Phenotypic Investigation of Biofilm Formation and Transcriptional Analysis of Invasive Growth of Commercial Wine Saccharomyces cerevisiae

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## Contents

Al	bstra	$\operatorname{ct}$	viii	
De	Declaration statement x			
A	cknov	vledgements	xi	
Tł	nesis	overview and structure	xii	
1	Lite	rature review	1	
	1.1	Commercial wine yeast in the vineyard and winery $\ldots \ldots \ldots \ldots$	2	
	1.2	Yeast's lifestyle as multicellular communities	3	
	1.3	Biofilm formation of S. cerevisiae	4	
	1.4	Biofilm-related phenotypes in <i>S. cerevisiae</i>	7	
	1.5	Mat formation in response to nutrient availability	9	
	1.6	Regulation of mat formation, filamentous growth and invasive growth	10	
	1.7	Cell-cell communication in morphological transitions	11	
	1.8	Quorum sensing in yeast	11	
	1.9	Hydrogen sulfide is a potential cell-cell signalling molecule in $S.$ cerevisiae	13	
	1.10	Research questions and objectives	14	
<b>2</b>	Wir	ne yeast biofilms	15	
	Cont	cextual statement	15	
	Stat	ement of Authorship	16	

Man	(mats)	: Evaluation of the ability of commercial wine yeasts to form biofilms and adhere to plastic: implications for the microbiota of the winery	
	enviro	nment	18
2.1	Abstra	act	19
2.2	Keywo	ords	19
2.3	Introd	luction	19
2.4	Mater	ials and Methods	21
	2.4.1	Yeast strains and media	21
	2.4.2	Mat formation assays	23
	2.4.3	Vitality and nuclear staining	24
	2.4.4	DNA preparation and PCR conditions	24
	2.4.5	Mat culture harvest and total RNA extraction	25
	2.4.6	Quantitative real-time PCR	25
	2.4.7	Plastic adhesion assays	26
	2.4.8	Winery hose adhesion assays	27
2.5	Result	S	27
	2.5.1	Prototrophic diploid $\Sigma$ 1278b as a laboratory reference	27
	2.5.2	Wine yeasts display diverse mat architectures	31
	2.5.3	Cell morphologies in the mat rim and mat body reveal distinct lifestyles	31
	2.5.4	Some wine strains grow invasively at the start of mat formation $\ .$ .	31
	2.5.5	Wine strain L2056 forms mats with a more rapidly expanding sector	34
	2.5.6	Plastic adhesion	36
	2.5.7	Wine yeast grow invasively and conduct fermentation on grape pulp soft agar	37
	2.5.8	Wine strain L2056 forms initial attachment on winery hose soft plastic	38
2.6	Discus	ssion	39

	2.7	Fundi	ng	41
	2.8	Ackno	wledgements	42
	2.9	Supple	ementary Data	42
		2.9.1	Methods	42
		2.9.2	Figures	43
3	Mat	t forma	ation in a low nitrogen medium	49
	Con	textual	statement	49
	Stat	ement o	of Authorship	50
	Man	uscript	Factors influencing filamentous and invasive growth of yeast cells in	
		mat fo	prmation in a low nitrogen environment	52
	3.1	Abstra	act	53
	3.2	Keywo	ords	53
	3.3	Introd	uction	53
	3.4	Mater	ials and Methods	54
		3.4.1	Yeast strains and media	54
		3.4.2	SLAD mat assays	56
		3.4.3	Microscopy imaging and image processing	56
		3.4.4	Conditioned medium mat assays	56
		3.4.5	Nitrogen and glucose measurement	57
	3.5	Result	S	57
		3.5.1	Nitrogen limitation induces filamentous and invasive growth in mats	57
		3.5.2	Mat size and biomass increases with increasing ammonium sulfate .	60
		3.5.3	Filamentous growth is inhibited by a neighbouring mat $\ . \ . \ .$ .	60
		3.5.4	Conditioned medium affects cell elongation in liquid culture but not invasive growth	61

		3.5.5	Effect of aromatic alcohols, ethanol, hydrogen sulfide and sulfite on	c o
			yeast growing on SLAD mat assays	02
	3.6	Discus	sion	66
	3.7	Fundi	ng	69
	3.8	Ackno	wledgements	69
	3.9	Supple	ementary Data	69
		3.9.1	Methods	69
4	Uno	lerstar	nding wine yeast invasive growth through transcriptional	
	ana	lysis		72
	Con	textual	statement	72
	Stat	ement o	of Authorship	73
	Mar	uscript	: Transcriptional analysis of invasively growing wine strains of	
		Sacche	nromyces cerevisiae	75
	4.1	Keywo	ords	76
	4.2	Summ	ary	76
	4.3	Introd	uction	76
	4.4	Result	s and Discussion	78
		4.4.1	Global change in gene expression between surface and invasively	
			growing cells	78
		4.4.2	Glucose import	80
		4.4.3	Carbohydrate metabolism / fungal-type cell wall organisation $\ . \ .$	80
		4.4.4	Medium-chain fatty acid biosynthesis pathway	81
		4.4.5	Genetic interaction network analysis predicts genes modulating invasive growth	82
		4.4.6	Protein interaction network analysis suggests Ssa2p as the major determinant of invasive growth	84
		4.4.7	Expression levels of transcription factor genes do not correlate with their previously reported involvement in invasive growth	86

		4.4.8	Cellular water homeostasis: aquaglyceroporin gene <i>FPS1</i> is required for invasive growth	88
	4.5	Conclu	usions	89
	4.6	Exper	imental Procedures	90
		4.6.1	Yeast strains	90
		4.6.2	Genomic DNA preparation and PCR conditions	90
		4.6.3	Low nitrogen invasive growth assays	91
		4.6.4	Sample harvest and RNA extraction	91
		4.6.5	RNA sequencing and analysis	92
		4.6.6	Network analysis	92
	4.7	Ackno	wledgements	93
	4.8	Suppo	rting Information	93
		4.8.1	Tables	93
		4.8.2	Figures	97
5	Con	clusio	ns	99
	5.1	Summ	ary of findings	99
	5.2	Contri	bution to knowledge	00
	5.3	Limita	ations and future directions	02
$\mathbf{A}_{j}$	ppen assa	dix A iys	Method development for mat formation and plastic adhesion	04
	A.1	Mat fo	prmation assays	04
		A.1.1	Reproduction of results by Reynolds and Fink (2001) and test mat formation ability of commercial wine yeast strains	04
		A.1.2	Evaluation of medium preparation methods for mat assays $\ldots \ldots 1$	05
		A.1.3	Evaluation of mat inoculation with cells at exponential growth phase1	07
	A.2	Plastic	e adhesion assays	08

A.2.1	Refinement of staining and washing methods
A.2.2	Determination of the maximum absorption of Crystal Violet 109
Appendix B	Method development for mat formation assays in a low
nitrogen n	nedium (SLAD) 111
B.0.1	Determination of inoculation rate
B.0.2	Preliminary study on the effect of sulfide on mat formation in ${\rm SLAD116}$
Appendix C	Attempt to construct $\triangle aqy1$ in AWRI796119
C.0.1	Transformation with homologous recombination
C.0.2	Construction of $Kan {\rm MX}$ gene replacement casset te from a plasmid . 120
Appendix D	Supporting information for Chapter 4 121
Bibliography	146

## Nomenclature

Term	Description	
Biofilm	Surface-attached multicellular communities with an extracellular matrix including any related biofilm-forming ability tests such as mat formation and plastic adhesion	
Mat	Thin layer of yeast biomass on low-density agar that resembles a film	
Filamentous growth	h Interchangeable with pseudohyphal growth, a form of growth as a colony that has a filamentous shape, usually contains chains of elongated cells	
Filamentous mat	A mat that has a filamentous periphery	
Invasive growth	A form of growth that penetrates agar	
Hub and spokes' mat A flat mat that has raised cables radiating from the hub		

 ${\bf NB}$  Filamentous growth and invasive growth are not ploidy-specific unless specified.

## Abstract

This study investigated the morphological properties, environmental effects on and gene expression of biofilms, more specifically referred to as mats, formed by laboratory and commercial wine strains of *Saccharomyces cerevisiae*. Two morphological assays were conducted: mat formation and plastic adhesion. Mat features varied between strains and included various architectures, cellular morphologies, and incidence of invasive growth. One commercial strain, L2056, formed mats where a sector produced a distinctive mat morphology, which was retained when subcultured. In considering the role of biofilms in winery conditions, mat formation assays were also performed with grape pulp and adhesion to the soft plastic of common winery hoses. All strains grew invasively on all agar media and appeared to conduct fermentation on the grape-pulp mat assay. Some strains also had the ability to adhere to winery hose plastic. When only limited nitrogen was available, both laboratory and commercial wine strains formed mats with a subpopulation of cells that switched to filamentous and invasive growth. Such invasive growth was influenced by nitrogen concentration, the presence of a neighbouring mat, and by the addition of yeast metabolites. Ethanol and hydrogen sulfide were found to enhance invasive growth of cells within mats exposed to low levels of nitrogen whereas tryptophol and 2-phenylethanol suppressed this enhancement. Sulfite was found to delay overall mat growth. In an effort to understand the cellular decision to switch morphology, changes in the transcriptome of invasively growing cells were studied. In this analysis, 272 genes were identified to be upregulated and 84 genes were downregulated in invasively growing cells. Of the ten largest differentially expressed genes, four were genes encoding hexose transporters (HXT3, HXT4, HXT6 and HXT7) which had an increase in transcript abundance up to 13-fold. One hypothetical gene (AWRI796\_5153) with a 6-fold increase in transcript abundance, has translation sequence homologous to an amidase domain. Following differential expression and Gene Ontology analysis, five GO categories represented the 37 significantly enriched GO terms in the upregulated gene set of invasively growing cells, these being glucose import, carbohydrate metabolic process, fungal-type cell wall organisation, medium-chain fatty acid biosynthetic process and cellular water homeostasis. Since cellular water homeostasis has not previously been associated with invasive growth, and four out of five genes in this group were found to be significantly upregulated in the invasively growing cells, further analysis of deletion mutants of each of these confirmed that FPS1, encoding the glycerol export protein, is required for invasive growth of yeast mats in low nitrogen conditions. In summary, this work reports the phenotypic properties of commercial wine yeast biofilms in

environments of both rich nutrient and low nitrogen, either in typical laboratory type agar media or in conditions simulating that of a grape or wine hose. The ability of these yeasts to form complex morphologies, grow invasively into grape solids and attach to winery hose plastic may confer their residency and survival in the vineyard and winery. The influence of different yeast metabolites and transcriptional changes in invasively growing cells provide further understanding of this morphogenetic program.

## **Declaration statement**

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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## Thesis overview and structure

Purposefully inoculated fermentations using commercial wine yeast are broadly implemented due to their success in completing fermentation efficiently and producing quality wine. Many commercial wine strains, usually *Saccharomyces cerevisiae*, were originally isolated from indigenous microflora of successful fermentations. These strains are now produced commercially and are widely available. Evidence suggests that the use of commercial strains leads to their presence and survival in the vineyard and winery. This could potentially lead to an alteration in native microflora in must and subsequently influence the regional character of wine. The mechanism of how these commercial yeasts remain in the winery environment is barely understood.

Biofilm formation is considered a survival strategy for many fungi and bacteria under harsh conditions. *S. cerevisiae* has been reported to be able to form biofilms, evidenced by the ability to grow into a mat and to adhere to plastic. These abilities have been investigated in not only laboratory strains, but also some clinical yeasts and yeasts isolated from wine grapes and must. It is likely that commercial wine yeasts also possess the ability to form biofilms which could drive their residency in the wine making environment. Current knowledge on yeast biofilms has focussed on the laboratory strain  $\Sigma$ 1278b which is not directly applicable to the understanding of wine yeast biofilms since they are substantially genetically different. The study of biofilm formation by commercial wine yeast and their characteristics is therefore warranted.

Given that biofilms are a multicellular growth form, and nitrogen is known to be essential for yeast proliferation, biofilm formation could be affected by nitrogen availability. Lack of nitrogen has been shown to induce a pseudohyphal (filamentous) and invasive growth response in yeast. Filamentous mats can be formed when cells are starved for glucose but the mat formation response to low nitrogen has not been reported. Filamentous and invasive growth responses can be manipulated by other environmental triggers and putative signalling molecules such as temperature, pH, atmosphere, preservatives and fusel alcohols. Studies have shown that cell-cell communication can occur in biofilm formation, but the involvement of this system in yeast, especially *S. cerevisiae* biofilms is poorly understood.

In a quest to understand wine yeast biofilms in greater details, the present study has three aims:

- 1. Investigation of commercial wine yeast biofilm-forming ability and characteristics;
- 2. Exploration of mat formation in low nitrogen conditions and the influence of putative quorum sensing or signalling molecules; and
- 3. Study the genetic regulation of wine yeast biofilms or related phenotypes.

This dissertation has been organised in several chapters to present background information, reports of studies to answer each of the aims, and a conclusion. Chapter 1 establishes the field of knowledge and summarises critical gaps for the present study. Areas of discussion include yeast biofilms and related morphological phenotypes, influence of nitrogen availability, and potential signalling molecules. Chapter 2 presents mat characteristics and plastic adhesion properties of commercial wine yeast strains which addresses Aim 1. Chapter 3 addresses Aim 2 where data involving mat formation of commercial wine yeast strains in a low nitrogen environment and the response to potential signalling molecules is presented. Based on the findings from Chapter 3 that describe invasive growth as the primary observation in low-nitrogen, Chapter 4 presents the investigation of transcriptional changes of invasively growing wine yeast. Chapter 5 is a summary of the main findings including how this work contributes to current knowledge, limitations and future directions of the work.

Chapters 2–4 are presented as unsubmitted work prepared in manuscript style. For consistency, the typing, format, and referencing styles have been adjusted. Numbering of figures and tables has also been modified according to the order in the dissertation. References from all chapters can be found in the Bibliography.

## Chapter 1

## Literature review

### **Contextual statement**

This literature review was mostly written within the first six months of candidature and only covers the literature up to August 2014. The purpose of this literature review was to provide the background information and establish a theoretical framework for this PhD project. For a more updated literature, please refer to the introduction section in Chapters 2 to 4.

### 1.1 Commercial wine yeast in the vineyard and winery

Traditional wine production takes advantage of indigenous yeasts in grape must and on winery equipment to carry out fermentation, *i.e.* uninoculated or indigenous fermentation as opposed to the relatively modern practice of purposeful inoculation with commercial wine yeast strains. The surface of intact grapes is predominantly colonised with species of Kloeckera, Hanseniaspora, Candida, Cryptococcus, Rhodotorula, Pichia, Kluyveromyces, and Hansenula, which usually dominate the early stage of alcoholic fermentation (Fleet and Heard, 1993). The composition of microflora on grapes fluctuates depending on climatic influences, soil and viticulture practices, grape variety, the incidence of physical damage of grapes, and potential insect vectors (Mortimer and Polsinelli, 1999; Pretorius et al., 1999; Stefanini et al., 2012). The yeast flora of must is further influenced by harvest method, transport time, grape temperature, and must treatment (Pretorius et al., 1999). Yeasts resident on winery equipment are likely introduced through contact with grapes, use of commercial starter cultures, and use of oak barrels with the source varying depending on the surface nature of equipment and cleaning and sanitisation practices (Martini, 1993; Ciani et al., 2004; Goddard et al., 2010). As the fermentation progresses, conditions usually favour the growth of Saccharomyces cerevisiae, which often becomes predominant at the later stage of the process. Due to a variety of factors that influence the microflora in must, the microbial composition of an uninoculated fermentation is highly variable between fermentations, vintages and subsequently is unpredictable.

The capacity to control and predict fermentation aids winemakers to produce quality products. This has led to the common use of commercial starter culture preparations. A large selection of wine yeast is manufactured in an active dry form and is available to inoculate grape must. For large-scale wineries, this method is beneficial since the use of commercial cultures is more likely to produce consistent, reliable and predictable outcomes. In addition to ethanol and nutrient stress tolerance, commercial wine yeast are also selected based on organoleptic properties such as desirable concentrations of organic acids and volatile thiols and little to no production of compounds generally considered as faults, such as hydrogen sulfide.

The use of commercial wine yeast for inoculation has become common practice since the second half of the twentieth century, however, there are some wineries that continue to use the traditional uninoculated method. This is mainly a result of the belief that the regional character will be retained in the wine, due to the presence of indigenous microbes from the region specific to the grapes (rather than inoculation with a purchased commercial yeast). An alternative that some winemakers choose is to use a reduced amount of a single culture or perform mixed culture inoculation with non-*Saccharomyces* species. Some studies report benefits of this practice, such as enhancing wine quality and complexity (Comitini et al., 2011; Azzolini et al., 2012; Gobbi et al., 2013). More specifically, the mixed culture fermentation of *Lachancea thermotolerans* and *S. cerevisiae* reduced pH and enhanced the production of 2-phenylethanol and glycerol, and the differences were also detected in sensory analysis (Gobbi et al., 2013). Also, the combination of *Metschnikowia pulcherrima* and *S. cerevisiae* enhanced medium-chain fatty acid, 2-phenylethanol and isoamyl acetate production and increased polysaccharide content in wine (Comitini et al., 2011). Furthermore, the use of *Torulaspora delbrueckii* with *S. cerevisiae* enhanced the aroma of Amarone wine by affecting alcohol, fermentative ester, fatty acid and lactone content (Azzolini et al., 2012).

The frequent use of commercial wine yeast in wineries may result in survival of residual yeast on winemaking equipment, or in wine lees (which is often disposed of in close proximity) even after sanitation. The impact of these yeast in the winery and vineyard ecosystem remains unknown. There is evidence that traces of commercial wine yeast are found in proximal vineyards. Three out of 13 such sites recovered commercial yeasts isolated from vineyards in the coastal regions of Western Cape, South Africa (Van der Westhuizen et al., 2000). A three-year study of Portuguese and French vinevards found that dissemination of commercial yeast was primarily restricted to short distances, with 94% of recovered commercial yeasts at 10 to 200 m from the winery (Valero et al., 2005). However, the population varied from year to year. Historical use of commercial yeast can also affect yeast microflora in uninoculated fermentation. For instance, a study spanning seven years of uninoculated fermentations in a winery that had routinely used commercial strains to inoculate fermentations reported that eight out of ten of the dominant yeasts isolated were commercial strains that had previously been used in the winery (Blanco et al., 2011). Furthermore, S. cerevisiae, the common commercial wine yeast, has been found inhabiting the winery surfaces prior to harvest (Bokulich et al., 2013). Residual commercial yeast can therefore survive in a winery or vineyard, and may consequently affect wine styles relying on fermentation with indigenous microflora. Information on how these yeast behave in a winery environment and mechanisms supporting their survival is limited.

### 1.2 Yeast's lifestyle as multicellular communities

Yeast cells experience many and varied nutritional challenges in both nature and industry where competition for nutrients leads to the selection of fitter individuals. For example, during alcoholic fermentation, nutrients are utilised and some, such as assimilable nitrogen, become limiting especially in high sugar and low nitrogen content grape juices. In other cases, yeast cells encounter physical or chemical stresses, such as high osmolarity, low pH and the presence of toxins (Bauer and Pretorius, 2000). In response to these environmental challenges, yeast often undergo morphological changes and develop diverse structured multicellular communities (Brückner and Mösch, 2012). The advantages of being in such a community are:

- (a) having better protection from a harmful environment,
- (b) having enhanced survival due to differentiation into specialised cell types, and
- (c) the provision of nutrients to the surviving cells in the community when supplies are limited (Palková and Váchová, 2006).

The architecture of multicellular communities can be influenced by their environment. For example, some industrial yeast cells flocculate after completing alcoholic fermentation and they can either form a sediment as flocs or float on the liquid surface as flors (Martínez et al., 1997; Verstrepen et al., 2003). Yeast can also form biofilms, which can be defined as a structured cell aggregate that is attached to a solid or semisolid surface and is encased by an extracellular matrix (Ramage et al., 2005; Zara et al., 2005). Previous investigations of yeast biofilms focussed on clinical implications, such as biofilms of *Candida spp.*, due to their association with virulence, pathogenesis and impact on human infections. *S. cerevisiae* also possesses common features of yeast and bacterial biofilms (Parsek and Singh, 2003; Hasan et al., 2009; Bojsen et al., 2012). Since *S. cerevisiae* is both a key industrial organism and one of the standard models of eukaryotic cellular biology, due to its genomic tractability, it is the preferred model to study mechanisms underlying yeast biofilm formation.

### 1.3 Biofilm formation of S. cerevisiae

Biofilms are communities of microorganisms attached to a surface, surrounded by selfproduced extracellular matrix, with the formation involving cell-surface and cell-cell interactions (O'Toole et al., 2000). The cycle of biofilm development consists of initial surface attachment, colonisation, biofilm maturation and cell detachment (Fig. 1.1; O'Toole et al., 2000).



Figure 1.1: Model of biofilm development cycle. Figure adapted from O'Toole et al. (2000).

Biofilm forming ability of *S. cerevisiae* was first reported by Reynolds and Fink (2001) where they demonstrated that *S. cerevisiae* was able to form a flat mat with 'hub and spokes' structure covering a large area across the surface of a YPD low-density agar medium (Fig. 1.2; Reynolds and Fink, 2001).



Figure 1.2: A mat formed by a haploid *S. cerevisiae*  $\Sigma$ 1278b strain on a 0.3% agar YPD plate after 13 days at 25 °C. Figure adapted from Reynolds and Fink (2001).

The group also showed that S. cerevisiae was able to adhere to plastic and adhesion was enhanced when cells were grown in low glucose conditions, indicating a nutrient-mediated response (Reynolds and Fink, 2001). Adhesion is an indication of the first step in biofilm development in pathogenic yeast and bacteria (O'Toole et al., 2000). Mat formation is thought to be similar to the ability to 'slide' on a low-density agar medium observed in non-flagellated *Mycobacterium spp.* which has a close connection with biofilm formation (Martínez et al., 1999; Recht et al., 2000). This form of translocation requires cell surface glycopeptidolipids. Similarly, both structured mat formation and plastic adhesion require Flo11p, a yeast adhesion-related cell surface glycoprotein. When *FLO11* was deleted, yeast cells adhered poorly to plastic and formed a poorly spreading mass of cells without complex structure (Reynolds and Fink, 2001). In this report, the term "biofilm" will be used to describe all forms of surface-attached multicellular communities with extracellular matrix and any related biofilm-forming ability tests such as adhesion, whereas "mat" refers specifically to the thin layer of yeast biomass that resembles a film on low-density agar.

Following the discovery of *S. cerevisiae*'s ability to form biofilms, many studies have investigated genes affecting mat formation and the regulatory pathways involved. Numerous signalling pathways have been identified that regulate the expression of *FLO11* (Fig. 1.3; Brückner and Mösch, 2012). Briefly, these include the mitogen-activated protein kinase (MAPK) pathway, the cAMP-PKA pathway, the SNF pathway, and the RIM pathway. A handful of *FLO11*-independent pathways have also been reported, such as the multivesicular body protein sorting pathway, the cell wall integrity pathway that is not via MAPK cascade, and the molecular chaperone of 70 kDa heat shock proteins (Martineau et al., 2007; Sarode et al., 2011, 2014).



Figure 1.3: Regulation of FLO11 expression. Arrows indicate positive regulation and inhibition is shown by bars. Figure adapted from Brückner and Mösch (2012).

So far, these S. cerevisiae biofilm studies have mainly been investigated in common laboratory strains. Wine strains of S. cerevisiae are known to be genetically different compared to laboratory strains, and this can affect their phenotypes (Borneman et al., 2008, 2011). The ability to form biofilms seems to be an inherent property for most microbes to survive in natural challenging environments (Costerton et al., 1995). Wine yeast are the dominant yeasts historically found in fermenting grape musts and where they have undergone natural selection driven by the conditions in grape juice fermentation (Querol et al., 1994). This may include the ability to form biofilms. Casalone and colleagues (2005) reported the ability of nine wine yeast isolates from grape and must to form mats that varied in size. S. cerevisiae has also been isolated from a mixed population biofilm developed on the rotating biological contactor disc in winery wastewater systems (Malandra et al., 2003). During the wastewater treatment process, industrial yeasts including S. cerevisiae adapted to the aerobic conditions in wastewater, combined with bacteria forming mixed population biofilms on the surface of the disc while degrading organic compounds (Malandra et al., 2003). This formation is beneficial because it can reduce the chemical oxygen demand of wastewater and bulking problems (Andreottola et al., 2005). The mechanism of this development is not completely understood. Therefore, an investigation of wine yeast biofilm formation is important within the context of the wine industry.

Biofilm formation might also serve as a model of survival to contribute to the understanding of how wine yeast respond to nutritional status such as during the course of fermentation. Even though *S. cerevisiae* is the principal organism for alcoholic fermentation, it is not usually prevalent on healthy intact grape berries, instead, it is commonly found on damaged grape berries and in the winery (Mortimer and Polsinelli, 1999; Bokulich et al., 2013). Damaged berries allow wine yeast to access rich nutrients from the semisolid grape interior, and hence encourage colonisation. An understanding of the successful yeast colonisation and behaviour on grapes would also be of great interest. More importantly,

the understanding of biofilm formation of commercial wine yeast could serve as the possible mechanisms for their residency and survival in the vineyard and winery.

### 1.4 Biofilm-related phenotypes in S. cerevisiae

In *S. cerevisiae*, the cell surface glycoprotein, Flo11p, required for mat formation and plastic adhesion, is also required for other phenotypes, such as invasive and filamentous growth (Lambrechts and Bauer, 1996; Lo and Dranginis, 1998). Invasive growth involves growing cells penetrating an agar medium. Haploid yeast cells have been shown to grow invasively in response to glucose depletion, which is termed haploid invasive growth (Cullen and Sprague, 2000). The authors also observed a filamentous morphology containing elongated cells that had a unipolar budding pattern associated with invasive growth (Fig. 1.4; Cullen and Sprague, 2000, 2002). Palecek and colleagues (2000) further showed that unipolar budding with or without cell elongation was sufficient to promote invasive growth.



Figure 1.4: (A) Microcolonies forming from single cells of  $\Sigma 1278b$  on Synthetic Complete medium lacking glucose after 24 h visualised by light microscopy at 200× magnification. Inset image represents an example of a microcolony formed in the presence of glucose. (B) A microcolony grown on SC medium lacking glucose visualised perpendicular to the plane of agar invasion by light microscopy. All images are at the same scale, with scale bar in (A) representing 40  $\mu$ m. Figure adapted from Cullen and Sprague (2000).

Diploid cells have been shown to respond to nitrogen depletion, by growing into colonies with filamentous form where the filamentous regions contained chains of elongated cells growing away from the colony (Fig. 1.5; Gimeno et al., 1992). This morphology is similar to the hyphae formed by filamentous fungi, and therefore has been termed diploid pseudohyphal growth. The authors also reported that cells of diploid pseudohyphal growth can invade into agar.



Figure 1.5: (A) Filamentous colonies formed by diploid cells of CGX19, a strain congenic to the  $\Sigma$ 1278b genetic background, on a low nitrogen medium after 11 days of incubation. Scale bar, 0.2 mm. (B) A high magnification view of a filamentous colony of the same strain on a low nitrogen medium after 2 days of incubation. Scale bar, 30  $\mu$ m. Figure adapted from Gimeno et al. (1992).

Filamentous growth (pseudohyphal growth) is a complex morphogenetic differentiation program that is tightly controlled with cell polarity, cell cycle and cell adhesion (Cullen and Sprague, 2012). At the start of a new cell cycle,  $G_1$ , cell polarity is determined by bud-site-selection proteins, which will direct bud growth (Park and Bi, 2007). Haploid cells bud in an axial pattern whereas diploid cells bud in a bipolar pattern (Chant and Pringle, 1995). When a cue to switch to filamentous growth is encountered, cells of both ploidy switch to a distal-unipolar budding pattern through Bud8p localisation (Gimeno et al., 1992; Harkins et al., 2001; Cullen and Sprague, 2002). When a bud emerges, actin cables extend into the bud and the bud grows apically (Pruyne and Bretscher, 2000). When nutrition is limiting, apical growth is prolonged at  $G_2$  phase, resulting in elongated cells (Kron et al., 1994). At the end of the cell cycle, cell adhesion proteins, such as Flo11p, enable cells to remain attached (Guo et al., 2000; Halme et al., 2004). Given that this differentiation occurs in response to nutrient limitation, it is widely believed that this represents a nutrient foraging response. Since nutrient fluctuation occurs in the winery environment, filamentous growth may also be employed for persistence and nutrient search.

Another biofilm-related phenotype is complex colony morphology or structured morphology from fluffy colonies, which also requires Flo11p (Granek and Magwene, 2010; Šťovíček et al., 2010). Fluffy colonies are raised but not smooth and have an aerial morphology. Colonies of such type produced a protective extracellular matrix, were more agar-adhesive, and may contain pseudohyphae and agar invasion ability (Šťovíček et al., 2010; Váchová et al., 2011). The extracellular matrix contained a glycoprotein of molecular weight > 200 kDa that is not related to flocculins (Kuthan et al., 2003). A genome-wide transcriptome analysis showed that the fluffy formation involved cell wall remodelling, secretion and modification of cell wall/membrane proteins, amino acid metabolism and nutrient transport (Šťovíček et al., 2014). This formation that harbours multiple transcriptomic and phenotypic modulations is seen as a protective mechanism to survive in the wild environment. Similar traits may be part of biofilm formation to support colonisation.

# 1.5 Mat formation in response to nutrient availability

Most mat formation studies of *S. cerevisiae* have been conducted in rich media. However, regulation of the key gene in mat formation, *FLO11*, involves pathways that are dependent on nutritional signals including glucose and nitrogen (Fig. 1.3). The impact of glucose on mat formation has been reported. For instance, formation of the 'hub and spokes' structure on mats was delayed if glucose concentration was increased (Reynolds et al., 2008). In contrast, *S. cerevisiae* formed a filamentous mat, a mat that has filamentous periphery, on a glucose-limiting medium (Fig. 1.6; Karunanithi et al., 2012). It appeared that both 'hub and spokes' structure on mats and filamentous growth are impacted by glucose availability. It is unclear if filamentous growth contributed to the 'hub and spokes' formation.



Figure 1.6: (A) Mats formed by  $\Sigma 1278b$  on 0.3% agar YEPD media (top panel; containing glucose) and 0.3% agar YEP media (bottom panel; lacking glucose). Photographs were taken after 4 days (YEPD) and 15 days (YEP) of incubation. Scare bar, 1 cm. (B) Microscopic examination of mat perimeters in (A). Scale bar, 100  $\mu$ m. Figure adapted from Karunanithi et al. (2012).

Nitrogen is well known to be essential for yeast growth, and therefore required for wine yeast to function efficiently for alcoholic fermentation (Bell and Henschke, 2005). Inadequate nitrogen in the second half of oenological fermentations is common and often results in stuck or sluggish fermentation. Yeast readily utilise all available nitrogen in a must fermentation, and thus they would be commonly exposed to a nitrogen-depleted environment. Since a mat is formed by expansion through the continuous cell division from the mat edge, nitrogen, or lack thereof may be an important factor for this expansion. This nutrient has not been thoroughly investigated for its influence on mat formation, although a few studies demonstrated that nitrogen limitation induced filamentous and invasive growth (Gimeno et al., 1992; Casalone et al., 2005; Chen and Fink, 2006). Filamentous and invasive growth can be affected by other metabolites or environmental factors. For example, fusel alcohols, by-products of amino acid catabolism via the Ehrlich pathway, have been shown to stimulate hyphal-like elongated cells, which could lead to multicellular filamentation (Dickinson, 1994, 1996). Other environmental factors that have been reported to affect invasive growth included salt, preservatives, pH, temperature, and modified atmosphere (Zupan and Raspor, 2010). This wide variety of stimulators suggest that there are many factors and biological pathways affecting this mode of growth and these may be synergetic or antagonistic in the presence of multiple stimulators.

# **1.6** Regulation of mat formation, filamentous growth and invasive growth

Although mat formation, filamentous growth and invasive growth require Flo11p and some shared core signalling pathways, large-scale studies have found specific genes regulating each phenotype (Jin et al., 2008; Ryan et al., 2012; Shively et al., 2013). To identify genes necessary for filamentous growth induced by butanol in S. cerevisiae, Jin and colleagues (2008) screened a library of 3,627 transposon insertion gene disruption constructs and 2,043 overexpression constructs in a haploid version of filamentous strain  $\Sigma$ 1278b. Of 487 genes identified as being necessary for filamentous growth, 243 were also necessary for haploid invasive growth. To study genes required for mat formation, filamentous growth as well as invasive growth, Ryan and colleagues (2012) screened both haploid and homozygous diploid  $\Sigma$ 1278b gene deletion libraries. The group identified 688, 600 and 577 genes required for mat formation in rich nutrient low-density agar medium, diploid pseudohyphal growth in nitrogen-limited medium, and haploid invasive growth in rich medium, respectively. Haploid invasive growth and mat formation were found to be significantly correlated, with 300 genes common to both phenotypes. Sixty-one core genes required for all three phenotypes, included *FLO11* and a number of genes encoding proteins involving in the regulation of FLO11 gene expression. These were components of the Rpd3L histone deacetylase complex, members of the Rim101 signalling pathway, and transcription factors for FLO11, Mit1p, Tec1p, Flo8p, and Mss11p. In contrast, Shively and colleagues (2013) found 551 genes that when overexpressed exaggerated diploid invasive growth in sufficient nitrogen and highlighted the potential role of nuclear Hog1p in repressing invasive growth. The findings from these studies suggest that each of these growth transitions have unique regulatory networks and these may be different for haploid and diploid cells.

### 1.7 Cell-cell communication in morphological transitions

Cell-cell communication via signals could lead to morphological changes and contribute to yeast community organisation (Honigberg, 2011). Intercellular communication such as quorum sensing (QS) is responsible for group motility, biofilm formation and maintenance, and production of virulence factors in bacteria (Miller and Bassler, 2001; Sperandio et al., 2002). This is similar to the communication system that controls physiological functions in multicellular organisms.

Cell-cell communication involves a chemical signalling molecule, secreted by one cell and sensed by another to trigger diverse behaviours (Youk and Lim, 2014). A wellstudied example is the yeast mating system (Cottier and Mühlschlegel, 2011). Pheromones are peptides, namely a-factor produced by MAT a cells and  $\alpha$ -factor produced by  $\alpha$  cells. Haploid cells of each mating type sense and respond to the opposite factor, triggering a morphological response, forming a structure called a 'shmoo', resulting in directional growth towards the other cell. When shmoos touch, mating occurs and diploid cells are formed.

A QS system, on the other hand, relies on a particular cell density to be reached before a function is triggered. The concentration of a signalling molecule increases as cell numbers increase. When the 'quorum' threshold is reached, the molecule induces intracellular signalling pathway(s), altering gene expression, and resulting in a population response. This is widely used and well researched in bacterial populations, and to some extent in yeast populations (Henke and Bassler, 2004; Albuquerque and Casadevall, 2012).

### 1.8 Quorum sensing in yeast

The QS phenomenon was not described in eukaryotes until the 21<sup>st</sup> century when farnesol was identified as a cell-density-dependent signalling molecule in *Candida albicans* (Hornby et al., 2001). Farnesol inhibits, while tyrosol initiates, the switch from yeast-form cells to hyphae formation at a threshold concentration (Hornby et al., 2001; Chen et al., 2004). However, there are conflicting reports on the molecular pathway acted on by farnesol to prevent this morphological switch. Davis-Hanna and colleagues (2008) and Hall and colleagues (2011) showed that farnesol inhibited genes involved in the Ras-cAMP pathway that leads to hyphal formation but Sato and colleagues (2004) showed no effect from farnesol on this pathway.

Quorum sensing in *S. cerevisiae* has more recently been proposed. Tryptophol and 2-phenylethanol have been described as QS molecules as their production and action is cell-density-dependent (Chen and Fink, 2006). These aromatic alcohols are generated from their corresponding amino acids, tryptophan and phenylalanine, via the Ehrlich pathway (Hazelwood et al., 2008). Similar to other fusel alcohols, they were shown to induce *S. cerevisiae* pseudohyphal formation (Dickinson, 1996; Chen and Fink, 2006). The effect was enhanced when nitrogen was scarce. For example, pseudohyphal formation was present on Synthetic Low Ammonium Dextrose agar which contained 50  $\mu$ M ammonium sulfate, but not on Synthetic Minimal Dextrose agar which contained 37 mM ammonium sulfate (Chen and Fink, 2006). The production of these aromatic alcohols was also greatly reduced when cells were grown in a medium containing greater than 500  $\mu$ M ammonium sulfate. Therefore, the production of tryptophol and 2-phenylethanol is regulated by both population density and the nutritional state of the environment independently.

Aromatic alcohols are important in wine production as they contribute to the complexity of wine aroma profiles (Francis and Newton, 2005). They are produced by yeast during fermentation in three enzymatic reactions comprising transamination, decarboxylation and dehydrogenation (Hazelwood et al., 2008). Two of the enzymes used in the transamination and decarboxylation steps, encoded by ARO9 and ARO10, are regulated by the transcription factor Aro80p (Iraqui et al., 1999). Tryptophol can upregulate ARO9 and ARO10 expression via Aro80p, resulting in a positive feedback loop to further stimulate aromatic alcohol synthesis (Chen and Fink, 2006). Likewise, high cell density also induces production via the same feedback regulation. On the contrary, ARO9 and ARO10 expression is repressed in high nitrogen conditions, consistent with the observation of low aromatic alcohol production when large amounts of ammonium sulfate are available (Chen and Fink, 2006).

Although the biosynthesis of tryptophol and 2-phenylethanol is understood, how these putative QS molecules induce the morphological transition from yeast-form to filamentous form is unclear. There are four signalling pathways known to regulate filamentous transition: the cAMP-PKA, MAPK, SNF and TOR pathways, which all target FLO11, the same genes required for mat formation, adhesion between cells, cell-to-surface adhesion, pseudohyphae formation and agar invasion (Sengupta et al., 2007; Cullen and Sprague, 2012). Both cAMP-PKA and MAPK pathways activate FLO11 while the SNF pathway regulates repressors of the FLO11 promoter (Kuchin et al., 2002; Braus et al., 2003). The FLO11 transcription factor regulated by the TOR pathway requires further investigation. Chen and Fink (2006) showed that tryptophol and 2-phenylethanol induced FLO11 expression and filamentation through Tpk2p, and its downstream Flo8p. Tpk2p is a subunit of PKA whereas Flo8p is the transcription factor of *FLO11*. The upstream elements of Tpk2p in the cAMP-PKA pathway were not required, neither were the elements of the MAPK pathway. Besides that, two transcription factors, Cat8p and Mig1p, were predicted to be responsible for regulating genes that were differentially expressed in response to the proposed QS aromatic alcohols (Wuster and Babu, 2010).

# 1.9 Hydrogen sulfide is a potential cell-cell signalling molecule in *S. cerevisiae*

Although work to fully describe a QS system is still underway for *S. cerevisiae*, there are molecules excreted by *S. cerevisiae* that result in a cell-cell signalling phenomenon and coordination, such as ammonia. Ammonia is produced by yeast colonies and is perceived as a signal by neighbouring colonies, resulting in growth inhibition towards the ammonium-producing neighbouring colonies (Palková et al., 1997). Another potential signalling molecule is hydrogen sulfide (H<sub>2</sub>S). This molecule is released during fermentation when nitrogen is deficient (Jiranek et al., 1995; Gardner et al., 2002; Wang et al., 2003). Stuck fermentations caused by insufficient nitrogen are often difficult to restart, even when additional nitrogen is supplied thereafter. Reports suggested that this is due to inactivation of glucose transporters (Busturia and Lagunas, 1986). The presence of signalling compounds, such as H<sub>2</sub>S, may prevent the re-activation of the glucose transport system but this requires further investigation.

The production of  $H_2S$  is part of the sulfate assimilation pathway that leads to the biosynthesis of sulfur-containing amino acids, cysteine, methionine and glutathione (Thomas and Surdin-Kerjan, 1997). Hydrogen sulfide is an undesirable product in wine due to its rotten egg aroma. However, intracellular  $H_2S$  is thought to be responsible for metabolic synchronisation in a yeast population (Murray et al., 2003). When pulses of Na<sub>2</sub>S were added, the respiratory cycle of the whole population spontaneously reset, suggesting that  $H_2S$  may act as a microbial cell-cell signalling molecule (Murray et al., 2003; Lloyd, 2006). Hydrogen sulfide signalling is recognised in animal systems to maintain oxygen homeostasis and mediate the response to nutritional status (Iranon and Miller, 2012).

In mammals, nitrogen restriction, particularly cysteine and methionine, delivers health benefits and longevity (Miller et al., 2005; Plaisance et al., 2011; Elshorbagy et al., 2013; Lees et al., 2014). Exposure of mice to  $H_2S$  resulted in the same health benefits as nitrogen restriction and offered protection against oxygen deprivation (Blackstone and Roth, 2007; Hine and Mitchell, 2015). Hydrogen sulfide also increased thermotolerance and lifespan in the nematode *Caenorhabditis elegans* (Miller and Roth, 2007). Similarly,  $H_2S$ -mediated biological benefits have also been reported in yeast. The deletion of *MET17* leads to  $H_2S$  accumulation and increased  $H_2S$  production on both BiGGY agar (containing bismuth salt and sulfite to be reduced to bismuth and sulfide forming brown to black precipitate) and synthetic grape juice medium (Linderholm et al., 2008). This mutant has an extended chronological lifespan when grown in minimal medium (Johnson and Johnson, 2014). Other benefits include increased resistance to heat shock, oxidative and heavy metal stresses, and metal chelate toxicity (Singh and Sherman, 1974; Brown et al., 2006; Hwang et al., 2007; Johnson et al., 2014). A microarray experiment has shown that there is significant overlap of gene expression in the presence of  $H_2S$  and a stress response (Jia et al., 2011). Since the formation of  $H_2S$  integrates with a number of stress responses, including nitrogen restriction,  $H_2S$  could potentially be a signalling molecule that influences other nitrogen-deficient responses such as filamentous and invasive growth. Further work is required for this to be confirmed.

### 1.10 Research questions and objectives

S. cerevisiae is the common commercial wine yeast used in inoculated fermentations. The persistence of this species in the vineyard and winery is an important question, especially as this would affect winemaking that pursuits geographical characteristics from the native microflora. S. cerevisiae has been shown to be capable of forming biofilms in both environmental (wine and medical) and laboratory strains. These properties have however not been fully evaluated in commercial wine yeast strains. It is not clear whether the biofilm-related phenotypes such as filamentous and invasive growth co-existed with wine yeast biofilms. The relatedness of biofilm assays to the winemaking context is also lacking. The first part of this study is to investigate biofilm traits of commercial wine yeast on the standard assays – mat formation on a rich medium and plastic adhesion in a low glucose medium. The investigation is extended to mat formation on grape pulp and adhesion to winery hose plastic. This knowledge will contribute to determining if and how the yeast colonise and survive in a winery/vineyard environment.

Yeast are frequently exposed to nutrient fluctuation including nitrogen depletion. Filamentous colonies are formed on nitrogen-limited solid agar and can be enhanced by the putative *S. cerevisiae* quorum sensing molecules, tryptophol and 2-phenylethanol (Chen and Fink, 2006). However, mat formation response (on semisolid agar) to low nitrogen conditions has not been investigated. The second part of this study will contribute to this knowledge and determine if quorum sensing and signalling molecules have a synergetic or antagonistic effect on mat formation in a low nitrogen environment.

Biofilm formation by laboratory *S. cerevisiae* requires Flo11p (Reynolds and Fink, 2001). There are several signalling pathways leading to the expression of *FLO11*. It is unknown if biofilms formed by commercial wine yeast also require Flo11p and whether it is regulated in a similar way. The final part of this study aims to understand the biological processes of commercial wine yeast biofilms or associated phenotypes using RNA-sequencing.

## Chapter 2

## Wine yeast biofilms

### **Contextual statement**

The manuscript in this chapter addresses the first aim; to investigate the biofilm-forming ability of commercial wine yeasts. The study was conducted based on methods described by Reynolds and colleagues (2001) in the first *S. cerevisiae* biofilm publication. During attempts to reproduce mat and adhesion assays as per published methods, it became evident that further optimisation of these methods was required. Therefore, preliminary studies were conducted to finalise the methodology used in this manuscript and are outlined in Appendix A.

# Statement of Authorship

Title of Paper	Evaluation of the ability of commercial wine yeasts to form biofilms (mats) and adhere to plastic: impact on microbiota in the winery environment	
Publication Status	Published     Submitted for Publication	<ul> <li>Accepted for Publication</li> <li>Unpublished and Unsubmitted work written in manuscript style</li> </ul>
Publication Details	Written in manuscript style for FEMS Microbiology Ecology	

#### **Principal Author**

Name of Principal Author (Candidate)	Ee Lin Tek
Contribution to the Paper	Performed all experiments and data analysis, interpreted data, and wrote manuscript.
Overall percentage (%)	70%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 04-/09/17-

#### **Co-Author Contributions**

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate in include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Joanna F. Sundstrom
Contribution to the Paper	Construction of <i>FLO11</i> deletion in prototrophic $\Sigma$ 1278b, L2056 and AWRI796, supervised development of work, helped in data interpretation and editing of the manuscript.
Signature	Date 4/00/2017

Name of Co-Author	Jennie M. Gardner
Contribution to the Paper	Supervised development of work, helped in data interpretation and editing of the manuscript.
Signature	Date 6/09/17

Name of Co-Author	Stephen G. Oliver	

	Supervised development of work, helped in	n data interpretati	on and editing of the manuscript.
Signature	r	Date	30.08.17

Contribution to the Paper	Supervised development of work, helped in data interpretation and editing of the manuscri		
Signature		Date	4.9.17
Please cut and paste additional co-auth	or panels here as required.		2

17

## Evaluation of the ability of commercial wine yeasts to form biofilms (mats) and adhere to plastic: implications for the microbiota of the winery environment

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### 2.1 Abstract

Commercially available active dried wine yeasts are regularly used by winemakers worldwide to achieve reliable fermentations and obtain quality wine. This practice has led to increased evidence of traces of commercial wine yeast in the vineyard, winery and uninoculated musts. The mechanism(s) that enables commercial wine yeast to persist in the winery environment and the impact on native microbial communities by this persistence is poorly understood. This study has investigated the ability of commercial wine yeasts to form biofilms and adhere to plastic. The results indicate that the biofilms formed by commercial yeasts consist of cells with a combination of different lifestyles (replicative and non-replicative) and growth modes including invasive growth, bud elongation, sporulation and a mat sectoring-like phenotype. Invasive growth was greatly enhanced on grape pulp regardless of strain, while adhesion on plastic varied between strains. The findings suggest a possible mechanism that allows commercial yeast to colonise and survive in the winery environment, which may have implications for the indigenous microbiota profile as well as the population profile in uninoculated fermentations if their dissemination is not controlled.

### 2.2 Keywords

Saccharomyces cerevisiae; wine yeast; biofilms; mats; plastic adhesion; invasive growth

### 2.3 Introduction

The fermentation of must with deliberately inoculated commercial strains of *Saccharomyces cerevisiae* is a common practice in winemaking throughout the world. This practice ensures consistent and reliable fermentations that achieve specific sensory outcomes. The alternative, the use of uninoculated musts in which 'wild' yeast species from the grapes and winery undertake the fermentation, is believed to bring out the regional character of wines since the indigenous yeast population will vary in different geographical locations (Gayevskiy and Goddard, 2012; Bokulich et al., 2014; Knight et al., 2015; Pinto et al., 2015). There is increased interest in using both methods in individual wineries, as well as using mixed starter cultures, to impart a regional character to the product of fermentations predominantly carried out by commercial yeast strains (Ciani et al., 2010). However, the frequent use of commercial strains without containment, prompts the question as to whether such practices could have an important impact on shaping the microbial ecology of the vineyard or the winery.

The country of New Zealand represents an island group that has only been inhabited by humans in comparatively recent time (800–1000 BP; Hurles et al., 2003).

Nevertheless, some S. cerevisiae isolates from uninoculated fermentation were found to be genotypically similar to isolates from a French oak barrel, suggesting that human activity has a role in affecting the endogenous yeast population and the resulting fermentations (Goddard et al., 2010). Reports show the prevalence and survival of commercial yeast strains in the winery, and in the vineyard at up to 700 m from the winery. While this suggests that the dissemination of such commercial strains to the environment has already occurred, their incidence was inconsistent from vintage to vintage (Valero et al., 2005, 2007; Cordero-Bueso et al., 2011; Martiniuk et al., 2016). Within a single vintage, the microbial communities residing winery surfaces at the University of California, Davis fluctuated during harvest (Bokulich et al., 2013). However, S. cerevisiae, one of the common inoculum in that winery, appeared to colonise the winery surfaces. A seven-year study of uninoculated fermentations in a winery that had routinely used commercial strains prior to this to inoculate fermentations, found that eight out of ten of the dominant yeasts isolated were commercial strains that had previously been used in the winery (Blanco et al., 2011). Whilst there is increasing evidence from different parts of the world to suggest commercial yeast remain in the winemaking environment, there is limited information on how such residual commercial yeasts behave and survive in this environment, and the properties that permit these yeasts to become members of the vineyard and/or winery microbiota remain unclear.

It is known that surface attachment and different modes of growth, such as biofilms, enable the long-term survival of fungi and bacteria in diverse ecological niches. The yeast S. cerevisiae is able to form biofilms as evidenced by two tests: mat formation on low density agar and adhesion to plastic (Reynolds and Fink, 2001). Both mat formation and plastic adhesion require the cell surface protein Flo11p. S. cerevisiae can also undergo nutrient-regulated filamentous and invasive growth, which are believed to be mechanisms used to forage for nutrients (Cullen and Sprague, 2000, 2012). These properties are not found in the universal laboratory reference strain S288C, due to a mutation in the FLO8 gene, whose product is required for FLO11 transcription (Liu et al., 1996; Rupp et al., 1999). In contrast, the laboratory strain  $\Sigma$ 1278b, like many wild yeasts, displays biofilm-forming ability, filamentation and invasive growth (Hope and Dunham, 2014). It has been suggested that the loss of biofilm-like characteristics was due to domestication in the laboratory where yeast are grown routinely in rich media (Kuthan et al., 2003). This suggests that biofilms, surface adhesion and filamentous/invasive growth may confer on wild S. cerevisiae strains the ability to invade and thrive in unfavourable nutrient environments.

Many wild S. cerevisiae isolates, from a variety of geographical niches including those from wine grapes and must, have been shown to form mats exhibiting a range of shapes and sizes (Hope and Dunham, 2014; Sidari et al., 2014). This is different to the commonly studied laboratory strain  $\Sigma$ 1278b that forms a large mat consisting of a central hub and spokes. This result challenges our understanding of the genetic basis and phenotypic roles of yeast biofilms in ecological contexts, since most studies that characterise yeast mats have been based on  $\Sigma 1278b$  (Reynolds, 2006; Martineau et al., 2007, 2010; Sarode et al., 2011, 2014; Chen et al., 2014).

Currently, limited information exists for the biofilm-forming ability of commercial wine yeast strains, which could be the mechanism enabling them to persist in the vineyard and winery (Zara et al., 2005; Rodriguez et al., 2014). To date, no research has addressed the details of mat formation for commercial wine yeast strains (such as cell and mat morphology, filamentation and invasive growth). Additionally, most biofilm studies on S. *cerevisiae* have been focused on mat formation of cells grown on the rich Yeast Extract Peptone Dextrose (YPD) medium and on adhesion to hard plastics. Little is known about how these yeast biofilm test results translate to survival in winery conditions. Sidari and colleagues (2014) investigated the biofilm formation of wild S. cerevisiae strains using deficient media for carbon and nitrogen such as SLAD and low glucose YPD to simulate fermentation conditions. The present study was undertaken to assess the mat-forming ability of commercial wine yeast strains as well as to investigate features of their mats, including structure, cellular morphology and any incidence of filamentous and invasive growth. Mats were grown on low-density (0.3%) agar to approximate the density of grape pulp. This study demonstrated how mat features change in response to grape pulp and the ability of commercial wine yeasts to adhere to the soft plastics of which hoses in the winery are made. We believe the results of this study provide a functional perspective on the role of commercial wine yeast biofilms in the wine ecosystem.

### 2.4 Materials and Methods

#### 2.4.1 Yeast strains and media

Yeast strains used in this study are listed in Table 2.1. Five wine yeasts and a derivative were selected from preliminary experiments in this laboratory suggesting diverse mat phenotypes. Yeast Peptone Dextrose broth (YPD, 1% yeast extract, 2% bacto peptone, 2% glucose) or YPD-agar (YPD with 0.3 or 2% agar) was used to grow yeast strains. Deletion of *FLO11* in prototrophic  $\Sigma 1278b$ , L2056 and AWRI796 strains was achieved by transformation (Gietz and Schiestl, 2007) with a *Kan*MX gene replacement cassette (Wach et al., 1994) generated by PCR using FLO11\_A and FLO11\_D primers (Table 2.2) and genomic DNA of the BY4741  $\Delta flo11$  strain (Winzeler et al., 1999). Positive transformants were selected using YPD-agar (2%) + 0.02% G418-sulfate (Astral, NSW, Australia). Homozygous diploid deletants were then isolated by sporulation using the PRE5 and SPO2 media (Codon et al., 1995), dissection and re-diploidisation, and verified by PCR amplification and sequencing using the primers FLO11\_783bpup\_F and FLO11\_506bpdown\_R (Table 2.2). Strain I1 was generated by transformation of the *Kan*MX cassette (generated with PCR using primers SUL1\_A and SUL1\_D, Table 2.2, and genomic DNA of the BY4741  $\Delta sul1$  strain; Winzeler et al., 1999) into the commercial wine yeast 'Distinction', followed

by sporulation, dissection and isolation of the re-diploidised wild type progeny.

Yeast strain	Genotype and comments	Reference
L2056	Commercial wine yeast strain; diploid	Lallemand Australia
EC1118	Commercial wine yeast strain; diploid	Lallemand Australia
AWRI796	Commercial wine yeast strain; diploid	Mauri Yeast Australia
PDM	Commercial wine yeast strain; diploid	Mauri Yeast Australia
Distinction	Commercial wine yeast strain; diploid	Mauri Yeast Australia
I1	Diploid derivative of Distinction	This study
Prototrophic $\Sigma 1278b$	Wild type laboratory strain; diploid	Ryan et al. (2012)
Auxotrophic	Y12958; $MATa/\alpha$	Dowell et al. $(2010)$
$\Sigma 1278b$	$can1\Delta$ :STE2pr-Sp-his5/CAN1	
	$lyp1\Delta$ ::STE3pr-LEU2/LYP1	
	$his 3::his 3G/his 3::his 3G\ leu 2\Delta/leu 2\Delta$	
	$ura3\Delta/ura3\Delta$	
P $\Sigma 1278b$	$flo11\Delta::KanMX/flo11\Delta::KanMX$	This study
$\Delta flo11/\Delta flo11$		
A $\Sigma 1278b$	Y12958;	Ryan et al. $(2012)$
$\Delta flo11 / \Delta flo11$	$flo11\Delta::KanMX/flo11\Delta::KanMX$	
L2056	$flo11\Delta$ :: $KanMX/flo11\Delta$ :: $KanMX$	This study
$\Delta flo11 / \Delta flo11$		
AWRI796	$flo11\Delta$ :: $KanMX/flo11\Delta$ :: $KanMX$	This study
$\Delta flo11/\Delta flo11$		
BY4741	$MATa\ his 3\Delta 1\ leu 2\Delta 0\ met 15\Delta 0\ ura 3\Delta 0$	Thermo Fisher
$\Delta flo11$	$flo11\Delta$ ::KanMX	Scientific Australia
BY4741	$MATa his3\Delta 1 \ leu2\Delta 0 \ met15\Delta 0 \ ura3\Delta 0$	Thermo Fisher
$\Delta sul1$	$sul1\Delta$ ::KanMX	Scientific Australia

Table 2.1: Yeast strains used in this study.

P = prototrophic; A = auxotrophic

Primer name	Sequence $(5' \text{ to } 3')$	Product size of BY4741 (bp)
FLO11_A	AATGTCCGTGTTCGAATTAAATAAA	$4666 \; (WT^1);$
FLO11_D	CCAATACTACCGGTACTTGTTCTTG	$2146 \; (del^2)$
FLO11_783bpup_F	TGTTGTCTTTTTTAACGGTCGTACTG	5394 (WT);
$FLO11_506bpdown_R$	CCTGGTCGAAGATTATTAGTTGTGC	2876 (del)
SUL_A	TCGAACACTGTCATTTGAAATTATG	3104 (WT);
SUL_D	GGACATTTGTAGAAAATAGGCTCAA	2108 (del)
<sup>1</sup> wild type		
2.1.1		

<sup>2</sup>deletion
#### 2.4.2 Mat formation assays

YPD-agar (0.3%) was prepared by mixing an equal volume of autoclaved 0.6% w/v bacteriological agar (Amyl Media; Cat No. RM250) and filter sterilised 2× YPD. 25 mL of medium was aliquoted per 90 mm plate, and then used within 24 h.

Exponential-phase cultures were prepared by inoculation of YPD broth with an overnight culture at  $1.25 \times 10^6$  cells mL<sup>-1</sup> and incubating for 5–7 h. The culture was diluted in Phosphate Buffered Saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) to  $1 \times 10^6$  cells mL<sup>-1</sup> and an aliquot of 5  $\mu$ L was spotted at the centre of a 90 mm YPD-agar (0.3%) plate. At least six replicate mats of each strain were prepared. The plates were wrapped in cling film and incubated with yeast inoculum side up at 25 °C for 13 days, unless otherwise indicated. To determine whether auxotrophy reduced spoke formation merely by reducing growth, 0.029% histidine, 0.117% leucine and 0.029% uracil were supplemented into YPD-agar (0.3%).

L2056 mats with a sectoring-like phenotype were subcultured to determine if each sector formed the same distinct mat structure. For direct subculturing, cells were picked up with a 1  $\mu$ L inoculation loop and transferred to a fresh YPD-agar (0.3%) plate. To remove any temporary stress-induced phenotypes, cells were subcultured after re-growing in YPD. For this method, cells were grown in YPD broth to stationary phase before being used to prepare exponential-phase cultures and plating as described above.

Where indicated, mats were washed off the plate with a gentle stream of water to reveal invasive growth specific to mat formation on 0.3% agar. Plates were first incubated at 4 °C for 30 min before washing as this prevented the agar from being removed during washing. Where indicated, to confirm adherence to agar, cells were also subjected to rubbing with a gloved finger. Invasive growth was confirmed by needing to break the agar to reach those cells.

Mats were photographed using either a Samsung Galaxy S3 camera, S5 camera or ProtoCOL 3 (Synbiosis). Mat areas were measured from ProtoCOL 3 images using Fiji software (Schindelin et al., 2012). Detailed steps for processing and measuring are in the Supplementary Data.

The morphology of cells obtained from mats mounted in PBS were observed and imaged at  $400 \times$  and  $1000 \times$  magnification using a Nikon Eclipse 50i microscope and an attached Digital Sight DS-2MBWc camera with NIS-Elements F3.0 imaging software (Nikon).

For the grape-pulp assay, organic table grapes were surface sterilised with 70% v/v ethanol before skinning. Pulp was homogenised with a stick blender. Grape pulp agar (0.3% w/v agar) was prepared by mixing homogenised pulp and autoclaved agar in a 3:1 ratio. 25 mL of medium was aliquoted per 90 mm plate, and then used within 24 h. Yeast

were inoculated at the centre of the agar using a toothpickwith cells cultured on YPD-agar (2%). Plates were wrapped and incubated at 25 °C as described above. Negative controls with no inoculum resulted in no contamination. Mat images were taken using a Nikon SMZ1270 stereomicroscope and an attached DS-Fi3 camera with the NIS-Elements F4.60 software. Mats were washed off as described above. Cross-section samples were prepared by slicing the agar with a scalpel blade and placed on a glass slide with the cut side facing up.

High-sugar YPD-agar (5% glucose, 5% fructose, 1% yeast extract, 2% bacto peptone, 0.3% agar) was prepared as described for YPD-agar (0.3%). Yeast were inoculated using a toothpick for this assay. Images were taken on Day 3 using the ProtoCOL 3.

#### 2.4.3 Vitality and nuclear staining

Cells with elongated buds were stained for vitality and nuclear DNA to visualise the physiological state. For vitality staining, yeast cells were resuspended in 20  $\mu$ L of 1× PBS containing 6.5  $\mu$ g mL<sup>-1</sup> propidium iodide (PI; Life Technologies, formerly Invitrogen; Cat No. P3566) and 4.75  $\mu$ g mL<sup>-1</sup> bis-(1,3-dibarbituric acid)-trimethine oxonol (DiBAC4(3); Sigma-Aldrich; Cat No. D8189) on a glass slide. The slide was incubated for 5 min in a black humid chamber. DAPI (Sigma-Aldrich; Cat No. D9542) staining was performed according to Meluh's Protocol (John Hopkins School of Medicine, 1999) for staining of the nucleus. Stained cells were observed using a Nikon Eclipse 50i microscope with an attached Nikon Intensilight C-HGFI illuminator and a suitable filter set. Filter sets used included G2-A (excitation 510–560, barrier 590) for PI, GFP-B (excitation 460–500, barrier 510–560) for DiBAC4(3) and UV-2A (excitation 330–380, barrier 420) for DAPI. Black and white fluorescence images were obtained. Fluorescence colours were then applied using the Fiji software (Schindelin et al., 2012).

### 2.4.4 DNA preparation and PCR conditions

Genomic DNA was extracted as described in Adams et al. (1998). Other DNA preparations for PCR amplification were carried out according to the Chelex-based procedure described by Antonangelo et al. (2013) with the heating step substituted with boiling for 10 min. 25  $\mu$ L PCR reactions consisted of 1× Hi-Fi Buffer, 1 mM dNTP Mix (Bioline; Cat No. BIO-39028), 0.2  $\mu$ M primer, 0.5 units polymerase (Bioline Velocity DNA Polymerase; Cat No. BIO-21098) and 2  $\mu$ L of the Chelex-extracted DNA. The thermocycling program was 98 °C for 2 min, followed by 30 cycles of 30 s at 98 °C, 30 s at 58 °C and 1.5 min at 72 °C, followed by 5 min at 72 °C. Primers used and the expected product sizes are listed in Table 2.2. PCR products were separated on a 0.8–1% w/v TAE-agarose gel containing GelRed nucleic acid stain (Biotin; Cat No. 41003). DNA fragments of deletion products were excised from the gel and purified using the *Wizard SV* Gel and PCR Clean-Up System

### 2.4.5 Mat culture harvest and total RNA extraction

Spoked and non-spoked mats of L2056 were harvested by using a cover slip with forceps to pick up cells across all regions from rim to centre. An inoculation loop was used to transfer and resuspend cells in 1 mL Trizol reagent (Life Technologies; Cat No. 15596-018). The sample was snap frozen in liquid nitrogen for 20 s. RNA extraction was performed using a combination of Trizol reagent and a Qiagen RNeasy Mini kit (Cat No. 74104). Samples were thawed on ice. Glass beads were added up to the halfway mark of the meniscus. Six cycles of 45 s of vortexing and 45 s of rest on ice were used to disrupt cells. Tubes were incubated at 65 °C for 3 min and 200  $\mu$ L of chloroform was added, followed by vortexing for 15 s before leaving at room temperature for 5 min. Tubes were centrifuged at 20,817 × g for 10 min at 4 °C. Supernatant was recovered to a fresh tube and an equal volume of 70% v/v ethanol was added, mixed by pipetting, before continuing according to the Qiagen RNeasy Mini kit manufacturer's instructions. RNA quality and quantity were checked using a NanoDrop ND-1000 UV-visible light spectrophotometer (Thermo Fisher Scientific) and on 1% TAE-agarose gel. The absence of genomic DNA contamination in RNA preparations was confirmed using RNA as a template in real-time PCR assays.

#### 2.4.6 Quantitative real-time PCR

Quantitative real-time PCR was performed to compare the two L2056 mat structures that resulted from subculturing and determine whether this was associated with differential gene expression of *FLO11*. Primers for reference genes and the gene of interest (Table 2.3) used in real-time PCR were as published in Teste et al. (2009) and Van Mulders et al. (2009). Two micrograms of total RNA was reverse-transcribed into cDNA using an iScript cDNA synthesis kit (Bio-Rad; Cat No. 1708891) in a 40  $\mu$ L reaction mixture. The RT-PCR reaction mix (10  $\mu$ L total volume) consisted of 5  $\mu$ L SsoFast EvaGreen Supermix (Bio-Rad; Cat No. 1725203), 0.2  $\mu$ M of each primer, 2  $\mu$ L water and 2  $\mu$ L of a 1:10 dilution of the cDNA preparation. Each reaction was done in triplicate. Triplicates of no template control were included for each primer pair run. The thermocycling program was 95 °C for 30 s, followed by 40 cycles of 5 s at 95 °C and 5 s at 60 °C, followed by a hold at 65 °C for 5 s before an end at 95 °C. The melt curve data was checked to confirm primer specificity and contamination.

	2
Target	Sequence
Reference gene	
ALG9	F: CACGGATAGTGGCTTTGGTGAACAATTAC
	R: TATGATTATCTGGCAGCAGGAAAGAACTTGGG
TAF10	F: ATATTCCAGGATCAGGTCTTCCGTAGC
	R: GTAGTCTTCTCATTCTGTTGATGTTGTTGTTG
UBC6	F: GATACTTGGAATCCTGGCTGGTCTGTCTC
	R: AAAGGGTCTTCTGTTTCATCACCTGTATTTGC
Gene of interest	
FLO11	F: GTTCAACCAGTCCAAGCGAAA
	R: GTAGTTACAGGTGTGGTAGGTGAAGTG
gDNA	
contamination	
verification	
ACT1	F: ATTATATGTTTAGAGGTTGCTGCTTTGG
	R: CAATTCGTTGTAGAAGGTATGATGCC

Table 2.3: Primer sequences for qRT-PCR.

A standard curve was used to determine the PCR reaction efficiency for each primer pair. Quantitative PCR was performed on a ten-fold serial dilution of cDNA samples over six points. Each concentration was done in triplicate. The standard curve for all primer pairs used in the study had 90–110% reaction efficiency and an  $r^2$  value > 0.980.

Three reference genes, ALG9, TAF10 and UBC6, were used for normalisation as suggested by Teste et al. (2009). Analysis of qRT-PCR reactions with qBase<sup>PLUS</sup> (Biogazelle) using all reference genes returned an M value below 1, an acceptable range of stable expression for heterogeneous sample according to Taylor et al. (2015) and Vandesompele et al. (2002). Results were imported to GraphPad Prism version 7.02 software for a two-way analysis of variance (ANOVA) with a Sidak multiple comparisons test.

#### 2.4.7 Plastic adhesion assays

Plastic adhesion was performed for auxotrophic  $\Sigma 1278b$ , prototrophic  $\Sigma 1278b$ , L2056, AWRI796 and prototrophic  $\Sigma 1278b \Delta flo11/\Delta flo11$  as described by Reynolds and Fink (2001) with slight modifications. Cells were grown in Synthetic Complete medium (SC; 0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate, 0.079% Complete Supplement Mixture, 0.5% ammonium sulfate) with 2% glucose overnight, washed with sterile ultrapure water, resuspended in 10 mL sterile ultrapure water and split into two 50 mL tubes. The cells were harvested and resuspended in SC with either 0.1% or 2% glucose to an OD<sub>600</sub> of 1.0. Six replicates of 100  $\mu$ L aliquots were transferred to 96-well non-treated polystyrene plates (Corning; Manufacturing No. 3370). The plates were incubated for 0, 1, 3 or 6 h at 28 °C. An equal volume of 1% v/v Crystal Violet solution (Sigma-Aldrich; Cat No. HT90132) was added to each well and removed after 15 min. This step was repeated before washing with 100  $\mu$ L once and 200  $\mu$ L twice with Reverse Osmosis water. 100  $\mu$ L of 10% sodium dodecyl sulfate (SDS) was added to each well to solubilise Crystal Violet for 30 min. Absorbance at 590 nm was measured after mixing with 100  $\mu$ L sterile ultrapure water.  $\Sigma 1278b \Delta flo11 / \Delta flo11$  was excluded in 2% glucose due to poor growth and therefore insufficient overnight culture for both conditions.

#### 2.4.8 Winery hose adhesion assays

This assay is a modified version of the plastic adhesion assay. A new winery hose (Red Heliflex composed of polyvinyl chloride, the most commonly used hose for wine transfer) was cut into half-circle strips and sterilised by dipping into 70% v/v ethanol. Four sterile hose strips were placed in a 90 mm plate. 10 mL Synthetic Low Ammonium Dextrose (SLAD; 0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate, 2% glucose, 50  $\mu$ M ammonium sulfate) cultures of two overnights were harvested and resuspended in 25 mL of fresh SLAD before being added to the plate. Plate were incubated at 30 °C for seven days. Sterile forceps were used to pick up strips and dip them in water to rinse off unattached cells. The strips were then observed with a light microscope for attached cells. Negative control with a blank medium showed no cells attached.

For the assay incorporating shaking, winery hose was cut into quarter strips and sterilised with 70% v/v ethanol. A strip was added to a 50 mL tube containing 10 mL SLAD after inoculation of yeast. Cultures were incubated at 30 °C with shaking at 130 rpm for four days. Cell attachment on strips was observed as above. Cells were imaged at  $400 \times$  magnification using the Nikon Eclipse 50i microscope with the attached camera and NIS-Elements F4.60 software.

## 2.5 Results

#### 2.5.1 Prototrophic diploid $\Sigma$ 1278b as a laboratory reference

 $\Sigma 1278b$  is the most commonly used strain in mat studies since, unlike S288C, it has a functional *FLO8* gene and is considered to have wild type adhesion and filamentation phenotypes. Since the wine yeast strains in this study were diploid, diploid  $\Sigma 1278b$  was selected as the reference strain. Furthermore, auxotrophic and prototrophic diploid strains produced different mats. Auxotrophic  $\Sigma 1278b$  formed a smaller mat (Fig. 2.1A and B; YPD) with fewer spokes, defined as raised cables radiating from the hub (Fig. 2.1C), compared to the prototrophic  $\Sigma 1278b$  mat. Deletion of *FLO11* in either background abolished spokes (Fig. 2.1A). Since auxotrophic  $\Sigma 1278b$  has been reported to form a spoked mat, the incubation time was extended to check for spoke formation. More spokes arose as the mats aged. Ten percent of the mats developed spokes by Day 16 compared to none on Day 11 (Fig. 2.1C), thus confirming the ability of auxotrophic  $\Sigma$ 1278b to form mats with spokes. However, the average number of spokes per mat was markedly less for auxotrophic than for prototrophic  $\Sigma$ 1278b (ca. 0.16 vs 5.74 after 16 days). Supplementation with histidine, leucine and uracil improved growth, as evidenced by increased mat areas (Fig. 2.1B) and indeed restored spoked mat features (Fig. 2.1D). Accordingly, in order to avoid the potential complication of exogenous amino acid supplementation on mat formation and given the similarity of its mat formation to that previously published, prototrophic  $\Sigma$ 1278b was selected as the laboratory strain reference in this study of wine yeast mat morphology.



Figure 2.1: Mat features of  $\Sigma 1278b$ . (A) Mats formed by prototrophic and auxotrophic  $\Sigma 1278b$  on YPD-agar (0.3%) and YPD-agar (0.3%) supplemented with 0.029% histidine, 0.117% leucine and 0.029% uracil. Last column shows mats of prototrophic and auxotrophic  $\Sigma 1278b \Delta flo11/\Delta flo11$  on YPD-agar (0.3%). Images were taken on Day 9. (B) Boxplot showing mat areas (cm<sup>2</sup>) of auxotrophic (black) and prototrophic (white)  $\Sigma 1278b$  growing on YPD-agar (0.3%) and supplemented YPD-agar (0.3%). Please refer to next page for (C) and (D).



Figure 2.1: (C) Number of spokes formed by 37 auxotrophic (black) and 38 prototrophic (white)  $\Sigma$ 1278b mats on YPD-agar (0.3%) on Day 11 and 16. (D) Number of spokes formed by auxotrophic (black) and prototrophic (white)  $\Sigma$ 1278b mats grown on YPD-agar (0.3%) (Day 12 for prototrophic and Day 21 for auxotrophic to normalise mat size) and supplemented YPD-agar (0.3%) (Day 12).

### 2.5.2 Wine yeasts display diverse mat architectures

Commercial wine yeast strains L2056, AWRI796, EC1118 and PDM formed similar sized mats to those of prototrophic  $\Sigma$ 1278b when they matured (Fig. 2.2A). Both L2056 and AWRI796 grew into circular mats and relatively smooth surfaces but those of L2056 had crinkled edges. In contrast, the mats formed by EC1118 and PDM had a petal-like shape, with curved spokes. 'Distinction', a commercial strain derived from PDM via ethyl methanesulfonate (EMS) mutagenesis (strain 22.1 in Cordente et al., 2009), formed a smaller petal-like mat, but without distinct spokes. I1, the product of a re-diploidised spore of 'Distinction', formed a round, smooth-surfaced mat similar to that of AWRI796 but smaller in size.

## 2.5.3 Cell morphologies in the mat rim and mat body reveal distinct lifestyles

The morphology of cells from different regions of each yeast mat, including the rim, centre, body and spokes (if present) was examined. In most cases, cells from the mat rim had a uniform, actively-dividing population (Fig. 2.2B, Fig. S1A). The cells from the mat body, centre or spokes each formed a non-uniform population made up of cells of various sizes and morphology; for example, cells with enlarged vacuoles, elongated buds and cells undergoing sporulation. The wine strains L2056 (arrows in Fig. 2.2B), EC1118 and Distinction had more sporulation events compared to other strains tested. In addition, cell-cell adhesion observed in PBS mount slides was more prevalent in the mat body compared to the mat rim (data not shown). Elongated buds of cells taken from mats were most likely non-viable as both vitality stains (DiBAC4(3) and PI) were readily taken up, DAPI staining also revealed that these contained no nuclear DNA (Fig. 2.2C).

## 2.5.4 Some wine strains grow invasively at the start of mat formation

Mats of  $\Sigma 1278b$  and the commercial wine strains tested were washed off with water to observe agar invasion events. All strains (as represented by  $\Sigma 1278b$  and 'Distinction' in Fig. 2.2D), except the strain I1, were able to grow invasively from 2 days after inoculation, indicating that agar invasion occurred at or soon after inoculation in the early stage of mat formation. Invasive growth was confirmed by needing to break the agar to reach those cells. Invasive growth only developed at the centre of the mat where the inoculum had been applied (boxes in Fig. 2.2D; plate). No correlation between mat size and agar invasion was observed. The invasive growth structures were similar between strains (Fig. 2.2D; micrograph). No filamentous cells were observed on the edge of the invasive structures.







Figure 2.2: Features of yeast mats on YPD-agar (0.3%), prototrophic  $\Sigma 1278b$  at Day 8, wine yeast at Day 13. Representative images were chosen to display the range of morphological features observed. (A) Images of mats typical of prototrophic  $\Sigma 1278b$  and each wine yeast strain. (B) Morphologies of cells from mat rim and mat body of  $\Sigma 1278b$  and L2056. Arrows indicate sporulation. Please refer to next two pages for (C), (D) and (E).

(C)







500 µm





Figure 2.2: (C) Fluorescence micrographs of prototrophic  $\Sigma 1278b$  cells with elongated buds stained with a combination of DiBAC4(3) (green) and PI (red) or L2056 cells with DAPI. Co-staining with both DiBAC4(3) and PI is visualised by an orange fluorescence. (D) Plate and micrograph images of invasively growing cells from washed yeast mats, with and without rubbing. Please refer to next page for (E).



Figure 2.2: (E) Mats formed by L2056  $\Delta flo11/\Delta flo11$  and AWRI796  $\Delta flo11/\Delta flo11$  (Day 13).

Compared to the mats formed by wine yeasts, the  $\Delta flo11/\Delta flo11$  strains had reduced mat size (compare images in Fig. 2.2E with those in Fig. 2.2A; the plate size and incubation time (13 days) were the same in both cases). The L2056 mutant had more petal structures than the AWRI796 mutant.

# 2.5.5 Wine strain L2056 forms mats with a more rapidly expanding sector

Some L2056 mats developed a sector that expanded across the agar more quickly than the rest of the mat. Of 38 biological replicates, 55% developed a sector with such growth (Fig. 2.3A). Cells were subcultured from the typical part of the mat and the expanding sector to fresh plates (primary direct subculturing) to compare mat morphologies. Cells from the expanding sector formed a  $\Sigma$ 1278b-like spoked mat, whilst cells from the standard part of the mat produced a smooth mat similar to the original L2056 mat (Fig. 2.3A). The spoked and smooth mat phenotypes, respectively, persisted when cells were subcultured from the primary direct subculture to fresh plates (secondary direct subculturing; Fig. 2.3A). This was independent of whether the inoculum came from the rim, body, spokes or centre (data not shown). After overnight growth of cells from the original L2056 mat in YPD broth, aimed to remove any temporary stress-induced phenotypes, the differences were still evident. However, when the inoculum came from the secondary direct subculture, the difference was minimal: here the expanding sector had more structured surfaces compared to the standard sector, which formed smooth surfaces. No distinct differences on cellular morphology between the two types of mats were observed (Fig. S1B).



Figure 2.3: Mat morphology of an L2056 'sectoring' mat and its subcultures on YPD-agar (0.3%). (A) An example of an original L2056 mat with a more rapidly expanding sector. Expanding and standard sectors from the original L2056 mat were subcultured directly onto YPD-agar (0.3%); primary direct subculture; n = 2). Cells from the rim, body, spokes (if any) and centre of the primary subculture mats were subcultured (secondary direct subculture, n = 4 for each mat section). Expanding and standard sectors from the original mat were also grown in YPD broth prior to plating on a fresh YPD-agar (0.3%; n = 5), as were cells from the mat body of the secondary subcultures (n = 4). (B) *FLO11* PCR products from genomic DNA isolated from L2056 mats, amplified with FLO11\_A and FLO11\_D primers. E = expanding sector; S = standard sector. (C) Relative fold change in *FLO11* gene expression between non-spoked and spoked mats produced by cells in the expanding and standard sector of an L2056 mat (n = 4). Each replicate is indicated by an enclosed circle. The long horizontal lines represent the mean and the error bars represent standard deviation. The difference is not statistically significant.

FLO11 is well known to affect cell adhesion and filamentation, and various gene sizes have been reported to affect biofilm-forming ability (Zara et al., 2009). Previous work in this laboratory had shown that PCR amplification of FLO11 from L2056 yields two amplicons. FLO11 was PCR amplified from cells within expanding and standard sectors of the original, primary and secondary subcultured mats to determine if these two amplicons were lost due to a meiotic event. Two products of expected sizes were amplified in each case (Fig. 2.3B), suggesting this had not occurred. FLO11 gene expression level was then compared between spoked and non-spoked mats produced by cells in the expanding sector and standard sector, respectively. Two out of four spoked mats showed increased FLO11gene expression by two- and three-fold compared to non-spoked mats (Fig. 2.3C).

#### 2.5.6 Plastic adhesion

Auxotrophic  $\Sigma 1278b$  showed the most adhesion to plastic in both low and sufficient glucose conditions (Fig. 2.4). Prototrophic  $\Sigma 1278b$  and L2056 displayed a modest increase in plastic adhesion ability in 0.1% glucose compared to that in 2% glucose, while AWRI796 was not affected by this nutrient change and showed less adhesion compared to  $\Sigma 1278b$  $\Delta flo11/\Delta flo11$  after 3 and 6 h in 0.1% glucose.



Figure 2.4: Plastic adhesion of laboratory and wine strains grown in SC medium with either 0.1 or 2% glucose. Absorbance at 590 nm was measured after 0, 1, 3 and 6 h of incubation. Each data point represents the mean of six samples: ( $\Box$ ) auxotrophic  $\Sigma$ 1278b, ( $\circ$ ) prototrophic  $\Sigma$ 1278b, ( $\triangle$ ) L2056, ( $\bullet$ ) AWRI796, ( $\blacktriangle$ ) prototrophic  $\Sigma$ 1278b  $\Delta flo11/\Delta flo11$ , ( $\blacksquare$ ) no cells (control). The error bars represent standard deviation and are included for all time points.

# 2.5.7 Wine yeast grow invasively and conduct fermentation on grape pulp soft agar

 $\Sigma$ 1278b and several commercial wine yeast strains were plated onto grape pulp agar for mat assays. Instead of forming a large mat, grape pulp induced fermentation. Bubble-forming mats were observed on Day 3. There was no structured morphology observed on the culture surfaces (Fig. 2.5A). On Day 9, gas was observed trapped underneath the agar (Fig. 2.5B) which raised the agar, resulting in some surface culture (e.g.  $\Sigma$ 1278b) coming into contact with the plate lid (Fig. 2.5A). Occurrence of cell adhesion and invasive growth can be seen after gently washing with water. Compared to the YPD mat assay, where the invasive growth only occurred in a few patches (Fig. 2.2D), the invasive growth in grape pulp agar was extensive (Fig. 2.5B; post-wash, cross-section).

The Brix of grape pulp was 17°, which means 75% pulp agar would have approximately 12.75° (12.7% sugar). To investigate whether the fermentation phenotype was solely induced by the high sugar concentration in grape pulp, YPD containing 10% total sugar (equimolar glucose and fructose) was prepared for mat assays. The high-sugar YPD-agar, however, did not induce the fermentation phenotype observed on grape pulp (Fig. 2.5C). Flat mats instead of bubble-forming mats were observed.



Figure 2.5: Grape-pulp mat assay. (A) Mat images of  $\Sigma 1278b$  and a representative wine strain, EC1118, on grape pulp agar (0.3%) at Day 9. (B) Images of EC1118 from the underside of the agar, post-wash and cross-section. Black arrows indicate invasively growing cells; white arrow indicates the grape pulp agar. (C) Day 3 image of EC1118 on high-sugar (10%) YPD-agar (0.3%).

# 2.5.8 Wine strain L2056 forms initial attachment on winery hose soft plastic

To begin to provide some insight into the potential significance of adhesion in a winemaking context, two assays were performed to investigate whether wine yeast are able to adhere to the soft plastics of commonly used winery hose. The first assay was modified from that used above to monitor plastic adhesion. All four strains tested,  $\Sigma 1278b$ , L2056, AWRI796 and prototrophic  $\Sigma 1278b \Delta flo11/\Delta flo11$  showed no adhesion. The second assay was performed in a 50 mL tube with shaking to imitate juice flowing through a winery hose.  $\Sigma 1278b$  and L2056 were observed to have initial attachment to the hose plastic, but this was not true for AWRI796 and prototrophic  $\Sigma 1278b \Delta flo11/\Delta flo11$  (Fig. 2.6). This matches the plastic adhesion result in plates, that AWRI796 did not adhere well,  $\Sigma 1278b$ had the most adhesion followed by L2056 (Fig. 2.4).



Figure 2.6: Cell adhesion on plastic of a common winery hose, Red Heliflex. Images of plastic after four days of incubation in SLAD culture with shaking and after rinsing with water to remove unbound cells.

## 2.6 Discussion

Similar to the wild S. cerevisiae strains isolated from wine grapes and must (Sidari et al., 2014), commercial wine yeast strains were found to form varied mat sizes with structured architecture that differed from those formed by the laboratory yeast,  $\Sigma 1278b$ . This may be explained by the genome differences between wine strains (Borneman et al., 2008, 2011, 2016). In this study, EC1118, PDM, Distinction and I1 are closely related whereas L2056 and AWRI796 are different from each other and from the others. Other wild veasts have also been shown to produce mats with morphologies that did not conform with the standard 'hub and spokes' structure. For example, the mats formed by wild flor strains, V80, V23 and M23 (Zara et al., 2009), did not fully cover the agar plate and had no spoke formation, similar to 'Distinction' and I1 in this study, but differing in rim shape. The baking yeast YS2 (Hope and Dunham, 2014) formed a relatively larger, smooth surface mat, like AWRI796. Highly complex mats, which were formed by soil yeast YPS128 and bee yeast UWOPS05-227.2 (Hope and Dunham, 2014) were distinct from each other as well as from any of the strains in this study. Deletion of the FLO11 gene in two wine strains, L2056 and AWRI796, resulted in smaller sized mats, confirming that FLO11 is also required for a full-sized wine yeast mat formation.

The ability to form mats by a panel of commercial wine yeast strains suggests the ability to adhere to surfaces in the wine environment, which could include equipment, grapevine and grape berries. It could also help these yeast to form associations with other microbes. The results in grape-pulp mat assay suggest that commercial wine yeast could adhere and even invade grapes for colonisation. The winery hose adhesion trial also provides an indication of an initial attachment to soft plastic by wine yeast. In addition to the ability to co-flocculate with other wine-associated yeast strains (Rossouw et al., 2015), the adhesion and invasion properties shown in this study could drive the microbial population resident in the vineyard and winery, and subsequently affecting the population in fermentations. This may explain the previous reports that commercial strains contribute to the yeast population in uninoculated fermentations (Hall et al., 2011; Martiniuk et al., 2016; Scholl et al., 2016).

Mat formation can be considered as an expansion of colony formation. Analysis of the cellular morphologies between the mat rim and body reveals distinct lifestyles between these populations. The replicative phase is characterised by many actively dividing cells and these were present in the mat rim of all yeast mats examined. The non-replicative phase as observed in mat body is when cells are no longer proliferating, similar to colonies growing on rich medium. Yeast biofilm colonies described by Váchová et al. (2011) also have distinct cell types on different parts of the structure: non-dividing cells on the surface of the aerial region and dividing cells inside the colonies. However, unlike biofilm colonies, mats are thin and have no aerial regions. The cell type differences were more distinct between mat body and rim. Cells of the mat body were heterogenous. They included cells that were sporulating, had elongated buds or enlarged vacuoles. This heterogeneous population has been described in aging yeast colonies, which consist of upper and lower regions with different stress resistances (Palková et al., 2014). The diversity of stress tolerance within a community arises from metabolic specialisation and cooperation between cells (Campbell et al., 2016). This mixture of differentiated cells within the mat body may contribute to supporting the survival of cells in the expanding edge *i.e.* mat rim.

An interesting cell type was found through microscopic observation in the mat body. Cells with an elongated bud were visualised with nuclear staining, the results suggesting the cell cycle arrested before nuclear migration (no nuclear DNA in elongated buds shown in Fig. 2.2C). Gladfelter and colleagues (2005) have shown that elongated bud morphology occurs due to Swe1p-mediated  $G_2$  arrest. Whilst other studies (Gladfelter et al., 2004; Homoto and Izawa, 2016) have shown this morphology is often associated with septin mislocalisation, Gladfelter and colleagues (2005) also showed  $G_2$  arrest could aggravate the effect of septin disorganisation. Ethanol has been shown to increase Swe1p expression, which inhibits Cdc28p kinase activity and subsequently causes  $G_2$  arrest cell cycle delay (Booher et al., 1993; Kubota et al., 2004). There may be environmental signals other than ethanol causing either  $G_2$  arrest or septin mislocalisation during mat formation, which requires further investigation.

Similar to the report by Rodriguez et al. (2014), L2056 formed crinkled-edge mats. In addition, this study also reports, for the first time, that mats formed by L2056 can be sectored (Fig. 2.3A). When the original L2056 mat was subcultured, cells from the expanding growth sector formed a typical  $\Sigma 1278b$  'hub and spokes' mat whereas the standard growth sector formed a non-spoked mat, similar to the original L2056 mat. Sectoring colonies have been observed in other fungi. For example, it is an indication of phenotypic switching in haploid *Candida tropicalis* (Porman et al., 2011), where the opaque sector shows more mating-competence. The fungus *Metarhizium anisopliae* forms a sector that has lost sporulation capacity, activity of certain enzymes, and changes in secondary metabolite profiles (Ryan et al., 2002). Sectoring can also occur when a fungus mutates and adapts to become drug resistance (He et al., 2014). In this study, two types of mats were formed persistently after two cycles of direct subculturing as well as subculturing after re-growing in rich medium. Since Flo11p is known to be important for forming 'hub and spokes' in  $\Sigma 1278b$  mats and the gene length is related to the biofilm-forming ability in flor strains (Zara et al., 2009), the length of FLO11 genes in cells of the two types of mats was studied. No difference in FLO11 allele sizes of either sectors in the original L2056 mat, primary or secondary subcultures was seen (Fig. 2.3B), suggesting no meiotic events had occurred. FLO11 expression levels were then compared between the two types of mats. Two out of four spoked mats showed an increase in expression compared to non-spoked mats (Fig. 2.3C), which suggests FLO11 may be involved in mat expansion and/or spoke formation through differential expression, similar to the model suggested by Regenberg et al. (2016). Differential expression of FLO11 in  $\Sigma 1278b$  was shown to generate  $Flo11^+$  and  $Flo11^-$  cells, containing adhesive and non-adhesive cells, in

mat formation (Regenberg et al., 2016). The differentiated mat had a spoked structure and was larger than the undifferentiated Flo11<sup>-</sup> mat. This may explain the formation of the rapidly expanding sector in the original L2056 mat, being due to the differentiated state. This differentiated state was carried on to the subcultures and resulted in spoked mats (Fig. 2.3A). Since L2056 has two unequal sized *FLO11* alleles, it is also possible that the differential expression involves switching of the expression of either allele.

While most mat studies use auxotrophic  $\Sigma 1278b$  strains, in this study auxotrophic  $\Sigma 1278b$  did not form mat structures as widely reported. However, the 'hub and spokes' structure was formed with extended incubation or nutrient supplementation (Fig. 2.1C and D). There have been cases when supplementation did not compensate for auxotrophies (Corbacho et al., 2011). The particular amino acids used for supplementation may also influence physiological regulation because some amino acid biosynthesis pathways are connected (Niederberger et al., 1981). In the plastic adhesion experiment, adhesion ability was affected by auxotrophies – the auxotrophic  $\Sigma 1278b$  was shown to be more adhesive than prototrophic  $\Sigma 1278b$  (Fig. 2.4). Changes in metabolic flux induced by auxotrophic vs prototrophic states, may interfere with system-wide regulatory processes (Grüning et al., 2010). Therefore, it is suggested that prototrophic strains are more representative of the natural state and should be used in future studies.

It is noticeable that the number of spokes formed by  $\Sigma 1278b$  in this study was less than those reported previously (Reynolds and Fink, 2001). This was probably due to the size of the inoculum. Toothpick inoculation (widely used in other mat publications) generated > 10 spokes while inoculation of 800 and 50 cells yielded almost none (Fig. S2). In order to control initial cell numbers, an inoculum size of 5,000 cells was chosen, which produced representative spokes.

Despite showing a variety of mat structures by commercial wine yeast, this study also demonstrated broad responses accompanied with mat features by visualising cell morphologies and growth modes. The findings contribute to a better understanding of commercial yeast lifestyle on biofilms and adhesion with respect to wine environments. The observations may provide an explanation for the survival of commercial strains which might influence the natural microflora in the vineyard and winery in the long-term.

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Conflict of interest: None declared.

## 2.8 Acknowledgements

Prototrophic  $\Sigma 1278b$ , auxotrophic  $\Sigma 1278b$  and A  $\Sigma 1278b \Delta flo11/\Delta flo11$  were kindly donated by Dr Charles Boone (University of Toronto). The authors thank the Australian Wine Research Institute for providing the Red Heliflex hose and access to ProtoCOL 3, and Dr Michelle Walker (University of Adelaide) for generating strain I1.

## 2.9 Supplementary Data

### 2.9.1 Methods

#### Mat area measurement from ProtoCOL 3 images

- 1. Capture images as described in ProtoCOL 3 manual. Exposure was set at 60 ms.
- 2. Download and install Fiji from http://imagej.net/Fiji/Downloads.
- 3. Open the application.
- 4. Open an image that needs to be measured.
- 5. Click Analyze from the menu, select Set Scale. Enter 583 pixels = 9 cm, check Global and press OK. Now the image and the following images opened within this session have been calibrated.
- 6. To convert into a black and white image, click **Image** from the menu, choose **Adjust** and select **Threshold**. Change the **Threshold colour** to **B**&**W**, press **Close**.
- 7. Click Wand (tracing) tools from the icon menu, move cursor to anywhere in the mat (black area), click on the mat. This will select the thresholded object mat.
- 8. Click **Analyze** from the menu, select **Set Measurement**. Check **Area** and press **OK**.
- 9. To measure, click **Analyze** from the menu, select **Measure**. A result table with the area measurement will pop up. Save the results for future use and close all the boxes.
- 10. To measure subsequent images in the same session, open a new image, **Image** > **Adjust** > **Threshold** > **Close**, click on the mat, **Analyze** > **Measure**.

## 2.9.2 Figures

Figure S1: Cellular morphology from yeast mats grown on YPD-agar (0.3%). (A) Prototrophic  $\Sigma$ 1278b and wine strains (B) L2056 subcultures. Scale bars, 50  $\mu$ m. N/A = not applicable.





PDM

Body – thick region



Body – thin region



Rim

Centre

00



Body – thick region





Body – thin region













Secondary Expanding

Secondary Standard

Figure S2: Mat formation of prototrophic  $\Sigma 1278b$  on YPD-agar (0.3%) with inocula from either a toothpick with cells or cell suspensions (800 or 50 cells per 5  $\mu$ L).



## Chapter 3

## Mat formation in a low nitrogen medium

## **Contextual statement**

Chapter 2 illustrated a variety of mat features for several commercial wine yeast strains. The manuscript in this chapter evaluated the response of wine yeast during mat formation under limiting nitrogen conditions and several factors that could affect the response, which corresponds to the second aim (p. xiii) of this project. Wine yeast assimilate nitrogen since it is an essential nutrient enabling growth. Fermenting wine musts commonly utilise all available nitrogen, often before all sugars are catabolised, the depletion of nitrogen from rotting grapes on the vine is expected to be similar. Thus, whether it be a fermenting must in a winery or grapes in the vineyard, exposure of yeast to an environment with significantly depleted nitrogen is common. Previous studies with a filamentation focus have used solid medium (2% agar) with very low nitrogen as it stimulates diploid cells to undergo pseudohyphal growth. The availability of nitrogen could also be an important factor in mat formation on less dense media (0.3% agar). Preliminary studies were performed to refine methodology used in this section of work, including optimal inoculation rate and the effect of a putative cell signalling compound (Appendix B), in order to establish the scope of work undertaken.

## Statement of Authorship

Title of Paper	Factors influencing filamentous and invasive growth of yeast cells in mat formation in a low nitrogen environment		
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## **Principal Author**

Name of Principal Author (Candidate)	Ee Lin Tek		
Contribution to the Paper	Performed all experiments and data analysis, interpreted data, and wrote manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature	Date 04/09/17		

## **Co-Author Contributions**

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate in include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Jennie M. Gardner		
Contribution to the Paper	Supervised development of work, helped in data interpretation and editing of the manuscript.		
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Contribution to the Paper	Supervised development of work, helped in data interpretation and editing of the manusc		
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	and for all restrictions		

Name of Co-Author	Stephen G. Oliver

Contribution to the Paper	Supervised development of work and helped in data interpretation.	
Signature	Date 30.08.17	
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Contribution to the Paper	Supervised development of work, helped in data interpretation and editing of the manuscript.	

Signature

Date 4.9.17

Please cut and paste additional co-author panels here as required.

## Factors influencing filamentous and invasive growth of yeast cells in mat formation in a low nitrogen environment

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## 3.1 Abstract

Saccharomyces cerevisiae forms complex mat structures on low agar YPD. In response to nutrient limitation, budding yeast can become adherent, switch to a filamentous form and grow invasively. Accordingly, mat structure is affected *i.e.* a filamentous mat is formed on a glucose-limited medium. In this study, a proportion of yeast in a mat switched to filamentous and invasive growth on a nitrogen-limited medium. Also, increasing nitrogen content increased cellular growth and mat size. The formation of filamentous and invasive foci within a mat was enhanced by ethanol and hydrogen sulfide but was inhibited by aromatic alcohols and sulfite. As previously reported, filamentous growth was also affected by neighbouring mats. This growth transition to filamentation and invasion, in low nitrogen low-density agar, may be a response adapted to the environmental niche typical of yeast, rotting fruit, which has low density and decreasing nutrients.

## 3.2 Keywords

Filamentous growth; invasive growth; low nitrogen; mats; aromatic alcohols; sulfide and sulfite

## 3.3 Introduction

Saccharomyces cerevisiae forms mats on a low-density agar (0.3%) medium (Reynolds and Fink, 2001). Mat structures are complex and diverse and depend on the strain background (Hope and Dunham, 2014). The laboratory yeast strain  $\Sigma 1278b$  generates a mat with characteristics of a central hub and radiating spokes. Hub and spoke formation can be delayed if glucose concentration is increased (Reynolds et al., 2008). Conversely, a filamentous mat is formed if glucose is removed (Karunanithi et al., 2012). The direction of filamentous growth has also been shown to be affected by the presence of a neighbouring mat (Karunanithi et al., 2012).

Yeast also undergo filamentous and invasive growth on medium-density agar (2%) when nitrogen is limited (Gimeno et al., 1992). Hyphal-like elongated cells can be stimulated by a number of fusel alcohols, which are by-products from catabolism of some amino acids (Dickinson, 1994, 1996). Conditioned medium from a  $\Sigma$ 1278b stationary-phase culture was reported to stimulate filamentous and invasive growth (Chen and Fink, 2006). The group demonstrated that tryptophol and 2-phenylethanol were acting as quorum sensing molecules that enhanced the filamentous and invasive phenotype on low nitrogen agar.

Hydrogen sulfide  $(H_2S)$  is associated with nitrogen deficiency during fermentation

(Jiranek et al., 1995). Nitrogen deficiency can cause a stuck fermentation. This is often difficult to restart even when additional nitrogen is supplied. Many reports have suggested that this is largely based on delayed nitrogen ameliorations being ineffective due to reduced hexose transporter activity. However, there is evidence that  $H_2S$  may also act as a cell-cell signalling molecule (Lloyd, 2006). Its presence has been postulated to alter the metabolic clock of the yeast population (Sohn et al., 2000). Microarrays have shown significant overlap of gene expression in the presence of  $H_2S$  and a stress response (Jia et al., 2011). It is possible that the presence of by-products such as  $H_2S$  could inhibit yeast cell metabolism resulting in reduced fermentation of residual sugars. Like tryptophol and 2-phenylethanol, H<sub>2</sub>S could potentially be a signalling molecule that affects other nitrogen-deficient responses such as filamentous and invasive growth. Interestingly, within the sulfate assimilation pathway, the precursor of sulfide is sulfite, which has been shown to block invasive growth (Zupan and Raspor, 2010). Several mechanisms have been proposed how sulfite inhibits growth (see review in Divol et al., 2012). Briefly, these include the reduction of intracellular ATP by activating ATPase, inhibition of key metabolic enzymes such as GAPDH, binding to co-enzymes, co-factors and to a number of metabolites including acetaldehyde (Schimz and Holzer, 1979; Schimz, 1980; Hinze and Holzer, 1986; Carmack et al., 1950; Rankine and Pocock, 1969). These could lead to cell death as energy metabolism is negatively impacted.

Since yeast cells are able to produce filamentous and invasive mats on glucoselimited low-density (0.3%) agar and limited nitrogen can induce this form of growth on medium-density (2%) agar, a hypothesis is made that filamentous and invasive mats would form when nitrogen is scarce on low-density agar. Agar of a lower density has similar consistency to the material where yeast are commonly found in nature; fruit, e.g. grape pulp, and thus is relevant when considering this microorganism's environmental niche. Yeast growing on grapes would deplete nutrients in their immediate vicinity and may switch to filamentous and invasive growth modes to enhance survival. With this in mind, this study investigated mat formation in a low nitrogen environment by *S. cerevisiae* including  $\Sigma$ 1278b and several wine yeast strains to see if the response is widespread for different genetic backgrounds. The addition of compounds or factors known or hypothesised to influence filamentous and invasive growth such as the presence of a neighbouring mat, tryptophol, 2-phenylethanol, H<sub>2</sub>S and sulfite was also evaluated.

## **3.4** Materials and Methods

#### 3.4.1 Yeast strains and media

Yeast strains used in this study are listed in Table 3.1. Strain I1 was generated by transformation (Gietz and Schiestl, 2007) of a *Kan*MX cassette (generated with PCR using primers 5'-TCGAACACTGTCATTTGAAATTATG-3' and 5'-GGACATTTGTAGAAAA

TAGGCTCAA-3', and genomic DNA of the BY4741  $\Delta sul1$  strain; Wach et al., 1994; Winzeler et al., 1999) into the commercial wine yeast 'Distinction', followed by sporulation, dissection and isolation of the re-diploidised wild type progeny.

Yeast strain	Genotype and comments	Reference
Prototrophic $\Sigma 1278b$	Wild type laboratory strain; diploid	Ryan et al. $(2012)$
L2056	Commercial wine yeast strain; diploid	Lallemand Australia
EC1118	Commercial wine yeast strain; diploid	Lallemand Australia
AWRI796	Commercial wine yeast strain; diploid	Mauri Yeast Australia
PDM	Commercial wine yeast strain; diploid	Mauri Yeast Australia
Distinction	Commercial wine yeast strain; diploid	Mauri Yeast Australia
I1	Diploid derivative of Distinction	This study
BY4741 $\Delta sul1$	$MATa\ his 3\Delta 1\ leu 2\Delta 0\ met 15\Delta 0$	Thermo Fisher
	$ura3\Delta 0 \ sul1\Delta$ ::KanMX	Scientific Australia

Table 3.1: Yeast strains used in this study.

Synthetic Low Ammonium Dextrose (SLAD; 0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate (Beckton Dickinson; Cat No. 233520), 2% glucose and 50  $\mu$ M ammonium sulfate) was filter sterilised as a 10× stock and diluted as required with sterile ultrapure water. Bacto agar (BD; Cat No. 260001) was washed twice in 800 mL ultrapure water in a 1 L Schott bottle, swirled to mix and rested for 15 min before decanting, followed by autoclaving. SLAD low-density agar (0.3%) used in mat assays was prepared by mixing equal volumes of 2× SLAD and 0.6% molten Bacto agar, and where indicated, the addition of one or more of the following chemicals: 0.05% v/v ethanol, 50  $\mu$ M tryptophol, 50  $\mu$ M 2-phenylethanol, 0.4 mg L<sup>-1</sup> sodium sulfide (Na<sub>2</sub>S.9H<sub>2</sub>O) and/or 0.01% w/v sodium metabisulfite (Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub>). Tryptophol and 2-phenylethanol stock solutions (50 mM) were prepared in 50 mL of 25% v/v ethanol making the final concentration of ethanol in SLAD agar containing aromatic alcohols 0.05%. All chemicals were sourced in ultrapure form from Sigma Aldrich. Aliquots of 10 mL were poured onto 60 mm polystyrene Petri dishes (Techno Plas; Cat No. S6014S10). Media were only made and poured one day prior to plating.

Conditioned medium (CM) was prepared as follows. Yeast were inoculated from glycerol stocks and cultured in 25 mL SLAD in two separate Erlenmeyer flasks for 48 h at 28 °C with agitation. Cultures were mixed and centrifuged at 20,817 × g for 10 min to collect the supernatant. Supernatant was tested for nitrogen (ammonia) and glucose content using enzymatic assays (see below). The supernatant was supplemented up to the original nitrogen (50  $\mu$ M ammonium sulfate) and glucose (2%) content in SLAD and filter sterilised.

#### 3.4.2 SLAD mat assays

Yeast were inoculated from glycerol stocks into 10 mL SLAD and incubated for 48 h at 28 °C with agitation. Cells were subsequently inoculated into 25 mL of fresh SLAD at  $1 \times 10^4$  cells mL<sup>-1</sup> and incubated for 16–18 h to obtain an exponential-phase culture.

The exponential-phase culture was diluted in Phosphate Buffered Saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) to  $2 \times 10^5$  cells mL<sup>-1</sup>. An aliquot of 5  $\mu$ L diluted culture was spotted at the centre of each plate (four to nine replicates per strain, per medium). Plates were incubated at 25 °C, yeast inoculum side up. Images of each mat were taken after 3, 6 and 9 days of incubation. To observe invasive growth structures, mats were washed with a gentle stream of water from a laboratory squeeze bottle after being kept at 4 °C for half an hour.

For paired SLAD mat assays, two diluted cultures were inoculated approximately 1 mm apart on SLAD agar.

### 3.4.3 Microscopy imaging and image processing

Mats were observed and imaged at  $40 \times$  magnification from the underside of the plates (with lid on) using a Nikon Eclipse 50i microscope and an attached Digital Sight DS-2MBWc camera with NIS-Elements F3.0 imaging software (Nikon). Four to six images were taken for each mat to capture all sectors. The sector images were stitched (Preibisch et al., 2009; Thévenaz and Unser, 2007) using Fiji software (Schindelin et al., 2012) to reconstruct a complete single mat image. The stitched image size of each mat was standardised using the stacking function in the same software. Detailed steps for image stitching and stacking are in the Supplementary Data.

#### 3.4.4 Conditioned medium mat assays

Yeast were cultured in 50 mL SLAD for 48 h. Cultures were washed twice with PBS before being inoculated into 20 mL of fresh SLAD or CM at  $1 \times 10^4$  cells mL<sup>-1</sup> and incubated for 16 h. Following incubation, cell images were taken at 400× magnification using the microscope and camera described above. Images were processed and cell elongation ratio (ratio of major to minor axis) was measured using Fiji software (Schindelin et al., 2012). Steps are described in the Supplementary Data. Cultures were also plated as described in SLAD mat assays.

Statistical analysis was performed with a Welch's T-test using GraphPad Prism version 7.02 software for Windows.

#### 3.4.5 Nitrogen and glucose measurement

Residual nitrogen and glucose were analysed spectrophotometrically with enzymatic methods. Nitrogen was analysed with a Megazyme Ammonia Assay Kit (Rapid; Cat No. K-AMIAR), according to the manufacturer's instructions. Glucose was measured according to the method of Mannheim (1989). For both assays,  $1 \times$  SLAD was used to generate a calibration curve.

## 3.5 Results

# 3.5.1 Nitrogen limitation induces filamentous and invasive growth in mats

Several commercial wine yeast strains and laboratory strain prototrophic  $\Sigma 1278b$  were tested for mat formation in SLAD to stimulate nitrogen limitation. Maximum mat size was approximately 4–5 mm in diameter, and this was often observed at the first time point analysed (3 days). In comparison, the maximum size of a colony grown from the same strains on SLAD with 2% agar reaches 1 mm (Joanna Sundstrom, pers. comm.). As the maximum mat size is at least 4-fold wider than colonies formed on SLAD with 2%agar, and cell growth occurs as a thin layer across the surface, these have been defined as mats rather than colonies. Mat expansion also extends beyond the boundary of the inoculum drop. This can be visualised in Appendix B, Figure B.1A, where growth of the mat inoculated with a single cell doubles in size between 3 and 9 days. The mats had no complex structures, but a subset of yeast cells grew filamentously and invaded the agar in all strains except I1 (Fig. 3.1A). The number of invasive foci varied between strains. Wine yeast had large, round invasive growth structures in comparison to  $\Sigma 1278b$  which were more filamentous (Fig. 3.1B). These structures can only be reached by breaking the agar, and thus were classified as invasive. The progression of filamentous, invasive mat formation for  $\Sigma 1278b$ , AWRI796 and EC1118 after 3, 6 and 9 days can be seen in Figure 3.1C. Interestingly, the size of the mat has limited changes, cells on the mat rim did not grow along the surface, yet the invasively growing cells continued to grow over time. Invasively growing cells at the mat rim grew much quicker than those inside the mat as shown by the different sizes of the structure.



Figure 3.1: SLAD mat assays on SLAD low-density agar (0.3%). (A) SLAD mat morphologies of  $\Sigma 1278b$  and selected wine yeast strains. Approximately 1000 cells were spotted on the plate. Four to six images were taken for each mat after 9 days and stitched to generate a single mat image. A typical representative image of each strain is shown. Scale bar, 1 mm. (B) Micrographs of mats post-wash showing invasive growth structures of  $\Sigma 1278b$  and the wine strain AWRI796. Please refer to next page for (C).


Figure 3.1: (C) Stitched mat images of  $\Sigma 1278b$  and the wine strains, AWRI796 and EC1118, after 3, 6 and 9 days. Note the same mat for each strain is imaged over the time series. Scale bar, 1 mm.

# 3.5.2 Mat size and biomass increases with increasing ammonium sulfate

Since nitrogen is a limiting growth factor and mat size on SLAD is reduced significantly compared to mats reported in rich medium, this section investigated whether there were any structural or size changes to mats as the amount of nitrogen increased. The filamentous invasive structures of  $\Sigma 1278b$  changed to a more rounded invasive structure when ten times more ammonium sulfate (500  $\mu$ M) was included in the medium (Fig. 3.2). The structures were still observed underneath the agar surface. The overall mat biomass and size increased with increasing ammonium sulfate for both  $\Sigma 1278b$  and AWRI796. With 100 times more ammonium sulfate (5 mM), the invasive structures of both strains were similar.



Figure 3.2: SLAD mat morphologies of  $\Sigma 1278b$  and AWRI796 after 3 days on SLAD low-density agar (0.3%) with 50  $\mu$ M, 500  $\mu$ M and 5 mM ammonium sulfate. The invasive structures of cells grown on 500  $\mu$ M and 5 mM ammonium sulfate could not be shown in the original brightness due to increased biomass. Therefore, brightness was increased to reveal the invasive structures.

#### 3.5.3 Filamentous growth is inhibited by a neighbouring mat

In order to test whether filamentous growth can be enhanced or inhibited by a neighbouring filamentous mat, paired SLAD mat assays were performed with cells inoculated at a distance of approximately 1 mm on the same plate. Filamentous growth was inhibited by

a neighbouring mat, regardless of whether it was the same or a different strain (Fig. 3.3). Cells at the rim away from a neighbouring inoculum continued to grow and were highly filamentous. When two inocula were close enough, filamentous growth was completely inhibited. In the case of a larger distance between inocula, filamentous growth was initiated but stopped when the gap reached approximately 3  $\mu$ m.



Figure 3.3: Paired SLAD mats at a close distance between  $\Sigma 1278b$  and AWRI796 (top panel) and  $\Sigma 1278b$  and  $\Sigma 1278b$  (bottom panel) on SLAD low-density agar (0.3%). Each whole mat is shown (left and right panels) while the middle panel shows a magnified view of the gap between the paired mats. Images were taken 18 days after inoculation. Each of the whole mat images has a scale of 1 mm and the middle images have a scale of 5  $\mu$ m.

## 3.5.4 Conditioned medium affects cell elongation in liquid culture but not invasive growth

Conditioned medium from a  $\Sigma 1278b$  stationary-phase culture has been previously shown to stimulate filamentous and invasive growth when it was used as the assay medium (Chen and Fink, 2006). This study tested if pre-exposure to conditioned medium would enhance the filamentous and invasive growth phenotype on SLAD agar. Stationary-phase cultures of  $\Sigma 1278b$  and AWRI796 were collected to prepare CM as described. These were then used to culture (in liquid) the same two strains overnight before analysis in a SLAD mat assay. After growing overnight in either CM, cell elongation ratio of both strains increased compared to an overnight culture in fresh SLAD (Fig. 3.4). The CM derived from AWRI796 resulted in a more enhanced response with longer cells formed by both strains (*p*-value < 0.0001). However, pre-culturing in CM from either strain did not result in enhanced filamentous invasive growth on SLAD agar for either strain (data not shown).



Figure 3.4: A boxplot showing cell elongation ratio (y-axis) of  $\Sigma 1278b$  and AWRI796 cultured in either SLAD,  $\Sigma 1278b$  CM or AWRI796 CM for 16 h. Cell elongation ratio is the ratio of major to minor axis of an ellipse (cell). For  $\Sigma 1278b$  cells, the mean cell elongation ratios were not significantly greater in  $\Sigma 1278b$  CM but significant in AWRI796 CM compared to in SLAD (*p*-values = 0.145 and 0.00984; df = 111.49 and 122.60). Mean cell elongation ratios of AWRI796 cells in  $\Sigma 1278b$  CM and AWRI796 CM were significantly greater than in SLAD (*p*-values = 1.291e-7 and 4.873e-10; df = 117.33 and 107.39).

## 3.5.5 Effect of aromatic alcohols, ethanol, hydrogen sulfide and sulfite on yeast growing on SLAD mat assays

Aromatic alcohols (tryptophol and 2-phenylethanol in combination) and sulfur compounds (H<sub>2</sub>S and sulfite) were examined for any effects on yeast mats growing on SLAD. Stock solutions of aromatic alcohols needed to be first dissolved in ethanol which has been reported to stimulate hyperfilamentation in diploid  $\Sigma 1278b$  (Lorenz et al., 2000). Therefore, the impact of ethanol alone was evaluated, in addition to aromatic alcohols, H<sub>2</sub>S (supplied as sodium sulfide) and sulfite. Without any additions to the medium,  $\Sigma 1278b$  had a larger proportion of cells that initiated agar invasion and filamentation compared to AWRI796 (Fig. 3.5A).  $\Sigma 1278b$  is commonly observed to rapidly undergo filamentation and invasive growth, and subsequently results in difficulty in deciphering differences in filamentous/invasive growth between conditions, except for the addition of sodium

metabisulfite (SMS) (Fig. 3.5A–E and F–J). SMS addition (0.01%) resulted in delays in both agar surface and invasive growth (Fig. 3.5F–J).

For AWRI796, the addition of 0.05% ethanol in SLAD increased the number of invasive foci and triggered early development of agar invasion (Fig. 3.5A and B). The presence of a total 100  $\mu$ M aromatic alcohols, however, suppressed this effect (Fig. 3.5C compared to B). The addition of 0.4 mg L<sup>-1</sup> sodium sulfide produced large numbers of invasive foci and reduced biomass on the agar surface (Fig. 3.5D). The effect remained with the combination of both aromatic alcohols and sulfide (Fig. 3.5E). All media containing SMS (Fig. 3.5F–J) resulted in reduced biomass (both surface and invasive). When ethanol, sodium sulfide and SMS were added, the majority of AWRI796 cells grew invasively (Fig. 3.5I). However, inclusion of aromatic alcohols returned cells to almost exclusively surface growth (Fig. 3.5J). Thus, it seems that the suppressive effect on invasive growth of aromatic alcohols is alleviated when sulfide is present and is supported in the presence of SMS, indicating an interplay between sulfide and sulfite.



Figure 3.5: SLAD mat morphologies of  $\Sigma$ 1278b and AWRI796 on Day 3 (D3), 6 (D6) and 9 (D9) on SLAD low-density agar (0.3%) (A) without any additions, with the addition of (B) ethanol (EtOH), (C) combination of ethanol and aromatic alcohols (AA), (D) combination of ethanol and sulfide (H<sub>2</sub>S), (E) combination of ethanol, aromatic alcohols and sulfide. Please refer to next page for (F–J).



Figure 3.5: (F–J) the same combinations as in A–E with the addition of sulfite (SMS).

#### 3.6 Discussion

Yeast grow into a large mat of a thin layer of cells across the surface of low-density YPD agar with some strains forming complex architecture (Reynolds and Fink, 2001; Hope and Dunham, 2014). Karunanithi and colleagues (2012) showed that filamentous mats can also be formed by  $\Sigma$ 1278b on YP medium in which glucose is limited. This study reports a different mat morphology formed by  $\Sigma$ 1278b and wine yeast strains on SLAD, where nitrogen is limited. Mat size was reduced, no complex architecture was formed and a proportion of cells switched to filamentous and invasive growth. This study further show that addition of some key yeast metabolic compounds affects invasive growth of these mats.

The low nitrogen conditions of SLAD agar limited cell spreading across the agar and instead, a subset of cells underwent pseudohyphal growth and invaded the agar. This response is consistent with the findings reported in other studies investigating nitrogen limitation on medium-density agar (Gimeno et al., 1992; Zupan and Raspor, 2010). Whilst there were no obvious morphology variations in the surface growing cells between strains, the number of invasive foci did vary between strains. The invasive foci of wine yeasts were more rounded in structure compared to those of  $\Sigma 1278b$  (Fig. 3.1B). This may simply represent more rapidly growing or less pseudohyphal cells. One reason may be that wine yeast have developed the ability to utilise nitrogen more efficiently, and thus be able to grow rapidly in the presence of limited nutrients compared to laboratory yeast, since they have evolved to survive multiple stresses in the winemaking environment. This is supported by the observations of increased nitrogen content in SLAD,  $\Sigma 1278b$  also grew into rounded invasive structures similar to wine yeasts (Fig. 3.2). This indicates that nutrient sufficiency may be reflected by rounded invasive structures. The increase in overall mat size also suggests that nitrogen is important for mat expansion, primarily for cellular growth.

Within each mat, (filamentous) invasive growth continued over time and was most rapid at the mat rim compared to the centre. This may be a result of less competition for nutrients at the rim due to lower cell density, and therefore cell division is supported and more likely to expand. In order to determine if filamentous growth can be induced by another filamentous mat in close proximity, cells of AWRI796 (less filamentous) and  $\Sigma 1278b$  (highly filamentous) were inoculated close to each other. In this experiment, filamentous growth was inhibited on the side closest to the neighbouring mat. The same occurred when two inocula of  $\Sigma 1278b$  were spotted next to each other, suggesting that the inhibition is neither strain dependent nor unidirectional. This observation is consistent with the repulsion between two neighbouring filamentous colonies of bacteria (Matsushita and Fujikawa, 1990). The inhibition was not evident in yeast surface filamentation colonies in other study in medium-density agar, but it may be due to the shorter incubation time *i.e.* three days (Liu et al., 1993). Non-filamentous yeast colony growth has been shown to be affected by the presence of a neighbouring colony. This was due to the release of ammonia via the amino acid permease Shr3p as a signalling molecule from colonies to prevent growth towards a neighbouring colony (Palková et al., 1997). The ammonia signalling may be involved in the filamentous growth inhibition between the two neighbouring mats.

In this study, several compounds were found to affect filamentous and invasive growth of mats on SLAD agar. However, the effect was not obvious for  $\Sigma 1278b$ . This strain readily forms filamentous and invasive cells, even in the control condition (SLAD).  $\Sigma 1278b$  is well known for its expeditious ability to invasively grow and is chosen for filamentous studies since it seems to be extremely sensitive to ammonia repression of nitrogen assimilation pathways (Rytka, 1975; Wiame et al., 1985; Gimeno et al., 1992).

For AWRI796, the transition to invasive growth in response to low nitrogen was enahnced by the addition of ethanol (Fig. 3.5A and B). FLO11 is required for filamentous and invasive growth during nitrogen starvation (Braus et al., 2003; Lo and Dranginis, 1998; Robertson and Fink, 1998). This gene can be regulated epigenetically by the binding of Sfl1p which requires histone deacetylase Hda1p (Halme et al., 2004; Octavio et al., 2009). Immunofluorescence analysis has shown that Flo11p was present on the cell surfaces of pseudohyphal or filament-forming cells and was silenced in the yeast form cells derived from the same clone (Guo et al., 2000; Halme et al., 2004). This may explain observations in this study that only a subset of cells transitioned to grow invasively. Ethanol has been previously shown to abolish Sf1p-mediated silencing (Octavio et al., 2009), which is consistent with the observation that ethanol addition to SLAD increased and induced an early development of agar invasion and filamentous growth. Interestingly, no enhancement of invasive growth by aromatic alcohols was observed, as was reported by Chen and Fink (2006) on 2% agar, but instead their addition suppressed the enhancement effect of ethanol (Fig. 3.5B and C). This may be due to variation in experimental preparation as Chen and Fink (2006) did not report the method for preparation of the aromatic alcohols.

The effect of addition of  $H_2S$  was also analysed, since evidence suggests that  $H_2S$  is a signalling mediator for stress resistance and longevity.  $H_2S$  offers protection against nutrient and oxygen deprivation in mammals (Blackstone and Roth, 2007; Hine and Mitchell, 2015). Similar  $H_2S$ -mediated biological benefits are also found in yeast. For example, the mutant  $\Delta met17$  has increased  $H_2S$  production and an extended chronological lifespan (Johnson and Johnson, 2014; Linderholm et al., 2008). The mutant also had increased resistance to heat shock, oxidative and heavy metal stresses, and metal chelate toxicity, suggesting that  $H_2S$  could lead to an adaptive response to environmental stresses (Singh and Sherman, 1974; Brown et al., 2006; Hwang et al., 2007; Johnson et al., 2014). This study reports a novel response to  $H_2S$ , which is to enhance invasive growth. Historically, invasive growth has been hypothesised to be a survival strategy adopted by yeast during nitrogen starvation that is believed to be a mode of action to forage for nutrients.  $H_2S$  is well known to be released by yeast upon nitrogen limitation, perhaps this extruded sulfide is sensed by nearby cells as a cue to switch to invasive growth. The mechanism that may

allow cells to respond to this putative  $H_2S$  cue is not yet known, however evidence supports involvement of the Retrograde-MAPK pathway. *RTG3* that activates the mitochondrial retrograde (RTG) signalling was shown to be required by a  $\Delta met17$  mutant to confer longevity and stress tolerance (Johnson and Johnson, 2014). The RTG pathway is usually activated when mitochondrial function is compromised, but the signalling pathway is unclear (Liu and Butow, 2006). Several eukaryotic models have demonstrated a tight inverse relationship between oxygen availability and H<sub>2</sub>S production in mitochondria, leading to the proposal of sulfide metabolism as an oxygen sensor (Doeller et al., 2005; Furne et al., 2008; Olson and Whitfield, 2010; Olson et al., 2010; Olson, 2012). Fission yeast, *Schizosaccharomyces pombe*, exposed to H<sub>2</sub>S results in downregulation of many mitochondrial genes and reduced mitochondrial oxygen consumption, which may result in RTG pathway activation (Jia et al., 2011). Many genes involved in the RTG pathway have been identified as positive regulators of filamentous MAPK cascade, thus supporting the enhancement of filamentous and invasive growth by H<sub>2</sub>S via the RTG-MAPK pathway (Chavel et al., 2014).

Exposure to sulfite delayed cellular and invasive growth in both  $\Sigma 1278b$  and AWRI796 (Fig. 3.5F–J). Less filamentous growth was also observed in  $\Sigma$ 1278b in the presence of sulfite. Cells undergoing filamentous growth display prolonged apical growth leading to highly polarised growth at the bud tip, and this is tightly regulated with cell cycle (Pruyne and Bretscher, 2000). A transcriptomics study of S. cerevisiae strain 3090-1d showed that sulfite exposure downregulated cell cycle and polarity-related genes including MYO1, BNR1 and PCL1 (Park and Hwang, 2008). This may reduce the hyperpolarisation event and in turn filamentous growth upon sulfite exposure. Growth, although delayed, did occur upon sulfite exposure suggesting adaption and tolerance to sulfite. Sulfite is produced as part of the sulfate assimilation pathway. The effect of sulfite is of interest to this study because sulfite is usually converted to sulfide and eventually metabolised into methionine, cysteine and glutathione. However, sulfite can be produced and excreted in the form of sulfur dioxide  $(SO_2)$ . Excess sulfite has antioxidant and antimicrobial activity and has previously been shown to reduce invasive growth (Zupan and Raspor, 2010). In comparison, this study used a lower concentration of sulfite (1.05 vs 9.61 mM) and hence did not completely block invasive growth.

Pre-exposure of CM was found to have no impact on invasive growth despite having triggered cell elongation in liquid culture (Fig. 3.4). This result suggests that cells decide their phenotypic fate based on an environmental trigger after inoculation onto agar plates. This supports the claim that yeast have developed mechanisms to quickly adapt to environmental changes. Several studies have investigated short and long term responses to the fluctuations in environmental factors. A transient transcriptional change was found immediately after temperature shifts before an adaptation to a new steady state of transcript levels (Gasch et al., 2000). Another transcriptomics study also showed that yeast re-programmed a number of metabolic networks rapidly towards nutritional perturbation (Dikicioglu et al., 2011). The findings from integrated data from both transcriptomics and metabolomics studies showed that glucose impulse in glucose-starved conditions provoked changes in carbon metabolism, purine and pyrimidine biosynthetic pathways, folate metabolism, superpathway of serine and glycine and the methionine biosynthetic pathways, and aspartate and glutamate biosynthetic pathways (Dikicioglu et al., 2012). Ammonium impulse in nitrogen-starved conditions also affected these metabolic pathways with different profiles. These reports suggest that in the present study, it could be possible that the yeast cells were re-programmed to elongate when cultured in CM, and quickly re-programmed again when transferred to SLAD agar.

The findings of this study build upon the current understanding of the effect of nitrogen limitation on mat formation and the influence of yeast metabolites, ethanol, aromatic alcohols,  $H_2S$  and sulfite. Further study should involve genetic analysis of the induction or inhibition of invasive growth by these compounds. These observations provide a further opportunity to study the physiological role of each of the metabolites and the pathways leading to the effect upon cellular differentiation.

## 3.7 Funding

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Conflict of interest: None declared.

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### 3.9 Supplementary Data

#### 3.9.1 Methods

#### Mat image stitching and stacking

- 1. Create a folder for stitching process, only import the group of images needing to be stitched one at one time.
- 2. Download and install Fiji from http://imagej.net/Fiji/Downloads.

- 3. Open the application.
- 4. Click **Plugins** from the menu, select **Stitching**, and select **Grid/Collection** stitching.
- 5. Choose Sequential Images from the drop-down menu for Type, click OK.
- 6. In the Directory, choose the path to the folder created for stitching containing images to be stitched, click **OK**.
- 7. Click **OK** to confirm image files to be stitched.
- 8. A stitched image will be generated. Save the image as a TIFF file.
- 9. For images that cannot be stitched using the method above, use MosaicJ to manually assemble the images.
- 10. Click Plugins from the menu, select Stitching, and select MosaicJ.
- 11. A new window will open.
- 12. Click File, select Open Image Sequence.
- 13. Select the first image in the stitching folder, click **Open**.
- 14. All images in the folder will be loaded.
- 15. Click an image, the image will be in the working space. Drag images to assemble at the correct position.
- 16. Click File from the menu, select Create Mosaic.
- 17. A stitched image will be generated. Save the image as a JPEG file as the TIFF image created using this method is very large.
- 18. For stacking, open the largest image which all other images will be normalised to.
- 19. Open other images to be normalised.
- 20. Click Image from the menu, select Stacks, and select Images to Stack.
- 21. Click OK. Images are now stacked.
- 22. Click Image from the menu, select Stacks, and select Stack to Images.
- 23. Individual images are shown with the normalised size.
- 24. To fill black area with background colour of the original image, click **Colour Picker** icon, and then click on the background to pick up colour. Click **Blood Fill Tool** icon, and click on the black area to fill with background colour.
- 25. Save the image.

#### Cell elongation ratio measurement

- 1. Open the image that needs to be measured (in this example, images were taken at  $400 \times$  magnification).
- 2. Click **Analyze** from the menu, select **Set Scale**. Enter 64 pixels = 10  $\mu$ m (Note: this needs to be checked for each individual microscope), check **Global** and click **OK**. Now the image and the following images opened within this session have been calibrated.
- 3. Click **Process** from the menu, select **Subtract Background**. Input **40** pixels for Rolling ball radius, check **Light background**, click **OK**.
- To convert into a black and white image, click Image from the menu, choose Adjust and select Threshold. Select Default and B&W from the drop-down menu, click Apply and Close.
- 5. Click **Process** from the menu, select **Binary**, and select **Fill Holes**.
- 6. Click **Process** from the menu, select **Binary**, and select **Watershed**.
- 7. To remove non-cell particles, click **Colour Picker** icon, and then click on the white area in the image. Click **Paintbrush Tool** icon, double right-click the icon to set brush width, and click **OK**.
- 8. Click (and drag) on the non-cell particles to be removed. Save the processed image if necessary.
- 9. To set measurements that need to be performed (only need to do this once), click Analyze from the menu, select Set Measurements. Check Area, Shape descriptors, Fit ellipse, Feret's diameter and Display label. Choose None for Redirect to and 3 for Decimal places. Click OK.
- To measure, click Analyze, select Analyze particles. Size (μm<sup>^2</sup>): 5–infinity; Circularity: 0–1; Show: Overlay Outlines; check Display results, Summarize, Exclude on edges, click OK.
- 11. Save Results table for later use. Save the outline image if necessary.
- 12. Use AR (aspect ratio) in the Results table as the cell elongation ratio for statistical analysis.

## Chapter 4

# Understanding wine yeast invasive growth through transcriptional analysis

## **Contextual statement**

Chapter 3 highlighted that nitrogen limitation leads to yeast invasive growth in low-density agar medium. This response was consistent across all strains except for one which had poor overall growth. Several environmental factors could manipulate this invasive growth. Chapter 4 addresses the final aim of the project: to investigate the triggers and regulation of wine yeast biofilms and invasive growth. The manuscript in this chapter presents genes and biological processes associated with invasive growth in low-nitrogen mat conditions.

## Statement of Authorship

Title of Paper	Transcriptional analysis of invasively growing wine strains of Saccharomyces cerevisiae		
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#### **Principal Author**

Name of Principal Author (Candidate)	Ee Lin Tek		
Contribution to the Paper	Performed all experiments and data analysis, interpreted data, and wrote manuscript.		
Overall percentage (%)	80%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature	Date 04/09/17		

#### **Co-Author Contributions**

By signing the Statement of Authorship, each author certifies that:

- i. the candidate's stated contribution to the publication is accurate (as detailed above);
- ii. permission is granted for the candidate in include the publication in the thesis; and
- iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Andrew R. Hesketh	Andrew R. Hesketh		
Contribution to the Paper	Supervised RNA-sequencing	data processing and analysis, ec	liting of the manuscript.	
Signature		Date	30th Aug.2017	

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Contribution to the Paper	Supervised development of work, helped in data interpretation and editing of the manuscript.				
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Signature	Date	6/09/17	
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Contribution to the Paper	Supervised development of work and helped in data inte	pretation.	
Signature	Date	30.08.17	
Signature Name of Co-Author	Date	30.08.17	
Signature Name of Co-Author Contribution to the Paper	Date     Date     Vladimir Jiranek     Supervised development of work, helped in data interpre	ation and editing of the manuscript.	

## Transcriptional analysis of invasively growing wine strains of *Saccharomyces cerevisiae*

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#### 4.1 Keywords

Invasive growth; diploid wine yeast; FPS1; low nitrogen; hexose transporter

#### 4.2 Summary

In response to unfavourable conditions such as nutritional and environmental stress, the yeast Saccharomyces cerevisiae can switch to filamentous growth and/or invasive growth by re-programing cellular systems. A number of signal transduction pathways have been identified that regulate these processes, some of which are common to both filamentous and invasive growth. Previous genome-wide studies have identified biological pathways specific to invasive growth, however, the regulation of these is yet to be completely understood. Using differential transcriptome analysis of surface and invasively growing cells of a diploid wine yeast strain, this study found genes and biological processes that have not previously been associated with invasive growth. The analysis identified 272 genes that were upregulated and 84 genes downregulated in invasively growing cells. Enriched Gene Ontology categories of upregulated genes included medium-chain fatty acid biosynthetic process, carbohydrate metabolic process, cellular water homeostasis, fungal-type cell wall organisation, and glucose import. Further analysis of deletion mutants confirmed that FPS1, encoding for the glycerol export protein, is required for invasive growth. This work also identified a hypothetical gene, that has a portion of translated gene sequence homologous to an amidase domain, may have a role in invasive growth.

#### 4.3 Introduction

The budding yeast *S. cerevisiae* is able to adapt in response to various nutritional stresses (e.g. glucose and nitrogen limitation) to aid survival. This yeast is dimorphic, in that it can undergo a developmental switch from round, single cells to an elongated (pseudohyphal) multicellular filamentous form (Gimeno et al., 1992; Kron et al., 1994). This results in both haploid and diploid cell types capable of invasion into agar (Gimeno et al., 1992; Cullen and Sprague, 2000). Since yeast cells are non-motile, it is believed that this change in morphology allows them to forage for nutrients. These modes of growth have also been shown to be influenced by strain genetic backgrounds and other physiochemical factors rather than simply nutrient depletion, for example the presence of fusel alcohols, pH, temperature, salt and atmosphere (Zupan and Raspor, 2010; Dickinson, 1996). Yeast can also switch from fluffy and smooth colonies in response to environmental conditions and is accompanied with change in gene expression (Kuthan et al., 2003).

Four signalling pathways shown to regulate pseudohyphal growth have been

well documented and reviewed by Cullen and Sprague (2012). These include the cyclic AMP-dependent protein kinase A (cAMP-PKA) pathway, the Snf1 pathway, the target of rapamycin (TOR) pathway and the mitogen-activated protein kinase (MAPK) pathway. Some proteins in these pathways are also required for invasive growth. For example, components of the MAPK pathway, required for pseudohyphal formation, Ste20p, Ste11p, Ste7p, Kss1p, and Ste12p, are also required for invasive growth (Roberts and Fink, 1994; Cook et al., 1997). Likewise, Tpk2p of the cAMP-PKA pathway is essential for both pseudohyphal and invasive growth (Robertson and Fink, 1998). In relation to the Snf1 pathway, yeast with a deletion of SNF1 were unable to invasively grow or undergo cell elongation (Vyas et al., 2003; Cullen and Sprague, 2000). Rapamycin inhibits invasive growth, which can be restored by overexpression of TAP42, showing that the TOR pathway is involved (Cutler et al., 2001). The cell surface flocculin, Flo11p, is widely regarded as the primary mechanism for cell-cell and cell-surface adhesion, important for pseudohyphal and invasive growth. Regulation of Flo11p is shared by the cAMP-PKA, Snf1 and MAPK pathways, *i.e.* Tpk2p, Snf1p, and Ste12p are all involved (Lo and Dranginis, 1998; Rupp et al., 1999; Kuchin et al., 2002; Pan and Heitman, 2002).

In diploid cells, pseudohyphal and invasive growth can occur simultaneously or independently, suggesting that apart from the shared core signalling pathways, it is likely there are invasive growth specific pathways. A number of studies have identified genes and biological pathways necessary for invasive growth, and these have involved screening of loss-of-function and overexpression mutants in a range of media (Jin et al., 2008; Ryan et al., 2012; Shively et al., 2013). Jin and colleagues (2008) screened a transposon insertion gene disruption and overexpression library in a haploid version of the filamentous strain of S. cerevisiae,  $\Sigma 1278b$ , to identify genes necessary for filamentous growth induced by butanol. They found 243 out of 487 genes were also necessary for haploid invasive growth. Ryan and colleagues (2012) screened both haploid and diploid  $\Sigma$ 1278b gene deletion libraries and identified 577 genes required for haploid invasive growth in rich medium, of these 132 were also required for diploid pseudohyphal growth. However, this group measured relative filamentous outgrowth to determine extent of pseudohyphal growth, which does not take into account agar invasion. In contrast, Shively and colleagues (2013) found 551 genes that when overexpressed exaggerated diploid invasive growth in sufficient nitrogen. The loss-of-function and overexpression studies were comprehensive, yet they are limited by the resources (*i.e.* number of mutants in the libraries versus number of verified ORFs) and they were studied in different media. Studying gene expression profiles offers an additional layer of information to understand active physiological processes that are associated with the condition of interest and potentially predict novel functions of genes.

In this study, RNA-sequencing technology was used to compare transcriptomes of diploid wine yeast cells collected from the agar surface to cells growing invasively which also included the filamentous phenotype. In the invasively growing population, 272 genes were upregulated and 84 genes were downregulated. Surprisingly, no transcripts from the mentioned four signalling pathways were differentially expressed. Enriched Gene Ontology (GO) terms of the upregulated gene sets showed that medium-chain fatty acid biosynthetic process, carbohydrate metabolic process, cellular water homeostasis, fungal-type cell wall organisation, and glucose import may be important processes for invasive growth. A number of the upregulated genes identified in this study are common to previous mutant screening studies. The association of cellular water homeostasis to invasive growth is novel, and thus further deletion analysis of genes within this group identified FPS1 as being necessary for invasive growth. Results from this work also uncovered a potential role for a hypothetical gene.

## 4.4 Results and Discussion

## 4.4.1 Global change in gene expression between surface and invasively growing cells

Total RNA was extracted from cells of the diploid wine yeast, AWRI796, collected from the surface and invasively growing into a low percentage (0.3%), low nitrogen agar medium. Three replicates for each growth type were used with an RNA-sequencing approach to profile global changes in gene expression between surface and invasively growing cells. Sequencing yielded between 35 and 37 million reads from each RNA sample. Differentially expressed genes were defined as those deemed to be statistically significant based on a t-test relative to a 1.3-fold-change threshold using the *limma* TREAT method of McCarthy and Smyth (2009). In the invasively growing population, 272 genes were upregulated and 84 genes downregulated, compared to surface growing cells (Table S1). Four of the ten genes having the largest change in expression were hexose transporter genes (HXT3, HXT4, HXT6 and HXT7; Table 4.1). Among these genes, HXT4 has been reported to affect filamentous growth and is required for invasive growth under butanol induction (Jin et al., 2008).

Gene Symbol	Name	$\log_2$ Fold Change	Adj. <i>p</i> -value
HXT7	HeXose Transporter	+3.78	1.39E-8
HXT4	HeXose Transporter	+3.04	1.58E-8
AWRI796_5153	Amidase	+2.69	3.30E-7
SSA2	Stress-Seventy subfamily A	+2.26	1.34E-7
HXT6	HeXose Transporter	+2.25	5.40 E-8
RGI1	Respiratory Growth Induced	+1.92	5.77E-8
AWRI796_2017	NA	+1.79	3.30E-7
HXT3	HeXose Transporter	+1.74	3.72E-7
CAR1	Catabolism of ARginine	+1.73	3.30E-7
NCE103	NonClassical Export	-1.72	6.93E-8

Table 4.1: Top 10 genes with the largest change in gene expression in invasively growing cells.

To investigate the biological processes that are associated with invasive growth, GO enrichment analysis was performed. This analysis revealed 37 enriched GO terms for upregulated genes, while none were identified for downregulated genes (Table S2). The enriched GO terms were summarised into five main groups by clustering similar GO terms using REVIGO (Supek et al., 2011), these were medium-chain fatty acid biosynthetic process, carbohydrate metabolic process, cellular water homeostasis, fungal-type cell wall organisation, and glucose import (Fig. 4.1). KEGG pathway analysis of the upregulated and downregulated genes did not identify any overrepresented pathways, including signal transduction pathways required for invasive growth. This may be due to strain differences *i.e.* wine yeast versus laboratory yeast. Wine yeast may have conserved signalling pathways involving genes that have not yet been characterised, and thus not represented in this transcriptomics analysis.



Figure 4.1: Enriched GO terms (37) of upregulated genes of the invasively growing cells submitted to the REVIGO program. Representative GO categories are shown by circles and visualised by clusters of semantically similar GO terms. Circle colour indicates  $\log_{10}$  adjusted *p*-value from the GO enrichment analysis whereas size represents the frequency of the GO term in the underlying GOA database (circles of more general terms are larger).

A number of interesting findings were obtained from differential expression analysis. Approximately 25% (67 genes) of upregulated genes have been reported in other studies as important for invasive growth or associated phenotypes (*i.e.* diploid pseudohyphal growth, haploid and diploid invasive growth, and differential expression between fluffy and smooth colonies; Table S3). Ten genes were found in more than one other data set, these being ARG8, ERG6, FLO11, IKI3, PMT2, SIN3, SLA1, MGA1, PUT4 and HXT4. However, this is not an exact comparison since: (1) each study used a different conditioned medium (e.g. rich YPD, low nitrogen SLAD, butanol treatment or alternative carbon source GMA); (2) strain ploidy and phenotypes assayed were different. The present study focuses on the differences between surface and invasive growth, regardless of elongated or ovoid cells, expecting to identify genes and/or biological processes that are important but not easily detected in single gene deletion or overexpression screen studies. Interestingly, a handful of upregulated genes identified in invasively growing cells are common to previously reported upregulated genes of fluffy and downregulated genes of smooth colonies (19 of 165 and 14 of 147, respectively; Kuthan et al., 2003). Yeast colonies with fluffy structure were suggested as a metabolic strategy in unfavourable conditions (Kuthan et al., 2003). This structure changed to smooth when culturing in laboratory conditions and accompanied with a change in gene expression. The presence of common genes expressed in fluffy colonies as well as in invasive growth may indicate a similar strategy used for hostile conditions.

#### 4.4.2 Glucose import

Four hexose transporter genes (HXT3, HXT4, HXT6 and HXT7) with increased in gene expression ranging from 3.34- to 13.7-fold in the invasively growing cells may be related to the availability of nutrients in the medium. Previous studies have shown that these genes are important to recover glucose uptake after ammonium supplementation following a nitrogen-limited sluggish fermentation (Palma et al., 2012). Luyten and colleagues (2002) showed that HXT6 and HXT7 are essential at the end of alcoholic fermentation where the uptake of sugar is often perturbed by the stressful environment of low nutrient and high ethanol. It seems that these genes encoding high-affinity glucose transporters are equally important in the SLADS (containing limited nitrogen supplied solely by ammonium sulfate) conditions of this study. Furthermore, the expression of these four genes was also associated with structured morphologies (Table S3), suggesting a strategy to cope with unfavourable conditions (Kuthan et al., 2003).

## 4.4.3 Carbohydrate metabolism / fungal-type cell wall organisation

Transcriptomics analysis showed genes of carbohydrate metabolic processes were significantly upregulated in invasively growing cells (Fig. 4.1, Table S2). These included glucose phosphorylation (*GLK1*, *HXK1*, *HXK2*), phosphoglucomutase (*PGM2*), galactokinase (*GAL1*), and maltose permease (*MAL31*). Some genes present in both carbohydrate metabolic process and fungal-type cell wall organisation GO categories were also upregulated in invasively growing cells. For example, *YEA4*, *EXG2*, *GAS1* and *GAS5* are involved in chitin and  $\beta$ -glucan maintenance. Other genes encoding cell wall components, especially fungal-type cell wall components (GO:0009277; p < 2.07E-5) were also upregulated in invasively growing cells (Table S2). Since yeast cells are non-motile, access to nutrients is largely dependent on a cell's access to proximal space. For cells growing on a surface, the expansion of a colony is limited by the agar concentration and nutrients (Chen et al., 2014). If cells were pushed upward, access to nutrients from solid medium becomes limited. Eventually, these cells undergo chronological aging differentiation (Palková et al., 2014). The action of invasion into a medium results in three dimensional access to nutrients, allowing cellular growth and division to continue. Many genes encoding cell wall components were upregulated in the invasively growing cells compared to surface growing cells (Fig. 4.1; Table S2). This suggests cell wall construction is strongly coordinated with cell cycle progress and cell growth activity in the invasively growing population (Klis et al., 2006). Together with the upregulation of genes involved in glucose import and carbohydrate metabolic processes (Fig. 4.1; Table S2), and the time series images of limited surface cell expansion whilst continual invasive population growth occurred (Fig. 4.6B and S2), data in this study supports the hypothesis that invading cells have greater access to nutrients, which aids growth.

AWRI796\_5153 is a hypothetical gene with the third largest change in expression (increased expression by 6.45-fold; Table 4.1). Interestingly, a portion of the gene sequence, when translated, is homologous to an amidase domain. Amidases (KEGG identifier EC 3.5.1.4) function by catalysing the hydrolysis of short-chain amides to organic acids, thereby releasing ammonia. Ammonia is known to be a yeast colony signalling molecule produced during starvation (Palková et al., 1997). To date, amidase is poorly characterised in yeast. However, in bacteria, this protein is normally used to breakdown peptidoglycan for cell wall recycling (Litzinger et al., 2010; Johnson et al., 2013). Other studies have also reported that amidase is involved in the regulation of quorum sensing, biofilms and virulence in bacteria (Ochiai et al., 2014; Clamens et al., 2017).

#### 4.4.4 Medium-chain fatty acid biosynthesis pathway

All three genes, *EEB1*, *EHT1* and *MGL2*, categorised under the medium-chain fatty acid biosynthetic process (GO:0051792; p < 0.015) were upregulated in invasively growing cells. *EEB1* is required for invasive growth upon butanol induction whereas *EHT1* is required for pseudohyphal growth (Jin et al., 2008; Ryan et al., 2012). Eeb1p and Eht1p are part of medium-chain fatty acid ethyl ester production (Saerens et al., 2006). Ethyl ester is known to contribute to aroma and flavour in alcoholic beverages, but its role in yeast is unclear (Swiegers and Pretorius, 2005; Swiegers et al., 2005). Several reasons have been given for ethyl ester production: (1) to reduce the toxicity of medium-chain fatty acids, (2) to regenerate free co-enzyme A, and (3) to attract *Drosophila* for dispersal in nature (Thurston et al., 1982; Bardi et al., 1998; Lilly et al., 2006; Saerens et al., 2010; Christiaens et al., 2014).

Overexpression of MGL2 reduced triacylglycerol and sterol ester but increased

phosphatidylcholine and phosphatidylethanolamine in the cell, indicating a change in lipid profile (Selvaraju et al., 2016). An increase in phospholipids as a result of increased MGL2expression may suggest a demand for plasma membrane synthesis, which may be due to an increase in cell size or cellular growth in the invasively growing population.

## 4.4.5 Genetic interaction network analysis predicts genes modulating invasive growth

Genetic interaction network analysis was used (see Experimental Procedures) to determine if any of the differentially expressed genes exhibited extensive connectivity. Network-based approaches are particularly useful in characterising complex biological systems (Barabási and Oltvai, 2004). By establishing a network graph using both differentially expressed genes in this study and interaction data from the Data Repository of Yeast Genetic Interactions, sum of interactions between genes in invasively growing cells can be shown at system-scale and this allowed determination of significant genes from their centrality and connectivity (Zotenko et al., 2008; Koh et al., 2010). In the hub of the largest connected network, three genes were shown to have high connectivity: MSC1, SIN3 and ARO1, with each having at least 30 edges (degree) connected to the node (Fig. S1). Of the three genes, SIN3, which encodes a component of Rpd3 historie deacetylase complexes, has also been identified as being required for pseudohyphal and invasive growth (Carrozza et al., 2005; Ryan et al., 2012). After analysing the network, the connectivity (Betweenness Centrality) value of each gene was examined with the change in expression level. This allowed the discovery of genes that have a larger role in modulating or association with invasive growth. Several genes have been highlighted to have a relatively large value in both Betweenness Centrality and change in expression (Fig. 4.2).



Figure 4.2: Relationship between Betweenness Centrality within the genetic interaction network of differentially expressed genes and changes in gene expression. Each circle represents a gene. Gene names are shown only for those meeting both criteria of absolute  $\log_2$  fold change greater than 0.7 and Betweenness Centrality greater than 0.015.

Some of these are related to previously identified enriched GO categories: (fungaltype) cell wall organisation and carbohydrate metabolic process such as HXK2, GAS1 and FKS1. Notably, ARO1 and ARO8 both upregulated in invasively growing cells, encode proteins that are involved in phenylalanine and tyrosine biosynthesis, which are precursors of suggested quorum sensing aromatic alcohols in S. cerevisiae (Chen and Fink, 2006). ARO1 is downregulated in smooth colonies compared to fluffy colonies (Kuthan et al., 2003). Genes IME1, MSH4 and EMI2 are involved in meiotic events. IME1 and MSH4 were downregulated in invasively growing cells, while *EMI2* was upregulated. *EMI2* has a paralog, GLK1, which encodes for glucokinase. In this case, the upregulation of EMI2 may not be due to meiotic function, but could be linked to glucose metabolism. The plot also identified genes related to mitochondria such as CIS1, NUM1 and CSF1, and genes related to cell cycle: DYN1, encodes for protein involved in spindle assembly and orientation and CLB2, encodes for cyclin involved in cell cycle progression. CLB2 overexpression delays  $G_2/M$  transition, which means apical growth is prolonged to induce invasive growth (Simpson-Lavy et al., 2009; Shively et al., 2013). Prolonged apical growth that is required for surface filamentation may also be involved in the induction of invasive growth.

RGI1, whose expression increased by 3.78-fold in the invasively growing cells (Table 4.1), has been reported to be involved in aerobic sugar metabolism (Rep et al., 2000; Domitrovic et al., 2010). Anaerobiosis within a nitrogen atmosphere inhibits invasive growth (Zupan and Raspor, 2010). Previous work in this laboratory has also shown that invasive growth is inhibited in the absence of oxygen as well as in 'petite' strains (Joanna

Sundstrom and Vladimir Jiranek, *pers. comm.*). Together with the upregulation of several genes related to or found in mitochondria, as identified from the plot in Figure 4.2 and *AIM17* (Table S1; Hess et al., 2009), this suggests that respiration may be required for invasive growth.

## 4.4.6 Protein interaction network analysis suggests Ssa2p as the major determinant of invasive growth

Protein-protein interactions were visualised (see Experimental Procedures) between the differentially expressed genes in a network graph using the *mentha* database (Calderone et al., 2013). Similar to the genetic network analysis, this enabled the discovery of high connectivity proteins, thereby reflecting their biological importance in invasive growth. In comparison to genetic interactions that provide information on how genes control cellular processes, protein interactions present functional connections. In the latter, Ssa2p was identified as the key node of the largest connected network, having the most number of edges (degree = 14) and the largest Betweenness Centrality value (0.77; Fig. 4.3). In addition, SSA2 had the fourth largest change in gene expression with nearly a five-fold increase in invasively growing cells (Table 4.1).



Figure 4.3: Protein-protein interaction network of differentially expressed genes between surface and invasively growing cells. A 25% fold-change threshold was applied for the differential expression analysis. The largest connected network with an interaction confidence score of at least 0.3 is shown. Red and green node colours represent upregulation and downregulation in invasively growing cells respectively; larger node size represents higher values of Betweenness Centrality, and vice versa.

SSA2 is required for pseudohyphal growth but not haploid invasive growth (Ryan et al., 2012). However, Shively and colleagues (2013) reported that it enhanced invasive growth (agar invasion score 0.96) when overexpressed in diploids. This protein is involved in protein folding and transport, suggesting that it may act as a checkpoint protein to ensure functionality of other proteins involving in invasive growth. The importance of this gene, along with *NOG2* and *HSP26*, is also shown in the relationship plot between their protein connectivity and change in expression (Fig. 4.4). Nog2p is a putative GTPase involved in exporting the large ribosomal subunit while Hsp26p is a chaperone which binds and prevents unfolded proteins forming large aggregates (Saveanu et al., 2001; White et al., 2006).



Figure 4.4: Relationship between the Betweenness Centrality of each gene in the protein interaction network and their corresponding change in gene expression. Each circle represents a single gene. Gene names are shown only for those meeting both criteria of absolute  $\log_2$  fold change greater than 0.8 and Betweenness Centrality greater than 0.125.

## 4.4.7 Expression levels of transcription factor genes do not correlate with their previously reported involvement in invasive growth

Interestingly, in this study the differentially expressed genes encoding transcription factors did not correlate with previous studies that highlighted their involvement in invasive growth (Jin et al., 2008; Van Mulders et al., 2009; Shively et al., 2013). Fifteen genes encoding known transcription factors increased expression and 12 decreased gene expression in invasively growing cells (Fig. 4.5).



Figure 4.5: Change in gene expression of genes encoding transcription factors in invasively growing cells compared to surface growing cells. Genes with black bars were shown to be involved in invasive growth in other studies (overexpression results in increased invasive growth or deletion results in no invasive growth).

Of these, MGA1, whose encoded protein restores filamentation defects by multicopy expression (Lorenz and Heitman, 1998), had the largest increase in expression. This was followed by *MTH1*, which encodes for a negative regulator of the glucose-sensing signal transduction pathway for HXT gene expression (Lafuente et al., 2000). The upregulation of many glucose transporter genes in the invasively growing cells suggests that MTH1 had released its repression on these genes. Similarly, NRG2, encoding a negative glucose regulator that interacts with Snf1p (Kuchin et al., 2002), was also upregulated in invasively growing cells. SNF2, encoding for a transcriptional activator of FLO11 under glucose limitation (Barrales et al., 2008), had increased expression. Conversely, ROX1, CIN5 and IME1 had the largest decrease in gene expression (approx. two-fold). When comparing to previously reported genes encoding transcription factor required for or to enhance invasive growth when overexpressed, four were upregulated in this study: MGA1, HMS2, FLO11 and RGT1 (Harashima and Heitman, 2002; Shively et al., 2013). Several genes reported to be involved in invasive or pseudohyphal growth had decreased in expression in invasively growing cells, these being MSN2, RSC30, PHD1, RME1 and CIN5.

## 4.4.8 Cellular water homeostasis: aquaglyceroporin gene *FPS1* is required for invasive growth

Cellular water homeostasis (GO:0009992; p < 0.0032) was one of the enriched GO categories identified in the upregulated gene set (Fig. 4.1). All five genes classified within this biological process showed an increase in gene expression (four were significant) in invasively growing cells compared to surface growing cells (AQY1, AQY2, YLL053C, FPS1 and AQY3; increased by 1.43–2.25 fold; Fig. 4.6A). None of these genes have previously been identified as being required for invasive growth, although AQY1 was reported to have increased gene expression in fluffy colonies compared to smooth colonies (Kuthan et al., 2003). To confirm if these genes are important for invasive growth, yeast deletants were constructed for each gene of interest and the deletants were screened for reduced invasive growth. Yeast deletants  $\Delta fps1$  and  $\Delta aqy3$  had a reduced number of invasive growth foci whereas  $\Delta aqy^2$  and  $\Delta yll053c$  had a similar number of invasive growth foci compared to the wild type AWRI796 on Day 4 (Fig. 4.6B). By Day 10, the invasive foci that had formed developed to a comparable size in all strains. Therefore, the decreased invasive growth observed for  $\Delta fps1$  and  $\Delta aqy3$  was more related to fewer foci rather than due to reduced or delayed growth. The reduced number of invasive growth foci was still observed on Day 10. These deletants and other progeny from the same spore were further evaluated to ensure the differences were due to the gene deletion and not spore variability. In this assay, the  $\Delta fps1$  progeny had less invasive growth than the wild type progeny whilst  $\Delta aqy3$  progeny gave mixed results (Fig. S2).  $\Delta aqy1$  was not evaluated in this analysis due to difficulties in generating the strain (Appendix C).



Figure 4.6: Evaluation of genes encoding proteins involved in cellular water homeostasis and their importance for invasive growth. (A) Comparison of aquaporin and aquaglyceroporin gene expression between invasively (white) and surface (black) growing cells SLADS-agar (0.3%). Please refer to next page for (B).



Figure 4.6: (B) Invasive growth evaluation of AWRI796 and homozygous deletants of indicated genes on SLADS-agar (0.3%). Images were photographed from the underside of the agar plates. Surface growing cells were in a large round mat. Multiple small spots on top of that were invasively growing cells. Scale bar, 1 mm.

The deletion analysis confirmed that *FPS1* is important for invasive growth. *FPS1* encodes a channel protein that regulates glycerol export (Oliveira et al., 2003). Lack of Fps1p results in cell wall stress due to high turgor pressure from accumulation of glycerol (Tamas et al., 1999). Cells then fortify their cell wall by displaying less sensitivity to the cell wall degrading enzyme, zymolyase (Beese et al., 2009). As the transcriptomics profile indicates that cell wall remodelling and organisation is actively performed in invasive growth, this fortified cell wall may have prevented the wall from being flexibly modified for growing invasively. This suggests that high turgor pressure or fortified cell wall may not be beneficial when growing invasively. Alternatively, invasive growth may also require the export of glycerol.

#### 4.5 Conclusions

In summary, this study has identified that genes of glucose import and carbohydrate metabolic process are upregulated in invasively growing cells compared to those growing on the agar surface, indicating improved access to nutrients. Fungal-type cell wall organisation is involved in invasive growth, potentially to enhance cell wall stability and adhesion. The protein of a hypothetical gene that has an amidase domain may have a role in invasive growth through the involvement of cell wall recycling process or cell-cell signalling. Ssa2p, which is involved in protein folding and transport, was identified as being significant in association with invasive growth from the protein interaction network analysis. Cellular water homeostasis is also important, especially the glycerol export channel protein Fps1p. Genes involved in medium-chain fatty acid biosynthetic process and ethyl ester production were upregulated in invasive growth but their roles in invasive growth remain elusive.

## 4.6 Experimental Procedures

#### 4.6.1 Yeast strains

Yeast strains used in this study are listed in Table 4.2. To generate deletants of genes of interest (GOI) in AWRI796, the corresponding gene deletant in a BY4741 background was used. The GOI KanMX gene replacement cassettes were generated by PCR using GOI specific primer pairs (GOI\_A and GOI\_D, Table S4) and genomic DNA of the corresponding BY4741 deletant (Wach et al., 1994; Winzeler et al., 1999). Deletion in AWRI796 was generated by transformation of the GOI KanMX gene replacement cassette, followed by selection on YPD agar (1% yeast extract, 2% bacto peptone, 2% glucose, 2% bacteriological agar) + 200 mg L<sup>-1</sup> G418-sulfate (Gietz and Schiestl, 2007). Homozygous deletants were isolated by sporulation, dissection and re-diploidisation, followed by verification with PCR amplification and sequencing using GOI specific primer pairs GOI\_A2 and GOI\_D2 (Table S4) located outside the original gene replacement cassette. AQY1 deletion in AWRI796 was omitted in this study due to difficulties in generating the correct strain (Appendix C).

Yeast strain	Genotype	Reference
AWRI796	Commercial wine yeast strain; diploid	Mauri Yeast Australia
AWRI796 $\Delta aqy2$	$aqy2\Delta::KanMX/aqy2\Delta::KanMX$	This study
AWRI796 $\Delta aqy3$	$aqy3\Delta::KanMX/aqy3\Delta::KanMX$	This study
AWRI796 $\Delta fps1$	$fps1\Delta::KanMX/fps1\Delta::KanMX$	This study
AWRI796 $\Delta y ll 053c$	$yll053c\Delta::KanMX/yll053c\Delta::KanMX$	This study
BY4741 $\Delta aqy2$	$MATa\ his 3\Delta 1\ leu 2\Delta 0\ met 15\Delta 0$ $ura 3\Delta 0\ aqy 2\Delta::Kan MX$	Thermo Fisher Scientific Australia
BY4741 $\Delta aqy3$	$MAT$ a $his3\Delta 1 \ leu2\Delta 0 \ met15\Delta 0$ $ura3\Delta 0 \ aqy3\Delta::KanMX$	Thermo Fisher Scientific Australia
BY4741 $\Delta fps1$	$MATa\ his3\Delta 1\ leu2\Delta 0\ met15\Delta 0$ $ura3\Delta 0\ fps1\Delta::KanMX$	Thermo Fisher Scientific Australia
BY4741 $\Delta yll053c$	$MATa\ his 3\Delta 1\ leu 2\Delta 0\ met 15\Delta 0$ $ura 3\Delta 0\ yll 053c\Delta::Kan MX$	Thermo Fisher Scientific Australia

Table 4.2: Yeast strains used in this study.

#### 4.6.2 Genomic DNA preparation and PCR conditions

Genomic DNA was extracted according to Lõoke et al. (2011). TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) was used to solubilise DNA, and 2  $\mu$ L was used as the template in a 50  $\mu$ L PCR reaction. PCR reactions were performed with Bioline Velocity DNA Polymerase (Cat No. BIO-21098), according to the manufacturer's instructions. PCR products were separated on a 0.8–1% TAE-agarose gel containing GelRed nucleic acid stain (Biotin; Cat No. 41003). DNA fragments of GOI KanMX gene replacement cassettes

were purified using the *Wizard SV* Gel and PCR Clean-Up System (Promega, Madison, WI; Cat No. A9282).

#### 4.6.3 Low nitrogen invasive growth assays

AWRI796 was grown in 10 mL SLAD (0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate, 2% glucose, 50  $\mu$ M ammonium sulfate, prepared according to Binder et al., 2015) for 48 h at 30 °C before re-inoculation into 25 mL SLAD at 1 × 10<sup>4</sup> cells mL<sup>-1</sup> in an Erlenmeyer flask and grown for a further 24 h. Prior to plating, the exponential-phase culture was diluted in Phosphate Buffered Saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) to 2 × 10<sup>5</sup> cells mL<sup>-1</sup>. An aliquot of 5  $\mu$ L was spotted at the centre of SLADS-agar (10 mL SLAD, 0.4 mg L<sup>-1</sup> sodium sulfide (Na<sub>2</sub>S.9H<sub>2</sub>O), 0.3% agar) in a 60 mm Petri dish (Techno Plas; Cat No. S6014S10). For RNA-sequencing, cells were harvested after 8 days of incubation at 25 °C. Three independent assays were conducted; the number of agar plates from which cells were harvested for each independent assay were 188, 190 and 192 respectively. For invasive growth screening of AWRI796 GOI deletants, invasive growth mats were imaged from the underside of the plate on Day 4 and 10 at 0.5 × 0.63 magnification using a Nikon SMZ1270 stereomicroscope and an attached DS-Fi3 camera with NIS-Elements F4.60 software.

#### 4.6.4 Sample harvest and RNA extraction

Cells growing on the agar surface were harvested with a disposable plastic inoculation loop and resuspended in 1 mL Trizol (Life Technologies; Cat No. 15596-018). Any remaining surface cells were rinsed off with ultrapure water. A scalpel blade was used to slice out the piece of agar containing invasively growing cells and transferred to a separate 1 mL Trizol. The cells from all agar plates making up each replicate were pooled for each of the three assays for both surface and invasively growing cells (*i.e.* six samples in total). Harvested cells in Trizol were heated at 60 °C for 3 min and then centrifuged at 3,824 × g for 30 s. Supernatant was removed and 1 mL fresh Trizol was added before being snap frozen in liquid nitrogen.

RNA extraction was performed using a combination of Trizol reagent and a Qiagen RNeasy Mini kit (Cat No. 74104). Samples were thawed on ice. Glass beads were added up to the halfway mark of the meniscus. Six cycles of 45 s of vortexing and 45 s of rest on ice were used to disrupt cells. Tubes were incubated at 65 °C for 3 min and 200  $\mu$ L of chloroform was added, followed by vortexing for 15 s before leaving at room temperature for 5 min. Tubes were centrifuged at 20,817 × g for 10 min at 4 °C. Supernatant was recovered to a fresh tube and an equal volume of 70% v/v ethanol was added, mixed by pipetting, before continuing according to the Qiagen RNeasy Mini kit manufacturer's instructions. RNA quality and quantity were checked using a Nanodrop

ND-1000 UV-visible light spectrophotometer (Thermo Fisher Scientific), separation on 1% TAE-agarose gel and an Experion Automated Electrophoresis System (Bio-Rad).

#### 4.6.5 RNA sequencing and analysis

RNA-sequencing was performed with an Illumina Hiseq instrument in one lane (Australian Genome Research Facility, Melbourne, Australia). Reads were mapped to the S. cerevisiae AWRI796 genome sequence located at the NCBI (GenBank assembly accession: GCA\_000190195.1) with TopHat (Trapnell et al., 2009). Reads were counted for each gene using *featureCounts* in the Rsubread software package (Liao et al., 2013). The translation sequence of genes annotated as "hypothetical protein" was used to search for any domain hits in the Pfam database (Finn et al., 2016). Gene Ontology IDs for the matched protein domains were extracted from InterPro database (Mitchell et al., 2015). These GO IDs were added to the latest gene association file downloaded from the Saccharomyces Genome Database (Date: 04/02/2016). This was used for downstream GO analysis. Counts and gene lengths were merged for genes that had sequences located next to each other in the genome and have matched part of the same domain. Genes with counts less than 50 were not considered in the downstream analysis. Differential expression analysis was performed using the *limma* package (Ritchie et al., 2015) in R with voom (Law et al., 2014) and TREAT (McCarthy and Smyth, 2009) functions, incorporating a 30% fold-change threshold in the hypothesis test. GOseq was used to perform GO enrichment and KEGG analysis (Young et al., 2010). The significantly enriched GO list was summarised and visualised using REVIGO (Supek et al., 2011).

#### 4.6.6 Network analysis

The interactions between significant differentially expressed genes were visualised using a network graph in Cytoscape (Shannon et al., 2003). The protein-protein interaction database of *S. cerevisiae* was downloaded from *mentha* (Calderone et al., 2013, Date: 24/04/2016) while genetic interaction data was obtained from the DRYGIN database (Costanzo et al., 2010; Koh et al., 2010). For protein-protein network graphs, interactions with a confidence score of at least 0.3 were used with the resulting differentially expressed genes from the hypothesis test with a 25% fold-change threshold. Basic network analysis was performed in Cytoscape. Some parameters were visualised in graphs: node color was mapped to up- or down-regulation whereas node size was mapped to Betweenness Centrality value which represents connectivity of each node in the graph. The Betweenness Centrality of each node was plotted with the change in gene expression value. The betweenness of a node v is obtained by counting the number of all shortest paths, connecting any pair of nodes within the network, which are going through that particular node v. The value is divided by the number of all shortest paths connecting two nodes. The *S. cerevisiae* transcription factor database was downloaded from YEASTRACT and was used to determine the differentially expressed transcription factor genes (Teixeira et al., 2014). A graph was plotted to show change in gene expression levels of differentially expressed transcription factor genes.

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The authors have no conflict of interest to declare.

## 4.8 Supporting Information

#### 4.8.1 Tables

Table S1: Due to the size of the table, this can be found in Appendix D.

Category	Number of	Numbe	er of GO Term	Ont	Adj. p -	Genes
	DE Genes	Genes	in	olog	value	
	in Catalogue	Catego	bry	У		
CO:0005886	Category	)	208 plasma membrane	CC	5.67E.06	AORI AOVI AOV2 ATO2 ATO3 BEM2 BNUI BULL CCHI
60.0003880	00	)	598 plasma memorane	cc	3.0/E-00	CIS3 CWHA3 CVR1 DIP5 DNF1 DNF2 DUR3 FNA1 FYG2
						EFT3 EKS1 ELO11 ELUI GAPI GAS1 GIK1 HKR1 HSP30
						HVT1 HVT2 HVT3 HVT4 HVT6 HVT7 INA1 KDE6 MCH5
						MEDI MMDI DDDIO DDDII DDDI5 DDD5 DHM7 DHOOO
						MEFI, MMFI, FDKIO, FDKII, FDKIJ, FDKJ, FDKJ, FHM7, FHO90,
						PUNI, PUI4, KGII, SLAI, SKU//, SSA2, SSUI, SII4, ICBS,
00.0000000	1.7		77.6 1. 11 11		2.0 cF 0.5	IDHI, IHI/, IOKI, IOK2, IPOI, IPO4, ILKI94C
GO:0009277	17		75 fungal-type cell wall	CC	2.06E-05	CIS3, CWP1, DSE2, EXG2, FII1, FII3, FLO5, GAS1, GAS5, PIRI,
CO 0005610	1.4		54 11 11	00	2.0CE 05	PRIS, SIMI, SSA2, SUN4, IDH1, ZPS1, ILR194C
GO:0005618	14	ł	54 cell wall	cc	2.06E-05	CISS, CWP1, DSE2, FII1, FII3, FLO11, FLO5, GAS1, GAS5,
				~~		PIRI, PRY3, SIMI, SSA2, SUN4
GO:000588/	22	2	95 integral component of plasma	CC	3.91E-05	AGP2, AQY1, AQY2, CWH43, DIP5, ENAI, FUSI, GAP1, HX11,
			membrane			HXT2, HXT3, HXT4, HXT6, HXT7, MAL31, MEP1, MMP1, PMC1,
						PUT4, YPK9, YFL054C, YLL053C
GO:0046323	10	)	22 glucose import	BP	6.14E-05	GLK1, HXK1, HXK2, HXT1, HXT2, HXT3, HXT4, HXT6, HXT7,
						MAL31
GO:0005353	6	5	7 fructose transmembrane transporter	MF	9.28E-05	HXT1, HXT2, HXT3, HXT4, HXT6, HXT7
			activity			
GO:0015578	6	5	7 mannose transmembrane transporte	er MF	9.28E-05	HXT1, HXT2, HXT3, HXT4, HXT6, HXT7
			activity			
GO:0005215	16	5	69 transporter activity	MF	9.28E-05	AQR1, AQY1, AQY2, DUR3, FUI1, HXT1, HXT2, HXT3, HXT4,
						HXT6, HXT7, MAL31, TH17, TPO4, YFL054C, YLL053C
GO:0055085	34	Ļ	205 transmembrane transport	BP	9.65E-05	AGP2, AQR1, AQY1, AQY2, ATO3, AVT6, CCH1, DIP5, DUR3,
						ECM3, ENA1, FUI1, GAP1, HXT1, HXT2, HXT3, HXT4, HXT6,
GO:0005576	15	5	81 extracellular region	CC	0.00064	CIS3, CWP1, DSE2, FIT1, FIT3, FLO11, FLO5, GAS1, GAS5,
			-			PIR1, PRY3, SIM1, SSA2, SUN4, ZPS1
GO:0008643	8	3	19 carbohydrate transport	BP	0.000789	HXT1, HXT2, HXT3, HXT4, HXT6, HXT7, MAL31, YEA4
GO:0005975	17	7	91 carbohydrate metabolic process	BP	0.001161	EMI2, EXG2, GAL1, GAS1, GAS5, GDB1, GLC3, GLK1, GPH1,
GO:0031225	11		49 anchored component of membrane	CC	0.00136	CWP1, DSE2, EXG2, FIT1, FIT3, FLO11, FLO5, GAS1, GAS5,
			1			PRY3, YLR194C
GO:0015146	4	Ļ	4 pentose transmembrane transporter	MF	0.003212	HXT1, HXT2, HXT4, HXT7
			activity			
GO:0009992	4	Ļ	5 cellular water homeostasis	BP	0.003212	AQY1, AQY2, YFL054C, YLL053C
GO:0015250	4	Ļ	5 water channel activity	MF	0.003212	AOY1, AOY2, YFL054C, YLL053C
GO:0015254	4	Ļ	5 glycerol channel activity	MF	0.003212	AOY1, AOY2, YFL054C, YLL053C
GO:0005355	7	1	18 glucose transmembrane transporter	MF	0.006459	HXT1, HXT2, HXT3, HXT4, HXT6, HXT7, MAL31
			activity			
GO:0001678	4	Ļ	5 cellular glucose homeostasis	BP	0.006772	EMI2, GLK1, HXK1, HXK2
GO:0004340	4	ł	5 glucokinase activity	MF	0.006772	EMI2, GLK1, HXK1, HXK2
GO:0004396	4	ł	5 hexokinase activity	MF	0.006772	EMI2, GLK1, HXK1, HXK2
GO:0008865	4	Ļ	5 fructokinase activity	MF	0.006772	EMI2, GLK1, HXK1, HXK2
GO:0019158	4	ł	5 mannokinase activity	MF	0.006772	EMI2, GLK1, HXK1, HXK2
GO:0008645	4	L	5 hexose transport	BP	0.006826	HXT3. HXT4. HXT6. HXT7
GO:0005351	7	7	19 sugar:proton symporter activity	MF	0.007095	HXT1 HXT2 HXT3 HXT4 HXT6 HXT7 MAL31
GO:0035428	7	7	19 hexose transmembrane transport	BP	0.007095	HXT1. HXT2. HXT3. HXT4. HXT6. HXT7. MAL31
GO:0031505	17	7	95 fungal-type cell wall organization	BP	0.009746	CIS3. CWH43. CWP1. EXG2. GAS1. HKR1. KRE6. MHP1. MYO3.
GO:0006833	3	}	3 water transport	BP	0.009746	AOY1 AOY2 YFL054C
GO:0051792	3	3	3 medium-chain fatty acid biosynthet	tic BP	0.011764	FFR1 FHT1 MGI2
00.0051772	5		process	ie bi	0.011704	
GO:0071555	15	i	79 cell wall organization	BP	0.014346	CIS3 DSF1 DSF2 ECM3 EXG2 EKS1 GAS1 GAS5 GSC2
00.0071555	15	,	/ ) con wan organization	DI	0.014540	KRF6 PIR1 SIM1 SUNA YPK2 YLR194C
GO:0006006	7	7	21 glucose metabolic process	BD	0.01701	DOG2 GLK1 HYK1 HYK2 PGM2 RGT1 TDH1
GO:0000000	1		13 ion transmembrane transport	DI	0.019770	40Y1 40Y2 CCH1 MCH5 YEL054C YLL053C
GO:0034220	7	, 1	23 substrate_specific transmembrane	ME	0.010//9	HYT1 HYT2 HYT3 HYTA HYT6 HVT7 MAL21
30.0022891	/		transporter activity	IVII'	0.022108	нан, пат, пат, пат, пат, пат, пат, маlэт
CO.0005525		1	7 glugoga hindin-	МГ	0.049257	EMID CLV1 HVV1 HVV2
GO.000536	4	•	12 structural constituent of coll 11	ME	0.048257	LIVIL2, ULA I, HAA I, HAAZ
GO:0003199	4	F	15 subctural constituent of cell Wall	ME	0.048257	UND, UWF1, FIK1, ILK194U
GO:0022857	1	-	20 transmemorane transporter activity	DP	0.048257	ΠΛΙΙ, ΠΛΙ2, ΠΛΙ3, ΠΛΙ4, ΠΛΙ0, ΠΛΙ7, ΜΑΔ3Ι
00:0046835	5	,	14 carbonyurate phosphorylation	вр	0.048257	EMIL, GALI, GLKI, HAKI, HAKZ

Table S2: List of enriched Gene Ontology terms from the upregulated gene set in invasively growing cells.
Table S3: Genes upregulated in invasively growing cells in this study and common to other related studies. Genes in bold are those that are reported in more than one of these studies.

Study	Phenotype	Medium	Common genes
Ryan	Haploid null mutant	YPD agar	ARG8, ERG6, FLO11, IKI3,
et al.	with reduced invasive		MEF2, <b>PMT2</b> , <b>PUT4</b> , <b>SIN3</b> ,
(2012)	growth		<b>SLA1</b> , YFL054C, ZPS1
Ryan	Diploid null mutant	SLAD + 2%	ARG8, BNI1, EHT1, ERG6,
et al.	with reduced	agar	<b>FLO11</b> , GCN1, <b>IKI3</b> , MMP1,
(2012)	pseudohyphal growth		NUM1, PEX25, <b>PMT2</b> , <b>SIN3</b> ,
			<b>SLA1</b> , SSA2, TPO1, YBL029W,
			YPK9
Shively	Overexpression in a	Nitrogen-	HMS2, <b>MGA1</b> , MTO3, NUP1,
et al.	diploid with increased	sufficient	NRD1, RGT1, TMA108
(2013)	invasive growth (score	minimal medium	
	> 0.99)	with galactose	
		induction	
Jin et al.	Haploid null mutant	Low nitrogen	<i>EEB1</i> , <b>HXT4</b> , <i>MEF2</i> , <i>MGR1</i> ,
(2008)	with no invasive	agar + 1%	NOG2, PDR5, PDR11, PWP2,
	growth	butanol	RPO21, UBR1
Kuthan	Upregulated genes in	GMA	AAH1, ALD4, AQY1, ATO3,
et al.	fluffy colonies		CAR1, DUR3, FAA3, FIT3,
(2003)			GAP1, GDH1, INO1, KRS1,
			MAL31, <b>MGA1</b> , <b>PUT4</b> , RRD1,
			SAH1, SSU1, THI7
Kuthan	Downregulated genes	GMA	ARG5,6, ARO1, FET3, HSP30,
et al.	in smooth colonies		<i>HXT3</i> , <b>HXT4</b> , <i>HXT6</i> , <i>HXT7</i> ,
(2003)			SPS100, SPS4, TOR1, YLR194C,
			YNL144C

Primer	Sequence $(5' \text{ to } 3')$
AQY2_A <sup>1</sup>	AATAATGACTTACCTCCTCCAATCC
$AQY2_D^1$	TTTCGGCATTAACGTATGTAGTGTA
$AQY3_A^1$	CTCCCTATTTGGTACTACGATACGA
$AQY3_D^1$	GCACAATTAGTTTATTTGGCAACTT
$FPS1_A^1$	TATATGTCGTGTAATACCGTCCCTT
$FPS1_D^1$	ATGTATAGTAGGTGACCAGGCTGAG
$YLL053C_A^1$	ATGTCTAACGAATCTAACGACCTTG
$YLL053C_D^1$	TACTTCACATCATCTTTGTTTCCAA
AQY2_A2	GCACCGTAACTACTACAGT
AQY2_D2	TACGATGGGAGCGTTATG
AQY3_A2	TTATCCAACTATGGTGACG
AQY3_D2	TGGTCAATCATACAGAACG
FPS1_A2	GGACGGAGAGAGTTACGGC
FPS1_D2	CGAATCGCTGCTTGATGTT
$YLL053C_A2$	TATCGTGAATCATATCTGCC
$YLL053C_D2$	GTCCTGGTTCCACTTCGTAG

Table S4: Primers used in this study for amplification of gene of interest KanMX gene replacement cassettes and sequencing.

<sup>1</sup>GOLA and GOLD primers are from Yeast Deletion Project (www-sequence.stanford.edu/group/yeast\_deletion\_project/deletions3.html).

#### 4.8.2 Figures

Figure S1: Genetic interaction network between differentially expressed genes (only the largest connected network is shown). Red and green node colours represent upregulated and downregulated genes in invasively growing cells; larger node size represents high value of Betweenness Centrality, and vice versa.



Figure S2: Invasive growth evaluation of strains from a tetrad set produced by sporulation and spontaneous re-diploidisation of a heterozygous deletion AWRI796 on SLADS-agar. Scale bar, 1 mm.



## Chapter 5

## Conclusions

#### 5.1 Summary of findings

The studies reported in this dissertation led to the following main conclusions:

- 1. Commercial wine yeast strains are able to form biofilms as determined by mat formation on a rich medium with low density (0.3%) agar. These mats consisted of replicative and non-replicative cells and featured invasive growth, bud elongation, sporulation, and in one case, a sector that was morphologically distinct. The use of a medium containing grape pulp resulted in enhanced invasive growth of yeast mats, while the ability to adhere to plastic was variable between strains.
- 2. Nitrogen limitation resulted in reduced mat sizes (maximum size was approximately 5 mm in diameter in comparison to up to a full 90 mm plate on rich medium) and induced filamentous and invasive growth. The mat regions where invasive growth occurred were in distinct foci, which did not increase in number markedly over the course of the experiment, indicating that invasively growing cells were triggered soon after inoculation. The number of invasive foci in each mat was strain dependent. Many factors were shown to influence the formation of filamentous invasive foci, these being nitrogen content, the presence of a neighbouring mat and the presence of exogenous yeast metabolites. For instance, ethanol and hydrogen sulfide enhanced filamentous invasive growth, while aromatic alcohols and sulfite inhibited their formation.
- 3. Differential expression analysis by RNA-sequencing of surface and invasively growing cells on low nitrogen, low density agar medium revealed genes associated with glucose import, carbohydrate metabolic processes, fungal-type cell wall organisation, medium-chain fatty acid biosynthesis and cellular water homeostasis were upregulated in invasively growing cells. Evaluation of deletants of genes involved in cellular water homeostasis confirmed that the aquaglyceroporin gene, *FPS1*, is important for

invasive growth of yeast mats in a low nitrogen medium.

#### 5.2 Contribution to knowledge

The characterisation of mat architecture and visualisation of cell morphologies formed by commercial wine yeast strains broaden the current knowledge beyond the typical and simple 'hub and spokes' description of mat structures. The complexity of cell morphologies within a mat suggests that cells may differentiate for different roles. This may be advantageous for the mat community since they are likely to adapt quicker to changing conditions as opposed to an individual cell morphology. This interesting feature challenges the current knowledge and calls for investigation of how each differentiated cell interacts with others and contributes to mat survival. The simple macro features (petal-like formation) of these mat structures have recently been described mathematically with a collaborating group determining algorithms that result in strikingly similar computer-generated patterns (Alexander Tam, pers. comm. 2017). It is important to understand the formation of these structures as this will aid knowledge of how non-laboratory yeast biofilms occur in nature. The findings of grape-pulp mat assays and winery hose plastic adhesion contribute to the knowledge of biofilm formation in relation to a winery/vineyard environment. The capability of commercial wine yeasts to form invasive biofilms on grape pulp agar supports this as a method for residency and persistence in and on damaged wine grapes, which might lead to a change in the profiles of indigenous microflora in vineyards and wineries in the long-term. The surprising ability of wine yeasts to adhere to commonly used winery hose suggests that in the absence of rigorous sanitation, yeast could remain in these hoses, and potentially be transferred between fermentations and wines. Other plastics or rubbers also commonly found in wineries have not been examined here. Plastic tanks, valve caps or internal components of pumps, presses and crushers warrant attention as these surfaces may also serve as areas for yeast residence.

This work is the first to report mat morphologies under low nitrogen conditions. Nitrogen limitation was chosen since this is a common environment that yeast in the vineyard and winery are exposed to. Previous studies as well as in this laboratory on non-mat (2% agar) conditions reported morphological changes when cells were limited for nitrogen, which lead to a hypothesis for this study that these conditions may also trigger similar responses to yeast mats (low density agar) enabling survival. Consistent with previous reports on 2% agar, yeast cells switched to filamentous and invasive growth in a low nitrogen environment on 0.3% agar. This morphological switch is believed to extend access to nutrients since limited proliferation prevents non-motile cells moving towards fresh nutrients. In order to develop filamentous and invasive growth, yeast cells undergo polarised growth, resulting in extension of the growth periphery and existence at a less crowded position, and thus having reduced competition for nutrients. In the vineyard, wine yeast that colonise damaged grapes may consume available nitrogen rapidly. As

nitrogen is depleted, these cells may differentiate into filamentous growth, accompanied with their ability to invade grape pulp, to access fresh nutrients, enhance residency and survival. In the winery, nitrogen is usually exhausted within the first 48 hours of must fermentation. Filamentous growth that will increase surface area to mass ratios of a cell may aid nutrient exposure and facilitate uptake during fermentation.

The invasive growth response can also be influenced by the presence of several yeast metabolites, such as ethanol (previously shown to induce elongation), aromatic alcohols (previously reported to enhance filamentous and invasive growth), hydrogen sulfide (novel), and sulfite (previously shown to inhibit growth). These were chosen since they are common metabolites produced by wine yeast in fermentation and have an effect on filamentous and/or invasive growth on 2% agar. The involvement of metabolites of the yeast sulfur metabolic pathway infers their involvement in the nutrient stress response. Particularly, hydrogen sulfide enhanced the morphological switch that occurs in response to nutrient starvation; the release of this compound is also linked to nutrient starvation, suggesting that this metabolite may be an intermediate signalling molecule enabling the response. This is important since hydrogen sulfide has so far been only linked to resistance to other stresses such as heat shock, heavy metal and oxidative stresses in yeast. The connection between nutrient starvation response and sulfur metabolism could help to understand hydrogen sulfide-related fermentation problems or biofilm formation occurring in a sulfur-rich environment.

The transcriptomics analysis revealed that many genes are upregulated in invasively growing cells and the processes involved were glucose import and carbohydrate metabolism, reflecting better access to fresh nutrients and potentially breakdown and utilisation of agar to allow invasive growth. Upregulation of genes involved in fungal-type cell wall organisation suggests biogenesis, assembly, re-arrangement and disassembly of cell wall components are associated with invasive growth, which infers certain composition or arrangement of the cell wall may be required for growing invasively. Upregulation of FLO11 also suggests the encoded protein is not just required for initial attachment to agar where invasive growth began, but is also required during growth inside the agar. The attachment facilitated by Flo11p may hold invaded cells in place to push daughter cells further into the agar instead of being replaced by the buds and moving away from the agar. A hypothetical gene (AWRI796\_5153) having part of its translation sequence homologous to an amidase, was one of the few genes with a large increase in transcript abundance (6.45-fold) in invasively growing cells, suggesting an important role in invasive growth. Amidases have not been linked to invasive growth or biofilm formation but have been shown to be involved in cell wall recycling processes in bacteria. The identification of several biological processes, previously not associated with invasive growth significantly expands the current knowledge of this field. In particular, gene deletant analysis revealed that *FPS1*, encoding the glycerol exporter, is important for invasive growth in low nitrogen agar. These are novel findings in nitrogen-limited conditions as opposed to glucose limitation in other genome-wide studies. As part of the transcriptomics studies, an RNA extraction

method has also been developed for yeast cells growing into agar.

#### 5.3 Limitations and future directions

The study of mat formation is challenging due to a variety of factors, including genetic variation between strains, occurrence of multiple events such as cell differentiation, cell-cell communication, adhesion and invasive growth. Future work could involve a deeper understanding of how a mat is formed, which factors influence their structure, and how cells choose their fate. Incorporation of mathematical modelling will help identify parameters resulting in the structures or formation of interest and make hypotheses for subsequent experimental design. The experimental approach could also include onsite experiments in a winery setting, followed by confirmation with laboratory experiments. The ultimate output would aid decision making in winemaking management regarding microbial impact.

For the first time, hydrogen sulfide is reported to enhance invasive growth in this study. Nitrogen limitation induces invasive growth and the liberation of hydrogen sulfide by yeast often occurs when nitrogen is insufficient. It has been suggested that hydrogen sulfide, alike to ammonia, may be involved in cell-cell signalling. Future work should investigate how hydrogen sulfide is detected by cells and the mechanism to stimulate invasive growth. The work could also be extended to confirm if hydrogen sulfide is a yeast cell-cell signalling molecule.

While assessing the impact of gene deletions involved in cellular water homeostasis on invasive growth, one deletant,  $\Delta aqy1$ , could not be generated, and hence was not evaluated. It appears that the gene replacement cassette was mis-targeted, and thus although transformants were obtained, unexpected amplified allele sizes were observed from spore progeny (Appendix C). Further work needs to be done to investigate why deletion of AQY1 was unsuccessful, for instance, study the AWRI796 genome sequence and investigate PCR conditions to optimise cassette generation and transformation.

Although the transcriptomics analysis has provided new information on invasive growth, there were limitations. For instance, transcriptomics analysis only examined gene expression, while post-translational modifications and protein activity were not examined. Assumptions have been made that an increase in gene expression results in an increase in protein levels. However, the regulation of many genes and their products can occur at many stages post transcription. Furthermore, gene expression may not directly support the phenotype observed. For example, ARO1 and ARO8, encoding for enzymes that are responsible for aromatic amino acid production, were upregulated in invasively growing cells. These amino acids are precursors to aromatic alcohols which were shown to inhibit invasive growth in this work. This result is contradictory to the findings that aromatic alcohols enhanced invasive growth as reported by Chen and Fink (2006), which could be due to different medium preparation such as 2% versus 0.3% agar or ethanol concentration

in preparation of aromatic alcohols. Metabolite analysis could be undertaken to identify if the amino acids were in fact produced. Future work could include both proteomics and metabolomics studies to gain a holistic view on the process.

Differential transcriptome analysis requires a well-annotated genome of a strain to be analysed. AWRI796 was chosen for this analysis since this strain has its genome annotated close to completion. The genome sequence is also very similar to the reference sequence of S288C. All currently annotated genes in AWRI796 are also present in the S288C genome. In addition, the AWRI796 genome has several hypothetical genes predicted. The translation sequence of these genes was blasted for homology to protein families. Most translated sequences were matched to a protein family, suggesting they are protein coding genes. GO information was obtained and used in the analysis. A number of the hypothetical genes, previously predicted to be individual genes, were merged based on the evidence of their location in the genome and the matching protein domain region. Further annotation of this reference sequence has contributed to an understanding of these hypothetical genes. Future work could evaluate the functions of these, including whether they appear in the commonly used S288C reference genome. This will continue to improve the annotation for the AWRI796 genome.

Deletion of *FPS1*, encoding the glycerol exporter led to reduced invasive growth. Previous work reports accumulation of glycerol inside the cell of *FPS1* deletants which could therefore alter turgor pressure and cause cell wall fortification that may disable invasive growth. Further analysis of deletants that similarly result in glycerol accumulation  $(\Delta adk1, \Delta sch9)$  would contribute to this understanding. Cells could also be stained with Calcofluor White to determine the intensity of chitin and  $\beta$ -glucan, and consequently examine if an increased glycerol accumulation results in fortification of the cell wall. Enzyme digestibility of cell walls could also be tested and compared between both wild type and mutant. Alternatively, the presence of glycerol in extracellular fluid may be important for invasive growth. Filamentous invasive growth was induced when glycerol was used to replace glucose in a medium (Palecek et al., 2002). Addition of glucose was shown to repress invasive growth in a dose-dependent manner. Future experiments could use glycerol in SLAD in the presence and absence of glucose to test if this would rescue invasive growth in the  $\Delta fps1$  deletant. This would also confirm the role of extracellular glycerol in invasive growth.

This study is significant since S. cerevisiae is a well-studied model organism, but processes such as mat formation are still not fully understood, especially for nonlaboratory strains. Mat formation forms the basic understanding of biofilm formation, which has implications such as persistence and survival of clinical yeasts in medical devices, invasiveness into human and plant tissues, interactions between microorganisms and the use of metabolites in wastewater, and in this study, survival of commercial wine yeasts in the wine environment. Many tantalising questions and possibilities have been revealed by this work.

## Appendix A

## Method development for mat formation and plastic adhesion assays

#### A.1 Mat formation assays

## A.1.1 Reproduction of results by Reynolds and Fink (2001) and test mat formation ability of commercial wine yeast strains

#### Background and methods

Mat formation methods described by Reynolds and Fink (2001) were reviewed in order to test if common commercial wine yeast strains also have the ability to form mats. Unfortunately, some steps were not detailed in this short form publication. For example, how the medium was prepared and whether the plates were incubated yeast inoculum side up or down since the agar concentration was very low. In this first preliminary study, the method reported was repeated with some modifications and additional details. Laboratory yeast strain prototrophic diploid  $\Sigma$ 1278b and two wine yeast strains, L2056 and AWRI796, were used. Yeast Peptone Dextrose (YPD; 1% yeast extract, 2% bacto peptone, 2% glucose) with 0.3% agar was made by dissolving all components in RO water and autoclaved to sterilise. Instead of inoculation with a toothpick as reported, to standardise initial cell number, this experiment used inoculation of 5  $\mu$ L of a diluted overnight YPD culture (1 × 10<sup>4</sup> cells mL<sup>-1</sup>). Plates were incubated yeast inoculum side down. Plate images were photographed after 16 days of incubation using a Samsung Galaxy S3 camera. Mats were washed with a stream of ultrapure water from a laboratory squeeze bottle for approximately 30 s to check for adhesive cells.

#### Results

Mats were small (< 40 mm; Fig. A.1) and  $\Sigma$ 1278b had a different mat structure (no spokes) compared to those reported by Reynolds and Fink (2001) where mats were described to have a 'hub and spokes' structure. Post-wash observations showed cells adhered to the agar near the mat rim.



Figure A.1: Mat formation pre- and post-wash by  $\Sigma 1278b$ , L2056 and AWRI796 on YPD with 0.3% agar on 90 mm Petri dishes on Day 16.

Liquid YPD discharged from agar during incubation, and the medium agar concentration may have increased, which may explain the differences in mat size and structure compared to those described by Reynolds and Fink (2001).

### A.1.2 Evaluation of medium preparation methods for mat assays

#### Background and methods

In this experiment, three approaches were tested to find the best solution to prepare dry low-density agar YPD:

- 1. YPD with 0.5% Bacto agar, autoclaved (increased agar concentration)
- 2. YPD with 0.5% agarose, autoclaved (alternative gelling agent)

- 3. Filtered 2× YPD and autoclaved 2× Bacto agar (0.6 %) (washed twice with 800 mL ultrapure water in a 1 L Schott bottle, swirled to mix and rested for 15 min before decanting), then mixed equal volumes before pouring into Petri dishes
- 4. Filtered  $2 \times$  YPD and autoclaved  $2 \times$  Bacto agar (0.6 %), then mixed equal volumes before pouring into Petri dishes

#### Results

Agar medium made using all approaches was dry even after two-week incubation. However, the  $\Sigma 1278b$  mat was smaller on YPD with 0.5% agar than 0.3% agar whereas the mat structure on YPD with 0.3% agar resembled those commonly reported (Fig. A.2).



Figure A.2: Mat formation by  $\Sigma 1278b$  on medium prepared using four different approaches: (A) autoclaved YPD with 0.5% Bacto agar; (B) autoclaved YPD with 0.5% agarose; (C) filtered YPD with 0.3% washed and autoclaved Bacto agar; (D) filtered YPD with 0.3% autoclaved Bacto agar. 90 mm Petri dishes were used.

#### Conclusions

The mat shape and size between washed and non-washed agar were similar. Therefore, approach '4' was used for the preparation of medium.

# A.1.3 Evaluation of mat inoculation with cells at exponential growth phase

#### Background and methods

Biological variability of mat size and structure had been observed across replications. In order to decrease this, mats were inoculated with cells standardised for initial cellular metabolic state. Following the initial overnight YPD culture, a fresh YPD culture was inoculated and incubated for a set amount of time to achieve exponential-phase cultures (instead of stationary phase). The following experiment was set up to identify the incubation time required after the re-inoculation. Overnight YPD cultures of prototrophic  $\Sigma 1278b$ , auxotrophic  $\Sigma 1278b$  and auxotrophic  $\Sigma 1278b$   $\Delta flo11/\Delta flo11$  were re-inoculated into 25 mL fresh YPD at  $1.25 \times 10^6$  cells mL<sup>-1</sup>. Cells were counted using a haemocytometer at several time points to determine the time required for cells to produce approximately three generations.

#### Results

All strains achieved three generations ( $\sim 1 \times 10^7$  cells mL<sup>-1</sup>) within 4.5 h and approximately four generations within 7 h, indicating rapid growth (Fig. A.3).



Figure A.3: Growth curve after re-inoculation of an overnight YPD culture into fresh YPD. Inoculation rate was  $1.25 \times 10^6$  cells mL<sup>-1</sup>. (•) Prototrophic  $\Sigma 1278b$ , ( $\blacksquare$ ) auxotrophic  $\Sigma 1278b$ , (•) auxotrophic  $\Sigma 1278b$   $\Delta flo11/\Delta flo11$ .

#### Conclusions

The mat assay method was modified to include a re-inoculation step using an overnight culture, inoculated into fresh YPD at a rate of  $1.25 \times 10^6$  cells mL<sup>-1</sup> and incubated for 6

h prior to inoculation of YPD agar (0.3%).

#### A.2 Plastic adhesion assays

#### A.2.1 Refinement of staining and washing methods

#### Background and methods

This experiment followed plastic adhesion methods described by Reynolds and Fink (2001) with modifications and additional details described below. Three strains used were kindly donated by J. Gardner (Uni. of Adelaide): (a) ISO C9 C (L2056  $MAT\alpha \ \Delta ho$ ); (b) ISO C9 D (L2056  $MATa \ \Delta ho$ ); and (c) ISO C9 C/D (L2056  $\Delta ho/\Delta ho$ ). In brief, these strains are derivatives of the commercial wine yeast strain L2056, considered to be very close to isogenic. They were created by three rounds of meiosis and re-diploidisation of the haploid wine yeast C9 (originally derived from L2056, as described in Walker et al., 2003). Homothallism was enabled by introduction of a functional HO gene carried on a plasmid also enabling G418-sulfate resistance. Loss of resistance and inability to sporulate were used to confirm plasmid loss. Mitochondrial RFLP analysis was used to check genetic similarity.

Media used were Synthetic Complete (SC; 0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate, 0.079% Complete Supplement Mixture, 0.5% ammonium sulfate, 0.1% glucose) and Synthetic Low Ammonium Dextrose (SLAD; 0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate, 50  $\mu$ M ammonium sulfate, 0.1% glucose). Following a one-hour incubation, cells were stained with 1% Crystal Violet solution for 15 min as described by Reynolds and Fink (2001). To remove unbound Crystal Violet, cells were washed with ultrapure water using a pipette to load water and a tipping and tapping motion to remove water. Cells adhered to the plastic were observed not to have been stained by the Crystal Violet. Therefore, cells were stained again for 20 min. For subsequent experiments, cells were stained twice for 20 min each time.

#### Results

The number of adhered cells was reflected by differences in absorbance values at 570 nm (Fig. A.4).



Figure A.4: Plastic adhesion of yeast strains, as measured by absorbance of Crystal Violet-stained residual cells post-washing at 570 nm. (•) ISO C9 C, (•) ISO C9 D, ( $\blacksquare$ ) ISO C9 C/D, and ( $\Box$ ) no cell control in SC and SLAD.

#### Conclusions

The plastic adhesion assay was modified with cells stained twice after incubation. Washing steps would include adding ultrapure water by pipetting, followed by inverting the microplate to tap the water out.

### A.2.2 Determination of the maximum absorption of Crystal Violet

#### Background and methods

Several publications have used either 570 or 590 nm to quantify absorbance of Crystal Violet (Reynolds and Fink, 2001; Zara et al., 2009; Gori et al., 2011; Granek et al., 2013). This experiment determined the best wavelength to use by performing an absorbance scan on three samples after one-hour incubation in specified medium: (a) no cell control in SLAD; (b) AWRI796 in SLAD; (c) L2056 in SC. The scan was performed using a Tecan Infinite M200 PRO microplate reader from 230 nm to 1000 nm in 10 nm increments.

#### Results

Maximum absorption of Crystal Violet was observed at 590 nm (Fig. A.5).



Figure A.5: Wavelength scan for Crystal Violet-stained cultures adhered to plastic. ( $\circ$ ) AWRI796 incubated in SLAD, ( $\Box$ )L2056 incubated in SC, ( $\bullet$ ) no cell control.

#### Conclusions

For all future assays, absorbance at 590 nm was chosen to quantify cell adhesion on plastic.

## Appendix B

## Method development for mat formation assays in a low nitrogen medium (SLAD)

#### B.0.1 Determination of inoculation rate

#### Background and methods

Mat formation was performed as described by Reynolds and Fink (2001) with the substitution of SLAD for YPD and several defined inoculation rates instead of the undefined toothpick inoculation.  $\Sigma 1278b$  and two commercial wine strains, L2056 and AWRI796, were tested in this experiment. Inoculation rates were 5  $\mu$ L of 2 × 10<sup>2</sup>, 2 × 10<sup>3</sup>, 2 × 10<sup>4</sup> and 2 × 10<sup>5</sup> cells mL<sup>-1</sup> of an overnight Synthetic Low Ammonium Dextrose (SLAD; 0.17% Yeast Nitrogen Base without amino acids and ammonium sulfate, 2% glucose, 50  $\mu$ M ammonium sulfate) culture, equating to approximately 1, 10, 100 and 1000 cells. Four to six images were photographed for each mat to capture all sectors on Day 3, 5, 7 and 9 using a Nikon Eclipse 50i microscope at 40× magnification and an attached Digital Sight DS-2MBWc camera with NIS-Elements F3.0 imaging software. The image of a whole mat was generated by stitching the sector images using Fiji software (Thévenaz and Unser, 2007; Preibisch et al., 2009; Schindelin et al., 2012).

#### Results

In general, a higher inoculation rate led to a greater number of invasive growth foci initiated at an early stage (Fig. B.1). Replications from higher inoculation rates produced more consistent results than lower inoculation rates (data not shown). Independent of the initial inoculum used, all mats reached a very similar maximum mat size, suggesting that growth is limited by a factor independent of initial cell numbers. This may be nitrogen availability. Maximum mat size was approximately 4–5 mm in diameter, and this was often observed at the first time point analysed (Day 3), except when only 1 cell was used as the inoculum. In comparison, the maximum size of a colony grown from the same strains on SLAD with 2% agar reaches approximately 1 mm (Joanna Sundstrom, *pers. comm.*). As the maximum mat size is at least 4-fold wider than colonies formed on SLAD with 2% agar, and cell growth occurs as a thin layer across the surface, these were defined as mats rather than colonies. Mat expansion also extends beyond the boundary of the inoculum drop. This can also be visualised in Figure B.1A, where growth of the mat inoculated with a single cell doubles in size between 3 and 9 days.

#### Conclusions

An inoculation rate of 5  $\mu$ L of 2 × 10<sup>5</sup> cells mL<sup>-1</sup> (1000 cells) was selected for all future SLAD mat assays. Use of rapidly growing cells (exponential phase) as the inoculum was also introduced as in the YPD mat assays to standardise the growth phase of cells. When capturing microscopic images, phase contrast was changed from dark field to the phase corresponding to the objective lens to obtain a white background image.



Figure B.1: Mat formation in a low nitrogen medium (SLAD with 0.3% agar) for a range of initial inoculum over time. (A) Prototrophic  $\Sigma 1278b$  inoculated with either 1, 10, 100 or 1000 cells. Scale bar, 1 mm. Please refer to next two pages for (B) and (C).



Figure B.1: (B) L2056 inoculated with either 1, 10, 100 or 1000 cells. Scale bar, 1 mm. Please refer to next page for (C).



Figure B.1: (C) AWRI796 inoculated with either 1, 10, 100 or 1000 cells. Scale bar, 1 mm.

## B.0.2 Preliminary study on the effect of sulfide on mat formation in SLAD

#### Background and methods

Sulfide is of interest to this project since it is a yeast metabolite that preliminary evidence from this laboratory suggests a possible role in cell signalling and it has a significant impact on wine quality. This experiment compared mats from a low nitrogen medium (SLAD) formed by prototrophic  $\Sigma 1278b$ , L2056, AWRI796, EC1118, PDM and Distinction with and without the influence of sulfide. Yeast cultures were grown in either SLAD or SLADS (SLAD with 0.4 mg L<sup>-1</sup> sodium sulfide) for two overnights before being re-inoculated into a fresh SLAD or SLADS at  $1 \times 10^4$  cells mL<sup>-1</sup> and incubated for 16 h. The cell concentration of cultures was adjusted to  $2 \times 10^5$  cells mL<sup>-1</sup> with PBS. A 5  $\mu$ L aliquot was spotted to Petri dishes containing SLAD or SLADS with 0.3% agar (*i.e.* 1000 cells were inoculated). EC1118, PDM and Distinction were evaluated in a separate experiment, and therefore there was a slight variation in the days where images were captured. Imaging and stitching methods were as described in Study B.0.1.

#### Results

All strains consistently showed an enhancement of invasive growth in the presence of exogenous sulfide (Fig. B.2A and B).  $\Sigma$ 1278b, EC1118, PDM and Distinction also had reduced surface growth.

#### Conclusions

Cell counts of liquid cultures confirmed that the re-inoculation rate and incubation period described achieved more than three generations and cultures had not reached saturation. Sulfide enhanced invasive growth and the addition would be tested in combinations with other compounds.

Figure B.2: Mat formation of yeast strains in a low nitrogen medium over time. (A)  $\Sigma$ 1278b, L2056, AWRI796, (B) EC1118, PDM and Distinction on SLAD and SLADS (containing 0.4 mg L<sup>-1</sup> sodium sulfide).





## Appendix C

# Attempt to construct $\triangle aqy1$ in AWRI796

#### C.0.1 Transformation with homologous recombination

#### Methods

A KanMX gene replacement cassette was generated by PCR from the genomic DNA of BY4741  $\Delta aqy1$  using the primers AQY1\_A (5'-TAGAAGTGGTAAATTGCAGGATAGC-3') and AQY1\_D (5'-TCAACCATATGACTACTTGGGATTT-3') (Winzeler et al., 1999; Wach et al., 1994). AQY1 deletion in AWRI796 was attempted by transformation of the cassette, followed by selection on YPD + 200 mg L<sup>-1</sup> G418-sulfate (Gietz and Schiestl, 2007). Homozygous deletants were isolated by sporulation, dissection and re-diploidisation, followed by verification with PCR amplification.

#### Results

The resulting progeny showed 2:2 segregation of the alleles with two corresponding to the wild type allele size and the other two corresponding to both wild type and deletion allele sizes (Fig. D.1A). When the alleles were amplified with the primer pairs located outside the gene replacement cassette, AQY1\_A2 (5'-TTCCAAGTGAATATCTGC-3') and AQY1\_D2 (5'-GATTCCTAGATCCTAACAT-3'), all four progenies showed wild type allele sizes (Fig. D.1B).

#### Conclusion

The results suggest that the KanMX cassette has a preferential recombination locus other than AQY1 in AWRI796.



Figure D.1: AQY1 PCR products from genomic DNA of four spore progenies (1–4), BY4741  $\Delta aqy1$  and AWRI796 using primers (A) AQY1\_A and AQY1\_D, (B) AQY1\_A2 and AQY1\_D2.

## C.0.2 Construction of KanMX gene replacement cassette from a plasmid

#### Methods

To increase specificity to AWRI796, a *Kan*MX gene replacement construct was PCR amplified from a plasmid (pBS418; kindly donated by M. Walker, Uni. of Adelaide) using a long primer pair with 40 bp specific to the AWRI796 sequence and 20 bp homologous to either the TEF promoter or terminator on the plasmid. These primers were AQY1\_TEFp\_fwd (5'-CCTTACACAGTAGGATTAGTCTAGAAGTGGTAAATTGAAGC CTTGACAGTCTTGACGTGC-3') and AQY1\_TEFt\_rev (5'-CGCACTTAACTTCGC ATCTGGACTACTTGGGATTTCAAGGACAAGATATACATCAACGAT-3').

#### Results

The PCR product quantity was low. When the product was used as a template in a new PCR reaction, no products were obtained.

## Appendix D

# Supporting information for Chapter 4

Table S1: Results of differential gene expression analysis comparing transcriptomes of cells growing invasively and on the surface of SLADS-agar (0.3%). Genes with low read counts (< 50) were excluded. Differentially expressed genes were selected on the basis of satisfying testing significance (FDR < 0.05) relative to a 1.3-fold-change threshold. Adjusted *p*-value is shown zero for less than  $1 \times 10^{-6}$ . Significantly upregulated, no change and downregulated genes in invasively growing cells are indicated by 1, 0 and -1 in the column Score.

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score		AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_1051	HXT7	3.781	0	1		AWRI796_2590	TDH1	0.731	0.002984	1
AWRI796_2159	HXT4	3.039	1.005.06	1	4	AWRI796_0070	PAU5	0.729	0.007187	1
AWRI796_5155 AWRI796_3083	AWR1/90_5155 SSA2	2.080	1.00E-06	1		AWRI796_1561 AWRI796_4656	IKC/ VPK9	0.729	0.003954	1
AWRI796 5256	HXT6	2.251	0	1		AWRI796 0144	YBL029W	0.720	4.40E-05	1
AWRI796_1349	RGI1	1.919	0	1		AWRI796_0617	DLD1	0.72	0.000194	1
AWRI796_2017	AWRI796_2017	1.789	1.00E-06	1		AWRI796_3327	HAP1	0.72	4.10E-05	1
AWRI796_5255	HXT3	1.742	1.00E-06	1	4	AWRI796_1703	SRM1	0.719	0.001178	1
AWRI796_4889	CAR1	1.727	1.00E-06	1	4	AWRI796_3329	GSY2	0.718	0.000191	1
AWR1/96_1052 AWR1796_1560	HXI3 HYK1	1.524	0	1	1	AWR1796_4406 AWR1796_0449	CSI2 MGP1	0.718	0.149065	1
AWRI796_0081	SR077	1.434	1.00E-06	1	1	AWRI796 4902	EEB1	0.713	0.003327	1
AWRI796_4257_58	AWRI796_4257_58	1.35	2.00E-06	1		AWRI796_1619	GCN1	0.712	7.00E-05	1
AWRI796_2502	CIS3	1.336	0.003327	1		AWRI796_4905	SSU1	0.712	0.002694	1
AWRI796_2813	PIR1	1.297	0.000181	1		AWRI796_4187	ATO2	0.71	0.000402	1
AWRI796_1614	ARO8	1.275	1.00E-06	1	4	AWRI796_1273	GLC3	0.708	0.000167	1
AWRI/96_0236	HSP26 CND1	1.245	2.90E-05	1		AWRI/96_40//	KRE33 PPD1	0.707	0.000697	1
AWRI796_2240 AWRI796_3653	FET3	1.21	1.80E-05	1		AWRI790_2284 AWRI796_3222	DIP2	0.705	0.000133	1
AWRI796 2160	HXT1	1.183	1.001 05	1	1	AWRI796 3647	ERB1	0.703	3.20E-05	1
AWRI796_3611	HXT2	1.183	1.00E-06	1		AWRI796_4752	DIP5	0.703	0.000359	1
AWRI796_3060	YLL053C	1.168	0.00017	1		AWRI796_1809	GSC2	0.701	0.000204	1
AWRI796_5047	TKL1	1.162	0	1	4	AWRI796_1530	FAB1	0.699	0.002214	1
AWRI/96_0102	YBL081W	1.139	0.000225	1	4	AWRI/96_30/9	IPOI PPA 100	0.699	0.000908	1
AWRI796_2308 AWRI796_1119B	HKR1	1.123	3 20E-05	1		AWRI796_4700	CAR2	0.099	0.000289	1
AWRI796 2201	SPS100	1.076	2.70E-05	1	1	AWRI796 0875	DOP1	0.695	0.00034	1
AWRI796_1107	PDR15	1.068	1.30E-05	1		AWRI796_4135	GCD10	0.687	0.000268	1
AWRI796_1988	MGA1	1.059	0.000144	1		AWRI796_0280	TPS1	0.684	0.000194	1
AWRI796_3020	YKR075C	1.044	0	1	4	AWRI796_2600	MHP1	0.684	0.000484	1
AWRI/96_32/4	YLR194C	1.044	4.10E-05	1	4	AWRI/96_1964	CCH1 DUS2	0.681	0.000408	1
AWRI796_4782	NUM1	1.038	1.00E-06	1	4	AWRI790_3438	BRR2	0.674	0.000331	1
AWRI796_3414	ELO3	1.009	1.00E-06	1	1	AWRI796 4995	RPA135	0.674	0.001538	1
AWRI796_4912	SEC16	0.996	1.00E-06	1		AWRI796_4837	OYE3	0.673	0.08583	0
AWRI796_3204	MDN1	0.987	1.00E-06	1		AWRI796_5151	ERR2	0.672	0.000955	1
AWRI796_1873	CLB1	0.96	0.000841	1		AWRI796_1402	DSE1	0.67	0.001535	1
AWRI796_3553	IMD4	0.954	0.000268	1	4	AWRI796_1771	ERG4	0.67	0.000631	1
AWRI/96_36//	YMR085W GUT1	0.951	8.00E-06	1		AWRI/96_2893	NUP100 VPP228C	0.668	0.00031	1
AWRI796_2041 AWRI796_5135	GDB1	0.944	5.00E-06	1		AWRI796_0578 AWRI796_3695	YPK2	0.007	0.003337	1
AWRI796 1463	YJL225C	0.94	4.00E-06	1		AWRI796 0983	EXG2	0.664	0.027963	1
AWRI796_2282	GUT2	0.94	2.00E-06	1		AWRI796_4003	POP1	0.66	0.000217	1
AWRI796_3447	INA1	0.927	0.001756	1		AWRI796_3309	THI7	0.657	0.001704	1
AWRI796_3186	CSF1	0.926	2.30E-05	1	4	AWRI796_0041	PMT2	0.656	0.000508	1
AWRI796_4670	MCH5	0.924	0.000387	1	4	AWRI796_2782	PTK1	0.656	0.000337	1
AWRI/96_1/30	PUS2 MTU1	0.92	0.00037	1	4	AWRI/96_1505	BLM10	0.655	0.001481	1
AWRI796_0993 AWRI796_4272	ZPS1	0.919	0.000214	1		AWRI796_4614 AWRI796_1323	SSF2 SAH1	0.653	0.143300	1
AWRI796 5083	CLB2	0.915	0.01243	1		AWRI796 4455	RSB1	0.651	0.084879	0
AWRI796_3696	PGM2	0.912	2.00E-06	1		AWRI796_0527	YCR061W	0.647	0.001089	1
AWRI796_4069	AAH1	0.909	7.00E-05	1	4	AWRI796_4705	PUT4	0.647	0.001156	1
AWRI796_1525	GSY1	0.907	3.60E-05	1		AWRI796_3978	NRD1	0.646	0.006427	1
AWRI796_3391	FKS1	0.904	1.10E-05	1	4	AWRI796_3679	YMR087W	0.641	0.001037	1
AWRI/96_46/6 AWRI796_0/32	SPS4 MAL31	0.901	0.018242	1	1	AWRI/96_5114 AWRI796_0534	GPH1 PSA4	0.641	0.001116	1
AWRI796_3061	AOY2	0.897	0.001162	1	1	AWRI796 5243	FL05	0.638	0.005408	1
AWRI796_3070	VPS13	0.896	4.00E-06	1		AWRI796_2311	SIM1	0.637	0.00837	1
AWRI796_0293	IRA1	0.892	1.00E-06	1		AWRI796_4495	NUP1	0.636	0.000773	1
AWRI796_4731	GDH1	0.888	1.70E-05	1	4	AWRI796_4503	LEU9	0.636	0.0013	1
AWRI796_2472	PHO90	0.871	1.00E-05	1	4	AWRI796_0524	PWP2	0.635	0.006427	1
AWRI796_3443	UTP21	0.87	0.000157	1		AWRI796_1883	MEPI	0.635	0.00084	1
AWR1796_4491 AWR1796_0464	EUM5 EUS1	0.858	3.00E-05 0.005336	1	1	AWRI/90_3801 AWRI706_1482	GUAI RIM15	0.635	0.000101	1
AWRI796 2204	DSE2	0.85	0.00219	1		AWRI796 4641	TPO4	0.633	0.004081	1
AWRI796_3893	GAS1	0.848	1.30E-05	1		AWRI796_0592	HEM3	0.632	0.006036	1
AWRI796_3482	FMP27	0.845	8.00E-06	1		AWRI796_3048	YKR104W	0.632	0.010288	1
AWRI796_0835	DNF2	0.839	5.00E-05	1		AWRI796_0197	RPL4A	0.63	0.000523	1
AWRI796_0286	AGP2	0.837	0.000168	1	4	AWRI796_0187	GAL1	0.629	0.016585	1
AWRI/96_2458 AWRI796_4285	ZNF1 ARG8	0.832	0.00017	1	1	AWRI/96_152/ AWRI796_11/1		0.629	0.225841	0
AWRI796_1087	ATO3	0.826	0.000901	1	1	AWRI796_1461	YRF1-7	0.625	0.036767	1
AWRI796_2780	TOR2	0.824	0.000131	1		AWRI796_0878	MKC7	0.624	0.140556	0
AWRI796_0496	HSP30	0.823	0.013751	1	4	AWRI796_2501	HSP150	0.623	0.574468	0
AWRI796_2626	CYR1	0.819	1.70E-05	1	4	AWRI796_4383	GAS5	0.622	0.002543	1
AWRI/96_3001	DYNI BNII	0.817	0.000283	1		AWRI/96_3461	SEN1 AWRI706 2845	0.62	0.000211	1
AWRI796_3301	SRB2	0.817	0.034664	1	-	AWRI796_3845 AWRI796_4356	MAM3	0.02	0.000937	1
AWRI796 4737	FIT3	0.813	0.001375	1		AWRI796 0130	FUI1	0.618	0.000327	1
AWRI796_4234	NOG2	0.808	2.60E-05	1		AWRI796_4132	SUN4	0.618	0.007419	1
AWRI796_1211	FIT1	0.807	0.002506	1	4	AWRI796_2119	FSH1	0.616	0.03499	1
AWRI796_0698	MRK1	0.804	2.90E-05	1	4	AWRI796_4334	PHM7	0.616	0.001008	1
AWRI/96_2868	CWPI AOV1	0.802	0.000162 7.00E.05	1	4	AWRI/96_2682	TORI	0.614	0.000803	1
AWRI796_5142 AWRI796_3149	AQYI YI R046C	0.802	7.00E-05 0.000265	1	4	AWRI796_3365 AWRI796_4688	ST14 PDR10	0.614	0.002408	1
AWRI796 2026	MAL31	0.798	0.037474	1		AWRI796_4000	CDC39	0.612	0.001107	1
AWRI796_0451	GLK1	0.797	3.10E-05	1		AWRI796_2008	SLH1	0.61	0.006695	1
AWRI796_2750	HMS2	0.797	0.003833	1		AWRI796_4255_56	AWRI796_4255_56	0.608	0.006771	1
AWRI796_2407	FAA3	0.793	4.10E-05	1		AWRI796_0024	CLN3	0.606	0.300806	0
AWRI/96_0166	UTP20 UDA7	0.789	0.000156	1		AWRI/96_2988	GAP1 CWI42	0.603	0.003409	1
AWRI790_0133 AWRI796_0789	ENA1	0.783	2.70E-05 0.003133	1		AWRI796_0493	CWII45 PMT1	0.601	0.003833	1
AWRI796 4998	YPR015C	0.774	0.001538	1		AWRI796 1368	AWRI796 1368	0.596	0.037497	1
AWRI796_4067	YNL144C	0.771	0.000204	1		AWRI796_0546	YCR087C-A	0.595	0.013038	1
AWRI796_1197	EMI2	0.769	6.10E-05	1		AWRI796_1761	STT3	0.594	0.00837	1
AWRI796_0864	ARO1	0.768	0.000131	1		AWRI796_3718	ECM16	0.594	0.002275	1
AWRI796_0007	BDH1	0.767	5.40E-05	1		AWRI796_4729	ALD4	0.594	0.006771	1
AWK1/90_2431	FLUII VRF1.6	0.767	0.006992	1		AWR1/90_3448	PUNI PPO31	0.593	0.011501	1
AWRI790_3193	YEA4	0.763	0.00011	1		AWRI796 2238	OYE2	0.591	0.005/03	1
AWRI796 4745	SAM4	0.759	0.006878	1		AWRI796 1351	ARG5.6	0.587	0.007087	1
AWRI796_3904	ADH6	0.75	0.000582	1		AWRI796_2798	FAS1	0.583	0.003833	1
AWRI796_3986	ATG2	0.748	0.000268	1		AWRI796_4287	RTC1	0.583	0.005287	1
AWRI796_2279	AIM20	0.747	0.017069	1		AWRI796_1935	UBR1	0.578	0.002163	1
AWRI796_2456	YFL054C	0.741	0.000139	1		AWRI796_3098	DRS1	0.578	0.016281	1
AWRI/96_0362	PYC2 HYK2	0.734	9.20E-05	1		AWRI/96_3855	YMR265C	0.578	0.018991	1
AWK1/90_13/1	11717	0.734	3.80E-05	1		AWK1/90_3812	KKPJ	0.577	0.0218/2	1

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_1325	ACA1 RPO21	0.576	0.002414	1	AWRI796_4592	SPR2 NDF2	0.494	0.081069	0
AWRI796_0229	NRG2	0.574	0.010703	1	AWRI796_2222	MPC2	0.493	0.049849	1
AWRI796_0300	YSW1	0.574	0.032219	1	AWRI796_0068	KIN3	0.49	0.499814	0
AWRI796_4574	ULS1	0.574	0.010703	1	AWRI796_1373	TSC11	0.489	0.077414	0
AWRI796_4746 AWRI796_0934	EBS1	0.573	0.003126	1	AWRI796_0822 AWRI796_1929	ATF2	0.488	0.998045	0
AWRI796_2225	PRP8	0.573	0.025081	1	AWRI796_3557	SUR7	0.488	0.419134	0
AWRI796_3891	SCW10	0.573	0.331383	0	AWRI796_4465	RPL3	0.488	0.085525	0
AWRI796_4655 AWRI796_0788	SINF2 KRS1	0.572	0.00618	1	AWRI796_1949 AWRI796_2016	ELP2 PXR1	0.487	0.339458	0
AWRI796_3594	ERG6	0.57	0.001099	1	AWRI796_3047	NFT1	0.487	1	0
AWRI796_4543	PDR5	0.57	0.011218	1	AWRI796_4636	HRK1	0.487	0.312734	0
AWRI/96_3864 AWRI796_0519	BULI ARE1	0.565	0.007286	1	AWRI796_0375 AWRI796_2306	VHCI TAO3	0.484	0.124417	0
AWRI796_1657	RPL9A	0.564	0.006771	1	AWRI796_0167	HTA2	0.482	0.384813	0
AWRI796_2546	MEF2	0.564	0.03499	1	AWRI796_5224	ENA1	0.482	0.081818	0
AWRI796_4111	NST1 TEE1	0.564	0.000841	1	AWRI796_1885	PPT1 DAL2	0.479	0.360763	0
AWRI796_3031 AWRI796_0327	EHT1	0.563	0.005577	1	AWRI796 2540	UTP10	0.479	0.136223	0
AWRI796_5141	QCR2	0.563	0.014402	1	AWRI796_4826	RTT10	0.477	0.707279	0
AWRI796_0513	TAF2	0.562	0.026974	1	AWRI796_5029	SMK1	0.477	0.728989	0
AWRI796_1085	LIHI	0.561	0.019518	1	AWRI796_1333 AWRI796_3046	FLO10	0.476	0.18098	0
AWRI796_2404	PDR11	0.56	0.006771	1	AWRI796_3436	SKI2	0.476	0.152831	0
AWRI796_1440	DNF1	0.559	0.002568	1	AWRI796_4203	ARE2	0.476	0.135731	0
AWRI/96_1//6 AWRI796_3388	VRP1	0.557	0.020233	1	AWR1/96_50/8 AWR1796_2088	ARG4	0.476	0.690813	0
AWRI796_0154	PEP1	0.553	0.011871	1	AWRI796_4792	FLC1	0.475	0.337584	0
AWRI796_4200	ACC1	0.553	0.005028	1	AWRI796_5011	CSR2	0.475	0.17496	0
AWRI796_2925 AWRI796_3528	MAE1 VMI 082W	0.551	0.112574	0	AWRI796_4602 AWRI796_2166	HER1 KIC1	0.471	0.214497	0
AWRI796_0215	REG2	0.55	0.097384	0	AWRI796_0424	SNF5	0.469	0.700881	0
AWRI796_2919	RGT1	0.549	0.005596	1	AWRI796_0428	SUL1	0.468	1	0
AWRI796_1299	SPC25	0.548	0.039632	1	AWRI796_0745	OSH2 SET5	0.468	0.38368	0
AWRI796 4133	AQR1	0.547	0.035965	1	AWRI796_2204 AWRI796_3454	DCK1	0.468	0.032044	0
AWRI796_5201	YRF1-7	0.547	0.378139	0	AWRI796_5096	RRP9	0.468	0.487406	0
AWRI796_1758	TRP5	0.545	0.025594	1	AWRI796_5181	CRH1	0.468	1	0
AWRI796_1933 AWRI796_2425	SOT1	0.543	0.011301	1	AWRI796_2995 AWRI796_3360	ECM38	0.467	0.112574	0
AWRI796_1565	YPS5	0.542	0.437484	0	AWRI796_3463	IMD3	0.465	0.16451	Ő
AWRI796_2105	NEL1	0.54	0.136688	0	AWRI796_0619	GLT1	0.464	0.366947	0
AWRI796_5158 AWRI796_1831	AWK1/96_5158 SPR3	0.539	0.23021	0	AWRI796_1577 AWRI796_2018	YOR1	0.464	0.159048	0
AWRI796_2298	TMA108	0.538	0.026549	1	AWRI796_0916	ATC1	0.462	0.965612	0
AWRI796_2507	INO1	0.538	0.013751	1	AWRI796_3064	YBT1	0.46	0.174723	0
AWRI/96_3180 AWRI796_3162	GAL2 FRS1	0.538	0 003954	0	AWRI/96_1137 AWRI796_0247	APT2 MIS1	0.459	0 455464	0
AWRI796_5113	KRE6	0.537	0.034567	1	AWRI796_1192	GNP1	0.458	1	0
AWRI796_0465	HBN1	0.536	0.252784	0	AWRI796_0263	VID24	0.457	0.464202	0
AWRI/96_1824 AWRI796_3053	FMP48 MMP1	0.536	0.014843	1	AWRI796_0355 AWRI796_0082	DURI,2 PKC1	0.457	0.438589	0
AWRI796_3299	IFH1	0.536	0.009361	1	AWRI796_3869	CAT8	0.456	0.540032	0
AWRI796_4409	SIN3	0.536	0.017069	1	AWRI796_4701	YOR342C	0.455	0.442478	0
AWRI/96_256/ AWRI796_0203	CHS2	0.535	0.813497	0	AWRI/96_4915 AWRI796_2525	MOTI URA2	0.455	0.281108	0
AWRI796_2054	AIM17	0.531	0.010209	1	AWRI796_3291	CRR1	0.454	0.668226	0
AWRI796_3424	IKI3	0.531	0.022776	1	AWRI796_1231	PRB1	0.453	0.690813	0
AWRI796_0548B	FIG2 PUE3	0.53	0.194484	0	AWRI796_2616	BBC1 RRB1	0.453	0.566396	0
AWRI796 4733	AMF1	0.53	0.158011	0	AWRI796 1259	SNU13	0.455	0.205540	0
AWRI796_0078	FLO1	0.528	0.526974	0	AWRI796_0841	ARX1	0.45	0.473642	0
AWRI796_5097	MEP3	0.528	0.424087	0	AWRI796_2447	IRC24 BANG	0.448	0.280158	0
AWRI796 1635	XRN1	0.526	0.040223	1	AWRI796_2291 AWRI796_0732	PUS9	0.446	0.751808	0
AWRI796_4888	PEX25	0.525	0.037714	1	AWRI796_2920	UGP1	0.446	0.278208	0
AWRI796_3257	CBF5	0.523	0.021297	1	AWRI796_0076	SWH1	0.445	0.538055	0
AWRI796_3878 AWRI796_2428	YIR016W	0.522	0.032219	1	AWRI796_0748 AWRI796_0172	GPI18	0.444	1	0
AWRI796_3067	FPS1	0.52	0.050173	0	AWRI796_0028	RBG1	0.441	0.52669	0
AWRI796_5191	YML133C	0.52	0.148872	0	AWRI796_3783	CLN1 DEM1	0.44	0 272255	0
AWRI796_0272 AWRI796_0455	GFD2	0.519	0.461205	0	AWRI796_1112 AWRI796_1586	MTO1	0.439	0.373233	0
AWRI796_2059	DUR3	0.519	0.032219	1	AWRI796_5251	PAU3	0.438	1	0
AWRI796_2117	AAP1	0.519	0.15397	0	AWRI796_0182	MNN2	0.437	0.52669	0
AWRI796_1342 AWRI796_0035	MYO4	0.518	0.03341	1	AWRI796_1466 AWRI796_3645	NUP116	0.437	1	0
AWRI796_1430	BEM2	0.517	0.011502	1	AWRI796_0600	SEC31	0.436	0.584003	0
AWRI796_2840	MYO3	0.517	0.022802	1	AWRI796_1650	AMS1	0.436	0 701 500	0
AWRI796_4028 AWRI796_0188	FUR4	0.517	0.419134	0	AWRI796_1915 AWRI796_1419	UBP5	0.436	0.701509	0
AWRI796_1839	ART5	0.515	0.135431	0	AWRI796_3606	PLB2	0.434	0.690813	Ő
AWRI796_1528	YFR018C	0.514	0.045177	1	AWRI796_4832	TRE1	0.434	0.686491	0
AWRI/96_2642 AWRI796_3095	ILV3 VEH1	0.514	0.050633	0	AWRI/96_4919 AWRI796_2029	ARN2	0.434	0.788578	0
AWRI796_3608	PLB1	0.514	0.091116	0	AWRI796_3034	TGL4	0.433	0.642353	0
AWRI796_1427	YER152C	0.513	0.020552	1	AWRI796_3255	YLR173W	0.432	0.743273	0
AWRI/96_3535 AWRI796_3537	HMGI TCB3	0.51	0.038446	1	AWRI/96_4236 AWRI796_2101	HOLI FRC1	0.432	0.891607	0
AWRI796_3780	YMR196W	0.51	0.161658	0	AWRI796_4192	LRO1	0.431	1	0
AWRI796_3981	RPA49	0.51	0.022479	1	AWRI796_3950	WSC2	0.43	1	0
AWRI/96_1397	AVT6 RAD54	0.509	0.017018	1	AWRI796_5230	YOR389W	0.43	0.95918	0
AWRI796_4640	YTM1	0.508	0.027963	1	AWRI796_2190	FUR1	0.429	3.268704	0
AWRI796_1683	PRP43	0.507	0.046019	1	AWRI796_3952	HCH1	0.429	1	0
AWRI/96_4735	FRE3 SLA1	0.507	0.770615	0	AWRI796_1243	GLY1 NAP1	0.428	1	0
AWRI796_3516	TSL1	0.505	0.474875	0	AWRI796_1450	ECM32	0.427	1	0
AWRI796_1708	NUP145	0.504	0.050633	0	AWRI796_1827	MUP1	0.425	1	0
AWRI796_1369	DOT6 HEA1	0.502	0.327954	0	AWRI796_4652	RRP36	0.425	1	0
AWRI796 3794	MGL2	0.5	0.045177	1	AWRI796 3564	RRN11	0.424	1	0
AWRI796_0273	TKL2	0.499	0.300067	0	AWRI796_2944	UFD4	0.423	1	0
AWRI796_4195	PRP2	0.499	0.083998	0	AWRI796_3569	AMD1	0.421	1	0
AWRI796_0518 AWRI796_1148	TOM1	0.498	0.223283 0.022507	1	AWRI796_1352	RNR1	0.419	1	0

AWRI796 Gene ID AWRI796 0242	Gene Name	log <sub>2</sub> Fold Change Adj. p -valu	e Score	AWRI796 Gene ID AWRI796 1098	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value Sco	ore
AWRI796_2153	RTC3	0.418	1 0	AWRI796_3521	UTP14	0.368	1	0
AWRI796_4787 AWRI796_2138	NEW1 ERG7	0.418 0.417	1 0 1 0	AWRI796_4747 AWRI796_0359	MDL2 SDS24	0.368	1	0
AWRI796_4673	NOP58	0.415	1 0	AWRI796_2265	BAT1	0.367	1	0
AWRI796_1438 AWRI796_0216	RFS1	0.414	1 0 1 0	AWRI796_3823 AWRI796_4075	YNL134C	0.367	1	0
AWRI796_1492	FRS2	0.413	1 0	AWRI796_4796	BMS1 DAT1	0.367	1	0
AWRI796_1030 AWRI796_1295	HEM14	0.412	1 0	AWRI796_1806	ERV1	0.365	1	0
AWRI796_0468 AWRI796_1384	AGP1 NUP157	0.411	1 0	AWRI796_2031 AWRI796_4186	YHL044W CIT1	0.365	1	0
AWRI796_0266	AIM3	0.409	1 0	AWRI796_2278	BNR1	0.364	1	0
AWRI796_1375 AWRI796_2091	RAD51 YHR022C	0.409 0.408	1 0 1 0	AWRI796_2346 AWRI796_3122	KTR7 MEU1	0.364 0.364	1	0
AWRI796_1994	COQ6	0.407	1 0	AWRI796_3223	ZRT2	0.364	1	0
AWRI796_4342 AWRI796_2442	YOL075C DAL7	0.406	1 0	AWRI796_4201 AWRI796_2368	YIL055C	0.364	1	0
AWRI796_2768	OXP1	0.405	1 0	AWRI796_3676	ADH3	0.363	1	0
AWRI796_0485 AWRI796_0942	ADR1	0.404	1 0	AWRI796_4032 AWRI796_4223	COQ2	0.363	1	0
AWRI796_1363	UTP7 PAU5	0.404	1 0	AWRI796_3052	MHT1 VPL 247C	0.362	1	0
AWRI796_0381	YBR241C	0.403	1 0	AWRI796_2015	SCW4	0.361	1	0
AWRI796_0901 AWRI796_2881	SEC7 TEF4	0.403	1 0	AWRI796_0561 AWRI796_2799	ADY3 PRS1	0.36	1	0
AWRI796_4858	PPT2	0.403	1 0	AWRI796_2736	NMD5	0.359	1	Ő
AWRI796_2829 AWRI796_3719	LTV1 POM152	0.402 0.402	1 0 1 0	AWRI796_2981 AWRI796_3968	SPO14 POL2	0.359	1	0
AWRI796_4337	IRA2	0.402	1 0	AWRI796_5102	ASN1	0.358	1	0
AWRI796_1808 AWRI796_1833	ADE6	0.4 0.399	1 0 1 0	AWRI796_2253 AWRI796_2405	TIR3	0.357	1	0
AWRI796_4237	BIO5	0.399	1 0	AWRI796_2862	APE1	0.357	1	0
AWRI796_2471	ACO2	0.399	1 0	AWRI796_2238 AWRI796_4642	MOD5	0.356	1	0
AWRI796_3929	KRI1 GCN2	0.397	1 0	AWRI796_4844	SVS1	0.356	1	0
AWRI796_1502	WWM1	0.396	1 0	AWRI796_1503	CDC4	0.354	1	0
AWRI796_0031 AWRI796_1664	FUN19 YGL 140C	0.395	1 0	AWRI796_3106 AWRI796_3232	YLR001C SUS1	0.354	1	0
AWRI796_3707	ASC1	0.395	1 0	AWRI796_4996	YPR011C	0.354	1	0
AWRI796_4821 AWRI796_2289	GUP2 SLN1	0.395 0.394	1 0	AWRI796_0730 AWRI796_1119A	PRM7 HKR1	0.353	1	0
AWRI796_1930	PBP1	0.393	1 0	AWRI796_1691	NSA1	0.353	1	Ő
AWRI796_2090 AWRI796_3233	YHR020W RRN5	0.393 0.393	1 0 1 0	AWRI796_2789 AWRI796_3195	DPH2 KIN2	0.353 0.353	1	0
AWRI796_3254	DPH5	0.393	1 0	AWRI796_3369	CDC25	0.353	1	0
AWRI796_0743 AWRI796_0958	GPM2 LYS4	0.392	1 0 1 0	AWRI796_4384 AWRI796_0668	TRM3	0.353	1	0
AWRI796_1344	GPP2	0.392	1 0	AWRI796_3514 AWRI796_2137	CAC2 TRM5	0.352	1	0
AWRI796_4653	MPD1	0.392	1 0	AWRI796_4753	YPL264C	0.351	1	0
AWRI796_4754 AWRI796_4806	KEL3 TYW1	0.392	1 0	AWRI796_1246 AWRI796_1886	GDA1 ASN2	0.348	1	0
AWRI796_3158	ERG3	0.391	1 0	AWRI796_3349	CTS1	0.348	1	0
AWRI796_4527 AWRI796_1403	BAG7 RSP5	0.391 0.39	1 0 1 0	AWRI796_1198 AWRI796_3258	GRH1 RFX1	0.347 0.347	1	0
AWRI796_1900	BTN2	0.39	1 0	AWRI796_3345	YLR278C	0.347	1	Ő
AWRI796_3800 AWRI796_0583	SKY1 PRR2	0.39 0.389	1 0 1 0	AWRI796_3888 AWRI796_1602	YME2 KIP3	0.346	1	0
AWRI796_1420	FTR1	0.389	1 0	AWRI796_1904	ECL1	0.345	1	0
AWRI796_2140 AWRI796_5076	MRD1	0.389	1 0 1 0	AWRI796_2945 AWRI796_0970	VHS1	0.343	1	0
AWRI796_0536 AWRI796_0121	SOL2 VBL055C	0.388	1 0	AWRI796_3774 AWRI796_0421	GCV2 APE3	0.343	1	0
AWRI796_0941	AHA1	0.387	1 0	AWRI796_4484	LPX1	0.342	1	0
AWRI796_1491 AWRI796_3123	BUD27 POM34	0.386 0.385	1 0 1 0	AWRI796_4526 AWRI796 4980	EFT2 NCR1	0.342 0.342	1	0
AWRI796_4504	INP53	0.385	1 0	AWRI796_4033	SWT21	0.341	1	0
AWRI796_3052 AWRI796_4293	ALR1	0.384	1 0 1 0	AWRI796_4353 AWRI796_0025	CYC3	0.341	1	0
AWRI796_1484 AWRI796_3330	AGX1 HSP60	0.383	1 0	AWRI796_2398 AWRI796_3141	HIS6 PAU23	0.34	1	0
AWRI796_3519	YML096W	0.383	1 0	AWRI796_5154_55	AWRI796_5154_55	0.34	1	0
AWRI796_4442 AWRI796_0756	AKR2 MCD1	0.383 0.382	1 0	AWRI796_2148 AWRI796_4063	KSP1 RPC31	0.339	1	0
AWRI796_1569	ZRT1	0.382	1 0	AWRI796_4333	ADH1	0.339	1	0
AWRI796_2254 AWRI796_3285	HRD3	0.382 0.382	1 0	AWRI796_0865 AWRI796_2746	PMT4	0.338	1	0
AWRI796_5008	ATH1 VCE1	0.382	1 0	AWRI796_2177	BZZ1 ECM5	0.337	1	0
AWRI796_1655	INO80	0.379	1 0	AWRI796_1341	FCY21	0.336	1	0
AWRI796_2043 AWRI796_3879	ECM29 TDA1	0.379	1 0	AWRI796_3000 AWRI796_0006	YSR3 BDH2	0.336	1	0
AWRI796_1677	SCS3	0.377	1 0	AWRI796_0159	SCT1	0.335	1	Ő
AWRI796_3018 AWRI796_3218	SIS2 YLR125W	0.377 0.377	1 0 1 0	AWRI796_2124 AWRI796_4321	WRS1	0.335	1	0
AWRI796_0733	GPR1	0.376	1 0	AWRI796_3963	LYP1	0.334	1	0
AWRI796_3790 AWRI796_4070	THO2	0.376	1 0 1 0	AWRI796_1133 AWRI796_1287	NUG1	0.333	1	0
AWRI796_2003 AWRI796_2102	YGR266W YHR033W	0.374	1 0 1 0	AWRI796_2161 AWRI796_4567	HXT5 LAS17	0.333	1	0
AWRI796_2396	TIM44	0.374	1 0	AWRI796_4364	SPE2	0.332	1	0
AWRI796_2628 AWRI796_0461	AVT1 HIS4	0.374 0.373	1 0 1 0	AWRI796_5209 AWRI796 1210	YML133C HSP31	0.332 0.331	1 1	0
AWRI796_0488	ADP1	0.373	1 0	AWRI796_3851	TPS3	0.331	1	0
AWRI796_0640 AWRI796_2331	DPH1	0.373	1 0 1 0	AWRI796_0223 AWRI796_1370	PTC2	0.33	1	0
AWRI796_1233	SDD1 NAP1	0.372	1 0	AWRI796_2321	SDP1 UTP25	0.33	1	0
AWRI796_1846	PAC10	0.37	1 0	AWRI796_2341 AWRI796_4713	CIR2	0.329	1	0
AWRI796_3496 AWRI796_4358	NDI1 ARG1	0.37	1 0 1 0	AWRI796_0168 AWRI796_2112	HTB2 NCP1	0.328	1	0
AWRI796_4963	MET12	0.37	1 0	AWRI796_2688	OPI3	0.328	1	0
AWRI796_3945 AWRI796 4839	PCL1 MEX67	0.369 0.369	1 0 1 0	AWRI796_2884 AWRI796 3413	DHR2 ROM2	0.328 0.328	1 1	0
AWRI796_0474	GBP2	0.368	1 0	AWRI796_4210	BUD17	0.328	1	Ő

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change A	dj. <i>p</i> -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_0008 AWRI796_0723	STP4	0.327	1	0	AWRI796_2974	DBP7	0.295	1	0
AWRI796_1862	VAS1	0.327	1	0	AWRI796_5101	NOC4	0.295	1	0
AWRI796_0540 AWRI796_0577	CDC13	0.326	1	0	AWRI796_3908	SAM3	0.294	1	0
AWRI796_2326	YIL108W	0.326	1	0	AWRI796_4402	PLB3	0.294	1	0
AWRI796_0231 AWRI796_1889	YGR127W	0.325	1	0	AWRI796_1984 AWRI796_4598	ODC2	0.293	1	0
AWRI796_2597	YJL045W	0.325	1	0	AWRI796_4849	TGS1	0.293	1	0
AWRI796_3239 AWRI796_4486	TCB1	0.325	1	0	AWRI796_2116 AWRI796_2575	UTP18	0.292	1	0
AWRI796_1195	YDR514C	0.324	1	0	AWRI796_2735	MNS1	0.292	1	0
AWRI796_3499 AWRI796 0116	SKT5	0.324	1	0	AWRI796_4084 AWRI796_0452	GID7	0.292	1	0
AWRI796_1143	YHP1	0.323	1	0	AWRI796_1428	PET122	0.291	1	0
AWRI796_4586 AWRI796_3133	RET1 ADE16	0.323 0.322	1	0	AWRI796_1950 AWRI796_2796	YGR201C SPE1	0.291 0.291	1	0
AWRI796_3767	RGM1	0.322	1	Õ	AWRI796_3444	VIP1	0.291	1	0
AWRI796_4743 AWRI796_4847	FDH1 CDC60	0.322	1	0	AWRI796_3513 AWRI796_0580	NUP188 TIM22	0.291	1	0
AWRI796_1254	MCM3	0.322	1	0	AWRI796_1958	YGR210C	0.29	1	0
AWRI796_2604	IRC18 PAV1	0.321	1	0	AWRI796_3078	FRA1	0.29	1	0
AWRI796_2032 AWRI796_4407	TOP1	0.321	1	0	AWRI796_1479	RPO41	0.29	1	0
AWRI796_0601	SNF3	0.32	1	0	AWRI796_3583	YML020W	0.289	1	0
AWRI796_0796 AWRI796 4425	HEM12 AWRI796 4425	0.32	1	0	AWRI796_4583 AWRI796_4726	GPB1	0.289	1	0
AWRI796_5156	AWRI796_5156	0.32	1	0	AWRI796_4945	NOP4	0.289	1	0
AWRI796_0257 AWRI796_1014	VPS15 BFR2	0.319	1	0	AWRI796_3138 AWRI796_3276	SMF3 PWP1	0.288	1	0
AWRI796_3467	DIF1	0.319	1	Õ	AWRI796_4214	SSK2	0.288	1	0
AWRI796_3470	SEC39 SIN4	0.319	1	0	AWRI796_5089	YPR127W PGD1	0.288	1	0
AWRI796_1410	PMD1	0.317	1	0	AWRI796_2395	YKE4	0.287	1	0
AWRI796_2058	YHL017W	0.317	1	0	AWRI796_3793	YMR209C	0.287	1	0
AWRI796_2233	ENO2	0.317	1	0	AWRI796_4441 AWRI796_0096	TEL1	0.287	1	0
AWRI796_4575	THI72	0.316	1	0	AWRI796_2443	DAL3	0.286	1	0
AWRI796_0306 AWRI796_0504	SNT1	0.315	1	0	AWRI/96_3422 AWRI796_3565	NAM2 CAT2	0.286	1	0
AWRI796_2451	AWRI796_2451	0.315	1	0	AWRI796_3806	FSH2	0.286	1	0
AWRI796_4094 AWRI796_4699	DBP2 RPA43	0.315	1	0	AWRI796_4696 AWRI796_0812	TEA1 RTR2	0.286	1	0
AWRI796_0712	TSR1	0.314	1	0	AWRI796_2449	YPS6	0.285	1	0
AWRI796_1238	AFG1 NNK1	0.314	1	0	AWRI796_2526	TRK1 PTC6	0.285	1	0
AWRI796_1940	HIP1	0.313	1	0	AWRI796_1887	YGR125W	0.284	1	0
AWRI796_3987	ZWF1	0.312	1	0	AWRI796_2277	POT1 MTD1	0.284	1	0
AWRI796_0274 AWRI796_2283	IMP2'	0.311	1	0	AWRI796_3023 AWRI796_3633	HOF1	0.284	1	0
AWRI796_2766	JEN1	0.311	1	0	AWRI796_4415	YSP3	0.284	1	0
AWRI796_3830 AWRI796_3831	GAD1 GTO3	0.311	1	0	AWRI796_4893 AWRI796_4997	CMR3	0.284	1	0
AWRI796_0045	FUN30	0.31	1	Õ	AWRI796_0043	CCR4	0.283	1	Ő
AWRI796_2445 AWRI796_2559	LYS1 ARG3	0.31	1	0	AWRI796_3555 AWRI796_2438	CYB2 DAL1	0.283	1	0
AWRI796_4350	RIB2	0.31	1	0	AWRI796_2664	ANB1	0.282	1	0
AWRI796_0351	COS111 MET13	0.309	1	0	AWRI796_3074	RIX7 SAS10	0.282	1	0
AWRI796_2564	IML2	0.309	1	0	AWRI796_3092	BPT1	0.281	1	0
AWRI796_2592	MTR4	0.309	1	0	AWRI796_0219	PRP6	0.28	1	0
AWRI796_0140 AWRI796_2608	HCA4	0.308	1	0	AWRI796_1926 AWRI796_3894	PSE1	0.28	1	0
AWRI796_2732	RSF2	0.308	1	0	AWRI796_0195	CDS1	0.279	1	0
AWRI796_3657 AWRI796_4403	RCL1	0.308	1	0	AWRI796_1162 AWRI796_1312	YPT31	0.279	1	0
AWRI796_2490	CPS1	0.307	1	0	AWRI796_1371	TRP2	0.279	1	0
AWRI796_3183 AWRI796_3433	CST9	0.307	1	0	AWR1796_2164 AWRI796_2904	TKAI TMA19	0.279	1	0
AWRI796_0598	GGC1	0.306	1	Õ	AWRI796_1282	MNN1	0.278	1	0
AWRI796_3007 AWRI796_3308	KTR2 TOP3	0.306	1	0	AWRI796_4021 AWRI796_1110	YNL200C SIZ1	0.278	1	0
AWRI796_4281	NOP8	0.306	1	0	AWRI796_2940	URB1	0.277	1	0
AWRI796_0184 AWRI796_2350	KAP104 THS1	0.305	1	0	AWRI796_4974 AWRI796_5022	RRP12 THP3	0.277	1	0
AWRI796_4718	PRT1	0.305	1	0	AWRI796_2224	DNA2	0.276	1	0
AWRI796_2109	MSC7 VMP144W	0.304	1	0	AWRI796_1309	MIG3	0.275	1	0
AWRI796_3732 AWRI796_4564	GAC1	0.304	1	0	AWRI796_4343	DSC2	0.273	1	0
AWRI796_0616	AIR2	0.303	1	0	AWRI796_5203	COS3	0.274	1	0
AWRI796_1025 AWRI796_2342	ICE2	0.303	1	0	AWRI796_2095	PPA1	0.273	1	0
AWRI796_4224	MVD1	0.303	1	0	AWRI796_0781	REG1	0.272	1	0
AWRI796_4791 AWRI796_0697	FMP40 THI3	0.303	1	0	AWRI796_1377 AWRI796_1982	UBP9 YAP1802	0.272	1	0
AWRI796_3529	TDA9	0.302	1	0	AWRI796_2155	GAR1	0.272	1	0
AWRI/96_4541 AWRI796_4820	RPB2 NAB3	0.302	1	0	AWRI/96_2/93 AWRI796_3862	ZDS1	0.272	1	0
AWRI796_1769	PUF4	0.301	1	0	AWRI796_3230	RKM5	0.271	1	0
AWRI/96_4016 AWRI796_4582	RIO2 HIS3	0.301	1	0	AWRI796_3261 AWRI796_2729	YLR1/9C YIR124C	0.271	1	0
AWRI796_4235	ESF2	0.299	1	Ő	AWRI796_3802	TRS130	0.27	1	0
AWRI796_4986 AWRI796_0567	CIT3 MFG1	0.299	1	0	AWRI796_4150 AWRI796_4852	LAP2 PEP4	0.27	1	0
AWRI796_3172	LAM6	0.298	1	0	AWRI796_5058	SRP54	0.27	1	0
AWRI796_4060 AWRI796_4166	YCK2 EFM6	0.298	1	0	AWRI796_0052 AWRI796_0434	CYS3 HMI ai pha?	0.269	1	0
AWRI796_1606	NCS6	0.298	1	0	AWRI796_1903	ENP2	0.269	1	0
AWRI796_5133	SEC23 PDX3	0.297	1	0	AWRI796_2086	YSC84 TOF2	0.269	1	0
AWRI796_0200 AWRI796_0389	ARO4	0.296	1	0	AWRI796_3895	NIP1	0.269	1	0
AWRI796_1613	KEX1	0.296	1	0	AWRI796_1270	EDC3	0.268	1	0
AWK1/96_3043 AWRI796_3128	IZH3	0.296	1	0	AWK1/96_1699 AWRI796_1890	UTP8	0.268 0.268	1	0
AWRI796_3380	PEX30	0.296	1	0	AWRI796_4355	PRS5	0.268	1	0
AWRI796_4320 AWRI796_4558	YRM1	0.296 0.296	1	0	AWRI796_1429 AWRI796_1598	EDC1	0.267 0.267	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_1720 AWRI796_1881	NUP57	0.267	1	0	AWRI796_3941	MSB3	0.241	1	0
AWRI796_4043 AWRI796_1183	NOP13 ITR1	0.267	1	0	AWRI796_4147 AWRI796_5050	ALG11 MRL1	0.241	1	0
AWRI796_1603	CLG1	0.266	1	0	AWRI796_0287	HSL7	0.24	1	0
AWRI796_2139 AWRI796_2656	OSH3 VIR039W	0.266	1	0	AWRI796_0759 AWRI796_1611	NTH1 CHC1	0.24	1	0
AWRI796_3884	PRC1	0.266	1	Ő	AWRI796_3460	CRN1	0.24	1	Ő
AWRI796_4228 AWRI796_2841	FPK1 PMU1	0.266 0.265	1	0	AWRI796_4269 AWRI796_4732	HPF1 ATF1	0.24 0.24	1	0
AWRI796_2873	YKL091C	0.265	1	0	AWRI796_0169	NTH2	0.239	1	0
AWRI796_5210 AWRI796_0550	YHL049C KIN82	0.265	1	0	AWRI796_0988 AWRI796_1116	HEL2 SYF1	0.239 0.239	1	0
AWRI796_1275	MIT1	0.264	1	0	AWRI796_2743	HIR3	0.239	1	0
AWRI796_3362 AWRI796_2714	ADO1	0.264	1	0	AWRI796_3938 AWRI796_0190	MON2 CHS3	0.239	1	0
AWRI796_0370	ROT2	0.262	1	0	AWRI796_0734	SLM3	0.238	1	0
AWRI796_2020 AWRI796_2167	SBE22	0.262	1	0	AWRI796_2228	MTG2	0.238	1	0
AWRI796_2822	YKL151C	0.262	1	0	AWRI796_3024	TRZ1	0.238	1	0
AWRI796_4456	ETT1	0.262	1	0	AWRI796_0202 AWRI796_0767	SNQ2	0.237	1	0
AWRI796_0217	YBR053C BPH1	0.261	1	0	AWRI796_2202	YHR140W SUC2	0.237	1	0
AWRI796_0505 AWRI796_2922	YKL033W-A	0.261	1	0	AWRI796_3900	PRE5	0.237	1	0
AWRI796_5111 AWRI796_0058	TDA6 SSA1	0.261	1	0	AWRI796_4101 AWRI796_4848	POL1 AIM44	0.237	1	0
AWRI796_0418	SSH1	0.26	1	0	AWRI796_0515	PER1	0.236	1	0
AWRI796_0623 AWRI796_2889	NRP1 STB6	0.26	1	0	AWRI796_2073 AWRI796_2797	TCD1 LOT5	0.236	1	0
AWRI796_4131	RPL9B	0.26	1	0	AWRI796_3027	NUP133	0.236	1	0
AWRI796_1391 AWRI796_0059	TMN3 VPS8	0.259	1	0	AWRI796_4378 AWRI796_0039	YOL036W MAK16	0.236	1	0
AWRI796_1050	YDR341C	0.258	1	0	AWRI796_2495	HAL5	0.235	1	Ő
AWRI796_2602 AWRI796_4298	NUP192 TRM11	0.258	1	0	AWRI796_2583 AWRI796_2680	YHC3 CCT5	0.235	1	0
AWRI796_4605	WTM1	0.258	1	0	AWRI796_4872	RPL5	0.234	1	0
AWRI796_1096 AWRI796 4559	SHE9 DCS2	0.257 0.257	1	0	AWRI796_0817 AWRI796_2120	IPT1 SMF2	0.233 0.233	1	0
AWRI796_0400	SHM1	0.256	1	0	AWRI796_0768	RPL4B	0.232	1	0
AWRI/96_0477 AWRI796 1587	ADE5,7	0.256	1	0	AWRI796_1936 AWRI796_2045	WSC4	0.232 0.232	1	0
AWRI796_1719	DBP3	0.256	1	0	AWRI796_2437	YVH1	0.232	1	0
AWRI796_1858 AWRI796_2128	GIC1	0.256	1	0	AWRI796_4624 AWRI796_0005	GDH3	0.232	1	0
AWRI796_4089	DCP2	0.256	1	0	AWRI796_1680	RPS2	0.231	1	0
AWRI796_4770	YPL245W	0.256	1	0	AWRI796_2007 AWRI796_3628	YMR027W	0.231	1	0
AWRI796_0238	PFF1 CAN1	0.255	1	0	AWRI796_3914	EGT2	0.231	1	0
AWRI796_2049	SNF6	0.255	1	0	AWRI796_0441	SPB1	0.231	1	0
AWRI796_2741	IML1 VKP015C	0.255	1	0	AWRI796_1300	ISC1	0.23	1	0
AWRI796_4802	NIP7	0.255	1	0	AWRI796_4020	PSY2	0.23	1	0
AWRI796_0539 AWRI796_2243	PAT1 KOG1	0.254	1	0	AWRI796_1636 AWRI796_3586	NUP49 PSP2	0.229	1	0
AWRI796_2712	URA8	0.254	1	0	AWRI796_4825	MRN1	0.229	1	0
AWRI/96_07/4 AWRI796 2349	GCV1 AIR1	0.253	1	0	AWRI796_0647 AWRI796_0896	RG12 TRM82	0.228	1	0
AWRI796_3260	TFS1	0.253	1	0	AWRI796_1121	SIP1	0.228	1	0
AWRI796_4046 AWRI796_1265	PSD1 PXP1	0.253	1	0	AWRI796_1800 AWRI796_4174	MILI HEF3	0.228	1	0
AWRI796_1531	ATG18	0.252	1	0	AWRI796_0548A	FIG2	0.227	1	0
AWRI796_2721 AWRI796_2860	SEG2	0.252	1	0	AWRI796_2806 AWRI796_4876	NAN1	0.227	1	0
AWRI796_3469	MRPL4 KTP5	0.252	1	0	AWRI796_0020	GCV3	0.226	1	0
AWRI796_4950	MET31	0.252	1	0	AWRI796_1792	SNU71	0.226	1	0
AWRI796_1128	CYM1 APN1	0.251	1	0	AWRI796_3209	HOG1 KREQ	0.226	1	0
AWRI796_4695	KRE5	0.251	1	0	AWRI796_4173	PUB1	0.225	1	0
AWRI796_1496 AWRI796_3951	LPD1 POP3	0.25	1	0	AWRI796_5036 AWRI796_0729	FCY1 NAT1	0.225	1	0
AWRI796_4887	YPL113C	0.25	1	0	AWRI796_2752	YJR149W	0.224	1	0
AWRI796_1667 AWRI796_2002	MES1	0.249	1	0	AWRI796_4766 AWRI796_5134	DPM1	0.224	1	0
AWRI796_2330	SHQ1	0.249	1	0	AWRI796_1656	ARO2	0.223	1	0
AWRI796_2896 AWRI796_3021	ECM4	0.249	1	0	AWRI796_2223 AWRI796_3697	YKU80	0.223	1	0
AWRI796_3866	FCP1 VCP4	0.249	1	0	AWRI796_4579	MCA1 PET2	0.223	1	0
AWRI796_4697	YOR338W	0.248	1	0	AWRI796_4520	RGA1	0.222	1	0
AWRI796_4704	PYK2 HEM25	0.248	1	0	AWRI796_4744	SAM3 CNE1	0.222	1	0
AWRI796_4001	ATG4	0.247	1	0	AWRI796_1416	COX15	0.221	1	0
AWRI796_0720 AWRI796_0833	SLC1 YDR089W	0.246	1	0	AWRI796_1946 AWRI796_0244	SNG1 SPT7	0.221	1	0
AWRI796_0903	HSP42	0.246	1	0	AWRI796_0793	HEM13	0.22	1	0
AWRI796_0956 AWRI796 3173	HEM1 RFU1	0.246	1	0	AWRI796_1115 AWRI796_1658	YDR415C RRT6	0.22 0.22	1	0
AWRI796_3306	BNA5	0.246	1	0	AWRI796_3277	NOP56	0.22	1	0
AWRI796_3/14 AWRI796_4025	EPUI YNL195C	0.246	1	0	AWRI796_3560 AWRI796_3786	KSE1 ERG2	0.22 0.22	1	0
AWRI796_2064	PRS3 FMP46	0.245	1	0	AWRI796_4022	GCR2	0.22	1	0
AWRI796_5166	AAD4	0.245	1	0	AWRI796_0106	ILS1	0.22	1	0
AWRI796_1100	UTP5 MDS3	0.244	1	0	AWRI796_0156	ACH1 RRN6	0.218	1	0
AWRI796_1917	TIF4631	0.244	1	0	AWRI796_2763	FRE2	0.218	1	0
AWRI796_3089_90 AWRI796_2663	AWRI796_3089_90 TAH11	0.244	1	0	AWRI796_3835 AWRI796_4155	ROY1 BDP1	0.218	1	0
AWRI796_3320	RCK2	0.243	1	0	AWRI796_4450	WHI2	0.218	1	0
AWRI796_3550 AWRI796_3953	NTE1 ERG24	0.243 0.243	1	0	AWRI796_1139 AWRI796 2083	YDR444W ARD1	0.217 0.217	1 1	0 0
AWRI796_0126	EDE1	0.241	1	0	AWRI796_2309	KGD1	0.217	1	0
AWRI796_1741 AWRI796_1912	PTI1	0.241	1	0	AWRI796_4638	VPH1	0.217 0.217	1	0

AWRI796 Gene ID AWRI796 0502	Gene Name SYP1	log <sub>2</sub> Fold Change Adj. p -valu	e Score	AWRI796 Gene ID AWRI796 3188	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value Scor	e 0
AWRI796_1748	HEM2	0.216	1 0	AWRI796_3663	NAT4	0.191	1	0
AWRI796_2367 AWRI796_2907	VHR1 ASK1	0.216 0.216	1 0 1 0	AWRI796_0295 AWRI796_0411	MAK5 RIF1	0.189 0.189	1	0 0
AWRI796_3171	RGR1	0.216	1 0	AWRI796_2908	YKL050C	0.189	1	0
AWRI796_3939 AWRI796_0388	MRX6 HIS7	0.216 0.215	1 0 1 0	AWRI796_1168 AWRI796_2480	MNN5	0.188	1	0
AWRI796_1324	ERG28	0.215	1 0	AWRI796_2569	NET1	0.188	1	0
AWRI796_2773 AWRI796_3081	UBA1 HSP104	0.215 0.215	1 0 1 0	AWRI796_2861 AWRI796_4517	UBP2	0.188 0.188	1	0
AWRI796_3685	UTP15	0.215	1 0	AWRI796_4615	PUS7	0.188	1	0
AWRI796_4325 AWRI796_1458	PUG1	0.215 0.214	1 0	AWRI796_1364 AWRI796_1952	AWR1/96_1364 ADE3	0.187	1	0
AWRI796_2107	PUT2	0.214	1 0	AWRI796_2118	YHK8	0.187	1	0
AWRI796_2193 AWRI796_3042	UBP11	0.214 0.214	1 0	AWRI796_3700	MYO5	0.187	1	0
AWRI796_3573	NDC1	0.214	1 0	AWRI796_0499	NPP1 PDI 10	0.186	1	0
AWRI796_0170	RER2	0.213	1 0	AWRI796_0921	RVB1	0.180	1	0
AWRI796_2103	YHR033W	0.212	1 0	AWRI796_1863	TPC1	0.185	1	0
AWRI796_3883	LCB1	0.212	1 0	AWRI796_3602	CDC5	0.185	1	0
AWRI796_1870	NOP7 MAL 33	0.211	1 0	AWRI796_3912	PEX6 NSA2	0.185	1	0
AWRI796_2466	LAA1	0.211	1 0	AWRI796_1404 AWRI796_1407	SAK1	0.184	1	0
AWRI796_3312 AWRI796_3919	VPS34 VNX1	0.211	1 0	AWRI796_1854 AWRI796_2286	PIL1 FSL1	0.184	1	0 0
AWRI796_3958	GOR1	0.21	1 0	AWRI796_2374	SYG1	0.184	1	0
AWRI796_4877 AWRI796_2978	KAP120 SAP190	0.21 0.209	1 0	AWRI796_4107 AWRI796_5015	YNL095C ARP7	0.184	1	0 0
AWRI796_3212	MSL5	0.209	1 0	AWRI796_4689	SCD5	0.183	1	0
AWRI796_4085 AWRI796_5160	NAF1 AWRI796 5160	0.209	1 0	AWRI796_4725 AWRI796_2097	MRS6 DAP2	0.183 0.182	1	0 0
AWRI796_0819	TPS2	0.208	1 0	AWRI796_4890	GDE1	0.182	1	0
AWRI796_4193 AWRI796_4934	NRM1 PDR12	0.208	1 0	AWRI796_5013 AWRI796_4521	SRO7 ADE2	0.182	1	0 0
AWRI796_1046	MSN5	0.207	1 0	AWRI796_0639	LDB17	0.18	1	0
AWRI796_1692 AWRI796_2370	CUE3 MMF1	0.207 0.207	1 0 1 0	AWRI796_1164 AWRI796_1891	SNF1 SYF2	0.18 0.18	1	0 0
AWRI796_3013	GPT2	0.207	1 0	AWRI796_3396	DIC1	0.18	1	0
AWRI796_3231 AWRI796 4618	NHA1 ENV9	0.207 0.207	1 0 1 0	AWRI796_3925 AWRI796_4948	EMW1 ISM1	0.18 0.18	1	0 0
AWRI796_2182	SET1	0.206	1 0	AWRI796_3982	YNL247W	0.179	1	0
AWRI796_2934 AWRI796_3738	SP123 IMP1	0.206 0.206	1 0 1 0	AWRI796_4612 AWRI796_0626	ABP140 CDC9	0.179 0.178	1	0
AWRI796_0568	BRE4	0.205	1 0	AWRI796_0955	COX20	0.178	1	0
AWRI796_0276 AWRI796_0395	RIB5	0.204 0.204	1 0	AWRI796_1679 AWRI796_1997	RAD2	0.178	1	0
AWRI796_2299	OM45	0.204	1 0	AWRI796_2666	UTR1	0.178	1	0
AWRI796_2037 AWRI796_4595	STE13	0.204 0.204	1 0 1 0	AWRI796_5062	SYT1	0.178	1	0
AWRI796_1009	DPL1	0.203	1 0	AWRI796_0597	YDL199C	0.177	1	0
AWRI796_1967 AWRI796_2504	SSY5	0.203	1 0	AWRI796_2102 AWRI796_3119	PPR1	0.177	1	0
AWRI796_0128	COR1 UPC2	0.202	1 0	AWRI796_1083	ARO10 THI74	0.176	1	0
AWRI796_1337	FCY2	0.202	1 0	AWRI796_1271	VAC8	0.176	1	0
AWRI796_3012 AWRI796_0387	CCP1 ENP1	0.202	1 0	AWRI796_1766 AWRI796_0491	ATE1 CTO1	0.176	1	0 0
AWRI796_2256	AIM46	0.201	1 0	AWRI796_0918	YDR186C	0.175	1	0
AWRI796_2675 AWRI796_2933	PTK2 MAK11	0.201	1 0	AWRI796_2325 AWRI796_3763	SEC24 YMR178W	0.175	1	0 0
AWRI796_3354	GCD7	0.201	1 0	AWRI796_4061	GIM3	0.175	1	0
AWRI796_3593 AWRI796_4536	SPT5 PNO1	0.201	1 0	AWR1796_4983 AWR1796_0406	ULA1 BIT2	0.175	1	0 0
AWRI796_1612	POX1	0.2	1 0	AWRI796_3022	MSA2	0.174	1	0
AWRI796_2281 AWRI796_3107	UBP/ NOC3	0.2 0.2	1 0 1 0	AWRI796_3096 AWRI796_3517	ARG81	0.174 0.174	1	0
AWRI796_4086	NMA111	0.2	1 0	AWRI796_1437	GCG1	0.173	1	0
AWRI796_4418 AWRI796_3174	BUD20	0.2	1 0 1 0	AWRI796_2322 AWRI796_2476	HOS4 YJL193W	0.173 0.173	1	0
AWRI796_4339	AVO1	0.199	1 0	AWRI796_2758	AAD10	0.173	1	0
AWRI796_4446 AWRI796_4952	PMA2	0.199	1 0	AWRI796_2095 AWRI796_2491	TOH1	0.172	1	0
AWRI796_2332	XBP1 RKP1	0.198	1 0	AWRI796_2963	PRY2 SPT8	0.172	1	0
AWRI796_4739	YOR385W	0.198	1 0	AWRI796_3374	TAD3	0.172	1	0
AWRI796_1251 AWRI796_1362	RAD23 SFR3	0.197	1 0	AWRI796_4188 AWRI796_5080	RPC34 RRG8	0.172	1	0
AWRI796_2411	CFD1	0.197	1 0	AWRI796_5110	TPO3	0.172	1	0
AWRI796_3272 AWRI796_1899	HCR1 VPS62	0.197 0.196	1 0	AWRI796_0516 AWRI796_4769	RRT12 RBD2	0.171	1	0 0
AWRI796_2110	BCD1	0.196	1 0	AWRI796_4931	ALD6	0.171	1	0
AWRI796_2262 AWRI796_3680	SCH9 VBA1	0.196 0.196	1 0 1 0	AWRI796_5140 AWRI796_0783	RPC82 PST2	0.171 0.17	1	0 0
AWRI796_0980	HSP78	0.195	1 0	AWRI796_2146	LAM4	0.17	1	0
AWRI796_2040 AWRI796_2250	EGD2	0.195	1 0	AWRI796_2320 AWRI796_4959	ERG10	0.17	1	0
AWRI796_2419	PAN1 KADO5	0.195	1 0	AWRI796_4181	LST8	0.169	1	0
AWRI796_3393 AWRI796_4464	YOR062C	0.195	1 0	AWRI796_4969	IRC15	0.169	1	0
AWRI796_0363	YBR220C RPP1A	0.194	1 0	AWRI796_0914 AWRI796_1743	CDC1	0.168	1	0
AWRI796_4573	SPR1	0.194	1 0	AWRI796_3753	MME1	0.168	1	0
AWRI796_0029 AWRI796_0202	FUN12 SCO1	0.193	1 0	AWRI796_1621 AWRI796_3985	IME4 SLA2	0.167	1	0 0
AWRI796_2388	BCY1	0.193	1 0	AWRI796_3176	FMP25	0.166	1	0
AWRI796_2541 AWRI796_3321	PRM10 YEF3	0.193	1 0 1 0	AWRI796_3600 AWRI796_4551	YML002W YRR1	0.166	1	0 0
AWRI796_4331	DUF1	0.193	1 0	AWRI796_5016	GLN1	0.166	1	0
AWRI796_0594 AWRI796_4921	ACK1 UBP16	0.192 0.192	1 0 1 0	AWRI796_5042 AWRI796_1928	SPE3 ERG1	0.166	1	0 0
AWRI796_5117	TIF3	0.192	1 0	AWRI796_2465	NUC1	0.165	1	0
AWRI796_0919 AWRI796_2640	GPI14	0.191 0.191	1 0 1 0	AWRI796_2771 AWRI796_2776	SACI EMC3	0.165 0.165	1	ມ 0
AWRI796_2954	PAP1	0.191	1 0	AWRI796_3177	BOS1	0.165	1	0
AWA1/90_3148	5104	0.191	. 0	AWK1/90_388/	AIMI	0.165	1	J

AWRI796 Gene ID AWRI796 4599	Gene Name	log <sub>2</sub> Fold Change Adj. p -val	ue Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change Adj. p -va	lue Score
AWRI796_1868	PCP1	0.164	1 0	AWRI796_2397	RPB3	0.146	1 0
AWRI796_3477 AWRI796_1481	FPR4 MIL1	0.164 0.163	1 0	AWRI796_2687 AWRI796_2774	NPA3 STE6	0.146	1 0
AWRI796_1760	PIB2	0.163	1 0	AWRI796_4231	LYS9	0.146	1 0
AWRI796_1892 AWRI796_2089	YGR130C DED81	0.163 0.163	1 0 1 0	AWRI796_0147 AWRI796_0595	NCLI TRM8	0.145 0.145	1 0
AWRI796_2163	SFB3	0.163	1 0	AWRI796_1823	SCM4	0.145	1 0
AWRI796_3297 AWRI796_3632	EIS1	0.163	1 0   1 0	AWRI796_5109 AWRI796_5005	EAF3	0.145	1 0 $     1 0$
AWRI796_4671	SLY41	0.163	1 0	AWRI796_0340	RIM2	0.144	1 0
AWRI796_0538 AWRI796_0641	YDL144C	0.162	1 0 1 0	AWRI796_0484 AWRI796_0620	UGA3	0.144	1 0
AWRI796_1568	ADH4	0.162	1 0	AWRI796_1297	BIM1 ENO1	0.144	1 0
AWRI796_2940 AWRI796_5115	SGV1	0.162	1 0	AWRI796_2154	RPF1	0.144	1 0
AWRI796_0353 AWRI796_0659	KTR3 UBP1	0.161	1 0	AWRI796_2251 AWRI796_3852	MDM31 YMR262W	0.144	1 0
AWRI796_2794	MTR2	0.161	1 0	AWRI796_4280	NOP8	0.144	1 0
AWRI796_3449 AWRI796_4232	VPS36 BRE5	0.161	1 0	AWRI796_0633 AWRI796_1422	MSH5 SCC4	0.143	1 0
AWRI796_0399	TAE1	0.16	1 0	AWRI796_1998	TNA1	0.143	1 0
AWRI796_0917 AWRI796_1563	UPS3 COS12	0.16	1 0	AWRI796_2135 AWRI796_2826	DYS1 AVT3	0.143 0.143	1 0
AWRI796_2023	BIO2	0.16	1 0	AWRI796_3150	FRE8	0.143	1 0
AWRI796_2577 AWRI796_2650	MPM1 GEA1	0.16 0.16	$     1  0 \\     1  0 $	AWRI796_3545 AWRI796_1625	TEM1 TPN1	0.143 0.142	1 0 1 0
AWRI796_4665	BUD7	0.16	1 0	AWRI796_2454	AAD4	0.142	1 0
AWRI796_1469 AWRI796_2060	RGD2 RPS20	0.159 0.159	1 0     1 0	AWRI796_2956 AWRI796_3886	YKR005C ADE4	0.141 0.141	1 0
AWRI796_3493	TUB3	0.159	1 0	AWRI796_4071	SRV2	0.141	1 0
AWRI796_3918 AWRI796_4180	SIS1	0.159	1 0 1 0	AWRI796_4490 AWRI796_0192	OLA1	0.141	1 0
AWRI796_2188	ANS1	0.158	1 0	AWRI796_1462	YPR204W	0.14	1 0
AWRI796_2373 AWRI796_4911	ELP3	0.158	1 0	AWRI796_2115 AWRI796_3747	YMR160W	0.14	1 0
AWRI796_1781	ERG26	0.157	1 0	AWRI796_1011	MHR1 CUS2	0.139	1 0
AWRI796_2323	COX5B	0.157	1 0	AWRI796_4230	MSO1	0.139	1 0
AWRI796_3097	PSR1 STV1	0.157	1 0	AWRI796_4461 AWRI796_5030	LPL1 SEC8	0.139	1 0
AWRI796_3050 AWRI796_3775	SGS1	0.157	1 0 1 0	AWRI796_0661	YFH1	0.139	1 0 1 0
AWRI796_0805 AWRI796_0866	TGL2 SAC6	0.156	1 0	AWRI796_0780 AWRI796_1054	VPS54 MRP1	0.138	1 0
AWRI796_2333	SGA1	0.156	1 0	AWRI796_1059	YPQ2	0.138	1 0
AWRI796_2553 AWRI796_3031	KHA1 PRP16	0.156	1 0	AWRI796_1298 AWRI796_2748	AFG3 RPS4B	0.138	1 0
AWRI796_4341	MDM20	0.156	1 0	AWRI796_2779	EAP1	0.138	1 0
AWRI796_4374 AWRI796_4650	NOP12 RDL1	0.156 0.156	1 0 1 0	AWRI796_2982 AWRI796_3159	DAL80 MNL2	0.138 0.138	1 0
AWRI796_0764	SOK1	0.155	1 0	AWRI796_3227	PDC5	0.138	1 0
AWRI796_3752 AWRI796_4124	IMP4	0.155	1 0	AWRI796_3973 AWRI796_0825	VPS41	0.138 0.137	1 0
AWRI796_4142	MSG5	0.155	1 0	AWRI796_0857	TRM1	0.137	1 0
AWRI796_0053	SWC3	0.155	1 0 1 0	AWRI796_1342 AWRI796_3937	CLA4	0.137	1 0 1 0
AWRI796_1948	PMT6 TOM71	0.154	1 0	AWRI796_4197	PHO91 SEA4	0.137	1 0
AWRI796_3005	TIF1	0.154	1 0	AWRI796_0321	NPL4	0.136	1 0
AWRI796_4068 AWRI796_4556	MEP2 GLN4	0.154	1 0	AWRI796_0635 AWRI796_1378	RPC53 PRS2	0.136	1 0
AWRI796_4896	FMP30	0.154	1 0	AWRI796_2157	MSR1	0.136	1 0
AWRI796_0047 AWRI796_0066	PSK1 BUD14	0.153 0.153	1 0	AWRI796_2580 AWRI796_2622	LAS21 NOP9	0.136	1 0
AWRI796_1049	YDR338C	0.153	1 0	AWRI796_2701	JSN1	0.136	1 0
AWRI796_2679 AWRI796 4413	RPA12 RRP6	0.153 0.153	1 0 1 0	AWRI796_2004 AWRI796_4053	FOL2 CBK1	0.135 0.135	1 0
AWRI796_1202	SPS1	0.152	1 0	AWRI796_4808	HRR25	0.135	1 0
AWRI796_1222 AWRI796_1489	BST1	0.152	1 0 1 0	AWRI796_4856 AWRI796_4904	NOG1	0.135	1 0
AWRI796_1740	TYW3 VI P252W	0.152	1 0	AWRI796_4949	YPL039W	0.135	1 0
AWRI796_3506	COQ5	0.152	1 0 1 0	AWRI796_2523	YJL132W	0.134	1 0 1 0
AWRI796_3684 AWRI796_4106	AIP1 PHO23	0.152	1 0	AWRI796_4136 AWRI796_0323	NOP2 SMY2	0.134	1 0
AWRI796_0786	ARO3	0.151	1 0	AWRI796_1142	UTP6	0.133	1 0
AWRI796_1249 AWRI796 2068	CYC7 STE20	0.151 0.151	$     1  0 \\     1  0 $	AWRI796_1405 AWRI796 1510	LCP5 DEG1	0.133 0.133	1 0     1 0
AWRI796_2338	LYS12	0.151	1 0	AWRI796_3298	UTP13	0.133	1 0
AWRI796_2576 AWRI796_2692	MIR1	0.151	1 0	AWRI796_3615 AWRI796_3890	UBP15	0.133	1 0
AWRI796_3071	ENT4	0.151	1 0	AWRI796_3993	YNL234W	0.133	1 0
AWRI796_4363	AIM39	0.151	1 0	AWRI796_0201	CSG2	0.133	1 0
AWRI796_2077	ERG11 NMD3	0.15	1 0	AWRI796_0403	REI1 FAL1	0.132	1 0
AWRI796_2409	YIA6	0.15	1 0	AWRI796_1005	RTT103	0.132	1 0
AWRI796_2552 AWRI796_2912	BCK1 DCW1	0.15	1 0	AWRI796_2226 AWRI796_2390	CDC23 SSM4	0.132	1 0
AWRI796_2987	KAE1	0.15	1 0	AWRI796_1182	PUF6	0.131	1 0
AWRI796_1269 AWRI796_1474	NPP2 FET5	0.149 0.149	1 0 1 0	AWRI/96_1909 AWRI796_1951	KSK1 PCT1	0.131 0.131	1 0 1 0
AWRI796_3526	TUB1	0.149	1 0	AWRI796_2517	RPB4	0.131	1 0
AWRI796_0521 AWRI796_0535	SSK22	0.148	1 0 1 0	AWRI796_2927 AWRI796_4112	RHO2	0.131	1 0 1 0
AWRI796_1545	RSC8	0.148	1 0	AWRI796_4216	ABZ1 CET1	0.131	1 0
AWRI796_3217	YPS3	0.148	1 0	AWRI796_1244	IES6	0.13	1 0
AWRI796_4771 AWRI796_2114	HUT1 DOG1	0.148	1 0	AWRI796_2837 AWRI796_4525	YKL133C VPS17	0.13	1 0
AWRI796_2424	STS1	0.147	1 0	AWRI796_0107	SSA3	0.129	1 0
AWRI796_2479 AWRI796_4581	SWE1 MRM1	0.147	1 0 1 0	AWRI796_1281 AWRI796_1406	IRC22 VFA1	0.129	1 0
AWRI796_4917	ATP4	0.147	1 0	AWRI796_1684	COQ8	0.129	1 0
AWRI/96_5128 AWRI796_0533	DPB2 CPR4	0.147 0.146	1 0 1 0	AWRI796_1925 AWRI796_3614	YIPI CLU1	0.129 0.129	1 0 1 0
AWRI796_0870	CCW12	0.146	1 0	AWRI796_4468	MSA1	0.129	1 0

AWRI796 Gene ID AWRI796 0227	Gene Name YBR063C	log <sub>2</sub> Fold Change Adj.	p-value Scor	re 0	AWRI796 Gene ID AWRI796 4463	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score	;
AWRI796_0354	FTH1	0.128	1	0	AWRI796_4482	TGL5	0.111	1	0	,
AWRI796_0993 AWRI796_1744	DON1 RIM8	0.128	1	0	AWRI796_0415 AWRI796_1512	SAF1 NIC96	0.11	1	. 0	1
AWRI796_2203	CHS7	0.128	1	Ő	AWRI796_1529	AWRI796_1529	0.11	1	0	)
AWRI796_2221 AWRI796_2496	YAP1801 TPK1	0.128	1	0	AWRI796_2046 AWRI796_2433	RIM101 SEC11	0.11	1	. 0	1
AWRI796_2668	OSM1	0.128	1	0	AWRI796_1538	PTR3	0.109	1	0	)
AWRI796_4964 AWRI796_1524	RAD1 CMK1	0.128	1	0	AWRI796_1840 AWRI796_2376	ROM1 PIG2	0.109	1	. 0	1
AWRI796_2001	SAY1	0.127	1	0	AWRI796_2870	YJU3	0.109	1	0	)
AWRI796_3832 AWRI796_0314	YMR252C TOS1	0.127	1	0	AWRI796_4649 AWRI796_5108	YOR283W PIN3	0.109	1	0	1
AWRI796_1132	PPZ2	0.126	1	0	AWRI796_0728	SIR2	0.109	1	0	)
AWRI796_1315	EDC2	0.126	1	0	AWRI796_0848	YDR109C	0.108	1	0	)
AWRI796_2080 AWRI796_2192	YHR131C	0.120	1	0	AWRI796_1867	MDR1	0.108	1	0	,
AWRI796_2362	EFM4	0.126	1	0	AWRI796_2245	GPI16	0.108	1	0	)
AWRI796_3139 AWRI796_1637	ROK1	0.126	1	0	AWRI796_3009 AWRI796_3125	YEH2	0.108	1	0	)
AWRI796_3431	ART10	0.125	1	0	AWRI796_0072	UIP3 CVP7	0.107	1	0	)
AWRI796_4302 AWRI796_1018	CPR5	0.123	1	0	AWRI796_0566 AWRI796_2562	ALY2	0.107	1	0	)
AWRI796_1245	YEL043W	0.124	1	0	AWRI796_3216	YPS1	0.107	1	0	)
AWRI796_1628 AWRI796_3870	GPI12	0.124 0.124	1	0	AWRI796_3322 AWRI796_3458	TDA5	0.107	1	. 0	)
AWRI796_4037	IPI3	0.124	1	0	AWRI796_3785	RAD14	0.107	1	0	)
AWRI796_4684 AWRI796_5048	PRO2 OPY2	0.124 0.124	1	0	AWRI796_4251 AWRI796_4629	PDR18 RPT4	0.107	1	. 0	, )
AWRI796_1144	PPN1	0.123	1	0	AWRI796_0946	GTB1	0.106	1	0	)
AWRI796_2772 AWRI796_2792	TRP3 PXA2	0.123 0.123	1	0	AWRI796_5214 AWRI796_0419	YCR102C YBR284W	0.106	1	. 0	/ )
AWRI796_3316	MAP1	0.123	1	0	AWRI796_0652	CDC53	0.105	1	0	)
AWRI796_3356 AWRI796_3073	GSP1 GRC3	0.123	1	0	AWRI796_0753 AWRI796_1069	PTC1 ESE1	0.105	1	. 0	1
AWRI796_3742	YMR155W	0.122	1	Ő	AWRI796_2654	RAD26	0.105	1	0	)
AWRI796_3799 AWRI796_4781	GAS3 SSO1	0.122	1	0	AWRI796_3713 AWRI796_3796	PKR1 FFR3	0.105	1	0	1
AWRI796_0101	ALG3	0.122	1	0	AWRI796_1537	CDC14	0.103	1	0	,
AWRI796_0379	ERT1	0.121	1	0	AWRI796_2021	ERV29	0.104	1	0	)
AWRI796_2380 AWRI796_0309	ICS2	0.121	1	0	AWRI796_2273 AWRI796_2473	UBP12	0.104	1	0	,
AWRI796_1240	RML2 PLM1	0.12	1	0	AWRI796_2985	CAF4	0.104	1	0	)
AWRI796_5129	BET2	0.12	1	0	AWRI796_4208	MPP6	0.104	1	0	)
AWRI796_0390	SPO23	0.119	1	0	AWRI796_4215	PPG1 POC2	0.104	1	0	)
AWRI796_1559 AWRI796_2657	GEF1	0.119	1	0	AWRI796_0122	TOD6	0.104	1	0	)
AWRI796_4149	YNL046W	0.119	1	0	AWRI796_0311	IFA38 MDH2	0.103	1	0	)
AWRI796_5017	VMA13	0.119	1	0	AWRI796_1007	SRP101	0.103	1	0	)
AWRI796_1426	UBP3	0.118	1	0	AWRI796_1445	RAD3	0.103	1	0	)
AWRI796_1343 AWRI796_1751	PNC1	0.118	1	0	AWRI796_1020 AWRI796_2308	STH1	0.103	1	0	)
AWRI796_1762	ALK1	0.118	1	0	AWRI796_2997	TRK2	0.103	1	0	)
AWRI796_1813 AWRI796_2132	RRP3	0.118	1	0	AWRI796_5264 AWRI796_4218	ARC35	0.103	1	0	)
AWRI796_2844	RRN3	0.118	1	0	AWRI796_4550	PNS1	0.103	1	0	)
AWRI796_3972 AWRI796_4606	MKK1	0.118	1	0	AWRI796_0038	DRS2	0.103	1	0	)
AWRI796_5014	HTS1	0.118	1	0	AWRI796_1646	AIM14	0.102	1	0	)
AWRI796_0149	PIM1	0.118	1	0	AWRI796_2801	COY1	0.102	1	0	)
AWRI796_0489	PGK1	0.117	1	0	AWRI796_2810	KKQ8	0.102	1	0	)
AWRI796_1223 AWRI796_1663	HUL5	0.117	1	0	AWRI796_4424	YOR012W	0.102	1	0	,
AWRI796_3113	PAM18 VML 110W	0.117	1	0	AWRI796_4968	CTF19 MMS1	0.102	1	0	)
AWRI796_4716	VTS1	0.117	1	0	AWRI796_0520	YCR051W	0.102	1	0	)
AWRI796_4840	MRX4	0.117	1	0	AWRI796_0663	CYK3 PMT7	0.101	1	0	)
AWRI796_0171 AWRI796_2767	URA1	0.116	1	0	AWRI796_1020	YSP2	0.101	1	0	)
AWRI796_4054	YGP1	0.116	1	0	AWRI796_2276	YIL161W	0.101	1	0	)
AWRI796_4894	SYH1	0.116	1	0	AWRI796_4519	IAH1	0.101	1	0	,
AWRI796_1483	HAC1 ECT1	0.115	1	0	AWRI796_0118 AWRI796_0342	SHP1	0.1	1	. 0	1
AWRI796_2050	SNF6	0.115	1	0	AWRI796_1507	VTC2	0.1	1	0	,
AWRI796_2751	BAT2 RPP2A	0.115	1	0	AWRI796_1551	DUG1 CDH1	0.1	1	0	)
AWRI796_4686	MYO2	0.115	1	0	AWRI796_2145	IRE1	0.1	1	0	)
AWRI796_4779	VMA11 SEC14	0.115	1	0	AWRI796_2932	CDC16	0.1	1	0	)
AWRI796_3795	DML1	0.114	1	0	AWRI796_4314	WSC3	0.1	1	0	)
AWRI796_4007	YNL217W	0.114	1	0	AWRI796_4799	THI6 ECM21	0.1	1	0	)
AWRI796_4657	YOR292C	0.114	1	0	AWRI796_0080 AWRI796_0410	CHK1	0.099	1	0	)
AWRI796_0407	EFM2	0.113	1	0	AWRI796_0742	GPD1	0.099	1	0	)
AWRI796_0508 AWRI796_0889	HOM2	0.113	1	0	AWRI796_1789 AWRI796_2497	YJL163C	0.099	1	0	)
AWRI796_0960	FMN1 DEV20	0.113	1	0	AWRI796_3457	TUS1	0.099	1	0	)
AWRI796_4381	OPI10	0.113	1	0	AWRI796_3750 AWRI796_3892	FKS3	0.099	1	. 0	)
AWRI796_5079	RGC1	0.113	1	0	AWRI796_0838	GRX3	0.098	1	0	)
AWRI796_0567	i dr225w PMT5	0.112 0.112	1	0	AWRI796_0974 AWRI796_1336	HIS1	0.098	1	. 0	)
AWRI796_1235	POL5	0.112	1	0	AWRI796_2069	MRP4	0.098	1	0	)
AWRI796_1944 AWRI796_3054	GTT2	0.112 0.112	1	0	AWRI796_2129 AWRI796_2655	HUL4	0.098	1	. 0	)
AWRI796_3198	ICT1	0.112	1	0	AWRI796_3662	AVO2	0.098	1	0	)
AWRI796_3237 AWRI796_3617	ERG5	0.112 0.112	1	0	AWRI/96_4/61 AWRI796_4774	HSP82	0.098 0.098	1	. 0	)
AWRI796_4594	RFC1	0.112	1	0	AWRI796_1756	SCW11	0.097	1	0	)
AWRI796_3273	UPS1	0.111	1	0	AWRI796_3710 AWRI796_4552	DDP1	0.097	1	. 0	)
AWRI796_3281 AWRI796_3983	MSS51 VPS75	0.111	1	0	AWRI796_4600 AWRI796_4719	RPB8 PRE10	0.097	1	0	)
	110/0	0.111	1	~			0.097	1	. 0	÷

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change Adj. p -valu	e Score	AWRI796 Gene ID AWRI796 4163	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value S	Score		
AWRI796_1595	VRG4	0.096	1 0	AWRI796_5061	ASR1	0.08	1	0		
AWRI796_3435 AWRI796_4755	AFG2 FUM1	0.096	1 0	AWRI796_5268 AWRI796_1610	FLP1 SPT16	0.08	1	0		
AWRI796_0271	RAD16	0.095	1 0	AWRI796_1945	FYV8	0.079	1	0		
AWRI796_1791 AWRI796_1901	YGR012W SKN1	0.095	1 0	AWRI796_4099 AWRI796_4184	LEU4 PET8	0.079	1	0		
AWRI796_2312	POG1	0.095	1 0	AWRI796_4453	RAT1	0.079	1	0		
AWRI796_3124 AWRI796_3420	PSR2 CSR1	0.095	1 0	AWRI796_0628 AWRI796_1923	DHH1 PSD2	0.078	1	0		
AWRI796_4212	YNR029C	0.095	1 0	AWRI796_3478	HMG2	0.078	1	0		
AWRI796_4219 AWRI796_4834	MRPS12 NIP100	0.095	1 0	AWRI796_0746 AWRI796_1242	CDC7 AWRI796 1242	0.077	1	0		
AWRI796_4943	CAM1	0.095	1 0	AWRI796_1382	SSA4	0.077	1	0		
AWRI796_1333	HOM3 CNP1	0.094	1 0	AWRI796_2764	COS9 STP2	0.077	1	0		
AWRI796_3575	USA1	0.094	1 0	AWRI796_1252	ANP1	0.076	1	0		
AWRI796_4306	PTH4	0.094	1 0	AWRI796_1434	BUR6	0.076	1	0		
AWRI796_4683	LDB19	0.094	1 0	AWRI796_2394 AWRI796_2734	STR2	0.076	1	0		
AWRI796_0143	PET9	0.093	1 0	AWRI796_3087	KNS1	0.076	1	0		
AWRI796_0532 AWRI796_0637	NOP14	0.093	1 0	AWRI796_4664	MBF1	0.076	1	0		
AWRI796_1045	SWR1	0.093	1 0	AWRI796_0859	KIN1	0.075	1	0		
AWRI796_1330 AWRI796_2055	OPI1	0.093	1 0	AWRI796_1851 AWRI796_3387	SGD1	0.075	1	0		
AWRI796_2237	AWRI796_2237	0.093	1 0	AWRI796_3964	PIK1	0.075	1	0		
AWRI796_2619 AWRI796_2966	MIC60	0.093	1 0	AWRI796_2261 AWRI796_3324	MNLI MCP2	0.074	1	0		
AWRI796_4204	ATP23	0.093	1 0	AWRI796_3741	RIM13	0.074	1	0		
AWRI796_0042 AWRI796_0110	FUN26 AST1	0.092 0.092	1 0 1 0	AWRI796_4213 AWRI796_4604	ALG12 WTM2	0.074 0.074	1	0		
AWRI796_0840	TVP15	0.092	1 0	AWRI796_0505	FEN1	0.074	1	0		
AWRI/96_1698 AWRI796_2414	RPL28 SGN1	0.092	1 0	AWRI796_0135 AWRI796_0718	APL3 MCH1	0.073	1	0		
AWRI796_5146	SGE1	0.092	1 0	AWRI796_2104	PIH1	0.073	1	0		
AWRI796_0806	MAK21 GCN20	0.091	1 0	AWRI796_2144	YHR078W VHR202W	0.073	1	0		
AWRI796_2172	GGA2	0.091	1 0	AWRI796_2378	CBR1	0.073	1	0		
AWRI796_2770	DOA1	0.091	1 0	AWRI796_3182	EMP70 PIO4	0.073	1	0		
AWRI796_4370	PSK2	0.091	1 0	AWRI796_4238	USV1	0.073	1	0		
AWRI796_1897	TPO2	0.09	1 0	AWRI796_4797	YPL216W	0.073	1	0		
AWRI796_2949 AWRI796_3235	DPH6	0.09	1 0	AWRI796_1250 AWRI796_1589	TAN1	0.072	1	0		
AWRI796_4815	APL5	0.09	1 0	AWRI796_1757	CWH41	0.072	1	0		
AWRI796_0556 AWRI796_2096	RPN1	0.089	1 0	AWRI796_2019 AWRI796_2199	ARO9	0.072	1	0		
AWRI796_2420	EGH1	0.089	1 0	AWRI796_3991	YTP1	0.072	1	0		
AWRI/96_2/17 AWRI796 3105	CPA2 DNM1	0.089 0.089	1 0	AWRI796_0693 AWRI796_0892	YDL086W ACL4	0.071 0.071	1	0		
AWRI796_4108	APP1	0.089	1 0	AWRI796_3532	CPR3	0.071	1	0		
AWRI796_0529 AWRI796_0578	HCM1 DTD1	0.088 0.088	1 0 1 0	AWRI796_4869 AWRI796_0392	ODC1 DUT1	0.071	1	0		
AWRI796_2234	FMO1	0.088	1 0	AWRI796_0849	FOB1	0.07	1	0		
AWRI796_3134 AWRI796_3542	RPL15A ERV41	0.088	1 0	AWRI796_0904 AWRI796_0574	SUP35 WHI4	0.07	1	0		
AWRI796_4198	YNR014W	0.088	1 0	AWRI796_1301	GPA2	0.069	1	0		
AWRI796_1937 AWRI796_3817	TFG1 RNA1	0.087 0.087	1 0	AWRI796_1554 AWRI796_2339	BNA6 RSM25	0.069	1	0		
AWRI796_3833	YMR253C	0.087	1 0	AWRI796_2636	TDH2	0.069	1	0		
AWRI796_4359 AWRI796_5026	YOL057W ATG11	0.087 0.087	1 0	AWRI796_3598 AWRI796_3989	GLO1 LAP3	0.069	1	0		
AWRI796_5152	AWRI796_5152	0.087	1 0	AWRI796_4459	NOB1	0.069	1	0		
AWRI796_2474 AWRI796_3419	ELO1 SEC61	0.086	1 0	AWRI796_4473 AWRI796_4530	SGO1 RUP1	0.069	1	0		
AWRI796_3544	ORC1	0.086	1 0	AWRI796_0279	PTC4	0.068	1	0		
AWRI796_4682 AWRI796_5004	PMT3 SDD4	0.086	1 0	AWRI796_0671 AWRI796_0968	YDL109C MNN10	0.068	1	0		
AWRI796_0357	NGR1	0.085	1 0	AWRI796_1120	ARO80	0.068	1	0		
AWRI796_0791	YDR042C	0.085	1 0	AWRI796_1826	YGR054W	0.068	1	0		
AWRI796_1478	TUB2	0.085	1 0	AWRI796_2710	RSM26	0.068	1	0		
AWRI796_3402	ILV5 PEK2	0.085	1 0	AWRI796_3861	SCS7 BNI5	0.068	1	0		
AWRI796_4724	RPS12	0.085	1 0	AWRI796_4288	BSC6	0.068	1	0		
AWRI796_5019	TIP41	0.085	1 0	AWRI796_0183	YBR016W GTE1	0.067	1	0		
AWRI796_2078	YHR007C-A	0.084	1 0	AWRI796_1983	LSC2	0.067	1	0		
AWRI796_2133	SSF1	0.084	1 0	AWRI796_2461	YJL213W	0.067	1	0		
AWRI796_2825 AWRI796_3063	LDB18	0.084	1 0	AWRI796_3348 AWRI796_4310	SHR5	0.067	1	0		
AWRI796_0296	SUP45	0.083	1 0	AWRI796_0093	MAP2	0.066	1	0		
AWRI796_2403 AWRI796_2457	MN13 DAK2	0.083	1 0 1 0	AWRI796_0653 AWRI796_0740	RTK1	0.066	1	0		
AWRI796_4901	PNG1	0.083	1 0	AWRI796_1056	YPS7	0.066	1	0		
AWRI796_0288 AWRI796_0445	YCL049C	0.082	1 0	AWRI796_2024 AWRI796_2065	IMAI ETP1	0.066	1	0		
AWRI796_0855	APC4	0.082	1 0	AWRI796_2566	SCP160	0.066	1	0		
AWRI796_1599 AWRI796_3376	NIF3 BUD6	0.082 0.082	1 0	AWRI796_3226 AWRI796_3265	TOS4	0.066	1	0		
AWRI796_4780	NSL1	0.082	1 0	AWRI796_3825	ZRC1	0.066	1	0		
AWRI/96_4838 AWRI796_0060	DAPI TFC3	0.082 0.081	1 0 1 0	AWRI/96_4129 AWRI796_4152	KPL16B BOP3	0.066	1	0		
AWRI796_0345	YBR197C	0.081	1 0	AWRI796_2518	YUR1	0.065	1	0		
AWRI796_1629 AWRI796_2042	ATG1 GOS1	0.081 0.081	1 0 1 0	AWRI796_2880 AWRI796_2976	KRP14 GCN3	0.065	1	0 0		
AWRI796_2883	SMY1	0.081	1 0	AWRI796_3579	YML6	0.065	1	0		
AWRI796_4002 AWRI796_0051	SSU72 DEP1	0.081 0.08	1 0 1 0	AWRI796_4156 AWRI796_4814	GPI15 OXR1	0.065	1	0		
AWRI796_0808	LCB2	0.08	1 0	AWRI796_4898	ATG21	0.065	1	0		
AWRI796_0885 AWRI796_2317	GIR2 PRM5	0.08	1 0	AWRI796_5060 AWRI796_0336	NVJ2 GDT1	0.065	1	0		
AWRI796_2510	RPA34	0.08	1 0	AWRI796_2673	YJR056C	0.064	1	0		
AWRI796_2960 AWRI796_3114	FOX2 RLP24	0.08	1 0	AWRI796_3501 AWRI796_3589	VAN1 TRM9	0.064	1	0		
AWRI796_3540	POB3	0.08	1 0	AWRI796_3638	CCS1	0.064	1	0		
AMERING, G.G.         ENG B	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change A	Adj. <i>p</i> -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
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ANERDER, ADA         ERCS         Order         I         O         ANERDER, ADA         Dist         O         O         D         O           ANERDER, ADA         FINA         ORG         I         I         ANERDER, ADA         ORG         I         I         I         ANERDER, JDI         I         I         ANERDER, JDI         ORG         I         I         I         ANERDER, JDI         I         I         ANERDER, JDI         I         I         ANERDER, JDI         I <td>AWRI796_3749</td> <td>DNF3</td> <td>0.064</td> <td>1</td> <td>0</td> <td>AWRI796_1591 AWRI796_1700</td> <td>SAP4 SEH1</td> <td>0.05</td> <td>1</td> <td>0</td>	AWRI796_3749	DNF3	0.064	1	0	AWRI796_1591 AWRI796_1700	SAP4 SEH1	0.05	1	0
APAPERSONE         APAPERSONE <thapapersone< th="">        APAPERSONE        APAPERSONE</thapapersone<>	AWRI796_3804	ERG8	0.064	1	0	AWRI796_2659	NUP85	0.05	1	0
ANDER-SOL         TEL         0.03         I         0         NEED-SOL         DASL <sup>2</sup> 0.05         I         0           ANDER-SOL         ILAT         0.00         I         0         NEED-SOL         TEL         0.00         I         0           ANTER-SOL         MPN1         0.00         I         0         NEED-SOL         0.00         I         0           ANTER-SOL         MPN1         0.00         I         0         NEED-SOL         0.00         I         0           ANTER-SOL         MPN1         0.00         I         0         NEED-SOL         0.00         I         0           ANTER-SOL         META         0.00         NEED-SOL         0         NEED-SOL         0.00        <	AWRI796_4765 AWRI796_4882	ATG41 DBP1	0.064	1	0	AWRI796_3045 AWRI796_3527	SIR1 YML083C	0.05	1	0
AMERTS         OTAL         ONA         AMERTS         OTAL         OTAL <thotal< th="">         OTAL         OTAL         &lt;</thotal<>	AWRI796_0193	ETR1	0.063	1	õ	AWRI796_4091	DMA2	0.05	1	0
AMERYS. 13.2         MPTO         0.00         1         0         MREPYS. 13.2         0.000         1         0           AMERYS. 13.2         DEC2         0.000         1         0         MREPYS. 10.2         0.000         1         0           AMERYS. 10.1         DEC2         0.000 <td>AWRI796_2081</td> <td>DIA4</td> <td>0.063</td> <td>1</td> <td>0</td> <td>AWRI796_4909</td> <td>YPL088W</td> <td>0.05</td> <td>1</td> <td>0</td>	AWRI796_2081	DIA4	0.063	1	0	AWRI796_4909	YPL088W	0.05	1	0
ANDERS.         DEAL         ONE         I         I         NUMBERS         DEAL         ONE         I         I         DEAL         ONE         I         I         DEAL         ONE         I         I         DEAL         DEAL         DEAL         DEEL         DEEL <thdeel< th="">         DEEL         DEEL         <thd< td=""><td>AWRI796_2629</td><td>MPP10</td><td>0.063</td><td>1</td><td>0</td><td>AWRI796_0088 AWRI796_0412</td><td>PPS1</td><td>0.049</td><td>1</td><td>0</td></thd<></thdeel<>	AWRI796_2629	MPP10	0.063	1	0	AWRI796_0088 AWRI796_0412	PPS1	0.049	1	0
AddIme         Line         Disk         Disk <thdisk< th="">         Disk         Disk         <t< td=""><td>AWRI796_3120</td><td>BRE2</td><td>0.063</td><td>1</td><td>0</td><td>AWRI796_0765</td><td>TRP1</td><td>0.049</td><td>1</td><td>0</td></t<></thdisk<>	AWRI796_3120	BRE2	0.063	1	0	AWRI796_0765	TRP1	0.049	1	0
AMERIES         CNG         L         AMERIES         CNG         DotAG         I         O           AMERIES         CAS         MAR         DOG         L         D         AMERIES         DOG         D         D         DOG         D         D         DOG         D         D         D         D         DOG         D         D         D         D         D         D         D         D         D         D         D         D         D         D         D        D         D         D </td <td>AWRI796_3160</td> <td>SHM2 BRO1</td> <td>0.063</td> <td>1</td> <td>0</td> <td>AWRI796_4328</td> <td>MSH2 TRE2</td> <td>0.049</td> <td>1</td> <td>0</td>	AWRI796_3160	SHM2 BRO1	0.063	1	0	AWRI796_4328	MSH2 TRE2	0.049	1	0
AMELTS, GL51         AMELTS, GL51         AMELTS, GL53         SULTS, GL53	AWRI796_0307	CNS1	0.062	1	0	AWRI796_0270	CYC8	0.048	1	0
AMARDER, S.S.         MEX.         USA         USA         AMARDER, S.S.         MEX.         USA         USA         USA         USA           AMARDER, S.S.         MEX.         USA         <	AWRI796_0547	ABP1	0.062	1	0	AWRI796_0475	SGF29	0.048	1	0
AMERTER, 2010         YELDEY         0.62         I         0         AMERTER, 2010         COLA         0.048         I         0           AMERTER, 2010         DATA         0.042         I         0         AMERTER, 2010         0.041         <	AWRI/96_0584 AWRI796_1358	NOP6 MRX1	0.062	1	0	AWRI796_1286 AWRI796_1622	CDC55	0.048	1	0
AMERIDS         AMERIDS         AMERIDS         AMERIDS         CALL         COLA         COLA <thcola< th="">         COLA         COLA</thcola<>	AWRI796_2340	YIL092W	0.062	1	õ	AWRI796_3452	URA4	0.048	1	0
AMENTER, 2119         MERI         ODD         I         OVARTING 2000         PCAT         ODD 7         I         O           AMENTES, 2120         COLO         ODD         I         O         AMENTES, 2140         COLO         ODD 7         I         O           AMENTES, 2140         COLO         ODD 7         I         O         AMENTES, 2140         COLO         ODD 7         I         O           AMENTES, 2140         COLO         ODD 7         I         ODD 7         I         O         AMENTES, 4000         COLO         I         O         AMENTES, 4000         DOD 7         I         O           AMENTES, 4000         TTT1         ODD 7         I         O         AMENTES, 4000         DOD 7         I         O           AMENTES, 4000         TTT1         ODD 1         O         AMENTES, 4000         DOT 7         I         O         O         AMENTES, 4000         DOT 7         I         O         AMENTES, 4000         DOT 7         DOT 7         I         O         AMENTES, 4000         DOT 7         I         O         AMENTES, 4000         DOT 7         I         O         AMENTES, 4000         DOT 7         I         DOT 7         I         DOT 7	AWRI796_2499	FMP33	0.062	1	0	AWRI796_4674	DGK1	0.048	1	0
ANBERS, 6254         PPAL         0.62         I         0         ANREPS, 6275         LEDI6         0.617         I         0           ANREPS, 6254         ALA         0.02         I         0         ANREPS, 6275         TYDAW         0.047         I         0           ANREPS, 6494         ALA         0.02         I         0         ANREPS, 6275         PKAL         0.047         I         0           ANREPS, 6272         ATCL         0.02         I         0         ANREPS, 6275         PKAL         0.047         I         0           ANREPS, 6272         ATCL         0.061         I         0         ANREPS, 6275         PKAL         0.047         I         0           ANREPS, 6272         ATCL         0.061         I         0         ANREPS, 6270         PKTA         0.047         I         0           ANREPS, 6270         PKTA         0.061         I         0         ANREPS, 6270         PKTA         0.064         I         0	AWRI796_2916 AWRI796_4183	HRB1	0.062	1	0	AWRI796_0008	ECM1	0.048	1	0
Add Prof.         Add Prof.         Dist.         Dist. <thdist.< th=""></thdist.<>	AWRI796_4284	PPM2	0.062	1	0	AWRI796_0478	LDB16	0.047	1	0
AMBRES, 649-4         ALAL         0.02         I         0         NARES, 175         PMAI         0.07         I         0           AMBRES, 649-4         LTE1         0.03         I         0         NARES, 2123         BARS         0.017         I         0           AMBRES, 649-5         REF2         0.011         I         0         NARES, 2235         TCR0         0.017         I         0           AMBRES, 649-5         REF2         0.011         I         0         NARES, 2355         TCR0         0.017         I         0           AMBRES, 619-5         TEAL         0.011         I         0         NARES, 2497         TCR0         0.016         I         0         NARES, 2497	AWRI796_4322	COQ3 NOC2	0.062	1	0	AWRI796_0544	TUPI VDI 124W	0.047	1	0
AWREPS, 6000         I.TI.         0.66         I         0         AWREPS, 1972         MSE2         0.617         I         0           AWREPS, 6105         REIG         0.66         I         0         AWREPS, 2012         NetWoil         0.617         I         0           AWREPS, 6105         REIG         0.66         I         0         AWREPS, 2012         NetWoil         0.617         I         0           AWREPS, 6105         VEAL         0.66         I         0         AWREPS, 2019         VEAL	AWRI796_4694	ALA1	0.062	1	0	AWRI796_1775	PMA1	0.047	1	0
AMERPS         BATCS         DOUG         I         O         AMERPS         DOUG         DOUG         I         O           AMERPS         DEF2         O.61         I         O         AMERPS         TCOMO         O.47         I         O           AMERPS         DEF2         O.61         I         O         AMERPS         TCOMO         O.47         I         O           AMERPS         DEF2         O.61         I         O         AMERPS         TCOMO         O.44         I         O           AMERPS         J.17         DEC         O.61         I         O         AMERPS         DES2         O.64         I         O           AMERPS         J.17         DEC         O.61         I         O         AMERPS         DES2         O.64         I         O           AMERPS         J.17         DET         O.61         I         O         AMERPS         DES2         O.64         I         O           AMERPS         J.17         DES1         O.66         I         O         AMERPS         J.18         DES2         O.64         I         O         AMERPS         J.18         DAMERPS         J.18	AWRI796_0040	LTE1	0.061	1	0	AWRI796_1793	MSB2	0.047	1	0
AVELPS, 0252         HEF2         0.061         1         0         AVELPS, 035         YET1         0.017         1         0           AVELPS, 0257         YEA1         0.061         1         0         AVELPS, 0459         YETT         0.017         1         0           AVELPS, 0457         YEA1         0.061         1         0         AVELPS, 0459         YEX7         0.046         1         0           AVELPS, 0457         YEX7         0.061         1         0         AVELPS, 0458         YEX7         0.046         1         0           AVELPS, 0477         TT2         0.061         1         0         AVELPS, 0488         YEX7         0.046         1         0           AVELPS, 0470         TT2         0.061         1         0         AVELPS, 0488         PARAL         0.046         1         0           AVELPS, 0475         TT2         0.061         1         0         AVELPS, 0488         PARAL         0.046         1         0           AVELPS, 0475         TT3         0.051         1         0         AVELPS, 0484         PARAL         0.046         1         0           AVELPS, 0475         TT3         0.051	AWRI/96_04/3 AWRI796_0532	BUD3 ATG15	0.061	1	0	AWRI796_2412 AWRI796_2785	INP51 MIA40	0.047	1	0
AMERPS, [084]         TEAP         OB1         I         O         AWERPS, [157]         COPB         O.017         I         O           AMERPS, [157]         DESTING, [257]         O.016         I         O         AWERPS, [157]         D.016         I         O         AWERPS, [157]         D.041         O.066         I         O         AWERPS, [157]         D.041         O         AWERPS, [157]         D.046         I         O	AWRI796_0925	REF2	0.061	1	õ	AWRI796_2895	YET1	0.047	1	0
ANRENDS 1279         LIBERA         0061         1         0         ANRENDS 137         USX1         0.064         1         0           ANRENDS 137         DC1         0.051         1         0         ANRENDS 137         0.061         1         0         ANRENDS 137         0.064         1         0         ANRENDS 137         0.064         1         0         ANRENDS 137         0.064         1         0         ANRENDS 137         CRAIN 4004         1         0         ANRENDS 137         ANRENDS 137         ANRENDS 137         ANREN	AWRI796_1084	YRA1	0.061	1	0	AWRI796_4828	TCO89	0.047	1	0
ARRPS.4.00         PIC1         0.051         I         0         AVRIPS.400         VCR32C         0.44         I         0           ARRPS.4.00         PIC1         0.051         I         0         AVRIPS.400         VCR32C         0.444         I         0           ARRPS.4.00         PIC1         0.051         I         0         AVRIPS.418         PIC1         0.444         I         0           ARRPS.4.00         PIC1         0.061         I         0         AVRIPS.418         PIC1         0.444         I         0           ARRPS.4.004         MTC3         0.06         I         0         AVRIPS.4165         CRAIN         0.046         I         0           ARRPS.107         STZ         0.06         I         0         AVRIPS.4215         DAAI         0.046         I         0           ARRPS.107         STZ         0.06         I         0         AVRIPS.4215         DAAI         0.046         I         0           ARRPS.107         0.05         I         0         AVRIPS.4215         DAAI         0.046         I         0           ARRPS.4181         DARRPS.4181         MARIPS.4111         MARIPS.4111         DA	AWRI796_1105 AWRI796_2579	MRPL8	0.061	1	0	AWRI796_5126 AWRI796_0409	UBX7	0.047	1	0
ANNERS, 400         IIIS         0.04         1         0         ANNERS, 450         CMC2         0.045         1         0           ANNERS, 450         HT2         0.06         1         0         ANNERS, 450         CMC2         0.045         1         0           ANNERS, 4897         ELF4         0.06         1         0         ANNERS, 450         CML1         0.046         1         0           ANNERS, 4897         ELF4         0.06         1         0         ANNERS, 450         CML1         0.046         1         0           ANNERS, 125         SPI1         0.06         1         0         ANNERS, 125         DNA1         0.064         1         0           ANNERS, 125         SPI1         0.066         1         0         ANNERS, 135         0.064         1         0           ANNERS, 135         DEP         0.06         1         0         ANNERS, 136         0.064         1         0           ANNERS, 135         RP10         0.06         1         0         ANNERS, 136         0.054         1         0           ANNERS, 1093         RP10         0.06         1         0         ANNERS, 136         0.045	AWRI796_3147	PDC1	0.061	1	0	AWRI796_0497	YCR023C	0.046	1	0
AMR 179, 4786         IFT2         O.06         I         O         AWR 179, 487         IPT3E         O.064         I         O           AWR 179, 694         MTC4, 694         MTC4, 694         O.06         I         O         AWR 179, 697         CMI         O.064         I         O           AWR 179, 694         MTC4, 694         MTC4         O.066         I         O         AWR 179, 127         DMAI         O.066         I         O         AWR 179, 1273         DMAI         O.066         I         O         AWR 179, 1273         DMAI         O.065         I         O           AWR 179, 1281         CCE 4         O.066         I         O         AWR 179, 4244         MAIX 2         O.055         I         O           AWR 179, 4388         RO1         O.066         I         O         AWR 179, 4444         BL1         O.055         I         O         AWR 179, 4443         BL1         O         AWR 179, 4443         BL1         O         AWR 179, 4443         BL1	AWRI796_4009 AWRI796_4270_71	IES2 AWRI796 4270 71	0.061	1	0	AWRI796_0588 AWRI796_1004	CWC2 NSF3	0.046	1	0
AMEPS, 6497         ILPA         0.06         I         0         AMERS, 0.94         0.05.4         I         0           AMERS, 0.94         MTCL         0.06         I         0         AMERS, 0.94         CMAI         0.05.6         I         0           AMERS, 0.97         SPI1         0.06         I         0         AMERS, 0.278         DMAI         0.056         I         0           AMERS, 0.923         MTOI         0.06         I         0         AMERS, 0.273         CDC3         0.046         I         0           AMERS, 0.923         MTOI         0.06         I         0         AMERS, 0.213         DBR         0.05.6         I         0           AMERS, 0.383         RPN         0.06         I         0         AMERS, 0.422         BDR         0.05.5         I         0           AMERS, 0.383         RPN         0.06         I         0         AMERS, 0.431         MES3         0.055         I         0           AMERS, 0.533         ROG         0.058         I         0         AMERS, 0.531         MES3         0.055         I         0           AMERS, 0.533         RAGA         0.058         I         0 <td>AWRI796_4736</td> <td>FIT2</td> <td>0.061</td> <td>1</td> <td>0</td> <td>AWRI796_1085</td> <td>RPP2B</td> <td>0.046</td> <td>1</td> <td>0</td>	AWRI796_4736	FIT2	0.061	1	0	AWRI796_1085	RPP2B	0.046	1	0
APKEP.0.195         NTL-1908         0.06         1         0         AVKEP.0.125         CARL         0.05.0         1         0           APKEP.0.125         SNT2         0.06         1         0         AVKEP.0.127         BAA1         0.046         1         0           APKEP.0.125         SNT2         0.06         1         0         AVKEP.2.125         CDC.2         0.046         1         0           APKEP.0.125         SNT2         0.06         1         0         AVKEP.2.125         CDC.2         0.046         1         0           APKEP.0.1255         CEP4         0.06         1         0         AVKEP.2.127         0.05         1         0           AVKEP.0.200         MEX3         0.059         1         0         AVKEP.2.127         0.058         1         0         AVKEP.2.127         0.058         1         0         AVKEP.2.127         0.058         1         0         AVKEP.2.127         0.044         1         0	AWRI796_4897	ELP4	0.061	1	0	AWRI796_1431	YER156C	0.046	1	0
AVREPS_[125]         SPI1         0.06         1         0         AVREPS_1278         DMA1         0.046         1         0           AVREPS_1002         NY101         0.06         1         0         AVREPS_2173         DCLS         0.046         1         0           AVREPS_102         NY101         0.06         1         0         AVREPS_2175         DCLS         0.046         1         0           AVREPS_105         SER         CP14         0.66         1         0         AVREPS_2165         0.045         1         0           AVREPS_1055         REP1         0.06         1         0         AVREPS_1545         REP1         0.055         1         0           AVREPS_0583         REP1         0.06         1         0         AVREPS_0517         NPF1         0.055         1         0           AVREPS_0583         GCD6         0.059         1         0         AVREPS_0517         NPF1         0.045         1         0           AVREPS_0583         GLO5         0.058         1         0         AVREPS_0518         VIR1         0.044         1         0           AVREPS_0583         GLO7         0.058         1	AWRI/96_0394 AWRI796_0611	MIC4 YDL180W	0.06	1	0	AWRI796_1965 AWRI796_2176	APF4	0.046	1	0
AWR179_0_202         SNT2         0.06         I         0         AWR179_0_204         BNA1         0.064         I         0           AWR179_0_205         GEP4         0.06         I         0         AWR179_537         CLC2         0.064         I         0           AWR179_0_385         RP90         0.06         I         0         AWR179_5438         BKR2         0.045         I         0           AWR179_5488         RP01         0.06         I         0         AWR179_5138         BOI2         0.045         I         0           AWR179_0_488         RE10         0.06         I         0         AWR179_5138         0.055         I         0         AWR179_5241         WR179         0.055         I         0         AWR179_5241         WR179         0.055         I         0         AWR179_5241         WR179         0.055         I         0         AWR179_5238         CAT1         0.044         I         0           AWR179_5175         PIG1         0.058         I         0         AWR179_5143         CAT1         0.044         I         0           AWR179_5175         PIG1         0.058         I         0         AWR179_5143	AWRI796_1425	SPI1	0.06	1	Ő	AWRI796_2178	DMA1	0.046	1	0
AVERUPS         DES         DEF         DEF <thdef< th="">         DEF         <thdef< th=""> <thdef< t<="" td=""><td>AWRI796_1672</td><td>SNT2</td><td>0.06</td><td>1</td><td>0</td><td>AWRI796_2648</td><td>BNA1</td><td>0.046</td><td>1</td><td>0</td></thdef<></thdef<></thdef<>	AWRI796_1672	SNT2	0.06	1	0	AWRI796_2648	BNA1	0.046	1	0
AVR1796_2385         GEP4         0.06         I         0         AVR1796_4371         MSB1         0.046         I         0           AVR1796_388         EK01         0.066         I         0         AVR1796_4394         BK12         0.064         I         0           AVR1796_4949         BK172         0.06         I         0         AVR1796_4949         BK17         0.045         I         0           AVR1796_4949         BK172         0.059         I         0         AVR1796_4154         MPH1         0.045         I         0           AVR1796_4949         BK172         0.059         I         0         AVR1796_4157         MPH1         0.045         I         0           AVR1796_4173         PL01         0.059         I         0         AVR1796_4173         URA3         0.044         I         0           AVR1796_4173         PL01         0.058         I         0         AVR1796_4173         MR1         0.044         I         0           AVR1796_4174         PL1         0.058         I         0         AVR1796_4174         NR1         0.044         I         0           AVR1796_4174         PL1         0.058	AWRI796_2092 AWRI796_2131	SSZ1	0.06	1	0	AWRI796_3373 AWRI796 4220	DBP6	0.046	1	0
AWR1796_389         RPP0         0.06         1         0         AWR1796_384         MRC2         0.044         1         0           AWR1796_084         BUT12         0.06         1         0         AWR1796_1844         MUD1         0.0445         1         0           AWR1796_0949         SUT12         0.066         1         0         AWR1796_1976         YGR07W         0.045         1         0           AWR1796_0948         CCD6         0.099         1         0         AWR1796_2111         VPS53         0.045         1         0           AWR1796_0948         CCD6         0.099         1         0         AWR1796_2510         VEAS WY         0.044         1         0           AWR1796_0457         PUP1         0.059         1         0         AWR1796_2517         KN11         0.044         1         0           AWR1796_0453         ALG7         0.058         1         0         AWR1796_2527         MR216         0.044         1         0           AWR1796_0543         YER15         0.044         1         0         AWR1796_2574         NR216         0.044         1         0           AWR1796_0545         MR176_0565 <t< td=""><td>AWRI796_2165</td><td>GEP4</td><td>0.06</td><td>1</td><td>0</td><td>AWRI796_4571</td><td>MSB1</td><td>0.046</td><td>1</td><td>0</td></t<>	AWRI796_2165	GEP4	0.06	1	0	AWRI796_4571	MSB1	0.046	1	0
AWR1796_282         RB4         0.06         1         0         AWR1796_1447         ISC10         0.045         1         0           AWR1796_0900         MRX3         0.059         1         0         AWR1796_0151         MPH1         0.045         1         0           AWR1796_0900         MRX3         0.059         1         0         AWR1796_0151         MPH1         0.045         1         0           AWR1796_0175         PH081         0.059         1         0         AWR1796_0131         CRA5         0.044         1         0           AWR1796_0132         ALT         0.048         1         0         AWR1796_0133         GAT1         0.044         1         0           AWR1796_0333         ALG7         0.058         1         0         AWR1796_0393         GAT1         0.044         1         0           AWR1796_0375         MET2         0.058         1         0         AWR1796_0373         NR216         0.044         1         0           AWR1796_0260         EXOS4         0.057         1         0         AWR1796_1421         NIS1         0.044         1         0           AWR1796_0260         FRC6         0.055	AWRI796_3389	RPP0 FRO1	0.06	1	0	AWRI796_4864	MKK2 BOI2	0.046	1	0
AWR1796_0949         SUT2         0.06         1         0         AWR1796_0076         V10079         0.0455         1         0           AWR1796_0078         GCD6         0.059         1         0         AWR1796_0077         SAN1         0.045         1         0           AWR1796_0079         PCR1         0.059         1         0         AWR1796_0077         SAN1         0.044         1         0           AWR1796_04577         PCR1         0.058         1         0         AWR1796_0433         CAT1         0.044         1         0           AWR1796_04577         RTG2         0.058         1         0         AWR1796_0238         V10R1         0.044         1         0           AWR1796_05172         RTG2         0.058         1         0         AWR1796_0238         V10R1         0.044         1         0           AWR1796_0528         V11         0.058         1         0         AWR1796_0537         PR12         0.044         1         0           AWR1796_0528         V11         0.077         1         0         AWR1796_0537         PR12         0.043         1         0           AWR1796_0512         V12         0.0757<	AWRI796_4282	RIB4	0.06	1	0	AWRI796_1454	ISC10	0.045	1	0
AWR179, (DM)         MCG3         U.B9         I         0         AWR170, (DM)         MCG3         U.B9         I         0         AWR170, (DM)         MCG3         U.B4         I         0.045         I         0           AWR179, (JS)         BK120, (DM)         BK120, (DM)         0         AWR179, (JS)         U.B43         0.045         I         0           AWR179, (JS)         RUTC         0.059         I         0         AWR179, (JS)         U.B43         I         0         AWR179, (JS)         U.B43         I         0         AWR179, (JS)         U.B43         I         0         AWR179, (JS)         0.044         I         0           AWR179, (JS)         KITC2         0.058         I         0         AWR179, (JS)         NR1         0.044         I         0           AWR179, (JS)         KITC2         0.058         I         0         AWR179, (JS)         NR1         0.044         I         0           AWR179, (JS)         FLC1         0.058         I         0         AWR179, (JS)         RADS         0.044         I         0           AWR179, (JS)         FLC1         0.058         I         0         AWR179, (JS)         RADS	AWRI796_4994	SUT2	0.06	1	0	AWRI796_1847	YGR079W	0.045	1	0
AWR1796_1975         PH081         0.059         1         0         AWR1796_4216         VIRA212/W         0.045         1         0           AWR1796_4547         PUPI         0.059         1         0         AWR1796_4687         SAN1         0.044         1         0           AWR1796_4183         ALG7         0.058         1         0         AWR1796_4183         GAT1         0.044         1         0           AWR1796_4184         RTG2         0.058         1         0         AWR1796_2384         VIRE1         0.044         1         0           AWR1796_53944         RTG3         0.058         1         0         AWR1796_3168         RAD5         0.044         1         0           AWR1796_5394         HC1         0.058         1         0         AWR1796_4734         RAD1         0.044         1         0           AWR1796_0182         YIR224W         0.057         1         0         AWR1796_4734         RD1         0.044         1         0           AWR1796_0182         YIR242W         0.057         1         0         AWR1796_4355         PRP4         0.043         1         0           AWR1796_175         UE11	AWRI796_0090 AWRI796_0938	MRX3 GCD6	0.059	1	0	AWRI796_2415 AWRI796_2611	MPH1 VPS53	0.045	1	0
AWR1796_5404         KSC2         0.059         1         0         AWR1796_4205         YNN21W         0.045         1         0           AWR1796_1083         ALG7         0.058         1         0         AWR1796_1087         GATI         0.044         1         0           AWR1796_1087         ART         0.048         1         0         AWR1796_1087         0.044         1         0           AWR1796_1314         VTH1         0.058         1         0         AWR1796_3343         YFK1         0.044         1         0           AWR1796_3344         WTH1         0.058         1         0         AWR1796_3737         CEF1         0.044         1         0           AWR1796_4757         PLC1         0.058         1         0         AWR1796_4737         NR15         0.044         1         0           AWR1796_0200         EX064         0.057         1         0         AWR1796_4737         PR246         0.044         1         0           AWR1796_1050         TFC6         0.056         1         0         AWR1796_4737         PR24         0.043         1         0           AWR1796_1060         TFC6         0.056         1	AWRI796_1975	PHO81	0.059	1	0	AWRI796_3510	URA5	0.045	1	0
AVRTPG, 12-35         L(7)         0.068         1         0         AVRTPG, 151         0.044         1         0           AVRTPG, 151         PI1         0.058         1         0         AVRTPG, 203         VIRI         0.044         1         0           AVRTPG, 2151         PI1         0.058         1         0         AVRTPG, 234         0.044         1         0           AVRTPG, 254         VSR1         0.058         1         0         AVRTPG, 2354         0.044         1         0           AVRTPG, 256         MET2         0.058         1         0         AVRTPG, 2375         0.044         1         0           AVRTPG, 2574         MET2         0.058         1         0         AVRTPG, 2375         0.044         1         0           AVRTPG, 2171         CDC12         0.057         1         0         AVRTPG, 2057         NR1         0.043         1         0           AVRTPG, 2171         CDC12         0.057         1         0         AVRTPG, 2057         NR4         0.043         1         0           AVRTPG, 2171         CDC12         0.056         0         AVRTPG, 2157         NR4         0.043         1	AWRI796_3404	RSC2	0.059	1	0	AWRI796_4205	YNR021W	0.045	1	0
AWR1796_1572         RTG2         0.058         1         0         AWR1796_2038         VMR1         0.044         1         0           AWR1796_334         YSH1         0.058         1         0         AWR1796_2345         YRV1         0.044         1         0           AWR1796_334         WR176_0344         RC2         0.058         1         0         AWR1796_2375         CEF1         0.044         1         0           AWR1796_0312         WR12         0.058         1         0         AWR1796_4313         USL         0.044         1         0           AWR1796_0312         WR124W         0.057         1         0         AWR1796_4353         RPH46         0.044         1         0           AWR1796_0312         UDA1         0.057         1         0         AWR1796_4353         RPH46         0.043         1         0           AWR1796_2702         UDA4         0.057         1         0         AWR1796_4313         TAP         0.043         1         0           AWR1796_2702         UBA1         0.056         1         0         AWR1796_4313         TAP         0.043         1         0           AWR1796_2811         DRA1 <td>AWRI796_4547 AWRI796_0383</td> <td>ALG7</td> <td>0.059</td> <td>1</td> <td>0</td> <td>AWRI796_0877 AWRI796_1493</td> <td>GAT1</td> <td>0.044</td> <td>1</td> <td>0</td>	AWRI796_4547 AWRI796_0383	ALG7	0.059	1	0	AWRI796_0877 AWRI796_1493	GAT1	0.044	1	0
AWR1796_2151         [PI]         0.058         1         0         AWR1796_2207         MRPL6         0.044         1         0           AWR1796_2344         RET2         0.058         1         0         AWR1796_2343         KPS1         0.044         1         0           AWR1796_2340         PEC1         0.058         1         0         AWR1796_1310         CMS1         0.044         1         0           AWR1796_0340         PEC1         0.057         1         0         AWR1796_1373         PBR1         0.044         1         0           AWR1796_0171         CD         AWR1796_0373         PBP2         0.043         1         0           AWR1796_2171         CDC12         0.057         1         0         AWR1796_0373         PBP2         0.043         1         0           AWR1796_2174         DAN4         0.057         1         0         AWR1796_01075         VPS74         0.043         1         0           AWR1796_1600         TFC5         0.056         1         0         AWR1796_1815         TAP9         0.042         1         0           AWR1796_2181         PK1         0.055         1         0         AWR1796_21	AWRI796_1572	RTG2	0.058	1	0	AWRI796_2038	VMR1	0.044	1	0
AWR1796_3344         RKC3         0.058         1         0         AWR1796_3136         RAD3         0.044         1         0           AWR1796_4749         PLC1         0.058         1         0         AWR1796_4121         NIS1         0.044         1         0           AWR1796_0205         EXOS44         0.057         1         0         AWR1796_1237         RBN1         0.044         1         0           AWR1796_0352         YBR242W         0.057         1         0         AWR1796_0373         PBP2         0.043         1         0           AWR1796_02702         BUD4         0.057         1         0         AWR1796_075         VRS74         0.043         1         0           AWR1796_0166         TFC0         0.056         1         0         AWR1796_175         VRS74         0.043         1         0           AWR1796_3191         DFS1         0.056         1         0         AWR1796_175         VRS74         0.042         1         0           AWR1796_3191         DFS1         0.056         1         0         AWR1796_379         DRV1         0.042         1         0           AWR1796_3199         EKG27         0.056	AWRI796_2151	IPI1 VSH1	0.058	1	0	AWRI796_2207	MRPL6 VPK1	0.044	1	0
AWR1796_3795         MET2         0.058         1         0         AWR1796_3797         CEFI         0.044         1         0           AWR1796_0260         EXO84         0.057         1         0         AWR1796_0273         PB24         0.044         1         0           AWR1796_0220         EXO84         0.057         1         0         AWR1796_0273         PB22         0.043         1         0           AWR1796_0273         DED4         0.057         1         0         AWR1796_0173         PF24         0.043         1         0           AWR1796_2174         DAMA         0.057         1         0         AWR1796_0173         PF42         0.043         1         0           AWR1796_0213         DTK3         0.056         1         0         AWR1796_1175         PAC11         0.042         1         0           AWR1796_03121         PML1         0.056         1         0         AWR1796_3173         PMV1         0.042         1         0           AWR1796_0378         SCI1         0         AWR1796_3174         WR1796_1175         PAC11         0.042         1         0           AWR1796_0378         SCI1         0.55         <	AWRI796_3944	RFC3	0.058	1	0	AWRI796_3136	RAD5	0.044	1	0
AWR1796_0249         PLCI         0.058         1         0         AWR1796_1211         N11         0.044         1         0           AWR1796_0352         YBR243W         0.057         1         0         AWR1796_173         PB12         0.044         1         0           AWR1796_0352         YBR243W         0.057         1         0         AWR1796_173         PB12         0.043         1         0           AWR1796_0175         PM24         0.043         1         0         AWR1796_1759         PM12         0.043         1         0           AWR1796_0176         TFC6         0.055         1         0         AWR1796_175         PM21         0.043         1         0           AWR1796_0170         TFC6         0.055         1         0         AWR1796_175         PAC11         0.042         1         0           AWR1796_3199         EKC37         0.056         1         0         AWR1796_2319         NUP159         0.042         1         0           AWR1796_0378         SC11         0.055         1         0         AWR1796_319         RKC0         0.042         1         0           AWR1796_0378         KK1         0.055 <td>AWRI796_3956</td> <td>MET2</td> <td>0.058</td> <td>1</td> <td>0</td> <td>AWRI796_3797</td> <td>CEF1</td> <td>0.044</td> <td>1</td> <td>0</td>	AWRI796_3956	MET2	0.058	1	0	AWRI796_3797	CEF1	0.044	1	0
AWR1796_0382         YBR242W         0.057         1         0         AWR1796_2485         PEPA6         0.044         1         0           AWR1796_2702         BUD4         0.057         1         0         AWR1796_0737         PEP2         0.043         1         0           AWR1796_2702         BUD4         0.057         1         0         AWR1796_075         VPS14         0.043         1         0           AWR1796_1204         DASA         0.056         1         0         AWR1796_105         VPS14         0.043         1         0           AWR1796_2811         TFK3         0.056         1         0         AWR1796_175         CLD1         0.042         1         0           AWR1796_3109         PFS1         0.056         1         0         AWR1796_2373         MDV1         0.042         1         0           AWR1796_03798         SCI1         0.055         1         0         AWR1796_3731         PK13         0.042         1         0           AWR1796_022         YER193W         0.055         1         0         AWR1796_3731         REG1         0.042         1         0           AWR1796_0350         MSK2         0.055	AWRI796_4749 AWRI796_0260	PLC1 FXO84	0.058	1	0	AWRI796_4121 AWRI796_4734	NIS1 RDR1	0.044	1	0
AWR1796_0171         CDCl2         0.057         1         0         AWR1796_0373         PBP2         0.043         1         0           AWR1796_0752         BUD4         0.057         1         0         AWR1796_0655         IVR1         0.043         1         0           AWR1796_0650         TFC6         0.056         1         0         AWR1796_102         0.043         1         0           AWR1796_1020         HDS2         0.056         1         0         AWR1796_301         0.043         1         0           AWR1796_3019         DPS1         0.056         1         0         AWR1796_3175         PAC11         0.042         1         0           AWR1796_3121         PML1         0.056         1         0         AWR1796_2319         NUD159         0.042         1         0           AWR1796_3108         RMR1796_5168         0.0356         1         0         AWR1796_2319         NUD159         0.042         1         0           AWR1796_05168         MSA1         0.055         1         0         AWR1796_2318         HMX1         0.042         1         0           AWR1796_0526         IMSA1         0.055         1 <t< td=""><td>AWRI796_0382</td><td>YBR242W</td><td>0.057</td><td>1</td><td>0</td><td>AWRI796_4855</td><td>PRP46</td><td>0.044</td><td>1</td><td>0</td></t<>	AWRI796_0382	YBR242W	0.057	1	0	AWRI796_4855	PRP46	0.044	1	0
AWK1796_2742         DAVA         0.037         1         0         AWK1796_075         IVS14         0.043         1         0           AWK1796_1066         ITC5         0.035         1         0         AWK1796_1075         VPS74         0.043         1         0           AWK1796_1060         ITC5         0.0356         1         0         AWK1796_1375         VPS74         0.043         1         0           AWK1796_2311         ITK5         0.056         1         0         AWK1796_1375         PAC11         0.042         1         0           AWK1796_3191         DFL1         0.056         1         0         AWK1796_5187         CLD1         0.042         1         0           AWK1796_5199         ERC27         0.056         1         0         AWK1796_2578         MD11         0.042         1         0           AWK1796_1756         NG1         0.055         1         0         AWK1796_2378         EGU2         0.042         1         0           AWK1796_1756         NG1         0.0555         1         0         AWK1796_2378         EGU2         0.042         1         0           AWK1796_1568         MWK1796_1575 <td< td=""><td>AWRI796_2171</td><td>CDC12 BUD4</td><td>0.057</td><td>1</td><td>0</td><td>AWRI796_0373</td><td>PBP2</td><td>0.043</td><td>1</td><td>0</td></td<>	AWRI796_2171	CDC12 BUD4	0.057	1	0	AWRI796_0373	PBP2	0.043	1	0
AWR1796_1066         TFC6         0.056         1         0         AWR1796_7818         TAP9         0.043         1         0           AWR1796_120         H0S2         0.056         I         0         AWR1796_1375         PAC11         0.042         I         0           AWR1796_1312         PML1         0.056         I         0         AWR1796_51875         CLD1         0.042         I         0           AWR1796_3121         PML1         0.056         I         0         AWR1796_51875         CLD1         0.042         I         0           AWR1796_3798         SCJ1         0.056         I         0         AWR1796_5373         MDV1         0.042         I         0           AWR1796_5188         AWR1796_5188         0.055         I         0         AWR1796_3319         RC20         0.042         I         0           AWR1796_5192         WBR130         0.055         I         0         AWR1796_378         SEG1         0.042         I         0           AWR1796_5184         DK51         0         AWR1796_5178         SEG1         0.042         I         0           AWR1796_5185         DK21         0.055         I	AWRI796_2702 AWRI796_2754	DAN4	0.057	1	0	AWRI796_0005 AWRI796_1075	VPS74	0.043	1	0
AWR1796_1620         HOS2         0.056         I         0         AWR1796_175         PA2         0.043         I         0           AWR1796_3091         DPS1         0.056         I         0         AWR1796_175         PACI1         0.042         I         0           AWR1796_3191         DPS1         0.056         I         0         AWR1796_2319         NUP159         0.042         I         0           AWR1796_3197         ERG27         0.056         I         0         AWR1796_3737         GPI13         0.042         I         0           AWR1796_036         MSS4         0.055         I         0         AWR1796_3783         SEGI         0.042         I         0           AWR1796_036         MSS4         0.055         I         0         AWR1796_3783         SEGI         0.042         I         0           AWR1796_3852         DKS2         0.055         I         0         AWR1796_4140         POR1         0.042         I         0           AWR1796_4855         DKS2         0.055         I         0         AWR1796_4343         MS11         0.041         I         0           AWR1796_016162         HIR1         0.055 <td>AWRI796_1066</td> <td>TFC6</td> <td>0.056</td> <td>1</td> <td>0</td> <td>AWRI796_3818</td> <td>TAF9</td> <td>0.043</td> <td>1</td> <td>0</td>	AWRI796_1066	TFC6	0.056	1	0	AWRI796_3818	TAF9	0.043	1	0
AWR1706_2001         DPS1         0.056         1         0         AWR1706_1875         CLD1         0.042         1         0           AWR1706_3121         PML1         0.056         1         0         AWR1706_2573         NUP19         0.042         1         0           AWR1706_3199         EGC37         0.056         1         0         AWR1705_537         MDV1         0.042         1         0           AWR1705_3798         SC11         0.056         1         0         AWR1705_3214         YLR18C         0.042         1         0           AWR1706_0222         YBR139W         0.055         1         0         AWR1796_3319         IRC20         0.042         1         0           AWR1796_0132         UVG1         0.055         1         0         AWR1796_3311         RPD3         0.042         1         0           AWR1796_3845         DSK2         0.055         1         0         AWR1796_5343         MS11         0.042         1         0           AWR1796_6384         DSK2         0.055         1         0         AWR1796_5343         MS11         0.041         1         0           AWR1796_0351         DK2         0 <td>AWRI796_1620</td> <td>HOS2 TPK3</td> <td>0.056</td> <td>1</td> <td>0</td> <td>AWRI796_4305</td> <td>PAP2 PAC11</td> <td>0.043</td> <td>1</td> <td>0</td>	AWRI796_1620	HOS2 TPK3	0.056	1	0	AWRI796_4305	PAP2 PAC11	0.043	1	0
AWR1796_3121         PML1         0.056         1         0         AWR1796_2139         NUP159         0.042         1         0           AWR1796_3798         SCI1         0.056         1         0         AWR1796_2377         GPI3         0.042         1         0           AWR1796_0378         SCI1         0         AWR1796_3214         YLR118C         0.042         1         0           AWR1796_0292         YBR139W         0.055         1         0         AWR1796_3283         HMX1         0.042         1         0           AWR1796_0319         HYG1         0.055         1         0         AWR1796_3678         SEG1         0.042         1         0           AWR1796_3822         CUS1         0.055         1         0         AWR1796_4140         POR1         0.042         1         0           AWR1796_0485         UME1         0.055         1         0         AWR1796_0343         MSI1         0.042         1         0           AWR1796_0485         UME1         0.055         1         0         AWR1796_0343         MSI1         0.041         1         0           AWR1796_0107         LHB1         0.054         1         0 </td <td>AWRI796_2091</td> <td>DPS1</td> <td>0.056</td> <td>1</td> <td>0</td> <td>AWRI796_1875</td> <td>CLD1</td> <td>0.042</td> <td>1</td> <td>0</td>	AWRI796_2091	DPS1	0.056	1	0	AWRI796_1875	CLD1	0.042	1	0
AWK1796_3199         EKG27         0.056         1         0         AWK1796_377         MDV1         0.042         1         0           AWK1796_5168         AWK1796_5168         0.056         1         0         AWK1796_313         0.042         1         0           AWK1796_092         YBK193W         0.055         1         0         AWK1796_3233         HMX1         0.042         1         0           AWK1796_0366         MSS4         0.055         1         0         AWK1796_3719         RC20         0.042         1         0           AWK1796_3822         CUS1         0.055         1         0         AWK1796_3719         RC20         0.042         1         0           AWK1796_3852         DSK2         0.055         1         0         AWK1796_0371         RPD3         0.042         1         0           AWK1796_0881         EKI1         0.055         1         0         AWK1796_0371         RK1         0.042         1         0           AWK1796_0871         WK1796_0475         UMK1796_0473         MSI1         0.041         1         0           AWK1796_0451         ISIN         0.054         1         0         AWK1796_137	AWRI796_3121	PML1	0.056	1	0	AWRI796_2319	NUP159	0.042	1	0
AWR1796_5168         AWR1796_5168         0.055         1         0         AWR1796_3214         YLR118C         0.042         1         0           AWR1796_0292         YBR139W         0.055         1         0         AWR1796_3283         HMX1         0.042         1         0           AWR1796_0366         MSS4         0.055         1         0         AWR1796_3678         SEG1         0.042         1         0           AWR1796_5385         DSK2         0.055         1         0         AWR1796_53678         SEG1         0.042         1         0           AWR1796_63865         DSK2         0.055         1         0         AWR1796_5044         POR1         0.042         1         0           AWR1796_0162         HIR1         0.055         1         0         AWR1796_0343         MS11         0.041         1         0           AWR1796_0162         HIR1         0.054         1         0         AWR1796_0372         CMC42         0.041         1         0           AWR1796_1305         YAT2         0.054         1         0         AWR1796_0372         SWC5         0.041         1         0           AWR1796_1454         ISN1	AWRI796_3199 AWRI796_3798	ERG27 SCJ1	0.056	1	0	AWRI796_2537 AWRI796_3077	GPI13	0.042	1	0
AWR1796_0292       YBR139W       0.055       1       0       AWR1796_3283       HMX1       0.042       1       0         AWR1796_1319       HVC1       0.055       1       0       AWR1796_3678       SEG1       0.042       1       0         AWR1796_3822       CUS1       0.055       1       0       AWR1796_3678       SEG1       0.042       1       0         AWR1796_3865       DSK2       0.055       1       0       AWR1796_0244       MSF1       0.042       1       0         AWR1796_0845       DKE1       0.055       1       0       AWR1796_0343       MSI1       0.041       1       0         AWR1796_0881       EK11       0.054       1       0       AWR1796_0377       CMK2       0.041       1       0         AWR1796_1305       YAT2       0.054       1       0       AWR1796_0372       SWC5       0.04       1       0         AWR1796_1435       ISN1       0.054       1       0       AWR1796_0372       SWC5       0.04       1       0         AWR1796_1457       LSB5       0.053       1       0       AWR1796_0137       DSU2       0.04       1       0	AWRI796_5168	AWRI796_5168	0.056	1	õ	AWRI796_3214	YLR118C	0.042	1	0
AWR1796_1319       HVG1       0.005       1       0       AWR1796_1319       HVG1       0.042       1       0         AWR1796_5822       CUS1       0.055       1       0       AWR1796_578       SEG1       0.042       1       0         AWR1796_5822       CUS1       0.055       1       0       AWR1796_5024       MSF1       0.042       1       0         AWR1796_6162       HIR1       0.054       1       0       AWR1796_6133       MSI1       0.041       1       0         AWR1796_0901       CCC2       0.054       1       0       AWR1796_036       FRT2       0.041       1       0         AWR1796_1305       YAT2       0.054       1       0       AWR1796_0326       ECM31       0.044       1       0         AWR1796_1305       YAT2       0.054       1       0       AWR1796_1459       MUA       1       0         AWR1796_1454       ISN1       0.053       1       0       AWR1796_1459       MUA       1       0         AWR1796_1459       YAT2       0.053       1       0       AWR1796_1459       MUA       1       0         AWR1796_1450       RPB7       0.053 <td>AWRI796_0292</td> <td>YBR139W</td> <td>0.055</td> <td>1</td> <td>0</td> <td>AWRI796_3283</td> <td>HMX1 IBC20</td> <td>0.042</td> <td>1</td> <td>0</td>	AWRI796_0292	YBR139W	0.055	1	0	AWRI796_3283	HMX1 IBC20	0.042	1	0
AWR1796_3822       CUS1       0.055       1       0       AWR1796_3140       POR1       0.042       1       0         AWR1796_3865       DSK2       0.055       1       0       AWR1796_5024       MSF1       0.042       1       0         AWR1796_0162       HIR1       0.054       1       0       AWR1796_0333       MSI1       0.041       1       0         AWR1796_0162       HIR1       0.054       1       0       AWR1796_0333       MSI1       0.041       1       0         AWR1796_0162       HIR1       0.054       1       0       AWR1796_0366       FRT2       0.044       1       0         AWR1796_1305       YAT2       0.054       1       0       AWR1796_0326       ECM31       0.044       1       0         AWR1796_1455       ISN1       0.053       1       0       AWR1796_1729       SWC5       0.044       1       0         AWR1796_108       SSD1       0.053       1       0       AWR1796_1799       YGK021W       0.04       1       0         AWR1796_108       SSD1       0.053       1       0       AWR1796_1299       AWR1796_1499       0.04       1       0	AWRI796_1319	HVG1	0.055	1	0	AWRI796_3678	SEG1	0.042	1	0
AWR1796_3865         DSK2         0.055         1         0         AWR1796_4140         POR1         0.042         1         0           AWR1796_0162         HIR1         0.055         1         0         AWR1796_0343         MSF1         0.042         1         0           AWR1796_0162         HIR1         0.054         1         0         AWR1796_0202         ZUO1         0.041         1         0           AWR1796_1305         YAT2         0.054         1         0         AWR1796_0236         FR12         0.041         1         0           AWR1796_1745         RNA15         0.054         1         0         AWR1796_0326         ECM31         0.044         1         0           AWR1796_10457         LSB5         0.053         1         0         AWR1796_01372         SWC5         0.04         1         0           AWR1796_2897         YKL063C         0.053         1         0         AWR1796_1459         AWR1796_1459         0.044         1         0           AWR1796_2897         YKL063C         0.053         1         0         AWR1796_2159         QIA4         1         0           AWR1796_2897         YKL063C         0.052	AWRI796_3822	CUS1	0.055	1	0	AWRI796_3911	RPD3	0.042	1	0
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	AWRI796_3865 AWRI796_4865	DSK2 UME1	0.055	1	0	AWRI796_4140 AWRI796_5024	POR1 MSF1	0.042	1	0
AWR1796_0881         EKI1         0.054         1         0         AWR1796_2022         ZU01         0.041         1         0           AWR1796_0991         CCC2         0.054         1         0         AWR1796_0036         FRT2         0.041         1         0           AWR1796_1305         YAT2         0.054         1         0         AWR1796_0326         ECM31         0.04         1         0           AWR1796_4545         ISN1         0.053         1         0         AWR1796_0132         DLD2         0.04         1         0           AWR1796_1650         SSD1         0.053         1         0         AWR1796_1459         0.044         1         0           AWR1796_2897         YKL063C         0.053         1         0         AWR1796_279         YGR021W         0.04         1         0           AWR1796_0264         ENB1         0.052         1         0         AWR1796_237         RPN15         0.04         1         0           AWR1796_0909         TCM62         0.052         1         0         AWR1796_5161         0.04         1         0           AWR1796_0909         TCM62         0.052         1         0	AWRI796_0162	HIR1	0.054	1	õ	AWRI796_0343	MSI1	0.041	1	0
AWR1796_02971         CCL2         0.004         1         0         AWR1796_1397         CMR2         0.071         1         0           AWR1796_1305         YAT2         0.054         1         0         AWR1796_0326         ECM31         0.04         1         0           AWR1796_1305         YAT2         0.054         1         0         AWR1796_0326         ECM31         0.04         1         0           AWR1796_0457         LSB5         0.053         1         0         AWR1796_1459         AWR1796_1459         0.044         1         0           AWR1796_1008         SD1         0.053         1         0         AWR1796_1459         AWR1796_1459         0.044         1         0           AWR1796_2897         YKL063C         0.053         1         0         AWR1796_2257         RPN10         0.04         1         0           AWR1796_0209         TCM62         0.052         1         0         AWR1796_5200         TH5         0.04         1         0           AWR1796_0209         TCM62         0.052         1         0         AWR1796_5161         0.04         1         0           AWR1796_1416         EM24         0.052	AWRI796_0881	EKI1	0.054	1	0	AWRI796_2022	ZUO1 CMK2	0.041	1	0
AWR1796_1745       RNA15       0.054       1       0       AWR1796_0326       ECM31       0.04       1       0         AWR1796_4545       ISN1       0.054       1       0       AWR1796_0372       SWC5       0.04       1       0         AWR1796_1008       SSD1       0.053       1       0       AWR1796_1459       AWR1796_1459       0.04       1       0         AWR1796_1008       SSD1       0.053       1       0       AWR1796_129       QGR021W       0.04       1       0         AWR1796_2097       YKL063C       0.053       1       0       AWR1796_2290       ATG32       0.04       1       0         AWR1796_0209       TCM62       0.052       1       0       AWR1796_3867       PRM15       0.04       1       0         AWR1796_054       IVY1       0.052       1       0       AWR1796_5161       AWR1796_5164       0.04       1       0         AWR1796_1391       MRP353       0.052       1       0       AWR1796_6137       0.039       1       0         AWR1796_1091       MRP3535       0.052       1       0       AWR1796_6137       D.04       1       0         AWR1796_	AWRI796 1305	YAT2	0.054	1	0	AWRI796_4397 AWRI796_0036	FRT2	0.041	1	0
AWR1796_4545       ISN1       0.054       1       0       AWR1796_0372       SWC5       0.04       1       0         AWR1796_1008       SSD1       0.053       1       0       AWR1796_1459       AWR1796_1459       0.04       1       0         AWR1796_1008       SSD1       0.053       1       0       AWR1796_1459       AWR1796_1459       0.04       1       0         AWR1796_2897       YKL063C       0.053       1       0       AWR1796_2290       ATG32       0.04       1       0         AWR1796_4264       ENB1       0.052       1       0       AWR1796_2290       ATG32       0.04       1       0         AWR1796_0954       IVY1       0.052       1       0       AWR1796_5020       TIF5       0.04       1       0         AWR1796_1616       EMP24       0.052       1       0       AWR1796_5020       TIF5       0.04       1       0         AWR1796_3071       DSL1       0.052       1       0       AWR1796_6131       0.039       1       0         AWR1796_057       CDC48       0.038       1       0       AWR1796_0657       CDC48       0.038       1       0	AWRI796_1745	RNA15	0.054	1	0	AWRI796_0326	ECM31	0.04	1	0
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	AWRI796_4545 AWRI796_0457	ISN1 I SB5	0.054	1	0	AWRI796_0372 AWRI796_0613	SWC5 DLD2	0.04	1	0
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	AWRI796_1008	SSD1	0.053	1	0	AWRI796_1459	AWRI796_1459	0.04	1	0
AWR1796_2897       YRL063C       0.053       1       0       AWR1796_2257       RPN10       0.04       1       0         AWR1796_0161       ALK2       0.053       1       0       AWR1796_2290       TG32       0.04       1       0         AWR1796_0209       TCM62       0.052       1       0       AWR1796_5200       TIF5       0.04       1       0         AWR1796_0209       TCM62       0.052       1       0       AWR1796_5020       TIF5       0.04       1       0         AWR1796_1616       EMP24       0.052       1       0       AWR1796_5020       TIF5       0.04       1       0         AWR1796_1616       EMP24       0.052       1       0       AWR1796_5020       TIF5       0.039       1       0         AWR1796_05161       0.052       1       0       AWR1796_633       POL31       0.039       1       0         AWR1796_0971       DSL1       0.052       1       0       AWR1796_633       POL31       0.038       1       0         AWR1796_0957       RTN1       0.051       1       0       AWR1796_0891       SSY1       0.038       1       0         AWR179	AWRI796_1105	RPB7	0.053	1	0	AWRI796_1799	YGR021W	0.04	1	0
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	AWRI/96_2897 AWRI796_4264	YKL063C ENB1	0.053	1	0	AWR1796_2257 AWR1796_2290	ATG32	0.04	1	0
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	AWRI796_0161	ALK2	0.052	1	õ	AWRI796_3867	PRM15	0.04	1	0
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	AWRI796_0209	TCM62	0.052	1	0	AWRI796_4244	YNR063W	0.04	1	0
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	AWRI796_1616	EMP24	0.052	1	0	AWRI796_5161	AWRI796_5161	0.04	1	0
Awk1/96_3004         GLG1         0.052         1         0         AWR1796_2633         POL31         0.039         1         0           AWR1796_3011         DSL1         0.052         1         0         AWR1796_0657         CDC48         0.038         1         0           AWR1796_0113         SEF1         0.051         1         0         AWR1796_0891         SSY1         0.038         1         0           AWR1796_0153         SFF1         0.051         1         0         AWR1796_1314         ZRG8         0.038         1         0           AWR1796_1255         SPF1         0.051         1         0         AWR1796_2361         RN83         0.038         1         0           AWR1796_1866         TEL2         0.051         1         0         AWR1796_3040         ESL2         0.038         1         0           AWR1796_2316         RH03         0.051         1         0         AWR1796_5056         ASA1         0.038         1         0           AWR1796_3006         UTP30         0.051         1         0         AWR1796_5156         ASA1         0.037         1         0           AWR1796_4034         UBP10         0.051 </td <td>AWRI796_1919</td> <td>MRPS35</td> <td>0.052</td> <td>1</td> <td>0</td> <td>AWRI796_1092</td> <td>SAC7</td> <td>0.039</td> <td>1</td> <td>0</td>	AWRI796_1919	MRPS35	0.052	1	0	AWRI796_1092	SAC7	0.039	1	0
AWR1796_0113         SEF1         0.051         1         0         AWR1796_0097         CDC40         0.058         1         0           AWR1796_0113         SEF1         0.051         1         0         AWR1796_0891         SSY1         0.038         1         0           AWR1796_0957         RTN1         0.051         1         0         AWR1796_1314         ZRG8         0.038         1         0           AWR1796_1255         SPF1         0.051         1         0         AWR1796_2082         VPS29         0.038         1         0           AWR1796_1866         TEL2         0.051         1         0         AWR1796_3040         ESL2         0.038         1         0           AWR1796_2316         RHO3         0.051         1         0         AWR1796_3040         ESL2         0.038         1         0           AWR1796_3006         UTP30         0.051         1         0         AWR1796_1040         ESL2         0.037         1         0           AWR1796_4034         UBP10         0.051         1         0         AWR1796_2141         PPE1         0.037         1         0           AWR1796_0982         SWM1         0.055 </td <td>AWRI796_3004 AWRI796_3071</td> <td>GLG1 DSI 1</td> <td>0.052</td> <td>1</td> <td>0</td> <td>AWRI796_2633 AWRI796_0657</td> <td>POL31 CDC48</td> <td>0.039</td> <td>1</td> <td>0</td>	AWRI796_3004 AWRI796_3071	GLG1 DSI 1	0.052	1	0	AWRI796_2633 AWRI796_0657	POL31 CDC48	0.039	1	0
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	AWRI796_0113	SEF1	0.052	1	0	AWRI796_0891	SSY1	0.038	1	0
Awr(1796_1255)         SPF1         0.051         1         0         AWR(1796_2082)         VPS29         0.038         1         0           AWR(1796_1866)         TEL2         0.051         1         0         AWR(1796_2082)         RNR3         0.038         1         0           AWR(1796_2316)         RHO3         0.051         1         0         AWR(1796_3040)         ESL2         0.038         1         0           AWR(1796_2316)         YAK1         0.051         1         0         AWR(1796_5056)         ASA1         0.038         1         0           AWR(1796_3006)         UTP30         0.051         1         0         AWR(1796_2141)         PPE1         0.037         1         0           AWR(1796_4034)         UBP10         0.051         1         0         AWR(1796_23959)         TOF1         0.037         1         0           AWR(1796_0982)         SWM1         0.05         1         0         AWR(1796_3959)         TOF1         0.037         1         0           AWR(1796_1366)         LV1         0.05         1         0         AWR(1796_474412)         PHO80         0.037         1         0           AWR(1796_1366	AWRI796_0957	RTN1	0.051	1	0	AWRI796_1314	ZRG8	0.038	1	0
AWR1796_2316         RHO3         0.051         1         0         AWR1796_3040         ESL2         0.038         1         0           AWR1796_2516         YAK1         0.051         1         0         AWR1796_3040         ESL2         0.038         1         0           AWR1796_2516         YAK1         0.051         1         0         AWR1796_5056         ASA1         0.038         1         0           AWR1796_3006         UTP30         0.051         1         0         AWR1796_1905         NAT2         0.037         1         0           AWR1796_4034         UBP10         0.051         1         0         AWR1796_3959         TOF1         0.037         1         0           AWR1796_0982         SWM1         0.05         1         0         AWR1796_474412         PHO80         0.037         1         0           AWR1796_1366         LIV1         0.05         1         0         AWR1796_474412         PHO80         0.037         1         0	AWK1/96_1255 AWRI796_1866	SPF1 TEL2	0.051	1	0 0	AWRI/96_2082 AWRI796_2361	VPS29 RNR3	0.038	1	0
AWR1796_2516         YAK1         0.051         1         0         AWR1796_5056         ASA1         0.038         1         0           AWR1796_2515         SDH1         0.051         1         0         AWR1796_1905         NAT2         0.037         1         0           AWR1796_3006         UTP30         0.051         1         0         AWR1796_1905         NAT2         0.037         1         0           AWR1796_4034         UBP10         0.051         1         0         AWR1796_3959         TOF1         0.037         1         0           AWR1796_0982         SWM1         0.05         1         0         AWR1796_4744         PHO80         0.037         1         0           AWR1796_1366         LLV1         0.05         1         0         AWR1796_4744         PHO80         0.037         1         0	AWRI796_2316	RHO3	0.051	1	õ	AWRI796_3040	ESL2	0.038	1	0
AWR179_025         SD11         0.051         1         0         AWR179_1205         NA12         0.057         1         0           AWR179_0306         UTP30         0.051         1         0         AWR179_2141         PPE1         0.037         1         0           AWR179_04034         UBP10         0.051         1         0         AWR179_3959         TOF1         0.037         1         0           AWR179_0982         SWM1         0.05         1         0         AWR179_412         PH080         0.037         1         0           AWR179_1366         LIV1         0.05         1         0         AWR179_47442         PH080         0.037         1         0	AWRI796_2516	YAK1 SDH1	0.051	1	0	AWRI796_5056	ASA1 NAT2	0.038	1	0
AWRI796_4034         UBP10         0.051         1         0         AWRI796_3959         TOF1         0.037         1         0           AWR1796_0982         SWM1         0.05         1         0         AWR1796_4412         PH080         0.037         1         0           AWR1796_1366         LLV1         0.05         1         0         AWR1796_4764         YAH1         0.037         1         0	AWRI796_2020 AWRI796_3006	UTP30	0.051	1	0	AWRI796_2141	PPE1	0.037	1	0
AWRI/96_0982         SWM1         0.05         1         0         AWRI796_4412         PHO80         0.037         1         0           AWRI796         1366         ILV1         0.05         1         0         AWR1796 4764         VAH1         0.037         1         0	AWRI796_4034	UBP10	0.051	1	0	AWRI796_3959	TOF1	0.037	1	0
	AWRI796_0982 AWRI796_1366	SWMI ILV1	0.05 0.05	1	0	AWRI/96_4412 AWRI796 4764	PHO80 YAH1	0.037 0.037	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change Adj. p -val	ue Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_2288	MLP2	0.036	1 0 1 0	AWRI796_3626	MRPL3	0.022	1	0
AWRI796_2740	MET5	0.036	1 0	AWRI796_5066	RPL11A TMT1	0.022	1	0
AWRI796_2855 AWRI796_3591	ERV25	0.036	1 0 1 0	AWRI796_1449 AWRI796_3129	UBR2	0.021	1	0
AWRI796_3616	BUD22	0.036	1 0	AWRI796_3500	ATR1	0.021	1	0
AWRI796_3927 AWRI796_4570	ZIM17 TUF1	0.036	1 0   1 0	AWRI796_4207 AWRI796_4498	SNF12 RAS1	0.021	1	0
AWRI796_2048	YHL026C	0.035	1 0	AWRI796_4827	CTI6	0.021	1	0
AWRI796_4227	TRM112 EPD2	0.035	1 0	AWRI796_4854	RRD2	0.021	1	0
AWRI796_0132 AWRI796_0269	YSA1	0.034	1 0	AWRI796_2324 AWRI796_2713	SOD1	0.02	1	0
AWRI796_2149	SAM35	0.034	1 0	AWRI796_3051	AYT1	0.02	1	0
AWRI/96_2174 AWRI796_2427	ERP5 YIR014W	0.034	1 0 $   1 0$	AWRI796_3115 AWRI796_3511	SEC65	0.02	1	0
AWRI796_2532	ALB1	0.034	1 0	AWRI796_3551	SML1	0.02	1	0
AWRI796_3017	DRE2 HBS1	0.034	1 0	AWRI796_3934	RPS19B FMP41	0.02	1	0
AWRI796_3353	COQ11	0.034	1 0	AWRI796_4058	NSG2	0.02	1	0
AWRI796_4918	GPI2	0.034	1 0	AWRI796_4507	CEX1	0.02	1	0
AWRI796_0444 AWRI796_0651	SRF1	0.033	1 0 $   1 0$	AWRI796_4936 AWRI796_1079	ARH1	0.02	1	0
AWRI796_1709	NBP35	0.033	1 0	AWRI796_3241	YLR149C	0.019	1	0
AWRI796_2328 AWRI796_3062	MOB1 FRE6	0.033	1 0   1 0	AWRI796_3561 AWRI796_4372	GSF2 NTG2	0.019	1	0
AWRI796_3286	SEC13	0.033	1 0	AWRI796_4907	RPS6B	0.019	1	0
AWRI796_3333	RED1	0.033	1 0	AWRI796_5081	YPR117W	0.019	1	0
AWRI796_0971	YDR248C	0.033	1 0	AWRI796_0758 AWRI796_0856	VBA4	0.018	1	0
AWRI796_1199	EUG1	0.032	1 0	AWRI796_1473	LAM5	0.018	1	0
AWRI/96_1262 AWRI796_1828	YEL023C RSC1	0.032	1 0 $   1 0$	AWRI796_2478 AWRI796_2696	RPS22A YJR084W	0.018	1	0
AWRI796_3189	XDJ1	0.032	1 0	AWRI796_0463	RNQ1	0.017	1	0
AWRI796_3200	APC9 DUS1	0.032	1 0	AWRI796_2970	VPS51 PTT109	0.017	1	0
AWRI796_3389	ADH2	0.032	1 0	AWRI796_3897	GLC8	0.017	1	0
AWRI796_4092	YNL115C	0.032	1 0	AWRI796_4920	YTA6	0.017	1	0
AWRI/96_4435 AWRI796_4005	ALG9	0.032	1 0   1 0	AWR1796_0402 AWR1796_0569	PTP1	0.016	1	0
AWRI796_4751	DIM1	0.031	1 0	AWRI796_0852	MRX14	0.016	1	0
AWRI796_4885	HOS3 PBV1	0.031	1 0	AWRI796_1439	PAB1 VIP5	0.016	1	0
AWRI796_0294	BMT2	0.03	1 0	AWRI796_2150	STE12	0.016	1	0
AWRI796_0377	PRP5	0.03	1 0	AWRI796_3665	TVP18	0.016	1	0
AWRI796_0844 AWRI796_2494	ERG20	0.03	1 0   1 0	AWRI796_0545	CSM1	0.016	1	0
AWRI796_2554	TOK1	0.03	1 0	AWRI796_1257	BUD16	0.015	1	0
AWRI796_4514 AWRI796_4870	RIO1 RDS2	0.03	1 0 1 0	AWRI796_1264 AWRI796_2130	URA3 PAN5	0.015	1	0
AWRI796_0127	PSY4	0.029	1 0	AWRI796_2581	NUP82	0.015	1	0
AWRI796_0935	UME6	0.029	1 0	AWRI796_2726	ATP2	0.015	1	0
AWRI796_2930	URA6	0.029	1 0	AWRI796_0716	MBP1	0.013	1	0
AWRI796_4151	YIP3	0.029	1 0	AWRI796_1436	RAD4	0.014	1	0
AWRI/96_4260 AWRI796_4668	YIR042C ISW2	0.029	1 0   1 0	AWRI796_1888 AWRI796_2239	YGR126W YHR182W	0.014	1	0
AWRI796_4925	YPL068C	0.029	1 0	AWRI796_2247	ERG9	0.014	1	0
AWRI796_1395 AWRI796_1669	RPL23B RPL1B	0.028	1 0	AWRI796_2838 AWRI796_3810	RMA1 TAF7	0.014	1	0
AWRI796_1864	ASK10	0.028	1 0	AWRI796_4122	APJ1	0.014	1	0
AWRI796_2212	SPO12	0.028	1 0	AWRI796_4182	MRP7	0.014	1	0
AWRI796_2344 AWRI796_3487	YML131W	0.028	1 0 1 0	AWRI796_0953	PCF11	0.014	1	0
AWRI796_0615	YDL176W	0.027	1 0	AWRI796_1588	SEC15	0.013	1	0
AWRI796_1812 AWRI796_2627	YGR035C OST1	0.027 0.027	$   1 0 \\   1 0 $	AWRI796_1961 AWRI796_2301	FLX1	0.013	1	0
AWRI796_4330	MPD2	0.027	1 0	AWRI796_3827	COA6	0.013	1	0
AWRI796_4408 AWRI796_4549	RPB11 MTR10	0.027	1 0	AWRI796_4414 AWRI796_0581	ALG6 RRI1	0.013	1	0
AWRI796_0089	BRN1	0.026	1 0	AWRI796_1006	HRQ1	0.012	1	0
AWRI796_0453	ATG22	0.026	1 0	AWRI796_1237	MAK10	0.012	1	0
AWRI796_1091 AWRI796_1596	SDT1	0.026	1 0   1 0	AWRI796_2047 AWRI796_2707	AWR1/96_2047 YJR098C	0.012	1	0
AWRI796_2318	HIS5	0.026	1 0	AWRI796_3127	SDO1	0.012	1	0
AWRI796_2514 AWRI796_3398	YJL144W NIT3	0.026	1 0	AWRI796_3654 AWRI796_3931	SEN15 MRPS18	0.012	1	0
AWRI796_3849	TRM732	0.026	1 0	AWRI796_5099	TAZ1	0.012	1	0
AWRI796_3949 AWRI796_4241	MRPL10 FRF4	0.026	1 0	AWRI796_0301 AWRI796_0320	ARA1 SSE2	0.011	1	0
AWRI796_5046	LTP1	0.026	1 0	AWRI796_0401	YPT10	0.011	1	0
AWRI796_5123	JIP5 DDI 22A	0.026	1 0	AWRI796_0738	MPS1 VDD1	0.011	1	0
AWRI796_0097 AWRI796_1532	ROG3	0.025	1 0   1 0	AWRI796_0760 AWRI796_1326	SPO73	0.011	1	0
AWRI796_1583	CSE1	0.025	1 0	AWRI796_2399	FAF1	0.011	1	0
AWRI796_2585 AWRI796_3225	IKSI USB1	0.025	1 0	AWRI796_2508 AWRI796_3335	SNA3 PDR8	0.011	1	0
AWRI796_4669	RRG7	0.025	1 0	AWRI796_3658	AEP1	0.011	1	0
AWRI796_1102	URH1 PPT13	0.024	1 0	AWRI796_3955	CAF120 TIR4	0.011	1	0
AWRI796_1548	SAP155	0.024	1 0	AWRI796_4458	VHS3	0.011	1	0
AWRI796_1796	YGR017W	0.024	1 0	AWRI796_4497	CRC1 MGM1	0.011	1	0
AWRI796_3271	PEX13	0.024	1 0	AWRI796_0164	LDB7	0.011	1	0
AWRI796_4470	VPS5	0.024	1 0	AWRI796_0758	RMD1	0.01	1	0
AWRI/96_4630 AWRI796_0735	GCD1 DBP10	0.024	1 0	AWRI796_0834 AWRI796_1876	KLII YGR111W	0.01	1	0
AWRI796_1268	GTT3	0.023	1 0	AWRI796_2731	VPS70	0.01	1	0
AWRI796_1963	GPI1 STP2	0.023	1 0	AWRI796_2943	CCE1 OMA1	0.01	1	0
AWRI796_4993	HAA1	0.023	1 0	AWRI796_4247	YNR066C	0.01	1	0
AWRI796_5034	ARO7	0.023	1 0	AWRI796_4654	YOR289W	0.01	1	0
AWRI796_0117 AWRI796_0175	DSF2	0.022	1 0 1 0	AWRI796_5006 AWRI796_0888	RPA14	0.01	1	0
AWRI796_1146	GUK1	0.022	1 0	AWRI796_3249	PUS5	0.009	1	0
AWRI/96_1726 AWRI796_2778	NPY1 LOS1	0.022	1 0	AWRI796_3635 AWRI796_4221	IMP2 ZRG17	0.009	1	0
AWRI796_2991	SHB17	0.022	1 0	AWRI796_4299	HRP1	0.009	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change Adj. p -val	1 Score	AWRI796 Gene ID AWRI796 5150	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value Score
AWRI796_4714	SNX3	0.009	1 0	AWRI796_1082	RGA2	-0.005	1 (
AWRI796_0317 AWRI796_1037	TYR1 YCG1	0.008	1 0	AWRI796_1535 AWRI796_1593	HIS2 VID30	-0.006	1 (
AWRI796_1367	AIM10	0.008	1 0	AWRI796_1753	MIG1	-0.006	1 (
AWRI796_1697 AWRI796_1723	VPS73 HSF1	0.008	1 0	AWRI796_1924 AWRI796_2142	MSM1 PTC7	-0.006	1 (
AWRI796_1818	NQM1	0.008	1 0	AWRI796_2169	YPT35	-0.006	1 (
AWRI796_2678 AWRI796_2783	NTA1 PEX1	0.008 0.008	$   1 0 \\   1 0 $	AWRI796_2236 AWRI796_3853	STB5 SAP30	-0.006 -0.006	1 (
AWRI796_2928	GPX1	0.008	1 0	AWRI796_4623	NAT5	-0.006	1 (
AWRI796_3907 AWRI796_4304	AWRI796_3907 MSN1	0.008 0.008	$   1 0 \\   1 0 $	AWRI796_0253 AWRI796_0277	PHO3 MRPL36	-0.007 -0.007	1 (
AWRI796_0589	NHP2	0.007	1 0	AWRI796_0325	SWD3	-0.007	1 (
AWRI796_2183 AWRI796_3278	MSH1 PBA1	0.007	$   1 0 \\   1 0 $	AWRI796_0446 AWRI796 1452	SPS22 PDA1	-0.007 -0.007	1 (
AWRI796_3724	ERG29	0.007	1 0	AWRI796_3346	YLR283W	-0.007	1 (
AWRI796_3828 AWRI796_4621	FAA4 TUM1	0.007 0.007	1 0 1 0	AWRI796_0224 AWRI796_0416	DUG2	-0.008 -0.008	1 (
AWRI796_4710	SOG2	0.007	1 0	AWRI796_0858	DPB4	-0.008	1 (
AWRI796_1307 AWRI796_2762	MCH2	0.006	$   1 0 \\   1 0 $	AWRI796_0932 AWRI796_2216	COQ4 LIN1	-0.008 -0.008	1 (
AWRI796_3581	APT1	0.006	1 0	AWRI796_2671	KCH1	-0.008	1 (
AWRI796_4233 AWRI796_5025	TAH18	0.006	$   1 0 \\   1 0 $	AWRI796_2852 AWRI796_3675	APN1 ISF1	-0.008	1 (
AWRI796_0826	PDC2	0.005	$\begin{array}{ccc} 1 & 0 \\ 1 & 0 \end{array}$	AWRI796_4661	YOR296W	-0.008	1 (
AWRI796_1061	TRP4	0.005	1 0 1 0	AWRI796_0603	ARF1	-0.008	1 (
AWRI796_2807	EBP2	0.005	1 0	AWRI796_0792	NRG1	-0.009	1 (
AWRI796_3220	APC2	0.005	1 0	AWRI796_2777	ADD66	-0.009	1 (
AWRI796_4387	MIM1	0.005	1 0	AWRI796_3253	APS1	-0.009	1 (
AWRI796_1033	ASP1 ASP1	0.004	1 0 1 0	AWRI796_0259	FES1	-0.09	1 (
AWRI796_1208	CAB1 CLE2	0.004	1 0	AWRI796_0948	CRF1	-0.01	1 (
AWRI796_1386 AWRI796_1418	DDI1	0.004	1 0 $   1 0$	AWRI796_2037 AWRI796_3210	AVL9	-0.01	1 (
AWRI796_1632	YGL176C VMP1	0.004	$\begin{array}{ccc} 1 & 0 \\ 1 & 0 \end{array}$	AWRI796_4015	YNL208W	-0.01	1 (
AWRI796_2996	FMP46	0.004	1 0	AWRI796_0376	ABD1	-0.011	1 (
AWRI796_3236	ACF2 LEM3	0.004	$\begin{array}{ccc} 1 & 0 \\ 1 & 0 \end{array}$	AWRI796_0528	BUD31 CCT4	-0.011	1 (
AWRI796_4467	CYT1	0.004	1 0	AWRI796_0727	PRP11	-0.011	1 (
AWRI796_5172	PAU6 SDS23	0.004	1 0	AWRI796_1316	ARB1 CAE16	-0.011	1 (
AWRI796_1767	KAP122	0.003	1 0	AWRI796_2125	MED6	-0.011	1 (
AWRI796_2725 AWRI796_4348	JHD2 HST1	0.003	1 0	AWRI796_3427 AWRI796_4817	REH1 RSA1	-0.011	1 (
AWRI796_0364	PDB1	0.002	1 0	AWRI796_5068	FHL1	-0.011	1 (
AWRI796_1263 AWRI796_1959	GEA2 ZPR1	0.002 0.002	1 0   1 0	AWRI796_0606 AWRI796_1226	PPH22 SIT1	-0.012	1 (
AWRI796_4366	GSH2	0.002	1 0	AWRI796_3428	STE23	-0.012	1 (
AWRI796_1280 AWRI796_3294	WBP1 CPR6	0.001 0.001	$   1 0 \\   1 0 $	AWRI796_3505 AWRI796_3668	BUL2 SDD2	-0.012 -0.012	1 (
AWRI796_3507	ZDS2	0.001	1 0	AWRI796_5216	COS3	-0.012	1 (
AWRI796_4647 AWRI796_4978	FSH3 CHL1	0.001	1 0 1 0	AWRI796_1060 AWRI796 1152	MRPL28	-0.013	1 (
AWRI796_0810	RPS13	0	1 0	AWRI796_1853	RPL11B	-0.013	1 (
AWRI796_1534 AWRI796_3304	ECM22	0	1 0 1 0	AWRI796_1962 AWRI796_3168	FYV7	-0.013	1 (
AWRI796_4762	HFI1 DKM2	0	1 0	AWRI796_3407	STE11	-0.013	1 (
AWRI796_0196 AWRI796_0312	CDC28	-0.001	1 0 1 0	AWRI796_4394	YOL019W	-0.013	1 (
AWRI796_0541	SRB8	-0.001	1 0	AWRI796_1615	MCM6	-0.014	1 (
AWRI796_1343	CEM1	-0.001	1 0 1 0	AWRI796_2719	YJR111C	-0.014	1 (
AWRI796_2506 AWRI796_2529	VPS35 GCD14	-0.001	$   1  0 \\   1  0 $	AWRI796_4146 AWRI796_4466	SFB2 VNG1	-0.014	1 (
AWRI796_2720	NNF1	-0.001	1 0	AWRI796_0208	QDR3	-0.015	1 (
AWRI796_2876 AWRI796_4789	CAB3 MMT2	-0.001	1 0   1 0	AWRI796_0422 AWRI796_0724	YBR287W SIT4	-0.015	1 (
AWRI796_0751	APC11	-0.002	1 0	AWRI796_1387	FLO8	-0.015	1 (
AWRI796_2722 AWRI796_3044	TDA4 SKG1	-0.002	1 0	AWRI796_1941 AWRI796_0012	TDH3 ACS1	-0.015	1 (
AWRI796_3381	YLR326W	-0.002	1 0	AWRI796_0669	RRP42	-0.016	1 (
AWRI796_4316 AWRI796_4385	ITR2 YAP7	-0.002 -0.002	$   1 0 \\   1 0 $	AWRI796_1396 AWRI796_1477	SHO1 YPT1	-0.016 -0.016	1 (
AWRI796_4906	GLR1	-0.002	1 0	AWRI796_2000	BUD32	-0.016	1 (
AWRI796_4941 AWRI796_0160	MNN9 YBL010C	-0.002 -0.003	$   1 0 \\   1 0 $	AWRI796_2067 AWRI796_2877	YHL008C CYT2	-0.016 -0.016	1 (
AWRI796_0256	YBR096W	-0.003	1 0	AWRI796_3782	CIK1	-0.016	1 (
AWRI796_0605 AWRI796_1973	PHB2	-0.003	$   1 0 \\   1 0 $	AWRI796_3811 AWRI796_4109	YPT53	-0.016	1 (
AWRI796_1995	GND2	-0.003	1 0	AWRI796_4115	MKT1	-0.016	1 (
AWRI796_2643	ESS1	-0.003	1 0 1 0	AWRI796_0305	RIB7	-0.018	1 (
AWRI796_3008	TFA2 MOT2	-0.003	1 0	AWRI796_0562	GUD1	-0.017	1 (
AWRI796_4199	SMM1	-0.003	1 0	AWRI796_1081 AWRI796_1451	BMH1	-0.017	1 (
AWRI796_5189	IMD2 RAD28	-0.003	1 0	AWRI796_1835	SPT4 PEX2	-0.017	1 (
AWRI796_2229	DBP8	-0.004	1 0	AWRI796_3342	MCM5	-0.017	1 (
AWRI796_3549 AWRI796_4294	OGG1 VPS68	-0.004 -0.004	1 0 1 0	AWRI796_3587 AWRI796_4958	PPZ1 SUV3	-0.017	1 (
AWRI796_1131	PPM1	-0.005	1 0	AWRI796_4967	VTC3	-0.017	1 (
AWRI/96_2108 AWRI796_2360	KRF1 YIL067C	-0.005 -0.005	1 0 1 0	AWRI796_0013 AWRI796_0559	flC2 AAD4	-0.018 -0.018	1 (
AWRI796_2848	OAC1	-0.005	1 0	AWRI796_1353	TDA2	-0.018	1 (
AWRI796_2911 AWRI796_3495	ANK2 GTR1	-0.005 -0.005	1 0 1 0	AWRI/96_1608 AWRI796_1750	MIG2 OCH1	-0.018 -0.018	1 (
AWRI796_3699	ILV2	-0.005	$\begin{array}{ccc} 1 & 0 \\ 1 & 0 \end{array}$	AWRI796_1786	TFG2	-0.018	1 (
AWRI796_3896 AWRI796_4045	APC1	-0.005	1 0 1 0	AWRI796_2697	YJR085C	-0.018	1 (
AWRI796_4082	FAR11 THP1	-0.005	$\begin{array}{ccc} 1 & 0 \\ 1 & 0 \end{array}$	AWRI796_2866	MTC2	-0.018	1 (
AWRI796_4388	LAG2	-0.005	1 0	AWRI796_0831	RRP1	-0.019	1 (
AWRI796_4956	PHO85	-0.005	1 0	AWRI796_0900	STB3	-0.019	1 (

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change A	Adj. p -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_1109 AWRI796_1174	ADE8 RIB3	-0.019 -0.019	1	0	AWRI/96_4168 AWRI796_0992	GLO2	-0.031 -0.032	1	0
AWRI796_2745	YJR142W	-0.019	1	0	AWRI796_1213	PAD1	-0.032	1	0
AWRI796_3016 AWRI796_3642	YKR070W ARG80	-0.019	1	0	AWRI796_1376 AWRI796_1540	SHC1 SMC2	-0.032	1	0
AWRI796_0391	MRPS5	-0.02	1	0	AWRI796_2220	PEX18	-0.032	1	0
AWRI796_0845	ARP10	-0.02	1	0	AWRI796_2387	CAP2	-0.032	1	0
AWRI796_2011 AWRI796_2730	ENT3	-0.02	1	0	AWRI796_3244 AWRI796_3622	FMS1	-0.032	1	0
AWRI796_4999	TIF6	-0.02	1	0	AWRI796_3755	CEP3	-0.032	1	Ő
AWRI796_5098	LOA1	-0.02	1	0	AWRI796_4279	CTR9	-0.032	1	0
AWRI796_0459 AWRI796_0557	YCR100C	-0.021	1	0	AWRI796_4942	DIG1	-0.032	1	0
AWRI796_1399	YER121W	-0.021	1	0	AWRI796_4946	SSN3	-0.032	1	0
AWRI/96_18/1 AWRI796_4851	SRB5 KIP2	-0.021	1	0	AWRI796_0264 AWRI796_0324	PHO88 UMP1	-0.033 -0.033	1	0
AWRI796_0472	DCC1	-0.022	1	0	AWRI796_0501	FEN2	-0.033	1	0
AWRI796_0667	ATG20	-0.022	1	0	AWRI796_0908	NGG1	-0.033	1	0
AWRI796_1068 AWRI796_1968	HSV2	-0.022 -0.022	1	0	AWRI796_1229 AWRI796_1687	SNF4	-0.033	1	0
AWRI796_2185	CIA2	-0.022	1	0	AWRI796_2755	DAL5	-0.033	1	0
AWRI796_3015	MET1 MEC2	-0.022	1	0	AWRI796_3509	PML39 GPX7	-0.033	1	0
AWRI796_3327	PSO2	-0.022	1	0	AWRI796_0181 AWRI796_1855	PDC6	-0.034	1	0
AWRI796_3765	CTL1	-0.022	1	0	AWRI796_3019	AIM29	-0.034	1	0
AWRI796_4866 AWRI796_4884	SPP1 ID11	-0.022	1	0	AWRI796_1032 AWRI796_1423	SWA2 SPT15	-0.035 -0.035	1	0
AWRI796_0176	FLR1	-0.023	1	0	AWRI796_2521	LCB3	-0.035	1	0
AWRI796_1127	TIF35	-0.023	1	0	AWRI796_3242	STM1	-0.035	1	0
AWRI/96_1308 AWRI796_1843	GAL83 PRP38	-0.023	1	0	AWR1796_3268 AWR1796_3270	MDL1 MMR1	-0.035 -0.035	1	0
AWRI796_2197	YCK1	-0.023	1	Ő	AWRI796_3434	VPS33	-0.035	1	0
AWRI796_2373	NEO1	-0.023	1	0	AWRI796_4006	MGS1	-0.035	1	0
AWRI796_3967 AWRI796_4103	MIC27	-0.023	1	0	AWRI796_0173 AWRI796_0225	TRM7	-0.036	1	0
AWRI796_4947	MRX11	-0.023	1	0	AWRI796_0289	MEC1	-0.036	1	0
AWRI796_0023	CDC24	-0.024	1	0	AWRI796_0344	PGI1	-0.036	1	0
AWRI796_1335 AWRI796_1546	GIP2 IRC5	-0.024 -0.024	1	0	AWRI796_0722 AWRI796_1640	KNH1 PMR1	-0.036 -0.036	1	0
AWRI796_1734	PKP2	-0.024	1	0	AWRI796_2052	NPR3	-0.036	1	Ő
AWRI796_2122	CIC1	-0.024	1	0	AWRI796_2100	RRM3	-0.036	1	0
AWRI796_3856 AWRI796_4000	SOS1	-0.024 -0.024	1	0	AWR1796_2232 AWR1796_2235	SPC97 YHR177W	-0.036	1	0
AWRI796_0245	UBC4	-0.025	1	Õ	AWRI796_2436	MND2	-0.036	1	Ő
AWRI796_1090	YDR387C	-0.025	1	0	AWRI796_2854	ABF1	-0.036	1	0
AWRI796_1218 AWRI796_1306	GCD11	-0.025	1	0	AWRI796_4300 AWRI796_0056	FUN14	-0.036	1	0
AWRI796_1798	VMA7	-0.025	1	0	AWRI796_0366	TDP1	-0.037	1	0
AWRI796_2531	MTC1	-0.025	1	0	AWRI796_1266	MMS21	-0.037	1	0
AWRI796_2044 AWRI796_3816	RNH1	-0.025	1	0	AWRI796_1330 AWRI796_2014	CWC22	-0.037	1	0
AWRI796_3850	TIF11	-0.025	1	0	AWRI796_2079	SOD2	-0.037	1	0
AWRI796_0240	SLM4	-0.026	1	0	AWRI796_2416	AIM21	-0.037	1	0
AWRI796_0713 AWRI796_1113	RRP17	-0.026	1	0	AWRI796_2874	CUE2	-0.037	1	0
AWRI796_1411	GLC7	-0.026	1	0	AWRI796_3439	SFP1	-0.037	1	0
AWRI796_1714	GUP1	-0.026	1	0	AWRI796_4004	ADE12	-0.037	1	0
AWRI796_3466	ECM30	-0.026	1	0	AWRI796_4093	RPC19	-0.037	1	0
AWRI796_3539	DAK1	-0.026	1	0	AWRI796_5057	SUA7	-0.037	1	0
AWRI796_4659	RRS1 PUP3	-0.026	1	0	AWRI796_0103	PET112 RET1	-0.038	1	0
AWRI796_1412	YER134C	-0.027	1	0	AWRI796_1236	RPL12A	-0.038	1	0
AWRI796_1820	TAM41	-0.027	1	0	AWRI796_1774	LEU1	-0.038	1	0
AWRI796_2485 AWRI796_3010	ATG27 OAE3	-0.027	1	0	AWRI796_2547 AWRI796_3139	GSH1 MI H2	-0.038	1	0
AWRI796_4405	COQ10	-0.027	1	0	AWRI796_3582	UNG1	-0.038	1	0
AWRI796_0714	USO1	-0.028	1	0	AWRI796_3860	RRN9	-0.038	1	0
AWRI796_0752 AWRI796_0967	RPT2 PEX5	-0.028	1	0	AWRI796_4518 AWRI796_4620	CAT5 CLP1	-0.038 -0.038	1	0
AWRI796_1706	PAN2	-0.028	1	0	AWRI796_0261	SIF2	-0.039	1	0
AWRI796_2389	ULP2	-0.028	1	0	AWRI796_0498	SLM5	-0.039	1	0
AWRI796_2747 AWRI796_2817	RCN1	-0.028	1	0	AWRI796_0811 AWRI796_0905	ARG82	-0.039	1	0
AWRI796_2887	MUD2	-0.028	1	0	AWRI796_3201	CDC45	-0.039	1	0
AWRI796_3430	CCW14 NUP53	-0.028	1	0	AWRI796_3567	YMD8	-0.039	1	0
AWRI796_3807	UBP8	-0.028	1	0	AWRI796_4957	TRM44	-0.039	1	0
AWRI796_5246	YNL284C-A	-0.028	1	0	AWRI796_0322	SEC66	-0.04	1	0
AWRI/96_1044 AWRI796_1095	SPT3	-0.029 -0.029	1	0	AWRI/96_0664 AWRI796_0986	NUP84 AKR1	-0.04	1	0
AWRI796_1715	SCY1	-0.029	1	0	AWRI796_1918	GTR2	-0.04	1	Ő
AWRI796_2986	SPC34	-0.029	1	0	AWRI796_2170	TRR2	-0.04	1	0
AWRI796_3792 AWRI796_3932	BXI1	-0.029	1	0	AWRI796_2828 AWRI796_3142	RIC1	-0.04	1	0
AWRI796_4938	KTR6	-0.029	1	0	AWRI796_3411	SSQ1	-0.04	1	0
AWRI796_0014	OAF1 SAC3	-0.03	1	0	AWRI796_3734	TIF34 VDC1	-0.04	1	0
AWRI796_1003	INM2	-0.03	1	0	AWRI796_5109	NCA2	-0.04	1	0
AWRI796_1093	UBA2	-0.03	1	0	AWRI796_0075	YAT1	-0.041	1	0
AWRI/96_1704 AWRI796_2788	TOS8 ACP1	-0.03	1	0	AWRI796_1393 AWRI796_2106	SPR6 BRL1	-0.041	1	0
AWRI796_3311	LIP2	-0.03	1	0	AWRI796_2314	QDR1	-0.041	1	0
AWRI796_3464	CNA1	-0.03	1	0	AWRI796_3712	RPL15B	-0.041	1	0
AWRI/96_3706 AWRI796_4472	MGR3 NRT1	-0.03	1	0	AWRI/96_4805 AWRI796_1605	KKMI VAM7	-0.041	1	0
AWRI796_5067	PRE2	-0.03	1	0	AWRI796_2307	MET18	-0.042	1	0
AWRI796_0134	MRPL16	-0.031	1	0	AWRI796_3130	SNF7	-0.042	1	0
AWRI/96_0867 AWRI796_1310	FINI SMB1	-0.031	1	0	AWRI/96_3313 AWRI796_4616	CSCI ESA1	-0.042	1	0
AWRI796_1860	DBF2	-0.031	1	0	AWRI796_4880	TFB2	-0.042	1	0
AWRI796_2621	VTC4	-0.031	1	0	AWRI796_4953	YPL034W	-0.042	1	0
AWRI796_2709 AWRI796_2998	YKR051W	-0.031 -0.031	1	0	AWRI796_5073 AWRI796_0092	RPL32	-0.042 -0.043	1	0
AWRI796_3072	PRP19	-0.031	1	0	AWRI796_0962	SEC26	-0.043	1	0
AWRI796_3558 AWRI796_3942	GAL80 PUS4	-0.031	1	0	AWRI796_1327 AWRI796_1441	SAP1 BCK2	-0.043	1	0
	. 004	-0.031	1	0	11111/0_1++1	DCI12	-0.045	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. <i>p</i> -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_1981 AWRI796_2527	PBS2	-0.043	1	0	AWRI796_3940	RIM21	-0.059	1	0
AWRI796_2556	GWT1 TIM50	-0.043	1	0	AWRI796_4841	REV3	-0.059	1	0
AWRI796_1013	ATP5	-0.043	1	0	AWRI796_1200	PRO3	-0.06	1	0
AWRI796_1470	FMP32 SWD2	-0.044	1	0	AWRI796_1770	PDR1	-0.06	1	0
AWRI796_4391	DIS3	-0.044	1	0	AWRI796_2631	SAG1	-0.06	1	0
AWRI796_4578	LIP5 CDC21	-0.044	1	0	AWRI796_3400	BUD8	-0.06	1	0
AWRI796_2595	RTT101	-0.044	1	0	AWRI796_4165 AWRI796_5065	SNT309	-0.06	1	0
AWRI796_2903	NUP120	-0.045	1	0	AWRI796_1034	MRPL35	-0.061	1	0
AWRI796_3066 AWRI796_3228	SLX4	-0.045	1	0	AWRI796_1852 AWRI796_2294	CCT2	-0.061	1	0
AWRI796_3315	GPN3	-0.045	1	0	AWRI796_2300	VHS2	-0.061	1	0
AWRI796_3522 AWRI796_4141	PRE8 VAC7	-0.045 -0.045	1	0	AWRI796_2803 AWRI796_3901	LST4 YMR315W	-0.061 -0.061	1	0
AWRI796_4431	HSP10	-0.045	1	0	AWRI796_4172	SIW14	-0.061	1	0
AWRI796_4489 AWRI796_5116	PTC5 ORC4	-0.045	1	0	AWRI796_4375 AWRI796_4816	RPS15 DDC1	-0.061	1	0
AWRI796_0141	HEK2	-0.046	1	0	AWRI796_5002	MCM4	-0.061	1	0
AWRI796_1624	COX4 SEN2	-0.046	1	0	AWRI796_0807	YDR061W	-0.062	1	0
AWRI796_3203 AWRI796_3874	NGL2	-0.046	1	0	AWRI796_1485	CAK1	-0.062	1	0
AWRI796_3902	DIA1	-0.046	1	0	AWRI796_2528	SPT10	-0.062	1	0
AWRI796_4249	YNR068C	-0.046	1	0	AWRI796_4097 AWRI796_4508	AZF1	-0.062	1	0
AWRI796_1580	YGL242C	-0.047	1	0	AWRI796_5138	MLC2	-0.062	1	0
AWRI796_2464 AWRI796_2865	CBP1 UTP11	-0.047 -0.047	1	0	AWRI796_0374 AWRI796_1790	ARC40 NMA2	-0.063	1	0
AWRI796_3397	ORM2	-0.047	1	0	AWRI796_3213	CLF1	-0.063	1	0
AWRI796_3498	NGL3 RCH1	-0.047	1	0	AWRI796_3371	MRPL15 TAF11	-0.063	1	0
AWRI796_3690	ATP25	-0.047	1	0	AWRI796_4117	SAL1	-0.063	1	0
AWRI796_0310	AMN1 EBC25	-0.048	1	0	AWRI796_4134	YDJ1	-0.063	1	0
AWRI796_1832 AWRI796_2992	UIP5	-0.048	1	0	AWRI796_5106 AWRI796_2244	IKI1	-0.063	1	0
AWRI796_3733	NDE1	-0.048	1	0	AWRI796_3140	YLR036C	-0.064	1	0
AWRI796_4400 AWRI796_5063	HKD1 YPR097W	-0.048 -0.048	1	0	AWR1796_3280 AWRI796_4603	MCP1	-0.064 -0.064	1	0
AWRI796_1609	SIP2	-0.049	1	0	AWRI796_1584	HAP2	-0.065	1	0
AWRI796_4048 AWRI796_4452	SKO1 STD1	-0.049 -0.049	1	0	AWRI796_3037 AWRI796_3757	SRP40 ALD2	-0.065	1	0
AWRI796_4809	TPK2	-0.049	1	0	AWRI796_3922	PFS2	-0.065	1	0
AWRI796_0778 AWRI796_1055	SES1 PAL1	-0.05	1	0	AWRI796_3974 AWRI796_4123	GIS2 MKS1	-0.065	1	0
AWRI796_1365	GET2	-0.05	1	0	AWRI796_4352	MET22	-0.065	1	0
AWRI796_1381	RPS8B CKP1	-0.05	1	0	AWRI796_4546	NFI1 MPPL 40	-0.065	1	0
AWRI796_2391	YNL284C-B	-0.05	1	0	AWRI796_0299	RTC2	-0.065	1	0
AWRI796_2563	TAX4	-0.05	1	0	AWRI796_0526	TAH1	-0.066	1	0
AWRI796_3111 AWRI796_4935	SUR1	-0.05	1	0	AWRI796_0963 AWRI796_1302	RPN3	-0.066	1	0
AWRI796_5137	PZF1	-0.05	1	0	AWRI796_2515	TIM17	-0.066	1	0
AWRI796_1724 AWRI796_1727	AFT1 SGF73	-0.051 -0.051	1	0	AWRI796_4644 AWRI796_4883	CAF20 MRP51	-0.066	1	0
AWRI796_1977	MIC26	-0.051	1	0	AWRI796_5035	JID1	-0.066	1	0
AWRI796_2252 AWRI796_2327	NVJ1 PFK26	-0.051	1	0	AWRI796_5159 AWRI796_1752	AWRI796_5159 YGL036W	-0.066	1	0
AWRI796_3943	MID1	-0.051	1	0	AWRI796_1986	CPD1	-0.067	1	0
AWRI796_4488 AWRI796_0119	VPS21 PTH2	-0.051	1	0	AWRI796_2352 AWRI796_4196	SEC28 URK1	-0.067	1	0
AWRI796_0303	APD1	-0.052	1	0	AWRI796_5059	YPR089W	-0.067	1	0
AWRI796_1523	IOC3 MLC1	-0.052	1	0	AWRI796_0996	RNH202	-0.068	1	0
AWRI796_1942	PDX1	-0.052	1	0	AWRI796_2382	TED1	-0.068	1	0
AWRI796_2533	RPE1	-0.052	1	0	AWRI796_3266	EMG1	-0.068	1	0
AWRI796_0538	FUB1	-0.053	1	0	AWRI796_3694	YMR102C	-0.068	1	0
AWRI796_2967	HEL1	-0.053	1	0	AWRI796_4018	SPS18	-0.068	1	0
AWRI796_3310 AWRI796_4044	MDG1	-0.053 -0.053	1	0	AWRI796_4040 AWRI796_4538	SPP2	-0.068	1	0
AWRI796_4283	RRP40	-0.053	1	0	AWRI796_5074	RPC40	-0.068	1	0
AWRI796_4989 AWRI796_1896	AIM45 LSB1	-0.053 -0.054	1	0	AWRI796_0672 AWRI796_0943	KIN28 RAD9	-0.069 -0.069	1	0
AWRI796_1913	CHO2	-0.054	1	0	AWRI796_1015	PRO1	-0.069	1	0
AWRI796_3534 AWRI796_4823	WAR1 MF(ALPHA)1	-0.054 -0.054	1	0	AWRI796_1674 AWRI796_4608	RSM23 KIN4	-0.069	1	0
AWRI796_0341	MED8	-0.055	1	0	AWRI796_0494	MAK32	-0.07	1	0
AWRI796_0737 AWRI796_3165	ARP2 ENV10	-0.055 -0.055	1	0	AWRI/96_0/47 AWRI796_1471	TSC13 SEC53	-0.07 -0.07	1	0
AWRI796_4041	MRPL22	-0.055	1	0	AWRI796_1972	SMI1	-0.07	1	0
AWRI796_4478 AWRI796_0237	BUD21 RDH54	-0.055	1	0	AWRI796_3737 AWRI796_0085	SWP1 SFT2	-0.07	1	0
AWRI796_0843	SPO71	-0.056	1	0	AWRI796_0485	SAT4	-0.071	1	0
AWRI796_1241	FRD1 SOL4	-0.056	1	0	AWRI796_0627	ENT1 PSM24	-0.071	1	0
AWRI796_2348	CAB2	-0.056	1	0	AWRI796_2587	YJL055W	-0.071	1	0
AWRI796_4301	RPS19A	-0.056	1	0	AWRI796_3979	RAD50 PRN10	-0.071	1	0
AWRI796_0018 AWRI796_0676	QRI7	-0.057	1	0	AWRI796_0140	STU1	-0.072	1	0
AWRI796_1400	GLO3	-0.057	1	0	AWRI796_0573	SHS1	-0.073	1	0
AWRI796_3820 AWRI796_4361	THI20	-0.057 -0.057	1	0	AWRI796_0691 AWRI796_1448	GRX4	-0.073	1	0
AWRI796_4533	LSC1	-0.057	1	0	AWRI796_2851	PRR1	-0.073	1	0
AWK1/96_4775 AWRI796 4777	1 AKI ENV7	-0.057 -0.057	1	0	AWRI/96_2935 AWRI796_3859	kam2 TMA23	-0.073	1	0
AWRI796_1180	RSM28	-0.058	1	0	AWRI796_0365	PCS60	-0.074	1	0
AWRI/96_1597 AWRI796_3858	COGI PRP24	-0.058 -0.058	1	0	AWRI/96_0522 AWRI796_1686	THR4 CDC20	-0.074	1	0
AWRI796_1039	SKP1	-0.059	1	0	AWRI796_3378	SFH1	-0.074	1	0
AWRI796_1380 AWRI796_1555	AST2 RMD8	-0.059	1	0	AWRI796_3819 AWRI796_3821	BCH1 RNT1	-0.074	1	0
AWRI796_2208	IMP3	-0.059	1	0	AWRI796_4031	SRP1	-0.074	1	0
AWRI796_2487 AWRI796_2544	SWI3 PAM16	-0.059	1	0	AWRI796_4609 AWRI796_0067	DFR1 ADE1	-0.074	1	0
AWRI796_2977	BCH2	-0.059	1	0	AWRI796_0622	SFA1	-0.075	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change A	dj. <i>p</i> -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_1256 AWRI796_1332	JHD1	-0.075	1	0	AWRI796_0700 AWRI796_1204	RBA50	-0.091	1	0
AWRI796_2477	SOP4	-0.075	1	0	AWRI796_1274	GCN4	-0.091	1	0
AWRI796_2690	HOC1	-0.075	1	0	AWRI796_1938 AWRI796_2964	YPT52	-0.091	1	0
AWRI796_4903	SEC62	-0.075	1	0	AWRI796_3994	BNI4	-0.091	1	0
AWRI796_0087 AWRI796_0802	PST1	-0.076	1	0	AWRI796_4010 AWRI796_4064	PEX17 PGA2	-0.091	1	0
AWRI796_2051	RIM4	-0.076	1	0	AWRI796_4130	FKH2	-0.091	1	0
AWRI796_2353 AWRI796_4338	RPN2 REX4	-0.076 -0.076	1	0	AWR1796_4275 AWRI796_4485	OST3	-0.091 -0.091	1	0
AWRI796_0688	UBX3	-0.077	1	0	AWRI796_4537	MDM32	-0.091	1	0
AWRI796_0989 AWRI796_1035	CIAI PEP7	-0.077 -0.077	1	0	AWRI796_0010 AWRI796_0629	GPB2 STE7	-0.092 -0.092	1	0
AWRI796_1497	MDJ1	-0.077	1	Õ	AWRI796_1205	HLR1	-0.092	1	Ő
AWRI796_2598 AWRI796_2618	GYP6 YIL016W	-0.077 -0.077	1	0	AWRI796_1673 AWRI796_1842	CEG1 UPF3	-0.092	1	0
AWRI796_2968	YKR018C	-0.077	1	0	AWRI796_2470	ECM25	-0.092	1	0
AWRI796_0987	PEX10 NSC1	-0.078	1	0	AWRI796_2821	GPM1	-0.092	1	0
AWRI796_2195 AWRI796_2434	DAL81	-0.078	1	0	AWRI796_4137 AWRI796_4483	WHI5	-0.092	1	0
AWRI796_3187	GAA1	-0.078	1	0	AWRI796_4793	RPL1A	-0.092	1	0
AWRI796_3282 AWRI796_3552	CMP2	-0.078	1	0	AWRI796_5130 AWRI796_0772	KCS1	-0.092	1	0
AWRI796_3644	IOC4	-0.078	1	0	AWRI796_0830	AFR1	-0.093	1	0
AWRI796_5107 AWRI796_0032	URN1 POP5	-0.078 -0.079	1	0	AWRI796_1097 AWRI796_1145	RPT3 TSA2	-0.093 -0.093	1	0
AWRI796_0131	PRE7	-0.079	1	0	AWRI796_1738	OLE1	-0.093	1	0
AWRI796_1641	CUP2 MNP1	-0.079	1	0	AWRI796_2044	OCA5 MNN11	-0.093	1	0
AWRI796_3386	NUP2	-0.079	1	0	AWRI796_3240	PEP3	-0.093	1	0
AWRI796_3405	ADE13	-0.079	1	0	AWRI796_3293	CDC123	-0.093	1	0
AWRI796_0824 AWRI796_0836	GIS1	-0.08	1	0	AWRI796_3476 AWRI796_4038	VMA6 YNL181W	-0.093	1	0
AWRI796_0863	SWF1	-0.08	1	0	AWRI796_4051	IBD2	-0.093	1	0
AWRI796_2623 AWRI796_2749	CCT8 RPS4A	-0.08 -0.08	1	0	AWRI796_4074 AWRI796_4382	FPR1 SIL1	-0.093 -0.093	1	0
AWRI796_2901	MPE1	-0.08	1	0	AWRI796_5077	PIS1	-0.093	1	0
AWRI796_2973	YKR023W MRPL 20	-0.08	1	0	AWRI796_0797	VMS1 CTA1	-0.094	1	0
AWRI796_1070	KEI1	-0.081	1	0	AWRI796_1138	SSN2	-0.094	1	0
AWRI796_3085	HIF1	-0.081	1	0	AWRI796_1294	PRP22	-0.094	1	0
AWRI796_3649 AWRI796_4239	BIO3	-0.081	1	0	AWRI796_1487 AWRI796_3099	LMO1	-0.094	1	0
AWRI796_4494	RKI1	-0.081	1	0	AWRI796_3669	RCO1	-0.094	1	0
AWRI796_5028 AWRI796_5070	NHP6A ISR1	-0.081 -0.081	1	0	AWRI796_4079 AWRI796_4554	SEY1	-0.094 -0.094	1	0
AWRI796_1801	MTL1	-0.082	1	Õ	AWRI796_4804	IPL1	-0.094	1	0
AWRI796_2181 AWRI796_2918	ORC6 PTM1	-0.082	1	0	AWRI796_5223 AWRI796_0285	ENA2 CC71	-0.094	1	0
AWRI796_3336	BOP2	-0.082	1	0	AWRI796_0708	IDP1	-0.095	1	0
AWRI796_0525	YIH1 KAD114	-0.083	1	0	AWRI796_1151	TFB3	-0.095	1	0
AWRI796_1381 AWRI796_1794	YGR015C	-0.083	1	0	AWRI796_2184 AWRI796_2715	ECM27	-0.095	1	0
AWRI796_2072	OSH7	-0.083	1	0	AWRI796_3442	YLR407W	-0.095	1	0
AWRI796_2538 AWRI796_2819	RSM22	-0.083	1	0	AWR1796_3762 AWR1796_3970	MM11 LTO1	-0.095 -0.095	1	0
AWRI796_3243	PCD1	-0.083	1	0	AWRI796_0868	YDR131C	-0.096	1	0
AWRI796_3728 AWRI796_4178	CIN4 IDP3	-0.083	1	0	AWRI796_1457 AWRI796_1668	TOG1 MRM2	-0.096 -0.096	1	0
AWRI796_4380	MSE1	-0.083	1	Ő	AWRI796_2606	TAD2	-0.096	1	0
AWRI796_4871	COX11 PD11	-0.083	1	0	AWRI796_2607	KAR2 MCM22	-0.096	1	0
AWRI796_0512	MATALPHA1	-0.084	1	0	AWRI796_2999	MRS4	-0.096	1	0
AWRI796_1074	CTS2	-0.084	1	0	AWRI796_3153	FCF2 MED7	-0.096	1	0
AWRI796_1200 AWRI796_3211	CFT2	-0.084	1	0	AWRI796_4290 AWRI796_4648	PLP2	-0.096	1	0
AWRI796_4584	GEP3	-0.084	1	0	AWRI796_0431	MAL33	-0.097	1	0
AWRI796_0275 AWRI796_0969	TRS23	-0.085 -0.085	1	0	AWR1796_0625 AWRI796_0861	YDR124W	-0.097 -0.097	1	0
AWRI796_1660	ROG1	-0.085	1	0	AWRI796_1631	MPT5	-0.097	1	0
AWRI796_3303 AWRI796_3406	ADY4 DCR2	-0.085 -0.085	1	0	AWRI796_1728 AWRI796_2952	ALG2 MET14	-0.097 -0.097	1	0
AWRI796_0034	GIP4	-0.086	1	Õ	AWRI796_4434	AHC1	-0.097	1	0
AWRI796_0650 AWRI796_1927	PPH21 CBP4	-0.086 -0.086	1	0	AWRI796_4487 AWRI796_4637	YVC1 PAC1	-0.097	1	0
AWRI796_4565	SYC1	-0.086	1	0	AWRI796_4886	BEM3	-0.097	1	0
AWRI796_1047	MRX8	-0.087	1	0	AWRI796_5027	MAK3 MCM2	-0.097	1	0
AWRI796_1652	LYS5	-0.087	1	0	AWRI796_0330	RPS6B	-0.098	1	0
AWRI796_2493	SET2	-0.087	1	0	AWRI796_0624	FAP7	-0.098	1	0
AWRI796_4829	PPQ1	-0.087 -0.087	1	0	AWRI796_1354 AWRI796_1550	IRC6	-0.098	1	0
AWRI796_1736	GEP7	-0.088	1	0	AWRI796_2304	FKH1	-0.098	1	0
AWRI796_1780 AWRI796_1989	ERP6 YGR250C	-0.088 -0.088	1	0	AWR1796_3758 AWR1796_3871	AEP2	-0.098 -0.098	1	0
AWRI796_2693	BNA2	-0.088	1	0	AWRI796_4416	UTP23	-0.098	1	0
AWRI796_2859 AWRI796_4531	AAT1 SFL 1	-0.088	1	0	AWRI796_5044 AWRI796_1467	YPR071W SWP82	-0.098	1	0
AWRI796_5232	HSP33	-0.088	1	Ő	AWRI796_2781	MNN4	-0.099	1	0
AWRI796_0033 AWRI796_0158	PRP45 FMT1	-0.089	1	0	AWRI796_3116 AWRI796_3759	LOT6 HOT1	-0.099	1	0
AWRI796_1902	THI4	-0.089	1	0	AWRI796_4553	GET4	-0.099	1	0
AWRI796_2765	SRY1 DCS1	-0.089	1	0	AWRI796_5124	BSP1	-0.099	1	0
AWRI796_3702	YMR111C	-0.089	1	0	AWRI796_0114 AWRI796_0872	RGP1	-0.1	1	0
AWRI796_3899	TGL3	-0.089	1	0	AWRI796_1206	QCR7	-0.1	1	0
AWK1796_4513 AWRI796_4622	TMA16	-0.089 -0.089	1	0	AWK1/96_1894 AWRI796 2364	PEA4 ARC15	-0.1 -0.1	1	0
AWRI796_0939	TCP1	-0.09	1	0	AWRI796_2551	MRPL49	-0.1	1	0
AWKI/96_1111 AWRI796 2189	SIE14 YHR127W	-0.09 -0.09	1	0	AWRI/96_3279 AWRI796 0438	YKE2 KRR1	-0.1 -0.101	1	0
AWRI796_2369	GPP1	-0.09	1	0	AWRI796_0572	GCS1	-0.101	1	0
AWRI796_2879 AWRI796_4451	MDH1 DBP5	-0.09 -0.09	1	0 0	AWRI/96_1073 AWRI796 1104	DX01 DIT1	-0.101 -0.101	1	0
AWRI796_4471	GYP1	-0.09	1	Ő	AWRI796_1169	CWC21	-0.101	1	0
AWRI796_0199	HMT1	-0.091	1	0	AWRI796_1978	YGR237C	-0.101	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value Score
AWRI796_0071	YAR023C	-0.102	1	0	AWRI796_2099	SLT2	-0.115	1 (
AWRI796_0514 AWRI796_0599	YCR043C ASF2	-0.102	1	0	AWRI796_3815 AWRI796_0207	TRI1 CST26	-0.115	1 (
AWRI796_0886	ENT5	-0.102	1	Ő	AWRI796_0250	IST2	-0.116	1 (
AWRI796_1130 AWRI796_1283	GPI17 NOP16	-0.102	1	0	AWRI796_1053 AWRI796_1057	SVF1 ATP22	-0.116	1 (
AWRI796_1340	PCL6	-0.102	1	0	AWRI796_1388	KAP123	-0.116	1 (
AWRI796_2674 AWRI796_3068	CDC8 ATG10	-0.102	1	0	AWRI796_2400 AWRI796_2733	VID28 FFM3	-0.116	1 (
AWRI796_3219	YLR126C	-0.102	1	0	AWRI796_3290	TUB4	-0.116	1 (
AWRI796_3284	ENT2 URE2	-0.102	1	0	AWRI796_3300	UCC1 VDI 129W	-0.116	1 (
AWRI796_4289	PFK27	-0.102	1	0	AWRI796_2392	EMC5	-0.117	1 (
AWRI796_4379	SMC5	-0.102	1	0	AWRI796_2894	YNK1 POM22	-0.117	1 (
AWRI796_2209	SKG6	-0.103	1	0	AWRI796_3403	ATG33	-0.117	1 (
AWRI796_2804	ZRT3	-0.103	1	0	AWRI796_3820	DFG5	-0.117	1 (
AWRI796_2971 AWRI796_3502	TAF8	-0.103	1	0	AWRI796_5885 AWRI796_4500	PIN2	-0.117	1 (
AWRI796_3689	MTG1	-0.103	1	0	AWRI796_0150	HAP3	-0.118	1 (
AWRI796_1038 AWRI796_2573	ARG2	-0.104	1	0	AWRI796_1703 AWRI796_3473	ECM7	-0.118	1 (
AWRI796_2847	DGR2	-0.104	1	0	AWRI796_0801	CDC34	-0.119	1 (
AWRI796_3103 AWRI796_4327	SPO21	-0.104	1	0	AWRI796_0821 AWRI796_1849	SLX9	-0.119	1 (
AWRI796_1178	IZH1	-0.105	1	0	AWRI796_2839	SHE2	-0.119	1 (
AWRI/96_1/13 AWRI796 1811	RPL26B	-0.105 -0.105	1	0	AWRI/96_3/84 AWRI796 4143	COX5A	-0.119 -0.119	1 (
AWRI796_1974	NAS6	-0.105	1	0	AWRI796_4420	SLG1	-0.119	1 (
AWRI796_3580 AWRI796_4023	NSE5 WHI3	-0.105 -0.105	1	0	AWRI796_4988 AWRI796_0750	GRX6	-0.119 -0.12	1 (
AWRI796_4169	CRZ1	-0.105	1	0	AWRI796_0775	DAS2	-0.12	1 (
AWRI796_4308 AWRI796_4914	MSB4 SEN54	-0.105	1	0	AWR1796_0979 AWR1796_4065	RKM4 ALF1	-0.12	1 (
AWRI796_4951	EGD1	-0.105	1	0	AWRI796_4426	RTS1	-0.12	1 (
AWRI796_0243 AWRI796_0832	SEC18	-0.106	1	0	AWRI796_0371 AWRI796_0393	OM14 TRS20	-0.121	1 (
AWRI796_0899	CDC37	-0.106	1	0	AWRI796_0604	UFD2	-0.121	1 (
AWRI796_3221	DCN1	-0.106	1	0	AWRI796_0837	MSH6	-0.121	1 (
AWRI796_3915	PFA3	-0.106	1	0	AWRI796_0895 AWRI796_1782	EFM5	-0.121	1 (
AWRI796_3954	PRM1 TMC1	-0.106	1	0	AWRI796_2305	ASG1	-0.121	1 (
AWRI796_1239	VMA8	-0.108	1	0	AWRI796_3194 AWRI796_3875	DSS1	-0.121	1 (
AWRI796_1288	PAC2	-0.107	1	0	AWRI796_5064	MRPL51	-0.121	1 (
AWRI796_3936 AWRI796 4206	MRPL50	-0.107 -0.107	1	0	AWRI796_5082 AWRI796_0862	ECM18	-0.121 -0.122	1 (
AWRI796_0063	SWD1	-0.108	1	0	AWRI796_2211	MTC6	-0.122	1 (
AWRI796_0368 AWRI796_0369	MCX1 SLX1	-0.108 -0.108	1	0	AWRI796_2227 AWRI796_2260	THP2 RPS4B	-0.122 -0.122	1 (
AWRI796_0470	LEU2	-0.108	1	0	AWRI796_4702	TYE7	-0.122	1 (
AWRI796_0923 AWRI796_1284	NUP42 PMI40	-0.108 -0.108	1	0	AWRI796_4818 AWRI796_0347	PRM3 KTR4	-0.122	1 (
AWRI796_1514	RPN11	-0.108	1	0	AWRI796_1476	ACT1	-0.123	1 (
AWRI796_2218 AWRI796_2975	KEL1 RPC37	-0.108	1	0	AWRI796_2950 AWRI796_3166	MRP17 SPC3	-0.123	1 (
AWRI796_3185	SMC4	-0.108	1	0	AWRI796_3250	SEC10	-0.123	1 (
AWRI796_4050 AWRI796_4128	YNL165W	-0.108	1	0	AWRI796_5033 AWRI796_0425	YMC1 BSD2	-0.123	1 (
AWRI796_0136	YBL036C	-0.109	1	0	AWRI796_0565	YPD1	-0.124	1 (
AWRI796_0804 AWRI796_0869	YOS9 YDR132C	-0.109 -0.109	1	0	AWRI796_1556 AWRI796_2613	PRE4 RNR2	-0.124	1 (
AWRI796_2036	CBP2	-0.109	1	0	AWRI796_2921	TUL1	-0.124	1 (
AWRI796_2219 AWRI796_2359	TDA11 SEC6	-0.109	1	0	AWRI796_2948 AWRI796_3772	BYE1 YMR187C	-0.124	1 (
AWRI796_2363	YRB2	-0.109	1	0	AWRI796_3854	CUE1	-0.124	1 (
AWRI796_4340	BRX1 SEC1	-0.109	1	0	AWRI796_4430	YOR019W MSD1	-0.124	1 (
AWRI796_1176	SLD5	-0.11	1	0	AWRI796_5164	AWRI796_5164	-0.124	1 (
AWRI796_1289	SEC3	-0.11	1	0	AWRI796_0278	TFC1 TPS21	-0.125	1 (
AWRI796_2039	SBP1	-0.11	1	0	AWRI796_3033	TVP38	-0.125	1 (
AWRI796_3475	YLR446W	-0.11	1	0	AWRI796_3620	YMR018W	-0.125	1 (
AWRI796_4113	TOP2	-0.11	1	0	AWRI796_0111	PRS4	-0.125	1 (
AWRI796_0030	MTW1	-0.111	1	0	AWRI796_0339	RPL21A	-0.126	1 (
AWRI796_0610 AWRI796_0675	NSE4	-0.111	1	0	AWRI796_2658	URB2	-0.126	1 (
AWRI796_0829	TVP23	-0.111	1	0	AWRI796_2863	HSL1	-0.126	1 (
AWRI/96_1682 AWRI796_1773	MPO1	-0.111 -0.111	1	0	AWRI/96_28/1 AWRI796_2969	IRS4	-0.126	1 (
AWRI796_2938	ATP7	-0.111	1	0	AWRI796_4516	LEO1	-0.126	1 (
AWRI796_2951 AWRI796_4417	DID4 DNL4	-0.111 -0.111	1	0	AWRI796_0055 AWRI796_1118	SPO7 RAD30	-0.127 -0.127	1 (
AWRI796_4822	POS5	-0.111	1	0	AWRI796_1293	PRE1	-0.127	1 (
AWRI796_5227 AWRI796_0211	AWRI796_5227 ZTA1	-0.111 -0.112	1	0	AWRI796_2809 AWRI796_3003	MRPL38 TRM2	-0.127	1 (
AWRI796_0456	GRX1	-0.112	1	0	AWRI796_3251	RPS31	-0.127	1 (
AWRI796_0725	FAD1 PI M2	-0.112	1	0	AWRI796_4596	RCN2 SPT14	-0.127	1 (
AWRI796_1258	VMA3	-0.112	1	0	AWRI796_0585	SHR3	-0.127	1 (
AWRI796_2739	TTI2 RCF1	-0.112	1	0	AWRI796_1509	SPB4 Hami	-0.128	1 (
AWRI796_4757	APM1	-0.112	1	0	AWRI796_3296	MSC3	-0.128	1 (
AWRI796_2800	RPL17A	-0.113	1	0	AWRI796_3429	ECM19 GCV1	-0.128	1 (
AWRI796_4991	ICL2	-0.113	1	0	AWRI796_4801	PUS1	-0.128	1 (
AWRI796_0112	UBP13	-0.114	1	0	AWRI796_0125	SEC17	-0.129	1 (
AWK1/96_10/8 AWRI796_1675	CWC23	-0.114 -0.114	1	0	AWRI/96_0/36 AWRI796 0809	AIM7	-0.129 -0.129	1 (
AWRI796_3432	ATP10	-0.114	1	0	AWRI796_1108	TRS120	-0.129	1 (
AWK1/96_3984 AWRI796_4139	OCA2	-0.114 -0.114	1	0 0	AWRI/96_2578 AWRI796_2805	TPO5	-0.129 -0.129	1 (
AWRI796_4860	NOP53	-0.114	1	Õ	AWRI796_2812	MCD4	-0.129	1 (
AWRI/96_0404 AWRI796_1511	MRPL37 LOC1	-0.115 -0.115	1	0 0	AWRI796_3808 AWRI796_4981	MREII AEP3	-0.129 -0.129	1 (

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. <i>p</i> -value	Score	AWRI796 Gene ID AWRI796 5010	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_5217	DDI2	-0.129	1	0	AWRI796_1955	CIR1	-0.145	1	0
AWRI796_0799	DET1 DEV2	-0.13	1	0	AWRI796_2012	RNH70 SMC2	-0.144	1	0
AWRI796_1165	SNM1	-0.13	1	0	AWRI796_2775	CBT1	-0.144	1	0
AWRI796_1276	YEA6	-0.13	1	0	AWRI796_3302	BUR2	-0.144	1	0
AWRI796_1966	TOS2	-0.13	1	0	AWRI796_5085 AWRI796_4164	RCM1	-0.144	1	0
AWRI796_2287	MCM10	-0.13	1	0	AWRI796_4613	MET7	-0.144	1	0
AWRI796_3011 AWRI796_3480	SST2	-0.13	1	0	AWRI796_0814	DOS2	-0.144	1	0
AWRI796_3969	ORC5	-0.13	1	0	AWRI796_1063	CNL1	-0.145	1	0
AWRI796_5266 AWRI796_0152	APN2	-0.13	1	0	AWRI796_2302 AWRI796_2336	BMT5	-0.145	1	0
AWRI796_0563	AIM6	-0.131	1	0	AWRI796_2614	RRN7	-0.145	1	0
AWRI796_1147 AWRI796_1401	YCK3	-0.131	1	0	AWRI796_3132 AWRI796_4830	CBC2	-0.145	1	0
AWRI796_2013	CAB4	-0.131	1	0	AWRI796_0926	CAB5	-0.146	1	0
AWRI796_2947 AWRI796_2958	MEH1	-0.131 -0.131	1	0	AWRI796_1125 AWRI796_2410	BNA/ EPS1	-0.146 -0.146	1	0
AWRI796_0222	UBP14	-0.132	1	0	AWRI796_2953	VPS1	-0.146	1	0
AWRI796_0649 AWRI796_1578	RDII RTF1	-0.132	1	0	AWRI796_3401 AWRI796_3512	MDM1	-0.146 -0.146	1	0
AWRI796_1763	GET1	-0.132	1	0	AWRI796_4966	ULP1	-0.146	1	0
AWRI796_2610 AWRI796_2641	MAD2 YIR015W	-0.132	1	0	AWRI796_4990 AWRI796_1653	HAL1 PEX14	-0.146 -0.147	1	0
AWRI796_2906	DEF1	-0.132	1	ő	AWRI796_1785	PEX31	-0.147	1	0
AWRI796_3492 AWRI796_3857	PGA3 PPA2	-0.132	1	0	AWRI796_3156 AWRI796_4711	OSW2 MSC6	-0.147	1	0
AWRI796_4634	RBL2	-0.132	1	0	AWRI796_0471	NFS1	-0.148	1	0
AWRI796_0500	RHB1	-0.133	1	0	AWRI796_0586	YDL211C	-0.148	1	0
AWRI796_3033 AWRI796_1227	AVT2	-0.133	1	0	AWRI796_0887	CPR1	-0.148	1	0
AWRI796_1331	RSM18	-0.134	1	0	AWRI796_2683	YAE1	-0.148	1	0
AWRI796_1050 AWRI796_2175	UBA4	-0.134	1	0	AWRI796_3193 AWRI796_3913	MDJ2	-0.148	1	0
AWRI796_2462	OPT1	-0.134	1	0	AWRI796_4324	RFC4	-0.148	1	0
AWRI796_4776 AWRI796_5043	SUI3 MED1	-0.134 -0.134	1	0	AWRI796_4715 AWRI796_0062	HAP5 ERP1	-0.148 -0.149	1	0
AWRI796_0385	ISW1	-0.135	1	0	AWRI796_0180	YBR013C	-0.149	1	0
AWRI796_0506 AWRI796_4014	RRP43 SSB2	-0.135	1	0	AWRI796_1409 AWRI796_3515	RPS26B CUE4	-0.149	1	0
AWRI796_5119	RHO1	-0.135	1	Ő	AWRI796_3754	MLH1	-0.149	1	0
AWRI796_0091	ROX3 CTP1	-0.136	1	0	AWRI796_4346	NBA1 VPS28	-0.149	1	0
AWRI796_1071	YPR1	-0.136	1	0	AWRI796_5052	GRS2	-0.149	1	0
AWRI796_1114	ERD1 GAR1	-0.136	1	0	AWRI796_0084	RTG3	-0.15	1	0
AWRI796_3536	FPR3	-0.136	1	0	AWRI796_0555 AWRI796_0785	LYS14	-0.15	1	0
AWRI796_3735	YMR147W	-0.136	1	0	AWRI796_1043	IRC3	-0.15	1	0
AWRI796_0204 AWRI796_0386	RRT2	-0.137	1	0	AWRI796_2002 AWRI796_3337	SEC22	-0.15	1	0
AWRI796_0614	YDL177C	-0.137	1	0	AWRI796_3370	ATG39	-0.15	1	0
AWRI796_1012 AWRI796_1029	OMS1	-0.137 -0.137	1	0	AWRI796_3704 AWRI796_3977	FOL3 MRPL17	-0.15	1	0
AWRI796_1749	YGL039W	-0.137	1	0	AWRI796_5090	ANT1	-0.15	1	0
AWRI796_2010 AWRI796_3456	SPP382	-0.137 -0.137	1	0	AWRI796_0590 AWRI796_1442	GLEI CCA1	-0.151 -0.151	1	0
AWRI796_3834	PET111	-0.137	1	0	AWRI796_3651	BUB2	-0.151	1	0
AWRI796_4313 AWRI796_4523	YOL107W ORT1	-0.137 -0.137	1	0	AWRI796_4502 AWRI796_5244	RGS2 YNL284C-B	-0.151 -0.151	1	0
AWRI796_4561	ALE1	-0.137	1	0	AWRI796_0405	SDH8	-0.152	1	0
AWRI796_0138 AWRI796_0440	POL12 KAR4	-0.138	1	0	AWRI796_0876 AWRI796_1234	PEX7 HAT2	-0.152	1	0
AWRI796_4119	EOS1	-0.138	1	Ő	AWRI796_2586	ZAP1	-0.152	1	0
AWRI796_4211 AWRI796_4691	CPR8 VMA4	-0.138	1	0	AWRI796_4447 AWRI796_4560	CKB2 MED4	-0.152	1	0
AWRI796_4767	GAL4	-0.138	1	Ő	AWRI796_4929	CWC27	-0.152	1	0
AWRI796_5041	HOS1 MUM2	-0.138	1	0	AWRI796_4975	TAF3 PAF1	-0.152	1	0
AWRI796_0531	SED4	-0.139	1	0	AWRI796_0648	ARF2	-0.153	1	0
AWRI796_0997	RRP45 STP1	-0.139	1	0	AWRI796_3546	RPS1B TIP2	-0.153	1	0
AWRI796_1623	RPS26A	-0.139	1	0	AWRI796_0021	PTA1	-0.155	1	0
AWRI796_1690	TAF6 REC107	-0.139	1	0	AWRI796_0109	RPS8A OCA6	-0.154	1	0
AWRI796_2698	EMC2	-0.139	1	0	AWRI796_0839	BMH2	-0.154	1	0
AWRI796_1129	NPL3	-0.14	1	0	AWRI796_1041	UBX5	-0.154	1	0
AWRI796_3681	YTA12	-0.14	1	0	AWRI796_3426	VAC14	-0.154	1	0
AWRI796_4347	NUF2	-0.14	1	0	AWRI796_5182	YNL284C-B	-0.154	1	0
AWRI796_4419 AWRI796_0769	PSF1	-0.14	1	0	AWRI796_0073 AWRI796_0153	POP8	-0.155	1	0
AWRI796_2453	THI13 DDI 17D	-0.141	1	0	AWRI796_0537	ERS1	-0.155	1	0
AWRI796_2480 AWRI796_3590	UBX2	-0.141	1	0	AWRI796_1080 AWRI796_1742	RPT6	-0.155	1	0
AWRI796_0417	MRPL27	-0.142	1	0	AWRI796_1754	RPL24A	-0.155	1	0
AWRI796_0481 AWRI796_0874	MTQ2	-0.142	1	0	AWRI796_2505 AWRI796_2831	SDH3	-0.155	1	0
AWRI796_1285	FMP52	-0.142	1	0	AWRI796_2885	YKL077W	-0.155	1	0
AWRI796_1906 AWRI796_2588	KPL24B TIM54	-0.142 -0.142	1	0	AWRI/96_3328 AWRI796_3385	TLK25/W RPS25B	-0.155 -0.155	1	0
AWRI796_3717	SAS2	-0.142	1	0	AWRI796_3625	MSS1	-0.155	1	0
AWRI/96_3/29 AWRI796 4512	RPT5	-0.142 -0.142	1	0 0	AWRI/96_3920 AWRI796 4191	YNL320W ATG3	-0.155 -0.155	1	0
AWRI796_4672	SNU66	-0.142	1	0	AWRI796_5072	RPN7	-0.155	1	0
AWRI/96_4970 AWRI796_0959	SW11 PRP42	-0.142 -0.143	1	0	AWRI/96_2205 AWRI796_2246	DCD1 PTH1	-0.156	1	0
AWRI796_2152	NAM8	-0.143	1	0	AWRI796_2691	CDC11	-0.156	1	0
AWRI/96_2196 AWRI796_2297	WSS1 TPM2	-0.143 -0.143	1	0	AWRI796_4404 AWRI796_0328	MDM12 FZO1	-0.156 -0.157	1	0
AWRI796_2422	MSL1	-0.143	1	Ő	AWRI796_0701	RXT3	-0.157	1	0
AWRI796_3372 AWRI796_3392	SPH1 GAS2	-0.143 -0.143	1	0 0	AWRI796_1190 AWRI796_1359	GMC1 ICP55	-0.157 -0.157	1	0
AWRI796_3459	MAG2	-0.143	1	Ő	AWRI796_3317	CDD1	-0.157	1	0
AWRI796_3610 AWRI796 4692	ADI1 MRS2	-0.143 -0.143	1	0 0	AWRI796_3450 AWRI796 4474	CDC73 CDC21	-0.157 -0.157	1	0

AWRI796 Gene ID AWRI796 5093	Gene Name SPN1	log <sub>2</sub> Fold Change Adj. p -value	e Score	AWRI796 Gene ID AWRI796 2343	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value Score
AWRI796_0882	KGD2	-0.158		AWRI796_2845	SSH4	-0.174	1 0
AWRI796_1644 AWRI796_1802	SUT1 THG1	-0.158 -0.158	1 0 1 0	AWRI796_3350 AWRI796 4795	YLR28/C SAR1	-0.174 -0.174	1 0
AWRI796_3056	JLP1	-0.158	1 0	AWRI796_0177	HHF1 KDF2	-0.175	1 0
AWRI796_4056 AWRI796_0973	PGA1 PAM1	-0.158	1 0	AWRI796_1170 AWRI796_1253	KRE2 HYP2	-0.175 -0.175	1 0
AWRI796_2329	SLM1 MID2	-0.159		AWRI796_1389	SWI4 CD11	-0.175	1 0
AWRI796_3384 AWRI796_3691	YMR099C	-0.159	1 0	AWRI796_1413 AWRI796_2917	NFU1	-0.175 -0.175	1 0
AWRI796_4159	YNL035C	-0.159		AWRI796_4088	NCS2	-0.175	1 0
AWRI796_4759 AWRI796_0016	GEM1	-0.159	1 0	AWRI796_0298	MRPS9	-0.175 -0.176	1 0
AWRI796_0990	MSW1	-0.16		AWRI796_0360	HPC2 VDP201C	-0.176	1 0
AWRI796_2255	AIM18	-0.16	1 0	AWRI796_1094 AWRI796_1272	UBC8	-0.176	1 0
AWRI796_2900	FBA1	-0.16		AWRI796_2522	MRS3	-0.176	1 0
AWRI796_3773	MRPS17	-0.161	1 0	AWRI796_3631	RSF1	-0.176	1 0
AWRI796_1117	RPL12B	-0.162		AWRI796_3701	HFD1	-0.176	1 0
AWRI796_1207	APA2	-0.162	1 0	AWRI796_4785	CET1	-0.176	1 0
AWRI796_1212	STL1 VPB30	-0.162		AWRI796_0061	NUP60 SDH7	-0.177	1 0
AWRI796_1778	RPN14	-0.162	1 0	AWRI796_2684	RFC2	-0.177	1 0
AWRI796_1877 AWRI796_2296	SHY1 REV7	-0.162		AWRI796_2820 AWRI796_3100	SRP102 MMM1	-0.177	1 0
AWRI796_4177	YNL010W	-0.162	1 0	AWRI796_0213	RPS11B	-0.178	1 0
AWRI796_4360 AWRI796_4632	GPM3 GPN2	-0.162	1 0	AWRI796_2677 AWRI796_2706	YJR061W YIR096W	-0.178 -0.178	1 0
AWRI796_0316	ARL1	-0.163	1 0	AWRI796_2761	YKL222C	-0.178	1 0
AWRI796_2210 AWRI796_2634	PEX28 SUI2	-0.163	1 0	AWRI796_2878 AWRI796_2937	SRX1 HCS1	-0.178 -0.178	1 0
AWRI796_2703	FIP1	-0.163	i Ö	AWRI796_4276	SPT20	-0.178	1 0
AWRI796_3167 AWRI796_3518	PET309 VPS9	-0.163	1 0	AWRI796_4922 AWRI796_0985	YPL071C DIN7	-0.178 -0.179	1 0
AWRI796_3876	HSH155	-0.163	i Ö	AWRI796_1328	CAJ1	-0.179	1 0
AWRI796_3910 AWRI796_4803	AAD14 SRP72	-0.163	101	AWRI796_1804 AWRI796_3178	RPS25A SIC1	-0.179 -0.179	1 0
AWRI796_1516	YFR006W	-0.164	1 0	AWRI796_3393	YLR345W	-0.179	1 0
AWRI796_4114 AWRI796_4874	TCB2 TBF1	-0.164 -0.164	1 0 1 0	AWRI796_5120 AWRI796_5149	MRP2 ARR3	-0.179 -0.179	1 0 1 0
AWRI796_0754	MED2	-0.165	1 0	AWRI796_1733	YBP2	-0.18	1 0
AWRI796_0965 AWRI796_1232	AMD2 PCM1	-0.165	1 0	AWRI796_1810 AWRI796_1822	UFD1	-0.18 -0.18	1 0
AWRI796_1433	YER158C	-0.165	1 0	AWRI796_0460	RRP7	-0.181	1 0
AWRI796_1834 AWRI796_2345	AIM19	-0.165	1 0	AWRI796_0976 AWRI796_1651	CHL4 CDC43	-0.181 -0.181	1 0
AWRI796_2535	NCA3	-0.165	1 0	AWRI796_2280	COA1	-0.181	1 0
AWRI796_3161 AWRI796_4448	GLO4	-0.165	1 0	AWRI796_2347 AWRI796_2574	SDS3 YJL070C	-0.181 -0.181	1 0
AWRI796_0423	APM3	-0.166	1 0	AWRI796_2875	MIF2	-0.181	1 0
AWRI796_1694 AWRI796_1859	PRP31	-0.166	1 0	AWRI796_4971 AWRI796_0254	PHO5	-0.181 -0.182	1 0
AWRI796_2056	APM2	-0.166		AWRI796_1960	SLI1 PPT1	-0.182	1 0
AWRI796_2194 AWRI796_4462	SLD7	-0.166	1 0	AWRI796_4377	PRE6	-0.182	1 0
AWRI796_4499	OST2 MRX9	-0.166		AWRI796_4432	SFM1 PTS2	-0.182	1 0
AWRI796_3055	YLL058W	-0.167	1 0	AWRI796_5023	MCM16	-0.182	1 0
AWRI796_3192 AWRI796_3343	NYV1 DBP9	-0.167	1 0	AWRI796_0191 AWRI796_1417	SCO2 MAG1	-0.183	1 0
AWRI796_3481	RIF2	-0.167	i Ö	AWRI796_1506	SEC4	-0.183	1 0
AWRI796_4008 AWRI796_0909	RAP1 UBC1	-0.167	1 0 1 0	AWRI796_1607 AWRI796_2446	YPT32 YIR035C	-0.183 -0.183	1 0
AWRI796_1670	PCL10	-0.168	i Ö	AWRI796_3131	SED5	-0.183	1 0
AWRI796_1784 AWRI796_1882	CUL3 COG2	-0.168	1 0 1 0	AWRI796_3736 AWRI796_5197	OSW5 COS3	-0.183 -0.183	1 0
AWRI796_1922	PUS6	-0.168	1 0	AWRI796_0644	BPL1	-0.184	1 0
AWRI796_3238 AWRI796 4469	SPE4 ALG8	-0.168 -0.168	1 0 1 0	AWRI796_0773 AWRI796 1520	YDR018C UBP6	-0.184 -0.184	1 0 1 0
AWRI796_4819	YPL191C	-0.168	1 0	AWRI796_1521	MIC19	-0.184	1 0
AWRI796_5003 AWRI796_0338	NTC20	-0.168	1 0	AWRI796_3574 AWRI796_3873	KCFI YKU70	-0.184 -0.184	1 0
AWRI796_1027	RAD34	-0.169	1 0	AWRI796_4548	PET123	-0.184	1 0
AWRI796_1856 AWRI796_2249	LNP1	-0.169	1 0	AWRI796_4569 AWRI796_0458	MXR2	-0.184 -0.185	1 0
AWRI796_2635	MHO1	-0.17	1 0	AWRI796_1357A	YER076C	-0.185	1 0
AWRI796_2869	YJU2	-0.17	1 0	AWRI796_2191	ARP1	-0.185	1 0
AWRI796_3756	ALD3 TPM12	-0.17		AWRI796_2406	DOT5	-0.185	1 0
AWRI796_0049	NTG1	-0.171	1 0	AWRI796_2550	SAP185	-0.185	1 0
AWRI796_0189 AWRI796_0380	POA1 THI2	-0.171		AWRI796_3720 AWRI796_4398	YMR130W IRC10	-0.185	1 0
AWRI796_1150	PFA5	-0.171	1 0	AWRI796_2126	FYV4	-0.186	1 0
AWRI796_2835 AWRI796_3730	APL2 SIP5	-0.171	1 0	AWRI796_4944 AWRI796_0476	VPS16 ILV6	-0.186 -0.187	1 0
AWRI796_4354	APM4	-0.171	1 0	AWRI796_0523	CTR86	-0.187	1 0
AWRI796_4740 AWRI796_5104	PHR1 YPR148C	-0.171	1 0	AWRI796_1517 AWRI796_1841	YFH7 ENV11	-0.187 -0.187	1 0
AWRI796_1582	DOC1	-0.172	1 0	AWRI796_2530	LSM1	-0.187	1 0
AWRI/96_1947 AWRI796_2371	YPP1 PCL7	-0.172 -0.172	10 10	AWRI796_0094 AWRI796_0124	MKP21 PIN4	-0.188 -0.188	1 0 1 0
AWRI796_2742	HOM6	-0.172	1 0	AWRI796_0384	GPX2	-0.188	1 0
AWRI796_4309 AWRI796_0099	BOI1	-0.172 -0.173	1 0 1 0	AWRI796_3646 AWRI796_3809	CSM3 YMR226C	-0.188 -0.188	1 0 1 0
AWRI796_0297	ADH5	-0.173		AWRI796_4026	YNL194C	-0.188	1 0
AWRI796_0704	YET3	-0.173	1 0	AWRI796_00081 AWRI796_0906	HMO1	-0.189 -0.189	1 0
AWRI796_1321	YEN1 FPT1	-0.173		AWRI796_1106	MRP20 LPP1	-0.189	1 0
AWRI796_2536	ASF1	-0.173	1 0	AWRI796_2534	PHO86	-0.189	1 0
AWRI796_2549 AWRI796_3568	CHS6 YML037C	-0.173		AWRI796_2676 AWRI796_2929	CBF1 PAN3	-0.189	1 0
AWRI796_0678	POL3	-0.175	1 0	AWRI796_3711	ADE17	-0.189	1 0
AWRI796_1161	PRP3	-0.174	1 0	AWRI796_4610	HES1	-0.189	1 0

AWRI796 Gene ID AWRI796 5012	Gene Name	log <sub>2</sub> Fold Change	Adj. <i>p</i> -value	Score	AWRI796 Gene ID AWRI796 3453	Gene Name RPN13	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_1042	GPI8	-0.19	1	0	AWRI796_3483	PDP3	-0.202	1	0
AWRI796_1398 AWRI796_1654	SCS2 NUT1	-0.19 -0.19	1	0	AWRI796_4845 AWRI796_0080	YPL162C MIX23	-0.202 -0.203	1	0
AWRI796_1772	SCL1	-0.19	1	0	AWRI796_0334	MBA1	-0.203	1	0
AWRI796_1836 AWRI796_1932	VHTT RNR4	-0.19 -0.19	1	0	AWRI796_1317 AWRI796_1702	USE1	-0.203	1	0
AWRI796_2385	CST6	-0.19	1	0	AWRI796_3287	PNP1	-0.203	1	0
AWRI796_2444 AWRI796_4410	PFA4	-0.19	1	0	AWRI796_3288 AWRI796_3923	ATP11	-0.203	1	0
AWRI796_4427	ERP4	-0.19	1	0	AWRI796_3976	TEX1	-0.203	1	0
AWRI796_0398	RGD1	-0.19	1	0	AWRI796_4568	SER1	-0.203	1	0
AWRI796_0703 AWRI796_0794	AHK1 RPC11	-0.191	1	0	AWRI796_5054 AWRI796_0349	MDM36 DFR1	-0.203	1	0
AWRI796_1879	SPT6	-0.191	1	0	AWRI796_1992	PUP2	-0.204	1	0
AWRI796_1920 AWRI796_2816	TRS65 KDX1	-0.191 -0.191	1	0	AWRI796_1996 AWRI796_3748	MTM1 HL11	-0.204	1	0
AWRI796_4226	PET494	-0.191	1	0	AWRI796_4772	SRP68	-0.204	1	0
AWRI796_5039 AWRI796_0194	UBA3 YPK3	-0.191 -0.192	1	0	AWRI796_4924 AWRI796_5136	BTS1 ATG13	-0.204 -0.204	1	0
AWRI796_0268	ALG1	-0.192	1	0	AWRI796_0469	KCC4	-0.205	1	0
AWRI796_0818 AWRI796_0920	SNF11 SLY1	-0.192 -0.192	1	0	AWRI796_0755 AWRI796_1291	YER010C	-0.205	1	0
AWRI796_1574	RMR1	-0.192	1	0	AWRI796_0549	YCR090C	-0.206	1	0
AWRI796_4429	ROD1	-0.192	1	0	AWRI796_2653	PET191	-0.206	1	0
AWRI796_1156	PKH3 NOT3	-0.193	1	0	AWRI796_3824	RPL20A	-0.206	1	0
AWRI796_2383 AWRI796_3571	SRC1	-0.193	1	0	AWRI796_4396	ESC8	-0.206	1	0
AWRI796_3667 AWRI796_4524	IRC21 YOR131C	-0.193	1	0	AWRI796_0019 AWRI796_0228	YAL044W-A FCM2	-0.207	1	0
AWRI796_0048	TPD3	-0.194	1	0	AWRI796_0894	CWC15	-0.207	1	0
AWRI796_0098 AWRI796_0206	YBL086C FAT1	-0.194 -0.194	1	0	AWRI796_1339 AWRI796_1815	PET117 KSS1	-0.207 -0.207	1	0
AWRI796_2572	PSF2	-0.194	1	0	AWRI796_3110	SSL1	-0.207	1	0
AWRI796_3224 AWRI796_3305	ACE2 CDC42	-0.194 -0.194	1	0	AWRI796_4881 AWRI796_4926	VPS30 YPL067C	-0.207 -0.207	1	0
AWRI796_3655	SAM37	-0.194	1	0	AWRI796_1320	GLN3	-0.208	1	Ő
AWRI796_4217 AWRI796_4662	SOL1 TIM18	-0.194 -0.194	1	0	AWRI796_2365 AWRI796_2555	SNP1 SRS2	-0.208 -0.208	1	0
AWRI796_5018	ERV2	-0.194	1	0	AWRI796_2560	TRL1	-0.208	1	0
AWRI796_1592 AWRI796_1845	PEX8	-0.195 -0.195	1	0	AWRI796_3771 AWRI796_4449	CUE5	-0.208	1	0
AWRI796_2689	MOG1	-0.195	1	0	AWRI796_4843	MLH3	-0.208	1	0
AWRI796_5555 AWRI796_4104	OCA1	-0.195	1	0	AWRI796_0045 AWRI796_0726	MTF2	-0.209	1	0
AWRI796_4658	RPS10A DIB1	-0.195	1	0	AWRI796_1247	YEF1 MRH4	-0.209	1	0
AWRI796_0319	PEX32	-0.195	1	0	AWRI796_3323	SYM1	-0.209	1	0
AWRI796_0884 AWRI796_1025	CTH1 SSF2	-0.196 -0.196	1	0	AWRI796_4189 AWRI796_4916	SWM2 RPL21B	-0.209	1	0
AWRI796_1181	VPS3	-0.196	1	0	AWRI796_0492	YCR016W	-0.21	1	0
AWRI796_1379 AWRI796_2630	UBC6 MRX12	-0.196 -0.196	1	0	AWRI796_0683 AWRI796_1600	RPN6 FRA2	-0.21 -0.21	1	0
AWRI796_3437	BDF1	-0.196	1	0	AWRI796_2143	NMD2	-0.21	1	0
AWRI796_3605 AWRI796_3624	UBC7	-0.196 -0.196	1	0	AWRI796_2215 AWRI796_4162	ARK1	-0.21	1	0
AWRI796_3777	GYL1	-0.196	1	0	AWRI796_5148	ARR2	-0.21	1	0
AWRI796_0682	SNU23	-0.198	1	0	AWRI796_0037 AWRI796_3215	SRN2	-0.211	1	0
AWRI796_1157	TLG1 VHR2	-0.197	1	0	AWRI796_3451	YLR419W VML053C	-0.211	1	0
AWRI796_1807	POP6	-0.197	1	0	AWRI796_2263	SKN7	-0.211	1	0
AWRI796_2121 AWRI796_2557	COX6 DPB11	-0.197 -0.197	1	0	AWRI796_2448 AWRI796_2615	GTT1 PFT130	-0.212	1	0
AWRI796_2649	YJR030C	-0.197	1	0	AWRI796_2728	RPS5	-0.212	1	0
AWRI796_2955 AWRI796_2994	OSH6 PET10	-0.197 -0.197	1	0	AWRI796_2926 AWRI796_3256	TFA1 IDP2	-0.212	1	0
AWRI796_4035	MRPL19	-0.197	1	0	AWRI796_3577	YOX1	-0.212	1	0
AWRI796_0560 AWRI796 1179	LRG1 MZM1	-0.198 -0.198	1	0	AWRI796_4098 AWRI796_4185	INP52 DOM34	-0.212 -0.212	1	0
AWRI796_1570	FZF1	-0.198	1	0	AWRI796_0246	TEC1	-0.213	1	0
AWRI796_2200 AWRI796_3648	FAR3	-0.198	1	0	AWRI796_0342 AWRI796_1357B	YER076C	-0.213	1	0
AWRI796_3760 AWRI796_4367	DDR48 RRT8	-0.198	1	0	AWRI796_1468 AWRI796_2413	EMP47 YII 001W	-0.213	1	0
AWRI796_4933	GRX5	-0.198	1	0	AWRI796_3075	IRC19	-0.213	1	0
AWRI796_0232 AWRI796_0576	BAP2 FMP45	-0.199 -0.199	1	0	AWRI796_4987 AWRI796_0479	PDH1 PGS1	-0.213 -0.214	1	0
AWRI796_2377	AGE2	-0.199	1	0	AWRI796_0702	BRE1	-0.214	1	0
AWRI796_3957 AWRI796_4588	BOR1 NPT1	-0.199 -0.199	1	0	AWRI796_0854 AWRI796_0897	TMA64 SEC5	-0.214 -0.214	1	0
AWRI796_4593	RUD3	-0.199	1	0	AWRI796_1907	YGR149W	-0.214	1	0
AWRI796_0231 AWRI796_0719	PBP4	-0.2	1	0	AWRI796_1956 AWRI796_1976	YHB1	-0.214	1	0
AWRI796_1549	ERJ5	-0.2	1	0	AWRI796_2061	YLF2 CPX8	-0.214	1	0
AWRI796_2857	SLD2	-0.2	1	0	AWRI796_4532	ARP8	-0.214	1	0
AWRI796_3928 AWRI796_3966	STB1 PDR17	-0.2	1	0	AWRI796_4539 AWRI796_4939	SMP3 OAZ1	-0.214	1	0
AWRI796_0632	CLB3	-0.201	1	0	AWRI796_5040	ISA2	-0.214	1	0
AWRI796_0873 AWRI796_3640	HPK1 YET2	-0.201 -0.201	1	0	AWRI/96_5075 AWRI796 0361	DBF20 YBP1	-0.214 -0.215	1	0
AWRI796_3686	CTF13	-0.201	1	0	AWRI796_0898	TAF10	-0.215	1	0
AWRI796_3779	ICY1	-0.201 -0.201	1	0	AWRI796_3715 AWRI796_3975	RTC4	-0.215 -0.215	1	0
AWRI796_4336	ATG19 KTR1	-0.201	1	0	AWRI796_4229	YNR048W STI1	-0.215	1	0
AWRI796_5100	KAR3	-0.201	1	0	AWRI796_0350	MCM7	-0.215	1	0
AWRI796_5125 AWRI796_0575	YPR172W HBT1	-0.201 -0.202	1	0	AWRI796_2168 AWRI796_2609	GRE3 BET4	-0.216	1	0
AWRI796_1338	HMF1	-0.202	1	0	AWRI796_3202	LCL2	-0.216	1	0
AWRI/96_1372 AWRI796 1432	MET6 COG3	-0.202 -0.202	1	0	AWRI796_0026 AWRI796_0618	CDC19 PAR32	-0.217 -0.217	1	0
AWRI796_2639	YJR012C	-0.202	1	0	AWRI796_3446	BER1	-0.217	1	0
AWAI/90_2913	1 K12	-0.202	1	0	AWK1/90_4120	1 1 1011	-0.21/	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change Adj. p -val	ie Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value Score
AWRI796_0486	RVS161	-0.218	1 0	AWRI796_0927	CBS2	-0.238	1 0
AWRI796_1122 AWRI796_1290	CAD1 NTF2	-0.218	1 0	AWRI796_2380 AWRI796_2855	GVP36 KTI12	-0.238	1 0
AWRI796_1848	TWF1	-0.218	1 0	AWRI796_3531	YML079W	-0.238	1 0
AWRI796_2334 AWRI796_2509	FMC1 DAS1	-0.218	1 0	AWRI796_3643 AWRI796_3995	MCM1 PDR16	-0.238	1 0
AWRI796_3234	PUT1	-0.218	1 0	AWRI796_4303	RRI2	-0.238	1 0
AWRI796_0741 AWRI796_2248	DIA3 CTF8	-0.219	1 0	AWRI796_0352 AWRI796_2941	LDH1 ARC19	-0.239	1 0
AWRI796_2565	ARP4	-0.219	1 0	AWRI796_3440	SEI1	-0.239	1 0
AWRI796_2647 AWRI796_3262	MDE1 SAM1	-0.219	1 0   1 0	AWRI796_0602 AWRI796_0621	NUS1 UGX2	-0.24	1 0
AWRI796_0179	IPP1	-0.22	1 0	AWRI796_1048	MRPS28	-0.24	1 0
AWRI796_0853	MRPL1	-0.22	1 0	AWRI796_2757	YJR154W	-0.24	1 0
AWRI796_2006	HUA1	-0.22	1 0 1 0	AWRI796_3980	MPA43	-0.24	1 0
AWRI796_2066	YAP3	-0.22	1 0	AWRI796_4222	YNR040W	-0.24	1 0
AWRI796_23108	CMS1	-0.22	1 0 $   1 0$	AWRI796_1028	IPK1	-0.241	1 0
AWRI796_4073	EAF7	-0.22	1 0	AWRI796_3947	SEC21	-0.241	1 0
AWRI796_5086	AXL1	-0.22	1 0 1 0	AWRI796_4675	RPL20B	-0.241	1 0
AWRI796_0880	SWI5	-0.221	1 0	AWRI796_4899	INA17	-0.241	1 0
AWRI796_0928 AWRI796_2231	ATG7	-0.221	1 0 1 0	AWRI796_1067 AWRI796_1167	DIG2	-0.242	1 0
AWRI796_2467	YJL206C	-0.221	1 0	AWRI796_1914	MTR3	-0.242	1 0
AWRI796_2596 AWRI796_4332	AIM22 AWRI796 4332	-0.221 -0.221	$   1 0 \\   1 0 $	AWRI796_2213 AWRI796_2850	SPO16 SBA1	-0.242 -0.242	1 0
AWRI796_2468	RCY1	-0.222	1 0	AWRI796_4125	MLF3	-0.242	1 0
AWRI796_2694 AWRI796_0054	AIM24 MDM10	-0.222 -0.223	$   1 0 \\   1 0 $	AWRI796_4932 AWRI796_0950	MFM1 HTA1	-0.242 -0.243	1 0
AWRI796_0690	NUR1	-0.223	1 0	AWRI796_0558	YCR101C	-0.244	1 0
AWRI796_1957 AWRI796_2421	TRX2 PRI1	-0.223 -0.223	1 0	AWRI796_1590 AWRI796_1765	EMC4 JAC1	-0.244	1 0
AWRI796_2942	PRP40	-0.223	1 0	AWRI796_1797	UGA1	-0.244	1 0
AWRI796_3275	NMT1 PMS1	-0.223	1 0	AWRI796_2028	COS8 EAE6	-0.244	1 0
AWRI796_0095	AVT5	-0.224	1 0	AWRI796_3471	RPS1A	-0.244	1 0
AWRI796_1133	GPI19	-0.224	1 0	AWRI796_4072	NAM9	-0.244	1 0
AWRI796_1665	FLC3	-0.224	1 0 1 0	AWRI796_0803	EMC10	-0.244	1 0
AWRI796_2270	AWRI796_2270	-0.224	$\begin{array}{ccc} 1 & 0 \\ 1 & 0 \end{array}$	AWRI796_3425	SWC7	-0.245	1 0
AWRI796_2888 AWRI796_3314	ARV1	-0.224 -0.224	1 0 1 0	AWRI796_4259 AWRI796_4679	FAA1	-0.245	1 0
AWRI796_3524	UFO1	-0.224	1 0	AWRI796_4846	BEM4	-0.245	1 0
AWRI796_1499 AWRI796_3672	CTF18	-0.225 -0.225	1 0 $   1 0$	AWRI796_0706 AWRI796 1135	LRS4	-0.246	1 0
AWRI796_4522	AFI1	-0.225	1 0	AWRI796_1544	CDC26	-0.246	1 0
AWRI796_0011 AWRI796 1693	PEX22 YGL108C	-0.226 -0.226	$     1 0 \\     1 0 $	AWRI796_1971 AWRI796_2381	DIE2 APQ12	-0.246 -0.246	1 0
AWRI796_3731	RPL13B	-0.226	1 0	AWRI796_2902	TOA2	-0.246	1 0
AWRI796_4557 AWRI796_4580	LCB4 BFR1	-0.226 -0.226	$     1 0 \\     1 0 $	AWRI796_2983 AWRI796_3595	DID2 YAP1	-0.246 -0.246	1 0
AWRI796_4985	HAT1	-0.226	1 0	AWRI796_3627	PEX12	-0.246	1 0
AWRI796_0596 AWRI796_1187	MGT1 SAM2	-0.227 -0.227	$   1 0 \\   1 0 $	AWRI796_0220 AWRI796_2435	YBR056W INA22	-0.247 -0.247	1 0
AWRI796_1455	FMP10	-0.227	1 0	AWRI796_2872	BUD2	-0.247	1 0
AWRI796_3082 AWRI796_0487	PAU17 ADY2	-0.227 -0.228	1 0	AWRI796_3722 AWRI796_0064	JLP2 RFA1	-0.247 -0.248	1 0
AWRI796_0761	RCR2	-0.228	1 0	AWRI796_0313	CSH1	-0.248	1 0
AWRI796_1747 AWRI796_2337	YGL041W-A PRK1	-0.228 -0.228	1 0   1 0	AWRI796_1490 AWRI796_2853	EPL1 RAD27	-0.248	1 0
AWRI796_3352	GUF1	-0.228	1 0	AWRI796_3126	IRC25	-0.248	1 0
AWRI796_3361 AWRI796_3520	EXG1 GIM5	-0.228	1 0   1 0	AWRI796_3366 AWRI796_4788	CDA1 VPI 225W	-0.248	1 0
AWRI796_4794	PCL8	-0.228	1 0	AWRI796_0282	ATG14	-0.249	1 0
AWRI796_1547 AWRI796_3996	OSW7 FLA1	-0.229	1 0	AWRI796_0721 AWRI796_0816	LHP1 PAA1	-0.249	1 0
AWRI796_5007	CCL1	-0.229	1 0	AWRI796_1031	MCM21	-0.249	1 0
AWRI796_1991	GCN5	-0.23	1 0	AWRI796_1980	PEX21 PAD52	-0.249	1 0
AWRI796_4055	AWK1790_3848 ASI2	-0.23	1 0 1 0	AWRI796_3618	SOK2	-0.249	1 0
AWRI796_4798	CBP3	-0.23	1 0	AWRI796_4307	SKM1	-0.249	1 0
AWRI796_2898	MSN4	-0.231	1 0	AWRI796_2429	MET28	-0.25	1 0
AWRI796_4225	AGA1	-0.231	1 0	AWRI796_3412	ARC18	-0.25	1 0
AWRI796_1552 AWRI796_3566	VPS71	-0.232	1 0 1 0	AWRI796_3088 AWRI796_3547	MFT1	-0.251	1 0
AWRI796_4601	ISU2	-0.232	1 0	AWRI796_4105	RAS2	-0.251	1 0
AWRI796_1010 AWRI796_2430	YAP5	-0.233	1 0 $   1 0$	AWRI796_4286 AWRI796_4879	RNY1	-0.251	1 0
AWRI796_2601	NSP1	-0.233	1 0	AWRI796_1746	DST1	-0.252	1 0
AWRI796_2711 AWRI796_3636	MIH1	-0.233 -0.233	$   1 0 \\   1 0 $	AWRI796_1934 AWRI796_4878	SPC29	-0.252 -0.252	1 0
AWRI796_3744	AIM36	-0.233	1 0	AWRI796_0709	PEX19	-0.253	1 0
AWRI796_4510 AWRI796_1639	SUA5	-0.233 -0.234	$   1 0 \\   1 0 $	AWRI796_0820 AWRI796_1019	YDR306C	-0.253	1 0
AWRI796_1908	CCM1	-0.234	1 0	AWRI796_3656	RNA14	-0.253	1 0
AWRI796_3355 AWRI796_3597	SEC/2 TRM12	-0.235 -0.235	$   1 0 \\   1 0 $	AWRI796_4145 AWRI796_4194	YNL050C CSE2	-0.253 -0.253	1 0
AWRI796_4681	GNT1	-0.235	1 0	AWRI796_4723	RAD17	-0.253	1 0
AWRI796_0348 AWRI796_1604	BEM1 SKI8	-0.236 -0.236		AWRI796_5188 AWRI796_0922	PHO12 HST4	-0.253 -0.254	1 0 1 0
AWRI796_1838	YGR067C	-0.236	1 0	AWRI796_1739	ERV14	-0.254	1 0
AWRI796_1939 AWRI796_2372	BUB1 DFG10	-0.236 -0.236	1 0 1 0	AWRI796_2173 AWRI796_5001	CTM1 RLF2	-0.254 -0.254	1 0 1 0
AWRI796_2795	ASH1	-0.236	1 0	AWRI796_1223	DSF1	-0.256	1 0
AWRI796_2892 AWRI796_2989	YKL069W YKR041W	-0.236	1 0   1 0	AWRI796_1296 AWRI796_1444	FAA2 ADK2	-0.256	1 0
AWRI796_3621	STB4	-0.236	1 0	AWRI796_2599	YJL043W	-0.256	1 0
AWRI796_3946 AWRI796_4386	CAF40 MDM38	-0.236	1 0	AWRI796_3358 AWRI796_3382	YLR297W NMA1	-0.256	1 0
AWRI796_4962	SKS1	-0.236	1 0	AWRI796_3768	SSO2	-0.256	1 0
AWRI796_0397 AWRI796_1173	YBR259W VPS60	-0.237	1 0	AWRI796_4076 AWRI796_4651	FYV6 RDI 2	-0.256	1 0
AWRI796_4323	HMI1	-0.237	1 0	AWRI796_0680	GET3	-0.257	1 0

AWRI796 Gene ID AWRI796 1558	Gene Name RPN12	log <sub>2</sub> Fold Change A	dj. <i>p</i> -value	Score	AWRI796 Gene ID AWRI796 4940	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_2423	DSN1	-0.257	1	0	AWRI796_0429	PCA1	-0.278	1	0
AWRI796_2744 AWRI796_4019	YJR141W SPS19	-0.257 -0.257	1	0	AWRI796_0777 AWRI796_0915	CIS1 PLP1	-0.278	1	0
AWRI796_4535	ELG1	-0.257	1	Ő	AWRI796_2539	GZF3	-0.278	1	0
AWRI796_5031 AWRI796_1460	TFB4 YER187W	-0.257	1	0	AWRI796_5147 AWRI796_0579	ARR1 YDL218W	-0.278	1	0
AWRI796_1825	YGR053C	-0.258	1	0	AWRI796_0689	RAM1	-0.279	1	0
AWRI796_1911 AWRI796_4436	CYS4 BUB3	-0.258	1	0	AWRI796_0762 AWRI796_3930	RAD57 MCK1	-0.279	1	0
AWRI796_1158	SDC1	-0.259	1	0	AWRI796_4619	APC5	-0.279	1	0
AWRI796_1659	TIP20 VBA2	-0.259	1	0	AWRI796_4635	PNT1 SNO2	-0.279	1	0
AWRI796_0543	TRX3	-0.26	1	0	AWRI796_4444	PEP12	-0.28	1	0
AWRI796_1261	RIP1 PEA2	-0.26	1	0	AWRI796_5094	MSS18 OTU1	-0.28	1	0
AWRI796_2408	NAS2	-0.26	1	0	AWRI796_1480	MOB2	-0.281	1	0
AWRI796_3601	YPT7	-0.26	1	0	AWRI796_2849	VPH2	-0.281	1	0
AWRI796_0050 AWRI796_0951	ADK1	-0.261	1	0	AWRI796_3289 AWRI796_4154	YNL040W	-0.281	1	0
AWRI796_2035	EFM1	-0.261	1	0	AWRI796_4157	IDH1 KABO	-0.281	1	0
AWRI796_2275 AWRI796_2484	ATP12	-0.261	1	0	AWRI796_1303	SRB4	-0.281	1	0
AWRI796_3170	XYL2	-0.261	1	0	AWRI796_2402	BAR1	-0.282	1	0
AWRI796_3383 AWRI796_4395	TLG2	-0.261	1	0	AWRI796_2737 AWRI796_4148	SLM2	-0.282 -0.282	1	0
AWRI796_4542	ATG40	-0.261	1	0	AWRI796_4712	GDS1	-0.282	1	0
AWRI796_4544 AWRI796_0879	SLP1 TAF12	-0.261 -0.262	1	0	AWRI/96_1662 AWRI796_2591	IRC8	-0.283 -0.283	1	0
AWRI796_1215	FDC1	-0.262	1	0	AWRI796_4176	YNL011C	-0.283	1	0
AWRI796_0430 AWRI796_3924	PHO89 DAL82	-0.263 -0.263	1	0	AWRI796_4633 AWRI796_4831	DSE3 CUP9	-0.283 -0.283	1	0
AWRI796_0267	CMD1	-0.264	1	0	AWRI796_0447	POF1	-0.284	1	0
AWRI796_1311 AWRI796_3776	CHZ1 SPG5	-0.264 -0.264	1	0	AWRI796_3630 AWRI796_2147	FAR8 LRP1	-0.284 -0.285	1	0
AWRI796_4263	CSS3	-0.264	1	0	AWRI796_3069	SDH2	-0.285	1	0
AWRI796_4428 AWRI796_4480	PET127 DIA2	-0.264	1	0	AWRI796_5231 AWRI796_0015	FEX1 AIM2	-0.285	1	0
AWRI796_4892	YPL107W	-0.264	1	0	AWRI796_2469	PRP21	-0.286	1	0
AWRI796_1990	NOP19 CTE2	-0.265	1	0	AWRI796_4707	MNE1	-0.286	1	0
AWRI796_3494	PHO84	-0.265	1	0	AWRI796_0000	SLX5	-0.287	1	0
AWRI796_0165	PDR3	-0.266	1	0	AWRI796_1334	PIC2	-0.287	1	0
AWRI796_0564 AWRI796_0692	LUC7	-0.266	1	0	AWRI796_1878 AWRI796_4017	RTT106	-0.287	1	0
AWRI796_1185	LCD1	-0.266	1	0	AWRI796_0913	SAS4	-0.288	1	0
AWRI796_5085 AWRI796_0815	DOA4	-0.266 -0.267	1	0	AWRI/96_3184 AWRI796_3191	SUL2	-0.288 -0.288	1	0
AWRI796_1872	VOA1	-0.267	1	0	AWRI796_3205	REX3	-0.288	1	0
AWRI796_2520 AWRI796_5207	GLG2 COS3	-0.267 -0.267	1	0	AWRI796_3247 AWRI796_5121	MAS1 MET16	-0.288 -0.288	1	0
AWRI796_0462	BIK1	-0.268	1	0	AWRI796_5131	HDA3	-0.288	1	0
AWRI796_0931 AWRI796_0964	RAV2 SNU56	-0.268	1	0	AWRI796_4274 AWRI796_4927	GRE2 RGL1	-0.289	1	0
AWRI796_1579	TAD1	-0.268	1	0	AWRI796_0656	PCL2	-0.29	1	0
AWRI796_2127	VMA22 DCG1	-0.268	1	0	AWRI796_0591	YDL206W VMP181C	-0.291	1	0
AWRI796_3152	YLR050C	-0.268	1	0	AWRI796_0290	YBR137W	-0.292	1	0
AWRI796_3548	PIF1	-0.268	1	0	AWRI796_0631	CMR1 FAU1	-0.292	1	0
AWRI796_4493	ARF3	-0.268	1	0	AWRI796_1814	ORM1	-0.292	1	0
AWRI796_0972	YDR249C FRO1	-0.269	1	0	AWRI796_1954	MVB12 UBX4	-0.292	1	0
AWRI796_2062	OTU2	-0.269	1	0	AWRI796_4824	UIP4	-0.292	1	0
AWRI796_3745	MRPS8	-0.269	1	0	AWRI796_1921	YGR168C	-0.293	1	0
AWRI796_0717	PSA1	-0.209	1	0	AWRI796_3486	COS3	-0.293	1	0
AWRI796_0851	PDS1	-0.27	1	0	AWRI796_5165	AWRI796_5165	-0.293	1	0
AWRI796_1415 AWRI796_2589	PEP8	-0.27	1	0	AWRI796_1721 AWRI796_4445	CYC2	-0.294	1	0
AWRI796_2681	ARP3	-0.27	1	0	AWRI796_5127	YPR174C	-0.294	1	0
AWRI796_3417 AWRI796_4029	DUG3	-0.27 -0.27	1	0	AWRI796_0356 AWRI796 1515	SAD1	-0.295 -0.295	1	0
AWRI796_4062	INN1	-0.27	1	0	AWRI796_4660	UAF30	-0.295	1	0
AWRI796_4335 AWRI796_5132	AOS1	-0.27 -0.27	1	0	AWRI796_5122 AWRI796_0413	DPB3	-0.295 -0.296	1	0
AWRI796_0265	IML3	-0.271	1	0	AWRI796_1191	GIN4	-0.296	1	0
AWRI796_1716 AWRI796_3093	YGL082W EMC6	-0.271	1	0	AWRI/96_2087 AWRI796_2315	YSC83 RPI1	-0.296	1	0
AWRI796_3490	RSC9	-0.271	1	0	AWRI796_3196	HRT3	-0.296	1	Ő
AWRI796_4080 AWRI796_1159	NRK1 UGO1	-0.271	1	0	AWRI796_4506 AWRI796_5179	YOR111W YLR410W-B	-0.296	1	0
AWRI796_2846	SRP21	-0.272	1	Ő	AWRI796_0304	SPP381	-0.297	1	0
AWRI796_3592 AWRI796_3770	RAD33 RTP1	-0.272	1	0	AWRI796_2084 AWRI796_3781	SPO13 VTI1	-0.298	1	0
AWRI796_4083	SPC98	-0.272	1	0	AWRI796_3813	PEP5	-0.298	1	0
AWRI796_0679 AWRI796_0120	DUN1 PTC3	-0.273	1	0	AWRI796_0302 AWRI796_2071	TBS1 HSF1	-0.299	1	0
AWRI796_1064	GGA1	-0.274	1	0	AWRI796_2401	SNL1	-0.299	1	0
AWRI796_1618	DSD1 VII 152W	-0.274	1	0	AWRI796_3146	TRX1 VBL028C	-0.299	1	0
AWRI796_0952	SIR4	-0.274	1	0	AWRI796_0827	STN1	-0.3	1	0
AWRI796_3295	COA4 HER2	-0.275	1	0	AWRI796_0929	VPS64 COG7	-0.3	1	0
AWRI796_4278	PSF3	-0.275	1	0	AWRI796_1916	RTS3	-0.3	1	0
AWRI796_4678	COT1 YOL 103W-P	-0.275	1	0	AWRI796_2354	SER33 ORI1	-0.3	1	0
AWRI796_1002	YDR286C	-0.275	1	0	AWRI796_2724	ILM1	-0.302	1	0
AWRI796_2651	CPR7 STE24	-0.276	1	0	AWRI796_3197	CHA4 SNE8	-0.302	1	0
AWRI796_0284	SHE3	-0.276	1	0	AWRI796_1594	MTC3	-0.302	1	0
AWRI796_0442	PBN1	-0.277	1	0	AWRI796_1710	LIF1 SPL 2	-0.303	1	0
AWRI796_0757 AWRI796_3484	NBP1	-0.277 -0.277	1	0	AWRI796_2198 AWRI796_3088	AWRI796_3088	-0.303	1	0
AWRI796_3629	TAP42	-0.277	1	0	AWRI796_4786	ALG5	-0.303	1	0
AWRI796_4096 AWRI796_4443	SHE4	-0.277 -0.277	1	0	AWRI796_3462 AWRI796_3916	FIG4	-0.304 -0.304	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change Adj. p -va	lue Score	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value S	core
AWRI796_4534 AWRI796_4773	IQG1	-0.304	1 0	AWRI796_2010	ELM1	-0.332 -0.333	1	0
AWRI796_0003	FLO5	-0.305	1 0	AWRI796_4371	PEX15	-0.334	1	0
AWRI796_2858 AWRI796_3339	CMG1	-0.305 -0.305	1 0	AWRI796_0554 AWRI796_1414	RTR1	-0.335 -0.335	1	0
AWRI796_0396	POP4	-0.306	1 0	AWRI796_3059	YLL054C	-0.335	1	0
AWRI796_2593	CHM7 PIP3	-0.306	1 0	AWRI796_3379	CWC24 TMA17	-0.335	1	0
AWRI796_2814 AWRI796_4365	GAL11	-0.306	1 0	AWRI796_1755	CGR1	-0.336	1	0
AWRI796_4587	PTP2	-0.306	1 0	AWRI796_1931	OKP1	-0.336	1	0
AWRI796_4758	THI21 FXO5	-0.307	1 0	AWRI796_4081	TEP1	-0.336	1	0
AWRI796_0443	LRE1	-0.308	1 0	AWRI796_4190	VPS27	-0.336	1	0
AWRI796_0638	RPN5	-0.308	1 0	AWRI796_1898	CBF2	-0.337	1	0
AWRI/96_1062 AWRI796_4439	DFG16	-0.308 -0.308	1 0	AWRI796_4540 AWRI796_4643	MRPL23 RIM20	-0.337	1	0
AWRI796_0945	MFB1	-0.309	1 0	AWRI796_2519	TIF2	-0.338	1	Ő
AWRI796_2856	HAP4 FLP6	-0.309	1 0	AWRI796_2074	NEM1 VD1	-0.339	1	0
AWRI796_3898 AWRI796 4153	COG6	-0.309	1 0	AWRI796_1313 AWRI796_0017	SPC72	-0.341	1	0
AWRI796_5084	CLB5	-0.309	1 0	AWRI796_1072	XRS2	-0.341	1	0
AWRI796_0234	ALG14 FAR1	-0.31	1 0	AWRI796_2959	RSC4 CUR1	-0.341	1	0
AWRI796_4373	NGL1	-0.31	1 0	AWRI796_0999	MRX10	-0.342	1	0
AWRI796_0509	BUD5	-0.311	1 0	AWRI796_1979	KEL2	-0.342	1	0
AWRI/96_2542 AWRI796_2769	IME2 YRA2	-0.311 -0.311	1 0	AWR1796_2632 AWR1796_2864	APL1 YPF1	-0.342 -0.342	1	0
AWRI796_2791	HYM1	-0.311	1 0	AWRI796_4401	HTZ1	-0.342	1	0
AWRI796_4961	AWRI796_4961	-0.311	1 0	AWRI796_4476	SKI7	-0.342	1	0
AWRI796_2886 AWRI796_2924	IXR1	-0.312 -0.312	1 0	AWRI796_1267 AWRI796_3363	EAF5 MET17	-0.344	1	0
AWRI796_3491	ERG13	-0.312	1 0	AWRI796_4127	RNH201	-0.344	1	0
AWRI796_3965 AWRI796_4242	IST1 VNR061C	-0.312	1 0	AWRI796_4607 AWRI796_0933	MGE1 MSC2	-0.344	1	0
AWRI796_4351	INP54	-0.312	1 0	AWRI796_3641	ARA2	-0.345	1	0
AWRI796_4800	LEA1	-0.312	1 0	AWRI796_4059	CUZ1	-0.345	1	0
AWRI/96_1196 AWRI796_5092	SLFI NAT3	-0.313	1 0	AWRI796_4317 AWRI796_2070	TPT1 LAG1	-0.346	1	0
AWRI796_0335	PCH2	-0.314	1 0	AWRI796_2418	IST3	-0.347	1	0
AWRI796_3585	YML018C	-0.314	1 0	AWRI796_3960	SEC2	-0.347	1	0
AWRI/96_4812 AWRI796_1361	YPL199C AIM9	-0.314 -0.315	1 0	AWRI796_4572 AWRI796_3541	IES4 ITT1	-0.347 -0.348	1	0
AWRI796_3359	YHC1	-0.315	1 0	AWRI796_0490	POL4	-0.349	1	0
AWRI796_4853	RAD53	-0.315	1 0	AWRI796_1022	GIC2	-0.349	1	0
AWRI796_0308 AWRI796_4708	SLI15 MEK1	-0.316 -0.316	1 0	AWRI796_1648 AWRI796_0115	RCK1 KIP1	-0.349 -0.35	1	0
AWRI796_4728	NUD1	-0.316	1 0	AWRI796_1021	SRB7	-0.35	1	0
AWRI796_0437	MRC1	-0.317	1 0	AWRI796_1689	SLD3	-0.35	1	0
AWR1796_0784 AWR1796_1712	MAD1	-0.317	1 0	AWRI796_1783 AWRI796_3181	SWC4 SRL2	-0.35	1	0
AWRI796_1803	YGR026W	-0.317	1 0	AWRI796_3307	EST1	-0.35	1	Ő
AWRI796_4277	PEX11	-0.317	1 0	AWRI796_3563	PRP39	-0.35	1	0
AWRI796_4328 AWRI796_4722	SCP1	-0.317	1 0	AWRI796_4012 AWRI796_2206	CRP1	-0.351	1	0
AWRI796_1318	KRE29	-0.318	1 0	AWRI796_2356	HOP1	-0.351	1	0
AWRI796_2272	YIL166C	-0.318	1 0	AWRI796_3716	DLT1	-0.351	1	0
AWRI796_1447	RAD24	-0.319	1 0	AWRI796_4126	MSK1	-0.351	1	0
AWRI796_2972	NTR2	-0.319	1 0	AWRI796_1149	HEH2	-0.352	1	0
AWRI796_3102 AWRI796_3671	ORC3 VPS20	-0.319	1 0	AWRI796_2824 AWRI796_3151	DBR1 VLR049C	-0.352	1	0
AWRI796_0763	MAF1	-0.32	1 0	AWRI796_3326	NDL1	-0.352	1	0
AWRI796_4030	YNL190W	-0.32	1 0	AWRI796_4087	TOM70	-0.352	1	0
AWRI796_4362 AWRI796_5105	NCE102	-0.32	1 0	AWRI796_4628 AWRI796_4863	FRK1	-0.352	1	0
AWRI796_5183	YOL103W-B	-0.32	1 0	AWRI796_1453	DMC1	-0.353	1	0
AWRI796_0050	SYN8 STE5	-0.321	1 0	AWRI796_3057	YLL056C	-0.353	1	0
AWRI796_2842	PGM1	-0.321	1 0	AWRI796_0420	YBR285W	-0.354	1	0
AWRI796_2979	SET3	-0.321	1 0	AWRI796_0731	BSC1	-0.354	1	0
AWRI796_3751	MSS11 TDA7	-0.321	1 0	AWRI796_3523	RPM2 ECM33	-0.354	1	0
AWRI796_4591	SAS5	-0.321	1 0	AWRI796_0902	YDR170W-A	-0.355	1	0
AWRI796_0609	YDL183C	-0.322	1 0	AWRI796_1171	VPS52	-0.355	1	0
AWR1796_2852 AWR1796_0823	SHU2	-0.322	1 0	AWRI796_1880 AWRI796_2584	BIT61	-0.355	1	0
AWRI796_2708	YUH1	-0.323	1 0	AWRI796_1536	ECO1	-0.356	1	Ő
AWRI796_2931	YKL023W SMD3	-0.323	1 0	AWRI796_3639	SUB1	-0.356	1	0
AWRI796_3805	YMR221C	-0.323	1 0	AWRI796_3538	COG8	-0.356 -0.357	1	0
AWRI796_5253	RDS1	-0.323	1 0	AWRI796_0930	SPC19	-0.359	1	0
AWRI796_1718 AWRI796_3331	KXD1 LCB5	-0.325	1 0	AWRI796_0233 AWRI796_1627	TAT1 STR3	-0.36 -0.36	1	0
AWRI796_4252	YNR071C	-0.325	1 0	AWRI796_1817	MTE1	-0.36	1	0
AWRI796_4836	COX10	-0.325	1 0	AWRI796_3603	MIX17	-0.36	1	0
AWRI/96_5045 AWRI796_1193	SMT3	-0.325	1 0	AWR1796_2498 AWR1796_4116	JJJ2 END3	-0.361 -0.361	1	0
AWRI796_4144	COG5	-0.326	1 0	AWRI796_1408	COM2	-0.362	1	0
AWRI796_0291	YBR138C SPT2	-0.327	1 0	AWRI796_1732	DUO1 BET2	-0.362	1	0
AWRI796 2661	VPS55	-0.327	1 0	AWRI790_5014 AWRI796 0100	CDC27	-0.362	1	0
AWRI796_3623	MAC1	-0.327	1 0	AWRI796_1634	BUD13	-0.365	1	Ő
AWRI796_4611	YOR238W HSM3	-0.327	1 0	AWRI796_3999	CNM67 STE4	-0.365	1	0
AWRI796_0142	SHE1	-0.328	1 0	AWRI796_4590 AWRI796_0459	STE50	-0.365	1	0
AWRI796_2266	CRG1	-0.329	1 0	AWRI796_2667	ISY1	-0.366	1	0
AWRI796_2830	MRP8 REC8	-0.329	1 0	AWRI796_4160	YNL034W TFB6	-0.366	1	0
AWRI796_0258	MMS4	-0.32	1 0	AWRI796_0860	INO2	-0.367	1	0
AWRI796_1017	RSC3	-0.33	1 0	AWRI796_2394	YIL024C	-0.367	1	0
AWRI/96_1518 AWRI796_2571	FAR7 JEM1	-0.33	1 0	AWRI796_2787 AWRI796_4479	SDS22 ATX2	-0.367	1	0
AWRI796_2957	MRPL13	-0.33	1 0	AWRI796_4577	SLK19	-0.367	1	0
AWRI796_3743	TPP1	-0.33	1 0	AWRI796_0630	YDL157C	-0.368	1	0
AWRI/96_416/ AWRI796_0281	SSIN8 VMA2	-0.33 -0.331	1 0 1 0	AWRI/96_16/1 AWRI796_2620	MAD3	-0.368 _0.368	1	0
AWRI796_3703	MED11	-0.332	1 0	AWRI796_1844	MRPL25	-0.369	1	0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change Adj. p	-value Sco	ore	AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_0037	SAW1	-0.309	1	0	AWRI796_1394	SLX8	-0.411	1	0
AWRI796_1865	ESP1	-0.37	1	0	AWRI796_3933	YPT11 PTT105	-0.411	1	0
AWRI796_2836	OCT1	-0.37	1	0	AWRI796_1385 AWRI796_2335	FYV10	-0.415	1	0
AWRI796_1488	STE2	-0.371	1	0	AWRI796_2524	AIM23	-0.414	1	0
AWRI796_2267 AWRI796_0123	SAS3	-0.371 -0.372	1	0	AWRI796_1101 AWRI796_0994	BSC2	-0.415 -0.416	1	0
AWRI796_4090	MLS1	-0.372	1	0	AWRI796_3778	MRPL24	-0.416	1	0
AWRI/96_44/5 AWRI796_1345	UFEI THO1	-0.372	1	0	AWRI796_4011 AWRI796_0555	GIT1	-0.417 -0.418	1	. 0
AWRI796_4389	IFM1	-0.373	1	Ő	AWRI796_2890	YKL071W	-0.418	1	0
AWRI796_4680	HSH49 VKT6	-0.373	1	0	AWRI796_0981	YAP6	-0.42	0 081777	0
AWRI796_2784 AWRI796_3604	MVP1	-0.375	1	0	AWRI796_0014 AWRI796_0912	SCC2	-0.421	0.981777	0
AWRI796_3705	YMR114C	-0.375	1	0	AWRI796_2187	NDT80	-0.421	1	0
AWRI796_0975 AWRI796 4100	MET4	-0.377	1	0	AWRI796_3639 AWRI796_3418	FBP1	-0.421	1	0
AWRI796_4955	SVL3	-0.377	1	0	AWRI796_5144	YPR196W	-0.423	1	0
AWRI/96_5087 AWRI796_0318	POP7	-0.377 -0.378	1	0	AWRI796_2558 AWRI796_4312	SIP4 INO4	-0.424 -0.424	1	. 0
AWRI796_1313	FIR1	-0.378	1	Ő	AWRI796_4295	YGK3	-0.425	0.816423	0
AWRI796_3002	RHO4 NPR 1	-0.378	1	0	AWRI796_1203	AGE1 AGP3	-0.426	0.748436	5 0
AWRI796_4875	HHO1	-0.378	1	0	AWRI796_4245	YNR064C	-0.426	1	0
AWRI796_2179	COX23	-0.379	1	0	AWRI796_2939	PUT3	-0.427	0 700651	0
AWRI796_2292 AWRI796_4867	GIP3	-0.379	1	0	AWRI796_1803 AWRI796_2511	MRX5	-0.428	0.709031	0
AWRI796_1089	MUS81	-0.38	1	0	AWRI796_3229	TIS11	-0.429	1	0
AWRI796_1390 AWRI796_5095	LSM4 CTF4	-0.38	1	0	AWRI796_3248 AWRI796_0582	SHH4 GDH2	-0.43	0.920426	5 U 0
AWRI796_1360	YER079W	-0.381	1	Ő	AWRI796_1123	SNX41	-0.432	0.838525	0
AWRI796_3246 AWRI796_3769	RNH203 ADD37	-0.381 -0.381	1	0	AWRI796_4078 AWRI796_4563	TOM22 MPC54	-0.432	0.929287	0
AWRI796_1136	DOT1	-0.382	1	Ő	AWRI796_3332	YPT6	-0.434	0.71729	0
AWRI796_3190	GEP5	-0.382	1	0	AWRI796_2033	YHL042W	-0.435	1	0
AWRI796_1850	TOM20	-0.384	1	0	AWRI796_3408	NMD4	-0.435	1	0
AWRI796_3038	PTR2	-0.385	1	0	AWRI796_5267	REP2	-0.438	0.715224	0
AWRI/96_3347 AWRI796_4937	LEE1	-0.385 -0.385	1	0	AWRI796_1504 AWRI796_3036	SMC1 SRL3	-0.439 -0.439	0.665738	5 U 0
AWRI796_0655	VCX1	-0.386	1	0	AWRI796_0329	DTR1	-0.441	1	0
AWRI796_2815 AWRI796_0924	YKL162C MSS116	-0.386 -0.387	1	0	AWRI796_3368 AWRI796_0333	IMH1 VBR184W	-0.441	0.534392	2 0
AWRI796_1821	TFC4	-0.387	1	0	AWRI796_2962	YKR011C	-0.444	0.699282	2 0
AWRI796_1830	PEF1 INP1	-0.387	1	0	AWRI796_5000	DSS4	-0.445	0 525651	. 0
AWRI796_2915	SPC42	-0.387	1	0	AWRI796_1985	BRF1	-0.440	0.428319	0
AWRI796_3666	ABF2	-0.388	1	0	AWRI796_3086	SPA2	-0.448	0.278208	8 0
AWRI796_4292 AWRI796_0331	SMP1	-0.388 -0.389	1	0	AWRI796_2475 AWRI796_2481	ATG36	-0.449	0.877099	, 0 ; 0
AWRI796_0646	SCM3	-0.389	1	0	AWRI796_2617	MPS3	-0.449	0.621808	6 0
AWRI796_1735 AWRI796_2303	RAD6 CSM2	-0.389 -0.389	1	0	AWRI796_3076 AWRI796_0210	YLL032C GIP1	-0.451 -0.452	0.467757	0
AWRI796_2561	EXO70	-0.389	1	Ő	AWRI796_1322	MXR1	-0.452	0.417917	0
AWRI796_3252	UPS2	-0.389	1	0	AWRI796_0790	RSM10	-0.453	0.253784	0
AWRI796_1026	PIB1	-0.39	1	0	AWRI796_4923	MUK1	-0.453	0.323065	5 0
AWRI796_0283	OPY1	-0.391	1	0	AWRI796_4631	RPN8	-0.454	0.378139	0 0
AWRI796_0198 AWRI796_2241	SSP1	-0.392	1	0	AWRI796_1633 AWRI796_3137	RSC58	-0.456	0.486132	2 0
AWRI796_2274	NIT1	-0.392	1	0	AWRI796_4240	MNT4	-0.456	0.266345	5 O
AWRI796_2786 AWRI796_3263	VTA1	-0.392 -0.392	1	0	AWRI796_3000 AWRI796_1910	GTO1	-0.457	0.286351	. U 5 O
AWRI796_4505	TFC7	-0.392	1	0	AWRI796_0337	AWRI796_0337	-0.46	1	0
AWRI/96_1857 AWRI796_2156	NNF2 YNG2	-0.393 -0.394	1	0	AWRI796_3814 AWRI796_3926	FUS2 SKP2	-0.46 -0.46	0.934274	- 0 - 0
AWRI796_3599	YML003W	-0.395	1	Ő	AWRI796_3637	MSN2	-0.461	0.180551	0
AWRI796_3708 AWRI796_3877	SPC24 AB72	-0.395	1	0	AWRI796_0850 AWRI796_0847	ALT2 TRS85	-0.462	0.27149	0 0
AWRI796_3164	PER33	-0.398	1	Ő	AWRI796_5194	COS7	-0.463	0.183422	0
AWRI796_4850	PRM4 VDR262W	-0.398	1	0	AWRI796_2545	GSM1 PTG1	-0.465	0.114261	0
AWRI796_1795	YGR016W	-0.399	1	0	AWRI796_0495	PET18	-0.47	0.189786	5 0
AWRI796_2834	CMC1 TMNI2	-0.399	1	0	AWRI796_0587	UGA4	-0.471	0.35127	0
AWRI796_1230	CIN8	-0.401	1	0	AWRI796_0073 AWRI796_0713	RAD59	-0.473	0.466597	0
AWRI796_3340	YCS4	-0.401	1	0	AWRI796_1086	NKP1	-0.473	0.129153	5 O
AWRI796_0105 AWRI796_0707	CBS1	-0.402	1	0	AWRI796_0214 AWRI796_3739	YIM1	-0.474 -0.476	0.050655	5 0
AWRI796_4250	BSC5	-0.402	1	0	AWRI796_2037	MUP3	-0.477	0.105831	0
AWRI796_4329 AWRI796_5225	HAL9 RSC30	-0.402 -0.402	1	0	AWRI796_4032 AWRI796_2909	KAR1 CSE4	-0.477 -0.48	0.340527	2 0
AWRI796_2891	YKL070W	-0.404	1	Ő	AWRI796_4311	ZEO1	-0.48	0.14794	0
AWRI796_3559	AIM32 SNO1	-0.404	1	0	AWRI796_2672	HIT1 VKR078W	-0.483	0.28633	5 0 5 0
AWRI796_4175	SPO1	-0.404	1	0	AWRI796_0436	VAC17	-0.486	0.131206	5 0
AWRI796_2358	MAM33	-0.405	1	0	AWRI796_2980	GMH1 VDI 220W	-0.486	0.632939	
AWRI796_3596	GIS4	-0.405	1	0	AWRI796_4790	GRE1	-0.489	0.319727	0
AWRI796_3682	YMR090W	-0.405	1	0	AWRI796_1220	YEL073C	-0.492	0.979922	0
AWRI796_0787	EHD3	-0.405	1	0	AWRI796_2075	GPA1	-0.492 -0.493	0.189159	) 0
AWRI796_1731	PYC1	-0.406	1	0	AWRI796_3698	SPG4	-0.493	0.044515	-1
AWK1/96_0230 AWRI796 1647	AWKI/96_0230 YGL159W	-0.407 -0.407	1	0 0	AWRI/96_1829 AWRI796 1124	LSI/ RPN9	-0.494 -0.495	0.125244 0.094225	+ 0 ; 0
AWRI796_2393	IRR1	-0.407	1	Õ	AWRI796_0770	RAD61	-0.496	0.087688	s 0
AWRI796_0178 AWRI796_2439	HHT1 DAL4	-0.408 _0.408	1	0 0	AWRI796_1221 AWRI796_2123	KMD6 RSC30	-0.497	0.045503	i -1
AWRI796_4027	YNL193W	-0.408	1	Ő	AWRI796_5069	COG4	-0.497	0.039632	-1
AWRI796_2543 AWRI796_2625	SET4 CTK2	-0.409	1	0	AWRI796_4345 AWRI796_4810	SDH5 AFT2	-0.499	0.027096	5 -1
AWRI796_3764	SPT21	-0.409	1	0	AWRI796_5184	YNL284C-A	-0.499	0.664532	1
AWRI796_0174	UGA2 OST6	-0.41	1	0	AWRI796_2582	BNA3	-0.504	0.084086	i 0
AWRI796_33881	JNM1	-0.41 -0.41	1	0	AWRI796_2005 AWRI796_1567	MNT2	-0.507	0.01/166	, -1 ; -1
AWRI796_4555	SWT1	-0.41	1	0	AWRI796_2802	STE3	-0.512	0.349035	5 0

AWRI796 Gene ID	Gene Name	log <sub>2</sub> Fold Change	Adj. p -value	Score
AWRI796_5163	AWRI796_5163	-0.512	0.151927	0
AWRI796_2914	PHD1	-0.514	0.159962	0
AWRI796_2660	POL32	-0.516	0.186218	0
AWRI/96_0910 AWRI796_3155	VI R053C	-0.518	0.133039	-1
AWRI796 3135	YLR030W	-0.519	0.052916	-1
AWRI796 4509	YOR114W	-0.52	0.062835	ő
AWRI796_2489	RFA3	-0.524	0.018505	-1
AWRI796_2053	SPO11	-0.525	0.523722	0
AWRI796_0212	FMP23	-0.527	0.020327	-1
AWRI796_0771	HED1	-0.527	0.523678	0
AWRI796_2295	AXL2	-0.527	0.016379	-1
AWRI796_3207	AHP1	-0.528	0.551449	0
AWRI/96_3245	ACS2 CIN1	-0.53	0.039159	-1
AWRI790_4700	ACM1	-0.53	0.01968	-1
AWRI796 2357	PCI8	-0.534	0 137441	0
AWRI796 1209	KRE28	-0.535	0.175397	ő
AWRI796 0027	YAL037W	-0.536	0.015263	-1
AWRI796_3489	MSC1	-0.537	0.007361	-1
AWRI796_0998	PHM6	-0.538	0.006341	-1
AWRI796_1356	PTP3	-0.539	0.050633	0
AWRI796_0435	CHA1	-0.54	0.007852	-1
AWRI796_0949	HTB1	-0.541	0.036087	-1
AWRI/96_0185	GAL/	-0.543	0.045655	-1
AWRI/96_4/05 AWRI796_4/33	XOR022C	-0.545	0.045655	-1
AWRI796_0530	RAD18	-0.544	0.010209	-1
AWRI796 4460	ASE1	-0.552	0.001968	-1
AWRI796 1189	PSP1	-0.555	0.001184	-1
AWRI796 5254	AAD3	-0.555	0.007255	-1
AWRI796_1216	IRC4	-0.56	0.18346	0
AWRI796_0065	SEN34	-0.561	0.008701	-1
AWRI796_0252	POL30	-0.562	0.020552	-1
AWRI796_1819	RME1	-0.563	0.000825	-1
AWRI796_3882	YMR295C	-0.566	0.00406	-1
AWRI/96_26/0	BFAI	-0.569	0.048402	-1
AWRI/96_4296	MDH2 PMD5	-0.57	0.016343	-1 1
AWRI790_0977	SPO20	-0.373	0.000823	-1
AWRI796_0218	VRO2	-0.577	0.0013	-1
AWRI796 3455	ATG17	-0.593	0.001116	-1
AWRI796 2217	REC104	-0.595	0.0706	0
AWRI796_1001	ZIP1	-0.602	0.005723	-1
AWRI796_3746	ATG16	-0.602	0.005336	-1
AWRI796_4066	YNL146W	-0.606	0.022802	-1
AWRI796_3693	SRT1	-0.612	0.057732	0
AWRI796_1970	AMA1	-0.613	0.016832	-1
AWRI/96_3109	THI/3	-0.614	0.000125	-1
AWRI/96_2214	CNN1	-0.615	0.00095	-1
AWRI790_1333 AWRI706_5178	VOR343W-B	-0.617	0.000731	-1
AWRI796_1533	PFS4	-0.017	0.065204	-1
AWRI796_3613	YDR210W-B	-0.629	0.050633	ő
AWRI796 0155	FUS3	-0.643	0.015286	-1
AWRI796_2899	BLI1	-0.644	0.000351	-1
AWRI796_0593	RTN2	-0.663	0.000181	-1
AWRI796_3041	PCK1	-0.667	0.001633	-1
AWRI796_3375	EST2	-0.668	8.30E-05	-1
AWRI796_3341	PIG1	-0.677	0.00095	-1
AWRI/96_5180	YOR192C-A	-0.679	0.042947	-1
AWRI/96_0551	MSH3 DEV 27	-0.085	0.000351	-1
AWRI796 1498	HSP12	-0.090	0.000283	-1
AWRI796 2646	LSM8	-0.721	0.000637	-1
AWRI796_3688	SNZ1	-0.721	7.00E-05	-1
AWRI796_1279	GIM4	-0.723	4.30E-05	-1
AWRI796_5177	YOR343W-A	-0.723	0.009878	-1
AWRI796_1884	YGR122W	-0.726	3.10E-05	-1
AWRI796_1277	VAB2	-0.731	0.002214	-1
AWRI/96_4667	CPAI	-0.731	0.000152	-1
AWRI/96_4039	KHO5	-0.738	0.001026	-1
AWRI/90_134/	COS2	-0.74	4.90E-03	-1
AWRI796_4201 AWRI796_3206	YLR108C	-0.75	7.00E-06	-1
AWRI796 0666	YDL114W	-0.769	0.005858	-1
AWRI796 4721	YOR365C	-0.773	0.005408	-1
AWRI796_0235	YBR071W	-0.8	6.40E-05	-1
AWRI796_3670	PDS5	-0.82	1.30E-05	-1
AWRI796_2705	SFC1	-0.823	1.20E-05	-1
AWRI796_3058	YCT1	-0.831	5.60E-05	-1
AWRI796_2032	ECM34	-0.833	0.008183	-1
AWKI/96_4868	ISUI SEO1	-0.897	6.40E-05	-1
AWR1/90_0002	AFB1	-0.907	0.001633	-1
AWRI796 3304	CISI	-0.911	2 000 05	-1
AWRI796 5038	ROX1	-0.994	0.000366	-1
AWRI796 4438	CIN5	-0.996	0	-1
AWRI796_1508	MSH4	-1.018	6.40E-05	-1
AWRI796_2704	IME1	-1.028	4.00E-06	-1
AWRI796_0947	YDR222W	-1.064	0	-1
AWRI796_0129	ECM13	-1.191	1.00E-06	-1
AWRI796_1837	YGR066C	-1.217	0	-1
AWKI/96_1077	PHO92 NCE102	-1.495	3.60E-05	-1
AWKI/70_4138	INCE103	-1./22	0	-1

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