**Proteomic analysis of *Saccharomyces cerevisiae*
grown on glucose or glycerol**

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Abstract

The goal of this research was to use two-dimensional electrophoresis to examine changes in abundance of enzymes of the glycolytic pathway in the yeast *Saccharomyces cerevisiae* grown on carbon sources that support either fermentation to ethanol or oxidative metabolism. Large-scale profiling of protein abundances (expression proteomics) often detects changes in protein abundance between physiological states. Such changes in enzyme abundance are often interpreted as evidence of metabolic change although most textbooks emphasise control of enzyme activities not enzyme amount. Two-dimensional difference gel electrophoresis (2D-DIGE) was therefore used to examine differences in protein abundance between *S. cerevisiae* strain BY4741 grown on either glucose (fermentation) or glycerol. Growth on 2% glucose, but not on glycerol, was accompanied by extensive production of ethanol. Doubling times for growth were 2 h 5 min in glucose and 9 h 41 min in glycerol.

Conditions for extraction and two-dimensional electrophoresis of proteins were established. One hundred and seventy nine proteins were identified by MALDI mass spectrometry of tryptic digests of protein spots excised from Coomassie stained gels. All of the enzymes for conversion of glucose to ethanol, except for the second enzyme of glycolysis phosphoglucose isomerase, were identified using two-dimensional electrophoresis of 100 µg of protein from cells grown on 2% glucose. Identification of proteins excised from the DIGE gels was more challenging, partly because of the lower amount of protein. Eight of the proteins that showed statistically significant differences in abundance (≥ 2 -fold, $p \leq 0.01$) between glucose and glycerol were identified by mass spectrometry of proteins excised from the 2D-DIGE gels, and a further 18 varying proteins were matched to proteins identified from the Coomassie stained gels. Of these total 26 identified or matched proteins, subunits of five of the enzymes for conversion of glucose to ethanol were more abundant from the fermentative cells grown on glucose. The more abundant glycolytic enzymes were phosphofructokinase 2, fructose-1,6-bisphosphate aldolase, triosephosphate isomerase and enolase, plus pyruvate decarboxylase that was required for conversion of the glycolytic product pyruvate to acetaldehyde. The

alcohol dehydrogenases Adh1 and Adh4 that convert acetaldehyde to ethanol were detected but did not vary significantly between growth on glucose or glycerol. The results confirmed that in this case changes in abundance of some enzymes were consistent with the altered metabolic output. Future studies should examine whether changes in the abundance and activity of these enzymes are responsible for the differences in metabolism.

Abbreviations

1D	One-dimensional
2D-DIGE	Two-dimensional-differential in gel electrophoresis
2DE	Two-dimensional electrophoresis
ACN	Acetonitrile
CBB G-250	Coomassie Brilliant Blue G-250
CHAPS	3-[(3-Cholamidopropyl)dimethylamonio]-1-propanesulfonate
CHCA	α -Cyano-4-hydroxycinnamic acid
DIGE	Differential in gel electrophoresis
DTT	Dithiothreitol
GE	Gel Electrophoresis
IAA	Iodoacetamide
IEF	Isoelectric focusing
IPG	Immobilized pH gradient
MALDI	Matrix assisted laser desorption ionisation
MS	Mass Spectrometry
<i>pI</i>	Isoelectric point
PMF	Peptide mass fingerprinting
SDS	Sodium dodecyl sulphate
TFA	Trifluoroacetic acid
Tris	Tris(hydroxymethyl)aminomethane

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1. Introduction

The goal of this project was to use two-dimensional electrophoresis (2DE)-based proteomics to examine changes in protein abundance between fermentative and oxidative metabolism in the yeast *Saccharomyces cerevisiae*. Metabolic pathways can be regulated by changing enzyme activities and enzyme amounts. Many proteomic studies, including examination of metabolic change, assume that change in enzyme abundance is related to change in metabolic output. Although this is sometimes the case (Rawson *et al.*, 2012) many enzymes are regulated by substrates, products, allosteric effectors or post-translational modification independent of enzyme amount. This project therefore uses proteomics to investigate potential differences in amount of enzymes that affect carbohydrate metabolism and ethanol synthesis under conditions when either ethanol production or oxidative metabolism predominates.

1.1. Proteomics

Proteomics is a set of techniques that can be used to profile the protein content of biological samples (Pham & Wright, 2007). Of particular interest for this project is quantitative proteomics, the ability to compare protein profiles under different conditions. Proteomic workflows can be simplified into the separation of complex protein mixtures coupled to protein identification. Separation of proteins can be divided into gel-based and gel-free techniques. Both have advantages and disadvantages as discussed later. Gel-based analysis by 2DE is used in this project. Protein identification is now commonly done by mass spectrometry with matching of sequence-based peptide masses to sequence databases.

1.2. Gel-based proteomics

1.2.1. Two-dimensional gel electrophoresis

In 1975 Patrick O'Farrell and Joachim Klose independently developed 2DE (Klose, 1975; O'Farrell, 1975). This technique uses two sequential episodes of protein separation. In the first phase of separation, proteins migrate to their *pI* using isoelectric focusing (IEF). The second dimension separates focused proteins according to their molecular mass using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The 2DE method is transformed by coupling pre-

electrophoretic labeling of protein samples with fluorescent Cy dyes allowing quantitative analysis of multiple sample sets (Unlu *et al.* (1997).

Isoelectric focusing (IEF) separates protein mixtures by applying an electric field to a pH gradient (Klose, 1975; O'Farrell, 1975). Proteins migrate to their isoelectric point where they are no longer charged. As they are amphoteric molecules their overall charge is determined by their side chains in that environment. Thus a protein with an overall positive charge would migrate towards the cathode and in a contrasting situation a protein with a net negative charge would migrate towards the anode.

Isoelectric focusing of proteins for 2DE was originally carried out in PAGE tube gels but was simplified by Angelika Gorg and Pier Righetti who independently developed immobilized pH gradients (IPG) (reviewed by Righetti and Bossi (1997)). IPG strips contain acid and basic buffering groups that are covalently incorporated into acrylamide gels attached to a thin layer of plastic. This development facilitated reproducible focusing over broad or narrow pH ranges including the highly acid and basic regions of the pH scale.

Size-based separation of proteins in the second dimension uses SDS-PAGE (Klose, 1975; O'Farrell, 1975). Proteins which have been fixed at their isoelectric point by IEF are equilibrated in an SDS solution for the formation of SDS-protein complexes. SDS is an anionic detergent that binds proteins and confers mass-based charge that allows size separation in polyacrylamide gels.

1.2.2. Difference gel electrophoresis (DIGE)

Prior to the development of DIGE in 1997 (Unlu *et al.*, 1997) the standard method for comparative 2DE was to match gels that had been stained for proteins after electrophoresis. Matching was limited by variability in protein loading and staining and the reproducibility of spot matching methods. Unlu *et al.* (1997) described DIGE using fluorescent cyanine (Cy) dyes containing an N-hydroxysuccinimidyl (NHS) ester reactive group for the labeling of protein samples prior to IEF. The Cy dyes Cy2, Cy3 and Cy5 are used to label up to three protein samples for analysis on one gel. The Cy dyes can be used for minimal or saturation (for low protein abundance samples) labeling of protein sets. For minimal labeling, the NHS moiety of the Cy dyes covalently bind to lysine residues of proteins by an amide bond. Cy dyes carry

a single intrinsic positive charge, as do lysine residues in acidic conditions, and upon attachment to a lysine the positive charge is replaced causing no significant change in the *pI* of the protein. It is often advantageous to incorporate an internal standard composed of equal amounts of all of the protein samples labeled with Cy2 to facilitate gel matching between replicates (Karp & Lilley, 2005). The Cy dyes are separately added to each condition, including the internal standard before they are separated in the first and second dimensions by 2DE (Figure 1). Computer-aided image analysis is used to determine differences in protein abundance among samples. There are several advantages of using 2DE combined with DIGE for comparative proteomic analysis. Two-dimensional electrophoresis allows visual and computational analysis of differences in protein abundance between samples, and of changes in protein spot position (mass or *pI*) in gels that can be due to posttranslational modification of proteins. Statistical analysis of changes in protein abundance is enhanced by uniform matching of protein spots by comparison with the internal standard protein mixture that is included in all gels. Disadvantages of gel-based methods include the time consuming aspects of the experiments and the difficulty to detect low abundance proteins (Pham & Wright, 2007).

Figure 1 - Summary of the DIGE labeling process. Protein extracts of each condition are labeled with Cy dyes. Images of each Cy dye labeled protein set are visualized by excitation at a certain wavelength and are overlaid for image analysis. An internal standard of all proteins included in the analysis is labeled with Cy2 and is used as the reference for spot matching on each gel. Copyright for use of this image was approved by GE Healthcare.

1.3. Gel-free proteomics

As an alternative or complementary strategy to 2DE, gel-free techniques use mass spectrometry based analysis of proteolytic digests of proteins (Pham & Wright, 2007). Protein samples are digested to give large sets of peptide fragments that are separated by liquid chromatography prior to tandem mass spectrometry for peptide and protein identification. The enzyme trypsin that cleaves peptide bonds on the C-terminal side of arginine or lysine is often used as the proteolytic reagent. This general approach can be called LC MS/MS, gel-free analysis, or shotgun proteomics. Quantitative methods include spectral counting (a label-free method) and isotopic labeling. In spectral counting the number of times a peptide detected is used as a

measure of protein abundance (Lundgren *et al.*, 2010). Alternatively, incorporation of isotopic labels for the tagging of proteins can be used to measure changes in protein abundance. Two of the commonly used methods of isotopic labeling are SILAC (Stable Isotope Labeling And Culture) and iTRAQ (Isobaric Tags for Relative and Absolute Quantification) (Brewis & Brennan, 2010).

SILAC was originally designed for mammalian samples but the technique has been adapted for yeast, bacteria and plant cultures (Ong & Mann, 2006). The technique involves the incorporation of a stable isotope-labeled essential amino acid into a cell culture lacking the same essential amino acid. For SILAC, cells are grown in medium containing amino acids such as arginine or lysine labeled with ^{13}C or ^{15}N stable isotopes and for comparison of protein change are mixed with cells grown in natural abundance (^{12}C or ^{14}N) amino acids. Shotgun mass spectrometry is then used to detect peptides that differ in the ratio of heavy and light isotopic labels that reflect differences in protein abundance between samples. A major advantage of isotopic labeling is the sensitivity of the tags and the ability to detect low abundance proteins that is a major limiting factor of 2D based techniques.

For iTRAQ, samples are chemically labeled with amine specific reagents containing stable isotopes and a mixing protocol is used to compare the abundances of digest peptides and hence their parent proteins from different samples. The first application of iTRAQ in yeast was done by Ross *et al.* (2004). The shotgun approach compared a wildtype strain with two mutant strains. Subsequently, Pham *et al.* (2006) examined the proteomes of wildtype yeast grown in media supplemented with 12%, 21% or 30% glucose. Protein separation and identification was completed using LC MS/MS resulting in detection of 413 proteins. Differences among enzymes involved in carbohydrate and amino acid metabolism were detected. Subsequent studies are outlined later in this Introduction.

1.4. Protein identification

Regardless of the method for protein separation, mass spectrometry has become the standard for protein identification (Pham & Wright, 2007). A common mass spectrometric method for the identification of proteins involves peptide mass fingerprinting (PMF) using MALDI TOF (matrix assisted laser desorption ionization

time of flight) or electrospray ionization (ESI) mass spectrometry (MS). Sequence-specific peptide fragments are created by cleaving proteins with an enzyme with high specificity. Trypsin is a commonly used proteolytic agent which cleaves proteins at the C-terminal residue of arginine or lysine. MS-based peptide masses are then used to match to sequence databases for probability based protein identification. More specific matching is obtained using MS/MS to measure sequence-based fragments of the peptides that are generated in the mass spectrometer.

1.5. *Saccharomyces cerevisiae*

The budding yeast *S. cerevisiae* is a well-studied organism. The single celled eukaryote had its genome sequence reported in April 1996 revealing a 16 kb genome with six chromosomes (Goffeau *et al.*, 1996). Thirty percent of the yeast genome is homologous with the human genome making it an excellent model for research using molecular biological tools such as the yeast gene deletion set (Giaever *et al.*, 2002), and red fluorescent protein (RFP), green fluorescent protein (GFP) (Bisson & Fraenkel, 1983) and TAP-tagged libraries (Babu *et al.*, 2009). Large-scale coverage of the yeast proteome has also been reported (de Godoy *et al.*, 2008; Picotti *et al.*, 2013; Shevchenko *et al.*, 1996). There are several online resources that are readily available including the Kyoto Encyclopedia of Genes and Genomes (KEGG, <http://www.genome.jp/kegg> and the Yeast Genome Database (SGD, <http://www.yeastgenome.org/>).

1.5.1. Carbohydrate metabolism in *S. cerevisiae*

S. cerevisiae has the ability to grow under a variety of conditions, in particular on many carbon substrates. One characteristic of this yeast is fermentation of glucose to ethanol under aerobic or anaerobic conditions. The specific conditions relevant to this thesis research are growth on glucose or glycerol. Growth on glucose utilizes the glycolytic pathway to produce pyruvate that can be metabolized to ethanol and carbon dioxide (fermentation). In contrast, fermentation does not occur during growth on glycerol. Glycerol can be used for synthesis of glucose (gluconeogenesis) or for energy production using glycolysis coupled to the citric acid cycle and oxidative phosphorylation.

S. cerevisiae can grow on a number of sugars such as fructose and mannose but prefers glucose. There are 18 hexose transporters and at least six are specific for glucose transport. Each glucose transporter has a different affinity for glucose to suit substrate availability. In high glucose conditions regardless of oxygen, glucose is directed into the cell via the low glucose affinity Hxt1 transporter and fermented to produce ethanol and carbon dioxide (Ozcan & Johnston, 1995). Metabolism of glucose is directed through glycolysis with the upregulation of genes involved in ethanol synthesis and the repression of genes responsible for other pathways of utilization (Figure 2). This is known as the glucose repression effect (Gancedo, 1992). In contrast, when glucose is limited, glucose is not fermented to produce ethanol but is fully oxidized to produce CO₂ via the TCA acid cycle (Postma *et al.*, 1989).

Figure 2 - A simplified diagram of glucose metabolism in *S. cerevisiae*. The diagram illustrates the metabolism of glucose towards ethanol synthesis via glycolysis and fermentation in glucose abundant conditions. The direction of glucose metabolism is supported by the upregulation of glycolytic genes and repression of genes involved in other pathways of utilization such as enzymes involved in the citric acid cycle. The copyrights of the original image (Johnston & Kim, 2005) for modification were granted.

1.6. Glycolysis and fermentation

1.6.1. Glycolysis

Glucose is metabolized to pyruvate (Lunt & Vander Heiden, 2011) by glycolysis in *S. cerevisiae* and in many other organisms as illustrated in Figure 3.

Figure 3 - Proteins and metabolites involved in glycolysis. Glucose is degraded to pyruvate through a series of reversible ($\updownarrow$) and irreversible ($\downarrow$) enzyme-catalyzed reactions. Enzyme names are shown using the symbols described in the text.

In the first, irreversible, step of glycolysis hexose is phosphorylated by either hexokinases Hxk1 and Hxk2 or glucokinase Glk1 to glucose-6-phosphate. Hxk2 is the main kinase with preference for glucose, fructose or mannose as substrates

(Bisson & Fraenkel, 1983; De Winde *et al.*, 1996). During growth on non-fermentable substrates transcript abundance for Hxk1 and Glk1 increases, while Hxk2 decreases (Rodriguez *et al.*, 2001). In the second step of glycolysis, glucose-6-phosphate is reversibly converted to fructose-6-phosphate by phosphoglucose isomerase (Pgi1). The third reaction of glycolysis is phosphorylation of fructose-6-phosphate by phosphofructokinase (Pfk) to fructose-1,6-bisphosphate. Phosphofructokinase is a heterooctamer composed of four alpha and four beta subunits (Pfk1 and Pfk2 respectively). This enzyme is low abundance and has not been reported in gel-based proteomic studies without genetic manipulation (Boucherie *et al.*, 1995).

The next reaction is the cleavage of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate by fructose-1,6-bisphosphate aldolase (Fba1). The subsequent interconversion of glyceraldehyde-3-phosphate to dihydroxyacetone phosphate is catalyzed by triosephosphate isomerase (Tpi1). The succeeding steps of glycolysis result in conversion of the three-carbon intermediates to pyruvate, plus generation of ATP by substrate-level phosphorylation. Glyceraldehyde-3-phosphate is oxidized and phosphorylated by the homotetramer glyceraldehyde-3-phosphate dehydrogenase (Tdh). There are three isozymes derived from three corresponding genes (*TDH1-3*). Another reaction which results in the generation of ATP is the catalysis of the transfer of a phosphoryl group from 1,3-bisphosphoglycerate to ADP by phosphoglycerate kinase (Pgc1). Subsequently, phosphoglycerate mutase (Pgm1) converts 3-phosphoglycerate to 2-phosphoglycerate, which is then metabolized to phosphoglycerate by enolase (Eno). The two isozymes Eno1 and Eno2 are encoded by the respective genes *ENO1* and *ENO2*. In glucose rich medium Eno2 protein is 20-times more abundant than Eno1 but Eno1 increases to Eno2 levels in cells grown on ethanol or lactate (McAlister & Holland, 1982). The last reaction of glycolysis is the irreversible catalysis of phosphoenolpyruvate to pyruvate by pyruvate kinase (Pyk). Pyk1 (Cdc19) is the primary enzyme. Its paralog, Pyk2, is repressed in glucose conditions (Boles *et al.*, 1997).

1.6.2. Fermentation

During fermentation in *S. cerevisiae* pyruvate is primarily converted to ethanol using a two-step process. First, pyruvate is converted to acetaldehyde by pyruvate decarboxylase. There are three pyruvate decarboxylases (Pdc1, 5 and 6) encoded by

different genes *PDC1*, *PDC5* and *PDC6*. *Pdc1* and *Pdc5* are induced in the presence of glucose. *Pdc6* is a low abundance isoform but increase when cells are grown on ethanol (Hohmann, 1991; Seeboth *et al.*, 1990).

Following the decarboxylation of pyruvate, acetaldehyde is reduced to ethanol by alcohol dehydrogenase. There are seven alcohol dehydrogenase genes *ADH1-7* (de Smidt *et al.*, 2008). Two of these (*ADH6* and *ADH7*) belong to the cinnamyl alcohol dehydrogenase (CAD) family and the remaining five are involved in ethanol metabolism (*ADH1-5*). The five dehydrogenases involved in ethanol metabolism are summarized in Table 1.

During growth on glucose *Adh1* is the primary dehydrogenase responsible for the oxidation of acetaldehyde to ethanol. The 348 aa enzyme is localized in the cytoplasm. *Adh2* is responsible for catalyzing the reverse reaction, reduction of ethanol to acetaldehyde. It is regulated by the transcription factor *Adr1*, which also has roles in glycerol metabolism. Expression of *ADH2* is repressed in glucose grown conditions (Stahlberg *et al.*, 2008). *Adh1* and *Adh2* have greater than 90% sequence similarity at the protein level and, due to only small differences in size and pI (Table 1), are expected to co-migrate on 2DE gels.

Adh3, 4 and 5 are also capable of catalyzing the oxidation of acetaldehyde but in more specific conditions. Of these, *ADH3* and *ADH5* are repressed in the presence of glucose (Young & Pilgrim, 1985). *ADH3* encodes an alcohol dehydrogenase which is localized to the mitochondrion (Wiesenfeld *et al.*, 1975). Wiesenfeld *et al.* (1975) reported that sequence homology between *Adh3* and *Adh1* or *Adh2* at the amino acid level was 79% and 80% respectively. Relatively little is known about the cytoplasmically localized *Adh5* whose encoding gene, *ADH5*, was discovered upon sequencing of chromosome II (Feldmann *et al.*, 1994). Smith *et al.* (2004) proposed that *Adh5* was the sole enzyme for ethanol production in an *ADH1 ADH3* double null mutant. *Adh4* is localized to the mitochondrion like *Adh3*, but is not repressed in cells grown on glucose. The pioneers of *Adh4* research were Paquin and Williamson (1986) and Walton *et al.* (1986). Early studies on *Adh4* implied *Adh4* was absent in the presence of *Adh1*. However, in 1999 Perrot *et al.* (1999) identified *Adh4* in the presence of *Adh1* and 2 using MAT α mating type yeast.

Table 1 - The five *S. cerevisiae* Adh isoforms involved in ethanol metabolism. The information was derived from ExPASy <http://www.expasy.org/>. The ExPASy records indicate that there is MS evidence of protein for Adh 1 and 2, but that there is not MS evidence for Adh3-5 protein in *S. cerevisiae*.

Adh	kDa	pI	Number of molecules per cell in log phase (from ExPASy)	Subcellular localization	Function	Role during fermentation on glucose
1	36.8	6.21	Not recorded	Cytoplasm	Aldehyde → alcohol	Major activity
2	36.7	6.26	1620	Cytoplasm	Alcohol → aldehyde	Glucose repressed
3	40.4	7.24	11600	Mitochondrion	Aldehyde → alcohol	Glucose repressed
4	41.1	5.76	125	Mitochondrion	Aldehyde → alcohol	Second acting aldehyde reducing dehydrogenase
5	37.6	5.94	1310	Cytoplasm nucleus	Aldehyde → alcohol	Glucose repressed

1.7. Glycerol metabolism and gluconeogenesis

Figure 4 - Proteins and metabolites involved in glycerol metabolism, gluconeogenesis/ glycolysis. Enzymes involved in glycerol metabolism ($\leftarrow$) and gluconeogenesis ($\uparrow$)/ glycolysis ($\downarrow$) are presented. Enzymes responsible for the catalysis of reversible gluconeogenic/ glycolytic reactions are represented by $\updownarrow$.

1.7.1. Glycerol metabolism

Utilization of glycerol by *S. cerevisiae* leads to entry into the glycolytic and gluconeogenic pathways. Glycerol is also a potential product of the metabolism of glucose. Two enzymes Gut1 (glycerol kinase) and Gut2 (glycerol-3-phosphate dehydrogenase) carry out the sequential reversible reactions converting glycerol to dihydroxyacetone phosphate, which is an intermediate of both glycolysis and gluconeogenesis (Figure 4). Gut1 is a cytoplasmic enzyme that phosphorylates glycerol to glycerol-3-phosphate. Glycerol-3-phosphate translocates to mitochondria where Gut2 catalyzes conversion to dihydroxyacetone phosphate.

Dihydroxyacetone phosphate is transported back into the cytoplasm where it enters the gluconeogenic/ glycolytic pathways. The genes of encoding both enzymes, *GUT1* and *GUT2* are repressed during fermentation of glucose (Sprague & Cronan, 1977). The mediator of *GUT1* is *Adr1*, which is also the transcriptional activator of *ADH2* (Grauslund *et al.*, 1999) .

1.7.2. Gluconeogenesis

Figure 4 also illustrates the reactions of glycolysis and gluconeogenesis showing the irreversible reactions that are common to both pathways and the irreversible reactions that are specific for one or the other of the two pathways. There are two irreversible reactions in glycolysis. *Pfk1* and *Pyk1* catalyze two of the glycolysis specific reactions. The corresponding irreversible steps of gluconeogenesis are catalyzed by fructose-1, 6-bisphosphatase (*Fbp1*) and a two-step reaction involving phosphoenolpyruvate carboxykinase (*Pck1*) and pyruvate carboxylase (*Pyc1* or *Pyc2*). *Pyc1* or its paralog *Pyc2* converts pyruvate to oxaloacetate. In yeast this reaction occurs in the cytoplasm unlike mammals where the reaction occurs in the mitochondrion. Oxaloacetate is catalyzed to phosphoenol pyruvate by *Pck1*. The gluconeogenic enzyme *Fbp1* converts fructose-1,6-bisphosphate to fructose-6-phosphate.

1.8. Regulation of glucose metabolism

The concentration of glucose plays a crucial role in glucose metabolism. It has influential effects on gene repression and induction. When there are high concentrations of extracellular glucose, greater than 1% (Yin *et al.*, 2003), low affinity hexose transporter (*HXT*) genes are expressed and allow glucose entry into the cell.

Extracellular glucose concentrations as low as 0.2% cause the cells to respire (Guidi *et al.*, 2010).

Two of the many pathways involved in glucose induction and repression are the Snf3-Rgt2 pathway required for glucose uptake by hexose transporters, and the Mig1-Snf1 pathway. Snf (sucrose nonfermenter) proteins affect transcription of a number of genes.

1.8.1. Snf3-Rgt2 pathway

The induction of hexose transporter genes is carried out by the Snf3-Rgt2 pathway. In high glucose conditions, glucose bound to low glucose affinity sensor Rgt2 (restores glucose transport), and Snf3 activates membrane bound yeast casein kinase 1 (Yck1) by a conformational change (Moriya & Johnston, 2004) (Figure 5 top). The activated Yck1 phosphorylates Mth1 (MSN three homolog) and Std1 (suppressor of Tbp deletion) which are bound to the C-terminal cytoplasmic tails of the glucose receptors. Subsequently, phosphorylated Mth1 and Std1 are ubiquitinated by the ubiquitin-ligase complex of Skp1 - Cul1 - F-box with Grr1 (SCF^{Grr1}) (Moriya & Johnston, 2004) and targets them for proteasome degradation. The transcriptional repressor Rgt1 loses its ability to bind DNA due to loss of Mth1, and Std1 (Kim *et al.*, 2003), becomes hyperphosphorylated and translocates to the cytoplasm allowing transcription of *HXT1* (Johnston & Kim, 2005; Kim *et al.*, 2003).

Whereas in low glucose conditions, intermediate and high glucose affinity transporters are induced to mediate glucose uptake (Figure 5 bottom) (Boles *et al.*, 1997). This process involves the high glucose affinity glucose receptor, Snf3. The intracellular signal generated from glucose detection is still unclear. However it is known that SCF^{Grr1} (Li & Johnston, 1997), Rgt1 and its co-repressors Tup1 and Ssn6, and Mth1 and Std1 (Lakshmanan *et al.*, 2003) are involved in the transcriptional activation of intermediate and high affinity hexose transporters, *HXT2*, *HXT3* and *HXT4* (Flick *et al.*, 2003; Ozcan & Johnston, 1995).

1.8.2. Mig1-Snf1 pathway

The repression or activation of genes involved in glucose utilization other than glycolysis is controlled by the Mig1-Snf1 pathway (Figure 5 right) (Johnston, 1999). The pathway involves Reg1-Glc7 protein phosphatase, Snf1 complex, and Mig1. The

pathway depends on the activity of the Snf1 complex which is regulated by glucose (Jiang & Carlson, 1996). In high glucose conditions, glucose is transported by Hxt1 and elevated intracellular glucose levels prevent Reg1-Glc7 from activating the Snf1 complex (Figure 5 top, right). The Snf1 complex has a catalytic (CD) and regulatory domain (RD). The catalytic domain is autoinhibited by binding to the regulatory domain resulting in an inactive Snf1 complex (Jiang & Carlson, 1996). As a result, transcriptional repressor Mig1 and its co-repressors Tup1 and Ssn6 are able to repress genes associated with more efficient means of glucose utilization as well as high affinity transporters, *HXT2*, *HXT3* and *HXT4* (Gancedo, 1998).

In contrast, when glucose is low Snf4 is able to bind to the regulatory domain of Snf1 causing the release of the catalytic domain (Jiang & Carlson, 1996) (Figure 5 bottom, left). The active Snf1 complex now has the ability to phosphorylate Mig1, which causes Mig1 to dissociate from its corepressors (Treitel *et al.*, 1998). As a result Mig1, Tup1 and Ssn6 are unable to repress transcription.

Figure 5 - The induction and repression of genes involved in the Snf3-Rgt2 (left) and Mig1-Snf1 (right) pathways of *S. cerevisiae*. The diagram shows cells grown in high (top) and low (bottom) glucose conditions. The above illustrations are modified diagrams from Gancedo (1998), Johnston and Kim (2005), and Rolland et al. (2002). The copyrights for the modifications of their images were approved.

1.9. Transcriptomic analysis of carbon substrate metabolism

In addition to study of individual genes and proteins both transcript and proteome analysis have been used to examine metabolism in *S. cerevisiae*. The power of transcript analysis is based on the ability to measure the effects of many genes.

Oxidative metabolism and fermentation of carbon substrates have been extensively studied at the level of the transcriptome. A recent interest is towards transcript studies focusing on substrate transition to get a greater understanding of the adaptation process. Roberts and Hudson (2006) used microarrays to examine the transition of cells grown to early log phase in glucose to a medium containing a non-fermentable substrate of glycerol or ethanol. They found the upregulation of the following gene clusters 'respiration', 'carbohydrate and fatty acid metabolism', 'stress', 'endocytosis, actin and stress', and 'protein localization'. The following biological process clusters were down regulated, 'respiration – oxidative phosphorylation, mitochondrial electron transport chain', 'transport', 'membrane and sphingolipid metabolism', and 'stress, electron transport chain'.

Transcriptional work by Dikicioglu *et al.* (2011) examined the effects of cells grown in a glucose-limited culture to a pulse of glucose. In response to the addition of glucose they found significant changes in 372 gene transcripts. These were genes involved in the following biological processes 'carboxylic acid metabolism', 'amino acid metabolism' 'purine metabolism', 'glycolysis', 'oxidative phosphorylation', 'alcoholic catabolism' and 'energy-coupled proton transport'. Genes involved in glycolysis that were mentioned to be upregulated during this action were not an immediate response. Five genes associated with alcohol metabolism, *PFK1*, *PFK2*, *ENO2*, *TKL1* and *CTS1*, were also upregulated.

1.10. Proteomic analysis

Proteomics complements measurements of mRNA transcripts through analysis of individual proteins. There is often a poor or only modest correlation between the amount of mRNA and the protein product. Wu *et al.* (2008) analyzed the relationship between transcriptome and proteome in *S. cerevisiae*. They concluded that protein degradation was a major factor affecting mRNA-protein ratios. Massoni *et al.* (2009) used pulse chase of *S. cerevisiae* strains grown in [³⁵S] methionine and 2DE analysis to examine changes in abundance of individual protein spots. Their study included

detection of post-translational modifications that resulted in change of protein spot position on the 2D gels, and analysis of differences in protein turnover. Post-translational modifications were identified by shift in *pI*, in most cases towards the acidic end of the pH scale. They also found proteolytic products (fragments) of proteins that resulted in changes in abundance of protein isoforms. For example, a decrease of pyruvate decarboxylase was complemented by an increase in abundance of its fragments Pdc1f1 and Pdc1f2.

Analysis of the *S. cerevisiae* proteome using 2DE or gel-free LC MS/MS has been described in several reports from Matthias Mann's research group. Initially, Shevchenko *et al.* (1996) described methods for identification of protein spots excised from 2D gels, resulting in identification of 150 proteins using a combination of MALDI TOF MS mass fingerprinting and ESI LC MS/MS of tryptic digests. Advances in mass spectrometer design since that time have led to major enhancements in resolution and sensitivity so that over 4000 yeast proteins can now be identified using gel free LC MS/MS of yeast extracts (Nagaraj *et al.*, 2012).

Several groups have used proteomics, with or without transcript analysis, to examine fermentative and oxidative metabolism in *S. cerevisiae*. Kolkman *et al.* (2005) compared the effects of glucose or ethanol-limitation on the transcriptome and 2DE proteome of cells grown under steady-state conditions in chemostat cultures. Protein identification by MALDI TOF MS or LC MS/MS found 44 proteins involved in central carbon metabolism including the functional categories glycolysis, pyruvate branch point, TCA cycle and gluconeogenesis and glyoxylate shunt. Fifteen proteins showed quantitative differences in abundance ($p \leq 0.02$) between ethanol or glucose-limited cells and a further two proteins (Hxk1, Adh1) were detected only during glucose-limitation compared to 12 proteins (mainly citric acid cycle and gluconeogenic enzymes) that were detected only during ethanol-limitation. Glycolytic enzymes shared between the two conditions were Glk1, Fba1, Tpi1, Tdh2, Tdh3, Pfk1, Gpm1, Eno1 and Eno2.

Usaite *et al.* (2008) used LC MS/MS shotgun analysis of tryptic digests for comparison of wildtype and Snf1 protein kinase mutant strains grown aerobically under steady-state conditions. Approximately 2400 proteins were quantified using

spectral counting and SILAC methods. The large increase in number of proteins detected compared with 2DE reflects the greater depth of analysis using gel-free MS methods although detection of processed protein fragments is not so straightforward. Approximately 15% of the measured proteins, including carbohydrate metabolism enzymes, varied ($p \leq 0.05$) between the wildtype and mutant strains. Calculated differences in protein abundance were a 2.3-fold increase in Adh1 and a 5.3-fold decrease in Adh2 in a Snf1-deficient strain compared to the wildtype, suggesting a shift towards ethanol production when Snf1 was deficient.

Bruckmann *et al.* (2009) used 2DE and transcript analysis to compare anaerobic and aerobic cultures grown in steady-state glucose-limited conditions. One hundred and ten identified protein spots, representing the products of 75 unique genes, differed by more than 2-fold between the cultures. The glycolytic enzymes Cdc19, Eno1, Hxk1, Pgc1, as well as proteins involved in pyruvate metabolism (Pdc1 and Adh1) were more abundant in anaerobic conditions. Changes in proteins specific to aerobic growth were associated with oxidative metabolism, including decreases in Adh2 and Ald4 and 6 for cells grown anaerobically. They also identified changes at the level of protein that were greater than predicted from transcripts.

Guidi *et al.* (2010) examined the metabolic adaptability of *S. cerevisiae* to three different concentrations of glucose. The three concentrations tested were 0.5% (low, glucose restriction), 2% (control) and 20% (excess). Proteins were separated by 2DE and abundances were measured using imaging of Coomassie stained gels, which is less accurate than DIGE. Twenty-one differentially expressed proteins were identified by MALDI mass fingerprinting. The abundance of some Pdc1, Pgc1, Eno2 and Tdh3 protein spots decreased in 0.5 and 20% glucose conditions compared to cells grown in 2% glucose. However, spots matching to the glycolytic enzymes Pdc1, Eno2 and Tdh3 increased in abundance at 20% glucose compared to control conditions. The analysis was complicated by detection of multiple protein spots for some of enzymes. For example, two protein spots matched to enolase 2 (Eno2). The most abundant form increased nearly 2-fold in 20% glucose, while a less abundant form showed a 3.5-fold increase in 2% compared to 0.5% glucose. Of two detected Adh1 protein spots, the most abundant did not show major change between 0.5% and 2% glucose but increased nearly 3-fold in 20% glucose. Measurement of glucose

use and alcohol production indicated complete conversion to ethanol at 0.5% glucose, and 63% and 38% conversion respectively at 2% and 20% glucose.

Transcript and proteome changes associated with nutrient limitation were examined by Stephen Oliver's research group Gutteridge *et al.* (2010). Carbon-limitation was carried out by a shift from 2.1% to 0.25% glucose in the medium. iTRAQ with LC MS/MS of tryptic digests was used for the proteomics analysis. Proteins that changed more than two-fold in abundance in glucose-limited medium included increases in hexokinase Hxk1 and aldehyde dehydrogenases Ald 1 and 3, and decreases of the hexose transporter Hxt3, and alcohol dehydrogenase 4 (Adh4).

Huang *et al.* (2012) used LC MS/MS of tryptic digests to compare the early- mid- and late-exponential phase (22.5, 33 and 44 h) proteins of *S. cerevisiae* grown in shake flask cultures with initial 4% glucose. Ethanol was the major metabolic product after 24 h, glycerol and acetic acid were not detected. Of the total 1389 proteins identified (representing 1331 unique genes) there were differences in protein abundance between stages of the growth phase, including lower amounts of ribosomal proteins associated with cell proliferation towards the end of the growth phase. Adh1 and the citric acid cycle enzymes were represented at all three stages of growth indicating capacity both for fermentation of glucose to ethanol and oxidative metabolism.

Most recently, Stobaugh *et al.* (2013) used LC MS/MS to compare the proteomes of *S. cerevisiae* grown on glucose or glycerol in shake flasks. Their emphasis was on comparison of fractionation by anion-exchange or reversed-phase chromatography of proteins prior to LC MS/MS of tryptic digests. Protein abundances were quantified using spectral counting, carbon substrate related changes were expected for 1.5-fold differences in protein abundance at $p \leq 0.05$. There were differences in the numbers of proteins detected and quantified between the two prefractionation protocols, however, the general conclusion was that the abundances of several glycolytic enzymes did not differ between growth on glucose or glycerol. Their results are summarized in Figure 6. In general, growth on glucose (dextrose) resulted in higher abundance of hexokinase B (Hxk2) and enzymes for synthesis of glycerol from dihydroxyacetone phosphate. Growth on glycerol favored production of hexokinase A (Hxk1) and glucokinase (Glk1), and enzymes involved in

gluconeogenesis, the use of glycerol and oxidative citric acid cycle activity. Although the amount of Adh1, that catalyzes synthesis of ethanol, did not vary significantly between growth on glucose or glycerol, use of glycerol as a substrate resulted in greater abundance of Adh2 and aldehyde dehydrogenases that catalyze the two-step pathway for synthesis of acetate from ethanol. The gluconeogenic enzyme fructose-1,6-biphosphatase was more abundant using glycerol as a substrate in samples prepared using reversed-phase chromatography for protein fractionation, but this enzyme was not detected using ion-exchange workup. Overall, the results illustrate the effect of carbon substrate on the abundances of proteins involved in pathways of carbon metabolism, and the importance of sample preparation protocols for protein detection and quantification.

Figure 6 - Proteomic analysis of *S. cerevisiae* grown in glucose (dextrose) or glycerol based media by Stobaugh et al. (2013). Samples were pre-fractionated by anion-exchange (a) or reversed-phase (b) chromatography before LC MS/MS.

Prefractionation of proteins by reversed-phase chromatography recovered more proteins than anion-exchange chromatography. Glycolytic proteins were found in both substrate conditions and between the two techniques. The copyrights to use this diagram were granted.

1.11. Goal of this research

The overall goal of this project was to examine the extent to which the abundances of enzymes involved in glucose metabolism varied between conditions that favored fermentation or respiration. Two-dimensional-DIGE was used for the comparison of protein abundances as many of the relevant enzymes are relatively high abundance and can be detected on 2D gels. One qualification for use of 2DE is that hydrophobic

proteins including membrane transporters are not soluble during the initial focusing step and are therefore not detected. The emphasis of the project was therefore on detection of soluble proteins including the enzymes of carbohydrate metabolism.

The four specific aims were to:

- Set up experimental conditions for extraction and separation of *S. cerevisiae* proteins
- Create a database of proteins identified by MALDI TOF/TOF MS of tryptic digests of proteins excised from 2DE gels
- Examine substrate conditions that favor fermentation or oxidation of the carbon source
- Use 2D-DIGE to compare the enzyme abundances between conditions that favor fermentation or oxidative metabolism of the carbon source

Growth of *S. cerevisiae* in glucose or glycerol in shake flask cultures was chosen for the DIGE analysis. At the time this decision was made there were no reported proteomic comparisons using these two substrates, although there is now the shotgun LC MS/MS analysis of Stobaugh *et al.* (2013). This Thesis is therefore the first 2DE study of protein variation using these growth conditions.

2. Methods

2.1. Yeast strains and storage

The haploid *Saccharomyces cerevisiae* strain BY4741 (MATa: *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*) was used in this project. The strain was stored in 15% glycerol and yeast peptone (YP) media at -80°C for long-term preservation. Single colonies were generated by streaking out cells on an YPD agar plate (1% yeast extract, 2% peptone, 2% D-glucose) using aseptic technique, then incubated at 30°C for 2 days, and kept at 4°C for up to 3 months.

2.2. Comparative growth experiments

To analyze the growth of BY4741 in fermentative and non-fermentative states cells were grown in (a) varying glucose concentrations with Synthetic Complete (SC) media and (b) a high glucose concentration versus glycerol supplemented SC media. The growth conditions for the two experiments are shown in Figure 7. For the glucose experiment, cultures supplemented with 0.2% (low), 0.5% (medium) or 2% (high) glucose and SC media were set up from precultured 2% glucose cultures in stationary phase (seeding culture). The comparative analysis of BY4741 grown in SC media supplemented with glycerol or glucose is described as the glycerol versus glucose experiment. Cultures of BY4741 supplemented with 3% glycerol (3% G2) or 2% glucose (2% D) were inoculated from previously grown stationary phase cultures of 3% glycerol (3% G1) in SC media. The growth of BY4741 using glycerol is commonly supplemented with ethanol. However, the addition of ethanol was excluded in order to simplify the measurement of ethanol production.

2.3. Growth conditions

Cells were grown in 25 mL of SC media supplemented with 2% glucose or 3% glycerol at 30°C, with shaking at 250 rpm (Bioline, NSW, Australia) as shown in Figure 7. Cultures were inoculated with 6.5×10^5 cells from previously grown stationary phase cultures, in conical flasks with a maximum depth of 1 cm medium to ensure aeration.

Figure 7 - Growth conditions of BY4741 for the analysis of fermentative and oxidative metabolism. Cells were grown in SC media supplemented with different glucose concentrations (top) or 3% glycerol vs. 2% glucose (bottom).

2.4. Growth Curves

Three 10 mL cultures of BY4741 in SC media with 2% glucose or 3% glycerol were prepared. The point at which cultures were inoculated with cells was time zero. An aliquot of each culture was taken, diluted 10X and the optical density (OD) measured at 600 nm using a Cary 300 Bio UV-Visible Spectrophotometer (Varian, VIC, Australia). The first measurement was taken at time zero. For glucose supplemented growth, the next measurement was taken at $t = 4.5$ h then every 1-1.5 h thereafter. For glycerol supplemented cultures, the next measurements were taken at $t = 12$ h and $t = 15$ h followed by a 9 h interval. From herein, time points were every 3 h for 15 h followed by a 9 h interval with the final measurement at $t = 96$ h.

2.5. Harvesting yeast cultures

Cells were collected at $OD_{600} = 0.5$ and were centrifuged using an Eppendorf Centrifuge 5810 (Hamburg, Germany) at 4,000 rpm for 5 min at 4°C. The culture supernatant was removed and stored at -80°C for glucose and ethanol analysis. The cells were washed three times in ddH₂O followed by centrifugation at 13,000 rpm, at 25°C for 5 min using a Beckman Coulter Microfuge®16 Centrifuge (Krefeld, Germany). The third wash had an extra addition of Complete Mini EDTA-free

Protease Inhibitor (1:100 w/v) (Roche, Mannheim, Germany). The yeast cells were stored at -80°C until they were lysed for protein extraction.

2.6. Protein extraction from cells

Two protein extraction methods were explored for proteomic analysis of BY4741. First, a Yeast Protein Extraction Buffer Kit from GE Healthcare (Uppsala, Sweden) was used. Harvested yeast cells were washed two times by the addition of 5X the volume of Yeast Suspension Buffer, mixed then centrifuged at 13,000 rpm at 4°C for 5 min. The supernatant was discarded after centrifugation. Cells were lysed in 2X the volume of the cell pellet with Yeast Protein Extraction Buffer. Protease inhibitor (1:100 w/v) was added, mixed then incubated on ice for 30 min. Cells were briefly incubated at 37°C for 2 min then centrifuged at 13,000 rpm for 30 min at 4°C. The supernatant was collected and stored at -80°C. The remaining cell pellet was discarded.

The second method used lysis buffer (4% w/v CHAPS, 2 M thiourea, 7 M urea, 30 mM Tris pH 8.8) and white quartz sand (Sigma-Aldrich, St. Louis, MO). One-times the volume of lysis buffer was added to the cell pellet then ground in a microcentrifuge tube for 60 sec with quartz sand using a mini pestle (GE Healthcare). Following cell grinding, cells were vortexed for 15 min at 4°C and centrifuged at 13,000 rpm at 4°C for 5 min. The supernatant was collected and stored at -20°C. The remaining cell pellet was discarded.

2.7. One-dimensional SDS PAGE

One-dimensional SDS PAGE was used for initial examination of the resulting cell lysates. The gel electrophoresis running buffer (1X) was prepared by mixing 760 mL of ddH₂O and 40 mL of NuPAGE® MOPS SDS running buffer (20X) (Invitrogen, Carlsbad, CA). A 10 or 12 well NuPAGE® 4-12% Novex Bis-Tris Zoom™ 1.0 mm gel (Invitrogen) was rinsed in ddH₂O and assembled in an XCellSureLock™ Novex Mini-Cell electrophoresis system (Invitrogen). Both the inner and outer chambers were filled with the 1X running buffer, and 500 µL of NuPAGE® Antioxidant (Invitrogen) was added to the inner chamber. Each protein sample (1 µL) was mixed with 2.5 µL of protein denaturant, 4X NuPAGE® LDS Sample Buffer (Invitrogen), and 1µL of 10X NuPAGE® Sample Reducing Agent (Invitrogen). These samples

were centrifuged (5 min, 25°C, 13,000 rpm) and heated at 70°C in an Ori-Block® OB-1 heat block (Techne, Cambridge, England) for 10 min. Samples were then loaded into separate lanes of the gel. Either Mark¹² (Invitrogen) or Rainbow 851 (GE Healthcare) was used as a protein standard and also loaded into a well. The gel was electrophoresed at 200 V for 55 min using an Electrophoresis Power Supply-EPS 301 (GE Healthcare).

2.8. Gel fixing, staining and scanning

Following electrophoresis, proteins were fixed by placing gels in 50% v/v ethanol, 3% v/v phosphoric acid for 30 min with shaking at 15 rpm on a Labnet Gyrotwister (Woodbridge, NJ). The gels were rinsed three times in ddH₂O for 30 min and transferred to 17% w/v ammonium sulfate, 34% v/v methanol, 3% v/v phosphoric acid. Gels were left in this solution for at least 60 min before the addition of Coomassie (CBB G-250) (Biorad, Hercules, CA). The gels were left in the staining solution for 2 days, and then washed three times in ddH₂O for 30 min each to remove excess CBB G-250. Washed gels were scanned at 12 bits per pixel with a 50 µm pixel size using a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA), and the gel images were viewed using ImageQuant™ software (Version 5.2, Molecular Dynamics). Images were saved as .gel files.

2.9. Protein concentration

The protein concentration of each cell lysate was measured using a GE Healthcare 2D-Quant kit. A standard curve was generated with duplicates of 0, 4, 6, 12 and 30 µg using BSA. Aliquots of 2 µL of each protein sample were tested in duplicate. First, proteins in the lysates and standard curve were precipitated by the addition of 500 µL 2D-Quant Precipitant, vortexed then incubated at room temperature for 3 min. Following this, 500 µL of Co-precipitant was added and mixed by inverting the microcentrifuge tubes. All samples were centrifuged at 13,000 rpm for 5 min at room temperature. The supernatant was discarded immediately, followed by a second period of centrifugation for 60 sec. The supernatant was decanted. To each protein pellet 500 µL of Copper Solution was added then vortexed. The addition of 1 mL of Working Colour Reagent was followed by an incubation period of 15-20 min at room temperature. The absorbance was measured at 480 nm using water as the blank.

2.10. Protein precipitation

Proteins in cell lysates were precipitated to remove unwanted cellular debris for better separation of proteins during electrophoresis and/or to concentrate samples. A ProteoExtract® (Calbiochem, La Jolla, CA) protein precipitation kit was employed. The precipitant was added to the protein samples and left at -20°C for at least 30 min. The mixture was centrifuged for 5 min at room temperature and the supernatant was discarded. The protein pellet was washed twice in the wash solution and centrifuged, the supernatant was discarded between washes. The pellet was left to dry in a laminar flow workstation for at least 30 min before the addition of rehydration buffer or lysis buffer.

2.11. Two-Dimensional Electrophoresis

2.11.1. First dimension

Three pH ranges were examined; a broad range pH 3-11 non-linear (3-11NL), an acidic range pH 4-7 and a basic range pH 6-11. Two methods of sample application were used, passive rehydration and cup loading. Sample application to pH 4-7 IPG strips was tested by passive rehydration and cup loading at the cathode. The pH 3-11 NL strips required passive rehydration of the samples. The rehydration buffer complemented the pI of the IPG strip used i.e. pH 4-7 rehydration buffer was used for pH 4-7 IPG strips. All Immobiline™ DryStrip gels (pI 4-7, 6-11 and 3-11 NL), IPG buffers, reswelling tray and all components of the Multiphor II system (paper wicks, cups, electrodes, tank and power supply) were from GE Healthcare.

2.11.2. Sample preparation for passive rehydration

To protein pellets for passive rehydration, 125 µL rehydration buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, 2% v/v IPG buffer (GE Healthcare), 2% w/v DTT (Merck) was added. This was left for 15 min before the sample was centrifuged. The sample was distributed over a 7 cm area in a well of an Immobiline™ DryStrip Reswelling Tray, and an IPG strip was placed gel side down over the sample. This was covered with 3 mL of Plus One DryStrip Cover Oil (GE Healthcare) and left overnight.

2.11.3. Sample preparation for cup loading

The preparation of samples for cup loading at the cathode or anode utilized the same protocol with the exception of the rehydration buffer. The rehydration buffer for pH 6-11 IPG strips contained 2 M thiourea, 7 M urea, 2.5% w/v DTT, 2% w/v CHAPS,

0.2% v/v pH 6-11 IPG buffer, 10% v/v isopropanol (Merck), 5% v/v glycerol. The protein pellet was reconstituted in 100 μ L of rehydration buffer. This was left for 15 min then centrifuged, and left overnight.

2.11.4. Isoelectric focusing using a Multiphor II system

For cup loaded samples, IPG strips were passively rehydrated the night before use, in 125 μ L of rehydration buffer, see section 2.11.2. Rehydrated IPG strips were placed gel side up in a Multiphor II IEF tank. Two paper wicks were soaked in 500 μ L of ddH₂O and placed over each end of the IPG strips. Electrodes were placed over the paper wicks. For cup loaded samples, cups were centred over the IPG strips before the tank was filled with Ondina oil (Shell, VIC, Australia). Cups were placed at the cathode or anode for focusing of pH 4-7 or pH 6-11 IPG strips respectively. For pH 6-11 IPG strips one paper wick was soaked in 500 μ L of pH 6-11 rehydration buffer and placed at the cathode end. IPG strips were electrophoresed as stated in Table 2 using an Electrophoresis Power Supply-EPS 3500 XL.

Table 2 - Settings of the Multiphor II system for isoelectric focusing using pH 3-11 NL, 4-7 or 6-11 IPG strips.

Step	Voltage	Time (h:m)	
		pH 4-7 and 3-11NL	pH 6-11
1	200	0:01	0:01
2	3500	1:30	1:30
3	3500	1:30	1:05

2.11.5. Second dimension - SDS PAGE

Prior to the second dimension of 2DE, IPG strips were reduced in 1% w/v DTT then subjected to alkylation (2.5% w/v IAA) in equilibration buffer (50 mM Tris pH 8.8, 6 M urea, 30% v/v glycerol, 2% w/v SDS), 15 min per incubation on a Platform Rocker (Bioline). The electrophoresis tank was prepared as described in section 2.7. The IPG strip was rinsed in the inner chamber, cut to fit within the well, and placed into the IPG well of a NuPAGE® 4-12% Novex Bis-Tris Zoom™ 1.0 mm IPG well gel. For reference purposes the anodic end of the IPG strip was placed closest to the

ladder lane. The gel was then electrophoresed at 120 V for 115 min. Gels were fixed, stained and scanned as described in section 2.8.

2.12. Protein spot picking, destaining of protein spots and trypsin digestion

Protein spots were manually excised from gels using a spot picker (OneTouch Plus Spot/Band picker PDM1.5, The Gel Company, San Francisco, CA). Each gel plug was placed into a separate well of a 96 well v-bottomed plate with 100 μL of 20 mM ammonium bicarbonate and left overnight at 4°C. An Ettan digester (GE Healthcare) was used for protein digestion. All solutions were made fresh and filtered through 0.2 μm membrane (Merck Millipore, Darmstadt, Germany). The digester software (version 1.10) method had a 3 phase wash cycle using 50 mM ammonium bicarbonate, high grade methanol (Merck): ddH₂O 1:1 v/v, followed by a drying period of at least 60 min. After the drying cycle 50 ng of modified sequencing grade trypsin (Roche) per well was added in 20 mM ammonium bicarbonate to cleave proteins into peptides. The peptides were extracted from the gel plugs in 0.1% v/v TFA (Fluka), ACN (Merck):ddH₂O 1:1 v/v and transferred to a new 96 well v-bottomed plate and left to dry overnight.

2.13. Preparation of samples for MALDI MS/MS

Digested peptides were mixed with fresh CHCA matrix solution (10 mg/mL CHCA (Sigma-Aldrich) in 0.25% v/v TFA. ACN: ddH₂O 1:1 v/v) and spotted onto MALDI plates. For internal calibration of masses, 0.7 μL of Calibration Mixture 2 (Calmix 2) was added to the fresh matrix solution. Calmix 2 contains angiotensin I, ACTH (clip 1-17), ACTH (clip 18-39) and ACTH (clip 7-38) with the monoisotopic masses of 1,296.68, 2,093.08, 2,465.19 and 3,657.92. The digested peptides were mixed in CHCA matrix with Calmix 2 before spotting. For external calibration of masses aliquots of Calmix 2 in CHCA matrix were spotted separately on MALDI plates.

2.14. MALDI TOF/TOF MS

An AB SCIEX 5800 mass spectrometer was used to acquire mass spectra for protein identification. Before data collection, the acquisition, processing and interpretation methods were tested on calibration spots to check for trypsin and calibration mixture peaks. Subsequently, the MALDI plate was calibrated. All spectra were collected in positive ion mode. The plate was set with continuous stage motion.

The details of the acquisition methods used for MS and MS/MS collection are as follows. For MS data collection, the selected mass range was 800-4,000 Da with a focus mass of 1,800 Da. Two hundred laser shots were fired per sub-spectrum. Five sequential pass rounds of sub-spectra collection were required for acceptance. Sub-spectra were accepted when peaks had a signal to noise ratio (s/n) ≥ 4 . The collective 1000 shots were merged to create one spectrum. For MS/MS data collection, collision induced dissociation (CID) was turned on, and air molecules filled the collision chamber. Acquiring spectra required 250 shots per sub-spectrum. Two rounds of sub-spectra accumulation were required to merge 500 shots per spectrum. All sub-spectra were accepted. The metastable suppressor was turned on. The processing method used required a minimum of three calibration peaks matched in each spectrum, and a mass tolerance of ± 0.5 m/z. The interpretation method was used to link the MS data for MS/MS spectra collection. From the MS data, the strongest peaks were examined first. Ten precursor ions per spot were selected for further processing.

There are three ways the plate can be calibrated; internal, external and default. The selected method of calibration was internal by adding calibrant to the matrix solution before mixing with samples. However, if calibration failed a default calibration method was used. In some cases, external calibration was used.

2.15. Protein identification

Peptide masses were searched using MASCOT (Version 2.4.01, Boston, MA) against the *S. cerevisiae* UniProtKB/Swiss-Prot database (Version 20081216, and 405,506 sequences; 6,743 *S. cerevisiae* sequences) with the peptide mass tolerance set at 40 ppm. The search criteria included allowance for one missed trypsin cleavage, complete modification of cysteine by carbamidomethyl, and variable methionine oxidation. The criteria for a match included the number of peptides matched, sequence coverage and significance scores. For proteins that were poorly annotated sequences were additionally searched using BLAST.

2.16. Gene Ontology

The gene ontology (GO) website Kyoto Encyclopedia of Genes and Genomes (KEGG) was used for GO annotations of proteins using www.genome.jp/kegg/tool/map_pathway2.html.

2.17. Spectrophotometric assays

Glucose and ethanol in cell culture supernatants were measured using spectrophotometric assays. A Cary spectrophotometer was used for all experiments.

2.17.1. Glucose assay

Glucose was measured using a glucose oxidase-peroxidase kit (Sigma-Aldrich) that coupled oxidation of glucose to H₂O₂ mediated oxidation of o-dianisidine. The volume of sample tested varied between conditions due to the glucose concentration. The supernatants of 0.2% and 0.5% glucose grown cells were diluted 100X, whilst the supernatants of the 2% glucose grown cells were diluted 1000X. Each set of assays included a media only blank and a glucose standard curve with 5-25 µg glucose. Reactions were started by addition of 660 µL of Assay Reagent then mixed by inversion and incubated at 37°C for 30 min. Reactions were stopped by adding 590 µL of 6 M H₂SO₄ followed by measuring the absorbances at 540 nm.

2.17.2. Ethanol assay

To measure the amount of ethanol produced by yeast cells an alcohol dehydrogenase ADH-NAD⁺ assay was used. Oxidation of ethanol by alcohol dehydrogenase was coupled to the reduction of NAD⁺ to NADH. The ADH-NAD⁺ assay reagent was prepared by reconstituting ADH-NAD⁺ in pH 9.0 0.5 M glycine buffer. An ethanol standard was made by diluting absolute ethanol to give a 20 µmol/µL stock and creating a 0.05-0.2 µmol standard curve. Proteins were precipitated from yeast supernatants by adding equal volumes of 10% TCA and sample supernatants, 10 µL of this was used for analysis. The reaction was started by the addition of 1 mL of NAD-ADH reagent, inverting the samples followed by an incubation period of 60 min at 37°C. Absorbances were measured at 340 nm.

2.18. Differential in Gel Electrophoresis (DIGE)

DIGE was used to compare the protein profiles of respiring and fermenting BY4741 cells. Cyanine (Cy) dye reagents were used for fluorescent labelling of samples for

comparative analysis. A protein concentration between 4-10 µg protein/µL was required for optimal Cy dye binding. For dilute protein samples, precipitation of protein was carried out to concentrate the sample (see section 2.10). However, instead of resuspending the protein pellet in rehydration buffer, a smaller volume of lysis buffer was added and protein concentrations were re-quantified (see section 2.9).

For labelling, the pH of protein (10 µg) samples was adjusted by the addition of 1.5 M Tris to pH ≥ 8.5. A 1 mM working stock solution was made for each Cy dye in N, N-dimethylformamide (Sigma-Aldrich), and each solution was vortexed and briefly centrifuged (10 sec). The labelling scheme for randomisation of dye labelling is shown in Table 3. For consistency between gels, a control was made using all eight samples (5 µg 3% G2a + 5 µg 3% G2b + 5 µg 3% G2c + 5 µg 3% G2d + 5 µg 2% Da + 5 µg 2% Db + 5 µg 2% Dc + 5 µg 3% Dd) and divided evenly between each replicate (10 µg used).

Samples were vortexed and centrifuged briefly (10 sec), before being left on ice in the dark for 30 min. The reaction was stopped by a 1 µL addition of 10 mM lysine, vortexed and centrifuged, and left for 15 min in the dark. Each sample set was then mixed together i.e. Cy2, Cy3 and Cy 5 labelled samples. This was then ready for protein precipitation as a clean-up step for preparation for the rehydration of samples for IEF (see section 2.10).

Table 3 - The DIGE labeling design for the comparison of glucose (D) and glycerol (G) grown cells. The second letter (a-d) shows the replicate used.

Gel number	Cy 3 labelled	Cy 5 labelled	Cy 2 standard
1	10 µg 3% G a	10 µg 2% D c	10 µg pool of all 4 samples
2	10 µg 3% G b	10 µg 2% D d	10 µg pool of all 4 samples
3	10 µg 3% G c	10 µg 2% D a	10 µg pool of all 4 samples
4	10 µg 3% G d	10 µg 2% D b	10 µg pool of all 4 samples

2.19. Fluorescent scanning

Gels containing Cy dye labelled samples were scanned immediately after SDS PAGE. Gel images were digitized using a Fujifilm Fluorescent Image Analyser FLA-5100

scanner (Fuji Photo Film Co., Ltd, Tokyo, Japan) at 16 bits per pixel with a 50 μm pixel size at each Cy dye excitation wavelength (Table 4), at 600 V. Post-fluorescent scanning, the gels were stained with CBB G-250(see section 2.8). Acquired images were saved as .tiff files.

Table 4 - Excitation wavelengths for each Cy dye and the type of emission filter used for each wavelength.

Cyanine dye	Wavelength (nm)	Emission filter
2	473	BPB1/ 530DF20
3	532	BPG1/ 570DF20
5	635	DBR1/ R665

2.20. Preparation of gel images for DeCyder™ analysis

Before the images were analyzed they were converted from .tiff files to .gel files using ImageQuant Tools™ software (Version 2.2, Molecular Dynamics). The .gel files were used to create a dataset file using Fluorochrome separation™ software (Multicolour version 2.2, Molecular Dynamics). This overlayed the Cy 2, 3 and 5 images for each gel set, i.e. one data set per replicate was created. The dataset images were cropped using ImageQuant Tools™, for exclusion of excess Cy dye that accumulated at the bottom and sides of the gel.

2.21. Data analysis using Decyder™ software

The scanned images of the Cy dye labelled gels were analyzed using the DeCyder™ software (Version 6.5, GE Healthcare). Protein spots were matched to a master gel, the one with the most spots. Gels were set to identify 2,500 protein spots although some detected 'spots' were due to noise generated from streaking or excess dye providing false spots. Detected spots were assessed to differentiate between protein spots (curved peaks) from noise (sharp peaks). The determination of proteins that varied between substrate conditions was done using a Student's *t*-test ($p \leq 0.01$) and selecting a ≥ 2 -fold change in protein abundance between samples. Protein abundances are log standardized by DeCyder™ software for comparative analysis. The degree of change of the standardized protein abundances between the conditions is represented by an average ratio. An average ratio greater or equal to a

2-fold change, positive or negative, is considered significant when using replicates of four (Karp & Lilley, 2005). The average ratio represents a change in protein abundance of one condition in relation to the other. To interpret changes in protein abundance for the glucose/glycerol experiment the degree of change was analyzed in relation to the glucose set. Therefore a positive average ratio indicates an increase in abundance in glucose and a negative ratio indicates an increase in protein abundance in the glycerol set.

3. Results

3.1. Growth analysis

S. cerevisiae divides by budding. The time it takes to create the daughter cell (budding/division time) is dependent on a number of factors such as the strain and growth conditions. *S. cerevisiae* naturally ferments when glucose is abundant. However, this yeast respires when grown on a non-fermentable carbon source such as glycerol. The aim of this set of experiments was to examine the growth of BY4741 in glucose and glycerol for proteomic analysis.

The growth of BY4741 in synthetic complete (SC) medium supplemented with glucose or glycerol as a carbon source is shown in Figure 8. The time taken for cell collection $OD_{600} = 0.5$ was approximately 14 h, the doubling time was 2 h 5 min.

The second experiment was designed to examine growth of BY4741 in the non-fermentative carbon source glycerol (Figure 8 bottom). The time taken to reach an OD_{600} of 0.5 was approximately 45 h. The doubling time of BY4741 in 3% glycerol supplemented SC media was calculated as 9 h 41 min, approximately 5X slower than when cells were grown in glucose. Figure 9 shows the Log_{10} growth of *S. cerevisiae* in 2% glucose (top) or 3% glycerol (bottom).

Figure 8 - Growth of BY4741 in 2% glucose (top) or 3% glycerol (bottom) and SC media. Aliquots of cultures were diluted (10X) and the absorbance was measured at 600 nm for glucose or glycerol grown cells. Cultures of BY4741 were grown in triplicate, error bars are standard deviations.

Figure 9 - Log₁₀ growth curves of BY4747 in 2% glucose (top) or 3% glycerol (bottom) and SC media. Aliquots of cultures were diluted (10X) and the absorbance was measured at 600 nm for glucose or glycerol grown cells. Cultures of BY4741 were grown in triplicate, error bars are standard deviations.

3.2. Extraction protocols and optimization of separation techniques for 2D analysis of a proteome

Three sets of experiments were designed to develop protocols for proteome analysis of BY4741. The parameters that were examined include methods for protein extraction, pH ranges for IEF, and optimization of protein separation by the method of application of sample to IPG strips.

3.2.1. Protein extraction

The first experiment compared two extraction methods; disruption of cells by homogenization in lysis buffer (4% w/v CHAPS, 2 M thiourea, 7 M urea, 30 mM Tris pH 8.8) with quartz sand, and use of a protein extraction kit (GE Healthcare). The resulting protein profiles were analyzed by 1D SDS PAGE (Figure 10). The yeast cells were grown to stationary phase ($OD \geq 1$) in 2% glucose in YP or SC media. The protein lysates produced using the kit protocol gave relatively few proteins > 55 kDa from BY4741 grown cultures in YP or SC media (Figure 10, lanes 2 and 3 respectively). The lysis buffer protocol gave better 1D SDS PAGE extraction profiles with proteins ranging from 6-200 kDa in both the YP or SC grown cells (Figure 10, lanes 4 and 5 respectively).

Figure 10 - Comparison of protein extraction methods analyzed by 1D SDS PAGE.

The two methods used were lysis buffer (4% w/v CHAPS, 2 M thiourea, 7 M urea, 30 mM Tris pH 8.8) with quartz sand (Lanes 4 & 5), and a protein extraction kit (GE Healthcare) (Lanes 2 & 3); 10 μ g of protein of each sample was used. Cells were grown in SCD (Lanes 2 & 4) or YPD: media (Lanes 3 & 5). Lane 1 contained Invitrogen Mark¹² ladder.

3.2.2. Separation of yeast proteins by 2DE using different pH ranges on 7 cm gels

A broad pH range of 3-11 NL (Figure 11, top) and two overlapping linear ranges pH 4-7 and 6-11 (Figure 11, bottom left and right, respectively) were used. Although loaded with the same amount of protein 2DE using pH 3-11 NL IPG strips in the first dimension gave less protein spots than the overlapping pH 4-7 and 6-11 ranges.

Figure 11 - Two-dimensional gel electrophoresis using three different 7 cm gradients for IEF. The gradients used were pH 3-11NL (top) and 4-7 and 6-11 (bottom; left and right). The Invitrogen Mark¹² ladder was used as a molecular weight marker.

Twenty micrograms of protein was loaded to each gel.

3.2.3. Optimization of 2D gels

This experiment focused on improving the separation of proteins in the first dimension by comparing methods for sample application to IPG strips and the effect of protein precipitation.

Figure 12 shows 2DE on 7 cm SDS PAGE gels. Three sample application methods were examined using precipitated protein (left column) in comparison to non-precipitated proteins (right column). Sample application by passive rehydration (top row) and cup loading at the cathode (middle row) on pH 4-7 IPG strips, and cup loading at the anode (bottom row) for pH 6-11 IPG strips were compared. During passive rehydration, samples are absorbed by the IPG strip overnight. In contrast, cup loading of protein at either the cathode or anode is a process where the sample is applied directly to the IPG strip during IEF. The method of cup loading of sample at the cathode was discarded due to loss of proteins in the mid pH range using either precipitated or non-precipitated protein samples (Figure 12, middle). Passive rehydration of sample for the separation of pH 4-7 proteins was therefore selected, and cup loading at the anode was used for focusing of basic proteins on pH 6-11 IPG strips. Protein precipitation was used for all subsequent sample preparations.

Figure 12 - Optimization of IEF. The experiment compared precipitated (left) against non-precipitated (right) protein samples. The first row are samples that were passively rehydrated, the middle row shows samples that were cup loaded at the cathode, and the bottom row shows samples which were cup loaded at the anode on a pH 6-11 gradient. The ladder used for molecular weight reference was Mark 12. Twenty micrograms of protein was used for each gel.

3.3. Protein identification

Analysis by 2D-DIGE typically requires small amounts (5 µg) of protein per sample for optimal binding of the Cy dye reagents. However, for the identification of proteins from gels, larger amounts of protein are often required. For the reference proteome experiment 100 µg of protein from cells grown in 2% glucose in SC media was analyzed.

3.3.1. 2D analysis of pI 4-7 proteins

The protein profile of BY4741 separated on a pH 4-7 IPG strip is shown in Figure 13. The horizontal axis displays the isoelectric range of the pH gradient (first dimension) and the vertical axis, separation on the basis of size. The 2D gel image shows 232 proteins visible by Coomassie staining that were selected for MALDI mass fingerprinting. Significant identifications required a match with a MASCOT score greater than 56 at $p \leq 0.05$. A total of 109 proteins were identified at the 5% level of significance (Table 5). Of these, 70 unique protein identifications were found. Thirty-nine identifications accounted for proteins with multiple spots or protein isoforms. Proteins with multiple spots include Adh1, Eno1, Eno2, Gdh1, Hsp82, Hxk2, Pdc1, Pdi1, Psa1, Rps12, Ssa1, Ssa2, Ssb2 and Vma2. Isoforms of proteins included Ade13, Ade5,7, Bmh1, Bmh2, Eno1, Eno2, Hsp104, Hsp26, Hsp60, Hsp82, Rps0A, Rps12, Rps21A, Rps5, Ssa1, Ssa2, Ssb1, Ssb2, Tif2, Tif6, Tub1, Tub2, Vma2, Vma4, Vma6 and Vma7. Of particular interest were the six glycolytic enzymes (Eno1, Eno2, Gdh1, Hxk2, Pdc1 and Pdi1) and Adh1 (alcohol dehydrogenase) present in multiple spots and isoforms.

Table 5 summarizes each protein that was matched including protein name, mass, isoelectric point, sequence length and coverage, subcellular localization, information about function and identity, GI and accession numbers. The last column, second matched protein, represents the first non-homologous protein match.

Figure 13 - The 2D gel image of the protein profile of *S. cerevisiae* BY4741 separated on a pH 4-7 linear gradient (horizontal axis). Separated proteins (100 μ g) were stained with Coomassie, and identified proteins are marked with an arrow. Numbers correspond to Table 5. The ladder used for molecular weight reference was Rainbow 851.

Table 5 - Summary of identified BY4741 proteins by MALDI mass spectrometry and separated by 2DE using a pH 4-7 gradient. Proteins (score ≥ 56 , $p \leq 0.05$) are alphabetized by gene name. The score and sequence coverage (%) values are representative of the significance of the match generated by the MASCOT search. The calculated mass, *pI*, GI, accession number and description of the protein were gathered from the NCBI protein website. The subcellular localization of the protein was found using Uniprot. The next matched protein was the next non-homologous protein matched. The spot position was cross checked with the calculated *pI* and *Mr* values. Differences between observed and predicted mass and / or *pI* are labeled A: acidic shift or B: basic shift in *pI* and/or I: increase or D: decrease in *Mr*.

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
177 ^{IA}	<i>ABP1</i>	65.59	4.59	592	178	21	gi 6319931	NP_010012	Cytoplasm > cytoskeleton > actin patch	Actin-binding protein of the cortical actin cytoskeleton, important for activation of the Arp2/3 complex that plays a key role actin in cytoskeleton organization.	AWRI1631_160620, gi 207340707 (33)
60 ^D	<i>ACT1</i>	41.89	5.44	375	246	19	gi 14318479	NP_116614	Cytoplasm > cytoskeleton	Actin, structural protein involved in cell polarization, endocytosis, and other cytoskeletal functions.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
26 ^B	<i>ADE13</i>	54.7	6.01	482	139	24	gi 6323391	NP_013463	Unknown	Adenylosuccinate lyase catalyzes two steps in the 'de novo' purine nucleotide biosynthetic pathway; expression is repressed by adenine and activated by Bas1p and Pho2p.	Erp6p, gi 6321436 (25)
190	<i>ADE5,7</i>	86.35	5.08	802	174	15	gi 6321203	NP_011280	Cytoplasm	Bifunctional enzyme of the 'de novo' purine nucleotide biosynthetic pathway contains aminoimidazoleribotide synthetase and glycinamideribotide synthetase activities.	Pau9p, gi 13129151 (36)
9 ^B	<i>ADH1</i>	37.15	6.21	347	295	26	gi 112491285	2HCY_A (NP_014555)	Cytoplasm	Chain A, Alcohol dehydrogenase I, fermentative enzyme.	Cdc53p, gi 6320070 (30)
10 ^B	<i>ADH1</i>	37.15	6.21	347	116	23	gi 112491285	2HCY_A (NP_014555)	Cytoplasm	Chain A, Alcohol dehydrogenase I.	(-)
31 ^D	<i>ADH1</i>	37.15	6.21	347	64	17	gi 112491285	2HCY_A (NP_014555)	Cytoplasm	Chain A, Alcohol dehydrogenase I.	(-)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
44 ^I	<i>ADK1</i>	24.29	5.98	222	101	18	gi 6320432	NP_010512	Cytoplasm Mitochondrion intermembrane space	Adenylate kinase, required for purine metabolism; lacks cleavable signal sequence.	(-)
201 ^B	<i>ALD6</i>	54.77	5.31	500	199	30	gi 6325196	NP_015264	Cytoplasm	Aldehyde dehydrogenase, activated by Mg ²⁺ and utilizes NADP ⁺ as the preferred coenzyme; required for conversion of acetaldehyde to acetate; constitutively expressed; locates to the mitochondrial outer surface upon oxidative stress	BSC4, gi 170963023 (27)
220 ^D _B	<i>ARO8</i>	56.37	5.68	500	149	9	gi 6321236	NP_011313	Cytoplasm	Aromatic aminotransferase I, expression is regulated by general control of amino acid biosynthesis.	Pau9p, gi 13129151 (36)
81	<i>ATP2</i>	51.09	5.11	478	85	24	gi 119389907	2HLD_D (NP_012655)	Mitochondrion	Chain D, Mitochondrial F1-ATPase	Igo1p, gi 6324172 (29)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
128 ^I	<i>BMH1</i>	30.18	4.82	267	92	12	gi 6321025	NP_011104	Nucleus Plasma membrane	14-3-3 protein, major isoform; controls proteome at post-transcriptional level, binds proteins and DNA, involved in regulation of many processes including exocytosis, vesicle transport, Ras/MAPK signaling, and rapamycin-sensitive signaling.	AWRI1631_141000, gi 207341878 (28)
127 ^I	<i>BMH2</i>	30.97	4.82	273	328	35	gi 151942087	EDN60443 (NP_010384)	Cytoplasm Nucleus	14-3-3 protein, minor isoform; controls proteome at post-transcriptional level, binds proteins and DNA, involved in regulation of many processes including exocytosis, vesicle transport, Ras/MAPK signaling, and rapamycin-sensitive signaling.	Pau9p, gi 13129151 (23)
185 ^B	<i>CCT5</i>	61.17	5.31	551	59	9	gi 567930	AAA53132 (NP_012598)	Cytoplasm	TCP1	SCY_2605, gi 151943234 (31)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
173 ^{IB}	<i>CDC48</i>	92.16	4.82	835	65	13	gi 6320077	NP_010157	ER Microsome	ATPase involved in ubiquitin-mediated protein degradation; Cdc48p-Npl4p-Ufd1p complex participates in ER-associated degradation (ERAD) while Cdc48p-Npl4p-Vms1p complex participates in mitochondria-associated degradation (MAD).	YKR101Wp-like protein, gi 20734331 1 (29)
110 ^D	<i>COF1</i>	15.94	5.05	143	108	36	gi 6322978	NP_013050	Cytoplasm >cytoskeleton Nucleus	Cofilin, promotes actin filament depolarization in a pH-dependent manner; binds both actin monomers and filaments and severs filaments; thought to be regulated by phosphorylation at SER4; ubiquitous and essential in eukaryotes.	Unnamed protein product, gi 4772 (27)
1 ^I	<i>CPR1</i>	17.49	6.9	162	309	37	gi 6320359	NP_010439	Cytoplasm	Peptidyl-prolylcis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues; binds the drug cyclosporin A.	Gas2p, gi 6323375 (35)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
11 ^{DB}	CYS3	42.51	6.06	394	277	20	gi 6319307	NP_009390	Cytoplasm	Cystathionine gamma-lyase catalyzes one of the two reactions involved in the transsulfuration pathway that yields cysteine from homocysteine with the intermediary formation of cystathionine.	Unnamed protein product, gi 4025 (35)
19 ^{IB}	<i>DPS1</i>	63.64	6.16	557	385	38	gi 6323011	NP_013083	Cytoplasm	Aspartyl-tRNA synthetase, primarily cytoplasmic; homodimeric enzyme that catalyzes the specific aspartylation of tRNA (Asp); class II aminoacyl tRNA synthetase; binding to its own mRNA may confer autoregulation.	Ade17p, gi 6323768 (51)
197 ^{IB}	<i>EFT2</i>	93.68	5.92	842	234	20	gi 6320593	NP_010673	Cytoplasm	Elongation factor 2 (EF-2), also encoded by EFT1; catalyzes ribosomal translocation during protein synthesis; contains diphthamide, the unique posttranslationally modified histidine residue specifically ADP-ribosylated by diphtheria toxin.	Type 2C protein phosphatase, gi 115361651 (31)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
17 ^{IB}	<i>ENO1</i>	46.84	6.16	437	131	7	gi 6321693	NP_011770	Cytoplasm	Enolase I, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is repressed in response to glucose.	Cor1p, gi 6319426 (52)
15 ^{IB}	<i>ENO1</i>	46.65	6.04	436	243	26	gi 157830958	1ELS_A (NP_011770)	Cytoplasm	Chain A, Catalytic Metal Ion Binding In Enolase-Mn2+-Phosphonoacetylhydroxamate Complex	Chain A, 3-Phosphoglycerate Kinase, Mutation R65q. (69)
224 ^{IB}	<i>ENO2</i>	46.94	5.67	437	371	37	gi 6321968	NP_012044	Cytoplasm	Phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	Pyruvate kinase, gi 4180 (76)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
41 ^{DB}	<i>ENO2</i>	46.94	5.67	437	196	16	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	AWRI1631_133840, gi 207342122 (24)
42 ^{DB}	<i>ENO2</i>	46.94	5.67	437	146	8	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	lcs2p, gi 30267748 (30)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
51 ^{IB}	<i>ENO2</i>	46.94	5.67	437	139	15	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	Chain A, Mata1MATA LPHA2-3a Heterodimer Bound To DNA, gi 21466066 (42)
162 ^{D_B}	<i>ENO2</i>	46.94	5.67	437	130	33	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	Hypothetical protein YDL124W, gi 6320079 (49)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
35 ^{DB}	<i>ENO2</i>	46.94	5.67	437	68	21	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	Tal1p, gi 6323386 (42)
120 ^D	<i>FBA1</i>	39.88	5.51	359	128	15	gi 6322790	NP_012863	Cytoplasm Mitochondrion	Fructose-1,6-bisphosphate aldolase, required for glycolysis and gluconeogenesis; catalyzes conversion of fructose-1,6-bisphosphate to glyceraldehyde-3-P and dihydroxyacetone-P; locates to mitochondrial outer surface upon oxidative stress.	Acp1p, gi 6322656 (30)
98 ^{DA}	<i>FPR1</i>	12.07	5.71	113	185	45	gi 157834293	1YAT_A (NP_014264)	Cytoplasm	Chain A, Improved Calcineurin Inhibition By Yeast Fkbp12-Drug Complexes	Kelch repeat-containing protein 1, gi 190405936 (35)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
157 ^I	<i>FUR1</i>	24.86	5.58	216	280	60	gi 37362660	NP_011996	Intracellular	Uracil phosphoribosyl transferase, synthesizes UMP from uracil; involved in the pyrimidine salvage pathway.	YNR045Wp-like protein, gi 207341541 (32)
77 ^{IB}	<i>GDH1</i>	49.89	5.55	453	129	22	gi 171594	AAA34642 (NP_015020)	Cytoplasm Nucleus	Glutamate dehydrogenase.	AWRI1631_41590, gi 207346975 (26)
76 ^{IB}	<i>GDH1</i>	49.89	5.55	453	68	22	gi 171594	AAA34642 (NP_015020)	Cytoplasm Nucleus	Glutamate dehydrogenase	(-)
221 ^B	<i>GLK1</i>	55.74	5.8	500	93	28	gi 6319809	NP_009890	Cytoplasm Plasma membrane	Glucokinase catalyzes the phosphorylation of glucose at C6 in the first irreversible step of glucose metabolism; one of three glucose phosphorylating enzymes; expression regulated by non-fermentable carbon sources.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
34 ^B	<i>GLN1</i>	42.13	5.92	370	83	18	gi 417061	P32288 (NP_015360.2)	Cytoplasm	Glutamine synthetase (GS), synthesizes glutamine from glutamate and ammonia; with Glt1p, forms the secondary pathway for glutamate biosynthesis from ammonia; expression regulated by nitrogen source and by amino acid limitation.	(-)
16 ^B	<i>GND1</i>	54.91	6.44	497	598	29	gi 157835612	2P4Q_A (NP_012053)	Cytoplasm	Chain A, Gnd1.	YPL083Cp-like protein, gi 207340534 (30)
24 ^{IB}	<i>GUA1</i>	58.73	6.05	525	128	16	gi 6323873	NP_013944	Cytoplasm	GMP synthase, an enzyme that catalyzes the second step in the biosynthesis of GMP from inosine 5'-phosphate (IMP); transcription is not subject to regulation by guanine but is negatively regulated by nutrient starvation.	Cdc19p, gi 6319279 (59)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
12 ^{IB}	<i>HOM2</i>	40.03	6.27	365	88	26	gi 6320362	NP_010442	Cytoplasm Nucleus Plasma membrane	Aspartic beta semi-aldehyde dehydrogenase catalyzes the second step in the common pathway for methionine and threonine biosynthesis; expression regulated by Gcn4p and the general control of amino acid synthesis.	Alcohol dehydrogenase isozyme I, gi 223142 (59)
119 ^{IB}	<i>HRI1</i>	27.54	5.1	244	163	26	gi 6323332	NP_013404	Cytoplasm Nucleus	Interacts with HRR25. May interact with SEC72.	Unnamed protein product, gi 4291 (42)
176 ^{D_B}	<i>HSC82</i>	80.82	4.76	705	591	22	gi 171723	AAA02813 (NP_013911)	Cytoplasm Mitochondrion	Hsc82 protein.	Bdh2p, gi 6319257 (33)
228 ^I	<i>HSP104</i>	102.4 6	5.62	908	94	16	gi 228154	1717391A (NP_013074)	Cytoplasm Nucleus	Protein Hsp104.	Pau9p, gi 13129151 (35)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
125 ^I	<i>HSP26</i>	23.86	5.31	214	58	31	gi 6319546	NP_009628	Cytoplasm Nucleus	Small heat shock protein (sHSP) with chaperone activity; forms hollow, sphere-shaped oligomers that suppress unfolded proteins aggregation; oligomer activation requires heat-induced conformational change; also has mRNA binding activity.	Mrpl38p, gi 6322678 (23)
168 ^A	<i>HSP60</i>	60.99	5.23	572	114	25	gi 6323288	NP_013360	Mitochondrion matrix	Tetradecameric mitochondrial chaperonin required for ATP-dependent folding of precursor polypeptides and complex assembly; prevents aggregation and mediates protein refolding after heat shock; role in mtDNA transmission; phosphorylated.	Conserved protein, gi 15194375 7 (24)
175 ^I	<i>HSP82</i>	77.92	5.06	677	344	15	gi 99031945	2CG9_A (NP_015084)	Cytoplasm	Chain A, Hsp90-Sba1 closed chaperone complex.	YNR045Wp-like protein, gi 20734154 1 (29)
174 ^I	<i>HSP82</i>	77.92	5.06	677	109	10	gi 99031945	2CG9_A (NP_015084)	Cytoplasm	Chain A, Hsp90-Sba1 closed chaperone complex.	(-)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
187 ^B	<i>HXK2</i>	54.13	5.16	486	226	35	gi 6321184	NP_011261	Cytoplasm Mitochondrion Nucleus	Hexokinase 2 catalyzes phosphorylation of glucose in the Cytoplasm; predominant hexokinase during growth on glucose; functions in the nucleus to repress expression of HXK1 and GLK1 and to induce expression of its own gene	Stress-seventy subfamily A protein, gi 151941146 (173)
82	<i>HXK2</i>	54.16	5.16	486	83	24	gi 3793	CAA27203	Cytoplasm Mitochondrion Nucleus	Hexokinase 2 catalyzes phosphorylation of glucose in the Cytoplasm; predominant hexokinase during growth on glucose; functions in the nucleus to repress expression of HXK1 and GLK1 and to induce expression of its own gene.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
178	KAR2	74.47	4.79	682	432	24	gi 6322426	NP_012500	ER lumen	ATPase involved in protein import into the ER, also acts as a chaperone to mediate protein folding in the ER and may play a role in ER export of soluble proteins; regulates the unfolded protein response via interaction with Ire1p.	(-)
222 ^{IB}	LYS21	49.02	5.88	440	61	17	gi 6320071	NP_010151	Mitochondrion	Homocitrate synthase isozyme, catalyzes the condensation of acetyl-CoA and alpha-ketoglutarate to form homocitrate, which is the first step in the lysine biosynthesis pathway; highly similar to the other isozyme, Lys20p.	(-)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
18 ^{IB}	OYE2	44.98	6.13	400	88	25	gi 6321973	NP_012049	Cytoplasm Mitochondrion Nucleus	Conserved NADPH oxidoreductase containing flavin mononucleotide (FMN), homologous to Oye3p with different ligand binding and catalytic properties; may be involved in sterol metabolism, oxidative stress response, and programmed cell death.	(-)
155 ^{IB}	PAA1	22.04	5.58	191	169	12	gi 6320276	NP_010356	Cytoplasm	Polyamine acetyltransferase; acetylates polyamines (e.g. putrescine, spermidine, spermine) and also aralkylamines (e.g. tryptamine, phenylethylamine); may be involved in transcription and/or DNA replication	Tif2p, gi 6322323 (39)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
217 ^D _B	<i>PDC1</i>	61.68	5.8	563	190	28	gi 6323073	NP_013145	Cytoplasm Nucleus	Major of three pyruvate decarboxylase isozymes, key enzyme in alcoholic fermentation, decarboxylates pyruvate to acetaldehyde; subject to glucose-, ethanol-, and autoregulation; involved in amino acid catabolism.	YGR087Cp-like protein, gi 207345151 (55)
218 ^D _B	<i>PDC1</i>	61.68	5.8	563	370	18	gi 7245976	1QPB_A (NP_013145)	Cytoplasm Nucleus	Chain A, Pyruvate decarboxylase (Form B) complexed with pyruvamide.	(-)
215 ^B	<i>PDC1</i>	61.68	5.8	563	135	11	gi 7245976	1QPB_A (NP_013145)	Cytoplasm Nucleus	Chain A, Pyruvate decarboxylase complexed with pyruvamide.	(-)
219 ^D _B	<i>PDC1</i>	61.68	5.8	563	117	15	gi 7245976	1QPB_A (NP_013145)	Cytoplasm Nucleus	Chain A, Pyruvate decarboxylase complexed with pyruvamide.	Unnamed protein product, gi 4114 (42)
27 ^{DB}	<i>PDC1</i>	61.68	5.8	563	110	15	gi 7245976	1QPB_A (NP_013145)	Cytoplasm Nucleus	Chain A, Pyruvate decarboxylase complexed with pyruvamide.	(-)
216 ^D _B	<i>PDC1</i>	61.68	5.8	563	80	8	gi 7245976	1QPB_A (NP_013145)	Cytoplasm Nucleus	Chain A, Pyruvate decarboxylase complexed with pyruvamide.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
158 ^{IA}	<i>PDI1</i>	56.67	4.38	504	172	26	gi 88192228	2B5E_A (NP_009887)	ER lumen	Chain A, Disulfide isomerase.	SCY_2605, gi 151943234 (24)
159 ^{IA}	<i>PDI1</i>	56.67	4.38	504	149	21	gi 88192228	2B5E_A (NP_009887)	ER lumen	Chain A, Disulfide Isomerase.	Tex1p, gi 6324076 (33)
200 ^{IB}	<i>PFK2</i>	105.1 ₃	6.09	959	71	7	gi 172140	AAA34860 (NP_013932)	Cytoplasm	Phosphofructokinase, a key regulatory enzyme in glycolysis, catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-biphosphate.	Ups3p, gi 6320391 (31)
13 ^{IB}	<i>PGK1</i>	44.6	6.73	415	167	16	gi 157833610	1QPG_A (NP_009938)	Cytoplasm	Chain A, 3-Phosphoglycerate kinase, mutation R65q.	(-)
223 ^{IB}	<i>PMI40</i>	48.44	5.66	429	235	14	gi 6320839	NP_010918	Cytoplasm	Mannose-6-phosphate isomerase, catalyzes the interconversion of fructose-6-P and mannose-6-P; required for early steps in protein mannosylation	Enolase II, gi 6321968 (82)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
164 ^D _B	<i>PRB1</i>	69.8	5.94	635	75	5	gi 6320775	NP_010854	Vacuole	Vacuolar proteinase B (yscB), a serine protease of the subtilisin family; involved in protein degradation in the vacuole and required for full protein degradation during sporulation; activity inhibited by Pbi2p.	YGL002W, gi 51013601 (29)
30 ^{DB}	<i>PSA1</i>	39.71	5.95	361	254	29	gi 6320148	NP_010228	Cytoplasm	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanylttransferase), synthesizes GDP-mannose from GTP and mannose-1-phosphate in cell wall biosynthesis; required for normal cell wall structure.	Chain B, Cytochrome Bc1 Complex (130)
29 ^{DB}	<i>PSA1</i>	39.71	5.95	361	172	19	gi 6320148	NP_010228	Cytoplasm	GDP-mannose pyrophosphorylase (mannose-1-phosphate guanylttransferase), synthesizes GDP-mannose from GTP and mannose-1-phosphate in cell wall biosynthesis; required for normal cell wall structure	Unknown protein, gi 808836 (36)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
153 ^{IB}	<i>PST2</i>	21.01	5.46	198	96	24	gi 6320235	NP_010315	Secreted	Protein with similarity to members of a family of flavodoxin-like proteins; induced by oxidative stress in a Yap1p dependent manner; the authentic, non-tagged protein is detected in highly purified mitochondria in high-throughput studies.	Tsa1p, gi 6323613 (49)
161 ^I	<i>RNA1</i>	46.07	4.53	407	107	19	gi 6323891	NP_013962	Cytoplasm	GTPase activating protein (GAP) for Gsp1p, involved in nuclear transport.	Ubiquitin carboxyl-terminal hydrolase 7, gi 190406367 (41)
139 ^{IA}	<i>RPS0A</i>	20.37	9.66	185	100	27	gi 49258822	1S1H_B (NP_011730)	Cytoplasm	Chain B, Ribosomal 80s-Eef2-Sordarin complex obtained by docking atomic models for RNA and protein components into a 11.7 Å Cryo-Em Map.	Ics2p, gi 30267748 (19)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
104 ^D _A	<i>RPS12</i>	15.46	4.68	143	103	24	gi 6324945	NP_015014	Cytoplasm	Protein component of the small (40S) ribosomal subunit; has similarity to rat ribosomal protein S12.	Ptc1p, gi 642800 (34)
105 ^D _A	<i>RPS12</i>	15.46	4.68	143	94	12	gi 6324945	NP_015014	Cytoplasm	Protein component of the small (40S) ribosomal subunit; has similarity to rat ribosomal protein S12.	Predicted protein, gi 190405789 (36)
97 ^{DA}	<i>RPS21A</i>	9.79	5.76	87	88	74	gi 6322910	NP_012983	Cytoplasm	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps21Bp and has similarity to rat S21 ribosomal protein.	Eno2p, gi 6321968 (70)
7 ^{IA}	<i>RPS5</i>	25.08	8.63	225	126	28	gi 6322583	NP_012657	Cytoplasm	Protein component of the small (40S) ribosomal subunit, the least basic of the non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; has similarity to E. coli S7 and rat S5 ribosomal proteins.	Fmp25p, gi 6323106 (35)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
95 ^I	<i>RPT5</i>	48.28	4.93	434	207	27	gi 6324691	NP_014760	Cytoplasm Nucleus	One of six ATPases of the 19S regulatory particle of the 26S proteasome involved in the degradation of ubiquitinated substrates; recruited to the GAL1-10 promoter region upon induction of transcription; similar to human TBP1	YOR117Wp-like protein, gi 207341153 (198)
36 ^B	<i>SAH1</i>	49.72	5.83	449	274	30	gi 6320882	NP_010961	Cytoplasm	S-adenosyl-L-homocysteine hydrolase, catabolizes S-adenosyl-L-homocysteine, which is formed after donation of the activated methyl group of S-adenosyl-L-methionine (AdoMet) to an acceptor.	Silent mating loci repressor, gi 151941641 (30)
86 ^I	<i>SAM1</i>	42.07	5.04	382	188	16	gi 6323209	NP_013281	Cytoplasm	S-adenosylmethionine synthetase, catalyzes transfer of the adenosyl group of ATP to the sulfur atom of methionine; one of two differentially regulated isozymes (Sam1p and Sam2p).	(-)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
118 ^A	<i>SEC53</i>	29.21	5.14	254	228	51	gi 14318474	NP_116609	Cytoplasm	Phosphomannomutase involved in synthesis of GDP-mannose and dolichol-phosphate-mannose; required for folding and glycosylation of secretory proteins in the ER lumen.	SCY_2344, gi 151944159 (39)
43 ^{IB}	<i>SOD1</i>	15.85	5.48	153	264	60	gi 6730103	1B4T_A (NP_012638)	Cytoplasm Mitochondrion intermembrane space	Chain A, H48c Cu(II)Zn superoxide dismutase.	Unnamed protein product (40)
65	<i>SPE3</i>	33.53	5.33	293	62	15	gi 6325326	NP_015394	Cytoplasm Nucleus	Spermidine synthase, involved in biosynthesis of spermidine and also in biosynthesis of pantothenic acid; spermidine is required for growth of wild-type cells.	Tetrameric tRNA splicing endonuclease 54 kDa subunit, gi 190407872 (36)
93	<i>SRP1</i>	59.46	4.8	530	126	14	gi 58177142	1WA5_B (NP_014210)	Cytoplasm perinuclear region	Chain B, Exportin Cse1p complexed with its cargo (Kap60p) and Rangtp.	(-)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
180	SSA1	69.78	5	642	1130	36	gi 144228166	NP_009396	Cytoplasm	ATPase involved in protein folding and nuclear localization signal (NLS)-directed nuclear transport; member of heat shock protein 70 (HSP70) family; forms a chaperone complex with Ydj1p.	(-)
188 ^{IB}	SSA1	69.78	5	642	60	15	gi 144228166	NP_009396	Cytoplasm Cell wall	ATPase involved in protein folding and nuclear localization signal (NLS)-directed nuclear transport; member of heat shock protein 70 (HSP70) family; forms a chaperone complex with Ydj1p.	(-)
179	SSA2	69.59	4.95	639	1100	35	gi 6323004	NP_013076	Cytoplasm	ATP binding protein involved in protein folding and vacuolar import of proteins; member of heat shock protein 70 (HSP70) family; associated with the chaperonin-containing T-complex.	Unnamed protein product. gi 3788 (32)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
116 ^D _A	SSA2	69.59	4.95	639	182	6	gi 6323004	NP_013076	Cytoplasm Cell wall	ATP binding protein involved in protein folding and vacuolar import of proteins; member of heat shock protein 70 (HSP70) family; associated with the chaperonin-containing T-complex.	Pau9p, gi 13129151 (35)
209	SSB1	66.73	5.32	613	173	30	gi 6319972	NP_010052	Cytoplasm	ATPase that is a ribosome-associated molecular chaperone, functions with J-protein partner Zuo1p; may be involved in folding of newly-made polypeptide chains; member of the HSP70 family; interacts with phosphatase subunit Reg1p	(-)
210	SSB2	66.66	5.37	613	775	39	gi 6324120	NP_014190	Cytoplasm	ATPase that is a ribosome-associated molecular chaperone, functions with J-protein partner Zuo1p; may be involved in the folding of newly-synthesized polypeptide chains; member of the HSP70 family; homolog of SSB1.	(-)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
211 ^D _B	SSB2	66.66	5.37	613	275	15	gi 6324120	NP_014190	Cytoplasm	ATPase that is a ribosome-associated molecular chaperone, functions with J-protein partner Zuo1p; may be involved in the folding of newly-synthesized polypeptide chains; member of the HSP70 family; homolog of SSB1.	(-)
189 ^A	SSC1	70.58	5.48	654	132	16	gi 6322505	NP_012579	Mitochondrion Nucleus	Hsp70 family ATPase, constituent of the import motor component of the Translocase of the Inner Mitochondrial membrane (TIM23 complex); involved in protein translocation and folding; subunit of Scel endonuclease.	AWRI1631_41900, gi 207346934 (33)
191 ^B	SSE1	77.6	5.12	693	835	36	gi 6325151	NP_015219	Cytoplasm	ATPase that is a component of the heat shock protein Hsp90 chaperone complex; binds unfolded proteins; member of the heat shock protein 70 (HSP70) family.	Pleiotropic drug resistance protein, gi 151944315 (32)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
96 ^{IA}	<i>SUP45</i>	48.55	5.01	432	89	7	gi 468605	CAA51935	Cytoplasm	Directs the termination of nascent peptide synthesis (translation) in response to the termination codons UAA, UAG and UGA	YOR117Wp-like protein, gi 207341153 (29)
5 ^{DB}	<i>TDH3</i>	35.83	6.46	332	59	7	gi 6321631	NP_011708	Cytoplasm	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3bis-phosphoglycerate.	(-)
181 ^D	<i>TFP1</i>	68.02	5.17	617	536	33	gi 3417405	CAA98761	Golgi Vacuolar membranes	V/A-type ATP synthase catalytic subunit A. These ATPases couple ATP hydrolysis to the buildup of a H ⁺ gradient, but V-type ATPases do not catalyze the reverse reaction.	YOL038C-A, gi 33438876 (32)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
147	<i>TIF2</i>	44.84	5.02	395	139	12	gi 6322323	NP_012397	Cytoplasm	Translation initiation factor eIF4A, identical to Tif1p; DEA (D/H)-box RNA helicase that couples ATPase activity to RNA binding and unwinding; forms a dumbbell structure of two compact domains connected by a linker; interacts with eIF4G.	YML070Wp-like protein, gi 207342537 (21)
131 ^l	<i>TIF6</i>	26.66	4.54	245	204	31	gi 6325273	NP_015341	Cytoplasm Nucleus > nucleolus	Constituent of 66S pre-ribosomal particles, has similarity to human translation initiation factor 6 (eIF6); may be involved in the biogenesis and or stability of 60S ribosomal subunits	YCR051W, gi 6319899 (56)
21 ^l	<i>TKL1</i>	73.8	6.48	680	78	8	gi 3212468	1AY0_A (NP_015399)	Cytoplasm	Chain A, Identification of catalytically important residues in transketolase.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
106 ^{IA}	<i>TMA19</i>	18.78	4.41	167	332	32	gi 6322794	NP_012867	Cytoplasm Mitochondrion	Protein that associates with ribosomes; homolog of translationally controlled tumor protein; green fluorescent protein (GFP)-fusion protein localizes to the cytoplasm and relocates to the mitochondrial outer surface upon oxidative stress.	Conserved protein, gi 151941105 (27)
45 ^{IB}	<i>TPI1</i>	26.76	5.75	247	181	21	gi 230405	1YPI_A (NP_010335)	Mitochondrion	Chain A, Triosephosphate isomerase	N1311, gi 600049 (32)
129	<i>TPM1</i>	23.52	4.58	199	197	53	gi 6324250	NP_014320	Cytoplasm > cytoskeleton	Major isoform of tropomyosin; binds to and stabilizes actin cables and filaments, which direct polarized cell growth and the distribution of several organelles; acetylated by the NatB complex and acetylated form binds actin most efficiently	YOL024W, gi 6324549 (21)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
115 ^l	<i>TSA1</i>	21.69	5.03	196	239	29	gi 6323613	NP_013684	Cytoplasm	Thioredoxin peroxidase, acts as both a ribosome-associated and free cytoplasmic antioxidant; self-associates to form a high-molecular weight chaperone complex under oxidative stress; deletion results in mutator phenotype	YML028Wp-like protein (144)
88 ^l	<i>TUB1</i>	50.39	4.96	447	82	9	gi 6323554	NP_013625	Cytoplasm > cytoskeleton	Alpha-tubulin; associates with beta-tubulin (Tub2p) to form tubulin dimer, which polymerizes to form microtubules.	Q0130, gi 2133224 (26)
94	<i>TUB2</i>	51.23	4.64	457	319	23	gi 14318481	NP_116616	Cytoplasm > cytoskeleton	Beta-tubulin; associates with alpha-tubulin (Tub1p and Tub3p) to form tubulin dimer, which polymerizes to form microtubules.	YKR101Wp-like protein, gi 20734331 1 (33)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
154 ^D _B	VMA2	57.77	4.95	517	64	10	gi 6319603	NP_009685	Vacuole membrane	Subunit B of the eight-subunit V1 peripheral membrane domain of the vacuolar H ⁺ -ATPase (V-ATPase), an electrogenic proton pump found throughout the endomembrane system; contains nucleotide binding sites.	Rib3p, gi 6320695 (52)
89	VMA2	57.77	4.95	517	57	11	gi 6319603	NP_009685	Vacuole membrane	Subunit B of the eight-subunit V1 peripheral membrane domain of the vacuolar H ⁺ -ATPase (V-ATPase), an electrogenic proton pump found throughout the endomembrane system; contains nucleotide binding sites.	Unnamed protein product, gi 395245 (20)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
124 ^l	VMA4	26.45	5.33	233	253	48	gi 6324907	NP_014977	Vacuole membrane	Subunit E of the eight-subunit V1 peripheral membrane domain of the vacuolar H ⁺ -ATPase (V-ATPase), an electrogenic proton pump found throughout the endomembrane system; required for the V1 domain to assemble onto the vacuolar membrane	YOR332Wp-like protein (133)
135	VMA6	40.1	4.48	345	81	12	gi 6323480	NP_013552	Vacuole membrane	Subunit d of the five-subunit V0 integral membrane domain of vacuolar H ⁺ -ATPase (V-ATPase), an electrogenic proton pump found in the endomembrane system; stabilizes VO subunits; required for V1 domain assembly on the vacuolar membrane.	Erp6p, gi 6321436 (24)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
101 ^D _A	<i>VMA7</i>	13.45	4.98	118	96	30	gi 6321457	NP_011534	Vacuole membrane	Subunit F of the eight-subunit V1 peripheral membrane domain of vacuolar H ⁺ -ATPase (V-ATPase), an electrogenic proton pump found throughout the endomembrane system; required for the V1 domain to assemble onto the vacuolar membrane.	Tex1p, gi 6324076 (32)
186 ^{IB}	<i>WTM1</i>	48.46	5.18	437	129	10	gi 6324804	NP_014873	Cytoplasm Nucleus	Transcriptional modulator involved in regulation of meiosis, silencing, and expression of RNR genes; required for nuclear localization of the ribonucleotide reductase small subunit Rnr2p and Rnr4p; contains WD repeats.	12 kDa heat shock protein, gi 18479209 (30)
20 ^B	<i>YDR341</i> _C	69.64	6.29	607	84	15	gi 190404719	EDV07986 (NP_010628)	Cytoplasm	Arginyl-tRNA synthetase.	Acp1p, gi 6322656 (27)

Spot No	Gene name	Mass [kDa]	p/ [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No.	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI No. (score)
193 ^B	<i>YHR020 W</i>	77.85	6.02	688	131	13	gi 6321808	NP_011884	Cytoplasm	Hypothetical protein that may interact with ribosomes, based on co-purification experiments; has similarity to proline-tRNA ligase; YHR020W is an essential gene	Mas2p, gi 6321813 (27)
99 ^I	<i>ZEO1</i>	12.58	5.43	113	443	60	gi 6324463	NP_014532	Plasma membrane	Interacts with Mid2p; regulates the cell integrity pathway mediated by Pkc1p and Slr2p; the authentic protein is detected in a phosphorylated state in highly purified mitochondria.	Cct8p, gi 6322452 (24)

3.3.2. 2D analysis of pI 6-11 proteins

The image of proteins separated by 2DE using pH 6-11 IEF is shown in Figure 14.

The Coomassie stained gel showed 152 visible proteins. The total number of proteins identified by PMF was 70 at the 5% level of significance. Table 6 shows pH 6-11 proteins identified by MALDI MS/MS. Thirty-three identifications corresponded to unique protein matches. The other 37 proteins represented multiple spots of proteins or protein isoforms. Proteins with multiple spots included Cdc19, Gpm1, Rps5, Tdh3 and Tef2. Identified isoforms included Atp1, Atp3, Cdc12, Cdc19, Imd2, Imd4, Rpl11A, Rpl12B, Rpl13B, Rpl13A, Rps15, Rps19B, Rps20, Rps22A, Rps27A, Rps3, Rps4B, Rps5, Rps7A, Shm1, Shm2, Tdh1, Tdh3, Tef2 and Tef4. The next protein match found 30.2% of the proteins that were next in line for a match were significant at the 5% level (score value ≥ 56). Six glycolytic proteins (Cdc19, Eno2, Gpm1, Pfk1, Tdh1 and Tdh3) were found. The abbreviation for pyruvate kinase is Cdc19.

Figure 14 - The 2DE image representing the protein (100 μ g) profile of BY4741 separated on a pH 6-11 gradient. Proteins were stained with Coomassie. Protein identification was done by MALDI mass spectrometry and significantly identified proteins are marked with an arrow (Table 6). The ladder used for molecular weight reference was Rainbow 851.

Table 6 - A summary of identified BY4741 proteins separated on a 6-11 pH gradient. Proteins were separated by 2DE and identified by MALDI mass spectrometry. Proteins (score ≥ 56 , $p \leq 0.05$) were alphabetized by gene name. The score and sequence coverage (%) values are representative of the significance of the match generated by the MASCOT search. The calculated mass, pI, GI, accession number and description of the protein are gathered from the NCBI protein website. The subcellular localization of the protein was found using Uniprot. The next matched protein was the next non-homologous protein matched. The spot position was cross checked with the calculated pI and Mr values. Alterations in protein positioning resulted in the labeling of A: acidic shift or B: basic shift in pI; I: increase or D: decrease in Mr.

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
109 ^{DA}	AAT2	47.99	8.89	432	140	17	gi 1360338	CAA97550	Cytoplasm Peroxisome	Aspartate aminotransferase family. This family belongs to pyridoxal phosphate (PLP)-dependent aspartate aminotransferase superfamily (fold I). Pyridoxal phosphate combines with an alpha-amino acid to form a compound called a Schiff base or aldimine.	Q0130, gi 2133224 (26)
138 ^I	ARC1	42.44	7.7	375	410	23	gi 1002712	AAC49072 (NP_011410)	Cytoplasm	G4p1 (GU4 nucleic binding-protein 1).	High-copy hsp90 suppress or, gi 151944268 (38)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
122	<i>ATP1</i>	54.96	6.73	510	499	33	gi 119389904	2HLD_A (NP_009453)	Mitochondrion inner membrane	Chain A, Mitochondrial F1-Atpase.	Imd3p, gi 6323464 (495)
81 ^A	<i>ATP3</i>	30.65	9.06	278	88	23	gi 119389910	2HLD_G (NP_009595)	Mitochondrion inner membrane	Chain G, Mitochondrial F1-Atpase.	YBR039 Wp-like protein, gi 207347765 (49)
108 ^{DA}	<i>BAT1</i>	43.79	9.01	393	77	23	gi 6322002	NP_012078	Mitochondrion matrix	Mitochondrial branched-chain amino acid aminotransferase, homolog of murine ECA39; highly expressed during logarithmic phase and repressed during stationary phase.	(-)
111 ^{IA}	<i>CAM1</i>	24.69	9.14	219	102	23	gi 28374020	1NHY_A (NP_015277)	Cytoplasm Nucleus	Chain A, GST-like domain of elongation factor 1-gamma.	Spc42p, gi 6322809 (29)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
137 ^I	<i>CDC12</i>	46.75	7.77	407	121	27	gi 6321899	NP_011975	Membrane Peripheral membrane bud neck	Component of the septin ring of the mother-bud neck that is required for cytokinesis; septins recruit proteins to the neck and can act as a barrier to diffusion at the membrane, and they comprise the 10nm filaments seen with EM.	Silent mating loci repressor, gi 151941641 (32)
140 ^A	<i>CDC19</i>	54.9	7.56	500	442	34	gi 6319279	NP_009362	Cytoplasm	Pyruvate kinase, converts phosphoenolpyruvate to pyruvate in glycolysis, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration.	Threonine deaminase, gi 190405649 (39)
119 ^{IA}	<i>CDC19</i>	54.9	7.56	500	362	31	gi 6319279	NP_009362	Cytoplasm	Pyruvate kinase, functions as a homotetramer in glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration.	YBR069C p-like protein, gi 207347739 (34)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
120 ^A	<i>CDC19</i>	54.9	7.56	500	310	24	gi 6319279	NP_009362	Cytoplasm	Pyruvate kinase, functions as a homotetramer in glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration.	ATPase 1 alpha subunit, gi 171116 (34)
130 ^{DA}	<i>CDC19</i>	54.96	8	499	259	21	gi 4180	CAA24631	Cytoplasm	Pyruvate kinase, functions as a homotetramer in glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration.	Branched-chain amino acid transaminase, gi 151945213 (176)
72 ^D	<i>CDC19</i>	54.96	8	499	156	14	gi 4180	CAA24631	Cytoplasm	Pyruvate kinase, functions as a homotetramer in glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration.	Rps5p, gi 6322583 (68)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
46 ^{DA}	<i>CDC19</i>	54.96	8	499	62	13	gi 4180	CAA24631	Cytoplasm	Pyruvate kinase, functions as a homotetramer in glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration.	Lsp1p, gi 6325253 (20)
114	<i>DBP2</i>	61.23	8.8	546	90	17	gi 160380611	A6ZRX0 (NP_014287.3)	Cytoplasm Nucleus	ATP-dependent RNA helicase involved nonsense-mediated mRNA decay and ribosome biogenesis through rRNA processing.	Cbr1p, gi 86558908 (26)
97 ^A	<i>DED1</i>	65.73	8.18	604	129	25	gi 227524	1705300A (NP_014847)	Cytoplasm	ATP dependent RNA helicase.	Chain A, Scpex13p Sh3 domain, gi 28948968 (25)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
143 ^{DA}	<i>EHT1</i>	51.5	7.64	451	174	23	gi 6319655	NP_009736	Lipid Particle Mitochondrion outer membrane	Acyl-coenzymeA: ethanol O-acyltransferase that plays a minor role in medium-chain fatty acid ethyl ester biosynthesis; possesses short-chain esterase activity; localizes to lipid particles and the mitochondrial outer membrane.	Shm2p, gi 6323087 (132)
8 ^{DB}	<i>ENO2</i>	46.94	5.67	437	295	33	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	Chain A, Mata1MA TALPHA2-3a heterodimer bound to DNA complex, gi 21466066 (26)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
146 ^A	<i>ERG10</i>	42.04	7.01	398	111	10	gi 6325229	NP_015297	Cytoplasm	Acetyl-CoA C-acetyltransferase (acetoacetyl-CoA thiolase), Cytoplasmic enzyme that transfers an acetyl group from one acetyl-CoA molecule to another, forming acetoacetyl-CoA; involved in the first step in mevalonate biosynthesis.	Chain C, Ribosomal 80s-Eef2-Sordarin complex, gi 49258841 (49)
76 ^{IA}	<i>GPM1</i>	26.68	8.27	237	269	48	gi 157884466	5PGM_E (NP_012770)	Cytoplasm Mitochondrion	Chain E, Phosphoglycerate mutase.	Fab1p, gi 14318541 (29)
70 ^I	<i>GPM1</i>	26.68	8.27	237	247	47	gi 157884466	5PGM_E (NP_012770)	Cytoplasm Mitochondrion	Chain E, Phosphoglycerate mutase.	Pau9p, gi 13129151 (36)
65 ^{DB}	<i>GPM1</i>	26.68	8.27	237	122	35	gi 157884466	5PGM_E (NP_012770)	Cytoplasm Mitochondrion	Chain E, Phosphoglycerate mutase.	Chain B, Sec23-Sar1 complexed with the active fragment of Sec31, gi 165761045 (30)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
71 ^I	<i>GPM1</i>	26.68	8.27	237	64	32	gi 157884466	5PGM_E (NP_012770)	Cytoplasm Mitochondrion	Chain E, Phosphoglycerate mutase.	(-)
94	<i>GUS1</i>	81.19	7.21	708	103	18	gi 37362649	NP_011269	Cytoplasm	Glutamyl-tRNA synthetase (GluRS), forms a complex with methionyl-tRNA synthetase (Mes1p) and Arc1p; complex formation increases the catalytic efficiency of both tRNA synthetases and ensures their correct localization to the cytoplasm.	Ptc1p, gi 642800 (33)
1 ^{DA}	<i>HSP10</i>	11.36	8.96	106	143	58	gi 6324594	NP_014663	Mitochondrion matrix	Mitochondrial matrix co-chaperonin that inhibits the ATPase activity of Hsp60p, a mitochondrial chaperonin; involved in protein folding and sorting in the mitochondria; 10 kD heat shock protein with similarity to <i>E. coli</i> groES.	Rps10ap, gi 6324867 (69)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
24 ^I	<i>HTB2</i>	14.09	10.07	130	71	36	gi 15988135	1ID3_D (NP_009553)	Chromosome Nucleus	Chain D, Nucleosome core particle reveals fundamental differences in inter-nucleosome interactions.	Gly1p, gi 585212 (42)
134 ^{DA}	<i>IDH1</i>	38.54	8.07	354	143	26	gi 167013430	3BLV_A (NP_014361)	Mitochondrion	Chain A, Isocitrate dehydrogenase with citrate bound in the regulatory subunits.	Conserve d protein, gi 151944262 (26)
112 ^A	<i>IDP1</i>	48.21	8.42	427	90	23	gi 193506511	2QFV_A (NP_010217)	Mitochondrion	Chain A, Mitochondrial NADP(+)-dependent isocitrate dehydrogenase in complex with NADP(+).	(-)
118 ^A	<i>ILV3</i>	63.39	7.92	585	192	19	gi 6322476	NP_012550	Mitochondrion	Dihydroxyacid dehydratase, catalyzes third step in the common pathway leading to biosynthesis of branched-chain amino acids.	YAL039C p-like protein, gi 207348032 (29)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
115 ^{DA}	<i>IMD2</i>	56.95	8.71	523	434	26	gi 6322012	NP_012088	Cytoplasm	Inosine monophosphate dehydrogenase catalyzes the rate-limiting step in GTP biosynthesis, expression is induced by mycophenolic acid resulting in resistance to the drug, and expression is repressed by nutrient limitation.	(-)
117 ^A	<i>IMD4</i>	56.87	8.39	524	313	25	gi 6323585	NP_013656	Cytoplasm	Inosine monophosphate dehydrogenase catalyzes the first step of GMP biosynthesis, member of a four-gene family in <i>S. cerevisiae</i> , constitutively expressed.	Ups3p, gi 6320391 (39)
139	<i>OLA1</i>	44.48	7.05	394	234	40	gi 6319499	NP_009581	Cytoplasm	P-loop ATPase with similarity to human OLA1 and bacterial YchF; identified as specifically interacting with the proteasome; protein levels are induced by hydrogen peroxide.	Ptc7p, gi 124263204 (28)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
151 ^{DA}	<i>PGK1</i>	44.76	7.11	416	274	45	gi 10383781	NP_009938	Cytoplasm	3-phosphoglycerate kinase, catalyzes transfer of high-energy phosphoryl groups from the acyl phosphate of 1,3-bisphosphoglycerate to ADP to produce ATP; key enzyme in glycolysis and gluconeogenesis.	Spindle pole component, gi 151942674 (33)
124I	<i>PGK1</i>	44.59	7.1	415	163	28	gi 157836867	3PGK_A (NP_009938)	Cytoplasm	Chain A, Phosphoglycerate kinase.	(-)
145 ^{DA}	<i>PGK1</i>	44.59	7.1	415	158	23	gi 157836867	3PGK_A (NP_009938)	Cytoplasm	Chain A, Phosphoglycerate kinase.	SCY_4031, gi 151940675 (26)
64	<i>PGK1</i>	44.6	6.73	415	154	16	gi 157833610	1QPG_A (NP_009938)	Cytoplasm	Chain A, 3-Phosphoglycerate kinase, mutation R65q.	Rps7ap, gi 6324670 (109)
147 ^{IA}	<i>PGK1</i>	44.6	6.73	415	115	18	gi 157833610	1QPG_A (NP_009938)	Cytoplasm	Chain A, 3-Phosphoglycerate kinase, mutation R65q.	Cit1p, gi 6324328 (102)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
35	<i>PHB2</i>	34.44	9.92	310	56	18	gi 50593217	NP_011747	Mitochondrion inner membrane	Subunit of the prohibitin complex (Phb1p-Phb2p), a 1.2 MDa ring-shaped inner mitochondrial membrane chaperone that stabilizes newly synthesized proteins; determinant of replicative life span; involved in mitochondrial segregation.	None
25 ^l	<i>RPL11A</i>	18.79	9.91	165	392	32	gi 119390530	2NOQ_H (NP_015427)	Cytoplasm	Protein of the large 60S ribosomal subunit, nearly identical to Rpl11Bp but expressed at twice the level; involved in ribosomal assembly; depletion causes degradation of 60S proteins and RNA; similar to E. coli L5 and rat L11.	FUN20, gi 5241 (47)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
28 ^I	<i>RPL12B</i>	17.86	9.43	165	99	27	gi 6320625	NP_010706	Cytoplasm	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl12Ap; rpl12a rpl12b double mutant exhibits slow growth and slow translation; has similarity to E. coli L11 and rat L12 ribosomal proteins.	(-)
75 ^{IA}	<i>RPL13A</i>	22.4	11.18	198	122	20	gi 190405088	EDV08355 (NP_010201)	Cytoplasm	Ribosomal protein L13A.	Tdh3p, gi 6321631 (62)
56 ^{IA}	<i>RPL13B</i>	22.51	11.08	199	82	35	gi 6323791	NP_013862	Cytoplasm	Protein component of the large (60S) ribosomal subunit, nearly identical to Rpl13Ap; not essential for viability; has similarity to rat L13 ribosomal protein	Pau9p, gi 13129151 (36)
18 ^{IA}	<i>RPS15</i>	8.88	9.9	80	70	16	gi 49258836	1S1H_S (NP_014602)	Cytoplasm	Chain S, Ribosomal 80s-Eef2-sordarin complex obtained by docking atomic models for RNA and protein components Into a 11.7 Å cryo-em map.	Rps15p, gi 6324533 (68)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
16	<i>RPS19 B</i>	15.88	9.61	144	205	61	gi 6324027	NP_014097	Cytoplasm	Protein component of the small (40S) ribosomal subunit, required for assembly and maturation of pre-40 S particles; mutations in human RPS19 are associated with Diamond Blackfan anemia; nearly identical to Rps19Ap.	Eno2p, gi 6321968 (123)
20 ^{IA}	<i>RPS20</i>	13.89	9.52	121	249	54	gi 6321772	NP_011848	Cytoplasm	Protein component of the small (40S) ribosomal subunit; overproduction suppresses mutations affecting RNA polymerase III-dependent transcription; has similarity to E. coli S10 and rat S20 ribosomal proteins.	AWRI1631_22090, gi 207347659 (33)
21 ^{IA}	<i>RPS22 A</i>	14.53	9.94	129	153	29	gi 49258827	1S1H_H (NP_012345)	Cytoplasm	Chain H, Ribosomal 80s-Eef2-Sordarin Complex obtained by docking atomic models for RNA and protein components into a 11.7 Å Cryo-Em Map.	Faf1p, gi 6322170 (33)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
9 ^{DB}	<i>RPS27A</i>	9.02	9.37	81	104	38	gi 2131116	CAA81997	Cytoplasm	40 S ribosomal protein S27-A.	YLR146W-A, gi 82795259 (26)
41 ^{IA}	<i>RPS3</i>	26.54	9.42	240	83	54	gi 6324151	NP_014221	Cytoplasm	Protein component of the small (40S) ribosomal subunit has apurinic/apyrimidinic (AP) endonuclease activity; essential for viability; has similarity to E.coli S3 and rat S3 ribosomal proteins.	Ics2p, gi 30267748 (30)
39 ^D	<i>RPS4B</i>	29.44	10.09	261	144	33	gi 6321997	NP_012073	Cytoplasm	Protein component of the small (40S) ribosomal subunit; identical to Rps4Ap and has similarity to rat S4 ribosomal protein.	Tex1p, gi 6324076 (37)
53 ^{IA}	<i>RPS5</i>	25.08	8.63	225	217	35	gi 6322583	NP_012657	Cytoplasm	Protein component of the small (40S) ribosomal subunit, the least basic of the non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; has similarity to E. coli S7 and rat S5 ribosomal proteins.	Rpl13bp, gi 6323791 (28)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
51 ^{IA}	<i>RPS5</i>	25.08	8.63	225	56	23	gi 6322583	NP_012657	Cytoplasm	Protein component of the small (40S) ribosomal subunit, the least basic of the non-acidic ribosomal proteins; phosphorylated in vivo; essential for viability; has similarity to <i>E. coli</i> S7 and rat S5 ribosomal proteins.	None
63 ^{IA}	<i>RPS7A</i>	21.6	9.83	190	161	22	gi 6324670	NP_014739	Cytoplasm Nucleus > nucleolus	Protein component of the small (40S) ribosomal subunit, nearly identical to Rps7Bp; interacts with Kti11p; deletion causes hypersensitivity to zymocin; has similarity to rat S7 and <i>Xenopus</i> S8 ribosomal proteins.	Rpl16bp, gi 6324260 (54)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
86 ^A	<i>RPT6</i>	45.47	9.09	405	57	22	gi 6321390	NP_011467	Cytoplasm Nucleus	One of six ATPases of the 19S regulatory particle of the 26S proteasome involved in the degradation of ubiquitinated substrates; bound by ubiquitin-protein ligases Ubr1p and Ufd4p; localized mainly to the nucleus throughout the cell cycle.	SUG1 gene, gi 228885 (52)
136 ^A	<i>SHM1</i>	53.88	8.83	490	79	13	gi 37362622	NP_009822	Mitochondrion	Mitochondrial serine hydroxymethyl transferase, converts serine to glycine plus 5,10methylenetetrahydrofolate; involved in generating precursors for purine, pyrimidine, amino acid, and lipid biosynthesis; reverse reaction generates serine.	Chain A, Pi-Scei, gi 3114410 (50)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
142 ^D	<i>SHM2</i>	52.47	6.98	469	248	34	gi 6323087	NP_013159	Cytoplasm	Cytoplasmic serine hydroxymethyl transferase, converts serine to glycine plus 5,10methylenetetrahydrofolate; major isoform involved in generating precursors for purine, pyrimidine, amino acid, and lipid biosynthesis.	YKL081W p-like protein, gi 207343526 (83)
87	<i>SIS1</i>	37.56	9.02	352	272	37	gi 6324321	NP_014391	Cytoplasm Nucleus	Type II HSP40 co-chaperone that interacts with the HSP70 protein Ssa1p; not functionally redundant with Ydj1p due to due to substrate specificity; shares similarity with bacterial DNAJ proteins.	Eis1p, gi 6323674 (33)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
80 ^{IA}	<i>STM1</i>	29.97	9.66	273	189	26	gi 6323179	NP_013251	Cytoplasm Nucleus	Protein required for optimal translation under nutrient stress; perturbs association of Yef3p with ribosomes; involved in TOR signaling; binds G4 quadruplex and purine motif triplex nucleic acid; helps maintain telomere structure.	Chain P, N-terminal domain of Tlg1 complexed with N-terminus of Vps51, gi 88192565 (30)
132 ^{DA}	<i>TDH1</i>	35.84	8.29	332	161	31	gi 6322409	NP_012483	Cytoplasm	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 1, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3bis-phosphoglycerate.	Tar1p, gi 23270399 (30)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
128 ^D	<i>TDH3</i>	35.83	6.46	332	110	25	gi 6321631	NP_011708	Cytoplasm	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3bis-phosphoglycerate.	(-)
43 ^{DB}	<i>TDH3</i>	35.83	6.46	332	89	12	gi 6321631	NP_011708	Cytoplasm	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3bis-phosphoglycerate.	Erp6p, gi 6321436 (31)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
152 ^D	<i>TDH3</i>	35.83	6.46	332	56	18	gi 6321631	NP_011708	Cytoplasm	Glyceraldehyde-3-phosphate dehydrogenase, isozyme 3, involved in glycolysis and gluconeogenesis; tetramer that catalyzes the reaction of glyceraldehyde-3-phosphate to 1,3bis-phosphoglycerate.	(-)
153 ^D	<i>TEF2</i>	50.4	9.14	458	328	40	gi 6319594	NP_009676	Cytoplasm	Translational elongation factor EF-1 alpha; also encoded by TEF1; functions in the binding reaction of aminoacyl-tRNA (AA-tRNA) to ribosomes; may also have a role in tRNA re-export from the nucleus.	Chain B, Sec23-Sar1 complexed with the active fragment of Sec31, gi 165761045 (32)
83 ^D	<i>TEF2</i>	50.4	9.14	458	88	9	gi 6319594	NP_009676	Cytoplasm	Translational elongation factor EF-1 alpha; also encoded by TEF1; functions in the binding reaction of aminoacyl-tRNA (AA-tRNA) to ribosomes; may also have a role in tRNA re-export from the nucleus.	YAR042 Wp, gi 30267774 (29)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
82 ^D	<i>TEF2</i>	50.4	9.14	458	59	8	gi 6319594	NP_009676	Cytoplasm	Translational elongation factor EF-1 alpha; also encoded by TEF1; functions in the binding reaction of aminoacyl-tRNA (AA-tRNA) to ribosomes; may also have a role in tRNA re-export from the nucleus.	(-)
68 ^{DB}	<i>TEF2</i>	41.54	8.36	376	114	15	gi 32693297	AAM83114 (NP_009676)	Cytoplasm	Translation elongation factor 1-alpha.	Chain B, Sec23-Sar1 complexed with the active fragment of Sec31, gi 165761045 (34)
85 ^{IB}	<i>TEF2</i>	31.6	7.22	289	103	13	gi 159895242	ABX09905 (NP_009676)	Cytoplasm	Translation elongation factor 1-alpha.	(-)
106 ^D	<i>TEF2</i>	40.56	8.13	367	66	23	gi 32693293	AAM83112 (NP_009676)	Cytoplasm	Translation elongation factor 1-alpha.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
123 ^{DA}	<i>TEF4</i>	46.5	7.63	411	182	23	gi 538258	AAA21473 (NP_012842)	Cytoplasm	Elongation factor 1-gamma	Chain A, 3-Phosphoglycerate kinase, mutation R65q, gi 157833610 (85)
100	<i>TKL1</i>	73.8	6.48	680	194	11	gi 3212468	1AY0_A (NP_015399)	Cytoplasm	Chain A, Transketolase.	Conserved protein, gi 151944795 (28)
141 ^I	<i>UGP1</i>	56.18	6.98	499	291	29	gi 6322815	NP_012889	Cytoplasm	UDP-glucose pyrophosphorylase (UGPase), catalyses the reversible formation of UDP-Glc from glucose 1-phosphate and UTP, involved in a wide variety of metabolic pathways, expression modulated by Pho85p through Pho4p.	Q0130, gi 2133224 (35)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/ Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
61 ^{DA}	<i>YNK1</i>	17.21	8.65	153	250	44	gi 6322783	NP_012856	Cytoplasm Mitochondrion intermembrane space	Nucleoside diphosphate kinase catalyzes the transfer of gamma phosphates from nucleoside triphosphates, usually ATP, to nucleoside diphosphates by a mechanism that involves formation of an autophosphorylated enzyme intermediate.	Rps17bp, gi 6320655 (81)

3.4. Gene ontology

Analysis of the function of identified proteins was done using the Kyoto Encyclopedia of Genes and Genomes (KEGG). Proteins were searched against the *S. cerevisiae* reference database, and the results are shown in Figure 15 (refer to supplementary file 1 for additional information).

The majority of proteins that were identified were classified as being involved in metabolism. Thirty-eight proteins were classified as participating in secondary metabolism (Aat2, Ade13, Adh1, Adk1, Ald6, Aro8, Bat1, Cdc19, Eno1, Eno2, Erg10, Fba1, Glk1, Gnd1, Gpm1, Gus1, Hom2, Hxk2, Idh1, Idp1, Ilv3, Imd2, Imd4, Pdc1, Pfk2, Pgc1, Pmi40, Psa1, Sam1, Sec53, Shm1, Shm2, Tdh1, Tdh3, Tkl1, Tpi1, Ugp1 and Ynk1). “Carbohydrate metabolism” contained 27 proteins (Adh1, Ald6, Cdc19, Eno1, Eno2, Erg10, Fba1, Glk1, Gln1, Gnd1, Gpm1, Hxk2, Idh1, Idp1, Lys21, Pdc1, Pfk2, Pgc1, Pmi40, Psa1, Sec53, Shm1, Shm2, Tdh1, Tdh3, Tkl1, Tpi1 and Ugp1), and included the subgroup “glycolysis/ gluconeogenesis” with 15 proteins (Adh1, Ald6, Cdc19, Eno1, Eno2, Fba1, Glk1, Gpm1, Hxk2, Pdc1, Pfk2, Pgc1, Tdh1, Tdh3 and Tpi1). Proteins involved in the central pathways of carbohydrate metabolism are shown in Table 7. Figure 16 shows the location of glycolytic enzymes on a 2DE gel. Some locations did not match the predicted Mr or pI, most likely due to the presence of proteolytic fragments of posttranslational modifications.

“Energy metabolism” included 16 proteins (Atp1, Atp2, Atp3, Eno1, Eno2, Fba1, Gdh1, Gln1, Gpm1, Pfk2, Shm1, Shm2, Vma2, Vma4, Vma6 and Vma7). Nucleotide metabolism was represented by 8 proteins (Ade13, Adk1, Cdc19, Fur1, Gua1, Imd2, Imd4 and Ynk1). There were 21 proteins involved in amino acid metabolism (Aat2, Ade13, Adh1, Ald6, Aro8, Bat1, Cys3, Erg10, Gdh1, Gln1, Gnd1, Gpm1, Hom2, Idp1, Ilv3, Lys21, Sah1, Sam1, Shm1, Shm2 and Spe3). “Metabolism of cofactors and vitamins” included 6 proteins: Aro8, Bat1, Gus1, Ilv3, Shm1 and Shm2. Erg10 was the only protein involved in metabolism of terpenoids and polyketides.

Twenty-seven proteins were found to be involved in transcription/translation. Four proteins were involved in transcription (Dbp2, Ssa1, Ssa2 and Ssb2), twenty-three proteins were involved in translation (Dps1, Gus1, Rna1, Rpl11A, Rpl12B, Rpl13A, Rps0A, Rps12, Rps15, Rps19B, Rps20, Rps21A, Rps22A, Rps27A, Rps3, Rps4B, Rps5,

Rps7A, Sup45, Tef2, Tif2, Tif6 and YHR020W). Sixteen proteins were involved in folding, sorting and degradation (Cdc48, Eno1, Eno2, Hsc82, Hsp60, Hsp82, Kar2, Pdi1, Ssa1, Ssa2, Ssb2, Ssc1, Sse1, Rpt5 and Rpt6). Twelve proteins were involved in transport and catabolism (Act1, Idp1, Sod1, Ssa1, Ssa2, Ssb2, Tub1, Tub2, Vma2, Vma4, Vma6 and Vma7). Thirty-four proteins were uncategorized: Abp1, Ade5, 7, Arc1, Bmh1, Bmh2, Cam1, Cct5, Cdc12, Cof1, Cpr1, Eft2, Eht1, Fpr1, Hri1, Hsp10, Hsp104, Htb2, Ola1, Oye2, Paa1, Phb2, Prb1, Pst2, Sis1, Srp1, Ssb1, Stm1, Tef4, Tfp1, Tma19, Tpm1, Tsa1, Wtm1 and Zeo1.

Figure 15 - Gene ontology annotations of the functions of protein identified from the reference protein maps (Figure 13 and Figure 14). The number of proteins listed is greater than the number identified due to multifunctional properties of some proteins.

Table 7 - Identified proteins from the reference protein map (Table 5 and Table 6) that were involved in central carbohydrate metabolism (Figure 15).

Gene name	Protein name	Gel A (pH 4-7, Figure 13) or gel B (pH 6-11, Figure 14) [Spot number]
<i>Glycolysis/ gluconeogenesis</i>		
<i>ADH1</i>	Alcohol dehydrogenase 1	A[9, 10 & 31]
<i>ALD6</i>	Aldehyde dehydrogenase 6	A[201]
<i>CDC19</i>	Pyruvate kinase 19	B[46, 72, 119, 120, 130 & 140]
<i>ENO1</i>	Enolase 1	A[15 & 17]
<i>ENO2</i>	Enolase 2	A[35, 41, 42, 51, 162 & 224] B[8]
<i>FBA1</i>	Fructose-1,6-bisphosphate aldolase 1	A[120]
<i>GLK1</i>	Glucokinase 1	A[221]
<i>GPM1</i>	Phosphoglycerate mutase 1	A[65, 70, 71 & 76]
<i>HXK2</i>	Hexose kinase 2	A[82 & 187]
<i>PDC1</i>	Pyruvate decarboxylase 1	A[27, 215 – 219]
<i>PFK2</i>	Phosphofructokinase (β chain)	A[200]
<i>PGK1</i>	3-phosphoglycerate kinase	A[13] B[64, 124, 145, 147 & 151]
<i>TDH1</i>	Glyceraldehyde-3-phosphate dehydrogenase 1	B[132]
<i>TDH3</i>	Glyceraldehyde-3-phosphate dehydrogenase 3	A[5] B[43, 128 & 152]
<i>TPI1</i>	Trisephosphate isomerase	A[45]
<i>Citrate acid cycle (TCA cycle)</i>		
<i>IDH1</i>	Mitochondrial NAD ⁺ dependent isocitrate dehydrogenase 1	B[134]
<i>IDP1</i>	Mitochondrial NADP specific isocitrate dehydrogenase 1	B[112]
<i>Pentose phosphate pathway</i>		
<i>FBA1</i>	Fructose-1,6-bisphosphate aldolase 1	A[120]
<i>GND1</i>	6-phosphoglycerate dehydrogenase 1	A[16]
<i>PFK2</i>	Phosphofructokinase (β chain)	A[200]
<i>TKL1</i>	Transketolase 1	B[100]

Figure 16 - Two-dimensional gel images of BY4741 proteins (100 μ g) separated on pH 4-7 (Figure 13, top) or pH 6-11 (Figure 14, bottom) IPG strips. The positions of glycolytic enzymes are shown. The ladder used for molecular weight reference was Rainbow 851.

3.5. Measurement of glucose use and ethanol production

The aim of this set of experiments was to determine conditions for growth of *S. cerevisiae* for 2D-DIGE comparative analysis of fermentative and non-fermentative growth. Two approaches were explored: using different glucose concentrations and use of glycerol as a carbon source.

Table 8 summarizes glucose consumption and ethanol production for cell cultures grown on 0.2–2% glucose (A), and growth on glucose or glycerol (B). Results include means and standard deviations for each $n = 4$ experiment. The first experiment, examined the use of low (0.2%), medium (0.5%) or high (2%) concentrations of glucose in SC media (Table 8A), and shows complete use of glucose at low and medium concentrations. Ethanol was only detected for cells grown at the high (2%) glucose concentration indicating use of glucose in the alternative pathways at the lower (0.2%, 0.5%) glucose concentrations. For the “high glucose” grown cells, glucose consumption was partial and the ratio of ethanol produced to glucose used was greater than expected for complete conversion of glucose to ethanol (2 ethanol per glucose). This suggested that other pathways may have contributed to ethanol production unless measurement errors were responsible for the > 2 ratio.

In the second experiment, comparing growth on glycerol, ethanol was only produced when glucose was the substrate (Table 8B). Therefore, fermentative growth was indicated by high ethanol production in aerobic conditions, and consumption of glucose.

Table 8 - Glucose consumption and ethanol production of 0.2% (low), 0.5% (medium) and 2% (high) glucose grown yeast cells (A) and 3% glycerol vs. 2% glucose grown cells (B). The second and third column displays the respective amount of glucose remaining and used. The last column represents the amount of ethanol produced in mmols as well as the ratio of glucose to ethanol used.

A	Glucose %(mmol)	Glucose remaining (mmol)	Glucose used (mmol)	Ethanol produced (mmol) [ethanol/glucose]
	0.2% (0.27)	0	0.27	0
	0.5% (0.67)	0	0.67	0
	2% (2.7)	1.09 $\pm$ 0.06	1.61	6.9 $\pm$ 0.44 [2.56]

B

Glucose or glycerol %(mmol)	Glucose remaining (mmol)	Glucose used (mmol)	Ethanol produced (ethanol/glucose)
3% glycerol (G1)	0	0	0
2% glucose (2.7)	1.29 ±0.11	1.41	6.19 ±1.19 [2.29]
3% glycerol (G2)	0	0	0

3.6. Comparative proteomics

2D-DIGE was used to compare differences in protein abundances between fermentative and non-fermentative growth using cells grown on glucose or glycerol.

3.6.1. Growth on glucose (D) or glycerol (G)

A 2D-DIGE experiment was used to compare the differences between glycerol (G2) and glucose (D) grown cells using pH 3-11 non-linear (3-11NL) IPG strips. The 2D-DIGE image showing the differences between glucose (red) and glycerol (blue) supplemented cells is shown in Figure 17. A total of 219 proteins changed greater than 2-fold at $p \leq 0.01$ (refer to supplementary file 2). Of these, 99 protein spots had an average ratio greater than 2, indicative of greater protein abundance in glucose grown conditions. The majority of proteins specific to fermentative growth had a neutral or acidic *pI*. The other 120 had an average ratio less than -2, indicating greater protein abundance in glycerol grown conditions. A large group of basic protein spots were associated with cells grown on glycerol. Only a small number of these proteins were identified by excision of CBB G-250 stained spots off the 2D-DIGE gels because of the small protein amounts used in DIGE analysis (indicated by a black arrow) (Table 8). The abundant proteins specific to glucose growth that were identified included Fba1, Eno2, Sec53, Ssa1/2, Sod1, Egd2, Crp1 and Fpr1 shown in Figure 17 and Table 9. Of note, several glycolysis/ gluconeogenesis proteins were found to be more abundant in glucose grown cultures (Fba1 and Eno2).

Figure 17 - Two-dimensional images of the DIGE experiment portraying the differences in protein abundance between BY4741 cells grown in glucose (red) and glycerol (blue). The DIGE experiment required fluorescent labeling of the cell cultures in 2% glucose or 3% glycerol with different dyes. The colored asterisks represent proteins that had a significant level of change ($\geq 2X$, $p \leq 0.01$). Proteins identified from the 2D-DIGE gels with a significant MASCOT score are marked with black asterisks.

Table 9 lists proteins that were identified by excision of protein spots from 2D-DIGE gels. For each identified protein the DeCyder™ spot number is given, with the average ratio (fold change) between replicates, the Student's *t*-test value and the gene name. Proteins that were identified were specific to growth on glucose. No proteins specific to non-fermentative growth (3% glycerol) were identified.

Table 9 - Proteins identified from the glucose versus glycerol grown DIGE experiment ($p \leq 0.01$). The data is sorted by the average ratio from lowest to highest fold (≥ 2 -fold) change. The average ratio is the value of change from condition 1 (glucose) in comparison to condition 2 (glycerol). A positive average ratio indicates an increase in protein abundance associated with growth on glucose.

Master No (Spot No)	Average ratio	<i>t</i> -test	Protein name	Gene name
1891 (16)	2.04	0.008	Phosphomannomutase	<i>SEC53</i>
1576 (37)	3	0.0037	Fructose-1,6- biphosphate aldolase	<i>FBA1</i>
1069 (137/138)	3.3	0.0046	Stress-seventy subfamily A	<i>SSA1/2</i>
2051 (7)	4.1	0.0012	Superoxide dismutase	<i>SOD1</i>
1916 (17)	4.28	0.0046	Enhancer of Gal4 DNA binding	<i>EGD2</i>
2194 (33)	6.04	0.0045	Cyclosporin A- sensitive proline rotamase	<i>CPR1</i>
1455 (97)	6.55	0.00021	Enolase	<i>ENO2</i>
2155 (150)	7.2	4.10E-05	Fk 506-senstive proline rotamase	<i>FPR1</i>

Subsequently, attempts were made to identify proteins that changed in abundance by matching proteins in the glucose vs. glycerol DIGE experiment (Figure 17) to the reference gels (Figure 13, Figure 14) using 'landmark patterns' on all gels. These proteins are listed in Table 10, and are possible matches only. Eighteen proteins were matched.

Table 10 - Possible protein identifications for the glucose vs. glycerol DIGE experiment. The reference pH 4-7 (Figure 13) and 6-11 (Figure 14) gels were used to match spots DeCyder™ assigned to the glucose versus glycerol 2D-DIGE gel (Figure 17).

	Master spot No.	Possible spot match	Protein name	Gene name
Glycerol	846	177	Actin binding protein 1	<i>ABP1</i>
	1762	128	Brain modulosignalin homologue 1	<i>BMH1</i>
	1713	127	Brain modulosignalin homologue 2	<i>BMH2</i>
	1408	77	Glutamate dehydrogenase	<i>GDH1</i>
	1267	24	Guanine auxotroph 1	<i>GUA1</i>
	1216	217	Pyruvate dehydrogenase	<i>PDC1</i>
	683	200	Phosphofructokinase	<i>PFK2</i>
	1538	30	GDP-mannose pyrophosphorylase	<i>PSA1</i>
	1843	139	40S ribosomal protein S0-A	<i>RPS0A</i>
	2042	104	40S ribosomal protein S12	<i>RPS12</i>
	1231	93	Importin subunit alpha	<i>SRP1</i>
	1047	189	Heat shock protein homolog SSC1	<i>SSC1</i>
	863	191	Heat shock protein homolog SSE1	<i>SSE1</i>
	1484	147	Translation initiation factor 2	<i>TIF2</i>
	1923	131	Translation initiation factor 6	<i>TIF6</i>
	1900	45	Triosephosphate isomerase	<i>TPI1</i>
	1027	20	Arginyl-tRNA synthase	<i>YDR341C</i>
	2101	99	Zeocin resistance protein	<i>ZEO1</i>

Figure 18 shows the GO annotations between the reference protein map and the proteins identified in the glucose versus glycerol DIGE experiment (refer to supplementary file 3 for additional information). The GO analysis of the glucose vs. glycerol 2D-DIGE experiment reflect a similar pattern, at a smaller scale, of functional groups compared to the GO annotations of the reference protein map. Carbohydrate metabolism is the largest category in both experiments. Figure 19 shows proteins involved in ethanol synthesis that were identified from the reference gels (red box) (Figure 13, Figure 14, Table 5 and Table 6) or changed in protein abundance between cells grown on glucose (D+) or glycerol (G+). Only four of the 11 proteins involved in ethanol synthesis were identified in the 2D-DIGE experiment between cells grown in glucose or glycerol.

Figure 18 - Comparative gene ontology annotations of the functions between proteins identified for the reference protein map (red) and proteins identified from the glucose vs. glycerol DIGE experiment (blue). The number of proteins listed is greater than the number identified due to multifunctional properties of some proteins.

Figure 19 - Identified proteins (red box) involved in glucose and glycerol metabolism. The diagram also shows identified proteins that changed in glycerol (G+) or glucose (D+) conditions.

Proteins that were matched by comparison with the reference gels (Figure 13, Figure 14). Proteins that were tentatively identified, not all of which were subject to specific growth conditions are shown in Figure 20 and Table 11. Twenty-seven protein spots were identified that corresponded to 20 unique protein identifications. Six glycolytic proteins were identified (Cdc19, Eno1, Eno2, Fba1, Pgk1 and Tpi1) as well as the fermentative enzyme Adh4. Adh2 and its transcriptional regulator Adr1 were detected, however they had Mascot scores ≤ 56 with low confidence intervals.

Figure 20 - Coomassie stained 2D-DIGE gel of the glucose vs. glycerol experiment. Proteins (30 μ g), which were identified by MALDI MS, are shown in Table 11. Four proteins (24, 39, 40, 45) had MASCOT scores less than 56 with low confidence interval.

Table 11 - Proteins identified from the simplified glucose vs. glycerol grown DIGE experiment. Proteins (30 µg) were separated on pH 3-11 NL IPG strips and identified using MALDI TOF/TOF. Proteins (score ≥ 56 , $p \leq 0.05$) are sorted alphabetically by their gene name. The score and sequence coverage (%) values are representative of the significance of the match generated by the MASCOT search. The calculated mass, pI, GI, accession number and description of the protein are gathered from the NCBI protein website. The subcellular localization of the protein was from UniProt. The next matched protein was the next non-homologous protein match.

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
24*	<i>ADH2</i>	37.17	6.26	348	20	20	gi 6323961	NP_014032	Cytoplasm	Glucose-repressible alcohol dehydrogenase II, catalyzes the conversion of ethanol to acetaldehyde; involved in the production of certain regulated by ADR1.	None
40*	<i>ADH2</i>	37.17	6.26	348	36	36	gi 6323961	NP_014032	Cytoplasm	Glucose-repressible alcohol dehydrogenase II, catalyzes the conversion of ethanol to acetaldehyde; involved in the production of certain regulated by ADR1.	None
36	<i>ADH4</i>	41.46	5.76	382	88	32	gi 205831682	P10127 (NP_011258)	Mitochondrion	Alcohol dehydrogenase 4	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
34	<i>ADH4</i>	41.46	5.76	382	56	30	gi 205831682	P10127 (NP_011258)	Mitochondrion	Alcohol dehydrogenase 4	(-)
39*	<i>ADR1</i>	15.72	6.27	139	20	20	gi 197253860	ACH54265 (NP_010502)	Nucleus	Carbon source-responsive zinc-finger transcription factor, required for transcription of the glucose-repressed gene ADH2, of peroxisomal protein genes, and of genes required for ethanol, glycerol, and fatty acid utilization.	None
45*	<i>ADR1</i>	15.72	6.27	139	22	22	gi 197253860	ACH54265 (NP_010502)	Nucleus	Carbon source-responsive zinc-finger transcription factor, required for transcription of the glucose-repressed gene ADH2, of peroxisomal protein genes, and of genes required for ethanol, glycerol, and fatty acid utilization	None

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
118	<i>CDC19</i>	54.91	7.56	500	73	24	gi 6319279	NP_009362	Cytoplasm	Pyruvate kinase, functions as a homotetramer in glycolysis to convert phosphoenolpyruvate to pyruvate, the input for aerobic (TCA cycle) or anaerobic (glucose fermentation) respiration.	Fat1p partial, AAP21758 (19)
77	<i>CPR1</i>	17.49	6.90	162	103	50	gi 6320359	NP_010439	Cytoplasm	Cytoplasmic peptidyl-prolylcis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues; binds the drug cyclosporin A.	unnamed protein product, CAA25036 (30)
33	<i>CPR1</i>	17.49	6.90	162	66	54	gi 6320359	NP_010439	Cytoplasm	Cytoplasmic peptidyl-prolylcis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues; binds the drug cyclosporin A.	Tom5p, NP_015459 (23)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
78	<i>CPR3</i>	20.02	8.81	182	71	44	gi 6323562	NP_013633	Mitochondrion matrix	Mitochondrial peptidyl-prolylcis-trans isomerase (cyclophilin), catalyzes the cis-trans isomerization of peptide bonds N-terminal to proline residues; involved in protein refolding after import into mitochondria.	unnamed protein product partial, CAA97190 (30)
17	<i>EGD2</i>	18.30	4.84	170	95	57	gi 172043	AAC15849 (NP_012063)	Cytoplasm Nucleus	Egd2p (Enhancer of GAL4 DNA binding).	Chain B, Ribosomal 80s-Eef2-Sordarin Complex, 1S1H_B (25)
90	<i>ENO1</i>	46.66	6.04	436	105	36	gi 157830958	1ELS_A (NP_011770)	Cytoplasm	Chain A, Enolase-Mn2+-Phosphonoacetohydroxamate	(-)
91	<i>ENO1</i>	46.68	6.17	436	67	31	gi 20151217	1L8P_A (NP_011770)	Cytoplasm	Chain A, Mg-Phosphonoacetohydroxamate Complex Of S39a Yeast Enolase 1	Ups3p, NP_010471 (27)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
97	<i>ENO2</i>	46.94	5.67	437	100	36	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	YGR254W p-like protein, EDZ71898 (42)
26	<i>ENO2</i>	46.94	5.67	437	56	25	gi 6321968	NP_012044	Cytoplasm	Enolase II, a phosphopyruvate hydratase that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis and the reverse reaction during gluconeogenesis; expression is induced in response to glucose.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
37	<i>FBA1</i>	39.88	5.51	359	130	40	gi 6322790	NP_012863	Cytoplasm Mitochondrion	Fructose-1,6-bisphosphate aldolase required for glycolysis and gluconeogenesis; catalyzes conversion of fructose-1, 6 bisphosphate to glyceraldehyde-3-P and dihydroxyacetone-P; locates to mitochondrial outer surface upon oxidative stress.	Glo4p, NP_014683 (22)
12	<i>FBA1</i>	39.88	5.51	359	92	30	gi 6322790	NP_012863	Cytoplasm Mitochondrion	Fructose 1,6-bisphosphate aldolase required for glycolysis and gluconeogenesis; catalyzes conversion of fructose 1,6 bisphosphate to glyceraldehyde-3-P and dihydroxyacetone-P; locates to mitochondrial outer surface upon oxidative stress.	Hsp26p, NP_009628 (68)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
150	<i>FPR1</i>	12.08	5.71	113	66	45	gi 157834293	1YAT_A (NP_014264)	Cytoplasm	Chain A, Improved Calcineurin Inhibition By Yeast Fkbp12-Drug Complexes. Crystallographic And Functional Analysis	Fpr1p, NP_014264 (66)
14	<i>HSP26</i>	23.87	5.31	214	121	52	gi 6319546	NP_009628	Cytoplasm Nucleus	Small heat shock protein (sHSP) with chaperone activity; forms hollow, sphere-shaped oligomers that suppress unfolded proteins aggregation; oligomer activation requires a heat-induced conformational change; not expressed in unstressed cells.	Cog7p, NP_011510 (40)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
15	<i>HSP26</i>	23.87	5.31	214	119	57	gi 6319546	NP_009628	Cytoplasm Nucleus	Small heat shock protein (sHSP) with chaperone activity; forms hollow, sphere-shaped oligomers that suppress unfolded proteins aggregation; oligomer activation requires a heat-induced conformational change; not expressed in unstressed cells.	YLR301W, NP_013404 (39)
13	<i>HSP26</i>	23.87	5.31	214	70	42	gi 6319546	NP_009628	Cytoplasm Nucleus	Small heat shock protein (sHSP) with chaperone activity; forms hollow, sphere-shaped oligomers that suppress unfolded proteins aggregation; oligomer activation requires a heat-induced conformational change; not expressed in unstressed cells.	PLC1, CAA98003 (20)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
87	<i>PGK1</i>	44.77	7.11	416	142	44	gi 10383781	NP_009938	Cytoplasm	3-phosphoglycerate kinase, catalyzes transfer of high-energy phosphoryl groups from the acyl phosphate of 1,3-bisphosphoglycerate to ADP to produce ATP; key enzyme in glycolysis and gluconeogenesis.	(-)
2	<i>RIB3</i>	22.90	5.46	208	83	51	gi 6320695	NP_010775	Cytoplasm Mitochondrial intermembrane space	3,4-dihydroxy-2-butanone-4-phosphate synthase (DHBP synthase), required for riboflavin biosynthesis from ribulose-5-phosphate, also has an unrelated function in mitochondrial respiration.	Vacuolar H ⁺ -ATPase, AAA66890 (38)
147	<i>RPS21A</i>	9.80	5.76	87	63	80	gi 6322910	NP_012983	Cytoplasm	Protein component of the small (40S) ribosomal subunit; nearly identical to Rps21Bp and has similarity to rat S21 ribosomal protein.	(-)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
16	<i>SEC53</i>	29.22	5.14	254	66	40	gi 14318474	NP_116609	Cytoplasm	Phosphomannomutase involved in synthesis of GDP-mannose and dolichol-phosphate-mannose; required for folding and glycosylation of secretory proteins in the ER lumen.	Hsp26p, NP_009628 (43)
7	<i>SOD1</i>	15.85	5.48	153	64	45	gi 6730103	1B4T_A (NP_012638)	Cytoplasm Mitochondrion intermembrane space	Chain A, Cu (II) Zn superoxide dismutase	(-)
137	<i>SSA1</i>	69.79	5.00	642	94	24	gi 144228166	NP_009396	Cytoplasm Cell wall Nucleus	ATPase involved in protein folding and nuclear localization signal (NLS)-directed nuclear transport; member of heat shock protein 70 (HSP70) family; forms a chaperone complex with Ydj1p.	AWRI1631_152250, EDZ69338 (26)

Spot No	Gene name	Mass [kDa]	pI [pH]	Sequence length [AA]	Score	Sequence Coverage [%]	GI No	Accession No [SwissProtKB/Swiss-Prot/ <i>S.cerevisiae</i>] (BLAST Accession No)	Subcellular localization	Description	Next match, GI no (score)
51	SSA1	37.59	5.24	339	61	31	gi 172713	AAA35095 (NP_009396)	Cytoplasm Cell wall	70kDa heat shock protein	YLL024Cp-like protein, partial, EDZ70769 (52)
138	SSA2	59.81	7.00	543	96	27	gi 207343245	EDZ70769 (NP_013076)	Cytoplasm Cell wall	YLL024Cp-like protein	AWRI1631_152250, EDZ69338 (25)
3	TPI1	26.76	5.75	247	98	32	gi 230405	1YPI_A (NP_010335)	Cytoplasm Mitochondrion Plasma membrane	Chain A, Triosephosphate Isomerase	YER186Cp-like protein, EDZ72486 (37)
149	TSA1	21.69	5.03	196	63	40	gi 6323613	NP_013684	Cytoplasm	Ubiquitous housekeeping thioredoxin peroxidase, reduces reactive oxygen, nitrogen and sulfur species using thioredoxin as hydrogen donor; mediates redox regulation of the nuclear localization of Yap1p; deletion results in mutator phenotype.	YML028Wp-like protein, partial, EDZ70249 (40)

4. Discussion

In this thesis, 2D-DIGE was used to search for proteins whose abundance differed between growth of *S. cerevisiae* on glucose or glycerol. The specific goal was to search for changes among enzymes associated with the metabolism of these substrates. The general background concerns the relationship between change in enzyme amount and metabolic output. One qualification is that 2DE generally detects only high abundance soluble and cytoskeletal proteins, although many of the enzymes of carbohydrate metabolism are detected using mammalian samples and appear to be relatively abundant proteins (Beddek *et al.*, 2008; Rawson *et al.*, 2012). At the time my research started there were no other proteomic comparisons of growth on glycerol versus glucose.

4.1. Proteins identified by MALDI MS

In this study 179 protein spots were identified from Coomassie stained gels of 100 µg protein from cells grown on 2% glucose. Typically, about 150-200 proteins can be identified by MALDI MS off the 7 cm gels used in my work (Beddek *et al.*, 2008; Rawson *et al.*, 2012). MS of protein spots excised from 2DE gels often results in identification of fewer proteins compared to shotgun LC MS/MS of digested cellular extracts, but comparison of abundances of individual proteins is well-established using 2D-DIGE.

The major GO categories of identified proteins were: protein folding, sorting and degradation, transcription, translation, and carbohydrate, amino acid, nucleotide and energy metabolism. The largest group of functionally categorized proteins were involved in carbohydrate metabolism including the sub groups glycolysis/gluconeogenesis, and fructose and mannose metabolism.

Proteins associated with oxidative phosphorylation and methane metabolism identified in the GO analysis (Figure 15) included seven subunits of the F- and V-type ATPases (Table 12). Proteins implicated in methane metabolism included the conversion of serine to glycine (Shm1 and Shm2), and glucose and fructose metabolism enzymes (Eno1, Eno2, Fba1, Gpm1 and Pfk2).

Table 12 - Components of complex V of the electron transport chain.

Protein name	Gene name	Description	Gel A (Figure 13) or B (Figure 14) [spot number]
ATP synthase subunit α	<i>ATP1</i>	α subunit of F-type H ⁺ transporting ATPase	B [122]
ATP synthase subunit β	<i>ATP2</i>	β subunit of F-type H ⁺ -transporting ATPase	A [81]
ATP synthase subunit γ	<i>ATP3</i>	γ subunit of F-type H ⁺ -transporting ATPase	B [81]
Vacuolar membrane ATPase subunit B	<i>VMA2</i>	Subunit B of V-type H ⁺ transporting ATPase	A [89 & 154]
Vacuolar membrane ATPase subunit E	<i>VMA4</i>	Subunit E of V-type H ⁺ transporting ATPase	A [124]
Vacuolar membrane ATPase subunit AC39	<i>VMA6</i>	Subunit AC39 of V-type H ⁺ -transporting ATPase	A [135]
Vacuolar membrane ATPase subunit F	<i>VMA7</i>	Subunit F of V-type H ⁺ -transporting ATPase	A [101]

Proteins involved in cysteine and methionine metabolism were Aat2, Aro8, Cys3, Hom2, Sah1, Sam1 and Spe3. Nucleotide metabolism included seven proteins involved in purine processing, Ade13, Adk1, Cdc19, Gua1, Imd2, Imd4 and Ynk1. Ten proteins were involved in protein processing in the endoplasmic reticulum, including Cdc48, Hsc82, Hsp26, Hsp82 and Sse1. Three proteins were components of the ubiquitin ligase complex (Ssa1, Ssa2 and Ssb1). Kar2, a part of a chaperone complex is associated with protein recognition and Pdi1 involved in protein targeting were also identified.

GO analysis indicated proteins associated with phagosomes including actin (Act1) and tubulins, although these high abundance cytoskeletal proteins have widespread functions. Act1 is involved in the initial internalisation and formation of phagosomes (phagocytic cup). Tubulin is involved in phagosome maturation (Tub1

and Tub2). Four proteins Vma2, Vma4, Vma6 and Vma7 are a part of the vATPase (early, mature and phagolysosome stages).

A large number of ribosomal proteins were identified. Ribosomes are organelles which have a significant role in protein synthesis. They are composed of large and small subunits. Three proteins associated with the large subunit were identified (Rpl11A, Rpl12B and Rpl13A). Twelve proteins associated with the small subunit were identified (Rps0A, Rps12, Rps15, Rps19B, Rps20, Rps21A, Rps22A, Rps27A, Rps3, Rps4B, Rps5 and Rps7A).

4.2. Carbohydrate and ethanol metabolism

The primary aim of this study was to examine the differences in abundance of enzymes involved in carbohydrate and ethanol metabolism between growth on glucose or glycerol. The major categories of proteins detected were involved in glycolysis/ gluconeogenesis and mannose metabolism. Proteins associated with the following subgroups were also identified: citric acid cycle, pentose phosphate pathway, galactose, starch and sucrose, amino sugar and nucleotide sugar, pyruvate, glyoxylate and dicarboxylate, propanoate, butanoate and inositol phosphate metabolism.

Most of the enzymes of the glycolytic pathway were identified except for Pgi1 that converts glucose-6-phosphate to fructose-6-phosphate (Figure 21). This protein has, however, been identified by several groups including Shevchenko *et al.* (1996) and Kolkman *et al.* (2005), using 2DE. Glycolytic enzymes that were detected included glucokinase Glk1, hexokinase Hxk2, phosphofructokinase Pfk2, fructose-bisphosphate aldolase Fba1, and triosephosphate isomerase Tpi1. Although many glycolytic enzymes have been reported in previous proteomic studies, my research seems to be the first detection of phosphofructokinase that has important regulatory properties but seems to be low abundance and not previously identified by 2DE.

As expected, for cells grown on glucose, enzymes required for ethanol synthesis were identified (Pdc1 and Adh1). An acetaldehyde dehydrogenase (Ald6) that catalyzes the conversion of aldehyde to acetate (Bruckmann *et al.*, 2009; Huang *et al.*, 2012; Kolkman *et al.*, 2005; Massoni *et al.*, 2009; Shevchenko *et al.*, 1996) was also detected. Proteins associated with metabolism of mannose included mannose-6-phosphate

isomerase Pmi40, mannose-1-phosphate guanyltransferase Psa1, and phosphomannomutase Sec53.

4.3. Effect of growth on glucose or glycerol

Two-dimensional-differential in gel electrophoresis detected proteins that changed in abundance between glucose or glycerol supplementation. Of the approximately 2500 possible protein features (spots) analyzed on the 3-11 DIGE gels, 219 showed ≥ 2 -fold ($p \leq 0.01$) change between carbon substrates but many were minor spots on the gels. Only eight proteins were directly identified by MS of spots excised from the 2D-DIGE gels, all were greater abundance from cells grown on glucose. Mass spectrometry of tryptic digests of proteins excised from DIGE gels is generally challenging because of the small amounts of proteins on these gels, and potentially also due to modification of some lysine residues by the DIGE reagents.

An additional 18 proteins that varied between cells grown on glucose or glycerol could be matched with reasonable certainty to the database of proteins that was derived using 100 μ g of protein from cells grown on 2% glucose (Table 5 and Table 6). GO analysis of the combined set of 26 proteins (Table 13) suggested that carbon substrate affected carbohydrate metabolism and other processes.

Five of the enzymes required for conversion of glucose to ethanol (Pfk2, Fba1, Tpi1, Eno2 and Pdc1) increased approximately 3-9 fold in cells grown on glucose compared to glycerol (Table 13 and Figure 20). The greatest change was for phosphofructokinase (8.8 fold). This result is consistent with that of Foy & Bhattacharjee (1978) who reported doubling of phosphofructokinase enzyme activity during fermentation compared to oxidative conditions. The final two steps of ethanol synthesis for conversion of pyruvate to acetaldehyde and ethanol are catalyzed by Pdc1 and Adh1 respectively. Pyruvate decarboxylase Pdc1 increased approximately 5-fold during metabolism of glucose but there was not a statistically significant change in the amount of the major ethanol synthesising isoform of alcohol dehydrogenase Adh1. There may have been less than 2-fold changes in enzyme amount that would not have been statistically significant using four biological replicates (Karp & Lilley, 2005) but the effects of small, or any, change in enzyme amount on metabolic output remains to be determined.

Several proteins were associated with protein synthesis and processing. Further functional analysis of each protein was obtained from the ExPASy database www.expasy.ch. Rps0A, Rps12, Tif2 and Tif6 have roles as part of the ribosome, RNA transport, and ribosome biogenesis. Rps0A and Rps12 are a part of the small subunit of the ribosome. Tif2 has a role in RNA transport while Tif6 is involved in ribosome biosynthesis. Egd2 is also involved in protein synthesis. The transcriptional protein Ssa1 is a part of splicesomes.

Proteins involved in folding, sorting and degradation were identified (Ssa1, Sse1, Ssc1). Ssa1 is a heat shock protein which plays a role in protein folding and is also a component of ER-associated degradation. Sse1 is a subunit of the ubiquitin ligase complex were the proteins which represent processing ER. Ssc1 is a mitochondrial heat shock protein.

Other proteins that showed changes in abundance between substrates included an actin binding protein Abp1, the 14-3-3 family proteins Bmh1 and Bmh2, two peptidyl-prolyl cis-trans isomerases Cpr1 and Fpr1 that are associated with protein folding, GMP synthase Gua1, superoxide dismutase (Sod1), the nuclear pore complex protein Srp1 that is implicated in transport of proteins between nucleus and cytoplasm, and Zeo1 that has been associated with cell stress.

Overall these changes suggest potential effects on protein synthesis and processing that may be associated with the experimental design when cells from glycerol cultures were transferred to either glucose or glycerol media. To investigate this potential "stress response" the design could be changed to compare the shift of cells grown in glucose to glycerol. Another approach would be to examine cells grown in either glucose or glycerol for two to three generations prior to proteomic analysis. The identification of proteins from cells grown on glucose should also be extended to MS of proteins from cells grown on glycerol to facilitate analysis of proteins that increase in abundance during metabolism of glycerol, including potentially the glycerol metabolising enzymes Gut1 and 2 that were not identified in the current study.

Table 13 - The twenty-six varying proteins associated with growth on glucose. Eight proteins were detected by DeCyder™ analysis (≥ 2 -fold, $p \leq 0.01$) and identified by MS (Table 9) of (30 μ g) proteins extracted from cells grown in 2% glucose or 3% glycerol. The other 18 were matched to spots on the reference gels (Table 10), 100 μ g proteins extracted from cells grown in 2% glucose. Average ratio and t-test values are associated with DeCyder™ data.

	Master No (Spot No)	Possible matches	Average ratio	t-test	Protein name (glycolytic enzymes are in bold)	Gene name
Identified proteins	1891 (16)	N/A	2.04	0.008	Phosphomannomutase	<i>SEC53</i>
	1576 (37)	N/A	3	0.0037	Fructose-1,6- biphosphate aldolase	<i>FBA1</i>
	1069 (137/138)	N/A	3.3	0.0046	Stress-seventy subfamily A	<i>SSA1/2</i>
	2051 (7)	N/A	4.1	0.0012	Superoxide dismutase	<i>SOD1</i>
	1916 (17)	N/A	4.28	0.0046	Enhancer of Gal4 DNA binding	<i>EGD2</i>
	2194 (33)	N/A	6.04	0.0045	Cyclosporin A- sensitive proline rotamase	<i>CPR1</i>
	1455 (97)	N/A	6.55	0.00021	Enolase	<i>ENO2</i>
	2155 (150)	N/A	7.2	4.10E-05	Fk 506-sensitve proline rotamase	<i>FPR1</i>
Matched proteins	846	177	5.56	0.0021	Actin-binding protein	<i>ABP1</i>
	1762	128	7.1	0.00021	Brain modulosignalin homologue 1	<i>BMH1</i>
	1713	127	5.88	0.00014	Brain modulosignalin homologue 2	<i>BMH2</i>
	1408	77	2.31	0.0084	Glutamate dehydrogenase	<i>GDH1</i>
	1267	24	2.58	0.0015	Guanine Auxotroph	<i>GUA1</i>
	1216	217	4.65	0.0045	Pyruvate decarboxylase	<i>PDC1</i>
	683	200	8.76	0.00021	Phosphofructokinase	<i>PFK2</i>
	1538	30	2.76	0.0079	GDP-mannose pyrophosphorylase	<i>PSA1</i>
	1843	139	7.35	0.00021	Ribosomal protein of	<i>RPS0A</i>

					the small subunit	
	2042	104	12.22	5.60E-05	Ribosomal protein of the small subunit	<i>RPS12</i>
	1231	93	3.81	0.0029	Supressor of Rpb1	<i>SRP1</i>
	1047	189	3.57	0.01	Stress-seventy subfamily C	<i>SSC1</i>
	863	191	3.68	0.0025	Stress-seventy subfamily E	<i>SSE1</i>
	1484	147	5.18	0.0017	Translation initiation factor 2	<i>TIF2</i>
	1923	131	20.5	1.60E-05	Translation initiation factor 6	<i>TIF6</i>
	1900	45	6.37	0.0019	Triosephosphate isomerase	<i>TPI1</i>
	1027	20	3.61	0.01	Arginyl-tRNA synthetase	<i>YDR341C</i>
	2101	99	16.3	1.60E-05	Zeocin resistance	<i>ZEO1</i>

Figure 21 - Proteins involved in glucose and glycerol metabolism. Identified proteins (red box) from cells grown on 2% glucose were excised from gels containing 100 µg protein (Figure 13 and Figure 14). Proteins detected in the 2D-DIGE experiment were labeled with D+ (glucose-based media) or G+ (glycerol-based media) (Table 13). Thirty micrograms of protein was used in the glucose versus glycerol 2D-DIGE experiment.

4.4. Changes associated with carbohydrate metabolism

Metabolic differences between *S. cerevisiae* during fermentation or oxidation of carbon substrates are well described including the proteomic studies surveyed in the Introduction. One aspect of this study was identification of Pfk2 that is rarely reported, if at all, using 2DE of mammalian or yeast cells. The identification of 26 proteins that changed between glucose or glycerol based media included seven that were involved in carbohydrate metabolism. All seven increased in abundance in cells grown on glucose.

My results are most comparable with those of Stobaugh *et al.* (2013) who used shotgun MS to examine protein change between shake flask cultures of *S. cerevisiae*. Although protein fractionation methods affected the results (see Introduction Figure 6) their combined results indicated that growth on glucose gave increased abundance of a hexokinase, triosephosphate isomerase and pyruvate decarboxylases, but there was not a significant increase in the detected Adh1 in this research. Also like my results, pyruvate decarboxylase Pdc1 increased with glucose compared to glycerol but they also found relative increase in Pdc5 and 6 for cells grown in glycerol. Other changes reported by Stobaugh *et al.* (2013) included increases in the final enzyme of the glycerol synthesis pathway. They detected phosphofructokinase and enolase but without significant change in abundance between grown on glucose and glycerol. Although proteomic methodology affects both detection and quantification of proteins, 2D-DIGE is a relatively robust method for comparison of protein abundances.

Similarly, Guidi *et al.* (2010) showed altered abundances of protein for several glycolytic enzymes between high (2%) glucose (fermentation) and low (0.5%) glucose conditions. They found greater abundance of five glycolytic proteins were associated with high glucose conditions including Eno2 and Pdc1, proteins which were detected in this study with growth on glucose. They also detected glyceraldehyde-3-phosphate dehydrogenase isozyme 3 Tdh3, phosphoglycerate kinase Pgk1, and Adh1. Therefore, the detection of these glycolytic enzymes, particularly Pdc1 and Adh1 would reasonably suggest greater abundance of enzymes involved in ethanol synthesis in high (2%) glucose conditions.

Gutteridge *et al.* (2010) reported an altered abundance of transcripts and proteins involved in the early stages of glucose metabolism in carbon limited conditions. They identified high glucose affinity transporters Hxt6/7 (glucose-limited conditions) and Hxk1 and Glk1 (linked with carbon sources other than glucose). Although their results are not directly comparable as conditions varied (chemostat cultures and supplemented with 0.25% glucose), they do provide an insight into other proteins likely to have changed with growth on glycerol.

4.5. Conclusions and future directions

The present study has given insight into the proteomic differences between growth on glucose or glycerol. Future studies could include the refinement of proteomics methods and modification of growth conditions. Changes in the proteomic workflow could include use of larger 2D gels to allow detection and identification of more proteins, or use of quantitative shotgun methods such as SILAC or iTRAQ. It is more likely that low abundance proteins would be detected using shotgun analysis. Use of larger numbers of biological replicates, for example $n = 10$, would give statistically significant detection of smaller changes in protein amount (Karp & Lilley, 2005). The growth conditions used showed a potential stress response affecting protein processing in cells shifted from glycerol to glucose. It would be interesting to find out if the transition from cells grown on glucose to glycerol gave a similar response. Subsequent research should investigate the *in vivo* consequences of change in enzyme amount, this potentially could be done using specific enzyme inhibitors or gene manipulation which is well established for *S. cerevisiae*. Future studies could therefore combine manipulation of enzyme amounts with proteomic and metabolite analysis.

The overall goal of this research was to examine whether enzyme amounts changed in a metabolically significant way between fermentative growth on glucose and oxidative growth on glycerol. The results showed selective increases among some of the enzymes for conversion of glucose to ethanol for cells grown on glucose. Although this is an expected result the relationship between change in protein amount and metabolic output requires more detailed analysis in many cellular systems.

5. References

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