GENE EXPRESSION PROFILE OF ETHANOL-STRESSED YEAST IN THE PRESENCE OF ACETALDEHYDE

A thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

BY

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DECLARATION

"I, Idris Mohammed, declare that the PhD thesis entitled, Gene Expression Profile of Ethanol-Stressed Yeast in the Presence of Acetaldehyde, is no more than 100,000 words in length, exclusive of tables, figures, appendices, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work"

Signature: Date: March 2007

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ABSTRACT

One of the major yeast stressors during fermentation is ethanol accumulation. Ethanol stress is associated with reduced cell growth and viability, consequently lowering yeast productivity. Although the underlying causes of ethanol inhibition of cells are yet to be identified, it has been discovered that yeast acclimatise more quickly to ethanol stress in the presence of low acetaldehyde concentrations; however, the biochemical processes underpinning this effect are unknown. The objective of this project was to identify the mechanisms associated with the acetaldehyde-mediated adaptation of yeast to ethanol stress, which may facilitate the development of yeast strains with improved ethanol tolerance and/or strategies for improving ethanol tolerance in yeast.

Gene array analysis was used to study gene expression in *Saccharomyces cerevisiae* during acclimatisation to non-lethal ethanol stress, in the presence and absence of acetaldehyde. Acetaldehyde caused significant changes in gene expression in ethanol-stressed yeast. For example, many genes associated with protein biosynthesis were more highly expressed, as were pyruvate decarboxylase genes. Interestingly, however, there was no significant increase in the expression of trehalose synthesis genes or genes encoding HSPs; genes which, in previous studies, appeared to be associated with acclimatisation to ethanol-stress. In addition, acetaldehyde did not have a major impact on gene expression in non-stressed cultures.

The results of this project are consistent with the speculation that the addition of acetaldehyde to ethanol-stressed *S. cerevisiae* primes glycolytic flux in ethanol-stressed cells by regenerating NAD^+ from accumulated NADH. This, in turn, stimulates glyceraldehyde-3-phosphate dehydrogenase activity and might account for the acetaldehyde-mediated increased expression levels of pyruvate decarboxylase genes; elevated levels of pyruvate would potentially increase the need for *PDC* activity. Overall, these speculated effects of acetaldehyde on ethanol-stressed yeast would increase glycolytic rate and energy production.

PUBLICATIONS AND PRESENTATIONS

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LIST OF ABBREVIATIONS

Organizations:

AWRI	Australian Wine Research Institute
CUB	Carlton & united breweries
VU	Victoria University
WEHI	Walter and Eliza Hall Institution

Chemicals and Units:

ADH	Alcohol dehydrogenase
ALDH	Aldehyde dehydrogenase
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
BLAST	Basic local alignment search tool
bp	Base pair
BSA	Bovine serum albumin
°C	Degree Celsius
cDNA	Complementary DNA
CoA	Coenzyme A
CO_2	Carbon dioxide
Ci/mmol	Curies per millimole
Cm	Centimeter
CP	Crossing point
Cy3	Cyanine dye 3
Cy5	Cyanine dye 5
ΔPDC1/5	BY4742*ΔPDC1/5*::*kanMX4*
ΔHXT4	BY4742*ΔHXT4*::*kanMX4*
ΔPHO84	BY4742*ΔPHO84*::*kanMX4*
ΔYLR364W	BY4742*ΔYLR364W::kanMX4*
DEPC	Diethyl pyrocarbonate
dATP	Deoxyadenosine 5'-triphosphate
dCTP	Deoxycytidine 5'-triphosphate
dGTP	Deoxyguanosine 5'-triphosphate
DIG	Digoxigenin
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTPs	Deoxynucleotide triphosphates (dATP, dTTP, dGTP, dCTP)
DTT	Dithiothreitol
dTTP	Deoxythymidine 5'-triphosphate
dUTP	Deoxyuridine 5'-triphosphate
EDTA	Ethylenediaminetetraacetic acid
FA	Fold alteration
g/l	Gram per litre
h	Hour
HCL	Hydrochloric Acid
HSE	Heat shock element

HSF	Heat shock factor
Hsp	Heat shock protein
KCl	Potassium choride
LHE	Less highly expressed
L	Litre
Mg	Milligram
MHE	More highly expressed
MIPS	Munich Information Centre for Protein Sequences
ml	Milliliter
mRNA	Messenger RNA
M	Molar
mM	Millimolar
MW	Molecular weight
$MgCl_2$	Magnesium chloride
µg	Microgram
µl	Microlitre
µm	Micrometer
µM	Micromolar
NaOH	Sodium hydroxide
nM	Nanomolar
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
$NaHCO_3$	Sodium bicarbonate
OD	Optical density
ORFs	Open reading frames
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
RNA	Ribonucleic acid
RNase	Ribonuclease
rRNA	Ribosomal RNA
rpm	Revolution per minute
RSAT	Regulatory sequence analysis tools
PCR	Polymerase chain reaction
RT-PCR	Peverse transcription polymerase chain reaction
SDS	Sodium dodecyl sulphate
SGD	*Saccharomyces* genome database
SSC	Sodium chloride-sodium citrate
SS-DNA	Salmon sperm DNA
STRE	stress response element
TRIS	Tris-(hydroxymethy)-aminomethane
UV	Ultra violet
V	Volt
v/v	Volume per volume
w/v	Weight per volume
YEASTRACT	Yeast Search for Transcriptional Regulators and Consensus Tracking
YEPD	Yeast extract, peptone and D-glucose
YMGV	Yeast microarray global viewer

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CHAPTER 1

General introduction

1.1 Introduction

Saccharomyces cerevisiae is an important organism in both fundamental and applied research. Along with *Escherichia coli* and *Drosophila melanogaster*, *S. cerevisiae* has been one of the central model organisms for studies in genetics, biochemistry, cell biology, and more recently molecular biology and systems biology. In fact, we probably know more about the biology of the yeast cell than any other eukaryote. This makes *S. cerevisiae* an ideal model organism for studies on environmental-stress responses and stress tolerance. Such studies are of huge economic importance because of the numerous industrial applications of yeast, including brewing, winemaking, baking and, in more recent years, bioethanol production. Global warming, conflicts in the Middle East and the finite supply of oil are contributing to increased interest in generating ethanol as a fuel. In all of these industries the costs associated with failed or suboptimal fermentations are significant, and one of the major causes of such problems is the environmental stresses that yeast cells encounter during fermentation (see for example Bisson and Block, 2002; Gasch *et al*., 2000).

Of the many environmental stresses encountered by yeast in industrial applications, exposure to accumulating levels of ethanol probably has the greatest negative impact. The effects of this self-inflicted stress include increased growth lag periods following re-pitching (a common brewing industry practice of recycling yeasts from one fermentation to the next), and a decrease in specific growth rate, vitality and yeast viability. These impacts result in lower productivity, suboptimal rates of fermentation, lower ethanol yields and ultimately less profit for the industries concerned (Ingram, 1986).

Although fermentation performance is inhibited by ethanol stress, yeast cells have built-in protective mechanisms to counteract some of the toxic effects of this alcohol, enabling them to acclimatise to this stress. The effectiveness of these mechanisms is however limited and, as ethanol levels increase in a fermentation, the yeast may ultimately succumb to the damaging and disruptive effect of its stressful environment. The principal mechanisms underpinning yeast acclimatisation to ethanol stress remain elusive, but greater knowledge in this field might enable the development of practices and/or yeast strains that will improve the efficiency and reliability of fermentation, thereby reducing costs to industry.

In yeast, acetaldehyde is the direct metabolic precursor of ethanol and acetic acid; and is therefore a key metabolite between the pathways of anaerobic and aerobic glucose metabolism. Research into the causes of ethanol toxicity in yeast has identified a role for acetaldehyde in stimulating the rate of adaptation of yeast to ethanol stress (Vriesekoop & Pamment 2005; Barber *et al*., 2002; Stanley *et al*., 1997; Stanley *et al*., 1993; Walker-Caprioglio and parks, 1987). These reports demonstrated that small amounts of added acetaldehyde markedly reduced the lag phase, and increased the specific growth rate, of yeast inoculated into ethanol-containing medium, however, the mechanism underpinning the stimulatory effect of added acetaldehyde on ethanol-stressed cultures is yet to be elucidated. Despite the amount of information that has been generated on the physiological and chemical changes in ethanol-stressed yeast during acetaldehyde stimulation, there is no published information on the yeast response to ethanol stress in the presence of acetaldehyde at the level of gene expression. The work in this thesis investigates the stimulatory effect of acetaldehyde on ethanol-stressed yeast at the molecular level.

CHAPTER 2

Literature review

2.1 Introduction

Fermentation uses living organisms in the production of foods and beverages such as bread, yoghurt, cheese, beer, wine and spirits. The exploitation of fermentation dates back several thousand years to when people inadvertently discovered the usefulness of this process. However millennia passed before yeast and bacteria were identified as the causative agents. The yeast *S. cerevisiae* is of particular importance in fermentation industries for the role that it plays in fermenting sugars into ethanol. There is no microorganism more closely associated with human life, from ancient to contemporary times, than this single-celled fungus. The Egyptians were the earliest recorded users of yeasts; they brewed beer and made wine. By medieval times, brewing technology had spread from the Middle East to Europe (Protz, 1998) and since then has evolved incrementally, but the basic process remains the same.

The role of microorganisms in fermentation was first identified in 1876 when Louis Pasteur proved that fermentation was due to living cells (Hardwick, 1995). The first pure yeast culture used in alcoholic beverage production was obtained by Emil Christian Hansen from the Carlsberg Brewery in 1883. A pure culture of wine yeast was subsequently obtained by Muller-Thurgau from Geisenheim (Germany) in 1890 (from Dequin, 2001). Prior to this, fermentations were considered spontaneous; yeasts were unknowingly transferred from brew to brew in fermentation vessels or from remnants of previous brews saved for starting the next brew. Brewing yeasts have in fact adapted over thousands of years from serial re-pitching (re-cycling) and this selection has led to the evolution of adaptive mechanisms that increase yeast tolerance to many of the physiological stresses encountered during brewing and industrial fermentations.

Of course ethanolic fermentation has great potential in other industries. For example, in a world of energy shortages, depleting and increasingly expensive non-renewable fossil fuels and global warning, bioethanol production is becoming an increasingly attractive energy option. As a result, in countries with large agricultural industries and limited access to fossil fuels, such as Brazil and South Africa, research is being conducted on improving the efficiency of production of ethanol from substrates such as lignocellulose. The objectives of such research are to improve substrate range, ethanol yield and productivity for use as fuel alcohol.

2.1.1 The problem of yeast stress during fermentation and associated processes

During fermentation sugars are converted into ethanol and carbon dioxide, but accumulating levels of ethanol become stressful for yeast cells. The exposure of yeast to this stress is thought to be responsible for a decline in yeast viability and vitality and this has a substantial impact on fermentation productivity by increasing growth/fermentation lag periods, reducing productivity, increasing fermentation turnover periods and, in a brewery context, limiting the life span of re-pitched (recycled) yeast. Of particular concern to the brewing and wine industries are stuck or suboptimal fermentations, where yeast growth and ethanol production can come to a virtual standstill, increasing processing times and in some cases leading to a complete failure of the fermentation process (Linko *et al*., 1998).

Improving stress tolerance in yeast may enable the cells to better withstand the stresses associated with fermentation, possibly leading to an increased rate of production and extend the life of re-pitched brewing yeast. This study was conducted to investigate the physiological and molecular responses of yeast to ethanol stress conditions.

2.2 The general stress response of *Saccharomyces cerevisiae*

When yeast cells are grown in suboptimal conditions, they exhibit a complex stress response. This stress response is a reprogramming of cellular activities to ensure survival, protect essential cell components, and to drive a resumption of cellular activities during recovery (Birch and Walker, 2000; Gasch *et al*., 2001; for reviews see

Mager and Hohmann, 1997; Attfield *et al*., 1997; Atttfield, 1997b; Mager and Moradas-Ferriera, 1993).

Yeast strains used for brewing, baking and winemaking are intrinsically tolerant to a range of extreme conditions including a level of ethanol that is toxic to most, if not all, competing microorganisms. This tolerance is presumably acquired by rapid molecular responses that protect against damage caused by ongoing exposure to the same or other forms of stress. These responses include changes in gene transcription, translational and post-translational modifications of stress-associated protein, and are triggered, at least in part, by stress-induced denaturation of proteins, disordering of membranes, DNA damage and metabolic disturbances (Mager and Moradas-Ferreira, 1993; Piper, 1993; Siderius and Mager, 1997).

Tolerance to stresses is acquired by means of protective biochemical processes which include the synthesis of osmolytes (e.g. glycerol), trehalose, heat shock proteins (HSPs), increased chaperone activity, enhanced radical oxygen scavenging, changes in redox control, increased proton pumping activity, adjustments in carbon/nitrogen balance and altered ion and water uptake (Piper, 1993; De Virgillio *et al.,* 1994; Parrou *et al*., 1997; Guldfeldt and Arneborg, 1998; Estruch, 2000; Yale *et al*., 2001). For example, yeast cells exposed to a salt shock of 300 mM NaCl for 45 minutes accumulate glycerol (Lewis *et al*., 1995) and heat shocked cells accumulate trehalose (Hottiger *et al*., 1987), suggesting that the accumulation of trehalose and glycerol have important roles in stress tolerance.

These stress response mechanisms not only initiate the repair of macromolecular damage caused by stress but presumably also establish a tolerant state, which helps prevent further damage. Central to these responses are the sensing and signaling pathways that communicate with the nucleus and facilitate necessary changes in gene expression. Stress responsive genes that are part of the general stress response machinery of yeast are presumed to encode proteins with functions that are necessary to cope with damage under various stress conditions. The expression of genes is controlled by specific regulatory factors up-stream of each gene. In *S. cerevisiae* there are two major independent stress responses: the general stress response (GSR) and heat shock response (HSR). The general stress response is induced by a wide variety of

stressing agents including heat, osmotic stress, oxidative stress, nitrogen starvation, ethanol, sorbate and low pH (Chatterjee *et al.,* 2000; Ruis and Schuller, 1995). Each GSR gene contains a stress-response promoter element that binds to transcription factors Msn2p and Msn4p. In contrast the heat shock response, induced when cells are exposed to any of a range of stresses including sublethal heat shock, requires the activation of a specific heat-shock transcription factor (Hsf1p) that binds to a specific conserved promoter sequence, the heat shock element (HSE) (Grably *et al*., 2002, Chatterjee *et al*., 2000).

2.2.1 Pre-treatment of yeast with Mild Stress

Pre exposure of yeast to a non-lethal stress stimulates an adaptive response resulting in transient resistance to higher levels of the same stress. The acquisition of tolerance to otherwise lethal levels of stress has been linked to stress protein synthesis during pre-exposure to the mild stress. For instance, yeast cells grown at 23°C develop enhanced tolerance to a lethal temperature of 51°C following prior incubation at 37°C for 20 minutes (Plesset *et al.*, 1982). This induction of thermo-tolerance has been observed in cells incubated at a series of sub-lethal temperatures, ranging between 37°C and 45°C (Coote *et al.*, 1991). Within this range of temperatures, a higher pre-stress heat shock produced a greater thermo-tolerance response (Coote *et al.*, 1991).

Davies *et al.* (1995), showed that the growth of yeast cells was arrested when exposed to 0.8 mM H_2O_2 (oxidative stress). But when pre-treated by exposure to 0.4 mM H_2O_2 for 45 min, they were better able to tolerate the subsequent 0.8 mM H_2O_2 stress and were able to grow and divide at a normal rate, i.e. the pre-treated yeast grew and divided at a 15-30% faster rate than the non-pretreated cells (Davies *et al.*, 1995). This pre-exposure effect is also true for stresses other than temperature and oxidative stress. A short pre-treatment of yeast with 0.7 M NaCl leads to an increase in the number of surviving cells when they are subsequently exposed to 1.4 M NaCl (Trollmo *et al.*, 1988; Varela *et al.*, 1992).

Thus, mild stress conditions may trigger cellular responses that prepare cells to cope with severe stress. Such investigations suggest that yeast and other microorganisms

have an inherent ability to improve their stress tolerance provided that the appropriate external and internal triggers are activated. A better understanding of these built-in molecular processes that underpin, and are a part of, the yeast stress response will greatly facilitate the development of strategies to improve yeast stress tolerance.

2.2.2 Mild stress, cross-stress protection and stress-specific responses

Yeast cells exposed to mild stress can develop tolerance not only to higher levels of the same stress, but also to stress caused by other agents. This phenomenon is called cross-protection and is caused by the expression of general stress-responsive genes under mild stress conditions (Chen *et al*., 2003). For example, a brief temperature shock not only increases yeast thermo-tolerance, but may also increase tolerance to other stressors such as ethanol (Watson and Caricchioli, 1983; Costa *et al.*, 1993), a high salt concentration and oxidative stress (Lewis *el al*., 1995). Steels *et al*., (1994) investigated the relationship between yeast tolerance to heat and oxidative stress, and found that a mild heat shock induced tolerance to an otherwise lethal temperature and H_2O_2 stress. Similarly, pre-treatment of yeast cells with a mild osmotic shock conferred increased resistance to heat shock (Trollmo *et al.,* 1988; Varela *et al.*, 1992) and the exposure of yeast to ethanol, sorbic acid and low external pH induced greater thermotolerance (Plesset *et al.,* 1982; Coote *et al.*, 1991). This phenomenon of cross-protection is consistent with commonality in the yeast cellular responses and protection to different forms of stress.

Although cross protection suggests commonality in stress responses there is a level of exclusivity. For example, a mild heat shock does not result in increased osmo-tolerance (Trollmo *et al.*, 1988; Varela *et al.*, 1992). Similarly, Steels *et al*. (1994) showed that pre-exposure of yeast to low temperature conferred resistance to both low temperature and oxidative stresses, but pre-treatment of cells with low concentration of H_2O_2 did not evoke resistance to heat stress. Thus, while a part of the stress response of yeast cells may be shared and lead to cross protection, there are also stress-specific responses. Studies of *S. cerevisiae* suggest that specific adaptive responses rely primarily on the increased synthesis of specialized stress proteins and/or organic solutes such as glycerol (Piper, 1993).

2.2.3 Heat shock proteins in stress tolerance

The heat shock response is the most extensively studied stress response in yeast and other organisms, and Heat Shock Protein (HSP) genes are among the best-characterized stress response genes (Mager and Moradas-Ferreira, 1993). Most HSPs are highly conserved across bacteria, fungi, plants and animals (Lindquist and Craig, 1988; Craig *et al*., 1993; Hartl, 1996) suggesting that they are of fundamental importance to cells. There is a number of documented studies that demonstrate the protective effect of HSPs to stress. For example, Plesset *et al*. (1982) investigated the effect of heat shock and ethanol stress on the survival of *S. cerevisiae*. Exponential parent cultures were shifted from 23°C to 37°C, 45°C, 49°C and 51°C, for 10 minutes, then viable counts were determined for each and for a control that was maintained at 23°C. Viability dropped rapidly above 37°C with 51°C being lethal. However, pre-treatment at 37°C for 20 minutes protected cells when subsequently shifted to 51°C, with greater than 70% viability. Cells were also pre-treated with mild ethanol stress (1.55 M) for 20 minutes then resuspended in fresh medium and incubated for 10 minutes at 49°C. This ethanol pre-treatment gave higher cell viability compared to non-pre-treated cells, but lower than that of a 37°C temperature pre-treatment (Plesset *et al*., 1982). A possible explanation for this was that pre-treatment with mild temperature or mild ethanol caused induction of HSPs and this resulted in the acquisition of thermotolerance. To investigate this possibility Plesset *et al*. (1982), used ^{35}S pulse-labeling and two-dimensional polyacrylamide gel electrophoresis to follow the induction of 'heat shock' protein synthesis after pre-incubations at 23°C (control), 37°C and in 1.55 M ethanol. Results showed the induction of the same 'heat shock' proteins [NB. the authors do not define which HSPs they followed in this work] for the 37°C and 1.55 M ethanol treatments. This result demonstrated the correlation between induction of putative HSPs and acquisition of thermotolerance.

Since the time of the above work of Plesset *et al.* (1982) a great deal of research has been conducted on stress response proteins. Of particular relevance in the context of stress tolerance are the Hsp70 and Hsp104 families; both have been shown to be involved in recovery from stress-induced damage (see, for example Parsell and

Lindquist, 1993 and Piper 1997). The Hsp70 family has been shown to be involved in the prevention of protein aggregation and the refolding of damaged proteins following heat shock (Piper, 1997), and Hsp104 cooperates with Hsp70 family members in refolding and reactivating previously denatured proteins (see http://db.yeastgenome.org/cgi-bin/locus.pl?locus=HSP104).

When wild-type and *hsp104Δ* mutant yeast cells were grown at 25°C and given a mild heat shock (30 minutes at 37°C) before exposure to 50°C, thermotolerance was induced in both strains. However, this tolerance was very transient in the Δ*hsp104* mutant since cells began to die at 100-1000 times the rate of the wild type (Parsell and Lindquist, 1993). In addition, cells with constitutive *HSP104* expression were found to have elevated thermotolerance in the absence of a pre-stress (Sanchez *et al.*, 1992). The acquirement of tolerance against high ethanol concentrations is similarly dependant on a functional *HSP104* gene (Sanchez *et al.*, 1992). Hsp104 is suggested to rescue heat-inactivated proteins directly from insoluble aggregates, which is not a function of other chaperones (Parsell *et al.*, 1994). There is also evidence that Hsp70 and Hsp104 may have complementary roles: *SSA* gene products (SSA stands for stress-seventy, sub family A; this is a sub family of the HSP70s) assume an important role in tolerance to extreme temperatures in the absence of Hsp104 while, in cells with low levels of Hsp70, Hsp104 assumes an important role in growth at normal temperatures (Sanchez *et al.*, 1993).

Recently, global gene array analysis of yeasts growing in stressful conditions has revealed that many heat shock genes are induced. During wine fermentation for example there was increased expression of *HSP*s *12, 26, 42, 78,* and *104* and the HSP70 family member *SSE2* (Rossignol *et al*., 2003). However, since there are so many environmental variables during a wine fermentation and conditions change enormously over time, it is difficult to determine what the yeast cells were responding to; it may have been osmotic stress, nutrient limitation, acidity, and/or accumulating alcohol. Yale and Bohnert, (2001) investigated global gene expression in *S. cerevisiae* exposed to 1.0 M NaCl osmotic stress. This work showed increased expression of *HSP*s *12, 26, 70, 78* and *104* at the 90 minute time point. Similarly, Alexandre *et al*. (2001) and Chandler *et al.* (2004) studied global expression in *S. cerevisiae* during ethanol stress. Their results

showed increased expression levels of *HSP*s *12*, *26*, *30*, *42*, *70, 78*, *82* and *104*. These *HSP* genes were also found to be up-regulated in *S. cerevisiae* following one hour exposure to toxic concentrations (1 g/l) of acetaldehyde (Aranda and Olmo, 2004). Thus, there is increased expression of *HSP* genes in response to various types of stresses; the roles of most of these genes (if any) in conferring stress-tolerance however remain to be determined.

2.2.4 Trehalose in stress tolerance

Trehalose is a non-reducing disaccharide that acts principally as a reserve or storage carbohydrate (Wiemken, 1990; Lillie and Pringle, 1980; Thevelein, 1984; Thevelein and Hohmann 1995). However, it has also been suggested that trehalose functions as a cellular protectant that is involved in stress tolerance (Rep *et al*., 2000; Parrou *et al*., 1999; Parrou *et al*., 1997; Kim *et al.,* 1996; Wiemken 1990; Van Laere, 1989). Large amounts of trehalose accumulate in *S. cerevisiae* cells during periods of adverse growth conditions such as high temperature (Eleutherio *et al.,* 1995; Hottiger *et al.*, 1987; Lewis *et al.*, 1995), freezing (Kim *et al.*, 1996), dehydration and desiccation (Eleutherio *et al*., 1993; Gadd *et al.*, 1987; D'Amore *et al.*, 1991), starvation (Lillie and Pringle, 1980), hyperosmotic shock (Hounsa *et al.*, 1998) and ethanol stress (Hounsa *et al.,* 1998, Soto *et al.,* 1999, Attfield, 1987; Kim *et al.*, 1996). Trehalose also accumulates when cells are exposed to copper sulphate or hydrogen peroxide and declines rapidly after the stress is removed (Attfield, 1987). High trehalose content in re-pitched yeast in brewing fermentations is known to improve cell viability and increase carbohydrate utilization during the initial stages of fermentation (Guldfelt and Arneborg, 1998). Similarly, a decline in trehalose content has been correlated with a loss of stress resistance (Van Dijck *et al.,* 1995). Soto *et al*. (1999) investigated the synthesis of trehalose in *S. pombe* and showed that mutant strains unable to synthesis trehalose were sensitive to temperature, freeze/thawing, dehydration, sodium chloride, and ethanol stresses. These authors speculated that trehalose is a key determinant in general stress tolerance.

The precise role of trehalose in stress tolerance, however, is unknown although it has been suggested that it acts to stabilize proteins in their native state and preserve the

integrity of cellular membranes during stress (Colaco *et al.*, 1994; Omdumeru *et al.*, 1993; Crowe *et al.*, 1984). Hottiger *et al.,* (1994) investigated thermal stability of purified glucose-6-phosphate dehydrogenase (Glc6PDH) *in vitro*. Trehalose was added at a concentration of 0.5 M to a range of solutions containing glucose-6-P dehydrogenase. These were heat shocked at a range of temperatures from 40°C to 60°C for eight minutes. After cooling, enzyme activity was measured and compared to activities for controls that were treated in the same way but without added trehalose. After a 55°C heat shock the activity of trehalose-treated enzyme preparations was 60% greater than the controls (Hottiger *et al.,* 1994). This suggests that trehalose increases the thermal stability of proteins *in vitro*; this finding is in line with that of De Virgilio *et al.* (1990)

However subsequent to the above, other researchers presented evidence that suggested trehalose may not have a protective role in stress tolerance. The proteins responsible for trehalose production are encoded by *TPS1* (trehalose-6-phosphate synthase), *TPS2* (trehalose-6-phosphate phosphatase) and *TSL1/TPS3* (these are regulatory subunits of the trehalose synthase complex). Mutations of the *TPS1* gene render yeast cells unable to produce trehalose, and the phenotype has increased sensitivity to heat stress (De Virgilio *et al.*, 1994). However, phenotypes of mutants lacking the *NTH1* gene, which is responsible for trehalose degradation, accumulate high levels of trehalose yet their ability to survive extreme heat is also reduced (Nwaka *et al.*, 1995a and Nwaka *et al.*, 1995b). This and other work led to doubts about the role of trehalose in protecting cells from heat stress (see review by Nwaka and Holzer, 1998). But it should be pointed out that the *Nth1*Δ mutant would have accumulated excessive trehalose and, as argued by Singer and Lindquist (1998), this would probably have interfered with other cellular functions including the activities of chaperones that are important for stress tolerance. The authors suggest that rapid early accumulation of trehalose, when cells are heat stressed, is needed only for a short time to stabilize proteins in their native state followed by rapid degradation of trehalose, necessary for full recovery from heat stress. The fact that trehalose acts as protein and plasma membrane stablizer and protectant for yeast cells under stressful conditions is well documented (Parro *et al*., 1999; Guldfelt and Arneborg, 1998; Parrou *et al*., 1997; Majara *et al*., 1996; Panek and Panek, 1990; Hottiger *et al.,* 1994; Odumeru *et al*., 1993; De Virgilio *et al.*, 1990).

2.3 Ethanol toxicity in S. cerevisiae

2.3.1 Overview

Sugars are the major carbon and energy source for yeast in natural habitats as well as in industrial fermentations, and ethanol is the major product of sugar catabolism in yeasts. Thus ethanol accumulates in the yeast cell's environment and this negatively impacts on cellular functions. The productivity and yield of fermentations is limited by the sensitivity of yeast to ethanol (Walker 1998). Many of the changes induced in yeast by 'stressful' levels of ethanol are similar to those resulting from sublethal heat stress. They include: increased frequency of petite mutations and the inhibition of metabolism (including fermentation), growth, nutrient intake and plasma membrane ATPase activity (Walker 1998; Casey and Ingledew, 1986; D'Amore *et al*., 1990; Ingram and Buttke, 1994; Jones 1989 and Misha, 1993).

It is believed that the main impact of high ethanol concentrations is the disruption of membrane structure, affecting membrane transport systems and leading to increased membrane fluidity, permeability and passive proton influx (Leao and Van Uden, 1984; Walker 1998; Sajbidor, 1997; Hallsworth, 1998 and Alexandre *et al.,* 2001). There is an associated loss of membrane potential and leakage of electrolytes, amino acids and ribose-containing compounds from the cell (Juroszek *et al*., 1987 and Salgueiro *et al*., 1988). In addition, ethanol inhibits the activity of key glycolytic enzymes and denatures proteins; this is probably due to a reduction in water availability (Hallsworth, 1998; Casey and Ingledew, 1986). There is also a lower rate of RNA (Walker, 1998 and Alexandre *et al*., 1993).

2.3.1.1 The yeast cell plasma membrane and ethanol toxicity

It has been proposed by several authors that the yeast cell membrane is the principal site of action of ethanol leading to its toxic effects (see for example Bisson and Block 2002; Piper, 1995; and Rose, 1993). The presence of ethanol around the phospholipid bilayer weakens the water-lattice structure of the membrane and decreases the strength of interactions between fatty acids, and this is thought to promote cell leakage and decrease the integrity of the membrane (Sajbidor *et al*., 1992). In support of this,

Mansure *et al*. (1994) found a correlation between ethanol stress and increased membrane leakage; interestingly, this leakage was reduced in the presence of trehalose.

Ethanol is thought to partition into the hydrophobic regions of the membrane where it increases the relative polarity of this microenvironment. It thereby increases the membrane's ability to solubilise other polar molecules (Ingram, 1986) and perturbs the functions of transport proteins in the membrane. This results in decreased nutrient uptake by the cells (van Uden, 1985; Pascual *et al*., 1988) and increased influx of protons, causing disruption of the proton-motive force (Ogawa *et al*., 2000; Walker, 1998) and intracellular acidification (Alexandre *et al*., 1998; Walker, 1998).

Ethanol-induced passive proton influx is thought to be the trigger for an increase in activity of the plasma membrane H^+-ATPase (Cartwright *et al*., 1987), the enzyme largely responsible for maintenance of the plasma membrane proton gradient (Serrano, 1991). Thus it might be expected that the cell would be able to quickly recover its proton gradient following ethanol stress, but this appears not to happen. Perhaps this is because Hsp30p synthesis is also induced in conditions of ethanol stress (Chandler *et al*., 2004 and Alexandre *et al*., 2001) and this protein is an inhibitor of the plasma membrane H^+-ATPase (Piper *et al*., 1997, Braley and Piper, 1997). To date, however, there has been no experimental work to test this in ethanol stressed cells.

2.3.1.2 The effect of ethanol on membrane fluidity and membrane transport

Jones and Greenfield (1987) used passive influx of undissociated acetic acid as an indicator of membrane fluidity for yeasts grown in batch and continuous culture, in medium containing between 20 – 50 gL^{-1} ethanol. Increases in ethanol concentration led to increased membrane permeability, and therefore it was inferred that membrane fluidity increased. Interestingly however, the membranes of cells acclimated to ethanol in continuous culture were less permeable than unacclimatised cells exposed to similar levels of ethanol in batch culture. The authors conclude that increased membrane permeability (and therefore fluidity) does not impart tolerance to ethanol. This landmark publication was very important for what it tells us about membrane permeability and acclimatisation to ethanol stress, however the assumption that

permeability to undissociated acetic acid is a measure of membrane fluidity is questionable.

Lloyd *et al*. (1993) measured membrane fluidity directly using electron spin resonance spectroscopy (ESR), for yeast cells exposed to 7-10% v/v ethanol. Membrane fluidity increased in microsomal fractions from yeast cells incubated with 9% (v/v) ethanol compared to no ethanol controls. Swan and Watson (1997) used fluorescence anistropy to measure membrane fluidity of stress-sensitive and stress-resistant strains of *S. cerevisiae*, and found that membrane fluidity increased slightly in all strains following treatment for 60 minutes with 17% ethanol. Whilst the increased fluidity was accompanied by a minor increase in cell survival, fluidity did not correlate with stress tolerance.

Membrane composition has also been shown to influence the uptake of nutrients in the presence of ethanol. Thomas and Rose (1979), examined the influence of membrane lipid components on the uptake of nutrients in the presence of 4.5 and 6% (v/v) ethanol. Cells were grown anaerobically with ergosterol and either monounsaturated oleic or polyunsaturated linoleic acid supplements. These were incubated with radiolabeled nutrients. Addition of ethanol was accompanied by an initial loss from the cells of glucose, glucosamine and lysine, and the growth rate was immediately reduced, particularly in cultures containing oleic rather than linoleic acid. Similarly, the accumulation rate within cells of labeled solutes glucose, glucosamine, lysine and arginine was reduced following ethanol addition, more so in oleic acid-supplemented cultures than polyunsaturated linoleic acid-supplemented cultures. The increased capacity of cells for solute uptake when enriched with polyunsaturated linoleyl residues compared to monounsaturated oleic acid residues was thought to be due to increased membrane fluidity.

2.3.2 The relationship between ethanol and oxidative stress

Dissolved oxygen and mitochondrial activity are important for the biosynthesis of unsaturated fatty acids and ergosterol (Casey *et al*., 1984; O'Connor-Cox *et al*., 1996; and Higgins *et al*., 2003). In brewing, for example, oxygenation of wort at pitching is

important for sterol, unsaturated fatty acid and lipid metabolism, and this impacts on yeast performance and beer flavour (Jahnke and Klein, 1983). However, growth under aerobic conditions exposes cells to oxidative stress due to the production of partially reduced forms of molecular oxygen known as reactive oxygen species (ROS). These highly reactive forms of 'oxygen', including the hydroxyl radical (OH^-), the superoxide anion (O_2^-) and peroxide (H_2O_2), are highly damaging to cellular components causing DNA lesions, lipid peroxidation, oxidation of proteins and perturbations to the cellular redox balance (Sies, 1986). ROS are formed during respiration, β-oxidation of fatty acids, and a range of other reactions. They are also produced by yeast cells exposed to ethanol or chemical stresses (Georgiou and Masip, 2003; Costa *et al.,* 1993; Steels *et al*., 1994 and Jamieson, 1998).

Cellular defenses that can inactivate ROS include the activities of a number of enzymes such as the cytoplasmic superoxide dismutase (Cu, ZnSOD) encoded by the *SOD1* gene, the mitochondrial superoxide dismutase (MnSOD) encoded by the *SOD2* gene, cytochrome *c* peroxidase (CCP) and cytoplasmic catalase T (*CTT1*).

Using respiratory deficient mutants as controls, Costa *et al*., (1997) showed that ethanol toxicity correlates with the production of ROS in the mitochondria and that the mitochondrial superoxide dismutase, MnSOD, is essential for ethanol tolerance in diauxic and post-diauxic-phase cells. Consistent with this, yeast lacking Sod1p (*SOD1*Δ) were found to have lower tolerance not only to oxidative stress but also to heat and ethanol stresses (Pereira *et al.,* 2003).

Many anti-oxidant genes are glucose-repressed, explaining why Gille *et al*., (1993) found that both intracellular and extracellular catalase activities in an aerated *S. cerevisiae* distillery strain were greater with ethanol as substrate than with glucose. The authors suggested that extracellular catalase acts as a protectant against the damaging effects of ethanol by oxidizing ethanol outside the cell.

Cytochrome P-450, an enzyme catalysing the oxidation of endogenous and exogenous substrates in *S. cerevisiae*, accumulates to a high level when yeast grows fermentatively on glucose and is also present when ethanol is added to cultures grown on low levels of glucose. Encoded by *ERG11*, this cytochrome oxidatively detoxifies ethanol.

Furthermore, studies by Gupta *et al*., (1994), indicated that ethanol might interfere with the antioxidant defense mechanisms of yeast cells and, as a result, catalase is unable to counter the toxic effects of ethanol. When *S. cerevisiae* cells were treated with ethanol, lipid peroxidation increased such that cells had decreased total lipids, phospholipids and free sterols. Ethanol-induced lipid peroxidation was associated with a decline in plasma membrane lipid order and interfered with catalase defensive activity, resulting in the deterioration of membrane integrity and loss of membrane impermeability (Gupta *et al*., 1994).

2.3.3 The effect of magnesium on ethanol tolerance

Magnesium is involved in many physiological functions, including growth, cell division, and enzyme activity. Magnesium ions also decrease proton and anion permeability of the plasma membrane by interacting with membrane phospholipids, resulting in stabilization of the membrane bilayer (Birch *et al*., 2000; Walker, 1994). Thus it is probably not surprising that magnesium is implicated in the relief of the detrimental effects of ethanol stress in yeast (Walker, 1994).

Birch *et al*. (2000) and Hu *et al*. (2003), demonstrated that increasing the extracellular availability of magnesium ions, increases physiological protection against temperature- and ethanol-stress. Hu *et al*. (2003), for example, showed that the exposure of yeast cells to 20% (v/v) ethanol for 9 hours resulted in the death of all cells, whereas over 50% of the cell population remained viable in the same ethanol concentration but in the presence of Mg^{2+}.

2.4 Response of *S. cerevisiae* to ethanol stress

As discussed previously in this literature review ethanol has a range of effects on cell biochemistry and physiology. The following will describe how yeast cells respond to ethanol assaults and in particular how they acclimatise to this stress. Factors thought to affect ethanol tolerance include the amount of ergosterol in cellular membranes, phospholipid biosynthesis, the degree of unsaturation of membrane fatty acids,

temperature, the activities of superoxide dismutase and plasma membrane ATPase, and trehalose production. These will be covered in the following sections.

2.4.1 Changes in plasma membrane composition in response to ethanol stress

Alterations in plasma membrane lipid composition in response to ethanol stress are thought to represent an adaptive mechanism to ethanol-induced, detrimental changes in plasma membrane function. Beaven *et al.* (1982), showed that when *S. cerevisiae* cultures were subjected to 6% (v/v) ethanol the fatty acid composition of the membrane was adjusted towards longer chain mono-unsaturated fatty acids. These adaptive changes enabled yeast cells to better tolerate and function in the presence of ethanol. Using fluorescence anisotropy Beaven *et al*., (1982) demonstrated that, in anaerobic *S. cerevisiae* cultures supplemented with 3.5 - 9% (v/v) ethanol, the proportion of mono-unsaturated fatty acids increased, particularly $C_{18:1}$ residue (oleic acid), whilst the proportion of saturated fatty acids decreased compared to cultures without ethanol. Similarly, Sajbidor and Grego (1992) observed an increase in the proportion of $C_{18:1}$ residues relative to $C_{16:1}$ residues in anaerobic cultures of *S. cerevisiae* strain CCY supplemented with up to 15% (v/v) ethanol. This appears to be due largely to a decline in the level of $C_{16:1}$ residues in all phospholipids tested thus there may have been no net synthesis of oleic acid ($C_{18:1}$) but a remodeling of the $C_{16:1}$ residues. These changes in membrane lipids are believed to improve ethanol tolerance by lowering membrane leakage.

Evidence suggests that the phospholipid and sterol composition of cell membranes influences yeast ethanol tolerance (see reviews by Bisson and Block, 2002; D'Amore *et al*., 1990; Mishra, 1993; Rose, 1993; Sajbidor, 1997). A common trend in the reported work is that exposure to ethanol leads to increased fatty acid length and increased proportions of unsaturated fatty acids and sterols in the cell membrane. In cells grown under anaerobic conditions, the levels of sterols were dramatically reduced with a concomitant increase of their squalene precursor, as compared to cells grown under aerobic conditions (Walker 1998, Paltauf *et al*., 1992). The presence of ethanol resulted in a decrease in sterol content under aerobic conditions (Alexandre *et al*., 1994). Under anaerobic conditions, however, the presence of ethanol resulted in a three-fold increase of total sterols, with lanosterol being the main constituent. It is suggested that

lanosterol in parallel with unsaturated fatty acids is responsible for maintaining membrane integrity of *S. pombe* cells growing in the presence of ethanol (Koukkou *et al.,* 1993).

Under anaerobic conditions, yeast cells are unable to synthesize unsaturated fatty acids because the yeast desaturase enzyme requires oxygen. Similarly many of the enzymes involved in sterol synthesis, in particular the conversion of squalene to ergosterol, require oxygen (Paltauf *et al*., 1992). Therefore, in anaerobic experiments unsaturated lipids must be imported into the cell from the growth medium. Under aerobic conditions however, in the presence of 10% (v/v) ethanol, membrane lipids of wine yeast strain *S. cerevisiae* 3079 were modulated towards a higher level of $C_{18:1}$ fatty acid residues with a corresponding decrease in palmitic acid residues ($C_{16:0}$) similarly to anaerobic cultures (Alexandre *et al*., 1993). As well as the increase in unsaturation of fatty acids, sterols were also modulated towards greater unsaturation in favour of ergosterol. You *et al*. (2003) reported that ethanol tolerance increased with addition of the unsaturated fatty acid oleic acid ($C_{18:1}$) which was incorporated to the plasma membrane and thereby effectively decreasing membrane fluidity.

Alexandre *et al.* (1994) used gas chromatography to measure the sterol composition of *S. cerevisiae* grown in the presence of 10% (v/v) ethanol. These authors measured ergosterol at concentrations of 59.1$\pm$2.4 mg/100mg dry wt cells in ethanol-stressed cultures compared to 40$\pm$0.7mg/100mg in non-stressed cultures. This was accompanied by a decrease in zymosterol concentration to 13.9$\pm$0.8mg/100mg in ethanol-stressed cultures from 31.9$\pm$0.9mg/100 mg in non-stressed cells. This suggests that ethanol tolerance in yeast correlates with increasing ergosterol levels. Swan and Watson (1999) explored the effects of ergosterol-supplementation on 17% (v/v) ethanol-stressed wild type *S. cerevisiae* strain S288C and a mutant (KD115) derived from the same strain that lacked Ole1p (stearoyl-CoA 9-desaturase) activity, (it is also called Fatty acid desaturase, required for monounsaturated fatty acid synthesis and for normal distribution of mitochondria). The membrane lipid composition of the wild type had 21% of $C_{16:0}$, 53% of $C_{16:1}$ and 16% of $C_{18:1}$ fatty acyl residues. When KD115 was supplemented with oleic acid ($C_{18:1}$) it produced a membrane that was enriched in $C_{16:0}$ (28%), $C_{16:1}$ (23%) and $C_{18:1}$ (30%) fatty acyl residues. When supplementation with linoleic ($C_{18:2}$) or linolenic ($C_{18:3}$) lipids resulted in the $C_{16:0}$ (30%), $C_{18:2}$ (54%) and $C_{18:3}$

(58%) fatty acid residues. The wild type had relatively less ergosterol (49%) content compared to the supplemented mutant (64-83%) strain; the content of ergosterol increased with increasing unsaturation of the lipid supplement. Measurement of cell viability showed less than 40% cell survival when the wild type strain was exposed to 17% ethanol stress for 5 min; there was a decrease in ethanol tolerance with an increase in the number of double bonds in the lipids i.e. cells supplemented with $C_{18:1}$ were more ethanol tolerant than cell supplemented with $C_{18:2}$ fatty acid residues. These data suggests that the degree of membrane lipid unsaturation may influence cellular stress tolerance to ethanol (Swan and Watson, 1999). KD115 cells enriched with $C_{18:1}$ were not only more ethanol tolerant than cells enriched with $C_{18:2}$ or $C_{18:3}$, they were significantly more tolerant than the wild type strains. These data clearly indicate a consistent correlation between membrane lipid composition and stress tolerance.

Chi and Arnebory (1999) compared the lipid composition of membranes from yeast strains with differing levels of ethanol-tolerance. The more ethanol-tolerant strains had a higher ergosterol:phospholipid ratio, a higher level of phosphatidylcholine, a lower level of phosphatidylethanolamine, a higher incorporation of long-chain fatty acids and a slightly higher level of unsaturated fatty acids relative to total phospholipid composition. Chi and Arnebory (1999), showed that the increased concentrations of ergosterol, long chain fatty acids and unsaturated fatty acids correlated with increased ethanol tolerance. However, the mechanisms by which ergosterol and long chain fatty acids affect ethanol tolerance of yeast cells was not discussed.

Although this section is dedicated to yeast, it is important to consider other microorganisms such as bacteria to further clarify the effect of ethanol on the plasma membrane. Graca *et al.* (2003), studied the effect of ethanol on the cytoplasmic membrane composition of bacterial (*Oenococcus oeni*) cells; ethanol tolerance was measured using carbonxyfluorescein (cF), which distinguishes intact membrane/viable cells that retain cF, whereas dead cells cannot retain cF.. Two cultures were prepared from the same parent culture, one was pretreated by exposing it to 8% ethanol (pretreated cells) to trigger an adaptive response, while the second culture was not pretreated. The pretreated cells which were subsequently exposed to 16% (v/v) ethanol were able to retain cF, while the non-pretreated culture exposed to 16% (v/v) ethanol had a rapid loss of cF, suggesting a failure of plasma membrane function. Membrane

leakage rate of cF in the pretreated cells was less than 50% of that observed in non-pretreated cells. The authors also measured the fatty acid composition, which showed that the addition of 8% (v/v) ethanol to the growth medium increased the degree of unsaturation in fatty acid composition of the ethanol-adapted cells, while the total lipid content markedly decreased. This was thought to be mainly due to a large decrease in $C_{16:0}$ levels and an increase in the level of $C_{16:1}$. The degree of unsaturation and the total amount of lipids were identical in the control cells and in cells pre-incubated in 12% (v/v) ethanol for 2 hours. These results suggest that adaptation at the membrane level to ethanol stress does not necessarily require a change in the membrane lipid composition. It is postulated that the physical state of the membrane, rather than membrane composition may determine ethanol tolerance. However, most other authors argue that there is a correlation between increased degree of unsaturated fatty acid composition and ethanol tolerance of *S. cerevisiae* (Mishra and Kaur, 1991). It will be important to conduct further studied on this aspect, to confirm or refute, these conflicting literature reports.

2.4.2 Proteins associated with ethanol-stress tolerance

As discussed in Section 2.2.3 there are numerous HSPs encoded in the yeast genome. Of these HSPs some have been shown to increase expression when yeast is exposed to ethanol stress. These include *Hsp104* (Chandler *et al*., 2004, Alexandre *et al.,* 2001; Piper *et al*., 1994; Sanchez *et al*., 1992), *Hsp26, Hsp30,* (Chandler *et al.*, 2004; Alexandre *et al*., 2001 and Piper *et al*., 1994), *Hsp82, Hsp70* (Piper *et al*., 1994) and *Hsp12* (Chandler *et al*., 2004, Praekelt and Meacock 1990 and Varela *et al*. 1995). Of these only Hsp104p (Parsell *et al*., 1991; Sanchez *et al*., 1992; Glover and Lindquist 1998) and Hsp12p (Sales *et al*., 2000) appear to influence yeast tolerance to ethanol. In the case of Hsp104, Sanchez *et al*. (1993) used an *hsp104*Δ mutant and tested the ethanol tolerance of this strain compared to its parent. These experiments demonstrated that heat-induced tolerance to 20% ethanol could not be achieved in the mutant but was inducible in the parent. Hsp12p is a membrane-associated protein that can protect liposomal membrane integrity against desiccation and ethanol stress. Sales *et al*. (2000) found that an *hsp12*Δ knockout mutant had a reduced growth rate over 24 hours in 10-12% ethanol compared to the wild type.

Another interesting protein that appears to be involved in conferring ethanol tolerance is Asr1p (Alcohol Sensitive Ring/PHD). Betz *et al.,* (2004) and Van Voorst *et al*., (2006) demonstrated that this protein constitutively shuttles between the nucleus and cytoplasm but accumulates in the nucleus upon exposure to alcohol. The localization of Asr1p in the nucleus is exclusive to alcohol stress; not being observed during other stress conditions such as oxidative, osmotic, nutrient limitation and heat stress (Betz *et al*., 2004). These authors hypothesized that the nuclear accumulation of Asr1p upon alcohol stress is the result of enhanced nuclear import or inhibition of nuclear export. The authors also speculated that Asr1p might be involved in a complex signal transduction pathway during ethanol stress that enables yeast to acclimatise to this stress, but this is yet to be tested.

2.4.3 Global gene expression response to ethanol stress

In a study by Alexandre *et al.,* (2001), *S. cerevisiae* S288C cells were grown in a rich medium at 30^{o}C in the presence and absence of 7% (v/v) ethanol, and the global expression level of genes was determined after 30 minutes. Ethanol stress altered the expression level of 395 ORFs. Of these 194 were up-regulated and 201 were down-regulated. Recently Chandler *et al*. (2004) tested and extended the findings of Alexandre *et al*. (2001) using *S. cerevisiae* PMY1.1 grown in defined medium with and without 5% (v/v) ethanol at 30°C in aerobic conditions. Global gene array analysis showed that 374 ORFs had altered expression levels after exposure to ethanol stress for one hour (Chandler *et al*., 2004). Among these 100 ORFs were up-regulated and 274 ORFs were down-regulated. Following three hours of exposure to ethanol stress, only 14 ORFs were up-regulated and 99 were down-regulated. Most of the ORFs changed expression level at the one hour time point were transient; only seven were common to both one and three hour time points.

In both of the above studies, genes encoding heat shock proteins *HSP12, HSP26, HSP78* and *HSP104*, were amongst the most highly up-regulated in the presence of ethanol stress; a finding that is consistent with previous work (Piper *et al*., 1994; 1995). Chandler *et al*. (2004) and Alexandre *et al*. (2001) also reported for the first time the

ethanol-induced up-regulation of members of the *HSP70* family (*SSA1, SSA2, SSA3, SSA4, SSE1*). Other up-regulated genes included *TPS1, TPS2* and *TLS1*, all three of which are associated with trehalose synthesis. This is consistent with the finding of other groups who have reported trehalose accumulation in response to ethanol stress (Mansure *et al.,* 1994, 1997 and Lucero *et al*., 2000) and under other stressfull conditions (Gasch *et al*., 200; Rep *et al*., 2000). The up-regulation of HSPs and trehalose synthesis genes is suggested to have a protective effect against damage caused by ethanol stress. As discussed previously trehalose is thought to impede protein denaturation during stress and reduce ethanol-induced membrane permeability (see Section 2.2.4).

Chandler *et al*., (2004) and Alexandre *et al*., (2001) also identified the up-regulation of glycolysis-associated genes, *GLK1, HXK1, TDH1 ALD4* and *PGM2*, and high affinity hexose transporter genes of *HXT6* and *HXT7*. This lead Chandler *et al*. (2004) to propose that when exposed to ethanol stress, yeast cells enter a pseudo starvation state since the molecular response to ethanol stress was similar to that when cells are starved of glucose; in this case, there was ample glucose in the medium but, because of the impact of ethanol, it was not accessible to cells. Other energy production-associated genes, *GPD1 HOR2, GRE3 HOR7* and *DAK1*, were also found to be up-regulated during ethanol stress in the work of Alexandre *et al*. (2001) but their up-regulation was not confirmed by Tamas *et al*. (2000) or Chandler *et al.* (2004).

One of the most interesting results at 30 minute and one hour time points in the studies of both Alexandre *et al.* (2001) and Chandler *et al*. (2004) was the down regulation of genes associated with protein biosynthesis, cell growth and RNA metabolism. It has been speculated that the down regulation of these genes reflected growth arrest during the stress conditions to allow energy conservation and cellular adaptation to the stress condition (Gasch *et al*., 2000).

It is clear from the above that there are similarities and differences between the results of Alexandre *et al.* (2001) and Chandler *et al.* (2004). This may be due to differences in experimental conditions (e.g. severity of ethanol stress, time-length of exposure to ethanol, media used and gene array methodology) or strain differences. Alexander *et al*. (2001) focused on a single time point using equalized RNA concentrations in the test

and control samples instead of RNA from equalized cell numbers (as used by Chandler and co-workers). It is well documented that stress reduces general transcription levels. Thus, if RNA concentrations were equalized in test and control samples, the concentration of mRNA in the stressed samples would be disproportionately increased compared to unstressed controls, increasing the likelihood of generating false positive results.

2.5 Effect of acetaldehyde on the growth of ethanol-stressed yeast cells

2.5.1 The effect of inoculum size on the lag period of yeast

There are number of studies reported in the literature on the ethanol-induced lag period in yeast (Walker-carpioglio *et al*., 1985; Walker-carpioglio and Parks, 1987; Stanley *et al*., 1993; Stanley *et al*., 1997; Barber *et al*., 2002 and Vriesekoop and Pamment, 2005). Walker-carpioglio *et al*., (1985) investigated the effect of a step change in ethanol concentration (between 1% to 8%) on the length of the lag period of *S. cerevisiae* X2180-1A under aerobic conditions. These authors showed that lag periods increased and growth rates decreased with increasing ethanol concentrations. The authors also investigated the effect of different inoculum sizes ranging from 10^3 to 10^6 cells/ml on the length of a 4% ethanol-induced lag period. They found that the lag period decreased as the inoculum size increased, demonstrating an inverse relationship between inoculum size and the lag period. This suggests that a lag-reducing metabolite(s) was present in the culture fluid, carried over with the inoculum, or excreted during the lag phase, or both (Walker-Caprioglio *et al*., 1985). Cells grown in culture filtrates also showed reduced lags.

This inoculum dependent ethanol-induced lag reduction was confirmed by Stanley *et al*. (1997); who used inocula comprising late exponential phase *S. cerevisiae* X2180-1A. The inoculum was washed to avoid carryover of conditioning factors that might have contributed to the reduction of the lag time. When initial cell populations of 5 x10^4 and 5 x 10^6 cells/ml were inoculated into rich medium containing 4% (v/v) ethanol, lag periods of 3.6 and 2.6 hours were observed respectively. Unlike Walker-Carpioglio *et*

al., (1985), Stanley *et al*. (1993) found that the inoculum-dependent lag reduction was observed only above a threshold cell population of around 10^5 cells/ml, below this the lag period was independent of inoculum size. Stanley *et al*. (1997) demonstrated that initial cell populations above 10^5 cells/ml were able to produce metabolite(s) that enables the ethanol-stressed culture to acclimatize more rapidly to ethanol stress and then commence growth. At initial cell populations below 10^5 cells/ml the metabolite(s) can be assumed to be present but at a concentration too low to have any effect on the lag period. One of the metabolites found to be partly responsible for the ethanol induced lag period reduction was identified as acetaldehyde (Walker-Carprioglio and Parks, 1987 and Stanley *et al*., 1997).

2.5.2 Stimulatory and inhibitory effect of acetaldehyde on ethanol-stressed yeast

Acetaldehyde is a natural by-product of yeast metabolism during alcoholic fermentation and it is the direct precursor to ethanol in the fermentative pathways of yeasts (Figure 2.3)**.** The effects of acetaldehyde on metabolism are complex and unclear due in part to acetaldehyde's high volatility and the difficulty in measuring its intracellular concentration. Acetaldehyde at high concentrations stops cell growth and is thought to contribute to the overall product inhibition effect in alcohol fermentation (Jones, 1989 and Stanley *et al*., 1993). Acetaldehyde is a known inhibitor of a wide range of metabolic activities and at times can be more toxic than ethanol. In fact, added acetaldehyde has been found to both inhibit and stimulate yeast growth in the presence of ethanol depending on its concentration (Stanley *et al*., 1993). Stanley *et al*. (1997) reported that acetaldehyde concentrations above 0.3 g/L inhibited *S. cerevisiae* cell growth if added to non-stressed cultures. Furthermore, acetaldehyde has been shown to inhibit growth rate and fermentation not only in yeast, but also in other organisms such as the bacterium *Zymomonas mobilis* under aerobic conditions (Ishikawa *et al*., 1990).

Acetaldehyde has a molecular weight of 44.1, which is similar to that of ethanol at 46.1; both are small, polar molecules. Acetaldehyde is, however, more polar than ethanol. For this reason, it passes more slowly through the hydrophobic core of cell membranes. This is consistent with the slower rate at which acetaldehyde diffuses out of the cells (Stanley and Pamment, 1993). As a result, acetaldehyde tends to accumulate inside

cells during fermentation, reaching concentrations of up ten times the prevailing extracellular concentration, with intracellular values reaching between 0.3-0.4 g/L (Stanley and Pamment, 1993). Although the intracellular acetaldehyde concentration in *S. cerevisiae* is estimated to be around 0.3 g/L (Martinez *et al.,* 1997; Stanley and Pamment, 1993), its level may reach up to around 1 g/L, depending on the strain and conditions. Rank *et al.,* (1995), investigated the concentrations of secondary metabolites such as ethanol, acetaldehyde and glycerol produced during an industrial fermentation of *S. cererevisiae* over the first 48 hours of fermentation. In this study the intracellular acetaldehyde level reached a peak value of about 0.3 g/L. Acetaldehyde is a very reactive compound and its intracellular accumulation might affect some structural components of cells. Hence, acetaldehyde is often better known for its more lethal effect on yeast metabolism than ethanol (Bandas, 1983).

Maiorella *et al*. (1983) studied the effect of added acetaldehyde on yeast cell growth under anaerobic conditions. Concentrations of 0.4 g/L acetaldehyde were found to inhibit cell growth, however, this inhibitory concentration was not found to affect the fermentation rate (ethanol production). This is in keeping with findings of Carlsen *et al*. (1991), both investigators concluding that high quantities of acetaldehyde did not inhibit fermentation under anaerobic conditions. When the effect of added acetaldehyde (0.55 g/L) on aerobically growing yeast cultures was studied, they found that aerobic fermentation was inhibited, showing a marked decrease in oxygen consumption and a slight decrease in CO_2 production. Carlsen's findings showed that the degree of inhibition of respiration and fermentation by acetaldehyde is quite distinct to that of ethanol, i.e. acetaldehyde was a more powerful inhibitor of respiration than fermentation, while ethanol is more detrimental to fermentation than respiration.

The toxicity of acetaldehyde in microorganisms was reviewed extensively by Jones (1989 and 1990), who suggested that acetaldehyde is more toxic to organisms of low ethanol tolerance; this was based on indirect evidence which demonstrated greater sensitivity of a variety of organisms to acetaldehyde compared to ethanol. Other more recent findings suggest that acetaldehyde has a direct effect on gene expression (Mayer *et al*., 1994). Ristow *et al*., (1995) and Obe and Ristow (1979), suggested that acetaldehyde causes DNA breakage and was mutagenic. The evidence presented by Obe and Ristow (1979) suggested that acetaldehyde is a more potent inhibitor of cells

than ethanol; the studies were conducted *in vitro* for RNA synthesis in the presence of 0.44 g/L acetaldehyde and *in vivo* for protein synthesis in the presence of 2.6 g/L acetaldehyde compared to 41.4 g/L and 21 g/L ethanol respectively. The results suggested a 50% greater inhibition of cellular processes by acetaldehyde compared to ethanol. Other studies using higher eukaryotes showed that the growth rate of mouse neuroblastoma cells was 50% inhibited at 0.22 g/L of acetaldehyde, whereas 9 g/L of ethanol had no noticeable effect (Syapin and Noble, 1979). However, it is important to note that the effect of acetaldehyde on different species might vary. For example yeast glucose transport permease is five times more tolerant to inhibition by acetaldehyde than the human intestinal glucose permease (Jones, 1990).

Acetaldehyde tolerance even within the same species can vary significantly. Aranda *et al*. (2002), analyzed the response of different *S. cerevisiae* strains to the sudden addition of acetaldehyde (1 g/L) into exponentially growing yeast cells in a complex medium for two hours at 20^0C. Their results showed that some strains are more acetaldehyde stress tolerant than others. For example, when investigating the response of three wine strains and four sherry wine strains during short-term exposure to acetaldehyde stress, the three wine strains had cell viabilities of less than 40% while the sherry strains had viabilities in the range 50% to 80%.

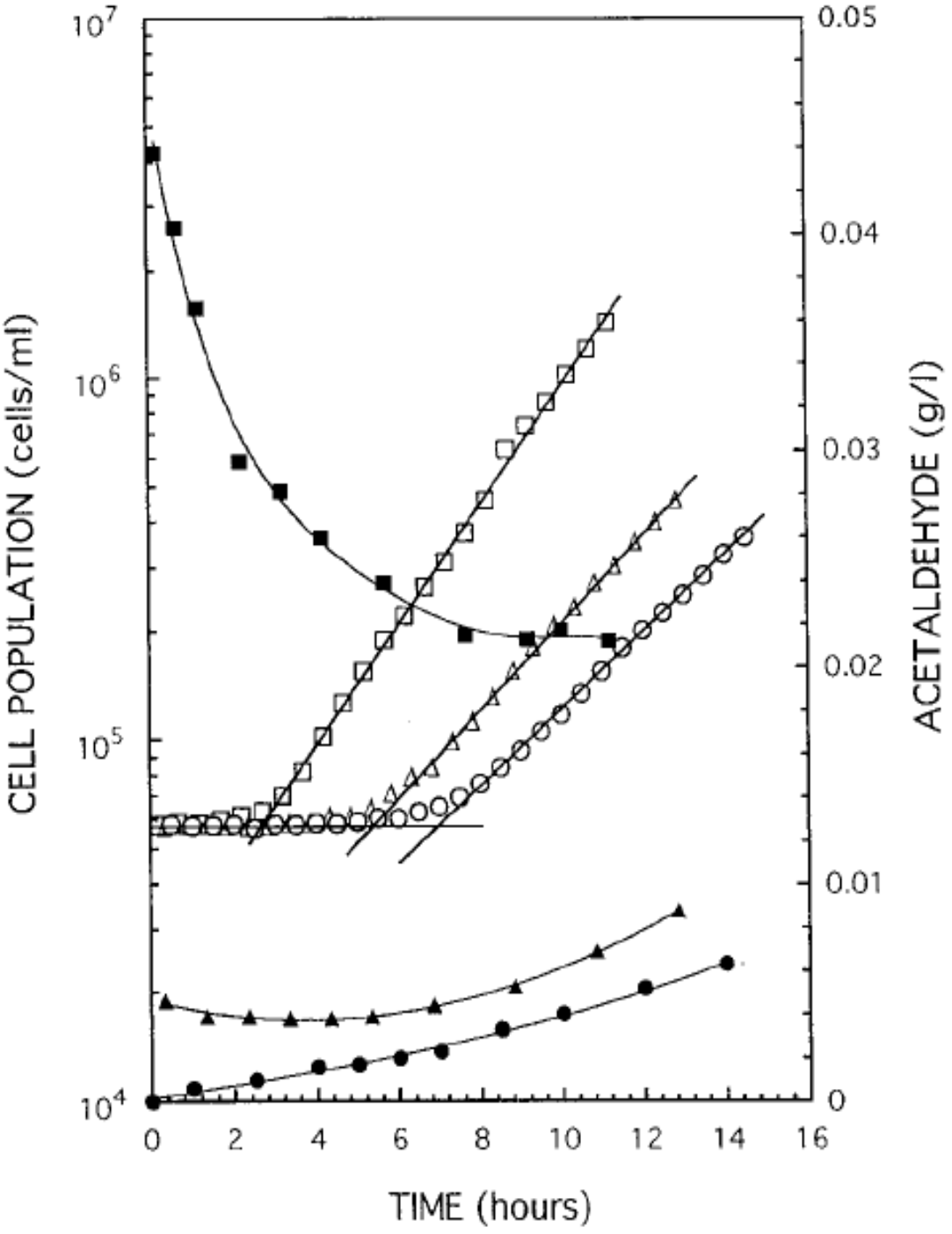

Figure 2.1: The stimulatory effect of added acetaldehyde on *S. cerevisiae* growth (open symbols) and added acetaldehyde concentration (closed symbols). Yeast cells were grown in rich medium with 4% (v/v) ethanol. Acetaldehyde concentration and corresponding lag period were: 0 g/l and 7 h lag (○, ●), 0.005 g/l and 5.4 h lag (Δ, ▲), 0.046 g/l and 2.7 h (□, ■) (from Stanley *et al.*, 1997).

Remize *et al*. (1999), investigated the overproduction of glycerol at the expense of low ethanol production and accumulation of acetaldehyde in *S. cerevisiae* cells. The *GPD1* gene that encodes for glycerol-3-phosphate dehydrogenase was engineered for glycerol overproduction in laboratory (L1 and L2) and industrial wine (W3, W6, W15, W18, W19, V5 and R) strains of *S. cerevisiae*, resulting in a two fold increase in glycerol production and a slight decrease in ethanol production (Remize *et al*., 1999). During the overproduction of glycerol in the industrial (wine strain), the cell biomass decreased which was attributed to high acetaldehyde production during the growth phase. The biomass increase by strains (W3, W6, W18 and R) were not affected for those that produced less the 300 mg/l acetaldehyde, while those strains (W15 and W19) that produced 400-500 mg/l and 600 mg/l (V5) of acetaldehyde exhibited a decrease in cell growth. The *S. cerevisiae* laboratory strains L1 and L2 produced about 4000 and 6000 mg/l of acetaldehyde respectively, and the growth of cells were severely affected compared to controls (wild type).

This result suggests a correlation between acetaldehyde concentration and its inhibitory effect. The results of Remize *et al*., (1999) are consistent with the role of acetaldehyde as a potent inhibitor of cell function if it is allowed to accumulate to levels above 300 mg/l (Stanley and Pamment 1993; Stanley *et al*., 1993; Aranda *et al*., 2002; Aranda and Olmo, 2003; 2004).

In contrast to this inhibitory role, acetaldehyde can have a growth stimulatory effect in ethanol-stressed cultures. Some initial evidence reported by Walker-Caprioglio and Parks (1987), showed that when yeast are inoculated into a complex medium containing a stressful level of ethanol, the resulting lag period was significantly reduced by the addition of small amounts of acetaldehyde. These positive effects of acetaldehyde on the growth of ethanol-stressed yeast was further investigated by Stanley *et al*. (1993 and 1997) and their results confirmed that the presence of small quantities (*ca* 0.01 and 0.046 g/L) of added acetaldehyde in ethanol-stressed yeast and *Zymononas mobilis* cultures significantly reduced the ethanol induced growth lag period and significantly increased the specific growth rate of the cultures after growth commenced (Figure 2.1).

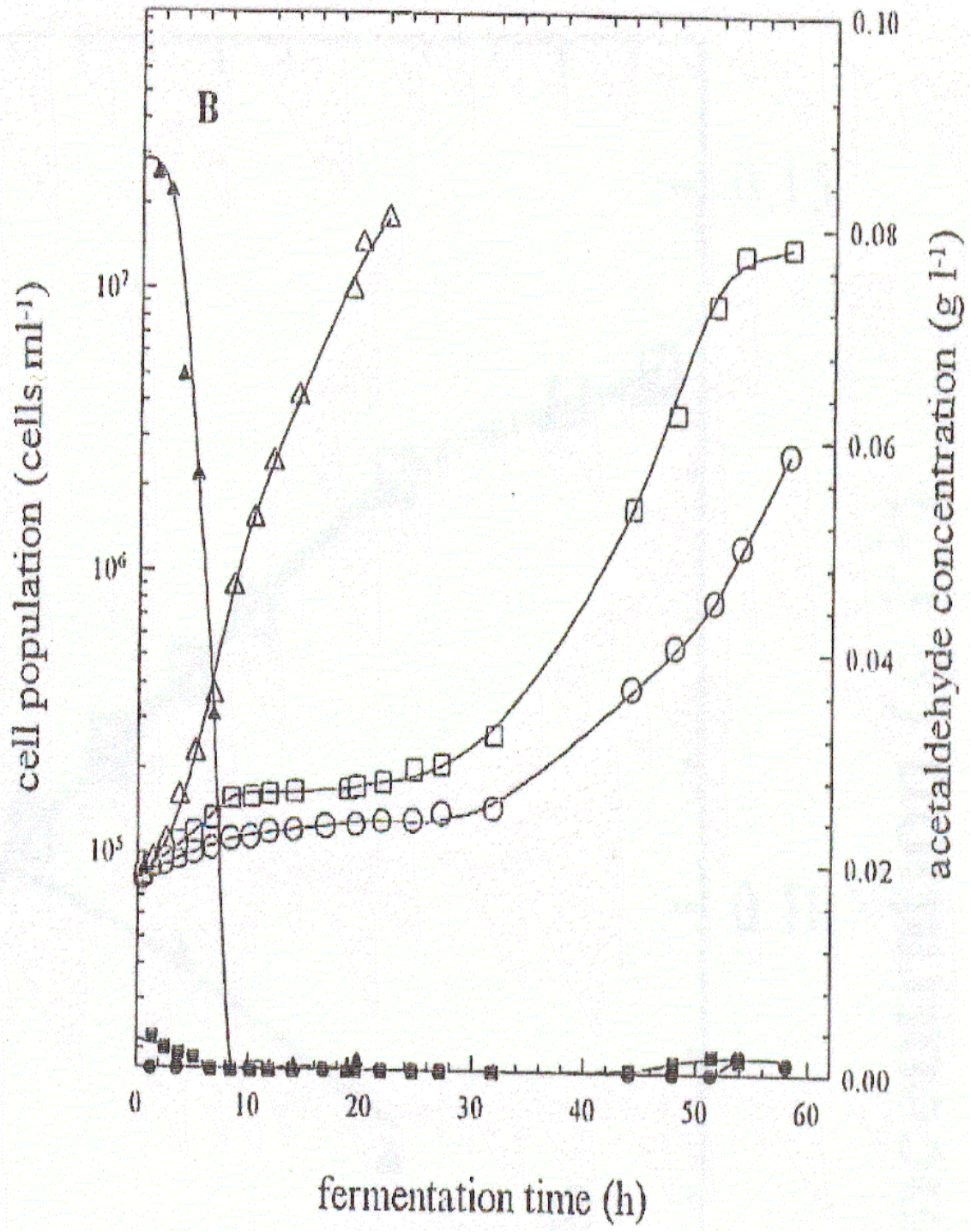

Figure 2.2: Effect of acetaldehyde addition on the growth of *S. cerevisiae* X2180-1A in defined medium containing 0.4% (v/v) n-propanol. Open symbols (cell numbers), closed symbols (acetaldehyde concentrations). Initial acetaldehyde concentrations (g/l): (○, ●) 0, (□, ■) 0.003, and (Δ, ▲) 0.085. The cultures were inoculated aerobically at 30C/140 rpm (from Barber *et al.*, 2002).

The effect of acetaldehyde in reducing the lag time of ethanol-stressed yeast cultures is also observed when yeast cells are stressed by other alcohol stressors such as propanol and butanol. Barber *et al.* (2002) reported that acetaldehyde (0.085 g/l) reduced the lag time of the propanol-inhibited *S. cerevisiae* culture from about 35 hours to only 2 hours (Figure 2.2). Barber *et al.* (2002) found that there is a clear difference between the acetaldehyde concentration profiles of ethanol-stressed cultures containing added acetaldehyde and those stressed using other alcohols. In the ethanol-stressed cultures, the extracellular acetaldehyde concentration decreased relatively slowly during lag and exponential growth period, while for the other alcohol-stressed cultures the concentration of extracellular acetaldehyde dropped significantly during the same period. As shown by Stanley *et al.* (1997), acetaldehyde evaporation is negligible under the conditions used by Barber *et al.* (2002) and acetaldehyde did not react chemically with the defined medium used in the experiment. Thus, the acetaldehyde loss was suggested to be due to its conversion to ethanol, therefore influencing the NAD+/NADH redox balance.

In support of this, Barber *et al*. (2002) found that when propanal (a three carbon aldehyde) was used instead of acetaldehyde to stimulate the ethanol stress response, an equivalent amount of propanol was produced relative to the amount of propanal lost (the ethanol-stressed cultures had a reduced lag period as with acetaldehyde), suggesting that the stimulatory effect may be due to aldehyde reduction to alcohol and its role in maintaining an NAD+/NADH redox balance.

The stimulatory effect of added acetaldehyde on chemically-inhibited cultures was not found to be universal. When *S. cerevisiae* was stressed by common food preservative acids at pH 5.2, the resulting lag was either not significantly affected or it was lengthened by the addition of acetaldehyde (0.005-0.055 g/l). Acetaldehyde at very low concentrations of 0.005 g/l, increased the inhibitory effect of lactic acid (Barber *et al*., 2002).

2.5.3 The stimulatory role of acetaldehyde in the physiological adaptation of yeast to ethanol stress

The mechanism underpinning the acetaldehyde-stimulated adaptation of *S. cerevisiae* to ethanol stress is unclear. There is considerable evidence suggesting that the plasma membrane could be the primary target of ethanol toxicity. Given this observation, Walker-Caprioglio and Parks (1987) studied yeast plasma membrane order using fluorescence anisotropy and found no difference between the plasma membrane of yeast in cultures containing acetaldehyde with 6% (v/v) ethanol and a control containing only 6% ethanol *i.e*. acetaldehyde did not reverse ethanol-induced changes to plasma membrane. Walker-Caprioglio and Parks (1987) concluded that the ethanol induced lag phase could not be due to the changes on the plasma membrane order, rather it could be due to ethanol-induced changes in cell metabolism. The authors also measured the concentration of ethanol in cultures with and without acetaldehyde and found that added acetaldehyde (0.078 g/l) did not affect the amount of ethanol produced, but still reduced the lag phase. This implies acetaldehyde did not significantly affect the metabolic rate of glycolysis.

Stanley *et al*. (1997), suggested the added acetaldehyde replaces intracellular acetaldehyde lost from the cell when the permeability of the plasma membrane is disturbed by ethanol stress. In an earlier publication, Stanley and Pamment (1993) demonstrated the intracellular acetaldehyde levels could be up to 10-fold higher than the extracellular concentration. The loss of intracellular acetaldehyde in ethanol-producing cells could lead to a cellular redox imbalance since regeneration of NAD^+ via alcohol dehydrogenase may be slowed. It was speculated that added acetaldehyde serves to replace intracellular acetaldehyde lost by diffusion when cells are inoculated into high ethanol concentration, permitting a more rapid rate of NAD^+ regeneration.

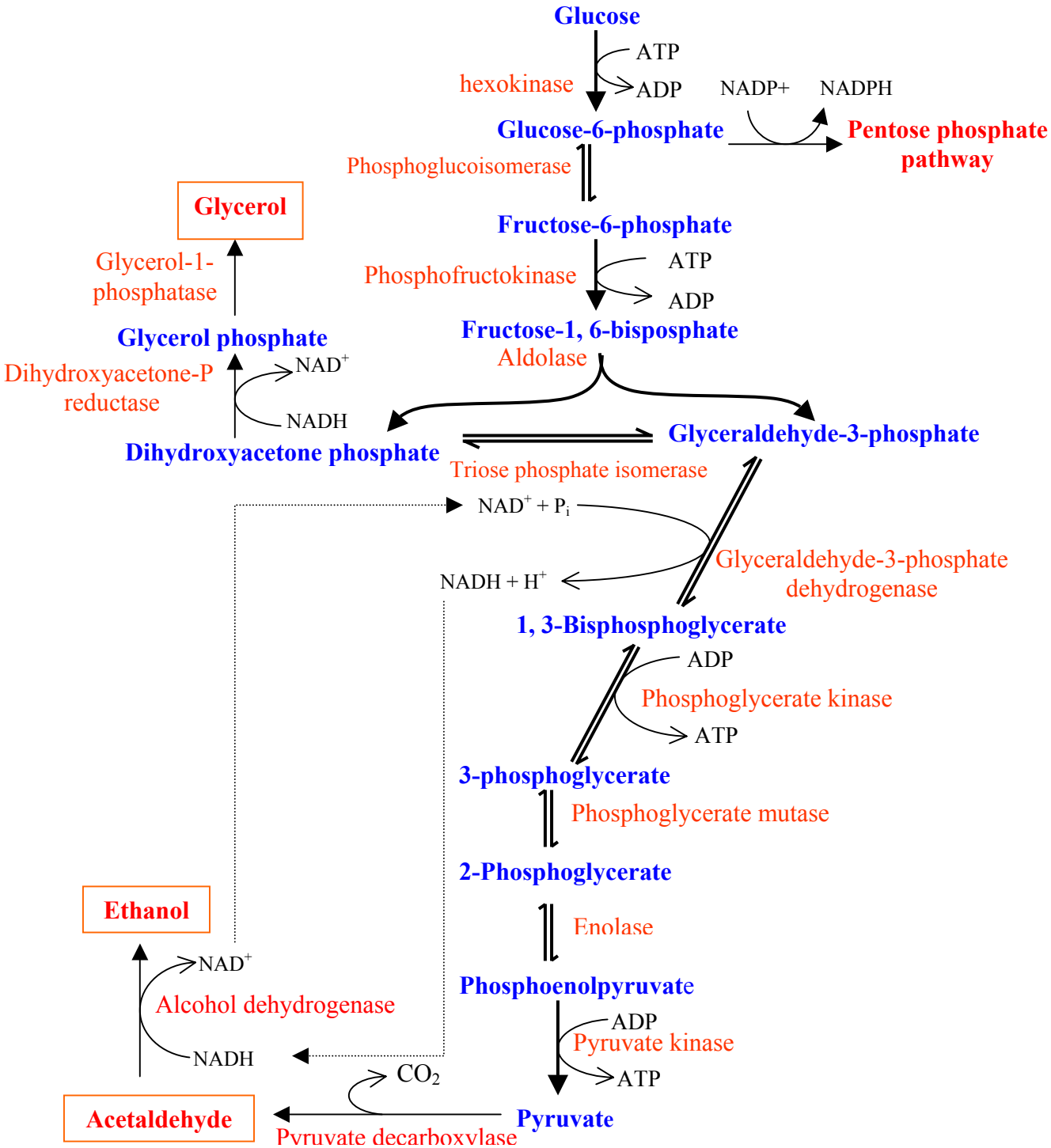

Figure 2.3: Schematic overview of glycolytic and fermentative pathways in yeast. If acetaldehyde is not reduced to ethanol, glycolysis would be limited by an NAD^+ deficiency, the reduction of dihydroxyacetone phosphate to form glycerol might help to balance the NAD^+ production.

2.5.4 Effect of acetaldehyde and glycerol on NAD^+/NADH ratio

During alcoholic fermentation, it is believed that one of the main roles of glycerol formation is to equilibrate the intracellular redox balance by converting the excess NADH generated during biomass formation to NAD^+ (Nordström, 1968) (Figure 2.3). It was previously shown that the increased utilization of NADH through glycerol formation led to a transient accumulation of pyruvate and acetaldehyde (Remize *et al.,* 1999 and Michnick *et al*., 1997). Most anabolic reductive reactions require NADPH rather than NADH. Since, ethanol production from glucose is a redox neutral process; glycerol production is the only other way of restoring the cytosolic redox balance under anaerobic conditions. Hence, substantial overproduction of glycerol is observed during alcoholic fermentation when reoxidation of glycolytically-formed NADH is restricted. This is the case, for instance, when the intermediate product, acetaldehyde, is trapped by bisulphate or amino acids in complex medium, so that it can no longer serve as an electron acceptor for cytosolic NADH (Barber *et al*., 2002; Stanley and Pamment, 1997; Hobley and Pamment, 1997).

In an anaerobic batch culture, Oura (1977) reported a huge production of glycerol compared to the amount produced during aerobic fermentation, and noted that glycerol production was linked to the fate of acetaldehyde and cellular redox balance (NAD^+/NADH). Furthermore, Oura (1977) suggested that there were two acetaldehyde-related factors that limit the progress of glycolysis. First, if acetaldehyde is not reduced to ethanol for some reason, glycolysis will tend to be limited due to a lack of NAD^+ (Figure 2.3). Second, the formation of acetate from acetaldehyde by an oxidation reaction that consumes NAD^+, further limiting the availability of NAD^+ for glycolysis. These effects might be resolved by the reduction of dihydroxyacetone phosphate to glycerol, which regenerates NAD^+ for upper glycolysis. Hence, redox balance is maintained either by the formation of ethanol or glycerol or both. The production of glycerol is linked to the metabolic fate of acetaldehyde.

Betz and Becker (1975), reported that acetaldehyde (0.009 g/L) added to an anaerobic yeast culture growing on glucose had markedly reduced the NADH levels. It was suggested that the added acetaldehyde was converted to ethanol, which was accompanied by the oxidation of NADH to NAD^+. Other studies, by Radler and Shutz

(1982), examined the concentration of glycerol, acetaldehyde and pyruvate in different strains of *S. cerevisiae*, growing in anaerobic conditions. They found that strains that produced a large amount of glycerol also produced a larger amount of acetaldehyde and pyruvate during fermentation. This suggests that in the absence of oxygen, and where there was a deficiency in alcohol dehydrogenase activity, acetaldehyde tends to accumulate.

The effect of added acetaldehyde on cellular metabolism can be more complex during aerobic growth. Iscaki (1975), reported that acetaldehyde added (0.01-0.07g/L) to an aerobic yeast culture reduced the consumption of glucose by glycolysis and increased utilization of glucose by the pentose phosphate pathway (Figure 2.3). Iscaki (1975), investigated the fate of added acetaldehyde using radioactively labeled glucose and found it to be oxidized to acetate by aldehyde dehydrogenase rather than reduced to ethanol. The acetaldehyde added into the aerobic culture was oxidized to acetate accompanied by the reduction of NAD^+ to NADH, which negatively affects glyceraldehyde 3-phosphate dehydrogenase (GAPDH) activity. Hence, this would not only increase the level of NADH in the cell, thereby inhibiting GAPDH, but also reduce the NAD^+ availability for glycolysis. As a result, glycolysis was partially inhibited by added acetaldehyde and the pentose phosphate pathway in which $NADP^+$, and not NAD^+ is used was activated. It could be concluded that when acetaldehyde is added to an anaerobic yeast culture, it appears to stimulate glycolysis by the reduction of acetaldehyde to ethanol. This increases the cellular levels of NAD^+ and deceases the glycerol production levels, as the purpose of glycerol is for the regeneration of NAD^+. However, when acetaldehyde is added to aerobic yeast cultures the acetaldehyde is oxidized to acetate instead of being reduced to ethanol. The acetate formation is accompanied by an accumulation of NADH, resulting in an NAD^+ deficiency for glycolysis.

2.5.5 Pre-treatment with mild ethanol-stress enhances the stimulatory effect of acetaldehyde

As described in section 2.2.1**,** pre-treatment of yeast cells with mild stress results in the acquisition of greater tolerance to a lethal dose of the same stress. It was therefore postulated that the combination of pre-treatment and the stimulatory effect of added

acetaldehyde could synergistically reduce the ethanol-induced lag period (Vriesekoop and Pamment, 2005). Vriesekoop and Pamment, (2005), inoculated *S. cerevisiae* TWY-397 into two flasks containing medium: one flask had 5% (v/v) ethanol and 0.09 g/l added acetaldehyde and the other flask had 5% (v/v) ethanol only. The ethanol-stressed culture had a lag period of nine hours, and the added acetaldehyde reduced this ethanol-induced lag period by six hours (67%); this finding is consistent with that of Stanley *et al*. (1993; 1997), Barber *et al*. (2000) and Barber *et al*. (2002). These workers then investigated the combined effect of pre-treatment of inocula with mild ethanol stress and the stimulatory effect of added acetaldehyde on the yeast ethanol stress response, *i.e.* the yeast inoculum was subjected to low-intensity stress of 1% (v/v) ethanol, compared to an inoculum without prestress (control), and both were inoculated into ethanol-containing medium in the presence and absence of acetaldehyde. The culture with a pre-treated inoculum showed a 70% reduction in the lag period compared to the non-pre-treated (control) culture containing only ethanol. When the combined effect of pre-treatment and added acetaldehyde was investigated the lag period was reduced by 90% (Vriesekoop and Pamment, 2005). Thus, the ethanol induced lag phase in yeast can be substantially reduced by the combined effect of pre-treatment with mild ethanol stress and added acetaldehyde. These results suggest that the stimulatory effects of prestressing or acetaldehyde addition on ethanol-stressed yeast operate by different and independent mechanisms.

2.5.6 The effect of acetaldehyde on gene expression

Acetaldehyde is a known inhibitor of a wide range of metabolic activities and is thought to be more toxic than ethanol (Jones 1988; 1990). Aranda *et al*. (2002) used Northern blots to study the expression profile of particular genes subject to acetaldehyde and ethanol stress. Exponentially growing yeast from the same parent culture were incubated with either 1 g/L added acetaldehyde or 12% ethanol and compared to untreated cultures, using different yeast strains (seven sherry wine strains, three wine strains and one laboratory strain). It was found that the sherry wine yeast strains had the highest viability to the acetaldehyde and ethanol stress. Among the *HSP* genes considered as stress indicators by Aranda *et al.* (2002) were HSP12/26/82/104 genes. The expression levels of these genes were analyzed at time points of 30 and 60 min after the addition of the stress agents; these time points were chosen because previous reports

have indicated that the maximal induction of *HSP* genes is achieved at approximately these times (Martinez-pastor *et al*., 1996; Treger *et al.,* 1998). Aranda *et al*., (2002) observed the up-regulation of all the above four genes, among these genes *HSP12* and *HSP104* exhibited the highest expression level for the sherry wine yeast strains. Higher induction levels were observed when yeast cells were exposed to acetaldehyde compared to ethanol stress cultures. The findings of Aranda *et al*. (2002) were in keeping with that of Shankar *et al*. (1996) and recently Aranda & Olmo (2003) and Aranda & Olmo (2004) who showed that several *HSP* genes were up-regulated at a high concentration of acetaldehyde-treated sherry wine *S. cerevisiae*. This strain dependent up-regulation of specific genes during exposure to a high concentration of acetaldehyde was also observed for *ALD* genes that encode aldehyde dehydrogenase proteins (Aranda & Olmo, 2003). The increased expression levels of *ALD* genes in response to acetaldehyde stress was expected, because the intrinsic cell response was anticipated to initiate a decrease in the toxic level of acetaldehyde by its oxidation to acetate. These results showed that the level of increase in gene expression depended on the yeast strain, and for all of the strains, maximal induction was achieved at around 1 hour after acetaldehyde treatment. Overall, the *HSP* and *ALD* gene expression pattern in different yeast strains showed correlation between resistance to acetaldehyde and ethanol stress (Aranda *et al*., 2002; Aranda & Olmo, 2003).

The adverse effect of acetaldehyde on gene expression was investigated using global gene expression analysis, to study the genome wide impact of acetaldehyde stress on yeast *S. cerevisiae*. *S. cerevisiae* was exposed to 1 g/L of acetaldehyde for one hour (Aranda and Olmo, 2004); the microarray result revealed 401 genes had significant shift in expression level, of these 273 were up-regulated and 128 genes were down-regulated at the one hour time point compared to the control. Among these genes activated by acetaldehyde are those involved in sulfur metabolism and some involved in polyamine transport *(TPO1-TPO4*), which are located in the plasma membrane, and which are involved in multi-drug transport activities. Microarray results showed a significant increase in the expression level of the *TPO2* gene, while *TPO3* was moderately induced and no significant expression levels of *TPO1* and *TPO4* was observed (Aranda and Olmo, 2004). The up-regulation of these *TPO* genes suggests that the product membrane proteins might be involved in the efflux of excess acetaldehyde from the cells. Hence, the increased expression of *TPO* genes may possibly have a role in

tolerance to acetaldehyde stress. Transcription of *TPO2* and *TPO3* genes, but not *TPO4* depends on the Haa1p transcription factor under acetaldehyde stress; these were confirmed by creating mutants for Haa1p gene (Aranda and Olmo, 2004).

Aranda and Olmo (2004) also observed a marked induction of sulfur metabolism genes (*MUP3, SAM3, MET3, SUL2, MUP1, MMP1 & MET3*) during acetaldehyde stress. Some of these genes are involved in the pathway from sulphate uptake to homocysteine synthesis and transport of sulphur compounds. The acetaldehyde induction of these genes depends on the transcription factor Met4p and partially on Met31p and Met32p. Both acetaldehyde and sulphite are toxic to yeast cells (Casalone *et al.,* 1991), hence, the induction of sulphur metabolism genes and *TPO* genes suggests their involvement in acetaldehyde and sulphite detoxification; either by eliminating (efflux) excess acetaldehyde or acetaldehyde binding with other molecules such as sulphite/sulphur or sulphur containing amino acids (e.g. cysteine & methionine) to produce non-toxic sulphate adducts and, subsequently, expelled by the *TPO* transporters (Aranda & Olmo, 2004).

2.6 Aims and objectives of this project

2.6.1 General aim

The primary objective of this research project was to investigate and characterise the molecular response of *S. cerevisiae* to ethanol stress in the presence of added acetaldehyde using global gene expression analysis. Information from this would be used to identify genes in yeast that are involved in the ethanol stress response and, in particular, are associated with the stimulatory effect of acetaldehyde on ethanol-stressed cultures.

2.6.2 Specific aims were to:

I. Compare, using gene array technology, mRNA profiles of ethanol-stressed yeast cells incubated in the presence and absence of acetaldehyde, to determine whether acetaldehyde induces a novel set of stress-response genes, whether it stimulates a more rapid expression of typical ethanol stress response genes and/or has a role in the generic up-regulation of gene expression during ethanol stress.

II. Using the data obtained in (i), develop a hypothesis that describes the mechanism by which acetaldehyde stimulates the growth of ethanol-stressed *Saccharomyces cerevisiae* cultures.

CHAPTER 3

Material and methods

3.1 Materials

3.1.1 General buffers and solutions

Analytical grade chemicals were used to prepare all buffers and solutions unless otherwise stated. Chemicals used were supplied by BDH (UK) or Sigma (USA) unless otherwise stated. All buffers and solutions were prepared using distilled and de-ionised Milli-Q water (Milli-Q Plus Ultra Pure Water System, Millipore, Billerica, MA, USA). Formulae for all buffers and solutions are provided in Appendix I, section 1.1. Buffers and solutions were sterilized by autoclaving at 121°C for 20 minutes or, where indicated, filter sterilized using a 0.22 μm or 0.45 μm Millipore membrane filter. All glassware and stainless steel components for preparing RNA solutions were baked at 180°C for at least 12 hours. Buffers for RNA work were prepared in RNase-free glassware using diethyl pyrocarbonate (DEPC)-treated water. DEPC-treated water (0.2%) was prepared with distilled and de-ionized Milli-Q water. A list of all enzymes, molecular weight markers, molecular biology kits and a list of suppliers are also provided in Appendix I, Table 1.1.

Table 3.1: Summary of *S. cerevisiae* strains used in the work described in this thesis.

S. cerevisiae STRAINS	DESCRIPTION	GENOTYPE	SOURCE
PMY1.1	Wild type	PMY1.1 (*MATa leu2, ura3, his4*)	P. Piper*
BY4742	Wild type	BY4742 (*MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*)	Provided by AWRI**
BY4742*ΔPDC5*::kanMX4	Knockout	BY4742 (*MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) ΔPDC5*::KanMX4	Provided by AWRI
BY4742*ΔPDC1*::kanMX4	Knockout	BY4742 (*MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) ΔPDC1*::KanMX4	Provided by AWRI
BY4742*ΔPHO84*::kanMX4	Knockout	BY4742 (*MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) ΔPHO84*::KanMX4	Provided by AWRI
BY4742*ΔHXT4*::kanMX4	Knockout	BY4742 (*MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) ΔHXT4*::KanMX4	Provided by AWRI
BY4742*ΔYLR364W*::kanMX4	Knockout	BY4742 *(MATalpha his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0) ΔYLR364W*::KanMX4	Provided by AWRI

Dr. Peter Piper*, University College, London, UK. **Australian Wine Research Institute, Adelaide, Australia.

3.1.2 Yeast strains

A haploid lab strain, *Saccharomyces cerevisiae* PMY1.1 (*MAT*a *leu2, ura3, his4*) and a haploid lab strain, *Saccharomyces cerevisiae* BY4742 (*MATalpha his3*Δ*1 leu2*Δ*0 lys2*Δ*0 ura3*Δ*0*) were used for the work described throughout this thesis. PMY1.1 was originally from the laboratory of Dr Peter Piper (University College, London, UK). Five knockout strains of *S. cerevisiae* BY4742 were used in this project. Each of these had one of the genes *PDC5, PDC1, PHO84, HXT4* or *YLR364W* replaced by the *kanMX4* module to generate five knockout strains, BY4742 (*MATalpha his3Δ1 leu2Δ0 lys2*Δ*0 ura3*Δ*0*) Δ*pdc5*::*kanMX4,* Δ*pdc1*::*kanMX4,* Δ*pho84*::*kanMX4,* Δ*hxt4*::*kanMX4* and Δ*ylr364w*::*kanMX4* (Table 3.1).

3.2 Microbiological work

3.2.1 Growth media

Yeast cultures were grown in a nutrient rich YEPD medium. Media and culture vessels were autoclaved at 121°C for 20 minutes. The glucose component of the rich YEPD medium was autoclaved separately. All water used in growth media was distilled and de-ionised Milli-Q water.

YEPD medium comprised of per litre: 10 g yeast extract, 20 g bacto-peptone, and 20 g D-glucose. Components were dissolved in distilled de-ionised water and autoclaved at 121°C for 20 minutes. For solid YEPD medium, bacto-agar was added to a final concentration of 15 g l^{-1} prior to autoclaving.

YEPD Geneticin plates for the selection of knockout strains with an integrated *kanMX4* cassette comprised YEPD medium with the addition of per litre: 15 g bacto-agar and 400 mg G418 Geneticin (Sigma). G418 was added when the medium had cooled to approximately 55°C.

Glycerol storage medium comprised of 2 x YEPD: yeast extract (20 g), bacto-peptone (40 g) and glucose (40 g), with the addition of 15% (v/v) glycerol. The dry components

were dissolved in distilled and de-ionised water and autoclaved at 121°C for 20 minutes. This medium was used for the storage of all yeast strains at –20°C or –80°C.

Control 1: Fresh medium with no added supplements.

Control 2: Fresh medium with added acetaldehyde.

Control 3: Fresh medium with added ethanol.

Experimental culture: fresh medium with added acetaldehyde and ethanol.

Figure 3.1: Experimental set-up in 2 litre sidearm flasks, all containing fresh medium with various supplements

3.2.2 Ethanol stress conditions during yeast growth

3.2.2.1 Standard culture conditions

Ethanol stress experiment: YEPD Liquid medium was added to clean sterile sidearm shaker flasks of appropriate volume as shown in Figure 3.1. The fresh medium was pre-warmed to 30°C to reduce the effect of temperature shock upon inoculum addition. The following four flasks containing fresh medium with or without acetaldehyde or ethanol were prepared immediately prior to the growth experiment: control flask 1 contained no supplements, control flask 2 contained acetaldehyde, control flask 3 contained added ethanol and experimental flask 4 contained added acetaldehyde and added ethanol. Yeast liquid cultures were grown under aerobic conditions at 30°C in an orbital shaker-incubator (Innova 4230 refrigerated, New Brunswick Scientific, Edison, New jersey) at 120 rpm. From the each flask samples for optical density, viability by plate counts and samples for RNA extraction were taken at regular intervals during incubations.

Preparation of acetaldehyde stock: Analytical grade acetaldehyde (BDH Ltd. Poole, England) was used in this research. Prior to the addition of acetaldehyde to the experimental cultures, acetaldehyde stock was prepared. As acetaldehyde is volatile (boiling point 20°C), it was necessary to prepare the stock solutions at 4°C to minimise evaporation loss. All glassware and solutions used to prepare the stock were autoclaved and pre-cooled. The procedure was conducted in a 4°C cold room. Stock solution was prepared by adding the appropriate amount of acetaldehyde to 50 ml of cold sterile water in a 100 ml volumetric flask. The pipette tip was immersed under the water to reduce evaporation when acetaldehyde was added. The weight of the added acetaldehyde was recorded and the volumetric flask filled up to mark with cold sterile water, stoppered and mixed.

3.2.3 Growth of yeast on plates and liquid media

3.2.3.1 Yeast storage

For long-term storage, yeast cultures were kept in glycerol storage medium at -20°C or -80°C. Transfers from glycerol stocks were undertaken aseptically using barrier pipette

tips. Yeast was streaked on YEPD agar plates for short-term storage. YEPD Geneticin plates were used for the selection and short-term storage of gene knockout strains.

3.2.3.2 Growth of yeast on plates

Yeast cells from -20°C glycerol vials were thawed to room temperature and aseptically streaked onto YEPD or YEPD Geneticin plates, and incubated at 30°C for 2 – 3 days.

3.2.3.3 Inoculum preparation and experimental cultures

To prepare inocula for growth curve experiments, a loopful of cells was taken from plates, and used to inoculate 200 ml of YEPD fresh medium in a sterile conical flask. The yeast cells were grown overnight at 30°C in an orbital shaker at 120 rpm. The optical density reading at 620 nm (OD_{620}) of these cultures was used to determine the inoculum size required for an initial OD_{620} reading of 0.1 when transferred to 300 ml of fresh medium in a conical flask (parent culture). The parent cultures for growth experiments were grown in 0.5 litre conical flasks, plugged with cotton wool stoppers. Late-exponential phase parent culture cells (OD_{620} of approximately 1.0) were collected by centrifugation at 4,000 rpm (3,313 g) in a swinging rotor centrifuge (Sorvall® RT 7) for 5 minutes. The supernatant was discarded and the cells washed in pre-warmed fresh medium. The temperature was maintained at 30°C, during the wash procedure and transfers were performed aseptically in a laminar flow cabinet under a bunsen burner flame.

Aliquots of the cell suspension were then inoculated, to an initial OD_{620} of 0.1 (approximately 2×10^6 cell ml^{-1}), into the control and experimental flasks containing pre-warmed medium. Once inoculated, the cultures were quickly transferred to the shaker incubator and grown under aerobic conditions at 30°C and 120 rpm.

3.2.3.4 Harvesting cells for molecular work

Yeast cells were harvested from each culture at regular intervals. Samples were removed through a clamped glass sidearm using a sterile syringe. An initial 5 ml of culture was discarded from the sampling line before the required volume was collected

and backflow of sample was prevented to avoid contamination. Each sample comprised 100 ml culture in a pair of 50 ml falcon tubes, which were pelleted by centrifugation for 5 minutes at 4°C in a swing rotor centrifuge (Sorvall® RT 7 Centrifuge) at 4,000 rpm (3, 313 g). The supernatant was poured off and the pelleted cells were frozen in liquid nitrogen and stored in -80°C until required for RNA isolation.

3.2.3.5 Cell population

Cell viability was measured using plate counts. Samples of 100 µl aliquots of culture were serially diluted in 900 µl of YEPD medium in microfuge tubes, and then 100 µl of diluted sample was spread onto duplicate YEPD agar plates. Plates were incubated at 30°C for 2 – 3 days and counted immediately. Plates with cell counts in the range of 30 – 300 cells per plate were counted. The readings for each set of duplicates were averaged and multiplied by the dilution factor to give the viable cell population of the culture. Optical density was determined using a spectrophotometer at a wavelength of 620 nm. Samples were diluted in such a way that the measured extinction was between 0.1 and 0.5

3.3 Molecular work

A list of all buffers, solutions, reagents and a list of suppliers for molecular work are provided in Appendix I, section 1.1.

3.3.1 RNA extraction: RNase-free procedures

For extraction of high quality total RNA, all chemicals, water, plastic-ware and glassware used for RNA preparation were RNase-free. Glassware and spatulas were covered in foil and baked at 180°C for at least 12 hours prior to use, while plastic containers, electrophoresis tanks, trays and combs were sprayed with RNase ERASE (ICN) and rinsed with DEPC treated water. Disposable plastic ware (pipette tips and eppendorf tubes) were purchased RNase-free, and autoclaved prior to use. RNase-free barrier tips were used for pipetting. Glass beads/Micro-Dismembrator of 0.4 µm (B. Braun Biotech International) were acid washed by stirring for 10 minutes and soaked overnight. The beads were then washed extensively under running water and then

rinsed with DEPC treated water, dried in the drying oven and then baked at 180°C overnight in a baking oven.

Centrifugation, vortexing, electrophoresis, optical density (OD) determinations and visualizing RNA in electrophoresis gels were performed using the following:

- A centrifuge 5415C Microfuge bench top centrifuge (Eppendorf).
- A vortex MT19DL Deluxe bench vortex (Chiltern Scientific).
- DNA grade Agarose (Progen).
- A 4054 UV/visible (Pharmacia Biotech) or DU® 530 UV/visible (Beckman) spectrophotometers.
- A UV/visible Darkroom (Pathtech Pty Ltd) connected to Lawork™ analysis software and Digital Graphic printer Up-D890 and Intelligent Dark box II.

3.3.1.1 Total RNA extraction from *S. cerevisiae*

Frozen cell pellets from section 3.2.3.4 were thawed on ice and the number of viable cells ml^{-1} in each sample was calculated. Cells were resuspended in RNA buffer to cell density of 2 x 10^8 viable cells ml^{-1}. Total RNA was extracted from *S. cerevisiae* using the glass bead extraction method, essentially as described by Ausubel *et al.*, (1997). *S. cerevisiae* cell pellets of 2 x 10^8 cells were resuspended in 300 µl (1 x) RNA buffer and added to approximately 300 µl of chilled, acid-washed 0.4 µm glass beads (Sigma). Samples were kept on ice throughout the procedure. The mixture was vortexed for 3 minutes (alternating one minute vortexing with one minute on ice x 3). Samples were centrifuged at 12,000 g for one minute to pellet cell debris and the upper phase transferred to a fresh microfuge tube. The following two extraction methods were used:

Trizol® Reagent (Invitrogen) extraction method: According the manufacture's instructions, supernatants containing 2 x 10^8 cells was mixed with 1.0 ml Trizol® Reagent (Invitrogen) and incubated for 5 minutes at room temperature. Chloroform (0.2 ml) was added and mixed by shaking vigorously for 15 seconds and then incubated at room temperature for 2 – 3 minutes. The mixture was centrifuged at 12,000 g for 15 minutes at 4°C. Following centrifugation, the mixture was separated into phases, the upper aqueous phase was transferred to a fresh tube and precipitated by the addition of

0.5 ml of isopropyl alcohol, incubated at room temperature for 10 minutes and centrifuged at 12,000 g at 4°C. Following the centrifugation the supernatant was discarded and the RNA pellet was washed (2 – 3 times) with 75% (v/v) ethanol. The washing was conducted by carefully resuspending the pellet with ice cold 75% ethanol and then centrifuging at 7,500 g for 5 minutes at 4°C. RNA pellets were air-dried at room temperature in a fume hood and resuspended in 25 µL RNase free water.

Phenol extraction method: Following glass bead extraction, the cell homogenate was centrifuged at 12,000 g for one minute to pellet debris and the supernatant was transferred to a fresh microfuge tube. An equal volume of phenol/choloroform/isoamyl alcohol (25:24:1) (pH 8.0) and 25 µl Tris buffer (0.5 M, pH 8.0) were added to the supernatant and the content was mixed by flicking it for 20 seconds; and then centrifuged for two minutes at 12,000 g to separate the precipitated protein phase. The upper phase was removed to a new microfuge tube and 3 volumes of chilled 100% ethanol and 25 µl sodium acetate (3.0 M) were added to precipitate the RNA. These solutions were mixed and allowed to precipitate for 2 hours at -80°C (or overnight) and centrifuged at 12,000 g for 10 min. Following centrifugation the supernatant was removed and the RNA pellets were washed 2 – 3 times with 500 µl of chilled 75% ethanol, re-centrifuged for 2 minutes at 12,000 g and the supernatant removed. The RNA pellets were air-dried and resuspended in 25 µl of RNase-free water.

To determine the quantity and quantity of RNA, 2 µl of total RNA solution was diluted with 598 µl of DEPC treated water and A_{260} and A_{280} values was determined. To test for purity and reproducibility of extraction across different time points RNA was visualized by electrophoresis of total RNA in RNase free 1% non-denaturing agarose gels in 1 x TAE buffer. A volume of 2 µl of ethidium bromide solution (1 g ml^{-1}) was added to agarose gel solutions. RNA samples (2.0 µl) were mixed with 2.0 µl of 6 x gel loading buffer and 4 µl of RNase free water and loaded onto the gel. Electrophoresis was conducted at 60 – 80 voltage typically for 45 – 60 minutes. Gels were viewed on a UV transilluminator and photographed using a UVP Laboratory Products gel documentation system. RNA samples were stored at -80°C.

3.3.1.2 DNase Treatment of Total RNA

Prior to cDNA synthesis all total RNA samples were DNase treated to remove any contaminating DNA. To 20 µl of RNA solution, 2 µl of 10 x DNase Buffer and 1.5 µl of DNase I enzyme (Ambion®) were added and mixed thoroughly by flicking the tube and then incubated at 37°C for 45 minutes. DNase I enzyme was then inactivated by the addition of 3 µl of DNase Inactivation Reagent each sample was mixed thoroughly with continued intermittent flicking during the 2 min incubation at room temperature. Samples were centrifuged at 12,000 g for 2 min and the supernatant was transferred to a sterile fresh eppendorf tube. To test the purity of DNase treated RNA, as in Section 3.3.1.1, 2.0 µl RNA gel loading buffer was added to 2.0 µl of DNase treated RNA and 4 µl of RNase free water. The RNA was then resolved electrophoretically in a 1% agarose gel. DNase treated RNA samples were stored at -80°C until required.

3.3.2 Gene expression analysis using Gene filter (Macro) arrays

Macroarray (Gene Filters) analysis was performed as described in the ResGen™ Technical Handbook GF100 (Research Genetics).

3.3.2.1 Gene array pre-hybridisation

The Yeast Index Gene Filters used in this work were purchased from Research Genetics. Filters were rinsed in boiling 0.5% SDS prior to use and placed in a hybridisation roller tube (35 x 150 mm) with the DNA side facing the interior of the tube. A volume of 5 ml MicroHyb solution (Research Genetics) was added to the roller tube containing the membranes. The MicroHyb solution was rolled around to saturate the membranes. Membranes were blocked by the addition of 5 µg PolydA (1 µg/µl) (Invitrogen). Any air bubbles between membranes and the tube were removed with forceps and the membranes placed so they were not overlapping. The membranes were pre-hybridised for 3 – 5 hours at 42°C in a XTRON HI 2002 hybridisation roller oven (Bartelt Instruments). The rotation of the tubes was set at approximately 10 rpm.

3.3.2.2 cDNA synthesis and labelling

Complementary DNA (cDNA) was prepared from RNA extracted using the Trizol method described in section 3.3.1.1. First strand cDNA synthesis was performed in 30 µl reaction volumes. Total RNA from 2 x 10^8 cells (approximately 1 µg for unstressed, control cells) and 5 µg oligo dT (1 µg/µl) was mixed in 8 µl of sterile distilled, de-ionized water. This was heated to 70°C then chilled on ice for 2 minutes. A volume of 6 µl First Strand Buffer (5x, Invitrogen), 1.5 µl of 20 µM dNTP mix (only dATP, dGTP and dTTP), 1.0 µl of 10 mM DTT, 1.5 µl of 300 U Superscript II reverse transcriptase (Invitrogen) and [α-^{33}P] dCTP (100 μCi, 3000 Ci/mmol; Perkin Elmer) were the added, mixed well and spun down. The mixture was incubated at 37°C for 90 minutes.

The cDNA was purified by passage through a sterile Bio-Spin 6 chromatography column (Bio-Rad); the volume was brought up to 100 µl with DEPC water. The Bio-spin 6 column was prepared for use by centrifugation at 1000 g in a bench top microfuge (Eppendorf) for 5 minutes. Column packing buffer was removed and the column placed into a new microfuge tube. The entire probe volume was loaded onto the Bio-Spin 6 column and centrifuged at 1000 g for 5 minutes. The purified probe was collected, denatured by heating to 100°C in a boiling water bath for 3 minutes, and chilled on ice for a further 2 minutes.

3.3.2.3 Hybridisation of labelled cDNA to probes on the gene filter

The purified and denatured cDNA from section 3.3.2.2 was pipetted into the pre-hybridisation mixture. The roller tube was vortexed thoroughly and allowed to hybridise overnight at 42°C. The rotation of the roller tube was set at approximately 10 rpm.

Following overnight hybridisation the hybridization solution was removed and the membranes were washed twice in 30 ml 2 x SSC, 1% SDS at 50°C for 20 minutes. These washes were performed in the hybridisation roller tube. A third wash was performed in 100 ml of 0.5 x SSC, 1% SDS in a plastic container at room temperature. The membranes were not allowed to overlap or adhere to the side of the container during the wash.

To prevent the membranes from drying after washing, they were placed on a piece of filter paper moistened with sterile distilled, de-ionized water and wrapped in plastic Cling Wrap®. All air bubbles were removed from between the plastic wrap and the filters.

3.3.2.4 Analysis of gene filters

Hybridised Gene Filters were placed in a cassette and carefully aligned with a BAS-MS 2340 phosphor-imaging screen (Fijifilm). Gene Filters were exposed to the phosphor-imaging screen for 48 hours to generate optimal signal intensities. Following this exposure, the phosphor-imaging screen was scanned using on FLA 3000 phosphor image analyser (Fujifilm) to obtain digital images, which were subsequently analysed using ArrayGauge™ software (version 1.3, Fujifilm). All spot intensities were normalised against the intensity of genomic DNA control spots. Comparisons of spot intensities for each time interval were calculated relative to the no-stress control.

3.3.2.5 Stripping gene filters for re-use

The gene filter membranes were stripped after analysis to facilitate their reuse. Membranes were placed into separate 500 ml solutions of boiling 0.5% SDS, covered, and agitated briskly for half hour. Following this, membranes were checked with a Geiger counter, placed on moistened filter paper and covered with plastic cling wrap. Again the filters were placed in a cassette, exposed to a phosphor-imaging screen for 48 hours, and scanned with a phosphor image analyser. The hybridisation intensity of the images was checked to make sure the stripping process was efficient. Following stripping, membranes were stored moist at 4°C until their next use. Gene filters were successfully stripped and reused a maximum of five times.

Table 3.2: Reagents for first strand cDNA synthesis (Mix 1): (supplier: Invitrogen).

Slide number	**1**	**2**	**3**	**4**	**5**
5 X first Strand Buffer (µl)	16.8	32.8	48.8	64.8	80.8
Oligo (dT)12-18 primer, 0.5 µg/µl (µl)	3.15	6.15	9.15	12.15	15.15
DTT, 0.1M (µl)	8.4	16.4	24.	32.4	40.4
Total Vol. (µl)	**28.35**	**55.35**	**82.35**	**109.35**	**136.35**

Table 3.3: Reagents for first strand cDNA synthesis (Mix 2): (supplier: Invitrogen).

Slide Number	**1**	**2**	**3**	**4**	**5**
*dNTPs {dATP, dCTP, dGTP) (10 mM)} (µl)	2.6	5.2	7.8	10.4	13
dTTP, 2.5 mM (µl)	3.4	6.8	10.2	13.6	17
Aminoallyl-dUTP 10 mM (µl)	1.8	3.6	5.4	7.2	9
Total Vol. (µl)	**13.0**	**15.6**	**23.4**	**31.2**	**39**

(Note: the stock concentrations of dNTPs (dATP, dCTP, dGTP and dTTP) was 100 mM. The dATP, dCTP and dGTP were diluted to 10 mM by taking 1 µl of stock in 9 µl of DEPC treated water). The dTTP was diluted to 2.5 mM by taking 1 µl of stock in 39 µl of DEPC treated water. *(For each dNTP 2.6 µl was added).

3.3.3 Gene expression analysis using glass chip (micro) arrays

Microarray analysis was performed as described in the following sections. All microarray slides were purchased from Clive and Vera Ramaciotti Centre for Gene Function Analysis, University of New South Wales.

3.3.3.1 cDNA synthesis for microarray analysis

First strand cDNA was prepared from phenol extracted RNA from yeast cell cultures, as described in section 3.3.1.1. A volume 18.7 µl (20-25 µg) of DNase-treated total RNA samples was mixed with 13.5 µl of **Mix 1** {according Table 3.2 reagent master Mix 1 of 28.35 µl was prepared for one slide (control and test sample)} and placed into PCR machine. The PCR machine was programmed at 65°C for 5 min and 42°C for 2 hrs and 15 min and final 65°C for 20 min. Following 65°C for 5 and 42°C for 5 min incubation the PCR reaction was paused and 5.95 µl of **Mix 2 (**from Table 3.3**)** and 2 µl of Superscript II enzyme were added directly to each sample the cycle was continued at 42°C for 2 hrs 15 min. Following this 4 µl EDTA (50 mM, pH 8.0) and 2 µl NaOH (10 M) were added, and the PCR cycler was set to 65°C for 20 min to hydrolyse the RNA. The reaction mixtures were then neutralized with 4 µl acetic acid (5 M).

3.3.3.2 Purification and labeling of PCR products

PCR products were purified on QIAquick® (QIAGEN) columns according the manufactures instructions. In brief, 150 µl PB buffer (phosphate buffer) was added to each PCR product and this was applied to the columns. An additional 150 µl PB buffer was used to rinse the tubes and this was also added to the columns. Columns were centrifuged at 3,185 g for 1 min, the flow through was discarded and a volume of 700 µl (70% v/v) ethanol was added to each column and incubated for 1 min. Following incubation, columns were centrifuged at 3,185 g for 1 min and the flow through was discarded (the washing step with 70% ethanol was repeated twice). Finally, columns were dried by centrifugation at 3,185 g for 1 min.

For elution of cDNA, columns were placed on fresh sterile microfuge tubes and 25 µl of DEPC-treated distilled water was added to each followed by incubation at room

temperature for 5 min. Columns were then centrifuged at 3,185 g for 1 min. Again 10 µl of DEPC-treated distilled water was added to each column and incubated at room temperature for 5 min, followed by centrifugation at 3,185 g for 1 min. A final volume of about 35 µl was collected. The collected cDNA samples were centrifuged in a Speedivac at a low speed for ~20 min to reduce the volume to 2 – 5 µl. Following the speedivac step, 9 µl of $NaHCO_3$ (0.1 M, pH 9) was added to each sample and mixed thoroughly by flicking it.

Following cDNA synthesis and purification, for indirect labeling aminoallyl-modified nucleotides (aminoally-dUTP) were used for the subsequent coupling to Cy3 and Cy5 fluorescent dyes. The fluorescent dyes, Cy3 and Cy5, that were used to label cDNA for microarray analysis were provided in pellet form by supplier (Amersham). Each dye was resuspended in 18 µL DMSO and aliquot of 2 µL were prepared into each eppendorf tubes, all aliquots were centrifuged in speedivac until dry pellets formed and stored at 4^0C until required. In darkness, 2 µl of DMSO stock solution was added to each Cy3 and Cy5 pellet, and resuspended by repetitive pipetting. In darkness 2 µl of Cy3 and Cy5 dyes were then added to corresponding tubes containing (2 – 5 µl PCR products plus 9 µL of $NaHCO_3$. The samples were mixed thoroughly by repetitive pipetting. Note that control samples were labeled with Cy3 (green) and experimental samples with Cy5 (red). The samples were incubated at room temperature in darkness for 1 hr 30 min.

3.3.3.3 Washes of labelled cDNA

Labelled cDNA was washed in darkness to remove unincorporated dyes. This was carried out by adding 65 µl of PB buffer to each sample, mixing thoroughly by repetitive pipetting and loading onto QIAquick® columns. Columns were centrifuged at 3,185 g for 1 min, and the flow through was discarded. To each column 700 µl of 70% (v/v) ethanol was added and incubated at room temperature for 1 min, then centrifuged at 3,185 g for 1 min, and the flow through discarded. The columns were dried by centrifugation at 3,185 g for 1 min. Columns were then placed in fresh sterile microfuge tubes, 25 µl of DEPC-treated distilled water was added to the centre of each column and incubated at room temperature for 5 min. The columns were then centrifuged at 3,185 for 1 min. Again, 10 µl of DEPC-treated distilled water was added

to the centre of each column and incubated at room temperature for 5 min. The columns were then centrifuged at 3,185 g for 1 min. The collected volumes of approx 35 µl of the labelled cDNA were reduced to 10 µl using a speedivac.

Finally, the two labelled cDNA preparations were combined in one tube to give total volume of 20 µl. These solutions were mixed thoroughly by repetitive pipetting. Each was then reduced from 20 µl to 5 µl using a speedivac at low speed for approximately 10 – 15 min. Finally, 85 µl of hybridisation solution was added to each tube (from Table 3.4) and incubated at 65°C for 5 min. These solutions were then cooled at room temperature in darkness. Samples of ~90 µl of the labelled cDNAs were loaded onto each slide in darkness and the slides were incubated in darkness at 37°C for 16 hrs.

Table 3.4: Hydridization solution mix per slide

Reagents	**Volume (µL)**
Dig Easy Hyb (filtered)	100
**Yeast tRNA (10 mg/ml)	5
*Salmon Herring Sperm (10 mg/ml)	2.75
Total vol.	**107.75**

Suppliers: *Invitrogen, **Ambion®

3.3.3.4 Washing and blocking microarray slides

Microarray slides were placed in a slide chamber which was then filled up with **solution 1** (0.1% Tritron X-100), and agitated gently for 5 min. Solution 1 was removed, then **solution 2** (4.38 mM HCl) was added and agitated gently for 2 min. Solution 2 was removed and fresh solution 2 was added and agitated gently for 2 min. Solution 2 was removed and **solution 3** (100 mM KCl) was added and agitated gently for 10 min. Solution 3 was removed and DEPC-treated distilled water was added and agitated gently for 1 min. DEPC-treated distilled water was removed and blocking buffer (25% Ethyene glycol and 0.01% HCl) was added and agitated gently at 50°C for 30 min. The blocking buffer was removed and DEPC-treated distilled water was added and agitated gently for 1 min. Slides were then air dried by centrifugation in falcon tubes at 830 g

for 10 min at 40°C using a swing out centrifuge rotor (Sorvall® RT 7). Dry slides were placed at room temperature in a light blocking slide box until required. Formula for all solutions used for washing are provided in Appendix I, section 1.1.

3.3.3.5 Hybridized slide washes

Following overnight incubation of the slides with the labeled cDNA, the cover-slip was removed by immersing in 1 x SSC + 0.1% SDS in a 50 ml falcon tube; the cover-slip was allowed to float off. The slide/s were then placed into 50 ml of 1 X SSC + SDS in a falcon tube, and this was gently agitated for 20 min. The wash solution was removed and this step repeated twice. Following the above three washes, further washes were performed in following solutions:

- 1 X SSC + SDS with gentle agitation for 15 min.
- 1 X SSC with gentle agitation for 10 min.
- 1 X SSC + Triton X-100 with gentle agitation for 10 min.
- 1 X SSC with gentle agitation for 10 min.
- 0.5 X SSC with gentle agitation for 10 min. This last step was repeated.

Washed slides were air dried by centrifuging at 830 g (using Sorvall® RT 7 Centrifuge) for 10 min at 40°C. Labelled slides were stored in the dark. Prior to scanning, the back of each slide was cleaned with 95% ethanol to remove any dust or smudges. Slides were scanned using a GenePix-Pro 4000, scanning machine (Axon), at the Australian Genomic Research Facility (AGRF).

3.3.3.6 Analysis of micorarray

Unreliable signals were filtered out and data normalized using GenePix Pro 5.0 software. GeneSpring® software was used to determine genes as more- or less-highly expressed when the difference in expression level between stressed and control cultures was reproducibly greater than three-fold in at least two replicates. Furthermore, to minimize systematic variations within or between slides (e.g. dye incorporation differences) in the measured gene expression levels of the two co-hybridized mRNA samples, the Locally Weighted Scatterplot Smoothing (LOWESS) normalization method was used. The *Saccharomyces cerevisiae* genome database (SGD;

http://www.yeastgenome.org) and yMGV (http://transcriptome.ens.fr/ymgv/) were used to group ORFs according their molecular and biological function.

3.3.4 First strand cDNA synthesis for PCR and Real-Time PCR analysis

cDNA was prepared from RNA extracted from equal number of cells of treated and untreated cells. According the reagent supplier's protocol (Invitrogen): 1 µl (1 ng – 5 µg) DNase treated total RNA, 1 µl oligo $(dT)_{12-18}$, 1 µl (10 mM) dNTP mix and 12 µl sterile distilled water were added and heated at 65°C for 5 min, and then quickly chilled on ice. The contents were collected by brief centrifugation and then 4 µl of 5 x First-strand Buffer, 2 µl (0.1 M) DTT and 1 µl RNase inhibitor were added and the contents gently mixed at 42°C for 2 min. Following the incubation, 1 µl (200 units) SuperScript™ II RT was added to give a final volume 23 µl. This was mixed by gentle pipetting then incubated at 42°C for 50 min. Finally, the reaction was inactivated by heating it at 70°C for 15 min. This cDNA was ready to be used as a template for amplification in PCR and Real Time PCR reactions.

3.3.5 Quantitative Real-Time (kinetic) PCR analysis

SYBR green is a dye that binds to double but not single stranded DNA is used in quantitative PCR reactions. All Real-Time PCR reactions were performed using a Roche LightCycler Instrument (Catalogue # 2 011 468). The reagents used were contained within the LightCycler FastStart DNA Master SYBR Green I kit (catalogue # 3 003 230). The kit components include: Taq DNA polymerase, reaction buffer, dNTP mix and SYBR Green I dye. Only template cDNA, primers, appropriate concentration of $MgCl_2$ and water was added. All Real-Time reactions were performed in a Roche LightCycler according to the manufacturer's instructions.

3.3.5.1 Specific primers for Real-Time PCR

Specific primers were designed to gene-specific target sequences within the sequences of transcripts to be assayed. These were designed using the *Saccharomyces cerevisiae* genome database (SGD;www.yeastgenome.org). These primers were 'tested' using the

BLASTn sequence alignment algorithm; primers were deemed suitable if they would not bind to a template other than the one of interest. These primers comprised 20 – 25 nucleotides with 50 – 60% G+C content, and the target amplicon length was between 100 and 400 bp. Quality of primers was further tested to avoid: primer dimmer and primer hairpin formation, and nonspecific binding using Primer3 website: (http://cbr-rbc.nrc-cnrc.gc.ca/cgi-bin/primer3_www.cgi). Primers were purchased from Invitrogen Custom Primers.

Two negative controls were prepared, one in which no Reverse Transcriptase enzyme was added to the reaction and the other in which no cDNA-template was added. For experiments in this thesis, RNA templates were derived from the same samples as those used in gene array experiments unless otherwise stated.

3.3.5.2 Magnesium chloride titration to optimise Real-Time PCR reaction

The optimal magnesium concentration for LightCycler Real-Time PCR varies between 2 – 5 mM. Hence, prior to starting an experiment, it was necessary to determine the concentration that provided optimal assay performance for each primer pair. The $MgCl_2$ concentration that gave the most efficient amplification PCR product value was chosen for subsequent experiments.

3.3.5.3 Real Time PCR reactions

cDNA for Real Time PCR reactions was prepared as described in section 3.3.4. It was used at a dilution of 1:20 and 2 μl of this was added to the Real-Time PCR reaction. The PCR amplification protocol was run using the following set-up program: 95°C for 10 min followed by 35 cycles of 95°C for 10 s, 60°C for 10 s, and 72°C for 15 s, then cooling down 40°C for 30 s.

3.3.5.4 Real-Time PCR data analysis

Once the melting curve confirmed that no primer dimer or non-specific products were formed, relative quantification analysis was conducted using the "quantification" mode. The amplification curve was displayed along with a table giving values for the crossover points. These values were indicative of the cycle at which amplification of

the product reached detectable threshold level. The more template present at the beginning of the reaction, the fewer the number of cycles it takes to reach a point at which a fluorescent signal can be recorded as statistically significant above background. In other words, the earlier the product was amplified, the greater the amount of template present. The relative concentration of transcript in each cDNA sample was quantified by direct comparison of cross point values between test and control samples employing the LightCycler logarithmic software. The fold alteration (FA) was calculated using the formula $FA = 2^{(CP1-CP0)}$, where the CP1 represents the crossing point[1] of RNA from the stressed cells sample and CP0 unstressed cells.

3.3.6 Promoter analysis

A region spanning 800 bp upstream of the transcription start sites of genes identified in array analysis as having increased expression was recovered using the Regulatory Sequence Analysis Tools (RSAT) databases (http://rsat.ulb.ac.be/rsat/) and www.yeastract.com. Upstream sequences were then searched for specific sequence motifs using RSAT.

3.3.7 Yeast DNA isolation

Frozen cell pellets containing approximately 10^8 cells were thawed on ice and were resuspended to a volume of 500 µl in DNA extraction buffer to which approximately 500µl of chilled, acid-washed 0.4 µm glass beads (Sigma) were added. Samples were kept on ice throughout the procedure. The mixture was vortexed for a total of 3 minutes (alternating one minute vortexing with one minute on ice). Samples were centrifuged at 12,000 g in a bench top microfuge (Eppendorf) for one minute to pellet cell debris and the upper phase was transferred to a fresh microfuge tube. Equal volumes of this upper phase cell pellets, Phenol/chloroform/isoamyl alcohol (25:24:1) and 5 µl Tris buffer (0.5M, pH 8.0) were combined and mixed by inversion and incubated for 5 min at room temperature. Precipitated proteins were removed from DNA by centrifugation for 5 minutes at 12,000 g. The upper aqueous layer was transferred to a new microfuge tube

[1] Crossing points and crossover values are when the log-linear part of the amplification curve crosses a fluorescence background threshold.

and 10 µl RNase (mg/ml) enzyme was added and incubated at 37°C for 40 min. Following incubation 1/10 volume (50 µl) of sodium acetate and 1.0 ml chilled 100% ethanol were added. The supernatant was removed, following three-minute centrifugation at 12,000 g, and 500 µl of ice-cold 70% ethanol was added, mixed, and centrifuged as previously. The supernatant was removed and the DNA pellet air-dried for 10 – 15 min. DNA was resuspended in 25 µl of autoclaved double distilled water. DNA was subsequently visualized on a 1% agarose gel (essentially as described in Section 3.3.1.1) and analysed by spectrophotometrically at 260 and 280 nm using an Ultraspec III UV/Vis spectrophotometer (Pharmacia). The reading at 260 nm allowed for calculation of nucleic acid concentration in the sample and the ratio of ODs at 260 nm and 280 nm was used to assess quality.

3.3.8 Confirmation of gene knockouts in deletion strains supplied for this project

The gene knockout yeast strains used for this work were from the EUROSCARF deletion collection and were kindly provided by the Australian Wine Research Institute as part of collaborative project. The EUROSCARF deletion strains were created by gene replacement, in which targeted genes are replaced with DNA cassette carrying a Kanamycin resistance that confers resistance not only to Kanamycin but also to Geneticin (G418).

The knockout status of the strains used for this project mutants were confirmed by plating onto medium containing G418 and by PCR. The primer pairs for such PCR reactions for the genes of interests and *KanMx4* were designed using *Saccharomyces cerevisiae* Genome Deletion Project website. http://www-sequence.stanford.edu/group/yeast_deletion_project/Enter_DB.html. Primers were purchased from Invitrogen Custom Primers.

For PCR reactions the genomic DNA was isolated as described in section 3.3.7 or cell colonies were picked with sterile pipette tips and smeared into a PCR tube containing 20 µl of sterile water. The cellular material was microwaved on high for 1.0 minute and immediately placed on ice. PCR-SuperMix (Invitrogen) reagent was used, it was added along with the following components into PCR tubes: 23 µl PCR SuperMix, 1 µl primer

mix and 1 µl DNA template. The PCR amplification protocol consisted of 2 minutes at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 90 s at 72°C. PCR product sizes were confirmed by gel electrophoresis on 1% agarose gel.

CHAPTER 4

The physiological role of acetaldehyde in promoting yeast tolerance to ethanol stress

4.1 Introduction

Yeast encounters a range of stresses during fermentation that compromise productivity of the brewing process and other processes. Some of the principal stresses that yeast cell encounter during fermentation are exposure to hyperosmotic stress and high ethanol concentrations. When yeast cells are exposed to high concentrations of ethanol there is a decrease in cell viability, ethanol yield and productivity (Chandler *et al*., 2004; Ingram, 1986 and Casey *et al*., 1984). If the underlying mechanisms responsible for the inhibitory effects of ethanol on yeast growth were understood, then this information could potentially be used to modify either the yeast environment or the genetic make-up of yeast strains to improve their tolerance to ethanol.

When actively growing yeast are exposed to a step change in ethanol concentration, cell division is halted, and provided the change is non-lethal, growth will recommence after a period of time. This growth lag period is recognized to be an adaptation period during which time the cells are undergoing structural and metabolic changes to adapt to their new environment. These physiological changes to the cell are usually prescribed by changes in gene expression. Recent research in this area has characterized the gene expression changes during the lag period that occur during ethanol stress and a large number of genes were observed to respond to ethanol stress (Alexandre *et al*., 2001, Chandler *et al*., 2004). Although some of these genes would have a crucial role in directing the important physiological changes in the cell that are necessary to adapt to the stress, many of the genes will most likely have only a minor or no role in stress adaptation. Identifying the important genes is challenging given the large number of genes affected by ethanol stress. It may be possible to reduce the number of candidate genes associated with ethanol stress response by examining yeast gene expression during ethanol stress in the presence of acetaldehyde. As described in Chapter 1 (section 2.5.3) the addition of acetaldehyde to ethanol-stressed yeast stimulated growth

recovery resulting in shorter lag periods. Comparing gene expression profiles in ethanol-stressed cultures in the presence and absence of acetaldehyde may identify a subset of genes that are differentially expressed in the acetaldehyde-stimulated cultures, and which may have a crucial role in yeast adaptation to ethanol stress. The aim of this research project is to determine gene expression levels during an ethanol-induced adaptation period (lag phase) in yeast in the presence and absence of acetaldehyde.

Before investigating the effect of acetaldehyde on gene expression during ethanol stress, it was necessary to determine the environmental conditions and methodology that induce a reproducible, non-lethal and clearly defined ethanol-induced growth lag period. This was achieved by inoculating late exponential phase yeast into medium containing a range of ethanol concentrations (0-9% v/v) and then measuring the resulting lag period. The induced lag period needed to be of sufficient duration (around 4-7 hours) to allow multiple time point sampling during the adaptation phase. It was also important to determine the appropriate concentration of acetaldehyde needed to reduce the lag period in the ethanol-stressed cultures. This chapter will focus on the growth of *S. cerevisiae* during ethanol stress in the presence and absence of acetaldehyde.

4.2 The effect of ethanol on the growth of *S. cerevisiae* PMY1.1

4.2.1 Experimental design and its parameters

For these experiments it was important to ensure that ethanol stress was the only adversary condition inducing the lag period in the ethanol-stressed cultures. To ensure this, all inocula for the experimental cultures were prepared from the same parent culture that was in the late exponential phase. The inoculum was washed with fresh medium at 30°C prior to inoculation to prevent the carryover of by-products from the parent culture that may influence the adaptation rate of yeast cells to ethanol. All experimental cultures had the same initial cell population at the time of inoculation. Experimental cultures were deemed to be ethanol-stressed when they exhibited a lag period compared to unstressed control cultures.

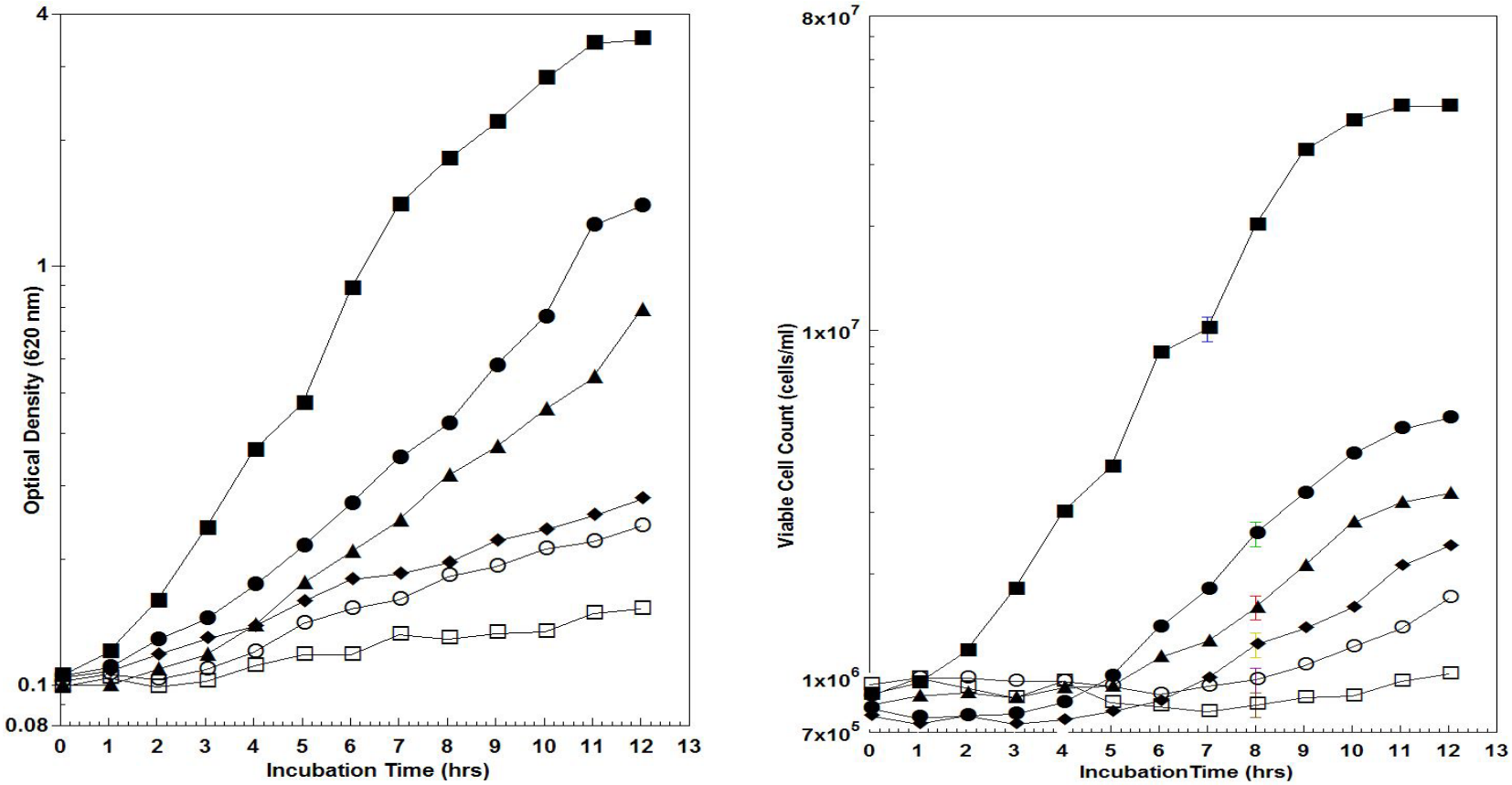

Figure 4.1: The effect of various ethanol concentrations on the growth of *S. cerevisiae* PMY1.1. Cells were inoculated into YEPD medium only (■), or medium containing 5% v/v (●), 6% v/v (▲), 7% v/v (♦), 8% v/v (○), or 9 % v/v (□) added ethanol. The cultures were incubated aerobically at 30°C/140 rpm.

4.2.2 Effect of ethanol on the lag period of *S. cerevisiae* PMY1.1

To determine the most suitable ethanol concentration for the stressed experimental cultures in this study, the growth profile of *S. cerevisiae* PMY1.1 was investigated in the presence of different ethanol concentrations in YEPD medium and grown batch-wise under aerobic conditions. Late exponential phase parent cells were washed and inoculated into fresh medium containing 0%, 5%, 6%, 7%, 8% and 9% (v/v) added ethanol. The growth of these cultures was monitored by OD_{620} readings and viable plate counts at hourly intervals over a 12-hour period. Most commentary on the growth profile of the yeast cultures are based on the viable plate count since they represent the viable cell population in the cultures.

The lag periods induced by step changes in ethanol concentration of 0%, 5%, 6%, 7%, 8% and 9% (v/v) are shown in Figure 4.1. This preliminary experiment with *S. cerevisiae* PMY1.1 indicated that ethanol concentrations in the range 5-9% (v/v) would induce a non-lethal growth lag period. According to the viable cell population, the lag periods, doubling times and growth rates were calculated from Figure 4.1. Yeast exposed to 5, 6, 7, and 8% (v/v) ethanol stress had lag periods of 4.6, 5.5, 6.1 and 7.8 hours respectively (Table 4.1) and doubling times of 2.3, 2.9, 3.6 and 5.8 hours respectively. In the presence of 9 % (v/v) ethanol the cells did not recover from the stress over the 12-hour period of the experiment, however, a constant viable cell population was maintained. In all five ethanol-stressed cultures the viable cell population either increased or was constant, suggesting that initial cell viability was not compromised even by the 9% step change in ethanol concentrations. The 9 % (v/v) ethanol stress appeared to be the upper threshold of cell recovery from ethanol stress over a 12-hour period. These experiments were repeated to determine the reproducibility and accuracy of the above experiments (duplicate experiments conducted), and similar results were obtained for the lag periods and growth rates (Appendix II, Figure 1).

Based on these results, the 6.1-hour lag period arising from a 7% (v/v) step change in ethanol concentration was considered to be of appropriate duration to allow the lag reducing effects of added acetaldehyde in future experiments to be clearly defined.

Yeast inoculated into medium containing 7% (v/v) ethanol did not have any apparent loss in viable cell population and the cells were able to recover sufficiently during the lag phase to enter a clearly defined exponential phase. It was therefore decided to use an ethanol concentration of 7% (v/v) to induce an ethanol stress in subsequent experiments.

Table 4.1: Effect of ethanol and acetaldehyde on the growth of *S. cerevisiae* PMY1.1

Fig.	Ethanol (v/v)	Added Acetaldehyde (g/l)	Lag Time (h)	Doubling Time (h)	Specific Growth Rate (h^{-1})	Reduction in lag (%)
4.1	0%	0.0	1.0	1.8±0.05	0.375±0.1	N/A
	5%	0.0	4.6	2.3±0.10	0.30±0.01	N/A
	6%	0.0	5.5	2.9±0.15	0.24±0.01	N/A
	7%	0.0	6.1	3.6±0.40	0.19±0.02	N/A
	8%	0.0	7.8	5.8±0.6	0.12±0.01	N/A
	9%	0.0	N/A	N/A	N/A	N/A
4.2	0%	0.0	0.5	1.9±0.06	0.37±0.01	N/A
	7%	0.0	6.2	3.5±0.15	0.20±0.015	N/A
	7%	0.01	5.0	3.0±0.15	0.23±0.02	19
	7%	0.05	4.4	2.7±0.04	0.26±0.015	29
	7%	0.10	2.0	2.5±0 09	0.28±0.01	68
	7%	0.20	4.6	2.8±0.01	0.25±0.015	26
	7%	0.30	5.2	2.8±0.10	0.25±0.01	16
	7%	0.40	6.4	3.2±0.20	0.22±0.01	N/A

N/A:- Not applicable

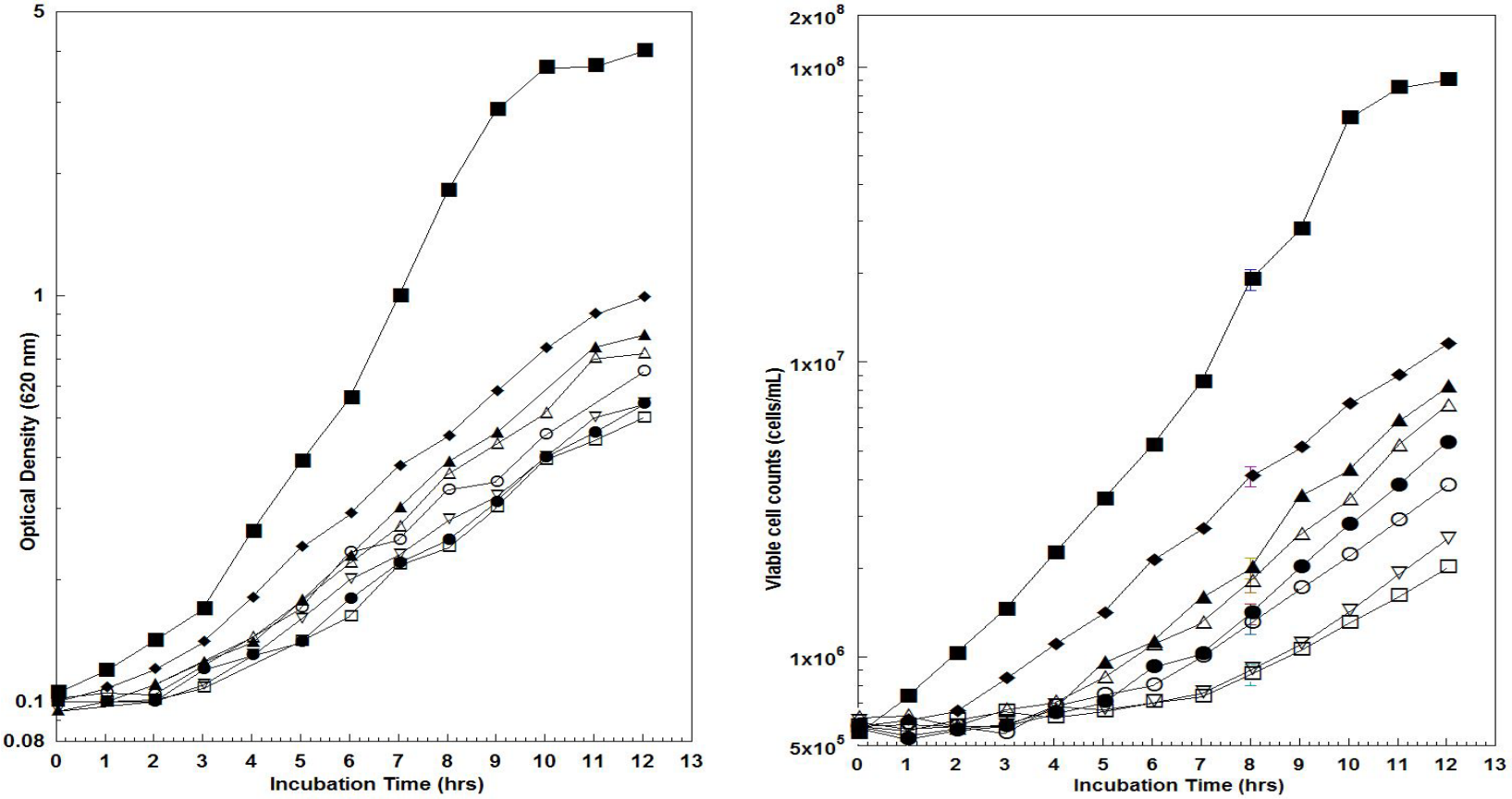

Figure 4.2: Effect of different concentrations of acetaldehyde on *S. cerevisiae* PMY1.1 growth in the presence of 7% (v/v) ethanol in YEPD medium: medium without ethanol or acetaldehyde (■), 7% (v/v) ethanol only (▽), 7% (v/v) ethanol & 0.01 g/l acetaldehyde (●), 7% (v/v) ethanol & 0.05 g/l acetaldehyde (▲), 7% (v/v) ethanol & 0.1 g/l acetaldehyde (♦), 7% (v/v) ethanol & 0.2 g/l acetaldehyde (Δ), 7% (v/v) ethanol & 0.3 g/l acetaldehyde (○) and 7% (v/v) ethanol & 0.4 g/l acetaldehyde (▽). The cultures were incubated aerobically at 30°C/140 rpm.

4.2.3 Effect of added acetaldehyde on the growth of ethanol-stressed *S. cerevisiae* PMY1.1

Having determined the appropriate ethanol concentration for the experimental work, the next step was to determine a concentration of added acetaldehyde that would cause a significant reduction in the ethanol-induced lag period. The added acetaldehyde concentrations (around 0.08 g/l) used to achieve this in the literature were approximated in the experiments in this project, as well as slightly higher and lower acetaldehyde concentrations to account for differences in environmental conditions and yeast strain.

The growth of 7% ethanol-stressed *S. cerevisiae* PMY1.1 cultures was investigated in the presence of added acetaldehyde concentrations in the range 0.01-0.4 g/l (Figure 4.2). The lag periods, doubling times and growth rates (Table 4.1) were calculated from Figure 4.2. The results showed that added acetaldehyde concentrations of 0.01, 0.05, 0.1 and 0.2 g/l reduced the ethanol-induced lag period by 19%, 29%, 68%, and 26% respectively, and increased the specific growth rate by 15%, 30%, 40% and 25% respectively. Acetaldehyde concentrations less than 0.05 g/l had only a slight stimulatory effect on the growth of ethanol stressed cultures, whilst concentrations above 0.3 g/l had no significant effect on the lag period or the specific growth rate. An acetaldehyde concentration of 0.1g/l had the largest stimulatory effect on the adaptation period and growth rate of ethanol-stressed yeast. These experiments were repeated to determine the reproducibility and accuracy of the above experiments and similar results were obtained as above.

The lag-reducing effect of 0.1 g/l added acetaldehyde on a 7% (v/v) ethanol-stressed culture of *S. cerevisiae* PMY1.1 in YEPD medium was repeated (Figures 4.3 and 4.4). The results show that both the lag period and exponential growth rate are significantly affected by the added acetaldehyde compared to the ethanol-stressed control. Cultures subjected to 7% (v/v) ethanol stress only, had a lag period of approximately 6.1 hours, however, when added acetaldehyde (0.1 g/l) was present in the ethanol-stressed cultures, the lag period was reduced to approximately 2 hours which

represents a lag period reduction of approximately 67% (Figures 4.3 and 4.4; Table 4.2). An acetaldehyde concentration of 0.1 g/l provided the optimum stimulatory effect on the ethanol-stressed yeast, yet it was not inhibitory to the yeast in the absence of ethanol. For these reasons 0.1g/l added acetaldehyde was chosen as the acetaldehyde concentration to be used for the remainder of the experimental work.

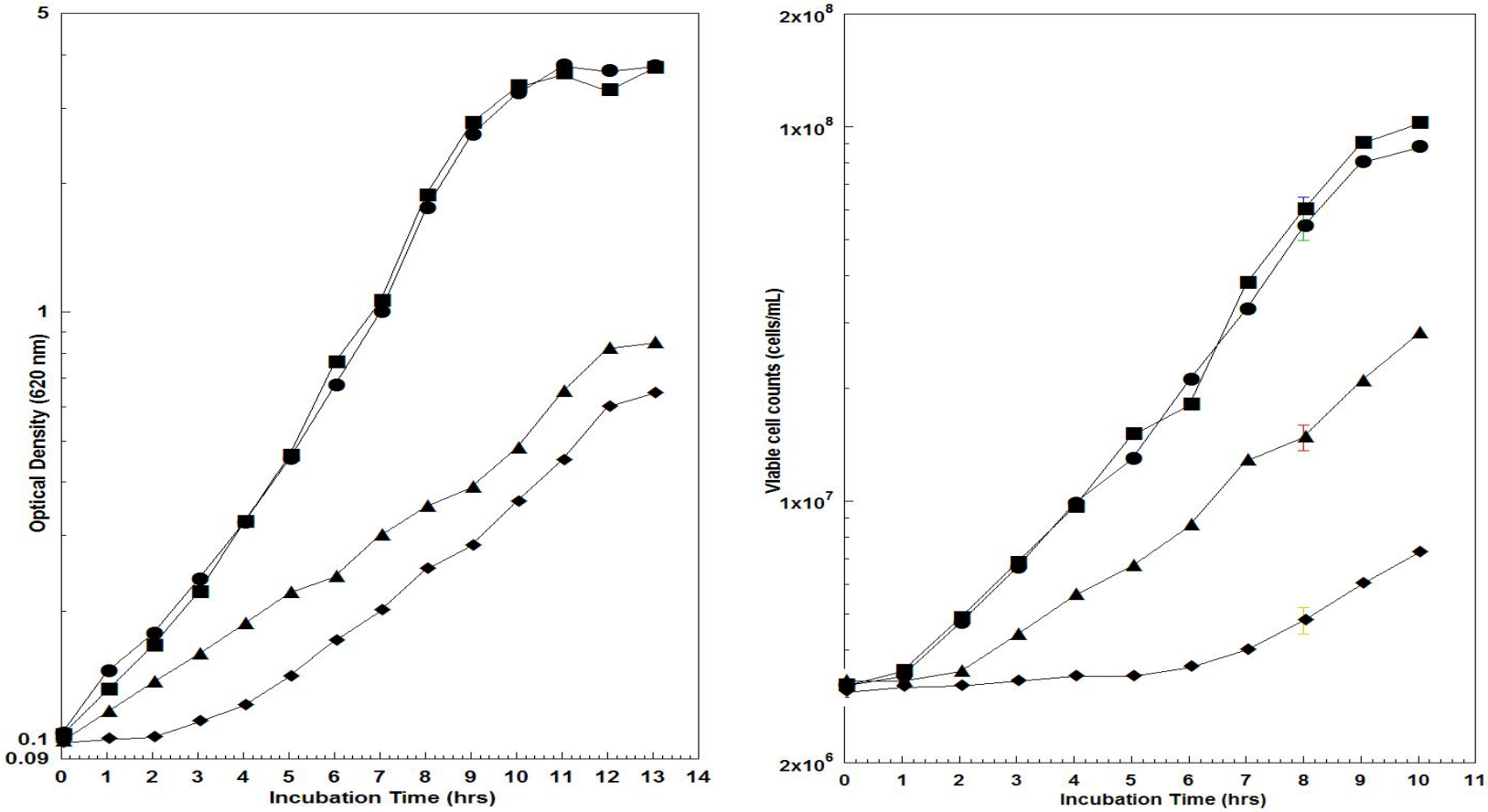

Figure 4.3: Effect of added acetaldehyde on *S. cerevisiae* PMY1.1 growth in the presence of 7% (v/v) ethanol in YEPD medium: YEPD medium without ethanol and acetaldehyde (■), YEPD with 0.1 g/l acetaldehyde (●), YEPD with 7% (v/v) ethanol (♦) and YEPD with 7% (v/v) ethanol & 0.1 g/l acetaldehyde (▲). The cultures were incubated aerobically at 30°C/140 rpm.

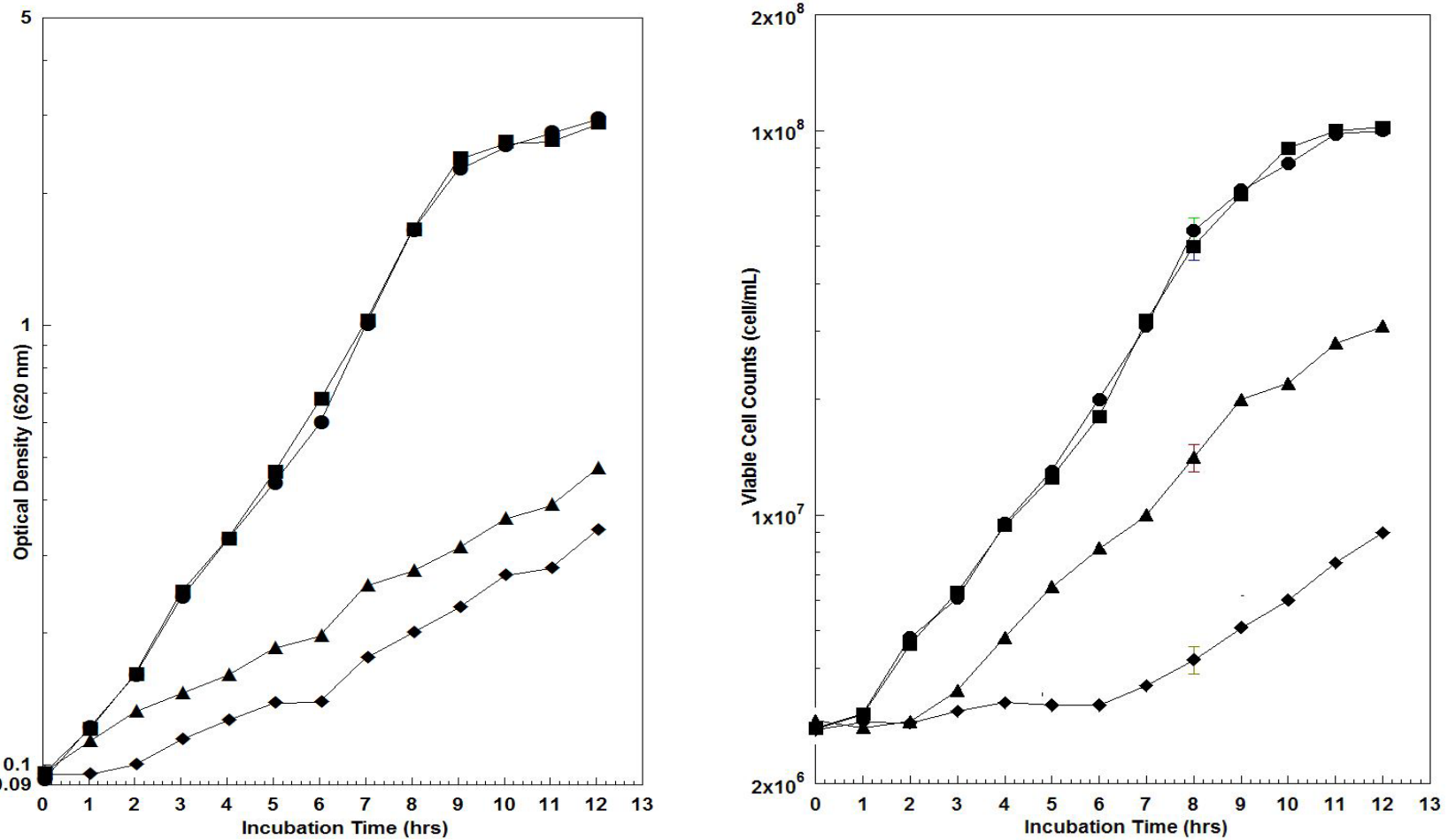

Figure 4.4: Effect of added acetaldehyde on *S. cerevisiae* PMY1.1 growth in the presence of 7% (v/v) ethanol in YEPD medium: YEPD medium without ethanol and acetaldehyde (■), YEPD with 0.1 g/l acetaldehyde (●), YEPD with 7% (v/v) ethanol (♦) and YEPD with 7% (v/v) ethanol & 0.1 g/l acetaldehyde (▲). The cultures were incubated aerobically at 30°C/140 rpm.

Table 4.2: Summary showing the effect of ethanol on the growth of *S. cerevisiae* in the presence and absence of acetaldehyde.

Figures	Ethanol (v/v)	Added acetaldehyde (g/l)	Lag Time (h)	Doubling Time (h)	Growth Rate (h^{-1})	Reduction in lag (%)
4.1	0%	0.0	1.0	1.8±0.05	0.375±0.1	N/A
	5%	0.0	4.6	2.3±0.10	0.30±0.01	N/A
	6%	0.0	5.5	2.9±0.15	0.24±0.01	N/A
	7%	0.0	6.1	3.6±0.40	0.19±0.02	N/A
	8%	0.0	7.8	5.8±0.6	0.12±0.01	N/A
	9%	0.0	N/A	N/A	N/A	N/A
4.2	0%	0.0	0.5	1.9±0.06	0.37±0.01	N/A
	7%	0.0	6.2	3.5±0.15	0.20±0.015	N/A
	7%	0.01	5.0	3.0±0.15	0.23±0.02	19
	7%	0.05	4.4	2.7±0.04	0.26±0.015	29
	7%	0.10	2.0	2.5±0 09	0.28±0.01	68
	7%	0.20	4.6	2.8±0.01	0.25±0.015	26
	7%	0.30	5.2	2.8±0.10	0.25±0.01	16
	7%	0.40	6.4	3.2±0.20	0.22±0.01	N/A
4.3	0%	0	0.8	1.9±0.10	0.37±0.01	N/A
	0%	0.1	0.8	1.9±0.10	0.37±0.01	N/A
	7%	0.0	6.1	3.5±0.23	0.20±0.015	N/A
	7%	0.1	2.0	2.6±0.20	0.27±0.010	67
4.4	0%	0.0	0.8	1.9±0.15	0.375±0.01	N/A
	0%	0.1	0.8	1.9±0.15	0.375±0.01	N/A
	7%	0.0	6.0	3.6±0.1	0.19±0.015	N/A
	7%	0.1	2.0	2.5±0.2	0.28±0.01	67

4.3 Discussion

4.3.1 Ethanol stress and acetaldehyde stimulation

Although there are many publications citing the effect of ethanol stress on yeast cells, comparing the results obtained in this project with published work must take into account the different experimental conditions used by other workers that can affect the response of yeast during an ethanol-induced lag phase. Some of these experimental variables such as yeast strain, inoculum size, medium type and other environmental conditions have been reported to have a significant influence on the length of an ethanol-induced lag period (Barber *et al.,* 2002; Emslie 2002, Stanley *et al*., 1997, 1993). The lag period is reduced when inoculum size is increased and when cells are grown in rich medium compared to defined medium (Barber *et al.,* 2002; Stanley *et al.,* 1997; Stanley and Pamment, 1993). For example, when *S. cerevisiae* X2180-1A from a late exponential phase parent culture was washed and inoculated at initial cell populations of 5 x 10^6 cells/ml and 5 x 10^4 cells/ml into rich medium containing 4% (v/v) ethanol, the subsequent lag periods were 2.6 and 3.6 hours respectively (Stanley *et al.,* 1997; Stanley and Pamment, 1993). When defined medium containing 4% ethanol was inoculated with the same strain to the same initial cell populations, the lag periods were around 3.4 and 4.86 hours respectively. The inoculum size-dependence of yeast cultures for ethanol stress-adaptation is an important observation since it indicates that extracellular factors may play a role in ethanol adaptation. Stanley *et al*., (1997), attributed the inoculum dependent lag-reducing effects to the accumulation of metabolites, which enable cultures to adapt to ethanol stress and recommence growth. When low inoculum sizes are used (below 10^5 cell/ml), the metabolite is either not produced or not present in sufficient concentration to affect the lag period. Walker-Caprioglio and Park (1987) and Stanley *et al*. (1997) speculated that the lag-reducing effect is due, in part, to acetaldehyde excretion.

It has been reported that the magnitude of the lag reducing effect of added acetaldehyde on ethanol-stressed yeast depends on the yeast strains being used (Barber *et al*., 2002 and Stanley *et al*., 1997). Barber *et al.* (2002), used *S. cerevisiae* strains (TWY-397, T2-3C, T2-3D and GG919) grown in complex medium containing 60 g/l ethanol under

aerobic conditions. All cultures showed that added acetaldehyde significantly reduced the lag period and increased specific growth rate. However, there was variability in the response of the different *S. cerevisiae* strains showing a lag period reduction ranging from 33-78% depending on the strains. Thus, the lag reducing ability of acetaldehyde on ethanol-stressed cultures varies widely according to yeast strains (Barber *et al*., 2002).

Given the many environmental variables that influence the extent of the lag reducing effect by acetaldehyde, it was important for this project that the effect of such environmental factors in the adaptation period been kept to a minimum. In this project, all inocula in the growth curve experiments were washed to avoid the carryover of lag affecting substances from the parent culture, and initial cell populations in the experimental cultures were approximately the same within each experiment and across experiments, although in the latter case there was a slightly greater variation in the initial cell populations (6×10^5 - 3×10^6 cell/ml).

4.3.2 Effect of ethanol on the growth of *S. cerevisiae* PMY1.1

Much of the early-published work on ethanol toxicity and stress was carried out by exposing cells to a relatively high ethanol concentration for a short period of time (Rosa and Sa-Correia 1991; Costa *et al*., 1997). In contrast, the ethanol-induced growth lag phase described in this project is a period for observing adaptative changes of cells exposed to non-lethal ethanol stress. The experiments conducted in this chapter were designed to determine a non-lethal ethanol concentration that would provide a level of stress sufficient to induce a 4-7 hour lag period. This condition subjects the cells to a growth inhibitory stress, which is not too severe as to prevent adaptive metabolic and molecular events to take place. There are few publications that examine the lag period adaptation of cells to non-lethal ethanol stress, of these Chandler *et al*. (2004) and Emslie (2002) used *S. cerevisiae* PMY1.1 (the same strain as the one used in this work). Chandler *et al*. (2004) reported that when cells were subjected to 5% and 7% (v/v) added ethanol concentrations in defined medium, it induced lag periods of 3 and 6 hours respectively, while cultures exposed to a 10% (v/v) ethanol stress did not recover from the stress over a 12 hour period, even though cell viability did not decrease during that

time. Similarly, Emslie (2002) investigated *S. cerevisiae* PMY1.1 in YEPD medium containing 2%, 3%, 4%, 5%, 6%, 7%, 8% and 10% (v/v) ethanol concentrations and its growth compared to a control culture without added ethanol. The results showed that cultures subjected to ethanol concentrations less than 4% (v/v) did not have a sufficient lag period for multiple sampling during the lag period for RNA analysis, whereas cultures containing greater than 8% ethanol had considerable lag period but had low subsequent growth rate. Cultures subjected to 4%, 5% or 6% ethanol concentrations induced lag periods of approximately 2.5, 3.5 and 4.5 hours respectively (Emslie, 2002). The findings of Chandler *et al*. (2004) and Emslie (2002) are consistent with the experimental results in this chapter, that showed the exposure of PMY1.1 to ethanol concentrations of 5%, 6%, 7% and 8% in rich medium induced lag periods of approximately 4.6, 5.5, 6.1 and 7.8 hours respectively (Figure 4.1).

One objective of this chapter was to determine an ethanol concentration that induces a lag period 4-7 hours which is sufficient for multiple sampling and allowing multiple time point analysis of gene expression, without compromising cell viability or recovery from the stress. The choice of 7% ethanol as stressor in this project satisfied the above requirement and is consistent with the findings of Chandler *et al*. (2004) and Alexandre *et al*. (2001).

4.3.3 Effect of acetaldehyde on the growth of ethanol-stressed *S. cerevisiae* PMY1.1

Experiments were performed in this study using various acetaldehyde concentrations (0.01, 0.05, 0.1, 0.2, 0.3 and 0.4 g/l) to determine the optimum growth stimulatory effect of acetaldehyde when added to 7% (v/v) ethanol-stressed cultures. These acetaldehyde concentrations were selected based on the previous studies of Walker-Caprioglio and Parks (1987), Stanley and Pamment (1993), Stanley *et al*., (1997), Barber *et al*. (2000) and Viresekoop and Pamment (2005). Added acetaldehyde concentrations of 0.01, 0.05, 0.1 and 0.2 g/l reduced the ethanol-induced lag period of *S. cerevisiae* PMY1.1 cultures by 19%, 29%, 68% and 26% respectively, whilst the concentrations of (0.3 to 0.4 g/l) showed no significant reduction in ethanol-induced lag period. The findings of this study are in keeping with that of Walker-Caprioglio and Parks (1987), and Stanley *et al*. (1993) who investigated the effect of different

concentrations of added acetaldehyde on the growth of yeast cells. Walker-Caprioglio and Parks (1987) inoculated *S. cerevisiae* X2180-1A into YEPD medium containing 6% (v/v) ethanol in the presence of a range of acetaldehyde concentrations 0, 0.08, 0.1, 0.4 and 1 g/l. The exogenously added acetaldehyde concentrations of 0.08 and 0.1 g/l reduced the ethanol-induced lag period, whilst concentrations 0.4 and 1 g/l showed no lag reducing effect, rather it further inhibited yeast growth and recovery from stress. Similarly, Stanley *et al.* (1993), studied the effect of added acetaldehyde concentrations (0-1g/l) on the growth of aerobic cultures of *S. cerevisiae* UNSW 706800; the added acetaldehyde became inhibitory once its concentration exceeded 0.15 g/l.

In this project it was found that the ethanol induced lag period was optimally reduced by the addition of 0.1g/l acetaldehyde, whereas, concentrations of acetaldehyde greater than 0.3 g/l has no stimulatory effect on yeast cell growth. The optimum acetaldehyde concentration for reducing the ethanol-induced lag period of 0.1 g/l found in this project agrees with the findings of other researchers (Viresekoop and Pamment 2005; Barber *et al*., 2002; Barber *et al*., 2000; Stanley *et al*., 1997, 1993; Walker-Caprioglio and Parks 1987). This is an interesting result given the different *S. cerevisiae* strains used in the various projects and the different response in lag period by each strain in the absence of acetaldehyde. It suggests that although various strains respond differently to the same ethanol concentration, they have a similar response to the same acetaldehyde concentration. However, this observation would need to be confirmed by conducting a comprehensive analysis of different *S. cerevisiae* strains and their response to acetaldehyde stimulation.

Based on the results of this study and previously published reports, an ethanol concentration of 7% (v/v) and acetaldehyde concentration of 0.1 g/l were selected as the optimum concentrations to be used in further experiments designed to investigate gene expression during ethanol stress in the presence of added acetaldehyde.

CHAPTER 5

Transcriptional Response of Ethanol-Stressed Yeast to the Presence of Acetaldehyde

5.1 Introduction

Experiments described in the previous chapter demonstrated the effect of added acetaldehyde on ethanol-stressed yeast cultures. In response to 7% (v/v) ethanol stress a lag period of around 6 hours was induced, which in the presence of a small amount of acetaldehyde, was reduced to 2 hours. In this chapter, the effects of ethanol stress and the stimulatory effect of added acetaldehyde were investigated at a molecular level.

The primary objective of work described in this chapter was to determine global gene expression profiles of *S. cerevisiae* during acclimatisation to ethanol stress in the presence and absence of acetaldehyde. Knowledge of differences in expression profiles between these two sets of conditions is important for our understanding of the mechanisms underpinning the acetaldehyde-induced amelioration of ethanol stress. This may, for example, inform the development of strategies for minimizing yeast stress in fermentation industries.

A secondary aim of the work described in this chapter was to test and extend findings reported by Chandler *et al*., (2004) and Alexandre *et al*., (2001), both of whom performed global gene expression analysis of ethanol-stressed yeast cells. Chandler *et al*., (2004) used the same yeast strain (*S. cerevisiae* PMY1.1) as the one used in this study, with 5% (v/v) ethanol and in minimal medium. Alexandre *et al*., (2001) used S. *cerevisiae* S288C grown in rich medium under 7% (v/v) ethanol stress. In the research conducted for this chapter a rich medium containing 7% (v/v) ethanol was used, as these conditions best suited the primary objective of the work.

The two most commonly used supports for global gene array analysis are nylon filters (known as macroarrays) and glass slides (known as microarrays). In each case the array is a reproducible pattern of probes in the form of oligonucleotides or PCR products,

each probe representing a different target gene sequence, and these are spotted onto the support. In a typical experiment, cDNA prepared from test and control mRNA is labeled with radioactive (^{33}P) for macroarrays or fluorescent dyes (Cy5 and Cy3) for glass slides. This is then hybridized to the immobilsed probes on the solid support. The array is then washed to remove non-specifically bound cDNA and is then read in an appropriate detector to determine which probes have labeled signal bound to them.

At the commencement of work for this thesis only nylon-based array technology was available to the laboratory at Victoria University and therefore initial experiments used only this approach. However glass slide arrays are more sensitive than filter arrays, enabling the detection of low abundance mRNAs (Bowtell, 1999). Therefore when resources for using slide-based arrays were accessible, some experiments were repeated using this technology.

There are no published reports on changes in gene expression associated with the stimulatory effects of added acetaldehyde to ethanol stressed yeast cultures. This data therefore provides important information for scientists working on cellular stress responses and will be of particular interest to those who work on ethanol stress and ethanol tolerance.

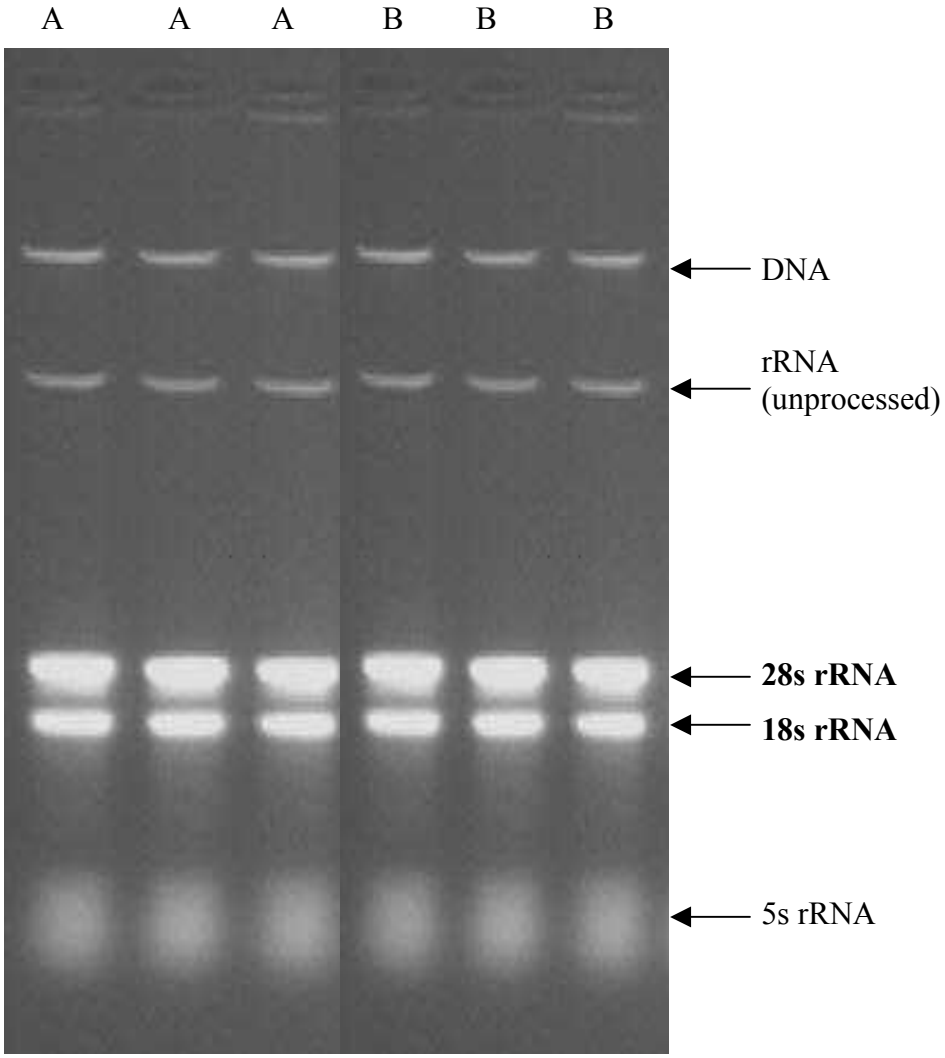

Figure 5.1: Total RNA was isolated from equal number (2 x 10^8) of cells from exponentially growing triplicates of two yeast cultures (A and B) and was visualized on a 1% ethidium bromide-stained agarose gel. The relatively constant yields of DNA and RNA in the replicates indicates that the RNA isolation method was consistent. The overall quality of the prepartions is apparent from the integrity of the bands. This experiment was repeated several times and always gave similar results.

5.2 Results and Discussion

5.2.1 RNA preparations: quality and reproducibility

Analysis of global gene expression requires the preparation of high quality template RNA. It is also important to consider the method of 'equalizing' template RNA from cultures that are to be compared; this is of particular importance when comparing transcriptomes from stressed and unstressed cells. The standard approach for most types of array experiment is to prepare total RNA, and equalize at this stage. However it is well documented that stress reduces overall transcription (Chandler *et al*., 2004; Mager and Moradas-Ferreira, 1993). Thus, if RNA concentrations from treated and untreated cultures were equalized the concentration of mRNA in the stressed samples would be disproportionately increased relative to unstressed controls, thus increasing the risk of generating false positive results. To minimize this risk for work described here, total RNA was extracted from equal numbers of cells (2×10^8) for the control and treated cultures, as described in Chandler *et al.* (2004). It was therefore important to make sure the RNA extraction efficiency was reproducible. The method used to achieve this end (as described in Section 3.3.1) was tested in several experiments by performing triplicate RNA extractions from various yeast cultures, and comparing the yields and quality of the product (see Figure 5.1).

5.2.2 Gene array analysis of ethanol-stressed and acetaldehyde-stimulated yeast cells using gene filter macroarrays

Macroarray analyses were performed using Yeast Index Gene Filters (Invitrogen). These filters carry 6,144 *S. cerevisiae* PCR-amplified Open Reading Frames (ORFs) spotted across two nylon membranes. cDNA was prepared from extracted RNA from equal numbers (2×10^8 cells) of cells taken from: unstressed (control) cultures, ethanol-stressed cultures, acetaldehyde-treated ethanol-stressed cultures and acetaldehyde-treated unstressed cultures; each of these were sampled at one and five hours post-inoculation. These time points corresponded to early and late stages in acclimatisation to ethanol stress (see Figures 4.3 & 4.4**)**. cDNA was synthesized from total RNA using Superscript® II (Invitrogen), according the manufacturer's instructions and was labelled

using [^{33}P]. Following hybridization, images of gene filters were produced using an FLA3000 Phosphor Imaging System (Fujifilm). Genes were considered up or down regulated when the difference in expression level between 'test' and 'control' cultures was greater than three-fold. All spot intensities were normalized to the intensity of genomic DNA control spots on the filters. Determining absolute changes in gene expression by normalizing against constitutively expressed genes, such as *ACT1*, was considered inappropriate because this (and other housekeeping genes) have consistently been found to be less highly expressed[1] (LHE) under ethanol stress in the laboratory at Victoria University (data not shown). Indeed *ACT1* was 5.1-fold LHE in the ethanol-stressed culture than it was in the control culture at the one-hour time point in experiments described here (Section 5.2.2.2). Hence, normalization against this housekeeping gene would have artificially increased the numbers of genes apparently more highly expressed[1] (MHE), in response to ethanol stress, potentially generating false positive results.

[1]From experiments described in this chapter it is not possible to determine whether a gene that is more highly expressed in a given set of conditions is induced, de-repressed or otherwise up-regulated. For this reason the term 'more highly expressed' (MHE) is used. For similar reasons the term 'less highly expressed' (LHE) is used to describe genes with reduced levels of expression.

Table 5.1: Summary of macroarray data: functional classes of genes with changed expression following one-hour exposure to 7% (v/v) ethanol in the presence and absence of acetaldehyde.

	Effect of ethanol stress		**Effect of acetaldehyde on ethanol-stressed cells**		**Effect of acetaldehyde on unstressed cells**	
Functional class of genes	**No. of ORFs MHE**	**No. of ORFs LHE**	**No. of ORFs MHE**	**No. of ORFs LHE**	**No. of ORFs MHE**	**No. of ORFs LHE**
Ribosomal Protein & Ribosomal subunit	-	148	75	-	-	-
Stress response	1	23	6	-	-	-
Cell cycle & growth	-	58	7	1	-	-
Protein metabolism	-	-	21	-	-	-
Transport genes	-	150	18	-	-	-
Transcription & translation factors	3	210	5	-	-	-
Energy utilization	7	44	4	1	1	1
Protein folding	1	141	6		-	-
Signal transduction	-	27	-	-	-	-
Cytoskeleton organization & maintenance	-	24	-	-	-	-
Cell wall & membrane proteins	-	20	3	-	-	-
Lipid metabolism		24	5			
Nucleotide metabolism	-	92	10	-	-	-
Miscellaneous	-	135	16	-	-	-
Unknown function	16	448	38	2	3	3
Total ORFs	**28**	**1544**	**214**	**4**	**4**	**4**

5.2.2.1 Overview of gene filter macroarray data

As mentioned previously, initial experiments were performed using macroarrays, and some of this work was subsequently repeated using microarrays. This section will focus only on data obtained from macroarray experiments; microarray data will be discussed in Section 5.2.3.

At the one-hour time point for the ethanol-stressed culture (Table 5.1), 1,572 ORFs displayed a change in expression level relative to an unstressed control. Of these, only 28 were more highly expressed (MHE), the rest being LHE. At the same time point, for cultures exposed to ethanol stress in the presence of added acetaldehyde compared to an ethanol-stressed culture, 218 ORFs displayed a shift in expression level. Of these, 214 ORFs were MHE, only 4 being LHE (see Table 5.1). When acetaldehyde was added to unstressed culture, there was a shift in expression of only 8 ORFs relative to the unstressed control; of these 4 were MHE.

At the five-hour time point for the ethanol-stressed culture, 1505 ORFs displayed a change in expression level relative to an unstressed control (See Table 5.2). Of these, 85 were MHE, the rest being LHE. At the same time point there were 396 ORFs that showed a shift in expression level for the acetaldehyde-treated ethanol-stressed culture compared to the ethanol-stressed culture. Of these 347 were MHE. When acetaldehyde was added to the unstressed culture there was a shift in expression level of only 3 ORFs, two of which were MHE (See Table 5.2).

5.2.2.2 Analysis of gene filter, macroarray, data for ethanol-stressed cells compared to unstressed, control cells.

Unlike previous reports on global gene expression of ethanol-stressed yeast cells there were very few genes highly expressed relative to the control at the early stages of stress (i.e. at the one-hour time point); only 28 ORFs were MHE in this work compared with 100 for Chandler *et al.* (2004) and 194 for Alexandre *et al*. (2001). Of the 28 ORFs that had increased expression in this study, 16 are of unknown function, 7 are involved in energy utilization, 3 are associated with RNA-directed DNA polymerase activity, 1 is associated with stress responses (*HSP26*) and 1 encodes a protein kinase.

It was interesting to find in this work that only one *HSP* gene was highly expressed; this is in stark contrast to findings of several other workers. For example Chandler *et al*. (2004) found that *HSP104, 78, 42, 30, 26, 12* and two members of the *HSP70* family, *SSA4* and *SSE2*, were more highly expressed. Similarly Alexandre *et al*. (2001) found *HSP104, 82, 78, 42, 30, 26, 12*, and several members of the *HSP70* family were MHE. These results from Chandler *et al*. (2004) and Alexandre *et al*. (2001) are consistent with much earlier work of Piper (1995) and Parsell and Lindquist (1993).

In the work for this thesis, of 7 MHE genes associated with energy utilization, *TDH1, ALD4, PYC1, and GLK1* were also reported as MHE in response to ethanol stress by Chandler *et al*. (2004) and Alexandre *et al*. (2001); Alexandre *et al*. also reported that *GLC3* was up-regulated. The higher level of expression of *DLD3* (associated with lactate metabolism) and *ACS1* (associated with acetyl-CoA biosynthesis) in response to ethanol stress at the one-hour time point, are reported for the first time in this study.

While there are many differences between the results presented in this thesis and the published results of Chandler *et al*. (2004) and Alexandre *et al*. (2001), it should be pointed out that there are also many differences between the result of Chandler *et al*. (2004) and Alexandre *et al*. (2001). These latter differences were acknowledged by Chandler *et al*. (2004), and were thought to be due to strain differences, stress severity, type of growth medium, time lengths of exposure to ethanol and other experimental conditions. Similar reasons can be offered for the differences between results presented in this thesis and those of the above authors. Alexandre *et al*. (2001) used a similar level of ethanol stress as in this study, but these authors used an earlier time point and a different strain. Chandler *et al*. (2001) used the same strain as the one used for this thesis, but different growth conditions (including different growth medium and different ethanol concentrations).

An interesting feature of the data presented in this thesis is the ethanol-stress-induced lower level of expression of many genes associated with anabolic processes, and this is consistent with the findings of Chandler *et al*. (2004) and Alexandre *et al*. (2001). It is evident from all three pieces of work that genes encoding proteins associated with anabolic functions such as ribosome synthesis, transport, transcription initiation, cell

cycle, energy utilization, DNA synthesis, and cell wall synthesis, are LHE in ethanol-stressed cultures. This however is typical of cells undergoing growth arrest as a result of encountering physiological stress (Gasch *et al*., 2000) and the reduced expression of genes encoding ribosomal proteins has previously been linked with cellular arrest (Warner, 1999). These observations indicate that reduced expression of ribosomal protein-genes is a general stress response rather than being specific to ethanol-stress (Causton *et al*., 2001. Synthesis of ribosomes is a major consumer of the cell's resources, thus, it is likely that transcription of ribosomal protein genes is reduced to conserve energy during stress (Warner, 1999).

During environmental insult lack of initiation of ribosome synthesis will block cell cycle. Consistent with this, the results presented here demonstrate reduced expression of genes associated with cell cycle and cytoskeleton (*TUB1, TUB2 TUB3 TUB4* and *ACT1*) (see Table 3.1b, in appendix III). Reduced expression of these genes would lead to a lower ATP demand. This is consistent with the decreased expression of genes associated with the lower part of the glycolysis (*PDA1* and *PDB1*) and fermentation (*PDC5, PDC1, ALD6* and *ADH6*). In fact the dramatic reduction in expression of ribosomal protein genes and genes associated with transcription initiation, energy utilization and cell cycle clearly correlates with the physiological status of the yeast cells, which is evident from the growth curves in Figures 4.3 & 4.4; the cultures these cells were derived from underwent a lag period of six hours.

In this study there were 150 transport-associated genes expressed at a reduced level in response to ethanol stress at the one-hour time point. Several of these (*HXT2, HXT3, HXT4, HXT6, HXT7, PHO3, PHO84*, and *PHO88*) are associated with hexose and phosphate transport and, of these, *HXT2* and *PHO3* were also reported by Chandler *et al*. (2004) to be LHE in response to ethanol stress (see Table 3.1b, in appendix III**).** However, the same authors found that high affinity hexose transport genes *HXT6* and *HXT7* were both MHE in response to 5% (v/v) ethanol stress.

Ethanol stress not only induced changes in expression of genes associated with energy utilization and transport but also genes encoding proteins associated with the plasma membrane. During ethanol stress 24 genes associated with lipid metabolism, including ergosterol and fatty acid metabolism, were LHE at one-hour time point; this is

consistent with the findings of Chandler *et al.,* (2004) and Alexandre *et al*., (2001). Previously it was reported that ethanol induces modifications of yeast membranes (Walker-Caprioglio *et al*., 1985 and Walker, 1998) that compromise the integrity of plasma membrane (Sections 2.3.1.1 and 2.3.1.2). It might be argued that global gene array analysis of *S. cerevisiae* PMY1.1 exposed to ethanol stress showed changes in expression of genes associated with energy utilization and genes associated with plasma membrane structure.

The data presented in this thesis also shows that the magnitude of the ethanol-stress response diminishes with time. As shown in Tables 5.1 and 5.2, at the one-hour time point there were 28 ORFs MHE whilst 1, 544 ORFs were LHE (Table 3.1a and 3.1b, in appendix III). When this is compared to the five-hour time point, there were 85 ORFs with increased expression and 1,420 ORFs with decreased expression (see Table 3.2a and 3.2b, in appendix III). Overall, the transcriptional response to ethanol stress demonstrated that the number of genes with altered expression was slightly diminished at the five-hour time point compared to the one-hour time point. In agreement with this finding, Chandler *et al*. (2004) also clearly demonstrated that many genes are transiently highly expressed in response to ethanol stress and a similar pattern has been observed for other stresses (Posas *et al.,* 2000 and Gasch *et al*., 2000). Thus, from previous published reports and results presented here, it is clear that the transcriptional response of genes with altered expression level occurs early in response to stress and is largely transient. In this study, the main categories of genes that were highly affected by ethanol stress were: genes associated with ribosome synthesis, transport, transcription factors, protein synthesis and genes of unknown function. The transient stress-induced repression of ribosomal protein genes has also been reported for stresses such as temperature shock (Warner, 1999; Eisen *et al.,* 1998 and Herruer *et al*., 1988) and osmotic stress (Gasch *et al*., 2000).

5.2.2.3 Analysis of gene filter, macroarray, data for ethanol-stressed cells in the presence and absence of added acetaldehyde

Although a growing number of studies have documented the changes in global gene expression in ethanol-stressed yeast cultures using global gene expression, there have been no such reports on the stimulatory effect of added acetaldehyde to ethanol-stressed

cultures. The following presents the first such data from gene filter, macroarray experiments.

At the one-hour time point there were 214 ORFs MHE in the acetaldehyde-treated ethanol-stressed culture than in the untreated ethanol-stressed culture, and only 4 ORFs had reduced expression (Tables 3.3a and 3.3b, in Appendix III). At the five-hour time point 347 were MHE and 49 were LHE (Tables 3.4a and Table 3.4b, in Appendix III).

One of the most interesting aspects of the array data is that, when acetaldehyde was added to the ethanol-stressed yeast cells, there was increased expression of many genes. Perhaps the most striking feature of the MHE ORFs was the large number that are associated with synthesis of ribosomal proteins, DNA synthesis, transcription and cell cycle, and this was evident at both time points (Tables 5.1 and 5.2). This is consistent with growth curve data shown in Figures 4.3 and 4.4, in which it clearly showed that cells are rescued from lag considerably faster when acetaldehyde is present in ethanol-containing cultures, relative to cultures without acetaldehyde; when acetaldehyde is present the cells commence division more rapidly.

Another notable feature of this study was that stress response genes and genes associated with trehalose synthesis were not highly expressed during acetaldehyde stimulation of ethanol-stressed cultures relative to untreated ethanol-stressed cultures. This finding is interesting, because the well-documented increased expression of *HSP* genes and trehalose synthesis genes in response to ethanol stress (Chandler *et al*., 2004; Alexandre *et al.,* 2001; Piper *et al.,* 1994 and Piper, 1995) has led to the proposal that stress response genes protect against the damaging effect of ethanol stress. This may be the case but results presented here suggest they are unlikely to have a role in acetaldehyde-induced tolerance to ethanol stress.

As described in Section 5.2.2.2 150 genes associated with transport were LHE in ethanol-stressed cultures relative to the unstressed control. In the presence of acetaldehyde 18 of these (along with two that were not affected by ethanol stress alone), displayed increased level of expression relative to ethanol stressed cells. This change in expression, brought about by the addition of acetaldehyde to ethanol stressed cultures, may represent a small fraction of the overall number of transport genes that were LHE

under ethanol stress, but it suggests that the cell is attempting to activate transport processes that are otherwise compromised in ethanol-stress conditions. For example, several *HXT* hexose and *PHO* phosphate transport genes were LHE under ethanol stress, a finding that is consistent with the findings of Chandler *et al*. (2004) and Alexandre *et al*. (2001). However, when acetaldehyde was added to the ethanol-stressed culture, *HXT3, PHO84, PHO3 and PHO88* showed increased expression levels at one hour time point, and *HXT1* at five hour time point. It has been proposed that cells under ethanol stress are likely to be in a pseudo-starvation state where nutrients, such as glucose, are present in the growth medium but are not accessible to the cell (Chandler *et al*., 2004). The increased expression of these transport genes following addition of acetaldehyde to the ethanol-stressed culture may ameliorate ethanol-induced stress by facilitating the transport of nutrients for normal cell metabolic activities.

Another group of genes that are noteworthy here because they were MHE when acetaldehyde was added to the ethanol-stressed culture are genes associated with plasma membrane structure and function. These include the ergosterol synthesis genes, *ERG3, ERG11, ERG25* and ERG26 at one-hour time point and *ERG3* and *ERG9* at five-hour time point. There was only one fatty acid metabolism gene, *FEN1* (*YCR034W)* with increased expression across both time points.

As discussed in Section 2.4.1, ethanol tolerance in yeast is thought by some workers to correlate with increasing ergosterol levels. Thus, increased expression of *ERG* genes in an ethanol-stressed culture with added acetaldehyde might be a significant factor in promoting ethanol tolerance. However, when Walker-Caprioglio *et al*. (1985) performed fluorescence anisotropy measurement of the plasma membrane during ethanol stress in the presence of small amounts of added acetaldehyde no reversal of the ethanol-induced changes to plasma membrane was observed; i.e. acetaldehyde did not reverse the ethanol-induced changes to plasma membrane. Thus, while the cell may be attempting to alter its membrane structure to better tolerate ethanol stress, actual changes in membrane composition may not be achieved. Membrane lipids were not analysed for work described in this thesis, therefore it is not possible to ascertain whether increased expression of *ERG* genes had any impact on the membrane.

Table 5.2: Summary of macroarray data: functional classes of genes with changed expression following five hours exposure to 7% (v/v) ethanol in the presence and absence of acetaldehyde.

	Effect of ethanol stress		**Effect of acetaldehyde on ethanol-stressed cells**		**Effect of acetaldehyde on unstressed cells**	
Functional class of genes	**No. of ORFs MHE**	**No. of ORFs LHE**	**No. of ORFs MHE**	**No. of ORFs LHE**	**No. of ORFs MHE**	**No. of ORFs LHE**
Ribosomal Protein & Ribosomal subunit	-	149	55	-	-	-
Stress response	7	18	8	-	-	-
Cell cycle & growth	-	25	13	1	1	-
Protein metabolism	1	34	-	-	1	1
Transport and translocation	3	91	30	4	-	-
Transcription & translation factors	5	125	30	3	-	-
Energy utilization	9	23	5	2	-	-
Protein synthesis & folding	-	28	24	-	-	-
Signal transduction	1	8	3	-	-	-
Cytoskeleton organization & maintenance	-	35	5	-	-	-
Cell wall & membrane proteins	4	49	8	2	-	-
Lipid metabolism	1	12	3	-		-
Nucleotide metabolism	9	109	13	5	-	-
Histone metabolism	-	12	-	-	-	-
Miscellaneous	9	308	47	2	-	-
Unknown function	36	394	103	30	-	-
Total ORFs	**85**	**1420**	**347**	**49**	**2**	**1**

5.2.2.4 Comparison of one- and five-hour time points using data from gene filter macroarray analysis of acetaldehyde-stimulated ethanol-stressed cultures

It was clearly shown in the previous chapter that the ethanol-induced lag period of six hours was reduced to two hours by the addition of a small quantity of acetaldehyde (Figures 4.3 & 4.4) and at the five-hour time point, the acetaldehyde-treated culture was clearly in exponential growth. From macroarray data the number of MHE genes in acetaldehyde-treated ethanol-stressed yeast cells relative to ethanol-stressed cells was greater at five hours than at one (Tables 5.1 and 5.2). This is perhaps not surprising because at five hours the acetaldehyde-treated, ethanol-stressed culture was fully recovered while the ethanol-stressed culture was still in lag phase. However, the amplitude of differences in gene expression was generally slightly reduced at the five-hour time point compared to one-hour. This might reflect the fact that, at five hours, both cultures had come out of the early 'shocked' state associated with changing environmental conditions, in which changes in gene expression tend to be far more dramatic (see for example Gasch *et al*., 2000), although this is not consistent with the lower number of genes exhibiting altered expression at the one-hour time point.

An interesting deviation from the above general pattern is found in the expression of ribosomal protein genes. In this case the number and magnitude of differences in expression both declined at the five-hour time point relative to the one-hour time point. The reason for this is unclear. One might have predicted that cells would require more ribosomal activity during exponential phase when they are involved in growth (and therefore anabolic) activities, than during lag phase. However, the result may simply reflect the fact that, at the five hours, the ethanol-only stressed cells had acclimatised to their environment and were moving into anabolism.

5.2.2.5 Analysis of gene filter, macroarray, data for acetaldehyde-treated, non-stressed yeast cells compared to unstressed control cells

As described in the previous chapter, small quantities (0.1 g/l) of added acetaldehyde did not affect the growth of non-stressed yeast cultures (Figures 4.3 & 4.4). Similarly, from array analysis a small quantity of added acetaldehyde did not have a major impact

on gene expression of non-stressed cultures; at the one-hour time point there were only 8 genes with a shift in expression level (Table 3.5a, in Appendix III). Of these, 4 were MHE. One gene of interest here is *PDC5,* which was 4.2-fold MHE. This gene was also found to be transiently MHE by 15.2-fold in the ethanol-stressed culture in the presence of a small quantity of added acetaldehyde relative to ethanol stress alone. At the five-hour time point three genes displayed shifts in expression level, two of these *MON2* and *PCL2* were MHE in the acetaldehyde-treated culture and one *VPS65* was LHE (Table 3.6a, in Appendix III).

Table 5.3: Summary of microarray data: functional classes of genes with changed expression following one hour exposure to 7% (v/v) ethanol, in the presence and absence of acetaldehyde.

	Effect of acetaldehyde on ethanol-stressed cells		Effect of acetaldehyde on unstressed cells	
Functional class of genes	**No. of ORFs MHE**	**No. of ORFs LHE**	**No. of ORFs MHE**	**No. of ORFs LHE**
Ribosomal Protein & Ribosomal subunit	95	-	-	-
Stress response	5	23	1	-
Cell cycle & growth	5	-	-	-
Protein synthesis and metabolism	9	3	-	-
Transport	6	15	1	-
Transcription and translation factors	28	29	1	-
Energy utilization	2	18	-	-
Protein folding	-	-	-	-
Signal transduction	-	-	-	-
Cytoskeleton organization & maintenance	2	2	1	-
Cell wall & membrane proteins	6	-	-	-
Lipid metabolism	3	-	-	-
Nucleotide metabolism	5	3	-	-
Transposable elements	-	11	-	-
Miscellaneous	33	27	4	-
Unknown function	40	86	17	-
Total ORFs	**239**	**217**	**25**	**0**

5.2.3 Overview of array analysis using glass slides (microarrays)

Validation of macroarray (gene filter) data described in previous sections of this chapter would have required repeat experiments. However, filter-based arrays became prohibitively expensive making it difficult to perform repeats (or use replicates). Fortunately, at the time of performing this work, slide-based microarray analysis became available to the laboratory at Victoria University, and because microarrays are more sensitive and more economical it was decided to perform the core experiments from the work described above, using microarray technology. This enabled the findings described above to be tested using different experimental tools.

The experimental design for performing microarray analyses was essentially the same as that used for the macroarrays. However, because of limited time and resources there were only two treatments used (ethanol with acetaldehyde and acetaldehyde alone) alongside an untreated control. The decision to prioritise the 'acetaldehyde/ethanol stress' experiment is justified on the grounds that the most important aspect of the work in this thesis is the impact of acetaldehyde on ethanol stressed cells; there is already considerable knowledge on the effects of ethanol stress on global gene expression in yeast cells.

Analysis was restricted to the one-hour time-point as this gave the most pronounced difference in gene expression profiles in the experiments described in the previous section and there was insufficient time and resources to assess multiple time points. However, the decision to prioritise this time point does not mean that it is the 'most important' or even 'most informative' time-point; there are good arguments that could be put for focusing on the five-hour time point, and this would be important to pursue in future studies.

In all experiments RNA was extracted from equal cell numbers (2×10^8 cells) and cDNA was labelled with fluorescent dyes (Cy5 and Cy3). The microarray slides

contained 6,528 *S. cerevisiae* PCR-amplified open reading frames spotted on each slide. Following hybridization, the glass slides were scanned using a GenePix-Pro 4000 scanner and analyzed using GeneSpring software.

For experiments on the effect of acetaldehyde on ethanol-stressed cells, triplicate cultures were used (and therefore triplicate arrays were performed). For experiments involving acetaldehyde treatment of unstressed cells, duplicates were used. In general, the replicates used in these experiments gave similar results (Table 4.1a, in Appendix IV)

Data analysis revealed that, after one hour, 93.06% ORFs were expressed at similar levels in the acetaldehyde-treated ethanol-stressed and ethanol-stressed cultures. As shown in Table 5.3, of the 454 ORFs that displayed a change in expression, 238 were MHE and have been grouped into functional categories (Tables 4.1a and 4.1b, in Appendix IV). Comparing expression in acetaldehyde-treated unstressed cultures to the controls (unstressed cultures), there were 25 ORFs MHE and none LHE in acetaldehyde treated cells (Table 4.2, in Appendix IV). Thus, as in the case of the macroarray analysis, there were very few changes in expression detected when cells were exposed to acetaldehyde alone.

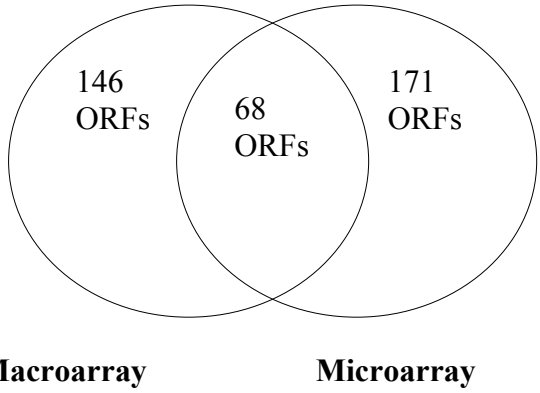

Figure 5.2: Venn diagram comparing the number of ORFs that were MHE using macroarray and microarray analysis of acetaldehye-treated ethanol-stressed cultures compared ethanol-stressed cultures at the one-hour time point.

5.2.4 Comparison of macroarray and microarray data, and general observations from array experiments

Results obtained using microarrays were similar to those from macroarrays in that similar groups of genes were affected by acetaldehyde treatment of ethanol-stressed cells. However, microarrays were generally more sensitive than macroarrays in that more differences in gene expression were detected using the former (Bowtell 1999). For example, from acetaldehyde-treated ethanol-stressed cells compared to untreated ethanol-stressed cells there were 239 ORFs MHE and 217 ORFs LHE in microarray analysis, compared with 214 ORFs MHE and 4 LHE for the macroarray. The huge difference in sensitivity when it came to detecting LHE ORFs is interesting but there is no obvious explanation for it. Time limitations meant that this discrepancy could not be explored further. Of the MHE ORFs, 68 were common to both types of array, and of the LHE ORFs, 3 (*SPS100, SPI1* and *HBT1*) were common to both (see Figure 5.2).

A consistent feature of microarray and macroarray data was the increased expression of genes associated with ribosome synthesis in acetaldehyde stimulated, ethanol-stressed cells at the one-hour time-point. Several genes associated with metabolism of pyruvate were also MHE in macro- and microarrays, but only one of these, *PDC5*, was found to be MHE in both. Another important group of genes found to be highly expressed in acetaldehyde-treated ethanol-stressed cells in both array-types was the group of genes encoding enzymes associated with lipid metabolism, in particular, *ERG25, ERG3 and FEN1*. As discussed previously, these genes may have an important role in ethanol tolerance (Section 5.2.2.3). Other genes that were MHE in both array-types were the transport genes *HXT3, KAP123, VRG4* and *PHO84* and four genes associated with protein synthesis, *SAM1*, *CYS1, THS1* and *HSL1*. In addition, three genes of unknown or uncharacterized function were MHE in both macro- and microarrays.

Of the 217 LHE ORFs in the microarray data, 23 were stress response genes, 7 of which encoded HSPs, including *HSP 26*, 30, *33*, *78*, *82*, *104*, and *YRO2* (a homolog of

HSP30). This study also found that, *SSA1, SSA3*, and *SSA4*, which belong to the *HSP70* family were LHE. This would suggest that stress-response genes do not play a part in acetaldehyde-induced tolerance to ethanol stress.

Of genes that fall into the energy metabolism grouping, 18 were LHE in response to acetaldehyde acting on ethanol-stressed cells. Among these were genes encoding glycolytic enzymes, *HXK1, GLK1, GPM2,* and *TDH1*. Other 'energy metabolism' genes found to be LHE in microarray analysis of acetaldehyde treated ethanol stressed cultures were those involved in trehalose synthesis: *TSL1, TPS2, TPS1,* and *UGP1*. Expression of these genes would be expected to result in the accumulation of trehalose in response to ethanol stress, and this has been reported to protect cells against the damaging effects of ethanol stress (Mansure *et al*., 1994); Chandler *et al*., (2004) found increased expression of genes associated with trehalose metabolism in response to ethanol stress. Thus, it is interesting that data presented here shows that genes involved in trehalose synthesis are LHE in ethanol-stressed cells in the presence of acetaldehyde. In fact, it seems from macroarray data (see section 5.2.2.3) and, even more so from microarray data, that trehalose does not play a part in the acetaldehyde-induced tolerance to ethanol stress.

Table 5.4: Specific primers designed for Real-Time PCR analysis.

Gene name	Primer sequences	Tm (°C)	Product size (bp)
ACT1	5' AGGTATCATGGTCGGTATGG 5' CGTGAGGTAGAGAGAAACCA	57 56	398 (123-520)
HSP30	5' GAACAAGGGCTCCAGATTGA 5' CAGGACAAGAACCAGGCAAT	60 60	216 (171-386)
HSP104	5' GCACGTCCACTGAACAGGTTA 5' ACCTAACGTGTCAGCCCCTA	61 59	207 (2473-2679)
TPO4	5' GCCATTAACTGTGGCATCCT 5' AACCCATGGAATTGGAATCA	60 60	166 (1446-1611)
TSL1	5' GTTCAGAGGCAGATTTGTTCG 5' TCTCCAACCCCTTGAGAATG	60 60	210 (2084-2293)
GPD2	5' CACAGAATTGCATTCCCATATC 5' TCAGGATCGGCCACTAGATT	59 59	162 (315-476)
SPS100	5' TTACAAAGCGAATCGTCTTCC 5' CCCACAGGAACAGTGTAAGGA	59 60	239 (235-473)
TOS1	5' CTTTGTCCCAGGCTCTACTAGC 5' CAGAACCGCTGGTGTCACT	59 60	326 (684-1009)
YER150W	5' TACGGCTCTCGGATTGGTAT 5' GAATGTAGCGCCATTCGTTA	59 59	226 (45-270)
HXT4	5' TCAAGCTTGTAACTGGATCTGG 5' GTTTGGTGGAACCAAGAAGGT	59 60	228 (1442-1649)
YLR364W	5' CCGACTGCGTCTATGCTAATTC 5' GGCAGAAGCCCGATTTTAGT	61 60	250 (77-326)
PDC5	5' GTTCCAATTACCAAGTCTACTCCA 5' GGACGATAGCGTATACATCTGTT	58 57	169 (1057-1225)
PHO84	5' CTGCCGCACAAGAACAAGAT 5' ACAGTGAAGACGGATACCCAGTA	61 60	376 (863 -1238)

5.2.5 Validation of array results using real-time-PCR analysis

To further test the validity of data from macroarray and microarray experiments and compare the two approaches, quantitative real-time PCR was used to analyse the level of expression of thirteen candidate genes (Table 5.4) for acetaldehyde-treated ethanol-stressed cultures compared to ethanol-stressed cells, and results from this were compared with macro- and micro-array data for the same genes. Some of the candidate genes registered similar changes in expression in macro- and micro-arrays whilst others were different across the two methods. This enabled the techniques to be compared for reliability.

To perform quantitative Real-time PCR reactions, specific primers were designed for the thirteen candidate genes using *Saccharomyces cerevisiae* genome database (SGD: www.yeastgenome.org). These primer sequences, their melting temperatures and product sizes are shown in Table 5.4.

Real Time PCR results largely confirmed microarray data but were less supportive of macroarray results. A possible explanation for this is that the RNA used as template for the Real Time PCR was the same as that used for one of the replicate microarrays (slide 1 in the data presented in Tables 4.1a and 4.1b, in Appendix IV). This does however raise questions about variation in gene expression between experiments or the validity of the macroarray data, and this should be tested in future work.

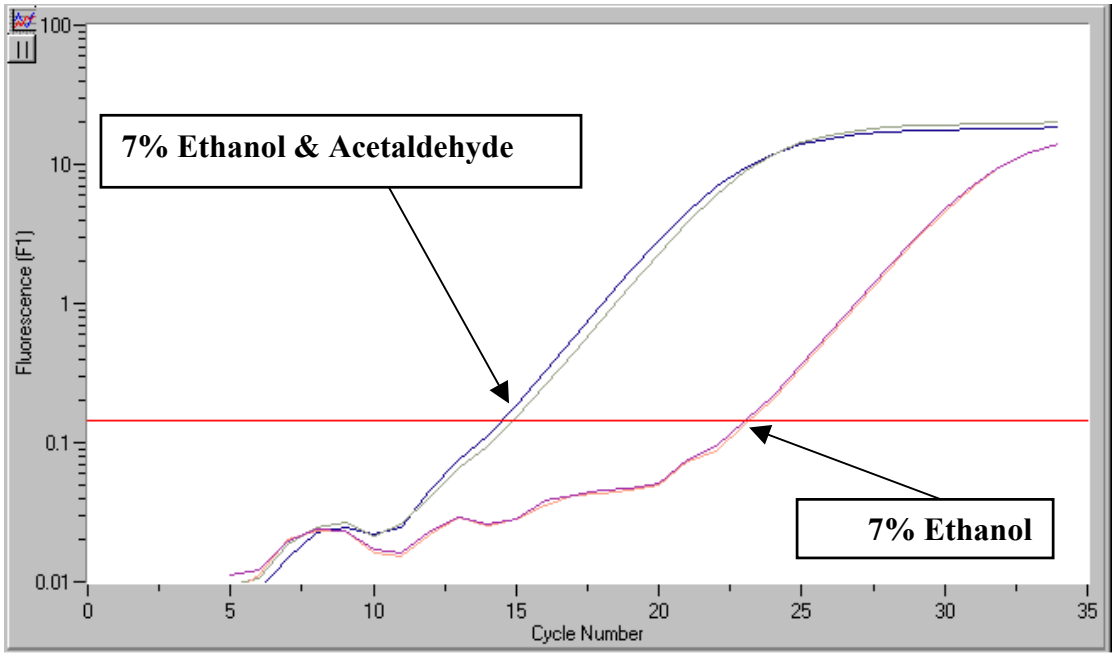

Figure 5.3: Fluorescence versus cycle number plot for PDC5 transcript in acetaldehyde-treated ethanol-stressed and ethanol-stressed cultures. Plots of similar quality were obtained for all genes analyzed using quantitative Real Time PCR.

To determine the relative levels of target transcripts in Real Time PCR, it was necessary to determine the crossing points (CPs) or crossing thresholds (CTs) for each transcript (Figure 5.3**)**. To determine the reproducibility and accuracy, as shown in Figure 5.3, duplicate real time PCR reactions were conducted for each ORF/gene. The CP is defined as the point at which fluorescence rises appreciably above background. Based on the CP, the relative expression level of treated sample versus a control sample for each candidate gene was calculated. A summary of the comparison between array and Real Time PCR data is given in Table 5.5.

From Table 5.5 it is apparent that ORFs identified as MHE or LHE in microarrays were confirmed as such by real-time PCR analysis. However, there are clear difference in magnitude between the microarray data and Real Time PCR. The reason(s) for this are unknown but perhaps reflect some of limitation inherent in the methodologies. For example, arrays rely largely on the affinity of the array probes for the labeled cDNA whereas Real Time PCR relies on the efficiency of PCR reactions, which in turn is dependent on the specificity and affinity of the primer used and the complex kinetics of a real time PCR reaction. It is important to note, that the discrepancies between the Real Time PCR and microarray were previously reported by Morey *et al*. (2006) and Wang et al. (2003). However, both reports showed overall significant correlation between microarray Real Time PCR. In general, as reporterd by *Wang et al*. (2003), one would expect Real Time PCR to be more precise and accurate than arrays, but this has not been tested for the experiments conducted here.

5.2.6 Promoter analysis of up-regulated genes

A systematic promoter analysis was performed to search for regulatory elements that were common to genes that were MHE in acetaldehyde-treated ethanol-stressed cells compared to cells from the untreated ethanol-stressed culture. While there is a large number of transcription factors and cognate promoter motifs in the yeast genome only three promoter elements were targeted in this analysis: STRE, HSE and ARE. These elements bind transcription factors Msn2/Msn4p, Hsf1p, and Yap1/2p respectively, and were selected based on previous studies by Van Voorst *et al*., (2006), Chandler *et al*. (2004); Alexandre *et al*., (2001); Gasch *et al*., (2000) and Moskvina *et al*., (1998). These authors found that STRE, HSE and ARE elements are common features of promoters associated with genes that are MHE in ethanol stressed cells. The aim of the work described here was to determine whether the same elements are associated with genes that are MHE in acetaldehyde-treated ethanol-stressed cells, and therefore may be important for the acetaldehyde-driven recovery from ethanol-stress.

Table 5.5: Comparison of gene expression measurements by macroarray, microarray and relative quantitative Real Time PCR for transcripts prepared from yeast cells exposed to 7% (v/v) ethanol-stress under the stimulatory condition of acetaldehyde.

Gene name	Macro array fold alteration	Microarray fold alteration	Real Time PCR
More highly expressed			
PDC5	15.2	70.0	256
PHO84	5.8	7.0	16
HXT4	NDD	17.0	32
TOS1	8.8	NDD	5.0
YLR364W	NDD	8	32
ACT1	NDD	NDD	NDD
Less highly expressed			
HSP30	NDD	78.0	64
HSP104	NDD	12.0	4
GPD2	NDD	6.0	4
TSL1	NDD	11.0	4
TPO4	NDD	6.0	2
SPS100	5.5	16	4
YER150W	NDD	32	8

NDD = No Detectable Difference in expression.

The Regulatory Sequence Analysis Tools (RSAT) (http://rsat.ulb.ac.be/rsat/) and www.yeastract.com databases were used to perform computer-aided pattern matching of the promoters of interest. The upstream regions of genes that were MHE in the acetaldehyde-treated ethanol-stressed cells compared to the untreated ethanol-stressed cells were searched for each of the above three motifs. Of the 214 ORFs identified at the one hour time point in macroarrays as MHE, 135 contained HSEs, 134 contained STREs and 44 had AREs (Table 3.3a, in Appendix III), while at five hour time point, of 347 ORFs identified as MHE in response to ethanol stress in the presence of acetaldehyde, 237 contained STREs, 213 contained HSEs and 50 contained AREs (Table 3.4a, in Appendix III). Similarly, of the 239 ORFs identified in microarrays as MHE in response to acetaldehyde treatment of ethanol stressed cells, 147 contained HSEs; 113 contained STREs and 92 contained AREs (Table 4.1a, in Appendix III). As shown in Table 3.3a, in appendix III, a detailed study of the macroarray result showed that, of the 75 ribosomal genes that were highly expressed by the stimulatory effect of acetaldehyde on ethanol-stressed cells, 44 contained HSEs, 47 had STREs and 17 had AREs sequences in their promoter regions. Of the 18 transport genes that were MHE, 13 contained HSEs, 13 contained STREs and 2 had AREs. This promoter analysis showed the central role of STRE and HSE elements in the likely activation of most of genes with increased expression levels and, to a lesser extent, ARE, in the response to ethanol stress in the presence of acetaldehyde.

5.3 Conclusion

In conclusion, the work presented here is largely consistent with previous work on the impact of ethanol stress on global gene expression in yeast reported by Chandler *et al*. (2004) and Alexandre *et al*. (2001). The general trend in these reports is that a large number of genes associated with anabolism are down regulated in response to ethanol stress, and the cell appears to enter into a pseudo starvation state.

However, in work presented in this thesis, when a small quantity of acetaldehyde was added to ethanol-stressed cultures there was a significant level of 'recovery' of expression of genes associated with anabolism and this is consistent with growth curves of the cultures; cells were clearly in recovery mode when acetaldehyde was present in the growth medium. Interestingly however, the recovery appears not to be due to increased expression of genes associated with trehalose synthesis or genes encoding HSPs.

When a small quantity (0.1 g/l) of acetaldehyde was added to a non-stressed yeast, its growth profile was not significantly different to that of an untreated control. Similarly, from array analysis a small quantity of added acetaldehyde did not have a major impact on gene expression of non-stressed cultures at the one and five hour time points.

Upstream promoter analysis of the ORFs that were MHE when ethanol-stressed cells were treated with acetaldehyde relative to ethanol-stressed cultures, demonstrated that HSE, STRE and ARE elements are common features of upstream regions of these ORFs. This may mean that at least some of the acetaldehyde-induced recovery to ethanol stress is mediated by typical stress response-like changes in the cell; i.e. acetaldehyde stimulates aspects of the stress-response to enable rapid acclimatisation to the stress. However this seems unlikely in light of the fact that acetaldehyde did not stimulate higher expression of HSP genes, in fact many HSP genes were LHE in ethanol-stressed cells following treatment with acetaldehyde. With hindsight the promoters of MHE ORFs should have been screened for common elements to see if there are any novel motifs in their promoters; this should be picked up in future work.

The time-course study conducted for this thesis showed that most of the differences in gene expression between various combinations of treatments were transient. This might reflect the fact that different genes are required at different stages of growth (Gasch *et al*., 2000) and may also reflect what has been observed elsewhere, that acclimatisation to imposed stress (or altered environmental conditions) is achieved in the first few hours of exposure to the changed conditions (Chandler *et al.,* 2004; Gasch *et al*., 2000).

The significance of the results presented in this chapter is discussed further, and in a broader context of the acetaldehyde-stimulated recovery from ethanol stress, in Chapter 7.

The significance of some of the changes identified at mRNA level using global array technology was further investigated using knockout strains for genes of interest. The phenotype performances of these strains were tested in ethanol-stressed cultures in presence and absence of acetaldehyde as described in the following chapter.

CHAPTER 6

Characterisatoin of the Phenotypes of *Saccharomyces cerevisiae* Strains with Targeted Knockouts of Genes Associated with Acetaldehyde-Mediated Amelioration of Ethanol-stress

6.1 Introduction

In the previous chapter a large number of genes were found to have significantly changed expression levels in response to ethanol stress in the presence of added acetaldehyde. While gene array technology allows the study of gene expression profile, the role of the products of genes with changed expression in response to ethanol stress in the presence of acetaldehyde remains unknown. To investigate the role of these gene products during ethanol stress, it was decided to determine the phenotype of yeast strains containing single knockouts of some genes identified in the previous chapter. In yeast, genes are deleted using gene replacement cassettes that can be generated by PCR, requiring as little as 30 bases of flanking homology to target recombination on either side of the gene of interest. The resulting mutant can then be examined under a number of different selective growth conditions to determine if it differs phenotypically from the parent (wild type).

PDC1, PDC5, PHO84, HXT4 and *YLR364W* were all observed to have higher expression levels during ethanol stress when in the presence of added acetaldehyde, relative to an ethanol-stressed culture. These five genes were chosen for further studies in view of their increased expression levels and their metabolic functions, which are described below.

Pyruvate decarboxylases (*PDC1* and *PDC5*): The first step in production of ethanol from pyruvate is the decarboxylation of pyruvate to acetaldehyde and CO_2 by the enzyme pyruvate decarboxylase. *Saccharomyces cerevisiae* has three structural genes encoding pyruvate decarboxylase enzymes (*PDC1, PDC5* and *PDC6*). Another *PDC*

gene, *PDC2,* encodes a positive regulator of the transcription of *PDC1* and *PDC5* (Hohmann and Cederberg, 1990). The finding that *PDC5* and *PDC1* were more highly expressed in acetaldehyde-treated ethanol-stressed cells relative to ethanol-stressed cells is supportive of a proposed model for the effect of acetaldehyde on ethanol-stressed yeast cells. Stanley *et al*. (1997) proposed that ethanol stressed cells leak acetaldehyde into the surrounding medium and thus lose the capacity to fully regenerate NAD^+, which is essential for glycolysis. Thus, according to this model, ethanol-stressed cells have a reduced capacity to generate ATP. Increased expression of the *PDC* genes would conceivably increase acetaldehyde production, perhaps compensating for acetaldehyde losses from the cell, and thereby maintaining glycolytic flux.

Hexose (*HXT4*) and Phosphate (*PHO84)* transporters: The first essential step of sugar utilization is the uptake of sugars by the yeast cells. Sugar transport across the plasma membrane is thought to be the limiting step in sugar (hexose) metabolism. There are twenty members of the hexose transport (*HXT*) gene family, only seven of which (*HXT1-HXT7*) are known to encode functional glucose transporters; these may operate in various environmental conditions to ensure that the cell receives an adequate supply of carbon and energy. *HXT4* encodes a hexose transporter with medium affinity (Reifenberger *et al*., 1997) and transcription of this gene depends completely on Gcr1p and partially on Gcr2p transcription factors (Turkel and Bisson, 1999).

The *PHO84* gene encodes a high affinity, transmembrane, inorganic phosphate transporter. It was previously reported that the *PHO84* gene product is the major transporter of cellular phosphate (Bun-Ya *et al*., 1991). However, studies on a *PHO84Δ* mutant found that such mutants can obtain their phosphate using five other phosphate transporters (*PHO87, 88, 89, 90,* and *91*); the deletion of all six *PHO* genes is lethal (Wykoff and O'Sheal, 2001).

Ethanol stress compromises nutrient transport activities across the plasma membrane (Leao and Van Uden, 1982; and Salmon *et al*., 1993), therefore the increased expression of *HXT and PHO84* genes may play a role in ethanol tolerance by increasing the availability of glucose and phosphate to cells.

Genes of unknown function (*YLR364W*): Many ORFs encoding for products with unknown function showed a change in expression when acetaldehyde was added to ethanol-stressed cells. *YLR364W* was chosen for further work because of its particularly high level of expression in acetaldehyde-treated ethanol-stressed cells (relative to ethanol-stressed cells).

6.2 Analysis of yeast knockout strains

The experiments described in this chapter investigated the phenotypes of ethanol-stressed *S. cerevisiae* knockout strains in the presence and the absence of added acetaldehyde. It must be noted that the yeast strain used in this chapter is different to that used in the preceding chapters. In previous chapters *S. cerevisiae* PMY1.1 was used to study the effect of added acetaldehyde on ethanol-stressed yeast cells. If this strain were to be used for studying the phenotype of yeast knockout strains then a different knockout strain of PMY1.1 would need to be constructed for each gene under investigation. This is a time consuming and unnecessary task given that a *S. cerevisiae* BY4742 gene knockout collection was available from the Australian Wine Research Institute for this project. With this in mind, the phenotype studies described in this chapter were conducted using *S. cerevisiae* BY4742 and single gene knockout strains derived from this.

Table 6.1: Selection of *S. cerevisiae* BY4742 knockout strains according to their growth on YEPD and YEPD geneticin (G418) plates.

Strain	Medium	Growth (Yes/No)
BY4742	YEPD	Yes
BY4742	YEPD + geneticin	No
ΔPDC1	YEPD	Yes
ΔPDC1	YEPD + geneticin	Yes
ΔPDC5	YEPD	Yes
ΔPDC5	YEPD + geneticin	Yes
ΔPHO84	YEPD	Yes
ΔPHO84	YEPD + geneticin	Yes
ΔHXT4	YEPD	Yes
ΔHXT4	YEPD + geneticin	Yes
ΔYLR364W	YEPD	Yes
ΔYLR364W	YEPD + geneticin	Yes

6.2.1 Confirmation of gene replacement

Five knockout strains in a *S. cerevisiae* background were used for this work: BY4742 *Δpdc5*::*kanMX4, Δpdc1*::*kanMX4, Δpho84*::*kanMX4, Δhxt4*::*kanMX4* and *Δylr364w*::*kanMX4*. These were kindly donated by The Australian Wine Research Institute (AWRI). The presence of a *kanMX* gene in each of these strains was confirmed by plating onto both YEPD and YEPD Geneticin plates and incubating at 30°C for 2 – 3 days. The YEPD Geneticin plates did not support the growth of the parent strain, whereas all of the knockout strains grew on YEPD and YEPD Geneticin plates (Table 6.1).

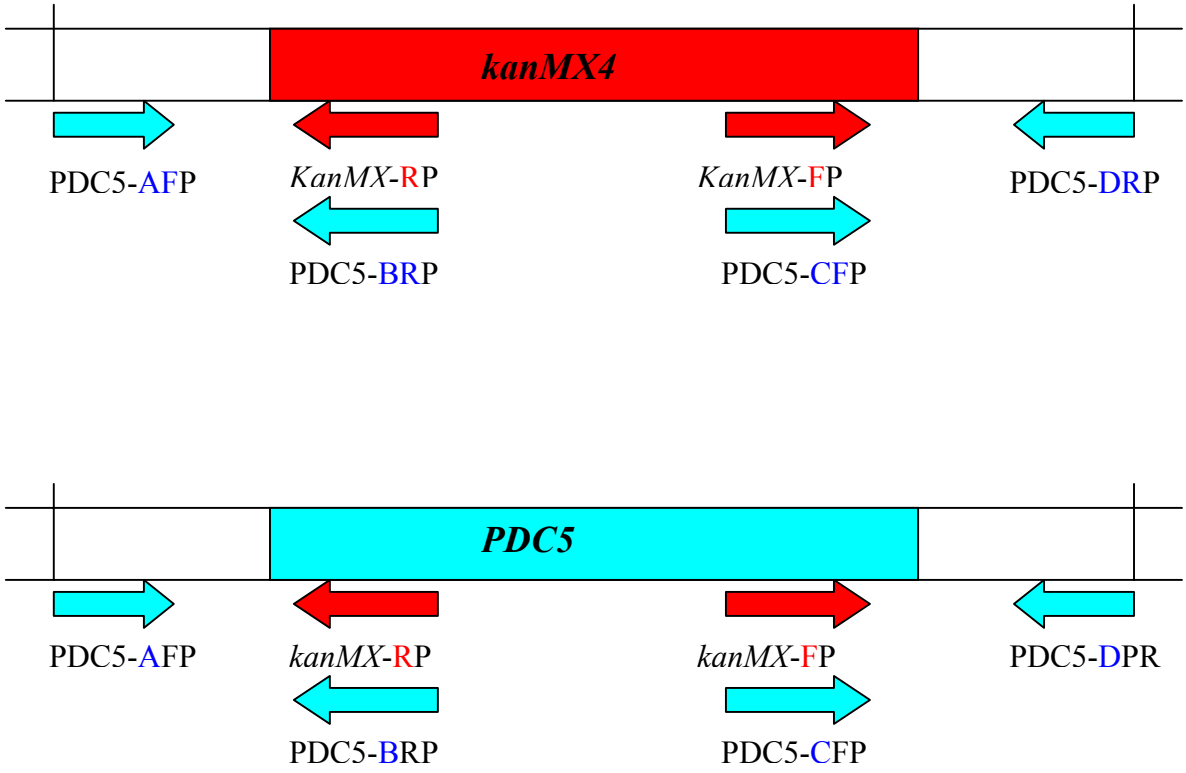

Figure 6.1: Schematic representation of PCR positioning primers for confirmation of ΔPDC5 knockout construct compared to the BY4742 wild type.

6.2.2 PCR-based confirmation of the presence of the *kanMX* cassette in BY4742 knockouts

The replacement of the *PDC1, PDC5, PHO84, HXT4* and *YLR364W* ORFs with the *kanMX4* module was confirmed by colony PCR. The presence and position of the *kanMX4* module was tested using a combination of four primers specific to each strain. For example, primers complementary to the upstream and downstream flanking regions of the *PDC5* ORF, *PDC5*-FP (forward primer) and *PDC5*-RP (reverse primer) were used in combination with upstream and downstream flanking regions of the *kanMX4* cassette (*kanMX*-FP and *kanMX*-RP). This is diagrammatically represented in Figure 6.1. The PCR reaction product was resolved on 1% agarose gel by electrophoresis at 60 V for 45-60 minutes (Figures 6.2 and 6.3).

The amplified product of PCR reactions that confirmed the integration of the *KanMX4* module in the place of *PDC5* and *PHO84* genes are shown in Figures 6.2 & 6.3. As shown in Figure 6.2 with primer combination of PDC5-AFP/PDC5-BRP no PCR product was amplified using DNA from *ΔPDC5* mutant cells. When PDC5-AFP/kanMX-RP primer combination was used with DNA from *ΔPDC5* mutant cells and wild type cells, this resulted in a 661 bp product and no PCR product respectively, but when PDC5-AFP/PDC5-BRP primer combination was used with DNA from wild type cells, it resulted in 850 bp PCR product. When PDC5-CFP/PDC5-DRP primer combination was used with DNA from Δ*PDC5* cells resulted in no PCR product amplification. When *kanMX*-FP and PDC5-DRP primer combination was used with DNA from *ΔPDC5* mutant cells and wild type cells result in 905 bp and no PCR product amplified respectively, but when PDC5-CFP/PDC5-DRP primer combination was used with DNA from wild type cells resulted in 515 bp PCR product (Figure 6.2). All knockout strains were confirmed.

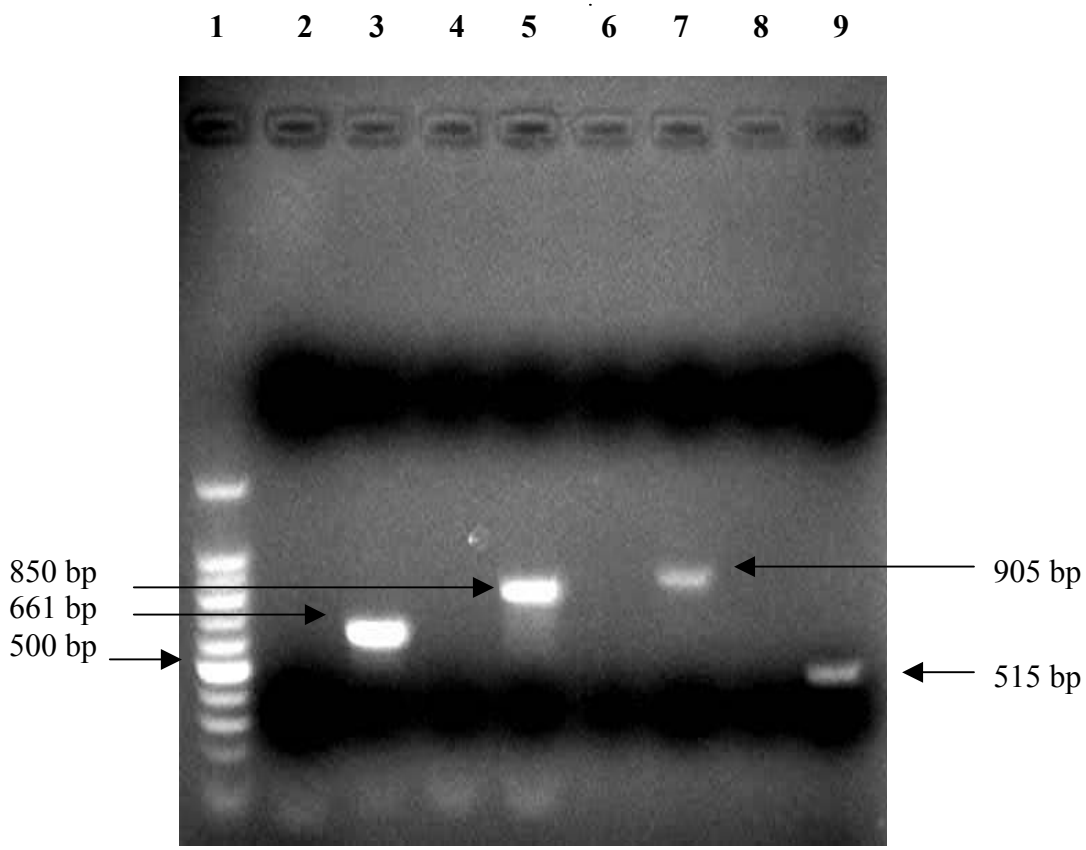

Figure 6.2: Agarose gel electrophoresis of PCR products to confirm the integration of the *KanMX4* module in the place of PDC5 gene. Lane 1: Marker DNA, Lane 2: No product was amplified from PDC5-AFP and PDC5-BRP primer combination using DNA from *ΔPDC5* mutant cells. Lane 3: A PCR product of 661 bp was amplified from PDC5-AFP and *kanMX*-RP primer combination using DNA from *ΔPDC5* mutant cells. Lane 4: No PCR product was amplified from PDC5-AFP and *kanMX*-RP primer combination using DNA from wild type cells. Land 5: A PCR product of 850 bp was amplified from PDC5-AFP and PDC5-BRP primer combination using DNA from wild type cells. Lane 6: No PCR product was amplified from PDC5-CFP and PDC5-DRP primer combination using DNA from Δ*PDC5* cells. Lane 7: A PCR product of 905 bp was amplified from *kanMX*-FP and PDC5-DRP primer combination using DNA from *ΔPDC5* mutant cells. 8: No PCR product was generated from *kanMX*-FP and PDC5-DRP primer combination using DNA from wild type cells. Lane 9: A PCR product of 515 bp was amplified from PDC5-CFP and PDC5-DRP primer combination using DNA from wild type cells.

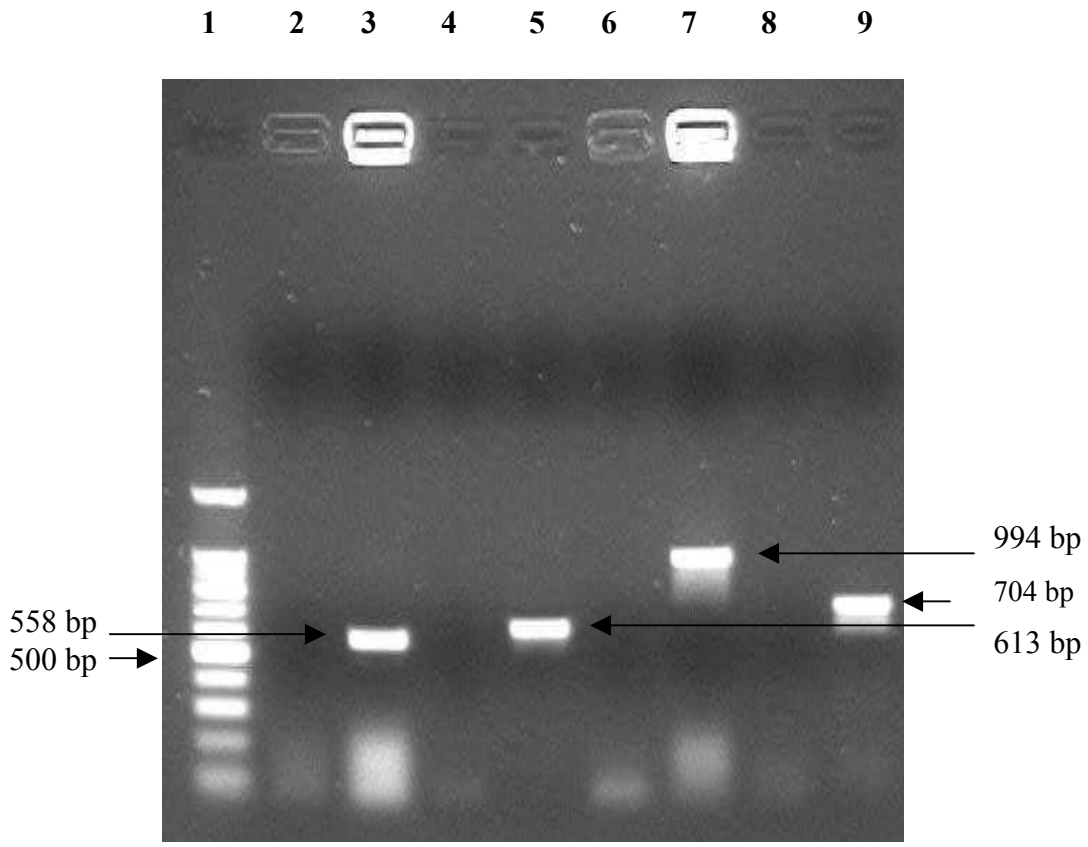

Figure 6.3: Agarose gel electrophoresis of PCR products to confirm the integration of the *KanMX4* module in the place of PHO84 gene. Lane 1: Marker DNA, Lane 2: No product was amplified from PHO84-AFP and PHO84-BRP primer combination using DNA from *ΔPHO84* mutant cells. Lane 3: A PCR product of 558 bp was amplified from PHO84-AFP and *kanMX*-RP primer combination using DNA from *ΔPHO84* mutant cells. Lane 4: No PCR product was amplified from PHO84-AFP and *kanMX*-RP primer combination using DNA from wild type cells. Land 5: A PCR product of 613 bp was amplified from PHO84-AFP and PHO84-BRP primer combination using DNA from wild type cells. Lane 6: No PCR product was amplified from PHO84-CFP and PHO84-DRP primer combination using DNA from *ΔPHO84* mutant cells. Lane 7: A PCR product of 994 bp was amplified from *kanMX*-FP and PHO84-DRP primer combination using DNA from PHO84 mutant cells. Lane 8: No PCR product was generated from *kanMX*-FP and PHO84-DRP primer combination using DNA from wild type cells. Lane 9: A PCR product of 704 bp was amplified from PHO84-CFP and PHO84-DRP primer combination using DNA from wild type cells.

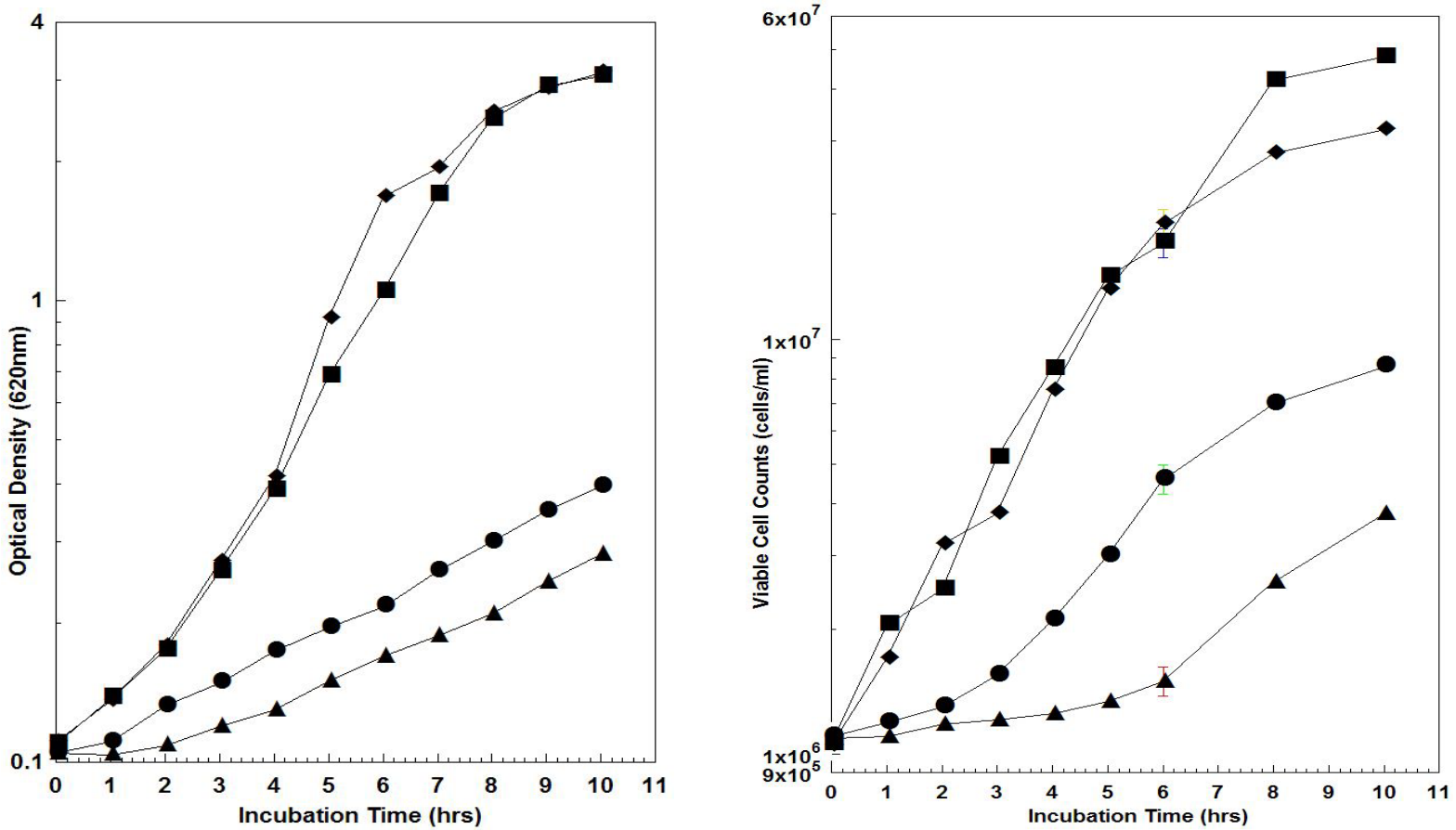

Figure 6.4: The stimulatory effect of acetaldehyde on ethanol-stressed *S. cerevisiae* BY4742 (Wt) strain. Late exponential phase culture was inoculated into YEPD only (■), or YEPD containing only 0.1 g/l acetaldehyde (♦), only 7.5% (v/v) ethanol (▲), or both 7.5% (v/v) ethanol & 0.1 g/l acetaldehyde (●). The cultures were incubated at 30°C/140 rpm, under aerobic conditions.

Table 6.2: Growth profiles of *S. cerevisiae* PMY1.1 and BY4742 strains during ethanol stress in presence or absence of acetaldehyde.

Figures	Strains	Ethanol (v/v)	Acetaldehyde (g/l)	Lag Time (h)	Doubling Time (h)	Growth Rate (h^{-1})
4.3	PMY1.1 (WT)	0%	0.0	0.8	1.9±0.10	0.37±0.01
		0%	0.1	0.8	1.9±0.10	0.37±0.01
		7%	0.0	6.1	3.5±0.23	0.20±0.015
		7%	0.1	2.0	2.6±0.20	0.27±0.010
6.4	BY4742 (WT)	0%	0.0	0.0	1.8±0.105	0.38±0.02
		0%	0.1	0.0	1.8±0.105	0.38±0.02
		7.5%	0.0	4.8	3.3±0.15	0.21±0.01
		7.5%	0.1	2.0	2.5±0.15	0.28±0.015

6.3 Characterizing the phenotypes of wild type and knockout strains

6.3.1 Growth profile of BY4742 strain compared to PMY1.1 strain

The experimental design used to characterize the phenotype of *S. cerevisiae* BY4742 and the corresponding knockout strains were based on that used for the growth studies in Chapters 3 and 4 (Sections 3.2.2 and 4.2**)**. To summarize, late exponential phase cells were inoculated into fresh YEPD medium containing either no ethanol or 7.5% (v/v) ethanol in the presence or absence of added acetaldehyde (0.1 g/l). Samples were taken regularly to monitor cell growth.

Before investigating the response of ethanol-stressed BY4742 knockout strains to added acetaldehyde, it was first necessary to compare the phenotype of ethanol-stressed *S. cerevisiae* BY4742 to that of *S. cerevisiae* PMY1.1. *S. cerevisiae* BY4742 was found to be slightly more ethanol-tolerant than strain PMY1.1 (Figures 4.3 and 6.4; Table 6.2). In the absence of ethanol and added acetaldehyde, both strains had similar growth rates and these were unaffected when in the presence of added acetaldehyde only. The lag period of PMY1.1 strain in the presence of 7% (v/v) ethanol was slightly longer than BY4742 when in the presence of a slightly higher ethanol concentration of 7.5% (v/v). Both strains had similar lag periods in the presence of added acetaldehyde and added ethanol (7% v/v for PMY1.1 and 7.5% v/v for BY4742). Given the similarities of the two strains in their response to ethanol stress and added acetaldehyde, *S. cerevisiae* BY4742 was considered to be a representative strain for determining the effect of selected gene deletions on the *S. cerevisiae* phenotype during ethanol stress and in the presence of added acetaldehyde.

S. cerevisiae BY4742 was inoculated from an overnight culture into fresh YEPD medium with and without an ethanol concentration of 7.5% (v/v) and in the presence or absence of acetaldehyde. Lag period, growth rates and doubling times were calculated from viable plate count data Figure 6.4. Strain BY4742 had no detectable lag period in the absence of

ethanol stress and a doubling time of 1.8 hrs and growth rate of 0.38 h^{-1}. In the presence of 7.5% ethanol stress strain BY4742 had a 4.8 h lag period, a 3.3 h doubling time and a growth rate of 0.21 h^{-1}. When a small quantity of acetaldehyde was present in the 7.5% ethanol-stressed cultures, the lag period was reduced to 2.0 hrs, the doubling time was 2.5 hrs and the growth rate was 0.28 h^{-1}. These results suggest that strains BY4742 and PMY1.1 have a similar physiological response to ethanol stress in the presence and absence of added acetaldehyde (Table 6.2); however BY4742 strain is slightly better able to acclimatize and recover from ethanol stress than strain PMY1.1.

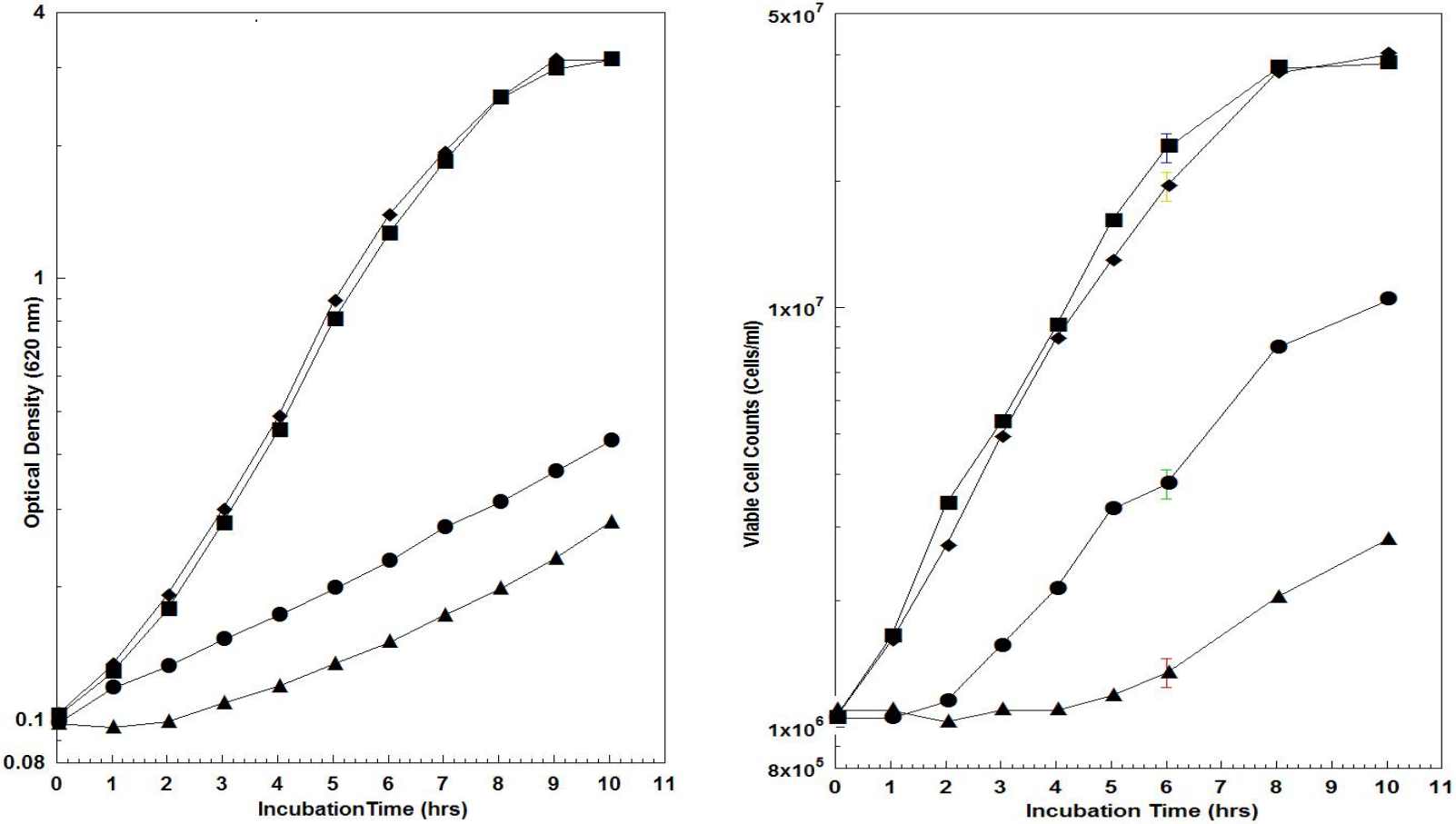

Figure 6.5: The stimulatory effect of acetaldehyde on ethanol-stressed *S. cerevisiae* ΔPDC1 strain. Late exponential phase culture was inoculated into YEPD only (■), or YEPD containing 0.1 g/l acetaldehyde only (♦), 7.5% (v/v) ethanol only (▲), or both 7.5% (v/v) ethanol & 0.1 g/l acetaldehyde (●). The cultures were incubated at 30°C/140 rpm, under aerobic conditions.

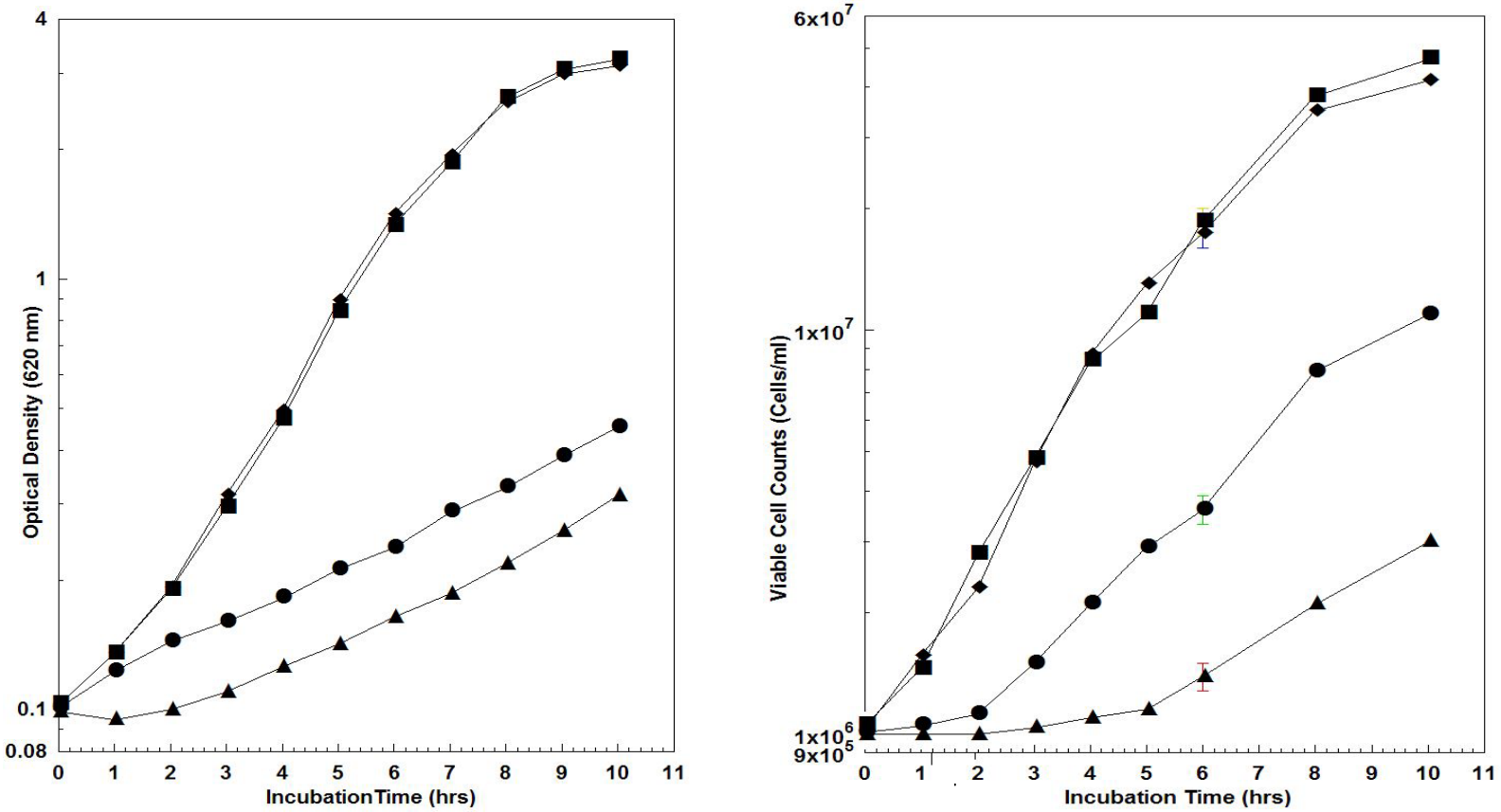

Figure 6.6: The stimulatory effect of acetaldehyde on ethanol-stressed *S. cerevisiae* ΔPDC5 strain. Late exponential phase culture was inoculated into YEPD only (■), or YEPD containing 0.1 g/l acetaldehyde only (♦), 7.5% (v/v) ethanol only (▲), or both 7.5% (v/v) ethanol & 0.1 g/l acetaldehyde (●). The cultures were incubated at 30°C/140 rpm, under aerobic conditions.

6.3.2 Phenotypes of *S. cerevisiae* BY4742 knockout strains

There was no significant difference in the growth profiles between parent strain BY4247 wild type compared to knockout strains, *ΔPDC1* and *ΔPDC5*, during 7.5% (v/v) ethanol stress in presence or absence of acetaldehyde (Figures 6.5 & 6.6, Table 6.3). Both *ΔPDC1* and *ΔPDC5* knockout strains grew similarly without a measurable lag period in the absence of ethanol stress. In the presence of a 7.5% ethanol stress, the lag periods were 4.8, 4.8 and 4.7 hours for the wild type BY4247, *ΔPDC1* knockout and *ΔPDC5* knockout strains respectively. In the ethanol-stressed cultures containing acetaldehyde (0.1g/l), the ethanol-induced lag period was reduced to 1.8 hours in both *ΔPDC1* knockout and *ΔPDC5* knockout strains (Table 6.3). There was no significant difference between the exponential growth rates of the parent strain and knockout strains during 7.5% ethanol stress in presence of added acetaldehyde.

Growth profiles of the three knockout strains *ΔPHO84, ΔHXT4 and ΔYLR364W* in YEPD medium containing 0% and 7.5% ethanol in presence and absence of acetaldehyde are shown in Figures 6.7, 6.8 and 6.9. There was no discernable difference in viable cell population profile for the knockout strain cultures compared to the viable cell population profile of the BY4247 strain grown under the same conditions (Table 6.3). In the absence of ethanol stress all three-knockout strains and the wild type commenced exponential growth without a detectable lag period. In the presence of 7.5% (v/v) ethanol stress, there were lag periods of 4.9±0.19 hours, when a small quantity of acetaldehyde (0.1 g/l) was added to the ethanol-stressed cultures the lag period was reduced to 1.9±0.14 hours (Figures 6.5, 6.6, 6.7, 6.8 and 6.9, Table 6.3). The knockout strains and wild type cells showed similar doubling time and growth rates compared to the wild type cells. These experiments were repeated to determine the reproducibility and accuracy of the above experiments, and similar results were obtained for each experiment.

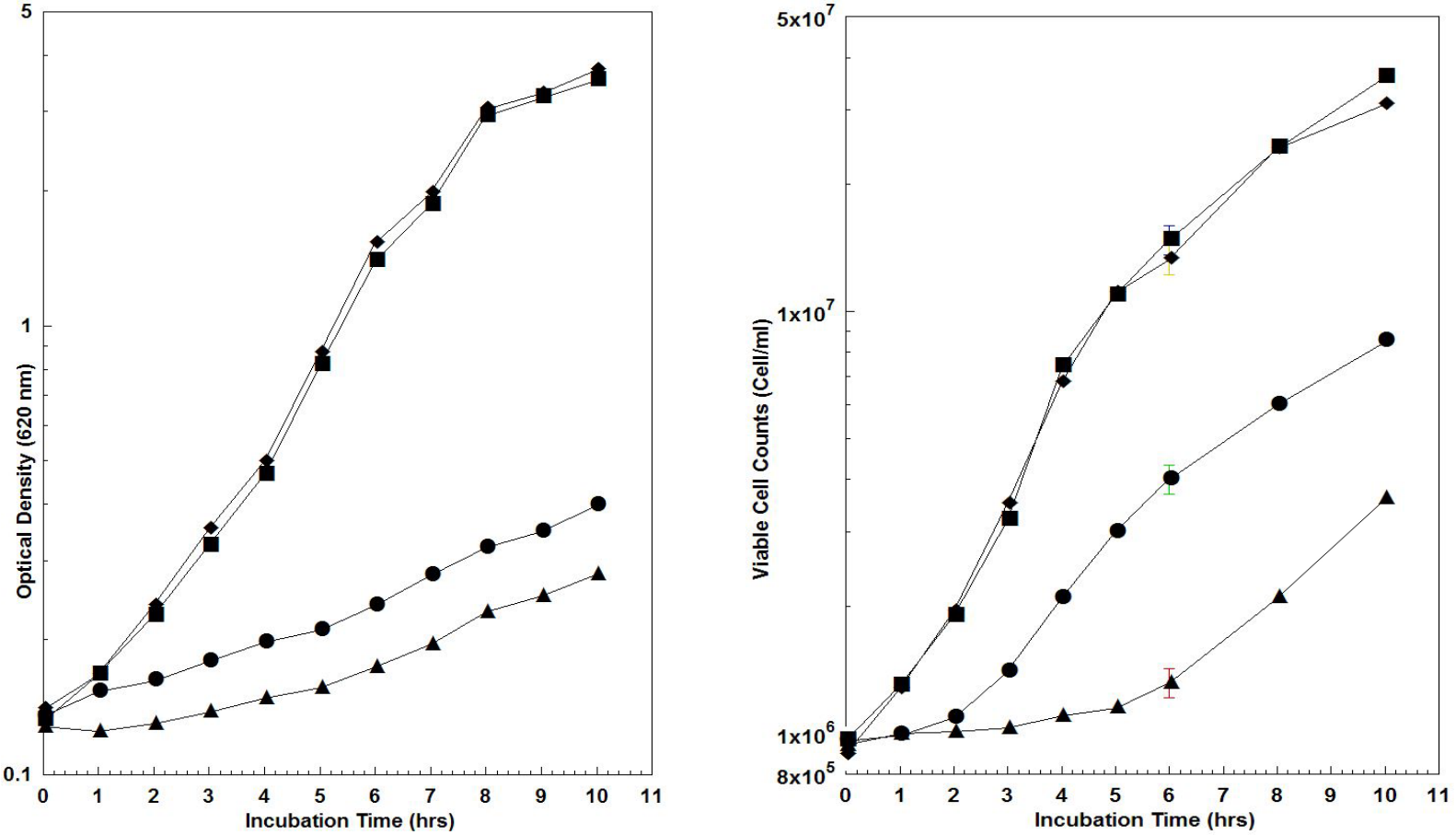

Figure 6.7: The stimulatory effect of acetaldehyde on ethanol-stressed *S. cerevisiae* ΔPHO84 strain. Late exponential phase culture was inoculated into YEPD only (■), or YEPD containing 0.1 g/l acetaldehyde only (♦), 7.5% (v/v) ethanol only (▲), or both 7.5% (v/v) ethanol & 0.1 g/l acetaldehyde (●). The cultures were incubated at 30°C/140 rpm, under aerobic conditions.

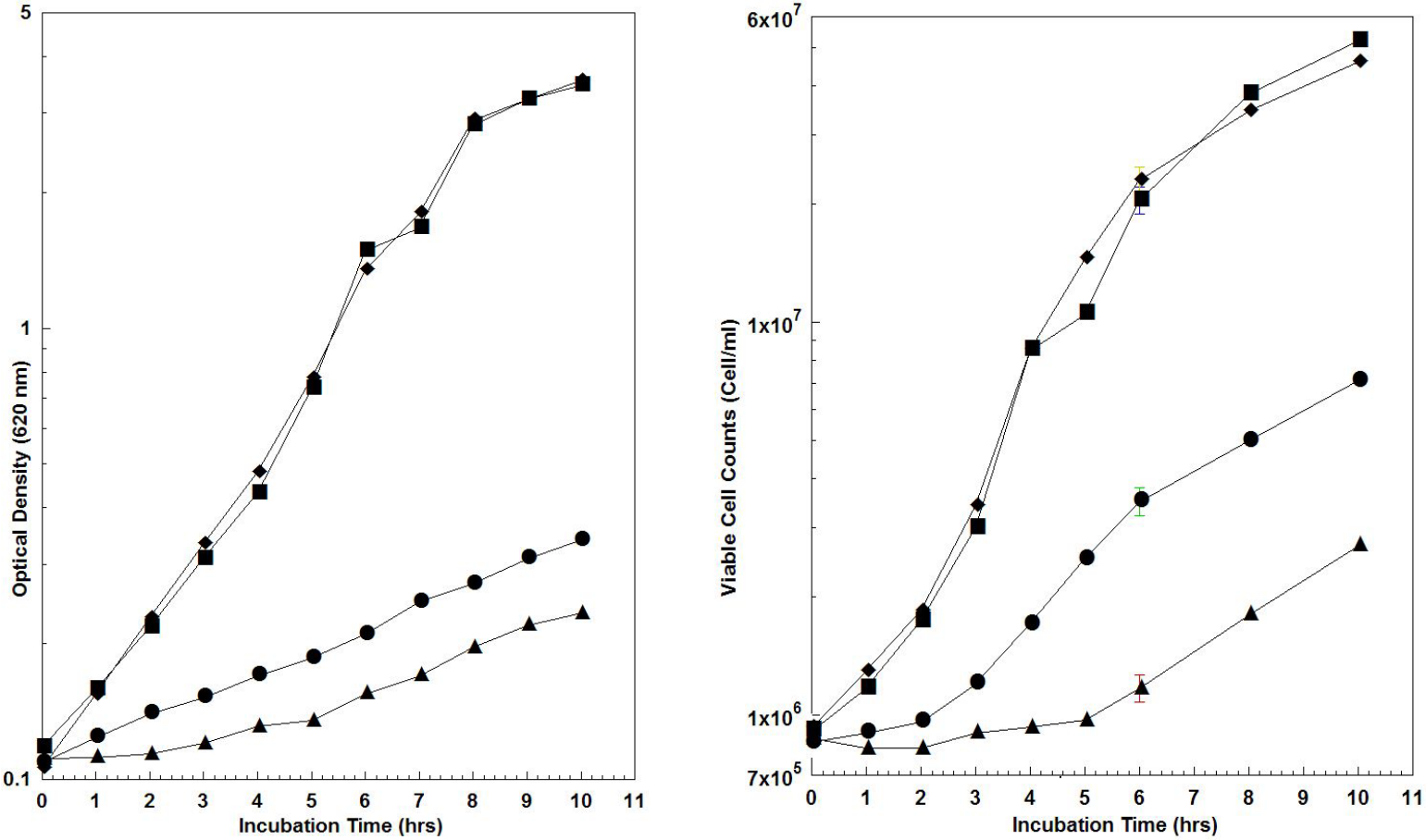

Figure 6.8: The stimulatory effect of acetaldehyde on ethanol-stressed *S. cerevisiae* ΔHXT4 strain. Late exponential phase culture was inoculated into YEPD only (■), or YEPD containing 0.1 g/l acetaldehyde only (♦), 7.5% (v/v) ethanol only (▲), or both 7.5% (v/v) ethanol & 0.1 g/l acetaldehyde (●). The cultures were incubated at 30°C/140 rpm, under aerobic conditions.

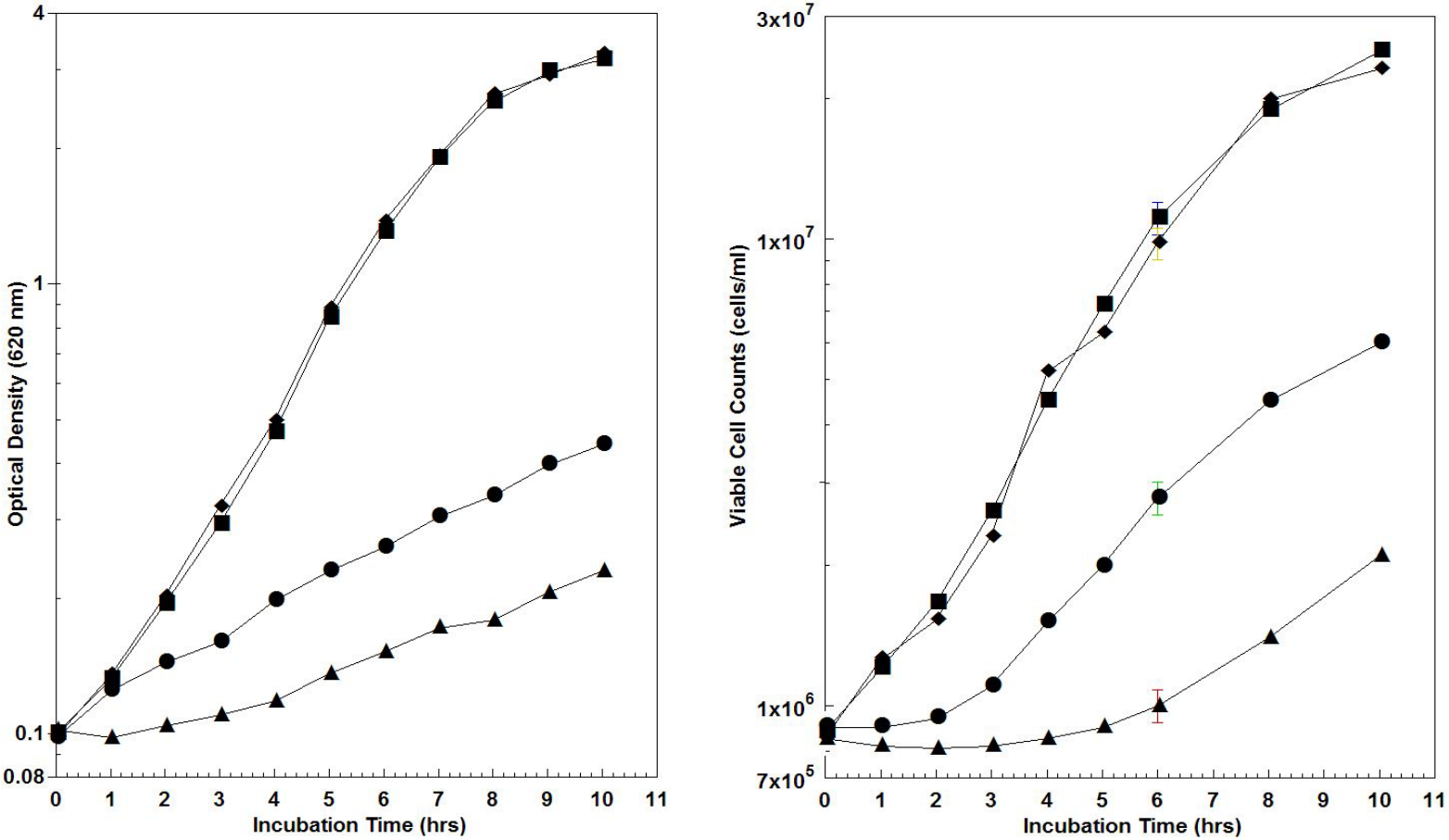

Figure 6.9: The stimulatory effect of acetaldehyde on ethanol-stressed *S. cerevisiae* ΔYLR346W strain. Late exponential phase culture was inoculated into YEPD only (■), or YEPD containing 0.1 g/l acetaldehyde only (♦), 7.5% (v/v) ethanol only (▲), or both 7.5% (v/v) ethanol & 0.1 g/l acetaldehyde (●). The cultures were incubated at 30°C/140 rpm, under aerobic conditions.

Table 6.3: Effect of ethanol stress on the growth of wild type and knockout mutant *S. cerevisiae* BY4742 stains in presence and absence of acetaldehyde.

Figures	Strains	Ethanol (v/v)	Acetaldehyde (g/l)	Lag Time (h)	Doubling Time (h)	Growth Rate (h^{-1})
6.4	BY4742 (Wild type)	0%	0.0	0.0	1.8±0.11	0.38±0.02
		0%	0.1	0.0	1.8±0.11	0.38±0.02
		7.5%	0.0	4.8	3.3±0.15	0.21±0.01
		7.5%	0.1	2.0	2.5±0.15	0.28±0.02
6.5	*ΔPDC1*	0%	0.0	0.0	1.9±0.12	0.38±0.01
		0%	0.1	0.0	1.9±0.12	0.38±0.01
		7.5%	0.0	4.8	3.5±0.10	0.20±0.01
		7.5%	0.1	1.8	2.2±0.10	0.32±0.01
6.6	*ΔPDC5*	0%	0.0	0.0	1.9±0.07	0.37±0.02
		0%	0.1	0.0	1.9±0.07	0.37±0.02
		7.5%	0.0	4.7	3.0±0.20	0.23±0.02
		7.5%	0.1	1.8	2.2±0.13	0.31±0.02
6.7	*ΔPHO84*	0%	0.0	0.0	1.8±0.01	0.38±0.02
		0%	0.1	0.0	1.8±0.01	0.38±0.02
		7.5%	0.0	4.9	3.3±0.15	0.21±0.01
		7.5%	0.1	2.0	2.2±0.06	0.31±0.01
6.8	*ΔHXT4*	0%	0.0	0.2	1.8±0.06	0.39±0.01
		0%	0.1	0.2	1.8±0.06	0.39±0.01
		7.5%	0.0	5.0	2.9±0.20	0.23±0.01
		7.5%	0.1	2.1	2.3±0.10	0.3±0.01
6.9	*ΔYLR346W*	0%	0.0	0.0	2.0±0.15	0.35±0.02
		0%	0.1	0.0	2.0±0.15	0.35±0.02
		7.5%	0.0	5.1	3.5±0.15	0. 20±0.01
		7.5%	0.1	1.8	2.4±0.10	0.29±0.01

6.4 Discussion

The ability of acetaldehyde to significantly reduce the adaptation period of *Saccharomyces cerevisiae* during ethanol stress is well proven; however the mechanism underpinning this effect is unknown. For the first time, this project has investigated the effect of acetaldehyde-mediated adaptation to ethanol stress on gene expression profile in *Saccharomyces cerevisiae*. Of the more than 200 genes found to have increased expression levels during ethanol stress in the presence of acetaldehyde (described in the previous chapter), five genes were selected for further investigation into the effect of their deletion on the phenotype of *S. cerevisiae* BY4742 mutants during ethanol stress in the presence and absence of acetaldehyde.

Four of the five chosen genes (*PDC1, PDC5, PHO84* and *HXT4*) were selected in part due to their relatively high expression levels during ethanol stress adaptation in the presence of acetaldehyde, and also due to their known roles, perceiving metabolism and potential influence on ethanol-stressed yeast cells. Chandler *et al.,* (2004) suggested that during ethanol-stress yeast are in a state of pseudo-starvation where nutrients such as glucose, although present in the extracellular medium, are not catabolized at a sufficient rate to meet cellular energy demands. This pseudo-starvation state could be due to ethanol inhibition of transporter activity, changes in the activity of enzymes involved in central metabolism, loss of intracellular metabolites or any combination of these or other events. With this in mind it was conceivable that genes stimulated by acetaldehyde during ethanol stress that produce proteins associated with central metabolism (*PDC1* and *PDC5*) and nutrient transport (*PHO84* and *HXT4*), were likely to have an important role in the acetaldehyde-mediated ethanol stress response of *S. cerevisiae*. The other gene, *YLR364W*, was selected because it had substantially increased expression levels during the ethanol stress response of *S. cerevisiae* in the presence of acetaldehyde and it has a product with unknown function, this combination of information stimulating curiosity into the possible role of *YLR364W* during ethanol stress.

The results of this chapter demonstrated that single deletions for each of the above five genes in *S. cerevisiae* BY4742 had no measurable effect on the growth phenotype of the

mutants during ethanol stress and in the presence of acetaldehyde. In the absence of each of these genes, the knockout strains had similar ethanol-induced lag periods to the parent strain and they also showed similar lag period reductions when acetaldehyde was added to the ethanol-stressed cells. This suggests that expression of each of the five genes singularly is not essential for acetaldehyde to have a stimulatory effect on ethanol-stressed yeast cells, these genes possibly have no role in the acetaldehyde effect on ethanol-stressed yeast cells.

A possible explanation for the gene deletions having no measurable effect on the acetaldehyde-stimulation of ethanol-stressed mutants is that the role of the deleted genes was compensated for by another gene. There is evidence in the literature to support this. Investigations into the role of *PDC1* and *PDC5* in *S. cerevisiae* have shown that in the absence of ethanol stress, the deletion of one *PDC* gene, either *PDC1* or *PDC5*, has no measurable effect on the growth profile of the knockout strain, the other *PDC* gene presumably compensating for the deletion (Hohmann and Cederberg, 1990); a double deletion of *PDC1* and *PDC5* resulted in a complete loss of *PDC* activity and the inability to ferment glucose. Interestingly a third *PDC* gene, known as *PDC2*, was found to be a positive regulator of both *PDC1* and *PDC5* genes (Hohmann, 1993). It was shown that a *PDC2* deletion resulted in a reduced expression level of *PDC1* and complete abolishment of *PDC5* expression (Hohmann and Cederberg, 1990; Nevoigt and Stahl, 1996). The role of the *PDC2* gene in regulating expression levels of both *PDC1* and *PDC5* presents an opportunity to further investigate the role of *PDC* activity in the effect of acetaldehyde on ethanol-stressed yeast cells. According to the above studies, a *PDC2* knockout strain would have no *PDC5* expression and a 70% reduction in *PDC1* expression levels. Investigations using a *PDC2* knockout strain may reduce the compensatory effect of *PDC1* for *PDC5*, and vice-versa, allowing the true effect of *PDC* deletions on the acetaldehyde stimulation of ethanol adaptation to be determined. This is recommended for further work.

PHO84 is a member of a set of isogenes (*PHO87, 88, 89, 90,* and *91*) that encode phosphate transporters. Wykoff and O'Sheal (2001) demonstrated that a ΔPHO84 knockout strain of *S. cerevisiae* had a similar growth profile to the parent strain when grown in the absence of ethanol stress, while the combined deletion of all *PHO* genes in a single strain was found to be lethal. The significantly increased expression levels of

PHO84 in response to acetaldehyde addition during ethanol stress (Section 5.2.2.3) suggested that *PHO84* may have a special role during ethanol stress, its protein product possibly being more resistant to the inhibitory effects of ethanol than other *PHO* gene products and therefore having a primary role in phosphate transport during ethanol stress. The work in this study has shown that the deletion of *PHO84* from *S. cerevisiae* have no measurable effect on the phenotype during ethanol stress in the presence of acetaldehyde. Based on previous studies by Wykoff and O'Sheal (2001) it is likely that the other *PHO* genes were compensating for the loss of *PHO84*. The importance of *PHO* activity during ethanol stress could be tested in further work by performing multiple knockouts of the *PHO* gene family and examining the phenotype of the multi-knockout strains during ethanol stress and in the presence of acetaldehyde.

To utilize glucose (hexose) as a carbon source cells must sense the presence of hexose sugar and transport it across the plasma membrane via the *HXT* gene family. The *HXT* gene family comprises seven isogenes (*HXT1, 2, 3, 4, 5, 6,* and *7*). It has previously been shown that *S. cerevisiae* strains containing multiple knockouts of all seven isogenes are not able to grow on glucose (Reifenberger *et al*., 1997) nor are they able to grow or ferment under winemaking conditions (Luyten *et al*., 2002). The *HXT4* gene has increased expression levels in the presence of low glucose concentrations, and to be repressed in the presence of high glucose concentrations (Ozcan and Johnston, 1995). It is therefore not surprising that the expression of *HXT4* is increased during periods of ethanol stress given that evidence suggests that ethanol-stressed *S. cerevisiae* cells are in a state of pseudo-starvation (Chandler *et al.,* 2004). Under such conditions the ethanol-stressed cell may 'believe' that it is in a low glucose environment and increase *HXT4* expression to compensate. The results of this project demonstrate that the deletion of *HXT4* from *S. cerevisiae* has no measurable effect on the phenotype during ethanol stress and in the presence of acetaldehyde. Once again it is likely that one or more of the other *HXT* genes is compensating for the loss of *HXT4* however this would need to be proven.

The deletion of *YLR364W* from *S. cerevisiae* has no measurable effect on the phenotype during ethanol stress and in the presence of acetaldehyde. Little is known about the function of the gene product in yeast and for this reason it is difficult to speculate on whether the gene has a role in the ethanol stress response of *S. cerevisiae*. Other genes

may compensate for *YLR364W* in the knockout strain or it may not have a crucial role in the adaptation of *S. cerevisiae* to ethanol stress.

6.4.1 Conclusion

The results in this chapter suggest that acetaldehyde-ameliorated recovery from ethanol-stress in *S. cerevisiae* does not rely on the singular expression of *PDC1, PDC5, PHO84, HXT4* or *YLR364W*. It is not suggested however that these genes do not have a role in this phenomenon, or indeed, in conferring ethanol-tolerance in the absence of acetaldehyde. It is possible, for example, that other genes may compensate for the loss of any one of five that were studied as knockout in the work described here. This would make sense from an evolutionary perspective since the important roles performed by each of the four known genes (*PDC1, PDC5, PHO84 and HXT4*) are unlikely to depend entirely on a single gene, rather, cell survival would be facilitated by having several genes able to perform similar roles.

The deletion of the above genes did not show a significant difference in viable population profile compared to the wild type strains. The specific roles of these genes in the ethanol stress response in presence of added acetaldehyde are yet to be determined, noting that the adaptation rate and growth rate were the only parameters tested in the work described here. It is possible that these genes may have a marginal influence on yeast adaptation to ethanol, or in their absence their role may be compensated by other genes. A more comprehensive and sensitive techniques are required to confirm or disprove this. These techniques are such as growth competition experiments, overexpresson and double/triple knockout strains. This should be investigated further, as will be discussed in the following chapter.

CHAPTER 7

General Discussion and Future Directions

7.1 Introduction

In this project, *S. cerevisiae* PMY1.1 was exposed to a 7% (v/v) ethanol stress, which induced a lag period of approximately 6 hours during which cells were acclimatizing to their environment. When a small quantity of acetaldehyde was added to ethanol-stressed cells, the lag period was reduced to approximately 2 hours. This result clearly demonstrated the acetaldehyde-induced amelioration of ethanol stress in yeast, in confirmation of the reports of Walker-Caprioglio and Parks (1987); Stanley and Pamment (1993); Stanley *et al.* (1997); Barber *et al*. (2002a); Vriesekoop and Pamment, (2005).

The mechanisms underlying the lag-reducing effect of acetaldehyde on ethanol-stressed yeast are unclear. However it was reasoned at the outset of this research project that the effect of added acetaldehyde on ethanol-stressed yeast would be likely to cause a change in expression of genes associated with the ethanol acclimatisation process, since changes in cell physiology are often the consequence of changes in gene expression. This project used both macroarray and microarray analyses to provide insights into the global pattern of gene expression during the acetaldehyde-stimulated recovery of ethanol-stressed cultures. Both technologies provided rapid, efficient, quantitative and a comprehensive analysis of gene expression profile. To-date, no molecular studies have been published that investigate the lag reducing effects of acetaldehyde on gene expression in ethanol-stressed cells

Part of this research study confirmed previous work reported by Chandler *et al*. (2004) and Alexandre *et al*. (2001) that showed a substantial number of genes associated with anabolism were LHE in response to ethanol stress compared to unstressed cultures. These genes encode proteins associated with ribosomes, transport, transcription

initiation, cell cycle, energy utilization, DNA synthesis, cell wall, nucleotide metabolism and lipid metabolism genes. The decreased expression levels of these genes could be expected for cells undergoing growth arrest to conserve energy expenditure during stress conditions. This response also correlates with growth curves that showed a lag period of approximately 6 hours, when yeast cultures were subjected to 7% ethanol stress. Overall, the array results of this study showed the decreased expression level of anabolic genes and increased expression level of some stress response genes (such as HSP genes and trehalose genes), are consistent with the findings of Chandler *et al*. (2004) and Alexandre *et al*. (2001), for ethanol-stressed yeast cells.

An interesting aspect of the array data is that, when acetaldehyde was added to ethanol-stressed yeast cells, there was increased expression of many genes. Perhaps the most striking feature was the clustering of MHE ORFs associated with the synthesis of ribosomal proteins, DNA synthesis, transcription and a cell cycle, and this was evident at both time points (See Tables 5.1 and 5.2). This is consistent with growth curve data shown in Figures 4.3 and 4.4, in which it is clear that cells are rescued from ethanol-induced lag considerably faster when added acetaldehyde is present in ethanol-containing cultures; when acetaldehyde is present cells commence division more rapidly.

Results presented in this thesis also show that acetaldehyde treatment of ethanol-stressed yeast cells decreases the expression level of *HSP* genes and genes associated with trehalose metabolism. On the one hand, the decreased expression levels of *HSP* and trehalose genes, in response to the stimulatory effect of added acetaldehyde to ethanol-stressed cells, may reflect that cell stress was alleviated by the addition of a small quantity of acetaldehyde and this stimulated growth; therefore cells did not need the protective 'inputs' of HSPs and trehalose. In this case, growth would not be a direct effect of acetaldehyde addition; i.e. acetaldehyde was not being used as a carbon source. On the other hand, in the presence of the acetaldehyde (for ethanol-stressed cells) there was increased expression of ribosomal protein genes, genes associated with transcription initiation and protein metabolism, and cell cycle genes, thus potentially stimulating cell growth and division. In light of this and the growth curves seen in Figures 4.3 and 4.4, it is clear that even in the presence of 'stressful levels' of ethanol, yeast cells were actively growing and dividing under the stimulatory effect of added

acetaldehyde. In the absence of acetaldehyde the ethanol-stressed cells were in growth arrest.

When a small quantity (0.1 g/l) of acetaldehyde was added to non-stressed yeast cultures, it had no detectable effect on the growth of yeast cells and it did not have a significant effect on gene expression in non-stressed cultures. This shows that the stimulatory effect of a small quantity of added acetaldehyde to yeast cultures and gene expression is only observed in cells subject to ethanol-stress. This is important to note because it suggests that the mechanism by which acetaldehyde stimulates the adaptation of yeast to ethanol is only functional in a stress-compromised cell.

The general patterns of gene expression using both macro- and microarrays appeared similar, except that microarrays seemed to be more sensitive, i.e. macroarray analysis revealed 214 ORFs were MHE and 4 ORFs were LHE whilst in microarrays 239 ORFs were MHE and 217 were LHE, in response to stimulatory effect of acetaldehyde to ethanol-stressed cultures compared to ethanol-stressed cultures, at one hour time point. This finding is in line with that of Bowtell, (1999), which showed microarrays were more sensitive than macroarrays in the detection of low abundance mRNAs.

To confirm macro- and microarray data, quantitative Real-Time PCR analysis was performed, and overall gave a similar trend as the microarray data. This suggests that the microarray data from this thesis is more reliable than that from macroarrays, which might be expected since microarrays were performed using replicate arrays; macroarrays were not. However, there are clear difference in magnitude between the microarray data and Real Time PCR. The reason(s) for this are unknown but perhaps reflect some of the limitations inherent in the methodologies. For example, the differences in sensitivity, efficiency and affinity of probes used in three different methodologies (macroarray, microarray and Real Time PCR), this requires further studies to be conducted.

This study showed, the upstream region of MHE ORFs in ethanol-stressed cells exposed to acetaldehyde contained regulatory elements in their promoters such as STRE/Msn2/4, HSE/Hf1p and ARE/Yap1/2p. The majority of MHE ORFs contained STRE and HSE sequence motifs, which are binding sites for Msn2/4p and Hsf1p transcription factors

respectively; few MHE ORFs had ARE sequence motifs, for binding Yap1/2p transcription factor. These transcription factors target individual genes to be turned on or off specifically. Thus, the correct transcription of cellular genes is one of the most critical factors of cell's normal development (Alberts *et al*., 1994).

7.2 Possible mechanism of acetaldehyde stimulatory effect

The reason for the lag reducing effect of acetaldehyde on ethanol-stressed cultures is unclear. It has been suggested however that, intracellular acetaldehyde lost from the cell by the ethanol-mediated disruption of the plasma membrane and associated increase of permeability, may cause a significant decrease it its intracellular concentration, thus limiting the rate of ethanol production and concurrent NAD^+ regeneration via alcohol dehydrogenase. This reduction in NAD^+ regeneration consequently affects glyceraldehyde-3-phosphate activity (which relies on NAD^+ as a cofactor), thus causing a bottleneck in glycolysis (Stanley *et al*., 1997). When acetaldehyde is exogenously added to ethanol-stressed cultures, it is speculated that it diffuses into the cells and increase the intracellular concentration, stimulating glycolysis and ATP production by increasing the rate of NAD^+ regeneration Stanley *et al.*, (1997).

The above hypothesis was further investigated by Barber *et al*., (2002a), using *S. cerevisiae* X2180-1A in complex medium containing 4% (v/v) ethanol under anaerobic conditions. These authors found that an ethanol-induced lag period of around 16 hours could be reduced to 2 hours by the addition of small quantities of acetaldehyde. This lag reducing effect by acetaldehyde was considerably more substantial than that observed for the same strain and conditions but grown aerobically. It was speculated that acetaldehyde was more effective under anaerobic conditions because the NAD^+/NADH redox balance and energy generation is entirely dependent on glycolysis and the fermentation pathway (i.e. substrate level phosphorylation); energy generation by respiration is absent. The authors also found that the lag period of ethanol-stressed cells could be reduced by the addition of propionaldehyde (3 carbon-based aldehyde) and that under such conditions the decrease in propionaldehyde concentration during the lag period stochiometrically matched the amount of propanol being formed by alcohol dehydrogenase. This provided further evidence that the mechanism of acetaldehyde

stimulation of ethanol-stressed cells is attributable to its role in restoring the NAD+/NADH redox balance.

The results of this project provide further evidence to support the role of acetaldehyde in restoring the NAD+/NADH redox balance in ethanol-stressed *S. cerevisiae*. The gene array data identified pyruvate decarboxlyase genes (*PDC1* and *PDC5*) in *S. cerevisiae* in the ethanol-stressed cells in the presence of added acetaldehyde. Pyruvate decarboxylase is involved in the decarboxylation of pyruvate to acetaldehyde, which is then reduced to ethanol (using NADH as a cofactor) by alcohol dehydrogenase (Then and Radler, 1970). It can be speculated that an increase in *PDC* activity would increase metabolic flux through the fermentative pathway thus increasing the regeneration rate of NAD^+, stimulating glyceraldehyde-3-phosphate dehydrogenase activity and metabolic flux through the upper glycolytic pathway; higher glycolytic flux would accelerate energy production which may assist the cell to more quickly overcome the ethanol stress.

Although the data supports an increase in *PDC* expression levels resulting from acetaldehyde addition, the mechanism behind this effect is uncertain. Acetaldehyde is a product of PDC activity therefore an increase in acetaldehyde concentration would theoretically decrease PDC activity (via feedback inhibition) rather than increase it. It is suggested that rather than directly impacting on *PDC* expression, the added acetaldehyde is directly stimulating NAD^+ regeneration (by reduction to ethanol, as shown by Barber *et al*., 2002) which in turn stimulates glyceraldehyde-3-phosphate dehydrogenase activity, consequently increasing glycolytic flux. This priming effect of added acetaldehyde on glycolytic flux in ethanol-stressed cells would reduce the metabolic bottleneck caused by low glyceraldehyde-3-phosphate dehydrogenase activity (due to low concentrations of its cofactor, NAD^+) and increase the concentrations of lower glycolytic intermediates, such as pyruvate. Since pyruvate is a substrate of pyruvate decarboxylase, an increase in its concentration might be expected to increase expression of *PDC* genes.

In summary, it is speculated that the addition of acetaldehyde to ethanol-stressed *S. cerevisiae* primes glycolytic flux by regenerating NAD^+ from accumulated NADH, which in turn helps to overcome the metabolic bottleneck caused by low

glyceraldehyde-3-phosphate dehydrogenase activity; the resulting increase in pyruvate concentration stimulating an increase in *PDC* activity and an overall increase in glycolytic rate. This proposed mechanism by which acetaldehyde stimulates the recovery of ethanol-stressed cells is in keeping with the observation that during ethanol stress, the cells are in a state of pseudo-starvation i.e. although there is excess glucose in the medium, the cells are unable to catabolise it at a sufficient rate to meet cellular energy demands (Chandler *et al.,* 2004)

However, in this project, the knockout studies could not detect a difference in phenotype in *S. cerevisiae* knockout strains (Δ*PDC1*, Δ*PDC5*, Δ*HXT4*, Δ*PHO48* and Δ*YLR134W*) compared to the parent strain during ethanol stress in the presence of acetaldehyde. Although this may at first appear not to support these genes having a crucial role in ethanol stress adaptation or the acetaldehyde effect, it is possible that other members of their isogene families compensated for the role of each of the genes in the knockout strains. This is discussed in chapter 6 and recommendations for further work with the knockout strains is given in the following section.

7.3 Concluding Remarks and Future Directions

7.3.1 Concluding Remarks

This project has for the first time shown that added acetaldehyde stimulates the expression of many genes in ethanol-stressed *S. cerevisiae*. This effect of acetaldehyde on ethanol-stressed yeast supports previous physiological-based investigations in this area. In particular, it has been shown that there are significant increases in the expression levels of groups of genes that are associated with particular, and important, functions in the stressed cell. Such functions include ribosomal processes, nutrient transport, cell cycle, energy production and lipid metabolism. All of these functions have a crucial role in restoring overall cell vitality during a period of ethanol stress.

In particular this project has identified an association between the addition of acetaldehyde to *S. cerevisiae* cultures and subsequent increases in the expression levels of pyruvate decarboxylase genes. Although acetaldehyde may directly influence the

level of expression of these genes, it seems unlikely that it would up-regulate them. Given that it is a product of pyruvate decarboxlyase activity, it is more likely that increased acetaldehyde concentrations would, in fact, lead to a decrease in pyruvate decarboxylase expression, if indeed it has any direct influence on it at all. Rather, it is proposed that acetaldehyde added to ethanol-stressed cells improves their stress adaptation rate by priming glycolysis thereby increasing glycolytic flux and increasing the levels of intracellular metabolites, improving the NAD^+/NADH ratio and increasing energy production. According to this proposed model, the observed changes in gene expression in ethanol-stressed cells following acetaldehyde addition is more likely a consequence of the effect of improved levels of glycolytic intermediates and improved energy production. This proposed model supports the more recent observations that ethanol-stressed yeast are primarily struggling to meet cellular energy demands due to low glycolytic flux and that the priority is to improve cellular energetics in order to facilitate the adaptation process.

7.3.2 Future Directions

1. The results presented in this thesis support the hypothesis that acetaldehyde stimulates the adaptation rate of yeast to ethanol stress by increasing the NAD^+/NADH ratio, consequently providing a greater supply of NAD^+ for upper glycolytic metabolism (Stanley *et al*., 1997). This could be investigated further by conducting metabolite analysis on ethanol-stressed yeast in the presence and absence of acetaldehyde. The concentrations of metabolites to be investigated include the NAD^+/NADH ratio, glyceraldehyde 3-phosphate and 1,3-diphosphoglycerate, which are the substrate and product respectively of glyceraldehyde 3-phosphate dehydrogenase, and pyruvate due to its role in stimulating the expression of *PDC* genes. It was reported by Martini *et al.,* (2006) that understanding complex systems of yeast cells needs understanding of cellular metabolitic processes resulting from activation, inhibition and feedback activities.

2. In light of advances in metabolomics over recent years, it would be informative to assess changes in the yeast metabolome in ethanol-stressed and in acetaldehyde-treated,

ethanol-stressed cells. This may give greater definition, over and above what is proposed in Point 1 above, to changes in metabolism.

3. In Chapter 6 of this thesis, phenotype analysis of selected knockout strains did not result in any significant difference in growth profile compared to the wild type under ethanol stress in presence or absence of acetaldehyde. To further test the functions of these genes, double and triple knockouts should be constructed to eliminate the possibility of compensatory effects by other genes. For example, screening of a *ΔPDC1ΔPDC5* knockout strain in ethanol-stressed cultures in the presence and absence of acetaldehyde would determine whether or not *PDC1* compensates for *PDC5* and *vice versa*.

4. The above work on double (and even triple knockouts) should be extended, as part of a long term aim, to include a synthetic lethality screen using a synthetic gene array as described in Tong *et al.,* (2004 and 2001), but using ethanol stress conditions. This would enable the identification of gene interactions between, for example, *PDC5*, and any other non-essential genes in the yeast genome. This is important because of the high probability that ethanol tolerance is affected by many genes, and thus gene interactions are very likely to be important.

5. The potential for compensatory effects by isogenes in the knockout strains could be investigated further by performing microarray analysis on the knockout cultures during ethanol stress. This may reveal changes in expression of compensating genes. Alternatively, expression levels of particular isogenes could be measured in ethanol-stressed knockout cultures using Real Time PCR. However, compensatory effects may not show up as changes in expression; changes in enzyme activities of compensating enzymes would have the same effect.

6. Investigation of the phenotype of knockout strains using conventional methodology such as measuring cell populations in batch growth experiments of pure cultures, has poor sensitivity. Growth competition experiments is a more sensitive technique for detecting small differences in fitness and this should be used with the knockout strains and the parent cultures described in Chapter 6 while in the presence of an ethanol stress.

7. Gene expression arrays measure changes in mRNA concentration, not protein levels. The assumption with such a technique is that mRNA production correlates with protein production. However, it is recognised that mRNA expression patterns do not always reflect changes in protein levels (Gygi *et al*., 1999). Given proteins are the ultimate arbiters of cell function, changes in protein levels are arguably more important in inferring alteration of function. It was recently reported that comparison of proteome analysis instead of mRNA analysis of yeast ale strain with lager brewing yeast strain showed better picture of expression level (Kobi *et al*., 2004). Thus, proteome analysis of ethanol-stressed yeast in the presence and absence of acetaldehyde is suggested for further work to more accurately quantify the changes in cell function brought about by acetaldehyde.

8. Athough *PDC1* and *PDC5* single gene deletions were found to have no significant effect on the ethanol-stress response of *S. cerevisiae* BY4742, a third *PDC* gene, known as *PDC2*, is known to be a positive regulator of both *PDC1* and *PDC5* genes (Hohmann, 1993). It was shown that a *PDC2* deletion resulted in a reduced expression level of *PDC1* and complete abolishment of *PDC5* expression (Hohmann and Cederberg, 1990; Nevoigt and Stahl, 1996). The role of the *PDC2* gene in regulating expression levels of both *PDC1* and *PDC5* presents an opportunity to further investigate the role of *PDC* activity in the effect of acetaldehyde on ethanol-stressed yeast cells. Accordingly, a *PDC2* knockout strain would have no *PDC5* expression and a 70% reduction in *PDC1* expression levels. Investigations using a *PDC2* knockout strain may reduce the compensatory effect of *PDC1* for *PDC5*, and vice-versa, allowing the true effect of *PDC* deletions on the acetaldehyde stimulation of ethanol adaptation to be determined.

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APPENDICES

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APPENDIX I

1.1 Buffers and solutions

Buffered phenol (for RNA isolation): was prepared using Special Grade phenol (Wako Pure Chemical Industries limited), RNA buffer (see below) and 0.1% (w/v) 8-hydroxyquinoline. Equal volumes of phenol and RNA buffer (5 x) were mixed with the 8-hydroxyquinoline in a brown (light proof) baked bottle with a stirring bar for 10 minutes. The phases were allowed to separate, the aqueous top phase removed and replaced with an equal volume of 1 x RNA buffer. The procedure was repeated using 1 x RNA buffer until the aqueous top phase was at pH 7.5 when tested with pH paper. The buffered phenol was stored at 4°C.

Buffered phenol (for DNA isolation): was prepared using Special Grade phenol (Wako Pure Chemical Industries limited), 50 mM Tris.Cl buffer (see below) and 0.1% (w/v) 8-hydroxyquinoline. Equal volumes of phenol and 50 mM Tris.Cl buffer were mixed with the 8-hydroxyquinoline in a brown (light proof) baked bottle with a stirring bar for 10 minutes. The phases were allowed to separate; the aqueous top phase removed and replaced with an equal volume of 50 mM Tris.Cl buffer. The procedure was repeated using 50 mM Tris.Cl buffer until the aqueous top phase was at pH 8.0 when tested with pH paper. The buffered phenol was stored at 4°C.

Chloroform/ Isoamyl alcohol (25:24): Chloroform (25 ml) and isoamyl alcohol (24 ml) added together and mixed well.

DEPC water: 0.1% DEPC and distilled de-ionized water were mixed well, allowed to stand overnight, and autoclaved.

EDTA 0.5 M: was prepared by dissolving 186.1 g EDTA in 800 ml of distilled de-ionized water. The solution was dissolved with gentle heating for several hours. The solution was cooled, the pH adjusted to 8.0 with NaOH and the volume adjusted to 1 L. The solution was autoclaved.

Ethidium Bromide: for non-denaturing RNA and DNA gels was prepared as a 10 mg/ml stock solution by dissolving ethidium bromide with distilled de-ionized water. The stock solution was stored in a baked lightproof glass bottle at 4°C. Ethidium bromide was added to a cooled agarose gel at a final concentration of 1 μg ml^{-1}.

Geneticin: A stock solution of 100 mg ml^{-1} was prepared by adding 1 ml of sterile distilled de-ionized water to 100 mg Geneticin G418 in a sterile bottle. The stock solution was dissolved and stored at 4°C. The solution was used at a final concentration of 0.2 mg ml^{-1}.

Gel loading buffer (6 x): 0.2% (w/v) bromophenol blue, 20% (w/v) Ficoll and 10 mM EDTA were dissolved in distilled de-ionized water and filter sterilized into a sterile glass bottle. The solution was stored at 4°C.

25 mM $MgCl_2$ (Perkin Elmer or Invitrogen)**:** used in PCR reactions and supplied with the enzyme, Amplitaq™ DNA Polymerase or Platinum Taq DNA Polymerase.

PCR buffer (10x): PCR buffer was supplied with the Taq DNA polymerase enzymes, Amplitaq™ (Perkin Elmer) or Platinum Taq (Invitrogen).

RNA lysis buffer (5 x): 2.5 M NaCl, 1 M Tris base and 50 mM EDTA were dissolved in distilled water. The pH was adjusted to 7.5 with HCl and the buffer filter sterilized through a 0.22 μm filter into a baked glass bottle.

RNA gel loading buffer (6 x): 0.2% (w/v) bromophenol blue, 20% (w/v) Ficoll and 10 mM EDTA were dissolved in DEPC treated water and filter sterilized into a baked glass bottle. The solution was stored at 4°C.

SDS 10%: Sodium dodecyl sulphate was dissolved in distilled de-ionized water by heating to 68°C. The solution was filter sterilized through a 0.45 μm filter into a sterile baked glass bottle.

3M Sodium acetate: Sodium acetate was dissolved in a small amount of DEPC treated water in baked glassware. The pH was adjusted to 5.3 with dilute glacial acetic acid and the solution filter sterilized through a 0.22 μm filter into a baked glass bottle.

Solution 1 (0.1% Tritron X-100, for washing microarray slides): 1 g of Tritron X-100 was dissolved in 1 liter filtered DEPC treated water.

Solution 2 (4.38 mM HCl, **for washing microarray slides):** 0.38 ml of concentrated HCL (36%, 11.64 M) was added into one litre DEPC treated water and then filtered.

Solution 3 (100 mM KCl, **for washing microarray slides):** 7.4551 g of KCL was added into one liter DEPC treated water and then filtered.

Blocking buffer: 25% Ethyene glycol and 0.01% HCl.

SSC (20 x): NaCl (175.3 g) and tri-sodium citrate (88.2 g) were dissolved in DEPC treated water. The pH was adjusted to 7.0 with 10M NaOH prior to autoclaving.

TAE buffer (10 x): Tris base (400 mM), 200 mM Sodium acetate, 20 mM EDTA (pH 8) were dissolved in DEPC treated water. pH was adjusted to approximately 7.2 with glacial acetic acid prior to autoclaving. The 10 x stock solution was diluted with DEPC treated water prior to use with RNA.

TBE buffer (10 x): Tris base (0.89 M), 0.89 M boric acid and 20 mM EDTA (pH 8) were dissolved in distilled de-ionized water and autoclaved. The 10 x stock was diluted with distilled se-ionized water prior to use.

TE buffer: contained 10 mM Tris.Cl (pH 7.4) and 1 mM EDTA (pH 8.0). The solution adjusted to pH 8.0 with HCl and was autoclaved.

Tris 1M: Tris base (121.1 g) was dissolved in distilled de-ionized water. The pH was adjusted to 7.5 with glacial acetic acid, the volume adjusted to 1 L and the solution autoclaved.

1.2 Enzymes, Molecular Weight Markers and Molecular Biology kits

Table 1.1: Reagents obtained from commercial sources

Reagents	Commercial sources
RNase ERASE Spray	ICN
DNase I, RNase free	Roche
Recombinant RNasin Ribonuclease inhibitor	Promega
RNA marker	Promega
Dig Easy Hybridisation solution	Roche
Yeast GeneFilters	Invitrogen
Radiolabel (γ ^{32}P ATP and α ^{33}P CTP)	PerkinElmer
SybGreen dye	Roche
Microarray slides Slide	The Clive & Vera Ramaciotti Centre for Gene function analysis
RT-PCR	Invitrogen

Enzymes: RNase-free DNase (Roche), Amplitaq (Perkin Elmer), Supermix (Invitrogen), RNase A (Epicentre Technologies), T4 Polynucleotide kinase (Ammersham Pharmacia Biotech), Superscript ^{TM} II RNase H$^-$ Reverse Transcriptase (Gibco BRL Life Technologies), Recombinant RNasin Ribonuclease Inhibitor (Promega), ABI Prism Cycle Sequencing.

Molecular Weight Markers: RNA markers, 0.28-6.58 kb (Promega), 100 bp DNA ladder (Promega), GeneRuler 100 bp DNA Ladder Plus (MBI Fermentas).

LIST OF SUPPLIERS

Applied Biosystems (Foster City, CA, USA)

Amersham Biosciences (Little Chalfont, Buckinghamshire, UK)

Bartelt Instruments Pty Ltd (Heidelberg, Victoria, Australia)

Beckmam Instruments (GmbH, Munchen, Germany)

Bio-Rad Laboratories (Hercules, CA, USA)

B. Braun Biotech International (Melsungen, Germany)

Bresatech Pty Ltd (Adelaide, South Australia)

Epicenter Technologies (Maddison, USA)

Invitrogen Corporation (Carlsbad, California, USA)

New England Biolabs, Inc. (Beverly, MA, USA)

PerkinElmer (Wellesley, MA, USA)

Promega Corporation (Maddison, USA)

Progen Industried Limited (Darra, Queensland, Australia)

Qiagen Pty Ltd (Clifton Hill, Victoria, Australia)

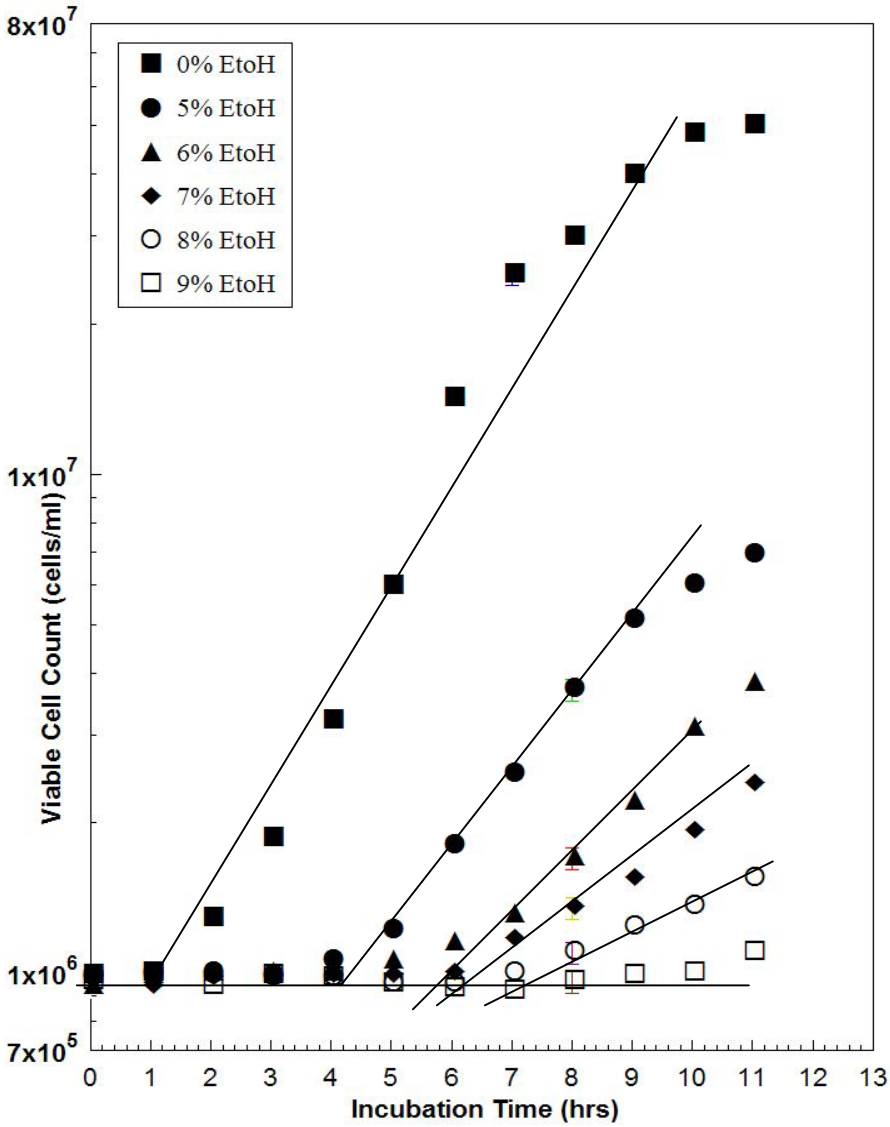
Roche Diagnostics (GmbH, Mannheim, Germany)

Sigma-Aldrich Corporation (St Louis, Missouri, USA)

APPENDIX II

2.1 Growth curves

This section contains replicates of growth curve figures and calculation of lag period, growth rate and double time.



Lag Period (h):

0% ethanol	1.0
5% ethanol	4.3
6% ethanol	5.4
7% ethanol	6.2
8% ethanol	7.5
9% ethanol	N/A

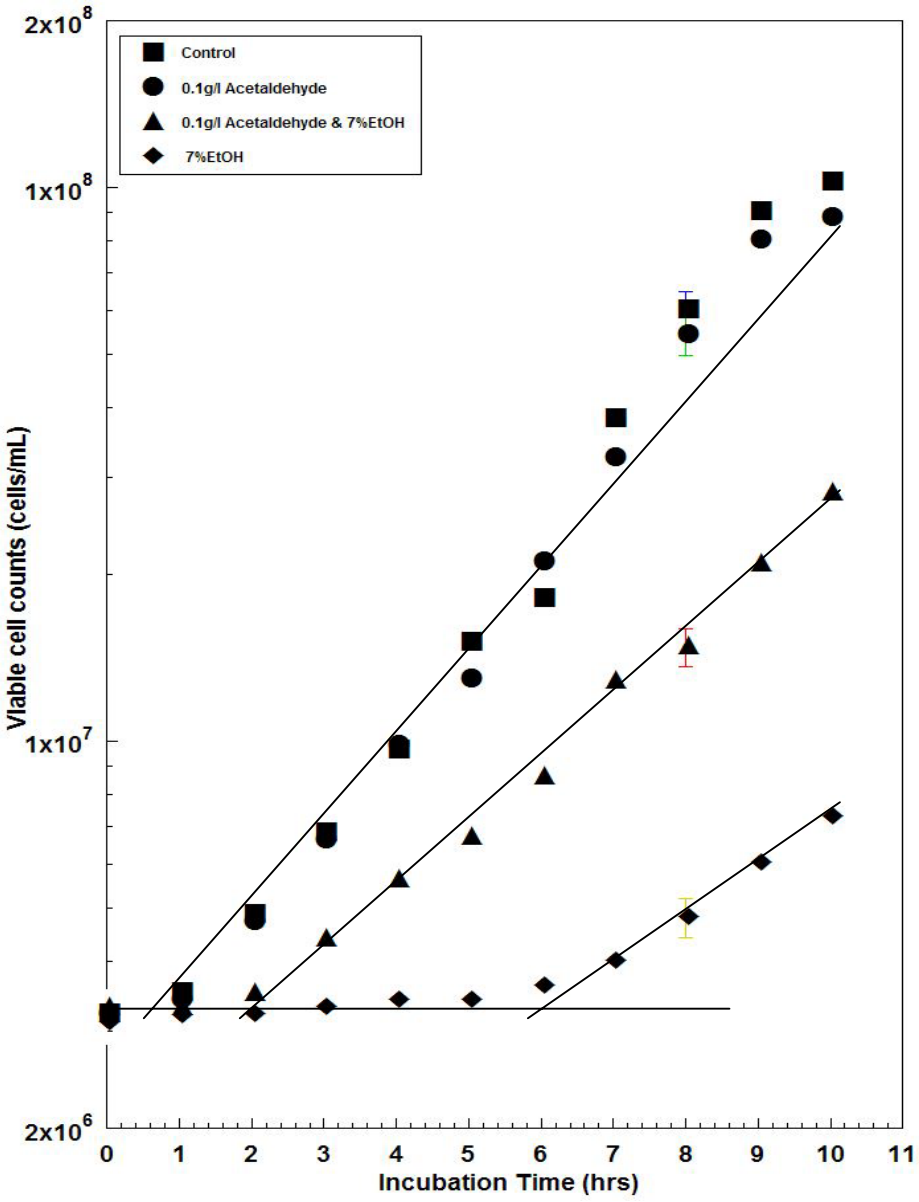
Growth Rate (h^{-1}):

0% ethanol	0.36
5% ethanol	0.29
6% ethanol	0.23
7% ethanol	0.18
8% ethanol	0.12

Doubling Time (h):

0% ethanol	1.9
5% ethanol	2.4
6% ethanol	3.0
7% ethanol	3.8
8% ethanol	5.8

Figure 1: Determination of lag period, growth rate and doubling time for *S. cerevisiae* PMY1.1 grown in YEPD medium: Medium only or medium containing different concentrations of ethanol



Lag Period (h):

0% ethanol	0.6
0.1 g/l acetaldehyde	0.6
7% ethanol	6.1
7% EtOH & 0.1g/l acet.	2.0

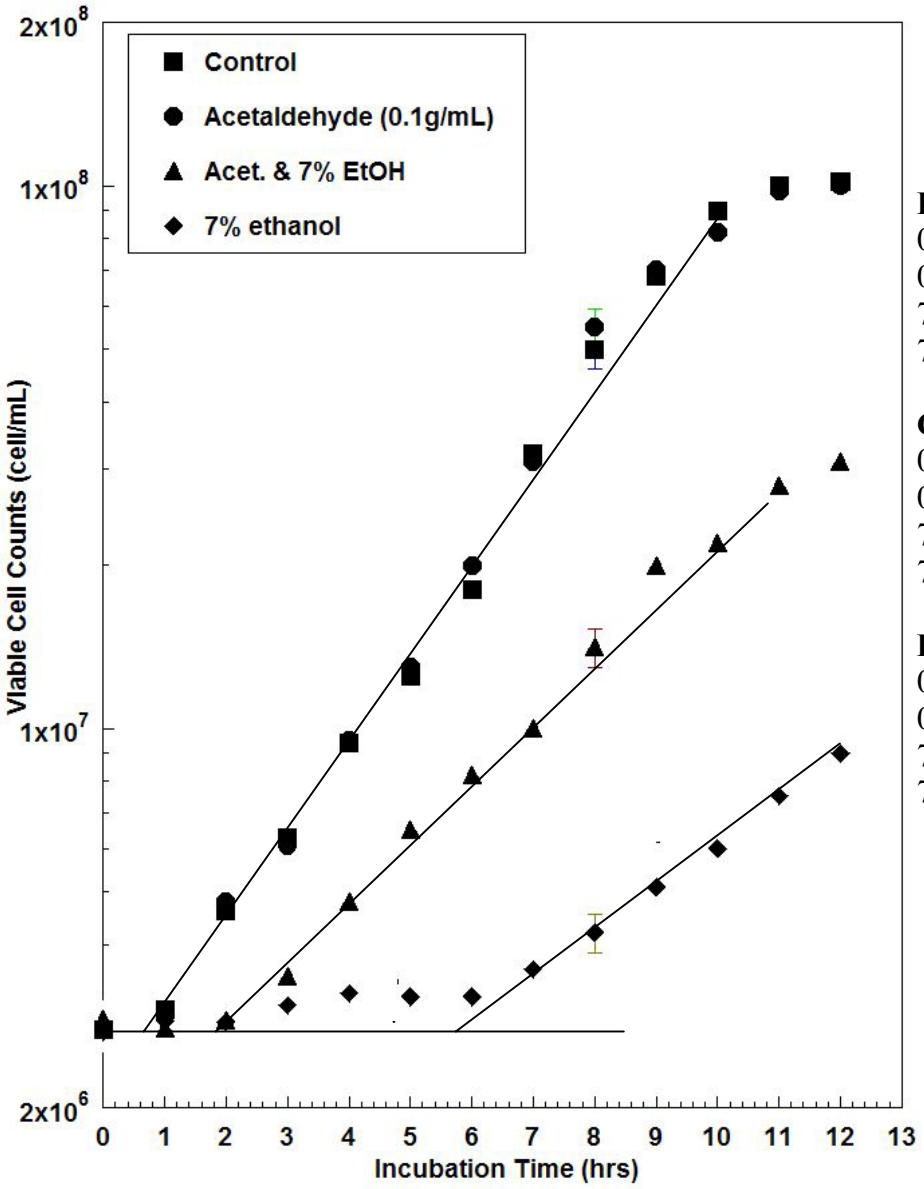
Growth Rate (h^{-1}):

0% ethanol	0.34
0.1g/l acetaldehyde	0.34
7% ethanol	0.19
7% EtOH & 0.1g/l acet.	0.25

Doubling Time (h):

0% ethanol	2.0
0.1 g/l acetaldehyde	2.0
7% ethanol	3.6
7% EtOH & 0.1 g/l acet.	2.8

Figure 2: Determination of lag period, growth rate and doubling time for *S. cerevisiae* PMY1.1 grown in YEPD medium: Medium only, or medium containing 0.1 g/l acetaldehyde, medium containing 7% (v/v) ethanol, or medium containing both 0.1 g/l acetaldehyde & 7% (v/v) ethanol.



Lag Period (h):

0% ethanol	0.8
0.1 g/l acetaldehyde	0.8
7% ethanol	6.0
7% EtOH & 0.1g/l acet.	2.0

Growth Rate (h^{-1}):

0% ethanol	0.36
0.1g/l acetaldehyde	0.36
7% ethanol	0.20
7% EtOH & 0.1 g/l acet.	0.28

Doubling Time (h):

0% ethanol	1.9
0.1 g/l acetaldehyde	1.9
7% ethanol	3.5
7% EtOH & 0.1 g/l acet.	2.7

Figure 3: Determination of lag period, growth rate and doubling time for *S. cerevisiae* PMY1.1 grown in YEPD medium: Medium only, or medium containing 0.1 g/l acetaldehyde, medium containing 7% (v/v) ethanol, or medium containing both 0.1 g/l acetaldehyde & 7% (v/v) ethanol.

APPENDIX III

Analysis of gene filter, macroarray, data for ethanol-stressed cells compared to unstressed, control cells.

This section of appendix contains the principal raw and calculated data obtained in the thesis, raw data that was not practical to tubulate, which was generated from macroarray and microarray analyses.

Table 3.1a: Macroarray data: Genes that were MHE following one hour exposure to 7% ethanol relative to control.

* Genes also found to be MHE in macroarray experiments for the same conditions at five-hour time point.

ORF/Gene name	Description of gene product	Fold increase	Putative Transcription factors
Stress response			
*HSP26	Heat shock protein	7.3	Msn2/4p, Hsf1p
Energy utilization genes			
TDH1	Glyceraldehyde 3-phosphate dehydrogenase (phosphorylating	7.1	Msn2/4p, Hsf1p, Yap1/2p
DLD3	Lactate metabolism (D-lactate dehydrogenase (cytochrome)	5.6	Hsf1p
ALD4	Aldehyde dehydrogenase (NAD+) activity (Ethanol metabolism)	5.4	Hsf1p, Yap1/2p
ACS1	Acetyl-CoA biosynthesis (acetate fermentation)	3.4	Msn2/4p, Hsf1p
PYC1	Gluconeogenesis (Pyruvate carboxylase)	3.3	Msn2/4p, Hsf1p
GLC3	Glycogen metabolism (1,4-alpha-glucan branching enzyme activity)	3.0	Msn2/4p
*GLK1	Carbohydrate metabolism (Glucokinase activity)	3.0	Msn2/4p, Hsf1p
Protein folding, synthesis, modification, translocation, degradation and complex assembly			
YDL025C	Protein kinase activity	4.0	Hsf1p
Transcription and translation factor and process			
*YBR012W-B	RNA-directed DNA polymerase activity	5.7	Hsf1p
YBL101W-B	RNA-directed DNA polymerase activity	3.4	Hsf1p
YCL019W	RNA-directed DNA polymerase activity	3.3	Msn2/4p, Hsf1p
Unknown function			
*YCL020W		12.5	Msn2/4p, Hsf1p
*YBL101W-A		10.1	Hsf1p
*YJR028W		7.7	Msn2/4p, Hsf1p
*YJR026W		7.2	Msn2/4p
*YMR051C		6.8	Msn2/4p, Hsf1p
*YCL042W		6.6	Msn2/4p, Hsf1p
HBT1		6.5	Msn2/4p, Hsf1p
*YBL005W-A		6.0	Msn2/4p, Hsf1p
*YBR012W-A		5.9	Hsf1p
*YAR010C		5.8	Msn2/4p, Hsf1p
*YMR046C		5.7	Msn2/4p, Hsf1p
*YML045W		5.7	Msn2/4p
*YML040W		5.5	Msn2/4p, Hsf1p
YMR050C		3.3	Msn2/4p, Hsf1p
YJR029W		3.2	Msn2/4p, Hsf1p
SPS100		3.0	Msn2/4p, Hsf1p, Yap1/2p

Table 3.1b: Macroarray data: Genes that were LHE following one hour exposure to 7% ethanol relative to control.

*Genes also found to be LHE in macro-array experiments for the same conditions at five-hour time point.

ORF/Gene name	Description of gene product	Fold decrease
Ribosomal proteins	**Protein synthesis**	
RPP0	Cytosolic small ribosomal subunit	-53.7
*RPS24A	Cytosolic small ribosomal subunit	-43.1
RPS0A	Cytosolic small ribosomal subunit	-39.0
RPS27B	Cytosolic small ribosomal subunit	-38.5
RPS1B	Cytosolic small ribosomal subunit	-37.5
RPL28A	Ribosomal large subunit biogenesis	-35.0
RPS26A	Cytosolic small ribosomal subunit	-34.5
RPS26B	Cytosolic small ribosomal subunit	-34.4
RPS20	Cytosolic small ribosomal subunit	-33.6
RPL24A	Ribosomal large subunit biogenesis	-30.5
RPL15A	Ribosomal large subunit biogenesis	-30.5
RPL1	Ribosomal large subunit biogenesis	-29.6
RPS30	Cytosolic small ribosomal subunit	-29.2
RPL30 / RPL32	Ribosomal large subunit biogenesis	-29.2
RPL4B	Ribosomal large subunit biogenesis	-27.3
RPL35A	Ribosomal large subunit biogenesis	-27.0
RPS2	Cytosolic small ribosomal subunit	-26.8
RPS14A	Cytosolic small ribosomal subunit	-26.5
RPS21	Cytosolic small ribosomal subunit	-26.5
RPL9A	Ribosomal large subunit biogenesis	-26.2
RPS5	Cytosolic small ribosomal subunit	-25.5
RPL35B	Ribosomal large subunit biogenesis	-25.3
RPS33A	Cytosolic small ribosomal subunit	-24.7
RPL20A	Ribosomal large subunit biogenesis	-24.4
RPS1B	Cytosolic small ribosomal subunit	-24.3
RPL46	Ribosomal large subunit biogenesis	-23.9
RPS6A	Cytosolic small ribosomal subunit	-23.9
RPS10A	Cytosolic small ribosomal subunit	-23.8
RPL1B	Ribosomal large subunit biogenesis	-23.4
UB12	Ribosomal large subunit biogenesis	-23.2
RPL6A	Ribosomal large subunit biogenesis	-23.1
SUP46	Ribosomal large subunit biogenesis	-22.4
RPL22A	Ribosomal large subunit biogenesis	-21.9
RPS11A	Cytosolic small ribosomal subunit	-21.7
RPS18B	Cytosolic small ribosomal subunit	-21.7
RPL26A	Ribosomal large subunit biogenesis	-21.6
SSM1	Ribosomal large subunit biogenesis	-21.2
RPL21B	Ribosomal large subunit biogenesis	-21.2
RPL30B	Ribosomal large subunit biogenesis	-21.1
RPL34B	Ribosomal large subunit biogenesis	-21.0
RPL4A	Ribosomal large subunit biogenesis	-20.9
RPS13C	Cytosolic small ribosomal subunit	-20.1
RPL3	Ribosomal large subunit biogenesis	-20.2
RPL34A	Ribosomal large subunit biogenesis	-19.9
RPL13A	Ribosomal large subunit biogenesis	-19.8
RPL16A	Ribosomal large subunit biogenesis	-19.8
RPS8B	Cytosolic small ribosomal subunit	-19.8
RPL17B	Ribosomal large subunit biogenesis	-19.7
RPL15B	Ribosomal large subunit biogenesis	-19.5
RPL18A1	Ribosomal large subunit biogenesis	-19.4
RPL6A	Ribosomal large subunit biogenesis	-19.3
RPS7B	Cytosolic small ribosomal subunit	-19.1

RPL28	Ribosomal large subunit biogenesis	-19.1
RPS29A	Cytosolic small ribosomal subunit	-18.7
RPL5B	Ribosomal large subunit biogenesis	-18.2
RPL17	Ribosomal large subunit biogenesis	-18.0
RPL10	Ribosomal large subunit biogenesis	-18.0
RPS31A	Cytosolic small ribosomal subunit	-17.4
RPS18B	Cytosolic small ribosomal subunit	-17.1
RPL14A	Ribosomal large subunit biogenesis	-17.0
RPL38	Ribosomal large subunit biogenesis	-17.0
RPL25	Ribosomal large subunit biogenesis	-16.9
RPS8A	Cytosolic small ribosomal subunit	-16.8
RPL14B	Ribosomal large subunit biogenesis	-16.7
RPL27	Ribosomal large subunit biogenesis	-16.6
RPS28A	Cytosolic small ribosomal subunit	-16.4
RPS10B	Cytosolic small ribosomal subunit	-16.3
RPL13A	Ribosomal large subunit biogenesis	-16.1
RPL5A	Ribosomal large subunit biogenesis	-16.1
RPS18A	Cytosolic small ribosomal subunit	-15.5
RPL28B	Ribosomal large subunit biogenesis	-14.8
RPL2B	Ribosomal large subunit biogenesis	-14.4
RPS16B	Ribosomal large subunit biogenesis	-13.4
RPS7A	Ribosomal large subunit biogenesis	-12.6
RPL2A	Ribosomal large subunit biogenesis	-12.4
RPS25	Cytosolic small ribosomal subunit	-12.0
RPS25B	Cytosolic small ribosomal subunit	-11.9
RPS17A	Cytosolic small ribosomal subunit	-11.6
RPL37B	Ribosomal large subunit biogenesis	-11.3
RPL43A	Ribosomal large subunit biogenesis	-11.3
RPS27A	Cytosolic small ribosomal subunit	-11.0
RPL35A	Ribosomal large subunit biogenesis	-10.9
RPS9A	Cytosolic small ribosomal subunit	-10.8
RPL19B	Ribosomal large subunit biogenesis	-10.6
RPS16A	Ribosomal large subunit biogenesis	-10.5
RPL9B	Ribosomal large subunit biogenesis	-10.2
RPS14B	Cytosolic small ribosomal subunit	-10.0
RPL27B	Ribosomal large subunit biogenesis	-9.8
RPS16B	Cytosolic small ribosomal subunit	-9.7
RPL13	Ribosomal large subunit biogenesis	-9.5
RPL45	Ribosomal large subunit biogenesis	-9.5
RPL19A	Ribosomal large subunit biogenesis	-9.2
RPL15B	Ribosomal large subunit biogenesis	-9.2
RPL37A	Ribosomal large subunit biogenesis	-9.1
RPS10A	Cytosolic small ribosomal subunit	-8.9
RPL2	Ribosomal large subunit biogenesis	-8.8
RPL41A	Ribosomal large subunit biogenesis	-8.7
RPLA3	Ribosomal large subunit biogenesis	-8.1
RPS3	Cytosolic small ribosomal subunit	-8.0
RPL35B	Ribosomal large subunit biogenesis	-8.0
RPS31	Cytosolic small ribosomal subunit	-7.3
RPP1A	Ribosomal large subunit biogenesis	-5.8
RPS24B	Cytosolic small ribosomal subunit	-5.7
RPL13B	Ribosomal large subunit biogenesis	-5.3
RP23	Ribosomal large subunit biogenesis	-5.1
MRPS18	Mitochondrial small ribosomal subunit	-4.8
RPS7B	Cytosolic small ribosomal subunit	-4.8
RPL17B	Ribosomal large subunit biogenesis	-4.3
RPS1B	Cytosolic small ribosomal subunit	-4.2
RPL11A	Ribosomal large subunit biogenesis	-4.1
RPL17A	Ribosomal large subunit biogenesis	-4.1
RPS19B	Cytosolic small ribosomal subunit	-3.9

RPS30A	Cytosolic small ribosomal subunit	-3.9
MRPL49	Ribosomal large subunit biogenesis	-3.9
RPS12	Cytosolic small ribosomal subunit	-3.8
RPL6B	Ribosomal large subunit biogenesis	-3.7
RPL47B	Ribosomal large subunit biogenesis	-3.7
MRP4	Mitochondrial ribosome small subunit component	-3.6
RSM10	Mitochondrial ribosome small subunit component	-3.6
MRP13	Mitochondrial ribosome small subunit component	-3.5
RPL47A	Ribosomal large subunit biogenesis	-3.3
MRPL31	Mitochondrial large ribosomal subunit	-3.2
MRP1	Mitochondrial large ribosomal subunit	-3.2
MRP51	Mitochondrial large ribosomal subunit	-3.1
MRPL7	Mitochondrial large ribosomal subunit	-3.1
MRPL11	Mitochondrial large ribosomal subunit	-3.1
RPS24B	Cytosolic small ribosomal subunit	-3.1
RPL43B	Ribosomal large subunit biogenesis	-3.0
MRP10	Mitochondrial ribosome small subunit component	-3.0
Ribosomal subunit		
MRT4	Ribosomal large subunit biogenesis	-10.6
BRX1	Ribosomal large subunit assembly and maintenance- rRNA primary transcript binding activity (5S RNA binding activity)	-10.3
RPF2	Ribosomal large subunit assembly and maintenance - rRNA, 5S RNA and 7S RNA binding activity	-10.2
EBP2	rRNA processing	-9.8
NOP2	rRNA processing- RNA methyltransferase	-6.8
RRB1	Ribosome biogenesis	-6.5
NOP7	Ribosomal large subunit biogenesis	-6.5
NOP8	rRNA processing	-5.4
RRP7	Ribosomal small subunit assembly andmaintenance (35S primary transcript processing)	-4.8
NIP7	rRNA processing	-4.8
YTM1	Ribosomal large subunit biogenesis	-4.2
LOC1	Ribosomal large subunit biogenesis- mRNA binding activity	-4.0
NSA1	Ribosomal large subunit biogenesis	-3.9
NOP15	Ribosomal large subunit biogenesis	-3.9
TSR1	Ribosome biogenesis and assembly	-3.8
RLP24	Ribosomal large subunit biogenesis	-3.6
MAK11	Ribosomal large subunit biogenesis	-3.1
NOC2	Ribosome assembly (ribosome nucleus export)	-3.0
SAT1	Ribosomal large subunit assembly and maintenance	-3.0
Cell cycle and growth		
HSL7	Regulation of cell cycle (G2/M transition of mitotic cell cycle)- Protein-arginine N-methyltransferase activity	-23.3
GIC1	Axial budding (cytokinesis) (cellular morphogenesis)- Phosphatidylinositol binding activity (structural constituent of cytoskeleton)	-6.8
YCK1	Cellular morphogenesis (cytokinesis) (endocytosis)- Casein kinase I activity	-6.8
GIC2	Axial budding- Small GTPase regulatory/interacting protein activity	-6.5
SPO12	Exit from mitosis (mitotic cell cycle) (Meiosis I)	-6.1
CLN3	G1/S transition of mitotic cell cycle (regulation of CDK activity)- Cyclin-dependent protein kinase, regulator activity	-6.1
NIS1	Regulation of mitosis	-5.8
ADK1	Cell proliferation- Adenylate kinase activity	-5.7
TAO3	Cellular morphogenesis (budding)	-5.6
UBC9	G2/M transition of mitotic cell cycle (protein modification)- Ubiquitin-like conjugating enzyme activity	-5.5
RSR1	Polar and axial budding (Bud site selection) (small GTPase mediated signal transduction)- RAS small monomeric GTPase activity (signal transducer activity) (small monomeric GTPase activity)	-5.4
SAP4	G1/S transition of mitotic cell cycle- Protein serine/threonine phosphatase activity	-5.2
CKS1	Regulation of cell cycle-	-5.0
CLG1	Cell cycle- Cyclin-dependent protein kinase, regulator activity	-4.9
ARC35	Cell growth and/or maintenance- Structural molecule activity	-4.9

CKA2	G2/M & G1/S transition of mitotic cell cycle- Protein kinase CK2 activity	-4.9
TOR1	G1 phase of mitotic cell cycle- Phosphatidylinositol 3-kinase activity	-4.8
PPH22	G1/S transition of mitotic cell cycle (protein biosynthesis) (protein amino acid dephosphorylation)- Protein phosphatase type 2A activity	-4.7
CMD1	Budding (cytoskeleton organization and biogenesis)	-4.7
YOR300W	Bud site selection-	-4.6
CLB5	Regulation of CDK activity (G1/S & G2/M transition of mitotic cell cycle) Cyclin-dependent protein kinase, regulator activity	-4.5
SAU5	Cell growth and/or maintenance	-4.5
BIK1	Mitotic anaphase B (mitotic spindle assembly)- Microtubule binding activity	-4.5
LAG1	Cell aging (ceramide biosynthesis)- Protein transporter activity	-4.3
YRB1	G1/S transition of mitotic cell cycle (Protein and RNA-nucleus export)- RAN protein binding activity	-4.2
CLB2	Regulation of CDK activity (G2/M transition of mitotic cell cycle)- Cyclin-dependent protein kinase, regulator activity	-4.0
GRC3	Cell growth and/or maintenance	-4.0
CDC28	G1/S transition of mitotic cell cycle (G2/M transition of mitotic cell cycle) (S phase of mitotic cell cycle) (protein amino acid phosphorylation) regulation of cell cycle- Cyclin-dependent protein kinase activity	-4.0
SIC1	Regulation of CDK activity (G1/S transition of mitotic cell cycle)- Kinase inhibitor activity (protein binding activity)	-3.9
SYF1	Cell cycle (mRNA splicing)	-3.9
SIR2	Cell aging (sensu Saccharomyces)- Histone binding activity (histone binding activity)	-3.8
SRO4	Axial budding (bud site selection)	-3.7
YBR276C	Regulation of S phase of mitotic cell cycle- Protein tyrosine/threonine phosphatase activity	-3.7
PCL1	Cell cycle- Cyclin-dependent protein kinase, regulator activity	-3.6
FKH1	Regulation of cell cycle- Transcription factor activity	-3.6
FIG2	Cellular morphogenesis during conjugation with cellular fusion	-3.6
STB1	G1/S transition of mitotic cell cycle	-3.5
TEM1	M phase of mitotic cell cycle (Signal transduction)- Small monomeric GTPase activity	-3.5
FER3	Cellular morphogenesis	-3.5
POG1	Re-entry into mitotic cell cycle after pheromone arrest- Specific RNA polymerase II transcription factor activity	-3.5
PCH2	Regulation of meiosis	-3.4
RIO1	S phase of mitotic cell cycle (processing of 20S pre-rRNA)- Protein kinase activity	-3.4
NDD1	G2/M-specific transcription in mitotic cell cycle- Transcriptional activator activity	-3.4
GRR1	G1/S transition of mitotic cell cycle (ubiquitin-dependent protein catabolism)- Protein binding activity (ubiquitin-protein ligase activity)	-3.4
PPH21	G1/S transition of mitotic cell cycle/ G1/S transition of mitotic cell cycle (protein amino acid Dephosphorylation) (protein biosynthesis)	-3.4
CKB1	G1/S transition of mitotic cell cycle (G2/M transition of mitotic cell cycle) (protein amino acid phosphorylation)- Protein kinase CK2 activity	-3.3
CLB3	G1/S transition of mitoticcell cycle (G2/M transition of mitotic cell cycle) (regulation of CDK activity)- Cyclin-dependent protein kinase, regulator activity	-3.2
WHI2	Regulation of growth (response to stress) Phosphatase activator activity	-3.1
CKB2	G2/M & G1/S transition of mitotic cell cycle- Protein kinase CK2 activity	-3.1
TOR2	G1 phase of mitotic cell cycle (phosphatidylinositol kinase involved in signaling activation of translation initiation)- Phosphatidylinositol 3-kinase activity	-3.1
RFC2	Cell cycle checkpoint- DNA clamp loader activity (purine nucleotide binding Activity)	-3.0
PRM4	Conjugation with cellular fusion	-3.0
ADY4	Sporulation	-3.0
PHO85	Cell cycle- Cyclin-dependent protein kinase activity	-3.0
TAP42	Cell growth and/or Maintenance (signal transduction)-Protein binding activity	-3.0
TAF12	G1-specific transcription in mitotic cell cycle (transcription initiation from Pol II promoter), protein amino acid and histone acetylation, chromatin modification- General RNA polymerase II transcription factor activity	-3.0
CLB1	G2/M transition of mitotic cell cycle (meiotic G2/MI transition)- Cyclin-dependent protein kinase, regulator activity	-3.0
PCL2	Cell cycle- Cyclin-dependent protein kinase regulator activity	-3.0

Stress response		
SNQ2	Response to drug- Xenobiotic-transporting ATPase activity	-15.0
MNN4	O-linked &N-linked glycosylation (response to stress)-	-9.6
YNL190W	Response to dessication	-8.5
ALO1	Response to oxidative stress- D-arabinono-1, 4-lactone oxidase activity	-7.6
IST2	Response to osmotic stress	-6.8
YPD1	Response to osmotic stress- Osmosensory signaling pathway via two-component system	-6.8
GRX5	Response to osmotic stress and oxidative stress- Thiol-disulfide exchange intermediate activity	-6.6
GPX2	Response to oxidative stress- Glutathione peroxides activity	-6.1
YBR016W	Response to dessication	-5.8
AGP2	Response to osmotic stress (fatty acid metabolism)- Hydrogen: amino acid symporter activity	-5.6
SVS1	Response to chemical substance	-5.5
ATC1	Response to stress	-5.3
SHO1/SSU81	Osmosensory signaling pathway via Sho1 osmosensor- osmosensor activity	-5.1
HAL1	Salinity response	-4.6
WWM1	Response to dessication	-4.4
CCP1	Response to oxidative stress	-4.3
YHB1	Response to stress	-4.2
YNL234W	Response to stress- Heme binding activity	-4.2
SNG1	Response to drug	-4.0
PSR2	Response to stress- Protein phosphatase activity	-3.8
CCT7	(Protein folding)- Chaperone activity	-3.3
HOG1	Hyperosmotic response- MAP kinase activity	-3.3
OCA1	Response to oxidative stress- Protein tyrosin phosphatase activity	-3.0
Transport & translocation		
HXT2	Hexose transport- Glucose, mannose and fructose transporter activity	-36.5
PHO3	Thiamine transport- Acid phosphatase	-35.3
HXT3	Hexose transport- Glucose transporter*	-26.7
VRG4/GOG5	Nucleotide-sugar transport - Nucleotide-sugar transporter activity	-20.4
SSH1	Protein transporter activity- Cotranslational membrane targeting	-18.6
Ato3	Nitrogen utilization (transport)- Transporter activity	-14.3
KAP123	Protein-nucleus import- Protein carrier activity	-12.5
VMA7	Vacuolar acidification- Hydrogen-transporting ATPase activity	-11.4
PMP3	Cation transport	-11.4
NTF2	Protein-nucleus import- RAN protein binding activity	-11.1
PMA1	Proton transport (regulation of PH)- Hydrogen-transporting ATPase activity	-10.6
PHO88	Phosphate transport- Phosphate transporter activity	-10.2
TPO2	Polyamine transport- Spermine transporter activity	-8.4
ANR2	Iron-siderochrome transport- Siderochrome-iron transporter activity	-8.4
SEC61	SRP-dependent cotranslational membrane targeting, translocation- Protein transporter activity	-8.1
PHO84	Phosphate transport- Inorganic phosphate transporter activity	-7.5
ARF1	ER to Golgi transport (intra-Golgi transport)- ARF small monomeric GTPase activity	-7.3
ZRT3	Zinc ion transport (zinc ion homeostasis)- Zinc ion transporter activity	-7.2
LAC1	Protein transporter activity- Ceramide biosynthesis (aging)	-7.0
EMP70	Transport- transporter activity	-6.9
PDR5	Drug transport (response to drug)- Xenobiotic-transporting ATPase activity	-6.9
SEC63	SRP-dependent cotranslational membrane targeting, translocation- Endoplasmic reticulum receptor activity	-6.8
OAC1	Oxaloacetate transport (sulfate transport)- Oxaloacetate carrier (sulfate porter)	-6.8
YPT1	ER to Golgi transport (protein complex assembly)- RAB small monomeric GTPase activity	-6.5
PPA1	Hydrogen-transporting ATPase activity- Vacuolar acidification	-6.5
VCX1	Calcium ion homeostasis (calium ion transport)- Calcium ion transporter activity	-6.5
FRE1	Iron and copper ion transport- Ferric-chelate reductase activity	-6.0
SEC13	ER to Golgi transport	-6.0
TOM22	Protein transporter activity- Mitochondrial translocation	-6.0
TOM6	Mitochondrial translocation- Protein transporter activity	-6.0
YCP4	Electron transporter activity	-5.9
PXA1	Fatty acid transport- ATP-binding cassette (ABC) transporter activity	-5.7

GOT1	ER to Golgi transport	-5.6
YGR257C/ MTM1	Transport- Transporter activity	-5.5
YKT6	Intra-Golgi transport (nonselective vesicle fusion)- v-SNARE activity	-5.4
ERV14	ER to Golgi transport (axial budding)	-5.3
KAP120	Protein-nucleus import- Structural constituent of nuclear pore	-5.3
GLO3	ER to Golgi transport (retrograde (Golgi to ER) Transport- ARF GTPase activaton activity	-5.3
SFH5	Phospholipid transport- Phosphatidylinositol transporter activity	-5.2
ARF2	ER to Golgi transport (intra-Golgi transport)- ARF small monomeric GTPase activity	-5.1
AGT1	Alpha-glucoside transporter, hexose transporter, maltose permease- Alpha-glucoside transport (trehalose transport)	-5.0
INP54	Exocytosis- Inositol-1, 4, 5-trisphosphate 5-phosphatase activity	-4.9
TIM50	Mitochondrial matrix protein import	-4.9
TIM13	Protein transporter activity- Mitochondrial translocation	-4.8
EMP24	ER to Golgi transport	-4.8
RET2	ER to Golgi transport	-4.8
SAR1	ER to Golgi transport- SAR small monomeric GTPase activity	-4.8
SVL3	Endocytosis	-4.8
DPB5	mRNA-nucleus export- RNA helicase activity	-4.7
PMP2	Cation transport	-4.7
HIP1	Histidine transport- Histidine transport	-4.7
KRE11	ER to Golgi transport	-4.7
CTP1	mitochondrial citrate transport- Tricarboxylate carrier activity	-4.6
VPH1	Hydrogen-transporting ATPase activity	-4.6
COT1	Cobalt ion transport- Cobalt ion transporter activity	-4.5
ITR2	Myo-inositol transport- Myo-inositol transporter activity	-4.5
FCY2	Cytosine transport (purine transport)- Cytosine-purine permease activity	-4.5
EMP47	ER to Golgi transport	-4.4
SFT2	Golgi to endosome transport	-4.4
VPS55	Late endosome to vacuole transport	-4.4
SOP4	ER to Golgi transport	-4.4
TRS33	ER to Golgi transport	-4.3
VMA8	Hydrogen-transporting ATPase activity- Vacuolar acidification	-4.3
SLY1	ER to Golgi transport- ER to Golgi transport	-4.2
CHS7	ER to Golgi transport (cell wall chitin biosynthesis)	-4.2
TRK1	Potassium ion homeostasis- Potassium ion transporter activity	-4.2
SCM2	Aromatic amino acid transport- Aromatic amino acid transporter activity	-4.1
SEC1	Exocytosis (nonselective vesicle docking and fusion)- SNARE binding activity	-4.1
ERV46	ER to Golgi transport	-4.1
HXT4	Hexose transport- Glucose, mannose and fructose transporter activity	-4.0
TRK2	Potassium ion homeostasis- Potassium ion transporter activity	-4.0
VMA4	Vacuolar acidification- Hydrogen-transporting ATPase activity	-4.0
BRF2	ER to Golgi transport	-4.0
CHS5	Golgi to plasma membrane transport	-3.9
MUP1	Sulfur amino acid transport- L-methionine porter activity	-3.9
AVT3	Neutral amino acid transport- Neutral amino acid transporter activity	-3.8
TOM7	Mitochondrial translocation- Protein transporter activity	-3.8
PMR1	Calcium and manganese ion transport- Calcium-transporting ATPase activity (manganese-transporting ATPase activity)	-3.8
ATP16	ATP synthesis coupled proton transport- Hydrogen-transporting ATPase activity	-3.8
HNM1	Choline transport- Choline transporter activity	-3.8
POM34	Nucleocytoplasmic transport	-3.7
ARF3	Intracellular protein transport-ARF small monomeric GTPase activ	-3.7
SSS1	Protein transporter activity- Cotranslational membrane targeting	-3.7
HXT6	Hexose transport- Fructose transporter activity (glucose transporter activity) (mannose transporter	-3.6
COG1	Intra-Golgi transport (retrograde (vesicle recycling within Golgi) transport)	-3.6
SEB1	Protein transporter activity- Cotranslational membrane targeting	-3.6
NUP57	mRNA, mRNA binding (hnRNP), ……see YGDB	-3.6
NUP84	mRNA, mRNA binding (hnRNP), ……see YGDB	-3.6
NCP1	Electron transporter activity	-3.6
ERV14	ER to Golgi transport	-3.6
PDR10	ATP-binding cassette (ABC) transporter activity- ATP-binding cassette (ABC) transporter activity	-3.6

YNL275W	Transport- Anion transporter activity	-3.5
TOM70	Mitochondrial translocation -Protein transporter activity	-3.5
PHO86	Phosphate transport	-3.5
YHL008C	Transporter activity	-3.5
FET5	Iron ion transport- Multicopper ferroxidase iron transport mediator activity	-3.5
GCS1	ER to Golgi transport- ARF GTPase activator activity	-3.5
SEC4	Golgi to plasma membrane transport (cytokinesis)- RAB small monomeric GTPase activity	-3.5
YPT31	Exocytosis (vesicle-mediated transport)- GTPase activity	-3.4
More 7	Transport- Hexose transporter	-3.4
ERP2	ER to Golgi transport	-3.4
YBT1	Bile acid transport- ATP-binding cassette (ABC) transporter activity (bile acid transporter activity)	-3.4
AVT1	Neutral amino acid transport- Neutral amino acid transporter activity	-3.4
ATX1	Copper ion transport- Copper chaperone activity	-3.4
AKR1	Endocytosis- Palmitoyltransferase activity	-3.4
TPO1	Polyamine transport- Spermidine transporter activity	-3.4
RFT1	Oligosaccharide transporter activity- Oligosaccharide transport (N-linked glycosylation)	-3.4
NUP145	mRNA, mRNA binding (hnRNP), ……see YGDB	-3.3
RNA1	Protein-nucleus import (rRNA-nucleus export) (ribosome nucleus export)- RNA GTPase activator activity	-3.3
YPR004C	Electron carrier activity	-3.3
NUP133	mRNA-nucleus export, mRNA-binding (hnRNP) protein-nucleus, NLS-bearing substrate-nucleus import, Import nuclear pore organization and biogenesis, (Structural molecule activity (nuclear pore complex subunit)	-3.3
TIP20	Retrograde (Golgi to ER) transport	-3.3
TIM22	Mitochondrial translocation- Protein transporter activity	-3.3
ERV29	ER to Golgi transport	-3.3
MCH5	Transport– Transport activity	-3.3
SRP1	Nucleocytoplasmic transport- Protein carrier activity	-3.3
FUN26	Nucleoside transport- Nucleoside transporter activity	-3.3
YAP1802	Endocytosis- Cytoskeletal adaptor activity	-3.3
TOM71	Protein transporter activity	-3.3
RLI1	ATP-binding cassette (ABC) transporter activity	-3.3
YIP3	ER to Golgi transport	-3.2
ZRC1	Zinc & cobalt ion transport- Zinc ion transporter activity	-3.2
SEC12	ER to Golgi transport- Guanyl-nucleotide exchange factor activity	-3.2
SYS1	Golgi to endosome transport (vesicle organization and Biogenesis	-3.2
ARL1	Vesicle-mediated transport- Small monomeric GTPase activity	-3.2
YHM1	Transporter activity- Mitochondrial genome maintenance (transport)	-3.1
ADP1	Transport- ATP-binding cassette (ABC) transporter activity	-3.1
ATX2	Manganese ion homeostasis- Manganese ion transporter activity	-3.1
FRE2	Iron and copper ion import- Ferric-chelate reductase activity	-3.1
ATP4	ATP synthesis coupled proton transport- Hydrogen-transporting ATPase activity	-3.1
HOL1	Transport- Transporter activity	-3.1
YLR004C	Transport- Transporter activity	-3.1
MID1	Calcium ion transport- Calcium channel activity	-3.1
CSE1	Protein-nucleus export- Importin-alpha export receptor activity	-3.1
MAL31	Alpha-glucoside transport- Alpha-glucoside:hydrogen symporter activity	-3.1
VID24	Vesicle-mediated transport	-3.1
APM4	Intracellular protein transport	-3.1
ASM4	mRNA, mRNA binding (hnRNP), ……see YGDB	-3.1
YER036C	ATP-binding cassette (ABC) transporter activity	-3.1
ZRT2	Low-affinity zinc ion transport- Low-affinity zinc ion transporter activity	-3.0
RET3	Retrograde (Golgi to ER) transport- Protein binding activity	-3.0
LST4	Golgi to plasma membrane transport- Protein transporter activity	-3.0
AQR1	Drug transport (monocarboxylic acid transport)– Drug transporter activity (monocarboxylic acid transporter activity)	-3.0
UBS1	Protein-nucleus export (protein ubiquitination	-3.0
ATP1	ATP synthesis coupled proton transport- Hydrogen-transporting ATPase activity	-3.0
SEC27	Retrograde (Golgi to ER) transport	-3.0
SEH1	mRNA, mRNA binding (hnRNP), ……see YGDB	-3.0
VPS53	Golgi to vacuole transport	-3.0

SNX41	Protein transporter- Protein transporter activity (Endosome)	-3.0
AGE2	ER to Golgi transport- ARF GTPase activator activity	-3.0
Energy utilization genes		
QCR9	Oxidative phosphorylation, ubiquinone to cytochrome c (aerobic respiration)- Ubiquinol-cytochrome C reductase activity	-17.7
ALD6	Acetate biosynthesis -aldehyde dehydrogenase activity	-11.0
IMD2/PUR5	GTP biosynthesis- IMP dehydrogenase activity	-9.5
SOL3	Pentose-phosphate shunt, oxidative branch (tRNA processing)	-9.4
NDEI	NADH oxidation (Ethanol fermentation)- NADH dehydrogenase activity	-8.8
MDH2	Gluconeogenesis (malate metabolism)- malic enzyme activity	-8.1
POS18/RPE1	Pentose-phosphate shunt- Ribulose-phosphate 3-epimerase activity	-8.1
PKI1	Pentose-phosphate shunt –Ribose-5-phosphate isomerase activity	-7.6
PDC1	Pyruvate metabolism (Ethanol fermentation)- Pyruvate decarboxylase activity	-7.2
GND1	Glucose metabolism- Phosphogluconate dehydrogenase (decarboxylating) activity	-7.1
PDB1	Pyruvate metabolism- Pyruvate dehydrogenase (lipoamide) activity	-6.2
PDA1	Pyruvate metabolism (alpha subunit of pyruvate dehydrogenase)- pyruvate dehydrogenase (lipoamide) activity	-5.7
TSA1	Regulation of redox homeostasis- Thioredoxin peroxidase activity	-5.6
PDC5	Pyruvate metabolism (ethanol fermentation)- Pyruvate decarboxylase activity	-5.5
MAL12	Maltose metabolism- Alpha-glucosidase activity	-5.5
EXG1	Glucan metabolism (cell wall organization and biogenesis)- Glucan 1,3-beta-glucosidase activity	-5.4
PFK27	Fructose 2,6-bisphosphate metabolism (regulation of glycolysis)- 6-phosphofructo-2-kinase activity	-5.1
STD1	Glucose metabolism (regulation of transcription from Pol II promoter)- Protein kinase activator activity	-5.1
COX9	Aerobic respiration- Cytochrome c oxidase activity	-4.6
TDH3	Gluconeogenesis (Glycolysis)- Glyceraldehyde 3-phosphate dehydrogenase (phosphorylating) activity	-4.6
QNS1	NAD+ synthase (glutamine-hydrolyzing) activity	-4.6
PSA1	GDP-mannose biosynthesis (cell wall mannoprotein biosynthesis)- Mannose-1-phosphate guanylyltransferase activity	-4.5
SDH2	Oxidative phosphorylation, succinate to ubiquinone- Succinate dehydrogenase activity	-4.1
KTI12	Carbon utilization (cell growth and/or maintenance)- Enzyme regulator activity	-4.0
ARA1	Carbohydrate metabolism- D-arabinose 1-dehydrogenase [NAD(P)] activity	-3.9
ADH6	Alcohol metabolism (aldehyde- Alcohol dehydrogenase (NADP+) activity	-3.8
GDH1	Glutamate biosynthesis, using glutamate dehydrogenase (NAD (P)+)- Glutamate dehydrogenasem (NADP+) activity	-3.8
VMA10	Glycogen metabolism- Hydrogen-transporting ATPase activity	-3.8
TAL1	Pentose-phosphate shunt- Transaldolase activity	-3.7
HRD3	ER-associated protein catabolism- Ubiquitin-protein ligase activity	-3.3
ACS2	Acetyl-CoA biosynthesis- Acetate-CoA ligase activity	-3.3
GFA1	Glucosamine-fructose-6-phosphate aminotransferase (isomerizing) activity	-3.2
MAE1	Malate dehydrogenase (oxaloacetate decarboxylating) activity- (pyruvate metabolism)	-3.2
PGM1	Glucose 6-phosphate utilization (glucose 1-phosphateutilization)- Phosphoglucomutase activity	-3.2
TKL1	Pentose-phosphate shunt- Transketolase activity	-3.2
FDH2	Formate catabolism- Formate dehydrogenase activity	-3.1
PHO80	Regulation of phosphate metabolism- Cyclin-dependent protein kinase, regulator activity	-3.1
COX5A	Aerobic respiration Cytochrome C oxidase activity	-3.1
APH1	Regulation of redox homeostasis- Thioredoxin peroxidase activity	-3.1
TKL2	Pentose-phosphate shunt- Transketolase activity	-3.1
TDH2	Glycolysis (gluconeogenesis)- Glyceraldehyde 3-phosphate dehydrogenase (phosphorylating) activity	-3.0
ERR2	Phosphopyruvate hydratase activity	-3.0
GLC3	Glycogen metabolism	-30
SHP1	Glycogen metabolism	-3.0
Protein folding, synthesis, modification, translocation, degradation and complex assembly		
SSB1	Chaperone- Protein biosynthesis	-30.0

SSB2	Protein biosynthesis- ATPase activity (chaperone activity)	-24.4
SSZ1	Chaperone activity- Protein biosynthesis	-14.0
ZUO1	Proteins folding (Z-DNA binding protein)- Chaperone activity	-12.3
THR1	Homoserine metabolism- Homoserine kinase activity	-12.1
MKC7	Proteolysis and peptidolysis- Aspartic-type signal peptidase activity	-12.0
CDC42	Rho subfamily of Ras-like proteins- Rho small monomeric GTPase activity	-11.4
SAM1	Methionine metabolism- Methionine adenosyltransferase activity	-11.1
DED81	Asparaginyl-tRNA aminoacylation- ATP binding activity (asparagine-Trna ligase activity)	-10.5
TWT1	Branched chain family amino acid biosynthesis (amino acid catabolism)- Branched-chain amino acid aminotransferase activity	-10.4
EGD2	Chaperone activity- Nascent polypeptide association	-10.5
YPL037C/EGD11	Nascent polypeptide association- Chaperone activity	-9.9
SAM4	Sulfur amino acid metabolism- Homocysteine S-methyltransferase activity	-9.9
SER3	Serine family amino acid biosynthesis- Phosphoglycerate dehydrogenase activity	-9.8
LYS9	Lysine biosynthesis, aminoadipic pathway- Saccharopine dehydrogenase (NADP+, L-glutamate forming) activity	-9.8
AAT2	Aspartate catabolism & biosynthesis- Aspartate aminotransferase activity	-9.7
SPE2	Pantothenate biosynthesis- Adenosylmethionine decarboxylase activity	-9.7
HOM6	Threonine and methionine metabolism- Homoserine dehydrogenase activity	-9.7
SWP1	N-linked glycosylation- Dolichyl-diphospho-oligosaccharide-protein glycosyltransferase activity	-9.6
UBR1	Protein monoubiquitination- Ubiquitin-protein ligase activity	-9.5
NMD3	Protein binding activity- RNA binding activity	-9.4
NAT5	Protein amino acid acetylation - Peptide alpha-N-acetyltransferase activity	-9.4
ILV5	Branched chain family amino acid biosynthesis- Ketol-acid reductoisomerase activity	-9.3
LEU1	leucine biosynthesis-3-isopropylmalate dehydratase activity	-8.8
CIC1	Protein catabolism- Protein binding activity, bridging	-8.7
HMT1	peptidyl-arginine modification- Protein-arginine N-methyltransferase activity	-8.5
APE3	Vacuolar protein catabolism- Aminopeptidase activity	-8.3
YHR020W	Proline-Trna ligase activity	-7.8
JAC1	Co-chaperone activity- Aerobic respiration (iron-sulfur cluster assembly)	-7.7
WRS1	Tryptophanyl-Trna aminoacylation- Tryptophan-tRNA ligase activity	-7.6
ARO8	Aromatic amino acid family metabolism- Aromatic amino acid transferase activity	-7.6
SHR3	Chaperone activity- ER to Golgi transport (amino acid transport	-7.5
MDN1	Protein complex assembly- ATPase activity	-7.4
DPS1	Protein biosynthesis- Aspartate-tRNA ligase activity	-7.0
TCP1	Protein folding (cytoskeleton organization and biogenesis)- Chaperone activity	-7.3
PRE9	ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-6.8
YRB30	Enzyme regulator activity (proteins binding activity)	-6.7
CYS3	cysteine metabolism (sulfur amino acidmetabolism) (transsulfuration)- cystathionine-gamma-lyase activity	-6.7
RRS1	protein biosynthesis- arginine-tRNA ligase activity	-6.6
NIN1	ubiquitin-dependentprotein catabolism- Proteasome endopeptidase activity	-6.6
AHA1	Protein folding (response to stress)- Chaperone activator activity	-6.5
CCT2	Protein folding- Chaperone activity	-6.4
CYS4	Cysteine biosynthesis- Cystathione beta-synthase activity	-6.4
SRP40	Nucleocytoplasmic transport- Chaperone activity	-6.0
MEU1	Glutamate biosynthesis	-5.9
UBC4	Protein monoubiquitination- Ubiquitin conjugating enzyme activity	-5.9
NMT1	N-terminal peptidyl-glycine N-myristoylation- Glycyl-peptide N-tetradecanoyltransferase activity	-5.7
ARO4	Aromatic amino acid family biosynthesis-2-dehydro-3-deoxyphosphoheptonate aldolase activity	-5.7
ASP3	Asparagine catabolism- Asparaginase activity	-5.5
MTR2	Poly (A)+ Mrna-nucleus export- Protein binding activity	-5.5
SES1	Amino acid activation- serine-tRNA ligase activity	-5.5
LEU9	Leucine biosynthesis-2-isopropylmalate synthase activity	-5.4
GPI16	Attachment of GPI anchor to protein- GPI-anchor transamidase activity	-5.4
CCT6	Protein folding (Cytoskeleton organization and biogenesis)- Chaperone activity	-5.4
FES1	Protein biosynthesis- Adenyl-nucleotide exchange factor activity	-5.4
PRE7	ubiquitin-dependent protein catabolism Proteasome endopeptidase activity	-5.4
STP22	Protein-membrane targeting (protein-vacuolar targeting- Protein binding	-5.3

	activity	
ARO3	Aromatic amino acid family biosynthesis-2-dehydro-3- Aromatic amino acid family biosynthesis-2-dehydro-3-deoxyphosphoheptonate aldolase activity	-5.3
HOM2	Methionine metabolism (threonine metabolism)- aspartate-semialdehyde dehydrogenase activity	-5.2
YMR226C	Serine metabolism- Oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor	-5.2
CPH1	Protein metabolism	-5.1
MRF1	Protein-ER retention	-5.0
VMA21	Protein complex assembly	-5.0
MAP2	Proteolysis and peptidolysis- Methionyl aminopeptidase activity	-4.9
TTP1	Protein amino acid glycosylation- Alpha-1,2-mannosyltransferase activity	-4.9
PPT1	Protein amino acid phosphorylation- Protein serine/threonine phosphatase	-4.9
PNG1	Misfolded or incompletely synthesized protein catabolism- Peptide-N4- (N-acetyl-beta glucosaminyl) asparagines amidase activity	-4.9
YFL035C	Protein kinase activator activity	-4.8
AAT1	Asparagine biosynthesis from oxaloacetate- Aspartate aminotransferase activity	-4.7
MNN11	Protein amino acid glycosylation Alpha-1, 6-mannosyltransferase activity	-4.7
MSD1	Protein biosynthesis- Aspartate-tRNA ligase activity	-4.7
PRE6	Ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-4.6
HIS1	Histidine biosynthesis- ATP phosphoribosyltransferase activity	-4.6
YTA2	Ubiquitin-dependent protein catabolism- ATPase activity (proteasome endopeptidase activity	-4.6
ASP1	Aspartate biosynthesis- Aspartate biosynthesis	-4.6
ASP3	Asparagine catabolism- Asparaginase activity	-4.5
PDS1	Protein binding activity	-4.5
PAD1	Aromatic compound catabolism- Carboxy-lyase activity	-4.3
MAK10	Amino acid N-acetyltransferase activity- N-terminal protein amino acid acetylation (virus-host interaction	-4.2
NOB1	Protein complex assembly- Chaperone activity	-4.2
ARO2	Aromatic amino acid family biosynthesis- Chorismate synthase activity	-4.2
TRP3	Tryptophan biosynthesis- Anthranilate synthase activity	-4.2
SSE1	Protein folding- Co-chaperone activity	-4.1
THS1	Protein biosynthesis- Threonine-tRNA ligase activity	-4.1
RPN3	ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-4.1
TRP4	Tryptophan biosynthesis- Anthranilate phosphoribosyltransferase activity	-4.1
RPN9	ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-4.1
TYS1	Tyrosyl-tRNA aminoacylation (amino acid activation)- Tyrosine-tRNA ligase activity	-4.0
THR4	Threonine metabolism- Threonine synthase activity	-4.0
CCT4	Chaperone activity- protein folding	-4.0
UBC6	Protein monoubiquitination (protein polyubiquitination)- Ubiquitin conjugating enzyme activity	-4.0
GNT1	Protein amino acid glycosylation- Acetylglucosaminyltransferase activity	-4.0
GLN4	Amino acid activation- Glutamine-tRNA ligase activity	-3.9
ASN2	Asparagine biosynthesis- Asparagine synthase (glutamine-hydrolyzing) activity	-3.9
ARG4	Arginine biosynthesis- Argininosuccinate lyase activity	-3.9
LYS2	Lysine biosynthesis, aminoadipic pathway- aminoadipate-semialdehyde dehydrogenase activity	-3.8
SAH1	Methionine metabolism (selenocysteine metabolism)- Adenosylhomocysteinase activity	-3.8
RPN5	ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-3.8
GSH1	Glutathione biosynthesis– Glutamate-cysteine ligase activity	-3.6
PRO2	Proline biosynthesis- Glutamate-5-semialdehyde dehydrogenase activity	-3.6
VPS45	Protein chaperone activity- protein assembly activity	-3.6
ASP3	Asparagine catabolism- Asparaginase activity	-3.6
DBF2	Protein amino acid phosphorylation- Protein kinase activity	-3.6
SLT2	MAP kinase activity- signal transduction)	-3.6
ILV2	Branched chain family amino acid biosynthesis- Threonine dehydratase activity	-3.6
TPK3	Protein amino acid phosphorylation- cAMP-dependent protein kinase activity	-3.5
SIS1	Chaperone activity- Translational initiation	-3.5
SCL1	ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-3.5
ANP1	N-linked glycosylation- Mannosyltransferase activity	-3.5
GDA1	Protein amino acid glycosylation- Guanosine diphosphatase activity (uridine diphosphatase activity)	-3.5

PIB1	Protein ubiquitination- ubiquitin-protein ligase activity	-3.5
RAM1	Protein amino acid farnesylation- Protein farnesyltransferase activity	-3.4
MSY1	Amino acid activation- Tyrosine-tRNA ligase activity	-3.4
UBA1	Ubiquitin cycle- Ubiquitin activating enzyme activity	-3.3
CDC26	Protein binding activity (ubiquitin-protein ligase activity)	-3.3
PCL10	Cyclin-dependent protein kinase, regulator activity- Regulation of glycogen biosynthesis (regulation of glycogen catabolism)	-3.3
YDR131C	ubiquitin-dependent protein catabolism- Protein binding activity	-3.3
IDP1	Glutamate biosynthesis (isocitrate metabolism)- Isocitrate dehydrogenase (NADP+) activity	-3.3
MES1	Amino acid activation- Methionine-tRNA ligase activity	-3.3
UFD2	Ubiquitin-dependent protein catabolism (stress response)	-3.3
CBS2	Protein biosynthesis	-3.3
TPD3	Protein biosynthesis (actin filament organization) (protein amino acid dephosphorylation) (bud growth)- Protein phosphatase type 2A activity	-3.2
LYS12	Lysine biosynthesis- Isocitrate dehydrogenase activity	-3.2
MNN5	Protein amino acid glycosylation- Alpha-1, 2-mannosyltransferase activity	-3.2
ARG1	arginine biosynthesis- argininosuccinate synthase activity	-3.2
TIM17	Mitochondrial translocation- Protein transporter activity	-3.2
MMS2	Ubiquitin-dependent protein catabolism- ubiquitin conjugating enzyme activity	-3.1
PRE4	ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-3.1
DAP2	Protein processing- Dipeptidyl-peptidase and tripeptidyl-peptidase activity	-3.1
PRE8	Ubiquitin-dependent protein catabolism- Proteasome endopeptidase activity	-3.1
YPS7	Aspartic-type endopeptidase activity	-3.1
SCC3	Protein binding activity- Peptidyl-prolyl cis-trans isomerase activity	-3.1
TOM1	Ubiquitin-protein ligase activity	-3.1
MPD2	Protein disulfide-isomerase reaction (protein folding)- Protein disulfide isomerase activity	-3.1
UMP1	Chaperone activity (chaperone activity)	-3.1
PNO1	Protein complex assembly- Chaperone activity	-3.0
YFR010W	Protein deubiquitination- Ubiquitin-specific protease activity	-3.0
MMF1	Isoleucine biosynthesis	-3.0
SRP14	Protein signal sequence binding activity/ Protein-ER targeting	-3.0
YGR052W	Kinase activity	-3.0
SGN1	Protein metabolism- Poly (A) binding activity	-3.0
Signal transduction proteins		
RAS2	RAS protein signal transduction (small GTP-binding protein)- RAS small monomeric GTPase activity	-22.9
MF(ALPHA)2	Response to pheromone during conjugation with cellular fusion- Pheromone activity (alpha mating factor)	-22.3
STE3	Signal transduction during conjugation with cellular fusion- Mating-type a-factor pheromone receptor activity	-12.0
HF(ALPHA)1	Response to pheromone during conjugation with cellular fusion- Pheromone activity	-11.6
STE24	Peptide pheromone maturation- Metalloendopeptidase activity	-7.3
RAS1	RAS protein signal transduction (G-protein signaling, adenylate cyclase activating pathway)- RAS small monomeric GTPase activity	-7.2
WSC3	Rho protein signal transduction- Transmembrane receptor activity	-6.9
CLA4	Rho protein signal transduction- Protein serine/threonine kinase activity	-6.8
YLR301W	Cotranslational membrane targeting-	-6.4
SRP21	Protein-ER targeting- Protein signal sequence binding activity	-6.4
SRP101	Signal recognition particle binding activity- Protein-ER targeting	-5.7
RHO2	Small GTPase mediate signal transduction- Rho small monomeric GTPase activity	-5.5
SMR1	Signal transducer activity- rRNA-nucleus export (ribosome nucleus export)	-5.5
RAM2	Protein amino acid farnesylation (peptide pheromone maturation)- Protein farnesyltransferase activity	-4.8
STE4	Signal transduction during conjugation with cellular fusion- Heterotrimeric G-protein GTPase activity	-4.8
BMH2	RAS protein signal transduction	-4.1
SRP68	Protein signal sequence binding activity- Protein-ER targeting	-3.9
PDE2	cAMP-mediated signaling- camp-specific phosphodiesterase activity	-3.9
CMP2	Adaptation to pheromone during conjugation with cellular fusion- Calcium-dependent protein serine/threonine phosphatase activity	-3.7
RGS2	G-protein signaling, coupled to cAMP nucleotide second messenger- GTPase activator activity	-3.9

GPA1	Signal transduction during conjugation with cellular fusion- Heterotrimeric G-protein GTPase activity	-3.9
RTG2	Intracellular signaling cascade	-3.5
MSO1	Nonselective vesicle docking	-3.5
MUC1	Signal transducer activity- Cell-cell adhesion (cell surface flocculin with structure similar to serine/threonine-rich GPI anchored cell wall proteins)	-3.3
SRV2	RAS protein signal Transduction (cytoskeleton organization & biogenesis)-Adenylate cyclase binding activity (cytoskeletal protein bindingactivity)	-3.1
PEA2	Cytoskeletal regulatory protein binding activity- Actin filament organization	-3.0
IRA2	RAS protein signal transduction- Ras GTPase activator activity	-3.0
Nucleotides Metabolism		
URA1	Pyrimidine base biosynthesis -Dihydroorotate dehydrogenase activity	-23.0
AAH1	Adenine catabolism- Adenine deaminase activity	-20.5
HTA2	Chromatin assembly /disassembly (Histone H2A)- DNA binding activity	-20.0
MPT4	Telomere maintenance (anti-apoptosis)- Telomeric DNA binding activity	-17.5
HTB2	Chromatin assembly /disassembly (histone H2B)- DNA binding activity	-16.4
HTB1	Chromatin assembly /disassembly (histone H2B)- DNA binding activity	-14.9
RNR2	DNA replication- Ribonucleoside-diphosphate reductase activity	-13.9
SML1	Response to DNA damage- Enzyme inhibitor activity	-13.6
HHT1	Chromatin assembly/disassembly- DNA binding activity	-13.3
YOR247W	Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	-13.1
TRX2	DNA dependent DNA replication (response to oxidative stress)- Thiol-disulfide exchange intermediate activity	-11.5
FUR1	Pyrimidine salvage- Uracil phosphoribosyltransferase activity	-11.3
HPT1	Purine nucleotide biosynthesis- Hypoxanthine phosphoribosyltransferase activity	-11.0
URA4	Pyrimidine nucleotide biosynthesis- Dihydroorotase activity	-10.5
GSP1	Nuclear organization and biogenesis- RAN small monomeric GTPase activity	-10.5
MCM3	DNA replication initiation- ATP dependent DNA helicase activity	-9.4
URA5	Pyrimidine base biosynthesis- Orotate phosphoribosyltransferase activity	-9.3
MSC7	Meiotic recombination	-8.9
URA7	Pyrimidine base biosynthesis- CTP synthase activity	-8.3
ADO1	Purine base metabolism- Adenosine kinase activity	-8.0
POL30	Base-excision repair (mismatch repair) (postreplication repair) (leading strand elongation)- DNA polymerase processivity factor activity	-7.6
YDL125C	Nucleotide metabolism- Hydrolase activity (nucleotide binding	-7.5
NPT1	Chromatin silencing at ribosomal DNA (rDNA) (nicotinate nucleotide biosynthesis, salvage pathway)- Nicotinate phosphoribosyltransferase activity	-7.0
RFC3	Leading strand elongation (mismatch repair)- DNA clamp loader (ATPase)	-6.8
IXR1	DNA repair- DNA binding activity	-6.7
PRI1	DNA replication initiation- Alpha DNA polymerase activity	-6.7
ASF1	Induction of apoptosis by DNA damage- Histone binding activity	-6.5
RFA3	DNA replication, priming, and elongation- DNA binding activity	-6.3
NUC1	DNA recombination- Endodeoxyribonuclease activity	-6.1
YLR003C	Regulation of DNA replication	-5.8
HHF1	DNA binding activity- Chromatin assembly/disassembly	-5.8
PHO13	Histone dephosphorylation (protein amino acid dephosphorylation-4-nitrophenylphosphatase activity (alkaline phosphatase activity)	-5.8
HUS2	Base-excision repair (lagging & leading strand elongation) (mismatch repair)-Delta DNA polymerase activity	-5.7
LIF1	Double-strand break repair via nonhomologous end-joining	-5.6
ADE13	Purine base metabolism- Adenylosuccinate lyase activity	-5.4
RAI1	RNA catabolism (processing of 27S pre-rRNA)- Enzyme regulator activity	-5.3
PAN3	Postreplication repair (DNA repair)- Poly (A)-specific ribonuclease activity	-5.1
MSI1	DNA repair (RAS signal transduction	-5.1
SAN1	Establishment and/or maintenance of chromatin architecture	-5.1
TRX1	DNA dependent DNA Replication (regulation of redox Homeostasis)- Thiol-disulfide exchange intermediate activity	-4.9
YFR038W	Helicase activity	-4.9
RNR4	DNA replication- Ribonucleoside-diphosphate reductase activity	-4.7
CTF8	Sister chromatid cohesion	-4.6
HHT2	Chromatin assembly/disassembly- DNA binding activity	-4.6
RAD57	DNA recombinase assembly- Protein binding activity	-4.4

CDC9	DNA ligation (DNA recombination) (base-excision repair) (base-excision repair)- DNA ligase (ATP) activity	-4.3
STS1	Chromosome segregation	-4.3
PAT1	Chromosome segregation (regulation of translational initiation)	-4.2
PMS1	Meiosis (mismatch repair)- ATP binding activity (ATPase activity)	-4.1
RFA1	DNA recombination (DNA replication, priming) (DNA strand elongation) (DNA unwinding) (double-strand break repair)- Damaged DNA binding activity	-4.1
SPC3	Signal peptide processing- Signal peptidase activity	-4.1
APA1	Nucleotide metabolism- Bis (5'-nucleosyl)-tetraphosphatase activity	-4.0
NOC3	DNA replication initiation (rRNA processing)- Chromatin binding activity (protein binding activity)	-3.9
MSH2	DNA recombination (mismatch repair)- ATP binding activity (ATPase activity)	-3.8
MGS1	DNA replication (regulation of DNA Replication)- ATPase activity (helicase activity)	-3.6
MCM1	DNA replication initiation (regulation of transcription from Pol II promoter)- DNA replication origin binding activity (DNA replication activity)	-3.5
ORC3	DNA replication initiation -DNA replication origin binding activity	-3.5
URA6	Nucleobase, nucleoside, nucleotide and nucleic acid metabolism- Uridine kinase activity	-3.5
SDT1	Pyrimidine base metabolism- Nucleotidase activity	-3.5
RGD2	Small GTPase mediated signal transduction- Rho GTPase activator activity	-3.4
CCE1	DNA recombination- Endodeoxyribonuclease activity	-3.4
RSC9	Chromatin modeling- Chromatin binding activity	-3.4
TBF1	Loss of chromatin silencing- DNA binding activity (transcription factor activity)	-3.4
ACT3	Establishment and/or maintenance of chromatin architecture- Chromatin binding activity (histone acetyltransferase activity)	-3.4
TRF4	DNA topological change- DNA-directed DNA polymerase activity	-3.3
HAMA	DNA repair	-3.3
RFC4	Leading strand elongation (mismatch repair)- DNA clamp loader activity (purine nucleotide binding Activity)	-3.3
SWD2	Histone methylation- Histone-lysine N-methyltransferase activity	-3.3
HTA1	DNA binding activity- Chromatin assembly/disassembly	-3.3
MSH6	Mismatch repair- ATP binding activity (ATPase activity) (DNA binding activity)	-3.3
SRL3	Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	-3.3
NAM7	mRNA catabolism, nonsense-mediated- ATP dependent helicase Activity (ATPase activity)	-3.3
PET309	RNA metabolism (protein biosynthesis)- Translation regulator activity	-3.3
AMD1	Purine nucleotide metabolism- AMP deaminase activity	-3.2
SER1	Purine base biosynthesis Phosphoserine aminotransferase activity	-3.2
REC102	Meiotic DNA double-strand break formation (meiotic recombination)- DNA binding activity	-3.2
CSM4	Meiotic chromosome segregation	-3.2
HRR25	DNA repair (cell growth or/and maintenance)- Casein kinase activity (protein kinase activity)	-3.2
DOT1	Chromatin silencing at telomere (histone methylation)- Protein-lysine N-methyltransferase activity	-3.2
RNH70	DNA replication and RNA processing-3'-5' exonuclease activity (ribonuclease H activity)	-3.2
CDC14	DNA dependent DNA replication (exit from mitosis)- Protein phosphatase activity	-3.2
HEX3	DNA recombinant ion- DNA binding activity	-3.2
POB3	Chromatin assembly/disassembly (DNA dependent DNA replication)- Chromatin binding activity	-3.1
TAH11	DNA replication licensing	-3.1
MUM2	Premeiotic DNA synthesis	-3.1
WSC4	Transmembrane receptor activity- Rho protein signal transduction and actin cytoskeleton organization and biogenesis and response to heat	-3.1
FCY1	Cytosine metabolism- Cytosine deaminase activity	-3.0
ADE1	Purine base metabolism- Phosphoribosylaminoimidazole-succinocarboxamide synthase activity	-3.0
SDS22	Chromosome segregation (protein targeting)- Enzyme regulator activity (proteiphosphatase type 1, regulator activity)	-3.0
NEJ1	DNA repair (double-strand break repair)	-3.0

RSC30	DNA binding- Regulation of transcription, DNA-dependent	-3.0
RPH1	DNA repair	-3.0
Transcription and translation factor and process		
TAF9	TFIID subunit (transcription initiation from Pol II promoter)	-10392
ASC1	Negative regulation of translation	-53.8
SNU13	mRNA splicing (processing of 20S pre-rRNA)- Pre-mRNA splicing factor activity	-19.9
EFB1	Translational elongation- Translation elongation factor activity	-18.2
MTF1	Transcription from mitochondrial promoter- Transcription cofactor activity	-17.8
TEF1	Translational elongation- Translation elongation factor activity	-16.7
ERG3	Translational elongation- Translation elongation factor activity	-16.5
CAF20	Negative regulation of translation- Translation regulator activity	-14.2
SIK1	35S primary transcript processing	-13.6
EFT1	Translational elongation- Translation+` elongation factor activity	-12.5
TEF2	Translational elongation- Translation elongation factor activity	-12.4
MIG2	Regulation of transcription from Pol II promoter (glucose metabolism)- Specific RNA polymerase II transcription factor activity	-12.1
CDC33	Translational initiation (regulation of cell cycle)- Translation initiation factor activity	-12.0
HRP1	mRNA polyadenylation and cleavage- Cleavage/polyadenylation specificity factor activity	-12.0
SUI2	Translation initiation- Translation initiation factor activity	-11.5
ALPHA1	Regulation of transcription from Pol II promoter- Transcription co-activator activity	-9.8
TIF1	Translational initiation- Translation initiation factor activity	-9.4
DRS1	35S primary transcript processing- ATP dependent RNA helicase activity	-9.4
GCD1	Translational initiation- Translation initiation factor activity	-9.1
HYP2	Translational initiation– Translation initiation factor activity	-9.0
SUB2	Lariat formation, 5'-splice site cleavage- ATP dependent RNA helicase activity (pre-Mrna splicing factor activity)	-8.8
GRS1	Glycyl-tRNA aminoacylation- Glycine-Trna ligase activity	-8.7
TIF2	Regulation of translational initiation (translation initiation)- RNA helicase activity (translation initiation factor activity)	-8.5
TIF34	Translation initiation- Translation initiation factor activity	-8.1
TEF4	Translational elongation- Translation elongation factor activity	-8.0
ADR1	Transcription (regulation of carbohydrate metabolism)- Transcription factor activity	-7.8
YLA1	tRNA processing- RNA binding activity	-7.8
KRS1	Lysyl-tRNA aminoacylation- lysine-tRNA ligase activity	-7.7
RRP42	35S primary transcript processing (mRNA catabolism-3'-5' exoribonuclease activity	-7.6
NOP58	35S primary transcript processing (processing of 20S pre-rRNA)	-7.6
NOP1	RNA methylation (35S primary transcript processing) (rRNA modification)- Methyltransferase activity	-7.5
TRM7	Protein biosynthesis (tRNA methylation)- tRNA methyltransferase activity	-7.4
TRA1	Histone acetylation (regulation of transcription from Pol II promoter)- Histone acetyltransferase activity	-7.3
TRM8	tRNA methylation- tRNA (guanine-N7-)-methyltransferase activity	-7.2
NHP2	35S primary transcript processing (rRNA modification- RNA binding activity	-7.0
GAR1	35S primary transcript processing (rRNA modification- RNA binding activity	-6.8
HIR3	G1/S-specific transcription in mitotic cell cycle- Transcription co-repressor	-6.7
RPA34	Transcription from Pol I promoter- DNA-directed RNA polymerase activity	-6.7
YEF3	Translational elongation- Translation elongation factor activity	-6.6
HMO1	RNA polymerase I transcription factor activity	-6.4
TRM1	tRNA methylation- tRNA (guanine-N2-)-methyltransferase activity	-6.2
IMP3	35S primary transcript processing- snoRNA binding activity	-6.1
DCP1	Deadenylation-dependent decapping- Hydrolase activity (mRNA binding activity)	-6.1
PAB1	Regulation of translational initiation- Poly (A) binding activity	-6.0
RPB8	Transcription from Pol I, II & III promoter- DNA-directed RNA Polymerase activity	-5.9
PWP2	Processing of 20S pre-rRNA (cytokinesis)- snoRNA binding activity	-5.9
SPT15	Transcription from Pol I, II and III promoter- DNA binding activity (RNA polymerase I, II, III transcription factor activity)	-5.8
CBF5	35S primary transcript processing- Pseudouridylate synthas activity	-5.8
IKI1	Regulation of transcription from Pol II promoter- Pol II transcription elongation	-5.6

	factor activity	
TIF5	Mature ribosome assembly (regulation of translational initiation)- GTPase activator activity (translation initiation factor activity)	-5.6
SUI1	Translational initiation- Translation initiation factor activity	-5.6
TIF35	Translational initiation- Translation initiation factor activity	-5.6
TFB2	Negative regulation of transcription from Pol II promoter, mitotic- General RNA polymerase II transcription factor activity	-5.5
SRM1	rRNA-nucleus export (ribosome nucleus export)– Signal transducer activity	-5.5
HEK2	mRNA localization, intracellular (telomerase-dependent telomere maintenance)- Mrna binding activity	-5.5
TRM82	tRNA methylation- tRNA (guanine-N7-)-methyltransferase activity	-5.5
USS1	mRNA splicing (rRNA processing)- Pre-mRNA splicing factor activity	-5.4
NAM8	mRNA splice site selection (mRNA splicing)- RNA binding activity (mRNA binding activity)	-5.4
RPO21	Transcription from Pol II promoter- DNA-directed RNA polymerase activity	-5.4
EFT2	Translational elongation- Translation elongation factor activity	-5.4
PRP38	mRNA splicing- Pre-mRNA splicing factor activity	-5.3
DIG1	Invasive growth- Transcription factor binding activity	-5.3
SNF11	General RNA polymerase II transcription factor activity	-5.2
GCN4	Regulation of transcription from Pol II promoter (amino acid biosynthesis)- Transcriptional activator activity (DNA binding activity)	-5.2
GRF10	Transcription (cellular response to phosphate starvation)- Transcription factor activity	-5.2
TFA1	Transcription II initiation from Pol II promoter- General RNA polymerase transcription factor activity	-5.1
MED2	RNA polymerase II Transcription mediator activity	-5.1
ALPHA1	Regulation of transcription from Pol II promoter (regulation of transcription, mating-type specific)	-5.1
RRN7	Transcription from Pol I promoter- RNA polymerase I transcription factor activity	-5.1
KRR1	rRNA processing (ribosome biogenesis)	-5.0
YRA1	mRNA-nucleus export- RNA binding activity	-5.0
TEC1	Positive regulation of transcription from Pol II promoter- Specific RNA polymerase II transcription factor activity	-4.9
SMM1	tRNA processing and modification- tRNA dihydrouridine synthase activity	-4.9
NOP13	RNA binding activity	-4.9
FRS2	Phenylalanyl-tRNA aminoacylation- Phenylalanine-tRNA ligase activity	-4.8
SUP45	Translation release factor activity, codon specific- Translational termination	-4.8
TIF11	Translational initiation- Translation initiation factor activity	-4.8
ANB1	Translational initiation- Translation initiation factor activity	-4.8
SNF6	General RNA polymerase II transcription factor activity	-4.8
RPC19	Transcription from Pol I Pol II promoter- DNA-directed RNA polymerase activity	-4.7
RRP3	mRNA splicing- ATP dependent RNA helicase activity	-4.6
KAR4	Positive regulation of transcription from Pol II promoter (meiosis and mitosis)- Transcription regulator activity	-4.6
PTI1	mRNA cleavage (mRNA polyadenylation)- Pre-mRNA cleavage factor activity	-4.6
ELC1	RNA elongation from Pol II promoter- Transcriptional elongation regulator activity	-4.5
DAT1	Negative regulation of transcription from Pol II promoter- AT DNA binding activity	-4.5
BUD21	Processing of 20S pre-rRNA- snoRNA binding activity	-4.5
MRS3	RNA splicing-carrier activity?	-4.5
CGR1	rRNA processing (ribosome biogenesis)	-4.4
GCD11	Translational initiation- Translation initiation factor activity	-4.4
RPB11	Transcription from Pol II promoter- DNA-directed RNA polymerase activity	-4.3
GCN20	Regulation of translational elongation	-4.3
UTP8	Processing of 20S pre-rRNA- snoRNA binding activity	-4.3
UTP4	Processing of 20S pre-rRNA- snoRNA binding activity	-4.3
UTP9	Processing of 20S pre-rRNA- snoRNA binding activity	-4.2
TPT1	tRNA splicing- Trna 2'-phosphotransferase activity	-4.2
PGD1	Transcription from Pol II promoter- RNA polymerase II transcription mediator activity	-4.2
TOS8	Transcription factor activity	-4.2
SLH1	Regulation of translation- RNA helicase activity	-4.2
MTR3	35S primary transcript processing-3'-5' exoribonuclease activity	-4.1
ENP1	Processing of 20S pre-rRNA) (rRNA processing)- snoRNA binding activity	-4.1

PDR1	Regulation of transcription from Pol II promoter (response to drug)- DNA binding activity (transcriptional activator activity)	-4.1
DBP3	35S primary transcript processing- TP dependent RNA helicase activity	-4.1
ARC1	tRNA-nucleus export (amino acid activation)- tRNA binding activity	-4.1
NOP14	Processing of 20S pre-Rrna- snoRNA binding activity	-4.0
RRP45	35S primary transcript processing (mRNA catabolism)- 3'-5' exoribonuclease activity	-4.0
RPL7	Processing of 27S pre-rRNA (ribosomal large subunit biogenesis)-rRNA binding activity	-4.0
BDF1	Transcription regulator activity	-4.0
PRP24	snRNP recycling (spliceosome assembly)- Pre-mRNA splicing factor activity	-4.0
GRC3	Transcription from Pol II promoter- RNA polymerase II transcription mediator activity	-3.9
TAF14	General RNA polymerase II transcription factor activity- Transcription initiation from Pol II promoter (G1-specific transcription in mitotic cell cycle)	-3.9
RTT106	Negative regulation of DNA transposition	-3.9
ERB1	Rrna processing	-3.9
TIF6	Processing of 27S pre-rRNA (ribosomal large subunit biogenesis)	-3.9
POP5 /FUN53	tRNA and rRNA processing - Ribonuclease MRP activity (rRNA processing)	-3.9
KEM1	35S primary transcript processing-5'-3' exoribonuclease activity (deoxyribonuclease activity)	-3.9
RAD3	General RNA polymerase II transcription factor activity (DNA helicase activity)- Transcription initiation from Pol II promoter (nucleotide-excision repair, DNA duplex unwinding)	-3.9
ELP2	Regulation of transcription from Pol II promoter- Pol II transcription elongation factor activity	-3.9
SNF5	General RNA polymerase II transcription factor activity	-3.9
GCN1	Regulation of translational elongation	-3.9
MSS116	RNA splicing- RNA helicase activity	-3.8
CDC36	Negative regulation of transcription from Pol II promoter (poly (A) tail shortening) (regulation of cell cycle)- 3'-5' exoribonuclease activity	-3.8
PRP8	mRNA splicing- Pre-mRNA splicing factor activity	-3.8
RPA14	Transcription from Pol I promoter- DNA-directed RNA polymerase activity	-3.8
TAF7	General RNA polymerase II transcription factor (TAS)- Transcription factor TFIID complex (TAS)	3.8
SUI3	Translational initiation- Translation initiation factor	-3.8
PRP19	mRNA splicing- Pre-mRNA splicing factor activity	-3.8
FRS1	Phenylalanyl-tRNA aminoacylation- Phenylalanine-tRNA ligase activity	-3.8
ARP9	General RNA polymerase II transcription factor activit	3.7
STO1	MRNA splicing- mRNA binding activity	-3.7
GCD7	Translational initiation- Translation initiation factor activity	-3.7
ARP7	General RNA polymerase II transcription factor activity	-3.7
MSS11	Specific RNA polymerase II transcription factor activity- Positive regulation of transcription from Pol II promoter	-3.7
RLM1	Positive regulation of transcription from Pol II promoter (signal transduction)	-3.7
RNC1	tRNA modification- Trna methyltransferase activity	-3.7
MSS51	Mrna processing (protein bioynthesis)	-3.7
HST1	Histidyl-tRNA aminoacylation- Histidine-Trna ligase activity	-3.7
SRB2	Transcription from Pol II promoter- RNA polymerase II transcription mediator activity	-3.7
RRP46	35S primary transcript processing-3'-5' exoribonuclease activity	-3.6
POP7	Ribonuclease P activity (tRNA processing)- Ribonuclease MRP activity (ribonuclease P activity)	-3.6
RPA12	Transcription from Pol I promoter- DNA-directed RNA polymerase activity	-3.6
RPC40	Transcription from Pol I & II promoter- DNA-directed RNA polymerase activity	-3.6
NAB3	Regulation of transcription from Pol II promoter- Poly (A) binding activity	-3.6
RRS1	rRNA processing (ribosome biogenesis)	-3.6
UTP11	Processing of 20S pre-Rrna- snoRNA binding activity	-3.5
DIM1	rRNA modification (35S primary transcript Processing)- rRNA (adenine-N6,N6-)- dimethyltransferase activity	-3.5
GCD6	Translational initiation- Translation initiation factor activity	-3.5
HCA4	35S primary transcript processing -ATP dependent RNA helicase activity	-3.5
KIN28	Negative regulation of transcription from Pol II promoter- Cyclin-dependent protein kinase activity (general RNA polymerase II transcription factor activity)	-3.5
SUP35	Translational termination- Translation release factor activity	-3.5
UFD1	mRNA processing	-3.4

SRB7	Transcription from Pol II promoter- RNA polymerase II transcription mediator activity	-3.4
GAL80	Transcription co-repressor activity- Galactose metabolism (regulation of transcription, DNA-dependent	-3.4
SMD3	mRNA splicing- mRNA binding activity (pre-mRNA splicing factor activity)	-3.4
HST1	Transcriptional gene silencing- NAD-dependent histone deacetylase activity (NAD-independent histone deacetylase activity)	-3.4
YJR014W	RNA binding activity	-3.4
CBC2	mRNA splicing- Pre-mRNA splicing factor activity	-3.4
PLP2	Positive regulation of transcription from Pol II promoter by pheromones- GTPase inhibitor activity	-3.4
NAF1	snoRNA metabolism (transport)- RNA binding activity (transporter activity)	-3.4
FUN12	Translational initiation- GTPase activity (translation initiation factor Activity)	-3.4
RPF1	Processing of 27S pre-Rrna (ribosomal large subunit assembly and maintenance)- rRNA primary transcript binding activity	-3.4
GCD10	tRNA methylation (translation initiation)- tRNA methyltransferase activity	-3.3
RPC34	Transcription from Pol III promoter- DNA-directed RNA polymerase activity	-3.3
HTZ1	Regulation of transcription from Pol II promoter- Chromatin binding activity	-3.3
YNL247W	Cysteine metabolism (cysteinyl-Trna aminoacylation)- Cysteine-tRNA ligase activity	-3.3
DBP10	35S primary transcript processing (ribosomal large subunit assembly and maintenance)- ATP dependent RNA helicase activity	-3.3
PRP43	Lariat formation, 5'-splice site cleavage- ATP dependent RNA helicase activity (pre-mRNA splicing factor) activity	-3.3
RRP1	rRNA processing	-3.3
PRP42	mRNA splicing- RNA binding activity	-3.2
RVB1	Regulation of transcription from Pol II promoter- ATPase activity	-3.2
PHD1	Specific RNA polymerase II transcription factor activity	-3.2
GAT3	Transcription -Transcription factor activity	-3.2
RPC37	Transcription from Pol III promoter- DNA-directed RNA polymerase activity	-3.2
EMG1	Processing of 20S pre-Rrna (ribosomal small subunit biogenesis)	-3.2
POP3	rRNA and tRNA processing- Ribonuclease MRP activity (ribonuclease P activity)	-3.2
MAK5	rRNA processing (ribosomal large subunit assembly and maintenance- ATP dependent RNA helicase activity	-3.2
PAN2	mRNA processing (postreplication repair)- Poly (A)-specific ribonuclease activity	-3.2
CBS1	Translation factor activity, nucleic acid binding	-3.2
REX2	RNA processing-3'-5' exonuclease activity	-3.1
MST1	Threonyl-tRNA aminoacylation- Threonine-tRNA ligase activity	-3.1
ALPHA2	Regulation of transcription from Pol II promoter- Transcription co-repressor activity	-3.1
RIM4	RNA binding activity- Meiotic recombination (and meiosis)	-3.0
GBP2	Poly (A)+ mRNA-nucleus export (telomere maintenance)- RNA binding activity, telomeric DNA binding activity	-3.0
RRN6	RNA polymerase I transcription factor activity- Transcription from Pol I promoter	-3.0
DBP8	ATP dependent RNA helicase activity-35S primary transcript processing	-3.0
RRP43	35S primary transcript processing-3'-5' exoribonuclease activity	-3.0
NCL1	tRNA methylation- tRNA (cytosine-5-)-methyltransferase activity	-3.0
MOT2	Poly (A) tail shortening (regulation of transcription from Pol II)- 3'-5' exoribonuclease activity (transcriptional repressor activity)	-3.0
DIS3	35S primary transcript processing (mRNA catabolism) 3'-5' exoribonuclease activity	-3.1
HMS2	Pseudohyphal growth- Transcription factor activity	-3.1
ROX1	Negative regulation of transcription from Pol II promoter- specific transcriptional repressor activity	-3.1
TIF4631	Translational initiation- Translation initiation factor activity	-3.1
SPB1	rRNA processing- RNA methyltransferase activity	-3.1
NSR1	valyl-tRNA aminoacylation- Valine-tRNA ligase activity- RNA binding activity (single-stranded DNA binding activity)	-3.1
VAS1	rRNA processing (ribosomal small subunit assembly and maintenance)	-3.1
REF2	mRNA processing- Cleavage /polyadenylation specificity factor activity	-3.1
SOH1	Transcription from Pol II promoter and DNA repair	-3.1
BUR6	Negative regulation of transcription from Pol II promoter- Transcription co-repressor activity	-3.1
YGL151W	Regulation of transcription from Pol II promoter	-3.1

CAM1	Regulation of translational elongation- Translation elongation factor activity	-3.0
GAT2	Transcription- Transcription factor activity	-3.0
LOS1	tRNA-nucleus export (tRNA splicing)- tRNA binding activity (RAN protein binding activity)	-3.0
POP1	tRNA and rRNA processing- Ribonuclease MRP activity (ribonuclease P activity)	-3.0
YNR048W	Transcription regulator activity	-3.0
NOT5	Poly (A) tail shortening-3'-5' exoribonuclease activity	-3.0
PRT1	Translational initiation- Translation initiation factor activity	-3.0
MED7	Transcription from Pol II promoter- RNA polymerase II transcription mediator activity	-3.0
RRP5	Processing of 20S pre-rRNA (Rrna processing)- RNA binding activity (snoRNA binding activity)	-3.0
POP2	Regulation of transcription from Pol II promoter (poly (A) tail shortening)- 3'-5' exoribonuclease activity	-3.0
CDC1	DNA recombinantion and bud growth	-3.0
RSC1	Positive regulation of transcription from Pol II promoter (high affinity ion iron transport)- Transcription factor activity	-3.0
Cytoskeleton organization and maintenance		
SFK1	Actin cytoskeleton organization and biogenesis	-13.8
#SIM1	Microtubule cytoskeleton organization and biogenesis	-10.7
TUB3	Mitotic and homologous chromosome segregation- Structural constituent of cytoskeleton	-5.6
TUB1	Homologous and mitotic chromosome segregation- Structural constituent of cytoskeleton	-5.5
CTS1	Cytokinesis, completion of separation- Chitinase activity	-5.5
RDI1	Actin filament organization	-5.2
ACT1	Structural constituent of cytoskeleton	-5.1
SHS1	Cytokinesis- Structural constituent of cytoskeleton	-4.8
TUB4	Microtubule nucleation- Structural constituent of cytoskeleton	-4.3
GIM5	Tubulin folding- Tubulin binding activity	-4.2
CHS2	Cytokinesis- Chitin synthase activity	-3.8
ENT1	Actin cortical patch assembly (actin filament organization- Cytoskeletal adaptor activity	-3.7
CCT5	Cytoskeleton organization and biogenesis (protein folding)- Chaperone activity	-3.6
ABP140	Actin cytoskeleton organization and biogenesis- Actin cross-linking activity	-3.6
NDC1	Microtubule nucleation (protein-nucleus import) (RNA-nucleus export)- Structural constituent of cytoskeleton	-3.3
YKL104C	Cell wall chitin biosynthesis	-3.2
ENT3	Actin filament organization (Golgi to endosome transport- Cytoskeletal adaptor activity	-3.2
SPC42	Microtubule nucleation- Structural constituent of cytoskeleton	-3.2
Dec1	Cytoskeleton organization and biogenesis- Cytoskeletal regulator activity	-3.2
DSK2	Spindle pole body duplication (sensu Saccharomyces)- Protein degradation tagging activity	-3.1
NUF2	Microtubule nucleation (chromosome segregation)- Structural constituent of cytoskeleton	-3.1
MAD1	Mitotic spindle checkpoint	-3.1
TUB2	Structural constituent of cytoskeleton- Homologous chromosome segregation (mitotic chromosome segregation)	-3.1**
APP1	Actin cytoskeleton organization and biogenesis	-3.0
Cell wall & membrane proteins		
CIS3	Cell wall organization and biogenesis- Structural constituent of cell wall	-20.1
#CWP2	Structural constituent of cell wall	-19.7
PIR1	Cell wall organization and biogenesis	-10.4
BGL2	Cell wall organization and biogenesis- Glucan 1,3-beta-glucosidas activity	-7.0
CWH43	Cell wall organization and biogenesis (signal transduction)	-7.7
KRE9	Cell wall organization and biogenesis	-6.5
CSR1	Cell wall organization and biogenesis- Phosphatidylinositol transporter activity	-5.9
SCW4	Conjugation with cellular fusion- Glucosidase activity	-5.8
SBE22	Cell wall organization and biogenesis-	-4.6
DSE2	Cell wall organization and biogenesis- Glucan 1,3-beta-glucosidase activity	-4.5
KRE1	Cell wall organization and biogenesis- Structural constituent of cell wall	-4.1
MTL1	Cell wall organization and biogenesis	-4.1
RMD7	Cell wall organization and biogenesis	-4.1

ECM14	Cell wall organization and biogenesis	-3.9
ECM3	Cell wall organization and biogenesis- ATPase activity	-3.8
MID2	Cell wall organization and biogenesis	-3.7
ECM25	Cell wall organization and biogenesis	-3.5
YOL155C	Cell wall organization and biogenesis- Glucosidase activity	-3.3
MYO3	Cell wall organization and biogenesis (endocytosis&exocytosis)-	-3.1
CWP1	Cell wall organization and biogenesis- Structural constituent of cell wall	-3.0
Lipid metabolism		
FEN1	Sphingolipid biosynthesis	-19.9
OLE1	Fatty acid desaturation- Stearoyl-CoA desaturase activity	-16.1
ERG13	Ergosterol biosynthesis- Hydroxymethylglutaryl-CoA synthase activity	-14.4
ERG27	Ergosterol biosynthesis-3-keto sterol reductase activity	-10.5
ERG11	Ergosterol biosynthesis- Sterol 14-demethylase activity	-9.3
YBR042C	Phospholipid biosynthesis- Acyltransferase activity	-8.5
ERG25	Ergosterol biosynthesis- C-4 methyl sterol oxidase activity	-8.0
ERG10	Ergo sterol biosynthesis- Acetyl-CoA C-acetyltransferase activity	-6.5
ERG9	Ergosterol biosynthesis- Farnesyl-diphosphate farnesyltransferase activity	-6.4
ERG1	Ergosterol biosynthesis- Squalene monooxygenase activity	-5.8
ACP1	Fatty acid biosynthesis- Acyl carrier activity	-5.6
PLB3	Phosphoinositide metabolism- Lysophospholipase activity	-5.4
ERG26	Ergosterol biosynthesis- C-3 sterol dehydrogenase (C-4 sterol decarboxylase) activity	-5.4
MVD1	Ergosterol biosynthesis (isoprenoid biosynthesis)- Diphosphomevalonate decarboxylase activity	-4.7
ERG6	Ergosterol biosynthesis- Delta (24)-sterol C-methyltransferase activity	-4.7
SUR2	Sphingolipid biosynthesis (sphingolipid metabolism)- Sphingosine hydroxylase activity	-4.7
ERG8	Ergosterol biosynthesis- Phosphomevalonate kinase activity	-4.6
SUR4	Fatty acid biosynthesis	-4.4
ELO1	Fatty acid elongation, unsaturated fatty acid	-4.3
ERG5	Ergosterol biosynthesis- C-22 sterol desaturase activity	-3.9
ECI1	Fatty acid beta-oxidation- Unknown dodecenoyl-CoA Delta-isomerase activity	-3.4
EHT1	Lipid metabolism	-3.4
ERG2	Ergosterol biosynthesis- C-8 sterol isomerase activity	-3.1
ERG7	Ergosterol biosynthesis- Lanosterol synthase activity	-3.0
Unknown function		
YOL109W		-42.1
YDL228C		-38.2
YGL102C		-31.7
YLL044W		-30.1
YJR115W		-29.1
UTR2		-28.3
YLR339C		-28.2
YBL109W		-27.5
YNL338W		-26.9
BUD28		-26.8
YMR244C-A		-26.2
BUD19		-23.9
YLR040C		-23.0
YDR544C		-19.8
YOR277C		-19.2
YDR544C		-18.9
YDR417C		-18.1
TOS1		-16.1
PRM7		-16.1
YPR044C		-16.1
YEL001C		-15.9
YARF1-1		-15.5
YLR076C		-15.1
YPL142C		-15.0
YLR391W		-14.9
YOR309C		-14.5
YNR046W		-14.4
YBR077C		-14.1

YKL202W	-13.5
YDR134C	-13.4
PKR1	-13.0
YDR442W	-12.8
YGL231C	-12.7
YGR106C	-12.6
YEL033W	-12.6
SNT2	-12.1
MKH1	-12.1
RIM	-11.8
YOR248W	-11.7
ECM33	-11.6
YNR021W	-11.3
WC23	-11.0
YOR271C	-11.0
YDR119W	-10.2
YCR025C	-10.1
NUG1	-9.8
ARX1	-9.8
YLR041W	-9.4
YER156C	-9.3
YOL092W	-9.2
YPL197C	-9.2
YGL072C	-9.0
YMR002W	-8.9
YKL056C	-8.7
YJR023C	-8.6
GAS5	-8.5
YML125C	-8.4
YRF1-6	-8.4
YMR321C	-8.3
FIT2	-8.1
YBR238C	-8.0
PRY2	-7.9
YLR194C	-7.7
TVP38	-7.7
ARR4	-7.7
FSH1	-7.6
YDR209C	-7.5
RTN1	-7.5
YKL030W	-7.5
YPR118W	-7.4
YJR114W	-7.4
RBS1	-7.3
YKR075C	-7.3
YIL110W	-7.2
YBR089W	-7.1
YDL050C	-7.0
STP4	-7.0
YHR045W	-6.9
YMR298W	-6.9
YOL124C	-6.9
YOL111C	-6.8
YDR157W	-6.7
YDR133C	-6.6
BCP1	-6.6
YPL272C	-6.6
PIL1	-6.5
NPC2	-6.5
YLR064W	-6.5
HSP32	-6.5
SVP26	-6.4
YDR539W	-6.3
YAR075W	-6.3
YHR115C	-6.2
YBL083C	-6.2
YOR305W	-6.2

YOR238W	-6.0
YGL068W	-6.0
YMR221C	-5.9
YOR286W	-5.9
YMR130W	-5.9
LSB1	-5.8
YIL096C	-5.8
YDR367W	-5.7
YHR100C	-5.7
YGR160W	-5.7
UTP30	-5.7
YFR044C	-5.6
YNL303W	-5.6
ILM1	-5.6
YMR252C	-5.6
YOL073C	-5.6
YER049W	-5.6
YBL077W	-5.5
SAM35	-5.5
YPL158C	-5.5
TIR3	-5.5
YGR151C	-5.4
HAS1	-5.3
YGR283C	-5.3
YDR071C	-5.3
YJL122W	-5.2
YPL014W	-5.2
YGR093W	-5.1
YBR187W	-5.1
USE1	-5.1
YHR151C	-5.1
HUR1	-5.0
YDL041W	-5.0
ERD1	-5.0
YER113C	-5.0
YIL105C	-5.0
YOR051C	-5.0
YJL193W	-5.0
YNL114C	-4.9
PWP1	-4.9
YLR414C	-4.9
YGL080W	-4.9
YGL139W	-4.9
SNA4	-4.9
YIL127C	-4.9
YGR137W	-4.9
YHR217C	-4.8
PAN5	-4.8
YOL022C	-4.8
YOR102W	-4.8
YFR039C	-4.7
YDR198C	-4.7
APT2	-4.6
YDR154C	-4.6
YDR411C	-4.6
YOR164C	-4.6
YLR236C	-4.5
YKL207W	-4.5
YNR061C	-4.5
YJR024C	-4.5
YHR214W-A	-4.5
UTR4	-4.5
YGL088W	-4.5
YDL237W	-4.4
YBR071W	-4.4
YDR094W	-4.4
RNH202	-4.4

YLR065C	-4.4
YKL177W	-4.4
TOS7	-4.4
YNL010W	-4.3
HUA2	-4.3
YLR198C	-4.3
YJR124C	-4.3
YNL010W	-4.3
YNL122C	-4.3
YHL039W	-4.3
YCR041W	-4.3
YBR025C	-4.3
YFR043C	-4.3
YDR066C	-4.3
ENP2	-4.2
TVP23	-4.2
YCL045C	-4.2
YJL097W	-4.2
YOR283W	-4.2
TOS6	-4.2
YKL206C	-4.2
YOR121C	-4.2
YLR230W	-4.2
PTM1	-4.2
YKR065C	-4.1
YNL043C	-4.1
YPL098C	-4.1
YJL123C	-4.1
YOR118W	-4.1
YER071C	-4.1
YDR365C	-4.1
GGA2	-4.1
YDR339C	-4.1
YLF2	-4.1
YDL089W	-4.0
ARP1	-4.0
YDR336W	-4.0
YCR051W	-4.0
TRM10	-4.0
YLR412W	-4.0
YLR202C	-4.0
YNR009W	-4.0
YPL264C	-4.0
YOR015W	-4.0
YOR331C	-4.0
YOR169C	-4.0
YLR073C	-4.0
YLR101C	-4.0
YLR021W	-4.0
FSH3	-4.0
YKR087C	-3.9
YPL066W	-3.9
YOL070C	-3.9
CTH1	-3.9
YGR266W	-3.9
YCR072C	-3.9
YDR132C	-3.9
YHR095W	-3.9
YBR287W	-3.9
YFR041C	-3.9
YIL169C	-3.9
YGR024C	-3.9
YDL037C	-3.8
YFR042W	-3.8
YBL081W	-3.8
YDL012C	-3.8
YHR121W	-3.8

VID22	-3.8
MIA1	-3.8
IPI3	-3.8
YPL073C	-3.8
YMR184W	-3.8
YOL107W	-3.7
YLR042C	-3.7
YKL027W	-3.7
YLR413W	-3.7
YKR089C	-3.7
YHL041W	-3.7
YBR219C	-3.7
YDL016C	-3.7
YHR036W	-3.7
YDR126W	-3.7
TVP15	-3.7
LIN1	-3.7
YHR087W	-3.7
YPI1	-3.7
YHR085W	-3.7
YBL028C	-3.6
SGI1	-3.6
YCL069W	-3.6
YDL121C	-3.6
CWC15	-3.6
VID21	-3.6
YDR370C	-3.6
YDL063C	-3.6
YCL065W	-3.6
YGR001C	-3.6
YCR016W	-3.6
YBR267W	-3.6
YGR026W	-3.6
SET6	-3.6
YNL305C	-3.6
YKR074W	-3.6
AKR2	-3.6
YPL041C	-3.6
YKL225W	-3.6
COS6	-3.6
YOR170W	-3.6
YLL025W	-3.6
LTV1	-3.5
YKL070W	-3.5
DRE2	-3.5
YOL079W	-3.5
YOL125W	-3.5
YLR050C	-3.5
YFL006W	-3.5
YSC83	-3.5
YHL026C	-3.5
YHR133C	-3.5
YCR026C	-3.5
YMR155W	-3.5
YML053C	-3.5
YAF9	-3.5
YOR240W	-3.5
YLR404W	-3.5
YOR252W	-3.4
YOR091W	-3.4
YLR243W	-3.4
YML119W	-3.4
YMR237W	-3.4
YJR054W	-3.4
YOL003C	-3.4
PRM10	-3.4
SGT2	-3.4

YKR033C	-3.4
IES3	-3.4
YMR099C	-3.4
YML096W	-3.4
YNL313C	-3.4
YOL024W	-3.4
YJL151C	-3.4
YHR149C	-3.4
BDF2	-3.4
YGL193C	-3.4
YHR032W	-3.4
YDL180W	-3.4
YGR163W	-3.3
YGR211W	-3.3
TOM71	-3.3
YHR202W	-3.3
YHL017W	-3.3
YGR111W	-3.3
YBR151W	-3.3
YDR056C	-3.3
YFL061W	-3.3
RNQ1	-3.3
PST1	-3.3
CHR1	-3.3
YEL010W	-3.3
IPI2	-3.3
YDL211C	-3.3
YHR192W	-3.3
YIL103W	-3.3
YDR210W	-3.3
TOS9	-3.3
YHR009C	-3.3
RTS2	-3.3
YOR292C	-3.3
YMR074C	-3.3
YNL184C	-3.3
YJR070C	-3.3
YMR299C	-3.3
MUK1	-3.3
YPL009C	-3.3
YNL265C	-3.3
YNL337W	-3.3
YPL245W	-3.3
YML020W	-3.3
YNL226W	-3.2
YOR062C	-3.2
YPL052W	-3.2
YLR317W	-3.2
PSY1	-3.2
YPL068C	-3.2
YOR072W	-3.2
YPL039W	-3.2
YOR200W	-3.2
YPR050C	-3.2
YPL279C	-3.2
YNL146W	-3.2
DCP3	-3.2
YJL022W	-3.2
YDR415C	-3.2
YDR371W	-3.2
YHL044W	-3.2
QRD2	-3.2
YDR458C	-3.2
YCR023C	-3.2
YGR035C	-3.2
YJL027C	-3.2
YBL009W	-3.2

YCR001W	-3.2
YDR307W	-3.2
YMR148W	-3.1
YNL047C	-3.1
YMR185W	-3.1
TIR4	-3.1
YMR181C	-3.1
YMR102C	-3.1
YJR015W	-3.1
YOL057W	-3.1
YLL012W	-3.1
YNR029C	-3.1
YPL199C	-3.1
YKR035C	-3.1
YOR131C	-3.1
YPL207W	-3.1
YOR315W	-3.1
YNL149C	-3.1
FRE7	-3.1
YOR152C	-3.1
YOL137W	-3.1
YPR013C	-3.1
YPL047W	-3.1
YLR140W	-3.1
YAR068W	-3.1
YGR294W	-3.1
HGH1	-3.1
YBR094W	-3.1
YGL114W	-3.1
YHR040W	-3.1
YER186C	-3.1
YDL027C	-3.1
YGR064W	-3.1
YEL059W	-3.1
YAL053W	-3.1
YDL221W	-3.1
AQR2	-3.1
FYV5	-3.1
YBR113W	-3.1
YBR141C	-3.1
AME1	-3.1
YDR319C	-3.1
RMD9	-3.1
MTM1	-3.0
YJR003C	-3.0
YNL056W	-3.0
YML079W	-3.0
YJR116W	-3.0
SCD6	-3.0
YPL144W	-3.0
YKR045C	-3.0
YNL116W	-3.0
YLR053C	-3.0
YOL007C	-3.0
YOL099C	-3.0
HSD1	-3.0
YNL129W	-3.0
YOR268C	-3.0
YLR251W	-3.0
YKL069W	-3.0
YNR071C	-3.0
YLR132C	-3.0
YOR073W	-3.0
YKL037W	-3.0
YOL029C	-3.0
YLR187W	-3.0
YOL085C	-3.0

YKL077W		-3.0
YGL152C		-3.0
YDR333C		-3.0
YGL177W		-3.0
YDR527W		-3.0
SHE1		-3.0
YCL044C		-3.0
YGL004C		-3.0
YCR082W		-3.0
YDL023C		-3.0
YHR097C		-3.0
YGR081C		-3.0
SOL4		-3.0
YIL039W		-3.0
GIR1		-3.0
YDR117C		-3.0
YBR004C		-3.0
YGL020C		-3.0
Miscellaneous		
SAG1	Agglutination during conjugation with cellular fusion- Cell adhesion receptor activity	-28.0
IMD4	IMP dehydrogenase activity	-19.1
AGA1	Agglutination during conjugation with cellular fusion- Cell adhesion receptor activity	-17.0
CCW12	Agglutination during conjugation with cellular fusion	-16.0
YGL039W	Dihydrokaempferol 4-reductase activity	-10.4
AUR1	Sphingolipid metabolism- Inositol phosphoceramide synthase activity	-10.0
CPR5	peptidyl-prolyl cis-trans isomerase activity	-9.9
SCS2	Myo-inositol metabolism-	-8.7
HOP2	Synapsis	-8.5
PMI40	GDP-mannose biosynthesis- Mannose-6-phosphate isomerase activity	-8.1
PIE3	Dephosphorylation (cell wall organization and biogenesis)- inositol-1,4,5-trisphosphate 5 phosphatase activity	-8.0
MNN9	N-linked glycosylation- mannosyltransferase activity	-8.0
SIZ1	Sumoylation- SUMO ligase activity	-7.8
SEC53	Protein-ER targeting- Phosphomannomutase activity	-7.8
STT3	N-linked glycosylation- Dolichyl-diphospho-oligosaccharide protein glycosyltransferase activity	-7.8
KTR1	O-linked glycosylation (N-glycan processing)- Alpha-1, 2-mannosyltransferase activity	-7.8
PMT4	O-linked glycosylation- Dolichyl-phosphate-mannose-protein mannosyltransferase activity	-7.4
OST1	N-linked glycosylation (N-linked glycosylation via asparagines)- Dolichyl-diphospho-oligosaccharide-protein glycosyltransferase activity	-7.0
HEM13	Heme biosynthesis- Coproporphyrinogen oxidase activity	-7.0
ALG8	Oligosaccharide-lipid intermediate assembly- Oligosaccharyl transferase activity	-6.9
RIB4	Vitamin B2 biosynthesis-6, 7 –dimethyl-8-ribityllumazine synthase activity	-6.7
PET9	ATP/ADP exchange- ATP/ADP antiporter activity	-6.4
TSC13	Very-long-chain fatty acid metabolism- Oxidoreductase activity	-6.4
SNF4	Peroxisome organization and biogenesis (peroxisome organization and biogenesis)- Protein kinase activator activity	-6.3
GP18	Attachment of GPI anchor to protein- GPI-anchor transamidase activity	-6.3
QCR6	Aerobic respiration (oxidative phosphorylation, ubiquinone to cytochrome c)- Ubiquinol-cytochrome c reductase activity	-6.3
NPI46	Peptidyl-prolyl cis-trans isomerase activity	-6.3
UTH1	Mitochondrion organization and biogenesis	-6.2
KRE2	N-glycan processing (O-linked glycosylation) (cell wall mannoprotein Biosynthesis)- Alpha-1, 2-mannosyltransferase activity	-6.1
ADE17	'de novo' IMP biosynthesis- IMP cyclohydrolase activity	-5.8
IPP1	Phosphate metabolism- Inorganic diphosphatase activity	-5.8
YOR084W	Peroxisome organization and biogenesis- Lipase activity	-5.7
DPH5	Peptidyl-diphthamide biosynthesis from peptidyl-histidine- Diphthine synthase activity	-5.6
KTR3	N-linked glycosylation (O-linked glycosylation)- Mannosyltransferase activity	-5.6
IPT1	Mannosyl diphosphorylinositol ceramide metabolism- Transferase activity,	-5.5

	transferring phosphorus-containing groups	
GPI11	GPI anchor biosynthesis- Phosphoethanolamine N-methyltransferase activity	-5.3
NUP49	Structural molecule activity- See SGD (nuclear pore complex subunit)	-5.2
PMT2	O-linked glycosylation- Dolichyl-phosphate-mannose-protein mannosyltransferase activity	-5.2
RHK1	Dolichol-linked oligosaccharide biosynthesis (protein amino acid glycosylation)- alpha-1, 3-mannosyltransferase activity	-5.0
MNS1	N-linked glycosylation- Mannosyl-oligosaccharide 1,2-alpha-mannosidase activity	-5.0
CPR8	Peptidyl-prolyl cis-trans isomerase activity	-4.9
SCC3	Peptidyl-prolyl cis-trans isomeras activity- Protein binding activity	-4.8
VPS75	Protein-vacuolar targeting	-4.8
GSH2	Glutathione biosynthesis- Glutathione synthase activity	-4.8
THI80	Thiamine biosynthesis- Thiamin pyrophosphokinase activity	-4.7
STE14	Peptide pheromone maturation- Protein-S-isoprenylcysteine O-methyltransferase activity	-4.7
ERG8	Ergosterol biosynthesis- Phosphomevalonate kinase activity	-4.6
YNL213C	Mitochondrion organization and biogenesis	-4.6
PH036	Membrane protein involved in zinc metabolism	-4.6
KES1	Steroid biosynthesis- Oxysterol binding activity	-4.5
TRF5	Sister chromatide cohesion- DNA-directed DNA polymerase activity	-4.5
YIM1	Mitochondrial processing- Peptidase activity	-4.5
OST2	N-linked glycosylation- Dolichyl-diphospho-oligosaccharide protein glycosyltransferase activity	-4.5
SSF1	Conjugation with cellular fusion (ribosomal large subunit assembly and maintenance)- rRNA binding activity	-4.5
PEX7/PAS7	Peroxisome organization and biogenesis (protein-peroxisome targeting)- Peroxisome targeting signal receptor activity (peroxisome targeting signal-2 receptor activity)	-4.4
YGR250C	RNA binding	-4.4
YGL142C	GPI anchor biosynthesis	-4.4
FPR1	Peptidyl-prolyl cis-trans isomerase activity	-4.3
DPP1	Phospholipid metabolism- Diacylglycerol pyrophosphate phosphatase activity	-4.3
PHB1	Proteolysis and peptidolysis (cell aging)	-4.3
DFR1	Folic acid and derivative metabolism- Dihydrofolate reductase activity	-4.2
ALG6	Dolichol-linked oligosaccharide biosynthesis- Transferase activity, transferring hexosyl groups	-4.2
PMT1	O-linked glycosylation- O-linked glycosylation	-4.1
OCH1	N-linked glycoprotein maturation- Alpha-1,6-mannosyltransferase activity (transferase activity, transferring glycosyl groups)	-4.1
SEC1	Exocytosis (nonselective vesicle docking and fusion)- SNARE binding activity	-4.1
SHM1	One-carbon compound metabolism- Glycine hydroxymethyltransferase activity	-4.1
SOD1	Copper, Zinc ion homeostasis (superoxide metabolism)- Copper, zinc superoxide dismutase activity	-4.1
ORD1	Pantothenate biosynthesis- Ornithine decarboxylase activity	-4.1
PMP27	Peroxisome organization and biogenesis	-4.0
SCT1	Phospholipid biosynthesis- Glycerol-3-phosphate O-acyltransferase activity	-4.0
GUA1	GMP metabolism- GMP synthase (glutamine hydrolyzing) activity	-3.9
GPI13	GPI anchor biosynthesis- Phosphoethanolamine N-methyltransferase activity	-3.9
ERP4	Secretory pathway	-3.9
MAP1	Proteolysis and peptidolysis- Methionyl aminopeptidase activity	-3.9
VPS1	Peroxisome organization and biogenesis- GTPase activity	-3.9
PMT6	O-linked glycosylation- Dolichyl-phosphate-mannose-protein mannosyltransferase activity	-3.9
FUS1	Conjugation with cellular fusion	-3.9
PRM5	Conjugation with cellular fusion	-3.8
EXG2	Glucan 1,3-beta-glucosidase activity	-3.8
YOR251C	Thiosulfate sulfurtransferase activity	-3.8
RMA1	Folylpolyglutamate synthase activity	-3.7
BTS1	Terpenoid biosynthesis- Farnesyltranstransferase activity	-3.7
SCW10	Conjugation with cellular fusion- Glucosidase activity	-3.7
MCK1	Meiosis (mitotic chromosome Segregation)- Glycogen synthase kinase 3 activity (protein threonine/tyrosine kinase activity)	-3.7
APT1	Hydrolase activity, acting on ester bonds	-3.7

FMN1	FMN biosynthesis- FMN adenylyltransferase activity (riboflavin kinase activity)	-3.7
QRI1	UDP-N-acetylglucosamine biosynthesis- UDP-N-acetylglucosamine pyrophosphorylase activity	-3.7
CDC20	Enzyme activator activity- Cyclin catabolism (mitotic chromosome segregation)	-3.7
SCS3	Phospholipid metabolism	-3.6
DEP1	Phospholipid metabolism	-3.6
YER078C	Xaa-Pro aminopeptidase activity	-3.5
VPS70	Protein-vacuolar targeting	-3.5
COX7	Aerobic respiration- Cytochrome c oxidase activity	-3.5
BCS1	Aerobic respiration- ATPase activity	-3.5
EPT1	Phosphatidylethanolamine biosynthesis- Ethanolaminephosphotransferase activity	-3.5
ARH1	Heme a biosynthesis (iron ion homeostasis)- NADPH-adrenodoxin reductase activity	-3.5
SMI1	Beta-1, 3 glucan biosynthesis	-3.4
TRR1	Regulation of redox homeostasis- Thioredoxin reductase (NADPH) activity	-3.4
OPT1	Sulfur metabolism- Oligopeptide transporter activity	-3.4
PRM10	Conjugation with cellular fusion	-3.4
FKS1	Beta-1, 3 glucan biosynthesis-1,3-beta-glucan synthase activity	-3.4
AYT1	Secondary metabolism- Trichothecene 3-O-acetyltransferase activity	-3.4
CYC2	Mitochondrial intermembrane space protein import	-3.4
DIA4	Aerobic respiration 9 invasive growth)- Serine-tRNA ligase activity	-3.4
FLO5	Flocculation- Cell adhesion molecule activity	-3.3
VTC1	Vacuole fusion (non-autophagic)	-3.3
CPR2	peptidyl-prolyl cis-trans isomerase activity	-3.3
AUT1	Autophagy (protein-vacuolar targeting)	-3.3
HEM15	Heme biosynthesis- Ferrochelatase activity	-3.3
VPS68	Protein-vacuolar targeting	-3.3
PNT1	Inner mitochondria membrane organization and biogenesis	-3.2
CDC43	Calcium ion homeostasis (small GTPase mediated signal transduction)- Protein geranylgeranyltransferase activity (signal transducer activity)	-3.2
FAD1	FAD biosynthesis- FMN adenylyltransferase activity	-3.2
ALG2	Oligosaccharide-lipid intermediate assembly- Glycolipid mannosyltransferase activity	-3.2
LSG1	Conjugation with cellular fusion	-3.2
UGA3	Nitrogen utilization (regulatio of transcription from Pol II promoter)- Specific RNA polymerase II transcription factor activity	-3.2
CCZ1	Autophagic vacuole fusion (protein-vacuolar targeting)- Guanyl-nucleotide exchange factor activity	-3.1
PMT3	O-linked glycosylation- Dolichyl-phosphate-mannose-protein mannosyltransferase activity	-3.1
SPE3	Pantothenate biosynthesis (spermidine biosynthesis)- Spermidine synthase activity	-3.1
PEX12	Peroxisome organization and biogenesis	-3.1
YLL057C	Sulfur metabolism- Sulfonate dioxygenase activity	-3.1
AHP1	Regulation of redox homeostasis- Thioredoxin peroxidase activity	-3.1
YLR294C	Cellular respiration	-3.1
YDR140W	S-adenosylmethionine-dependent methyltransferase activity	-3.1
CSG2	Calcium ion homeostasis	-3.1
ALG7	N-linked glycosylation- UDP-N-acetylglucosamine-dolichyl-phosphate N-acetylglucosamine-1-phosphate transferase activity	-3.1
YLL057C	Sulfur metabolism- Sulfonate dioxygenase activity	-3.1
EBS1	Telomerase-dependent telomere maintenance	-3.0
ECM39	Mannosyltransferase- Alpha-1, 6-mannosyltransferase activity	-3.0
HRK1	Cell ion homeostasis- Protein kinase activity	-3.0
FPR4	Peptidyl-prolyl cis-trans isomerase activity	-3.0
PRS5	'de novo' IMP biosynthesis (ribose-phosphate pyrophosphokinase)- Ribose-phosphate pyrophosphokinase activity	-3.0
YDR115W	Aerobic respiration- Structural constituent of ribosome	-3.0
HEM2	Heme biosynthesis- Porphobilinogen synthase activity	-3.0
KTR4	N-linked glycosylation- Mannosyltransferase	-3.0

Table 3.2a: Macroarray data: Genes that were MHE following five hour exposure to 7% ethanol relative to control.

* Genes also found to be MHE in macroarray experiments for the same conditions at one-hour time point.

ORF/Gene name	Description of gene product	Fold increase	Putative Transcription factors
Stress response			
*YBR072W/HSP26	Small HSP of unknown function	20.4	Msn2/4p, Hsf1p
YJL159W / HSP150	Heat shock protein, secretory glycoprotein	6.8	Msn2/4p, Yap1/2p
YER103W/SSA4	HSP of the HSP70 family, cytosolic	4.2	Msn2/4p, Hsf1p, Yap1/2p
YNL160W / YGP1	Glycoprotein synthesized in response to nutrient limitation	4.1	Msn2/4p, Hsf1p, Yap1/2p
YAL005C/SSA1	Heat shock protein of HSP70 family	4.0	Msn2/4p, Hsf1p, Yap1/2p
YDR533C/ HSP31	Heat shock protein	3.5	Msn2/4p, Hsf1p, Yap1/2p
YLL026W / HSP104	Heat shock protein 104	3.1	Msn2/4p, Hsf1p, Yap1/2p
Energy utilization			
YLR134W / PDC5	Pyruvate decarboxylase	5.9	Msn2/4p, Hsf1p, Yap1/2p
YCR012W/PGK1	Phosphoglycerate kinase activity - gluconeogenesis	4.2	Msn2/4p, Hsf1p, Yap1/2p
YFR053C/HXK1	Hexokinase activity - fructose metabolism	4.2	Msn2/4p, Hsf1p
YBR020W/GAL1	Galactokinase activity - galactose metabolism	4.0	Yap1/2p
*YCL040W/GLK1	Glucokinase activity - carbohydrate metabolism	4.0	Msn2/4p
YBR019C/GAL10	Galactose metabolism	3.7	Hsf1p, Yap1/2p
YBL030C/PET9	ATP/ADP antiporter activity - ATP/ADP exchange	3.5	Msn2/4p, Hsf1p
YML100W / TSL1	Similar to TPS3 gene product, trehalose-6-phosphate synthase/phosphatase complex	3.1	Msn2/4p, Hsf1p
YLR258W / GSY2	Glycogen synthase (UDP-glucose-starch glucosyltransferase)	3.1	Msn2/4p, Hsf1p, Yap1/2p
Transport & translocation			
YDR432W/NPL3	mRNA binding - mRNA-nucleus export	7.8	Msn2/4p, Hsf1p,
YIL166C	Transporter activity	7.5	Msn2/4p, Hsf1p, Yap1/2p
YMR058W / FET3	Multicopper oxidase-transport	3.8	Msn2/4p
Lipid metabolism			
YMR008C / PLB1	Phospholipase B	6.5	Msn2/4p
Protein metabolism			
YDR019C/GCV1	Glycine dehydrogenase (decarboxylating) activity- Glycine metabolism as nitrogen source	3.2	Yap1/2p
Signal transduction proteins			
YAL056W/GPB2	Signal transducer activity - signal transduction	3.2	Msn2/4p, Hsf1p, Yap1/2p
Transcription and translation factor and process			
YMR053C / STB2	Transcriptional repressor	3.9	Msn2/4p, Hsf1p
YMR042W / ARG80	Transcription factor	3.1	Msn2/4p, Hsf1p, Yap1/2p
YJL076W / NET1	Ribosomal DNA (rDNA) binding	3.0	Msn2/4p
YOL004W / SIN3	DNA binding protein, involved in transcriptional regulation	3.0	Msn2/4p, Hsf1p
YOR290C / SNF2	Transcriptional regulator	3.0	Msn2/4p, Hsf1p
Nucleotide Metabolism			
*YBR012W-B	RNA-directed DNA polymerase activity - ribonuclease activity	9.6	Yap1/2p
YLR466W / YRF1-4	Y'-helicase protein 1	6.0	-

YPL283C / YRF1-7	Y'-helicase protein 1	5.2	-
YLR398C / SKI2	RNA helicase	5.0	-
YHL050C	Helicase activity	4.4	-
YKR048C / NAP1	Nucleosome assembly protein I	4.0	Msn2/4p, Hsf1p
YLR467W / YRF1-5	Y'-helicase protein 1	3.8	Hsf1p, Yap1/2p
YOR204W / DED1	RNA helicase	3.5	Msn2/4p, Hsf1p, Yap1/2p
YFR037C/RSC8	Chromatin modeling	3.3	Hsf1p, Yap1/2p
Cell wall & membrane proteins			
YJL079C / PRY1	Nuclear membrane	7.2	Msn2/4p, Hsf1p,
YDR077W/SED1	Structural constituent of cell wall - cell wall organization and biogenesis	6.7	Msn2/4p
YJR034W / PET191	Mitochondrial inner membrane	5.3	Msn2/4p, Hsf1p
YNL283C / WSC2	Contains novel cysteine motif, integral membrane protein (putative	3.3	-
Miscellaneous			
YDL025C	Protein kinase activity	7.8	Msn2/4p, Hsf1p, Yap1/2p
YMR287C / MSU1	3'-5' exonuclease complex component	7.2	Hsf1p, Yap1/2p
YMR038C / LYS7	Copper chaperone for superoxide dismutase Sod1p	7.2	-
YCR008W/SAT4	Protein kinase activity - G1/S transition of mitotic cell cycle	5.7	Msn2/4p, Yap1/2p
YKL103C / LAP4	Vacuolar aminopeptidase ysc1	5.0	Msn2/4p, Hsf1p, Yap1/2p
YJR036C / HUL4	Ubiquitin ligase (E3)	4.9	Msn2/4p, Yap1/2p
YHR008C/SOD2	Manganese superoxide dismutase activity - oxygen and reactive oxygen species metabolism	4.3	Msn2/4p, Hsf1p, Yap1/2p
YDL024C/DIA3	Acid phosphatase activity	3.2	Msn2/4p, Hsf1p, Yap1/2p
YCR005C/CIT2	Citrate (SI)-synthase activity - citrate metabolism	3.2	Msn2/4p
Unknown function			
*YCL042W		25.2	Msn2/4p, Yap1/2p
*YBL005W-A		11.6	Msn2/4p, Yap1/2p
YHR219W		11.5	Msn2/4p, Hsf1p, Yap1/2p
YBL113C		11.2	Msn2/4p, Hsf1p, Yap1/2p
*YCL020W		11.2	Hsf1p
YFL067W		11.0	Msn2/4p
*YBR012W-A		9.9	Yap1/2p
*YML045W		9.6	-
*YML040W		9.4	Msn2/4p, Yap1/2p
*YBL101W-A		9.3	-
*YJR026W		9.0	Yap1/2p
*YAR010C		8.9	Hsf1p
*YMR046C		8.6	Msn2/4p, Yap1/2p
*YJR028W		8.4	Msn2/4p, Yap1/2p
*YMR051C		8.2	Msn2/4p
YDL023C		7.1	Hsf1p
YLL067C		6.5	Hsf1p, Yap1/2p
YBR054W/YRO2		6.2	Msn2/4p, Yap1/2p
YAL004W		5.8	Hsf1p, Yap1/2p
YML133C		5.8	Hsf1p, Yap1/2p
YER188W		5.7	Msn2/4p
YLL066C		5.3	Hsf1p, Yap1/2p
YJL116C / NCA3		5.1	Msn2/4p, Hsf1p,
YEL077C		4.6	Yap1/2p
YER190W/YRF1-2		4.4	Msn2/4p, Yap1/2p
YLR465C / BSC3		4.2	Msn2/4p, Hsf1p, Yap1/2p
YOL106W		4.1	Hsf1p, Yap1/2p
YEL076C-A		4.3	Msn2/4p, Yap1/2p
YJL225C		3.3	Msn2/4p, Hsf1p, Yap1/2p
YNR042W		3.3	-

YLR162W	3.3	Hsf1p, Yap1/2p
YBR209W	3.2	Msn2/4p, Hsf1p
YLR279W	3.1	Msn2/4p, Hsf1p, Yap1/2p
YLR190W / MMR1	3.1	Msn2/4p, Hsf1p
YBL012C	3.0	Msn2/4p, Hsf1p
YHR145C	3.0	Yap1/2p

Table 3.2b: Macroarray data: Genes that were LHE following five hour exposure to 7% ethanol relative to control.

* Genes also found to be LE in macroarray experiments for the same conditions at one-hour time point.

ORF/Gene name	Description of gene product	Fold decrease
Ribosomal proteins		
YGL147C/RPL9A	Cytosolic large ribosomal subunit	-18.7
YER131W/RPS26B	Cytosolic small ribosomal subunit	-17.3
YKL156W / RPS27A	Cytosolic small ribosomal subunit	-17.1
YDL061C/RPS29B	Cytosolic small ribosomal subunit	-17.0
YDR450W/RPS18A	Cytosolic small ribosomal subunit	-16.2
YBR084C-A/RPL19B	Cytosolic large ribosomal subunit	-15.7
YGL031C/RPL24A	Cytosolic large ribosomal subunit	-15.5
YER056C-A/RPL34A	Cytosolic large ribosomal subunit	-15.4
YOR096W / RPS7A	Cytosolic small ribosomal subunit	-15.2
YGR214W/RPS0A	Cytosolic small ribosomal subunit	-14.6
YDR471W/RPL27B	Cytosolic large ribosomal subunit	-14.5
YOL077C / BRX1	Ribosomal large subunit assembly and maintenance	-14.2
YJL190C / RPS22A	Cytosolic small ribosomal subunit	-14.2
YLR340W / RPP0	Cytosolic small ribosomal subunit	-13.9
YHL015W / RPS20	Cytosolic small ribosomal subunit	-13.7
YOR167C / RPS28A	Cytosolic small ribosomal subunit	-13.6
YDL083C / RPS16B	Cytosolic small ribosomal subunit	-13.6
YKR094C / RPL40B	Cytosolic large ribosomal subunit	-13.2
YBR191W / RPL21A	Cytosolic large ribosomal subunit	-13.2
YGR103W / NOP7	Ribosomal large subunit biogenesis	-13.1
YKL009W / MRT4	Ribosomal large subunit biogenesis	-12.9
YBL027W / RPL19B	Cytosolic large ribosomal subunit	-12.9
YGR118W / RPS23A	Cytosolic small ribosomal subunit	-12.8
YML026C / RPS18B	Cytosolic small ribosomal subunit	-12.8
YPL079W / RPL21B	Cytosolic large ribosomal subunit	-12.8
YGL123W / RPS2	Cytosolic small ribosomal subunit	-12.6
YML073C / RPL6A	Cytosolic large ribosomal subunit	-12.5
YGR148C / RPL24B	Cytosolic large ribosomal subunit	-12.5
YDL191W / RPL35A	Cytosolic large ribosomal subunit	-12.4
YHR021C / RPS27B	Cytosolic small ribosomal subunit	-12.4
YOR312C / RPL20B	Cytosolic large ribosomal subunit	-12.4
YMR242C / RPL20A	Cytosolic large ribosomal subunit	-12.4
YNL162W / RPL42A	Cytosolic large ribosomal subunit	-12.4
YHL001W / RPL14B	Cytosolic large ribosomal subunit	-12.0
YLR388W / RPS29A	Cytosolic small ribosomal subunit	-11.9

YJL177W / RPL17B	Cytosolic large ribosomal subunit	-11.9
YJL136C / RPS21B	Cytosolic small ribosomal subunit	-11.8
YLR344W / RPL26A	Cytosolic large ribosomal subunit	-11.8
YGR085C / RPL11B	Cytosolic large ribosomal subunit	-11.7
YIL052C / RPL34B	Cytosolic large ribosomal subunit	-11.7
YHR010W / RPL27A	Cytosolic large ribosomal subunit	-11.5
YDL082W / RPL13A	Cytosolic large ribosomal subunit	-11.5
YDR418W / RPL12B	Cytosolic large ribosomal subunit	-11.4
YLR167W / RPS31	Cytosolic small ribosomal subunit	-11.4
YIL148W / RPL40A	Cytosolic large ribosomal subunit	-11.3
YJL189W / RPL39	Cytosolic large ribosomal subunit	-11.2
YML024W / RPS17A	Cytosolic small ribosomal subunit	-11.2
YLR061W / RPL22A	Cytosolic large ribosomal subunit	-11.2
YMR143W / RPS16A	Cytosolic small ribosomal subunit	-11.0
YKL006W / RPL14A	Cytosolic large ribosomal subunit	-11.0
YDR025W / RPS11A	Cytosolic small ribosomal subunit	-11.0
YOR234C / RPL33B	Cytosolic large ribosomal subunit	-11.0
YML063W / RPS1B	Cytosolic small ribosomal subunit	-10.9
YDL136W / RPL35B	Cytosolic large ribosomal subunit	-10.8
YDL075W / RPL31A	Cytosolic large ribosomal subunit	-10.8
YKR057W / RPS21A	Cytosolic small ribosomal subunit	-10.8
YOL127W / RPL25	Cytosolic large ribosomal subunit	-10.8
YKL180W / RPL17A	Cytosolic large ribosomal subunit	-10.6
YHR141C / RPL42B	Cytosolic large ribosomal subunit	-10.6
YOL040C / RPS15	Cytosolic small ribosomal subunit	-10.5
YOL121C / RPS19A	Cytosolic small ribosomal subunit	-10.5
YLR029C / RPL15A	Cytosolic large ribosomal subunit	-10.5
YGL135W / RPL1B	Cytosolic large ribosomal subunit	-10.5
YBR048W / RPS11B	Cytosolic small ribosomal subunit	-10.5
YGR027C / RPS25A	Cytosolic small ribosomal subunit	-10.3
YOL144W / NOP8	Ribosomal large subunit assembly and maintenance	-10.1
YBR189W / RPS9B	Cytosolic small ribosomal subunit	-10.1
YEL054C / RPL12A	Cytosolic large ribosomal subunit	-10.1
YBL092W / RPL32	Cytosolic large ribosomal subunit	-10.0
YOR293W / RPS10A	Cytosolic small ribosomal subunit	-10.0
YNL067W / RPL9B	Cytosolic large ribosomal subunit	-9.9
YGL189C / RPS26A	Cytosolic small ribosomal subunit	-9.8
YGL103W / RPL28	Cytosolic large ribosomal subunit	-9.7
YPL090C / RPS6A	Cytosolic small ribosomal subunit	-9.6
YKR081C / RPF2	Ribosomal large subunit assembly and maintenance	-9.5
YLR441C / RPS1A	Cytosolic small ribosomal subunit	-9.3
YDR447C / RPS17B	Cytosolic small ribosomal subunit	-9.2
YGL111W / NSA1	Cytosolic large ribosomal subunit	-9.2
YMR121C / RPL15B	Cytosolic large ribosomal subunit	-9.0
YDR064W / RPS13	Cytosolic small ribosomal subunit	-8.7
YIL069C / RPS24B	Cytosolic small ribosomal subunit	-8.7
YLR325C / RPL38	Cytosolic large ribosomal subunit	-8.7
YPL220W / RPL1A	Cytosolic large ribosomal subunit	-8.7
YMR230W /	Cytosolic small ribosomal subunit	-8.6

RPS10B		
YLR048W / RPS0B	Cytosolic small ribosomal subunit	-8.3
YOL039W / RPP2A/RPLA2	Cytosolic large ribosomal subunit	-8.2
YDR500C / RPL37B	Cytosolic large ribosomal subunit	-8.1
YBR181C / RPS6B	Cytosolic small ribosomal subunit	-8.1
YGR095C / RRP46	Cytosolic small ribosomal subunit	-8.0
YCL031C / RRP7	Cytosolic small ribosomal subunit	-7.9
YNL178W / RPS3	Cytosolic small ribosomal subunit	-7.5
YPL081W / RPS9A	Cytosolic small ribosomal subunit	-7.4
YPL143W / RPL33A	Cytosolic large ribosomal subunit	-7.3
YER074W / RPS24A	Cytosolic small ribosomal subunit	-7.3
YLR333C / RPS25B	Cytosolic small ribosomal subunit	-7.3
YGL030W / RPL30	Cytosolic large ribosomal subunit	-7.2
YOR063W / RPL3	Ribosomal large subunit assembly and maintenance	-7.2
YBR031W / RPL4A	Cytosolic large ribosomal subunit	-7.1
YPR102C / RPL11A	Ribosomal large subunit assembly and maintenance	-7.1
YDR012W / RPL4B	Cytosolic large ribosomal subunit	-6.8
YJR123W / RPS5	Ribosomal protein S5 (S2) (rp14) (YS8)	-6.8
YOL120C / RPL18A	Ribosomal protein L18A (rp28A)	-6.7
YBL087C / RPL23A	Cytosolic large ribosomal subunit	-6.7
YIL018W / RPL2B	Cytosolic large ribosomal subunit	-6.3
YJR145C / RPS4A	Cytosolic small ribosomal subunit	-6.2
YHL033C / RPL8A	Cytosolic large ribosomal subunit	-6.0
YHR203C / RPS4B	Cytosolic small ribosomal subunit	-6.0
YKR006C / MRPL13	Cytosolic large ribosomal subunit	-5.9
YLR186W / EMG1	Ribosome biogenesis	-5.9
YLR075W / RPL10	Cytosolic large ribosomal subunit	-5.9
YIL133C / RPL16A	Cytosolic large ribosomal subunit	-5.8
YJL191W / RPS14B	Cytosolic small ribosomal subunit	-5.8
YLR406C / RPL31B	Cytosolic small ribosomal subunit	-5.7
YLL045C / RPL8B	Cytosolic large ribosomal subunit	-5.6
YLR287C-A / RPS30A	Cytosolic small ribosomal subunit	-5.5
YNL069C / RPL16B	Cytosolic large ribosomal subunit	-5.4
YHR147C / MRPL6	Mitochondrial large ribosomal subunit	-5.2
YJL096W / MRPL49	Mitochondrial large ribosomal subunit	-5.2
YMR142C / RPL13B	Cytosolic large ribosomal subunit	-5.2
YPL131W / RPL5	Ribosomal large subunit biogenesis	-5.1
YNL002C / RLP7	Ribosomal large subunit biogenesis	-5.1
YCR031C / RPS14A	Cytosolic small ribosomal subunit	-4.8
YER050C / RSM18	Mitochondrial ribosome small subunit component	-4.8
YNL096C / RPS7B	Cytosolic small ribosomal subunit	-4.8
YNL301C / RPL18B	Cytosolic large ribosomal subunit	-4.8
YDR041W / RSM10	Mitochondrial ribosome small subunit component	-4.6
YFR031C-A / RPL2A	Cytosolic large ribosomal subunit	-4.3
YDR312W / SSF2	Ribosomal large subunit assembly and maintenance	-4.2
YDR382W / RPP2B	Cytosolic small ribosomal subunit	-4.2
YKR085C / MRPL20	Mitochondrial large ribosomal subunit	-4.2
YBL090W / MRP21	Mitochondrial ribosome small subunit component	-4.1
YER117W / RPL23B	Cytosolic large ribosomal subunit	-4.1
YER102W / RPS8B	Cytosolic small ribosomal subunit	-4.0
YDR347W / MRP1	Mitochondrial ribosomal protein	-3.9
YLR185W / RPL37A	Ribosomal protein L37A (L43) (YL35)	-3.8
YNR022C / MRPL50	Mitochondrial large ribosomal subunit	-3.8
YGL076C / RPL7A	Cytosolic large ribosomal subunit	-3.8
YBL072C / RPS8A	Cytosolic small ribosomal subunit	-3.7
YDL130W / RPP1B	Cytosolic small ribosomal subunit	-3.6
YBR282W /	Cytosolic large ribosomal subunit	-3.6

MRPL27		
YJL063C / MRPL8	Ribosomal protein	-3.5
YIR012W / SQT1	Ribosomal large subunit assembly and maintenance	-3.4
YBL038W / MRPL16	Mitochondrial large ribosomal subunit	-3.4
YER126C / NSA2	Ribosomal large subunit biogenesis	-3.4
YPR016C / TIF6	Ribosomal large subunit biogenesis	-3.3
YNL302C / RPS19B	Ribosomal protein S19B	-3.3
YOR369C / RPS12	Ribosomal protein S12	-3.2
YBR146W / MRPS9	Mitochondrial small ribosomal subunit	-3.0
YKL003C / MRP17	Mitochondrial small ribosomal subunit	-3.0
Energy utilization		
YML106W / URA5	Orotate phosphoribosyltransferase 1	-7.8
YOR095C / RKI1	Ribose-5-phosphate ketol-isomerase	-7.3
YOL058W / ARG1	Arginosuccinate synthetase	-6.8
YHR183W / GND1	Glucose metabolism	-6.7
YOR067C / ALG8	Glycosyl transferase	-5.5
YOR002W / ALG6	Glucosyltransferase	-5.3
YBR221C / PDB1	Pyruvate dehydrogenase beta subunit	-4.9
YGR192C / TDH3	Glyceraldehyde-3-phosphate dehydrogenase	-4.8
YLL041C / SDH2	Succinate dehydrogenase (ubiquinone) iron-sulfur protein subunit	-4.5
YOL126C / MDH2	Malate dehydrogenase	-4.5
YBL058W / SHP1	Glycogen metabolism	-4.4
YBR196C / PGI1	Glucose-6-phosphate isomerase	-4.4
YIL074C / SER33	3-phosphoglycerate dehydrogenase	-4.3
YJL167W / ERG20	Arnesyl diphosphate synthetase (FPP synthetase)	-4.1
YKL016C / ATP7	ATP synthase d subunit	-3.9
YOL136C / PFK27	Fructose 2,6-bisphosphate metabolism	-3.7
YJR131W / MNS1	Alpha-mannosidase	-3.6
YBR149W / ARA1	D-arabinose dehydrogenase	-3.4
YOR360C / PDE2	High affinity cAMP phosphodiesterase	-3.4
YIL162W / SUC2	Invertase (sucrose hydrolyzing enzyme)	-3.3
YBR084W / MIS1	C1-tetrahydrofolate synthase	-3.2
YDR368W / YPR1	Arabinose metabolism	-3.1
YPL061W / ALD6	Aldehyde dehydrogenase	-3.1
Stress response		
YDL229W / SSB1	HSP70 family	-11.0
YNL209W / SSB2	SSB1 homolog, heat shock protein of HSP70 family	-7.5
YDL235C / YPD1	Response to osmotic stress	-6.5
YIL053W / RHR2	Glycerol-1-phosphatase -response to osmotic stress	-6.2
YML028W / TSA1	Response to oxidative stress	-5.2
YDL120W / YFH1	Iron homeostasis	-5.2
YIL153W / RRD1	Response to osmotic stress	-4.9
YGL097W / SRM1	Pheromone response pathway suppressor	-4.9
YLR043C / TRX1	Response to oxidative stress	-4.5
YER118C / SHO1	Transmembrane osmosensor	-4.4
YHR064C / SSZ1	HSP70 family	-4.0
YDL166C / FAP7	Response to oxidative stress	-4.0
YML086C / ALO1	Response to oxidative stress	-3.8
YBR244W / GPX2	Response to oxidative stress	-3.4
YDR214W / AHA1	Hsp90 system cochaperone; Aha1 binds to the middle domain of Hsp90 and improves client protein activation in vivo	-3.1
YFL017C / GNA1	Glucosamine-phosphate N-acetyltransferase	-3.0
YGR209C / TRX2	Response to oxidative stress	-3.0
YJR147W / HMS2	Heat shock transcription factor homolog	-3.0
Cell cycle and growth		

YPL187W	Mating factor alpha	-14.7
YLR062C / BUD28	Bud site selection	-12.0
YJL188C / BUD19	Bud site selection	-10.3
YOR212W / STE4	G protein beta subunit, coupled to mating factor receptor	-8.9
YPR120C / CLB5	B-type cyclin	-8.7
YNL078W / NIS1	Regulation of mitosis	-7.8
YBR247C / ENP1	Cell growth and/or maintenance	-7.1
YGL134W / PCL10	Cyclin-dependent protein kinase, regulator	-6.1
YHR152W / SPO12	Protein- positive regulator of exit from M-phase in mitosis and meiosis	-5.7
YBR057C / MUM2	Premeiotic DNA synthesis	-5.2
YOL001W / PHO80	Pho80p cyclin	-4.7
YBR160W / CDC28	Cyclin-dependent protein kinase	-4.5
YGL215W / CLG1	Cyclin-dependent protein kinase holoenzyme complex	-4.5
YDR303C / RSC3	Regulation of cell cycle	-4.2
YBL084C / CDC27	Anaphase promoting complex (APC) subunit	-3.9
YLR210W / CLB4	B-type cyclin	-3.7
YBR135W / CKS1	Regulation of cell cycle	-3.5
YLR226W / BUR2	Cyclin-dependent protein kinase, regulator	-3.5
YDL155W / CLB3	Cyclin-dependent protein kinase, regulator	-3.4
YIR025W / MND2	Anaphase-promoting complex	-3.4
YJL098W / SAP185	G1/S transition of mitotic cell cycle	-3.4
YGL169W / SUA5	Cell growth and/or maintenance	-3.2
YPL256C / CLN2	G1 cyclin	-3.2
YKR063C / LAS1	Bud growth	-3.1
YLR074C / BUD20	Bud site selection	-3.1
Transport & translocation		
YMR011W / HXT2	High affinity hexose transporter-2	-25.7
YHR094C/HXT1	Hexose transport	-16.1
YHL047C / ARN2	Triacetylfusarinine C transporter	-12.5
YER009W / NTF2	Nuclear transport factor	-11.1
YDR011W / SNQ2	ABC transporter	-10.8
YHR026W / PPA1	Hydrogen-transporting ATPase	-10.1
YEL051W / VMA8	Hydrogen-transporting ATPase	-9.9
YKL186C / MTR2	mRNA transport regulator	-9.5
YBR291C / CTP1	Citrate tranporter	-9.3
YDR441C / APT2	Adenine phosphoribosyltransferase	-9.2
YKL120W / OAC1	Oxaloacetate transport protein	-8.8
YFL048C / EMP47	Golgi apparatus	-7.7
YKL196C / YKT6	V-SNARE-intra-Golgi transport	-7.5
YDR276C / PMP3	Cation transport	-7.2
YDR384C / ATO3	Transmembrane protein-transport protein	-7.1
YGR289C / MAL11	Alpha-glucoside transporter, hexose transporter, maltose permease	-7.0
YBL102W / SFT2	Golgi to endosome transport	-7.0
YLR078C / BOS1	v-SNARE - ER to Golgi transport	-7.0
YDR425W / SNX41	Protein transport	-6.9
YDR002W / YRB1	RNA-nucleus export	-6.8
YOR115C / TRS33	ER to Golgi transport	-6.4
YJL145W / SFH5	Phospholipid transport	-6.1
YHR110W / ERP5	p24 protein involved in membrane trafficking	-6.1
YAL014C / SYN8	Syntaxin family	-6.1
YNL044W / YIP3	ER to Golgi transport	-6.1
YGR082W / TOM20	20 kDa mitochondrial outer membrane protein import receptor	-5.9
YML067C / ERV41	ER to Golgi transport	-5.9
YOR079C / ATX2	Manganese-trafficking protein	-5.8
YML077W / BET5	ER to Golgi transport	-5.7
YLR130C / ZRT2	Low affinity zinc transport protein	-5.6
YBR104W / YMC2	Mitochondrial inner membrane-transport	-5.6
YHL003C / LAG1	Protein transport	-5.6
YLR411W / CTR3	Copper transporter (	-5.6

YOR153W / PDR5	Multidrug resistance transporter	-5.6
YFR051C / RET2	ER to Golgi transport	-5.2
YLR208W / SEC13	Nuclear pore complex subunit, protein involved in release of transport vesicles from the ER	-5.1
YDR395W / SXM1	Protein carrier	-5.1
YNR039C / ZRG17	Zinc ion transport	-5.0
YKL175W / ZRT3	Zinc ion transporter	-5.0
YDR202C / RAV2	Hydrogen-transporting ATPase V1 domain	-4.8
YJL133W / MRS3	Carrier protein	-4.7
YDR091C / RLI1	ATP-binding cassette (ABC) transporter	-4.6
YBR106W / PHO88	Phosphate transporter	-4.6
YJR117W / STE24	Zinc metallo-protease	-4.6
YKR014C / YPT52	Protein-vacuolar targeting	-4.5
YDL198C / YHM1	Mitochondrial inner membrane-transporter	-4.5
YGR055W / MUP1	High affinity methionine permease	-4.4
YJL024C / APS3	Golgi to vacuole transport	-4.4
YDR166C / SEC5	Exocyst complex 107 kDa component	-4.4
YJR044C / VPS55	Involved in Golgi to vacuolar targeting	-4.3
YLR026C / SED5	ER to Golgi transport	-4.1
YJL129C / TRK1	High affinity potassium transporter	-4.1
YKR068C / BET3	Transport protein particle (TRAPP) component	-4.1
YGL077C / HNM1	Transporter (permease) for choline and nitrogen mustard; share homology with UGA4	-4.1
YDR345C / HXT3	Low affinity glucose transporter	-4.0
YBR132C / AGP2	Plasma membrane carnitine transporter	-3.9
YDR299W / BFR2	ER to Golgi transport	-3.9
YKL080W / VMA5	Hydrogen-transporting ATPase V1 domain	-3.9
YLR214W / FRE1	Cupric reductase, ferric reductase-iron transport	-3.8
YOR075W / UFE1	t-SNARE (ER)	-3.7
YLR093C / NYV1	Vacuolar v-SNARE	-3.7
YFR009W / GCN20	ATP-binding cassette (ABC) family	-3.6
YBR017C / KAP104	Protein-nucleus import	-3.6
YDL090C / RAM1	Farnesyltransferase beta subunit	-3.6
YGL136C / MRM2	2'O-ribose methyltransferase	-3.6
YDL018C / ERP3	P24 protein involved in membrane trafficking	-3.6
YGL223C / COG1	Golgi transport complex	-3.6
YOL103W / ITR2	Myo-inositol transporter	-3.5
YCR004C / YCP4	Electron transporter	-3.5
YGL161C / YIP5	ER to Golgi transport	-3.5
YBL089W / AVT5	Transporter	-3.5
YML130C / ERO1	Electron carrier	-3.5
YGL002W / ERP6	P24 protein involved in membrane trafficking	-3.4
YOR016C / ERP4	Protein involved in membrane trafficking	-3.4
YLR309C / IMH1	Vesicle-mediated transport	-3.3
YGR166W / KRE11	ER to Golgi transport	-3.3
YER145C / FTR1	Iron permease	-3.3
YHR092C / HXT4	High affinity glucose transporter	-3.2
YHR117W / TOM71	Protein transporter	-3.2
YOR244W / ESA1	Acetyltransferase in the SAS gene family, NuA4 complex component	-3.2
YGL145W / TIP20	Transport protein that interacts with Sec20p; required for protein transport from the endoplasmic reticulum to the golgi apparatus"	-3.1
YGL233W / SEC15	Exocyst complex 113kDa component	-3.1
YJL054W / TIM54	Translocase for the insertion of proteins into the mitochondrial inner membrane	-3.1
YIL022W / TIM44	Protein involved in mitochondrial protein import	-3.1
YAL007C / ERP2	P24 protein involved in membrane trafficking	-3.1
YLR380W / CSR1	Phosphatidylinositol transporter	-3.1
YPL252C / YAH1	Iron-sulfur protein similar to human adrenodoxin	-3.1
YLL027W / ISA1	Iron transport	-3.1
YKL019W / RAM2	CAAX farnesyltransferase alpha subunit	-3.0

YGR057C / LST7	Protein transport	-3.0
YOL020W / TAT2	Tryptophan permease, high affinity (transport)	-3.0
Lipid metabolism		
YLR056W / ERG3	Ergosterol biosynthesis- C-5 sterol desaturase	-8.3
YML008C / ERG6	Ergosterol biosynthesis	-8.2
YGR060W / ERG25	C-4 sterol methyl oxidase	-7.8
YPL028W / ERG10	Acetoacetyl CoA thiolas	-5.5
YLR100W / ERG27	3-keto sterol reductase	-5.4
YDL052C / SLC1	Lipid particle	-4.6
YNR043W / MVD1	Ergosterol biosynthesis	-4.6
YPR128C / ANT1	Fatty acid beta-oxidation	-4.2
YDR036C / EHD3	Fatty acid beta-oxidation	-4.2
YML126C / ERG13	3-hydroxy-3-methylglutaryl coenzyme A synthase	-3.9
YHR190W / ERG9	Squalene synthetase	-3.8
YHR007C / ERG11	Cytochrome P450 lanosterol 14a-demethylase	-3.4
Protein metabolism		
YNR050C / LYS9	Lysine biosynthesis, aminoadipic pathway	-10.0
YIL020C / HIS6	Histidine biosynthesis	-8.4
YHR052W / CIC1	Protein catabolism	-8.0
YHR025W / THR1	Homoserine kinase	-7.4
YHR181W / SVP26	Integral membrane protein	-7.3
YHR208W / BAT1	Branched-chain amino acid transaminase	-6.9
YBR133C / HSL7	Has homology to arginine methyltransferases	-6.6
YFR052W / RPN12	Ubiquitin-dependent protein catabolism	-6.4
YGR155W / CYS4	Cystathionine beta-synthase	-6.3
YIR034C / LYS1	Lysine biosynthesis	-5.4
YCR053W / THR4	Threonine synthase	-5.4
YER052C / HOM3	Aspartate kinase	-4.9
YIL116W / HIS5	Histidinol-phosphate aminotransferase	-4.9
YBR173C / UMP1	20S proteasome maturation factor	-4.8
RRS1 / YDR341C	Arginine-tRNA ligase	-4.8
YBR034C / HMT1	Arginine methyltransferase	-4.7
YDR390C / UBA2	SUMO activating enzyme	-4.6
YER081W / SER3	Serine family amino acid biosynthesis	-4.6
YJL101C / GSH1	Gamma-glutamylcysteine synthetase	-4.6
YPR033C / HTS1	Histidine-tRNA ligase	-4.4
YER069W / ARG5,6	Arginine biosynthesis	-4.4
YJR023C	Protein disulfide isomerase related protein	-4.3
YDR158W / HOM2	Aspartic beta semi-aldehyde dehydrogenase	-4.0
YLR027C / AAT2	Aspartate aminotransferase	-4.0
YKL106W / AAT1	Aspartate aminotransferase	-4.0
YDL131W / LYS21	Lysine biosynthesis	-3.9
YBL080C / PET112	Protein biosynthesis	-3.6
YDR354W / TRP4	Tryptophan biosynthesis	-3.5
YBL091C / MAP2	Methionine aminopeptidase	-3.3
YMR164C / MSS11	Amino acid polypeptide with poly-glutamine and poly-asparagine domains	-3.3
YCL030C / HIS4	Histidinol dehydrogenase	-3.2
YGL202W / ARO8	Aromatic amino acid aminotransferase	-3.2
YNL161W / CBK1	Serine/threonine protein kinase	-3.2
YLR017W / MEU1	Glutamate biosynthesis	-3.1
Protein folding, synthesis, modification, translocation, degradation and complex assembly		
YGL018C / JAC1	Co-chaperone	-11.2
YGR135W / PRE9	proteasome component Y13	-11.8
YBR155W / CNS1	Protein folding	-8.0
YIL034C / CAP2	Capping protein beta subunit	-6.5
YHR111W / UBA4	Protein modification	-6.4
YHL002W / HSE1	Endosome	-6.3

YHR135C / YCK1	Casein kinase I homolog	-6.2
YGR185C / TYS1	Tyrosine-tRNA ligase	-6.2
YHR170W / NMD3	Factor required for a late assembly step of the 60S subunit	-6.2
YER003C / PMI40	Mannose-6-phosphate isomerase	-6.2
YBR121C / GRS1	Glycine-tRNA ligase	-6.1
YJL111W / CCT7	Chaperonin containing T-complex subunit seven component	-5.9
YEL053C / MAK10	Amino acid N-acetyltransferase	-5.9
YDR321W / ASP1	Asparaginase I, intracellular isozyme	-5.9
YFR028C / CDC14	Protein phosphatase	-5.6
YGL229C / SAP4	Protein serine/threonine phosphatase	-5.6
YIL131C / FKH1	Forkhead protein	-5.6
YDR212W / TCP1	Chaperonin subunit alpha	-5.4
YFR040W / SAP155	Protein serine/threonine phosphatase	-5.3
YOR089C / VPS21	Small GTP-binding protein	-5.1
YDR007W / TRP1	Tryptophan biosynthesis	-4.7
YGL035C / MIG1	C2H2 zinc finger protein that resembles	-4.0
YKL117W / SBA1	HSP90 associated co-chaperone	-3.7
YER133W / GLC7	Protein phosphatase type I	-3.4
YDR300C / PRO1	Glutamate 5-kinase	-3.3
YJL008C / CCT8	Chaperonin containing T-complex subunit eight component	-3.2
YJR064W / CCT5	Chaperonin subunit epsilon subunit	-3.2
YDL040C / NAT1	N-terminal acetyltransferase	-3.1
Signal transduction proteins		
YKL122C / SRP21	Signal recognition particle component	-7.2
YPL243W / SRP68	Signal recognition particle component	-6.4
YLR066W / SPC3	Signal peptidase complex	-6.1
YDL092W / SRP14	Signal recognition particle	-5.2
YDL135C / RDI1	Signal transducer	-5.2
YDR142C / PEX7	Beta-transducin-related (WD-40) protein family	-4.4
YDR364C / CDC40	Beta transducin family	-3.1
YDR103W / STE5	Signal transduction during conjugation with cellular fusion	-3.0
Transcription and translation factor and process		
YPL037C / EGD1	Pol II transcribed genes regulator	-12.9
YGR104C / SRB5	RNA polymerase II transcription mediator	-11.1
YER148W / SPT15	TFIID subunit	-11.0
YCL067C / HMLALPHA2	Transcription co-repressor	-9.7
DR429C / TIF35	Translation initiation factor eIF3 subunit	-9.2
YGL099W / LSG1	Ribosome nucleus export	-9.1
YKR059W / TIF1	Translation initiation factor eIF4A subunit	-8.9
YBR088C / POL30	Proliferating Cell Nuclear Antigen	-8.7
YEL034W / HYP2	Translation initiation factor eIF-5A	-8.7
YGL078C / DBP3	ATP dependent RNA helicase	-8.5
YCR039C / MATALPHA2	Ranscription co-repressor	-8.5
YBR083W / TEC1	Specific RNA polymerase II transcription factor	-8.4
YMR005W / TAF4	Transcription factor TFIID complex	-8.0
YJL138C / TIF2	Translation initiation factor eIF4A subunit	-8.0
YOR168W / GLN4	Glutamine-tRNA ligase	-7.8
YPR041W / TIF5	Translation initiation factor eIF5	-7.8
YCR040W / MATALPHA1	Transcription co-activator	-7.8
YBR154C / RPB5	DNA-directed RNA polymerase II, core complex	-7.6
YJR007W / SUI2	Translation initiation factor eIF-2 alpha subunit	-7.3
YCL066W / HMLALPHA1	Involved in the regulation of alpha-specific genes, transcription factor	-7.3
YOR260W / GCD1	Ulator in the general control of amino acid biosynthesis, translation initiation factor eIF2B subunit	-7.2

YOL097C / WRS1	Tryptophan-tRNA ligase	-7.1
YJL148W / RPA34	DNA-directed RNA polymerase	-7.1
YKL110C / KTI12	Elongator associated protein	-6.9
YKR026C / GCN3	Translation initiation factor	-6.9
YEL009C / GCN4	Transcriptional activator of amino acid biosynthetic genes	-6.8
YAL003W / EFB1	Translation elongation factor EF-1beta	-6.8
YGL209W / MIG2	Contains zinc fingers very similar to zinc fingers in Mig1p	-6.7
YNL255C / GIS2	Transcription factor	-6.5
YOR224C / RPB8	DNA-directed RNA polymerase II, core complex	-6.4
YGL043W / DST1	Positive transcription elongation factor	-6.3
YDL150W / RPC53	RNA polymerase III subunit	-6.3
YHR088W / RPF1	rRNA primary transcript binding	-6.2
YCL055W / KAR4	Transcription regulator	-6.2
YNL229C / URE2	Glutathione transferase (putative), transcriptional regulator, prion	-6.1
YMR260C / TIF11	Translation initiation factor eIF1A	-6.0
YER025W / GCD11	Translational initiation factor eIF-2 gamma subunit	-5.8
YKR056W / TRM2	tRNA methyltransferase	-5.7
YGL070C / RPB9	RNA polymerase II core subunit	-5.7
YDR120C / TRM1	N2-dimethylguanosine-specific tRNA methyltransferase	-5.5
YGL025C / PGD1	- RNA polymerase II transcription mediator	-5.4
YDR023W / SES1	Serine-tRNA ligase	-5.4
YNL244C / SUI1	Translation initiation factor eIF1	-5.4
YKL081W / TEF4	Translation elongation factor EF-1gamma	-5.4
YDR005C / MAF1	Negative regulation of transcription from Pol III promoter	-5.3
YDR143C / SAN1	Transcriptional regulator (putative)	-5.3
YER165W / PAB1	Poly (A) binding protein	-5.2
YDR279W / RNH202	Required for RNase H2 activity - one of three subunits	-5.2
YKR099W / BAS1	Transcription factor	-5.1
YGL105W / ARC1	Methionyl glutamyl tRNA synthetase complex	-5.0
YBL057C / PTH2	aminoacyl-tRNA hydrolase	-5.0
YOL005C / RPB11	RNA polymerase II core subunit	-4.8
YKL205W / LOS1	tRNA splicing and binding	-4.8
YAL033W / POP5	RNase MRP subunit (putative), RNase P integral subunit	-4.7
YDR216W / ADR1	Positive transcriptional regulator	-4.7
YDR311W / TFB1	Transcription initiation factor IIb,	-4.7
YDR172W / SUP35	Translation termination factor eRF3	-4.6
YMR043W / MCM1	(MCM1, AG, DEFAm SRF)-box motif within its DNA binding domain, plays a central role inthe formation of both repressor and activator complexes, transcription factor	-4.5
YLR005W / SSL1	RNA polymerase transcription factor TFIIH component	-4.5
YBR049C / REB1	RNA polymerase I enhancer binding protein	-4.5
YJR047C / ANB1	Translation initiation factor eIF-5A, anaerobically expressed form	-4.5
YKL144C / RPC25	RNA polymerase III subunit	-4.5
YNL206C / RTT106	Negative regulation of DNA transposition	-4.5
YPL016W / SWI1	Zinc finger transcription factor	-4.4
YER127W / LCP5	Small nucleolar ribonucleoprotein complex	-4.4
YJL025W / RRN7	RNA polymerase I transcription factor complex	-4.3
YDL005C / MED2	RNA polymerase II holoenzyme/mediator subunit	-4.3
YGL013C / PDR1	Zinc finger transcription factor of the Zn(2)-Cys(6) binuclear cluster domain type	-4.3
YGR083C / GCD2	Translation initiation factor eIF2B subunit	-4.2
YDR037W / KRS1	Lysine-tRNA ligase	-4.2
YGR271W / SLH1	Regulation of translation	-4.1
YGR162W / TIF4631	Translation initiation factor	-4.1
YNL199C / GCR2	Transcription factor	-4.1
YMR146C / TIF34	Translation initiation factor eIF3 p39 subunit	-4.1
YPL169C / MEX67	a poly(A)+RNA binding protein	-4.0
YOR340C / RPA43	DNA dependent RNA polymerase I subunit A43	-4.0

YHR187W / IKI1	Transcription elongation factor complex	-4.0
YGL195W / GCN1	Translational activator of GCN4 through activation of GCN2 in response to starvation	-4.0
YGR044C / RME1	Zinc finger protein, negative regulator of meiosis; directly repressed by a1-alpha 2 regulator	-4.0
YHL009C / YAP3	Transcription factor	-4.0
YDR190C / RVB1	Regulation of transcription from Pol II promoter	-3.9
YIL021W / RPB3	RNA polymerase II 45 kDa subunit	-3.9
YNL016W / PUB1	Poly(A) binding protein	-3.9
YPL212C / PUS1	tRNA pseudouridine synthase	-3.9
YOR281C / PLP2	Positive regulation of transcription from Pol II promoter by pheromones	-3.8
YPL129W / TAF14	Transcription initiation factor TFIIF small subunit	-3.8
YJR006W / HYS2	DNA polymerase delta 55 kDa subunit	-3.8
YCR077C / PAT1	Regulation of translational initiation	-3.8
YDL031W / DBP10	ATP dependent RNA helicase	-3.8
YJR063W / RPA12	RNA polymerase I A12.2 subunit	-3.7
YDR289C / RTT103	Negative regulation of DNA transposition	-3.7
YIR023W / DAL81	Transcriptional activator for allantoin and GABA catabolic genes	-3.7
YJL087C / TRL1	tRNA ligase	-3.7
YPL122C / TFB2	TFIIH subunit	-3.7
YLR182W / SWI6	Transcription factor	-3.6
YPL190C / NAB3	Polyadenylated RNA binding protein, polyadenylated single strand DNA-binding protein	-3.6
YHR058C / MED6	RNA polymerase II transcription mediator	-3.6
YGR276C / RNH70	Ribonuclease H	-3.6
YDR404C / RPB7	RNA polymerase II dissociable subunit	-3.6
YJR140C / HIR3	Transcription co-repressor	-3.5
YDR045C / RPC11	TFIIS-like small Pol III subunit C11	-3.4
YER169W / RPH1	Specific transcriptional repressor	-3.4
YOL051W / GAL11	RNA polymerase II holoenzyme complex component, positive and negative transcriptional regulator	-3.4
YJL140W / RPB4	RNA polymerase II fourth largest subunit	-3.4
YER159C / BUR6	Transcription co-repressor	-3.3
YDR228C / PCF11	Cleavage/polyadenylation specificity factor of mRNA	-3.3
YBR112C / CYC8	Transcription co-activator	-3.3
YOL148C / SPT20	Transcription cofactor	-3.3
YPL101W / ELP4	RNA polymerase II Elongator protein subunit	-3.3
YML080W / DUS1	tRNA dihydrouridine synthase	-3.3
YKL125W / RRN3	DNA independent RNA polymerase I transcription factor	-3.2
YGL096W / TOS8	Transcription factor	-3.2
YBR212W / NGR1	Glucose-repressible RNA binding protein	-3.2
YBR143C / SUP45	Translation release factor	-3.2
YKL025C / PAN3	Pab1p-dependent poly(A) ribonuclease (PAN) 76 kDa subunit	-3.2
YDL108W / KIN28	Transcription factor TFIIH complex	-3.1
YDR448W / ADA2	ADA and SAGA component, two transcriptional adaptor/HAT	-3.1
YKR002W / PAP1	poly(A) polymerase	-3.1
YNR052C / POP2	Transcription factor (putative)	-3.1
YJL124C / LSM1	mRNA cap complex	-3.1
YDR145W / TAF12	Transcription factor TFIID subunit	-3.0
YDR167W / TAF10	Transcription factor TFIID subunit	-3.0
YGL162W / SUT1	Specific RNA polymerase II transcription factor	-3.0
YBR188C / NTC20	Pre-mRNA splicing factor	-3.0
YGL071W / RCS1	Positive regulation of transcription from Pol II promoter	-3.0
Nucleotic metabolism		
YOR276W / CAF20	mRNA cap complex	-17.7
YGL090W/LIF1	Double-strand break repair via nonhomologous end-joining	-17.1
YBL003C/HTA2	DNA binding - chromatin assembly/disassembly	-14.9
YHR089C / GAR1	RNA binding	-14.0
YER006W / NUG1	rRNA processing	-13.3

YCR028C-A / RIM1	DNA binding protein	-13.0
YEL026W / SNU13	U3 snoRNP protein	-12.8
YOL149W / DCP1	Deadenylation-dependent decapping	-12.8
YCL059C / KRR1	RRNA processing	-11.9
YDL051W / LHP1	RNA binding	-11.8
YHR163W / SOL3	TRNA processing	-11.5
YNL141W / AAH1	Adenine aminohydrolase (adenine deaminase)	-11.0
YCR057C / PWP2	U3 snoRNP protein	-10.9
YFR001W / LOC1	MRNA binding	-10.9
YGR158C / MTR3	Nuclear exosome	-9.1
YOL139C / CDC33	mRNA cap binding protein eIF-4E	-8.9
YDL084W / SUB2	ATP-dependent RNA helicase	-8.9
YOL123W / HRP1	Cleavage and polyadenylation factor CF I component involved in pre-mRNA 3'-end processing	-8.8
YOL041C / NOP12	rRNA metabolism	-8.8
YDL192W / ARF1	ADP-ribosylation factor	-8.5
YGL171W / ROK1	ATP dependent RNA helicase	-8.5
YHR196W / UTP9	U3 snoRNP protein	-8.4
YHR066W / SSF1	RNA binding	-7.9
YDR409W / SIZ1	Chromatin protein; SUMO1/Smt3 ligase	7.7
YMR290C / HAS1	RNA-dependent helicase (putative)	-7.6
YDR280W / RRP45	3'->5' exoribonuclease	-7.5
YFR005C / SAD1	Pre-mRNA splicing factor	-7.4
YGR280C / PXR1	Possible telomerase regulator or RNA-binding protein	-7.3
YDR195W / REF2	mRNA processing	-7.2
YLR321C / SFH1	Snf5p homolog, chromatin remodeling complex member	-7.0
YDR324C / UTP4	U3 snoRNP protein	-7.0
YKR008W / RSC4	Nucleosome remodeling complex	-6.9
YDR087C / RRP1	RRNA processing	-6.7
YCL050C / APA1	Nucleotide metabolism	-6.7
YDR381W / YRA1	RNA-binding RNA annealing protein	-6.7
YLR002C / NOC3	DNA replication initiation	-6.7
YHR069C / RRP4	mRNA catabolism	-6.6
YOR078W / BUD21	U3 snoRNP protein	-6.5
YDL111C / RRP42	3'-5' exoribonuclease	-6.4
YNL339C / YRF1-6	Y'-helicase protein 1	-6.4
YDR545W / YRF1-1	Y'-helicase protein 1	-6.4
YGL094C / PAN2	Post-replication repair	-6.3
YGL128C / CWC23	Spliceosome complex	-6.3
YBR061C / TRM7	tRNA methyltransferase	-6.3
YNL175C / NOP13	Nucleoplasm	-6.2
YLR150W / STM1	Purine motif triplex-binding protein	-6.1
YJL121C / RPE1	D-ribulose-5-Phosphate 3-epimerase	-5.1
YDR083W / RRP8	Nucleolar protein required for efficient processing of pre-rRNA at site A2	-5.1
YDL125C / HNT1	Nucleotide binding	-4.9
YGR159C / NSR1	Nuclear localization sequence binding protein	-4.9
YHR065C / RRP3	ATP dependent RNA helicase	-5.8
YOR046C / DBP5	RNA helicase	-5.7
YJL033W / HCA4	RNA helicase (putative)	-5.6
YER056C / FCY2	Purine-cytosine permease	-5.4
YBR142W / MAK5	ATP dependent RNA helicase	-5.2
YIR008C / PRI1	DNA primase p48 polypeptide	-5.1
YLR033W / RSC58	Subunit of RSC Chromatin Remodeling Complex	-5.1
YML113W / DAT1	Datin, oligo(dA).oligo(dT)-binding protein	-4.9
YMR153W / NUP53	Nuclear pore complex subunit	-4.8
YHR146W / CRP1	Cruciform DNA binding protein	-4.7
YDR416W / SYF1	Spliceosome complex	-4.7
YPR065W / ROX1	HMG-domain site-specific DNA binding protein	-4.7
YOL146W / PSF3	a subunit of the GINS complex required for chromosomal DNA replication	-4.7
YNL061W / NOP2	RNA methyltransferase	-4.7

YPL178W / CBC2	Nuclear cap binding complex subunit	-4.6
YJR068W / RFC2	Replication factor C subunit 2, similar to human RFC 37 kDa subunit	-4.6
YHL034C / SBP1	RNA binding	-4.6
YGR285C / ZUO1	Z-DNA binding protein (putative	-4.5
YOR048C / RAT1	5'-3' exoribonuclease	-4.5
YOL093W / TRM10	tRNA (guanine) methyltransferase	-4.4
YBR167C / POP7	RNase MRP subunit (putative), RNase P integral subunit	-4.5
YOR304W / ISW2	ATPase component of a two subunit chromatin remodeling complex	-4.4
YEL032W / MCM3	DNA replication initiation	-4.4
YGL207W / SPT16	Alpha DNA polymerase:primase complex	-4.3
YGL120C / PRP43	RNA helicase	-4.3
YDR254W / CHL4	Chromosome segregation	-4.3
YGL213C / SKI8	mRNA catabolism	-4.2
YNL110C / NOP15	Ribosome biosynthesis	-4.1
YMR061W / RNA14	Cleavage and polyadenylation factor CF I component involved in pre-mRNA 3'-end processing	-4.1
YIR001C / SGN1	mRNA metabolism	-4.1
YML069W / POB3	DNA polymerase delta binding protein	-4.1
YDR021W / FAL1	RNA helicase (putative), dead box protein	-4.1
YGR030C / POP6	RNase P integral subunit, subunit of RNase MRP (putative)	-4.0
YDL060W / TSR1	Ribosome biogenesis and assembly	-4.0
YER161C / SPT2	Non-specific DNA binding protein	-4.0
YGL224C / SDT1	Pyrimidine base metabolism	-4.0
YER171W / RAD3	Nucleotide excision repair factor 3 complex	-3.9
YGR074W / SMD1	Small nuclear ribonucleoprotein complex	-3.9
YDL013W / HEX3	DNA binding	-3.9
YGL087C / MMS2	DNA repair	-3.8
YLR059C / REX2	RNA processing	-3.8
YDR305C / HNT2	Nucleoside catabolism	-3.8
YGR156W / PTI1	pre-mRNA cleavage factor	-3.7
YGR169C / PUS6	Pseudouridylate synthase	-3.7
YBL068W / PRS4	Ribose-phosphate pyrophosphokinase	-3.7
YBL039C / URA7	CTP synthase	-3.7
YNL062C / GCD10	RNA-binding protein, subunit of tRNA	-3.5
YHR169W / DBP8	ATP dependent RNA helicase	-3.5
YNR038W / DBP6	RNA helicase (putative	-3.5
YDL030W / PRP9	RNA splicing factor	-3.4
YBL024W / NCL1	tRNA:m5C-methyltransferase	-3.4
YNR012W / URK1	Uridine kinase	-3.4
YBR060C / ORC2	DNA replication origin binding	-3.3
YDR325W / YCG1	Mitotic chromosome condensation	-3.2
YHL025W / SNF6	Chromatin remodeling Snf/Swi complex subunit	-3.2
YBL052C / SAS3	Chromatin silencing at telomere	-3.2
YDR439W / LRS4	Chromatin silencing at ribosomal DNA	-3.2
YNR015W / SMM1	tRNA dihydrouridine synthase	-3.2
YDR194C / MSS116	RNA helicase DEAD box	-3.0

Cytoskeleton organization and maintenance

YNL166C / BNI5	Septin ring (sensu Saccharomyces	-7.5
YML094W / GIM5	Tubulin binding	-6.3
YLL050C / COF1	Actin binding and severing protein	-5.5
YOR239W / ABP140	Actin filament binding protein	-5.4
YCR009C / RVS161	Cytoskeletal protein binding	-5.2
YDL225W / SHS1	Structural constituent of cytoskeleton	-5.1
YBR156C / SLI15	Kinetochore microtubule	-5.1
YML124C / TUB3	Alpha-tubulin	-5.1
YER016W / BIM1	Structural constituent of cytoskeleton	-4.8
YJL030W / MAD2	Spindle checkpoint complex subunit	-4.8
YFL037W / TUB2	Beta-tubulin	-4.8
YJR125C / ENT3	Actin cortical patch	-4.6

YGR241C / YAP1802	Actin cortical patch	-4.4
YKL042W / SPC42	Spindle pole body component	-4.3
YOR326W / MYO2	Class V myosin	-4.3
YOL076W / MDM20	Cytoskeletal regulator	-4.2
YEL061C / CIN8	Microtubule motor	-4.0
YLR175W / CBF5	Major low affinity 55 kDa centromere/microtubule binding protein	-3.9
YPR034W / ARP7	Actin related protein, chromatin remodeling Snf/Swi complex subunit	-3.9
YPL253C / VIK1	Cik1p homolog	-3.9
YHR129C / ARP1	Structural constituent of cytoskeleton	-3.8
YBR117C / TKL2	Transketolase, similar to TKL1	-3.7
YLR227C / ADY4	Spindle pole body	-3.7
YDR188W / CCT6	Cytoskeleton organization	-3.6
YGL093W / SPC105	Spindle pole component	-3.6
YGR080W / TWF1	Twinfilin A, an actin monomer sequestering protein	-3.6
YGR109C / CLB6	Cyclin-dependent protein kinase, regulator	-3.5
YDR309C / GIC2	Actin cap (sensu Saccharomyces	-3.5
YIL095W / PRK1	Actin filament organization	-3.4
YGL061C / DUO1	Structural constituent of cytoskeleton	-3.4
YFL039C / ACT1	Actin cable	-3.4
YDL161W / ENT1	Actin cortical patch	-3.4
YEL003W / GIM4	Tubulin binding	-3.4
YHR107C / CDC12	10 nm filament component of mother-bud neck, septin	-3.3
YDR016C / DAD1	Structural constituent of cytoskeleton	-3.3
Histone metabolism		
YBL002W / HTB2	Histone H2B	-9.5
YHR099W / TRA1	Histone acetylation	-8.2
YBR009C / HHF1	Histone H4 (HHF1 and HHF2 code for identical proteins)	-5.2
YDR224C / HTB1	Histone H2B (HTB1 and HTB2 code for nearly identical proteins)	-5.2
YJL168C / SET2	istone-lysine N-methyltransferase	-5.0
YDL042C / SIR2	Histone deacetylase	-4.4
YGL066W / SGF73	Subunit of SAGA histone acetyltransferase complex	-4.4
YBR010W / HHT1	Histone H3 (HHT1 and HHT2 code for identical proteins)	-4.3
YOL068C / HST1	histone deacetylase complex	-3.9
YEL056W / HAT2	Histone acetyltransferase subunit	-3.8
YDR225W / HTA1	Histone H2A (HTA1 and HTA2 code for nearly identical proteins	-3.4
YDR181C / SAS4	H3/H4 histone acetyltransferase	-3.3
Cell wall & membrane proteins		
YEL040W/UTR2	Cell wall organization and biogenesis	-36.2
YJR004C / SAG1	Alpha-agglutinin, cell wall, cell adhesion receptor	-34.5
YNR044W / AGA1	a-agglutinin anchorage subunit, cell wall, cell adhesion receptor	-31.7
YHR143W / DSE2	Cell wall organization and biogenesis	-14.6
YOR254C / SEC63	Endoplasmic reticulum membrane	-7.9
YHR103W / SBE22	Cell wall organization and biogenesis	-7.3
YER083C / RMD7	Cell wall organization and biogenesis	-6.7
YOR327C / SNC2	Vesicle-associated membrane protein	-6.7
YPL065W / VPS28	Protein-membrane targeting	-6.4
YCR002C / CDC10	Cell wall structure	-6.2
YKR088C / TVP38	Integral membrane protein	-6.2
YMR292W / GOT1	Membrane protein	-6.0
YKL051W / SFK1	Plasma membrane	-5.8
YPR028W / YOP1	Membrane organization and biogenesis	-5.1
YGR120C / COG2	Peripheral membrane protein of membrane fraction	-4.9
YJL208C / NUC1	Mitochondrial inner membrane	-4.9
YGL200C / EMP24	Type I transmembrane protein	-4.8
YJL073W / JEM1	Peripheral membrane protein of membrane fraction	-4.8
YLR110C / CCW12	Cell wall mannoprotein	-4.8
YJL174W / KRE9	Cell wall organization and biogenesis	-4.7

YMR013C / SEC59	Membrane protein, required for core glycosylation	-4.4
YJL201W / ECM25	Cell wall organization and biogenesis	-4.3
YLR330W / CHS5	Cell wall chitin catabolism	-4.3
YOR311C / HSD1	ER membrane protein	-4.2
YOR099W / KTR1	Type II transmembrane protein	-4.0
YER031C / YPT31	GTPase, YPT32 homolog, ras homolog	-3.9
YKL039W / PTM1	Membrane protein (putative)	-3.9
YDR351W / SBE2	Cell wall organization and biogenesis	-3.7
YER157W / COG3	Peripheral membrane protein of membrane fraction	-3.7
YGR152C / RSR1	Plasma membrane	-3.6
YHR195W / NVJ1	Nuclear membrane	-3.6
YCR044C / PER1	Vacuolar membrane	-3.6
YIL005W / EPS1	Endoplasmic reticulum membrane	-3.5
YBR205W / KTR3	Membrane and cell wall organization and biogenesis	-3.5
YNL154C / YCK2	Plasma membrane	-3.4
YIL041W / GVP36	Peripheral membrane protein	-3.4
YML052W / SUR7	Integral membrane protein	-3.4
YLR083C / EMP70	Membrane fraction	-3.4
YDR057W / YOS9	Membrane-associated glycoprotein	-3.3
YDR126W / SWF1	Integral to membrane	-3.3
YOR049C / RSB1	Plasma membrane	-3.3
YDR292C / SRP101	Endoplasmic reticulum membrane	-3.2
YMR200W / ROT1	Membrane protein (putative)	-3.2
YIL140W / AXL2	Integral to plasma membrane	-3.1
YOR176W / HEM15	Ferrochelatase (protoheme ferrolyase)	-3.1
YLR220W / CCC1	Transmembrane Ca2+ transporter (putative)	-3.1
YBL020W / RFT1	Integral membrane protein	-3.0
YGR216C / GPI1	Membrane-proteins	-3.0
YLR390W / ECM19	Cell wall organization and biogenesis	-3.0
Miscellaneous		
YGL089C/ MF(ALPHA)2	Pheromone activity -response to pheromone during conjugation with cellular fusion	-78.4
YDL014W/NOP1	Methyltransferase activity	-25.1
YKL178C / STE3	a-factor receptor	-19.3
YJL088W / ARG3	Ornithine carbamoyltransferase	-17.4
YKL216W / URA1	Dihydroorotate dehydrogenase	-12.9
YLR197W / SIK1	U3 snoRNP protein	-11.8
YER122C / GLO3	Zinc finger protein	-11.2
YDR144C / MKC7	Aspartyl protease	-11.1
YGR119C / NUP57	Nuclear pore protein	-11.1
YDR372C / VPS74	Protein-vacuolar targeting	-11.0
YDL212W / SHR3	ER integral membrane component	-10.6
YGL115W / SNF4	Associates with Snf1p	-10.6
YGR183C / QCR9	Ubiquinol cytochrome c oxidoreductase complex	-10.6
YKL172W / EBP2	Nucleolar protein	-10.6
YHR005C / GPA1	G protein alpha subunit,	-10.2
YDR410C / STE14	Farnesyl cysteine-carboxyl methyltransferase	-10.0
YLR355C / ILV5	Acetohydroxyacid reductoisomerase	-10.0
YLR286C / CTS1	Endochitinase	-9.9
YHR193C / EGD2	GAL4 enhancer protein	-9.9
YGL098W	SNAP receptor	-9.9
YGL054C / ERV14	Endoplasmic reticulum membrane (IDA)	-9.9
YOR253W / NAT5	N-acetyltransferase	-9.7
YDR165W / TRM82	Protein binding	-9.6
YLR106C / MDN1	Midasin	-9.4
YOL143C / RIB4	6,7-dimethyl-8-ribityllumazine synthase	-9.3
YDL208W / NHP2	HMG-like protein	-9.3
YER112W / LSM4	U6 snRNA associated protein	-9.3
YHR063C / PAN5	Pantothenate biosynthesis	-9.1

YOR184W / SER1	Phosphoserine transaminase	-8.9
YGR167W / CLC1	Clathrin light chain	-8.5
YOR232W / MGE1	GrpE homolog	-8.5
YGL143C / MRF1	Mitochondrial polypeptide chain release factor	-8.4
YGR123C / PPT1	Protein amino acid phosphorylation	-8.3
YLR293C / GSP1	GTP-binding protein	-8.3
YGL009C / LEU1	Isopropylmalate isomerase	-8.2
YCL027W / FUS1	Plasma membrane	-8.1
YGL148W / ARO2	Chorismate synthase	-8.1
YER021W / RPN3	19S proteasome regulatory particle	-8.1
YDR098C / GRX3	Glutaredoxin	-7.9
YDL064W / UBC9	SUMO-conjugating enzyme	-7.9
YNL131W / TOM22	Mitochondrial import receptor protein	-7.5
YGL238W / CSE1	Nuclear membrane	-7.5
YIL076W / SEC28	Epsilon-COP coatomer subunit	-7.5
YML074C / FPR3	Peptidyl-prolyl cis-trans isomerase (PPIase)	-7.4
YDR044W / HEM13	Coproporphyrinogen III oxidase	-7.4
YHR216W / IMD2	IMP dehydrogenase homolog	-7.4
YOR056C / NOB1	Associated with the 26S proteasome	-7.3
YMR149W / SWP1	Oligosaccharyl transferase glycoprotein complex, delta subunit	-7.3
YDL226C / GCS1	ADP-ribosylation factor GTPase-activating protein	-7.3
YGL100W / SEH1	Nuclear pore complex subunit	-7.3
YDR399W / HPT1	Hypoxanthine guanine phosphoribosyltransferase	-7.2
YBR165W / UBS1	Protein ubiquitination	-7.2
YPL050C / MNN9	Required for complex glycosylation	-7.2
YOR310C / NOP58	U3 snoRNP protein	-6.9
YPL204W / HRR25	Casein kinase I isoform	-6.9
YIL044C / AGE2	ARF GAP with effector function(s)	-6.9
YBR101C / FES1	Hsp70 nucleotide exchange factor	-6.9
YDR174W / HMO1	High mobility group (HMG) family	-6.8
YDL097C /RPN6	Regulatory Particle Non-ATPase	-6.8
YGL086W /MAD1	Coiled-coil protein involved in the spindle-assembly checkpoint	-6.8
YOL052C / SPE2	Adenosylmethionine decarboxylase	-6.7
YNL213C	Mitochondrion organization and biogenesis	-6.7
YBR171W / SEC66	Protein transporter- an integral endoplasmic reticulum membrane protein complex	-6.7
YKL184W / SPE1	Ornithine decarboxylase	-6.7
YHR061C / GIC1	Incipient bud site	-6.6
YCL011C / GBP2	Contains RNA recognition motifs	-6.6
YHR074W / QNS1	Glutamine-dependent NAD synthetase	-6.6
YOR145C / PNO1	Associated with Nob1	-6.6
YOR209C / NPT1	Nicotinate phosphoribosyltransferase	-6.6
YDR151C / CTH1	CCCH zinc finger protein family that has two or more repeats of a novel zinc finger motif	-6.5
YGR128C / UTP8	U3 snoRNP protein	-6.5
YFR004W / RPN11	Endopeptidase	-6.5
YBR186W / PCH2	ATPase (putative)	-6.5
YPL218W / SAR1	ARF family, GTP-binding protein	-6.4
YDL153C / SAS10	U3 snoRNP protein	-6.2
YDL148C / NOP14	U3 snoRNP protein	-6.2
YHR060W / VMA22	Endoplasmic reticulum membrane	-6.1
YHR148W / IMP3	U3 snoRNP protein	-6.1
YBR015C / MNN2	Golgi alpha-1,2-mannosyltransferase (putative)	-6.1
YGR163W / GTR2	Similar to Gtr1, small GTPase (putative)	-6.1
YOR103C / OST2	Oligosaccharyl transferase complex	-6.0
YJL110C / GZF3	GATA zinc finger protein 3 homologous to Dal80 in structure and function	-6.0
YEL017C-A / PMP2	Proteolipid associated with plasma membrane H(+)-ATPase (Pma1p)	-6.0
YIL062C / ARC15	Mitochondrial membrane	-6.0
YFL045C / SEC53	Phosphomannomutase	-6.0
YOR039W / CKB2	Protein kinase CK2, beta' subunit	-6.0

YNR026C / SEC12	Guanine nucleotide exchange factor for Sar1p	-6.0
YBR290W / BSD2	Endoplasmic reticulum	-5.9
YGL011C / SCL1	Proteasome subunit YC7alpha/Y8 (protease yscE subunit 7)	-5.9
YER107C / GLE2	Nuclear pore complex subunit, rae1 S. pombe homolog	-5.9
YDL039C / PRM7	Integral to membrane	-5.8
YER136W / GDI1	GDP dissociation inhibitor	-5.8
YOR043W / WHI2	Phosphatase activator	-5.8
YOR323C / PRO2	Gamma-glutamyl phosphate reductase	-5.8
YNL231C / PDR16	Pdr17p homolog, Sec14p homolog	-5.7
YOR236W / DFR1	Dihydrofolate reductase	-5.7
YHR133C / YIG1	Potential homolog to mammalian Insig1	-5.7
YDR177W / UBC1	Ubiquitin-conjugating enzyme	-5.7
YDL207W / GLE1	Nuclear pore complex subunit,	-5.7
YOL062C / APM4	Clathrin associated protein complex medium subunit	-5.7
YPL125W / KAP120	Structural constituent of nuclear pore	-5.6
YOR061W / CKA2	Protein kinase CK2 alpha' subunit	-5.6
YPL273W / SAM4	AdoMet-homocysteine methyltransferase	-5.6
YER174C / GRX4	Glutaredoxin	-5.6
YHR013C / ARD1	Alpha-acetyltransferase major subunit	-5.6
YDL015C / TSC13	Endoplasmic reticulum membrane	-5.6
YGR184C / UBR1	Ubiquitin-protein ligase	-5.6
YDR189W / SLY1	t-SNARE-interacting protein that functions in ER-to-Golgi traffic	-5.6
YER123W / YCK3	Casein kinase I homolog	-5.6
YKL166C / TPK3	cAMP-dependent protein kinase catalytic subunit	-5.5
YDL188C / PPH22	Protein phosphatase type 2A complex	5.5
YIL117C / PRM5	Integral to membrane	-5.5
YDR331W / GPI8	Endoplasmic reticulum membrane,	-5.5
YCR034W / FEN1	1,3-beta-glucan synthase subunit (putative),	-5.5
YER100W / UBC6	Ubiquitin-conjugating enzyme	-5.5
YLR229C / CDC42	Rho subfamily of Ras-like proteins	-5.4
YOR101W / RAS1	ras homolog	-5.4
YKL004W / AUR1	Golgi apparatus	-5.4
YBR257W / POP4	Ribonuclease P complex	-5.4
YER120W / SCS2	Endoplasmic reticulum	-5.4
YDR414C / ERD1	Protein-ER retention	-5.3
YDR427W / RPN9	Proteasome regulatory particle subunit	-5.3
YJL053W / PEP8	Vacuolar protein similar to mouse gene H58	-5.3
YGL225W / VRG4	Golgi apparatus	-5.3
YDL147W / RPN5	Proteasome regulatory particle subunit	-5.3
YKL007W / CAP1	Capping protein	-5.3
YDR113C / PDS1	Nuclear securin	-5.2
YJR105W / ADO1	Adenosine kinase	-5.1
YLR399C / BDF1	Two bromodomains	-5.1
YOR321W / PMT3	Dolichyl phosphate-D-mannose: protein O-D-mannosyltransferase	-5.1
YDR240C / SNU56	U1 snRNP protein	-5.1
YGL142C / GPI10	Alpha 1,2 mannosyltransferase (putative)	-5.1
YGR105W / VMA21	Endoplasmic reticulum	-5.1
YJL001W / PRE3	20S proteasome subunit	-5.1
YBR039W / ATP3	ATP synthase gamma subunit	5.0
YHR046C / INM1	Myo-inositol-1(or 4)-monophosphatase	-5.0
YHR076W / PTC7	Type 2C Protein Phosphatase	-5.0
YDR100W / TVP15	Integral membrane protein	-5.0
YHR144C / DCD1	dCMP deaminase	-5.0
YJL002C / OST1	Endoplasmic reticulum lumen	-5.0
YHR201C / PPX1	Exopolyphosphatase	-5.0
YGR132C / PHB1	Phb2p homolog, mitochondrial protein	-5.0
YBR158W / AMN1	Protein binding	-5.0
YKR028W / SAP190	Type 2A-related protein phosphatase	-4.9
YJR139C / HOM6	L-homoserine: NADP oxidoreductase, homoserine dehydrogenase	-4.9
YMR120C / ADE17	5-aminoimidazole-4-carboxamide ribonucleotide	-4.9

YDR302W / GPI11	Endoplasmic reticulum	-4.9
YDR073W / SNF11	SWI/SNF global transcription activator complex component	-4.9
YHR188C / GPI16	Endoplasmic reticulum membrane, intrinsic protein	-4.9
YGL172W / NUP49	Nuclear pore complex subunit	-4.9
YGL065C / ALG2	Glycosyltransferase	-4.9
YMR116C / ASC1	G-beta like protein	-4.9
YDR235W / PRP42	U1 snRNP protein	-4.8
YEL060C / PRB1	Vacuolar protease B	-4.8
YBR119W / MUD1	U1 snRNP A protein	-4.8
YOR119C / RIO1	Protein serine kinase	-4.8
YPL059W / GRX5	Glutaredoxin	-4.8
YJR066W / TOR1	Phosphatidylinositol kinase homolog	-4.8
YOR108W / LEU9	Alpha-isopropylmalate synthase (2-isopropylmalate synthase)	-4.7
YKL099C / UTP11	U3 snoRNP protein	-4.7
YKL012W / PRP40	U1 snRNP protein	-4.7
YDR371W / CTS2	Sporulation-specific chitinase	-4.7
YDR313C / PIB1	Vacuolar membrane	-4.7
YER082C / UTP7	U3 snoRNP protein	-4.6
YER068W / MOT2	Zinc finger protein (putative)	-4.6
YBL032W / HEK2	Telomerase-dependent telomere maintenance	-4.6
YLL018C / DPS1	Aspartyl-tRNA synthetase	-4.6
YKL018W / SWD2	Compass (complex proteins associated with Set1p) component	-4.6
YLR181C / VTA1	Class E Vacuolar-Protein Sorting	-4.5
YOL094C / RFC4	Replication factor C subunit 4, similar to human RFC 40 kDa subunit	-4.5
YPR106W / ISR1	Protein kinase	-4.5
YCR035C / RRP43	Exosome 3->5 exoribonuclease complex	-4.5
YGL187C / COX4	Cytochrome c oxidase subunit IV	-4.5
YFL038C / YPT1	GTP-binding protein, ras homolog, similar to mammalian Rab1A protein	-4.5
YFR002W / NIC96	96 kDa nucleoporin-interacting component, nuclear pore complex subunit	-4.4
YGR172C / YIP1	Golgi apparatus	-4.4
YGL095C / VPS45	Protein complex assembly	-4.4
YDR236C / FMN1	Riboflavin kinase	-4.4
YBR199W / KTR4	Alpha-1, 2-mannosyltransferase (putative)	-4.4
YOR320C / GNT1	N-acetylglucosaminyltransferase	-4.4
YMR006C / PLB2	Lysophospholipase, phospholipase B	-4.3
YJR104C / SOD1	Cu, Zn superoxide dismutase	-4.3
YPR073C / LTP1	Phosphotyrosine phosphatase	-4.3
YJR086W / STE18	G protein gamma subunit, coupled to mating factor receptor	-4.3
YGL058W / RAD6	Ubiquitin-conjugating enzyme	-4.3
YDR072C / IPT1	Inositolphosphotransferase 1	-4.3
YBR166C / TYR1	Prephenate dehydrogenase (NADP+)	-4.3
YGR020C / VMA7	Vacuolar ATPase V1 domain subunit F	-4.3
YLR180W / SAM1	Methionine adenosyltransferase	-4.3
YFR033C / QCR6	Ubiquinol cytochrome C oxidoreductase subunit 6 (17 kDa)	-4.2
YHR057C / CPR2	Cyclophilin, peptidyl-prolyl cis-trans isomerase	-4.2
YBR283C / SSH1	Endoplasmic reticulum membrane	-4.2
YJL031C / BET4	Geranylgeranyltransferase type II alpha subunit	-4.2
YDR297W / SUR2	Sphingosine hydroxylase	-4.2
YOR261C / RPN8	Proteasome regulatory particle subunit	-4.2
YOR319W / HSH49	Mammalian splicing factor/U2 snRNP protein homolog	-4.1
YOR047C / STD1	MTH1 homolog	-4.1
YML121W / GTR1	Small GTPase (putative)	-4.1
YDR192C / NUP42	Protein associated with nuclear pore complexes	-4.1
YIL149C / MLP2	Coiled-coil protein (putative), similar to myosin and TPR)	-4.1
YAL041W / CDC24	Guanine nucleotide exchange factor	-4.1
YGR234W / YHB1	flavohemoglobin	-4.1
YBR109C / CMD1	Calcium ion binding	-4.1
YDL123W / SNA4	vacuolar membrane	-4.1
YBR153W / RIB7	Vitamin B2 biosynthesis	-4.0
YGL154C / LYS5	Alpha aminoadipate reductase phosphopantetheinyl transferase	-4.0

YBL036C	Alanine racemase	-4.0
YDR483W / KRE2	Alpha-1,2-mannosyltransferase	-4.0
YCL052C / PBN1	Protease B nonderepressible form	-4.0
YFL008W / SMC1	SMC chromosomal ATPase family member	-4.0
YER027C / GAL83	Protein amino acid phosphorylation	-4.0
YLR420W / URA4	Dihydrooratase	-4.0
YOR241W / MET7	Folylpolyglutamate synthetase	-4.0
YKL032C / IXR1	Intrastrand crosslink recognition protein	-3.9
YMR012W / CLU1	Sometimes copurifies with translation initiation factor eIF3	-3.9
YCL008C / STP22	Putative ubiquitin receptor	-3.9
YFR050C / PRE4	Necessary for peptidyl glutamyl peptide hydrolyzing activity	-3.9
YHR200W / RPN10	26S proteasome component, mammalian S5a protein homolog	-3.9
YAR002W / NUP60	Nuclear pore complex subunit	-3.9
YEL036C / ANP1	Mannosyltransferase complex	-3.9
YHR019C / DED81	Asparaginyl-tRNA synthetase	-3.9
YBR185C / MBA1	Aerobic respiration (IMP)	-3.9
YER072W / VTC1	S. pombe Nrf1p homolog	-3.9
YER177W / BMH1	Member of conserved eukaryotic 14-3-3 gene family	-3.9
YOR126C / IAH1	Isoamyl acetate-hydrolyzing esterase	-3.9
YOL056W / GPM3	Phosphoglycerate mutase	-3.9
YMR229C / RRP5	U3 snoRNP protein	-3.9
YPL117C / IDI1	Isopentenyl diphosphate:dimethylallyl diphosphate isomerase	-3.9
YNR035C / ARC35	Arp2/3 protein complex	-3.8
YMR264W / CUE1	Ubc7p binding and recruitment protein	-3.8
YDL236W / PHO13	p-nitrophenyl phosphatase	-3.8
YGR007W / MUQ1	Choline phosphate cytidylyltransferase	-3.8
YOR057W / SGT1	Ubiquitin ligase complex	-3.7
YLR359W / ADE13	Adenylosuccinate lyase	-3.7
YNR006W / VPS27	Cysteine rich putative zinc finger essential for function, hydrophilic protein	-3.7
YOL049W / GSH2	Glutathione synthetase	-3.7
YHR142W / CHS7	Endoplasmic reticulum membrane	-3.7
YBR274W / CHK1	Protein kinase	-3.7
YIL119C / RPI1	Ras inhibitor	-3.7
YER012W / PRE1	Proteasome subunit	-3.7
YDR226W / ADK1	Adenylate kinase	-3.7
YDR304C / CPR5	Cyclophilin D, peptidyl-prolyl cis-trans isomerase	-3.7
YBL026W / LSM2	snRNA-associated protein	-3.7
YGR202C / PCT1	Cholinephosphate cytidylyltransferase	-3.7
YPL031C / PHO85	Cyclin-dependent protein kinase	-3.6
YOL102C / TPT1	tRNA 2'-phosphotransferase	-3.6
YOR122C / PFY1	Actin polymerization and/or depolymerization	-3.6
YOR106W / VAM3	Syntaxin family	-3.6
YMR217W / GUA1	GMP synthase	-3.6
YCL028W / RNQ1	Transferable epigenetic modifier	-3.6
YIL118W / RHO3	GTP-binding protein, ras homolog	-3.6
YBL082C / RHK1	Dol-P-Man dependent alpha (1-3) mannosyltransferase	-3.5
YFR036W / CDC26	Anaphase-promoting complex	-3.5
YIL094C / LYS12	Homo-isocitrate dehydrogenase	-3.5
YBR011C / IPP1	Inorganic pyrophosphatase	-3.5
YAL016W / TPD3	Protein phosphatase 2A regulatory subunit A	-3.5
YGL210W / YPT32	GTPase, YPT31 homolog, ras homolog	-3.5
YHR011W / DIA4	Aerobic respiration	-3.5
YNL085W / MKT1	Retroviral protease signature protein	-3.5
YOR267C / HRK1	Protein kinase similar to Npr1	-3.4
YPL091W / GLR1	Glutathione oxidoreductase	-3.4
YDL116W / NUP84	Nuclear pore complex subunit	-3.4
YDL045C / FAD1	FAD biosynthesis	-3.4
YHR165C / PRP8	U5 snRNP and spliceosome component	-3.4
YDR004W / RAD57	RecA homolog, interacts with Rad 55p by two-hybrid analysis	-3.4
YGL092W / NUP145	Nuclear pore complex subunit	-3.4

YOR094W / ARF3	GTP-binding ADP-ribosylation factor	-3.3
YEL013W / VAC8	Armadillo repeat-containing protein	-3.3
YDR097C / MSH6	Human GTBP protein homolog	-3.3
YKL011C / CCE1	Cruciform cutting endonuclease	-3.3
YMR235C / RNA1	GTPase activating protein (GAP) for Gsp1p	-3.2
YPR113W / PIS1	Phosphatidylinositol synthase	-3.2
YGL019W / CKB1	Protein kinase CK2 beta subunit	-3.2
YBR256C / RIB5	Vitamin B2 biosynthesis	-3.2
YGR003W / CUL3	Ubiquitin-protein ligase	-3.2
YBL056W / PTC3	Protein phosphatase type 2C	-3.2
YHR051W / COX6	Cytochrome c oxidase subunit	-3.2
YCR052W / RSC6	Nucleosome remodeling complex	-3.2
YBR164C / ARL1	ADP-ribosylation factor-like protein 1	-3.2
YDL137W / ARF2	ADP-ribosylation factor 2	-3.2
YDR538W / PAD1	Phenylacrylic acid decarboxylase	-3.2
YDL074C / BRE1	Ubiquitin-protein ligase	-3.2
YHR086W / NAM8	snRNP U1	-3.2
YMR152W / YIM1	Protease, similar to E. coli leader peptidase	-3.2
YOR117W / RPT5	19S proteasome regulatory particle	-3.2
YOR136W / IDH2	NAD-dependent isocitrate dehydrogenase	-3.2
YDR099W / BMH2	Member of conserved eukaryotic 14-3-3 gene family	-3.1
YGL016W / KAP122	Karyopherin beta family member	-3.1
YGR195W / SKI6	RNAse PH homolog	-3.1
YHR123W / EPT1	sn-1,2-diacylglycerol ethanolamine- and cholinephosphotranferase	-3.1
YHR215W / PHO12	Biological_process unknown (ND)	-3.1
YER055C / HIS1	Histidine biosynthesis	-3.1
YNL207W / RIO2	Protein kinase	-3.1
YOL021C / DIS3	3'-5' exoribonuclease complex subunit	-3.1
YJR032W / CPR7	Cyclophilin 40, peptidyl-prolyl cis-trans isomerase (PPIase	-3.1
YLR395C / COX8	Cytochrome c oxidase chain VIII	-3.1
YOL065C / INP54	Inositol polyphosphate 5-phosphatase	-3.1
YMR183C / SSO2	t-SNARE	-3.1
YKL008C / LAC1	LAG1 longevity gene homolog	-3.1
YNL079C / TPM1	Tropomyosin I	-3.1
YOL038W / PRE6	20S proteasome alpha-type subunit	-3.1
YKL204W / EAP1	Functionally analogous to mammalian 4E-BPs functional and limited sequence similarity to CAF20	-3.0
YBR249C / ARO4	3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP) synthase isoenzyme	-3.0
YDR197W / CBS2	Cytochrome b translational activator	-3.0
YIL145C / PAN6	Pantothenate synthase	-3.0
YDL101C / DUN1	Protein kinase	-3.0
YHR068W / DYS1	Deoxyhypusine synthase	-3.0
YDL134C / PPH21	Protein phosphatase type 2A complex	-3.0
Unknown function		
YOR277C		-16.7
YOL109W		-16.2
YGL131C		-15.7
YDL228C		-13.4
YOR309C		-13.4
YMR321C		-12.9
YBR151W / APD1		-12.7
YDR417C		-12.6
YBL077W		-12.5
R361C / BCP1		-12.5
YER156C		-12.1
YGL039W		-11.9
YGL102C		-11.9
YDR442W		-11.8
YGR081C		-11.7

YNR046W	-11.6
YIL096C	-11.6
YHR039C / MSC7	-11.5
YLR040C	-11.2
YPL156C / PRM4	-11.0
YGL072C	-10.8
YHR128W / FUR1	-10.8
YDL050C	-10.6
YDR209C	-10.2
YLR221C / RSA3	-10.1
YGL033W / HOP2	-10.1
YIL080W	-10.0
YGL139W	-9.9
YPL142C	-9.8
YOR302W	-9.7
YKR060W / UTP30	-9.7
YPR044C	-9.5
YGL088W	-9.4
YDR101C / ARX1	-9.4
YOR169C	-9.4
YLR076C	-9.3
YAL036C / FUN11	-9.3
YBR042C	-9.3
YOR051C	-8.9
YHL039W	-8.9
YFR043C / YFR043C	-8.7
YDL121C	-8.6
YDR346C	-8.6
YNR061C	-8.5
YIL051C / MMF1	-8.5
YIR011C / STS1	-8.5
YDR367W	-8.2
YDL201W / TRM8	-8.2
YGL168W / HUR1	-8.1
YAR075W	-8.1
YIL110W	-8.0
YIL129C / TAO3	-7.9
YHR020W	-7.9
YNR021W	-7.9
YGL232W	-7.9
YGL080W	-7.9
YDR184C / ATC1	-7.8
YGL231C	-7.8
YOR091W	-7.8
YIL127C	-7.7
YOR164C	-7.6
YGL127C / SOH1	-7.6
YDR339C	-7.6
YHR083W	-7.5
YDR071C	-7.5
YHR100C	-7.4
YGR001C	-7.4
YDR133C	-7.4
YBL109W	-7.3
YHR081W / LRP1	-7.3
YEL001C	-7.2
YHR045W	-7.2
YMR049C / ERB1	-7.1
YGR283C	-7.1

YJR114W	-7.0
YCR096C / HMRA2	-7.0
YCR041W	-7.0
YJL122W	-6.9
YPR118W	-6.9
YJR115W	-6.8
YGR093W	-6.8
YLR198C	-6.8
YDR365C	-6.7
YDR094W	-6.7
YKL206C	-6.7
YJL169W	-6.7
YBR089W	-6.6
YKR071C / DRE2	-6.6
YMR123W / PKR1	-6.6
YLR073C	-6.5
YPL032C / SVL3	-6.5
YHR040W / BCD1	-6.5
YDR119W	-6.5
YBR096W	-6.5
YMR130W	-6.5
YPL146C	-6.4
YGR145W / ENP2	-6.4
YHR085W / IPI1	-6.4
YKR074W	-6.4
YJL097W	-6.3
YKR074W	-6.3
TOS9 / YEL007W	-6.3
YDR544C	-6.3
YJL097W	-6.3
YNL338W	-6.2
YLR003C	-6.2
YGL068W	-6.1
YBR014C	6.0
YGL069C	-6.0
YNL303W	-6.1
YPL199C	-6.1
YMR074C	-6.0
YHR192W	-5.9
YDL193W	-5.9
YMR298W	-5.9
YLL044W	-5.9
YBR273C	-5.8
YCL065W	-5.7
YHR214W-A	-5.7
YMR131C / RRB1	-5.7
YOR118W	-5.7
YJR118C / ILM1	-5.7
YGL107C / RMD9	-5.6
YDR157W	-5.6
YHR162W	-5.6
YIL027C / KRE27	-5.6
YGR151C	-5.6
YNL190W	-5.6
YOR305W	-5.5
YLR064W	-5.5
YDR458C	-5.5
YCR016W	-5.5
YGR106C	-5.5
YLR230W	-5.4

YOL111C	-5.4
YML125C	-5.4
TOS11 / YOR248W	-5.4
YDR117C	-5.4
YDR415C	-5.4
YGR173W / GIR1	-5.3
YEL033W	-5.3
YOR247W / SRL1	-5.2
YMR226C	-5.2
YJL217W	-5.2
YDL100C / ARR4	-5.2
YCL045C	-5.2
YBL083C	-5.1
YGL042C	5.1
YDR370C	-5.1
YDL099W	-5.1
YNL010W	-5.1
YKR030W / GMH1	-5.1
YLR243W	-5.0
YPR129W / SCD6	-5.0
YDR210W	-5.0
YBR159W	-5.0
YOL022C	-5.0
YLR301W	-5.0
YOR004W	-5.0
YJL151C / SNA3	-5.0
YNL174W	-4.9
YBR219C	-4.9
YNR009W	-4.9
YLR101C	-4.9
YML096W	-4.9
YOR271C	-4.9
YPL197C	-4.8
YLR022C	-4.8
YMR185W	-4.8
YHL012W	-4.8
YHR121W	-4.8
YOR015W	-4.7
YCR025C	-4.7
YBL006C / LDB7	-4.7
YPR126C	-4.6
YNL246W / VPS75	-4.6
YOL125W	-4.6
YNL043C	-4.6
YOR021C	-4.6
YGL079W	-4.6
YGL159W	-4.6
YDR132C	-4.6
YFR006W	-4.6
YGL083W / SCY1	-4.5
YGR038W / ORM1	-4.5
YHR059W / FYV4	-4.5
YJL023C / PET130	-4.5
YHL014C / YLF2	-4.5
YDR333C	-4.5
YLR050C	-4.5
YOR252W	-4.5
YKL177W	-4.5
YOR287C	-4.5
YOR238W	-4.4

YDL089W	4.4
YGR026W	-4.4
YGR024C	-4.4
YKR047W	-4.4
YNR051C / BRE5	-4.4
YKL084W	-4.3
YJR023C	-4.3
YPL144W	-4.3
YKR065C	-4.3
YBL051C / PIN4	-4.3
YDR198C	-4.3
YHL017W	-4.3
YGR086C / PIL1	-4.3
YDR182W / CDC1	-4.2
YER049W	-4.2
YIL091C	-4.2
YFL006W	-4.2
YBR141C	-4.2
YBR162W-A / YSY6	-4.2
YBL028C	-4.2
YDL211C	-4.2
YHR036W	-4.2
YLR065C	-4.2
YLR196W / PWP1	-4.2
YNL337W	-4.2
YLR172C / DPH5	-4.2
YPL068C	-4.2
YLR104W	-4.1
YLR041W	-4.1
YMR099C	-4.1
YHR132C / ECM14	-4.1
YDR134C	-4.1
YGR137W	-4.1
YDR063W	-4.1
YGL050W	-4.1
YHL043W / ECM34	-4.0
YHL008C	-4.0
YDL177C	-4.0
YEL048C	-4.0
YDR336W	-4.0
YDL237W	-4.0
YEL059W	-4.0
YBR190W	-4.0
YGR111W	-4.0
YLR412W	-4.0
YOL029C	-4.0
YOL124C	-4.0
YLR021W	-4.0
YGL174W / BUD13	-3.9
YGL219C / MDM34	-3.9
YBR161W	-3.9
YFR011C	-3.9
YCR090C	-3.9
YBR004C	-3.9
YBR293W	-3.9
YBL054W	-3.9
YGR035C	-3.9
YJR070C	-3.9
YOR286W	-3.9

YKR087C	-3.9
YKR007W	-3.8
YPL158C	-3.8
YOL014W	-3.8
YOL003C	-3.8
YLR250W / SSP120	-3.8
YHR032W	-3.8
YIL105C / LIT2	-3.8
YEL015W / EDC3	-3.8
YDR068W / DOS2	-3.8
YGR002C / GOD1	-3.8
YGR042W	-3.8
YDL172C	-3.8
YIL130W	-3.8
YCL005W	-3.7
YGR073C	-3.7
YGL010W	-3.7
YDR327W	-3.7
YOL092W	-3.7
YJR142W	-3.7
YOR355W / GDS1	-3.7
YJL152W	-3.7
YNL107W / YAF9	-3.7
YMR010W	-3.7
YNL074C / MLF3	-3.7
YOR131C	-3.7
YMR126C / DLT1	-3.7
YKL040C / NFU1	-3.7
YBL081W	-3.7
YJR124C	-3.7
YKL030W	-3.6
YLR261C / VPS63	-3.6
YGR125W	-3.6
YDR288W	-3.6
YBL009W	-3.6
YGL081W	-3.6
YDR352W	-3.6
YDR222W	-3.6
YBR025C	-3.6
YDR049W	-3.6
YLR257W	-3.6
YLR183C / TOS4	-3.6
YLR042C	-3.6
YGL193C	-3.5
YCL075W	-3.5
YDR090C	-3.5
YBL059W	-3.5
YIL092W	-3.5
YDR411C	-3.5
YDR219C	-3.5
YML082W	-3.5
YJR024C	-3.5
YKR079C	-3.5
YML056C / IMD4	-3.5
YOR088W	-3.5
YOL099C	-3.5
YFR041C	-3.4
YNL140C	-3.4
YGL110C / CUE3	-3.4
YEL006W	-3.4

YBR238C	-3.4
YIL103W	-3.4
YCL033C	-3.4
YGR272C	-3.4
YGR117C	-3.4
YDR486C / VPS60	-3.4
YER186C	-3.4
YEL029C / BUD16	-3.4
YHR151C	-3.4
YLR294C	-3.4
YNL182C / IPI3	-3.4
YOR315W	-3.4
YOL101C	-3.4
YPL229W	-3.4
YPL052W	-3.4
YML079W	-3.3
YOR29-24 / YOR073W	-3.3
YJR097W	-3.3
YOR342C	-3.3
YOR121C	-3.3
YMR091C / NPL6	-3.3
YOR102W	-3.3
YDR066C	-3.3
YGL101W	-3.3
YHL026C	-3.3
YGR136W / LSB1	-3.3
YDR539W	-3.3
YBR016W	-3.3
FSH1 / YHR049W	-3.3
YER080W	-3.3
YBR022W	-3.3
YFR020W	-3.3
YIL161W	-3.3
YDR154C	-3.3
YMR134W	-3.3
YNL234W	-3.2
YML053C	-3.2
YOR322C	-3.2
YKR075C	-3.2
YEL074W	-3.2
YER113C	-3.2
YDR360W	-3.2
YGR102C	-3.2
YDR295C / PLO2	-3.2
YGR033C	-3.2
YGR294W	-3.2
YGL082W	-3.2
YDL063C	-3.2
YGL188C	-3.2
YDR056C	-3.2
YDR466W	-3.1
YBL107C	-3.1
YGR071C	-3.1
YGL181W / GTS1	-3.1
YGL057C	-3.1
YBR233W / PBP2	-3.1
YBR242W	-3.1
YGL060W / YBP2	-3.1
YDR149C	-3.1

YGR187C / HGH1	-3.1
YOR112W	-3.1
YLL037W	-3.1
YKL179C / COY1	-3.1
YNR054C	-3.1
YJR134C / SGM1	-3.1
YMR184W	-3.1
YHR115C	-3.0
YFR042W	-3.0
YDR344C	-3.0
YGL152C	-3.0
YER057C / HMF1	-3.0
YJR013W	-3.0
YJR126C / VPS70	-3.0
YOR390W	-3.0
YJR129C	-3.0
YJL193W	-3.0
YMR073C	-3.0
YKL174C	-3.0
YLR202C	-3.0
YPL056C	-3.0
YOR200W	-3.0

Table 3.3a: Macroarray data: genes that were MHE after one hour exposure to 7% ethanol in the presence of acetaldehyde.

*Genes that also found to be MHE in the five-time point experiments for the same conditions (in the macroarray experiments).
#Genes also found to be MHE in microarray experiments for the same conditions at one -hour time point.

Names of Gene /ORF	Description of Genes	Fold Increase	Putative Transcription Factors
Ribosomal proteins			
RPL10	Ribosomal large subunit biogenesis	8.1	Hsf1p
RPS8A	Cytosolic small ribosomal subunit	7.6	Msn2/4p, Hsf1p, Yap1/2p
#RPL15A	Ribosomal large subunit biogenesis	7.6	Msn2/4p, Hsf1p
#RPS4A	Cytosolic small ribosomal subunit	7.6	Msn2/4p, Hsf1p
#RPL6A	Ribosomal large subunit biogenesis	7.2	Msn2/4p
#RPS26A	Cytosolic small ribosomal subunit	6.8	Msn2/4p, Hsf1p, Yap1/2p
*RPS31	Cytosolic small ribosomal subunit	6.8	Hsf1p
#RPL21A	Ribosomal large subunit biogenesis	6.4	Msn2/4p, Hsf1p
*RPL9A	Ribosomal large subunit biogenesis	6.4	Msn2/4p
#RPL5	Ribosomal large subunit biogenesis	6.4	Msn2/4p
*RPL35A	Ribosomal large subunit biogenesis	6.3	Hsf1p
#*RPS1B	Cytosolic small ribosomal subunit	6.3	Msn2/4p, Hsf1p, Yap1/2p
#RPS1A	Cytosolic small ribosomal subunit	6.2	Msn2/4p, Hsf1p, Yap1/2p
*RPL1B	Ribosomal large subunit biogenesis	6.2	Hsf1p
RPL35B	Ribosomal large subunit biogenesis	6.1	Hsf1p
RPL40A	Ribosomal large subunit biogenesis	6.1	Hsf1p, Yap1/2p
#RPS8B	Cytosolic small ribosomal subunit	6.0	Hsf1p, Yap1/2p
#RPL2A	Ribosomal large subunit biogenesis	5.9	Msn2/4p
RPL15B	Ribosomal large subunit biogenesis	5.8	-
#RPS7A	Cytosolic small ribosomal subunit	5.7	Hsf1p
RPS2	Cytosolic small ribosomal subunit	5.7	Msn2/4p, Hsf1p, Yap1/2p
#*RPL1A	Ribosomal large subunit biogenesis	5.7	Msn2/4p, Hsf1p, Yap1/2p
#RPS0A	Cytosolic small ribosomal subunit	5.6	Hsf1p, Yap1/2p
#*RPL30	Ribosomal large subunit biogenesis	5.5	-
#RPS24A	Cytosolic small ribosomal subunit	5.5	Hsf1p
#RPL2B	Ribosomal large subunit biogenesis	5.4	Msn2/4p, Hsf1p

#RPS5	Ribosomal large subunit biogenesis	5.4	Msn2/4p, Hsf1p
*RPL24A	Ribosomal large subunit biogenesis	5.2	Yap1/2p
#RPL16A	Ribosomal large subunit biogenesis	5.2	Hsf1p, Yap1/2p
RPS14A	Cytosolic small ribosomal subunit	5.2	Hsf1p
*RPL32	Ribosomal large subunit biogenesis	5.1	Msn2/4p, Hsf1p
#*RPL34A	Ribosomal large subunit biogenesis	5.1	-
RPL18A	Ribosomal large subunit biogenesis	5.0	-
#*RPS15	Cytosolic small ribosomal subunit	4.8	Msn2/4p, Hsf1p
RPS13	Cytosolic small ribosomal subunit	4.7	Msn2/4p, Hsf1p
#*RPL34B	Ribosomal large subunit biogenesis	4.7	Msn2/4p
#RPS10A	Cytosolic small ribosomal subunit	4.7	Hsf1p
*RPL25	Ribosomal large subunit biogenesis	4.7	Msn2/4p, Hsf1p
#RPL8B	Ribosomal large subunit biogenesis	4.6	Msn2/4p, Hsf1p
RPL3	Ribosomal large subunit biogenesis	4.5	-
#*RPS26B	Cytosolic small ribosomal subunit	4.5	Msn2/4p, Yap1/2p
#RPL8A	Ribosomal large subunit biogenesis	4.5	Hsf1p
#*RPL28	Ribosomal large subunit biogenesis	4.4	Hsf1p
#RPS4B	Cytosolic small ribosomal subunit	4.5	Msn2/4p
#RPS10B	Cytosolic small ribosomal subunit	4.4	Msn2/4p
RPL4B	Ribosomal large subunit biogenesis	4.3	-
*RPP0	Ribosomal large subunit biogenesis	4.3	Hsf1p
#*RPL17B	Ribosomal large subunit biogenesis	4.3	Msn2/4p, Yap1/2p
#*RPS27B	Cytosolic small ribosomal subunit	4.2	Msn2/4p, Yap1/2p
#RPS0B	Cytosolic small ribosomal subunit	4.2	Msn2/4p
*RPS29B	Cytosolic small ribosomal subunit	4.1	Msn2/4p, Hsf1p
#*RPS9B	Cytosolic small ribosomal subunit	4.0	Msn2/4p
RPL21B	Ribosomal large subunit biogenesis	4.0	Msn2/4p
*RPS20	Cytosolic small ribosomal subunit	4.0	Msn2/4p, Hsf1p
RPL24B	Ribosomal large subunit biogenesis	4.0	Hsf1p
RPL17A	Ribosomal large subunit biogenesis	4.0	Msn2/4p, Hsf1p,
#*RPS18B	Cytosolic small ribosomal subunit	4.0	Msn2/4p, Yap1/2p
RPL13A	Ribosomal large subunit biogenesis	3.9	Msn2/4p, Hsf1p
RPL14B	Ribosomal large subunit biogenesis	3.8	Msn2/4p
#*RPL20B	Ribosomal large subunit biogenesis	3.8	Hsf1p
#RPL11A	Ribosomal large subunit biogenesis	3.8	Msn2/4p
#RPL38	Ribosomal large subunit biogenesis	3.8	Msn2/4p, Hsf1p
RPS28S	Cytosolic small ribosomal subunit	3.7	-
#*RPL39	Ribosomal large subunit biogenesis	3.6	-
RPL42B	Ribosomal large subunit biogenesis	3.6	Msn2/4p
#RPL22A	Ribosomal large subunit biogenesis	3.6	Msn2/4p
#*RPL27A	Ribosomal large subunit biogenesis	3.4	Msn2/4p
#RPL11B	Ribosomal large subunit biogenesis	3.4	Msn2/4p
#RPL20A	Ribosomal large subunit biogenesis	3.4	Msn2/4p, Hsf1p, Yap1/2p
#RPL19A	Ribosomal large subunit biogenesis	3.3	Msn2/4p, Hsf1p
#RPS19A	Cytosolic small ribosomal subunit	3.3	Msn2/4p
#RPL14A	Ribosomal large subunit biogenesis	3.2	Msn2/4p, Hsf1p
#RPL31A	Ribosomal large subunit biogenesis	3.2	Msn2/4p, Hsf1p
#RPL19B	Ribosomal large subunit biogenesis	3.2	Hsf1p, Yap1/2p
RPS21A	Cytosolic small ribosomal subunit	3.1	Msn2/4p, Hsf1p
Stress response			
ALO1	Response to oxidative stress-D-arabinono-1,4-lactone oxidase activity	3.4	Msn2/4p, Hsf1p, Yap1/2p
HSP82	Stress response (heat shock protein)	3.3	Hsf1p
#SVS1	Response to chemical substance	3.3	Hsf1p
#SSZ1/YHR064C	Chaperone activity- protein biosynthesis	3.7	Msn2/4p, Hsf1p
SSA3	Stress response (Heat shock protein)	3.1	-
TRX2	Oxidative stress response (Thiol-disulfide exchange intermediate)	3.0	Yap1/2p

Transport			
#*VRG4	Nucleotide-sugar transporter activity	6.7	Msn2/4p, Hsf1p
#PHO84	Inorganic phosphate transporter (transmembrane protein)	5.8	Msn2/4p
#HXT3	Inorganic phosphate transport	5.2	Hsf1p
TFP1	Glucose transporter	4.6	Msn2/4p, Hsf1p
#KAP123	Hydrogen-transporting two-sector ATPase	4.5	Msn2/4p, Hsf1p
PHO3	Protein carrier activity- protein-nucleus import	4.3	Msn2/4p, Yap1/2p
YPT1	Thiamine transport (acid phosphatase)	3.4	Msn2/4p, Hsf1p
PMA1	Plasma membrane H+-ATPase	3.4	Msn2/4p, Hsf1p
CPR1	Electron transporter activity	3.3	Msn2/4p
TOM20	Endocytosis (actin cross-linking)	3.2	Msn2/4p, Hsf1p
SEC13	Mitochondrial translocation (protein transporter)	3.2	Hsf1p
ATP1	ER to Golgi transport* (M.F Unknown)	3.1	Msn2/4p, Hsf1p
PHO88	ATP synthesis coupled proton transport (Hydrogen-transporting two-sector ATPase)	3.1	Hsf1p
SSH1	Phosphate transport	3.1	Msn2/4p, Yap1/2p
OAC1	Co-translational membrane targeting (protein transport)	3.1	Hsf1p
SEC61	Sulfate transporte	3.1	Msn2/4p
ERV29	SRP-dependent, co-translational membrane targeting, translocation (transporter)	3.1	Msn2/4p, Hsf1p
SEC63	ER to Golgi transport	3.1	Hsf1p
Energy utilization			
#PDC5	Pyruvate decarboxylase activity-pyruvater metabolims	15.2	Msn2/4p, Hsf1p, Yap1/2p
PDA1	Pyruvate dehydrogenase (Lipoamide)	4.7	Hsf1p, Yap1/2p
PDC1	Pyruvate decarboxylase- pyruvate metabolism (Glucose and ethanol fermentation)	4.7	Msn2/4p, Hsf1p
TRR1	Thioredoxin reductase (NADPH) activity-regulation of redox hemostasis	3.1	Msn2/4p, Hsf1p
Protein metabolism			
EFT1	Protein synthesis elongation	6.2	Hsf1p
TEF1	Protein synthesis elongation)	5.6	Msn2/4p, Hsf1p
EFT2	Protein synthesis elongation	5.4	Msn2/4p, Hsf1p
LEU1	leucine biosynthesis	5.4	Msn2/4p, Hsf1p, Yap1/2p
#YEF3	Protein synthesis elongation	5.0	Msn2/4p
#YPS3	Protein metabolism and modification	5.0	Msn2/4p, Hsf1p
THS1	Protein biosynthesis –threonine-tRNA ligase activity	4.9	Msn2/4p, Yap1/2p
#HSL1	Protein animo acid phosphorylation	4.0	Msn2/4p, Yap1/2p
#SAM1	Methionine metabolism	3.8	Msn2/4p, Yap1/2p
PRE6	Ubiquitin-dependent protein degradation	3.4	Msn2/4p
#GRS1	glycine-tRNA ligase activity	3.3	Msn2/4p
CYS3	Cystathionine-gamma-lyase	3.3	Yap1/2p
DPS1	Protein biosynthesis (Aspartate-tRNA ligase)	3.2	Hsf1p
AAT2	Aspartate catabolism (Aspartate aminotransferase)	3.2	Hsf1p
LYS2	Amino acid biosynthesis (Aminoadipate-semialdehyde dehydrogenase)	3.1	Hsf1p
PRE9	ubiquitin-dependent protein degradation (Proteasome endopeptidase)	3.1	Hsf1p
KRS1	Lysine-tRNA ligase activity	3.1	Hsf1p, Yap1/2p
SES1	Serine-tRNA ligase activity	3.1	-
HSL7	Bud growth (Protein kinase inhibitor)	3.0	Msn2/4p, Hsf1p
ASN2	Asparagine synthase (glutamine-hydrolyzing)	3.0	Hsf1p, Yap1/2p
ILV5/ YLR355C	Branched chain family amino acid biosynthesis – ketol-acid reductoisomerase activity	3.0	Msn2/4p, Hsf1p
Protein folding			
KAR2	Proteins folding (m.f. Adenosinetriphosphatase*)	5.3	Msn2/4p
SSB2	Protein biosynthesis (m.f. chaperone)	4.6	Hsf1p
SSE1	Proteins folding (MF chaperone)	4.0	Msn2/4p, Hsf1p
ZUO1	Protein folding (MF chaperone)	3.5	Msn2/4p, Hsf1p
EFB1	Protein synthesis elongation (MF. Translation elongation factor)	3.5	Msn2/4p, Hsf1p

STI1	Protein folding (m.f. Unknown)	3.5	
Transcription factor			
RSC30	Transcription regulation- DNA binding	3.8	Hsf1p
CLU1	Protein synthesis initiation (Translation initiation factor)	3.5	Msn2/4p, Hsf1p
KEM1	35S primary transcript processing* (MF. 5'-3' exoribonuclease)	3.5	Msn2/4p, Hsf1p
MSS116	RNA splicing (MF. RNA helicase)	3.4	Hsf1p, Yap1/2p
ADR1	Transcription factor activity	3.3	Msn2/4p, Hsf1p
Histone and DNA /chromosome synthesis, repair, replication and modification			
HTA1	Histone H2A (Chromatin assembly/disassembly)	6.3	Hsf1p
*HTB2	Histone H2B (Chromatin assembly/disassembly)	5.1	Hsf1p
STM1	Telomeric DNA binding activity- telomere maintenance	5.0	Hsf1p
HTB1	Histone H2B (Chromatin assembly/disassembly)	4.8	Hsf1p
*POL30	Nucleotide-excision repair	4.6	Msn2/4p, Hsf1p
RFA1	Nucleotide-excision repair (RNA binding)	4.3	Msn2/4p, Hsf1p
*HTA2	Histone H2A (Chromatin assembly/disassembly)	4.0	Hsf1p
MSH2	DNA repair	4.0	Msn2/4p, Hsf1p
#RTT107/YHR154W	Negative regulation of DNA transposition	3.7	Hsf1p
RNR2	Ribonucleoside-diphosphate reductase	3.5	Msn2/4p, Hsf1p
Cell wall organization			
#CWP2	Structural constituent of cell wall- Cell wall organization and biogenesis	5.3	Msn2/4p, Hsf1p
YBR078W (ECM33)	Cell wall organization and biogenesis	4.2	Msn2/4p, Hsf1p
BGL2	Cell wall organization and biogenesis	3.9	Hsf1p, Yap1/2p
Cell cycle and growth			
BUD19	Bud site selection	4.5	-
BUD28	Bud site selection	4.4	Msn2/4p, Hsf1p
*PMI40	Catalyzers the interconversion of fructose-6-P and mannose-6-P	4.0	Msn2/4p, Hsf1p
AXL2	Axial budding	3.2	-
SHS1	Establishment of cell polarity (Structural constituent of cytoskeleton)	3.2	Msn2/4p
CUP1-2	Response to copper ion-required for cell growth	3.1	Hsf1p, Yap1/2p
RSR1	Polar budding (Signal tranducer)	3.1	Msn2/4p, Hsf1p, Yap1/2p
Lipid metabolism			
#ERG25	Ergosterol biosynthesis	4.1	Msn2/4p, Hsf1p
#*ERG3	Ergosterol biosynthesis	4.1	Msn2/4p
#*FEN1	Fatty acid biosynthesis	3.6	Msn2/4p, Hsf1p, Yap1/2p
ERG11	Ergosterol biosynthesis	3.5	Msn2/4p
ERG26	Ergosterol biosynthesis	3.3	Msn2/4p, Hsf1p
Miscellaneous			
SIM1	Microtubule cytoskeleton organization and biogenesis	5.1	Msn2/4p, Hsf1p, Yap1/2p
STE24	Zinc metallo-protease	4.8	Msn2/4p, Hsf1p
SCS2	Myo-inositol metabolism	4.3	-
STT3	Protein amino acid glycosylation	4.0	-
#DED81	Cytosolic asparaginyl-tRNA synthetase	3.9	Msn2/4p
BAT1	Branched-chain amino acid aminotransferase	3.9	Hsf1p
GND1	Phosphogluconate dehydrogenase (decarboxylating)	3.8	Msn2/4p
PMT1	O-linked glycosylation	3.6	Msn2/4p, Hsf1p
MSB2	Establishment of cell polarity	3.5	Msn2/4p
VAS1	Valine-tRNA ligase activity (MF. Valine-tRNA ligase)	3.5	Msn2/4p, Hsf1p
QRI1	UDP-N-acetylglucosamine biosynthesis (MF. UDP-N-acetylglucosamine pyrophosphorylase)	3.3	Hsf1p
*CPR5	Peptidyl-prolyl cis-trans isomerase activity	3.3	Msn2/4p, Yap1/2P

*PSA1	Synthesizes GDP-mannose from GTP and mannose-1-phosphate.	3.3	Hsf1p
HOM6	Homoserine dehydrogenase activity)- catalyzes third step in common pathway for methionine and threonine biosynthesis	3.2	Msn2/4p
UBA1	ubiquitin activating enzyme	3.2	Msn2/4p, Hsf1p
MCD4	GPI anchor biosynthesis	3.1	Hsf1p
Unknown function			
YHR049W	Gene of unknown function	11.4	Msn2/4p, Hsf1p
TOS1	Gene of unknown function	8.8	Msn2/4p, Hsf1p
ASC1	Gene of unknown function	7.6	-
AHA1	Gene of unknown function	7.1	Msn2/4p, Hsf1p
YLL044W	Gene of unknown function	6.8	Msn2/4p, Hsf1p
YLR076C	Gene of unknown function	6.2	Msn2/4p, Hsf1p
*YGL102C	Gene of unknown function	6.1	Hsf1p
*PRY2	Gene of unknown function	6.0	Msn2/4p
YBR077C	Gene of unknown function	5.8	-
SGT2/ YOR007C	Gene of unknown function	5.5	Hsf1p
*YDR417C	Gene of unknown function	5.4	Msn2/4p, Yap1/2p
*YDL228C	Gene of unknown function	5.2	Msn2/4p, Hsf1p
YFR044C	Gene of unknown function	4.8	Msn2/4p
YLR339C	Gene of unknown function	4.8	Hsf1p
*YOL109W	Gene of unknown function	4.8	Hsf1p
*YBR089W	Gene of unknown function	4.2	-
#*YEL001C	Gene of unknown function	4.2	-
YGR106C	Gene of unknown function	3.7	Msn2/4p, Hsf1p, Yap1/2p
*YML133C	Gene of unknown function	3.7	Msn2/4p
ARR4/YDL100 C	Gene of unknown function	3.6	Msn2/4p
#YGR151C	Gene of unknown function	3.5	Msn2/4p, Yap1/2p
YKL030W	Gene of unknown function	3.5	-
*YPL197C	Gene of unknown function	3.5	Yap1/2p
FIT2/YOR382W	Gene of unknown function	3.5	-
YKL056C	Gene of unknown function	3.5	Hsf1p
YOP1/YPR028 W	Gene of unknown function	3.4	-
YBR025C	Gene of unknown function	3.4	Msn2/4p, Hsf1p
*YGL131C	Gene of unknown function	3.4	Msn2/4p, Hsf1p
TRA1	Gene of unknown function	3.4	Msn2/4p, Hsf1p, Yap1/2p
YDR154C	Gene of unknown function	3.3	Msn2/4p
#YHR095W	Gene of unknown function	3.3	Msn2/4p
YFL066C	Gene of unknown function	3.3	Msn2/4p
*YBL109W	Gene of unknown function	3.3	Msn2/4p
YKR012C	Gene of unknown function	3.3	Msn2/4p, Hsf1p
YNL134C	Gene of unknown function	3.3	Msn2/4p, Yap1/2p
DRE2/YKR071 C	Gene of unknown function	3.1	Msn2/4p, Hsf1p, Yap1/2p
*YJR115W	Gene of unknown function	3.1	Msn2/4p, Hsf1p
TOS4/YLR183C	Gene of unknown function	3.0	Msn2/4p, Hsf1p

*ORFs without identified transcription factors

Table 3.3b: Macroarray data: genes that were LHE after one hour exposure to 7% ethanol in the presence of acetaldehyde.

#Genes also found to be MHE in microarray experiments for the same conditions.

Names of Gene /ORF	Description of Genes	Fold Decrease	Putative Transcription Factors
Energy utilization			
RHR2	Glycerol-1-phosphatase activity-response to	4.3	Msn2/4p

	osmotic shock		
Cell cycle and growth			
#SPS100	Spore wall assembly (sensus Saccharomyces)	5.5	Msn2/4p, Hsf1pYap1/2p
Unknown function			
#SPI1	Gene of unknown function	3.0	Msn2/4p, Yap1/2p
#YDL223C/ HBT1	Gene of unknown function	3.5	Msn2/4p, Hsf1p

Table 3.4a: Macroarray data: genes that were MHE after five hour exposure to 7% ethanol in the presence of acetaldehyde.

*Genes that also found to be MHE in the one-hour time point experiment for the same conditions.

ORF/Gene Name	Description of Gene Product	Fold Increase	Putative Transcription Factors
Ribosomal proteins			
RPS27A	Cytosolic small ribosomal subunit	5.2	Msn2/4p
*RPL1A	Ribosomal large subunit biogenesis	5.0	Msn2/4p, Hsf1p, Yap1/2p
*RPS20	Cytosolic small ribosomal subunit	4.6	Msn2/4p, Hsf1p
YRPS18A	Cytosolic small ribosomal subunit	4.4	Yap1/2p
*RPL9A	Ribosomal large subunit biogenesis	4.2	Msn2/4p, Hsf1p
*RPL30	Ribosomal large subunit biogenesis	4.2	-
RPL27B	Ribosomal large subunit biogenesis	4.1	Msn2/4p, Hsf1p
*RPL39	Ribosomal large subunit biogenesis	4.0	-
RPL33B	Ribosomal large subunit biogenesis	4.0	Msn2/4p, Hsf1p
RPL22B	Ribosomal large subunit biogenesis	3.9	Hsf1p
*RPS18B	Cytosolic small ribosomal subunit	3.8	Msn2/4p
RPS7A	Cytosolic small ribosomal subunit	3.6	Msn2/4p, Hsf1p
*RPL25	Ribosomal large subunit biogenesis	3.6	Msn2/4p, Hsf1p
RPS16B	Cytosolic small ribosomal subunit	3.6	Msn2/4p, Hsf1p
RSM25	Mitochondrial small ribosomal subunit	3.6	-
RPS23A	Cytosolic small ribosomal subunit	3.6	Msn2/4p, Hsf1p
*RPL34A	Ribosomal large subunit biogenesis	3.5	-
*RPS31	Cytosolic small ribosomal subunit	3.5	Hsf1p
RPS28A	Cytosolic small ribosomal subunit	3.4	Msn2/4p, Hsf1p
RPS2	Cytosolic small ribosomal subunit	3.4	Msn2/4p, Hsf1p, Yap1/2p
RPS25A	Cytosolic small ribosomal subunit	3.4	Yap1/2p
MRP21	Mitochondrial small ribosomal subunit	3.4	Msn2/4p
RPS17B	Cytosolic small ribosomal subunit	3.4	Hsf1p
*RPL1B	Ribosomal large subunit biogenesis	3.3	Hsf1p
*RPL34B	Ribosomal large subunit biogenesis	3.3	Msn2/4p
*RPS26B	Cytosolic small ribosomal subunit	3.3	Msn2/4p, Yap1/2p
*RPL28	Ribosomal large subunit biogenesis	3.3	Hsf1p
*RPL20B	Ribosomal large subunit biogenesis	3.3	Hsf1p
RPS17A	Cytosolic small ribosomal subunit	3.3	Msn2/4p
*RPP0	Protein biosynthesis	3.2	Hsf1p
*RPL24A	Ribosomal large subunit biogenesis	3.2	Yap1/2p
*RPL16A	Ribosomal large subunit biogenesis	3.2	Hsf1p, Yap1/2p
*RPS29B	Cytosolic small ribosomal subunit	3.2	Msn2/4p, Hsf1p
RPL40B	Ribosomal large subunit biogenesis	3.2	Msn2/4p, Hsf1p, Yap1/2p
*RPL17B	Ribosomal large subunit biogenesis	3.2	Msn2/4p, Yap1/2p

*RPS1B	Cytosolic small ribosomal subunit	3.2	Msn2/4p, Hsf1p, Yap1/2p
*RPL32	Ribosomal large subunit biogenesis	3.1	Msn2/4p, Hsf1p
*RPS27B	Cytosolic small ribosomal subunit	3.1	Msn2/4p, Yap1/2p
*RPL27A	Ribosomal large subunit biogenesis	3.1	Msn2/4p
RPS11B	Cytosolic small ribosomal subunit	3.1	Hsf1p, Yap1/2p
*RPL35A	Cytosolic small ribosomal subunit	3.1	Hsf1p
RPS21B	Cytosolic small ribosomal subunit	3.1	Msn2/4p, Hsf1p
RPL42A	Ribosomal large subunit biogenesis	3.1	Hsf1p
RPL3	Ribosomal large subunit biogenesis	3.1	-
RPS11A	Cytosolic small ribosomal subunit	3.0	Msn2/4p, Hsf1p
*RPS9B	Cytosolic small ribosomal subunit	3.0	Msn2/4p
*RPS15	Cytosolic small ribosomal subunit	3.0	Msn2/4p, Hsf1p
Ribosomal subunit			
NOP1	Methyltransferase activity-RNA methylation	6.8	Msn2/4p, Hsf1p
MRT4	Ribosomal large subunit assembly and maintenance	5.0	Msn2/4p
NUG1	rRNA processing	4.2	Hsf1p
KRR1	rRNA processing	3.8	Msn2/4p,
NSA1	Ribosomal large subunit biogenesis	3.4	Msn2/4p, Hsf1p, Yap1/2p
ARX1	Ribosomal large subunit	3.3	Hsf1p
SQT1	Ribosomal large subunit assembly and maintenance	3.2	Msn2/4p
BRX1	Ribosomal large subunit assembly and maintenance	3.2	-
Cell cycle and growth			
MSC7	Meiotic recombination	4.8	Msn2/4p, Hsf1p
RHC21	Mitotic chromosome condensation	4.3	Msn2/4p, Hsf1p
PCL2	Cell cycle-cyclin-dependent protein kinase, regulator activity	4.2	Msn2/4p
STS1	Chromosome segregation	4.1	Msn2/4p
WHI2	Regulation to growth/response to stress-phosphatase activator activity	3.7	Hsf1p, Yap1/2p
CDC28	Regulation of cell cycle- cyclin-dependent protein kinase activity	3.6	Msn2/4p, Hsf1p
AMN1	Negative regulation of exit from mitosis	3.4	Msn2/4p, Hsf1p, Yap1/2p
UBC9	G2/M transition of mitotic cell cycle- ubiquitin-like conjugating enzyme activity	3.3	Msn2/4p, Hsf1p
CLB5	Cyclin-dependent protein kinase, regulator activity - G1/S and G2/M transition of mitotic cell cycle	3.3	Msn2/4p
BUD28	Bud site selection	3.2	Msn2/4p, Hsf1p
YRB1	G1/S transition of mitotic cell cycle	3.1	Hsf1p
TOR1	G1 phase of mitotic cell cycle	3.1	Msn2/4p
CKB2	G1/S & G2/M transition of mitotic cell cycle-protein kinase CK2 activity	3.0	Hsf1p
Stress response			
YNL234W	Response to stress-heme binding	4.5	Msn2/4p, Hsf1p
CUP1-1	Response to copper ion	4.3	Hsf1p, Yap1/2p
GRX3	Response to oxidative stress- thiol-disulfide exchange intermediate activity	4.2	Msn2/4p, Hsf1p
HOR2	Glycerol-1-phosphatase activity- glycerol biosynthesis (Response to osmotic shock)	4.0	Hsf1p
CUP1-2	Response to copper ion	4.0	Hsf1p, Yap1/2p
HAL1	Salinity response		Msn2/4p, Hsf1p
RHR2	Glycerol-1-phosphatase activity- glycerol biosynthesis (Response to osmotic stress)	3.1	Msn2/4p, Hsf1p
SHO1	Osmosensor activity- osmosensory signaling pathway via Sho1 osmosensor	3.0	-
Transport & translocation			
MRS3	Carrier activity- Transport	4.0	Msn2/4p, Hsf1p
YIP3	ER to Golgi transport	3.8	Msn2/4p
ARN2	siderochrome-iron transporter activity	3.7	Msn2/4p

MTR2	Poly (A)+ mRNA-nucleus export-Protein binding	3.7	Msn2/4p, Hsf1p
YKT6	v-SNARE activity-intra Golgi transport	3.7	Msn2/4p
HXT1	hexose transport-fructose, glucose, mannose and galactose transporter activity	3.6	Hsf1p
*VRG4	Nucleotide-sugar transport-activity	3.6	Msn2/4p, Hsf1p
VID24	Vesicle-mediated transport	3.6	Hsf1p
TOM20	Protein transport activity- mitochondrial translocation	3.5	Msn2/4p, Hsf1p
PEP8	Retrograde (endosome to Golgi) transport	3.5	Msn2/4p
NTF2	RNA protein binding- nucleocytoplasmic transport	3.5	Msn2/4p, Hsf1p
SAR1	SAR small monomeric GTPase activity- ER to Golgi transport	3.5	Msn2/4p
BOS1	v-SNARE activity-intra Golgi transport	3.4	-
ATP7	ATP synthesis coupled proton transport- ATP synthesis coupled proton transport	3.3	Msn2/4p, Hsf1p, Yap1/2p
SCO2	Copper transport	3.3	Msn2/4p, Hsf1p
LSM4	Pre-mRNA splicing factor activity-mRNA splicing and rRNA processing	3.2	Msn2/4p, Hsf1p, Yap1/2p
YDR061W	ATP-binding cassette (ABC) transporter activity	3.2	Hsf1p
ZRT3	Zinc ion transporter activity	3.2	Msn2/4p
OAC1	Oxaloacetate transport- oxaloacetate carrier activity	3.2	Hsf1p
ERV41	ER to golgi transport	3.2	Msn2/4p, Hsf1p
VPS55	Late endosome to vacuole transport	3.2	Msn2/4p, Hsf1p
ERV14	ER to golgi transport	3.1	Hsf1p
COG2	Protein binding- ER to golgi transport	3.1	Msn2/4p, Hsf1p
YIP1	Vesicle-mediated transport	3.1	Hsf1p
VMA7	Hydrogen-exporting ATPase activity	3.1	Msn2/4p
ERO1	Electron carrier activity	3.1	Msn2/4p, Hsf1p, Yap1/2p
CHS7	ER to Golgi transport	3.0	Msn2/4p
DDI1	SNARE binding- vesicle-mediated transport	3.0	Msn2/4p
GLO3	ER to Golgi transport	3.0	Msn2/4p
BET5	ER to Golgi transport	3.0	Msn2/4p
Energy utilization			
OYE2	NADPH dehydrogenase activity	3.3	Msn2/4p, Hsf1p, Yap1/2p
SDH3	succinate dehydrogenase activity - oxidative phosphorylation, succinate to ubiquinone	3.3	-
SER33	Phosphoglycerate dehydrogenase activity- serine family amino acid biosynthesis	3.2	Msn2/4p
TRR1	Regulation of redox homeostasis- thioredoxin reductase (NADPH) activity	3.1	Msn2/4p Hsf1p
*PMI40	Mannose-6-phosphate isomerase activity	3.1	Msn2/4p Hsf1p
Protein folding, synthesis, modification, translocation, degradation and complex assembly			
YPT1	Protein complex assembly-RAB small monomeric GTPase activity	4.2	Msn2/4p, Hsf1p
VMA22	Protein complex assembly-Chaperone activity	3.8	Msn2/4p, Hsf1p
HIS6	Histidine biosynthesis- 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino) methylideneamino]imidazole-4-carboxamide isomerase activity	3.7	-
MRF1	Protein biosynthesis- translation release factor activity	3.7	Msn2/4p,
SAP4	Protein serine/threonine phosphatase activity-G1/S transition mitotic cell cycle	3.7	Msn2/4p
MKC7	Aspartic-type signal peptidase activity- proteolysis and peptidolysis	3.7	Hsf1p, Yap1/2p
SER1	Serine biosynthesis - phosphoserine transaminase activity	3.7	Msn2/4p,
HSL7	Protein-arginine N-methyltransferase activity-regulation of cell cycle	3.6	Msn2/4p, Hsf1p
ARO2	Aromatic amino acid family biosynthesis	3.6	Msn2/4p, Hsf1p
SHR3	Chaperone activity	3.5	Msn2/4p, Hsf1p
GIC1	Small GTPase regulatory/interacting protein activity	3.5	Msn2/4p
EGD1	Chaperone activity	3.5	Msn2/4p, Hsf1p
MGE1	Chaperone activity- mitochondrial translocation	3.4	-

GIC2	Small GTPase regulatory/interacting protein activity	3.4	Msn2/4p, Hsf1p
RPN4	ubiquitin-dependent protein catabolism-endopeptidase activity	3.3	Msn2/4p, Hsf1p, Yap1/2p
UMP1	proteasome activator activity- protein catabolism	3.3	Hsf1p
HSE1	Protein binding	3.2	Msn2/4p, Hsf1p
CYS9	Lysine biosynthesis, aminoadipic pathway	3.2	Hsf1pYap1/2p
PBN1	Protein processing	3.1	-
LYS1	Lysine biosynthesis, aminoadipic pathway	3.1	Hsf1p
SSB1	Protein biosynthesis	3.1	-
CYS4	Cysteine biosynthesis-cystathione beta-synthase activity	3.0	Msn2/4p, Hsf1p
EGD2	Chaperone activity	3.0	Msn2/4p, Yap1/2p
PPH21	Protein phosphatase type 2A activity- protein amino acid dephosphorylation	3.0	Hsf1p, Yap1/2p
Signal transduction proteins			
STE4	Signal transduction during conjugation with cellular fusion - heterotrimeric G-protein GTPase activity	3.1	Msn2/4p
RSR1	RAS small monomeric GTPase activity-signal transducer activity	3.0	Msn2/4p, Hsf1p, Yap1/2p
PEX7	Peroxisome targeting signal receptor activity	3.0	Msn2/4p, Hsf1p
Transcription and translation factor and process			
URE2	Transcription co-repressor activity	4.5	Msn2/4p
GCN4	Transcriptional activator activity- regulation of transcription from Pol II promoter	4.1	Msn2/4p, Hsf1p, Yap1/2p
RRP33	ATP dependent RNA helicase activity-35S primary transcript processing Or mRNA splicing	4.1	-
	Transcriptional repressor activity- regulation of	4.1	
NRG1	transcription from Pol II promoter		Msn2/4p
SNU13	Pre-mRNA splicing factor activity-mRNA splicing-processing 20S pre-rRNA	4.0	Msn2/4p, Hsf1p
ELP4	Pol II transcription elongation factor activity-regulation of transcription from Pol II promoter	4.0	Msn2/4p
MED6	RNA polymerase II transcription mediator activity-transcription from Pol II promoter	3.9	Msn2/4p, Hsf1p
YOR302W	Translation regulator activity- regulation of protein biosynthesis	3.9	Msn2/4p
CAF20	Translation regulation activity/ negative regulation of translation	3.5	Msn2/4p, Hsf1p
IKI1	Pol II transcriptional elongation factor activity-regulation of transcription from Pol II promoter	3.4	Msn2/4p
MTR3	35S primary transcript processing and mRNA catabolism- 3'-5' exoribonuclease activity	3.4	Msn2/4p, Hsf1p, Yap1/2p
PXR1	35S primary transcript processing-RNA bingding	3.3	Msn2/4p, Hsf1p
SOH1	Transcription from Pol II promoter	3.3	-
RPB8	Transcription from Pol I, II and III promoter	3.3	Hsf1p
RPP1	rRNA and rRNA processing- ribonuclease MRP activity	3.2	Hsf1p
GAR1	35S primary transcript processing-RNA binding	3.2	Msn2/4p, Hsf1p
UTP1	SnoRNA binding	3.2	-
RRP45	3'-5' exoribonuclease activity- 35S primary transcript processing	3.2	Msn2/4p, Hsf1p
SNF4	Protein kinase activator activity- regulation of transcription from Pol II promoter	3.2	Msn2/4p, Hsf1p
LHP1	RNA binding and tRNA pocessing	3.2	Msn2/4p
CDC33	Translation initiation - translation initiation factor activity	3.2	Msn2/4p, Hsf1p
PGD1	RNA polymerase II transcription mediator activity	3.1	Msn2/4p
SAD1	RNA polymerase II transcription mediator activity-transcription from Pol II promoter	3.1	Msn2/4p
IMP3	Pre-mRNA splicing factor activity-mRNA splicing	3.1	Msn2/4p
HMLALPHA2	35S primary transcript processing-snoRNA binding	3.0	Msn2/4p
CUP2	Transcription co-repressor activity-regulation of transcription from Pol II promoter	3.0	Hsf1p

ELP2	ligand-regulatedtranscription factor activity-transcription initiation from Pol II promoter (response to copper ion)	3.0	Msn2/4p, Hsf1p
SMD3	Pol II transcription elongation factor activity-regulation of transcription from Pol II promoter	3.0	Hsf1p
TIF2	mRNA splicing – pre-mRNA splicing factor activity	3.0	Msn2/4p, Hsf1p
USE1	ER to Golgi vesicle-mediated transport	3.0	Msn2/4p, Hsf1p
Nucleotide Metabolism			
*HTB2	DNA binding-chromatin assembly/disassembly	4.2	Hsf1p
*HTA2	DNA binding- chromatin assembly/disassembly	4.0	Hsf1p
FCY2	Cytosine-purine permease activity-purine transport/Cytosine transport	3.8	Msn2/4p, Hsf1p
*POL30	DNA polymerase processivity factor activity	3.6	Msn2/4p, Hsf1p
BMH1	DNA binding	3.5	Hsf1p
CDC1	DNA recombinant ion	3.5	Msn2/4p, Yap1/2p
HEX3	DNA binding/DNA recombinantion/response to DNA damage	3.4	Hsf1p, Yap1/2p
HRR25	DNA repair - protein and casein kinase activity	3.4	Msn2/4p
AAH1	Adenine catabolism- adenine deaminase activity	3.3	Msn2/4p
FUR1	Uracil phosphoribosyltransferase activity-pyrimidine salvage	3.2	Hsf1p
SRL1	Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	3.2	Msn2/4p, Hsf1p
PRI1	Alpha DNA polymerase –DNA replication and priming activity	3.2	Msn2/4p, Hsf1p
ADO1	Purine base metabolism- adenosine kinase activity	3.1	Yap1/2p
Cytoskeleton organization and maintenance			
CDC10	Structural constituent of cytoskeleton- Cytokinesis	3.3	Msn2/4p, Hsf1p
ARC15	Actin binding-actin cortical patch assembly	3.1	Hsf1p
BN15	Cytokinesis	3.1	Hsf1p
ENT4	Actin cortical patch assembly - cytoskeletal adaptor activity	3.1	Hsf1p
SPC34	Structural constituent of cytoskeleton - microtubule nucleation	3.0	-
Cell wall organization			
DSE2	Glucan 1,3-beta-glucosidase activity-cell wall organization and biogenesis	4.6	-
CWP2	Structural constituent of cell wall	3.6	Msn2/4p, Hsf1p
KTR6	Cell wall organization and biogenesis-mannosylphosphate transferase activity	3.6	Msn2/4p, Hsf1p
KTR7	Cell wall organization and biogenesis-mannosyltransferase activity	3.5	Msn2/4p, Hsf1p
ECM34	Cell wall organization and biogenesis	3.4	-
SBE22	Cell wall organization and biogenesis	3.2	Msn2/4p, Hsf1p
UTR2	Cell wall organization and biogenesis	3.2	Hsf1p
CWH43	Cell wall organization and biogenesis	3.1	Msn2/4p, Hsf1p
Lipid metabolism			
*ERG3	Ergosterol biosynthesis-C-5 sterol desaturase activity	4.5	Msn2/4p
ERG9	Farnesyl-diphosphate farnesyltransferase activity-ergosterol biosynthesis	3.3	Msn2/4p, Hsf1p
*FEN1	Fatty acid elongation	3.0	Msn2/4p, Hsf1pYap1/2P
Miscellaneous			
QCR9	Ubiquinol-cytochrome C reductase activity-oxidative phosphorylation, ubiquinone to cytochrome C	5.2	-
CTS1	Chitinase activity- cytokinesis, completion of separation	4.3	Msn2/4p
YBR159W	Ketoreductase activity-fatty acid elongation	4.3	Msn2/4p

FPR1	Peptidyl-prolyl cis-trans isomerase activity	4.0	-
MF(APHA)1	Pheromone activity-response to pheromone during conjugation with cellular fusion	3.9	Msn2/4p, Hsf1p
MMS2	Ubiquitin conjugating enzyme activity- ubiquitin-dependent protein catabolism	3.8	Hsf1p
PRE9	Endopeptidase activity- ubiquitin-dependent protein catabolism	3.8	Hsf1p
SWP1	Dolichyl-diphospho-oligosaccharide-protein glycosyltransferase activity-N-linked glycosylation	3.8	Msn2/4p
URA1	Dihydroorotate dehydrogenase activity - pyrimidine base biosynthesis	3.6	Msn2/4p, Hsf1p, Yap1/2P
ERP3	Secretory pathway	3.5	Hsf1p
YGL039W	Dihydrokaempferol 4-reductase activity	3.5	Msn2/4p, Hsf1p
SAS4	Acetyltransferase activity	3.5	Msn2/4p, Hsf1p
ERP5	Secretory pathway	3.5	Hsf1p
RIB1	Vitamin B2 biosynthesis- cyclohydrolase activity	3.5	Msn2/4p
CPR2	Peptidyl-prolyl cis-trans isomerase activity	3.5	Msn2/4p
VPS74	Protein-vacuolar targeting	3.4	Msn2/4p, Hsf1p
*PSA1	Mannose-1-phosphate guanylyltransferase activity-GDP-mannose biosynthesis	3.3	Msn2/4p, Hsf1p
SHP1	Glycogen metabolism	3.3	Msn2/4p, Yap1/2P
DFG10	Pseudohyphal growth	3.3	Msn2/4p, Hsf1p
CSN9	Adaptation to pheromone during conjugation with cellular fusion	3.3	Msn2/4p, Hsf1p
SCC3	Peptidyl-prolyl cis-trans isomerase activity	3.3	-
SOL3	pentose-phosphate shunt, oxidative branch	3.3	Msn2/4p, Hsf1p
YFR006W	Xaa-Pro aminopeptidase activity	3.2	Msn2/4p, Hsf1p
BIO2	Biotin synthase activity-Biotin biosynthesis	3.2	Msn2/4p, Hsf1p
YBR042C	Acyltransferase activity-phospholipid biosynthesis	3.2	Msn2/4p, Hsf1p
OCH1	Alpha-1,6-mannosyltransferase activity- N-linked glycoprotein maturation	3.2	Msn2/4p, Hsf1p
YDR140W	S-adenosylmethionine-dependent methyltransferase activity	3.2	Msn2/4p, Hsf1p, Yap1/2P
*CPR5	Peptidyl-prolyl cis-trans isomerase activity	3.1	Msn2/4p, Yap1/2P
NUP57	Essential subunit of the nuclear pore complex	3.1	Hsf1p, Yap1/2P
NUP42	Essential subunit of the nuclear pore complex	3.1	-
SPE1	Ornithine decarboxylase activity - pantothenate biosynthesis	3.1	Hsf1p
YPL176C	Receptor activity	3.1	Hsf1p
OST2	Dolichyl-diphospho-oligosaccharide-protein glycosyltransferase activity	3.1	Msn2/4p
PDS1	Mitotic sister chromatid separation	3.1	Hsf1p
YFL035C	Protein kinase activity	3.1	-
UBR1	Ubiquitin-protein ligase activity-protein monoubiquitination	3.1	Msn2/4p, Hsf1p
YDC1	Ceramidase activity or metabolims	3.1	Msn2/4p
NFU1	Iron ion homeostasis	3.1	Msn2/4p, Hsf1p
MG(ALPHA) 2	Pheromone activity-response to pheromone during conjugation with cellular fusion	3.0	Msn2/4p, Hsf1p, Yap1/2P
TSC13	Oxidoreductase activity-very-long-chain fatty acid metabolism	3.0	Msn2/4p, Hsf1p
EXG2	Glucan 1,3-beta-glucosidase activity	3.0	Msn2/4p, Yap1/2P
LSG1	Conjugation with cellular fusion	3.0	Msn2/4p, Hsf1p
PLB2	Glycerophospholipid metabolism - lysophospholipase activity	3.0	Msn2/4p
NPT1	Nicotinate phosphoribosyltransferase activity - nicotinate nucleotide biosynthesis, salvage pathway	3.0	Msn2/4p, Hsf1p
VPS28	Protein-membrane targeting/ protein-vacuolar targeting	3.0	Msn2/4p, Hsf1p
HEM15	Ferrochelatase activity/ heme biosynthesis	3.0	Hsf1p
OPI3	Phosphatidylcholine biosynthesis	3.0	Msn2/4p
Unknown function			
YOL007C		5.9	Hsf1p
YDR133C		5.7	Msn2/4p, Hsf1p
FYV4		5.0	Msn2/4p
*YBR089W		4.8	-

*PRY2	4.7	Msn2/4p
*YGL131C	4.6	Msn2/4p, Hsf1p
SCD6	4.5	Msn2/4p
YJR114W	4.4	Msn2/4p
YGR042W	4.2	Msn2/4p
YRF1-1	4.1	Msn2/4p
YDR346C	4.0	Hsf1p
APT2	4.0	Hsf1p
YHR040W	4.0	Msn2/4p, Yap1/2P
YPR126C	4.0	Msn2/4p
YBL083C	4.0	Msn2/4p, Hsf1p
SNA3	4.0	Msn2/4p, Hsf1p
YNL213C	4.0	Msn2/4p, Yap1/2P
YGR151C	3.9	Msn2/4p, Yap1/2P
YGL231C	3.9	Msn2/4p, Hsf1p
YBL077W	3.8	Msn2/4p, Hsf1p
SYN8	3.8	Hsf1p
YBR096W	3.8	Msn2/4p, Hsf1p, Yap1/2P
*YDR417C	3.8	Msn2/4p, Yap1/2P
GIR2	3.8	-
SYP1	3.7	Msn2/4p
*YBL109W	3.7	Msn2/4p
YFR043C	3.7	Msn2/4p, Hsf1p
YKR087C	3.7	Msn2/4p
YNL337W	3.7	-
*YJR115W	3.7	Msn2/4p, Hsf1p
YGR081C	3.6	Msn2/4p, Hsf1p
YCR025C	3.6	Msn2/4p
YBR141C	3.6	Msn2/4p, Hsf1p
YGL188C	3.6	Hsf1p
YLR073C	3.6	Hsf1p
YKL206C	3.6	-
YER156C	3.5	Msn2/4p
APD1	3.5	Msn2/4p
YDR544C	3.5	Msn2/4p
YIL012W	3.5	Msn2/4p, Hsf1p
YDR209C	3.5	Msn2/4p, Hsf1p
BCP1	3.5	Msn2/4p, Hsf1p, Yap1/2P
TOSII	3.5	Msn2/4p, Hsf1p
YMR304C-A	3.5	Msn2/4p
YOR277C	3.4	Msn2/4p, Hsf1p
YNR046W	3.4	Msn2/4p
*YEL001C	3.4	-
SWF1	3.4	Msn2/4p, Hsf1p, Yap1/2P
YGR283C	3.4	Yap1/2P
YGL139W	3.4	Msn2/4p, Hsf1p
YGL193C	3.4	Hsf1p
YHL012W	3.4	Msn2/4p, Hsf1p
YGL117W	3.4	Msn2/4p, Hsf1p, Yap1/2P
YHR083W	3.4	Msn2/4p, Hsf1p
YPR130C	3.4	Msn2/4p, Hsf1p
NCE103	3.4	Msn2/4p
*YPL197C	3.4	Yap1/2P
YOR051C	3.3	Hsf1p
YDR442W	3.3	Hsf1p
YER049W	3.3	Hsf1p
YFR035C	3.3	Hsf1p
YGL168W	3.3	Msn2/4p
CWC23	3.3	-
YJL122W	3.3	Msn2/4p, Hsf1p
YOL003C	3.3	Msn2/4p, Hsf1p, Yap1/2P
YPL014W	3.3	Msn2/4pYap1/2P
TOS6	3.2	Msn2/4p
YDL050C	3.2	Msn2/4p
YDR317W	3.2	Msn2/4p, Hsf1p
YDR370C	3.2	Msn2/4p
*YDL228C	3.2	Msn2/4p, Hsf1p

YGR079W	3.2	Msn2/4p, Hsf1p
FUN11	3.2	Msn2/4p, Hsf1p
YHR032W	3.2	-
YDR015C	3.2	-
SNA4	3.2	Msn2/4p, Hsf1p
YKL207W	3.2	-
*YOL109W	3.2	Hsf1p
YNL043C	3.2	Msn2/4p, Hsf1p
TVP38	3.2	Msn2/4p, Hsf1p, Yap1/2P
YML096W	3.2	Msn2/4p, Hsf1p, Yap1/2P
RSA3	3.2	-
PSP2	3.1	Msn2/4p, Hsf1p
YEL023C	3.1	-
YDR134C	3.1	-
YFR042W	3.1	Msn2/4p
YIL083C	3.1	Msn2/4p
YGR001C	3.1	-
YGR102C	3.1	Msn2/4p, Hsf1p
YNR009W	3.1	Msn2/4p, Hsf1p
YMR027W	3.1	Msn2/4p, Hsf1p
YNL144C	3.1	Msn2/4p, Hsf1p
YLR199C	3.1	Msn2/4p
YPL144W	3.1	Msn2/4p, Hsf1p
YOL125W	3.1	Msn2/4p, Hsf1p
*YGL102C	3.0	Hsf1p
YBR273C	3.0	Msn2/4p
YGR290W	3.0	Hsf1p
FSH1	3.0	Msn2/4p, Hsf1p
YEL059W	3.0	Msn2/4p, Hsf1p
YBR267W	3.0	-
YLR194C	3.0	Msn2/4p, Hsf1p
HSD1	3.0	Hsf1p

Table 3.4b: Macroarray data: genes that were LHE after five-hour exposure to 7% ethanol in the presence of acetaldehyde.

*Genes that also found to be MHE in the macroarray experiments at one hour time point for the same conditions.

ORF/Gene Name	Description of Gene Product	Fold Decrease
Cell cycle and growth		
SAT4	Protein kinase activity - G1/S transition of mitotic cell cycle	-3.1
Transport & translocation		
YIL166C	Transporter activity	-3.3
FET3	Multicopper ferroxidase iron transport mediator activity/high affinity iron ion transport	-3.5
YLYS7	Superoxide dismutase copper chaperone activity- intracellular copper ion transport	-3.7
NPL3	Protein carrier in mRNA export-mRNA nucleus export	-7.0
Energy utilization		
GAL10	Galactose metabolism	-3.1
GAL1	Galactokinase activity / galactose metabolism	-.7
Transcription and translation factor and process		
ARG80	Regulation of translational initiation - translation initiation factor activity	-3.2
DED1	Specific RNA polymerase II transcription factor activity	-3.3

SKI2	RNA helicase activity- translational initiation	-5.2
Nucleotide Metabolism		
YBR012W-B	RNA-directed DNA polymerase activity / peptidase activity/ ribonuclease activity	-14.7
MSU1	Exoribonuclease II activity / RNA catabolism / mRNA splicing	-6.4
YHL050C	Helicase activity	-6.3
YDR334W	Helicase activity	-3.9
SCP160	RNA binding/ chromosome segregation	-3.2
Cell wall organization		
BBC1	Myosin I binding /actin cytoskeleton organization and biogenesis	-5.3
SSD1	Cell wall organization and biogenesis	-6.5
Miscellaneous		
HUL4	ubiquitin-protein ligase activity / protein monoubiquitination protein polyubiquitination	-4.0
YDL025C	Protein kinase activity	-3.9
Unknown function		
YRF1-5	Genes of unknown function	-3.1
YDR348C	Genes of unknown function	-3.1
YOL098C	Genes of unknown function	-3.5
YHR145C	Genes of unknown function	-3.6
YRF1-3	Genes of unknown function	-3.6
YBL012C	Genes of unknown function	-3.6
YRF1-7	Genes of unknown function	-3.9
YEL077C	Genes of unknown function	-3.9
YRF1-4	Genes of unknown function	-4.3
YOL106W	Genes of unknown function	-4.4
YAL004W	Genes of unknown function	-5.0
YRF1-2	Genes of unknown function	-5.6
YLL067C	Genes of unknown function	-5.8
YLL066C	Genes of unknown function	-6.4
*YML133C	Genes of unknown function	-7.4
YBL113C	Genes of unknown function	-8.1
YHR219W	Genes of unknown function	-10.0
YBL101W-A	Genes of unknown function	-11.5
YJR026W	Genes of unknown function	-13.9
YAR010C	Genes of unknown function	-13.9
YJR028W	Genes of unknown function	-14.2
YFL067W	Genes of unknown function	-14.6
YBL005W-A	Genes of unknown function	-15.4
YBR012W-A	Genes of unknown function	15.6
YMR051C	Genes of unknown function	16.4
YCL020W	Genes of unknown function	16.8
YMR046C	Genes of unknown function	17.2
YCL042W	Genes of unknown function	17.3
YML040W	Genes of unknown function	17.9
YML045W	Genes of unknown function	19.9

Table 3.5a: Macroarray data: genes that were MHE and LHE after one-hour exposure to acetaldehyde only.

* Genes that are also MHE in the 'ethanol and acetaldehyde' conditions relative to ethanol control macroarray experiments at one-hour time point.

Genes/ORF	Description of Gene Products	Fold Increase/Decrease	Putative Transcription Factors
Energy utilizaton			
*PDC5	Pyruvate decarboxylase activity - pyruvate metabolism	4.2	Msn2/4p, Hsf1p, Yap1/2p
GPM1	Phosphoglycerate mutase activity – gluconeogenesis or Glycolysis	-3.0¶	Msn2/4p
Unknown function			
YNL179C	Protein of unknown function	3.7	Msn2/4p, Hsf1p
YRF1-5	Protein of unknown function	3.3	Msn2/4p
YHR219W	Protein of unknown function	3.2	Msn2/4p, Hsf1p
YKL153W	Protein of unknown function	-3.0¶	Msn2/4p, Hsf1p
YGL088W	Protein of unknown function	-3.0¶	Msn2/4p Hsf1p, Yap1/2p
YLR064W	Protein of unknown function	-3.0¶	Hsf1p

Legend - = ¶Fold decrease

Table 3.6a: Macroarray data: Shift in gene expression level following five-hour exposure to acetaldehyde only.

Genes/ORF	Description of Gene Products	Fold Increase/Decrease	Putative Transcription Factors
MON2	Protein-vascular targeting	3.4	Msn2/4p, Hsf1p
PCL1	Cell cycle - cyclin-dependent protein kinase, regulator activity	3.3	Msn2/4p
VPS65	Protein-vascular targeting	-3.0¶	Hsf1p

Legend - = ¶Fold decrease

APPENDIX IV

4.0 Analysis of glass slides, microarray, data for ethanol-stressed cells compared to unstressed, control cells.

Table 4.1a: Microarray data: genes that were MHE after one-hour exposure to 7% ethanol in the presence of acetaldehyde.

The following table contains genes that appeared to be MHE in at least two of the three replicates. Genes that were apparently MHE in only one of the replicates were not considered to have changed expression and therefore are not included in this table.

#Genes also found to be MHE in macroarray experiments for the same conditions.
*Genes that also found to be MHE in the five hour time point experiments for the same conditions.
♣Genes that are also MHE in the 'acetaldehyde only' conditions relative to an untreated control

Open-reading frame	Gene name	Function	Fold increase			Putative Transcription Factors
			Slide 1	Slide 2	Slide 3	
Ribosomal proteins						
YDL184C	RPL41A	Protein component of the large (60S) ribosomal subunit	-	3.8	9.6	Msn2/4p, Hsf1p
YDL133C-A	RPL41B	Protein component of the large (60S) ribosomal subunit	-	3.6	9.2	Hsf1p, Yap1p
YFR032C-A	RPL29	Protein component of the large (60S) ribosomal subunit	-	7.9	5.0	Msn2/4p, Hsf1p, Yap1p
#YIL052C	RPL34B	Protein component of the large (60S) ribosomal subunit	-	7.9	3.4	Msn2/4p, Hsf1p
#YKL180W	RPL17A	Protein component of the large (60S) ribosomal subunit,	3.4	6.6	3.2	Hsf1p
YOR182C	RPS30B	Protein component of the small (40S) ribosomal subunit	-	3.8	6.8	Hsf1p, Yap1p
#YLR048W	RPS0B	Protein component of the small (40S) ribosomal subunit (Ribosomal protein S0B)	7.1	6.3	-	Hsf1p
#YML063W	RPS1B	Ribosomal protein 10 (rp10) of the small (40S) subunit	6.9	7.3	4.4	Msn2/4p, Hsf1p, Yap1p
#YGR214W	RPS0A	Protein component of the small (40S) ribosomal subunit	6.5	8.5	-	Msn2/4p, Yap1p
YNL067W	RPL9B	Protein component of the large (60S) ribosomal subunit	6.4	7.2	-	Msn2/4p
#YLR029C	RPL15A	Protein component of the large (60S) ribosomal subunit	6.3	4.5	6.9	Msn2/4p, Hsf1p

#YGL123W	RPS2	Protein component of the small (40S) subunit	6.3	6.4	-	Msn2/4p, Yap1p
YLR448W	RPL6B	Protein component of the large (60S) ribosomal subunit	6.1	7.0	-	Msn2/4p, Hsf1p, Yap1p
YDR025W	RPS11A	Protein component of the small (40S) ribosomal subunit	5.9	4.4	-	Hsf1p
#YHL033C	RPL8A	Ribosomal protein L4 of the large (60S) ribosomal subunit	5.7	4.7	-	Msn2/4p, Hsf1p,
YNL248C	RPA49	RNA polymerase I subunit A49	5.7	3.6	-	Hsf1p
#YER074W	RPS24A	Protein component of the small (40S) ribosomal subunit	5.6	4.3	4.0	Hsf1p
YGL076C	RPL7A	Protein component of the large (60S) ribosomal subunit	5.5	5.0	-	Yap1p
#YBR189W	RPS9B	Protein component of the small (40S) ribosomal subunit	5.4	5.0	-	Hsf1p
#YLL045C	RPL8B	Ribosomal protein L4 of the large (60S) ribosomal subunit,	5.4	6.5	-	Msn2/4p
YMR229C	RRP5	Part of small ribosomal subunit (SSU)	5.4	3.2	-	Hsf1p
YPL198W	RPL7B	Protein component of the large (60S) ribosomal subunit,	5.4	7.3	-	Yap1p
#YKL006W	RPL14A	Protein component of the large (60S) ribosomal subunit	5.3	4.3	3.1	Hsf1p
YGR034W	RPL26B	Protein component of the large (60S) ribosomal subunit	5.2	4.5	3.5	Hsf1p
#YOL121C	RPS19A	Protein component of the small (40S) ribosomal subunit	5.2	4.0	-	Hsf1p, Yap1p
#YJR145C	RPS4A	RNA polymerase I subunit 190 (alpha)	5.1	4.5	3.3	Msn2/4p, Hsf1p, Yap1p
YOR341W	RPA190	RNA polymerase I subunit alpha; largest subunit of RNA polymerase I	5.1	4.0	-	-
YLR406C	RPL31B	Protein component of the large (60S) ribosomal subunit	-	6.2	4.4	Yap1p
YEL054C	RPL12A	Protein component of the large (60S) ribosomal subunit	4.0	4.5	3.3	Hsf1p
#YHR010W	RPL27A	Ribosomal protein L27A	-	6.1	4.5	Msn2/4p, Hsf1p
#YJL189W	RPL39	Protein component of the large (60S) ribosomal subunit	-	5.9	7.4	Hsf1p, Yap1p
YJR094W-A	RPL43B	Protein component of the large (60S) ribosomal subunit	-	4.2	6.2	Hsf1p
#YLR325C	RPL38	Protein component of the large (60S) ribosomal subunit	-	3.9	6.0	Msn2/4p, Hsf1p
#YML073C	RPL6A	N-terminally acetylated protein component of the large (60S) ribosomal subunit,	3.1	5.8	-	-
YLR287C-A	RPS30A	Protein component of the small (40S) ribosomal subunit	-	3.5	5.6	Hsf1p
YML024W	RPS17A	Ribosomal protein S17A (rp51A)	-	5.2	4.1	
YPR043W	RPL43A	Protein component of the large (60S) ribosomal subunit	-	5.1	8.1	Msn2/4p
#YGL030W	RPL30	Protein component of the large (60S) ribosomal subunit	-	4.4	5.1	Hsf1p
YPL090C	RPS6A	Protein component of the small (40S) ribosomal subunit	4.8	5.0	3.4	Hsf1p
YBR181C	RPS6B	Protein component of the small (40S) ribosomal subunit	4.9	3.4	3.4	Msn2/4p, Hsf1p
YJL190C	RPS22A	Protein component of the small (40S) ribosomal subunit	4.8		3.6	Hsf1p, Yap1p
YBL087C	RPL23A	Protein component of the large (60S) ribosomal subunit	4.7	4.3	4.1	-
#YLR441C	RPS1A	Ribosomal protein 10 (rp10) of the small (40S) subunit	4.7	4.1	-	Hsf1p, Yap1p
YNL096C	RPS78	Protein component of the small (40S) ribosomal subunit	4.7	3.6	-	-

#YOR312C	RPL20B	Protein component of the large (60S) ribosomal subunit	4.7	4.3	-	Msn2/4p, Yap1p
#YOR096W	RPS7A	Protein component of the small (40S) ribosomal subunit	4.7	4.0	-	Msn2/4p, Yap1p
#YHR021C	RPS27B	Protein component of the large (60S) ribosomal subunit	-	3.9	4.7	Msn2/4p, Hsf1p, Yap1p
#YIL133C	RPL16A	Protein component of the large (60S) ribosomal subunit	4.6	3.7	-	Msn2/4p, Hsf1p, Yap1p
#YER102W	RPS8B	Protein component of the small (40S) ribosomal subunit	4.5	4.9	-	Msn2/4p, Hsf1p, Yap1p
YOR369C	RPS12	Protein component of the large (60S) ribosomal subunit	-	4.9	4.5	-
YNL162W	RPL42A	Protein component of the large (60S) ribosomal subunit	-	4.3	4.4	Hsf1p
YLR388W	RPS29A	Protein component of the large (60S) ribosomal subunit	-	4.7	4.4	Hsf1p
YDR450W	RPS18A	Protein component of the small (40S) ribosomal subunit	4.3	4.7	-	Msn2/4p
YDR500C	RPL37B	Protein component of the large (60S) ribosomal subunit	-	3.1	4.3	Hsf1p,
#YJR123W	RPS5	Protein component of the small (40S) ribosomal subunit	4.2	4.9	3.2	Msn2/4p, Hsf1p
YBR048W	RPS11B	Protein component of the small (40S) ribosomal subunit	4.2	4.2	-	Hsf1p, Yap1p
#YHR203C	RPS4B	Protein component of the small (40S) ribosomal subunit	4.2	3.3	-	Hsf1p
YJL136C	RPS21B	Protein component of the small (40S) ribosomal subunit	-	4.6	4.1	Msn2/4p, Hsf1p
YDR418W	RPL12B	Protein component of the large (60S) ribosomal subunit	4.0	4.4	3.3	Msn2/4p
#YML026C	RPS18B	Protein component of the small (40S) ribosomal subunit	4.0	4.0	3.5	Msn2/4p, Hsf1p, Yap1p
#YBL027W	RPL19B	Protein component of the large (60S) ribosomal subunit	4.0	4.6	3.7	Hsf1p, Yap1p
YER117W	RPL23B	Protein component of the large (60S) ribosomal subunit	3.9	-	3.3	Msn2/4p, Yap1p
#YOR293W	RPS10A	Protein component of the small (40S) ribosomal subunit	3.8	3.6	-	Msn2/4p, Hsf1p, Yap1p
#YMR230W	RPS10B	Protein component of the small (40S) ribosomal subunit	-	4.3	3.8	Hsf1p
YLR197W	SIK1	Component of the small (ribosomal) subunit (SSU) processosome	3.5	3.7	3.7	Msn2/4p, Hsf1p
#YGL103W	RPL28	Ribosomal protein L29 of the large (60S) ribosomal subunit	3.5	4.0	-	Msn2/4p
#YPL220W	RPL1A	Protein component of the large (60S) ribosomal subunit	3.5	4.9	-	Hsf1p, Yap1p
YDL083C	RPS16B	Protein component of the large (60S) ribosomal subunit	3.5	4.1	3.1	Msn2/4p, Hsf1p
#YJL177W	RPL17B	Protein component of the large (60S) ribosomal subunit	3.4	4.5	4.7	Msn2/4p, Hsf1p, Yap1p
YNL302C	RPS19B	Protein component of the small (40S) ribosomal subunit	3.4	4.6	3.7	Hsf1p
YNL069C	RPL16B	Protein component of the large (60S) ribosomal subunit	3.3	4.3	-	Hsf1p
YPR132W	RPS23B	Ribosomal protein 28 (rp28) of the small (40S) ribosomal subunit	3.3	-	3.5	Hsf1p
#YMR242C	RPL20A	Protein component of the large (60S) ribosomal subunit	3.3	4.6	-	Msn2/4p, Yap1p
#YPR102C	RPL11A	Protein component of the large (60S) ribosomal subunit	3.2	3.1	6.1	Hsf1p
YLR344W	RPL26A	Protein component of the large (60S) ribosomal subunit	3.2	4.4	-	Hsf1p
YLR264W	RPS28B	Protein component of the small (40S) ribosomal subunit	3.2	4.0	3.0	Msn2/4p, Hsf1p, Yap1p
#YFR031C-A	RPL2A	Protein component of the large (60S) ribosomal subunit	3.2	4.0	3.7	Msn2/4p, Hsf1p
YMR143W	RPS16A	Protein component of the small (40S) ribosomal subunit	-	4.5	3.7	Yap1p
YLR185W	RPL37A	Protein component of the large (60S) ribosomal subunit	-	4.6	3.6	Msn2/4p, Hsf1p

YOL039W	RRP2A	Protein component of the large (60S) ribosomal subunit	-	4.6	3.6	-
#YER056C-A	RPL34A	Protein component of the large (60S) ribosomal subunit	-	4.9	3.6	Msn2/4p, Hsf1p
#YBR191W	RPL21A	Protein component of the large (60S) ribosomal subunit	-	4.9	3.4	Hsf1p
#YGL189C	RPS26A	Protein component of the small (40S) ribosomal subunit	-	4.8	3.4	Hsf1p, Yap1p
#YER131W	RPS26B	Protein component of the small (40S) ribosomal subunit	-	4.9	3.4	Msn2/4p, Hsf1p, Yap1p
YKR094C	RPL40B	Protein component of the large (60S) ribosomal subunit	-	3.4	3.3	Yap1p
#YGR085C	RPL11B	Protein component of the large (60S) ribosomal subunit	3.2	3.6	-	Msn2/4p, Hsf1p
#YIL018W	RPL2B	Protein component of the large (60S) ribosomal subunit	3.1	4.6	-	-
#YLR061W	RPL22A	Protein component of the large (60S) ribosomal subunit	3.1	3.7	3.0	-
YDR471W	RPL27B	Protein component of the large (60S) ribosomal subunit	3.1	3.7	3.1	-
#YBR084C-A	RPL19A	Protein component of the large (60S) ribosomal subunit	3.1	4.5	-	Msn2/4p, Hsf1p
YPL143W	RPL33A	Protein component of the large (60S) ribosomal subunit	-	3.4	3.1	Yap1p
YGR118W	RPS23A	Ribosomal protein 28 (rp28) of the small (40S) ribosomal subunit	3.0	3.4	6.2	Msn2/4p
#YPL131W	RPL5	In component of the large (60S) ribosomal subunit	3.0	3.2	-	Yap1p
#YOL040C	RPS15	Protein component of the small (40S) ribosomal subunit	3.0	4.0	-	Msn2/4p
#YDL075W	RPL31A	Protein component of the large (60S) ribosomal subunit	-	3.4	3.4	Msn2/4p, Hsf1p
Stress response						
YGR234W	YHB1	May play a role in the oxidative stress response	5.0	7.4	3.3	Hsf1p, Yap1p
YKL143W	LTV1	Protein required for growth at low temperature	-	5.7	3.0	Msn2/4p, Hsf1p
#YPL163C	SVS1	Cell wall and vacuolar protein, required for wild-type resistance to vanadate	-	6.7	4.7	Hsf1p
#YHR064C	SSZ1	Unfolded protein binding	-	3.6	3.4	-
YFR034C	PHO4	Cellular response to phosphate starvation	3.0	8	-	Msn2/4p, Hsf1p
Energy utilization						
*#YLR134W	PDC5	Minor isoform of pyruvate decarboxylase, key enzyme in alcoholic fermentation, decarboxylate pyruvate to acetaldehyde	70.1	30.0	-	Msn2/4p, Hsf1p
YER073W	ALD5	Aldehyde dehydrogenase activity	3.5	3.8	-	Msn2/4p, Yap1p
Cell cycle and growth						
YPL256C	CLN2	Role in cell cycle START; G (sub)1 cyclin	7.5	7.8	-	-
YNL247W		Protein required for cell viability	-	6.7	4.2	Msn2/4p, Hsf1p
YDL003W	MCD1	Mitotic sister chromatid cohesion	-	5.2	15.8	Hsf1p

YDR241W	BUD26	Bud site selection	-	3.7	8.0	Hsf1p, Yap1p
YMR199W	CLN1	Cyclin-dependent protein kinase regulator activity	3.1	4.2	-	Msn2/4p
Protein metabolism						
#YLR121C	YPS3	Aspartic protease	7.5	3.5	-	Yap1p
YGR155W	CYS4	Cysteine biosynthesis	6.9	3.5	-	Msn2/4p, Yap1p
YOR108W	LEU9	Leucine biosynthesis	4.2	3.7	-	Yap1p
#YLR180W	SAM1	Methionine synthetase	9.1	3.8	-	Yap1p
YOR335C	ALA1	Alanine-tRNA ligase activity	4.1	3.7	-	Hsf1p
YHR025W	THR1	Homoserine kinase activity	4.1	3.7	-	Msn2/4p
#YKL101W	HSL1	Protein kinase activity (G2/M transition of mitotic cell cycle)	3.1	7.2	6.1	Hsf1p, Yap1p
YDR502C	SAM2	Methionine metabolism	4.0	-	3.3	Msn2/4p, Hsf1p, Yap1p
#YBR121C	GRS1	Glycine-tRNA ligase activity	3.1	4.1	-	Msn2/4p, Hsf1p
Cytoskeleton genes						
YGR245C	SDA1	Actin cytoskeleton organization and biogenesis	-	3.1	3.4	Hsf1p
YPL241C	CIN2	Microtubule-based process	-	5.0	3.0	Hsf1p, Yap1p
Transcription and translation						
YGL043W	DST1	General transcription elongation factor TFIIS, enables RNA polymerase II to read	28.1	3.6	-	Msn2/4p, Hsf1p
YOR340C	RPA43	RNA polymerase I subunit A43	5.3	4.4	-	Msn2/4p, Hsf1p
YOR310C	NOP58	Protein involved in pre-rRNA processing, 18S rRNA synthesis	5.6	4.5	-	Hsf1p, Yap1p
YPL160W	CDC60	Leucine-tRNA ligase activity	4.9	3.6	-	Hsf1p, Yap1p
YPL266W	DIM1	rRNA (adenine-N6,N6-)-dimethyltransferase activity	4.5	3.4	4.5	Hsf1p, Yap1p
YJL109C	UTP10	Processing of 20S pre-rRNA	4.4	-	5.1	Msn2/4p, Hsf1p
YMR260C	TIF11	Translation initiation factor eIF1A	-	3.8	4.1	-
YOR095C	RKI1	Ribose-5-phosphate ketol-isomerase	4.0	-	3.5	Msn2/4p, Hsf1p, Yap1p
YLL008W	DRS1	ATP-dependent RNA helicase activity	-	4.5	4.0	Hsf1p
YOR207C	RET1	DNA-directed RNA polymerase activity	3.9	3.4	-	Msn2/4p, Hsf1p, Yap1p
YGR162W	TIF4631	Translation initiation factor activity	3.9	3.6	-	Msn2/4p, Hsf1p, Yap1p
YJL050W	MTR4	ATP-dependent RNA helicase activity	3.8	-	3.7	Hsf1p, Yap1p
YDR120C	TRM1	Trna (guanine-N2-)-methyltransferase activity	3.7	4.8	-	-
YDL112W	TRM2	tRNA (guanosine) methyltransferase activity	3.6	-	3.0	Hsf1p

YPL043W	NOP4	RNA processing	3.6	3.9	-	Msn2/4p, Hsf1p, Yap1p
YKL078W	DHR2	RNA helicase activity	3.5	3.3	-	Hsf1p
YHR020W		Proline-tRNA ligase activity	3.5	4.1	-	Yap1p
YPR010C	RPA135	DNA-directed RNA polymerase activity	3.5	4.8	-	Msn2/4p, Yap1p
YOL077C	BRX1	rRNA primary transcript binding	3.4	-	3.0	Hsf1p
YJL148W	RPA34	RNA polymerase I subunit A34.5	3.4	-	4.3	Msn2/4p, Hsf1p
YLR409C	UTP21	Processing of 20S pre-rRNA	3.3	3.7	3.8	Msn2/4p
#YLR249W	YEF3	Translation elongation factor activity	3.3	-	3.3	Msn2/4p, Hsf1p, Yap1p
YEL055C	POL5	rRNA transcription	3.2	3.4	3.3	Yap1p
YJL011C	RPC17	DNA-directed RNA polymerase activity	3.1	3.6	-	Msn2/4p, Hsf1p
YGR158C	MTR3	3'-5'-exoribonuclease activity	3.0	4.0	-	Hsf1p, Yap1p
YNL062C	GCD10	tRNA methyltransferase activity	3.0	4.5	-	Msn2/4p, Hsf1p
YPL217C	BMS1	35S primary transcript processing	3.0	4.6	4.0	Hsf1p, Yap1p
YDL208W	NHPS	35S primary transcript processing	3.0	3.2	-	-
Nucleotide metabolism						
#YER070W	RNR1	Ribonucleotide-diphosphate reductase (RNR), large subunit	6.6	5.9	9.2	Hsf1p
YOR074C	CDC21	Thymidylate synthase activity	-	3.6	8.2	Msn2/4p, Hsf1p
YHR089C	GAR1	RNA binding	5.0	5.2	-	Msn2/4p, Hsf1p
YDL227C	HO	Endonuclease activity	4.2	4.9	4.5	Hsf1p, Yap1p
YNL262W	POL2	Epsilon DNA polymerase activity	3.1	3.6	-	Msn2/4p, Hsf1p
Transport						
YHR092C	HXT4	High-affinity glucose transporter of the major facilitator superfamily	24.7	10.5	8.1	Msn2/4p
#YML123C	PHO84	Inorganic phosphate (Pi) transporter	9.7	3.9		Msn2/4p
#YDR345C	HXT3	Low affinity glucose transporter of the major facilitator superfamily,	5.4	-	3.4	Msn2/4p, Hsf1p
#YER110C	KAP123	Protein carrier activity	-	5.5	3.8	Hsf1p, Yap1p
YJL145W	SFH5	Phosphatidylinositol transporter activity	3.7	4.9	-	Msn2/4p, Hsf1p, Yap1p
#YGL225W	VRG4	Nucleotide-sugar transporter activity	3.0	-	3.4	Msn2/4p, Hsf1p
Cell Wall						
YKL163W	PIR3	Structural constituent of cell wall	12.1	6.0	-	Msn2/4p
YGL028C	SCW11	Cell wall protein with similarity to glucanases	7.4	4.0	-	-
YGR189C	CRH1	Putative glycosidase of the cell wall, may have a role in cell wall	6.7	3.5	-	Hsf1p, Yap1p

		architecture				
#YKL096W-A	CWP2	Structural constituent of cell wall	-	5.0	3.8	Msn2/4p, Hsf1p
YBR038W	CHS2	Chitin synthase activity	4.3	-	3.3	Msn2/4p, Yap1p
YML066C	SMA2	Spore Membrane Assembly	3.0	4.7	-	Msn2/4p, Hsf1p
Lipid metabolism						
#*YGR060W	ERG25	C-4 methylsterol oxidase activity	5.0	5.7		-
#*YLR056W	ERG3	C-5 sterol desaturase	4.4	5.1	6.5	Msn2/4p, Hsf1p
#*YCR034W	FEN1	Fatty acid elongase, involved in sphingolipid biosynthesis	-	6.3	6.1	Msn2/4p, Yap1p
Miscellaneous						
YPR002W	PDH1	Mitochondrial protein that participates in respiration	9.4	3.6	4.3	Msn2/4p, Yap1p
YCL054W	SPB1	Suppressor of PaB1 mutant	7.2	-	5.1	Hsf1p
YMR095C	SNO1	Biological and molecular functions unknown	5.3	6.4	-	Yap1p
YBR244W	GPX2	Glutathione peroxidase activity	5.3	4.9	-	Msn2/4p, Hsf1p, Yap1p
#YHR019C	DED81	Cytosolic asparaginyl-tRNA synthetase	5.3	4.0	-	Msn2/4p, Hsf1p, Yap1p
YLR401C	DUS3	tRNA dihydrouridine synthase activity	5.2	4.0	-	Msn2/4p, Hsf1p
YMR290C	HAS1	Putative RNA-dependent helicase	5.1	6.4	-	Msn2/4p
#YHR154W	RTT107	Regulator of Ty1 Transposition	-	5.4	4.0	Hsf1p
YPL093W	NOG1	GTPase activity	4.7	3.3	-	-
YDR144C	MKC7	Aspartic-type signal peptidase activity	-	3.6	4.7	Msn2/4p, Hsf1p, Yap1p
YLR342W	FKS1	1,3-beta-glucan synthase activity	4.6	3.4	-	Hsf1p, Yap1p
YLL022C	HIF1	Chromatin silencing at telomere	-	3.2	-4.4	Hsf1p
YJR143C	PMT4	Dolichyl-phosphate-mannose-protein mannosyltransferase activity	4.3	3.1	-	Msn2/4p, Hsf1p
YDL014W	NOP1	Methyltransferase activity	4.0		3.3	Msn2/4p, Hsf1p, Yap1p
YDR097C	MSH6	ATP binding (IDA) (ATPase activity)	-	3.1	3.6	Msn2/4p, Hsf1p, Yap1p
YGL148W	ARO2	Aromatic amino acid family biosynthesis	3.5	4.7	-	Msn2/4p, Yap1p
YGR109C	CLB6	Cyclin-dependent protein kinase regulator activity	3.4	4.5	7.0	Hsf1p
YCL024W	KCC4	Protein kinase activity	3.3	-	5.3	Msn2/4p, Hsf1p, Yap1p
YCL026C-A	FRM2	Negative regulation of fatty acid metabolism	3.3	3.2	-	Msn2/4p
YDR507C	GIN4	Protein kinase activity	3.2	4.1	3.6	Msn2/4p, Hsf1p, Yap1p
YIL066C	RNR3	Ribonucleoside-diphosphate reductase activity	3.2	3.9	-	Msn2/4p
YER001W	MNN1	Alpha-1, 3-mannosyltransferase activity	-	3.1	3.1	Hsf1p, Yap1p
YDL219W	DTD1	Hydrolase activity, acting on ester bonds	-	3.3	3.1	Msn2/4p, Yap1p

YBR084W	MIS1	Mitochondrial C1-tetrahydroflate synthase	3.0	-	3.8	Msn2/4p, Hsf1p, Yap1p
YLR172C	DHP5	Diphthine synthase activity	3.0	4.9	-	-
#YIL140W	AXL2	Axial bud site selection	3.0	4.6	4.3	-
YLR348C	DIC1	Dicarboxylic acid transporter activity	3.0	3.3	-	-
YLR153C	ACS2	Acetate-CoA ligase activity	3.0	3.3	-	Msn2/4p
YOL147C	PEX11	Peroxisome organization and biogenesis	3.0	4.0	-	-
YLR024C	UBR2	Ubiquitin-protein ligase activity	3.0	3.4	-	Msn2/4p, Yap1p
YOR243C	PUS7	Pseudouridine synthase activity	3.0	3.1	-	-
YHR170W	NMD3	Ribosomal large subunit assembly and maintenance	3.0	3.8	-	Hsf1p
Unknown function						
YLR303W-R			38.9	4.1	-	-
YLR180W-R			11.8	4.5	-	-
YGL034C			3.9	-	9.5	Msn2/4p, Hsf1p
YLR364W			9.8	5.2	9.5	Hsf1p, Yap1p
#YHR095W			8.6	8.5	-	Msn2/4p
YIL141W			4.2	6.2	-	Hsf1p
YKR075C			6.0	4.0	-	Msn2/4p
YOL007C			5.6	9.6	-	Msn2/4p
YDR345C-R			5.0	-	4.0	-
YNL132W	KREE33		4.9	-	4.2	Hsf1p, Yap1p
#YGR151C			-	4.3	4.8	Hsf1p, Yap1p
YOL141W	PPM2		4.7	-	4.0	Yap1p
YKL014C	URB1		-	3.9	4.5	Hsf1p
YCL036W	GFD2		4.2	3.8	-	-
YOR325W			4.1	3.6	-	Yap1p
YGR145W	ENP2		-	4.6	4.1	Hsf1p, Yap1p
YPL267W	ACM1		-	3.8	4.1	Msn2/4p, Hsf1p
YHR153C	SPO16		3.9	4.4	7.5	Hsf1p, Yap1p
YML037C			-	3.0	4.0	Msn2/4p,
YJL118W			-	3.6	4.0	-
YOR287C			3.9	4.0	-	Hsf1p
YOL124C	TRM11		3.9	3.7	3.7	Msn2/4p, Hsf1p
YOL131W			3.8	-	3.0	Hsf1p
YJR146W			3.7	3.1	-	Msn2/4p, Hsf1p, Yap1p

YGL165C			3.7	4.3	-	-
YGR103W	NOP7		-	3.0	3.7	Hsf1p, Yap1p
YOR195W	SLK19		-	3.2	3.7	Msn2/4p
YJL043W			3.6	-	10.7	Hsf1p
YGR269W			3.5	3.7	-	Msn2/4p, Yap1p
YLR331C			3.5	3.3	3.6	Yap1p
YCL065W			3.5	-	5.7	?
YGR141W	VPS62		3.4	-	3.6	Msn2/4p, Hsf1p
YJL122W			3.4	3.3	-	Msn2/4p, Hsf1p
YGL196W			-	4.2	3.4	-
YGL081W			-	4.9	3.4	-
YIL064W			-	4.1	3.2	Msn2/4p
YMR305C-R			3.1	3.3	-	-
#YEL001C			3.1	3.3	-	Msn2/4p, Hsf1p
YIL096C			-	3.5	3.1	Msn2/4p, Hsf1p, Yap1p
YBR134W			3.0	3.6	7.3	Hsf1p

Table 4.1b: Microarray data: genes that were LHE after one-hour exposure to 7% ethanol in the presence of acetaldehyde.

The following table contains genes that appeared to be LHE in at least two of the three replicates. Genes that were apparently LHE in only one of the replicates were not considered to have changed expression and therefore are not included in this table.

*Genes that are also LE in the 'acetaldehyde only' conditions relative to an untreated control
#Genes also found to be LE in macroarray experiments for the same conditions.

Open reading frame	Genes	Function	Fold decrease		
			Slide 1	Slide 2	Slide 3
Stress response protein folding genes					
YCR021C	HSP30	Response to stress	96.3	48.2	24.1
YDR258C	HSP78	Unfolded protein binding	36.2	22.9	5.0
YOL052C-A	DDR2	Response to stress	-	21.5	13.5
YNL007C	SIS1	HSP40 family chaperone	18.9	15.8	3.5
*YBR067C	TIP1	Cold and heat-shock induced protein of the Srp1p/Tip1p family	-	17.7	26.5
YNL077W	APJ1	Unfolded protein binding	14.8	4.0	-
YBR054W	YRO2	Putative plasma membrane protein (homolog to HSP30)	3.9	11.6	10.5
YBR072W	HSP26	Unfolded protein binding	-	13.1	5.6
YER103W	SSA4	Unfolded protein binding	13.2	15.5	6.1
YGR088W	CTT1	Cytoplasmic catalase T	12.0	-	3.6
YLL026W	HSP104	Heat shock protein that cooperates with Ydj1p (Hsp40) and Ssa1p (Hsp70) to	11.0	13.6	-
YBR169C	SSE2	HSP70 family member, highly homologous to Sse1p	10.8	12.7	-
YML130C	ERO1	Glycoprotein required for oxidative protein folding in the endoplasmic reticulum	10.4	6.8	-
YPL240C	HSP82	Protein folding (response to stress)	6.8	7.4	3.4
YFL016C	MDJ1	Chaperone binding (unfolded protein binding)	6.1	6.5	-
YNL160W	YGP1	Response to nutrients (response to stress)	-	5.7	6.3
YOR027W	STI1	Heat shock protein also induced by canavanine and entry into stationary phase	3.8	5.6	4.0
YAL005C	SSA1	Heat shock protein of HSP70 family,	3.7	5.0	3.6

YOR267C	HRK1	Protein kinase with a role in ion homeostasis	3.5	4.2	3.5
YMR186W	HSC82	Constitutively expressed heat shock protein	-	3.8	3.2
YOR391C	HSP33	Possible chaperone and cysteine protease with similarity to E. coli Hsp31 and S. cerevisiae Hsp32p, Hsp33p, and Sno4p	-	3.6	-
YBL075C	SSA3	Heat-inducible cytosolic member of the 70 kDa HSP family	-	3.6	3.0
YMR251W-A	HOR7	Hyperosmotic stress response	3.1	3.3	-
Energy utilization					
YML100W	TSL1	Trehalose-6-phosphate synthase\/phosphatase	11.6	10.1	6.8
YDL021W	GPM2	Converts 3-phosphoglycerate to 2 phosphoglycerate in glycolysis	9.2	9.6	-
YFR053C	HXK1	Hexokinase activity	4.8	8.0	-
YIL053W	GPP1	Glycerol biosynthesis (response to osmotic stress)	6.4	4.3	4.9
YDL022W	GPD1	Glycerol-3-phosphate dehydrogenase	-	7.4	5.6
YOL059W	GPD2	Glycerol-3-phosphate dehydrogenase (NAD+) activity	5.0	7.2	5.0
YGR254W	ENO1	Catalyses the first common step of glycolysis and gluconeogenesis	-	4.3	8.2
YMR105C	PGM2	Phosphoglucomutase activity	-	6.5	3.6
YDR074W	TPS2	Trehalose-6-phosphate phosphatase	-	5.9	3.0
YPR184W	GDB1	Glycogen debranching enzyme containing glucanotranferase	-	5.7	4.0
YER081W	SER3	phosphoglycerate dehydrogenase activity	-	5.5	-
YLR258W	GSY2	Glycogen (starch) synthase activity	-	-	3.9
YGR248W	SOL4	6-phosphogluconolactonase activity	-	5.5	3.5
YNR034W	SOL1	6-phosphogluconolactonase activity	3.7	-	3.3
YKL035W	UGP1	UTP-glucose-1-phosphate uridylyltransferase activity	-	4.9	6.1
YJL052W	TDH1	Glyceraldehyde-3-phosphate dehydrogenase 1	-	4.4	7.9
YCL040W	GLK1	Glucokinase, catalyzes the phosphorylation of glucose at C6 in the first irreversible step of glucose metabolism	-	3.8	5.3
YMR303C	ADH2	Alcohol dehydrogenase activity (ethanol metabolism)	3.7	-	4.2
Cytoskeleton genes					
YPR154W	PIN3	Actin cytoskeleton organization and biogenesis	17.1	10.8	9.8
YBL078C	ATG8	Microtubule binding	3.5	5.2	-
Transcription and translation					
YOR344C	TYE7	Transcription factor activity	30.8	6.3	4.3

YKL043W	PHD1	Specific RNA polymerase II transcription factor activity	13.6	6.7	8.1
YDL020C	RPN4	Transcription factor that stimulates expression of proteasome genes	11.3	5.9	-
YDR043C	NRG1	Transcriptional repressor activity	10.5	-	3.5
YOR298C-A	MBF1	Transcriptional co-activator that bridges the DNA-binding region of Gcn4p and	7.5	8.8	3.2
YOR178C	GAC1	Regulatory subunit for Glc7p (protein phosphatase I) for glycogen synthesis	7.5	-	4.3
YDL005C	MED2	RNA Polymerase II transcriptional regulation mediator	8.1	-	3.7
YJL035C	TAD2	tRNA-specific adenosine-34 deaminase subunit Tad2p	6.6	3.3	-
YDR040C	ENA1	P-type ATPase sodium pump, involved in Na+ and Li+ efflux to allow salt tolerance	5.9	-	3.9
YMR070W	MOT3	Nuclear transcription factor with two Cys2-His2 zinc fingers; involved in repression	5.9	3.4	-
YBR066C	NRG2	Transcriptional repressor activity	5.8	5.5	-
YNL251C	NRD1	RNA-binding protein that interacts with the C-terminal domain of the RNA	5.6	-	7.8
YPL177C	CUP9	Specific RNA polymerase II transcription factor activity	4.4	-	3.7
YPL075W	GCR1	Transcriptional activator of genes involved in glycolysis	4.3	4.8	-
YOR028C	CIN5	Basic leucine zipper transcriptional factor of the yAP-1 family	4.3	3.3	-
YER045C	ACA1	Basic leucine zipper (bZIP) transcription factor of the ATF/CREB family,	4.1	4.1	-
YDR073W	SNF11	Component of SWI/SNF global transcription activator complex	4.0	3.4	-
YGL096W	TOS8	Transcription factor activity	3.8	4.5	-
YOL081W	IRA2	Inhibitory Regulator of the RAS-cAMP pathway	3.8	3.4	-
YIL122W	POG1	Specific RNA polymerase II transcription factor activity	-	3.9	3.8
YPR008W	HAA1	Specific RNA polymerase II transcription factor activity	-	3.7	3.9
YKR034W	DAL80	Negative regulator of genes in multiple nitrogen degradation pathways	3.6	3.3	-
YKL185W	ASH1	Specific transcriptional repressor activity	-	3.6	3.6
YFR001W	LOC1	mRNA localization, intracellular	-	3.5	3.2
YMR136W	GAT2	Protein containing GATA family zinc finger motifs	3.5	-	3.1
YFL031W	HAC1	Specific RNA polymerase II transcription factor activity	3.0	4.0	6.6
YLR228C	ECM22	Sterol regulatory element binding protein, regulates transcription of the sterol biosynthetic genes ERG2 and ERG3	-	3.2	3.1
YOR230W	WTM1	Transcription corepressor activity	-	3.1	3.1
YFL021W	GAT1	Specific RNA polymerase II transcription factor activity	-	3.0	7.2
Protein metabolism					
YJR036C	HUL4	Ubiquitin-protein ligase activity	3.5	4.6	-
YEL012W	UBC8	Ubiquitin conjugating enzyme activity	3.4	3.6	-
YBR082C	UBC4	Ubiquitin conjugating enzyme activity	3.2	4.4	-

Nueleotied mtabolism					
YBR214W	SDS24	DNA metabolism	4.8	5.0	-
YNL107W	YAF9	Chromatin remodelling	4.4	4.0	-
YKL032C	IXR1	DNA binding	3.4	3.7	-
Transport					
YGR142W	BTN2	Intracellular protein transport	-	48.6	4.3
YNL006W	LST8	Protein required for transport of permeases from the Golgi to the plasma membrane	24.7	17.4	5.6
YEL039C	CYC7	Electron carrier activity	18.4	30.3	4.4
YGR121C	MEP1	Ammonium transporter activity	8.2	4.4	3.2
YOR161C	PNS1	Choline transporter activity	8.6	10.1	3.6
YNL142W	MEP2	Ammonia transport protein	6.2	-	6.5
YOR273C	TPO4	Spermidine transporter activity (Polyamine transport protein)	4.2	8.0	3.5
YER053C	PIC2	Inorganic phosphate transporter activity	4.1	6.0	4.1
YKR039W	GAP1	General amino acid permease	4.1	-	3.9
YCL025C	AGP1	Amino acid transport	3.8	4.2	6.6
YDR358W	GGA1	Golgi to vacuole transport	3.4	3.2	3.4
YGR009C	SEC9	Golgi to plasma membrane transport	3.2	4.1	-
YPR138C	MEP3	Ammonium transporter activity	3.0	4.6	5.9
YHR096C	HXT5	Hexose transport	3.0	3.2	8.8
YOR348C	PUT4	Amino acid permease activity	-	3.1	5.1
Transposable element gene					
YDR034C-D	-	TyB Gag-Pol protein. Gag processing	-	6.4	5.4
YLR410W-B	-	TyB Gag-Pol protein. Gag processing	-	6.0	4.9
YFL002W-A	-	TyB Gag-Pol protein. Gag processing	3.5	5.5	6.8
YGR161W-B	-	TyB Gag-Pol protein. Gag processing	4.5	5.0	6.9
YDR261W-B	-	TyB Gag-Pol protein. Gag processing	4.3	3.6	7.5
YBL101W-B	-	TyB Gag-Pol protein. Gag processing	4.3	3.8	-
YDR210W-A	-	TyB Gag-Pol protein. Gag processing	3.9	4.4	6.5
YNL054W-B	-	TyB Gag-Pol protein. Gag processing	3.6	-	3.3
YBL101W-A	-	TyB Gag-Pol protein. Gag processing	-	3.5	3.7
YPL257W-A	-	TyB Gag-Pol protein. Gag processing	-	3.3	3.9

YDR261W-A	-	TyB Gag-Pol protein. Gag processing	-	3.3	5.0
Miscellaneous					
YGR161C	RTS3	Protein phosphatase type 2A activity	39.5	10.3	4.8
YHR055C	CUP1-2	Copper ion binding	15.3	14.2	3.5
YGR211W	ZPR1	Zinc finger protein	11.7	3.3	-
#YHR139C	SPS100	Involved in spore development; sporulation-specific wall maturation protein	9.1	32.1	8.0
YPR149W	NCE102	Involved in secretion of proteins that lack classical secretory signal sequences	8.2	8.2	12.4
YHR053C	CUP1-1	Copper ion binding	7.6	7.5	3.4
YJL034W	KAR2	ATPase activity	7.3	4.4	-
YIL113W	SDP1	MAP kinase phosphatase activity	7.0	7.7	3.7
YPL003W	ULA1	NEDD8 activating enzyme activity	6.8	10.3	3.1
YGR008C	STF2	ATPase stabilizing factor	-	5.5	3.4
YGR052W	FMP48	Kinase activity	5.1	3.0	-
YIR032C	DAL3	Allantoin catabolism	4.9	4.9	3.1
YLR178C	TFS1	Carboxypeptidase Y inhibitor; (putative) lipid binding protein	-	4.7	5.3
YJL166W	QCR8	Ubiquinol cytochrome-c reductase subunit 8	4.8	4.5	-
YML128C	MSC1	Meiotic recombination	-	4.6	4.1
YOR193W	PEX27	Involved in peroxisome proliferation	4.5	3.2	-
YGR144W	THI4	Biosynthetic pathway of thiamin biosynthesis	-	4.3	3.9
YJL141C	YAK1	Protein kinase activity	-	4.1	6.9
YMR232W	FUS2	Plasma membrane fusion	3.9	3.1	-
YJR059W	PTK2	Protein kinase activity	-	3.9	3.3
YML054C	CYB2	Expression is repressed by glucose and anaerobic conditions	-	3.9	6.0
YGR007W	MUQ1	Ethanolamine-phosphate cytidylyltransferase activity	3.7	3.1	4.6
YDR516C	EMI2	Sporulation (sensu Fungi)	3.4	3.8	4.2
YIL099W	SGA1	Sporulation (sensu Fungi)	3.3	3.6	3.8
YGR028W	MSP1	40 kDa putative membrane-spanning ATPase	3.2	-	3.2
YGR143W	SKN1	Involved in (1->6)-beta-glucan biosynthesis (Cell wall organization)	-	3.3	3.4
Unknown function					
#YER150W	SPI1		34.1	30.1	5.6
YOL032W	-		33.9	14.8	4.5

YNL194C	-		23.7	14.0	-
YBR099C	-		23.1	27.8	-
YPR158W	-		20.6	13.9	3.1
YOL014W	-		19.1	4.3	-
YOL084W	PHM7		18.6	13.7	4.8
YPL250C	ICY2		17.5	9.3	4.8
*ARA13	-		17.2	10	-
YNL195C	-		14.5	22.1	-
YNR034W-A	-		-	13.9	6.3
YLR413W	-		11.5	3.0	-
YKL044W	-		10.9	8.6	11.7
YMR320W	-		10.4	7.0	4.6
YER054C	-		-	9.6	3.4
YJL144W	-		9.4	-	3.2
YHR087W	-		8.4	10.7	7.0
YOR220W	-		8.2	-	6.5
YMR316W	DIA1		7.9	3.2	-
YAR020C	PAU7		7.8	4.0	5.7
YOL161C	-		7.6	4.0	-
YJL016W	?-		7.5	7.5	6.7
YER053C-A	-		7.3	-	3.9
YJL017W	?-		-	7.1	3.4
YOL114C	-		7.0	4.2	-
YAL068C	-		6.8	3.5	-
YPL230W	-		6.8	5.0	-
YER067W	-		6.5	17.3	6.8
YNL193W	-		6.5	4.4	-
YER053C-A			6.4	4.4	6.2
YOR173W	DCS2		-	7.8	3.1
YMR325W	-		6.2	9.1	4.6
YLR461W	PAU4		6.0	3.6	-
YNR076W	PAU6		6.0	5.5	-
YIL176C	-		5.9	4.0	-
YER091C-A	-		5.9	3.0	-

YLR177W	-		5.8	6.4	5.5
YJL223C	PAU1		5.8	4.5	-
YBR085C-A	-		5.7	14.0	3.9
YNR068C	-		5.7	8.5	3.6
YLR327C	-		5.6	3.1	
YDR070C	FMP16		5.6	12.0	6.3
YFL020C	PAU5		3.9	5.4	-
YMR040W	YET2		5.3	-	5.0
YMR087W	-		5.2	5.6	-
YOL123W-R	?		-	5.2	-
YGL261C	-		4.9	3.6	3.0
YBR012C	-		4.5	3.3	-
#YDL223C	HBT1		-	4.5	5.0
YMR324C	-		4.4	3.8	-
YDR185C	-		4.4	3.1	-
YIL056W	-		4.3	3.4	-
YLR311C	-		4.3	3.3	3.4
YLR312C	-		4.3	3.1	-
*YJL142C	-		4.3	3.0	-
YOL154W	ZPS1		3.9	3.1	-
YMR084W	-		3.8	4.4	-
YHL021C	FMP12		3.8	4.2	4.9
YOR009W	TIR4		3.7	3.4	-
YBR285W	-		3.7	3.9	-
YBR090C	-		3.7	4.1	-
YLR064W	-		3.7	-	3.1
YLL064C	-		3.6	4.3	-
YMR265C	-		3.6	3.3	3.7
YIR014W	-		3.6	-	4.9
YOR393W	ERR1		-	3.9	3.6
YOR186W	-		3.5	4.2	3.0
YIL055C	-		3.5	4.2	-
YHR054C	-		3.5	3.6	-
YLR149C	-		3.5	3.1	-
YLR437C-R	?		3.4	-	3.1

YLR446W	-		3.4	-	3.4
YBL086C	-		3.4	3.6	-
YMR181C	-		3.4	3.4	-
YCR100C	-		3.3	3.2	-
YIL130W	-		3.3	3.3	-
YDR276C-R	?		3.3	4.1	-
YPL168W	-		3.2	3.5	-
YPR150W	-		3.2	3.4	-
YPL247C	-		-	3.2	4.0
YJL163C	-		-	3.2	3.4
YPR035W-R	-		-	3.2	3.0
YLR219W	MSC3		3.1	-	3.1
YPL054W	LEE1		-	3.1	9.4
YDR482C	CWC21		3.0	3.0	5.5
YCL042W	-		-	3.0	3.6

Table 4.2: Microarray data: genes that were MHE after one-hour exposure to acetaldehyde only.

*Genes that are also MHE in the 'ethanol-stressed cells in presence of acetaldehyde' conditions relative to an ethanol stress.

Open reading frame	Genes	Function	Fold increase		Putative Transcription Factors
			Slide 1	Slide 2	
Stress response					
YHR106W	TRR2	Response to oxidative stress (IMP)	3.3	28.5	Msn2/4p, Hsf1p
					Msn2/4p, Hsf1p, Yap1p
Cytoskeleton genes					
YLR319C	BUD6	Cytoskeletal regulatory protein binding	3.9	3.2	Hsf1p, Yap1p
Transcription and translation					
YGL035C	MIG1	Specific RNA polymerase II transcription factor activity	4.9	4.3	-
Transport					
YAL002W	VPS8	Late endosome to vacuole transport	5.3	53.6	Msn2/4p, Hsf1p, Yap1p
Miscellaneous					
YBR136W	MEC1	Protein kinase activity	5.1	3.1	-
YCR005C	CIT2	Citrate (Si)-synthase activity	4.9	3.8	Msn2/4p
YLR321C	SFH1	Protein binding	4.3	4.1	Yap1p
YGL194C	HOS2	NAD-dependent histone deacetylase activity	3.3	3.5	Msn2/4p, Hsf1p, Yap1p
Unknown function					
*YFR048W	RMD8		17.7	7.1	Yap1p
YCR025C			14.8	3.9	-
YGL007W			8.9	7.5	Msn2/4p, Hsf1p, Yap1p
YGR139W			8.4	5.9	Msn2/4p
YER139C			8.3	3.4	-
YBR178W			7.6	3.8	Msn2/4p, Hsf1p
YMR086C-A			7.3	4.0	Msn2/4p, Hsf1p
YDR417C			5.9	5.0	Hsf1p, Yap1p

YFR023W	PES4		4.9	21.7	Yap1p
YDR522C	SPS2		4.6	4.0	Msn2/4p, Hsf1p
YFL061W	DDI2		4.6	3.5	Msn2/4p, Hsf1p, Yap1p
YPR195C			4.5	4.9	Yap1p
YJL043W			4.4	4.7	Hsf1p, Yap1p
YBR134W			4.4	3.7	Hsf1p
YBR113W			4.1	6.2	Hsf1p
YMR118C			3.9	4.1	Yap1p
YFL032W			3.8	3.4	Yap1p