEN1, LSB1, DAL5, UGA4, YOR1, FLO1, NRT1, PHO84
membrane-enclosed lumen	32 of 87 genes, 36.8%	1284 of 7166 genes, 17.9%	0.00198	0.00	0.00	NAN1, CYB2, MAK5, DUS3, UTP15, MAK21, MAE1, UTP10, FUN19, RRP5, NOP4, ALD4, MDN1, DBP10, BMS1, GAP1, RRP8, UTP20, JIP5, MAK16, NSR1, BFR2, IFH1, ARG8, PWP2, UTP6, UTP23, ENP2, RRN6, HEM1, FAF1, CIR2
organelle lumen	32 of 87 genes, 36.8%	1284 of 7166 genes, 17.9%	0.00198	0.00	0.00	NAN1, CYB2, MAK5, DUS3, UTP15, MAK21, MAE1, UTP10, FUN19, RRP5, NOP4, ALD4, MDN1, DBP10, BMS1, GAP1, RRP8, UTP20, JIP5, MAK16, NSR1, BFR2, IFH1, ARG8, PWP2, UTP6, UTP23, ENP2, RRN6, HEM1, FAF1, CIR2
intracellular organelle lumen	32 of 87 genes, 36.8%	1284 of 7166 genes, 17.9%	0.00198	0.00	0.00	NAN1, CYB2, MAK5, DUS3, UTP15, MAK21, MAE1, UTP10, FUN19, RRP5, NOP4, ALD4, MDN1, DBP10, BMS1, GAP1, RRP8, UTP20, JIP5, MAK16, NSR1, BFR2, IFH1, ARG8, PWP2, UTP6, UTP23, ENP2, RRN6, HEM1, FAF1, CIR2
integral component of plasma membrane	7 of 87 genes, 8.0%	71 of 7166 genes, 1.0%	0.00203	0.00	0.00	SUL1, FTR1, GAP1, UGA4, DUR3, PHO84, FET3
t-UTP complex	3 of 87 genes, 3.4%	7 of 7166 genes, 0.1%	0.00549	0.31	0.04	NAN1, UTP15, UTP10
preribosome, large subunit precursor	7 of 87 genes, 8.0%	87 of 7166 genes, 1.2%	0.00762	0.29	0.04	NOP4, RRP8, NEW1, MDN1, MAK16, MAK21, DBP10

Cellular Component enriched by SDRGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
nucleotide-excision repair complex	4 of 51 genes, 7.8%	23 of 7166 genes, 0.3%	0.00153	0.00	0.00	SSL2, RAD16, RAD7, RAD2
DNA repair complex	4 of 51 genes, 7.8%	36 of 7166 genes, 0.5%	0.00951	1.00	0.02	SSL2, RAD16, RAD7, RAD2

Molecular Function enriched by SURGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
catalytic activity	73 of 113 genes, 64.6%	2432 of 7166 genes, 33.9%	3.07e-09	0.00	0.00	PGM2, ADH3, GLC3, GAL1, SPS19, DAK2, LSC1, IMO32, YMR315W, HEM14, YMR027W, ACH1, PCK1, GAL10, IMA1, YJL068C, LSC2, PST2, YKL151C, TIP1, RTK1, CBR1, YJR096W, URE2, GPH1, YBR053C, MGM1, YLR345W, CYM1, PNC1, INO1, GLK1, SOR1, MHT1, ADH6, LAT1, GLR1, ETR1, UGA2, RIM11, GSY1, PDA1, GAL7, MLS1, SUR1, PDX1, POX1, MDH1, ADH7, ICL1, SOL3, NTH1, SDH1, BDH1, UBC7, PKP1, YDR109C, CWH41, ROT2, SCO1, HXK1, SUC2, MSW1, YLL056C, CLD1, LIP5, GPD1, FOX2, AAT2, PXA1, LIP2, PDB1, GUT1
oxidoreductase activity	23 of 113 genes, 20.4%	340 of 7166 genes, 4.7%	3.06e-07	0.00	0.00	CBR1, ADH3, POX1, MDH1, ADH7, YJR096W, URE2, ADH6, SPS19, SDH1, GLR1, BDH1, YMR315W, HEM14, ETR1, UGA2, SCO1, PDA1, GPD1, FOX2, SOR1, PST2, PDB1
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	9 of 113 genes, 8.0%	78 of 7166 genes, 1.1%	0.00049	0.00	0.00	ADH3, MDH1, ADH7, YJR096W, ADH6, FOX2, GPD1, SOR1, BDH1
carbohydrate kinase activity	5 of 113 genes, 4.4%	16 of 7166 genes, 0.2%	0.00052	0.00	0.00	GAL1, YLR345W, GLK1, YDR109C, HXK1
oxidoreductase activity, acting on CH-OH group of donors	9 of 113 genes, 8.0%	84 of 7166 genes, 1.2%	0.00092	0.00	0.00	ADH3, MDH1, ADH7, YJR096W, ADH6, FOX2, GPD1, SOR1, BDH1
carbohydrate binding	5 of 113 genes, 4.4%	26 of 7166 genes, 0.4%	0.00693	0.33	0.02	GAL1, GAL10, ROT2, GLK1, HXK1

Molecular Function enriched by SURGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR	False Positives	Genes annotated to the term
oxidoreductase activity	20 of 76 genes, 26.3%	340 of 7166 genes, 4.7%	2.42e-08	0.00	0.00	YDL124W, CYB2, ALD5, GOR1, ADH7, MET10, ADH6, FDH1, SPS19, BDH1, ETR1, PDA1, GDH1, PUT2, DLD1, ALD4, FOX2, SOR1, PST2, PDB1
oxidoreductase activity, acting on CH-OH group of donors	10 of 76 genes, 13.2%	84 of 7166 genes, 1.2%	1.90e-06	0.00	0.00	YDL124W, CYB2, GOR1, ADH7, DLD1, FDH1, ADH6, FOX2, SOR1, BDH1
catalytic activity	49 of 76 genes, 64.5%	2432 of 7166 genes, 33.9%	6.05e-06	0.00	0.00	CYB2, UTR2, ALD5, GOR1, MET10, GAL1, ADH6, SPS19, ENA1, LAT1, LSC1, IMO32, ETR1, IAH1, ACH1, PDA1, MYO1, GAL7, GDH1, PUT2, YGR012W, DLD1, ALD4, GAL10, OXP1, NCE103, HER2, PDX1, LSC2, PST2, YDL124W, ARO10, ADH7, RKI1, FDH1, NTH1, BDH1, UGA1, YDR109C, GLY1, YLR345W, HXK1, SUC2, YLL056C, PRD1, CLD1, FOX2, SOR1, PDB1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR	False Positives	Genes annotated to the term
oxidoreductase activity, acting on the aldehyde or oxo group of donors	6 of 76 genes, 7.9%	27 of 7166 genes, 0.4%	3.81e-05	0.00	0.00	FDH1, ALD4, YDL124W, ALD5, PDA1, PDB1
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	8 of 76 genes, 10.5%	78 of 7166 genes, 1.1%	0.00018	0.00	0.00	YDL124W, GOR1, ADH7, FDH1, ADH6, FOX2, SOR1, BDH1
cofactor binding	10 of 76 genes, 13.2%	152 of 7166 genes, 2.1%	0.00052	0.00	0.00	CYB2, GOR1, ARO10, YLL056C, DLD1, FDH1, YGR012W, LSC1, PST2, UGA1
carbohydrate kinase activity	4 of 76 genes, 5.3%	16 of 7166 genes, 0.2%	0.00254	0.00	0.00	GAL1, YLR345W, HXK1, YDR109C
alcohol dehydrogenase (NADP+) activity	3 of 76 genes, 3.9%	7 of 7166 genes, 0.1%	0.00513	0.00	0.00	ADH6, YDL124W, ADH7
aldo-keto reductase (NADP) activity	3 of 76 genes, 3.9%	8 of 7166 genes, 0.1%	0.00815	0.22	0.02	ADH6, YDL124W, ADH7

Molecular Function enriched by SDRGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
transporter activity	25 of 87 genes, 28.7%	476 of 7166 genes, 6.6%	2.58e-08	0.00	0.00	GAP1, YCT1, TPN1, YMC1, THI73, FEN2, SUL1, AMF1, MAL31, FCY2, MCH2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, SEO1, JEN1, DAL5, YOR1, UGA4, NRT1, PHO84
transmembrane transporter activity	22 of 87 genes, 25.3%	379 of 7166 genes, 5.3%	6.28e-08	0.00	0.00	GAP1, YCT1, TPN1, YMC1, FEN2, SUL1, AMF1, MAL31, FCY2, MCH2, FET3, FTR1, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, YOR1, UGA4, NRT1, PHO84
substrate-specific transmembrane transporter activity	20 of 87 genes, 23.0%	338 of 7166 genes, 4.7%	3.29e-07	0.00	0.00	GAP1, YCT1, TPN1, YMC1, FEN2, SUL1, AMF1, MAL31, FCY2, FET3, FTR1, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, UGA4, NRT1, PHO84
substrate-specific transporter activity	21 of 87 genes, 24.1%	410 of 7166 genes, 5.7%	1.62e-06	0.00	0.00	GAP1, YCT1, TPN1, YMC1, FEN2, SUL1, AMF1, MAL31, FCY2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, UGA4, NRT1, PHO84
snoRNA binding	7 of 87 genes, 8.0%	26 of 7166 genes, 0.4%	2.44e-06	0.00	0.00	NAN1, PWP2, UTP6, UTP15, UTP10, RRP5, BMS1
ion transmembrane transporter activity	17 of 87 genes, 19.5%	282 of 7166 genes, 3.9%	4.86e-06	0.00	0.00	GAP1, YCT1, YMC1, FEN2, SUL1, AMF1, MAL31, FET3, FTR1, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, UGA4, PHO84
symporter activity	8 of 87 genes, 9.2%	50 of 7166 genes, 0.7%	1.77e-05	0.00	0.00	DAL4, MAL31, UGA4, DUR3, STL1, MCH2, PHO84, JEN1
cation transmembrane transporter activity	14 of 87 genes, 16.1%	206 of 7166 genes, 2.9%	2.11e-05	0.00	0.00	FTR1, GAP1, DAL4, MEP2, DUR3, STL1, YCT1, YMC1, JEN1, AMF1, UGA4, MAL31, PHO84, FET3
secondary active transmembrane transporter activity	9 of 87 genes, 10.3%	93 of 7166 genes, 1.3%	0.00023	0.00	0.00	SUL1, DAL4, MAL31, UGA4, DUR3, STL1, MCH2, PHO84, JEN1
cofactor binding	11 of 87 genes, 12.6%	152 of 7166 genes, 2.1%	0.00028	0.00	0.00	DUS3, CYB2, YDL144C, MAE1, ARO10, DLD1, FMO1, HEM1, CIR2, ARG8, OYE2
mRNA binding	12 of 87 genes, 13.8%	188 of 7166 genes, 2.6%	0.00034	0.00	0.00	NEW1, DUS3, NAN1, PWP2, UTP20, MAK21, RRP5, NOP4, YBR238C, NSR1, IMD4, BMS1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
carboxylic acid transmembrane transporter activity	8 of 87 genes, 9.2%	74 of 7166 genes, 1.0%	0.00039	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4
organic acid transmembrane transporter activity	8 of 87 genes, 9.2%	76 of 7166 genes, 1.1%	0.00048	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4
oxidoreductase activity	16 of 87 genes, 18.4%	347 of 7166 genes, 4.8%	0.00048	0.00	0.00	CYB2, FMO1, BNA2, PRO3, IMD4, JLP1, FET3, OYE2, DUS3, GCV2, YDL144C, MAE1, PRX1, DLD1, ALD4, CIR2
anion transmembrane transporter activity	9 of 87 genes, 10.3%	103 of 7166 genes, 1.4%	0.00054	0.00	0.00	GAP1, YCT1, YMC1, JEN1, FEN2, SUL1, DAL5, UGA4, PHO84
organic anion transmembrane transporter activity	8 of 87 genes, 9.2%	87 of 7166 genes, 1.2%	0.00134	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4
solute: cation symporter activity	6 of 87 genes, 6.9%	43 of 7166 genes, 0.6%	0.00168	0.00	0.00	DAL4, MAL31, UGA4, STL1, PHO84, JEN1
monocarboxylic acid transmembrane transporter activity	4 of 87 genes, 4.6%	13 of 7166 genes, 0.2%	0.00195	0.00	0.00	FEN2, DAL5, UGA4, JEN1
ammonium transmembrane transporter activity	4 of 87 genes, 4.6%	16 of 7166 genes, 0.2%	0.00485	0.00	0.00	AMF1, UGA4, MEP2, DUR3
U3 snoRNA binding	3 of 87 genes, 3.4%	6 of 7166 genes, 0.1%	0.00495	0.00	0.00	NAN1, RRP5, BMS1
active transmembrane transporter activity	10 of 87 genes, 11.5%	170 of 7166 genes, 2.4%	0.00526	0.00	0.00	DAL4, DUR3, STL1, JEN1, SUL1, UGA4, MAL31, YOR1, MCH2, PHO84

Molecular Function enriched by SDRGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
ion binding	29 of 51 genes, 56.9%	1583 of 7166 genes, 22.1%	8.14e-06	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, CRZ1, MAK5, BNA2, YOL098C, GLT1, ZAP1, BIO2, NEW1, TPA1, IDP1, MAE1, NAR1, RAD2, DBP1, SSL2, YOR1, FAS2, PAP1, NOB1, CIR2, YGR109W-B
cation binding	19 of 51 genes, 37.3%	824 of 7166 genes, 11.5%	0.00018	0.00	0.00	ADE5,7, ACS1, RAD16, CRZ1, BNA2, YOL098C, GLT1, ZAP1, BIO2, TPA1, IDP1, MAE1, NAR1, RAD2, FAS2, PAP1, NOB1, CIR2, YGR109W-B
metal ion binding	18 of 51 genes, 35.3%	817 of 7166 genes, 11.4%	0.00075	0.00	0.00	ADE5,7, RAD16, CRZ1, BNA2, YOL098C, GLT1, ZAP1, BIO2, TPA1, IDP1, MAE1, NAR1, RAD2, FAS2, PAP1, NOB1, CIR2, YGR109W-B
catalytic activity	33 of 51 genes, 64.7%	2439 of 7166 genes, 34.0%	0.00083	0.00	0.00	ADE3, RAD16, KNS1, BNA4, MAK5, YOL098C, RAD7, BIO2, MAE1, RAD2, DBP1, SSL2, TRM2, NOB1, PAP1, ACS1, ADE5,7, IME2, YAT2, ATG1, BNA2, GLT1, SNZ1, NEW1, GCV2, TPA1, IDP1, SPE2, NAR1, YOR1, FAS2, CIR2, YGR109W-B
small molecule binding	19 of 51 genes, 37.3%	933 of 7166 genes, 13.0%	0.00119	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, MAK5, GLT1, NEW1, TPA1, IDP1, MAE1, SSL2, DBP1, YOR1, PAP1, YGR109W-B
nucleotide binding	18 of 51 genes, 35.3%	870 of 7166 genes, 12.1%	0.00182	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, MAK5, GLT1, NEW1, IDP1, MAE1, SSL2, DBP1, YOR1, PAP1, YGR109W-B
nucleoside phosphate binding	18 of 51 genes, 35.3%	870 of 7166 genes, 12.1%	0.00182	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, MAK5, GLT1, NEW1, IDP1, MAE1, SSL2, DBP1, YOR1, PAP1, YGR109W-B
oxidoreductase activity	11 of 51 genes, 21.6%	347 of 7166 genes, 4.8%	0.00289	0.00	0.00	ADE3, GCV2, TPA1, IDP1, MAE1, BNA4, NAR1, BNA2, GLT1, FAS2, CIR2

Biological Process enriched by SURGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
acyl-CoA metabolic process	7 of 38 genes, 18.4%	20 of 7166 genes, 0.3%	1.08e-09	0.00	0.00	LAT1, LSC1, ACH1, PDX1, PDA1, LSC2, PDB1
thioester metabolic process	7 of 38 genes, 18.4%	20 of 7166 genes, 0.3%	1.08e-09	0.00	0.00	LAT1, LSC1, ACH1, PDX1, PDA1, LSC2, PDB1
small molecule metabolic process	21 of 38 genes, 55.3%	812 of 7166 genes, 11.3%	1.05e-08	0.00	0.00	CAT8, ADH7, GAL1, ADH6, SPS19, LAT1, LSC1, BDH1, ETR1, YDR109C, YLR345W, ACH1, PDA1, GAL7, HXK1, GAL10, FOX2, SOR 1, LSC2, PDX1, PDB1
acetyl-CoA biosynthetic process from pyruvate	4 of 38 genes, 10.5%	4 of 7166 genes, 0.1%	1.50e-07	0.00	0.00	LAT1, PDX1, PDA1, PDB1
monocarboxylic acid metabolic process	11 of 38 genes, 28.9%	185 of 7166 genes, 2.6%	3.70e-07	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, PDX1, ETR1, PDB1
carbohydrate metabolic process	13 of 38 genes, 34.2%	314 of 7166 genes, 4.4%	7.73e-07	0.00	0.00	YLR345W, CAT8, PDA1, GAL7, MPH2, HXK1, SUC2, GAL1, GAL10, SOR1, NTH1, YDR109C, PDB1
acetyl-CoA metabolic process	5 of 38 genes, 13.2%	13 of 7166 genes, 0.2%	8.92e-07	0.00	0.00	LAT1, ACH1, PDX1, PDA1, PDB1
carbohydrate catabolic process	9 of 38 genes, 23.7%	120 of 7166 genes, 1.7%	1.85e-06	0.00	0.00	YLR345W, PDA1, GAL7, HXK1, GAL1, SUC2, GAL10, NTH1, PDB1
acetyl-CoA biosynthetic process	4 of 38 genes, 10.5%	6 of 7166 genes, 0.1%	2.24e-06	0.00	0.00	LAT1, PDX1, PDA1, PDB1
monosaccharide metabolic process	8 of 38 genes, 21.1%	99 of 7166 genes, 1.4%	7.76e-06	0.00	0.00	YLR345W, CAT8, GAL7, HXK1, GAL1, GAL10, SOR1, YDR109C
thioester biosynthetic process	4 of 38 genes, 10.5%	8 of 7166 genes, 0.1%	1.03e-05	0.00	0.00	LAT1, PDX1, PDA1, PDB1
acyl-CoA biosynthetic process	4 of 38 genes, 10.5%	8 of 7166 genes, 0.1%	1.03e-05	0.00	0.00	LAT1, PDX1, PDA1, PDB1
carboxylic acid metabolic process	13 of 38 genes, 34.2%	433 of 7166 genes, 6.0%	3.61e-05	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, LSC1, PDX1, LSC2, ETR1, PDB1
pyruvate metabolic process	6 of 38 genes, 15.8%	52 of 7166 genes, 0.7%	5.62e-05	0.00	0.00	YLR345W, LAT1, PDX1, PDA1, PDB1, HXK1
oxoacid metabolic process	13 of 38 genes, 34.2%	450 of 7166 genes, 6.3%	5.66e-05	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, LSC1, PDX1, LSC2, ETR1, PDB1
organic acid metabolic process	13 of 38 genes, 34.2%	451 of 7166 genes, 6.3%	5.81e-05	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, LSC1, PDX1, LSC2, ETR1, PDB1
hexose metabolic process	7 of 38 genes, 18.4%	88 of 7166 genes, 1.2%	6.86e-05	0.00	0.00	GAL1, YLR345W, GAL10, CAT8, SOR1, GAL7, HXK1
coenzyme metabolic process	9 of 38 genes, 23.7%	185 of 7166 genes, 2.6%	8.02e-05	0.00	0.00	YLR345W, ACH1, PDA1, HXK1, LAT1, LSC1, LSC2, PDX1, PDB1
carbohydrate phosphorylation	4 of 38 genes, 10.5%	14 of 7166 genes, 0.2%	0.00014	0.00	0.00	GAL1, YLR345W, YDR109C, HXK1
galactose catabolic process via UDP-galactose	3 of 38 genes, 7.9%	5 of 7166 genes, 0.1%	0.00030	0.00	0.00	GAL1, GAL10, GAL7
oxidation-reduction process	12 of 38 genes, 31.6%	454 of 7166 genes, 6.3%	0.00048	0.00	0.00	PDA1, ADH7, ADH6, FOX2, SPS19, SOR1, LSC1, BDH1, LSC2, PST2, ETR1, PDB1
cofactor metabolic process	9 of 38 genes, 23.7%	232 of 7166 genes, 3.2%	0.00053	0.00	0.00	YLR345W, ACH1, PDA1, HXK1, LAT1, LSC1, LSC2, PDX1, PDB1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
galactose catabolic process	3 of 38 genes, 7.9%	8 of 7166 genes, 0.1%	0.00169	0.00	0.00	GAL1, GAL10, GAL7
sulfur compound metabolic process	7 of 38 genes, 18.4%	146 of 7166 genes, 2.0%	0.00210	0.00	0.00	LAT1, LSC1, ACH1, PDX1, PDA1, LSC2, PDB1
hexose catabolic process	3 of 38 genes, 7.9%	9 of 7166 genes, 0.1%	0.00253	0.00	0.00	GAL1, GAL10, GAL7
cellular carbohydrate metabolic process	8 of 38 genes, 21.1%	215 of 7166 genes, 3.0%	0.00290	0.00	0.00	YLR345W, CAT8, MPH2, HXK1, GAL1, SUC2, NTH1, YDR109C
glycolytic process	4 of 38 genes, 10.5%	33 of 7166 genes, 0.5%	0.00551	0.00	0.00	YLR345W, PDA1, PDB1, HXK1
ATP generation from ADP	4 of 38 genes, 10.5%	33 of 7166 genes, 0.5%	0.00551	0.00	0.00	YLR345W, PDA1, PDB1, HXK1
succinyl-CoA metabolic process	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00613	0.00	0.00	LSC1, LSC2
nucleoside diphosphate phosphorylation	4 of 38 genes, 10.5%	35 of 7166 genes, 0.5%	0.00700	0.00	0.00	YLR345W, PDA1, PDB1, HXK1
galactose metabolic process	3 of 38 genes, 7.9%	13 of 7166 genes, 0.2%	0.00849	0.00	0.00	GAL1, GAL10, GAL7

Biological Process enriched by SDRGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
transmembrane transport	8 of 19 genes, 42.1%	471 of 7166 genes, 6.6%	0.00123	0.00	0.00	GAP1, STL1, TPN1, THI73, UGA4, YOR1, MCH2, PHO84

Cellular Component enriched by SURGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
mitochondrial pyruvate dehydrogenase complex	4 of 38 genes, 10.5%	7 of 7166 genes, 0.1%	1.46e-06	0.00	0.00	LAT1, PDX1, PDA1, PDB1
pyruvate dehydrogenase complex	4 of 38 genes, 10.5%	7 of 7166 genes, 0.1%	1.46e-06	0.00	0.00	LAT1, PDX1, PDA1, PDB1
mitochondrion	17 of 38 genes, 44.7%	1210 of 7166 genes, 16.9%	0.00338	0.67	0.02	RCF2, TMA10, LAT1, LSC1, IMO32, ETR1, ACH1, PDA1, FMP37, HXK1, SUC2, CLD1, LEU5, LSC2, PDX1, PST2, PDB1
oxidoreductase complex	4 of 38 genes, 10.5%	43 of 7166 genes, 0.6%	0.00450	0.50	0.02	LAT1, PDX1, PDA1, PDB1

Molecular Function enriched by SURGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
carbohydrate kinase activity	4 of 38 genes, 10.5%	16 of 7166 genes, 0.2%	0.00010	0.00	0.00	GAL1, YLR345W, YDR109C, HXK1
oxidoreductase activity	10 of 38 genes, 26.3%	347 of 7166 genes, 4.8%	0.00078	0.00	0.00	PDA1, ADH7, ADH6, FOX2, SPS19, SOR1, BDH1, ETR1, PST2, PDB1
catalytic activity	26 of 38 genes, 68.4%	2439 of 7166 genes, 34.0%	0.00136	0.00	0.00	ADH7, GAL1, ADH6, SPS19, LAT1, NTH1, LSC1, IMO32, BDH1, ETR1, YDR109C, YLR345W, ACH1, PDA1, GAL7, HXK1, SUC2, YLL 056C, GAL10, FOX2, CLD1, SOR1, PDX1, LSC2, PST2, PDB1
pyruvate dehydrogenase (acetyl-transferring) activity	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00246	0.00	0.00	PDA1, PDB1
succinate-CoA ligase activity	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00246	0.00	0.00	LSC1, LSC2
succinate-CoA ligase (ADP-forming) activity	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00246	0.00	0.00	LSC1, LSC2
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	5 of 38 genes, 13.2%	78 of 7166 genes, 1.1%	0.00457	0.00	0.00	ADH6, FOX2, SOR1, BDH1, ADH7
oxidoreductase activity, acting on CH-OH group of donors	5 of 38 genes, 13.2%	84 of 7166 genes, 1.2%	0.00654	0.00	0.00	ADH6, FOX2, SOR1, BDH1, ADH7
pyruvate dehydrogenase activity	2 of 38 genes, 5.3%	3 of 7166 genes, 0.0%	0.00736	0.22	0.02	PDA1, PDB1

Molecular Function enriched by SDRGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
symporter activity	4 of 19 genes, 21.1%	50 of 7166 genes, 0.7%	0.00043	0.00	0.00	UGA4, STL1, MCH2, PHO84
transporter activity	8 of 19 genes, 42.1%	476 of 7166 genes, 6.6%	0.00081	0.00	0.00	GAP1, STL1, TPN1, THI73, UGA4, YOR1, MCH2, PHO84
transmembrane transporter activity	7 of 19 genes, 36.8%	379 of 7166 genes, 5.3%	0.00183	2.00	0.06	GAP1, YOR1, UGA4, STL1, TPN1, MCH2, PHO84
active transmembrane transporter activity	5 of 19 genes, 26.3%	170 of 7166 genes, 2.4%	0.00364	2.00	0.08	YOR1, UGA4, STL1, MCH2, PHO84
secondary active transmembrane transporter activity	4 of 19 genes, 21.1%	93 of 7166 genes, 1.3%	0.00514	1.60	0.08	UGA4, STL1, MCH2, PHO84
solute: proton symporter activity	3 of 19 genes, 15.8%	37 of 7166 genes, 0.5%	0.00672	1.33	0.08	UGA4, STL1, PHO84
cytoplasm	100 of 113 genes, 88.5%	4739 of 7166 genes, 66.1%	3.27e-06	0.00	0.00	PGM2, ADH3, CTR1, GLC3, GAL1, MPM1, HOL1, SPS19, SSU1, YNL144C, LSC1, IMO32, YMR315W, HEM14, VHR1, RTC3, YMR02 7W, VBA4, ATX1, YKR070W, ACH1, TSR4, MDM31, PCK1, GAL10, IMA1, SDS24, YJL068C, LSC2, PST2, CLU1, YKL151C, TIP1, RTK 1, CBR1, SDH8, NAS6, YJR096W, URE2, GPH1, MGM1, YLR345W, CYM1, VBA1, PNC1, INO1, GLK1, LEU5, PIG2, MHT1, RCF2, LA T1, GLR1, ETR1, YBR096W, UGA2, RIM11, GSY1, PDA1, GAL7, MLS1, HXT13, SUR1, REG1, RGI2, PDX1, COA1, CAT8, BSC6, DIP5, POX1, MDH1, PAB1, ICL1, TMA10, SOL3, SDH1, NTH1, BDH1, UBC7, PKP1, CWH41, ROT2, HXT6, SCO1, ETP1, FMP37, HXK1, M SW1, SUC2, CLD1, LIP5, GPD1, FOX2, AAT2, PXA1, LIP2, HXT7, PDB1, GUT1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
oxidoreductase complex	7 of 113 genes, 6.2%	42 of 7166 genes, 0.6%	0.00032	0.00	0.00	GPD1, LAT1, SDH1, PDA1, PDX1, PKP1, PDB1
peroxisome	9 of 113 genes, 8.0%	87 of 7166 genes, 1.2%	0.00074	0.00	0.00	MGM1, POX1, MLS1, PNC1, GPD1, FOX2, PXA1, SPS19, AAT2
microbody	9 of 113 genes, 8.0%	87 of 7166 genes, 1.2%	0.00074	0.00	0.00	MGM1, POX1, MLS1, PNC1, GPD1, FOX2, PXA1, SPS19, AAT2
mitochondrial part	24 of 113 genes, 21.2%	629 of 7166 genes, 8.8%	0.00315	0.00	0.00	COA1, CBR1, ADH3, SDH8, MDH1, RCF2, MPM1, LAT1, SDH1, LSC1, PKP1, HEM14, ETR1, MGM1, CYM1, SCO1, PDA1, FMP37, MDM31, MSW1, CLD1, LEU5, PDX1, PDB1

Cellular Component enriched by SURGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
mitochondrial pyruvate dehydrogenase complex	4 of 76 genes, 5.3%	7 of 7166 genes, 0.1%	4.15e-05	0.00	0.00	LAT1, PDA1, PDX1, PDB1
pyruvate dehydrogenase complex	4 of 76 genes, 5.3%	7 of 7166 genes, 0.1%	4.15e-05	0.00	0.00	LAT1, PDA1, PDX1, PDB1
mitochondrion	30 of 76 genes, 39.5%	1210 of 7166 genes, 16.9%	0.00022	0.00	0.00	CYB2, ALD5, GCN1, GOR1, RCF2, TMA10, LAT1, LSC1, IMO32, ETR1, ADY2, ACH1, PDA1, FMP37, HXK1, JEN1, PUT2, SUC2, YGR 012W, DLD1, PRD1, ALD4, CLD1, NCE103, LEU5, PDX1, LSC2, HER2, PST2, PDB1
mitochondrial part	19 of 76 genes, 25.0%	629 of 7166 genes, 8.8%	0.00213	0.00	0.00	CYB2, ALD5, RCF2, LAT1, LSC1, ETR1, PDA1, FMP37, PUT2, DLD1, YGR012W, PRD1, ALD4, CLD1, NCE103, LEU5, PDX1, HER2, P DB1

Cellular Component enriched by SDRGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
preribosome	18 of 87 genes, 20.7%	186 of 7166 genes, 2.6%	4.75e-10	0.00	0.00	RRP8, NAN1, UTP20, MAK16, BFR2, NEW1, UTP23, UTP6, PWP2, UTP15, MAK21, ENP2, UTP10, RRP5, NOP4, MDN1, DBP10, BM S1
nucleolus	22 of 87 genes, 25.3%	386 of 7166 genes, 5.4%	5.70e-08	0.00	0.00	RRP8, NAN1, JIP5, UTP20, MAK16, MAK5, NSR1, IFH1, BFR2, UTP6, PWP2, UTP23, UTP15, MAK21, ENP2, RRN6, UTP10, RRP5, N OP4, FAF1, DBP10, BMS1
small-subunit processome	10 of 87 genes, 11.5%	54 of 7166 genes, 0.8%	5.96e-08	0.00	0.00	UTP23, NAN1, PWP2, UTP6, UTP20, UTP15, ENP2, UTP10, RRP5, BFR2
90S preribosome	12 of 87 genes, 13.8%	92 of 7166 genes, 1.3%	7.31e-08	0.00	0.00	RRP8, NAN1, PWP2, UTP6, UTP20, UTP15, ENP2, MAK21, UTP10, RRP5, BFR2, BMS1
plasma membrane	23 of 87 genes, 26.4%	532 of 7166 genes, 7.4%	4.42e-06	0.00	0.00	GAP1, YCT1, TPN1, THI73, FEN2, SUL1, AMF1, FCY2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, JEN1, LSB1, DAL5, YOR1, UG A4, FLO1, NRT1, PHO84
cell part	86 of 87 genes, 98.9%	5844 of 7166 genes, 81.6%	3.44e-05	0.00	0.00	NAN1, CYB2, YCT1, MCH2, DYS1, OYE2, FTR1, MET3, YDL144C, MAK21, MAE1, UTP10, RRP5, SYO1, ALD4, DBP10, BMS1, GAP1, RRP8, MAK16, YMC1, PSD1, ARO10, SUL1, FMO1, BNA2, IFH1, ARG8, NEW1, DAL4, JEN1, YOR1, UGA4, FLO1, CIR2, YGR109W-B, TPN1, TRM732, AMF1, MAK5, HEF3, IMD4, JLP1, FET3, DUS3, MEP2, UTP15, STL1, FUN19, SEO1, LSB1, PRX1, DLD1, NOP4, YL R278C, YBR238C, OXP1, MET2, SWE1, MDN1, HER2, NRT1, UTP20, JIP5, THI73, FEN2, HXK2, MAL31, NSR1, FCY2, MSG5, PRO3, BFR2, PDR16, UTP6, PWP2, UTP23, GCV2, DUR3, ENP2, RRN6, DAL5, HEM1, FAF1, FUR1, PHO84

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
cell	86 of 87 genes, 98.9%	5846 of 7166 genes, 81.6%	3.54e-05	0.00	0.00	NAN1, CYB2, YCT1, MCH2, DYS1, OYE2, FTR1, MET3, YDL144C, MAK21, MAE1, UTP10, RRP5, SYO1, ALD4, DBP10, BMS1, GAP1, RRP8, MAK16, YMC1, PSD1, ARO10, SUL1, FMO1, BNA2, IFH1, ARG8, NEW1, DAL4, JEN1, YOR1, UGA4, FLO1, CIR2, YGR109W-B, TPN1, TRM732, AMF1, MAK5, HEF3, IMD4, JLP1, FET3, DUS3, MEP2, UTP15, STL1, FUN19, SEO1, LSB1, PRX1, DLD1, NOP4, YLR278C, YBR238C, OXP1, MET2, SWE1, MDN1, HER2, NRT1, UTP20, JIP5, THI73, FEN2, HXK2, MAL31, NSR1, FCY2, MSG5, PRO3, BFR2, PDR16, UTP6, PWP2, UTP23, GCV2, DUR3, ENP2, RRN6, DAL5, HEM1, FAF1, FUR1, PHO84
cell periphery	25 of 87 genes, 28.7%	803 of 7166 genes, 11.2%	0.00053	0.00	0.00	GAP1, YCT1, TPN1, THI73, FEN2, SUL1, AMF1, MAL31, FCY2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, SEO1, JEN1, LSB1, DAL5, UGA4, YOR1, FLO1, NRT1, PHO84
membrane-enclosed lumen	32 of 87 genes, 36.8%	1284 of 7166 genes, 17.9%	0.00198	0.00	0.00	NAN1, CYB2, MAK5, DUS3, UTP15, MAK21, MAE1, UTP10, FUN19, RRP5, NOP4, ALD4, MDN1, DBP10, BMS1, GAP1, RRP8, UTP20, JIP5, MAK16, NSR1, BFR2, IFH1, ARG8, PWP2, UTP6, UTP23, ENP2, RRN6, HEM1, FAF1, CIR2
organelle lumen	32 of 87 genes, 36.8%	1284 of 7166 genes, 17.9%	0.00198	0.00	0.00	NAN1, CYB2, MAK5, DUS3, UTP15, MAK21, MAE1, UTP10, FUN19, RRP5, NOP4, ALD4, MDN1, DBP10, BMS1, GAP1, RRP8, UTP20, JIP5, MAK16, NSR1, BFR2, IFH1, ARG8, PWP2, UTP6, UTP23, ENP2, RRN6, HEM1, FAF1, CIR2
intracellular organelle lumen	32 of 87 genes, 36.8%	1284 of 7166 genes, 17.9%	0.00198	0.00	0.00	NAN1, CYB2, MAK5, DUS3, UTP15, MAK21, MAE1, UTP10, FUN19, RRP5, NOP4, ALD4, MDN1, DBP10, BMS1, GAP1, RRP8, UTP20, JIP5, MAK16, NSR1, BFR2, IFH1, ARG8, PWP2, UTP6, UTP23, ENP2, RRN6, HEM1, FAF1, CIR2
integral component of plasma membrane	7 of 87 genes, 8.0%	71 of 7166 genes, 1.0%	0.00203	0.00	0.00	SUL1, FTR1, GAP1, UGA4, DUR3, PHO84, FET3
t-UTP complex	3 of 87 genes, 3.4%	7 of 7166 genes, 0.1%	0.00549	0.31	0.04	NAN1, UTP15, UTP10
preribosome, large subunit precursor	7 of 87 genes, 8.0%	87 of 7166 genes, 1.2%	0.00762	0.29	0.04	NOP4, RRP8, NEW1, MDN1, MAK16, MAK21, DBP10

Cellular Component enriched by SDRGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
nucleotide-excision repair complex	4 of 51 genes, 7.8%	23 of 7166 genes, 0.3%	0.00153	0.00	0.00	SSL2, RAD16, RAD7, RAD2
DNA repair complex	4 of 51 genes, 7.8%	36 of 7166 genes, 0.5%	0.00951	1.00	0.02	SSL2, RAD16, RAD7, RAD2

Molecular Function enriched by SURGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
catalytic activity	73 of 113 genes, 64.6%	2432 of 7166 genes, 33.9%	3.07e-09	0.00	0.00	PGM2, ADH3, GLC3, GAL1, SPS19, DAK2, LSC1, IMO32, YMR315W, HEM14, YMR027W, ACH1, PCK1, GAL10, IMA1, YJL068C, LSC2, PST2, YKL151C, TIP1, RTK1, CBR1, YJR096W, URE2, GPH1, YBR053C, MGM1, YLR345W, CYM1, PNC1, INO1, GLK1, SOR1, MHT1, ADH6, LAT1, GLR1, ETR1, UGA2, RIM11, GSY1, PDA1, GAL7, MLS1, SUR1, PDX1, POX1, MDH1, ADH7, ICL1, SOL3, NTH1, SDH1, BDH1, UBC7, PKP1, YDR109C, CWH41, ROT2, SCO1, HXK1, SUC2, MSW1, YLL056C, CLD1, LIP5, GPD1, FOX2, AAT2, PXA1, LIP2, PDB1, GUT1
oxidoreductase activity	23 of 113 genes, 20.4%	340 of 7166 genes, 4.7%	3.06e-07	0.00	0.00	CBR1, ADH3, POX1, MDH1, ADH7, YJR096W, URE2, ADH6, SPS19, SDH1, GLR1, BDH1, YMR315W, HEM14, ETR1, UGA2, SCO1, PDA1, GPD1, FOX2, SOR1, PST2, PDB1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR	False Positives	Genes annotated to the term
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	9 of 113 genes, 8.0%	78 of 7166 genes, 1.1%	0.00049	0.00	0.00	ADH3, MDH1, ADH7, YJR096W, ADH6, FOX2, GPD1, SOR1, BDH1
carbohydrate kinase activity	5 of 113 genes, 4.4%	16 of 7166 genes, 0.2%	0.00052	0.00	0.00	GAL1, YLR345W, GLK1, YDR109C, HXK1
oxidoreductase activity, acting on CH-OH group of donors	9 of 113 genes, 8.0%	84 of 7166 genes, 1.2%	0.00092	0.00	0.00	ADH3, MDH1, ADH7, YJR096W, ADH6, FOX2, GPD1, SOR1, BDH1
carbohydrate binding	5 of 113 genes, 4.4%	26 of 7166 genes, 0.4%	0.00693	0.33	0.02	GAL1, GAL10, ROT2, GLK1, HXK1

Molecular Function enriched by SURGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR	False Positives	Genes annotated to the term
oxidoreductase activity	20 of 76 genes, 26.3%	340 of 7166 genes, 4.7%	2.42e-08	0.00	0.00	YDL124W, CYB2, ALD5, GOR1, ADH7, MET10, ADH6, FDH1, SPS19, BDH1, ETR1, PDA1, GDH1, PUT2, DLD1, ALD4, FOX2, SOR1, PST2, PDB1
oxidoreductase activity, acting on CH-OH group of donors	10 of 76 genes, 13.2%	84 of 7166 genes, 1.2%	1.90e-06	0.00	0.00	YDL124W, CYB2, GOR1, ADH7, DLD1, FDH1, ADH6, FOX2, SOR1, BDH1
catalytic activity	49 of 76 genes, 64.5%	2432 of 7166 genes, 33.9%	6.05e-06	0.00	0.00	CYB2, UTR2, ALD5, GOR1, MET10, GAL1, ADH6, SPS19, ENA1, LAT1, LSC1, IMO32, ETR1, IAH1, ACH1, PDA1, MYO1, GAL7, GDH 1, PUT2, YGR012W, DLD1, ALD4, GAL10, OXP1, NCE103, HER2, PDX1, LSC2, PST2, YDL124W, ARO10, ADH7, RKI1, FDH1, NTH1, BDH1, UGA1, YDR109C, GLY1, YLR345W, HXK1, SUC2, YLL056C, PRD1, CLD1, FOX2, SOR1, PDB1
oxidoreductase activity, acting on the aldehyde or oxo group of donors	6 of 76 genes, 7.9%	27 of 7166 genes, 0.4%	3.81e-05	0.00	0.00	FDH1, ALD4, YDL124W, ALD5, PDA1, PDB1
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	8 of 76 genes, 10.5%	78 of 7166 genes, 1.1%	0.00018	0.00	0.00	YDL124W, GOR1, ADH7, FDH1, ADH6, FOX2, SOR1, BDH1
cofactor binding	10 of 76 genes, 13.2%	152 of 7166 genes, 2.1%	0.00052	0.00	0.00	CYB2, GOR1, ARO10, YLL056C, DLD1, FDH1, YGR012W, LSC1, PST2, UGA1
carbohydrate kinase activity	4 of 76 genes, 5.3%	16 of 7166 genes, 0.2%	0.00254	0.00	0.00	GAL1, YLR345W, HXK1, YDR109C
alcohol dehydrogenase (NADP+) activity	3 of 76 genes, 3.9%	7 of 7166 genes, 0.1%	0.00513	0.00	0.00	ADH6, YDL124W, ADH7
aldo-keto reductase (NADP) activity	3 of 76 genes, 3.9%	8 of 7166 genes, 0.1%	0.00815	0.22	0.02	ADH6, YDL124W, ADH7

Molecular Function enriched by SDRGs in AWRI1499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
transporter activity	25 of 87 genes, 28.7%	476 of 7166 genes, 6.6%	2.58e-08	0.00	0.00	GAP1, YCT1, TPN1, YMC1, THI73, FEN2, SUL1, AMF1, MAL31, FCY2, MCH2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, SEO1, JEN1, DAL5, YOR1, UGA4, NRT1, PHO84

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
transmembrane transporter activity	22 of 87 genes, 25.3%	379 of 7166 genes, 5.3%	6.28e-08	0.00	0.00	GAP1, YCT1, TPN1, YMC1, FEN2, SUL1, AMF1, MAL31, FCY2, MCH2, FET3, FTR1, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, YOR1, UGA4, NRT1, PHO84
substrate-specific transmembrane transporter activity	20 of 87 genes, 23.0%	338 of 7166 genes, 4.7%	3.29e-07	0.00	0.00	GAP1, YCT1, TPN1, YMC1, FEN2, SUL1, AMF1, MAL31, FCY2, FET3, FTR1, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, UGA4, NRT1, PHO84
substrate-specific transporter activity	21 of 87 genes, 24.1%	410 of 7166 genes, 5.7%	1.62e-06	0.00	0.00	GAP1, YCT1, TPN1, YMC1, FEN2, SUL1, AMF1, MAL31, FCY2, FET3, FTR1, PDR16, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, UGA4, NRT1, PHO84
snoRNA binding	7 of 87 genes, 8.0%	26 of 7166 genes, 0.4%	2.44e-06	0.00	0.00	NAN1, PWP2, UTP6, UTP15, UTP10, RRP5, BMS1
ion transmembrane transporter activity	17 of 87 genes, 19.5%	282 of 7166 genes, 3.9%	4.86e-06	0.00	0.00	GAP1, YCT1, YMC1, FEN2, SUL1, AMF1, MAL31, FET3, FTR1, DAL4, MEP2, STL1, DUR3, JEN1, DAL5, UGA4, PHO84
symporter activity	8 of 87 genes, 9.2%	50 of 7166 genes, 0.7%	1.77e-05	0.00	0.00	DAL4, MAL31, UGA4, DUR3, STL1, MCH2, PHO84, JEN1
cation transmembrane transporter activity	14 of 87 genes, 16.1%	206 of 7166 genes, 2.9%	2.11e-05	0.00	0.00	FTR1, GAP1, DAL4, MEP2, DUR3, STL1, YCT1, YMC1, JEN1, AMF1, UGA4, MAL31, PHO84, FET3
secondary active transmembrane transporter activity	9 of 87 genes, 10.3%	93 of 7166 genes, 1.3%	0.00023	0.00	0.00	SUL1, DAL4, MAL31, UGA4, DUR3, STL1, MCH2, PHO84, JEN1
cofactor binding	11 of 87 genes, 12.6%	152 of 7166 genes, 2.1%	0.00028	0.00	0.00	DUS3, CYB2, YDL144C, MAE1, ARO10, DLD1, FMO1, HEM1, CIR2, ARG8, OYE2
mRNA binding	12 of 87 genes, 13.8%	188 of 7166 genes, 2.6%	0.00034	0.00	0.00	NEW1, DUS3, NAN1, PWP2, UTP20, MAK21, RRP5, NOP4, YBR238C, NSR1, IMD4, BMS1
carboxylic acid transmembrane transporter activity	8 of 87 genes, 9.2%	74 of 7166 genes, 1.0%	0.00039	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4
organic acid transmembrane transporter activity	8 of 87 genes, 9.2%	76 of 7166 genes, 1.1%	0.00048	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4
oxidoreductase activity	16 of 87 genes, 18.4%	347 of 7166 genes, 4.8%	0.00048	0.00	0.00	CYB2, FMO1, BNA2, PRO3, IMD4, JLP1, FET3, OYE2, DUS3, GCV2, YDL144C, MAE1, PRX1, DLD1, ALD4, CIR2
anion transmembrane transporter activity	9 of 87 genes, 10.3%	103 of 7166 genes, 1.4%	0.00054	0.00	0.00	GAP1, YCT1, YMC1, JEN1, FEN2, SUL1, DAL5, UGA4, PHO84
organic anion transmembrane transporter activity	8 of 87 genes, 9.2%	87 of 7166 genes, 1.2%	0.00134	0.00	0.00	GAP1, YCT1, YMC1, JEN1, SUL1, FEN2, DAL5, UGA4
solute: cation symporter activity	6 of 87 genes, 6.9%	43 of 7166 genes, 0.6%	0.00168	0.00	0.00	DAL4, MAL31, UGA4, STL1, PHO84, JEN1
monocarboxylic acid transmembrane transporter activity	4 of 87 genes, 4.6%	13 of 7166 genes, 0.2%	0.00195	0.00	0.00	FEN2, DAL5, UGA4, JEN1
ammonium transmembrane transporter activity	4 of 87 genes, 4.6%	16 of 7166 genes, 0.2%	0.00485	0.00	0.00	AMF1, UGA4, MEP2, DUR3
U3 snoRNA binding	3 of 87 genes, 3.4%	6 of 7166 genes, 0.1%	0.00495	0.00	0.00	NAN1, RRP5, BMS1
active transmembrane transporter activity	10 of 87 genes, 11.5%	170 of 7166 genes, 2.4%	0.00526	0.00	0.00	DAL4, DUR3, STL1, JEN1, SUL1, UGA4, MAL31, YOR1, MCH2, PHO84

Molecular Function enriched by SDRGs in CBS2499 – Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
ion binding	29 of 51 genes, 56.9%	1583 of 7166 genes, 22.1%	8.14e-06	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, CRZ1, MAK5, BNA2, YOL098C, GLT1, ZAP1, BIO2, NEW1, TPA1, IDP1, MAE1, NAR1, RAD2, DBP1, SSL2, YOR1, FAS2, PAP1, NOB1, CIR2, YGR109W-B
cation binding	19 of 51 genes, 37.3%	824 of 7166 genes, 11.5%	0.00018	0.00	0.00	ADE5,7, ACS1, RAD16, CRZ1, BNA2, YOL098C, GLT1, ZAP1, BIO2, TPA1, IDP1, MAE1, NAR1, RAD2, FAS2, PAP1, NOB1, CIR2, YGR109W-B
metal ion binding	18 of 51 genes, 35.3%	817 of 7166 genes, 11.4%	0.00075	0.00	0.00	ADE5,7, RAD16, CRZ1, BNA2, YOL098C, GLT1, ZAP1, BIO2, TPA1, IDP1, MAE1, NAR1, RAD2, FAS2, PAP1, NOB1, CIR2, YGR109W-B
catalytic activity	33 of 51 genes, 64.7%	2439 of 7166 genes, 34.0%	0.00083	0.00	0.00	ADE3, RAD16, KNS1, BNA4, MAK5, YOL098C, RAD7, BIO2, MAE1, RAD2, DBP1, SSL2, TRM2, NOB1, PAP1, ACS1, ADE5,7, IME2, YAT2, ATG1, BNA2, GLT1, SNZ1, NEW1, GCV2, TPA1, IDP1, SPE2, NAR1, YOR1, FAS2, CIR2, YGR109W-B
small molecule binding	19 of 51 genes, 37.3%	933 of 7166 genes, 13.0%	0.00119	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, MAK5, GLT1, NEW1, TPA1, IDP1, MAE1, SSL2, DBP1, YOR1, PAP1, YGR109W-B
nucleotide binding	18 of 51 genes, 35.3%	870 of 7166 genes, 12.1%	0.00182	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, MAK5, GLT1, NEW1, IDP1, MAE1, SSL2, DBP1, YOR1, PAP1, YGR109W-B
nucleoside phosphate binding	18 of 51 genes, 35.3%	870 of 7166 genes, 12.1%	0.00182	0.00	0.00	ADE3, ADE5,7, ACS1, RAD16, IME2, KNS1, BNA4, ATG1, MAK5, GLT1, NEW1, IDP1, MAE1, SSL2, DBP1, YOR1, PAP1, YGR109W-B
oxidoreductase activity	11 of 51 genes, 21.6%	347 of 7166 genes, 4.8%	0.00289	0.00	0.00	ADE3, GCV2, TPA1, IDP1, MAE1, BNA4, NAR1, BNA2, GLT1, FAS2, CIR2

Biological Process enriched by SURGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
acyl-CoA metabolic process	7 of 38 genes, 18.4%	20 of 7166 genes, 0.3%	1.08e-09	0.00	0.00	LAT1, LSC1, ACH1, PDX1, PDA1, LSC2, PDB1
thioester metabolic process	7 of 38 genes, 18.4%	20 of 7166 genes, 0.3%	1.08e-09	0.00	0.00	LAT1, LSC1, ACH1, PDX1, PDA1, LSC2, PDB1
small molecule metabolic process	21 of 38 genes, 55.3%	812 of 7166 genes, 11.3%	1.05e-08	0.00	0.00	CAT8, ADH7, GAL1, ADH6, SPS19, LAT1, LSC1, BDH1, ETR1, YDR109C, YLR345W, ACH1, PDA1, GAL7, HXK1, GAL10, FOX2, SOR1, LSC2, PDX1, PDB1
acetyl-CoA biosynthetic process from pyruvate	4 of 38 genes, 10.5%	4 of 7166 genes, 0.1%	1.50e-07	0.00	0.00	LAT1, PDX1, PDA1, PDB1
monocarboxylic acid metabolic process	11 of 38 genes, 28.9%	185 of 7166 genes, 2.6%	3.70e-07	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, PDX1, ETR1, PDB1
carbohydrate metabolic process	13 of 38 genes, 34.2%	314 of 7166 genes, 4.4%	7.73e-07	0.00	0.00	YLR345W, CAT8, PDA1, GAL7, MPH2, HXK1, SUC2, GAL1, GAL10, SOR1, NTH1, YDR109C, PDB1
acetyl-CoA metabolic process	5 of 38 genes, 13.2%	13 of 7166 genes, 0.2%	8.92e-07	0.00	0.00	LAT1, ACH1, PDX1, PDA1, PDB1
carbohydrate catabolic process	9 of 38 genes, 23.7%	120 of 7166 genes, 1.7%	1.85e-06	0.00	0.00	YLR345W, PDA1, GAL7, HXK1, GAL1, SUC2, GAL10, NTH1, PDB1
acetyl-CoA biosynthetic process	4 of 38 genes, 10.5%	6 of 7166 genes, 0.1%	2.24e-06	0.00	0.00	LAT1, PDX1, PDA1, PDB1

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
monosaccharide metabolic process	8 of 38 genes, 21.1%	99 of 7166 genes, 1.4%	7.76e-06	0.00	0.00	YLR345W, CAT8, GAL7, HXK1, GAL1, GAL10, SOR1, YDR109C
thioester biosynthetic process	4 of 38 genes, 10.5%	8 of 7166 genes, 0.1%	1.03e-05	0.00	0.00	LAT1, PDX1, PDA1, PDB1
acyl-CoA biosynthetic process	4 of 38 genes, 10.5%	8 of 7166 genes, 0.1%	1.03e-05	0.00	0.00	LAT1, PDX1, PDA1, PDB1
carboxylic acid metabolic process	13 of 38 genes, 34.2%	433 of 7166 genes, 6.0%	3.61e-05	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, LSC1, PDX1, LSC2, ETR1, PDB1
pyruvate metabolic process	6 of 38 genes, 15.8%	52 of 7166 genes, 0.7%	5.62e-05	0.00	0.00	YLR345W, LAT1, PDX1, PDA1, PDB1, HXK1
oxoacid metabolic process	13 of 38 genes, 34.2%	450 of 7166 genes, 6.3%	5.66e-05	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, LSC1, PDX1, LSC2, ETR1, PDB1
organic acid metabolic process	13 of 38 genes, 34.2%	451 of 7166 genes, 6.3%	5.81e-05	0.00	0.00	YLR345W, CAT8, ACH1, PDA1, HXK1, FOX2, SPS19, LAT1, LSC1, PDX1, LSC2, ETR1, PDB1
hexose metabolic process	7 of 38 genes, 18.4%	88 of 7166 genes, 1.2%	6.86e-05	0.00	0.00	GAL1, YLR345W, GAL10, CAT8, SOR1, GAL7, HXK1
coenzyme metabolic process	9 of 38 genes, 23.7%	185 of 7166 genes, 2.6%	8.02e-05	0.00	0.00	YLR345W, ACH1, PDA1, HXK1, LAT1, LSC1, LSC2, PDX1, PDB1
carbohydrate phosphorylation	4 of 38 genes, 10.5%	14 of 7166 genes, 0.2%	0.00014	0.00	0.00	GAL1, YLR345W, YDR109C, HXK1
galactose catabolic process via UDP-galactose	3 of 38 genes, 7.9%	5 of 7166 genes, 0.1%	0.00030	0.00	0.00	GAL1, GAL10, GAL7
oxidation-reduction process	12 of 38 genes, 31.6%	454 of 7166 genes, 6.3%	0.00048	0.00	0.00	PDA1, ADH7, ADH6, FOX2, SPS19, SOR1, LSC1, BDH1, LSC2, PST2, ETR1, PDB1
cofactor metabolic process	9 of 38 genes, 23.7%	232 of 7166 genes, 3.2%	0.00053	0.00	0.00	YLR345W, ACH1, PDA1, HXK1, LAT1, LSC1, LSC2, PDX1, PDB1
galactose catabolic process	3 of 38 genes, 7.9%	8 of 7166 genes, 0.1%	0.00169	0.00	0.00	GAL1, GAL10, GAL7
sulfur compound metabolic process	7 of 38 genes, 18.4%	146 of 7166 genes, 2.0%	0.00210	0.00	0.00	LAT1, LSC1, ACH1, PDX1, PDA1, LSC2, PDB1
hexose catabolic process	3 of 38 genes, 7.9%	9 of 7166 genes, 0.1%	0.00253	0.00	0.00	GAL1, GAL10, GAL7
cellular carbohydrate metabolic process	8 of 38 genes, 21.1%	215 of 7166 genes, 3.0%	0.00290	0.00	0.00	YLR345W, CAT8, MPH2, HXK1, GAL1, SUC2, NTH1, YDR109C
glycolytic process	4 of 38 genes, 10.5%	33 of 7166 genes, 0.5%	0.00551	0.00	0.00	YLR345W, PDA1, PDB1, HXK1
ATP generation from ADP	4 of 38 genes, 10.5%	33 of 7166 genes, 0.5%	0.00551	0.00	0.00	YLR345W, PDA1, PDB1, HXK1
succinyl-CoA metabolic process	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00613	0.00	0.00	LSC1, LSC2
nucleoside diphosphate phosphorylation	4 of 38 genes, 10.5%	35 of 7166 genes, 0.5%	0.00700	0.00	0.00	YLR345W, PDA1, PDB1, HXK1
galactose metabolic process	3 of 38 genes, 7.9%	13 of 7166 genes, 0.2%	0.00849	0.00	0.00	GAL1, GAL10, GAL7

Biological Process enriched by SDRGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
transmembrane transport	8 of 19 genes, 42.1%	471 of 7166 genes, 6.6%	0.00123	0.00	0.00	GAP1, STL1, TPN1, THI73, UGA4, YOR1, MCH2, PHO84

Cellular Component enriched by SURGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
mitochondrial pyruvate dehydrogenase complex	4 of 38 genes, 10.5%	7 of 7166 genes, 0.1%	1.46e-06	0.00	0.00	LAT1, PDX1, PDA1, PDB1
pyruvate dehydrogenase complex	4 of 38 genes, 10.5%	7 of 7166 genes, 0.1%	1.46e-06	0.00	0.00	LAT1, PDX1, PDA1, PDB1
mitochondrion	17 of 38 genes, 44.7%	1210 of 7166 genes, 16.9%	0.00338	0.67	0.02	RCF2, TMA10, LAT1, LSC1, IMO32, ETR1, ACH1, PDA1, FMP37, HXK1, SUC2, CLD1, LEU5, LSC2, PDX1, PST2, PDB1
oxidoreductase complex	4 of 38 genes, 10.5%	43 of 7166 genes, 0.6%	0.00450	0.50	0.02	LAT1, PDX1, PDA1, PDB1

Molecular Function enriched by SURGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
carbohydrate kinase activity	4 of 38 genes, 10.5%	16 of 7166 genes, 0.2%	0.00010	0.00	0.00	GAL1, YLR345W, YDR109C, HXK1
oxidoreductase activity	10 of 38 genes, 26.3%	347 of 7166 genes, 4.8%	0.00078	0.00	0.00	PDA1, ADH7, ADH6, FOX2, SPS19, SOR1, BDH1, ETR1, PST2, PDB1
catalytic activity	26 of 38 genes, 68.4%	2439 of 7166 genes, 34.0%	0.00136	0.00	0.00	ADH7, GAL1, ADH6, SPS19, LAT1, NTH1, LSC1, IMO32, BDH1, ETR1, YDR109C, YLR345W, ACH1, PDA1, GAL7, HXK1, SUC2, YLL 056C, GAL10, FOX2, CLD1, SOR1, PDX1, LSC2, PST2, PDB1
pyruvate dehydrogenase (acetyl-transferring) activity	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00246	0.00	0.00	PDA1, PDB1
succinate-CoA ligase activity	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00246	0.00	0.00	LSC1, LSC2
succinate-CoA ligase (ADP-forming) activity	2 of 38 genes, 5.3%	2 of 7166 genes, 0.0%	0.00246	0.00	0.00	LSC1, LSC2
oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	5 of 38 genes, 13.2%	78 of 7166 genes, 1.1%	0.00457	0.00	0.00	ADH6, FOX2, SOR1, BDH1, ADH7
oxidoreductase activity, acting on CH-OH group of donors	5 of 38 genes, 13.2%	84 of 7166 genes, 1.2%	0.00654	0.00	0.00	ADH6, FOX2, SOR1, BDH1, ADH7
pyruvate dehydrogenase activity	2 of 38 genes, 5.3%	3 of 7166 genes, 0.0%	0.00736	0.22	0.02	PDA1, PDB1

Molecular Function enriched by SDRGs in common between AWRI1499 & CBS2499 - Long response

Gene Ontology term	Cluster frequency	Genome frequency	Corrected P-value	FDR %	False Positives	Genes annotated to the term
symporter activity	4 of 19 genes, 21.1%	50 of 7166 genes, 0.7%	0.00043	0.00	0.00	UGA4, STL1, MCH2, PHO84
transporter activity	8 of 19 genes, 42.1%	476 of 7166 genes, 6.6%	0.00081	0.00	0.00	GAP1, STL1, TPN1, THI73, UGA4, YOR1, MCH2, PHO84
transmembrane transporter activity	7 of 19 genes, 36.8%	379 of 7166 genes, 5.3%	0.00183	2.00	0.06	GAP1, YOR1, UGA4, STL1, TPN1, MCH2, PHO84
active transmembrane transporter activity	5 of 19 genes, 26.3%	170 of 7166 genes, 2.4%	0.00364	2.00	0.08	YOR1, UGA4, STL1, MCH2, PHO84
secondary active transmembrane transporter activity	4 of 19 genes, 21.1%	93 of 7166 genes, 1.3%	0.00514	1.60	0.08	UGA4, STL1, MCH2, PHO84
solute: proton symporter activity	3 of 19 genes, 15.8%	37 of 7166 genes, 0.5%	0.00672	1.33	0.08	UGA4, STL1, PHO84

Appendix 4

(Scientific products)

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The vintage effect overcomes the *terroir* effect: a three year survey on the wine yeast biodiversity in Franciacorta and Oltrepò Pavese, two northern Italian vine-growing areas

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A three year survey on the dominant yeast populations in samples of air, must and wine in different vineyards and cellars of two northern Italian vine-growing territories (six sites in Franciacorta and eight sites in Oltrepò Pavese areas) was carried out. A total of 505 isolates were ascribed to 31 different species by RFLP analysis of the ITS1–5.8SrRNA–ITS2 region and partial sequence analysis of the 26S rRNA gene. The most commonly found species were *Saccharomyces cerevisiae* (frequency, $F'=58.7\,\%$; incidence, $I'=53.5\,\%$), *Hanseniaspora uvarum* ($F'=14.3\,\%$; $I'=5.3\,\%$), *Metschnikowia fructicola* ($F'=11.1\,\%$; $I'=5.0\,\%$) and *Torulaspora delbrueckii* ($F'=10.3\,\%$; $I'=3.8\,\%$). Among 270 *S. cerevisiae* new isolates, 156 (57.8 %) revealed a different genetic pattern through polymorphism analysis of the interdelta regions by capillary electrophoresis, while 47 isolates (17.4 %) were clones of starter cultures. By considering the Shannon–Wiener index and results of principal component analysis (PCA) analyses, the year of isolation (vintage) proved to be a factor that significantly affected the biodiversity of the yeast species, whereas the geographical site (*terroir*) was not. Seventy-five per cent of *S. cerevisiae* isolates gathered in a unique cluster at a similarity level of 82 %, while the remaining 25 % were separated into minor groups without any evident relationship between δ-PCR profile and territory, year or source of isolation. However, in six cases a similar strain appeared at the harvesting time both in Franciacorta and Oltrepò Pavese areas, whereas surprisingly no strain was reisolated in the same vineyard or cellar for consecutive years.

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INTRODUCTION

In winemaking, yeasts are essential for the transformation of grape sugars into ethanol and carbon dioxide through alcoholic fermentation; nonetheless, due to their specific enzymic activities and cell autolysis, they can also generate typical sensorial characteristics in wine, like secondary flavours and smoothness (Romano *et al.*, 2003a). Although selected *Saccharomyces* strains are usually added by oenologists as starter cultures to control the fermentative process, several micro-organisms enter the must from the vineyard environment, winery facilities and cellar equipment, and these can affect the quality of the end product. Nowadays, for a certain style of wines, the use of the 'so called' autochthonous yeasts is considered essential in providing for the valorization and preservation of the environmental microbial biodiversity (Pretorius, 2000). In

†These authors contributed equally to this paper.

Abbreviations: ADY, active dry yeast; CE, capillary electrophoresis; ITS, internal transcribed spacer; LSD, least significant difference; PCA, principal component analysis; UPGMA, unweighted pair group method with arithmetic means.

Two supplementary figures are available with the online Supplementary Material.

frontiers in Microbiology

ORIGINAL RESEARCH
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Indigenous Georgian Wine-Associated Yeasts and Grape Cultivars to Edit the Wine Quality in a Precision Oenology Perspective

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In Georgia, one of the most ancient vine-growing environment, the homemade production of wine is still very popular in every rural family and spontaneous fermentation of must, without addition of chemical preservatives, is the norm. The present work investigated the yeast biodiversity in five Georgian areas (Guria, Imereti, Kakheti, Kartli, Ratcha-Lechkhumi) sampling grapes and wines from 22 different native cultivars, in 26 vineyards and 19 family cellars. One hundred and eighty-two isolates were ascribed to 15 different species by PCR-ITS and RFLP, and partial sequencing of D1/D2 domain 26S rDNA gene. *Metschnikowia pulcherrima* ($F' = 0.56$, $I' = 0.32$), *Hanseniaspora guilliermondii* ($F' = 0.49$, $I' = 0.27$), and *Cryptococcus flavescens* ($F' = 0.31$, $I' = 0.11$) were the dominant yeasts found on grapes, whereas *Saccharomyces cerevisiae* showed the highest prevalence into wine samples. Seventy four isolates with fermentative potential were screened for oenological traits such as ethanol production, resistance to SO_2, and acetic acid, glycerol and H_2S production. Three yeast strains (*Kluyveromyces marxianus* UMY207, *S. cerevisiae* UMY255, *Torulaspora delbrueckii* UMY196) were selected and separately inoculated in vinifications experiments at a Georgian cellar. Musts were prepared from healthy grapes of local varieties, Goruli Mtsvane (white berry cultivar) and Saperavi (black berry cultivar). Physical (°Brix) and microbial analyses (plate counts) were performed to monitor the fermentative process. The isolation of indigenous *S. cerevisiae* yeasts beyond the inoculated strains indicated that a co-presence occurred during the vinification tests. Results from quantitative GC-FID analysis of volatile compounds revealed that the highest amount of fermentation flavors, such as 4-ethoxy-4-oxobutanoic acid (monoethyl succinate), 2-methylpropan-1-ol, ethyl 2-hydroxypropanoate, and 2-phenylethanol, were significantly more produced in fermentation conducted in Saperavi variety inoculated with *K. marxianus*, whereas other aromatic compounds like 3-methylbutyl acetate, ethyl hexanoate and dihydrofuran-2(3H)-one (γ- butyrolactone)

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Cloning the putative gene of vinyl phenol reductase of *Dekkera bruxellensis* in *Saccharomyces cerevisiae*

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ABSTRACT

Vinylphenol reductase of *Dekkera bruxellensis*, the characteristic enzyme liable for "Brett" sensory modification of wine, has been recently recognized to belong to the short chain dehydrogenases/reductases family. Indeed, a preliminary biochemical characterisation has conferred to the purified protein a dual significance acting as superoxide dismutase and as a NADH-dependent reductase. The present study aimed for providing a certain identification of the enzyme by cloning the VPR gene in *S. cerevisiae*, a species not producing ethyl phenols. Transformed clones of *S. cerevisiae* resulted capable of expressing a biologically active form of the heterologous protein, proving its role in the conversion of 4-vinyl guaiacol to 4-ethyl guaiacol. A VPR specific protein activity of 9 ± 0.6 mU/mg was found in crude extracts of *S. cerevisiae* recombinant strain. This result was confirmed in activity trials carried out with the protein purified from transformant cells of *S. cerevisiae* by a his-tag purification approach; in particular, VPR-enriched fractions showed a specific activity of 1.83 ± 0.03 U/mg at pH 6.0. Furthermore, in agreement with literature, the purified protein behaves like a SOD, with a calculated specific activity of approximatively 3.41 U/mg. The comparative genetic analysis of the partial VPR gene sequences from 17 different *D. bruxellensis* strains suggested that the observed polymorphism (2.3%) and the allelic heterozygosity state of the gene do not justify the well described strain-dependent character in producing volatile phenols of this species. Actually, no correlation exists between genotype membership of the analysed strains and their capability to release off-flavours. This work adds valuable knowledge to the study of *D. bruxellensis* wine spoilage and prepare the ground for interesting future industrial applications.

1. Introduction

Biological origin of wine spoilage can be attributed to the activity of yeasts and bacteria. Although technological advances in oenology and the implementation of Good Manufacturing Practices in winemaking have led to a significant reduction in the risk of wine alteration by bacteria, spoilage linked to yeast contamination is still a potential threat. Most common alterations involve the formation of films or sediments, turbidity, gas production and sensory injuries such as the release of volatile phenols. The responsibility of this latter issue is particularly directed toward the development of yeasts belonging to *Dekkera bruxellensis* species (or its anamorph form, *Brettanomyces bruxellensis*). The negative impact of these compounds on wine has been largely revised in literature (Aguilar-Uscanga et al., 2003; Caruso et al., 2002; Chatonnet et al., 1992, 1995, 1997; Dias et al., 2003a; Fugelsang et al., 1993; Fugelsang, 1997; Joseph and Bisson, 2004; Loureiro and Malfeito-Ferreira, 2003; Mansfield et al., 2002; Romano, 2007; Snowdon et al., 2006) Because of cellar treatments involving the use of sulfur dioxide are not working on resistant strains (Barata et al., 2008; Agnolucci et al., 2010; Curtin et al., 2012a,b; Vigentini et al., 2013), preventing actions against the propagation of *Dekkera bruxellensis* in wine have been proposed, such as the use of ozone (Guzzon et al., 2013) and heat treatments of barrels (Fabrizio et al., 2015) or the application to wine of low electric current (Lustrato et al., 2010) and biocontrol agents (Mehlomakulu et al., 2014). However, the damage caused by *B. bruxellensis* in wines is still persisting and a better understanding of volatile phenol production in this species is required. Volatile phenols originate from

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Use of Native Yeast Strains for In-Bottle Fermentation to Face the Uniformity in Sparkling Wine Production

*Ileana Vigentini[1], Shirley Barrera Cardenas[1], Federica Valdetara[1], Monica Faccincani[2], Carlo A. Panont[3], Claudia Picozzi[1] and Roberto Foschino[1]**

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The in-bottle fermentation of sparkling wines is currently triggered by few commercialized *Saccharomyces cerevisiae* strains. This lack of diversity in *tirage* yeast cultures leads to a prevalent uniformity in sensory profiles of the end products. The aim of this study has been to exploit the natural multiplicity of yeast populations in order to introduce variability in sparkling wines throughout the re-fermentation step. A collection of 133 *S. cerevisiae* strains were screened on the basis of technological criteria (fermenting power and vigor, SO_2 tolerance, alcohol tolerance, flocculence) and qualitative features (acetic acid, glycerol and H_2S productions). These activities allowed the selection of yeasts capable of dominating the in-bottle fermentation in actual cellar conditions: in particular, the performances of FX and FY strains (isolated in Franciacorta area), and OX and OY strains (isolated in Oltrepò Pavese area), were compared to those of habitually used starter cultures (IOC18-2007, EC1118, Lalvin DV10), by involving nine wineries belonging to the two Consortia of Appellation of Origin. The microbiological analyses of samples have revealed that the indigenous strains showed an increased latency period and a higher cultivability along the aging time than the commercial starter cultures do. Results of chemical analyses and sensory evaluation of the samples after 18 months *sur lies* have shown that significant differences ($p < 0.05$) were present among the strains for alcoholic strength, carbon dioxide overpressure and pleasantness, whereas they were not observed for residual sugars content, titratable acidity or volatile acidity. Indigenous *S. cerevisiae* exhibited comparable values respect to the commercial starter cultures. The ANOVA has also proven that the base wine formulation is a key factor, by significantly affecting ($p < 0.01$) some oenological parameters of wine, like alcoholic strength, volatile acidity, carbon dioxide overpressure, titratable acidity and dry extract. The use of native yeast strains for the re-fermentation step can be considered a convenient way for introducing differentiation to the final product without modifying the traditional technology. In a perspective of "precision enology," where the wine is designed on specific vine cultivars and microorganisms, this work underlines that exploring yeast biodiversity is a strategic activity to improve the production.

Keywords: Franciacorta, in-bottle fermentation, Oltrepò Pavese, *Saccharomyces cerevisiae*, sparkling wine, yeast strain selection

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A Response Surface Methodology Approach to Investigate the Effect of Sulfur Dioxide, pH, and Ethanol on *DbCD* and *DbVPR* Gene Expression and on the Volatile Phenol Production in *Dekkera/Brettanomyces bruxellensis* CBS2499

Federica Valdetara[1], Daniela Fracassetti[1], Alessia Campanello[1], Carlo Costa[2], Roberto Foschino[1], Concetta Compagno[1] and Ileana Vigentini[1]*

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Dekkera/Brettanomyces bruxellensis, the main spoilage yeast in barrel-aged wine, metabolize hydroxycinnamic acids into off-flavors, namely ethylphenols. Recently, both the enzymes involved in this transformation, the cinnamate decarboxylase (*DbCD*) and the vinylphenol reductase (*DbVPR*), have been identified. To counteract microbial proliferation in wine, sulfur dioxide (SO_2) is used commonly to stabilize the final product, but limiting its use is advised to preserve human health and boost sustainability in winemaking. In the present study, the influence of SO_2 was investigated in relation with pH and ethanol factors on the expression of *DbCD* and *DbVPR* genes and volatile phenol production in *D. bruxellensis* CBS2499 strain under different model wines throughout a response surface methodology (RSM). In order to ensure an exact quantification of *DbCD* and *DbVPR* expression, an appropriate housekeeping gene was sought among *DbPDC*, *DbALD*, *DbEF*, *DbACT*, and *DbTUB* genes by GeNorm and Normfinder algorithms. The latter gene showed the highest expression stability and it was chosen as the reference housekeeping gene in qPCR assays. Even though SO_2 could not be commented as main factor because of its statistical irrelevance on the response of *DbCD* gene, linear interactions with pH and ethanol concurred to define a significant effect ($p < 0.05$) on its expression. The *DbCD* gene was generally downregulated respect to a permissive growth condition (0 mg/L mol. SO_2, pH 4.5 and 5% v/v ethanol); the combination of the factor levels that maximizes its expression (0.83-fold change) was calculated at 0.25 mg/L mol. SO_2, pH 4.5 and 12.5% (v/v) ethanol. On the contrary, *DbVPR* expression was not influenced by main factors or by their interactions; however, its expression is maximized (1.80-fold change) at the same conditions calculated for *DbCD* gene. While no linear interaction between factors

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Wild Grape-Associated Yeasts as Promising Biocontrol Agents against *Vitis vinifera* Fungal Pathogens

Gustavo Cordero-Bueso[1], Nicola Mangieri[2], David Maghradze[3], Roberto Foschino[2], Federica Valdetara[2], Jesús M. Cantoral[1] and Ileana Vigentini[2]*

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The increasing level of hazardous residues in the environment and food chains has led the European Union to restrict the use of chemical fungicides. Thus, exploiting new natural antagonistic microorganisms against fungal diseases could serve the agricultural production to reduce pre- and post-harvest losses, to boost safer practices for workers and to protect the consumers' health. The main aim of this work was to evaluate the antagonistic potential of epiphytic yeasts against *Botrytis cinerea*, *Aspergillus carbonarius*, and *Penicillium expansum* pathogen species. In particular, yeast isolation was carried out from grape berries of *Vitis vinifera* ssp sylvestris populations, of the Eurasian area, and *V. vinifera* ssp *vinifera* cultivars from three different farming systems (organic, biodynamic, and conventional). Strains able to inhibit or slow the growth of pathogens were selected by *in vitro* and *in vivo* experiments. The most effective antagonist yeast strains were subsequently assayed for their capability to colonize the grape berries. Finally, possible modes of action, such as nutrients and space competition, iron depletion, cell wall degrading enzymes, diffusible and volatile antimicrobial compounds, and biofilm formation, were investigated as well. Two hundred and thirty-one yeast strains belonging to 26 different species were isolated; 20 of them, ascribed to eight species, showed antagonistic action against all molds. Yeasts isolated from *V. vinifera* ssp sylvestris were more effective (up to 50%) against *B. cinerea* rather than those isolated from *V. vinifera* ssp vinifera. Six strains, all isolated from wild vines, belonging to four species (*Meyerozyma guilliermondii*, *Hanseniaspora uvarum*, *Hanseniaspora clermontiae*, and *Pichia kluyveri*) revealed one or more phenotypical characteristics associated to the analyzed modes of antagonistic action.

Keywords: yeasts, molds, *V. vinifera* ssp sylvestris, biocontrol, fungal diseases

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INTRODUCTION

Plants provide over 80% of the human diet. Just three cereal crops (i.e., rice, maize, and wheat) and two fruit crops (grape-berries and citrus fruits) provide 70% of energy intake and cope the production of 80% of the fermented beverages in the world (FAO, 2011). Since the 1900s, around 75% of crop diversity has been lost from farmers' fields. Regarding harvest products, many losses (up to 25% of total production in industrialized

Valdetara F. (2010)

Molecular investigation on stress responses and enzymatic activities of *Brettanomyces/Dekkera bruxellensis* in wine conditions.

XX Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology

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1st Annual PhD Report

Molecular investigation on stress responses and enzymatic activities of *Brettanomyces/Dekkera bruxellensis* in wine conditions

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This PhD project has the main goals of improving the basic knowledge on the wine spoilage yeast *Brettanomyces/Dekkera bruxellensis* and developing possible applications to prevent or reduce its detrimental activity due to the production of volatile phenols. Different strains of *B./D. bruxellensis* will be characterize for both the genetic polymorphism and the expression of the gene encoding the vinyl phenol reductase enzyme. This task will release useful data for the developing of molecular probes for a reliable and rapid detection of this yeast by Real-Time PCR. Furthermore, some strains will be analysed for their molecular response to sulphur dioxide exposure.

Studio della risposta allo stress e delle attività enzimatiche di *Brettanomyces/Dekkera bruxellensis* in condizioni enologiche attraverso metodi molecolari

Questo progetto di dottorato ha lo scopo principale di approfondire le conoscenze di base riguardo *Brettanomyces/Dekkera bruxellensis*, lievito contaminante del vino, la e di sviluppare possibili applicazioni che aiutino a prevenirne o almeno a ridurne la deleteria attività di produzione di fenoli volatile. Differenti ceppi di *B./D. bruxellensis* saranno caratterizzati per il polimorfismo genetico e l'espressione del gene codificante l'enzima vinilfenolo reduttasi. Ciò porterà all'ottenimento di dati utili allo sviluppo di sonde molecolari specifiche, per una identificazione ripetibile e rapida di questa specie di lievito attraverso Real-Time PCR. Alcuni ceppi saranno inoltre valutati in merito alla risposta molecolare allo stress da solforosa.

1. State-of-the-Art

Brettanomyces/Dekkera bruxellensis has been isolated from aged wines worldwide, vineyards and grapes being important source of contamination. The presence of this yeast has been correlated with an increase of phenolic aromas in wine (Godoy et al., 2008). The sensorial spoilage caused in wines by the formation of volatile phenols is a serious economic problem, due to large economic losses (Suarez et al., 2007). The main compounds responsible for this decrease in quality are overall volatile phenols, 4-ethyl phenol and 4-ethyl guaiacol (Chatonnet et al., 1992), but *B./D. bruxellensis* is also able to produce detrimental amounts of acetic acid as well as other minor compounds. Sensory descriptors associated with these contaminants compounds, usually generically named off-flavours, are "leathery", "pharmaceutical", "burned plastic", "smoky", "barnyard", "horse", "stable", "wet dog". Ethyl phenols production is a two-step metabolic pathway whereby hydroxycinnamic acids (free form) are first decarboxylated to vinyl phenols by the enzyme hydroxycinnammate decarboxylase (HCD). This step can be performed by different microorganisms of enological interest, such as *Saccharomyces, Pichia, Torulaspora* and *Brettanomyces* (Suarez et al., 2007). Subsequent conversion of vinyl phenols to ethyl phenols by a vinyl phenol reductase (VPR) can be accomplish, in wine, by *B./D. bruxellensis*. Godoy et al. (2008) purified a p-coumarate decarboxylase from *B./D. bruxellensis* and identified a "putative gene" of a VPR from the same strain. Granato et al. (2014) purified from the strain *D. bruxellensis* CBS4481 a protein with VPR activity, being probably a moonlighting superoxide dismutase.

The VPR gene represents a suitable target to approach the early detection of *B./D. bruxellensis* in wine contamination control. Till now few studies are focused on the development of Real-Time protocols for the detection of this species (Tofalo et al. 2012; Willenburg & Divol, 2012).

The management of microbial contamination during the winemaking process is mainly carried out through the use of SO_2. The SO_2 tolerance between wine yeasts is various and strain specific. *B./D. bruxellensis* has been shown to be able, in particular during the wine aging, to counteract the normal dosage of SO_2 and consequently to damage the products. In some cases, depending on the strain and the SO_2 amount, it enters a Viable But Not Culturable (VBNC) phase in which it seems to be able to produce ethyl phenols (Serpaggi et al., 2012). In *S. cerevisiae* the response to SO_2 stress is achieved by an increasing of the expression of *SSU1* and *FZF1* genes, being the sulphites efflux pump and the positive regulator protein respectively (Nardi et al., 2010). Therefore, we would like to understand if *B./D. bruxellensis* uses similar mechanisms, or the one of other closely related yeasts.

2. PhD Thesis Objectives and Milestones

A1) Molecular characterization of the vinyl phenol reductase enzyme.

Evaluation of VPR gene polymorphism through the screening of strains from different sources and with variable ethyl phenols production. Cloning of VPR gene in *S. cerevisiae*. The transcriptional VPR gene regulation under different oenological stimuli will be investigated.

A2) Development of a Q-PCR protocol for *B./D. bruxellensis* detection.

Setting the molecular probes for Real-Time PCR using the VPR gene as target. Challenge test with spiked samples to validate the protocol. Survey on real musts and wines to test the possible contamination.

A3) Evaluation of the molecular mechanisms involved in the stress response to SO_2 exposure.

Tolerance to increasing amounts of SO_2 by different strains. Determination of the global gene expression, before and after the treatment with SO_2, through RNA-Seq approach in a wine-model medium under oenological conditions.

A4) Writing of PhD thesis and peer-reviewed papers

Table 2 *Gantt diagram for this PhD thesis project.*

Activities		Months																							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
A1)	**Molecular characterization of the vinyl phenol reductase**																								
	VPR gene polymorphism																								
	Cloning of VPR gene																								
	VPR gene regulation																								
A2)	**Development of a Q-PCR protocol for *B./D. bruxellensis* detection**																								
	Set-up of Real-Time PCR																								
	Challenge test																								
	Survey on real musts and wines																								
A3)	**Evaluation of molecular mechanisms involved in stress response to SO2 exposure**																								
	SO_2 tolerance																								
	RNA sequencing																								
A4)	**Thesis and Paper Preparation**																								

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Molecular investigation on stress responses and enzymatic activities of *Brettanomyces/Dekkera bruxellensis* in wine conditions

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Tutor: Prof. Roberto Foschino

Introduction

This PhD project has the main goals of improving the basic knowledge on the wine spoilage yeast *Brettanomyces/Dekkera bruxellensis* and developing possible applications to prevent or reduce its detrimental activity due to the production of volatile phenols. Different strains of *B./D. bruxellensis* will be characterize for both the genetic polymorphism and the expression of the gene encoding the vinyl phenol reductase enzyme. This task will release useful data for the developing of molecular probes for a reliable and rapid detection of this yeast by Real-Time PCR. Furthermore, some strains will be analysed for their molecular response to sulphur dioxide exposure.

State of the art, milestones, specific objectives and preliminary results

Molecular characterization of the vinyl phenol reductase enzyme

A putative ORF of the VPR gene from *D. bruxellensis* CBS4481, the characteristic enzyme involved in ethyl phenol production in wine, has been recently described by Granato et al. (2014).

Specific objectives: Evaluation of VPR genetic/phenotypic polymorphism; Cloning of VPR gene in *Saccharomyces*/non *Saccharomyces* yeast and his deletion in natural host; Investigation of transcriptional VPR level under different oenological stimuli.

Milestones: HPLC training; optimization of molecular tools for non-*Saccharomyces* and *Brettanomyces* yeasts; setup of an experimental design for VPR expression under different oenological conditions.

Preliminary results: Specific molecular probes for the amplification of the VPR gene has been designed and tested on different genetic background, such as *D. bruxellensis* CBS4481 (a, Fig. 1), *S. cerevisiae* W303 (b, Fig. 1), and *S. cerevisiae* W303 overexpressing the VPR gene (c, Fig. 1). Results confirmed that the gene is expressed by transformed *S. cerevisiae* cells.

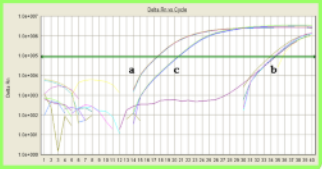

Fig. 1

Evaluation of the molecular mechanisms involved in the stress response to SO_2 exposure

In *S. cerevisiae* the response to SO_2 stress is achieved by an over-expression of *SSU1* and *FZF1* genes, being the sulphite efflux pump and the positive regulator protein, respectively (Nardi et al., 2010).

Specific objectives: Assessment of SO_2 tolerance by the sequenced strains *D./B. bruxellensis* CBS2499 and AWRI1499; Determination of the global gene expression, before and after SO_2treatment, through RNA-Seq approach in a wine-model medium.

Milestones: Management of *B./D. bruxellensis* in bioreactor; bioinformatic elaboration of raw data sequencing.

Preliminary results: Fermentative results (Fig. 2) confirmed that *B. bruxellensis* AWRI1499 shows variability in the growth-no-growth interface between 0,5 and 0,6 mg/L of molecular SO_2 (Curtin et al., 2012).

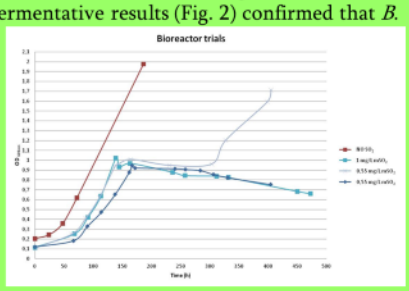

Fig. 2

Development of a Q-PCR protocol for *B./D. bruxellensis* detection

The VPR gene could represent a suitable target to approach the early detection of *D./B. bruxellensis* in wine contamination control (Tofalo et al., 2012; Willenburg et al., 2012).

Specific objectives: Development of a Real-Time PCR protocol using the VPR gene as target; Absolute quantification of *Brettanomyces* contamination in model wine; Accuracy evaluation of q-PCR analysis on real must and wines.

Milestone: Validation of Real-Time protocols for the detection of at least one strain of *D./B. bruxellensis* species.

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Unravelling SO_2 stress response in

***Brettanomyces/Dekkera bruxellensis* using RNA-seq.**

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Awaji Yumebutai, Hyogo, JAPAN.

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Unravelling SO_2 stress response in *Brettanomyces/Dekkera bruxellensis* using RNA-seq

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Introduction: Wine spoilage by *Brettanomyces/Dekkera bruxellensis* has increased in frequency because of the use of less-severe processing conditions, the great variety of diverse vinification tecniques and the tendency to reduce the use of preservatives, such as sulphur dioxide. Due to its antioxidant and antimicrobial effect, SO_2 is a very common additive in many foods; however, it is also known for increased allergic reactions in humans and therefore consumer pressure to reduce the levels. The capability of *B./D. bruxellensis* to survive and to grow in wine can be partially ascribed to its high resistance to SO_2, but no data are available on mechanisms involved in this metabolic trait.

Results and Discussion:
Triplicate batch fermentations were conducted in a wine-like medium using *D. bruxellensis* AWRI1499. Cells in exponential phase of growth were exposed to a molecular SO_2 concentration of 0.35 mg/L and samples were collected for RNA-Seq analysis: *i*) before the SO_2 pulse; *ii*) 5h thereafter; and *iii*) at cell growth recovery. Of the 4861 genes submitted to the gene set enrichment analysis (GSEA), 720 gene sets resulted upregulated. In particular, two distinct groups of gene sets resulted significantly enriched ($p<0.01$) in conditions *ii*) and *iii*), respectively. This result indicates that an early- and late response can be actuated by the yeast as a sequential strategy occurring under SO_2 stress. Interestingly, *SSU1*, the main mechanism of sulphite detoxification in *Saccharomyces cerevisiae*, increased its expression during the late response up to 50 times. On the other hand, the data show that a long-term response, involving the carbohydrate biosynthesis and the sulphur compound metabolism, is activated and maintained by the yeast to counteract SO_2 exposure.

KEYWORDS: *Brettanomyces/Dekkera bruxellensis*, wine spoilage, sulphur dioxide, stress response

Unravelling SO_2 stress response in *Brettanomyces/Dekkera bruxellensis* using RNA-Seq

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Yeasts for the Sustainability in Viticulture and Oenology (YeSVitE)

Introduction

Yeast spoilage in beverages has become of increasing importance because of the use of less-severe processing conditions, the great variety of diverse vinification techniques and the tendency to reduce the use of preservatives, such as sulfur dioxide (SO_2). Sulfur dioxide is widely used as an antioxidant agent and an antimicrobial compound in oenology. Nevertheless, it is known that it has allergenic effects on humans, however carcinogenicity has been not found. It would be important to reduce the quantity of SO_2 in wine to avoid a cumulative effect since it is a very common additive in many foods.

Winemaking process can involve dozen different microorganisms some of which usually produce a partial fermentation of sugars, increasing in volatile acidity and releasing of unpleasant aromas. The spoilage yeasts, showing the ability to tolerate the concentration of sulfite normally used during wine production, include *Brettanomyces/Dekkera*, *Pichia*, *Schizosaccharomyces* and *Zygosaccharomyces*. In particular, *Brettanomyces bruxellensis* can survive and grow in barrel-aged wine partially because of its high resistance to SO_2. Although a recent transcriptomic approach identified genes involved in SO_2-induced VBNC (Viable But Non Culturable) state in *B. bruxellensis* (Capozzi et al., 2016), main molecular mechanisms involved of SO_2 tolerance in this species are still unknown.

In *S. cerevisiae* sulfite is a normal metabolite produced during reductive sulfate assimilation pathway. The question of how endogenous toxicity is avoided can be linked to an efficient regulation system that would minimize pools of intermediates, sulfite among them. Metabolic and genetic studies in yeast suggest that there are at least three distinct mechanisms for protection against sulfur dioxide (Divol et al., 2012): a) an important feature of sulfite is its chemical reactivity, particularly with carbonyl groups. This fact explains why a high production of acetaldehyde by a yeast strain leads to increased sulfite resistance; (b) regarding sulfur metabolism, yeast is able to import sulfate from the medium and reduce it first into sulfite and then into sulfide to finally incorporate it into sulfur amino acids such as methionine and cysteine; (c) adenine plays a role in sulfur metabolism and sulfite resistance and its presence enhances sulfite resistance; (d) the main protein involved in sulfite resistance is the sulfite pump, encoded by SSU1 gene, that mediates sulfite efflux. Deletion of this gene leads to sulfite sensitivity. This membrane protein is regulated at the transcriptional level by the transcription factor Fzf1. Mutations in this gene also lead to sulfite sensitivity

Research Rationale

Relevance of the study: This study will increase the knowledge about the capability of *D./B. bruxellensis* to tolerate high levels of SO_2 during winemaking. The recent advances in *D./B. bruxellensis* genome sequences, becoming readily available, is allowing for a more in-depth view into this yeast.

Significance and Impact of the study: The understanding of the main molecular mechanisms involved in sulfite tolerance in *D./B. bruxellensis* will help scientists to propose innovative and sustainable approaches useful to reduce spoilage yeasts and decrease the level of sulfite in wines. As a consequence, improved procedures in the management of sulfur dioxide addition during wine production could be setup and be available for winemakers.

Experimental Plan

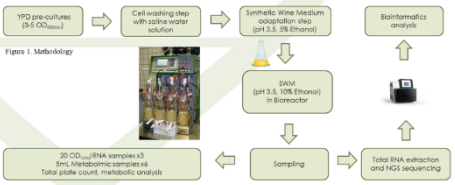


Figure 1. Methodology

Triplicate batch fermentations were conducted in a wine-like medium using *D. bruxellensis* AWRI1499. Cells in exponential phase of growth were exposed to a molecular SO_2 concentration of 0.35 mg/L and samples were collected for RNA-Seq analysis: *i*) before the SO_2 pulse (T0h); *ii*) 5h thereafter (T5h); and *iii*) at cell growth recovery (when sugar were almost exhausted, TF). Extracted RNAs underwent to a quality control throughout the evaluation of the RNA Integrity Number (RIN value>8, Agilent 2100 bioanalyzer). After library preparation, including quality control and quantification, sample were submitted to the sequencing (Illumina NextSeq 500). Bioinformatics analysis included: quality evaluation of reads (trimmed of adapters) with FastQC, reads mapping to the reference genome of *D./B. bruxellensis* AWRI1499 (hisat2), reads quantification - CUFF QUANT, normalization - CUFF NORM, average for the three replicates of FPKM (fragments per kilobase of exon per million fragments mapped) values and Δaverage FPKM VALUE within conditions filtered and correspondent genes used for GO term finder analysis.

Yeast fermentations

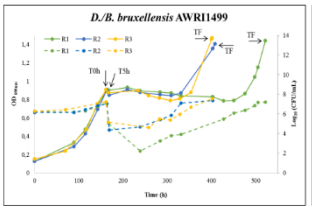


Figure 2. Kinetics of growth of the biological replicates (R1, R2 and R3). Dashed lines: effect of SO_2 treatment on culturability. Continuous lines: effect of SO2 on growth. Cultures were grown in bioreactor in SWM. T0h, before the SO_2 pulse, T5h, 5h thereafter and TF, early stationary phase.

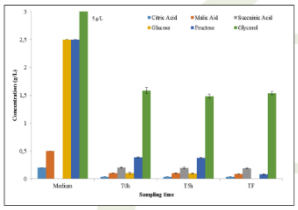


Figure 3. Dosages of sugars, glycerol and organic acids in the supernatants.

Preliminary Cellular overview

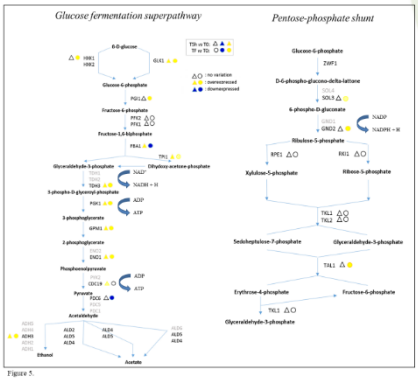
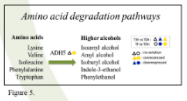


Figure 5.



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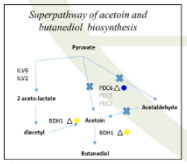

Figure 6.

Divol B. et al. Appl Microbiol Biotechnol (2012) 95:601–613
Vigentini et al. FEMS Yeast Res 13 (2013) 597–608
Capozzi V. et al. Food Microbiology 59 (2016) 1996204

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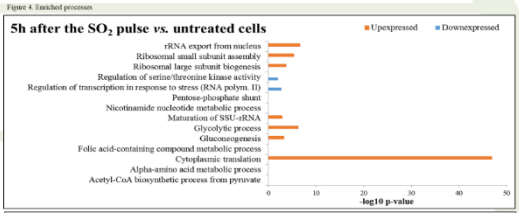
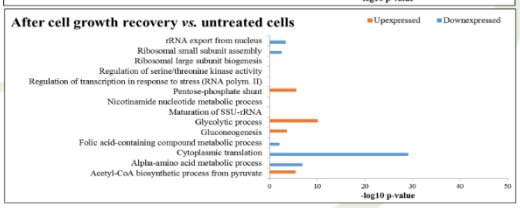
Gene Set Enrichment Analysis

Of the 4861 genes submitted to the gene set enrichment analysis (GSEA), 720 gene sets resulted differentially expressed. In particular, two distinct groups of gene sets resulted significantly enriched ($p<0.01$) in conditions *ii*) and *iii*), respectively. This result indicates that an early- and late response can be actuated by the yeast as a sequential strategy occurring under SO_2 stress. On the other hand, the data show that a long-term response, involving the carbohydrate biosynthesis and the sulfur compound metabolism, is activated and maintained by the yeast to counteract SO_2 exposure (Figure 4). Interestingly, SSU1, the main mechanism of sulfite detoxification in *S. cerevisiae*, increased its expression during the late response up to 50 times.

Figure 4. Enriched processes





Carbon metabolism results significantly affected by SO_2 exposure. In the early response (T5h), glycolysis appears mainly activated probably because of the yeast energy requirement under the occurred stress condition. A similar expression profile was observed in adapted cells, although a few genes resulted downexpressed. On the other hand, pentose-phosfate shunt is enriched in three genes of its way at TF. This could serve the cells for the generation of reducing equivalents, in the form of NADPH, for reductive biosynthesis within the cells or be useful to supply the cell of ribose-5-phosphate for the synthesis of nucleotides and nucleic acids or to improve the production of erythrose 4-phosphate, a key precursor in the synthesis of amino acids (Figure 5). Indeed, in TF the expression for the degradation of same amino acids resulted upregulated leading to a possible accumulation of superior alcohols (Figure 6). These compounds could have a role in protecting cellular components to the oxidative stress exerted by SO_2 in balancing the cellular redox. Actually, the same function could be associated to polyols in *D./B. bruxellensis* (Vigentini et al., 2013); in the condition TF, pyruvate appears deviated to butanediol production throughout acetoin (Figure 6). Moreover, this last shunt could exclude acetaldehyde as a strategy to increase the SO_2 tolerance in *D./B. bruxellensis*, thought an overproduction of acetoin could act similarly to acetaldehyde, blocking sulfite throughout its carbonyl group.

Valdetara F., Fracassetti D., Bottelli P., Foschino R., Vigentini I. (2016)

Yeast selection for the production of novel "mixed-fruit wines"

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Yeast selection for the production of novel "mixed-fruit wines"

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Introduction: Statistics of the global wine consumption has shown that Europe is consuming less and less wine and, on the market, have been appearing new alternatives of wine that are inexpensive and easy drinking, such as the "flavored" wines with low alcohol content and obtained blending wines and fruit juices or flavoring wine with artificial or natural aromas. Although there is an ancient art to obtain fruit wines (not grape-based), no literature is available regarding the production of beverages that are obtained by the co-fermentation of grape and fruit. The formulation of new mixed-fruit wines could represent the basis for reducing post-harvest fruit losses and contribute to the economy of the existing wine industry. For this purpose, the selection of useful yeasts is advised to drive the alcoholic fermentation (AF) and, meantime, to enrich the final wines in active bio-functional compounds.

Results and Discussion:
Must fermentations were carried out inoculating *Saccharomyces cerevisiae* EC1118 commercial wine yeast, in four musts obtained by blending grape must (Chardonnay and Cabernet Sauvignon) and strawberry fresh juice at two different proportion, 80:20 and 60:40. *S. cerevisiae* EC1118 was able to exhaust the available sugars in all trials in 144h from the inoculum, as a maximum. In comparison to a theoretical alcoholic degree of about 15% and 11.5% (v/v) (calculated from the initial sugar concentration of the red and white grape musts alone, respectively), a significant reduction of ethanol was obtained in the mixed-fruit wines. In particular, on the basis of grape: strawberry proportion, alcohol was 3-4.5% less in wines prepared with red grape and 6-7% (v/v) less in case of white grape. Only negligible difference was found for the pH. The total acidity was higher for mixed-fruit wines obtained with must from red grape as higher concentrations of organic acids, namely tartaric, malic and citric, were found. The higher levels of citric acid detected for the proportion 60:40 could protect the wine components, bio-functional compounds included, against the oxidation. *Torulaspora delbruekii* showed a good fermentation performance as well, suggesting its potential use as starter yeast.

KEYWORDS: *Saccharomyces cerevisiae*, non-*Saccharomyces* yeasts, wine, fruit wines

Yeast selection for the production of novel "mixed-fruit wines"

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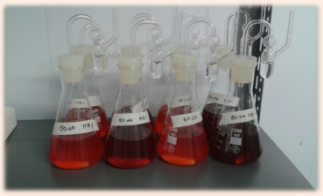
DeFENS

Introduction

Winemakers, and all the stakeholders of the wine World, must reflect in front of the global wine consumption statistics of the last fifteen years. Since 2000 the global tendency has been upwards, increasing from 226 Mhl in 2000, reached a peak at 255 Mhl in 2007 when it went down to just over 243 Mhl in 2012. In the last three years it kept stable at 240 Mhl. Europe is declining, consuming less and less wine. The traditional consumer countries resumed their downward trend, to the advantage of new consumption areas; today 40% of wine production is consumed outside of European countries, compared with 31% in 2000 [1]. On the market have been appearing new alternatives of wine that are inexpensive and easy drinking. An example is given by the "flavored" wines (red lollipop, peach, grapefruit, mandarin or black currant) with medium alcohol content (from 8% to 10.5%) and obtained blending wines and fruit juices or flavoring wines with artificial or natural aromas. The main consumers of these products are younger people between 18 to 34 years old, 33% drinking flavored wines as an aperitif. The market is at an ever-growing rate. However, there is an ancient art of fermenting fruits to obtain wines with different taste and nutritive values, providing numerous health benefits [2]. Actually, many of the fruits are consumed fresh, but some quantities of harvested fruits are wasted, overall during peak harvest periods. Therefore, winemaking from their juice could be intended as an alternative to exploit the surplus of over-ripe fruit volumes for generating additional revenues for the fruit growers. Nowadays, no literature is available regarding the production of beverages that are obtained by the co-fermentation of grape and fruit. The formulation of new mixed-fruit wines obtained from grape and surplus summer fruits in mixed fermentation processes will represent the basis for the standardization of technologies for reducing post-harvest losses and contributes to the economy of the existing wine industry.

1. General aims and specific expected results

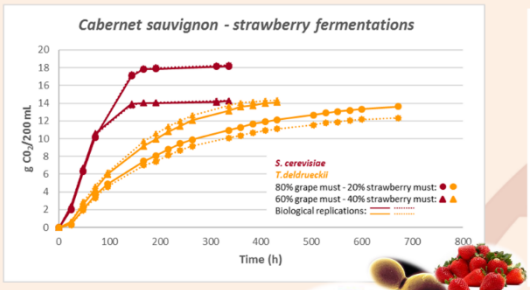
Purpose of the proposed research is to reduce post-harvest losses and surplus of summer fruits by addressing wine industry to alternative beverages. This action aims to formulate and optimize new mixed-fruit wines obtained from the co-fermentation of grape (or rectified concentrated grape must, MCR) and fruit musts. More specifically, the expected results of the project are the: i) identification of summer fruits, concerned by a constant surplus production per year, able to co-ferment with grape musts (fresh or obtained from MCR); ii) formulation of healthy products by the obtainment of low alcohol beverages enriched with phytochemical bioactive molecules; iii) assessment of consumer preferences for the potential placing on the market of final products.



2. Production of "mixed-fruit wines" in laboratory scale

The activity will involve the setup of fermentation trials using grape (or MCR) and fruit musts at different proportions. At this scope summer fruits, usually representing the major food surplus, such as peaches, strawberries, cherries, melons and watermelons will be used to prepare juices by a fruit extractor. Depending on the chosen fruit, the must will follow a different processing before the start of fermentation. The alcoholic fermentation will be controlled by the inoculation of different *Saccharomyces* and non-*Saccharomyces* yeasts with the aim of select most performant strains in terms of growth rate. Priority will be given to microbial strains able to produce bio-functional compounds such as glutathione, melatonin, etc. [3]. Standard chemical parameters (i.e. pH, ethanol, acetic acid, acetaldehyde, glycerol and other organic acids) will be evaluated. If necessary, the malolactic fermentation (MF) driven by selected strains of *Oenococcus oeni*, or other strains belonging to *Lactobacillus* species, will be exploited for the disacidification of base wines.

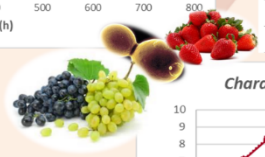
3. Grape-strawberry fermentations results



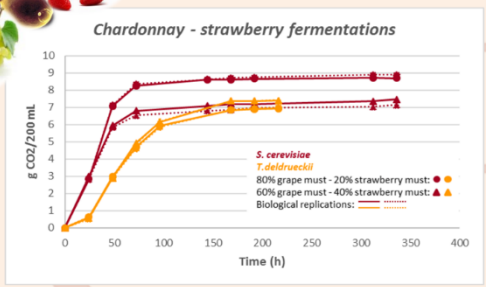
Cabernet sauvignon - strawberry musts and fermentations

	T0		AF		MF	
	80:20	60:40	80:20	60:40	80:20	60:40
pH	3.01	3.05	3.1	3.2		
	2.97	3.05	3.0	3.1		
Total acidity	5.85	6.30	7.5	6.8		
	5.10	5.25	8.8	9.5		
Tartaric acid	4.72	1.91	4.27	2.79	5.98	3.76
	8.34	4.35	4.45	2.58		
Malic acid	4.24	4.42	4.14	5.72	nd	nd
	4.14	5.12	4.91	6.43		
Lactic acid	nd	nd	nd	nd	5.07	5.02
	nd	nd	nd	nd		
Citric acid	1.63	2.72	3.60	5.09	nd	nd
	4.32	8.57	3.71	6.23		
Acetic acid	nd	nd	nd	nd	3.30	3.8
	nd	nd	1.88	2.37		
Succinic acid	Trace	Trace	2.78	3.02	4.48	4.97
	1.89	1.60	2.73	4.07		
Ethanol	-	-	10.2	7.8		
	-	-	6.7	7.12		

- Fermentations were conducted adjusting APA level to 200 mg/L
- As expected fermentations with *T. delbrueckii* went slower
- Content in ethanol were similar, within red and white most tipe, with the exeption of 80:20 combination
- Replicates fermentations show the same curves
- Acids content in tables is expressed in g/L



- T0: initial time
- AF: alcoholic fermentation
- MF: malolactic fermentation



Chardonnay - strawberry musts and fermentations

	T0		AF		MF	
	80:20	60:40	80:20	60:40	80:20	60:40
pH	3.13	3.17	3.1	3.2		
	3.08	3.08	2.9	3.0		
Total acidity	5.10	5.70	6.0	6.3		
	4.80	6.00	6.9	7.7		
Tartaric acid	2.95	2.74	2.65	1.89	3.01	2.38
	4.78	4.64	2.69	2.46		
Malic acid	4.07	4.49	4.53	4.67	nd	nd
	7.00	7.60	5.44	6.34		
Lactic acid	nd	nd	nd	nd	5.07	5.02
	nd	nd	nd	nd		
Citric acid	2.08	4.19	3.56	4.95	nd	Nd
	4.47	6.66	3.69	5.72		
Acetic acid	nd	nd	nd	nd	2.40	3.3
	nd	nd	nd	nd		
Succinic acid	1.14	1.88	3.60	3.56	4.16	4.46
	1.67	2.12	3.47	4.42		
Ethanol	-	-	5.77	4.85		
	-	-	3.98	4.04		

- Consistent acid decrese is shown after malolactic fermentation, with complete transformation of malic into lactic acid

4. Conclusions and future works

In Italy, according to ISTAT, about 3% of the total Italian agricultural production remained in the field; fresh fruit represents 10%. Indeed, when the supply is greater than demand it is possible that farmers prefer to leave the product in the field, because is more convenient than collect it in cost terms. This situation can increase the risk of plant diseases, determining the increase in chemical inputs consequently and, again, costs. The surplus of fruit production does not represent a waste but a production waste since the edibility of fruit is not impair. To transform this surplus in another product such as a mixed-fruit wine could represent a sustainable solution, reducing costs of waste management (**short-term impact**). Moreover, the global wine consumption is stable and there is an increase request for quality wines; with the introduction of this type of products the cellar could decide to process to wine only the grape responding to certain quality criteria, thus limiting the production of low quality wines potentially increasing the quote of unsold wines (**short-term impact**). The remaining grape amount could be transformed in MCR for long time storage. The social and economic context of the wine sector can also profit from this product, with growth in employment and increase of the number of the companies and supplier involved (**medium-term impact**). Finally, an increasing number of consumers will be able to find remarkable advantages in these products because there is evidence that the intake of fruit is crucial for maintaining health, probably because of the antioxidant compounds present in fruits. Fruit containing high amounts of vitamin C, vitamin E and polyphenols, may be helpful in reducing risk factors related with cardiovascular disease. Also, the release by yeasts of bio-functional compounds (glutathione, melatonin, etc.) during the fermentation can represent another economic benefit adding a value to these new products (**long-term impact**). In conclusion, the whole productive chain, from fruit growers to the consumer can benefit in economic terms from the production of mixed-fruit wines.

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[1] http://www.oiv.int/public/medias/2246/press-release-2015-bilan-vin-en-oiv.pdf
[2] Umesh, B., et al. 2015. Wines from fruits other than grapes: Current status and future prospectus. Food Bioscien, 9, 80–96.
[3] Vigentini et al. 2015. Yeast contribution in melatonin, melatonin isomers and tryptophan-ethylester during alcoholic fermentation of grape musts. J Pin Res, 58, 388-396.

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Production of novel "mixed-fruit wines" as a sustainable approach to reduce food losses

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Production of novel "mixed-fruit wines" as a sustainable approach to reduce food losses

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Statistics of the global wine consumption have shown that Europe is consuming less wine and, on the market, have been appearing new alternatives of alcoholic beverages that are inexpensive and easy drinking, such as the "flavored" wines with low alcohol content and obtained blending wines and fruit juices or flavoring wine with artificial or natural aromas. Although there is an ancient art to obtain fruit wines (not grape-based), no literature is available regarding the production of beverages that are obtained by the co-fermentation of grape and juices of other fruits. The formulation of new mixed-fruit wines could represent the basis for reducing post-harvest fruit losses and contribute to the economy of the existing wine industry. For this purpose, the proposed winemaking is kept under control in order to achieve the regular proceeding of the alcoholic fermentation (AF) and, meantime, to enrich the final wines in active bio-functional compounds.
Must fermentations were carried out inoculating *Saccharomyces cerevisiae* EC1118 commercial wine yeast, in four musts obtained by blending grape must (Chardonnay and Cabernet Sauvignon) and strawberry fresh juice at two different proportion, 80:20 and 60:40. *S. cerevisiae* EC1118 was able to exhaust the available sugars in all trials in 6 days from the inoculum, as a maximum. In comparison to a theoretical alcoholic degree of about 15% and 11.5% (v/v) (calculated from the initial sugar concentration of the red and white grape musts alone, respectively), a significant reduction of ethanol was obtained in the mixed-fruit wines. In particular, on the basis of grape/strawberry proportion, alcohol was 3-4.5% (v/v) less in wines prepared with red grape and 6-7% (v/v) less in case of white grape. Only negligible difference was found for the pH. The total acidity was higher for mixed-fruit wines obtained with must from red grape (20.0% and 7.7% more for 80:20 and 60:40 proportions, respectively) as higher concentrations of organic acids, namely tartaric, malic and citric, were found. The higher levels of citric acid detected for the proportion 60:40 grape/strawberry could protect the wine components, bio-functional compounds included, against the oxidation. *Torulaspora delbruekii* showed a good fermentation performance as well, suggesting its potential use as starter yeast.

KEYWORDS: wine, fruit wines, sustainability, winemaking, alcoholic fermentation.

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Production of novel "mixed-fruit wines" as a sustainable approach to reduce food losses

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INTRODUCTION

- ✓ The global tendency of wine consumption has been upwards, increasing from 226 Mhl in 2000, reached a peak at 255 Mhl in 2007 when it went down to just over 243 Mhl in 2012, and it kept stable at 240 Mhl in the last three years.
- ✓ The traditional consumer countries, Europe included, resumed their downward trend, to the advantage of new consumption areas; today 40% of wine production is consumed outside of European countries, compared with 31% in 2000 [1].
- ✓ On the market have been appearing new alternatives of wine that are inexpensive and easy drinking, such as the "flavored" wines with medium alcohol content (8-10.5%) and obtained by blending wines and fruit juices or flavoring wines with artificial or natural aromas.
- ✓ The art of fermenting fruits to obtain wines with different taste and nutritive values is an ancient practice and it can provide numerous health benefits [2].
- ✓ Actually, many of the fruits are consumed fresh, but some quantities of harvested fruits are wasted, overall during peak harvest periods. Therefore, winemaking from their juice could be intended as an alternative to exploit the surplus of over-ripe fruit volumes for generating additional revenues for the fruit growers, reducing post-harvest losses and contributing to the economy of the existing wine industry.

RESEARCH RATIONALE

✓ To reduce post-harvest losses and surplus of summer fruits by addressing wine industry to alternative beverages. → ✓ To formulate and optimize new mixed-fruit wines obtained from the co-fermentation of grape must and fruit juice.

AIMS

- ✓ To produce "mixed-fruit wines" with the fruits representing the major food surplus, such as strawberries, peaches, cherries, melons.
- ✓ To formulate healthy products by the obtainment of low alcohol beverages enriched with phytochemical bioactive molecules.
- ✓ To assess consumer preferences for the potential placing on the market of final products.

MATERIALS AND METHODS

Production of "mixed-fruit wines" in laboratory scale

- The alcoholic fermentation (AF) was carried out by the inoculation of *Saccharomyces cerevisiae* and *Torulaspora delbrueckii*;
- The AF trend was monitored and standard chemical parameters (i.e. pH, ethanol, total acidity, organic acids) were evaluated.
- The malolactic fermentation (MLF) was performed.

"Grape - strawberry wine"

- Strawberry juice was obtained by a fruit extractor.
- The juice was added to Chardonnay and Carbernet Sauvignon musts at two different proportion, 80:20 and 60:40 grape must:strawberry juice.
- Chemical parameters of strawberry juice (pH, total acidity, organic acids, readily assimilable nitrogen) were determined.

RESULTS – "GRAPE - STRAWBERRY WINES"

Wine produced with Cabernet Sauvignon must

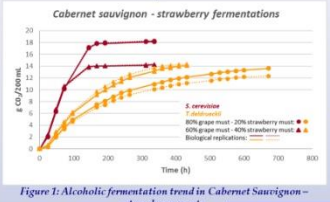

Figure 1: Alcoholic fermentation trend in Cabernet Sauvignon – strawberry musts

- ✓ The AF was complete in case of *S. cerevisiae* for both the conditions tested (80:20 and 60:40).
- ✓ As expected, *T. delbrueckii* went slowly in both cases.

Parameter	80:20		60:40	
	Must	Wine	Must	Wine
pH	3.1	3.1	3.2	3.2
Total acidity (g tartaric acid/L)	5.9	7.5	6.3	6.8
Tartaric acid (g/L)	4.72	4.27	1.91	2.79
Malic acid (g/L)	4.24	4.14	4.42	5.72
Lactic acid (g/L)	n.d.	n.d.	n.d.	n.d.
Citric acid (g/L)	1.63	3.60	2.72	5.09
Acetic acid (g/L)	n.d.	n.d.	n.d.	n.d.
Succinic acid (g/L)	trace	2.78	trace	3.02
Ethanol (%)	---	8.5	---	7.5

Table 1: Chemical parameters of Cabernet Sauvignon – strawberry musts and wines.

- ✓ The ethanol was lower than the theoretical alcoholic (about 15%).
- ✓ The total acidity was slightly higher with the proportion 60:40
- ✓ The FML was complete as no malic acid was detected; the lactic acid was 5.0 g/L in both wines.

Wine produced with Chardonnay must

- ✓ As observed for the Cabernet Sauvignon must, *S. cerevisiae* was able to completely ferment the two Chardonnay-strawberry musts.
- ✓ *T. delbrueckii* showed a higher fermentation rate.

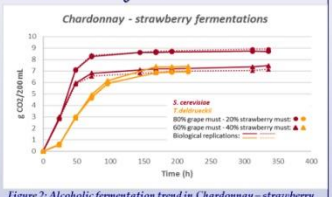

Figure 2: Alcoholic fermentation trend in Chardonnay – strawberry musts

Parameter	80:20		60:40	
	Must	Wine	Must	Wine
pH	3.1	3.1	3.2	3.2
Total acidity (g tartaric acid/L)	5.1	6.0	5.7	6.3
Tartaric acid (g/L)	2.95	2.65	2.74	1.89
Malic acid (g/L)	4.07	4.53	4.49	4.67
Lactic acid (g/L)	n.d.	n.d.	n.d.	n.d.
Citric acid (g/L)	2.08	3.56	4.19	4.85
Acetic acid (g/L)	n.d.	n.d.	n.d.	n.d.
Succinic acid (g/L)	1.14	3.60	1.88	3.56
Ethanol (%)	---	4.9	---	6.9

Table 2: Chemical parameters of Chardonnay – strawberry musts and wines.

- ✓ The ethanol was 6-7% less than the theoretical alcoholic (about 11.5%).
- ✓ Higher levels of citric acid were observed for the proportion 60:40.
- ✓ The complete FML led to 6.0 g/L and 5.3 g/L of lactic acid for the proportions 80:20 and 60:40, respectively.

CONCLUSIONS AND FUTURE PERSPECTIVES

✓The production of mixed-fruit wines can represent a sustainable solution as both the food losses and the costs for the waste management can be reduced.

✓The consumers can take advantages from these beverages as there is evidence that the intake of fruit is crucial for maintaining health, probably because of the antioxidant compounds present in fruits. The release by yeasts of bio-functional compounds (glutathione, melatonin, etc.) during the fermentation can represent another economic benefit adding a value to these new products.

✓The feasibility of mixed-fruit wines production by blending grape must and other fruits representing the major food surplus, such as peaches, cherries and melons, will be investigated.

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[2] Umesh, B., et al. 2015. Wines from fruits other than grapes: Current status and future prospectus. Food Bioscien, 9, 80-96.
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Yeasts for the Sustainability in Viticulture and Oenology (YeSVitE)

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Appendix 5

(Abstracts)

English version

Biological spoilage of wine arises from yeasts and bacteria metabolic activity. *Brettanomyces/Dekkera bruxellensis,* one of the main contaminating yeast, is able to produce unpleasant compounds, such as vinyl and ethyl phenols (VPs). These off-flavors define the so called "Brett" character. Two enzymes, the cinnamate decarboxylase (CD) and the vinylphenol reductase (VPR), are involved in the spoilage activity. Sulphur dioxide (SO_2) is the most common additive used to prevent and/or control microbial contamination in many foods, but decreasing its use is advisable both for limiting the detrimental cumulative effects on human health and improving the sustainability in winemaking. The first aim of this study was to provide a certain identification of the VPR enzyme, by cloning the gene in a species not producing ethyl phenols, such as *Saccharomyces cerevisiae*. The role of this enzyme in the conversion of 4-vinyl guaiacol into 4-ethyl guaiacol was proven by the expression, of a biologically active form of the heterologous protein. A VPR specific activity of 9 ± 0.6 mU/mg is found in crude extracts of transformed clones of *S. cerevisiae*. A his-tag purification approach allowed to confirm the results in activity trials carried out in the enriched fraction of the protein purified from recombinant cells of *S. cerevisiae*; in particular, a VPR specific activity of 1.83 ± 0.03 U/mg at pH 6.0 is measured. Furthermore, the strain-dependent character of the species regarding the VP production was investigated at sequence level in 17 different *D. bruxellensis* strains. Since the observed polymorphism (2.3%) and the allelic heterozygosity state of the gene do not correlate with the different release in off-flavors, this could indicate that transcriptional/post-translational mechanisms might affect the final production. In addition, the expression of the two genes involved in VP production was investigated as a three-factor variation response using a Response Surface Methodology approach. As first, a proper house-keeping-gene was identified to allow the analysis of different SO_2, pH and ethanol concentrations on VP production under oenological conditions. While statistical irrelevance as far SO_2 lead this to not be commented as main factor affecting *CD* expression, the linear interaction with pH and ethanol concurr to define a significant effect ($p < 0.05$) on it. Considering the permissive growth condition (0 mg/L mol.SO_2, pH 4.5 and 5% v/v EtOH), *CD* is generally downregulated. The combination of factor levels maximizing (0.83 fold-change) *CD* expression is: 0.25 mg/L mol. SO_2, pH 4.5 and 12.5% v/v EtOH. Contrariwise, *VPR* expression does not seem to be influenced by any main factor nor by their interactions, but its expression is maximized (1.80 fold-change) at the same conditions calculated for *CD* gene. Finally, the study of the genetic mechanisms involved in the SO_2 stress response of two *B./D. bruxellensis* strains (AWRI1499 and CBS2499) was carried out. In particular, a RNA-Seq based approach was applied on cells grew in a

wine-model environment. Results confirm the ability of the species of growing in such severe conditions and suggest that the environmental adaptation observed might be due to a detoxification activity that include the sulphur metabolic process. Indeed, this metabolic strategy has been observed to be one of the main mechanisms of resistance to sulphur dioxide stress in *S. cerevisiae*. A relative up-regulation of genes involved in the last step of the Sequence of Reduction of Sulfate (SRS) and a high up-regulation of *SSU1* gene (up to 4-fold and 47-fold in the CBS2499 and AWRI1499 strains, respectively), encoding a plasmamembrane sulfite pump, are observed.

Italian version

Nel vino l'attività di alcuni lieviti e batteri può portare al deterioramento del prodotto. *Brettanomyces/Dekkera bruxellensis,* uno dei principali lieviti contaminanti, è in grado di produrre composti sensorialmente sgradevoli, come vinil ed etil fenoli. Questi off-flavors definiscono il cosiddetto carattere "Brett". La produzione di questi fenoli volatili, a partire dagli acidi idrossicinnamici presenti nelle uve, avviene attraverso l'azione sequenziale di due enzimi, una cinnammato decarbossilasi (CD) ed una vinilfenolo reduttasi (VPR). L'anidride solforosa (SO_2) risulta l'additivo più diffusamente impiegato a livello alimentare al fine di prevenire e/o controllare la contaminazione microbiologica e stabilizzare il prodotto. Tuttavia, una riduzione dei quantitativi utilizzati è fortemente auspicabile sia per il suo impatto negativo sulla salute umana, sia al fine di migliorare la sostenibilità produttiva. La proteina coinvolta a livello della trasformazione dei vinil fenoli in etil fenoli è stata recentemente purificata, permettendo in questo lavoro la sua clonazione in *S. cerevisiae*, specie non produttrice di etilfenoli. Un' attività vinilfenolo reduttasica di 9 ± 0.6 mU/mg è stata osservata negli estratti cellulari dei cloni ricombinanti. La purificazione ottenuta dall'estratto grazie all' his-tag, ha consentito di confermare l'attività VPR nella frazione arricchita della proteina purificata (1.83 ± 0.03 U/mg di attività specifica misurata a pH 6.0). Inoltre, la produzione ceppo-dipendente di fenoli volatile è stata analizzata a livello di sequenza del gene VPR in 17 ceppi. Il polimorfismo genico osservato (2.3%) e lo stato di eterozigosi allelica non risultano correlate ai diversi livelli di produzione di fenoli. Questo potrebbe indicare che la variabilità nella produzione di off-flavors potrebbe essere ascrivibil a differenze a livello di meccanismi di regolazione trascrizionale. In seguito, attraverso un'analisi di risposta di superficie è stata valutata l'influenza di tre fattori (SO_2, pH ed etanolo), sull'espressione dei due enzimi coinvolti nel pathway di formazione dei fenoli volatili. Innanzitutto, è stato identificato un gene house-keeping idoneo a consentire l'analisi di differenti concentrazioni di SO_2, pH ed etanolo sulla formazione di fenoli volatili in condizini enologiche. Mentre il fattore SO_2, non risultando significativo, non può essere descritto come impattante l'espressione del gene *CD*, l'interazione lineare di pH ed etanolo determinano un effetto significativo ($p < 0.05$). considerando la condizione di crescita "permissiva" (0 mg/L mol.SO_2, pH 4.5 e 5% v/v etanolo), il gene *CD* risulta generalmete down-regolato. La combinazione dei fattori 0.25 mg/L mol. SO_2, pH 4.5 e 12.5% v/v EtOH massimizza l'espressione di entrambi i geni *CD* (0.83 fold-change) e *VPR* (1.80 fold-change). Diversamente, l'espressione del gene VPR non sembra essere influenzato da alcuno dei fattori presi in analisi, né dalla loro interazione. Infine, due ceppi di *B./D. bruxellensis* (AWRI1499 e CBS2499) sono stati sottoposti a sequenziamento dell'RNA al fine di valutare i

meccanismi di risposta allo stress da SO_2. Cellule derivanti da colture ottenute in ambiente simil-vino sono state analizzate. I risultati confermano la capacità della specie di resistere e crescere in condizioni ambientali restrittive e suggeriscono che l'adattamento osservato potrebbe essere ascrivibile ad un'azione di detossificazione che coinvolge il metabolismo dello zolfo. Questa strategia metabolica è stata osservata come uno dei principali meccanismi di resistenza allo stress da anidride solforosa in *S. cerevisiae*. Una relativa up-regolazione dei geni coinvolti nell'ultimo step della sequenza di riduzione del solfato e un'elevato incremento di espressione del gene *SSU1* (rispettivamente valutato pari a 4 e 47 volte nei ceppi CBS2499 e AWRI1499), codificante per una pompa di membrana per l'estrusione dei solfiti, sono osservate.

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