

Proteomic Analysis of Chinese Hamster Ovary Cells Producing Glycosylated Monoclonal Antibodies

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Therapeutic monoclonal antibodies (MAb) are produced as secreted complex glycoproteins from mammalian cell systems and represent one of the most important classes of therapeutic medicines for the treatment of a variety of human diseases. Their benefit in health care and high economic impact provide the driving force for the development of improved production levels with the focus of optimizing clinical efficacy. One important issue is the optimization of monoclonal antibody production. A frequent approach used to address this challenge is the engineering of mammalian cell lines to increase antibody production levels through genetic manipulation. Valuable information can then be obtained by monitoring the effects of genetic changes on the biochemistry of the cell associated with MAb production. Global protein expression profiling of mammalian cells used for the production of biopharmaceuticals may reveal key biochemical characteristics associated with MAb-producing cell lines. A better understanding of these characteristics can in turn lead to more rational strategies for cell line and process development.

The proposed research relates to a larger NSERC Strategic Network (MAbNet) Grant to develop and establish a novel platform for the large-scale manufacture of specific glycoforms of therapeutic monoclonal antibodies. The efficacy of these recombinant MAbs will be enhanced by the control of their glycosylation profiles. The work presented in this thesis will assist MAbNet in meeting their objectives. Specifically, we use 2D-Differential In-Gel Electrophoresis (2D-DIGE) to quantify protein expression differences between EG2-hFc1-producing Chinese Hamster Ovary cells (CHO-1A7) with its parental cell line (CHO-BRI). Here, we identified 34 unique differentially expressed proteins associated with EG2-hFc1 production that relate to various biological processes including protein processing, carbohydrate metabolism, amino acid metabolism, energy metabolism, apoptosis, and cell proliferation pathways.

The majority of identified significant protein expression changes and their associated metabolic processes seem to prioritize energy production in CHO-1A7 cells. Due to the metabolic load of recombinant antibody production, the CHO-1A7 cell line attempts to meet the energy requirements needed for recombinant protein biosynthesis while maintaining cell viability and efficient protein folding mechanisms.

A 2-D proteome reference map was also constructed for the CHO-BRI host cell line containing 131 identified protein spots. The map provides information that will further expand our understanding of this particular cell line. It will be a useful tool for studies investigating physiological responses and protein expression patterns of CHO-BRI to genetic and environmental perturbations.

The set of identified differentially expressed proteins provides data on the downstream changes in protein expression due to genetic manipulation, and furthermore can provide targets for cell-line specific optimization of antibody production. The work described in this thesis furthers our understanding of antibody production in a specific CHO cell line.

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List of Abbreviations

2D-DIGE	Two-Dimension Differential In-Gel Electrophoresis
2D-GE	Two-Dimension Gel Electrophoresis
SDS-PAGE	Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis
ACN	acetonitrile
Aco2	Aconitate hydratase
Acot2	Acyl-coenzyme A thioesterase 2
Actb	β -actin
ADCC	antibody-dependent cell-mediated cytotoxicity
ALC	Average of Local Confidence
Aldh	Aldehyde dehydrogenase
Ast	Aspartate aminotransferase
Atp5b	ATP synthase subunit beta
BRI	Biotechnology Research Institute
BSA	bovine serum albumin
BVA	Biological Variation Analysis
CDC	complement-dependent cytotoxicity
CDR	complementarity-determining regions
CHAPS	3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulphonate
CHO	chinese hamster ovary
Chp	Calcium-binding protein p22
Cs	Citrate synthase
CID	collision-induced dissociation
CMV	cytomegalovirus
Da	Dalton
DAVID	The database for annotation, visualization, and integrated discovery
DB	database
DHFR	dihydrofolate reductase
DI	deionized water
DIA	Differential In Gel Analysis
Dld	Dihydrolipoyl dehydrogenase
DTT	dithiothreitol
EGFR	Epidermal Growth Factor Receptor
ER	endoplasmic reticulum
ESI	electrospray ionization
FDR	False Discovery Rate
Fv	variable fragment
Gluc I	α -1,2 glucosidase I
Gluc II	α -1,3 glucosidase II
GlcNAc	<i>N</i> -acetylglucosamine
GO	Gene ontology
Gpd2	Glycerol-3-phosphate dehydrogenase
GRP75	Heat shock 70 kDa protein 9/Mortalin

GRP94	Endoplasmic
hFc	human constant fragment (crystallizable fragment)
Hibadh	3-hydroxyisobutyrate dehydrogenase
Hist1h2ab	Histone H2A type 1
Hsd17b10	3-hydroxyacyl-CoA dehydrogenase type-2
Hsp90aa1	Heat shock protein HSP 90-alpha
Hspd1	60 kDa Heat shock protein
IAA	iodoacetamide
ICAT	isotope-coded affinity tag
IDs	identifications
IEF	isoelectric focusing
IgG	immunoglobulin G
IPG	immobilized pH gradient
iTRAQ	isobaric tag for relative and absolute quantitation
LC	liquid chromatography
LFQ	label-free quantification
Lmna	Lamin A
Lmnb1	Lamin B1
LOESS	Local regression normalization
MAb	monoclonal antibody
MALDI	matrix-assisted laser desorption ionization
Man I	mannosidase I
MS	mass spectrometry
Mthfd1l	Methylenetetrahydrofolate dehydrogenase
MvA	plot of the variability of the data (M) as a function of mean (A)
Ndufs3	NADH dehydrogenase
OST	oligosaccharyl transferase
PBS	phosphate buffered saline
Pcna	Proliferating cell nuclear antigen
Pdcd6	Programmed cell death 6
PDIA6	Protein disulfide-isomerase A6
Phb	Prohibitin
pI	isoelectric point
Plod3	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3
PMF	peptide mass fingerprint
PMT	photomultiplier tube
Polr2e	DNA-directed RNA polymerase I, II, and III subunit RPABC1
ppm	parts per million
PTM	post-translational modification
Rplp0	60S Acidic ribosomal protein P0
SDS	sodium dodecyl sulfate
SILAC	stable isotope labeling by amino acids in cell culture
SPIDER	Software Protein Identifier
TCA Cycle	Tricarboxylic Acid Cycle
TEMED	tetramethylethylenediamine
TLC	Total of Local Confidence

Trap1
Vim

Heat shock 75 kDa protein/Tumor necrosis factor receptor-associated protein 1
Vimentin

Chapter 1

Introduction

The following sections (1.2.1, 1.2.2, 1.2.3 are based on a published review by Raymond Ho and Brendan J. McConkey (2011), Modulating glycosylation of recombinant monoclonal antibodies for therapeutic applications, Current Topics in Biotechnology, Vol. 6, pages 57-69. [1]

The concept of a cell can be seen as analogous to a manufacturing plant: an intricate network of blueprints and protocols, which are mediated by a number of personnel working in a concerted fashion to produce high-value products. As with the manufacture of any goods, it is essential to optimize the production process by regulating and fine-tuning the various steps within the production pipeline. This is possible by improving our understanding of the complex biological processes - including gene and protein expression, metabolism, host cell engineering, and post-translational modifications - that govern these biological manufacturing platforms. As a result, not only do commercial industries benefit from such research, but groups involved in health care are also impacted due to the availability of these products targeted towards therapeutic and diagnostic applications.

Mammalian cell expression systems are the most widely used manufacturing platform for the production of recombinant proteins. A variety of alternative expression systems exist, such as transgenic yeasts, fungi, plants, and insect cell lines, that offer some advantages over mammalian systems, such as high titers of protein expression and relatively inexpensive growth media [2-14]. However, the optimization of cost, productivity, efficacy, and potential for immunogenicity remains a challenging task for the biopharmaceutical industry for both mammalian and non-mammalian systems. With the advancements in recombinant DNA technology, media design, bioprocessing development, and metabolic engineering techniques, Chinese hamster ovary (CHO) cells have become the mammalian cell line of choice, and has become the industry's workhorses for nearly 70% of all recombinant therapeutic proteins produced today [15]. Significant improvements in protein production in CHO cells have lead to titers up to 10 g/L in order to satisfy the growing market demands for recombinant therapeutics [16].

1.1 Chinese Hamster Ovary Cells (CHO Cells)

CHO cells are a cell line derived from the ovary of the Chinese hamster (*Cricetulus griseus*), and although they are often used in biological and medical research, they are most commonly known for use in biopharmaceutical production. The first immortalized Chinese hamster ovary cell line was isolated from a primary tissue culture by Dr. Theodore T. Puck in 1957 [17]. Since then, several cell lines have been derived from the original, including the CHO-K1 cell line, which is a widely used proline-auxotrophic strain. The CHO-K1 strain was mutagenized to derive the CHO-DUKX, a cell line with a deletion of one *dhfr* allele, resulting in a deficiency in dihydrofolate reductase (DHFR) activity [18]. Similarly, a deletion in both *dhfr* alleles from the CHO-pro3- strain, another derivative of the original CHO cell line that requires proline, yielded the CHO-DG44 cell line resulting in a more stable DHFR-minus cell line [19].

To develop CHO cell line with the ability to produce a protein of interest, clonal selection is performed by co-transfection of the gene of interest with a functional copy of the DHFR gene, followed by growth in media devoid of glycine, hypoxanthine, and thymidine. To enhance chances of securing a high producing strain, the transgene can be efficiently amplified by culturing transfected CHO cells in high levels of methotrexate (MTX), which decreases DHFR activity. The mere presence of an exogenous DHFR gene is insufficient for survival; rather, by applying this selection pressure, the transfected CHO cells adapt by amplifying the copy number of the DHFR locus, which in turn co-amplifies the transgene [15].

Several reasons can be attributed to the popularity of CHO cells as a recombinant protein manufacturing platform. Firstly, since CHO cells have been developed under Good Manufacturing Practice conditions [20], it may be easier to obtain market approval of therapeutic proteins from regulatory agencies such as the FDA. Secondly, powerful gene amplification systems allow for increased specific productivity in a mammalian system such as CHO cells. Thirdly, CHO cells have greater capacity for proper protein folding and appropriate post-translational modifications, producing recombinant proteins with glycosylation patterns that are compatible with and similar to those produced in human glycoproteins. Finally, CHO cultures can be adapted to and grown in serum-free suspension conditions, which can facilitate large-scale batch cultures in bioreactors. The use of serum-free cell culture media provides several beneficial reasons over the use of serum-based media including the cost of the serum, the

undefined nature of the serum, and the batch-to-batch variability in composition of the serum. Additionally, it is less difficult to purify recombinant proteins produced by a cell line from a serum-free supernatant [21].

1.2 Monoclonal Antibodies

CHO cells continue to be the dominant eukaryotic expression system for the production of complex recombinant proteins due to a number of reasons including the ability to perform post-translational modifications and ensure proper folding of the final protein product. As a result, CHO cells have become the predominant expression vehicle for the production of recombinant monoclonal antibodies (MAbs).

For therapeutic applications, MAbs represent one of the most important classes of therapeutic medicines for the treatment of a variety of human diseases. Their benefit in health care and high economic impact provides the driving force for the development of improved production levels with the focus of optimizing clinical efficacy. Presently, there are 20 rMAbs licensed and marketed, six of which are known as blockbuster therapeutic monoclonal antibody products (i.e. Avastin, Herceptin, Remicade, Rituxan, Humira and Erbitux) with more than \$25 billion in annual sales [22]. Thus, large-scale processes must be established, with respect to bioreactor designs and culture parameters associated with high productivity levels, that meet market demands while at the same time reducing costs [23; 24].

The clinical success of therapeutic antibodies continues to drive development, with a focus on optimizing clinical efficacy which may potentially lead to a lowered cost of treatment by reducing the dose and duration of treatment. However, optimization of antibodies for production of next-generation therapeutics is still a nascent field.

1.2.1 Structure and Function of MAbs

Structure

Currently licensed MAbs are derived from the immunoglobulin G (IgG) class [22]. In the simplest case, the structure of an IgG molecule consists of two identical heavy chains and two identical light chains that associate with each other through hydrogen bonding, van der Waals interactions and

disulfide linkages (**Figure 1-1**). Each individual light chain consists of a variable domain (V_L) and a constant domain (C_L), whereas each individual heavy chain consists of a variable domain (V_H) and three constant domains (C_{H1} , C_{H2} , and C_{H3}). The discrete domains within antibody molecules have various functions and may be specifically targeted for genetic manipulation. Two identical Fab fragments, each of which contains a light chain and the V_H and C_{H1} domains of the heavy chain, confer antigen specificity. Here, the Fv fragment (consisting of the V_H and V_L regions as depicted in **Figure 1-1**) houses complementarity-determining regions (CDRs) that are highly variable in their amino acid sequences. This hypervariable region results in an antibody's specificity for a single epitope (i.e. the part on an antigen recognized by an antibody). The Fc fragment consists of two heavy chain fragments, each containing C_{H2} and C_{H3} domains interlinked through disulfide bonds. The Fc fragment has been shown to confer effector function through interactions with lymphocyte receptors (Fc γ Rs) and can subsequently trigger antibody-dependent cell-mediated cytotoxicity (ADCC), a mechanism by which a cell-mediated immune response is activated against target cells [25]. The overall mass of a typical IgG molecule is approximately 150 kDa.

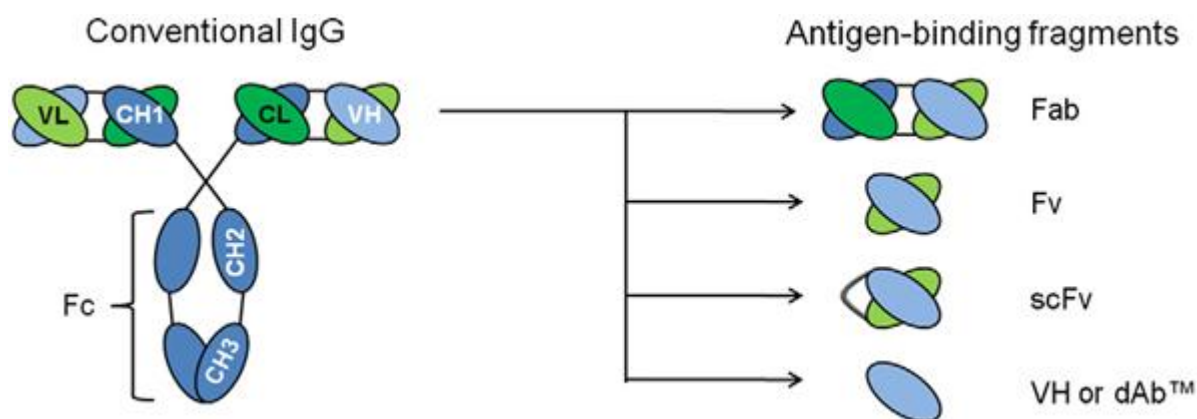


Figure 1-1. Structure of an IgG antibody molecule. Conventional IgG shown on the left with two symmetric Fab (antigen-binding) fragments and one Fc (constant) fragment. Heavy chains (shown in blue) and light chains (shown in green) are interlinked by disulfide bonds. Conserved sites of N-glycosylation occur on Asn297 residues of the heavy chain CH2 domains. Antigen-binding fragments shown on the right represent different types of antibodies variants under research and development for various therapeutic applications. Fc = crystallisable fragment, Fab = Fragment antigen-binding, Fv = Fragment variable, scFv = single-chain fragment variable, VH = Heavy chain [26].

All human-derived IgG molecules contain a single conserved *N*-glycosylation site (Asn297) within the Fc fragment at each CH2 domain of both heavy chains (**Figure 1-1**). The presence or absence of oligosaccharides at this constant Fc region of an IgG monoclonal antibody has been observed to have a profound impact on the antibody's effector functions and efficacy [27-29]. Oligosaccharides attached in the Fab region where antigen binding occurs may also affect antigen specificity [30-32]. Electrospray ionization mass spectrometry coupled with endoglycosidases have been used for glycan analysis of both Fab and Fc, which may contribute to the development of therapeutic IgG with customized glycan profiles [32]. Therefore, it is important to develop methods that allow the production of recombinant MAbs, particularly for therapeutics, with specific and consistent glycosylation profiles.

Function

Antibodies are a crucial part of the immune system, in which they identify and neutralize foreign objects. An antibody is a protein produced by B-cells that recognizes an antigen, a unique part that is displayed on the surfaces of foreign and/or potentially harmful invaders such as bacteria and viruses. The therapeutic potential of monoclonal antibodies lies in their high specificity to their target antigen. The therapeutic antibody initially forms an antigen-antibody immune complex that can directly neutralize its target, for example, by blocking a part of the microbe that is essential for its invasion and survival. Alternatively, an antibody can tag a microbe for attack by subsequent activation of a series of downstream biological cascades, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cell cytotoxicity (CDC) that result in the elimination of the target [25].

In ADCC, surface antigens are bound by antibodies, which then recruit effector cells (e.g. natural killer cells, macrophages, neutrophils, and eosinophils) of the immune system that actively lyses the target cell. In CDC, the binding of antibodies to surface antigens of a bacteria, for example, initiates activation of the complement system, which can eliminate the microbe in one of two ways. First, the binding of the antibody marks the microbe for ingestion by phagocytes in a process called opsonization. Secondly, components of the complement system forms the membrane attack complex, which are transmembrane channels that disrupt the phospholipid bilayer of target cells, resulting in cell lysis and death.

1.2.2 Glycosylation and its Importance in Protein Therapeutics

Glycosylation of proteins is a fundamental biological process, and its impact on mechanisms of action, treatment costs, and clinical efficacy in recombinant antibody-based therapeutics research is of ongoing interest [25; 33-35]. Glycosylation is a post-translational modification process where varying glycan structures are attached to a number of glycosylation sites on a protein. There are two forms of glycosylation that can occur on a given protein: *N*-glycosylation and *O*-glycosylation. *O*-linked glycans, commonly of the mucin-type, are initially catalyzed by an *N*-acetylgalactosamine transferase enzyme, which transfers an *N*-acetylgalactosamine to a serine or threonine residue on the fully folded protein and confers protein stability [36]. Protein *N*-glycosylation initially occurs in the endoplasmic reticulum (ER) through various sequential oligosaccharide additions or removal before transported to the Golgi apparatus for further processing.

The Golgi *N*-glycan processing pathways are not always pursued to completion in a reaction pathway, resulting in a heterogeneous mixture of glycoprotein products. Many secreted glycoproteins are glycosylation pathway intermediates, producing a pool of different glycoforms. For a given glycoprotein, the distribution of glycans is collectively referred to as the glycoform profile. Macroheterogeneity refers to the variation in the site-occupancy of *N*-glycans at *N*-glycosylation sites on a protein. In other words, a protein may have multiple *N*-glycosylation sites, but a particular site may or may not be occupied by an *N*-glycan. A number of aspects can influence macroheterogeneity. For example, a study demonstrated that the extent of core *N*-glycosylation of tissue plasminogen activator is dependent on disulfide bond formation. Covalent bonds occur co-translationally in the ER and may hinder the ability of glycosylating enzymes to access the particular glycosylation site, leading to macroheterogeneity [37]. By contrast, the varying glycan structure within the same glycosylation site of glycoprotein molecules is referred to as microheterogeneity and has been broadly investigated in mammalian cell systems used to produce recombinant proteins [38].

Depending on the types and number of glycan structures attached to a MAb, it has been shown to impact its biological properties, including potency [25; 33; 39-42], clearance rate [43-46], solubility [47; 48], secretion [43], and immunogenicity [43; 49; 50]. For example, a reduction of fucosylation by genetic engineering methods have been attributed to enhanced levels of ADCC [25; 33; 51-55]. Similarly, the

presence of terminal sialic acid residues on glycan branches extends a glycoprotein's *in vivo* circulatory half-life [45; 46; 56].

For any given glycoprotein, the glycan profile depends on a number of factors including: (i) the particular host cell line used and its corresponding complement of glycosylation enzymes [10; 13; 43; 57; 58]; (ii) medium composition [43; 59]; (iii) culture conditions [60]; and (iv) method of downstream processing. In addition, the protein primary structure, such as the number and location of glycosylation sites, will directly influence the glycosylation profile [33].

Thus, numerous strategies have attempted to control and enhance the resulting glycosylation patterns of glycoproteins by implementing metabolic control strategies (ie: media supplementation and nucleotide sugar precursor feeding) [61-68], employing cell-engineering methods to reduce fucosylation [28; 41; 69; 70] in addition to increasing sialylation [45; 46], as well as monitoring cell culture conditions [60; 71-75].

Due to the impact of varying glycosylation patterns on the pharmacokinetics and efficacy of therapeutic recombinant MAbs, a thorough understanding of the glycosylation pathways and the enzymes involved is vital for the production of glycoprotein drugs with consistent glycoform profiles.

1.2.3 Mechanism of Glycosylation

During the protein elongation phase of protein translation in the ER, *N*-glycosylation can occur by the transfer of a dolichol-linked oligosaccharide precursor, Glucose₃Mannose₉*N*-acetylglucosamine₂ (Glc₃Man₉GlcNAc₂) by oligosaccharyl transferase (OST) to an available consensus sequence Asn-*X*-Ser/Thr, where *X* can be any amino acid except proline (**Figure 1-2**). In IgG, this consensus sequence is conserved at Asn297 within the C_{H2} domain of the Fc fragment. The oligosaccharides attached are composed of a core pentasaccharide with added variable units of fucose, galactose, *N*-acetylglucosamine (GlcNAc) and sialic acid.

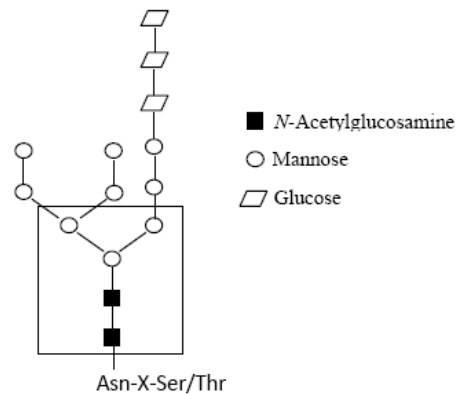


Figure 1-2. *N*-glycan Precursor. Glc₃Man₉GlcNAc₂ attached to Asn-X-Ser/Thr consensus sequence. The box indicates the core pentasaccharide.

After attachment of the initial Glc₃Man₉GlcNAc₂ oligosaccharide, this precursor is further processed by α -1,2 glucosidase I (Gluc I), which removes the terminal glucose, followed by α -1,3 glucosidase II (Gluc II), which sequentially removes the next two glucose monosaccharide subunits. Following glycoprotein folding, ER resident mannosidase I (Man I) removes at least one mannose monosaccharide from the attached glycans, forming the core structure, before the glycoprotein is actively transported to the Golgi apparatus [43; 76] (**Figure 1-3**).

The Golgi itself contains several enzyme groups that are involved in the *N*-glycosylation pathway that catalyzes the addition of various sugars to the existing attached glycan structure thereby forming high-mannose, hybrid, or complex-type glycans (**Figure 1-3**). High-mannose structures consist of multiple mannose residues attached to the common tri-mannosyl core structure. Complex-type glycans have two or more GlcNAc residues as well as variable numbers of galactose and sialic acid residues attached to the nonreducing end of the tri-mannosyl core. Finally, the hybrid type has branches of both the high-mannose and complex type glycans [8]. The number of branches determines the antennarity of the glycan structure. *N*-acetylglucosaminyltransferase catalyzes the addition of GlcNAc to the mannose sugars of the glycan core structure. The terminal ends of the glycan structure can be extended through addition of galactose and sialic acid by galactosyltransferase and sialyltransferase respectively. Fucosyltransferase catalyzes the addition of fucose to GlcNAc proximal to the protein, or attached to the outer arm GlcNAc further down the glycan branch.

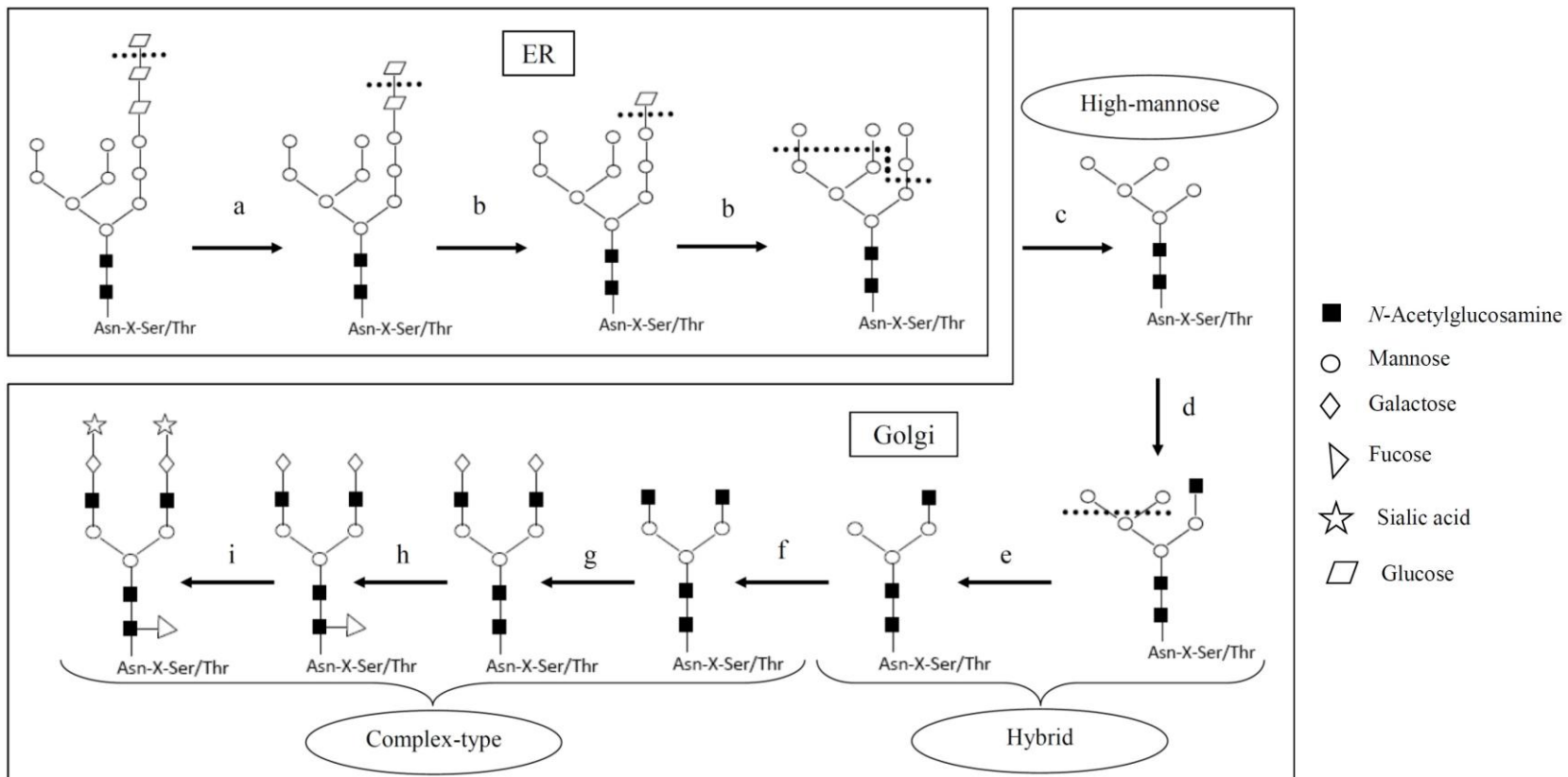


Figure 1-3. Processing of N-Glycans in the Glycosylation Pathway. Enzymes: (a) α -1,2 glucosidase I, (b) α -1,3 glucosidase II, (c) mannosidase I, (d) *N*-acetylglucosaminyltransferase I, (e) mannosidase II, (f) *N*-acetylglucosaminyltransferase II, (g) β -1,4-galactosyltransferase, (h) α -1,6-fucosyltransferase, (i) α -2,3-sialyltransferase. Incomplete Golgi transferase reactions often lead to the export of *N*-glycan intermediates from the Golgi resulting in a heterogeneous mixture of glycoforms. Adapted from [43].

1.3 Proteomics

As genomics is associated with the study of genes, proteomics is the large-scale study of proteins. In general, proteomics involves the qualitative and quantitative comparative analysis of an organism's complete complement of proteins (i.e. the proteome) to promote our understanding of various biological processes. In other words, proteomics involves the analysis of changes in an organism's protein expression profile when subjected to various biological perturbations. The utility of proteomic studies is realized when, for example, global expression profiling of mammalian cells give insights for genetic intervention to possibly create better host cell lines, or even provide rational strategies for cell line and process development.

Coupling large-scale proteomics with mass spectrometry, thousands of proteins can be identified from a complex protein extract. For this to be successful, however, sufficient protein separation is required for unambiguous identifications. One major approach to protein separation widely used in proteomics is the gel-based method known as two-dimensional gel electrophoresis (2D-GE).

1.3.1 2D-GE

Two-dimensional gel electrophoresis (2D-GE) has been a traditional workhorse of proteomics for analyzing whole cell proteomes. In this method the proteins are separated on a polyacrylamide gel in two separate steps. The first dimension step, isoelectric focusing, separates the proteins horizontally across a gel strip according to their isoelectric points (pI). In other words, the proteins are subjected to electric currents that cause them to migrate along the pH gradient of the gel strip, and stops at the point where the protein has an overall net charge of zero. The second dimension step follows a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) procedure that separates proteins vertically down a polyacrylamide gel from the gel strip according to their molecular weight. Following separation, proteins are visualized by either Coomassie or silver staining and relevant spots can be subsequently identified by mass spectrometry. Ideally, each spot on the resulting gel corresponds to one protein in the original proteome. However, this is not always the case due to proteins of similar size, pI, and/or fragments that may resolve to the same location on the 2D array. Though 2D-GE is a relatively simple visual method for mapping differences in protein expression, this approach is limited due to its low

detection sensitivity, poor solubility of membrane proteins, relatively low throughput, and low reproducibility of gels [77]. Two-dimension differential in-gel electrophoresis (2D-DIGE) circumvents many of the issues associated with traditional 2D-GE, such as reproducibility and limited dynamic range, and allows for more accurate and sensitive quantitative proteomic studies.

1.3.2 2D-DIGE

2D-DIGE is an important proteomics technique that allows relative quantitation of each protein spot after the simultaneous separation of thousands of proteins within a protein extract. The technique relies on the labeling of samples, prior to separation, with one of three spectrally resolvable fluorescent CyDyes (Cy2, Cy3, and Cy5), allowing multiplexing of samples into the same gel. Protein spots are detected and quantitated based on the intensity of the fluorescent emission when they are scanned with a specific wavelength corresponding to each CyDye. The scanned gel images and the corresponding intensity values of each protein spot can be analyzed using DeCyder (GE Healthcare, Uppsala, Sweden) and subjected to statistical analysis to generate a list of differentially expressed protein spots.

This system enables the expression levels of two or more different complex protein samples to be directly compared on the same 2D gel, which substantially reduces gel-to-gel variability as experienced in the traditional method. In addition, the sensitivity of fluorescent labeling is superior compared to colorimetric methods. The detection limit is in the order of 150 – 500 pg for a single protein, with a linear dynamic range of five orders of magnitude. In comparison, silver-staining has a detection limit of 1 ng of protein and Coomassie Brilliant Blue G250 staining with sensitivity around 10 ng, with linear dynamic ranges of one- or two- orders of magnitude [78].

Each CyDye DIGE fluor contains an N-hydroxy succinimidyl ester reactive group, which can covalently bond with the terminal amino group of lysine side chains forming an amide linkage. A minimal labeling method was employed in the present work to ensure that visualized proteins are labeled with a single dye molecule only. The three fluors are mass matched and add approximately 500 Da to the labeled protein, and therefore the increase in molecular weight between protein samples remains consistent. Additionally, the pI of the protein is maintained upon labeling as all three fluors contain an intrinsic charge of +1. In order to maximize the amount of protein available for MS, coomassie-stained

preparative gels are generated, and selected protein spots are excised and subjected to protein identification via MS.

1.3.3 Protein Identification via Mass Spectrometry

To determine the identity of a protein, the excised gel spot is first subjected to the method of in-gel digestion, where the protein spot of interest is digested proteolytically. The resulting peptide mixtures are then further resolved and enriched by liquid chromatography (LC) or directly analyzed by MS. A mass spectrometer consists of three main parts: an ion source for sample ionization to generate gas phase ions; a mass analyzer for separation of ions according to their mass to charge (m/z) ratios; and a detector, which measures the intensity of the ions [79].

Two common ionization methods employed are electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), which produce ions in the gas phase from macromolecules such as proteins and peptides. The list of the experimentally measured masses of the resulting proteolytic peptides, referred to as a peptide mass fingerprint (PMF), are used as input to search against a protein database of predicted masses that would arise from digestion of known proteins. Identification is achieved when there is a match between the PMF of the target protein and a specific protein candidate in the database. However, very little structural information can be gained from the simple mass spectrum obtained from one round of MS [80].

In tandem MS, the ionized peptides (parent ions) from the first round of MS are selected and fragmented via bombardment with an inert gas, resulting in fragmented ions (daughter ions), generating MS/MS spectra that reveals structural information that is related to the sequence of the peptide [79]. This MS/MS data can be used by algorithms such as PEAKS [81] to sequence the target peptides by method of *de novo* sequencing.

Due to the lack of available genomic information, previous CHO proteomic studies were performed by searching against protein databases of related species including *Mus musculus* (mouse), *Rattus norvegicus* (rat) and *Homo sapiens*, though this leads to a reduction in stringency for the protein identifications. In 2011, the first draft genomic sequence of the CHO-K1 cell line was published [82], simplifying CHO proteomic analyses. The 2.45 gigabase long genome features 24,383 predicted genes on

21 chromosomes. The availability of the CHO genomic sequence facilitated a complementary large-scale proteomic study in which 6164 proteins were identified [83]. Additionally, another study showed a considerable improvement in both the number of proteins identified and the confidence of those identifications by searching against a CHO-specific protein database [84]. The new sequence information thus paves the way for improved interpretation of proteomic results from CHO cells.

1.3.4 Data Analysis Using PEAKS Studio 5.3 Proteomic Mass Spectrometry Software

PEAKS Studio 5.3 is a complete software package that is commercially available from Bioinformatics Solutions Inc (ON, Canada) for proteomics mass spectrometry data analysis. It can incorporate raw mass spectrometry data from all major mass spectrometry vendors, and analysis is performed in a user-friendly easy-to-use graphical interface. PEAKS is an effective tool for peptide and protein identification by employing multiple analytical algorithms such as the combination of auto *de novo* sequencing with database searching. The inChorus tool, which combines the search results of multiple search engines, can help improve the overall protein coverage as well as the confidence of protein identification. The software can also characterize PTMs and potential mutations. For a target organism that does not have a protein database, the SPIDER tool performs homology searches on a database of a closely related species. Additionally, data resulting from a variety of quantitative proteomic techniques (ICAT, iTRAQ, SILAC, LFQ, and user defined labels) can also be analyzed. Finally, PEAKS can handle large datasets by utilizing high-throughput workflows (**Figure 1-4**) whereby multiple tasks can be set to run automatically upon the completion of a previously assigned task. Results can then be visualized, validated, and reported.

Peptide *de novo* sequencing is PEAKS' best-known function, whereby a peptide sequence is derived directly from tandem mass spectra, without the use of a protein database. The *de novo* sequencing algorithm of PEAKS was evaluated and compared with four other commonly used algorithms, and was found to perform best with the greatest sensitivity in analyzing QSTAR and LCQ mass spectrometer data [85]. For each amino acid in the *de novo* sequence, a local confidence score that ranges from 0% to 99% is assigned, indicating how confident the algorithm is with a particular amino acid at a particular position. The ALC score is the average of all the local confidence scores of each individual amino acid within a peptide sequence, and this reflects the average correct ratio of amino acids in the sequence. The TLC

score reflects the expected total number of correct amino acids in a particular sequence. The two aforementioned measurements can be used to evaluate the correctness of the *de novo* peptide sequence.

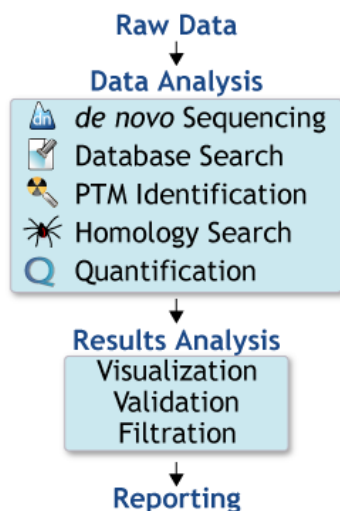


Figure 1-4. PEAKS General MS Data Analysis Workflow. Filtered raw MS scans undergo *de novo* sequencing followed by database searching for peptide and protein identifications. Further searches and refinement can also be performed with the SPIDER and PTM identification tools.
(<http://www.bioinfor.com/peaks/features/overview.html>)

By incorporating PEAKS' *de novo* sequencing results into the PEAKS DB search tool, the accuracy and sensitivity of the peptide identification is significantly improved [86]. PEAKS DB functions by the conventional database search approach where a corresponding spectrum is searched against a database of known spectra. The accuracy is measured by the false discovery rate (FDR), which reports the expected percentage of false positives in all identifications above the score threshold within a given dataset. Typically, PEAKS DB is run in conjunction with a target-decoy database to estimate the FDR. This method combines a target database with a decoy database of the same size. The search is then conducted on the two combined databases and the number of false positives can be estimated by the search engine's outcome. The user can define the FDR score threshold such that increasing the score would increase accuracy. However, this will at the same time reduce sensitivity. Therefore, the setting for the FDR score threshold needs to be considered with respect to an acceptable expected number of false positives.

The inChorus function is another useful tool that combines the database search results of PEAKS DB with other search engines (Mascot, X!Tandem, Omssa, and Proteome Discoverer (Sequest)) as shown in **Figure 1-5**. Since the search engines use independent algorithms, they can be used to confirm each other's results. As a consequence, the confidence of the identification as well as the overall coverage is enhanced. Result validation by employing the FDR is also applied to determine each engine's contribution to the final result. PEAKS offers a summary view that provides a comprehensive visual examination of the analysis results with subsequent result statistics. Score thresholds can also be specified in the summary view to quickly and efficiently filter results. Final filtered results can be exported for publication or result sharing with non-PEAK users.

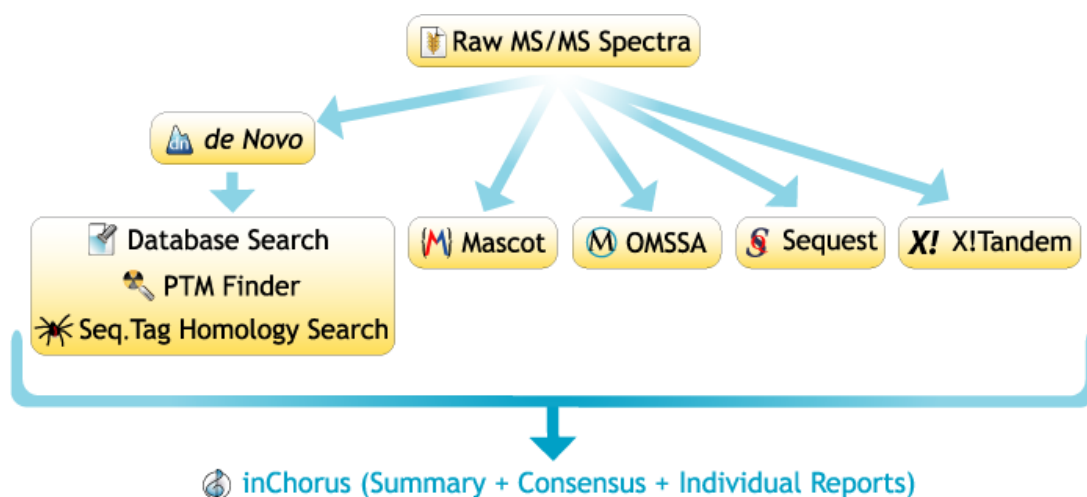


Figure 1-5. PEAKS inChorus Multi-Engine ID Tool. A built-in tool that combines the results from different database search engines to enhance the accuracy and sensitivity of peptide identifications. (<http://www.bioinfor.com/peaks/features/inchorus.html>)

Chapter 2

Comparative Proteomic Analysis of Antibody-Producing CHO Cells

2.1 Introduction

The present work provides a better understanding of the *in vivo* biochemical changes within CHO cells that are involved in recombinant protein production via a proteomics approach. The parental non-producing strain, termed CHO-BRI developed at the Biotechnology Research Institute (Montreal, Canada), was derived from the CHO-DXB11 subtype, a cell line lacking dihydrofolate reductase activity. Transfection of CHO-BRI resulted in a cell line that expresses a chimeric heavy chain camelid MAb known as EG2-hFc1. This recombinant cell line was termed CHO-1A7. The objective of performing a comparative proteomic analysis is to study systemic changes at the protein level between CHO-BRI and CHO-1A7 and to identify important mechanisms related to these changes that are associated with EG2-hFc1 production. Differentially expressed proteins will be examined and identified. These may include proteins that are related to protein translation and protein folding machinery as a response to the production of a recombinant product.

EG2-hFc1, as described previously by Zhang et al. (2009), is a chimeric heavy chain antibody constructed by fusing a single domain portion named EG2 to the fragment crystallizable region of human IgG1 (hFc1), resulting in a bivalent 84 kDa construct [87]. The EG2 domain originates from a heavy chain antibody collection that is produced in *Camelidae* (llamas, camels, alpacas) immunized with a variant of EGFR, a protein that is widely known as a tumor marker [88]. Features of camelid single domain antibodies include high thermostability [89], relatively high proteolytic resistance [90], high expression yields in various expression systems[87], and relatively small size, making them ideal candidates as therapeutic agents. Although this kind of antibody lacks a light chain and is composed of a heavy chain dimer only, it is fully functional in antigen recognition.

EG2-hFc1 targets cells that express a receptor tyrosine kinase known as epidermal growth factor receptor (EGFR). EGFR plays a fundamental role in the control of certain cellular processes including growth, proliferation, and angiogenesis; and mutations in the EGFR gene are associated with a variety of human cancers [91-93]. A recent *in vivo* study tested the tumour-targeting properties of EG2-hFc to

glioblastoma tumours. The study demonstrated high apparent affinity of EG2-hFc1 to EGFR (sub-nM range), long plasma half-life and slow clearance rate (12 hours), and enhanced tumour penetration [93]. These results suggest that EG2-hFc1 may be an excellent therapeutic candidate for targeting EGFR-overexpressed cells in various tumours. As such, it is important to implement strategies to enhance both the specific productivity and quality of EG2-hFc1 in CHO cells, particularly if it is to be used as a therapeutic agent.

By performing a comparative proteome analysis between CHO-BRI (non-producer) and CHO-1A7 (EG2-hFc1-producer), the cellular proteins identified associated with EG2-hFc1 production may provide potential cell-engineering targets in an attempt to develop more efficient CHO cell expression systems. Additionally, the protein expression patterns can be correlated with particular glycoform profiles associated with the CHO-1A7 strain.

2.2 Materials and Methods

2.2.1 Cell Lines

Cell lines were kindly provided by Dr. Yves Durocher at the Biotechnology Research Institute (BRI) (Montréal, Canada). The CHO-1A7 cell line was engineered for the stable expression of the chimeric heavy chain antibody EG2-hFc1. It originates from CHO-BRI cells that are a suspension-adapted and serum-free subtype of DXB11-CHO cells, a cell line lacking dihydrofolate reductase activity [18; 94].

The development of the cell lines, cell culture and transfection was contributed by Mr. Igor Slivac from Dr. Yves Durocher's lab at the BRI. The cell line was cultured in suspension culture conditions inside a humidified incubator at 37°C and 5% of CO₂. The EG2-hFc1 cDNA was cloned into the pTT44 vector, which is a modified pTT5 vector [95] containing a puromycin expression cassette. The sequence expressing EG2-hFc1 is under the control of a cytomegalovirus (CMV) promoter. The parental strain of CHO-BRI cells (at density 2.0×10^6 cells/ml and 99% viability) were transfected with the linearized construct pTT44-EG2hFc1 using PEI_{max} as a transfection reagent in 2 ml of CD DG44 media (Invitrogen, ON, Canada). The following day, the cells were put under puromycin selection (10 µg/ml) until the pool of resistant transfectants finally reached 80-90% viability. Single cell dilution in 96-well plates was applied to isolate the best-producing clone. After routine microscopic investigation of the colony development and a dot blot analysis, the clone named CHO-1A7 was chosen and expanded for cryopreservation. The clone was found to have a specific growth rate of about 0.03 h⁻¹ and a doubling time of around 24 hours. Clone stability was subsequently assessed by continuous maintenance of 25 ml cell culture for 64 days in PowerCHO-2CD media (Lonza, ON, Canada) supplemented with glutamine (8 mM). During 8 days batch cultivation, the volumetric productivity was 50-60 mg/l of EG2-hFc1.

Culture media samples from parental CHO-BRI cells and the newly established CHO-1A7 cell line that constitutively produces EG2-hFc1 were harvested after 8 days of batch cultivation. Six parallel cultivations were carried out in 250 mL shaker flasks (*Corning*) for both cell lines. *PowerCHO-2CD* culture medium was inoculated at 0.25×10^6 cells/mL with highly viable cells (99%) in exponential growth phase for both cell lines. The shaker flasks, each containing 60 mL of cell culture, were kept in

suspension culture (120 rpm) in a humidified incubator at 37°C with 5% CO₂ for eight days. Both cell lines and their replicates were grown under the same culture conditions. Cell density and viability were routinely checked every other day. On day 8, culture media samples were harvested (at density 6.75×10^6 cells/mL for CHO-BRI, and 7.50×10^6 cells/mL for CHO-1A7), clarified by centrifugation and filtered through a 0.45 µm membrane. Cell pellets were collected and kept frozen until analysis.

2.2.2 Cell Lysis and Protein Extraction

Frozen cell pellets from each cell line were obtained from the BRI (Montréal, Canada). Approximately 2×10^7 cells were pelleted at 1000 rpm for 5 minutes, followed by aspiration and removal of the growth media. The resulting cell pellet was washed three times with sterile ice cold 1X phosphate-buffered saline (PBS) solution (137mM sodium chloride (NaCl) (Merck, Darmstadt, Germany), 2.7mM potassium chloride (KCl) (VWR, Mississauga, Canada), 10mM sodium phosphate dibasic (Na₂HPO₄) (Merck, Darmstadt, Germany), 17.6mM potassium phosphate monobasic (KH₂PO₄) (BioShop Canada Inc., Burlington, Canada). During each wash, the PBS-cell mixture was centrifuged at 1000 rpm for 5 minutes followed by the removal of the PBS wash buffer. After the third wash, cell pellets were stored at -80°C or immediately lysed as described below.

For whole cell protein extraction, the cell pellets were re-suspended in 500 µL lysis buffer containing 7M urea (Fisher, ON, Canada), 2M thiourea (Fisher, ON, Canada), 50mM Tris pH 8.0 (Fisher, ON, Canada), 4% CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulphonate) (Fisher, ON, Canada), and 15µL protease inhibitor cocktail (Sigma, ON, Canada). Cell suspensions were then vortexed and homogenized by sonication (Microson Ultrasonic Cell Disrupter, Misonix, NY, USA) two times for 30 seconds each at a power level of 4 at 4°C. Following sonication, the cell homogenates were incubated on a rotator at 4°C for 3.5 hours to ensure complete cell lysis. The samples were clarified by centrifugation for 30 minutes at 14,000 rpm at 4°C to remove insoluble cell debris. The protein-containing supernatants were transferred into new microcentrifuge tubes and stored at -20°C until protein concentration determination.

Bio-Rad Protein Assay

The protein concentrations were measured using the Bio-Rad protein assay (Bio-Rad Laboratories LTD, ON, Canada), which is based on the Bradford method [96]. For each Bio-Rad protein

assay run, a linear standard curve was constructed using bovine serum albumin (BSA) (Sigma, ON, Canada) as a standard at concentrations of 2, 4, 6, 8, and 10 µg/mL. The protein samples and BSA standards were measured in triplicates to ensure accuracy of the assay. The absorbances were measured at 595nm (Varian Cary 50 Bio UV-Visible Spectrophotometer), and the protein concentrations were calculated.

2.2.3 Protein Sample Clean-Up

To remove interfering substances such as detergents, salts, lipids, phenolics and nucleic acids, the protein sample was subjected to the Amersham 2-D Clean-Up Kit (GE Healthcare, Uppsala, Sweden). A volume of 200 µL containing approximately 400 µg of protein was added to 450 µL of precipitant, vortexed, and incubated on ice for 15 minutes. Following incubation, 450 µL of co-precipitant was added to the sample mixture and briefly vortexed. The sample was then centrifuged at 14,000 rpm for 5 minutes at 4°C and the supernatant was removed. 40 µL of co-precipitant was then added to the pellet, incubated on ice for 5 minutes, followed by centrifugation at 14,000 rpm for 5 minutes at 4°C. The supernatant was removed and the resulting pellet was re-suspended in 40 µL of MilliQ water, 1 mL of wash buffer, and 5 µL of wash additive. The sample was then vortexed and incubated for 40 minutes at -20°C, vortexing every 10 minutes. After incubation, the sample was centrifuged for 5 minutes at 14,000 rpm and the supernatant was removed. The pellet was then re-suspended in 100 µL lysis buffer and incubated at room temperature for 1 hour to ensure complete re-suspension.

The protein concentration of the above samples, which were subjected to the protein sample clean-up procedure, were determined by the Bio-Rad protein assay as per the protocol outlined in Section 2.2.1. Hence, the protein concentration of the samples were determined before and after sample clean-up.

2.2.4 Two-Dimension Differential In-Gel Electrophoresis

2.2.4.1 Protein Sample Labeling

Two-Dimension Differential In-Gel Electrophoresis (2D-DIGE) was employed to detect differentially expressed proteins. Six biological replicate gels were prepared for comparative proteomic analysis between CHO-1A7 and CHO-BRI cell lines. **Table 2-1** shows the labeling strategy adopted in the present work. For each gel, 2 protein samples and an internal standard were each labeled with a

different CyDye DIGE Fluor minimal dye (GE Healthcare, Uppsala, Sweden). The internal standard was generated by pooling an equal amount of all protein samples and was used to aid in spot-matching in a 2-D image analysis software. Potential dye bias was taken into account by employing a dye swap procedure during the labeling of protein replicates (**Table 2-1**).

Table 2-1. 2D-DIGE Experimental Design.

Gel #	Cy3	Cy5	Cy2
1	CHO-1A7-1	CHO-BRI-1	Internal Std
2	CHO-1A7-2	CHO-BRI-2	Internal Std
3	CHO-1A7-3	CHO-BRI-3	Internal Std
4	CHO-BRI-4	CHO-1A7-4	Internal Std
5	CHO-BRI-5	CHO-1A7-5	Internal Std
6	CHO-BRI-6	CHO-1A7-6	Internal Std

Fifty µg of each protein sample was labeled with 1µL of 200pmol/µL CyDye DIGE Fluor minimal dye according to **Table 2-1**. Following labeling, the samples were incubated on ice in the dark for 30 minutes. To stop further labeling, 1µL of 10mM lysine was added, vortexed, and incubated on ice in the dark for 10 minutes. The three protein sample tubes were then combined for a total of 150µg protein mixture loaded per gel. Finally, sufficient rehydration buffer (7M urea (Fisher, ON, Canada), 2M thiourea (Fisher, ON, Canada), 4% CHAPS (Fisher, ON, Canada), 20 mM dithiothreitol (DTT) (Fisher, ON, Canada), 0.5% pH 3-10 IPG buffer ampholytes (GE Healthcare, Uppsala, Sweden)) was added to the combined protein mixture for a total sample volume of 450µL per gel.

2.2.4.2 First Dimension Separation

Gel Strip Rehydration

The combined protein samples containing rehydration buffer were each pipetted onto individual lanes on the Immobiline Drystrip Reswelling Tray (GE Healthcare, Uppsala, Sweden). Immobiline DryStrip, pH 3-10NL, 24cm immobilized pH gradient (IPG) gel strips (GE Healthcare, Uppsala, Sweden) were warmed at room temperature for 5 minutes and then applied to a lane of the reswelling tray, gel side down. The strips were placed on the surface of the sample solution and adjusted so that any air bubbles were removed and the gel was fully coated with sample. Approximately 2.5mL of PlusOne DryStrip Cover Fluid (GE Healthcare, Uppsala, Sweden) was added to each lane to prevent gel drying and urea

crystallization. The reswelling tray was verified to be level with the surface and then wrapped with aluminum foil. The strips were allowed to rehydrate in the dark overnight, from 12 to 20 hours maximum.

Isoelectric Focusing

First-dimensional isoelectric focusing (IEF) was performed using the Ettan IPGphor II system (GE Healthcare, Uppsala, Sweden) at 20°C. First, a ceramic manifold tray (GE Healthcare, Uppsala, Sweden) was placed atop the Ettan IPGphor II apparatus, which was then overlaid with 130mL of PlusOne DryStrip Cover Fluid (mineral oil) (GE Healthcare, Uppsala, Sweden). The strips were removed from the reswelling tray and transferred to the ceramic manifold tray with the gel side facing up. The strips were aligned so that the black lines at the top of the gel were just below the top dot of the ceramic manifold. Two paper wicks, each wetted with 150µL MilliQ water, were partially placed over the + and – ends of the strip. Once all the strips were positioned, electrodes were placed on top of the tray so that they were in contact with the paper wicks and the top surface of the IEF apparatus. IEF was carried out using the following protocol:

- Step 1. 100V (1 hr)
- Step 2. 500V (2 hr)
- Step 3. Gradient to 1000V (2 hrs)
- Step 4. Gradient to 3000V (3 hrs)
- Step 5. 3000V (2 hrs)
- Step 6. Gradient to 8000V (3 hrs)
- Step 7. 8000V (10:00 hrs)
- Step 8. 500V (24 hrs)

IEF was completed at Step 7 and the strips were maintained at 500V until second dimension separation.

2.2.4.3 Second Dimension Separation

Gel Casting

All DIGE non-fluorescent clear glass plates, plastic spacers (sheets and blocks), and gel caster were washed thoroughly using MilliQ water with Sparkleen detergent (Fisher, ON, Canada) and deionized (DI) water. They were further rinsed with 70% ethanol then methanol and wiped dry with

KimWipes. For each gel, a plate sandwich was made by aligning and pressing a large and a short plate together firmly while ensuring the edges were straight. The plate sandwiches and plastic spacers were added alternatively into the DALTsix gel caster (GE Healthcare, Uppsala, Sweden). Plastic spacers were added to ensure the plates sit flush with the height of the edge of the gel caster. Spacer blocks were used when there were less than 6 plate sandwiches. To complete assembly, the faceplate was added and tightened with screws. On both sides of the faceplate, six spring clamps were used to completely seal the gel caster.

Fresh 12% polyacrylamide gel solution (30% acrylamide mix (BioShop Canada Inc., Burlington, Canada), 1.5M Tris pH 8.8 (Fisher, ON, Canada), 10% sodium dodecyl sulfate (SDS) (BioShop Canada Inc., Burlington, Canada), 10% ammonium persulfate (Fisher, ON, Canada), tetramethylethylenediamine (TEMED) (Sigma, ON, Canada)) was made after the gel caster had been assembled and immediately poured into the gel caster, ensuring air bubbles were removed and the gel solution was level within the caster. More gel solution was added until the gel level was 0.5cm below the lower edge of the short plate. To prevent the gel from drying, 2mL of MilliQ water was layered ontop of the gel between the plate sandwiches. Once the gels solidified, the gel caster was disassembled and the plate sandwiches were removed and rinsed with DI water to eliminate any gel residues. The top surfaces of the gels were layered again with DI water and the outer surfaces dried until ready for use in second dimension separation.

Equilibration

Following completion of IEF, the strips were removed and placed in an equilibration tube with the plastic side in contact with the tube. Each strip was reduced in 10mL equilibration buffer (6M urea (Fisher, ON, Canada), 30% glycerol (Fisher, ON, Canada), 50 mM Tris, pH 8.8 (Fisher, ON, Canada), 2% SDS (BioShop Canada Inc., ON, Canada)) with 1% w/v DTT (Fisher, ON, Canada) for 15 minutes, followed by alkylation in 10mL equilibration buffer with 2.5% w/v iodoacetamide (IAA) (Sigma, ON, Canada) for 15 minutes.

Electrophoresis

Following the equilibration step, each immobilized pH gradient (IPG) strip was inserted into a plate sandwich and aligned with the top edge of the gel. Bubbles between the strip and the gel were removed. The water layer on top of the gel was then decanted and replaced with an agarose sealing

solution (25mM Tris base (Fisher, ON, Canada), 192mM glycine (Fisher, ON, Canada), 0.1% SDS (BioShop Canada Inc., Burlington, Canada), 0.5% agarose (BioShop Canada Inc., Burlington, Canada), 0.002% bromophenol blue (Fisher, ON, Canada)). A maximum of 6 gel plate sandwiches were placed into the cassette carrier, and any unused slots were filled with spacer blocks. The upper buffer chamber was fitted firmly onto the carrier. Prior to inserting the cassette carrier assembly, the lower buffer chamber of the DALTsix Electrophoresis unit (GE Healthcare, Uppsala Sweden) was filled with 4.5L of 1X running buffer (25mM Tris base (Fisher, ON, Canada), 192mM glycine (Fisher, ON, Canada), 0.1% SDS (BioShop Canada Inc., Burlington, Canada)) and cooled to 10°C using the MultiTemp III Thermostatic Circulator (GE Healthcare, Uppsala, Sweden). Once the 1X running buffer was cooled to the desired temperature, the cassette carrier assembly was then positioned in the electrophoresis unit. The upper buffer chamber was filled with 2X running buffer (50 mM Tris base (Fisher, ON, Canada), 384mM glycine (Fisher, ON, Canada), 0.2% SDS (BioShop Canada Inc., Burlington, Canada)) above the "MIN" line indicator. The gel apparatus lid was added and electrophoresis began at 1 watt/gel for 30 minutes. After half an hour, the settings were increased to 17.5 watts/gel and electrophoresis continued until the blue dye front was about 0.5cm away from the bottom of the gel. The 2D-DIGE gel plate sandwiches were removed from the cassette carrier, rinsed with DI water and wiped dry prior to gel scanning.

2.2.4.4 Gel Scanning and Analysis

The plate sandwiches containing 2D-DIGE gels were scanned under fluorescence acquisition mode using the Typhoon 9400 scanner (GE Healthcare, Uppsala Sweden). The PMT was set to 575V and the scanning resolution was set to 200µm with a focal plane of +3mm. The filters used for each channel were: 580 BP30 Cy3 and 670 BP30 Cy5.

All the resulting gel images were cropped to the same size using PDQuest Advanced 2-D Analysis Software v8.0.1 (Bio-Rad Laboratories LTD, ON, Canada) and then uploaded into DeCyder v7.0 software (GE Healthcare, Uppsala, Sweden). The gel images were first processed in the DeCyder Differential In Gel Analysis (DIA) module by detecting the number of spots per gel image. The spot detection procedure uses an arbitrary number inputted by the user to guide the detection. The estimate number was set to 2500 to detect as many spots as possible on a gel image. Gel-to-gel matching was then performed by matching detected spots across all gel images using the DeCyder Biological Variation Analysis (BVA) module. Only spots that matched across all six replicate gels were considered and these

were assigned master spot numbers that could be shared between gels. Their relative spot intensity levels were extracted from DeCyder v7.0 and imported into the statistical package R v2.12.0 [97] for statistical analysis.

Statistical Analysis using R

Differences in protein abundance were assessed based on the intensity ratios of the paired samples. However, dye bias may arise due to differences between the labeling efficiencies or scanning properties of the fluors. As such, the spot intensity levels must first be normalized to adjust for effects arising from variation in the experimental system rather than from biological differences between protein samples. R was used for normalization as well as to generate a spot-picking list composed of proteins that showed a statistically significant change in relative protein abundance (paired *t*-test; FDR corrected $p < 0.05$).

Intra-gel normalization was first performed on the relative spot intensity levels of all spots within a particular gel image to account for dye bias. The underlying assumption is that the bulk of the signals between two treatments to be compared should have signal intensities that are closely matched, and that biological differences should only be seen in a small proportion of proteins. The method of normalization was based on LOESS normalization [98]. To achieve this, an MvA plot was generated by plotting the log-transformed sum intensity of the two signals in each channel (Cy3, Cy5) against the log-transformed difference between those signals (x-axis: $\log_2(\text{Cy5}) + \log_2(\text{Cy3})$; y-axis: $\log_2(\text{Cy3}) - \log_2(\text{Cy5})$). The smoothing function LOESS, which uses a locally weighted least squares estimate of a regression fit, was used to fit a regression line to this data plot. The function operates by taking small sections of the data at a time and fitting a linear regression line to that area of data. This function is then applied using a moving window strategy across the whole intensity range. By performing the smoothing function, each window undergoes local fitting that is adjusted by its relevant scaling factor. The result is a smoothed curvilinear regression line that represents the deviation of the data away from the zero axis. With the above assumption in mind, this deviation is due to experimental artefact rather than biological differences. As such, a correction was applied to align the data back to the zero axis by adjusting the log difference values.

Inter-gel normalization was then performed on the LOESS normalized data by method of quantile normalization [99], to account for bias in terms of gel-specific effects. The expected distribution of intensity levels should be similar between gels, or in other words, the distribution of effect sizes should be more or less consistent across samples. Quantile normalization adjusts intensities on each gel so that all gels have similar distributions of intensity levels.

Once adjusted and log-transformed, the relative spot intensity levels were subjected to a paired *t*-test and an initial list of p-values was produced. This list was subjected to the False Discovery Rate (FDR) procedure of Benjamini and Hochberg [100]. Proteins were considered differentially expressed if they had a calculated FDR below 10%.

2.2.5 Protein Identification via Mass Spectrometry

Protein spots that showed statistically significant differential expression between CHO-BRI and CHO-1A7 were excised from coomassie-stained preparative gels. These spots were subjected to in-gel tryptic digestion followed by LC-MS/MS analysis. The resulting mass spectrometry data was analyzed using PEAKS Studio v5.3 (Bioinformatics Solutions Inc., ON, Canada) for protein identification.

2.2.5.1 Colloidal Coomassie Stained Preparative Gels

To visualize protein spots, coomassie-stained gels were prepared for spot excision and identification. A total of 800µg containing a pool of all 12 protein samples were loaded onto 24cm pH 3-10NL IPG strips. The procedure for first and second dimension separation using 12% polyacrylamide gels was similar to that of 2D-DIGE (see section 2.2.4.2 and section 2.2.4.3) with a few modifications. CyDye labeling of protein samples was not used for running preparative gels.

After second dimension separation was completed, the cassette carrier assembly was removed from the DALTsix Electrophoresis unit and the gel plate sandwich was removed. A slanted ruler edge was used to carefully open the gel plate sandwich without breaking the gel piece. Using the ruler to help free the edges, the gel piece was then transferred into a container with MilliQ water and placed on a rocking platform for 5 minutes. After rinsing, the water was removed and proteins were fixed with 10% acetic acid/50% methanol for 10 minutes. The gels were further rinsed with MilliQ water and stained overnight

with GelCode® Blue Stain Reagent (Thermo Scientific, ON, Canada) at room temperature followed by destaining for 5 hours in MilliQ water on a rocking platform. The resulting stained gel was scanned using the EXQuest Spot Cutter (Bio-Rad Laboratories LTD, ON, Canada). Spot matching between 2D-DIGE and preparative gels was performed using PDQuest Advanced 2-D Analysis Software v8.0.1 (Bio-Rad Laboratories LTD, ON, Canada) prior to protein spot excision.

2.2.5.2 Multiwell In-Gel Trypsin Digestion

To minimize keratin contamination, all equipment used was cleaned thoroughly and all procedures were followed carefully. The flow hood was sprayed and wiped down with 70% ethanol, and the UV light was activated for 30 minutes prior to digesting samples. All pipette tips and 96-well plates were rinsed twice with MilliQ water, methanol, and 100% acetonitrile (ACN) and let dry overnight in a flow hood.

This method is based on a multiwell in-gel protein digestion protocol developed by Pluskal et al., 2002 [101] with some modifications. Briefly, this protocol takes advantage of 96-well plates that allows up to 96 protein gel spots to be processed simultaneously. A 96-well perforated filter plate (Cat# CB080, Thermo Scientific, ON, Canada) was stacked atop a 96-well collecting plate (Cat# AB-1058, Thermo Scientific, ON, Canada) such that the protein gel spots were processed in the first plate and the washes were collected in the second plate. Note that during the destaining, reduction, and alkylation steps, washes were collected in the second plate, by centrifugation, and discarded. Once proteins were fully digested, centrifugation was applied to draw the extracted digests into a new clean collection plate.

Protein Spot Excision

Spots of interest were excised from the preparative gel using a 1.5mm cutter tip size in the EXQuest Spot Cutter (Bio-Rad Laboratories LTD, ON, Canada). The gel pieces were transferred to a 96-well perforated filter plate. A volume of MilliQ water covered the gel pieces to prevent from drying, and the plate was stored at 4°C until ready for protein digestion.

Destaining

To remove coomassie blue stains, the gel pieces were washed with 30µL of 50 mM ammonium bicarbonate (NH_4HCO_3) (Fisher, ON, Canada) and mixed on a rocking platform for 5 minutes. The waste

was removed and discarded by centrifugation. The above wash and removal steps were repeated another two times.

The gel pieces were then shrunk with 30µL of 50% ACN/25mM NH_4HCO_3 , and mixed on a rocking platform for 10 minutes. The waste was removed and discarded by centrifugation. The dehydration and removal steps were repeated another two times.

Reduction and Alkylation

After destaining, the gel pieces were reduced with 30µL of 10 mM DTT in 50 mM NH_4HCO_3 for 30 minutes at 37°C. The supernatant liquid was then removed by centrifugation and discarded. Following reduction, the gel pieces were alkylated with 30µL of 100mM IAA in 50 mM NH_4HCO_3 for 15 minutes in the dark at room temperature. The supernatant liquid was then removed by centrifugation and discarded. Next, the gel pieces were dehydrated with 50µL of 50% ACN/25mM NH_4HCO_3 , and mixed on a rocking platform for 15 minutes. All liquid was removed via centrifugation.

Trypsin Digestion

A 0.2µg/µL trypsin stock solution was freshly prepared by adding 100µL of Promega resuspension buffer to 20µg of lyophilized Promega Modified Sequencing Grade Trypsin (Promega Corporation, Madison, USA). From this trypsin stock solution, a 13ng/µL trypsin working solution was prepared in 50mM NH_4HCO_3 . The gel pieces were covered with 40µL of the 13ng/µL trypsin working solution and incubated overnight at 37°C.

Peptide Extraction

A new clean 96-well collection plate was used to collect the supernatant. The 96-well perforated filter plate containing the protein digest was removed from incubation and the liquid was transferred to the receiving wells of the 96-well collection plate by centrifugation. The gel pieces were then washed with 20µL of 25mM NH_4HCO_3 , mixed on a rocking platform for 10 minutes, and collected using the same collection plate. Next, the gel pieces were alternatively reswollen and dehydrated twice with 20µL of 5% formic acid (Fisher, ON, Canada) and 20µL of 100% acetonitrile (ACN), while mixing and collecting the supernatants in between steps. The extracted peptides were subsequently stored at -20°C

until ready to evaporate down. When ready for LC-MS/MS analysis, the protein digests were evaporated down to dryness and reconstituted in 5µL of 0.1% formic acid in water.

2.2.5.3 Mass Spectrometry (LC-MS/MS)

The digested peptides were sent to the Hospital for Sick Children Mass Spectrometry Facility (ON, Canada) for mass spectrometry (MS) analysis. The procedure is outlined below.

The samples were loaded onto a 150µm ID pre-column (Magic C18, Michrom Biosciences, Auburn, CA) at 4µl/min and separated over a 75µm ID analytical column. Using an EASY n-LC nano-chromatography pump (Proxeon Biosystems, Odense Denmark), the peptides were eluted over 60 minutes at 300nL/min in a 0 to 40% ACN gradient in 0.1% formic acid. The peptides were eluted into a LTQ linear ion trap mass spectrometer (Thermo-Fisher, San Jose, CA) operated in a data dependent mode. Six MS/MS scans were obtained per MS cycle.

2.2.5.4 Database Searching and Protein Identification

Protein identification was performed using PEAKS Studio v5.3 (Bioinformatics Solutions Inc., ON, Canada) which combines auto *de novo* sequencing with database searching. A draft version of the genome sequence of the CHO-K1 cell line was recently established, hence all mass spectra were searched against the corresponding CHO protein database [82]. A study recently showed a considerable improvement in both the number of proteins identified and the confidence of those identifications (as determined by searching against a CHO-specific protein database) [84].

Instrument parameters and settings selected in PEAKS Studio v5.3 are found in **Table 2-2**. A refining filter was initially applied to the raw MS data to filter scans with a PEAKS quality value of 0.65 or greater. Auto *de novo* sequencing was then performed on filtered scans using the parameters from **Table 2-3**. The *de novo* sequencing results were searched against the CHO protein database using the inChorus function within PEAKS, which combines the results of two search engines, namely: PEAKS DB and X!Tandem. The use of two unique search engines helps in increasing overall coverage and confidence of the protein identifications by utilizing different search algorithms. The database search parameters for each search engine are listed in **Table 2-4** and **Table 2-5**, respectively.

Table 2-2. PEAKS Instrument Parameter Settings.

Parameter	Setting
Manufacturer	Thermo Scientific
Ion source	ESI (nano-spray)
Fragmentation type	CID, CAD, IRMPD (y and b ions)
MS precursor scan	Linear ion trap
MS/MS product scan	Linear ion trap

Table 2-3. PEAKS Auto *de novo* Sequencing Parameter Settings.

Parameter	Setting
Parent ion error tolerance	2.0 Da
Fragment ion error tolerance	0.5 Da
Enzyme	Trypsin
Fixed PTM	Carbamidomethylation (57.02 Da)
Variable PTM	Oxidation (M) (15.99 Da)
Maximum allowed variable PTM per peptide	3
# of candidates reported per spectrum	Up to 5

Table 2-4. PEAKS DB Search Engine Parameter Settings.

Parameter	Setting
Parent mass error tolerance	2.0 Da
Fragment mass error tolerance	0.5 Da
Enzyme	Trypsin
Max missed cleavages	1
Fixed modifications	Carbamidomethylation (57.02 Da)
Variable modifications	Oxidation (M) (15.99 Da)
Max variable PTM per peptide	3
Decoy performed	True (FDR decoy set)

Table 2-5. X!Tandem Search Engine Parameter Settings.

Parameter	Setting
Fragment mass error tolerance	0.5 Da
Residue, modification mass	57.02@C
User defined potential modification	15.99@M
Protein cleavage sites	[RK][P]

For PEAKS DB, the results were evaluated based on a percentage system. The higher the percentage score, the more reliable and accurate the identification. The FDR was also applied and estimated with decoy sequences. A protein identification was considered successful when the protein score was $\geq 90\%$ with ≥ 2 unique peptides and an FDR below 1%. Additionally, at least 2 unique peptides must also be identified in the X!Tandem search engine. For some samples, there was more than one confident protein ID. The molecular weight (MW) and isoelectric points (pI) of these protein IDs were compared with their relative spot locations on the gel to confirm and/or reject an identification.

2.2.5.5 Functional Annotation Analysis Using DAVID

The resulting list of differentially expressed proteins (gene list) was compiled and uploaded onto the DAVID (the database for annotation, visualization, and integrated discovery) bioinformatics resources for functional and pathway analysis. DAVID is a publicly available web-based tool (<http://david.abcc.ncifcrf.gov/>) that is able to extract meaningful biological information associated with large gene lists that is important to a particular biological study [102; 103]. It operates by mapping systematically a large number of interesting genes in a given list to their associated biological annotation (e.g., gene ontology terms), and statistically highlight those that are overrepresented. Due to many redundant biological annotation terms, the Functional Annotation Clustering tool found within DAVID was used to cluster enriched related terms into a single group. DAVID calculates a *p*-value to determine the significance of each gene-term enrichment by using a modified Fisher's exact test (EASE score). This way, DAVID only looks for the most overrepresented terms and more attention can be given to groups with enrichment scores greater than or equal to 1.3 (enrichment score of 1.3 is equivalent to non-log scale 0.05) [103].

Due to the fairly recent publication of the CHO-K1 genome sequence, the DAVID knowledgebase has not yet integrated the gene/protein identifiers and their corresponding biological annotations for CHO cells. As an alternative, the gene list was compiled using gene accession numbers of mouse homologs. As such, the gene population background that was used was the whole mouse genome for gene-term enrichment analysis.

2.3 Results

A comparative proteomic analysis was conducted between the antibody producing CHO-1A7 strain with its corresponding non-producing parental strain CHO-BRI. In the present study, six biological replicate cell cultures from each strain were collected and subjected to cell lysis and protein extraction. The differences in expression at the protein level were examined with 2D-DIGE by detecting and quantifying the relative abundance of the protein spots resolved on a gel. Mass spectrometry was performed to identify proteins that showed statistically significant differential expression.

2.3.1 Cell Growth and Viability

The CHO-1A7 line, which expresses and secretes EG2-hFc1 under the control of a CMV promoter, was created through the transfection of CHO-BRI cells with the pTT44-EG2-hFc1 construct. In the present work, the clone was found to have a specific growth rate of about 0.03 h^{-1} and a doubling time of around 24 hours.

The newly designed clone, after being checked for its stability, was used for batch production of EG2-hFc1. In order to collect an adequate amount of cell biomass as well as to avoid accumulation of cellular debris in media, the process was finished after 8 days when the cell growth reached stationary phase and the viability decreased to approximately 90% (**Figure 2-1**). On day 8, harvested culture media samples achieved a density of 6.75×10^6 cells/mL for CHO-BRI, and 7.50×10^6 cells/mL for CHO-1A7.

2.3.2 Cell Lysis and Protein Extraction

Whole cell protein extracts were obtained from CHO samples after cell lysis by sonication. Cell lysates were subjected to a clean-up procedure to remove interfering substances. For protein concentration determination, BSA was used to generate the standard curve depicted in **Figure A 1** via the Bio-Rad protein assay.

The protein concentration (as shown in **Table A 1**) was used to determine the amount that was required for sample labeling (50 μg per sample) as shown in section 2.2.4.1.

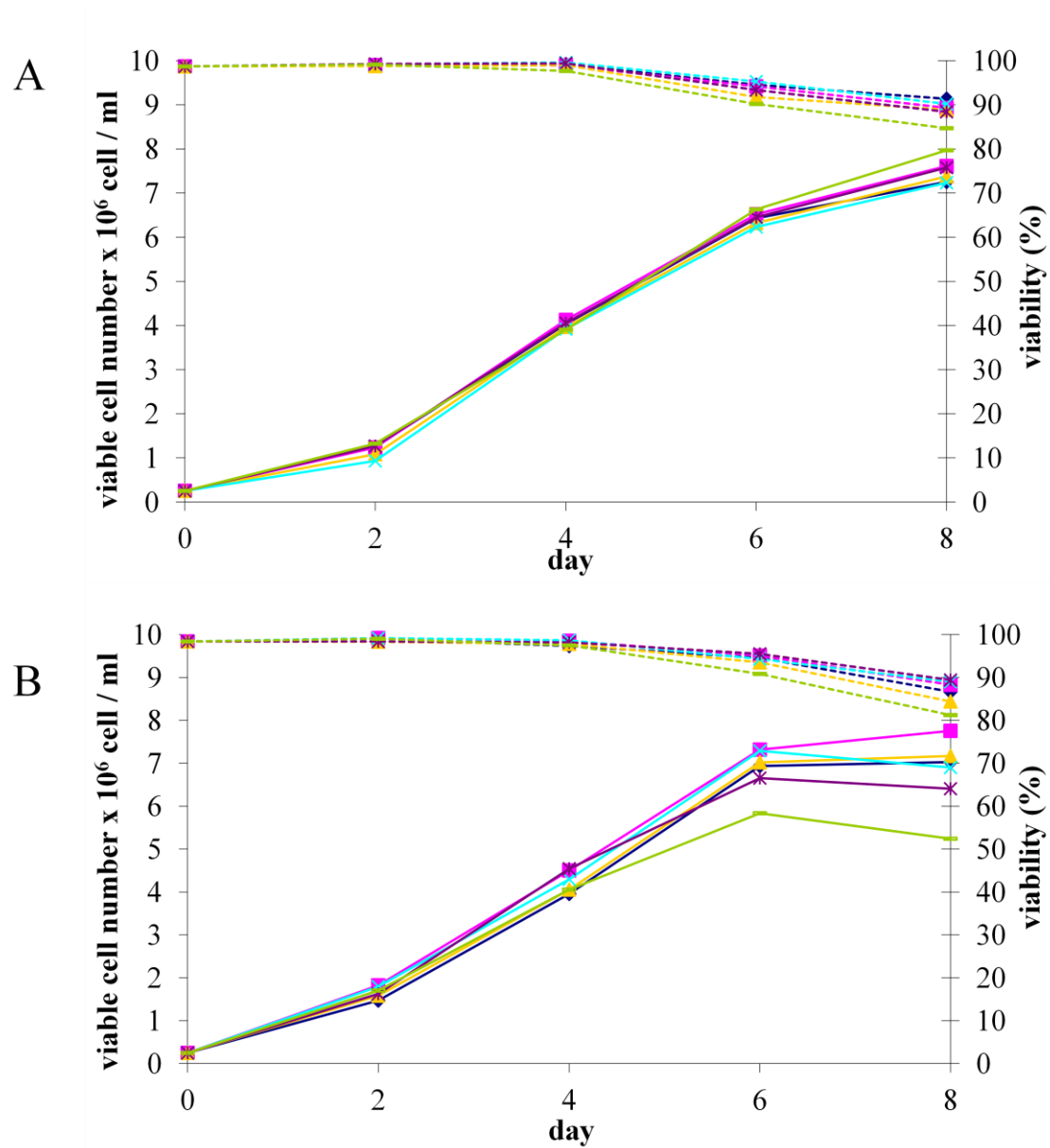


Figure 2-1. Growth and viability curve during 8 days batch cultivation in (A) CHO-1A7 and for the production of EG2-hFc1 in (B) CHO-BRI. Solid lines represent cell densities whereas dashed lines represent cell viabilities for each biological replicate. (Igor Slivac, BRI, Montréal, Canada).

2.3.3 Gel Scanning and Analysis

2D-DIGE gels were scanned and the relative quantities of proteins were measured by fluorescence. The gels were scanned at 580nm and 670nm, which corresponded to Cy3 and Cy5 channels respectively. Due to technical difficulties, scanning the gel at 488nm, which corresponded to the internal standard labeled with Cy2, was not possible. However, this did not pose a problem as the internal standard was only meant to aid spot-matching in DeCyder.

Protein spots labeled with Cy3 appear green, whereas protein spots labeled with Cy5 appear red. **Figure 2-2** shows a representative 2D-DIGE protein expression map generated from one of the six biological replicates. All 6 gels showed fairly uniform spot separation, suggesting the electrophoresis process was reproducible and that the resulting spot maps were comparable. Spots that appear yellow indicates that the two protein samples have roughly equal amounts of the corresponding protein. With most proteomic studies, the relative abundance of the majority of proteins within the same cell line do not differ significantly. As expected, the majority of spots in all 6 gels were yellow, indicating no significant differences in abundance. Spots that appear more red or green indicates greater abundance of that particular protein in a sample depending on the labeling design. These differences are difficult to visualize by the naked eye; therefore, the images were processed using the gel analysis software DeCyder v7.0.

After cropping all the gel images to the same size, they were then processed in DeCyder. Firstly, the DIA module was applied to detect and identify protein spots in conjunction with the first-level-of-matching (within gel). Next, the images were processed in the BVA module to match spots between gels. The number of spots detected per gel as well as the number of spots matched across all 6 gels are shown in **Table 2-6**.

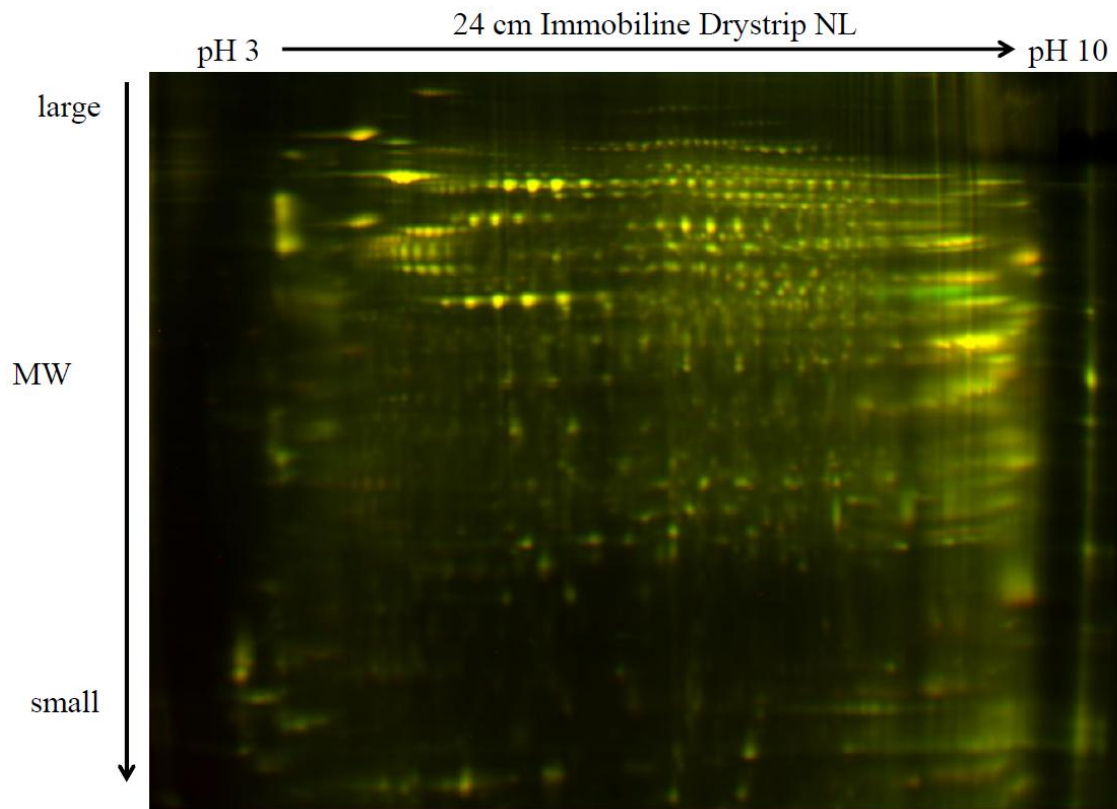


Figure 2-2. Sample Image of 2D-DIGE Gel 1. CHO-BRI-1 was labeled with Cy5 and CHO-1A7-1 was labeled with Cy3. Arrows indicate the direction of protein separation. Proteins were separated initially in the first dimension on a non-linear gradient pH 3 -10, and subsequently separated in the second dimension on a 12% polyacrylamide gel. Green and red fluorescence were measured independently (532 nm for Cy3 and 635 nm for Cy5), and protein spots with equal abundance between samples appear yellowish in the combined image above.

Table 2-6. Spot Detection in 2D-DIGE Gel Images.

Gel Number	Samples (Fluorescent Label)	# of Spots Detected	# of Spots Matched Across All Gels
1	CHO-BRI-1 (Cy5)	1844	912
	CHO-1A7-1 (Cy3)		
2	CHO-BRI-2 (Cy5)	1824	
	CHO-1A7-2 (Cy3)		
3	CHO-BRI-3 (Cy5)	1871	
	CHO-1A7-3 (Cy3)		
4	CHO-BRI-4 (Cy3)	1826	
	CHO-1A7-4 (Cy5)		
5	CHO-BRI-5 (Cy3)	1885	
	CHO-1A7-5 (Cy5)		
6	CHO-BRI-6 (Cy3)	1789	
	CHO-1A7-6 (Cy5)		

2.3.4 Statistical Analysis in the Generation of a Spot-Picklist

A compilation of a list of differentially expressed protein spots was generated as a subset from the 912 spots that were present in all 6 gels. This list was known as the spot-picklist.

Differences in fold change are generally difficult to interpret. Subtle fold changes may reflect proteins that were regulated slightly in response to changes occurring in cell cultures. Therefore, using a fold change threshold may not be a good indicator of differentially expressed proteins as biologically relevant proteins that undergo small fold changes would be filtered out. To establish the spot-picklist, a statistical threshold and a fixed FDR threshold must both be passed in order for a spot to be considered differentially expressed. A paired *t*-test was used, in assessing differences in protein abundance between CHO-BRI and CHO-1A7, to generate an initial list of spots with $p < 0.05$. The complete *p*-value list was subjected to the FDR procedure, generating a list of significant spots for which the expected FDR is at most 10% as shown in **Table 2-7**.

Table 2-7. Differentially Expressed Protein Spots. Raw fold change was calculated as the average intensity ratios between paired samples. A green label indicates spots that were selected for LC-MS/MS.

Spot ID	Raw Fold Change	p-Value	FDR (< 0.1)	Spot ID	Raw Fold Change	p-Value	FDR (< 0.1)
679	1.99	2.70E-05	1.84E-02	929	-1.38	3.84E-03	6.64E-02
1210	-1.39	4.04E-05	1.84E-02	1804	1.14	3.87E-03	6.64E-02
663	1.55	6.85E-05	2.08E-02	705	1.21	3.92E-03	6.64E-02
1779	-1.25	1.35E-04	2.59E-02	1089	-1.08	3.94E-03	6.64E-02
1215	-1.31	1.42E-04	2.59E-02	1622	-1.31	4.06E-03	6.64E-02
918	1.05	1.77E-04	2.69E-02	1473	1.26	4.11E-03	6.64E-02
1815	1.09	2.75E-04	2.82E-02	651	1.21	4.19E-03	6.64E-02
1299	-1.20	3.17E-04	2.82E-02	1027	1.09	4.20E-03	6.64E-02
681	-1.17	3.22E-04	2.82E-02	1274	1.22	4.21E-03	6.64E-02
511	1.18	3.41E-04	2.82E-02	635	-1.14	4.22E-03	6.64E-02
841	-1.57	3.41E-04	2.82E-02	577	-1.25	4.31E-03	6.65E-02
575	-1.12	4.63E-04	3.52E-02	1607	-1.07	4.44E-03	6.65E-02
854	-1.07	5.86E-04	4.01E-02	549	1.10	4.45E-03	6.65E-02
524	1.07	6.31E-04	4.01E-02	566	1.12	4.61E-03	6.77E-02
1572	-1.26	6.60E-04	4.01E-02	1251	-1.14	5.09E-03	7.37E-02
187	-1.28	7.71E-04	4.40E-02	1336	-1.13	5.39E-03	7.61E-02
359	1.08	9.76E-04	5.23E-02	1095	-1.09	5.62E-03	7.61E-02
1505	1.44	1.10E-03	5.28E-02	379	-1.07	5.64E-03	7.61E-02
1184	1.15	1.13E-03	5.28E-02	980	1.09	5.64E-03	7.61E-02
372	1.11	1.32E-03	5.28E-02	1234	1.11	5.68E-03	7.61E-02
909	1.11	1.43E-03	5.28E-02	545	1.14	5.96E-03	7.69E-02
1026	-1.22	1.47E-03	5.28E-02	1194	1.11	5.96E-03	7.69E-02
153	1.16	1.48E-03	5.28E-02	959	-1.20	6.00E-03	7.69E-02
1494	1.44	1.49E-03	5.28E-02	313	-1.12	6.07E-03	7.69E-02
835	1.10	1.52E-03	5.28E-02	893	1.20	6.40E-03	7.84E-02
231	1.25	1.56E-03	5.28E-02	881	-1.09	6.41E-03	7.84E-02
1202	-1.31	1.56E-03	5.28E-02	832	1.16	6.47E-03	7.84E-02
1683	-1.15	1.68E-03	5.48E-02	1791	1.14	6.56E-03	7.84E-02
1159	1.17	1.86E-03	5.56E-02	1306	-1.26	6.62E-03	7.84E-02
529	1.06	2.00E-03	5.56E-02	1228	-1.25	6.74E-03	7.88E-02
364	1.05	2.08E-03	5.56E-02	410	1.08	6.97E-03	8.05E-02
423	-1.10	2.09E-03	5.56E-02	234	1.09	7.31E-03	8.33E-02
428	1.11	2.13E-03	5.56E-02	807	-1.06	7.40E-03	8.33E-02
726	-1.16	2.15E-03	5.56E-02	465	-1.06	7.70E-03	8.56E-02

Spot ID	Raw Fold Change	p-Value	FDR (< 0.1)	Spot ID	Raw Fold Change	p-Value	FDR (< 0.1)
1301	-1.32	2.35E-03	5.56E-02	1098	-1.26	7.90E-03	8.68E-02
1437	1.17	2.36E-03	5.56E-02	1304	-1.18	8.49E-03	9.22E-02
1211	-1.17	2.41E-03	5.56E-02	1711	1.16	8.63E-03	9.26E-02
245	1.12	2.44E-03	5.56E-02	1540	-1.12	8.74E-03	9.27E-02
1522	-1.46	2.46E-03	5.56E-02	487	-1.16	8.93E-03	9.36E-02
543	1.11	2.49E-03	5.56E-02	240	1.05	9.16E-03	9.50E-02
1321	-1.28	2.50E-03	5.56E-02	727	-1.12	9.39E-03	9.62E-02
660	1.32	2.79E-03	6.06E-02	369	1.05	9.66E-03	9.63E-02
1526	-1.19	3.18E-03	6.64E-02	837	-1.08	9.79E-03	9.63E-02
1493	-1.10	3.24E-03	6.64E-02	1028	-1.14	9.82E-03	9.63E-02
1208	-1.41	3.34E-03	6.64E-02	1691	-1.15	9.88E-03	9.63E-02
1519	-1.49	3.43E-03	6.64E-02	316	-1.06	9.92E-03	9.63E-02
1570	1.38	3.58E-03	6.64E-02	544	-1.12	1.02E-02	9.70E-02
1543	-1.33	3.72E-03	6.64E-02	181	-1.20	1.02E-02	9.70E-02
				1138	1.03	1.03E-02	9.70E-02

From **Table 2-7**, 97 protein spots passed both the statistical and FDR thresholds. This initial spot-pick list was further manually screened and visually inspected to filter out artifactual spots due to errors in spot detecting, splitting, or matching. Consequently, a total of 86 spots that exhibited changes in abundance were excised and identified by LC-MS/MS.

2.3.5 Colloidal Coomassie Stained Preparative Gels

After the analysis of the 2D-DIGE gel images and the generation of a spot-picklist consisting of spots of interests, coomassie stained gels were prepared for spot excision and subsequent MS analysis. All 12 CHO protein samples were pooled together to ensure all proteins were represented on the preparative gel. The protein concentration of the pooled sample was first determined via the protein standard curve in **Figure A 2**.

The protein concentration was determined to be $4.64 \pm 0.83 \mu\text{g}/\mu\text{L}$ for the CHO-Pooled protein sample. Approximately 800 μg of the CHO-Pooled protein sample was loaded onto IPG strips to develop preparative gels for protein identification.

2.3.6 Protein Identification

Several preparative gels of the CHO-Pooled protein sample were run for spot excision and protein identification. The assigned spot IDs from the spot picklist generated from the analysis of 2D-DIGE gels were matched to their corresponding spots on the preparative gels using PDQuest Advanced 2-D Analysis Software v8.0.1.

Out of the 86 selected spots, a total of 52 protein spots were successfully identified by LC-MS/MS (**Table 2-8**). Their relative locations on a gel are shown in **Figure 2-3**. Of these, 26 protein spots were down-regulated, and 26 were up-regulated, resulting in 34 unique protein identifications. The unique protein identifications stem from the observation where multiple spots that were identified were matched to the same protein. This reflects the fact that certain proteins have many isoforms within the cell, which can be observed as a "train" of spots on a 2D gel (**Figure 2-3**). These isoforms have similar molecular weight but varying pI due to PTMs such as phosphorylation. A proportion of spots were not identified (orange dots with no annotations in **Figure 2-3**), however, this generally occurs with many other 2D-DIGE studies. Another preparative gel could be run in an attempt to identify them. However, a first pass with 60% of the selected spots identified was sufficient for an initial analysis of the global expression changes in CHO-1A7.

Of the 34 unique protein IDs, 15 protein spots were consistently down-regulated, and 18 were consistently up-regulated, and 1 showed mixed behaviour. The identified proteins were classified into five broad categories according to their biological function in **Table 2-8**.

Table 2-8. List of Identified Differentially Expressed Proteins Associated with EG2-hFc1-Producing CHO Strain. The relative abundance ratios subjected to a paired t-test that pass a p-value of 0.05 and an FDR cutoff of 0.10 were considered to be statistically significant. Multiple spots representing different protein isoforms were identified and matched to the same protein. Fold Change < 1, is shown as -1/FC in table. A fold change of 1 corresponds to no change.

Protein Name	Short Name	Spot ID	Accession no.	PEAKS Score	p-value	MW (kDa)*	pI*	% Coverage	# of Peptides Matched	Fold change	Biological process
Protein processing											
Heat shock 75 kDa protein	Trap1	364	EGV93626	99.2	2.08E-03	80.3	6.64	43	22	1.05	Chaperone - Protein folding
Heat shock 70 kDa protein 9	Grp75	372	EGW06687	99.2	1.32E-03	65.8	5.83	57	29	1.11	Chaperone - Protein folding
		359		98.2	9.76E-04			7	3	1.08	
		369		99.2	9.66E-03			59	31	1.05	
Serpin H1	Serpinh1	635	EGW02226	99.2	4.22E-03	46.6	8.74	38	12	-1.14	Chaperone - Protein folding for collagen
60 kDa Heat shock protein	Hspd1	465	EGW00783	99.2	7.70E-03	56.5	6.90	52	19	-1.06	Chaperone - Protein folding
Heat shock protein HSP 90-alpha	Hsp90aa1	929	EGV94858	98.9	3.84E-03	38.1	4.58	22	5	-1.38	Chaperone - Protein folding
Endoplasmin	GRP94	841	EGW09701	99.1	3.41E-04	92.6	4.74	15	8	-1.57	Chaperone - Protein processing and transport
60S Acidic ribosomal protein P0	Rplp0	893	EGV93157	99.2	6.40E-03	29.9	8.68	38	7	1.20	Protein biosynthesis
Protein disulfide-isomerase A6	Pdia6	1026	EGW08481	99.0	1.47E-03	28.4	4.80	27	5	-1.22	Chaperone - Protein folding
Structural proteins											
Beta-actin	Actb	726	EGW06240	99.1	2.15E-03	41.7	5.29	29	7	-1.16	Cytoskeletal component
		1028		99.1	9.82E-03			27	5	-1.14	
		1228		98.9	6.74E-03			26	5	-1.25	
		837		99.1	9.79E-03			25	7	-1.08	

Protein Name	Short Name	Spot ID	Accession no.	PEAKS Score	p-value	MW (kDa)*	pI*	% Coverage	# of Peptides Matched	Fold change	Biological process
Vimentin	Vim	1202	EGW02055	99.2	1.56E-03	53.7	5.05	38	9	-1.31	Intermediate filaments for maintaining cell integrity
		1208		99.2	3.34E-03			30	12	-1.41	
		1210		99.2	4.04E-05			41	12	-1.39	
		1215		99.2	1.42E-04			34	7	-1.31	
		1301		98.6	2.35E-03			8	4	-1.32	
		1522		98.2	2.46E-03			17	5	-1.46	
		544		99.2	1.02E-02			62	25	-1.12	
Lamin B1	Lmnb1	379	EGV92912	99.1	5.64E-03	55.0	4.99	33	11	-1.07	Provides framework for nuclear envelope
Lamin A	Lmna	410	EGW07066	99.2	6.97E-03	64.0	7.75	60	33	1.08	Provides framework for nuclear envelope
		428		99.2	2.13E-03			64	39	1.10	
Calcium-binding protein p22	Chp	1304	EGV97270	98.7	8.49E-03	22.5	4.97	25	4	-1.18	Regulates Na ⁺ /H ⁺ exchangers which regulate intracellular pH and cytoskeletal organization
Metabolic enzymes											
Aconitate hydratase	Aco2	313	EGW13953	98.8	6.07E-03	85.4	8.07	10	4	-1.12	TCA cycle
Aldehyde dehydrogenase	Aldh	543	EGW07431	99.2	2.49E-03	45.9	5.55	44	15	1.11	Oxidation of aldehydes to carboxylic acids
		545		99.2	5.96E-03			59	20	1.14	
		549		99.2	4.45E-03			44	16	1.10	
Acyl-coenzyme A thioesterase 2	Acot2	651	EGW01631	99.1	4.19E-03	49.8	8.27	25	9	1.21	Lipid metabolism
Citrate synthase	Cs	679	EGW12503	99.1	2.70E-05	51.7	8.70	32	5	1.99	Carbohydrate metabolism/TCA cycle
		663		99.0	6.85E-05			22	5	1.55	
Aspartate aminotransferase	Ast	705	EGW01755	99.1	3.92E-03	47.4	9.13	23	7	1.21	Amino acid metabolism
3-hydroxyacyl-CoA dehydrogenase type-2	Hsd17b10	1234	EGW02330	99.2	5.68E-03	27.2	7.70	72	12	1.11	Oxidation of fatty acids, alcohols, and steroids
Methylenetetrahydrofolate dehydrogenase	Mthfd1l	234	EGV95936	99.1	7.31E-03	105.4	7.02	23	15	1.09	Folate metabolism

Protein Name	Short Name	Spot ID	Accession no.	PEAKS Score	p-value	MW (kDa)*	pI*	% Coverage	# of Peptides Matched	Fold change	Biological process
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3	Plod3	187	EGW01863	99.1	7.71E-04	37.7	5.88	13	8	-1.28	Hydroxylation of lysyl residues in collagen-like peptides Catabolism of branched-chain amino acids
		181		99.2	1.02E-02			19	9	-1.20	
		316		98.9	9.92E-03			20	4	-1.06	
		524		99.2	6.31E-04			30	12	1.07	
		529		99.2	2.00E-03			44	14	1.06	
3-hydroxyisobutyrate dehydrogenase	Hibadh	1027	EGW08863	99.1	4.20E-03	31.7	5.17	33	6	1.09	Amino acid catabolism
Glycerol-3-phosphate dehydrogenase	Gpd2	660	EGW05701	99.2	2.79E-03	80.7	5.92	34	17	1.32	Carbohydrate/Lipid metabolism
Apoptosis/Cell Proliferation											
Proliferating cell nuclear antigen	Pcna	959	EGV92404	99.1	6.00E-03	28.8	4.57	41	9	-1.20	Acts as a processivity factor DNA polymerase in eukaryotic DNA replication Regulate transcription, DNA repair, DNA replication, and chromosomal stability Catalyze transcription of DNA Role in regulating proliferation, inhibits DNA synthesis Induction of apoptosis by extracellular signals
Histone H2A type 1	Hist1h2ab	1779	EGV96120	98.9	1.35E-04	28.5	11.04	24	4	-1.25	
DNA-directed RNA polymerase I, II, and III subunit RPABC1	Polr2e	1159	EGV99483	98.3	1.86E-03	25.4	5.52	27	3	1.17	
Prohibitin	Phb	245	EGW01773	98.9	2.44E-03	29.8	5.57	21	4	1.12	
Programmed cell death 6	Pdcd6	1505	EGW09040	99.0	1.10E-03	25.5	5.56	27	5	1.44	
Energy Metabolism											
SPRY domain-containing protein 4	Spryd4	1299	EGW12495	98.9	3.17E-04	23.1	8.85	30	5	-1.20	Uncharacterized
ATP-binding cassette sub-family G member 3	Abcd3	918	EGV92982	99.1	1.77E-04	69.6	5.43	13	6	1.05	Peptide-transporter across extra- and intra-cellular membranes
NADH dehydrogenase	Ndufv1	566	EGW12368	99.1	4.61E-03	50.6	7.53	21	8	1.12	Oxidative phosphorylation

Protein Name	Short Name	Spot ID	Accession no.	PEAKS Score	p-value	MW (kDa)*	pI*	% Coverage	# of Peptides Matched	Fold change	Biological process
NADH dehydrogenase	Ndufs3	1138	EGW03953	99.1	1.03E-02	30.1	7.78	34	7	1.03	Oxidative phosphorylation
ATP synthase subunit beta	Atp5b	575	EGW12490	99.2	4.63E-04	56.4	5.18	45	17	-1.12	ATP synthesis

* MW and pI were calculated from the amino acid sequence, derived from the accession no., using the ExPASy Compute pI/MW tool [104] and compared with gel location.

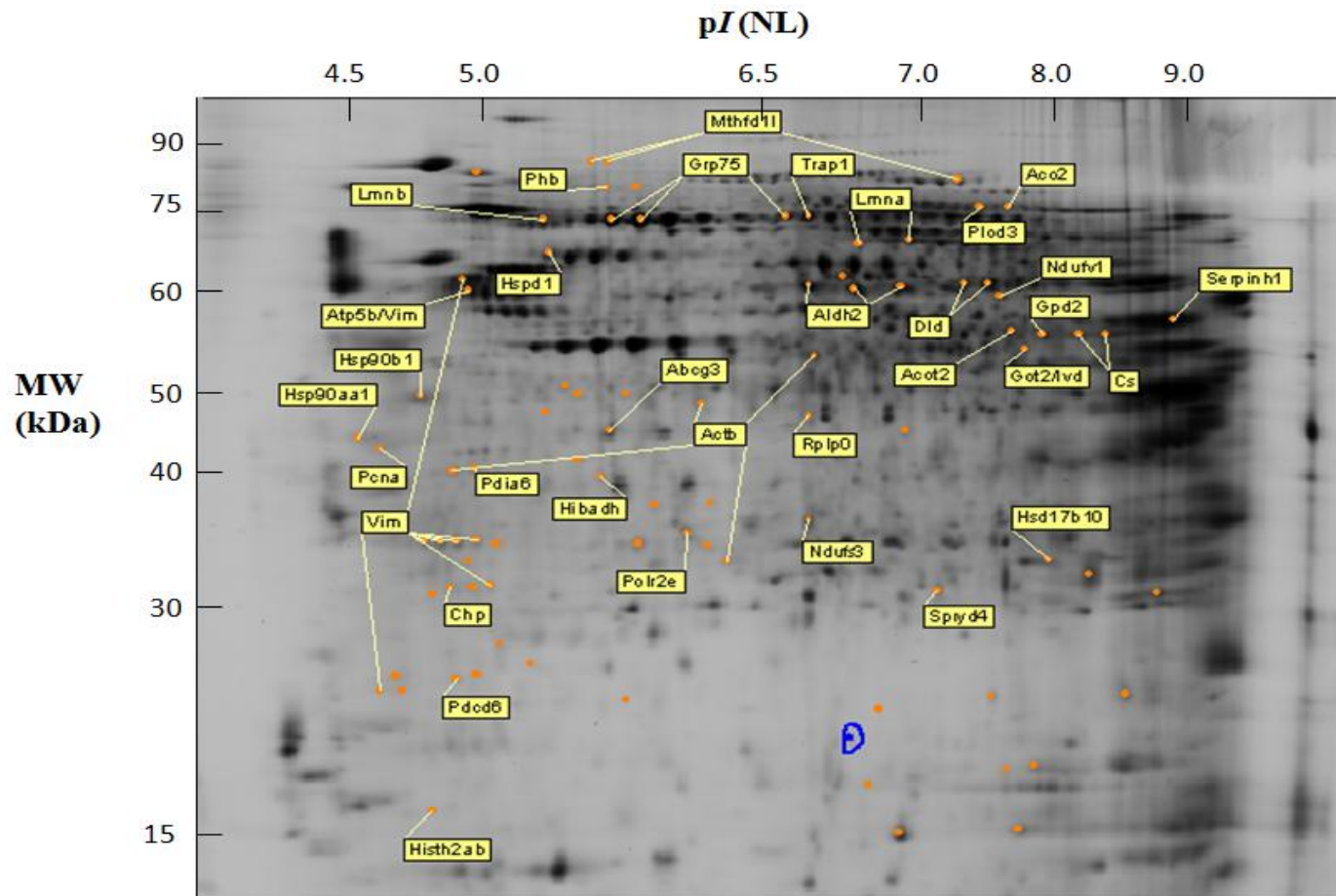


Figure 2-3. Preparative 2-D Gel of Whole Protein Extract from CHO-Pooled Protein Samples. Successfully identified protein spots are labeled and annotated according to their short name format from Table 2-9. Orange dots with no annotations were unsuccessful in protein identification. The relative estimates of the molecular weight and pI were obtained based on the protein identifications. There is no significant meaning regarding the selected blue spot; DeCyder functions by having a spot selected at all times.

2.3.7 Functional Annotation Analysis Using DAVID

The compiled gene list was subjected to DAVID's various analytical modules to explore enriched functionally related gene groups and their relationship to antibody production. Given an abnormal biological process in a given study, genes that are related to the abnormality should have a higher potential (enriched) to be selected as a relevant group by high-throughput screening technologies. The degree of enrichment is decided by performing a comparison against a set gene population background. Thus, the enrichment can be quantitatively measured by statistical methods such as Fisher's exact test. An enrichment score (for each group) is determined by a geometric mean of all the enrichment *p*-values of each annotation term in the group followed by a minus log transformation that is applied on the averaged *p*-values. The enrichment score is a relative score used to rank overall importance of annotation term groups. DAVID only looks for the most overrepresented terms and more attention can be given to groups with enrichment scores greater than or equal to 1.3 [103]. Application of the aforementioned tool revealed a total of 3 clusters with enriched gene ontology (GO) terms that are involved in a number of biological processes, as represented in **Table 2-9**.

Table 2-9. Enriched Functional Annotation Clusters Observed from the Corresponding Gene List.

Annotation Cluster #	Enrichment Score	Enriched Terms	# of Genes Involved	<i>p</i> -value
1	3.08	Nad	6	1.40E-05
		Generation of precursor metabolites and energy	6	2.40E-04
		Oxidoreductase activity, acting on NADH or NADPH	3	5.90E-03
		Electron transport chain	3	2.50E-02
2	2.98	Chaperone	6	7.70E-06
		Unfolded protein binding	5	1.70E-05
		Stress response	4	7.70E-05
		Protein folding	5	1.70E-04
3	2.39	Generation of precursor metabolites and energy	6	2.40E-04
		Cellular respiration	3	7.50E-03
		Citrate cycle (TCA cycle)	3	7.80E-03
		Energy derivation by oxidation of organic compounds	3	2.00E-02

Analysis of the proteomic data by Functional Annotation Clustering revealed the enrichment of a number of GO terms including protein folding and processing, oxidative phosphorylation, and carbohydrate metabolism. Additionally, based on the uploaded gene list, DAVID identified a number of genes that play an important role in two related metabolic network pathways as represented in **Table 2-10**.

Table 2-10. Genes Associated with Two Metabolic Pathways.

KEGG Pathway	<i>p</i> -value	Genes Involved
Valine, leucine and isoleucine degradation	9.80E-04	Aldehyde dehydrogenase Dihydrolipoamide dehydrogenase 3-hydroxyacyl-CoA dehydrogenase type-2 3-hydroxyisobutyrate dehydrogenase
Citrate cycle (TCA cycle)	7.80E-03	Aconitase 2 Citrate synthase Dihydrolipoamide dehydrogenase

The proteins identified reflect observations of metabolic changes within the proteome of CHO-1A7 as compared with that of CHO-BRI. Certain heat shock proteins (Trap1, Grp75) were up-regulated whereas others (Hspd1, Hsp90aa1) were down-regulated. Molecular ER chaperones (Serpinh1, Grp94, Pdia6) were differentially down-regulated in EG2-hFc1-producing cells. The up-regulation of Rplp0, a component involved in protein biosynthesis, was also observed while the majority of proteins (Actb, Vim, Lmnb1, Chp) related to the structural integrity of the cell showed down-regulation. Enzymes involved in various metabolic processes including carbohydrate (Aco2, Aldh, Cs, Gpd2), amino acid (Ast, Plod3, Dld, Hibadh), and lipid (Acot2) metabolism were generally up-regulated. Proteins involved in DNA repair, DNA replication and transcription, apoptosis, and cellular respiration were also found to be differentially expressed. Two examples of spots that were observed to be differentially regulated are shown in **Figure 2-4**.

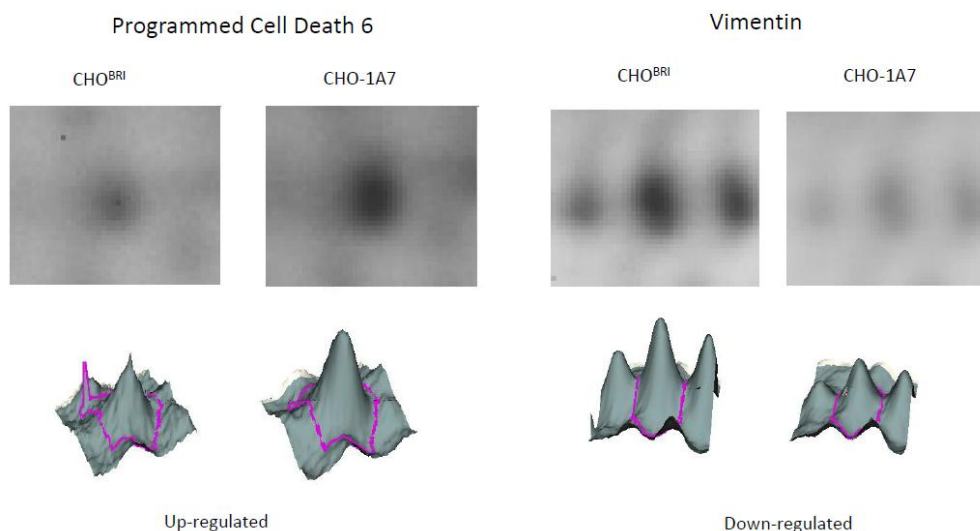


Figure 2-4. Sample Differentially Regulated Protein Spots. A visual observation of spots that showed up-regulation (top left, Pcd6, Spot ID 1505) and down-regulation (top-right, Vim, Spot IDs 1208, 1210, 1215). (bottom) The "3-D volume view" from the DeCyder analysis showing the same spots that were up- and down-regulated based on spot volume.

A few of the identified proteins (Grp75, Actb, Vim, Lmna, Aldh, Cs, Mthfd1l, Dld) were observed to appear in multiple spot locations on the resulting 2-D gel (**Figure 2-3**). There was no indication of the appearance of the protein spot unique to EG2-hFc1 at the approximate location of 84 kDa (dimer form) and theoretical pI of 7.73. Additionally, EG2-hFc1 was not among the list of differentially expressed proteins detected by 2D-DIGE. This is likely due to secretion of EG2-hFc1 into the media following protein biosynthesis, which was confirmed by SDS-PAGE (**Figure 2-5**).

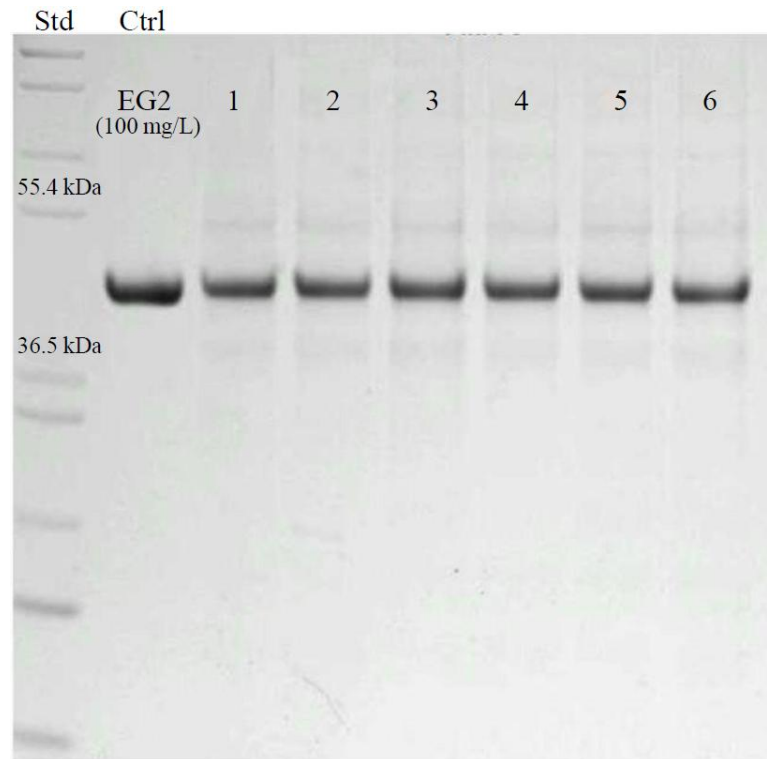


Figure 2-5. Coomassie Stained SDS-PAGE Gel of EG2-hFc1 Purified from an 8 Day Batch Cultivation of CHO-1A7 Media Samples. Std represents the protein standard ladder. Ctrl represents the control sample with 100 mg/L of purified EG2-hFc1. Biological replicates are numbered 1 to 6. The stained bands represent the monomer form of EG2-hFc1 (42 kDa) (Igor Slivac, BRI, Montréal, Canada).

2.4 Discussion

In this study, a derivative CHO-BRI cell line was engineered to produce the EG2-hFc1 chimeric heavy chain monoclonal antibody, producing a recombinant cell line denoted CHO-1A7. The cell lines were cultured and provided by Dr. Yves Durocher's group at the Biotechnology Research Institute (Montreal, Canada). The method of 2D-DIGE was employed to examine the effects of EG2-hFc1 expression on the proteome profiles between the producing (derivative) and non-producing (parental) strains. These two cell types were each partitioned and cultured in separate replicate batch processes, and sampled near early stationary phase (Day 8) for pairwise proteomic analysis. A cell density of approximately 6.75×10^6 cells/mL for CHO-BRI, and 7.50×10^6 cells/mL for CHO-1A7 resulted at harvest.

It is often difficult to infer changes in expression levels of any one particular individual protein and its effect on the biological state of the cell including recombinant protein production. This becomes increasingly difficult when the results of expression levels for individual proteins are subtle. We attempt to gain a systems-level understanding by grouping identified proteins based on their functional biological processes which provide a greater insight on the major cellular processes that can be affected as a result of recombinant EG2-hFc1 expression.

Apoptosis/Cell-Proliferation

A cellular response that involved proteins in the apoptotic and cell proliferation pathways was observed. The production of EG2-hFc1 may potentially be a stress factor for the cell, as some of its energy is directed towards recombinant protein biosynthesis as a result of using a strong promoter. Hsp60 and Hsp90, which are protein chaperones that exhibit anti-apoptotic activity [105; 106], were down-regulated in CHO-1A7. In contrast, mitochondrial heat shock proteins, Trap1 and Grp75, were observed to be up-regulated in the same cell line. Trap1 has been shown to be linked to various biological pathways and its up-regulation promotes cell proliferation [107]. Grp75 is predominantly localized in the mitochondria, suggesting that it does not play a direct role in protein folding during the expression of EG2-hFc1. Also known as mortalin, Grp75 is an inducible chaperone that is responsive to stress, and is also believed to influence cell proliferation and ageing [108; 109]. As a

result, the up-regulation of Trap1 and Grp75 may be indicative of CHO-1A7 changes consistent with prolonging or maintaining cellular life span.

Other apoptotic/cell proliferation-related proteins, including prohibitin (Phb), proliferating cell nuclear antigen (Pcna), aldehyde dehydrogenase (Aldh), NADH dehydrogenase [ubiquinone] iron-sulfur protein 3 (Ndufs3), and programmed cell death 6 protein (Pdc6), were found to be differentially regulated in this study. Phb over-expression has been shown to increase cell survival by arresting cells in the G0/G1 phase, which leads to the inhibition of cell proliferation [110]. Pcna enhances the processivity of DNA polymerases in replication-linked processes by recruiting crucial players to the replication fork [111]. Aldh is an enzyme responsible for the oxidation of a wide variety of aldehydes to their corresponding carboxylic acids using NAD or NADP as a coenzyme. A number of studies have observed a decrease in cell proliferation as a consequence of Aldh inhibition [112-114]. Both Pdc6 and Ndufs3 are positive regulators of apoptosis, the latter also being a subunit of mitochondrial complex I. Inhibition of Ndufs3 has been shown to provide resistance to IFN- β /Retinoic acid-induced apoptosis [115; 116]. Phb was up-regulated in this study while Pcna was down-regulated, both of which coincide with the increased viability observed at 8 day cultivation in CHO-1A7 in comparison to CHO-BRI cells. A number of studies have shown an increase in recombinant protein production in growth-arrested CHO cells [117-119], and this suggests a shift away from cell proliferation processes due to EG2-hFc1 production. The up-regulation of Ndufs3 and Pdc6 may suggest the initiation of apoptosis in a portion of cells by the end of the batch cultivation process. The expression of many anti- and pro-apoptotic proteins may reflect changes in cell signalling influencing apoptosis and viability, and may therefore reflect an increase of the population of dying cells.

Molecular Chaperones

Intracellular perturbations caused by a variety of stressors, including oxidative stress, glucose and amino acid deprivation, protein over-expression, and inhibition of glycosylating enzymes, can lead to protein misfolding and aggregation within the ER [120]. If aberrant protein folding becomes overwhelming, the unfolded protein response (UPR) can be activated in eukaryotes, which attempts to restore protein folding as well as proper post-translational modifications. UPR proteins were observed to be differentially regulated in a recent proteomic study that investigated apoptosis in a MAb-

producing CHO cell line during prolonged cultivation [121]. However, in this study, the key molecular chaperones of the UPR pathway (Erp29, Erp72, Hsp70, Calnexin, Calreticulin, Grp78/Bip, Xbp1) [122] did not show any significant differential expression. The discrepancy could in part be due to the investigation of differentially expressed proteins associated with apoptosis in the former study rather than MAb production in our study. Similarly, there was a lack of apparent changes in these proteins in earlier studies on recombinant CHO cells producing high and low levels of *dhfr*-GFP fusion protein [123] as well as with CHO cells producing human growth hormone [124]. This might indicate that the stress on the protein folding machinery is insufficient to invoke the UPR response and any differences may be cell-line specific or may be associated with levels of proteins produced.

Two ER-resident proteins, protein disulfide-isomerase (PDI) and GRP94 showed down-regulation in the EG2-hFc1-producing CHO-1A7 strain. PDI is a thiol oxidoreductase involved in the formation, isomerization, and reduction of disulfide bonds [125]. PDI also exhibits chaperone activity, where it aids wrongly folded proteins to reach a correctly folded state. GRP94, also known as endoplasmic reticulum chaperone, is implicated to be involved with chaperoning secretory proteins [126]. GRP94 was also shown to associate with unassembled immunoglobulin chains exerting chaperone activity [127]. Down-regulation of PDI and GRP94 in this present work could suggest more efficient protein folding in CHO-1A7 cells. It is possible that the endogenous levels of PDI and GRP94 are adequate for the post-translational processing requirements of EG2-hFc1. Additionally, the reduced expression of Hspd1 and Hsp90aa1 further support this hypothesis. Both Hspd1 and Hsp90aa1 are heat shock proteins that exhibit chaperone activity essential for proper protein folding within the mitochondria and cytosol, respectively [127; 128]. Hsp90aa1 is a member of the Hsp90 family of proteins known for their key roles in signal transduction, protein folding, and protein degradation. They participate in the regulation of stress response and, in association with co-chaperones, function to stabilize and refold denatured or misfolded proteins after stress [129]. Hspd1 and Hsp90aa1 are located in different cellular compartments and are not directly involved with MAb production. As the cell's energy is partly redirected towards MAb production, this may result in a reduced demand for certain chaperones, with a resultant drop in expression level. Although other studies noted an increased expression of Hspd1 in high-producing GS-NS0 cell lines [127; 130], this contrasting result may be due to the differences in the cell lines used and suggest that CHO cells may have a greater efficiency in sustaining protein synthesis and folding.

Structural Proteins

Cytoskeletal proteins that are related to the structural integrity of the cell, including vimentin (Vim) and β -actin (Actb), generally showed reduced expression in CHO-1A7 cells. Vimentin was recently hypothesized to contribute to mitochondrial morphology and organization by interacting with the mitochondria and its associated microtubules [131]. Knockdown of vimentin resulted in mitochondrial fragmentation and reorganization that might ultimately disturb ATP generation [131]. Down-regulation of vimentin in CHO-1A7 cells could be related to the down-regulation of proteins involved in ATP synthesis (ie: Atp5b) and thus EG2-hFc1-producing cells harvested after 8 day batch cultivation could have expended most of their energy towards protein biosynthesis compared to the control. However, further investigation into the role and interaction of this protein with the mitochondria are warranted.

Several studies have accumulated evidence of a close association between the cellular microfilament network and the structure and organization of the cellular protein translation apparatus [132-137]. Perturbations of actin filaments have been shown to affect the protein synthesis machinery in CHO cells, with disruption of the actin filament network resulting in a reduction in protein translation [138]. In addition, a subsequent study observed that eukaryotic translation elongation factor 1A (eEF1A) acts as a regulator that affects translation initiation through the actin cytoskeleton [137]. Although antibody titers were not measured throughout the batch cultivation process, the down-regulation of actin may reflect a reduction in protein biosynthesis owing to the cells having been harvested at stationary phase.

Metabolic Enzymes

Various metabolic enzymes are differentially regulated in association with EG2-hFc1 expression, including proteins related to carbohydrate, amino acid, and lipid metabolism. Citrate synthase (Cs) catalyzes the condensation of acetyl-CoA and oxaloacetate to form citrate. It is a key enzyme in the citric acid cycle that provides energy for cellular functions including protein biosynthesis. During the process of catalyzing the irreversible conversion of glycerol-3-phosphate to dihydroxyacetone phosphate, the enzyme glycerol-3-phosphate dehydrogenase (Gpd2) concurrently transfers electrons from flavin adenine dinucleotide (FAD) to the electron transport chain [139]. Aconitate hydratase (Aco2) is sensitive to the accumulation of reactive oxygen species (ROS)

resulting from increased electron flow through the respiratory chain [140], and thus regulates citrate accumulation by reversible isomerisation of citrate to isocitrate. The decreased levels of Aco2 observed in this present work may reflect increased expression of Cs and Gpd2, and possibly correspond to higher levels of citrate and subsequent levels of electron flux towards ATP synthesis. Similarly, the up-regulation of dihydrolipoyl dehydrogenase (Dld), a component of various multi-enzyme systems, suggests a shift in metabolic flux towards energy generation via the pyruvate dehydrogenase complex (PDC), the α -ketoglutarate dehydrogenase complex (KGDC), and the branched-chain α -keto acid dehydrogenase complex (BCKDC). The PDC plays a key role in linking the glycolysis pathway to the citric acid cycle by converting pyruvate into acetyl-CoA and concurrently yielding NADH for cellular respiration [141]. The KGDC catalyzes the decarboxylation of α -ketoglutarate into succinyl-CoA in the citric acid cycle [142]. Finally, the BCKDC is responsible for regulating homeostatic levels of branched chain amino acids (BCAA) – isoleucine, leucine, and valine - through catabolic activities that yield end-products that eventually lead to the citric acid cycle for energy metabolism [143]. For example, BCKDC catalyzes the irreversible degradation of valine via a two-step mechanism to initially yield 3-hydroxyisobutyrate (HIB). Next, 3-hydroxyisobutyrate dehydrogenase (Hibadh), also observed to be up-regulated in this study, enzymatically converts HIB to succinyl-CoA [143].

Aspartate aminotransferase (Ast) is involved in an anapleurotic reaction that replenishes TCA intermediates via a reversible reaction forming oxaloacetate (OAA), and thus is important in both the TCA cycle and amino acid metabolism. Many of the differentially regulated metabolic enzymes discussed above can be associated with the TCA cycle. The TCA cycle is the central hub of metabolism and is crucial for energy production and subsequent biosynthesis. The metabolic load, which is the amount of energy required to express a particular gene within a cell, may have increased as a response to recombinant antibody production. The differential expression of enzymes noted in this study seem to generally point towards an increase in energy generation as a means to meet the energy demands of increased recombinant protein biosynthesis.

2.5 Conclusion

The present study monitored global changes in the protein expression profiles between CHO cells engineered to produce a chimeric heavy chain monoclonal antibody EG2-hFc1 and the corresponding non-producer parental cell line. A total of 86 protein spots were selected for subsequent MS sequencing, which yielded 34 unique protein identifications. Through functional analyses, the production of EG2-hFc1 has shown evidence of pathway changes associated with chaperone activity, cytoskeletal elements involved in protein translation machinery, carbohydrate and amino acid metabolism. Additionally, the major cellular processes affected include the regulation of the apoptotic and cell proliferation pathways as well as energy derivation. The upregulation of metabolic enzymes suggests increased energy production in CHO-1A7 cells. This is thought to be a direct response of the CHO-1A7 cell line to meet the energy requirements needed for recombinant protein biosynthesis while maintaining cell viability.

Chapter 3

Development of a 2-D CHO Protein Reference Map via Multiplexing

3.1 Introduction

Presently, the costs of mass spectrometry analysis of protein samples are fairly affordable if there are a low number of samples to be analyzed. The costs range from \$85 to \$150 per sample at various facilities in Ontario, Canada that offer MS/MS services. The price can be reduced to as low as \$60 per sample if all the sample handling and data analysis is performed by the investigators who use those services. To develop comprehensive 2-D proteome reference maps, however, the identification of hundreds of protein spots would become costly, time-consuming, and laborious. This would discourage researchers from expanding the 2-D gel database, which would otherwise provide beneficial tools for proteomic studies. Hence, in the following studies, we developed a multiplexing scheme that would facilitate the development of 2-D protein reference maps at a significantly reduced cost, while identifying a large number of protein spots. This method would allow up to 528 protein spots to be identified at a cost of approximately \$34 per sample.

CHO proteome reference maps have been established as efforts to better understand CHO cell physiology and protein expression changes caused by genetic or culture environmental perturbations. In 1999, the first comprehensive 2-D CHO proteome reference map was published by Champion and colleagues [144]. Whole cell protein extracts from a CHO-DUKX host cell line were silver-stained and the investigators identified 25 cellular proteins by MS and MS/MS. Using fluorescence staining, Hayduk et al (2004) significantly improved the number of protein identifications to 224 identifications in a CHO-K1 host cell line [145]. The most recently published protein reference map, in 2010, belongs to the dihydrofolate reductase-deficient CHO-DG44 host cell line [146]. Composing of over 1400 silver-stained protein spots, a total of 179 spots, which represented 106 unique proteins, were identified [146].

Though several 2-D proteome reference maps have been published for CHO cells, the studies mentioned were performed at a time when the proteome database of CHO cells was rather limited. As a result, the researchers performed protein database searching from related species including *Mus*

musculus (mouse), *Rattus norvegicus* (rat) and *Homo Sapiens* leading to a loss of stringency in the identifications. Secondly, an attempt to comprehensively identify over 1000 gel spots from a given whole cell protein extract, by mass spectrometry, would be relatively costly. Finally, the CHO 2-D protein reference maps published to date contain annotation data within the articles themselves, thereby limiting its usefulness when one relies on visual comparison of one's gels to the images displayed in the manuscript.

The present study addresses these issues. A 2-D proteome reference map was generated for the CHO-BRI host cell line to further expand our understanding of this particular cell line. It will be a useful tool for studies investigating physiological responses and protein expression patterns of CHO-BRI to genetic and environmental modifications. Mass spectral data analysis was performed against a CHO-specific protein database to enhance the confidence of protein identifications. A multiplexed design was developed to maximize the number of protein identifications while minimizing the cost from gel-based maps. The proof of principle of this unique experimental design was tested with CHO-BRI cells and further developed using *Escherichia coli* (strain MG1655) cells. Finally, the resulting CHO-BRI 2-D proteome reference map and its corresponding proteomic data can be deposited into the World-2DPAGE Repository (a public database for gel-based proteomics data linked to protein identifications published in literature). These mapping efforts would contribute to optimizing the growth of CHO cells and efficient cell line engineering initiatives which could lead to enhanced recombinant protein production.

3.2 Materials and Methods

3.2.1 Multiplex Experimental Design

A paired gel design was devised in a multiplexed fashion that allows up to 528 spots to be identified by mass spectrometry as seen in **Figure 3-1**. The design incorporated two preparative gels that were produced from a single whole cell protein extract sample. Spots were matched between the two gels and assigned spot IDs using PDQuest Advanced 2-D Analysis Software v8.0.1. Spots were then excised into the wells of a 96-well perforated filter plate, with increasing abundant spots going down the rows of the plate, such that two of the same spot ID (1 from each gel) appears in two different wells of a given row. For example, according to **Figure 3-2**, spot #1 was excised into wells H1 and H2. Spot #30 was excised into wells H6 and H9. Similarly, spot #55 was excised into wells H7 and H12. Each unique spot ID appears in a unique pair of wells that only correspond to that specific spot ID. After mass spectrometry analysis, the proteins within each well were identified. By observing for pairs of protein IDs within each row and the combination of wells they are found in, this was mapped back to their corresponding spot ID, which subsequently mapped back to the corresponding protein spot on the preparative gel.

In this multiplex design, 11 spots are placed into each well, which corresponds to 66 unique protein spots per row. With 8 rows per 96-well perforated filter plate, this would correspond to a total of 528 protein spots that could potentially be identified from a preparative gel of a target organism. The proof of concept was initially confirmed using the CHO-BRI cell line to develop a 2-D CHO protein reference map. The method was further developed using the *Escherichia coli* (*E. coli*) strain MG1655 as a model organism. For the *E. coli* samples, a control plate was also run that contained 96 single protein spots excised into each well from a third preparative gel to facilitate comparison. Specifically, 12 protein spots for each row (1 per well) of the singlet plate were randomly selected from the list of 66 unique protein spots in the multiplex design to keep in accordance with the increasing abundant levels down the plate (**Figure 3-3**). This also facilitates comparison to see if the proteins identified in the singlet plate would also be identified in the multiplex plate on a per row basis.

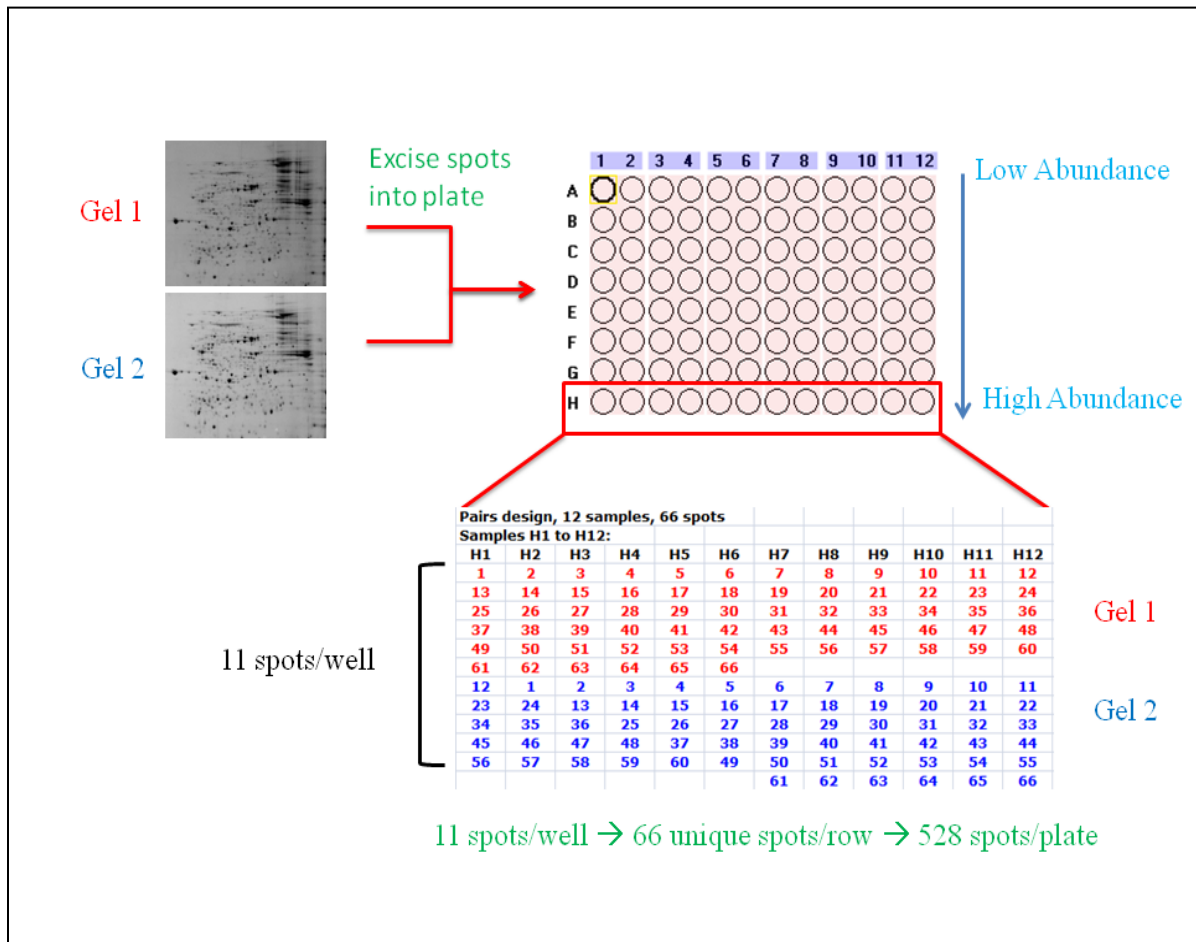


Figure 3-1. Multiplex Paired Gel Design (A). Two preparative gels were run from a single protein sample. Protein spots were excised into the wells of a 96-well perforated filter plate. Each well consists of 11 protein spots. Each row contains 66 unique spots that result in 528 unique spots across the entire plate.

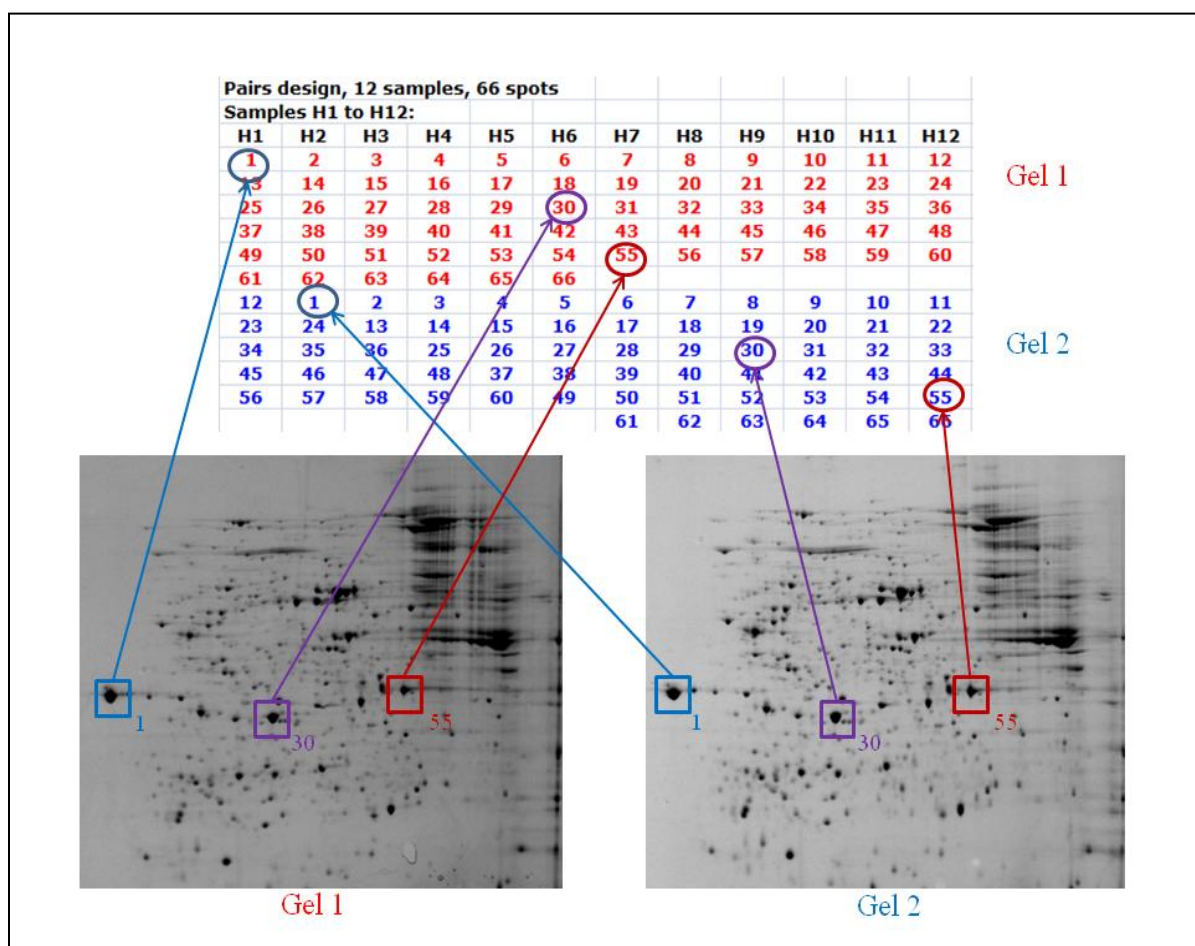


Figure 3-2. Multiplex Paired Gel Design (B). A schematic example: each unique spot ID appears in a pair of unique wells that only corresponds to that spot ID. Pairs of protein IDs can then be mapped back to the corresponding pair of wells which maps back to the location of the protein spot on the preparative gel. This schematic is applied to rows A through H.

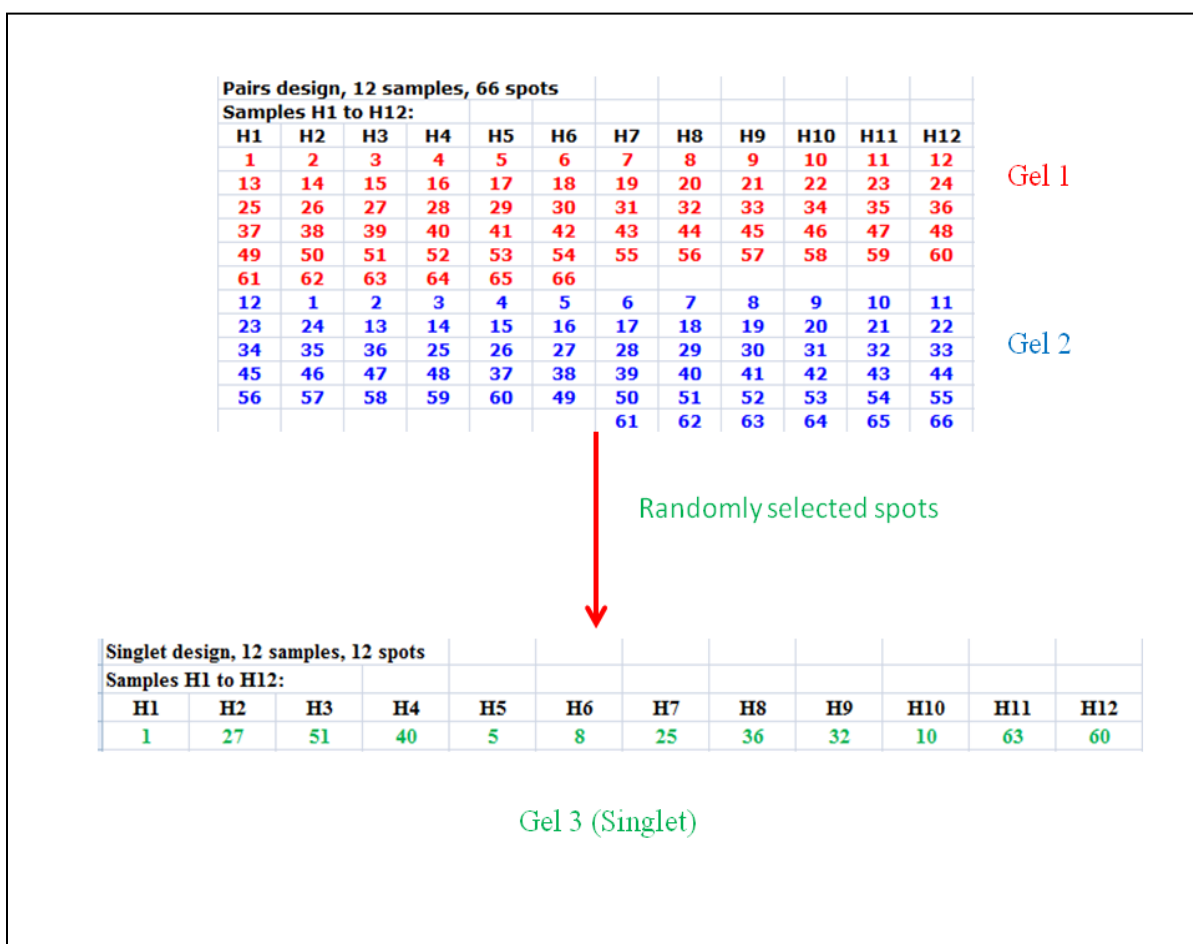


Figure 3-3. Singlet Gel Design (Control). A schematic example: For a given row, 12 spots were randomly selected from the list of 66 unique protein spots generated in the multiplex design. This second plate was denoted the 'singlet plate' and consisted of a total of 96 protein spots.

3.2.2 Cell Lysis and Protein Extraction

CHO-BRI

CHO-BRI protein samples from the comparative proteomic study described in Chapter 2 were used, with cell lysis and protein extraction as previously described. The six CHO-BRI biological replicate protein samples were pooled together (total volume: ~700µL) and subjected to the Bio-Rad protein assay prior to protein sample clean-up.

MG1655

E. coli strain MG1655 was kindly provided by Dr. Matthew Scott from the University of Waterloo. The cells were grown in minimal M63 media with 20mM NH_4^+ as the nitrogen source and 0.2% glucose for the carbon source. The doubling time was roughly 1 hour.

The method for cell lysis and protein extraction was similar to that of section 2.2.2 with minor changes. After washing with PBS, MG1655 was re-suspended in 1000 μL lysis buffer and 24 μL protease inhibitor. Nucleic acids were removed from the extract by addition of 1% protamine sulfate (Calbiochem, Billerica, USA). Due to high cell density, the extracted protein samples underwent 20x dilution prior to protein concentration determination by the Bio-Rad protein assay.

3.2.3 Protein Sample Clean-Up

The protein sample clean-up procedure was similar to that of section 2.2.3 with a few modifications.

CHO-BRI

The pooled protein sample with a volume of approximately 700 μL was split into 150 μL aliquots for protein sample clean-up. After clean-up, the aliquots were pooled back together and re-suspended in 600 μL lysis buffer. The protein concentration was determined once more by the Bio-Rad protein assay (see section 2.2.2).

MG1655

A volume of 25 μL from the MG1655 protein extract was aliquoted into 10 microcentrifuge tubes for sample clean-up. After clean-up, the aliquots were pooled together and re-suspended in 1mL lysis buffer. The protein concentration was determined once more by the Bio-Rad protein assay (see section 2.2.2).

3.2.4 2-D SDS-PAGE

CHO-BRI

Due to a limited amount of sample, three colloidal coomassie stained preparative gels were initially prepared as per the protocol in section 2.2.5.1.

MG1655

Six colloidal coomassie stained preparative gels were initially prepared as per the protocol in section 2.2.5.1.

3.2.5 Gel Scanning and Analysis

The resulting stained gels were scanned using the EXQuest Spot Cutter camera. All the gel images were cropped to the same size and the spot detection wizard within PDQuest Advanced 2-D Analysis Software v8.0.1 was used to facilitate spot matching. A maximum value was chosen for each spot detection parameter resulting in an increase in the sensitivity of the spot detection wizard while minimizing the detection of image noise. Additionally, optional settings to remove horizontal and vertical streaks, speck removal, and background subtraction were selected.

The number of matched spots was then determined between each pair of gel images (Gel 1 vs 2, Gel 1 vs 3, Gel 2 vs 3 etc). The pair of gels that resulted in the greatest number of matched spots were selected for the multiplex experiment due to increased coverage of the entire proteome. In the MG1655 experiment, a third gel that had the most number of spots detected was used as the single gel control. Next, the list of spot IDs for the matched spots between paired gels, including their relative x- and y- coordinates on the gel as well as their spot intensity levels, were exported into MATLAB v7.5.0 (The MathWorks, Inc) to generate spot-picking lists totaling 528 unique protein spots. The algorithm generated 13 pick lists (6 for each gel in the paired design and 1 for the singlet control). The pick lists were generated in such a way that row A of the plate contained the least abundant protein spots (as determined by their intensity levels), with increasing abundance towards row H in order to ensure consistency in abundance levels between each row. This is to minimize masking of low abundant spots by high abundant spots which may not be seen during protein identification steps.

Subsequently, the spot-picking lists were imported into PDQuest as several .txt files and the manual excision function was used to excise spots of interest. Due to the differences in the positioning of the gel inside the EXQuest Spot Cutter, the x- and y-coordinates of each spot of interest must be manually screened and corrected to ensure the correct spot of interest was excised. Spot excision was carried out as per section 2.2.5.2 under 'Protein Spot Excision'.

3.2.6 Multiwell In-Gel Trypsin Digestion

The multiwell in-gel trypsin digestion was performed according to section 2.2.5.2 following protein spot excision.

3.2.7 Mass Spectrometry (LC-MS/MS)

The digested peptides were sent to the Hospital for Sick Children Mass Spectrometry Facility (ON, Canada) for mass spectrometry (MS) analysis. The CHO-BRI samples were analyzed according to section 2.2.5.3.

To improve accuracy, the MG1655 samples were run on an LTQ Orbitrap instrument with a UPLC system, which has ± 10 ppm accuracy on the parent ions. This speeds up database searches and end up with a lower false positive rate. The procedure was performed by Mr. Paul Taylor for the MG1655 samples and is outlined below:

The digested peptides were loaded onto a 75 μ m x 2cm C18 (3 μ m particle size) pre-column (Thermo-Fisher Scientific, ON, Canada) at 4 μ l/min and separated over a 75 μ m ID x 15cm analytical column packed with C18 (2 μ m) beads (Thermo-Fisher Scientific, ON, Canada) fitted with an emitter tip. The peptides were eluted over 120 minutes at 250 nL/min using a 0 to 35% ACN gradient in 0.1% formic acid using an EASY n-LC 1000 nano-chromatography pump (Proxeon Biosystems, Odense Denmark). The peptides were eluted into a LTQ-Orbitrap hybrid mass spectrometer (Thermo-Fisher, Bremen, Germany) operated in a data dependant mode. MS/MS was carried out in the linear ion trap. Six MS/MS scans were obtained per MS cycle.

3.2.8 Database Searching and Protein Identification

The raw data files were searched using PEAKS Studio v5.3. Protein identification was performed similarly to section 2.2.5.4 with a minor change to the instrument parameters, *de novo* parameters, and PEAKS DB search parameters for MG1655.

CHO-BRI

The instrument parameters, *de novo* parameters, and PEAKS DB search parameters were the same as section 2.2.5.4.

MG1655

The MS precursor scan was set to FT-ICR/Orbitrap and the parent ion/mass error tolerance was set to 20 ppm in the *de novo* and PEAKS DB search parameters because the data was obtained from analyzing on a more sensitive instrument. The *de novo* sequencing results were searched against the UniProt database under the *E. coli* taxa.

3.2.9 Cross-matching Between Protein IDs and Spot IDs

In PEAKS DB, proteins identified by a shared subset of peptides are characterized into the same group. Occasionally, one or more peptides will map back to several protein identifiers all of which correspond to the same actual protein sequence (eg: homologs in different strains). This poses a problem as the matching algorithm relies on this for identifying pairs of the exact same protein identifiers. To circumvent this issue, all protein identifiers were queried in UniRef (UniProt Reference Clusters) to identify a unique and universal UniRef ID that represents a given protein. In other words, all identical sequences and subfragments with 11 or more residues are placed into a single record, which would facilitate proper matching.

R was used to perform the cross-matching for each multiplexed experiment. We looked for successful intersections where a particular UniRefID appears in 2 and only 2 of the 12 wells for a given row.

3.3 Results

3.3.1 Cell Lysis and Protein Extraction

Following cell lysis and protein sample clean-up, the protein concentrations of CHO-BRI and MG1655 samples were determined by the Bio-Rad protein assay. BSA was used as a protein standard to generate the standard curves depicted in **Error! Reference source not found..**

The protein concentrations were calculated for CHO-BRI and MG1655 samples as shown in **Table B 1**. The protein concentration was used to determine the volume of sample required to load approximately 800µg of protein per preparative gel.

3.3.2 Gel Scanning and Analysis

Coomassie stained preparative gels were scanned and the number of spots were detected for each gel. Combinations of pairs of gels were then analyzed to determine the pair that gave the highest number of spots matched. As indicated in **Table 3-1**, Gel 1 vs 3 was chosen for the CHO-BRI sample, and Gel 3 vs 4 was chosen for the MG1655 sample. The initial multiplex run was performed on CHO-BRI samples (Gel 1 and Gel 3) to confirm the proof-of-principle. The *E.coli* MG1655 data was performed as a comprehensive follow-up with modifications to the protocol, such as an inclusion of a single gel control plate and use of a more sensitive mass spectrometer to facilitate comparison. Gel 5 contained the highest number of detected spots, as shown in **Table 3-2**, and was chosen to be the singlet control plate for MG1655. Once the third gel was chosen, it was matched with the previously selected gel pair (Gel 3 vs 4) to assign the same spot IDs for all 3 gels.

Table 3-1. Spot Matching Comparison Between Each Combination of Gel Pairs. Highlighted rows with the highest number of spots matched indicate pairs of gels that were chosen for the paired gel multiplex experiment. A third gel that has the next most number of spots detected was chosen as the single control plate for MG1655, and was matched with the previously selected gel pair to assign the same spot IDs for all 3 gels, as highlighted in blue.

	Gel Comparison	# of Spots Matched
CHO-BRI	Gel 1 vs 2	673
	Gel 1 vs 3	685
	Gel 2 vs 3	600
MG1655	Gel 1 vs 2	895
	Gel 1 vs 3	884
	Gel 1 vs 4	946
	Gel 1 vs 5	927
	Gel 1 vs 6	782
	Gel 2 vs 3	862
	Gel 2 vs 4	907
	Gel 2 vs 5	860
	Gel 2 vs 6	738
	Gel 3 vs 4	981
	Gel 3 vs 5	924
	Gel 3 vs 6	863
	Gel 4 vs 5	977
	Gel 4 vs 6	888
	Gel 5 vs 6	853
	Gel 3 vs 4 vs 5	782

Table 3-2. Spot Detection in MG1655 Gels for Multiplex. The highlighted row indicates the third gel that was chosen for the single control plate.

	Gel #	# of Spots Detected
MG1655	1	1370
	2	1342
	3	1356
	4	1481
	5	1497
	6	1311

3.3.3 Database Searching and Protein Identification

After spots from each gel were excised according to the spot-picking lists generated through MATLAB v7.5.0, they were identified using PEAKS Studio 5.3. **Table B 2**, **Table B 3**, and **Table B 4** in **Appendix B** shows these identifications for both CHO-BRI and MG1655 multiplexed experiments as well as the MG1655 singlet control, respectively.

3.3.4 Cross-matching Between Protein IDs and Spot IDs

Protein IDs were cross-matched to look for successful intersections where a particular UniRefID appears in 2 and only 2 of the 12 wells for a given row. The following tables (**Table 3-3** and **Table 3-4**) display these results for CHO-BRI and MG1655 samples, respectively.

CHO-BRI

Table 3-3. Successful Protein ID Cross-Matches for CHO-BRI.

Wells	Spot ID	Cluster ID	MW (kDa)/pI*	MW (kDa)/pI^	Protein ID	
A01	A08	3217	UniRef90_P31937	29.0/5.7	31.7/5.2	3-hydroxyisobutyrate dehydrogenase, mitochondrial
A01	A09	4606	UniRef90_Q12874	70.4/5.8	58.8/5.2	Splicing factor 3A subunit 3
			UniRef90_G3IA58	70.4/5.8	185.7/6.1	Kinesin-like protein KIF27
A01	A10	2201	UniRef90_P63101	26.7/4.8	27.8/4.7	14-3-3 protein zeta/delta
			UniRef90_P31946	26.7/4.8	28.1/4.8	14-3-3 protein beta/alpha
A01	A11	1504	UniRef90_Q16576	51.6/4.6	47.7/4.9	Histone-binding protein RBBP7
						NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial
A02	A06	5101	UniRef90_G3HZ25	23.7/6.3	25.5/5.0	26S proteasome non-ATPase regulatory subunit 13
A02	A11	4306	UniRef90_G3IND2	42.5/5.7	30.9/5.4	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
A02	A12	6601	UniRef90_P31040	72.1/6.9	68.0/6.3	Cytosol aminopeptidase
A04	A06	5504	UniRef90_G3HR08	55.5/6.3	50.3/6.4	Stress-70 protein, mitochondrial
A05	A07	4709	UniRef90_G3HEZ0	73.0/5.8	65.8/5.8	Annexin A5
A05	A09	2207	UniRef90_P08758	32.2/5.4	35.8/4.9	Triosephosphate isomerase
A05	A11	6212	UniRef90_G3HNV4	25.1/8.5	16.4/8.5	Triosephosphate isomerase
			UniRef90_G3I216	25.1/8.5	20.4/8.9	17beta-hydroxysteroid dehydrogenase type 10/short chain L-3-hydroxyacyl-CoA dehydrogenase
A06	A07	7101	UniRef90_Q99N15	24.1/8.6	27.3/8.9	Poly(rC)-binding protein 1
A06	A12	6304	UniRef90_Q15365	42.3/7.7	37.5/6.7	28S ribosomal protein S22, mitochondrial
			UniRef90_Q9CXW2	42.3/7.7	41.2/8.6	Actin, cytoplasmic 2
A07	A08	1112	UniRef90_G3I6E6	18.7/4.7	32.0/5.2	Actin, cytoplasmic 1
			UniRef90_P60709	18.7/4.7	41.7/5.3	Mitochondrial-processing peptidase subunit beta
A07	A10	4406	UniRef90_G3HE28	50.7/5.8	40.1/5.4	Prohibitin
A07	A11	4010	UniRef90_P35232	15.5/5.9	29.8/5.6	Proliferating cell nuclear antigen
A07	A12	1206	UniRef90_P12004	33.0/4.6	28.8/4.6	Protein disulfide-isomerase A3
A08	A11	603	UniRef90_G3H0U6	71.3/3.8	50.1/5.5	Nucleophosmin
B02	B05	4011	UniRef90_G3IBF1	14.6/6.2	31.1/4.5	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
B02	B06	6603	UniRef90_P31040	72.1/7.0	68.0/6.3	Tropomyosin alpha-3 chain
B02	B10	1205	UniRef90_G3HC01	30.2/4.5	35.4/4.7	Ran GTPase-activating protein 1
B03	B04	3710	UniRef90_P46061	72.9/5.6	63.4/4.6	Protein-glutamine gamma-glutamyltransferase 2
			UniRef90_P21981	72.9/5.6	76.9/5.0	

B03	B06	6407	UniRef90_G3HNG2	47.3/7.0	49.8/8.3	Acyl-coenzyme A thioesterase 2, mitochondrial
B03	B09	6303	UniRef90_G3HHK0	39.5/7.1	35.4/7.1	28S ribosomal protein S31, mitochondrial
B03	B11	7102	UniRef90_Q99N15	24.2/8.6	27.3/8.9	17beta-hydroxysteroid dehydrogenase type 10/short chain L-
B03	B12	4105	UniRef90_G3GTF9	22.1/5.7	18.9/5.5	3-hydroxyacyl-CoA dehydrogenase
B04	B08	2104	UniRef90_Q99653	22.7/4.9	22.5/5.0	ATP synthase subunit d, mitochondrial
B04	B10	4404	UniRef90_G3HE28	50.5/5.7	40.1/5.4	Calcineurin B homologous protein 1
B04	B11	1507	UniRef90_Q16576	53.2/4.6	47.7/4.9	Mitochondrial-processing peptidase subunit beta
B04	B12	6202	UniRef90_G3I2M0	31.9/6.9	25.5/8.6	Histone-binding protein RBBP7
B05	B07	4119	UniRef90_P02794	20.4/6.2	21.2/5.3	Sulfatase-modifying factor 2
B06	B07	613	UniRef90_Q14697	71.8/3.9	104.0/5.6	Ferritin heavy chain
B06	B10	1320	UniRef90_P07910	40.9/4.7	33.5/5.0	Neutral alpha-glucosidase AB
B07	B10	6204	UniRef90_P35232	28.2/7	29.8/5.6	Heterogeneous nuclear ribonucleoproteins C1/C2
B07	B11	4706	UniRef90_Q9CQN1	78.7/5.7	73.8/5.7	Prohibitin
B07	B12	402	UniRef90_Q9DB77	45.7/3.8	46.6/9.0	Heat shock protein 75 kDa, mitochondrial
			UniRef90_G3HQI8	45.7/3.8	229.3/8.1	Cytochrome b-c1 complex subunit 2, mitochondrial
B08	B10	4612	UniRef90_P17987	71.5/6.2	60.3/5.8	Myosin-Vb
B09	B11	3717	UniRef90_A4FUY3	75.6/5.6	83.2/5.1	T-complex protein 1 subunit alpha
C01	C09	2202	UniRef90_G3HEQ3	25.1/5.1	25.2/5.1	Prolyl 3-hydroxylase 1
C01	C10	2705	UniRef90_Q14568	76.4/5.2	39.4/4.6	Ran-specific GTPase-activating protein
C02	C03	4210	UniRef90_P35232	28.3/5.7	29.8/5.6	Putative heat shock protein HSP 90-alpha A2
C02	C10	1101	UniRef90_P56537	24.8/4.5	26.6/4.6	Prohibitin
C04	C06	2608	UniRef90_P30153	71.5/5.1	65.3/5.0	Eukaryotic translation initiation factor 6
			UniRef90_P30154	71.5/5.1	66.2/4.8	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
C04	C07	6104	UniRef90_G3HNV4	24.7/7.1	16.4/8.5	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform
			UniRef90_G3I216	24.7/7.1	20.4/8.9	Triosephosphate isomerase
C05	C12	5705	UniRef90_O00571	72.7/6.5	73.1/6.7	Triosephosphate isomerase
C06	C08	1207	UniRef90_G3HC01	29.3/4.6	35.4/4.7	ATP-dependent RNA helicase DDX3X
C06	C11	2612	UniRef90_Q9R0A0	71.1/5.3	41.1/5.0	Tropomyosin alpha-3 chain
C07	C10	4913	UniRef90_Q8CGK3	98.8/6.1	98.9/5.7	Peroxisomal membrane protein PEX14
C08	C10	6302	UniRef90_P11021	36.2/7.0	70.5/5.0	Lon protease homolog, mitochondrial
C08	C11	1211	UniRef90_B9A067	29.3/4.6	79.0/6.6	78 kDa glucose-regulated protein
						Mitochondrial inner membrane protein
D01	D03	5701	UniRef90_Q8BMF4	72.7/6.3	58.8/5.7	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
D01	D07	4509	UniRef90_Q02790	69.0/6.0	51.8/5.4	Peptidyl-prolyl cis-trans isomerase FKBP4
			UniRef90_G3IAS8	69.0/6.0	185.7/6.1	Kinesin-like protein KIF27
D02	D07	5901	UniRef90_P05091	92.8/6.3	54.4/5.7	Aldehyde dehydrogenase, mitochondrial
D02	D12	6507	UniRef90_P09622	56.1/7.2	50.2/6.5	Dihydrolipoyl dehydrogenase, mitochondrial
D03	D05	3718	UniRef90_O95302	72.5/5.6	60.4/4.8	Peptidyl-prolyl cis-trans isomerase FKBP9
D03	D06	6505	UniRef90_G3IE04	65.2/7.2	53.4/6.1	Pre-mRNA-processing factor 19
D03	D11	1204	UniRef90_P56537	24.9/4.5	26.6/4.6	Eukaryotic translation initiation factor 6
D05	D07	4407	UniRef90_O96019	49.3/5.8	47.3/5.4	Actin-like protein 6A
D06	D12	4412	UniRef90_G3HE28	50.8/6.1	40.1/5.4	Mitochondrial-processing peptidase subunit beta
			UniRef90_G3HKP3	50.8/6.1	40.6/5.6	Cytochrome b-c1 complex subunit 1, mitochondrial
D07	D08	408	UniRef90_Q8CGK3	50.4/3.9	98.9/5.7	Lon protease homolog, mitochondrial
D07	D10	6412	UniRef90_G3HNG2	47.7/7.2	49.8/8.3	Acyl-coenzyme A thioesterase 2, mitochondrial
D07	D12	207	UniRef90_Q3V3R1	29.6/4.4	102.2/6.2	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
E01	E03	5209	UniRef90_O88696	26.3/6.6	24.1/5.6	Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial
E01	E04	5614	UniRef90_Q9CQN1	72.3/6.7	73.8/5.7	Heat shock protein 75 kDa, mitochondrial
E01	E09	3101	UniRef90_Q3V3R1	21.1/5.5	102.2/6.2	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
E01	E12	3410	UniRef90_Q7TP98	45.8/5.6	51.4/5.4	Interleukin enhancer-binding factor 2
E02	E04	5006	UniRef90_G3HDS1	14.6/6.7	14.9/11.0	Histone H4
			UniRef90_B9W7J9	14.6/6.7	11.6/11.4	Histone H4
			UniRef90_P62805	14.6/6.7	11.2/11.4	Histone H4
			UniRef90_G3HPV6	14.6/6.7	28.5/11.0	Histone H2A type 1
E02	E05	503	UniRef90_P34897	53.9/3.9	52.6/8.1	Serine hydroxymethyltransferase, mitochondrial
E02	E08	6406	UniRef90_G3HKP3	51.4/7.0	40.6/5.6	Cytochrome b-c1 complex subunit 1, mitochondrial
E02	E09	407	UniRef90_Q9DB77	46.1/3.9	46.6/9.0	Cytochrome b-c1 complex subunit 2, mitochondrial
			UniRef90_P00505	46.1/3.9	44.7/9.0	Aspartate aminotransferase, mitochondrial
			UniRef90_G3HQI8	46.1/3.9	229.3/8.1	Myosin-Vb
E03	E04	2315	UniRef90_P07910	40.0/5.2	33.5/5.0	Heterogeneous nuclear ribonucleoproteins C1/C2

E03	E10	5703	UniRef90_B9A067	73.0/6.5	79.0/6.6	Mitochondrial inner membrane protein
E04	E05	6503	UniRef90_Q9Y265	53.1/7.0	50.2/6.0	RuvB-like 1
E04	E06	4710	UniRef90_P28331	72.6/5.9	77.0/5.4	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
E04	E09	4508	UniRef90_Q9GZL7	53.0/6.0	47.7/5.6	Ribosome biogenesis protein WDR12
E04	E11	4111	UniRef90_P20108	53.0/6.0	52.2/5.6	Glutathione synthetase
				23.3/5.9	21.6/5.7	Thioredoxin-dependent peroxide reductase, mitochondrial
			UniRef90_Q8K3J1	23.3/5.9	20.4/5.1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial
E04	E12	6403	UniRef90_Q9UQ80	48.5/6.9	43.7/6.1	Proliferation-associated protein 2G4
E05	E07	6004	UniRef90_P09622	12.1/7.1	50.2/6.5	Dihydrolipoyl dehydrogenase, mitochondrial
E05	E08	609	UniRef90_Q71U36	71.3/3.8	50.1/4.9	Tubulin alpha-1A chain
			UniRef90_G3IAS8	71.3/3.8	185.7/6.1	Kinesin-like protein KIF27
E05	E09	4501	UniRef90_G3HZY1	52.2/5.7	57.2/5.4	RuvB-like 2
			UniRef90_Q9Z1N5	52.2/5.7	49.0/5.4	Spliceosome RNA helicase Ddx39b
E06	E07	6611	UniRef90_Q16822	71.7/7.4	67.1/6.6	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
			UniRef90_P49368	71.7/7.4	60.5/6.1	T-complex protein 1 subunit gamma
E07	E10	1410	UniRef90_G3ILN5	44.4/4.6	34.4/4.7	Reticulocalbin-3
			UniRef90_G3H584	44.4/4.6	28.2/4.8	SPARC
E07	E12	2708	UniRef90_Q9Z0X1	75.7/5.3	55.5/7.3	Apoptosis-inducing factor 1, mitochondrial
E08	E10	2302	UniRef90_G3HJN3	35.7/4.8	51.8/5.5	Clusterin
E09	E11	1110	UniRef90_G3GVX1	24.8/4.7	21.7/8.7	Ribosome-binding protein 1
			UniRef90_G3HJN14	24.8/4.7	23.8/5.7	Transitional endoplasmic reticulum ATPase
F01	F03	204	UniRef90_G3HNG5	25.2/4.2	50.4/6.4	Acyl-coenzyme A thioesterase 1
F01	F06	3915	UniRef90_G3I6E6	138.4/5.6	32.0/5.2	Actin, cytoplasmic 2
F01	F11	6420	UniRef90_P49411	49.9/7.9	45.0/6.3	Elongation factor Tu, mitochondrial
F02	F03	7002	UniRef90_G3I129	12.2/8.8	48.7/8.8	Ubiquitin
			UniRef90_P0CG83	12.2/8.8	19.2/6.6	Polyubiquitin (Fragment)
F02	F06	3901	UniRef90_Q16822	103.5/5.4	70.7/6.6	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
F03	F04	1416	UniRef90_G3HJN3	51.0/4.7	51.8/5.5	Clusterin
F03	F05	4609	UniRef90_G3IAS8	71.2/6.0	185.7/6.1	Kinesin-like protein KIF27
F03	F06	5514	UniRef90_G3HR08	57.8/6.7	50.3/6.4	Cytosol aminopeptidase
F03	F09	3507	UniRef90_G3HZY1	52.0/5.6	57.2/5.4	RuvB-like 2
F03	F10	5114	UniRef90_P20108	23.5/6.8	21.6/5.7	Thioredoxin-dependent peroxide reductase, mitochondrial
						Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
F03	F11	4207	UniRef90_P62873	34.0/5.7	37.2/5.6	Calreticulin
F04	F05	2502	UniRef90_P27797	52.9/4.8	48.1/4.3	Tubulin alpha-1A chain
F04	F07	4502	UniRef90_Q71U36	53.3/5.7	50.1/4.9	Aspartate aminotransferase, mitochondrial
F05	F10	5112	UniRef90_P00505	22.4/6.7	44.7/9.0	Peroxisome oxidoreductase
F05	F11	4117	UniRef90_P32119	22.3/6.1	21.8/5.7	28S ribosomal protein S31, mitochondrial
F06	F08	5309	UniRef90_G3HHK0	38.9/6.7	35.4/7.1	Pyruvate dehydrogenase protein X component, mitochondrial
F07	F09	5501	UniRef90_Q8BKZ9	53.2/6.2	48.0/5.8	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
F07	F10	5207	UniRef90_H0X554	25.6/6.5	30.8/6.3	Elongation factor G, mitochondrial
F07	F11	7201	UniRef90_Q8K0D5	25.5/8.5	79.4/5.9	Thioredoxin domain-containing protein 5
F10	F12	4405	UniRef90_Q91W90	48.7/5.7	43.0/5.2	
G01	G02	2407	UniRef90_P81117	50.4/5.1	47.4/5.0	Nucleobindin-2
G02	G04	5515	UniRef90_G3H0U6	68.1/6.7	50.1/5.5	Protein disulfide-isomerase A3
G02	G10	4503	UniRef90_Q9Z1N5	51.7/5.7	49.0/5.4	Spliceosome RNA helicase Ddx39b
G02	G12	6414	UniRef90_P49411	49.5/7.3	49.6/7.7	Elongation factor Tu, mitochondrial
						Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
G03	G12	4216	UniRef90_P62873	34.5/6.0	37.4/5.6	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
G04	G05	3715	UniRef90_P28331	72.4/5.6	79.8/5.9	Dihydrolipoyl dehydrogenase, mitochondrial
G04	G06	6508	UniRef90_P09622	53.4/7.2	54.1/8.0	Serine hydroxymethyltransferase, mitochondrial
			UniRef90_P34897	53.4/7.2	55.9/8.7	Ferritin heavy chain
G04	G08	4108	UniRef90_P02794	21.5/5.7	21.2/5.3	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial
G05	G06	5401	UniRef90_G3GS40	44.4/6.3	38.8/4.9	Reticulocalbin-3
G06	G07	1310	UniRef90_G3ILN5	41.0/4.6	34.4/4.7	Galectin-1
G07	G12	3007	UniRef90_P09382	15.0/5.7	14.8/5.5	Aspartate aminotransferase, mitochondrial
G08	G10	6415	UniRef90_P00505	44.9/7.3	47.4/9.1	Actin, cytoplasmic 2
G09	G10	5102	UniRef90_G3I6E6	23.4/6.3	32.0/5.2	

G11	G12	6701	UniRef90_B9A067	73.1/6.9	79.3/6.4	Mitochondrial inner membrane protein
H01	H09	2602	UniRef90_P81117	70.4/4.9	50.3/5.1	Nucleobindin-2
H01	H12	3704	UniRef90_G3HSM8	72.4/5.5	67.7/5.2	Lamin-B2
						Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
H02	H04	5301	UniRef90_P50213	39.6/6.3	39.6/6.5	Glucosidase 2 subunit beta
H03	H04	717	UniRef90_G3H697	73.2/4.4	62.4/4.4	Heat shock cognate 71 kDa protein
H03	H08	4601	UniRef90_G3IGQ3	70.9/5.7	23.1/8.9	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial
H04	H07	3409	UniRef90_G3GS40	50.0/5.6	38.8/4.9	Inhibitor of nuclear factor kappa-B kinase-interacting protein
H04	H10	3501	UniRef90_G3I2N0	42.5/5.2	42.5/5.2	Dihydrolipoyl dehydrogenase, mitochondrial
H05	H06	6510	UniRef90_P09622	54.1/8.0	54.1/8.0	Aldehyde dehydrogenase, mitochondrial
			UniRef90_P05091	56.5/7.5	56.5/7.5	T-complex protein 1 subunit epsilon
H06	H07	3610	UniRef90_P48643	71.0/5.6	59.5/5.3	Galectin-1
H06	H08	3001	UniRef90_P09382	14.8/5.5	14.8/5.5	Nucleophosmin
H06	H10	1314	UniRef90_G3IBF1	31.0/4.5	31.0/4.5	Protein disulfide-isomerase A4
H08	H11	3611	UniRef90_P08003	72.0/5.6	72.0/5.6	

*MW and pI of the identified proteins were estimated from their gel locations and compared to ^calculated values using ExPASy Compute pI/MW tool [104]. Spot pattern and location were further cross-referenced with available CHO 2D reference maps [124; 145].

According to **Table 3-3**, a total of 131 spots were successfully identified. The relative locations of the identified spots for the CHO-BRI multiplexed sample are shown below (**Figure 3-4**).

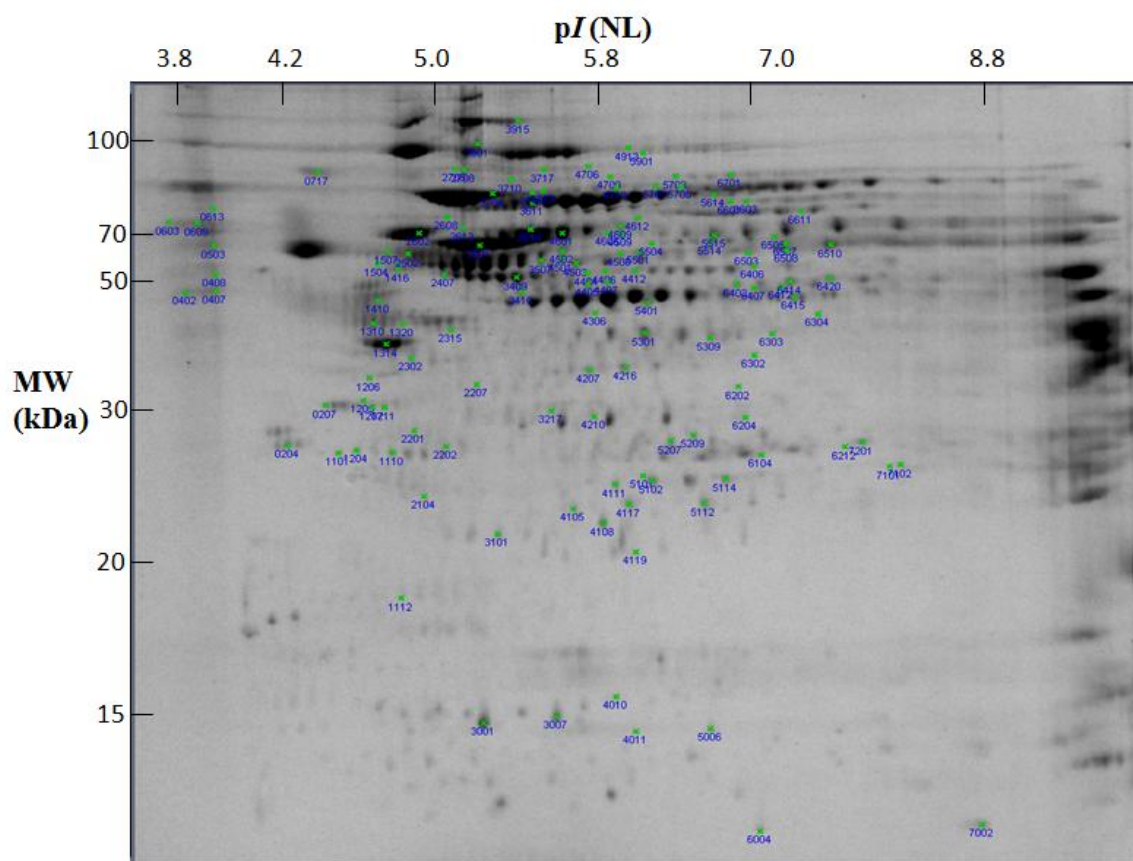


Figure 3-4. (CHO-BRI Multiplex) Identified Spots and Their Relative Locations According to Table 3-3.

MG1655

Table 3-4. Successful Protein ID Cross-Matches for MG1655. Highlighted in green are protein IDs that were also identified in the singlet control plate, refer to **Table A-3 in Appendix A.**

Wells	Spot ID	Cluster ID	MW (kDa)/pI*	MW (kDa)/pI^	Protein ID
A01	A06	4001	UniRef90_A7ZPW1	14.2/5.9	15.5/5.5
A01	A11	2605	UniRef90_B5F6U1	50.5/5.3	49.8/5.3
A02	A04	3706	UniRef90_A8ACN8	55.9/5.7	55.3/5.8
		UniRef90_P23847	55.9/5.7	57.4/5.7	Periplasmic dipeptide transport protein
A02	A09	2516	UniRef90_A7ZHR7	35.1/5.3	88.4/4.9
A02	A10	2220	UniRef90_A7ZPU1	22.5/5.3	22.5/5.3
A03	A04	2406	UniRef90_P0A797	35.0/5.3	34.8/5.5
					6-phosphofructokinase isozyme 1
A03	A06	3209	UniRef90_P22731	26.3/5.6	26.3/5.6
					High-affinity branched-chain amino acid transport ATP-binding protein LivF
A04	A06	4810	UniRef90_P33363	78.8/6.0	81.4/5.8
A04	A09	14	UniRef90_P0A6G4	16.4/5.1	17.6/5.1
					Periplasmic beta-glucosidase
					Protein YgaD

A05	A06	4211	UniRef90_P38489	26.7/6.0	23.9/5.8	Oxygen-insensitive NAD(P)H nitroreductase
A05	A09	2714	UniRef90_A7ZPV7	53.7/5.3	47.0/5.7	Histidine--tRNA ligase
A05	A10	1606	UniRef90_B7UMJ8	44.3/5.2	31.6/8.8	ATP synthase gamma chain
A05	A11	2206	UniRef90_P0AEN3	27.3/5.3	26.1/5.3	NAD(P)H-flavin reductase
A06	A07	3304	UniRef90_B5FTC5	29.7/5.5	27.7/5.9	Deoxyribose-phosphate aldolase
A06	A11	4301	UniRef90_P0A9L8	30.3/5.9	28.1/5.6	Pyrroline-5-carboxylate reductase
B01	B02	2403	UniRef90_A7ZHR7	35.0/5.3	88.4/4.9	Outer membrane protein assembly factor BamA
B01	B03	1611	UniRef90_P02930	51.7/5.2	51.5/5.2	Outer membrane protein tolC
B01	B06	4405	UniRef90_P33030	34.7/6.0	36.1/6.0	Uncharacterized protein YeiR
B01	B10	3512	UniRef90_Q8X4S6	43.6/5.7	43.6/5.7	Acetylornithine/succinyl-diaminopimelate aminotransferase
B02	B07	1803	UniRef90_P06971	78.7/5.2	78.7/5.1	Ferrichrome-iron receptor
B02	B12	3303	UniRef90_A7ZH92	34.4/5.5	33.6/5.5	Homoserine kinase
B04	B07	1109	UniRef90_P0A9W9	20.7/5.2	20.2/5.3	Protein YrdA
B05	B06	215	UniRef90_Q32EF3	24.1/5.1	26.1/5.1	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
B05	B10	2302	UniRef90_P23917	34.4/5.3	32.5/5.5	Fructokinase
B06	B07	1302	UniRef90_B5F769	34.3/5.2	31.4/4.8	Bis(5'-nucleosyl)-tetraphosphatase, symmetrical
B09	B10	2312	UniRef90_P58603	31.2/5.3	33.5/5.3	Protease 7
C02	C04	2311	UniRef90_P0ADS7	34.5/5.3	26.6/6.1	Uncharacterized protein yggE
C02	C05	610	UniRef90_P02930	51.1/5.4	51.5/5.2	Outer membrane protein tolC
C02	C09	2306	UniRef90_A8ALS4	29.1/5.3	28.7/5.7	Dihydrodipicolinate reductase
C02	C12	3505	UniRef90_A8AKV5	39.4/5.5	42.1/5.8	tRNA (uracil(54)-C(5))-methyltransferase
			UniRef90_P00509	39.4/5.5	43.6/5.5	Aspartate aminotransferase
			UniRef90_Q0T204	39.4/5.5	41.0/6.3	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
C03	C08	3517	UniRef90_A7ZSR4	35.6/5.8	38.9/5.7	3-dehydroquinate synthase
C04	C10	4804	UniRef90_P0A928	85.1/6.0	31.4/4.9	Nucleoside-specific channel-forming protein tsx
C04	C11	2518	UniRef90_B7MI93	43.0/5.3	42.3/5.5	Acetylornithine deacetylase
C05	C10	2513	UniRef90_Q8X732	35.1/5.3	35.9/5.6	N-acetyl-gamma-glutamyl-phosphate reductase
C06	C08	1309	UniRef90_P25906	31.7/5.2	30.7/5.3	Putative oxidoreductase YdbC
C06	C11	4203	UniRef90_P75736	26.7/5.9	28.4/5.9	Esterase ybfF
C07	C10	4513	UniRef90_A8ACN8	39.6/6.1	55.3/5.8	ATP synthase subunit alpha
C07	C12	3113	UniRef90_Q3Z3B7	21.8/5.8	21.2/5.9	Flavoprotein WrbA
C08	C11	4112	UniRef90_B7UN33	20.0/6.0	19.0/6.1	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
C09	C10	15	UniRef90_P0AEB8	10.3/5.1	12.5/5.0	RutC family protein yoaB
C09	C11	2802	UniRef90_P08331	64.4/5.3	68.9/5.4	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase
C10	C11	1016	UniRef90_P12994	15.5/5.2	17.1/5.3	UPF0098 protein ybhB
C10	C12	2314	UniRef90_P58603	31.0/5.3	33.5/5.3	Protease 7
C11	C12	4206	UniRef90_P09126	27.8/6.0	27.8/6.0	Uroporphyrinogen-III synthase
			UniRef90_P76034	27.8/6.0	27.6/6.0	Uncharacterized HTH-type transcriptional regulator YciT
D01	D06	2511	UniRef90_P00888	36.8/5.3	38.8/5.4	Phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive
D01	D09	3307	UniRef90_A8ALC4	34.5/5.6	29.9/5.5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
			UniRef90_A7ZN88	34.5/5.6	31.1/5.6	Molecular chaperone Hsp31 and glyoxalase 3
D01	D10	408	UniRef90_P0AFK9	34.9/5.1	36.5/4.9	Spermidine/putrescine-binding periplasmic protein
D01	D11	1408	UniRef90_P66794	35.0/5.2	36.7/5.3	Selenide, water dikinase
D02	D04	3705	UniRef90_Q3Z3B7	52.4/5.6	21.2/5.9	Flavoprotein WrbA
D02	D05	2101	UniRef90_Q57M35	21.3/5.2	20.5/5.4	NADH-quinone oxidoreductase subunit I
D02	D07	2309	UniRef90_B5FTC5	29.0/5.3	27.7/5.9	Deoxyribose-phosphate aldolase
D02	D09	3407	UniRef90_P31550	34.9/5.8	34.2/5.9	Thiamine-binding periplasmic protein
D03	D06	1603	UniRef90_P02930	51.4/5.2	51.5/5.2	Outer membrane protein tolC
D03	D07	3518	UniRef90_B7MG93	38.3/5.8	39.2/6.0	Chorismate synthase
D03	D08	4510	UniRef90_P0A9K5	40.7/6.0	39.0/5.7	PhoH-like protein
D03	D10	611	UniRef90_A7ZVS5	47.3/5.1	47.2/5.2	Thymidine phosphorylase
D04	D09	4215	UniRef90_P0A9D3	22.4/6.0	22.9/5.9	Glutathione S-transferase GstA
D05	D06	2515	UniRef90_P0A9R0	37.0/5.3	40.0/5.4	Aspartate-semialdehyde dehydrogenase
D05	D08	3310	UniRef90_P0ADS7	34.5/5.7	26.6/6.1	Uncharacterized protein yggE
			UniRef90_Q8X4S5	34.5/5.7	34.0/5.9	Arabinose 5-phosphate isomerase GutQ
D05	D10	2703	UniRef90_Q8X9Z2	54.3/5.3	53.3/5.4	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-

						diaminopimelate ligase
D05	D12	2507	UniRef90_P0AEJ3	41.6/5.3	42.9/5.5	Isochorismate synthase entC
D06	D09	505	UniRef90_B5Y275	43.7/5.1	44.3/5.1	Phosphopentomutase
D06	D11	2603	UniRef90_B7N5L4	44.4/5.2	44.4/5.6	CinA-like protein
D07	D08	3220	UniRef90_A7FGQ6	27.0/5.9	25.8/5.8	NADH-quinone oxidoreductase subunit B
D07	D10	1209	UniRef90_P09551	27.2/5.2	25.8/5.2	Lysine-arginine-ornithine-binding periplasmic protein
D08	D10	1802	UniRef90_P06971	78.1/5.2	78.7/5.1	Ferrichrome-iron receptor
D08	D11	3522	UniRef90_Q9S5G5	41.9/5.8	40.3/5.9	Histidine biosynthesis bifunctional protein HisB
D10	D12	4507	UniRef90_Q0T204	39.6/6.0	41.0/6.3	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
D11	D12	603	UniRef90_A8ACN6	51.6/5.0	50.3/4.9	ATP synthase subunit beta
E02	E03	1801	UniRef90_P06971	77.6/5.1	78.7/5.1	Ferrichrome-iron receptor
E02	E05	1306	UniRef90_A7ZHM4	29.3/5.2	28.2/5.2	3-methyl-2-oxobutanoate hydroxymethyltransferase
E02	E06	3	UniRef90_A9MHF3	10.8/4.6	12.4/4.6	50S ribosomal protein L7/L12
E02	E09	2201	UniRef90_P21367	22.5/5.2	23.1/5.2	Uncharacterized protein ycaC
E03	E04	109	UniRef90_P76576	20.5/5.1	22.2/5.1	UPF0070 protein yfgM
E03	E05	213	UniRef90_A4WC74	26.9/5.1	28.4/5.0	Imidazole glycerol phosphate synthase subunit HisF
E03	E06	2210	UniRef90_A7FGQ6	26.6/5.3	25.8/5.8	NADH-quinone oxidoreductase subunit B
E03	E08	4410	UniRef90_B5F446	34.7/6.0	34.4/5.9	Aspartate carbamoyltransferase
E03	E11	4306	UniRef90_P0AAY0	34.4/6.0	30.9/5.6	Probable L,D-transpeptidase YbiS
E03	E12	2505	UniRef90_A1AC10	43.5/5.3	42.4/5.5	Phosphoribosylglycinamide formyltransferase 2
E04	E07	4309	UniRef90_P0AEZ1	34.3/6.0	33.1/6.0	5,10-methylenetetrahydrofolate reductase
E04	E08	4110	UniRef90_B7UN33	19.9/6.0	19.0/6.1	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
E04	E10	4209	UniRef90_P12758	27.3/6.0	27.0/5.8	Uridine phosphorylase
E04	E12	3504	UniRef90_A7ZSR4	35.1/5.4	38.9/5.7	3-dehydroquinase synthase
E05	E06	3513	UniRef90_Q46856	36.3/5.7	42.1/5.7	Alcohol dehydrogenase YqhD
E05	E08	1310	UniRef90_P16703	34.4/5.2	32.7/5.4	Cysteine synthase B
E05	E09	310	UniRef90_P23905	34.4/5.1	33.4/5.3	D-galactose-binding periplasmic protein
E05	E10	4119	UniRef90_A7ZKL2	20.0/6.1	21.2/6.1	UPF0227 protein ycfP
E05	E11	2415	UniRef90_P26646	34.6/5.3	34.7/5.6	Putative quinone oxidoreductase YhdH
E06	E09	2308	UniRef90_P33362	34.1/5.3	30.2/5.6	Putative osmoprotectant uptake system substrate-binding protein osmF
E07	E08	2512	UniRef90_Q8X732	35.1/5.3	35.9/5.6	N-acetyl-gamma-glutamyl-phosphate reductase
E07	E09	2209	UniRef90_A8ANW8	27.5/5.3	28.0/5.5	Phosphoadenosine phosphosulfate reductase
E08	E11	2707	UniRef90_P25526	53.2/5.3	51.7/5.4	Succinate-semialdehyde dehydrogenase [NADP(+)]
E09	E11	2713	UniRef90_P23847	54.6/5.3	57.4/5.7	GabD
			UniRef90_A8ALC4	54.6/5.3	29.9/5.5	Periplasmic dipeptide transport protein
E09	E12	217	UniRef90_P67271	24.4/5.1	26.9/5.4	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
E10	E11	705	UniRef90_P77804	54.3/5.1	52.8/5.0	UPF0135 protein ybgI
E10	E12	210	UniRef90_Q32EF3	23.2/5.1	26.1/5.1	Protein ydgA
F01	F03	3204	UniRef90_P0AFU8	22.4/5.4	23.4/5.6	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase
F01	F04	1212	UniRef90_P0AET9	25.3/5.2	26.8/5.2	Riboflavin synthase alpha chain
			UniRef90_B7MIX6	25.3/5.2	27.0/5.6	7-alpha-hydroxysteroid dehydrogenase
F01	F05	2408	UniRef90_Q3Z3B7	35.0/5.3	21.2/5.9	Thiazole synthase
F01	F06	219	UniRef90_A8ALC9	25.9/5.1	24.3/5.1	Flavoprotein WrbA
F01	F08	4412	UniRef90_P0AB92	35.0/6.0	38.0/6.1	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
F02	F04	1410	UniRef90_P0A9P5	34.6/5.2	34.5/5.3	Phospho-2-dehydro-3-deoxyheptonate aldolase, Phe-sensitive
F02	F05	2208	UniRef90_A7ZUD3	26.8/5.3	27.0/5.6	Thioredoxin reductase
F02	F06	4004	UniRef90_A8API1	11.0/5.9	11.0/5.9	Triosephosphate isomerase
F02	F07	2606	UniRef90_A8AIH5	44.4/5.3	46.0/5.3	Probable Fe(2+)-trafficking protein
F02	F08	2808	UniRef90_P00904	66.4/5.3	56.7/6.1	3-phosphoshikimate 1-carboxyvinyltransferase
F02	F11	3701	UniRef90_P19480	57.3/5.4	55.9/5.6	Anthranilate synthase component II
F03	F04	704	UniRef90_P77804	54.0/5.1	52.8/5.0	Alkyl hydroperoxide reductase subunit F
F03	F05	3210	UniRef90_A7ZIN4	27.8/5.7	23.6/5.6	Protein ydgA
			UniRef90_Q57RI5	27.8/5.7	28.5/5.8	Adenylate kinase
F03	F06	3604	UniRef90_A7FDW9	46.0/5.6	44.8/5.7	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
F03	F08	3110	UniRef90_P0ACS1	20.4/5.8	20.6/5.8	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
						Transcriptional repressor mprA

F03	F09	4217	UniRef90_P67082	26.8/6.1	25.8/6.1	UPF0001 protein yggS
			UniRef90_P10346	26.8/6.1	26.7/6.1	Glutamine transport ATP-binding protein GlnQ
F04	F05	3410	UniRef90_P16657	34.6/5.8	27.6/5.6	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI
			UniRef90_P0AE20	34.6/5.8	29.3/5.6	Methionine aminopeptidase
			UniRef90_P0ADS7	34.6/5.8	26.6/6.1	Uncharacterized protein yggE
F04	F06	1417	UniRef90_A7ZNI1	34.6/5.2	33.4/5.5	ATP phosphoribosyltransferase
F04	F07	4009	UniRef90_P0ADN4	16.1/6.1	13.0/6.1	UPF0438 protein yifE
F04	F08	4713	UniRef90_B7UPI6	59.6/6.0	61.5/5.9	Glucose-6-phosphate isomerase
F04	F11	406	UniRef90_Q3Z606	34.9/5.0	35.2/5.1	Transaldolase 1
F05	F09	3218	UniRef90_P38489	25.7/5.8	23.9/5.8	Oxygen-insensitive NAD(P)H nitroreductase
F05	F10	1608	UniRef90_P39099	49.8/5.2	44.4/5.4	Periplasmic pH-dependent serine endoprotease DegQ
F05	F11	3404	UniRef90_P0A797	35.0/5.6	34.8/5.5	6-phosphofructokinase isozyme 1
F06	F09	3711	UniRef90_P07003	62.0/5.9	62.0/5.9	Pyruvate dehydrogenase [ubiquinone]
F07	F10	607	UniRef90_A8ACN6	49.7/5.1	50.3/4.9	ATP synthase subunit beta
			UniRef90_Q8FG52	49.7/5.1	46.1/5.1	Histidinol dehydrogenase
F08	F10	2701	UniRef90_P25526	52.9/5.2	51.7/5.4	Succinate-semialdehyde dehydrogenase [NADP(+)] GabD
F09	F10	3312	UniRef90_P09030	34.4/5.8	31.0/5.8	Exodeoxyribonuclease III
			UniRef90_P33362	34.4/5.8	30.2/5.6	Putative osmoprotectant uptake system substrate-binding protein osmF
F09	F11	3301	UniRef90_P0A9L8	29.1/5.4	28.1/5.6	Pyrroline-5-carboxylate reductase
F10	F11	3521	UniRef90_P0A9K5	40.1/5.8	39.0/5.7	PhoH-like protein
G01	G02	4708	UniRef90_P0ADG8	59.4/6.0	52.0/6.0	Inosine-5'-monophosphate dehydrogenase
G01	G08	1203	UniRef90_A1AFA2	22.4/5.2	24.3/5.5	Ribose-5-phosphate isomerase A
G01	G09	3214	UniRef90_P0A957	22.3/5.8	22.3/5.6	KHG/KDPG aldolase
			UniRef90_P0A9D3	22.3/5.8	22.9/5.9	Glutathione S-transferase GstA
G01	G11	4006	UniRef90_B5FI12	14.3/6.0	15.2/5.9	Ribosome-binding factor A
			UniRef90_Q8FK07	14.3/6.0	16.0/6.0	Universal stress protein G
G02	G03	1202	UniRef90_A7ZHM4	28.7/5.2	28.2/5.2	3-methyl-2-oxobutanoate hydroxymethyltransferase
G02	G04	3609	UniRef90_A7FDW9	46.1/5.8	44.8/5.7	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
G02	G05	2804	UniRef90_Q8XEG2	66.0/5.3	66.8/5.6	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]
G02	G09	1125	UniRef90_P0A4L6	21.7/5.2	21.1/5.4	Thiol:disulfide interchange protein DsbA
G03	G04	4305	UniRef90_Q8XBT6	30.1/6.0	31.1/5.9	2,5-diketo-D-gluconic acid reductase A
G03	G07	4311	UniRef90_A7ZPS4	34.5/6.1	31.3/6.0	Dihydrodipicolinate synthase
G03	G09	4113	UniRef90_B7UN33	19.8/6.0	19.0/6.1	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
G03	G11	3516	UniRef90_Q9S5G5	42.8/5.8	40.3/5.9	Histidine biosynthesis bifunctional protein HisB
			UniRef90_A7MH28	42.8/5.8	43.2/5.9	Acetate kinase
G04	G05	116	UniRef90_P0A252	21.2/5.1	20.6/5.0	Alkyl hydroperoxide reductase subunit C
G04	G06	4303	UniRef90_P02925	29.0/5.9	28.5/6.0	D-ribose-binding periplasmic protein
G04	G07	4611	UniRef90_P78067	46.3/6.1	45.8/6.0	Thiosulfate sulfurtransferase YnjE
G04	G09	1011	UniRef90_P0AEN0	16.4/5.2	26.1/5.3	Cystine-binding periplasmic protein
			UniRef90_P0A919	16.4/5.2	16.4/5.3	Outer membrane protein X
G04	G11	3104	UniRef90_P0AGD5	21.6/5.5	21.1/5.6	Superoxide dismutase [Fe]
G04	G12	4512	UniRef90_P33570	35.0/6.0	73.0/5.9	Transketolase 2
G05	G08	111	UniRef90_P0AG09	22.2/5.1	24.6/5.1	Ribulose-phosphate 3-epimerase
			UniRef90_P0ADK2	22.2/5.1	25.7/6.1	Uncharacterized protein yiaF
G05	G09	2204	UniRef90_A7ZPU1	22.4/5.3	22.5/5.3	Uracil phosphoribosyltransferase
G05	G10	2414	UniRef90_P0A797	35.0/5.3	34.8/5.5	6-phosphofructokinase isozyme 1
G05	G11	3217	UniRef90_Q3Z3B7	22.4/5.8	21.2/5.9	Flavoprotein WrbA
G06	G08	3703	UniRef90_P09832	55.3/5.5	51.9/5.5	Glutamate synthase [NADPH] small chain
			UniRef90_P23847	55.3/5.5	57.4/5.7	Periplasmic dipeptide transport protein
G06	G09	1206	UniRef90_P0ACA5	26.6/5.2	24.2/5.2	Stringent starvation protein A
G06	G12	2315	UniRef90_A7ZN88	34.5/5.3	31.1/5.6	Molecular chaperone Hsp31 and glyoxalase 3
G07	G08	3509	UniRef90_P0A9K5	39.1/5.6	39.0/5.7	PhoH-like protein
G07	G09	1312	UniRef90_P58603	30.6/5.2	33.5/5.3	Protease 7
G07	G11	405	UniRef90_P0AFK9	34.9/5.0	36.5/4.9	Spermidine/putrescine-binding periplasmic protein
G08	G09	1610	UniRef90_A8AIH5	44.0/5.2	46.0/5.3	3-phosphoshikimate 1-carboxyvinyltransferase
G08	G11	1201	UniRef90_P0AET9	24.6/5.2	26.8/5.2	7-alpha-hydroxysteroid dehydrogenase
G08	G12	4111	UniRef90_A7ZHY1	21.8/6.0	20.8/6.0	Phosphoheptose isomerase
G09	G12	4118	UniRef90_B5XNU6	19.4/6.1	19.4/6.1	UPF0304 protein KPK_1463
G10	G11	3216	UniRef90_B7MUC3	25.2/5.8	26.2/5.8	Orotidine 5'-phosphate decarboxylase
G11	G12	1208	UniRef90_Q9S5G3	22.5/5.2	22.8/5.1	Histidine biosynthesis bifunctional protein HisIE
H01	H02	2405	UniRef90_P0A797	34.9/5.3	34.8/5.5	6-phosphofructokinase isozyme 1

H01	H03	2115	UniRef90_P0A4L6	21.7/5.3	21.1/5.4	Thiol:disulfide interchange protein DsbA
H01	H07	1507	UniRef90_P18815	37.6/5.2	40.5/7.0	Maltose-binding periplasmic protein
			UniRef90_Q57RI5	37.6/5.2	28.5/5.8	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
H01	H09	107	UniRef90_P0A252	21.6/5.1	20.6/5.0	Alkyl hydroperoxide reductase subunit C
H01	H10	3215	UniRef90_P12758	27.2/5.8	27.0/5.8	Uridine phosphorylase
H02	H03	4506	UniRef90_Q9S5G5	42.3/6.0	40.3/5.9	Histidine biosynthesis bifunctional protein HisB
H02	H07	1213	UniRef90_P0AFM7	26.5/5.2	25.4/5.4	Phage shock protein A
H02	H08	1121	UniRef90_P0AFH9	20.2/5.2	18.2/5.4	Osmotically-inducible protein Y
H03	H04	4104	UniRef90_A7ZII0	19.9/5.9	18.3/5.9	UPF0234 protein yajQ
H03	H07	4502	UniRef90_A7MH28	43.4/5.9	43.2/5.9	Acetate kinase
			UniRef90_P0A6F2	43.4/5.9	41.4/5.9	Carbamoyl-phosphate synthase small chain
H03	H09	2413	UniRef90_A7ZSD0	34.7/5.3	32.3/5.6	Malate dehydrogenase
			UniRef90_P00805	34.7/5.3	34.6/5.7	L-asparaginase 2
H04	H05	605	UniRef90_Q8FG52	49.2/5.1	46.1/5.1	Histidinol dehydrogenase
H04	H07	4605	UniRef90_A7FDW9	45.9/6.0	44.8/5.7	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
			UniRef90_P00370	45.9/6.0	48.6/6.0	NADP-specific glutamate dehydrogenase
H04	H09	2107	UniRef90_P0AGD5	21.5/5.3	21.1/5.6	Superoxide dismutase [Fe]
H04	H11	2007	UniRef90_P0C0L2	14.4/5.3	15.0/5.6	Peroxioredoxin osmC
H05	H07	221	UniRef90_P09551	27.0/5.4	25.8/5.2	Lysine-arginine-ornithine-binding periplasmic protein
H05	H11	1402	UniRef90_Q57T38	34.6/5.2	30.4/5.1	Elongation factor Ts
H07	H08	1217	UniRef90_A8ALS4	28.2/5.2	28.7/5.7	Dihydrodipicolinate reductase
H07	H11	1115	UniRef90_A7ZPU1	22.2/5.2	22.5/5.3	Uracil phosphoribosyltransferase
H08	H09	2301	UniRef90_A7ZH92	34.4/5.2	33.6/5.5	Homoserine kinase
H08	H10	1405	UniRef90_Q7N590	34.6/5.2	34.2/5.4	Ribose-phosphate pyrophosphokinase
H09	H10	309	UniRef90_P0AEU2	29.4/5.1	26.2/5.2	Histidine-binding periplasmic protein
H09	H11	216	UniRef90_B7UH18	27.0/5.1	14.3/5.1	Autonomous glycyl radical cofactor
H10	H11	3411	UniRef90_P33570	35.0/5.9	73.0/5.9	Transketolase 2

*MW and pI of the identified proteins were estimated from their gel locations and compared to ^calculated values using ExPASy Compute pI/MW tool [104].

In **Table 3-4**, the protein IDs highlighted in green were also matched to the spot and protein IDs selected in the singlet plate (**Table B 4 in Appendix B**). A total of 69 out of 96 spots were successfully identified within the MG1655 singlet plate. In contrast, 184 spots were successfully identified in the MG1655 multiplexed plate (**Table 3-4**), indicating approximately a 2.7 fold increase in the number of identifications. Some spot IDs have more than 1 corresponding protein ID as a result of having more than 1 protein that is common between the same pair of wells. This is likely due to proteins or protein fragments of similar MW and pI that co-migrate to the same location on the 2-D gel.

The relative locations of the identified MG1655 spots for both the multiplexed experiment and the singlet control are shown in **Figure 3-5** and **Figure 3-6**.

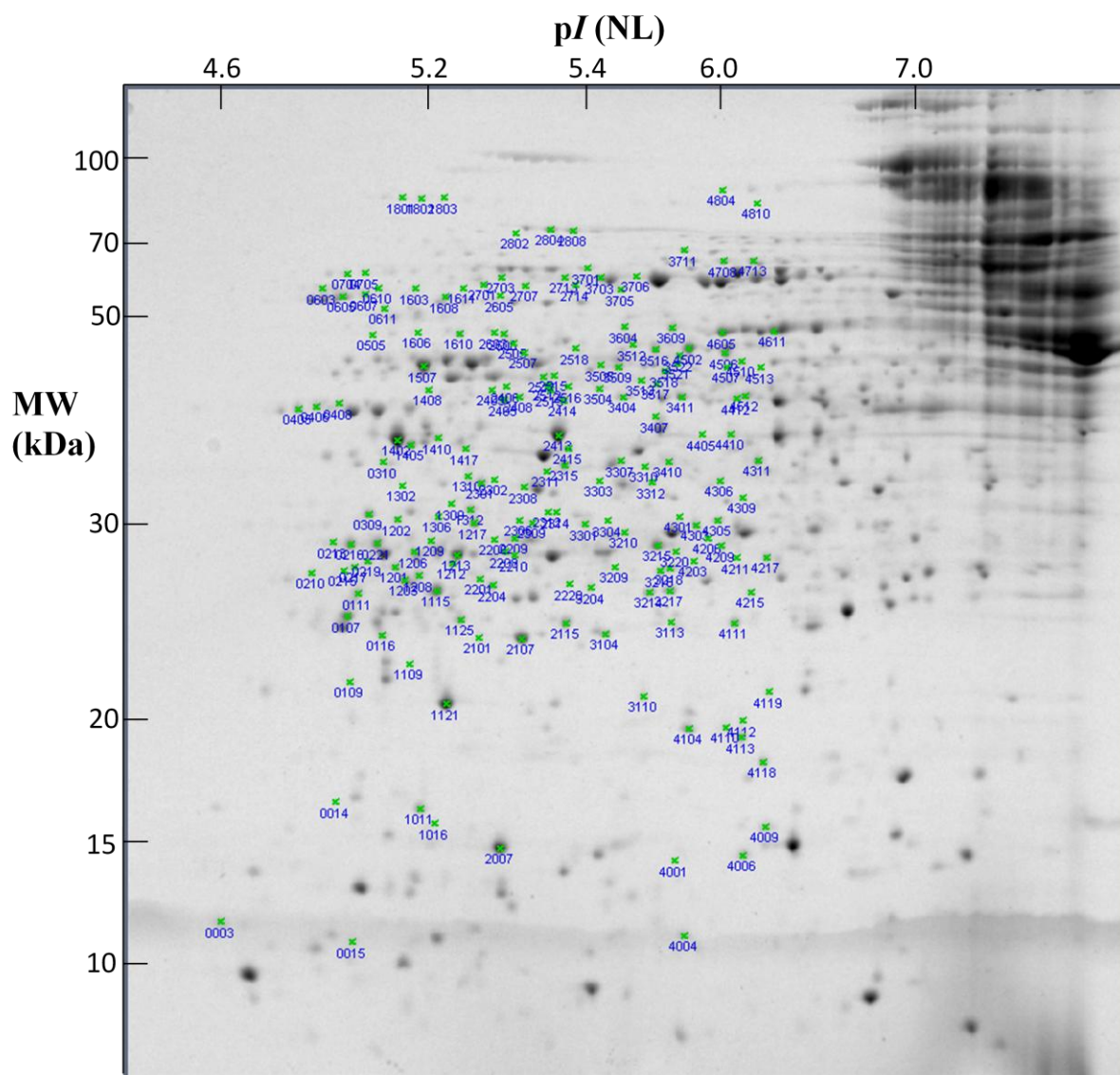


Figure 3-5. (MG1655 Multiplex) Identified Spots and Their Relative Locations According to Table 3-4.

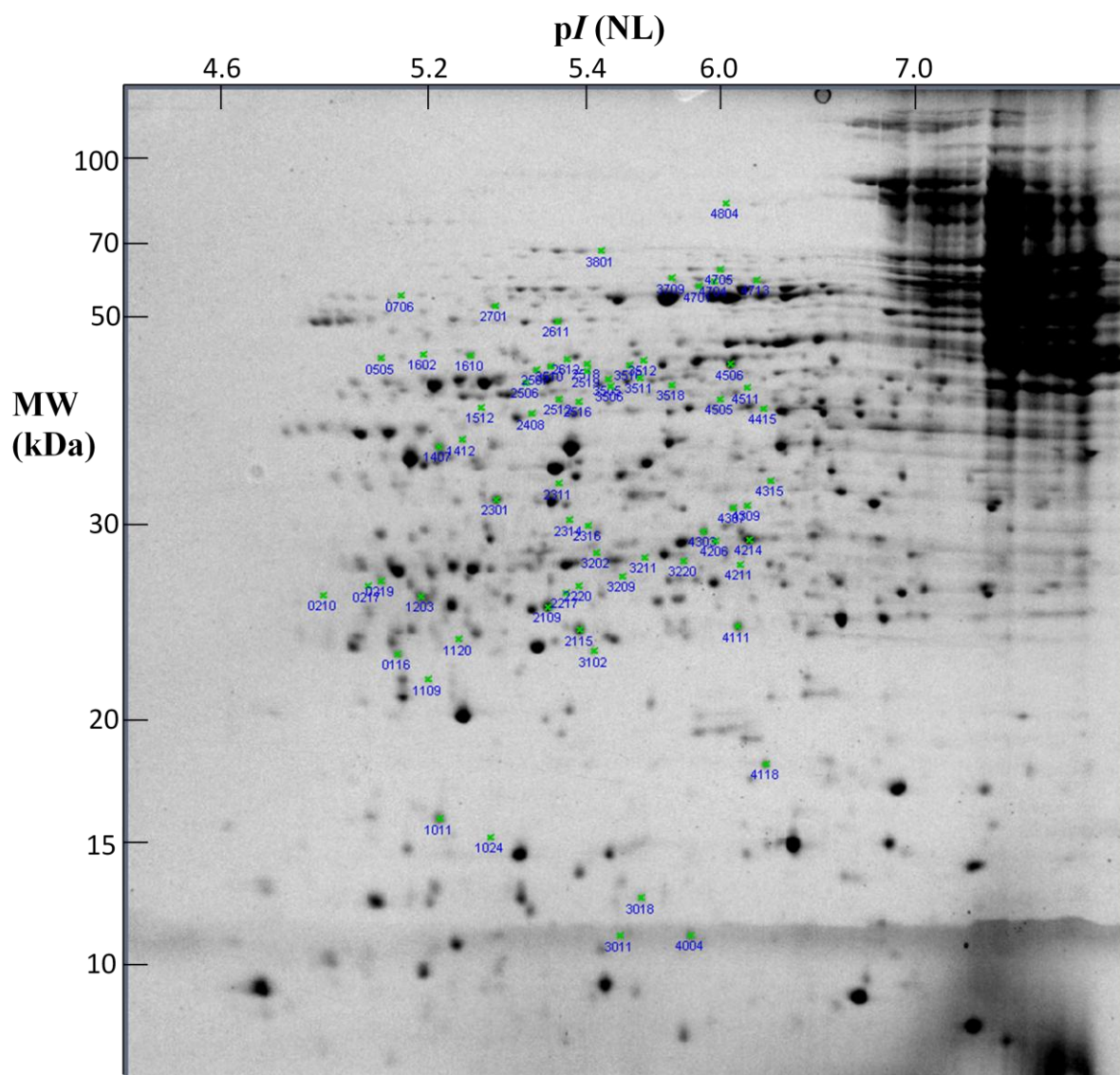


Figure 3-6. (MG1655 Singlet) Identified Spots and Their Relative Locations According to Table B 4 in Appendix B.

3.4 Discussion

Two similar experiments were performed and analyzed within this Chapter. The first corresponds to the development of a proteome reference map from CHO-BRI protein extracts. Further method development was conducted in the second experiment using *E. coli* strain MG1655 protein extracts.

3.4.1 2-D CHO-BRI Proteome Reference Map

A CHO-BRI proteome reference map, which contains 131 identified protein spots, representing 90 different proteins was constructed using a multiplexed experimental design. The proteins identified in this map represent several cellular compartments, including the cytosol, mitochondria, and endoplasmic reticulum, which indicates that the methodology is able to provide a degree of representation of all cellular compartments. The identified proteins were categorized based on their functional annotation as described from the UniProtKB database. Identified proteins were distributed within the following categories: oxidoreductase activity (7%), transcription (10%), amino acid metabolism (9%), cell proliferation and apoptosis (11%), translation (9%), lipid metabolism (2%), energy metabolism (4%), protein processing/folding/degradation (20%), carbohydrate metabolism (9%), cytoskeleton organization (7%), transport (2%), metabolism of cofactors and vitamins (1%), signal transduction (1%), calcium ion homeostasis (1%), nuclear envelope (1%), and unknown (6%) (**Figure 3-7**).

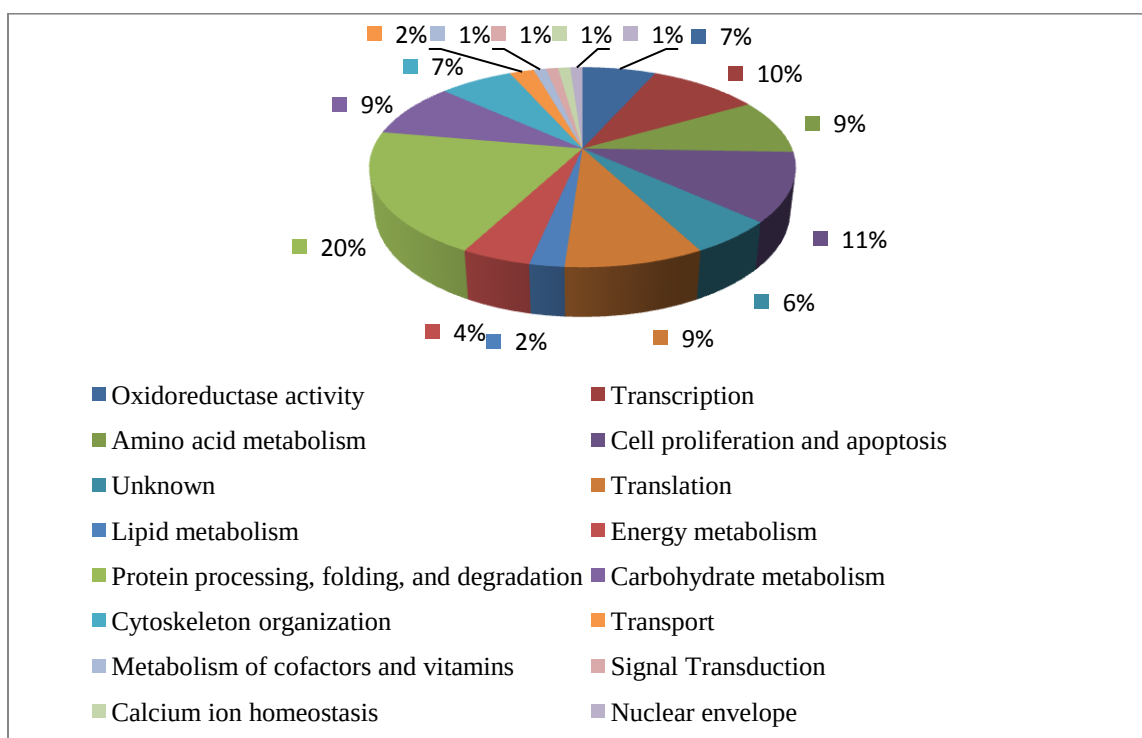


Figure 3-7. Distribution of CHO-BRI Proteins Identified by MS/MS and Organized into Functional Categories.

The developed 2D CHO-BRI protein reference map was related back to those differentially regulated proteins identified from the previous proteomic analysis study (Chapter 2) to look for common spots with respect to their identifications and relative spot locations. Nine spots (Trap1 (364), Actb (1228), Aldh (549), Acot2 (651), Dld (524 and 529), Hibadh (1027), Pcna (959), Ndufs3 (1138)) from the proteomic study match well with the relative spot locations identified in the CHO protein reference map. The low number of matches could be due to several reasons. One reason could be the simple fact that those spots were not selected for excision and identification (of the 528 unique spots), and was therefore omitted. A more likely reason is that the relative spots may not have been identified in the reference map, and therefore comparison is not possible. The fact that certain spots were not identified could be due to 'spot trains' on the gel. The appearance of 'spot trains' (several spots aligned horizontally one after another indicating similar molecular weight with different pI) on the gel for a single gene product is due to post-translational modifications that may arise from different extents of protein phosphorylation widespread on mammalian proteins or the presence of

multiple protein subunits. This is an issue during cross-matching: if a particular protein ID appears in multiple wells of a given row, it would result in several combinations of matched pairs of protein IDs. If this happens, then it would not be possible to accurately map the protein IDs back to their corresponding spot location on the gel. We decided to select for pairs of protein IDs that appear in '2 and only 2' of the 12 wells, for a given row, to simplify the cross-matching process as well as to determine a baseline of the number of spots that could be accurately identified using this methodology. It was unlikely that spots that were part of a particular 'spot train' would pass this selection criteria, and would, therefore, be omitted. As some of the proteins identified in the proteomic study are observed to be part of a spot train (e.g. Grp75, Vim), it was therefore unlikely that these would also be identified in the CHO 2D protein reference map.

Following the cross-matching procedure, the identified proteins match well according to their estimated MW and pI with respect to their locations on the 2D gel (**Table 3-3**). Spot pattern and location were further cross-referenced with available CHO 2D reference maps [124; 145].

By multiplexing gel samples in a 96-well plate, we have developed a method for accurately identifying a large number of protein samples derived from a coomassie stained 2-D gel. Using this method, we have constructed a proteome 2-D reference map of the CHO-BRI cell line, which will enable investigation of systematic responses of this cell line to various environmental signals. A CHO-specific protein database was used for database searching and protein identification, which further improves the confidence of the spots identified compared to CHO protein reference maps published to date that use homologous protein sequence databases. The proteome reference map developed in this study will be an informative addition to the current CHO 2-D gel database. Furthermore, the resulting CHO-BRI 2-D proteome reference map developed in the present work can be deposited into the World-2DPAGE Repository to further facilitate its utility in complementing other CHO genomic and proteomic studies.

3.4.2 Using MG1655 for Development of 2-D Gel Multiplexing Design

To improve on the 2-D gel multiplexing design, *Escherichia coli* strain MG1655 was used as the test model organism. This cell line was provided by Dr. Matthew Scott from the University of Waterloo. MG1655 is a descendent from the original *E. coli* K-12 wild-type laboratory strain.

Originally isolated in 1922, the K-12 strain has become the primary model organism for basic biology, molecular genetics and physiology of bacteria, as well as a prominent platform for applications in the biotechnology industry. The accuracy and completeness of information regarding *E. coli* has provided inferences of gene functions to other prokaryotic and eukaryotic genomes through analogy based on genomic and protein sequence similarities. The entire genome of MG1655 has been sequenced and is well-characterized [147]. The wealth of information and the availability of MG1655 aided in the development of this protein identification technique from 2-D gels. In addition, as a prokaryote, it does not possess post-translational modifications that lead to 'spot trains' on a 2-D gel. This further simplifies the cross-matching procedure.

A total of six 2-D gels were prepared to ensure that we had adequate protein samples for MS analysis. They were also used for 'back-up' in the event that a gel was ripped during handling, and therefore unusable. With a higher number of 2-D gels prepared, a singlet control containing 96 protein gel pieces was also analyzed in conjunction with the multiplexed plate to facilitate comparison. To improve the accuracy of MS analysis and subsequent protein identifications, the MG1655 samples were run on an LTQ Orbitrap instrument with a UPLC system, which gives ± 20 ppm accuracy on parent ions. This speeds up database searches and ends up with a lower false positive rate.

With the changes mentioned above, 184 protein spots were successfully identified in the multiplexed plate. The estimated MW and pI of these spots match well with calculated values as shown in **Table 3-4**. In comparison to the singlet plate, of which 69 out of 96 protein spots were successfully identified, this corresponds to an approximate 2.7 fold increase in the number of protein identifications in a single run for the cost of a single 96-well plate. In other words, by utilizing the multiplexing approach, we were able to identify almost 3 times as many protein spots as compared to a single gel approach using a 96-well plate format. Furthermore, according to **Table 3-4**, 31 of the proteins identified in the multiplexed plate, including their relative spot locations as denoted by their spot IDs, matches with the ones identified in the singlet control plate (highlighted in green). This match represents approximately 50% of the number of proteins identified in the singlet control. This value is acceptable considering the present work was performed as a first pass through to test the proof-of-principle resulting in preliminary results. The loss of the other half of matches could

partially be attributed to some of the wells that returned pure noise (i.e. no protein identifications were obtained) due to problems with sample handling. It could also be due to highly abundant proteins that may have appeared in more than 2 wells of a given row, and was therefore not taken into account during the matching process. However, the results still suggests that the principle works and requires further refinement in combination with the changes already made. Thus the results provide groundwork for further research and development to improve the multiplex strategy for developing 2-D protein reference maps.

The samples were analyzed by mass spectrometry at the Hospital for Sick Children MS facility. In terms of costs, the facility charges approximately \$85 per sample for an MS/MS run, which would amount to \$8160 per 96 samples. In contrast, analysis of a 96-well plate costs \$3264, which corresponds to \$34 per sample when the investigators perform their own data analysis. The combination of the multiplexing method developed here using 96-well plates significantly reduces the cost of analysis on a per sample basis allowing up to 528 protein spots to be identified for the cost of a single 96-well plate. This results in a cost savings percentage of approximately 82%. In the present study, 184 protein spots were identified via multiplexing while 69 protein spots were identified in the single gel control plate. This results in a cost savings percentage of approximately 62% per identification.

Additionally, in-gel digestion is performed on all samples simultaneously using a multi-channel pipette, which saves a considerable amount of benchwork time. However, a bottleneck with this method comes from mass spectrometry data analysis, which generates a large amount of data that is time-consuming to analyze. Protein identification must be performed for each well, which takes up computing time and is dependent on the specifications of the computer used. However, the multiplex method described in this chapter significantly reduces the labour time in comparison with the traditional method of performing in-gel digestion on each individual gel piece per sample tube followed by MS analysis. Another advantage is that contaminants are virtually screened and filtered during the cross-matching procedure. For example, keratin is a contaminant that poses a problem for MS analyses as it is highly abundant and is almost certain to appear during the protein identification step. With the multiplex method, it is likely that keratin will be identified in several sample wells for each row. However, since the cross-matching step looks for protein IDs that only appear in a pair of

unique wells (i.e. appear in 2 and only 2 of the 12 wells per row), contaminants such as keratin will be screened out.

3.5 Conclusion

The present work describes a novel method developed for identifying a large number of protein spots from a representative 2D gel. Using this method, a protein reference map, consisting of 131 identified protein spots, was generated for the CHO-BRI strain. Similarly, a protein reference map consisting of 184 identified protein spots was generated for *E. coli* strain MG1655.

Some refinement could further improve the number of successful identifications and reduce analysis time. If PDQuest incorporated a function that generates the picklists in accordance to the specifications of the script developed in MATLAB, this would further reduce the time of the gel excision runs as it eliminates the differences in the x- and y- coordinates of each spot of interest due to the repositioning of the gel inside the EXQuest Spot Cutter following gel scanning. This would also prevent the gel from drying out due to prolonged exposure to air. A method for distinguishing the spots within spot trains as a result of post-translational modifications would aid in identification: possibly by separating each spot into different rows of the 96-well plate. Automating the in-gel digestion procedure would improve the robustness of this method while reducing the loss of sample due to sample handling.

The multiplexing scheme described in this chapter allows for the identification of a large number of protein spots from a preparative 2-D gel at a significantly reduced cost and time-consumption. The method illustrated herein would therefore make the expansion of the current 2-D gel database more feasible and applicable to a number of organisms that have a limited number of, or non-existent, 2-D protein reference maps.

Chapter 4

Conclusions and Future Directions

4.1 Proteomic Changes in EG2-hFc1-Producing CHO Cells

A comparative proteomic analysis between an EG2-hFc1-producing CHO strain (CHO-1A7) and its parental cell line (CHO-BRI) was carried out where 86 protein spots were selected for subsequent MS analysis. The successful MS analyses of 52 protein spots yielded 34 unique protein identifications. These include proteins involved in apoptosis/cell proliferation regulation, protein processing, cell structural integrity, and enzymes related to various metabolic pathways. Functional annotation analyses revealed enriched metabolic pathways that are important for the generation of precursor metabolites and energy in CHO-1A7.

A number of differentially regulated proteins were found to be related to the apoptotic and cell proliferation processes. Hsp60 and Hsp90 were observed to be down-regulated in CHO-1A7. These heat shock proteins are primarily responsible for maintaining the conformation of the cell's proteins, particularly in response to environmental stress. Their down-regulation may lead to apoptotic processes that result in cell death. Further supporting this hypothesis, Nudfs3 and Pdcd6 showed an increase in abundance. Ndufs3 and Pdcd6 are positive regulators of apoptosis and their up-regulation suggests an initiation of apoptosis in a portion of cells by the end of the batch cultivation process. In contrast, Grp75 and Trap1 which were observed to be up-regulated in CHO-1A7, are known to influence cell proliferation processes, while the over-expression of Phb and under-expression of Pcna in CHO-1A7 suggests an inhibition of cell proliferation by arresting CHO cells in the G0/G1 phase [110]. Growth-arrested CHO cells have been observed to influence increased recombinant protein production [117; 119]. The trends of the differentially regulated proteins related to apoptosis and cell proliferation seems to have conflicting influences on the fate of CHO cells. Whether this is due to harvesting the cells at the end of the batch cultivation process or an effect as a result of recombinant protein production is unclear. Nonetheless, the differential expression of anti- and pro-apoptotic proteins provides an indication of pathway changes related to cell growth and viability in CHO-1A7 that may play a role in recombinant protein production. Further studies are

warranted to investigate the connection between apoptotic/cell proliferation proteins with respect to antibody production.

Various molecular chaperones were also observed to be differentially regulated in CHO-1A7. PDI, GRP94, Hspd1, and Hsp90aa1 showed down-regulation in CHO-1A7. Both PDI and GRP94 are ER-resident chaperones involved in protein processing. PDI catalyzes the formation and breakage of cysteine residues within proteins, thus aids in finding the correct arrangement of disulfide bonds during protein folding. GRP94 plays a role in the processing and transport of secreted proteins, and it has also been implicated to assert chaperoning activity on unassembled immunoglobulin chains. Hspd1 is essential for the folding and assembly of newly imported proteins in the mitochondria, while Hsp90aa1 functions in the cytosol in the maintenance of properly folded proteins upon stress. The drop in expression levels of a number of molecular chaperones may indicate an adequate level of efficiency and capacity in sustaining protein translation and folding within the CHO-1A7 cell line. Though recombinant protein production is a stressor upon the cell, it may not be at a level high enough that would warrant the induction of chaperones.

Cytoskeletal proteins, including vimentin and β -actin showed reduced expression in CHO-1A7. They are more commonly known for their involvement with maintaining the integrity of the cell, and their down-regulation may indirectly affect ATP generation and protein biosynthesis. Vimentin may play a role in maintaining mitochondrial morphology and organization, and its down-regulation was shown to contribute to mitochondrial fragmentation that may affect energy derivation [131]. β -actin was also shown in studies to be related to the protein translation machinery. The disruption of the actin filament network in CHO cells resulted in a reduction in protein translation [138]. Although antibody titers were not measured throughout the batch cultivation process, the down-regulation of β -actin and vimentin may reflect a reduction in protein biosynthesis owing to the cells having been harvested at stationary phase.

Metabolic enzymes related to carbohydrate, amino acid, and lipid metabolism were also observed to be differentially expressed in EG2-hFc1-producing CHO cells. The enzymes involved relate to the cell's capacity for deriving energy and metabolites from organic compounds in order to sustain the production of EG2-hFc1. This was evident in the up-regulation of a number of enzymes

(Cs, Gpd2, Dld, Aldh, Ast, Hibadh, Ndufs3, Ndufv1) that are involved in energy generating metabolic pathways. These metabolic pathways were also found to be enriched following functional annotation analysis using DAVID which is a tool that looks for overrepresented terms from an input gene list. The observations noted in the present work suggests a shift in metabolic flux towards energy production to meet the energy demands of EG2-hFc1 production.

From a systems-level perspective, taking into account all the cellular processes involved, it appears that CHO-1A7 attempts to increase energy production in order to sustain EG2-hFc1 productivity while maintaining cell viability. Subsequently, protein processing and protein folding are operating at a sufficient level that does not warrant the induction of enzymes involved in those biological functions in the production of EG2-hFc1.

For future studies, a few technical modifications could be employed to increase the number of protein identifications. A smaller pI range (ie: pH 4 – 7) could be used to better separate overlapping proteins with similar pIs, thereby increasing the resolution of the resulting gels. Further resolution can be obtained by separating proteins in a gradient gel, rather than a homogenous 12% SDS-PAGE gel during second dimension separation. Automated in-gel trypsin digestion would reduce labour time, minimize exposure of gel pieces to air, as well as minimize human errors (e.g. pipetting) due to sample handling.

The present work discusses changes in protein expression levels in CHO-1A7 associated with EG2-hFc1 production. It serves as a reference for future studies performed using this particular cell line. Based on this study, targeted over-expression of enzymes involved in the generation of energy in CHO-1A7 may improve EG2-hFc1 productivity. A comparative proteomic analysis of EG2-hFc1-producing CHO-BRI derived cell lines with differing productivities would also provide insights into cellular changes and biomarkers that correlate with productivity levels. Finally a time-lapse study of CHO-1A7 will provide information in regards to the metabolic changes associated with growth via a batch process.

4.2 Development of 2-D CHO Proteome Reference Map

A multiplexing scheme was developed to generate proteome reference maps using 2-D gel-based methods. The proof of concept was tested using both CHO-BRI and *E.coli* MG1655 as the model cell line and organism respectively. Under this scheme, 131 protein spots were identified in CHO-BRI, while 184 protein spots were identified in MG1655. These maps can serve as reference markers for cell-line specific 2-D gel studies.

To date, CHO protein reference maps found in literature were generated without CHO genomic sequence. Thus, protein identifications were searched against protein databases of related species including *Mus musculus* (mouse), *Rattus norvegicus* (rat) and *Homo Sapiens*, with a resultant loss of stringency in the identifications. In the present work, database searching and protein identifications were performed against a CHO-specific sequence database corresponding to the recent publication of the CHO-K1 genome. A total of 131 protein spots were successfully identified, consisting of 90 different proteins distributed over several cellular compartments. The resulting CHO-BRI 2-D proteome reference map, with its corresponding proteomic data will be deposited into the World-2DPAGE Repository to complement future CHO genomic and proteomic studies.

Following the mapping efforts of the CHO-BRI cell line, the multiplexing method was further tweaked using MG1655 as the model organism. MG1655 is a well-characterized bacterial strain that contains a wealth of information in literature. In addition, due to the lack of post-translational modifications of its proteins which would otherwise lead to 'spot trains' on a 2-D gel, the cross-matching procedure of the multiplex design is further simplified. A control plate consisting of 1 gel spot per well of a 96 well-plate allowed the successful identification of 69 protein spots. In comparison, 184 protein spots were successfully identified under the multiplexing methodology, which represents a 2.7-fold increase in the number of protein identifications in a single run. Approximately 50% of the number of proteins identified in the control plate were also identified in the multiplex plate and matched well according to their relative locations on the 2-D gels.

Though the multiplex design could facilitate the identification of a large number of protein spots at a reduced cost and time-consumption compared to traditional methods, it could still use

further refinement. A method is needed to distinguish between post-translationally modified spots of the same protein, possibly by separating each spot within a spot train into different rows of the 96-well plate. Automating the in-gel trypsin digestion protocol would improve the robustness of this method and reduce errors resulting from sample handling. Incorporating a function within PDQuest to generate the picklists according to the specifications of the multiplex design would facilitate faster excision times, and minimize the amount of time the 2-D gels are exposed to the open environment potentially leading to contamination. The analyses of the MS data presents a major bottleneck of this method. A single MS run on a 96-well plate generates a large amount of data to analyze. Nonetheless, automating gel spot-excision and using a 96-well plate format with multiple gel pieces per well significantly reduces the time spent on wet-lab work, as compared to traditional methods, while having the capacity to identify a large number of spots. Additionally, the costs of MS analysis is reduced, therefore making the expansion of 2-D gel database more feasible and applicable to a number of organisms that have a limited number of, or non-existent, 2-D protein reference maps.

4.3 Contribution to MAbNet

This work is a component of a larger project that seeks to establish a novel platform for the large-scale manufacture of specific glycoforms of therapeutic monoclonal antibodies. EG2-hFc1 represents one candidate that is under investigation. The ability to control the glycosylation profiles of recombinant MAbs could lead to enhanced therapeutic efficacy. It would also allow the generation of recombinant MAbs with various desired properties for other types of studies. The work presented in this thesis will assist MAbNet in meeting their objectives, by providing the proteome profile of CHO-1A7 that corresponds to its glycosylation profile. Although proteins that directly modulate glycosylation were not apparent and could not be identified as differentially expressed from the proteomic analyses, this is not surprising given the non-specific nature of whole cell proteome analysis. In other words, the set up of this experiment was not to specifically look for changes in expression of glycosylating enzymes, but to identify systematic changes between an EG2-hFc1 producing CHO strain and its parental non-producing counterpart as a whole. Thus, the DIGE results provides a baseline of the proteome profile of CHO-1A7, where the set of differentially regulated proteins associated with the various biological processes and metabolic pathways should be considered in the optimization of EG2-hFc1 production. For example, targeted overexpression of proteins involved in the TCA cycle may lead to an increase in the production of precursor metabolites

and energy required for increased MAb production while ensuring maintenance of cell survival. This work can, therefore, serve as a reference check for future proteomic studies on this particular cell line. For example, cell-engineering strategies can be employed to reduce core fucosylation of EG2-hFc1 in CHO-1A7 and its corresponding proteome profile can then be compared with the results of the present study. Additionally, studying the changes to the subcellular proteomes, such as the proteome within the ER, may provide information in regards to glycosylation enzymes. The development of a 2-D gel protein reference map, specifically from the CHO-BRI cell line, contributes to future proteomic studies of this particular cell line, including the investigation of physiological responses and protein expression patterns as a result of genetic and environmental modifications.

The enhanced tumour-targeting properties associated with EG2-hFc1 suggest it to be a viable therapeutic candidate for the treatment of various cancers. Thus, it is important to optimize the production of EG2-hFc1 from its host cell line. The list of differentially expressed proteins identified in this study provides possible candidates for targeted metabolic engineering to improve EG2-hFc1 production in CHO-1A7 cells. Overall, the present work shows the potential of utilizing a proteomics approach in understanding the intracellular changes occurring in MAb-producing CHO cells. This will provide a key step towards building a fundamental framework for optimizing MAb production.

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Appendix A

Proteomic Raw Data and Analysis

The following **Figures** and **Tables** represent standard curves for the determination of protein concentration in the CHO 2D-DIGE experiment.

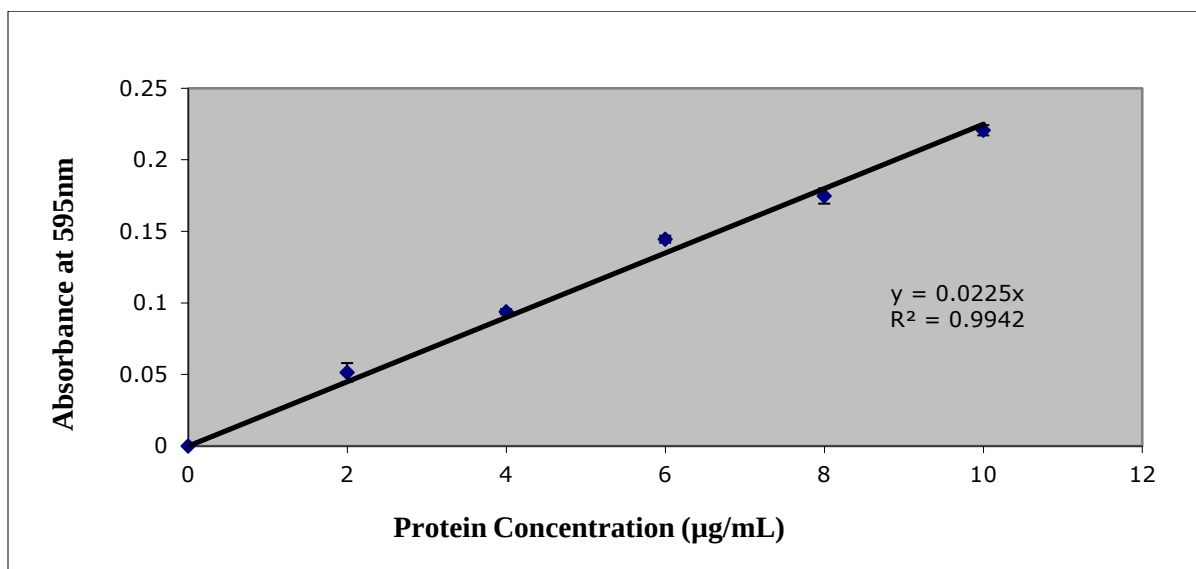


Figure A 1. Standard Curve for CHO 2D-DIGE Experiment. Data points represent the average absorbances from triplicate samples with their corresponding standard deviations, using BSA as a standard. The equation for the linear regression line ($y = 0.0225x$) was used to calculate the protein concentrations of the CHO-BRI and CHO-1A7 samples.

By utilizing the standard curve, the following protein concentrations were determined for each protein sample (**Table A 1**) prior to sample labeling.

Table A 1. Protein Concentration and Cell Density of CHO Samples for 2D-DIGE. Calculated protein concentrations that represent the average and standard deviation of three replicates post sample clean-up.

Sample	Protein Concentration ($\mu\text{g}/\mu\text{L}$)	Cell Density (10^6 cells/mL) on day 8 harvest
CHO-BRI-1	1.66 ± 0.25	7.029
CHO-BRI-2	1.69 ± 0.10	7.753
CHO-BRI-3	1.55 ± 0.11	7.166
CHO-BRI-4	2.07 ± 0.15	6.899
CHO-BRI-5	1.69 ± 0.13	6.403
CHO-BRI-6	2.21 ± 0.06	5.243
CHO-1A7-1	1.63 ± 0.02	7.257
CHO-1A7-2	1.52 ± 0.21	7.613
CHO-1A7-3	2.53 ± 0.13	7.377
CHO-1A7-4	2.24 ± 0.10	7.235
CHO-1A7-5	1.72 ± 0.07	7.576
CHO-1A7-6	1.45 ± 0.25	7.970

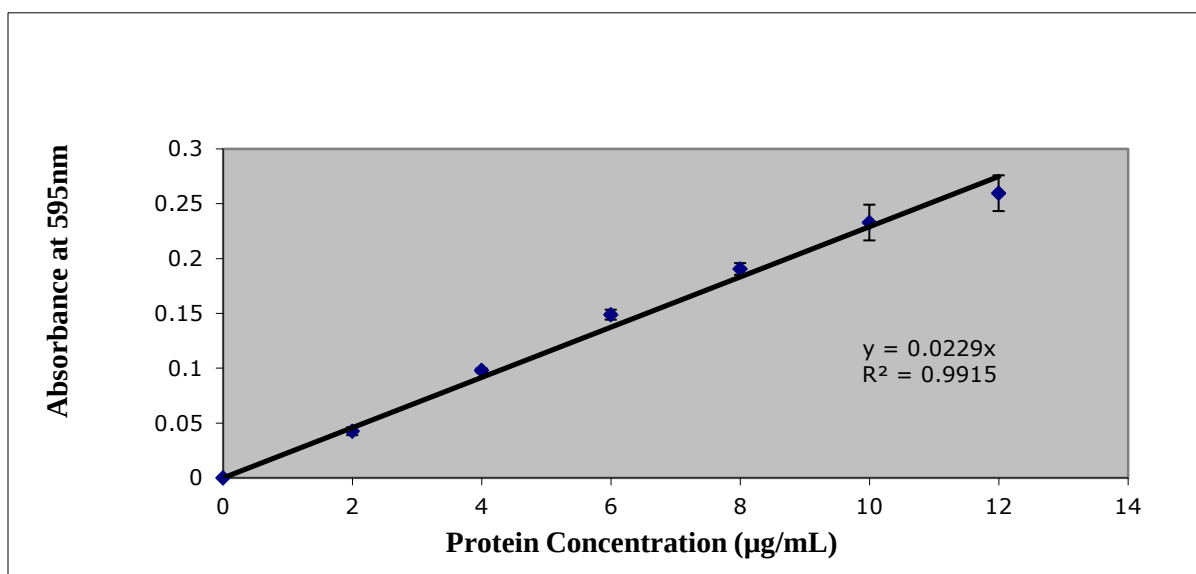


Figure A 2. Standard Curve for CHO Preparative Gel. Data points represent the average absorbances from triplicate samples with their corresponding standard deviations, using BSA as a standard. The equation for the linear regression line ($y = 0.0229x$) was used to calculate the protein concentration of the pooled CHO protein sample.

Appendix B

Multiplex Raw Data and Analysis

The following **Figure** and **Table** represent standard curves for the determination of protein concentration for CHO-BRI and MG1655 in the multiplex experiment.

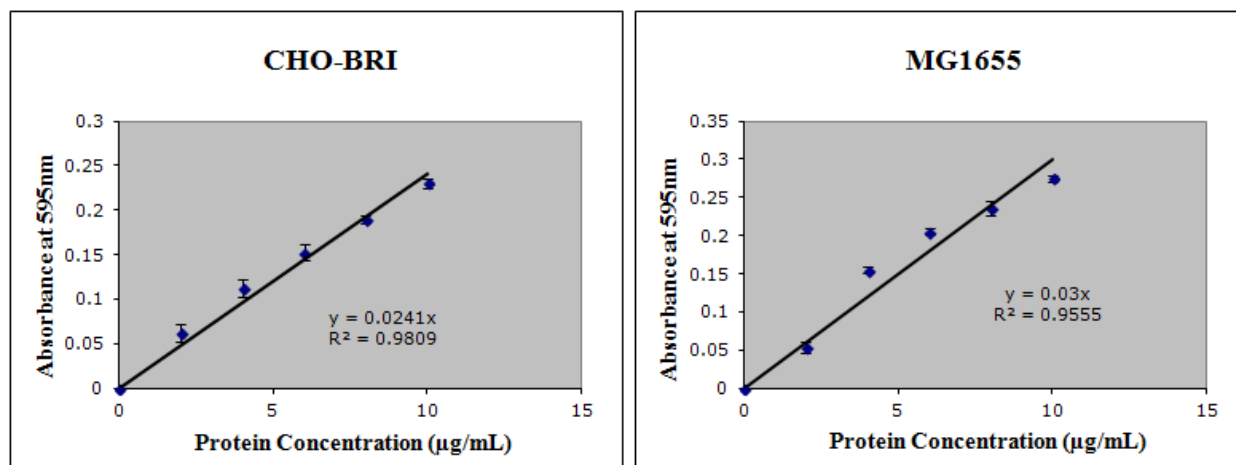


Figure B 1. Standard Curves for CHO-BRI and MG1655 Multiplex Experiments. Data points represent the average absorbances from triplicate samples with their corresponding standard deviations, using BSA as a standard. The equation for the linear regression line (CHO-BRI: $y = 0.0241x$; MG1655: $y = 0.03x$) was used to calculate the protein concentrations of the CHO-BRI and MG1655 samples respectively.

By utilizing the standard curve, the following protein concentrations were determined for each protein sample (Table B 1).

Table B 1. Protein Concentration of CHO-BRI and MG1655 for Multiplex. Calculated protein concentrations that represent the average and standard deviation of three replicates post sample clean-up.

Sample	Protein Concentration ($\mu\text{g}/\mu\text{L}$)
CHO-BRI	2.49 ± 0.31
MG1655	3.17 ± 0.85

The following tables show identified proteins based on PEAKS analyses for both CHO-BRI and MG1655 samples. The identifications in each well are organized on a per row basis from a 96-well plate.

Table B 2. Identified CHO Proteins (Multiplex). Each table denotes a specific row of a 96-well plate with identifications shown in each well.

Row A	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
A01	UniRef90_P11021	72.4	99.2	19	10	78 kDa glucose-regulated protein
	UniRef90_P08670	53.7	99.1	25	8	Vimentin
	UniRef90_Q3V3R1	105.4	99.1	18	13	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_G3IAS8	18.6	99.0	5	5	Kinesin-like protein KIF27
	UniRef90_Q16576	47.0	98.9	11	4	Histone-binding protein RBBP7
	UniRef90_P31946	27.9	98.9	23	3	14-3-3 protein beta/alpha
	UniRef90_P63101	27.7	98.8	22	2	14-3-3 protein zeta/delta
	UniRef90_P78371	57.5	98.8	11	4	T-complex protein 1 subunit beta
	UniRef90_P31937	31.7	98.8	21	4	3-hydroxyisobutyrate dehydrogenase, mitochondrial
	UniRef90_Q12874	58.8	98.5	12	3	Splicing factor 3A subunit 3
A02	UniRef90_P14625	92.6	99.2	20	10	Endoplasmin
	UniRef90_P08670	53.7	99.1	31	8	Vimentin
	UniRef90_P02545-2	64.0	99.1	25	6	Isoform C of Prelamin-A/C
	UniRef90_G3HZ25	25.5	98.5	21	3	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial
	UniRef90_G3IND2	30.9	98.4	14	3	26S proteasome non-ATPase regulatory subunit 13
A03	UniRef90_P31040	71.7	91.1	5	2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P14625	92.6	99.2	27	20	Endoplasmin
	UniRef90_P08670	53.7	99.1	31	9	Vimentin
	UniRef90_G3GVX1	21.7	99.1	9	6	Ribosome-binding protein 1
	UniRef90_Q96J01	41.9	99.0	17	6	THO complex subunit 3
	UniRef90_G3HN14	237.6	99.0	4	4	Transitional endoplasmic reticulum ATPase
	UniRef90_G3I220	36.4	98.9	21	5	Cytoskeleton-associated protein 4
	UniRef90_G3I2Q7	58.0	98.2	6	3	Transcription intermediary factor 1-beta
A04	UniRef90_G3HJE0	36.9	97.8	13	3	Mitochondrial import inner membrane translocase subunit TIM50 (Fragment)
	UniRef90_P14625	92.6	99.2	25	13	Endoplasmin
	UniRef90_G1SLD5	111.5	99.2	20	12	Hypoxia up-regulated protein 1
	UniRef90_P08670	53.7	99.2	44	13	Vimentin
	UniRef90_P78371	57.5	99.2	36	14	T-complex protein 1 subunit beta
	UniRef90_G3HR08	50.3	98.8	11	4	Cytosol aminopeptidase
	UniRef90_G3I2M0	25.5	98.8	20	4	Sulfatase-modifying factor 2
	UniRef90_P62814	49.9	98.4	14	3	V-type proton ATPase subunit B, brain isoform
A05	UniRef90_A4FUY3	73.1	98.3	5	3	Prolyl 3-hydroxylase 1
	UniRef90_P08670	53.7	99.1	34	7	Vimentin
	UniRef90_P14625	92.6	99.1	16	8	Endoplasmin
	UniRef90_P08758	36.1	99.0	26	5	Annexin A5
	UniRef90_G3I2M0	25.5	98.8	24	4	Sulfatase-modifying factor 2
	UniRef90_P56537	26.6	98.8	29	4	Eukaryotic translation initiation factor 6
	UniRef90_P02545-2	64.0	98.8	9	3	Isoform C of Prelamin-A/C
	UniRef90_G3HNV4	16.4	98.8	35	3	Triosephosphate isomerase
A05	UniRef90_G3I2I6	20.4	98.7	21	3	Triosephosphate isomerase

	UniRef90_Q8BMF4	68.0	98.5	12	3	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
	UniRef90_P30153	59.0	98.5	17	3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
	UniRef90_Q9Z0X1	66.8	98.4	10	3	Apoptosis-inducing factor 1, mitochondrial
	UniRef90_G3H8Y5	89.0	98.3	9	3	Collagen alpha-1(VI) chain
	UniRef90_Q9WTX8	83.2	98.1	9	3	Mitotic spindle assembly checkpoint protein MAD1
	UniRef90_G3HEZ0	65.8	98.1	7	3	Stress-70 protein, mitochondrial
	UniRef90_P07910	33.7	98.0	23	3	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_G3HFL6	69.6	91.8	5	2	ATP-binding cassette sub-family G member 3
A06	UniRef90_G3HFL6	69.6	98.8	13	4	ATP-binding cassette sub-family G member 3
	UniRef90_G3HR08	50.3	99.1	27	7	Cytosol aminopeptidase
	UniRef90_G3HTU8	42.8	99.1	19	6	Galactokinase
	UniRef90_G3HZ25	25.5	99.1	37	6	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial
	UniRef90_G3I220	36.4	99.0	24	6	Cytoskeleton-associated protein 4
	UniRef90_P14625	92.6	98.3	8	3	Endoplasmic
	UniRef90_P30153	59.0	98.3	8	3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
	UniRef90_P78371	57.5	97.9	9	3	T-complex protein 1 subunit beta
	UniRef90_Q15365	37.5	98.8	18	4	Poly(rC)-binding protein 1
	UniRef90_Q3V3R1	105.4	99.2	18	15	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q99N15	27.2	98.3	20	3	17beta-hydroxysteroid dehydrogenase type 10/short chain L-3-hydroxyacyl-CoA dehydrogenase
	UniRef90_Q9CXW2	41.2	97.7	8	3	28S ribosomal protein S22, mitochondrial
A07	UniRef90_G1SLD5	111.5	99.1	18	6	Hypoxia up-regulated protein 1
	UniRef90_G3HE28	40.1	98.9	14	4	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HEZ0	65.8	99.1	22	8	Stress-70 protein, mitochondrial
	UniRef90_G3I6E6	32.0	98.3	13	3	Actin, cytoplasmic 2
	UniRef90_P08670	53.7	99.0	20	4	Vimentin
	UniRef90_P11021	72.4	99.2	24	10	78 kDa glucose-regulated protein
	UniRef90_P12004	28.8	99.1	40	6	Proliferating cell nuclear antigen
	UniRef90_P14625	92.6	99.2	19	10	Endoplasmic
	UniRef90_P35232	29.8	98.2	15	3	Prohibitin
	UniRef90_P60709	41.7	98.3	10	3	Actin, cytoplasmic 1
	UniRef90_Q14697	107.0	99.1	19	16	Neutral alpha-glucosidase AB
	UniRef90_Q61576	64.7	98.8	14	4	Peptidyl-prolyl cis-trans isomerase FKBP10
	UniRef90_Q99N15	27.2	99.2	60	10	17beta-hydroxysteroid dehydrogenase type 10/short chain L-3-hydroxyacyl-CoA dehydrogenase
A08	UniRef90_G3H0U6	50.1	98.9	25	4	Protein disulfide-isomerase A3
	UniRef90_G3HRM9	25.2	99.0	26	5	Prostaglandin E synthase 3 (Fragment)
	UniRef90_G3I220	36.4	98.2	10	3	Cytoskeleton-associated protein 4
	UniRef90_G3I2L9	7.8	98.3	59	4	Sulfatase-modifying factor 2
	UniRef90_G3I2M0	25.5	98.8	19	4	Sulfatase-modifying factor 2
	UniRef90_G3I6E6	32.0	98.4	20	3	Actin, cytoplasmic 2
	UniRef90_P08670	53.7	99.2	42	13	Vimentin
	UniRef90_P10809	56.5	99.1	15	7	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.1	20	12	Endoplasmic
	UniRef90_P31937	31.7	99.0	23	5	3-hydroxyisobutyrate dehydrogenase, mitochondrial
	UniRef90_P60709	41.7	98.4	18	3	Actin, cytoplasmic 1
A09	UniRef90_G3H0L9	37.5	98.8	15	4	Cathepsin B
	UniRef90_G3HJN3	51.8	98.5	13	3	Clusterin
	UniRef90_G3I1Y9	27.4	99.1	39	7	Sulfated glycoprotein 1
	UniRef90_G3I220	36.4	99.1	29	6	Cytoskeleton-associated protein 4
	UniRef90_G3IAS8	185.7	99.0	6	5	Kinesin-like protein KIF27
	UniRef90_P02545-2	64.0	99.0	24	5	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	44	16	Vimentin
	UniRef90_P08758	36.1	99.2	36	10	Annexin A5

	UniRef90_P10809	56.5	96.6	9	2	60 kDa heat shock protein, mitochondrial
	UniRef90_P11021	72.4	99.0	11	5	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.1	20	13	Endoplasmic
	UniRef90_P27797	48.2	99.0	19	5	Calreticulin
	UniRef90_P30153	59.0	99.1	20	7	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
	UniRef90_Q12874	58.8	91.0	14	2	Splicing factor 3A subunit 3
	UniRef90_Q9JLH7	51.4	92.2	11	2	CDK5 regulatory subunit-associated protein 3
A10	UniRef90_G1SLD5	111.5	99.2	19	14	Hypoxia up-regulated protein 1
	UniRef90_G3HC84	47.8	98.7	14	4	Heat shock protein HSP 90-beta
	UniRef90_G3HE28	40.1	99.1	21	5	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HFL6	69.6	98.7	7	4	ATP-binding cassette sub-family G member 3
	UniRef90_P02545-2	64.0	99.1	23	8	Isoform C of Prelamin-A/C
	UniRef90_P14625	92.6	99.1	20	12	Endoplasmic
	UniRef90_P31946	27.9	98.2	16	2	14-3-3 protein beta/alpha
	UniRef90_P50213	39.7	98.7	13	4	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P62873	37.4	91.6	9	2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
	UniRef90_P63101	27.7	98.9	26	3	14-3-3 protein zeta/delta
	UniRef90_Q14568	38.1	98.4	12	3	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q14697	107.0	99.1	15	9	Neutral alpha-glucosidase AB
A11	UniRef90_G3H0U6	50.1	98.1	11	2	Protein disulfide-isomerase A3
	UniRef90_G3HNV4	16.4	91.6	29	2	Triosephosphate isomerase
	UniRef90_G3I216	20.4	91.9	28	2	Triosephosphate isomerase
	UniRef90_G3IND2	30.9	98.4	14	3	26S proteasome non-ATPase regulatory subunit 13
	UniRef90_P08670	53.7	97.9	10	2	Vimentin
	UniRef90_P10809	56.5	99.1	12	6	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.1	19	11	Endoplasmic
	UniRef90_P35232	29.8	98.3	11	3	Prohibitin
	UniRef90_Q16576	47.0	98.3	8	3	Histone-binding protein RBBP7
A12	UniRef90_G3HFP0	15.5	98.2	18	3	Adrenodoxin, mitochondrial
	UniRef90_G3I220	36.4	98.8	23	4	Cytoskeleton-associated protein 4
	UniRef90_P08670	53.7	98.4	10	3	Vimentin
	UniRef90_P10809	56.5	98.3	9	3	60 kDa heat shock protein, mitochondrial
	UniRef90_P12004	28.8	99.1	40	7	Proliferating cell nuclear antigen
	UniRef90_P14625	92.6	99.2	24	17	Endoplasmic
	UniRef90_P30153	59.0	98.9	21	4	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
	UniRef90_P30154	66.0	98.1	10	2	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform
	UniRef90_P31040	71.7	98.8	11	4	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_Q14697	107.0	99.1	11	5	Neutral alpha-glucosidase AB
	UniRef90_Q15365	37.5	98.9	21	5	Poly(rC)-binding protein 1
	UniRef90_Q3V3R1	105.4	90.4	4	2	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q9CXW2	41.2	98.3	8	3	28S ribosomal protein S22, mitochondrial

Row B	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
B01	UniRef90_G3HEZ0	65.8	99.0	12	5	Stress-70 protein, mitochondrial
	UniRef90_G3I6E6	32.0	98.4	21	3	Actin, cytoplasmic 2
	UniRef90_H0X554	30.1	98.9	28	5	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P08670	53.7	99.1	30	6	Vimentin
	UniRef90_P11021	72.4	99.1	17	10	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	24	17	Endoplasmic
	UniRef90_P60709	41.7	98.4	16	3	Actin, cytoplasmic 1
	UniRef90_Q99798	85.5	99.1	23	11	Aconitate hydratase, mitochondrial

B02	UniRef90_F1MDN4	29.9	99.0	26	5	60S acidic ribosomal protein P0
	UniRef90_G3HC01	35.4	98.7	16	3	Tropomyosin alpha-3 chain
	UniRef90_G3HEZ0	65.8	96.5	6	2	Stress-70 protein, mitochondrial
	UniRef90_G3IBF1	31.1	96.0	16	2	Nucleophosmin
	UniRef90_P11021	72.4	99.1	16	6	78 kDa glucose-regulated protein
	UniRef90_P31040	71.7	99.1	12	6	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_Q99798	85.5	98.5	10	3	Aconitate hydratase, mitochondrial
B03	UniRef90_G3GTF9	18.9	98.8	32	6	ATP synthase subunit d, mitochondrial
	UniRef90_G3HHK0	35.4	98.7	16	4	28S ribosomal protein S31, mitochondrial
	UniRef90_G3HNG2	49.8	98.8	10	4	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_G3HNG5	50.4	98.7	9	4	Acyl-coenzyme A thioesterase 1
	UniRef90_G3I6E6	32.0	98.2	13	3	Actin, cytoplasmic 2
	UniRef90_H0X554	30.1	98.3	21	3	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P21981	77.2	98.8	12	4	Protein-glutamine gamma-glutamyltransferase 2
	UniRef90_P46061	63.1	99.1	22	9	Ran GTPase-activating protein 1
	UniRef90_P50213	39.7	98.5	15	4	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P60709	41.7	98.2	10	3	Actin, cytoplasmic 1
	UniRef90_Q99N15	27.2	99.1	43	9	17beta-hydroxysteroid dehydrogenase type 10/short chain L-3-hydroxyacyl-CoA dehydrogenase
B04	UniRef90_G3HB04	28.4	98.9	32	5	Protein disulfide-isomerase A6
	UniRef90_G3HE28	40.1	99.1	26	9	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HEZ0	65.8	99.1	33	13	Stress-70 protein, mitochondrial
	UniRef90_G3HJA9	19.9	98.1	18	3	Eukaryotic translation initiation factor 3 subunit K (Fragment)
	UniRef90_G3I2M0	25.5	98.8	24	5	Sulfatase-modifying factor 2
	UniRef90_H0X554	30.1	99.0	33	6	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P08670	53.7	99.1	21	5	Vimentin
	UniRef90_P11021	72.4	99.0	13	5	78 kDa glucose-regulated protein
	UniRef90_P21981	77.2	99.1	19	6	Protein-glutamine gamma-glutamyltransferase 2
	UniRef90_P46061	63.1	99.1	18	10	Ran GTPase-activating protein 1
	UniRef90_Q16576	47.0	98.4	11	4	Histone-binding protein RBBP7
	UniRef90_Q99653	22.5	99.1	49	6	Calcineurin B homologous protein 1
B05	UniRef90_G3H0L9	37.5	99.0	29	5	Cathepsin B
	UniRef90_G3I6E6	32.0	98.9	22	5	Actin, cytoplasmic 2
	UniRef90_G3IBF1	31.1	98.3	16	3	Nucleophosmin
	UniRef90_G3ILN5	34.4	98.9	18	5	Reticulocalbin-3
	UniRef90_H0X554	30.1	99.1	38	6	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P02545-2	64.0	99.1	24	9	Isoform C of Prelamin-A/C
	UniRef90_P02794	21.5	98.7	26	4	Ferritin heavy chain
	UniRef90_P08670	53.7	99.1	27	6	Vimentin
	UniRef90_P50213	39.7	98.8	17	4	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P60709	41.7	98.9	17	5	Actin, cytoplasmic 1
B06	UniRef90_G3HNG2	49.8	99.1	30	8	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_G3HPK0	56.3	98.7	10	3	Hydroxysteroid dehydrogenase-like protein 2
	UniRef90_G3I6E6	32.0	99.1	42	5	Actin, cytoplasmic 2
	UniRef90_G3ILN5	34.4	98.9	19	5	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.1	28	12	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.0	25	2	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.1	24	6	Vimentin
	UniRef90_P11021	72.4	99.2	43	20	78 kDa glucose-regulated protein
	UniRef90_P31040	71.7	99.2	34	16	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P60709	41.7	99.1	35	6	Actin, cytoplasmic 1
	UniRef90_Q14697	107.0	99.2	23	17	Neutral alpha-glucosidase AB
	UniRef90_Q16822	70.8	99.0	15	5	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
	UniRef90_Q9CWG8	48.8	98.6	11	3	NADH dehydrogenase [ubiquinone] complex I,

	UniRef90_Q9Z0X1	66.8	98.9	12	4	assembly factor 7 Apoptosis-inducing factor 1, mitochondrial
B07	UniRef90_G3HC79	102.8	99.0	9	5	Putative alanyl-tRNA synthetase, mitochondrial
	UniRef90_G3HEZ0	65.8	99.2	36	11	Stress-70 protein, mitochondrial
	UniRef90_G3HQL8	229.3	98.7	4	4	Myosin-Vb
	UniRef90_P02545-2	64.0	99.2	47	22	Isoform C of Prelamin-A/C
	UniRef90_P02794	21.5	99.1	43	7	Ferritin heavy chain
	UniRef90_P08670	53.7	98.0	11	2	Vimentin
	UniRef90_P11021	72.4	99.2	36	19	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	14	9	Endoplasmic
	UniRef90_P35232	29.8	99.1	44	6	Prohibitin
	UniRef90_P55302	41.2	98.1	15	3	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	99.0	20	5	Actin, cytoplasmic 1
	UniRef90_Q14697	107.0	99.2	28	22	Neutral alpha-glucosidase AB
	UniRef90_Q3V3R1	105.4	99.1	14	7	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q8CGK3	95.0	99.1	17	9	Lon protease homolog, mitochondrial
	UniRef90_Q9CQN1	80.3	99.1	19	5	Heat shock protein 75 kDa, mitochondrial
	UniRef90_Q9DB77	48.3	99.0	20	5	Cytochrome b-c1 complex subunit 2, mitochondrial
B08	UniRef90_G3HEZ0	65.8	99.1	28	11	Stress-70 protein, mitochondrial
	UniRef90_G3HZ25	25.5	99.0	29	5	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial
	UniRef90_G3I6E6	32.0	97.1	17	2	Actin, cytoplasmic 2
	UniRef90_P02545-2	64.0	99.2	37	15	Isoform C of Prelamin-A/C
	UniRef90_P11021	72.4	99.2	34	13	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.1	14	9	Endoplasmic
	UniRef90_P17987	60.3	99.1	25	11	T-complex protein 1 subunit alpha
	UniRef90_P60709	41.7	97.1	13	2	Actin, cytoplasmic 1
	UniRef90_Q99653	22.5	99.1	36	6	Calcineurin B homologous protein 1
	UniRef90_A4FUY3	73.1	98.3	7	3	Prolyl 3-hydroxylase 1
B09	UniRef90_G3GWC4	30.2	97.3	15	2	GrpE protein-like 1, mitochondrial
	UniRef90_G3HHK0	35.4	98.8	20	4	28S ribosomal protein S31, mitochondrial
	UniRef90_G3HN14	237.6	98.8	4	3	Transitional endoplasmic reticulum ATPase
	UniRef90_G3HZ25	25.5	98.4	33	3	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial
	UniRef90_G3IAS8	185.7	98.9	5	4	Kinesin-like protein KIF27
	UniRef90_G3ILN5	34.4	99.0	18	5	Reticulocalbin-3
	UniRef90_I3MCK6	29.3	98.3	25	3	Reticulocalbin-1
	UniRef90_P08670	53.7	99.0	18	4	Vimentin
	UniRef90_P09382	14.8	98.0	28	2	Galectin-1
	UniRef90_P50213	39.7	98.3	10	3	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_Q9UJZ1	38.2	98.8	20	4	Stomatin-like protein 2
	UniRef90_F1MDN4	29.9	98.9	24	5	60S acidic ribosomal protein P0
	UniRef90_G3H1B3	55.0	97.9	8	2	Lamin-L(I)
	UniRef90_G3HB04	28.4	99.0	36	5	Protein disulfide-isomerase A6
	UniRef90_G3HC01	35.4	99.1	23	6	Tropomyosin alpha-3 chain
B10	UniRef90_G3HE28	40.1	99.1	21	8	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3I6E6	32.0	98.2	11	3	Actin, cytoplasmic 2
	UniRef90_G3ILG6	14.5	98.3	37	3	Inorganic pyrophosphatase 2, mitochondrial (Fragment)
	UniRef90_G3ILN5	34.4	98.7	18	4	Reticulocalbin-3
	UniRef90_P07910	33.7	99.1	26	4	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P17987	60.3	99.2	43	17	T-complex protein 1 subunit alpha
	UniRef90_P35232	29.8	99.1	38	7	Prohibitin
	UniRef90_P50213	39.7	98.8	18	4	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P60709	41.7	98.2	8	3	Actin, cytoplasmic 1
	UniRef90_Q02218	104.5	90.0	4	2	2-oxoglutarate dehydrogenase, mitochondrial
	UniRef90_Q3V3R1	105.4	99.1	15	8	Monofunctional C1-tetrahydrofolate synthase, mitochondrial

B11	UniRef90_A4FUY3	73.1	99.0	12	6	Prolyl 3-hydroxylase 1
	UniRef90_G3HB04	28.4	99.1	31	6	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.1	29	12	Stress-70 protein, mitochondrial
	UniRef90_G3HRE2	313.8	94.1	4	2	Apoptotic chromatin condensation inducer in the nucleus
	UniRef90_P08670	53.7	97.8	20	2	Vimentin
	UniRef90_P11021	72.4	99.1	20	5	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.1	16	9	Endoplasmic
	UniRef90_P50454	46.6	94.9	9	2	Serpin H1
	UniRef90_Q14568	38.1	97.7	13	2	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q16576	47.0	98.8	9	4	Histone-binding protein RBBP7
	UniRef90_Q99798	85.5	99.1	12	6	Aconitate hydratase, mitochondrial
	UniRef90_Q99N15	27.2	99.1	41	6	17beta-hydroxysteroid dehydrogenase type 10/short chain L-3-hydroxyacyl-CoA dehydrogenase
	UniRef90_Q9CQN1	80.3	99.0	12	4	Heat shock protein 75 kDa, mitochondrial
B12	UniRef90_F1MDN4	29.9	98.2	12	3	60S acidic ribosomal protein P0
	UniRef90_G3GTF9	18.9	98.8	31	5	ATP synthase subunit d, mitochondrial
	UniRef90_G3HDY7	29.9	98.2	12	3	60S acidic ribosomal protein P0
	UniRef90_G3HQL8	229.3	99.0	4	5	Myosin-Vb
	UniRef90_G3HW62	35.5	98.7	17	4	Elongation factor Ts, mitochondrial
	UniRef90_G3HZ25	25.5	99.0	31	5	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial
	UniRef90_G3I2M0	25.5	98.7	19	3	Sulfatase-modifying factor 2
	UniRef90_G3I6E6	32.0	99.1	31	5	Actin, cytoplasmic 2
	UniRef90_P14625	92.6	99.1	20	11	Endoplasmic
	UniRef90_P55735	35.5	98.7	18	4	Protein SEC13 homolog
	UniRef90_P60709	41.7	99.1	27	5	Actin, cytoplasmic 1
	UniRef90_Q3V3R1	105.4	99.1	19	12	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q9DB77	48.3	98.3	11	3	Cytochrome b-c1 complex subunit 2, mitochondrial

Row C	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
C01	UniRef90_G3H0U6	50.1	99.2	27	10	Protein disulfide-isomerase A3
	UniRef90_G3H697	62.4	90.3	5	2	Glucosidase 2 subunit beta
	UniRef90_G3HC84	47.8	99.1	20	8	Heat shock protein HSP 90-beta
	UniRef90_G3HE28	40.1	98.3	11	2	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HEQ3	25.2	98.3	17	3	Ran-specific GTPase-activating protein
	UniRef90_G3HQX0	19.7	98.9	33	5	40S ribosomal protein SA
	UniRef90_O43681	38.8	98.3	11	3	ATPase ASNA1
	UniRef90_P07910	33.7	99.0	22	3	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.1	32	8	Vimentin
	UniRef90_P14625	92.6	99.2	28	20	Endoplasmic
	UniRef90_P20108	28.1	98.8	19	4	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_Q14568	38.1	98.9	16	5	Putative heat shock protein HSP 90-alpha A2
C02	UniRef90_F1MDN4	29.9	98.3	16	3	60S acidic ribosomal protein P0
	UniRef90_G3HEZ0	65.8	99.1	25	10	Stress-70 protein, mitochondrial
	UniRef90_G3HFL6	69.6	98.9	13	4	ATP-binding cassette sub-family G member 3
	UniRef90_G3HQX0	19.7	98.6	36	3	40S ribosomal protein SA
	UniRef90_G3I6E6	32.0	98.7	19	4	Actin, cytoplasmic 2
	UniRef90_G3ILN5	34.4	98.7	16	4	Reticulocalbin-3
	UniRef90_O43681	38.8	99.1	28	6	ATPase ASNA1
	UniRef90_P07910	33.7	98.8	22	2	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	39	13	Vimentin
	UniRef90_P08758	36.1	98.9	18	5	Annexin A5
	UniRef90_P35232	29.8	98.8	15	4	Prohibitin
	UniRef90_P56537	26.6	98.8	34	4	Eukaryotic translation initiation factor 6

	UniRef90_P60709	41.7	98.8	17	4	Actin, cytoplasmic 1
C03	UniRef90_G3H0L9	37.5	98.0	14	3	Cathepsin B
	UniRef90_G3HBY4	24.5	99.0	29	2	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_G3I6E6	32.0	98.2	17	3	Actin, cytoplasmic 2
	UniRef90_P07910	33.7	99.2	42	7	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08758	36.1	99.0	23	6	Annexin A5
	UniRef90_P14625	92.6	99.1	18	12	Endoplasmic
	UniRef90_P35232	29.8	98.8	19	4	Prohibitin
	UniRef90_P60709	41.7	98.2	16	3	Actin, cytoplasmic 1
C04	UniRef90_G3GT05	22.2	98.3	21	3	T-complex protein 1 subunit theta
	UniRef90_G3H0U6	50.1	99.1	40	16	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	99.0	38	5	Protein disulfide-isomerase A6
	UniRef90_G3HBZ7	12.2	98.2	32	3	HCLS1-associated protein X-1
	UniRef90_G3HKP3	40.6	96.1	10	3	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HNV4	16.4	98.9	39	3	Triosephosphate isomerase
	UniRef90_G3HQX0	19.7	98.8	41	2	40S ribosomal protein SA
	UniRef90_G3I216	20.4	99.1	32	4	Triosephosphate isomerase
	UniRef90_G3IAS8	185.7	98.6	5	3	Kinesin-like protein KIF27
	UniRef90_G3ILN5	34.4	99.0	19	6	Reticulocalbin-3
	UniRef90_O43681	38.8	99.1	24	6	ATPase ASNA1
	UniRef90_P06733	46.7	98.3	14	3	Alpha-enolase
	UniRef90_P07910	33.7	99.1	31	5	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.1	22	5	Vimentin
	UniRef90_P27797	48.2	96.0	9	2	Calreticulin
	UniRef90_P30153	59.0	99.2	40	13	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
	UniRef90_P30154	66.0	99.0	14	5	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform
C05	UniRef90_F8VPV9	56.9	97.9	6	3	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	98.0	10	3	Protein disulfide-isomerase A3
	UniRef90_G3H697	62.4	97.9	5	3	Glucosidase 2 subunit beta
	UniRef90_G3HE28	40.1	99.1	20	6	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HKP3	40.6	98.9	22	3	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_O00571	73.2	95.9	7	2	ATP-dependent RNA helicase DDX3X
	UniRef90_P02545-2	64.0	99.2	43	21	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	98.0	13	3	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.1	44	18	Vimentin
	UniRef90_P08758	36.1	98.9	23	5	Annexin A5
	UniRef90_P10809	56.5	98.8	11	4	60 kDa heat shock protein, mitochondrial
	UniRef90_P20108	28.1	98.9	19	5	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P28331	79.8	99.1	9	7	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_Q96I25	45.3	94.0	8	2	Splicing factor 45
	UniRef90_Q99798	85.5	98.4	6	3	Aconitate hydratase, mitochondrial
	UniRef90_Q9CQN1	80.3	99.0	11	5	Heat shock protein 75 kDa, mitochondrial
C06	UniRef90_F8VPV9	56.9	99.2	25	10	ATP synthase subunit beta
	UniRef90_G3HC01	35.4	98.3	12	3	Tropomyosin alpha-3 chain
	UniRef90_G3I1Y9	27.4	98.5	20	3	Sulfated glycoprotein 1
	UniRef90_G3I220	36.4	91.5	12	2	Cytoskeleton-associated protein 4
	UniRef90_P07910	33.7	92.1	12	2	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	49	13	Vimentin
	UniRef90_P10809	56.5	99.0	14	5	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	21	16	Endoplasmic
	UniRef90_P27797	48.2	97.6	9	2	Calreticulin
	UniRef90_P30153	59.0	99.1	17	6	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
	UniRef90_P30154	66.0	98.5	14	3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform
	UniRef90_P31946	27.9	98.0	14	3	14-3-3 protein beta/alpha
	UniRef90_P31947	27.7	98.0	10	3	14-3-3 protein sigma
	UniRef90_P63101	27.7	98.0	10	3	14-3-3 protein zeta/delta

	UniRef90_P68254	27.7	98.0	10	3	14-3-3 protein theta
	UniRef90_Q3V3R1	105.4	98.3	8	3	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q9R0A0	35.2	99.0	22	4	Peroxisomal membrane protein PEX14
	UniRef90_Q9Y224	12.3	98.3	37	3	UPF0568 protein C14orf166
C07	UniRef90_A4FUY3	73.1	99.0	15	5	Prolyl 3-hydroxylase 1
	UniRef90_G3HNV4	16.4	98.6	30	3	Triosephosphate isomerase
	UniRef90_G3HPV6	28.5	97.3	13	2	Histone H2A type 1
	UniRef90_G3HRE2	138.8	98.7	4	4	Apoptotic chromatin condensation inducer in the nucleus
	UniRef90_G3I216	20.4	98.4	21	3	Triosephosphate isomerase
	UniRef90_P07910	33.7	99.1	31	4	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	48	15	Vimentin
	UniRef90_P14625	92.6	99.2	28	17	Endoplasmic
	UniRef90_P31937	31.7	94.6	12	2	3-hydroxyisobutyrate dehydrogenase, mitochondrial
	UniRef90_Q8CGK3	95.0	97.3	4	2	Lon protease homolog, mitochondrial
C08	UniRef90_B9A067	79.3	97.8	8	3	Mitochondrial inner membrane protein
	UniRef90_F1MDN4	29.9	99.1	36	8	60S acidic ribosomal protein P0
	UniRef90_F6T365	7.9	98.2	56	3	14-3-3 protein epsilon
	UniRef90_F8VPV9	56.9	98.3	11	3	ATP synthase subunit beta
	UniRef90_G3H697	62.4	99.0	12	6	Glucosidase 2 subunit beta
	UniRef90_G3HC01	35.4	98.3	10	3	Tropomyosin alpha-3 chain
	UniRef90_G3HC84	47.8	97.9	14	2	Heat shock protein HSP 90-beta
	UniRef90_G3HEZ0	65.8	99.1	23	10	Stress-70 protein, mitochondrial
	UniRef90_G3HFL6	69.6	98.9	10	5	ATP-binding cassette sub-family G member 3
	UniRef90_G3I6E6	32.0	98.1	11	3	Actin, cytoplasmic 2
	UniRef90_G3IIE7	76.0	98.3	6	3	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1
	UniRef90_G3ILN5	34.4	98.8	14	4	Reticulocalbin-3
	UniRef90_O43681	38.8	99.0	21	5	ATPase ASNA1
	UniRef90_P02545-2	64.0	99.0	17	4	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.2	44	8	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.1	26	7	Vimentin
	UniRef90_P11021	72.4	99.2	29	12	78 kDa glucose-regulated protein
	UniRef90_P31946	27.9	98.2	10	2	14-3-3 protein beta/alpha
	UniRef90_P31947	27.7	98.2	10	2	14-3-3 protein sigma
	UniRef90_P60709	41.7	98.1	8	3	Actin, cytoplasmic 1
	UniRef90_P63101	27.7	98.2	10	2	14-3-3 protein zeta/delta
	UniRef90_P68254	27.7	98.2	10	2	14-3-3 protein theta
	UniRef90_P99027	11.7	98.4	46	4	60S acidic ribosomal protein P2
	UniRef90_Q07955	27.7	98.1	13	3	Serine/arginine-rich splicing factor 1
	UniRef90_Q8K0D5	83.3	97.9	6	3	Elongation factor G, mitochondrial
	UniRef90_Q99798	85.5	99.2	23	13	Aconitate hydratase, mitochondrial
	UniRef90_Q9CQN1	80.3	98.8	10	3	Heat shock protein 75 kDa, mitochondrial
C09	UniRef90_F8VPV9	56.9	99.2	26	11	ATP synthase subunit beta
	UniRef90_G3HB04	28.4	98.8	22	4	Protein disulfide-isomerase A6
	UniRef90_G3HEQ3	25.2	98.3	20	3	Ran-specific GTPase-activating protein
	UniRef90_G3HFL6	69.6	98.3	10	3	ATP-binding cassette sub-family G member 3
	UniRef90_P02545-2	64.0	98.6	9	3	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.2	41	7	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	59	15	Vimentin
	UniRef90_P10809	56.5	98.2	6	3	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.1	18	6	Endoplasmic
	UniRef90_P27797	48.2	97.7	13	2	Calreticulin
	UniRef90_P28331	79.8	99.1	17	8	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P62879	30.5	98.7	14	4	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2
	UniRef90_Q16576	47.0	98.7	9	4	Histone-binding protein RBBP7
	UniRef90_Q61576	64.7	98.8	15	4	Peptidyl-prolyl cis-trans isomerase FKBP10
	UniRef90_Q71U36	50.1	99.1	23	3	Tubulin alpha-1A chain
	UniRef90_Q96I25	45.3	98.8	15	3	Splicing factor 45
C10	UniRef90_F1MDN4	29.9	98.8	18	4	60S acidic ribosomal protein P0

	UniRef90_F8VPV9	56.9	98.4	7	3	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.1	20	8	Protein disulfide-isomerase A3
	UniRef90_G3HC84	47.8	98.8	13	5	Heat shock protein HSP 90-beta
	UniRef90_P07910	33.7	99.2	23	4	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	98.9	15	4	Vimentin
	UniRef90_P11021	72.4	99.0	12	5	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.1	16	11	Endoplasmic
	UniRef90_P28331	79.8	99.0	12	6	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P56537	26.6	98.3	18	3	Eukaryotic translation initiation factor 6
	UniRef90_Q14568	38.1	98.9	18	5	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q14697	107.0	98.8	9	5	Neutral alpha-glucosidase AB
	UniRef90_Q16576	47.0	98.7	11	4	Histone-binding protein RBBP7
	UniRef90_Q8CGK3	95.0	91.1	5	2	Lon protease homolog, mitochondrial
C11	UniRef90_B9A067	79.3	99.1	18	10	Mitochondrial inner membrane protein
	UniRef90_G1SLD5	111.5	99.1	14	7	Hypoxia up-regulated protein 1
	UniRef90_G3H0U6	50.1	99.2	39	16	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	98.8	23	4	Protein disulfide-isomerase A6
	UniRef90_G3HE28	40.1	98.6	14	3	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HEZ0	65.8	99.2	39	16	Stress-70 protein, mitochondrial
	UniRef90_G3HKP3	40.6	99.0	24	6	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_P02545-2	64.0	99.1	19	5	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.1	50	17	Vimentin
	UniRef90_P10809	56.5	99.1	18	6	60 kDa heat shock protein, mitochondrial
	UniRef90_P28331	79.8	99.1	19	9	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P31946	27.9	94.6	10	2	14-3-3 protein beta/alpha
	UniRef90_P31947	27.7	94.6	10	2	14-3-3 protein sigma
	UniRef90_P63101	27.7	94.6	10	2	14-3-3 protein zeta/delta
	UniRef90_P68254	27.7	94.6	10	2	14-3-3 protein theta
	UniRef90_Q16576	47.0	98.3	8	4	Histone-binding protein RBBP7
	UniRef90_Q99798	85.5	99.1	23	14	Aconitate hydratase, mitochondrial
	UniRef90_Q9CQN1	80.3	98.3	9	3	Heat shock protein 75 kDa, mitochondrial
	UniRef90_Q9R0A0	35.2	98.9	22	4	Peroxisomal membrane protein PEX14
C12	UniRef90_F8VPV9	56.9	97.9	6	3	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	98.0	10	3	Protein disulfide-isomerase A3
	UniRef90_G3H697	62.4	97.9	5	3	Glucosidase 2 subunit beta
	UniRef90_G3HE28	40.1	99.1	20	6	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HKP3	40.6	98.9	22	3	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_O00571	73.2	95.9	7	2	ATP-dependent RNA helicase DDX3X
	UniRef90_P02545-2	64.0	99.2	43	19	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	98.0	13	3	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.1	44	18	Vimentin
	UniRef90_P08758	36.1	98.9	23	5	Annexin A5
	UniRef90_P10809	56.5	98.8	11	4	60 kDa heat shock protein, mitochondrial
	UniRef90_P20108	28.1	98.9	19	5	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P28331	79.8	99.1	9	7	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_Q96I25	45.3	94.0	8	2	Splicing factor 45
	UniRef90_Q99798	85.5	98.4	6	3	Aconitate hydratase, mitochondrial
	UniRef90_Q9CQN1	80.3	99.0	11	5	Heat shock protein 75 kDa, mitochondrial

Row D	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
D01	UniRef90_A4FUY3	73.1	98.9	11	4	Prolyl 3-hydroxylase 1
	UniRef90_G3GT05	22.2	96.1	14	2	T-complex protein 1 subunit theta
	UniRef90_G3GYD5	23.7	98.3	22	4	Complement component 1 Q subcomponent-binding protein, mitochondrial

	UniRef90_G3H0U6	50.1	96.9	14	2	Protein disulfide-isomerase A3
	UniRef90_G3H584	28.2	99.0	27	5	SPARC
	UniRef90_G3HRE2	138.8	98.2	5	3	Apoptotic chromatin condensation inducer in the nucleus
	UniRef90_G3IAS8	185.7	99.1	6	6	Kinesin-like protein KIF27
	UniRef90_G3ILN5	34.4	98.9	19	5	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.1	28	7	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	38	13	Vimentin
	UniRef90_P10809	56.5	98.5	10	3	60 kDa heat shock protein, mitochondrial
	UniRef90_P28331	79.8	99.1	24	10	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P62873	37.4	99.0	17	5	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
	UniRef90_Q02790	46.6	98.1	13	3	Peptidyl-prolyl cis-trans isomerase FKBP4
	UniRef90_Q14568	38.1	98.9	23	5	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q8BMF4	68.0	99.1	24	11	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
D02	UniRef90_A4FUY3	73.1	99.0	13	6	Prolyl 3-hydroxylase 1
	UniRef90_G3HC01	35.4	99.1	28	4	Tropomyosin alpha-3 chain
	UniRef90_G3HC84	47.8	90.9	14	2	Heat shock protein HSP 90-beta
	UniRef90_G3HEZ0	65.8	99.1	21	6	Stress-70 protein, mitochondrial
	UniRef90_G3HN14	237.6	98.4	3	3	Transitional endoplasmic reticulum ATPase
	UniRef90_G3HR08	50.3	98.9	20	5	Cytosol aminopeptidase
	UniRef90_G3HRE2	138.8	98.6	5	4	Apoptotic chromatin condensation inducer in the nucleus
	UniRef90_G3HSM8	67.7	99.1	18	5	Lamin-B2
	UniRef90_G3ILN5	34.4	98.7	14	4	Reticulocalbin-3
	UniRef90_I3MCK6	29.3	98.3	28	3	Reticulocalbin-1
	UniRef90_P05091	45.9	99.1	35	8	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08003	72.6	98.9	17	4	Protein disulfide-isomerase A4
	UniRef90_P08670	53.7	99.2	50	22	Vimentin
	UniRef90_P09622	54.1	99.1	27	8	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P11021	72.4	99.1	20	9	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	90.1	7	2	Endoplasmic
	UniRef90_P28331	79.8	99.1	18	7	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P34897	55.9	98.5	16	3	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_Q14568	38.1	99.1	34	9	Putative heat shock protein HSP 90-alpha A2
D03	UniRef90_G3H0U6	50.1	99.0	16	6	Protein disulfide-isomerase A3
	UniRef90_G3HEZ0	65.8	99.2	28	12	Stress-70 protein, mitochondrial
	UniRef90_G3HFL6	69.6	99.1	12	5	ATP-binding cassette sub-family G member 3
	UniRef90_G3HSM8	67.7	99.1	20	8	Lamin-B2
	UniRef90_G3IE04	53.4	99.0	14	6	Pre-mRNA-processing factor 19
	UniRef90_G3ILN5	34.4	99.0	19	5	Reticulocalbin-3
	UniRef90_O95302	63.0	98.2	10	3	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P02545-2	64.0	99.1	39	16	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	98.7	17	4	Vimentin
	UniRef90_P11021	72.4	99.2	23	8	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	23	15	Endoplasmic
	UniRef90_P28331	79.8	99.2	18	10	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P56537	26.6	98.8	22	4	Eukaryotic translation initiation factor 6
	UniRef90_P60709	41.7	98.7	11	4	Actin, cytoplasmic 1
	UniRef90_P78371	57.5	99.1	21	6	T-complex protein 1 subunit beta
	UniRef90_Q8BMF4	68.0	99.2	28	12	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
	UniRef90_Q91W90	46.4	99.2	37	12	Thioredoxin domain-containing protein 5
	UniRef90_Q9CQN1	80.3	98.9	14	5	Heat shock protein 75 kDa, mitochondrial
D04	UniRef90_A4FUY3	73.1	98.8	11	4	Prolyl 3-hydroxylase 1
	UniRef90_G1SLD5	111.5	98.7	7	4	Hypoxia up-regulated protein 1
	UniRef90_G3H0U6	50.1	99.1	26	9	Protein disulfide-isomerase A3
	UniRef90_G3HNJ3	51.8	98.8	11	4	Clusterin

	UniRef90_G3HRE2	138.8	97.7	3	3	Apoptotic chromatin condensation inducer in the nucleus
	UniRef90_G3ILN5	34.4	98.8	15	4	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.0	16	6	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.1	22	5	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.1	20	7	Vimentin
	UniRef90_P14625	92.6	99.2	15	11	Endoplasmic
	UniRef90_Q14568	38.1	99.0	18	5	Putative heat shock protein HSP 90-alpha A2
D05	UniRef90_G3HEZ0	65.8	99.2	44	21	Stress-70 protein, mitochondrial
	UniRef90_G3HFL6	69.6	98.9	10	4	ATP-binding cassette sub-family G member 3
	UniRef90_G3HHK0	35.4	99.1	33	8	28S ribosomal protein S31, mitochondrial
	UniRef90_G3HSM8	67.7	98.9	18	4	Lamin-B2
	UniRef90_O95302	63.0	98.9	15	5	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_O96019	47.5	98.3	10	3	Actin-like protein 6A
	UniRef90_P08003	72.6	99.2	24	13	Protein disulfide-isomerase A4
	UniRef90_P27797	48.2	99.0	18	6	Calreticulin
	UniRef90_P49821	50.6	93.7	8	2	NADH dehydrogenase [ubiquinone] flavoprotein 1, mitochondrial
	UniRef90_P50213	39.7	98.5	14	4	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P62873	37.4	99.0	21	6	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
	UniRef90_Q07955	27.7	98.2	12	3	Serine/arginine-rich splicing factor 1
	UniRef90_Q16576	47.0	99.1	13	7	Histone-binding protein RBBP7
	UniRef90_Q91W90	46.4	99.1	27	8	Thioredoxin domain-containing protein 5
D06	UniRef90_Q9Y265	50.2	98.6	16	4	RuvB-like 1
	UniRef90_G3H0U6	50.1	99.2	32	11	Protein disulfide-isomerase A3
	UniRef90_G3H2T7	29.6	98.6	17	2	Histone H3
	UniRef90_G3H584	28.2	98.4	24	3	SPARC
	UniRef90_G3HAE2	7.0	98.2	58	3	ATP synthase subunit alpha, mitochondrial
	UniRef90_G3HDS3	26.7	98.6	19	2	Histone H2A
	UniRef90_G3HE28	40.1	98.9	14	4	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HKP3	40.6	99.2	33	11	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3IE04	53.4	99.1	23	5	Pre-mRNA-processing factor 19
	UniRef90_G3ILN5	34.4	99.0	32	8	Reticulocalbin-3
	UniRef90_H0X554	30.1	99.2	44	10	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_I3MCK6	29.3	98.9	32	4	Reticulocalbin-1
	UniRef90_P07910	33.7	99.0	25	3	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	31	9	Vimentin
D07	UniRef90_P10809	56.5	99.2	28	10	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	16	10	Endoplasmic
	UniRef90_Q9GZL7	47.6	98.1	11	3	Ribosome biogenesis protein WDR12
	UniRef90_A6QR15	30.7	98.9	28	6	Tropomyosin beta chain
	UniRef90_G3H0U6	50.1	99.2	32	11	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	98.2	17	3	Protein disulfide-isomerase A6
	UniRef90_G3HFX6	19.0	98.7	31	4	Stromal cell-derived factor 2-like protein 1
	UniRef90_G3HNG2	49.8	99.1	21	9	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_G3HR08	50.3	98.7	11	4	Cytosol aminopeptidase
	UniRef90_G3IAS8	185.7	99.2	6	8	Kinesin-like protein KIF27
	UniRef90_H0X554	30.1	99.1	27	7	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_O88696	29.8	99.1	40	8	Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial
	UniRef90_O96019	47.5	98.3	10	3	Actin-like protein 6A
	UniRef90_P02545-2	64.0	99.1	19	8	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	98.2	11	3	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.1	22	6	Vimentin
	UniRef90_P10809	56.5	98.3	9	3	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	27	22	Endoplasmic
	UniRef90_P60709	41.7	99.1	31	7	Actin, cytoplasmic 1
	UniRef90_Q02790	46.6	99.0	18	6	Peptidyl-prolyl cis-trans isomerase FKBP4
	UniRef90_Q14697	107.0	99.1	11	9	Neutral alpha-glucosidase AB

	UniRef90_Q3V3R1	105.4	99.1	18	11	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q8CGK3	95.0	99.1	16	6	Lon protease homolog, mitochondrial
	UniRef90_Q91W90	46.4	99.0	6	3	Thioredoxin domain-containing protein 5
D08	UniRef90_B9A067	79.3	99.1	12	6	Mitochondrial inner membrane protein
	UniRef90_F8VPV9	56.9	98.3	8	3	ATP synthase subunit beta
	UniRef90_G3HEZ0	65.8	99.2	32	17	Stress-70 protein, mitochondrial
	UniRef90_G3HSM8	67.7	99.1	19	7	Lamin-B2
	UniRef90_H0X554	30.1	99.1	32	8	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P02545-2	64.0	99.1	36	16	Isoform C of Prelamin-A/C
	UniRef90_P08003	72.6	99.1	18	11	Protein disulfide-isomerase A4
	UniRef90_P08670	53.7	99.2	40	17	Vimentin
	UniRef90_P11021	72.4	98.3	9	3	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	21	16	Endoplasmic
	UniRef90_P27797	48.2	99.1	29	12	Calreticulin
	UniRef90_P28331	79.8	99.1	18	10	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P49411	49.6	98.4	12	3	Elongation factor Tu, mitochondrial
	UniRef90_P50454	46.6	97.4	9	3	Serpin H1
	UniRef90_Q16576	47.0	98.8	13	5	Histone-binding protein RBBP7
	UniRef90_Q8CGK3	95.0	99.1	12	5	Lon protease homolog, mitochondrial
	UniRef90_Q8K0D5	83.3	99.1	14	6	Elongation factor G, mitochondrial
	UniRef90_Q91W90	46.4	98.9	18	5	Thioredoxin domain-containing protein 5
	UniRef90_Q99798	85.5	99.2	17	9	Aconitate hydratase, mitochondrial
	UniRef90_Q9CQN1	80.3	99.1	21	9	Heat shock protein 75 kDa, mitochondrial
	UniRef90_Q9Y3B8	26.8	96.7	16	3	Oligoribonuclease, mitochondrial
D09	UniRef90_A6QR15	30.7	99.0	28	6	Tropomyosin beta chain
	UniRef90_B4DJ75	58.7	98.2	5	3	Serine/threonine-protein phosphatase
	UniRef90_B6UV57	49.4	98.9	11	5	Cysteine desulfurase, mitochondrial
	UniRef90_F8VPV9	56.9	98.4	13	4	ATP synthase subunit beta
	UniRef90_G2HG05	17.1	98.3	32	3	Interleukin enhancer-binding factor 2
	UniRef90_G3GXA9	21.1	98.9	39	4	Protein disulfide-isomerase
	UniRef90_G3H0U6	50.1	99.1	27	9	Protein disulfide-isomerase A3
	UniRef90_G3H298	34.1	98.2	9	3	Serine/threonine-protein phosphatase
	UniRef90_G3HJN3	51.8	99.0	17	5	Clusterin
	UniRef90_G3HSM8	67.7	97.6	7	3	Lamin-B2
	UniRef90_G3I5H3	36.6	99.0	26	5	Elongation factor 1-delta
	UniRef90_P07910	33.7	99.2	37	7	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.0	19	4	Vimentin
	UniRef90_P10809	56.5	99.1	23	11	60 kDa heat shock protein, mitochondrial
	UniRef90_P27797	48.2	98.2	9	3	Calreticulin
	UniRef90_P38159	42.1	98.8	20	4	Heterogeneous nuclear ribonucleoprotein G
	UniRef90_P50213	39.7	99.2	23	9	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P60709	41.7	99.1	28	6	Actin, cytoplasmic 1
	UniRef90_Q16576	47.0	99.1	13	3	Histone-binding protein RBBP7
	UniRef90_Q7TP98	12.8	98.7	37	4	Interleukin enhancer-binding factor 2
	UniRef90_Q91W90	46.4	99.2	36	12	Thioredoxin domain-containing protein 5
D10	UniRef90_F8VPV9	56.9	98.9	10	4	ATP synthase subunit beta
	UniRef90_G3H1B3	55.0	98.0	6	2	Lamin-L(I)
	UniRef90_G3HC84	47.8	99.1	21	4	Heat shock protein HSP 90-beta
	UniRef90_G3HEZ0	65.8	97.6	11	2	Stress-70 protein, mitochondrial
	UniRef90_G3HNG2	49.8	99.1	17	6	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_G3HNG5	50.4	99.1	16	6	Acyl-coenzyme A thioesterase 1
	UniRef90_P02545-2	64.0	99.1	31	13	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	50	22	Vimentin
	UniRef90_P10809	56.5	99.0	12	5	60 kDa heat shock protein, mitochondrial
	UniRef90_P11021	72.4	99.1	23	9	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.1	17	10	Endoplasmic
	UniRef90_P27797	48.2	99.1	20	7	Calreticulin
	UniRef90_P50213	39.7	99.1	28	10	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_Q14568	38.1	99.0	23	6	Putative heat shock protein HSP 90-alpha A2

	UniRef90_Q16576	47.0	98.8	11	4	Histone-binding protein RBBP7
D11	UniRef90_A4FUY3	73.1	98.4	10	3	Prolyl 3-hydroxylase 1
	UniRef90_F8VPV9	56.9	98.7	11	4	ATP synthase subunit beta
	UniRef90_G3H584	28.2	99.0	27	5	SPARC
	UniRef90_G3H937	58.8	92.1	5	2	Histone-binding protein RBBP4
	UniRef90_G3HC84	47.8	98.9	15	5	Heat shock protein HSP 90-beta
	UniRef90_G3HFL6	69.6	98.8	10	4	ATP-binding cassette sub-family G member 3
	UniRef90_G3HRE2	138.8	98.6	5	4	Apoptotic chromatin condensation inducer in the nucleus
	UniRef90_G3I7A8	131.3	98.3	5	3	Sortilin (Fragment)
	UniRef90_G3ILN5	34.4	98.9	19	6	Reticulocalbin-3
	UniRef90_I3MCK6	29.3	98.4	35	3	Reticulocalbin-1
	UniRef90_P08670	53.7	99.2	64	25	Vimentin
	UniRef90_P14625	92.6	99.1	17	12	Endoplasmic
	UniRef90_P27797	48.2	98.2	14	3	Calreticulin
	UniRef90_P56537	26.6	99.0	31	5	Eukaryotic translation initiation factor 6
	UniRef90_P62873	37.4	97.8	10	3	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
	UniRef90_Q14568	38.1	99.1	26	6	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q16576	47.0	98.4	8	3	Histone-binding protein RBBP7
D12	UniRef90_A6QR15	30.7	99.1	29	8	Tropomyosin beta chain
	UniRef90_G3HE28	40.1	98.7	14	3	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HEZ0	65.8	99.1	21	6	Stress-70 protein, mitochondrial
	UniRef90_G3HGS2	96.2	98.9	8	4	Propionyl-CoA carboxylase alpha chain, mitochondrial
	UniRef90_G3HKP3	40.6	99.1	24	6	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HJN3	51.8	98.4	9	3	Clusterin
	UniRef90_G3HR08	50.3	98.8	13	4	Cytosol aminopeptidase
	UniRef90_P02545-2	64.0	99.1	28	9	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.1	37	8	Vimentin
	UniRef90_P09622	54.1	99.2	26	9	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P14625	92.6	99.2	23	18	Endoplasmic
	UniRef90_P54652	69.7	98.8	10	4	Heat shock-related 70 kDa protein 2
	UniRef90_Q3V3R1	105.4	98.8	11	4	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q9CQN1	80.3	99.2	27	15	Heat shock protein 75 kDa, mitochondrial

Row E	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
E01	UniRef90_G3HEZ0	65.8	98.0	7	3	Stress-70 protein, mitochondrial
	UniRef90_O88696	29.8	99.1	27	6	Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial
	UniRef90_P02545-2	64.0	98.4	11	3	Isoform C of Prelamin-A/C
	UniRef90_P14625	92.6	99.1	18	9	Endoplasmic
	UniRef90_P27797	48.2	99.0	19	6	Calreticulin
	UniRef90_P31040	71.7	99.0	11	5	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P54652	69.7	98.2	5	3	Heat shock-related 70 kDa protein 2
	UniRef90_P60709	41.7	98.9	15	5	Actin, cytoplasmic 1
	UniRef90_Q02218	104.5	95.6	4	2	2-oxoglutarate dehydrogenase, mitochondrial
	UniRef90_Q3V3R1	105.4	98.3	9	3	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q7TP98	12.8	98.0	27	3	Interleukin enhancer-binding factor 2
	UniRef90_Q8BMF4	68.0	99.2	28	10	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
	UniRef90_Q8CGK3	95.0	99.1	11	6	Lon protease homolog, mitochondrial
	UniRef90_Q91W90	46.4	99.1	22	7	Thioredoxin domain-containing protein 5
	UniRef90_Q9CQN1	80.3	99.1	29	15	Heat shock protein 75 kDa, mitochondrial
E02	UniRef90_B9W7J9	11.4	98.3	27	3	Histone H4

	UniRef90_F8VPV9	56.9	99.2	27	9	ATP synthase subunit beta
	UniRef90_G2HG05	17.1	98.4	32	3	Interleukin enhancer-binding factor 2
	UniRef90_G3HC84	47.8	99.1	23	6	Heat shock protein HSP 90-beta
	UniRef90_G3HDS1	14.9	98.3	21	3	Histone H4
	UniRef90_G3HKP3	40.6	99.0	20	7	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HPV6	28.5	98.3	11	3	Histone H2A type 1
	UniRef90_G3HPV7	21.8	98.3	14	3	Histone H4
	UniRef90_G3HQI8	229.3	98.7	2	4	Myosin-Vb
	UniRef90_H0X554	30.1	99.2	51	11	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P00505	47.4	98.8	17	5	Aspartate aminotransferase, mitochondrial
	UniRef90_P05091	45.9	99.1	21	6	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.1	30	8	Vimentin
	UniRef90_P27797	48.2	99.1	24	7	Calreticulin
	UniRef90_P34897	55.9	98.3	12	3	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_P55302	41.2	99.1	28	8	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	99.2	36	10	Actin, cytoplasmic 1
	UniRef90_P62805	10.8	98.3	29	3	Histone H4
	UniRef90_Q14568	38.1	99.1	29	6	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q91W90	46.4	99.1	37	10	Thioredoxin domain-containing protein 5
	UniRef90_Q9DB77	48.3	98.8	16	4	Cytochrome b-c1 complex subunit 2, mitochondrial
E03	UniRef90_B9A067	79.3	99.2	28	16	Mitochondrial inner membrane protein
	UniRef90_F8VPV9	56.9	98.2	7	3	ATP synthase subunit beta
	UniRef90_G3HEZ0	65.8	99.2	51	28	Stress-70 protein, mitochondrial
	UniRef90_G3IDL7	22.7	99.2	45	4	Heat shock cognate 71 kDa protein
	UniRef90_G3IE04	53.4	98.8	12	4	Pre-mRNA-processing factor 19
	UniRef90_G3IGQ3	23.1	99.0	33	2	Heat shock cognate 71 kDa protein
	UniRef90_O88696	29.8	99.0	31	5	Putative ATP-dependent Clp protease proteolytic subunit, mitochondrial
	UniRef90_P07910	33.7	99.1	30	6	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	55	20	Vimentin
	UniRef90_P27797	48.2	99.2	24	10	Calreticulin
	UniRef90_P55302	41.2	99.0	19	6	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P78371	57.5	99.0	17	5	T-complex protein 1 subunit beta
	UniRef90_Q8BMF4	68.0	98.6	9	4	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
	UniRef90_Q99798	85.5	98.7	12	4	Aconitate hydratase, mitochondrial
E04	UniRef90_B9W7J9	11.4	98.4	39	3	Histone H4
	UniRef90_G3HDS1	14.9	98.4	30	3	Histone H4
	UniRef90_G3HEZ0	65.8	99.1	21	8	Stress-70 protein, mitochondrial
	UniRef90_G3HFQ4	33.2	98.2	16	3	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HGS2	96.2	98.9	11	4	Propionyl-CoA carboxylase alpha chain, mitochondrial
	UniRef90_G3HPV6	28.5	98.4	15	3	Histone H2A type 1
	UniRef90_G3HPV7	21.8	98.4	20	3	Histone H4
	UniRef90_G3HR08	50.3	99.1	23	7	Cytosol aminopeptidase
	UniRef90_G3IE04	53.4	99.0	12	5	Pre-mRNA-processing factor 19
	UniRef90_H0X554	30.1	99.1	34	8	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P02545-2	64.0	99.1	29	8	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.2	37	6	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P20108	28.1	99.1	36	7	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P28331	79.8	99.2	27	16	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P43304	80.7	98.7	16	3	Glycerol-3-phosphate dehydrogenase, mitochondrial

	UniRef90_P49411	49.6	99.2	44	16	Elongation factor Tu, mitochondrial
	UniRef90_P51855	52.2	97.1	12	2	Glutathione synthetase
	UniRef90_P62805	10.8	98.4	41	3	Histone H4
	UniRef90_Q61576	64.7	96.8	12	2	Peptidyl-prolyl cis-trans isomerase FKBP10
	UniRef90_Q757K0	11.3	98.3	29	3	Histone H4.1
	UniRef90_Q8K3J1	23.1	98.7	23	5	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial
	UniRef90_Q9CQN1	80.3	99.2	30	20	Heat shock protein 75 kDa, mitochondrial
	UniRef90_Q9GZL7	47.6	95.6	9	2	Ribosome biogenesis protein WDR12
	UniRef90_Q9UQ80	43.7	96.1	13	2	Proliferation-associated protein 2G4
	UniRef90_Q9Y265	50.2	98.6	20	3	RuvB-like 1
E05	UniRef90_F8VPV9	56.9	99.2	28	11	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.1	25	7	Protein disulfide-isomerase A3
	UniRef90_G3HC79	102.8	97.6	6	2	Putative alanyl-tRNA synthetase, mitochondrial
	UniRef90_G3HZY1	57.2	98.9	12	5	RuvB-like 2
	UniRef90_G3I5W2	22.6	98.8	24	4	TAR DNA-binding protein 43
	UniRef90_G3I6E6	32.0	99.0	25	3	Actin, cytoplasmic 2
	UniRef90_G3IAS8	185.7	94.4	2	2	Kinesin-like protein KIF27
	UniRef90_P05091	45.9	99.2	26	8	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.2	50	20	Vimentin
	UniRef90_P09622	54.1	98.3	11	3	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P10809	56.5	99.2	27	10	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	18	9	Endoplasmic
	UniRef90_P27797	48.2	98.9	18	4	Calreticulin
	UniRef90_P34897	55.9	97.9	18	2	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_P35232	29.8	99.2	54	12	Prohibitin
	UniRef90_P60709	41.7	99.1	22	3	Actin, cytoplasmic 1
	UniRef90_Q13148	21.8	99.0	31	4	TAR DNA-binding protein 43
	UniRef90_Q14697	107.0	99.1	18	10	Neutral alpha-glucosidase AB
	UniRef90_Q71U36	50.1	98.5	25	7	Tubulin alpha-1A chain
	UniRef90_Q8CGK3	95.0	99.1	18	10	Lon protease homolog, mitochondrial
	UniRef90_Q9CWWG8	48.8	94.1	9	2	Protein midA-like, mitochondrial
	UniRef90_Q9Y265	50.2	99.2	30	10	RuvB-like 1
	UniRef90_Q9Z1N5	48.7	94.4	11	2	Spliceosome RNA helicase Ddx39b
E06	UniRef90_F8VPV9	56.9	99.2	21	9	ATP synthase subunit beta
	UniRef90_G3HEZ0	65.8	99.2	43	21	Stress-70 protein, mitochondrial
	UniRef90_H0X554	30.1	99.2	36	9	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P02545-2	64.0	99.1	20	6	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	58	26	Vimentin
	UniRef90_P14625	92.6	99.1	17	11	Endoplasmic
	UniRef90_P28331	79.8	99.2	24	14	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P31040	71.7	99.2	21	12	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P35232	29.8	98.3	13	3	Prohibitin
	UniRef90_P49368	60.6	97.8	11	3	T-complex protein 1 subunit gamma
	UniRef90_P60709	41.7	99.1	24	7	Actin, cytoplasmic 1
	UniRef90_Q13509	49.7	99.0	28	8	Tubulin beta-3 chain
	UniRef90_Q16822	70.8	99.1	18	8	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
	UniRef90_Q8CGK3	95.0	98.7	8	4	Lon protease homolog, mitochondrial
E07	UniRef90_F8VPV9	56.9	99.1	21	6	ATP synthase subunit beta
	UniRef90_G3H584	28.2	99.0	24	5	SPARC
	UniRef90_G3HAE2	7.0	98.2	58	3	ATP synthase subunit alpha, mitochondrial
	UniRef90_G3HC84	47.8	99.1	19	6	Heat shock protein HSP 90-beta
	UniRef90_G3HEZ0	65.8	98.7	11	4	Stress-70 protein, mitochondrial
	UniRef90_G3HJB6	71.8	97.6	6	3	Ras and Rab interactor-like protein
	UniRef90_G3HR08	50.3	99.1	29	8	Cytosol aminopeptidase
	UniRef90_G3I129	48.7	98.9	11	4	Ubiquitin
	UniRef90_G3I834	8.6	94.6	32	3	Cytochrome c oxidase subunit 5A, mitochondrial
	UniRef90_G3IE04	53.4	99.1	19	8	Pre-mRNA-processing factor 19
	UniRef90_G3ILN5	34.4	99.0	19	6	Reticulocalbin-3
	UniRef90_O43175	56.6	98.2	9	3	D-3-phosphoglycerate dehydrogenase

	UniRef90_P02545-2	64.0	99.2	50	23	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	58	24	Vimentin
	UniRef90_P09622	54.1	98.2	9	3	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P0CG83	17.3	98.9	32	4	Polyubiquitin (Fragment)
	UniRef90_P14625	92.6	99.1	17	14	Endoplasmic
	UniRef90_P27797	48.2	99.0	20	5	Calreticulin
	UniRef90_P31040	71.7	99.2	21	10	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P49368	60.6	98.4	11	3	T-complex protein 1 subunit gamma
	UniRef90_P52825	74.5	93.9	6	2	Carnitine O-palmitoyltransferase 2, mitochondrial
	UniRef90_Q13217	57.6	98.8	13	4	DnaJ homolog subfamily C member 3
	UniRef90_Q13509	49.7	98.6	17	3	Tubulin beta-3 chain
	UniRef90_Q14568	38.1	99.0	19	5	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q16822	70.8	98.3	11	3	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
	UniRef90_Q8BMF4	68.0	99.2	26	14	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
	UniRef90_Q9Z0X1	66.8	99.2	23	7	Apoptosis-inducing factor 1, mitochondrial
E08	UniRef90_F8VPV9	56.9	99.2	26	10	ATP synthase subunit beta
	UniRef90_G3GXA9	21.1	98.6	36	4	Protein disulfide-isomerase
	UniRef90_G3H0U6	50.1	99.1	36	13	Protein disulfide-isomerase A3
	UniRef90_G3H697	62.4	98.2	7	3	Glucosidase 2 subunit beta
	UniRef90_G3HC84	47.8	99.0	18	6	Heat shock protein HSP 90-beta
	UniRef90_G3HFAQ	33.2	99.1	26	6	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HKKP3	40.6	99.1	32	9	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HNN3	51.8	98.8	13	4	Clusterin
	UniRef90_G3HR08	50.3	99.2	42	14	Cytosol aminopeptidase
	UniRef90_G3IAS8	185.7	98.3	4	3	Kinesin-like protein KIF27
	UniRef90_G3IE04	53.4	98.2	7	3	Pre-mRNA-processing factor 19
	UniRef90_H0X554	30.1	99.1	38	9	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_Q43175	56.6	98.9	11	5	D-3-phosphoglycerate dehydrogenase
	UniRef90_P02545-2	64.0	99.2	29	11	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.1	18	6	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.1	36	11	Vimentin
	UniRef90_P10809	56.5	99.2	25	11	60 kDa heat shock protein, mitochondrial
	UniRef90_P27797	48.2	99.0	18	6	Calreticulin
	UniRef90_P78371	57.5	99.2	31	13	T-complex protein 1 subunit beta
	UniRef90_Q14568	38.1	99.0	25	5	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q71U36	50.1	97.3	10	2	Tubulin alpha-1A chain
E09	UniRef90_F8VPV9	56.9	99.0	15	5	ATP synthase subunit beta
	UniRef90_G3GVX1	216.7	96.4	2	2	Ribosome-binding protein 1
	UniRef90_G3HC84	47.8	99.1	21	5	Heat shock protein HSP 90-beta
	UniRef90_G3HEZ0	65.8	99.1	20	8	Stress-70 protein, mitochondrial
	UniRef90_G3HFAQ	33.2	98.4	13	3	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HN14	237.6	98.9	3	5	Transitional endoplasmic reticulum ATPase
	UniRef90_G3HQI8	229.3	98.3	2	4	Myosin-Vb
	UniRef90_G3HZH3	32.5	98.9	21	2	Spliceosome RNA helicase BAT1
	UniRef90_G3HZY1	57.2	99.2	25	14	RuvB-like 2
	UniRef90_G3I6E6	32.0	98.7	17	4	Actin, cytoplasmic 2
	UniRef90_P00505	47.4	95.9	6	2	Aspartate aminotransferase, mitochondrial
	UniRef90_P08670	53.7	99.2	38	12	Vimentin
	UniRef90_P10809	56.5	98.3	10	3	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.1	20	16	Endoplasmic
	UniRef90_P51855	52.2	97.5	8	2	Glutathione synthetase
	UniRef90_P60709	41.7	98.9	16	5	Actin, cytoplasmic 1
	UniRef90_Q14568	38.1	99.1	29	8	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q14697	107.0	99.1	14	9	Neutral alpha-glucosidase AB

	UniRef90_Q3V3R1	105.4	99.1	12	7	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q8CGK3	95.0	99.1	19	11	Lon protease homolog, mitochondrial
	UniRef90_Q8VVDL4	53.9	99.0	13	5	ADP-dependent glucokinase
	UniRef90_Q9DB77	48.3	98.7	11	4	Cytochrome b-c1 complex subunit 2, mitochondrial
	UniRef90_Q9GZL7	47.6	97.6	8	3	Ribosome biogenesis protein WDR12
	UniRef90_Q9Y5S9	19.5	98.2	15	3	RNA-binding protein 8A
	UniRef90_Q9Z1N5	32.5	98.9	21	2	Spliceosome RNA helicase Ddx39b
E10	UniRef90_B9A067	79.3	99.0	10	6	Mitochondrial inner membrane protein
	UniRef90_G3H584	28.2	98.8	16	4	SPARC
	UniRef90_G3HEZ0	65.8	99.1	26	14	Stress-70 protein, mitochondrial
	UniRef90_G3HJN3	51.8	98.4	12	4	Clusterin
	UniRef90_G3I6E6	32.0	98.7	17	4	Actin, cytoplasmic 2
	UniRef90_G3ILN5	34.4	98.2	14	3	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.1	24	12	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	51	23	Vimentin
	UniRef90_P14625	92.6	99.2	17	13	Endoplasmic
	UniRef90_P17987	60.3	99.1	23	8	T-complex protein 1 subunit alpha
	UniRef90_P49411	49.6	99.2	35	12	Elongation factor Tu, mitochondrial
	UniRef90_P60709	41.7	98.7	19	4	Actin, cytoplasmic 1
	UniRef90_Q14697	107.0	98.7	9	4	Neutral alpha-glucosidase AB
	UniRef90_Q61112	41.2	97.6	8	3	45 kDa calcium-binding protein
	UniRef90_Q91W90	46.4	99.1	34	12	Thioredoxin domain-containing protein 5
E11	UniRef90_F8VPV9	56.9	98.9	10	5	ATP synthase subunit beta
	UniRef90_G3GVX1	216.7	98.8	6	4	Ribosome-binding protein 1
	UniRef90_G3H0U6	50.1	98.7	13	4	Protein disulfide-isomerase A3
	UniRef90_G3HC84	47.8	98.4	10	3	Heat shock protein HSP 90-beta
	UniRef90_G3HN14	237.6	98.9	4	4	Transitional endoplasmic reticulum ATPase
	UniRef90_G3HR08	50.3	99.1	29	10	Cytosol aminopeptidase
	UniRef90_G3IG44	41.9	96.7	13	3	Tubulin beta-2C chain
	UniRef90_Q43175	56.6	98.1	8	3	D-3-phosphoglycerate dehydrogenase
	UniRef90_P02545-2	64.0	99.2	29	10	Isoform C of Prelamin-A/C
	UniRef90_P04350	49.6	96.7	11	3	Tubulin beta-4A chain
	UniRef90_P08670	53.7	99.2	51	22	Vimentin
	UniRef90_P10809	56.5	99.0	12	5	60 kDa heat shock protein, mitochondrial
	UniRef90_P20108	28.1	96.9	15	2	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P27797	48.2	97.9	9	3	Calreticulin
	UniRef90_P78371	57.5	99.2	25	10	T-complex protein 1 subunit beta
	UniRef90_Q13509	49.7	96.7	14	3	Tubulin beta-3 chain
	UniRef90_Q14568	38.1	97.3	22	3	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q8K3J1	23.1	92.8	14	2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial
E12	UniRef90_F8VPV9	56.9	98.9	15	4	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.1	21	8	Protein disulfide-isomerase A3
	UniRef90_G3HC84	47.8	99.1	21	7	Heat shock protein HSP 90-beta
	UniRef90_G3HFQ4	33.2	99.0	19	5	Dihydrolipoylysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HPK0	56.3	98.2	8	3	Hydroxysteroid dehydrogenase-like protein 2
	UniRef90_G3I6E6	32.0	98.3	13	3	Actin, cytoplasmic 2
	UniRef90_P02545-2	64.0	98.4	8	3	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	46	15	Vimentin
	UniRef90_P10809	56.5	99.0	15	5	60 kDa heat shock protein, mitochondrial
	UniRef90_P31040	71.7	99.1	13	6	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P35232	29.8	99.0	23	6	Prohibitin
	UniRef90_P49411	49.6	98.8	15	5	Elongation factor Tu, mitochondrial
	UniRef90_P55302	41.2	99.1	27	10	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	98.2	8	3	Actin, cytoplasmic 1
	UniRef90_Q14568	38.1	99.0	18	5	Putative heat shock protein HSP 90-alpha A2
	UniRef90_Q7TP98	12.8	96.2	37	3	Interleukin enhancer-binding factor 2

UniRef90_Q9UQ80	43.7	98.5	10	4	Proliferation-associated protein 2G4
UniRef90_Q9Z0X1	66.8	99.0	12	5	Apoptosis-inducing factor 1, mitochondrial

Row F	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
F01	UniRef90_G3HEZ0	65.8	99.1	18	9	Stress-70 protein, mitochondrial
	UniRef90_G3HNG2	49.8	93.9	9	3	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_G3HNG5	50.4	93.9	9	3	Acyl-coenzyme A thioesterase 1
	UniRef90_G3I1P6	33.3	98.5	20	4	Nucleosome-binding protein 1
	UniRef90_G3I6E6	32.0	98.8	23	4	Actin, cytoplasmic 2
	UniRef90_G3ILN5	34.4	99.0	19	6	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.0	18	7	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.2	27	11	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.2	42	13	Vimentin
	UniRef90_P28331	79.8	99.2	27	16	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P31040	71.7	99.1	18	10	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P34897	55.9	98.7	10	4	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_P49411	50.0	99.1	18	7	Elongation factor Tu, mitochondrial
	UniRef90_P60709	41.7	98.8	18	4	Actin, cytoplasmic 1
	UniRef90_Q16576	47.0	99.0	14	7	Histone-binding protein RBBP7
	UniRef90_Q61576	64.7	99.1	19	7	Peptidyl-prolyl cis-trans isomerase FKBP10
	UniRef90_Q9Z0X1	66.8	98.7	12	4	Apoptosis-inducing factor 1, mitochondrial
F02	UniRef90_B9A067	79.3	99.2	28	15	Mitochondrial inner membrane protein
	UniRef90_G3H0U6	50.1	99.2	41	16	Protein disulfide-isomerase A3
	UniRef90_G3I129	48.7	98.3	9	3	Ubiquitin
	UniRef90_P02545-2	64.0	99.2	43	19	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.2	41	14	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.2	53	19	Vimentin
	UniRef90_P0CG83	17.3	98.3	25	3	Polyubiquitin (Fragment)
	UniRef90_P10809	56.5	99.1	19	7	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	14	9	Endoplasmic
	UniRef90_P28331	79.8	98.7	7	4	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P31040	71.7	99.2	28	12	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P34897	55.9	98.9	18	4	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_P55302	41.2	99.1	29	8	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_Q13148	21.8	98.2	20	3	TAR DNA-binding protein 43
	UniRef90_Q16822	70.8	98.9	14	5	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
	UniRef90_Q9Z0X1	66.8	96.1	13	2	Apoptosis-inducing factor 1, mitochondrial
F03	UniRef90_F8VPV9	56.9	99.2	22	10	ATP synthase subunit beta
	UniRef90_G3GT05	22.2	98.1	21	3	T-complex protein 1 subunit theta
	UniRef90_G3HNG2	49.8	98.9	14	3	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_G3HNG5	50.4	98.9	14	3	Acyl-coenzyme A thioesterase 1
	UniRef90_G3HJN3	51.8	94.0	8	2	Clusterin
	UniRef90_G3HR08	50.3	99.1	17	7	Cytosol aminopeptidase
	UniRef90_G3HZY1	57.2	98.7	8	4	RuvB-like 2
	UniRef90_G3I129	48.7	98.3	8	3	Ubiquitin
	UniRef90_G3IAS8	185.7	98.9	4	5	Kinesin-like protein KIF27
	UniRef90_G3IE04	53.4	99.0	12	5	Pre-mRNA-processing factor 19
	UniRef90_O43402	23.5	98.2	13	3	ER membrane protein complex subunit 8
	UniRef90_P08670	53.7	99.2	41	16	Vimentin
	UniRef90_P0CG83	17.3	98.3	22	3	Polyubiquitin (Fragment)
	UniRef90_P10809	56.5	99.1	21	9	60 kDa heat shock protein, mitochondrial
	UniRef90_P20108	28.1	98.9	19	5	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P62873	37.4	99.0	23	6	Guanine nucleotide-binding protein

						G(I)/G(S)/G(T) subunit beta-1
F04	UniRef90_F8VPV9	56.9	99.2	38	14	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.1	48	15	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	98.7	23	4	Protein disulfide-isomerase A6
	UniRef90_G3HJN3	51.8	98.0	10	3	Clusterin
	UniRef90_P02545-2	64.0	99.2	51	21	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.2	40	9	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	61	22	Vimentin
	UniRef90_P14625	92.6	99.2	28	19	Endoplasmic
	UniRef90_P27797	48.2	99.2	32	12	Calreticulin
	UniRef90_P34897	55.9	98.6	8	3	Serine hydroxymethyltransferase, mitochondrial
F05	UniRef90_Q16576	47.0	99.1	13	6	Histone-binding protein RBBP7
	UniRef90_Q71U36	50.1	99.0	20	2	Tubulin alpha-1A chain
	UniRef90_B9A067	79.3	99.1	16	10	Mitochondrial inner membrane protein
	UniRef90_F8VPV9	56.9	99.1	20	6	ATP synthase subunit beta
	UniRef90_G3GTF9	18.9	99.1	59	9	ATP synthase subunit d, mitochondrial
	UniRef90_G3H0U6	50.1	98.9	16	4	Protein disulfide-isomerase A3
	UniRef90_G3HEZ0	65.8	99.1	22	7	Stress-70 protein, mitochondrial
	UniRef90_G3IAS8	185.7	99.1	7	6	Kinesin-like protein KIF27
	UniRef90_P00505	47.4	96.1	11	2	Aspartate aminotransferase, mitochondrial
	UniRef90_P02545-2	64.0	98.8	4	4	Isoform C of Prelamin-A/C
F06	UniRef90_P08670	53.7	99.2	58	22	Vimentin
	UniRef90_P10809	56.5	99.1	24	8	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	23	15	Endoplasmic
	UniRef90_P27797	48.2	98.5	19	3	Calreticulin
	UniRef90_P28331	79.8	99.2	39	21	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P32119	21.8	98.2	17	3	Peroxisomal
	UniRef90_P55302	41.2	99.1	36	11	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_Q14697	107.0	98.9	9	4	Neutral alpha-glucosidase AB
	UniRef90_Q16576	47.0	98.2	6	3	Histone-binding protein RBBP7
	UniRef90_Q3V3R1	105.4	99.1	15	10	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
F07	UniRef90_Q61576	64.7	98.8	18	4	Peptidyl-prolyl cis-trans isomerase FKBP10
	UniRef90_Q99798	85.5	99.1	12	7	Aconitate hydratase, mitochondrial
	UniRef90_F8VPV9	56.9	99.2	29	12	ATP synthase subunit beta
	UniRef90_G1SLD5	111.5	99.2	35	24	Hypoxia up-regulated protein 1
	UniRef90_G3HHK0	35.4	99.0	23	5	28S ribosomal protein S31, mitochondrial
	UniRef90_G3HR08	50.3	99.1	26	7	Cytosol aminopeptidase
	UniRef90_G3I6E6	32.0	99.0	34	5	Actin, cytoplasmic 2
	UniRef90_G3IE04	53.4	99.1	25	7	Pre-mRNA-processing factor 19
	UniRef90_P02545-2	64.0	99.2	43	15	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.1	47	9	Heterogeneous nuclear ribonucleoproteins C1/C2
F08	UniRef90_P08670	53.7	99.2	54	23	Vimentin
	UniRef90_P14625	92.6	99.1	23	12	Endoplasmic
	UniRef90_P31040	71.7	99.2	25	11	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P50213	39.7	99.0	15	5	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P60709	41.7	99.0	30	5	Actin, cytoplasmic 1
	UniRef90_Q13509	49.7	99.0	24	6	Tubulin beta-3 chain
	UniRef90_Q14697	107.0	99.1	18	11	Neutral alpha-glucosidase AB
	UniRef90_Q16822	70.8	99.0	13	4	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
	UniRef90_Q3V3R1	105.4	99.2	18	12	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_B9A067	79.3	99.2	41	25	Mitochondrial inner membrane protein
F09	UniRef90_F8VPV9	56.9	99.2	26	11	ATP synthase subunit beta
	UniRef90_G3ILN5	34.4	99.0	19	6	Reticulocalbin-3
	UniRef90_H0X554	30.1	99.1	30	6	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial
	UniRef90_P02545-2	64.0	99.2	40	15	Isoform C of Prelamin-A/C
	UniRef90_P05091	46.0	99.2	38	13	Aldehyde dehydrogenase, mitochondrial

	UniRef90_P08670	53.7	98.8	14	3	Vimentin
	UniRef90_P09622	54.1	97.4	8	2	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P14625	92.6	99.2	24	17	Endoplasmic
	UniRef90_P34897	55.9	99.0	16	5	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_Q14697	107.0	99.1	15	10	Neutral alpha-glucosidase AB
	UniRef90_Q3V3R1	105.4	99.2	20	13	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
	UniRef90_Q71U36	50.1	99.0	14	3	Tubulin alpha-1A chain
	UniRef90_Q8BKZ9	54.2	99.0	12	5	Pyruvate dehydrogenase protein X component, mitochondrial
	UniRef90_Q8CGK3	95.0	98.7	7	3	Lon protease homolog, mitochondrial
	UniRef90_Q8K0D5	83.3	99.2	17	8	Elongation factor G, mitochondrial
	UniRef90_Q99798	85.5	99.2	19	13	Aconitate hydratase, mitochondrial
	UniRef90_Q9CQN1	80.3	98.7	9	3	Heat shock protein 75 kDa, mitochondrial
	UniRef90_Q9Y265	50.2	99.0	19	6	RuvB-like 1
F08	UniRef90_B9A067	79.3	99.2	33	18	Mitochondrial inner membrane protein
	UniRef90_G3HEZ0	65.8	99.1	29	8	Stress-70 protein, mitochondrial
	UniRef90_G3HF59	25.4	98.3	17	3	DNA-directed RNA polymerases I, II, and III subunit RPABC1
	UniRef90_G3HHK0	35.4	99.1	31	6	28S ribosomal protein S31, mitochondrial
	UniRef90_G3HW13	45.7	95.9	9	2	Torsin-3A
	UniRef90_G3ILN5	34.4	99.0	25	6	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.1	26	8	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.2	34	11	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.2	41	13	Vimentin
	UniRef90_P14625	92.6	99.1	24	12	Endoplasmic
	UniRef90_P28331	79.8	99.2	42	22	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P50213	39.7	98.9	13	4	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P55302	41.2	99.1	32	8	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_Q61576	64.7	99.1	23	8	Peptidyl-prolyl cis-trans isomerase FKBP10
	UniRef90_Q99798	85.5	99.2	25	12	Aconitate hydratase, mitochondrial
F09	UniRef90_F7G3R3	45.2	98.0	11	3	Heterogeneous nuclear ribonucleoprotein H (Fragment)
	UniRef90_F8VPV9	56.9	99.2	28	11	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.1	23	6	Protein disulfide-isomerase A3
	UniRef90_G3HFQ4	33.2	99.0	22	5	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HZY1	57.2	99.2	28	11	RuvB-like 2
	UniRef90_G3ILN5	34.4	98.0	9	3	Reticulocalbin-3
	UniRef90_O43681	38.8	99.0	25	5	ATPase ASNA1
	UniRef90_P02545-2	64.0	99.2	50	23	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.2	45	14	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P07910	33.7	99.1	40	7	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	47	18	Vimentin
	UniRef90_P10809	56.5	99.0	16	6	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	33	25	Endoplasmic
	UniRef90_P28331	79.8	98.4	9	3	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P34897	55.9	99.1	24	8	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_P50213	39.7	99.1	29	10	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_Q16576	47.0	99.2	26	10	Histone-binding protein RBBP7
	UniRef90_Q8BKZ9	54.2	98.4	9	3	Pyruvate dehydrogenase protein X component, mitochondrial
F10	UniRef90_F8VPV9	56.9	98.3	7	3	ATP synthase subunit beta
	UniRef90_G3GTF9	18.9	99.1	40	8	ATP synthase subunit d, mitochondrial
	UniRef90_G3H5Z5	59.0	98.8	13	4	Cleavage stimulation factor 64 kDa subunit
	UniRef90_G3HQJ8	229.3	99.0	5	6	Myosin-Vb
	UniRef90_G3IE04	53.4	99.1	15	7	Pre-mRNA-processing factor 19
	UniRef90_H0X554	30.1	99.2	56	11	NADH dehydrogenase [ubiquinone] iron-sulfur

	UniRef90_O43175	56.6	99.1	16	8	protein 3, mitochondrial
	UniRef90_P00505	47.4	98.5	9	3	D-3-phosphoglycerate dehydrogenase
	UniRef90_P02545-2	64.0	99.2	52	26	Aspartate aminotransferase, mitochondrial
	UniRef90_P11021	72.4	98.3	7	3	Isoform C of Prelamin-A/C
	UniRef90_P20108	28.1	99.1	24	6	78 kDa glucose-regulated protein
						Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P28331	79.8	99.2	38	18	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P31040	71.7	98.7	12	4	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P55302	41.2	99.1	28	8	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	99.0	21	5	Actin, cytoplasmic 1
	UniRef90_Q61576	64.7	98.5	15	3	Peptidyl-prolyl cis-trans isomerase FKBP10
	UniRef90_Q91W90	46.4	99.1	34	8	Thioredoxin domain-containing protein 5
	UniRef90_Q9DB77	48.3	98.4	16	3	Cytochrome b-c1 complex subunit 2, mitochondrial
F11	UniRef90_B9A067	79.3	99.2	23	10	Mitochondrial inner membrane protein
	UniRef90_G3GTF9	18.9	98.9	48	6	ATP synthase subunit d, mitochondrial
	UniRef90_P02545-2	64.0	99.1	16	7	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.2	43	14	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.2	31	8	Vimentin
	UniRef90_P32119	21.8	98.1	18	3	Peroxisoredoxin-2
	UniRef90_P49411	49.6	99.1	31	7	Elongation factor Tu, mitochondrial
	UniRef90_P50213	39.7	99.2	29	10	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P62873	37.4	99.1	33	6	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
	UniRef90_Q8K0D5	83.3	98.3	7	3	Elongation factor G, mitochondrial
F12	UniRef90_Q99798	85.5	99.1	22	13	Aconitate hydratase, mitochondrial
	UniRef90_G3GRH9	39.4	98.1	9	3	Tropomodulin-3
	UniRef90_G3HNG2	49.8	99.2	27	8	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_G3ILN5	34.4	99.0	19	6	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.2	46	22	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.2	36	6	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	36	14	Vimentin
	UniRef90_P14625	92.6	99.2	22	12	Endoplasmic
	UniRef90_P31040	71.7	99.1	18	7	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P55302	41.2	99.1	21	6	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	99.0	32	5	Actin, cytoplasmic 1
	UniRef90_Q16576	47.0	98.6	9	3	Histone-binding protein RBBP7
	UniRef90_Q91W90	46.4	99.1	32	6	Thioredoxin domain-containing protein 5
	UniRef90_Q9Z0X1	66.8	99.0	13	5	Apoptosis-inducing factor 1, mitochondrial

Row G	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
G01	UniRef90_A6QR15	30.7	99.1	34	7	Tropomyosin beta chain
	UniRef90_G3HB04	28.4	99.0	35	5	Protein disulfide-isomerase A6
	UniRef90_G3HKP3	40.6	99.1	31	10	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_P02545-2	64.0	99.2	51	24	Isoform C of Prelamin-A/C
	UniRef90_P06733	46.7	99.1	35	10	Alpha-enolase
	UniRef90_P07910	33.7	99.2	46	8	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P55302	41.2	99.0	29	5	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	99.2	51	9	Actin, cytoplasmic 1
	UniRef90_P81117	50.3	98.3	11	3	Nucleobindin-2
	UniRef90_P8VPV9	56.9	98.4	10	3	ATP synthase subunit beta

	UniRef90_G3H0U6	50.1	99.2	52	19	Protein disulfide-isomerase A3
	UniRef90_G3H1B3	55.0	99.2	46	14	Lamin-L(I)
	UniRef90_G3HB04	28.4	99.1	33	6	Protein disulfide-isomerase A6
	UniRef90_G3HFQ4	33.2	99.1	26	7	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HKP3	40.6	99.1	20	5	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HNG2	49.8	98.3	10	3	Acyl-coenzyme A thioesterase 2, mitochondrial
	UniRef90_P02545-2	64.0	99.2	53	21	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	67	26	Vimentin
	UniRef90_P11021	72.4	94.5	7	2	78 kDa glucose-regulated protein
	UniRef90_P49411	49.6	99.1	28	9	Elongation factor Tu, mitochondrial
	UniRef90_P81117	50.3	94.9	10	2	Nucleobindin-2
	UniRef90_Q16576	47.0	98.9	12	5	Histone-binding protein RBBP7
	UniRef90_Q9Z1N5	48.7	97.8	8	3	Spliceosome RNA helicase Ddx39b
G03	UniRef90_A6QR15	30.7	99.1	43	8	Tropomyosin beta chain
	UniRef90_F8VPV9	56.9	98.8	13	4	ATP synthase subunit beta
	UniRef90_G3H4E2	32.0	98.2	14	3	tRNA modification GTPase GTPBP3, mitochondrial
	UniRef90_G3HE28	40.1	98.2	8	3	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HFQ4	33.2	98.3	13	3	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_P02545-2	64.0	99.2	60	30	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.1	30	8	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.2	58	21	Vimentin
	UniRef90_P14625	92.6	99.1	20	11	Endoplasmic
	UniRef90_P50213	39.7	99.2	23	9	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P60709	41.7	99.1	34	3	Actin, cytoplasmic 1
	UniRef90_P62873	37.4	99.1	27	7	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
G04	UniRef90_G3H0U6	50.1	99.2	42	16	Protein disulfide-isomerase A3
	UniRef90_G3H1B3	55.0	98.8	10	3	Lamin-L(I)
	UniRef90_G3HEZ0	65.8	99.2	55	26	Stress-70 protein, mitochondrial
	UniRef90_G3HFQ4	33.2	99.1	26	7	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HKP3	40.6	99.2	31	12	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HR08	50.3	96.6	9	2	Cytosol aminopeptidase
	UniRef90_G3HSM8	67.7	99.2	52	25	Lamin-B2
	UniRef90_G3HZY1	57.2	98.3	11	3	RuvB-like 2
	UniRef90_G3IDL7	22.7	99.0	33	2	Heat shock cognate 71 kDa protein
	UniRef90_O95302	63.0	99.1	20	9	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P02545-2	64.0	99.2	60	28	Isoform C of Prelamin-A/C
	UniRef90_P02794	21.5	99.1	39	6	Ferritin heavy chain
	UniRef90_P05091	45.9	99.2	41	13	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P06733	46.7	99.2	41	10	Alpha-enolase
	UniRef90_P07910	33.7	99.2	46	14	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08003	72.6	99.2	37	14	Protein disulfide-isomerase A4
	UniRef90_P09622	54.1	98.2	9	3	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P11021	72.4	99.2	43	21	78 kDa glucose-regulated protein
	UniRef90_P28331	79.8	99.1	14	7	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P34897	55.9	99.0	11	6	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_P50213	39.7	99.2	30	10	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P78371	57.5	98.4	18	3	T-complex protein 1 subunit beta
	UniRef90_Q9CQN1	80.3	99.2	28	14	Heat shock protein 75 kDa, mitochondrial
G05	UniRef90_G3GS40	38.8	99.0	28	5	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial
	UniRef90_G3H1B3	55.0	99.2	43	17	Lamin-L(I)

	UniRef90_G3H5Z5	59.0	91.5	8	2	Cleavage stimulation factor 64 kDa subunit
	UniRef90_G3HEZ0	65.8	99.2	38	18	Stress-70 protein, mitochondrial
	UniRef90_G3HSM8	67.7	99.2	46	20	Lamin-B2
	UniRef90_G3I1Y9	27.4	98.8	27	4	Sulfated glycoprotein 1
	UniRef90_O75531	10.1	98.1	43	3	Barrier-to-autointegration factor
	UniRef90_O95302	63.0	99.1	17	7	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P02545-2	64.0	99.2	48	19	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	92.4	16	2	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08003	72.6	99.2	30	11	Protein disulfide-isomerase A4
	UniRef90_P08670	53.7	99.2	64	26	Vimentin
	UniRef90_P10809	56.5	99.0	15	5	60 kDa heat shock protein, mitochondrial
	UniRef90_P11021	72.4	99.2	24	8	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	31	25	Endoplasmic
	UniRef90_P28331	79.8	98.3	8	3	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial
	UniRef90_P54652	69.7	99.0	12	3	Heat shock-related 70 kDa protein 2
	UniRef90_Q12874	58.8	98.8	13	4	Splicing factor 3A subunit 3
	UniRef90_Q71U36	50.1	98.1	15	3	Tubulin alpha-1A chain
G06	UniRef90_A6QR15	30.7	99.1	30	7	Tropomyosin beta chain
	UniRef90_G3GS40	38.8	99.2	53	15	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial
	UniRef90_G3HEZ0	65.8	99.2	38	18	Stress-70 protein, mitochondrial
	UniRef90_G3HKP3	40.6	99.2	44	14	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HSM8	67.7	99.1	20	5	Lamin-B2
	UniRef90_G3ILN5	34.4	99.1	25	7	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.2	70	36	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.1	39	9	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.0	20	3	Vimentin
	UniRef90_P09622	54.1	98.7	13	4	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P11021	72.4	99.2	26	8	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	28	22	Endoplasmic
	UniRef90_P34897	55.9	99.0	18	5	Serine hydroxymethyltransferase, mitochondrial
	UniRef90_Q91W90	46.4	99.1	51	16	Thioredoxin domain-containing protein 5
	UniRef90_Q9Y265	50.2	98.4	10	3	RuvB-like 1
G07	UniRef90_F8VPV9	56.9	99.2	38	13	ATP synthase subunit beta
	UniRef90_G3HEZ0	65.8	99.2	36	15	Stress-70 protein, mitochondrial
	UniRef90_G3HGS2	96.2	92.7	5	2	Propionyl-CoA carboxylase alpha chain, mitochondrial
	UniRef90_G3HZY1	57.2	98.6	8	4	RuvB-like 2
	UniRef90_G3ILN5	34.4	98.9	21	6	Reticulocalbin-3
	UniRef90_P02545-2	64.0	99.0	13	5	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.2	32	8	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P07910	33.7	99.2	41	13	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	42	12	Vimentin
	UniRef90_P09382	14.8	99.0	47	6	Galectin-1
	UniRef90_P14625	92.6	99.2	35	27	Endoplasmic
	UniRef90_P20108	28.1	99.1	31	7	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P55302	41.2	99.1	30	7	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	99.2	35	3	Actin, cytoplasmic 1
	UniRef90_Q13509	49.7	98.2	8	3	Tubulin beta-3 chain
	UniRef90_Q16576	47.0	99.2	32	11	Histone-binding protein RBBP7
	UniRef90_Q9CQN1	80.3	99.2	32	15	Heat shock protein 75 kDa, mitochondrial
G08	UniRef90_F8VPV9	56.9	99.0	12	5	ATP synthase subunit beta
	UniRef90_G3HFQ4	33.2	99.1	26	7	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HKP3	40.6	99.1	22	8	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HZY1	57.2	99.2	29	11	RuvB-like 2
	UniRef90_G3IBF1	31.1	98.8	33	4	Nucleophosmin
	UniRef90_P00505	47.4	98.6	12	3	Aspartate aminotransferase, mitochondrial

	UniRef90_P02794	21.5	99.1	43	7	Ferritin heavy chain
	UniRef90_P07910	33.7	99.2	46	9	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	62	24	Vimentin
	UniRef90_P14625	92.6	99.2	37	29	Endoplasmic
	UniRef90_P55302	41.2	99.1	46	14	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_Q3V3R1	105.4	98.1	9	3	Monofunctional C1-tetrahydrofolate synthase, mitochondrial
G09	UniRef90_F8VPV9	56.9	99.2	43	16	ATP synthase subunit beta
	UniRef90_G1SLD5	111.5	99.1	15	8	Hypoxia up-regulated protein 1
	UniRef90_G3H1B3	55.0	98.2	9	2	Lamin-L(I)
	UniRef90_G3HNV4	16.4	98.3	30	3	Triosephosphate isomerase
	UniRef90_G3HZY1	57.2	98.9	11	5	RuvB-like 2
	UniRef90_G3I6E6	32.0	98.8	19	4	Actin, cytoplasmic 2
	UniRef90_P02545-2	64.0	98.2	7	2	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.1	26	8	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	98.5	13	2	Vimentin
	UniRef90_P14625	92.6	99.1	16	8	Endoplasmic
	UniRef90_P20108	28.1	99.1	36	7	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_P50213	39.7	99.1	39	11	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P55302	41.2	99.1	33	8	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	98.8	14	4	Actin, cytoplasmic 1
G10	UniRef90_Q71U36	50.1	99.0	17	2	Tubulin alpha-1A chain
	UniRef90_Q91W90	46.4	99.2	38	14	Thioredoxin domain-containing protein 5
	UniRef90_F8VPV9	56.9	99.1	20	9	ATP synthase subunit beta
	UniRef90_G3H1B3	55.0	99.0	12	4	Lamin-L(I)
	UniRef90_G3HB04	28.4	98.8	21	4	Protein disulfide-isomerase A6
	UniRef90_G3HE28	40.1	98.1	8	2	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HEZ0	65.8	99.1	21	6	Stress-70 protein, mitochondrial
	UniRef90_G3HFQ4	33.2	98.2	13	3	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HKP3	40.6	99.1	29	8	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HRG8	29.7	98.1	19	3	Endoplasmic reticulum resident protein ERp44 (Fragment)
	UniRef90_G3HSM8	67.7	99.2	46	21	Lamin-B2
	UniRef90_G3I6E6	32.0	98.7	17	4	Actin, cytoplasmic 2
	UniRef90_O95302	63.0	99.0	12	6	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P00505	47.4	98.9	16	4	Aspartate aminotransferase, mitochondrial
	UniRef90_P02545-2	64.0	99.2	54	23	Isoform C of Prelamin-A/C
	UniRef90_P06733	46.7	99.2	25	9	Alpha-enolase
	UniRef90_P07910	33.7	99.2	49	9	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08003	72.6	98.5	12	4	Protein disulfide-isomerase A4
	UniRef90_P08670	53.7	99.2	36	10	Vimentin
	UniRef90_P11021	72.4	99.2	24	12	78 kDa glucose-regulated protein
	UniRef90_P50213	39.7	97.2	9	3	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P55302	41.2	99.1	39	11	Alpha-2-macroglobulin receptor-associated protein
	UniRef90_P60709	41.7	98.7	13	4	Actin, cytoplasmic 1
	UniRef90_Q16576	47.0	99.1	9	9	Histone-binding protein RBBP7
	UniRef90_Q8VDL4	53.9	91.1	8	2	ADP-dependent glucokinase
	UniRef90_Q91W90	46.4	98.1	8	3	Thioredoxin domain-containing protein 5
	UniRef90_Q9Z1N5	48.7	96.6	8	2	Spliceosome RNA helicase Ddx39b
G11	UniRef90_B9A067	79.3	99.1	11	8	Mitochondrial inner membrane protein
	UniRef90_G3H1B3	55.0	98.3	10	2	Lamin-L(I)
	UniRef90_G3HB04	28.4	99.0	29	5	Protein disulfide-isomerase A6
	UniRef90_G3HE28	40.1	98.7	15	3	Mitochondrial-processing peptidase subunit beta
	UniRef90_G3HEZ0	65.8	99.2	32	14	Stress-70 protein, mitochondrial
	UniRef90_G3HFQ4	33.2	98.8	19	4	Dihydrolipoyllysine-residue succinyltransferase

	UniRef90_G3HKP3	40.6	99.1	29	10	component of 2-oxoglutarate dehydrogenase complex, mitochondrial
	UniRef90_G3HZY1	57.2	99.1	19	8	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_P02545-2	64.0	99.2	52	29	RuvB-like 2
	UniRef90_P06733	46.7	98.7	14	4	Isoform C of Prelamin-A/C
	UniRef90_P07910	33.7	99.1	48	9	Alpha-enolase
	UniRef90_P08670	53.7	99.2	59	22	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P11021	72.4	99.2	23	11	Vimentin
	UniRef90_P20108	28.1	99.0	24	6	78 kDa glucose-regulated protein
	UniRef90_Q71U36	50.1	99.0	16	2	Thioredoxin-dependent peroxide reductase, mitochondrial
	UniRef90_Q9CQN1	80.3	98.8	9	4	Tubulin alpha-1A chain
G12	UniRef90_B9A067	79.3	99.2	22	14	Heat shock protein 75 kDa, mitochondrial
	UniRef90_F8VPV9	56.9	99.2	42	15	Mitochondrial inner membrane protein
	UniRef90_G3HB04	28.4	98.3	16	3	ATP synthase subunit beta
	UniRef90_G3HKP3	40.6	99.1	31	11	Protein disulfide-isomerase A6
	UniRef90_G3HPK0	56.3	98.2	10	3	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3HSM8	67.7	98.8	13	3	Hydroxysteroid dehydrogenase-like protein 2
	UniRef90_P02545-2	64.0	99.1	26	9	Lamin-B2
	UniRef90_P06733	46.7	98.6	21	3	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	27	10	Alpha-enolase
	UniRef90_P09382	14.8	99.1	59	6	Vimentin
	UniRef90_P11021	72.4	99.2	21	10	Galectin-1
	UniRef90_P31040	71.7	99.1	21	7	78 kDa glucose-regulated protein
	UniRef90_P49411	49.6	99.2	29	9	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
	UniRef90_P62873	37.4	99.1	26	4	Elongation factor Tu, mitochondrial
	UniRef90_Q16576	47.0	99.2	22	8	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1
	UniRef90_Q16822	70.8	98.7	10	4	Histone-binding protein RBBP7
	UniRef90_Q99798	85.5	98.4	9	4	Phosphoenolpyruvate carboxykinase [GTP], mitochondrial
						Aconitate hydratase, mitochondrial

Row H	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
H01	UniRef90_F8VPV9	56.9	99.2	39	16	ATP synthase subunit beta
	UniRef90_G3GXA9	21.1	99.1	49	10	Protein disulfide-isomerase
	UniRef90_G3H0U6	50.1	99.2	44	14	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	99.1	35	5	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	38	15	Stress-70 protein, mitochondrial
	UniRef90_G3HSM8	67.7	98.5	17	3	Lamin-B2
	UniRef90_G3IDL7	22.7	99.1	41	2	Heat shock cognate 71 kDa protein
	UniRef90_P08670	53.7	99.0	15	5	Vimentin
	UniRef90_P11021	72.4	99.2	51	24	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	29	22	Endoplasmic reticulum chaperone
	UniRef90_P43304	80.7	98.6	8	4	Glycerol-3-phosphate dehydrogenase, mitochondrial
	UniRef90_P60709	41.7	99.2	38	9	Actin, cytoplasmic 1
	UniRef90_P81117	50.3	93.4	8	2	Nucleobindin-2
	UniRef90_Q9CQN1	80.3	98.4	7	3	Heat shock protein 75 kDa, mitochondrial
H02	UniRef90_F8VPV9	56.9	99.2	34	13	ATP synthase subunit beta
	UniRef90_G3GXA9	21.1	99.1	42	7	Protein disulfide-isomerase
	UniRef90_G3H0U6	50.1	99.1	34	14	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	98.2	18	3	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	54	29	Stress-70 protein, mitochondrial
	UniRef90_G3HKP3	40.6	99.1	22	8	Cytochrome b-c1 complex subunit 1, mitochondrial
	UniRef90_G3IDL7	22.7	99.0	34	3	Heat shock cognate 71 kDa protein

	UniRef90_P02545-2	64.0	99.2	66	37	Isoform C of Prelamin-A/C
	UniRef90_P06733	46.7	98.9	17	4	Alpha-enolase
	UniRef90_P08670	53.7	99.2	62	27	Vimentin
	UniRef90_P10809	56.5	99.1	30	13	60 kDa heat shock protein, mitochondrial
	UniRef90_P50213	39.7	99.2	28	10	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P54652	69.7	99.0	13	2	Heat shock-related 70 kDa protein 2
	UniRef90_P60709	41.7	99.2	58	6	Actin, cytoplasmic 1
	UniRef90_Q16576	47.0	99.0	17	6	Histone-binding protein RBBP7
	UniRef90_Q9CQN1	80.3	99.1	17	6	Heat shock protein 75 kDa, mitochondrial
H03	UniRef90_F8VPV9	56.9	99.2	46	16	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.1	37	15	Protein disulfide-isomerase A3
	UniRef90_G3H1B3	55.0	98.8	16	4	Lamin-L(I)
	UniRef90_G3H697	62.4	98.8	13	4	Glucosidase 2 subunit beta
	UniRef90_G3HB04	28.4	99.2	46	8	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	60	34	Stress-70 protein, mitochondrial
	UniRef90_G3IDL7	22.7	99.2	50	4	Heat shock cognate 71 kDa protein
	UniRef90_G3IGQ3	23.1	98.8	27	2	Heat shock cognate 71 kDa protein
	UniRef90_P08670	53.7	99.2	38	15	Vimentin
	UniRef90_P10809	56.5	99.2	49	21	60 kDa heat shock protein, mitochondrial
	UniRef90_P60709	41.7	99.1	29	8	Actin, cytoplasmic 1
	UniRef90_Q12874	58.8	98.4	14	3	Splicing factor 3A subunit 3
	UniRef90_Q13509	49.7	99.1	24	6	Tubulin beta-3 chain
	UniRef90_Q16576	47.0	99.1	22	9	Histone-binding protein RBBP7
	UniRef90_Q71U36	50.1	98.8	14	2	Tubulin alpha-1A chain
H04	UniRef90_F8VPV9	56.9	99.2	44	16	ATP synthase subunit beta
	UniRef90_G3GS40	38.8	96.4	12	2	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial
	UniRef90_G3H697	62.4	99.1	16	8	Glucosidase 2 subunit beta
	UniRef90_G3HB04	28.4	99.1	35	7	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	61	32	Stress-70 protein, mitochondrial
	UniRef90_G3I2N0	42.5	98.2	15	3	Inhibitor of nuclear factor kappa-B kinase-interacting protein
	UniRef90_Q95302	63.0	95.5	7	2	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P07910	33.7	98.4	17	3	Heterogeneous nuclear ribonucleoproteins C1/C2
	UniRef90_P08670	53.7	99.2	68	29	Vimentin
	UniRef90_P11021	72.4	99.2	56	28	78 kDa glucose-regulated protein
	UniRef90_P35232	29.8	99.2	59	15	Prohibitin
	UniRef90_P50213	39.7	99.2	32	10	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial
	UniRef90_P54652	69.7	99.1	14	2	Heat shock-related 70 kDa protein 2
	UniRef90_P60709	41.7	99.0	25	5	Actin, cytoplasmic 1
	UniRef90_Q13509	49.7	98.2	10	3	Tubulin beta-3 chain
H05	UniRef90_F8VPV9	56.9	98.8	13	4	ATP synthase subunit beta
	UniRef90_G3H1B3	55.0	99.2	30	9	Lamin-L(I)
	UniRef90_G3HB04	28.4	99.1	33	5	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	54	28	Stress-70 protein, mitochondrial
	UniRef90_G3HRG8	29.7	98.6	33	3	Endoplasmic reticulum resident protein ERp44 (Fragment)
	UniRef90_Q95302	63.0	99.1	25	10	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P02545-2	64.0	99.2	58	26	Isoform C of Prelamin-A/C
	UniRef90_P05091	45.9	99.1	23	7	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.2	64	27	Vimentin
	UniRef90_P09622	54.1	99.0	14	5	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P10809	56.5	99.2	42	17	60 kDa heat shock protein, mitochondrial
	UniRef90_P11021	72.4	99.2	54	30	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	37	30	Endoplasmic
	UniRef90_Q12874	58.8	98.5	8	3	Splicing factor 3A subunit 3
	UniRef90_Q71U36	50.1	98.3	16	3	Tubulin alpha-1A chain
H06	UniRef90_B9W7J9	11.4	98.3	39	3	Histone H4
	UniRef90_G3HB04	28.4	99.1	35	7	Protein disulfide-isomerase A6
	UniRef90_G3HDS1	14.9	98.3	30	3	Histone H4
	UniRef90_G3HPV6	28.5	98.3	15	3	Histone H2A type 1
	UniRef90_G3HPV7	21.8	98.3	20	3	Histone H4

	UniRef90_G3I1Y9	27.4	99.2	49	8	Sulfated glycoprotein 1
	UniRef90_G3IBF1	31.1	99.0	30	4	Nucleophosmin
	UniRef90_P02545-2	64.0	99.2	67	37	Isoform C of Prelamin-A/C
	UniRef90_P05091	46.0	99.2	43	11	Aldehyde dehydrogenase, mitochondrial
	UniRef90_P08670	53.7	99.0	20	4	Vimentin
	UniRef90_P09382	14.8	99.0	36	5	Galectin-1
	UniRef90_P09622	54.1	99.1	22	7	Dihydrolipoyl dehydrogenase, mitochondrial
	UniRef90_P10809	56.5	99.2	31	12	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	38	28	Endoplasmic
	UniRef90_P27797	48.2	99.2	48	16	Calreticulin
	UniRef90_P35232	29.8	99.2	59	14	Prohibitin
	UniRef90_P48643	59.5	98.2	9	3	T-complex protein 1 subunit epsilon
	UniRef90_P60709	41.7	99.2	55	5	Actin, cytoplasmic 1
	UniRef90_P62736	49.5	99.1	31	2	Actin, aortic smooth muscle
	UniRef90_P62805	10.8	98.3	41	3	Histone H4
	UniRef90_P63267	81.6	99.1	17	2	Actin, gamma-enteric smooth muscle
	UniRef90_P68032	42.0	99.2	39	2	Actin, alpha cardiac muscle 1
	UniRef90_Q9CQN1	80.3	99.0	15	5	Heat shock protein 75 kDa, mitochondrial
H07	UniRef90_F8VPV9	56.9	99.2	37	13	ATP synthase subunit beta
	UniRef90_G3GS40	38.8	99.1	26	7	Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial
	UniRef90_G3GXA9	21.1	99.1	53	11	Protein disulfide-isomerase
	UniRef90_G3H1B3	55.0	99.2	38	16	Lamin-L(I)
	UniRef90_G3HB04	28.4	99.2	40	7	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	98.9	9	5	Stress-70 protein, mitochondrial
	UniRef90_G3I1Y9	27.4	98.8	28	4	Sulfated glycoprotein 1
	UniRef90_G3I2Q7	58.0	96.7	4	2	Transcription intermediary factor 1-beta
	UniRef90_P08670	53.7	99.1	40	8	Vimentin
	UniRef90_P10809	56.5	99.2	31	13	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	53.7	99.1	40	8	Endoplasmic
	UniRef90_P17980	29.5	98.3	14	3	26S protease regulatory subunit 6A
	UniRef90_P48643	59.5	99.1	16	8	T-complex protein 1 subunit epsilon
	UniRef90_P54652	69.7	98.8	8	4	Heat shock-related 70 kDa protein 2
	UniRef90_P60709	41.7	99.2	52	9	Actin, cytoplasmic 1
H08	UniRef90_F8VPV9	56.9	99.2	44	17	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.1	40	17	Protein disulfide-isomerase A3
	UniRef90_G3H1B3	55.0	99.2	42	16	Lamin-L(I)
	UniRef90_G3HB04	28.4	99.0	33	5	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	53	25	Stress-70 protein, mitochondrial
	UniRef90_G3I1Y9	27.4	94.9	23	3	Sulfated glycoprotein 1
	UniRef90_G3IDL7	22.7	99.1	45	3	Heat shock cognate 71 kDa protein
	UniRef90_G3IGQ3	23.1	98.8	31	2	Heat shock cognate 71 kDa protein
	UniRef90_O95302	63.0	98.8	9	5	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P08003	72.6	98.7	7	4	Protein disulfide-isomerase A4
	UniRef90_P08670	53.7	99.2	67	30	Vimentin
	UniRef90_P09382	14.8	98.9	36	6	Galectin-1
	UniRef90_P10809	56.5	99.2	51	22	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.2	30	22	Endoplasmic
	UniRef90_Q13509	49.7	98.1	13	3	Tubulin beta-3 chain
	UniRef90_Q71U36	50.1	98.2	9	3	Tubulin alpha-1A chain
H09	UniRef90_F5H6R6	29.3	98.2	15	3	Heterogeneous nuclear ribonucleoprotein D0
	UniRef90_F8VPV9	56.9	99.2	35	13	ATP synthase subunit beta
	UniRef90_G3GXA9	21.1	99.1	48	9	Protein disulfide-isomerase
	UniRef90_G3HB04	28.4	99.2	33	6	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	55	21	Stress-70 protein, mitochondrial
	UniRef90_G3IDC2	26.1	97.2	20	2	Heat shock cognate 71 kDa protein
	UniRef90_G3IDL7	22.7	99.1	50	4	Heat shock cognate 71 kDa protein
	UniRef90_P02545-2	64.0	99.2	57	29	Isoform C of Prelamin-A/C
	UniRef90_P08670	53.7	99.2	62	24	Vimentin
	UniRef90_P10809	56.5	99.2	51	22	60 kDa heat shock protein, mitochondrial
	UniRef90_P11021	72.4	99.2	25	11	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	17	11	Endoplasmic
	UniRef90_P27797	48.2	96.9	9	2	Calreticulin
	UniRef90_P60709	41.7	99.2	44	12	Actin, cytoplasmic 1

	UniRef90_P81117	50.3	96.7	6	2	Nucleobindin-2
	UniRef90_Q71U36	50.1	96.8	9	2	Tubulin alpha-1A chain
	UniRef90_Q8BMF4	68.0	99.1	19	7	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
	UniRef90_Q9CQN1	80.3	99.1	11	5	Heat shock protein 75 kDa, mitochondrial
H10	UniRef90_F8VPV9	56.9	99.2	45	15	ATP synthase subunit beta
	UniRef90_G3H0U6	50.1	99.2	35	14	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	99.1	37	6	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	28	10	Stress-70 protein, mitochondrial
	UniRef90_G3I2N0	42.5	98.6	18	3	Inhibitor of nuclear factor kappa-B kinase-interacting protein
	UniRef90_G3IBF1	31.1	98.7	23	3	Nucleophosmin
	UniRef90_P08670	53.7	99.2	62	26	Vimentin
	UniRef90_P10809	56.5	99.2	54	19	60 kDa heat shock protein, mitochondrial
	UniRef90_P14625	92.6	99.1	22	13	Endoplasmic reticulum chaperone
	UniRef90_P35232	29.8	99.1	35	10	Prohibitin
	UniRef90_P60709	41.7	99.2	34	11	Actin, cytoplasmic 1
	UniRef90_Q13509	49.7	98.4	16	3	Tubulin beta-3 chain
	UniRef90_Q16576	47.0	98.3	12	3	Histone-binding protein RBBP7
	UniRef90_Q8BMF4	68.0	99.2	25	10	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial
H11	UniRef90_F8VPV9	56.9	99.1	14	5	ATP synthase subunit beta
	UniRef90_G3GXA9	21.1	99.1	41	7	Protein disulfide-isomerase
	UniRef90_G3H1B3	55.0	99.1	23	9	Lamin-L(I)
	UniRef90_G3HEZ0	65.8	99.2	58	29	Stress-70 protein, mitochondrial
	UniRef90_G3IDL7	22.7	99.1	45	4	Heat shock cognate 71 kDa protein
	UniRef90_O95302	63.0	99.0	17	6	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P02545-2	64.0	99.2	57	22	Isoform C of Prelamin-A/C
	UniRef90_P08003	72.6	96.0	8	2	Protein disulfide-isomerase A4
	UniRef90_P08670	53.7	99.2	26	9	Vimentin
	UniRef90_P10809	56.5	99.2	42	17	60 kDa heat shock protein, mitochondrial
	UniRef90_P11021	72.4	98.7	9	2	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	42	32	Endoplasmic reticulum chaperone
	UniRef90_P35232	29.8	99.2	51	11	Prohibitin
	UniRef90_P60709	41.7	99.2	38	7	Actin, cytoplasmic 1
	UniRef90_Q12874	58.8	92.8	10	2	Splicing factor 3A subunit 3
	UniRef90_Q9CQN1	80.3	99.0	12	4	Heat shock protein 75 kDa, mitochondrial
H12	UniRef90_F8VPV9	56.9	99.2	45	16	ATP synthase subunit beta
	UniRef90_G3GXA9	21.1	99.1	49	8	Protein disulfide-isomerase
	UniRef90_G3H0U6	50.1	99.1	37	15	Protein disulfide-isomerase A3
	UniRef90_G3HB04	28.4	98.3	20	3	Protein disulfide-isomerase A6
	UniRef90_G3HEZ0	65.8	99.2	52	19	Stress-70 protein, mitochondrial
	UniRef90_G3HSM8	67.7	99.0	19	4	Lamin-B2
	UniRef90_G3IDL7	22.7	98.9	28	2	Heat shock cognate 71 kDa protein
	UniRef90_O95302	63.0	96.1	9	2	Peptidyl-prolyl cis-trans isomerase FKBP9
	UniRef90_P08670	53.7	99.2	22	7	Vimentin
	UniRef90_P10809	56.5	99.0	12	4	60 kDa heat shock protein, mitochondrial
	UniRef90_P11021	72.4	99.2	33	14	78 kDa glucose-regulated protein
	UniRef90_P14625	92.6	99.2	31	22	Endoplasmic reticulum chaperone
	UniRef90_P27797	48.2	99.1	26	12	Calreticulin
	UniRef90_P60709	41.7	99.2	55	12	Actin, cytoplasmic 1
	UniRef90_Q8BMF4	68.0	99.1	16	7	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial

*MW values were calculated from their amino acid sequence, derived from the accession no., using the ExPASy Compute pI/MW tool [103].

Table B 3. Identified MG1655 Proteins (Multiplex). Each table denotes a specific row of a 96-well plate with identifications shown in each well.

Row A	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
A01	UniRef90_A7MQX5	41.4	99.0	16	6	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_A7ZPW1	15.5	82.8	18	2	Nucleoside diphosphate kinase
	UniRef90_A7ZJD2	41.4	82.8	5	2	Galactokinase
	UniRef90_P00946	42.8	82.9	14	2	Mannose-6-phosphate isomerase
	UniRef90_B5F6U1	49.9	98.0	7	3	Argininosuccinate synthase
	UniRef90_A1AFA2	24.3	78.7	7	2	Ribose-5-phosphate isomerase A
	UniRef90_A7ZR34	41.4	73.0	5	2	Phosphoglycerate kinase
A02	UniRef90_A7ZHR7	90.6	99.1	13	11	Outer membrane protein assembly factor BamA
	UniRef90_A8ACN8	55.2	99.1	16	8	ATP synthase subunit alpha
	UniRef90_A8ALC4	29.9	98.7	19	4	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_P23847	60.3	98.7	8	4	Periplasmic dipeptide transport protein
	UniRef90_A8AIH6	39.9	98.2	9	3	Phosphoserine aminotransferase
	UniRef90_A8ACZ6	50.2	82.8	5	2	Xaa-Pro dipeptidase
	UniRef90_P09832	52.0	82.7	5	2	Glutamate synthase [NADPH] small chain
	UniRef90_B7NUD2	23.3	82.2	18	2	Peptide methionine sulfoxide reductase MsrA
	UniRef90_P08200	45.8	82.8	6	2	Isocitrate dehydrogenase [NADP]
	UniRef90_A7ZPU1	22.5	59.2	4	2	Uracil phosphoribosyltransferase
A03	UniRef90_P0A797	34.8	99.0	16	6	6-phosphofructokinase isozyme 1
	UniRef90_P75960	26.7	98.7	21	4	NAD-dependent protein deacylase
	UniRef90_C4K4F8	43.3	98.2	8	3	Elongation factor Tu
	UniRef90_P22731	26.3	83.0	8	2	High-affinity branched-chain amino acid transport ATP-binding protein LivF
	UniRef90_A7MQE1	97.3	85.7	4	2	Translation initiation factor IF-2
	UniRef90_P75792	30.4	72.3	6	2	Sugar phosphatase YbiV
A04	UniRef90_A8ACN8	55.2	99.0	12	6	ATP synthase subunit alpha
	UniRef90_P33363	83.5	99.0	7	6	Periplasmic beta-glucosidase
	UniRef90_A7MQX5	41.4	99.0	19	7	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_P66794	36.7	98.7	11	4	Selenide, water dikinase
	UniRef90_P23847	60.3	98.6	8	3	Periplasmic dipeptide transport protein
	UniRef90_P0A797	34.8	99.0	16	5	6-phosphofructokinase isozyme 1
	UniRef90_A8AIH6	39.9	98.2	9	3	Phosphoserine aminotransferase
	UniRef90_P58603	35.6	98.2	8	3	Protease 7
	UniRef90_P00509	43.6	98.2	8	3	Aspartate aminotransferase
	UniRef90_P04693	43.5	82.9	6	2	Aromatic-amino-acid aminotransferase
	UniRef90_P0A6G4	17.6	82.9	15	2	Protein YgaD
	UniRef90_P37344	37.0	86.3	8	2	Psp operon transcriptional activator
A05	UniRef90_A7ZPV7	47.0	99.1	23	9	Histidine--tRNA ligase
	UniRef90_P04425	35.4	98.9	23	5	Glutathione synthetase
	UniRef90_P0AEN3	26.2	98.7	21	4	NAD(P)H-flavin reductase
	UniRef90_P00888	38.8	98.2	8	3	Phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive
	UniRef90_P12281	44.1	83.0	11	2	Molybdopterin molybdenumtransferase
	UniRef90_P75736	28.4	98.2	15	3	Esterase ybfF
	UniRef90_Q3Z606	35.2	98.2	9	3	Transaldolase 1
	UniRef90_A7ZN88	31.2	83.0	8	2	Molecular chaperone Hsp31 and glyoxalase 3
	UniRef90_Q8X7W8	25.5	83.0	11	2	PKHD-type hydroxylase ybiX
	UniRef90_B7UMJ8	31.6	83.0	7	2	ATP synthase gamma chain
	UniRef90_P38489	23.9	59.6	5	2	Oxygen-insensitive NAD(P)H nitroreductase
A06	UniRef90_B5FTC5	27.8	98.9	22	5	Deoxyribose-phosphate aldolase
	UniRef90_P0A9L8	28.1	98.7	18	4	Pyrroline-5-carboxylate reductase
	UniRef90_B7LNW7	37.2	98.9	18	5	Outer membrane protein A
	UniRef90_P39099	47.2	98.9	12	5	Periplasmic pH-dependent serine endoprotease DegQ
	UniRef90_P33363	83.5	98.2	4	3	Periplasmic beta-glucosidase

	UniRef90_P0AFU8	23.4	98.7	18	4	Riboflavin synthase alpha chain
	UniRef90_P12282	26.7	85.5	13	2	Molybdopterin-synthase adenyllyltransferase
	UniRef90_P0AE20	29.3	82.9	16	2	Methionine aminopeptidase
	UniRef90_P22731	26.3	82.9	14	2	High-affinity branched-chain amino acid transport ATP-binding protein LivF
	UniRef90_A7ZPW1	15.4	82.8	18	2	Nucleoside diphosphate kinase
	UniRef90_P38489	23.9	59.5	5	2	Oxygen-insensitive NAD(P)H nitroreductase
A07	UniRef90_B5FTC5	27.8	98.3	18	3	Deoxyribose-phosphate aldolase
	UniRef90_A8AIH6	39.9	98.2	9	3	Phosphoserine aminotransferase
	UniRef90_P0C8J9	47.0	98.2	9	3	D-tagatose-1,6-bisphosphate aldolase subunit gatZ
	UniRef90_Q8XDJ8	33.6	98.0	12	3	Murein tetrapeptide carboxypeptidase
	UniRef90_P07003	62.0	84.1	5	2	Pyruvate dehydrogenase [ubiquinone]
A08	NA	NA	NA			NA
A09	UniRef90_A7ZPV7	47.0	99.1	23	9	Histidine--tRNA ligase
	UniRef90_P00509	43.6	99.0	17	6	Aspartate aminotransferase
	UniRef90_A7ZHR7	90.6	99.0	9	7	Outer membrane protein assembly factor BamA
	UniRef90_Q8FBC3	56.2	98.3	9	3	Glycerol kinase
	UniRef90_A7MQX5	41.4	99.0	16	5	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_Q0T204	40.7	98.7	11	4	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
	UniRef90_P0A6G4	17.6	82.7	16	2	Protein YgaD
	UniRef90_A8AIH6	39.9	82.8	10	2	Phosphoserine aminotransferase
A10	UniRef90_P27833	41.9	98.9	15	5	Lipopolysaccharide biosynthesis protein RffA
	UniRef90_P00509	43.6	99.0	19	8	Aspartate aminotransferase
	UniRef90_A8AJD6	63.5	98.9	10	5	Glutamine--tRNA ligase
	UniRef90_P16703	32.7	82.7	7	2	Cysteine synthase B
	UniRef90_B7UMJ8	31.6	82.6	8	2	ATP synthase gamma chain
	UniRef90_A7ZPU1	22.5	82.4	12	2	Uracil phosphoribosyltransferase
A11	UniRef90_A7ZLK8	21.6	98.2	18	3	FMN-dependent NADH-azoreductase
	UniRef90_P15047	26.2	83.0	11	2	2,3-dihydro-2,3-dihydroxybenzoate dehydrogenase
	UniRef90_P0A9L8	28.1	83.0	8	2	Pyrroline-5-carboxylate reductase
	UniRef90_P0AEN3	26.2	83.0	10	2	NAD(P)H-flavin reductase
	UniRef90_A7ZPS1	27.0	82.9	7	2	Phosphoribosylaminoimidazole-succinocarboxamide synthase
	UniRef90_B5F6U1	50.0	63.3	6	2	Argininosuccinate synthase
A12	UniRef90_P18815	43.4	82.7	5	2	Maltose-binding periplasmic protein

Row B	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
B01	UniRef90_B7LNW7	37.2	99.0	19	6	Outer membrane protein A
	UniRef90_P02930	53.7	99.0	14	6	Outer membrane protein tolC
	UniRef90_P33030	36.1	98.7	15	4	Uncharacterized protein YeiR
	UniRef90_P0ABK6	34.5	98.7	17	4	Cysteine synthase A
	UniRef90_Q8X4S6	43.8	98.2	7	3	Acetylornithine/succinyldiaminopimelate aminotransferase
	UniRef90_A7ZHR7	90.6	98.7	5	4	Outer membrane protein assembly factor BamA
	UniRef90_P75682	32.5	82.9	8	2	Probable 2-keto-3-deoxy-galactonate aldolase YagE
	UniRef90_P0AEY7	21.9	82.8	10	2	Modulator of drug activity B
	UniRef90_A7ZIN4	23.6	98.1	16	3	Adenylate kinase
	UniRef90_A7ZHR7	90.6	99.1	9	8	Outer membrane protein assembly factor BamA
B02	UniRef90_A7ZKW9	63.7	98.7	10	4	Periplasmic trehalase
	UniRef90_P06971	82.2	98.2	4	3	Ferrichrome-iron receptor
	UniRef90_P09029	39.5	98.7	10	4	N5-carboxyaminoimidazole ribonucleotide synthase
	UniRef90_P76270	18.1	95.1	15	2	Free methionine-R-sulfoxide reductase
	UniRef90_B7UJV9	29.5	82.9	7	2	Transcriptional regulator NanR
	UniRef90_A7ZH92	33.6	82.7	7	2	Homoserine kinase
	UniRef90_P02930	53.7	98.7	8	4	Outer membrane protein tolC

	UniRef90_A8AHY1	33.5	86.6	19	2	3-oxoacyl-[acyl-carrier-protein] synthase 3
	UniRef90_P37985	61.2	96.4	5	2	30S ribosomal protein S1
	UniRef90_A7ZHM3	31.6	98.0	9	3	Pantothenate synthetase
B04	UniRef90_A7ZSD0	32.3	99.1	39	9	Malate dehydrogenase
	UniRef90_P0A9W9	20.2	98.9	27	5	Protein YrdA
B05	UniRef90_P0ADG8	52.0	98.3	9	3	Inosine-5'-monophosphate dehydrogenase
	UniRef90_P23917	32.5	98.9	19	5	Fructokinase
	UniRef90_P0A9P5	34.6	98.6	13	3	Thioredoxin reductase
	UniRef90_Q32EF3	26.0	98.2	11	3	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
B06	UniRef90_A8ACN6	50.3	99.0	15	6	ATP synthase subunit beta
	UniRef90_Q32EF3	26.0	99.0	24	5	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
	UniRef90_A7ZPS4	31.3	98.7	15	4	Dihydrodipicolinate synthase
	UniRef90_P33030	36.1	98.2	11	3	Uncharacterized protein YeiR
	UniRef90_A8AIH6	39.9	98.2	9	3	Phosphoserine aminotransferase
	UniRef90_B5F769	31.3	98.2	9	3	Bis(5'-nucleosyl)-tetraphosphatase, symmetrical
B07	UniRef90_P06971	82.2	99.1	12	9	Ferrichrome-iron receptor
	UniRef90_P0ABZ8	47.3	98.7	11	4	Chaperone SurA
	UniRef90_Q57TE8	49.9	98.2	9	3	3-isopropylmalate dehydratase large subunit 1
	UniRef90_Q8X621	42.9	98.2	8	3	Uncharacterized protein ydhQ
	UniRef90_P00370	48.6	82.8	6	2	NADP-specific glutamate dehydrogenase
	UniRef90_A7ZNJ3	39.4	99.0	15	6	Histidinol-phosphate aminotransferase
	UniRef90_P33363	83.5	98.2	4	3	Periplasmic beta-glucosidase
	UniRef90_A1AIG4	23.0	82.8	11	2	Thiamine-phosphate synthase
	UniRef90_P0A9W9	20.2	97.9	16	3	Protein YrdA
	UniRef90_A8AFI0	22.1	82.8	10	2	Holliday junction ATP-dependent DNA helicase RuvA
	UniRef90_B5F769	31.3	82.8	6	2	Bis(5'-nucleosyl)-tetraphosphatase, symmetrical
B08	UniRef90_P07003	62.0	99.0	12	5	Pyruvate dehydrogenase [ubiquinone]
	UniRef90_B5Y275	44.4	98.2	8	3	Phosphopentomutase
	UniRef90_P08200	45.8	99.0	12	5	Isocitrate dehydrogenase [NADP]
	UniRef90_P59735	27.2	98.2	14	3	3'(2'),5'-bispophosphate nucleotidase CysQ
	UniRef90_P0AFG9	99.7	82.7	2	2	Pyruvate dehydrogenase E1 component
	UniRef90_P0AF09	39.9	82.7	5	2	Protein mrp
B09	UniRef90_Q8XBL0	30.8	99.0	17	5	Pyridoxine kinase
	UniRef90_P30138	27.0	82.7	14	2	Sulfur carrier protein ThiS adenyllyltransferase
	UniRef90_P58603	35.6	98.2	8	3	Protease 7
	UniRef90_P69784	18.3	82.7	15	2	Glucose-specific phosphotransferase enzyme IIA component
B10	UniRef90_P58603	35.6	99.1	31	2	Protease 7
	UniRef90_P23917	32.5	98.7	19	4	Fructokinase
	UniRef90_P12758	27.2	98.2	13	3	Uridine phosphorylase
	UniRef90_P23905	35.7	82.7	9	2	D-galactose-binding periplasmic protein
	UniRef90_Q8X4S6	43.8	98.2	7	3	Acetylornithine/succinyldiaminopimelate aminotransferase
	UniRef90_P66794	36.7	82.5	5	2	Selenide, water dikinase
B11	UniRef90_P08331	70.8	89.0	5	2	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase
	UniRef90_P0A2K8	32.8	83.3	7	2	UTP--glucose-1-phosphate uridylyltransferase
B12	UniRef90_P77804	54.7	83.1	4	2	Protein ydgA
	UniRef90_A7ZH92	33.6	78.5	7	2	Homoserine kinase

Row B	Cluster ID	MW (kDa)*	Score %	Protein Name
B01	UniRef90_B7LNW7	37.2	99.0	Outer membrane protein A
	UniRef90_P02930	53.7	99.0	Outer membrane protein tolC
	UniRef90_P33030	36.1	98.7	Uncharacterized protein YeiR
	UniRef90_P0ABK6	34.5	98.7	Cysteine synthase A

	UniRef90_Q8X4S6	43.8	98.2	Acetylornithine/succinyldiaminopimelate aminotransferase
	UniRef90_A7ZHR7	90.6	98.7	Outer membrane protein assembly factor BamA
	UniRef90_P75682	32.5	82.9	Probable 2-keto-3-deoxy-galactonate aldolase YagE
	UniRef90_P0AEY7	21.9	82.8	Modulator of drug activity B
	UniRef90_A7ZIN4	23.6	98.1	Adenylate kinase
B02	UniRef90_A7ZHR7	90.6	99.1	Outer membrane protein assembly factor BamA
	UniRef90_A7ZKW9	63.7	98.7	Periplasmic trehalase
	UniRef90_P06971	82.2	98.2	Ferrichrome-iron receptor
	UniRef90_P09029	39.5	98.7	N5-carboxyaminoimidazole ribonucleotide synthase
	UniRef90_P76270	18.1	95.1	Free methionine-R-sulfoxide reductase
	UniRef90_B7UJV9	29.5	82.9	Transcriptional regulator NanR
	UniRef90_A7ZH92	33.6	82.7	Homoserine kinase
B03	UniRef90_P02930	53.7	98.7	Outer membrane protein tolC
	UniRef90_A8AHY1	33.5	86.6	3-oxoacyl-[acyl-carrier-protein] synthase 3
	UniRef90_P37985	61.2	96.4	30S ribosomal protein S1
	UniRef90_A7ZHM3	31.6	98.0	Pantothenate synthetase
B04	UniRef90_A7ZSD0	32.3	99.1	Malate dehydrogenase
	UniRef90_P0A9W9	20.2	98.9	Protein YrdA
B05	UniRef90_P0ADG8	52.0	98.3	Inosine-5'-monophosphate dehydrogenase
	UniRef90_P23917	32.5	98.9	Fructokinase
	UniRef90_P0A9P5	34.6	98.6	Thioredoxin reductase
	UniRef90_Q32EF3	26.0	98.2	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
B06	UniRef90_A8ACN6	50.3	99.0	ATP synthase subunit beta
	UniRef90_Q32EF3	26.0	99.0	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
	UniRef90_A7ZPS4	31.3	98.7	Dihydrodipicolinate synthase
	UniRef90_P33030	36.1	98.2	Uncharacterized protein YeiR
	UniRef90_A8AIH6	39.9	98.2	Phosphoserine aminotransferase
	UniRef90_B5F769	31.3	98.2	Bis(5'-nucleosyl)-tetraphosphatase, symmetrical
B07	UniRef90_P06971	82.2	99.1	Ferrichrome-iron receptor
	UniRef90_P0ABZ8	47.3	98.7	Chaperone SurA
	UniRef90_Q57TE8	49.9	98.2	3-isopropylmalate dehydratase large subunit 1
	UniRef90_Q8X621	42.9	98.2	Uncharacterized protein ydhQ
	UniRef90_P00370	48.6	82.8	NADP-specific glutamate dehydrogenase
	UniRef90_A7ZNJ3	39.4	99.0	Histidinol-phosphate aminotransferase
	UniRef90_P33363	83.5	98.2	Periplasmic beta-glucosidase
	UniRef90_A1AIG4	23.0	82.8	Thiamine-phosphate synthase
	UniRef90_P0A9W9	20.2	97.9	Protein YrdA
	UniRef90_A8AFI0	22.1	82.8	Holliday junction ATP-dependent DNA helicase RuvA
	UniRef90_B5F769	31.3	82.8	Bis(5'-nucleosyl)-tetraphosphatase, symmetrical
B08	UniRef90_P07003	62.0	99.0	Pyruvate dehydrogenase [ubiquinone]
	UniRef90_B5Y275	44.4	98.2	Phosphopentomutase
	UniRef90_P08200	45.8	99.0	Isocitrate dehydrogenase [NADP]
	UniRef90_P59735	27.2	98.2	3'(2'),5'-bisphosphate nucleotidase CysQ
	UniRef90_P0AFG9	99.7	82.7	Pyruvate dehydrogenase E1 component
	UniRef90_P0AF09	39.9	82.7	Protein mrp
B09	UniRef90_Q8XBL0	30.8	99.0	Pyridoxine kinase
	UniRef90_P30138	27.0	82.7	Sulfur carrier protein ThiS adenyltransferase
	UniRef90_P58603	35.6	98.2	Protease 7
	UniRef90_P69784	18.3	82.7	Glucose-specific phosphotransferase enzyme IIA component
B10	UniRef90_P58603	35.6	99.1	Protease 7
	UniRef90_P23917	32.5	98.7	Fructokinase
	UniRef90_P12758	27.2	98.2	Uridine phosphorylase
	UniRef90_P23905	35.7	82.7	D-galactose-binding periplasmic protein
	UniRef90_Q8X4S6	43.8	98.2	Acetylornithine/succinyldiaminopimelate aminotransferase
	UniRef90_P66794	36.7	82.5	Selenide, water dikinase
B11	UniRef90_P08331	70.8	89.0	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase
	UniRef90_P0A2K8	32.8	83.3	UTP--glucose-1-phosphate uridylyltransferase
B12	UniRef90_P77804	54.7	83.1	Protein ydgA
	UniRef90_A7ZH92	33.6	78.5	Homoserine kinase

Row C	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
C01	UniRef90_P04425	35.4	98.9	22	5	Glutathione synthetase
	UniRef90_Q3Z606	35.2	98.5	13	3	Transaldolase 1
	UniRef90_P0AFK9	38.9	98.9	14	4	Spermidine/putrescine-binding periplasmic protein
	UniRef90_P0AF94	13.6	83.2	17	2	Enamine/imine deaminase
C02	UniRef90_A8ALC4	29.9	99.1	35	9	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_P00509	43.6	99.1	28	11	Aspartate aminotransferase
	UniRef90_A8ALS4	28.7	99.0	31	5	Dihydrodipicolinate reductase
	UniRef90_P02930	53.7	99.1	19	8	Outer membrane protein tolC
	UniRef90_B5FTC5	27.7	98.7	19	4	Deoxyribose-phosphate aldolase
	UniRef90_P0ADS7	26.6	98.7	19	5	Uncharacterized protein yggE
	UniRef90_Q0T204	40.7	98.7	15	4	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
	UniRef90_A8AKV5	42.0	82.8	6	2	tRNA (uracil(54)-C(5))-methyltransferase
	UniRef90_P07023	42.0	82.8	6	2	T-protein
	UniRef90_P67097	20.1	82.8	14	2	Phosphodiesterase yfcE
C03	UniRef90_A7MQX5	41.4	98.7	13	4	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_A7ZSR4	38.9	98.7	13	4	3-dehydroquinase synthase
	UniRef90_A8ANW8	28.0	98.2	13	3	Phosphoadenosine phosphosulfate reductase
	UniRef90_A8AIH6	40.0	82.7	6	2	Phosphoserine aminotransferase
	UniRef90_P0AGD5	21.3	98.7	20	4	Superoxide dismutase [Fe]
	UniRef90_A7ZL78	28.8	82.7	7	2	Tryptophan synthase alpha chain
	UniRef90_A7ZHR7	90.6	82.3	2	2	Outer membrane protein assembly factor BamA
C04	UniRef90_Q3Z606	35.2	99.1	26	8	Transaldolase 1
	UniRef90_A7ZTD9	50.6	99.0	14	6	L-seryl-tRNA(Sec) selenium transferase
	UniRef90_P0ADS7	26.6	98.7	17	5	Uncharacterized protein yggE
	UniRef90_A8AIH6	39.9	98.7	13	4	Phosphoserine aminotransferase
	UniRef90_P0AF94	13.6	98.2	24	3	Enamine/imine deaminase
	UniRef90_P0ACLO	28.0	98.2	13	3	Glycerol-3-phosphate regulon repressor
	UniRef90_P0A928	33.6	82.7	8	2	Nucleoside-specific channel-forming protein tsx
	UniRef90_B7MI93	42.3	85.8	12	2	Acetylornithine deacetylase
C05	UniRef90_A7ZTD9	50.6	99.1	19	8	L-seryl-tRNA(Sec) selenium transferase
	UniRef90_P02930	53.7	99.0	16	7	Outer membrane protein tolC
	UniRef90_A8AIH6	39.9	99.0	17	5	Phosphoserine aminotransferase
	UniRef90_A7ZKW9	63.7	98.9	10	4	Periplasmic trehalase
	UniRef90_Q8X732	35.9	98.7	15	4	N-acetyl-gamma-glutamyl-phosphate reductase
	UniRef90_Q57TE8	49.9	82.8	5	2	3-isopropylmalate dehydratase large subunit 1
	UniRef90_A7ZHY1	20.8	82.7	10	2	Phosphoheptose isomerase
	UniRef90_A8AJD6	63.5	97.5	5	2	Glutamine--tRNA ligase
C06	UniRef90_Q8XDS0	52.4	99.1	15	6	Aspartate ammonia-lyase
	UniRef90_P25906	30.7	98.2	13	3	Putative oxidoreductase YdbC
	UniRef90_P75736	28.4	83.2	10	2	Esterase ybfF
	UniRef90_P24232	43.8	76.1	5	2	Flavoheporein
C07	UniRef90_A8ACN8	55.2	99.1	14	7	ATP synthase subunit alpha
	UniRef90_P06999	32.5	98.9	19	5	6-phosphofructokinase isozyme 2
	UniRef90_Q3Z3B7	20.8	98.8	25	4	Flavoprotein WrbA
	UniRef90_Q9S5G3	22.8	83.2	14	2	Histidine biosynthesis bifunctional protein HisIE
	UniRef90_P0AGD5	21.3	98.2	16	3	Superoxide dismutase [Fe]
C08	UniRef90_P16657	27.9	98.7	15	4	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI
	UniRef90_A7ZR71	20.7	98.1	20	3	UPF0301 protein yqgE
	UniRef90_A7ZSR4	38.9	98.3	11	3	3-dehydroquinase synthase
	UniRef90_P25906	30.7	98.1	12	3	Putative oxidoreductase YdbC
	UniRef90_Q57TE8	49.9	82.6	6	2	3-isopropylmalate dehydratase large subunit 1
	UniRef90_A7MH28	43.3	82.6	6	2	Acetate kinase
	UniRef90_B7UN33	19.0	98.1	16	3	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
	UniRef90_A7ZTD9	50.6	82.6	5	2	L-seryl-tRNA(Sec) selenium transferase
	UniRef90_P0A252	20.8	82.6	17	2	Alkyl hydroperoxide reductase subunit C

C09	UniRef90_B5Y275	44.4	99.0	20	8	Phosphopentomutase
	UniRef90_Q3Z606	35.2	99.0	35	13	Transaldolase 1
	UniRef90_A8ALS4	28.7	99.0	27	6	Dihydrodipicolinate reductase
	UniRef90_Q8XDS0	52.4	99.0	16	8	Aspartate ammonia-lyase
	UniRef90_P0AF94	13.6	98.1	24	3	Enamine/imine deaminase
	UniRef90_P08331	70.8	98.1	5	3	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase
	UniRef90_P38489	23.9	82.4	10	3	Oxygen-insensitive NAD(P)H nitroreductase
	UniRef90_P0AEB8	12.5	82.4	22	2	RutC family protein yoaB
	UniRef90_P46837	85.1	99.1	18	12	Protein yhgF
	UniRef90_A8ACN8	55.2	99.1	18	8	ATP synthase subunit alpha
C10	UniRef90_P58603	35.6	99.1	28	2	Protease 7
	UniRef90_P36938	58.4	99.0	12	6	Phosphoglucomutase
	UniRef90_P0AEB8	12.5	98.2	33	3	RutC family protein yoaB
	UniRef90_P0A928	33.6	82.8	8	2	Nucleoside-specific channel-forming protein tsx
	UniRef90_P0AEJ3	42.9	98.2	8	3	Isochorismate synthase entC
	UniRef90_Q8X732	35.9	98.2	8	3	N-acetyl-gamma-glutamyl-phosphate reductase
	UniRef90_P0ABQ3	30.4	82.8	8	2	2-hydroxy-3-oxopropionate reductase
	UniRef90_A7ZTA0	35.4	82.8	8	2	Glyoxylate/hydroxypyruvate reductase B
	UniRef90_P12994	17.1	82.7	11	2	UPF0098 protein ybhB
	UniRef90_A8ALC4	29.9	99.1	41	10	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
C11	UniRef90_Q8XDS0	52.4	99.0	13	6	Aspartate ammonia-lyase
	UniRef90_Q57TE8	49.9	98.2	9	3	3-isopropylmalate dehydratase large subunit 1
	UniRef90_P75736	28.4	98.2	15	3	Esterase ybfF
	UniRef90_P76034	27.6	98.7	14	4	Uncharacterized HTH-type transcriptional regulator YciT
	UniRef90_P08331	70.8	82.7	3	2	2',3'-cyclic-nucleotide 2'-phosphodiesterase/3'-nucleotidase
	UniRef90_B7UN33	19.0	98.2	16	3	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
	UniRef90_B7MI93	42.4	82.7	6	2	Acetylornithine deacetylase
	UniRef90_P12994	17.1	78.6	11	2	UPF0098 protein ybhB
	UniRef90_P09126	27.8	82.6	7	2	Uroporphyrinogen-III synthase
	UniRef90_P00509	43.6	99.1	26	10	Aspartate aminotransferase
C12	UniRef90_A8ALC4	29.9	98.9	22	5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_A8AKV5	42.0	98.2	12	3	tRNA (uracil(54)-C(5))-methyltransferase
	UniRef90_P76034	27.6	98.7	16	4	Uncharacterized HTH-type transcriptional regulator YciT
	UniRef90_Q3Z3B7	20.8	98.2	16	3	Flavoprotein WrbA
	UniRef90_Q0T204	40.7	98.7	11	4	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
	UniRef90_P58603	35.6	98.3	15	3	Protease 7
	UniRef90_P0AGD5	21.3	98.7	20	4	Superoxide dismutase [Fe]
	UniRef90_P09126	27.8	89.6	9	2	Uroporphyrinogen-III synthase
	UniRef90_P0AF94	13.6	82.9	14	2	Enamine/imine deaminase
	UniRef90_P0AAY0	33.3	82.8	6	2	Probable L,D-transpeptidase YbiS

Row D	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
D01	UniRef90_P46837	85.1	99.1	16	13	Protein yhgF
	UniRef90_P0AFK9	38.9	99.0	28	9	Spermidine/putrescine-binding periplasmic protein
	UniRef90_P34209	29.7	98.9	24	5	Protein ydcF
	UniRef90_A8ALC4	29.9	98.9	23	5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_P33570	35.7	99.0	22	6	Transketolase 2
	UniRef90_P66794	36.7	98.9	15	5	Selenide, water dikinase
	UniRef90_P00888	38.8	98.2	12	4	Phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive

	UniRef90_A8AEK3	39.4	99.0	17	6	Histidinol-phosphate aminotransferase
	UniRef90_A7ZN88	31.2	82.8	8	2	Molecular chaperone Hsp31 and glyoxalase 3
	UniRef90_A8ANW8	28.0	82.8	9	2	Phosphoadenosine phosphosulfate reductase
	UniRef90_Q3Z606	35.2	83.8	9	2	Transaldolase 1
	UniRef90_P0A4L6	23.1	97.8	13	3	Thiol:disulfide interchange protein DsbA
D02	UniRef90_A7ZSD0	32.3	99.1	29	9	Malate dehydrogenase
	UniRef90_A7ZPV7	47.0	99.0	17	6	Histidine--tRNA ligase
	UniRef90_P19926	45.7	98.9	14	5	Glucose-1-phosphatase
	UniRef90_B5FTC5	27.7	98.7	19	4	Deoxyribose-phosphate aldolase
	UniRef90_A8ANW8	28.0	98.9	22	5	Phosphoadenosine phosphosulfate reductase
	UniRef90_P31550	36.2	98.2	15	3	Thiamine-binding periplasmic protein
	UniRef90_B7US97	36.8	98.2	12	3	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_P00370	48.6	83.0	6	2	NADP-specific glutamate dehydrogenase
	UniRef90_Q57M35	20.5	98.2	16	3	NADH-quinone oxidoreductase subunit I
	UniRef90_Q3Z3B7	20.8	83.0	12	2	Flavoprotein WrbA
D03	UniRef90_A7ZVS5	47.2	99.1	33	11	Thymidine phosphorylase
	UniRef90_P02930	53.7	99.0	20	8	Outer membrane protein tolC
	UniRef90_Q3Z606	35.2	99.0	19	6	Transaldolase 1
	UniRef90_A1AIG4	23.0	98.1	21	3	Thiamine-phosphate synthase
	UniRef90_P0A9K5	39.0	98.1	10	3	PhoH-like protein
	UniRef90_B7MG93	39.2	82.5	8	2	Chorismate synthase
D04	UniRef90_A7ZPV7	47.0	99.1	28	9	Histidine--tRNA ligase
	UniRef90_P22715	37.3	98.9	21	6	UDP-glucose 4-epimerase
	UniRef90_A8ANW8	28.0	99.1	34	8	Phosphoadenosine phosphosulfate reductase
	UniRef90_B7US97	36.8	99.0	21	6	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_Q3Z606	35.2	99.0	23	7	Transaldolase 1
	UniRef90_Q3Z3B7	20.8	98.7	25	4	Flavoprotein WrbA
	UniRef90_P0ABZ8	47.3	82.8	5	2	Chaperone SurA
	UniRef90_P0AGD5	21.3	98.7	20	4	Superoxide dismutase [Fe]
	UniRef90_P0A9D3	22.9	98.2	15	3	Glutathione S-transferase GstA
	UniRef90_A7ZIN4	23.6	82.8	11	2	Adenylate kinase
	UniRef90_P76014	22.6	82.7	9	2	PTS-dependent dihydroxyacetone kinase, ADP-binding subunit dhaL
	UniRef90_A7ZL78	28.7	82.8	7	2	Tryptophan synthase alpha chain
	UniRef90_P0AEJ3	42.9	99.0	15	6	Isochorismate synthase entC
D05	UniRef90_Q8X9Z2	53.4	98.2	7	3	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase
	UniRef90_A8ALA3	35.3	98.2	11	3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha
	UniRef90_Q8X4S5	34.0	98.7	13	4	Arabinose 5-phosphate isomerase GutQ
	UniRef90_P0A9R0	40.0	82.8	9	2	Aspartate-semialdehyde dehydrogenase
	UniRef90_Q57M35	20.5	98.2	16	3	NADH-quinone oxidoreductase subunit I
	UniRef90_Q0TIY7	21.7	82.8	11	2	Maf-like protein yceF 1
	UniRef90_P58409	38.9	82.8	9	2	Uncharacterized oxidoreductase ybiC
	UniRef90_P0ADS7	26.6	82.7	7	2	Uncharacterized protein yggE
	UniRef90_P02930	53.7	99.1	19	8	Outer membrane protein tolC
	UniRef90_B5Y275	44.4	98.9	16	5	Phosphopentomutase
D06	UniRef90_P00888	38.8	98.7	12	4	Phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive
	UniRef90_P0A9R0	40.0	82.9	9	2	Aspartate-semialdehyde dehydrogenase
	UniRef90_A8AIH6	39.9	82.9	6	2	Phosphoserine aminotransferase
	UniRef90_B7N5L4	44.2	82.9	6	2	CinA-like protein
	UniRef90_P22715	37.3	82.8	6	2	UDP-glucose 4-epimerase
	UniRef90_P09551	28.0	99.0	26	6	Lysine-arginine-ornithine-binding periplasmic protein
	UniRef90_B5FTC5	27.7	99.0	22	5	Deoxyribose-phosphate aldolase
	UniRef90_A7ZJN3	39.4	99.0	15	6	Histidinol-phosphate aminotransferase
	UniRef90_B7US97	36.8	82.8	8	2	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_A7FGQ6	25.1	82.8	10	2	NADH-quinone oxidoreductase subunit B
D07	UniRef90_B7MG93	39.2	82.8	7	2	Chorismate synthase
	UniRef90_P22715	37.3	96.5	9	2	UDP-glucose 4-epimerase
	UniRef90_A8AJD6	63.5	82.6	3	2	Glutamine--tRNA ligase
	UniRef90_Q9S5G5	40.3	99.0	15	6	Histidine biosynthesis bifunctional protein HisB

	UniRef90_P06971	82.2	98.7	6	4	Ferrichrome-iron receptor
	UniRef90_Q8X4S5	34.0	98.9	18	6	Arabinose 5-phosphate isomerase GutQ
	UniRef90_B2U2C1	36.4	99.0	12	4	Adenosine deaminase
	UniRef90_P0ADS7	26.6	98.1	16	4	Uncharacterized protein yggE
	UniRef90_P0A9K5	39.0	98.1	10	3	PhoH-like protein
	UniRef90_A7FGQ6	25.1	82.3	10	2	NADH-quinone oxidoreductase subunit B
D09	UniRef90_A8ALC4	29.9	98.9	24	5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_A7MH28	43.3	98.7	14	4	Acetate kinase
	UniRef90_P31550	36.2	98.7	15	4	Thiamine-binding periplasmic protein
	UniRef90_A8ANW0	16.9	82.6	19	2	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase
	UniRef90_B5Y275	44.4	98.1	8	3	Phosphopentomutase
	UniRef90_A7ZN88	31.2	82.5	8	2	Molecular chaperone Hsp31 and glyoxalase 3
	UniRef90_B2VCV8	15.8	82.6	17	2	50S ribosomal protein L9
	UniRef90_P0AEX1	33.8	82.4	5	2	1-phosphofructokinase
	UniRef90_P0C0L2	15.1	82.5	14	2	Peroxiredoxin osmC
	UniRef90_P0A9D3	22.9	82.4	10	2	Glutathione S-transferase Gsta
D10	UniRef90_Q0T204	40.7	99.1	23	9	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
	UniRef90_A7ZVS5	47.2	99.1	23	8	Thymidine phosphorylase
	UniRef90_P06971	82.2	99.0	10	6	Ferrichrome-iron receptor
	UniRef90_Q8X769	50.5	99.0	13	6	Fumarate hydratase class II
	UniRef90_P09551	28.0	99.0	26	6	Lysine-arginine-ornithine-binding periplasmic protein
	UniRef90_P19926	45.7	98.7	11	4	Glucose-1-phosphatase
	UniRef90_Q8X9Z2	53.4	98.7	9	4	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase
	UniRef90_P0AFK9	38.9	99.0	19	7	Spermidine/putrescine-binding periplasmic protein
	UniRef90_A7ZPV7	47.0	83.2	8	2	Histidine--tRNA ligase
	UniRef90_Q3Z606	35.2	82.7	7	2	Transaldolase 1
D11	UniRef90_P19926	45.7	98.7	13	4	Glucose-1-phosphatase
	UniRef90_P77804	54.7	98.9	11	5	Protein ydgA
	UniRef90_Q9S5G5	40.3	98.1	10	3	Histidine biosynthesis bifunctional protein HisB
	UniRef90_A8ACN6	50.3	82.5	5	2	ATP synthase subunit beta
	UniRef90_P66794	36.7	82.5	6	2	Selenide, water dikinase
	UniRef90_B7N5L4	44.2	82.5	6	2	CinA-like protein
D12	UniRef90_A8ACN6	50.3	98.9	16	5	ATP synthase subunit beta
	UniRef90_A8ANW8	28.0	98.7	18	4	Phosphoadenosine phosphosulfate reductase
	UniRef90_P0AEJ3	42.9	98.7	11	4	Isochorismate synthase entC
	UniRef90_A1AC10	42.4	98.7	11	4	Phosphoribosylglycinamide formyltransferase 2
	UniRef90_Q0T204	40.7	82.8	6	2	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase

Row E	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
E01	NA	NA	NA			NA
E02	UniRef90_B7US97	36.8	99.1	32	10	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_P06971	82.2	99.0	11	8	Ferrichrome-iron receptor
	UniRef90_A7ZJN1	33.4	98.9	18	5	ATP phosphoribosyltransferase
	UniRef90_A7ZHM4	28.2	98.2	13	3	3-methyl-2-oxobutanoate hydroxymethyltransferase
	UniRef90_A9MHF3	12.3	98.2	26	3	50S ribosomal protein L7/L12
	UniRef90_P0AD98	39.1	82.6	6	2	Leu/Ile/Val-binding protein
	UniRef90_A8AIH6	39.8	82.6	6	2	Phosphoserine aminotransferase
	UniRef90_P21367	23.1	82.6	9	2	Uncharacterized protein ycaC
	UniRef90_Q8FF18	26.4	82.3	9	2	Pyridoxine 5'-phosphate synthase
E03	UniRef90_B5F446	34.4	99.0	30	8	Aspartate carbamoyltransferase
	UniRef90_A1AC10	42.4	99.1	26	9	Phosphoribosylglycinamide formyltransferase 2
	UniRef90_A7FGQ6	25.1	98.9	26	5	NADH-quinone oxidoreductase subunit B

	UniRef90_P0ABK6	34.5	98.7	15	4	Cysteine synthase A
	UniRef90_P06971	82.2	98.7	6	4	Ferrichrome-iron receptor
	UniRef90_P0AD98	39.1	98.5	13	3	Leu/Ile/Val-binding protein
	UniRef90_A4WC74	28.4	98.7	20	4	Imidazole glycerol phosphate synthase subunit HisF
	UniRef90_P76576	22.2	98.1	18	3	UPF0070 protein yfgM
	UniRef90_A8AIH6	39.8	82.6	6	2	Phosphoserine aminotransferase
	UniRef90_P66794	36.7	82.5	5	2	Selenide, water dikinase
	UniRef90_P0AAY0	33.3	98.1	12	3	Probable L,D-transpeptidase YbiS
E04	UniRef90_P0AEZ1	33.1	99.0	22	6	5,10-methylenetetrahydrofolate reductase
	UniRef90_A7ZSR4	38.9	98.7	14	4	3-dehydroquinase synthase
	UniRef90_P76576	22.2	98.2	18	3	UPF0070 protein yfgM
	UniRef90_B5F6U1	49.9	98.7	15	4	Argininosuccinate synthase
	UniRef90_B7UN33	19.0	98.2	22	3	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
	UniRef90_P0AD98	39.1	82.6	6	2	Leu/Ile/Val-binding protein
	UniRef90_P12758	27.2	82.6	8	2	Uridine phosphorylase
	UniRef90_A8AIH6	39.8	82.6	6	2	Phosphoserine aminotransferase
	UniRef90_Q83IW8	26.7	98.1	10	3	Uncharacterized protein yigA
	UniRef90_P16703	32.7	99.1	33	8	Cysteine synthase B
E05	UniRef90_P26646	34.7	98.2	12	3	Putative quinone oxidoreductase YhdH
	UniRef90_A4WC74	28.4	98.2	14	3	Imidazole glycerol phosphate synthase subunit HisF
	UniRef90_A7ZHM4	28.2	98.2	13	3	3-methyl-2-oxobutanoate hydroxymethyltransferase
	UniRef90_A8ALS4	28.7	82.8	9	2	Dihydrodipicolinate reductase
	UniRef90_P23905	35.7	82.8	9	2	D-galactose-binding periplasmic protein
	UniRef90_A7ZJN1	33.4	82.7	7	2	ATP phosphoribosyltransferase
	UniRef90_A7ZKL2	21.2	98.1	12	3	UPF0227 protein ycfP
	UniRef90_Q46856	42.1	82.8	5	2	Alcohol dehydrogenase YqhD
	UniRef90_Q46868	11.3	82.7	17	2	Uncharacterized protein yqiC
	UniRef90_A7FGQ6	25.1	82.7	10	2	NADH-quinone oxidoreductase subunit B
E06	UniRef90_P33362	32.6	98.8	15	4	Putative osmoprotectant uptake system substrate-binding protein osmF
	UniRef90_Q46856	42.1	98.7	11	4	Alcohol dehydrogenase YqhD
	UniRef90_A9MHF3	12.3	82.7	19	2	50S ribosomal protein L7/L12
	UniRef90_A7ZHY1	20.8	73.5	10	2	Phosphoheptose isomerase
	UniRef90_A8ANW8	28.0	99.1	35	9	Phosphoadenosine phosphosulfate reductase
E07	UniRef90_A8AIH6	39.8	98.9	17	5	Phosphoserine aminotransferase
	UniRef90_P0AEZ1	33.1	98.9	18	5	5,10-methylenetetrahydrofolate reductase
	UniRef90_P22731	26.3	98.9	23	5	High-affinity branched-chain amino acid transport ATP-binding protein LivF
	UniRef90_A7ZPX4	45.1	98.7	12	4	Cysteine desulfurase
	UniRef90_P0AD98	39.1	98.1	8	3	Leu/Ile/Val-binding protein
	UniRef90_Q8X732	35.9	98.2	8	3	N-acetyl-gamma-glutamyl-phosphate reductase
	UniRef90_P09551	28.0	82.7	9	2	Lysine-arginine-ornithine-binding periplasmic protein
	UniRef90_P0ADU3	11.5	82.7	17	2	Probable quinol monooxygenase ygiN
	UniRef90_A7ZIJ5	23.2	82.4	7	2	ATP-dependent Clp protease proteolytic subunit
	UniRef90_B5F446	34.4	99.1	32	9	Aspartate carbamoyltransferase
E08	UniRef90_P0AEN0	29.0	99.0	28	7	Cystine-binding periplasmic protein
	UniRef90_P16703	32.7	98.9	22	5	Cysteine synthase B
	UniRef90_P25526	51.7	99.0	13	6	Succinate-semialdehyde dehydrogenase [NADP(+)] GabD
	UniRef90_P08200	45.8	98.9	12	5	Isocitrate dehydrogenase [NADP]
	UniRef90_P0A9L3	22.2	98.2	18	3	FKBP-type 22 kDa peptidyl-prolyl cis-trans isomerase
	UniRef90_P11880	47.4	98.2	9	3	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase
	UniRef90_B7UN33	19.0	98.7	22	4	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
	UniRef90_Q8X732	35.9	98.2	8	3	N-acetyl-gamma-glutamyl-phosphate reductase
	UniRef90_P09029	39.5	82.4	5	2	N5-carboxyaminoimidazole ribonucleotide

						synthase
E09	UniRef90_B7UPI6	61.5	99.1	17	8	Glucose-6-phosphate isomerase
	UniRef90_P33362	32.6	98.9	19	5	Putative osmoprotectant uptake system substrate-binding protein osmF
	UniRef90_A1AFA2	22.9	98.9	27	5	Ribose-5-phosphate isomerase A
	UniRef90_P67271	26.9	98.7	26	4	UPF0135 protein ybgI
	UniRef90_A8ALC4	29.9	98.9	23	5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_A8ANW8	28.0	98.9	22	5	Phosphoadenosine phosphosulfate reductase
	UniRef90_A7ZI01	39.1	98.9	14	5	Glutamate 5-kinase
	UniRef90_P23847	60.3	98.2	6	3	Periplasmic dipeptide transport protein
	UniRef90_P34209	29.7	82.7	9	2	Protein ydcF
	UniRef90_P21367	23.1	98.2	18	3	Uncharacterized protein ycaC
E10	UniRef90_P23905	35.7	82.7	7	2	D-galactose-binding periplasmic protein
	UniRef90_Q8FBC3	56.2	99.0	12	6	Glycerol kinase
	UniRef90_P77804	54.7	99.0	13	6	Protein ydgA
	UniRef90_A8ADK6	34.7	98.7	15	4	Glucokinase
	UniRef90_B7MI93	42.3	98.7	18	4	Acetylornithine deacetylase
	UniRef90_P0AD98	39.1	98.6	13	3	Leu/Ile/Val-binding protein
	UniRef90_P12758	27.2	98.2	12	3	Uridine phosphorylase
	UniRef90_Q32EF3	26.0	98.2	14	3	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
	UniRef90_P60596	21.6	82.7	11	2	Imidazole glycerol phosphate synthase subunit HisH
	UniRef90_A7ZKL2	21.2	73.8	8	2	UPF0227 protein ycfP
E11	UniRef90_P26646	34.7	99.1	33	8	Putative quinone oxidoreductase YhdH
	UniRef90_P77804	54.7	99.1	19	7	Protein ydgA
	UniRef90_A8ALC4	29.9	98.7	17	4	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_P25526	51.7	98.7	10	4	Succinate-semialdehyde dehydrogenase [NADP(+)] GabD
	UniRef90_P0AFG9	99.7	99.0	6	6	Pyruvate dehydrogenase E1 component
	UniRef90_P23847	60.3	98.7	9	4	Periplasmic dipeptide transport protein
	UniRef90_A7ZPS1	27.0	82.8	7	2	Phosphoribosylaminoimidazole-succinocarboxamide synthase
	UniRef90_P37648	55.5	83.0	5	2	Protein yhjJ
	UniRef90_A7ZNJ1	33.4	82.9	7	2	ATP phosphoribosyltransferase
	UniRef90_P0AAY0	33.3	87.6	9	2	Probable L,D-transpeptidase YbiS
E12	UniRef90_A1AC10	42.3	99.1	27	8	Phosphoribosylglycinamide formyltransferase 2
	UniRef90_P0A9S4	37.4	99.1	24	8	Galactitol-1-phosphate 5-dehydrogenase
	UniRef90_P67271	26.9	98.9	30	5	UPF0135 protein ybgI
	UniRef90_A7ZNJ1	33.4	99.0	20	6	ATP phosphoribosyltransferase
	UniRef90_Q32EF3	26.0	99.0	33	5	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino]imidazole-4-carboxamide isomerase
	UniRef90_P0ABU1	31.6	98.4	15	3	1,4-Dihydroxy-2-naphthoyl-CoA synthase
	UniRef90_A8AHY1	33.5	83.0	7	2	3-oxoacyl-[acyl-carrier-protein] synthase 3
	UniRef90_A7ZSR4	38.9	83.0	6	2	3-dehydroquinate synthase
	UniRef90_Q8XBL0	30.8	98.0	9	3	Pyridoxine kinase
	UniRef90_A8AIH6	39.8	76.5	6	2	Phosphoserine aminotransferase

Row F	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
F01	UniRef90_Q0T204	40.7	99.1	40	14	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
	UniRef90_B7MIX6	26.9	99.1	41	9	Thiazole synthase
	UniRef90_P07023	42.0	42.0	28	11	T-protein
	UniRef90_P33570	35.7	99.1	31	8	Transketolase 2
	UniRef90_Q57T38	30.4	99.1	33	9	Elongation factor Ts
	UniRef90_P0ADG8	52.0	98.7	11	4	Inosine-5'-monophosphate dehydrogenase

	UniRef90_A8ALC9	24.4	98.5	26	3	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
	UniRef90_P0AB92	38.0	98.9	18	5	Phospho-2-dehydro-3-deoxyheptonate aldolase, Phe-sensitive
	UniRef90_P0AET9	26.8	98.9	22	5	7-alpha-hydroxysteroid dehydrogenase
	UniRef90_P00509	43.6	99.1	17	7	Aspartate aminotransferase
	UniRef90_P0AFU8	23.4	98.9	24	5	Riboflavin synthase alpha chain
	UniRef90_P0C8J9	47.1	98.9	13	5	D-tagatose-1,6-bisphosphate aldolase subunit gatZ
	UniRef90_P0AGD5	21.3	98.7	20	4	Superoxide dismutase [Fe]
	UniRef90_Q7N590	34.2	83.1	8	2	Ribose-phosphate pyrophosphokinase
	UniRef90_Q3Z3B7	20.8	83.1	12	2	Flavoprotein WrbA
F02	UniRef90_A8AIH5	46.1	99.1	27	11	3-phosphoshikimate 1-carboxyvinyltransferase
	UniRef90_P0A9P5	34.6	99.1	27	7	Thioredoxin reductase
	UniRef90_P0A252	20.8	98.9	38	5	Alkyl hydroperoxide reductase subunit C
	UniRef90_P0ADG8	52.0	98.4	9	3	Inosine-5'-monophosphate dehydrogenase
	UniRef90_Q57T38	30.4	99.1	26	8	Elongation factor Ts
	UniRef90_P19480	56.2	99.1	16	7	Alkyl hydroperoxide reductase subunit F
	UniRef90_B7N6B0	46.2	98.9	12	5	Peptidase B
	UniRef90_P0AGD5	21.3	98.9	40	5	Superoxide dismutase [Fe]
	UniRef90_P00904	56.9	98.2	7	3	Anthranilate synthase component II
	UniRef90_A7ZUD3	27.0	98.7	16	4	Triosephosphate isomerase
	UniRef90_P0A7B0	19.7	98.7	22	4	Inorganic pyrophosphatase
	UniRef90_P23847	60.3	83.0	4	2	Periplasmic dipeptide transport protein
	UniRef90_A8API1	11.0	98.2	27	3	Probable Fe(2+)-trafficking protein
	UniRef90_P0A9T9	24.5	83.0	9	2	Uncharacterized ABC transporter ATP-binding protein YbbA
	UniRef90_A7ZLL8	62.8	83.0	3	2	Glucans biosynthesis protein D
F03	UniRef90_B7N6B0	46.2	99.1	33	13	Peptidase B
	UniRef90_A7FDW9	44.8	99.1	27	10	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
	UniRef90_P77804	54.7	99.1	20	8	Protein ydgA
	UniRef90_A8AHY1	33.5	99.1	29	7	3-oxoacyl-[acyl-carrier-protein] synthase 3
	UniRef90_P0AFU8	23.4	99.0	38	6	Riboflavin synthase alpha chain
	UniRef90_Q57RI5	28.6	98.9	22	5	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	UniRef90_P76422	28.6	98.7	23	4	Hydroxymethylpyrimidine/phosphomethylpyrimidine kinase
	UniRef90_P0AGD5	21.3	99.0	37	6	Superoxide dismutase [Fe]
	UniRef90_A7ZIN4	23.6	98.7	21	4	Adenylate kinase
	UniRef90_P10346	26.7	83.0	9	2	Glutamine transport ATP-binding protein GlnQ
	UniRef90_A7ZNJ3	39.4	98.7	10	4	Histidinol-phosphate aminotransferase
	UniRef90_P67082	25.8	97.8	15	3	UPF0001 protein yggS
	UniRef90_P0ACS1	20.6	81.5	12	2	Transcriptional repressor mprA
	UniRef90_B7LNW7	37.2	99.1	31	11	Outer membrane protein A
	UniRef90_P77804	54.7	99.1	30	11	Protein ydgA
	UniRef90_P0AFK9	38.9	99.1	38	13	Spermidine/putrescine-binding periplasmic protein
F04	UniRef90_Q7N590	34.2	99.0	26	6	Ribose-phosphate pyrophosphokinase
	UniRef90_B7MIX6	26.9	99.1	34	8	Thiazole synthase
	UniRef90_P0A9P5	34.6	99.0	25	7	Thioredoxin reductase
	UniRef90_Q57T38	30.0	99.1	33	10	Elongation factor Ts
	UniRef90_P0ADG8	52.0	98.9	13	5	Inosine-5'-monophosphate dehydrogenase
	UniRef90_P0AET9	26.8	98.9	22	5	7-alpha-hydroxysteroid dehydrogenase
	UniRef90_P0AE20	29.3	98.7	16	4	Methionine aminopeptidase
	UniRef90_Q3Z606	35.2	98.7	12	4	Transaldolase 1
	UniRef90_B7UPI6	61.5	98.9	11	5	Glucose-6-phosphate isomerase
	UniRef90_P16657	27.9	98.2	10	3	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI
	UniRef90_P0ADS7	26.6	98.2	10	3	Uncharacterized protein yggE
	UniRef90_A7ZNJ1	33.4	82.8	5	2	ATP phosphoribosyltransferase
	UniRef90_P0ADN4	13.1	82.3	12	2	UPF0438 protein yifE
	UniRef90_B7US97	36.8	99.1	41	14	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_P0ADG8	52.0	99.0	17	7	Inosine-5'-monophosphate dehydrogenase

	UniRef90_A7ZTA0	35.4	99.1	27	8	Glyoxylate/hydroxypyruvate reductase B
	UniRef90_Q9S5G5	40.3	99.0	23	7	Histidine biosynthesis bifunctional protein HisB
	UniRef90_P39099	47.2	99.0	15	7	Periplasmic pH-dependent serine endoprotease DegQ
	UniRef90_P16657	27.9	98.9	19	5	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI
	UniRef90_A7ZUD3	27.0	98.9	20	4	Triosephosphate isomerase
	UniRef90_P0AD98	39.1	98.2	10	3	Leu/Ile/Val-binding protein
	UniRef90_Q3Z3B7	20.8	98.2	16	3	Flavoprotein WrbA
	UniRef90_P0AE20	29.3	98.2	12	3	Methionine aminopeptidase
	UniRef90_P0A797	34.8	98.9	13	5	6-phosphofructokinase isozyme 1
	UniRef90_P00509	43.6	98.7	10	4	Aspartate aminotransferase
	UniRef90_A7ZIN4	23.6	98.2	16	3	Adenylate kinase
	UniRef90_Q57RI5	28.6	98.2	13	3	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	UniRef90_P0AEG7	25.6	82.8	9	2	Thiol:disulfide interchange protein DsbC
	UniRef90_P0ADS7	26.6	82.8	7	2	Uncharacterized protein yggE
	UniRef90_P38489	23.9	59.5	5	2	Oxygen-insensitive NAD(P)H nitroreductase
F06	UniRef90_B7LNNW7	37.2	99.0	24	8	Outer membrane protein A
	UniRef90_A7FDW9	44.8	99.1	20	8	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
	UniRef90_P07003	62.0	99.1	19	9	Pyruvate dehydrogenase [ubiquinone]
	UniRef90_P0AEZ1	33.1	99.0	27	6	5,10-methylenetetrahydrofolate reductase
	UniRef90_A8AEK3	39.4	99.1	20	8	Histidinol-phosphate aminotransferase
	UniRef90_A8ALC9	24.4	82.9	10	2	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
	UniRef90_A8API1	11.0	98.2	27	3	Probable Fe(2+)-trafficking protein
	UniRef90_P0AAY0	33.3	89.5	8	2	Probable L,D-transpeptidase YbiS
	UniRef90_A7ZNJ1	33.4	62.6	6	2	ATP phosphoribosyltransferase
F07	UniRef90_P18815	43.4	99.1	38	15	Maltose-binding periplasmic protein
	UniRef90_Q8FG52	46.1	99.1	23	9	Histidinol dehydrogenase
	UniRef90_A8AIH5	46.1	99.1	26	10	3-phosphoshikimate 1-carboxyvinyltransferase
	UniRef90_Q8XEG2	66.9	99.1	16	8	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]
	UniRef90_A7ZII0	18.3	99.0	47	6	UPF0234 protein yajQ
	UniRef90_A7ZPX4	45.1	99.0	18	6	Cysteine desulfurase
	UniRef90_A8AEK3	46.1	99.1	26	9	Histidinol-phosphate aminotransferase
	UniRef90_B7US97	36.8	98.7	15	4	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_A8ACN6	50.3	98.9	12	5	ATP synthase subunit beta
	UniRef90_P0AGD5	21.3	99.0	35	6	Superoxide dismutase [Fe]
	UniRef90_A7MH28	43.3	83.0	6	2	Acetate kinase
	UniRef90_P07023	42.0	82.8	5	2	T-protein
	UniRef90_P0ADN4	13.1	75.0	12	2	UPF0438 protein yifE
F08	UniRef90_Q8XEG2	66.9	99.1	24	13	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]
	UniRef90_P0AFK9	38.9	99.1	33	11	Spermidine/putrescine-binding periplasmic protein
	UniRef90_P25526	51.7	99.1	19	9	Succinate-semialdehyde dehydrogenase [NADP(+)] GabD
	UniRef90_B7N6B0	46.2	99.0	18	7	Peptidase B
	UniRef90_P0AB92	38.0	98.9	18	5	Phospho-2-dehydro-3-deoxyheptonate aldolase, Phe-sensitive
	UniRef90_P07023	42.0	99.1	27	10	T-protein
	UniRef90_P33570	35.7	99.0	20	6	Transketolase 2
	UniRef90_P0ADG8	52.0	98.7	9	4	Inosine-5'-monophosphate dehydrogenase
	UniRef90_P00904	56.9	98.9	11	5	Anthranilate synthase component II
	UniRef90_A8AEK3	39.4	99.1	22	8	Histidinol-phosphate aminotransferase
	UniRef90_P0ACE1	62.5	98.7	7	4	Hydrogenase-2 large chain
	UniRef90_P00893	63.0	98.9	8	5	Acetolactate synthase isozyme 3 large subunit
	UniRef90_A8AKV5	42.0	82.9	6	2	tRNA (uracil(54)-C(5))-methyltransferase
	UniRef90_B7UPI6	61.5	98.2	5	3	Glucose-6-phosphate isomerase
	UniRef90_P0ACS1	20.6	78.3	9	2	Transcriptional repressor mprA
F09	UniRef90_B7N6B0	46.2	99.1	26	9	Peptidase B
	UniRef90_P0A9L8	28.1	99.0	26	6	Pyrroline-5-carboxylate reductase

	UniRef90_Q7N590	34.2	98.9	22	5	Ribose-phosphate pyrophosphokinase
	UniRef90_P0AEZ1	33.1	99.0	21	6	5,10-methylenetetrahydrofolate reductase
	UniRef90_P07003	62.0	99.0	13	6	Pyruvate dehydrogenase [ubiquinone]
	UniRef90_Q8XEG2	66.9	99.0	10	6	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]
	UniRef90_P10346	26.7	98.2	15	3	Glutamine transport ATP-binding protein GlnQ
	UniRef90_P38489	23.9	83.0	10	3	Oxygen-insensitive NAD(P)H nitroreductase
	UniRef90_P33362	32.6	98.9	15	5	Putative osmoprotectant uptake system substrate- binding protein osmF
	UniRef90_P76004	23.7	83.0	11	2	Uncharacterized protein ycgM
	UniRef90_Q57T38	30.4	98.9	20	5	Elongation factor Ts
	UniRef90_Q8X4S6	43.8	83.0	5	2	Acetylornithine/succinyldiaminopimelate aminotransferase
	UniRef90_P0AFH9	21.1	83.0	10	2	Osmotically-inducible protein Y
	UniRef90_P09030	31.0	83.0	9	2	Exodeoxyribonuclease III
	UniRef90_P67082	25.8	83.0	9	2	UPF0001 protein yggS
F10	UniRef90_P07023	42.0	99.1	39	17	T-protein
	UniRef90_B7N6B0	46.2	99.1	28	10	Peptidase B
	UniRef90_P39099	47.2	99.1	26	11	Periplasmic pH-dependent serine endoprotease DegQ
	UniRef90_B7LNW7	37.2	99.1	26	9	Outer membrane protein A
	UniRef90_P25526	51.7	99.1	19	10	Succinate-semialdehyde dehydrogenase [NADP(+)] GabD
	UniRef90_Q8FG52	46.1	99.0	19	6	Histidinol dehydrogenase
	UniRef90_Q0T204	40.7	99.0	17	7	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
	UniRef90_A8ALG8	32.3	98.7	19	4	Spermidine synthase
	UniRef90_P09030	31.0	98.9	18	5	Exodeoxyribonuclease III
	UniRef90_P0A9K5	39.0	98.7	12	5	PhoH-like protein
	UniRef90_P00509	43.6	98.9	12	5	Aspartate aminotransferase
	UniRef90_P33362	32.6	98.7	15	4	Putative osmoprotectant uptake system substrate- binding protein osmF
	UniRef90_B5F769	31.3	98.2	9	3	Bis(5'-nucleosyl)-tetraphosphatase, symmetrical
	UniRef90_P0AF09	39.9	83.0	5	2	Protein mrp
	UniRef90_A8ACN6	55.2	79.4	5	2	ATP synthase subunit beta
F11	UniRef90_Q0T204	40.7	99.1	45	15	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
	UniRef90_P0AFK9	38.9	99.1	33	11	Spermidine/putrescine-binding periplasmic protein
	UniRef90_B7US97	36.8	98.9	18	5	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_P33570	35.7	99.1	21	7	Transketolase 2
	UniRef90_P0A797	34.8	99.1	22	7	6-phosphofructokinase isozyme 1
	UniRef90_P0A9K5	39.0	98.9	16	6	PhoH-like protein
	UniRef90_P0A9L8	28.1	98.2	14	3	Pyrroline-5-carboxylate reductase
	UniRef90_A8AEK3	39.4	99.1	20	8	Histidinol-phosphate aminotransferase
	UniRef90_P19480	56.2	99.0	14	6	Alkyl hydroperoxide reductase subunit F
	UniRef90_P0AEZ1	33.1	98.7	12	4	5,10-methylenetetrahydrofolate reductase
	UniRef90_Q3Z606	35.2	62.6	9	2	Transaldolase 1
	UniRef90_P76569	11.3	59.7	11	2	Uncharacterized protein YfgD
F12	NA	NA	NA	NA	NA	NA

Row G	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
G01	UniRef90_P19480	56.2	99.1	26	12	Alkyl hydroperoxide reductase subunit F
	UniRef90_Q57T38	30.4	99.1	33	10	Elongation factor Ts
	UniRef90_A1AFA2	22.9	98.9	27	5	Ribose-5-phosphate isomerase A
	UniRef90_P0AEU2	28.5	98.7	18	5	Histidine-binding periplasmic protein
	UniRef90_P0A9J9	43.1	98.9	12	6	P-protein
	UniRef90_A8ACN8	55.2	99.0	12	6	ATP synthase subunit alpha
	UniRef90_P0ADG8	52.0	83.0	5	2	Inosine-5'-monophosphate dehydrogenase
	UniRef90_P0A957	22.2	98.2	15	3	KHG/KDPG aldolase

	UniRef90_P0A9D3	22.9	98.2	16	3	Glutathione S-transferase Gsta
	UniRef90_P21367	23.1	98.2	14	3	Uncharacterized protein ycaC
	UniRef90_Q8FK07	15.9	98.2	26	3	Universal stress protein G
	UniRef90_B5FI12	15.2	98.1	21	3	Ribosome-binding factor A
G02	UniRef90_A8ACN6	50.3	99.1	40	14	ATP synthase subunit beta
	UniRef90_A7ZHG6	57.3	99.1	24	12	2-isopropylmalate synthase
	UniRef90_B7US97	36.8	99.1	29	9	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_A7FDW9	44.8	99.1	24	10	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
	UniRef90_Q8XEG2	66.9	99.1	19	10	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]
	UniRef90_P00370	48.6	83.1	6	2	NADP-specific glutamate dehydrogenase
	UniRef90_A7ZIN4	23.6	98.9	25	5	Adenylate kinase
	UniRef90_Q57RI5	28.6	98.2	13	3	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	UniRef90_P0ADG8	52.0	83.1	5	2	Inosine-5'-monophosphate dehydrogenase
	UniRef90_P21367	23.1	98.2	14	3	Uncharacterized protein ycaC
	UniRef90_A7ZHM4	28.2	83.1	9	2	3-methyl-2-oxobutanoate hydroxymethyltransferase
	UniRef90_P0A4L6	23.1	98.2	13	3	Thiol:disulfide interchange protein DsbA
	UniRef90_P19480	56.2	98.2	5	3	Alkyl hydroperoxide reductase subunit F
	UniRef90_P0ACE1	62.5	98.2	5	3	Hydrogenase-2 large chain
	UniRef90_P00893	63.0	98.9	8	5	Acetolactate synthase isozyme 3 large subunit
	UniRef90_A7ZML6	43.7	82.7	4	2	Succinylornithine transaminase
	UniRef90_P36938	58.4	82.7	3	2	Phosphoglucomutase
G03	UniRef90_Q57T38	30.4	99.1	41	11	Elongation factor Ts
	UniRef90_P0A6C9	27.0	99.1	37	8	Acetylglutamate kinase
	UniRef90_A7MH28	43.3	99.0	20	6	Acetate kinase
	UniRef90_P00370	48.6	99.0	19	6	NADP-specific glutamate dehydrogenase
	UniRef90_P0AEU2	28.5	98.7	18	5	Histidine-binding periplasmic protein
	UniRef90_A7ZPZ4	45.3	99.1	17	7	Serine hydroxymethyltransferase
	UniRef90_Q8XBT6	31.1	99.1	29	8	2,5-diketo-D-gluconic acid reductase A
	UniRef90_P0C8J9	47.1	99.0	13	6	D-tagatose-1,6-bisphosphate aldolase subunit gatZ
	UniRef90_A7ZPS4	31.3	98.7	15	4	Dihydrodipicolinate synthase
	UniRef90_B7UN33	19.0	98.7	27	4	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
	UniRef90_P0A9D6	29.3	98.7	14	4	Serine acetyltransferase
	UniRef90_A7ZHM4	28.2	83.1	9	2	3-methyl-2-oxobutanoate hydroxymethyltransferase
	UniRef90_Q9S5G5	28.5	98.7	5	2	Histidine biosynthesis bifunctional protein HisB
	UniRef90_P0A9T2	44.2	83.0	5	2	D-3-phosphoglycerate dehydrogenase
G04	UniRef90_P00370	48.6	99.1	36	15	NADP-specific glutamate dehydrogenase
	UniRef90_P33570	35.7	99.1	42	12	Transaldolase A
	UniRef90_P02925	31.0	99.0	26	7	D-ribose-binding periplasmic protein
	UniRef90_P0A252	20.8	99.0	44	6	Alkyl hydroperoxide reductase subunit C
	UniRef90_A7FDW9	44.8	99.1	19	8	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
	UniRef90_P78067	48.2	99.0	17	5	Thiosulfate sulfurtransferase YnjE
	UniRef90_Q57RI5	28.6	98.9	22	4	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	UniRef90_Q8XBT6	31.1	99.1	25	7	2,5-diketo-D-gluconic acid reductase A
	UniRef90_P0A919	18.6	98.2	22	3	Outer membrane protein X
	UniRef90_P0AGD5	21.3	99.0	45	6	Superoxide dismutase [Fe]
	UniRef90_A8AK39	16.2	98.7	39	4	6,7-dimethyl-8-ribityllumazine synthase
	UniRef90_P0A9T2	44.2	98.9	11	5	D-3-phosphoglycerate dehydrogenase
	UniRef90_P0ABC8	45.5	98.2	10	3	Protein HflK
	UniRef90_A7ZPZ4	45.3	98.6	8	4	Serine hydroxymethyltransferase
	UniRef90_P0AB92	38.0	82.9	6	2	Phospho-2-dehydro-3-deoxyheptonate aldolase, Phe-sensitive
	UniRef90_B7US97	36.8	83.1	6	2	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_P0AEN0	29.0	98.6	13	3	Cystine-binding periplasmic protein
	UniRef90_A7ZIN4	23.6	83.0	10	2	Adenylate kinase
	UniRef90_P37769	27.1	83.0	9	2	2-dehydro-3-deoxy-D-gluconate 5-dehydrogenase

G05	UniRef90_P0A6C9	27.0	99.1	44	9	Acetylglutamate kinase
	UniRef90_P0C8J9	47.1	99.1	25	10	D-tagatose-1,6-bisphosphate aldolase subunit gatZ
	UniRef90_B7UH18	14.3	99.1	55	8	Autonomous glycyl radical cofactor
	UniRef90_P0A252	20.8	98.7	27	4	Alkyl hydroperoxide reductase subunit C
	UniRef90_P04816	39.4	99.0	18	6	Leucine-specific-binding protein
	UniRef90_P0A797	34.8	99.1	22	8	6-phosphofructokinase isozyme 1
	UniRef90_Q3Z3B7	20.8	98.9	31	4	Flavoprotein WrbA
	UniRef90_A7ZPU1	22.5	98.7	20	5	Uracil phosphoribosyltransferase
	UniRef90_P0ADK2	25.7	98.2	14	3	Uncharacterized protein yiaF
	UniRef90_Q8XEG2	66.9	98.2	5	3	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]
G06	UniRef90_P0AG09	24.6	82.9	8	2	Ribulose-phosphate 3-epimerase
	UniRef90_A8ACN6	50.3	99.1	34	13	ATP synthase subunit beta
	UniRef90_A8ACN8	55.2	99.1	28	12	ATP synthase subunit alpha
	UniRef90_P19480	56.2	99.1	31	14	Alkyl hydroperoxide reductase subunit F
	UniRef90_A7ZPZ4	45.3	99.1	17	7	Serine hydroxymethyltransferase
	UniRef90_P0ADS7	26.6	99.0	25	7	Uncharacterized protein yggE
	UniRef90_P0ACA5	24.3	98.9	25	5	Stringent starvation protein A
	UniRef90_P02925	31.0	98.9	21	5	D-ribose-binding periplasmic protein
	UniRef90_P21367	23.1	98.7	23	4	Uncharacterized protein ycaC
	UniRef90_B7UH18	14.3	99.0	42	7	Autonomous glycyl radical cofactor
	UniRef90_P00370	48.6	98.2	7	3	NADP-specific glutamate dehydrogenase
	UniRef90_A7ZN88	31.2	94.4	12	2	Molecular chaperone Hsp31 and glyoxalase 3
	UniRef90_P0A9T2	44.2	98.3	9	3	D-3-phosphoglycerate dehydrogenase
	UniRef90_P23847	60.3	83.1	4	2	Periplasmic dipeptide transport protein
	UniRef90_P0AFM7	25.5	97.3	11	2	Phage shock protein A
	UniRef90_P0A6C9	27.0	83.1	7	2	Acetylglutamate kinase
	UniRef90_Q8FG52	46.2	98.2	6	3	Histidinol dehydrogenase
	UniRef90_A7ZHG6	57.3	83.1	4	2	2-isopropylmalate synthase
	UniRef90_P09832	52.0	83.0	8	2	Glutamate synthase [NADPH] small chain
	UniRef90_P30859	26.9	83.3	14	2	Putative ABC transporter arginine-binding protein 2
G07	UniRef90_P00370	48.6	99.1	33	14	NADP-specific glutamate dehydrogenase
	UniRef90_A8ACN6	50.3	99.1	28	11	ATP synthase subunit beta
	UniRef90_A7ZSD0	32.3	99.1	29	9	Malate dehydrogenase
	UniRef90_A7ZPS4	31.3	99.0	26	6	Dihydrodipicolinate synthase
	UniRef90_P19480	56.2	99.1	21	10	Alkyl hydroperoxide reductase subunit F
	UniRef90_P04816	39.4	99.1	23	7	Leucine-specific-binding protein
	UniRef90_P58603	35.6	99.1	34	10	Protease 7
	UniRef90_P0AFK9	38.9	99.1	28	10	Spermidine/putrescine-binding periplasmic protein
	UniRef90_P0A9K5	39.0	99.0	22	7	PhoH-like protein
	UniRef90_A7ZHG6	57.3	99.0	14	6	2-isopropylmalate synthase
	UniRef90_P0AEU2	28.5	98.7	17	4	Histidine-binding periplasmic protein
	UniRef90_P78067	48.2	96.8	6	2	Thiosulfate sulfurtransferase YnjE
	UniRef90_A7ZPZ4	45.3	98.2	6	3	Serine hydroxymethyltransferase
	UniRef90_P16657	27.9	83.0	7	2	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI
G08	UniRef90_B7US97	36.8	99.1	48	16	Phenylalanine--tRNA ligase alpha subunit
	UniRef90_P0AET9	26.8	99.1	36	8	7-alpha-hydroxysteroid dehydrogenase
	UniRef90_A8ACN8	55.2	99.1	25	11	ATP synthase subunit alpha
	UniRef90_P0A9K5	39.0	99.1	25	8	PhoH-like protein
	UniRef90_A8AIH5	46.1	99.1	21	9	3-phosphoshikimate 1-carboxyvinyltransferase
	UniRef90_A1AFA2	22.9	98.9	27	5	Ribose-5-phosphate isomerase A
	UniRef90_P0C8J9	47.1	99.1	24	9	D-tagatose-1,6-bisphosphate aldolase subunit gatZ
	UniRef90_A7ZHY1	20.8	99.0	28	6	Phosphoheptose isomerase
	UniRef90_P0ADK2	25.7	98.2	14	3	Uncharacterized protein yiaF
	UniRef90_P23847	60.3	98.7	8	4	Periplasmic dipeptide transport protein
	UniRef90_P30859	26.9	98.2	12	3	Putative ABC transporter arginine-binding protein 2
	UniRef90_P0AG09	24.6	98.2	27	5	Ribulose-phosphate 3-epimerase
	UniRef90_P09832	52.0	81.0	3	2	Glutamate synthase [NADPH] small chain

G09	UniRef90_P58603	35.6	99.1	40	2	Protease 7
	UniRef90_P0ACA5	24.3	99.0	32	6	Stringent starvation protein A
	UniRef90_A7ZPU1	22.5	99.0	36	7	Uracil phosphoribosyltransferase
	UniRef90_P0A919	18.6	98.7	32	4	Outer membrane protein X
	UniRef90_B5XNU6	19.6	99.1	49	8	UPF0304 protein KPK_1463
	UniRef90_A8AIH5	46.1	99.1	21	8	3-phosphoshikimate 1-carboxyvinyltransferase
	UniRef90_P04816	39.4	98.9	14	5	Leucine-specific-binding protein
	UniRef90_B7UN33	19.0	98.9	27	5	3-hydroxydecanoyl-[acyl-carrier-protein] dehydratase
	UniRef90_P0A957	22.3	98.7	20	4	KHG/KDPG aldolase
	UniRef90_P0AEU2	28.5	98.7	21	4	Histidine-binding periplasmic protein
	UniRef90_P0AE54	17.6	98.2	24	3	Putative peroxiredoxin bcp
	UniRef90_P0A4L6	23.1	98.2	13	3	Thiol:disulfide interchange protein DsbA
	UniRef90_P0A6C9	27.0	83.1	7	2	Acetylglutamate kinase
	UniRef90_P0ADI8	20.5	83.1	11	2	Uncharacterized isochorismatase family protein yecD
	UniRef90_P30859	26.9	83.0	7	2	Putative ABC transporter arginine-binding protein 2
	UniRef90_P0AEN0	29.0	82.9	6	2	Cystine-binding periplasmic protein
	UniRef90_P0A9D3	22.9	83.1	10	2	Glutathione S-transferase GstA
G10	UniRef90_Q57T38	30.4	99.1	53	14	Elongation factor Ts
	UniRef90_A7ZSD0	32.3	99.1	35	9	Malate dehydrogenase
	UniRef90_P0A797	34.8	99.1	27	9	6-phosphofructokinase isozyme 1
	UniRef90_B7MUC3	26.4	99.1	37	8	Orotidine 5'-phosphate decarboxylase
	UniRef90_B7UH18	14.3	99.1	61	8	Autonomous glycyl radical cofactor
	UniRef90_A7ZIN4	23.6	99.1	44	8	Adenylate kinase
	UniRef90_A7ZTA0	35.4	98.2	10	3	Glyoxylate/hydroxypyruvate reductase B
G11	UniRef90_A8ACN6	50.3	99.1	38	14	ATP synthase subunit beta
	UniRef90_P0AFK9	38.9	99.1	29	11	Spermidine/putrescine-binding periplasmic protein
	UniRef90_P04816	39.4	99.0	21	6	Leucine-specific-binding protein
	UniRef90_P0AET9	26.8	99.0	26	6	7-alpha-hydroxysteroid dehydrogenase
	UniRef90_Q3Z3B7	20.8	98.9	31	5	Flavoprotein WrbA
	UniRef90_B7MUC3	26.4	99.1	31	7	Orotidine 5'-phosphate decarboxylase
	UniRef90_P0AGD5	21.3	99.0	37	6	Superoxide dismutase [Fe]
	UniRef90_Q57RI5	28.6	98.7	19	4	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	UniRef90_Q8FK07	15.9	83.2	16	3	Universal stress protein G
	UniRef90_B5FI12	15.2	98.7	28	4	Ribosome-binding factor A
	UniRef90_Q9S5G3	40.3	83.0	5	2	Histidine biosynthesis bifunctional protein HisIE
	UniRef90_A7MH28	43.3	83.2	6	2	Acetate kinase
	UniRef90_B5FTC5	27.7	97.9	12	3	Deoxyribose-phosphate aldolase
	UniRef90_Q9S5G5	40.3	83.0	5	2	Histidine biosynthesis bifunctional protein HisB
	UniRef90_P0AFU8	23.4	80.7	9	2	Riboflavin synthase alpha chain
G12	UniRef90_P33570	35.7	99.1	22	7	Transketolase 2
	UniRef90_A7ZHG6	57.3	99.1	15	7	2-isopropylmalate synthase
	UniRef90_B5XNU6	19.6	99.1	42	8	UPF0304 protein KPK_1463
	UniRef90_B7UH18	14.3	99.0	45	6	Autonomous glycyl radical cofactor
	UniRef90_A7ZHY1	20.8	99.0	30	6	Phosphoheptose isomerase
	UniRef90_Q57T38	30.4	99.1	27	9	Elongation factor Ts
	UniRef90_A7ZN88	31.2	98.7	16	4	Molecular chaperone Hsp31 and glyoxalase 3
	UniRef90_Q9S5G3	22.8	98.1	17	3	Histidine biosynthesis bifunctional protein HisIE
	UniRef90_A7ZSD0	32.3	98.2	12	3	Malate dehydrogenase

Row H	Cluster ID	MW (kDa)*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
H01	UniRef90_P0AEN0	29.0	99.1	49	11	Cystine-binding periplasmic protein
	UniRef90_Q57RI5	28.6	99.1	34	8	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	UniRef90_A7MQX5	41.4	99.1	31	11	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_P0A797	34.8	99.1	27	9	6-phosphofructokinase isozyme 1

	UniRef90_P18815	43.4	99.1	25	10	Maltose-binding periplasmic protein
	UniRef90_P04693	43.5	99.1	21	7	Aromatic-amino-acid aminotransferase
	UniRef90_P0AD98	39.1	99.0	19	6	Leu/Ile/Val-binding protein
	UniRef90_P23847	60.3	99.0	15	6	Periplasmic dipeptide transport protein
	UniRef90_P12758	27.2	98.9	19	5	Uridine phosphorylase
	UniRef90_P0A252	20.8	98.7	29	4	Alkyl hydroperoxide reductase subunit C
	UniRef90_P30860	26.8	98.2	12	3	ABC transporter arginine-binding protein 1
	UniRef90_Q3Z606	35.2	83.1	6	2	Transaldolase 1
	UniRef90_P0A4L6	23.1	93.4	21	2	Thiol:disulfide interchange protein DsbA
H02	UniRef90_Q3Z606	35.2	99.1	49	17	Transaldolase 1
	UniRef90_A8ALC4	29.9	99.1	46	12	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_B7LWN7	37.2	99.1	31	11	Outer membrane protein A
	UniRef90_P0AD98	39.1	99.0	19	7	Leu/Ile/Val-binding protein
	UniRef90_P0AFM7	25.5	99.0	27	6	Phage shock protein A
	UniRef90_P0A797	34.8	99.1	22	8	6-phosphofructokinase isozyme 1
	UniRef90_Q8X505	27.3	98.7	17	4	NADP-dependent L-serine/L-allo-threonine dehydrogenase ydfG
	UniRef90_A7MQX5	41.4	99.0	19	6	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_B5QX24	46.0	98.7	8	4	Protein TolB
	UniRef90_P0AFH9	21.1	98.2	17	3	Osmotically-inducible protein Y
	UniRef90_P0ACA5	24.3	98.2	17	3	Stringent starvation protein A
	UniRef90_Q9S5G5	40.3	98.9	13	5	Histidine biosynthesis bifunctional protein HisB
	UniRef90_P23847	60.3	98.7	8	4	Periplasmic dipeptide transport protein
	UniRef90_P0AD60	16.9	83.1	10	2	Inhibitor of vertebrate lysozyme
H03	UniRef90_B7LWN7	37.2	99.1	33	12	Outer membrane protein A
	UniRef90_P0AEN0	29.0	99.1	41	10	Cystine-binding periplasmic protein
	UniRef90_A7ZSD0	32.3	99.1	35	10	Malate dehydrogenase
	UniRef90_P00805	36.9	99.1	25	8	L-asparaginase 2
	UniRef90_A7ZII0	18.3	99.1	55	8	UPF0234 protein yajQ
	UniRef90_B5QX24	46.0	99.1	21	7	Protein TolB
	UniRef90_A7MH28	43.3	99.0	22	6	Acetate kinase
	UniRef90_P0A957	22.3	99.0	34	6	KHG/KDPG aldolase
	UniRef90_P0AE20	29.3	99.0	27	6	Methionine aminopeptidase
	UniRef90_Q9S5G5	40.3	99.0	15	6	Histidine biosynthesis bifunctional protein HisB
	UniRef90_A7ZUD3	27.0	98.7	17	4	Triosephosphate isomerase
	UniRef90_A7ZN88	31.2	98.2	12	3	Molecular chaperone Hsp31 and glyoxalase 3
	UniRef90_P0A6F2	41.4	98.2	9	3	Carbamoyl-phosphate synthase small chain
	UniRef90_P0A2K8	32.8	83.2	7	2	UTP--glucose-1-phosphate uridylyltransferase
	UniRef90_A8ALA3	35.2	83.2	6	2	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha
	UniRef90_P16657	27.9	83.2	7	2	Enoyl-[acyl-carrier-protein] reductase [NADH] FabI
	UniRef90_P0A4L6	23.1	83.0	10	2	Thiol:disulfide interchange protein DsbA
	UniRef90_P00509	43.6	68.0	5	2	Aspartate aminotransferase
H04	UniRef90_Q3Z606	35.2	99.1	55	17	Transaldolase 1
	UniRef90_P00370	48.6	99.1	33	13	NADP-specific glutamate dehydrogenase
	UniRef90_P0AEN0	29.0	99.1	41	10	Cystine-binding periplasmic protein
	UniRef90_A7ZII0	18.3	99.0	45	7	UPF0234 protein yajQ
	UniRef90_P0AGD5	21.3	99.0	35	6	Superoxide dismutase [Fe]
	UniRef90_Q8FG52	46.2	99.0	14	6	Histidinol dehydrogenase
	UniRef90_P0ADE7	16.1	99.0	40	6	Uncharacterized protein ygaU
	UniRef90_P0AD98	39.1	99.0	19	6	Leu/Ile/Val-binding protein
	UniRef90_P0C0L2	15.1	98.7	27	4	Peroxioredoxin osmC
	UniRef90_P00509	43.6	99.0	15	6	Aspartate aminotransferase
	UniRef90_P04693	43.5	98.7	10	4	Aromatic-amino-acid aminotransferase
	UniRef90_A8AFH1	27.8	98.0	19	3	tRNA (cmo5U34)-methyltransferase
	UniRef90_P0ACG0	15.5	98.7	30	4	DNA-binding protein H-NS
	UniRef90_A7FDW9	44.8	98.2	5	3	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
H05	UniRef90_A7MQX5	41.4	99.1	51	19	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_A8ACN8	55.2	99.1	33	15	ATP synthase subunit alpha
	UniRef90_Q57T38	30.4	99.1	46	13	Elongation factor Ts
	UniRef90_A8ALC4	29.9	99.1	41	11	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-

	UniRef90_Q8FG52	46.1	99.1	21	8	succinyltransferase
	UniRef90_P09551	28.0	99.1	35	8	Histidinol dehydrogenase
	UniRef90_P0A957	22.3	99.0	34	6	Lysine-arginine-ornithine-binding periplasmic protein
	UniRef90_A7ZPS1	27.0	99.1	38	8	KHG/KDPG aldolase
	UniRef90_P0A9R0	40.0	99.0	19	5	Phosphoribosylaminoimidazole-succinocarboxamide synthase
	UniRef90_P04693	43.5	99.0	16	6	Aspartate-semialdehyde dehydrogenase
	UniRef90_A8ACN6	50.3	99.0	15	6	Aromatic-amino-acid aminotransferase
	UniRef90_Q3Z3B7	20.8	98.2	16	3	ATP synthase subunit beta
	UniRef90_P0AD98	39.1	83.0	5	2	Flavoprotein WrbA
H06	NA	NA	NA			Leu/Ile/Val-binding protein
H07	UniRef90_P00370	48.6	99.1	31	12	NA
	UniRef90_Q57R15	28.6	99.1	34	8	NADP-specific glutamate dehydrogenase
	UniRef90_P18815	43.4	99.1	33	12	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
	UniRef90_A8ALS4	28.7	99.1	33	7	Maltose-binding periplasmic protein
	UniRef90_P0AD98	39.1	99.0	21	6	Dihydrodipicolinate reductase
	UniRef90_P09551	28.0	99.1	35	8	Leu/Ile/Val-binding protein
	UniRef90_P0A6F2	41.4	99.0	19	6	Lysine-arginine-ornithine-binding periplasmic protein
	UniRef90_A7ZPU1	22.5	99.0	39	7	Carbamoyl-phosphate synthase small chain
	UniRef90_P0AFM7	25.5	99.1	34	8	Uracil phosphoribosyltransferase
	UniRef90_A7MH28	43.3	98.9	16	5	Phage shock protein A
	UniRef90_B5QX24	46.0	99.1	18	7	Acetate kinase
	UniRef90_A7ZPS1	27.0	99.0	25	5	Protein TolB
	UniRef90_P0A7B0	19.7	99.0	34	6	Phosphoribosylaminoimidazole-succinocarboxamide synthase
	UniRef90_A7ZIM8	19.9	98.7	25	4	Inorganic pyrophosphatase
	UniRef90_Q3Z3B7	20.8	98.2	16	3	Adenine phosphoribosyltransferase
	UniRef90_P0A957	22.3	98.7	19	4	Flavoprotein WrbA
	UniRef90_A7FDW9	44.8	99.0	13	6	KHG/KDPG aldolase
	UniRef90_P0A9T2	44.2	98.2	7	3	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
H08	UniRef90_P0ADG8	52.0	99.1	35	11	D-3-phosphoglycerate dehydrogenase
	UniRef90_Q3Z606	35.2	99.1	40	13	Inosine-5'-monophosphate dehydrogenase
	UniRef90_Q7N590	34.2	99.1	34	8	Transaldolase 1
	UniRef90_A8ALS4	28.7	99.1	34	7	Ribose-phosphate pyrophosphokinase
	UniRef90_P23847	60.3	99.1	19	10	Dihydrodipicolinate reductase
	UniRef90_P00509	43.6	99.1	32	12	Periplasmic dipeptide transport protein
	UniRef90_Q3Z3B7	20.8	98.9	31	6	Aspartate aminotransferase
	UniRef90_P0AD98	39.1	99.0	19	6	Flavoprotein WrbA
	UniRef90_A8ACN8	55.2	99.1	15	7	Leu/Ile/Val-binding protein
	UniRef90_A7ZUD3	27.0	98.9	21	6	ATP synthase subunit alpha
	UniRef90_P0AFH9	21.1	98.7	22	4	Triosephosphate isomerase
	UniRef90_A7ZH92	33.6	99.1	22	7	Osmotically-inducible protein Y
	UniRef90_P0A9R0	40.0	98.7	15	4	Homoserine kinase
	UniRef90_P04693	43.5	98.7	11	4	Aspartate-semialdehyde dehydrogenase
	UniRef90_P60596	21.6	98.1	15	3	Aromatic-amino-acid aminotransferase
H09	UniRef90_A8ALC4	29.9	99.1	46	12	Imidazole glycerol phosphate synthase subunit HisH
	UniRef90_A7ZSD0	32.3	99.1	42	10	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
	UniRef90_P0AD98	39.1	99.0	19	6	Malate dehydrogenase
	UniRef90_P0A252	20.8	98.9	38	5	Leu/Ile/Val-binding protein
	UniRef90_B7UH18	14.3	99.0	46	5	Alkyl hydroperoxide reductase subunit C
	UniRef90_A7ZH92	33.6	99.0	20	6	Autonomous glycyl radical cofactor
	UniRef90_P0AGD5	21.3	99.0	37	6	Homoserine kinase
	UniRef90_A7ZPS1	27.0	98.8	23	4	Superoxide dismutase [Fe]
	UniRef90_P0A9R0	40.0	98.7	14	4	Phosphoribosylaminoimidazole-succinocarboxamide synthase
	UniRef90_P0A7B0	19.7	99.0	34	6	Aspartate-semialdehyde dehydrogenase
	UniRef90_P0AEU2	28.5	98.2	14	3	Inorganic pyrophosphatase
						Histidine-binding periplasmic protein

	UniRef90_A7MQX5	41.4	87.3	8	2	Succinyl-CoA ligase [ADP-forming] subunit beta
	UniRef90_P00805	36.9	83.2	6	2	L-asparaginase 2
H10	UniRef90_Q3Z606	35.2	99.1	49	16	Transaldolase 1
	UniRef90_B7L7NW7	37.2	99.1	35	12	Outer membrane protein A
	UniRef90_P33570	35.7	99.1	29	12	Transketolase 2
	UniRef90_Q7N590	34.2	99.1	32	7	Ribose-phosphate pyrophosphokinase
	UniRef90_P12758	27.2	99.0	26	6	Uridine phosphorylase
	UniRef90_P0AEU2	28.5	98.9	28	5	Histidine-binding periplasmic protein
	UniRef90_P21599	51.4	98.9	10	5	Pyruvate kinase II
	UniRef90_P23847	60.3	98.7	8	4	Periplasmic dipeptide transport protein
	UniRef90_P0AF94	13.6	98.2	24	3	Enamine/imine deaminase
	UniRef90_P0A7B0	19.7	98.8	27	4	Inorganic pyrophosphatase
	UniRef90_A8ACN8	55.2	82.8	6	2	ATP synthase subunit alpha
	UniRef90_A7ZUD3	27.0	70.7	7	2	Triosephosphate isomerase
H11	UniRef90_P0AEN0	29.0	99.1	41	10	Cystine-binding periplasmic protein
	UniRef90_P33570	35.7	99.1	34	13	Transketolase 2
	UniRef90_B7L7NW7	37.2	99.1	21	8	Outer membrane protein A
	UniRef90_Q57T38	30.4	99.1	33	9	Elongation factor Ts
	UniRef90_P0ABZ8	47.3	99.1	22	8	Chaperone SurA
	UniRef90_A7ZPU1	22.5	99.0	36	7	Uracil phosphoribosyltransferase
	UniRef90_A7ZPS1	27.0	99.0	30	6	Phosphoribosylaminoimidazole-succinocarboxamide synthase
	UniRef90_B7UH18	14.3	99.1	65	6	Autonomous glycol radical cofactor
	UniRef90_Q3Z3B7	20.8	98.9	31	4	Flavoprotein WrbA
	UniRef90_P0C0L2	15.1	98.2	20	3	Peroxiredoxin OsmC
	UniRef90_A7ZPZ4	45.3	98.1	6	3	Serine hydroxymethyltransferase
	UniRef90_P0A957	22.3	82.7	8	2	KHG/KDPG aldolase
H12	NA	NA	NA	NA	NA	NA

*MW values were calculated from their amino acid sequence, derived from the accession no., using the ExPASy Compute pI/MW tool [103]

Table B 4. Identified MG1655 Proteins (Singlet). A 96-well plate with 1 gel piece per well. The table displays the identifications in each well as analyzed using PEAKS. Highlighted in green are protein IDs that were also confirmed in the multiplex plate, refer to **Table 3-4**. Samples where identification was unsuccessful are denoted as NA.

	Spot Id	Cluster ID	MW (kDa)*	pI*	Score %	Sequence Coverage (%)	# of Unique Peptides	Protein Name
A01	4212	NA	NA	NA	NA	NA	NA	NA
A02	2506	UniRef90_P18815	38.9	5.3	99.0	22	7	Maltose-binding periplasmic protein
A03	1512	UniRef90_P66794	35.0	5.2	99.0	19	6	Selenide, water dikinase
		UniRef90_P58603	35.0	5.2	99.0	21	6	Protease 7
A04	3506	UniRef90_P00888	37.9	5.5	98.2	8	3	Phospho-2-dehydro-3-deoxyheptonate aldolase, Tyr-sensitive
		UniRef90_A8AIH6	37.9	5.5	83.0	6	2	Phosphoserine aminotransferase
A05	2705	NA	NA	NA	NA	NA	NA	NA
A06	2516	UniRef90_A7ZHR7	35.1	5.3	99.0	12	10	Outer membrane protein assembly factor BamA
		UniRef90_P0A797	35.1	5.3	99.0	16	6	6-phosphofructokinase
		UniRef90_A8AIH6	35.1	5.3	82.4	6	2	Phosphoserine aminotransferase
A07	3511	UniRef90_P27833	39.7	5.7	98.2	9	3	Lipopolysaccharide biosynthesis protein RffA

		UniRef90_Q0T204	39.7	5.7	98.2	8	3	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
		UniRef90_P0A9K5	39.7	5.7	82.6	5	2	PhoH-like protein
A08	3209	UniRef90_P22731	26.3	5.6	83.2	14	2	High-affinity branched-chain amino acid transport ATP-binding protein LivF
A09	2519	UniRef90_P04693	41.3	5.3	98.2	8	3	Aromatic-amino-acid aminotransferase
A10	2220	UniRef90_A7ZPU1	22.5	5.3	98.2	12	4	Uracil phosphoribosyltransferase
A11	4211	UniRef90_P75736	26.7	6.0	98.7	18	4	Esterase ybfF
A12	4720	NA	NA	NA	NA	NA	NA	NA
B01	1024	UniRef90_A8AK39	15.3	5.2	83.3	18	2	6,7-dimethyl-8-ribityllumazine synthase
B02	1602	UniRef90_B5Y275	44.6	5.2	83.0	5	2	Phosphopentomutase
B03	1109	UniRef90_P0A9W9	20.7	5.2	99.0	38	6	Protein YrdA
B04	1116	NA	NA	NA	NA	NA	NA	NA
B05	3211	UniRef90_P59735	27.1	5.7	98.9	22	5	3'(2'),5'-bisphosphate nucleotidase CysQ
		UniRef90_A7ZUD3	27.1	5.7	83.0	12	2	Triosephosphate isomerase
B06	4602	NA	NA	NA	NA	NA	NA	NA
B07	4704	UniRef90_P0ADG8	59.1	6.0	99.0	15	6	Inosine-5'-monophosphate dehydrogenase
		UniRef90_P23847	59.1	6.0	98.3	7	3	Periplasmic dipeptide transport protein
B08	3202	UniRef90_P12758	27.2	5.4	98.1	11	3	Uridine phosphorylase
		UniRef90_P30843	27.2	5.4	87.3	17	2	Transcriptional regulatory protein BasR
B09	706	UniRef90_P77804	54.6	5.1	98.2	6	3	Protein ydgA
B10	2012	NA	NA	NA	NA	NA	NA	NA
B11	3512	UniRef90_A7MH28	43.6	5.7	82.9	6	2	Acetate kinase
		UniRef90_Q8X4S6	43.6	5.7	82.9	5	2	Acetylornithine/succinyldiaminopimelate aminotransferase
B12	3806	NA	NA	NA	NA	NA	NA	NA
C01	4808	NA	NA	NA	NA	NA	NA	NA
C02	3011	UniRef90_P0AF94	10.9	5.6	98.2	24	3	Enamine/imine deaminase
C03	2314	UniRef90_P0AEJ3	31.0	5.3	98.7	11	4	Isochorismate synthase entC
		UniRef90_P58603	31.0	5.3	99.0	27	8	Protease 7
C04	2311	UniRef90_A8ALC4	34.5	5.3	99.0	33	9	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
		UniRef90_P0ADS7	34.5	5.3	98.9	19	5	Uncharacterized protein yggE
C05	4714	NA	NA	NA	NA	NA	NA	NA
C06	4206	UniRef90_P76034	27.8	6.0	99.0	32	7	Uncharacterized HTH-type transcriptional regulator YciT
		UniRef90_P09126	27.8	6.0	99.0	30	7	Uroporphyrinogen-III synthase
		UniRef90_Q57RI5	27.8	6.0	98.1	13	3	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
		UniRef90_A7ZIN4	27.8	6.0	82.5	19	2	Adenylate kinase
C07	4804	UniRef90_P46837	85.1	6.0	98.7	5	4	Protein yhgF
C08	2518	UniRef90_P0AEJ3	43.0	5.3	98.9	13	5	Isochorismate synthase entC
C09	4313	NA	NA	NA	NA	NA	NA	NA
C10	3102	UniRef90_P67097	21.3	5.4	98.2	23	3	Phosphodiesterase yfcE
C11	3505	UniRef90_P00509	39.4	5.5	99.1	33	13	Aspartate aminotransferase
		UniRef90_Q0T204	39.4	5.5	99.1	25	9	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase
		UniRef90_A8AKV5	39.4	5.5	98.9	15	5	tRNA (uracil(54)-C(5))-methyltransferase
		UniRef90_P07023	39.4	5.5	98.7	10	4	T-protein
		UniRef90_P76223	39.4	5.5	98.4	10	3	Protein ynjB
		UniRef90_P27830	39.4	5.5	82.6	6	2	dTDP-glucose 4,6-dehydratase 2
C12	1120	UniRef90_P0AGD5	21.6	5.2	98.1	14	3	Superoxide dismutase [Fe]
D01	4415	UniRef90_P22715	35.0	6.1	98.0	9	3	UDP-glucose 4-epimerase

D02	4505	UniRef90_A7ZVR0	35.1	6.0	98.7	12	4	Ribosomal RNA small subunit methyltransferase C
		UniRef90_B7US97	35.1	6.0	82.5	8	2	Phenylalanine--tRNA ligase alpha subunit
D03	2612	UniRef90_A1AC10	43.9	5.3	99.0	19	7	Phosphoribosylglycinamide formyltransferase 2
		UniRef90_A8AIH5	43.9	5.3	82.7	5	2	3-phosphoshikimate 1-carboxyvinyltransferase
D04	3801	UniRef90_Q8XEG2	66.8	5.4	80.7	3	2	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]
D05	2217	UniRef90_A1AIG4	22.5	5.3	83.1	11	2	Thiamine-phosphate synthase
D06	505	UniRef90_B5Y275	43.7	5.1	98.7	11	4	Phosphopentomutase
D07	3510	UniRef90_A7MH28	43.0	5.6	83.0	6	2	Acetate kinase
		UniRef90_B5QX24	43.0	5.6	83.0	4	2	Protein TolB
		UniRef90_Q9S5G5	43.0	5.6	82.9	5	2	Histidine biosynthesis bifunctional protein HisB
D08	3220	UniRef90_A7FGQ6	27.0	5.9	99.0	34	7	NADH-quinone oxidoreductase subunit B
		UniRef90_P0AEN0	27.0	5.9	97.1	9	2	Cystine-binding periplasmic protein
D09	2507	UniRef90_A7MQX5	41.6	5.3	99.1	32	11	Succinyl-CoA ligase [ADP-forming] subunit beta
		UniRef90_P0AEJ3	41.6	5.3	99.1	25	9	Isochorismate synthase entC
D10	3518	UniRef90_P0AF09	38.3	5.8	99.0	19	6	Protein mrp
		UniRef90_B7MG93	38.3	5.8	82.6	6	2	Chorismate synthase
D11	1412	UniRef90_P0AEX1	34.8	5.2	98.2	8	3	1-phosphofructokinase
D12	1706	NA	NA	NA	NA	NA	NA	NA
E01	705	NA	NA	NA	NA	NA	NA	NA
E02	4511	UniRef90_A7ZI01	37.6	6.0	99.0	16	6	Glutamate 5-kinase
		UniRef90_P0A9S4	37.6	6.0	81.9	6	2	Galactitol-1-phosphate 5-dehydrogenase
E03	3709	UniRef90_P0ADG8	59.5	5.8	98.7	11	4	Inosine-5'-monophosphate dehydrogenase
E04	4315	UniRef90_A8ADK6	34.5	6.0	99.0	21	6	Glucokinase
E05	2707	NA	NA	NA	NA	NA	NA	NA
E06	217	UniRef90_P67271	24.4	5.1	83.1	13	2	UPF0135 protein ybgI
E07	3018	UniRef90_Q46868	12.5	5.7	98.2	25	3	Uncharacterized protein yqiC
E08	210	UniRef90_Q32EF3	23.2	5.1	98.7	18	4	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase
E09	2510	UniRef90_P00509	42.4	5.3	98.9	12	5	Aspartate aminotransferase
		UniRef90_P0AD98	42.4	5.3	98.5	13	3	Leu/Ile/Val-binding protein
E10	2316	UniRef90_Q8FF18	30.5	5.3	98.2	14	3	Pyridoxine 5'-phosphate synthase
E11	4309	UniRef90_P0AEZ1	34.3	6.0	98.9	20	5	5,10-methylenetetrahydrofolate reductase
E12	2512	UniRef90_P09029	35.1	5.3	99.0	17	5	N5-carboxyaminoimidazole ribonucleotide synthase
		UniRef90_Q8X732	35.1	5.3	98.2	8	3	N-acetyl-gamma-glutamyl-phosphate reductase
		UniRef90_P58603	35.1	5.3	98.2	8	3	Protease 7
F01	4202	NA	NA	NA	NA	NA	NA	NA
F02	219	UniRef90_A8ALC9	25.9	5.1	98.2	18	3	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
F03	3410	NA	NA	NA	NA	NA	NA	NA
F04	2002	NA	NA	NA	NA	NA	NA	NA
F05	4705	UniRef90_P07003	62.0	6.0	98.7	7	4	Pyruvate dehydrogenase [ubiquinone]
F06	4701	UniRef90_A8ACN8	57.8	5.9	99.1	18	8	ATP synthase subunit alpha
		UniRef90_P0ADG8	57.8	5.9	82.8	5	2	Inosine-5'-monophosphate dehydrogenase
		UniRef90_P23847	57.8	5.9	98.7	8	4	Periplasmic dipeptide transport protein

F07	4713	UniRef90_P0ADG8	59.6	6.0	99.0	15	6	Inosine-5'-monophosphate dehydrogenase
		UniRef90_P0ACE1	59.6	6.0	98.9	10	5	Hydrogenase-2 large chain
F08	2408	UniRef90_A7ZTA0	35.0	5.3	83.1	8	2	Glyoxylate/hydroxypyruvate reductase B
F09	2808	NA	NA	NA	NA	NA	NA	NA
F10	2701	UniRef90_P25526	52.9	5.2	98.7	8	4	Succinate-semialdehyde dehydrogenase [NADP(+)] GabD
F11	4307	UniRef90_P0AEZ1	33.1	6.0	98.9	16	5	5,10-methylenetetrahydrofolate reductase
F12	4004	UniRef90_A8API1	11.0	5.9	83.0	20	2	Probable Fe(2+)-trafficking protein
G01	1407	UniRef90_Q57T38	34.7	5.2	99.0	33	10	Elongation factor Ts
G02	4303	UniRef90_P02925	29.0	5.9	98.9	21	5	D-ribose-binding periplasmic protein
G03	2611	UniRef90_P0C8J9	50.2	5.3	99.0	17	7	D-tagatose-1,6-bisphosphate aldolase subunit gatZ
G04	1203	UniRef90_A1AFA2	22.4	5.2	98.9	27	5	Ribose-5-phosphate isomerase A
G05	1011	UniRef90_P0A919	16.4	5.2	98.2	22	3	Outer membrane protein X
G06	8	NA	NA	NA	NA	NA	NA	NA
G07	2009	NA	NA	NA	NA	NA	NA	NA
G08	4111	UniRef90_A7ZHY1	21.8	6.0	99.1	51	9	Phosphoheptose isomerase
G09	1101	NA	NA	NA	NA	NA	NA	NA
G10	116	UniRef90_P0A252	21.2	5.1	98.7	29	4	Alkyl hydroperoxide reductase subunit C
G11	1610	UniRef90_A8AIH5	44.0	5.2	99.1	24	10	3-phosphoshikimate 1-carboxyvinyltransferase
		UniRef90_B7N6B0	44.0	5.2	82.7	5	2	Peptidase B
G12	4118	UniRef90_B5XNU6	19.4	6.1	99.0	42	8	UPF0304 protein KPK_1463
H01	2013	NA	NA	NA	NA	NA	NA	NA
H02	2509	NA	NA	NA	NA	NA	NA	NA
H03	3006	NA	NA	NA	NA	NA	NA	NA
H04	4214	UniRef90_Q57RI5	28.1	6.0	99.0	34	8	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase
H05	2301	UniRef90_A7ZH92	34.4	5.2	99.0	22	7	Homoserine kinase
		UniRef90_A8ALG8	34.4	5.2	98.2	13	3	Spermidine synthase
		UniRef90_A8ALC4	34.4	5.2	98.2	12	3	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
H06	3704	NA	NA	NA	NA	NA	NA	NA
H07	1507	NA	NA	NA	NA	NA	NA	NA
H08	309	NA	NA	NA	NA	NA	NA	NA
H09	4506	UniRef90_B5QX24	42.3	6.0	99.1	25	9	Protein TolB
		UniRef90_Q9S5G5	42.3	6.0	99.0	20	7	Histidine biosynthesis bifunctional protein HisB
		UniRef90_A7MH28	42.3	6.0	98.2	12	3	Acetate kinase
		UniRef90_P08390	42.3	6.0	82.7	7	2	USG-1 protein
		UniRef90_P00509	42.3	6.0	82.7	6	2	Aspartate aminotransferase
H10	5	NA	NA	NA	NA	NA	NA	NA
H11	2109	UniRef90_Q3Z3B7	22.2	5.3	99.0	40	7	Flavoprotein WrbA
		UniRef90_P0A957	22.2	5.3	98.7	20	4	KHG/KDPG aldolase
		UniRef90_P60596	22.2	5.3	98.6	23	4	Imidazole glycerol phosphate synthase subunit HisH
H12	2115	UniRef90_P0A4L6	21.7	5.3	98.2	13	3	Thiol:disulfide interchange protein DsbA
		UniRef90_A7ZIJ5	21.7	5.3	82.6	7	2	ATP-dependent Clp protease proteolytic subunit

*MW and pI of the identified proteins were estimated from their gel locations and compared to calculated values using ExPASy Compute pI/MW tool [104].