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QUANTITATIVE YEAST PHYSIOLOGY AND NITROGEN METABOLISM DURING HETEROLOGOUS PROTEIN PRODUCTION

by

Johann Ferdinand Görgens

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Promoters: Profs. J.H. Knoetze, W.H. van Zyl and B. Hahn-Hägerdal

Stellenbosch, South Africa

April 2003

DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

Signature:

Johann Ferdinand Görgens

Date: 28 February 2003

University of Stellenbosch

Abstract

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By Johann F. Görgens

The physiology and nitrogen metabolism of the yeast, *Saccharomyces cerevisiae*, during heterologous xylanase production in a defined medium was quantified by the comparison of isogenic yeast strains, whereby several potential limitations in the production of the heterologous xylanase could be identified. The presence of global sensing and regulatory mechanisms, by which the yeast is able to actively regulate both heterologous gene expression and the physiological response to the process, was also investigated.

The deleterious effects of heterologous xylanase production on the physiology of the recombinant host were disproportionately large with respect to the amount of foreign protein produced. The cellular processes involved in this response were identified by the transcriptional profiling of isogenic recombinant strains, in a novel analytical approach to investigating foreign protein production by *S. cerevisiae*. Heterologous gene expression affected a combination of cellular processes and induced the yeast stringent stress response. The corresponding loss of metabolic functionality resulted in the disproportionate physiological effects of foreign protein production, similar to previous observations in recombinant *Escherichia coli*, and a possible reduction in attainable production levels. Reducing the propensity of recombinant gene expression to introduce metabolic stress may therefore increase production levels of foreign proteins by yeast. The metabolic vitality of transformed strains was also reduced by the presence of multiple copies of active, plasmid-based *PGK1*-promoters in the cell without expression of the heterologous gene. The negative effect was caused by an increase in the biosynthetic and glycolytic capacity of the strain at the expense of other processes.

Production levels of heterologous xylanase were influenced by expression vector selection and the presence of auxotrophic mutations in transformed strains of *S. cerevisiae*. The increased transcription levels obtained with the multicopy plasmid-

based YEp-type expression system, compared to the integrative YIp-type expression system, resulted in higher levels of xylanase production. Heterologous xylanase production thus did not saturate the secretory capacity of the host strain. The genetic stability of the autoselective YEp-type expression system in long-term chemostat culture was also demonstrated. High levels of heterologous xylanase production by transformed *S. cerevisiae* strains containing auxotrophic markers required the stabilisation of nitrogen metabolism via saturation of yeast cells with an excess of imported amino acids. By the removal of excessive auxotrophic markers, high levels of xylanase production by a prototrophic transformant in defined medium without amino acid addition could be obtained. Heterologous xylanase production by the prototrophic transformant was further enhanced by increasing the availability of preferred amino acids or succinate in the defined medium, indicating an additional requirement for metabolic precursors and building blocks for foreign protein synthesis. Comparable levels of heterologous xylanase production were obtained in high cell density cultures of the alternative yeast, *Pichia stipitis*, by the proper induction of the native *ADH2*-promoter, the control of oxygenation, and addition of an amino acid mixture to the defined medium, indicating the presence of generic limitations in transcription, nutrient availability and the yeast biosynthetic capacity for foreign protein production by various yeasts.

The presence of global sensing and regulatory mechanisms was confirmed by the physiological response of *S. cerevisiae* to heterologous protein production, which included the downregulation of biosynthesis and growth, and the induction of various processes involved in the stringent stress response. Additionally, heterologous xylanase production was actively regulated on a posttranscriptional level by the auxotrophic transformants in response to the level of amino acid availability. The biosynthetic capacity for foreign protein production by both recombinant *S. cerevisiae* and *P. stiptis* was also regulated in response to the physiological state of the yeast and the availability of nutrients. The presence of these regulatory mechanisms complicated the manipulation of cellular biosynthesis at will.

Universiteit van Stellenbosch

Opsomming

KWANTITATIEWE GIS-FISIOLOGIE EN -STIKSTOF METABOLISME GEDURENDE HETEROLOË PROTEÏEN PRODUKSIE

Deur Johann Ferdinand Görgens

Die fisiologie en stikstof-metabolisme van die gis, *Saccharomyces cerevisiae*, gedurende heteroloë xilanase produksie in 'n gedefiniëerde medium is gekarakteriseer deur isogeniese gis-rasse te vergelyk, waardeur verskeie moontlike beperkings in die produksie van die heteroloë xilanase uitgewys kon word. Die teenwoordigheid van globale sensoriese- en beheer-meganismes, wat die gis in staat stel om beide heteroloë geen uitdrukking en die fisiologiese respons op die proses aktief te reguleer, is ook ondersoek.

Die nadelige effekte van heteroloë xilanase produksie op die fisiologie van die rekombinante gasheer-organisme was uitermatig groot in vergelyking met die hoeveelheid vreemde proteïen wat geproduseer is. Die sellulêre prosesse verantwoordelik vir hierdie respons is identifiseer deur die transkripsionele profiele van isogeniese rekombinante rasse te vergelyk, in 'n nuwe analitiese benadering tot die bestudering van vreemde proteïen produksie deur *S. cerevisiae*. Heteroloë geen uitdrukking het 'n kombinasie van sellulêre prosesse geaffekteer en die gis se algemene voedingstres-respons geaktiveer. Die gepaardgaande verlies aan metaboliese funksie het die uitermatige fisiologiese effek van vreemde proteïen produksie veroorsaak, soortgelyk aan vorige waarnemings met rekombinante *Escherichia coli*. Die haalbare produksie-vlakke is moontlik ook verlaag deur hierdie respons. 'n Verlaging van die geneigdheid van rekombinante geen uitdrukking om metaboliese stres te veroorsaak, mag dus die produksievlakke van vreemde proteïene in gis verbeter. Die metaboliese groei-potensiaal van die getransformeerde rasse is ook verlaag deur die teenwoordigheid van etlike aktiewe kopieë van plasmied-gebaseerde *PGK1*-promotors in die sel, sonder uitdrukking van die heteroloë geen, deur 'n toename in die biosintetiese en glikolitiese kapasiteit ten koste van die ander sellulêre prosesse.

Die produksievlakke van heteroloë xilanase is deur die keuse van uitdrukkings-sisteem en die teenwoordigheid van autotrofiese mutasies in die getransformeerde rasse van *S.*

cerevisiae beïnvloed. Die verhoogde transkripsie vlakke wat met die multi-kopie, plasmied-gebaseerde YEp-tipe uitdrukkingsisteem, eerder as die geïntegreerde YIp-tipe sisteem, verkry is, het tot verhoogde xilanase produksie gelei. Heteroloë xilanase produksie het dus nie die uitskeidingskapasiteit van die gasheer versadig nie. Die genetiese stabiliteit van die autoselektiewe, YEp-tipe uitdrukkingsisteem in langtermyn chemostaat-kulture is ook gedemonstreer. Hoë vlakke van xilanase produksie deur getransformeerde *S. cerevisiae* rasse met autotrofiese merkers het die stabilisering van die stikstof metabolisme, deur die versadiging van die sel met ingevoerde aminosure, vereis. Die verwydering van oormatige autotrofiese merkers het tot hoë vlakke van xilanase produksie deur die prototrofiese transformant in gedefinieerde medium sonder aminosuur byvoeging gelei. Heteroloë xilanase produksie deur die prototrofiese transformant kon verder verbeter word deur die byvoeging van voorkeur-aminosure of suksinaat tot die gedefinieerde medium, en ‘n addisionele behoefte aan metaboliese voorloper-molekules en bou-blokke vir vreemde proteïensintese het dus bestaan. Vergelykbare vlakke van heteroloë xilanase produksie is in kulture met hoë sel-digthede van die alternatiewe gis, *Pichia stipitis*, verkry deur die doeltreffende induksie van die eiesoortige *ADH2*-promotor en die byvoeging van ‘n aminosuur-mengsel tot die gedefinieerde medium, wat die teenwoordigheid van generiese beperkinge in transkripsie, voedingstof-beskikbaarheid en biosintetiese kapasiteit van die gis vir vreemde proteïen produksie deur verskeie giste uitgewys het.

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Die teenwoordigheid van globale sensoriese- en beheer-meganismes is bevestig deur die fisiologiese respons van *S. cerevisiae* tot heteroloë proteïen produksie, wat die afwaartse regulering van biosintese en groei, en die induksie van verskeie prosesse betrokke by die algemene voedingstres-respons, ingesluit het. Heteroloë xilanase produksie is ook op ‘n na-transkripsionele vlak aktief gereguleer deur die autotrofiese transformante in reaksie tot die vlak van aminosuur beskikbaarheid. Die biosintetiese kapasiteit vir vreemde proteïen-produksie van beide rekombinante *S. cerevisiae* en *P. stipitis* is ook in reaksie tot die fisiologiese toestand van die gis en die beskikbaarheid van voedingstowwe gereguleer. Die teenwoordigheid van hierdie regulatoriese meganismes het die willekeurige manipulasie van sellulêre proteïen-biosintese bemoeilik.

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Ecclesiastes 11:1

Cast your bread upon the waters,
For you will find it after many days

Stellenbosch, South Africa
April 2003

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Chapter 1

INTRODUCTION

Heterologous protein production by yeast expression systems

1.1. WHAT IS HETEROLOGOUS PROTEIN PRODUCTION?

"Heterologous protein production" refers to the cloning, transfer and expression of genes from a specified microorganism into a host organism of choice, enabling the alternative host to produce the proteins encoded by the cloned genes. Also referred to as "Recombinant gene expression," this method enables the production of a variety of foreign proteins in a chosen host, provided that the necessary genetic tools and expression system are available. The two major applications of heterologous protein production in industrial biotechnology is, (a) the development of microbial expression systems able to overproduce valuable proteins in a convenient manner, and (b) the engineering of new or improved pathways in microorganisms for the production of metabolites or proteins, also known as "metabolic engineering" (Bailey, 1991). The alternative host organism may be better suited to the industrial production of a heterologous protein than the native host, due to factors such as improved growth and higher cell densities, a lower background of native protein levels resulting in the production of a more pure product, higher expression levels of the specific protein due to genetic manipulation and increased environmental acceptance of the organism due to reduced toxicity. Some pharmaceutical proteins of human origin, such as interferons and interleukins cannot be produced otherwise than with a heterologous host (Swinkels et al., 1993; Walsh, 2000). The profitability of heterologous proteins produced in such a manner for medical use is significant, with the industry producing these pharmaceuticals recently valued at US$12 billion (Walsh, 2000). Other examples of human proteins produced in large volumes for medical use are: Interferon-α, Hepatitis B vaccine, granulocyte colony-stimulating factor (GC-SF) and tissue plasminogen activator (Koths, 1995; Walsh, 2000).

In the current work, the application of heterologous protein production for metabolic engineering applications – i.e. the development of a new or improved functionality in a host organism – was not considered, but rather the relationship between the physiology of the *S. cerevisiae* yeast host and production levels of a model recombinant protein, *Trichoderma reesei* xylanase II.

1.2. WHY XYLANASE PRODUCTION?

The cloning of *T. reesei* endo-1,4-xylanase II (*XYN2* gene) and expression in yeast via transformation enabled heterologous xylanase production by *S. cerevisiae* (also known as baker's or brewer's yeast), which possesses no native xylanase genes (La Grange et al., 1996). Xylanase is a hydrolytic enzyme that cleaves the polymeric backbone of the xylan hemicellulose, which is second only to cellulose in natural abundance as carbohydrate.

The catalytic activity of endo-1,4-xylanases have found application in several industries, of which the most effective current use is in the prebleaching of kraft pulp, where environmental regulations restrict the use of chlorine (Buchert et al., 1992; Viikari et al., 1994). Xylanase pretreatment has been shown to minimize the use of harsh chemicals in subsequent treatment steps and resulted in greater brightness of the final paper product. The use of xylanases produced by yeast in these processes is especially advantageous, since these preparations contain no cellulase activity that will reduce the strength of the final paper product. Extensive purification of xylanase from fungal sources is required for the removal of cellulase activity prior to application in bleaching processes (Bajpai, 1999; Kulkarni et al., 1999; Beg et al., 2001).

Xylanases are also used in the baking industry due to the presence of hemicellulose in the raw material. Xylanase addition to wheat flour has improved dough handling and quality of baked products (bread volume) (Maat et al., 1992; Randez-Gill et al., 1999; Kulkarni et al., 1999). The use of baking yeasts expressing heterologous xylanase can also eliminate the need for baking additives and thereby improve profit margins (Randez-Gill et al., 1999). The nutritional properties of agricultural silage and grain feed, especially for poultry, can also be improved by the addition of xylanase (Bedford and Classen, 1992; Kudah and Singh, 1993). Xylanases are also used in combination

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with pectinase and cellulase for clarification of fruit juices (Biely, 1985; Kulkarni et al., 1999), and in wine making where it has been shown that the fruity aroma of wine, made with recombinant yeasts producing *Aspergillus nidulans* xylanase A, was improved (Ganga et al., 1998; Ganga et al., 1999). Xylanases are also useful in coffee making, oil recovery and the extraction of other organic compounds such as plant oils and starch (Wong and Saddler, 1992; Kulkarni et al., 1999). Other possible applications of xylanase are in the production of liquid or gaseous fuels via renewable resource utilisation (Olsson and Hahn-Hägerdal, 1996), where the expression of xylanase genes in fermentative organisms could enable these organisms to directly convert xylan residue into liquid fuels (Kulkarni et al., 1999).

In particular, the heterologous xylanases from *T. reesei* and *Cryptococcus albidus* were selected as model proteins in the present study due to the efficient secretion of the proteins by their yeast hosts, *S. cerevisiae* and *P. stipitis*, respectively. Because of an absence of catalytic activity in metabolism, the actual production of these heterologous proteins could thus be studied in isolation from effects introduced by the enzymatic activity of the produced recombinant protein.

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1.3. REASONS FOR USING YEAST PRODUCTION SYSTEMS

Heterologous gene expression may be obtained in a variety of host strains, ranging from simpler prokaryotes, such as recombinant *E. coli*, to lower eukaroyes, yeasts in particular, and higher eukaryotes, such as mammalian or insect cells. A brief comparison of yeast expression systems with either bacterial or higher eukaryotic cells reveal a number of specific reasons why the former may be preferred.

Heterologous gene expression by *S. cerevisiae* was first described in 1981 (Hitzeman et al., 1981). Along with the "alternative" yeast species (most notably *Pichia pastoris* and *Hansenula polymorpha*), *S. cerevisiae* has become a strong favourite for the production of foreign proteins (Hinnen et al., 1995). As with the frequently-used bacterial systems, this has partly been due to the prior experience with the microorganisms at industrial scale and the possibility to use cheap substrates, which allows the efficient scale-up of yeast expression systems into commercial processes (Swinkels et al., 1993; Schultz et al., 1994; Cereghino and Cregg, 1999). Yeasts also offer the ease of gene manipulation

found in bacteria, as yeast genetics are relatively well known (Schultz et al., 1994; Cereghino and Cregg, 1999; Calado et al., 2002). In contrast to bacterial systems, however, the ability of several yeast species to perform the posttranslational processing and secretion of foreign proteins is particularly advantageous, since exploiting the capabilities of the eukaryotic secretory apparatus of yeast is a direct means of producing a purer protein product with a high fidelity to the naturally occurring species (Wittrup et al., 1994; Schultz et al., 1994; Hinnen et al., 1995; Calado et al., 2002). The latter is possible since yeasts, being a lower eukaryote, possess an intracellular environment with the ability to perform many eukaryotic-specific posttranslational modifications, such as proteolytic processing, folding, disulfide bridge formation and glycosylation, which is required to obtain a protein with the correct biological activity in higher eukaryotes (e.g. mammalian cells) (Moreau et al., 1992; Romanos et al., 1992; Swinkels et al., 1993; Schultz et al., 1994; Cereghino and Cregg, 1999; Rosenfeld, 1999). Yeasts therefore have a significant advantage over bacteria, where the *in vitro* unfolding and refolding of the recombinant product is usually required to obtain biological activity (Lee et al., 1999). Export of the protein product from the host cell also reduces the risk of protein degradation by intracellular proteases (Calado et al., 2002), and since only a very small fraction (0.5%) of the native yeast proteins are secreted, a reasonably pure secreted product can be obtained, which simplifies downstream processing (Mendoza-Vega et al., 1994; Schultz et al., 1994; Vasavada, 1995; De Baetselier and Van Broekhoven, 1998; Calado et al., 2002). The yeast *S. cerevisiae* in particular does not produce endotoxins, contrary to several of the bacterial expression systems, and is considered safe for the production of health care and food products, and FDA approval of production processes can thus be obtained more readily (Romanos et al., 1992; Schultz et al., 1994; Vasavada, 1995; Kulkarni et al., 1999). The GRAS (Generally Regarded As Safe) status of this organism is based on a long history of safe use as food organisms and this yeast is therefore more acceptable for the production of pharmaceutical or food proteins than e.g. prokaryotes (bacteria) or mammalian cells, which may pose the potential problems of pyrogenic endotoxins and oncogenic DNA, respectively (Swinkels et al., 1993). *S. cerevisiae* has been used by at least 4 pharmaceutical companies in production processes, including human insulin production by Novo Nordisk (Denmark) (Alberghina et al., 1993). Yeasts also offer faster growth rates than more complex (mammalian) expression systems, which result in less contamination and shorter production times, along with the ability for

propagation in large quantities with ease and at low cost (Vasavada, 1995; Law et al., 1998; Cereghino and Cregg, 1999).

However, the foremost reason for using yeast expression systems, i.e. the ability to perform the posttranslational modification and secretion of foreign proteins, may also represent their greatest limitation: due to the complexity of the protein secretion process, production levels of secreted proteins are likely to be lower than that of proteins produced intracellularly (Vasavada, 1995). Thus, although the need for *in vitro* posttranslational maturation can be a limiting factor in the production of recombinant proteins with bacteria such as *E. coli* (Ljubijankic et al., 1999), yeast has not been used as frequently as *E. coli* for the production of therapeutic proteins due to the lower production levels usually obtained (Lee et al., 1999). The selection of a bacterial, yeast or mammalian expression system for production of a recombinant protein depends on the nature of the protein, the required posttranslational modifications, the quantity needed and the cost of production (Vasavada, 1995), and is empirical in several cases (Bathurst, 1994). The yeasts *S. cerevisiae* and *P. pastoris* are most frequently used for heterologous protein expression (Cereghino and Cregg, 1999) (see Chapter 2 for comparison of yeast species).

1.4. LIMITATIONS IN THE PRODUCTION OF HETEROLOGOUS XYLANASES BY YEASTS

To be suited for industrial cultivation, a microbial production system should possess strict genetic stability, leading to stable production levels and high yields in repeated fermentations, especially if an expensive substrate or very valuable protein is produced. In the present heterologous protein production system, however, low yields of xylanase production were initially observed, prompting a fundamental investigation into the factors that may limit the production of heterologous proteins by yeasts. In an effort to identify possible limitations in the production and secretion of heterologous xylanase by *S. cerevisiae*, the physiology and nitrogen metabolism of isogenic, transformed strains were compared during cultivation under well-controlled conditions.

The experimental approaches represented in the following chapters were based on the careful quantification of the yeast physiology (Fig. 1.1). The heterologous xylanase

produced by *S. cerevisiae* was first purified to allow estimation of the mass amount of foreign protein produced (Chapter 3). The yeast physiology was subsequently quantified in terms of the additional "load" of heterologous gene expression introduced in the metabolism, which reduced the metabolic vitality of the production strain and affected the cultivation performance thereof negatively (Chapter 4). The deleterious effects of heterologous protein production on the physiology of the transformed strains can reduce the levels of biomass formation, while increasing the time required for completion of an industrial production process, thus affecting the overall productivity negatively. The observed "metabolic load" was further investigated on a transcriptional level, whereby the nature of the physiological stress responsible for the reduced metabolic vitality could be identified (Chapter 5). The potential limitation of heterologous xylanase production levels due to the choice of host genetic background or foreign gene expression system was investigated by the comparison of three potential production systems, whereby the influence of auxotrophic requirements and foreign gene copy number was demonstrated (Chapter 6). The limited availability of nutrients that specifically enhance heterologous xylanase production may also reduce the production levels obtained with recombinant *S. cerevisiae* (Chapter 7). The effect of nutrient availability and cultivation conditions on the level of promoter induction and foreign gene expression was also demonstrated in novel yeast host for heterologous protein production, *P. stipitis* (Chapter 8).

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The aforementioned experimental approaches provided data to support the central theme of the present dissertation: Does the yeast possess global mechanisms for the sensing and active regulation of heterologous protein production and/or the physiological response to the production process? Regulatory mechanisms in cellular metabolism may determine the physiological response of the yeast host to heterologous protein production, and determine the eventual production levels of heterologous proteins, and may therefore have a significant effect on the productivity of recombinant strains in industrial fermentations. The identification of such regulatory mechanisms is therefore critical for understanding and improving the production of heterologous proteins by yeast.

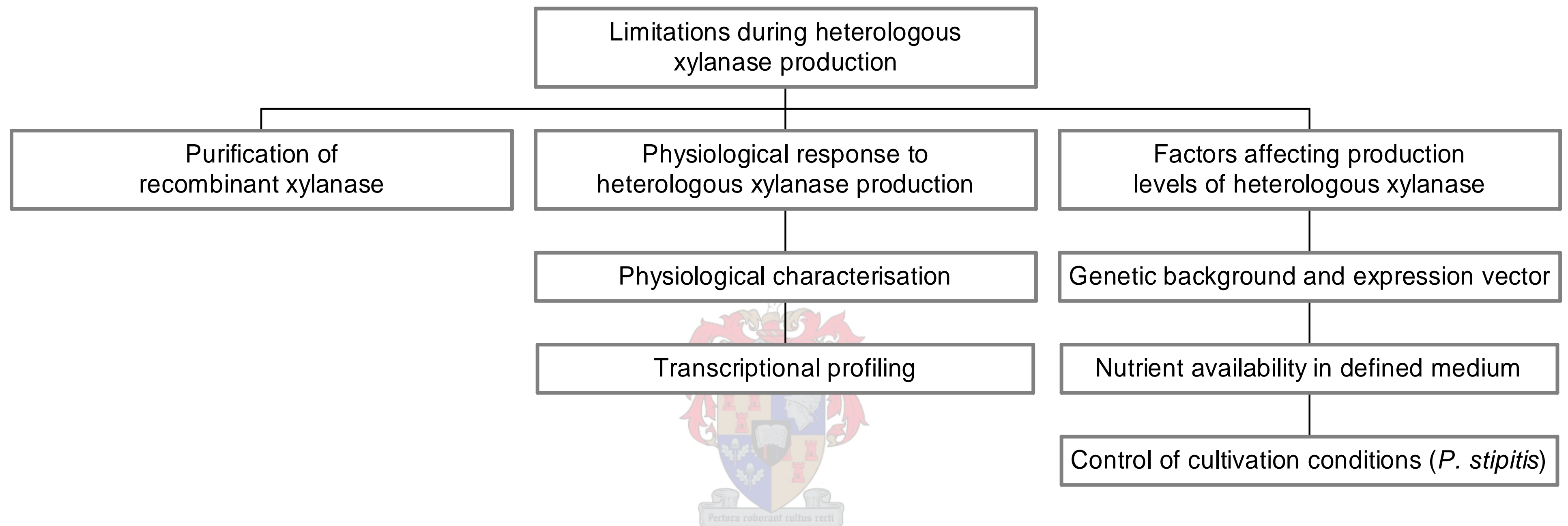

Figure 1.1. Layout of experimental work

Besides providing experimental data on the possible limitations in yeast metabolism during heterologous xylanase production, these investigations also demonstrated the importance of reproducible experimental procedures and accurate analytical techniques for investigating microbial physiology. The experimental results obtained in Chapter 4 to 8 were largely dependent on the reproducibility of the yeast cultures, and, more importantly, on the availability of analytical techniques such as transcriptional profiling, amino acid analysis, fermentation off-gas analysis and high performance liquid chromatography (HPLC). Without adequate analytical techniques, the mechanistic investigation of microbial physiology and assimilation of a holistic view of metabolism would not be possible.

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Chapter 2

Review of Literature: RATIONAL APPROACHES FOR IMPROVING HETEROLOGOUS PROTEIN PRODUCTION BY YEAST

Addressing known limitations in yeast expression systems

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2.1. INTRODUCTION - Quantitative aspects of recombinant protein production in yeast

The production of a secreted heterologous protein by yeast proceeds via three major steps:

- During transcription mRNA (messenger RNA) molecules, which are complementary to the template DNA (the foreign gene being encoded), are synthesized in a process catalysed by the yeast RNA polymerase II.
- Translation proceeds after transport of mRNA to the ribosomes, where proteins are synthesised via the addition of individual amino acids in sequential manner. Amino acids are transported to the ribosomes by the tRNA (transport RNA) molecules.
- Posttranslational modification and secretion involves the processing of the synthesised protein through the yeast secretory apparatus, to obtain an extracellular, properly folded, mature protein with biological activity.

The mechanisms that determine the efficiency of the native apparatus towards the production of a foreign protein are not well understood, and the presence of several limitations and large variations in production levels are evident from previous reports. Reported expression levels of foreign proteins in *P. pastoris* range from m g.l^{-1} to g.l^{-1} (d'Anjou and Daugulis, 2001), whereas similar large variations in the levels of foreign protein secretion by *S. cerevisiae* may be attributed to variations in the characteristics of individual production systems (Ichikawa et al., 1989). It should also be noted that production levels reported in the literature are typically from laboratory-scale experiments, not large-scale production (Hensing et al., 1995a).

General factors known to influence the production level of a heterologous protein by a yeast expression system include (Calado et al., 2002; Bae et al., 1998; Shiba et al., 1994; Weber et al., 1992; Ichikawa et al., 1989; Sleep et al., 1991; Romanos et al., 1992; Vasavada, 1995):

- Transcriptional efficiency and the availability of transcription factors
- Nature and strength of promoter and terminator

- Type, stability and copy number (gene dosage) of expression vector (plasmid)
- Efficiency of translation, based on the untranslated mRNA leader sequence, and codon bias
- Efficiency of secretion
- Signal/leader sequence and chaperone availability
- Posttranslational modification and proteolytic protein processing
- Nature and stability of the proteins produced
- Host strain characteristics (some strain are known to overexpress or overproduce recombinant proteins)
- Interaction between the host cell physiology, metabolism and the expression vector (plasmid)
- Environmental factors such as bioreactor operational strategy and medium composition

When attempting gene expression and secretion of a heterologous protein, barriers can therefore be encountered at numerous stages, from transcription through to the stability of secreted proteins (Romanos et al., 1992). Versatile expression systems such as *S. cerevisiae* thus need to be optimised to ensure high production levels of heterologous proteins (Alberghina et al., 1993). Due to the complex interaction of the factors that determine the efficiency of the expression and secretion process, the prediction of the yield of a recombinant protein is not possible, and most approaches for addressing the critical parameter(s) in production systems remain empirical in nature (Sleep et al., 1991; d'Anjou and Daugulis, 2001). As success with the production of a heterologous protein can seldom be ensured beforehand, the most suitable host-vector and gene expression systems for production of a given protein are usually determined by comparing the various candidate expression systems (Uchiyama et al., 1995). Despite the highly empirical nature of heterologous protein production, the application of the various known "rules of thumb" can result in the improvement of heterologous protein production by yeast. The inclusion of multiple genetic improvements in this manner frequently has cumulative effects on the production level of heterologous protein (Schultz et al., 1994). In this review, the application of new and existing rational approaches for improving the levels of heterologous protein production by yeast are presented on the basis of several case studies.

2.2. TRANSCRIPTION

As the first step in the synthesis of a foreign protein by yeast, transcription may either present the cell with an "excess" of heterologous mRNA molecules, or limit production of the protein due to inefficient mRNA production. Various factors determine the eventual level of heterologous transcripts in the cell.

2.2.1. Type of expression system

For heterologous protein production in *S. cerevisiae*, three major types of expression systems can be employed. Yeast Integrative plasmids (YIp) are based on the homologous recombination of sections in the expression vector at single sites in the yeast chromosome. The use of YIp typically results in the integration of a single or few copies of the desired heterologous gene maintained with great stability during cell division (Mendoza-Vega et al., 1994a; Gellissen and Hollenberg, 1997). Integrative plasmids in general have the advantage of not necessitating the inclusion of bacterial DNA sequences, which are potentially toxic to other organisms and may decrease vector stability (Lopes et al., 1996).

Alternatively, yeast episomal plasmids (YEp) are stable genetic sequences maintained independent of chromosomal DNA within the nucleus of the cell, by the inclusion of the native yeast 2 μm sequence in the plasmid construct. Typical components in a YEp vector are a selection marker for which the yeast is auxotrophic, the expression cassette and the bacterial sequences for amplification in *E. coli*. YEp expression vectors are normally present at higher copy numbers than YIp vectors (Mendoza-Vega et al., 1994a), in the order of 50-60 (Shuster et al., 1989) or 60-100 (Bae et al., 1998) copies per cell. The copy number of the YEp expression vectors may also be increased by inclusion of the *LEU2(d)* selection marker in a leucine-auxotrophic background. This partially defective gene is expressed at low levels, forcing the cell to maintain high copy numbers of the plasmid to ensure sufficient leucine availability (Erhart and Hollenberg, 1983). The increased copy number of the YEp vectors, resulting in an increased "gene dosage" of the foreign gene, may lead to an increase (sometimes proportional) in the production levels of the recombinant protein (Weber et al., 1992; Ljubijankic et al., 1999; Park et al., 2000). However, the genetic and physiological background of the

host strain may also overshadow the effects of increased copy number, as is discussed in the remainder of this review (Porro et al., 1992). In *Kluyveromyces lactis*, production of a heterologous protein has also been increased through the use of a multicopy plasmid-based expression system (pKD1), rather then chromosomal integration (Fleer et al., 1991). A large variation in the heterologous protein production levels between individual transformants may frequently be observed when the yeast 2 μm vectors are employed (Mendoza-Vega et al., 1994a). Despite the presence of selection markers on the recombinant plasmid, YEp vectors may also suffer from severe segregational instability, resulting in the loss of the recombinant plasmid during cell division (Harashima, 1998). The copy number and stability of these plasmids may therefore vary, depending on the selection marker, plasmid/promoter properties, gene expression level, expressed protein and growth conditions (Kjeldsen, 2000; Nacken et al., 1996; Buckholtz, 1993; Janes et al., 1990). Nitrogen-limitation may improve the stability of the plasmid-containing population (Gupta and Mukherjee, 2002; Gupta et al., 2001).

Due to the frequent instability of YEp vectors and the low copy number of YIp vectors (1-5), integrative expression systems based on repetitive chromosomal DNA sequences have been developed (Lopes et al., 1996; Gellissen and Hollenberg, 1997; Buckholtz, 1993). These expression systems offer a high copy number and high mitotic stability, which can be essential for a high expression level of heterologous proteins (Shiba et al., 1998; Castelli et al., 1994; Fleer, 1992). A novel vector system based on the integration of the expression cassette in the repetitive ribosomal DNA of the yeast, resulted in a high copy number, though the mitotic stability of these vectors decreased sharply for heterologous genes larger than 9-10 kb (Lopes et al., 1996). Similar integrative expression systems based either on the repetitive *Ty1* DNA sequences (Lee and Da Silva, 1996a; Lee and Da Silva, 1996b) or the repetitive δ sequences (Parekh et al., 1996; Lee and Da Silva, 1997; Kim et al., 2001) have also been developed for *S. cerevisiae*. Transcription from integrated expression cassettes may be more efficient than from YEp type vectors. A recombinant *S. cerevisiae* strain harbouring 8 integrated copies of a heterologous expression cassette secreted a larger amount of recombinant protein than a strain containing the same cassette on the 2μm plasmid, the copy number of which can be several hundreds (Nomura et al., 1995a).

The transformation of *P. pastoris* is possible by integration only, because of the absence of endogenous plasmid sequences. Multiple insertions of the expression cassette can occur at a single locus, with transformants containing gene copy numbers up to 30 recovered by selection for increased G418 resistance (Rosenfeld, 1999; Pennell and Eldin, 1998; Sreekrishna et al., 1997). In some cases a single integrated copy of a heterologous gene is sufficient for optimal production, whereas for other cases a high copy number is essential for the production of high levels of heterologous protein (Cereghino and Cregg, 2000; Romanos, 1995; Sreekrishna et al., 1997). The type of expression system may therefore influence both the efficiency of transcription and the level of foreign mRNA in the cell. However, the maximal level of recombinant protein production may be elicited by an optimal rather than maximal copy number of the expression vector (Rosenfeld, 1999).

2.2.2. Autoselection systems

Because of the ease of genetic manipulation, the 2 μm plasmids may, in some cases, still be the preferred expression vector for heterologous protein production in *S. cerevisiae* (Alberghina et al., 1993). Several autoselection systems have therefore been developed, to ensure adequate plasmid stability and genetically stable recombinant strains. Although YEp expression vectors normally contain a selection marker for which the host is auxotrophic, frequent instabilities have been observed, presumably due to uptake of the metabolite for which an auxotrophic requirement exists from the medium (Meinander, 1997). To obtain "autoselection," these single selection systems are complimented by an additional selection system, where either the uptake of a required metabolite from the cultivation medium is incapacitated, or an essential structural gene is disrupted on the chromosome and complimented on the recombinant plasmid, to fabricate double or triple selection.

The first such autoselection system for *S. cerevisiae* was described by Loison et al. (1986), which utilized a disruption of the native *FUR1*-gene in combination with insertion of the *URA3* gene on the recombinant plasmid, in a host strain that contains a mutation in *URA3*. The reduced uracil uptake from the medium obtained by *FUR1*-disruption, resulted in a transformant that showed excellent genetic stability, also in continuous culture (Marquet et al., 1987). This autoselection system was further

improved by the additional disruption of the *URID-K* gene, which may also function in nucleotide uptake (Wang and Da Silva, 1993), and transformants were grown in complex medium without significant plasmid loss. A double selection system based on the complementation of *URA3*-selection with *FBA1*-selection (*FBA1* encodes the FDP aldose enzyme) has also been developed and tested (Compagno et al., 1993; Compagno et al., 1996). A similar autoselection system that ensures plasmid stability in recombinant *K. lactis* has also been described (Hsieh and Da Silva, 1998). These autoselection systems have greatly improved the genetic stability and stability of transformants containing YEp type vectors, whereby stable production levels under controlled cultivation conditions could be ensured.

2.2.3. Comparison of promoters

The characteristics of the promoter used to drive the expression of a recombinant gene can also influence production levels, as the strength of the promoter is an additional determinant of the transcription level of a heterologous gene (Kjeldsen, 2000; Park et al., 1993; Ruohonen et al. 1995; Park et al., 2000). Although both constitutive and regulated promoters may be used for heterologous gene expression, tight regulation becomes essential if the produced protein has a strong deleterious effect on cellular metabolism (Buckholtz, 1993). In *S. cerevisiae*, such regulated promoters are frequently used, since they are typically stronger, support a faster rate of transcription and restrict the time during which the recombinant protein is produced (Buckholtz and Gleeson, 1991; Fleer, 1992; Alberghina et al., 1993). In comparison to the constitutive *SUC2* promoter, for example, use of the regulatable *GPD* promoter resulted in a higher maximum protein production rate, although use of the *SUC2* promoter supported a higher final product concentration (Park and Ramirez, 1990). A further example of a regulated promoter is the yeast alcohol dehydrogenase (*ADH2*) promoter, which is normally inactive and requires positive activation for derepression (Beier et al., 1985). For this promoter, however, the availability of positive transcription factors for activation of the *ADH2* promoter may limit gene expression from multicopy plasmids (Irani et al., 1987), and increased expression has been observed by overexpression of the Adr1p transcriptional activator (Price et al., 1990). Similarly, the low availability of the *GAL4* transcriptional activator is normally rate-limiting for the maximal induction of *GAL* promoters, and significant increases in the level of transcription, and heterologous protein production, from *GAL* promoters have been obtained by

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overexpression of *GAL4*, both for integrated and plasmid-based expression systems (Neeper et al., 1990; Porro et al., 1992; Alberghina et al., 1993; Schultz et al., 1994; Pedersen et al., 1996; Lee et al., 1999). Overexpression of *GAL4* increased foreign protein production to similar levels in several yeast strains tested, despite differences in physiological and genetic background, indicating that a major generic limitation had been addressed (Porro et al., 1992). In general, limitations in the availability of transactivators for expression from multicopy plasmids may thus be overcome by overexpression (Fleer, 1992; Buckholtz, 1993). Several investigations have thus confirmed the potential effect of promoter characteristics on the final production level of heterologous protein.

2.2.4. Additional factors

In a groundbreaking example it was shown that homologous Pgk1p produced from a plasmid-expression system accumulated to 80% of the total soluble cellular protein, whereas heterologous protein production levels seldom exceed 1-5% of the total cellular protein, far below the theoretically attainable values. The significant difference in production levels was explained by the lower steady-state levels of heterologous mRNAs in the cell (Chen et al., 1984; Mellor et al. 1985; Chen and Hitzeman, 1987), indicating that transcription may limit heterologous protein production. Other examples of the influence of mRNA transcript levels on the level of foreign protein production have been reported (Aho et al., 1996; Skory et al., 1996).

Besides the aspects of gene copy number and promoter strength, levels of heterologous transcripts are also influenced by the exact positioning of the heterologous gene on the recombinant plasmid, and the presence of foreign non-coding sequences at the 3'-end of the heterologous cDNA (Joseph-Liauzun et al., 1995). 3' untranslated mRNA sequences may influence mRNA stability and the efficiency of transcription termination (Fleer, 1992) and removal of these regions has significantly increased foreign mRNA levels (Joseph-Liauzun et al., 1995; Lang and Looman, 1995; Broker et al., 1991). Similarly, murine interleukin-2 expression in yeast increased 10-fold by deleting the major part of the mammalian 3' untranslated region, which was apparently responsible for rapid degradation of the murine IL-2 mRNA (Demolder et al., 1992).

In *P. pastoris*, insertion of the first ATG-site of the heterologous coding sequence as close as possible to the ATG position of the *AOX1* generally also improves expression levels (Cereghino and Cregg, 2000). In another yeast, *H. polymorpha*, a change in the site of integration increased production levels two-fold, demonstrating the possible effect of chromosomal topography on gene expression (Kang et al., 2001).

Efficient termination of foreign gene transcription also influences gene expression in yeast, as was demonstrated by a 7-10 fold and 3-5 fold increase in production levels due to increased transcriptional termination efficiency (Bijvoet et al., 1991; Kanai et al., 1996; Cho et al., 1997). Stability of the multicopy pMIRY1 integrative vectors was also dramatically improved by including the transcription-terminating sequence of the rDNA units where it was integrated (Lopes et al., 1996).

2.3. TRANSLATION

Although the efficiency of translation may also limit heterologous protein production, relatively few investigations on the kinetics of the various processes involved in foreign protein synthesis have been undertaken. An exception is the potential effect of codon bias on the efficient translation and production of a heterologous protein, which was recognised during the development of the early recombinant protein production systems. The highly efficiency expression of a foreign gene may result in a mismatch between the demand and supply in the protein synthetic machinery, especially if the codon bias and/or amino acid content of the heterologous gene is significantly different to that of the production host. In such a case the demands placed on host protein synthetic apparatus are not matched to its normal tRNA population, which can limit the synthesis of the protein, or result in significant translational errors (Kurland and Gallant, 1996). Native sequences for heterologous genes and signal peptides are therefore sometimes replaced by synthetically generated genes, based on the yeasts preferred codon usage (Bennetzen and Hall, 1982) to ensure the most efficient translation possible (e.g. Pohlig et al., 1996; Lee et al., 1999). However, some reports have also questioned the likelihood of codon usage preventing high level of gene expression, unless extreme requirements are placed on the tRNA population (Fleer, 1992). Adverse effects may also result from the proposed "optimisation of codon usage," since changing the primary sequence of a gene may alter the primary and secondary structure

of the mRNA, which may influence translation negatively (Fleer, 1992). Neither does the drive for higher expression levels by recombinant protein production systems always lead to lower quality or authenticity of the protein product's sequence or structure (Olins, 1996).

A worthy consideration, however, is the sensitivity of translation initiation in yeast to the secondary structure of the 5'-untranslated region (UTR) of mRNA (Fleer, 1992), and the potential effect on translation efficiency. The native *SDH1* and *SUC2* 5' UTRs were capable of conferring glucose-sensitive mRNA instability to other genes expressed in *S. cerevisiae*, and could also control the turnover of the transcripts (De la Cruz et al., 2002; Cereghino et al., 1995). These changes in the mRNA stability due to the manipulation of the 5' UTR were correlated with changes in the translational efficiency for the corresponding transcripts, indicating a direct relationship (De la Cruz et al., 2002). An increase in levels of heterologous protein production by *S. cerevisiae* was also obtained by either the modification or removal of the 5'-end of the heterologous gene sequence (Joseph-Liauzun et al., 1995; Lang and Looman, 1995; Broker et al., 1991). An apparent correlation between the predicted stability of the local secondary structure within the region of translation initiation at the 5'-end of the heterologous cDNA, and the level of recombinant protein production was also observed (Joseph-Liauzun et al., 1995). Furthermore, the removal of the 5'-UTR from the recombinant human serum albumin (HSA) cDNA has led to a 5-fold increase in the production of the recombinant protein by *H. polymorpha*. Differences in production levels due to changes in gene dosage, shown to increase the production levels prior to deletion of the 5'-UTR, were thereby abolished, resulting in maximal production levels from a single integration copy, indicating that the effect of gene dosage was strongly related to inefficient translation (Kang et al., 2001). For expression in *P. pastoris* the 5'-UTR of the heterologous cDNA should be as close as possible to *AOX1* mRNA to ensure high production levels, as 50-fold changes in the expression levels by these adjustments have been observed (Sreekrishna et al., 1997).

2.4. POSTTRANSLATIONAL MODIFICATIONS AND SECRETION

The advantages of the secretory production of foreign proteins, compared to intracellular production, rely heavily on the efficient functioning of the posttranslation

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and secretion machinery. The exploitation of the ability of the yeast intracellular environment to perform many eukaryotic-specific posttranslational modifications, such as proteolytic processing, disulfide bridge formation and glycosylation, is a direct means of producing a protein product with high fidelity and biological activity in higher eukaryotes (Wittrup et al., 1994; Schultz et al., 1994; Calado et al., 2002; Moreau et al., 1992; Romanos et al., 1992; Swinkels et al., 1993; Schultz et al., 1994; Cereghino and Cregg, 1999; Rosenfeld, 1999). Biological activity is of such importance that the authenticity of the mature protein product in general, represents a more important consideration than the highest level of expression (Eckart and Bussineau, 1996).

2.4.1. Maximum expression ≠ maximum secretion

Although, for the production of an intracellular protein, maximum synthesis generally results in maximum accumulation, secretion of a heterologous protein often does not increase proportionally with increased expression (i.e. higher levels of foreign mRNA). The rate-limiting step in protein secretion is generally not synthesis (transcription and translation), but posttranslational processing, which represents a kinetic bottleneck (Wittrup et al., 1994; Romanos et al., 1992; Robinson et al., 1994). Large amounts of heterologous protein synthesis may saturate the secretory pathway, thus resulting in a reduced overall production level (Harmsen et al., 1993; Wittrup et al., 1994; Parekh et al., 1995; Robinson and Wittrup, 1995).

2.4.2. Summary of the secretory pathway

To improve the secretion of a foreign protein, understanding and manipulating the secretory pathway is essential (Shuster, 1991; Tuite and Freedman, 1994). Secretion and posttranslational modification of newly synthesised protein proceeds via several distinct steps (Kjeldsen, 2000; Ellgaard et al., 1999; Tuite and Freedman, 1994), as is presented in Fig. 2.1.

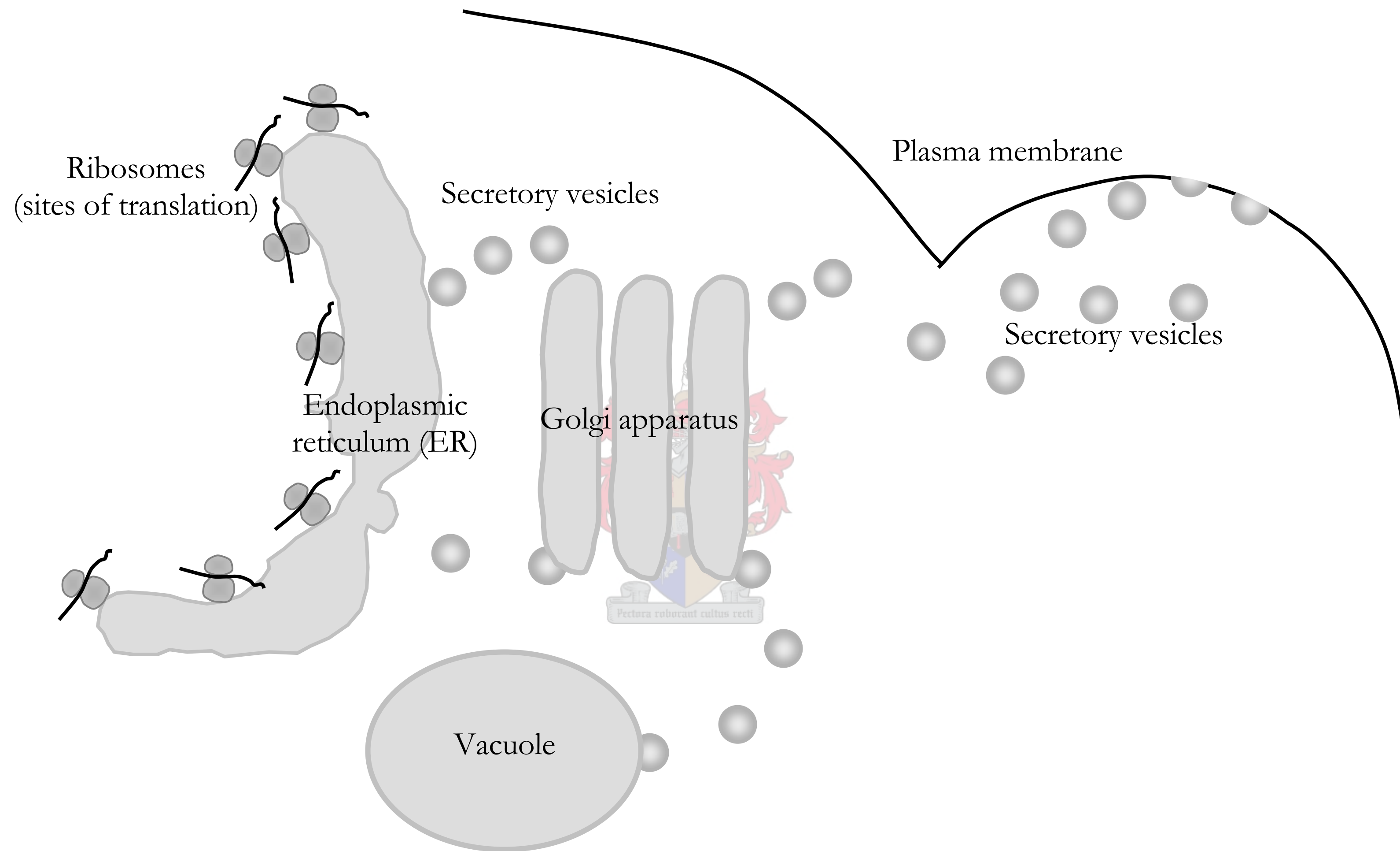

Figure 2.1. Eukaryotic secretory pathway

Translocation from the cytoplasm (where proteins are synthesised) across the membrane of the endoplasmic reticulum (ER) proceeds during or after translation (depending on the type of protein being produced), where the attachment of N-linked carbohydrate chains, folding and maturation of proteins are performed. The formation of secretory vesicles subsequently allows for transport from the ER to the Golgi apparatus, where further posttranslational modifications are completed. Export from the Golgi to the cell (plasma) membrane and into the periplasmic space, from where the proteins are finally exported to the extracellular medium, is also facilitated by secretory vesicles. Proteins destined for secretion typically feature a signal peptide (secretion signal) at the N-terminus, which facilitates the targeting of the protein from the cytoplasm to the ER. Translocation into the ER requires the specific action of the ER's resident chaperone BiP/Kar2p ("heavy chain binding protein"). The posttranslational modifications performed in the ER include removal of the N-terminus signal peptide by the signal peptidases (such as Kex2p), the formation of disulfide bonds by protein disulfide isomerase (PDI), the attachment of core glycosylation, and folding. Protein folding and tertiary structure formation are also completed to an advanced stage in the ER, as the presence of several quality control mechanisms ensures that only folded proteins are transported to the Golgi. The primary quality control mechanism involves the association of newly folded proteins with chaperones and folding enzymes in the ER (e.g. BiP and PDI) which not only assist the folding and assembly process, but also retain immature proteins. Degradation of misfolded proteins is an essential function of the quality control system of the ER, and the sorting of misfolded soluble proteins to the vacuole for this purpose, contributes to a stringent quality control system in *S. cerevisiae*. The machineries in the ER and Golgi appear to recognize distinct structural features on misfolded heterologous proteins, and thereby guide them to different degradation pathways (Holkeri and Makarow, 1998). The various foldases and chaperones thus have protein-specific activities and exert their effect on selected proteins or protein families. Vesicular transport from the ER to the Golgi is also highly regulated. In the Golgi the core carbohydrate chains of glycoproteins are further modified by outer-chain glycosylation. Transport to the plasma membrane is generally considered to be the default route while retention in the secretory pathway or sorting to cellular compartments requires additional sorting signals.

The secretory pathway of *S. cerevisiae* exhibits much of the structure and function of the mammalian secretory system. However, the yeast has a smaller amount of ER structures, Golgi apparatus and secretory vesicles than plant and mammalian cells, and secretion is thus characterised by rapid transit times and low levels of precursors (Demolder et al., 1994; Parekh and Wittrup, 1997). The observed difficulties in the folding and processing of (heterologous) proteins from other eukaryotic sources are therefore indicative of the measure of differentiation between different cell types (Ellgaard et al., 1999; Rokkones et al., 1994). The lower intrinsic secretory capacity of yeasts compared to mammalian cells is generally compensated for by the ease of high cell density cultivation (Parekh and Wittrup, 1997).

Despite these apparent limitations, some examples of complex posttranslational modifications performed *S. cerevisiae* have been reported. In one case the three polypeptides of human fibrogen was successfully combined into a complete molecule, which was secreted at higher levels than by mammalian cells (Roy et al., 1995). *S. cerevisiae* was also able to assemble multimeric human procollagen from the individual polypeptides, synthesised by a four-gene expression system, into a proper secondary structure (Toman et al., 2000). A similar molecule was also produced by recombinant *P. pastoris* using single copy integrants (Vuorela et al., 1999; Myllyharju et al., 2000) with production levels by both yeast expression systems significantly higher than levels obtained with mammalian cell culturing. Co-expressed α-globin and β-globin chains were also processed correctly and assembled into fully functional, tetrameric hemoglobin A by *S. cerevisiae* (Coghlan et al., 1992).

2.4.3. Optimal expression

Maximal use of the limited secretory capacity of *S. cerevisiae* for heterologous proteins may also require the application of optimal, rather than maximal, expression of the heterologous gene. In some cases, large overexpression has resulted in the saturation of the secretory pathway and the accumulation of unfolded heterologous protein in the ER (Wittrup et al., 1994; Tuite and Freedman, 1994; Parekh et al., 1995; Parekh and Wittrup, 1997). Overexpression has also resulted in a sharp decrease in the level of heterologous protein secretion, despite constant mRNA levels. In one case, the optimal tuning of the expression level has resulted in an order of magnitude increase in

secretion, compared to levels obtained with maximal expression (Parekh and Wittrup, 1997). The sub-optimal use of the *S. cerevisiae* secretory capacity may also be responsible for the lower levels of heterologous protein production compared to other yeasts, such as *P. pastoris* (Kjeldsen et al., 1999; Cereghino and Cregg, 1999). There have also been reports that high-level expression in *P. pastoris* may overwhelm the posttranslational machinery of the cell, causing a significant proportion of the foreign protein to be misfolded, unprocessed or mislocalised (Cereghino and Cregg, 2000).

2.4.4. Limiting components in the secretory pathway

The limited capacity of *S. cerevisiae* for the secretion of some heterologous proteins is evident from the above-mentioned reports. However, the production of foreign proteins may be increased by a rational approach to increasing the folding-capacity of components of the secretory pathway via overexpression (Tuite and Freedman, 1994).

The exit of proteins from the ER into the Golgi is generally believed to be the major rate-limiting step in protein secretion, as this is the point where the stringent quality control is exerted. Quality control determines whether misfolded proteins or aggregates are retained in the ER and destroyed by the proteasome (Buckholtz, 1993; Harmsen et al., 1993; Robinson et al., 1994; Tuite and Freedman, 1994; Robinson and Wittrup, 1995; Cha and Yoo, 1996). The capacity of *S. cerevisiae* to process unnaturally large fluxes of heterologous proteins may therefore be limited by its ability to produce adequate quantities of lumenal (ER) chaperones and foldases to ensure proper folding and processing of the foreign protein (Robinson et al., 1994). Two ER–resident, candidate proteins for overexpression are the heavy chain binding protein (BiP/Kar2p) and protein disulfide isomerase (PDI). These proteins either associates transiently with normal proteins and form a more stable interaction with mutant or misfolded proteins (Buckholtz, 1993; Robinson and Wittrup, 1995), and catalyses disulfide bond formation within the oxidizing environment of the ER, along with chaperone functionality, respectively. Levels of BiP and PDI were shown to decrease, probably due to titration, during constitutive high-level expression of heterologous proteins (Robinson and Wittrup, 1995). Simultaneous overexpression of these proteins, which acted synergistically, together with optimisation of the level of foreign gene expression, has been undertaken. This resulted in the first example of recombinant single-chain antibody fragments (scFv) secretion by *S. cerevisiae*, in a process that was previously

thought impossible. Cumulative modifications to the expression temperature, vector system, and BiP and PDI levels, resulted in a 50-fold increase in scFv specific productivity (Shusta et al., 1998). Increasing the BiP concentration in the ER increased heterologous protein secretion up to a certain point, beyond which increasing BiP levels provided no significant benefit (Robinson et al., 1996; Harmsen et al., 1996). Negative effects associated with BiP overexpression have also been observed (Van der Heide et al., 2002). Several examples of increased secretion of heterologous proteins due to PDI overexpression have been reported (Robinson et al., 1994; Schultz et al., 1994), which may be particularly relevant for the production of disulfide-bonded heterologous proteins (Robinson et al., 1994; Wittrup, 1995). Overexpression of the ER-resident Ssa1p has also resulted in a 5-fold increase in extracellular production of human interferon β by *S. cerevisiae* (Demolder et al., 1994). Polyubiquitin, though normally active in the targeting of abnormal or short-lived proteins for degradation, also functioned as a chaperone during overproduction, which increased the secretion level of a human leucocyte protease inhibitor by *S. cerevisiae*, despite constant mRNA levels (Chen et al., 1994). Disruption of the Ca^{2+}-ATPase *PMR1* in *S. cerevisiae* has caused an increase in the secretion of several heterologous proteins, possibly due to the involvement of Ca^{2+} in the transport of secretory proteins from the ER, though secretion was unaffected in other cases, despite alterations in the glycosylation pattern of the protein (Smith et al., 1985; Harmsen et al., 1993). Variable responses in the production of heterologous proteins due to disruption of *PMR1* in the yeast *Yarrowia lipolytica*, depending on the nature of the protein, was also reported (Sohn et al., 1998).

The secretory capacity of *S. cerevisiae* for overproduced proteins may also be increased by overexpression of secretory components active in the later stages of protein secretion. Overexpression of the yeast syntaxins, Sso1p and Sso2p, which function at the targeting/fusion of Golgi-derived secretory vesicles to the plasma membrane, resulted in a proportional increase in secreted heterologous α-amylase levels and both the native- and over-expression production levels of extracellular invertase. Transcriptional levels of the heterologous proteins were unaffected (Ruohonen et al., 1997).

Alternative means of increasing the secretory capacity of yeast have also been sought, such as the induction of membrane proliferation in *S. cerevisiae* by overexpression of the canine p180 ribosome binding protein, which resulted in a marked increase in secretory capacity through up-regulation of the entire secretory pathway (Becker et al., 1999). Posttranslational modification of a heterologous protein can also be temperature-dependent as is evident from higher secretion rates found at lower cultivation temperature (Ljubijankic et al., 1999; Cassland and Jönsson, 1999). Induction of heat shock protein and chaperone synthesis by heat shock treatment may also improve heterologous protein secretion (Rocha et al., 1996). Finally, the secretion efficiency of heterologous proteins may also be increased by the addition of components such as glycerol (10%), which acts as a chemical chaperone to facilitate secretion (Figler et al., 2000) and Tween 80, which apparently interacts with the plasma membrane of *S. cerevisiae*, to the cultivation medium (Bae et al., 1998). Cumulatively the range of case studies has indicated several possibilities for the rational manipulation of the secretory pathway of yeast to obtain higher levels of foreign protein production.

2.4.5. Changing signal peptide and Kex2p activity

Secretion of a protein leads to the *in vivo* complete removal of the N-terminal amino acid residue (signal peptide or secretion signal) of the synthesized foreign protein by signal peptidase activity in the ER. This prevents the expressed proteins from being degraded by host cellular proteases (Lee et al., 1999). Both the alternative yeasts and *S. cerevisiae* can clip off the signal peptide correctly, resulting in a fully processed protein (Buckholtz and Gleeson, 1991). The presence of a signal sequence is therefore essential for the extracellular production of a heterologous protein by yeast (Takahashi et al., 1998) and selection of the correct secretion signal can affect product yields (Sleep et al., 1990; Buckholtz, 1993). Several signal sequences of the yeast secretory proteins have been employed to ensure the secretion of foreign proteins, with the *S. cerevisiae MF*α secretion signal used most frequently. This signal sequence has been shown to direct secretion of heterologous proteins in all yeast systems tested so far (Shuster et al., 1989; Harmsen et al., 1993; Gellissen and Hollenberg, 1997). Other examples are the *S. cerevisiae* invertase signal sequence (Pohlig et al., 1996), the secretion signal of the *K. lactis* killer toxin (Fusetti et al., 1996), and the secretion signal derived from the *Kluyveromyces marxianus* inulinase (Kang et al., 1996; Chung et al., 1996). In many

cases the native secretion signal of secretory proteins will also function correctly in most yeast expression systems (Gellissen and Hollenberg, 1997). Several methods for improving the secretion of a recombinant protein via molecular engineering of the secretion signals have been demonstrated (Kjeldsen, 2000).

The processing of the secretion signal by the ER-resident signal peptidases (such as Kex2p) may also limit the secretion of a heterologous protein (Buckholtz, 1993; Zhang et al., 2001). Insertion of a spacer peptide between the start of the heterologous ORF and the peptidase signal cleavage site has resulted in a significant increase in the production levels of the secreted heterologous proteins by *S. cerevisiae*, due to the improved efficiency of the signal peptidase (Degryse et al., 1992; Parekh et al., 1995; Kjeldsen et al., 1996). Insertion of the N-terminal fragment of human interleukin-1β, which is secreted efficiently by *S. cerevisiae*, between the existing leader peptide and the *KEX2* cleavage site of heterologous protein, enhanced secretion 3- to 4-fold (Bae et al., 1998; Lee et al., 1999). Changing the level of Kex2p in the strain may also alter the *KEX2*-processing pattern of secreted proteins (Takahashi et al., 1999).

2.4.6. Properties of the secreted protein

Despite the successful secretion of some heterologous proteins, poor secretion of a variety of other heterologous secretory proteins from *S. cerevisiae* may be attributed to the recognition and retention of these proteins in the endoplasmic reticulum (ER) via strong binding of ER molecular chaperones (Kowalski et al., 1998a; Ellgaard et al., 1999). The biophysical properties of the heterologous protein may thus partly determine its sorting in the degradation/secretory pathway (Zhang et al., 2001). However, the intrinsic biophysical property of the protein that determines the sorting thereof is not obvious (Kowalski et al., 1998b). In one case, the fermentation yield of insulin analogue precursors expressed in yeast correlated with the folding stability of the corresponding insulin analogues (Kjeldsen, 2000). In another example, the secretion of bovine pancreatic trypsin inhibitor was directly correlated with the *in vitro* unfolding temperature and the thermodynamic stability of the folded protein, though no relationship was observed between secretion efficiency and *in vitro* folding or unfolding rates of heterologous protein (Kowalski et al., 1998a). Increasing the hydrophobicity of a heterologous cutinase, through fusion with various peptide tails, also significantly

reduced the secretion of the recombinant products (Calado et al., 2002). Fusion of human apolipoprotein E (hApoE) to either *Rhizomucor* rennin or human serum albumin, both of which are secreted efficiently by *S. cerevisiae*, also contributed to the efficient secretion and protection from proteolysis of the fusion protein heterologous hApoE (Nomura et al., 1995a; Nomura et al., 1995b). In *P. pastoris* secretion of the heterologous protein is usually reserved for proteins that are normally secreted by their native hosts (Cereghino and Cregg, 2000). The intrinsic properties of the heterologous protein may thus be a major cause for low levels of secretory expression.

2.4.7. Hyperglycosylation

In some cases, the hyperglycosylation of heterologous proteins during secretion by yeasts, *S. cerevisiae* in particular, may significantly alter the biological activity of the produced protein. Hyperglycosylation can reduce the antigenic activity of pharmaceutical proteins or the catalytic activity of recombinant enzymes and thereby negate any advantage that *S. cerevisiae* may have over *E. coli* and mammalian expression systems for the production of such proteins (De Baetselier and Van Broekhoven, 1998; Crabbe et al., 1996; Hodgson, 1993). The ability of particular antibodies to bind to the heterologous protein may thus be lost due to hyperglycosylation, which requires the use of site-directed mutagenesis to remove the sites of hyperglycosylation, without affecting the activity of structure of the heterologous protein (Malissard et al., 1996). Several mutations for the manipulation of glycosylation patterns have been identified, including disruption of the *MNN1* and/or *MNN9* genes, which represents an alternative approach to avoiding the problems associated with hyperglycosylation. These mutations may, however, affect the growth characteristics of the recombinant strains in large-scale cultures (Buckholtz, 1993; Schultz et al., 1994). Finally, although the correct glycosylation pattern may be essential for the biological activity of some proteins, several of the enzymes produced by *S. cerevisiae* have retained their native kinetic properties, despite hyperglycosylation (e.g. Valmaseda et al., 1992).

2.4.8. *S. cerevisiae* lacks capacity for posttranslational folding of all proteins

Posttranslational processing of malaria surface molecules during production in *S. cerevisiae* resulted in a series of stable conformers, each containing a different pattern of disulfide bond formation. These observations indicate that *S. cerevisiae* might not

have the necessary machinery to correctly fold some proteins that require complex posttranslational processing (Stowers et al., 2001). The availability of a sufficient secretory capacity, and optimal use thereof through molecular adaptation, is therefore a major factor that determines the extracellular production levels heterologous proteins.

2.5. PRODUCT INSTABILITY AND PROTEOLYTIC DEGRADATION

The proteolytic activity of the yeast expression host may significantly degrade a heterologous protein product, thereby reducing the quality and quantity of product obtained (Mendoza-Vega et al., 1994a). Since *S. cerevisiae* has more than 20 proteinases (mostly intracellular; Ogrydziak, 1993; Harashima, 1998), methods to develop protease-deficient strains and to inhibit or inactivate the proteolytic activity of the production organism have been investigated.

2.5.1. Intracellular proteases

The effect of intracellular proteases on production levels may be either due to the intracellular degradation of heterologous proteins during posttranslational processing – i.e. the proteolytic fragmentation of complete degradation of products; some products are rapidly degraded during or shortly after synthesis – or due to the release of intracellular proteases by cell lysis during high-cell density or long-term fermentation (Harashima, 1998; Cereghino and Cregg, 2000). It is generally accepted that intracellular proteases are not directly involved in the hydrolysis of (extracellular) protein products, already secreted from the host, except through release by cell lysis (Rose, 1987). Multiple factors affect the susceptibility of foreign proteins to proteolytic degradation, including the linear amino acid sequence and conformational structure (Gimenez et al., 2000). Peptides shorter than 100 amino acids are typically subjected to significant intracellular proteolysis when expressed in *S. cerevisiae,* unless the peptide is constrained by a large number of disulfide bonds (Egel-Mitani et al., 2000).

Wingfield and Dickinson (1992) first reported a 90% reduction in the activity of the major vacuolar proteinases, protease A and B, carboxypeptidase Y, amino peptidases and some RNAse species, in *S. cerevisiae* by disruption of the *PEP4* gene. The corresponding decrease in the expression of intracellular proteases resulted in higher production levels of a recombinant protein, without deleterious effects on cell growth.

However, the highest production levels were not obtained with the lowest vacuolar peptidase activity, but by tailoring the host strain peptidase activity to the heterologous protein (Wingfield and Dickinson, 1993). More recently, the production of heterologous α-amylase increased 20-30 fold due to *PEP4*-disruption, though some deleterious growth effects were observed (Chen et al., 2000). Figler et al. (2000) also observed an increase in the yield of a heterologous membrane protein due to *PEP4*-disruption, whereas the production of two heterologous tonoplast intrinsic proteins in *S. cerevisiae* could only be detected in *PEP4*-disrupted strains (Inoue et al., 1997). Degradation of the heterologous fusion proteins were also reduced by using a host strain deficient in vacuolar protease activity (Nomura et al., 1995a). However, these examples of increased production by *PEP4*-deficient strains of *S. cerevisiae* should be considered as exceptions, since *PEP4*-disruption do not normally appear to offer an advantage in their product yield of recombinant protein (Romanos et al., 1992; Copley et al., 1998). Protease-deficient strains also exhibit a lower proliferative ability than wild-type strains, both for *S. cerevisiae* (sometimes dramatically lower) and *P. pastoris* (Chen et al., 1999; Romanos et al., 1992; Copley et al., 1998; Cereghino and Cregg, 2000). Production of heterologous proteins in *P. pastoris* more frequently benefits from protease deficiency than is the case for *S. cerevisiae*. However, due to the corresponding negative growth effects, these strains should be used only when other measures to reduce proteolysis have yielded unsatisfactory results (Sreekrishna et al., 1997; Rosenfeld, 1999). The proliferative ability of *PEP4*-disruption *S. cerevisiae* cells may apparently be restored by using asparagine as a nitrogen source, rather than ammonium sulphate (Chen et al., 2000).

Zhang et al. (1997a) reported that the yapsin 1 (*YPS1* or *YAP3*) and yapsin 2 (*MKC7* or *YPS2*) proteases act in the late Golgi secretory pathway of *S. cerevisiae*. These proteases cleave proteins at basic (Arg and Lys) residues, which can result in the incorrect proteolytic cleavage of heterologous proteins, especially peptides. Intracellular proteolytic fragmentation of the heterologous peptides such as recombinant human albumin (rHA) fused to human albumin growth hormone (especially at high cell density), human parathyroid, insect diuretic hormone, leptin and neuropeptide was significantly reduced by disruption of *YPS1* (*YAP3*) and/or *MKC7* (*YPS2*) (Kerry-Williams et al., 1998; Kang, 1998; Song and Chung, 1999; Copley et al., 1998; Egel-

Mitani et al., 2000). It is thus advisable to use *YPS1*-deficient mutants, or multiple mutants, to produce high amounts of recombinant proteins containing basic residues. Some peptides may also be cleaved by the Kex2p protease at Lys and Arg, necessitating the disruption thereof, or by the presence of uncharacterised endopeptidases (Kerry-Williams et al., 1998; Rourke et al., 1997).

2.5.2. Extracellular proteases

The extracellular proteolytic degradation of several heterologous proteins produced by *S. cerevisiae* has frequently been observed, despite earlier claims that this yeast produces no or very few extracellular proteases (Ogrykziak, 1993). The release of intracellular proteases through cell lysis may therefore represent an alternative source of extracellular proteases. Extracellular protease activity is affected by nutritional conditions, and may increase due to glucose exhaustion or carbon starvation (Gimenez et al., 2000; Mendoza-Vega et al., 1994a). The use of protease-deficient host strains and medium supplements may thus be required to significantly reduce extracellular proteolytic degradation (Vad et al., 1998). The addition of complex medium components, such as casein hydrolysate (casamino acids), peptides, amino acids, skim milk or bovine serum albumin (BSA), to the culture medium of recombinant *S. cerevisiae* and *P. pastoris* strains has shown to decrease the degradation of the heterologous proteins (Coppella and Djurjati, 1989; Nomura et al., 1995a; Kozlov et al., 1995; Aho et al., 1996; Sreekrishna et al., 1997; Werten et al., 1999; Shiba et al., 1998; Choi et al., 2000; Boze et al., 2001; Juge et al., 2001; Goodrick et al., 2001). These medium components apparently reduce the protease activity towards a heterologous protein of interest by providing large amounts of protein substrate. The production of extracellular proteases may also be reduced in a medium that is rich in nitrogen sources. Addition of the amino acids arginine and lysine to cultures of *S. cerevisiae* in defined medium has also decreased proteolysis of the extracellular recombinant protein, probably due to inhibition of the proteolytic enzymes targeted to basic amino acid sites in the protein (Choi et al., 2000; Kang et al., 2000; Chung and Park, 1998). Specific protease inhibitors, such as PMSF (phenyl methyl sulfonyl fluoride) may also be added to cultures, though only during the production phase since cell growth can be inhibited by their presence (Kim and Kang, 1996). Kobayashi et al. (2000a) also observed that levels of ammonium lower than 0.3 $mg.l^{-1}$ in the fermentation broth during *P. pastoris* cultivation significantly increased the activity of proteolytic enzymes, indicating an

association with nitrogen starvation. Buffering the cultivation medium to a pH where protein degradation is minimised can also reduce the breakdown of heterologous proteins (Rosenfeld, 1999; Shiba et al., 1998; Nomura et al., 1995a; Kobayashi et al., 2000a; Cox et al., 2000; Juge et al., 2001). The higher levels recombinant protein production by *P. pastoris* were also observed at lower cultivation temperature, which was associated with better product stability, smaller release of proteases from dead cells and improved folding of the product (Hong et al., 2002). Siegel and Brierly (1990) devised a cell recycle reactor whereby the recombinant product could be removed quickly from the fermentation broth, thus avoiding proteolytic degradation. An extracellular heterologous protein may also lose activity due to polymerisation with other proteins, which can be avoided by adding 0.2% (w/v) Tween 80 to the medium and changing the pH thereof (Bae et al., 1999).

2.6. NATURE OF HETEROLOGOUS PROTEIN

Despite numerous references to the influence of the nature/structure of the heterologous protein on production levels attainable with the various yeast expression systems, no systematic study on the intrinsic characteristics of the protein that determine these levels has been undertaken. Levels of accumulation of heterologous proteins in *S. cerevisiae* vary widely depending on the foreign gene expressed, and in some cases protein engineering techniques may also be required to achieve efficient expression in a host organism (Mendoza-Vega et al., 1994a; Fusetti et al., 1996). Use of the *P. pastoris* expression system will not always result in high-level production of all proteins either, due to the influence of unspecific properties such as the amino acid sequence, codon bias, tertiary structure and the required posttranslational modifications and the site of expression (Sreekrishna et al., 1997; Loewen et al., 1999).

2.7. HOST STRAINS

The production levels of heterologous proteins are highly dependent on the genetic background of the host strain, as specific characteristics of the host may influence the level of heterologous transcript, translational and secretory efficiency, protein quantity and quality, plasmid stability, and plasmid copy number (Park et al., 2000; Eckart and Bussineau, 1996). The magnitude of these differences between strains can render cross-

species comparisons very difficult (Fleer, 1992). During screening for a suitable strain of *S. cerevisiae* for production of a heterologous glucose oxidase, a 100-fold difference in production levels was observed, with the maximum production eventually representing one of the highest levels of heterologous protein secretion by this yeast (De Baetselier et al., 1991). Schultz et al. (1994) observed a 10-fold difference in production levels of a foreign protein during a similar screening of host strains with different genetic backgrounds. Despite these large differences, the effect of host cell metabolism on the synthesis of heterologous proteins has not been systematically investigated, and the improvement of the genetic characteristics of the host strain has seldom been reported (Mendoza-Vega et al., 1994a; Chen et al., 1999). To facilitate systematic investigations, the microbial physiology of the host strain, especially in terms of nutritional requirements, auxotrophic markers and protease activities, should be characterized prior to transformation (Mendoza-Vega et al., 1994a).

2.7.1. Auxotrophic requirements in transformed strains

An aspect of host strain physiology that has gone largely unnoticed is the potential influence of auxotrophic markers in transformant strains on the production levels of heterologous proteins. Most laboratory strains of *S. cerevisiae* that have been used for recombinant protein production, originated from a series of genetic crosses of the S288C strain with other strains, as part of the early studies on the molecular biology and genetics of this yeast (Guthrie and Fink, 1991). These laboratory strains were adopted for use in recombinant protein production, often without further modification, and are currently still used as host strains in numerous studies. During the early genetic studies, however, the presence of auxotrophic mutations in these strains were not considered as a major limitation during cultivation, an idea that has been transferred to numerous molecular biologists of today. Host strains with excessive auxotrophic mutations are therefore still used for recombinant protein production, resulting in transformant strains containing uncomplimented auxotrophic markers. The effect of these auxotrophic markers in transformant strains has frequently been disregarded in modern molecular biology, as is evident from the plethora of physiological investigations conducted with such strains (Table 2.1).

Table 2.1 Examples of the effect of auxotrophic markers in *S. cerevisiae* transformants disregarded during cultivation and recombinant protein production

Shiba et al., 1998	Morosoli et al., 1992
Choi et al., 2000	Moreau et al., 1992
Rao et al., 2000	Ganga et al., 1998
Song and Chung, 1999	Lee et al., 1999
Takahashi et al., 1999	Pohlig et al., 1996
Roy et al., 1995	Porro et al., 1992
Janes et al., 1990	Okada et al., 1998
Giuseppin et al., 1993	Kanai et al., 1997
Beretta et al., 1991	Stone et al., 1995
De Baetselier et al., 1991	Shuster et al., 1989
Mendoza-Vega et al., 1996	Castelli et al., 1994
Mendoza-Vega et al., 1994b	Wingfield and Dickinson, 1993
Monfort et al., 1999	Nomura et al., 1995a
Nieto et al., 1999 – industrial strain	Inoue et al., 1997
O'Kennedy et al., 1995	Neeper et al., 1990
Rossini et al., 1993	Law et al., 1998
Pignatelli et al., 1998	Papakonstantinou et al., 2000
Zurbriggen et al., 1989	Zigova et al., 1999
Lang and Looman, 1995	Zigova, 2000
Lang et al., 1997 – pilot scale	Joseph-Liauzun et al., 1995
VanDusen et al., 1997	Ljubijankic et al., 1999
Parekh and Wittrup, 1997	Pyun et al., 1999
Parekh et al., 1995	Cook et al., 1998
Parekh et al., 1996	Robinson and Wittrup, 1995
Takahashi et al., 1999	Robinson et al., 1994
Fieschko et al., 1987	Robinson et al., 1996
Wittrup et al., 1994	Wittrup and Benig, 1994

However, the use of recombinant strains with auxotrophic markers can result in problems with growth, protein production and genetic stability at high cell densities and during scale-up (Mendoza-Vega et al., 1994a). The auxotrophic markers in the host strains that remain uncomplimented in transformant strains firstly require a sufficient

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availability of the corresponding metabolite in the complex or defined cultivation medium. Since yeasts have long been known to accumulate amino acids tenaciously (Eddy, 1982), the metabolite for which an auxotrophic requirement exists may be consumed beyond the amount that is stoichiometrically required for biomass formation, and result in a nutrient-deficiency towards the end of a fermentation. An overconsumption of adenine, for which an auxotrophic requirement in the transformed strain existed, was demonstrated by VanDusen et al. (1997) whom observed a maximal consumption level of more than three times the amount required for maximal biomass formation. Without considering the effect of metabolite overconsumption, however, most defined media used for shake-flask, or even large-scale, cultures still do not contain enough of the required metabolite to support minimum levels of biomass formation (Pronk et al., 1996). Auxotrophy can thus result in a significant limitation of cell growth if a sufficient amount of the corresponding growth factor (amino acid) is not present in the medium (Chopra et al., 1999). In such a case, the biomass yield of an auxotrophic recombinant strain will be strongly reduced, as has been demonstrated for histidine auxotrophy (Beretta et al., 1991). The stress imposed by limiting concentrations of auxotrophic requirements during the cultivation of auxotrophic strains, will also result in an increase in the mutation and reversion rates of the corresponding markers (Korogodin et al., 1991; Çakar et al., 1999; Heidenreich and Wintersberger, 1997). Auxotrophic strains may also have physiological alterations and sensitivities that are not generally recognized, and it is not clear exactly how much of the required substrate should be added to avoid these (Çakar et al., 1999).

Besides growth defects, heterologous protein production may also be negatively influenced by auxotrophic mutations in the yeast host. In one such example, Zigova et al. (1999) and Zigova (2000) observed a very low cell yield (0.25 $g_{dry\ cell\ mass} \cdot g_{glucose\ consumed}^{-1}$) during the aerobic cultivation of an auxotrophic *S. cerevisiae* transformant on pilot plant scale, indicating that the availability of auxotrophic amino acids could very well have been limiting. Further supplementation of the fermentation broth with amino acids also resulted in an increase in the specific production level of heterologous protein, indicating possible nitrogen limitation for protein synthesis. The metabolic changes that occur when cloned genes are expressed in auxotrophic strains, may well arise from peculiarities of the auxotrophic host rather than from activity of the cloned proteins (Kozlov et al., 1995; Çakar et al., 1999). VanDusen et al. (1997) observed that

maximum consumption of adenine also resulted in low levels of heterologous protein production, indicating that the concentrations of adenine required for biomass formation and maximum heterologous protein production were different. Auxotrophic strains grown in nutrient supplemented medium are thus NOT necessarily physiologically equivalent to the complemented transformants (Pronk et al., 1996; Chopra et al., 1999). Excessive auxotrophic markers should preferentially be removed prior to transformation of the parental strain, as demonstrated by Motwani et al. (1996), since the advantages of using prototrophic transformants for physiological studies on laboratory, pilot plant and production scale should be evident from these results.

Despite the disadvantages to the use of auxotrophic strains, two of the highest production levels of heterologous protein reported in *S. cerevisiae* were obtained by cultivating an auxotrophic transformant in complex medium (De Baetselier et al., 1991; Lee et al., 1999). Chung et al. (1997) also did not observe any difference in production levels due to removal of excessive auxotrophic markers. The effect of auxotrophic mutations in transformants on the production of heterologous proteins is thus not well understood.

2.7.2. Comparison of auxotrophic *S. cerevisiae* transformants

Several comparisons of production levels obtained with auxotrophic *S. cerevisiae* and "fully functional," prototrophic versions of other yeasts have been reported, although the effect of auxotrophic mutations in the former on heterologous protein production was not clear. Higher production levels of recombinant anticoagulant peptide (AcAP-5) were observed for *P. pastoris*, compared to those reported for a *S. cerevisiae* transformant retaining an adenine-requirement (Neeper et al., 1990; Inan et al., 1999). A similar observation was made during the comparative production of human glutamic acid decarboxylase (GAD) by these two yeasts: superior production levels for *P. pastoris* compared to an auxotrophic *S. cerevisiae* (Papakonstantinou et al., 2000). Giuseppin et al. (1993) compared guar-α-galactosidase production in defined medium by an auxotrophic *S. cerevisiae* transformant to production by a prototrophic *H. polymorpha* transformant, which produced more of the heterologous protein. Both strains carried multiple integrations of the heterologous gene.

2.7.3. Selection of improved hosts

Screening and selection of transformants or mutants that show increased production and secretion levels of heterologous proteins, is an alternative to the use of rational approaches, aimed at specific steps in synthesis and secretion, for improving heterologous protein production. Smith et al. (1985) and Sakai et al. (1988) first described such mutant strains, demonstrating the potential of random selection procedures for increasing heterologous protein production. Such random selection is especially useful in recombinant protein production since the discovery and rational improvement of important host cell properties for heterologous protein production remains difficult (Kozlov et al., 1995). Despite more than 20 years of experience with heterologous protein production in yeast, the extensive knowledge of *S. cerevisiae* molecular genetics is yet to be fully exploited towards to development of more efficient production systems (Fleer, 1992; Harashima, 1998). Secretion of a heterologous protein can be improved by a combination of both a rational approach and isolation of mutant strains that show elevated levels of production (Tuite and Freedman, 1994).

Classical methods of strain improvement, such as random mutagenesis, are thus frequently used in industry due to the significant benefits (Fleer, 1992). Park et al. (2000) recently used UV mutagenesis to increase the yield of recombinant protein production by *S. cerevisiae*, without negatively affecting the growth of the recombinant strain. Conversely, previous investigations have reported a decrease in the growth rate of over-producing transformants obtained from a screening of random mutants (Aho et al., 1996). Sleep et al. (1991) also used several rounds of chemical mutagenesis to obtain stable mutants able to produce elevated levels of intracellular heterologous proteins. Adaptation of host strains under constant selection pressure is also an effective tool for host strain improvement, with one set of mutants showing a two-fold increase in glucoamylase production after 7 days of cultivation on solid maltose medium (Zhang et al., 1997b). Similarly, stable mutants of *PEP4*-disrupted transformants, resistant to the negative effects of heterologous HBsAG protein production and having growth characteristics similar to the parental strains, were selected after long-term cultivation (25 days). These strains also produced higher levels of recombinant α-amylase than the non-mutated transformants, though the mechanism for improved growth and recombinant protein production was not clear (Chen et al.,

1999). Long-term continuous cultivation of parental *E. coli* strains also resulted in improved physiological characteristics and stress resistance (Weikert et al., 1997). These strains showed 2- to 3-fold higher production levels when used for the production of heterologous proteins, indicating the importance of physiological robustness for recombinant protein production (Weikert et al., 1998). Similarly, Gill et al. (2001) demonstrated that the "conditioning" of recombinant *E. coli* cells, by stimulating the cellular stress response prior to induction of heterologous protein synthesis, resulted in higher production levels of recombinant protein. Though increased production levels of a specific heterologous protein may be obtained, isolated mutants do not always show enhanced production of other proteins, and limited analysis of overproducing mutants has not implicated mutations in genes encoding components of the secretory pathway (Harashima, 1998; Tuite and Freedman, 1994). Much therefore remains to be learned about the major rate-limiting steps of the secretory pathways of eukaryotic hosts.

2.8. METABOLIC BURDEN

The situation where cells are challenged to produce abnormal quantities of a foreign protein is physiologically not well understood (Vasavada, 1995). Transformants expressing heterologous proteins often exhibit growth retardation, reduction of survival rate and instability of the product yield (Romanos et al., 1992). The observed "metabolic burden" or "load" has adverse effects on the productivity of large-scale bioprocesses, since the accumulation of biomass for heterologous protein production is retarded, and overcoming these factors to obtain high cell densities is not trivial (Vasavada, 1995). The burden associated with the overproduction of a heterologous protein also causes the spontaneous creation of mutant populations with increased growth rates and reduced production rates, which are frequently detected after many generation times (Zelder and Hauer, 2000). Strong selection pressure in favour of cells producing less recombinant protein may also result in structural instability in transformants (Fleer, 1992; Lopes et al., 1996).

The origin of the "metabolic burden" associated with heterologous protein production is not clear. In comparing two isogenic strains producing either a homologous (Pgk1p) and heterologous (prochymosin) protein from identical expression plasmids, Gopal et al. (1989) observed a reduced biomass yield for both transformants compared to the

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host strain, though the production levels of heterologous protein was 10-fold lower than homologous proteins. Heterologous protein production apparently imposed a greater drain on cellular energetic resources than homologous protein production. In a similar case, overproduction of Pgk1p from a multicopy integrative expression system to 47% of the total soluble cellular protein, resulted in a 40% decrease in the growth rate of the host strain (Van der Aar et al., 1990). Further examples of the deleterious effects of heterologous protein production on the host cell metabolism are:

- Decreased growth rates for recombinant yeast strains producing various heterologous proteins, compared to the parental strain (Shuster et al., 1989; Da Silva and Bailey, 1991; Dequin and Barre, 1994; Giuseppin et al., 1993; Janes et al., 1990; Okada et al., 1998; Meinander, 1997; Snoep et al., 1995).
- Decreased growth rates and biomass yields for mutants selected on the basis of a 10-fold increase in heterologous protein production (Aho et al., 1996).
- Decreased growth rates for clones selected on the basis of improved heterologous protein production (Skory et al., 1996).
- Decreased the critical dilution rate of recombinant *S. cerevisiae* strains overproducing invertase from a multicopy plasmid-based expression system (Pyun et al., 1999)
- Increased size and more complex internal structure of recombinant cells (Chau et al., 2001; Peterson and Patkar, 1992).

However, for both plasmid-based and integrative expression systems, mutant strains partially able to overcome the deleterious effects of heterologous protein production and producing high levels of various heterologous proteins could be selected (Shuster et al., 1989), indicating the ability of yeast strains to adapt to heterologous protein production.

No clear conclusions on a possible correlation between the observation of deleterious metabolic effects of heterologous protein production on *S. cerevisiae* strains, and the presence of auxotrophic requirements in these strains can be made from available literature. Although in some cases a “metabolic burden” was observed for strains containing auxotrophic requirements (Van der Aar et al., 1990; Skory et al., 1996; Shuster et al., 1989; Pyun et al., 1999; Janes et al., 1990; Giuseppin et al., 1993; Da Silva and Bailey, 1991; Aho et al., 1996; Vad et al., 1998), in other cases no deleterious

effects were associated with heterologous protein production, despite the presence of these markers (Okada et al., 1998; Papakonstantinou et al., 2000). Examples of the presence of a “metabolic burden” in prototrophic production strains have also been reported, even during growth in complex medium where parental and transformed strains are usually more similar (Van Hoek et al., 1998; Dequin and Barre, 1994; Gopal et al., 1989; Lyness and Meaden, 1997). The frequent presence of auxotrophic requirements in *S. cerevisiae* transformant strains exhibiting the deleterious effects of heterologous protein production is probably an artefact of the frequent use of auxotrophic strains for the production of heterologous proteins (see Section 2.7).

2.9. CULTIVATION CONDITIONS AND NUTRIENT AVAILABILITY

Microbial physiology and fermentation optimisation are important steps in developing a yeast-based system for heterologous protein production, since optimal cultivation conditions for the production of a foreign protein can be highly specific to the expression system used (Stouthamer and Van Verseveld, 1987; Bae et al., 1998). It is also essential to consider that conditions for maximal biomass formation do not necessarily facilitate maximal production of the heterologous protein production, which is the aim of any optimisation procedure. A first major consideration is the composition of the cultivation medium, which can affect both cell growth and product yield, depending on the strain and heterologous protein. Medium composition should thus be balanced according to growth and production requirements, and should be matched to large-scale requirements (Vasavada, 1995; Rosenfeld, 1999; Mendoza-Vega et al., 1994a). Preference is usually given to the use of defined medium, which often allows for rapid scale-up and the production of a purer product (Choi et al., 1996; Greasham and Herber, 1997; Rosenfeld, 1999). Besides medium composition, other cultivation parameters, such as temperature, pH, aeration and feeding profile may also affect production of heterologous proteins (Rosenfeld, 1999). The examples presented here of improved heterologous protein production in response to altered cultivation conditions, were limited to cases where the changes in cultivation conditions had no direct effect on protease activity (discussed in Section 2.5), thus indicating a stimulation of microbial metabolism towards heterologous protein production. However, despite the demonstrated advantages such optimisations, a review of literature would indicate that molecular genetics and the molecular adaptation of expression systems are more

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powerful for increasing the production levels of heterologous proteins. Whereas several examples of orders of magnitude increases in production levels through the latter approaches have been demonstrated, improvements smaller than 10-fold are typically obtained via the optimisation of cultivation conditions.

2.9.1. Medium components

To allow for high cell density fermentations and maximal heterologous protein production, the required medium components should be available for the duration of the bioprocess, and the use of a balanced medium is thus essential (Mendoza-Vega et al., 1994a; Mendoza-Vega et al., 1994b; Vasavada, 1995). The defined medium used most often for the cultivation of *S. cerevisiae* strains, comprises Yeast Nitrogen Base (YNB) (without amino acids; Difco) and glucose, and is frequently supplemented with casein hydrolysate (casamino acids) to improve the production of heterologous proteins. However, YNB medium without casamino acids (and possibly inisitol) are not able to support oxidative growth on ethanol, thus allowing only fermentative growth in batch culture (Chen et al., 1993; Gu et al., 1991; Vasavada, 1995). YNB medium also contains several vitamins (biotin, thiamine, inisitol and pantothenic acid) and mineral trace elements that are required for the cultivation of certain strains of *S. cerevisiae*, and are considered as essential "growth factors" in the medium. Amino acids required for the cultivation of microorganisms containing auxotrophic requirements are also considered as growth factors and should be added to the medium (Greasham and Herber, 1997).

The addition of complex medium components (yeast extract, peptone, casamino acids, etc.) to the cultivation medium has frequently improved biomass formation and heterologous protein production by *S. cerevisiae*, both for the production of heterologous xylanase (Donald et al., 1994; Pérez-González et al., 1996; Nuyens et al., 2001) and other heterologous proteins (Chiruvolu et al., 1996; Choi et al., 1996; Hensing et al., 1995b; Vasavada, 1995; Wang and Da Silva, 1993; Castelli et al., 1994; Kim et al., 2000; Kapat et al., 2001; Toman et al., 2000; Chauhan et al., 1999; Boze et al., 2001; Gupta and Mukherjee, 2002). [Although the amino acid composition of casamino acids (i.e. casein hydrolysate) is frequently known, its complex origin and the interaction between different components do not allow for classification as a defined medium component.] In some cases, however, strong increases in biomass yields due

to addition of these compounds are accompanied by a decrease in the specific productivity (amount of heterologous protein produced per gram of biomass), with varying effects on the overall productivity (Vasavada, 1995; Vad et al., 1998). Gupta and Mukherjee (2002) also observed an increase in plasmid stability and productivity when the availability of yeast extract was reduced to limit the growth of the production strain. The nature of the complex nitrogen sources may thus influence the metabolic flux to the production of the recombinant protein and segregational stability (Mendoza-Vega et al., 1994a). The optimal concentration of complex medium components should always be determined (Chang et al., 1998).

The concentration of the various components in defined media for the cultivation of yeast strains may also influence growth and heterologous protein production of the yeast, most notably sources of nitrogen, vitamins and trace elements (Boze et al., 2001; Mendoza-Vega et al., 1994a; Blondeau et al., 1994; De Kock et al., 2000; Jung et al., 1991). Nitrogen sources most strongly preferred by *S. cerevisiae* include glutamine, asparagine and ammonium (Dubois and Messenguy, 1997), though Wittrup and Benig (1994) observed increased production of heterologous proteins due to several amino acids. For the production of heterologous proteins for pharmaceutical use, the addition of pure amino acids to increase production levels may provide additional benefits, as it avoids the regulatory requirements associated with raw materials from animal origin (Mendoza-Vega et al., 1994a). The beneficial effect of supplementing an existing defined medium with individual amino acids on the physiology of the cultivated yeast strain has been demonstrated (Toman et al., 2000; Albers et al., 1996; Blechl et al., 1992). Although frequently used in defined media, ammonium has been shown to inhibit production of α-amylase by *S. cerevisiae*, whereas its utilisation also results in the acidification of the medium (Chen et al., 2000; Hensing et al., 1995b). Alternative nitrogen sources such as urea and asparagine have thus been suggested (Hensing et al., 1995b; Chen et al., 2000). Other defined components shown to increase heterologous protein production by yeast were succinate (Cha et al., 1998) and oleic acid (Kobayashi et al., 2000b).

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2.9.2. pH and cultivation temperature

Both the pH and temperature during various stages in the cultivation of a recombinant yeast strain can have an effect on the production level of heterologous protein, and should thus be optimised to improve physiological conditions for heterologous protein production (Kozlov et al., 1995; Kim and Kang, 1996). The results of such empirical optimisations are highly specific to the production system being investigated, due to the plethora of cellular processes affected by changes in temperature and pH, besides the possible reduction in protease activity. Blondeau et al. (1994), for example, observed separate pH optima for recombinant protein secretion (6.5) and biomass production (pH 6.0) by *K. lactis*. Since the posttranslational modification of a heterologous protein can be temperature-dependent, higher secretion rates may also be obtained by lowering the cultivation temperature (Ljubijankic et al., 1999; Cassland and Jönsson, 1999; Hong et al., 2002; Nagashima et al., 1994).

2.9.3. Aeration

Supply of oxygen to the growing cell population is the rate-limiting step in many aerobic processes, being mostly influenced by the speed of agitation and aeration rate (Kapat et al., 2001). In high cell density bioreactors the oxygen supply towards the end of the fermentation may also become limiting and subject recombinant yeast to considerable stress, which can affect the quality of the produced protein and lead to proteolytic degradation, the incorporation of incorrect amino acids or incorrect posttranslational modifications (Roecklin et al., 1997; Rao et al., 1999). As both heterologous protein production and glucose consumption by yeast are generally dependent on the level of dissolved oxygen during cultivation, optimal levels should be determined empirically for each production system (Alberghina et al., 1993; Calado et al., 2002; Blondeau et al., 1994; Pyun et al., 1999). However, the potential benefits of improved oxygenation may not be realisable on large scale, since high oxygen consumption will lead to significant metabolic heat generation in bioreactors (Rosenfeld, 1999). The generation of large amounts of heat will escalate the operational cost of fermentations due to an increased requirement for cooling.

2.9.4. Feeding profile

Heterologous protein production on industrial scale is frequently undertaken in fed-batch cultures, though continuous cultures have also found some application. The flow

rate and composition of the feed to a fed-batch fermentation can have a significant effect on the productivity of the bioprocess and selection of an optimal feeding profile is thus essential for high levels of heterologous protein production. The relevant aspects of the physiology of the production strain, such as yields, medium requirements, growth rates and kinetics, should be determined prior to the design of a large-scale, high cell density fed-batch fermentation and can be measured in continuous culture (Alberghina et al., 1993; Hensing et al., 1995c; Blondeau et al., 1994; Mendoza-Vega et al., 1994b). Feeding should be designed to ensure that the required nutrients are available at optimal concentrations, whereas the addition of components that may exhibit a toxic effect due to accumulation in fed-batch cultures should be avoided. Possible changes in the physiology of the production strain during the fed-batch fermentation should also be accounted for. For cultures of *S. cerevisiae* the control of both glucose and ethanol levels are essential for high level of heterologous protein, since ethanol formation inhibits biomass formation and possibly also heterologous protein production (Shiba et al., 1994; Vasavada, 1995; Kapat et al., 1997; Noronha et al., 1999). The short perturbation of cultivation by exposure to high levels of glucose early in the fermentation will result in a significant reduction in the biomass yield, due to the long-term nature of Crabtree effect (Vasavada, 1995). Multiple production levels may also be observed at a single growth rate or dilution rate, whereas repeated fed-batch cultures may significantly improve productivity (Da Silva and Bailey, 1991; Ibba et al., 1993). For the methylotrophic yeasts, *P. pastoris* and *H. polymorpha* the feeding profile of glycerol and/or methanol during various phases of a fed-batch cultivation should be optimised, and may be complimented by the accurate control of the methanol concentration (Loewen et al., 1999; Inan et al., 1999; Hong et al., 2002). Heterologous protein production may also be optimised by using model-based feed control to determine the required flow rate. The model used for control should describe both biomass formation and heterologous protein production (Zhang et al., 2000). The use of more complex feeding profiles, such as a cyclic feed, may also improve heterologous protein production by yeast (Chang et al., 1998).

2.10. COMPARISON OF YEASTS

With regards to the choice of yeast species for production of a foreign protein, *S. cerevisiae* and *P. pastoris* remain the most frequently used hosts (Cereghino and Cregg, 1999). However, public literature on heterologous protein production with yeast is increasingly being dominated by reports on the successful production of various foreign proteins using the *P. pastoris* expression system, though this may not necessarily reflect the situation in industry (Hodgson, 1993). Besides having a well-established reputation for high-level secretion of foreign proteins, the use of this system is facilitated by the commercial availability of suitable expression vectors and strains (Invitrogen) (Cereghino and Cregg, 2000; Rosenfeld, 1999; Gellissen and Hollenberg, 1997; Buckholtz and Gleeson, 1991). Examples of low production levels or failure to express heterologous proteins with the *P. pastoris* system are also accumulating, though usually not reported (Romanos, 1995). Despite several reports of "lower production levels," *S. cerevisiae* is still widely used as host, mostly due to its suitability for the production of health care and food grade proteins, its usefulness in industrial biofuel processes, the extensive knowledge on its molecular genetics and the ability to manipulate them, and the previous establishment of production processes using this organism. Fundamental breakthroughs in understanding the genetic and physiological requirements of a host strain for the production of high levels of heterologous protein, may only be possible in a host such as *S. cerevisiae* that facilitates investigations on a genomic, transcriptional, metabolite, biochemical and physiological basis. Despite the problems associated with the hyperglycosylation of heterologous proteins and ethanol production during aerobic growth on glucose, *S. cerevisiae* was therefore the preferred for the fundamental studies presented in Chapters 4 to 7. Recent information on the comparative aspects of the various yeast species for heterologous protein production (including *H. polymorpha*, *Y. lipolytica*, *Schizosaccharomyces pombe* and *P. stipitis*) is presented below.

2.10.1. *S. cerevisiae*

S. cerevisiae was the first yeast to be used for heterologous protein production, mostly due to knowledge on its molecular genetics and long-time use in industrial processes (Swinkels et al., 1993). Due to its GRAS status *S. cerevisiae* remains a favourite for the production of health care products, therapeutic proteins and food products (Kleman and Strohl, 1994; Vasavada, 1995; De Baetselier and Van Broekhoven, 1998; Romanos et

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al., 1992). High cell densities (> 50 $g.l^{-1}$) can also be obtained with *S. cerevisiae* when cultivated on glucose under optimised conditions (Fieschko et al., 1987; Shiba et al., 1994; Alberghina et al., 1991; Mendoza-Vega et al., 1994a; Mendoza-Vega et al., 1994b; Kerry-Williams et al., 1998; Yang et al., 1997). However, limited oxygen availability or too high growth rates may induce ethanol accumulation in these fermentations, resulting in a loss of glucose-substrate and a possible repression of strong glycolytic promoters (such as *PGK1*) used to drive heterologous gene expression (Kappeli, 1986). Although production levels attainable with *S. cerevisiae* seldom exceed 1-5% to the total cellular protein, a few notable examples of heterologous proteins produced to levels above 1 $g.l^{-1}$ have been reported (Buckholtz and Gleeson, 1991; Mendoza-Vega et al., 1994a):

- Roecklin et al. (1997): 6 $g.l^{-1}$ of heterologous glutathione S-transferase produced intracellularly during high cell density (78 $g.l^{-1}$) cultivation
- De Baetselier et al. (1991): 3 $g.l^{-1}$ of heterologous glucose oxidase produced extracellularly after screening of host strains
- Lee et al. (1999): 1.3 $g.l^{-1}$ of extracellular human growth hormone by using an optimised, plasmid-based expression system.
- Fusetti et al. (1996): More than 1 $g.l^{-1}$ of *Candida rugosa* Lipase I was accumulated in the fermentation medium
- Alberghina et al. (1993): After optimisation intracellular heterologous protein production up to 30% of total cellular protein was attained.
- Sleep et al., 1991: By screening a number of mutants it was possible to identify a transformant in which the heterologous protein constituted 40% of the total soluble protein

A significant disadvantage to the use of *S. cerevisiae* for the production of heterologous proteins may be the extensive overglycosylation of secreted proteins, which can reduce the binding and activity of heterologous hydrolytic enzymes on crystalline surfaces (Boer et al., 2000). For mammalian proteins, however, glycosylation is often not essential for biological activity (Olins, 1996). An example of therapeutic proteins produced with *S. cerevisiae*, besides those mentioned in the Chapter 1, is recombinant human granulocyte colony-stimulating factor (hG-CSF), which has been established by

international standard as preferential to products from other yeasts, based on in vitro bioassays and immunoassays (Bae et al., 1999).

Limitations in the expression of heterologous proteins by yeast may sometimes be overcome by using well advanced molecular techniques to modify the host or the expression systems, as has been presented elsewhere in this review. The completion of the Yeast Genome Project (Goffeau et al., 1996) and information furnished by Functional Genomics (Kowalczuk et al., 1999) has led to an increased interest in the use of *S. cerevisiae* as host for the production of various recombinant proteins. Besides the frequent direct functionality of *S. cerevisiae* molecular genetics in alternative yeasts, the extensive physiological and genetic knowledge available on this yeast can provide a scientific knowledge base for heterologous protein production. *S. cerevisiae* may thus be considered as a development platform for genetic engineering in yeast.

2.10.2. *P. pastoris* and *H. polymorpha*

Heterologous gene expression in the methyloptrophic yeasts *P. pastoris* and *H. polymorpha* is based on the utilisation of the promoter of the methanol oxidase gene for methanol regulation of gene expression, although alternative promoters have also been developed more recently. Expression of heterologous genes in these hosts occurs exclusively by integration of the expression vector into the host genome, resulting in stable transformants. However, methanol induction for heterologous gene expression may be problematic on industrial scale, since methanol is a potential fire hazard, necessitates the use of expensive explosion proof fermentation equipment and is not suitable for the production of food grade products (Cereghino and Cregg, 2000; Cereghino and Cregg, 1999; Swinkels et al., 1993). The major advantages of *P. pastoris* are: The preference for respiratory growth, the proven ability for high cell density fermentations, the ease of scale-up and the proven ability for secretion of correctly folded proteins (Cereghino and Cregg, 2000; Rosenfeld, 1999; Olins, 1996). An impressive list of proteins has been produced to relatively high levels in *P. pastoris* (Cereghino and Cregg, 2000). Some examples of high levels of recombinant protein production by the methylotrophic yeasts are:

- Werten et al. (1999): 14.8 $g.l^{-1}$ of heterologous gelatin produced extracellularly by *P. pastoris* after inactivation of proteolytic activity.

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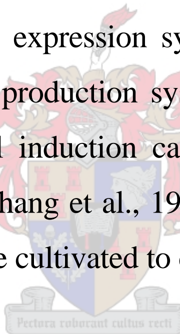
- Clare et al. (1991): 12 $g.l^{-1}$ tetanus toxin produced by *P. pastoris* transformants containing multiple integrations of the expression cassette
- Barr et al. (1992): 4 $g.l^{-1}$ human serum albumin secreted by *P. pastoris*
- Mayer et al. (1999): Phytase production levels of 13.5 $g.l^{-1}$ obtained by cultivation of recombinant *H. polymorpha*

2.10.3. *K. lactis*

K. lactis is suited to the production of food grade products due to its GRAS status, and is considered to be a major alternative to *S. cerevisiae* in this regard (Bonekamp and Oosterom, 1994; Swinkels et al., 1993). Accumulation of heterologous protein production to levels corresponding to "several gram per litre" (Fleer et al., 1991) and 30% of the total cellular protein (Faber et al., 1996) have been reported.

2.10.4. *Y. lipolytica*

Early development of *Y. lipolytica* expression systems was based on the use of the native *XPR2*-promoter. However, production systems suffer from the complexity of regulation of this promoter as full induction can only be obtained by the addition proteose peptone to the medium (Chang et al., 1997). Kim et al. (2000) demonstrated the ease with which this yeast can be cultivated to cell densities over 100 $g.l^{-1}$ by using a one-step feeding process.



2.10.5 S. pombe

Next to *S. cerevisiae*, *S. pombe* represents the yeast with the best-characterised molecular genetics. However, the potential of this yeast to produce eukaryotic mature proteins in a form closer to their natural conformation, due to several characteristics that are more similar to mammalian cells than other yeasts, has not been fully utilised due to slow progress in the development of an effective foreign-gene expression system (Giga-Hama and Kumagai, 1999).

2.10.6. *P. stipitis*

P. stipitis has seldom been used as a host for the production of heterologous proteins. This xylose-fermenting yeast is Crabtree-negative, with ethanol-production induced only in response to oxygen limitation (Du Preez et al., 1989). Growth of the yeast is not

inhibited by glucose concentrations of up to 50 g.l^{-1} (Du Preez et al., 1986). Production of a heterologous cellulase under control of the native *XYL1* promoter, and heterologous xylanases under control of the native *XYL1*, *TKL* and *ADH2* promoters, has been reported (Piontek et al., 1998; Den Haan and Van Zyl, 2001; Passoth and Hahn-Hägerdal, 2000).

2.10.7. Experimental comparisons of expression systems

Conclusions on the suitability of different yeast hosts for the production of a heterologous protein should be based on direct experimental comparison of transformants for the production of a specific protein, as is presented here. For the production of recombinant single chain Fv (scFv) antibody fragments, *P. pastoris* was long considered to be the only option, since the secretion of scFv fragments in *S. cerevisiae* was hampered by the formation of large intracellular protein aggregates (Pennell and Eldin, 1998; Frenken et al., 1998). However, optimisation of the expression system and overproduction of some of the secretory components has led to the successful secretion of reasonable amounts of scFv fragments by *S. cerevisiae* (Shusta et al., 1998). Recombinant *P. pastoris* has also secreted functional Fab fragments at a level of 40 mg.l^{-1}, which is similar to the production levels obtained for other heterodimeric biologically active proteins (Lange et al., 2001). Conversely, production levels of heterologous β-glucosidase levels in *S. cerevisiae* were superior to those obtained with *P. pastoris*, though only a single copy of the expression cassette was present in the latter (Skory et al., 1996). Improved production levels of recombinant anticoagulant peptide (AcAP-5) by *P. pastoris* compared to production by *S. cerevisiae* may also have been an artefact of the adenine-auxotrophic mutation of the latter transformant, which can influence heterologous protein production negatively (Neeper et al., 1990; Inan et al., 1999).

With regard to the other "alternative" yeasts, a comparison of production levels of six fungal proteins by *S. cerevisiae*, *H. polymorpha*, *K. lactis*, *S. pombe* and *Y. lipolytica* revealed inferior production levels by *S. cerevisiae* transformants in all cases (Müller et al., 1998). These sentiments were echoed by the increased production levels of heterologous β-lactoglobulin (Rocha et al., 1996) and interleukin 1β (Blondeau et al., 1994) obtained with *K. lactis*, compared to *S. cerevisiae*, and improved production of

heterologous receptor protein (Sander et al., 1994) and *T. reesei* endoglucanase (Okada et al., 1998) by *S. pombe*, also compared to *S. cerevisiae*. In several additional cases, the use of the yeasts *K. lactis* and *H. polymorpha* as expression hosts have resulted in increased yields of a better-quality product (Gellissen and Hollenberg, 1997).

2.11. CONCLUSIONS

Though by no means extensive, this review has identified potential limitations in most of the steps involved in the production of heterologous proteins by yeasts, specifically *S. cerevisiae*. Though several examples of improved production through the application of rational improvements to these limitations were presented, no single limitation could be identified as determining production levels of heterologous proteins. Variations in the strains, expression systems, heterologous proteins and media composition render such a conclusion impossible in the light of the biological complexity of microorganisms. However, the application of various rational approaches in an empirical manner has frequently resulted in significant improvements in heterologous protein production, both in terms of quantity and quality of the heterologous protein produced. The nature of the limitations in heterologous protein production is such that the task of addressing them lies more strongly in the hands of the molecular biologist, since the significant improvements attainable with improved fermentation technology are frequently much smaller than the order-of-magnitude improvements observed due to changes based on molecular genetics. The improvement of strains for the production of heterologous proteins should be based on rational approaches to molecular genetics and selection of transformants with higher production levels, with quantitative microbial physiology applied for the rigorous characterisation of improvements.

Most notable among the presented limitations, was firstly the possible effect of proteolytic degradation of synthesised products shortly after translation, during posttranslational processing or after secretion, which may represent a generic limitation in the production of heterologous proteins. Furthermore, the much emphasised “limited secretory capacity” of yeasts, though more specifically *S. cerevisiae*, also requires the optimisation of gene expression and improvement of the secretory machinery via rational approaches to obtain improved production levels. However, vague references to the effect of “host strain characteristics” and the “intrinsic protein characteristics”

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remain to be quantified. The use of *S. cerevisiae* as a host strain for heterologous protein production also remains advantageous due to the applicability of transformants to established processes, such as brewing, baking and wine making, and the production of bioethanol. Basic studies on the molecular genetics of heterologous protein production is possible for *S. cerevisiae*, since it has the most comprehensive yeast genetic tool kit available, thus facilitating fundamental breakthroughs (Shusta et al., 1998).

Three aspects of microbial physiology that may influence heterologous protein production levels and are yet to be fully explored are the effect of heterologous gene expression on the physiology of the host strain, the uncharacterised effect of auxotrophic markers on heterologous protein production and the effect of medium composition on the cellular carbon- and nitrogen-metabolism. Changes in the host metabolism and physiology due to the production of a heterologous protein specifically may represent an untapped well of information on the limitations in microbial metabolism during foreign protein production and secretion, and was further investigated in Chapters 4 and 5. The possible effect of auxotrophic requirements may also represent a host strain characteristic that is important for heterologous protein production, and was studied in comparison to prototrophic production strains (Chapter 6), whereas a systematic determination of the effect of medium composition on heterologous protein production may reveal limitations in resource availability for heterologous protein production (Chapter 7). The applicability of these results to the use of an alternative yeast host (*P. stipitis*) for heterologous protein production was also studied (Chapter 8). Clarification of these and other fundamental aspects of microbial physiology are required prior to scale-up to ensure an optimal production process.

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Chapter 3

PURIFICATION OF HETEROLOGOUS XYLANASE PRODUCED BY *S. CEREVISIAE* AND *ASPERGILLUS NIGER*

3.1. INTRODUCTION

A proper investigation of the microbial physiology during heterologous protein production required the ability to quantify the mass amount of heterologous xylanase produced by *S. cerevisiae*. As production levels of recombinant xylanase were determined as activity measurements (typically $nkat.ml^{-1}$) obtained with the DNS-method (Bailey et al., 1992), it was necessary to determine the specific activity of the pure recombinant xylanase ($nkat.mg^{-1}$), and thereby calculate the amount of recombinant protein (e.g. $mg.l^{-1}$) produced. The purification of the recombinant xylanase was therefore necessary. Pure recombinant xylanase could also be used for raising polyclonal rabbit antibodies, which are useful for detecting small amounts of recombinant protein in protein mixtures, using Western blots.

The purification of the β-1,4-xylanase II of *T. reesei* from a fungal fermentation broth was reported previously (Törrönen et al., 1992; Tenkanen et al., 1992). This purification method was selected as a starting point for the purification of recombinant xylanase in the present study, by assuming that the recombinant xylanase and the native protein would have similar isoelectric characteristics (Henrik Stålbrand; Evodia Setati, personal communication). The basic steps in the purification protocol by Törrönen et al. (1992) were empirically optimised for the purification of heterologous xylanase from each of the two microbial sources.

3.2. PRODUCTION OF RECOMBINANT β-XYLANASE

The recombinant *T. reesei* β-xylanase produced by both *S. cerevisiae* (La Grange et al., 1996) and *A. niger* (Rose and Van Zyl, 2002) was purified, to account for possible changes in the specific activity due to the hyperglycosylation of the recombinant

xylanase by the former. Recombinant *S. cerevisiae* was cultivated in a chemically defined medium (Verduyn et al., 1992), supplemented with seven amino acids (Chapter 4) whereas the recombinant *A. niger* was cultivated in a semi-defined medium (Rose and Van Zyl, 2002). Cultures were performed in shake-flasks at 30ºC in semi-batch mode, with a single addition of fresh medium subsequent to the consumption of the glucose in the medium used for inoculation. The purity of the recombinant xylanase samples was determined by silver-staining (Biorad Silverstain Kit) of SDS-PAGE gels (Laemmeli, 1970).

3.3. SAMPLE PREPARATION

Approximately 3L of fermentation broth obtained from each of the microbial cultures was separated from cells by centrifugation at 4ºC, and the proteins therein precipitated by the slow addition of crystalline $(NH_4)_2SO_4$ to the cooled sample. To obtain a saturated solution, $(NH_4)_2SO_4$ was added to a final concentration of approximately 0.57 $g.ml^{-1}$. The samples were incubated overnight at 8ºC to obtain complete precipitation.

The precipitated protein from the two samples were collected separately by centrifugation, and re-dissolved in the cation exchange buffer. Proteins smaller than 14 kDa were removed by dialysis, using a membrane with a 12-14 kDa cut-off point. Precipitation with $(NH_4)_2SO_4$ resulted in an approximately 30-fold concentration of the proteins in the original fermentation broth sample. The volumetric xylanase activity in the *A. niger* concentrated sample was higher than in the *S. cerevisiae* sample (Table 3.1), indicating a significantly higher production level of recombinant xylanase during cultivation. However, the xylanase in the *A. niger* sample was also less pure than in the *S. cerevisiae* sample, indicating a significantly higher level of native proteins secreted by the former.

For *S. cerevisiae* two dominants forms of the recombinant protein were visible in the concentrated sample, i.e. 21 kDa, which is close to the native protein, and a more heavily glycosylated form of ≈26 kDa (Fig. 3.1). For the sample obtained from *A. niger*, only a single glycosylation form was visible, i.e. 21 kDa, and the extracellular xylanase protein was thus not hyperglycosylated (Lane 1; Fig. 3.5).

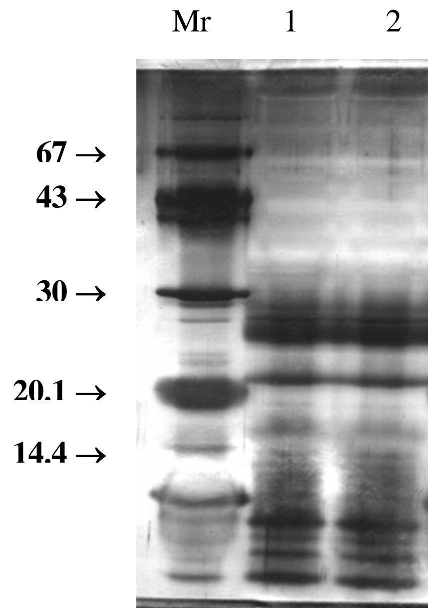

Figure 3.1. Concentrated sample from *S. cerevisiae* fermentation broth (Lanes 1 and 2).

3.4. CATION EXCHANGE

Cation exchange was selected as the first purification step due to the relatively high isoelectric point of the native β-xylanase (pI = 9.0; Tenkanen et al., 1992; Törrönen et al., 1992). Sephadex Fast Flow cation exchange gel (Amersham-Pharmacia Biotech, Uppsala, Sweden) was therefore used to "capture" the majority of the recombinant xylanase from the mixture of native proteins in the concentrated sample. The most effective pH for cation exchange was determined in a small-scale experiment with the *S. cerevisiae* protein sample (Fig. 3.2). 1.0 ml of cation exchange gel was equilibrated to different pH values using either a 5 or 50 mM buffer, and loaded with 0.5 ml of sample in the same buffer. The bound xylanase was eluted with a B buffer at the same pH and ionic strength, though also containing 1M NaCl. To cover the range of pH values investigated, two different buffers [Tris (pH 7.2-8.0) and phosphate-citrate (pH 4.0-7.0)] were used.

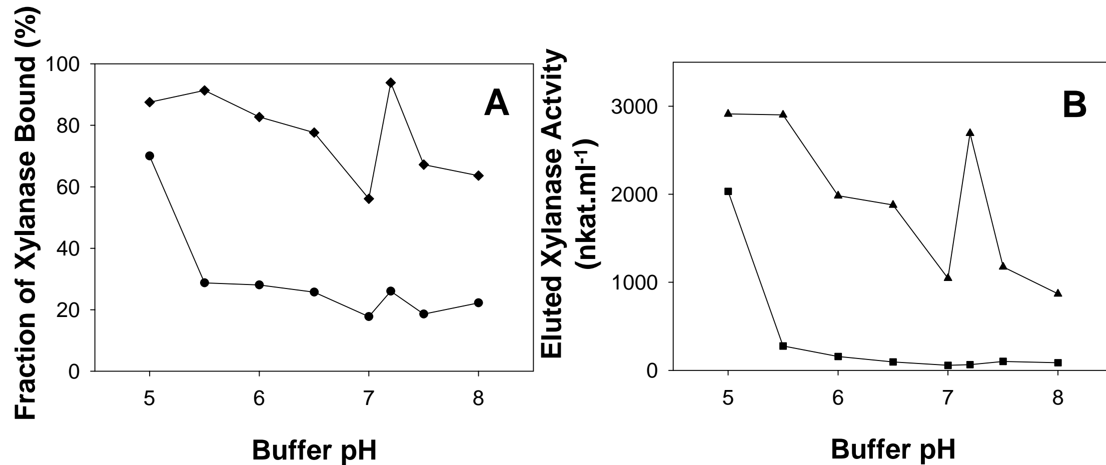

Figure 3.2. Small-scale cation exchange with a *S. cerevisiae* protein sample. A. Percentage of xylanase in the loaded sample bound to the gel. B. Xylanase activity eluted from gel during washing with B buffer. (♦) Percentage of xylanase bound when using a 5 mM buffer. (●) Percentage of xylanase bound when using 50 mM buffer. (▲) Xylanase activity eluted from gel after loading using a 50 mM buffer. (■) Xylanase activity eluted from gel after loading using a 50 mM buffer.

Although the percentage of the xylanase bound to the cation exchange gel increased with a decrease in pH, the most dramatic improvement in the binding was obtained by reducing the ionic strength of the buffer from 50 mM to 5 mM (Fig 3.2A). The xylanase bound to the gel could be recovered by eluting the gel with the corresponding B buffers (Fig. 3.2B). The increased concentration of cations in the 50 mM buffers apparently out-competed the weak binding of the positively charged xylanase protein to the gel. A large difference in the binding efficiency and eluted activity at pH 7.2 (Tris) and pH 7.0 (phosphate-citrate) was observed. This may have been due to the presence of cations in the phosphate-citrate buffer that competed with xylanase for binding to the cation exchange gel. A 5 mM Tris buffer at pH 7.2 was therefore the most efficient for cation exchange using the *S. cerevisiae* protein sample. A similar small-scale cation exchange experiment was performed using the *A. niger* protein sample, with equilibration of the gel over the pH range 7.2-8.0 (Tris) and 4.0-7.0 (phosphate-citrate) using only 5 mM buffers (Fig. 3.3). The 5 mM Tris buffer at pH 7.2, however, poorly facilitated cation exchange with the *A. niger* sample, compared to its performance with the *S. cerevisiae* sample. The poor binding was also confirmed when loading the *A.*

niger sample to a cation-exchange column equilibrated with the 5 mM Tris buffer at pH 7.2. The 5 mM phosphate-citrate buffer at pH 7.0 was thus selected for cation exchange when using the *A. niger* sample.

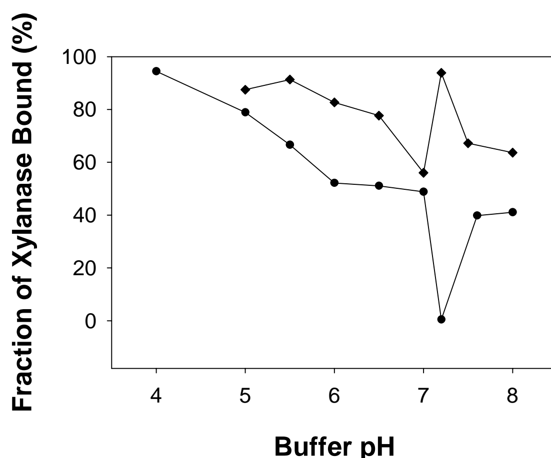

Fig. 3.3. Small-scale cation exchange with an *A. niger* protein sample. The percentage of xylanase in the loaded sample bound to the gel at different pH values when using a 5 mM buffer. (●) Percentage of xylanase from *A. niger* protein sample bound to gel when using 5 mM buffer. (◆) Percentage of xylanase from *S. cerevisiae* sample bound to gel when using a 5 mM buffer.

Subsequent cation exchange was performed on a 16 mm diameter column, containing 140 mm of packed gel (Packing volume: 28 ml gel; Gel capacity: 1.407 g per 28 ml; Maximum load: 280 mg protein bound to the gel). Elution was performed with a B-buffer containing 2M NaCl, which was applied to the column without a gradient. For the *S. cerevisiae* sample, the specific xylanase activity was higher in the fractions from cation exchange than in the original sample (Table 3.1), indicating that the xylanase protein was concentrated during cation exchange. The recombinant xylanase from *S. cerevisiae* was also visibly cleaner after cation exchange (Figs. 3.1 and 3.4). Cation exchange with the *A. niger* sample was less efficient. Despite the optimisation of the cation exchange buffer, the proper binding of the recombinant xylanase to the cation exchange column could not be obtained. The cation exchange column instead only retarded the movement of the recombinant xylanase through the column, relative to the other proteins, and thereby facilitated a partial separation of the xylanase from these proteins. The concentration of xylanase in the resulting eluent was lower than in the original sample. However, a reasonable amount of the xylanase in the original sample

was removed during the cation exchange procedure (compare lanes 1 and 2; Fig. 3.5). The large increase in the specific activity of the recombinant xylanase sample vouched for a significant increase in the purity of the xylanase protein during cation exchange (Table 3.1). The additional difficulties observed during cation exchange using an *A. niger* concentrated sample might be attributed to either the large amount of other proteins present in the sample, that could hamper the binding of the recombinant xylanase to the column, or a decrease in the binding capacity of the gel due to ageing.

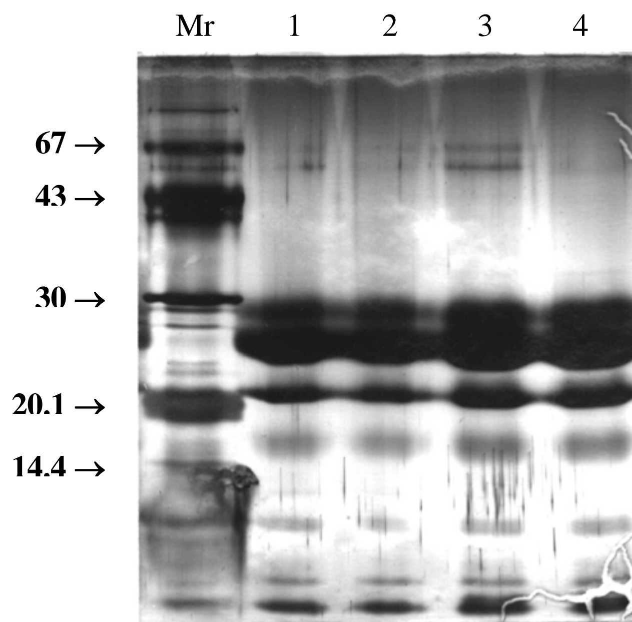

Figure 3.4. Eluent from cation exchange performed with a *S. cerevisiae* protein sample. Lane 1 to 4: Purest fractions collected from cation exchange. Mr: Molecular weight marker.

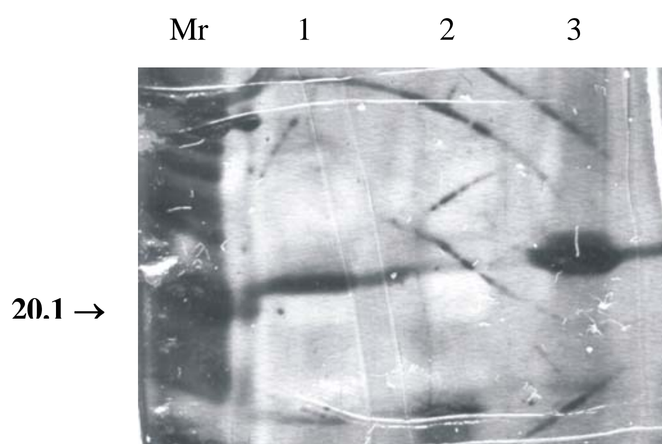

Fig 3.5. Eluent from cation exchange performed with *A. niger* protein sample. Lane 1: Concentrated sample. Lane 2: Concentrated sample after cation exchange. Lane 3: Eluent from cation exchange (concentrated). Mr: Molecular weight marker.

3.5. GELFILTRATION

Gelfiltration was applied as a final purification and "polishing" step, to obtain a pure protein. Gelfiltration was performed on a pre-packed Sephadex 200 HR 16/60 column (Amersham-Pharmacia Biotech, Uppsala, Sweden) equilibrated with a 50 mM phosphate buffer at pH 6.0. The cation exchange fractions were concentrated using a ultrafiltration membrane device (Amicon Centricon) and applied as a 1 ml sample to the gelfiltration column. For the *S. cerevisiae* sample a single gelfitration run was sufficient to obtain a protein with apparent electrophoretic purity (Fig. 3.6), whereas for the *A. niger* sample two subsequent runs were performed to ensure the purity of the final product (Fig. 3.7). In the final *S. cerevisiae* sample three distinct glycosylation patterns could be identified: 21 kDa, ≈26 kDa and ≈28 kDa, with the latter two representing hyperglycosylated versions of the recombinant xylanase. For the *A. niger* sample only a single 21 kDa form of the protein was observed, corresponding to the size of the native xylanase (Törrönen et al., 1992; Tenkanen et al., 1992).

The specific activity of the final *S. cerevisiae* sample was slightly lower than the sample obtained after cation exchange, despite a visible improvement in the electrophoretic purity of the protein (compare Figs. 3.4 and 3.6). This decrease could be attributed either to a destabilisation of the xylanase protein due to the removal of other proteins in the solution, or xylanase activity present in some of the impurities removed during gelfiltration. However, the specific activities obtained for the pure *S. cerevisiae* and *A. niger* samples were very similar, indicating that the purification procedures resulted in proteins of similar purity, and that these values were reliable for the conversion of activity measurements to amount of recombinant protein. In Table 3.1 the progress in the various steps in the purification procedure for recombinant xylanase from *S. cerevisiae* and *A. niger* respectively, are compared.

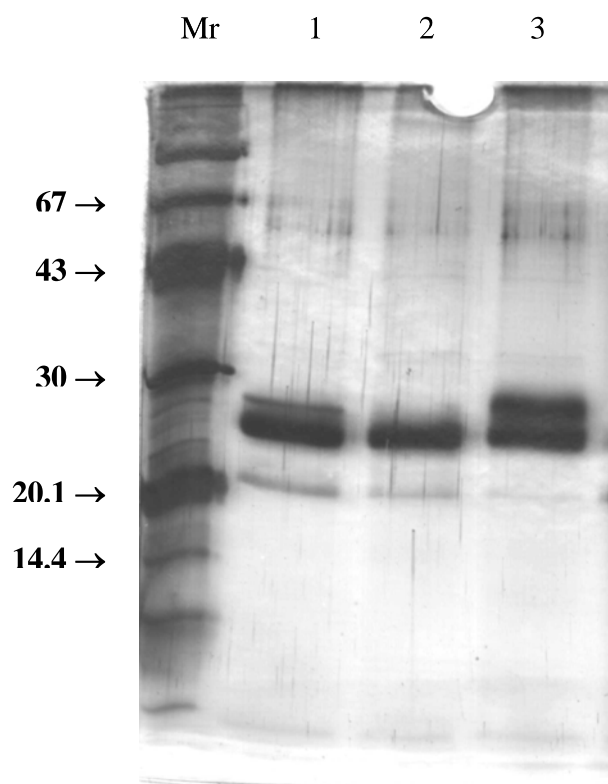

Figure 3.6. Final product from gelfiltration performed with *S. cerevisiae* sample. Lanes 1 to 3: Purest fractions. Mr: Molecular weight marker

Table 3.1 Activity measurements from steps in the purification of recombinant xylanase

Product from =>	***S. cerevisiae***		***A. niger***	
	Volumetric* **nkat.ml^{-1}**	**Specific*** **nkat.µg$_{protein}^{-1}$**	**Volumetric*** **nkat.ml^{-1}**	**Specific*** **nkat. µg$_{protein}^{-1}$**
Sample Preparation	5450	31.6	30900	14
Cation Exchange	47200	36.4	1750	30.8
Gelfiltration	3000	30.2	1460	33.3

* Activity Measurements

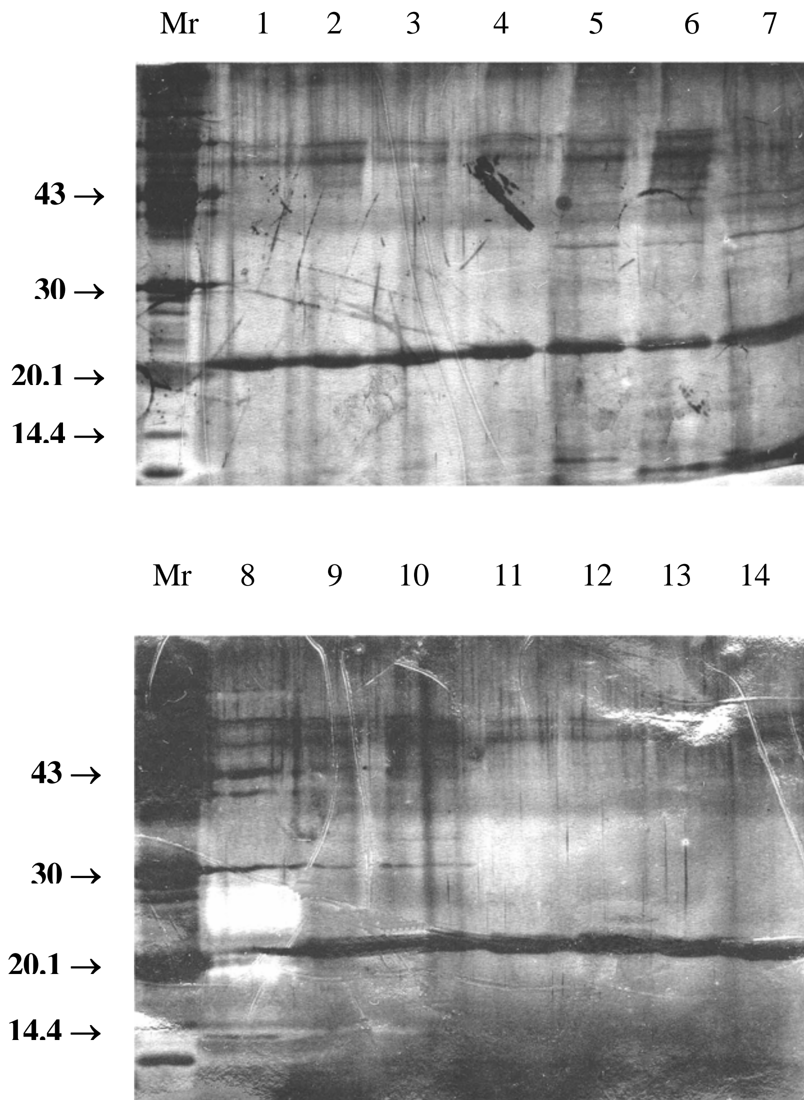

Figure 3.7. Final product from gelfiltration performed with *A. niger* sample. Lanes 1-4 and 11 to 14: Purest fractions. Lanes 5 to 10: Less pure fractions.

Both the *S. cerevisiae* and *A. niger* pure xylanase protein samples were used for raising polyclonal rabbit antibodies. The antibody obtained from the *S. cerevisiae* sample mostly bound to the ≈26 kDa form of the recombinant protein [visualised with Western blot (Fig. 3.8)], indicating that binding might be strongest on the outer mannose glycosylation chains. The *A. niger*-antibody was also of sufficient quality for use in Western blotting (data not shown).

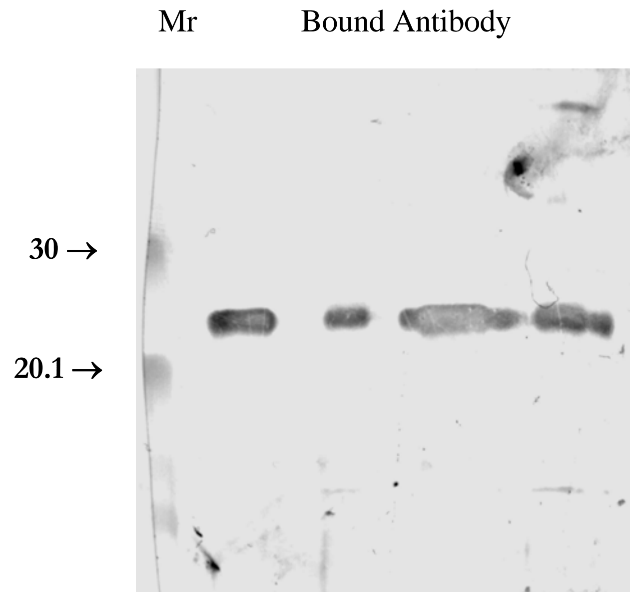

Figure 3.8. Western blot with detection of xylanase in a protein mixture using an antibody raised against a pure xylanase protein produced by *S. cerevisiae*.

3.6. CONCLUSIONS

The recombinant xylanases produced by *S. cerevisiae* and *A. niger* were purified in two steps, resulting in electrophetretically pure proteins. The specific activities of these proteins were very similar, indicating that the expression host had little effect on the functionality of the proteins. These specific activities were subsequently used to determine the mass amount of heterologous protein produced by *S. cerevisiae* during physiological studies.

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Stålbrand, H. Department of Biochemistry, Lund University, Sweden.

Setati, E. Department of Microbiology, Stellenbosch University, South Africa.

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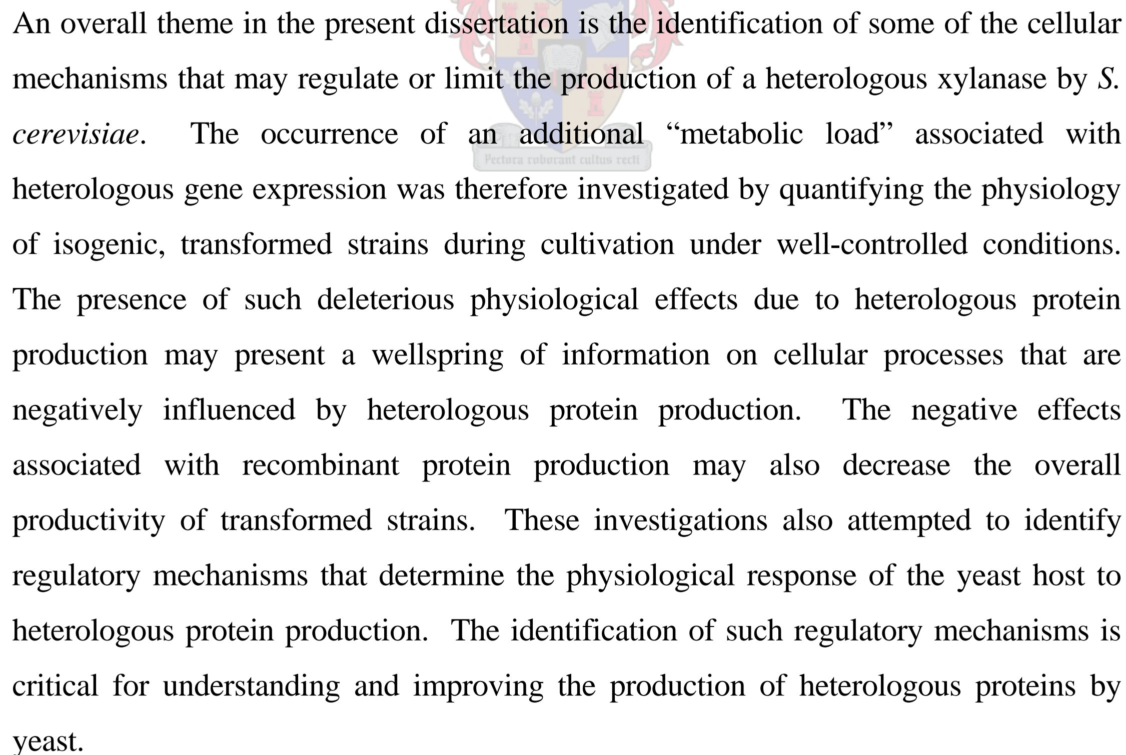
Chapter 4

PHYSIOLOGICAL RESPONSE OF *S. CEREVISIAE* TO HETEROLOGOUS XYLANASE PRODUCTION

Published as: Görgens, J.F., Van Zyl, W.H., Knoetze, J.H., Hahn-Hägerdal, B. (2001) The metabolic burden of the *PGK1* and *ADH2* promoter systems for heterologous xylanase production by *Saccharomyces cerevisiae* in defined medium. Biotechnology and Bioengineering 73(3): 238-245.

Please Note: Although the fermentation work presented in this chapter was completed as part of a previous master's degree, the discussion and interpretation of the data, as presented here, was performed as part of the Ph.D. degree.

4.1. INTRODUCTION



An overall theme in the present dissertation is the identification of some of the cellular mechanisms that may regulate or limit the production of a heterologous xylanase by *S. cerevisiae*. The occurrence of an additional "metabolic load" associated with heterologous gene expression was therefore investigated by quantifying the physiology of isogenic, transformed strains during cultivation under well-controlled conditions. The presence of such deleterious physiological effects due to heterologous protein production may present a wellspring of information on cellular processes that are negatively influenced by heterologous protein production. The negative effects associated with recombinant protein production may also decrease the overall productivity of transformed strains. These investigations also attempted to identify regulatory mechanisms that determine the physiological response of the yeast host to heterologous protein production. The identification of such regulatory mechanisms is critical for understanding and improving the production of heterologous proteins by yeast.

Due to the importance of recombinant gene expression in yeasts and other microorganisms for metabolic engineering (Bailey, 1991) and the commercial

production of heterologous proteins (Hensing et al., 1995), expression systems for a number of yeasts have been developed (Hensing et al., 1995), especially in *S. cerevisiae*, which has served as a host for the production of numerous foreign proteins (Hadfield et al., 1993; Heinisch and Hollenberg, 1993). The presence of a non-specific "metabolic burden" (Bentley et al., 1990; Janes et al., 1990) or "protein burden" (Snoep et al., 1995) has been observed in numerous host organisms due to the expression of foreign genes, affecting the growth thereof negatively. For recombinant *S. cerevisiae* strains, reductions in the maximum specific growth rate, biomass yield, respiratory capacity and stability of the recombinant plasmid, due to heterologous gene expression, have been observed (Srienc et al., 1986; Marquet et al., 1987; Sardonini and DiBiasio, 1987; Gopal et al., 1989; Zurbriggen et al., 1989; Janes et al., 1990; Da Silva and Bailey, 1991, Giuseppin et al., 1993; Dequin and Barre, 1994; Nacken et al., 1996). A decrease in the flux through glycolysis of the host strain (Snoep et al., 1995; Van Hoek et al., 1998) and an increase in the maintenance energy requirement (Stouthamer and Van Verseveld, 1987; Bhattacharya and Dubey, 1995) have also been observed. The metabolic burden of recombinant protein production increased with increasing production levels, either due to plasmid copy number amplification or an increase in the strength of the recombinant promoter (Seo and Bailey, 1985; Srienc et al., 1986; Betenbaugh et al., 1989; Bentley et al., 1990; Janes et al., 1990; Ryan and Parulekar, 1991; Snoep et al., 1995; Gu et al., 1996; Nacken et al., 1996). This non-specific effect can be separated from the catalytic activity of the protein being produced (Snoep et al., 1995), and is often disregarded in studies concerning heterologous gene expression in *S. cerevisiae* (Romanos et al., 1992).

The metabolic burden of recombinant gene expression has been associated with the allocation of cellular resources to plasmid-related activities (Peretti and Bailey, 1987; Gopal et al., 1989; Ryan and Parulekar, 1991; Gu et al., 1996), either for plasmid replication or the production of the cloned gene product (Bentley et al., 1990; Da Silva and Bailey, 1991; Ryan and Parulekar, 1991; Bailey, 1993). The effect of cloned gene expression has been associated with either the energetic cost of extra protein synthesis (Gopal et al., 1989; Bailey, 1993; Snoep et al., 1995) or the competitive effect of extra protein synthesis (Shuster, 1989; Gopal et al., 1989; Van der Aar et al., 1992; Snoep et al., 1995). The "dilution" of native proteins, i.e. the reduction of the activity of native proteins by recombinant gene expression, was also proposed as a major mechanism

Pectora roborant cultus recti

causing a decrease in the flux through glycolysis and a decrease in the maximum specific growth rate of the host organism (Snoep et al., 1995; Van Hoek et al., 1998). Other proposed mechanisms are: the effect of foreign DNA synthesis during plasmid replication (Ibba et al., 1993; Lang and Looman, 1995; Lopes et al., 1996 and Carlsen et al., 1997), the "toxicity" of recombinant proteins to the cell (Ibba et al., 1993) and the negative consequences of small amounts of read-through transcripts (Janes et al., 1990).

In the present investigation, the metabolic burden introduced by the individual genetic components in a model expression system for heterologous xylanase production in *S. cerevisiae* was quantified, in an effort to identify some of the mechanisms that may limit heterologous protein production. The *T. reesei* ß-1,4-xylanase II encoding gene, *XYN2*, was expressed from a multicopy, 2µm plasmid under regulation of either the yeast glycolytic phosphoglyceratekinase (*PGK1)* or alcoholdehydrogenase II (*ADH2)* promoters (La Grange et al., 1996). The two xylanase-producing strains were compared quantitatively with three references strains, where either the heterologous *XYN2* gene, or the heterologous gene and the promoter and terminator were omitted from the recombinant plasmid. The five recombinant yeast strains were cultivated under identical conditions in aerobic batch culture. In batch culture, the *PGK1* promoter is maximally induced by the presence of glucose (Shuster, 1989; Kingsman et al., 1990; Romanos et al., 1992), whereas the *ADH2* promoter is repressed during growth on glucose and derepressed during the transition to growth on ethanol (Shuster, 1989; Price et al., 1990; Noronha et al., 1998). As the produced xylanase had no known catalytic function in yeast metabolism and was secreted efficiently by the recombinant strains, the quantified metabolic burden was strictly related to the presence and activity of the recombinant expression system (Van der Aar et al., 1990a; Van der Aar et al., 1990b; Van Hoek et al., 1998). Stable maintenance of the recombinant plasmids in non-selective cultivation media has been ensured through the inclusion of the *fur1 ura3* autoselective system (Loison et al., 1986) in the recombinant strains (La Grange et al., 1996).

4.2. MATERIALS AND METHODS

4.2.1. Strains and plasmids

The *S. cerevisiae* strains selected for this study are presented in Table 4.1. For the construction of autoselective strains the method of Loison et al. (1986) has been used. The strain stocks were stored in a 15% glycerol solution at –80ºC.

Table 4.1. Strains used in this study

Strain	**Genotype**			**Source**
S. cerevisiae Y294	*ura3, leu2, trp1,his3*			La Grange et al. (1996)
	Plasmid	**Promoter**	**Gene**	
Y294 [Host]*	*YEp352*	-	-	This study
Y294 [PGK1]*	*pJC1*	*PGK1*	-	Crous et al. (1995)
Y294 [PGK1-XYN]*	*pDLG6*	*PGK1*	*XYN2*	La Grange et al. (1996)
Y294 [ADH2]*	*pDLG1*	*ADH2*	-	La Grange et al. (1996)
Y294 [ADH2-XYN]*	*pDLG5*	*ADH2*	*XYN2*	La Grange et al. (1996)

* [ ] Indicates the content of the recombinant plasmid.

4.2.2. Medium and inoculum

Batch fermentations were conducted in a defined medium (Verduyn et al., 1992) containing 20 g.l^{-1} glucose as the carbon source. The medium was also supplemented with amino acids, both according to the auxotrophic requirements of the yeast strains [histidine (165 mg.l^{-1}), leucine (870 mg.l^{-1}) and tryptophan (664 mg.l^{-1})] and to enhance heterologous enzyme production [aspartate (257 mg.l^{-1}), glutamate (64 mg.l^{-1}), glycine (33 mg.l^{-1}) and serine (108 mg.l^{-1})]. The concentrations of amino acids were calculated according to biosynthetic requirements (unpublished results).

A two-step procedure was used for inoculum preparation. A preculture (5 ml) was inoculated with a small amount of cells from a –80ºC culture and grown overnight (12 h) at 30ºC in a Gallenkamp INR-200 orbital incubator (Leicester, UK) at 150 rpm. The preculture was subsequently transferred to 200 ml of medium in a 1L baffled shake flask and grown for 12-15 h (depending on the growth rate of the strain), at 30ºC. Both the preculture and the inoculum were prepared in the same medium as used in the fermenter. Cell densities in all cultures were estimated as absorbance (optical density)

measurements at 620 nm (A_{620}) with a Hitachi U-1100 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The volume of shake-flask culture, required to inoculate the fermenter to an A_{620} of 0.5, was centrifuged at 5000 rpm for 6 min in a Beckman J2-21 centrifuge (Geneva, Switzerland) and the cells re-suspended in 80 ml of medium, to obtain the final inoculum.

4.2.3. Fermentations

Fully aerobic batch fermentations were conducted in a computer-controlled glass fermenter (Belach Bioteknik AB, Stockholm, Sweden) with a total volume of 1000 ml and a working volume of 800 ml. The temperature and pH of the cultures were controlled at 30ºC and pH 5.0 (by the addition of 2M NaOH or 2M HCl), respectively. The fermentation broth was magnetically agitated in the range of 350 to 450 rpm and aerated with a 0.5 $l.min^{-1}$ airflow (standard conditions). The level of dissolved oxygen was monitored with a dissolved oxygen probe (Belach Bioteknik AB, Stockholm, Sweden) and maintained at a minimum of 50% of air saturation by adjusting the agitation speed when necessary. The outlet gas from the fermenter was cooled in a condenser, through which a water/methanol mixture at 2ºC was circulated. Dow Corning anti-foam (BDH) was added to the fermenter to control foaming, although this was not necessary during batch growth on glucose. All fermentations were repeated at least three times.

4.2.4. Analytical methods

Samples for the determination of cell density, substrate consumption and product formation were taken from fermentations at 45 to 60 min intervals. Samples for substrate and product analysis were centrifuged for 3 min at 14 000 rpm in a Force 14 microfuge (Denver Instruments, Denver, CO) within 1 min after sampling. The supernatant was collected in microfuge tubes, rapidly frozen at -80ºC and stored at –20ºC for analysis.

Samples for the determination of cell density were kept on ice during analyses and diluted with 9 $g.l^{-1}$ NaCl into the 0.05 - 0.2 linear absorbance detection range of the spectrophotometer. A_{620} measurements were completed in duplicate within 4 min of sampling. During the late exponential phase of each fermentation the dry weight (Meinander et al., 1996) and absorbance (A_{620}) measurements were also calibrated.

The fraction of non-viable cells in each sample was determined using fluorescence microscopy (Rapoport and Meysel, 1985). Samples were first diluted for cell counting in a haemocytometer, using 9 $g.l^{-1}$ NaCl for the initial dilutions and an equal volume of a 1 $mg.ml^{-1}$ primulin dye solution (Sigma) for the final 1:2 dilution. Primulin dye caused fluorescence of the non-viable cells. Plasmid stability (fraction or percentage of plasmid containing cells) was determined throughout a typical fermentation for each strain. Approximately 100 yeast colonies were transferred from a complex medium (YPD) plate to a $SC^{-ura-Leu}$ selective plate (Rose et al., 1990), using sterile, dried toothpicks and the percentage of colonies that grew on the selective medium was determined (Da Silva and Bailey, 1991).

4.2.5. Substrate consumption and product formation

Glucose, ethanol, glycerol, acetate and succinate concentrations were determined by column liquid chromatography (CLC) in a Gilson CLC system (Middletown, WI). The compounds were separated on an HPX87-H column (Biorad, Richmond, CA) at 65ºC, with 5 mM H_2SO_4 at 0.6 $ml.min^{-1}$ as mobile phase, and detected with a Shimadzu RID6A refractive index detector (Kyoto, Japan). Samples were assayed for xylanase activity according to Bailey et al. (1992). The substrate (1% birchwood xylan [Sigma] suspended in 50 mM pH 6.0 citrate buffer) and enzyme (diluted with 50 mM pH 6.0 citrate buffer) mixtures were incubated for 5 min at 60ºC, and the reducing sugar determined (Miller et al., 1960). By diluting the enzyme preparations, xylanase activity could be measured within the linear range of the assay. 1 unit of enzyme activity (1 U) corresponded to 1 μmole of reducing sugar released per minute. All enzyme activities were converted to protein amounts (mg) by use of the conversion factor 1.812 $U.\mu g_{pure\ xylanase}^{-1}$, obtained by protein purification (Chapter 3).

4.2.6. Calculations

Specific growth rates were calculated at individual points on the growth curve [ln (cell density) vs. time] by using the four surrounding points (two on each side) on the curve to determine the slope at the specific point. The maximum of these specific growth rates for each fermentation was selected, and an average calculated for each strain. The specific rates of cell death were also calculated for the fraction of non-viable cells in the

population. These rates were defined as the rate of increase in the density of non-viable cells in the culture, and were also estimated from slope determinations in the natural logarithmic domain. The yields of biomass and fermentation products on glucose were estimated from the slopes of straight-line sections in the product concentration vs. glucose concentration curves. The rates of glucose consumption and ethanol production were calculated by fitting a limited population growth model (Hirsch and Smale, 1974) to fermentation data (unpublished results). Specific substrate consumption and product formation rates were calculated from the time-based derivatives of the model. The significance of differences between average values, for all of the reported variables, was estimated with Student's independent T-test, using the SigmaPlot® (© SPSS Inc.) software package.

4.3. RESULTS

Two recombinant *S. cerevisiae* strains, Y294 [PGK1-XYN] and Y294 [ADH2-XYN], producing heterologous xylanase from a 2µm plasmid expression system, controlled either by the *PGK1* or the *ADH2* promoters, were characterized quantitatively. The strains were compared with three reference strains, where either the heterologous *XYN2* gene (Y294 [PGK1] and Y294 [ADH2]) or the heterologous gene and the promoter and terminator (Y294 [Host]) were omitted from the plasmid (Table 4.1). The recombinant *S. cerevisiae* Y294 [Host] strain was preferred as a reference strain to the parental *S. cerevisiae* Y294 strain, as the latter did not grow in the defined medium. The quantitative differences between the autoselective, recombinant *S. cerevisiae* strains in aerobic batch culture, were verified statistically.

4.3.1. Substrate consumption and product formation

During batch cultivation the growth, substrate consumption and product formation of the recombinant strains were monitored and typical data for the [PGK1-XYN] and [ADH2-XYN] strains are presented in Figure 4.1. Initial growth on glucose continued for 12-14 h, with ethanol and glycerol as the major by-products. Growth continued after glucose depletion with ethanol and glycerol as carbon sources.

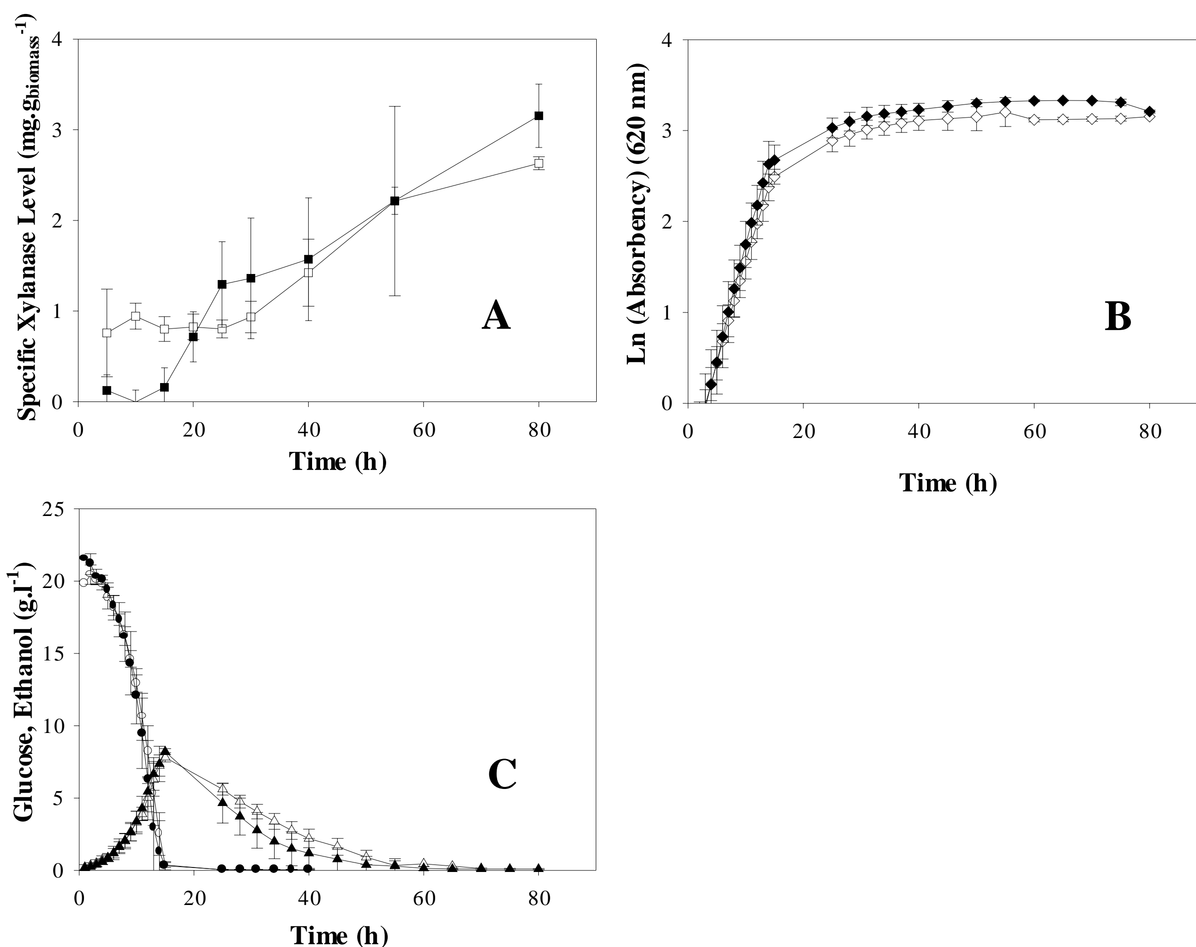


Figure 4.1. Time course for substrate consumption and product-formation by the Y294 [ADH2-XYN] (closed symbols) and Y294 [PGK1-XYN] (open symbols) strains during batch culture in optimised defined medium. (A) (■,□) Xylanase production (mg.g$_{biomass}$$^{-1}$). (B) (♦,◊) biomass formation (absorbance, 620 nm). (C) (●,O) glucose concentration (g.l^{-1}) and (▲,Δ) ethanol concentration (g.l^{-1}).

4.3.2. Heterologous protein production levels

Levels of heterologous enzyme production were significantly affected by the characteristics of the promoter used. During exponential growth on glucose and ethanol, heterologous enzyme production by the [PGK1-XYN] strain was growth associated, whereas during the stationary phase production continued without biomass

formation (Fig. 4.1). For the [ADH2-XYN] strain, heterologous enzyme production as fully repressed during growth on glucose and derepressed during the transition to growth on ethanol (Fig. 4.1). Heterologous enzyme production during the stationary phase was also observed.

Overall production levels of the [ADH2-XYN] strain were slightly higher than for the [PGK1-XYN] strain. After 80 h of cultivation time, specific xylanase production levels of 3.2 and 2.6 $mg.g_{biomass}^{-1}$ were obtained with these strains, respectively (Fig. 4.1A), based on a specific activity of 1810 $U.mg^{-1}$ for the pure recombinant xylanase obtained during protein purification. Approximately 2 $mg_{xylanase}.g_{cellular\ protein}^{-1}$ xylanase protein was produced by the [PGK1-XYN] strain during growth on glucose, assuming a biomass composition containing circa 50% cellular protein (Albers et al., 1996). This amount of xylanase production corresponds to maximally 0.2% of the total cellular protein produced during this growth phase.

4.3.3. Growth rate

The maximum specific growth rates of the five recombinant yeast strains during growth on glucose, and subsequent growth on ethanol, were compared (Tables 4.2 and 4.5). During growth on glucose, the maximum specific growth rate of the Y294 [Host] strain was significantly higher than the other strains, also confirmed with hypothesis testing (Table 4.5). The growth rate of the [PGK1] strain was also significantly higher than the [PGK1-XYN] strain, whereas the [ADH2] and [ADH2-XYN] strains had similar growth rates. The [PGK1-XYN] strain thus grew significantly slower on glucose than the [ADH2-XYN] strain. During growth on ethanol, the growth rates of all strains were approximately 10-fold lower than during growth on glucose. During this second growth phase, the maximum specific growth rate of the [ADH2-XYN] strain was significantly lower than the [ADH2] strain, similar to the difference observed between the [PGK1-XYN] and [PGK1] strains during growth on glucose. The [PGK1-XYN] and [ADH2-XYN] strains grew at similar growth rates during this growth phase (Table 4.5). For all strains, the fraction of plasmid containing cells in the population remained at 100% throughout fermentations. The fractions of non-viable cells for the individual strains were also similar (1-5%) and consequently the rates of cellular death were not significantly different, irrespective of the content of the recombinant plasmid.

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Table 4.2. Maximum specific growth rates on glucose and ethanol in defined medium

Strain	μ_{max} on glucose	μ_{max} on ethanol
Y294 [Host]	0.33 ± 0.030	0.026 ± 0.005
Y294 [PGK1]	0.28 ± 0.010	0.035 ± 0.002
Y294 [PGK1-XYN]	0.25 ± 0.003	0.030 ± 0.003
Y294 [ADH2]	0.29 ± 0.004	0.032 ± 0.001
Y294 [ADH2-XYN]	0.27 ± 0.020	0.028 ± 0.002

4.3.4. Biomass and by-product yields

The yields of biomass and by-products on glucose were determined during the initial exponential growth phase (Tables 4.3 and 4.5). The biomass yield of the Y294 [Host] strain was significantly larger than the other recombinant strains. However, the differences in the biomass yields between the [PGK1-XYN] and [PGK1] strains, and the [ADH2] and [ADH2-XYN] strains, respectively, were less significant than the differences observed in the maximum specific growth rates between these strains. Though the difference between the [ADH2-XYN] and the [PGK1-XYN] strains during growth on glucose was not significant, the overall level of biomass formation for the [ADH2-XYN] was higher (Fig. 4.1B). The reduced significance of differences in the biomass yields (based on the statistical t-test) was attributed to inaccuracy in the estimation of biomass concentrations, leading to large variations in individual values between fermentations. Little difference was observed between the yields of ethanol and glycerol on glucose for individual strains whereas the values obtained for succinate and acetate were small and therefore not reliable (Appendix A, Table A.1).

Table 4.3. Yields on glucose in defined medium

Strain	Yield on glucose ($g_{product}{\cdot}g_{glucose}^{-1}$)		
	Biomass	Ethanol	Glycerol
Y294 [Host]	0.123 ±0.008	0.37 ±0.04	0.059 ±0.023
Y294 [PGK1]	0.114 ±0.007	0.40 ±0.02	0.061 ±0.009
Y294 [PGK1-XYN]	0.109 ±0.008	0.39 ±0.01	0.055 ±0.006
Y294 [ADH2]	0.110 ±0.004	0.37 ±0.02	0.063 ±0.007
Y294 [ADH2-XYN]	0.112 ±0.007	0.36 ±0.04	0.047 ±0.008

4.3.4. Rates of substrate consumption and product formation

The specific glucose consumption rate of the Y294 [Host] strain was compared to the other recombinant strains (Tables 4.4 and 4.5). The glucose consumption rates of the [PGK1] and [ADH2] strains were decreased compared to the Y294 [Host] strain. Further decreases in the maximum specific glucose consumption rates of the [PGK1-XYN] and [ADH2-XYN] strains, compared to the [PGK1] and [ADH2] strains, respectively, were also observed, though the specific glucose consumption rate of the [ADH2-XYN] was higher than the [PGK1-XYN] strain. The specific rates of ethanol formation for the recombinant strains were also compared (Table 4.4) and the differences between the strains were qualitatively similar to differences in the specific glucose consumption rates.

Table 4.4. Specific rates of glucose consumption and ethanol formation after 9 h in defined medium

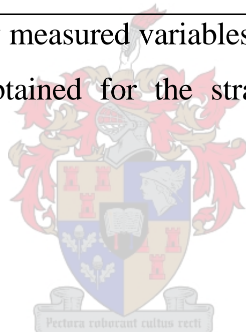
Strain	Specific glucose consumption rate ($g_{glucose}\cdot g_{biomass}^{-1}.h^{-1}$)	Specific ethanol formation rate ($g_{ethanol}\cdot g_{biomass}^{-}1.h^{-1}$)
Y294 [Host]	2.34 ±0.23	0.90 ±0.02
Y294 [PGK1]	2.10 ±0.04	0.86 ±0.04
Y294 [PGK1-XYN]	1.85 ±0.18	0.72 ±0.06
Y294 [ADH2]	2.19 ±0.15	0.86 ±0.07
Y294 [ADH2-XYN]	2.13 ±0.21	0.79 ±0.08

Table 4.5. Significance of differences in calculated average values (Tables 4.2 to 4.4) according to Student's T-test.

Difference tested between		Significance (%) **				
		μ_{max} on Glucose	μ_{max} on Ethanol	Biomass Yield	Ethanol Yield	Glucose Consumption Rate
[Host]	**[PGK1]**	93	95	80	82	85
[PGK1]	**[PGK1-XYN]**	99	95	*N. s.*	80	94
[Host]	**[PGK1-XYN]**	99.6	*N. s.*	93	*N. s.*	98
[Host]	**[ADH2]**	89	87	93	*N. s.*	*N. s.*
[ADH2]	**[ADH2-XYN]**	*N. s.*	97	*N. s.*	*N. s.*	*N. s.*
[Host]	**[ADH2-XYN]**	96	*N. s.*	87	*N. s.*	84
[PGK1]	**[ADH2]**	*N. s.*	96	*N. s.*	86	*N. s.*
[PGK1-XYN]	**[ADH2-XYN]**	94	*N. s.*	*N. s.*	*N. s.*	89

** For each of the experimentally measured variables, the significance of the difference between the average values, obtained for the strains mentioned on the left, was determined.

N. s. Not significant



4.4. DISCUSSION

Five recombinant *S. cerevisiae* strains were cultivated under identical conditions to quantify the molecular basis of the metabolic burden of heterologous gene expression, and to evaluate proposed mechanisms for the metabolic burden. The study was designed to quantitatively estimate the metabolic effect of different genes and differently regulated promoters in the heterologous expression system.

4.4.1. Plasmid replication

The maintenance and replication of multiple copies of the 2µm YEp352 plasmid by the Y294 [Host] strain did not affect the growth of the host organism significantly, as the maximum specific growth rates of the parental Y294 and Y294 [Host] strains were similar in complex medium (Appendix A, Table A.2). The effect of 2µm plasmid replication on growth was previously shown to be too small for experimental

observation (Mead et al., 1986; Van Hoek et al., 1998), though simulation studies had proposed a 1.5-3% reduction in the maximum specific growth rate due to plasmid replication in defined medium (Mead et al., 1986). Notions toward the effect of foreign DNA synthesis during plasmid replication on cellular activities were also disproved by similar growth rates and biomass yields for the [ADH2] and [ADH2-XYN] strains during growth on glucose.

4.4.2. Plasmid-based glycolytic promoter

Conversely to plasmid replication, the inclusion of either the *PGK1* or *ADH2* promoter on the recombinant plasmid introduced a significant metabolic burden on the host organism. The observed reductions in the maximum specific growth rate (12-15%), biomass yield on glucose (8-11%) and specific glucose consumption rate (6-10%) of the [PGK1] and [ADH2] strains, compared to the Y294 [Host] strain (Tables 4.2, 4.3 and 4.5), were quantitatively more significant than the effect of plasmid replication (Mead et al., 1986; Van Hoek et al., 1998). This effect of a plasmid-based promoter has not been reported in *S. cerevisiae* before.

As the metabolic effect of the plasmid-based *PGK1* and *ADH2* promoters was not influenced by the regulatory characteristics of the promoters, it might be attributed to the regulation of transcription, i.e. the synthesis of transcription machinery and subsequent binding to the promoter, from a large number of plasmid-based glycolytic promoters. The observed effect may also be caused by significant changes in the plasmid copy number and/or read-through transcription.

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4.4.3. Heterologous gene expression

The presence of the heterologous *XYN2* gene on the recombinant plasmid introduced a metabolic burden in the [PGK1-XYN] and [ADH2-XYN] strains only during active gene expression. During growth on glucose, the maximum specific growth rate (11%), biomass yield (4%) and specific glucose consumption rate (12%) of the [PGK1-XYN] strain was reduced (Tables 4.2, 4.3 and 4.4), whereas during growth on ethanol the maximum specific growth rate (14%) of the [ADH2-XYN] strain was decreased (Table 4.2); compared to the [PGK1] and [ADH2] strains. Similar reductions have been observed for other foreign gene expression systems in *S. cerevisiae* (Da Silva and Bailey, 1991; Dequin and Barre, 1994).

The metabolic burden of heterologous gene expression was disproportionally large with respect to the amount of heterologous protein produced, as also noted by Koch (1983). The energetic cost for the synthesis of recombinant protein to the equivalent of 0.2% cellular protein during growth on glucose could not directly be translated into the 11% reduction in the maximum specific growth rate of the [PGK1-XYN] strain (Table 4.2). Reduced production levels for heterologous gene expression, compared to homologous gene expression, have also been observed for production from the same expression system (Chen et al., 1984; Gopal et al., 1989), apparently due to a higher energetic demand for heterologous gene expression (Gopal et al., 1989).

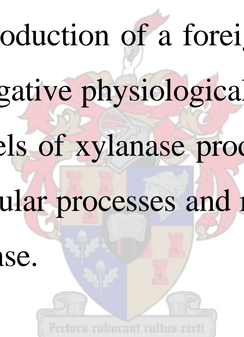
Alternatively, the "dilution" of native proteins (Snoep et al., 1995) may have been caused by a competition for available transcription factors during active heterologous gene expression, as the cells mostly secreted the heterologous xylanase. For the *ADH2* promoter the availability of the Adr1p transcription factor has become limiting when a large number of plasmid-based *ADH2* promoters were present in the cell, which subsequently reduced the expression levels of the chromosomal *ADH2* (Irani et al., 1987). As Adr1p is a limiting factor in *ADH2* transcription (Kramer et al., 1984; Price et al., 1990) the overexpression of *ADR1* has improved the expression level of the chromosomal *ADH2* (Irani et al., 1987) and has also increased the production levels of *ADH2*-regulated recombinant protein production (Price et al., 1990). Similarly, for the *PGK1* promoter, available evidence indicates that the transcription factor essential for the activation of the promoter, Gcr1p, is produced at extremely low levels (Baker, 1986; Huie and Baker, 1996). A limitation in the availability of essential transcription factors for both the *PGK1* and *ADH2* promoters may therefore influence the expression of the glycolytic enzymes and thereby affect the growth of the host organism. The disproportionally large effect of foreign gene expression may also be attributed to a competition for limiting amounts of translation factors, biosynthetic precursors or metabolic energy (Shuster, 1989; Janes et al., 1990; Van der Aar et al., 1992).

Heterologous xylanase production levels by the [ADH2-XYN] and [PGK1-XYN] strains during stationary phase were similar and were apparently not affected by the regulatory characteristics of the promoters. Continued heterologous protein production, during the post-exponential phase, both by expression systems containing the *PGK1*

and the *ADH2* promoter has been reported (Shuster, 1989; Price et al., 1990; Cartwright et al., 1994; Dickson and Brown, 1998). Conversely, the regulatory characteristics of both promoter systems, associated with their function in primary metabolism, were retained during the major growth phases (Shuster, 1989; Kingsman et al., 1990; Price et al., 1990; Romanos et al., 1992; Noronha et al., 1998). The observed metabolic burden of heterologous xylanase production was directly related to the induction of gene expression by either of these promoters. Results from this study have quantitatively justified the use of a promoter for heterologous protein production in *S. cerevisiae* that is not active during the major growth phase, based on a decreased metabolic burden during biomass formation.

4.5. CONCLUSIONS

The present study has confirmed the presence of a disproportional yeast response to heterologous xylanase production. This was associated with the ability of the cell to sense and respond to both the production of a foreign protein and the presence of the plasmid-based promoter. The negative physiological effect of foreign protein synthesis may also limit the attainable levels of xylanase production. Further investigation was required to identify the exact cellular processes and regulatory mechanisms involved in the observed physiological response.



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Chapter 5

TRANSCRIPTIONAL RESPONSE OF *S. CEREVISIAE* TO HETEROLOGOUS XYLANASE PRODUCTION

5.1. INTRODUCTION

Low production levels of heterologous proteins secreted by yeasts limit the advantages associated with the capacity of these hosts for posttranslational processing and secretion of foreign proteins (Hinnen et al., 1995). Posttranslational processing and secretion of foreign proteins allow for correctly folded proteins with full biological activity to be produced in the extracellular medium, thus simplifying purification of the product. Evidence of possible limitations in the production and secretion of a heterologous xylanase by *S. cerevisiae* was given by the presence of a "metabolic burden" on the host physiology due to foreign gene expression (Chapter 4). In the present chapter the major cellular processes and regulatory mechanisms associated with the observed physiological response were therefore identified, by using genome-wide transcriptional profiling. Further illumination of a global sensing and regulation mechanism for heterologous protein by yeast, representing a possible limitation to the attainable production levels, could thus be obtained. A potential relationship between the physiological response and the level of heterologous xylanase production also resulted in a general strategy for improving recombinant protein production.

Microarray technology allows for the quantification of the transcriptional response of cellular processes to a variety of environmental and genetic changes, such as high salinity (Yale and Bohnert, 2001; Posas et al., 2000), aerobic and anaerobic culture (Ter Linde et al., 1999), the diauxic shift (DeRisi et al., 1997), progression through the mitotic cell cycle (Cho et al., 1998; Chu et al., 1998), different levels of copper availability (Gross et al., 2000), rapamycin treatment (Shamji et al., 2000; Cardenas et al., 1999), amino acid starvation (Natarajan et al., 2001), treatment with an alkylating agent (Jelinsky and Samson, 1999), deletion of the *snf/swi* complex components, *SNF2* or *SWI1* (Sudarsanam et al., 2000), deletion of *CDC73* (Kerkmann and Lehming, 2001), deletion of the *GCR1* transcription factor (Lopez and Baker, 2000), reduced cell wall

integrity (Jung and Levin, 1999), aging (Lin et al., 2001), drug treatments and mutations affecting ergosterol synthesis (Bammert and Fostel, 2000; DeRisi et al., 2000) and long-term physiological adaptation (Ferea et al., 1999). The genes required for meiosis and spore formation (Rabitsch et al., 2001) and iron homeostasis (Yun et al., 2000; Foury and Talibi, 2001) have also been identified using genome-wide transcription profiling, whereas the function of several unclassified genes has been identified (Hughes et al., 2000). Microarray technology has also been used to investigate recombinant protein production by *E. coli*, where significant changes in the expression of glycolytic and pentose phosphate pathways, biosynthesis, respiration, and heat shock and chaperone genes were observed (Oh and Liao, 2000; Gill et al., 2000; Gill et al., 2001).

In the present investigation, the transcriptional response of *S. cerevisiae* to individual genetic components in a model expression system for heterologous xylanase production was quantified. The *T. reesei* ß-1,4-xylanase II encoding gene, *XYN2*, was expressed from a multicopy, 2µm plasmid under regulation of the yeast glycolytic phosphoglyceratekinase (*PGK1)* promoter (La Grange et al., 1996), which is maximally induced in aerobic batch culture by the presence of glucose (Shuster, 1989; Chambers et al., 1989; Kingsman et al., 1990; Romanos et al., 1992; Hauf et al., 2000). The xylanase-producing strain was compared to two references strains, where either the heterologous *XYN2* gene, or the heterologous gene and the promoter and terminator sequences were omitted from the recombinant plasmid. As the produced xylanase had no known catalytic function in yeast metabolism and was secreted efficiently by the recombinant strains, the transcriptional response associated with both the presence of a large number of plasmid-based glycolytic *PGK1* promoters and the expression of heterologous *XYN2* gene under control of the *PGK1* promoter from the 2µm plasmid (Chapter 4), was strictly related to the presence and activity of the recombinant expression system (Van der Aar et al., 1990a; Van der Aar et al., 1990b; Van Hoek et al., 1998). Stable maintenance of the recombinant plasmids in non-selective cultivation media was ensured through the inclusion of the *fur1 ura3* autoselective system in the recombinant strains (Loison et al., 1986).

5.2. MATERIALS AND METHODS

5.2.1. Strains and plasmids

The *S. cerevisiae* strains selected for this study are presented in Table 5.1. For the construction of autoselective strains the method of Loison et al. (1986) was used (La Grange et al., 1996). The strain stocks were stored at –80ºC in a 15% glycerol solution.

Table 5.1. Strains used in this study

Strain	**Genotype**			**Source**
S. cerevisiae Y294	*ura3, leu2, trp1,his3*			La Grange et al. (1996)
	Plasmid	**Promoter**	**Gene**	
Y294 [Host]*	*YEp352*	-	-	Chapter 4
Y294 [PGK1]*	*pJC1*	*PGK1*	-	Crous et al. (1995)
Y294 [PGK1-XYN]*	*pDLG6*	*PGK1*	*XYN2*	La Grange et al. (1996)

* [ ] Indicates the content of the recombinant plasmid.

5.2.2. Medium, inoculum and fermentations

Fully aerobic batch fermentations were conducted in a chemically defined medium (Verduyn et al., 1992) supplemented with the amino acids [histidine (165 mg.l^{-1}), leucine (870 mg.l^{-1}), tryptophan (664 mg.l^{-1}), aspartate (257 mg.l^{-1}), glutamate (64 mg.l^{-1}), glycine (33 mg.l^{-1}) and serine (108 mg.l^{-1})], as described previously (Chapter 4). Cultures were inoculated to a low cell density using a 5 ml pre-culture, containing cells in the late-exponential phase, which improves the reproducibility of batch fermentations (A. Eliasson, personal communication). Cell densities in all cultures were estimated as optical density (absorbance) measurements at 620 nm (A_{620}) with a Hitachi U-1100 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The fermentor was controlled at 30ºC and pH 5.0.

5.2.3. Analytical methods

Cultures were sampled regularly and the concentrations of glucose and ethanol, and extracellular xylanase activity determined as previously (Chapter 4). Cell samples for the characterisation of the transcriptional profile were taken during mid-exponential growth, when the cell density reached an absorbance (620 nm) of 5.0. 75 ml of fermentation broth was rapidly cooled by adding approximately 40 ml of ice,

centrifuged promptly and washed twice with ice-cold sodium-acetate (NaAc) buffer (Schmitt et al., 1990). The final, concentrated sample was suspended in NaAc buffer, flash-frozen and stored at –80ºC until analysis.

5.2.4. Quantification of transcriptional response

The transcriptional profiles of individual recombinant strains were quantified using the Affymetrix GeneChip system for *S. cerevisiae*. Total yeast RNA was isolated in triplicate according to Schmitt et al. (1990) and these triplicates were pooled according to strains, followed by mRNA isolation using the Qiagen mRNA Kit. Subsequent steps in the preparation of a hybridisation mixture were performed according to the manufacturer's protocol, and checked using gel electrophoresis. The fermentation, sample collection and preparation, and hybridisation steps were done in duplicate for the [PGK1-XYN] strain, whereas the procedure was completed once for the reference strains.

5.2.5. Data processing

Data sets obtained from the scanning of GeneChips were compared using the Affymetrix software package, whereby comparisons of the Y294 [PGK1-XYN] and Y294 [PGK1] strains to the reference host strain (Y294 [Host]) could be obtained. For the [PGK1-XYN] strain, the geometric average of the duplicate comparisons to the [PGK1] and [Host] strains, obtained from individual hybridisations, were calculated. All of the changes in the expression of genes in the [PGK1-XYN] strain mentioned in this report were reproducible between the two hybridisations.

5.3. RESULTS

The genome-wide transcriptional profile of a recombinant *S. cerevisiae* strain (Y294 [PGK1-XYN]), producing a heterologous xylanase from a 2µm plasmid-based expression system controlled by the *PGK1* promoter, was generated using Affymetrix microarray technology. The transcriptional profile was compared to the profiles of two reference strains where the heterologous *XYN2* gene (Y294 [PGK1]) or both the heterologous gene and the *PGK1* promoter and terminator (Y294 [Host]) were omitted from the recombinant plasmid (Table 5.1).

5.3.1. Aerobic batch fermentations

The reproducibility of aerobic batch fermentations and physiological differences between these strains were previously demonstrated (Chapter 4), with similar trends in the maximum specific growth rates observed during the present cultures (Table 5.2). Typical patterns of substrate-consumption, and biomass- and product-formation, during batch cultivation on glucose are presented in Figure 5.1. Samples for transcriptional profiling were rapidly removed when the cell density (Absorbance) in the fermentor reached A_{620} = 5.0, which corresponded to mid-exponential growth [A final cell density of $A_{620} \approx 12$ was normally reached during growth on glucose (Chapter 4)].

Table 5.2 – Physiological Comparison of Recombinant Strains

	Maximum Specific Growth Rate[a], μ_{max}, [h^{-1}]	**Glucose Uptake Rate[b] [$g_{glucose} \cdot g_{biomass} \cdot h^{-1}$]**
Y294 [Host]	0.43	2.34 ±0.23
Y294 [PGK1]	0.36	2.10 ±0.04
Y294 [PGK1-XYN]	0.29	1.85 ±0.18

[a] Present study. Similar trend was observed in Chapter 4

[b] Chapter 4

5.3.2. Comparison of transcriptional profiles

Clustering of genes according to similar transcriptional responses and expression patterns has shown that genes with similar functionality are usually co-regulated (DeRisi et al., 1997; Eisen et al., 1998; Gasch et al., 2000), allowing the global analysis of transcription data on the basis of cumulative changes in gene expression in broad functional families (Nau et al., 2000). In the present study a total of 1014 genes showing a significant change in their expression level were sorted into functional groups according to the MIPS classification (Mewes et al., 2000), and the individual *S. cerevisiae* strains compared on the basis of general trends in the major cellular processes/functions (Table 5.3).

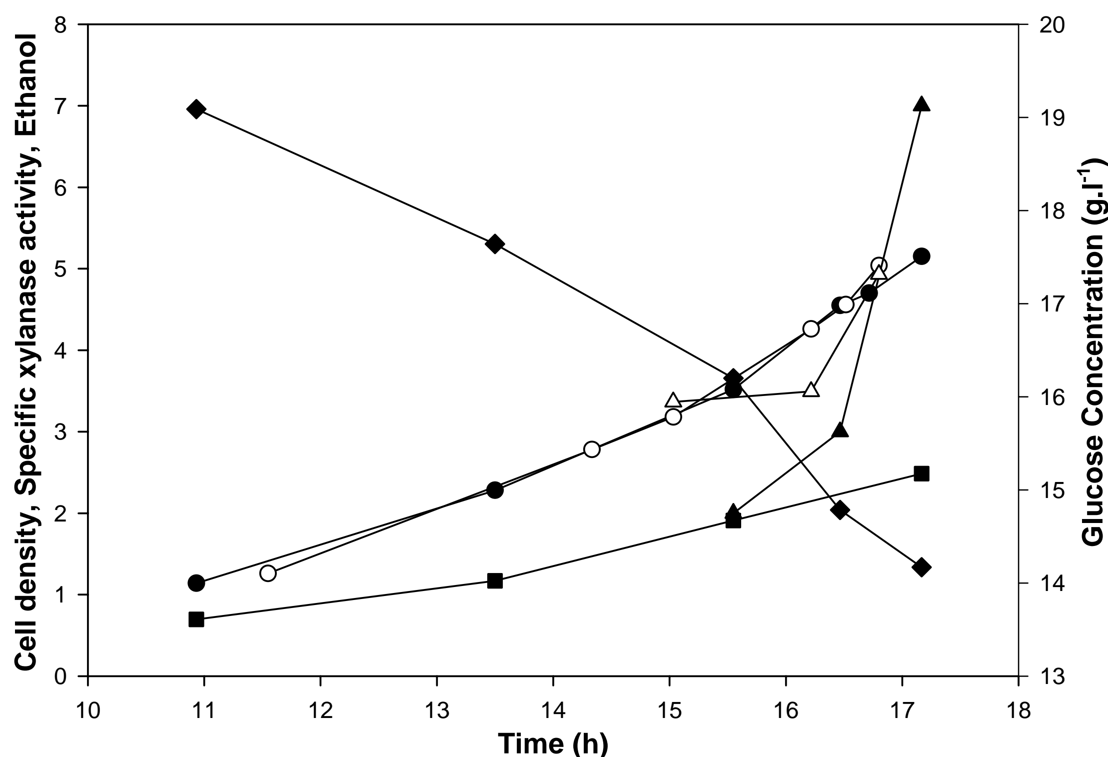

Figure 5.1. Aerobic batch cultivation of the [PGK1-XYN] strain in defined medium prior to final sampling for transcriptional profiling. (●,O) Cell density in absorbance units (620 nm), (▲,△) specific xylanase activity, (♦) glucose concentration ($g.l^{-1}$) and (■) ethanol concentration ($g.l^{-1}$). Open symbols indicate duplicate fermentations

5.3.2.1. Significant changes in transcription level

The significance of changes in transcription level was assessed using two criteria. Primary selection of genes was based solely on the fold-change in expression level, with changes larger than two-fold considered as significant (as suggested by Affymetrix). However, fold-changes larger than two were observed almost exclusively for genes with low expression levels, which do not fulfil central functions in metabolism. The tighter regulation of the more central genes in metabolism, which also had higher expression levels, apparently resulted in much smaller fold-changes. To include the latter types of genes in the analysis, the significance of smaller changes in mRNA levels were evaluated on the basis of a "Significance factor", defined as the [(Absolute value of fold change)-1] multiplied by the average expression level of the gene between two strains. A minimum value of 100 for the Significance factor was chosen for this secondary selection criterion, as this represented an inflection point in

the plot of Number of genes selected versus Minimum "Significance factor" (Fig. 5.2). The number of selected genes increased exponentially when a criteria below 100 was used, indicating the selection of the numerous genes in yeast with low expression levels. The inflection point in Figure 5.2 at a Significance factor of 100 thus represented the transition from highly expressed genes with small fold-changes, to genes with low expression levels. The secondary selection was therefore limited to more highly expressed genes.

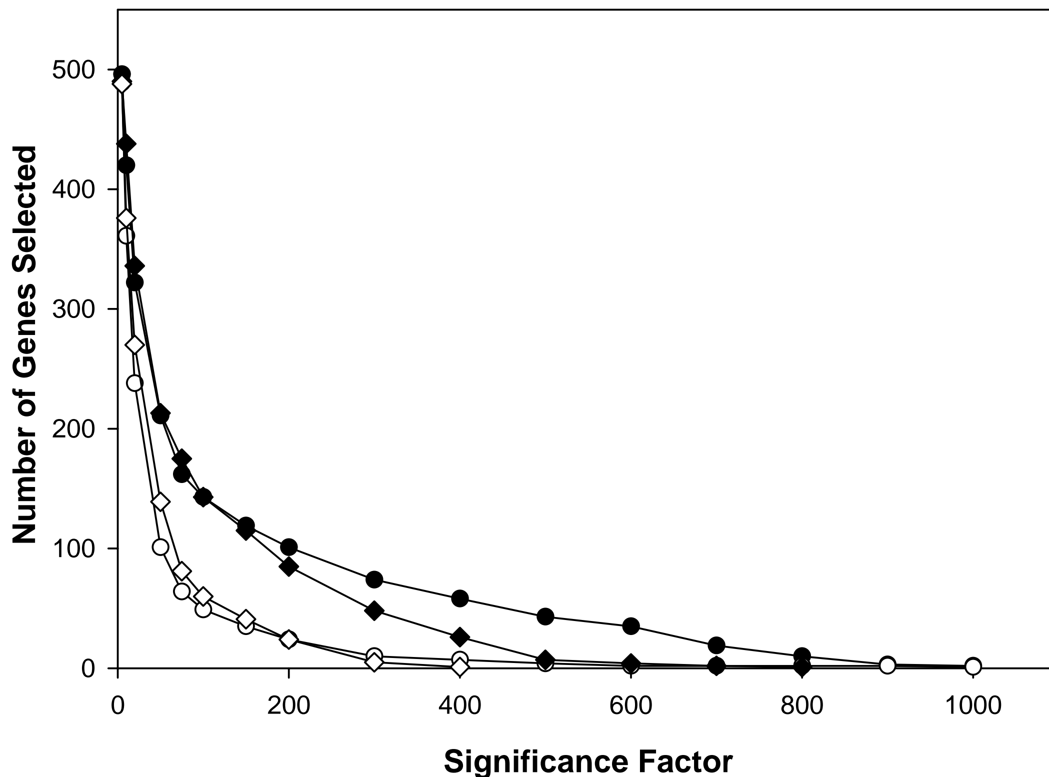

Figure 5.2. Number of genes in each strain-comparison for which the change in expression level satisfied a "Minimum Significance Factor" criterion. (•,o) Y294 [PGK1-XYN] vs. Y294 [PGK1] and (◆,◇) Y294 [PGK1-XYN] vs. Y294 [Host]. Closed symbols are genes of known function, and open symbols are genes of unknown function.

Overall changes in the individual functional categories were quantified by comparing the sum-totals of both the number of genes up- or down-regulated and the sum-totals of the "Significance factors," as presented in Table 5.3. The "Overall changes" in Table 5.3 represent the total number of genes with significant changes in expression level in

each functional group, either in the [PGK1] or [PGK1-XYN] strain. The "Magnitude of … changes" represents specific data for either the [PGK1] or [PGK1-XYN] strain, with the sum totals for up-regulation ("UP") and down-regulation ("DOWN") presented separately. Both the total number of genes with changed expression level ("# ORFs") and the sum-total of the "Significance factors" ("Σ Significance") are presented. For the summation based on the "Significance factor," negative values were assigned to the genes that were down-regulated, resulting in the negative values associated with the "Σ Significance" total for down-regulated groups.

In some functional categories, several changes in the expression of genes were observed without any clear overall trend. This "shuffling around" of gene expression was associated with the large amount of redundancy present in metabolism, by which it obtains robustness (Cornish-Bowden and Cardenas, 2000). Differential expression of isoenzymes, possibly due to differences in the properties of these enzymes, is also frequently observed in response to environmental changes (Gasch et al., 2000).

5.3.3. Cellular processes affected by heterologous gene expression

The cellular functions most strongly affected by changes in the content of the recombinant plasmids were: Metabolism (mostly amino acids and ammonium), energy conservation (glycolysis, respiration, TCA cycle), protein synthesis (ribosome biogenesis, translation and transcription), transport facilitation (mechanisms, ionic and amino acid transport, and cellular import), and cell rescue and virulence. The presentation of the overall changes in cellular processes is based mostly on the results presented in Table 5.3, though conclusions were also confirmed by the original data presented in Appendix D. Further data on changes in the expression of individual genes, obtained mostly from the results presented in Appendix D, are discussed in some of the functional categories to confirm the extent of transcriptional changes.

Table 5.3. Cellular processes significantly changed in the [PGK1] or [PGK1-XYN] strains

Cellular Process / Function	Overall Changes[a]			Magnitude of [PGK1-XYN] Changes				Magnitude of [PGK1] Changes			
	# ORFs		% ORFs	# ORFs		Σ Significance		# ORFs		Σ Significance	
	Changed	Total	Changed	UP	DOWN	UP	DOWN	UP	DOWN	UP	DOWN
A. METABOLISM	360	1219	30	154	136	290358	-13861	122	66	65825	-3606
A.1. Amino acid metabolism	89	215	41	60	13	274678	-4029	53	5	62569	-729
Amino acid biosynthesis	53	119	45	31	10	29088	-2583	31	3	12533	-436
Regulation of AA metabolism	8	33	24	6	2	7236	-286	2	2	1177	-294
AA Transport	14	32	44	13	0	233410	0	9	0	46586	0
AA Degradation	14	35	40	10	2	4944	-1161	11	0	2273	0
A.2. Nitrogen and sulphur utilisation	22	67	33	7	9	3736	-2607	7	2	1493	-105
A.3. Nucleotide metabolism	52	155	34	10	32	4053	-7225	10	18	1763	-2772
Purine ribonucleotide metabolism	23	45	51	2	15	566	-2796	8	5	601	-1200
Pyrimidine ribonucleotide metabolism	10	29	34	4	6	11155	-1942	0	5	0	-603
Deoxyribonucleotide metabolism	6	11	55	2	4	40	-685	1	2	104	-351
B. ENERGY CONSERVATION	99	264	38	45	32	16813	-9447	27	29	9267	-4725
B.1. Glycolysis	17	35	49	1	14	137	-4381	10	4	4661	-724
B.2. Pentose-phosphate pathway	7	9	78	2	3	99	-500	2	1	219	-47
B.3. Tricarboxylic-acid pathway (Krebs cycle, TCA cycle)	10	25	40	6	2	914	-741	0	4	0	-600
B.4. Respiration	39	92	42	25	3	4239	-514	4	13	852	-2139
B.5. Fermentation	9	33	27	4	4	10283	-1429	6	2	3432	-274
B.6. Glyoxylate cycle	3	6	50	1	2	310	-1105	0	2	0	-319

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Table 5.3. Cellular processes significantly changed in the [PGK1] or [PGK1-XYN] strains (continued)

Cellular Process / Function	**Overall Changes[a]**			**Magnitude of [PGK1-XYN] Changes**				**Magnitude of [PGK1] Changes**			
	# ORFs		**% ORFs**	**# ORFs**		**Σ Significance**		**# ORFs**		**Σ Significance**	
	Changed	**Total**	**Changed**	**UP**	**DOWN**	**UP**	**DOWN**	**UP**	**DOWN**	**UP**	**DOWN**
C. TRANSCRIPTION, PROTEIN SYNTHESIS AND FATE	408	1895	22	122	234	19883	30854	178	60	49955	935
C.1. Transcription	120	861	14	53	39	6126	6028	39	16	5579	1651
rRNA transcription	22	113	19	8	7	1061	989	10	1	1578	114
tRNA transcription	11	84	13	4	5	373	641	4	1	381	142
mRNA transcription	79	580	14	34	26	3469	4053	25	14	3240	1395
C.2. Protein Synthesis	175	372	47	23	145	6424	36793	115	18	39257	2810
Ribosome biogenesis	135	223	61	15	85	4671	27583	85	7	33458	1600
Translation	22	64	34	3	16	492	3643	6	5	1704	614
Aminoacyl-tRNA-synthetases	12	37	32	2	7	537	1177	4	2	719	316
tRNA Expression				0	36	0	4077	19	2	2898	24
C.3. Protein Fate (folding, modification, destination)	113	662	17	46	50	7333	11967	24	26	5120	5396
Protein folding and stabilization	15	60	25	6	6	972	1890	1	5	360	1318
Protein targeting, sorting and translocation	21	147	14	7	12	1157	3369	6	6	1148	1675
Protein modification	29	188	15	14	10	2656	1548	8	2	1312	306
Assembly of protein complexes	20	95	21	6	12	914	3345	4	7	1356	1287
Proteolytic degradation	20	164	12	12	9	1453	1573	5	6	943	810

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Table 5.3. Cellular processes significantly changed in the [PGK1] or [PGK1-XYN] strains (continued)

Cellular Process / Function	**Overall Changes[a]**			**Magnitude of [PGK1-XYN] Changes**				**Magnitude of [PGK1] Changes**			
	# ORFs		**% ORFs**	**# ORFs**		**Σ Significance**		**# ORFs**		**Σ Significance**	
	Changed	**Total**	**Changed**	**UP**	**DOWN**	**UP**	**DOWN**	**UP**	**DOWN**	**UP**	**DOWN**
D. CELLULAR TRANSPORT	302	1116	27	154	190	496554	29292	120	85	104349	9919
D.1. Intracellular Transport and Transport	112	541	21	43	50	222095	12220	36	22	46741	4431
Nuclear transport	6	59	10	1	4	107	926	1	1	132	867
Mitochondrial transport	27	81	33	13	5	2572	1323	7	10	1196	1666
Vesicular transport (Golgi network, etc.)	21	129	16	6	13	575	3214	5	3	568	537
Vacuolar transport	16	56	29	4	12	732	3157	3	4	641	610
Cellular import	29	101	29	12	12	218109	3600	15	2	44205	751
D.2. Transport Facilitation/ Interaction with Environment	190	575	33	111	140	274459	17073	84	63	57608	5488
D.2.1. Ionic homeostasis	44	137	32	16	19	3943	4412	11	11	2036	1734
Homeostasis of cations	40	123	33	14	19	3539	4412	9	10	1760	1582
Homeostasis of metal ions (Na, K, Ca etc.)	*20*	*63*	*32*	*13*	*8*	*3299*	*1547*	*3*	*1*	*444*	*168*
Homeostasis of protons	*19*	*36*	*53*	*1*	*10*	*241*	*2865*	*4*	*9*	*1200*	*1414*
D.2.2. Facilitation of Ion Transport	44	86	51	90	115	270515	12660	71	45	55572	3754
Cation transporters	34	65	52	12	20	15733	5545	5	5	1816	1038
Heavy metal ion transporters	*14*	*25*	*56*	*10*	*4*	*15419*	*684*	*1*	*1*	*112*	*168*
Other cation transporters (Na, K, Ca , NH4, etc.)	*19*	*39*	*49*	*1*	*16*	*215*	*4861*	*4*	*4*	*1704*	*871*
Anion transporters (Cl, SO4, PO4, etc.)	10	21	48	6	2	1194	1240	3	1	353	413
D.2.3. Amino acid transporters	14	25	56	12	1	233362	109	11	0	46770	0
D.2.4. Drug transporters	10	35	29	9	1	9154	167	6	2	1119	121
D.2.5. Transport mechanism	29	74	39	4	26	1041	3784	6	11	2027	1513
Transport ATPases	23	45	51	2	13	376	3411	7	9	1830	1414
ABC transporters	6	28	21	2	13	665	373	1	2	198	99

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Table 5.3. Cellular processes significantly changed in the [PGK1] or [PGK1-XYN] strains (continued)

Cellular Process / Function	**Overall Changes[a]**			**Magnitude of [PGK1-XYN] Changes**				**Magnitude of [PGK1] Changes**			
	# ORFs		**% ORFs**	**# ORFs**		**Σ Significance**		**# ORFs**		**Σ Significance**	
	Changed	**Total**	**Changed**	**UP**	**DOWN**	**UP**	**DOWN**	**UP**	**DOWN**	**UP**	**DOWN**
E. CELL RESCUE, DEFENSE AND VIRULENCE	79	291	27	42	19	36274	4008	24	18	10628	2951
E.1. Stress response	50	176	28	25	12	25207	2561	14	14	8208	2682
E.2. Detoxification	28	105	27	17	7	11052	1447	10	4	2421	269
F. CELL FATE, DNA PROCESSING AND CELL CYCLE	191	1347	14	80	64	23738	15073	45	57	13469	7896
F.1. Cell Fate	100	558	18	42	33	8732	8158	32	29	5963	4461
Cell growth / morphogenesis	17	96	18	6	9	1393	2012	3	7	692	1390
(Fungal) Cell differentiation	78	448	17	34	27	6687	6146	27	20	4711	2914
F.2. DNA Processing and Cell Cycle	91	789	12	38	31	15006	6915	13	28	7506	3435
DNA processing	26	281	9	6	15	304	2188	1	8	305	1174
Cell cycle	63	502	13	30	16	4915	4828	11	20	3694	2261
Mitotic cell cycle and cell cycle control	*38*	*356*	*11*	*18*	*11*	*3060*	*3704*	*9*	*12*	*3592*	*1803*
Meiosis	*19*	*108*	*18*	*11*	*3*	*1625*	625	*1*	*6*	*103*	459

[a] Indicates the total number of genes with significant changes in expression levels, either in the [PGK1] or the [PGK1-XYN] strain.

Pectora roborant cultus recti

5.3.3.1. Metabolism

Gene expression for various processes in amino acid metabolism (biosynthesis, transport and degradation) increased strongly in both the [PGK1-XYN] and [PGK1] strains, with 45% of the genes in amino acid biosynthesis affected. Several changes in the utilisation of nitrogenous compounds were also observed. Though extracellular nitrogen availability was identical during the cultivation of the individual strains, differences in the physiology of the strain can lead to different transcriptional outputs using the same nitrogen sources (Ter Schure et al., 2000).

Aspartate biosynthetic family (Asp, Asn, Thr, Met, Ile)

In Y294 [PGK1] the availability of asparagine and methionine was apparently limited. Expression of the genes for the biosynthesis of asparagine (*ASN1*, *ASN2*) and methionine (*MET6* and *MET17*) were up-regulated, along with methionine permease (*MUP1*), S-adenosylmethionine (AdoMet, SAM) supply (*SAM1*, *SAM2* and *SAM4*), sulphate uptake and sulphur transfer. This could be due to decreased intracellular levels of S-adenosylmethionine (AdoMet, SAM), induction by the general amino acid control (*GCN4*) system or induction by the Ssy1p extracellular amino acid sensor (Hinnebusch, 1992; Forsberg et al., 2001).

In Y294 [PGK1-XYN] the systems for asparagine synthesis, sulphur transfer and AdoMet supply were additionally up-regulated (*ASN1, ASN2*, *MUP1*, *SAM4* and the genes for sulphate uptake and transfer). However, methionine biosynthesis was down-regulated (*MET14, MET3, MET6*), probably due to a sufficient supply thereof (Hinnebusch, 1992), or an improper compartmentalisation of AdoMet to the vacuole where it is normally stored (Forsberg et al., 2001). Decreased expression of thioredoxin reductase (*TRR1*) also indicated a reduction in sulphur utilisation towards methionine synthesis by the [PGK1-XYN] strain.

Glutamate biosynthetic family (Glu, Gln, Pro, Arg, Lys)

An apparent increase in the requirement for arginine in both the [PGK1] and [PGK1-XYN] strains increased the expression of *ARG1* (both strains), and *ARG5,6* and *CPA2* ([PGK1-XYN] only) (Hilger et al., 1973; Hinnebusch, 1992). Expression of *ARG1* is also up-regulated by Ssy1p (Forsberg et al., 2001), whereas *CPA2* expression is regulated solely by the *GCN4*-system (Messenguy, 1979). *PRO3* expression, required for proline synthesis and arginine degradation (Jones and Fink, 1982), was down-

regulated in both the [PGK1] and [PGK1-XYN] strains, though more severely in the latter.

Gene expression for lysine biosynthesis increased more severely in the [PGK1] strain (*LYS1, LYS9, LYS12, LYS20* expression increased) than in the [PGK1-XYN] strain (*LYS1, LYS20* expression increased). All of the genes involved in lysine biosynthesis are regulated by the general amino acid control (Gcn4p) (Hinnebusch, 1992; Natarajan et al., 2001), though expression of *LYS20* and *LYS9* is also up-regulated in response to Ssy1p (Forsberg et al., 2001). Changes in the expression of *GLT1*, *GLN1*, *GDH1* and *GDH2* related strongly to the utilisation of ammonium as nitrogen source (discussed below).

Aromatic biosynthetic family (Phe, Tyr, Trp)

Genes in tryptophan biosynthesis (*TRP1*, *TRP3*, *TRP4*, *TRP5*) and the precursor pathway (*ARO3*) were up-regulated in both the [PGK1] and [PGK1-XYN] strains, with an additional increase in expression in the latter. Biosynthesis of the enzymes required for the synthesis of aromatic amino acids is mostly controlled at transcriptional level, with the expression of *ARO3*, *TRP3*, *TRP4* and *TRP5* regulated by the Gcn4p activator (Braus, 1991). Both strains apparently experienced a mild increase in the requirement for amino acids, as the expression level of *ARO3* was not increased under conditions of severe intracellular amino acid limitation (Paravicini et al., 1989).

Serine biosynthetic family (Ser, Gly, Cys)

Expression of genes for cysteine uptake (*MUP1*), glycine biosynthesis (*GLY1*), serine degradation (*SRY1*, *SDL1*, *CHA1*) and glycine degradation (*GCV1*, *GLY1*) was increased, with higher expression levels in the [PGK1-XYN] than in the [PGK1] strain.

Pyruvate biosynthetic family (Ala, Val, Leu)

The biosynthetic genes *LEU2* and *LEU4*, as well as several of the *ILV* genes were up-regulated in both the [PGK1] and [PGK1-XYN] strains, with higher expression levels in the latter. Gene expression for branched chain amino acid synthesis is repressed in response to leucine availability (Forsberg et al., 2001). The biosynthesis of leucine, valine and isoleucine, also respond to a general amino acid limitation, confirmed by patterns of *LEU4* expression (Natarajan et al., 2001; Hinnebusch, 1992).

Histidine biosynthesis (His)

Three genes for histidine biosynthesis were up-regulated (*HIS3*, *HIS4*, *HIS5*) to approximately equal levels in the [PGK1-XYN] and [PGK1] strains. Due to the absence of histidine-specific transcriptional repression in yeast, these inductions were solely due to the action of the *GCN4*-transcriptional activator (Natarajan et al., 2001).

Amino acid and ammonium transport

An apparent increase in the requirement for amino acid uptake caused a strong increase in the expression of the permeases for Val, Cys, Leu, Ile, Asp, Glu, Met, Gln and Tyr in both Y294 [PGK1] and Y294 [PGK1-XYN], though more severely in the latter. Expression of the general *AGP3* and *LYP1* lysine permeases were also up-regulated, though to similar levels in the [PGK1-XYN] and [PGK1] strains. In Y294 [PGK1-XYN] additional increases in the expression of the permeases for tryptophan (*TAT2*), amino acids in general (*AGP1*, *AGP2*) and sulphur amino acids (*MMP1*) were also observed. Since uptake systems are generally active only when their substrates are both available in the medium and useful to the cell, the increased permease expression was probably aimed at increasing the uptake of amino acids from the medium (Horák, 1997; Sophianopoulou and Diallinas, 1995; Grenson, 1992; Eddy, 1980). The intracellular availability of either individual amino acids or amino acids in general may have been limiting, since several of the amino acid permeases (*AGP1*, *BAP2*, *BAP3*, *PTR2*, *DIP5*, *TAT1*, *TAT2*, *GNP1*, *CAR1*) are co-ordinately regulated via the Ssy1p transcriptional factor (Klasson et al., 1999; Regenberg et al., 1999; Forsberg et al., 2001; Bernard and Andre, 2001). The very strong increase in the expression of the permeases for branched-chain amino acids (*BAP2*, *BAP3* and *TAT1*) indicated the potential utilisation of the excess of leucine supplied in the medium. High concentrations of a particular amino acid usually results in it being taken up, deaminated and secreted as fusel oil (Cooper, 1982; Grenson, 1992).

A significant down-regulation of the lower affinity, high capacity (*MEP1*) and high affinity, low capacity (*MEP2*) ammonia transport systems were also observed in the [PGK1-XYN] strain, whereas only the expression of *MEP2* was slightly down-regulated in Y294 [PGK1]. Both permeases are repressed in the presence of a good nitrogen source (Marini et al., 1997), whereas *MEP2* is also involved in the response to ammonium limitation (Lorenz and Heitman, 1998). In the [PGK1-XYN] strain the coordination of increased amino permease expression with a deliberate decrease in

ammonium uptake corresponded to the repression of amino acid transport by ammonium ions (Horák, 1986; Slaughter et al., 1990; Horák, 1997). Amino acid-induced signals may cross-talk with signals derived from sensors monitoring other nitrogen sources, to co-ordinate gene expression in response to nutrient availability (Forsberg et al., 2001).

General regulation of amino acid metabolism

Gene expression for both the biosynthesis and uptake of amino acids was increased in the [PGK1] and [PGK1-XYN] strains, though more severely in the latter. The expression of several important activators of amino acid biosynthesis was increased, including *ARO9* and *SSY1* ([PGK1] and [PGK1-XYN]), and *ARG80*, *ARO8*, *MET28* and *MCM*1 ([PGK1-XYN] only). The expression of a significant fraction (30/36) of a selection of genes known to be controlled by the *GCN4*-system, was increased in the [PGK1] strain, whereas the fraction of up-regulated genes was smaller (22/36) in the [PGK1-XYN] strain, though still significant. The level of *GCN4* transcripts was unaffected.

Nitrogen utilisation

Gene expression for ammonium utilisation was strongly altered in the [PGK1-XYN] strain, as the expression of *GDH1* (NADP-specific glutamate dehydrogenase) was down-regulated whilst *GDH2* (NAD-specific glutamate dehydrogenase) expression was up-regulated. Expression of the ammonium permeases (*MEP1* and *MEP2*) was also decreased. Decreased *GDH1* activity has been associated with carbon or nitrogen starvation (Cooper, 1982; Gancedo and Serrano, 1989). *GDH2* expression is also derepressed during nitrogen limitation (Cooper, 1982; Coschigano et al., 1991), or carbon limitation (See below; Donnini et al., 1990; Coschigano et al., 1991; Ter Schure et al., 2000). Maximal *GDH2* derepression also required the catabolic utilisation of leucine, serine or valine when ammonia was the primary nitrogen source (Coschigano et al., 1991; Forsberg et al., 2001). Catabolic leucine utilisation was observed during chemostat cultivation of an isogenic strain in an identical defined medium (Chapter 6).

Decreased *GDH1* expression is also related to the TCA cycle and respiration via its control by the *HAP* system, which is also required for mitochondrial biosynthesis (Dang et al., 1996; DeLuna et al., 2001; De Winde and Grivell, 1995) and the activation of respiration, e.g. during the diauxic shift (Bourgarel et al., 1999) when *GDH1* expression

decreases (Dang et al., 1996). The combined decrease in the expression of *GDH1* and *ACO1* (TCA cycle) may also indicate an overall decrease in the synthesis of glutamate, for which α-ketoglutarate is required (Dang et al., 1996; Forsburg and Guarente, 1989; Liu and Butow, 1999; DeLuna et al., 2001). *GDH1* expression is also reduced in the presence of leucine due to its relation with branched-chain amino acid synthesis via the Leu3p transcription factor (Dang et al., 1996), which corresponds to the increased uptake of leucine from the medium (see above). *GDH2* does not have a function in carbon metabolism, and expression is not affected by the *HAP* system (Coschigano et al., 1991; Dang et al., 1996).

Up-regulation of *GDH2* expression in the presence of decreased *GDH1* expression in the [PGK1-XYN] strain may thus have been caused by an increase in leucine uptake (Dang et al., 1996; Coschigano et al., 1991; Forsberg et al., 2001), an excess of NADH in the cell, a limitation in the availability of NADPH for biosynthesis, or a decrease in ammonium uptake. Overexpression of *GDH2* in a Δ*gdh1* strain will restore the efficiency of ammonium utilisation to that of the parental strain (Roon et al., 1974; Miller and Magasanik, 1990; Nissen et al., 2000), and will increase the rate of NADH utilisation, the availability of NADPH and the biomass yield of the strain (Nissen et al., 2000). Such a strategy may have balanced an increase in NADH production in the cell due to the up-regulation of amino acid biosynthesis in the [PGK1-XYN] strain (Albers, 2000). Alternatively, the decreased expression of the *MEP* ammonium permeases may have mimicked an increase in the extracellular ammonium concentration, which elicited very similar changes in *GDH* expression during continuous cultivation (Ter Schure et al., 1995; Ter Schure et al., 2000). The down-regulation of *GLT1*/*GLN1* expression in [PGK1-XYN] will also decrease ATP-consumption during ammonium utilisation. *GDH* and *GLN1*/*GLT1* activity may therefore have been varied to tune the redox balance in the cell (Albers, 2000), as no transhydrogenase activity exists in yeast (Gancedo and Serrano, 1989).

Arginase (*CAR1*) expression was increased in both the [PGK1] and [PGK1-XYN] strains (more severely in the latter). This has been associated with nitrogen limitation (Dubois and Messenguy, 1997), or an increase in the availability of either arginine (Klasson et al., 1999) or micromolar concentrations of a variety of amino acids (Dubois and Wiame, 1976) in the cell. Arginine, together with allantoin and allantoate, are present at high concentrations (1-10 mM) in the vacuole, and may be released during

amino acid limitation, causing the induction of cytosolic arginase expression (Cooper, 1982; Davis, 1986). *DAL1* and *DAL2* expression for allantoin degradation (Rai et al., 1999; Cooper, 1982) was also increased in Y294 [PGK1].

Nucleotide metabolism

Gene expression for nucleotide biosynthesis was decreased in both the [PGK1-XYN] and [PGK1] strains, which corresponded to the observed decreases in growth rate. The expression of *URA3* (present on the recombinant plasmid) for pyrimidine biosynthesis was decreased in Y294 [PGK1] and Y294 [PGK1-XYN], though more severely in the latter and in combination with reduced *URA5* expression. The apparent limitation in pyrimidine availability in Y294 [PGK1-XYN] was complimented by a strong up-regulation of the genes for the supply of uracil (*FUR1*, *FUI1*, *FUR4* and *URA2*) (Grenson, 1992; Reece, 2000).

5.3.3.2. Energy Conservation

The conservation of energy was significantly changed in both the [PGK1-XYN] and [PGK1] strains, as the rate of glucose uptake was decreased (Chapter 4). The observed physiological change was reflected on a transcriptional level by significant changes in glycolysis and the pentose phosphate pathway (strongly up-regulated in [PGK1]; strongly down-regulated in [PGK1-XYN]), respiration and the TCA cycle (strongly up-regulated in [PGK1-XYN] and down-regulated in [PGK1]), the glyoxylate cycle (down regulated in both [PGK1-XYN] and [PGK1]) and fermentation (up-regulated in [PGK1]). Patterns of gene expression in glycolysis and respiration were highly consistent between the individual genes in the group, indicating the presence of an overall regulatory strategy and expression as co-ordinated groups (Eisen et al., 1998; Gasch et al., 2000).

Glycolysis and PPP

The observed decrease in the glucose uptake rate (Table 5.2) and glycolytic gene expression in the [PGK1-XYN] strain corresponded to the apparent limitation in nitrogen availability, which can cause a reduction in sugar uptake and the glycolytic flux (Gancedo and Serrano, 1989). The reduced glycolytic flux of the [PGK1-XYN] strain most likely decreased the proportion of sugars fermented versus respired during respirofermentative growth and thus caused respiration to become more important (Lagunas, 1986), resulting in its up-regulation. The down-regulation of hexose

transport, and up-regulation of respiration and mitochondrial gene expression strongly correlated with the transcription profile of cells undergoing a nutritional (carbon or nitrogen) downshift or glucose-deprivation (Shamji et al., 2000). Decreased sugar uptake will also reduce the intracellular glucose concentration, which will diminish the repression of respiration by glucose (Alexander and Jeffries, 1990; Meijer et al., 1998). Conversely to the situation in the [PGK1-XYN] strain, the decrease in the glucose uptake rate (Chapter 4), and apparent limitation in intracellular nitrogen availability of the [PGK1] strain (not as severe as in the [PGK1-XYN] strain; see above) was associated with an increase in the expression of the glycolytic enzymes, a slightly decreased expression of respiration and increased expression of the fermentative pathway (*ADH1* and *ASC1*).

The pentose phosphate pathway (PPP) is the major source of NADPH required for biosynthesis during growth in defined medium (Gancedo and Serrano, 1989). In the [PGK1-XYN] strain the flux through the PPP was apparently unaltered, as transcriptional changes only affected the ratios between constituent enzymes (*GND1*/*GND2*; *RPE1*/*TAL1*). However, a net increase in the expression of two other NADPH-producing reactions was observed: the cytosolic malic enzyme (*MDH2*) and aldehyde dehydrogenase (*ALD5*/*ALD6*). Changes in ammonium assimilation also indicated a possible shortage of NADPH in the cell (see above). Changes in the expression of isoenzymes is a frequent response of yeast cells to changes in environmental conditions, possibly due to differences in the properties of these enzymes (Gasch et al., 2000). The flux through the PPP was apparently changed in the [PGK1] strain, as the decreased expression of *ZWF1* was complimented by an increase in the expression of *PGI1* in glycolysis, indicating a strategy for decreasing the fraction of carbon shunted to the PPP. A net increase in the expression of aldehyde dehydrogenase (*ALD5*/*ALD6*) for additional NADPH generation, along with the increased expression of transketolase (*TKL1*) and *RPE1*, was also observed.

TCA cycle and respiration

Expression of *ACO1* decreased more severely in the [PGK1-XYN] strain than in the [PGK1] strain in the early TCA cycle was mimicked by the decreased expression of *CIT2* in the glyoxylate cycle. In the [PGK1-XYN] strain the expression of the genes in the latter part of the TCA (*SDH2*, *SDH4*, *FUM1*) was increased, while the ratio between the isoenzymes *LSC1*/*LSC2* and *MDH1*/*MDH3* in the glyoxylate cycle was apparently

changed. Gene expression for respiration also increased strongly in this strain, as was confirmed by the increase in mitochondrial gene expression and the increased expression amino acid permeases, which is related to the respiratory capacity of the strain (Horák, 1997). Respiration is also up-regulated in response to limited nitrogen availability, as it seems to conserve nitrogen sources better than fermentative growth (Backhus et al., 2001). Increased respiratory capacity was also evident from the strong decrease in *CIT2* and *DLD3* expression, whose expression levels are sharply increased in cells with dysfunctional mitochondria (limited respiratory capacity) (Liu and Butow, 1999; Robinson and Lopes, 2000) and the induction of *HSP26* expression (Meunier and Choder, 1999). Changes in ammonium assimilation also indicated a strategy for reducing the excess of NADH in the cell (see above), possibly due to increased amino acid biosynthesis (Albers, 2000). The reduced expression of *TPI1*, *TDH* and *ACO1* (iron-dependent) in the [PGK1-XYN] strain would slowdown the rate of NADH production, while decreasing the glycolytic flux (Krieger and Ernst, 1994). In the [PGK1] strain the decreased expression of *ACO1* and *CIT2* were accompanied by reduced expression of *SDH2* and subunits of the alpha-ketoglutarate dehydrogenase complex (*KGD2* and *LPD1*) in the latter part of the TCA. Gene expression in both the TCA cycle and respiration thus decreased in this strain, as was confirmed by the decrease in mitochondrial gene expression. The overall decrease in the TCA flux indicated either that the increase in glycolytic gene expression (see above) caused the decrease expression of respiration and the TCA, or that the imported amino acids were directly incorporated into protein and reduced the requirement for TCA metabolites. No additional strategy for reducing the NADH level in [PGK1] cells was apparent.

5.3.3.3. Transcription, protein synthesis and protein fate

Large, overall changes in the expression of genes for protein synthesis, both in the [PGK1-XYN] and [PGK1] strains, were complimented by smaller changes in transcription and the fate of the synthesised protein.

Transcription

Changes in the expression of 14% of the genes encoding transcription machinery resulted in stronger increases in gene expression in the [PGK1] strain than in the [PGK1-XYN] strain. Stronger up-regulation in the [PGK1] strain was evident for the functions rRNA transcription (strongly up-regulated in [PGK1]; unchanged in [PGK1-

XYN]), tRNA transcription (up-regulated in [PGK1] and slightly down-regulated in [PGK1-XYN]) and mRNA transcription (up-regulated in [PGK1] only).

Protein synthesis

The expression of 47% of the genes involved in the synthesis of proteins was changed in either the [PGK1] or the [PGK1-XYN] strain. Overall, expression was strongly down-regulated in the [PGK1-XYN] strain and up-regulated in the [PGK1] strain, as was exemplified by the functions of ribosome biogenesis (ribosomal proteins), translation (machinery), aminoacyl-tRNA-synthetases and tRNA expression. Ribosomal gene expression, ribosomal biogenesis and protein synthesis is regulated almost entirely at transcriptional level (Warner, 1989; Tuite, 1989; Planta, 1997; DeRisi et al., 1997; Li et al., 1999). The different ribosomal proteins are transcribed and synthesised in approximately equimolar amounts, and transcription is co-ordinately regulated to match the synthesis of rRNA and other translational components (Oliver and Warmington, 1989; Warner, 1989; Tuite, 1989; Planta, 1997; Li et al., 1999). Cells can adjust the production of all ribosomal proteins in a precise and concerted fashion to meet the physiological demands under varying environmental conditions (Galego et al., 1993; Planta, 1997).

Protein fate (folding, modification, destination)

Gene expression for the folding, modification and sorting of proteins in the cell were not strongly affected in either the [PGK1-XYN] or [PGK1] strains. Protein folding, stabilization, targeting, sorting and translocation was down-regulated in both the [PGK1-XYN] and [PGK1] strains, whereas protein modification was up-regulated in both. The assembly of protein complexes was down-regulated in [PGK1-XYN], whilst no net change in the expression of genes involved in proteolytic degradation was observed. The composition of the proteasome may therefore have been changed without affecting the level of proteases. A net increase in the expression of the stress response in Y294 [PGK1-XYN] may be related to the secretion of the heterologous protein, as numerous heat shock proteins (HSPs) and stress response proteins also function as molecular chaperones (Morano et al., 1998). The decrease in *UBI4* expression in Y294 [PGK1] corresponds to the increased in fermentation relative to respiration in this strain (Cheng et al., 1994).

5.3.3.4. Cellular transport and transport mechanisms, interaction with the cellular environment and transport facilitation

Intracellular transport and transport mechanisms

Intra-organellular transport was up-regulated in both the [PGK1-XYN] and [PGK1] strains. In the [PGK1-XYN] strain nuclear transport, vesicular transport (Golgi network, etc.) and vacuolar transport was down-regulated, whereas mitochondrial transport was up-regulated. Cellular import was strongly increased in [PGK1] and [PGK1-XYN], mostly due to the large increases in the expression of amino acid transporters (*BAP3*, *BAP2*, *MUP1*, *TAT1*, *TAT2* and *LYP1*), and changes in the ratios between hexokinase and glucokinase isoenzymes (carbon uptake). Virtually no decreases in the expression of amino acid transporters were observed.

Transport facilitation and interaction with the cellular environment

Ionic homeostasis was strongly altered in both the [PGK1-XYN] and [PGK1] strains, with the expression of more than 50% of the ion and amino acid transport facilitators affected. In [PGK1-XYN], expression of the ORFs required for the homeostasis of metal ions was increased, which was mostly caused by a large increase in the expression of the transporters for heavy metals and dominated by altered expression of the genes involved in iron transport at the plasma membrane and in vesicles (*FIT*, *FRE*, *FET* and *ARN*) (Georgatsou and Alexandraki, 1994; Yun et al., 2001; Yun et al., 2000). Iron and copper transporters are induced during limited metal ion availability, and is regulated at transcriptional level (Georgatsou and Alexandraki, 1994; Martins et al., 1998; Georgatsou and Alexandraki, 1999). The expression of remaining ORFs for cation (Na^+, K^+, Ca^{2+}, NH_4^+, etc.) and proton homeostasis was decreased. In the [PGK1] strain, an increase in the expression of ORFs for metal transport was also observed, though at lower levels than in Y294 [PGK1-XYN] and without increasing the expression of the heavy metal transporters. Homeostasis of protons was also decreased, though the changes were smaller in magnitude than in Y294 [PGK1-XYN]. Changes in the expression of anion transporters were insignificant in both strains. The expression of drug transporters was also increased in both Y294 [PGK1] and Y294 [PGK1-XYN], though more strongly in the latter. The general transport mechanisms (including ATPases and ABC transporters) were down-regulated in both the [PGK1-XYN] (more strongly) and [PGK1] strains, consistent with the decreased homeostasis

of protons, the glucose-dependency thereof (Gancedo and Serrano, 1989) and the observed decreases in the glucose uptake rate of these strains (Chapter 4).

5.3.3.5. Cell rescue, defence and virulence

The expression of 27% of the genes involved in cell rescue, defence and virulence were changed in either the [PGK1-XYN] or [PGK1] strains, with Y294 [PGK1-XYN] experiencing significantly more stress than Y294 [PGK1]. Some of the genes in metabolism that are partially controlled by stress response elements (STREs) were up-regulated in Y294 [PGK1-XYN] (*ACS1*, *GLC7*, *GLK1*, *MDH2*, *TPS2*, *SGA1*), whereas others were down-regulated in Y294 [PGK1] (*GAC1*, *GLK1*, *HXK1*, *PGM2*) (Moskvina et al., 1998). The induction of the general stress response was apparent in both the [PGK1-XYN] and [PGK1] strains, though less obviously in the latter. Three genes involved in the response to nutrient limitation or starvation (*SNZ1*, *SNO1* and *YGP1*; Padilla et al., 1998) were strongly increased in both Y294 [PGK1-XYN] and Y294 [PGK1]. Detoxification and the expression of various drug resistance genes increased in both the [PGK1] and [PGK1-XYN] strains, though more strongly in the latter. In the [PGK1-XYN] strain, strong increases in the expression of the *ARN* transporters, associated with vesicular iron transport, as well as an increase in *ATX1* expression, was observed as part of this response. Changes in the glucose and energy metabolism of a cell can alter its lifespan, and the shift in the metabolism of the [PGK1-XYN] strain away from glycolysis towards a more respiratory metabolism (see above), and decreased transcription of tRNAs, partially corresponds to an increase in cellular ageing (Lin et al., 2001).

5.3.3.6. Summary

The presented overall changes in the various metabolic processes are summarised in Table 5.4.

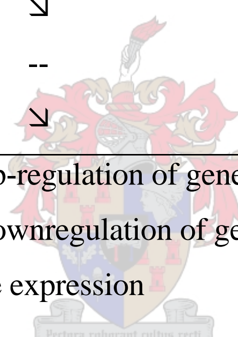
Table 5.4. Summary of overall changes in cellular processes

Cellular Process	[PGK1] vs. [Host]	[PGK1-XYN] vs. [Host]	[PGK1-XYN] vs. [PGK1]
Amino acid requirement	↑	↑↑	↑
NH_4^+ utilisation	--	↓	↓
μ_{max}	↓	↓↓	↓
Glucose uptake rate	↓	↓↓	↓
Glycolysis	↑	↓	↓↓
Respiration and TCA	↘	↑	↑
Iron uptake	--	↑	↑
Fermentation	↑	--	↓
Transcription machinery	↑	↑	↓
Protein synthesis	↑	↓	↓↓
General stress response	↑	↑↑	↑
URA3 expression	↘	↓	↓
Pyrimidine uptake	--	↑	↑
Plasmid copy number	↘	↓	↓

↑, ↑↑: Increased measure of up-regulation of gene expression

↓, ↓↓: Increased measure of downregulation of gene expression

↘: Slight downregulation of gene expression



5.4. DISCUSSION

Genome-wide transcriptional profiles of three recombinant *S. cerevisiae* strains were compared under identical cultivation conditions, to identify the cellular processes involved in the physiological response to foreign protein production. The transcriptional effect of a large number of glycolytic *PGK1* promoters, present on the recombinant plasmid in the Y294 [PGK1] strain, could also be determined. An increase in the abundance of a particular transcript was interpreted as an increase in the capacity of the cell to synthesise the corresponding protein (Backhus et al., 2001; Gasch et al., 2000). Though the changes in the transcriptional profiling of *S. cerevisiae* due to various environmental and genetic factors have been reported previously, the present investigation is the first to quantify of the transcriptional response to the production of a heterologous protein.

5.4.1. Data analysis

The tight regulation of genes with high expression levels resulted in smaller changes their expression level. In response to this, a "Significance factor" was defined, which afforded significance to these changes. The reported changes in the expression of several genes in glycolysis, respiration and ribosomal protein synthesis would otherwise not have been observed, despite the presence of clear regulatory trends in the transcriptional response. The overall regulatory pattern for gene expression in these three categories was strongly mimicked by the individual genes. The changes in expression levels observed in the present investigation were generally smaller in magnitude than in several previous reports, probably due to the transient nature of the transcriptional response (Gasch et al., 2000). The present cultures were sampled after several hours of exponential, balanced growth, and the transcriptional response therefore represent quasi-steady-state changes in gene expression, which are typically smaller than the changes observed within a short period after a sudden change (Gasch et al., 2000). The physiological changes due to the presence of the heterologous gene expression were also marginal, typically smaller than 15% (Chapter 4).

5.4.2. Apparent amino acid limitation

Although an excess of ammonium and amino acids was available during the course of batch fermentations, the transcriptional profiles of both the [PGK1] and [PGK1-XYN] strains were similar to cells experiencing nitrogen limitation. This was indicated by the up-regulation of amino acid biosynthesis and uptake from the medium, which was more severe in the [PGK1-XYN] than in the [PGK1] strain, and the increased expression of the *SNZ1*, *SNO1* and several of the *GCN4*-regulated genes in both strains (Padilla et al., 1998; Natarajan et al., 2001). The expression of the genes in respiration was also up-regulated in the [PGK1-XYN] strain, whilst glycolytic gene expression was down-regulated. Both confirmed the increased severity of the apparent nitrogen limitation in this strain (Backhus et al., 2001; Gancedo and Serrano, 1989). The increase in gene expression for amino acid uptake in the [PGK1-XYN] strain was complimented by a decrease in ammonium uptake, further supporting an apparent increase in the dependency on amino acid uptake. Changes in the expression of the glutamate dehydrogenases, *GDH1* and *GDH2*, in the [PGK1-XYN] strain also indicated an increase in the intracellular leucine availability.

The transcriptional response was similar to that of cells experiencing nitrogen limitation despite the various transcriptional strategies aimed at increasing amino acid availability. All three of the isogenic transformants compared in the present study, retained the *his3* and *trp1* auxotrophic markers, while the *leu2* marker was complimented by the disruption *fur1::LEU2*. Gene expression in the biosynthetic pathways for these three amino acids also increased in both the [PGK1] and [PGK1-XYN] strains. However, despite the potential disturbance of amino acid metabolism by the auxotrophic mutations, the apparent amino acid limitation in these strains was solely due to changes in the content of the recombinant plasmids, as these strains were isogenic to the reference strain (Y294 [Host]).

5.4.3. Transcriptional response to plasmid-based *PGK1* promoter

In the [pJC1] plasmid, present in the [PGK1] strain, the site of transcription initiation in the *PGK1* promoter was separated from the site of transcription termination in the *PGK1* terminator by 101 base pairs (Crous et al., 1995; Hitzeman et al., 1982). A large number of non-translatable transcripts could therefore have been synthesised without subsequent protein production. The transcriptional response of the [PGK1] strain was thus associated with both the presence of a large number of active, plasmid-based *PGK1* promoters on the recombinant plasmid (50-100; Shuster et al., 1989; Bae et al., 1998), and the synthesis of small mRNA molecules.

The transcriptional changes observed in response to the addition of the *PGK1* promoter and terminator to the recombinant plasmid are summarised in Table 5.4. Most notable were the increased expression of the genes in glycolysis, fermentation and protein synthesis compared to the [Host] strain, despite the observed decrease in the maximum specific growth rate and glucose uptake rate (Table 5.2). However, decoupling between glycolytic enzyme expression and the glycolytic flux has been demonstrated (Hauf et al., 2000; Schaaff et al., 1989), probably due to the involvement of the sugar uptake rate in high-level glycolytic flux control (Pritchard and Kell, 2002). Glycolytic and fermentative gene expression may rather have been increased in response to the presence of numerous transcriptionally active *PGK1* promoters in the cell, as overproduction of Pgk1p caused a significant increase in the glycolytic flux, fermentative activity and respiratory capacity of *S. cerevisiae* during respirofermentative growth (Van der Aar et al., 1990a; Van der Aar et al., 1990b). The cell may thus have responded to an apparent increase in the glycolytic flux, indicated by

transcription from a large number of *PGK1* promoters in the cell. The down-regulation of gene expression in the TCA cycle and respiration, confirmed by the decrease in mitochondrial gene expression, further substantiated an apparent increase in the glycolytic flux observed by the cell. Since ribosomal protein synthesis functions as an indicator for the potential to grow, rather than the rate of growth itself (Donovan and Pearson, 1986; Ju and Warner, 1994; Pernambuco et al., 1996; Crauwels et al., 1997), the increase in ribosomal gene expression in the [PGK1] strain may similarly be part of a strategy to create spare translational capacity. This would allow the yeast to increase the rate of protein synthesis instantaneously during a nutritional up-shift (Oliver and Warmington, 1989; Tuite, 1989). However, both glycolytic and ribosomal gene expression place high demands on the transcriptional apparatus of the host strain. Ribosomal protein mRNAs are among the most abundant mRNAs in the cell, despite their short half-life, whilst the transcription of rRNA genes represents about 60% of the total transcription of a growing cell (Warner, 1989; Planta, 1997; Li et al., 1999). The biosynthetic capacity of *S. cerevisiae* for the overproduction of glycolytic enzymes is also limited (Hauf et al., 2000). The increased ribosomal and glycolytic gene expression, associated with an expected increase in the glycolytic flux, would thus occupy a large fraction of the available transcriptional apparatus of the host strain (Planta, 1997; Li et al., 1999; Jelinsky and Samson, 1999). The resulting decrease in the availability of transcriptional machinery may therefore have caused a decrease in the growth rate and glucose uptake rate of the [PGK1] strain. The apparent amino acid limitation in the [PGK1] strain may also be related to the general stress response to foreign gene expression, which is discussed below. However, the severity of the general stress response and the apparent amino acid limitation was insufficient to dominate the transcriptional profile of the [PGK1] strain.

5.4.4. Cellular processes affected by foreign protein production

The transcriptional response to the production of translatable foreign mRNA molecules, the synthesis of the foreign protein during translation and the secretion of the produced protein was determined in isolation from other metabolic effects, by the comparison of the [PGK1-XYN] strain to the [PGK1] and [Host] strains (Table 5.4). Foreign protein production caused an apparent amino acid limitation in the cell, which corresponded to the dependency of recombinant protein production on amino acid availability reported either in *S. cerevisiae* (Wittrup and Benig, 1994; Van der Aar et al., 1990a) or bacterial systems (*E. coli*: Ramirez and Bentley, 1993; *Bacillus brevis*: Park et al. 1996;

Staphylococcus: Gupta et al., 1999), where production levels increased significantly by the addition of amino acids to the cultivation medium. Metabolite balancing during recombinant protein production has also identified a drain on biosynthetic precursors in the TCA cycle during recombinant protein production (Jin et al., 1997). The increased expression level for the general stress response, specifically due to production of the foreign protein, indicated a greater severity of stress in the [PGK1-XYN] than in the [PGK1] strain (Gasch et al., 2000). Gene expression for protein synthesis also decreased in response to the production of the foreign xylanase protein, which is normally associated with general starvation/stress conditions (Cardenas et al., 1999; Chu et al., 1998; DeRisi et al., 1997; Jelinsky and Samson, 1999; Natarajan et al., 2001; Planta, 1997; Eisen et al., 1998; Planta and Mager, 1998; Li et al., 1999; Gasch et al., 2000) or nitrogen limitation (Warner and Gorenstein, 1978; Oliver and Warmington, 1989; Warner, 1989; Moehle and Hinnebusch, 1991; Cardenas et al., 1999). However, the decreased expression of ribosomal proteins may also be a concerted effort to divert transcription to the expression of other genes, since gene expression for ribosomal protein synthesis places large demands on the transcriptional apparatus of the yeast (see above; Jelinsky and Samson, 1999). A strong correlation between the growth rate of the strain and the expression level of the genes for ribosomal proteins also exists (Tuite, 1989; Lopez and Baker, 2000). The expression of ribosomal proteins was thus related to the decrease in glycolytic gene expression, the glycolytic flux and the maximum specific growth rate of the [PGK1-XYN] strain compared to the [PGK1] strain (Tables 5.2 to 5.4). The secretory capacity of the host strain was apparently not saturated by foreign protein expression (see also Chapter 6), as was reported for other production systems (Wittrup et al., 1994; Tuite and Freedman, 1994; Parekh et al., 1995; Parekh and Wittrup, 1997), since the expression of the genes that facilitate the sorting, degradation and secretion of the proteins in the cell was not significantly changed in the [PGK1-XYN] strain. This also indicated low levels of erroneously translated protein production (Kurland and Gallant, 1996). The up-regulation of respiration in the [PGK1-XYN] strain was related to the apparent limitation in amino acid availability, as exhibited by other yeast systems (Lagunas, 1986; Shamji et al., 2000; Backhus et al., 2001). The increased requirement for iron in the [PGK1-XYN] strain also corresponded to the increase in gene expression for respiration and the electron transport chain (cytochromes), which are critically dependent on its availability (Georgatsou and Alexandraki, 1994; Krieger and Ernst, 1994; Martins et al., 1998; Georgatsou and Alexandraki, 1999; Foury and Talibi, 2001). Decreased levels of

URA3 transcripts indicated a reduction in the copy number of the recombinant plasmid in the [PGK1-XYN] strain, with the resulting decrease in pyrimidine biosynthesis complimented by increasing the expression of the pyrimidine permeases. The stability and copy number of the 2 μm plasmid was previously shown to decrease in response to active gene expression (Srienc et al., 1986; Janes et al., 1990; Da Silva and Bailey, 1991).

The observed transcriptional trends in the [PGK1-XYN] strain were very similar to the *E. coli* stringent response, where nutrient limitation decreased ribosome synthesis and increased respiration, amino acid uptake and amino acid biosynthesis (Moehle and Hinnebusch, 1991). Nitrogen limitation and a nutritional downshift was previously shown to elicit a similar stringent response in *S. cerevisiae* (Backhus et al., 2001; Perez-Ortin, 2002; McEntee et al., 1994; Yang et al., 2000; Natarajan et al., 2001).

To the knowledge of the author no previous study on the transcriptional response of yeast to recombinant protein production has been undertaken, though several investigators have reported the physiological and transcriptional profiling of recombinant *E. coli*. Decreases in the growth rate (Oh and Liao, 2000; Dong et al., 1995) and glucose consumption rates, were complimented by a partial repression of glycolytic and PPP gene expression, and the transcriptional induction of respiration (Oh and Liao, 2000). Measured decreases in the levels of ribosomes, the ribosomal proteins and those involved in translation and protein folding (Jürgen et al., 2000; Rinas, 1996; Dong et al., 1995) reduced the levels of normal cellular protein synthesis, and thereby decreasing glycolytic gene expression and activity (Dong et al., 1995; Snoep et al., 1995). Increased transcription of various stress-related (heat shock) and chaperone genes in response to recombinant protein production has also been reported (Oh and Liao, 2000; Dong et al., 1995; Gill et al., 2000; Gill et al., 2001), along with the induction of the *E. coli* stringent response, associated with an increase in intracellular proteolytic activity (Harcum and Bentley, 1993).

Although the level of heterologous protein production by *S. cerevisiae* reported here (less than 1% of the total cellular protein production; Chapter 4) was considerably lower than typical production levels in bacterial systems (up to 40% of the total cellular protein production), very similar physiological and transcriptional responses were observed. The magnitude of these physiological and transcriptional responses to

heterologous protein production by yeast was also disproportionate to the amount of foreign protein produced (Chapter 4). Similar observations for recombinant *E. coli* strains overproducing heterologous proteins with no apparent catalytic activity in metabolism was associated with a strong decrease in translation due to the coordinate reduction in the rate of normal protein synthesis, the accumulation of heat shock proteins, and the degradation of rRNA and ribosomes (Kurland and Dong, 1996). In response, the recombinant bacteria behaved as if experiencing amino acid starvation or antibiotic inhibition (Kurland and Dong, 1996). In the present investigation, the production of heterologous xylanase by a transformed strain of *S. cerevisiae* apparently resulted in a similar stress response, by which the cells lost the ability to grow in an optimal, balanced fashion. The loss of functionality by the cell due to foreign protein production may thus have caused the observed disproportionate metabolic effects. The severity of the stress response to heterologous protein synthesis was sufficient to dominate the transcriptional profile of the strain.

5.5. CONCLUSIONS

The physiological response of recombinant *S. cerevisiae* strains to the presence of an expression system for heterologous protein production were related to the presence of multiple copies of the glycolytic *PGK1*-promoter and terminator, which apparently caused the cell to increase its biosynthetic capacity at the expense of other processes, and the synthesis of the foreign protein, which resulted in a stringent stress response by the host strain. The introduction of physiological stress by heterologous gene expression thus caused a loss of functionality in the host strain, causing the disproportionate physiological effects of heterologous protein production. The active up-regulation of the stringent stress response due to the synthesis and secretion of a foreign protein further confirmed the presence of a global sensing and regulatory mechanism, able to sense and respond to heterologous protein production. The loss of biosynthetic capacity associated with the stringent stress response may also have limited the ability of the host strain to produce heterologous xylanase, resulting in lower production levels. Reducing the propensity of recombinant gene expression to introduce metabolic stress may therefore increase production levels of foreign proteins by yeast.

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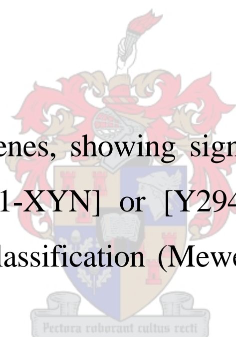
5.7 APPENDIX



The classification of the 1014 genes, showing significant change in their expression level in either the Y294 [PGK1-XYN] or [Y294 [PGK1] strains, into functional categories according the MIPS classification (Mewes et al., 2000) is presented in the Appendix D.

Chapter 6

COMPARISON OF THREE EXPRESSION SYSTEMS FOR HETEROLOGOUS XYLANASE PRODUCTION BY *S. CEREVISIAE*

6.1. INTRODUCTION

In the previous two chapters the presence of a regulated stress response by the yeast cell to heterologous protein production was presented. However, besides the effect of metabolic stress, the production levels of heterologous proteins may also be influenced by other genetic and physiological factors. In the present study, the influence of expression vector selection and uncomplimented auxotrophic markers in transformant strains on the production levels of a heterologous protein were investigated. The presence of global sensing and regulatory mechanisms, which may determine the production level of heterologous xylanase in response to these factors, was thus investigated.

Both the copy number and stability of the expression cassette, and the genetic and physiological characteristics of the host strain, have been shown to affect the production levels of a heterologous protein by *S. cerevisiae* (Weber et al., 1992; Ljubijankic et al., 1999; Park et al., 2000; Nacken et al., 1996; Janes et al., 1990; Lopes et al., 1996; Park et al., 2000; Eckart and Bussineau, 1996; Fleer, 1992; Schultz et al., 1994; Mendoza-Vega et al., 1994; Porro et al., 1992). Two types of expression vectors commonly used for heterologous protein production in *S. cerevisiae*, are the yeast episomal plasmids (YEp), based on the endogenous, extra-chromosomal 2 μm plasmid, and yeast integrative plasmids (YIp), based on homologous integration at specific chromosomal positions. These vectors represent alternative methods for maintaining foreign DNA within a host cell. With regard to expression level, the former benefits from higher copy number (up to 100 copies per cell), resulting in a higher foreign gene dosage, whereas the latter provides robust genetic stability. Disadvantages are the frequent segregational instability of YEp vectors, resulting in the loss of the recombinant plasmid during cell division despite the presence of selection markers on the recombinant plasmid, and the low copy number of YIp vectors, resulting in low levels of expression (Harashima, 1998; Mendoza-Vega et al., 1994; Gellissen and Hollenberg,

1997; Lopes et al., 1996; Shuster et al., 1989; Bae et al., 1998; Weber et al., 1992; Ljubijankic et al., 1999; Park et al., 2000). To improve the stability of YEp-type vectors, several autoselection systems, based on double or triple selection, have been developed (Loison et al., 1986; Wang and Da Silva, 1993; Compagno et al., 1993). One such system, based on the disruption of the native *FUR1*-gene in combination with insertion of the *URA3* gene on the recombinant plasmid, in a host that contains a *URA3* mutation, resulted in excellent genetic stability, also in continuous culture (Loison et al., 1986; Marquet et al., 1987).

The presence of auxotrophic mutations in host strains for recombinant protein production is often essential for obtaining genetically stable transformants, through the use of selection strategies such as those presented above. However, during the early studies of yeast genetics and molecular biology, the effect of uncomplimented auxotrophic mutations, not complimented by the genetic components in the recombinant gene expression system, on the growth and metabolism of laboratory strains were disregarded, or considered as insignificant. This idea has been transferred to numerous molecular biologists of today. Host strains with excessive auxotrophic mutations are still frequently used for recombinant protein production, resulting in transformant strains containing uncomplimented auxotrophic markers (See Table 2.1). The presence of uncomplimented auxotrophic markers in transformant strains requires the availability of a sufficient amount of the required metabolite in the complex or defined cultivation medium. Some investigators have observed that such transformants may overconsume the required metabolite (amino acid or nucleotide) and can have difficulties in growth, protein production and genetic stability (Mendoza-Vega et al., 1994; VanDusen et al., 1997; Pronk et al., 1996; Chopra et al., 1999; Beretta et al., 1991; Korogodin et al., 1991; Çakar et al., 1999; Kozlov et al., 1995; L. Gustafsson, personal communication). Auxotrophic strains grown in nutrient supplemented medium are thus not necessarily physiologically equivalent to the complemented transformants (Pronk et al., 1996; Chopra et al., 1999).

The production of several heterologous xylanases by *S. cerevisiae* transformants based on the YEp-type expression vectors containing auxotrophic markers has been reported, though no mention of the effect of host strain genetics and type of expression vector was made (La Grange et al., 1996; Ganga et al., 1998; Crous et al., 1995; Nuyens et al., 2001; Li and Ljungdahl, 1996; Pérez-González et al., 1996). The effect of expression

vector selection and auxotrophic mutations on heterologous xylanase production by *S. cerevisiae* in batch and continuous culture were thus determined. A *trp1 his3* auxotrophic transformant, expressing *T. reesei* xylanase II (*XYN2*) from a YEp expression vector, stabilised by the autoselection system of Loison et al. (1986), was cultivated in a chemically defined medium containing various concentrations of the required amino acids. Heterologous protein production by this strain was compared to two prototrophic transformants, containing either the same autoselection-YEp expression system, or an alternative YIp-type of expression vector. The expression cassette construct (promoter-heterologous xylanase-terminator) was identical in these strains. Cultivation of auxotrophic transformants in the presence of an excess of the required amino acids resulted in a dramatic increase in levels of recombinant xylanase production, comparable to production levels obtained with a similar protrophic transformant. The potential benefits of utilising the autoselective YEp expression system in a prototrophic background were thus demonstrated.

6.2. MATERIALS AND METHODS

6.2.1. Strains and plasmids

The construction of the recombinant *S. cerevisiae* Y294 [*ura3/URA3, leu2::LEU2, trp1, his3*] strains producing a heterologous ß-1,4-xylanase, through expression of a plasmid-based *XYN2* gene, was previously reported (La Grange et al., 1996). Strain stocks were stored in a 15% glycerol solution at –80ºC. Manipulation of plasmid DNA and transformation of strains were according to standard protocols. The genetic stability of the transformants were determined by replica plating from complex (YPD) medium to selective, defined medium (without the amino acids used for selection) and counting the percentage of growing colonies (Da Silva and Bailey, 1991).

6.2.2. Fermentations

Batch fermentations in a defined medium (Verduyn et al., 1992) containing 20 $g.l^{-1}$ glucose as the carbon source and amino acid supplementation as discussed below, were conducted in a high performance bioreactor, as previously reported (Chapter 4). For glucose-limited chemostat cultures, new medium was fed to the fermenters at a constant rate of 60 $ml.h^{-1}$ and the working volume maintained at 600 ml by controlling the weight of the fermentation set-up. Glucose-limitation with a defined feed medium containing 15 $g.l^{-1}$ of glucose was ensured by measuring the biomass yield in the same medium with 20 or 22 $g.l^{-1}$ glucose feed. No apparent decrease in the biomass yield

was observed, confirming that biomass production was limited by glucose rather than other nutrients. Fermentations were aerated with oxygen-enriched air at 0.5 $l.min^{-1}$ (standard conditions) and the level of dissolved oxygen concentration maintained at a minimum of 60% to ensure fully aerobic growth at low dilution rates. Steady-state measurements were taken after the carbon dioxide, biomass and recombinant xylanase production level had stabilised; the latter usually after approximately 7-8 residence times.

6.2.3. Sampling

Samples for the determination of cell density, substrate consumption and product formation during continuous cultures were removed directly from the fermentation broth. The supernatant was collected by filtration through a 0.2 µm filter before storage at -20ºC. Samples for the determination of cell density by absorbance (620 nm) were diluted with 9 $g.l^{-1}$ NaCl into the 0.05 - 0.2 linear absorbance detection range of the spectrophotometer. Samples for dry weight measurements, made in parallel to absorbance (A_{620}) measurements for all steady-state samples, were taken directly from the fermentation broth and kept on ice during the analysis. For the quantification of *XYN2* and *GCN4* RNA levels, 10 ml of fermentation broth was rapidly sampled, added to a tube containing approximately 5 ml of ice, centrifuged for 2 min and washed twice with cold Acetate buffer (50 mM NaAc; 10 mM EDTA), before storage at -80ºC. Samples for extracellular amino acids were prepared by addition of an equal volume of 4% Trichloroacetic acid (TCA) to a supernatant sample and storage at -20ºC. Samples for the quantification of intracellular xylanase activity were taken from the outlet of the cultivation. Approximately 15 ml of fermentation broth was collected on ice, washed twice with 9 $g.l^{-1}$ NaCl and the cellular protein extracted with Y-PER (Yeast Protein Extraction Reagent; Pierce). Samples for the determination of the total cellular content of protein were also taken from the outlet. The cells were collected by centrifugation, washed twice with 9 $g.l^{-1}$ NaCl and resuspended to a final concentration of 5 $g.l^{-1}$ (dry weight). Cell extracts and cell samples were frozen at -80ºC and stored at -20 ºC until analysis. Sampling for cell density, substrate consumption and product formation during batch fermentation were similarly performed.

6.2.4. Substrate consumption and product formation

Glucose, ethanol, glycerol, acetate and succinate concentrations, as well as xylanase activity were determined as previously described (Chapter 4). Xylanase enzyme

activities were converted to protein amounts (mg) by use of the conversion factor 1.812 $U.\mu g_{pure\ xylanase}^{-1}$, obtained for the purified protein (Chapter 3). The specific intracellular xylanase activity ($mg_{active\ xylanase}.g_{cell\ protein}^{-1}$) was determined by measuring both the xylanase activity ($U.ml^{-1}$; as in Chapter 4) and the total protein concentration, using the MicroBCA kit (Pierce), in the cell extract made for this purpose. Amino acid analyses were performed at the Department of Biochemistry and Nutrition, Denmark Technical University, Lyngby, Denmark. Total cellular protein content was determined with the modified biuret method (Herbert et al., 1971), whereas total yeast protein secretion was measured with the MicroBCA Kit (Pierce), according to manufacturers specifications. Due to the potential effect of extracellular free amino acids on total protein measurements, samples were first treated by dialysis in pure water using a dialysis membrane with a very low molecular weight cut-off (1.5 kDa).

6.2.5. *XYN2* and *GCN4* expression levels

Quantitative Reverse-Transcriptase PCR (QRT-PCR) was used for the quantification of *XYN2* and *GCN4* RNA levels. The total RNA content of cell samples was extracted according to Schmitt et al. (1990), with slight modifications to account for volume differences. Extracted total RNA was used as template for cDNA synthesis using the Reverse Transcriptase II enzyme (Gibco), according to manufacturer's specifications. Synthesised cDNA was quantified using real-time PCR performed by the LightCycler® (Roche). Levels of *XYN2*, *GCN4* and *ACT1* cDNA in each 20 µl sample (containing 4 µl cDNA mixture) were quantified, with the latter used as standard. Each measurement was repeated at least three times using different dilutions of the cDNA mixture (1:1, 1:4, 1:16), and the results analysed using the Roche LightCycler® software package.

6.3. RESULTS

Heterologous xylanase production by auxotrophic (*trp1* and *his3*) transformants from a YEp expression vector, stabilised by an autoselection system not involving tryptophan or histidine (Loison et al., 1986), in a chemically defined medium was compared to xylanase production by prototrophic transformants, containing either the same autoselection-YEp expression system, or an alternative YIp-type of expression vector. The concentrations of amino acids in the defined medium were varied.

6.3.1. Construction and screening of prototrophic transformants

For the construction of prototrophic versions of the recombinant *S. cerevisiae* strains producing heterologous xylanase, the excessive auxotrophic markers were removed either from the host strain prior to transformation (YIp-type strains), or directly from the corresponding auxotrophic transformants (YEp-type strains; La Grange et al., 1996). Functional versions of the *LEU2*, *TRP1* and *HIS3* yeast open reading frames were isolated from the plasmids, YIplac128, YIplac204 and YDp-H (Berben et al., 1991), respectively. Transformation of either the host strain or the auxotrophic transformants with the *HIS3* and *TRP1* fragments for the removal of *his3* and *trp1* mutations were performed in a single step, with a good success rate. Removal of the *leu2* mutation prior to integration required a further transformation of the host strain *S. cerevisiae* Y294 [*ura3 leu2*] with the *LEU2* fragment to obtain the host strain *S. cerevisiae* Y294 [*ura3*].

6.3.1.1 Construction and screening of prototrophic YEp-based transformants

Transformation of the two existing auxotrophic, YEp-based recombinant strains (see Chapter 4; La Grange et al., 1996) resulted in two prototrophic, xylanase producing strains: Y294 [PGK1-XYN] PlasPro and Y294 [ADH2-XYN] PlasPro. Additional strain selection through the isolation of stable colonies from selective plating was also performed, prior to isolation of the final transformants. Heterologous xylanase production and genetic stability of several transformants were tested in two subsequent rounds of screening in shake-flask cultures. Xylanase production by the prototrophic YEp-based transformants at similar levels to those obtained with the auxotrophic strains in the defined medium supplemented with seven amino acids (see Chapter 4) was observed (Fig. 6.1). Due to the intrinsic lack of reproducibility of shake-flask cultures, little significance was associated with the differences in production levels from individual transformants (Fig. 6.1).

Although the prototrophic transformants originating from the *S. cerevisiae* Y294 [ADH2-XYN] strain was completely stable during the screening procedure, significant genetic instability was present in the prototrophic plasmid-based [PGK1-XYN] PlasPro transformants. After 96 hours of cultivation in shake-flasks, a significant percentage (10-100%) of the growing transformant colonies had lost their prototrophic characteristics, despite two successive rounds of positive selection.

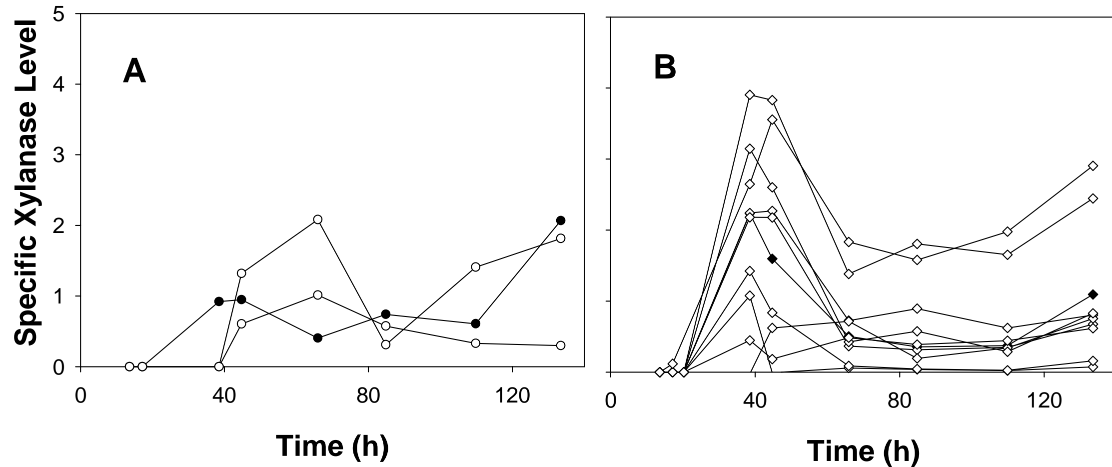


Figure 6.1. Comparison of original auxotrophic strains (closed symbols) with several of the generated prototrophic transformants (open symbols). (A) [PGK1-XYN] PlasPro strains and (B) [ADH2-XYN] PlasPro strains. (●,♦) Specific xylanase level ($mg.g_{biomass}^{-1}$).

6.3.1.2. Construction of prototrophic YIp-based transformants

Prototrophic, integrative transformants, based on a YIp-type expression vector, were obtained by rearranging sections of the YEp-expression vector and integration of the resulting plasmid into the *ura3* locus of the Y294 [*ura3*] host strain. These procedures are described in detail below.

6.3.1.2.A. Construction of integration cassette

Rearrangement of both the *pDLG5* and *pDLG6* YEp-expression vectors (Fig. 6.2) were attempted, to provide flanking of the expression cassettes by the *URA3* selection marker, as depicted in Figure 6.3, and thereby allow for homologous recombination at the *URA3* locus of the host strain. Several potential strategies of Polymerase Chain Reaction (PCR) amplification were applied, with limited success in some cases.

PCR AMPLIFICATION

The Expand High Fidelity PCR System (Roche) was used for the amplification of the final integrative fragments, as the final fragments were relatively large (approximately 3800 bp). The PCR reactions were optimised by varying the annealing temperature (formaldehyde addition), the concentration of Mg^{2+} ions and the template DNA

concentration. Primers for the PCR reactions were designed using the following general rules:

- GC content of the primers should be 40-60%.
- Melting point (temperature at which binding between the primer and the template is disturbed) of the primers used in the same reaction should be similar.
- The 3' end of the primer should contain at least 2 G and/or C base pairs to facilitate strong binding.

RESTRICTION CUTTING OF PLASMIDS

The expression cassette and *URA3*-marker were cut from the original *pDLG5* and *pDLG6* plasmids (La Grange et al., 1996) using the restriction enzymes, NdeI, SmaI and Alw44I (Fig. 6.2). The fragments were purified using gel electroporation.

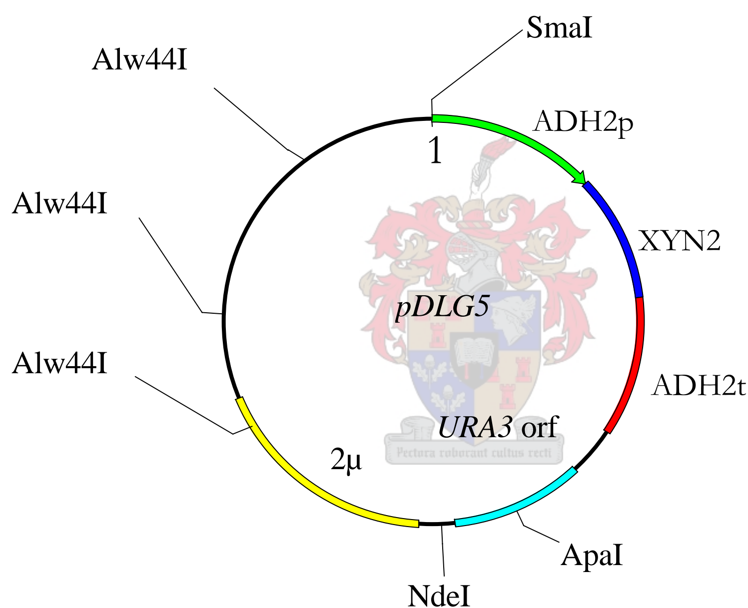


Figure 6.2. Restriction enzyme digestion of the *pDLG5* plasmid. Fragment containing the expression cassette was isolated. The *pDLG6* plasmid was treated in a similar manner.

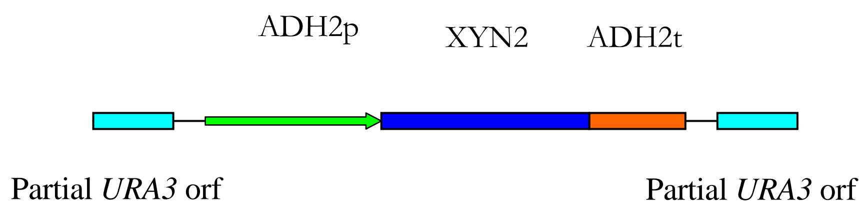


Figure 6.3. Required integrative fragment (*pDLG5* shown here) for transformation of Y294 [*ura3*] host.

PCR STRATEGY ONE

The fragments isolated from the *pDLG5* and *pDLG6* plasmids, containing only the expression cassettes and *URA3* marker, possessed one blunt end (SmaI) and one sticky end (NdeI). The latter were removed by a blunt ending procedure using T4 DNA polymerase (Gibco), followed by ligation of the blunts ends using T4 DNA ligase (Gibco). Hereby the reconstruction of the two "mini-plasmids", each containing only an expression cassette and a *URA3* marker, was attempted (Fig. 6.4). In a final step an integrative fragment with *URA3* flanking the expression cassettes would then be generated using PCR amplification (note arrows in Fig. 6.4). Primers were designed to start amplification in the middle of the *URA3* marker, the efficiency of these primers was confirmed using plasmid DNA (YIplac211) as template. Primers binding inside the *URA3* gene were: Ura_L: 5'-TGT GGT GGG CCC AGG TAT TGT TAG CGC TTT GAA G-3'; Ura_R: 5'-AAT ACC TGC GCC CAC CAC ACC GTG TG-3'. However, Strategy One was not successful, most likely because of the inefficiency of ligations using two blunt ends. The low success rate of this procedure apparently compromised the rest of the approach.

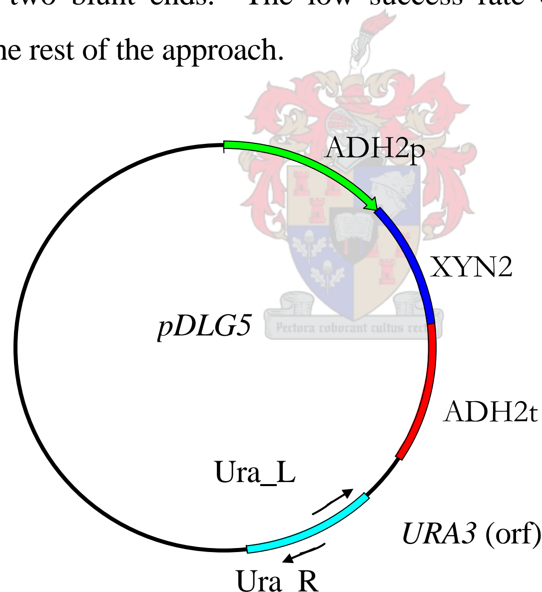


Figure 6.4. Strategy One for amplification of expression cassette, resulting in an integrative fragment. Ura_R and Ura_L show the binding sites and directions of the primers used for PCR.

PCR STRATEGY TWO

In the second approach, the isolated fragments containing the expression cassettes were amplified in two PCR steps, resulting in two primary PCR products: The Long product extended from the middle of the *URA3* gene to the end of the expression cassette,

whereas the remainder of the *URA3* gene was amplified as the Short product. The same *URA3* primers as used in Strategy One were employed, though in combination with two additional primers, which were designed as follows: A BamHI restriction site was introduced on the 5' end of the primers which bind to the end of the expression cassette, whereas for the new primers binding to the end of the *URA3* gene, a 19 bp extension on the 5' end was introduced. This extension contained a BamHI restriction site, closest to the binding region, whereas the base pairs corresponding the end of the expression cassette was introduced in the remainder of the 19 bp extension (Fig. 6.5). The additional primers were: pDLG5: 3'-GCG GAT CCC ATC GTC CAT TC-5' and 5'-GAA TGG ACG ATG GGA TCC GAA ACA TGA AAT TGC CCA G-3' (with extension); pDLG6: 5'-GCG GAT CCT TAA CGA ACG CAG AAT TTT CGA G-3' and 5'-CTC GAA AAT TCT GCG TTC GTT AAG GAT CCG CCC AGT ATT CTT AAC CC-3' (with extension).

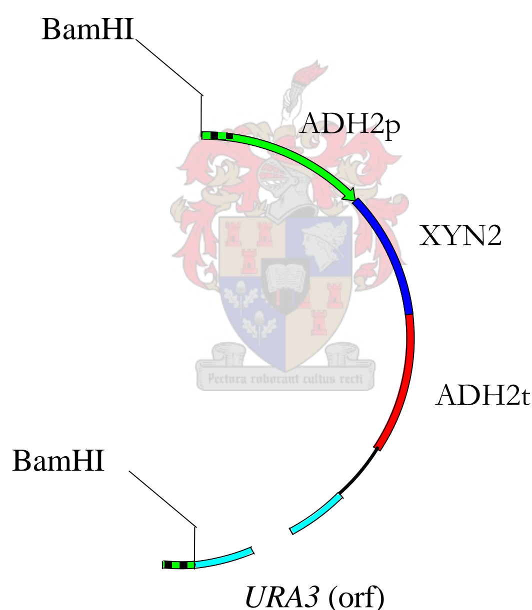


Figure 6.5. Primary PCR products obtained with Strategy Two.

Two options for using the two primary (purified) PCR products to generate integrative fragments were investigated: Firstly, the two primary products were used concurrently as template DNA in a single PCR reaction with the primers binding in the middle of the *URA3* gene. The overlapping regions (19 bp extension) would bind during the annealing and elongation steps, and thereby facilitate the generation of a single product with *URA3* flanking of the expression cassette (See Fig. 6.6A). Alternatively (secondly) the two primary PCR products were first cut with BamHI, producing

compatible sticky ends on both fragments. Ligation of fragments was attempted, again using T4 DNA Ligase, as the probability of a successful sticky end ligation should be much higher than that of a blunt end ligation. Successful PCR amplification of the ligation product, also using the primers that bind to the middle of the *URA3* gene, would produce the required integrative fragments (See Fig. 6.6B).

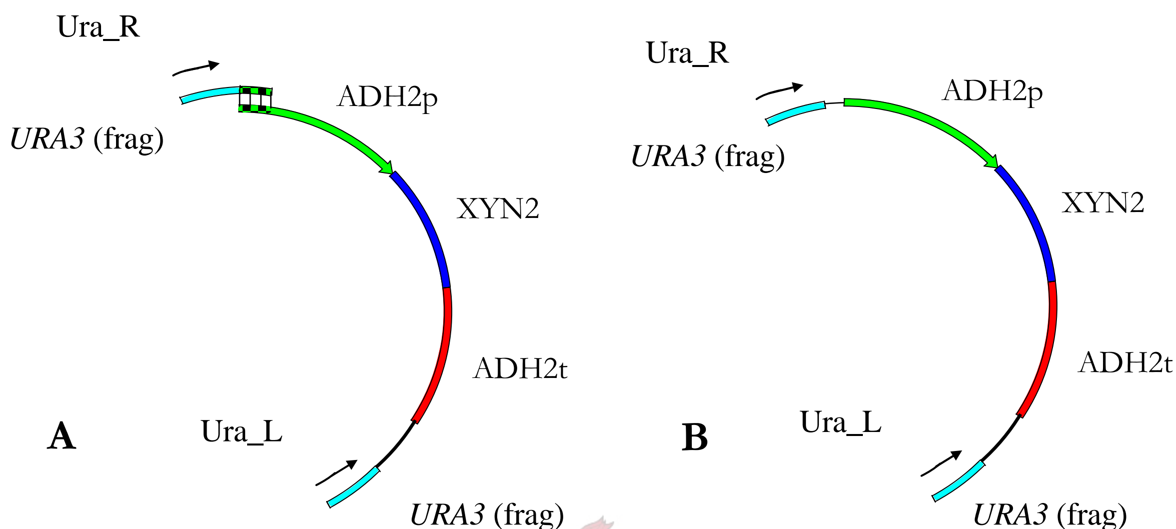


Figure 6.6. Use of primary PCR products in Strategy Two to obtain integrative fragments.

For both the *pDLG5* and *pDLG6* cassettes the generation of the Short primary PCR products were trivial. However, generation of the Long PCR products (middle of the *URA3* gene to the end of the expression cassette) was more problematic and required much optimisation of the reaction conditions. The Long product was eventually obtained for the *pDLG5* cassette ([ADH2-XYN]), though not for the *pDLG6* cassette. One of the primers in the primary reaction for the *pDLG6* cassette was also replaced with a primer binding further from the end of the expression cassette: 5'-GCG GAT CCA TTA AAA GAT AAA TAA TAG TCT ATA TAT ACG-3'. The primary PCR product could still not be obtained.

The attempted "Overlap PCR" (Fig. 6.6A) with the Long and Short products from the *pDLG5* cassette produced a smear of amplification products, which indicated improper binding in the overlap region resulting in unspecific amplification products. Further optimisation did not improve the product quality. PCR amplification of the ligated *pDLG5* fragments was also unsuccessful, despite attempted optimisations. The reverse primer binding to the middle of the *URA3* gene was also replaced, though no final

PCR product could be obtained with the new, ligated primary products. A severe limitation in the latter approach was the reliance on ligation products, as the generated amount of these products is very small. It is therefore not possible to determine prior to the PCR reaction whether the ligation was successful, which compromises rational approaches to optimisation of the procedure.

PCR STRATEGY THREE

In the final approach for obtaining integrative fragments, a single PCR amplification of the original template was performed using two new primers: The first primer was designed to bind 50 bp from the end of the Open Reading Frame (ORF) of the *URA3* gene, whereas the second annealed to the other end of the expression cassette. The latter primer contained the 50 bp between the start of the *URA3* ORF and the position where the first primer annealed, as an extension on the 5' end. A successful PCR amplification product would thus contain the expression cassette with 50 bp flanking of the *URA3* gene ORF on the one side, and the remainder of the *URA3* ORF on the other side (Fig. 6.7). The final primers were: *pDLG5*: 5'-TGC TGC CAA GCT ATT TAA TAT CAT GCA CG-3' and 5'-ACA GGA CTA GGA TGA GTA GCA GCA CGT TCC TTA TAT GTA GCT TTC GAC ATG GAT CCC ATC GTC CAT TC-3' (with 50 bp extension); *pDLG6*: 5'-TGC ACG AGT TAT AAT ATA TCG GGT GAC AC-3' and 5'-GCG GAT CCA TTA AAA GAT AAA TAA TAG TCT ATA TAT ACG GCA GCA CGT TCC TTA TAT GTA GCT TTC GAC ATG GAT CCC ATC GTC CAT TC -3' (with 50 bp extension).

PCR amplification of the *pDLG5* expression cassette with the newly designed primers proved trivial, whereas numerous optimisation efforts did not result in a PCR product with the *pDLG6* cassette. The *pDLG6* plasmid was also sequenced in the areas close to the binding sites of the primers, which proved to match the designed primers exactly. The addition of "PCR enhancers" such as BSA, DMSO and Tween 20 also did not result in the generation of a proper PCR product, and no explanation for the poor performance of the *pDLG6* plasmid as PCR template could be afforded.

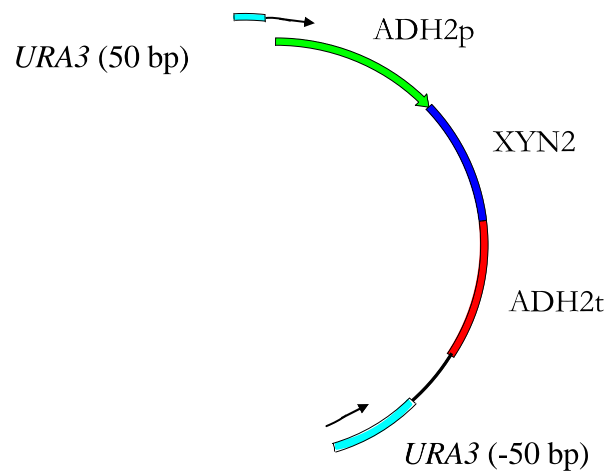


Figure 6.7. Strategy Three to obtain integrative fragments containing an expression cassette.

6.3.1.2.B. Transformation of S. cerevisiae *Y294 [*ura3*]*

The *S. cerevisiae* Y294 [*ura3*] host strain was transformed with the integrative fragment generated by PCR from the *pDLG5* expression cassette, containing only DNA from yeast origin. Recombination of the non-functional *URA3* gene ORF on the yeast chromosome with the functional fragments of the ORF contained in the integrative plasmid (double cross-over procedure) resulted in a single functional version of the *URA3* gene ORF on the chromosome (Fig. 6.8). Transformants were selected on the basis of prototrophy.

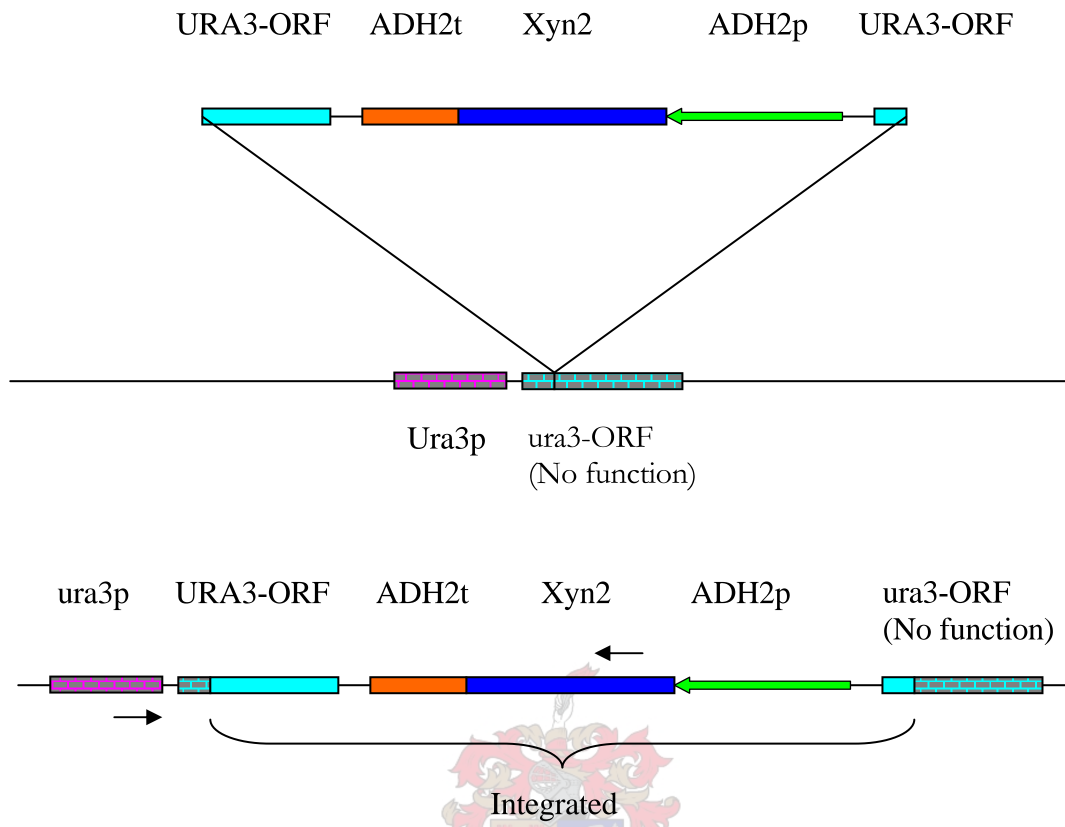


Figure 6.8. Recombination of chromosomal *ura3* ORF with integrative plasmid *URA3* ORF. Native *ura3* promoter was used for regulation of *URA3* expression. Binding sites of PCR primers used to verify the presence of the *XYN2* expression cassette are indicated.

To confirm the presence of the ADH2-XYN expression cassette and the *XYN2* gene on the chromosome, the transformants were cultivated in shake-flasks and the presence of xylanase activity confirmed (Appendix B, Table B.1). The total genomic DNA content of several transformants was also isolated, and the presence of the *XYN2* gene confirmed with PCR (Fig. 6.9). The functional version of the *URA3* ORF was integrated into the chromosome of the *S. cerevisiae* Y294 [*ura3*] host strain downstream from the original *ura3* promoter (Fig. 6.8). The generation of a PCR product using the genomic DNA as template and primers that bind inside the *ura3* promoter and inside the *XYN2* gene (indicated by arrows in Fig. 6.8) thus proved that the *XYN2* gene was present on the chromosome (Fig. 6.9). The resulting strain was named [ADH2-XYN] Int.

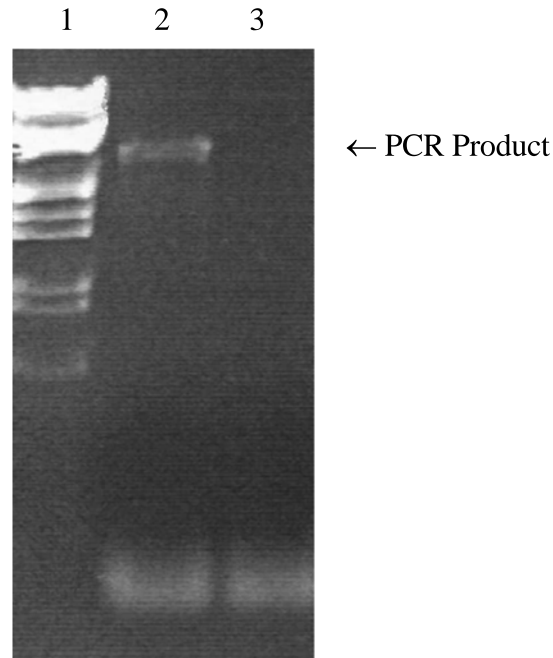


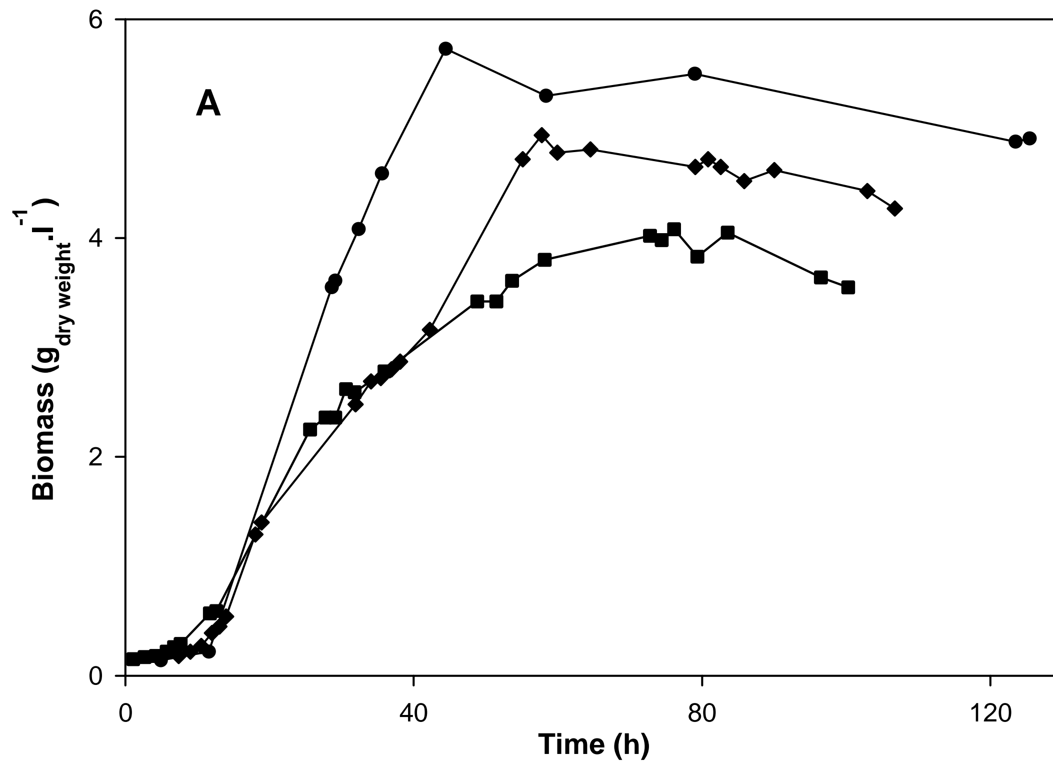
Figure 6.9. Use of PCR to confirm integration of the ADH2-XYN expression cassette (rearranged from plasmid *pDLG5*) in *S. cerevisiae* Y294 [ADH2-XYN] Int. Lane 1: Molecular weight marker; Lane 2: Successful integration and PCR, showing a 2.7 kbp product; Lane 3: Unsuccessful integration.

6.3.2. Comparison of prototrophic and auxotrophic transformants in batch culture

Prototrophic and auxotrophic strains were compared in batch culture to determine the effect of expression vector selection and auxotrophic requirements on the production level of heterologous xylanase and the physiology of the transformant strain.

6.3.2.1. Comparison of prototrophic strains

The growth and xylanase production of the constructed prototrophic strains ([ADH2-XYN] Int, [ADH2-XYN] PlasPro, [PGK1-XYN] PlasPro) were first compared in batch cultivation in defined medium without amino acids (Fig. 6.10).



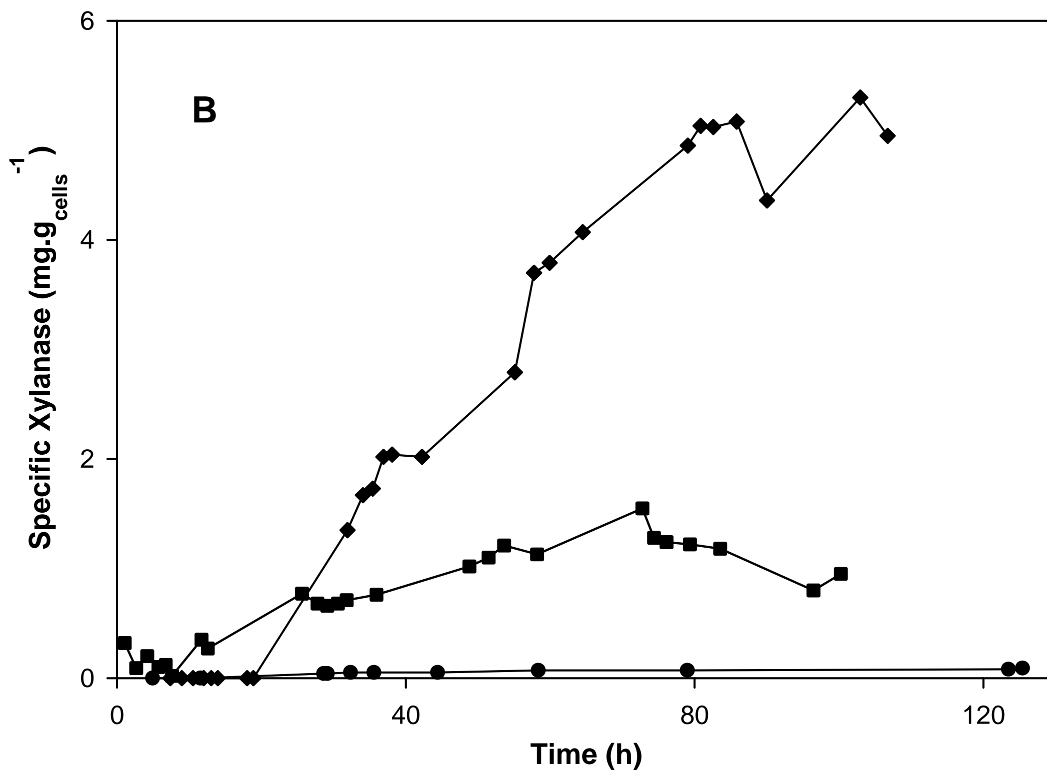


Figure 6.10. (A) Growth and (B) Specific xylanase production by the prototrophic recombinant *S. cerevisiae* strains in defined medium without amino acids. (♦) [ADH2-XYN] PlasPro, (•) [ADH2-XYN] Int, and (■) [PGK1-XYN] PlasPro strains.

During the cultivation of the prototrophic transformants, the patterns of heterologous xylanase production from the YEp-based expression vectors corresponded to the typical characteristics of the *PGK1* and *ADH2* promoters employed (Fig. 6.10). The characteristics of these promoters were discussed in Chapter 4. Xylanase production by the [PGK1-XYN] PlasPro strain was also inferior to the [ADH2-XYN] PlasPro strain, as was observed for the auxotrophic versions of the strains (Chapter 4), although the genetic instability of the former may have attributed to this effect. Though xylanase production by the [ADH2-XYN] Int strain was measurable, it was very poor compared to the YEp-based prototrophic strains. The [ADH2-XYN] Int strain apparently grew faster and to a higher biomass yield than the other strains, with the [PGK1-XYN] PlasPro strain growing the slowest and to the lowest final biomass yield. Due to the presence of genetic instabilities, no further investigation of the [PGK1-XYN] PlasPro strain was undertaken, and the comparison of auxotrophic and prototrophic strains was thus limited to expression systems regulated by the *ADH2* promoter.

6.3.2.2. Comparison of plasmid-based auxotrophic and prototrophic transformants with ADH2*-regulated expression systems in batch culture*

The auxotrophic and prototrophic versions of the YEp-based [ADH2-XYN] strain were also compared in batch culture. To support the growth of the auxotrophic strain, the chemically defined medium was first supplemented only with the amino acids, histidine (165 $mg.l^{-1}$), leucine (870 $mg.l^{-1}$) and tryptophan (664 $mg.l^{-1}$). Whereas histidine and tryptophan represent auxotrophic requirements, leucine was added because of the high levels of consumption in defined medium previously reported (Albers, 2000; Kozlov et al., 1995; L. Gustafsson, personal communication). The concentrations of these amino acids were selected by a rational approach, based on the requirements for biomass formation (Greasham and Herber, 1997; unpublished results), with the total amino acid concentration in the medium corresponding to 10.8 mM. During shake-flask cultivation a medium with a total amino acid content of 4.3 mM was able to support the maximum level of biomass formation by the auxotrophic strain (Appendix B, Fig. B.1). At a total concentration of 10.8 mM, an excess of amino acids were thus available for biomass formation and heterologous protein production, justifying its use as the maximum level of addition. The effect of reducing the total amino acid concentration to 4.3 and 1.9 mM on the general fermentation performance of the strain, while keeping the ratios between the individual amino acids constant, was also evaluated. The influence the total auxotrophic amino acid concentration on the maximum specific growth rate of the

auxotrophic [ADH2-XYN] strain, compared to the prototrophic strain cultivated in defined medium without amino acids, is presented in Table 6.1.

Table 6.1. Effect of total auxotrophic amino acid concentration on the growth of the auxotrophic [ADH2-XYN] strain.

Total Amino Acid Concentration [mM]	**Maximum Specific Growth Rate, μ_{max} [h^{-1}]**
10.8	0.22
4.3	0.30
1.9	0.41
0.0**	0.32

** [ADH2-XYN] PlasPro strain

Decreasing the total amino acid concentration in the defined medium increased the growth rate of the auxotrophic strain, resulting in a fairly fast growth rate for the auxotrophic strain in a medium containing only 1.9 mM of amino acids. The substrate consumption and product formation during these cultures are presented in Figures 6.11 and 6.12. The highest levels of biomass formation, and xylanase production per unit of biomass, were observed during cultivation of the auxotrophic strain in a medium supplemented with the maximum level of amino acids (10.8 mM). Growth and xylanase production in the latter cultures were comparable to levels obtained with the prototrophic strain in the medium without amino acids, and were significantly improved compared with cultures with 4.3 or 1.9 mM of amino acids (Fig. 6.11A and B). The increase in biomass and xylanase production in the former two cultures was accompanied by a decrease in ethanol formation (Fig. 6.11D) and an increase in CO_2 production (Fig. 6.11E; Fig. 6.12C), though the rate of glucose uptake (i.e. the glycolytic flux) was unaffected (Fig. 6.12A). The decreased biomass yield of the auxotrophic strain in the presence of 4.3 mM amino acids thus resulted in increased ethanol, carbon dioxide and glycerol formation (Fig. 6.11A, D and E; Fig. 6.12C and D).

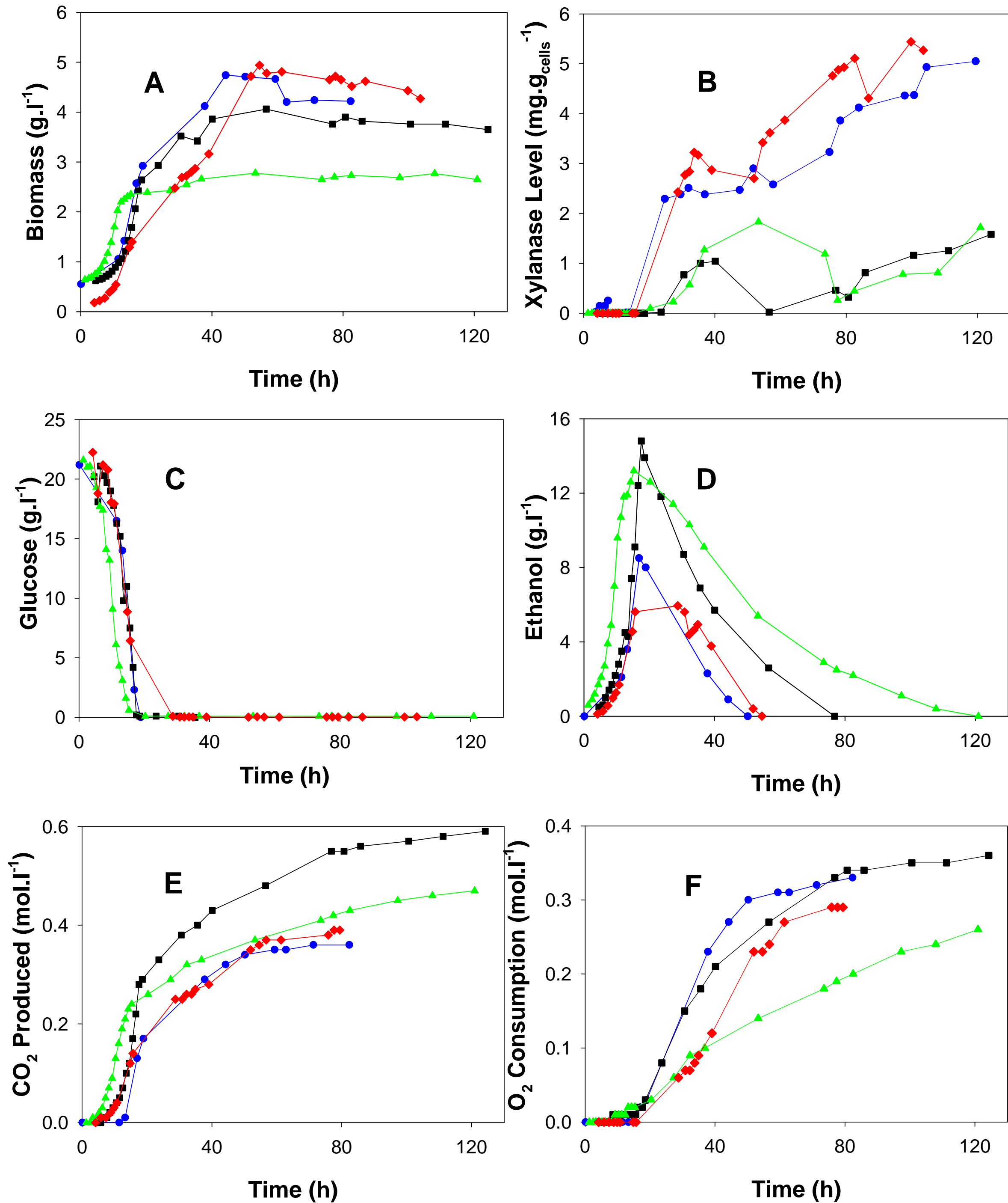


Figure 6.11. Substrate consumption and production formation by the auxotrophic [ADH2-XYN] and prototrophic [ADH2-XYN] PlasPro strain during batch cultivation in defined medium. The [ADH2-XYN] PlasPro strain (◆) was cultivated in a defined medium without amino acids, whereas the auxotrophic strain was cultivated in a medium containing the amino acids, histidine, leucine and tryptophan at a total concentration of 10.8 mM (●), 4.3 mM (■) and 1.9 mM (▲).

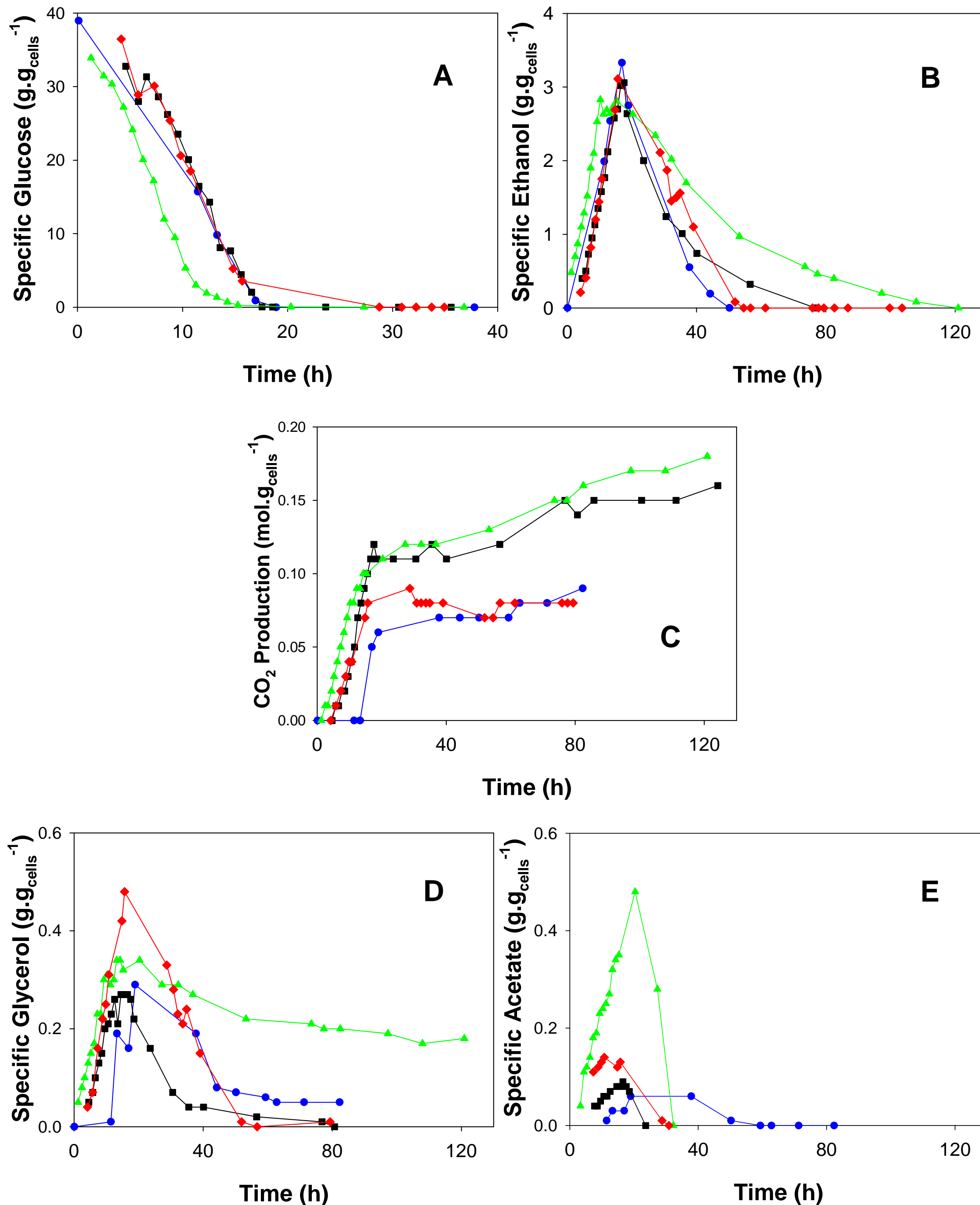


Figure 6.12. Substrate consumption and production formation by the auxotrophic [ADH2-XYN] and prototrophic [ADH2-XYN] PlasPro strain during batch cultivation in defined medium. The [ADH2-XYN] PlasPro strain (◆) was cultivated in a defined medium without amino acids, whereas the auxotrophic strain was cultivated in a medium containing the amino acids, histidine, leucine and tryptophan at a total concentration of 10.8 mM (●), 4.3 mM (■) and 1.9 mM (▲).

Decreasing the total amino acid concentration below 4.3 mM limited biomass formation by the auxotrophic strain, as the final biomass yield in the cultivation with 1.9 mM of amino acids was significantly reduced (Fig 6.11A). Severe amino acid limitation apparently set in before glucose was completely consumed, resulting in immediate cessation of biomass formation (Fig. 6.11 A and C), though ethanol formation may have continued for a short while after amino acid depletion. Despite the severe amino acid limitation of biomass formation, the xylanase production levels during cultivation in the presence of 1.9 and 4.3 mM of amino acids were approximately equal. However, these levels were significantly lower than those obtained with the prototrophic strain in defined medium without amino acids, or with the auxotrophic strain during cultivation in the presence of an excess of amino acids (10.8 mM) (Fig. 6.11A and B). The faster growth rate of the auxotrophic strain in the presence of 1.9 mM of amino acids was reflected by an increased rate of glucose consumption, and carbon dioxide and ethanol formation (Fig. 6.11C, D and E; Fig. 6.12A, B and C). Ethanol and glycerol consumption after the diauxic shift in the presence of severe amino acid limitation (1.9 mM amino acids) was apparently much reduced, though high levels of carbon dioxide formation was observed during this metabolic phase (Fig. 6.11D and E; Fig. 6.12B, C and D). The degree of reduction- and carbon -balances also indicated that evaporation contributed significantly to the time-wise decrease in ethanol levels after severe amino acid limitation, rather than metabolic consumption (Appendix B, Table B.2). The level of acetate formation was significantly increased during severe amino acid limitation (Fig. 6.12E).

6.3.2.3. Effect of amino acid supplementation on the prototrophic YEp- and YIp-transformants in batch culture

In an effort to further improve xylanase production by the auxotrophic strain, the amino acid content of the defined medium was increased, prior to cultivation in glucose-limited chemostat, to include the amino acids aspartate (257 $mg.l^{-1}$), glutamate (64 $mg.l^{-1}$), glycine (33 $mg.l^{-1}$) and serine (108 $mg.l^{-1}$), in addition to those already present at maximum concentration in the defined medium. These amino acids and their concentrations were also selected by considering biosynthetic requirements (suggested by Greasham and Herber, 1997; Chapter 4). The effect on the prototrophic strains of supplementing the defined medium with all seven amino acids was tested in batch culture (Fig. 6.13).

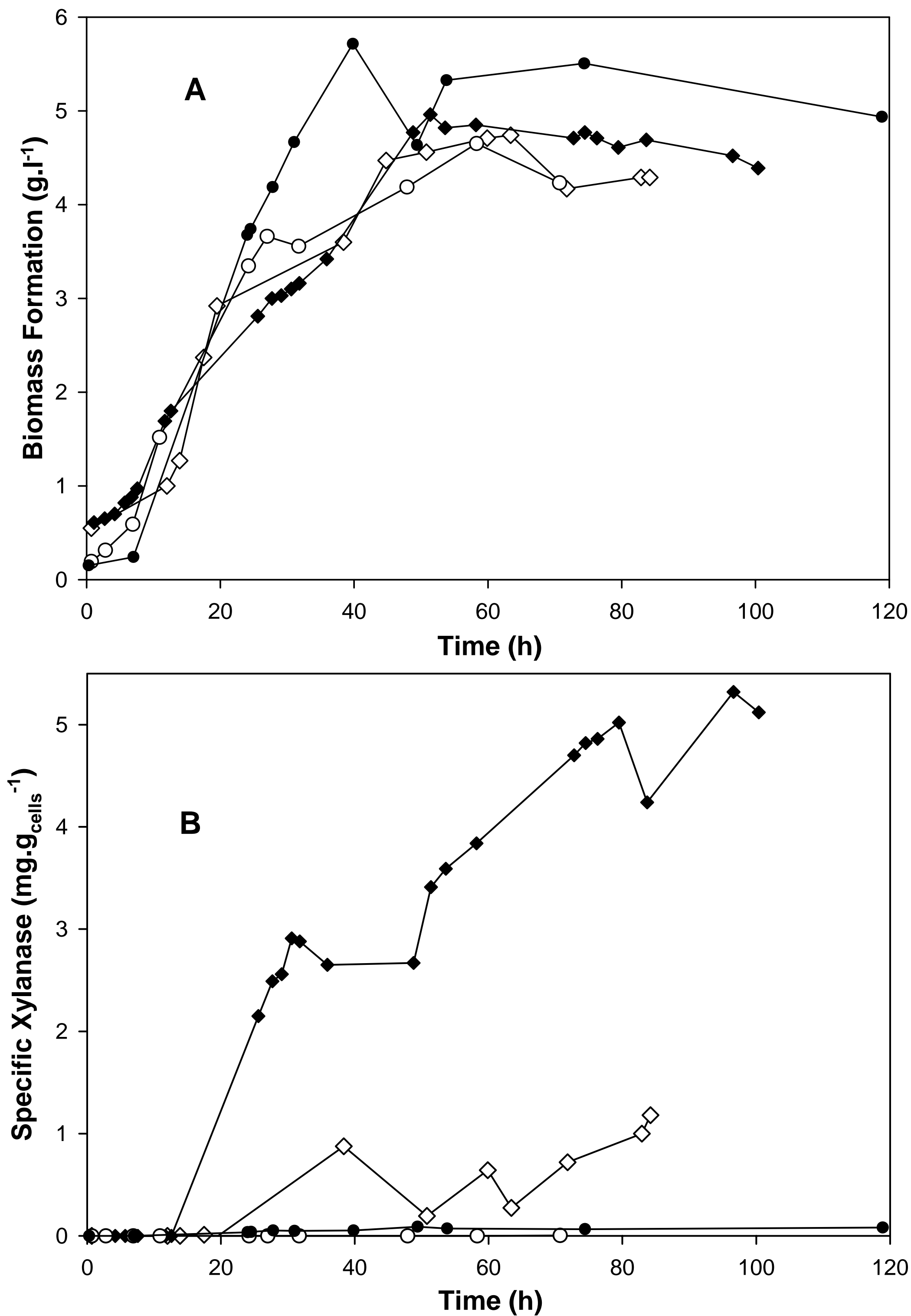


Figure 6.13. Growth and xylanase production by the prototrophic [ADH2-XYN] PlasPro (◆,◇) and [ADH2-XYN] Int (●,O) strains during batch cultivation in defined medium. Open symbols indicate cultivation in medium with the seven amino acid mixture, and closed symbols in the medium without. (A) Biomass formation ($g.l^{-1}$) and (B) specific xylanase production ($mg.g_{cells}^{-1}$).

Addition of the selected amino acid mixture to the defined medium significantly inhibited xylanase production by both the [ADH2-XYN] PlasPro and [AND-XYN] Int prototrophic strains, though the growth of the strains was not severely affected (Fig. 6.13). Xylanase production levels by the [ADH2-XYN] Int strain in defined medium without amino acids was measurable, though in the medium with amino acids no production could be measured.

6.3.3. Comparison of prototrophic and auxotrophic transformants in glucose-limited chemostat culture

The growth and heterologous xylanase production of the prototrophic [ADH2-XYN] PlasPro and [ADH2-XYN] Int strains were compared to the performance of two auxotrophic strains, [ADH2-XYN] and [PGK1-XYN], in chemostat culture. Cultivation of the strains at low dilution rates (0.1 h^{-1} or below) under aerobic conditions resulted in a fully respiratory metabolism, characterised by high levels of biomass and CO_2 formation with very little ethanol production. The stabilisation of the YEp-expression vector by the *ura3 fur1* autoselection system resulted in excellent genetic stability in continuous cultivation, with no significant decrease in xylanase production or plasmid stability by the auxotrophic [ADH2-XYN] and [PGK1-XYN] strains during 1150 hours of growth, corresponding to more than 100 generations.

6.3.3.1. Oscillatory behaviour during fully aerobic, glucose-limited growth in chemostat culture

In some chemostat cultures, the attainment of steady-state levels of carbon dioxide production and oxygen consumption was complicated by cell cycle synchronisation in the yeast population, resulting in oscillatory changes in the CO_2 and O_2 concentrations in the gas-outlet from the fermenter (Fig. 6.14). This phenomenon has previously been associated with fully aerobic metabolism in chemostat culture (Beuse et al., 1999; Sohn et al., 2000; Murray et al., 1998). The period of the oscillations varied between 4.5h ([AND2-XYN] Int) and 6-8h ([ADH2-XYN] PlasPro).

Frequent sampling during oscillatory behaviour confirmed that oscillations had no measurable effect on the production level of heterologous xylanase, whereas representative steady-state values for the CO_2 and O_2 levels in the outlet during these oscillations were obtained by averaging over several residence times. The effect of

oscillations on the steady-state readings could further be minimised by comparing HPLC-measured concentrations of glucose and extracellular metabolites in the fermenter during the final three residence times prior to steady-state sampling.

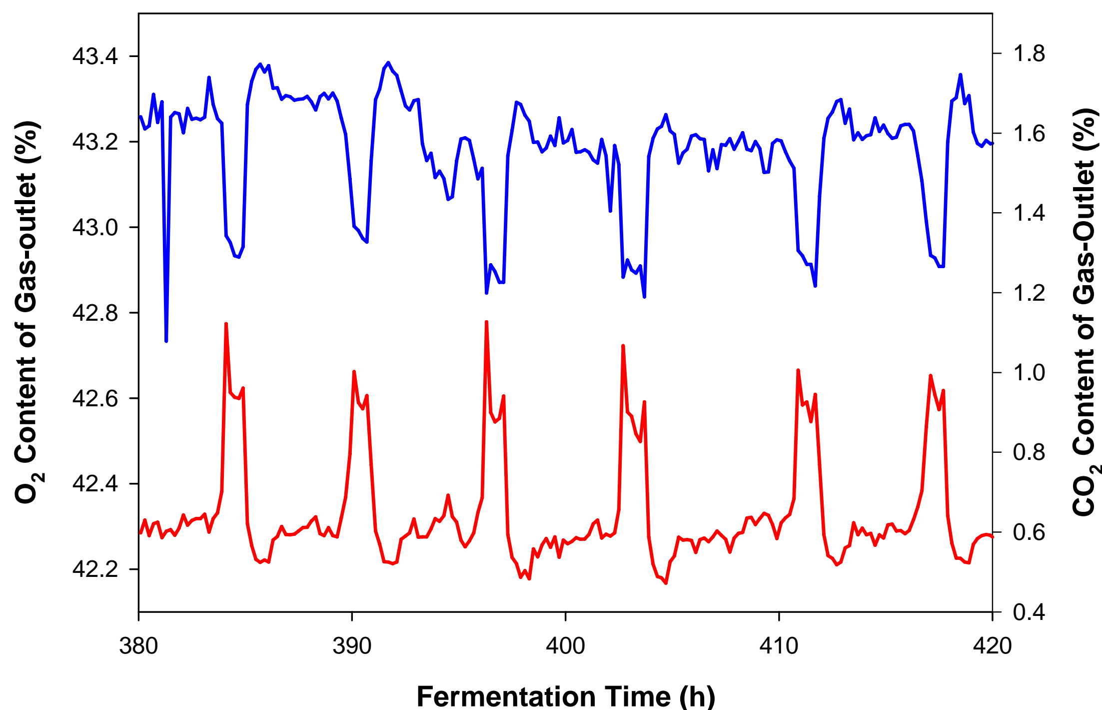


Figure 6.14. Oscillatory behaviour of [ADH2-XYN] PlasPro yeast population during fully aerobic growth in glucose-limited chemostat culture, maintained at a dilution rate of 0.1 (h^{-1}) by feeding of chemically defined medium without amino acids. Graph indicates changes in the O_2 (upper, blue line) and CO_2 (lower, red line) content (%) of the gas-outlet from the fermenter.

6.3.3.2. Comparison of auxotrophic and prototrophic transformants producing heterologous xylanase in glucose-limited chemostat culture

For the cultivation of the auxotrophic strains in glucose-limited chemostat culture, the defined feed medium, containing 15 $g.l^{-1}$ of glucose, was supplemented with seven amino acids at the maximum concentrations (see above). The effect of varying the total amino acid concentration in the feed was investigated by reducing the concentration of glucose in the feed from 15 to 5 $g.l^{-1}$, which subsequently decreased the C/N ratio ($g_{glucose}.mmol_{free\ amino\ nitrogen}^{-1}$) of the feed and the concentration of biomass in the fermenter, while increasing the total amino acid uptake per gram of biomass (Fig. 6.15).

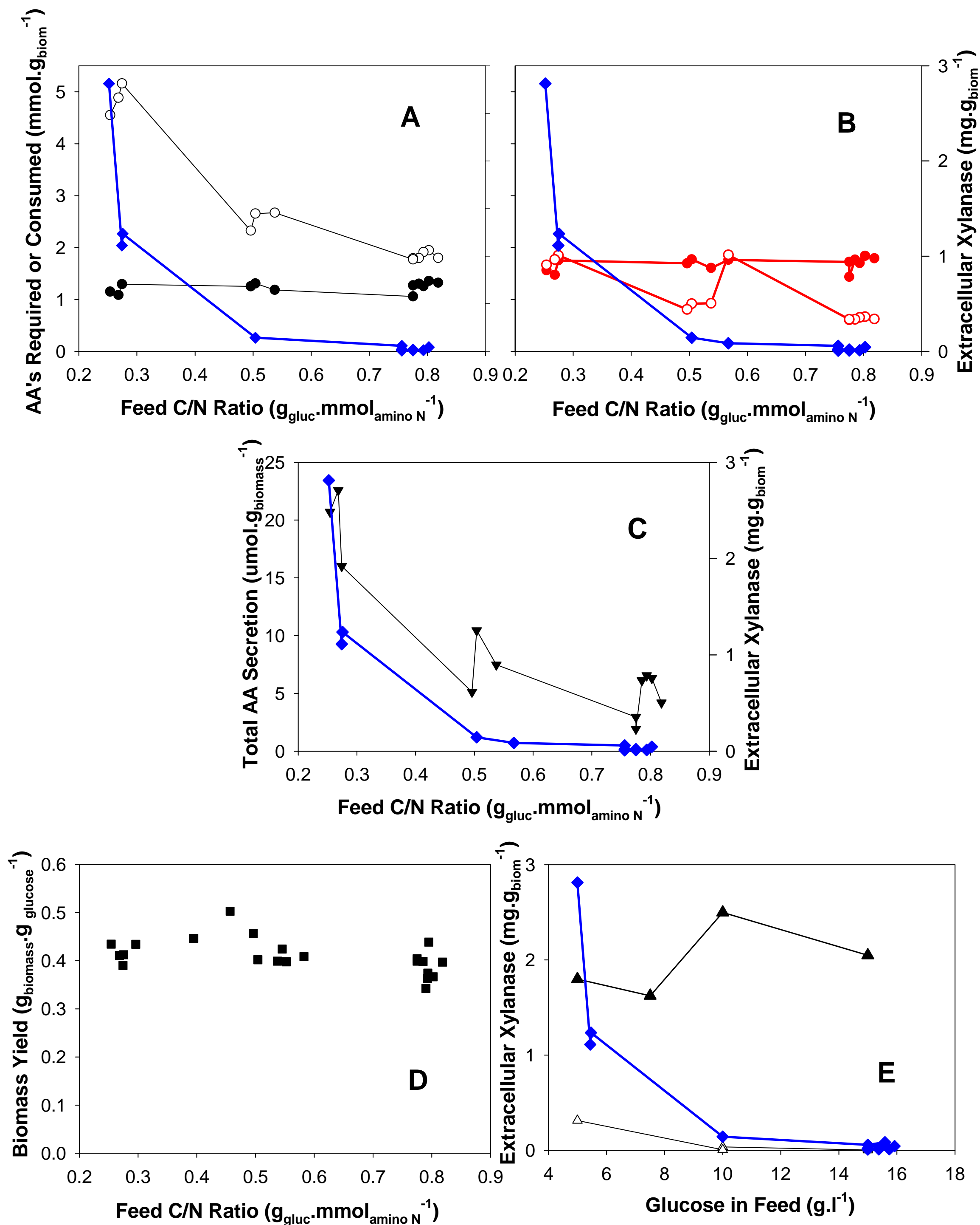
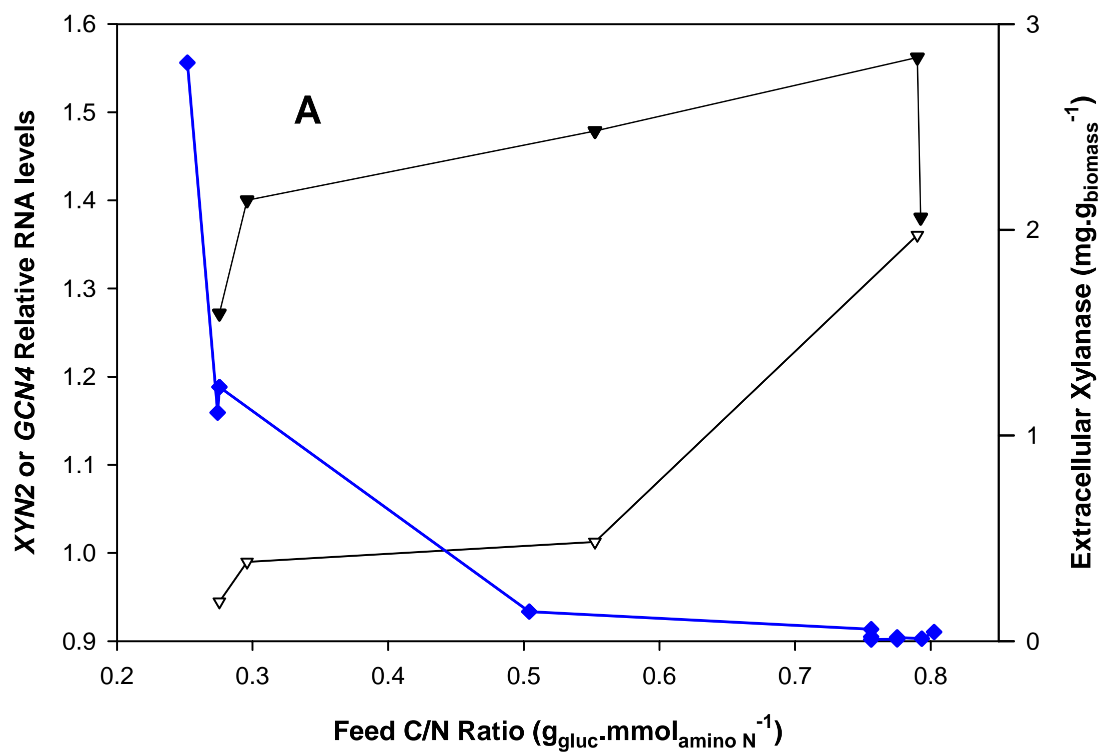


Figure 6.15. Xylanase production, biomass formation and amino acid metabolism of the auxotrophic [ADH2-XYN] and [PGK1-XYN] strains in glucose-limited chemostat culture. (◆) Extracellular xylanase production (mg.g$_{biomass}^{-1}$). (A) Consumption of Trp, His and Leu (●,O) and (B) consumption of Asp, Glu, Gly and Ser (●,O). (●,●) Total requirement for biomass formation and (O,O) actual amino acid consumption. (C) (▼) Total amino acid secretion (µmol.g$_{biomass}^{-1}$). (D) (■) Biomass yield (g$_{biomass}$.g$_{glucose\ consumed}^{-1}$). (E) For comparison, xylanase production by [ADH2-XYN] PlasPro in defined medium with seven amino acids (△) or defined medium without amino acids (▲).



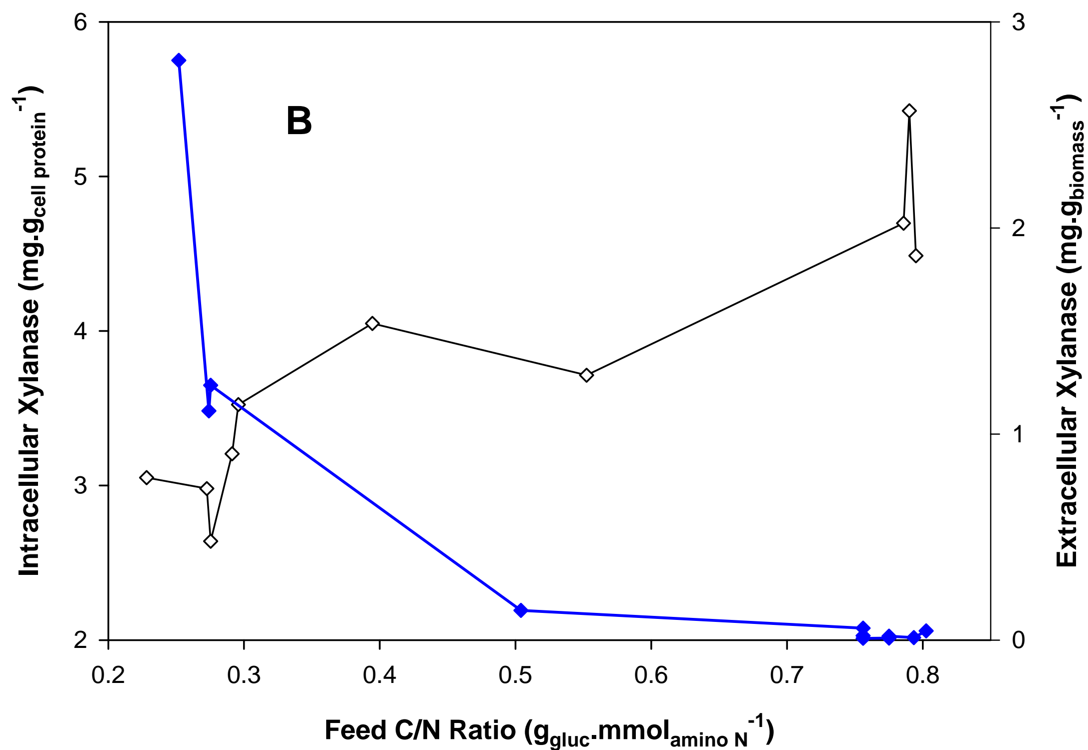


Figure 6.16. Expression and processing of Xyn2p by auxotrophic [ADH2-XYN] in glucose-limited chemostat culture. (◆) Extracellular xylanase production (mg.$g_{biomass}^{-1}$). (A) *XYN2* (▼) and *GCN4* (▽) total RNA levels, quantified with RT-PCR. (B) (◇) Intracellular level of xylanase protein (mg.$g_{protein}^{-1}$), based on activity measurements.

Heterologous xylanase production levels by both auxotrophic strains were radically dependent on the C/N ratio of the feed medium, and thus on the level of amino acid uptake per gram of biomass (Fig. 6.15). The dramatic improvement in specific xylanase production demonstrated in Fig. 6.15 (A and B) was caused solely by changing the ratio between glucose and amino acids in the feed, since changing the concentrations of other defined medium components (ammonium, mineral salts, vitamins and trace elements) did not have a similar effect (Appendix B, Table B.3). Differences in the xylanase production levels of the [ADH2-XYN] and [PGK1-XYN] auxotrophic strains were overshadowed by the dramatic increase in production levels in response to high levels of amino acid uptake, indicating that the effect was strongly related to the host strain physiology and genetics. Increased production of xylanase was apparently caused by the increased uptake of leucine, histidine and tryptophan, since increasing the concentration of the other four amino acids (aspartate, glutamate, glycine and serine) did not cause a similar increase in production levels (Fig. 6.15B; C/N ratio ≈ 0.6). Levels of leucine, histidine and tryptophan uptake were constantly in excess of the stoichiometric requirement for biomass formation (Fig. 6.15A). Amino acid analysis also revealed a low level of secretion of the 13 amino acids not supplemented to the defined medium (Fig. 6.15C). The dramatic increase in extracellular xylanase production corresponded to a significant increase in the total amino acid secretion per gram of biomass (Fig. 6.15C). Although the biomass yields of the auxotrophic strains increased slightly when the C/N ratio of the feed was reduced from 0.8 to 0.55, no further increases were observed during the shift to the lowest C/N ratio (Fig. 6.15D), which corresponded to the maximum amino acid uptake and xylanase production. Increased amino acid uptake thus did not improve biomass formation. Changes in the extracellular levels of heterologous xylanase were also associated with decreases in the total *XYN2* and *GCN4* RNA-content of the auxotrophic [ADH2-XYN] cells (Fig. 6.16A). Fairly significant decreases in the intracellular xylanase level (based on activity measurements) were also observed in response to the decrease in the C/N ratio that resulted in high levels of extracellular xylanase (Fig. 6.16B). The dramatic increase in the level of extracellular xylanase production was not mimicked by a similar improvement in total protein secretion, although accurate measurements of total protein secretion was complicated by the low production levels and the dilution of samples during dialysis (Appendix B, Fig. B.2).

The formation of metabolic products by the auxotrophic [ADH2-XYN] strain was analysed by means of a carbon-balance, assisted by a degree of reduction balance (Table 6.2). Due to difficulties with the measurement of the O_2-content of the outlet, an RQ value of 1.07 (Sonnleitner and Käppeli, 1986) was used to calculate the O_2-consumption. Since the increase in xylanase production at low C/N ratios was not accompanied by a significant increase in the levels of metabolic products, the carbon skeletons of the amino acids imported by the cell were most likely not utilised, but rather secreted (Cooper, 1982). The utilisation of carbon skeletons would have resulted in a significant increase in ethanol production and evaporation (approximately 0.39 $cmol.cmol_{glucose\ consumed}^{-1}$), which is highly unlikely since the production of biomass and other metabolic products were unaffected.

The effect of high levels of amino acids in the defined medium on heterologous xylanase production by the prototrophic [ADH2-XYN] PlasPro and [ADH2-XYN] Int strains in chemostat cultivation was also determined. Supplementation of the defined medium with all seven amino acids significantly inhibited xylanase production by the [ADH2-XYN] PlasPro strain (Fig. 6.15E), similar to the observation made during batch cultivation in defined medium (Fig. 6.13). Xylanase production levels by the [ADH2-XYN] PlasPro strain during chemostat cultivation in the same defined medium without amino acids were very similar to the maximum levels produced by the auxotrophic [ADH2-XYN] strain during chemostat cultivation in the defined medium supplemented with maximum levels of amino acids. Although cell cycle synchronisation and metabolic oscillations in chemostat was observed more frequently with the prototrophic than with the auxotrophic strains, no oscillatory behaviour was observed for the [ADH2-XYN] PlasPro strain when cultivated in the presence of all seven amino acids. The biomass yield of the [ADH2-XYN] PlasPro strain during fully aerobic cultivation was also negatively affected by the presence of these amino acids (Table 6.3). Although the levels of xylanase production by the [ADH2-XYN] Int strain in chemostat culture were too low for quantification, biomass formation was negatively affected by the presence of all seven amino acids (Table 6.3). Even in the presence of maximum amino acid levels, the biomass yield of the auxotrophic strain was lower than for the prototrophic strain in defined medium without amino acids (Table 6.3). For both the [ADH2-XYN] PlasPro and [ADH2-XYN] Int strains, no significant genetic instability was observed during continuous cultivation up to 690 and 400 hours, respectively.

Table 6.2. Carbon-balance* for the auxotrophic [ADH2-XYN] during glucose-limited chemostat cultivation in defined medium.

Feed		Co-substrates		Metabolic products [cmol]							Degree of	Carbon
C/N ratio[a]	Glucose[b]	AA's [cmol]	O_2 [amol][c]	Biomass	CO_2	Glycerol	Acetate	Succinate	Ethanol[d]	Xylanase [mg]	reduction	balance
0.802	15.9	-0.187	-1.11	0.405	0.592	0.001	0.0	0.006	0.012	0.49	0.0	1.02
0.793	15.7	-0.188	-1.06	0.413	0.568	0.001	0.001	0.004	0.021	0.13	0.0	1.01
0.793	15.7	-0.188	-1.16	0.401	0.621	0.008	0.002	0.010	0.008	0.23	0.0	1.02
0.790	15.7	-0.189	-1.18	0.379	0.630	0.008	0.002	0.011	0.008	0.60	0.0	1.02
0.775	15.4	-0.188	-1.02	0.445	0.546	0.008	0.0	0.004	0.008	0.23	0.0	1.01
0.775	15.4	-0.188	-1.03	0.440	0.549	0.009	0.001	0.004	0.009	0.11	0.0	1.01
0.567	15.6	-0.238	-1.10	0.491	0.589	0.008	0.0	0.003	0.016	1.13	0.0	1.01
0.552	11.0	-0.261	-1.03	0.441	0.551	0.015	0.0	0.003	0.014	1.71	0.0	1.01
0.395	7.8	-0.366	-0.90	0.495	0.481	0.016	0.0	0.003	0.008	30.48	0.0	1.00
0.296	5.9	-0.475	-0.86	0.484	0.462	0.024	0.0	0.003	0.021	37.14	0.0	0.99
0.291	11.6	-0.495	-0.88	0.452	0.473	0.015	0.0	0.003	0.043	27.61	0.0	0.99
0.275	5.5	-0.509	-0.97	0.459	0.518	0.029	0.0	0.002	0.001	15.47	0.0	1.01
0.274	5.4	-0.530	-1.05	0.433	0.563	0.0	0.0	0.003	0.012	13.15	0.0	1.01
0.273	10.8	-0.529	-1.19	0.470	0.635	0.014	0.0	0.003	0.018	27.69	0.0	1.02

*Note: Except for Feed Glucose in [$g.l^{-1}$], the units of measurements are reported on the basis of one cmol of glucose consumed.

[a] $g_{glucose}.mmol_{amino\ N}^{-1}$ [b] $g.l^{-1}$ [c] Moles of O-atoms

[d] Corrected for evaporation, as calculated with degree of reduction balance

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Table 6.3. Biomass yield of auxotrophic and prototrophic strains in chemostat culture.

Strain	Amino acid supplementation	Biomass yield
[ADH2-XYN] Aux	7 AA's (Max)	0.42 ± 0.04
[ADH2-XYN] PlasPro	None	0.53 ± 0.04
[ADH2-XYN] PlasPro	7 AA's (Max)	0.45 ± 0.03
[ADH2-XYN] Int	7 AA's (Low)	0.57 ± 0.02
[ADH2-XYN] Int	7 AA's (Max)	0.40

6.4. DISCUSSION

The growth and heterologous xylanase production of a *trp1 his3* auxotrophic *S. cerevisiae* transformant, from a YEp-based expression vector stabilised by an autoselection system (Loison et al., 1986), was characterised in a chemically defined medium containing various concentrations of the required amino acids. The performance of the strain was compared to two prototrophic transformants, containing either the identical autoselection-YEp expression system, or an alternative YIp-type expression vector with the same expression cassette. The effect of expression vector selection and auxotrophic mutations on the growth and heterologous xylanase production of recombinant *S. cerevisiae* in batch and continuous culture could thus be determined.

6.4.1. Choice of expression vector

The choice of expression vector significantly affected production levels of heterologous xylanase. Production levels by the YIp-type expression vector were largely inferior to levels obtained with the YEp-type vectors, both in batch and continuous culture. The increased gene dosage obtained with the YEp-type vectors thus dramatically improved heterologous xylanase production, as has been reported for other heterologous protein production systems (Smith et al., 1985; Bitter et al., 1987; Denis and Drouin, 1987; Kaisho et al., 1989; Janes et al., 1990; Weber et al., 1992; Mendoza-Vega et al., 1994; Compagno et al., 1996; Lopes et al., 1996; Nacken et al., 1996; Gellissen and Hollenberg, 1997; Ljubijankic et al., 1999; Park et al., 2000; Vassileva et al., 2001; Kim et al., 2001). The increase in the growth rate and biomass yield of the [ADH2-XYN]

Int strain compared to the YEp-based strains in batch culture (Fig. 6.10) was apparently related to the absence of the "metabolic loads" associated with the retention of multiple copies of the 2μm plasmid (Mead et al., 1986) and the production of the heterologous xylanase (Chapter 4).

The high level of foreign gene expression from the multi-copy YEp-type vector did not saturate the secretory capacity of the host strain for proteins, though in other cases saturation has been observed for similar expression systems (Wittrup et al., 1994; Tuite and Freedman, 1994; Parekh et al., 1995; Parekh and Wittrup, 1997). The excellent genetic stability of the present YEp-based transformants during chemostat cultivation demonstrated the ability of the *ura3 fur1* autoselection system to stabilise YEp-type vectors (Marquet et al., 1987), and cast doubt on the necessity of extending the system by inclusion of *urid-k* disruption (Wang and Da Silva, 1993). The total xylanase production of 24 $mg.l^{-1}$ by the prototrophic [ADH2-XYN] PlasPro strain at low biomass concentration (approximately 5 $g.l^{-1}$) in batch cultivation was of the same order of magnitude as the 60 $mg.l^{-1}$ of heterologous xylanase obtained with *P. pastoris* under similar conditions (Berrin et al., 2000). Use of autoselection-YEp-based expression vectors may be preferred to multi-copy integration systems, such as the *loxP* or repetitive DNA-targeting methods (Lee and Da Silva, 1996; Güldener et al., 1996; Parekh et al., 1996), due to ease of molecular biology techniques (Alberghina et al., 1993).

6.4.2. Influence of auxotrophic requirements

The effect of uncomplimented auxotrophic markers on the production levels of heterologous proteins by transformed strains remain unclear, since two of the highest production levels of heterologous protein reported in *S. cerevisiae* were obtained by cultivating an auxotrophic transformant in complex medium (De Baetselier et al., 1991; Lee et al., 1999). However, despite these examples of high production levels with auxotrophic transformants, convincing evidence was given in the present investigation of the potential negative effects of these markers.

6.4.2.1. Effect on growth

A limited availability of the required amino acids significantly reduced biomass formation by the auxotrophic [ADH2-XYN] strain in batch culture, similar to previous observations (Beretta et al., 1991; VanDusen et al., 1997; Shiba et al., 1998; Zigova et al., 1999). Increasing the concentration of these amino acids in the defined medium increased the final biomass concentration, until the maximum biomass yield was attained, whilst decreasing the growth rate of the transformant strain. The latter observation is consistent with the frequent over-accumulation of amino acids by yeast, even to the point of inhibition of its own growth (Eddy, 1982). Poor growth on ethanol during severe amino acid limitation indicated that the activity of the enzymes required for gluconeogenesis was also reduced.

6.4.2.2. Effect on heterologous xylanase production

Supplementation of the defined medium with an excess of leucine, histidine and tryptophan, compared to the requirements for biomass formation, dramatically increased heterologous xylanase production by the auxotrophic [ADH2-XYN] and [PGK1-XYN] strains, both in batch and chemostat cultivation. Extremely low levels of heterologous xylanase production by auxotrophic, YEp-based transformants in defined media without amino acid excess, were previously reported (Donald et al., 1994; Pérez-González et al., 1996; Nuyens et al., 2001), along with increased production of various heterologous proteins by auxotrophic transformants due to increased amino acid availability (Carty et al. 1987; Wittrup and Benig, 1994; Shiba et al., 1998; Zigova et al., 1999; Rao et al., 2000). Wittrup and Benig (1994) observed a 8-fold increase in foreign protein production by a *leu2 ura3* auxotrophic transformant in batch cultivation due to supplementation with an excess of amino acids. Surprisingly, the level of total free amino nitrogen in the two defined media reportedly supporting higher production levels (37 and 19 mM; Wittrup and Benig, 1994) would have resulted in an amino acid availability of approximately 15 and 7.9mM.$g_{biomass}^{-1}$ in batch culture (assuming a biomass yield of 0.12 g.$g_{glucose\ consumed}^{-1}$), both of which are larger than the availability of 7.8 mM.$g_{biomass}^{-1}$ required for increased xylanase production in the present glucose-limited chemostat cultures. The effect of growth rate changes in chemostat culture on the levels of heterologous xylanase production by the auxotrophic strains were marginal compared to the effect of amino acid concentration in the feed (Appendix B, Fig. B.3).

The observation of the "amino acid effect" for both the *ADH2*- and *PGK1*-regulated expression systems, indicated a strong relation to the host strain characteristics.

The utilisation of an excess of auxotrophic amino acids, resulting in maximal levels of heterologous xylanase production by the auxotrophic strain, did not increase the level of biomass formation significantly. The excess of amino acids was therefore not incorporated into cellular protein, as observed in previous reports (Wittrup and Benig, 1994; Albers et al., 1996). The excess of amino acids was probably catabolised by de- or trans-amination, resulting in the utilisation of the amino groups and secretion of the carbon skeleton (Cooper, 1982). This occurs more readily when the amino acids are present at a high concentration (Grenson, 1992), as was the case when the C/N ratio of the feed medium was below 0.3 ($g_{glucose}.mmol_{amino\ N}^{-1}$) (Fig. 6.15A). Both metabolite balancing using the Metabolic Network Analysis software package (SPAD IT, The Netherlands), as well as the carbon- and degree of reduction-balances (Table 6.2), indicated that the carbon skeletons of the amino acids were probably not utilised.

The increased xylanase production at a C/N ratio below 0.3 ($g_{glucose}.mmol_{amino\ N}^{-1}$) in glucose-limited chemostat cultivation was associated with the increased secretion of the 13 amino acids not supplied in the defined medium, indicating a saturation of the auxotrophic yeast cells with amino acids (Fig. 6.15C). Although uptake and accumulation of most amino acids from the external medium seems irreversible, amino acids are excreted into the medium whenever available above a given threshold in yeast cells (Grenson, 1992). A saturation of auxotrophic yeast cells by excessive of amino acid uptake at low C/N ratio's was thus required to support maximal production of the heterologous xylanase by the auxotrophic strain. Improved xylanase production at a low C/N ratio was reflected in a marginal change of the level of total *XYN2* RNA (Fig. 6.16A), indicating that xylanase production was strongly regulated during posttranslational processing and secretion. Decreased levels of intracellular xylanase also indicated an improved secretion of the heterologous protein at a C/N ratio below 0.3 ($g_{glucose}.mmol_{amino\ N}^{-1}$). In Chapter 5, a significant induction of the *S. cerevisiae* stringent response in reaction to heterologous xylanase production was described, which has also been associated with an increase in intracellular proteolytic activity in other microorganisms (Harcum and Bentley, 1993). The increased production of heterologous xylanase in response to saturation of the auxotrophic cells with amino

acids, would indicate that the inhibition of proteolytic degradation of the foreign protein during posttranslational processing, may have caused the increased production levels observed for the auxotrophic [ADH2-XYN] strain at lower C/N ratio's.

Maximum levels of xylanase production obtained with the auxotrophic YEp-based strains during amino acid excess, both in chemostat (C/N ratio below 0.3) and batch (10.8 mM total amino acid level) cultivation, were similar to production levels obtained with the prototrophic YEp-based [ADH2-XYN] PlasPro strain during the same cultivation, though in defined medium without amino acids. The presence of auxotrophic mutations in transformants thus caused a physiological defect during the cultivation of the recombinant yeast, which severely reduced heterologous protein production. These excessive auxotrophic requirements either made it more difficult for the yeast to cope with the additional burden of heterologous protein production, or introduced irregularities into the amino acid metabolism. The auxotrophic transformants also required significant amino acid overconsumption to stabilise the nitrogen metabolism, and were more able to cope with these excessive amounts of extracellular amino acids, as was demonstrated by the inhibition of both growth and heterologous xylanase production of the prototrophic strains by high amino acid concentrations. The irregularities introduced by the presence of auxotrophic mutations in transformants thus reduced heterologous protein production severely, which supported a previous suggestion: Completely avoid the use of auxotrophic strains for heterologous protein production and metabolic engineering (Çakar et al., 1999).

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6.5. CONCLUSIONS

The present investigation has demonstrated the ability of an autoselection-YEp-based expression system to support increased levels of heterologous protein production by prototrophic transformants. The deleterious effects of auxotrophic mutations in transformants on heterologous xylanase production were also demonstrated, which required either the removal of these markers, or supplementation of the cultivation medium with an excess of the appropriate amino acids. The use of auxotrophic strains should be completely avoided.

Further evidence of the global sensing and regulation of heterologous protein production by the cell was presented by the response of auxotrophic recombinant strains to amino acid availability below the levels required for saturation of the cell, which resulted in a posttranscriptional down-regulation of production levels. The general physiological state of the yeast host and the availability of nutrients thus affected the biosynthetic capacity of *S. cerevisiae* towards the production of a heterologous protein.

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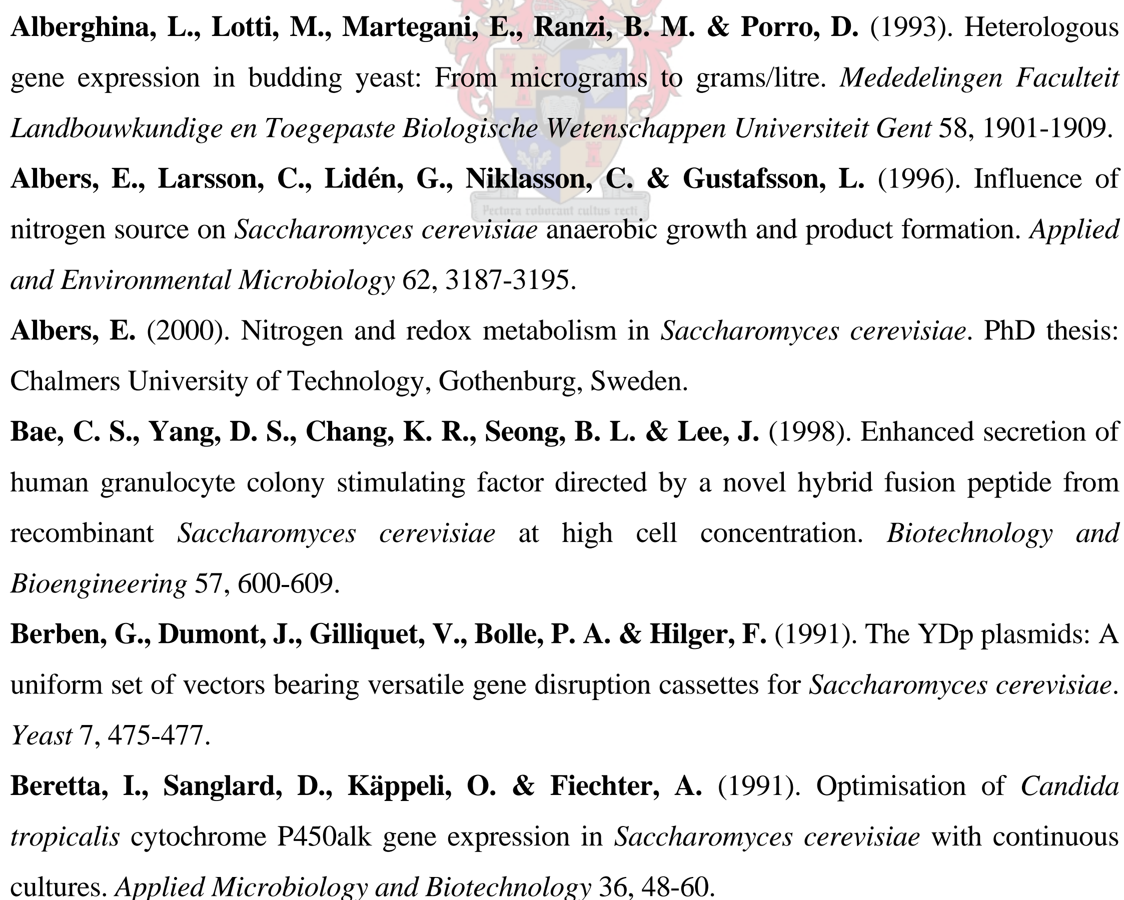

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Chapter 7

DEFINED MEDIUM REQUIREMENTS FOR IMPROVED HETEROLOGOUS XYLANASE PRODUCTION BY A PROTOTROPHIC TRANSFORMANT OF *S. CEREVISIAE*

7.1. INTRODUCTION

Although the use of a defined medium in processes for heterologous protein production is popular, due to easier purification of the product and the propensity towards rapid scale-up (Greasham and Herber, 1997), very low production levels of heterologous xylanases compared to complex medium have been reported (Donald et al., 1994; Pérez-González et al., 1996; Nuyens et al., 2001; unpublished results). The experimental results presented in Chapter 6 also indicated that the saturation of auxotrophic transformants with amino acids could dramatically improve heterologous xylanase production levels. In the present work, the existence of an increased requirement for amino acid and/or carbon metabolite availability during heterologous xylanase production by a prototrophic transformant was thus investigated. The possible use of all 20 amino acids or succinate, as an additional carbon source, for the enhancement of heterologous xylanase production by the prototrophic [ADH2-XYN] PlasPro strain (see Chapter 6) in defined medium, was investigated.

Supplementation of defined medium with exogenous nitrogen sources may improve the general physiology of yeast strains, specifically during fully respirative growth on ethanol in batch culture after the diauxic shift, which is not supported by some defined media (Chen et al., 1993; Gu et al., 1991). Supplementation of a defined medium with a mixture of amino acids has increased the level of biomass formation, the maximum specific growth rate and the glucose uptake rate by *S. cerevisiae*, due to direct incorporation of the consumed amino acids into biomass (Albers et al., 1996). Similar positive effects on growth and fermentation by *S. cerevisiae* due to amino acid supplementation has been reported elsewhere (Thomas and Ingledew, 1990; Thomas and Ingledew, 1992). Imbalances and deficiencies in the supply of assimilable nitrogen compounds are also the most common causes of fermentation faults in the wine

industry (Jiranek et al., 1995). Nitrogen sources most strongly preferred by *S. cerevisiae* include glutamine, asparagine and ammonium. These nitrogen sources lead to higher growth rates, when present in cultivation medium as the sole nitrogen source, than less preferred nitrogen sources and can prevent other nitrogen sources from being utilised (Ter Schure et al., 2000; Dubois and Messenguy, 1997; Wiame et al., 1985; Cooper, 1982).

The availability of amino acids may also limit the production of heterologous proteins, as the transcriptional profile of a recombinant *S. cerevisiae* strain producing heterologous xylanase is very similar to yeast experiencing amino acid limitation (Chapter 5). Nitrogen sources such as amino acids are also known to improve production levels of heterologous proteins (Mendoza-Vega et al., 1994), as has been demonstrated in several studies (Wittrup and Benig, 1994; Toman et al., 2000; Blechl et al., 1992). Supplementation of a defined medium with amino acids may also reduce the proteolytic degradation of the heterologous protein product, which is a frequent limitation in the attainment of high production levels by yeast (Chapter 2). Addition of the amino acids arginine and lysine to cultures of *S. cerevisiae* has decreased proteolysis of the extracellular recombinant protein, probably due to inhibition of the proteolytic enzymes targeted to basic amino acid sites in the protein (Choi et al., 2000; Kang et al., 2000; Chung and Park, 1998). Supplementation of defined media with casein hydrolysate (casamino acids) has frequently inhibited extracellular proteolysis of heterologous protein products by yeast (Coppella and Dhurjati, 1989; Boze et al., 2001; Werten et al., 1999; Goodrick et al., 2001; Sreekrishna et al., 1997), although it was excluded from the present study due to its complex origin (Mendoza-Vega et al., 1994). Metabolite balancing during recombinant protein production has also identified a drain on amino acids and biosynthetic precursors from the TCA cycle during recombinant protein production (Jin et al., 1997). The addition of succinate was previously shown to increase levels of recombinant glucoamylse production in this manner, though possibly in part due to the improved buffering of the defined medium (Cha et al., 1998).

In the present investigation, the effect of supplementation with amino acids and succinate on the production of heterologous xylanase by a prototrophic *S. cerevisiae* transformant was determined. The *T. reesei* ß-1,4-xylanase II encoding gene, *XYN2*, was expressed from a multicopy, 2µm plasmid under regulation of the yeast glycolytic

alcoholdehydrogenase II (*ADH2)* promoter by the prototrophic [ADH2-XYN] PlasPro transformant (Chapter 6). Due to the derepression of heterologous xylanase production by this strain during growth on ethanol (Chapters 4 and 6), the additional medium components were added at a time close to that of glucose depletion. Supplementation was thus aimed at stimulating the TCA cycle through succinate addition, which provides the cell with precursors for biosynthesis, or increasing the availability of complete amino acids for biosynthesis. The aim of these experiments was to identify possible limitations in the availability of metabolic nutrients for the synthesis of heterologous xylanase.

7.2 MATERIALS AND METHODS

7.2.1. Strains and plasmids

Construction of the prototrophic recombinant *S. cerevisiae* Y294 [*ura3/URA3, leu2::LEU2*] strain, [ADH2-XYN] PlasPro, producing a heterologous ß-1,4-xylanase through expression of a plasmid-based *XYN2* gene under control of the native *ADH2*-promoter, and verification of its genetic stability were previously described (Chapter 6). Strain stocks were stored in a 15% glycerol solution at –80ºC.

7.2.2. Shake-flasks screening of medium components

The screening of potential medium components was performed in shake-flask cultures. The prototrophic yeast strain was cultivated in baffled shake-flasks, using a defined medium (Verduyn et al., 1992) containing 20 $g.l^{-1}$ glucose as the carbon source until glucose depletion. Supplementary medium components were added to individual shake-flask cultures at the start of the subsequent diauxic shift, thus increasing the availability of the components during growth on ethanol and the production phase for heterologous xylanase (Chapter 4). Growth on ethanol in shake-flask cultures was performed at either pH 5.0, by using a 50 mM citrate buffer, or at pH 3.0, by allowing the acidity of the broth to increase during growth on glucose in unbuffered defined medium, due to ammonium utilisation (Hensing et al., 1995; Greasham and Herber, 1997). Amino acids were added to the cultures in powder form without sterilisation, though no subsequent contamination was observed, probably due to the purity of individual amino acid preparations.

7.2.3. Fermentations

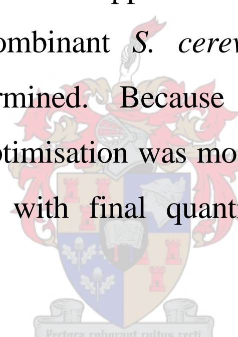
Batch and continuous cultivation in the defined medium, with supplementation as presented below, were performed as previously (Chapter 6). The pH of the fermentations was controlled at 5.0, unless otherwise specified.

7.2.4. Sampling and analytical methods

Sampling for both batch and continuous cultures, and the analytical methods employed, were previously described (Chapter 6). Ammonium concentration was determined using the Boehringer Mannheim Ammonia test kit (Cat. Nr. 1112732) adapted for use with the Cobas Mira autoanalyser.

7.3. RESULTS

The effect of various potential medium supplements on the production of heterologous xylanase and biomass by recombinant *S. cerevisiae* in shake-flask, batch and continuous cultivation was determined. Because of the relatively large number of experiments required, medium optimisation was mostly done in shake-flask cultivation (Greasham and Herber, 1997), with final quantification performed in controlled fermentation equipment.


Pectora roborant cultus recti

7.3.1. Supplementation with amino acids

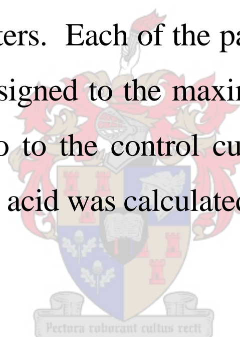
7.3.1.1. Shake-flask cultivation

All 20 amino acids were initially screened in shake-flask cultivation, to identify those that either had a positive effect on yeast growth and/or xylanase production, or did not influence metabolism negatively. The aim was to select a small number of amino acids that may be added as mixtures to the defined medium, and thereby obtain an improved defined medium. The individual amino acids were added to shake-flask cultures at a time close to that of glucose depletion to maximise their availability during the xylanase production phase of the [ADH2-XYN] PlasPro strain. During the screening of amino acids the effect of supplementation at a concentration of approximately 4 mM was determined at both pH 3.0 and 5.0.

Supplementation of the defined medium with individual amino acids during cultivation at pH 3.0 resulted in dramatic differences in the production levels of heterologous

xylanase (Fig. 7.1). Under these cultivation conditions, a significant time-wise loss of xylanase activity in the extracellular medium was observed, probably due to the activity of acidic proteases. The addition of some of the amino acids, most notably Arg, Ala, Asn, Glu, Gln and Gly, significantly retarded the extracellular proteolytic degradation of the xylanase protein and thus improved the stability of the heterologous protein (Fig. 7.1).

The overall effect of individual amino acids on the extracellular xylanase production was quantified by means of a single parameter, defined as the "Relative xylanase productivity" (see Tables 7.1 and 7.2). During the course of a particular cultivation, the maximum volumetric xylanase activity ($nkat.ml^{-1}$) and the maximum specific xylanase activity ($nkat.g_{cells}^{-1}$) were estimated. The time-wise integrals of the specific xylanase activity and the cell density were also determined for each shake-flask culture. The quantitative influence of each amino acid on the overall productivity was thus represented by these four parameters. Each of the parameters was subsequently scaled, with an arbitrary value of 100 assigned to the maximum value among the 20 different amino acids, and a value of zero to the control cultivation. The "Relative xylanase productivity" for a specific amino acid was calculated by summing the four productivity parameters.



The scaled results obtained during growth on ethanol at pH 3.0 are presented in Table 7.1, which confirmed the efficiency of Arg, Ala, Asn, Glu, Gln and Gly for improving the xylanase productivity (Table 7.1; "Relative productivity"). Of specific interest was arginine, which was the most efficient amino for improving xylanase production at pH 3.0 on all accounts. Some of the amino acids significantly inhibited biomass formation during growth on ethanol, most notably Ile, Trp, Met, Cys, Phe, Thr, Leu and Tyr (Table 7.1; "Cell density integral"). Supplementation of the defined medium with several of the amino acids, including Cys, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Tyr and Val, inhibited xylanase production by the prototrophic [ADH2-XYN] strain under these conditions (Table 7.1).

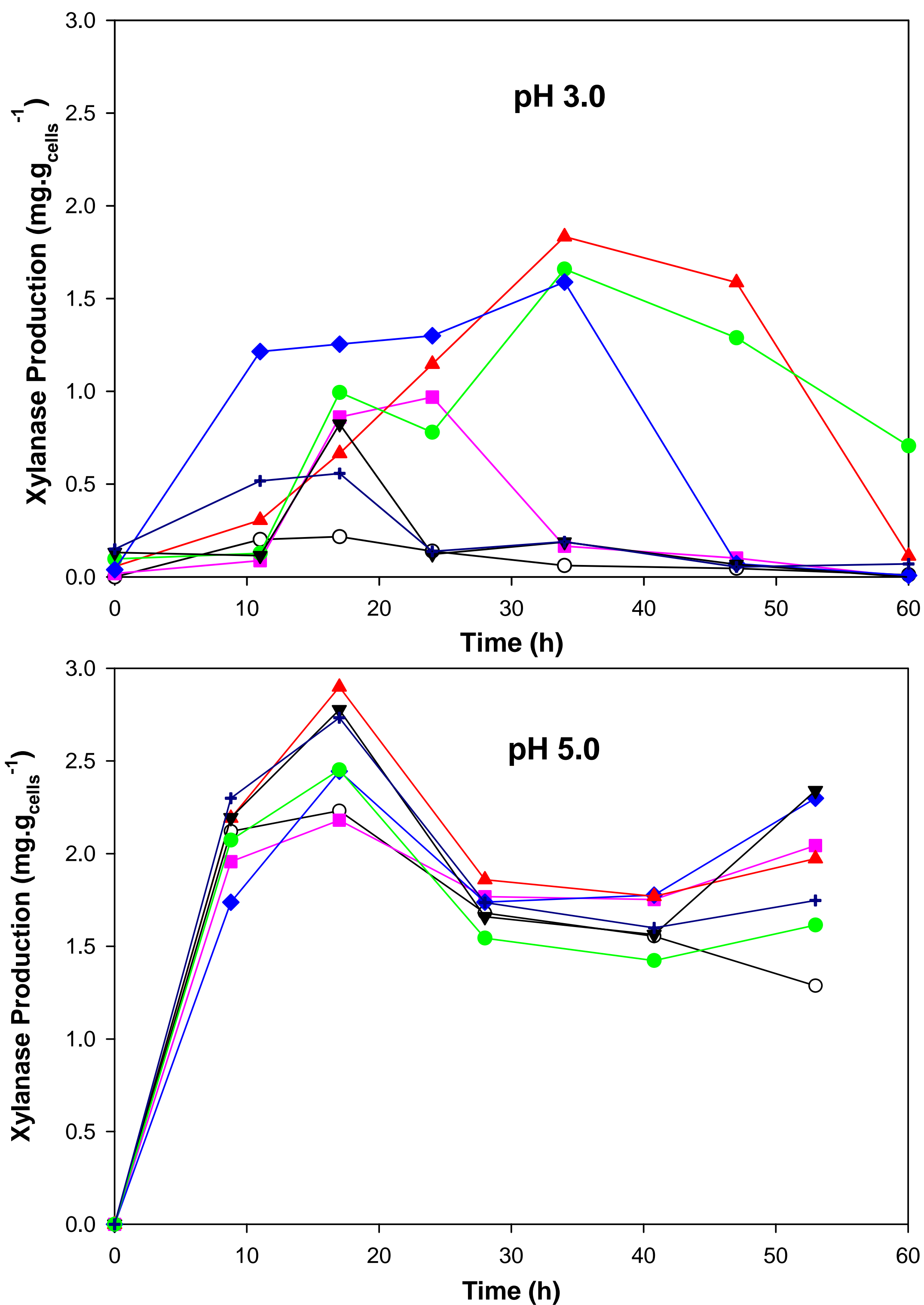


Figure 7.1. Effect of individual amino acids on xylanase production in shake-flask culture during growth on ethanol at pH 3.0 and pH 5.0. (O) Control without amino acid addition, (●) glutamine, (▲) asparagine, (◆) arginine, (■) alanine and (+) glycine. (▼) Glutamate (pH 3.0) or aspartate (pH 5.0).

Table 7.1. Effect of individual amino acids on the xylanase productivity of the prototrophic [ADH2-XYN] PlasPro strain at pH 3.0 in shake flask culture.

Amino acid [a]	**Cell density integral**	**Volumetric activity**	**Specific activity**	**Specific activity integral**	**Relative productivity** [b]
Alanine	29	54	64	60	207
Arginine	100	100	100	100	400
Asparagine	45	85	88	90	308
Aspartate	3	-21	8	7	-4
Cysteine	-74	-94	-80	-34	-282
Glutamate	40	54	71	82	246
Glutamine	64	96	98	91	349
Glycine	25	30	44	25	124
Histidine	-1	-36	-3	-6	-46
Isoleucine	-132	-105	-99	-71	-407
Leucine	-28	-68	-49	-26	-171
Lysine	-4	-47	-19	-17	-86
Methionine	-110	-103	-83	-41	-338
Phenylalanine	-69	-85	-56	-34	-244
Proline	21	-8	27	15	55
Serine	14	-11	16	12	31
Threonine	-50	-77	-54	-32	-212
Tryptophan	-116	-104	-85	-45	-350
Tyrosine	-22	-64	-37	-23	-146
Valine	-6	-53	-27	-18	-104

[a] Amino acids were added to a final concentration of approximately 4 mM.

[b] The maximum value corresponds to a 2-5 fold increase in the specific xylanase activity ($U.mg_{biomass}^{-1}$).

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The effect of amino acid supplementation on extracellular xylanase levels was less dramatic during cultivation at pH 5.0, with relatively small improvements in the production levels due to addition of the individual amino acids (Fig. 7.1). However, the overall levels of heterologous xylanase production at pH 5.0 were noticeably higher than at pH 3.0, as extracellular xylanase was significantly more stable (Fig. 7.1). The

effect of interaction between amino acids and extracellular proteases on the production of heterologous xylanase was thus much less pronounced at pH 5.0. Similar to observations during cultivation at pH 3.0, several of the amino acids significantly inhibited biomass formation at pH 5.0 (Cys, His, Ile, Leu, Met, Phe, Ser, Thr, Trp, Tyr and Val; Table 7.2). However, very few of the amino acids, namely Cys, Ile, Trp, Tyr and Val, inhibited xylanase production in the defined medium during cultivation at pH 5.0 (Table 7.2). The amino acids that improved xylanase production by the [ADH2-XYN] PlasPro strain under these cultivation conditions, without inhibiting biomass formation, were Ala, Arg, Asn, Asp, Gln, Gly and Lys (Table 7.2).

Table 7.2. Effect of individual amino acids on the xylanase productivity of the prototrophic [ADH2-XYN] PlasPro strain at pH 5.0 in shake flask culture.

Amino acid [a]	**Cell density integral**	**Volumetric activity**	**Specific activity**	**Specific activity integral**	**Relative productivity**
Alanine	54	44	-4	4	98
Arginine	46	83	10	27	165
Asparagine	42	67	39	45	193
Aspartate	66	100	23	36	226
Cysteine	-477	-213	-12	-213	-916
Glutamate	34	-4	7	0	37
Glutamine	100	26	5	2	133
Glycine	11	81	28	50	170
Histidine	-282	53	69	84	-76
Isoleucine	-430	-261	-59	-292	-1042
Leucine	-83	18	17	11	-37
Lysine	15	73	19	22	128
Methionine	-308	33	80	25	-170
Phenylalanine	-75	53	29	-8	0
Proline	92	60	2	-10	145
Serine	-47	27	38	76	94
Threonine	-389	61	100	94	-135
Tryptophan	-172	-54	4	-1	-223
Tyrosine	-116	-36	26	21	-104
Valine	-276	-9	51	100	-134

[a] Amino acids were added to a final concentration of approximately 4 mM.

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The effect of individual amino acids at pH 3.0 and 5.0 on the relative xylanase productivity are summarised in Table 7.3. The amino acids were differentiated according to their influence on productivity under both conditions, resulting in the selection of Ala, Arg, Asn, Glu, Gln and Gly for further testing during cultivation of the [ADH2-XYN] PlasPro strain under controlled conditions in defined medium.

Table 7.3. Summary of relative xylanase productivity of the [ADH2-XYN] PlasPro strain at pH 3.0 and 5.0.

Amino acid [a]	**Relative xylanase productivity**		
	pH 3.0	pH 5.0	Total
Arginine	400	165	565
Asparagine	308	193	501
Glutamine	349	133	482
Alanine	207	98	305
Glycine	124	170	294
Glutamate	246	37	284
Aspartate	-4	226	222
Proline	55	145	199
Serine	31	94	125
Lysine	-86	128	42
Histidine	-46	-76	-123
Leucine	-171	-37	-208
Valine	-104	-134	-238
Phenylalanine	-244	0	-244
Tyrosine	-146	-104	-251
Threonine	-212	-135	-347
Methionine	-338	-170	-508
Tryptophan	-350	-223	-573
Cysteine	-282	-916	-1198
Isoleucine	-407	-1042	-1449

[a] Amino acids were added to a final concentration of approximately 4 mM.

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7.3.1.2. Quantification in batch culture

The influence of the selected amino acids on growth and xylanase production by the [ADH2-XYN] PlasPro strain was tested during growth on ethanol in batch cultivation. Cultures were supplemented either with arginine (40 mM), a four amino acid mixture (Arg, Asn, Ala, Gly; 20 mM) or a six amino acid mixture (Arg, Asn, Gly, Ala, Gln, Glu; 20 mM) after the diauxic shift, during the onset of ethanol consumption. The concentrations in brackets indicate the total free amino nitrogen content of the medium after supplementation (Fig. 7.2; Table 7.4). The product yields presented in Table 7.4 were calculated for the duration of growth on ethanol.

Definite increases in the levels of biomass formation, xylanase production, and ammonium consumption were observed due to supplementation of the defined medium with the four or six amino acid mixtures (Fig. 7.2A, B and E). The complete consumption of the available ethanol during the first 33 hours after the diauxic shift in these supplemented cultures also indicated that the rate of carbon consumption was increased (Fig. 7.2C and D). The final levels of biomass and xylanase production in the medium supplemented with the four and six amino acid mixtures were comparable to those observed during cultivation in complex medium (Fig. 7.2A and B). Supplementation of the medium with arginine did not affect the yeast physiology significantly, whereas biomass and xylanase production were inhibited during cultivation at pH 3.0 (Fig. 7.2A and B), as was observed in shake-flask cultures (Fig. 7.1).

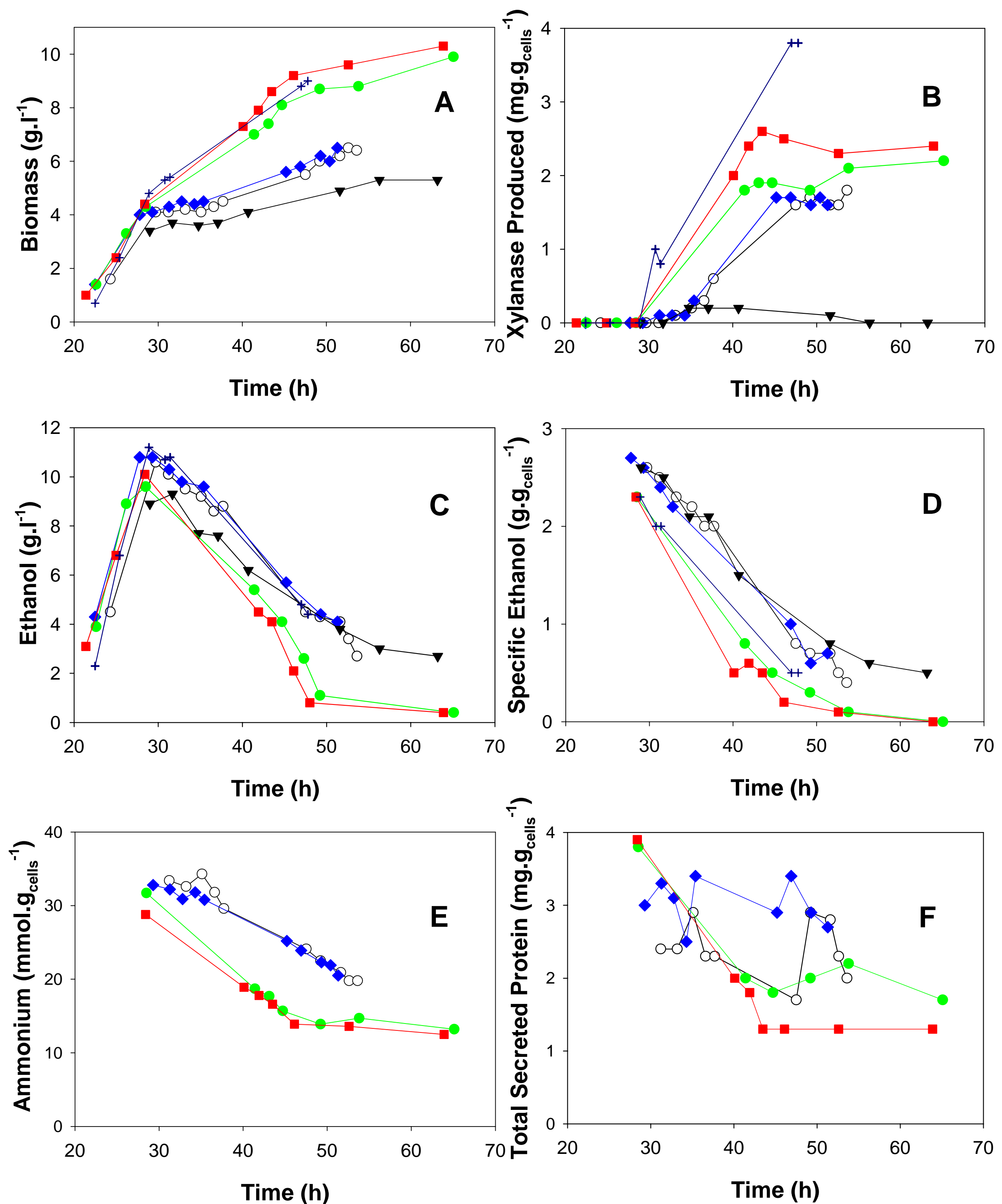


Figure 7.2. Effect of defined medium supplementation with amino acids (at different total free amino nitrogen concentrations) during growth on ethanol in batch culture on (A) biomass, (B) xylanase and (C and D) ethanol production, (E) ammonium consumption and (F) total protein secretion. (O) pH 5.0 and (▼) pH 3.0 controls without amino acid addition, (●) Arg, Asn, Ala, Gly mixture (20 mM) (◆) arginine (40 mM), (■) Arg, Asn, Gly, Ala, Gln, Glu mixture (20 mM), and (+) complex (YPD) medium control.

Table 7.4. Product yields during growth on ethanol after the diauxic shift in batch culture ($cmol_{product}.cmol_{ethanol\ consumed}^{-1}$)[Ψ]

Supplementation	Maximum specific growth rate [h^{-1}]	Biomass	Glycerol	Acetate	CO_2	Total amino acids [a]
No amino acids, pH 5.0	0.025	0.50	-0.18	-0.07	0.75	0.00
No amino acids, pH 3.0	0.019	0.49	-0.24	--	0.75	0.00
Arginine (40 mM)	0.020	0.59	-0.20	-0.10	0.84	-0.13
Arg, Asn, Ala, Gly (20 mM)	0.038	0.62	-0.04	-0.05	0.66	-0.09
Arg, Asn, Gly, Ala, Gln, Glu (20 mM)	0.043	0.71	-0.04	-0.18	0.64	-0.13
Complex medium (YPD)	0.049	0.81	-0.04	-0.45	1.09	-0.40

[Ψ] Yields were corrected for ethanol evaporation, which was calculated from the degree of reduction balance

[a] Total co-consumption of amino acids ($cmol_{amino\ acid\ consumed}.cmol_{ethanol\ consumed}^{-1}$).

Addition of the four or six amino acid mixture also increased the growth rate of the recombinant *S. cerevisiae* strain during ethanol consumption (Table 7.4). The co-consumption of glycerol during growth on ethanol was reduced by supplementation of the four or six amino acid mixtures and was similar to levels observed during growth on complex medium. Accurate measurement of the total protein secretion by the yeast was not possible due low production levels (Fig. 7.2 F), and no conclusion with regards to the effect of amino acid supplementation on the total protein secretion by the [ADH2-XYN] PlasPro strain could thus be made. The effect of amino acid supplementation on the intracellular levels of xylanase was determined in samples taken from the respective cultures at a single time point, corresponding to 20h after the depletion of glucose and supplementation of the defined medium (Table 7.5). The intracellular xylanase activity increased in response to supplementation of the defined medium with the four and six amino acid mixtures.

Table 7.5. Specific intracellular xylanase activity 20h after supplementation

Supplementation	Specific intracellular xylanase activity ($mg_{active\ xylanase} \cdot g_{cell\ protein}^{-1}$)
No amino acids, pH 5.0	2.49
Arginine (40 mM)	2.46
Arg, Asn, Ala, Gly (20 mM)	3.49
Arg, Asn, Gly, Ala, Gln, Glu (20 mM)	3.80

7.3.1.3. Quantification in continuous culture

The effect of various amino acid mixtures on heterologous xylanase production was also investigated during steady-state growth in glucose-limited chemostat culture (Table 7.7). Supplementation of defined medium with two mixtures of pure amino acids, “Casamino acids” and “SD-Optimised”, previously enhanced heterologous protein production by *S. cerevisiae* (defined and tested by Wittrup and Benig, 1994). The effect of these mixtures on heterologous xylanase production in continuous culture were compared to the seven amino acid mixture (see Chapters 4 and 6) and various mixtures of the six amino acids selected from shake-flask screening. In some chemostat cultures, the level of amino acid consumption at steady-state was measured (Table 7.7; “Amino acid uptake”).

Surprisingly, supplementation of the feed medium with the "Casamino acids" and "SD-Optimised" mixtures significantly inhibited xylanase production by the prototrophic strain, despite the positive effect of "Casamino acids" on biomass formation. The similar negative effect of the seven amino acid mixture for the auxotrophic [ADH2-XYN] strain on xylanase and biomass production by the prototrophic [ADH2-XYN] PlasPro strain was also observed in Chapter 6. Addition of arginine by itself to the defined medium at 8 and 20 mM free amino nitrogen concentration did not affect xylanase production by the prototrophic strain, although at 40 mM xylanase production was inhibited. Biomass formation was slightly improved by arginine supplementation, as was also observed during the screening of amino acids in shake-flasks at pH 5.0 (see above). The combination of arginine with asparagine or glutamine also resulted in some inhibition of xylanase production at 20 mM level, though the arginine-asparagine mixture at 40mM stimulated both biomass and xylanase production. Addition of the four amino acid mixture improved heterologous xylanase production, despite the low level of supplementation, as was also observed during batch cultivation. Supplementation with the six amino acid mixture significantly improved both biomass and xylanase production during growth on glucose. Consumption of arginine was relatively poor when added as a single amino acid, or in a bi-component mixture. However, in the six amino acid mixture all of the amino acids were utilised effectively by the prototrophic strain (Table 7.6).

Pectora roborant cultus recti

Table 7.6. Effect of amino acid supplementation of the feed to glucose-limited chemostat cultures on product yields ($cmol_{product}.cmol_{glucose\ consumed}^{-1}$)

Amino acid supplementation [§]	Total amino-N (mM) [§]	Amino acid uptake	Biomass	CO_2	Ethanol evaporation	Xylanase [Ψ]
None	0	0	0.55	0.35	0.055	31.86
Casamino acids [a]	20	--	0.74	0.39	0.054	12.23
SD-optimised [a]	20	--	0.59	0.42	0.054	3.81
Auxotrophic 7 AA mixture [b]	20	--	0.43	0.59	0.035	0.23
Auxotrophic 7 AA mixture [b,c]	20	--	0.40	0.55	0.030	3.42
Arginine	8	--	0.58	0.38	0.028	32.93
Arginine (59.1) [d]	20	0.051	0.63	0.37	0.025	31.97
Arginine	40	--	0.63	0.46	0.047	22.14
Arg (48.1), Asn (99.8) [d]	20	0.069	0.63	0.48	0.00	24.85
Arg (59.1), Gln (97.9) [d]	20	0.081	0.60	0.38	0.054	29.31
Arg, Asn	40	--	0.66	0.51	0	38.07
Arg, Asn, Ala, Gly	2.5	--	0.56	0.34	0.064	37.22
Arg (99.4), Asn (99.8), Gly (95.1), Ala (82.7), Gln (99.1), Glu (99.1) [d]	20	0.132	0.68	0.41	0.016	49.29

[§] Different amino acid mixtures added to a feed medium containing 10 $g.l^{-1}$ of glucose as carbon source.

[Ψ] ($mg_{xylanase}.cmol_{glucose\ consumed}^{-1}$)

[a] Pure amino acids added according to Wittrup and Benig (1994) [b] Chapter 6 [c] Feed medium contained only 5 $g.l^{-1}$ of glucose

[d] Values in brackets indicate the percentage of the amino acid supply in feed that was utilised by the yeast.

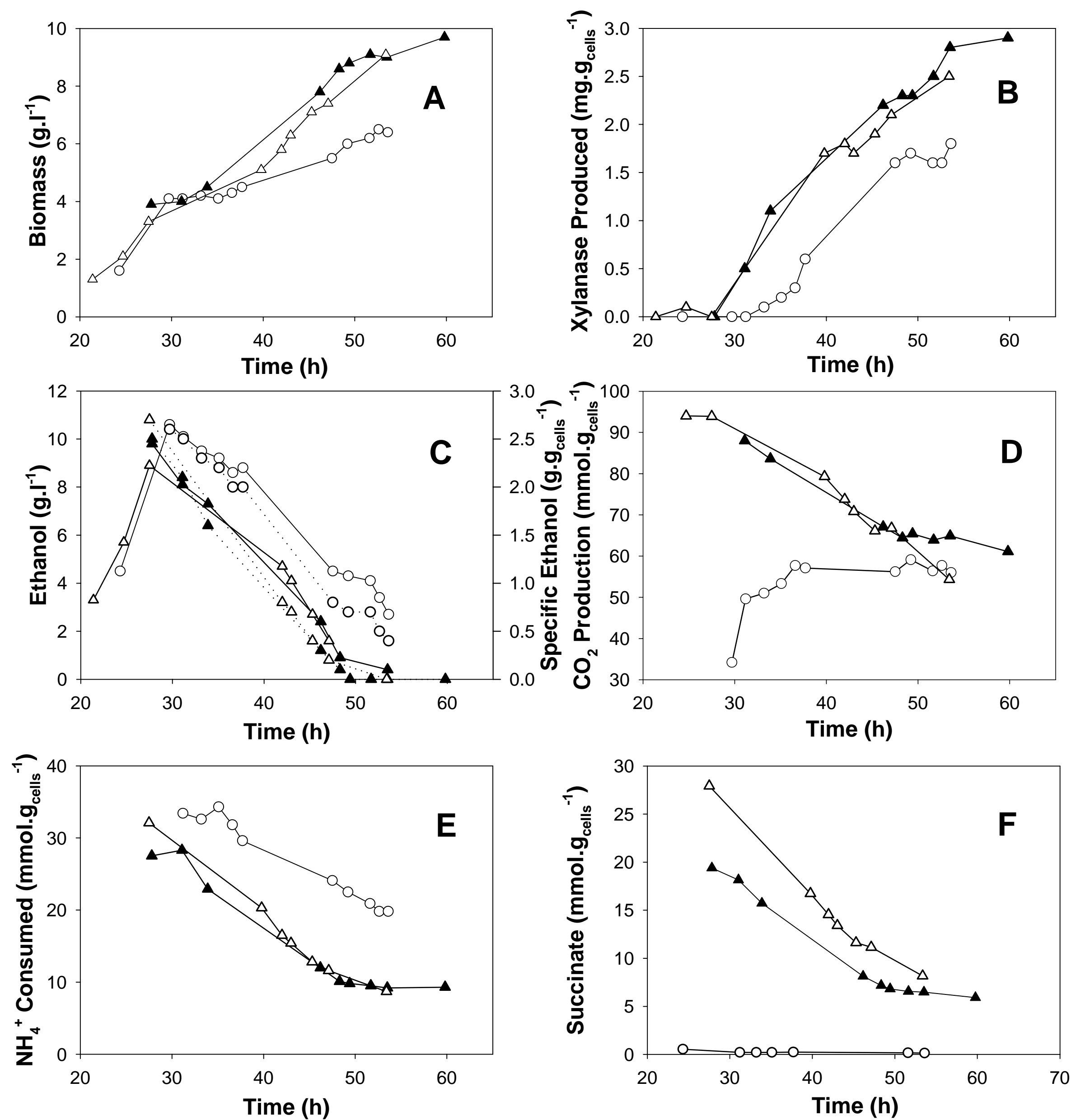


Fig. 7.3. Effect of succinate addition and consumption (F) on the production of (A) biomass, (B) xylanase and (C and D) ethanol, and the consumption of (E) ammonium. (O) Defined medium without supplementation. Succinate was supplemented at a concentration of (▲) 9 $g.l^{-1}$ or (△) 11 $g.l^{-1}$. (C) Dotted line represents the Specific Ethanol level ($g_{EtOH}.g_{cells}^{-1}$).

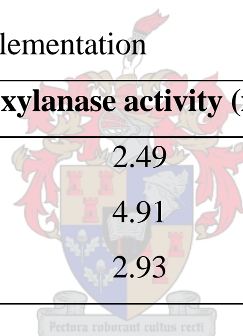
Table 7.7. Product yields during growth on ethanol after the diauxic shift in batch culture ($cmol_{product}.cmol_{ethanol\ consumed}^{-1}$)$^{\Psi}$

Supplementation	Maximum specific growth rate (h^{-1})	Biomass	Glycerol	Acetate	Succinate	CO_2
Defined only	0.025	0.50	-0.18	-0.07	0.00	0.75
Low succinate	0.045	0.67	-0.12	-0.11	-0.14	0.69
High succinate	0.043	0.73	-0.25	-0.05	-0.14	0.70

$^{\Psi}$ Yields were corrected for ethanol evaporation, which was calculated from the degree of reduction balance

Table 7.8. Specific intracellular xylanase activity 20h after supplementation

Supplementation	Specific intracellular xylanase activity ($mg_{active\ xylanase}.g_{cell\ protein}^{-1}$)
Defined only	2.49
Low succinate	4.91
High succinate	2.93



7.3.2. Addition of succinate

The effect of succinate addition on biomass formation and xylanase production in defined medium during growth on ethanol in batch culture was also tested (Fig. 7.3 and Table 7.7). The defined medium was supplemented with succinate after the diauxic shift to final levels of 9 and 11 $g.l^{-1}$. The majority of the added succinate was consumed during approximately 32 hours of growth on ethanol (Fig. 7.3F), although the rate of ethanol consumption was not significantly affected by the consumption of succinate. Succinate consumption resulted in a higher final level of biomass formation, and increased xylanase production and ammonium consumption (Fig. 7.3A, B and E). The increase in xylanase production was partly due to a decrease in the lag phase after derepression of the *ADH2*-promoter, whereas the increase in the biomass yield during growth on ethanol due to succinate consumption was accompanied by an increase in the maximum specific growth rate (Table 7.7). The improvement in extracellular xylanase production due to supplementation with succinate was apparently associated with an increase in the intracellular xylanase activity (Table 7.8).

7.4 DISCUSSION

The influence of supplementing a chemically defined medium with individual amino acids, mixtures of selected amino acids or succinate, on the production of heterologous xylanase by the prototrophic, recombinant *S. cerevisiae* strain, [ADH2-XYN] PlasPro via native *ADH2*-regulation, was investigated. Amino acids were first screened in shake-flask culture, prior to the combination of potential medium components in mixtures. The aim of these experiments was to determine whether limitations in the availability of metabolic precursors in both the carbon- and nitrogen metabolism affected the production of heterologous xylanase.

7.4.1. Effect of amino acids

7.4.1.1. Screening of amino acids

Six amino acids (Arg, Asn, Gly, Ala, Gln and Glu) were selected on the basis of improved xylanase production and/or biomass formation during growth on ethanol in shake-flask culture at pH 3.0 and 5.0. The effects of these amino acids on metabolism, whether individually or in mixtures, were observed in the presence of an excess ammonium in the defined medium, which is a general inhibitor of amino acid uptake in

yeast (Slaughter et al., 1990; Jiranek et al., 1995). The positive effect of the preferred nitrogen sources Gln and Asn on heterologous xylanase production may thus be related to the ability of yeast to utilise these compounds in the presence of ammonium (Slaughter et al., 1990; Jiranek et al., 1995; Ter Schure et al., 2000; Wiame et al., 1985; Cooper, 1982). The presence of ammonium in the defined medium was advantageous, however, as it may prevent the induction of catabolic enzymes for amino acid utilisation, thus allowing for anabolic assimilation of amino acids (Cooper, 1982; Stanbrough and Magasanik, 1995).

One of the reasons for the “Preferred Nitrogen Source” status of some of the amino acids is their relative richness in amino nitrogen – one mol of asparagine or glutamine contains twice the amount of amino nitrogen than most of the other amino acids. The high nitrogen content of arginine and glutamate may similarly be responsible for their preferential utilisation from amino acid mixtures (Jiranek et al., 1995; Herriaz and Ough, 1993), and ability to improve heterologous xylanase and biomass production. The effects of glutamate addition may be related to its central role in nitrogen metabolism (Watson, 1976). The positive effects of glycine and alanine observed during amino acid screening could not be explained similarly, since neither contains large amounts of amino nitrogen, whilst glycine is poorly metabolised from amino acid mixtures (Jiranek et al., 1995; Herriaz and Ough, 1993).

The beneficial effect of the selected amino acids may also be related to the inhibition of extracellular proteases, which dominated the screening of amino acids in shake-flasks at pH 3.0, while also having some effect at pH 5.0 (Fig. 7.1). The ability of arginine to improve heterologous protein production in such a manner has been demonstrated, either when added as a single amino acid to defined medium (Kang et al., 2000), or along with glycine and glutamate in casamino acid mixtures (Coppella and Dhurjati, 1989; Boze et al., 2001; Werten et al., 1999; Goodrick et al., 2001; Sreekrishna et al., 1997). As the stability of extracellular xylanase was significantly improved by increasing the cultivation pH to 5.0, the effect of the amino acid addition in the latter cultures were most strongly related to their utilisation in metabolism.

Growth and heterologous xylanase production by recombinant *S. cerevisiae* was improved by supplementation of the defined medium with the amino acids

preferentially stored in the vacuole after utilisation. The basic amino acids (Arg and Lys) are strongly preferred for storage in the vacuole due to their richness in nitrogen, along with Gly, Ala, Asn and Gln (Messenguy et al., 1980; Wiame et al., 1985; Grenson, 1992; Horàk, 1997). Free amino acids accumulated in this manner may be stored either in the cytoplasm, where it is rapidly utilised for protein synthesis, or in the vacuole, where it can remain for longer periods (Messenguy et al., 1980). The acidic amino acids (aspartate and glutamate) are not accumulated in the vacuole (Grenson, 1992).

7.4.1.2. Supplementation with amino acid mixtures

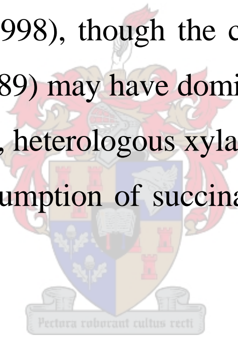
The supplementation of defined medium with amino acids in controlled batch and continuous cultures were investigated at a cultivation pH of 5.0. The addition of the four and six amino acid mixtures during batch growth on ethanol improved the yield of biomass and heterologous xylanase, with ethanol and ammonium consumption rates increased as a result of the higher growth rate. Improved biomass formation due to amino acid supplementation was previously observed (Albers et al., 1996). The availability of nitrogen apparently limited protein production and biomass formation during growth on ethanol, as has been reported in other defined media (Chen et al., 1993; Gu et al., 1991). The positive effect of amino acid addition of xylanase production was thus dependent on a balance in the availability of preferred nitrogen sources. During chemostat cultivation the amino acids arginine, asparagine and glutamine also did not have a positive effect on heterologous xylanase production if not present in the mixture of at least four amino acids. The synergism between the individual amino acids in these two mixtures was therefore essential for the improvement of heterologous xylanase and biomass production.

During growth on glucose in continuous culture, the presence of the amino acids, His, Ile, Leu, Met, Phe, Ser, Thr, Tyr and Val in the seven amino acid, "Casamino acid" and "SD-Optimised" mixtures apparently reduced xylanase production levels (Table 7.6; see above). The disparity between the reported stimulation of heterologous protein production by the latter two mixtures (Wittrup and Benig, 1994) and the inhibition of xylanase production observed in the present study, emphasised the need for the empirical optimisation of defined medium for each production system. The negative effect of the seven amino acid mixture on biomass formation in the medium containing

only 5 $g.l^{-1}$ of glucose (Table 7.7) may further be related to the inability of the yeast to utilise the carbon-skeletons derived from the respective amino acids (Thomas and Ingledew, 1990). No further rational origins for the inhibition of xylanase production by individual amino acids were evident from the analysis.

7.4.2. Supplementation with succinate

The majority of the succinate supplemented to the defined medium was consumed during batch growth on ethanol (Fig. 7.3F), despite previous reports that extracellular TCA intermediates cannot support the growth on *S. cerevisiae* (Kaclikova et al., 1992). Succinate consumption increased the final levels of biomass formation and stimulated heterologous xylanase production, which identified a limitation in the availability of TCA cycle intermediates during growth on ethanol. The addition of succinate apparently improved the synthesis and intracellular level of heterologous xylanase (Table 7.8). Improvement of heterologous protein production by succinate addition was previously reported (Cha et al., 1998), though the capacity of succinate to buffer the defined medium (Adams et al., 1989) may have dominated the reported observation. In the present investigation, however, heterologous xylanase production by recombinant *S. cerevisiae* was improved by consumption of succinate as an additional carbon-source alone.



7.5 CONCLUSIONS

Heterologous xylanase production by *S. cerevisiae* in defined medium was partially limited by the availability of metabolic precursors for protein synthesis, since both the addition of suitable amino acid mixtures and succinate improved heterologous xylanase production. This supports the notion that foreign protein production may drain metabolic resources from the central pathways (Jin et al., 1997; Ramirez and Bentley, 1993). The biosynthetic capacity of a recombinant yeast strains for heterologous protein production may thus be regulated actively in response to the availability of sufficient resources for protein synthesis. However, the improvements in xylanase production due to medium supplementation observed in the present study were typically smaller than two-fold, indicating that heterologous protein production may also limited by other factors besides the availability of amino nitrogen and TCA metabolites.

7.6. ACKNOWLEDGEMENTS

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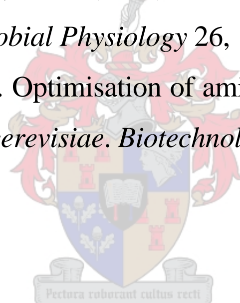


Chapter 8

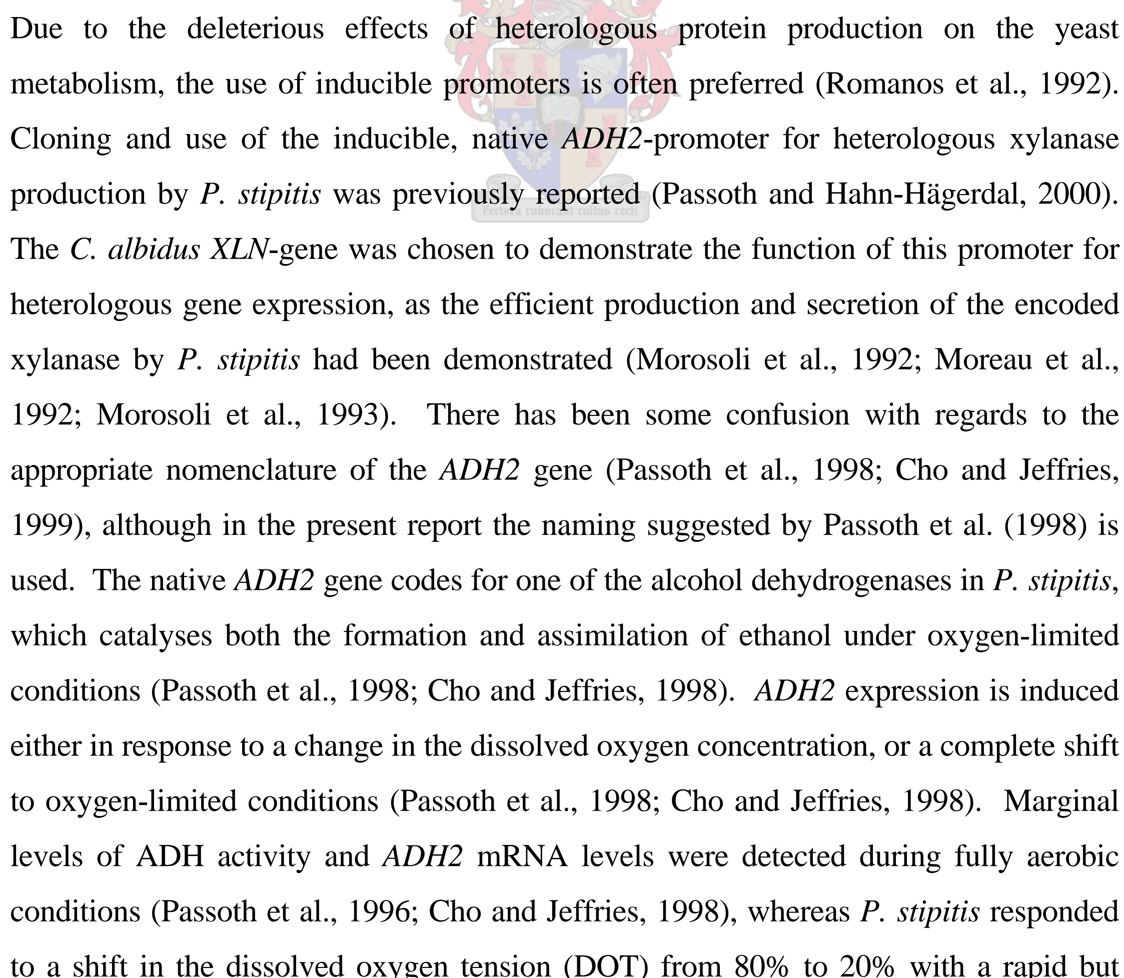
EFFECT OF OXYGENATION AND AMINO ACID SUPPLEMENTATION ON HETEROLOGOUS XYLANASE PRODUCTION BY *P. STIPITIS*

8.1. INTRODUCTION

Limitations in the attainable production levels of heterologous protein by yeasts represent a major hurdle in the application of these hosts in industrial processes (Ljubijankic et al., 1999). In the previous chapters the influence of the regulatory characteristics of the promoter (Chapter 4), the presence of auxotrophic markers (Chapter 6) and the availability of sufficient nutrients (Chapter 7) on the production of a heterologous xylanase by recombinant *S. cerevisiae* was demonstrated. In the present study, the presence of similar limitations during heterologous xylanase production by an alternative yeast, *P. stipitis*, was investigated. A strong influence of the cultivation conditions, promoter induction and supplementation of the medium with amino acids would heterologous protein production by a *his3* auxotrophic transformant would confirm the generic nature of the limitations previously observed in *S. cerevisiae*. Finally, the characteristics of the selected yeast host strain is known to influence the levels of foreign gene transcription, translation and secretory efficiency, protein quantity and quality, plasmid stability and plasmid copy number (Park et al., 2000; Eckart and Bussineau, 1996; Mendoza-Vega et al., 1994). The development of *P. stipitis* as an alternative host for heterologous xylanase production is therefore of interest for further elucidating the relationship between yeast host and expression level.

P. stipitis is a xylose-fermenting yeast for which the production of heterologous proteins (one cellulase and two xylanases) under control of the native *XYL1*, *TKL* and *ADH2* promoters has been reported (Den Haan and Van Zyl, 2001; Passoth and Hahn-Hägerdal, 2000; Piontek et al., 1998; Morosoli et al., 1992; Moreau et al., 1992). A major advantage of this yeast is its preference for respiratory growth due to Crabtree-negative status; with ethanol formation induced strongly by oxygen-limited conditions

and not in response to high glucose concentrations during aerobic conditions (Skoog and Hahn-Hägerdal, 1990; Du Preez, 1994; Cho and Jeffries, 1999; Passoth et al., 1996). No fermentation will thus occur under fully aerobic conditions in the presence of high glucose concentrations, resulting in high growth rates and high biomass yields in the presence of glucose levels up to 50 g.l^{-1} (Passoth et al., 1996; Skoog and Hahn-Hägerdal, 1990; Du Preez, 1994; Du Preez et al., 1989). These characteristics make *P. stipitis* ideal for large-scale fermentations, where high levels of biomass production can be obtained in the presence of excess glucose in batch culture, without the need for stringent feed control to avoid ethanol formation. Although *P. stipitis* produces ethanol from glucose or xylose at low dissolved oxygen concentrations, it is also able to re-assimilate ethanol under the same conditions, depending on the levels of sugar depletion and ethanol formation (Passoth et al., 1998; Du Preez et al., 1989). The ability to grow on xylose is also advantageous since D-xylose is the most predominant pentose sugar in hemicellulose, which is an abundant renewable carbon source (Jeffries and Jin, 2000; Piontek et al., 1998).



Due to the deleterious effects of heterologous protein production on the yeast metabolism, the use of inducible promoters is often preferred (Romanos et al., 1992). Cloning and use of the inducible, native *ADH2*-promoter for heterologous xylanase production by *P. stipitis* was previously reported (Passoth and Hahn-Hägerdal, 2000). The *C. albidus XLN*-gene was chosen to demonstrate the function of this promoter for heterologous gene expression, as the efficient production and secretion of the encoded xylanase by *P. stipitis* had been demonstrated (Morosoli et al., 1992; Moreau et al., 1992; Morosoli et al., 1993). There has been some confusion with regards to the appropriate nomenclature of the *ADH2* gene (Passoth et al., 1998; Cho and Jeffries, 1999), although in the present report the naming suggested by Passoth et al. (1998) is used. The native *ADH2* gene codes for one of the alcohol dehydrogenases in *P. stipitis*, which catalyses both the formation and assimilation of ethanol under oxygen-limited conditions (Passoth et al., 1998; Cho and Jeffries, 1998). *ADH2* expression is induced either in response to a change in the dissolved oxygen concentration, or a complete shift to oxygen-limited conditions (Passoth et al., 1998; Cho and Jeffries, 1998). Marginal levels of ADH activity and *ADH2* mRNA levels were detected during fully aerobic conditions (Passoth et al., 1996; Cho and Jeffries, 1998), whereas *P. stipitis* responded to a shift in the dissolved oxygen tension (DOT) from 80% to 20% with a rapid but

transient induction of ADH activity (Passoth et al., 1996). During a shift from aerobic to oxygen-limited (0% DOT) conditions, a more permanent induction of *ADH2* expression was observed, with a large increase in *ADH2* mRNA (Passoth et al., 1998; Cho and Jeffries, 1999). Effective induction of *ADH2*-regulated heterologous xylanase expression, during a shift from aerobic to oxygen-limited conditions, was previously demonstrated for a low cell density culture of recombinant *P. stipitis* (Passoth and Hahn-Hägerdal, 2000). Although *P. stipitis* grows reasonably quickly under aerobic conditions, growth is severely retarded during oxygen-limited growth (Du Preez et al., 1989; Skoog and Hahn-Hägerdal, 1990; Du Preez, 1994; Passoth et al., 1996; Passoth and Hahn-Hägerdal, 2000; Rizzi et al., 1989). However, low growth rates during the production phase of cultures producing heterologous proteins are preferred, due to the decrease in substrate consumption. The level of oxygenation and the oxygen transfer rate during oxygen-limited conditions strongly influence the rate of ethanol production (Du Preez, 1994) and therefore possibly also the expression level of the *ADH2*-gene. As the extent of oxygen limitation increases, the level of ethanol production increases with a concomitant decrease in the cell yield, growth rate, and sugar uptake rate (Skoog and Hahn-Hägerdal, 1990; Du Preez, 1994), and *P. stipitis* therefore needs a minimum low aeration rate to sustain growth (Rizzi et al., 1989; Du Preez, 1994). Consumption of amino acids from the cultivation medium has also decreased ethanol formation during oxygen-limited conditions (Guebel et al., 1992).

Pectora roborant cultus recti

In the present investigation, *P. stipitis* was grown to higher cell densities than previously employed (Passoth and Hahn-Hägerdal, 2000) in the presence of excess glucose, prior to induction of heterologous xylanase production. The proper control of promoter induction, the level of oxygenation and the medium composition during the oxygen-limited production phase significantly improved production levels of the heterologous xylanase.

8.2. MATERIALS AND METHODS

8.2.1. Strains and medium

Transformation of the *P. stipitis* PJH53 [*trp5-10*, *his3-1*] strain with the *pVPA2CaXLN* episomal plasmid, containing the *Cryptococcus albidus XLN*-gene under control of the *P. stipitis ADH2*-promoter and the *P. stipitis HIS3* gene as selection marker, was previously reported (Passoth and Hahn-Hägerdal, 2000). Growth medium for the

plating of the recombinant strain contained glucose (20 $g.l^{-1}$), yeast nitrogen base without amino acids (YNB; 6.7 $g.l^{-1}$), tryptophan (120 $mg.l^{-1}$) and agar (20 $g.l^{-1}$). Glucose and agar were autoclaved together, whereas YNB and tryptophan were filter-sterilised separately and added to the medium once the autoclaved solution had cooled down sufficiently. The basic growth medium for liquid cultivation was similarly prepared and contained: KH_2PO_4 (18.75 $g.l^{-1}$), $(NH_4)_2HPO_4$ (6 $g.l^{-1}$), $MgSO_4*7H_2O$ (1.13 $g.l^{-1}$), YNB without amino acids (6.5 $g.l^{-1}$), tryptophan (400 $mg.l^{-1}$) and glucose 30 $g.l^{-1}$. The pH of the medium was adjusted to 5.0 prior to sterilisation (Dellweg et al., 1984; Passoth and Hahn-Hägerdal, 2000). The growth medium was also supplemented with an amino acid mixture during the course of the fermentation, as discussed below.

8.2.2. Batch fermentations

Batch fermentations were conducted in a Braun Biotech Biostat® fermenter with a total volume of 2000 ml and a working volume of 1500 ml. Fermenters contained 1350 ml of Millipore water during autoclaving, with 150 ml of a 10-fold concentrate of the liquid cultivation medium added during inoculation. The temperature and pH of the cultures were controlled at 30ºC and pH 5.0 (by the addition of 2M NaOH or 2M HCl), respectively. The fermentation broth was agitated in the range of 350 to 600 rpm and aerated with a 0.5 $l.min^{-1}$ airflow (standard conditions). The level of dissolved oxygen was monitored with a dissolved oxygen probe (Braun Biotech) and controlled by manual adjustment of the agitation speed and level of oxygen-enrichment of the inlet gas flow. The outlet gas from the fermenter was cooled in a condenser, through which cold tap water was circulated. Dow Corning anti-foam (BDH) was added to the fermenter to control foaming, since excessive foaming removed the relatively small yeast cells from the fermentation broth.

The transformant strain was cultivated on solid medium prior to inoculation of a 5 ml liquid pre-culture. The pre-culture was grown overnight at 30ºC in a Gallenkamp INR-200 orbital incubator (Leicester, UK) at 150 rpm and transferred to 200 ml of liquid medium in a 1l baffled shake-flask, which was similarly incubated until a sufficient cell density was reached. Both the pre-culture and inoculum were prepared in the same medium as used in the fermenter. Cell densities in all cultures were estimated as absorbance (optical density) measurements at 620 nm (A_{620}) with a Hitachi U-1100 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The volume of shake-flask culture,

required to inoculate the fermenter to an A_{620} of 0.5, was centrifuged at 5000 rpm for 6 min in a Beckman J2-21 centrifuge (Geneva, Switzerland) and the cells re-suspended in 150 ml of the 10-fold medium concentrate, which was rapidly used to inoculate the fermenter.

The basic batch cultivation proceeded in at least two steps (Table 8.1). During the aerobic growth phase, the dissolved oxygen tension was maintained at 100% by manual adjustment of the level of oxygen-enrichment in the airflow and the agitation speed during the first 31 hours of fermentation. Approximately 150 ml of sample was removed during fully aerobic conditions. Prior to the transfer to oxygen limitation, to induce heterologous gene expression, a further 150 ml of the 10-fold concentrate of the liquid medium was added to the fermenter, to ensure sufficient nutrient availability during heterologous protein production. A rapid transfer from fully aerobic conditions in the fermenter to oxygen-limitation was undertaken once the cell density reached a level of A_{620} = 30, at which time most of the glucose in the cultivation medium had been utilised. The fermenter was first sparged with 100% N_2, until the dissolved oxygen level had decreased to 0%, with the airflow subsequently re-admitted to fermenter either containing the normal level of oxygen (21%), resulting in the "High oxygenation" condition, or containing a significantly lower percentage of oxygen, resulting in the (very) "Low oxygenation" condition. The level of dissolved oxygen was kept at 0% for the remainder of both types of culture, despite the differences in the oxygen transfer rate, thus ensuring that oxygen-limited conditions were maintained. After 43h of cultivation two of the cultures, one with "High" and one with "Low" oxygenation were also supplemented with a mixture of amino acids (see below). During one of the cultures, supplied with both a higher level of oxygenation and amino acid addition, a shift from oxygen-limited conditions back to fully aerobic conditions was performed once the glucose was completely consumed.

Table 8.1. Modes of batch fermentation by recombinant *P. stipitis* in YNB medium

Time	Oxygenation	Oxygen content of gas inlet	Nutrients / Supplements
0 – 31 h	Fully aerobic	21 – 27%	Glucose
31h	Anaerobic	0%	Glucose added
31h – end	Oxygen limited, "High"	21%	Glucose
31h – end	Oxygen limited, "Low"	5 to 0.5%	Glucose
43h – end [a]	Oxygen limited	21 – 0.5% [b]	Glucose/Ethanol + Amino acid mixture
63h – end [c]	Fully aerobic	34%	Ethanol

[a] Amino acid mixtures were added to one of the "High" and one of the "Low" oxygenation cultures.

[b] Depending on whether "High" or "Low" oxygenation was maintained

[c] Only one of the "High oxygenation" cultures with amino acid addition was shifted back to fully aerobic growth after glucose consumption.

8.2.3. Analytical methods

Samples for the determination of cell density, substrate consumption and product formation were removed regularly from fermentations, as described previously (Chapter 4). Samples for the determination of cell density (Absorbance at 620 nm) were diluted with 9 $g.l^{-1}$ NaCl into the 0.05 - 0.2 linear absorbance detection range of the spectrophotometer. A calibration of the dry weight measurements (Meinander et al., 1996) to absorbance (A_{620}) was employed to calculate levels of biomass formation during the various stages of cultivation.

8.2.4. Substrate consumption and product formation

Glucose, ethanol, glycerol, acetate and succinate concentrations were determined as previously (Chapter 4). To circumvent the problems associated with the presence of excess amounts of a reducing sugar (glucose) in the cultivation medium, samples were assayed for xylanase activity by using RBB-dyed xylan (Remazol brilliant blue) dissolved in acetate buffer (50mM; pH 5.4) as substrate, as described by Biely et al. (1985). Samples were incubated for 2-6 hours at 30ºC, precipitated with 2 volumes of 96% ethanol, and the absorbance (595 nm) measured. Enzyme activity was calculated

by multiplying the rate of change in Ab_{595} (dAb_{595}/dt) by the number of RBB molecules per xylan unit (20) and dividing by the molar extinction coefficient (9.25 $mM^{-1}.cm^{-1}$).

8.3. RESULTS

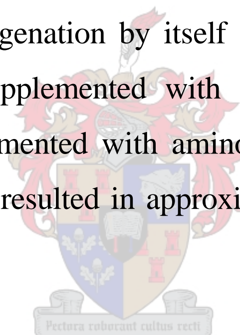
The relationship between the metabolic state of recombinant *P. stipitis* and the level of heterologous xylanase production, regulated by the native *ADH2* promoter, was investigated in batch culture. Biomass formation, substrate consumption and product formation by the recombinant strain are presented in Figure 8.1 and Tables 8.2 and 8.3. Levels of biomass formation during fully aerobic cultivation were approximately 15-fold higher than those obtained in previous cultures with the same strain (Passoth and Hahn-Hägerdal, 2000), without measurable ethanol formation (Fig. 8.1A and E; Table 8.2). The maximum specific growth rate of the transformant during this growth phase was 0.154 ±0.013 (h^{-1}).

A rapid induction of heterologous xylanase production by the *ADH2*-promoter was obtained by changing the conditions the fermenter from fully aerobic to oxygen-limited conditions after 31 hours of cultivation (Fig. 8.1B). In contrast to results with lower cell densities reported previously (Passoth and Hahn-Hägerdal, 2000), no apparent delay in the secretion of the foreign protein occurred (Fig. 8.1C and D). Biomass formation significantly decreased during oxygen-limited conditions, as evident from a significantly reduced biomass yield (Fig. 8.1A; Table 8.2). The consumption of glucose during oxygen-limitation resulted mostly in ethanol and carbon dioxide formation, with the latter calculated by assuming that 1 mole of CO_2 was formed for each mole of ethanol or acetic acid formed (Skoog and Hahn-Hägerdal, 1990). This approach resulted in the closure of the carbon balance during oxygen-limited growth (Table 8.2).

Aeration is a critical variable for the cultivation of *P. stipitis*, due to the dependency of glucose fermentation on the oxygen transfer rate (Laplace et al., 1991; Guebel et al., 1992; Du Preez et al., 1989; Du Preez, 1994). However, the estimation of the effect of oxygenation on yeast physiology is hampered by the difficulty controlling oxygen consumption accurately, due to the poor sensitivity of dissolved oxygen probes (Du Preez, 1994) and the need for on-line estimation and control of the rate of O_2-

consumption per unit of biomass. In the present investigation two constant oxygen transfer rates were employed during oxygen-limited conditions, with an inevitable decrease in the oxygen-consumption per gram biomass during the course of the fermentation, to determine the effect of host physiology and heterologous protein production.

The different oxygenation strategies during oxygen-limited conditions resulted in two different levels of oxygen uptake by the recombinant yeast, as was confirmed by significant differences in the yeast physiology: Increased oxygenation resulted in increased biomass formation (Fig. 8.1A; Table 8.2), an increased rate of glucose consumption (Table 8.3), a decrease in CO_2 production (Table 8.2), decreased glycerol formation (Fig 8.1F). Ethanol consumption during oxygen-limited conditions was not observed when the recombinant strain was cultivated with the lower level of oxygenation (Fig. 8.1E). However, no clear effect on the level of recombinant xylanase production due to increased oxygenation by itself was observed, as was particularly evident for the cultures not supplemented with amino acids (Fig. 8.1C and D). However, for the culture supplemented with amino acids the increased oxygenation under oxygen-limited conditions resulted in approximately 3 times more heterologous xylanase production.



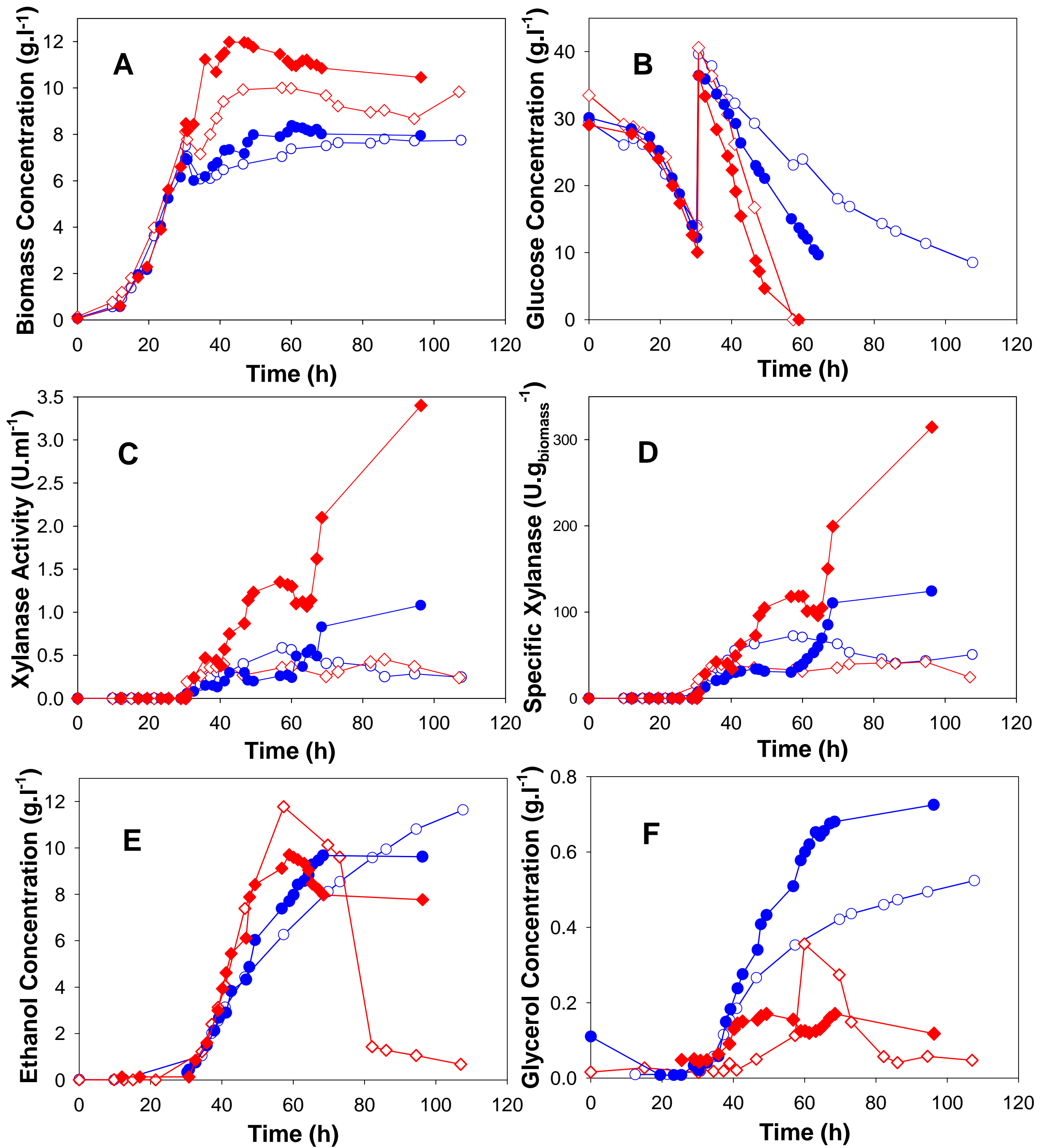


Figure 8.1. Biomass, xylanase, ethanol and glycerol production in batch cultivation of recombinant *P. stipitis*, switched from fully aerobic conditions to oxygen-limitation after 31h. "Low" (●,O) or "High" (◆,◇) oxygenation was supplied during oxygen limitation. The amino acid mixture was added after 43h to the cultures represented by the closed symbols. The culture with high oxygenation and amino acid addition was shifted to fully aerobic growth after 63h.

Table 8.2. Product formation during growth of recombinant *P. stipitis* on glucose under fully aerobic and oxygen-limited conditions

Level of oxygenation	Yields on glucose ($cmol_{product\ formed}.cmol_{glucose\ consumed}^{-1}$)											
	Biomass			Ethanol			Carbon dioxide (calculated)			Carbon balance		
	Aerobic[a]	O_2-lim[b]	+AA's[c]	Aerobic	O_2-lim	+AA's	Aerobic	O_2-lim	+AA's	Aerobic	O_2-lim	+AA's
Low	0.71	0.07	--	0.00	0.48	--	0.02	0.49	--	0.74	1.06	--
High	0.61	0.19	--	0.00	0.40	--	0.02	0.42	--	0.64	1.02	--
Low +AA's	0.45	0.09	0.12	0.00	0.44	0.41	0.05	0.45	0.41	0.55	1.01	0.96
High +AA's	0.50	0.23	0.00	0.00	0.34	0.41	0.00	0.35	0.42	0.51	0.94	0.85

[a] Fully aerobic growth on glucose prior to the induction of heterologous xylanase production (0 – 31h; Table 8.1)

[b] Cultivation under oxygen-limited conditions after the induction of heterologous xylanase production, prior to amino acid addition (31-43h; Table 8.1).

[c] Cultivation under oxygen-limited conditions after amino acid addition (43h – 63h; Table 8.1)

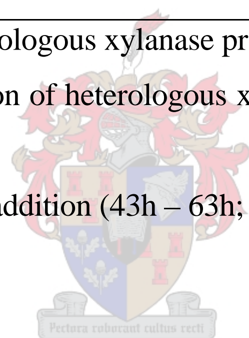


Table 8.3. Rate of glucose consumption during oxygen-limited cultivation

Level of oxygenation	Rate of glucose consumption ($g_{glucose}.l^{-1}.h^{-1}$)	
	O_2-lim[a]	+AA's[b]
Low	0.52	--
High	1.53	--
Low +AA's	0.78	0.77
High +AA's	1.48	1.61

[a] Cultivation under oxygen-limited conditions after the induction of heterologous xylanase production, prior to amino acid addition (31-43h; Table 8.1).

[b] Cultivation under oxygen-limited conditions after amino acid addition (43h – 63h; Table 8.1)

The addition of an amino acid mixture to the cultivation medium during oxygen-limited conditions had positive effects on the productivity of the recombinant strain (Fig. 8.1 and Table 8.2). The composition of the amino acid mixture was designed on the basis of a rational approach, whereby a 40-60% of the amino acids required for biosynthesis were supplied exogenously (Table 8.4; Greasham and Herber, 1997). The amino acids were selected to ensure representation of all the amino acid groups in yeast biosynthesis (Jones and Fink, 1982), and did not contain histidine due to its use as selection marker for plasmid maintenance. The amino acid mixture was added as a concentrate to the cultures after 43 hours of cultivation, and resulted in a significant increase in biomass formation during both low and high oxygenation (Fig. 8.1A). The rate of glucose consumption during oxygen-limited conditions was not affected by the amino acid addition (Table 8.3). The specific level of heterologous xylanase production was significantly improved by the synergistic action of increased oxygenation and amino acid addition in the 43-63h window (Fig. 8.1D), whereas the overall production level also benefited from the additional biomass formation (Fig. 8.1C). Amino acid consumption had variable effects on ethanol formation (Fig. 8.1E).

Ethanol consumption after glucose depletion in oxygen-limited cultivation was observed only when the higher level of oxygenation was supplied, though no biomass formation due to ethanol consumption was evident. Shifting back from oxygen-limited to fully aerobic conditions after glucose depletion did not increase biomass formation or

ethanol consumption appreciably, although heterologous xylanase production substantially increased (Fig. 8.1 A, C, D and E). Consumption of ethanol was accompanied by a small measure of glycerol (Fig. 8.1 E-F), acetate and succinate co-consumption (Appendix C, Fig. C.1).

Table 8.4. Amino acid mixtures added to some cultures during oxygen limitation

Amino acid	**Final concentrations (mg.l^{-1})**	
	Low oxygenation	**High oxygenation**
Alanine	679	971
Asparagine	590	842
Glutamine	744	1116
Arginine	296	443
Glycine	338	507

8.4. DISCUSSION

Heterologous protein production under control of the oxygen regulated *ADH2*-promoter was successfully demonstrated in *P. stipitis* (Passoth and Hahn-Hägerdal, 2000). In an attempt to improve production levels of heterologous xylanase, the effect of oxygenation, amino acid supplementation and high cell density was determined. The presence of generic limitations in the production of heterologous proteins, similar to those observed with recombinant *S. cerevisiae* (Chapters 4 to 7), could thus be investigated.

The ability of *P. stipitis* to grow to high cell densities in aerobic culture the presence of excess glucose, without measurable ethanol formation, was demonstrated (Fig. 8.1A, B and E). The maximum specific growth rate of the transformant during fully aerobic growth was significantly lower than the μ_{max} of 0.3 (h^{-1}) previously reported for a wild-type *P. stipitis* strain under similar conditions (Laplace et al., 1991). The reduction in growth rate may have been caused by the loss of the recombinant plasmid during fully aerobic growth, or the negative effect of the auxotrophic *trp5* marker. A general

reduction in the metabolic activity of a *P. stipitis* strain due production of the present heterologous xylanase was also reported (Morosoli et al., 1992).

In the present investigation, the induction of heterologous xylanase production by the transfer from fully aerobic conditions to oxygen limitation was almost immediate, compared to the 6-8 h lag phase prior to the detection of extracellular xylanase previously reported (Fig. 8.1C and D; Passoth and Hahn-Hägerdal, 2000). The increase in cell density prior to induction, or the accuracy of dissolved oxygen control, may have contributed to this observation. Heterologous xylanase production continued for up to 70h after the shift to oxygen-limited conditions in the cultures supplemented with amino acids (Fig. 8.1C and D), indicating that the induction of the *ADH2*-promoter under these conditions were reasonably constant, despite the transient reduction in native *ADH2* mRNA-levels previously reported (Cho and Jeffries, 1999). The quantification of *XLN* mRNA levels is required to confirm the maintenance of heterologous gene transcription during oxygen-limited cultivation.

Increased oxygenation during oxygen-limited conditions improved the general physiology of the host strain, resulting in more biomass formation, decreased CO_2 production, decreased glycerol formation and ethanol consumption after glucose depletion during oxygen-limitation (Fig. 8.1 and Table 8.2), corresponding to previous reports on the dependency of the growth and physiology on the aeration rate (Du Preez et al., 1989; Skoog and Hahn-Hägerdal, 1990; Du Preez, 1994; Passoth and Hahn-Hägerdal, 2000). However, the observed increase in the rate of glucose consumption and biomass formation due to higher oxygenation may not be beneficial in heterologous protein production processes, since the amount of substrate required to sustain the culture during the production phase is significantly increased. The onset of ethanol re-assimilation under oxygen-limited conditions in high aeration cultures also coincided with glucose depletion, as was previously reported (Fig. 8.1B and E; Passoth et al., 1998). The inability of low oxygenation cultures to consume ethanol may therefore have been an artefact of the retardation of glucose consumption. Increased oxygenation did not have a significant effect on heterologous xylanase production in cultures without amino acid supplementation (Fig. 8.1C and D). The level of heterologous gene expression directed by the plasmid-based *ADH2*-promoter was apparently unaffected by the increase in oxygen consumption during oxygen-limited conditions, despite

substantial improvements in the rate of carbon utilisation and biomass formation by the recombinant strain.

The availability of exogenous amino acids for the enhancement of heterologous protein synthesis was critical at both oxygenation rates, since xylanase production was only observed during the 40-100 h window in cultures supplemented with the amino acid mixture (Fig. 8.1C and D). Either the severe effect of oxygen limitation on yeast growth and biosynthesis (Fig. 8.1A; Table 8.2; Du Preez et al., 1989; Skoog and Hahn-Hägerdal, 1990; Du Preez, 1994; Passoth et al., 1996; Passoth et al., 1998), or the aforementioned presence of the *trp5* auxotrophic marker, apparently limited the biosynthetic capacity of the transformant substantially, as was also evident from the inability to produce biomass from consumed ethanol. Auxotrophic yeast transformants were also shown to require excess amounts of exogenous amino acids to stabilise metabolism and to allow for high levels of heterologous protein production (Chapter 6). The increased production of heterologous xylanase by the combined effect of higher oxygenation and amino acid supplementation, both of which also increased biomass formation, thus indicated the requirement for improved resource availability and biosynthetic capacity to support heterologous protein production (Fig. 8.1C and D). The positive effect of amino acids on heterologous protein production was probably not related to the presence of extracellular proteolytic activity, as reported by Den Haan and Van Zyl (2001), since no degradation of extracellular xylanase between 40-100 hours of cultivation was observed in cultures not supplemented with amino acids (Fig. 8.1C and D).

Heterologous xylanase production under regulation of the oxygen-sensitive *ADH2* promoter was also improved by a shift from oxygen-limited to fully aerobic growth during ethanol assimilation, though no additional biomass formation or ethanol consumption was observed (Fig. 8.1 C-E). The induction of the *ADH2* promoter of *P. stipitis* under these conditions, known to be required for both ethanol production and assimilation (Passoth et al., 1998; Cho and Jeffries, 1999), has not been reported previously.

Ethanol production during the induction phase (i.e. oxygen-limitation) of cultures based on the present transformant seems inevitable. However, adequate control of ethanol

formation and consumption for maximal heterologous protein production by *S. cerevisiae* has been demonstrated (Badziong et al., 1999; Noronha et al., 1998; Noronha et al., 1999). Although the current low production levels of *C. albidus* xylanase by the *ADH2*-based expression system could not be compared directly to production by *P. stipitis* via *XYL1*-regulation (Morosoli et al., 1992; Morosoli et al., 1993), or by *S. cerevisiae* (Moreau et al., 1992), due to different enzyme assays, the levels obtained with *S. cerevisiae* were fairly poor, and corresponded to approximately 1% of the total secreted protein (Moreau et al., 1992; Morosoli et al., 1992; Morosoli et al., 1993). In defined medium the production levels of the *T. reesei* xylanase II by *P. stipitis* compared favourably to levels obtained with *S. cerevisiae* (Den Haan and Van Zyl, 2001; La Grange et al., 1996). *P. stipitis* may therefore represent a viable alternative to the more traditional yeasts for heterologous xylanase production.

8.5. CONCLUSIONS

In the present investigation the production of heterologous xylanase by recombinant *P. stipitis*, under regulation of the native *ADH2* promoter, could be improved by cultivating the yeast to higher cell densities, increasing the level of oxygenation under oxygen-limited conditions, supplementing the defined medium with amino acids and reverting to fully aerobic conditions during ethanol consumption. The production level of heterologous xylanase was strongly influenced by the level of induction from the *ADH2* promoter, as was also observed during the cultivation of recombinant *S. cerevisiae* (Chapter 4). The requirement for exogenous amino acids to improve heterologous xylanase production by an auxotrophic transformant was demonstrated in both *P. stipitis* and *S. cerevisiae* (Chapter 6). The general physiological state of the yeast host and the availability of nutrients affected the biosynthetic capacity of both *S. cerevisiae* and *P. stipitis* for the production of a heterologous protein (Chapter 6 and 7). The active regulation of heterologous xylanase production by both yeasts was thus demonstrated.

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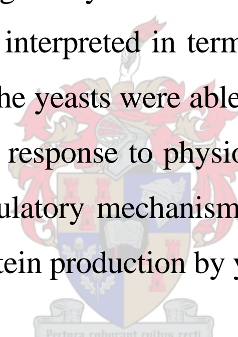
Chapter 9

CONCLUSIONS ON YEAST PHYSIOLOGY AND NITROGEN METABOLISM DURING HETEROLOGOUS PROTEIN PRODUCTION

9.1. INTRODUCTION

The low yields of heterologous xylanase production observed with an *S. cerevisiae* expression system prompted an investigation into the factors that may limit heterologous protein production by yeast. Several experimental strategies were therefore designed to identify specific aspects of yeast genetics, physiology and nitrogen metabolism that may negatively influence yeast productivity in bioprocesses. The experimental data were also interpreted in terms of a possible global sensing and regulatory mechanism, whereby the yeasts were able to actively regulate the production level of heterologous xylanase in response to physiological and environmental factors. The identification of general regulatory mechanisms is of importance for the rational improvement of heterologous protein production by yeast.



9.2. FACTORS THAT MAY LIMIT YEAST PRODUCTIVITY

In the previous chapters, the negative effect of several factors on the productivity of recombinant yeast for heterologous protein production was demonstrated. The disproportionate "metabolic burden" of heterologous protein production may affect yields in a bioprocess negatively by reducing biomass formation and increasing the fermentation time (Chapter 4). The additional metabolic "load" of foreign protein production was strongly related to induction of the yeast stringent stress response (Chapter 5). The corresponding loss of metabolic functionality, due to the downregulation of glycolysis, translation and protein synthesis, the apparent nitrogen limitation and the up-regulation of respiration, resulted in the disproportionate physiological effects of foreign protein production. The loss of biosynthetic capacity associated with the stringent stress response may therefore have limited the ability of the host strain to produce heterologous xylanase, resulting in lower production levels.

The ability of the yeast strain to cope with the metabolic stress associated with the production of a heterologous protein may thus be crucial for obtaining a high level of foreign protein production. The production level of a particular protein could be linked to its propensity to introduce metabolic stress when expressed in a heterologous host organism, which may explain the large variation in production of various heterologous proteins in a particular organism (Chapter 2).

The production levels of heterologous xylanase were significantly influenced by expression vector selection and the presence of auxotrophic mutations in transformed strains of *S. cerevisiae* (Chapter 6). Increasing the gene dosage of the heterologous xylanase with the multicopy YEp-type expression system, dramatically improved xylanase production compared to the integrative, low copy number YIp-type expression system. The level of foreign gene transcription in the cell thus limited heterologous xylanase production, whereas the secretory capacity of the host strain was not saturated. The genetic stability of the autoselective YEp-type expression system in long-term chemostat culture was also demonstrated, as is required for repetitive cultivation in bioprocesses. The autoselection-YEp-based expression system was able to support high levels of heterologous protein production by prototrophic transformants.

The presence of auxotrophic requirements in the transformed *S. cerevisiae* strains substantially reduced heterologous xylanase production (Chapter 6). The retention of uncomplimented auxotrophic markers in transformants required the stabilisation of nitrogen metabolism via the saturation of yeast cells with imported amino acids, to allow for high levels of heterologous xylanase production. The addition of amino acids to the cultivation medium also improved heterologous xylanase production by an auxotrophic *P. stipitis* transformant (Chapter 8). The presence of excessive auxotrophic mutations apparently caused a physiological defect in transformants that severely reduced production levels of heterologous xylanase. The auxotrophic requirements either made it more difficult for the yeast to cope with the additional burden of heterologous protein production, or introduced irregularities into the amino acid metabolism. The removal of excessive auxotrophic markers resulted in high levels of xylanase production by a prototrophic transformant in defined medium without amino acid addition. As the irregularities introduced by the presence of auxotrophic mutations in transformants may cripple the productivity of a bioprocess for heterologous protein

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production severely, the use of auxotrophic strains should be avoided completely (Çakar et al., 1999).

Heterologous xylanase production by *S. cerevisiae* in defined medium was partially limited by the availability of metabolic precursors for protein synthesis, since both the addition of suitable amino acid mixtures and succinate improved heterologous xylanase production (Chapter 7). This supported the notion that foreign protein production may drain metabolic resources from the central pathways (Jin et al., 1997; Ramirez and Bentley, 1993). An additional requirement for metabolic precursors and building blocks may thus exist in cellular metabolism for the synthesis of a foreign protein. Although the addition of these components to the defined medium may increase the productivity of a bioprocess, the magnitude of improvements were typically smaller than two-fold, indicating the presence of other limiting factors besides the availability of amino nitrogen and TCA metabolites. The order of magnitude improvement obtained by the removal of the auxotrophic requirements from the transformed strains indicated that molecular genetics and the adaptation of the expression system has a superior potential for increasing the production levels of heterologous proteins, rather than the simple optimisation of cultivation conditions.

The production of heterologous xylanase by recombinant *P. stipitis*, under regulation of the native *ADH2* promoter, could be improved by cultivating the yeast to high cell densities, increasing the level of oxygenation under oxygen-limited conditions, supplementing the defined medium with amino acids and reverting to fully aerobic conditions during ethanol consumption (Chapter 8). The production level of heterologous xylanase was thus strongly influenced by the level of induction from the oxygen-sensitive *ADH2* promoter, similar to observations during the cultivation of recombinant *S. cerevisiae* (Chapter 4). The requirement for exogenous amino acids to improve heterologous xylanase production by auxotrophic transformants of both *S. cerevisiae* (Chapter 6) and *P. stipitis* (Chapter 8) was also demonstrated. Changes in the general physiological state of the yeast host and nutrient availability influenced the total biosynthetic capacity of both *S. cerevisiae* and *P. stipitis* and thus affected production levels of heterologous xylanase (Chapters 6 to 8). The presence of several generic limitations in the production of heterologous xylanase by the yeasts, *S. cerevisiae* and *P. stipitis*, was therefore identified, including transcription level, nutrient

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availability, the metabolic vitality of the host and the biosynthetic capacity. Due to ability to grow to high cell density in the presence of excess glucose in batch culture without the formation of ethanol, *P. stipitis* remains an attractive host for the development of bioprocesses for heterologous protein production.

9.3. THE DESIGN OF LIFE

The present investigations have provided proof for the existence of global sensing and regulatory mechanisms whereby the yeast may actively regulate both the production of heterologous proteins and the physiological response to this process. Yeast cells were able to down-regulate biomass formation in response to heterologous protein production, indicating the presence of cellular mechanisms able to sense and respond to the production of a foreign protein (Chapter 4). Yeast also responded actively to the production of heterologous xylanase by inducing the stringent stress response, which dominated the physiology and transcriptional profile of *S. cerevisiae* during foreign protein production. The active induction of the stringent stress response due to the synthesis and secretion of a foreign protein confirmed the presence of a global sensing and regulatory mechanism, similar to a virtual "cellular intelligence," that determined the response to heterologous protein production. The production levels of heterologous xylanase may also be down-regulated by the loss of functionality (biosynthetic capacity) associated with the stringent response.

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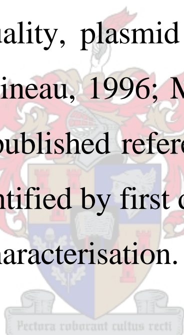
The global and apparently "intelligent" regulation of heterologous protein production by the yeast cell was also demonstrated by the response of auxotrophic *S. cerevisiae* strains to a limited availability of amino acids, below the levels required for the saturation of the cell. The production level of the foreign protein was actively down-regulated without reducing the level of xylanase transcripts in the cell (Chapter 6). A strong relationship between the availability of resources in nitrogen metabolism and the willingness of the yeast to produce a foreign protein was thus demonstrated. The general physiological state of the yeast host and the availability of nutrients also affected the biosynthetic capacity of both prototrophic *S. cerevisiae* and auxotrophic *P. stipitis* for the production of a heterologous protein (Chapters 6 to 8). The biosynthetic capacity of a recombinant yeast strain for heterologous protein production may thus be actively regulated in response to the availability of sufficient resources for protein

synthesis. The heterologous protein production from a plasmid-based expression system was thus subjected to a number of regulatory mechanisms in the yeast cell, which complicated the manipulation of the cellular biosynthesis of a foreign protein at will.

9.4. RECOMMENDATIONS

Two aspects of yeast expression systems for heterologous protein production that require extensive clarification are the influence of host strain and foreign protein characteristics on the attainable production levels. In the present dissertation, the influence of host strain characteristics on heterologous xylanase was demonstrated by the large difference in production levels obtained with the auxotrophic and prototrophic transformants of *S. cerevisiae*. However, the specific characteristics of different yeast strains that determine the levels of foreign gene transcription, translation and secretory efficiency, protein quantity and quality, plasmid stability and plasmid copy number (Park et al., 2000; Eckart and Bussineau, 1996; Mendoza-Vega et al., 1994) have not been identified, despite numerous published references to their influence (see Chapter 2). Such characteristics may be identified by first developing and/or selecting improved host strains, followed by rigorous characterisation.



Several investigations have also referred to the influence of the biochemical characteristics of a specific recombinant protein on the attainable production levels (see Chapter 2). In the present dissertation reference was made to the potential relationship between the measure of metabolic stress introduced by the production of a particular protein, and the final production level (Chapter 5). The measurement of the intracellular proteolytic activity towards various heterologous proteins may thus be useful for determining the relevance of the stringent proteolytic response in heterologous protein production. The magnitude of the stringent stress response by a particular host strain towards various heterologous proteins may also be compared via transcriptional profiling, whereby a possible relationship between the biochemical characteristics of a protein and the corresponding levels of stress response and extracellular protein production can be established. Furthermore, the elucidation of the specific characteristics of recombinant proteins that may limit their production in heterologous hosts will provide valuable information on the most suitable host for the

production of a particular protein. A systematic investigation of the influence of host strains and recombinant protein characteristics that determine production levels will allow for the rational, rather than empirical, design of future production systems.

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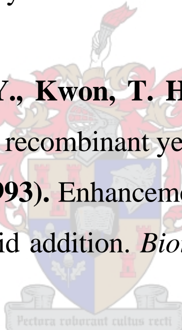

Appendix A

Additional results from Chapter 4

Table A.1 Product yields on glucose during batch cultivation of auxotrophic strains in defined medium

Strain	**Yield on glucose ($g_{product} \cdot g_{glucose}^{-1}$)**	
	Acetate	**Succinate**
[Host]	0.012 ±0.004	0.0015 ±0.0001
[PGK1]	0.012 ±0.004	0.0019 ±0.0004
[PGK1-XYN]	0.014 ±0.006	0.0013 ±0.0003
[ADH2]	0.008 ±0.001	0.0011 ±0.0005
[ADH2-XYN]	0.011 ±0.001	0.0011 ±0.0002

Table A.2 Maximum specific growth rate (μ_{max}, h^{-1}) during growth on glucose in complex medium (YPD)

Strain	**Maximum specific growth rate (μ_{max}, h^{-1})**
***Sc* Y294**	0.37 ± 0.04
***Sc* Y294 [Host]**	0.38 ± 0.03

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Appendix B

Additional results from Chapter 6

Table B.1 Xylanase production by the prototrophic [ADH2-XYN] Int transformants during shake-flask cultivation in defined medium

Transformant #	Xylanase production (μg.l^{-1})	
	3 days cultivation	5 days cultivation
2	98	91
7	10	77
8	16	96
10	27	21
13	23	110
19	28	85
20	38	44
23	127	0
25	42	99
31	22	68
37	19	122
40	0	132
45	29	65
46	111	78
56	25	84
57	85	120

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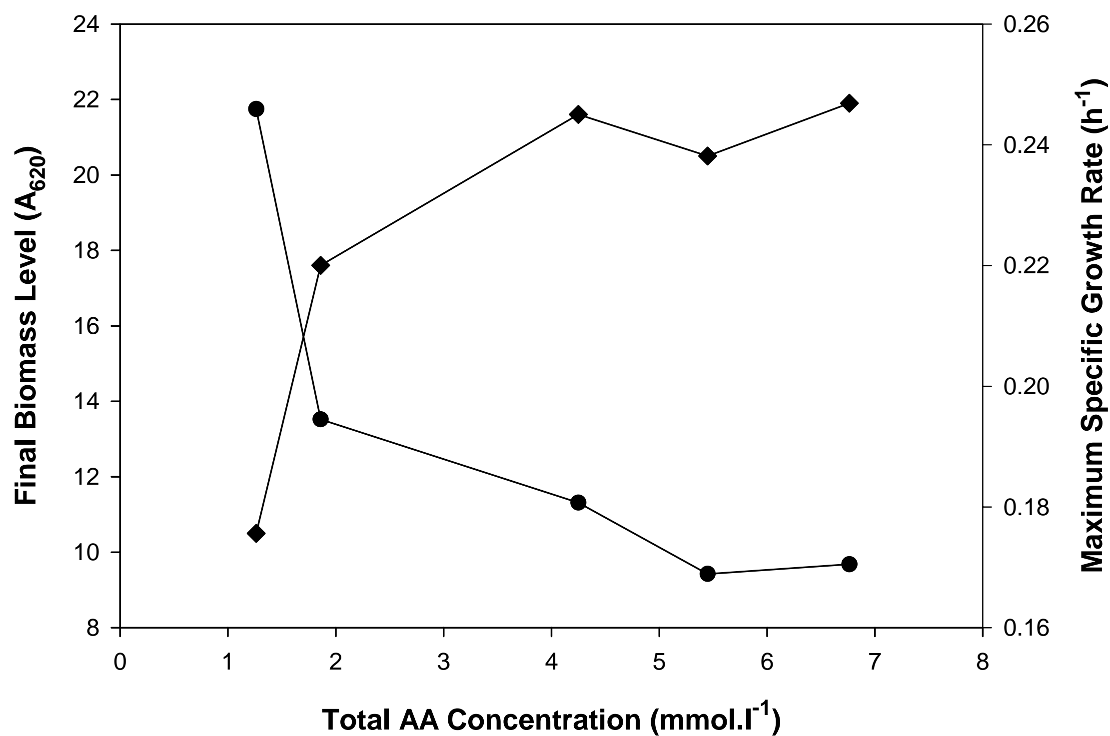

Figure B.1. Biomass formation by the auxotrophic [ADH2-XYN] strain during shake-flask cultivation in defined medium, supplemented with His, Trp and Leu at different total concentrations. (♦) Final level of biomass formation (Absorbance, 620 nm) and (●) maximum specific growth rate (μ_{max}, h^{-1}).

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Table B.2. Carbon- and degree of reduction balance during batch cultivation of the auxotrophic [ADH2-XYN] strain in defined medium containing different total levels of amino acids

Total AA Concentration [mM]	Product formation or substrate consumption ($cmol.cmol_{ethanol\ consumed}^{-1}$)								Degree of Reduction	C-balance
	Biomass	Glycerol	Acetate	Succinate	CO_2	O_2 *	Amino acids	EtOH Evaporated		
10.8	0.24	-0.05	-0.02	0.00	0.47	-1.50	-0.12	0.48	0.00	1.00
4.3	0.28	-0.05	-0.04	0.00	0.76	-2.38	-0.10	0.15	0.00	1.00
1.9	0.05	-0.03	-0.05	0.01	0.72	-2.10	0.00	0.32	0.00	1.00
0.0 **	0.34	-0.12	0.00	0.00	0.45	-1.30	0.00	0.33	0.00	1.00

* Reported as $amol.cmol_{ethanol\ consumed}^{-1}$

** [ADH2-XYN] PlasPro strain

Table B.3. Effect of components besides amino acids in the defined medium on xylanase production by the auxotrophic [ADH2-XYN] strain in chemostat culture

Feed C/N Ratio	**Changes in medium components**		**Xylanase**
($g_{glucose}$.$mmol_{amino\ N}^{-1}$)	**$NH4^+$ and Salts**	**Vitamins and Trace Elements**	**[$mg.l^{-1}$]**
0.25	1	1	8.3
0.25	½	1	10.6
0.25	½	½	9.8

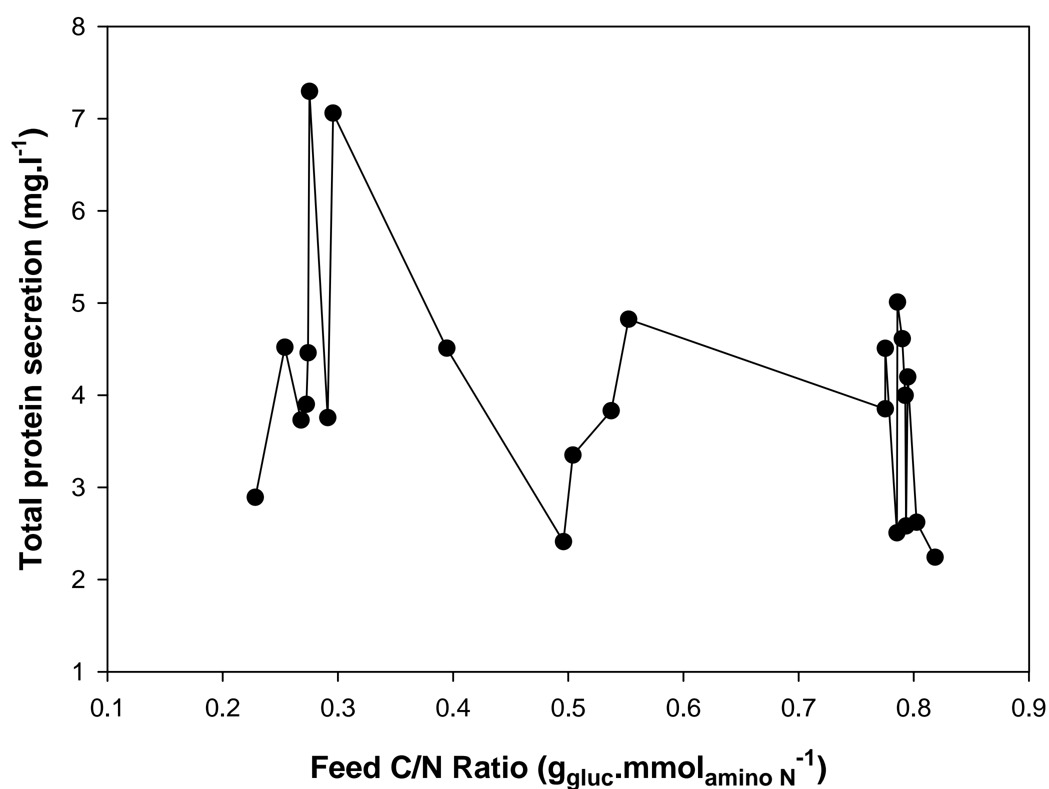

Figure B.2. Total extracellular protein production by the auxotrophic [ADH2-XYN] strain during chemistat cultivation in defined medium containing seven amino acids. (•) Total extracellular protein production ($mg.l^{-1}$).

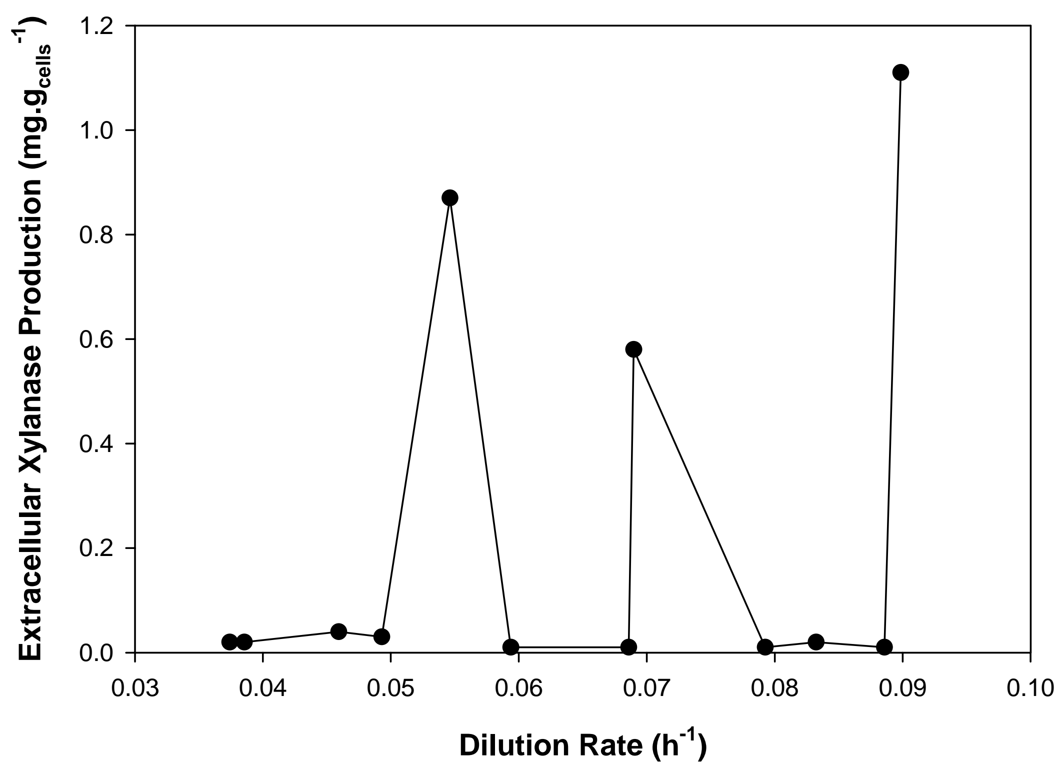

Figure B.3. No clear correlation between dilution rate and xylanase production by the auxotrophic [ADH2-XYN] strain during chemistat cultivation in defined medium containing seven amino acids. Outliers of high xylanase production correspond to media with low C/N ratio's. (•) Extracellular xylanase production (mg.$g_{biomass}^{-1}$).

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Appendix C

Additional results from Chapter 8

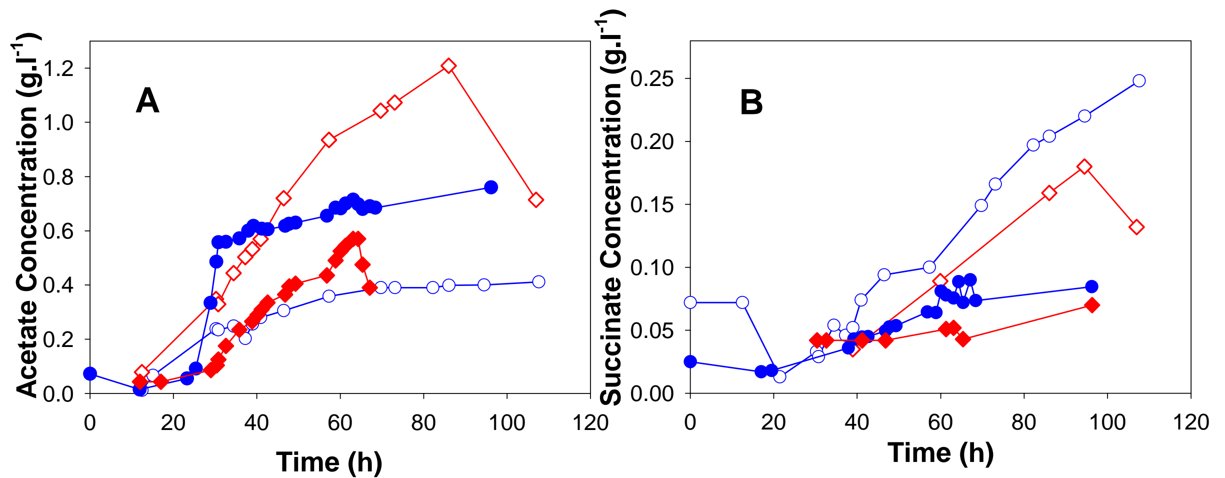

Figure C.1. Acetate and succinate production in batch cultivation of recombinant *P. stipitis*, switched from fully aerobic conditions to oxygen-limitation after 31h. "Low" (●,O) or "High" (◆,◇) oxygenation was supplied during oxygen limitation. The amino acid mixture was added after 43h to the cultures represented by the closed symbols. The culture with high oxygenation and amino acid addition was shifted to fully aerobic growth after 63h.

Appendix D

Functional classification of the gene expression data from Chapter 5.

The classification of the 1014 genes, with a significant change in their expression level in either the *S. cerevisiae* Y294 [PGK1-XYN] or Y294 [PGK1] strains, into the MIPS functional categories (Mewes et al., 2000) is presented below.

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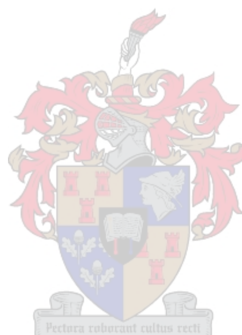

All Genes showing Significant Change (1014), sorted into MIPS categories

Information presented in the Sequence:	ORF Name	Gene Nam	Gene Alias	Identifier	Function	pDLG6 vs pJC1 Comparison			pDLG6 vs YEp352 Comparison			pJC1 vs pDLG6 Comparison			pJC1 vs YEp352 Co	
						Expr Level	Fold Chng	Signific. Factor	Expr Level	Fold Chng	Signific. Factor	Expr Level	Fold Chng	Signific. Factor	Expr Level	Fold Chng
METABOLISM																
amino acid metabolism																
amino acid biosynthesis																
Glutamate Family (Glut, Glum, Pro, Arg, Lys)																
	YOL058W	*ARG1*	*ARG10*	8593_at	YOL058W	1917.4	1.1	191.7	1917.4	1.6	1150.4	1715.0	-1.1	171.5	1715.0	1.5
	YDL182W	*LYS20*		6683_g_at	YDL182W	2082.9	-1.0	101.7	2082.9	1.1	310.2	2189.0	1.0	106.8	2189.0	1.2
	YIR034C	*LYS1*		4070_at	YIR034C s	1335.0	-1.0	65.2	1335.0	1.2	332.4	1385.0	1.0	67.6	1385.0	1.3
	YNR050C	*LYS9*	*LYS13*	8808_at	YNR050C .	0.0	0.0	0.0	0.0	0.0	0.0	1516.0	1.1	151.6	1516.0	1.2
	YIL094C	*LYS12*		4172_at	YIL094C H	1052.7	-1.2	206.1	1052.7	-1.0	0.0	1219.6	1.2	238.8	1219.6	1.2
	YJR109C	*CPA2*		10902_at	YJR109C c	778.2	1.1	115.9	778.2	1.2	193.8	0.0	0.0	0.0	0.0	0.0
	YER069W	*ARG5,6*		5642_at	YER069W	592.0	1.2	118.4	592.0	1.0	28.9	499.6	-1.2	99.9	499.6	-1.1
	YDL171C	*GLT1*		6649_at	YDL171C (	204.4	-1.7	142.5	204.4	-2.2	244.8	344.1	1.7	239.9	344.1	-1.3
	YPR035W	*GLN1*		7698_at	YPR035W	1270.0	-1.4	508.0	1270.0	-1.2	316.2	1721.7	1.4	688.7	1721.7	1.1
	YER023W	*PRO3*	*ORE2*	5678_at	YER023W	771.7	-1.2	192.1	771.7	-1.4	346.6	961.5	1.2	239.4	961.5	-1.2
Aromatic Family (Phen, Tyr, Tryp)																
	YDR035W	*ARO3*		6450_at	YDR035W	1396.4	1.1	139.6	1396.4	1.3	418.9	1273.6	-1.1	127.4	1273.6	1.2
	YKL211C	*TRP3*		10801_at	YKL211C a	465.6	1.2	115.9	465.6	1.7	348.9	337.3	-1.2	84.0	337.3	1.4
	YDR354W	*TRP4*		6095_at	YDR354W	321.6	1.2	80.1	321.6	1.5	176.6	0.0	0.0	0.0	0.0	0.0
	YGL026C	*TRP5*		5036_at	YGL026C t	1026.9	-1.2	201.1	1026.9	-1.1	98.0	1215.8	1.2	238.1	1215.8	1.1
	YDR007W	*TRP1*		6468_at	YDR007W	233.8	1.4	105.0	233.8	1.1	34.8	0.0	0.0	0.0	0.0	0.0
Serine Family (Ser, Gly, Cys)																
	YGR055W	*MUP1*		4936_at	High affinity	968.3	2.0	968.3	968.3	9.0	7745.5	486.4	-2.0	486.4	486.4	4.5
	YEL046C	*GLY1*		5789_at	Threonine .	560.0	2.2	699.6	560.0	3.1	1175.9	248.0	-2.2	309.9	248.0	1.4
	YGR155W	*CYS4*	*NHS5, STI*	4856_at	YGR155W	911.2	1.1	135.7	911.2	1.3	273.3	789.1	-1.1	117.5	789.1	1.1
	YFL018C	*LPD1*	*HPD1*	5367_at	YFL018C c	871.1	1.2	216.9	871.1	-1.0	0.0	700.8	-1.2	174.5	700.8	-1.2
Aspartate Family (Asp, Asgn, Thr, Meth, Ileu)																
	YGR055W	*MUP1*		4936_at	High affinity	968.3	2.0	968.3	968.3	9.0	7745.5	486.4	-2.0	486.4	486.4	4.5
	YPR145W	*ASN1*		7588_at	YPR145W	1054.4	1.3	368.1	1054.4	2.6	1685.0	757.0	-1.3	264.2	757.0	1.9
	YPL273W	*SAM4*		8022_at	YPL273W .	1143.8	1.4	457.5	1143.8	1.7	800.6	810.0	-1.4	324.0	810.0	1.2
	YGR124W	*ASN2*		4869_at	YGR124W	938.4	1.3	281.5	938.4	1.5	469.2	735.3	-1.3	220.6	735.3	1.2
	YBR294W	*SUL1*		7067_at	Putative su	97.3	1.3	33.9	97.3	4.0	291.6	70.8	-1.3	24.7	70.8	2.9
	YJR130C	*STR2*		10878_at	YJR130C (	347.7	1.2	86.6	347.7	1.7	260.5	272.6	-1.2	67.9	272.6	1.4
	YGL184C	*STR3*		5196_at	YGL184C (	243.4	1.3	72.1	243.4	1.9	229.8	0.0	0.0	0.0	0.0	0.0
	YGL125W	*MET13*	*MET11*	5117_at	YGL125W	579.3	1.1	86.3	579.3	1.3	173.8	0.0	0.0	0.0	0.0	0.0
	YLR092W	*SUL2*		10256_at	YLR092W	560.9	1.1	56.1	560.9	1.2	112.2	545.7	-1.1	54.6	545.7	1.1
	YDR158W	*HOM2*		6304_at	YDR158W	1615.5	-1.1	240.6	1615.5	1.0	78.9	1816.5	1.1	270.5	1816.5	1.1
	YJR148W	*BAT2*	*TWT2, EC*	10896_at	YJR148W	0.0	0.0	0.0	0.0	0.0	0.0	204.4	-2.0	214.5	204.4	1.7
	YER052C	*HOM3*	*BOR1, SIL*	5664_at	YER052C .	0.0	0.0	0.0	0.0	0.0	0.0	821.6	1.1	82.2	821.6	1.2
	YLR303W	*MET17*	*MET15, MI*	10018_at	YLR303W	2391.4	-1.1	356.1	2391.4	-1.0	0.0	2776.8	1.1	413.5	2776.8	1.2
	YCR053W	*THR4*		6841_at	YCR053W	0.0	0.0	0.0	0.0	0.0	0.0	802.0	1.1	80.2	802.0	1.3
	YER091C	*MET6*		5620_at	YER091C \	966.8	-1.2	240.7	966.8	-1.1	96.7	1207.0	1.2	300.5	1207.0	1.1
	YLR180W	*SAM1*	*ETH10*	10161_g_a	YLR180W	3444.8	-1.2	689.0	3444.8	-1.0	168.1	4328.7	1.2	865.7	4328.7	1.2
	YJR010W	*MET3*		11032_at	YJR010W .	902.1	-1.2	180.4	902.1	-1.2	180.4	1098.4	1.2	219.7	1098.4	1.0

	YKL001C	*MET14*		10563_at	YKL001C a	1314.9	-1.2	327.4	1314.9	-1.1	195.8	1620.4	1.2	403.5	1620.4	1.0
	YLR027C	*AAT2*	*ASP5*	10323_at	YLR027C a	1551.3	-1.1	231.0	1551.3	-1.1	231.0	1722.6	1.1	256.5	1722.6	-1.0
	YJR139C	*HOM6*		10887_at	YJR139C H	2306.2	-1.2	461.2	2306.2	-1.1	343.4	2917.6	1.2	583.5	2917.6	1.0
Pyruvate Family (Ala, Val, Leu)																
	YNL104C	*LEU4*		8973_at	YNL104C a	1720.8	1.1	172.1	1720.8	2.0	1720.8	1616.3	-1.1	161.6	1616.3	1.8
	YLR089C			10253_at	YLR089C a	820.2	2.2	984.2	820.2	2.5	1230.2	348.3	-2.2	418.0	348.3	1.1
	YLR355C	*ILV5*		9979_at	YLR355C a	1911.6	1.0	93.3	1911.6	1.1	284.7	0.0	0.0	0.0	0.0	0.0
	YJR016C	*ILV3*		10993_at	YJR016C d	1257.3	1.1	125.7	1257.3	1.2	251.5	0.0	0.0	0.0	0.0	0.0
	YMR108W	*ILV2*	*SMR1, TH*	9550_at	YMR108W	502.3	1.1	74.8	502.3	1.4	225.6	448.3	-1.1	66.8	448.3	1.3
	YCL018W	*LEU2*		6909_at	YCL018W	1041.6	1.3	312.5	1041.6	1.2	208.3	805.3	-1.3	241.6	805.3	-1.0
	YJR148W	*BAT2*	*TWT2, EC*	10896_at	YJR148W	0.0	0.0	0.0	0.0	0.0	0.0	204.4	-2.0	214.5	204.4	1.7
	YCL009C	*ILV6*		6871_at	YCL009C S	1187.4	-1.1	176.8	1187.4	-1.0	0.0	1351.5	1.1	201.3	1351.5	1.1
	YER086W	*ILV1*	*ISO1*	5614_at	YER086W	655.1	-1.0	0.0	655.1	1.2	131.0	0.0	0.0	0.0	0.0	0.0
Histidine (His)																
	YCL030C	*HIS4*		6902_at	YCL030C H	1793.9	-1.1	179.4	1793.9	1.2	358.8	1904.8	1.1	190.5	1904.8	1.3
	YIL116W	*HIS5*		4195_at	YIL116W h	582.6	1.2	145.1	582.6	1.6	378.2	441.8	-1.2	110.0	441.8	1.4
	YOR202W	*HIS3*		8359_at	YOR202W	673.6	1.1	67.4	673.6	1.1	100.3	0.0	0.0	0.0	0.0	0.0
	YGR204W	*ADE3*		4814_at	YGR204W	724.6	-1.2	144.9	724.6	-1.2	180.4	901.8	1.2	180.4	901.8	-1.0
General																
	YPR074C	*TKL1*		7694_at	YPR074C T	1097.1	-1.1	163.4	1097.1	-1.0	53.5	1280.0	1.1	190.6	1280.0	1.1
	YMR250W	*GAD1*		9388_at	YMR250W	67.9	2.2	81.3	67.9	1.7	50.9	31.1	-2.2	37.2	31.1	-1.2
	YHR068W	*DYS1*		4449_at	YHR068W	603.4	-1.4	241.4	603.4	-1.3	181.0	781.4	1.4	312.6	781.4	1.1
regulation of amino acid metabolism																
	YHR137W	*ARO9*		4386_at	Aromatic a	1112.7	2.2	1332.6	1112.7	6.9	6610.5	513.9	-2.2	615.5	513.9	3.2
	YDR160W	*SSY1*	*SHR10*	6306_at	YDR160W	35.6	1.1	3.6	35.6	2.5	55.2	33.2	-1.1	3.3	33.2	2.4
	YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2
	YGL202W	*ARO8*		5223_at	YGL202W	1059.9	1.2	263.9	1059.9	1.3	318.0	843.6	-1.2	210.1	843.6	1.0
	YIR017C	*MET28*		4098_at	YIR017C T	353.7	1.2	88.1	353.7	1.3	106.1	0.0	0.0	0.0	0.0	0.0
	YMR042W	*ARG80*	*ARGR1*	9575_at	Regulator c	57.3	1.8	45.8	57.3	1.9	51.5	31.3	-1.8	25.0	31.3	1.0
	YCR028C	*FEN2*		6863_at	YCR028C	375.2	-1.2	93.4	375.2	-1.4	148.7	465.8	1.2	116.0	465.8	-1.1
	YEL009C	*GCN4*	*AAS3, AR*	5737_at		1433.2	1.0	70.0	1433.2	-1.1	136.8	1390.5	-1.0	67.9	1390.5	-1.1
	YDR328C	*SKP1*	*MGO1*	6160_at	YDR328C	487.2	1.2	121.3	487.2	-1.1	48.7	386.5	-1.2	96.2	386.5	-1.4
amino acid transport																
	YDR046C	*BAP3*		6415_at	Valine tran	670.0	2.6	1072.0	670.0	178.1	118656.5	297.2	-2.6	475.5	297.2	81.3
	YBR068C	*BAP2*		7291_at	Major AA p	646.7	2.3	839.2	646.7	133.8	85884.2	280.3	-2.3	363.8	280.3	58.7
	YPL265W	*DIP5*		8030_at	Dicarboxyli	911.4	2.2	1085.3	911.4	18.4	15885.5	416.4	-2.2	495.9	416.4	8.5
	YGR055W	*MUP1*		4936_at	High affinity	968.3	2.0	968.3	968.3	9.0	7745.5	486.4	-2.0	486.4	486.4	4.5
	YNL268W	*LYP1*		9126_at	YNL268W	580.1	-1.0	0.0	580.1	2.3	782.8	570.8	-1.0	0.0	570.8	2.3
	YDR508C	*GNP1*		5977_at	High-affinity	434.0	1.7	302.5	434.0	5.8	2102.6	256.4	-1.7	178.7	256.4	3.5
	YBR069C	*TAT1*	*VAP1, WA*	7292_at	Amino acid	164.5	3.1	345.1	164.5	11.2	1685.2	53.1	-3.1	111.4	53.1	4.1
	YKR039W	*GAP1*		10511_at	YKR039W	46.7	-2.4	65.2	46.7	-1.4	21.0	110.8	2.4	154.9	110.8	1.7
	YCL025C	*AGP1*	*YCC5*	6907_at	YCL025C A	249.7	1.3	87.2	249.7	1.8	212.1	186.9	-1.3	65.2	186.9	1.4
	YOL020W	*TAT2*	*LTG3, SAB*	8587_at	YOL020W	216.0	1.7	151.2	216.0	2.3	291.5	127.2	-1.7	89.0	127.2	1.4
	YFL055W	*AGP3*		5425_at	General an	16.0	1.6	10.4	16.0	4.8	61.6	9.6	-1.6	6.2	9.6	3.0
	YKL124W	*SSH4*	*MLF4*	10704_at	YKL124W s	28.2	2.2	33.4	28.2	2.7	47.9	13.1	-2.2	15.5	13.1	1.5
	YBR132C	*AGP2*		7223_at	Amino acid	13.8	3.5	34.3	13.8	3.5	34.4	4.0	-3.5	9.9	4.0	-1.2

Category	ORF	Gene	Alias	Probe	Description												
	YLL061W	*MMP1*		10415_at	YLL061W s	16.8	2.5	25.1	16.8	2.2	20.1	6.6	-2.5	9.9	6.6	-1.2	
amino acid degradation (catabolism)																	
Glutamate Family (Glut, Glum, Pro, Arg, Lys)																	
	YPL111W	*CAR1*	*LPH15*	7869_at	Arginase /	304.8	2.1	350.3	304.8	3.8	853.4	142.4	-2.1	163.7	142.4	1.9	
	YDL215C	*GDH2*		6693_at	YDL215C N	199.9	1.8	169.7	199.9	2.2	249.7	107.7	-1.8	91.5	107.7	1.2	
	YOR375C	*GDH1*	*URE1*	8174_at	YOR375C	662.8	-2.4	927.9	662.8	-2.5	994.1	1441.3	2.4	2017.8	1441.3	-1.0	
Aromatic Family (Phen, Tyr, Tryp)																	
Serine Family (Ser, Gly, Cys)																	
	YEL046C	*GLY1*		5789_at	Threonine	560.0	2.2	699.6	560.0	3.1	1175.9	248.0	-2.2	309.9	248.0	1.4	
	YKL218C	*SRY1*		10794_at	YKL218C S	517.3	1.6	335.8	517.3	2.7	878.4	307.0	-1.6	199.3	307.0	1.6	
	YDR019C	*GCV1*	*GSD1*	6480_at	YDR019C	443.3	1.4	199.1	443.3	1.2	86.8	318.4	-1.4	143.0	318.4	-1.2	
	YIL167W	*SDL1*		4284_at	Serine deh	13.4	4.2	42.7	13.4	3.8	37.9	1.6	-4.2	5.1	1.6	-1.5	
	YCL064C	*CHA1*		6915_at	YCL064C c	14.8	-1.1	2.1	14.8	2.3	19.0	16.6	1.1	2.3	16.6	2.6	
Aspartate Family (Asp, Asgn, Thr, Meth, Ileu)																	
	YLR180W	*SAM1*	*ETH10*	10161_g_a	YLR180W	3444.8	-1.2	689.0	3444.8	-1.0	168.1	4328.7	1.2	865.7	4328.7	1.2	
	YPL273W	*SAM4*		8022_at	YPL273W	1143.8	1.4	457.5	1143.8	1.7	800.6	810.0	-1.4	324.0	810.0	1.2	
	YJR148W	*BAT2*	*TWT2, EC*	10896_at	YJR148W	0.0	0.0	0.0	0.0	0.0	0.0	204.4	-2.0	214.5	204.4	1.7	
	YDR502C	*SAM2*	*ETH2*	5971_at	YDR502C	988.2	-1.2	197.6	988.2	-1.0	48.2	1185.2	1.2	237.0	1185.2	1.1	
	YCL064C	*CHA1*		6915_at	YCL064C c	14.8	-1.1	2.1	14.8	2.3	19.0	16.6	1.1	2.3	16.6	2.6	
	YDR321W	*ASP1*		6152_at	YDR321W	670.4	-1.3	234.0	670.4	-1.2	166.9	901.4	1.3	314.7	901.4	1.1	
Pyruvate Family (Ala, Val, Leu)																	
	YJR148W	*BAT2*	*TWT2, EC*	10896_at	YJR148W	0.0	0.0	0.0	0.0	0.0	0.0	204.4	-2.0	214.5	204.4	1.7	
Histidine (His)																	
General																	
	YJR025C	*BNA1*	*HAD1*	11003_at	YJR025C 3	1095.0	1.1	163.1	1095.0	1.6	710.9	940.5	-1.1	140.1	940.5	1.4	
nitrogen and sulfur metabolism																	
nitrogen and sulfur utilization																	
	YOL058W	*ARG1*	*ARG10*	8593_at	YOL058W	1917.4	1.1	191.7	1917.4	1.6	1150.4	1715.0	-1.1	171.5	1715.0	1.5	857.5
	YPR035W	*GLN1*		7698_at	YPR035W	1270.0	-1.4	508.0	1270.0	-1.2	316.2	1721.7	1.4	688.7	1721.7	1.1	172.2
	YDR353W	*TRR1*		6139_at	YDR353W	869.2	-1.2	173.8	869.2	-1.1	129.4	1042.3	1.2	208.5	1042.3	1.1	104.2
	YIR029W	*DAL2*	*ALC1*	4065_at	Allantoicas	2.1	-3.1	4.3	2.1	-1.2	0.4	22.3	3.1	46.2	22.3	3.0	44.6
	YLR089C			10253_at	YLR089C a	820.2	2.2	984.2	820.2	2.5	1230.2	348.3	-2.2	418.0	348.3	1.1	34.8
	YDL215C	*GDH2*		6693_at	YDL215C N	199.9	1.8	169.7	199.9	2.2	249.7	107.7	-1.8	91.5	107.7	1.2	21.5
	YIR027C	*DAL1*		4063_at	Allantoinas	-2.7	-3.1	-5.5	-2.7	1.3	-0.9	1.4	3.1	2.9	1.4	2.6	2.2
	YJR010W	*MET3*		11032_at	YJR010W	902.1	-1.2	180.4	902.1	-1.2	180.4	1098.4	1.2	219.7	1098.4	1.0	0.0
	YKL001C	*MET14*		10563_at	YKL001C a	1314.9	-1.2	327.4	1314.9	-1.1	195.8	1620.4	1.2	403.5	1620.4	1.0	0.0
	YLR027C	*AAT2*	*ASP5*	10323_at	YLR027C a	1551.3	-1.1	231.0	1551.3	-1.1	231.0	1722.6	1.1	256.5	1722.6	-1.0	0.0
	YOR375C	*GDH1*	*URE1*	8174_at	YOR375C	662.8	-2.4	927.9	662.8	-2.5	994.1	1441.3	2.4	2017.8	1441.3	-1.0	0.0
	YIR032C	*DAL3*		4068_at	Ureidoglyc	27.7	-5.8	132.3	27.7	-7.0	166.2	143.8	5.8	686.9	143.8	-1.2	28.8
	YDL171C	*GLT1*		6649_at	YDL171C G	204.4	-1.7	142.5	204.4	-2.2	244.8	344.1	1.7	239.9	344.1	-1.3	103.2
regulation of nitrogen and sulphur utilization																	
	YPL111W	*CAR1*	*LPH15*	7869_at	Arginase /	304.8	2.1	350.3	304.8	3.8	853.4	142.4	-2.1	163.7	142.4	1.9	128.2
	YFL021W	*GAT1*	*NIL1*	5409_at	YFL021W t	52.6	-2.5	81.2	52.6	-2.1	60.2	119.0	2.5	183.9	119.0	1.2	23.8
	YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2	10.9
	YIR017C	*MET28*		4098_at	YIR017C T	353.7	1.2	88.1	353.7	1.3	106.1	0.0	0.0	0.0	0.0	0.0	0.0
	YMR042W	*ARG80*	*ARGR1*	9575_at	Regulator c	57.3	1.8	45.8	57.3	1.9	51.5	31.3	-1.8	25.0	31.3	1.0	0.0
	YKR034W	*DAL80*	*UGA43*	10551_at	YKR034W	0.0	0.0	0.0	0.0	0.0	0.0	1.8	2.0	1.9	1.8	-2.0	1.8
	YCR028C	*FEN2*		6863_at	YCR028C	375.2	-1.2	93.4	375.2	-1.4	148.7	465.8	1.2	116.0	465.8	-1.1	46.6
nucleotide metabolism																	
purine ribonucleotide metabolism																	

	YMR120C	*ADE17*		9519_at	YMR120C	1034.4	-1.3	361.1	1034.4	-1.1	103.4	1366.7	1.3	477.1	1366.7	1.2	273.3
	YMR300C	*ADE4*		9306_at	YMR300C	0.0	0.0	0.0	0.0	0.0	0.0	183.2	1.3	62.6	183.2	1.8	146.6
	YNL220W	*ADE12*	*BRA9*	9083_at	YNL220W	1163.7	-1.1	173.3	1163.7	-1.0	0.0	1347.0	1.1	200.6	1347.0	1.1	134.7
	YML056C	*IMD4*		9700_at	YML056C	602.7	1.5	331.0	602.7	1.7	451.6	360.6	-1.5	198.0	360.6	1.2	72.1
	YIR029W	*DAL2*	*ALC1*	4065_at	Allantoicas	2.1	-3.1	4.3	2.1	-1.2	0.4	22.3	3.1	46.2	22.3	3.0	44.6
	YIR027C	*DAL1*		4063_at	Allantoinas	-2.7	-3.1	-5.5	-2.7	1.3	-0.9	1.4	3.1	2.9	1.4	2.6	2.2
	YAR073W	*IMD1*		11263_f_at	YAR073W	255.3	1.3	89.1	255.3	1.4	114.7	0.0	0.0	0.0	0.0	0.0	0.0
	YNL141W	*AAH1*		9027_at	YNL141W	190.1	-1.2	38.0	190.1	-1.5	104.4	0.0	0.0	0.0	0.0	0.0	0.0
	YDR399W	*HPT1*	*BRA6*	6050_at	YDR399W	662.7	-1.1	98.7	662.7	-1.2	132.5	810.6	1.1	120.7	810.6	-1.0	0.0
	YGL234W	*ADE5,7*		5234_at	YGL234W	946.7	-1.2	235.7	946.7	-1.1	141.0	1163.0	1.2	289.6	1163.0	1.0	0.0
	YAR015W	*ADE1*		11330_at	YAR015W	804.0	-1.1	119.7	804.0	-1.2	160.8	952.4	1.1	141.8	952.4	-1.0	0.0
	YGR204W	*ADE3*		4814_at	YGR204W	724.6	-1.2	144.9	724.6	-1.2	180.4	901.8	1.2	180.4	901.8	-1.0	0.0
	YLR058C	*SHM2*	*SHMT2*	10267_at	YLR058C	1548.0	-1.1	230.5	1548.0	-1.1	230.5	1780.5	1.1	265.1	1780.5	-1.0	0.0
	YML035C	*AMD1*	*AMD3*	9674_at	YML035C	0.0	0.0	0.0	0.0	0.0	0.0	5.9	-1.3	1.9	5.9	-2.4	8.3
	YIR032C	*DAL3*		4068_at	Ureidoglyc	27.7	-5.8	132.3	27.7	-7.0	166.2	143.8	5.8	686.9	143.8	-1.2	28.8
	YBR263W	*SHM1*	*SHMT1, TI*	7083_at	YBR263W	338.5	-1.2	84.3	338.5	-1.5	169.2	423.2	1.2	105.4	423.2	-1.2	84.6
	YMR217W	*GUA1*		9399_at	YMR217W	937.7	-1.1	139.6	937.7	-1.2	233.5	1069.8	1.1	159.3	1069.8	-1.1	107.0
	YLR028C	*ADE16*		10324_at	YLR028C	511.1	1.4	229.5	511.1	1.0	24.9	345.5	-1.4	155.2	345.5	-1.4	138.2
	YOR128C	*ADE2*		8420_at	YOR128C	752.3	-1.0	0.0	752.3	-1.2	150.5	778.8	-1.0	0.0	778.8	-1.2	155.8
	YHR216W	*IMD2*	*PUR5*	4323_f_at	YHR216W	765.5	-1.0	37.4	765.5	-1.2	149.9	797.7	1.0	38.9	797.7	-1.2	159.5
	YGR061C	*ADE6*		4942_at	YGR061C	543.9	-1.1	81.0	543.9	-1.5	298.7	604.9	1.1	90.1	604.9	-1.3	181.5
	YML022W	*APT1*		9688_at	YML022W	901.0	-1.0	0.0	901.0	-1.2	224.3	928.0	-1.0	0.0	928.0	-1.2	185.6
	YJR105W	*ADO1*		10898_at	YJR105W	2356.6	-1.1	350.9	2356.6	-1.1	350.9	2638.0	1.1	392.8	2638.0	-1.1	263.8
pyrimidine ribonucleotide metabolism																	
	YBR021W	*FUR4*		7334_at	Uracil perm	208.8	4.1	656.1	208.8	6.4	1124.9	41.4	-4.1	130.1	41.4	1.5	20.7
	YBL042C	*FUI1*		7410_at	High affinity	254.7	7.2	1578.8	254.7	7.4	1629.8	31.8	-7.2	197.2	31.8	1.0	0.0
	YJL130C	*URA2*		11165_at	YJL130C c	1459.3	1.4	655.4	1459.3	1.3	509.4	928.3	-1.4	416.9	928.3	-1.0	0.0
	YPR062W	*FCY1*		7682_at	YPR062W	1068.9	-1.1	106.9	1068.9	-1.2	213.8	0.0	0.0	0.0	0.0	0.0	0.0
	YML106W	*URA5*	*PYR5*	9736_at	YML106W	1266.1	-1.3	375.0	1266.1	-1.3	442.0	1643.2	1.3	486.6	1643.2	-1.0	0.0
	YHR128W	*FUR1*		4422_at	Uracil phos	441.7	48.6	21042.7	441.7	18.9	7891.4	9.1	-48.6	433.6	9.1	-2.8	16.4
	YKL024C	*URA6*		10583_at	YKL024C	314.8	1.1	30.0	314.8	-1.3	93.2	273.6	-1.1	26.1	273.6	-1.4	109.4
	YJR133W	*XPT1*		10881_at	YJR133W	632.7	-1.0	30.9	632.7	-1.3	189.8	671.5	1.0	32.8	671.5	-1.2	134.3
	YEL021W	*URA3*		5724_at	Orotidine-5	1100.9	-1.5	604.6	1100.9	-1.7	767.4	1703.4	1.5	935.5	1703.4	-1.1	170.3
	YBR252W	*DUT1*		7117_at	YBR252W	525.9	-1.1	52.6	525.9	-1.4	236.2	574.6	1.1	57.5	574.6	-1.3	172.4
deoxyribonucleotide metabolism																	
	YDR353W	*TRR1*		6139_at	YDR353W	869.2	-1.2	173.8	869.2	-1.1	129.4	1042.3	1.2	208.5	1042.3	1.1	104.2
	YOR074C	*CDC21*	*CRT9, TMI*	8152_f_at	YOR074C	29.7	1.1	4.0	29.7	2.0	31.0	29.4	-1.1	3.9	29.4	1.8	23.2
	YGR180C	*RNR4*		4835_at	YGR180C	942.0	-1.0	46.0	942.0	-1.1	140.3	0.0	0.0	0.0	0.0	0.0	0.0
	YIL066C	*RNR3*	*DIN1, RIR*	4045_s_at	YIL066C R	7.3	2.2	8.5	7.3	-1.1	1.0	2.2	-2.2	2.6	2.2	-1.2	0.4
	YJL026W	*RNR2*	*CRT6*	11041_at	YJL026W	665.7	-1.1	66.6	665.7	-1.3	232.4	774.7	1.1	77.5	774.7	-1.2	154.9
	YER070W	*RNR1*	*CRT7, RIR*	5643_at	YER070W	457.9	1.0	22.3	457.9	-1.4	183.1	391.7	-1.0	19.1	391.7	-1.5	195.9
regulation of nucleotide metabolism																	
	YER056C	*FCY2*	*BRA7*	5668_at	YER056C	1819.5	-1.1	238.3	1819.5	1.0	19.9	2117.5	1.1	277.4	2117.5	1.1	311.1
	YIR023W	*DAL81*	*UGA35*	4059_at	YIR023W T	21.9	-1.0	0.0	21.9	1.3	6.6	21.4	-1.0	0.0	21.4	1.2	4.3
	YLR014C	*PPR1*		10310_at	YLR014C	34.7	1.1	5.2	34.7	1.2	6.9	30.4	-1.1	4.5	30.4	1.0	0.0
	YNL098C	*RAS2*		8979_at	YNL098C	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3	143.6
polynucleotide degradation																	
	YKL009W	*MRT4*		10598_at	YKL009W	766.7	-1.1	76.7	766.7	-1.2	153.3	0.0	0.0	0.0	0.0	0.0	0.0
	YIL078W	*THS1*		4186_at	YIL078W T	799.3	-1.1	79.9	799.3	-1.1	119.0	0.0	0.0	0.0	0.0	0.0	0.0
	YEL034W	*HYP2*	*TIF51A*	5756_at	YEL034W	2636.4	-1.2	656.5	2636.4	-1.1	392.6	3320.4	1.2	826.8	3320.4	1.1	332.0
nucleotide transport																	

YBR085W	*AAC3*	*ANC3*	7263_at	YBR085W	47.0	-2.0	48.6	47.0	-1.9	43.8	99.8	2.0	103.3	99.8	1.1	10.0
YER056C	*FCY2*	*BRA7*	5668_at	YER056C	1819.5	-1.1	238.3	1819.5	1.0	19.9	2117.5	1.1	277.4	2117.5	1.1	311.1
YOR222W	*ODC2*		8334_at	YOR222W	506.1	-1.3	149.9	506.1	-1.5	274.6	689.7	1.3	204.3	689.7	-1.2	137.9
other nucleotide-metabolism activities																
YKL067W	*YNK1*	*NDK1*	10629_at	YKL067W	846.5	1.1	126.1	846.5	-1.0	0.0	677.9	-1.1	100.9	677.9	-1.1	67.8
YKR091W	*SRL3*		10470_at	YKR091W	39.2	2.4	56.8	39.2	1.9	35.3	15.7	-2.4	22.8	15.7	-1.4	6.3
YDR226W	*ADK1*		6237_at	YDR226W	1116.1	-1.1	166.2	1116.1	-1.1	166.2	1457.8	1.1	217.1	1457.8	1.0	0.0
YDL125C	*HNT1*		6605_at	YDL125C	1355.2	-1.1	135.5	1355.2	-1.1	201.8	0.0	0.0	0.0	0.0	0.0	0.0
YDR454C	*GUK1*	*PUR5*	6013_at	YDR454C	909.7	-1.2	178.1	909.7	-1.5	493.7	1122.7	1.2	219.9	1122.7	-1.3	336.8
phosphate metabolism																
phosphate utilization																
YBR092C	*PHO3*		7273_at	Acid phosp	356.2	1.4	141.2	356.2	4.4	1226.2	257.9	-1.4	102.2	257.9	3.2	567.4
YBR011C	*IPP1*		7370_at	YBR011C	1251.3	-1.2	250.3	1251.3	-1.2	250.3	1505.0	1.2	301.0	1505.0	1.0	0.0
regulation of phosphate utilization																
YFR034C	*PHO4*		5333_at	YFR034C	9.9	2.3	12.4	9.9	1.4	4.0	3.3	-2.3	4.1	3.3	-1.2	0.7
YBR106W	*PHO88*		7242_at	YBR106W	1301.9	-1.2	260.4	1301.9	-1.2	324.2	1545.7	1.2	309.1	1545.7	-1.0	0.0
phosphate transport																
YJL117W	*PHO86*		11132_at	YJL117W	0.0	0.0	0.0	0.0	0.0	0.0	473.2	1.0	23.1	473.2	1.3	142.0
YER053C	*??*		5665_at	YER053C	227.7	1.6	136.6	227.7	1.3	79.5	141.7	-1.6	85.0	141.7	-1.2	28.3
YLR348C	*DIC1*		9972_at	YLR348C	231.9	1.5	115.9	231.9	1.3	69.6	0.0	0.0	0.0	0.0	0.0	0.0
YJR077C	*MIR1*		10961_at	YJR077C	1731.2	1.1	257.8	1731.2	1.0	84.5	1524.8	-1.1	227.1	1524.8	-1.1	152.5
YBR106W	*PHO88*		7242_at	YBR106W	1301.9	-1.2	260.4	1301.9	-1.2	324.2	1545.7	1.2	309.1	1545.7	-1.0	0.0
C-compound and carbohydrate metabolism																
C-compound and carbohydrate utilization																
YDR380W	*ARO10*		6077_at	Similarity t	883.1	2.3	1191.7	883.1	11.5	9314.3	375.6	-2.3	506.9	375.6	5.0	1502.4
YGR192C	*TDH3*	*GLD1, HSI*	4802_i_at	Glyceralde	3727.1	-1.2	898.1	3727.1	-1.0	165.7	4578.3	1.2	1103.2	4578.3	1.2	915.7
YJR009C	*TDH2*	*GLD2*	11031_s_a	YJR009C	3547.9	-1.1	528.3	3547.9	-1.0	0.0	4151.9	1.1	618.3	4151.9	1.2	830.4
YHR174W	*ENO2*		4334_i_at	YHR174W	2988.8	-1.3	1043.3	2988.8	-1.1	445.1	4037.7	1.3	1409.5	4037.7	1.2	807.5
YLR044C	*PDC1*		10296_at	YLR044C	2283.0	-1.2	456.6	2283.0	-1.0	0.0	2763.9	1.2	552.8	2763.9	1.2	552.8
YDL182W	*LYS20*		6683_g_at	YDL182W	2082.9	-1.0	101.7	2082.9	1.1	310.2	2189.0	1.0	106.8	2189.0	1.2	437.8
YBR145W	*ADH5*		7236_at	YBR145W	1213.1	1.0	59.2	1213.1	1.3	423.4	1149.0	-1.0	56.1	1149.0	1.3	344.7
YBR196C	*PGI1*	*CDC30*	7152_at	Glucose-6-	1683.3	-1.2	419.1	1683.3	-1.0	82.2	2100.2	1.2	522.9	2100.2	1.2	420.0
YAL023C	*PMT2*	*FUN25*	11344_at	YAL023C	1047.0	1.1	104.7	1047.0	1.3	365.5	859.8	-1.1	86.0	859.8	1.2	172.0
YCR012W	*PGK1*		6890_at	3-Phospho	3009.0	-1.2	589.2	3009.0	-1.1	287.2	3629.6	1.2	710.8	3629.6	1.1	363.0
YKL152C	*GPM1*		10721_at	Phosphogl	3118.6	-1.1	464.4	3118.6	-1.0	152.2	3538.3	1.1	526.9	3538.3	1.1	353.8
YOL126C	*MDH2*		8663_at	Cytosolic m	126.7	1.9	113.7	126.7	3.4	309.9	66.1	-1.9	59.3	66.1	1.8	52.9
YKL085W	*MDH1*		10654_at	YKL085W	1063.9	1.1	158.4	1063.9	1.2	264.9	866.5	-1.1	129.0	866.5	1.1	86.7
YKL104C	*GFA1*		10680_at	YKL104C	276.7	1.6	179.6	276.7	1.9	262.6	169.2	-1.6	109.9	169.2	1.1	16.9
YLR300W	*EXG1*	*BGL1*	10015_at	YLR300W	1585.6	1.0	77.4	1585.6	1.1	236.1	0.0	0.0	0.0	0.0	0.0	0.0
YDL095W	*PMT1*		6591_at	YDL095W	415.6	1.4	166.2	415.6	1.4	166.2	299.5	-1.4	119.8	299.5	1.0	0.0
YJL174W	*KRE9*		11209_at	YJL174W	643.5	1.2	128.7	643.5	1.2	160.2	581.8	-1.2	116.4	581.8	1.0	0.0
YGR282C	*BGL2*		4712_at	YGR282C	1370.4	-1.1	204.1	1370.4	-1.0	66.9	1589.9	1.1	236.8	1589.9	1.1	159.0
YOR067C	*ALG8*		8495_at	YOR067C	598.7	-1.2	149.1	598.7	-1.0	0.0	730.4	1.2	181.9	730.4	1.2	146.1
YMR083W	*ADH3*		9569_at	YMR083W	1156.0	-1.2	231.2	1156.0	-1.0	56.4	1403.8	1.2	280.8	1403.8	1.1	140.4
YCL040W	*GLK1*	*HOR3*	6937_at	YCL040W	686.5	1.6	411.9	686.5	1.2	137.3	437.3	-1.6	262.4	437.3	-1.3	131.2
YOL059W	*GPD2*	*GPD3*	8592_at	YOL059W	166.1	1.7	115.8	166.1	1.8	132.4	98.2	-1.7	68.5	98.2	1.1	9.8
YGR244C	*LSC2*		4764_at	YGR244C	330.9	1.5	164.3	330.9	1.4	131.2	0.0	0.0	0.0	0.0	0.0	0.0
YPR074C	*TKL1*		7694_at	YPR074C	1097.1	-1.1	163.4	1097.1	-1.0	53.5	1280.0	1.1	190.6	1280.0	1.1	128.0
YAL054C	*ACS1*		11356_at	YAL054C	350.3	1.2	87.2	350.3	1.3	122.3	0.0	0.0	0.0	0.0	0.0	0.0
YGL156W	*AMS1*		5179_at	Alpha man	58.3	4.3	192.3	58.3	2.7	102.0	12.2	-4.3	40.2	12.2	-1.6	7.3
YGR143W	*SKN1*		4843_at	YGR143W	145.7	1.8	123.7	145.7	1.7	102.0	79.0	-1.8	67.1	79.0	-1.1	7.9

YOR377W	ATF1		8176_at	YOR377W	98.6	2.0	103.5	98.6	1.8	83.7	44.1	-2.0	46.3	44.1	-1.1	4.4
YGR043C	??		4969_at	Transaldol:	42.7	2.6	70.2	42.7	2.9	80.7	16.0	-2.6	26.3	16.0	1.1	1.6
YIL099W	SGA1		4212_g_at	Intracellula	40.4	2.4	56.6	40.4	2.3	52.4	25.6	-2.4	35.8	25.6	-1.0	1.1
YKR061W	KTR2		10532_at	YKR061W	52.6	2.3	70.7	52.6	1.9	49.6	22.7	-2.3	30.5	22.7	-1.2	4.5
YPL053C	KTR6	MNN6	7790_at	YPL053C r	0.0	0.0	0.0	0.0	0.0	0.0	406.6	1.2	101.2	406.6	1.1	40.7
YGR256W	GND2		4732_at	YGR256W	12.0	2.4	17.3	12.0	2.5	18.5	0.0	0.0	0.0	0.0	0.0	0.0
YPR001W	CIT3		7753_at	YPR001W	11.9	2.6	18.9	11.9	1.4	5.3	5.5	-2.6	8.8	5.5	-1.8	4.4
MEL1	MEL1		3933_s_at	MEL1 Requ	0.0	0.0	0.0	0.0	0.0	0.0	3.7	2.0	3.7	3.7	2.1	4.1
YFL056C	AAD6		5424_at	YFL056C a	4.7	2.1	5.2	4.7	1.5	2.5	2.3	-2.1	2.6	2.3	-1.5	1.2
YHR104W	GRE3		4442_at	YHR104W	279.7	1.4	111.9	279.7	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
YMR306W	FKS3		9313_at	1,3-Beta-gl	-11.2	5.3	-47.9	-11.2	3.7	-30.7	-19.1	-5.3	-82.1	19.1	-2.7	32.5
YNL241C	ZWF1	MET19, PO	9108_at	Glucose-6-	206.9	1.3	62.1	206.9	-1.0	0.0	157.1	-1.3	47.1	157.1	-1.3	47.1
YML054C	CYB2		9702_at	YML054C (	95.6	2.7	166.8	95.6	1.2	18.7	37.7	-2.7	65.8	37.7	-2.4	52.8
YDR178W	SDH4		6279_at	YDR178W	695.1	1.2	139.0	695.1	1.0	33.9	586.8	-1.2	117.4	586.8	-1.1	58.7
YDR074W	TPS2	HOG2, PFI	6398_at	YDR074W	224.7	1.5	111.6	224.7	-1.0	11.0	140.6	-1.5	69.8	140.6	-1.6	84.4
YMR105C	PGM2		9547_at	YMR105C	0.0	0.0	0.0	0.0	0.0	0.0	157.8	-1.3	55.1	157.8	-1.6	94.7
YDR148C	KGD2		6339_at	YDR148C	0.0	0.0	0.0	0.0	0.0	0.0	206.5	-1.2	41.3	206.5	-1.5	103.3
YDR368W	YPR1		6110_at	YDR368W	0.0	0.0	0.0	0.0	0.0	0.0	260.2	-1.0	12.7	260.2	-1.4	104.1
YOR142W	LSC1		8389_at	YOR142W	701.9	-1.0	34.3	701.9	-1.1	104.5	0.0	0.0	0.0	0.0	0.0	0.0
YJL002C	OST1	NLT1	11019_at	YJL002C 6	1148.9	-1.1	171.1	1148.9	-1.1	114.9	1315.8	1.1	195.9	1315.8	1.0	0.0
YDL078C	MDH3		6565_at	YDL078C r	270.4	-1.0	13.2	270.4	-1.4	121.4	258.3	1.0	12.6	258.3	-1.4	103.3
YAL060W	BDH1		11388_at	YAL060W	844.2	-1.1	84.4	844.2	-1.1	125.7	0.0	0.0	0.0	0.0	0.0	0.0
YDL022W	GPD1	DAR1, HO	6485_at	YDL022W	763.2	1.1	113.7	763.2	-1.0	0.0	647.3	-1.1	96.4	647.3	-1.2	129.5
YJL052W	TDH1	GLD3	11061_g_a	YJL052W (	1811.4	-1.0	77.3	1811.4	-1.1	134.2	2067.3	-1.0	88.2	2067.3	1.0	0.0
YJL121C	RPE1	EPI1, POS	11128_at	YJL121C D	688.8	-1.3	240.4	688.8	-1.2	137.8	905.1	1.3	315.9	905.1	1.1	90.5
YLR354C	TAL1		9978_at	YLR354C T	946.7	-1.0	46.2	946.7	-1.1	141.0	0.0	0.0	0.0	0.0	0.0	0.0
YEL002C	WBP1		5744_at	YEL002C c	953.0	-1.0	46.5	953.0	-1.1	141.9	0.0	0.0	0.0	0.0	0.0	0.0
YCR034W	FEN1	ELO2, GN	6869_at	YCR034W	728.3	-1.1	72.8	728.3	-1.2	145.7	0.0	0.0	0.0	0.0	0.0	0.0
YGL253W	HXK2	HEX1, HKI	5260_at	YGL253W	751.4	-1.3	262.3	751.4	-1.2	150.3	981.0	1.3	342.4	981.0	1.1	98.1
YMR149W	SWP1		9504_at	YMR149W	783.3	-1.1	116.6	783.3	-1.2	153.4	883.5	1.1	131.6	883.5	-1.1	88.4
YJR143C	PMT4		10891_at	YJR143C c	771.0	-1.1	77.1	771.0	-1.2	154.2	0.0	0.0	0.0	0.0	0.0	0.0
YFR053C	HXK1		5307_at	YFR053C I	271.0	1.0	13.2	271.0	-1.5	135.5	259.3	-1.0	12.7	259.3	-1.6	155.6
YER003C	PMI40	PMI	5702_at	YER003C r	630.6	-1.1	63.1	630.6	-1.2	157.0	694.4	1.1	69.4	694.4	-1.2	138.9
YGL062W	PYC1		5090_at	YGL062W	142.4	-1.7	106.7	142.4	-2.3	185.1	291.0	1.7	218.0	291.0	-1.3	87.3
YFL045C	SEC53	ALG4	5435_at	YFL045C p	1356.7	-1.1	202.0	1356.7	-1.1	202.0	1549.8	1.1	230.8	1549.8	-1.0	0.0
YHR183W	GND1		4343_at	YHR183W	1485.6	-1.1	221.2	1485.6	-1.1	221.2	1682.4	1.1	250.5	1682.4	1.0	0.0
YLR342W	FKS1		9966_at	YLR342W	726.7	-1.1	69.4	726.7	-1.3	253.7	861.6	1.1	82.2	861.6	-1.2	172.3
YGR240C	PFK1		4760_at	Phosphofru	1005.6	-1.2	250.4	1005.6	-1.3	301.7	1258.0	1.2	313.2	1258.0	-1.1	125.8
YLR286C	CTS1		10045_at	YLR286C E	2323.9	-1.1	346.1	2323.9	-1.1	346.1	2400.2	1.1	357.4	2400.2	-1.1	240.0
YDR050C	TPI1		6419_at	Triosephos	2559.9	-1.2	637.4	2559.9	-1.1	381.2	3338.3	1.2	831.2	3338.3	1.1	333.8
YFL014W	HSP12		5372_at	YFL014W	625.4	1.8	493.3	625.4	-1.2	122.5	350.2	-1.8	276.3	350.2	-2.1	385.2
YKL060C	FBA1		10636_at	Aldolase	3014.1	-1.2	750.5	3014.1	-1.1	448.8	3801.9	1.2	946.7	3801.9	1.1	380.2
YCR005C	CIT2		6883_at	YCR005C	1341.0	-1.1	199.7	1341.0	-1.3	468.1	1588.0	1.1	236.5	1588.0	-1.1	158.8
YDL055C	PSA1	MPG1, VIG	6543_at	YDL055C r	1894.9	-1.2	471.8	1894.9	-1.2	471.8	2378.1	1.2	592.1	2378.1	1.0	0.0
YGR254W	ENO1	HSP48	4730_s_at	Enolase I	2833.6	-1.2	493.5	2833.6	-1.2	493.5	3244.5	1.2	565.1	3244.5	1.0	158.4
YMR205C	PFK2		9432_at	YMR205C	1287.5	-1.2	252.1	1287.5	-1.4	510.4	1558.7	1.2	305.2	1558.7	-1.2	311.7
YOL086C	ADH1	ADC1	8657_at	YOL086C /	3553.5	-1.2	695.9	3553.5	-1.1	529.2	4293.4	1.2	840.8	4293.4	1.1	429.3
YAL038W	CDC19	PYK1	11371_at	Pyruvate ki	3016.0	-1.2	751.0	3016.0	-1.2	590.6	3721.2	1.2	926.6	3721.2	1.0	0.0
YLR304C	ACO1	GLU1	10019_at	YLR304C /	1066.5	-1.4	479.0	1066.5	-1.6	636.6	1602.0	1.4	719.5	1602.0	-1.1	160.2

regulation of C-compound and carbohydrate utilization

	YLR094C	*GIS3*		10258_at	Cyclin C ar	40.0	3.8	111.6	40.0	6.0	199.5	9.8	-3.8	27.4	9.8	1.6	5.9
	YOR344C	*TYE7*	*SGC1*	8188_at	33 kDa ser	66.1	2.8	121.6	66.1	3.6	174.1	23.0	-2.8	42.3	23.0	1.3	6.9
	YGR288W	*MAL13*		4718_at	YGR288W	60.3	1.7	45.1	60.3	2.7	105.4	30.5	-1.7	22.9	30.5	1.6	18.3
	YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2	10.9
	YGL066W	*SGF73*		5086_at	YGL066W	60.2	1.4	23.8	60.2	2.4	86.4	0.0	0.0	0.0	0.0	0.0	0.0
	YER133W	*GLC7*	*CID1, DIS2*	5573_at	protein phc	242.7	1.2	40.7	242.7	1.3	84.2	217.2	-1.2	36.4	217.2	1.1	30.4
	YGL209W	*MIG2*	*MLZ1*	5216_at	YGL209W	54.7	2.0	54.5	54.7	2.3	73.5	0.0	0.0	0.0	0.0	0.0	0.0
	YML048W	*GSF2*	*ECM6*	9709_at	YML048W	0.0	0.0	0.0	0.0	0.0	0.0	33.1	1.3	9.2	33.1	2.2	39.7
	YDR216W	*ADR1*		6273_at	YDR216W	13.6	1.5	7.4	13.6	2.7	23.0	8.6	-1.5	4.7	8.6	1.8	6.9
	YOL067C	*RTG1*		8630_at	YOL067C	11.2	1.1	1.7	11.2	2.7	19.5	9.6	-1.1	1.4	9.6	2.4	13.4
	YCL010C	*SGF29*		6870_at	YCL010C	11.0	2.0	11.1	11.0	1.5	5.7	5.6	-2.0	5.7	5.6	-1.3	1.7
	YOR178C	*GAC1*		8380_at	YOR178C	5.2	1.7	3.5	5.2	-1.3	1.8	3.1	-1.7	2.1	3.1	-2.1	3.4
	YNL098C	*RAS2*		8979_at	YNL098C I	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3	143.6
	YCR028C	*FEN2*		6863_at	YCR028C	375.2	-1.2	93.4	375.2	-1.4	148.7	465.8	1.2	116.0	465.8	-1.1	46.6
	YGL253W	*HXK2*	*HEX1, HKI*	5260_at	YGL253W	751.4	-1.3	262.3	751.4	-1.2	150.3	981.0	1.3	342.4	981.0	1.1	98.1
	YDR328C	*SKP1*	*MGO1*	6160_at	YDR328C	487.2	1.2	121.3	487.2	-1.1	48.7	386.5	-1.2	96.2	386.5	-1.4	154.6
	YPR165W	*RHO1*		7563_at	Ras homol	819.3	-1.0	0.0	819.3	-1.2	163.9	828.3	-1.0	0.0	828.3	-1.2	165.7
	YHR193C	*EGD2*		4353_at	YHR193C	1137.1	-1.1	169.3	1137.1	-1.2	283.1	1294.9	1.1	192.8	1294.9	-1.1	129.5
	YGR240C	*PFK1*		4760_at	Phosphofru	1005.6	-1.2	250.4	1005.6	-1.3	301.7	1258.0	1.2	313.2	1258.0	-1.1	125.8
	YMR205C	*PFK2*		9432_at	YMR205C	1287.5	-1.2	252.1	1287.5	-1.4	510.4	1558.7	1.2	305.2	1558.7	-1.2	311.7
	YPL037C	*EGD1*		7806_at	YPL037C (	1962.5	-1.2	392.5	1962.5	-1.3	588.8	2272.2	1.2	454.4	2272.2	-1.1	227.2
C-compound, carbohydrate transport																	
	YDR497C	*ITR1*		5966_at	Myo-inosito	1187.5	-1.2	237.5	1187.5	-1.0	0.0	1358.0	1.2	271.6	1358.0	1.2	271.6
	YMR011W	*HXT2*		9633_at	YMR011W	809.5	-1.3	242.8	809.5	-1.0	0.0	1029.2	1.3	308.8	1029.2	1.2	205.8
	YHR094C	*HXT1*	*HOR4*	4430_at	YHR094C	540.4	-1.0	0.0	540.4	1.3	162.1	525.9	-1.0	0.0	525.9	1.3	157.8
	YDR345C	*HXT3*		6131_at	YDR345C	1351.7	-1.2	264.7	1351.7	-1.1	129.0	1606.0	1.2	314.5	1606.0	1.1	160.6
	YCL040W	*GLK1*	*HOR3*	6937_at	YCL040W	686.5	1.6	411.9	686.5	1.2	137.3	437.3	-1.6	262.4	437.3	-1.3	131.2
	YLR348C	*DIC1*		9972_at	YLR348C r	231.9	1.5	115.9	231.9	1.3	69.6	0.0	0.0	0.0	0.0	0.0	0.0
	YFL011W	*HXT10*		5377_at	YFL011W I	4.7	-1.9	4.1	4.7	1.1	0.6	10.8	1.9	9.4	10.8	2.0	10.8
	YDL247W	*??*		6709_i_at	YDL247W	2.0	2.3	2.6	2.0	-1.0	0.1	-2.2	-2.3	2.9	-2.2	-2.1	2.4
	YDR342C	*HXT7*		6128_f_at	YDR342C	1141.2	-1.1	114.1	1141.2	-1.2	228.2	0.0	0.0	0.0	0.0	0.0	0.0
	YDR343C	*HXT6*		6129_f_at	YDR343C	1105.6	-1.1	110.6	1105.6	-1.4	442.2	1197.1	1.1	119.7	1197.1	-1.3	359.1
lipid, fatty-acid and isoprenoid metabolism																	
lipid, fatty-acid and isoprenoid biosynthesis																	
	YLR372W	*SUR4*	*ELO3, SRE*	9953_at	YLR372W	1590.9	-1.2	318.2	1590.9	-1.0	0.0	1910.0	1.2	382.0	1910.0	1.2	382.0
	YMR272C	*SCS7*	*FAH1*	9366_at	YMR272C	774.6	-1.0	37.8	774.6	1.2	151.7	845.3	1.0	41.3	845.3	1.3	253.6
	YHR007C	*ERG11*		4525_at	YHR007C	0.0	0.0	0.0	0.0	0.0	0.0	1087.9	1.0	53.1	1087.9	1.2	217.6
	YNL280C	*ERG24*		9159_at	YNL280C s	0.0	0.0	0.0	0.0	0.0	0.0	400.7	1.4	180.0	400.7	1.4	160.3
	YOR221C	*MCT1*		8332_at	YOR221C	88.7	2.0	88.7	88.7	2.8	159.7	55.8	-2.0	55.8	55.8	1.5	27.9
	YKL004W	*AUR1*		10560_at	YKL004W	618.5	-1.2	123.7	618.5	-1.0	0.0	749.2	1.2	149.8	749.2	1.2	149.8
	YDR062W	*LCB2*	*SCS1, TSC*	6431_at	YDR062W	668.6	-1.1	99.6	668.6	1.0	32.6	730.5	1.1	108.8	730.5	1.2	146.1
	YGR187C	*HGH1*		4797_at	YGR187C	238.6	1.1	35.5	238.6	1.5	119.3	0.0	0.0	0.0	0.0	0.0	0.0
	YMR246W	*FAA4*		9386_at	YMR246W	383.4	1.3	115.0	383.4	1.3	115.0	0.0	0.0	0.0	0.0	0.0	0.0
	YLR100W	*ERG27*		10219_at	YLR100W	555.6	1.3	166.7	555.6	1.2	111.1	433.9	-1.3	130.2	433.9	-1.1	43.4
	YCR048W	*ARE1*	*SAT2*	6837_at	YCR048W	178.6	1.3	62.3	178.6	1.6	107.2	0.0	0.0	0.0	0.0	0.0	0.0
	YMR296C	*LCB1*	*END8, TSC*	9347_at	YMR296C	0.0	0.0	0.0	0.0	0.0	0.0	262.5	1.0	12.8	262.5	1.4	105.0
	YHR190W	*ERG9*		4350_at	YHR190W	534.8	1.1	79.6	534.8	1.2	104.7	390.9	-1.1	58.2	390.9	1.0	0.0
	YPL145C	*KES1*	*LPI3, OSH*	7880_at	YPL145C I	405.0	1.2	100.8	405.0	-1.0	19.8	0.0	0.0	0.0	0.0	0.0	0.0
	YGR175C	*ERG1*		4830_at	YGR175C	828.8	-1.1	123.4	828.8	-1.0	40.5	926.4	1.1	138.0	926.4	1.1	92.6
	YCL004W	*PGS1*	*PEL1*	6875_at	17-kDa Ph	6.6	3.2	14.7	6.6	1.4	2.5	0.0	-3.2	0.0	0.0	-2.4	0.0
	YNR019W	*ARE2*	*SAT1*	8867_at	YNR019W	2.1	-2.1	2.4	2.1	-1.7	1.6	8.4	2.1	9.6	8.4	1.2	1.7

YDL015C	*TSC13*		6492_at	YDL015C	641.7	-1.2	128.3	641.7	-1.1	95.5	767.0	1.2	153.4	767.0	1.1	76.7
YGR157W	*CHO2*	*PEM1*	4858_at	Phosphatic	579.5	-1.1	86.3	579.5	-1.1	57.9	665.4	1.1	99.1	665.4	1.0	0.0
YGL001C	*ERG26*		5016_at	YGL001C	333.4	-1.0	16.3	333.4	-1.3	100.0	0.0	0.0	0.0	0.0	0.0	0.0
YBR265W	*TSC10*		7085_at	YBR265W	220.6	-1.0	10.8	220.6	-1.5	109.6	0.0	0.0	0.0	0.0	0.0	0.0
YBR177C	*EHT1*		7179_at	YBR177C	369.1	-1.0	0.0	369.1	-1.3	110.7	335.1	-1.0	0.0	335.1	-1.3	100.5
YML075C	*HMG1*		9724_at	YML075C	412.1	-1.1	39.3	412.1	-1.3	122.0	0.0	0.0	0.0	0.0	0.0	0.0
YPR113W	*PIS1*		7645_at	YPR113W	962.8	-1.1	143.4	962.8	-1.1	143.4	1132.6	1.1	168.7	1132.6	1.0	0.0
YCR034W	*FEN1*	*ELO2, GN*	6869_at	YCR034W	728.3	-1.1	72.8	728.3	-1.2	145.7	0.0	0.0	0.0	0.0	0.0	0.0
YBR263W	*SHM1*	*SHMT1, TI*	7083_at	YBR263W	338.5	-1.2	84.3	338.5	-1.5	169.2	423.2	1.2	105.4	423.2	-1.2	84.6
YGR037C	*ACB1*		4963_at	YGR037C	0.0	0.0	0.0	0.0	0.0	0.0	923.3	-1.0	45.1	923.3	-1.2	184.7
YPL028W	*ERG10*	*LPB3, TSM*	7770_at	YPL028W	1419.8	-1.1	211.4	1419.8	-1.1	211.4	1632.9	1.1	243.2	1632.9	-1.0	0.0
YNL130C	*CPT1*		8992_at	YNL130C	553.9	-1.4	221.6	553.9	-1.4	221.6	791.4	1.4	316.6	791.4	1.0	0.0
YLR058C	*SHM2*	*SHMT2*	10267_at	YLR058C	1548.0	-1.1	230.5	1548.0	-1.1	230.5	1780.5	1.1	265.1	1780.5	-1.0	0.0
YKL182W	*FAS1*		10781_at	YKL182W	802.1	-1.0	39.1	802.1	-1.3	237.5	931.3	1.0	45.5	931.3	-1.2	186.3
YGL012W	*ERG4*		5005_at	YGL012W	985.3	-1.1	98.5	985.3	-1.2	245.3	1049.9	1.1	105.0	1049.9	-1.2	210.0
YJL196C	*ELO1*		11230_at	YJL196C E	1330.9	-1.2	331.4	1330.9	-1.2	266.2	1651.1	1.2	411.1	1651.1	1.0	0.0
YPL231W	*FAS2*		7973_at	YPL231W	396.9	1.0	19.4	396.9	-1.7	297.4	377.5	-1.0	18.4	377.5	-1.8	302.0
YMR202W	*ERG2*		9429_at	YMR202W	1288.1	-1.1	191.8	1288.1	-1.3	449.6	1456.0	1.1	216.8	1456.0	-1.2	291.2
YNR016C	*ACC1*		8864_at	YNR016C	893.1	-1.2	178.6	893.1	-1.5	490.5	1077.2	1.2	215.4	1077.2	-1.3	323.2
YJR073C	*OPI3*	*PEM2*	10957_at	Methylene-	1629.4	-1.3	568.8	1629.4	-1.4	651.8	2223.8	1.3	776.3	2223.8	-1.0	0.0
breakdown of lipids, fatty acids and isoprenoids				4												
YMR008C	*PLB1*		9630_at	YMR008C	605.9	1.4	272.1	605.9	1.2	150.9	379.7	-1.4	170.5	379.7	-1.1	38.0
YNL012W	*SPO1*		8883_at	YNL012W	10.8	2.2	13.1	10.8	1.3	3.2	4.3	-2.2	5.3	4.3	-1.8	3.4
YDR281C	*PHM6*		6201_at	Phosphate	6.4	-2.4	9.0	6.4	-3.3	14.7	16.9	2.4	23.7	16.9	-1.2	3.4
YER037W	*PHM8*		5692_at	YER037W	31.7	-1.3	11.1	31.7	-2.3	41.1	0.0	0.0	0.0	0.0	0.0	0.0
lipid, fatty-acid and isoprenoid utilization																
YMR246W	*FAA4*		9386_at	YMR246W	383.4	1.3	115.0	383.4	1.3	115.0	0.0	0.0	0.0	0.0	0.0	0.0
YDL078C	*MDH3*		6565_at	YDL078C	270.4	-1.0	13.2	270.4	-1.4	121.4	258.3	1.0	12.6	258.3	-1.4	103.3
YDR410C	*STE14*		6061_at	YDR410C	325.8	-1.4	130.3	325.8	-1.2	81.1	459.0	1.4	183.6	459.0	1.1	45.9
YFL014W	*HSP12*		5372_at	YFL014W	625.4	1.8	493.3	625.4	-1.2	122.5	350.2	-1.8	276.3	350.2	-2.1	385.2
regulation of lipid, fatty-acid and isoprenoid metabolism																
YNL038W	*GPI15*		8903_at	Glysosyl PI	112.7	1.1	16.8	112.7	6.8	658.5	83.4	-1.1	12.4	83.4	5.9	408.7
YFL031W	*HAC1*	*ERN4, IRE*	5318_s_at	bZIP Trans	398.7	1.9	355.4	398.7	2.2	484.5	284.7	-1.9	253.8	284.7	1.2	53.7
YNL231C	*PDR16*		9117_at	YNL231C i	264.8	1.4	105.9	264.8	1.6	158.9	0.0	0.0	0.0	0.0	0.0	0.0
YDR123C	*INO2*	*DIE1, SCS*	6358_at	Transcripti	18.7	2.4	26.3	18.7	8.1	132.6	9.1	-2.4	12.8	9.1	4.9	35.5
YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2	10.9
YHR079C	*IRE1*	*ERN1*	4461_r_at	Ire1p, is im	6.0	1.3	1.8	6.0	1.3	1.5	3.9	-1.3	1.2	3.9	-1.0	0.1
YCR028C	*FEN2*		6863_at	YCR028C	375.2	-1.2	93.4	375.2	-1.4	148.7	465.8	1.2	116.0	465.8	-1.1	46.6
YDR284C	*DPP1*		6204_at	YDR284C	276.7	-1.2	55.3	276.7	-1.5	151.9	0.0	0.0	0.0	0.0	0.0	0.0
lipid and fatty-acid transport																
YDR497C	*ITR1*		5966_at	Myo-inosit	1187.5	-1.2	237.5	1187.5	-1.0	0.0	1358.0	1.2	271.6	1358.0	1.2	271.6
YNL231C	*PDR16*		9117_at	YNL231C i	264.8	1.4	105.9	264.8	1.6	158.9	0.0	0.0	0.0	0.0	0.0	0.0
YMR246W	*FAA4*		9386_at	YMR246W	383.4	1.3	115.0	383.4	1.3	115.0	0.0	0.0	0.0	0.0	0.0	0.0
YGR037C	*ACB1*		4963_at	YGR037C	0.0	0.0	0.0	0.0	0.0	0.0	923.3	-1.0	45.1	923.3	-1.2	184.7
YOR153W	*PDR5*	*LEM1, YDI*	8400_at	Multidrug r	707.7	-1.3	209.6	707.7	-1.3	209.6	916.0	1.3	271.3	916.0	1.0	0.0
other lipid, fatty-acid and isoprenoid metabolism activities																
YKL055C	*OAR1*		10641_at	YKL055C r	27.3	2.6	43.5	27.3	1.8	21.8	7.8	-2.6	12.5	7.8	-1.4	3.1
YNL106C	*INP52*	*SJL2*	8971_at	Inositol pol	15.3	1.3	4.6	15.3	3.1	32.9	11.8	-1.3	3.5	11.8	2.4	16.5
YDR213W	*UPC2*	*MOX4*	6270_at	YDR213W	4.0	-1.1	0.4	4.0	-2.5	6.0	4.5	1.1	0.4	4.5	-2.2	5.4
YBR067C	*TIP1*		7290_at	YBR067C	1652.2	-1.1	246.0	1652.2	-1.2	411.4	1934.7	1.1	288.1	1934.7	-1.1	193.5
YMR307W	*GAS1*	*CWH52, G*	9315_at	YMR307W	2126.2	-1.2	425.2	2126.2	-1.2	425.2	2549.2	1.2	509.8	2549.2	-1.0	0.0

metabolism of vitamins, cofactors, and prosthetic groups																
biosynthesis of vitamins, cofactors, and prosthetic groups																
YJR025C	*BNA1*	*HAD1*	11003_at	YJR025C 3	1095.0	1.1	163.1	1095.0	1.6	710.9	940.5	-1.1	140.1	940.5	1.4	376.2
YOL143C	*RIB4*		8691_at	YOL143C (	566.7	-1.0	0.0	566.7	1.4	226.7	547.7	-1.0	0.0	547.7	1.3	164.3
YGL125W	*MET13*	*MET11*	5117_at	YGL125W	579.3	1.1	86.3	579.3	1.3	173.8	0.0	0.0	0.0	0.0	0.0	0.0
YDR487C	*RIB3*		6001_at	YDR487C :	662.0	1.1	66.2	662.0	1.2	164.8	537.7	-1.1	53.8	537.7	1.2	107.5
YKR069W	*MET1*	*MET20*	10494_at	YKR069W	506.9	1.3	152.1	506.9	1.3	152.1	382.0	-1.3	114.6	382.0	-1.0	0.0
YNL333W	*SNZ2*		9198_s_at	YNL333W	159.9	1.9	143.4	159.9	1.8	134.9	86.0	-1.9	77.2	86.0	-1.1	8.6
YBR256C	*RIB5*		7121_at	YBR256C I	221.1	1.4	88.4	221.1	1.5	121.4	0.0	0.0	0.0	0.0	0.0	0.0
YBR153W	*RIB7*		7199_at	YBR153W	219.2	1.5	120.4	219.2	1.3	65.8	125.2	-1.5	68.8	125.2	-1.1	12.5
YBL033C	*RIB1*		7419_at	YBL033C (	196.2	1.6	117.7	196.2	1.4	78.5	122.3	-1.6	73.4	122.3	-1.1	12.2
YGR010W	*NMA2*		4982_at	YGR010W	34.6	2.1	39.7	34.6	1.3	12.1	14.4	-2.1	16.6	14.4	-1.6	8.6
YDR044W	*HEM13*		6413_at	YDR044W	202.2	-1.2	50.3	202.2	-1.5	101.1	0.0	0.0	0.0	0.0	0.0	0.0
YML110C	*COQ5*	*DBI56*	9777_at	YML110C I	0.0	0.0	0.0	0.0	0.0	0.0	729.9	1.1	108.7	729.9	1.1	73.0
YGR286C	*BIO2*		4716_at	YGR286C	182.3	-1.3	63.6	182.3	-1.7	127.1	0.0	0.0	0.0	0.0	0.0	0.0
YGR204W	*ADE3*		4814_at	YGR204W	724.6	-1.2	144.9	724.6	-1.2	180.4	901.8	1.2	180.4	901.8	-1.0	0.0
YGL037C	*PNC1*		5025_at	YGL037C		732.7	1.3	255.7	732.7	-1.2	182.4	0.0	0.0	0.0	0.0	0.0	0.0
YER043C	*SAH1*		5698_at	YER043C		774.2	-1.1	115.3	774.2	-1.2	192.8	893.0	1.1	133.0	893.0	-1.1	89.3
utilization of vitamins, cofactors, and prosthetic groups																
YBR092C	*PHO3*		7273_at	Acid phosp	356.2	1.4	141.2	356.2	4.4	1226.2	257.9	-1.4	102.2	257.9	3.2	567.4
secondary metabolism																
biosynthesis of secondary products derived from primary amino acids																
biosynthesis of amines																
YKL184W	*SPE1*	*ORD1, SP*	10779_at	YKL184W	159.9	1.8	127.9	159.9	2.4	231.8	89.2	-1.8	71.4	89.2	1.4	
YPR069C	*SPE3*		7689_at	YPR069C		167.8	-1.4	75.4	167.8	-1.6	100.2	242.2	1.4	108.8	242.2	-1.1	
ENERGY CONSERVATION																
glycolysis and gluconeogenesis																
YGR192C	*TDH3*	*GLD1, HSI*	4802_i_at	Glyceralde	3727.1	-1.2	898.1	3727.1	-1.0	165.7	4578.3	1.2	1103.2	4578.3	1.2	915.7
YJR009C	*TDH2*	*GLD2*	11031_s_a	YJR009C ç	3547.9	-1.1	528.3	3547.9	-1.0	0.0	4151.9	1.1	618.3	4151.9	1.2	830.4
YHR174W	*ENO2*		4334_i_at	YHR174W	2988.8	-1.3	1043.3	2988.8	-1.1	445.1	4037.7	1.3	1409.5	4037.7	1.2	807.5
YBR196C	*PGI1*	*CDC30*	7152_at	Glucose-6-	1683.3	-1.2	419.1	1683.3	-1.0	82.2	2100.2	1.2	522.9	2100.2	1.2	420.0
YKL060C	*FBA1*		10636_at	Aldolase	3014.1	-1.2	750.5	3014.1	-1.1	448.8	3801.9	1.2	946.7	3801.9	1.1	380.2
YCR012W	*PGK1*		6890_at	3-Phospho	3009.0	-1.2	589.2	3009.0	-1.1	287.2	3629.6	1.2	710.8	3629.6	1.1	363.0
YKL152C	*GPM1*		10721_at	Phosphogl	3118.6	-1.1	464.4	3118.6	-1.0	152.2	3538.3	1.1	526.9	3538.3	1.1	353.8
YDR050C	*TPI1*		6419_at	Triosephos	2559.9	-1.2	637.4	2559.9	-1.1	381.2	3338.3	1.2	831.2	3338.3	1.1	333.8
YGR254W	*ENO1*	*HSP48*	4730_s_at	Enolase I	2833.6	-1.2	493.5	2833.6	-1.2	493.5	3244.5	1.2	565.1	3244.5	1.0	158.4
YGL253W	*HXK2*	*HEX1, HKI*	5260_at	YGL253W	751.4	-1.3	262.3	751.4	-1.2	150.3	981.0	1.3	342.4	981.0	1.1	98.1
YJL052W	*TDH1*	*GLD3*	11061_g_a	YJL052W (	1811.4	-1.0	77.3	1811.4	-1.1	134.2	2067.3	-1.0	88.2	2067.3	1.0	0.0
YAL038W	*CDC19*	*PYK1*	11371_at	Pyruvate ki	3016.0	-1.2	751.0	3016.0	-1.2	590.6	3721.2	1.2	926.6	3721.2	1.0	0.0
YGL062W	*PYC1*		5090_at	YGL062W	142.4	-1.7	106.7	142.4	-2.3	185.1	291.0	1.7	218.0	291.0	-1.3	87.3
YGR240C	*PFK1*		4760_at	Phosphofru	1005.6	-1.2	250.4	1005.6	-1.3	301.7	1258.0	1.2	313.2	1258.0	-1.1	125.8
YCL040W	*GLK1*	*HOR3*	6937_at	YCL040W	686.5	1.6	411.9	686.5	1.2	137.3	437.3	-1.6	262.4	437.3	-1.3	131.2
YFR053C	*HXK1*		5307_at	YFR053C I	271.0	1.0	13.2	271.0	-1.5	135.5	259.3	-1.0	12.7	259.3	-1.6	155.6
YMR205C	*PFK2*		9432_at	YMR205C	1287.5	-1.2	252.1	1287.5	-1.4	510.4	1558.7	1.2	305.2	1558.7	-1.2	311.7
pentose-phosphate pathway																
YPR074C	*TKL1*		7694_at	YPR074C '	1097.1	-1.1	163.4	1097.1	-1.0	53.5	1280.0	1.1	190.6	1280.0	1.1	128.0
YJL121C	*RPE1*	*EPI1, POS*	11128_at	YJL121C D	688.8	-1.3	240.4	688.8	-1.2	137.8	905.1	1.3	315.9	905.1	1.1	90.5
YGR043C	*??*		4969_at	Transaldol	42.7	2.6	70.2	42.7	2.9	80.7	16.0	-2.6	26.3	16.0	1.1	1.6
YGR256W	*GND2*		4732_at	YGR256W	12.0	2.4	17.3	12.0	2.5	18.5	0.0	0.0	0.0	0.0	0.0	0.0

YLR354C	*TAL1*		9978_at	YLR354C T	946.7	-1.0	46.2	946.7	-1.1	141.0	0.0	0.0	0.0	0.0	0.0	0.0
YHR183W	*GND1*		4343_at	YHR183W	1485.6	-1.1	221.2	1485.6	-1.1	221.2	1682.4	1.1	250.5	1682.4	1.0	0.0
YNL241C	*ZWF1*	*MET19, P(*	9108_at	Glucose-6-	206.9	1.3	62.1	206.9	-1.0	0.0	157.1	-1.3	47.1	157.1	-1.3	47.1
tricarboxylic-acid pathway (citrate cycle, Krebs cycle, TCA cycle)																
YKL085W	*MDH1*		10654_at	YKL085W	1063.9	1.1	158.4	1063.9	1.2	264.9	866.5	-1.1	129.0	866.5	1.1	86.7
YGR244C	*LSC2*		4764_at	YGR244C	330.9	1.5	164.3	330.9	1.4	131.2	0.0	0.0	0.0	0.0	0.0	0.0
YOR142W	*LSC1*		8389_at	YOR142W	701.9	-1.0	34.3	701.9	-1.1	104.5	0.0	0.0	0.0	0.0	0.0	0.0
YPR001W	*CIT3*		7753_at	YPR001W	11.9	2.6	18.9	11.9	1.4	5.3	5.5	-2.6	8.8	5.5	-1.8	4.4
YDR178W	*SDH4*		6279_at	YDR178W	695.1	1.2	139.0	695.1	1.0	33.9	586.8	-1.2	117.4	586.8	-1.1	58.7
YPL262W	*FUM1*		8033_at	YPL262W	770.2	1.2	150.8	770.2	1.1	73.5	627.8	-1.2	122.9	627.8	-1.1	62.8
YDR148C	*KGD2*		6339_at	YDR148C (	0.0	0.0	0.0	0.0	0.0	0.0	206.5	-1.2	41.3	206.5	-1.5	103.3
YFL018C	*LPD1*	*HPD1*	5367_at	YFL018C c	871.1	1.2	216.9	871.1	-1.0	0.0	700.8	-1.2	174.5	700.8	-1.2	140.2
YLR304C	*ACO1*	*GLU1*	10019_at	YLR304C A	1066.5	-1.4	479.0	1066.5	-1.6	636.6	1602.0	1.4	719.5	1602.0	-1.1	160.2
YLL041C	*SDH2*		10390_at	YLL041C S	1047.5	1.6	628.5	1047.5	1.2	209.5	654.7	-1.6	392.8	654.7	-1.3	196.4
respiration																
YLR044C	*PDC1*		10296_at	YLR044C p	2283.0	-1.2	456.6	2283.0	-1.0	0.0	2763.9	1.2	552.8	2763.9	1.2	552.8
YDL130W	*STF1*		6600_at	YDL130W	1812.2	-1.0	89.9	1812.2	-1.0	11.0	2258.3	1.3	635.7	2258.3	1.1	148.1
YBL099W	*ATP1*		7487_at	YBL099W	1009.5	-1.1	150.3	1009.5	-1.0	0.0	1258.4	1.1	187.4	1258.4	1.1	125.8
YKL016C	*ATP7*		10591_at	YKL016C A	689.5	1.2	171.7	689.5	1.3	240.7	546.0	-1.2	136.0	546.0	1.1	54.6
YOR221C	*MCT1*		8332_at	YOR221C	88.7	2.0	88.7	88.7	2.8	159.7	55.8	-2.0	55.8	55.8	1.5	27.9
YNL237W	*YTP1*		9111_at	Yeast puta	28.1	2.5	41.9	28.1	8.2	201.5	11.1	-2.5	16.6	11.1	3.3	25.5
YBR046C	*ZTA1*		7314_at	Homolog to	129.6	2.6	207.0	129.6	3.3	297.8	49.3	-2.6	78.8	49.3	1.3	14.8
YMR145C	*NDE1*	*NDH1*	9500_at	YMR145C	1003.9	1.5	502.0	1003.9	1.5	551.3	664.3	-1.5	332.2	664.3	1.0	0.0
YMR256C	*COX7*		9350_at	YMR256C	1167.2	1.1	116.7	1167.2	1.2	233.4	0.0	0.0	0.0	0.0	0.0	0.0
YLR038C	*COX12*		10290_at	YLR038C s	1869.2	1.1	278.3	1869.2	1.1	186.9	1626.8	-1.1	242.3	1626.8	-1.0	0.0
YOR065W	*CYT1*	*CTC1*	8493_at	YOR065W	269.6	1.5	133.9	269.6	1.5	148.0	0.0	0.0	0.0	0.0	0.0	0.0
YLR395C	*COX8*		9930_at	YLR395C C	1205.1	1.1	179.5	1205.1	1.1	115.0	1054.3	-1.1	157.0	1054.3	-1.0	0.0
YLL009C	*COX17*		10334_at	YLL009C in	551.2	1.2	110.2	551.2	1.1	82.1	0.0	0.0	0.0	0.0	0.0	0.0
YLR205C	*HMX1*		10143_at	YLR205C H	53.9	1.7	40.3	53.9	2.1	61.9	0.0	0.0	0.0	0.0	0.0	0.0
YFR033C	*QCR6*	*COR3, UC*	5332_at	YFR033C u	563.1	1.2	112.6	563.1	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Q0185	*??*		4001_at	Cytochrom	22.9	2.3	30.5	22.9	2.2	28.1	10.6	-2.3	14.1	10.6	-1.0	0.2
YKL055C	*OAR1*		10641_at	YKL055C r	27.3	2.6	43.5	27.3	1.8	21.8	7.8	-2.6	12.5	7.8	-1.4	3.1
YKL150W	*MCR1*		10723_at	YKL150W	476.9	1.6	286.1	476.9	1.5	238.4	291.2	-1.6	174.7	291.2	-1.1	29.1
YBL045C	*COR1*	*QCR1*	7407_at	YBL045C 4	624.4	1.3	184.9	624.4	1.1	93.0	476.8	-1.3	141.2	476.8	-1.1	47.7
YPR191W	*QCR2*	*COR2, UC*	7543_at	YPR191W	497.9	1.9	448.1	497.9	1.5	273.4	238.5	-1.9	214.7	238.5	-1.2	47.7
YML054C	*CYB2*		9702_at	YML054C C	95.6	2.7	166.8	95.6	1.2	18.7	37.7	-2.7	65.8	37.7	-2.4	52.8
YDR178W	*SDH4*		6279_at	YDR178W	695.1	1.2	139.0	695.1	1.0	33.9	586.8	-1.2	117.4	586.8	-1.1	58.7
YGR008C	*STF2*		4980_at	YGR008C	783.8	1.3	235.1	783.8	1.1	116.7	605.4	-1.3	181.6	605.4	-1.1	60.5
YML120C	*NDI1*		9765_at	YML120C r	207.2	1.9	186.5	207.2	1.2	41.4	108.0	-1.9	97.2	108.0	-1.6	64.8
YGR183C	*QCR9*	*UCR9*	4838_at	YGR183C	1046.0	1.1	155.8	1046.0	1.0	51.1	903.1	-1.1	134.5	903.1	-1.1	90.3
YBR039W	*ATP3*		7307_at	YBR039W	630.5	1.2	126.1	630.5	-1.0	0.0	526.3	-1.2	105.3	526.3	-1.2	105.3
YGL191W	*COX13*		5188_at	YGL191W	0.0	0.0	0.0	0.0	0.0	0.0	560.7	-1.1	83.5	560.7	-1.2	112.1
YEL024W	*RIP1*		5766_at	YEL024W	767.0	1.4	344.5	767.0	1.2	191.0	600.6	-1.4	269.8	600.6	-1.2	120.1
YHR051W	*COX6*		4481_at	YHR051W	904.0	1.3	271.2	904.0	1.1	90.4	652.9	-1.3	195.9	652.9	-1.2	130.6
YER141W	*COX15*		5581_at	YER141W	491.1	-1.0	0.0	491.1	-1.2	122.3	484.7	-1.0	0.0	484.7	-1.3	145.4
YNL052W	*COX5A*		8935_at	YNL052W	606.5	1.2	121.3	606.5	-1.0	29.6	501.4	-1.2	100.3	501.4	-1.3	150.4
YLR295C	*ATP14*		10010_at	YLR295C A	630.6	1.6	376.4	630.6	1.1	93.9	394.9	-1.6	235.7	394.9	-1.4	158.0
YDR322C	*TIM11*		6154_at	YDR322C s	975.4	1.2	195.1	975.4	1.1	97.5	799.4	-1.2	159.9	799.4	-1.2	159.9
YDL004W	*ATP16*		6503_at	YDL004W	720.0	1.2	179.3	720.0	1.0	35.1	558.7	-1.2	139.1	558.7	-1.3	167.6
YPL271W	*ATP15*	*ATPEPSIL*	8024_at	YPL271W	1021.8	1.1	152.2	1021.8	-1.0	49.9	899.9	-1.1	134.0	899.9	-1.2	180.0
Q0310			3976_at	F1F0-ATPa	804.9	1.2	200.4	804.9	-1.0	39.3	644.5	-1.2	160.5	644.5	-1.3	193.4

Category	ORF	Gene	Alias	Probe	Description												
	YGL187C	*COX4*		5193_at	YGL187C s	759.1	1.1	75.9	759.1	-1.2	151.8	691.0	-1.1	69.1	691.0	-1.3	207.3
	YJR048W	*CYC1*		10977_at	YJR048W	827.3	1.1	123.2	827.3	-1.1	79.0	697.0	-1.1	103.8	697.0	-1.3	209.1
	YDL067C	*COX9*		6531_at	YDL067C S	687.3	-1.0	33.5	687.3	-1.3	239.9	707.7	1.0	34.5	707.7	-1.3	212.3
fermentation																	
	YDR380W	*ARO10*		6077_at	Similarity tc	883.1	2.3	1191.7	883.1	11.5	9314.3	375.6	-2.3	506.9	375.6	5.0	1502.4
	YLR044C	*PDC1*		10296_at	YLR044C p	2283.0	-1.2	456.6	2283.0	-1.0	0.0	2763.9	1.2	552.8	2763.9	1.2	552.8
	YOL086C	*ADH1*	*ADC1*	8657_at	YOL086C A	3553.5	-1.2	695.9	3553.5	-1.1	529.2	4293.4	1.2	840.8	4293.4	1.1	429.3
	YMR116C	*ASC1*	*CPC2*	9512_at	YMR116C	2863.4	-1.2	572.7	2863.4	-1.1	286.3	3505.4	1.2	701.1	3505.4	1.1	350.5
	YBR145W	*ADH5*		7236_at	YBR145W	1213.1	1.0	59.2	1213.1	1.3	423.4	1149.0	-1.0	56.1	1149.0	1.3	344.7
	YER073W	*ALD5*		5646_at	YER073W	453.5	1.2	109.3	453.5	2.2	540.1	360.2	-1.2	86.8	360.2	1.7	252.1
	YFL056C	*AAD6*		5424_at	YFL056C a	4.7	2.1	5.2	4.7	1.5	2.5	2.3	-2.1	2.6	2.3	-1.5	1.2
	YHR039C	*MSC7*	*VMA10*	4514_at	YHR039C	783.6	-1.3	235.1	783.6	-1.4	351.9	1017.3	1.3	305.2	1017.3	-1.1	101.7
	YPL061W	*ALD6*		7828_at	YPL061W	750.5	-1.1	111.8	750.5	-1.3	262.0	859.1	1.1	127.9	859.1	-1.2	171.8
metabolism of energy reserves (glycogen, trehalose)																	
	YPL240C	*HSP82*	*HSP83, HS*	8010_i_at	YPL240C H	0.0	0.0	0.0	0.0	0.0	0.0	514.6	-1.0	0.0	514.6	1.2	102.9
	YER133W	*GLC7*	*CID1, DIS2*	5573_at	protein pho	242.7	1.2	40.7	242.7	1.3	84.2	217.2	-1.2	36.4	217.2	1.1	30.4
	YKL128C	*PMU1*		10700_at	YKL128C P	585.1	1.3	175.5	585.1	1.3	175.5	0.0	0.0	0.0	0.0	0.0	0.0
	YOR178C	*GAC1*		8380_at	YOR178C	5.2	1.7	3.5	5.2	-1.3	1.8	3.1	-1.7	2.1	3.1	-2.1	3.4
	YDR074W	*TPS2*	*HOG2, PFI*	6398_at	YDR074W	224.7	1.5	111.6	224.7	-1.0	11.0	140.6	-1.5	69.8	140.6	-1.6	84.4
	YMR105C	*PGM2*		9547_at	YMR105C	0.0	0.0	0.0	0.0	0.0	0.0	157.8	-1.3	55.1	157.8	-1.6	94.7
	YDR214W	*AHA1*		6271_at	YDR214W	0.0	0.0	0.0	0.0	0.0	0.0	295.8	-1.1	28.2	295.8	-1.4	118.3
glyoxylate cycle																	
	YOL126C	*MDH2*		8663_at	Cytosolic m	126.7	1.9	113.7	126.7	3.4	309.9	66.1	-1.9	59.3	66.1	1.8	52.9
	YCR005C	*CIT2*		6883_at	YCR005C	1341.0	-1.1	199.7	1341.0	-1.3	468.1	1588.0	1.1	236.5	1588.0	-1.1	158.8
	YLR304C	*ACO1*	*GLU1*	10019_at	YLR304C A	1066.5	-1.4	479.0	1066.5	-1.6	636.6	1602.0	1.4	719.5	1602.0	-1.1	160.2
oxidation of fatty acids																	
	YBR046C	*ZTA1*		7314_at	Homolog tc	129.6	2.6	207.0	129.6	3.3	297.8	49.3	-2.6	78.8	49.3	1.3	14.8
other energy generation activities				6	16												
	YAL054C	*ACS1*		11356_at	YAL054C i	350.3	1.2	87.2	350.3	1.3	122.3	0.0	0.0	0.0	0.0	0.0	0.0
	YPL171C	*OYE3*	*ZRG6*	7944_at	NAD(P)H o	23.0	3.4	54.7	23.0	2.7	38.7	6.8	-3.4	16.2	6.8	-1.3	2.0
	YML035C	*AMD1*	*AMD3*	9674_at	YML035C	0.0	0.0	0.0	0.0	0.0	0.0	5.9	-1.3	1.9	5.9	-2.4	8.3
	YDL078C	*MDH3*		6565_at	YDL078C r	270.4	-1.0	13.2	270.4	-1.4	121.4	258.3	1.0	12.6	258.3	-1.4	103.3
	YEL071W	*DLD3*		5813_at	YEL071W	934.3	-1.4	419.6	934.3	-1.5	513.1	1352.6	1.4	607.5	1352.6	-1.1	135.3
	YDL181W	*INH1*		6684_at	YDL181W	140.3	1.2	28.1	140.3	-2.0	140.3	113.1	-1.2	22.6	113.1	-2.4	158.3
CELL CYCLE AND DNA PROCESSING																	
DNA processing																	
DNA synthesis and replication																	
	YAL040C	*CLN3*	*DAF1, FUN*	11369_at	YAL040C (	188.8	1.2	37.8	188.8	1.7	132.1	0.0	0.0	0.0	0.0	0.0	0.0
	YIL066C	*RNR3*	*DIN1, RIR3*	4045_s_at	YIL066C R	7.3	2.2	8.5	7.3	-1.1	1.0	2.2	-2.2	2.6	2.2	-1.2	0.4
	YAL033W	*POP5*	*FUN53*	11333_at	YAL033W	232.6	-1.3	69.8	232.6	-1.4	104.5	0.0	0.0	0.0	0.0	0.0	0.0
	YBR088C	*POL30*		7266_at	YBR088C p	191.6	-1.2	38.3	191.6	-1.6	115.0	0.0	0.0	0.0	0.0	0.0	0.0
	YGR180C	*RNR4*		4835_at	YGR180C	942.0	-1.0	46.0	942.0	-1.1	140.3	0.0	0.0	0.0	0.0	0.0	0.0
	YCR028C	*FEN2*		6863_at	YCR028C	375.2	-1.2	93.4	375.2	-1.4	148.7	465.8	1.2	116.0	465.8	-1.1	46.6
	YER070W	*RNR1*	*CRT7, RIR*	5643_at	YER070W	457.9	1.0	22.3	457.9	-1.4	183.1	391.7	-1.0	19.1	391.7	-1.5	195.9
	YMR241W	*YHM2*		9380_at	YMR241W	739.4	-1.3	221.8	739.4	-1.3	221.8	974.2	1.3	292.3	974.2	1.0	0.0
	YJL026W	*RNR2*	*CRT6*	11041_at	YJL026W s	665.7	-1.1	66.6	665.7	-1.3	232.4	774.7	1.1	77.5	774.7	-1.2	154.9
DNA recombination																	
	YCR014C	*POL4*	*POLX*	6891_at	DNA polym	15.3	-3.0	30.5	15.3	1.4	6.1	67.8	3.0	135.1	67.8	5.5	305.1
	YMR167W	*MLH1*	*PMS2*	9479_at	YMR167W	10.2	2.7	17.6	10.2	1.2	2.4	3.0	-2.7	5.2	3.0	-2.1	3.3
	YMR190C	*SGS1*		9461_at	YMR190C	0.0	0.0	0.0	0.0	0.0	0.0	11.3	-1.4	4.5	11.3	-2.1	12.4

		YLR466W	*YRF1-4*	*YRF1*	3735_f_at	YLR466W	437.7	-1.3	131.3	437.7	-1.6	262.6	635.6	1.3	190.7	635.6	-1.3	190.7
		YFL003C	*MSH4*		5385_at	YFL003C r	5.6	2.2	6.9	5.6	-1.2	0.9	0.0	0.0	0.0	0.0	0.0	0.0
		YJR021C	*REC107*	*MER2*	10999_at	YJR021C M	2.3	2.0	2.4	2.3	2.4	3.2	0.0	0.0	0.0	0.0	0.0	0.0
		YGR063C	*SPT4*		4944_at	YGR063C	239.6	-1.2	59.6	239.6	-1.4	107.6	0.0	0.0	0.0	0.0	0.0	0.0
		YBR088C	*POL30*		7266_at	YBR088C p	191.6	-1.2	38.3	191.6	-1.6	115.0	0.0	0.0	0.0	0.0	0.0	0.0
	DNA repair																	
		YAL015C	*NTG1*	*FUN33*	11352_at	YAL015C D	144.8	1.7	108.5	144.8	2.0	144.4	82.6	-1.7	61.9	82.6	1.1	8.3
		YIL066C	*RNR3*	*DIN1, RIR3*	4045_s_at	YIL066C R	7.3	2.2	8.5	7.3	-1.1	1.0	2.2	-2.2	2.6	2.2	-1.2	0.4
		YFL014W	*HSP12*		5372_at	YFL014W	625.4	1.8	493.3	625.4	-1.2	122.5	350.2	-1.8	276.3	350.2	-2.1	385.2
		YGR180C	*RNR4*		4835_at	YGR180C	942.0	-1.0	46.0	942.0	-1.1	140.3	0.0	0.0	0.0	0.0	0.0	0.0
		YOL053C	*??*		8599_at	YOL053C I	0.0	0.0	0.0	0.0	0.0	0.0	206.7	-2.3	268.3	206.7	-2.1	227.4
	DNA restriction or modification																	
		YOR144C	*ELG1*	*RTT110*	8391_at	YOR144C	40.1	-1.1	6.0	40.1	-2.0	42.1	0.0	0.0	0.0	0.0	0.0	0.0
		YPR204W	*??*		3542_f_at	YPR204W	374.9	-1.3	128.1	374.9	-1.4	144.6	0.0	0.0	0.0	0.0	0.0	0.0
		chromatin modification																
			YGR063C	*SPT4*		4944_at	YGR063C	239.6	-1.2	59.6	239.6	-1.4	107.6	0.0	0.0	0.0	0.0	0.0
			YML010W	*SPT5*		9655_at	YML010W	15.2	2.9	29.0	15.2	1.6	9.6	3.0	-2.9	5.7	3.0	-2.3
cell cycle																		
	mitotic cell cycle and cell cycle control																	
		YLR075W			10239_at	YLR075W	2845.6	-1.2	708.6	2845.6	-1.0	138.9	3518.0	1.2	876.0	3518.0	1.2	703.6
		YDR524C	*AGE1*	*SAT1*	5926_g_at	YDR524C .	3729.0	-1.3	1015.6	3729.0	-1.1	539.0	4720.7	1.3	1285.7	4720.7	1.1	703.0
		YKR042W	*UTH1*		10514_at	YKR042W	1982.9	-1.0	0.0	1982.9	1.2	396.6	2155.3	-1.0	0.0	2155.3	1.2	431.1
		YFR031C	*SMC2*		5329_s_at	YFR031C (	3309.1	-1.2	824.0	3309.1	-1.1	492.8	4068.5	1.2	1013.1	4068.5	1.1	406.9
		YJL034W	*KAR2*	*BIP, GRP7*	11078_at	Homologue	1978.5	1.1	188.8	1978.5	1.2	492.6	1802.2	-1.1	172.0	1802.2	1.2	360.4
		YLR175W	*CBF5*		10155_at	YLR175W	795.5	-1.0	0.0	795.5	1.4	318.2	829.9	-1.0	0.0	829.9	1.4	332.0
		YHR071W	*PCL5*		4452_at	YHR071W	0.0	0.0	0.0	0.0	0.0	0.0	251.9	-1.6	151.1	251.9	2.1	277.1
		YDL155W	*CLB3*		6620_at	G(sub)2-sp	84.2	1.0	4.1	84.2	3.1	180.9	79.0	-1.0	3.9	79.0	2.9	150.1
		YGL215W	*CLG1*		5210_at	YGL215W	190.2	1.0	9.3	190.2	2.1	208.7	136.2	-1.0	6.6	136.2	1.9	122.6
		YMR094W	*CTF13*	*CBF3C*	9536_at	58 kd comp	30.0	1.4	12.0	30.0	8.2	215.6	21.4	-1.4	8.6	21.4	5.9	104.9
		YCL004W	*PGS1*	*PEL1*	6875_at	17-kDa Pho	6.6	3.2	14.7	6.6	1.4	2.5	0.0	-3.2	0.0	0.0	-2.4	0.0
		YCL024W	*KCC4*		6762_s_at	YCL024W	12.4	2.4	17.2	12.4	1.2	2.4	5.1	-2.4	7.1	5.1	-2.0	5.1
		YPL255W	*BBP1*		7994_at	YPL255W	0.0	0.0	0.0	0.0	0.0	0.0	4.1	-1.5	2.0	4.1	-2.4	5.7
		YJL157C	*FAR1*		11181_at	YJL157C F	346.1	-1.3	120.8	346.1	-1.6	206.5	476.4	1.3	166.3	476.4	-1.2	95.3
		YNL307C	*MCK1*		9177_at	YNL307C 4	624.7	-1.1	93.0	624.7	-1.3	218.1	701.8	1.1	104.5	701.8	-1.2	140.4
		YJL080C	*SCP160*		11122_at	YJL080C M	403.0	-1.1	60.0	403.0	-1.5	221.3	472.9	1.1	70.4	472.9	-1.3	141.9
		YNL098C	*RAS2*		8979_at	YNL098C F	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3	143.6
		YGL048C	*RPT6*	*CIM3, CRL*	5059_at	YGL048C a	697.5	-1.0	0.0	697.5	-1.1	103.9	734.5	-1.0	0.0	734.5	-1.2	146.9
		YDR328C	*SKP1*	*MGO1*	6160_at	YDR328C	487.2	1.2	121.3	487.2	-1.1	48.7	386.5	-1.2	96.2	386.5	-1.4	154.6
		YNL064C	*YDJ1*	*MAS5*	8924_at	YNL064C y	979.7	1.0	47.8	979.7	-1.1	145.9	959.6	-1.0	46.8	959.6	-1.2	191.9
		YDL126C	*CDC48*		6604_at	YDL126C a	655.9	-1.1	65.6	655.9	-1.3	229.0	652.5	1.1	65.3	652.5	-1.3	195.8
		YLR229C	*CDC42*		10121_at	Member of	1147.0	-1.0	56.0	1147.0	-1.3	400.4	1103.3	1.0	53.9	1103.3	-1.3	331.0
		YFL037W	*TUB2*	*ARM10, SH*	5394_at	YFL037W I	727.1	1.1	108.3	727.1	1.3	218.1	0.0	0.0	0.0	0.0	0.0	0.0
		YMR200W	*ROT1*		9427_at	YMR200W	276.0	1.5	138.0	276.0	1.5	151.6	0.0	0.0	0.0	0.0	0.0	0.0
		YAL040C	*CLN3*	*DAF1, FUN*	11369_at	YAL040C C	188.8	1.2	37.8	188.8	1.7	132.1	0.0	0.0	0.0	0.0	0.0	0.0
		YGR029W	*ERV1*		3946_at	YGR029W	370.4	1.2	82.3	370.4	1.3	129.3	307.9	-1.2	68.4	307.9	1.1	30.8
		YNL007C	*SIS1*		8888_at	YNL007C s	635.8	1.2	127.2	635.8	1.1	63.6	519.0	-1.2	103.8	519.0	-1.1	51.9
		YHR030C	*SLT2*	*BYC2, MPK*	4504_at	YHR030C	187.6	1.6	111.9	187.6	1.6	121.8	118.8	-1.6	70.9	118.8	1.1	11.9
		YNL172W	*APC1*		9041_at	YNL172W	102.1	1.3	34.9	102.1	2.2	121.6	76.1	-1.3	26.0	76.1	1.6	45.7
		YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2	10.9
		YER133W	*GLC7*	*CID1, DIS2*	5573_at	protein pho	242.7	1.2	40.7	242.7	1.3	84.2	217.2	-1.2	36.4	217.2	1.1	30.4
		YER123W	*YCK3*	*CKI3*	5608_at	YER123W	35.8	1.4	16.1	35.8	2.3	48.2	0.0	0.0	0.0	0.0	0.0	0.0

Category	ORF	Gene	Alias	Probe	Description												
	YNL012W	*SPO1*		8883_at	YNL012W	10.8	2.2	13.1	10.8	1.3	3.2	4.3	-2.2	5.3	4.3	-1.8	3.4
	YCR091W	*KIN82*		6786_at	YCR091W	5.7	1.4	2.3	5.7	2.0	5.8	0.0	0.0	0.0	0.0	0.0	0.0
	YBR109C	*CMD1*		7245_at	YBR109C (	758.7	-1.1	75.9	758.7	-1.2	151.7	0.0	0.0	0.0	0.0	0.0	0.0
	YLR043C	*TRX1*	*LMA1*	10295_at	YLR043C t	1339.6	-1.2	267.9	1339.6	-1.1	199.5	1597.3	1.2	319.5	1597.3	1.0	0.0
	YFL039C	*ACT1*	*ABY1, ENI*	5392_at	YFL039C A	2061.4	-1.2	403.7	2061.4	-1.2	403.7	2477.1	1.2	485.1	2477.1	1.0	0.0
cell cycle checkpoints (checkpoints of morphogenesis, DNA-damage,-replication, mitotic phase and spindle)											1	27 ORFs					
		YER177W	*BMH1*		5525_at	YER177W	1125.3	-1.1	112.5	1125.3	-1.3	392.8	1252.2	1.1	125.2	1252.2	-1.2
meiosis																	
	YPL240C	*HSP82*	*HSP83, HS*	8010_i_at	YPL240C I	0.0	0.0	0.0	0.0	0.0	0.0	514.6	-1.0	0.0	514.6	1.2	102.9
	YDR523C	*SPS1*		5947_at	YDR523C (	5.0	2.0	5.2	5.0	-1.2	0.9	2.0	-2.0	2.1	2.0	-2.3	2.6
	YMR167W	*MLH1*	*PMS2*	9479_at	YMR167W	10.2	2.7	17.6	10.2	1.2	2.4	3.0	-2.7	5.2	3.0	-2.1	3.3
	YPL255W	*BBP1*		7994_at	YPL255W	0.0	0.0	0.0	0.0	0.0	0.0	4.1	-1.5	2.0	4.1	-2.4	5.7
	YDR214W	*AHA1*		6271_at	YDR214W	0.0	0.0	0.0	0.0	0.0	0.0	295.8	-1.1	28.2	295.8	-1.4	118.3
	YNL307C	*MCK1*		9177_at	YNL307C 4	624.7	-1.1	93.0	624.7	-1.3	218.1	701.8	1.1	104.5	701.8	-1.2	140.4
	YBR078W	*ECM33*		7302_at	YBR078W	1606.6	-1.2	321.3	1606.6	-1.2	400.0	1885.9	1.2	377.2	1885.9	-1.1	188.6
	YER175C	*TMT1*	*TAM1*	5523_at	Trans-acor	320.3	2.5	477.9	320.3	3.9	925.2	107.8	-2.5	160.8	107.8	1.5	53.9
	YFL037W	*TUB2*	*ARM10, SI*	5394_at	YFL037W I	727.1	1.1	108.3	727.1	1.3	218.1	0.0	0.0	0.0	0.0	0.0	0.0
	YLR094C	*GIS3*		10258_at	Cyclin C ar	40.0	3.8	111.6	40.0	6.0	199.5	9.8	-3.8	27.4	9.8	1.6	5.9
	YHL027W	*RIM101*		4581_at	YHL027W	326.7	1.2	65.3	326.7	1.4	130.7	0.0	0.0	0.0	0.0	0.0	0.0
	YER133W	*GLC7*	*CID1, DIS2*	5573_at	protein phc	242.7	1.2	40.7	242.7	1.3	84.2	217.2	-1.2	36.4	217.2	1.1	30.4
	YDL154W	*MSH5*		6621_at	YDL154W	20.5	1.7	15.2	20.5	2.1	23.3	0.0	0.0	0.0	0.0	0.0	0.0
	YHR124W	*NDT80*		4418_at	YHR124W	11.8	1.3	4.1	11.8	2.2	14.7	0.0	0.0	0.0	0.0	0.0	0.0
	YLL005C	*SPO75*		10338_at	YLL005C S	6.0	2.3	8.0	6.0	2.7	9.8	1.8	-2.3	2.4	1.8	1.4	0.7
	YOL091W	*SPO21*	*MPC70*	8652_at	YOL091W	5.1	1.4	2.0	5.1	2.9	9.4	0.0	0.0	0.0	0.0	0.0	0.0
	YFL003C	*MSH4*		5385_at	YFL003C r	5.6	2.2	6.9	5.6	-1.2	0.9	0.0	0.0	0.0	0.0	0.0	0.0
	YJR021C	*REC107*	*MER2*	10999_at	YJR021C I	2.3	2.0	2.4	2.3	2.4	3.2	0.0	0.0	0.0	0.0	0.0	0.0
	YNL204C	*SPS18*	*SPX18*	9055_at	YNL204C s	4.2	-1.8	3.4	4.2	-2.7	7.2	0.0	0.0	0.0	0.0	0.0	0.0
cytokinesis (cell division)																	
	YNL271C	*BNI1*	*PPF3*	9123_at	Cytoskeleta	94.5	1.3	28.0	94.5	3.4	229.7	108.6	-1.3	32.2	108.6	2.1	119.5
	YPL255W	*BBP1*		7994_at	YPL255W	0.0	0.0	0.0	0.0	0.0	0.0	4.1	-1.5	2.0	4.1	-2.4	5.7
	YDR284C	*DPP1*		6204_at	YDR284C	276.7	-1.2	55.3	276.7	-1.5	151.9	0.0	0.0	0.0	0.0	0.0	0.0
	YLR286C	*CTS1*		10045_at	YLR286C I	2323.9	-1.1	346.1	2323.9	-1.1	346.1	2400.2	1.1	357.4	2400.2	-1.1	240.0
nuclear and chromosomal cycle																	
chromosome segregation																	
		YJR135C	*MCM22*		10883_at	YJR135C F	0.0	0.0	0.0	0.0	0.0	0.0	6.5	-1.8	5.2	6.5	-2.4
other cell division and DNA synthesis activities																	
	YMR096W	*SNZ1*		9538_at	Encodes hi	1883.5	1.5	941.7	1883.5	6.2	9787.9	1095.7	-1.5	547.9	1095.7	4.2	3506.2
	YFL034C			5396_at	YFL034C F	503.0	1.2	100.6	503.0	1.2	100.6	0.0	0.0	0.0	0.0	0.0	0.0
TRANSCRIPTION																	
rRNA transcription																	
rRNA synthesis																	
	YNL039W	*BDP1*	*TFC5*	8902_at	Subunit of	62.2	1.2	15.5	62.2	3.2	136.5	56.3	-1.2	14.0	56.3	2.5	84.5
	YOR224C	*RPB8*		8336_at	YOR224C	710.6	-1.1	105.8	710.6	-1.1	105.8	816.1	1.1	121.5	816.1	-1.0	0.0
	YOR341W	*RPA190*	*RRN1*	8185_at	YOR341W	328.5	-1.1	48.9	328.5	-1.4	130.2	383.7	1.1	57.1	383.7	-1.2	76.7
	YPR187W	*RPO26*	*RPB6*	7539_at	YPR187W	335.7	-1.2	83.6	335.7	-1.4	150.8	429.1	1.2	106.8	429.1	-1.2	85.8
	YPR010C	*RPA135*	*RPA2, RRI*	7762_at	YPR010C I	352.9	-1.0	17.2	352.9	-1.4	139.9	379.1	1.0	18.5	379.1	-1.3	113.7
rRNA processing																	
	SNR73			9193_at	SNR73 Sm	36.6	-1.8	28.8	36.6	4.0	110.9	63.4	1.8	50.0	63.4	7.3	399.4
	YLR175W	*CBF5*		10155_at	YLR175W	795.5	-1.0	0.0	795.5	1.4	318.2	829.9	-1.0	0.0	829.9	1.4	332.0
	YOR310C	*NOP58*	*NOP5*	8247_at	YOR310C	757.9	-1.2	151.6	757.9	-1.0	37.0	888.7	1.2	177.7	888.7	1.1	88.9

Category	ORF	Gene	Alias	Probe	Description												
	YHR089C	*GAR1*		4425_at	YHR089C :	1304.6	-1.1	194.3	1304.6	-1.0	63.7	1525.6	1.1	227.2	1525.6	1.1	152.6
	RPR1			5413_at	RPR1 RNa	0.0	0.0	0.0	0.0	0.0	0.0	155.0	1.9	139.1	155.0	1.2	31.0
	YLR197W	*SIK1*		10132_at	YLR197W	0.0	0.0	0.0	0.0	0.0	0.0	402.0	1.1	40.2	402.0	1.3	120.6
	YHL034C	*SBP1*	*SSBR1*	4574_at	YHL034C S	0.0	0.0	0.0	0.0	0.0	0.0	743.6	1.1	110.7	743.6	-1.1	74.4
	SNR36			8059_at	SNR36 snR	14.4	-1.2	3.5	14.4	3.0	29.4	18.5	1.2	4.5	18.5	3.9	53.7
	YCR018C	*SRD1*		6755_i_at	YCR018C	1.6	-2.4	2.2	1.6	-1.7	1.2	5.7	2.4	8.0	5.7	1.3	1.7
	YNL282W	*POP3*		9157_at	Structural c	21.9	1.4	9.4	21.9	5.6	101.6	20.1	-1.4	8.6	20.1	3.5	50.3
	YNR053C	*NOG2*		8812_at	YNR053C	66.8	2.0	66.8	66.8	2.6	106.8	0.0	0.0	0.0	0.0	0.0	0.0
	YAL033W	*POP5*	*FUN53*	11333_at	YAL033W .	232.6	-1.3	69.8	232.6	-1.4	104.5	0.0	0.0	0.0	0.0	0.0	0.0
	YDR432W	*NPL3*	*MTR13, M*	6039_g_at	YDR432W	442.1	1.2	106.8	442.1	1.1	53.2	326.8	-1.2	78.9	326.8	-1.2	54.8
	YNL112W	*DBP2*		8965_at	YNL112W	510.9	-1.2	102.2	510.9	-1.3	153.3	604.6	1.2	120.9	604.6	-1.1	60.5
	YDL208W	*NHP2*		6700_at	YDL208W	821.0	-1.1	122.3	821.0	-1.2	204.4	930.8	1.1	138.6	930.8	-1.1	93.1
other rRNA-transcription activities				1	6												
	YMR131C	*RRB1*		9484_at	YMR131C	431.3	1.3	150.6	431.3	1.1	64.2	322.8	-1.3	112.7	322.8	-1.2	64.6
tRNA transcription																	
tRNA synthesis																	
	YNL039W	*BDP1*	*TFC5*	8902_at	Subunit of	62.2	1.2	15.5	62.2	3.2	136.5	56.3	-1.2	14.0	56.3	2.5	84.5
	YOR224C	*RPB8*		8336_at	YOR224C	710.6	-1.1	105.8	710.6	-1.1	105.8	816.1	1.1	121.5	816.1	-1.0	0.0
	YPR187W	*RPO26*	*RPB6*	7539_at	YPR187W	335.7	-1.2	83.6	335.7	-1.4	150.8	429.1	1.2	106.8	429.1	-1.2	85.8
tRNA processing																	
	YIL075C	*RPN2*	*SEN3*	4189_at	YIL075C R	0.0	0.0	0.0	0.0	0.0	0.0	385.5	1.2	77.1	385.5	1.4	154.2
	RPR1			5413_at	RPR1 RNa	0.0	0.0	0.0	0.0	0.0	0.0	155.0	1.9	139.1	155.0	1.2	31.0
	YDR381W	*YRA1*	*SHE11*	6078_at	YDR381W	400.7	1.1	59.7	400.7	-1.2	99.8	355.9	-1.1	53.0	355.9	-1.4	142.4
	YNL282W	*POP3*		9157_at	Structural c	21.9	1.4	9.4	21.9	5.6	101.6	20.1	-1.4	8.6	20.1	3.5	50.3
	YML091C	*RPM2*		9754_at	Rpm2p is a	187.9	1.7	131.5	187.9	1.4	75.1	120.0	-1.7	84.0	120.0	-1.2	24.0
	YAL033W	*POP5*	*FUN53*	11333_at	YAL033W .	232.6	-1.3	69.8	232.6	-1.4	104.5	0.0	0.0	0.0	0.0	0.0	0.0
tRNA modification																	
	YBL013W	*FMT1*		7394_at	YBL013W	2.8	1.1	0.3	2.8	2.3	3.6	2.6	-1.1	0.3	2.6	2.2	3.1
	YGR204W	*ADE3*		4814_at	YGR204W	724.6	-1.2	144.9	724.6	-1.2	180.4	901.8	1.2	180.4	901.8	-1.0	0.0
mRNA transcription																	
mRNA synthesis																	
general transcription activities																	
	YCR042C	*TSM1*	*TAF150*	6830_at	"Essential g	31.2	-1.0	0.0	31.2	6.3	165.3	34.4	-1.0	0.0	34.4	6.1	
	YML010W	*SPT5*		9655_at	YML010W	15.2	2.9	29.0	15.2	1.6	9.6	3.0	-2.9	5.7	3.0	-2.3	
	YOR151C	*RPB2*	*RPB150, R*	8398_at	YOR151C	378.0	1.2	74.0	378.0	-1.1	56.3	281.6	-1.2	55.1	281.6	-1.4	
	YLR094C	*GIS3*		10258_at	Cyclin C ar	40.0	3.8	111.6	40.0	6.0	199.5	9.8	-3.8	27.4	9.8	1.6	
	YDL140C	*RPO21*	*RPB1, RPI*	6635_at	YDL140C R	247.9	1.2	61.7	247.9	1.5	123.1	0.0	0.0	0.0	0.0	0.0	
	YOR224C	*RPB8*		8336_at	YOR224C	710.6	-1.1	105.8	710.6	-1.1	105.8	816.1	1.1	121.5	816.1	-1.0	
	YGR063C	*SPT4*		4944_at	YGR063C	239.6	-1.2	59.6	239.6	-1.4	107.6	0.0	0.0	0.0	0.0	0.0	
	YHR041C	*SRB2*	*HRS2*	4470_at	YHR041C	188.0	-1.6	112.2	188.0	-1.6	112.2	343.7	1.6	205.1	343.7	1.0	
	YDR404C	*RPB7*		6055_at	YDR404C (	422.4	-1.1	42.2	422.4	-1.3	126.7	0.0	0.0	0.0	0.0	0.0	
	YPR187W	*RPO26*	*RPB6*	7539_at	YPR187W	335.7	-1.2	83.6	335.7	-1.4	150.8	429.1	1.2	106.8	429.1	-1.2	
transcriptional control																	
	YDR225W	*HTA1*	*H2A1, SPT*	6236_i_at	YDR225W	1800.8	-1.1	268.2	1800.8	1.1	268.2	2018.3	1.1	300.6	2018.3	1.3	
	YNL031C	*HHT2*		8910_at	YNL031C H	2640.6	-1.1	393.2	2640.6	-1.0	128.9	3123.3	1.1	465.1	3123.3	1.1	
	YNL030W	*HHF2*		8911_s_at	YNL030W	0.0	0.0	0.0	0.0	0.0	0.0	1477.0	1.1	147.7	1477.0	1.2	
	YFL021W	*GAT1*	*NIL1*	5409_at	YFL021W t	52.6	-2.5	81.2	52.6	-2.1	60.2	119.0	2.5	183.9	119.0	1.2	
	YCR042C	*TSM1*	*TAF150*	6830_at	"Essential g	31.2	-1.0	0.0	31.2	6.3	165.3	34.4	-1.0	0.0	34.4	6.1	
	YDR224C	*HTB1*	*SPT12*	6235_at	YDR224C	0.0	0.0	0.0	0.0	0.0	0.0	839.3	1.1	83.9	839.3	1.2	

Pectora roborant cultus recti

	YKL190W	*CNB1*		10772_at	YKL190W	0.0	0.0	0.0	0.0	0.0	0.0	575.0	1.1	57.5	575.0	1.2
	YJR147W	*HMS2*		10895_at	Heat shock	8.6	-2.0	9.0	8.6	2.0	8.7	16.7	2.0	17.5	16.7	4.2
	YDR160W	*SSY1*	*SHR10*	6306_at	YDR160W	35.6	1.1	3.6	35.6	2.5	55.2	33.2	-1.1	3.3	33.2	2.4
	YDR123C	*INO2*	*DIE1, SCS*	6358_at	Transcriptio	18.7	2.4	26.3	18.7	8.1	132.6	9.1	-2.4	12.8	9.1	4.9
	YMR182C	*RGM1*		9452_at	Putative tra	14.8	1.9	12.9	14.8	6.1	75.1	7.3	-1.9	6.4	7.3	4.1
	YOL067C	*RTG1*		8630_at	YOL067C	11.2	1.1	1.7	11.2	2.7	19.5	9.6	-1.1	1.4	9.6	2.4
	YDR216W	*ADR1*		6273_at	YDR216W	13.6	1.5	7.4	13.6	2.7	23.0	8.6	-1.5	4.7	8.6	1.8
	YKR034W	*DAL80*	*UGA43*	10551_at	YKR034W	0.0	0.0	0.0	0.0	0.0	0.0	1.8	2.0	1.9	1.8	-2.0
	YDR213W	*UPC2*	*MOX4*	6270_at	YDR213W	4.0	-1.1	0.4	4.0	-2.5	6.0	4.5	1.1	0.4	4.5	-2.2
	YDR213W	*UPC2*	*MOX4*	6270_at	YDR213W	4.0	-1.1	0.4	4.0	-2.5	6.0	4.5	1.1	0.4	4.5	-2.2
	YBL066C	*SEF1*		7430_at	Putative tra	9.5	4.0	28.5	9.5	1.8	7.6	5.2	-4.0	15.6	5.2	-2.2
	YHR193C	*EGD2*		4353_at	YHR193C	1137.1	-1.1	169.3	1137.1	-1.2	283.1	1294.9	1.1	192.8	1294.9	-1.1
	YEL009C	*GCN4*	*AAS3, AR*	5737_at		1433.2	1.0	70.0	1433.2	-1.1	136.8	1390.5	-1.0	67.9	1390.5	-1.1
	YBL002W	*HTB2*		7359_at	YBL002W	541.0	-1.1	54.1	541.0	-1.5	270.5	614.9	1.1	61.5	614.9	-1.3
	YPL037C	*EGD1*		7806_at	YPL037C C	1962.5	-1.2	392.5	1962.5	-1.3	588.8	2272.2	1.2	454.4	2272.2	-1.1
	YFL031W	*HAC1*	*ERN4, IRE*	5318_s_at	bZIP Trans	398.7	1.9	355.4	398.7	2.2	484.5	284.7	-1.9	253.8	284.7	1.2
	YOR344C	*TYE7*	*SGC1*	8188_at	33 kDa ser	66.1	2.8	121.6	66.1	3.6	174.1	23.0	-2.8	42.3	23.0	1.3
	YMR039C	*SUB1*	*TSP1*	9617_at	YMR039C	306.3	1.1	29.2	306.3	1.4	137.6	0.0	0.0	0.0	0.0	0.0
	YHL027W	*RIM101*		4581_at	YHL027W	326.7	1.2	65.3	326.7	1.4	130.7	0.0	0.0	0.0	0.0	0.0
	YIR017C	*MET28*		4098_at	YIR017C T	353.7	1.2	88.1	353.7	1.3	106.1	0.0	0.0	0.0	0.0	0.0
	YGR288W	*MAL13*		4718_at	YGR288W	60.3	1.7	45.1	60.3	2.7	105.4	30.5	-1.7	22.9	30.5	1.6
	YKL109W	*HAP4*		10675_at	YKL109W	192.2	1.8	153.2	192.2	1.5	95.4	106.7	-1.8	85.1	106.7	-1.2
	YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2
	YGL066W	*SGF73*		5086_at	YGL066W	60.2	1.4	23.8	60.2	2.4	86.4	0.0	0.0	0.0	0.0	0.0
	YGL209W	*MIG2*	*MLZ1*	5216_at	YGL209W	54.7	2.0	54.5	54.7	2.3	73.5	0.0	0.0	0.0	0.0	0.0
	YMR042W	*ARG80*	*ARGR1*	9575_at	Regulator c	57.3	1.8	45.8	57.3	1.9	51.5	31.3	-1.8	25.0	31.3	1.0
	YPR168W	*NUT2*	*MED10*	7566_at	YPR168W	14.1	2.2	17.6	14.1	2.0	14.7	6.3	-2.2	7.9	6.3	-1.1
	YCL010C	*SGF29*		6870_at	YCL010C S	11.0	2.0	11.1	11.0	1.5	5.7	5.6	-2.0	5.7	5.6	-1.3
	YFR034C	*PHO4*		5333_at	YFR034C p	9.9	2.3	12.4	9.9	1.4	4.0	3.3	-2.3	4.1	3.3	-1.2
	YNL012W	*SPO1*		8883_at	YNL012W	10.8	2.2	13.1	10.8	1.3	3.2	4.3	-2.2	5.3	4.3	-1.8
	YLR055C	*SPT8*		10264_at	YLR055C t	0.0	0.0	0.0	0.0	0.0	0.0	22.9	-2.0	22.7	22.9	-1.7
	YIL084C	*SDS3*		4182_at	YIL084C F	4.6	2.0	4.8	4.6	-1.2	0.9	0.0	0.0	0.0	0.0	0.0
	YNL204C	*SPS18*	*SPX18*	9055_at	YNL204C s	4.2	-1.8	3.4	4.2	-2.7	7.2	0.0	0.0	0.0	0.0	0.0
	YJL206C	*??*		11221_at	YJL206C ir	427.0	-1.1	63.6	427.0	-1.2	106.3	0.0	0.0	0.0	0.0	0.0
	YBL003C	*HTA2*	*H2A2*	7358_s_at	YBL003C H	1320.7	-1.2	258.6	1320.7	-1.2	328.9	1555.2	1.2	304.5	1555.2	-1.0
	YBR010W	*HHT1*	*BUR5, SIN*	7368_i_at	YBR010W	2568.7	-1.1	382.5	2568.7	-1.1	382.5	2912.9	1.1	433.8	2912.9	-1.0
mRNA processing (splicing, 5'-, 3'-end processing)																
YKL009W	*MRT4*		10598_at	YKL009W	mRNA turn	-1.2	153.33		D6		766.65	-1.1	76.665	766.65	-1.2	153.33
splicing																
	SNR128			10782_at	SNR128 sn	133.2	-1.8	113.1	133.2	-1.5	73.1	246.2	1.8	209.1	246.2	1.3
	SNR56			6950_at	SNR56 snR	67.6	-2.1	74.3	67.6	-1.9	60.8	141.5	2.1	155.7	141.5	1.1
	SNR13			5807_at	SNR13 snR	0.0	0.0	0.0	0.0	0.0	0.0	278.5	1.5	153.0	278.5	1.3
	YEL026W	*SNU13*		5764_at	YEL026W	1087.2	-1.2	270.7	1087.2	-1.1	161.9	1391.4	1.2	346.5	1391.4	1.1
	SNR68			4031_at	SNR68 snR	0.0	0.0	0.0	0.0	0.0	0.0	172.7	1.1	17.3	172.7	1.7
	SNR63			5804_at	SNR63 snR	0.0	0.0	0.0	0.0	0.0	0.0	145.2	1.7	108.0	145.2	-1.0
	SNR4			5417_at	SNR4 snRI	182.7	-1.7	127.9	182.7	-1.3	54.8	316.8	1.7	221.8	316.8	1.3
	SNR62			8058_r_at	SNR62 snR	16.7	-1.2	2.8	16.7	2.6	26.0	17.9	1.2	3.0	17.9	3.0
	YBL026W	*LSM2*	*SMX5, SN*	7380_f_at	YBL026W	6.6	1.2	1.0	6.6	2.8	12.1	5.5	-1.2	0.9	5.5	2.6
	SNR190			10783_at	SNR190 sn	0.0	0.0	0.0	0.0	0.0	0.0	3.7	2.4	5.1	3.7	-1.3
	YDR378C	*LSM6*		6075_at	YDR378C	285.1	-1.1	42.4	285.1	-1.3	99.5	333.1	1.1	49.6	333.1	-1.1
	YGR222W	*PET54*		4788_at	YGR222W	0.0	0.0	0.0	0.0	0.0	0.0	136.2	-1.4	54.0	136.2	-1.7

				YPR182W	*SMX3*		7580_at	YPR182W	0.0	0.0	0.0	0.0	0.0	0.0	206.3	-1.0	10.1	206.3	-1.5
				SNR67			5448_at	SNR67 snR	0.0	0.0	0.0	0.0	0.0	0.0	182.1	1.8	145.7	182.1	-1.9
				YER146W	*LSM5*		5586_at	YER146W	523.1	-1.1	77.9	523.1	-1.6	312.2	612.3	1.1	91.2	612.3	-1.3
				YOR204W	*DED1*	*SPP81*	8361_at	YOR204W	325.4	1.8	260.3	325.4	1.3	97.6	169.7	-1.8	135.8	169.7	-1.3
				YDL087C	*LUC7*		6554_at	YDL087C L	14.4	2.0	15.0	14.4	1.7	10.8	8.2	-2.0	8.6	8.2	-1.1
				SNR64			10407_at	SNR64 snR	9.7	-1.4	3.5	9.7	-2.0	9.8	0.0	0.0	0.0	0.0	0.0
				SNR74			9192_at	SNR74 snR	14.6	-2.0	15.3	14.6	-2.5	22.5	29.3	2.0	30.7	29.3	-1.3
				SNR17a			8061_at	snr17a snR	26.1	-2.2	32.4	26.1	-2.1	29.8	52.0	2.2	64.7	52.0	1.0
				SNR6			9794_at	SNR6 snRI	27.4	-1.9	24.4	27.4	-2.5	40.6	51.4	1.9	45.7	51.4	-1.3
				SNR75			9191_at	SNR75 snR	62.7	-1.4	25.1	62.7	-2.1	68.9	0.0	0.0	0.0	0.0	0.0
				SNR70			7475_at	SNR70 snR	293.8	-1.5	145.9	293.8	-1.3	102.5	443.7	1.5	220.4	443.7	1.1
				YDL084W	*SUB2*		6557_at	YDL084W	678.8	-1.2	135.8	678.8	-1.3	203.6	823.9	1.2	164.8	823.9	-1.1
		other mRNA-transcription activities																	
			YNL016W	*PUB1*		8879_at	YNL016W	329.2	1.6	197.5	329.2	1.9	296.2	203.9	-1.6	122.3	203.9	1.2	40.8
			Q0205			4004_at	probable m	3.9	2.3	5.0	3.9	1.8	3.0	0.0	0.0	0.0	0.0	0.0	0.0
	RNA transport																		
		YDR432W	NPL3	MTR13, M	6039_g_at	YDR432W	442.1	1.2	106.8	442.1	1.1	53.2	326.8	-1.2	78.9	326.8	-1.2	54.8	
		YDR002W	YRB1	CST20, HT	6463_at	YDR002W	912.6	-1.2	182.5	912.6	-1.2	227.2	0.0	0.0	0.0	0.0	0.0	0.0	
	other transcription activities			6	57	ORFs													
		YNL004W	*HRB1*	*TOM34*	8845_at	Heterogen	232.1	1.3	69.6	232.1	3.6	614.9	160.5	-1.3	48.2	160.5	2.7	272.9	
		YOL010W	*RCL1*		8552_at	YOL010W	220.6	1.2	54.9	220.6	1.9	208.2	178.3	-1.2	44.4	178.3	1.6	107.0	
		YCL033C	*??*		6899_at	YCL033C	256.1	1.3	76.8	256.1	1.6	166.2	0.0	0.0	0.0	0.0	0.0	0.0	
		YOR230W	*WTM1*		8342_at	YOR230W	812.5	1.0	39.7	812.5	1.1	121.0	0.0	0.0	0.0	0.0	0.0	0.0	
		YPL230W	*??*		7974_at	YPL230W	4.2	2.5	6.2	4.2	1.1	0.2	1.8	-2.5	2.6	1.8	-1.8	1.4	
		YNL255C	*GIS2*		9139_at	YNL255C (	604.0	-1.1	57.6	604.0	-1.2	118.3	0.0	0.0	0.0	0.0	0.0	0.0	
PROTEIN SYNTHESIS																			
	ribosome biogenesis																		
		RDN5-1			3768_i_at	RDN5-1 5S	633.1	-4.4	2165.4	633.1	-2.8	1137.0	2544.8	4.4	8703.9	2544.8	1.5	1397.6	
		YJR145C			10893_s_a	YJR145C R	2528.9	-1.2	505.8	2528.9	1.1	376.6	3067.1	1.2	613.4	3067.1	1.4	1226.8	
		YPL079W			7811_at	YPL079W	3889.3	-1.2	968.4	3889.3	-1.0	189.8	4786.7	1.2	1191.9	4786.7	1.2	957.3	
		YLR344W			9968_s_at	YLR344W	3362.5	-1.2	837.2	3362.5	-1.0	164.1	4066.5	1.2	1012.6	4066.5	1.2	813.3	
		YNL137C	*NAM9*	*MNA6*	8985_at	Structural c	251.5	1.2	49.3	251.5	4.9	990.3	215.5	-1.2	42.2	215.5	4.3	711.2	
		YLR075W			10239_at	YLR075W	2845.6	-1.2	708.6	2845.6	-1.0	138.9	3518.0	1.2	876.0	3518.0	1.2	703.6	
		YKL180W			10738_i_at	YKL180W	2817.6	-1.2	563.5	2817.6	-1.0	0.0	3486.2	1.2	697.2	3486.2	1.2	697.2	
		YNL005C	*MRP7*		8890_at	Peptidyltrar	200.5	1.3	70.0	200.5	7.0	1212.7	151.3	-1.3	52.8	151.3	5.4	665.7	
		YHR021C			4494_at	YHR021C -	2555.9	-1.2	636.4	2555.9	-1.0	124.7	3188.4	1.2	793.9	3188.4	1.2	637.7	
		YLR340W			9964_at	YLR340W	2536.8	-1.2	631.6	2536.8	-1.0	123.8	3106.9	1.2	773.6	3106.9	1.2	621.4	
		YDR382W			6080_at	YDR382W	2413.6	-1.3	714.8	2413.6	-1.1	230.4	3071.7	1.3	909.7	3071.7	1.2	614.3	
		YJL136C			11158_i_at	YJL136C R	3299.5	-1.2	808.3	3299.5	-1.0	79.6	4054.7	1.2	993.3	4054.7	1.1	603.8	
		YGL189C			5191_f_at	YGL189C I	2355.7	-1.3	636.4	2355.7	-1.1	230.2	2883.3	1.3	778.9	2883.3	1.2	564.6	
		YDL075W			6569_at	YDL075W	1960.6	-1.4	777.2	1960.6	-1.1	283.4	2750.1	1.4	1090.2	2750.1	1.2	550.0	
		YOR293W			8269_f_at	YOR293W	1286.0	-1.2	320.2	1286.0	1.1	122.7	1609.6	1.2	400.8	1609.6	1.3	549.9	
		YPR043W			7708_f_at	YPR043W	1791.2	-1.2	446.0	1791.2	-1.1	44.6	2186.7	1.2	544.5	2186.7	1.1	54.4	
		YMR143W			9497_i_at	YMR143W	849.7	-1.2	211.6	849.7	1.2	175.1	1025.4	1.2	255.3	1025.4	1.5	533.1	
		YIL052C			4165_i_at	YIL052C R	2049.3	-1.3	606.9	2049.3	-1.0	0.0	2582.5	1.3	764.8	2582.5	1.2	516.5	
		YLR441C			9890_s_at	YLR441C R	2182.4	-1.2	431.9	2182.4	-1.1	42.2	2569.5	1.2	508.5	2569.5	1.1	48.5	
		YMR242C			9381_s_at	YMR242C	1408.7	-1.2	281.7	1408.7	1.0	68.8	1685.4	1.2	337.1	1685.4	1.3	505.6	
		YGL147C			5142_i_at	YGL147C I	472.5	-1.4	173.6	472.5	1.4	173.6	629.9	1.4	231.5	629.9	1.8	503.9	

YNL301C			9183_s_at	YNL301C F	1971.2	-1.2	490.8	1971.2	-1.0	47.5	2419.5	1.2	602.5	2419.5	1.2	483.9
YEL054C			5781_i_at	YEL054C F	1913.3	-1.2	476.4	1913.3	-1.0	93.4	2365.3	1.2	589.0	2365.3	1.2	473.1
YHL001W			4515_i_at	YHL001W	1047.4	-1.3	285.3	1047.4	1.1	65.1	1278.9	1.3	348.3	1278.9	1.4	470.0
YMR230W			9413_f_at	YMR230W	798.7	-1.2	126.4	798.7	1.3	219.4	958.6	1.2	151.7	958.6	1.5	450.2
YML063W			9691_i_at	YML063W	1232.7	-1.2	306.9	1232.7	1.0	60.2	1493.1	1.2	371.8	1493.1	1.3	447.9
YLR333C			10002_i_at	YLR333C F	1534.6	-1.2	267.3	1534.6	1.0	65.4	1832.1	1.2	319.1	1832.1	1.2	441.5
YGL031C			5031_at	YGL031C I	1705.7	-1.3	505.1	1705.7	-1.1	162.8	2163.7	1.3	640.8	2163.7	1.2	432.7
YGR148C			4848_at	YGR148C	3354.9	-1.2	835.4	3354.9	-1.1	499.6	4259.1	1.2	1060.5	4259.1	1.1	425.9
YGL030W			5032_at	YGL030W	1709.5	-1.2	411.9	1709.5	-1.0	0.0	2125.5	1.2	512.2	2125.5	1.2	425.1
YKL006W			10558_s_a	YKL006W	2624.3	-1.3	687.1	2624.3	-1.1	293.1	3341.8	1.3	874.9	3341.8	1.1	423.1
YGR027C			5001_f_at	YGR027C	1532.4	-1.2	340.4	1532.4	-1.1	24.5	1862.8	1.2	413.8	1862.8	1.1	61.6
YLR061W			10270_at	YLR061W	3375.8	-1.2	840.6	3375.8	-1.1	337.6	4118.2	1.2	1025.4	4118.2	1.1	411.8
YFR031C			5329_s_at	YFR031C I	3309.1	-1.2	824.0	3309.1	-1.1	492.8	4068.5	1.2	1013.1	4068.5	1.1	406.9
YHR010W			4528_i_at	YHR010W	1525.0	-1.2	332.7	1525.0	-1.1	23.9	1850.0	1.2	403.6	1850.0	1.2	79.0
YBR031W			7344_i_at	YBR031W	903.3	-1.5	411.8	903.3	-1.2	172.4	1175.7	1.5	536.0	1175.7	1.3	401.6
YLR048W			10301_at	YLR048W	2484.7	-1.2	533.5	2484.7	-1.1	159.3	3017.4	1.2	647.8	3017.4	1.1	399.4
YNL162W			9006_s_at	YNL162W	1629.0	-1.2	325.8	1629.0	-1.0	79.5	1986.6	1.2	397.3	1986.6	1.2	397.3
YLR287C			10047_f_at	YLR287C F	3167.8	-1.2	788.8	3167.8	-1.0	154.6	3855.8	1.2	960.1	3855.8	1.1	385.6
YML073C			9726_at	YML073C I	1002.1	-1.2	249.5	1002.1	1.0	48.9	1281.1	1.2	319.0	1281.1	1.3	384.3
YLR406C			9897_f_at	YLR406C F	1821.9	-1.2	453.6	1821.9	-1.1	147.9	2302.1	1.2	573.2	2302.1	1.2	381.4
YDR418W			6069_i_at	YDR418W	1443.6	-1.4	524.7	1443.6	-1.1	199.9	1942.5	1.4	706.0	1942.5	1.2	380.4
YDL083C			6559_f_at	YDL083C F	1335.4	-1.2	299.5	1335.4	-1.1	44.6	1641.7	1.2	368.2	1641.7	1.0	18.0
YOR182C			8384_f_at	YOR182C	2871.4	-1.2	715.0	2871.4	-1.1	274.1	3570.6	1.2	889.1	3570.6	1.1	357.1
YMR116C	*ASC1*	*CPC2*	9512_at	YMR116C	2863.4	-1.2	572.7	2863.4	-1.1	286.3	3505.4	1.2	701.1	3505.4	1.1	350.5
YLR388W			9923_f_at	YLR388W	2857.6	-1.1	425.5	2857.6	-1.0	139.5	3370.6	1.1	501.9	3370.6	1.1	337.1
YDR012W			6473_i_at	YDR012W	1178.7	-1.4	529.4	1178.7	-1.2	293.5	1664.0	1.4	747.4	1664.0	1.2	332.8
YNL069C			8918_at	YNL069C F	2660.9	-1.2	662.6	2660.9	-1.1	396.2	3325.4	1.2	828.0	3325.4	1.1	332.5
YLL045C			10386_s_a	YLL045C F	1659.8	-1.2	289.1	1659.8	-1.1	26.4	1906.1	1.2	332.0	1906.1	1.0	14.8
YBR191W			7147_at	YBR191W	2492.3	-1.3	738.1	2492.3	-1.2	488.1	3246.8	1.3	961.5	3246.8	1.1	324.7
YER074W			5647_s_at	YER074W	1173.0	-1.3	409.4	1173.0	-1.1	174.7	1562.3	1.3	545.4	1562.3	1.2	312.5
YER056C	*FCY2*	*BRA7*	5668_at	YER056C p	1819.5	-1.1	238.3	1819.5	1.0	19.9	2117.5	1.1	277.4	2117.5	1.1	311.1
YOL039W			8613_at	YOL039W	2361.0	-1.2	587.9	2361.0	-1.2	472.2	2988.9	1.2	744.2	2988.9	1.1	298.9
YDR500C			5969_at	YDR500C (	2208.7	-1.3	654.1	2208.7	-1.1	328.9	2914.7	1.3	863.2	2914.7	1.1	291.5
YML024W			9686_s_at	YML024W	2444.4	-1.1	364.0	2444.4	-1.0	119.3	2901.7	1.1	432.1	2901.7	1.1	290.2
YBL072C			7425_s_at	YBL072C F	2290.7	-1.2	570.4	2290.7	-1.1	341.1	2779.9	1.2	692.2	2779.9	1.1	278.0
YOR369C			8168_at	YOR369C	2272.3	-1.2	445.0	2272.3	-1.0	110.9	2671.6	1.2	523.2	2671.6	1.1	267.2
YGL123W			5119_at	YGL123W	2085.1	-1.2	519.2	2085.1	-1.1	310.5	2617.9	1.2	651.9	2617.9	1.1	261.8
YMR194W			9420_i_at	YMR194W	2154.9	-1.2	527.9	2154.9	-1.2	375.3	2714.2	1.2	664.9	2714.2	1.1	259.1
YNL302C			9182_s_at	YNL302C F	2205.2	-1.1	328.4	2205.2	-1.0	107.6	2531.0	1.1	376.9	2531.0	1.1	253.1
YNL096C			8981_at	YNL096C F	1165.4	-1.0	56.9	1165.4	1.1	173.5	1260.1	1.0	61.5	1260.1	1.2	252.0
YLR185W			10166_at	YLR185W	1831.8	-1.3	639.4	1831.8	-1.2	366.4	2462.9	1.3	859.7	2462.9	1.1	246.3
YPL220W			7984_s_at	YPL220W	2002.5	-1.2	498.6	2002.5	-1.1	298.2	2442.9	1.2	608.3	2442.9	1.1	244.3
RDN5-3			3770_i_at	RDN5-3 5S	99.6	-5.9	485.9	99.6	-4.2	322.3	580.0	5.9	2829.7	580.0	1.4	232.0
YOL120C			8668_at	YOL120C I	1845.5	-1.2	459.5	1845.5	-1.2	361.4	2308.9	1.2	574.9	2308.9	1.1	230.9
YMR286W			9335_at	YMR286W	496.5	1.0	24.2	496.5	1.6	296.3	456.1	-1.0	22.3	456.1	1.5	228.1
YPL143W			7882_f_at	YPL143W	1752.7	-1.2	436.4	1752.7	-1.1	261.0	2153.6	1.2	536.2	2153.6	1.1	215.4
YKR094C			10474_s_a	YKR094C I	1812.2	-1.2	358.7	1812.2	-1.1	266.2	2208.2	1.2	437.0	2208.2	1.1	210.8
YIL148W			4253_i_at	YIL148W F	822.0	-1.2	198.1	822.0	-1.0	0.0	1017.9	1.2	245.3	1017.9	1.2	203.6
YOR167C			8369_f_at	YOR167C	1763.8	-1.2	352.8	1763.8	-1.0	38.8	2079.9	1.2	416.0	2079.9	1.1	198.5
YJL189W			11237_at	YJL189W F	1683.5	-1.2	336.7	1683.5	-1.1	168.3	1974.3	1.2	394.9	1974.3	1.1	197.4
YGL103W			5094_at	YGL103W	1673.9	-1.2	334.8	1673.9	-1.1	167.4	1962.7	1.2	392.5	1962.7	1.1	196.3

YOR096W			8479_at	YOR096W	1643.4	-1.1	244.7	1643.4	-1.0	80.2	1915.7	1.1	285.3	1915.7	1.1	191.6
YPR102C			7632_i_at	YPR102C I	1550.4	-1.2	310.1	1550.4	-1.1	155.0	1849.3	1.2	369.9	1849.3	1.1	184.9
YCR031C			6866_at	YCR031C	1455.3	-1.2	362.4	1455.3	-1.1	216.7	1839.9	1.2	458.1	1839.9	1.1	184.0
YOL127W			8662_at	YOL127W	1394.8	-1.2	347.3	1394.8	-1.1	139.5	1828.8	1.2	455.4	1828.8	1.1	182.9
YPL090C			7845_s_at	YPL090C F	1431.8	-1.2	286.4	1431.8	-1.1	143.2	1768.0	1.2	353.6	1768.0	1.1	176.8
YFR032C			5331_at	YFR032C I	1564.0	-1.2	389.4	1564.0	-1.1	232.9	1715.2	1.2	427.1	1715.2	1.1	171.5
YMR142C			9496_at	YMR142C	1229.5	-1.3	429.2	1229.5	-1.2	306.1	1708.6	1.3	596.4	1708.6	1.1	170.9
YDR471W			5985_at	YDR471W	1396.6	-1.2	279.3	1396.6	-1.1	208.0	1697.3	1.2	339.5	1697.3	1.1	169.7
YGR214W			4780_at	YGR214W	1457.7	-1.2	285.4	1457.7	-1.1	217.1	1570.5	1.2	307.5	1570.5	1.1	157.1
YDL130W	*RPP1B*	*RPL44', RI*	6600_at	YDL130W	1812.2	-1.0	89.9	1812.2	-1.0	11.0	2258.3	1.3	635.7	2258.3	1.1	148.1
YDL184C			6680_s_at	YDL184C F	1060.2	-1.3	318.1	1060.2	-1.2	212.0	1389.1	1.3	416.7	1389.1	1.1	138.9
YOL077C	*BRX1*		8621_at	YOL077C I	485.6	-1.0	0.0	485.6	1.1	48.6	508.0	-1.0	0.0	508.0	1.2	101.6
Q0325			3979_i_at	mitochondr	2.4	1.5	1.1	2.4	-2.4	3.4	0.8	-1.5	0.4	0.8	-2.9	1.5
YGL068W			5084_at	YGL068W	0.0	0.0	0.0	0.0	0.0	0.0	283.9	-1.2	56.8	283.9	-1.4	113.6
YNR036C			8839_at	YNR036C s	0.0	0.0	0.0	0.0	0.0	0.0	590.3	-1.1	87.9	590.3	-1.3	177.1
YGL076C			5075_i_at	YGL076C I	1397.4	-1.0	68.2	1397.4	-1.3	380.6	1462.8	1.0	71.4	1462.8	-1.1	217.8
YBL087C			7454_s_at	YBL087C F	2647.6	-1.1	394.3	2647.6	-1.2	659.3	2964.6	1.1	441.5	2964.6	-1.1	296.5
YML026C			9684_s_at	YML026C I	2948.1	-1.1	439.0	2948.1	-1.2	734.1	3458.0	1.1	514.9	3458.0	-1.1	345.8
YBL027W			7378_s_at	YBL027W	1885.8	-1.2	377.2	1885.8	-1.3	658.3	2238.6	1.2	447.7	2238.6	-1.2	447.7
YGL224C	*SDT1*	*SSM1*	5246_at	YGL224C s	264.2	1.5	131.2	264.2	2.0	263.5	0.0	0.0	0.0	0.0	0.0	0.0
YGL125W	*MET13*	*MET11*	5117_at	YGL125W	579.3	1.1	86.3	579.3	1.3	173.8	0.0	0.0	0.0	0.0	0.0	0.0
YBR282W			7100_at	YBR282W	266.4	1.3	91.0	266.4	1.5	144.6	0.0	0.0	0.0	0.0	0.0	0.0
YBR146W			7237_at	YBR146W	214.7	1.2	42.9	214.7	1.5	107.3	0.0	0.0	0.0	0.0	0.0	0.0
YOR158W	*PET123*		8405_at	YOR158W	210.3	1.2	42.1	210.3	1.5	105.2	0.0	0.0	0.0	0.0	0.0	0.0
YFL034C			5396_at	YFL034C F	503.0	1.2	100.6	503.0	1.2	100.6	0.0	0.0	0.0	0.0	0.0	0.0
YDR405W			6056_at	YDR405W	27.5	1.3	9.6	27.5	2.3	35.5	0.0	0.0	0.0	0.0	0.0	0.0
RDN18-1			3767_s_at	RDN18-1 1	0.8	-21.7	16.5	0.8	-13.4	10.0	61.1	21.7	1263.5	61.1	1.6	36.7
YBR189W			7191_f_at	YBR189W	1255.6	-1.2	312.6	1255.6	-1.1	46.6	1693.8	1.2	421.8	1693.8	1.1	42.2
YKL156W			10717_at	YKL156W -	1167.3	-1.1	173.8	1167.3	-1.1	116.7	1337.0	1.1	199.1	1337.0	1.0	0.0
YNL255C	*GIS2*		9139_at	YNL255C (	604.0	-1.1	57.6	604.0	-1.2	118.3	0.0	0.0	0.0	0.0	0.0	0.0
YER131W			5571_at	YER131W	475.1	-1.2	93.0	475.1	-1.3	135.2	563.2	1.2	110.3	563.2	-1.0	0.0
YKL009W	*MRT4*		10598_at	YKL009W	766.7	-1.1	76.7	766.7	-1.2	153.3	0.0	0.0	0.0	0.0	0.0	0.0
YPL081W			7854_i_at	YPL081W	563.9	-1.3	163.7	563.9	-1.3	176.7	717.0	1.3	208.2	717.0	-1.0	35.0
YBR048W			7316_s_at	YBR048W	1977.1	-1.1	294.4	1977.1	-1.1	188.7	2248.8	1.1	334.9	2248.8	1.0	0.0
YHL015W			4547_at	YHL015W	1285.1	-1.2	251.7	1285.1	-1.1	191.4	1504.1	1.2	294.5	1504.1	1.0	0.0
YPR132W			7619_s_at	YPR132W	2790.2	-1.1	415.5	2790.2	-1.1	200.5	3197.7	1.1	476.2	3197.7	1.0	156.1
YOR234C			8300_f_at	YOR234C	1443.6	-1.2	288.7	1443.6	-1.1	215.0	1742.0	1.2	348.4	1742.0	1.0	0.0
YJR123W			10916_at	YJR123W	2208.1	-1.1	328.8	2208.1	-1.1	220.8	2589.4	1.1	385.6	2589.4	1.0	0.0
YDL061C			6537_f_at	YDL061C F	1608.4	-1.2	321.7	1608.4	-1.1	239.5	2021.8	1.2	404.4	2021.8	1.0	0.0
YLR325C			9994_at	YLR325C F	2428.1	-1.1	361.6	2428.1	-1.1	242.8	2763.9	1.1	411.6	2763.9	1.0	0.0
YLR264W			10068_i_at	YLR264W	2584.2	-1.1	384.8	2584.2	-1.1	252.5	2952.9	1.1	439.7	2952.9	1.0	144.1
YNL067W			8921_s_at	YNL067W	1726.3	-1.0	84.3	1726.3	-1.1	257.1	0.0	0.0	0.0	0.0	0.0	0.0
YOR063W			8491_at	YOR063W	1309.3	-1.1	130.9	1309.3	-1.2	261.9	0.0	0.0	0.0	0.0	0.0	0.0
YPL249C			8000_i_at	YPL249C F	907.7	-1.3	268.8	907.7	-1.3	268.8	1184.0	1.3	350.6	1184.0	1.0	0.0
YJL191W			11235_at	YJL191W F	642.2	-1.4	288.4	642.2	-1.4	288.4	945.5	1.4	424.7	945.5	1.0	0.0
YLR448W			9852_at	YLR448W	1941.5	-1.1	194.2	1941.5	-1.1	289.1	0.0	0.0	0.0	0.0	0.0	0.0
YBL092W			7448_at	YBL092W	1463.1	-1.2	292.6	1463.1	-1.2	292.6	1879.3	1.2	375.9	1879.3	1.0	0.0
YDL191W			6672_s_at	YDL191W	1556.5	-1.2	304.8	1556.5	-1.2	304.8	1877.9	1.2	367.7	1877.9	-1.0	0.0
RDN37-1			3807_s_at	RDN37-1 3	89.7	-5.0	356.7	89.7	-4.5	309.7	238.4	5.0	947.9	238.4	1.2	36.4
YGR118W			4909_f_at	YGR118W	2127.7	-1.1	316.8	2127.7	-1.1	316.8	2514.3	1.1	374.4	2514.3	1.0	0.0
YDR064W			6433_at	YDR064W	1722.3	-1.2	344.5	1722.3	-1.2	344.5	2085.3	1.2	417.1	2085.3	1.0	0.0

	YOL040C			8612_at	YOL040C 4	1952.9	-1.2	390.6	1952.9	-1.2	390.6	2270.9	1.2	454.2	2270.9	-1.0	0.0	
	YHL033C			4575_i_at	YHL033C F	1955.4	-1.1	195.5	1955.4	-1.2	391.1	0.0	0.0	0.0	0.0	0.0	0.0	
	YPL131W			7894_at	YPL131W	2684.5	-1.2	536.9	2684.5	-1.1	399.7	3209.3	1.2	641.9	3209.3	1.0	0.0	
	YDL081C			6561_at	YDL081C /	2074.7	-1.2	499.9	2074.7	-1.2	406.3	2727.7	1.2	657.3	2727.7	1.0	0.0	
	YJL177W			11205_i_at	YJL177W F	2100.3	-1.2	523.0	2100.3	-1.2	420.1	2639.5	-1.2	657.2	2639.5	1.0	128.8	
	YDL082W			6560_at	YDL082W	1468.9	-1.2	365.8	1468.9	-1.3	435.0	1816.6	1.2	452.3	1816.6	-1.0	0.0	
	YLR029C			10325_at	YLR029C F	1856.7	-1.2	416.4	1856.7	-1.3	505.7	2276.3	1.2	510.5	2276.3	-1.0	111.1	
	YIL133C			4223_at	YIL133C R	2311.2	-1.2	575.5	2311.2	-1.2	575.5	2889.0	1.2	719.4	2889.0	1.0	0.0	
	YJL190C			11236_at	YJL190C F	3932.3	-1.1	585.6	3932.3	-1.1	585.6	4680.2	1.1	696.9	4680.2	1.0	0.0	
	YKR057W			10529_i_at	YKR057W	4839.1	-1.2	947.6	4839.1	-1.2	947.6	5831.4	1.2	1141.9	5831.4	1.0	0.0	
	RDN25-1			3765_s_at	RDN25-1 1	58.5	-17.2	946.6	58.5	-19.2	1062.1	602.7	17.2	9755.3	602.7	-1.0	22.0	
	YGR085C			4921_i_at	YGR085C	3199.3	-1.2	796.6	3199.3	-1.4	1268.3	3846.1	1.2	957.7	3846.1	-1.2	769.2	
translation																		
	YPR080W	*TEF1*		7656_s_at	YPR080W	2055.9	-1.3	608.9	2055.9	-1.1	196.2	2619.5	1.3	775.8	2619.5	1.2	523.9	
	YMR116C	*ASC1*	*CPC2*	9512_at	YMR116C	2863.4	-1.2	572.7	2863.4	-1.1	286.3	3505.4	1.2	701.1	3505.4	1.1	350.5	
	YEL034W	*HYP2*	*TIF51A*	5756_at	YEL034W	2636.4	-1.2	656.5	2636.4	-1.1	392.6	3320.4	1.2	826.8	3320.4	1.1	332.0	
	YJL138C	*TIF2*		11156_s_a	YJL138C tı	1972.0	-1.1	293.7	1972.0	-1.0	96.3	2291.9	1.1	341.3	2291.9	1.1	229.2	
	YKL081W	*TEF4*	*EFC1*	10658_at	YKL081W	389.0	-1.3	130.3	389.0	1.2	74.6	471.5	1.3	157.9	471.5	1.3	134.1	
	YJR047C	*ANB1*	*HYP1, TIF*	10976_at	YJR047C a	180.8	-1.8	153.6	180.8	-1.6	108.5	332.5	1.8	282.4	332.5	1.2	66.5	
	YNL014W	*HEF3*	*ZRG7*	8881_at	Translation	3.5	1.5	1.8	3.5	-2.4	4.9	-1.9	-1.5	-1.0	-1.9	-3.7	-5.1	
	YPL226W	*NEW1*		7978_at	YPL226W	453.2	-1.1	67.5	453.2	-1.3	158.2	519.5	1.1	77.4	519.5	-1.2	103.9	
	YOR187W	*TUF1*		8343_at	YOR187W	687.5	1.2	137.5	687.5	-1.0	0.0	586.9	-1.2	117.4	586.9	-1.2	117.4	
	YMR146C	*TIF34*		9501_at	YMR146C	380.6	-1.0	18.6	380.6	-1.4	170.9	385.5	1.0	18.8	385.5	-1.4	154.2	
	YBR143C	*SUP45*	*SAL4, SUF*	7234_at	YBR143C	569.1	-1.0	0.0	569.1	-1.4	227.6	583.2	-1.0	0.0	583.2	-1.4	233.3	
	YOR204W	*DED1*	*SPP81*	8361_at	YOR204W	325.4	1.8	260.3	325.4	1.3	97.6	169.7	-1.8	135.8	169.7	-1.3	50.9	
	YNL007C	*SIS1*		8888_at	YNL007C s	635.8	1.2	127.2	635.8	1.1	63.6	519.0	-1.2	103.8	519.0	-1.1	51.9	
	YMR260C	*TIF11*		9354_at	YMR260C	392.1	-1.3	136.9	392.1	-1.2	97.6	539.5	1.3	188.3	539.5	1.1	54.0	
	YOR276W	*CAF20*	*CAF2, CAI*	8252_at	YOR276W	722.3	-1.1	72.2	722.3	-1.2	179.8	0.0	0.0	0.0	0.0	0.0	0.0	
	YNL244C	*SUI1*	*MOF2, RF*	9105_at	YNL244C t	939.7	-1.1	94.0	939.7	-1.2	187.9	0.0	0.0	0.0	0.0	0.0	0.0	
	YOL139C	*CDC33*	*TIF45*	8695_at	YOL139C ı	1080.1	-1.1	108.0	1080.1	-1.2	216.0	0.0	0.0	0.0	0.0	0.0	0.0	
	YOR133W	*EFT1*		8425_s_at	YOR133W	1558.5	-1.1	232.1	1558.5	-1.2	388.1	1852.7	1.1	275.9	1852.7	-1.0	0.0	
	YDL081C			6561_at	YDL081C /	2074.7	-1.2	499.9	2074.7	-1.2	406.3	2727.7	1.2	657.3	2727.7	1.0	0.0	
	YLR249W	*YEF3*	*TEF3*	10097_at	YLR249W	2805.7	-1.1	417.8	2805.7	-1.1	417.8	3188.9	1.1	474.9	3188.9	1.0	0.0	
	initiation																	
		YKL204W	*EAP1*		10762_at	YKL204W	56.4	1.1	7.9	56.4	2.9	104.7	48.1	-1.1	6.7	48.1	2.4	67.3
	elongation																	
		YAL003W	*EFB1*	*TEF5*	11320_at	YAL003W	1673.1	-1.2	331.1	1673.1	-1.1	203.9	2018.1	1.2	399.4	2018.1	1.0	98.5
translational control																		
	YLR203C	*MSS51*		10141_at	YLR203C F	244.8	1.7	171.3	244.8	1.3	85.4	144.6	-1.7	101.2	144.6	-1.3	43.4	
	YER133W	*GLC7*	*CID1, DIS2*	5573_at	protein pho	242.7	1.2	40.7	242.7	1.3	84.2	217.2	-1.2	36.4	217.2	1.1	30.4	
	YGR222W	*PET54*		4788_at	YGR222W	0.0	0.0	0.0	0.0	0.0	0.0	136.2	-1.4	54.0	136.2	-1.7	95.3	
aminoacyl-tRNA-synthetases																		
	YCR024C	*PMP1*		6858_f_at	YCR024C	897.8	-1.3	313.4	897.8	-1.0	0.0	1190.8	1.3	415.7	1190.8	1.3	357.2	
	YHR020W	*??*		4493_at	YHR020W	644.3	-1.2	160.4	644.3	-1.1	64.4	807.4	1.2	201.0	807.4	1.1	80.7	
	YDR037W	*KRS1*	*GCD5*	6452_at	YDR037W	756.0	-1.2	148.0	756.0	-1.0	36.9	901.7	1.2	176.6	901.7	1.1	90.2	
	YNL073W	*MSK1*		8914_at	YNL073W	54.4	1.2	10.9	54.4	2.9	103.3	50.6	-1.2	10.1	50.6	2.4	70.8	
	YLR060W	*FRS1*		10269_at	YLR060W	1379.2	-1.1	205.4	1379.2	-1.2	275.8	1572.9	1.1	234.2	1572.9	-1.1	157.3	
	YPR033C	*HTS1*	*TSM4572*	7741_at	YPR033C (	720.9	-1.1	72.1	720.9	-1.3	216.3	795.2	1.1	79.5	795.2	-1.2	159.0	
	YLL018C	*DPS1*		9868_at	YLL018C a	496.3	1.7	328.2	496.3	1.9	433.5	408.8	-1.7	270.3	408.8	1.1	60.9	
	YBL076C	*ILS1*		7421_at	YBL076C (	367.9	-1.1	35.1	367.9	-1.3	109.0	0.0	0.0	0.0	0.0	0.0	0.0	

	YIL078W	*THS1*		4186_at	YIL078W T	799.3	-1.1	79.9	799.3	-1.1	119.0	0.0	0.0	0.0	0.0	0.0	0.0
	YBR121C	*GRS1*		7257_at	YBR121C (	709.4	-1.2	141.9	709.4	-1.2	141.9	776.5	1.2	155.3	776.5	1.0	0.0
	YOR335C	*ALA1*		8225_at	YOR335C	579.8	-1.2	144.4	579.8	-1.2	144.4	722.2	1.2	179.8	722.2	-1.0	0.0
	YGL245W	*??*		5268_at	YGL245W	569.8	-1.2	114.0	569.8	-1.3	170.9	669.4	1.2	133.9	669.4	-1.1	66.9
tRNAs																	
	TQ(UUG)D1			3436_f_at	TQ(UUG)D	204.9	-1.7	153.5	204.9	-1.4	92.0	360.4	1.7	270.0	360.4	1.2	72.1
	TQ(UUG)E1			3376_f_at	TQ(UUG)E	270.3	-1.6	162.2	270.3	-1.5	135.1	431.0	1.6	258.6	431.0	1.1	43.1
	TQ(UUG)D2			3439_f_at	TQ(UUG)D	225.3	-1.6	146.2	225.3	-1.3	78.6	373.1	1.6	242.2	373.1	1.2	74.6
	TA(AGC)D			3473_f_at	TA(AGC)D	245.5	-1.6	147.3	245.5	-1.3	73.6	373.8	1.6	224.3	373.8	1.2	74.8
	TS(UGA)E			3378_f_at	TS(UGA)E	99.4	-2.2	119.7	99.4	-1.4	42.6	178.0	2.2	214.4	178.0	1.5	89.0
	TA(AGC)J			3885_f_at	TA(AGC)J	234.2	-1.6	140.5	234.2	-1.3	70.3	352.3	1.6	211.4	352.3	1.2	70.5
	TA(AGC)K2			3830_f_at	TA(AGC)K	289.6	-1.4	130.0	289.6	-1.2	72.1	412.7	1.4	185.4	412.7	1.2	82.5
	TG(GCC)D2			3382_f_at	TG(GCC)D	0.0	0.0	0.0	0.0	0.0	0.0	105.4	2.5	158.5	105.4	1.5	52.7
	TA(AGC)M2			3688_f_at	TA(AGC)M	290.1	-1.4	116.0	290.1	-1.2	58.0	369.4	1.4	147.8	369.4	1.1	36.9
	TG(GCC)E			3358_f_at	TG(GCC)E	0.0	0.0	0.0	0.0	0.0	0.0	93.1	2.5	143.3	93.1	1.1	9.3
	TA(AGC)P			3537_f_at	TA(AGC)P	273.5	-1.5	150.2	273.5	-1.1	40.7	418.5	1.5	229.8	418.5	1.3	125.6
	TA(AGC)L			3743_f_at	TA(AGC)L	0.0	0.0	0.0	0.0	0.0	0.0	350.1	1.3	122.2	350.1	1.2	70.0
	TA(AGC)M1			3710_f_at	TA(AGC)M	241.3	-1.6	144.8	241.3	-1.2	60.1	355.7	1.6	213.4	355.7	1.3	106.7
	TA(AGC)K1			3819_f_at	TA(AGC)K	236.6	-1.5	129.9	236.6	-1.2	47.3	344.3	1.5	189.1	344.3	1.3	103.3
	TA(UGC)L			3796_f_at	TA(UGC)L	0.0	0.0	0.0	0.0	0.0	0.0	144.9	1.7	101.4	144.9	1.3	43.5
	TA(UGC)O			3574_f_at	TA(UGC)O	0.0	0.0	0.0	0.0	0.0	0.0	150.5	1.6	90.3	150.5	1.4	60.2
	TG(UCC)O			3633_f_at	TG(UCC)C	0.0	0.0	0.0	0.0	0.0	0.0	87.2	1.8	73.6	87.2	1.2	17.4
	TR(CCG)L			3760_at	TR(CCG)L	0.0	0.0	0.0	0.0	0.0	0.0	69.9	1.9	61.2	69.9	1.9	62.9
	TP(AGG)C			3451_f_at	tRNA-Pro	4.3	-9.6	36.8	4.3	-4.6	15.6	50.6	9.6	433.5	50.6	2.1	55.7
	TG(CCC)D			3397_f_at	TG(CCC)D	8.1	-1.3	2.7	8.1	-2.4	11.5	5.6	1.3	1.8	5.6	-2.7	9.5
	TG(CCC)O			3604_f_at	TG(CCC)C	10.1	-1.1	1.1	10.1	-2.9	19.6	10.8	1.1	1.2	10.8	-2.3	14.0
	TI(AAU)L1			3753_s_at	TI(AAU)L1	9.4	-2.3	12.4	9.4	-2.0	9.6	21.6	2.3	28.6	21.6	1.1	2.2
	TT(AGU)J			3929_s_at	TT(AGU)J	6.6	-1.9	5.8	6.6	-2.6	10.6	0.0	0.0	0.0	0.0	0.0	0.0
	TP(AGG)N			3668_f_at	tRNA-Pro	7.6	-8.9	59.8	7.6	-5.0	30.5	64.9	8.9	510.3	64.9	1.8	51.9
	TG(GCC)O2			3645_f_at	tRNA-Gly	9.9	-2.1	11.4	9.9	-4.1	30.6	73.7	2.1	84.7	73.7	-1.1	7.4
	TG(GCC)B			3508_f_at	tRNA-Gly	17.8	-5.5	80.5	17.8	-2.9	34.4	58.5	5.5	265.4	58.5	1.9	52.7
	TG(GCC)O1			3640_f_at	TG(GCC)C	42.3	-1.9	38.4	42.3	-2.1	46.4	130.0	1.9	118.0	130.0	1.3	39.0
	TG(GCC)J2			3842_f_at	TG(GCC)J	30.3	-1.7	20.4	30.3	-2.9	57.8	135.3	1.7	91.1	135.3	1.2	27.1
	TG(GCC)J1			3869_f_at	tRNA-Gly	27.5	-2.2	34.2	27.5	-3.3	63.7	111.5	2.2	138.8	111.5	1.4	44.6
	TS(UGA)P			3562_f_at	TS(UGA)P	65.9	-2.8	118.1	65.9	-2.0	66.9	172.3	2.8	308.9	172.3	1.4	68.9
	TL(UAA)J	*SUP51*	*SUP52*	3878_s_at	tRNA-Leu /	31.2	-3.3	71.3	31.2	-3.3	72.4	90.2	3.3	206.2	90.2	-1.1	9.0
	TG(GCC)D1			3472_f_at	TG(GCC)D	47.7	-1.8	40.0	47.7	-2.5	72.9	119.5	1.8	100.2	119.5	1.4	47.8
	TL(GAG)G			3243_at	TL(GAG)G	84.4	-2.0	87.3	84.4	-2.0	87.3	171.0	2.0	176.9	171.0	1.0	0.0
	TQ(UUG)E2			3364_f_at	TQ(UUG)E	235.6	-1.7	164.2	235.6	-1.4	93.4	395.0	1.7	275.3	395.0	1.2	79.0
	TQ(UUG)L			3804_f_at	TQ(UUG)L	217.0	-1.6	140.9	217.0	-1.5	108.5	350.4	1.6	227.5	350.4	1.1	35.0
	TG(GCC)P1			3561_f_at	tRNA-Gly	32.7	-2.0	33.4	32.7	-4.4	111.9	119.9	2.0	122.3	119.9	1.2	24.0
	TD(GUC)J1			3905_s_at	TD(GUC)J	235.1	-1.4	105.6	235.1	-1.5	129.1	338.7	1.4	152.1	338.7	-1.1	33.9
	TE(CUC)D			3383_f_at	TE(CUC)D	176.7	-1.6	114.7	176.7	-1.7	132.4	285.4	1.6	185.3	285.4	-1.1	28.5
	TQ(UUG)C			3462_f_at	TQ(UUG)C	272.7	-1.6	163.6	272.7	-1.5	136.3	432.5	1.6	259.5	432.5	1.1	43.3
	TQ(UUG)B			3478_f_at	TQ(UUG)B	328.3	-1.6	213.1	328.3	-1.4	147.5	531.9	1.6	345.3	531.9	1.1	53.2
	TQ(UUG)D3			3405_f_at	TQ(UUG)D	302.1	-1.5	165.9	302.1	-1.5	151.0	472.8	1.5	259.7	472.8	1.0	0.0
	TE(UUC)K			3813_f_at	TE(UUC)K	362.8	-1.5	199.2	362.8	-1.4	162.9	559.5	1.5	307.3	559.5	1.0	0.0
	TE(UUC)P			3594_f_at	TE(UUC)P	418.9	-1.5	208.0	418.9	-1.4	166.0	621.0	1.5	308.4	621.0	1.1	62.1
	TR(ACG)O			3598_f_at	TR(ACG)O	95.7	-2.4	138.6	95.7	-2.7	167.3	261.5	2.4	379.0	261.5	-1.1	26.2
	TR(ACG)L			3803_f_at	TR(ACG)L	93.8	-2.6	150.1	93.8	-2.9	178.2	266.1	2.6	425.8	266.1	-1.1	26.6

TR(ACG)D			3438_f_at	TR(ACG)D	98.0	-2.3	131.8	98.0	-2.8	180.9	257.2	2.3	346.0	257.2	-1.2	51.4
TR(ACG)J			3906_f_at	tRNA-Arg	85.0	-2.8	157.1	85.0	-3.1	182.6	240.8	2.8	445.4	240.8	-1.1	24.1
TE(UUC)J			3884_f_at	TE(UUC)J	375.0	-1.5	205.9	375.0	-1.5	187.5	578.6	1.5	317.8	578.6	1.0	0.0
TE(UUC)C			3491_f_at	TE(UUC)C	383.3	-1.6	230.0	383.3	-1.5	191.7	605.3	1.6	363.2	605.3	1.0	0.0
TE(UUC)L			3758_f_at	TE(UUC)L	437.9	-1.4	193.6	437.9	-1.4	193.6	629.7	1.4	278.5	629.7	1.0	0.0
TE(UUC)E1			3371_f_at	TE(UUC)E	400.5	-1.4	177.1	400.5	-1.5	198.9	577.7	1.4	255.5	577.7	-1.0	0.0
TE(UUC)B			3482_f_at	TE(UUC)B	414.4	-1.6	248.6	414.4	-1.5	207.2	659.3	1.6	395.6	659.3	1.1	65.9
TR(ACG)K			3827_f_at	TR(ACG)K	124.2	-2.3	160.3	124.2	-2.7	209.0	285.9	2.3	369.2	285.9	-1.2	57.2
TE(UUC)M			3709_f_at	TE(UUC)M	384.2	-1.5	211.0	384.2	-1.5	211.0	598.0	1.5	328.4	598.0	1.0	0.0
other protein-synthesis activities																
YDL219W	*DTD1*		6689_at	YDL219W	284.5	-1.1	21.1	284.5	2.4	395.4	309.6	1.1	22.9	309.6	2.5	478.4
YLL039C	*UBI4*	*SCD2*	10392_at	YLL039C u	432.6	1.3	151.0	432.6	-1.1	64.4	321.2	-1.3	112.1	321.2	-1.5	160.6
YNL209W	*SSB2*		9094_s_at	YNL209W	2100.0	-1.1	312.7	2100.0	-1.1	312.7	2488.7	1.1	370.6	2488.7	1.0	0.0
PROTEIN FATE (folding, modification, destination)																
protein folding and stabilization																
YJL034W	*KAR2*	*BIP, GRP7*	11078_at	Homologue	1978.5	1.1	188.8	1978.5	1.2	492.6	1802.2	-1.1	172.0	1802.2	1.2	360.4
YDR188W	*CCT6*	*HTR3, TCI*	6290_at	YDR188W	379.4	1.3	132.4	379.4	1.2	75.9	0.0	0.0	0.0	0.0	0.0	0.0
YLR090W	*XDJ1*		10254_at	YLR090W	106.3	1.5	53.2	106.3	2.2	132.3	0.0	0.0	0.0	0.0	0.0	0.0
YFL016C	*MDJ1*		5370_at	YFL016C D	158.6	1.6	95.2	158.6	1.7	111.0	108.1	-1.6	64.9	108.1	1.1	10.8
YKL073W	*LHS1*	*CER1, SSI*	10668_at	YKL073W	144.8	1.6	94.0	144.8	1.2	36.0	77.9	-1.6	50.6	77.9	-1.3	23.4
YMR154C	*RIM13*	*CPL1*	9465_at	YMR154C	8.5	2.1	9.5	8.5	-1.0	0.0	2.7	-2.1	3.0	2.7	-1.9	2.4
YNL328C	*MDJ2*		9202_at	YNL328C F	0.0	0.0	0.0	0.0	0.0	0.0	4.5	-1.7	3.3	4.5	-2.5	6.8
YMR161W	*HLJ1*		9473_at	YMR161W	124.7	2.0	126.6	124.7	1.2	22.8	96.8	-2.0	98.2	96.8	-2.1	106.5
YDR214W	*AHA1*		6271_at	YDR214W	0.0	0.0	0.0	0.0	0.0	0.0	295.8	-1.1	28.2	295.8	-1.4	118.3
YDR304C	*CPR5*	*CYP5*	6178_at	YDR304C	962.7	-1.1	96.3	962.7	-1.1	143.4	0.0	0.0	0.0	0.0	0.0	0.0
YDR155C	*CPR1*	*CYP1, CPI*	6301_at	YDR155C (	1671.5	-1.2	327.3	1671.5	-1.1	159.5	0.0	0.0	0.0	0.0	0.0	0.0
YDR212W	*TCP1*	*CCT1*	6269_at	YDR212W	560.2	-1.2	112.0	560.2	-1.4	224.1	677.8	1.2	135.6	677.8	-1.1	67.8
YLR259C	*HSP60*	*CPN60, MI*	10061_at	YLR259C I	1822.6	-1.1	182.3	1822.6	-1.1	271.4	0.0	0.0	0.0	0.0	0.0	0.0
YLL024C	*SSA2*		10362_at	YLL024C n	1750.5	-1.3	525.2	1750.5	-1.4	700.2	2199.2	1.3	659.8	2199.2	-1.1	219.9
YAL005C	*SSA1*	*YG100*	11315_i_at	YAL005C I	2682.4	-1.0	127.0	2682.4	-1.1	391.6	2804.4	1.0	132.8	2804.4	-1.3	866.8
protein targeting, sorting and translocation																
YJL034W	*KAR2*	*BIP, GRP7*	11078_at	Homologue	1978.5	1.1	188.8	1978.5	1.2	492.6	1802.2	-1.1	172.0	1802.2	1.2	360.4
YNL070W	*TOM7*	*MOM7, YC*	8917_at	YNL070W	828.9	-1.1	123.4	828.9	1.1	123.4	927.8	1.1	138.2	927.8	1.3	278.3
YOR045W	*TOM6*	*ISP6, MOI*	8518_at	YOR045W	1407.9	-1.1	209.6	1407.9	-1.1	140.8	1605.3	1.1	239.0	1605.3	1.1	160.5
YDR086C	*SSS1*		6410_at	YDR086C	557.7	-1.3	165.2	557.7	-1.1	83.0	727.0	1.3	215.3	727.0	1.2	145.4
YGR028W	*MSP1*	*YTA4*	4956_at	YGR028W	231.7	1.5	127.2	231.7	1.2	46.3	147.3	-1.5	80.9	147.3	-1.3	44.2
YLR168C	*MSF1*		10148_at	YLR168C p	269.3	1.4	107.7	269.3	1.4	107.7	0.0	0.0	0.0	0.0	0.0	0.0
YDR432W	*NPL3*	*MTR13, M*	6039_g_at	YDR432W	442.1	1.2	106.8	442.1	1.1	53.2	326.8	-1.2	78.9	326.8	-1.2	54.8
YJL054W	*TIM54*		11058_at	YJL054W T	105.3	1.4	41.7	105.3	2.0	105.0	0.0	0.0	0.0	0.0	0.0	0.0
YKL073W	*LHS1*	*CER1, SSI*	10668_at	YKL073W	144.8	1.6	94.0	144.8	1.2	36.0	77.9	-1.6	50.6	77.9	-1.3	23.4
YLL040C	*VPS13*	*SOI1, VPT*	10391_at	YLL040C v	0.0	0.0	0.0	0.0	0.0	0.0	61.3	-1.3	18.4	61.3	-2.0	61.3
YOR327C	*SNC2*		8217_at	YOR327C	431.6	-1.1	64.3	431.6	-1.2	107.5	0.0	0.0	0.0	0.0	0.0	0.0
YGR181W	*TIM13*		4836_at	YGR181W	412.8	1.0	20.1	412.8	-1.2	102.8	359.6	-1.0	17.6	359.6	-1.3	107.9
YDL212W	*SHR3*	*APF1*	6696_at	YDL212W	1059.4	-1.2	211.9	1059.4	-1.1	157.8	1263.7	1.2	252.7	1263.7	1.0	0.0
YCR075C	*ERS1*		6816_at	YCR075C	185.0	-1.2	33.9	185.0	-1.9	162.0	0.0	0.0	0.0	0.0	0.0	0.0
YBR283C	*SSH1*		7101_at	YBR283C p	700.9	-1.3	244.7	700.9	-1.2	174.5	938.8	1.3	327.7	938.8	1.1	93.9
YNL064C	*YDJ1*	*MAS5*	8924_at	YNL064C y	979.7	1.0	47.8	979.7	-1.1	145.9	959.6	-1.0	46.8	959.6	-1.2	191.9
YMR292W	*GOT1*		9342_at	YMR292W	760.7	-1.4	341.6	760.7	-1.3	228.2	1098.6	1.4	493.4	1098.6	1.1	109.9
YOR270C	*VPH1*		8291_at	YOR270C	1344.7	-1.3	403.4	1344.7	-1.3	469.4	1771.5	1.3	531.5	1771.5	-1.0	0.0

YPL037C	*EGD1*		7806_at	YPL037C (	1962.5	-1.2	392.5	1962.5	-1.3	588.8	2272.2	1.2	454.4	2272.2	-1.1	227.2
YLL024C	*SSA2*		10362_at	YLL024C n	1750.5	-1.3	525.2	1750.5	-1.4	700.2	2199.2	1.3	659.8	2199.2	-1.1	219.9
YAL005C	*SSA1*	*YG100*	11315_i_at	YAL005C H	2682.4	-1.0	127.0	2682.4	-1.1	391.6	2804.4	1.0	132.8	2804.4	-1.3	866.8
protein modification																
YBR082C	*UBC4*		7260_at	YBR082C (	946.7	1.1	94.7	946.7	1.5	473.3	840.4	-1.1	84.0	840.4	1.3	252.1
YNL238W	*KEX2*	*QDS1, VM*	9110_at	Ca2+-depe	189.7	1.2	37.1	189.7	3.0	385.1	156.4	-1.2	30.6	156.4	2.5	234.6
YAL023C	*PMT2*	*FUN25*	11344_at	YAL023C c	1047.0	1.1	104.7	1047.0	1.3	365.5	859.8	-1.1	86.0	859.8	1.2	172.0
YML130C	*ERO1*		9802_at	YML130C (	365.0	2.0	383.0	365.0	1.8	292.0	129.8	-2.0	136.2	129.8	-1.1	13.0
YLR120C	*YPS1*		10193_at	YLR120C (	331.0	1.8	281.1	331.0	1.8	281.1	178.2	-1.8	151.3	178.2	-1.0	0.0
YIR039C	*YPS6*		4075_at	YIR039C G	112.8	1.9	107.0	112.8	2.6	186.0	58.1	-1.9	55.2	58.1	1.4	23.2
YDL095W	*PMT1*		6591_at	YDL095W	415.6	1.4	166.2	415.6	1.4	166.2	299.5	-1.4	119.8	299.5	1.0	0.0
YOR067C	*ALG8*		8495_at	YOR067C	598.7	-1.2	149.1	598.7	-1.0	0.0	730.4	1.2	181.9	730.4	1.2	146.1
YPL154C	*PEP4*	*PHO9, PR*	7916_at	YPL154C v	1033.3	-1.2	206.7	1033.3	-1.1	103.3	1221.5	1.2	244.3	1221.5	1.1	122.2
YNL172W	*APC1*		9041_at	YNL172W	102.1	1.3	34.9	102.1	2.2	121.6	76.1	-1.3	26.0	76.1	1.6	45.7
YLR163C	*MAS1*	*MIF1*	10188_at	YLR163C r	166.2	1.7	115.8	166.2	1.2	41.4	85.4	-1.7	59.5	85.4	-1.3	25.6
YMR246W	*FAA4*		9386_at	YMR246W	383.4	1.3	115.0	383.4	1.3	115.0	0.0	0.0	0.0	0.0	0.0	0.0
YBR034C	*HMT1*		7348_at	YBR034C r	340.4	1.3	100.8	340.4	1.2	84.8	0.0	0.0	0.0	0.0	0.0	0.0
YKL201C	*MNN4*		10445_s_a	YKL201C r	0.0	0.0	0.0	0.0	0.0	0.0	251.0	1.2	50.2	251.0	1.4	100.4
YGL259W	*YPS5*		5253_g_at	YGL259W	39.2	1.3	13.4	39.2	2.1	41.3	18.8	-1.3	6.4	18.8	1.6	11.0
YOL141W	*PPM2*		8693_at	YOL141W	9.5	1.3	3.1	9.5	2.3	12.8	0.0	0.0	0.0	0.0	0.0	0.0
YMR154C	*RIM13*	*CPL1*	9465_at	YMR154C	8.5	2.1	9.5	8.5	-1.0	0.0	2.7	-2.1	3.0	2.7	-1.9	2.4
YPL053C	*KTR6*	*MNN6*	7790_at	YPL053C r	0.0	0.0	0.0	0.0	0.0	0.0	406.6	1.2	101.2	406.6	1.1	40.7
YEL042W	*GDA1*		5748_at	YEL042W	223.0	-1.1	33.2	223.0	-1.5	111.5	0.0	0.0	0.0	0.0	0.0	0.0
YJL002C	*OST1*	*NLT1*	11019_at	YJL002C 6	1148.9	-1.1	171.1	1148.9	-1.1	114.9	1315.8	1.1	195.9	1315.8	1.0	0.0
YDR410C	*STE14*		6061_at	YDR410C	325.8	-1.4	130.3	325.8	-1.2	81.1	459.0	1.4	183.6	459.0	1.1	45.9
YEL002C	*WBP1*		5744_at	YEL002C c	953.0	-1.0	46.5	953.0	-1.1	141.9	0.0	0.0	0.0	0.0	0.0	0.0
YMR149W	*SWP1*		9504_at	YMR149W	783.3	-1.1	116.6	783.3	-1.2	153.4	883.5	1.1	131.6	883.5	-1.1	88.4
YJR143C	*PMT4*		10891_at	YJR143C c	771.0	-1.1	77.1	771.0	-1.2	154.2	0.0	0.0	0.0	0.0	0.0	0.0
YDR328C	*SKP1*	*MGO1*	6160_at	YDR328C	487.2	1.2	121.3	487.2	-1.1	48.7	386.5	-1.2	96.2	386.5	-1.4	154.6
YDL212W	*SHR3*	*APF1*	6696_at	YDL212W	1059.4	-1.2	211.9	1059.4	-1.1	157.8	1263.7	1.2	252.7	1263.7	1.0	0.0
YLR043C	*TRX1*	*LMA1*	10295_at	YLR043C t	1339.6	-1.2	267.9	1339.6	-1.1	199.5	1597.3	1.2	319.5	1597.3	1.0	0.0
YFL045C	*SEC53*	*ALG4*	5435_at	YFL045C p	1356.7	-1.1	202.0	1356.7	-1.1	202.0	1549.8	1.1	230.8	1549.8	-1.0	0.0
YDR139C	*RUB1*		6329_at	YDR139C	465.7	-1.0	22.7	465.7	-1.4	209.1	503.7	1.0	24.6	503.7	-1.3	151.1
assembly of protein complexes																
YLR075W	*??*		10239_at	YLR075W	2845.6	-1.2	708.6	2845.6	-1.0	138.9	3518.0	1.2	876.0	3518.0	1.2	703.6
YLR048W	*??*		10301_at	YLR048W	2484.7	-1.2	533.5	2484.7	-1.1	159.3	3017.4	1.2	647.8	3017.4	1.1	399.4
YPR191W	*QCR2*	*COR2, UC*	7543_at	YPR191W	497.9	1.9	448.1	497.9	1.5	273.4	238.5	-1.9	214.7	238.5	-1.2	47.7
YMR256C	*COX7*		9350_at	YMR256C	1167.2	1.1	116.7	1167.2	1.2	233.4	0.0	0.0	0.0	0.0	0.0	0.0
YLR038C	*COX12*		10290_at	YLR038C s	1869.2	1.1	278.3	1869.2	1.1	186.9	1626.8	-1.1	242.3	1626.8	-1.0	0.0
YLL009C	*COX17*		10334_at	YLL009C ir	551.2	1.2	110.2	551.2	1.1	82.1	0.0	0.0	0.0	0.0	0.0	0.0
YLR393W	*ATP10*		9928_at	YLR393W	8.6	1.9	8.1	8.6	2.5	12.8	0.0	0.0	0.0	0.0	0.0	0.0
YER141W	*COX15*		5581_at	YER141W	491.1	-1.0	0.0	491.1	-1.2	122.3	484.7	-1.0	0.0	484.7	-1.3	145.4
YLR447C	*VMA6*		9851_at	YLR447C 3	508.6	-1.0	24.8	508.6	-1.3	150.6	518.0	1.0	25.3	518.0	-1.3	155.4
YDR322C	*TIM11*		6154_at	YDR322C s	975.4	1.2	195.1	975.4	1.1	97.5	799.4	-1.2	159.9	799.4	-1.2	159.9
YLL039C	*UBI4*	*SCD2*	10392_at	YLL039C u	432.6	1.3	151.0	432.6	-1.1	64.4	321.2	-1.3	112.1	321.2	-1.5	160.6
YKL080W	*VMA5*	*CSL5, VAT*	10660_at	YKL080W	713.6	-1.3	249.1	713.6	-1.2	177.7	957.0	1.3	334.1	957.0	1.1	95.7
YGR214W			4780_at	YGR214W	1457.7	-1.2	285.4	1457.7	-1.1	217.1	1570.5	1.2	307.5	1570.5	1.1	157.1
YDL067C	*COX9*		6531_at	YDL067C S	687.3	-1.0	33.5	687.3	-1.3	239.9	707.7	1.0	34.5	707.7	-1.3	212.3
YPL231W	*FAS2*		7973_at	YPL231W a	396.9	1.0	19.4	396.9	-1.7	297.4	377.5	-1.0	18.4	377.5	-1.8	302.0

YOR332W	*VMA4*		8222_at	YOR332W	978.3	-1.3	341.5	978.3	-1.3	341.5	1290.1	1.3	450.3	1290.1	-1.0	0.0
YPL234C	*TFP3*	*CLS9, VM/*	7970_at	YPL234C 1	1186.4	-1.3	355.9	1186.4	-1.3	355.9	1511.7	1.3	453.5	1511.7	-1.1	151.2
YDL137W	*ARF2*		6638_at	YDL137W	1842.3	-1.1	175.8	1842.3	-1.2	360.8	0.0	0.0	0.0	0.0	0.0	0.0
YDL192W	*ARF1*		6671_at	YDL192W	3045.6	-1.2	609.1	3045.6	-1.1	453.5	3689.1	1.2	737.8	3689.1	1.0	0.0
YOR270C	*VPH1*		8291_at	YOR270C	1344.7	-1.3	403.4	1344.7	-1.3	469.4	1771.5	1.3	531.5	1771.5	-1.0	0.0
proteolytic degradation																
YLR423C	*APG17*		9914_at	YLR423C I	65.2	2.9	127.0	65.2	2.9	123.8	21.9	-2.9	42.7	21.9	1.0	0.0
YKL158W	*APE2*	*YKL157W*	10715_at	Leucyl ami	14.5	6.7	82.6	14.5	5.9	70.7	1.8	-6.7	10.3	1.8	1.4	0.7
YNR069C	*??*		8781_at	Similarity t	13.7	5.1	56.5	13.7	5.3	58.9	0.0	-5.1	0.0	0.0	1.7	0.0
YMR119W	*ASI1*		9517_g_at	YMR119W	361.6	-1.2	90.0	361.6	-1.3	126.2	414.2	1.2	103.1	414.2	-1.1	41.4
YNL015W	*PBI2*	*YSCB, I2B*	8880_at	YNL015W	683.7	1.3	205.1	683.7	1.0	33.4	507.9	-1.3	152.4	507.9	-1.3	152.4
cytoplasmic and nuclear degradation																
YBR082C	*UBC4*		7260_at	YBR082C (	946.7	1.1	94.7	946.7	1.5	473.3	840.4	-1.1	84.0	840.4	1.3	252.1
YIL148W			4253_i_at	YIL148W F	822.0	-1.2	198.1	822.0	-1.0	0.0	1017.9	1.2	245.3	1017.9	1.2	203.6
YIL075C	*RPN2*	*SEN3*	4189_at	YIL075C R	0.0	0.0	0.0	0.0	0.0	0.0	385.5	1.2	77.1	385.5	1.4	154.2
YNL172W	*APC1*		9041_at	YNL172W	102.1	1.3	34.9	102.1	2.2	121.6	76.1	-1.3	26.0	76.1	1.6	45.7
YER098W	*UBP9*		5629_at	YER098W	13.9	1.9	12.4	13.9	2.4	20.0	0.0	0.0	0.0	0.0	0.0	0.0
YER094C	*PUP3*	*SCS32*	5625_at	YER094C :	928.9	-1.1	92.9	928.9	-1.1	138.3	0.0	0.0	0.0	0.0	0.0	0.0
YGL048C	*RPT6*	*CIM3, CRL*	5059_at	YGL048C a	697.5	-1.0	0.0	697.5	-1.1	103.9	734.5	-1.0	0.0	734.5	-1.2	146.9
YDR328C	*SKP1*	*MGO1*	6160_at	YDR328C	487.2	1.2	121.3	487.2	-1.1	48.7	386.5	-1.2	96.2	386.5	-1.4	154.6
YLL039C	*UBI4*	*SCD2*	10392_at	YLL039C u	432.6	1.3	151.0	432.6	-1.1	64.4	321.2	-1.3	112.1	321.2	-1.5	160.6
YDL126C	*CDC48*		6604_at	YDL126C a	655.9	-1.1	65.6	655.9	-1.3	229.0	652.5	1.1	65.3	652.5	-1.3	195.8
YKR094C			10474_s_a	YKR094C I	1812.2	-1.2	358.7	1812.2	-1.1	266.2	2208.2	1.2	437.0	2208.2	1.1	210.8
lysosomal and vacuolar degradation																
YBR105C	*VID24*		7241_at	YBR105C i	89.7	2.0	89.7	89.7	2.8	165.9	43.4	-2.0	43.4	43.4	1.4	17.4
YPL154C	*PEP4*	*PHO9, PR/*	7916_at	YPL154C v	1033.3	-1.2	206.7	1033.3	-1.1	103.3	1221.5	1.2	244.3	1221.5	1.1	122.2
YKL054C	*VID31*		10642_at	YKL054C V	199.3	1.2	39.9	199.3	1.6	119.6	0.0	0.0	0.0	0.0	0.0	0.0
YBL078C	*AUT7*	*APG8, CV*	7464_at	YBL078C F	230.6	1.4	102.0	230.6	1.5	114.5	167.6	-1.4	74.1	167.6	1.0	0.0
YNR007C	*AUT1*	*APG3*	8855_at	AUT1 is es	44.1	1.5	22.1	44.1	2.5	68.3	28.9	-1.5	14.5	28.9	1.6	17.3
YGL180W	*APG1*	*AUT3*	5200_at	YGL180W	21.5	1.7	16.1	21.5	2.1	24.7	0.0	0.0	0.0	0.0	0.0	0.0
YJR044C	*VPS55*		10973_at	YJR044C V	773.9	-1.0	0.0	773.9	-1.1	115.2	0.0	0.0	0.0	0.0	0.0	0.0
YBR286W	*APE3*	*APY1*	7059_at	YBR286W	1238.0	-1.2	242.4	1238.0	-1.2	242.4	1456.7	1.2	285.3	1456.7	-1.0	0.0
YMR297W	*PRC1*	*LBC1*	9348_at	YMR297W	999.2	-1.1	148.8	999.2	-1.2	248.8	1135.5	1.1	169.1	1135.5	-1.1	113.6
other proteolytic degradation																
YBR201W	*DER1*		3940_at	YBR201W	96.1	1.7	71.7	96.1	2.0	92.2	58.1	-1.7	43.4	58.1	1.1	6.9
other protein fate related activities																
YDR258C	*HSP78*		6223_at	YDR258C	201.3	1.2	50.1	201.3	1.9	180.6	0.0	0.0	0.0	0.0	0.0	0.0
YDL185W	*TFP1*	*CLS8, VM/*	6679_at	YDL185W	690.5	-1.3	207.2	690.5	-1.3	241.0	892.6	1.3	267.8	892.6	-1.0	0.0
CELLULAR TRANSPORT AND TRANSPORT MECHANISM																
nuclear transport																
YOR098C	*NUP1*		8435_at	YOR098C	0.0	0.0	0.0	0.0	0.0	0.0	87.8	1.5	48.2	87.8	2.5	131.7
YDR432W	*NPL3*	*MTR13, M*	6039_g_at	YDR432W	442.1	1.2	106.8	442.1	1.1	53.2	326.8	-1.2	78.9	326.8	-1.2	54.8
YMR308C	*PSE1*	*KAP121*	9316_at	YMR308C	734.5	-1.1	109.4	734.5	-1.2	143.8	822.2	1.1	122.4	822.2	-1.1	82.2
YER009W	*NTF2*		5709_at	YER009W	834.6	-1.2	163.4	834.6	-1.2	163.4	957.1	1.2	187.4	957.1	1.0	0.0
YDR002W	*YRB1*	*CST20, H1*	6463_at	YDR002W	912.6	-1.2	182.5	912.6	-1.2	227.2	0.0	0.0	0.0	0.0	0.0	0.0
YAL005C	*SSA1*	*YG100*	11315_i_at	YAL005C I	2682.4	-1.0	127.0	2682.4	-1.1	391.6	2804.4	1.0	132.8	2804.4	-1.3	866.8
mitochondrial transport																
YNL055C	*POR1*	*OMP2*	8932_at	YNL055C (	1947.9	1.1	194.8	1947.9	1.2	485.0	0.0	0.0	0.0	0.0	0.0	0.0

YDL198C	*YHM1*		6665_at	YDL198C I	982.5	-1.0	0.0	982.5	1.4	441.3	964.8	-1.0	0.0	964.8	1.4	385.9
YKL120W	*OAC1*		10709_at	YKL120W (	519.5	1.3	181.3	519.5	1.5	285.3	376.9	-1.3	131.6	376.9	1.1	37.7
YNL070W	*TOM7*	*MOM7, YC*	8917_at	YNL070W	828.9	-1.1	123.4	828.9	1.1	123.4	927.8	1.1	138.2	927.8	1.3	278.3
YKL016C	*ATP7*		10591_at	YKL016C A	689.5	1.2	171.7	689.5	1.3	240.7	546.0	-1.2	136.0	546.0	1.1	54.6
YPR058W	*YMC1*		7678_at	YPR058W	624.9	1.1	62.5	624.9	1.3	187.5	569.7	-1.1	57.0	569.7	1.2	113.9
YOR045W	*TOM6*	*ISP6, MOM*	8518_at	YOR045W	1407.9	-1.1	209.6	1407.9	-1.1	140.8	1605.3	1.1	239.0	1605.3	1.1	160.5
YOR316C	*COT1*		8206_at	YOR316C	280.8	1.4	111.3	280.8	1.5	152.4	0.0	0.0	0.0	0.0	0.0	0.0
YER053C	*??*		5665_at	YER053C i	227.7	1.6	136.6	227.7	1.3	79.5	141.7	-1.6	85.0	141.7	-1.2	28.3
YGR028W	*MSP1*	*YTA4*	4956_at	YGR028W	231.7	1.5	127.2	231.7	1.2	46.3	147.3	-1.5	80.9	147.3	-1.3	44.2
YBL099W	*ATP1*		7487_at	YBL099W	1009.5	-1.1	150.3	1009.5	-1.0	0.0	1258.4	1.1	187.4	1258.4	1.1	125.8
YLR348C	*DIC1*		9972_at	YLR348C r	231.9	1.5	115.9	231.9	1.3	69.6	0.0	0.0	0.0	0.0	0.0	0.0
YJL054W	*TIM54*		11058_at	YJL054W T	105.3	1.4	41.7	105.3	2.0	105.0	0.0	0.0	0.0	0.0	0.0	0.0
YPL134C	*ODC1*		7891_at	YPL134C r	139.0	1.7	104.2	139.0	1.4	62.4	80.6	-1.7	60.4	80.6	-1.2	16.1
YOR100C	*CRC1*		8437_at	Mitochondr	30.0	1.4	11.9	30.0	3.2	67.3	21.3	-1.4	8.4	21.3	2.3	27.7
YBR085W	*AAC3*	*ANC3*	7263_at	YBR085W	47.0	-2.0	48.6	47.0	-1.9	43.8	99.8	2.0	103.3	99.8	1.1	10.0
YIL114C	*POR2*		4197_at	YIL114C vo	123.8	-1.8	105.1	123.8	-1.8	105.1	0.0	0.0	0.0	0.0	0.0	0.0
YBR039W	*ATP3*		7307_at	YBR039W	630.5	1.2	126.1	630.5	-1.0	0.0	526.3	-1.2	105.3	526.3	-1.2	105.3
YJR077C	*MIR1*		10961_at	YJR077C i	1731.2	1.1	257.8	1731.2	1.0	84.5	1524.8	-1.1	227.1	1524.8	-1.1	152.5
YLR295C	*ATP14*		10010_at	YLR295C A	630.6	1.6	376.4	630.6	1.1	93.9	394.9	-1.6	235.7	394.9	-1.4	158.0
YDR322C	*TIM11*		6154_at	YDR322C s	975.4	1.2	195.1	975.4	1.1	97.5	799.4	-1.2	159.9	799.4	-1.2	159.9
YDL004W	*ATP16*		6503_at	YDL004W	720.0	1.2	179.3	720.0	1.0	35.1	558.7	-1.2	139.1	558.7	-1.3	167.6
YPL271W	*ATP15*	*ATPEPSIL*	8024_at	YPL271W	1021.8	1.1	152.2	1021.8	-1.0	49.9	899.9	-1.1	134.0	899.9	-1.2	180.0
YNL064C	*YDJ1*	*MAS5*	8924_at	YNL064C y	979.7	1.0	47.8	979.7	-1.1	145.9	959.6	-1.0	46.8	959.6	-1.2	191.9
Q0310			3976_at	F1F0-ATPa	804.9	1.2	200.4	804.9	-1.0	39.3	644.5	-1.2	160.5	644.5	-1.3	193.4
YOR222W	*ODC2*		8334_at	YOR222W	506.1	-1.3	149.9	506.1	-1.5	274.6	689.7	1.3	204.3	689.7	-1.2	137.9
YLL024C	*SSA2*		10362_at	YLL024C n	1750.5	-1.3	525.2	1750.5	-1.4	700.2	2199.2	1.3	659.8	2199.2	-1.1	219.9

vesicular transport (Golgi network, etc.)

YNL272C	*SEC2*		9122_at	Protein with	57.0	1.0	2.8	57.0	3.8	159.2	56.4	-1.0	2.8	56.4	3.8	157.9
YNL006W	*LST8*		8889_at	YNL006W	316.8	1.5	158.4	316.8	1.2	63.4	242.1	-1.5	121.1	242.1	-1.2	48.4
YKR068C	*BET3*		10493_at	YKR068C I	0.0	0.0	0.0	0.0	0.0	0.0	657.0	-1.0	0.0	657.0	1.2	131.4
YDL195W	*SEC31*	*WEB1*	6668_at	YDL195W	233.6	1.4	104.9	233.6	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
YPL145C	*KES1*	*LPI3, OSH*	7880_at	YPL145C I	405.0	1.2	100.8	405.0	-1.0	19.8	0.0	0.0	0.0	0.0	0.0	0.0
YNL304W	*YPT11*		9180_at	YNL304W	28.2	1.1	2.7	28.2	2.8	51.8	27.4	-1.1	2.6	27.4	2.5	41.1
YNL044W	*YIP3*		3949_i_at	YNL044W	299.9	-1.2	66.6	299.9	-1.0	0.5	427.8	1.2	95.0	427.8	1.2	78.4
YLR262C	*YPT6*		10066_s_a	YLR262C I	408.9	-1.1	40.9	408.9	-1.2	101.8	0.0	0.0	0.0	0.0	0.0	0.0
YOR327C	*SNC2*		8217_at	YOR327C	431.6	-1.1	64.3	431.6	-1.2	107.5	0.0	0.0	0.0	0.0	0.0	0.0
YER031C	*YPT31*	*YPT8*	5686_at	YER031C r	252.5	-1.0	12.3	252.5	-1.4	113.4	0.0	0.0	0.0	0.0	0.0	0.0
YML001W	*YPT7*	*AST4, VAM*	9622_at	YML001W	822.3	-1.0	40.1	822.3	-1.1	122.4	0.0	0.0	0.0	0.0	0.0	0.0
YFL005W	*SEC4*	*SRO6*	5383_at	Ras-like sn	416.8	-1.0	0.0	416.8	-1.3	123.4	416.5	-1.0	0.0	416.5	-1.3	125.0
YPL218W	*SAR1*		7986_at	YPL218W	590.6	-1.2	118.1	590.6	-1.1	59.1	712.8	1.2	142.6	712.8	1.1	71.3
YPR028W	*YOP1*	*YIP2*	7735_at	YPR028W	874.0	-1.2	196.0	874.0	-1.2	171.1	1064.0	1.2	238.6	1064.0	1.0	0.0
YNL064C	*YDJ1*	*MAS5*	8924_at	YNL064C y	979.7	1.0	47.8	979.7	-1.1	145.9	959.6	-1.0	46.8	959.6	-1.2	191.9
YML012W	*ERV25*		9653_at	YML012W	1340.8	-1.1	134.1	1340.8	-1.1	199.7	0.0	0.0	0.0	0.0	0.0	0.0
YGL225W	*VRG4*	*VAN2, GO*	5245_at	YGL225W	800.7	-1.3	279.5	800.7	-1.3	237.1	1065.2	1.3	371.8	1065.2	1.0	0.0
YDL137W	*ARF2*		6638_at	YDL137W	1842.3	-1.1	175.8	1842.3	-1.2	360.8	0.0	0.0	0.0	0.0	0.0	0.0
YGL200C	*EMP24*	*BST2*	5225_at	YGL200C t	1255.6	-1.1	125.6	1255.6	-1.3	376.7	0.0	0.0	0.0	0.0	0.0	0.0
YDL192W	*ARF1*		6671_at	YDL192W	3045.6	-1.2	609.1	3045.6	-1.1	453.5	3689.1	1.2	737.8	3689.1	1.0	0.0
YLL024C	*SSA2*		10362_at	YLL024C n	1750.5	-1.3	525.2	1750.5	-1.4	700.2	2199.2	1.3	659.8	2199.2	-1.1	219.9

vacuolar transport

YBR082C	*UBC4*		7260_at	YBR082C	946.7	1.1	94.7	946.7	1.5	473.3	840.4	-1.1	84.0	840.4	1.3	252.1
YBR105C	*VID24*		7241_at	YBR105C i	89.7	2.0	89.7	89.7	2.8	165.9	43.4	-2.0	43.4	43.4	1.4	17.4
YNR007C	*AUT1*	*APG3*	8855_at	AUT1 is es	44.1	1.5	22.1	44.1	2.5	68.3	28.9	-1.5	14.5	28.9	1.6	17.3
YGL180W	*APG1*	*AUT3*	5200_at	YGL180W	21.5	1.7	16.1	21.5	2.1	24.7	0.0	0.0	0.0	0.0	0.0	0.0
YER031C	*YPT31*	*YPT8*	5686_at	YER031C	252.5	-1.0	12.3	252.5	-1.4	113.4	0.0	0.0	0.0	0.0	0.0	0.0
YGR020C	*VMA7*		4993_at	YGR020C	462.1	-1.4	184.8	462.1	-1.3	138.6	648.7	1.4	259.5	648.7	1.1	64.9
YLR447C	*VMA6*		9851_at	YLR447C	508.6	-1.0	24.8	508.6	-1.3	150.6	518.0	1.0	25.3	518.0	-1.3	155.4
YKL080W	*VMA5*	*CSL5, VA7*	10660_at	YKL080W	713.6	-1.3	249.1	713.6	-1.2	177.7	957.0	1.3	334.1	957.0	1.1	95.7
YPR036W	*VMA13*	*CLS11*	7699_at	YPR036W	437.6	1.1	43.8	437.6	-1.3	152.7	403.1	-1.1	40.3	403.1	-1.5	201.6
YDL185W	*TFP1*	*CLS8, VM*	6679_at	YDL185W	690.5	-1.3	207.2	690.5	-1.3	241.0	892.6	1.3	267.8	892.6	-1.0	0.0
YBR127C	*VMA2*	*ATPVS, V*	7218_at	YBR127C	734.2	-1.5	367.1	734.2	-1.4	329.7	1021.3	1.5	510.7	1021.3	1.0	0.0
YEL027W	*CUP5*		5763_at	YEL027W	2242.6	-1.3	664.1	2242.6	-1.1	334.0	2929.6	1.3	867.6	2929.6	1.1	293.0
YOR332W	*VMA4*		8222_at	YOR332W	978.3	-1.3	341.5	978.3	-1.3	341.5	1290.1	1.3	450.3	1290.1	-1.0	0.0
YHR039C	*MSC7*	*VMA10*	4514_at	YHR039C	783.6	-1.3	235.1	783.6	-1.4	351.9	1017.3	1.3	305.2	1017.3	-1.1	101.7
YPL234C	*TFP3*	*CLS9, VM*	7970_at	YPL234C 1	1186.4	-1.3	355.9	1186.4	-1.3	355.9	1511.7	1.3	453.5	1511.7	-1.1	151.2
YOR270C	*VPH1*		8291_at	YOR270C	1344.7	-1.3	403.4	1344.7	-1.3	469.4	1771.5	1.3	531.5	1771.5	-1.0	0.0

extracellular transport, exocytosis and secretion

YDR524C	*AGE1*	*SAT1*	5926_g_at	YDR524C	3729.0	-1.3	1015.6	3729.0	-1.1	539.0	4720.7	1.3	1285.7	4720.7	1.1	703.0
YNL272C	*SEC2*		9122_at	Protein with	57.0	1.0	2.8	57.0	3.8	159.2	56.4	-1.0	2.8	56.4	3.8	157.9
YNL036W	*NCE103*		8905_at	YNL036W	705.6	1.2	141.1	705.6	1.1	70.6	596.1	-1.2	119.2	596.1	-1.1	59.6

cellular import

YDR046C	*BAP3*		6415_at	Valine trans	670.0	2.6	1072.0	670.0	178.1	118656.5	297.2	-2.6	475.5	297.2	81.3	23865.2
YBR068C	*BAP2*		7291_at	Major AA p	646.7	2.3	839.2	646.7	133.8	85884.2	280.3	-2.3	363.8	280.3	58.7	16173.3
YGR055W	*MUP1*		4936_at	High affinity	968.3	2.0	968.3	968.3	9.0	7745.5	486.4	-2.0	486.4	486.4	4.5	1702.4
YBR069C	*TAT1*	*VAP1, WA*	7292_at	Amino acid	164.5	3.1	345.1	164.5	11.2	1685.2	53.1	-3.1	111.4	53.1	4.1	164.6
YMR058W	*FET3*		9588_at	YMR058W	900.7	2.1	988.6	900.7	2.4	1259.1	422.2	-2.1	463.4	422.2	1.1	42.2
YBR021W	*FUR4*		7334_at	Uracil perm	208.8	4.1	656.1	208.8	6.4	1124.9	41.4	-4.1	130.1	41.4	1.5	20.7
YNL268W	*LYP1*		9126_at	YNL268W	580.1	-1.0	0.0	580.1	2.3	782.8	570.8	-1.0	0.0	570.8	2.3	742.0
YDR524C	*AGE1*	*SAT1*	5926_g_at	YDR524C	3729.0	-1.3	1015.6	3729.0	-1.1	539.0	4720.7	1.3	1285.7	4720.7	1.1	703.0
YER056C	*FCY2*	*BRA7*	5668_at	YER056C	1819.5	-1.1	238.3	1819.5	1.0	19.9	2117.5	1.1	277.4	2117.5	1.1	311.1
YBR294W	*SUL1*		7067_at	Putative su	97.3	1.3	33.9	97.3	4.0	291.6	70.8	-1.3	24.7	70.8	2.9	134.5
YOL020W	*TAT2*	*LTG3, SAE*	8587_at	YOL020W	216.0	1.7	151.2	216.0	2.3	291.5	127.2	-1.7	89.0	127.2	1.4	50.9
YDR497C	*ITR1*		5966_at	Myo-inosito	1187.5	-1.2	237.5	1187.5	-1.0	0.0	1358.0	1.2	271.6	1358.0	1.2	271.6
YMR011W	*HXT2*		9633_at	YMR011W	809.5	-1.3	242.8	809.5	-1.0	0.0	1029.2	1.3	308.8	1029.2	1.2	205.8
YGL077C	*HNM1*		5074_at	YGL077C	544.7	-1.5	272.3	544.7	-1.2	108.9	924.4	1.5	462.2	924.4	1.2	184.9
YHR094C	*HXT1*	*HOR4*	4430_at	YHR094C	540.4	-1.0	0.0	540.4	1.3	162.1	525.9	-1.0	0.0	525.9	1.3	157.8
YDR345C	*HXT3*		6131_at	YDR345C	1351.7	-1.2	264.7	1351.7	-1.1	129.0	1606.0	1.2	314.5	1606.0	1.1	160.6
YKR039W	*GAP1*		10511_at	YKR039W	46.7	-2.4	65.2	46.7	-1.4	21.0	110.8	2.4	154.9	110.8	1.7	77.6
YCL040W	*GLK1*	*HOR3*	6937_at	YCL040W	686.5	1.6	411.9	686.5	1.2	137.3	437.3	-1.6	262.4	437.3	-1.3	131.2
YHR175W	*CTR2*		4335_at	YHR175W	453.1	1.2	112.8	453.1	1.2	88.7	0.0	0.0	0.0	0.0	0.0	0.0
YFL011W	*HXT10*		5377_at	YFL011W	4.7	-1.9	4.1	4.7	1.1	0.6	10.8	1.9	9.4	10.8	2.0	10.8
YER031C	*YPT31*	*YPT8*	5686_at	YER031C	252.5	-1.0	12.3	252.5	-1.4	113.4	0.0	0.0	0.0	0.0	0.0	0.0
YMR243C	*ZRC1*	*OSR1*	9382_at	YMR243C	815.0	-1.0	39.8	815.0	-1.1	121.4	0.0	0.0	0.0	0.0	0.0	0.0
YML001W	*YPT7*	*AST4, VAM*	9622_at	YML001W	822.3	-1.0	40.1	822.3	-1.1	122.4	0.0	0.0	0.0	0.0	0.0	0.0
YBR109C	*CMD1*		7245_at	YBR109C	758.7	-1.1	75.9	758.7	-1.2	151.7	0.0	0.0	0.0	0.0	0.0	0.0
YGR121C	*MEP1*	*AMT1*	4866_at	Ammonia p	42.9	-4.6	155.0	42.9	-5.4	189.0	195.4	4.6	706.8	195.4	-1.2	39.1
YDR342C	*HXT7*		6128_f_at	YDR342C	1141.2	-1.1	114.1	1141.2	-1.2	228.2	0.0	0.0	0.0	0.0	0.0	0.0
YFL039C	*ACT1*	*ABY1, ENL*	5392_at	YFL039C	2061.4	-1.2	403.7	2061.4	-1.2	403.7	2477.1	1.2	485.1	2477.1	1.0	0.0
YDR343C	*HXT6*		6129_f_at	YDR343C	1105.6	-1.1	110.6	1105.6	-1.4	442.2	1197.1	1.1	119.7	1197.1	-1.3	359.1

		YNL142W	*MEP2*		9026_at	Ammonia t	75.9	-17.2	1229.5	75.9	-14.8	1051.2	1305.0	17.2	21139.5	1305.0	1.2	261.0	
	cytoskeleton-dependent transport																		
		YBL078C	*AUT7*	*APG8, CV*	7464_at	YBL078C F	230.6	1.4	102.0	230.6	1.5	114.5	167.6	-1.4	74.1	167.6	1.0	0.0	
		YCR068W	*CVT17*	*AUT5*	6809_at	Teter et al.	24.0	5.6	110.5	24.0	4.7	89.4	5.6	-4.4	18.9	5.6	-1.2	1.1	
		YIL062C	*ARC15*		4155_at	YIL062C A	655.4	1.0	32.0	655.4	-1.2	128.3	617.0	-1.0	30.1	617.0	-1.3	185.1	
	other intracellular transport activities																		
		YKR093W	*PTR2*		10472_at	YKR093W	148.2	2.7	258.7	148.2	5.8	718.0	48.2	-2.7	84.2	48.2	2.1	53.0	
		YJL034W	*KAR2*	*BIP, GRP7*	11078_at	Homologue	1978.5	1.1	188.8	1978.5	1.2	492.6	1802.2	-1.1	172.0	1802.2	1.2	360.4	
		YDR086C	*SSS1*		6410_at	YDR086C	557.7	-1.3	165.2	557.7	-1.1	83.0	727.0	1.3	215.3	727.0	1.2	145.4	
		YKL198C	*PTK1*	*KKT8, STK*	3948_s_at	YKL198C p	6.7	2.2	8.4	6.7	2.6	10.6	4.3	-2.2	5.4	4.3	1.5	2.2	
		YNL079C	*TPM1*		8953_at	YNL079C t	713.2	1.0	34.8	713.2	-1.2	142.6	676.8	-1.0	33.0	676.8	-1.2	135.4	
		YCR075C	*ERS1*		6816_at	YCR075C	185.0	-1.2	33.9	185.0	-1.9	162.0	0.0	0.0	0.0	0.0	0.0	0.0	
CELLULAR COMMUNICATION/SIGNAL TRANSDUCTION MECHANISM																			
	intracellular signalling																		
		unspecified signal transduction																	
			YIL071C	*PCI8*	*YIH1*	4148_at	YIL071C P	9.3	-1.0	0.0	9.3	2.1	9.8	0.0	0.0	0.0	0.0	0.0	0.0
			YER177W	*BMH1*		5525_at	YER177W	1125.3	-1.1	112.5	1125.3	-1.3	392.8	1252.2	1.1	125.2	1252.2	-1.2	250.4
		enzyme mediated signal transduction																	
			G-protein mediated signal transduction																
				YOR107W	*RGS2*		8444_at	YOR107W	5.8	2.9	11.1	5.8	2.3	7.8	2.1	-2.9	4.0	2.1	-1.3
				YNL098C	*RAS2*		8979_at	YNL098C F	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3
				YPR165W	*RHO1*		7563_at	Ras homol	819.3	-1.0	0.0	819.3	-1.2	163.9	828.3	-1.0	0.0	828.3	-1.2
				YLR229C	*CDC42*		10121_at	Member of	1147.0	-1.0	56.0	1147.0	-1.3	400.4	1103.3	1.0	53.9	1103.3	-1.3
		other intracellular signal transduction activities																	
			YFL031W	*HAC1*	*ERN4, IRE*	5318_s_at	bZIP Trans	398.7	1.9	355.4	398.7	2.2	484.5	284.7	-1.9	253.8	284.7	1.2	53.7
			YNL138W	*SRV2*		8984_at	YNL138W	473.5	1.1	70.5	473.5	1.9	424.9	409.0	-1.1	60.9	409.0	1.6	245.4
			YNL128W	*TEP1*		8994_at	Tyrosine pl	15.9	1.3	4.8	15.9	14.6	216.4	12.5	-1.3	3.8	12.5	13.6	157.5
			YDL212W	*SHR3*	*APF1*	6696_at	YDL212W	1059.4	-1.2	211.9	1059.4	-1.1	157.8	1263.7	1.2	252.7	1263.7	1.0	0.0
	transmembrane signal transduction				1	4	ORFs												
		YKL178C	*STE3*		10741_at	YKL178C a	887.2	-1.1	132.1	887.2	-1.4	351.7	1048.9	1.1	156.2	1048.9	-1.2	209.8	
CELL RESCUE, DEFENSE AND VIRULENCE																			
	stress response																		
		YMR096W	*SNZ1*		9538_at	Encodes hi	1883.5	1.5	941.7	1883.5	6.2	9787.9	1095.7	-1.5	547.9	1095.7	4.2	3506.2	
		YMR095C	*SNO1*		9537_at	Upstream f	887.2	2.0	887.2	887.2	9.8	7806.9	439.8	-2.0	439.8	439.8	5.0	1759.2	
		YKR042W	*UTH1*		10514_at	YKR042W	1982.9	-1.0	0.0	1982.9	1.2	396.6	2155.3	-1.0	0.0	2155.3	1.2	431.1	
		YMR251W	*HOR7*		9390_at	YMR251W	3669.8	-1.1	546.5	3669.8	-1.0	179.1	4175.2	1.1	621.7	4175.2	1.1	417.5	
		YMR186W	*HSC82*		9456_at	YMR186W	1536.6	-1.2	300.9	1536.6	-1.0	0.0	1859.1	1.2	364.1	1859.1	1.2	371.8	
		YDR077W	*SED1*		6401_at	YDR077W	1872.7	1.3	561.8	1872.7	1.5	1028.5	1464.7	-1.3	439.4	1464.7	1.2	292.9	
		YER011W	*TIR1*	*SRP1*	5711_at	YER011W	690.6	-1.2	172.0	690.6	1.1	69.1	684.8	1.2	170.5	684.8	1.4	273.9	
		YBR082C	*UBC4*		7260_at	YBR082C (	946.7	1.1	94.7	946.7	1.5	473.3	840.4	-1.1	84.0	840.4	1.3	252.1	
		YLR109W	*AHP1*	*cTPxIII*	10228_at	YLR109W	1813.1	-1.2	451.4	1813.1	-1.2	362.6	2293.7	1.2	571.1	2293.7	1.1	229.4	
		YJL159W	*HSP150*	*CCW7, OF*	11179_at	Kex2-proce	1519.4	1.0	74.2	1519.4	1.1	226.3	1451.1	-1.0	70.8	1451.1	1.1	145.1	
		YNL160W	*YGP1*		9008_at	YNL160W	1172.5	2.3	1524.2	1172.5	2.8	2168.5	474.9	-2.3	617.4	474.9	1.3	142.5	
		YOR010C	*TIR2*	*SRP2*	8527_at	YOR010C	0.0	0.0	0.0	0.0	0.0	0.0	244.1	1.1	24.4	244.1	1.5	122.1	
		YIL011W	*TIR3*		4114_at	YIL011W s	0.0	0.0	0.0	0.0	0.0	0.0	357.9	1.3	107.4	357.9	1.1	35.8	
		YPL240C	*HSP82*	*HSP83, HS*	8010_i_at	YPL240C I	0.0	0.0	0.0	0.0	0.0	0.0	514.6	-1.0	0.0	514.6	1.2	102.9	
		YJR147W	*HMS2*		10895_at	Heat shock	8.6	-2.0	9.0	8.6	2.0	8.7	16.7	2.0	17.5	16.7	4.2	53.4	

YNL328C	*MDJ2*		9202_at	YNL328C F	0.0	0.0	0.0	0.0	0.0	0.0	4.5	-1.7	3.3	4.5	-2.5	6.8
YNL241C	*ZWF1*	*MET19, P(*	9108_at	Glucose-6-	206.9	1.3	62.1	206.9	-1.0	0.0	157.1	-1.3	47.1	157.1	-1.3	47.1
YIR037W	*HYR1*	*GPX3*	4073_at	YIR037W p	436.4	-1.1	43.6	436.4	-1.3	130.9	482.3	1.1	48.2	482.3	-1.2	96.5
YIR038C	*GTT1*		4074_at	YIR038C G	252.0	-1.0	12.3	252.0	-1.5	138.4	251.6	1.0	12.3	251.6	-1.4	100.6
YOR027W	*STI1*		8545_at	YOR027W	0.0	0.0	0.0	0.0	0.0	0.0	288.3	-1.1	42.9	288.3	-1.4	115.3
YDR214W	*AHA1*		6271_at	YDR214W	0.0	0.0	0.0	0.0	0.0	0.0	295.8	-1.1	28.2	295.8	-1.4	118.3
YPL106C	*SSE1*	*LPG3, MSI*	7874_at	YPL106C H	672.7	-1.0	0.0	672.7	-1.2	134.5	633.8	-1.0	0.0	633.8	-1.2	126.8
YDL022W	*GPD1*	*DAR1, HO*	6485_at	YDL022W	763.2	1.1	113.7	763.2	-1.0	0.0	647.3	-1.1	96.4	647.3	-1.2	129.5
YNL098C	*RAS2*		8979_at	YNL098C F	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3	143.6
YLL039C	*UBI4*	*SCD2*	10392_at	YLL039C u	432.6	1.3	151.0	432.6	-1.1	64.4	321.2	-1.3	112.1	321.2	-1.5	160.6
YNL064C	*YDJ1*	*MAS5*	8924_at	YNL064C y	979.7	1.0	47.8	979.7	-1.1	145.9	959.6	-1.0	46.8	959.6	-1.2	191.9
YBR067C	*TIP1*		7290_at	YBR067C (	1652.2	-1.1	246.0	1652.2	-1.2	411.4	1934.7	1.1	288.1	1934.7	-1.1	193.5
YFL014W	*HSP12*		5372_at	YFL014W	625.4	1.8	493.3	625.4	-1.2	122.5	350.2	-1.8	276.3	350.2	-2.1	385.2
YAL005C	*SSA1*	*YG100*	11315_i_at	YAL005C H	2682.4	-1.0	127.0	2682.4	-1.1	391.6	2804.4	1.0	132.8	2804.4	-1.3	866.8
YBR054W	*YRO2*		7322_at	Homolog to	334.3	2.3	451.1	334.3	3.6	869.2	153.8	-2.3	207.5	153.8	1.5	76.9
YKL163W	*PIR3*	*CCW8*	10756_at	Protein cor	144.4	4.2	461.2	144.4	3.3	338.7	34.4	-4.2	109.9	34.4	-1.1	3.4
YMR173W	*DDR48*	*FSP*	9442_s_at	YMR173W	852.0	1.5	401.1	852.0	1.4	333.2	596.9	-1.5	281.0	596.9	-1.0	29.1
YDR258C	*HSP78*		6223_at	YDR258C	201.3	1.2	50.1	201.3	1.9	180.6	0.0	0.0	0.0	0.0	0.0	0.0
YAL015C	*NTG1*	*FUN33*	11352_at	YAL015C D	144.8	1.7	108.5	144.8	2.0	144.4	82.6	-1.7	61.9	82.6	1.1	8.3
YGR234W	*YHB1*	*YHB4*	4754_at	YGR234W	274.2	1.4	121.3	274.2	1.5	136.2	0.0	0.0	0.0	0.0	0.0	0.0
YNL333W	*SNZ2*		9198_s_at	YNL333W	159.9	1.9	143.4	159.9	1.8	134.9	86.0	-1.9	77.2	86.0	-1.1	8.6
YAL040C	*CLN3*	*DAF1, FUN*	11369_at	YAL040C C	188.8	1.2	37.8	188.8	1.7	132.1	0.0	0.0	0.0	0.0	0.0	0.0
YKL164C	*CCW6*	*PIR1*	10755_at	Ccw protein	1316.5	1.1	131.7	1316.5	1.0	64.3	1226.1	-1.1	122.6	1226.1	-1.0	0.0
YDL025C	*YDL025C*		6482_at	YDL025C s	237.2	1.5	130.3	237.2	1.3	82.8	0.0	0.0	0.0	0.0	0.0	0.0
YPL188W	*POS5*		7926_at	YPL188W	136.1	1.6	81.7	136.1	1.9	122.5	97.0	-1.6	58.2	97.0	1.3	29.1
YHR030C	*SLT2*	*BYC2, MPI*	4504_at	YHR030C	187.6	1.6	111.9	187.6	1.6	121.8	118.8	-1.6	70.9	118.8	1.1	11.9
YHR104W	*GRE3*		4442_at	YHR104W	279.7	1.4	111.9	279.7	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
YDR074W	*TPS2*	*HOG2, PFI*	6398_at	YDR074W	224.7	1.5	111.6	224.7	-1.0	11.0	140.6	-1.5	69.8	140.6	-1.6	84.4
YFL016C	*MDJ1*		5370_at	YFL016C D	158.6	1.6	95.2	158.6	1.7	111.0	108.1	-1.6	64.9	108.1	1.1	10.8
YBR072W	*HSP26*		7295_at	YBR072W	88.4	2.2	105.9	88.4	1.4	39.7	41.9	-2.2	50.2	41.9	-1.5	21.0
YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2	10.9
YDR404C	*RPB7*		6055_at	YDR404C (	422.4	-1.1	42.2	422.4	-1.3	126.7	0.0	0.0	0.0	0.0	0.0	0.0
YDR155C	*CPR1*	*CYP1, CPI*	6301_at	YDR155C (	1671.5	-1.2	327.3	1671.5	-1.1	159.5	0.0	0.0	0.0	0.0	0.0	0.0
YER057C	*HMF1*		5672_at	YER057C I	665.2	-1.1	63.5	665.2	-1.2	165.6	0.0	0.0	0.0	0.0	0.0	0.0
YLR259C	*HSP60*	*CPN60, MI*	10061_at	YLR259C H	1822.6	-1.1	182.3	1822.6	-1.1	271.4	0.0	0.0	0.0	0.0	0.0	0.0

detoxification

YOL109W	*ZEO1*		8679_at	YOL109W	2052.6	-1.0	100.2	2052.6	1.1	305.7	2118.1	1.0	103.4	2118.1	1.2	423.6
YBR145W	*ADH5*		7236_at	YBR145W	1213.1	1.0	59.2	1213.1	1.3	423.4	1149.0	-1.0	56.1	1149.0	1.3	344.7
YLL028W	*TPO1*		10358_at	YLL028W p	714.9	-1.0	0.0	714.9	1.5	357.4	613.7	-1.0	0.0	613.7	1.5	306.9
YEL027W	*CUP5*		5763_at	YEL027W V	2242.6	-1.3	664.1	2242.6	-1.1	334.0	2929.6	1.3	867.6	2929.6	1.1	293.0
YOL158C	*ENB1*	*ARN4*	8723_at	YOL158C I	881.4	1.4	395.8	881.4	2.0	924.9	600.5	-1.4	269.7	600.5	1.4	240.2
YML116W	*ATR1*	*SNQ1*	9771_at	YML116W	507.3	1.1	50.7	507.3	1.6	329.4	459.7	-1.1	46.0	459.7	1.5	229.9
YBR043C	*AQR2*		7311_at	YBR043C A	373.6	1.5	186.8	373.6	2.5	560.4	256.8	-1.5	128.4	256.8	1.7	179.8
YNL065W	*AQR1*		8923_at	A(acids, az	200.2	1.9	180.2	200.2	4.5	700.5	105.2	-1.9	94.7	105.2	2.4	147.3
YNL296W	*KRE25*		9143_at	Killer toxin Resistant			0.0			0.0	11.2	2.1	12.3	11.2	3.0	22.4
YPR156C	*TPO3*		7599_i_at	Polyamine	1.7	-3.0	3.4	1.7	-1.0	0.1	8.1	3.0	16.2	8.1	2.9	15.4
YIL120W	*QDR1*		4236_at	Multidrug r	41.4	9.5	351.8	41.4	3.6	109.7	3.3	-9.5	28.0	3.3	-2.6	5.3
YNL241C	*ZWF1*	*MET19, P(*	9108_at	Glucose-6-	206.9	1.3	62.1	206.9	-1.0	0.0	157.1	-1.3	47.1	157.1	-1.3	47.1
YIR038C	*GTT1*		4074_at	YIR038C G	252.0	-1.0	12.3	252.0	-1.5	138.4	251.6	1.0	12.3	251.6	-1.4	100.6
YGR138C	*TPO2*		4884_i_at	YGR138C	146.9	-1.3	50.2	146.9	-2.1	166.8	193.0	1.3	65.9	193.0	-1.6	115.8

ORF	Gene	Alias	Probe	Description												
YEL065W	*SIT1*	*ARN3*	5769_at	Siderophor	407.0	6.4	2204.5	407.0	12.4	4625.1	67.8	-6.4	367.3	67.8	1.8	54.2
YHL040C	*ARN1*		4568_at	Siderochro	271.7	5.7	1288.9	271.7	6.7	1546.6	43.8	-5.7	207.8	43.8	1.3	13.1
YPL092W	*SSU1*	*LPG16*	7843_at	YPL092W	279.1	1.3	83.7	279.1	2.3	362.8	0.0	0.0	0.0	0.0	0.0	0.0
YHR053C	*CUP1-1*	*CUP1*	4483_s_at	YHR053C	1985.9	1.2	397.2	1985.9	1.1	295.7	0.0	0.0	0.0	0.0	0.0	0.0
YNL259C	*ATX1*		9135_at	YNL259C	701.7	1.1	70.2	701.7	1.3	210.5	0.0	0.0	0.0	0.0	0.0	0.0
RTM1	*RTM1*		3934_at	RTM1 Prot	55.2	2.2	69.0	55.2	3.5	140.7	22.1	-2.2	27.6	22.1	1.6	13.3
YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2	10.9
YFL007W	*BLM3*		5381_at	YFL007W	34.7	2.7	59.9	34.7	2.5	52.0	12.9	-2.7	22.3	12.9	-1.0	0.0
YNR070W	*PDR18*		8782_at	YNR070W	6.7	2.3	8.6	6.7	2.8	12.3	3.7	-2.3	4.7	3.7	1.5	1.9
YMR243C	*ZRC1*	*OSR1*	9382_at	YMR243C	815.0	-1.0	39.8	815.0	-1.1	121.4	0.0	0.0	0.0	0.0	0.0	0.0
YLR043C	*TRX1*	*LMA1*	10295_at	YLR043C t	1339.6	-1.2	267.9	1339.6	-1.1	199.5	1597.3	1.2	319.5	1597.3	1.0	0.0
YOR153W	*PDR5*	*LEM1, YDI*	8400_at	Multidrug r	707.7	-1.3	209.6	707.7	-1.3	209.6	916.0	1.3	271.3	916.0	1.0	0.0
YJR104C	*SOD1*	*CRS4*	10897_at	YJR104C (	1864.3	-1.1	186.4	1864.3	-1.1	277.6	0.0	0.0	0.0	0.0	0.0	0.0
detoxification involving cytochrome P450																
YHR007C	*ERG11*		4525_at	YHR007C	0.0	0.0	0.0	0.0	0.0	0.0	1087.9	1.0	53.1	1087.9	1.2	217.6
other cell rescue activities																
YGR213C	*RTA1*		4778_at	YGR213C	10.4	2.4	15.0	10.4	1.7	7.2	4.2	-2.4	6.1	4.2	-1.4	1.7

REGULATION OF / INTERACTION WITH CELLULAR ENVIRONMENT

ionic homeostasis

homeostasis of cations

ORF	Gene	Alias	Probe	Description												
YDR276C	*PMP3*	*SNA1*	6196_at	YDR276C	1952.2	-1.2	486.1	1952.2	-1.0	95.3	2416.2	1.2	601.6	2416.2	1.1	241.6
homeostasis of metal ions (Na, K, Ca etc.)				20	63 ORFs											
YMR058W	*FET3*		9588_at	YMR058W	900.7	2.1	988.6	900.7	2.4	1259.1	422.2	-2.1	463.4	422.2	1.1	
YPL135W	*ISU1*	*NUA1*	7890_at	YPL135W	907.5	1.9	861.5	907.5	1.7	635.3	464.8	-1.9	441.3	464.8	-1.1	
YLR034C	*SMF3*		10286_at	YLR034C F	700.6	1.1	104.3	700.6	1.3	244.5	596.7	-1.1	88.9	596.7	1.2	
YNL259C	*ATX1*		9135_at	YNL259C	701.7	1.1	70.2	701.7	1.3	210.5	0.0	0.0	0.0	0.0	0.0	
YOR316C	*COT1*		8206_at	YOR316C	280.8	1.4	111.3	280.8	1.5	152.4	0.0	0.0	0.0	0.0	0.0	
YDR270W	*CCC2*		6190_at	Accessory	150.0	1.9	135.0	150.0	1.9	135.0	92.0	-1.9	82.8	92.0	1.0	
YER145C	*FTR1*		5585_at	YER145C	260.0	1.1	30.5	260.0	1.5	124.6	143.9	-1.1	18.6	143.9	1.4	
YER145C	*FTR1*		5585_at	YER145C	260.0	1.1	30.5	260.0	1.5	124.6	143.9	-1.1	18.6	143.9	1.4	
YLL009C	*COX17*		10334_at	YLL009C ir	551.2	1.2	110.2	551.2	1.1	82.1	0.0	0.0	0.0	0.0	0.0	
YOR381W	*FRE3*		8180_at	Cell surfac	42.4	2.8	77.9	42.4	3.2	92.6	14.9	-2.8	27.4	14.9	1.1	
YHR175W	*CTR2*		4335_at	YHR175W	453.1	1.2	112.8	453.1	1.2	88.7	0.0	0.0	0.0	0.0	0.0	
YLR214W	*FRE1*		10106_at	Ferric (and	107.8	1.5	59.2	107.8	1.7	80.8	63.6	-1.5	34.9	63.6	1.1	
YJR049C	*UTR1*		10978_at	Shown to a	32.6	1.2	7.9	32.6	2.2	40.3	26.7	-1.2	6.4	26.7	1.8	
YCR044C	*PER1*		6832_at	YCR044C	324.0	-1.2	64.8	324.0	-1.3	113.1	0.0	0.0	0.0	0.0	0.0	
YMR243C	*ZRC1*	*OSR1*	9382_at	YMR243C	815.0	-1.0	39.8	815.0	-1.1	121.4	0.0	0.0	0.0	0.0	0.0	
YLR411W	*CTR3*		9902_at	YLR411W	78.5	-1.8	62.4	78.5	-2.9	145.5	135.3	1.8	107.5	135.3	-1.6	
YLR130C	*ZRT2*		10204_at	YLR130C I	498.4	-1.5	249.2	498.4	-1.3	174.0	750.0	1.5	375.0	750.0	1.1	
YKL080W	*VMA5*	*CSL5, VA1*	10660_at	YKL080W	713.6	-1.3	249.1	713.6	-1.2	177.7	957.0	1.3	334.1	957.0	1.1	
YOR153W	*PDR5*	*LEM1, YDI*	8400_at	Multidrug r	707.7	-1.3	209.6	707.7	-1.3	209.6	916.0	1.3	271.3	916.0	1.0	
YGL255W	*ZRT1*		5258_at	YGL255W	147.7	-1.7	103.0	147.7	-2.6	243.1	279.2	1.7	194.6	279.2	-1.6	
YLR109W	*AHP1*	*cTPxIII*	10228_at	YLR109W	1813.1	-1.2	451.4	1813.1	-1.2	362.6	2293.7	1.2	571.1	2293.7	1.1	
homeostasis of protons																
YGL008C	*PMA1*		5009_at	YGL008C	0.0	0.0	0.0	0.0	0.0	0.0	2122.2	1.1	212.2	2122.2	1.2	
YCR024C	*PMP1*		6858_f_at	YCR024C	897.8	-1.3	313.4	897.8	-1.0	0.0	1190.8	1.3	415.7	1190.8	1.3	
YKL016C	*ATP7*		10591_at	YKL016C	689.5	1.2	171.7	689.5	1.3	240.7	546.0	-1.2	136.0	546.0	1.1	
YBL099W	*ATP1*		7487_at	YBL099W	1009.5	-1.1	150.3	1009.5	-1.0	0.0	1258.4	1.1	187.4	1258.4	1.1	
YBR039W	*ATP3*		7307_at	YBR039W	630.5	1.2	126.1	630.5	-1.0	0.0	526.3	-1.2	105.3	526.3	-1.2	
YGR020C	*VMA7*		4993_at	YGR020C	462.1	-1.4	184.8	462.1	-1.3	138.6	648.7	1.4	259.5	648.7	1.1	

	ORF	Gene	Alias	Probe	Description											
	YLR447C	*VMA6*		9851_at	YLR447C 3	508.6	-1.0	24.8	508.6	-1.3	150.6	518.0	1.0	25.3	518.0	-1.3
	YLR295C	*ATP14*		10010_at	YLR295C /	630.6	1.6	376.4	630.6	1.1	93.9	394.9	-1.6	235.7	394.9	-1.4
	YDL004W	*ATP16*		6503_at	YDL004W	720.0	1.2	179.3	720.0	1.0	35.1	558.7	-1.2	139.1	558.7	-1.3
	YPL271W	*ATP15*	*ATPEPSIL*	8024_at	YPL271W	1021.8	1.1	152.2	1021.8	-1.0	49.9	899.9	-1.1	134.0	899.9	-1.2
	Q0310			3976_at	F1F0-ATPa	804.9	1.2	200.4	804.9	-1.0	39.3	644.5	-1.2	160.5	644.5	-1.3
	YPR036W	*VMA13*	*CLS11*	7699_at	YPR036W	437.6	1.1	43.8	437.6	-1.3	152.7	403.1	-1.1	40.3	403.1	-1.5
	YDL185W	*TFP1*	*CLS8, VMA*	6679_at	YDL185W	690.5	-1.3	207.2	690.5	-1.3	241.0	892.6	1.3	267.8	892.6	-1.0
	YBR127C	*VMA2*	*ATPVS, VA*	7218_at	YBR127C I	734.2	-1.5	367.1	734.2	-1.4	329.7	1021.3	1.5	510.7	1021.3	1.0
	YEL027W	*CUP5*		5763_at	YEL027W	2242.6	-1.3	664.1	2242.6	-1.1	334.0	2929.6	1.3	867.6	2929.6	1.1
	YOR332W	*VMA4*		8222_at	YOR332W	978.3	-1.3	341.5	978.3	-1.3	341.5	1290.1	1.3	450.3	1290.1	-1.0
	YHR039C	*MSC7*	*VMA10*	4514_at	YHR039C	783.6	-1.3	235.1	783.6	-1.4	351.9	1017.3	1.3	305.2	1017.3	-1.1
	YPL234C	*TFP3*	*CLS9, VMA*	7970_at	YPL234C 1	1186.4	-1.3	355.9	1186.4	-1.3	355.9	1511.7	1.3	453.5	1511.7	-1.1
	YOR270C	*VPH1*		8291_at	YOR270C	1344.7	-1.3	403.4	1344.7	-1.3	469.4	1771.5	1.3	531.5	1771.5	-1.0
homeostasis of other cations																
	YKL190W	*CNB1*		10772_at	YKL190W	0.0	0.0	0.0	0.0	0.0	0.0	575.0	1.1	57.5	575.0	1.2
homeostasis of anions																
homeostasis of sulfates																
	YBR294W	*SUL1*		7067_at	Putative su	97.3	1.3	33.9	97.3	4.0	291.6	70.8	-1.3	24.7	70.8	2.9
	YLR092W	*SUL2*		10256_at	YLR092W	560.9	1.1	56.1	560.9	1.2	112.2	545.7	-1.1	54.6	545.7	1.1
homeostasis of phosphate																
	YJL117W	*PHO86*		11132_at	YJL117W F	0.0	0.0	0.0	0.0	0.0	0.0	473.2	1.0	23.1	473.2	1.3
	YJR077C	*MIR1*		10961_at	YJR077C i	1731.2	1.1	257.8	1731.2	1.0	84.5	1524.8	-1.1	227.1	1524.8	-1.1
cellular sensing and response																
chemoperception and response																
perception of nutrients and nutritional adaptation																
	YKL198C	*PTK1*	*KKT8, STK*	3948_s_at	YKL198C p	6.7	2.2	8.4	6.7	2.6	10.6	4.3	-2.2	5.4	4.3	1.5
	YPL106C	*SSE1*	*LPG3, MSI*	7874_at	YPL106C H	672.7	-1.0	0.0	672.7	-1.2	134.5	633.8	-1.0	0.0	633.8	-1.2
	YNL098C	*RAS2*		8979_at	YNL098C F	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3
pheromone response																
	YIL117C	*PRM5*		4194_at	YIL117C pl	67.2	1.2	16.2	67.2	2.7	113.0	59.1	-1.2	14.2	59.1	2.2
	YDR461W	*MFA1*		6021_f_at	YDR461W	31.6	2.2	39.3	31.6	1.9	28.3	11.8	-2.2	14.7	11.8	-1.1
	YIL037C	*PRM2*		4134_at	Pheromone	7.6	-4.0	22.6	7.6	-2.6	11.9	29.4	4.0	87.5	29.4	1.8
	YAR031W	*PRM9*		11293_at	YAR031W	10.8	-1.0	0.5	10.8	2.7	18.1	11.4	1.0	0.5	11.4	2.9
	YJL157C	*FAR1*		11181_at	YJL157C F	346.1	-1.3	120.8	346.1	-1.6	206.5	476.4	1.3	166.3	476.4	-1.2
	YKL178C	*STE3*		10741_at	YKL178C a	887.2	-1.1	132.1	887.2	-1.4	351.7	1048.9	1.1	156.2	1048.9	-1.2
	YLR229C	*CDC42*		10121_at	Member of	1147.0	-1.0	56.0	1147.0	-1.3	400.4	1103.3	1.0	53.9	1103.3	-1.3
osmosensing																
	YHR030C	*SLT2*	*BYC2, MPK*	4504_at	YHR030C	187.6	1.6	111.9	187.6	1.6	121.8	118.8	-1.6	70.9	118.8	1.1
	YDL022W	*GPD1*	*DAR1, HOI*	6485_at	YDL022W	763.2	1.1	113.7	763.2	-1.0	0.0	647.3	-1.1	96.4	647.3	-1.2
	YFL014W	*HSP12*		5372_at	YFL014W	625.4	1.8	493.3	625.4	-1.2	122.5	350.2	-1.8	276.3	350.2	-2.1

CELL FATE

cell growth / morphogenesis

ORF	Gene	Probe	Description												
YLR175W	*CBF5*	10155_at	YLR175W	795.5	-1.0	0.0	795.5	1.4	318.2	829.9	-1.0	0.0	829.9	1.4	332.0
YNL138W	*SRV2*	8984_at	YNL138W	473.5	1.1	70.5	473.5	1.9	424.9	409.0	-1.1	60.9	409.0	1.6	245.4
YKL190W	*CNB1*	10772_at	YKL190W	0.0	0.0	0.0	0.0	0.0	0.0	575.0	1.1	57.5	575.0	1.2	115.0
YOR027W	*STI1*	8545_at	YOR027W	0.0	0.0	0.0	0.0	0.0	0.0	288.3	-1.1	42.9	288.3	-1.4	115.3
YNL079C	*TPM1*	8953_at	YNL079C t	713.2	1.0	34.8	713.2	-1.2	142.6	676.8	-1.0	33.0	676.8	-1.2	135.4
YER177W	*BMH1*	5525_at	YER177W	1125.3	-1.1	112.5	1125.3	-1.3	392.8	1252.2	1.1	125.2	1252.2	-1.2	250.4

Pectora roborant cultus recti

YPR052C	*NHP6A*		7717_at	YPR052C	530.1	-1.1	65.8	530.1	-1.6	328.4	596.6	1.1	74.1	596.6	-1.4	265.9
YLR300W	*EXG1*	*BGL1*	10015_at	YLR300W	1585.6	1.0	77.4	1585.6	1.1	236.1	0.0	0.0	0.0	0.0	0.0	0.0
YJL174W	*KRE9*		11209_at	YJL174W (	643.5	1.2	128.7	643.5	1.2	160.2	581.8	-1.2	116.4	581.8	1.0	0.0
YAL040C	*CLN3*	*DAF1, FUI*	11369_at	YAL040C (	188.8	1.2	37.8	188.8	1.7	132.1	0.0	0.0	0.0	0.0	0.0	0.0
YCR034W	*FEN1*	*ELO2, GN*	6869_at	YCR034W	728.3	-1.1	72.8	728.3	-1.2	145.7	0.0	0.0	0.0	0.0	0.0	0.0
YDR284C	*DPP1*		6204_at	YDR284C	276.7	-1.2	55.3	276.7	-1.5	151.9	0.0	0.0	0.0	0.0	0.0	0.0

directional cell growth (morphogenesis)

YHR030C	*SLT2*	*BYC2, MP*	4504_at	YHR030C	187.6	1.6	111.9	187.6	1.6	121.8	118.8	-1.6	70.9	118.8	1.1	11.9
YPR165W	*RHO1*		7563_at	Ras homol	819.3	-1.0	0.0	819.3	-1.2	163.9	828.3	-1.0	0.0	828.3	-1.2	165.7
YLR229C	*CDC42*		10121_at	Member of	1147.0	-1.0	56.0	1147.0	-1.3	400.4	1103.3	1.0	53.9	1103.3	-1.3	331.0

other morphogenetic activities

YPL106C	*SSE1*	*LPG3, MSI*	7874_at	YPL106C I	672.7	-1.0	0.0	672.7	-1.2	134.5	633.8	-1.0	0.0	633.8	-1.2
YBR109C	*CMD1*		7245_at	YBR109C (	758.7	-1.1	75.9	758.7	-1.2	151.7	0.0	0.0	0.0	0.0	0.0

cell differentiation

fungal cell differentiation

budding, cell polarity and filament formation

YLR372W	*SUR4*	*ELO3, SRE*	9953_at	YLR372W	1590.9	-1.2	318.2	1590.9	-1.0	0.0	1910.0	1.2	382.0	1910.0	1.2
YNL138W	*SRV2*		8984_at	YNL138W	473.5	1.1	70.5	473.5	1.9	424.9	409.0	-1.1	60.9	409.0	1.6
YGR214W			4780_at	YGR214W	1457.7	-1.2	285.4	1457.7	-1.1	217.1	1570.5	1.2	307.5	1570.5	1.1
YJL159W	*HSP150*	*CCW7, OR*	11179_at	Kex2-proce	1519.4	1.0	74.2	1519.4	1.1	226.3	1451.1	-1.0	70.8	1451.1	1.1
YNL271C	*BNI1*	*PPF3*	9123_at	Cytoskeleta	94.5	1.3	28.0	94.5	3.4	229.7	108.6	-1.3	32.2	108.6	2.1
YLR267W	*BOP2*		10072_at	Bypass of I	21.8	1.8	18.5	21.8	7.2	136.2	12.0	-1.8	10.2	12.0	4.3
YCL068C	*??*		6957_s_at	YCL068C E	27.3	1.3	7.2	27.3	2.5	41.8	20.9	-1.3	5.5	20.9	2.0
YMR063W	*RIM9*		9593_at	Required fo	14.5	3.3	33.8	14.5	6.0	72.3	5.6	-3.3	13.1	5.6	2.6
YIL159W	*BNR1*		4242_at	Bni1p-relat	3.3	-2.0	3.3	3.3	-1.7	2.2	6.5	2.0	6.5	6.5	1.1
YPL255W	*BBP1*		7994_at	YPL255W	0.0	0.0	0.0	0.0	0.0	0.0	4.1	-1.5	2.0	4.1	-2.4
YJL157C	*FAR1*		11181_at	YJL157C F	346.1	-1.3	120.8	346.1	-1.6	206.5	476.4	1.3	166.3	476.4	-1.2
YFL005W	*SEC4*	*SRO6*	5383_at	Ras-like sn	416.8	-1.0	0.0	416.8	-1.3	123.4	416.5	-1.0	0.0	416.5	-1.3
YNL079C	*TPM1*		8953_at	YNL079C t	713.2	1.0	34.8	713.2	-1.2	142.6	676.8	-1.0	33.0	676.8	-1.2
YPR165W	*RHO1*		7563_at	Ras homol	819.3	-1.0	0.0	819.3	-1.2	163.9	828.3	-1.0	0.0	828.3	-1.2
YPR052C	*NHP6A*		7717_at	YPR052C	530.1	-1.1	65.8	530.1	-1.6	328.4	596.6	1.1	74.1	596.6	-1.4
YLR229C	*CDC42*		10121_at	Member of	1147.0	-1.0	56.0	1147.0	-1.3	400.4	1103.3	1.0	53.9	1103.3	-1.3
YJL174W	*KRE9*		11209_at	YJL174W (	643.5	1.2	128.7	643.5	1.2	160.2	581.8	-1.2	116.4	581.8	1.0
YAL040C	*CLN3*	*DAF1, FUI*	11369_at	YAL040C (	188.8	1.2	37.8	188.8	1.7	132.1	0.0	0.0	0.0	0.0	0.0
YHR030C	*SLT2*	*BYC2, MP*	4504_at	YHR030C	187.6	1.6	111.9	187.6	1.6	121.8	118.8	-1.6	70.9	118.8	1.1
YER133W	*GLC7*	*CID1, DIS2*	5573_at	protein pho	242.7	1.2	40.7	242.7	1.3	84.2	217.2	-1.2	36.4	217.2	1.1
YOR030W	*DFG16*	*ECM41, ZI*	8503_at	YOR030W	4.4	2.1	4.8	4.4	2.2	5.5	1.1	-2.1	1.2	1.1	1.3
YMR238W	*DFG5*		9377_at	YMR238W	292.8	-1.0	14.3	292.8	-1.4	116.1	0.0	0.0	0.0	0.0	0.0
YCR034W	*FEN1*	*ELO2, GN*	6869_at	YCR034W	728.3	-1.1	72.8	728.3	-1.2	145.7	0.0	0.0	0.0	0.0	0.0
YBR109C	*CMD1*		7245_at	YBR109C (	758.7	-1.1	75.9	758.7	-1.2	151.7	0.0	0.0	0.0	0.0	0.0
YFL039C	*ACT1*	*ABY1, ENL*	5392_at	YFL039C A	2061.4	-1.2	403.7	2061.4	-1.2	403.7	2477.1	1.2	485.1	2477.1	1.0

pheromone response, mating-type determination, sex-specific proteins

YCL067C	*HMLALPH*	*ALPHA2, M*	6958_s_at	Mating type	210.9	1.0	10.3	210.9	6.2	1106.8	201.1	-1.0	9.8	201.1	6.0
YLR441C			9890_s_at	YLR441C F	2182.4	-1.2	431.9	2182.4	-1.1	42.2	2569.5	1.2	508.5	2569.5	1.1
YPL187W	*MF(ALPHA)1*		7927_at	YPL187W	2437.9	-1.1	363.0	2437.9	-1.0	119.0	2754.2	1.1	410.1	2754.2	1.1
YBR082C	*UBC4*		7260_at	YBR082C (	946.7	1.1	94.7	946.7	1.5	473.3	840.4	-1.1	84.0	840.4	1.3
YNL238W	*KEX2*	*QDS1, VM*	9110_at	Ca2+-depe	189.7	1.2	37.1	189.7	3.0	385.1	156.4	-1.2	30.6	156.4	2.5
YJR004C	*SAG1*	*AG(ALPHA*	11026_at	YJR004C a	1562.2	-1.3	545.3	1562.2	-1.2	312.4	2131.1	1.3	743.9	2131.1	1.1
YNR044W	*AGA1*		8802_at	YNR044W	473.1	-1.3	140.1	473.1	-1.0	23.1	650.6	1.3	192.7	650.6	1.2
YNL145W	*MFA2*		9023_at	Mating a-fa	22.9	1.0	1.0	22.9	7.0	137.4	21.9	-1.0	1.0	21.9	6.8

			YNL271C	*BNI1*	*PPF3*	9123_at	Cytoskeleta	94.5	1.3	28.0	94.5	3.4	229.7	108.6	-1.3	32.2	108.6	2.1
			YKL190W	*CNB1*		10772_at	YKL190W '	0.0	0.0	0.0	0.0	0.0	0.0	575.0	1.1	57.5	575.0	1.2
			YPL240C	*HSP82*	*HSP83, HS*	8010_i_at	YPL240C H	0.0	0.0	0.0	0.0	0.0	0.0	514.6	-1.0	0.0	514.6	1.2
			YCR097W	*HMRA1*	*YCR097W*	6792_s_at	YCR097W	0.0	0.0	0.0	0.0	0.0	0.0	2.4	1.2	0.4	2.4	2.1
			YJL157C	*FAR1*		11181_at	YJL157C F	346.1	-1.3	120.8	346.1	-1.6	206.5	476.4	1.3	166.3	476.4	-1.2
			YDR214W	*AHA1*		6271_at	YDR214W	0.0	0.0	0.0	0.0	0.0	0.0	295.8	-1.1	28.2	295.8	-1.4
			YNL079C	*TPM1*		8953_at	YNL079C t	713.2	1.0	34.8	713.2	-1.2	142.6	676.8	-1.0	33.0	676.8	-1.2
			YNL307C	*MCK1*		9177_at	YNL307C 4	624.7	-1.1	93.0	624.7	-1.3	218.1	701.8	1.1	104.5	701.8	-1.2
			YKL178C	*STE3*		10741_at	YKL178C a	887.2	-1.1	132.1	887.2	-1.4	351.7	1048.9	1.1	156.2	1048.9	-1.2
			YLR229C	*CDC42*		10121_at	Member of	1147.0	-1.0	56.0	1147.0	-1.3	400.4	1103.3	1.0	53.9	1103.3	-1.3
			YJL212C	*OPT1*	*HGT1*	11260_at	Oligopeptic	238.5	2.1	274.1	238.5	3.4	571.9	113.1	-2.1	130.0	113.1	1.6
			YKL104C	*GFA1*		10680_at	YKL104C (	276.7	1.6	179.6	276.7	1.9	262.6	169.2	-1.6	109.9	169.2	1.1
			YLR300W	*EXG1*	*BGL1*	10015_at	YLR300W	1585.6	1.0	77.4	1585.6	1.1	236.1	0.0	0.0	0.0	0.0	0.0
			YJL174W	*KRE9*		11209_at	YJL174W (	643.5	1.2	128.7	643.5	1.2	160.2	581.8	-1.2	116.4	581.8	1.0
			YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2
			YDR461W	*MFA1*		6021_f_at	YDR461W	31.6	2.2	39.3	31.6	1.9	28.3	11.8	-2.2	14.7	11.8	-1.1
			YBR040W	*FIG1*		7308_at	Integral me	18.7	-5.3	81.1	18.7	-2.7	32.6	99.9	5.3	434.4	99.9	1.9
			YDL227C	*HO*		6725_at	YDL227C H	163.5	-1.4	73.4	163.5	-1.8	138.9	234.2	1.4	105.2	234.2	-1.3
			YCR034W	*FEN1*	*ELO2, GNS*	6869_at	YCR034W	728.3	-1.1	72.8	728.3	-1.2	145.7	0.0	0.0	0.0	0.0	0.0
			YBR109C	*CMD1*		7245_at	YBR109C (	758.7	-1.1	75.9	758.7	-1.2	151.7	0.0	0.0	0.0	0.0	0.0
			YGL089C	*MF(ALPHA)2*		5108_at	YGL089C a	1468.1	-1.5	729.2	1468.1	-1.4	659.4	2220.7	1.5	1102.9	2220.7	1.0
		sporulation and germination																
			YBR082C	*UBC4*		7260_at	YBR082C u	946.7	1.1	94.7	946.7	1.5	473.3	840.4	-1.1	84.0	840.4	1.3
			YKL165C	*MCD4*	*ZRG16*	10754_at	YKL165C M	637.9	1.2	127.6	637.9	1.4	255.2	584.3	-1.2	116.9	584.3	1.2
			YPL240C	*HSP82*	*HSP83, HS*	8010_i_at	YPL240C H	0.0	0.0	0.0	0.0	0.0	0.0	514.6	-1.0	0.0	514.6	1.2
			YNR019W	*ARE2*	*SAT1*	8867_at	YNR019W	2.1	-2.1	2.4	2.1	-1.7	1.6	8.4	2.1	9.6	8.4	1.2
			YMR063W	*RIM9*		9593_at	Required fo	14.5	3.3	33.8	14.5	6.0	72.3	5.6	-3.3	13.1	5.6	2.6
			YBR148W	*YSW1*		7194_at	YBR148W	7.5	1.0	0.3	7.5	2.5	11.4	6.2	-1.0	0.2	6.2	2.4
			YDR523C	*SPS1*		5947_at	YDR523C (	5.0	2.0	5.2	5.0	-1.2	0.9	2.0	-2.0	2.1	2.0	-2.3
			YBL066C	*SEF1*		7430_at	Putative tra	9.5	4.0	28.5	9.5	1.8	7.6	5.2	-4.0	15.6	5.2	-2.2
			YDR214W	*AHA1*		6271_at	YDR214W	0.0	0.0	0.0	0.0	0.0	0.0	295.8	-1.1	28.2	295.8	-1.4
			YNL307C	*MCK1*		9177_at	YNL307C 4	624.7	-1.1	93.0	624.7	-1.3	218.1	701.8	1.1	104.5	701.8	-1.2
			YNL098C	*RAS2*		8979_at	YNL098C F	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3
			YLL039C	*UBI4*	*SCD2*	10392_at	YLL039C u	432.6	1.3	151.0	432.6	-1.1	64.4	321.2	-1.3	112.1	321.2	-1.5
			YBR078W	*ECM33*		7302_at	YBR078W	1606.6	-1.2	321.3	1606.6	-1.2	400.0	1885.9	1.2	377.2	1885.9	-1.1
			YLR300W	*EXG1*	*BGL1*	10015_at	YLR300W	1585.6	1.0	77.4	1585.6	1.1	236.1	0.0	0.0	0.0	0.0	0.0
			YCR048W	*ARE1*	*SAT2*	6837_at	YCR048W	178.6	1.3	62.3	178.6	1.6	107.2	0.0	0.0	0.0	0.0	0.0
			YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2
			YIL099W	*SGA1*		4212_g_at	Intracellula	40.4	2.4	56.6	40.4	2.3	52.4	25.6	-2.4	35.8	25.6	-1.0
			YGL180W	*APG1*	*AUT3*	5200_at	YGL180W	21.5	1.7	16.1	21.5	2.1	24.7	0.0	0.0	0.0	0.0	0.0
			YNL012W	*SPO1*		8883_at	YNL012W	10.8	2.2	13.1	10.8	1.3	3.2	4.3	-2.2	5.3	4.3	-1.8
			YMR154C	*RIM13*	*CPL1*	9465_at	YMR154C	8.5	2.1	9.5	8.5	-1.0	0.0	2.7	-2.1	3.0	2.7	-1.9
			YIL084C	*SDS3*		4182_at	YIL084C F	4.6	2.0	4.8	4.6	-1.2	0.9	0.0	0.0	0.0	0.0	0.0
			YNL204C	*SPS18*	*SPX18*	9055_at	YNL204C s	4.2	-1.8	3.4	4.2	-2.7	7.2	0.0	0.0	0.0	0.0	0.0
			YCR034W	*FEN1*	*ELO2, GNS*	6869_at	YCR034W	728.3	-1.1	72.8	728.3	-1.2	145.7	0.0	0.0	0.0	0.0	0.0
			YOL139C	*CDC33*	*TIF45*	8695_at	YOL139C r	1080.1	-1.1	108.0	1080.1	-1.2	216.0	0.0	0.0	0.0	0.0	0.0
cell death																		
	YKR042W	*UTH1*		10514_at	YKR042W	1982.9	-1.0	0.0	1982.9	1.2	396.6	2155.3	-1.0	0.0	2155.3	1.2	431.1	
	YJL116C	*NCA3*		11133_at	YJL116C V	138.4	2.0	144.6	138.4	2.8	255.5	67.9	-2.0	70.9	67.9	1.4	27.2	
	YMR190C	*SGS1*		9461_at	YMR190C	0.0	0.0	0.0	0.0	0.0	0.0	11.3	-1.4	4.5	11.3	-2.1	12.4	

Category	ORF	Gene	Alias	Probe	Description													
	YKL008C	*LAC1*		10599_at	YKL008C L	420.6	-1.2	104.7	420.6	-1.1	62.6	518.8	1.2	129.2	518.8	1.1	51.9	
	YNL098C	*RAS2*		8979_at	YNL098C F	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3	143.6	
CONTROL OF CELLULAR ORGANIZATION																		
cell wall																		
	YKL184W	*SPE1*	*ORD1, SP*	10779_at	YKL184W	159.9	1.8	127.9	159.9	2.4	231.8	89.2	-1.8	71.4	89.2	1.4	35.7	
	YAL054C	*ACS1*		11356_at	YAL054C i	350.3	1.2	87.2	350.3	1.3	122.3	0.0	0.0	0.0	0.0	0.0	0.0	
	YGR008C	*STF2*		4980_at	YGR008C	783.8	1.3	235.1	783.8	1.1	116.7	605.4	-1.3	181.6	605.4	-1.1	60.5	
	YER150W	*SPI1*		5544_at	YER150W	194.0	1.6	115.8	194.0	1.1	28.9	121.0	-1.6	72.2	121.0	-1.4	48.4	
	YML048W	*GSF2*	*ECM6*	9709_at	YML048W	0.0	0.0	0.0	0.0	0.0	0.0	33.1	1.3	9.2	33.1	2.2	39.7	
	YOR030W	*DFG16*	*ECM41, ZI*	8503_at	YOR030W	4.4	2.1	4.8	4.4	2.2	5.5	1.1	-2.1	1.2	1.1	1.3	0.3	
	YCL004W	*PGS1*	*PEL1*	6875_at	17-kDa Ph	6.6	3.2	14.7	6.6	1.4	2.5	0.0	-3.2	0.0	0.0	-2.4	0.0	
	YPR069C	*SPE3*		7689_at	YPR069C		167.8	-1.4	75.4	167.8	-1.6	100.2	242.2	1.4	108.8	242.2	-1.1	24.2	
	YPL053C	*KTR6*	*MNN6*	7790_at	YPL053C r	0.0	0.0	0.0	0.0	0.0	0.0	406.6	1.2	101.2	406.6	1.1	40.7	
	YBR078W	*ECM33*		7302_at	YBR078W	1606.6	-1.2	321.3	1606.6	-1.2	400.0	1885.9	1.2	377.2	1885.9	-1.1	188.6	
	YLR249W	*YEF3*	*TEF3*	10097_at	YLR249W	2805.7	-1.1	417.8	2805.7	-1.1	417.8	3188.9	1.1	474.9	3188.9	1.0	0.0	
	YMR307W	*GAS1*	*CWH52, G*	9315_at	YMR307W	2126.2	-1.2	425.2	2126.2	-1.2	425.2	2549.2	1.2	509.8	2549.2	-1.0	0.0	
	YDL055C	*PSA1*	*MPG1, VIC*	6543_at	YDL055C r	1894.9	-1.2	471.8	1894.9	-1.2	471.8	2378.1	1.2	592.1	2378.1	1.0	0.0	
	YLR110C	*CCW12*		10229_at	YLR110C c	4285.1	-1.2	1067.0	4285.1	-1.1	638.1	5335.7	1.2	1328.6	5335.7	1.1	533.6	
cytoskeleton																		
	YPL250C	*ICY2*		7999_at	YPL250C I	734.1	1.1	109.3	734.1	1.8	587.3	630.2	-1.1	93.8	630.2	1.5	315.1	
	YLR175W	*CBF5*		10155_at	YLR175W	795.5	-1.0	0.0	795.5	1.4	318.2	829.9	-1.0	0.0	829.9	1.4	332.0	
	YMR195W	*ICY1*		9422_at	YMR195W	815.3	1.3	244.6	815.3	1.3	244.6	645.9	-1.3	193.8	645.9	1.0	0.0	
	YKL004W	*AUR1*		10560_at	YKL004W	618.5	-1.2	123.7	618.5	-1.0	0.0	749.2	1.2	149.8	749.2	1.2	149.8	
	YLL038C	*ENT4*		10393_at	YLL038C c	4.3	2.2	5.4	4.3	-1.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0	
Golgi																		
	YDL137W	*ARF2*		6638_at	YDL137W	1842.3	-1.1	175.8	1842.3	-1.2	360.8	0.0	0.0	0.0	0.0	0.0	0.0	
nucleus																		
organization of chromosome structure					2	19 ORFs												
		YLR453C	*RIF2*		9857_at	YLR453C I	12.2	2.0	12.8	12.2	2.3	16.5	5.9	-2.0	6.2	5.9	1.1	0.6
		YBL032W	*HEK2*	*KHD1*	7420_at	YBL032W	115.2	-1.6	69.1	115.2	-2.0	120.9	0.0	0.0	0.0	0.0	0.0	0.0
mitochondrion																		
	YIL051C	*MMF1*	*IBM1*	4166_at	YIL051C M	768.9	-1.0	37.5	768.9	1.2	150.6	816.6	1.0	39.9	816.6	1.3	245.0	
	YCL004W	*PGS1*	*PEL1*	6875_at	17-kDa Ph	6.6	3.2	14.7	6.6	1.4	2.5	0.0	-3.2	0.0	0.0	-2.4	0.0	
other control of cellular organization				1	7													
	YMR131C	*RRB1*		9484_at	YMR131C	431.3	1.3	150.6	431.3	1.1	64.2	322.8	-1.3	112.7	322.8	-1.2	64.6	
SUBCELLULAR LOCALISATION																		
cell wall																		
	YKL097W	*??*		10688_at	YKL097W (	3109.4	-1.2	608.9	3109.4	1.0	151.8	3690.9	1.2	722.8	3690.9	1.2	738.2	
	YGR189C	*CRH1*		4799_at	YGR189C	582.5	1.2	116.5	582.5	1.6	378.2	470.8	-1.2	94.2	470.8	1.3	141.2	
	YKL163W	*PIR3*	*CCW8*	10756_at	Protein cor	144.4	4.2	461.2	144.4	3.3	338.7	34.4	-4.2	109.9	34.4	-1.1	3.4	
	YER011W	*TIR1*	*SRP1*	5711_at	YER011W	690.6	-1.2	172.0	690.6	1.1	69.1	684.8	1.2	170.5	684.8	1.4	273.9	
	YJL159W	*HSP150*	*CCW7, OF*	11179_at	Kex2-proce	1519.4	1.0	74.2	1519.4	1.1	226.3	1451.1	-1.0	70.8	1451.1	1.1	145.1	
	YGR282C	*BGL2*		4712_at	YGR282C	1370.4	-1.1	204.1	1370.4	-1.0	66.9	1589.9	1.1	236.8	1589.9	1.1	159.0	
	YKL164C	*CCW6*	*PIR1*	10755_at	Ccw protei	1316.5	1.1	131.7	1316.5	1.0	64.3	1226.1	-1.1	122.6	1226.1	-1.0	0.0	
	YOR010C	*TIR2*	*SRP2*	8527_at	YOR010C	0.0	0.0	0.0	0.0	0.0	0.0	244.1	1.1	24.4	244.1	1.5	122.1	
	YBR040W	*FIG1*		7308_at	Integral me	18.7	-5.3	81.1	18.7	-2.7	32.6	99.9	5.3	434.4	99.9	1.9	89.9	
	YAR050W	*FLO1*	*FLO2, FLC*	11301_i_at	Putative ce	2.3	3.0	4.7	2.3	-3.2	5.1	-3.7	-3.0	-7.5	-3.7	-5.3	-15.9	

YMR305C	*SCW10*		9312_at	YMR305C	1138.9	-1.0	0.0	1138.9	-1.2	227.8	1022.2	-1.0	0.0	1022.2	-1.2	204.4
YGR279C	*SCW4*		4709_at	YGR279C	1742.7	-1.1	259.5	1742.7	-1.1	259.5	2027.2	1.1	301.9	2027.2	1.0	0.0
YBR067C	*TIP1*		7290_at	YBR067C (	1652.2	-1.1	246.0	1652.2	-1.2	411.4	1934.7	1.1	288.1	1934.7	-1.1	193.5
YLR110C	*CCW12*		10229_at	YLR110C (	4285.1	-1.2	1067.0	4285.1	-1.1	638.1	5335.7	1.2	1328.6	5335.7	1.1	533.6

plasma membrane

YDR046C	*BAP3*		6415_at	Valine tran	670.0	2.6	1072.0	670.0	178.1	118656.5	297.2	-2.6	475.5	297.2	81.3	23865.2
YBR068C	*BAP2*		7291_at	Major AA p	646.7	2.3	839.2	646.7	133.8	85884.2	280.3	-2.3	363.8	280.3	58.7	16173.3
YPL265W	*DIP5*		8030_at	Dicarboxyli	911.4	2.2	1085.3	911.4	18.4	15885.5	416.4	-2.2	495.9	416.4	8.5	3123.0
YGR055W	*MUP1*		4936_at	High affinity	968.3	2.0	968.3	968.3	9.0	7745.5	486.4	-2.0	486.4	486.4	4.5	1702.4
YBR069C	*TAT1*	*VAP1, WA*	7292_at	Amino acid	164.5	3.1	345.1	164.5	11.2	1685.2	53.1	-3.1	111.4	53.1	4.1	164.6
YBL042C	*FUI1*		7410_at	High affinity	254.7	7.2	1578.8	254.7	7.4	1629.8	31.8	-7.2	197.2	31.8	1.0	0.0
YMR058W	*FET3*		9588_at	YMR058W	900.7	2.1	988.6	900.7	2.4	1259.1	422.2	-2.1	463.4	422.2	1.1	42.2
YBR021W	*FUR4*		7334_at	Uracil perm	208.8	4.1	656.1	208.8	6.4	1124.9	41.4	-4.1	130.1	41.4	1.5	20.7
YDR077W	*SED1*		6401_at	YDR077W	1872.7	1.3	561.8	1872.7	1.5	1028.5	1464.7	-1.3	439.4	1464.7	1.2	292.9
YNL268W	*LYP1*		9126_at	YNL268W	580.1	-1.0	0.0	580.1	2.3	782.8	570.8	-1.0	0.0	570.8	2.3	742.0
YKR093W	*PTR2*		10472_at	YKR093W	148.2	2.7	258.7	148.2	5.8	718.0	48.2	-2.7	84.2	48.2	2.1	53.0
YGL008C	*PMA1*		5009_at	YGL008C p	0.0	0.0	0.0	0.0	0.0	0.0	2122.2	1.1	212.2	2122.2	1.2	424.4
YPL092W	*SSU1*	*LPG16*	7843_at	YPL092W s	279.1	1.3	83.7	279.1	2.3	362.8	0.0	0.0	0.0	0.0	0.0	0.0
YEL017C	*PMP2*		5728_i_at	YEL017C P	1819.7	-1.3	488.1	1819.7	-1.1	214.0	2277.6	1.3	611.0	2277.6	1.1	339.2
YML116W	*ATR1*	*SNQ1*	9771_at	YML116W	507.3	1.1	50.7	507.3	1.6	329.4	459.7	-1.1	46.0	459.7	1.5	229.9
YER056C	*FCY2*	*BRA7*	5668_at	YER056C p	1819.5	-1.1	238.3	1819.5	1.0	19.9	2117.5	1.1	277.4	2117.5	1.1	311.1
YBR294W	*SUL1*		7067_at	Putative su	97.3	1.3	33.9	97.3	4.0	291.6	70.8	-1.3	24.7	70.8	2.9	134.5
YOL020W	*TAT2*	*LTG3, SAE*	8587_at	YOL020W	216.0	1.7	151.2	216.0	2.3	291.5	127.2	-1.7	89.0	127.2	1.4	50.9
YLR120C	*YPS1*		10193_at	YLR120C (	331.0	1.8	281.1	331.0	1.8	281.1	178.2	-1.8	151.3	178.2	-1.0	0.0
YDR497C	*ITR1*		5966_at	Myo-inosito	1187.5	-1.2	237.5	1187.5	-1.0	0.0	1358.0	1.2	271.6	1358.0	1.2	271.6
YDR276C	*PMP3*	*SNA1*	6196_at	YDR276C (	1952.2	-1.2	486.1	1952.2	-1.0	95.3	2416.2	1.2	601.6	2416.2	1.1	241.6
YCL025C	*AGP1*	*YCC5*	6907_at	YCL025C A	249.7	1.3	87.2	249.7	1.8	212.1	186.9	-1.3	65.2	186.9	1.4	74.8
YMR011W	*HXT2*		9633_at	YMR011W	809.5	-1.3	242.8	809.5	-1.0	0.0	1029.2	1.3	308.8	1029.2	1.2	205.8
YCR011C	*ADP1*		6888_at	YCR011C	0.0	0.0	0.0	0.0	0.0	0.0	282.6	1.0	13.8	282.6	1.7	197.8
YGL077C	*HNM1*		5074_at	YGL077C	544.7	-1.5	272.3	544.7	-1.2	108.9	924.4	1.5	462.2	924.4	1.2	184.9
YHR094C	*HXT1*	*HOR4*	4430_at	YHR094C	540.4	-1.0	0.0	540.4	1.3	162.1	525.9	-1.0	0.0	525.9	1.3	157.8
YDR345C	*HXT3*		6131_at	YDR345C	1351.7	-1.2	264.7	1351.7	-1.1	129.0	1606.0	1.2	314.5	1606.0	1.1	160.6
YKR039W	*GAP1*		10511_at	YKR039W	46.7	-2.4	65.2	46.7	-1.4	21.0	110.8	2.4	154.9	110.8	1.7	77.6
YMR008C	*PLB1*		9630_at	YMR008C	605.9	1.4	272.1	605.9	1.2	150.9	379.7	-1.4	170.5	379.7	-1.1	38.0
YNR044W	*AGA1*		8802_at	YNR044W	473.1	-1.3	140.1	473.1	-1.0	23.1	650.6	1.3	192.7	650.6	1.2	130.1
YER145C	*FTR1*		5585_at	YER145C I	260.0	1.1	30.5	260.0	1.5	124.6	143.9	-1.1	18.6	143.9	1.4	58.6
YLR214W	*FRE1*		10106_at	Ferric (and	107.8	1.5	59.2	107.8	1.7	80.8	63.6	-1.5	34.9	63.6	1.1	6.4
YER123W	*YCK3*	*CKI3*	5608_at	YER123W	35.8	1.4	16.1	35.8	2.3	48.2	0.0	0.0	0.0	0.0	0.0	0.0
YCL068C	*??*		6957_s_at	YCL068C E	27.3	1.3	7.2	27.3	2.5	41.8	20.9	-1.3	5.5	20.9	2.0	20.9
YFL011W	*HXT10*		5377_at	YFL011W I	4.7	-1.9	4.1	4.7	1.1	0.6	10.8	1.9	9.4	10.8	2.0	10.8
YMR243C	*ZRC1*	*OSR1*	9382_at	YMR243C	815.0	-1.0	39.8	815.0	-1.1	121.4	0.0	0.0	0.0	0.0	0.0	0.0
YFL005W	*SEC4*	*SRO6*	5383_at	Ras-like sn	416.8	-1.0	0.0	416.8	-1.3	123.4	416.5	-1.0	0.0	416.5	-1.3	125.0
YNL098C	*RAS2*		8979_at	YNL098C F	0.0	0.0	0.0	0.0	0.0	0.0	478.7	-1.1	47.9	478.7	-1.3	143.6
YCR034W	*FEN1*	*ELO2, GN*	6869_at	YCR034W	728.3	-1.1	72.8	728.3	-1.2	145.7	0.0	0.0	0.0	0.0	0.0	0.0
YGR121C	*MEP1*	*AMT1*	4866_at	Ammonia p	42.9	-4.6	155.0	42.9	-5.4	189.0	195.4	4.6	706.8	195.4	-1.2	39.1
YOR153W	*PDR5*	*LEM1, YDI*	8400_at	Multidrug r	707.7	-1.3	209.6	707.7	-1.3	209.6	916.0	1.3	271.3	916.0	1.0	0.0
YDR342C	*HXT7*		6128_f_at	YDR342C	1141.2	-1.1	114.1	1141.2	-1.2	228.2	0.0	0.0	0.0	0.0	0.0	0.0
YLR342W	*FKS1*		9966_at	YLR342W	726.7	-1.1	69.4	726.7	-1.3	253.7	861.6	1.1	82.2	861.6	-1.2	172.3
YJR004C	*SAG1*	*AG(ALPHA*	11026_at	YJR004C a	1562.2	-1.3	545.3	1562.2	-1.2	312.4	2131.1	1.3	743.9	2131.1	1.1	213.1
YKL178C	*STE3*		10741_at	YKL178C a	887.2	-1.1	132.1	887.2	-1.4	351.7	1048.9	1.1	156.2	1048.9	-1.2	209.8

	YLR229C	*CDC42*		10121_at	Member of	1147.0	-1.0	56.0	1147.0	-1.3	400.4	1103.3	1.0	53.9	1103.3	-1.3	331.0
	YMR307W	*GAS1*	*CWH52, G*	9315_at	YMR307W	2126.2	-1.2	425.2	2126.2	-1.2	425.2	2549.2	1.2	509.8	2549.2	-1.0	0.0
	YDR343C	*HXT6*		6129_f_at	YDR343C	1105.6	-1.1	110.6	1105.6	-1.4	442.2	1197.1	1.1	119.7	1197.1	-1.3	359.1
	YNL142W	*MEP2*		9026_at	Ammonia t	75.9	-17.2	1229.5	75.9	-14.8	1051.2	1305.0	17.2	21139.5	1305.0	1.2	261.0
cytoplasm																	
	YHR128W	*FUR1*		4422_at	Uracil phos	441.7	48.6	21042.7	441.7	18.9	7891.4	9.1	-48.6	433.6	9.1	-2.8	16.4
	YNL104C	*LEU4*		8973_at	YNL104C a	1720.8	1.1	172.1	1720.8	2.0	1720.8	1616.3	-1.1	161.6	1616.3	1.8	1293.0
	YPR145W	*ASN1*		7588_at	YPR145W	1054.4	1.3	368.1	1054.4	2.6	1685.0	757.0	-1.3	264.2	757.0	1.9	681.3
	YJR145C			10893_s_a	YJR145C F	2528.9	-1.2	505.8	2528.9	1.1	376.6	3067.1	1.2	613.4	3067.1	1.4	1226.8
	YOL058W	*ARG1*	*ARG10*	8593_at	YOL058W	1917.4	1.1	191.7	1917.4	1.6	1150.4	1715.0	-1.1	171.5	1715.0	1.5	857.5
	YPL079W			7811_at	YPL079W	3889.3	-1.2	968.4	3889.3	-1.0	189.8	4786.7	1.2	1191.9	4786.7	1.2	957.3
	YGR192C	*TDH3*	*GLD1, HSI*	4802_i_at	Glyceralde	3727.1	-1.2	898.1	3727.1	-1.0	165.7	4578.3	1.2	1103.2	4578.3	1.2	915.7
	YPL111W	*CAR1*	*LPH15*	7869_at	Arginase /	304.8	2.1	350.3	304.8	3.8	853.4	142.4	-2.1	163.7	142.4	1.9	128.2
	YJR009C	*TDH2*	*GLD2*	11031_s_a	YJR009C g	3547.9	-1.1	528.3	3547.9	-1.0	0.0	4151.9	1.1	618.3	4151.9	1.2	830.4
	YLR344W			9968_s_at	YLR344W	3362.5	-1.2	837.2	3362.5	-1.0	164.1	4066.5	1.2	1012.6	4066.5	1.2	813.3
	YHR174W	*ENO2*		4334_i_at	YHR174W	2988.8	-1.3	1043.3	2988.8	-1.1	445.1	4037.7	1.3	1409.5	4037.7	1.2	807.5
	YLR075W			10239_at	YLR075W	2845.6	-1.2	708.6	2845.6	-1.0	138.9	3518.0	1.2	876.0	3518.0	1.2	703.6
	YKL180W			10738_i_at	YKL180W	2817.6	-1.2	563.5	2817.6	-1.0	0.0	3486.2	1.2	697.2	3486.2	1.2	697.2
	YHR021C			4494_at	YHR021C	2555.9	-1.2	636.4	2555.9	-1.0	124.7	3188.4	1.2	793.9	3188.4	1.2	637.7
	YLR340W			9964_at	YLR340W	2536.8	-1.2	631.6	2536.8	-1.0	123.8	3106.9	1.2	773.6	3106.9	1.2	621.4
	YDR382W			6080_at	YDR382W	2413.6	-1.3	714.8	2413.6	-1.1	230.4	3071.7	1.3	909.7	3071.7	1.2	614.3
	YJL136C			11158_i_at	YJL136C F	3299.5	-1.2	808.3	3299.5	-1.0	79.6	4054.7	1.2	993.3	4054.7	1.1	603.8
	YGL189C			5191_f_at	YGL189C I	2355.7	-1.3	636.4	2355.7	-1.1	230.2	2883.3	1.3	778.9	2883.3	1.2	564.6
	YLR303W	*MET17*	*MET15, MI*	10018_at	YLR303W	2391.4	-1.1	356.1	2391.4	-1.0	0.0	2776.8	1.1	413.5	2776.8	1.2	555.4
	YLR044C	*PDC1*		10296_at	YLR044C p	2283.0	-1.2	456.6	2283.0	-1.0	0.0	2763.9	1.2	552.8	2763.9	1.2	552.8
	YDL075W			6569_at	YDL075W	1960.6	-1.4	777.2	1960.6	-1.1	283.4	2750.1	1.4	1090.2	2750.1	1.2	550.0
	YOR293W			8269_f_at	YOR293W	1286.0	-1.2	320.2	1286.0	1.1	122.7	1609.6	1.2	400.8	1609.6	1.3	549.9
	YMR143W			9497_i_at	YMR143W	849.7	-1.2	211.6	849.7	1.2	175.1	1025.4	1.2	255.3	1025.4	1.5	533.1
	YPR080W	*TEF1*		7656_s_at	YPR080W	2055.9	-1.3	608.9	2055.9	-1.1	196.2	2619.5	1.3	775.8	2619.5	1.2	523.9
	YIL052C			4165_i_at	YIL052C R	2049.3	-1.3	606.9	2049.3	-1.0	0.0	2582.5	1.3	764.8	2582.5	1.2	516.5
	YMR242C			9381_s_at	YMR242C	1408.7	-1.2	281.7	1408.7	1.0	68.8	1685.4	1.2	337.1	1685.4	1.3	505.6
	YGL147C			5142_i_at	YGL147C I	472.5	-1.4	173.6	472.5	1.4	173.6	629.9	1.4	231.5	629.9	1.8	503.9
	YNL301C			9183_s_at	YNL301C F	1971.2	-1.2	490.8	1971.2	-1.0	47.5	2419.5	1.2	602.5	2419.5	1.2	483.9
	YBR082C	*UBC4*		7260_at	YBR082C	946.7	1.1	94.7	946.7	1.5	473.3	840.4	-1.1	84.0	840.4	1.3	252.1
	YEL054C			5781_i_at	YEL054C F	1913.3	-1.2	476.4	1913.3	-1.0	93.4	2365.3	1.2	589.0	2365.3	1.2	473.1
	YHL001W			4515_i_at	YHL001W	1047.4	-1.3	285.3	1047.4	1.1	65.1	1278.9	1.3	348.3	1278.9	1.4	470.0
	YMR230W			9413_f_at	YMR230W	798.7	-1.2	126.4	798.7	1.3	219.4	958.6	1.2	151.7	958.6	1.5	450.2
	YML063W			9691_i_at	YML063W	1232.7	-1.2	306.9	1232.7	1.0	60.2	1493.1	1.2	371.8	1493.1	1.3	447.9
	YLR333C			10002_i_at	YLR333C F	1534.6	-1.2	267.3	1534.6	1.0	65.4	1832.1	1.2	319.1	1832.1	1.2	441.5
	YLL018C	*DPS1*		9868_at	YLL018C a	496.3	1.7	328.2	496.3	1.9	433.5	408.8	-1.7	270.3	408.8	1.1	60.9
	YGL031C			5031_at	YGL031C I	1705.7	-1.3	505.1	1705.7	-1.1	162.8	2163.7	1.3	640.8	2163.7	1.2	432.7
	YGL030W			5032_at	YGL030W	1709.5	-1.2	411.9	1709.5	-1.0	0.0	2125.5	1.2	512.2	2125.5	1.2	425.1
	YKL006W			10558_s_a	YKL006W	2624.3	-1.3	687.1	2624.3	-1.1	293.1	3341.8	1.3	874.9	3341.8	1.1	423.1
	YBR196C	*PGI1*	*CDC30*	7152_at	Glucose-6-	1683.3	-1.2	419.1	1683.3	-1.0	82.2	2100.2	1.2	522.9	2100.2	1.2	420.0
	YDR035W	*ARO3*		6450_at	YDR035W	1396.4	1.1	139.6	1396.4	1.3	418.9	1273.6	-1.1	127.4	1273.6	1.2	254.7
	YIR034C	*LYS1*		4070_at	YIR034C s	1335.0	-1.0	65.2	1335.0	1.2	332.4	1385.0	1.0	67.6	1385.0	1.3	415.5
	YBR031W			7344_i_at	YBR031W	903.3	-1.5	411.8	903.3	-1.2	172.4	1175.7	1.5	536.0	1175.7	1.3	401.6
	YLR048W			10301_at	YLR048W	2484.7	-1.2	533.5	2484.7	-1.1	159.3	3017.4	1.2	647.8	3017.4	1.1	399.4
	YNL162W			9006_s_at	YNL162W	1629.0	-1.2	325.8	1629.0	-1.0	79.5	1986.6	1.2	397.3	1986.6	1.2	397.3
	YLR287C			10047_f_at	YLR287C F	3167.8	-1.2	788.8	3167.8	-1.0	154.6	3855.8	1.2	960.1	3855.8	1.1	385.6

YML073C			9726_at	YML073C I	1002.1	-1.2	249.5	1002.1	1.0	48.9	1281.1	1.2	319.0	1281.1	1.3	384.3
YLR406C			9897_f_at	YLR406C F	1821.9	-1.2	453.6	1821.9	-1.1	147.9	2302.1	1.2	573.2	2302.1	1.2	381.4
YDR418W			6069_i_at	YDR418W	1443.6	-1.4	524.7	1443.6	-1.1	199.9	1942.5	1.4	706.0	1942.5	1.2	380.4
YMR186W	*HSC82*		9456_at	YMR186W	1536.6	-1.2	300.9	1536.6	-1.0	0.0	1859.1	1.2	364.1	1859.1	1.2	371.8
YCR012W	*PGK1*		6890_at	3-Phospho	3009.0	-1.2	589.2	3009.0	-1.1	287.2	3629.6	1.2	710.8	3629.6	1.1	363.0
YOR182C			8384_f_at	YOR182C	2871.4	-1.2	715.0	2871.4	-1.1	274.1	3570.6	1.2	889.1	3570.6	1.1	357.1
YKL152C	*GPM1*		10721_at	Phosphogl	3118.6	-1.1	464.4	3118.6	-1.0	152.2	3538.3	1.1	526.9	3538.3	1.1	353.8
YMR116C	*ASC1*	*CPC2*	9512_at	YMR116C	2863.4	-1.2	572.7	2863.4	-1.1	286.3	3505.4	1.2	701.1	3505.4	1.1	350.5
YKL211C	*TRP3*		10801_at	YKL211C a	465.6	1.2	115.9	465.6	1.7	348.9	337.3	-1.2	84.0	337.3	1.4	134.9
YLR388W			9923_f_at	YLR388W	2857.6	-1.1	425.5	2857.6	-1.0	139.5	3370.6	1.1	501.9	3370.6	1.1	337.1
YDR012W			6473_i_at	YDR012W	1178.7	-1.4	529.4	1178.7	-1.2	293.5	1664.0	1.4	747.4	1664.0	1.2	332.8
YER074W			5647_s_at	YER074W	1173.0	-1.3	409.4	1173.0	-1.1	174.7	1562.3	1.3	545.4	1562.3	1.2	312.5
YER056C	*FCY2*	*BRA7*	5668_at	YER056C p	1819.5	-1.1	238.3	1819.5	1.0	19.9	2117.5	1.1	277.4	2117.5	1.1	311.1
YOL126C	*MDH2*		8663_at	Cytosolic m	126.7	1.9	113.7	126.7	3.4	309.9	66.1	-1.9	59.3	66.1	1.8	52.9
YNR050C	*LYS9*	*LYS13*	8808_at	YNR050C	0.0	0.0	0.0	0.0	0.0	0.0	1516.0	1.1	151.6	1516.0	1.2	303.2
YNL016W	*PUB1*		8879_at	YNL016W	329.2	1.6	197.5	329.2	1.9	296.2	203.9	-1.6	122.3	203.9	1.2	40.8
YHR053C	*CUP1-1*	*CUP1*	4483_s_at	YHR053C	1985.9	1.2	397.2	1985.9	1.1	295.7	0.0	0.0	0.0	0.0	0.0	0.0
YML024W			9686_s_at	YML024W	2444.4	-1.1	364.0	2444.4	-1.0	119.3	2901.7	1.1	432.1	2901.7	1.1	290.2
YOR369C			8168_at	YOR369C	2272.3	-1.2	445.0	2272.3	-1.0	110.9	2671.6	1.2	523.2	2671.6	1.1	267.2
YKL104C	*GFA1*		10680_at	YKL104C C	276.7	1.6	179.6	276.7	1.9	262.6	169.2	-1.6	109.9	169.2	1.1	16.9
YOR204W	*DED1*	*SPP81*	8361_at	YOR204W	325.4	1.8	260.3	325.4	1.3	97.6	169.7	-1.8	135.8	169.7	-1.3	50.9
YNL302C			9182_s_at	YNL302C F	2205.2	-1.1	328.4	2205.2	-1.0	107.6	2531.0	1.1	376.9	2531.0	1.1	253.1
YNL096C			8981_at	YNL096C F	1165.4	-1.0	56.9	1165.4	1.1	173.5	1260.1	1.0	61.5	1260.1	1.2	252.0
YDL215C	*GDH2*		6693_at	YDL215C N	199.9	1.8	169.7	199.9	2.2	249.7	107.7	-1.8	91.5	107.7	1.2	21.5
YCR053W	*THR4*		6841_at	YCR053W	0.0	0.0	0.0	0.0	0.0	0.0	802.0	1.1	80.2	802.0	1.3	240.6
YJL138C	*TIF2*		11156_s_a	YJL138C tr	1972.0	-1.1	293.7	1972.0	-1.0	96.3	2291.9	1.1	341.3	2291.9	1.1	229.2
YNL259C	*ATX1*		9135_at	YNL259C A	701.7	1.1	70.2	701.7	1.3	210.5	0.0	0.0	0.0	0.0	0.0	0.0
YCL018W	*LEU2*		6909_at	YCL018W	1041.6	1.3	312.5	1041.6	1.2	208.3	805.3	-1.3	241.6	805.3	-1.0	0.0
YIL148W			4253_i_at	YIL148W R	822.0	-1.2	198.1	822.0	-1.0	0.0	1017.9	1.2	245.3	1017.9	1.2	203.6
YOR167C			8369_f_at	YOR167C	1763.8	-1.2	352.8	1763.8	-1.0	38.8	2079.9	1.2	416.0	2079.9	1.1	198.5
YJL189W			11237_at	YJL189W F	1683.5	-1.2	336.7	1683.5	-1.1	168.3	1974.3	1.2	394.9	1974.3	1.1	197.4
YGL103W			5094_at	YGL103W	1673.9	-1.2	334.8	1673.9	-1.1	167.4	1962.7	1.2	392.5	1962.7	1.1	196.3
YJR109C	*CPA2*		10902_at	YJR109C c	778.2	1.1	115.9	778.2	1.2	193.8	0.0	0.0	0.0	0.0	0.0	0.0
YOR096W			8479_at	YOR096W	1643.4	-1.1	244.7	1643.4	-1.0	80.2	1915.7	1.1	285.3	1915.7	1.1	191.6
YPR102C			7632_i_at	YPR102C I	1550.4	-1.2	310.1	1550.4	-1.1	155.0	1849.3	1.2	369.9	1849.3	1.1	184.9
YOL127W			8662_at	YOL127W	1394.8	-1.2	347.3	1394.8	-1.1	139.5	1828.8	1.2	455.4	1828.8	1.1	182.9
YPL090C			7845_s_at	YPL090C F	1431.8	-1.2	286.4	1431.8	-1.1	143.2	1768.0	1.2	353.6	1768.0	1.1	176.8
YDR354W	*TRP4*		6095_at	YDR354W	321.6	1.2	80.1	321.6	1.5	176.6	0.0	0.0	0.0	0.0	0.0	0.0
YNL272C	*SEC2*		9122_at	Protein witl	57.0	1.0	2.8	57.0	3.8	159.2	56.4	-1.0	2.8	56.4	3.8	157.9
YPL262W	*FUM1*		8033_at	YPL262W	770.2	1.2	150.8	770.2	1.1	73.5	627.8	-1.2	122.9	627.8	-1.1	62.8
YDL130W	*RPP1B*	*RPL44', RI*	6600_at	YDL130W	1812.2	-1.0	89.9	1812.2	-1.0	11.0	2258.3	1.3	635.7	2258.3	1.1	148.1
YJR148W	*BAT2*	*TWT2, EC*	10896_at	YJR148W	0.0	0.0	0.0	0.0	0.0	0.0	204.4	-2.0	214.5	204.4	1.7	143.1
YCL040W	*GLK1*	*HOR3*	6937_at	YCL040W	686.5	1.6	411.9	686.5	1.2	137.3	437.3	-1.6	262.4	437.3	-1.3	131.2
YGR234W	*YHB1*	*YHB4*	4754_at	YGR234W	274.2	1.4	121.3	274.2	1.5	136.2	0.0	0.0	0.0	0.0	0.0	0.0
YKL081W	*TEF4*	*EFC1*	10658_at	YKL081W	389.0	-1.3	130.3	389.0	1.2	74.6	471.5	1.3	157.9	471.5	1.3	134.1
YDR188W	*CCT6*	*HTR3, TCI*	6290_at	YDR188W	379.4	1.3	132.4	379.4	1.2	75.9	0.0	0.0	0.0	0.0	0.0	0.0
YAL040C	*CLN3*	*DAF1, FUI*	11369_at	YAL040C C	188.8	1.2	37.8	188.8	1.7	132.1	0.0	0.0	0.0	0.0	0.0	0.0
YPR074C	*TKL1*		7694_at	YPR074C	1097.1	-1.1	163.4	1097.1	-1.0	53.5	1280.0	1.1	190.6	1280.0	1.1	128.0
YNL007C	*SIS1*		8888_at	YNL007C s	635.8	1.2	127.2	635.8	1.1	63.6	519.0	-1.2	103.8	519.0	-1.1	51.9
YKL067W	*YNK1*	*NDK1*	10629_at	YKL067W	846.5	1.1	126.1	846.5	-1.0	0.0	677.9	-1.1	100.9	677.9	-1.1	67.8
YGL026C	*TRP5*		5036_at	YGL026C t	1026.9	-1.2	201.1	1026.9	-1.1	98.0	1215.8	1.2	238.1	1215.8	1.1	121.6

YER091C	*MET6*		5620_at	YER091C v	966.8	-1.2	240.7	966.8	-1.1	96.7	1207.0	1.2	300.5	1207.0	1.1	120.7
YKL190W	*CNB1*		10772_at	YKL190W T	0.0	0.0	0.0	0.0	0.0	0.0	575.0	1.1	57.5	575.0	1.2	115.0
YBL078C	*AUT7*	*APG8, CV*	7464_at	YBL078C F	230.6	1.4	102.0	230.6	1.5	114.5	167.6	-1.4	74.1	167.6	1.0	0.0
YDR074W	*TPS2*	*HOG2, PF*	6398_at	YDR074W	224.7	1.5	111.6	224.7	-1.0	11.0	140.6	-1.5	69.8	140.6	-1.6	84.4
YLL009C	*COX17*		10334_at	YLL009C ir	551.2	1.2	110.2	551.2	1.1	82.1	0.0	0.0	0.0	0.0	0.0	0.0
YBR072W	*HSP26*		7295_at	YBR072W	88.4	2.2	105.9	88.4	1.4	39.7	41.9	-2.2	50.2	41.9	-1.5	21.0
YDR007W	*TRP1*		6468_at	YDR007W	233.8	1.4	105.0	233.8	1.1	34.8	0.0	0.0	0.0	0.0	0.0	0.0
YPL240C	*HSP82*	*HSP83, HS*	8010_i_at	YPL240C h	0.0	0.0	0.0	0.0	0.0	0.0	514.6	-1.0	0.0	514.6	1.2	102.9
YPL145C	*KES1*	*LPI3, OSH*	7880_at	YPL145C k	405.0	1.2	100.8	405.0	-1.0	19.8	0.0	0.0	0.0	0.0	0.0	0.0
YOR202W	*HIS3*		8359_at	YOR202W	673.6	1.1	67.4	673.6	1.1	100.3	0.0	0.0	0.0	0.0	0.0	0.0
YDR037W	*KRS1*	*GCD5*	6452_at	YDR037W	756.0	-1.2	148.0	756.0	-1.0	36.9	901.7	1.2	176.6	901.7	1.1	90.2
YCR068W	*CVT17*	*AUT5*	6809_at	Teter et al.	24.0	5.6	110.5	24.0	4.7	89.4	5.6	-4.4	18.9	5.6	-1.2	1.1
YER133W	*GLC7*	*CID1, DIS2*	5573_at	protein pho	242.7	1.2	40.7	242.7	1.3	84.2	217.2	-1.2	36.4	217.2	1.1	30.4
YIL167W	*SDL1*		4284_at	Serine deh	13.4	4.2	42.7	13.4	3.8	37.9	1.6	-4.2	5.1	1.6	-1.5	0.8
YGL180W	*APG1*	*AUT3*	5200_at	YGL180W	21.5	1.7	16.1	21.5	2.1	24.7	0.0	0.0	0.0	0.0	0.0	0.0
YFR034C	*PHO4*		5333_at	YFR034C p	9.9	2.3	12.4	9.9	1.4	4.0	3.3	-2.3	4.1	3.3	-1.2	0.7
YIL066C	*RNR3*	*DIN1, RIR3*	4045_s_at	YIL066C R	7.3	2.2	8.5	7.3	-1.1	1.0	2.2	-2.2	2.6	2.2	-1.2	0.4
YOR178C	*GAC1*		8380_at	YOR178C	5.2	1.7	3.5	5.2	-1.3	1.8	3.1	-1.7	2.1	3.1	-2.1	3.4
YBR189W			7191_f_at	YBR189W	1255.6	-1.2	312.6	1255.6	-1.1	46.6	1693.8	1.2	421.8	1693.8	1.1	42.2
YNL241C	*ZWF1*	*MET19, PO*	9108_at	Glucose-6-	206.9	1.3	62.1	206.9	-1.0	0.0	157.1	-1.3	47.1	157.1	-1.3	47.1
YMR105C	*PGM2*		9547_at	YMR105C	0.0	0.0	0.0	0.0	0.0	0.0	157.8	-1.3	55.1	157.8	-1.6	94.7
YMR260C	*TIF11*		9354_at	YMR260C	392.1	-1.3	136.9	392.1	-1.2	97.6	539.5	1.3	188.3	539.5	1.1	54.0
YDR044W	*HEM13*		6413_at	YDR044W	202.2	-1.2	50.3	202.2	-1.5	101.1	0.0	0.0	0.0	0.0	0.0	0.0
YJR047C	*ANB1*	*HYP1, TIF*	10976_at	YJR047C a	180.8	-1.8	153.6	180.8	-1.6	108.5	332.5	1.8	282.4	332.5	1.2	66.5
YBL076C	*ILS1*		7421_at	YBL076C c	367.9	-1.1	35.1	367.9	-1.3	109.0	0.0	0.0	0.0	0.0	0.0	0.0
YKL024C	*URA6*		10583_at	YKL024C u	314.8	1.1	30.0	314.8	-1.3	93.2	273.6	-1.1	26.1	273.6	-1.4	109.4
YKL156W			10717_at	YKL156W -	1167.3	-1.1	173.8	1167.3	-1.1	116.7	1337.0	1.1	199.1	1337.0	1.0	0.0
YDR214W	*AHA1*		6271_at	YDR214W	0.0	0.0	0.0	0.0	0.0	0.0	295.8	-1.1	28.2	295.8	-1.4	118.3
YIL078W	*THS1*		4186_at	YIL078W T	799.3	-1.1	79.9	799.3	-1.1	119.0	0.0	0.0	0.0	0.0	0.0	0.0
YDL022W	*GPD1*	*DAR1, HO*	6485_at	YDL022W	763.2	1.1	113.7	763.2	-1.0	0.0	647.3	-1.1	96.4	647.3	-1.2	129.5
YJL052W	*TDH1*	*GLD3*	11061_g_a	YJL052W C	1811.4	-1.0	77.3	1811.4	-1.1	134.2	2067.3	-1.0	88.2	2067.3	1.0	0.0
YPL106C	*SSE1*	*LPG3, MSI*	7874_at	YPL106C H	672.7	-1.0	0.0	672.7	-1.2	134.5	633.8	-1.0	0.0	633.8	-1.2	126.8
YER131W			5571_at	YER131W	475.1	-1.2	93.0	475.1	-1.3	135.2	563.2	1.2	110.3	563.2	-1.0	0.0
YJL121C	*RPE1*	*EPI1, POS*	11128_at	YJL121C D	688.8	-1.3	240.4	688.8	-1.2	137.8	905.1	1.3	315.9	905.1	1.1	90.5
YLR354C	*TAL1*		9978_at	YLR354C T	946.7	-1.0	46.2	946.7	-1.1	141.0	0.0	0.0	0.0	0.0	0.0	0.0
YBR121C	*GRS1*		7257_at	YBR121C (	709.4	-1.2	141.9	709.4	-1.2	141.9	776.5	1.2	155.3	776.5	1.0	0.0
YOR335C	*ALA1*		8225_at	YOR335C	579.8	-1.2	144.4	579.8	-1.2	144.4	722.2	1.2	179.8	722.2	-1.0	0.0
YGL253W	*HXK2*	*HEX1, HKI*	5260_at	YGL253W	751.4	-1.3	262.3	751.4	-1.2	150.3	981.0	1.3	342.4	981.0	1.1	98.1
YNL015W	*PBI2*	*yscB, I2B,*	8880_at	YNL015W	683.7	1.3	205.1	683.7	1.0	33.4	507.9	-1.3	152.4	507.9	-1.3	152.4
YFR053C	*HXK1*		5307_at	YFR053C I	271.0	1.0	13.2	271.0	-1.5	135.5	259.3	-1.0	12.7	259.3	-1.6	155.6
YER003C	*PMI40*	*PMI*	5702_at	YER003C r	630.6	-1.1	63.1	630.6	-1.2	157.0	694.4	1.1	69.4	694.4	-1.2	138.9
YDR155C	*CPR1*	*CYP1, CPI*	6301_at	YDR155C	1671.5	-1.2	327.3	1671.5	-1.1	159.5	0.0	0.0	0.0	0.0	0.0	0.0
YLL039C	*UBI4*	*SCD2*	10392_at	YLL039C u	432.6	1.3	151.0	432.6	-1.1	64.4	321.2	-1.3	112.1	321.2	-1.5	160.6
YER009W	*NTF2*		5709_at	YER009W	834.6	-1.2	163.4	834.6	-1.2	163.4	957.1	1.2	187.4	957.1	1.0	0.0
YDR226W	*ADK1*		6237_at	YDR226W	1116.1	-1.1	166.2	1116.1	-1.1	166.2	1457.8	1.1	217.1	1457.8	1.0	0.0
YDR321W	*ASP1*		6152_at	YDR321W	670.4	-1.3	234.0	670.4	-1.2	166.9	901.4	1.3	314.7	901.4	1.1	90.1
YMR146C	*TIF34*		9501_at	YMR146C	380.6	-1.0	18.6	380.6	-1.4	170.9	385.5	1.0	18.8	385.5	-1.4	154.2
YPL081W			7854_i_at	YPL081W	563.9	-1.3	163.7	563.9	-1.3	176.7	717.0	1.3	208.2	717.0	-1.0	35.0
YOR276W	*CAF20*	*CAF2, CAI*	8252_at	YOR276W	722.3	-1.1	72.2	722.3	-1.2	179.8	0.0	0.0	0.0	0.0	0.0	0.0
YGR204W	*ADE3*		4814_at	YGR204W	724.6	-1.2	144.9	724.6	-1.2	180.4	901.8	1.2	180.4	901.8	-1.0	0.0
YGL062W	*PYC1*		5090_at	YGL062W	142.4	-1.7	106.7	142.4	-2.3	185.1	291.0	1.7	218.0	291.0	-1.3	87.3

YNL244C	*SUI1*	*MOF2, RF*	9105_at	YNL244C t	939.7	-1.1	94.0	939.7	-1.2	187.9	0.0	0.0	0.0	0.0	0.0	0.0
YBR048W			7316_s_at	YBR048W	1977.1	-1.1	294.4	1977.1	-1.1	188.7	2248.8	1.1	334.9	2248.8	1.0	0.0
YHL015W			4547_at	YHL015W	1285.1	-1.2	251.7	1285.1	-1.1	191.4	1504.1	1.2	294.5	1504.1	1.0	0.0
YNL064C	*YDJ1*	*MAS5*	8924_at	YNL064C y	979.7	1.0	47.8	979.7	-1.1	145.9	959.6	-1.0	46.8	959.6	-1.2	191.9
YPR132W			7619_s_at	YPR132W	2790.2	-1.1	415.5	2790.2	-1.1	200.5	3197.7	1.1	476.2	3197.7	1.0	156.1
YFL045C	*SEC53*	*ALG4*	5435_at	YFL045C p	1356.7	-1.1	202.0	1356.7	-1.1	202.0	1549.8	1.1	230.8	1549.8	-1.0	0.0
YAL003W	*EFB1*	*TEF5*	11320_at	YAL003W	1673.1	-1.2	331.1	1673.1	-1.1	203.9	2018.1	1.2	399.4	2018.1	1.0	98.5
YJL157C	*FAR1*		11181_at	YJL157C F	346.1	-1.3	120.8	346.1	-1.6	206.5	476.4	1.3	166.3	476.4	-1.2	95.3
YDR471W			5985_at	YDR471W	1396.6	-1.2	279.3	1396.6	-1.1	208.0	1697.3	1.2	339.5	1697.3	1.1	169.7
YPL028W	*ERG10*	*LPB3, TSM*	7770_at	YPL028W	1419.8	-1.1	211.4	1419.8	-1.1	211.4	1632.9	1.1	243.2	1632.9	-1.0	0.0
YDL184C			6680_s_at	YDL184C F	1060.2	-1.3	318.1	1060.2	-1.2	212.0	1389.1	1.3	416.7	1389.1	1.1	138.9
YOL139C	*CDC33*	*TIF45*	8695_at	YOL139C r	1080.1	-1.1	108.0	1080.1	-1.2	216.0	0.0	0.0	0.0	0.0	0.0	0.0
YPR033C	*HTS1*	*TSM4572*	7741_at	YPR033C	720.9	-1.1	72.1	720.9	-1.3	216.3	795.2	1.1	79.5	795.2	-1.2	159.0
YCR031C			6866_at	YCR031C	1455.3	-1.2	362.4	1455.3	-1.1	216.7	1839.9	1.2	458.1	1839.9	1.1	184.0
YGR214W			4780_at	YGR214W	1457.7	-1.2	285.4	1457.7	-1.1	217.1	1570.5	1.2	307.5	1570.5	1.1	157.1
YJR123W			10916_at	YJR123W	2208.1	-1.1	328.8	2208.1	-1.1	220.8	2589.4	1.1	385.6	2589.4	1.0	0.0
YHR183W	*GND1*		4343_at	YHR183W	1485.6	-1.1	221.2	1485.6	-1.1	221.2	1682.4	1.1	250.5	1682.4	1.0	0.0
YDR212W	*TCP1*	*CCT1*	6269_at	YDR212W	560.2	-1.2	112.0	560.2	-1.4	224.1	677.8	1.2	135.6	677.8	-1.1	67.8
YDR002W	*YRB1*	*CST20, HT*	6463_at	YDR002W	912.6	-1.2	182.5	912.6	-1.2	227.2	0.0	0.0	0.0	0.0	0.0	0.0
YLR058C	*SHM2*	*SHMT2*	10267_at	YLR058C s	1548.0	-1.1	230.5	1548.0	-1.1	230.5	1780.5	1.1	265.1	1780.5	-1.0	0.0
YLR027C	*AAT2*	*ASP5*	10323_at	YLR027C a	1551.3	-1.1	231.0	1551.3	-1.1	231.0	1722.6	1.1	256.5	1722.6	-1.0	0.0
YJL026W	*RNR2*	*CRT6*	11041_at	YJL026W s	665.7	-1.1	66.6	665.7	-1.3	232.4	774.7	1.1	77.5	774.7	-1.2	154.9
YFR032C			5331_at	YFR032C I	1564.0	-1.2	389.4	1564.0	-1.1	232.9	1715.2	1.2	427.1	1715.2	1.1	171.5
YBR143C	*SUP45*	*SAL4, SUF*	7234_at	YBR143C	569.1	-1.0	0.0	569.1	-1.4	227.6	583.2	-1.0	0.0	583.2	-1.4	233.3
YKL182W	*FAS1*		10781_at	YKL182W	802.1	-1.0	39.1	802.1	-1.3	237.5	931.3	1.0	45.5	931.3	-1.2	186.3
YDL061C			6537_f_at	YDL061C F	1608.4	-1.2	321.7	1608.4	-1.1	239.5	2021.8	1.2	404.4	2021.8	1.0	0.0
YLR325C			9994_at	YLR325C F	2428.1	-1.1	361.6	2428.1	-1.1	242.8	2763.9	1.1	411.6	2763.9	1.0	0.0
YBR011C	*IPP1*		7370_at	YBR011C I	1251.3	-1.2	250.3	1251.3	-1.2	250.3	1505.0	1.2	301.0	1505.0	1.0	0.0
YLR264W			10068_i_at	YLR264W	2584.2	-1.1	384.8	2584.2	-1.1	252.5	2952.9	1.1	439.7	2952.9	1.0	144.1
YNL067W			8921_s_at	YNL067W	1726.3	-1.0	84.3	1726.3	-1.1	257.1	0.0	0.0	0.0	0.0	0.0	0.0
YPL143W			7882_f_at	YPL143W	1752.7	-1.2	436.4	1752.7	-1.1	261.0	2153.6	1.2	536.2	2153.6	1.1	215.4
YOR063W			8491_at	YOR063W	1309.3	-1.1	130.9	1309.3	-1.2	261.9	0.0	0.0	0.0	0.0	0.0	0.0
YPL061W	*ALD6*		7828_at	YPL061W	750.5	-1.1	111.8	750.5	-1.3	262.0	859.1	1.1	127.9	859.1	-1.2	171.8
YKR094C			10474_s_a	YKR094C I	1812.2	-1.2	358.7	1812.2	-1.1	266.2	2208.2	1.2	437.0	2208.2	1.1	210.8
YPL249C			8000_i_at	YPL249C F	907.7	-1.3	268.8	907.7	-1.3	268.8	1184.0	1.3	350.6	1184.0	1.0	0.0
YLR060W	*FRS1*		10269_at	YLR060W	1379.2	-1.1	205.4	1379.2	-1.2	275.8	1572.9	1.1	234.2	1572.9	-1.1	157.3
YJR104C	*SOD1*	*CRS4*	10897_at	YJR104C C	1864.3	-1.1	186.4	1864.3	-1.1	277.6	0.0	0.0	0.0	0.0	0.0	0.0
YHR193C	*EGD2*		4353_at	YHR193C	1137.1	-1.1	169.3	1137.1	-1.2	283.1	1294.9	1.1	192.8	1294.9	-1.1	129.5
YJL191W			11235_at	YJL191W F	642.2	-1.4	288.4	642.2	-1.4	288.4	945.5	1.4	424.7	945.5	1.0	0.0
YLR448W			9852_at	YLR448W	1941.5	-1.1	194.2	1941.5	-1.1	289.1	0.0	0.0	0.0	0.0	0.0	0.0
YBL092W			7448_at	YBL092W	1463.1	-1.2	292.6	1463.1	-1.2	292.6	1879.3	1.2	375.9	1879.3	1.0	0.0
YPL220W			7984_s_at	YPL220W	2002.5	-1.2	498.6	2002.5	-1.1	298.2	2442.9	1.2	608.3	2442.9	1.1	244.3
YGR061C	*ADE6*		4942_at	YGR061C	543.9	-1.1	81.0	543.9	-1.5	298.7	604.9	1.1	90.1	604.9	-1.3	181.5
YGR240C	*PFK1*		4760_at	Phosphofru	1005.6	-1.2	250.4	1005.6	-1.3	301.7	1258.0	1.2	313.2	1258.0	-1.1	125.8
YPL231W	*FAS2*		7973_at	YPL231W	396.9	1.0	19.4	396.9	-1.7	297.4	377.5	-1.0	18.4	377.5	-1.8	302.0
YDL191W			6672_s_at	YDL191W	1556.5	-1.2	304.8	1556.5	-1.2	304.8	1877.9	1.2	367.7	1877.9	-1.0	0.0
YMR142C			9496_at	YMR142C	1229.5	-1.3	429.2	1229.5	-1.2	306.1	1708.6	1.3	596.4	1708.6	1.1	170.9
YGL123W			5119_at	YGL123W	2085.1	-1.2	519.2	2085.1	-1.1	310.5	2617.9	1.2	651.9	2617.9	1.1	261.8
YNL209W	*SSB2*		9094_s_at	YNL209W	2100.0	-1.1	312.7	2100.0	-1.1	312.7	2488.7	1.1	370.6	2488.7	1.0	0.0
YPR035W	*GLN1*		7698_at	YPR035W	1270.0	-1.4	508.0	1270.0	-1.2	316.2	1721.7	1.4	688.7	1721.7	1.1	172.2
YGR118W			4909_f_at	YGR118W	2127.7	-1.1	316.8	2127.7	-1.1	316.8	2514.3	1.1	374.4	2514.3	1.0	0.0

YDR500C			5969_at	YDR500C (	2208.7	-1.3	654.1	2208.7	-1.1	328.9	2914.7	1.3	863.2	2914.7	1.1	291.5
YLL045C			10386_s_a	YLL045C F	1659.8	-1.2	289.1	1659.8	-1.1	26.4	1906.1	1.2	332.0	1906.1	1.0	14.8
YBL072C			7425_s_at	YBL072C F	2290.7	-1.2	570.4	2290.7	-1.1	341.1	2779.9	1.2	692.2	2779.9	1.1	278.0
YDR064W			6433_at	YDR064W	1722.3	-1.2	344.5	1722.3	-1.2	344.5	2085.3	1.2	417.1	2085.3	1.0	0.0
YER023W	*PRO3*	*ORE2*	5678_at	YER023W	771.7	-1.2	192.1	771.7	-1.4	346.6	961.5	1.2	239.4	961.5	-1.2	192.3
YOL120C			8668_at	YOL120C I	1845.5	-1.2	459.5	1845.5	-1.2	361.4	2308.9	1.2	574.9	2308.9	1.1	230.9
YLR185W			10166_at	YLR185W	1831.8	-1.3	639.4	1831.8	-1.2	366.4	2462.9	1.3	859.7	2462.9	1.1	246.3
YDL083C			6559_f_at	YDL083C F	1335.4	-1.2	299.5	1335.4	-1.1	44.6	1641.7	1.2	368.2	1641.7	1.0	18.0
YMR194W			9420_i_at	YMR194W	2154.9	-1.2	527.9	2154.9	-1.2	375.3	2714.2	1.2	664.9	2714.2	1.1	259.1
YGL076C			5075_i_at	YGL076C I	1397.4	-1.0	68.2	1397.4	-1.3	380.6	1462.8	1.0	71.4	1462.8	-1.1	217.8
YDR050C	*TPI1*		6419_at	Triosephos	2559.9	-1.2	637.4	2559.9	-1.1	381.2	3338.3	1.2	831.2	3338.3	1.1	333.8
YOR133W	*EFT1*		8425_s_at	YOR133W	1558.5	-1.1	232.1	1558.5	-1.2	388.1	1852.7	1.1	275.9	1852.7	-1.0	0.0
YOL040C			8612_at	YOL040C 4	1952.9	-1.2	390.6	1952.9	-1.2	390.6	2270.9	1.2	454.2	2270.9	-1.0	0.0
YHL033C			4575_i_at	YHL033C F	1955.4	-1.1	195.5	1955.4	-1.2	391.1	0.0	0.0	0.0	0.0	0.0	0.0
YEL034W	*HYP2*	*TIF51A*	5756_at	YEL034W	2636.4	-1.2	656.5	2636.4	-1.1	392.6	3320.4	1.2	826.8	3320.4	1.1	332.0
YNL069C			8918_at	YNL069C F	2660.9	-1.2	662.6	2660.9	-1.1	396.2	3325.4	1.2	828.0	3325.4	1.1	332.5
YPL131W			7894_at	YPL131W	2684.5	-1.2	536.9	2684.5	-1.1	399.7	3209.3	1.2	641.9	3209.3	1.0	0.0
YHR010W			4528_i_at	YHR010W	1525.0	-1.2	332.7	1525.0	-1.1	23.9	1850.0	1.2	403.6	1850.0	1.2	79.0
YDL081C			6561_at	YDL081C A	2074.7	-1.2	499.9	2074.7	-1.2	406.3	2727.7	1.2	657.3	2727.7	1.0	0.0
YGR027C			5001_f_at	YGR027C	1532.4	-1.2	340.4	1532.4	-1.1	24.5	1862.8	1.2	413.8	1862.8	1.1	61.6
YLR249W	*YEF3*	*TEF3*	10097_at	YLR249W	2805.7	-1.1	417.8	2805.7	-1.1	417.8	3188.9	1.1	474.9	3188.9	1.0	0.0
YJL177W			11205_i_at	YJL177W F	2100.3	-1.2	523.0	2100.3	-1.2	420.1	2639.5	-1.2	657.2	2639.5	1.0	128.8
YDL082W			6560_at	YDL082W	1468.9	-1.2	365.8	1468.9	-1.3	435.0	1816.6	1.2	452.3	1816.6	-1.0	0.0
YPR043W			7708_f_at	YPR043W	1791.2	-1.2	446.0	1791.2	-1.1	44.6	2186.7	1.2	544.5	2186.7	1.1	54.4
YKL060C	*FBA1*		10636_at	Aldolase	3014.1	-1.2	750.5	3014.1	-1.1	448.8	3801.9	1.2	946.7	3801.9	1.1	380.2
YOL039W			8613_at	YOL039W	2361.0	-1.2	587.9	2361.0	-1.2	472.2	2988.9	1.2	744.2	2988.9	1.1	298.9
YBR191W			7147_at	YBR191W	2492.3	-1.3	738.1	2492.3	-1.2	488.1	3246.8	1.3	961.5	3246.8	1.1	324.7
YGR254W	*ENO1*	*HSP48*	4730_s_at	Enolase I	2833.6	-1.2	493.5	2833.6	-1.2	493.5	3244.5	1.2	565.1	3244.5	1.0	158.4
YGR148C			4848_at	YGR148C	3354.9	-1.2	835.4	3354.9	-1.1	499.6	4259.1	1.2	1060.5	4259.1	1.1	425.9
YLR029C			10325_at	YLR029C F	1856.7	-1.2	416.4	1856.7	-1.3	505.7	2276.3	1.2	510.5	2276.3	-1.0	111.1
YLR441C			9890_s_at	YLR441C F	2182.4	-1.2	431.9	2182.4	-1.1	42.2	2569.5	1.2	508.5	2569.5	1.1	48.5
YMR205C	*PFK2*		9432_at	YMR205C	1287.5	-1.2	252.1	1287.5	-1.4	510.4	1558.7	1.2	305.2	1558.7	-1.2	311.7
YEL071W	*DLD3*		5813_at	YEL071W	934.3	-1.4	419.6	934.3	-1.5	513.1	1352.6	1.4	607.5	1352.6	-1.1	135.3
YOL086C	*ADH1*	*ADC1*	8657_at	YOL086C A	3553.5	-1.2	695.9	3553.5	-1.1	529.2	4293.4	1.2	840.8	4293.4	1.1	429.3
YIL133C			4223_at	YIL133C R	2311.2	-1.2	575.5	2311.2	-1.2	575.5	2889.0	1.2	719.4	2889.0	1.0	0.0
YJL190C			11236_at	YJL190C R	3932.3	-1.1	585.6	3932.3	-1.1	585.6	4680.2	1.1	696.9	4680.2	1.0	0.0
YAL038W	*CDC19*	*PYK1*	11371_at	Pyruvate ki	3016.0	-1.2	751.0	3016.0	-1.2	590.6	3721.2	1.2	926.6	3721.2	1.0	0.0
YLR304C	*ACO1*	*GLU1*	10019_at	YLR304C A	1066.5	-1.4	479.0	1066.5	-1.6	636.6	1602.0	1.4	719.5	1602.0	-1.1	160.2
YBL027W			7378_s_at	YBL027W	1885.8	-1.2	377.2	1885.8	-1.3	658.3	2238.6	1.2	447.7	2238.6	-1.2	447.7
YBL087C			7454_s_at	YBL087C F	2647.6	-1.1	394.3	2647.6	-1.2	659.3	2964.6	1.1	441.5	2964.6	-1.1	296.5
YML026C			9684_s_at	YML026C I	2948.1	-1.1	439.0	2948.1	-1.2	734.1	3458.0	1.1	514.9	3458.0	-1.1	345.8
YAL005C	*SSA1*	*YG100*	11315_i_at	YAL005C H	2682.4	-1.0	127.0	2682.4	-1.1	391.6	2804.4	1.0	132.8	2804.4	-1.3	866.8
YKR057W			10529_i_at	YKR057W	4839.1	-1.2	947.6	4839.1	-1.2	947.6	5831.4	1.2	1141.9	5831.4	1.0	0.0
YOR375C	*GDH1*	*URE1*	8174_at	YOR375C	662.8	-2.4	927.9	662.8	-2.5	994.1	1441.3	2.4	2017.8	1441.3	-1.0	0.0
YGR085C			4921_i_at	YGR085C	3199.3	-1.2	796.6	3199.3	-1.4	1268.3	3846.1	1.2	957.7	3846.1	-1.2	769.2
cytoskeleton																
YNL138W	*SRV2*		8984_at	YNL138W	473.5	1.1	70.5	473.5	1.9	424.9	409.0	-1.1	60.9	409.0	1.6	245.4
YNL271C	*BNI1*	*PPF3*	9123_at	Cytoskeleta	94.5	1.3	28.0	94.5	3.4	229.7	108.6	-1.3	32.2	108.6	2.1	119.5
YFL037W	*TUB2*	*ARM10, SI*	5394_at	YFL037W I	727.1	1.1	108.3	727.1	1.3	218.1	0.0	0.0	0.0	0.0	0.0	0.0
YCR091W	*KIN82*		6786_at	YCR091W	5.7	1.4	2.3	5.7	2.0	5.8	0.0	0.0	0.0	0.0	0.0	0.0

YIL159W	*BNR1*		4242_at	Bni1p-relat	3.3	-2.0	3.3	3.3	-1.7	2.2	6.5	2.0	6.5	6.5	1.1	0.7
YCL024W	*KCC4*		6762_s_at	YCL024W	12.4	2.4	17.2	12.4	1.2	2.4	5.1	-2.4	7.1	5.1	-2.0	5.1
YNL079C	*TPM1*		8953_at	YNL079C t	713.2	1.0	34.8	713.2	-1.2	142.6	676.8	-1.0	33.0	676.8	-1.2	135.4
YBR109C	*CMD1*		7245_at	YBR109C (	758.7	-1.1	75.9	758.7	-1.2	151.7	0.0	0.0	0.0	0.0	0.0	0.0
YPR165W	*RHO1*		7563_at	Ras homol	819.3	-1.0	0.0	819.3	-1.2	163.9	828.3	-1.0	0.0	828.3	-1.2	165.7
YIL062C	*ARC15*		4155_at	YIL062C A	655.4	1.0	32.0	655.4	-1.2	128.3	617.0	-1.0	30.1	617.0	-1.3	185.1
YFL039C	*ACT1*	*ABY1, ENI*	5392_at	YFL039C /	2061.4	-1.2	403.7	2061.4	-1.2	403.7	2477.1	1.2	485.1	2477.1	1.0	0.0
centrosome																
YPL255W	*BBP1*		7994_at	YPL255W	0.0	0.0	0.0	0.0	0.0	0.0	4.1	-1.5	2.0	4.1	-2.4	5.7
YER018C	*SPC25*		5718_at	YER018C :	0.0	0.0	0.0	0.0	0.0	0.0	25.0	-1.7	18.7	25.0	-2.9	47.5
YBR109C	*CMD1*		7245_at	YBR109C (	758.7	-1.1	75.9	758.7	-1.2	151.7	0.0	0.0	0.0	0.0	0.0	0.0
endoplasmic reticulum																
YJL034W	*KAR2*	*BIP, GRP7*	11078_at	Homologue	1978.5	1.1	188.8	1978.5	1.2	492.6	1802.2	-1.1	172.0	1802.2	1.2	360.4
YAL023C	*PMT2*	*FUN25*	11344_at	YAL023C c	1047.0	1.1	104.7	1047.0	1.3	365.5	859.8	-1.1	86.0	859.8	1.2	172.0
YML130C	*ERO1*		9802_at	YML130C r	365.0	2.0	383.0	365.0	1.8	292.0	129.8	-2.0	136.2	129.8	-1.1	13.0
YHR007C	*ERG11*		4525_at	YHR007C (	0.0	0.0	0.0	0.0	0.0	0.0	1087.9	1.0	53.1	1087.9	1.2	217.6
YDL095W	*PMT1*		6591_at	YDL095W	415.6	1.4	166.2	415.6	1.4	166.2	299.5	-1.4	119.8	299.5	1.0	0.0
YIL075C	*RPN2*	*SEN3*	4189_at	YIL075C R	0.0	0.0	0.0	0.0	0.0	0.0	385.5	1.2	77.1	385.5	1.4	154.2
YOR067C	*ALG8*		8495_at	YOR067C	598.7	-1.2	149.1	598.7	-1.0	0.0	730.4	1.2	181.9	730.4	1.2	146.1
YDR086C	*SSS1*		6410_at	YDR086C	557.7	-1.3	165.2	557.7	-1.1	83.0	727.0	1.3	215.3	727.0	1.2	145.4
YHR190W	*ERG9*		4350_at	YHR190W	534.8	1.1	79.6	534.8	1.2	104.7	390.9	-1.1	58.2	390.9	1.0	0.0
YKL073W	*LHS1*	*CER1, SSI*	10668_at	YKL073W	144.8	1.6	94.0	144.8	1.2	36.0	77.9	-1.6	50.6	77.9	-1.3	23.4
YGR175C	*ERG1*		4830_at	YGR175C	828.8	-1.1	123.4	828.8	-1.0	40.5	926.4	1.1	138.0	926.4	1.1	92.6
YBR201W	*DER1*		3940_at	YBR201W	96.1	1.7	71.7	96.1	2.0	92.2	58.1	-1.7	43.4	58.1	1.1	6.9
YGR157W	*CHO2*	*PEM1*	4858_at	Phosphatic	579.5	-1.1	86.3	579.5	-1.1	57.9	665.4	1.1	99.1	665.4	1.0	0.0
YBR265W	*TSC10*		7085_at	YBR265W	220.6	-1.0	10.8	220.6	-1.5	109.6	0.0	0.0	0.0	0.0	0.0	0.0
YBR177C	*EHT1*		7179_at	YBR177C I	369.1	-1.0	0.0	369.1	-1.3	110.7	335.1	-1.0	0.0	335.1	-1.3	100.5
YKL065C	*YET1*		10631_at	YKL065C Y	635.4	1.2	127.1	635.4	-1.0	0.0	573.3	-1.2	114.7	573.3	-1.2	114.7
YJL002C	*OST1*	*NLT1*	11019_at	YJL002C 6	1148.9	-1.1	171.1	1148.9	-1.1	114.9	1315.8	1.1	195.9	1315.8	1.0	0.0
YML075C	*HMG1*		9724_at	YML075C :	412.1	-1.1	39.3	412.1	-1.3	122.0	0.0	0.0	0.0	0.0	0.0	0.0
YDR410C	*STE14*		6061_at	YDR410C '	325.8	-1.4	130.3	325.8	-1.2	81.1	459.0	1.4	183.6	459.0	1.1	45.9
YER094C	*PUP3*	*SCS32*	5625_at	YER094C :	928.9	-1.1	92.9	928.9	-1.1	138.3	0.0	0.0	0.0	0.0	0.0	0.0
YEL002C	*WBP1*		5744_at	YEL002C c	953.0	-1.0	46.5	953.0	-1.1	141.9	0.0	0.0	0.0	0.0	0.0	0.0
YDR304C	*CPR5*	*CYP5*	6178_at	YDR304C	962.7	-1.1	96.3	962.7	-1.1	143.4	0.0	0.0	0.0	0.0	0.0	0.0
YPR113W	*PIS1*		7645_at	YPR113W	962.8	-1.1	143.4	962.8	-1.1	143.4	1132.6	1.1	168.7	1132.6	1.0	0.0
YGL048C	*RPT6*	*CIM3, CRL*	5059_at	YGL048C a	697.5	-1.0	0.0	697.5	-1.1	103.9	734.5	-1.0	0.0	734.5	-1.2	146.9
YMR149W	*SWP1*		9504_at	YMR149W	783.3	-1.1	116.6	783.3	-1.2	153.4	883.5	1.1	131.6	883.5	-1.1	88.4
YJR143C	*PMT4*		10891_at	YJR143C c	771.0	-1.1	77.1	771.0	-1.2	154.2	0.0	0.0	0.0	0.0	0.0	0.0
YDL212W	*SHR3*	*APF1*	6696_at	YDL212W	1059.4	-1.2	211.9	1059.4	-1.1	157.8	1263.7	1.2	252.7	1263.7	1.0	0.0
YCR075C	*ERS1*		6816_at	YCR075C	185.0	-1.2	33.9	185.0	-1.9	162.0	0.0	0.0	0.0	0.0	0.0	0.0
YNL064C	*YDJ1*	*MAS5*	8924_at	YNL064C y	979.7	1.0	47.8	979.7	-1.1	145.9	959.6	-1.0	46.8	959.6	-1.2	191.9
YJL080C	*SCP160*		11122_at	YJL080C M	403.0	-1.1	60.0	403.0	-1.5	221.3	472.9	1.1	70.4	472.9	-1.3	141.9
YNL130C	*CPT1*		8992_at	YNL130C s	553.9	-1.4	221.6	553.9	-1.4	221.6	791.4	1.4	316.6	791.4	1.0	0.0
YMR202W	*ERG2*		9429_at	YMR202W	1288.1	-1.1	191.8	1288.1	-1.3	449.6	1456.0	1.1	216.8	1456.0	-1.2	291.2
YNR016C	*ACC1*		8864_at	YNR016C :	893.1	-1.2	178.6	893.1	-1.5	490.5	1077.2	1.2	215.4	1077.2	-1.3	323.2
YJR073C	*OPI3*	*PEM2*	10957_at	Methylene-	1629.4	-1.3	568.8	1629.4	-1.4	651.8	2223.8	1.3	776.3	2223.8	-1.0	0.0
Golgi																
YNL238W	*KEX2*	*QDS1, VM*	9110_at	Ca2+-depe	189.7	1.2	37.1	189.7	3.0	385.1	156.4	-1.2	30.6	156.4	2.5	234.6

YMR272C	*SCS7*	*FAH1*	9366_at	YMR272C	774.6	-1.0	37.8	774.6	1.2	151.7	845.3	1.0	41.3	845.3	1.3	253.6
YLR034C	*SMF3*		10286_at	YLR034C F	700.6	1.1	104.3	700.6	1.3	244.5	596.7	-1.1	88.9	596.7	1.2	119.3
YDR270W	*CCC2*		6190_at	Accessory	150.0	1.9	135.0	150.0	1.9	135.0	92.0	-1.9	82.8	92.0	1.0	0.0
YKR068C	*BET3*		10493_at	YKR068C I	0.0	0.0	0.0	0.0	0.0	0.0	657.0	-1.0	0.0	657.0	1.2	131.4
YGR143W	*SKN1*		4843_at	YGR143W	145.7	1.8	123.7	145.7	1.7	102.0	79.0	-1.8	67.1	79.0	-1.1	7.9
YKR061W	*KTR2*		10532_at	YKR061W	52.6	2.3	70.7	52.6	1.9	49.6	22.7	-2.3	30.5	22.7	-1.2	4.5
YER123W	*YCK3*	*CKI3*	5608_at	YER123W	35.8	1.4	16.1	35.8	2.3	48.2	0.0	0.0	0.0	0.0	0.0	0.0
YLR262C	*YPT6*		10066_s_a	YLR262C F	408.9	-1.1	40.9	408.9	-1.2	101.8	0.0	0.0	0.0	0.0	0.0	0.0
YEL042W	*GDA1*		5748_at	YEL042W	223.0	-1.1	33.2	223.0	-1.5	111.5	0.0	0.0	0.0	0.0	0.0	0.0
YER031C	*YPT31*	*YPT8*	5686_at	YER031C r	252.5	-1.0	12.3	252.5	-1.4	113.4	0.0	0.0	0.0	0.0	0.0	0.0
YPR113W	*PIS1*		7645_at	YPR113W	962.8	-1.1	143.4	962.8	-1.1	143.4	1132.6	1.1	168.7	1132.6	1.0	0.0
YPR165W	*RHO1*		7563_at	Ras homol	819.3	-1.0	0.0	819.3	-1.2	163.9	828.3	-1.0	0.0	828.3	-1.2	165.7
YNL130C	*CPT1*		8992_at	YNL130C s	553.9	-1.4	221.6	553.9	-1.4	221.6	791.4	1.4	316.6	791.4	1.0	0.0
YGL225W	*VRG4*	*VAN2, GO*	5245_at	YGL225W	800.7	-1.3	279.5	800.7	-1.3	237.1	1065.2	1.3	371.8	1065.2	1.0	0.0
YDL137W	*ARF2*		6638_at	YDL137W	1842.3	-1.1	175.8	1842.3	-1.2	360.8	0.0	0.0	0.0	0.0	0.0	0.0
YDL192W	*ARF1*		6671_at	YDL192W	3045.6	-1.2	609.1	3045.6	-1.1	453.5	3689.1	1.2	737.8	3689.1	1.0	0.0
intracellular transport vesicles																
YBR105C	*VID24*		7241_at	YBR105C i	89.7	2.0	89.7	89.7	2.8	165.9	43.4	-2.0	43.4	43.4	1.4	17.4
YNL272C	*SEC2*		9122_at	Protein witl	57.0	1.0	2.8	57.0	3.8	159.2	56.4	-1.0	2.8	56.4	3.8	157.9
YGR284C	*ERV29*		4714_at	YGR284C	0.0	0.0	0.0	0.0	0.0	0.0	639.9	-1.0	0.0	639.9	1.2	128.0
YIL117C	*PRM5*		4194_at	YIL117C pl	67.2	1.2	16.2	67.2	2.7	113.0	59.1	-1.2	14.2	59.1	2.2	70.9
YDL195W	*SEC31*	*WEB1*	6668_at	YDL195W	233.6	1.4	104.9	233.6	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
YAR031W	*PRM9*		11293_at	YAR031W	10.8	-1.0	0.5	10.8	2.7	18.1	11.4	1.0	0.5	11.4	2.9	21.7
YIL037C	*PRM2*		4134_at	Pheromone	7.6	-4.0	22.6	7.6	-2.6	11.9	29.4	4.0	87.5	29.4	1.8	23.5
YOR327C	*SNC2*		8217_at	YOR327C	431.6	-1.1	64.3	431.6	-1.2	107.5	0.0	0.0	0.0	0.0	0.0	0.0
YML001W	*YPT7*	*AST4, VAI*	9622_at	YML001W	822.3	-1.0	40.1	822.3	-1.1	122.4	0.0	0.0	0.0	0.0	0.0	0.0
YFL005W	*SEC4*	*SRO6*	5383_at	Ras-like sn	416.8	-1.0	0.0	416.8	-1.3	123.4	416.5	-1.0	0.0	416.5	-1.3	125.0
YPL218W	*SAR1*		7986_at	YPL218W	590.6	-1.2	118.1	590.6	-1.1	59.1	712.8	1.2	142.6	712.8	1.1	71.3
YML012W	*ERV25*		9653_at	YML012W	1340.8	-1.1	134.1	1340.8	-1.1	199.7	0.0	0.0	0.0	0.0	0.0	0.0
YDL137W	*ARF2*		6638_at	YDL137W	1842.3	-1.1	175.8	1842.3	-1.2	360.8	0.0	0.0	0.0	0.0	0.0	0.0
YGL200C	*EMP24*	*BST2*	5225_at	YGL200C t	1255.6	-1.1	125.6	1255.6	-1.3	376.7	0.0	0.0	0.0	0.0	0.0	0.0
nucleus																
YCL067C	*HMLALPH*	*ALPHA2, N*	6958_s_at	Mating type	210.9	1.0	10.3	210.9	6.2	1106.8	201.1	-1.0	9.8	201.1	6.0	1005.5
YDR524C	*AGE1*	*SAT1*	5926_g_at	YDR524C .	3729.0	-1.3	1015.6	3729.0	-1.1	539.0	4720.7	1.3	1285.7	4720.7	1.1	703.0
YJL130C	*URA2*		11165_at	YJL130C c	1459.3	1.4	655.4	1459.3	1.3	509.4	928.3	-1.4	416.9	928.3	-1.0	0.0
YFL031W	*HAC1*	*ERN4, IRE*	5318_s_at	bZIP Trans	398.7	1.9	355.4	398.7	2.2	484.5	284.7	-1.9	253.8	284.7	1.2	53.7
YDL182W	*LYS20*		6683_g_at	YDL182W	2082.9	-1.0	101.7	2082.9	1.1	310.2	2189.0	1.0	106.8	2189.0	1.2	437.8
YLR175W	*CBF5*		10155_at	YLR175W	795.5	-1.0	0.0	795.5	1.4	318.2	829.9	-1.0	0.0	829.9	1.4	332.0
YCR014C	*POL4*	*POLX*	6891_at	DNA polym	15.3	-3.0	30.5	15.3	1.4	6.1	67.8	3.0	135.1	67.8	5.5	305.1
YNL016W	*PUB1*		8879_at	YNL016W	329.2	1.6	197.5	329.2	1.9	296.2	203.9	-1.6	122.3	203.9	1.2	40.8
YOR204W	*DED1*	*SPP81*	8361_at	YOR204W	325.4	1.8	260.3	325.4	1.3	97.6	169.7	-1.8	135.8	169.7	-1.3	50.9
YLR094C	*GIS3*		10258_at	Cyclin C ar	40.0	3.8	111.6	40.0	6.0	199.5	9.8	-3.8	27.4	9.8	1.6	5.9
YCR042C	*TSM1*	*TAF150*	6830_at	"Essential (	31.2	-1.0	0.0	31.2	6.3	165.3	34.4	-1.0	0.0	34.4	6.1	175.4
YOR344C	*TYE7*	*SGC1*	8188_at	33 kDa ser	66.1	2.8	121.6	66.1	3.6	174.1	23.0	-2.8	42.3	23.0	1.3	6.9
YIL075C	*RPN2*	*SEN3*	4189_at	YIL075C R	0.0	0.0	0.0	0.0	0.0	0.0	385.5	1.2	77.1	385.5	1.4	154.2
YHR089C	*GAR1*		4425_at	YHR089C :	1304.6	-1.1	194.3	1304.6	-1.0	63.7	1525.6	1.1	227.2	1525.6	1.1	152.6
YMR039C	*SUB1*	*TSP1*	9617_at	YMR039C	306.3	1.1	29.2	306.3	1.4	137.6	0.0	0.0	0.0	0.0	0.0	0.0
YNL039W	*BDP1*	*TFC5*	8902_at	Subunit of	62.2	1.2	15.5	62.2	3.2	136.5	56.3	-1.2	14.0	56.3	2.5	84.5
YNL220W	*ADE12*	*BRA9*	9083_at	YNL220W	1163.7	-1.1	173.3	1163.7	-1.0	0.0	1347.0	1.1	200.6	1347.0	1.1	134.7

YDR123C	*INO2*	*DIE1, SCS*	6358_at	Transcripti	18.7	2.4	26.3	18.7	8.1	132.6	9.1	-2.4	12.8	9.1	4.9	35.5
YOR098C	*NUP1*		8435_at	YOR098C	0.0	0.0	0.0	0.0	0.0	0.0	87.8	1.5	48.2	87.8	2.5	131.7
YHL027W	*RIM101*		4581_at	YHL027W	326.7	1.2	65.3	326.7	1.4	130.7	0.0	0.0	0.0	0.0	0.0	0.0
YNL007C	*SIS1*		8888_at	YNL007C s	635.8	1.2	127.2	635.8	1.1	63.6	519.0	-1.2	103.8	519.0	-1.1	51.9
YDL140C	*RPO21*	*RPB1, RPI*	6635_at	YDL140C F	247.9	1.2	61.7	247.9	1.5	123.1	0.0	0.0	0.0	0.0	0.0	0.0
YNL172W	*APC1*		9041_at	YNL172W	102.1	1.3	34.9	102.1	2.2	121.6	76.1	-1.3	26.0	76.1	1.6	45.7
YOR230W	*WTM1*		8342_at	YOR230W	812.5	1.0	39.7	812.5	1.1	121.0	0.0	0.0	0.0	0.0	0.0	0.0
YLR197W	*SIK1*		10132_at	YLR197W	0.0	0.0	0.0	0.0	0.0	0.0	402.0	1.1	40.2	402.0	1.3	120.6
YDR432W	*NPL3*	*MTR13, M*	6039_g_at	YDR432W	442.1	1.2	106.8	442.1	1.1	53.2	326.8	-1.2	78.9	326.8	-1.2	54.8
YIR017C	*MET28*		4098_at	YIR017C T	353.7	1.2	88.1	353.7	1.3	106.1	0.0	0.0	0.0	0.0	0.0	0.0
YBR072W	*HSP26*		7295_at	YBR072W	88.4	2.2	105.9	88.4	1.4	39.7	41.9	-2.2	50.2	41.9	-1.5	21.0
YGR288W	*MAL13*		4718_at	YGR288W	60.3	1.7	45.1	60.3	2.7	105.4	30.5	-1.7	22.9	30.5	1.6	18.3
YNL282W	*POP3*		9157_at	Structural c	21.9	1.4	9.4	21.9	5.6	101.6	20.1	-1.4	8.6	20.1	3.5	50.3
YBR034C	*HMT1*		7348_at	YBR034C r	340.4	1.3	100.8	340.4	1.2	84.8	0.0	0.0	0.0	0.0	0.0	0.0
YKL109W	*HAP4*		10675_at	YKL109W	192.2	1.8	153.2	192.2	1.5	95.4	106.7	-1.8	85.1	106.7	-1.2	21.3
YMR043W	*MCM1*	*FUN80*	9576_at	Putative tra	90.2	1.6	58.6	90.2	2.0	94.7	54.3	-1.6	35.3	54.3	1.2	10.9
YGL066W	*SGF73*		5086_at	YGL066W	60.2	1.4	23.8	60.2	2.4	86.4	0.0	0.0	0.0	0.0	0.0	0.0
YMR182C	*RGM1*		9452_at	Putative tra	14.8	1.9	12.9	14.8	6.1	75.1	7.3	-1.9	6.4	7.3	4.1	22.6
YMR042W	*ARG80*	*ARGR1*	9575_at	Regulator c	57.3	1.8	45.8	57.3	1.9	51.5	31.3	-1.8	25.0	31.3	1.0	0.0
YER123W	*YCK3*	*CKI3*	5608_at	YER123W	35.8	1.4	16.1	35.8	2.3	48.2	0.0	0.0	0.0	0.0	0.0	0.0
YOR074C	*CDC21*	*CRT9, TMI*	8152_f_at	YOR074C	29.7	1.1	4.0	29.7	2.0	31.0	29.4	-1.1	3.9	29.4	1.8	23.2
YDL154W	*MSH5*		6621_at	YDL154W	20.5	1.7	15.2	20.5	2.1	23.3	0.0	0.0	0.0	0.0	0.0	0.0
YDR216W	*ADR1*		6273_at	YDR216W	13.6	1.5	7.4	13.6	2.7	23.0	8.6	-1.5	4.7	8.6	1.8	6.9
YLR055C	*SPT8*		10264_at	YLR055C t	0.0	0.0	0.0	0.0	0.0	0.0	22.9	-2.0	22.7	22.9	-1.7	16.0
YOL067C	*RTG1*		8630_at	YOL067C	11.2	1.1	1.7	11.2	2.7	19.5	9.6	-1.1	1.4	9.6	2.4	13.4
YLR453C	*RIF2*		9857_at	YLR453C N	12.2	2.0	12.8	12.2	2.3	16.5	5.9	-2.0	6.2	5.9	1.1	0.6
YPR168W	*NUT2*	*MED10*	7566_at	YPR168W	14.1	2.2	17.6	14.1	2.0	14.7	6.3	-2.2	7.9	6.3	-1.1	0.6
YNL012W	*SPO1*		8883_at	YNL012W	10.8	2.2	13.1	10.8	1.3	3.2	4.3	-2.2	5.3	4.3	-1.8	3.4
YFR034C	*PHO4*		5333_at	YFR034C p	9.9	2.3	12.4	9.9	1.4	4.0	3.3	-2.3	4.1	3.3	-1.2	0.7
YBL026W	*LSM2*	*SMX5, SN*	7380_f_at	YBL026W	6.6	1.2	1.0	6.6	2.8	12.1	5.5	-1.2	0.9	5.5	2.6	8.8
YML010W	*SPT5*		9655_at	YML010W	15.2	2.9	29.0	15.2	1.6	9.6	3.0	-2.9	5.7	3.0	-2.3	3.9
YFL003C	*MSH4*		5385_at	YFL003C r	5.6	2.2	6.9	5.6	-1.2	0.9	0.0	0.0	0.0	0.0	0.0	0.0
YCL010C	*SGF29*		6870_at	YCL010C S	11.0	2.0	11.1	11.0	1.5	5.7	5.6	-2.0	5.7	5.6	-1.3	1.7
YIL084C	*SDS3*		4182_at	YIL084C F	4.6	2.0	4.8	4.6	-1.2	0.9	0.0	0.0	0.0	0.0	0.0	0.0
YJR021C	*REC107*	*MER2*	10999_at	YJR021C M	2.3	2.0	2.4	2.3	2.4	3.2	0.0	0.0	0.0	0.0	0.0	0.0
YCR097W	*HMRA1*	*YCR097W*	6792_s_at	YCR097W	0.0	0.0	0.0	0.0	0.0	0.0	2.4	1.2	0.4	2.4	2.1	2.6
YKR034W	*DAL80*	*UGA43*	10551_at	YKR034W	0.0	0.0	0.0	0.0	0.0	0.0	1.8	2.0	1.9	1.8	-2.0	1.8
YCR018C	*SRD1*		6755_i_at	YCR018C	1.6	-2.4	2.2	1.6	-1.7	1.2	5.7	2.4	8.0	5.7	1.3	1.7
YMR167W	*MLH1*	*PMS2*	9479_at	YMR167W	10.2	2.7	17.6	10.2	1.2	2.4	3.0	-2.7	5.2	3.0	-2.1	3.3
YBL066C	*SEF1*		7430_at	Putative tra	9.5	4.0	28.5	9.5	1.8	7.6	5.2	-4.0	15.6	5.2	-2.2	6.2
YNL204C	*SPS18*	*SPX18*	9055_at	YNL204C s	4.2	-1.8	3.4	4.2	-2.7	7.2	0.0	0.0	0.0	0.0	0.0	0.0
YFL021W	*GAT1*	*NIL1*	5409_at	YFL021W	52.6	-2.5	81.2	52.6	-2.1	60.2	119.0	2.5	183.9	119.0	1.2	23.8
YPR182W	*SMX3*		7580_at	YPR182W	0.0	0.0	0.0	0.0	0.0	0.0	206.3	-1.0	10.1	206.3	-1.5	103.2
YAL033W	*POP5*	*FUN53*	11333_at	YAL033W	232.6	-1.3	69.8	232.6	-1.4	104.5	0.0	0.0	0.0	0.0	0.0	0.0
YOR224C	*RPB8*		8336_at	YOR224C	710.6	-1.1	105.8	710.6	-1.1	105.8	816.1	1.1	121.5	816.1	-1.0	0.0
YGR063C	*SPT4*		4944_at	YGR063C	239.6	-1.2	59.6	239.6	-1.4	107.6	0.0	0.0	0.0	0.0	0.0	0.0
YKL024C	*URA6*		10583_at	YKL024C u	314.8	1.1	30.0	314.8	-1.3	93.2	273.6	-1.1	26.1	273.6	-1.4	109.4
YHL034C	*SBP1*	*SSBR1*	4574_at	YHL034C S	0.0	0.0	0.0	0.0	0.0	0.0	743.6	1.1	110.7	743.6	-1.1	74.4
YHR041C	*SRB2*	*HRS2*	4470_at	YHR041C	188.0	-1.6	112.2	188.0	-1.6	112.2	343.7	1.6	205.1	343.7	1.0	0.0
YOR151C	*RPB2*	*RPB150, F*	8398_at	YOR151C	378.0	1.2	74.0	378.0	-1.1	56.3	281.6	-1.2	55.1	281.6	-1.4	112.6
YBR088C	*POL30*		7266_at	YBR088C p	191.6	-1.2	38.3	191.6	-1.6	115.0	0.0	0.0	0.0	0.0	0.0	0.0

	YDR404C	*RPB7*		6055_at	YDR404C (	422.4	-1.1	42.2	422.4	-1.3	126.7	0.0	0.0	0.0	0.0	0.0	0.0	
	YOR341W	*RPA190*	*RRN1*	8185_at	YOR341W	328.5	-1.1	48.9	328.5	-1.4	130.2	383.7	1.1	57.1	383.7	-1.2	76.7	
	YER094C	*PUP3*	*SCS32*	5625_at	YER094C :	928.9	-1.1	92.9	928.9	-1.1	138.3	0.0	0.0	0.0	0.0	0.0	0.0	
	YDL227C	*HO*		6725_at	YDL227C H	163.5	-1.4	73.4	163.5	-1.8	138.9	234.2	1.4	105.2	234.2	-1.3	70.3	
	YEL009C	*GCN4*	*AAS3, ARC*	5737_at		1433.2	1.0	70.0	1433.2	-1.1	136.8	1390.5	-1.0	67.9	1390.5	-1.1	139.1	
	RPR1			5413_at	RPR1 RNa	0.0	0.0	0.0	0.0	0.0	0.0	155.0	1.9	139.1	155.0	1.2	31.0	
	YPR010C	*RPA135*	*RPA2, RRI*	7762_at	YPR010C I	352.9	-1.0	17.2	352.9	-1.4	139.9	379.1	1.0	18.5	379.1	-1.3	113.7	
	YGR180C	*RNR4*		4835_at	YGR180C	942.0	-1.0	46.0	942.0	-1.1	140.3	0.0	0.0	0.0	0.0	0.0	0.0	
	YDR381W	*YRA1*	*SHE11*	6078_at	YDR381W	400.7	1.1	59.7	400.7	-1.2	99.8	355.9	-1.1	53.0	355.9	-1.4	142.4	
	YMR308C	*PSE1*	*KAP121*	9316_at	YMR308C	734.5	-1.1	109.4	734.5	-1.2	143.8	822.2	1.1	122.4	822.2	-1.1	82.2	
	YGL048C	*RPT6*	*CIM3, CRL*	5059_at	YGL048C a	697.5	-1.0	0.0	697.5	-1.1	103.9	734.5	-1.0	0.0	734.5	-1.2	146.9	
	YGL253W	*HXK2*	*HEX1, HKI*	5260_at	YGL253W	751.4	-1.3	262.3	751.4	-1.2	150.3	981.0	1.3	342.4	981.0	1.1	98.1	
	YPR187W	*RPO26*	*RPB6*	7539_at	YPR187W	335.7	-1.2	83.6	335.7	-1.4	150.8	429.1	1.2	106.8	429.1	-1.2	85.8	
	YNL112W	*DBP2*		8965_at	YNL112W	510.9	-1.2	102.2	510.9	-1.3	153.3	604.6	1.2	120.9	604.6	-1.1	60.5	
	YDR328C	*SKP1*	*MGO1*	6160_at	YDR328C	487.2	1.2	121.3	487.2	-1.1	48.7	386.5	-1.2	96.2	386.5	-1.4	154.6	
	YLL039C	*UBI4*	*SCD2*	10392_at	YLL039C u	432.6	1.3	151.0	432.6	-1.1	64.4	321.2	-1.3	112.1	321.2	-1.5	160.6	
	YER009W	*NTF2*		5709_at	YER009W	834.6	-1.2	163.4	834.6	-1.2	163.4	957.1	1.2	187.4	957.1	1.0	0.0	
	YOR310C	*NOP58*	*NOP5*	8247_at	YOR310C	757.9	-1.2	151.6	757.9	-1.0	37.0	888.7	1.2	177.7	888.7	1.1	88.9	
	YDL208W	*NHP2*		6700_at	YDL208W	821.0	-1.1	122.3	821.0	-1.2	204.4	930.8	1.1	138.6	930.8	-1.1	93.1	
	YJL157C	*FAR1*		11181_at	YJL157C F	346.1	-1.3	120.8	346.1	-1.6	206.5	476.4	1.3	166.3	476.4	-1.2	95.3	
	YPR033C	*HTS1*	*TSM4572*	7741_at	YPR033C (	720.9	-1.1	72.1	720.9	-1.3	216.3	795.2	1.1	79.5	795.2	-1.2	159.0	
	YJL080C	*SCP160*		11122_at	YJL080C M	403.0	-1.1	60.0	403.0	-1.5	221.3	472.9	1.1	70.4	472.9	-1.3	141.9	
	YER146W	*LSM5*		5586_at	YER146W	523.1	-1.1	77.9	523.1	-1.6	312.2	612.3	1.1	91.2	612.3	-1.3	183.7	
	YFR031C	*SMC2*		5329_s_at	YFR031C (	3309.1	-1.2	824.0	3309.1	-1.1	492.8	4068.5	1.2	1013.1	4068.5	1.1	406.9	
	YPL037C	*EGD1*		7806_at	YPL037C (	1962.5	-1.2	392.5	1962.5	-1.3	588.8	2272.2	1.2	454.4	2272.2	-1.1	227.2	
	chromosome																	
		YDR225W	*HTA1*	*H2A1, SPT*	6236_i_at	YDR225W	1800.8	-1.1	268.2	1800.8	1.1	268.2	2018.3	1.1	300.6	2018.3	1.3	605.5
		YNL031C	*HHT2*		8910_at	YNL031C H	2640.6	-1.1	393.2	2640.6	-1.0	128.9	3123.3	1.1	465.1	3123.3	1.1	312.3
		YNL030W	*HHF2*		8911_s_at	YNL030W	0.0	0.0	0.0	0.0	0.0	0.0	1477.0	1.1	147.7	1477.0	1.2	295.4
		YMR094W	*CTF13*	*CBF3C*	9536_at	58 kd comp	30.0	1.4	12.0	30.0	8.2	215.6	21.4	-1.4	8.6	21.4	5.9	104.9
		YDR224C	*HTB1*	*SPT12*	6235_at	YDR224C	0.0	0.0	0.0	0.0	0.0	0.0	839.3	1.1	83.9	839.3	1.2	167.9
		YOL012C	*HTZ1*	*HTA3*	8550_at	YOL012C /	193.0	-1.0	9.4	193.0	-1.7	144.6	204.4	1.0	10.0	204.4	-1.7	143.1
		YMR241W	*YHM2*		9380_at	YMR241W	739.4	-1.3	221.8	739.4	-1.3	221.8	974.2	1.3	292.3	974.2	1.0	0.0
		YGR285C	*ZUO1*		4715_at	YGR285C	650.8	-1.2	130.2	650.8	-1.3	227.2	776.2	1.2	155.2	776.2	-1.1	77.6
		YBL002W	*HTB2*		7359_at	YBL002W	541.0	-1.1	54.1	541.0	-1.5	270.5	614.9	1.1	61.5	614.9	-1.3	184.5
		YPR052C	*NHP6A*		7717_at	YPR052C	530.1	-1.1	65.8	530.1	-1.6	328.4	596.6	1.1	74.1	596.6	-1.4	265.9
		YBL003C	*HTA2*	*H2A2*	7358_s_at	YBL003C H	1320.7	-1.2	258.6	1320.7	-1.2	328.9	1555.2	1.2	304.5	1555.2	-1.0	0.0
		YBR010W	*HHT1*	*BUR5, SIN*	7368_i_at	YBR010W	2568.7	-1.1	382.5	2568.7	-1.1	382.5	2912.9	1.1	433.8	2912.9	-1.0	0.0
mitochondrion																		
	YNL104C	*LEU4*		8973_at	YNL104C a	1720.8	1.1	172.1	1720.8	2.0	1720.8	1616.3	-1.1	161.6	1616.3	1.8	1293.0	
	YNL005C	*MRP7*		8890_at	Peptidyltrai	200.5	1.3	70.0	200.5	7.0	1212.7	151.3	-1.3	52.8	151.3	5.4	665.7	
	YNL137C	*NAM9*	*MNA6*	8985_at	Structural c	251.5	1.2	49.3	251.5	4.9	990.3	215.5	-1.2	42.2	215.5	4.3	711.2	
	YMR145C	*NDE1*	*NDH1*	9500_at	YMR145C	1003.9	1.5	502.0	1003.9	1.5	551.3	664.3	-1.5	332.2	664.3	1.0	0.0	
	YER073W	*ALD5*		5646_at	YER073W	453.5	1.2	109.3	453.5	2.2	540.1	360.2	-1.2	86.8	360.2	1.7	252.1	
	YNL055C	*POR1*	*OMP2*	8932_at	YNL055C (	1947.9	1.1	194.8	1947.9	1.2	485.0	0.0	0.0	0.0	0.0	0.0	0.0	
	YDL198C	*YHM1*		6665_at	YDL198C H	982.5	-1.0	0.0	982.5	1.4	441.3	964.8	-1.0	0.0	964.8	1.4	385.9	
	YDR035W	*ARO3*		6450_at	YDR035W	1396.4	1.1	139.6	1396.4	1.3	418.9	1273.6	-1.1	127.4	1273.6	1.2	254.7	
	YMR286W			9335_at	YMR286W	496.5	1.0	24.2	496.5	1.6	296.3	456.1	-1.0	22.3	456.1	1.5	228.1	
	YKL120W	*OAC1*		10709_at	YKL120W (	519.5	1.3	181.3	519.5	1.5	285.3	376.9	-1.3	131.6	376.9	1.1	37.7	
	YLR355C	*ILV5*		9979_at	YLR355C a	1911.6	1.0	93.3	1911.6	1.1	284.7	0.0	0.0	0.0	0.0	0.0	0.0	

YNL070W	*TOM7*	*MOM7, YC*	8917_at	YNL070W	828.9	-1.1	123.4	828.9	1.1	123.4	927.8	1.1	138.2	927.8	1.3	278.3
YPR191W	*QCR2*	*COR2, UC*	7543_at	YPR191W	497.9	1.9	448.1	497.9	1.5	273.4	238.5	-1.9	214.7	238.5	-1.2	47.7
YKL085W	*MDH1*		10654_at	YKL085W	1063.9	1.1	158.4	1063.9	1.2	264.9	866.5	-1.1	129.0	866.5	1.1	86.7
YJR016C	*ILV3*		10993_at	YJR016C c	1257.3	1.1	125.7	1257.3	1.2	251.5	0.0	0.0	0.0	0.0	0.0	0.0
YKL016C	*ATP7*		10591_at	YKL016C /	689.5	1.2	171.7	689.5	1.3	240.7	546.0	-1.2	136.0	546.0	1.1	54.6
YKL150W	*MCR1*		10723_at	YKL150W	476.9	1.6	286.1	476.9	1.5	238.4	291.2	-1.6	174.7	291.2	-1.1	29.1
YMR256C	*COX7*		9350_at	YMR256C	1167.2	1.1	116.7	1167.2	1.2	233.4	0.0	0.0	0.0	0.0	0.0	0.0
YMR108W	*ILV2*	*SMR1, TH*	9550_at	YMR108W	502.3	1.1	74.8	502.3	1.4	225.6	448.3	-1.1	66.8	448.3	1.3	134.5
YLL041C	*SDH2*		10390_at	YLL041C S	1047.5	1.6	628.5	1047.5	1.2	209.5	654.7	-1.6	392.8	654.7	-1.3	196.4
YDR019C	*GCV1*	*GSD1*	6480_at	YDR019C	443.3	1.4	199.1	443.3	1.2	86.8	318.4	-1.4	143.0	318.4	-1.2	63.7
YEL024W	*RIP1*		5766_at	YEL024W	767.0	1.4	344.5	767.0	1.2	191.0	600.6	-1.4	269.8	600.6	-1.2	120.1
YPR058W	*YMC1*		7678_at	YPR058W	624.9	1.1	62.5	624.9	1.3	187.5	569.7	-1.1	57.0	569.7	1.2	113.9
YLR038C	*COX12*		10290_at	YLR038C s	1869.2	1.1	278.3	1869.2	1.1	186.9	1626.8	-1.1	242.3	1626.8	-1.0	0.0
YDR258C	*HSP78*		6223_at	YDR258C	201.3	1.2	50.1	201.3	1.9	180.6	0.0	0.0	0.0	0.0	0.0	0.0
YGL125W	*MET13*	*MET11*	5117_at	YGL125W	579.3	1.1	86.3	579.3	1.3	173.8	0.0	0.0	0.0	0.0	0.0	0.0
YLR203C	*MSS51*		10141_at	YLR203C F	244.8	1.7	171.3	244.8	1.3	85.4	144.6	-1.7	101.2	144.6	-1.3	43.4
YML054C	*CYB2*		9702_at	YML054C (	95.6	2.7	166.8	95.6	1.2	18.7	37.7	-2.7	65.8	37.7	-2.4	52.8
YOR045W	*TOM6*	*ISP6, MOM*	8518_at	YOR045W	1407.9	-1.1	209.6	1407.9	-1.1	140.8	1605.3	1.1	239.0	1605.3	1.1	160.5
YGR183C	*QCR9*	*UCR9*	4838_at	YGR183C	1046.0	1.1	155.8	1046.0	1.0	51.1	903.1	-1.1	134.5	903.1	-1.1	90.3
YOR316C	*COT1*		8206_at	YOR316C	280.8	1.4	111.3	280.8	1.5	152.4	0.0	0.0	0.0	0.0	0.0	0.0
YPL262W	*FUM1*		8033_at	YPL262W	770.2	1.2	150.8	770.2	1.1	73.5	627.8	-1.2	122.9	627.8	-1.1	62.8
YDL130W	*STF1*		6600_at	YDL130W	1812.2	-1.0	89.9	1812.2	-1.0	11.0	2258.3	1.3	635.7	2258.3	1.1	148.1
YOR065W	*CYT1*	*CTC1*	8493_at	YOR065W	269.6	1.5	133.9	269.6	1.5	148.0	0.0	0.0	0.0	0.0	0.0	0.0
YBR282W			7100_at	YBR282W	266.4	1.3	91.0	266.4	1.5	144.6	0.0	0.0	0.0	0.0	0.0	0.0
YMR083W	*ADH3*		9569_at	YMR083W	1156.0	-1.2	231.2	1156.0	-1.0	56.4	1403.8	1.2	280.8	1403.8	1.1	140.4
YDR178W	*SDH4*		6279_at	YDR178W	695.1	1.2	139.0	695.1	1.0	33.9	586.8	-1.2	117.4	586.8	-1.1	58.7
YCL009C	*ILV6*		6871_at	YCL009C S	1187.4	-1.1	176.8	1187.4	-1.0	0.0	1351.5	1.1	201.3	1351.5	1.1	135.2
YOL059W	*GPD2*	*GPD3*	8592_at	YOL059W	166.1	1.7	115.8	166.1	1.8	132.4	98.2	-1.7	68.5	98.2	1.1	9.8
YML091C	*RPM2*		9754_at	Rpm2p is a	187.9	1.7	131.5	187.9	1.4	75.1	120.0	-1.7	84.0	120.0	-1.2	24.0
YER086W	*ILV1*	*ISO1*	5614_at	YER086W	655.1	-1.0	0.0	655.1	1.2	131.0	0.0	0.0	0.0	0.0	0.0	0.0
YGR029W	*ERV1*		3946_at	YGR029W	370.4	1.2	82.3	370.4	1.3	129.3	307.9	-1.2	68.4	307.9	1.1	30.8
YGR028W	*MSP1*	*YTA4*	4956_at	YGR028W	231.7	1.5	127.2	231.7	1.2	46.3	147.3	-1.5	80.9	147.3	-1.3	44.2
YBL099W	*ATP1*		7487_at	YBL099W	1009.5	-1.1	150.3	1009.5	-1.0	0.0	1258.4	1.1	187.4	1258.4	1.1	125.8
YAL054C	*ACS1*		11356_at	YAL054C i	350.3	1.2	87.2	350.3	1.3	122.3	0.0	0.0	0.0	0.0	0.0	0.0
YER069W	*ARG5,6*		5642_at	YER069W	592.0	1.2	118.4	592.0	1.0	28.9	499.6	-1.2	99.9	499.6	-1.1	50.0
YGR008C	*STF2*		4980_at	YGR008C	783.8	1.3	235.1	783.8	1.1	116.7	605.4	-1.3	181.6	605.4	-1.1	60.5
YLR348C	*DIC1*		9972_at	YLR348C r	231.9	1.5	115.9	231.9	1.3	69.6	0.0	0.0	0.0	0.0	0.0	0.0
YLR163C	*MAS1*	*MIF1*	10188_at	YLR163C r	166.2	1.7	115.8	166.2	1.2	41.4	85.4	-1.7	59.5	85.4	-1.3	25.6
YLR395C	*COX8*		9930_at	YLR395C (	1205.1	1.1	179.5	1205.1	1.1	115.0	1054.3	-1.1	157.0	1054.3	-1.0	0.0
YFR033C	*QCR6*	*COR3, UC*	5332_at	YFR033C (	563.1	1.2	112.6	563.1	-1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
YDR074W	*TPS2*	*HOG2, PF*	6398_at	YDR074W	224.7	1.5	111.6	224.7	-1.0	11.0	140.6	-1.5	69.8	140.6	-1.6	84.4
YFL016C	*MDJ1*		5370_at	YFL016C D	158.6	1.6	95.2	158.6	1.7	111.0	108.1	-1.6	64.9	108.1	1.1	10.8
YLL009C	*COX17*		10334_at	YLL009C ir	551.2	1.2	110.2	551.2	1.1	82.1	0.0	0.0	0.0	0.0	0.0	0.0
YLR168C	*MSF1*		10148_at	YLR168C p	269.3	1.4	107.7	269.3	1.4	107.7	0.0	0.0	0.0	0.0	0.0	0.0
YBR146W			7237_at	YBR146W	214.7	1.2	42.9	214.7	1.5	107.3	0.0	0.0	0.0	0.0	0.0	0.0
YOR158W	*PET123*		8405_at	YOR158W	210.3	1.2	42.1	210.3	1.5	105.2	0.0	0.0	0.0	0.0	0.0	0.0
YJL054W	*TIM54*		11058_at	YJL054W T	105.3	1.4	41.7	105.3	2.0	105.0	0.0	0.0	0.0	0.0	0.0	0.0
YNL073W	*MSK1*		8914_at	YNL073W	54.4	1.2	10.9	54.4	2.9	103.3	50.6	-1.2	10.1	50.6	2.4	70.8
YML120C	*NDI1*		9765_at	YML120C r	207.2	1.9	186.5	207.2	1.2	41.4	108.0	-1.9	97.2	108.0	-1.6	64.8
YBL045C	*COR1*	*QCR1*	7407_at	YBL045C 4	624.4	1.3	184.9	624.4	1.1	93.0	476.8	-1.3	141.2	476.8	-1.1	47.7
YDR405W			6056_at	YDR405W	27.5	1.3	9.6	27.5	2.3	35.5	0.0	0.0	0.0	0.0	0.0	0.0

	Q0185	*??*		4001_at	Cytochrom	22.9	2.3	30.5	22.9	2.2	28.1	10.6	-2.3	14.1	10.6	-1.0	0.2
	YPR001W	*CIT3*		7753_at	YPR001W	11.9	2.6	18.9	11.9	1.4	5.3	5.5	-2.6	8.8	5.5	-1.8	4.4
	YLR393W	*ATP10*		9928_at	YLR393W	8.6	1.9	8.1	8.6	2.5	12.8	0.0	0.0	0.0	0.0	0.0	0.0
	YBL013W	*FMT1*		7394_at	YBL013W	2.8	1.1	0.3	2.8	2.3	3.6	2.6	-1.1	0.3	2.6	2.2	3.1
	YCL004W	*PGS1*	*PEL1*	6875_at	17-kDa Ph	6.6	3.2	14.7	6.6	1.4	2.5	0.0	-3.2	0.0	0.0	-2.4	0.0
	Q0325			3979_i_at	mitochondr	2.4	1.5	1.1	2.4	-2.4	3.4	0.8	-1.5	0.4	0.8	-2.9	1.5
	YNL328C	*MDJ2*		9202_at	YNL328C F	0.0	0.0	0.0	0.0	0.0	0.0	4.5	-1.7	3.3	4.5	-2.5	6.8
	YAR035W	*YAT1*		11297_at	Outer carni	48.8	-1.4	21.6	48.8	-1.3	16.7	79.9	1.4	35.3	79.9	1.1	8.0
	YBR085W	*AAC3*	*ANC3*	7263_at	YBR085W	47.0	-2.0	48.6	47.0	-1.9	43.8	99.8	2.0	103.3	99.8	1.1	10.0
	YGR222W	*PET54*		4788_at	YGR222W	0.0	0.0	0.0	0.0	0.0	0.0	136.2	-1.4	54.0	136.2	-1.7	95.3
	YDR148C	*KGD2*		6339_at	YDR148C	0.0	0.0	0.0	0.0	0.0	0.0	206.5	-1.2	41.3	206.5	-1.5	103.3
	YIL114C	*POR2*		4197_at	YIL114C vo	123.8	-1.8	105.1	123.8	-1.8	105.1	0.0	0.0	0.0	0.0	0.0	0.0
	YBR039W	*ATP3*		7307_at	YBR039W	630.5	1.2	126.1	630.5	-1.0	0.0	526.3	-1.2	105.3	526.3	-1.2	105.3
	YIL070C	*MAM33*		4149_at	YIL070C 33	369.8	1.4	147.9	369.8	-1.0	0.0	270.4	-1.4	108.2	270.4	-1.4	108.2
	YML110C	*COQ5*	*DBI56*	9777_at	YML110C I	0.0	0.0	0.0	0.0	0.0	0.0	729.9	1.1	108.7	729.9	1.1	73.0
	YGL191W	*COX13*		5188_at	YGL191W	0.0	0.0	0.0	0.0	0.0	0.0	560.7	-1.1	83.5	560.7	-1.2	112.1
	YGL068W			5084_at	YGL068W	0.0	0.0	0.0	0.0	0.0	0.0	283.9	-1.2	56.8	283.9	-1.4	113.6
	YOR187W	*TUF1*		8343_at	YOR187W	687.5	1.2	137.5	687.5	-1.0	0.0	586.9	-1.2	117.4	586.9	-1.2	117.4
	YHR051W	*COX6*		4481_at	YHR051W	904.0	1.3	271.2	904.0	1.1	90.4	652.9	-1.3	195.9	652.9	-1.2	130.6
	YFL018C	*LPD1*	*HPD1*	5367_at	YFL018C c	871.1	1.2	216.9	871.1	-1.0	0.0	700.8	-1.2	174.5	700.8	-1.2	140.2
	YER141W	*COX15*		5581_at	YER141W	491.1	-1.0	0.0	491.1	-1.2	122.3	484.7	-1.0	0.0	484.7	-1.3	145.4
	YCR028C	*FEN2*		6863_at	YCR028C	375.2	-1.2	93.4	375.2	-1.4	148.7	465.8	1.2	116.0	465.8	-1.1	46.6
	YNL052W	*COX5A*		8935_at	YNL052W	606.5	1.2	121.3	606.5	-1.0	29.6	501.4	-1.2	100.3	501.4	-1.3	150.4
	YJR077C	*MIR1*		10961_at	YJR077C i	1731.2	1.1	257.8	1731.2	1.0	84.5	1524.8	-1.1	227.1	1524.8	-1.1	152.5
	YLR295C	*ATP14*		10010_at	YLR295C A	630.6	1.6	376.4	630.6	1.1	93.9	394.9	-1.6	235.7	394.9	-1.4	158.0
	YDL181W	*INH1*		6684_at	YDL181W	140.3	1.2	28.1	140.3	-2.0	140.3	113.1	-1.2	22.6	113.1	-2.4	158.3
	YDR322C	*TIM11*		6154_at	YDR322C s	975.4	1.2	195.1	975.4	1.1	97.5	799.4	-1.2	159.9	799.4	-1.2	159.9
	YDL004W	*ATP16*		6503_at	YDL004W	720.0	1.2	179.3	720.0	1.0	35.1	558.7	-1.2	139.1	558.7	-1.3	167.6
	YBR263W	*SHM1*	*SHMT1, TI*	7083_at	YBR263W	338.5	-1.2	84.3	338.5	-1.5	169.2	423.2	1.2	105.4	423.2	-1.2	84.6
	YPL271W	*ATP15*	*ATPEPSIL*	8024_at	YPL271W	1021.8	1.1	152.2	1021.8	-1.0	49.9	899.9	-1.1	134.0	899.9	-1.2	180.0
	Q0310			3976_at	F1F0-ATPa	804.9	1.2	200.4	804.9	-1.0	39.3	644.5	-1.2	160.5	644.5	-1.3	193.4
	YGL187C	*COX4*		5193_at	YGL187C s	759.1	1.1	75.9	759.1	-1.2	151.8	691.0	-1.1	69.1	691.0	-1.3	207.3
	YJR048W	*CYC1*		10977_at	YJR048W	827.3	1.1	123.2	827.3	-1.1	79.0	697.0	-1.1	103.8	697.0	-1.3	209.1
	YMR241W	*YHM2*		9380_at	YMR241W	739.4	-1.3	221.8	739.4	-1.3	221.8	974.2	1.3	292.3	974.2	1.0	0.0
	YDL067C	*COX9*		6531_at	YDL067C S	687.3	-1.0	33.5	687.3	-1.3	239.9	707.7	1.0	34.5	707.7	-1.3	212.3
	YLR259C	*HSP60*	*CPN60, MI*	10061_at	YLR259C h	1822.6	-1.1	182.3	1822.6	-1.1	271.4	0.0	0.0	0.0	0.0	0.0	0.0
	YLR304C	*ACO1*	*GLU1*	10019_at	YLR304C A	1066.5	-1.4	479.0	1066.5	-1.6	636.6	1602.0	1.4	719.5	1602.0	-1.1	160.2
peroxisome																	
	YAL054C	*ACS1*		11356_at	YAL054C i	350.3	1.2	87.2	350.3	1.3	122.3	0.0	0.0	0.0	0.0	0.0	0.0
	YDL078C	*MDH3*		6565_at	YDL078C r	270.4	-1.0	13.2	270.4	-1.4	121.4	258.3	1.0	12.6	258.3	-1.4	103.3
	YLR027C	*AAT2*	*ASP5*	10323_at	YLR027C a	1551.3	-1.1	231.0	1551.3	-1.1	231.0	1722.6	1.1	256.5	1722.6	-1.0	0.0
	YLR109W	*AHP1*	*cTPxIII*	10228_at	YLR109W	1813.1	-1.2	451.4	1813.1	-1.2	362.6	2293.7	1.2	571.1	2293.7	1.1	229.4
	YCR005C	*CIT2*		6883_at	YCR005C	1341.0	-1.1	199.7	1341.0	-1.3	468.1	1588.0	1.1	236.5	1588.0	-1.1	158.8
vacuole or lysosome																	
	YLR034C	*SMF3*		10286_at	YLR034C F	700.6	1.1	104.3	700.6	1.3	244.5	596.7	-1.1	88.9	596.7	1.2	119.3
	YHR026W	*PPA1*	*VMA16*	4500_at	YHR026W	1541.2	-1.3	462.3	1541.2	-1.1	154.1	1942.4	1.3	582.7	1942.4	1.1	194.2
	YPL154C	*PEP4*	*PHO9, PR*	7916_at	YPL154C v	1033.3	-1.2	206.7	1033.3	-1.1	103.3	1221.5	1.2	244.3	1221.5	1.1	122.2
	YGL156W	*AMS1*		5179_at	Alpha man	58.3	4.3	192.3	58.3	2.7	102.0	12.2	-4.3	40.2	12.2	-1.6	7.3
	YIL099W	*SGA1*		4212_g_at	Intracellula	40.4	2.4	56.6	40.4	2.3	52.4	25.6	-2.4	35.8	25.6	-1.0	1.1

		YCR044C	*PER1*		6832_at	YCR044C	324.0	-1.2	64.8	324.0	-1.3	113.1	0.0	0.0	0.0	0.0	0.0	0.0	
		YGR020C	*VMA7*		4993_at	YGR020C	462.1	-1.4	184.8	462.1	-1.3	138.6	648.7	1.4	259.5	648.7	1.1	64.9	
		YLR447C	*VMA6*		9851_at	YLR447C	508.6	-1.0	24.8	508.6	-1.3	150.6	518.0	1.0	25.3	518.0	-1.3	155.4	
		YKL080W	*VMA5*	*CSL5, VAT*	10660_at	YKL080W	713.6	-1.3	249.1	713.6	-1.2	177.7	957.0	1.3	334.1	957.0	1.1	95.7	
		YPR036W	*VMA13*	*CLS11*	7699_at	YPR036W	437.6	1.1	43.8	437.6	-1.3	152.7	403.1	-1.1	40.3	403.1	-1.5	201.6	
		YDL185W	*TFP1*	*CLS8, VM*	6679_at	YDL185W	690.5	-1.3	207.2	690.5	-1.3	241.0	892.6	1.3	267.8	892.6	-1.0	0.0	
		YBR286W	*APE3*	*APY1*	7059_at	YBR286W	1238.0	-1.2	242.4	1238.0	-1.2	242.4	1456.7	1.2	285.3	1456.7	-1.0	0.0	
		YMR297W	*PRC1*	*LBC1*	9348_at	YMR297W	999.2	-1.1	148.8	999.2	-1.2	248.8	1135.5	1.1	169.1	1135.5	-1.1	113.6	
		YBR127C	*VMA2*	*ATPVS, V*	7218_at	YBR127C	734.2	-1.5	367.1	734.2	-1.4	329.7	1021.3	1.5	510.7	1021.3	1.0	0.0	
		YEL027W	*CUP5*		5763_at	YEL027W	2242.6	-1.3	664.1	2242.6	-1.1	334.0	2929.6	1.3	867.6	2929.6	1.1	293.0	
		YOR332W	*VMA4*		8222_at	YOR332W	978.3	-1.3	341.5	978.3	-1.3	341.5	1290.1	1.3	450.3	1290.1	-1.0	0.0	
		YHR039C	*MSC7*	*VMA10*	4514_at	YHR039C	783.6	-1.3	235.1	783.6	-1.4	351.9	1017.3	1.3	305.2	1017.3	-1.1	101.7	
		YPL234C	*TFP3*	*CLS9, VM*	7970_at	YPL234C	1186.4	-1.3	355.9	1186.4	-1.3	355.9	1511.7	1.3	453.5	1511.7	-1.1	151.2	
		YOR270C	*VPH1*		8291_at	YOR270C	1344.7	-1.3	403.4	1344.7	-1.3	469.4	1771.5	1.3	531.5	1771.5	-1.0	0.0	
	extracellular / secretion proteins																		
		YNL160W	*YGP1*		9008_at	YNL160W	1172.5	2.3	1524.2	1172.5	2.8	2168.5	474.9	-2.3	617.4	474.9	1.3	142.5	
		YBR092C	*PHO3*		7273_at	Acid phosp	356.2	1.4	141.2	356.2	4.4	1226.2	257.9	-1.4	102.2	257.9	3.2	567.4	
		YPL187W	*MF(ALPHA)1*		7927_at	YPL187W	2437.9	-1.1	363.0	2437.9	-1.0	119.0	2754.2	1.1	410.1	2754.2	1.1	275.4	
		YLR300W	*EXG1*	*BGL1*	10015_at	YLR300W	1585.6	1.0	77.4	1585.6	1.1	236.1	0.0	0.0	0.0	0.0	0.0	0.0	
		YJL159W	*HSP150*	*CCW7, OF*	11179_at	Kex2-proce	1519.4	1.0	74.2	1519.4	1.1	226.3	1451.1	-1.0	70.8	1451.1	1.1	145.1	
		YJL174W	*KRE9*		11209_at	YJL174W	643.5	1.2	128.7	643.5	1.2	160.2	581.8	-1.2	116.4	581.8	1.0	0.0	
		YNL145W	*MFA2*		9023_at	Mating a-fa	22.9	1.0	1.0	22.9	7.0	137.4	21.9	-1.0	1.0	21.9	6.8	127.0	
		YDR461W	*MFA1*		6021_f_at	YDR461W	31.6	2.2	39.3	31.6	1.9	28.3	11.8	-2.2	14.7	11.8	-1.1	1.2	
		YLR286C	*CTS1*		10045_at	YLR286C	2323.9	-1.1	346.1	2323.9	-1.1	346.1	2400.2	1.1	357.4	2400.2	-1.1	240.0	
		YGL089C	*MF(ALPHA)2*		5108_at	YGL089C	1468.1	-1.5	729.2	1468.1	-1.4	659.4	2220.7	1.5	1102.9	2220.7	1.0	0.0	
	other subcellular localisation																		
		YGR175C	*ERG1*		4830_at	YGR175C	828.8	-1.1	123.4	828.8	-1.0	40.5	926.4	1.1	138.0	926.4	1.1	92.6	
		YBR177C	*EHT1*		7179_at	YBR177C	369.1	-1.0	0.0	369.1	-1.3	110.7	335.1	-1.0	0.0	335.1	-1.3	100.5	
PROTEIN ACTIVITY REGULATION																			
	target of regulation																		
		regulator of G-protein signalling																	
			YOR107W	*RGS2*			8444_at	YOR107W	5.8	2.9	11.1	5.8	2.3	7.8	2.1	-2.9	4.0	2.1	-1.3
TRANSPORT FACILITATION																			
	channel / pore class transporters																		
		ion channels																	
			YLL052C	*AQY2*		10378_at	YLL052C a	50.0	1.0	2.4	50.0	2.6	80.6	55.1	-1.0	2.7	55.1	2.0	55.1
			YPR192W	*AQY1*		7544_at	YPR192W	0.0	0.0	0.0	0.0	0.0	0.0	6.2	-1.7	4.0	6.2	-2.4	8.7
			YIL114C	*POR2*		4197_at	YIL114C vo	123.8	-1.8	105.1	123.8	-1.8	105.1	0.0	0.0	0.0	0.0	0.0	0.0
	ion transporters																		
		cation transporters																	
			YOR087W	*YVC1*		8468_at	Calcium-ac	40.1	4.9	154.9	40.1	3.5	98.4	10.2	-4.9	39.5	10.2	-1.6	6.2
			heavy metal ion transporters (Cu, Fe, etc.)																
				YOR382W	*FIT2*		8181_at	Facilitator	727.5	11.0	7240.4	727.5	12.0	7996.5	62.0	-11.0	617.1	62.0	1.1
				YEL065W	*SIT1*	*ARN3*	5769_at	Siderophor	407.0	6.4	2204.5	407.0	12.4	4625.1	67.8	-6.4	367.3	67.8	1.8
				YMR058W	*FET3*		9588_at	YMR058W	900.7	2.1	988.6	900.7	2.4	1259.1	422.2	-2.1	463.4	422.2	1.1
				YOR383C	*FIT3*		8182_at	YOR383C	832.9	1.5	413.6	832.9	1.8	702.8	559.8	-1.5	278.0	559.8	1.2

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ORF	Gene	Alias	Probe	Description												
YNL259C	*ATX1*		9135_at	YNL259C /	701.7	1.1	70.2	701.7	1.3	210.5	0.0	0.0	0.0	0.0	0.0	
YOR316C	*COT1*		8206_at	YOR316C	280.8	1.4	111.3	280.8	1.5	152.4	0.0	0.0	0.0	0.0	0.0	
YDR270W	*CCC2*		6190_at	Accessory	150.0	1.9	135.0	150.0	1.9	135.0	92.0	-1.9	82.8	92.0	1.0	
YER145C	*FTR1*		5585_at	YER145C I	260.0	1.1	30.5	260.0	1.5	124.6	143.9	-1.1	18.6	143.9	1.4	
YER145C	*FTR1*		5585_at	YER145C I	260.0	1.1	30.5	260.0	1.5	124.6	143.9	-1.1	18.6	143.9	1.4	
YHR175W	*CTR2*		4335_at	YHR175W	453.1	1.2	112.8	453.1	1.2	88.7	0.0	0.0	0.0	0.0	0.0	
YMR243C	*ZRC1*	*OSR1*	9382_at	YMR243C	815.0	-1.0	39.8	815.0	-1.1	121.4	0.0	0.0	0.0	0.0	0.0	
YLR411W	*CTR3*		9902_at	YLR411W	78.5	-1.8	62.4	78.5	-2.9	145.5	135.3	1.8	107.5	135.3	-1.6	
YLR130C	*ZRT2*		10204_at	YLR130C L	498.4	-1.5	249.2	498.4	-1.3	174.0	750.0	1.5	375.0	750.0	1.1	
YGL255W	*ZRT1*		5258_at	YGL255W	147.7	-1.7	103.0	147.7	-2.6	243.1	279.2	1.7	194.6	279.2	-1.6	
other cation transporters (Na, K, Ca , NH4, etc.)																
YGL008C	*PMA1*		5009_at	YGL008C p	0.0	0.0	0.0	0.0	0.0	0.0	2122.2	1.1	212.2	2122.2	1.2	
YCR024C	*PMP1*		6858_f_at	YCR024C :	897.8	-1.3	313.4	897.8	-1.0	0.0	1190.8	1.3	415.7	1190.8	1.3	
YEL017C	*PMP2*		5728_i_at	YEL017C F	1819.7	-1.3	488.1	1819.7	-1.1	214.0	2277.6	1.3	611.0	2277.6	1.1	
YGR065C	*VHT1*		4946_at	YGR065C	433.6	1.3	128.4	433.6	1.5	215.4	0.0	0.0	0.0	0.0	0.0	
YHR026W	*PPA1*	*VMA16*	4500_at	YHR026W	1541.2	-1.3	462.3	1541.2	-1.1	154.1	1942.4	1.3	582.7	1942.4	1.1	
YGR020C	*VMA7*		4993_at	YGR020C	462.1	-1.4	184.8	462.1	-1.3	138.6	648.7	1.4	259.5	648.7	1.1	
YLR447C	*VMA6*		9851_at	YLR447C 3	508.6	-1.0	24.8	508.6	-1.3	150.6	518.0	1.0	25.3	518.0	-1.3	
YKL080W	*VMA5*	*CSL5, VA7*	10660_at	YKL080W 1	713.6	-1.3	249.1	713.6	-1.2	177.7	957.0	1.3	334.1	957.0	1.1	
YGR121C	*MEP1*	*AMT1*	4866_at	Ammonia p	42.9	-4.6	155.0	42.9	-5.4	189.0	195.4	4.6	706.8	195.4	-1.2	
YPR036W	*VMA13*	*CLS11*	7699_at	YPR036W	437.6	1.1	43.8	437.6	-1.3	152.7	403.1	-1.1	40.3	403.1	-1.5	
YOR153W	*PDR5*	*LEM1, YDI*	8400_at	Multidrug r	707.7	-1.3	209.6	707.7	-1.3	209.6	916.0	1.3	271.3	916.0	1.0	
YDL185W	*TFP1*	*CLS8, VMA*	6679_at	YDL185W	690.5	-1.3	207.2	690.5	-1.3	241.0	892.6	1.3	267.8	892.6	-1.0	
YBR127C	*VMA2*	*ATPVS, VA*	7218_at	YBR127C I	734.2	-1.5	367.1	734.2	-1.4	329.7	1021.3	1.5	510.7	1021.3	1.0	
YEL027W	*CUP5*		5763_at	YEL027W 1	2242.6	-1.3	664.1	2242.6	-1.1	334.0	2929.6	1.3	867.6	2929.6	1.1	
YOR332W	*VMA4*		8222_at	YOR332W	978.3	-1.3	341.5	978.3	-1.3	341.5	1290.1	1.3	450.3	1290.1	-1.0	
YHR039C	*MSC7*	*VMA10*	4514_at	YHR039C	783.6	-1.3	235.1	783.6	-1.4	351.9	1017.3	1.3	305.2	1017.3	-1.1	
YPL234C	*TFP3*	*CLS9, VMA*	7970_at	YPL234C 1	1186.4	-1.3	355.9	1186.4	-1.3	355.9	1511.7	1.3	453.5	1511.7	-1.1	
YOR270C	*VPH1*		8291_at	YOR270C	1344.7	-1.3	403.4	1344.7	-1.3	469.4	1771.5	1.3	531.5	1771.5	-1.0	
YNL142W	*MEP2*		9026_at	Ammonia t	75.9	-17.2	1229.5	75.9	-14.8	1051.2	1305.0	17.2	21139.5	1305.0	1.2	
anion transporters (Cl, SO4, PO4, etc.)																
YBR294W	*SUL1*		7067_at	Putative su	97.3	1.3	33.9	97.3	4.0	291.6	70.8	-1.3	24.7	70.8	2.9	134.5
YKL120W	*OAC1*		10709_at	YKL120W (	519.5	1.3	181.3	519.5	1.5	285.3	376.9	-1.3	131.6	376.9	1.1	37.7
YAL067C	*SEO1*		11379_at	Suppresso	92.1	1.6	59.8	92.1	3.7	252.9	50.8	-1.6	33.0	50.8	2.5	76.2
YJL117W	*PHO86*		11132_at	YJL117W I	0.0	0.0	0.0	0.0	0.0	0.0	473.2	1.0	23.1	473.2	1.3	142.0
YER053C	*??*		5665_at	YER053C i	227.7	1.6	136.6	227.7	1.3	79.5	141.7	-1.6	85.0	141.7	-1.2	28.3
YLR348C	*DIC1*		9972_at	YLR348C r	231.9	1.5	115.9	231.9	1.3	69.6	0.0	0.0	0.0	0.0	0.0	0.0
YLR092W	*SUL2*		10256_at	YLR092W	560.9	1.1	56.1	560.9	1.2	112.2	545.7	-1.1	54.6	545.7	1.1	54.6
YJR077C	*MIR1*		10961_at	YJR077C i	1731.2	1.1	257.8	1731.2	1.0	84.5	1524.8	-1.1	227.1	1524.8	-1.1	152.5
YGR121C	*MEP1*	*AMT1*	4866_at	Ammonia p	42.9	-4.6	155.0	42.9	-5.4	189.0	195.4	4.6	706.8	195.4	-1.2	39.1
YNL142W	*MEP2*		9026_at	Ammonia t	75.9	-17.2	1229.5	75.9	-14.8	1051.2	1305.0	17.2	21139.5	1305.0	1.2	261.0
C-compound and carbohydrate transporters																
YDL198C	*YHM1*		6665_at	YDL198C I	982.5	-1.0	0.0	982.5	1.4	441.3	964.8	-1.0	0.0	964.8	1.4	385.9
YDR497C	*ITR1*		5966_at	Myo-inosito	1187.5	-1.2	237.5	1187.5	-1.0	0.0	1358.0	1.2	271.6	1358.0	1.2	271.6
YMR011W	*HXT2*		9633_at	YMR011W	809.5	-1.3	242.8	809.5	-1.0	0.0	1029.2	1.3	308.8	1029.2	1.2	205.8
YHR094C	*HXT1*	*HOR4*	4430_at	YHR094C	540.4	-1.0	0.0	540.4	1.3	162.1	525.9	-1.0	0.0	525.9	1.3	157.8
YDR345C	*HXT3*		6131_at	YDR345C	1351.7	-1.2	264.7	1351.7	-1.1	129.0	1606.0	1.2	314.5	1606.0	1.1	160.6
YLR348C	*DIC1*		9972_at	YLR348C r	231.9	1.5	115.9	231.9	1.3	69.6	0.0	0.0	0.0	0.0	0.0	0.0
YFL011W	*HXT10*		5377_at	YFL011W I	4.7	-1.9	4.1	4.7	1.1	0.6	10.8	1.9	9.4	10.8	2.0	10.8
YDL247W	*??*		6709_i_at	YDL247W	2.0	2.3	2.6	2.0	-1.0	0.1	-2.2	-2.3	2.9	-2.2	-2.1	2.4

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	YDR342C	*HXT7*		6128_f_at	YDR342C	1141.2	-1.1	114.1	1141.2	-1.2	228.2	0.0	0.0	0.0	0.0	0.0	0.0	
	YGL225W	*VRG4*	*VAN2, GO*	5245_at	YGL225W	800.7	-1.3	279.5	800.7	-1.3	237.1	1065.2	1.3	371.8	1065.2	1.0	0.0	
	YDR343C	*HXT6*		6129_f_at	YDR343C	1105.6	-1.1	110.6	1105.6	-1.4	442.2	1197.1	1.1	119.7	1197.1	-1.3	359.1	
amino-acid transporters																		
	YDR046C	*BAP3*		6415_at	Valine tran	670.0	2.6	1072.0	670.0	178.1	118656.5	297.2	-2.6	475.5	297.2	81.3	23865.2	
	YBR068C	*BAP2*		7291_at	Major AA p	646.7	2.3	839.2	646.7	133.8	85884.2	280.3	-2.3	363.8	280.3	58.7	16173.3	
	YPL265W	*DIP5*		8030_at	Dicarboxyli	911.4	2.2	1085.3	911.4	18.4	15885.5	416.4	-2.2	495.9	416.4	8.5	3123.0	
	YGR055W	*MUP1*		4936_at	High affinity	968.3	2.0	968.3	968.3	9.0	7745.5	486.4	-2.0	486.4	486.4	4.5	1702.4	
	YDR508C	*GNP1*		5977_at	High-affinit	434.0	1.7	302.5	434.0	5.8	2102.6	256.4	-1.7	178.7	256.4	3.5	641.0	
	YBR069C	*TAT1*	*VAP1, WA*	7292_at	Amino acid	164.5	3.1	345.1	164.5	11.2	1685.2	53.1	-3.1	111.4	53.1	4.1	164.6	
	YNL268W	*LYP1*		9126_at	YNL268W	580.1	-1.0	0.0	580.1	2.3	782.8	570.8	-1.0	0.0	570.8	2.3	742.0	
	YOL020W	*TAT2*	*LTG3, SAE*	8587_at	YOL020W	216.0	1.7	151.2	216.0	2.3	291.5	127.2	-1.7	89.0	127.2	1.4	50.9	
	YCL025C	*AGP1*	*YCC5*	6907_at	YCL025C	249.7	1.3	87.2	249.7	1.8	212.1	186.9	-1.3	65.2	186.9	1.4	74.8	
	YGL077C	*HNM1*		5074_at	YGL077C	544.7	-1.5	272.3	544.7	-1.2	108.9	924.4	1.5	462.2	924.4	1.2	184.9	
	YKR039W	*GAP1*		10511_at	YKR039W	46.7	-2.4	65.2	46.7	-1.4	21.0	110.8	2.4	154.9	110.8	1.7	77.6	
	YFL055W	*AGP3*		5425_at	General an	16.0	1.6	10.4	16.0	4.8	61.6	9.6	-1.6	6.2	9.6	3.0	19.2	
	YBR132C	*AGP2*		7223_at	Amino acid	13.8	3.5	34.3	13.8	3.5	34.4	4.0	-3.5	9.9	4.0	-1.2	0.8	
	YLL061W	*MMP1*		10415_at	YLL061W s	16.8	2.5	25.1	16.8	2.2	20.1	6.6	-2.5	9.9	6.6	-1.2	1.3	
nucleotide transporters																		
	YBR021W	*FUR4*		7334_at	Uracil perm	208.8	4.1	656.1	208.8	6.4	1124.9	41.4	-4.1	130.1	41.4	1.5	20.7	
	YER056C	*FCY2*	*BRA7*	5668_at	YER056C	1819.5	-1.1	238.3	1819.5	1.0	19.9	2117.5	1.1	277.4	2117.5	1.1	311.1	
	YPL134C	*ODC1*		7891_at	YPL134C r	139.0	1.7	104.2	139.0	1.4	62.4	80.6	-1.7	60.4	80.6	-1.2	16.1	
	YBR085W	*AAC3*	*ANC3*	7263_at	YBR085W	47.0	-2.0	48.6	47.0	-1.9	43.8	99.8	2.0	103.3	99.8	1.1	10.0	
	YOR222W	*ODC2*		8334_at	YOR222W	506.1	-1.3	149.9	506.1	-1.5	274.6	689.7	1.3	204.3	689.7	-1.2	137.9	
allantoin and allantoate transporters																		
	YGR260W	*TNA1*		4736_at	YGR260W	549.7	-1.1	55.0	549.7	-1.2	109.9	186.9	-1.3	65.2	186.9	1.4	74.8	
	YCR028C	*FEN2*		6863_at	YCR028C	375.2	-1.2	93.4	375.2	-1.4	148.7	465.8	1.2	116.0	465.8	-1.1	46.6	
drug transporters																		
	YEL065W	*SIT1*	*ARN3*	5769_at	Siderophor	407.0	6.4	2204.5	407.0	12.4	4625.1	67.8	-6.4	367.3	67.8	1.8	54.2	
	YHL040C	*ARN1*		4568_at	Siderochro	271.7	5.7	1288.9	271.7	6.7	1546.6	43.8	-5.7	207.8	43.8	1.3	13.1	
	YOL158C	*ENB1*	*ARN4*	8723_at	YOL158C f	881.4	1.4	395.8	881.4	2.0	924.9	600.5	-1.4	269.7	600.5	1.4	240.2	
	YNL065W	*AQR1*		8923_at	A(acids, az	200.2	1.9	180.2	200.2	4.5	700.5	105.2	-1.9	94.7	105.2	2.4	147.3	
	YBR043C	*AQR2*		7311_at	YBR043C	373.6	1.5	186.8	373.6	2.5	560.4	256.8	-1.5	128.4	256.8	1.7	179.8	
	YLL028W	*TPO1*		10358_at	YLL028W p	714.9	-1.0	0.0	714.9	1.5	357.4	613.7	-1.0	0.0	613.7	1.5	306.9	
	YML116W	*ATR1*	*SNQ1*	9771_at	YML116W	507.3	1.1	50.7	507.3	1.6	329.4	459.7	-1.1	46.0	459.7	1.5	229.9	
	YIL120W	*QDR1*		4236_at	Multidrug r	41.4	9.5	351.8	41.4	3.6	109.7	3.3	-9.5	28.0	3.3	-2.6	5.3	
	YPR156C	*TPO3*		7599_i_at	Polyamine	1.7	-3.0	3.4	1.7	-1.0	0.1	8.1	3.0	16.2	8.1	2.9	15.4	
	YGR138C	*TPO2*		4884_i_at	YGR138C	146.9	-1.3	50.2	146.9	-2.1	166.8	193.0	1.3	65.9	193.0	-1.6	115.8	
transport mechanism																		
	transport ATPases																	
		YGL008C	*PMA1*		5009_at	YGL008C p	0.0	0.0	0.0	0.0	0.0	0.0	2122.2	1.1	212.2	2122.2	1.2	424.4
		YCR024C	*PMP1*		6858_f_at	YCR024C	897.8	-1.3	313.4	897.8	-1.0	0.0	1190.8	1.3	415.7	1190.8	1.3	357.2
		YEL017C	*PMP2*		5728_i_at	YEL017C F	1819.7	-1.3	488.1	1819.7	-1.1	214.0	2277.6	1.3	611.0	2277.6	1.1	339.2
		YEL027W	*CUP5*		5763_at	YEL027W	2242.6	-1.3	664.1	2242.6	-1.1	334.0	2929.6	1.3	867.6	2929.6	1.1	293.0
		YHR026W	*PPA1*	*VMA16*	4500_at	YHR026W	1541.2	-1.3	462.3	1541.2	-1.1	154.1	1942.4	1.3	582.7	1942.4	1.1	194.2
		YBL099W	*ATP1*		7487_at	YBL099W	1009.5	-1.1	150.3	1009.5	-1.0	0.0	1258.4	1.1	187.4	1258.4	1.1	125.8
		YKL080W	*VMA5*	*CSL5, VA1*	10660_at	YKL080W	713.6	-1.3	249.1	713.6	-1.2	177.7	957.0	1.3	334.1	957.0	1.1	95.7

	ORF	Gene	Alias	Probe	Description												
	YHR039C	*MSC7*	*VMA10*	4514_at	YHR039C	783.6	-1.3	235.1	783.6	-1.4	351.9	1017.3	1.3	305.2	1017.3	-1.1	101.7
	YBR039W	*ATP3*		7307_at	YBR039W	630.5	1.2	126.1	630.5	-1.0	0.0	526.3	-1.2	105.3	526.3	-1.2	105.3
	YPL234C	*TFP3*	*CLS9, VM/*	7970_at	YPL234C 1	1186.4	-1.3	355.9	1186.4	-1.3	355.9	1511.7	1.3	453.5	1511.7	-1.1	151.2
	YLR447C	*VMA6*		9851_at	YLR447C 3	508.6	-1.0	24.8	508.6	-1.3	150.6	518.0	1.0	25.3	518.0	-1.3	155.4
	YLR295C	*ATP14*		10010_at	YLR295C /	630.6	1.6	376.4	630.6	1.1	93.9	394.9	-1.6	235.7	394.9	-1.4	158.0
	YDL004W	*ATP16*		6503_at	YDL004W	720.0	1.2	179.3	720.0	1.0	35.1	558.7	-1.2	139.1	558.7	-1.3	167.6
	YPL271W	*ATP15*	*ATPEPSIL*	8024_at	YPL271W	1021.8	1.1	152.2	1021.8	-1.0	49.9	899.9	-1.1	134.0	899.9	-1.2	180.0
	Q0310			3976_at	F1F0-ATPa	804.9	1.2	200.4	804.9	-1.0	39.3	644.5	-1.2	160.5	644.5	-1.3	193.4
	YPR036W	*VMA13*	*CLS11*	7699_at	YPR036W	437.6	1.1	43.8	437.6	-1.3	152.7	403.1	-1.1	40.3	403.1	-1.5	201.6
	YKL016C	*ATP7*		10591_at	YKL016C /	689.5	1.2	171.7	689.5	1.3	240.7	546.0	-1.2	136.0	546.0	1.1	54.6
	YDR270W	*CCC2*		6190_at	Accessory	150.0	1.9	135.0	150.0	1.9	135.0	92.0	-1.9	82.8	92.0	1.0	0.0
	YGR020C	*VMA7*		4993_at	YGR020C	462.1	-1.4	184.8	462.1	-1.3	138.6	648.7	1.4	259.5	648.7	1.1	64.9
	YDL185W	*TFP1*	*CLS8, VM/*	6679_at	YDL185W	690.5	-1.3	207.2	690.5	-1.3	241.0	892.6	1.3	267.8	892.6	-1.0	0.0
	YBR127C	*VMA2*	*ATPVS, V/*	7218_at	YBR127C I	734.2	-1.5	367.1	734.2	-1.4	329.7	1021.3	1.5	510.7	1021.3	1.0	0.0
	YOR332W	*VMA4*		8222_at	YOR332W	978.3	-1.3	341.5	978.3	-1.3	341.5	1290.1	1.3	450.3	1290.1	-1.0	0.0
	YOR270C	*VPH1*		8291_at	YOR270C	1344.7	-1.3	403.4	1344.7	-1.3	469.4	1771.5	1.3	531.5	1771.5	-1.0	0.0
ABC transporters																	
	YPL058C	*PDR12*		7831_at	Multidrug r	266.9	1.6	159.3	266.9	3.4	653.0	167.9	-1.6	100.2	2.2	0.0	-2.2
	YCR011C	*ADP1*		6888_at	YCR011C	0.0	0.0	0.0	0.0	0.0	0.0	282.6	1.0	13.8	282.6	1.7	197.8
	YNR070W	*PDR18*		8782_at	YNR070W	6.7	2.3	8.6	6.7	2.8	12.3	3.7	-2.3	4.7	3.7	1.5	1.9
	YNL014W	*HEF3*	*ZRG7*	8881_at	Translation	3.5	1.5	1.8	3.5	-2.4	4.9	-1.9	-1.5	-1.0	-1.9	-3.7	-5.1
	YPL226W	*NEW1*		7978_at	YPL226W	453.2	-1.1	67.5	453.2	-1.3	158.2	519.5	1.1	77.4	519.5	-1.2	103.9
	YOR153W	*PDR5*	*LEM1, YDI*	8400_at	Multidrug r	707.7	-1.3	209.6	707.7	-1.3	209.6	916.0	1.3	271.3	916.0	1.0	0.0
other transport facilitators																	
	YPR194C	*OPT2*		7546_at	Oligopeptic	78.8	2.8	141.8	78.8	31.3	2387.5	34.1	-2.8	61.4	34.1	12.7	399.0
	YNL125C	*ESBP6*	*MCH3*	8997_at	Protein witl	566.5	2.5	848.6	566.5	4.4	1953.0	225.6	-2.5	337.9	225.6	1.8	180.5
	YER064C	*??*		5635_at	Mutation le	121.6	3.7	328.3	121.6	7.5	796.5	38.1	-3.7	102.9	38.1	2.5	57.2
	YKR093W	*PTR2*		10472_at	YKR093W	148.2	2.7	258.7	148.2	5.8	718.0	48.2	-2.7	84.2	48.2	2.1	53.0
	YJL034W	*KAR2*	*BIP, GRP7*	11078_at	Homologue	1978.5	1.1	188.8	1978.5	1.2	492.6	1802.2	-1.1	172.0	1802.2	1.2	360.4
	YNL055C	*POR1*	*OMP2*	8932_at	YNL055C (	1947.9	1.1	194.8	1947.9	1.2	485.0	0.0	0.0	0.0	0.0	0.0	0.0
	YNL070W	*TOM7*	*MOM7, YC*	8917_at	YNL070W	828.9	-1.1	123.4	828.9	1.1	123.4	927.8	1.1	138.2	927.8	1.3	278.3
	YAL067C	*SEO1*		11379_at	Suppresso	92.1	1.6	59.8	92.1	3.7	252.9	50.8	-1.6	33.0	50.8	2.5	76.2
	YGR065C	*VHT1*		4946_at	YGR065C	433.6	1.3	128.4	433.6	1.5	215.4	0.0	0.0	0.0	0.0	0.0	0.0
	YOL119C	*MCH4*		8669_at	YOL119C ı	179.2	1.7	134.2	179.2	2.0	188.0	100.7	-1.7	75.5	100.7	1.2	20.1
	YPR058W	*YMC1*		7678_at	YPR058W	624.9	1.1	62.5	624.9	1.3	187.5	569.7	-1.1	57.0	569.7	1.2	113.9
	YOR045W	*TOM6*	*ISP6, MOM*	8518_at	YOR045W	1407.9	-1.1	209.6	1407.9	-1.1	140.8	1605.3	1.1	239.0	1605.3	1.1	160.5
	YDR086C	*SSS1*		6410_at	YDR086C	557.7	-1.3	165.2	557.7	-1.1	83.0	727.0	1.3	215.3	727.0	1.2	145.4
	YJL054W	*TIM54*		11058_at	YJL054W T	105.3	1.4	41.7	105.3	2.0	105.0	0.0	0.0	0.0	0.0	0.0	0.0
	YDR322C	*TIM11*		6154_at	YDR322C :	975.4	1.2	195.1	975.4	1.1	97.5	799.4	-1.2	159.9	799.4	-1.2	159.9

mparison Signific. Factor
857.5
437.8
415.5
303.2
243.9
0.0
50.0
103.2
172.2
192.3
254.7
134.9
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121.6
0.0
1702.4
99.2
78.9
140.2
1702.4
681.3
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147.1
134.5
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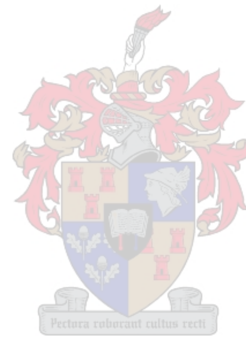

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6.2
78.1

1130.6
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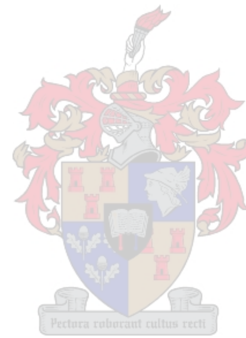

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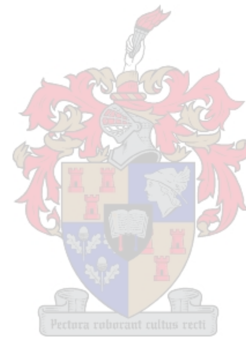
376.2



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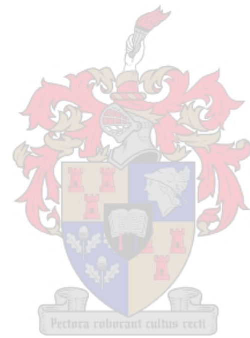
35.7
24.2



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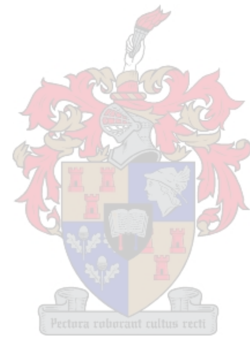

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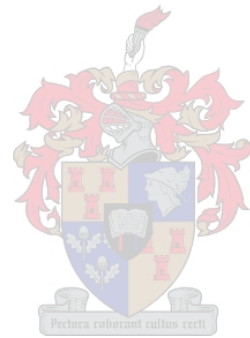

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139.1
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18.3
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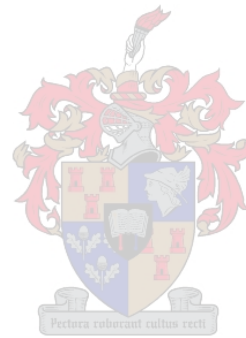

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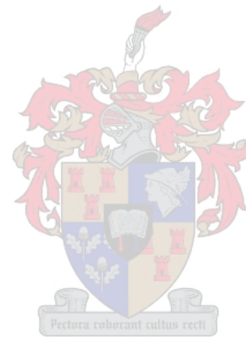

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0.6
143.6
165.7
331.0

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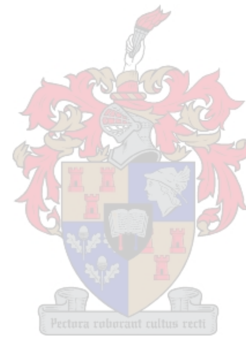

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105.3
64.9

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152.5

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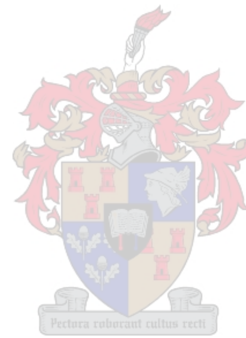
385.2



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165.7
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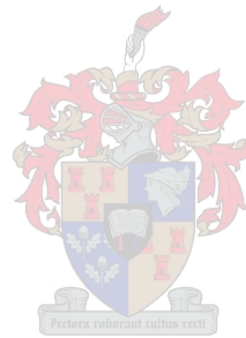

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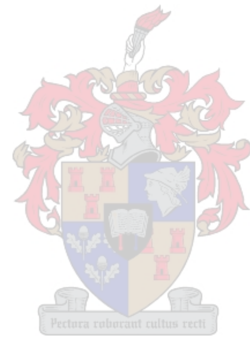

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75.0
167.5

424.4
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194.2
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201.6
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